Human Proteome Organization
17th Annual World Congress

HUPO 2018

17th Annual World Congress of the Human Proteome Organization

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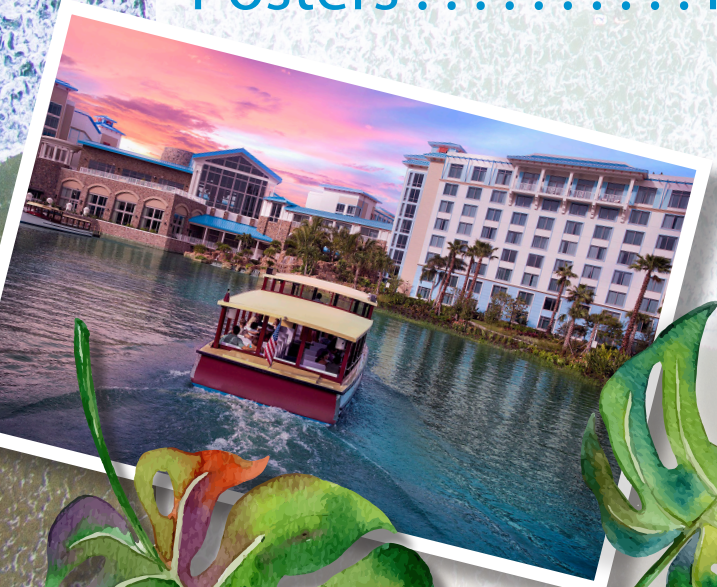
BOOK OF ABSTRACTS



10:30 am - 12:20 pm Monday
AFFINITY, PROXIMITY, & SPATIAL PROTEOMICS
Session Chairs: Anne-Claude Gingras and Michael Washburn
Oceana 8-10

MOA am 10:30
Capturing the RNA binding proteome in time and space
Kathryn Lilley
University of Cambridge, Cambridge, United Kingdom

mRNA species are spatially located and translated in a highly controlled manner and is reliant on the interaction of cis- and trans- acting factors with RNA-binding proteins (RBPs). Aberrant translation of proteins in the wrong location underpins multiple disease states. In order to characterise intracellular RNA location, efficient extraction of RNA species and their coordinating protein binding partners is essential. RBP capture has centred round UV crosslinking RNA to protein coupled and enrichment with oligo(dT)-coupled beads, an approach which favours mRNAs over other RNA species. More recent methods have employed the use of specialised bases incorporated into RNA during in vivo transcription followed by biotinylation.

Here we present a simple method to extract UV crosslinked RBPs that does not require the incorporation of specialised bases or the presence of 3' poly A tails. This highly efficient unbiased method, the orthogonal organic phase separation (OOPS) method, enables reproducible recovery of RBPs or protein-bound RNA (PBR), free protein and free RNA, from a single sample, and is compatible with downstream proteomics and RNA sequencing. We demonstrate, using OOPS, that all long RNAs in the cell are bound by proteins and identify protein-binding sites across the transcriptome in a high throughput manner. Furthermore, we reveal that RBPs are present across all membrane-bound organelles. As OOPS does require the presence of 3' poly A tails, we show its versatility by cataloguing bacterial RBPs. As OOPS dramatically reduces the amount of sample required per experiment, we also demonstrate that we can access changes in the RNA-binding proteome upon cell cycle arrest, shedding light on cellular mechanisms that are influenced by the dynamic interactions of RNA and protein.

Finally, we show that subcellular fractionation methods such are LOPIT (localisation of organelles proteins using isotope tagging), are compatible with OOPS, obtaining spatial information for RBPs in UV-cross-linked cells.

MOA am 10:50
Invited talk for Affinity, Proximity, and Spatial Proteomics
Bernd Wollscheid
ETH Zurich, Zurich, Switzerland

MOA am 11:10
Proximity Assays to Annotate Oncogenic Signaling-Associated Complexes: A Path towards Clinical Implementation
Matthew Smith[1]; Brian Kelly[2]; Nathan Polaske[2]; Yuri Belosludstev[2]; Theresa Boyle[1]; Y. Ann Chen[1]; Eric Haura[1]
[1]Moffitt Cancer Center, Tampa, Florida; [2]TRED, Roche Tissue Diagnostics, Tuscon, AZ

Protein complexes are major biological effectors and often dysregulated in cancer. However, the ability to detect and annotate protein complexes in human tissues has been technically challenging. Using proximity-based readouts, we have previously shown proof-of-principle that detection of signaling-associated complexes may predict response to targeted therapeutics in lung cancer. Fluorescence-based proximity ligation assay approaches revealed a predictive capacity of EGFR:GRB2 signaling complexes for response to EGFR inhibitors (Science Signaling 2015) and MET:GRB2 signaling complexes could identify response to MET inhibition in cell lines, PDX models and a patient treated at our institute (Clinical Cancer Research 2018). However, fluorescence-based readouts are difficult to implement in a clinical setting and are not conducive to pathology laboratory workflow. Here, we demonstrate a novel proximity-based technology platform utilizing a "caged hapten" strategy. Reagents consist of mouse and rabbit secondary antibodies conjugated to alkaline phosphatase and a hapten modified by an enzyme-labile caging group, which when in proximity to alkaline phosphatase is detectable by chromogenic immunohistochemistry. These reagents and assays are fully automated on the Ventana DISCOVERY ULTRA autostainer instruments currently used worldwide in pathology labs. We provide proof of concept for multiple proximity pairs in formalin-fixed paraffin embedded (FFPE) cell blocks, PDX models, and lung cancer patient specimens. We confirm assay specificity through technical controls involving reagent omission experiments and treatment with small molecule kinase inhibitors that disrupt kinase:adaptor interactions. We reveal heterogeneous EGFR and MET signaling in MET-amplified PDX specimens. For EGFR:GRB2, we developed a pathologist-guided ordinal scoring system and evaluated inter-rater concordance. We show predictive capacity of EGFR:GRB2 for identifying response to cetuximab in a PDX cohort (N=9). Current work is focused on establishing the reproducibility and sensitivity of the assay with the future goal of performing molecular correlate studies in annotated clinical trial-associated specimen sets.

MOA am 11:22
Interactomic analysis of VAV1, a key signaling molecules of the TCR signaling pathway in primary T cells
Guillaume Gaud[2]; Romain Roncagalli[3]; Karima Chaoui[1]; Céline Colacios[2]; Sahar Kassem[2]; Bernard Monsarrat[1]; Odile Burlet-Schiltz[1]; Anne Gonzalez De Peredo[1]; Bernard Malissen[3]; Abdelhadi Saoudi[2]
[1]Institute of Pharmacology and Structural Biology, Toulouse, France; [2]Centre de Physiopathologie de Toulouse Purpan, Toulouse, France; [3]Centre d'Immunologie de Marseille-Luminy, Marseille, France

The T-cell receptor (TCR) pathway is essential for the development and function of T cells, and involves a complex network of signaling cascades, tightly regulated to ensure immune tolerance. Although many proteins involved in TCR signaling have been identified, the general organization of the TCR signalosome and its temporal dynamics remain incompletely understood. Proteomics methods based on affinity purification coupled to mass spectrometry allow the systematic mapping of protein-protein interactions networks, and we used them to obtain a detailed picture of the assembly and the internal organization of key molecular complexes formed upon TCR engagement (1,2,3).

Here, we present more specifically the proteomic characterization of protein complexes dynamically associating around VAV1, a key TCR signaling molecule. We used genetically modified mice that express the VAV1 bait protein bearing a C-terminal One-Strep tag (OST) to purify the protein

complexes that assemble around the bait at different time points following activation of primary CD4+ T cells. Label-free quantitative comparison with appropriate controls prepared from WT mice allowed to characterize specific binding partners, which were clustered according to their binding kinetic profiles over 600 seconds of TCR activation. Major clusters contained interactors that bound to VAV1 between 30 s and 2 min, including kinases and adaptor proteins involved in signal initiation and propagation, while late clusters were mainly composed of phosphatases likely involved in the termination of TCR responses. We also detected a very early, transient, and previously unreported interaction with CD226, a costimulatory molecule of immune cells. Further studies indicated that engagement of CD226 induces the tyrosine phosphorylation of VAV1 and synergizes with TCR signals to specifically enhance IL-17 production by human primary CD4 T cells (4).

1. Reginald, J.Immunology 2015

2. Voisinne, Mol Syst Biol 2016

3. Zvezdova, Science Signal 2016

4. Gaud, Science Signal, in press

MOA am 11:34

Complex complexes: diversification of Sin3 HDAC complexes via protein paralogs and isoforms.

Mark Adams[1]; Charles Banks[1]; Mihaela Sardiu[1]; Janet Thornton[1]; Cassandra Eubanks[1]; Md Sayem Miah[1]; Laurence Florens[1]; Michael Washburn[1, 2]

[1]Stowers Institute for Medical Research, Kansas City, MO; [2]University of Kansas Medical Center, Kansas City, KS

Accessibility of genetic loci to transcriptional machinery is tightly regulated by the acetylation status of histones. The removal of acetyl groups from histone lysine residues is classically associated with transcriptional repression and, within yeast, is catalyzed by the Sin3 HDAC complex. Named for the organizing protein of the complex, the Sin3 complex is well studied within yeast; however, humans possess two Sin3 paralogs that share only 68% sequence similarity. Previous findings indicate that SIN3A and SIN3B have non-redundant functions in vertebrates and have contrasting influences on metastasis. Despite being targets of FDA-approved chemotherapeutic agents, few studies have sought to delineate the properties of the individual SIN3A and SIN3B HDAC complexes. The current study was undertaken to define the properties and functional attributes of HDAC complexes containing the two human Sin3 proteins. Our results demonstrate that SIN3A and SIN3B share a common core of interaction network components but also highlight the existence of paralog-specific interaction partners. Combining proteomics approaches with functional assays, we find that alternative splicing and alternative start codons have the capacity to dramatically influence the catalytic potentials and subcellular localization patterns of SIN3B complexes. Insights gained through this study strengthen our understanding of the biological pathways influenced by Sin3 proteins and assist in defining the mechanisms through which chemotherapeutic HDAC inhibitors function. Together, our results stress the importance of considering paralog- and isoform-specific properties when assessing protein function.

MOA am 11:46

An enhanced proximity biotinylation method for characterisation of large protein complexes

Vincent Geoghegan

University of York, York, United Kingdom

Proximity biotinylation is emerging as a powerful approach to investigating protein complexes that are beyond the reach of more traditional methods such as co-immunoprecipitation. The approach relies on genetically tagging a protein of interest with a promiscuous biotin ligase, interacting and proximal proteins are then biotinylated *in vivo*, providing a covalent record of their proximity. These can then be enriched using streptavidin prior to identification by mass spectrometry. We have developed an enhanced proximity biotinylation based method for greater coverage of large protein complexes in cells. We apply this method to investigate the composition of kinetochores in the human pathogen *Leishmania.* Kinetochores are large essential complexes that connect chromosomes to microtubules for faithful segregation during mitosis and are challenging to study with existing proteomics methods. Our method provided substantially increased coverage of kinetochores compared to a standard proximity biotinylation workflow or co-immunoprecipitation. From our mass spectrometry data, we were able to calculate relative intra-complex distances between proteins, enabling us to build a topological model of the *Leishmania* kinetochore.

MOA am 11:58

Antibodypedia – an open access resource of validated antibodies

Cristina Al-Khalili Szigyarto; Lukas Persson; Kalle von Feilitzen; Mathias Uhlen

KTH-Royal Institute of Technology, Stockholm, Sweden

There is a need for validated antibodies to study human biology in health and disease. Antibodypedia (www.antibodypedia.com) is a free, open source repository of antibodies that enables scientists to compare and select the most appropriate antibody for a given application based on experimental evidence. A vast number of antibodies are available through Antibodypedia, such as 1 225, 592 for Immunohistochemistry and 298, 491 for Flow cytometry. Currently the database comprises more than 3 million antibodies and 1.3 million validation experiments.

Although the number of antibodies produced world-wide for research purposes is increasing, their quality as reagents varies due to several factors ,such as then the ability to recognize and interact with the target protein and the level of off-binding to other proteins present in the sample. Antibodypedia has therefore adopted the validation strategies and standards conceptualized by the International Working Group for Antibody Validation (1) to aid user in selection of validated antibodies.

While hundreds or even thousands of antibodies are available for some proteins, there are other target proteins with few or no antibodies. An important mission for the future is thus to provide more validated antibodies for target proteins with few or no antibodies. However, in the present version, there are more than 10 antibodies to 88,6 % of all the 20,320 protein coding genes and Antibodypedia is thus a valuable resource for researchers interested in studying the human proteins with an antibody-based method.

1. Uhlen et al 2016 “A proposal for validation of antibodies”, *Nature Methods* volume 13, pages 823–827.

10:30 am - 12:20 pm Monday
INFECTIOUS DISEASE
Session Chair: Doug Sheeley
Oceana 7

MOB am 10:30
Antibiotic resistance and pathogenicity: mechanistic insights from the interactome

James Bruce; Xuefei Zhong; Juan Chavez; Jared Mohr; Andrew Keller; Martin Mathay; Devin Schweppe; Xia Wu
University of Washington, Seattle, WA

Acinetobacter baumannii (AB) is an opportunistic human pathogen that predominantly infects critically ill patients causing pneumonia, wound infections, urinary tract infections, bacteremia and meningitis. Of critical concern with AB is its desiccation resistance that enables long term persistence on dry surfaces, resistance to host defenses, and highly frequent multi-drug resistant (MDR) infections that account for 45% of all AB infections globally and as high as 70% in some countries. This MDR fraction is nearly four times higher than that observed for other Gram-negative pathogens, often requiring use of last resort antibiotics such as carbapenems. In the US, detection of carbapenem-resistant AB isolates increased at an alarming rate from 20.6% in 2002 to 49.2% in 2008. Because of its rapid dissemination of MDR strains, the CDC categorized MDR Acinetobacter spp. As a serious threat and the WHO has included carbapenem-resistant AB in the critical group of bacteria presenting the greatest threat to human health, prioritizing research and development efforts for new antimicrobial treatments.

New proteome technologies enable large-scale visualization of protein-protein interactions (PPIs) and conformations. Our lab has focused on development and application of in vivo chemical cross-linking Protein Interaction Reporter (PIR) technologies. In vivo PIR applications to a carbapenem-resistant AB clinical strain revealed that this isolate has evolved previously undiscovered PPIs that confer increased carbapenem resistance. Site-directed mutagenesis based on identified PPI interfaces was observed to increase carbapenem sensitivity in this strain. In addition, PIR application to human cells during AB infection can provide unique insight on host-pathogen PPIs. This presentation will highlight interactome discoveries made with PIR applications to AB cells and AB-infected human lung epithelial cells, with emphasis on PPIs relevant to antibiotic resistance and host cell invasion.

MOB am 10:50
Peroxisome maintenance en route to virus replication: Integrated proteomics, lipidomics, microscopy and mathematical modeling

Ileana Cristea
Princeton University, Princeton, NJ

Viral proteins have evolved to target cellular organelles and usurp their functions for virus replication. Despite the knowledge of these critical functions for several organelles, little is known about peroxisomes during infection. Peroxisomes are primarily metabolic organelles with important functions in lipid metabolism. Here, using integrative proteomics, lipidomics, microscopy, mathematical modeling, and genetic knockouts, we establish a previously unrecognized function for peroxisomes in viral infection. We discover that medically relevant enveloped viruses, human cytomegalovirus (HCMV) and herpes simplex virus (HSV-1), induce peroxisome biogenesis and unique morphological changes to support viral replication. Targeted proteomic quantification revealed a global viral-induced upregulation of peroxisome proteins. Mathematical modeling and microscopy structural analysis show that infection triggers peroxisomes growth and fission, leading to increased peroxisome numbers and irregular disc-like structures. We determine the functional relevance of these changes by generating a series of CRISPR knockouts in primary human fibroblasts and using pharmacological peroxisome proliferators to perturb peroxisome abundance and morphology. We demonstrate that HCMV-induced peroxisome biogenesis increases plasmalogen production, enhancing virus production. We further provide clinical relevance by analyzing cells from patients with peroxisome disorders. Since plasmalogens are enriched in other enveloped human viruses, our findings uncover a role of peroxisomes in viral pathogenesis with likely implications on viral infectivity across multiple viral species. In a broader context, we define the relationship between peroxisome morphology and metabolic function in a context of cellular pathology. The mechanism we describe also has implications beyond viral infection, as peroxisomes numbers are regulated in diverse biological processes, such as cell division and development.

MOB am 11:10
Remodeling of the Glyco-phenotype of T Cell Surface Proteins with Antisense RNA of Human Immunodeficiency Virus

Weiming Yang[1]; Minghui Ao[1]; Fabio Romerio[2]; Hui Zhang[1]
[1]Johns Hopkins University, Baltimore, MD; [2]University of Maryland, Baltimore, MD

Human immunodeficiency virus (HIV) infection is not curable due to viral latency and identifying T cells with HIV latent infection is a crucial step to the cure. Compelling reports and our recent study suggested that there is a distinct profile of cell surface proteins that can be used for targeting latently infected cells. To identify which HIV-specific component may play a role in remodeling cell surface proteins, we established stable T cell lines that expressed two different HIV derived RNAs including HIV trans-activation-responsive (TAR) RNA and viral antisense transcript (Ast). Analysis of glycoproteins from the stable lines using mass spectrometry revealed that expression of Ast drastically changed the expression of a range of glycoproteins. In sharp contrast, expression of TAR RNA had little effect to the glycoproteome pointing to that the change seen in Ast expressing cells was selective. Additionally changed glycoproteins in the Ast expressing cells were identified using glycoproteomic analysis of de-glycosylated peptides. Downstream pathway analysis and antibody study confirmed that glyco-phenotype of T cells was reshaped by the expression of HIV Ast. The significance of the observation in the context of current HIV cure effort will be discussed.

MOB am 11:22
HBx: Hepatitis B Virus Swiss Army Knife enabling successful infection

Emanuela Milani[1]; Bingqian Qu[2]; Stephan Urban[2]; Bernd Wollscheid[1]
[1]Dep. of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland; [2]University Clinic Heidelberg, Heidelberg, Deutschland

Viruses are obligatory intracellular organisms. Their survival and propagation rely on the interplay with host proteins and the efficient hijacking of host signaling machineries. A systems-level understanding of virus-host signaling networks and a

detailed knowledge on how molecular interactions drive infection phenotypes and insure viral propagation is lacking in the hepatitis B virus (HBV). To gain a comprehensive and quantitative understanding of the **infection proteotype** we applied a **DIA/SWATH**-based strategy. The quantitative data generated provides direct evidence that HBV hijacks concurrently several molecular signaling networks. To gain a molecular understanding on how HBV exactly rewires host cellular signaling networks upon infection we elucidated the dynamic viral-host protein interaction map of HBV, in particular of the viral protein HBx which plays an essential role in HBV pathogenesis and HBV-induced hepatocellular carcinoma.

A combination of affinity purification (**AP-MS**) and proximity-dependent mass spectrometry (**BioID**) technologies provides an unprecedented view of the dynamic **HBx-host interactome,** indicating the likely existence of several co-existing HBx **proteoform-specific subcomplexes**. The obtained data confirms and expands our knowledge about known HBx interactions, showing now in molecular detail that HBx rewires four cellular modules, including proteasome-dependent degradation, transcriptional regulation, chromatin remodeling, and cell cycle regulation. The data further provides detailed molecular cues for HBx as HBV's swiss army knife – one viral protein with pleiotropic activities hijacking numerous signaling pathways. To specifically interrogate the functional relevance of the identified interactomes in the viral life cycle, we modulated the expression of selected targets using shRNA-based strategies in a virus susceptible hepatic cell model. Monitoring the efficiency of viral replication revealed that targets involved in chromatin remodeling and mRNA processing act as potential HBV host restriction factors.

Together, we present the HBx molecular interaction network, which acts as viral swiss army knife and led to the discovery of targets for possible pharmaceutical intervention.

MOB am 11:34
Proteolytic events regulate virulence processes of the human pathogen Salmonella as shown by quantitative degradomics.

Geremy Clair; Ryan Sontag; Joshua Hansen; Aaron Wright; Joshua Adkins
Pacific Northwest National Laboratory, Richland, WA

Salmonella enterica serovar Typhimurium uses of an arsenal of regulatory mechanisms that allows it to rapidly sense and adapt to minute micro-environmental changes within the human body. When extracellular contactwith target host cells occurs, Salmonella produces a Type 3 secretion system (T3SS) encoded by Salmonella pathogenicity island (Spi) 1 to inject virulence factors within the cytoplasm of host cells to induce phagocytosis. When engulfed intracellularly in phagosomal vacuoles, the bacterium produces a second T3SS encoded by Spi-2. The conditions that induce these different mechanisms can be mimicked by using appropriate environmental cues (e.g. low magnesium concentration and low pH). Transferring Salmonella from rich growth medium to an infection mimicking state causes a global reorganization of the proteome (PMID:22900174) and major changes in multiple Post-Translational Modifications (PTM) states including cysteine-thiolations (PMID: 23720318) and proteolytic events (PMID: 17228056). The potential involvement of these PTMs in the regulation of virulence has remained largely unclear.

To explore the role of proteolysis in Salmonellosis, we leveraged a combination of proteomics technologies: (i) we used bottom-up and quantitative top-down proteomics to identify proteolytic events modulated in the phagocytic condition; (ii) we used activity-based probing to identify new putative serine proteases; and (iii) we created a set of differentially abundant protease Knock-Out (KO) mutants to identify the role of individual proteases in phagocytosis adaptation.

In this work, we have revealed specific proteolytic events in the intra-phagosomal lifestyle. Activity-based proteomics led to the identification of several putative proteases that may participate in these proteolytic activities. Bottom-up quantitative proteomics of our KO mutants allowed us to identify multiple proteolytic driven processes. In particular, we have demonstrated that an ATP-dependent protease controls the intracellular pathogenicity of the bacterium by modulating simultaneously the abundance of transcription factors, the intracellular type 3 secretion system and various secreted effectors.

MOB am 11:46
Systematic Identification of Mycobacterium tuberculosis Effectors Reveals that BfrB Suppresses Innate Immunity

Xiang He; Sheng-Ce Tao
Shanghai Jiao Tong University, Shanghai, China

Mycobacterium tuberculosis (Mtb) has evolved multiple strategies to counter the human immune system. The effectors of Mtb play important roles in the interactions with the host. However, due to the lack of highly-efficient strategies, there are only a handful of known Mtb effectors, thus hampering our understanding of Mtb pathogenesis. In this study, we probed the Mtb proteome microarray with non-denatured, biotinylated whole cell lysates of human macrophages, and identified 27 Mtb membrane proteins and secreted proteins that bind to macrophage proteins. Using the identified Mtb proteins as bait, we enriched binders from macrophage through GST pull-down and then identified the binders with mass spectrometry. We refer this proteome microarray-based strategy as SOPHIE (Systematic unlOck Pathogen and Host Interacting Effectors). We then investigated a newly identified effector, the iron storage protein, BfrB (Rv3841), further in functional assays. We found that BfrB inhibits NF-κB-dependent transcription through binding and reducing the nuclear abundance of ribosomal protein S3 (RPS3), which is a functional subunit of NF-κB. Furthermore, overexpression of BfrB in Mycobacterium smegmatis promotes survival of mycobacteria in macrophages. The set of Mtb effectors identified in this work will greatly facilitate the understanding of the pathogenesis of Mtb, possibly leading to additional potential molecular targets in the battle against tuberculosis. Also, SOPHIE is generally applicable for the study of other pathogens.

10:30 am - 12:20 pm Monday
CANCER
Session Chairs: Yu-Ju Chen and Sudhir Srivastava
Oceana 6

MOC am 10:30
Invited talk for Cancer session

Nickolas Papadopoulos
Johns Hopkins School of Medicine, Baltimore, MD

MOC am 10:50

The sTRA glycan is complementary to CA19-9 as a serological biomarker of pancreatic cancer

Ben Staal[1]; Daniel Barnett[1]; Zonglin He[2]; Ying Huang[2]; Katie Partyka[1]; Aatur Singhi[3]; Richard Drake[4]; Anirban Maitra[5]; Randall Brand[3]; Brian Haab[1]

[1]Van Andel Research Institute, Grand Rapids, MI; [2]Fred Hutchinson Cancer Research Center, Seattle, WA; [3]University of Pittsburgh Medical Center, Pittsburgh, PA; [4]Medical University of South Carolina, Charleston, SC; [5]MD Anderson Cancer Center, Houston, TX

The CA19-9 biomarker is elevated in a substantial group of patients with pancreatic cancer, but not enough to be reliable for the detection or diagnosis of the disease. We hypothesized that a glycan called sTRA complements CA19-9, and that the two biomarkers in combination provide added information over either biomarker individually. We first asked whether the secreted levels of each marker reflected tissue expression. In cell culture and PDX mouse models of pancreatic ductal adenocarcinoma (PDAC), the amount of CA19-9 or sTRA in the media or serum, respectively, was proportional to the expression in tissue, with each model expressing primarily one or the other. We observed a similar relationship in matched sera and primary tumors from 52 patients with PDAC. Next, we tested whether a biomarker panel comprising CA19-9 and two versions of sTRA performed better than either marker individually for distinguishing PDAC from benign pancreatic diseases. In a training set of 197 subjects (97 cancer from all stages and 100 benign disease), a panel optimized for specificity gave 65%/97% sensitivity/specificity compared to 47%/97% for CA19-9, and a panel optimized for sensitivity gave 96%/67%, compared to 96%/14% for CA19-9. The application of the markers to an independent, blinded set of 147 subjects gave 54%/95% and 94%/38% for the panels, relative to 30%/97% and 87%/34% for CA19-9 at the preset thresholds. Adjustments of the marker thresholds upon unblinding of the data yielded 70%/97% and 96%/37%, relative to 31%/97% and 96%/9% for the equivalently-optimized CA19-9 thresholds. Thus, sTRA and CA19-9 are complementary serological biomarkers that in combination improve performance over CA19-9 and that potentially identify distinct subgroups of tumors.

MOC am 11:10

A proteomic contexture of exosomes isolated from viable renal cell carcinoma tissues, toward development of cancer liquid biopsy diagnostics

Atsushi Ikeda[1]; Kentaro Jingushi[2]; Naomi Ohnishi[1]; Motohide Uemura[2]; Kazutake Tsujikawa[3]; Koji Ueda[1]

[1]Japanese Found. for Can. Res., Koto-Ku, Japan; [2]Dep. Therap. Uro. Onco, Osaka Univ, Osaka, Japan; [3]Lab. Mol. Cell. Phys, Osaka Univ, Osaka, Japan

Early detection of cancer is one of the most fundamental strategies to improve therapeutic outcomes and reduce cancer-related mortality rate. Here we propose a new strategy to explore targets for cancer EV diagnostics, which allowed high-purity EV isolation even from a tiny viable tissue section of early staged cancer.

We extracted tissue-exudative EVs (Te-EVs) from serum-free media of freshly resected renal cell carcinoma (RCC) tissues and adjacent normal tissues using ultracentrifugation method (n = 20). Te-EV proteome was then comprehensively identified and quantified by high resolution LC/MS system and Expressionist proteomics server. A couple of RCC-EV specific proteins were further validated by serum EV sandwich ELISA (n = 104) and analyzed individually for their biological significance.

Comprehensive LC/MS analysis identified 3,871 Te-EV proteins, in which 106 proteins showed significant upregulation in EVs from RCC tissue (p < 0. 05, fold-change > 2. 0) compared to those from kidney normal tissues. Particularly, azurocidin (AZU1) and TME19 exhibited highly RCC-specific load on EVs ($p = 2.85 \times 10^{-3}$, fold-change = 31. 6 and $p = 1.18 \times 10^{-4}$, fold-change = 17. 4, respectively). Importantly, serum EV-AZU1 level demonstrated stage-dependent escalation in EV sandwich ELISA even from stage-I. AZU1^{++}-EVs drastically collapsed vascular endothelial cell sheet structure, suggesting that EV-AZU1 may promote hematogenous metastasis of RCC (Int J Cancer, 142: 607, 2018). On the other hand, EV-TME19 directly induced transformation from patient-derived renal fibroblasts to cancer associated fibroblasts (CAFs).

Our Te-EV proteome catalog can provide lots of new and reliable insights regarding relationship between behaviors of EVs and cancer biology, which could lead to development of novel diagnostics and therapy of cancer.

MOC am 11:22

Multi-omic characterization of pathway abnormalities in high grade serous ovarian cancer

Osama Arshad[1]; Jason McDermott[1]; Vladislav Petyuk[1]; Samuel Payne[1]; Marina Gritsenko[1]; Therese Clauss[1]; Ronald Moore[1]; Matthew Monroe[1]; Mathangi Thiagarajan[2]; Christopher Kinsinger[3]; Henry Rodriguez[3]; Richard Smith[1]; Tao Liu[1]; Karin Rodland[1]

[1]Pacific Northwest National Laboratory, Richland, WA; [2]Frederick National Laboratory for Cancer Research, Frederick, MD; [3]National Cancer Institute, Bethesda, MD

High-grade serous ovarian cancer (HGSOC) is the most aggressive histotype accounting for an overwhelming majority of cases of ovarian cancer with poor long-term survival. Furthering our understanding of the molecular underpinnings of this disease is necessary for improving treatment outcomes. Towards this end, The Cancer Genome Atlas (TCGA) comprehensively characterized the genomic and transcriptomic landscape of this lethal malignancy. Under the umbrella of the Clinical Proteomic Tumor Analysis Consortium (CPTAC), we have recently published an extensive proteomic and phosphoproteomic analysis of a subset of the TCGA tumors. Although this analysis provided additional insights, sample acquisition was not designed with proteomics in mind. We have prospectively requisitioned 110 new patient samples following a strict protocol optimized for proteomic analyses by controlling for warm ischemia, shown to be a confounding factor for proteomics in the TCGA collection. Moreover, this collection incorporates matched normal tissues for proteomics which were lacking in the retrospective TCGA cohort. We profiled this prospective collection with multiple omics platforms including whole-exome sequencing, RNA-Seq and tandem mass tagging (TMT)-based global proteomics and phosphoproteomics. We analyzed these datasets to validate the findings of the retrospective study in this independent cohort and to investigate what new insights are revealed by these fit-for-purpose samples. A number of previous observations were validated including mRNA-protein correlation and a survival signature. We leveraged the new

normal samples to interrogate the proteomic and phosphoproteomic datasets to determine differences in the relative expression profiles of the malignant and normal cases. The proteomics tumor-normal comparison identified increased proliferation and decreased immune response in tumors. Kinase-substrate enrichment analysis on tumor-normal relative phosphoprotein abundance revealed the dysregulation of a number of kinase signaling pathways in HGSOC. Our comprehensive multi-modal characterization of HGSOC has been made publicly available.

MOC am 11:34
Proteogenomic landscape of medulloblastoma subgroups

Antoine Forget[1, 2]; Loredana Martignetti[3, 4]; Stephanie Puget[5]; Laurence Calzone[3, 4]; Sebastian Brabetz[6, 7]; Daniel Picard[8, 9]; Arnau Montagud[3, 4]; Stephane Liva[3, 4]; Alexandre Sta[3, 4]; Florent Dingli[10]; Guillaume Arras[10]; Jaime Rivera[10]; Damarys Loew[10]; Aurore Besnard[11]; Joëlle Lacombe[11]; Mélanie Pagès[11]; Pascale Varlet[11]; Christelle Dufour[12]; Hua Yu[1, 2]; Audrey Mercier[1, 2]; Sophie Leboucher[1, 13]; Laura Sieber[6, 7]; Kevin Beccaria[5]; Michael Gombert[9]; Frauke Meyer[8, 9]; Nan Qin[8, 9]; Jasmin Bartl[8, 9]; Lukas Chavez[6, 7]; Konstantin Okonechnikov[6, 7]; Tanvi Sharma[6, 7]; Venu Thatikonda[6, 7]; Franck Bourdeaut[14]; Celio Pouponnot[1, 2]; Vijay Ramaswamy[15]; Andrey Korshunov[16]; Arndt Borkhardt[9]; Guido Reifenberger[17]; Patrick Poullet[3, 4]; Michael D. Taylor[18]; Marcel Kool[6, 7]; Stefan M. Pfister[6, 7]; Daisuke Kawauchi[6, 7]; Emmanuel Barillot[3, 4]; Marc Remke[8, 9]; Olivier Ayrault[1, 2]

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Extensive high-throughput sequencing has led to the characterization of four Medulloblastoma (MB) subgroups (WNT, SHH, Group 3 and Group 4), delineated with distinct molecular signatures and clinical outcomes. However, to date these analyses have not given a global comprehension of their dynamic network complexity. Indeed, the underlying biology of Group 3 and 4 MB is still not well understood. In order to get a comprehensive view of all MB subgroups we employed a multi-scale analysis to integrate genomic copy number (CN), DNA methylation, mRNA, protein and phosphorylation levels across 38 primary human MBs (5 WNT, 10 SHH, 10 Group 3 and 13 Group 4). Our analysis revealed that each MB subgroups can be distinguished at the proteomic level. Moreover, Group 4 MB are particularly distinguishable from the other subgroups based on their phosphorylation profile. Moreover, integration of all omic layer by similarity network fusion allowed precise definition of the four subgroups. Importantly, protein levels were poorly predicted by corresponding mRNA levels with consequence on active pathway prediction. Notably, mRNA consistently over predicted protein levels in Group 3 while it was the reverse in Group 4. This observation was of particular importance in Group 4 MB in which protein and phosphorylation levels allowed the identification of pathways that were unable to be predicted by RNA data. Aberrant activation of this identified pathway during mouse cerebellar development lead to tumor formation closely mimicking Group 4 human tumor providing a murine model of Group 4 MB. Altogether, combined multi-scale analyses of MB have allowed us to identify and prioritize novel molecular drivers involved in human MB genesis and progression.

MOC am 11:46
Proteomic-based machine learning computational analysis discovered biomarkers of aberrant vesicle-exosomal trafficking to determine chemotherapeutic responses in the FFPE-human breast cancer sample

Han Suk Ryu; Dohyun Han; Kyung-min Lee; Kwangsoo Kim

Seoul National University Hospital, Seoul, South Korea

The chemotherapy has become a critical standard therapeutic strategy for patients with breast cancers. However, the therapeutic response is still low and the need for a biomarker predictive to overcome chemo-resistance is becoming all the more necessary to enhance therapeutic efficacy. We performed quantitative proteomics mass spectrometry in twenty paired FFPE biopsy breast cancer samples consist of non-responsive and responsive groups to chemotherapy. A total of 6,424 proteins were identified and 254 were confirmed to be significantly altered proteins related to chemotherapeutic response. From the patient group with chemo-resistance, we featured 56 significantly upregulated proteins in six closely related subcellular organelles concerning intra- and extracellular transportation system based on domain knowledge for text-mining and public network databases for network analysis. In the first validation step, 10 human breast cancer cell lines enrolled and verified biological functions for 56 protein candidates through molecular biology-driven assays, including RNAi, celltiter-glo luminescent assay, mitochondrial membrane potential assay (MMPA), immunofluorescence, exosome uptake assay, time-lapse live cell imaging system and the 3D tumor spheroid-based function assays for target validation. As a result, 14 intracellular exosomal transportation markers were identified to control chemo-sensitivity through siRNA array panel assay. MMPA revealed altered metabolic and radical oxygen networks and live cell images showed altered exosomal trafficking in cell lines manipulated by candidate markers. Through the second verification step by r-caret recursive feature elimination, we selected five markers and applied them to an independent cohort with 50 FFPE biopsy samples to discover the most optimal combination of immunohistochemical biomarkers to predict chemo-responsiveness. The present study provides the first evidence to identify a predictive biomarker for chemotherapeutic response based on in-depth proteomics. We expect that the newly discovered biomarkers and biological evidence can provide the novel insight to overcome chemo-resistance in breast cancer.

MOC am 11:58
Proteomics in PreCancer Atlas: an Evidence Based Biomarkers Discovery
Sudhir Srivastava
National Cancer Institute, NIH, Bethesda, MD

10:30 am - 12:20 pm Monday
HPP: PARTNERING WITH PATHOLOGY TOWARDS PRECISION MEDICINE
Session Chairs: Daniel W. Chan and Ed Nice
Oceana 3-5

MOD am 10:30
Can proteomic pathology deliver solutions for unmet clinical needs?
Stephen Pennington[1, 2]
[1]UCD Conway Institute, School of Medicine, Dublin, Ireland; [2]University College Dublin, Dublin, Ireland
In moving to an era of precision medicine and healthcare it is pertinent to ask how proteomics can contribute to the development of new molecular pathology strategies? Clearly, there is a huge opportunity to improve on current clinical tests for the diagnosis, prognosis and monitoring of disease. Drawing on examples of significant unmet clinical needs in prostate cancer and psoriatic arthritis this talk will seek to highlight how some of these opportunities may be addressed. In particular, some of key issues faced in the development of multiplexed protein assays and strategies for the delivery and implementation of these assays in a diagnostic setting will be discussed.

MOD am 10:50
Adoption of Proteomics into Clinical Practice- Barriers and Opportunities
Peter Stewart[1, 2]
[1]Royal Prince Alfred and Liverpool Hospitals, Sydney, Australia; [2]University of Sydney, Sydney, Australia
Protein measurements in clinical laboratories are widely used to diagnose, monitor disease and response to treatment. In general this consists of measuring individual proteins (e.g. albumin, transferrin) or small groups (serum EPG). Methodologies such as dye binding, immunoassay, and electrophoresis are well-established clinical laboratory techniques. Currently comprehensive protein identification and quantitation (proteomics) is not performed in the clinical laboratory. What are the opportunities and likelihood of this occurring?

Proteomics is in a discovery phase and relying on current basic research to fuel innovation. The time frame to widespread clinical utility is uncertain as is indeed is the overall impact of proteomics.

There remain major barriers to uptake and examples of these include, the very complexity of the technology, standardisation of assays, quality control and quality assurance of testing, analytical sensitivity of the technology and ability to precisely quantify, throughput, and not least cost. There will need to be enhancement in informatics to handle the data generation. Proteomics will also compete in the clinical space with established technology such as immunoassay that in some areas may have a clinical advantage.

Nevertheless proteomics does offer opportunities to identify new biomarkers for disease diagnosis and management that are likely to be more specific than some currently available. It also may be used to better indicate early treatment responses through capturing individual variability.

Genomics is now well established in clinical laboratories and the process of adoption of this technology provides a useful case model to clinical proteomics.

MOD am 11:10
Immuno-Proteomics of Colon Cancer
Michael Roehrl
MSKCC, New York, NY
Discovery of tissue-based protein biomarkers for early diagnosis has the potential to improvetargeted therapy outcomes through judicious selection of colon cancer patients in clinical trials. The immune system of cancer patients can sense aberrant proteins as tumor-associated antigens and has the ability to produce autoantibodies against these antigens.

We developed cancer patient serum antibody screening (immuno-proteomics) to discover and characterizenovel tissue-resident tumor-specific antigens. Furthermore, we characterized a matched set of primary and metastatic colorectal cancers by pan-omic profiling: (1) whole genome sequencing, (2) RNA-Seq, (3) DNA methylation, (4) miRNA sequencing, (5) ChIP-Seq genome-wide enhancer profiling, and (6) deep proteome profiling.

Mass spectrometry-driven immuno-proteomics is a novel powerful technique for cancer biomarker discovery and may have important implications for immunotherapeutic monitoring and therapy selection.

MOD am 11:22
Comparison of fractionated versus unfractionated plasma lipoproteome in the context of vascular contributions to Alzheimer's disease
Danni Li
University of Minnesota, Minneapolis, <Not Specified>
Scientific evidence continues to support vascular contributions (i.e., cerebrovascular) to development of Alzheimer's disease (AD). Plasma lipoproteins play important roles in cerebrovascular integrity. Plasma lipoproteins are comprised of four major types: very-low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high-density lipoprotein (HDL). Because of the heterogeneous distribution of proteins across these four plasma lipoprotein types and their distinctive cerebrovascular contributions, we hypothesize that fractionated plasma lipoproteome, measured in individual plasma lipoproteins, will provide molecular insights that cannot be revealed by unfractionated plasma lipoproteome, measured in unfractionated plasma. This study applied a targeted selected reaction monitoring (SRM) method to measure 79 proteins in VLDL, IDL, LDL and HDL (fractionated by sequential gradient ultracentrifugation) and in unfractionated plasma (removed of albumin and IgG by immunoaffinity depletion) in a case-control study of five AD cases and five sex and age-matched controls. Within-day and between-days technical precisions of the targeted SRM, and between-days technical precisions (mean [SD]) of the entire work flow (including sequential gradient ultracentrifugation, proteomics sample preparation, and

targeted SRM analysis) were 3.95% (2.65), 9.31%(5.59), and 27.90%(14.61), respectively. The un-adjusted p values of peptide level comparisons between the AD cases and controls for all the 79 proteins showed that HDL, LDL, IDL, and VLDL had generally had lower p values compared to unfractionated plasma. Since lower p values indicated stronger evidence of a difference between the cases and controls, the observation provides an initial support for the conceptualization that there may be a higher probability for plasma lipoproteome to be different between AD cases and controls when measured in plasma lipoprotein fractions than unfractionated plasma in the context of vascular contributions to Alzheimer's disease.

MOD am 11:34
Proteomics of Kidney Biopsy Tissue and Urine Liquid Biopsy Towards Precision Medicine

Tadashi Yamamoto[1, 2]; Keiko Yamamoto[1]; Bo Xu[1]; Amr Elguoshy[1]; Yoshitoshi Hirao[1]
[1]Biofluid Biomarker Center, Niigata University, Niigata, Japan; [2]Shinrakuen Hospital, Niigata, Japan

Chronic kidney disease (CKD) is one of the most common but serious diseases these days. Development and progression of CKD are highly associated with life-styles of the patients. The patients are clinically diagnosed with CKD by persistent proteinuria mostly and treated with dialysis or kidney transplantation at the advanced end-stage. Progression of CKD patients is variable since CKD is histologically differentiated into several diseases, such as diabetic nephropathy, chronic glomerulonephritis, nephrosclerosis and others and individual life-styles are also different. Although the histological diagnosis is currently made by kidney biopsy examination, information obtained by this is not enough for precise information on molecular pathophysiological conditions of individual patients. To obtain the molecular information of the individual kidney biopsy specimens and to make it possible to provide suitable therapy to each CKD patient as personalized or precision medicine, proteomic analysis of biological samples from patients, such as kidney biopsy tissues, urine and plasma, are expected to be significant.

Therefore, we have established a platform for proteomics of kidney biopsy tissues and urine samples to grasp disease condition of each patient. The formalin-fixed paraffin-embedded kidney tissues from CKD patients were used after histological examination for MS-based proteomics of the glomerulus and the cortex separated by laser micro dissection. Peptides were prepared from these tissue sections and proteomes were analyzed individually by LC-MS to compare with those of normal controls. Urine samples were also analyzed to understand injury severity of kidney nephron segments and other kidney sites by antibody-based measurement for newly identified urine biomarkers (as liquid biopsy), which have been selected by our previous studies. By combining the proteomics data of kidney biopsy tissues and urine liquid biopsy, information on molecular changes of individual kidney injury was obtained to predict establishment of precision medicine for CKD in the near future.

MOD am 11:46
An approach to spatiotemporally resolve protein interaction networks in living cells

Ruth Hüttenhain[1, 2]; Braden T. Lobingier[1]; Kelsie Eichel[1]; Alice Y. Ting[3]; Brian Shoichet[1]; Mark von Zastrow[1]; Nevan J. Krogan[1, 2]
[1]UCSF, San Franciso, CA; [2]Gladstone Institutes, San Francisco, CA; [3]Stanford University, Stanford, CA

Biological processes are mediated by protein interaction networks that are organized in space and remodel over time. While temporal dynamics of protein interaction networks have been previously characterized, a major challenge remains largely unmet: how to interrogate protein interaction networks engaged by a specific protein while capturing both the spatial and temporal context in which these interactions occur.

Protein proximity labeling holds exciting potential to elucidate these networks in living cells. However, methods that offer sufficient labeling activity are typically thought to be limited in spatial resolution due to labeling of nearby off-pathway proteins. Here, we show that this 'nonspecific' signal is actually advantageous, and can be used to extract higher-order spatial information. We developed a novel analytical pipeline combining APEX-based proximity labeling with quantitative proteomics and a system of spatial references, which allows distinguishing protein interaction network components from nearby off-pathway proteins. This pipeline delivers, with sub-minute temporal resolution, local protein interaction networks and subcellular location for a target protein of interest.

We applied this strategy to interrogate how protein interaction networks engaged by GPCRs respond to ligand-induced activation. We not only validated capture of proteins known to interact with the receptors, including those with transient or low affinity interactions, but demonstrated that our pipeline can be used to discover new network components regulating receptor function. We reported a previously unrecognized ubiquitin network that controls homeostatic down-regulation of the delta opioid receptors. Currently, we extend this approach to examine the protein interaction networks engaged by the mu-type opioid receptor after stimulation with full, partial, and G protein biased agonists.

In summary, we described and validated a methodology that extends the utility of APEX-mediated proximity labeling to achieve spatiotemporally resolved protein network interrogation in intact cells.

MOD am 11:58
Improved Survival Prognostication of Node-Positive Malignant Melanoma Patients – A Proteogenomics Study Guided by Histopathological Characterization

Lazaro Betancourt; Krzysztof Pawlowski; Jonatan Eriksson; Marcell Szasz; Shamik Mitra; Indira Pla Parada; Charlotte Welinder; Henrik Ekedahl; Per Broberg; Roger Appelqvist; Maria Yakovleva; Yutaka Sugihara; Kenichi Miharada; Christian Ingvar; Lotta Lundgre; Bo Baldetorp; Håkan Olsson; Melinda Rezeli; Elisabet Wieslander; Peter Horvatovich; Johan Malm; Göran Jönsson; György Marko-Varga
Lund University, Lund, Sweden

Metastatic melanoma is one of the most common deadly cancers, and robust biomarkers are still needed. Here, protein expression analysis of one hundred eleven melanoma lymph node metastases with 68 men and 43 women among the investigated cases. Average age ± standard deviation (range) at diagnosis of lymph node metastasis was 62.4±13.7 (25-89) years. The time elapsed to progression from primary tumor to lymph node metastasis was 5.0±5.6 (0-18.0) years and overall survival was 7.9±6.8 (0.2-43.0) years. The dominant histotypes of primary tumors were Superficial Spreading Melanoma (SSN) and Nodular Melanoma (NM). In this study high performance

mass spectrometry is coupled with in-depth histopathology analysis, clinical data and genomics profiles. This broad view of protein expression allowed to identify novel candidate protein markers that allowed prediction of survival in melanoma patients. The protein data is matched to genomic analysis of the same tumor tissue. This information coupled with extensive clinical information on each subject provides an excellent opportunity to identify novel protein markers to predict progression and survival of melanoma. Some of the prognostic proteins have not been reported in melanoma context before, and few of them exhibit unexpected relationship to survival, which exemplifies complexity of melanoma heterogeneity disease presentation.

10:30 am - 12:20 pm Monday
STATISTICS IN EXPERIMENTAL DESIGN
Session Chairs: Olga Vitek and Pei Wang
Oceana 1-2

MOE am 10:30
Experimental design and data-analysis in label-free quantitative MS-based proteomics

Lieven Clement
Ghent University, Ghent, Belgium

Label-free mass spectrometry based proteomics is routinely used for protein quantification and differential analysis. However, extracting relevant information from the massive amounts of data in contemporary proteomics studies remains challenging. The reproducibility and accuracy of the results critically depends on the experimental design and the data analysis.

With this respect, we will introduce the key sources of variability in label-free shotgun proteomics experiments, their implications for study design and how to correctly account for it in the data-analysis. We also elaborate on the different levels of replication, i.e. peptide level, technical and biological repeats, batches and blocks, repeated or longitudinal measures, that are widespread in the proteomics literature. We will introduce these concepts by real data examples and show how to set-up powerful data analysis workflows that correctly account for the complex hierarchical dependence structure of peptide-level data in modern proteomics studies using our open-source software tool MSqRob.

MOE am 10:50
Components of reproducible quantitative mass spectrometry-based research: a statistician's perspective

Olga Vitek
Northeastern University, Boston, MA

This talk presents a statistical perspective on reproducible quantitative mass spectrometry-based proteomics. Statistical components of reproducibility include experimental design, from both biological perspective (which proteins and samples, and how many, do we need to quantify?) and technological perspective (are the assays appropriate for the task? Do the experimental steps run properly?). Statistical components of reproducibility also include data processing (which features should we use to quantify a protein?) and downstream statistical analysis (how to detect changes in protein abundance? Are our conclusions consistent with prior results?). Answer these questions requires the availability of statistical methods, and but also of publicly available data that help understand the advantages and the limitations of the methods. This talk will highlight the contributions of our lab to these components of reproducible research.

MOE am 11:10
Empirical peptide-level statistics allow robust and sensitive differential expression detection in MS-proteomics data

Constantin Ammar; Gergely Csaba; Markus Gruber; Ralf Zimmer
LMU Munich, Munich, Germany

One of the ubiquitous problems underlying current MS based proteomics setups is the large fraction of distorted peptide intensities used for quantification, in particular for lower intensity peptides. While numerous instrumental setups aim at the reduction of intensity distortion, current computational methods only implicitly account for this problem.

We introduce a new empirical model to account for measurement-inherent noise. The only assumption we have to make in our model is consistency between replicates. For a given quantitative MS data set, we derive tailored empirical distributions for each peptide, which are used for individual weighing of peptide intensities in the context of differential expression analysis. We show that this is an effective way to deal with low-intensity high-noise peptides and demonstrate significantly improved sensitivity (up to two-fold) in the detection of differentially expressed proteins in comparison to current state-of-the-art methods.

We derive a new in-silico approach to assess the differential quantification performance of common quantitative MS setups such as LFQ, TMT, SILAC and DIA. This allows proteomics researchers to easily assess, via the measurement of replicate standard samples, how many differentially expressed proteins up to which fold change they can expect to detect with their own MS setups.

MOE am 11:22
A statistical framework for relative quantification of post-translational modifications in global proteomics experiments

Tsung-Heng Tsai[1]; Lilian Phu[2]; Yi Zeng[2]; Donald Kirkpatrick[2]; Erik Verschueren[2]; Olga Vitek[1]
[1]Northeastern University, Boston, MA; [2]Genentech, Inc., South San Francisco, CA

Post-translational modifications (PTMs) play a crucial role in dynamically altering proteomes and are key regulators for a multitude of complex processes in eukaryotic cells. Affinity enrichment followed by quantitative Mass Spectrometry (MS) is currently the most successful approach to systematically identify PTMs and quantify their relative abundance with great depth and throughput. Relative changes in PTM site abundance, quantified by MS, are traditionally modeled with a two-sample t-test, comparing either the mean intensity or the modified to unmodified ratio of representative peptides. However, the interpretation of changes at a single modification site in a typical bottom-up proteomics workflow is complicated by sparse coverage and confounded by both changes in overall protein abundance and variability in enrichment efficiency. We propose here an alternative statistical approach to model relative abundance changes for modification sites, which explicitly incorporates major sources of variability and confounding factors present in PTM experiments. Moreover, the general statistical framework underlying the proposed

approach allows for natural extensions to complex experimental designs including multiple conditions and multiple batches. We evaluated our proposed approach by comparing it to the results of a 'naïve' t-test using computer simulations, a custom designed benchmark experiment and real biological datasets of enriched ubiquitinated peptides. Compared to the t-test, the proposed approach improved the reproducibility and accuracy of the estimated abundance changes, resulted in a better calibrated type I error rate, and improved statistical power of detecting differentially modified PTM sites. Furthermore, for analyses of datasets with multiple batches, the proposed approach improved the overall performance under various forms of batch effects. Finally, our proposed framework also facilitates the design of new experiments aiming to quantify relative PTM changes by providing minimal sample size calculations and power analysis. All algorithms and statistical tests are implemented in the 'MSstatsPTM' R package.

MOE am 11:34

Bayesian Confidence Intervals for Multiplexed Proteomics Integrate Ion Statistics with Peptide Quantification Concordance

Leonid Peshkin[2]; Meera Gupta[1]; Lillia Ryazanova[1]; Martin Wühr[1]

[1]Princeton University, Princeton, NJ; [2]Harvard Medical School, Boston, MA

Multiplexed proteomics has emerged as a powerful tool to measure protein expression levels across multiple conditions. The relative protein abundances are inferred by comparing the signal generated by reporter ions. Intuitively, the trust associated with a protein measurement depends on the similarity of ratios from different peptides and the signal level of these measurements. So far, peptide-level information has typically not been integrated into confidence, and only the most likely results for relative protein abundances are reported. If confidence is reported, it is based on protein-level measurement agreement between replicates. We show that a hierarchical Dirichlet-Multinomial model can be used to adequately reflect uncertainty in quantitative multiplexed proteomics measurements. We demonstrated how to estimate a calibration multiplier for a given instrument and mass resolution and then use that multiplier to convert a continuous MS signal value into discrete event counts suitable for the Dirichlet-Multinomial modeling. We call our method BACIQ (Bayesian Approach to Confidence Intervals for protein Quantification). BACIQ integrates peptide intensities and peptide-measurement agreement into confidence intervals for protein ratios. The main advantages of BACIQ are: 1) it removes the need to threshold reported peptide signal based on an arbitrary cut-off, thereby reporting more measurements from a given experiment; 2) confidence can be assigned without replicates; 3) for repeated experiments BACIQ provides confidence intervals for the union, not the intersection, of quantified proteins; 4) for repeated experiments, BACIQ confidence intervals are more predictive than confidence intervals based on protein measurement agreement. To demonstrate the power of BACIQ we reanalyzed previously published data on subcellular protein movement upon Exportin-1 inhibition. Compared to our previous analysis, with BACIQ we detect 227% more highly significant movers, down to changes of 2.5%. Thus, our method drastically increases the value obtainable from quantitative proteomics experiments helping researchers to interpret their data and prioritize resources.

MOE am 11:46

MSstatsTMT: Statistical detection of differentially abundant proteins in mass spectrometry experiments with isobaric labeling

Ting Huang[1]; Meena Choi[1]; Manuel Tzouros[2]; Nikhil Pandya[2]; Balazs Banfai[2]; Tom Dunkley[2]; Olga Vitek[1]

[1]Northeastern University, Boston, MA; [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland

Isobaric labeling (iTRAQ and TMT) mass spectrometry is a popular technology for protein quantification since it allows multiple samples to be analyzed simultaneously in a single experiment. However, the design of multiple iTRAQ or TMT experiments can be very complex due to different allocations of samples and conditions to channels and experiments. Different designs introduce distinct sources of variation in the reporter ion intensity. Statistical approaches for accurately estimating the components of variation and incorporating them to identify differentially abundant proteins are still lacking.

In this study, we develop a statistical approach for detecting differentially abundant proteins in isobaric labeling proteomic experiments. It first measures relative protein abundance by robustly summarizing reporter ion intensities. This summarization addresses the issue of missing values across multiple experiments, where a large proportion of peptides are only identified in a subset of experiments. Next, protein abundances are normalized based on reference channels to allow comparison across multiple experiments. This normalization also makes our approach able to analyze data from both balanced and unbalanced design. Then different linear models are fitted based on the underlying experimental design, including one or multiple experiments with or without technical replicates and fractionation. Finally, model-based inference is carried out to identify differentially abundant proteins. In particular, the inference procedure is adjusted by applying an empirical Bayes shrinkage when the sample size is small. We implement the statistical approach in an open-source R package, named MSstatsTMT.

MSstatsTMT is evaluated using a controlled mixtures dataset comprising 15 TMT10-plex experiments with 48 Sigma UPS1 spiked-in proteins. The results show that our approach produces best sensitivity with controlled FDR. It also demonstrates the benefit of using robust summarization to improve the accuracy of fold change estimation. We also illustrate the application of MSstatsTMT using two publicly available biological datasets.

2:00 - 3:50 pm Monday
AGING
Session Chairs: Birgit Schilling and Paola Sebastiani
Oceana 8-10

MOA pm 2:00

Proteomics Analysis of Skeletal Muscle in Healthy Human

Ceereena Ubaida-Mohien; Alexey Lyashkov; Ravi Tharakan; Marta Gonzalez-Freire; Michelle Shardell; Ruin Moaddel; Chee Chia; Luigi Ferrucci

National Institute on Aging, Baltimore, MD

Introduction:

Muscle degeneration is a substantial cause of weakness and frailty and in older persons, but the cause of such degeneration remains unknown and no previous study assessed the human skeletal muscle proteome over the course of aging. We have examined the proteome of human skeletal muscle utilizing a

TMT-based protein quantification approach in very healthy persons dispersed over a wide age-range.

Methods:

Skeletal muscle biopsies were collected from 60 healthy human donors ranging in age from 22 to 87 years. Tandem Mass Tag (TMT) 6+ was used for relative protein quantification. Skeletal muscle proteins were extracted, trypsin digested, reduced and alkylated. MS/MS peaks from the samples were searched and analyzed. For final representation, spectra were normalized by median polish and median sweep, protein identifications were quantified and annotated.

Results:

Relationships of protein levels with aging were examined by the linear regression model. Several functional classes of proteins were found to be altered during aging, including contractile proteins, mitochondrial proteins, metabolism proteins, assembly complex proteins, innate immune proteins and proteins involved in gene expression. Proteins that maintain cellular physiology, biogenesis and cell cycle were also dysregulated with the course of aging. We found an age-dependent decline of TCA cycle, respiratory chain, glycolysis and the electron transport pathway proteins. An age-dependent increase in major spliceosome complex proteins was also evident.

Conclusion:

Our preliminary analysis shows that the skeletal muscle proteome undergoes substantial changes with healthy aging, indicating profound changes in energy metabolism and spliceosome complex.

MOA pm 2:20
May mitochondrial dysfunction predispose for cancer?

Christopher Gerner
University Of Vienna, ,

The contribution of mitochondrial dysfunction to carcinogenesis has been a question of debate for decades. However, the Warburg effect and its consequences are now recognized as hallmark of cancer, and a causative role of metabolic stress for carcinogenesis is emerging. Taking a perspective that tumor cells do not invent new but rather take advantage of evolutionary programmed mechanisms established for other purposes, mitochondrial events observed upon stimulation of normal T-cells may represent a model for cancer cells. Here, normal cells may get the privilege for an energy burst required for fast anabolism at potentially adverse metabolic conditions such as hypoxia. Mitochondrial proteome signatures of several kinds of cancer are actually highly reminiscent to that of activated T cells. However, such an energy burst may come at a price, eventually causing activation-induced apoptosis in normal cells. Here I suggest that mitochondrial adaptation to the associated metabolic conditions may cause dysregulation of the reactive oxygen species management and thus select for tumor-promoting conditions affecting cell de-differentiation and apoptosis control. Combining proteomics with lipidomics and metabolomics, my lab is collecting evidence for systemic metabolic stress associated with chronic inflammation and aging, also involving platelets as relevant tumor-promoting entities related to mitochondrial dysfunction. In line, since chronic inflammation is known to increase with aging and may promote such mitochondrial dysregulation, chronic inflammation seems to cause increased cancer risk via mitochondrial adaptation.

MOA pm 2:40
Mouse and human models of longevity indicate altered response to diet induced free radical damage

Nadia Ashrafi[1, 2]; Wendy E. Heywood[1]; Marie-Stéphanie Clerget-Froidevaux[2]; Barbara Demeneix[2]; Diana van Heemst[3]; Raymond Noordam[3]; Kevin Mills[1]
[1]University College London, London, United Kingdom; [2]Evolution of Endocrine Regulations, Paris, France; [3]Leiden University Medical Center, Leiden, Netherland

The aim of this study was to perform multi-organ proteomic profiling of a mouse model of longevity and understand its response to high fat diet. We subsequently validated key findings using a human longevity model. Two mouse strains BL (normal life span) and WSB (long-lived) mice were investigated and fed a high fat diet (HFD) for a period of three days to eight weeks. Label free proteomics was used to establish proteomic profiles of 8 different mouse organs.

A greater change from normal diet (baseline) was observed in the liver proteome after three days of HFD and more so than 8 weeks. Less change was observed in normal strain: liver (3-4%) compared to long lived strain: liver (7-8%). The greatest changes were observed in white and brown fat tissue. Thirty percent of the proteome altered after 3 days and 33% after eight weeks of HFD in brown adipose tissue. The long-lived mouse proteome changes faster than the normal-lived mouse on a high fat diet. Ingenuity pathway analysis showed reduced detoxification and response to reactive oxygen species in the liver of long-lived mice in response to high fat diet.

To confirm changes in ‘detoxification’ and ‘response to reactive oxygen species occurs in humans as well we measured modified urinary vitamin E metabolites (known biomarkers of oxidative stress). This was performed in a human model of longevity namely from offspring of centenarians (age > 100yrs). Markers of oxidative stress were found higher in normal lived subject’s than centenarian offspring. This confirms the mouse model observations and indicates in response to diet long lived people have reduced oxidative stress burden through an improved ability to deal with free radical damage compared to average lived people. We conclude that better mechanisms of detoxification are likely a key factor in longevity.

MOA pm 2:52
Representation of neonatal and paediatric proteins in current plasma proteome databases

Conor McCafferty[1]; Jochen M. Schwenk[2]; Vera Ignjatovic[1, 3]
[1]Murdoch Children's Research Institute, Parkville, Australia; [2]KTH Royal Institute of Technology, Stockholm, Sweden; [3]The University of Melbourne, Parkville, Australia

Age-specific differences in the plasma proteome have recently been determined, covering the age spectrum from newborns to adults [1]. However, the majority of the publicly available data and resources focus on adults. Considering the plasma proteome as a dynamic milieu, this study aimed to understand the representation of the age-specific proteins within the currently existing lists of plasma proteins.

We used the 107 age-specific plasma proteins that were previously identified using a SWATH-MS approach [1], and compared these to three plasma centric protein databases. Databases used were: (i) the recent draft of the Human Plasma Proteome [2] listing MS-based non-glycosylated plasma proteins in PeptideAtlas; (ii) plasma proteins detectable by MS and immunoassays and (iii) a list of the most popular proteins described in a literature mining study, ranked by publication number and citation rate [3]. Specifically, these databases were searched to determine if they previously contained any of the 107 proteins detected to have age-specific differences.

The number of age-specific proteins matched to each database was: 57 in the Plasma Peptide Atlas (n=3,694); 51 in the MS/immunoassay lists (n=1087); and 38 in recent literature mining (n=8,122). A set of 22 of these proteins was common to all databases, reflecting their importance in the human proteome.

This study serves to advance the knowledge of the plasma proteome as related to development, providing suggestions of the biological processes that affect the bioavailability of plasma proteins or interacting components. Understanding the age-specific progression in protein expression is integral for early recognition and prevention of diseases specific for neonates and children or those generally associated with aging.

1. Bjelosevic, S., et al., Molecular & Cellular Proteomics, 2017;16:924-935

2. Schwenk, J.M., et al., Journal of Proteome Research, 2017;16:4299-4310

3. Yu, K.-H., et al., Journal of Proteome Research, 2018;17:1383-1396

MOA pm 3:04
Identification and Quantification of Biomarkers for Senescent Cells Using Mass Spectrometry

Nathan Basisty; Abhijit Kale; Herbert Kasler; Eric Verdin; Judith Campisi; Birgit Schilling
The Buck Institute for Research on Aging, Novato, CA

Background: Cellular senescence is a stress response that causes an irreversible arrest of cell proliferation and development of a senescence-associated secretory phenotype (SASP). Pre-clinical studies have demonstrated that targeted removal of senescent cells is beneficial for cardiac, metabolic, neurological, and musculoskeletal functions, including improvements in mobility and frailty. Major efforts are underway to develop drugs that selectively induce apoptosis in senescent cells. However, translating these therapies requires a means to measure the senescent cell burden in humans.

Methods: Senescence-associated proteins were characterized by analyzing the secreted soluble, exosome, and cell-surface proteins of oncogene-induced (RAS) or irradiation-induced senescent (SEN) human fibroblasts versus non-senescent control (CTL) cells. These proteomes were compared with proteins detected in human plasma and plasma-derived exosomes to establish biomarker candidates. Secreted proteins and exosomes were obtained from the medium of cells cultured for 24 hours in serum-free conditions. Cell-surface proteins were isolated using Cell-Surface Capture, a biotinylation/affinity enrichment approach. Data-independent acquisitions (DIA) were performed on a TripleTOF 6600.

Results: We identified >1500 secreted proteins in the culture supernatants, with 592 and 1136 significantly altered in abundance in radiation-induced and RAS-induced senescence, respectively. Over 120 of these secreted SASP (sSASP) factors were also present in human plasma. In the exosome SASP (eSASP), 470 proteins were significantly changed in SEN versus CTL cells, including proteins involved in RAGE, syndecan-1, and notch signaling. 145 eSASP proteins were also present in plasma-derived exosomes. Finally, 100 cell-surface proteins were differentially expressed in SEN vs CTL cells, mapping to pathways in cell death, mRNA processing, and TGFb signaling. Early validation of these biomarkers in human aging will be presented.

Conclusions: Our comprehensive proteomics approach has revealed novel senescence-associated secreted and cell-surface proteins. We will further focus on validating biomarker candidates and assessing their utility for removal of human senescent cells *in vivo*.

MOA pm 3:16
Establishing a Roadmap for Brain-based Protein Biomarkers in Alzheimer's Disease

Maotian Zhou; Duc Duong; Lingyan Ping; Eric Dammer; Marla Gearing; James Lah; Allan Levey; Nicholas Seyfried
Emory School of Medicine, Atlanta, <Not Specified>

There is a need for novel biomarkers of Alzheimer's disease (AD) and other neurodegenerative disorders that are minimally invasive and that more broadly serve as accurate indicators of the underlying pathophysiological processes in brain. The Accelerating Medicine Partnership AMP-AD target discovery consortium is performing large scale multi-omics profiling and systems level integration of more than 2,000 postmortem human brains, establishing an unprecedented understanding of the pathophysiological processes driving cognitive decline, pathological burden, and other disease traits. The Emory AMP-AD team has focused on large scale proteomic analyses using unbiased mass spectrometry methods to quantify thousands of proteins in brains from several different cohorts. Systems based network approaches reveal highly conserved modules of co-expressed proteins, many of which correlate strongly with clinical and pathological phenotypes, including those reflecting key mechanisms strongly correlated with impaired neuronal and synaptic function, neuroinflammation, and neurodegeneration. Because the protein modules directly relate to various pathophysiological processes, the hub proteins within each module may also serve as novel biomarkers of these processes. We performed preliminary studies to determine whether hub proteins representing these brain-based modules are found in cerebrospinal fluid (CSF). Following albumin depletion, we analyzed CSF samples from well-characterized AD and non-AD control patients and reliably quantified ~2,000 proteins by mass spectrometry across all samples. Of these, ~70% of the proteins were also identified in brain tissue, including members of phenotype-associated modules. We are now developing targeted proteomic assays (PRM and SRM) in CSF using stable isotopic labeled peptides standards to directly quantify hub proteins from AD brain modules most associated with disease traits. Hence, large-scale proteomics with systems analyses provides a comprehensive dataset of brain-based protein changes linked to AD and other neurodegenerative diseases. This establishes a novel pipeline for targeting brain-based proteins in CSF as biomarkers for diagnosis, staging and therapeutic responses.

MOA pm 3:28
Investigating the relationship between protein glycation and stability in cells and tissues

Simone Di Sanzo; Joanna Kirkpatrick; Nicolas Huber; Alessandro Ori
Leibniz Institute on Aging, Jena, Germany

The homeostasis of the proteome of cells is required to maintain the function of organs and it was shown to decline during aging. The proteostasis is influenced by changes in protein localization, protein abundance and post-translational modification. The formation of advanced glycation end products (AGEs) is a non-enzymatic posttranslational modification that has been shown to influence the activity and aggregation propensity of both extracellular and intracellular proteins. The formation of highly reactive α-oxoaldeheyde (methylglyoxal, glyoxal and 3-deoxyglucosome) determinant for AGEs production, has been shown to have a key role in Drosophila metabolism and hence in the progression of type 2 diabetes. On the other hand, increased endogenous level of methylglyoxal has been shown to extend lifespan and healthspan in *C. Elegans* through a proteohormesis mechanism. Although a handful of specific AGE-modified proteins have been identified for example in aging skeletal muscle, a detailed characterization of the targets of AGEs and the relationship between this modification and protein stability are still missing. Aiming at identifying the preferential targets of AGEs, we developed a proteomic workflow based on selective enrichment of AGE-modified proteins coupled to mass spectrometry for protein identification. In parallel, we investigated the relationship between protein glycation and stability by monitoring proteome-wide changes in protein thermal stability induced by exposure of primary cells to glyoxal. By measuring changes of glycation state, protein abundance and stability in the same system, we aim to reveal whether modification by AGEs is mechanistically linked to changes in protein stability, and whether a specific protein expression is induced in response to this specific proteotoxic stress. In the future, we plan to extend our work to tissue to study the targets of AGEs during aging across different tissues, and the effect of dietary interventions and obesity on the extent and specificity of protein glycation.

2:00 - 3:50 pm Monday
NUTRITION AND FOOD
Session Chairs: Subhra Chakraborty and Paola Roncada
Oceana 7

MOB pm 2:00
MPK4 interacting proteins in crop immunity response

Sixue Chen
University of Florida, GAINESVILLE, FL

MAP kinase 4 (MPK4) has been proposed to be a negative player in plant immunity, and it is also activated by pathogen-associated molecular patterns (PAMPs), such as flg22. The molecular mechanisms by which MPK4 is activated and regulates plant defense remain elusive. In this study, we investigated plant defense against a bacterial pathogen Pseudomonas syringae pv. tomato (Pst) DC3000 when the MPK4 is overexpressed. We showed an increase in pathogen resistance and suppression of jasmonic acid (JA) signaling in the MPK4 overexpressing (OE) plants. We also showed that the OE plants are very sensitive to flg22-triggered reactive oxygen species (ROS) burst in guard cells, which resulted in an enhanced stomatal closure, compared to wild-type (WT). During flg22 activation, dynamic phosphorylation events within and outside of the conserved TEY activation loop were observed. To elucidate how MPK4 functions during the defense response, we used immunoprecipitation coupled with mass spectrometry (IP-MS) to identify MPK4 interacting proteins in the absence and presence of flg22. Quantitative proteomic analysis revealed a shift in the MPK4-associated protein network, providing insight into the molecular functions of MPK4 at the systems level.

MOB pm 2:20
Omics approaches to uncover tolerant mechanism in soybean at the initial flooding stress

Setsuko Komatsu
Fukui University of Technology, Fukui, Japan

Global climate changes influence the magnitude and frequency of hydrological fluctuations and cause unfavorable environment for plant growth and development. Soybean is sensitive to flooding stress, which markedly reduces its growth. To identify the mechanism of flooding tolerance at initial stage in early-stage soybean, proteomic, transcriptomic, and metabolomic techniques were used. Flooding tolerant mutant line and abscisic acid-treated soybean, which exhibited flooding tolerant phenotype, were used as materials. Early-stage soybeans were treated during initial stage of flooding stress and roots were collected for proteomic as well as metabolomic and transcriptomic analyses. Data were analyzed using functional categorization, cluster separation, and in silico protein-protein interaction. Furthermore, commonly changed metabolites, proteins, and genes between mutant and abscisic acid-treated soybeans were considered as flooding-tolerance related candidate factors. Finally, omics results were integrated to analyze the flooding tolerant mechanism in soybean and confirmed using biochemical and biological techniques. These results suggest that flooding tolerance at initial stage in early-stage soybean might be through protecting newly synthesized proteins and enhancing activities of antioxidative enzymes to remove reactive oxygen species. Furthermore, an integrated approach of proteomics and computational genetic modification effectiveness analysis was applied to explore flood-tolerant genes in soybean, suggesting that proteins related to energy metabolism might play an essential role to confer flood tolerance in soybeans.

MOB pm 2:40
Nutraceuticals, deciphering mechanism of actions of novel bioactive compounds by thermal proteome profiling

Ana M Carrasco del Amor[1]; Olatz Fresnedo[2]; Siegfried Ussar[4]; Ralph Urbatzka[3]; Susana Cristobal[1]
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Nutraceuticals are comestible substances that could provide some physiological benefit against chronical diseases. Nutraceutical discovery strategy is based on phenotyping screening. However the direct targets of the bioactive compounds are mainly unknown and this is still the biggest field challenges. A mass spectrometry based method has been recently developed that study the perturbation of the thermal stability of the proteome. The thermal proteome profiling (TPP) has been successfully applied to study drug targets and off-

target. We hypothesized that this method could be applied to assess the target of any bioactive compound.

Identification of targets is the key to decipher the mechanism of action of a bioactive compound, predict the mode of action as well as possible harmful effects. TPP is Thermal is a very promising strategy to assess thousands of candidate protein targets in parallel in a physiologically relevant context. Our lab has tested the hypothesis in the context of a project focused on novel nutraceutical for obesity. We have analyzed novel bioactive compound extracted from cyanobacteria. The selected compounds arises from phenotypic screening for their capabilities to reduce triglycerides level in zebrafish embryo. TPP profiling has been applied to elucidate possible targets and mode of actions. Results from the analysis on hepatocytes and brown adipocytes cell lines indicates that regulation of lipid and carbohydrate metabolism are the principal targets. Considering the compound chemical structure a possible enzymatic inhibition will be discussed. The nutraceutical mode of action against obesity will be decipher from the integrated map of network pathways. This knowledge is the first step to elucidate the interactions of the different protein targets in a living cells that is the realistic scenario. Additional experiments will be performed to determine compound potency and to infer a rank of potency of the different protein targets at the cellular context.

MOB pm 2:52

Single shot DIA profiling of >1500 plasma proteomes of the weight loss and maintenance study DiOGenes

Roland Bruderer[1]; Jan Muntel[1]; Sebastian Müller[1]; Oliver M. Bernhardt[1]; Tejas Gandhi[1]; Polina Mironova[2]; Ondine Walter[2]; Jérôme Carayol[2]; Arne Astrup[3]; Wim H.M. Saris[4]; Jörg Hager[2]; Armand Valsesia[2]; Loic Dayon[2]; Lukas Reiter[1]

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Comprehensive, robust, high-throughput analysis of the plasma proteome has the potential to enable analysis of the health state. Up to now, analysis of the plasma proteome was achieved using delicate nano-flow setups. To reduce this limitation, we established a robust capillary-flow LC-MS-DIA setup capable of 32 proteomes a day and acquired the samples of a weight loss and maintenance plasma sample study (DiOGenes).

Plasma samples were randomized and prepared with an optimized in-solution digestion protocol and spiked with Biognosys' PlasmaDeepDive. The samples were acquired on a Thermo Fusion Lumos connected to a Waters M-class and a Waters CSH 1.7µm column. DDA runs were performed using HPRP fractionation. Analysis of DIA runs was performed using Spectronaut Pulsar.

After testing several combinations, we obtained optimized conditions using a 300 µm inner diameter column. This setup has the advantage of offering increased flow rates (5 µl/min) resulting in small injection-to-injection overhead times (i.e., 5 min). A gradient of 40 min was determined as an optimal gradient length, resulting in 90% of the maximally achieved identifications with longer gradients. Further characterization of the system showed precise quantification of 47 FDA approved plasma biomarkers (CVs below 20%).

We successfully used this capillary LS-MS DIA setup to acquire 1,508 samples of the DiOGenes project. We identified in average 450 proteins per samples and achieved a dataset completeness of 77% at the protein level. Proteins of the inflammation system were the most variable and proteins involved in blood coagulation were the least variable protein groups. Unsupervised clustering showed that the weight loss was most severe intervention accompanied with significant protein changes in plasma.

Differential abundance analysis identified proteins to be significantly differential between all of the time points revealing biology. Non-enzymatic addition of glucose to proteins (i.e., glycation) was detected and could be followed over the time course of the study.

MOB pm 3:04

Comparative Proteomics of Low Oxalate Tomatoes during Post-harvest Storage

Sudip Ghosh; Kanika Narula; Pooja Aggarwal; Niranjan Chakraborty; Subhra Chakraborty

National Institute of Plant Genome Research, New Delhi, India

Post-harvest storage has a profound impact on nutritional quality and economic loss in food crops that is governed by combinatorial properties of sensorial attributes, organoleptic parameters and physiological characteristics. Organic acid composition of storage organs forms the molecular and biochemical basis of organoleptic and nutritional qualities with metabolic specialization. Of these, oxalic acid (OA), determines the post-harvest quality in fruits. Tomato (*Solanum lycopersicum*) ranks first among fruits and vegetables with 16% of the total production worldwide, however, loss due to post-harvest storage is 10%. We have earlier shown that tomato plants overexpressing an oxalate decarboxylase (*FvOXDC*) in the fruit, leads to decrease in the oxalic acid content besides increasing fungal tolerance and better storage effect. Here, we elucidate the mechanistic correlation between oxalic acid downregulation and maintenance of organoleptic and textural properties in tomato during post-harvest storage by comparative protein profiling of wild-type and low oxalate tomato in a temporal manner. The MS/MS analyses led to the identification of 32 and 39 differentially abundant proteins in wild-type and low oxalate tomatoes, associated with primary and secondary metabolism, assimilation, and development. Correlation network analysis identified significant functional hubs pointing toward storage related coinciding processes. Furthermore, physiochemical analyses revealed reduced oxalic acid content with concomitant increase in citric acid, lycopene and pectinesterase activity in low oxalate fruit. The comparative analysis of proteome from wild-type and low oxalate fruits identified shared and discrete pathways and regulatory modules related to storage. The study for the first time explained how low oxalate leads to the modulation of fruit quality typically linked to post-harvest storage and may reduce loss due to patho-stress.

MOB pm 3:16

Identification of functional peptides with tolerogenic potential in a partially hydrolysed infant formula

Joost Gouw[1]; Juandy Jo[2, 3]; Laura Meulenbroek[1, 3]; Sam Heijjer[1, 3]; Erica Kremer[1]; Elena Sandalova[2, 3]; André Knulst[4]; Sergio Oliviera[1]; Jan Knol[1, 5]; Johan Garssen[1, 3]; Anneke Rijnierse[1]; Léon Knippels[1, 3]

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Oral tolerance is the default response of the immune system to innocuous food proteins and is characterized by regulation of local and systemic immune responses to these proteins. Failure to induce oral tolerance to food proteins results in food allergy. International prevention guidelines recommend the use of partial hydrolysed cow's milk-based infant formula in infants with increased risk of developing allergic diseases, when breastfeeding is limited or absent. The aim of this study was to investigate whether a specific partial hydrolysed whey-based infant formula contains unique peptides that might act as functional human T-cell epitopes to support the development of oral tolerance to whey.

First, a liquid chromatography-mass spectrometry (LC-MS) method was developed to characterise b-lactoglobulin (BLG)-derived peptides present in a whey-based hydrolysed formula with a particular focus on AA13-48 of the mature BLG protein, a region which has previously been described to contain T-cell epitopes with tolerogenic potential. Second, the same formula was subjected to the ProImmune ProPresent® antigen presentation assay to identify HLA-DRB1-restricted, BLG-derived T-cell epitopes. Third, synthetic peptides identical to the BLG-derived peptides identified by LC-MS were tested on human cow's milk-specific T-cell lines to determine T-cell recognition.

Thirteen BLG-derived peptides of minimal 9AAs long that overlap with the region of AA13-48 of mature BLG were identified. Six of them were found across all batches analysed. It was further confirmed that these peptides were internalized, processed and presented by human dendritic cells. The identified HLA-DRB1-restricted T-cell epitopes were correlated to AA11-30 and AA23-39 of mature BLG. Importantly, the T-cell proliferation assay showed that the synthetic peptides were recognized by cow's milk-specific T-cell lines and induced T-cell proliferation.

This study demonstrates that the developed LC-MS method allows for the detection of functional HLA-DRB1-restricted T-cell epitopes which have the potential to support the development of oral tolerance.

MOB pm 3:28

Culture independent label free method for milk metaproteome and resistome analysis

Cristian Piras[1]; Alessio Soggiu[1]; Viviana Greco[2]; Luigi Bonizzi[1]; Alfonso Zecconi[1]; Andrea Urbani[3]; Claudia Gusmara[1]; Domenico Britti[4]; Paola Roncada[4]

[1]DIMEVET - University of Milan, Milano, Italy; [2]Fondazione Santa Lucia, Rome, Italy; [3]Catholic University of Sacred Heart, Rome, Italy; [4]Università Magna Græcia, Catanzaro, Italy

Dairy cow mastitis (CM) is the cause of a great financial loss for animal production industry. The average cost of a single case of CM is around $ 179. Late and imprecise diagnose lead to costs increase and to a more difficult therapeutic intervention.

Milk microbiota and, more widely, milk cellular fraction that includes as well somatic cells of mammary gland, represent a great source of biological information.

The main aim of this work is to analyze through proteomics both the bacterial and cellular compartment of raw milk in order to gain information about the whole bacterial composition and about the index of antibiotic resistance.

The cellular fraction isolated from raw milk has been collected and subjected to bead beating to achieve bacterial lysis. After quantitation, precipitated proteins have been digested using Filter-aided sample preparation (FASP) and analyzed through Label-free in ion mobility–enhanced data-independent acquisition (DIA) proteomics analysis by Expression configuration mode (HDMSe). Raw data were analyzed with ProteinLynx Global Server v. 3.0.2 (PLGS, Waters Corp.) against two different protein databases.

Results highlighted the identification of 400 proteins in total, of this ones, 285 proteins were identified as Bos taurus proteins, 115 as bacterial proteins and, 11 of the identified proteins were related to the CARD database and therefore actively involved in antibiotic resistant processes.

This method allowed the culture free analysis of raw milk bacterial consortia and provided an index of intrinsic antimicrobial resistance that could be consistently related to antibiotic contamination in the environment.

2:00 - 3:50 pm Monday
POSTTRANSLATIONAL MODIFICATIONS (PTMs)
Session Chair: Karolin Luger and Yingming Zhao
Oceana 6

MOC pm 2:00

Quantitative Proteomics for Understanding Cancer Epigenetics

Benjamin Garcia

University of Pennsylvania School of Medicine, Philadelphia, PA

Histones are small proteins that package DNA into chromosomes, and a large number of studies have showed that several single post-translational modification (PTM) sites on the histones are associated with both gene activation and silencing. Along with DNA and small non-coding RNA, histone PTMs make up epigenetic mechanisms that control gene expression patterns outside of DNA sequence mutations. Dysregulation of the epigenetic networks underlie several human diseases such as cancer. Here we use mass spectrometry based proteomics to survey the histone PTM landscape in a specific sarcoma cancer, identifying several histone marks that are reprogrammed in the cancer state. Global large scale proteomics further identified signaling pathways that were over or underexpressed in the disease state. Our results point to a combination of DNA methylation and histone modification pathways as being crucial for maintain the oncogenic state.

MOC pm 2:20

Linear Ubiquitin Control Identified by Positional Proteomics leading to Discovery of a Molecular Corrector to Rescue NFkB Activation in Immunodeficiency

Christopher Overall

University of British Columbia, Vancouver, BC

Upregulated proteases are attractive drug targets in a variety of malignancies, inflammatory and autoimmune diseases. However, increasing protease activity to correct delayed or insufficient protease activity in disease is chemically and therapeutically challenging. MALT1 is central for transducing B and T lymphocyte antigen receptor activation of NF-κB. Overactivity can lead to lymphomas whereas underactivity can lead to immunodeficiency.

We identified a patient homozygous for a MALT1 Trp580Ser mutation who suffered from reduced NFkB activation and combined immunodeficiency. The mutation weakened the interaction between the paracaspase and C-terminal immunoglobulin domains of MALT1 resulting in protein instability (Tm 45.9°C versus 52.5°C) and consequently reduced MALT1 function, protein levels. TAILS enrichment of mature protein and cleaved neo-N-termini identified MALT1 downregulation of LUBAC and essential linear ubiquitination in the NF-κB pathway that was impaired in immunodeficiency.

We discovered nanomolar, selective allosteric inhibitors of MALT1 paracaspase activity that bind by replacing the side-chain of Trp580 and locking the protease in an inactive conformation. Due to this specific mode of action and the coincident location of the MALT1-Trp580Ser mutation in the MALT1 immunodeficient patient, we reasoned that such allosteric compounds may stabilize the MALT1-Trp580Ser mutant protein by replacing the absent tryptophan residue. We found that the new allosteric inhibitors bind MALT1-Trp580Ser, stabilizing the mutant protein and increasing the Tm to that of wild-type MALT1. In so doing the small molecule allosteric nanomolar inhibitors restored MALT1 protein levels in MALT1mut/mut lymphocytes, rescuing canonical NF-κB and JNK signaling, and improving the proteolytic activity of MALT1-Trp580Ser to restore LUBAC regulation by increasing HOIL1 cleavage and hence function. Thus, a pharmacological molecular corrector compound rescued protease deficiency by substituting for the mutated residue to restore NFkB function and increase cleavage activity, inspiring potential precision therapies to increase mutant enzyme activity in a variety of diseases.

MOC pm 2:40
Lysine benzoylation is a new type of histone mark regulated by SIRT2

He Huang; Di Zhang; Mathew Perez-Neut; Yingming Zhao
The University of Chicago, Chicago, <Not Specified>

We report the identification and characterization of a previously undescribed histone mark, lysine benzoylation (Kbz). First, we identified a mass shift of +104.0268 Da at histone H2BK5 residue. MS/MS and coelution analysis of the in vivo peptide and corresponding synthetic peptide demonstrated that this mass shift is caused by a new type of PTM, Kbz. Next, we detected Kbz histones from HepG2 cells, mouse liver, and Drosophila S2 cells by western blot, indicating Kbz is an evolutionarily conserved histone mark in mammalian and insect cells. Second, to identify in vivo histone Kbz marks, we carried out proteomic analysis using histone proteins extracted from HepG2 and RAW cells that were treated by 5 mM of Sodium Benzoate (SB). We identified 21 unique histone Kbz sites, in which 18Kbz sites were mainly located on core histone N-terminal tails. In addition, the mass spectrometry-based quantification experiment showed an increase of D5-benzoyl-CoA in a dose-dependent manner upon D5-SB treatment, suggesting that SB is a precursor for the synthesis of benzoyl-CoA in the lysine benzoylation reaction. Third, quantitative proteomics results indicated that at 5 mM (~0.07%) of SB, a concentration lower than the maximum allowed percentage in food, dramatically increased the abundance of histone Kbz. For example, H3K23bz and H4K8bz sites in HepG2 cells, as well as H4K5bz and H2AK13bz sites in RAW cells, increased ranging from 26.25- to 49.49-fold, while H2AK9bz in both HepG2 and RAW cells increased more than 50-fold. Finally, we demonstrate that histone Kbz marks are associated with gene expression and have unique physiological relevance from histone acetylation. In addition, we demonstrate that SIRT2, a NAD+-dependent protein deacetylase, removes histone Kbz both in vitro and in vivo. Together, this study reveals a new type of physiologically relevant histone mark and identifies non-canonical functions of a widely used chemical food preservative.

MOC pm 2:52
Reanalysis of Global Proteomic and Phosphoproteomic Data Identified a Large Number of Glycopeptides

Yingwei Hu; Punit Shah; David J. Clark; Minghui Ao; Hui Zhang
Johns Hopkins University, Baltimore, Maryland

Protein glycosylation plays fundamental roles in many cellular processes, and previous reports have shown dysregulation to be associated with several human diseases, including diabetes, cancer, and neurodegenerative disorders. Despite the vital role of glycosylation for proper protein function, the analysis of glycoproteins has been lagged behind to other protein modifications. In this study, we describe the re-analysis of global proteomic data from breast cancer xenograft tissues using recently developed software package GPQuest 2.0, revealing a large number of previously unidentified N-linked glycopeptides. More importantly, we found that using immobilized metal affinity chromatography (IMAC) technology for the enrichment of phosphopeptides had co-enriched a substantial number of sialoglycopeptides, allowing for a large-scale analysis of sialoglycopeptides in conjunction with the analysis of phosphopeptides. Collectively, combined MS/MS analyses of global proteomic and phosphoproteomic datasets resulted in the identification of 6,724 N-linked glycopeptides from 617 glycoproteins derived from two breast cancer xenograft tissues. Next, we utilized GPQuest 2.0 for the re-analysis of global and phosphoproteomic data generated from 108 human breast cancer tissues that were previously analyzed by Clinical Proteomic Analysis Consortium (CPTAC). Reanalysis of the CPTAC dataset resulted in the identification of 2,683 glycopeptides from the global proteomic dataset and 4,554 glycopeptides from phosphoproteomic data set, respectively. Together, 11,292 N-linked glycopeptides corresponding to 1,731 N-linked glycosites from 883 human glycoproteins were identified from the two datasets. This analysis revealed an extensive number of glycopeptides hidden in the global and enriched in IMAC-based phosphopeptide-enriched proteomic data, information which would have remained unknown from the original study otherwise. The reanalysis described herein can be readily applied to identify glycopeptides from already existing datasets, providing insight into many important facets of protein glycosylation in different biological, physiological, and pathological processes.

MOC pm 3:04
Phosphoproteomic landscapes of cancer cell lines predict drug response

Martin Frejno[1]; Benjamin Ruprecht[1, 2]; Chen Meng[1]; Alexander Hogrebe[1, 3]; Jana Zecha[1, 4]; Dominic Helm[1, 5]; Thomas Oellerich[6, 7]; Sebastian Scheich[7]; Hans-Michael Kvasnicka[7]; Enken Drecoll[1]; Wilko Weichert[1]; Bernhard Kuster[1, 8]
[1]Technical University of Munich, Freising, Germany; [2]Merck & Co., Boston, MA; [3]University of Copenhagen, Copenhagen, Denmark; [4]German Cancer Consortium (DKTK), Munich, Germany; [5]EMBL, Heidelberg, Germany; [6]Cambridge University, Cambridge, United Kingdom; [7]Goethe University, Frankfurt, Germany; [8]Center for Integrated Protein Science (CIPSM), Munich, Germany

In recent years, proteomic profiling of cancer cell lines combined with quantification of their response to drugs has proven to be useful for the identification of protein biomarkers of drug sensitivity and resistance. However, given that phosphorylation-based signalling is known to play a major role in cancer biology and is the target of many modern drugs, predicting drug sensitivity of cancer cell lines by focusing on their activity landscapes may be a promising approach to discover molecular markers or alternative therapeutic options. Here, we profiled the proteomes and phosphoproteomes of 125 cancer cell lines using label-free mass spectrometry to a depth of >10,000 proteins and >55,000 phosphorylation sites (p-sites). We integrated this data with publicly available drug sensitivity measurements, identify proteomic and phosphoproteomic markers of drug response and suggest novel functional relationships. We validated selected proteins predicting drug resistance by immunohistochemistry, which revealed their negative influence on overall survival in a small cohort of patients treated with a certain class of drugs. Subsequent in vitro experiments showed that these proteins can indeed metabolize these drugs, thereby inactivating them. Using concordance analysis, we were able to model the response to drugs with similar sensitivity profiles simultaneously as a function of our phosphoproteomic data. This led to the identification of known and novel p-sites correlated with the response to groups of drugs sharing a common mode of action. Outlier analysis of our phosphoproteomic data suggests that co-occurrence of phosphorylation on pairs of receptor tyrosine kinases in specific cell lines is indicative of their benefit from co-inhibition of these proteins. Finally, correlation network analysis of our proteomic and phosphoproteomic data revealed known functional relationships (e.g. CDKs and cyclins), while also suggesting novel ones.

MOC pm 3:16
A tandem affinity enrichment method identifies macroH2A1 as a BRCA1/BARD1 E3 ligase substrate

Beom-Jun Kim[1]; Doug Chan[1]; Sung Jung[1]; Yue Chen[1]; Jun Qin[1, 2]; Yi Wang[1, 2]
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Purpose

Develop a MS-based method to identify E3 ubiquitin ligase substrates and apply it to identify BRCA1 substrates

Methods

The recombinant GST fused to 4 UBA domains was used to first enrich ubiquitinated proteins; then a Lys-□-Gly-Gly (diGly) antibody was used to enrich ubiquitinated tryptic peptides, followed by identification by mass spectrometry.

Results

MS analysis identified 5563 ubiquitinated peptides belonging to 2482 gene products from the control pcDNA3 and BRCA1/BARD1 transfected cells. Among them, 108 peptides from 101 proteins showed an increase of more than 3-fold upon BRCA1/BARD1 overexpression. Three proteins (HLTF, GADD45GIP1 and macroH2A1) that were known to be involved in DNA damage response and transcription were validated by transfection/western blotting. We further validated that BRCA1/BARD1 ubiquitinated macroH2A1 at lysine 123 in vitro and in vivo, and that primary human fibroblasts stably expressing ubiquitination-deficient macroH2A1mutant were defective in cellular senescence comparing to its wild-type counterpart.

Conclusions

A tandem affinity enrichment method was established that allowed for the identification of putative E3 ligase substrates. Further biochemical experiments demonstrated that K123 of macroH2A1 is ubiquitinated in a BRCA1/BARD1-dependent manner, and that this ubiquitination event plays an important role in cellular senescence.

MOC pm 3:28
Temporal-spatial orchestration of protein acetylation in antiviral response and immunity

Laura Murray; Xinlei Sheng; Ileana Cristea
Princeton University, Princeton, NJ

Virus-host interactions are at the core of the tug-of-war between host defense mechanisms and viral evasion of these responses. Emerging evidence points to protein acetylation as a nexus at this interface. Enzymes regulating protein acetylation are known to critically modulate metabolism and transcription during infection. However, the temporal control and function of acetylation in the context of infection remains poorly understood. Here, we present the first temporal-spatial characterization of acetylation during infection with human cytomegalovirus (HCMV). Acetylation-mediated functions were further investigated by integrating proteomic, virology, genetic, and microscopy tools. The dynamics of protein acetylation were defined at early (24 hpi), delayed early (48 hpi), and late (72, 96 hpi) stages of HCMV infection. By using acetyl-peptide enrichment, we identified approximately 5000 acetylation events on cellular and viral proteins with a wide-range of cellular abundances. As infection alters the cellular proteome, temporal proteome analyses were performed in parallel. The discovered acetylation events were placed in the context of subcellular localization using our analysis of density-fractionated organelle proteomes during infection. This highlighted organelles with increased acetylation (e.g., mitochondria). As mitochondrial acetylation can be enzymatically and non-enzymatically regulated, we used immunoaffinity purifications to monitor the interactions of the main mitochondrial deacetylase, SIRT3, with its substrates. Mitochondrial membrane potential and pH were assessed in parallel. Further, by live-cell microscopy and protein mutants, we identified acetylation driven mechanisms within the nucleus and at the nuclear periphery that suppress viral immune evasion and replication. Strikingly, we also discovered acetylations on viral proteins from all three classes of temporal expression, none of which were previously known. To determine if acetylations may already be present in infectious

virus particles, we enriched virions and performed an acetyl-peptide enrichment. By generating mutant virus strains with charge- or acetyl-mimics, we demonstrated that viral acetylation impedes viral replication.

2:00 - 3:50 pm Monday
HPP: TARGETING THE PROTEOME IN WOMEN'S HEALTH
Session Chair: Jennifer Van Eyk
Oceana 3-5

MOD pm 2:00
Invited talk for HPP: Targeting the Proteome in Women's Health
Tony Whetton
University of Manchester, Manchester, UK

MOD pm 2:30
Invited talk for HPP: Targeting the Proteome in Women's Health
Nur Yucer
Cedars Sinai Medical Center, Los Angeles, CA

MOD pm 3:00
Location is everything: protein translocation as a virus replication strategy
Katelyn C Cook; Pierre M Jean Beltran; Michelle A Kennedy; Ileana M Cristea
Princeton University, Princeton, NJ

Viruses, obligate intracellular parasites, are master manipulators of cellular machinery. Accordingly, cellular organelles, as specialized biological compartments, are often targeted by viruses during an infection process. This can cause broad alterations in organelle composition, structure, and localization, disrupting organelle function to facilitate the viral cycle. We integrated quantitative proteomics, bioinformatics, multidimensional image analysis, and functional analyses to gain a systems-level view of organelle regulation as a function of infection time. We applied this strategy to human cytomegalovirus (HCMV) infection, a beta-herpesvirus with nearly 90% worldwide seroprevalence and a significant health concern for pregnant women and immunocompromised patients. While HCMV is known to cause changes in protein abundance and organelle structure, our study uncovers acute virus-induced translocations between organelles for hundreds of proteins. We further defined the directionality and temporality of these events. Further investigation using CRISPR-mediated knockouts, live cell microscopy, and molecular virology revealed that translocations of cellular proteins contribute to both host defense and virus replication processes. Specifically, we found that lysosomal identity, virus assembly, and trafficking of infectious particles are dynamically regulated by translocating cellular proteins. Additionally, we discovered the translocation of previously uncharacterized viral proteins as a mean to inhibit host defense mechanisms. Our integrative study underlines the importance of localization-dependent protein function and adds a new dimension to understanding how viruses manipulate their hosts.

MOD pm 3:15
Improvement of detection sensitivity of nLC-MS towards the single-cell proteomics in the era of precision medicine
Qing Kay Li[1]; Chuanzi Ouyang[2]; Hui Zhang[2]
[1]Johns Hopkins Hospitals, Baltimore, <Not Specified>; [2]Johns Hopkins University, Baltimore, Maryland

Introduction: Both tumor heterogeneity and the tumor cell percentage have a potential effect on proteomic analysis of tumor tissue. When minute tumor specimen is analyzed, these factors could lead to false identification of biomarkers. Thus, the challenge still remains as how to identify proteins from a minimal number of tumor cells obtained from tissue slices. We have developed an approach to improve the detection sensitivity of nLC-MS for proteomics profiling at single-cell level.

Methods: the Tandem Mass Tag (TMT) was used. Two fold serial dilutions of NCI-7 tryptic peptides with Angiotensin II as the carrier peptide were used to label across 10-plex TMT set in a concentration of 65 ng/uL. The proteins in this channel play the role as flags for other channels which contain critically lower cell contents. For the TMT labelling reaction, zero concentration of NCI-7 was used in channel 126 with increasing concentration towards higher channels. After TMT labelling, 1 ug of the 10-plex mixture was loaded onto Q-Exactive for data acquisition.

Result: Among the 1ug of sample, we calculated the corresponding NCI-7 peptide in each channel: 3 cells equivalent of peptides in channel 127N, 6 cells equivalent of peptides in channel 127C, and with 2-fold increasing to channel 131, which contains 768 cells equivalent of NCI-7 peptides. We were able to see the 2-fold dilution pattern with quantification results. With the highest concentration channel 131 containing 768 cell equivalent peptides, we could identify over 2500 proteins. Even for channel 129 C, which containing 96 cell equivalent, we could still identify 2000 proteins.

Conclusion: Our approach not only demonstrated a good labelling efficiency across all the concentrations of the setup even for sub-nanogram level, but also provides a potential tool for proteomic analysis of clinical sample at single-cell levels.

MOD pm 3:30
Evaluating Mitra® microsampling devices for remote monitoring of apolipoproteins in patients at risk for cardiac events
Kelly Mouapi[1, 2]; Irene van den Broek[1, 2]; Mitra Mastali[1, 2]; Qin Fu[1, 2]; Garth Fuller[4]; Sandy Young[1, 3]; Shivani Dhawan[1, 3]; Mayra Lopez[4]; Chrisandra Shufelt[1, 3]; Brennan Spiegel[4]; Noel Bairey Merz[1, 3]; Jennifer Van Eyk[1, 2]
[1]Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA; [2]Advanced Clinical Biosystems Research Institute, Los Angeles, CA; [3]Barbra Streisand Women's Heart Center, Smidt Heart, Los Angeles, CA; [4]Cedars-Sinai Center for Outcomes Research(CS-CORE), Los Angeles, CA

Cardiovascular disease remains a leading cause of death in the nation and worldwide; among established patients, the majority of unexpected death occurs in those with stable ischemic heart disease. The need for early prediction of major adverse cardiac events (MACE) is therefore necessary. In this population study, we assess the feasibility of remote longitudinal blood sample collection using Mitra® microsampling devices coupled with a mass spectrometry protein biomarker panel. We evaluated

compliance and Mitra® tip quality in stable ischemic heart disease patients (n=200). Blood was collected from a fingerpick with the Mitra® microsampling device at four time-points: baseline (t0), one-month (t30), two-months (t60) and three-months (t90). At t0 and t90, samples were collected during outpatient visits and venous blood draws were also obtained. At t30 and t60, samples were collected by patients from home and mailed to the laboratory. We obtained greater than 80% compliance from home and 90 % in hospital setting. We also evaluated process quality control and the ratio apolipoprotein B (apoB) to apolipoprotein A-I (apoA-I), a known risk indicator for cardiovascular risk. ApoB/ApoA-1 ratios were measured from Mitra® tips using a targeted multiple reaction monitoring (MRM) mass spectrometry approach. Blood proteins were extracted and digested from Mitra® tips and plasma samples using our automated workflow. The analytical validity of apoA-I and apoB measurements was assessed by evaluating recovery, reproducibility, linearity, and stability, including storage and shipment. Recovery of apoA-I and apoB from the Mitra® device at three different hematocrit levels ranged between 85.3-96.7%, and 94.7-111.2% respectively. Analysis of the dried blood samples collected over a three-month period showed an average intra-individual %CV in apoB/apoA-I ratio of 9.5 %, whereas the inter-individual %CV was 37.5 % (n=40). We demonstrated the feasibility of remotely monitoring apoB/apoA-I ratios in stable ischemic heart disease patients using the Mitra® microsampling device.

2:00 - 3:50 pm Monday
PROTEOGENOMICS
Session Chair: Henry Rodriguez
Oceana 1-2

EditedMOE pm 2:00
Antibiotics Discovery: From Peptidogenomics to Genome Mining to Spectral Networks

Pavel A. Pevzner
University of California, San Diego, San Diego, CA

Genomics studies revealed numerous antibiotics-encoding genes across a wide range of bacterial and fungal species, including various species in the human microbiome. However, little is known about antibiotics (including many cyclopeptides) produced by microorganisms in the gut, despite the fact that humans are chronically exposed to them. Exploration of this *meta-antibiome* critically depends on a transition from the current one-off process of antibiotics analysis to a high-throughput antibiotics identification and *de novo* sequencing using tandem mass spectrometry. I will discuss recent advances in computational antibiotics discovery that span bioinformatics techniques ranging from peptidogenomics to genome mining to spectral networks. Specifically, I will describe how the recently developed tools SpecNets (Wang et al., *Nature Biotechnology* 2016), Dereplicator (Mohimani et al., *Nature Chemical Biology* 2017), VarQuest (Gurevich et al., *Nature Microbiology* 2018) and CycloNovo (Behsaz et al., submitted) revealed a vast hidden cyclopeptidome in the human gut and other environments. Moreover, they revealed a wealth of anti-microbial cyclopeptides from food that survive the complete human gastrointestinal tract, raising the question of how these cyclopeptides might affect the human microbiome.

This is a joint work with Bahar Behsaz (UCSD), Pieter Dorrestein (UCSD), Mark Fisher (University of Western Australia) Alexey Gurevich (Saint Petersburg University), Anton Korobeynikov (Saint Petersburg University), Hosein Mohimani (CMU), Joshua Mylne (University of Western Australia), and Alexander Shlemov (Saint Petersburg University).

MOE pm 2:20
Finding Functional Similarities: Proteogenomics and Pathway Analysis

Karin Rodland
Pacific Northwest National Laboratory, Richland, WA

MOE pm 2:40
Impact of Alternative Splicing on Proteoisoforms and Epigenetic Regulation during T cell Stimulation

Laura Agosto[1, 2]; Michael J. Mallory[1]; Simone Sidoli[1, 2]; Amber K. Weiner[1, 2]; Kristen W. Lynch[1]; Benjamin A. Garcia[1, 2]
[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Penn Epigenetics Institute, Philadelphia, PA

During stimulation, T cells undergo multiple changes in protein expression that arise through numerous mechanisms, including altered gene transcription, pre-mRNA alternative splicing (AS) and epigenetic marks on chromatin. While once studied in isolation, the interplay between splicing, transcription and epigenetics is now beginning to be appreciated. The extent and which differentially spliced mRNA isoforms are successfully translated into proteins is still an unanswered question in the RNA and proteomics fields. Likewise, we want to specifically interrogate how changes in splicing impact epigenetics. Here, we used RASL-Seq to quantify changes in splicing patterns upon T cell stimulation and applied bottom-up mass spectrometry (MS) based proteomics to correlate changes in splicing to changes in protein isoform expression. In a systematic comparison of unstimulated vs stimulated T cells, we identified 5,376 proteins and 35,878 peptides across all samples, of which 57 proteins and 280 peptides correspond to proteins functionally associated to chromatin that also have significant changes in splicing according to RASL-Seq. We focused on HDAC7, a histone deacetylase, because it has one of the largest changes in splicing and had been previously shown to play key roles in T cell function. We demonstrate that the splicing of HDAC7 controls the level of the active protein. By direct manipulation of the splicing pattern, we further show that this directly impacts expression of HDAC7-sensitive genes. Finally, we have identified some of the RNA binding proteins that regulate HDAC7 splicing in activated T cells. Together, we present a novel integration of high throughput RNA sequencing paired with quantitative mass spectrometry interrogation in the interest of detecting successful translation of alternatively spliced mRNAs. In addition, we provide new evidence for how regulation of pre-mRNA splicing alters epigenetics and transcription, and reveal another example of how alternative splicing contributes to the function of activated T cells.

MOE pm 2:52
Proteogenomic subtypes of squamous cell lung cancer

Paul Stewart; Eric Welsh; Robbert Slebos; Bin Fang; Victoria Izumi; Matthew Chambers; Guolin Zhang; Ling Cen; Fredrik Pettersson; Yonghong Zhang; Zhihua Chen; Chia-Ho Cheng; Katherine Fellows; Jewel Francis; Tania Mesa; Chaomei Zhang; Sean Yoder; Gina DeNicola; Amer Beg; Theresa Boyle; Jamie Teer; Ann Chen; John Koomen; Steven Eschrich; Eric Haura
Moffitt Cancer Center, Tampa, FL

Genomic analyses yielded a number of breakthroughs in our understanding of non-small cell lung cancers, but unlike lung

adenocarcinoma, transforming this understanding into benefit for squamous cell lung cancer (SCC) patients has yet to be achieved. Here, we present proteogenomic analysis (DNA, mRNA, protein) of 108 surgically resected SCC samples with full clinical and follow up data. Proteomic analysis was performed using TMT6 labeling, 12-fraction bRPLC separation, and UPLC-MS/MS (RSLC and Q Exactive Plus, Thermo). Database searches were performed with MyriMatch, Comet, and MS-GF+ against RefSeq (v.78). Data was summarized by an in-development version of IDPicker and in-house pipelines, which allowed for detection of >8,000 protein groups (Protein FDR < 5%). Consensus clustering of protein expression levels grouped tumors into three subtypes. The first subtype, **Inflamed**, reflected neutrophil biology and expressed MHC proteins. The second subtype, **Redox**, reflected reduction-oxidation biology and contained the majority of tumors with NFE2L2/KEAP1 genomic alterations. The third subtype, **Mixed**, reflected protein expression consistent with Wnt signaling and had enriched APC mutations. Integrating with RNA-seq in conjunction with ESTIMATE and CIBERSORT that infer immunological cell types, we found Inflamed had increased memory B-cells, CD8 T-cells, neutrophils, and monocytes. Examination of the tumor architecture and histological features of these tumors identified aggregates of lymphoid cells consistent with tertiary lymphoid structures (TLS). TLS were significantly enriched in the Inflamed subtype, and the presence of TLS was associated with decreased tumor recurrence and improved overall survival across all patients. Integrative analysis of copy number variation, mRNA expression, and protein expression combined with shRNA screen data identified novel drug targets in SCC, including TP63 and NFE2L2-related targets: AKR1C3 (aldoketoreductase) and PSAT1 (catalyzes serine biosynthesis). Insights gained from our proteogenomic analysis have the potential to produce a molecular classification linked to tumor biology and develop novel treatment strategies for this disease.

MOE pm 3:04
Proteogenomics discovery of human coding regions and cancer neoantigens

Janne Lehtiö[1, 2]; Yafeng Zhu[1]; Husen Umer[1]; Rui Branca[1]
[1]Karolinska Institutet, Stockholm, Sweden; [2]SciLifeLab, Stockholm, Sweden

The explosion of genomics data in recent years has greatly improved our understanding of human genetic variations and diseases related to genome changes. However, the human proteome annotation is not complete and knowledge on how genomic aberrations affect the functional proteome is still limited. To enable effective annotation of the human protein coding genome by using the mass spectrometry data, we combined experimental peptide pI data, from HiRIEF LC-MS/MS, to stringent data-analysis workflow to map back peptides to the human genome (Branca R, Nature Methods 2014). Moreover, by integrating proteomics and transcriptomics data to standard genome annotation tools, we demonstrate how proteogenomics can substantially improve gene annotation (Zhu Y., NAR 2017). To study the impact of sequence variants, we have improved the detection and curation of single amino acid variants (SAAV) containing peptides, by a tool for automated inspection of MS/MS spectra (SpectrumAI) and by incorporating orthogonal validation levels to generate an integrated proteogenomics analysis workflow (IPAW) (Zhu Y., Nature Communications, 2018). The results provide evidence for the translation of pseudogenes, lncRNAs, short ORFs, alternative ORFs, N-terminal extensions, intronic sequences as well as high confidence detection of sequence variants at protein level. Moreover, our quantitative analysis indicates that protein production from particular pseudogenes and lncRNAs is tissue specific. In cancer, genomic instability leads to aberrations of coding and non-coding regions. We will also present the modification and use of above mentioned tolls for cancer proteogenomics analysis to discover tumor specific proteins, so called neoantigens.

MOE pm 3:16
Proteogenomic integration for systematic identification and prioritization of tumor neoantigens

Bo Wen; Yun Zhang; Noel Namai; Yongchao Dou; Bing Zhang
Baylor College of Medicine, Houston, TX

Personalized neoantigen vaccine prompts strong anti-tumor response in cancer patients and provides a promising means for cancer immunotherapy. In silico analysis plays a critical role in selecting candidate neoantigens from genome-wide omics data for vaccine design. Protein expression is required for the generation of neoantigens, but existing bioinformatics approaches fail to incorporate proteomics data. Here we present a proteogenomic strategy that integrates whole exome sequencing (WES), RNA Sequencing (RNA-Seq), and mass spectrometry (MS)-based proteomics data from individual cancer patients to systematically identify and prioritize neoantigen targets for personalized vaccine design. In this strategy, WES data was used to identify somatic mutations and to determine human leukocyte antigen (HLA) type. NetMHCpan was used to predict the binding to HLA molecules. RNA-Seq and proteomics data were used to assess the expression of the mutated alleles at the RNA and protein levels, respectively. Neoantigen targets were prioritized by combining estimated HLA binding affinity and mRNA and protein expression data. The workflow was implemented as a reproducible pipeline using Docker and Nextflow. We applied this pipeline to a large colon cancer cohort with 106 patients. Using a stringent filter combining high predicted HLA binding affinity and positive mRNA and protein expression evidence, we identified candidate neoantigens for 31 out of the 106 patients (29%). Using one patient as an example, we identified 2051 somatic mutations, among which 865 had the potential to produce mutant peptides with a high HLA binding affinity. mRNA expression evidence was observed for 315 out of the 865 mutations, protein expression evidence was observed for 11, and nine mutations were supported by both mRNA and protein expression evidence. In conclusion, we have developed a proteogenomics pipeline for systematic identification and prioritization of tumor neoantigens. We demonstrated the utility of this pipeline using a large colon cancer cohort.

MOE pm 3:28
The Proteome Data Commons in the context of the NCI Cancer Research Data Commons

Christopher R Kinsinger[1]; Izumi Hinkson[1]; Ratna R Thangudu[2]; Michael Holck[2]; Deepak Singhal[2]; Karen Ketchum[2]; Paul A Rudnick[3]; Nathan J Edwards[4]; Michael J MacCoss[5]; Anand Basu[2]
[1]National Cancer Institute, Bethesda, Maryland; [2]ESAC, Rockville, MD; [3]Spectragen-Informatics, Bainbridge Island, WA; [4]Georgetown University Medical Center, Washington, DC; [5]University of Washington Genome Sciences, Seattle, WA

One recommendation of the 2016 Beau Biden Cancer Moonshot[SM] initiative was the creation of a National Cancer Data Ecosystem which would establish data science

infrastructure necessary to connect repositories, analytical tools, and knowledge bases. These resources would allow data to be aggregated, queried, analyzed, and visualized in unique and powerful ways within and across data types.

To this end, the National Cancer Institute (NCI) has begun the development of the NCI Cancer Research Data Commons (CRDC). To date, the CRDC contains a Data Commons Framework, a Genome Data Center, Cloud Resources, and a Proteome Data Commons. In the near future, the CRDC will launch an Imaging Data Commons and a Cancer Data Aggregator.

As a node in the CRDC, the overarching goal of the NCI Proteomic Data Commons (PDC) project is to democratize access to cancer-related proteomic datasets as well as to provide sustainable computational support to the cancer research community. To foster collaboration and accelerate discovery, NCI aims to enhance data sharing within the cancer proteomics community and beyond. The PDC seeks to enable and empower the cancer-research community, including scientists working in both intramural and extramural laboratories, with the necessary informatics capabilities to carry out large-scale, multi-omic data analysis. As a Node of the NCI CRDC, the PDC will overcome current data access limitations by co-localizing proteomics data and analysis tools within a larger data ecosystem that supports multiple research and clinical data types.

This presentation will describe the current status of the PDC, including: data sets, application programming interfaces, analysis pipelines, data model, underlying architecture, integration with the CRDC, and the overall philosophy of the PDC.

10:30 am - 12:20 pm Tuesday
SYSTEMS BIOLOGY
Session Chair: Robert Moritz
Oceana 8-10

TOA am 10:30
Pathology from the Molecular Scale on Up

Garry P. Nolan
Stanford University School of Medicine, Stanford, CA

High parameter single cell analysis has driven deep understanding of immune processes. Using a next-generation single-cell “mass cytometry” platform we quantify surface and cytokine or drug responsive indices of kinase target with 45 or more parameter analyses (e.g. 45 antibodies, viability, nucleic acid content, and relative cell size). Similarly, we have developed two advanced technologies termed MIBI and CODEX that enable deep phenotyping of solid tissue in both fresh frozen and FFPE formats (50 – 100 markers). Collectively, the systems allows for subcellular analysis from the 70nm resolution scale to whole tissue in 3D.

I will present evidence of deep internal order in immune functionality demonstrating that differentiation and immune activities have evolved with a definable “shape”. Further, specific cellular neighborhoods of immune cells are now definable with unique abilities to affect cellular phenotypes—and these neighborhoods alter in various cancer disease states. In addition to cancer, these shapes and neighborhoods are altered during immune action and “imprinted” during, and after, pathogen attack, traumatic injury, or auto-immune disease. Hierarchies of functionally defined trans-cellular modules are observed that can be used for mechanistic and clinical insights in cancer and immune therapies.

TOA am 10:50
Systems genetics approaches to map the functional organization of the proteome

Jasmin Coulombe-Huntington; Thierry Bertomeu; Linnea Olofsson; Caroline Huard; Daniel St-Cyr; Lily Zhang; Andrew Chatr-ayramontri; Mike Tyers
Université de Montréal, Montréal, Canada

The phenotype of a given cell or organism is governed by a vast landscape of genetic interactions. This landscape is underpinned by myriad protein interactions that form the structural and information processing systems of the cell. Deciphering the totality of this landscape - referred to as the genotype-to-phenotype problem - has been dramatically accelerated by functional genomic and proteomic technologies. Recently, CRISPR-based strategies have enabled systematic genetic screens to uncover genotype-phenotype relationships in human cells. To undertake CRISPR-based genetic screens, we generated an extended knockout (EKO) library pool of 278,754 different gRNAs that targets RefSeq genes, alternatively spliced isoforms, and hypothetical genes. We used the EKO library to correlate genetic fitness defects with different features of the human proteome. The universal set of genes essential for viability across different cell lines is surprisingly small but many genes exhibit context-specific fitness defects. Essential protein complexes frequently contain non-essential subunits that reflect the structural organization and evolution of protein complexes. We also established trends across the proteome at the single residue level for evolutionary conservation, structural burial, modular domains, and protein interaction interfaces and other features. To explore the contextual specificity of genotype-phenotype relationships, we screened the EKO library against more than 300 drugs, toxins and other bioactive agents in a human pre-B cell line. These screens identified a matrix of >8,000 high-confidence chemical-gene interactions. Pairs of genes with similar chemical-genetic profiles tended to share functions and encode physically interacting proteins. The dataset identified general chemical resistance and sensitization mechanisms, including drug transport, apoptosis, protein translation and lipid metabolism. Examples of novel mechanisms of action for bioactive compounds will be presented. These approaches demonstrate that unbiased genome-wide genetic screens can be used to map the functional organization of the human proteome.

TOA am 11:10
Towards the human co-receptome: a systematic exploration of the Immunoglobulin Superfamily Interactome

Nadia Martinez
Genentech, South San Francisco, CA

Deciphering the ensemble of receptor-ligand interactions that take place in the extracellular milieu is vital to understand cellular communication and signaling during homeostasis and disease. Although extensive data have been generated in the last decade, our understanding of the cell surface receptor interactomes is still limited, fundamentally due to the technical challenges that these proteins present for most proteomics approaches. This is well illustrated by the Immunoglobulin Superfamily (IgSF), one of the largest gene families in the human genome, which remains greatly uncharacterized

despite representing a prime source of therapeutically relevant targets. With the long-term goal of elucidating the complex networks that define the human co-receptome, we have implemented an automated, oligomerization-based method for unbiased, cell-independent, and high sensitivity detection of receptor interactions. Using this technology, hundreds of IgSF proteins were interrogated for binding to a newly established library consisting of nearly all single transmembrane (STM) receptors, so far evaluating more than 600,000 pairwise IgSF-STM receptor interactions. This effort has resulted in over 1,000 predicted interactions, most of which were previously unrecognized, including new interacting partners for prominent immune receptors that we have confirmed using orthogonal technologies. This study represents the first systematic evaluation of the IgSF Interactome, knowledge that is central to defining the players and molecular processes that drive cell function.

TOA am 11:22

Systems pharmacology dissection of cell-specific cholesterol regulation mechanisms reveals large pharmacodynamic variability

Peter Blattmann[1]; David Henriques[2]; Michael Zimmermann[1]; Fabian Frommelt[1]; Uwe Sauer[1]; Julio Saez-Rodriguez[3, 4]; Ruedi Aebersold[1, 5]

[1]ETH Zurich, Zurich, Switzerland; [2]Spanish Council for Scientific Research, Vigo, Spain; [3]RWTH-Aachen University, Aachen, Germany; [4]European Molecular Biology Laboratory - European B, Hinxton, United Kingdom; [5]University of Zurich, Zurich, Switzerland

In individuals, heterogeneous drug response phenotypes result from a complex interplay of dose, drug specificity, genetic background, and environmental factors, thus challenging our understanding of the underlying processes. Here we present an approach to explain drug response differences in a panel of cell lines combining mass spectrometry-based quantification of molecular phenotypes with subsequent integration of the quantitative data using logic modeling. The approach was applied to cellular cholesterol regulation, a biological process with high clinical relevance. The phenotypes elicited by various targeted pharmacologic or genetic treatments were profiled by quantifying >3000 proteins and >1000 metabolites using mass spectrometry. Based on this data and a prior knowledge network, we generated cell-line-specific models that quantified the processes beneath the idiotypic intracellular drug responses and could identify which processes in the model varied between the cell lines. The models revealed that in addition to drug uptake and metabolism further cellular processes showed significant pharmacodynamic response variability between cell lines, resulting in cell-line-specific drug response phenotypes. This study demonstrates the importance of integrating different types of quantitative systems-level molecular measurements with modeling to understand the effect of pharmacological perturbations on complex biological processes.

TOA am 11:34

Building a Global Map of Human Protein Complexes: Synthesis of >15k Mass Spectrometry Experiments

Kevin Drew; John B. Wallingford; Edward Marcotte

University of Texas at Austin, Austin, TX

Understanding the underlying molecular network of the cell is crucial to our ability to develop therapeutics for human diseases. Unfortunately, we currently lack knowledge of the composition, formation and function of the human molecular network. We recently integrated > 9k mass spectrometry experiments from published datasets and construct one of the most comprehensive human protein complex maps to date covering over a third of the proteome and producing over 4k complexes (Drew et al. MolSystBiol. 2017). Our integrated complex map, Hu.MAP (http://proteincomplexes.org) identified many new complexes including ones enriched for cilia related developmental disorders (e.g. Joubert Syndrome, Bardet-Biedl Syndrome, Meckel-Gruber Syndrome) that have novel members suggesting candidate disease genes, which we experimentally validated *in vivo*. Here, we present an even more complete and accurate human complex map resulting from our application of > 15k mass spectrometry experiments to our machine learning pipeline. This new map recovers many known protein complexes as well as identifies new complexes suggesting novel biology to be discovered. The expansiveness and accuracy of this human protein complex map yields greater understanding of cellular function and provides avenues for better disease characterization.

This work was funded by NIH F32 GM112495 and K99 HD092613.

TOA am 11:46

Charting of endothelin B receptor signaling using phosphoproteomics discovers critical kinases for endothelin induced cell migration

Alexander Schaefer[1]; Richard W. D. Welford[2]; Imke Renz[2]; Francois Lehembre[2]; Peter M. A. Groenen[2]; Enio Gjerga[3]; Julio Saez-Rodriguez[3, 4]; Ruedi Aebersold[1, 5]; Matthias Gstaiger[1]

[1]ETH Zurich, Zurich, Switzerland; [2]Idorsia Pharmaceuticals, Allschwil, Switzerland; [3]RWTH Aachen University, Aachen, Germany; [4]European Molecular Biology Laboratory, Hinxton, UK; [5]Faculty of Science, University of Zürich, Zurich, Switzerland

Introduction and Objectives: Endothelin (EDN) peptide hormones activate a G-Protein coupled receptor signalling system and contributes to several diseases, including the progression of several cancer types. EDN is the most potent vasoconstrictor in humans and EDN inhibition is a key therapeutic strategy for pulmonary arterial hypertension. In melanoma, EDNRB has been implicated in progression and resistance development. Current knowledge about EDN signalling is derived from fragmentary evidence, with no systems level model available. We address this need by studying endothelin B receptor (ENDRB) signalling in melanoma cell lines using state-of-the-art phosphoproteomics, network modelling and functional validation.

Methods: Time resolved EDN signalling was studied in melanoma cells using a SILAC global internal standard followed by LC-MS/MS phosphoproteomics. Two cell lines with five timepoints and a CRISPR/Cas9 knockout of EDNRB were assessed in triplicate,. Activated kinases were predicted based on high confidence EDN target sites and validated by Western blot. Target sites and kinases were assembled into a network model using prior knowledge based optimization and kinase inhibition was used to link kinases to EDN induced cell migration.

Results and Discussion: 5240 unique phosphopeptides were quantified, of which 641 were robustly affected by EDN. All observed phosphopeptide changes were strictly dependent on the presence of EDNRB. From these sites, 19 target kinases were predicted, whose activation was validated by Western

blot. The first comprehensive endothelin signalling network was constructed. Targeting the kinases in our network with inhibitors, four kinases controlling EDN induced cell migration were identified.

Conclusions: A global network model of the EDNRB signalling pathway was obtained and the functional relevance of central kinases in the network to cell migration is demonstrated. Our model will aid the understanding of the mechanisms underlying the pleiotropic effects of EDN and support the selection of kinase inhibitors for combination treatments with endothelin receptor antagonists.

TOA am 11:58

A high-resolution organellar proteomics approach to study subcellular distribution and dynamics of proteins in cells

Johannes Jordan[1]; Wolfgang Bildl[1]; Clara Steinbrueck[1]; Alexander Haupt[1]; Maciej Kocylowski[1]; Astrid Kollewe[1]; Bernd Fakler[1, 2]; Uwe Schulte[1, 3]

[1]University of Freiburg, Germany, Freiburg, Germany; [2]Center for Biological Signaling Studies (BIOSS), Freiburg, Germany; [3]Logopharm GmbH, March-Buchheim, Germany

It is meanwhile well established that proteins exert their biological function through protein-protein interactions mostly within protein (super)complexes and networks. Despite considerable progress in their identification, the biogenesis, cellular distribution and remodeling of these protein assemblies remain poorly characterized. As potential solution '*organellar proteomics*' was established as a systemic approach that combines subcellular fractionation, quantitative mass spectrometry and bioinformatic analysis. We largely extended this approach by introducing multidimensional fractionation and advanced tools for relative protein quantification, cluster analysis and visualization by dimensionality reduction (t-SNE) to resolve protein clusters with unprecedented resolution. For reference, a primary epithelial cell line was sub-fractionated into 40 samples analyzed by comprehensive LC-MS/MS yielding >6000 high confidence protein distribution profiles. Cosine-distance based hierarchical clustering revealed more than 30 distinct entities representing classical cellular organelles and/or substructures, populations of trafficking vesicles, proteins with dual localization, multiprotein machineries and cytoskeletal networks. Results from unbiased clustering were highly consistent with the distribution of >1000 published bona fide organellar markers which allowed training of a classifier that achieved a per-protein recognition rate of over 85% to extend clustal/subcellular assignment to the remaining identified proteins. Furthermore, cluster analysis revealed a number of details on functional relationships such as ER-Golgi-plasma membrane transits during protein biogenesis, endosome formation, recycling and lysosomal degradation, as well as exclusive versus shared or dynamic components of known protein machineries and interactomes.

In summary, our approach provided a comprehensive view on the subcellular proteome distribution with detailed information on the protein composition of subcellular structures and on functional relationships. Thus, it allows for identification and verification of subcellular markers, classification of protein interactomes, unbiased definition of subcellular proteomes, characterization of subcellular localization determinants and analysis of spatial dynamics of proteins/-assemblies.

10:30 am - 12:20 pm Tuesday
IMMUNOLOGY
Session Chair: Pierre Thibault
Oceana 7

TOB am 10:30

Advanced immunopeptidomics based discovery engine for the development of personalized cancer immunotherapy

Michal Bassani-Sternberg

Unil-Chuv, ,

The remarkable clinical efficacy of the immune checkpoint blockade therapies has motivated researchers to discover immunogenic epitopes and exploit them for personalized vaccines. Mutated human leukocyte antigen binding peptides (HLAp) are currently the leading targets for T-cell recognition of cancer cells. We have shown that the direct identification of tissue derived neoantigens by mass spectrometry is feasible. However, many tumors have low mutational load, and therefore, the straightforward and confident identification of other classes of tumor antigens, remains necessary.

We have recently designed a novel high-throughput, reproducible and sensitive method for sequential immuno-affinity purification of HLA-I and -II peptides, suitable for both cell lines and tissues. In addition, we have developed dedicated proteo-genomic informatics pipelines to interrogate the presentation of potentially clinically relevant targets, such as shared tumor antigens, neoantigens, as well as proteasomal-spliced peptides. Importantly, spike-in targeted MS experiments of labeled synthetic counterparts facilitate their confident validation. We have compiled a large immunopeptidomics database across dozens of tumor tissues and HLA allotypes that is highly valuable. For example, we have shown that by taking advantage of co-occurring HLA-I allotypes we can rapidly and accurately identify HLA-I binding motifs. Consequently, training HLA-I ligand predictors on refined motifs significantly improves the identification of neoantigens. In addition, our database captures the global nature of the in vivo peptidome averaged over many HLA alleles, and therefore, reflects the propensity of peptides to be presented on HLA complexes, which is complementary to the existing neoantigen prediction features. We have shown as a proof of concept that our immunopeptidomics MS-based features improved neoantigen prioritization by up to 50%. Overall, immunopeptidomics facilitates direct identification, as well as improved prediction, of clinically relevant targets applicable for personalized anti-cancer immunotherapy.

TOB am 10:50

Allegedly non-coding regions are the main source of tumor-specific antigens

Claude Perreault

IRIC-Université de Montréal, Montreal, Canada

Tumor-specific antigens (TSAs) represent ideal targets for cancer immunotherapy. However, very few TSAs have been identified thus far using a variety of approaches focusing on MHC-associated peptides coded by mutated exonic sequences. We therefore developed a novel proteogenomic approach that enables high-throughput discovery of TSAs coded by all reading frames of any genomic regions. In two murine cancer cell lines and seven primary human tumors, we identified numerous TSAs, 90% of which derived from allegedly non-coding regions and would have been missed by standard approaches. Moreover, about 70% of these TSAs derived from

non-mutated yet cancer-restricted transcripts (e.g., endogenous retroelements) that can be shared by multiple tumors. In mice, the strength of antitumor responses following TSA vaccination was influenced by TSA expression level and the frequency of TSA-specific T cells in the pre-immune repertoire. In conclusion, the strategy reported herein is readily applicable to human tumors and should considerably facilitate the identification of actionable TSAs.

TOB am 11:10
Systematic profiling of HLA class I peptide epitopes by LC-MS/MS in mono-allelic cells improves neoantigen binding prediction algorithms

Susan Klaeger[1]; Derin Keskin[2, 3]; Siranush Sarkizova[4]; Karl R Clauser[1]; Oliver Spiro[1]; Hasmik Keshishian[1]; Christina Hartigan[1]; Nir Hacohen[1, 5]; Catherine J Wu[1, 2]; Steven A Carr[1]
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Highly polymorphic class I HLA molecules present short peptide sequences from endogenous or foreign proteins to cytotoxic T cells. Each allele is estimated to bind and present 1000 – 10000 unique peptides, however the rules of antigen presentation are not fully understood. Mass spectrometry allows for direct identification of endogenously processed and presented peptides. Using a single allele expressing cell line, the underlying criteria for antigen presentation can be systematically studied.

The HLA class I deficient B721.221 cell line was transfected with HLA allele expression vectors representing a single allele each. Up to 50 million cells were immunoprecipitated, peptides were acid eluted and analyzed by high-resolution LC-MS/MS. Mass spectra were interpreted with the Spectrum Mill software.

To date, we have generated MHC I binding data on over 70 alleles from various HLA class I A, B, C and G alleles covering more than 70% of the most frequently occurring alleles in the population. Over 140,000 peptides were identified overall, >40,000 peptides for HLA-A and HLA-C alleles and >59,000 for HLA-B alleles. This unique, comprehensive dataset enables peptide-binding and proteasomal cleavage motifs to be elucidated on a single allele basis.

The data we generate is used to train neural network models to predict potential neoantigens/MHC-binders from WES and RNAseq data. These models show superior performance in predicting immunogenic epitopes compared to the currently used predictors. The predictive value of the models is determined using MHC I peptide binding data generated by LC-MS/MS from primary tumor samples as well as by performing targeted analyses for specific peptides of interest.

In conclusion, our strategy provides a rapid and scalable method to generate rules for the substantially diverse set of human HLA alleles. Moreover, data obtained by LC-MS/MS of mono-allelic MHC expressing cell lines improves the success rate of prediction algorithms for neo-antigen prediction.

TOB am 11:22
Integrated proteome and HLA peptidome quantitation with tandem mass tags

Patrick Murphy[1]; Prathyusha Konda[1]; Joao Paulo[3]; Heiko Schuster[2]; Daniel Kowalewski[2]; Youra Kim[1]; Derek Clements[1]; Michael Giacomantonio[1]; Stefan Stevanovic[2]; Steve Gygi[3]; Shashi Gujar[1]
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Adaptive immune responses require interaction between $CD8^+$ T-cells and class I HLA molecules containing HLA peptides of pathogen, self, or tumor origin. Although comparing both protein and HLA peptide levels across experimental conditions may offer insight into how proteome changes are reflected in the immunopeptidome (and ultimately T-cell responses) these approaches have not been fully developed. Here, we established a strategy to quantify HLA peptides and their source proteins with tandem mass tags (TMT) to achieve accurate, multiplexed, immunopeptidome analysis. The data are integrated with multiplexed proteome measurements in colon cancer cells treated with the chemotherapy drug doxorubicin.

Adjustments to HLA immunoprecipitation and database searching enabled us to perform multiplexed analysis with 10-plex TMT. We use doxorubicin treatment of cancer cells as a model, resulting in a dataset of 3353 HLA peptides and 6588 proteins both measured in duplicate across 5 time points of 36 hours. Differences in the immunopeptidome were mostly observed by 24 hours doxorubicin treatment, after which we observed induction of 134 peptides by more than 2-fold. By integrating immunopeptidome data with quantitative proteome measurements, 2737 peptides (81%) could be matched to proteins in the proteome dataset. Among the protein-matched peptides, we observed no correlation (R=.01) between source protein and immunopeptide changes was observed albeit several interesting immunopeptide and source protein changes emerged. For example, 2 HLA peptides from the E3 ligase for p53, MDM2, increased 12.1 and 2.9 fold in doxorubicin-treated cells alongside the levels of MDM2 and p53 protein which were both increased (> 4 fold) in doxorubicin-treated cells. We suggest that integrating proteome and immunopeptidome dynamics using TMT will lead to a greater understanding of how HLA peptides are formed from source proteins and how this process affects CD8+ T cell-mediated adaptive immunity.

TOB am 11:34
Is hybrid peptide formation a new post-translational modification that drives autoimmunity?

Timothy Wiles; Anita Hohenstein; Thomas Delong
University of Colorado Skaggs School of Pharmacy, Aurora, CO

Autoreactive T cells that target insulin-producing beta-cells are considered to play a central role in the development of type 1 diabetes (T1D). Progress has been made in identifying T cell ligands in T1D, yet the causal antigens have remained elusive. We recently discovered a new family of autoantigens that is targeted by autoreactive T cells found in residual pancreatic islets of T1D organ donors as well as diabetes-triggering T cells in an animal model for T1D. These antigens contain insulin fragments that are linked through peptide bonds to other protein fragments found in beta-cell secretory granules. The potential number of hybrid peptides that may form is several orders of magnitude higher than the total number of proteins found in an organisms' proteome, making the identification of hybrid peptides by mass spectrometry a challenging task. We computer generated large hybrid peptide databases and in combination with mass spectrometric analyses of pancreatic islet extracts identified a list of new hybrid peptides. The discovery of hybrid peptides as T cell epitopes has challenged a paradigm in T1D research. Hybrid peptides are likely formed

in many tissues and could provide explanations for various autoimmune diseases. Demonstration that autoreactive T cells in T1D patients react to HIPs that form in human islets could be used to establish a T cell reactivity "signature" unique for patients as well as pre-diabetic individuals. T cells from human subjects could be rapidly tested for reactivity to a peptide panel to establish an autoreactive T cell disease-risk profile. The benefits of such a system could be twofold, in that the autoreactive T cells could serve as biomarkers of disease-activity and as targets for therapeutic approaches involving antigen-specific tolerance induction.

TOB am 11:46
Citrullinated glucose-regulated protein 78: an autoantigen in human type 1 diabetes

Lut Overbergh[1]; Inne Crèvecoeur[1]; Fernanda MC Sodré[1]; Aïsha Callebaut[1]; Gabriele B fagan[2]; Mei-Ling Yang[3]; David Arribas-Layton[2]; Meghan Marré[4]; Dana P Cook[1]; Etienne Waelkens[1]; Roberto Mallone[5]; Jon Piganelli[4]; Rita Derua[1]; Mark Mamula[3]; Eddie A James[2]; Chantal Mathieu[1]; Mijke Buitinga[1]

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The beta-cell has become recognized as a central player in the pathogenesis of type 1 diabetes (T1D), with the generation of neo-antigens in response to inflammatory stress as potential triggers for breaking immune tolerance. The identification of new autoantigens is critical to better understand disease pathology, to discover new biomarkers for patient stratification, and to develop novel disease interventions. We have previously shown that the endoplasmic reticulum chaperone GRP78 is an autogantigen in non-obese diabetic mic, when citrullinated. Here, we aimed to translate these findings to the human situation and report on citrullinated GRP78 as an autoantigen in human T1D.

2DIGE analysis of cytokine-exposed human islet proteins revealed an increase in post-translationally modified (PTM) residues in GRP78 (in 3 out of 5 islet preparations). Further evaluation by LC-MS/MS (Orbitrap Q Exactive) identified 2 out of 28 R-residues within the GRP78 protein sequence as citrullinated, again being increased in abundance upon cytokine exposure. Next, we evaluated the antigenicity of citrullinated GRP78 in T1D patients. Citrullination led to the generation of neo-epitopes that could effectively be presented by HLA-DRB1*04:01 molecules. Evaluation by HLA-DRB1*04:01 class II tetramer assays showed significantly elevated levels of CD4+ T-cells directed against citrullinated GRP78 epitopes in T1D patients compared to healthy controls (n=8-15, $p<0.02$). Interestingly, patients with T1D had a predominantly higher percentage of central memory cells and a lower percentage of effector memory cells directed against citrullinated GRP78, as compared to the native epitope. In addition, 33% of the T1D patients had autoantibodies against citrullinated GRP78, whereas only 5.6% of the healthy control subjects were autoantibody positive ($p<0.001$), as evaluated by ELISA. These results strongly suggest that citrullination of beta-cell proteins, exemplified here by the citrullination of GRP78, contributes to loss of self-tolerance towards beta-cells in human T1D.

TOB am 11:58
Cytoplasmic interactions of the pathogen recognition receptor cGAS modulate Type I IFN induction during herpesvirus infection

Krystal Lum; Bokai Song; Joel Federspiel; Benjamin Diner; Ileana Cristea

Princeton University, Princeton, <Not Specified>

It has long been understood that mammalian cells can distinguish pathogen-derived DNA from genomically-stable host DNA to elicit innate immune responses. One prominent host defense factor is the DNA sensor cyclic-GMP-AMP synthase (cGAS). Upon binding to viral DNA, cGAS catalyzes the production of cyclic dinucleotides, which activate STING to induce type I interferons. Our lab recently established that cGAS also induces apoptosis in a STING-dependent manner during certain infections with herpes simplex virus 1 (HSV-1). Despite this understood critical role for cGAS in sensing viral DNA, little is known about how cGAS activity is initiated and regulated upon infection. How cGAS is homeostatically maintained in an inactive state and whether these rely on certain protein interactions remain unknown. Its protein interactions during immune response are yet to be investigated. Additionally, our understanding of the global impact of HSV-1 infection on the cellular proteome is limited. Here, we provide the first unbiased characterization of cytoplasmic cGAS protein interactions during 1) cellular states of active immune signaling via infection with HSV-1 in primary human fibroblasts and, 2) transfection with vaccinia virus-derived DNA in epithelial cells. We validate interactions using microscopy and reciprocal isolations. Furthermore, we use a series of functional assays to define the function of interactions. These assays include domain construction, CRISPR-mediated knockouts, antiviral cytokine measurements, and targeted mass spectrometry quantification. We discover a specific interaction that inhibits cGAS as a regulatory negative feedback loop for cytokine induction. We place this knowledge of interactions in the context of proteome alterations during infection by using isobaric-labeling (TMT)-MS. Mutant HSV-1 strains that induce strong cytokine responses and apoptosis allow us to present the most in-depth characterization of proteomes during HSV-1 replication. Our findings provide an explanation for how cGAS may be inactively maintained and contribute mechanistic insight into its regulation during immune signaling.

10:30 am - 12:20 pm Tuesday
STRUCTURAL PROTEOMICS
Session Chairs: Patrick Griffin and Carol Robinson
Oceana 6

TOC am 10:30
Different Means to Solubilize Membrane Proteins for MS Analysis: Going More Native

Nina Morgner; Oliver Peetz; Nils Hellwig

Goethe University Frankfurt/Main, Frankfurt/Main, Germany

Membrane proteins are of high interest, but still underrepresented in research due to the inherent difficulties for many investigation methods, arising from their hydrophobic nature. Depending on the feature of the membrane protein complex the researcher is interested in (protein stoichiometry, lipid affinity, annular lipids…), different means of solubilisation can enable MS analysis.

LILBID (Laser Induced Liquid Bead Ion Desorption) is an ionization method which employs a droplet generator to produce analyte droplets of 30-50um diameter at a frequency

of 10Hz. These droplets are irradiated by a mid-IR laser leading to the explosive expansion of the droplet. The solvated ions are set free and are mass analyzed.

We investigated the usability of artificial membranes for the study of membrane protein complexes with LILBID-MS. Lipid bilayer mimics as nanodiscs and liposomes allow the analysis of the oligomerization state of a protein complex. Cell-free expressed membrane proteins can be solvated by lipid bilayer mimics without ever being in contact with detergents. This allows for example the study of lipid dependent oligomerization. Even SMALPs, which allow to cut protein complexes out of living cells preserving their annular lipid belt, can be used to analyze complexes, in dependence of growth conditions of the cells.

LILBID-MS is well suited for the analysis of membrane proteins complexes solubilized by detergent or different lipid bilayer mimics, such as nanodiscs, liposomes or SMALPs. This allows analysis of the complexes' constituting proteins, stoichiometries, oligomerization states as well as their dependence on specific lipids or ligands.

TOC am 10:50

Photo-Crosslinking Mass Spectrometry and Integrative Modeling Enables Rapid Screening of Antigen Interactions Involving Bacterial Transferrin Receptors

Daniel S. Ziemianowicz; Dixon Ng; Anthony B. Schryvers; David Schriemer

University of Calgary, Calgary, Canada

Structure-based approaches that map antigens for vaccine development have a throughput requirement that is difficult to meet in practice with conventional methods of structure determination. Here we present a strategy for rapid and accurate structure generation in support of antigen engineering programs. The approach is developed around the modeling of interactions between host transferrin (Tf) and the bacterial vaccine target transferrin binding protein B (TbpB), from Gram-negative pathogens such as *Neisseria meningitidis*. Using methods based only on crosslinking mass spectrometry (XL-MS), monomeric structural models and the Integrative Modeling Platform (IMP), we will show that converged representations of the Tf:TbpB interactions can be returned that accurately reflect the binding interface and the relative orientation of the monomeric units, with the capacity to scale to the analysis of interactions from any number of additional strains. A key element to accurate modeling in this context involves the use of heterobifunctional crosslinkers, which incorporate fast-acting photoactivatable diazirines. We will present our strategy for detecting crosslinked residues and accommodating higher ambiguity data in modeling, and compare our approach with similar strategies using conventional homobifunctional reagents. We demonstrate that these conventional, long-lived reagents kinetically trap non-representative interactions and negatively compromise modeling, in a manner that highlights concern around the use of these reagents in integrative structural biology in general.

TOC am 11:10

Deciphering the role of histone H2A proteolysis during stem cell differentiation and its consequence in nucleosome stability

Mariel Coradin[1]; Kelly Karch[1, 2]; Enrique Lin-Shiao[1, 2]; Simone Sidoli[1, 2]; Shelley Berger[1, 2]; Benjamin A. Garcia[1, 2]

[1]University of Pennsylvania, Epigenetics Institute, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA

Histone proteolysis is a poorly understood process by which the N-terminal tails get irreversible cleaved (clipped). This process has been described in cellular senescence, inflammation and stem cell differentiation, where its role remains unclear. In this study we combined Top-down proteomics and hydrogen deuterium exchange coupled to mass spectrometry (HDX-MS) to interrogate the functional role of clipped H2A (cH2A) during stem cells differentiation and assess its consequences on nucleosome stability. Previous studies have found that during stem cell differentiation, the lysosomal protease Cathepsin L cleaves histone H3, but whether other histones such as H2A are also cleaved is still unknown. To address this, we differentiated mouse embryonic stem cells (mESCs) into embryoid bodies, and performed Top-down MS. Our data showed that H2A also gets cleaved upon differentiation. Additionally, we were able to map the major cleavages sites to be at L23 and G44 (cH2A). Our *in vitro* studies using recombinant nucleosomes, confirm that some of these sites are facilitated by Cathepsin L. Furthermore, cells treated with Cathepsin L inhibitors show lower levels of cH2A, indicating that Cathepsin L also serves as H2A protease *in vivo*. Using RNA-sequencing, we found that inhibition of this enzyme leads to upregulation of genes involved in endoderm formation. Finally, we sought to probe the structural consequences of cH2A, which lacks the alpha-1 helix. Using *in vitro* reconstituted dimers, we compared the deuterium exchange rate of full-length H2A and cH2A containing complexes. Our findings reveal that cH2A/H2B dimers are less stable than full-length H2A dimers. In fact, we were unable to reconstitute cH2A containing nucleosomes, indicating that the lack of the alpha-1 helix in H2A is critical for nucleosome assembly. Taken together, our data suggest that histone proteolysis could be a novel mechanism for nucleosome eviction during mammalian development.

TOC am 11:22

Cell-deep structural biology insights from surface-exposed biotins

David-Paul Minde; Manasa Ramakrishna; Kathryn Lilley

University of Cambridge, Cambridge, United Kingdom

Cellular protein folding is essential in all kingdoms of life. Aberrations from normal protein folding are linked to cancer, neurodegeneration and heart diseases. Most studies of protein folding have been conducted *in vitro* and *in silico*, with predictions of unstructured or "intrinsically disordered" regions (IDRs) ranging between 10% and 80% of predicted proteome sequences from Archaea to human. Experimental validation is still lacking for the presence of IDRs *in vivo* despite mounting interest in IDRs due to their enrichment in disease-linked phospho-proteins. The task of interrogating membrane-extrinsic IDRs of integral membrane proteins is likely to be an even greater challenge due to low solubility. We postulate that lysines and tyrosines in IDRs are more accessible to *in vivo* biotinylation than lysine and tyrosine residues in more structured protein domains. To test this hypothesis and use existing biotin-based proximity tagging data to test protein structure models, we sought to perform the first systematic *in vivo* validation of current IDR models and condition-specific comparisons. Indeed, we observed a statistically significant preference for partially and highly disordered proteins and their IDRs in our structural re-analysis of >20 000 *in vivo* biotinylation sites in >4000 (including >400 membrane) proteins in human cells from four large-scale proximity proteomics studies that

report direct detections of biotinylation sites. The significant implications of our findings for quantitative *in vivo* structural proteomics and proximity proteomics will be discussed.

TOC am 11:34

Structure and Protein Interaction-based Gene Ontology Annotations Reveal Likely Functions of Uncharacterized Proteins on Human Chromosome 17

Chengxin Zhang; Gilbert Omenn; Yang Zhang

University of Michigan, Ann Arbor, <Not Specified>

Our understanding of protein functions is far from complete. As of neXtProt release 2018-01-17, for example, 1260 proteins among the 17470 PE1 protein-coding genes have unknown or insufficiently specific function annotation (uPE1). To reveal the function of poorly annotated proteins, we developed a hybrid pipeline that creates protein structure prediction using I-TASSER and infers functional insights for the target protein from the functional templates recognized by COFACTOR. As a case study, we applied the pipeline to all 66 uPE1 proteins on human chromosome 17. For a benchmark set of 100 well-characterized proteins randomly selected from the same chromosome, our pipeline shows high Gene Ontology (GO) term prediction accuracies of 0.69, 0.57, and 0.67 for molecular function (MF), biological process (BP) and cellular component (CC), respectively, even after excluding templates sharing > 30% sequence identity in structure and function modeling processes. While the COFACTOR algorithm derives consensus function predictions from sequence homologs, protein-protein interaction network, and structure templates, detailed analyses show that structure template detection using low-resolution protein structure prediction made the major contributions to the sensitivity and precision of the annotation predictions, especially for the cases that do not have sequence-level homologous templates. For the 66 Chr 17 uPE1 proteins, the I-TASSER/ COFACTOR pipeline confidently assigned MF, BP and CC for 13, 33, and 49 proteins, respectively, with predicted functions ranging from sphingosine N-acyl transferase and sugar transferase transmembrane transporters to cytoskeleton constitution. This novel computational approach to systematically annotate protein function in the human proteome provides useful insights to guide experimental design and follow-up validation studies of these uncharacterized proteins and is a significant contribution to the C-HPP uPE1 Challenge.

TOC am 11:46

Novel strategies for enrichment of membrane proteins and structural characterization by top-down mass spectrometry with ultra-violet photodissociation (UVPD)

Julian Whitelegge

Semel Institute, UCLA, Los Angeles, CA

Detailed proteomic characterization of integral membrane protein complexes requires dedicated strategies for extraction and enrichment of membrane proteins from biological lipid-protein bilayers. We are interested in protocols that preserve non-covalent lipid-protein interactions of functional significance, to better understand the role of 'structural lipids'. Native mass spectrometry has provided an important new conduit for presenting membrane proteins to the mass spectrometer typically using positive ion electrospray-ionization. We will compare and contrast native versus non-native mass spectrometric approaches for accurate structural characterization addressing primary through tertiary structural features. Versatile modern mass spectrometers present opportunities for novel top-down strategies that improve our ability to rapidly focus on important subunits and their domains of structural interest. I will consider the role of middle-down versus MS3 approaches. Dissociation options are expanding as we better understand membrane protein requirements. With instrumentation for ultra-violet photodissociation (UVPD) we test the comparative efficacy of this technique applied to polytopic membrane proteins under non-native conditions using an Orbitrap instrument equipped with a solid state UV laser (213 nm) enabling comparison with collisional and electron-based dissociation chemistry (ECD/ETD/CAD/CID/HCD).

TOC am 11:58

Conformational switching of the MLKL pseudokinase domain promotes MLKL tetramerization and cell death by necroptosis

Jarrod Sandow[1]; Emma Petrie[1]; Annette Jacobson[1]; Brian Smith[3]; Michael Griffin[2]; Isabelle Lucet[1]; Weiwen Dai[1]; Samuel Young[1]; Maria Tanzer[1]; Ahmad Wardak[1]; Lung-Yu Liang[1]; Angus Cowan[1]; Joanne Hildebrand[1]; Wilhelmus Kersten[1]; Guillaume Lessene[1]; John Silke[1]; Peter Czabotar[1]; Andrew Webb[1]; James Murphy[1]

[1]Walter & Eliza Hall Institute, Parkville, Australia; [2]The University of Melbourne, Parkville, Australia; [3]LaTrobe University, Bundoora, Australia

Necroptosis is a cell death mechanism characterised by permeabilisation of the inner plasma membrane, with subsequent release of cellular contents initiating an inflammatory response. The pseudokinase, Mixed Lineage Kinase-domain Like (MLKL) is the most terminal known effector of necroptotic cell death. MLKL is a multi-domain protein in which the N-terminal four-helix bundle (4HB) executes cell death via lipid engagement and is tethered to the C-terminal pseudokinase domain (PsKD) by a two-helix linker. Following cell death stimuli, MLKL is phosphorylated by Receptor Interacting Protein Kinase-3 promoting activation and oligomer formation, which is essential for necroptosis. The stoichiometry, structure and dynamics of MLKL during transition to an active oligomer remains unclear. Using native mass spectrometry, we determined that MLKL assembles as a tetramer where as a mutant MLKL remains monomeric. To determine the structural changes between monomeric and tetrameric MLKL, we utilised cross-linking mass spectrometry with constraint mapping and small angle x-ray scattering to model the monomer and tetramer MLKL structures. By combining these results with hydrogen-deuterium exchange mass spectrometry we mapped the dynamic changes that occur as MLKL transitions to an active state. This analysis revealed that the PsKD is the molecular switch that constrains the 4HB, while the linker facilitates oligomerisation upon activation. We next validated our observations by reconstituting MLKL-/- cell lines with mutant MLKL proteins designed to disrupt interaction sites identified from our analysis. Together, this work describes how the PsKD of MLKL regulates the transition to an active tetramer during necroptotic cell death and identifies potential pharmacological targets in inflammatory pathologies.

10:30 am - 12:20 pm Tuesday
HPP: METABOLIC REMODELING AND HUMAN DISEASE
Session Chairs: Fernando Corrales and Ferdinando Cerciello
Oceana 3-5

TOD am 10:30

Nonalcoholic fatty liver disease diversity: learning from mouse models

Cristina Alonso
OWL Metabolomics, Derio, Spain

Non-alcoholic fatty liver disease (NAFLD) affects an estimated 30% of the general population, being considered a direct consequence of the rising global epidemic of obesity and closely associated with type 2 diabetes.

The hallmark of NAFLD is the excessive accumulation of triglycerides in hepatocyte lipid droplets, but it encompasses a spectrum of conditions, ranging from hepatic fat accumulation, to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. The ectopic accumulation of fat in the liver can be considered a physiological adaptation, an evolutionary advantage to anticipate periods of prolonged food shortage rather than a condition increasing the risk of developing liver injury. However, NASH is a histological definition that groups together defects in diverse biochemical processes causing hepatic fat accumulation, inflammation, necrosis and fibrosis. Increasing evidence points to different subtypes of NAFLD which progress to NASH and fibrosis at different rates and may respond differently to treatment. This large inter-patient variability not only affects the disease severity, but also the rate of progression.

We have followed a translational approach to unravel this NAFLD diversity, studying three different mouse models: Germline methionine adenosyltransferase 1a knockout (Mat1a-/-), WT mice fed a methionine, choline deficient (MCD) diet, and high-fat fed Ldlr-/-.Leiden mice. Then, murine serum metabolomic signatures were used as comparators to serum metabolome of a cohort of biopsied NAFLD patients.

The findings of the comparison between murine and human metabolome indicate the existence of two major human NAFLD phenotypes, each of them characterized by a specific metabolic alteration. The first phenotype comprised 40% of the patients, showing a metabolic profile compatible with low hepatic S-adenosylmethionine (SAMe), impaired VLDL secretion, increased fatty acid uptake and normal de novo lipogenesis (DNL). The second phenotype included 32% of the patients, showing a metabolic profile compatible with normal SAMe and VLDL secretion, and increased DNL.

TOD am 10:50

Technology for Clinical Proteomics and Its Application to Liver Disease

Matthias Mann
Max Planck Institute of Biochemistry, Martinsried, Germany

For a number of years, our laboratory has been developing technologies for translational applications of proteomics. In this talk, I will briefly summarize, where these efforts stand, in particular focusing on novel LC technology, scan modes and different types of mass analyzers. These different workflows are applied to the analysis of body fluids such as plasma, as well as the analysis of small number of cells in cancer patients.

Fatty liver disease (NAFLD) is the manifestation of the metabolic syndrome in this organ. Due to the obesity epidemic a large percentage of the population is affected and the disease can progress to liver fibrosis and cirrhosis, which has very poor prognosis. In our group, we use proteomics to study diverse aspects of NAFLD. Protein correlation profiling at the proteome and phospho-proteome level of normal and high fat diet mice revealed a striking rearrangement of cellular organelles. In particular, the Golgi apparatus is almost completely sequestered to lipid droplets, dramatically affecting the liver secretome. We used proteomics to create a cell type resolved human liver atlas, which can serve as a background to discover alterations in liver disease progression. Finally, we used plasma proteome profiling on several small cohorts of NAFLD and cirrhotic patients, revealing potential biomarkers of progressive liver disease.

TOD am 11:10

Quantitative targeted proteomic analysis of One-Carbon Metabolism Proteins in human liver cancer

Fernando Corrales[1]; Alberto Paradela[4]; Verónica Ambao[2]; Ignacio Granero[4]; Bruno Sangro[3]
[1]Centro Nacional de Biotecnología, CSIC; CIBEREHD, Madrid, Spain; [2]Centro de Investigaciones Endocrinológicas CONICET, Buenos Aires, Argentina; [3]Clínica Universidad de Navarra-IDISNA and CIBEREHD, Pamplona, Spain; [4]Centro Nacional de Biotecnología, CSIC, Madrid, Spain

Background:Primary liver cancer (HCC) is recognized as the fifth most common neoplasm and the second leading cause of cancer death worldwide. Although most risk factors are known, and the molecular pathogenesis has been widely studied the underlying molecular mechanisms remain to be unveiled. This is a central issue to facilitate the definition of novel biomarkers and clinical targets for more effective patient management. We utilize the B/D-HPP popular protein strategy to detect proteins that have been associated to liver cancer in previous studies. Several enzymes highlight the known metabolic remodeling of liver cancer cells, four of which participate in one-carbon metabolism (1CM). This pathway is central to the maintenance of differentiated hepatocytes, as it is considered the connection between intermediate metabolism and epigenetic regulation.

Methods: We designed a targeted selective reaction monitoring (SRM) method to track quantitatively 15 different 1CM proteins in human liver samples (control, HCC and cirrhotic). This method allows systematic monitoring of one-carbon metabolism and could prove useful in the follow-up of HCC and of chronically liver-diseased patients (cirrhosis) at risk of HCC.

Results: Relevant changes occur at the quantitative level when non-diseased and tumor liver samples are compared. A more complex quantitative pattern was found from samples obtained from cirrhotic livers, suggesting that these particular samples are more heterogeneous than previously expected.

Conclusions: Significant and reliable quantitative changes found for several 1CM-specific proteins could be useful for the diagnosis and prognosis of human liver cancer.

TOD am 11:22

Systems analysis reveals phosphatidylcholine metabolism changes in relapsed multiple myeloma

Ahmed Mohamed[1]; Joel Collins[3, 4]; Hui Jiang[2]; Jeffrey Molendijk[2]; Thomas Stoll[2]; Kate Markey[1, 5]; Michelle Hill[1, 2]
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Multiple myeloma (MM) is a haematological malignancy characterised by the clonal expansion of abnormal plasma cells

within the bone marrow. MM has traditionally been considered incurable, with relapse occurring in almost all patients. Currently, patients are stratified using the Revised International Staging System (R-ISS), but MM within the same risk group show heterogenous behaviour, and R-ISS is unable to predict risk of relapse. Obesity has been linked with higher MM risk and worse outcome, but there are limited studies on the lipid metabolism changes in MM. We performed proteomics and lipidomics analysis on plasma cells isolated from a small cohort of patients, then analysed integratively with a larger public transcriptomic dataset from Multiple Myeloma Research Consortium reference collection. Interestingly, while both proteomics and lipidomics separated relapse from non-relapse patients, the molecular profiles of plasma cells were less distinguishable among R-ISS risk groups. Pathway analysis revealed 76 pathways significantly enriched in both proteomic and transcriptomic data including the up-regulation of TCR and NF-kB signalling in relapsed plasma cells. A down-regulation trend was observed in relapse compared to non-relapse patients in both proteomics and lipidomics profiles. Out of ~4100 identified proteins, 123 were down-regulated while 45 were up-regulated. Untargeted lipidomics showed a similar pattern with 187 features down-regulated and 94 up-regulated. In agreement, targeted lipidomics indicated significant down-regulation of phosphatidylcholines (PCs). Focussing on lipid metabolism pathways, we found PC metabolic network gene expression to be exclusively correlated in relapsed patients. Within this correlated network, LPBD1, which is a phospholipase B degrading PC, was up-regulated at the protein level. In summary, through systems analysis of plasma cells, we found corroborating evidence of PCs down-regulation in relapsed MM. Future studies in larger cohorts are needed to confirm candidate biomarkers and new therapeutic approaches to prevent MM relapse.

TOD am 11:34

NEDDylated proteome in Non Alcoholic Fatty Liver Disease

Marina Serrano-Maciá[1]; Mikel Azkargorta[1, 4]; Jorge Simón[1]; Naroa Goikoetxea-Usandizaga[1]; Teresa Cardoso[1]; David Fernandez-Ramos[1]; Virginia Gutierrez de Juan[1]; Marta Varela-Rey[1, 5]; Pablo Fernandez-Tussy[1]; Fernando Lopitz-Otsoa[1]; Patricia Aspichueta[2]; Paula Iruzubieta[2]; Javier Crespo[2]; Selly C Lu[3]; José M. Mato[1]; Felix Elortza[1, 4]; María Luz Martinez-Chantar[1, 5]

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Non-alcoholic fatty liver disease (NAFLD) is a clinical term that includes a set of pathological conditions, from lipid accumulation as a simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC). NAFLD is the most frequent liver disorder with a 25% prevalence worldwide. Most of the treatments are limited to lifestyle modifications while alternative pharmacological approaches are still limited. As NAFLD includes a wide spectrum of secondary conditions, a therapy of reprogramming hepatic metabolism could be the perfect option of treatment. Post-translational modifications (PTMs) of the proteome provide a faster mechanism for the activation or inhibition of metabolic pathways. Neddylation is an ubiquitin-like reversible PTM characterized by the conjugation of Nedd8 (neural precursor cell expressed developmentally down-regulated 8) to its target proteins by promoting their stabilization. It is involved in several processes such as cell growth, viability and development. Preliminary data of our laboratory showed an increase of hepatic and circulating global neddylated proteome from steatosis and NASH mice model induced by different diets. Importantly, overrepresentation of this PTM was detected in a small obese cohort diagnosed with liver steatosis and NASH. Owing to the importance of neddylation in premalignant and malignant stages, Takeda Pharmaceutical developed MLN4924 (Pevodenistat). It is an inhibitor of Nedd8-activating enzyme E1 (NAE), involved in the first step of the neddylation process. We have performed a preclinical study treating NASH mice model (methionine and choline-deficient diet (MCDD)) with Pevonedistat. Remarkably, neddylation inhibition reverts reduces steatosis, inflammation and fibrosis. In addition, we describe also the reversion of neddylation levels in serum samples after Pevonedistat administration. β-oxidation and ketone bodies levels were increased. Moreover, proteomics analysis showed a high regulation of Acox1, Pparα and RICTOR cellular pathways. Interestingly, comparison between RNAseq results (transcripcional level) with proteomics results (translational level) showed a perfectly compensatory response as an autoregulation.

TOD am 11:46

Comparative proteomic and lipidomic profiling reveals broad dysregulation of lipid metabolism in triple-negative breast cancer development

Ling Lin[1]; Songping Lin[2]; Huali Shen[1]; Pengyuan Yang[1]

[1]Fudan University, Shanghai, China; [2]Affiliated Union Hospital of Fujian Medical Univer, Fujian, China

Triple Negative Breast Cancer (TNBC) is an aggressive disease, accounting for 15% -23.8% of all breast cancers. TNBCs are associated with poor long-term outcomes compared with other breast cancer subtypes. By quantitative proteomic analyses, we discovered that lipid metabolism was activated in TNBC progression using different pathological grade of TNBC tumor and corresponding para-tumor tissues. Due to the complexity and diversity of lipid molecules, along with the challenges in analytical techniques development and comprehensive lipid database construction, altered lipid composition and reprogrammed lipid metabolism have not been fully elucidated during cancer progression. Based on the shotgun lipidomics platform, we identified 1556 intact lipids in TNBC specimens. Palmitic acyl (C16:0)-containing glycerophospholipids (GPs) were significantly reduced in tumor tissues compared with adjacent nontumor tissues. We further examined the effects of palmitic acid treatment on cell proliferation and invasion ability in vitro as well as tumor growth and metastasis in mouse xenograft models via MDA-MB-231 cell line. Besides, we explored the regulatory roles of palmitic acid in energy metabolism and endoplasmic reticulum stress signaling. This research will provide the essential theoretical and experimental basis for diagnosing and treating TNBCs by targeting the altered lipid metabolism.

TOD am 11:58

A molecular portrait of ground state pluripotency

Ana Martinez-Val[1]; Cian Lynch[2, 3]; Manuel Serrano[2, 3]; Javier Muñoz[1]

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Mouse embryonic stem cells cultured in serum fluctuate between a primed and a *naïve* state of pluripotency. Whilst the primed state is prone to differentiation, the *naïve* state shows

higher self-renewal capacity and is considered as the ground state of pluripotency (an *in vitro* surrogate of pre-implantation embryos). Dual inhibition of GSK3 and ERK (aka 2i) is commonly used to capture this ground state in culture. Most recently, inhibition of CDK8/19 (negative regulators of the Mediator complex in enhancers) has been also shown to stabilize this condition. Whether these two mechanisms (2i and CDK8/19i) converge in a similar *naïve* state is not clear. Here, we used quantitative mass spectrometry to fully characterize these two transitions from three different angles: proteome, metabolome and phosphoproteome.

First, we profiled proteome dynamics comprehensively across seven time points in four different cell lines. These analyses revealed that both treatments induce a similar and synchronized transcriptional response characteristic of the inner cell mass. Among many others, we found several proteins involved in metabolism consistently altered. To further investigate this, we next analysed their metabolomes in long-term adapted cultures and confirmed a switch towards oxidative phosphorylation in the *naïve* state compared to the more glycolytic primed cells. Finally, given that these events are initiated by kinases inhibition, we sought to delineate the phosphorylation cascades triggered in the early phases of the process and monitored ~14,000 phosphosites within the first 6 hours of treatment. We found that both GSK3i/ERKi and CDK8/19i induce a rapid alteration of phosphorylation networks, mainly affecting pluripotency transcription factors (Sall4, Sox2, Tbx3) and the transcriptional machinery (Suz12, Ezh2, RNAPolII).

Our results demonstrate that the establishment of the ground state by stimulation of two different routes, proliferation/self-renewal (2i) and enhancer function (CDK8/19i), undergo similar mechanisms, suggesting that these pathways are highly interconnected in pluripotent cells.

10:30 am - 12:20 pm Tuesday
RARE DISEASES
Session Chair: Nicholas J. Schork
Oceana 1-2

TOE am 10:30
Integrated Approaches to Patient-Specific Research

Nicholas J. Schork[1, 2]
[1]Translational Genomics Research Institute (TGen), Phoenix, AZ; [2]City of Hope/TGen IMPACT Center, Duarte, CA

Individualized medicine, or the tailoring of therapeutic interventions to a patient's unique genetic, biochemical, physiological, exposure and behavioral profile, has been enhanced, if not enabled, by modern biomedical technologies such as high-throughput DNA sequencing platforms, induced pluripotent stem cell assays, biomarker discovery protocols, imaging modalities, and wireless monitoring devices. Despite successes in the isolated use of these technologies, however, it is arguable that their combined and integrated use in focused studies of individual patients is the best way to not only tailor interventions for those patients, but also shed light on treatment strategies for patients with similar conditions. This is particularly true for individuals with rare diseases since, by definition, they will require study without recourse to other individuals, or at least without recourse to many other individuals. Such integration and focus will require new biomedical scientific paradigms and infrastructure, including the creation of databases harboring study results, the formation of dedicated multidisciplinary research teams and new training programs. I consider the motivation and potential for such integration, point out areas in need of improvement, and argue for greater emphasis on improving patient health via technological innovations, not merely improving the technologies themselves. I provide concrete examples involving rare forms of Alzheimer's disease and argue that the paradigm described can, in theory, be extended to the study of individuals with more common diseases.

TOE am 10:50
Pediatric Cancer: A Genomics-based Study of a Rare Disease

Elaine R. Mardis
Nationwide Children's Hospital, Columbus, OH

The relative incidence of pediatric cancers compared to adult cancers defines them as rare diseases, which invokes a unique set of challenges and opportunities. Baseline genomic characterizations of pediatric cancers have been completed by several large studies, thereby defining the pediatric cancer genome as having a paucity of mutations, multiple fusion protein drivers, and a higher contribution from germline mutations than previously thought. Our Institute has opened a genomics-based study of pediatric cancers to begin to define how genomic characterization in the clinical setting may have value to informing prognosis, diagnosis and therapeutic options. I will highlight our study to-date and illustrate the contribution of genomics to clinical benefit in the pediatric setting.

TOE am 11:10
Quantitative Proteomic Analyses of Uterine Leiomyomas from Hereditary Leiomyomatosis and Renal Cell Cancer Patients.

Thomas Conrads[1]; Christopher Tarney[2]; Nicholas Bateman[2]; Niyati Parikh[2]; Ming Zhou[1]; Kelly Conrads[2]; James Segars[3]; Paul Driggers[3]; Yovanni Casablanca[2]; Chad Hamilton[2]; G. Larry Maxwell[4]
[1]Inova Schar Cancer Institute, Falls Church, VA; [2]Gynecologic Cancer Center of Excellence, Annandale, VA; [3]Johns Hopkins School of Medicine, Baltimore, MD; [4]Obstetrics and Gynecology, Inova Fairfax Hospital, Falls Church, VA

Introduction: Mutation in the fumarate hydratase (FH) gene causes hereditary leiomyomatosis and renal cell cancer (HLRCC). Impaired FH activity leads to accumulation of cellular fumarate causing increased post-translation modifications, such as the formation of 2-succinyl-cysteine (2SC) residues. We performed quantitative proteomic analyses of ULMs collected from HLRCC versus non-syndromic patients to decipher novel mechanistic insights that will improve the management of ULMs in HLRCC patients.

Methods: ULMs from HLRCC (n=17) and non-syndromic (n=14) patients were obtained from a single institution. Multiplexed, tandem mass tags (TMT-10) quantitative proteomics was performed on tryptic digests generated from cryopulverized tissues using a pressure cycle-assisted lysis and digestion strategy followed by LC-MS/MS analyses on an Orbitrap Fusion Lumos Tribrid MS (Thermo). Proteomic database searches included variable modifications for 2SC residues. Functional inference was performed using IPA (Qiagen).

Results: Differential analyses of 5,506 total proteins quantified revealed 394 as significantly altered between HLRCC and non-syndromic ULMs (FDR<0.05, ~2-fold altered). Pathway analysis suggested marked alteration of mitochondrial activity in HLRCC versus non-syndromic ULMs as reflected by increased expression of electron transport and ATP synthase proteins. Analyses of 2SC post-translational modifications identified 364 modified peptides corresponding to 240 proteins, 31 of which have been previously described as altered in FH-mutated cancer cell lines. Pathway analysis of 2SC-modified protein targets revealed altered regulation of cytoskeletal organization, cell death and cell migration signaling in HLRCC ULMs. Quantitative analyses revealed 63 unique 2SC-modified peptides were ~2.45 ± 0.03-fold elevated in HLRCC versus non-syndromic patients. These candidates included a peptide modified on C106 of Parkin 7 (PARK7), a potent cellular deglycase and sensor of oxidative stress.

Conclusions: Quantitative proteomics revealed protein alterations and modifications impacting mitochondrial and oxidative stress signaling in HLRCC versus non-syndromic ULMs. These findings define proteomic alterations that may lead to improved management of ULMs in HLRCC patients.

TOE am 11:22

Plasma Proteomic Profiling to Identify Potential Biomarkers for Early Diagnosis of Multiple Myeloma in Premalignant Disease

Yurany Moreno[1]; Nicola J Weston-Bell[1]; Kate Vandyke[3]; Duncan Hewett[3]; Andrew Zannettino[3]; Spiros Garbis[2]; Surinder S Sahota[1]

[1]Tumour Immunogenetics Group, University of Southampton, United Kingdom; [2]Proteomics Group, University of Southampton, United Kingdom; [3]Myeloma Research Laboratory, The University of Adelaide, Australia

Multiple myeloma (MM) is a lethal hematological malignancy characterized by monoclonal plasma cell expansion in the bone marrow, almost invariably preceded by asymptomatic monoclonal gammopathy of undetermined significance (MGUS). MM is diagnosed by CRAB features (hypercalcemia, renal insufficiency, anemia and bone lesions) to then initiate therapy. However, diagnosing MM based on end-organ damage is a major concern, and novel biomarkers that can identify early detection of malignant transformation to MM in MGUS offer considerable scope to improve therapeutic outcomes, in the absence of morbidity associated with organ failure.

To identify potential biomarkers able to distinguish MGUS-MM disease, we examined the plasma proteome in discovery cohorts (6 IgG-MGUS, 6 IgG-MM, 6 age-matched healthy). We utilized multiplex isobaric labelling and three-dimensional liquid chromatography tandem mass spectrometry (TMT-3DLC-MS/MS) based quantitative proteomics analysis of non-depleted plasma. Proteome Discoverer v.1.4 was used for identification and quantification of plasma proteins.

We identified and fully quantified 1084 proteins with high statistical confidence. When compared with healthy controls, 474 proteins were differentially expressed in MM and 416 in MGUS, of which 317 common to both disease states. Several proteins identified as dysregulated in MM have been previously reported, including B2M and OSTP. Of these, 45 proteins were differentially regulated between MGUS and MM.

Emerging from the data, 28 proteins were found dysregulated *exclusively* in MM, including ECM1, BCHE, ICAM2, ADAMTS2, SAA2 and BST1. Gene ontology enrichment analysis of MM-specific dysregulated proteins was associated with inflammatory and immune response, vesicle-mediated transport and proteolysis.

We report a compendium of biomarkers that can now be interrogated further on a more targeted basis to elucidate which plasma proteins demarcate onset of malignant MM in MGUS. Such biomarkers have enormous potential to progress early detection and clinical management of lethal MM disease.

TOE am 11:34

Angiotensin II signature proteins as non-invasive markers of fibrosis in kidney transplant recipients

Zahraa Mohammed-Ali[1]; Tomas Tokar[1]; Ihor Batruch[2]; Shelby Reid[3]; Alexandre Tavares-Brum[4]; Paul Yip[1]; Héloïse Cardinal[4]; Marie-Josée Hébert[4]; Yanhong Li[1]; S. Joseph Kim[1, 3]; Igor Jurisica[1, 3]; Rohan John[1]; Ana Konvalinka[1]

[1]University Health Network, Toronto, ON, Canada; [2]Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; [3]University of Toronto, Toronto, ON, Canada; [4]Centre Hospitalier de l'Université de Montréal, Montréal, QC, Canada

Introduction

Interstitial fibrosis/ tubular atrophy (IFTA) is inducible by angiotensin(Ang)II, the main effector of the renin-angiotensin system (RAS), and leads to kidney allograft loss. However, there are no clinical, non-invasive markers of AngII activity or IFTA. We examined whether the urine excretion of 6 AngII-regulated proteins (BST1, GLUL, LAMB2, LYPLA1, RHOB and TSP1), established from our previous studies, could reflect kidney IFTA and RAS activity in kidney allograft recipients.

Methods

Our study samples were obtained via the Canadian National Transplant Research Program and comprised: 1) 16 patients with IFTA and 19 stable controls with concomitant urine and biopsy samples, and 2) 19 patients with urine samples before and after RAS inhibition. Urine excretion of 6 AngII--regulated proteins was quantified using selected reaction monitoring and normalized by urine creatinine. Immunohistochemistry staining analysis was used to assess protein expression of AngII-regulated proteins, TSP1 and GLUL, in kidney biopsies from IFTA vs. control kidney allograft recipients.

Results

The urine excretion of AngII--regulated proteins was higher in IFTA compared to control patients (in log2 [fmol/µmol of creatinine], BST1: 3.7 vs. 3.0, p=0.03; GLUL: 1.1 vs. -0.3,

p=0.05; LAMB2: 6.0 vs. 5.4, p=0.09; LYPLA1: 2.1 vs. 0.6, p=0.002; RHOB: 1.1 vs. -0.1, p=0.01; TSP1_GGV: 2.5 vs. 2.0; p=0.3; TSP1_TIV: 1.9 vs. 0.6; p=0.0009). Urine AngII-regulated proteins were decreased in response to RAS inhibitor treatment (ln log2 [fmol/µmol of creatinine], comparing pre-treatment vs. post-treatment, BST1: 3.9 vs. 3.0, p=0.03; GLUL: 1.3 vs. -0.4, p=0.03; LAMB2: 6.5 vs. 5.3, p=0.006; LYPLA1: 2.4 vs. 0.8, p=0.002; RHOB: 1.4 vs. 0.1, p=0.003; TSP1_GGV: 3.6 vs. 2.0, p=0.003; TSP1_TIV: 3.1 vs. 0.6, p=0.00004). GLUL protein expression was significantly increased in IFTA vs. control kidneys (p=0.04).

Conclusion

Our AngII-regulated proteins may represent markers of kidney fibrosis and potentially guide therapy with RAS inhibitors.

TOE am 11:46

Aptamer-based Proteomics Identifies Potential Predictive Biomarkers of Doxorubicin-induced Cardiotoxicity

Li-Rong Yu[1]; Jaclyn Daniels[1]; Zhijun Cao[1]; Richard Beger[1]; Issam Makhoul[2]; Angela Pennisi[2]; Jeanne Wei[2]; Jane Bai[3]; Julia Lathrop[4]; Jinong Li[5]; Valentina Todorova[2]

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Treatment of cancer patients with doxorubicin (DOX) can cause cumulative dose-dependent cardiotoxicity. It is crucial to detect potential cardiotoxicity early before onset of symptomatic cardiac dysfunction or heart failure. Currently there are no qualified clinical biomarkers for the prediction of cardiotoxicity caused by cancer treatment. In this study, 70 breast cancer patients were enrolled under an IRB-approved protocol and treated with a combination of DOX (60 mg/m2) and cyclophosphamide (600 mg/m2). Blood samples were collected prior to treatment (T0), after the first (T1), and the second (T2) cycles of DOX treatment. Cardiac function was assessed by a multi-gated acquisition (MUGA) scan before the start of DOX treatment and at completion of four cycles of chemotherapy. SOMAscan® proteomic platform was used to profile 1,305 proteins in each plasma sample. Statistical analysis (Welch's *t*-test) of the proteomic data identified abundance changes (fold change ≥ 1.2 and $p < 0.05$) in 263 proteins at T0, 15 proteins at T1, and 11 proteins at T2 in the group of patients with cardiotoxicity (reduction of >10% in left ventricular ejection fraction) as compared to the patients with normal cardiac functions after completion of DOX treatment. Myeloperoxidase was increased with DOX treatment and its levels were 1.32-fold higher at T2 in the cardiotoxicity group. When patients were compared to their own baseline levels, 25 proteins at T1 and 70 proteins at T2 were changed after DOX treatment and were significantly different in patients with cardiotoxicity as compared to the patients with normal cardiac functions. A 2-fold increase of insulin at T2 was observed in the cardiotoxicity group. These putative biomarkers may potentially predict which patients are vulnerable to DOX-induced cardiotoxicity; further validation is needed using a larger cohort of patients.

TOE am 11:58

Targeted Proteomic Analysis of Formalin-Fixed Paraffin Embedded Prostate Biopsies with Outcomes Data to Identify Candidate Biomarkers for Aggressive Prostate Cancer

Yuqian Gao[1]; Hui Wang[1]; Denise Young[2]; Jennifer Cullen[2, 3]; Yingjie Song[2]; Yongmei Chen[2]; Athena Schepmoes[1]; Gyorgy Petrovics[2, 3]; Thomas Fillmore[1]; Tujin Shi[1]; Wei-Jun Qian[1]; Richard Smith[1]; Sudhir Srivastava[4]; Jacob Kagan[4]; Albert Dobi[2, 3]; Inger Rosner[2]; Karin Rodland[1]; Isabell Sesterhenn[5]; Shiv Srivastava[2]; Tao Liu[1]

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Although the majority of screen-detected prostate cancers are indolent and pose minimal risk for progression, advanced stage prostate cancer is a lethal disease with 5-year survival rates around 29%. The challenge is to identify biomarkers for early detection of aggressive disease, when it is still organ confined. Such markers would be also used to better select patients with indolent and low risk cancers for active surveillance. In order to identify a panel of proteins with the potential to predict prostate cancer progression, we developed sensitive high-pressure, high-resolution separations coupled with intelligent selection and multiplexing-selected reaction monitoring (PRISM-SRM) assays for 52 protein candidates selected from existing prostate cancer genomics data sets and validated lists of known cancer drivers. The PRISM-SRM assays used heavy isotope-labeled synthetic peptides as internal standards for quantitative proteomics analysis of 105 formalin-fixed paraffin embedded (FFPE) tissue samples (scraped 10 m sections from slides) with clinical outcomes data: 20 primary tumors from patients showing metastatic progression, 37 primary tumors from patients who showed biochemical recurrence (BCR), and 48 primary tumors from patients with no BCR or metastatic progression after more than ten years of follow-up after radical prostatectomy. Overall, PRISM-SRM analyses of the FFPE tissue samples enabled the detection of 42 out of 52 biomarker candidates; in comparison regular LC-SRM without the front-end chromatographic enrichment could detect only 21 of these candidates at the protein level. Kruskal-Wallis test was used for statistical evaluation of the PRISM-SRM results and comparison of relative protein levels between the "no progression", BCR and "metastatic progression" groups. Several prostate differentiation/androgen receptor signaling related proteins (FOLH1, PSA and NCOA) and tumor progression-related proteins (TGFB1, CCND1 and SPRC) had significantly different expression levels between the three groups. These biomarker candidates are currently under evaluation in an independent cohort of 234 patient samples having outcomes data.

2:00 - 3:50 pm Tuesday
CARDIOLOGY
Session Chairs: Peipei Ping and Pothur Srinivas
Oceana 8-10

TOA pm 2:00

Top-Down Proteomics in Cardiac Disease and Regeneration

Ying Ge

U OF WISCONSIN MADISON, Madison, WI

Heart diseases remain the leading causes of death in developed countries for both men and women. Altered post-translational modifications (PTMs) and sequence variations of key regulatory cardiac proteins have been implicated as causative factors for cardiac diseases. Nevertheless, the

disease mechanisms are highly heterogeneous and poorly understood. To understand the molecular mechanisms in various cardiac diseases, we need to obtain a global qualitative and quantitative view of the combinatorial PTM-amino acid sequence variant landscape of the cardiac proteome. Mass spectrometry (MS)-based top-down proteomics is the most powerful technology for deciphering PTM codes together with sequence variations, providing essential insight into the structure and function of proteoforms, the effectors of all biological processes. Herein, we aim to develop and implement novel top-down proteomics technologies to characterize the human heart at the proteoform level to deepen our knowledge of the human heart proteome. We have identified previously unknown phosphoproteins and large proteins (> 250 kDa) in the cardiac sarcomere using our lab's novel top-down proteomics technology enabled by serial size exclusion chromatography and ultra-high resolution MS. Using label-free quantitative top-down proteomics, we have unveiled a reversal of deleterious PTMs following cardiac injury and treatment with human cardiac muscle patches from induced-pluripotent stem cells. Additionally, a new quantitative proteomics platform developed in our lab has allowed for label-free quantification of protein expression levels of isoforms for assessing maturation of iPSC-derived cardiomyocytes. Our novel top-down proteomics platforms will enable an increased understanding of heart disease and regeneration.

TOA pm 2:20
Oxidative Stress Post-translational Modification Landscape in Cardiac Hypertrophy Revealed By Machine Learning Approaches

Peipei Ping
UCLA, Los Angeles, CA

Introduction: Oxidative stress is an established pathological stimulus for heart failure, yet its molecular actions and their scope, targets, as well as impact are largely undefined. We performed a multi-faceted proteomics study, combined with a state-of-the-art computational pipeline, to elucidate key oxidative stress-sensitive post-translational modifications (O-PTMs) of molecular signatures and pathways in cardiac remodeling and heart failure progression.

Methods: We performed a large-scale proteomic characterization to define O-PTM regulation in the cardiac proteomes with respect to their modification type, site, and occupancy. To achieve deep molecular phenotyping via O-PTM signatures, we devised a novel analytical workflow that implemented supervised and unsupervised machine-learning (ML) approaches to interrogate spectral data with that of functional analysis. We studied 6 mouse genetic strains and validated part of our findings in proteomes of failing human hearts. Specifically, we identified O-PTM signatures representative of the cardiac hypertrophy phenotype using feature selection algorithms and dissected their temporal dynamics using cubic spline-based clustering.

Results: We report that cardiac dysfunction impacted 6,521 of 8,227 murine cardiac proteins with 25,472 sites in 549 cellular pathways. Our analysis produced four unique O-PTM signatures that robustly represent the cardiac hypertrophy phenotype in mice. These signatures predominantly operate under two mitochondrial pathways well-established in heart failure: tricarboxylic-acid cycle and respiratory electron transport chain (TCA) and fatty acid beta-oxidation (FABO). Intriguingly, when we investigated the temporal dimension of their O-PTM alterations, proteins in TCA, FABO, calcium regulation, and branched chain amino acid catabolism (BCAA) pathways displayed diverse regulatory patterns, corroborating their molecular network organization and biological degeneracy. To understand the translational value of the identified O-PTM signatures and pathways, we examined O-PTM landscapes in heart failure patients and identified 475 of 898 mitochondrial cardiac proteins with 2,301 sites in 307 pathways. Notably, TCA, FABO, and BCAA pathways were identified in both mouse and human samples, yet with distinct O-PTM and protein abundance patterns.

Conclusions: Our findings provide a molecular map that can serve as an avenue for biomarker discovery toward potential therapies and drugs. Our novel ML-based integrative analytics approaches present a powerful means to monitor biomarker panels for disease progression and drug response.

TOA pm 2:40
Proteomic Mapping Reveals Differences in the Bioenergetics of the Heart

Wendy Heywood[1]; Richard Collis[2, 4]; Jonathan Searle[1]; Ivan Doykov[1]; Caroline Coats[2]; Michael Ashworth[3]; Perry Elliott[2, 4]; Kevin Mills[1]
[1]UCL Great Ormond Street Institute of Child Health, London, United Kingdom; [2]UCL Institute of Cardiovascular Science, London, UK; [3]Great Ormond Street Hospital, London, UK; [4]Barts Hospital, London, UK

Diseases of the heart are a leading cause of debilitating illness and death. Understanding of the heart proteome will be a fundamental tool in elucidating disease mechanisms. Proteomic studies often explore between whole organs and systems however within an organ itself a more subtle variation exists. Differences between specific anatomical regions have previously been described. This study aims to look at the overall proteome in more depth including inter-regional differences in the cardiac proteome. A total of 133 sections were isolated from across a bovine heart. Each section was digested and analysed using label free proteomics. Data was searched against the bovine and human reference proteomes and analysed using Progenesis QI, SIMCA and an excel mapping script. Multivariate analysis revealed three distinct proteomes. When mapped to the extracted sections cluster 1 corresponded largely to the outer wall of the right ventricle (largest variation), cluster 2 to the outer wall of the left ventricle, right atrial appendage tricuspid and mitral valves, modulator band and parts of the left atrium. Cluster 3 corresponded to the inner walls of the left and right ventricles, septum and left atrial appendage. Gene ontology analysis indicated differences in the energy metabolism of each region. Organelle composition analysis showed that mitochondrial proteins consisted of 20-26% of the proteome across the organ. However cluster 1 has a lower proportion of mitochondrial proteins. Complexomic analysis revealed variable abundances of complexes I-V between the clusters indicating differences in the bioenergetics of the different sub-proteomes. Cluster 1 revealed greatest correlation of the mitochondrial complex levels with anti-oxidant proteins ALDH2 and PRDX3. Overall this analysis reveals that differences in the heart proteome are not confined to anatomical regions and the greatest variation is actually between the outer walls to the inner tissue of the heart.

TOA pm 2:52

Circulating concentrations of MYDGF in healthy individuals and patients with myocardial infarction as assessed by a new, quantitative LC-MS assay

Andreas Pich[1]; Felix Polten[1]; Marc Reboll[1]; Kerstin Bethmann[1]; Christian Widera[1]; Evangelos Giannitsis[2]; Jochen Tillmanns[1]; Tibor Kempf[1]; Johann Bauersachs[1]; Kai Wollert[1]
[1]Hannover Medical School, Hannover, Germany; [2]UNiversity of Heidelberg, Heidelberg, Germany

MYDGF is a monocyte- and macrophage-derived secreted growth factor mediating tissue repair after myocardial infarction in mice. Here, we developed a quantitative assay to measure MYDGF in patients and healthy volunteers.

An MRM assay was developed to measure MYDGF in human plasma samples. Two unique tryptic peptides were selected and stable isotope-labeled standards were spiked into gel-fractionated plasma samples to enable absolute quantification. The preanalytic characteristics of MYDGF were assessed and plasma MYDGF concentrations were measured in 120 apparently healthy individuals and 101 patients admitted with myocardial infarction.

The assay had a limit of detection of 0.6 ng/mL, a limit of quantification of 0.8 ng/mL, an intraassay imprecision of 12.6%, and an interassay imprecision of 17.0%. MYDGF was stable for up to 4 h in whole blood containing anticoagulants and was resistant to 3 freeze-thaw cycles in plasma samples. MYDGF concentrations in EDTA, lithium heparin, and citrate plasma did not differ significantly; serum was not suitable for quantification of MYDGF. Healthy individuals had a median MYDGF plasma concentration of 3.3 ng/mL. MYDGF concentrations decreased with age (p=0.004). After myocardial infarction, MYDGF plasma concentrations were elevated (median, 8.9 ng/mL). MYDGF plasma concentrations after myocardial infarction were associated with the inflammatory biomarkers GDF-15 and CRP and with eGFR. During follow-up, MYDGF levels above the median were associated with increased cardiovascular mortality after myocardial infarction.

This assay will enable further studies into the role of MYDGF in myocardial infarction and other cardiovascular disease states. Further MS-based analyses are underway in our laboratory to elucidate the functions of MYDGF.

TOA pm 3:04

Getting to the heart of the matter: Multispecies heart tissue proteome characterization

Joel Federspiel[1]; Caralynn Wilczewski[2]; Laura Herring[2]; Samvida Venkatesh[1]; Lauren Wasson[2]; Frank Conlon[2]; Ileana Cristea[1]
[1]Princeton University, Princeton, NJ; [2]University of North Carolina at Chapel Hill, Chapel Hill, NC

A leading cause of infant mortality in the US and Europe is congenital heart disease (CHD) deriving from structural birth defects. *Xenopus*, mouse, and pig model systems have been used to study CHD, but it remains unknown what proteins and pathways are conserved between these species and human. Furthermore, proteome expression differences between three-chambered (*Xenopus*) and four-chambered (mammalian) hearts are unknown. Comparative proteomics of heart tissue from species at different evolutionary points can reveal molecular processes underlying heart function. Therefore, we examined heart tissue proteomes of *Xenopus tropicalis*, *Xenopus laevis, Mus musculus*, and *Sus scrofa* and assessed protein abundance changes in the context of pathways, protein complexes, and enrichment of corresponding genes in human heart diseases. To do this, heart tissues were dissected and subjected to differential solubilization and were then gel fractionated, digested, and analyzed on a Q Exactive HF (ThermoScientific). Differential heart proteome assessment was performed by label-free quantitation using Proteome Discoverer 2.1 and Scaffold. Over 9,000 proteins were identified across all four species analyzed, and were mapped to orthologous human proteins to assist with bioinformatic analysis. The quantitative differences revealed numerous species-specific enrichments in selected pathways. One example is a pronounced enrichment of cell cycle associated proteins in *Xenopus laevis* compared to the other species, which we confirmed via targeted mass spectrometry. We further developed a computational approach to interrogate the conservation and relative levels of functional protein complexes across species. Additionally, our dataset can be used to select a model system for a disease pathology based on protein expression. For example, our results suggest that mouse is an adequate model for analyzing arrhythmogenic right ventricular cardiomyopathy and *Xenopus* for studying atrial fibrillation. This dataset represents one of the only multispecies heart proteome resources and gives insight into evolutionarily directed functional differences among important model systems.

TOA pm 3:16

Phosphoproteomic profiling reveals perturbed cardiac signaling in dilated cardiomyopathy patients

Sina Hadipour-Lakmehsari[1]; Uros Kuzmanov[1]; Andrew Emili[1]; Gavin Oudit[2]; Anthony Gramolini[1]
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Dilated cardiomyopathy (DCM) is a debilitating disease characterized by thinning of the left ventricular wall and systolic dysfunction; individuals with dilated cardiomyopathy are at high risk of heart failure. Despite being a serious and increasingly prevalent disease, the cardiac signaling defects are not well known. Phosphorylation events are a very common method of signaling within cells and, thus, were our main investigative focus. Using 10-plex TMT labelling strategies, HPLC-HILIC chromatography fractionation, and TiO_2 pulldown for phosphopeptide enrichment, we performed mass spectrometry analyses to investigate the global proteomic and phospho-proteomic changes in 4 healthy and 4 adult DCM explanted cardiac ventricular tissue samples. We identified 23120 unique peptides and 7419 phosphorylation sites (phosphosites) which corresponded to 3078 proteins and 1791 phophoproteins. Global statistical enrichment analysis of the differential phosphoprotein patterns revealed selective perturbation of signalling pathways regulating cardiovascular activity in DCM. Statistical analysis revealed 1494 significant different phosphosites and 680 significantly different proteins in DCM patients. 2D principal component analysis showed much tighter clustering of DCM patient samples compared to healthy individuals. Hierarchical clustering of the significantly different merged data shows remarkable changes in proteins involved in sarcomere organization, cardiac chamber development, mitochondrial biogenesis, and extracellular matrix organization. Changes in proteins involved in Ras signal transduction, Myc targets, and EGFR signaling pathways were also observed. The distribution of significantly altered phosphoproteins shows altered signaling in metabolic processes (primarily protein metabolic processes), G-protein coupled receptor pathways, and MAPK signaling cascades. In addition, we performed gene set enrichment analysis (GSEA) to reveal dysregulated

pathways in DCM. Notably, pathways involved in electron transport respiration, mitochondrial translation termination, and fatty acid b-oxidation were perturbed in DCM. These data reveal previously unknown signaling and protein perturbations in DCM involving pathways essential for proper cardiac function.

TOA pm 3:28
Global Proteomic and Transcriptomic Analyses Identify a Profile that Distinguishes Advanced Heart Failure Patients Capable of Cardiac Recovery Following LVAD-Unloading

Christopher Tracy; Aman Makaju; Rachit Badolia; Sutip Navankasattusas; Dinesh Ramadurai; Anna Bakhtani; Lauren McCreath; Nikolaos Diakos; Craig Selzman; Stavros Drakos; Sarah Franklin
University of Utah, Salt Lake City, <Not Specified>

Left ventricular assist devices (LVADs) are increasingly used in everyday clinical practice as either a bridge to heart transplantation (B-T-T) for end-stage heart failure patients or as a permanent (destination) therapy. Evidence from many prospective studies indicate that a subset of patients with LVAD implants can significantly improve the structure and function of their heart and undergo LVAD explantation (i.e. “responders”) while another subset doesn’t experience such recovery, remaining B-T-T candidates (i.e. “non-responders”). This intriguing phenomenon provides great promise for heart failure patients, although the underlying mechanisms driving this recovery are largely unknown. To identify global changes in the normal and failing human heart we performed phosphopeptide profiling from cardiac tissue of 38 patients [10 donor controls, 6 recovered heart failure patients (responders), and 22 patients that did not respond to LVAD therapy (non-responders)] and RNA-Sequencing of 96 patients [9 donor controls, 26 responders and 61 non-responders]. Mass spectrometry-based analyses identified 15,816 unique phosphopeptides, and label-free quantitation further classified 288 peptides that distinguish control tissue from those in heart failure. Most intriguing however, was our analyses of heart failure patients at the time of LVAD implantation which, using a signature of 93 peptides (71 proteins), allowed complete separation of responder and non-responder samples via statistical analysis. In addition, RNA-Seq analysis identified 29 genes differentially expressed between responders and non-responders. Thus, this panel of 93 phosphopeptides and 29 transcripts enabled us to determine which patients would experience cardiac recovery following ventricular unloading, prior to LVAD implantation. Bioinformatic analyses of these proteins and transcripts, highlight key players in adherens junctions, cytoskeletal remodeling, cell cycle regulation and Jun signaling. Overall this study characterizes unique molecular changes in the normal and failing human heart and specifies those which define hearts capable of cardiac recovery, which may guide strategies to improving current heart failure therapies.

2:00 - 3:50 pm Tuesday
COMPUTATIONAL ADVANCES
Session Chairs: Nuno Bandeira and David Fenyo
Oceana 7

TOB pm 2:00
Imputing missing values in Proteomics data from mass spectrometry based experiments

Pei Wang
Icahn School of Medicine at Mount Sinai, New York, NY

Due to the dynamic nature of the mass spectrometry instruments, mass spectrometry based proteomics (especially PTMs) data often contain a large number of missing values. This imposes a great challenge to proteomics data analyses, as many (advanced) data analysis tools/models cannot deal with missing values. Moreover, missing events in mass spectrometry based proteomics data often are not missing at random. Thus simply ignoring the NA values or imputing with constants will lead to biased analysis results.

In a recent NCI-CPTAC-Dream Challenge, together with the challenge participants, we developed a new imputation algorithm for proteomics data through crowd-learning. With this new imputation tool, we expect that information from a large number of proteins (and PTMs) can be rescued in proteomics data analyses,

In this talk, I will first review the design and result of the aforementioned dream challenge on proteomic data imputation. I will then introduce the final imputation method learnt in this challenge based on aggregating a few leading winning algorithms. The key idea of these imputation methods is to make use of the correlation structure among proteins to “estimate” missing data points, as proteins interacting with each other or belonging to the same protein complex usually have similar (or highly correlated) abundance profiles. In the end, I will demonstrate the impact of proper imputation on proteomic data analysis capabilities through real data examples.

TOB pm 2:20
Low Abundance Peptide Sequencing by Deep Learning

Lei Xin[1]; Hieu Tran[2]; Xin Chen[1]; Rui Qiao[2]; CY Liu[2]; Baozhen Shan[1]; Ming Li[2]
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Personalized immunotherapy in ideal case should depend on the neoantigens present on the cancer cell surface, of one person, one tumor, and one time. A few research groups reported direct identification of mutated peptides isolated from human leucocyte antigens (HLA) by LC-MS. Until recently, MS technologies were not sensitive enough to do this. The key challenge is to deal with the low abundance of these peptides. DIA technology promises to capture the low abundance data. However, there is no working algorithm/system to de novo sequence DIA data. Most database search method for DIA spectra also depend on spectrum library that significantly limits their abilities to find mutated peptides. For the first time, we have introduced deep learning into peptide de novo sequencing with DIA data.

Our deep learning system combines two convolutional networks and a long short-term memory (LSTM) recurrent neural network for de novo peptide sequencing. In this model, the first convolutional neural network, called ion-CNN, learns local alternative peak features of all possible next amino acid candidates given the currently predicted partial peptide. The second convolutional neural network, called spectrum-CNN, learns general features of the spectrum and passes them to the LSTM network. The LSTM learns sequence patterns of the currently predicted partial peptide in association with the spectrum features from the spectrum-CNN. The information learned by the ion-CNN and LSTM networks is then integrated to make the final prediction.

This talk will present the testing results with seven LC-MS data sets with DIA approach. The results showed that the deep learning system could handles DIA data, and de novo sequence several peptides from each DIA spectrum, with comparable accuracy to DDA data. Further, the system solves the problem of peptide identification for DIA spectra, combining database search and de novo sequencing to identify low abundance peptides.

TOB pm 2:40
A combined identification and quantification error model of label-free protein quantification

Matthew The; Lukas Käll
KTH - Royal Inst of Technology, Stockholm, Sweden

Protein quantification by label-free shotgun proteomics experiments is plagued by a multitude of error sources. Typical pipelines for identifying differentially expressed proteins use intermediate filters in an attempt to control the error rate. However, they often ignore certain error sources and, moreover, regard filtered lists as completely correct in subsequent steps. These two indiscretions can easily lead to a loss of control of the false discovery rate (FDR). We propose a probabilistic graphical model, Triqler, that propagates error information through all steps, employing distributions in favor of point estimates, most notably for missing value imputation. The model outputs posterior probabilities for fold changes between treatment groups, highlighting uncertainty rather than hiding it.

We analyzed three engineered datasets with Triqler and achieved FDR control and high sensitivity, and we found that the method assigned low concentrations for truly absent proteins. For the iPRG2015 dataset, all 6 spiked-in proteins had PEP<1e-4 for |log2(fold-change)|>0.5, with the most differential background protein of PEP=0.7. For the iPRG2016 dataset, we recovered 347 out of 383 spiked-in PrESTs at 5% reported FDR for |log2(fold-change)|>1.0, with an observed FDR of 4.4%. For the UPS-Yeast mixture, we recovered 45 out of 48 UPS proteins at 5% reported FDR for |log2(fold-change)|>1.0, with an observed FDR of 4.2%.

Further, we analyzed two biological datasets. On a hepatitis C virus dataset (PXD001474) we obtained 21 significant proteins at 5% FDR for |log2(fold-change)|>0.5, whereas the original study found no significant proteins at 5% quantitative FDR (but 70 proteins with p-value<0.05). In a bladder cancer clinical dataset (PXD002170) we discovered 57 proteins at 5% FDR, with the original study discovering none at this threshold. Compellingly, these proteins showed enrichment for known cancer-related functional annotation terms.

In summary, Triqler combines identification and quantification FDRs as well as quantitative levels into readily interpretable and well-calibrated results for label-free data.

TOB pm 2:52
See Deeper with iSwathX: An Effortless Approach to Combine DDA Data Libraries for DIA Data Analysis

Zainab Noor[1]; Abidali Mohamedali[1]; Mark S. Baker[2]; Shoba Ranganathan[1]
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Data-independent acquisition (DIA)-based mass spectrometry (MS) is considered as highly accurate and reproducible technique for quantitative proteomics. This technique requires the availability of a large number of comprehensive reference spectral libraries, generated using tandem mass spectrometry (MS/MS) in a data-dependent acquisition mode (DDA), to identify and quantify large proteome maps generated through DIA. However, the generation, availability and compatibility of these reference libraries with full coverage and depth is crucial and remains a laborious task, requiring a significant expenditure of computational and experimental resources which often leads to data redundancy. In this study, we developed and deployed a platform to integrate spectral data found in different DDA-based libraries stored in large proteomics databases and repositories by extending the functions of previously available methods of SwathXtend. This open-source web-based interactive user interface 'iSwathX' provides a fully-automated processing of reference assay libraries by normalizing and combining the spectra from different DDA-based libraries to generate extended libraries. The interface analyses the libraries stored in PeakView, Spectronaut, OpenSWATH and Skyline formats, which makes it compatible with commonly used DIA analysis software. It provides an automated pipeline for library combination which requires only a modern web browser and no programming knowledge. This strategy can be used to design large-scale and comprehensive spectral libraries from publically available repositories which can be employed for significant and in-depth quantitative analysis of DIA-based proteome profiles. It can enable the extraction of DIA data that has been previously inaccessible, potentially leading to new insights for biomedical and therapeutic studies.

TOB pm 3:04
Topological Scoring of Protein Interaction Networks

Mihaela Sardiu[1]; Joshua Gilmore[2]; Brad Groppe[3]; Arnob Dutta[4]; Laurence Florens[1]; Michael Washburn[1, 5]
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Extensive efforts in multiple organisms have been made to identify protein interactions and protein complexes using affinity purification and quantitative proteomics. The computational approaches used to analyze these datasets typically results in the assignment of a statistical value, to determine the confidence one has in one protein associating with another protein. However, an individual protein can interact with many other proteins and proteins can be part of large protein complexes. Methods are therefore needed to better define individual protein associations within any given dataset. Here we demonstrate the topological scoring (TopS) platform for the analysis of quantitative proteomic based protein interaction network datasets. By analyzing a transient human DNA repair protein interaction network and a yeast chromatin remodeling protein interaction network we find that high scores in individual runs capture direct protein interactions and modules within protein complexes. The TopS approach provides a rapid method for the efficient and informative computational analysis of protein interaction datasets that is complementary to existing protein interaction pipelines and provides new insights into protein interaction networks.

TOB pm 3:16
The functional human phospho-proteome

David Ochoa; Andrew Jarnuczak; Pedro Beltrao; Juan Antonio Vizcaino

EMBL-European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom

Over the years we have developed an infrastructure to enable robust data sharing of mass spectrometry proteomics data in the public domain, including the world-leading PRIDE database (www.ebi.ac.uk/pride), open data standards and the worldwide ProteomeXchange Consortium (http://www.proteomexchange.org/). Thanks, among other efforts, to the success of PRIDE and ProteomeXchange, the community is now widely embracing open data policies. This plethora of data is being increasingly reused by the community, e.g. in proteogenomics approaches, creation of spectral libraries and innovative *meta*-analysis studies. In this context, we have created a comprehensive and, as a key point, functionally relevant version of the human phospho-proteome, using PRIDE public datasets as the base.

First, we manually selected and curated 118 phospho-enriched PRIDE datasets coming from human cell lines and tissues. We re-analysed all the datasets together (>6,000 raw files) using MaxQuant in a highly stringent set-up. We obtained ~120,000 identified phospho-peptides across all samples and demonstrated the importance of aggregating so many datasets from different origins in order to achieve a greater analysis reliability and depth. Reported phosphosites were compared with those available in PhosphoSitePlus.

However, it is well-known that phosphorylation sites are not equally relevant from a functional point of view. We developed a machine-learning approach (different for Ser/Thr and Tyr phosphorylation) to create a functional ranking score for each phosphosite. The features used were selected based on different groups of properties: regulatory information, the amino acid sequence, the three-dimensional structure, the available MS evidence from the analysis, and taxonomic data. The scoring system was then applied to the human proteome as a whole, highlighting well-known biologically-relevant sites, but very interestingly, many new potential functional ones as well.

This study highlights just one of the many possible directions in reuse of public proteomics data, providing a scoring system with many potential future applications.

TOB pm 3:28

Functional 5′ UTR motif discovery with LESMoN: Local Enrichment of Sequence Motifs in biological Networks

Mathieu Lavallée-Adam[1, 2]; Philippe Cloutier[3]; Benoit Coulombe[3, 4]; Mathieu Blanchette[1]

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Introduction

Protein-protein interaction networks are rich representations of protein relationships. These networks have become increasingly complete, thanks to various high-throughput network mapping experimental approaches. This allowed the application of computational methods for the identification of protein complexes and the functional inference of uncharacterized proteins. However, protein-protein interaction networks have yet to be exploited for the discovery of functional sequence motifs.

Methods

Here, we propose a novel computational method that uses such networks to guide the search for functional sequence motifs. Specifically, we introduce Local Enrichment of Sequence Motifs in biological Networks (LESMoN), an enumerative motif discovery algorithm that identifies 5′ untranslated region (UTR) sequence motifs whose associated proteins form unexpectedly dense clusters in a given protein-protein interaction network. Should the proteins associated with a given 5' UTR motif be significantly clustered, this would suggest that the motif is linked directly or indirectly to the mechanism causing the clustering of the associated proteins.

Results

When applied to the human protein–protein interaction network from BioGRID, LESMoN identifies 1,873 significantly clustered 5′ UTR sequence motifs, including both previously known motifs and uncharacterized ones. The vast majority of these motifs are evolutionary conserved and the genes containing them are significantly enriched for various Gene Ontology terms, suggesting new associations between 5′ UTR motifs and a number of biological processes. We show that LESMoN outperforms a standard network analysis approach involving the Markov clustering algorithm and the MEME software package. We validate in vivo the role in protein expression regulation of three uncharacterized 5' UTR motifs discovered by LESMoN.

Conclusion

Finally, LESMoN demonstrates that protein-protein interaction networks can be used to discover novel functional sequence motifs. It illustrates another approach by which biological networks can provide a better understanding of the regulatory mechanisms of genes and proteins in the cell.

2:00 - 3:50 pm Tuesday
NEW TECHNOLOGICAL ADVANCEMENTS IN PROTEOMICS
Session Chairs: Kimberly Lee and Julian Saba
Oceana 6

TOC pm 2:00

Scratching the Surface: Ligand-Based Receptor Capture Methodologies to Explore the surfaceome of Living Cells and Its Interactors

Maria Pavlou; Laura A. Lopez-Garcia; Sandra Marder; Levent Demiray; Paul Helbling

Dualsystems Biotech AG, Schlieren, Switzerland

If the plasma membrane is considered the gateway through which cells communicate and interact with their environment, then proteins associated with the surface – also known as surfaceome – can be seen as the gatekeepers. The original concept, depicting the plasma membrane as a homogeneous fluid bilayer with freely diffusing proteins, has been evolved to another depicting a highly organised and crowded mosaic of interacting lipids and glycoproteins. This higher organisation modulates the biological processes occurring on the cell surface, exemplified by receptors being active only when they form dimers or higher order oligomers. Although the importance of protein interaction networks is now well-recognised, protein interactions taking place at the cell surface are generally under-represented.

To shed light particularly on interactions between extracellular ligands and the cell surface, the ligand-based receptor capture (LRC) methodologies have been developed. The key component of LRC methodologies is a tri-functional reagent with three moieties; one that binds ligands containing a primary amine, a second that binds glycosylated receptors on living cells and a function for purifying the target proteins for MS-based identification and quantitation. The LRC methodologies are unique in several ways. First, they can be applied in a multitude of ligands ranging from small molecules, to peptides, proteins and even whole viruses. Second, they identify the targets of these ligands on the surface of living cells under physiological conditions. Third, no genetic manipulation is required and therefore can be applied on a multitude of cell lines – including primary cells. Fourth, they are hypothesis-free meaning that no previous knowledge or speculation on the target is required. Finally, they allow the discovery one-to-many and many-to-many interactions in a single experiment.

The above mentioned advantages will be illustrated by presenting case studies where the LRC methodologies have been applied to deorphanize extracellular ligands.

TOC pm 2:20
Integrating cryo electron microscopy and LC-MS/MS for in-depth characterization of viral glycoproteins

Joost Snijder[1]; David Veesler[2]
[1]Snijder Bioscience, Arnhem, The Netherlands; [2]University of Washington, Seattle, WA

Viral glycoproteins play an important role in the viral replication cycle and constitute the primary antigens in natural infection and vaccination. Both N- and O-linked glycosylation of viral proteins play a pivotal role in determining cell attachment/entry, host tropism and antigenicity. Cryo electron microscopy can yield high-resolution structural information on viral glycoproteins, whereas mass spectrometry can yield valuable information on site-specific glycosylation patterns. Recent developments in automation, microscope stability, detector technology and data processing have improved cryoEM analyses to near-atomic resolutions, while new fragmentation techniques and software tools in MS have enabled vastly improved glycopeptide analysis. When used in combination, cryoEM and MS offer exciting new opportunities to obtain qualitative confirmation of glycosylation sites to aid in model-building, assign site-specific glycan compositions, determine glycan conformations, and offer mechanistic insight into virus-host interactions and antibody-mediated neutralization of infection. The utility of cryoEM with complementary MS will be illustrated for recombinant subunits and whole-virions, taking examples from important human viral pathogens such as HIV, Epstein Barr Virus, and SARS and MERS coronaviruses. Key details of the experimental protocol will be discussed alongside current limitations and outstanding challenges.

TOC pm 2:40
Evaluation of a novel LC system that embeds analytes in pre-formed gradients for rapid, ultra-robust proteomics

Nicolai Bache[1]; Philipp E. Geyer[2]; Dorte B. Bekker-Jensen[3]; Ole Hoerning[1]; Lasse Falkenby[1]; Peter V. Treit[2]; Sophia Doll[2]; Igor Paron[2]; Florian Meier[2]; Jesper V. Olsen[3]; Ole Vorm[1]; Matthias Mann[2]
[1]EVOSEP Biosystems, Odense, Denmark; [2]Max Planck Institute of Biochemistry, Martinsried, Germany; [3]University of Copenhagen, Copenhagen, Denmark

Background

Mass spectrometry-based proteomics and metabolomics are fast growing and powerful technologies, with the potential to revolutionize health care and precision medicine. However, available separation systems have so far limited throughput and robustness and thereby prevented omic technologies from being fully integrated and routinely applied in clinical settings. Here, we evaluate a conceptually novel liquid chromatography (LC) system that significantly increases robustness and sample throughput while maintaining the sensitivity of current nano-flow LC.

Methods

The new system, called Evosep One, uses four low-pressure pumps in parallel to elute samples from a disposable and single use trap column while also forming a chromatographic gradient. The sample and gradient are moved into a storage loop that subsequently is switched in-line with a single high-pressure pump and an analytical separation column for separation.

Results

We have characterized the performance of the new system regarding cross contaminations (<0.07%), retention time shifts and peak width (<3 sec) in over 1500 HeLa runs. The short overhead time of approximately 2 min allows us to efficiently measure 200, 100, 60 or 30 samples per day with corresponding gradient lengths of 5.5, 11.5, 21 and 45 minutes, respectively. The performance and applicability in various proteomics LC-MS strategies was evaluated for simple, medium and complex sample types. From fractionated HeLa cell lysates, deep proteomes covering more than 130,000 sequence unique peptides and around 10,000 proteins were rapidly acquired (18 h total instrument time). Using this data as a library for data independent acquisition, we demonstrate the quantitation of 5200 proteins in only 21 min.

Conclusion

We evaluated and benchmarked how to use the Evosep One in cutting edge LC-MS strategies to significantly increase overall performance and throughput. We also demonstrate how this can be applied to clinical research workflows that require uninterrupted analysis of thousands of crude biological samples.

TOC pm 2:52
Efficient top-down characterization of native proteoforms by Electron Capture dissociation at chromatographic speeds with Q-ToF and Orbitrap Instruments

Joseph S Beckman[1]; Valery G. Voinov[2]; Yury Vasil'ev[2]; Blaine Roberts[3]; Jared Shaw[4]
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Electron capture dissociation (ECD) is well known to produce uncluttered spectra of intact proteins with labile post translational modifications preserved. However, ECD has only been practical in FTICR-MS. We have developed a device that yields efficient ECD of proteins and can be reversibly retrofitted into both Q-ToFs or the Orbitrap Exactive family in one hour without diminishing performance. Once the ECD cell has been optimized to maximize fragmentation of small peptides like substance P, the same parameters worked well for fragmenting native and unfolded proteins. Nearly complete sequence

coverage was obtained with “native”-folded proteins such as the 5+and 6+charge states of ubiquitin. Sequence coverage of 80-95% was obtained for small proteins like ubiquitin and a-synuclein (14 kDa) during UPLC separations from peaks lasting 3-5 seconds. For carbonic anhydrase (29kDa), sequence coverage was 93% (half of the human proteome is smaller than 30kDa). Greater than 90% sequence coverage for a monoclonal antibody was obtained from analysis of 0.1 ug of an IdeS-digest (LC, Fd and Fc/2 subunits) using a thirty-minute nanoflow separation. The protein spectra consisted primarily of cand zions, though the ECD cell also produced a substantial number of dand wsidechain fragments. These side-chain fragments allow leucine/isoleucine or lysine/glutamine pairs to be distinguished, facilitating de novosequencing. Labile post-translational modifications were also retained. All eight phosphoserines were retained in alpha-casein (23 kDa) with 87% sequence coverage. The copper and zinc cofactors in superoxide dismutase (17 kDa) remained bound to their respective binding sites in ECD fragments. Deuterium labeling of ubiquitin enabled top-down hydrogen/deuterium exchange with residue-specific resolution at rates consistent with NMR. The simpler fragmentation patterns made possible with the ECD device allows existing mass spectrometers to characterize mid-sized proteins even using fast front-end separations.

TOC pm 3:04
Mass Spectrometry Imaging as Tool in Cancer Diagnostics and Cancer Drug Testing Platform

Peter Hoffmann[1]; Parul Mittal[2]; Mitchell Acland[2]; Georgia Arentz[1]; Gurjeet Kaur[3]; Martin K. Oehler[4]
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Mass Spectrometry Imaging (MSI) is typically used to determine the distribution of proteins in fresh frozen tissue. Formalin-fixed paraffin embedded (FFPE) tissue can be analyzed after antigen retrieval and enzymatic digest on tissue for imaging tryptic peptides and glycans. Imaging tryptic peptides and glycans in FFPE tissue has multiple advantages over imaging of intact proteins in fresh frozen tissue. These includes the identification of peptides and glycans by matching accurate m/z from the MSI experiment with in situ MS/MS on tissue and high quality LC-MS/MS data obtained through in solution digestion of relevant laser dissected tissue. A novel method for investigating tissue-specific N-linked glycans was recently developed by our group on FFPE tissue.

Here we present the latest developments within our group, including up-to-date methods for analysis of FFPE tissue (e.g. tryptic peptide and PNGase F released glycans) and the use of tissue micro arrays. We present that MSI can spatially profile glycoforms in tissue-specific regions, while through LC-ESI-MS/MS the corresponding glycol compositions are structurally characterized. These methods are applied to endometrial and ovarian cancer FFPE tissues to potentially make diagnostic decisions in order to improve treatment of cancer patients.

We also show the testing of cancer drugs using 3 dimensional ovarian cancer spheroids and show distribution of the drugs and their efficacy by using embedding, sectioning and MSI analysis.

TOC pm 3:16
Digging deeper into the plasma proteome, a novel nanoflow LCMS approach using micro pillar array columns (µPAC™)

Jeff Op De Beeck[1]; Paul Jacobs[1]; Natalie Van Landuyt[1]; Wim De Malsche[2]; Gert Desmet[2]; Jarne Pauwels[3]; An Staes[3]; Francis Impens[3]; Kris Gevaert[3]
[1]PharmaFluidics, Zwijnaarde, Belgium; [2]Vrije Universiteit Brussel, Brussel, Belgium; [3]VIB Proteomics Expertise Center, UGent, Gent, Belgium

In bottom-up proteomics, 50 to 100 µm ID capillaries packed with sub 2 µm C18 functionalized particles are routinely used in combination with a high resolution mass spectrometer to analyze protein samples from tissues, body fluids or cell lysates. Typically, micrograms of samples are separated in 30 to 240 min LC gradients on columns with lengths up to 75 cm.

PharmaFluidics’ µPAC™ technology (micro Pillar Array Column) is a unique and novel approach to a chromatographic support structure and builds upon micromachining chromatographic separation beds into silicon, with exceptional properties that result in excellent chromatographic performance with high-resolution and high sensitivity. The freestanding nature of the pillars also leads to much lower back pressure allowing the use of very long columns (up to 200 cm) at only a fraction of the pressure needed to operate packed bed alternatives.

To validate the µPAC™ column performance for bottom-up proteomics, tryptic digest samples obtained from HEKT cell lysates were prepared over a wide concentration range (100 ng/µl to 3 µg/µl) and separated using a 200 cm µPAC™ column as well as a 40 cm long pulled tip packed bed capillary LC column. Using a Thermo Orbitrap Elite mass spectrometer for detection, on average 25% more proteins could be identified when working with the µPAC™ column, and this over the entire concentration range. Peak width data extracted from the .raw files clearly pointed out that the rate at which peak width increases according to gradient is much lower on the µPAC column as compared to the packed bed column. For a 10h gradient, average peak widths below 20s were observed, resulting in consistent identification of over 5000 proteins. The combination of these long gradients and this new type of column resulted in very deep plasma proteome coverage.

TOC pm 3:28
Comprehensive single-shot proteomics experiments with LC-FAIMS-MS/MS

Alexander S. Hebert[1]; Satendra Prasad[2]; Michael W. Belford[2]; Derek J. Bailey[2]; Susan E. Abbatiello[2]; Romain Huguet[2]; Graeme C. McAlister[2]; Eloy R. Wouters[2]; Jean-Jacques Dunyach[2]; Dain Brademan[1]; Michael S Westphall[1]; Joshua J. Coon[1, 3]
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Limitations in separation peak capacity and dynamic range presently restrict identifications in single shot proteomics experiments to <7,000 protein groups from human cells. Liquid chromatography pre-fractionation can circumvent these issues; however, while effective, this approach is laborious, requires considerable sample amount, and can be cumbersome. Here we demonstrate that High Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) coupled with a Thermo Orbitrap

Fusion Lumos mass spectrometer can increase protein identifications from a single-shot experiment to >8,000, from just over 100,000 peptide identifications. We describe a next generation cylindrical electrode, interfaced between the nanoESI-emitter and MS inlet, with improved ion sampling at the ESI-FAIMS interface, increased electric field strength, and helium free ion transport gas. This electrode enables fast intra-analysis CV switching (<20ms), producing multiple unique gas phase fractions, which are analyzed simultaneously over the course of an MS/MS analysis. We have comprehensively demonstrated how this device performs for bottom-up proteomics experiments as well as characterizing the effects of peptide m/z, and z, mass loading, and analysis time. We will offer recommendations for the number of CVs and which CVs to use for different types of experiments. The performance of FAIMS enabled mass spectrometry experiments were benchmarked against single shot and LC fractions analyzed without FAIMS. In single shot 4 hour LFQ experiments of a human cell line, we quantified 7,818 proteins with FAIMS using intra-analysis CV switching compared to 6,809 without FAIMS. Single shot FAIMS results also compare favorably with LC fractionation experiments. A 6 hour single shot FAIMS experiment generates 8,007 protein identifications, while 4 fractions analyzed for 1.5 hours each, produces 7,776 protein identifications. We conclude that the redesigned FAIMS hardware can accomplish gas-phase separations that enable the collection of single shot proteomic data with comparable depth to conventional two-dimensional LC approaches, with substantially less difficulty.

2:00 - 3:50 pm Tuesday
HPP: HARNESSING THE IMMUNE SYSTEM TO FIGHT DISEASE
Session Chairs: Arie Admon and Ileana Cristea
Oceana 3-5

TOD pm 2:00
A tissue-based draft map of the murine MHC class I Immunopeptidome

Heiko Schuster[2]; Wenguang Shao[1]; Tobias Weiss[3]; Patrick Pedrioli[1]; Patrick Roth[3]; Michael Weller[3]; David Campbell[4]; Eric Deutsch[4]; Robert Moritz[4]; Oliver Planz[2]; Hans-Georg Rammensee[2]; Ruedi Aebersold[1]; Etienne Caron[1]

[1]ETH Zurich, Zurich, Switzerland; [2]University of Tübingen, Tübingen, Germany; [3]University Hospital Zürich, Zürich, Switzerland; [4]Institute for Systems Biology, Seattle, USA

The large array of peptides presented to CD8+ T cells by major histocompatibility complex (MHC) class I molecules is referred to as the MHC class I immunopeptidome. Although the MHC class I immunopeptidome is ubiquitous in mammals and represents a critical component of the immune system, very little is known, in any species, about its composition across most tissues and organs in vivo. We applied mass spectrometry (MS) technologies to draft the first tissue-based atlas of the murine MHC class I immunopeptidome in health. Peptides were extracted from 19 normal tissues from C57BL/6 mice and prepared for MS injections, resulting in a total number of 28,448 high-confidence $H2D^b/K^b$-associated peptides identified and annotated in the atlas. This atlas provides initial qualitative data to explore the tissue-specificity of the immunopeptidome and serves as a guide to identify potential tumor-associated antigens from various cancer models. Our data were integrated into SWATH Atlas and the SysteMHC Atlas project, which support the comprehensive mapping of the immunopeptidome and the consistent measurement of large immunopeptidomic sample cohorts. We anticipate that this atlas will be further expanded in the future and will find wide applications in basic and translational immunology.

TOD pm 2:20
Targeted Proteomics-Driven Computational Modeling of Macrophage Microbial Sensing Pathways

Nathan Manes; Jessica Mann; Pauline Kaplan; Martin Meier-Schellersheim; Iain Fraser; Ronald Germain; Aleksandra Nita-Lazar

National Institutes of Health, BETHESDA, <Not Specified>

Toll-like receptor (TLR) signaling in macrophages is essential for generating effective innate immune responses. Quantitative differences dependent on the dose and timing of the stimulus critically affect cell function and have been found to involve proteins that are not components of widely shared transduction pathways. Mathematical modeling is an important approach to better understand how these signaling networks function in time and space.

We have successfully modeled the S1P signaling pathway in macrophages using selected reaction monitoring (SRM) to measure the absolute abundance of the pathway proteins and were able to use the resulting values as parameters in a computational pathway model. RNA-seq was performed to identify expressed transcripts. Shotgun mass spectrometry was used to identify proteotypic peptides. Now, to model the TLR signaling networks SRM assays for the canonical TLR signaling pathway and related proteins and phosphoproteins have been developed. SRM with heavy-labeled internal peptide standards was used to quantify protein and phosphorylated protein molecule numbers per cell in both untreated and LPS-stimulated macrophages. These absolute protein abundance values were entered into a model of the TLR pathway that has been developed using Simmune, the rule-based modeling tool with a visual interface.

This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH.

TOD pm 2:40
Comparison of the HLA peptidome of primary and PDX human tumors enables identification of neoepitopes of potential for personalized immunotherapy

Nataly Nataly Rijensky[1]; Netta Shraga[2]; Eilon Barnea[1]; Eitan Rubin[3]; Yitzhak Haviv[2]; Arie Admon[1]

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HLA peptidomics provides useful information about the presentation of neoepitope and tumor antigens by tumor cells, potentially useful as vaccine candidates for immunotherapy. Here, we employed patient-derived xenografts (PDX), of primary or metastatic tumor tissues, as a source for HLA peptidome analyses. The HLA molecules were purified by immunoaffinity, followed by nano-capillary-LC-MS/MS analysis of the extracted HLA peptides. The HLA peptides were identified using databanks of all human protein sequences, supplemented with sequences of the mutated proteins, as elucidated from exome data of the tumors. Thus, a number of neoepitope HLA peptides could be identified, including neoepitopes that were not detected in the original tumors. The HLA peptidomes of the PDX tumors were very similar to those of the original patients' tumors. Furthermore, identical HLA peptidomes were obtained from multiple PDX tumors grown in

separate mice and originating from the same human tumors, even after three generations of re-grafting, and these were very similar to same patient's original tumors. The majority of the identified peptides were likely true ligands of the patient's HLA since they possess their typical sequence motifs. Thus, PDX tumors are advantageous also for HLA peptidome analysis since they maintain closer similarities to the original tumors and their microenvironments, significantly better than in-vitro cultured cells. In addition, the expansion of tumors in PDX overcomes the limitations imposed by the available amounts of original tumors tissues for analysis.

TOD pm 2:52
Microscale Cell Surface Capture Technology for Discovery of Cell Surface *N*-Glycoproteins and its Application to Human Primary Cardiomyocytes

Rachel A Jones Lipinski; Matthew Waas; Ranjuna Weerasekera; Theodore R. Keppel; Christopher Ashwood; Rebekah L. Gundry
Medical College of Wisconsin, Milwaukee, WI

Cell surface proteins, glycoproteins, and glycans play critical roles in maintaining cellular structure and adhesion, and act as gatekeepers controlling how cells send and receive exogenous signals. Therefore, the collection of these molecules (*i.e.* surfaceome) is a rich source of accessible targets for developing new tools and strategies to identify, study, and manipulate specific cell types of interest, from immunophenotyping to immunotherapy. Classic Cell Surface Capture Technology (CSC) is a chemoproteomic approach that enables selective enrichment and identification of extracellular domains of cell surface *N*-glycoproteins. Since 2007, CSC has been applied to identify >3000 cell surface *N*-glycoproteins from >125 human and rodent cell types, providing unique surfaceome views in a cell-type specific manner to reveal new immunophenotyping markers and proteins involved in development and disease. However, while the classic CSC approach is highly specific for cell surface *N*-glycoproteins, the method requires >80 million cells on average to produce high quality results, precluding its application to rare cell types. Moreover, classic CSC is labor intensive and quantitation is challenging. In this study, we adapted an automated liquid handling workstation for CSC sample processing, which minimizes human intervention and decreases processing time from 5 days to 50 hours. By incorporating magnetic beads for glycopeptide enrichment and clean-up, all previous filter-based separations are avoided, reducing sample losses and benefitting reproducibility. This new µCSC method successfully identifies >500 cell surface proteins from just 5-10 million cells with >90% specificity. We have applied µCSC to directly map the surfaceome of primary isolated human cardiomyocytes, the first study of its kind. Results demonstrate the power of this approach for identifying proteins involved in cardiac disease and discovering new markers for stem cell derived cardiomyocyte characterization. Current efforts focus on incorporating data independent acquisition for robust quantitation and mapping surfaceome glycan structures to complement the proteomic data.

TOD pm 3:04
Gas phase ion fractionation provides unparalleled sensitivity for proteomic and immunopeptidomic analyses

Sibylle Pfammatter[1]; Eric Bonneil[1]; Joel Lanoix[1]; Marie-Pierre Hardy[1]; Satendra Prasad[2]; Michael Belford[2]; Jean-Jacques Dunyach[2]; Claude Perreault[1]; Pierre Thibault[1]
[1]Universite de Montreal, Montreal, Canada; [2]Thermo Fisher Scientific, San Jose, CA

The depth of proteomic analyses is often limited by the overwhelming proportion of confounding background ions that not only compromise proteome coverage, but also undermine the quantification of low abundance proteins. Here, we present the application of gas phase sequential ion fractionation (SIFT) using a new differential ion mobility interface to expand the comprehensiveness of proteomic analyses and improve the accuracy and dynamic range of quantitative proteomics. In large-scale proteomic analyses, the use of SIFT enabled a 30% increase in peptide identification and extended the limit of detection by almost an order of magnitude compared to traditional LC-MS/MS experiments. The reduction in chimeric MS/MS spectra using SIFT also improved the precision and the number of quantifiable peptides when using isobaric labeling with tandem mass tag (TMT) 10-plex reagent. We compared quantitative proteomic measurements for LC-MS/MS analyses performed using synchronous precursor selection (SPS) and SIFT to profile the temporal changes in protein abundance of HEK293 cells following heat shock for periods up to 9h. SIFT provided 2.5-fold increase in the number of quantifiable peptides compared to SPS experiments (30848 peptides from 2646 proteins for SIFT vs. 12400 peptides from 1229 proteins with SPS). The improved sensitivity of SIFT enabled the identification of more than 500 dynamic protein profiles to better define the sequential events and cellular pathways involved in the regulation of the toxic effects of protein aggregates, alteration in protein synthesis, increased catabolic activities and targeted translation of HSP gene products. We also demonstrate the application of SIFT and isobaric labeling to the identification of low abundance peptides presented by major histocompatibility complex class I (MHC I) and the targeted identification of minor histocompatibility antigens present in in B-lymphoblastoid cell lines and patient B-ALL cells, and further discuss the benefits of SIFT in the context of cancer immunotherapy.

TOD pm 3:16
Longitudinal Omics and Biomolecular Network Analysis Dissect Host-specific Immune Dynamics in Multihost Fungal disease

Pooja Aggarwal; Kanika Narula; Sudip Ghosh; Rajul Tayal; Niranjan Chakraborty; Subhra Chakraborty
National Institute of Plant Genome Research, New Delhi, India

Morbidity and mortality associated with fungal infections and emergence of resistant fungal strains necessitate study of fungal pathogenesis and host innate immunity. Innate immune response is governed by conserved cellular events in phylogenetically diverse hosts. However, the underlying molecular mechanisms by which this process is regulated against multi-host pathogen remains unknown. *Fusarium oxysporum,* a medically and agronomically important multi-host pathogen is known to be associated with neuronal stress in humans and vascular wilt in plants, while *Fusarium*-mediated killing of worm has recently been described. To elucidate regulatory framework of *Fusarium*-associated disease and immune response, we analyzed the gene and protein expression during infection, integrated temporal expressions and network analysis with genetic inactivation data in worm and plant. Longitudinal spatiotemporal multiomics analyses and the

derived biomolecular networks revealed organ and organelle function in diverse kingdoms during fungal invasion. Results indicate that a ubiquitous response occurs during *Fusarium* infection mediated by highly conserved regulatory components and pathways. Furthermore, our data identified disease responsive genes conserved and unique among animal and plant. Finally, this study for the first time provides novel insight on cross-species immune signaling that impinge upon the surveillance mechanism of innate immunity in multi-host pathogen response and may facilitate discovery of cellular therapeutic targets for *Fusarium*-associated disease.

TOD pm 3:28
Computational immunomics reveals differential human immune recognition of the *Candida albicans* cell surface proteome during dimorphic transition in invasive candidiasis

Aida Pitarch; César Nombela; Concha Gil
Complutense University, Madrid, Spain

Dimorphic transition (the ability of *Candida albicans* to reversibly switch between yeast and hyphal growth under specific host environmental stimuli) is important for fungal virulence. Both morphological forms exhibit differences in their cell surfaces that affect the host-pathogen interaction. Although the *C. albicans* cell surface-associated proteomes from yeasts and hyphae have been characterized, their antibody-mediated immune recognition in invasive candidiasis (IC) has barely been explored. We examined the antibody responses to the *C. albicans* yeast and hyphal surface-associated proteomes in IC patients using computational immunomics. A total of 27 cell surface-associated proteins (CSPs) were differentially immunorecognized in yeasts and hyphae during IC. Capture ELISAs on selected CSPs substantiated serological proteome analysis data. Unsupervised clustering analysis uncovered two IgG antibody-reactivity signatures that separated IC sera hybridized with yeast and hyphal CSPs into two distinct groups. Coordinated IgG antibody responses to two repertoires of CSPs as a function of the growth form were observed in IC. Pairwise correlation and gene ontology term enrichment analyses uncovered discrete subgroups of functionally related CSPs that displayed opposing IgG antibody-mediated immune recognition patterns in yeasts and hyphae during IC. Changes in the antigenicity of the identified yeast and hyphal CSPs in IC resulted in significant topological reorganization of their immune co-recognition networks. By testing the effect of CSP abundance normalization on reactivity changes, we found that antigenicity of CSPs was modulated in IC by changes in their relative abundance and potential post-translational modifications in their epitopes upon dimorphic transition. Further follow-up functional assays revealed that specific post-translational modifications influenced the antigenicity of a subset of CSPs upon dimorphic transition in IC. This study sheds new light on human antibody-mediated immune recognition of the *C. albicans* surface-associated proteomes from yeasts and hyphae in IC. This work was supported by BIO-2015-65147-R, PRB3 (IPT17/0019-ISCIII-SGEFI/ERDF) and RD16/0016/0011.

2:00 - 3:50 pm Tuesday
MICROBIOME AND PATHOGEN INFECTIONS
Session Chair: Frank Schmidt and Concha Gil
Oceana 1-2

TOE pm 2:00
Ionbot: a novel, fully data-driven search engine for open modification and mutation searches with applications in quantitative (meta-)proteomics

Sven Degroeve; Lennart Martens
VIB-UGent Center for Medical Biotechnology, Ghent, Belgium

Modern shotgun proteomics is entirely dependent on accurate search engine tools to match observed spectra to the peptide sequences that generated them. Here we focus on the widely applied search engine approach that is based on a target database that contains all peptides expected to be in the sample under consideration. Such an approach consists of a filter function to select candidate peptides from the target database and a peptide-spectrum-match (PSM) scoring function that ranks these candidate peptides. The filter function is important as the target database can become extremely large (allowing thousands of potential protein modifications including mutations, glycosylation, and more). Similarly, the PSM scoring function needs to be accurate enough to distinguish the true peptide from all other (similar) peptides. Current search engines simply cannot handle these kinds of extremely large target databases, struggling greatly to deliver either sensitivity or specificity (Colaert et al., Journal of Proteome Research, 2011).

Here, Ionbot, a completely new and highly powerful search engine, is presented as a solution for precisely this problem. Ionbot applies data-driven methods to learn both the filter and the PSM scoring function form large amounts of spectral data. Ionbot is shown to be extremely reliable, delivering exquisite false discovery rate (FDR) control, while maintaining a very high degree of sensitivity. Ionbot will be demonstrated to perform very well in open modification and mutation searches. In addition, Ionbot can be coupled to the moFF software for label-free quantification (Argentini et al., Nature Methods, 2016), which has recently been upgraded to cope well with the specific challenges of metaproteomics data quantification.

TOE pm 2:20
Pathogen-Specific Monoclonal Antibodies Maintain Short-Chain Fatty Acids (SCFA) And The Intestinal Microbiome

Sonja Hess
Medimmune, ,

The microbiome plays a key role in human health and its perturbation is increasingly recognized as contributing to many human diseases. Although antibiotic therapy for bacterial infections has revolutionized medicine, it is now clear that broad-spectrum antibiotics alter the composition and function of the host microbiome. This adverse effect on the microbiome coupled with the emergence of multi-drug resistant pathogens has spurred development of pathogen-specific strategies, such as monoclonal antibodies (mAbs), to combat bacterial infection. We hypothesized that pathogen-specific mAbs against prevalent antibiotic resistant pathogens would not significantly disrupt the intestinal microbiota as compared to conventional antibiotics. We treated 7-week-old, female, C57BL/6 mice with either a single systemic mAb dose or (saline as a control) or human equivalent doses of the antibiotics vancomycin, linezolid, ciprofloxacin, meropenem, or levofloxacin for 5 consecutive days. Independent experiments were conducted for each indication. Short-chain fatty acid (SCFA) metabolic and metagenomic studies were performed using fecal pellets collected from individual animals prior to treatment and for up to 14 days.

SCFA and the taxonomic abundance and diversity of the bacterial genera in the fecal pellets from mice treated with pathogen-specific mAbs was minimally affected, and was similar to that in the control groups. In contrast, as expected, SCFA were drastically reduced in antibiotic-treated mice (12 hr, and day 7). This correlated with dramatic changes in the relative abundance, as well as alpha- and beta-diversity in the feces of all antibiotic treated groups. While the SCFA showed some restoration at day 14, changes in the microbiome persisted until the end of the study. Taken together, these results indicate that pathogen-specific mAbs do not alter the fecal microbiome like broad-spectrum antibiotic therapy does, and may therefore represent an approach to antibacterial therapy that leaves a healthy microbiome intact.

TOE pm 2:40

Multi-Omics Comparative Analysis Reveals Host Signaling Pathways Affected by the Gut Microbiota

Nathan Manes[1]; Natalia Shulzhenko[2]; Arthur Nuccio[1]; Sara Azeem[1]; Andrey Morgun[2]; Aleksandra Nita-Lazar[1]

[1]National Institutes of Health, Bethesda, MD; [2]Oregon State University, Corvallis, OR

Mammals are hosts to communities of trillions of microbes from thousands of species, whose effects on health and development have only recently begun to be appreciated. In this investigation, proteomics and transcriptomics were integrated to quantitatively compare the terminal ilia from conventional and germfree mice. The microbiota primarily caused upregulation of immunological pathways and downregulation of metabolic pathways within the conventional mice. For example, the microbiota caused the interferon signaling and antigen presentation pathways to be concordantly upregulated in the transcriptome and proteome, and the serotonin degradation and glutathione-mediated detoxification pathways to be concordantly downregulated. Female and male mice responded similarly to the microbiota, but C57BL/10A mice responded much more strongly than BALB/c mice at both the transcriptome and proteome levels. Of the pathways that were affected at both the transcriptome and proteome level, most were affected concordantly. However, we discovered significant transcriptome-proteome discordance caused by the microbiota, demonstrating the importance of using an integrative approach to study host-microbiota interaction, and that transcript-level studies are insufficient for predicting protein level adaptation to the microbiota. The discordant pathways were not principally involved in the immune system but instead were related to metabolism, oxidative phosphorylation, and protein translation, transport, and turnover. To broaden the discovery of affected host pathways, a meta-analysis using OMiCC (https://omicc.niaid.nih.gov/) was performed using intestinal transcriptomics data from previously published studies of germfree versus conventional mice with globally diverse microbiota populations. Similar transcriptomic responses to the microbiota were discovered, and many additional affected host pathways were revealed. This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH.

TOE pm 2:52

Quantifying Functional Microbiomes using MetaQuant: An integrated, quantitative metaproteomics tool reveals connections between taxa, function and protein expression in microbiomes.

Pratik Jagtap[1]; Caleb Easterly[1]; Nadia Szeinbaum[2]; Andrea Argentini[3]; Subina Mehta[1]; Ray Sajulga[1]; Bart Mesuere[4]; James Johnson[1]; Carolin Kolmeder[5]; Praveen Kumar[1]; Jennifer Glass[2]; Joel Rudney[1]; Lennart Martens[3]; Brook Nunn[6]; Timothy Griffin[1]

[1]University of Minnesota, Minneapolis, Minnesota; [2]Georgia Tech, Atlanta, GA; [3]VIB-UGent Center for Medical Biotechnology, Ghent, Belgium; [4]University of Ghent, Ghent, Belgium; [5]University of Helsinki, Helsinki, Finland; [6]University of Washington, Seattle, WA

Metaproteomics identifies proteins that are actively expressed by the microbial community and characterizes the functional responses of a given microbiome to its surrounding. However, metaproteomics data based on spectral counts alone, offers limited quantitative information. Moreover, current software tools lack the means to elucidate the relationship between taxonomic groups and function. Here, we demonstrate the use of MetaQuant, a tool that enables quantitative analysis of microbiomes and the interplay between taxa and function.

For this study, we used : (1) clinical datasets from microcosms inoculated with dental plaque samples from cavity-prone children (DOI:10.1186/s40168-015-0136-z) and (2) environmental microbial datasets from sediments of an anoxic lake incubated in bioreactors.

Bioinformatic analyses of mass spectral data were performed within the Galaxy framework. Microbial peptides were identified by searching against their respective metaproteomics databases. Subsequently, the datasets were subjected to MS1 quantitation (moFF), taxonomic assignments (Unipept) and functional analysis (EggNOG mapper). These outputs from each analysis were integrated into a peptide tabular file.

The MetaQuant software tool processes the tabular file by i) normalizing the peptide quantitation values; ii) assigning them to taxonomical and functional groups and iii) generating lists of differentially expressed values for proteins, taxa and functional groups. MetaQuant also generates outputs that can be visualized by interactive visualization tools that provide deeper biological insight into microbiome studies.

Our initial application of this quantitative metaproteomics workflow reveals connections between specific taxa and their contributions to the functional profile of the system, measuring changes in abundance of peptides derived from specific classes of microbes. It also quantifies and aids in the characterization of ‘unassigned’ peptides by clustering them using expression values of known functions. We anticipate that this study will lay the groundwork for an advanced multi-omics integration that will enable a quantitative, higher resolution study of the expressed functional microbiome.

TOE pm 3:04

Strength in numbers: Impact of oligomerization of antiviral proteins in immune response

Timothy Howard; Krystal Lum; Catherina Pan; Ileana Cristea

Princeton University, Princeton, NJ

Innate immune responses to invading pathogens rely on the ability of specific host proteins to recognize pathogen molecules and induce signals. The interferon-inducible protein IFI16 was shown to recognize pathogenic DNA after a variety of viral infections, including herpesviruses and HIV, as well as bacterial infections. Given the expression of IFI16 in numerous

tissues relevant to host-pathogen interactions, including skin, nasal and oral mucosa, this protein is thought to play a critical role in maintaining balanced immune responses for a healthy system. IFI16 has two functions in host defense against dsDNA viruses herpes simplex virus type I (HSV-1) and human cytomegalovirus (HCMV). It induces antiviral cytokine expression and suppresses viral gene expression. At the core of these antiviral responses are its functional domains: a PYRIN domain (PYD) that mediates its oligomerization and HIN-200 domains that bind pathogenic DNA. Our lab has previously demonstrated that PYD is required for IFI16 localization to incoming viral DNA at the nuclear periphery. However, the properties and downstream consequences of PYD-dependent IFI16 oligomerization during viral infection remained unknown.

Here, we used an integrative approach of molecular biology, virology, and quantitative proteomics to explore the role of IFI16 oligomerization during herpesvirus infection. We first identify several solvent-exposed charged amino acid residues that regulate IFI16 oligomerization. Through mutagenesis assays combined with immunoaffinity purifications and parallel reaction monitoring (PRM) MS/MS we establish the necessity of IFI16 oligomerization for immune signaling. Oligomerization also mediates interactions with other critical antiviral proteins. We also find that IFI16 oligomerization-incompetent cells permit more productive infections and result in increased virus gene and protein expression. Altogether, we provide mechanistic insights into the contributions of PYD oligomerization in viral DNA sensing for innate immune response. Furthermore, we provide experimental evidence for the conservation of pyrin domain antiviral functions within several other human oligomerizing proteins.

TOE pm 3:16
Exploring the anti-staphylococcal antibody response using a bead-based array approach

Tanja Meyer; Stephan Michalik; Barbara Bröker; Uwe Völker
University Medicine Greifswald, Greifswald, Germany

About 30% of the human population is asymptomatically colonized with *Staphylococcus aureus*. However, this Gram-positive bacterium can also act as a pathogen causing a wide range of severe diseases like sepsis or toxic shock syndrome. Both carriers and non-carriers display a broad range of anti-staphylococcal antibodies with pronounced inter-individual variations. Studies of bacteremia patients indicate that these antibodies might have protective potential decreasing the disease severity but are not able to fully prevent infection. With increasing rates of antibiotic-resistant strains the treatment of *S. aureus*-related infections becomes more difficult and expensive making a preventive strategy by vaccination very desirable. Unfortunately, immunization trials using specific surface antigens of *S. aureus* have failed so far. Therefore, a multi-antigen mixture might increase the protective potential. To gain a better understanding of the anti-staphylococcal antibody repertoire in the human population and to estimate the immunogenicity of potential candidates we included 148 extracellular and surface-associated staphylococcal proteins in a pilot study targeting blood samples from healthy subjects as well as from patients with known *S. aureus*-associated diseases (e. g. bacteremia, Cystic fibrosis, Atopic dermatitis, Asthma, Epidermolysis bullosa, dialysis). The antigens were coupled to xMAP®-beads (Luminex®) and incubated as a multiplexing bead mix with dilutions of the serum samples. Bound antigen-specific serum antibodies were quantified using detection antibodies against human total IgG, IgA, and IgG4. The complex dataset was quantitatively evaluated with a newly developed analysis pipeline in R. From the results of the pilot study we selected a smaller panel of 77 candidates for a population based approach including about 1,000 plasma samples of subjects from the Study of Health in Pomerania. The resulting comprehensive dataset allows correlation analyses on a large scale showing age-dependent and sex-specific overall trends but also sheds light on antibody profiles for specific antigens.

TOE pm 3:28
Bacterial proteotyping using Machine Learning defined peptide signatures and validation on Q-Exactive HF-X coupled to Capillary flow liquid chromatography

Florence Roux-Dalvai[1]; Clarisse Gotti-Barban[1]; Mickaël Leclercq[1]; Frédéric Fournier[1]; Marie-Claude Hélie[2]; Judith Marcoux[1]; Isabelle Kelly[1]; Tabiwang N. Arrey[4]; Julie Bestman-Smith[3]; Claire Dauly[5]; Maurice Boissinot[2]; Michel G. Bergeron[2]; Arnaud Droit[1]
[1]Proteomics Platform - CHU Quebec Laval University, Quebec, Canada; [2]Infectiology CHU Quebec Laval University, Quebec, Canada; [3]Enfant Jesus Hospital CHU Quebec ULaval, Quebec, Canada; [4]Thermo Fisher Scientific, Bremen, Germany; [5]Thermo Fisher Scientific, Paris, France

Bacterial typing (i.e identification of bacterial species) is essential to many applications in biology, health, and environment.

Low cost and fast MALDI-TOF MS technology has become an approach of choice for bacterial typing. But it has several drawbacks: it requires a long step of bacterial culture prior to analysis (24h), it has a low specificity and is not quantitative.

We have developed a new 3-steps strategy for the identification of bacterial species in biological samples using LC-MSMS peptide signature. First, deep proteome coverage of bacteria of interest is performed in Data Independent Acquisition (DIA) mode. In a second step, Machine Learning algorithms are applied to define peptides the most susceptible to distinguish each bacterial species from the other. Finally, validation of this peptide signature is done in targeted proteomics. This method, which allows the bacterial proteotyping in less than 4h, has been applied to 15 species representing more than 90% of all Urinary Tract Infections (UTIs).

More than 23000 peptides in 200 samples were quantified by DIA and analyzed by Machine Learning algorithms to identify a signature of 85 peptides and build prediction models able to classify the 15 bacteria. This signature was validated for its use in clinical laboratories using Parallel Reaction Monitoring on a capillary flow chromatography coupled to a Thermo Scientific™ Q-Exactive HF-X instrument. Linearity and reproducibility of the method were demonstrated as well as its accuracy on donor specimens: our method was able to efficiently predict, in less than 4h, the bacteria infecting the sample in 95% of cases above the 1x105 CFU/mL threshold commonly used by clinical laboratories.

This work demonstrates the efficiency of our method for bacterial typing of UTIs and could be extended in the future to other biological specimens and to bacteria having specific virulences or resistances.

For Research Use Only

10:30 am - 12:20 pm Wednesday
ACTIVITY / CHEMICAL PROTEOMICS
Session Chairs: Marcus Bantscheff and Christopher Overall
Oceana 8-10

WOA am 10:30
Expanding the cancer cell DUBome using advanced chemoproteomics

Adan Pinto-Fernandez[1]; Abigail Schofield[1]; Eidarus Salah[1]; Sebastian Mathea[2]; Simon Davis[1]; Philip Charles[1]; Roman Fischer[1]; Benedikt M. Kessler[1]
[1]University of Oxford, Oxford, UK; [2]Goethe-Universität, Frankfurt, Germany

Enzymes that bind and process ubiquitin, a small 76 amino acid protein, have been recognized as pharmacological targets in oncology, immunological disorders and neurodegeneration. Selection of molecular targets relevant for human diseases is a crucial step in drug development. Mass spectrometry technology has now reached the capacity to cover the proteome in the ten thousand range, and ubiquitylation can be profiled in the thousands, covering enough depth to interrogate DUB and E3 ligase substrates. We have recently characterized the breast cancer cell (MCF-7) deep proteome by detecting ~14,000 proteins including more than 90 different posttranslational modifications. Within this data set, we can detect endogenous expression of >60 deubiquitylating enzymes (DUBs). We have combined advanced mass spectrometry technology with the use of classical and more potent ubiquitin active-site probes with propargyl based electrophiles to profile >40 DUBs in MCF-7 crude extract material. Experiments combined with cysteine alkylating agents and ubiquitin probe labelling revealed the proportion of active cellular DUBs directly engaged with ubiquitin probes by label-free quantitative (LFQ) mass spectrometry. In addition, we noted targeting of active cysteine residues encoded by other ubiquitin binding proteins, most likely reflecting the intrinsic capacity to form transient ubiquitin-thioester intermediates. Our extended chemoproteomics workflow increases depth of covering the active DUBome, and provides the framework for more comprehensive cell-based small molecule DUB selectivity profiling and the discovery of novel proteins capable of forming covalent ubiquitin intermediates

WOA am 10:50
Deciphering the cell-specific redoxome by chemical proteomics

Jing Yang
National Center for Protein Sciences, Beijing, China

Reactive oxygen species (ROS), such as hydrogen peroxide (H2O2) generated in a wide range of physiological and pathological processes can promote cell damage, but also can activate cell regulatory and signaling pathways as a signaling molecule. Site-specific modification of cysteinyl thiols on oxidation-sensitive proteins represents a unique molecular mechanism for transducing H2O2 signals into biological responses.

Recent advances in chemical proteomics have greatly expanded the repertoire of redox-sensitive cysteines, the so-called 'redoxome'. To understand how the redoxome as a whole control upon oxidative stress, it is essential to globally and site-specifically profile cysteine reactivity in complex proteomes. In this talk, I will focus on a chemical proteomic platform recently developed in our lab (http://www.yangresearchlab.com/), termed as QTRP (Quantitative Thiol Reactivity Profiling). Using this QTRP approach, we first site-specifically map and quantify the reactivity of thousands of cysteines toward H2O2 in human cells. Overall, we identified >900 H2O2-sensitive cysteines, which are defined as the H2O2-dependent redoxome. This analysis has substantially expanded the scope of the observable redoxome and suggests a change in functional paradigm from a small set of conserved switches to a much larger, adaptable and cell type specific system. Our data set provides a basis for greatly expanded exploration of the complex networks controlled by H2O2-induced redox sensing, transduction and cellular adaptive responses. More recently, we applied the QTRP method to more physiological relevant context, in which we identified NO66 as one of functional substrates of the ovary-specific thioredoxin Deadhead (DHD), a key redox regulator at the oocyte-to-embryo transition in Drosophila. The talk will summarize what we have learned from these findings in the particular context of redoxome remodeling in development and evolution.

WOA am 11:10
Proteome analysis using label-free DARTS and LC-MS/MS method reveals a target protein of small molecule inhibitor of autophagy

Hui-Yun Hwang[1]; Yoon Sun Cho[1]; Jin Young Kim[2]; Ki Na Yun[2]; Jong Shin Yoo[2]; György Marko-Varga[1, 3]; Ho Jeong Kwon[1]
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Autophagy inhibitors that induce autophagic cell death have unrealized potential as anti-cancer drugs. Previously, we identified a novel autophagy regulating small molecule, APZ, based on its ability to induce autophagosome (AP)-like vesicles. APZ induces apoptosis-independent cell death by binding an unknown target via an unknown mechanism. Using a combination of label-free DARTS (drug affinity response target stability) and liquid chromatography/tandem mass spectrometry (LC-MS/MS), thirty-five candidate protein targets showed reasonable sequence coverage values that increased significantly upon APZ treatment compared to controls in the absence of APZ. Following heatmap analysis and filtering based on function by known autophagy inhibiting phenotype narrowed down functional target to APZ binding protein (APZBP). For further characterization of APZBP as a potential target of APZ, the protein was applied for sequence-based peptide analysis. Rescue of sequence coverage in specific locations suggested binding specificity and possible binding sites between a small molecule and its protein target. Entire domains can be protected; in this case, protection was generally observed at the level of protein domains, the nucleotide binding domain (NBD, ATPase domain). Sequence coverage analysis revealed that specific peptide fragments in the NBD of APZBP were protected by APZ in the pronase-treated proteome, indicating that APZ specifically binds to this domain of APZBP. To validate the biological relevance of APZBP as a target protein of APZ, target protein inhibition through siRNA knockdown or a specific inhibitor, inhibited autophagy in HeLa cells, whereas APZBP overexpression prevented inhibition. Moreover, APZBP inhibition affects fusion of lysosomes with autophagosomes, as demonstrated by GFP-RFP double-tagged LC3 transfection. These findings

demonstrate the mode of action of APZ to induce autophagic cell death and warrant its development as a promising candidate of anticancer drug. Additionally, we showed combined DARTS and LC-MS/MS-based target identification is effective for protein target identification of small molecule with target-unknown.

WOA am 11:22
Photoaffinity probes and quantitative proteomics enable assessment of target engagement and compound potency in live cells

H.Christian Eberl; Johanna Vappiani; Anne J. Wagner; Stephanie Lehmann; Marcel Muelbaier; Marcus Bantscheff
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Photo affinity labelling (PAL) extends conventional chemoproteomics methods by establishing a covalent linkage between target proteins and small molecules in live cells. PAL probes are designed as trifunctional molecules consisting of a small molecule ligand binding to the target protein, a photo reactive moiety for covalent labelling and an affinity tag for enrichment. These probes allow the specific labelling of target proteins in complex mixtures and within cells as well as enrichment and subsequent identification by mass spectrometry. We designed generic building blocks for synthesis of PAL probes and established a workflow to determine the affinity of small molecules to their target proteins inside cells.

Modular building blocks, consisting of a photo reactive group, a spacer and trans-cyclo octene (TCO) for subsequent enrichment were synthesized. These building blocks were attached to a functionalized HDAC inhibitor and a functionalized inhibitor of the G-Protein coupled receptor (GPCR) ADRB2. Target proteins were labelled by irradiation at 365 nm and the bioorthogonal reaction between TCO and tetrazine was utilized for subsequent enrichment. Dose-dependent reduction of target binding by competition with parent inhibitor was monitored by isobaric mass tag-enabled quantitative mass spectrometry on a Qexactive platform. Photo affinity labelling using PAL-modified Vorinostat retrieved the expected targets: HDACs and their interactors. Further, experiments in cells revealed Tetratricopeptide repeat protein 38 as specific binder of Vorinostat. Monitoring small molecule interactions with G-protein coupled receptors (GPCRs) is particularly challenging with non-covalent methods. A modular PAL-probe with a desisopropyl propranolol warhead targeting ADRB2 efficiently captured this target from cell systems with and without ADRB2 over-expression.Dose-dependent competition experiments with cyanopindolol revealed nanomolar affinities with this compound.

In summary, a modular PAL strategy combined with quantitative mass spectrometry enables identification of transmembrane and other challenging drug targets as well as determination of binding affinities.

WOA am 11:34
Chemical proteomics unravel protein targets and binding sites of sphingolipid like small molecules with anticancer properties

Peter Kubiniok[1]; Alison McCracken[2]; Brendan Finicle[2]; Lorenzo Sernissi[1]; Stephen Hanessian[1]; Aimee Edinger[2]; Pierre Thibault[1]
[1]University of Montreal, Montreal, Canada; [2]University of California, Irvine, Irvine, CA

Abstract Removed. Contact the HUPO 2018 office for information.

WOA am 11:46
Inside-out: targeting matrix metalloproteinases and the surrounding proteome in the healing skin wound by hybrid degradomics

Simonas Savickas[1, 3]; Tobias Kockmann[2]; Ulrich auf dem Keller[1, 3]
[1]Technical University of Denmark, Lyngby, Denmark; [2]Functional Genomics Center Zurich, Zurich, Switzerland; [3]Institute of Molecular Health Science, Zurich, Switzerland

Matrix metalloproteinases (MMPs) contribute to skin homeostasis, cutaneous wound repair, and skin carcinogenesis. Thereby, they do not only remodel the extracellular matrix, but they also play pivotal roles in immune cell recruitment, angiogenesis, and epithelial cell proliferation. At the wound edge, MMPs are secreted by migrating keratinocytes and fibroblasts and form an interdependent zymogen activation network. In this project, we apply a novel hybrid proteomics approach to map this MMP activation network and its interconnections on the molecular, cellular and tissue level.

By using recombinant mouse proMMPs 2, 3, 7, 8, 9, 10, and 13 we have developed parallel reaction monitoring (PRM) assays for detection of these proteases in complex biological matrices with very high specificity and sensitivity. The power of these assays was uniquely increased by including discriminative features for latent and active MMPs that allow monitoring zymogen removal. Next, we exploited our newly established PRM assays to assess pairwise co-activation of MMPs and generated a first model of interconnected MMP activation. Furthermore, we have developed a strategy combining the sensitivity of Parallel Reaction Monitoring and the scalable nature of data independent acquisition to observe MMP 3 and 10 confirming their function as ‘activator’ MMPs in MMP activation networks and in the same run monitor surrounding 10,000 peptides.

Harnessing the power of hybrid proteomics, we have built a framework for the detection of endogenous MMP activity and their surrounding proteome to study their interdependent activation in cell secretomes from keratinocytes and fibroblasts as well as in mouse wound epidermis and skin papilloma. Ultimately, the delineation of the MMP activation network by hybrid proteomics will elucidate how its components temporally and spatially shape the proteome at epithelial-mesenchymal interfaces in conditions of controlled and uncontrolled cell proliferation.

WOA am 11:58
Ubiquitinome dynamics upon proteasome modulation

Lennart Van der Wal; Karel Bezstarosti; Karen Sap; Dick Dekkers; Erikjan Rijkers; Jeroen A.A. Demmers
Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands

The 26S proteasome is a highly conserved protein complex that is involved in the degradation of unneeded, damaged and misfolded proteins. Such proteins are flagged by the small protein ubiquitin, which serves as the trigger signal to target them to the proteasome. Malfunctioning of this ubiquitin–proteasome system (UPS) has been implicated in diseases such as cancer and neurodegenerative disorders.

In this study, the molecular mechanisms of the UPS were dissected by monitoring the dynamic global proteome and the deep ubiquitinome upon selective inactivation of specific proteasome modules by RNAi knockdown or CRISPR/Cas knockout or by small molecule inhibitors. We used a quantitative mass spectrometry workflow based on SILAC and TMT, combined with diGly peptide enrichment.

We observed that dysfunctioning of the proteasome results in a largely affected global proteome: after inactivation by chemical inhibitors or by subunit depletion, the abundances of several 100s of proteins were upregulated, including stress response, cell cycle regulation, apoptosis and UPS proteins. This was even more pronounced for the ubiquitinome, which was dramatically remodeled upon proteasome modulation. Although the far majority of proteins became increasingly ubiquitinated, many proteins showed heterogeneous ubiquitination patterns on different lysine residues within their sequences. This heterogeneity of ubiquitination suggests that a substantial part of the identified ubiquitinome reflects functions other than targeting proteins for proteasomal degradation, e.g. in intracellular signaling events.

Next, we selectively inactivated the three proteasome associated deubiquitinases (DUBs) and observed remarkably different effects on the proteome, suggesting specific and unique functions or target specificities for these proteasome embedded enzymes.

Finally, using an improved and powerful combination of peptide fractionation, economical use of diGly antibody beads and an efficient Orbitrap decision tree in which least intense peptides are fragmented first, we were able to routinely identify >23,000 diGly peptides from a single sample.

10:30 am - 12:20 pm Wednesday
BIOMARKERS, NON-CANCER
Session Chairs: Karin Rodland and Hui Zhang
Oceana 7

WOB am 10:30
Cardiovascular Disease: Moving to Precision Medicine and Health
Jennifer Van Eyk
Cedars Sinai Medical Center, Los Angeles, CA

WOB am 10:50
Plasma protein biomarker discovery and down selection for subsequent validation in The Environmental Determinants of Diabetes in the Young cohort
Bobbie-Jo Webb-Robertson[1]; Ernesto Nakayasu[1]; Charles Ansong[1]; Lisa Bramer[1]; Marina Gritsenko[1]; Therese Clauss[1]; Paul Piehowski[1]; Athena Schepmoes[1]; Bryan Stanfill[1]; Daniel Orton[1]; Ronald Moore[1]; Brigitte Frohnert[2]; Marian Rewers[2]; Richard Smith[1]; Jeffrey Krischer[3]; Thomas Metz[1]
[1]Pacific Northwest National Laboratory, Richland, WA; [2]University of Colorado School of Medicine, Denver, CO; [3]University of South Florida, Tampa, FL
Type 1 diabetes (T1D) is an autoimmune disease that results in the loss of the pancreatic beta cells. A significant gap in the study of the onset of the autoimmune response and its progression is the lack of biomarkers that can be used to accurately predict and monitor these processes. The Environmental Determinants of Diabetes in the Young (TEDDY) study aims to identify new biomarkers for and obtain a mechanistic understanding of the autoimmune response leading to T1D. As a first step towards achieving these goals, we have performed an in-depth plasma proteomics analysis of children carrying the human leukocyte antigen genotypes associated with higher risk of developing T1D. Individuals were further selected based on the appearance of autoimmune antibodies and whether the child developed T1D or not. Age- and gender-matched children that did not progress to autoimmunity or T1D served as controls. We describe the experimental design, different points of sample/data quality check and the statistical and informatics approaches for prioritizing proteins for further investigation in an expanded, next-phase validation study. From 1,724 proteins identified in the biomarker discovery process, 133 had significantly different relative abundances in children with autoimmunity/T1D vs controls. Based on the results of statistical analysis, literature evidence, machine learning approaches and physical-chemical properties of the peptides, 811 peptides from 167 proteins were selected for developing selected reaction monitoring mass spectrometry assays. Assays were successfully developed for 96.7% (784 peptides), which will allow the validation of identified biomarker candidates in a large cohort of samples from the TEDDY study. This approach allowed the discovery of a large number of biomarker candidates which will be further validated in their association with the risk of developing T1D.

WOB am 11:10
Mitochondrial proteins as Parkinson's Disease circulatory biomarkers – a translational study
Sandra I. Anjo[1, 2]; Patrícia Valério dos Santos[3]; Maria Luiza Constante Rosado[4, 5]; Graça Baltazar[4]; Mário Grãos[1, 6]; Bruno Manadas[1]
[1]Center for Neuroscience and Cell Biology, UC, Coimbra, Portugal; [2]Faculty of Medicine, UC, Coimbra, Portugal; [3]Centro Hospitalar de Setúbal, Setúbal, Portugal; [4]Faculty of Health Sciences, UBI, Covilhã, Portugal; [5]Centro Hospitalar Cova da Beira, E.P.E., Covilhã, Portugal; [6]Biocant, Biotechnology Transfer Association, Cantanhede, Portugal
The identification of circulating biomarkers that closely correlate with PD has failed several times in the past. Nevertheless, using a translational approach we could monitor two mitochondria-related proteins in plasma samples, which in combination lead to a powerful model with potential diagnostic value to discriminate the PD patients from matched controls.

This translational approach was initiated by the analysis of secretomes from cells cultured under control or oxidative stress conditions, from which several mitochondria-related proteins were found to be released in higher amounts under oxidative stress. This screening, performed by SWATH-MS, was translated to the analysis of plasma samples from 28 control and 31 PD patients, and two of these proteins were found to be significantly changed in PD cohort. A linear discriminant analysis of the results obtained for these two proteins originates a model with potential diagnostic value to discriminate PD patients. The model obtained has a specificity of 77.4%, a sensitivity of 78.6%; cross-validation of 76.3% and a ROC analysis with an area under the curve (AUC) of 0.872.

These two proteins are associated with apoptotic mitochondrial changes, which may correspond to potential indicators of cell death and have never been reported as blood biomarkers for PD. In fact, to the best of our knowledge, one of these proteins

was identified in plasma samples for the first time, and the other protein was already reported to be altered in as Alzheimer's patients but not in PD patients. In this sense, we believe that the novelty and success of our results arise from the combination of: i) a translational research pipeline, where plasma samples were interrogated with previous knowledge from cell secretome under oxidative stress, and ii) the use of the quantification approach SWATH-MS associated with the use of a biofluid optimized normalization method.

WOB am 11:22

Development a of novel targeted proteomic plasma biomarker panel for hypertrophic cardiomyopathy

Wendy Heywood[4]; Gabriella Captur[2]; Caroline Coats[2]; Stefania Rosmini[2]; Vimal Patel[3]; Richard Collis[2]; Nina Patel[4]; Petros Syrris[3]; Paul Bassett[1]; Ben O'Brian[2]; James Moon[2]; Perry Elliott[3]; Kevin Millls[4]

[1]UCL, London, United Kingdom; [2]Barts Heart Centre, St Bartholemews Hospital, London, UK; [3]UCL Institute of Cardiovascuslar Science, London, UK; [4]UCL Great Ormond Street Institute of Child Health, London, UK

There is a need for new and better biomarkers for hypertrophic cardiomyopathy (HCM) which correlate more closely with disease progression as determined by clinical imaging and biohumoral information. Myocardial tissue and plasma samples from patients with HCM and healthy volunteers (controls) were screened using label free proteomics. Twenty-six potential biomarkers were identified from the proteomics screens and developed into a multiplexed targeted proteomic assay. Their association with clinical phenotypes was tested in plasma samples collected from 207 prospectively recruited participants: 110 patients with HCM (50.1 ± 15.0 years, 70% male; 48 [44%] with identified genetic mutations) and 97 controls (49.6 ± 13.4 years, 58% male), randomly split into training (80 HCM, 67 controls) and validation datasets (30 HCM, 30 controls). Six markers associated with oxidative stress response, scaffolding and immune response were significantly increased (P<0.006) in the plasma of HCM patients compared to controls in the training dataset. These markers correlated with left ventricular (LV) wall thickness, LV mass and % myocardial scar on cardiovascular magnetic resonance imaging. Using supervised machine learning (ML) this panel differentiated HCM from controls (area under the curve: 0.89 in the training dataset, sensitivity 96%, 95% confidence interval [CI] 77–93; specificity 87%, 95%CI 77–94; and 0.87 in the validation dataset, sensitivity 97%, 95%CI 83–100; specificity 77%, 95%CI 58–90). Four of the biomarkers as well as the composite ML score of the plasma proteome correlated with the presence of nonsustained ventricular tachycardia and the estimated 5-year risk of sudden cardiac death. By developing a high-throughput, multiplex, and targeted proteomic plasma assay we identified 6 biomarkers that correlate with the presence of disease and with clinical risk score for sudden cardiac death.

WOB am 11:34

A novel and robust method for urinary proteome profiling applied in an exploratory case-control study

Ireshyn Govender[1, 2]; Stoyan Stoychev[1]; Demetra Mavri-Damelin[2]; Ebrahim Variava[2]; Faheem Seedat[2]; Neil Martinson[2]; Dalu Mancama[1]

[1]Council for Scientific and Industrial Research, Pretoria, South Africa; [2]University of the Witwatersrand, Johannesburg, South Africa

Background: South African patients account for ~10% of the global HIV/AIDS burden. In response, the country has implemented the largest antiretroviral therapy (ART) program in the world. However, not all patients respond positively and it has been observed that 8-10% of patients on first-line ART experience acute kidney injury (AKI). Current tests for AKI are unreliable and only reflect following significant kidney damage. Hence there is a dire need to identify biomarkers for early detection of AKI. The urinary proteome provides a large and diverse source of information pertaining to a patient's health status. To date, there is no - published - standard operating protocol for reproducible collection and processing of urine for mass spectrometry-based clinical proteomics.

Methods: Acetone precipitation, of urinary protein, followed by FASP was compared to two novel sample preparation techniques using MagReSyn® HILIC and Norgen Biotek Corporation Urine Kits. The kit-based method was applied to a cohort of 74 HIV positive patients. Their urinary proteomes were compared with the aim of detecting markers associated with AKI due to first-line ART using SWATH-MS.

Results and Discussion: Using MagReSyn® HILIC was the easiest, fastest and cheapest method whilst generating the most comprehensive data. The kit-based method was the most easily implemented at the point-of-care clinic and generated data that was comparable to the MagReSyn® HILIC method. Using the kit based method, we identified eight proteins (≥ 4-fold change, q value ≤ 0.01, ≥ 2 unique peptides), previously reported in literature, that have an association with kidney dysfunction. Furthermore, novel, putative markers for AKI have been identified.

Conclusion: The methods developed in this study can serve as the gold-standard for future urinary proteomics experiments. Additionally, following assessment in a blinded verification cohort, previously reported and novel proteins have the potential to be used as early markers for AKI.

WOB am 11:46

Astroglial Injury-Defined Biomarkers for Assessment of Traumatic Brain Injury

Ina-Beate Wanner; Julia Halford; Sean Shen; Joseph Loo

University of California, Los Angeles, Los Angeles, CA

Traumatic brain injury (TBI) is an expanding public health epidemic with pathophysiology that is difficult to diagnose and thus treat. TBI biomarkers should assess patients across severities and reveal pathophysiology, but currently, their kinetics and specificity are unclear. No single ideal TBI biomarker exists to date. Our primary objective has been to select candidate neurotrauma biomarkers that are robustly released by trauma and that are brain specific. Astrocytes are known for neurotrauma biomarkers and for reactive astrogliosis with scar formation; yet they have distinct pathophysiologies. To differentiate these injury stages, we deciphered proteomic astrocyte-release signatures, called the "traumatome", from a unique trauma culture model. Subsequently, a targeted proteomic screen and proteome mining of cerebrospinal fluid from traumatic brain injury patients (including data from LC-MS/MS measurements), enabled the selection of astroglial-specific trauma-release markers. These markers include aldolase C (ALDOC), brain lipid binding protein (BLBP), astrocytic phosphoprotein (PEA15), glutamine synthetase, and breakdown products of ALDOC and GFAP. Their levels increase over four orders of magnitude in severe TBI CSF. During the first post-injury week, ALDOC levels are markedly high and stable. Short-lived BLBP and PEA15 relate to injury

progression. ALDOC, BLBP and PEA15 appear hyperacutely and are similarly robust in blood samples from severe and mild TBI subjects. Delayed cell death corresponded with GFAP release and proteolysis into small GFAP-BDPs. CSF and blood levels of these astrocyte injury-defined biomarkers capture the entire spectrum of mild-severe neurotrauma and document their potential translational use for patient diagnosis and outcome prediction.

WOB am 11:58
Remodeling of the Glyco-phenotype of T Cell Surface Proteins with Antisense RNA of Human Immunodeficiency Virus

Weiming Yang[1]; Minghui An[1]; Fabio Romerio[2]; Hui Zhang[1]
[1]Johns Hopkins University, Baltimore, MD; [2]University of Maryland, Baltimore, MD

Human immunodeficiency virus (HIV) infection is not curable due to viral latency and identifying T cells with HIV latent infection is a crucial step to the cure. Compelling reports and our recent study suggested that there is a distinct profile of cell surface proteins that can be used for targeting latently infected cells. To identify which HIV-specific component may play a role in remodeling cell surface proteins, we established stable T cell lines that expressed two different HIV derived RNAs including HIV trans-activation-responsive (TAR) RNA and viral antisense transcript (Ast). Analysis of glycoproteins from the stable lines using mass spectrometry revealed that expression of Ast drastically changed the expression of a range of glycoproteins. In sharp contrast, expression of TAR RNA had little effect to the glycoproteome pointing to that the change seen in Ast expressing cells was selective. Additionally changed glycoproteins in the Ast expressing cells were identified using glycoproteomic analysis of de-glycosylated peptides. Downstream pathway analysis and antibody study confirmed that glyco-phenotype of T cells was reshaped by the expression of HIV Ast. The significance of the observation in the context of current HIV cure effort will be discussed.

10:30 am - 12:20 pm Wednesday
SINGLE CELL PROTEOMICS
Session Chair: Nikolai Slavov
Oceana 6
This session is generously supported by

WOC am 10:30
Dissecting the spatiotemporal subcellular distribution of the human proteome

Emma Lundberg
KTH Royal Institute of Technology, Stockholm, Sweden

Presentation content: Compartmentalization of biological reactions is an important mechanism to allow multiple cellular reactions to occur in parallel. Resolving the spatial distribution of the human proteome at a subcellular level increases our understanding of human biology and disease. We have generated a high-resolution map of the subcellular distribution of the human proteome as part of the open access Human Protein Atlas database. We have shown that as much as half of all proteins localize to multiple compartments. Such proteins may have context specific functions and 'moonlight' in different parts of the cell, thus increasing the functionality of the proteome and the complexity of the cell from a systems perspective. I will present how this spatial data can complement quantitative omics data for improved functional read-out. Furthermore, I will present unpublished data on the extent of single cell variations of the human proteome, in correlation to cell cycle progression and other deterministic factors, as well as the overlap with observed variations at the RNA level. In summary, I will demonstrate the importance of spatial proteomics data for improved single cell biology.

WOC am 10:50
Single-molecule protein sequencing

Jagannath Swaminathan; Alexander A. Boulgakov; Erik T. Hernandez; Angela M. Bardo; James L. Bachman; Joseph Marotta; Amber M. Johnson; Eric V. Anslyn; Edward M. Marcotte
University of Texas at Austin, Austin, TX

Introduction: The identification and quantification of proteins lags behind DNA sequencing methods in scale, sensitivity and dynamic range. Currently, mass spectrometry is the method of choice for large-scale protein identification, but it is limited in its ability to analyze low-abundance samples and map rare amino acid variants. These limitations could in principle be addressed by highly parallel single-molecule protein sequencing, a concept analogous to nucleic acid technologies that sequence millions to billions of oligonucleotides in complex mixtures in parallel. Such an approach would offer more than a million-fold improvement in sensitivity over conventional technologies and allow millions of distinct peptide molecules to be sequenced in parallel, identified and digitally quantified.

Results: Here, we describe an approach, termed fluorosequencing, for directly visualizing individual fluorescently labeled peptide or protein molecules as they are subjected to a classic protein sequencing chemistry, Edman degradation. This required developing instrumentation and methods, extensive testing of fluorophores, microfluidic design, chemistry of peptide immobilization and Edman degradation, image processing algorithms for monitoring individual peptide's fluorescent intensities, and classifying and modeling the sources of errors. We analyze peptide samples of increasing complexity, both individually and in zeptomole-scale peptide mixtures, and distinguish specific phosphoserine post-translational modifications. We measured >93% efficiencies for dye labeling, survival, and cleavage; further improvements should empower studies of increasingly complex proteomic mixtures, with the high sensitivity and digital quantification offered by single molecule sequencing.

Conclusions: We have shown that sparse amino acid sequence information can be obtained for individual protein molecules for thousands to millions of molecules in parallel. Single-molecule protein sequencing combines aspects from DNA sequencing, mass spectrometry proteomics, and classic Edman sequencing, and can potentially improve the sensitivity and throughput of proteomics experiments by orders of magnitude, as well as offering digital quantification (by counting molecules).

WOC am 11:10
Automated sample preparation for high-throughput single-cell proteomics

Harrison Specht[1]; Guillaume Harmange[1]; David Perlman[1]; Ed Emmott[1]; Zach Niziolek[2]; Bogdan Budnik[2]; Nikolai Slavov[1]

[1]Northeastern University, Boston, <Not Specified>; [2]Harvard University, Cambridge, MA

A major limitation to applying quantitative mass spectrometry-based proteomics to small samples, such as single cells, are the losses during sample cleanup. To relieve this limitation, we developed a Minimal ProteOmic sample Preparation (mPOP) method for culture-grown mammalian cells. mPOP obviates cleanup, and thus eliminates cleanup-related losses while simplifying and expediting sample preparation. Bulk SILAC samples processed by mPOP or by conventional urea-based methods indicated that mPOP results in complete cell lysis and accurate relative quantification. Combining mPOP with cell-sorting and liquid handling of U-937, HEK293 and Jurkat cells, we can prepare hundreds of Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS) samples per day and can process 12 such samples, equivalent to 96 single cells, per day per instrument. Using this approach, we quantify thousands of proteins across 96 single cells. Likewise, mPOP enables protein measurements in 10, 20, and 100 cell samples with unprecedented breadth and throughput.

WOC am 11:22
Comparison of Novel Quantitative Methods for Single Cell Proteomics

Akos Vegvari[1]; Christian Beusch[1]; Alexandra Alexandridou[1]; Amirata S Dibavar[1]; Jaakko S Teppo[2]; Roman A Zubarev[1]
[1]Karolinska Institutet, Stockholm, Sweden; [2]University of Helsinki, Helsinki, Finland

Single-cell transcriptomics has revolutionized biology unravelling an unforeseen molecular diversity of cells, providing new insights into intricate details of essential cellular processes. However, mRNA levels do not always reflect protein abundances, and totally miss posttranslational modifications, necessitating their direct measurements at single cell level resulting more reliable readouts of the functional state of cells.

Mass spectrometry based single-cell proteomic (SCP) analysis using tandem-mass tag (TMT) labeling and “carrier proteome” has recently been introduced in Boston, yet the methodology is still under development. We established two novel SCP approaches promising improved sensitivity. One of the methods employs a combination of TMT-duplex labeled “carrier proteome” and 10-plex labeled single-cell proteomes. In each cycle of data-dependent acquisition, MS/MS was triggered by “carrier proteome” peptides followed by MS/MS of their corresponding peptides with an isolation window off-set of 4Da (2Th for 2+ and 1.33Th for 3+ peptides). The second method is based on the phenomenon of stochastic resonance. Hypothesizing that SCP sensitivity could be increased by adding an above-threshold constant signal, a background 10-plexed proteome of N cells (N=5-20) was added to traditionally prepared 10-plexed SC proteome.

Both approaches were able to detect several hundred proteins in HTC-116 cells and human monocytes and to distinguish drug treated cells from their untreated controls, quantifying different levels of affected proteins. The introduction of parallel processing of single cells in plates allowed for reduction of statistical fluctuations due to sample preparation. However, variances of protein abundances between the technical replicates in pseudo-SCP analyses were still relatively high (≥20%) indicating a need for further improvements.

While the initial results with the present methods are encouraging, more robust sample preparation techniques and novel ways to improve sensitivity are highly desirable. Additionally, the analytical throughput must be increased toward 1000s cells/project to afford statistical power sufficient to answer biological questions.

WOC am 11:34
Cellular and Subcellular Heterogeneity of Metabolites, Lipids, and Peptides in Selected and Perturbed Cells Explored by Single-Cell Mass Spectrometry

Linwen Zhang; Nikkita Khattar; Akos Vertes
The George Washington University, Washington, DC

Individual cells within isogenic cell populations and complex biological tissues exhibit large variations in their molecular compositions. Single-cell analysis helps to uncover the cellular chemical compositions correlated with their functions and phenotypes. Analysis on a subcellular level is necessary for understanding molecular functions within subcellular compartments. Here, we apply capillary microsampling electrospray ionization (ESI) mass spectrometry (MS) with ion mobility separation (IMS) for metabolic and lipidomic analysis of single human hepatocellular carcinoma and neuroblastoma cells, and subcellular analysis of peptide distributions in the F group (Fgp) neurons of *Lymnaea stagnalis*. For human hepatocytes, 29 metabolite and 54 lipid ions were identified within a cell. The levels and distributions of cellular adenylate energy charge (AEC), were determined in rotenone treated and vehicle control cells. A dramatic drop of AEC level from 0.82±0.12 for the control group to 0.16±0.12 for the rotenone treated cells was observed. Subpopulations in different mitotic stages, selected by fluorescence microscopy, exhibited increased AEC in metaphase and increased [GTP]/[GDP] in cytokinesis compared to prometaphase. Furthermore, using capillary microsampling ESI-IMS-MS, distinct subcellular distributions of neuropeptides within the cytoplasm and nucleus of single Fgp neurons from *L. stagnalis* central nervous system (CNS) were observed. Due to its simplicity, the CNS of pond snail, *L. stagnalis,* is a widely used model system for neuroscience studies. Distinct sets of cardioexcitatory FMRFamide neuropeptides were detected in the cytoplasms of two types of Fgp neurons with alternative mRNA splicing. Nine peptides, e.g., GDPFLRFamide, were identified in the Type 2 Fgp cytoplasm spectra, and six of them were also detected in the nucleus. A new neuropeptide with 28 amino acid residues was identified by single cell tandem MS. This technique was also applied for analyzing single neuroblastoma cells. Electrical stimulation followed by single-cell MS will be implemented for studying neural responses due to external perturbations.

WOC am 11:46
Cell-type resolved Proteome and Lipidome of Human Lung at Population and Single Cell Level.

Geremy Clair; Jennifer Kyle; Ying Zhu; Lisa Bramer; Paul Piehowski; Ryan Kelly; Charles Ansong
PNNL, Richland, <Not Specified>

The lung is a complex organ comprised of more than 40 cell types each playing overlapping and niche roles in facilitating normal lung development and function. Proteins and lipids perform the biochemical activities required for biological functions and are only moderately correlated with mRNA abundance. Thus characterization of the proteome and lipidome of individual lung cell types during development will help to better understand the specification mechanisms that drive normal lung formation and function.

Mass spectrometry-based proteomics and lipidomics were used to characterize the proteome and lipidome of four major lung cell types (mesenchymal, epithelial, endothelial and mixed immune) isolated from 12 human donors from birth to adulthood. Proteins and lipids were extracted using a modified Folch extraction method (metabolite, protein, and lipid extraction, MPLEx) and then introduced into a LC-MS/MS platform for population proteomics and lipidomics analyses. For single cell analyses, single epithelial and fibroblast cells were prepared using our novel nanoPOTS platform prior LC-MS/MS platform for proteomics analyses.

Our integrative multi-omics approaches identified ~5,300 proteins and ~400 lipids from the cell-type resolved analyses. The populations differentially retained expression of well-known markers typically used to discriminate lung cell type (SFTPC, NKX2.1, EPCAM, ABCA3, CD11c, CD288, PDGFRb, PECAM/CD31, etc.) and identified several potential "signature proteins" enriched in specific cell types. The dynamic of the proteome across the different ages and cell types allowed us to explore regulatory processes that could be essential for the normal lung developmental process. Cell-type lipidomics supported the presence of lipofibroblasts in the human lung and revealed cellular cooperation in lung function. Finally our nanowell-LC-MS/MS platform was able to identify ~500 proteins from single lung cells in an unbiased label-free manner, and PCA analysis partitioned the single cells based on protein expression alone.

WOC am 11:58
PASEF for ultra-sensitive shotgun proteomics

Romano Hebeler[1]; Heiner Koch[1]; Christopher M. Adams[2]; Scarlet Koch[1]; Markus Lubeck[1]; Florian Meier[3]; Andreas Brunner[3]; Matthias Mann[3]
[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Bruker Daltonics Inc., San Jose, USA; [3]Max Planck Institute of Biochemistry, Martinsried, Germany

Time and space focusing of ion clusters focused by their collisional cross section in trapped ion mobility spectrometry (TIMS) boosts the sensitivity of QTOF MS. In addition, TIMS enables parallel accumulation–serial fragmentation (PASEF), which couples high sequencing speed (>120 Hz) at 100% duty cycle without sacrificing spectral quality. We show the combined increase in sensitivity and speed can be used for deep proteome coverage using very low sample amounts (< 10 ng).

A dilution series of a tryptic digest from a human cancer cell line (HeLa) was used. LC was performed on a nanoElute (Bruker Daltonics) using nano-flow HPLC and a 25 cm column with integrated emitter (IonOpticks, Australia) and a 60 min gradient. Data was generated using the PASEF acquisition mode with a cycle time of 1.1 s. Data analysis was performed using PEAKS studio (Bioinformatics Solution Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry).

Sensitivity of the timsTOF Pro instrument with PASEF was evaluated on sample concentrations of ~ 3 ng and up to 100 ng on column. Using the lowest amount on column, which corresponds to 10 HeLa cells (~ 3 ng), more than 1,650 protein families from 7,000 unique peptide sequences were identified. A linear increase in ID numbers was observed up to the identification of 5,091 and a sample amount of 100 ng. This linear increase is a testament to the 100% duty cycle coupled with fast sequencing speed. We tested four different admixtures of HeLa and E.coli where the final protein mix was 100 ng and the ratios varied from 3/2, 3/1, 7/1 and 16/1, respectively. Proteome admixtures showed good reproducibility and sensitivity. Applying PASEF on the timsTOF Pro mass spectrometer provides deep proteome coverage at low sample concentrations.

10:30 am - 12:20 pm Wednesday
HPP: HUMAN CHEMOSENSATION: OLFACTION AND TASTE
Session Chairs: Mark Baker and Jong Shin Yoo
Oceana 3-5

WOD am 10:30
Odorant Receptors and the Perception of Odors

Andreas Keller
Rockefeller University, New York, NY

I will present the results of our psychophysical investigation of odor perception. These results have shed light on our capacity to detect and discriminate smells as well as on the relation between the structure and smell of odor molecules. Humans smell the volatile molecules in their environment with around 400 different receptors and the odorant receptor genes show unusually high genetic variability with many receptors present as functional as well as non-functional variants. Testing smell perception in subjects with sequenced odorant receptor genes therefore gives insights into the perceptual consequences of variability in the odorant receptor repertoire. The overarching goal of this research is to understand the olfactory code: how does the activation of different sets of odorant receptors encode the many different smells we can perceive?

WOD am 10:50
Bitter Sweet Taste Proteins

Danielle Reed
Monell Chemical Senses Center, Philadelphia, PA

Eating is dangerous because foods can contain poisons or pathogens and taste is the final sensory system that protects us from consuming toxins. Taste is also important because the pleasure from food motivates us to eat and helps us bond with family and friends. There are approximately twenty five human bitter receptor proteins that respond to different chemical classes and there is a smaller family of three proteins that in combination allow us to perceive sweet and savory foods. These proteins have many variant forms in the global human population which affect how well or how poorly the receptor functions and hence how well each person can perceive foods and drinks such as goitrins in cruciferous vegetables and high-potency sweeteners. Each person lives in their own sensory world, owing in part to the protein structure of taste receptors as determined by their inborn genetic makeup.

WOD am 11:10
Comprehensive Proteomic Approaches for Membrane Proteins Analysis

Jing Gao; Yanting Zhou; Hongwen Zhu; Zhenyun Zhu; Hu Zhou
Shanghai Institute of Materia Medica, Chinese Acad, Shanghai, China

Despite their importance in many biological processes, membrane proteins are underrepresented in proteomic analysis because of their poor solubility (hydrophobicity) and often low abundance. Identification of plasma membrane proteins was improved via our comprehensive proteomic

approaches that include: (i) a simplified centrifugation-based membrane protein extraction strategy, (ii) an integrative workflow for evaluation of detergent and their effects on peptide identification, (iii) a novel one-dimensional online pH gradient-eluted strong cation exchange-nanoelectrospray ionization-tandem mass spectrometry (SCX-nano-ESI-MS/ MS) method facilitates in increasing membrane protein sequence coverage. By reducing the rotational speed to 35000 × g, we offered a more convenient option without rigorous requirement on experimental equipment, while identifying higher proportion of membrane proteins, integral proteins and transmembrane proteins in membrane fraction (76.6%, 48.1%, and 40.6%) than in total cell lysate (41.6%, 16.4%, and 13.5%), respectively. We also developed a multiple reaction monitoring (MRM)-based LC-MS workflow for the quantification of detergents and salt additives (i.e., Tris, urea, CHAPS, SDS, SDC, and Triton X-100) in proteomic samples and systematically evaluated the relationships between the level of remaining additive after sample processing and the number of peptides/proteins identified by mass spectrometry. Our results revealed that FASP outperformed acetone precipitation followed by in-solution digestion and strong cation exchange-based centrifugal proteomic reactors, in terms of peptide/protein identification. Furthermore, SCXLC− MS/MS method is an excellent alternative to the RPLC− MS/MS method for analysis of membrane proteomes. The two methods were highly complementary with only ≤ 30% of the peptides and ∼ 55% of the proteins overlapping between the two methods. The combination of two methods could significantly increase sequence coverage and the concomitant confidence level in membrane protein identification. Thus, these proteomic approaches may offer new analytical options for structure and function studies of membrane proteins.

WOD am 11:22

Changes in proteome of age-related maculopathy susceptibility protein 2 (ARMS2) edited retinal pigment epithelial cells in response to oxidative stress

Meleha Ahmad [1]; Todd Greco[2]; Marisol Cano[1]; James Handa[1]; Karl Wahlin[1]; Donald Zack[1]; Srinivas Sripathi[1]; Ileana Cristea[2]; Richard Semba[1]

[1]Wilmer Eye Institute, Baltimore, MD; [2]Princeton University, Princeton , NJ

Age-related macular degeneration (AMD), one of the leading causes of blindness in the developed world, remains poorly understood on a molecular level. Age-related maculopathy susceptibility protein 2 (ARMS2) is strongly associated with a high risk of AMD, but its role in the pathogenesis of AMD has not been well characterized. To study the expression of drusen-associated proteins and other proteins that are up- or down-regulated in response to cellular stress, we exposed iPS-RPE wild type and CRISPR/cas9 edited ARMS2 A69S variant cells to cigarette smoke extract (CSE) and quantified changes in the global proteome using isobaric tandem mass tagging (TMT) quantitative mass spectrometry. A total of 5,914 proteins were identified using a two-peptide match. There were 11 proteins significantly upregulated and 11 proteins significantly downregulated between wild type and A69S variant iPS-RPE cells. In wild type cells exposed to CSE, there were 22 proteins significantly upregulated and 58 proteins down-regulated. In ARMS2 A69S iPS-RPE exposed to CSE, there were 22 proteins significantly upregulated and 20 proteins downregulated. CSE was strongly associated with upregulation of proteins involved in antioxidant and free radical responses (e.g. heme oxygenase 1 and cystine/glutamate transporter), ubiquitin-dependent protein degradation and DNA repair (e.g. sequestosome 1), and cytoskeletal regulation. There were no major drusen-associated proteins that were differentially expressed between wild type and ARMS2 A69S iPS-RPE cells or between these cell types exposed to CSE.

WOD am 11:34

Identification of missing proteins and alternative splicing variants from human olfactory epithelial tissue.

Heeyoun Hwang[1]; Ji Eun Jeong[1, 2]; Hyun Kyoung Lee[1, 2]; Ki Na Yun[1]; Hyun Joo An[2]; Bonghee Lee[3]; Young-Ki Paik[4]; Gi Taek Yee[5]; Jin Young Kim[1]; Jong Shin Yoo[1, 2]

[1]Korea Basic Science Institute, Chungju-Si, South Korea; [2]Chungnam National University, Daejeon, South Korea; [3]Gachon University, Incheon, South Korea; [4]Yonsei University, Seoul, South Korea; [5]Gil Medical Center, Incheon, South Korea

In order to map all human proteins with expanding the understanding of each protein coding gene on each chromosome, the chromosome-centric human proteome project (C-HPP) is looking for dark proteins with missing proteins (MPs), alternative splicing variants (ASVs) and single amino acid variants (SAAV). We (Chr #11 team of C-HPP groups) first analyzed human olfactory epithelial tissue, which contains olfactory sensory neurons expected to be found many kinds of MPs and ASVs of membrane proteins including G protein-coupled receptors (GPCRs), and olfactory receptors, and components of olfactory signal transductions. Using the next-generation proteomic pipeline (nextPP), we found 4,000 more proteins including five MPs and 59 ASVs, with < 1% false discovery rate at protein and < 0.1% at peptide level. We checked the tissue and cellular expression specificity through all found proteins, where seven GPCRs were found. We also report strong evidence of 5 missing proteins through peptide spectrum matching by validation with MS/MS fragmentation from their corresponding synthetic peptides. From these, P46721 (SLCO1A2) and Q8N434 (SVOPL) have 12 and 10 transmembrane domains, respectively. P59826 (BPIFB3) which has a function of recognition and binding specific class of odorants, and transporting odorants across to receptors, was also found as a missing protein in our study. Reticulon-4 (Q9NQC3 -2 and -5), and integrin beta-4 with or without cytoplasmic domain, P16144 -3 or -1, respectively, were also identified as the ASVs with transmembrane domains. In particular a neural cell adhesion molecule 1 (NCAM1) ASV with 70% of N-terminal loss and insertion of a novel exon translating as 276 amino acids but maintaining a transmembrane domain that was identified from human brain samples, was rediscovered in this study. We will further study their biological functions of missing proteins and ASVs related in olfactory signal transduction.

WOD am 11:46

Identifiable Human Olfactory Receptor Proteome Using High-Stringency Mass Spectrometry.

Subash Adhikari; Samridhi Sharma; Seong Beom Ahn; Mark S. Baker

Macquarie University, Sydney, AU

Olfactory receptors (ORs) are 7-transmembrane domain (TMD) GPCRs that constitute a significant 19% of the missing human proteome (404 out 2,186) according to the neXtProt 2018 update for PE2-4 proteins. None of the ORs meet the stringent mass spectrometry (MS) requirements set by the 2017 metrics of the Human Proteome Project (HPP). Shotgun approaches to identify proteins often requires generation of tryptic peptides where the availability/accessibility of arginine (R) and lysine (K) residues in domains determines cleavage. Sequence and

domain topology (UniProtKB) analyses demonstrate that extremely few helical TMDs contain either R or K residues. Equally, these residues have not proven accessible as the hydrophobicity of the plasma membrane environment hinders trypsin cleavage. In addition, requirement of two cleavage site in either of ecto or endo domain to release peptide further hinders soluble peptide generation. In silico tryptic digestion (with no missed cleavages) of the soluble ecto- and endo- OR domains (i.e., trypsin-accessible) were sorted according to >2 unitypic peptides of length >9 amino acids after digestion. This study demonstrates that that only 264 of a potential 404 ORs can generate peptides qualifying number, length and unitypicity stringency to qualify as PE1s. By default, the remaining ORs may never be re-classified as PE1 proteins solely on MS based evidence. We discuss potential strategies to enable potential assignment of these 140 PE2-4 OR proteins as PE1 proteins - including decreased numbers of unitypic peptides and/or length of those detected peptides and/or provision of additional non-MS biological evidence. Equally, the use of specialized methods to disrupt, release and allow MS identification of TMD peptides (e.g., use of heated columns, cyanogen bromide cleavage at M residues) in ORs identification may be warranted.

10:30 am - 12:20 pm Wednesday
NEURODEGENERATIVE DISEASES
Session Chair: Nicholas Seyfried
Oceana 1-2

WOE am 11:10
Large-scale proteomic analysis of alternative open reading frames reveals novel proteins in neurodegenerative diseases such as Amyotrophic Lateral Sclerosis

Marie Brunet; Jennifer Raisch; Mylene Brunelle; Jean-Francois Lucier; Jean-Fancois Jacques; Nathalie Rivard; Xavier Roucou
University of Sherbrooke, Sherbrooke, Canada

Current genome annotations underestimate the proteomic landscape, and numerous studies demonstrated the expression and function of alternative open reading frames (altORFs). We analyzed large-scale mass spectrometry and ribosome profiling data using OpenProt. OpenProt is a freely available database of predicted altORFs, collecting evidence of conservation, translation and expression across 10 species. Our analyses annotated hundreds of novel proteins, and we describe here two examples involved in Amyotrophic Lateral Sclerosis (ALS). ALS is a fatal neurodegenerative disease, characterized by autophagy, proteostasis and mitochondrial function dysregulation. Yet, pathological mechanisms remain mostly unknown.

We identified a novel protein (altFZD1), encoded in an altORF of the *FZD1* gene. FZD1 is the canonical receptor for the Wnt/β-catenin pathway altered in ALS. AltFZD1 is a well-conserved nuclear protein. Its over-expression led to increased chromatin-associated β-catenin and transcriptional activity. It promoted anchorage independent growth and cellular proliferation. AP-MS studies demonstrated altFZD1 interactions with β-catenin and fragile X proteins complex, pivotal in neurodegenerative diseases. This suggests altFZD1 role in Wnt signaling as a transcriptional regulator.

Another novel protein is altFUS, encoded in an altORF of the *FUS* gene. FUS is a RNA-binding protein involved in ALS. AltFUS is a well-conserved mitochondrial protein endogenously expressed in cell lines and tissues, as validated with a custom antibody. AltFUS is necessary for the proteostasis, autophagy and mitochondrial function dysregulation observed in ALS. FUS-linked ALS drosophila models without altFUS expression displayed a significantly lessened ALS phenotype. Mutations altering altFUS, but not FUS, were found in patients. These mutations led to mitochondrial alterations, highlighting altFUS role in ALS.

Discovered through proteomics analyses using OpenProt, altFUS and altFZD1 are novel functional proteins that will further our understanding of pivotal pathways involved in ALS. This highlights the polycistronic nature of mammalian genes and importance of expanded databases as new tools for discoveries in functional proteomics.

WOE am 11:22
Hydralazine induces stress resistance and extends C. elegans lifespan by activating the NRF2/SKN-1 signalling pathway

Hamid Mirzaei[1, 2]
[1]Department of Biochemistry, UT Southwestern, Dallas, TX; [2]Center for Alzheimer's disease, UT Southwestern, Dallas, TX

Nuclear factor (erythroid-derived 2)-like 2 and its Caenorhabditiselegans ortholog, SKN-1, are transcription factors that have a pivotal role in the oxidative stress response, cellular homeostasis, and organismal lifespan. Similar to other defense systems, the NRF2-mediated stress response is compromised in aging and neurodegenerative diseases. Here, we report that the FDA approved drug hydralazine is a bona fide activator of the NRF2/SKN-1 signaling pathway. We demonstrate that hydralazine extends healthy lifespan (~25%) in wild type and tauopathy model C. elegans at least as effectively as other anti-aging compounds, such as curcumin and metformin. We show that hydralazine-mediated lifespan extension is SKN-1 dependent, with a mechanism most likely mimicking calorie restriction. Using both in vitro and in vivo models, we go on to demonstrate that hydralazine has neuroprotective properties against endogenous and exogenous stressors. Our data suggest that hydralazine may be a viable candidate for the treatment of age-related disorders.

WOE am 11:34
Urine proteomics for biomarker discovery in neurodegenerative disease

Jenny Hällqvist[1]; Ross W. Paterson[2]; Robert Clayton[1]; Nick Fox[2]; Jonathan M. Schott[2]; Henrik Zetterberg[3, 5]; Amanda Heslegrave[3, 4]; Wendy Heywood[1]; Kevin Mills[1]
[1]GOS Institute of Child Health, UCL, London, UK; [2]Dementia Research Centre, UCL IoN, London, UK; [3]Department of Molecular Neuroscience, UCL IoN, London, UK; [4]UK Dementia Research Institute at UCL, London, UK; [5]Clinical Neurochemistry Laboratory, SUH, Mölndal, Sweden

Alzheimer's disease (AD) is a progressive neurodegenerative condition characterised by the build-up of amyloid plaques and neurofibrillary tangles in the brain. It is the most common type of dementia and close to 50 million people live with the disease. AD is predicted to affect an ever increasing percentage of an ageing population, the vast majority sporadic rather than inherited cases. Along with lifestyle factors, the *APOE* ε4 genotype and variation in genes involved in cholesterol metabolism, immune response and endocytosis have been found to increase the risk. Even though AD was described more

than a century ago, there are no approved disease modifying therapies yet. This is partly due to the lack of early diagnostic biomarkers which make pre-symptomatic clinical trials challenging. Biomarkers are also valuable for measuring target engagement and predicting disease progression.

In this work, we set out to investigate whether urine can be used for biomarker discovery in neurodegenerative disease. Urine is easily and non-invasively collected and in plentiful supply. It contains information from nearby tissues and blood perfusing distant organs. Urine from six AD patients and five healthy controls were analysed using a refined deep phenotyping approach with 10 fractions 2D-LC-MSMS bottom-up MS^e proteomics. The run time for each sample was 10 hours and more than 1500 proteins were detected using only 2 mL of urine.

33 proteins were observed to be differentially expressed between AD and control, several previously described in AD studies. Using Ingenuity pathway analysis, we found that the top enriched disease was AD ($p < 0.0001$). Validation of these markers were confirmed by the development of a targeted multiplexed LC-MS assay. Two proteomic biomarkers were confirmed to be significantly downregulated in AD patients compared to controls ($p < 0.05$). The development of the assay and relevance of the biomarkers will be discussed.

WOE am 11:46
Autoantigen Anoctamin 2 associates with increased risk for multiple sclerosis

Peter Nilsson
KTH - Royal Institute of Technology, Solna, Sweden

Multiple sclerosis (MS) is a chronic demyelinating and inflammatory disease of the central nervous system which is likely driven by autoimmune mechanisms and caused by a combination of genetic predispositions and environmental factors. We have previously identified the calcium activated chloride-channel protein Anoctamin 2 (ANO2) as an autoantigen associated to MS 1,2. Here we have in a validation study determined IgG reactivity towards ANO2 and the Epstein-Barr virus nuclear antigen 1 (EBNA1) using a suspension bead array immunoassay in 7600 additional MS cases and 6200 controls and confirmed increased ANO2 autoantibody levels in MS. The ANO2-seropositivity was here defined as 5.2% in MS cases and 1.9% in controls resulting in an odds ratio for MS of 2.2.

The odds ratio for MS in individuals carrying the three MS risk factors: ANO2-seropositivity, HLA-DRB1*15:01 and high antibody reactivity against EBNA1 was 16. This was twice as high compared to the OR for MS in individuals with the first two factors but without ANO2-seropositivity.

[1] *Ayoglu 2013. Autoantibody profiling in multiple sclerosis using arrays of human protein fragments. Mol Cell Proteomics.*

[2] *Ayoglu 2016. Anoctamin 2 identified as an autoimmune target in multiple sclerosis. Proc Natl Acad Sci USA.*

WOE am 11:58
An update on the HUPO Brain Protein Project (HBPP)

Klaus Oliver Schubert[1, 2]; Katrin Marcus[4]; Robert McCollumsmith[5]; Daniel Martins-De-Souza[6]; Charlotte Teunissen[7]; Peter Nilsson[3]

[1]University of Adelaide, Adelaide, Australia; [2]Northern Adelaide Mental Health Service, Salisbury , Australia ; [3]KTH Royal Institute of Technology, Stockholm, Sweden; [4]Ruhr University, Bochum , Germany; [5]University of Toledo, Toledo, OH; [6]University of Campinas (UNICAMP), Campinas, Brazil; [7]VU University, Amsterdam, Netherlands

The HUPO Brain Protein Project is an initiative under the B/D HPP umbrella, aiming to bring together neuroproteomic researchers to promote and disseminate large-scale and targeted state-of-the-art proteome studies on human brain, brain-related body fluids such as CSF, and pre-clinical models to decipher the role of proteins in brain development, health and disease.

The HBPP promotes an annual Spring Workshop every year and a neuroproteomics event at every HUPO meeting. At these interactive meetings, delegates share scientific and technological updates, network, and form ideas for future collaborations or academic exchange programmes. Coordinated by an international steering committee of scientists from 4 continents and 6 countries, HBPP encourages participation of young neuroproteomics scientists and clinicians to accelerate capacity building and clinical translation.

This presentation gives an overview and update of the recent activities of HBPP.

2:00 - 3:50 pm Wednesday
DRUG DISCOVERY
Session Chair: Mike Tyers
Oceana 8-10

WOA pm 2:00
Protein Acylation is a General Regulatory Mechanism in Biosynthetic Pathway of Acyl-CoA-Derived Bioactive Natural Products

Minjia Tan
Shanghai Institute of Materia Medica,, Shanghai, China

Coenzyme A (CoA) esters of short fatty acids (acyl-CoAs) function as key precursors for the biosynthesis of various bioactive natural products and the dominant donors for lysine acylation. Herein, we investigated the functional interplay between beneficial and adverse effects of acyl-CoA supplements on the production of acyl-CoA-derived natural products in microorganisms by using erythromycin biosynthesized Saccharopolyspora erythraea as a model: accumulation of propionyl-CoA benefited erythromycin biosynthesis, but lysine propionylation inhibited the activities of important enzymes involved in biosynthetic pathways of erythromycin. The results showed that the overexpression of NAD + -dependent deacylase could circumvent the inhibitory effects of high acyl-CoA concentrations. In addition, we demonstrated the similar lysine acylation mechanism in other acyl-CoA-derived natural product biosynthesis, such as malonyl-CoA-derived alkaloid and butyryl-CoA-derived bioalcohol. These observations systematically uncovered the important role of protein acylation on interaction between the accumulation of high concentrations of acyl-CoAs and the efficiency of their use in metabolic pathways.

WOA pm 2:20
Interactome rewiring following pharmacological targeting of BET bromodomains

Jean-Philippe Lambert[1]; Sarah Picaud[2]; Takao Fujisawa[3]; Huayun Hou[4]; Pavel Savitsky[2]; Liis Uusküla-Reimand[4]; Gagan D. Gupta[1]; Hala Abdouni[1]; Zhen-Yuan Lin[1]; Monika

Tucholska[1]; James D.R. Knight[1]; Beatriz Gonzalez-Badillo[1]; Nicole St-Denis[1]; Joseph A. Newman[2]; Manuel Stucki[5]; Laurence Pelletier[1, 4]; Nuno Bandeira[6]; Michael D. Wilson[4, 4]; Panagis Filippakopoulos[2, 3]; Anne-Claude Gingras[1, 4]
[1]Lunenfeld-Tanenbaum Research Inst at Mt Sinai Hosp, Toronto, Canada; [2]Structural Genomics Consortium, Oxford, UK; [3]Ludwig Institute for Cancer Research, Oxford, UK; [4]University of Toronto, Toronto, Canada; [5]University of Zurich, Schlieren, Switzerland; [6]University of California, San Diego, San Diego, CA

Targeting bromodomains (BRDs) of the bromo-and extra-terminal (BET) family has offered opportunities for the therapeutic intervention of cancer and other diseases. However, there has not been a systematic study of the consequences of BET BRD inhibition on the interactions established by members of this important protein scaffolding family. Here, we profile the interactomes of BRD2, BRD3, BRD4 and BRDT during a time course of treatment with the pan-BET BRD inhibitor JQ1, revealing broad rewiring of the interaction landscape, with three distinct classes of behaviour for the 603 unique interactors identified. We report a group of proteins that associate with BET BRDs through canonical and new binding modes that dissociate upon inhibitor treatment. We further establish two classes of extra-terminal (ET)-domain binding motifs that mediate acetylation-independent interactions, offering attractive possibilities for specific therapeutic modulation. Lastly, we identify an unexpected increase in several interactions following JQ1 treatment. These increased interactions allow us to define new negative functions for BRD3 in the regulation of rRNA synthesis and potentially Pol II-dependent gene expression that result in decreased cell proliferation. Together, our data outline the contributions of BET protein modules to their interactomes and allow for a better understanding of pharmacological rewiring in response to JQ1.

WOA pm 2:40
Multipronged Quantitative Proteomics Analyses Reveal Alterations in Kinase Activities as a Fundamental Mechanism of Action of Circadian Period Altering Drugs

Sandipan Ray[1, 2]; Akhilesh B. Reddy[1]
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Circadian clocks exist in almost all living organisms and play a fundamental role in regulating daily physiological and behavioural processes. Drugs or small molecules that are capable of modulating circadian rhythms, could be important in developing therapeutic approaches directed towards diverse types of human diseases associated with circadian dysfunctions, such as shift-work disorder, jet lag, and sleep disorders. In this study, we carried out an integrated global proteome and phosphoproteome analysis, thermal proteome profiling (TPP), and kinome profiling to decipher the molecular targets of seven circadian period modulating drugs in the human osteosarcoma cell line – a robust and well-characterized circadian model system. Luminescence rhythms of Per2-dluc cells indicated that Longdaysin drastically lengthened circadian period. Roscovitine, Purvalanol A, and SP600125 also introduced an increase in period length, while treatment with Chir99021, 1-Azakenpaullone, and Indirubin-3′ oxime resulted shorter circadian periods. TMT-based quantitative proteomics analysis revealed increased expressions of kinase inhibitors and reduced levels of cyclin-dependent and other kinases (Casein kinases, Calcium/calmodulin-dependent protein kinases, Serine/threonine protein kinases and GSK-3β) after treatment with these drugs, which were validated further by kinase enrichment assays using desthiobiotin nucleotide probes. An integrated phosphoproteomics analysis indicated altered phosphorylation levels of several proteins associated with diverse signalling pathways and circadian rhythmicity. Moreover, TPP analysis revealed direct binding of some of these drugs with clock regulatory kinases and their modulators. Notably, radioactivity-based biochemical assays indicated inhibition of CAMK1/2, CDK2/7, CK1d, TNIK and STK26 activity as a common mechanism for the circadian period lengthening drugs. Taken together, our findings reveal that these compounds modulate circadian period through inhibition of multiple kinases. We anticipate that this comprehensive study will contribute towards a better understanding of the mechanism of action of these circadian period altering drugs. This is critical in clearly defining molecular targets in order to modulate daily rhythms for therapeutic benefit.

WOA pm 2:52
Limited Proteolysis as a Target Deconvolution Strategy in Mammals

Nigel Beaton[1]; Roland Bruderer[1]; Ilaria Piazza[2]; Paola Picotti[2]; Lukas Reiter[1]
[1]Biognosys, Schlieren, Switzerland; [2]ETH Zurich, Zurich, Switzerland

High attrition rates in target-centric approaches, as well as a limited number of targets have shifted the focus of drug development back towards phenotypic screening[1]. In parallel novel proteomics-based target deconvolution approaches to drug target identification, like thermal proteome profiling, have gained popularity[2]. Limited proteolysis (LiP) is a new target deconvolution technique that exploits protein conformational changes driven by drug binding. A major advantage of LiP is its unique focus on novel ‘conformotypic’ peptides that are generated by a limited, unspecific digestion and subsequently identified by proteomic analysis[3]. To date LiP has been used to identify novel drug and metabolite targets in yeast and bacteria[4]. However, to find widespread use in commercial drug development it is imperative that LiP’s applicability is broadened, including to mammalian systems, which are substantially more complex. We demonstrate its reproducibility and performance in human cell lysate.

Rapamycin, a potent immunosuppressant, inhibits mTOR via FKBP1A binding[5]. Herein, we demonstrate in HeLa lysate that our modified LiP approach reproducibly identified several conformotypic FKBP1A peptides with high confidence, a finding yet to be shown in a mammalian context. Notably, these same peptides displayed a strong dose-response correlation with increasing rapamycin concentrations. A similar dose-response relationship could be shown for the protein targets of the phosphatase inhibitor Calyculin A, a compound that has never been used in target deconvolution. Importantly, we were also able to confirm known target affinities via extrapolation of IC50 values. Additionally, we identified a previously unknown target of Calyculin A among the same phosphatase family.

Collectively, our data demonstrates that LiP can be used to effectively identify protein targets in mammalian cells and characterize their binding properties (e.g. IC50). These capabilities make LiP a powerful target deconvolution strategy

with the potential to become an essential part of the drug development pipeline.

WOA pm 3:04
Drug effects on protein homeostasis

Nico Zinn[1]; Maria Faelth-Savitiski[1]; Mikhail Savitski[2]; Giovanna Bergamini[1]; Marcus Bantscheff[1]
[1]Cellzome, A GSK company, Heidelberg, Germany; [2]EMBL, Heidelberg, Germany

Protein degradation plays important roles in biological processes and is tightly regulated. Further, targeted proteolysis is an emerging research tool and therapeutic strategy. However, proteome-wide technologies to investigate the causes and consequences of protein degradation in biological systems are lacking. We recently developed 'multiplexed proteome dynamics profiling', mPDP[1], a mass spectrometry-based approach combining dynamic-SILAC labelling with isobaric mass tagging for multiplexed analysis of protein degradation and synthesis. When applied in combination with other quantitative proteomics approaches such as chemoproteomics and thermal protein profiling, mPDP provides unique insights in drug mechanism-of-action. The presentation will focus on the MoA of targeted protein degraders (PROTACs) and describes a novel approach for identifying deubiquitinase substrates and targets of DUB inhibitors.

1. Savitski MM et al., Cell 173, 260-274 (2018)

WOA pm 3:16
Building a toolkit for studying the orphan kinome

Laurie Parker[1]; Minervo Perez[1, 2]; John Blankenhorn[1]; Lindsay Breidenbach[1]; Hannah Peterson[1]
[1]University of Minnesota, Minneapolis, <Not Specified>; [2]Purdue University, West Lafayette, IN

Protein phosphorylation by kinases is a major mechanism of cell signaling, and is involved in almost all aspects of cell biology. Kinase dysregulation is a key factor in diseases like cancer, and kinases are one of the major drug targets in oncology. However, despite decades of research and billions of dollars in drug discovery efforts on kinases, relatively few are well characterized. The majority of the ~90 tyrosine kinases are considered "orphans," for which few to no substrates, and thus few details about biological pathways and roles, are known. Without substrates to use as activity probes, inhibitors for use as tool compounds or potential therapeutics cannot be discovered. We have developed an adaptation of the Kinase Assay Linked with Phosphoproteomics (KALIP) strategy (originally from Andy Tao's lab) to incorporate empirically-determined substrate profiling data into our KINATEST-ID bioinformatics pipeline to efficiently tackle the orphan kinase problem, determine substrate preferences and design novel substrate tools. Protease-digested peptides from cell lysates are stripped of pre-existing phosphates, then re-phosphorylated with a kinase of interest. The resulting phosphopeptides are enriched and analyzed using mass spectrometry. Phosphopeptide sequences are extracted from the peptide ID list and funneled through the KINATEST-ID pipeline using a set of scripts implemented in GalaxyP, to define substrate sequence preferences and propose candidate optimal substrate peptides. Those are then synthesized and tested for phosphorylation efficiency by the target kinase. Using this approach, we have characterized substrate preferences for several understudied kinases for which few validated substrates were known, including FLT3 and two clinically relevant mutants, ALK and two clinically relevant fusion variants, and BTK. Current and future efforts are to broaden the scope of kinases characterized using this streamlined phosphoproteomics/bioinformatics pipeline and proceed with systematically defining substrate information and developing novel tools for the rest of the orphaned kinases in the kinome.

2:00 - 3:50 pm Wednesday
NEW MASS SPEC TECHNOLOGIES
Session Chairs: Lissa Anderson and John Yates
Oceana 7

Livia Eberlin's invited talk is generously supported by

WOB pm 2:00
Development of the MasSpec Pen for Tissue Analysis and Cancer Diagnosis

Livia Eberlin
University of Texas at Austin, Austin, TX

Implementation of new technologies that provide precise molecular diagnosis of tissues is highly desirable to guide treatment strategies and improve patient care. Tissue diagnosis is critical during surgical removal of solid cancers for surgical margin evaluation and optimal excision. Molecular technologies offer the exciting opportunity to incorporate cancer-specific biomarkers into clinical decision making for improved cancer detection and diagnosis. In particular, mass spectrometry techniques provide the specificity and sensitivity necessary for near real time assessment of molecular signatures. We have recently reported the development of an innovative handheld MS device, termed the "MasSpec Pen", for rapid and non-destructive tissue analysis and cancer diagnosis. The MasSpec Pen is a handheld device that employs a single water droplet to gently extract molecules from tissues upon contact. The discrete water droplet is then transferred through a tubing system to a high-performance orbitrap mass spectrometer, where analysis and statistical classification are performed to provide a rapid predictive diagnosis. The non-destructive nature of the MasSpec Pen allows direct, gentle, and rapid analysis of tissues (<10 seconds) and the unparalleled analytical performance provided by mass spectrometry further enables untargeted and accurate molecular diagnosis. In this presentation, I will describe the development of this method, the key features of the MasSpec Pen platform, and recent advances in design and automation. In addition, I will describe key applications, results, and validation studies using the MasSpec Pen for in vivo and ex vivo tissue identification and cancer diagnosis.

WOB pm 2:20
The Important Role of Proteoforms in Human Health and Disease:The Significance and Relevance of the Cell-Based Human Proteome Project

Neil Kelleher
Northwestern University, Evanston, United States

The top-down, intact analysis of proteins under either denaturing or native conditions has progressively led to the recognition that the new currency for proteomic analysis should be the proteoform. In order to catalog a protein with complete molecular specificity (i.e. proteoform identification and characterization), top-down proteomic approaches will have to be performed and adopted by a larger portion of the proteomic community. A proteoform includes not only the observed amino

acid sequence of a protein, but also includes any amino acid substitutions (i.e. mutations) and any post-translational modifications and the co-occurring combinations of these protein "decorations." This top-down approach introduces multiple possible avenues for new and interesting hypotheses about the central role of proteoforms in basic cellular biochemistry. With top-down proteomics, we have mapped 11 unique proteoforms from the oncogene KRAS from colorectal cancer. These proteoforms expand our understanding of how oncogenic mutations of KRAS affect post-translational modifications elsewhere on the same protein molecule. Finally, we have applied top-down proteomic analysis to FACS-sorted human lymphocytes to identify multiple proteoforms that differentiate cell populations as a part of funding from the Paul G. Allen Frontiers Group and all identified proteoforms from this project are a part of the Allen Proteoform Atlas and the Consortium for Top-Down Proteomics. Taken together, these projects and results underscore the importance of leveraging the significant gains made with the Human Proteome Project (HPP) and pivoting toward a Cell-Based Human Proteome Project (CB-HPP). Just as the Human Genome Project has sparked a revolution in analysis of nucleic acids, a cell type-specific, comprehensive human proteoform atlas could advance society's understanding of how proteoforms influence human health and disease.

WOB pm 2:40

Trapped ion mobility mass spectrometry for improved sensitivity and fastest proteomics

Oliver Raether[1]; Markus Lubeck[1]; Heiner Koch[1]; Scarlet Koch[1]; Florian Meier[2]; Andreas Brunner[2]; Juergen Cox[2]; Matthias Mann[2]

[1]Bruker Daltonik GmbH, Bremen, Germany; [2]Max Planck Institute of Biochemistry, Martinsried, Germany

Background: With the previously introduced parallel accumulation - serial fragmentation method (PASEF, Meier et al., JPR 2015) for combined trapped ion mobility spectrometry (TIMS) quadrupole time of flight (QTOF) instruments, five to ten times faster data dependent acquisition of fragment ion spectra became possible. With a distinguished two-dimensional precursor selection algorithm using mass and ion mobility information, the limit of quantitation (LOQ) could be reduced by directing multiple PASEF MS/MS scans to low abundant species.

Methods: Low peptide sample amounts (12 - 100 ng) of a HeLa protein digest and IMAC phosphopeptide enrichments from 200 µg proteolytic digests were HPLC separated (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) and analyzed on a timsTOF Pro instrument (Bruker Daltonics) with different LC gradients from 30 - 90 min; gradients down to 5 min were used with the Evosep One HPLC (Evosep, Denmark). Proteomics ID, label free quantitation and isobaric labeling data analysis are performed using DataAnalysis (Bruker), Mascot (www.matrixscience.com), PEAKS studio (Bioinformatics Solution Inc.) and MaxQuant (Cox group, MPI of Biochemistry).

Results: We show very fast acquisition speed of more than 100 Hz enabling the identification of hundreds of peptides per minute; instrument performance on low sample amounts, offering new possibilities to investigate samples at high sensitivity; high-throughput DDA measurements with remarkable depth at 30 - 90 min gradient length resulting in identifications of more than 50000 unique peptides and up to 6000 protein groups; more than 4 orders of magnitude quantification range for accurate label free analysis of different samples. Shorter gradients enable 200 samples per day, each with 7000 unique peptides identified.

WOB pm 2:52

EASI-tag enables accurate multiplexed and interference-free MS2-based proteome quantification

Sebastian Virreira Winter; Florian Meier; Christoph Wichmann; Juergen Cox; Matthias Mann; Felix Meissner

Max Planck Institute of Biochemistry, Martinsried/Munich, Germany

We developed EASI-tag (Easily Abstractable Sulfoxide-based Isobaric tag), a new generation of amine-derivatizing and sulfoxide-containing isobaric labelling reagents, which dissociate at low collision energy and generate peptide-coupled, interference-free reporter ions with high yield. Efficient isolation of 12C precursors and quantification at the MS2 level enable accurate determination of quantitative differences between currently up to six multiplexed samples. EASI-tag makes accurate quantification by isobaric labeling applicable to widely-used mass spectrometers.

WOB pm 3:04

Molecularly Imprinted Polymers (MIPs) for the detection of low abundance proteins as biomarkers for lung cancer

Rachel Norman; Sergey Piletsky; Leong Ng; Antonio Guerreiro; Francesco Canfarotta; Donald Jones

University of Leicester, Leicester, United Kingdom

MIPs are like plastic antibodies which are produced by polymerising a monomer such as acrylamide in the presence of a template. Once the template is removed, the MIP is capable of rebinding it. MIPs could be used to enable the detection of low abundance proteins by specifically enriching them in biological samples. Thus, early diagnosis of diseases such as lung cancer could be achieved. As most patients present with advanced disease which is more difficult to treat, lung cancer prognosis is poor. It is currently the most common cause of cancer-related death but prompt detection would improve survival. However, many potential biomarkers are present at sub-ng/mL levels and are undetectable by current methods. KRAS is a low abundance protein which is involved in the early development of many cancers. Single amino acid substitutions, predominantly at position 12, result in constitutive activation of the protein which leads to aberrant cell growth.

MIPs were produced using a C-terminal peptide from the KRAS protein as the template then immobilised on glass beads. Plasma samples from non-small cell lung cancer (NSCLC) patients with known KRAS mutation status were incubated with the MIPs on Spin X centrifugal filters. After washing to remove other proteins, KRAS was digested on the MIPs before selected reaction monitoring (SRM) LC-MS/MS analysis on a Waters NanoACQUITY UPLC coupled to a Waters Xevo triple quadrupole mass spectrometer. Initial results in plasma showed that wild-type KRAS could be detected in a healthy control sample and the G12C mutant peptide was detected in a patient plasma sample, corroborating with the DNA mutation data for the same sample. Results from further clinical studies will be presented. It is hoped that MIPs can be used for a panel of biomarkers for early diagnosis of NSCLC and to predict response to treatment.

WOB pm 3:16

Protein analysis using sub-nanopore sensors

Mikhail Kolmogorov[1]; Gregory Timp[2]; Pavel Pevzner[1]

[1]UC San Diego, La Jolla, California; [2]University of Notre Dame, Notre Dame, IN

Recent advances in top-down mass spectrometry enabled identification of intact proteins, but this technology still faces challenges. For example, top-down mass spectrometry suffers from a lack of sensitivity since the ion counts for a single fragmentation event are often low. In contrast, nanopore technology is exquisitely sensitive to single intact molecules. The electrical blockade current that develops when a single molecule immersed in electrolyte is impelled by an electric field through a nanopore can inform on the molecular structure. So far, DNA has been sequenced this way. In this report, we explore the potential of single molecule protein analysis using a sub-nanometer-diameter pore, i.e. a sub-nanopore, sensor. First, we show that fluctuations in the blockade current, associated with the translocation of a denatured protein through the sub-nanopore, are related to the primary structure of the protein. Next, we present different computational models for protein translocation through a sub-nanopore, that generates the electrical current blockade signal (nanospectrum). Finally, we discuss the related algorithmic challenges, such as noise reduction and protein identification.

WOB pm 3:28

MaxQuant software for ion mobility enhanced shotgun proteomics

Nikita Prianichnikov[1]; Christoph Wichmann[1]; Scarlet Beck[2]; Heiner Koch[2]; Markus Lubeck[2]; Romano Hebeler[2]; Juergen Cox[1]

[1]Max Planck Institute of Biochemistry, Martinsried, Germany; [2]Bruker Daltonik GmbH, Bremen, Germany

Ion mobility adds a dimension to LC-MS based shotgun proteomics which potentially can boost proteome coverage, quantification accuracy and dynamic range. Required for this is suitable software that extracts the information contained in the four-dimensional data space spanned by m/z, retention time, ion mobility and signal intensity. Here we describe the ion mobility enhanced MaxQuant software, which utilizes the added data dimension. It offers an end to end computational workflow for the identification and quantification in LC-IMS-MS/MS shotgun proteomics data. Here we apply it to trapped ion mobility spectrometry (TIMS) coupled to a quadrupole time-of-flight (QTOF) analyzer. Application to benchmark datasets show unprecedented identification depth in shingle shot HeLa runs and precise ratio quantification in datasets with known ground truth.

We adapted the complete MaxQuant shotgun proteomics workflow to process data with the added ion mobility dimension. Most adaptations were done in the feature detection workflow which now produces 4D peaks. De-isotoping and assembling of MS1 labeling multiplets utilize intensity profile correlations also over the ion mobility direction. Matching MS1 features between runs, the transfer of identifications without MS/MS identifications, is making use of the ion mobility coordinates to become more specific. Peptide identification, FDR, protein assembly, label-free quantification and isobaric labeling quantification algorithms are included.

We performed benchmark measurements on a Bruker timsTOF Pro instrument with 'Parallel Accumulation followed by SErial Fragmentation' (PASEF) functionality for the acquisition of MS/MS spectra. We generated benchmark datasets in which HeLa and E. coli proteomes were mixed in replicates in defined ratios of 1:2 and 1:5 which were compared to 1:1 replicates with the MaxLFQ algorithm for label-free quantification. The resulting measured ratios were found to be uncompressed and precise. We provide a LC-TIMS-MS data viewer that shows map views of any slice of the data cube for inspecting the 4D features.

2:00 - 3:50 pm Wednesday
METABOLISM
Session Chair: Robert Moritz
Oceana 6

WOC pm 2:00

Lipids: Why Bother? From R&D to Clinical Applications

Anne K. Bendt; Tze Ping Loh; Markus R. Wenk

National University of Singapore, Singapore, -

Due to their central role in physiology and metabolism, lipids and their corresponding enzymes present us with powerful opportunities to understand, describe and influence human health and diseases. Not surprisingly, they have been exploited as drug targets and diagnostic markers for decades i.e. via therapeutic statins and cholesterol biomarkers.

With the recent advances in technology, mass spectrometry has become a key driver for lipid analysis. At SLING, the Singapore Lipidomics Incubator anchored at the National University of Singapore, we capitalize primarily on LC-MS/MS to develop robust assays to quantitate lipids and other small molecules from biological specimen such as human plasma. A key aim is to understand how lipid levels vary already between and within healthy individuals, i.e. natural biological variation. Being embedded in the multi-racial environment of Singapore, we have a special focus on ethnicity.

Results from our ongoing efforts to describe natural biological variation in cohorts comprising our three main ethnicities (Chinese, Indian and Malay) will be discussed.

Secondly, the harmonization and standardization of the developed LC-MS-based workflows are of crucial importance to enable comparability and traceability of derived data across time and space. A community-initiated position paper to develop accepted guidelines has just been published (Burla et al., JLR).

To now drive the translation of R&D findings into clinical applications, we created a model for engagement of key stakeholders. Our team comprises of four core competencies: top-notch lipidomics R&D, data integration, clinical trial capabilities and last but not least laboratory medicine as the end user. Importantly, the selection of target molecules is needs-driven: by close interaction with clinicians we move from a mere technical 'push' towards a clinician's 'pull', i.e. identifying current gaps in the clinical pathway and developing appropriate methods to address unmet diagnostic needs, with a clear application in mind.

WOC pm 2:20
Healthy Lipidome is a Resource for Understanding Metabolic Diseases

Andrej Shevchenko
MPI of Molecular Cell Biology and Genetics, Dresden, Germany

A typical blood test reports more than 30 clinically relevant indexes and qualified physicians compare the determined values with corresponding ranges established for the physiological norm. Although human blood plasma lipidome has been extensively used in sheer biomarker discovery efforts, its basal composition received little attention. No generally accepted reference values for individual lipids are available and the impact of gender, age, ethnic background and staple diet is poorly understood. Not surprisingly, lipidomic measurements are of potential interest, yet little direct relevance for the broad community of clinicians.

We employed quantitative shotgun lipidomics on a high resolution mass spectrometer to determine the absolute (molar) concentrations of 281 molecular species from 27 major lipid classes and established their reference values and ranges of variation for a representative cohort of healthy young Caucasians of both genders. We found that gender is a major and previously underestimated lipidomic factor that alters the concentrations of 112 plasma lipids and the total abundance of 21 lipid classes. Within healthy gender-restricted cohorts lipidomics revealed a compositional signature correlating with clinical trends towards developing a metabolic syndrome. We reasoned that accurate quantification of the healthy plasma lipidome followed up by longitudinal observational studies could reveal minor, yet highly specific signs of emerging metabolic abnormalities, which are later obscured by massive changes triggered by unfolding metabolic syndrome. These trends were further correlated with metabolic signatures revealed by shotgun analysis of more than 350 human liver biopsies from patients diagnosed with NAFLD.

WOC pm 2:40
Plasma Biomarkers of Cardiovascular Disease Associated with Type 1 Diabetes in Children

Chantal Attard[1]; Stefan Bjelosevic[2]; Jasmine Wong[1]; Fergus Cameron[1, 3]; Paul Monagle[2, 3]; Vera Ignjatovic[1, 2]
[1]Murdoch Children's Research Institute, Parkville, Australia; [2]The University of Melbourne, Parkville, Australia; [3]Royal Children's Hospital, Parkville, Australia

There has been a rising incidence of Type 1 diabetes (T1D) in both children and adults worldwide. Studies have shown that having T1D is an important risk factor for Cardiovascular Disease (CVD) in adults, however, there are limited studies focusing on this concept in children. Detection of CVD markers in children with T1D provides opportunities for early intervention and prevention of disease progression. This study aimed to identify early markers of Cardiovascular Disease in children with Type 1 diabetes.

Blood samples were collected from a total of 20 participants aged 8-11 years old, with 10 samples from T1D children and 10 samples from healthy age-matched controls. Sampling involved exclusive selection of children with sub-optimally controlled diabetes (HbA1C > 7.5 %) since this specific population is at the highest risk of developing T1D related complications (including CVD). The expression of 92 proteins known to be associated with CVD was evaluated using the Proseek® Multiplex CVD I96×96 immunoassay panel in children from both groups. Dynamic principle component analysis (PCA) was performed to determine differentially expressed proteins before the two groups.

Of the 92 CVD markers investigated, 12 proteins were differentially expressed in the T1D group compared to healthy children. These were: Kallikrein-6, Agouti-related protein, Platelet endothelial cell adhesion molecule, Pentraxin-related protein PTX3, TNF-related activation-induced cytokine (TRANCE), Follistatin, Beta-nerve growth factor (β-NGF), Prolactin, Angiopoietin-1-receptor, Resistin, N-terminal pro-B-type natriuretic peptide, and Galectin-3.

We suggest that differentially expressed proteins identified in this study may serve as early markers of CVD in children with T1D, a population that is at high risk of developing CVD in adulthood. The proteins identified to have the greatest potential for clinical use are β-NGF, Galectin-3, and TRANCE.

WOC pm 2:52
Multi-omic profiling of the liver in a rat model of type 2 diabetes

Desmond Li[1]; Lauren Smith[1]; Yen Chin Koay[1, 2]; Holly McEwen[1, 3]; Anthony Don[1, 3]; John O'Sullivan[1, 2]; Stuart Cordwell[1]; Melanie White[1]
[1]University of Sydney, Sydney, Australia; [2]Heart Research Institute, Sydney, Australia; [3]ACRF Centenary Cancer Research Centre, Sydney, Australia

Altered glucose metabolism via insulin resistance is a hallmark of type 2 diabetes (T2D), clinically observed as the inability to maintain postprandial blood glucose levels (BGL). Associated with energetic excess arising from caloric overload, T2D is linked to excess non-esterified fatty acid production and rising nutrient levels, which influence metabolic processes. The liver plays a pivotal role in the pathogenesis of T2D, via elevated gluconeogenesis, whereby glycogen stores are liberated, further elevating BGL. It is important to understand the molecular adaptations of the liver to the metabolic flux and insulin resistance arising from T2D. To achieve this we performed a multi-omic analysis including proteomics, lipidomics and metabolomics in a rat model of T2D combining the effects of high fat diet feeding (calorie overload) and streptozotocin (elevated BGL). To quantify alterations in protein abundance, samples were isobarically tagged prior to mass spectrometry (MS). Discovery lipidomics was achieved with relative quantitation by comparison with synthetic standards. Targeted metabolomics was performed using multiple reaction monitoring, in the presence of deuterated metabolite standards. We quantified close to 7,000 proteins, 300 lipid species and 100 metabolites in the course of this study. Proteomics revealed increased levels of proteins regulating phospholipid biosynthesis and fatty acid metabolism. A concurrent decrease in proteins involved in steroid biosynthesis was observed. Lipid analysis show increased sphingomyelin levels and decreased levels of phosphatidylcholines in T2D, both of which are components of cell membranes and can play a role in metabolic and apoptotic signalling. Elevated levels of branched chain amino acids as well as changes in metabolites indicative of altered energy and amino acid metabolism were detected by metabolomics. The current study has identified changes in protein, lipid composition and metabolite levels indicative of dysregulated energy utilisation and molecular adaptations that contribute to the pathogenesis of T2D.

WOC pm 3:04
Urinary peptidomic analysis reveals bioactive uromodulin peptides in early type 1 diabetes

Julie Van[1]; Sergi Clotet Freixas[2]; Xiaohua Zhou[1]; Ihor Batruch[3]; Etienne Sochett[1]; Farid Mahmud[1]; Eleftherios Diamandis[1, 3]; James Scholey[1, 2]; Ana Konvalinka[1, 2]
[1]University of Toronto, Toronto, Canada; [2]University Health Network, Toronto, Canada; [3]Mount Sinai Hospital, Toronto, Canada

Background: Diabetic nephropathy is the leading cause of kidney disease worldwide. Yet, current treatments cannot prevent the progressive nature of the disease, exposing our limited understanding of the early kidney response to chronic hyperglycemia. In this study, we examined the urine peptidomes of youths with type 1 diabetes without evidence of nephropathy and age/sex-matched healthy controls, in order to determine early changes in protein processing in the hyperglycemic kidney.

Methods: The study population comprises two separate cohorts: a discovery cohort (N = 30) and an internal validation cohort (N = 30). Urines were normalized to creatinine and underwent 10kDa-filter centrifugation to isolate naturally occurring peptides. For discovery, filtered peptides were then fractionated by strong cation exchange liquid chromatography and analyzed on a Q-Exactive mass spectrometer. For verification, parallel reaction monitoring (PRM) assays were developed and performed on a Q-Exactive HFX. Maxquant and Skyline were used for peptide identification and quantification.

Results: A total of 6349 urinary peptides from 750 proteins were quantified from 15 cases with type 1 diabetes and 15 healthy controls. Of the 15 differentially excreted peptides ($P < 0.05$), five remained significant after Benjamini-Hochberg adjustment ($q < 0.05$). Seven of these top 15 peptides derive from the C-terminus of uromodulin (a kidney-specific protein), which contains the functional domain that regulates uromodulin polymerization. Differential excretions of six uromodulin peptides were validated using PRM. Two of the differentially excreted uromodulin peptides significantly induced NF-κB and AP-1 signaling in HK-2 cells, suggesting that these peptides may mediate inflammation in kidney cells.

Conclusions: Differences between youths with type 1 diabetes and healthy controls are reflected in the urinary peptidome before the development of microalbuminuria. Uromodulin peptides we discovered may play a significant role in the early injury in a diabetic kidney and may represent a therapeutic target.

WOC pm 3:16
Elucidating changes in plasma protein profiles related to bariatric surgery: an IMI DIRECT study.

Ragna Häussler[1]; Matilda Dale[1]; Mun-Gwan Hong[1]; Cecilia Thomas[1, 2]; Johann Gassenhuber[3]; Violeta Raverdy[4]; Francois Pattou[4]; Jochen Schwenk[1]
[1]Science for Life Laboratory, Solna, Sweden; [2]The Novo Nordisk Foundation Center, Copenhagen, Denmark; [3]Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany; [4]Inserm U1190, Lille, France

One major risk factor for the development and progression of type 2 diabetes (T2D) is obesity. Bariatric surgery induces significant and often lasting weight reduction and T2D remission. To study the imminent effects of surgery, we profiled the plasma proteomes of patients before and after surgery. Conducted within the EU IMI project DIRECT, we used three affinity proteomics platforms: a 384-plexed antibody bead array (SBA), a 4-plexed microfluidic sandwich assay (MSA), and a 90-plexed proximity extension assay (PEA). The study comprised four study sets with samples collected before and 3-months after surgery: Serum from two sets with 67 and 74 subjects was used for discovery; for validation, sets of EDTA-plasma from two mixed-meal-tolerance-studies (MMTT) with 7 and 24 subjects was analyzed. We used slopes of quantitative changes between baseline and 3-months samplings to describe how individuals responded to treatment and recorded Bonferroni-corrected p-values.

Using SBA assays for both discovery sets, we identified IGFBP-2 and others with differential abundance before and after surgery. We validated our findings using the MSA platform and expanded our analysis in MMTT plasma samples using PEA assays. In the latter, we confirmed significant consistent increases after surgery for IGFBP-2 ($p<0.0008$), OPN ($p<0.015$), IGFBP-1 and NT-proBNP. We found a decrease of plasma levels for SELE, t-PA, MB and DLK-1 ($p<0.005$). In addition we observed a notable change for patients' plasma proteome profiles in response to surgery.

Using affinity proteomics assays, we observed changes of plasma proteins after bariatric surgery. The current candidates are proteins related to insulin binding and their regulation, which have previously been brought into context of T2D development and disease manifestation hence require further referencing to other T2D-related parameters. Monitoring changes in plasma abundance of metabolic markers upon surgery might provide valuable leads for elucidating how individuals will respond to the treatment longer-term.

WOC pm 3:28
A systematic map of protein-metabolite interactions reveals principles of chemical communication

Ilaria Piazza; Paola Picotti
ETH Zurich, Zürich, Switzerland

Metabolite-protein interactions control a variety of cellular processes, thereby playing a major role in maintaining cellular homeostasis. Different types of functional interactions between proteins and metabolites have been reported and involve binding of metabolites to the active site of enzymes as substrates, cofactors, or products of enzymatic reactions. Metabolites comprise the largest fraction of molecules in cells, but our knowledge of the metabolite-protein interactome lags behind our understanding of protein-protein or protein-DNA interactomes, since most characterized protein-metabolite interactions have been discovered via hypothesis-driven experiments that rely on *in vitro* activity assays.

We devised LiP-SMap a proteomic workflow for the systematic identification of metabolite protein-interactions directly in their native environment. LiP-SMap combines limited proteolysis (LiP) with DIA (Data Independent Acquisition) mass spectrometry in the presence of unmodified metabolites to enable a systematic analysis, unbiased with regard to both metabolites and proteins. With LiP-SMap we identified a network of known and novel interactions and binding sites in *Escherichia coli*, and we demonstrated the functional relevance of a number of newly identified interactions. Our data enabled identification of new enzyme-substrate relationships and cases of metabolite-induced remodeling of protein complexes. Our metabolite-protein interactome consists of 1678 interactions

and 7345 putative binding sites. Moreover the dataset reveals functional and structural principles of chemical communication, shed light on the prevalence and mechanisms of enzyme promiscuity, and enable extraction of quantitative parameters of metabolite binding on a proteome-wide scale. We will further present our latest applications for the study of protein-ligand interactomes and drug target deconvolution on a cell-wide scale.

2:00 - 3:50 pm Wednesday
HPP: UNRAVELLING TISSUE PATHOLOGY THROUGH CELL MAPPING
Session Chairs: Justyna Fert-Bober and Emma Lundberg
Oceana 3-5

WOD pm 2:00
Highly multiplexed imaging of tissues with subcellular resolution by imaging mass cytometry.
Bernd Bodenmiller
University of Zurich, Zurich, Switzerland

Cancer is a tissue disease where heterogeneous tumor cells, stromal cells and immune cells form a dynamic ecosystem that evolves to support tumor expansion and ultimately tumor spread. The complexity of this system is the main obstacle to treat cancer. The study of the tumor ecosystem and its cell-to-cell communications is thus essential to enable an understanding of tumor biology, to define new biomarkers, and to identify new therapeutic routs and targets.

To understand the workings of the tumor ecosystem, highly multiplexed image information of tumor tissues is essential. Such multiplexed images will reveal which cell types are present in a tumor, their functional states, and how they interact together. To enable multiplexed tissue imaging, we developed imaging mass cytometry (IMC), a novel imaging modality that uses metal isotopes as reporters and currently allows to visualize over 50 antibodies and DNA probes simultaneously with subcellular resolution. In the near future, we expect that over 100 markers can be visualized. We applied IMC for the analysis of breast cancer samples in a quantitative manner. To extract biological meaningful data and potential biomarkers from this dataset, we developed a novel computational pipeline called histoCAT geared for the interactive and automated analysis of large scale, highly multiplexed tissue image datasets. Our analysis reveals a surprising level of inter and intra-tumor heterogeneity and identify new diversity within known human breast cancer subtypes as well as a variety of stromal cell types that interact with them.

In summary, our results show that IMC provides targeted, high-dimensional analysis of cell types, cell states and cell-to-cell interactions within the tumor ecosystem. Spatial relationships of complex cell states of cellular assemblies can be inferred and potentially used as biomarkers. We envision that IMC will enable a systems biology approach to understand and diagnose disease and to guide treatment.

WOD pm 2:30
Integrated omics for spatial mapping of the human proteome - understanding the molecular repertoire in health and disease
Cecilia Lindskog
Uppsala University, Uppsala, Sweden

In the evolving era of “big data”, integration of datasets from different omics technologies, such as genomics, transcriptomics and proteomics have received increased attention. The Human Protein Atlas database, www.proteinatlas.org, constitutes a comprehensive knowledge resource for spatial localization of proteins in organs, tissues, cells and organelles, as well as the consequence of all human genes on patient survival. The tissue mapping is based on genome-wide mRNA expression data, which is summarized on the interactive webpage and used for categorization of all human genes based on expression level and tissue distribution. The analysis is combined with tissue microarray-based immunohistochemistry covering all major normal human organs and cancer types, and in the most recent update of the database, a large effort has been put into enhanced antibody validation strategies. The publicly available datasets and high-resolution images allow for further exploration of missing proteins, and detailed analysis of protein expression patterns in different cell types within the same tissue. A recently added in-depth characterization in human tissues is single cell evaluation of >500 proteins elevated in testis, which contributes to further understanding of the biology and molecular repertoire of normal and pathological spermatogenesis. Knowledge of the architecture of every human cell aids in identification of candidate proteins that may accelerate research in molecular medicine, and contributes to further knowledge of underlying disease mechanisms.

WOD pm 3:00
Mass Spectrometry Imaging of the Metabolome and Lipidome Through Development of an Innovative Ionization Method
David Muddiman
North Carolina State University, Raleigh, NC

Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI) is a powerful analytical platform for the visualization of analytes within tissues, plants, textiles, hair, and numerous other matrices. Application of MSI methodologies to drug distribution studies over the last decade has provided invaluable insight to the distribution of pharmaceuticals in preclinical and clinical trials, but quantitative MSI data is often unable to be achieved. Through optimization and careful definition of analytical figures of merit, IR-MALDESI MSI provides absolute quantification of small molecule drugs in tissue. In this MSI ionization method, an IR laser ablates a voxel of material at each rastered position, providing a complete and reproducible volume of a tissue section to be sampled with subsequent ionization of analytes by ESI. MSI variability from tissue microenvironments is reduced with the uniform incorporation of an internal standard for normalization of analyte on a per-voxel basis. Absolute quantification MSI is accomplished through the inclusion of a spatial calibration curve in the MSI analysis allowing direct correlation of observed ion abundance in MSI to absolute analyte concentration. The absolute quantification procedure has been automated in the MSiQuantification software tool, available within the open source, vendor neutral MSI software MSiReader v1.0. Lipid profiles provide invaluable information for understanding the biological basis of many diseases. Due to their remarkable structural diversity, different lipid classes exhibit significant differences in their ionization efficiency. Therefore, analyzing tissue sections in positive and negative modes is essential for obtaining comprehensive lipid coverage. Data from the domestic hen model for spontaneous development of ovarian cancer will be presented. Healthy and

cancerous hen ovarian tissue sections were analyzed using this method. Differences in spatial distribution and relative abundance of more than 700 analytes between the two tissue sections were simultaneously monitored.

WOD pm 3:20
A high-precision tissue-based mouse proteome with BoxCar and data-independent acquisition

Florian Meier[1]; Oliver Bernhardt[2]; Marta Murgia[1]; Catherine Vasilopoulou[1]; Michael Wierer[1]; Lynn Verbeke[2]; Tejas Gandhi[2]; Lukas Reiter[2]; Matthias Mann[1]
[1]MPI of Biochemistry, Martinsried, Germany; [2]Biognosys AG, Schlieren, CH

Mammals exhibit a variety of cell types, tissues and organs. Resolving the proteome at this level will greatly benefit our understanding of health and disease. Pioneering tissue-based proteomic maps built upon extensive fractionation techniques and mined community data to maximize proteome coverage. However, while achieving unprecedented depths, these approaches are inherently limited in their quantitative accuracy and spatial resolution. We have recently reported an acquisition method (termed BoxCar) that increases the MS1 level performance of Orbitrap mass analyzers to a level at which over 10,000 proteins were detected in a single 100 min run (Meier et al., Nat. Methods 2018). In parallel, data-independent acquisition (DIA) methods are rapidly evolving and offer very high quantitative accuracy and reproducibility at the MS2 level.

Here, we devised an acquisition strategy that unifies the distinct advantages of BoxCar and DIA for deep, quantitative analysis of the mouse proteome in single runs. We adapted the data analysis pipeline in Spectronaut (Biognosys) to make full use of the novel acquisition method. Our draft proteome comprises 28 tissues from four animals that were processed in a high-throughput manner in 96-well format, and measured within ten days of LC-MS time on a Q Exactive HF-X mass spectrometer. Our initial analysis employed a project-specific peptide library from fractionated and pooled tissue samples comprising over 200,000 unique peptide sequences and over 14,000 protein groups. To increase the proteome coverage further, we complemented our library with tissue-specific community datasets. Notably, the increased dynamic range of BoxCar makes library-free peptide identification directly from DIA runs very attractive. This enabled us to capture tissue-specific proteins that were absent in the original library.

We assembled all BoxCar/DIA single runs to the first in-depth and quantitative map of the tissue-resolved mouse proteome, a rich resource for systems biology that also opens new perspectives for proteogenomics.

2:00 - 3:50 pm Wednesday
PERSONALIZED WELLNESS
Session Chairs: Sanjeeva Srivastava and Mike Snyder
Oceana 1-2

WOE pm 2:00
The Value of Imputing -Omics Data into Biobanks Linked to Electronic Health Records

Nancy J. Cox
Vanderbilt University, Nashville, TN

Biobanks in which there are hundreds of thousands to millions of subjects with both large-scale genome interrogation and rich phenome data such as electronic health records going back > 20 years offer an outstanding opportunity for discovery. The fact that many additional sources of -omics data (transcriptomes, methylomes, metabolomes, proteomes) are both expensive and highly heritable raises the possibility of using imputation based on reference -omics data and genome variation as a way to probe the association of these -omics technologies in large-scale clinical data. I will describe results of such studies in BioVU, the Biobank at Vanderbilt University with more than 250,000 DNA samples linked to a high quality electronic health record going back on average 10-15 years for these subjects, and more than 20 years in some individuals. We have imputed transcriptomes in all data using reference data from GTEx and are imputing metabolomes based on other large-scale publicly available data. These imputed -omics provide outstanding opportunities for discovery of the biology of human disease because they aggregate individual DNA variants into more biologically relevant sets that allow us to probe the function of genes for these clinical human phenotypes in ways that provide insight into mechanism and yield an easy-to-interpret direction of effect.

WOE pm 2:20
Proteomics Analysis in Context of Personal, Dense, Dynamic Data Clouds from Thousands of People

Nathan Price
Institute for Systems Biology, Seattle, WA

Healthcare is becoming more proactive and data-rich than anything before possible. Lee Hood and I have recently launched a data-rich wellness project that integrates proteomics, genomics, metabolomics, microbiomes, clinical chemistries and wearable devices of the quantified self to monitor wellness and disease. These resulting personal, dense, dynamic data (PD3) clouds enable the creation of a field we term "scientific wellness" that aims to help individuals take informed actions to enhance their wellness and help reduce their risk for disease — informed by PD3 clouds. In essence, scientific wellness becomes a key to understanding disease because it provides a framework in which to detect the earliest transition states in a data-rich context. Analyses of these data — individually and in aggregate — will enable us to identify scientifically-validated metrics for wellness, see early warning signs of disease, and develop approaches to reverse disease in its early stages. I will present results from our proof-of-concept pilot study in a set of 108 individuals (the Pioneer 100 study) as well as data from thousands of subsequent individuals that have been profiled to date. I will show how the interpretation of these data led to actionable findings for individuals to improve health and reduce risk drivers of disease, and how they are giving us insights into human biology. I will also give views of how this endeavor relates to the future of health and big data analyses for biology and medicine.

WOE pm 2:40
Integrative Proteomics and Transcriptomics for Personalized Wellness: Using Saliva and Blood to Monitor Immune Response in Individuals

George Mias
Michigan State University, East Lansing, MI

Individualized wellness has emerged as a new field in precision medicine, built on rapid advancements in genome sequencing and mass spectrometry. The majority of ongoing studies have focused on static genomic variant information, which allows us to successfully infer genomic disease risk. However, for any future medical implementation, we must also incorporate dynamically changing molecular components, by integrating multiple omics (e.g. proteomes and transcriptomes) that reflect the continuously changing physiological state of an individual patient. Such dynamic monitoring is essential for true precision

wellness, where we investigate individualized departures from a personalized baseline over time, indicating the onset of disease and other deviations from wellness.

We will present the latest results from our clinical trials that monitor individual immune responses, pre and post pneumococcal vaccination, from collected saliva and blood sample omics profiling. Our investigation represents one of the most extensive saliva proteomics and trascriptomics profiling in an individual, where we have generated samples and data from more than 100 timepoints in one year. In conjunction with saliva, we will also show results from peripheral blood mononuclear cells (PBMCs, 25 timepoints). For all timepoints we have profiled full proteomes (mass spectrometry un-targeted profiling, with Tandem Mass Tag quantitation), and corresponding transcriptomes (RNA-sequencing). Following integrative analysis with MathIOmica, we have observed that saliva omics show distinct trends corresponding to departures from wellness, including the onset of both innate and adaptive immune responses post pneumococcal vaccination.

Our research represents one of the first systematic implementations of integrative personalized wellness monitoring in saliva. The study is an extensible paradigm, that we are expanding to other diseases and immunizations. Our investigation exemplifies the potential for non-invasive saliva diagnostics and provides novel proteomics/transcriptomics resources to both the clinical as well as the systems biology communities.

WOE pm 2:52
Longitudinal Multi-omics Profiling in Insulin Resistant and Sensitive Prediabetic Population

Sara Ahadi; Wenyu Zhou; Reza Sailani; Kevin Contrepois; Mike Snyder
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Prevention of type 2 diabetes(T2D) requires a better understanding of its mechanism in human body. Research suggests that 1 out of 3 adults in US has prediabetes, which emphasizes the importance of studying paths that lead to development of T2D. Beside insulin resistance that is known to increase the risk of prediabetes, physiological stress can also help in advancing T2D. This study longitudinally tracks a cohort of prediabetic individuals and healthy controls for over 3 years. These individuals were sampled during healthy and respiratory viral infection (RVI) periods. In addition, particular events such as immunization, antibiotic treatment and other health perturbations were sampled to obtain an overall view of changes of biological molecules of the cohort over time.

We developed a novel high-throughput proteomics pipeline that is capable of analyzing >100 samples per week, has low technical variation, is depletion- and separation-free, and is able to quantify 450 proteins in plasma, with single-shot data-independent-acquisition (DIA) method. We applied this pipeline to our study cohort of >1000 samples to generate personalized, longitudinal proteomics profiles of prediabetics under various physiological stresses. Our deep longitudinal measurements constitute one of the largest proteomics datasets ever produced, and enable the application of intense proteome profiling to advance precision medicine.

We then used steady-state plasma glucose (SSPG) test to characterize participants of the study into IR or IS groups. Integrated multi-omics analysis of proteome data with other -omics data and clinical labs identified 99 features significantly correlated with insulin resistance. Long chain and polyunsaturated fatty acids, indole lactic acid, hippuric acid, multiple genera within the gut microbiome, immune proteins, as well as lipoproteins were identified as significantly correlated with SSPG levels. We have also identified new molecular pathways associated with RVI and studied their differences between IR and IS individuals.

WOE pm 3:04
Advancing Mass Spectrometry-based Large-Cohort Proteomics for Precision Medicine – An International Cancer Moonshot Multi- Site Study

Yue Xuan[1, 2]; Nicholas W. Bateman[3]; Sebastien Gallien[2, 14]; Yue Zhou[15]; Niyati Parikh[3]; Mo Hu[15]; Pedro Navarro[1]; Yuju Chen[4]; Albert Sickmann[5]; Bernd Wollscheid[6]; Connie R. Jimenez[7]; Martin R. Larsen[8]; Hu Zhou[9]; Siqi Liu[10]; Zhinan Chen[11]; Thomas Kislinger[12]; Ben Crossett[13]; Brian Hood[3]; Reta Birhanu Kitata[4]; Christin Lorenz[5]; Christina Loosse[5]; Sandra Goetze[6]; Sander Piersma[7]; Davide Chiasserini[7]; Muhammad Tahir[8]; Hongwen Zhu[9]; Guixue Hou[10]; Xiuxuan Sun[11]; Andrew Macklin[12]; Amanda Khoo[12]; Benjamin L. Parker[16]; Stuart J. Cordwell[16]; Thomas P. Conrads[3, 17]
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A high-throughput analytical workflow using high resolution MS1-based quantitative data-independent acquisition (HRMS1–DIA) mass spectrometry was standardized with well-defined experimental steps and systematically applied to a set of test samples. The study was benchmarked across multiple Cancer Moonshot sites worldwide utilizing similar instrument platforms, procedures, and software, and was demonstrated to be stable in a 24/7 operation mode for 7 consecutive days.

A HELA cell digest quality control (QC) sample was routinely analyzed. Two mixed proteome samples with different ratios of three proteomes (HELA, Yeast, and E.coli) were analyzed to evaluate the label-free quantitation performance. >280 QC files and >380 mixed proteome sample files were acquired by 11 participated laboratories.

With the 1 hour capillary LC-HRMS1 DIA workflow, >5,000 protein groups from >40,000 peptides were consistently identified from the QC sample across all sites and all days (1%

FDR). The peptide intensity correlation among all QC files from the entire study was 0.9, demonstrating ultra-high inter-laboratory and inter-day reproducibility. The normalized median coefficient of variation among all identified precursors across 7days was <20% at the same site.

With the label-free quantitation sample, >7,200 up to ~8,600 protein groups were identified (1% FDR) across all 11 labs. In addition to the 1% FDR, a rollup strategy was developed to filter none-reliable peptides from protein quantitation, successfully enhancing the quantitative precision to >0.9. As a result, an average of >6,000 protein groups from the mixed proteome sample, including >4,000 Human proteins, ~2,000 yeast proteins, and >300 E.coli proteins, were precisely quantified amongst all labs and all days. ~80% protein groups were identified and quantified in common across all the laboratories, and >85% were quantified in common across different days at the same site. Empirical label-free quantitation ratios of the three mixed proteomes accurately reflected the ratios anticipated at each site.

POSTER 001

Subcellular Proteome Analysis of the Pancreatic Beta Cell lines: INS1e and MIN6

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Diabetes is a global health epidemic affecting more than 400 million people. Pancreatic beta cells are the primary regulators of diabetes physiology, as they secrete insulin in response to blood glucose levels. In order to prevent and cure diabetes we must gain a fundamental understanding of the beta cells' integral components, including its proteome. In this report, we provide comprehensive proteomes for the mammalian pancreatic beta cell lines INS1 (rat) and MIN6 (mouse), as well as an analysis of beta cell proteome changes in response to glucose stimulation and starvation.

POSTER 002

What are the 'Popular Proteins' in Rheumatic and Autoimmune Diseases?

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The Rheumatic and Autoimmune Diseases (RAD) initiative of the Human Proteome Project (RAD-HPP) was launched at the 2017 HUPO meeting with the aim of tackling the several unmet clinical needs in RAD and improve their management. One of the immediate goals of this initiative, under the frame of the biology and disease-centric strategy of the HPP (B/D-HPP) has been to assemble prioritized lists of proteins clinically relevant to RAD using the 'popular proteins' strategy and text mining software.

The PubPular v3 tool was used to retrieve publications and rank the corresponding most popular proteins related to representative RADs. These RADs include rheumatic diseases (osteoarthritis, rheumatoid arthritis, spondyloarthropathies) and autoimmune connective tissue disorders (systemic lupus erythematosus, systemic sclerosis, Sjögren's syndrome). Additionally, the PURPOSE engine was employed to estimate global numbers of proteins associated with RAD.

Literature mining identified 2810 proteins related to rheumatic diseases and 1246 related to autoimmune connective tissue disorders. These two topic areas shared more than 1000 proteins, primarily from known immune and inflammatory response pathways. The most popular proteins in osteoarthritis were involved in cartilage extracellular matrix organization and dissasembly. In rheumatoid arthritis, proteins related to key pathogenic processes such as citrullination or T-cell regulation were ranked at the top of the list. In spondyloathropathies, our strategy highlighted the importance of well-known pathways as the IL23/IL17 axis, or the HLB27 antigen. Additionally, it provided several candidates of genetic studies for which the role of the corresponding proteins has not been elucidated yet, such as Late Cornified Envelope (LCE) proteins in psoriatic arthritis. Similar findings were seen in the autoimmune connective tissue disorders, with immune system proteins as IRF5 and STAT4 at the top.

Altogether, this study shows the utility of the 'Popular Proteins' strategy to prioritize relevant proteins in the context of rheumatic and autoimmune diseases.

POSTER 003

Targeting Endothelial Erk1/2-Akt Axis as a Regeneration Strategy to Bypass Fibrosis during Chronic Liver Injury

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Liver sinusoidal endothelial cells (LSECs) have great capacity for liver regeneration, and this capacity can easily switch to profibrotic phenotype, which is still poorly understood. In this study, we elucidated a potential target in LSECs for regenerative treatment that can bypass fibrosis during chronic liver injury. Proregenerative LSECs can be transformed to profibrotic phenotype after 4 weeks of carbon tetrachloride administration or 10 days of bile duct ligation. This phenotypic alternation of LSECs was mediated by Erk1/2-Akt axis switch in LSECs during chronic liver injury; Erk1/2 was normally associated with maintenance of the LSEC proregenerative phenotype, inhibiting hepatic stellate cell (HSC) activation and promoting tissue repair by enhancing NO/ ROS ratio and increasing expression of HGF and Wnt2. Alternatively, Akt induced LSEC profibrotic phenotype, which mainly stimulated HSC activation and concomitant senescence by reducing NO/ROS ratio and decreasing HGF/Wnt2 expression. LSEC-targeted adenovirus or drug particle to promote Erk1/2 activity can alleviate liver fibrosis, accelerate fibrosis resolution, and enhance liver regeneration. This study demonstrated that the Erk1/2-Akt axis acted as a switch to regulate the proregenerative/ profibrotic phenotypes of LSECs, and targeted therapy promoted liver regeneration while bypassing fibrosis, providing clues for a more effective treatment of liver diseases.

POSTER 004

An update on the Human Plasma Proteome

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The analysis of plasma samples provides important insights about human health and disease. In order to collect the proteins detectable in this bo dy fluid, the Human Plasma Proteome Project (HPPP) was started by HUPO in 2002. With continuing advances of proteomics technologies, the previous collection of 1.900 proteins (Farrah et al, 2011, MCP) has recently been updated (Schwenk et al, 2017, JPR).

We gathered data from > 170 shotgun MS experiments in the Human Plasma PeptideAtlas Build 2017-04 to describe the currently most comprehensive composition of the plasma proteome. The data suggests that > 3.500 proteins can be reliably identified by two peptides. This is based on 43 million peptide spectrum matches with > 120.000 distinct peptide

sequences at a 1% protein-level FDR and application of the HPP data interpretation guidelines. In addition, we describe the plasma proteins detectable by traditional as well as multiplexed immunoassay-based methods. Of the currently ~ 2.000 proteins available from different assay platforms, there were ~ 1.000 proteins in common with MS-based identifications.

To this date, about 17% of the predicted human proteome is detectable in plasma by shotgun MS. New methods and approaches will certainly lead to a further expansion of that list to > 5000 proteins (25%) in the near future. In addition to expanding our technological possibilities to study the plasma proteome, considerations regarding sample collection and integrity as well as study design need to be further emphasized. This can then lead to a more precise annotation and use of the plasma proteome in relation to variations of and heterogeneity among individuals and study sets.

POSTER 005

SWATH-MS with internal landmarks for quantitative proteomics of liver cirrhosis patient urine

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Cirrhosis of the liver (LC) often has no signs or symptoms until damages were found extensivelyand 3-4 million people are suffered from LC in the world. To understand pathophysiological pathways in liver cirrhosis, SWATH-MS analysis of urine samples from the patients and healthy volunteers (HV) was performed in our current study.

Urine from both HV and LC patients were collected and stored at -20°C until use. An optimized precipitation method was used to extract urine proteins. Tryptic urine peptides samples were analyzedunder DDA and DIA mode in SCIEX 5600+ system, alternately. A consensus library was created from search results of ProteinPilot™, in which tryptic peptides were mainly selected. SWATH-MS data was analyzed by using SWATH™ MicroApp in PeakView™ at default settings. RT landmarks were selected from 11 proteins as internal standard. In total, 1958 proteins were included into the library, in which 389 and 314 proteins were uniquely included from proteins identified in HV and LC patient urine, respectively. More than 85% of peptide peaks matched well and 1425 proteins were reliably quantified, 415 proteins were significantly changed more than 5 times in LC patient urine. Since these proteins may be involved in the onset or progression of LC, further pathway analysis and enrichment analysis were done. Serine-type endopeptidase activity was the most significant over-represented molecular function. Glycolysis/gluconeogenesis pathway was enriched clearly. Proteins decreased in LC patient urine more than 5 times than HV urine revealed to associate with cell adhesion, immune response, and proteolysis.

Optimization of the library and landmarks enhanced our quantitative accuracy of peptides and proteins of HV and LC patient urine. We hope our results may provide progression of urine targeted proteome research using internal standards.

POSTER 006

Immunoproteomics Profiling of Citrullinated AAgeome Reveals Next-Generation Biomarkers for Rheumatoid Arthritis

Wei Yu[1]; Hongye Wang[1]; Xiaolong Guan[2]; Haiyong Wang[2]; Fei Wang[2]; Lei Song[1]; Mingwei Liu[1]; Haoyu Wang[3]; Liuhui Yang[1]; Jiayu Dai[1]; Te Liang[1]; Hu Duan[1]; Dong Li[1]; Yuan Liu[1]; Zhonglin Fu[1]; Xiaoling Yan[2]; Guorui Liu[2]; Linghui Li[2]; Andrea Throop[4]; Joshua LaBaer[4]; Xiao-Jun Li[2]; Jun Qin[1]; Bei Zhen[1]; Xiaobo Yu[1]

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Post-translational modification (PTM) has been increasingly recognized with a role in immune recognition and detection of autoimmune diseases as biomarkers. However, the immunogenic properties and translational potential of most proteins' PTM are unknown without a highly effecient proteomics platform. Here, we report an immunoproteomics pipeline that enables the proteome-wide identification of PTM related autoantigens (AAgs), verification of their antigenicity, and validation as disease biomarkers in high-throughput. With citrulliation as a model, we identified the largest set of citrullinated AAgs that participate in a variety of biological processes and signaling pathways related to the immunity. We further verified 86 citrullinated peptides interactions using high-density peptide microarrays and validated the selected biomarkers utilizing bead-based multiplexed immunoassays. With the training (n=157) and validation (n=177) sets, we found that the AAb signatures constructed by logistic regression model enable the detection of α- CCP+ (rheumatoid arthritis) RA patients with the sensitivity of 90.57 % and 81.30 % at the specificity of 100% , and provided the unprecedented sensitivity of 22.64 % and 19.67 % for α-CCP- RA patients at the specificity of 98%, respectively. All the results demonstrate the utilitary of our platform to study PTM AAgeome, and in the overall understanding of human immunity and identification of autoimmune biomarkers.

POSTER 007

Early candidate urine biomarkers for detecting Alzheimer's disease before amyloid-β plaque deposition in an APP (swe)/PSEN1dE9 transgenic mouse model

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Alzheimer's disease (AD) is an incurable age-associated neurodegenerative disorder that is characterized by irreversible progressive cognitive deficits and extensive brain damage. The identification of candidate biomarkers before amyloid-β plaque deposition occurs is therefore of great importance for the early intervention of AD. Urine, which is not regulated by homeostatic mechanisms, theoretically accumulates changes associated with AD earlier than cerebrospinal fluid and blood. In this study, an APP (swe)/PSEN1dE9 transgenic mouse model was used to identify candidate biomarkers for early AD. Urine samples were collected from 4-, 6-, and 8-month-old transgenic mouse models, and the urinary proteomes were profiled using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The levels of 29 proteins differed significantly between wild type and 4-month-old mice, which had not started to deposit amyloid-β plaque. Among these proteins, 13 have been associated with the mechanisms of AD, while 9 have been

suggested as AD biomarkers. Our results indicated that urine proteins enable detecting AD before amyloid-β plaque deposition, which may present an opportunity for intervention.

Key words: Alzheimer's disease (AD), urine proteome, early diagnosis, APP (swe)/PSEN1dE9

POSTER 008

Characterization of human multipotent stromal cells secretome in response to in vitro passaging

Ramavati Pal

Food and Drug Administration, Silver Spring, <Not Specified>

Multipotent Stromal Cells (MSCs) also known as mesenchymal stem cells have a remarkable potential in regenerative medicine for their immune suppressive ability, migration ability to sites of injury, ability to differentiate into different cell types and easy accessibility. Increasing evidence suggests that MSCs might exert their therapeutic benefits through secreted factors, termed the secretome. The secretome has become a subject of intensive proteomic profiling in the search for released proteins and microvesicles that might be applicable to regenerative medicine. In this study, we used a label-free protein quantitation method to investigate the molecular composition of the MSC secretome from 3 commercially available donor cell lines (P110877, RB12 and RB16) at different passages. Over 196 proteins were quantified and a list of differentially expressed proteins was compiled. Preliminary results show that the protein composition of the secretome of MSC lines P110877 and RB12 was similar, however the RB16 secretome showed differences. A number of proteins demonstrate variable isoform secretion patterns at different passages for e.g., collagen alpha isoforms including CO1A1, CO1A2, CO2A1, CO3A1, CO4A2, CO6A1, CO6A3; insulin-like growth factor binding proteins IBP4, IBP6, IBP 7; fibronectin isoforms FINC 4, FINC 5, FINC 6, FINC 10, FINC12; transgelin isoforms TAGL and TAGL2; actin and tropomyosin isoforms and 14-3-3 protein isoforms. Bioinformatics-based analysis revealed that proteins involved in biogenesis, metabolic processes, biological regulation and the immune system were largely upregulated in early passage. We also observed significant proteomic alterations in functional categories such as molecular binding, structural molecule activity and catalytic activity following in vitro cell passaging. These analytical results may aid in identifying secreted factors that may be responsible for biological activity of MSCs in vitro and in vivo.

POSTER 009

A spectral-library based quantitative study of protein signatures to predict response of pancreatic cancer patients receiving chemotherapy

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer characterized by its poor prognosis, rapid progression and development of drug resistance. Chemotherapy is a vital treatment option for most of PDAC patients. Stratification of PDAC patients, who would have a higher likelihood of responding to chemotherapy, could facilitate treatment selection and patient management. We developed a spectral library-based platform that was specifically tailored for analysis of clinical plasma samples, which are subject to significant heterogeneity and protein abundant difference. The platform is composed of a DDA protocol for MS interrogation and a targeted data analysis strategy for candidate identification and quantification. In this quantitative proteomics study, we used the platform to characterize the protein profiles in the plasma of PDAC patients undergoing chemotherapy to determine if specific biomarkers could be used to predict likelihood of therapeutic response. By comparing the plasma proteome of the PDAC patients with positive therapeutic response and longer overall survival (Good-responders) to those who did not respond as well (Limited-responders), we identified differential proteins that could effectively segregate Good-responders from Limited-responders. Functional clustering and pathway analysis further suggested that many of these differential proteins were involved in pancreatic tumorigenesis. Four protein candidates, including vitamin-k dependent protein Z (PZ), zinc-alpha-2-glycoprotein (AZGP1), sex hormone-binding globulin (SHBG), and von Willebrand factor (VWF), were further evaluated as single or composite predictive biomarker with/without CA 19-9. Two composite biomarkers were generated that could distinguish Good-responders from Limited-responders with AUC values of 0.95 and 0.90 for stage III and stage IV PDAC patients, respectively. The study suggests that stratification of PDAC patients in terms of their tumor stages and histological types could potentially enhance the performances of the biomarkers.

POSTER 010

MIF induce Th17-related cytokines secretion in PBMC from rheumatoid arthritis patients: analysis through the Heat Map method

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Introduction: Rheumatoid arthritis (RA) is a highly disabling autoimmune disease. Particularly, the T-helper (Th) 17 cells subset plays an important role in the development and establishment of RA. These cells produce interleukin (IL)-17, IL-21 and IL-22 and required previously stimulation of IL-6 and IL-23. It has been reported that macrophage migration inhibitory factor (MIF) induces IL-6 and IL-17 secretion.

Aim: To evaluate the effect of recombinant human MIF (rhMIF) on IL-6, IL-17A, IL-17F, IL-21, IL-22 and IL-23 (Th17-related cytokines) secretion in peripheral blood mononuclear cells (PBMC) from control subjects (CS) and RA patients.

Methodology: We performed cell cultures of human PBMC from CS and RA patients. The PBMC were stimulated with rhMIF and besides this with LPS as positive control for 24 hours. The Th17-related cytokines were quantified from culture supernatants by means of a magnetic beads-based multiplex immunoassay method using the Bio-Plex Pro Human Th17 Cytokine Panel (Bio-Rad Laboratories, Inc.). We analyzed the results through heat map method using R v3.4.1 software and the differences between groups were determined by Mann-Whitney U test using SPSS v22.

Results: The rhMIF promote a significant increase in the IL-17A, IL-17F, IL-21, IL-22 and IL-23 levels after stimulation in the PBMC from RA patients but not in PBMC from CS. A statistical significant increase was observed on the secretion of IL-6, IL-17A, IL-17F, IL-21, IL-22 and IL-23 in PBMC from CS stimulated with LPS and in PBMC from patient's similar results were found, except for IL-23. PBMC from CS secreted higher IL-6 and IL-23 levels than PBMC from RA patients after LPS stimulation.

Conclusion: MIF induce the Th17-related cytokines secretion in PBMC from RA patients but not in PBMC from CS, therefore, MIF can play an important role in the modulation of RA immune response.

POSTER 011
Preliminary steps towards the generation of a rat plasma spectral library

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Introduction:

Animal models can be utilised to identify biomarkers to monitor disease pathogenesis and drug response. This preclinical information can be used to inform clinical trial design. For many animal species, reliable spectral libraries do not exist. Here we describe the construction of a rat plasma spectral library.

Method:

Blood samples were collected from the tail vein of Han Wistar rats. Immune-depleted and non-depleted aliquots of plasma proteins were denatured and alkylated prior to fractionation by gel electrophoresis. Peptides were generated by in-gel tryptic digestion. Samples were separated (NanoLC™ 425) and analysed (TripleTOF® 6600 system, SCIEX) in both micro and nano flow. Data dependent acquisition (DDA) was performed. DDA files were searched against a rat database (fasta acquired from Uniprot). A 1% expectation value filter was applied. Peptides (and corresponding proteins) that reached this threshold were retained.

Results:

The number of unique proteins identified was assessed using two different depletion kits (albumin and top-12); the former performed more effectively. Over 600 proteins were detected using both micro and nano flow methodologies. Depletion-specific and flow-specific proteins were identified suggesting that there was no single superior acquisition method, but a combined approach enhanced the proteome coverage. Abundance plots of these combination-unique proteins were also generated by cross-referencing against the PaxDb protein abundance database.

Conclusion:

The results demonstrated the importance of utilising both micro and nano flow methods to generate a spectral library. The micro flow method generated a library with greater coverage of protein identifications. In contrast, the nano flow method allowed a greater number of low abundant protein identifications, which may have been missed using micro flow. To achieve the most comprehensive library for rat plasma, identification of unique proteins from both immune depleted and non-depleted samples is recommended.

Keywords: rat plasma, spectral library, Data dependent acquisition (DDA), biomarkers

POSTER 012
Differences of saliva composition in relation to tooth decay and gender

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Most people worldwide suffer from dental caries. Only a small part of the population is caries-resistant and the reason for this resistance is unknown. Unstimulated whole saliva samples were collected from healthy females and males volunteers (caries-susceptible and caries-free people). The samples were centrifuged and were divided on two parts of samples: supernatant and pellet and then the proteins of oral fluids were separated by two-dimensional electrophoresis, by two-dimensional difference gel electrophoresisi and by Label-Free Quantitative analysis. Our results demostrate that the observed differences in the protein levels might have an influence on anti-caries resistance. Newly detected potential protein markers of dental caries can be a good basis for further research and for possible future therapeutic use.

POSTER 013
Developing Serum Multi-marker Panels for Diagnosing Hepatocellular Carcinoma using Multiple Reaction Monitoring-Mass Spectrometry

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BACKGROUND : Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. It has a poor prognosis as evidenced by being the third most common cause of cancer-related deaths. Hepatitis B virus (HBV) and liver cirrhosis (LC) are the most relevant risk factors of HCC. Early detection of HCC amongst high-risk groups is paramount in improving prognosis. We aimed to identify and assess a serum biomarker combination that could detect the presence of clinical and preclinical hepatocellular carcinoma in high-risk patients.

METHODS : A total of 300 serum samples from HBV (n=100), LC (n=100), and HCC patients (n=100) were analyzed via multiple reaction monitoring-mass spectrometry (MRM-MS) to verify HCC biomarkers and to develop multi-marker panels.

The HCC biomarkers and multi-marker panels were validated in 140 independent samples (57 HBV, 43 LC, and 40 HCC).

RESULTS : Multi-marker panels displayed superior diagnostic power in comparatively analyzing HBV vs. HCC, LC vs. HCC, and high-risk group (LC+HBV). The serum proteins of patients with HCC were distinct from those with HBV, LC, and high-risk group (LC+HBV) as demonstrated by the area under the receiver operating characteristic (AUROC) curves of 0.970 (accuracy=0.850), 0.940 (accuracy=0.950), and 0.970 (accuracy=0.933) in the training set, where 0.851 (accuracy=0.773), 0.923 (accuracy=0.783), and 0.928 (accuracy=0.821) in the validation set, respectively. These panels showed higher accuracy than AFP (cut-off 20 ng/mL) in distinguishing individuals with hepatocellular carcinoma from HBV (AUC=0.790), LC (AUC=0.737), and high-risk group (LC+HBV; AUC=0.760) in the validation set.

CONCLUSIONS : We have been able to identify a panel of diagnostic biomarkers of HCC from serum proteins. These multi-marker panels have the potential to predict the risk of HCC development in high-risk populations prior to clinical diagnosis, which is meaningful for the surveillance of patients with preclinical HCC.

POSTER 014

Discovery of Serum Biomarkers for Pancreatic Cancer by Lectin Affinity Capture Coupled with iTRAQ-Based Quantitative Glycoproteomics Approach

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Pancreatic cancer (PC) has high mortality rate and represents the most aggressive cancer type. Because of diagnosis at late stage, the five-year survival rate for PC is only 3%. Carbohydrate antigen 19-9 (CA19-9) is a currently used glycobiomarker for PC detection. However, its poor sensitivity and limited use in early detection warrant the need for developing other useful biomarkers. Aberrant protein fucosylation has been reported in PC. In this study, we used lectin (aleuria aurantia lectin, AAL)-based glycoprotein/glycopeptide enrichment coupled with iTRAQ quantitative proteomics approach to discover novel PC glycobiomarkers. From 417 glycopeptides, we selected 49 glycopeptides whose levels were significantly higher in the serum samples of PC patients than patients with gallstone (GS). Consequently, we chose PC03 for further validation by lectin ELISA in 132 serum samples including 50 GS, 71 PC, and 7 pancreatitis, 4 postoperative. These analyses uncovered the significantly higher levels of fucosylated PC03 in serum samples from PC patients as compared to GS patients; however, its specificity was still far lower than that of CA19-9 (AUC = 0.914) and exhibited poor ability to discriminate between GS and PC patients (AUC = 0.652). To improve the verification of potential glycobiomarkers, we will try to establish a high throughput platform of accurate inclusion mass screening (AIMS) for more accurate quantification of intact glycopeptides in body fluid samples.

Keyword: Pancreatic cancer, glycobiomarker, AAL, iTRAQ-based quantitativeproteome, fucosylated PC03

POSTER 015

Discovery of prognostic biomarkers for hepatocellular carcinoma by mass spectrometry-based phosphoproteomics approach

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Hepatocellular carcinoma (HCC) accounts for almost 90% liver cancer which is one of the most common cancers worldwide. Patients can be treated curatively if diagnosed early. However, most of HCC patients are still diagnosed at advanced stage, and at this stage, Sorafenib, a multi-tyrosine kinase inhibitor, is the major treatment option. Unfortunately, the prognosis of HCC is still poor. Previous studies observed that phosphorylation catalyzed various vital signaling cascades which may contribute to poor prognosis of HCC patients. Therefore, these phosphorylation-related events have generally been considered cancer biomarkers and/or viable therapeutic targets for HCC. In the present study, we aim to discover potential phosphorylation-related biomarkers for predicting prognosis and/or therapeutic targets for developing new treatment option for HCC patients. First, we established a pre-fractionation workflow using strong anion-exchange chromatography (SAX) under continuous pH gradient, which can efficiently increase the number of identified proteins and phosphopeptides. Next, we applied this pre-fraction workflow and iTRAQ-based labeling strategy to analyze/quantify proteome and phosphoproteome of paired tissue samples (cancerous and adjacent non-cancerous tissues) from HCC patients with good or poor prognosis. In the four pooled tissue samples, this analysis identified and quantified 22,002 phosphopeptides and 9,310 proteins. Among the identified proteins and phosphoproteins, up-regulated phosphoproteins in poor prognosis patients were correlated with metabolic-related pathways, and the up-regulated proteins might take part in ECM-receptor interaction, DNA replication and cell cycle process pathways. Additionally, MAPK, CaMK, CK1 and TGFbR were predicted as upstream kinases of up-regulated phosphopeptides in poor prognosis patients, implying that these kinases might be highly active in liver tissue from patients with poor prognosis. Characterizing tissue proteome/phosphoproteome between HCC patients with poor and good prognosis provide a good chance to identify a useful bio-signature to precisely predict outcome of HCC and/or potential therapeutic targets for developing new treatment strategy for HCC patients.

POSTER 016

Identification of potential serum protein biomarkers for recurrence in gastric cancer patients using a quantitative multiple reaction monitoring approach

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Despite improvements in clinical therapies of gastric cancer (GC), the recurrence rate of GC patients remains high (~55%) with advanced stage of the disease. Therefore, it is essential to elucidate the GC recurrence mechanisms that would help effective clinical application for GC diagnosis and prognosis. Here, we aimed to identify the potential multi-biomarker panel for GC recurrence with a quantitative multiple reaction monitoring (MRM) approach using GC patients' serum

samples. To establish the multi-biomarker panel, we first employed both the global proteome profiling and the preliminary MRM analysis using GC patient serum samples. As a result, we determined 94 proteins as final MRM targets and then conducted the quantitative MRM analysis with 180 individual patients divided into the two groups, i.e. response group (n=133) and recurrence group (n=47), who received chemotherapy after D2 lymph node dissection in both groups, as a training set. By a stringent statistical analysis with training set's quantitative MRM data, 6-marker panel, consisting of APOA2, APOC1, CLU, ITIH4, LRG1, and SERPINA3, was constructed. These proteins showed the differentially expressed levels (p-value < 0.05) between the two groups with an area under the curve (AUC) value of 0.810 and high prediction rates in both groups (95.5% and 61.7% in response and recurrence groups, respectively). To verify the 6-marker panel, we further applied MRM analysis with independent patient samples (n=64), i.e. response group (n=43) and recurrence group (n=21), as a test set. We demonstrated that 6 marker proteins showed the correlated expression patterns as in a training set with statistical significance (p-value < 0.05). We propose that these proteins can serve as diagnostic signatures to identify the recurrence in GC patients and our quantitative MRM assay based serum biomarker development platform could serve as a valuable tool in the clinical biomarker discovery-verification process.

POSTER 017

PRM and MRM methods to identify prognostic biomarkers of Tocilizumab

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Rheumatoid arthritis (RA) is one of the most common chronic and systemic autoimmune diseases that cause inflammation of the tissue around the joints. Interleukin-6 (IL-6) plays a vital role in activation of local synovial leukocyte production and induction of chronic inflammation. IL-6, therefore, is an attractive therapeutic target for RA and its humanized anti-IL-6 receptor antibody, Tocilizumab (TCZ), has shown to be very effective in the treatment of patients with RA. Alothough TCZ has proven to be efficacious in patients who did not respond to other RA therapeutics, some patiens show a partial respond or resistant to the therapeutic agent. In this study, we used a proteomic approach to discover prognostic biomarkers for TCZ response from serum samples of TCZ responder and non-resoponder groups of RA patients. Potential biomarker candidates identified from serum protein profile data and public database were verified by both multiple reaction monitoring (MRM) and parallel reaction monitoring (PRM) approaches. Based on our results, it is expected that those two different quantification analyses would be utilized as complementary to each other. TCZ response predicton biomarkers candidates were further compared with prognostic biomarkers of anti-tumor necrosis facter alpha (TNF-a) to discover clinical relations between the two biologics, which could complement and reinforce a beneficial therapy for RA paitens at the end.

Keywords: Rheumatoid arthritis, Tocilizumab, Interleukin-6, tumor necrosis factor alpha, Proteomics, Biomarker, Tendom mass spectrometry, Muliple reaction monitoring, Parallel reaction monitoring

POSTER 018

Quantitative proteomic analysis of pancreatic cyst fluid proteins associated with malignancy in intraductal papillary mucinous neoplasms

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Background: The application of advanced imaging technologies for identifying pancreatic cysts has become widespread. However, accurately differentiating between low-grade dysplasia (LGD), high-grade dysplasia (HGD), and invasive intraductal papillary mucinous neoplasms (IPMNs) remains a diagnostic challenge with current biomarkers, necessitating the development of novel biomarkers that can distinguish IPMN malignancy.

Methods: Cyst fluid samples were collected from 9 IPMN patients (3 LGD, 3 HGD, and 3 invasive IPMN) during their pancreatectomies. An integrated proteomics approach that combines filter-aided sample preparation, Stage Tip-based high-pH fractionation, and high-resolution MS was applied to acquire in-depth proteomic data of pancreatic cyst fluid and discover marker candidates for IPMN malignancy. Biological processes of differentially expressed proteins that are related to pancreatic cysts and aggressive malignancy were analyzed using bioinformatics tools such as Gene ontology analysis and Ingenuity pathway analysis. In order to confirm the validity of the marker candidates, 19 cyst fluid samples were analyzed by western blot.

Results: A dataset of 2992 proteins was constructed from pancreatic cyst fluid samples. A subsequent analysis found 2963 identified proteins in individual samples, 2837 of which were quantifiable. Differentially expressed proteins between histological grades of IPMN were associated with pancreatic diseases and malignancy according to Ingenuity pathway analysis. Eighteen biomarker candidates that were differentially expressed across IPMN histological grades were discovered—7 DEPs that were upregulated and 11 that were downregulated in more malignant grades. HOOK1 and PTPN6 were validated by western blot in an independent cohort, the results of which were consistent with our proteomic data.

Conclusions: This study demonstrates that novel biomarker candidates for IPMN malignancy can be discovered through proteomic analysis of pancreatic cyst fluid.

Keywords: pancreatic cyst fluid, intraductal papillary mucinous neoplasm (IPMN), IPMN dysplasia, biomarker candidates, LC-MS/MS

POSTER 019

Variability Assessment of 90 Salivary Proteins in Intraday and Interday Samples from Healthy Donors by Multiple Reaction Monitoring-Mass Spectrometry

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Purpose: Saliva is an attractive sample source for the biomarker-based testing of several diseases, especially oral cancer. Here, we sought to apply multiplexed LC-MRM-MS to precisely quantify 90 disease-related proteins and assess their intra- and interindividual variability in saliva samples from healthy donors.

Experimental design: We developed two multiplexed LC-MRM-MS assays for 122 surrogate peptides representing a set of disease-related proteins. Saliva samples were collected from 10 healthy volunteers at three different time points (Day 1 morning and afternoon, and Day 2 morning). Each sample was spiked with a constant amount of a 15N-labeled protein and analyzed by MRM-MS in triplicate. Quantitative results from LC-MRM-MS were calculated by single-point quantification with reference to a known amount of internal standard (heavy peptide).

Results: The CVs for assay reproducibility and technical variation were 13 and 11%, respectively. The average concentrations of the 99 successfully quantified proteins ranged from 0.28 ± 0.58 ng mL−1 for profilin-2 (PFN2) to 8.55 ± 8.96 μg mL−1 for calprotectin (S100A8). For the 90 proteins detectable in >50% of samples, the average CVs for intraday, interday, intraindividual, and interindividual samples were 38%, 43%, 45%, and 69%, respectively. The fluctuations of most target proteins in individual subjects were found to be within ± twofold.

Conclusions and clinical relevance: Our study elucidated the intra- and interindividual variability of 90 disease-related proteins in saliva samples from healthy donors. The findings may facilitate the further development of salivary biomarkers for oral and systemic diseases.

POSTER 020
Development of an automated immuno-MALDI mass spectrometry assay for detection of interstitial collagenase in dried saliva spot sample.

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Oral squamous cell carcinoma (OSCC), which is the most common subtype of oral cavity cancer, accounts for more than 90% of oral cancer cases. Over 60% of patients present with stage III and IV disease, and that OSCC has a higher rate of second primary tumors. Thus, we urgently need new approaches that will enable the early detection and monitoring of OSCC. Salivary MMP1 is one of the most promising candidate biomarkers for OSCC detection and worth to be validated in large cohort of sample. For this purpose, we developed a workflow, which comprises dry saliva spot sampling and immuno-enrichment coupled to MALDI-TOF (i-MALDI-TOF) analysis, to assay the salivary MMP1 in this study. First, we demonstrated that MALDI-TOF could precisely (CV<15%) and accurately (accuracy within 80-100%) detect the pure peptide in the range from 0.26 fmol to 100 fmol. While the immune-enrichment exhibited 32.2±4.3% recovery rate, the i-MALDI-TOF could detect the target peptide in the background of 100 μl saliva with the limit of quantification (LOQ) at 5.68 fmol, which refer to 3.07 ng/ml. We have also optimized the automatic immune-enrichment platform with using antibody-coupling protein G beads and magnetic particle processor. In addition to TOF-MS, the FT-MS could provide more accurate quantification which indicated the MALDI ionization method is capable of handling the enriched sample without LC separation. We further demonstrated that the intra-day and inter-day variation of i-MALDI-TOF assay applied in dry saliva spot sample were less than 20%, and the stability of MMP1 stored at dried saliva spot remain almost 100% for 3 months at least. Using this workflow, we successfully detect the overexpressed MMP1 in all of 9 OSCC cases. Development of a convenient, rapid, and high-throughput workflow would benefit the further application of salivary MMP1 in early detection and screening of OSCC.

POSTER 021
Comprehensive proteomic profiling of serum exosomes identifies novel biomarkers for early detection of gastric cancer

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Gastric cancer (GC) is the third leading cause of cancer-related deaths worldwide. To reduce the mortality and improve early detection rate for gastric cancer, we comprehensively explored novel biomarker proteins on circulating exosomes isolated from gastric cancer patient's sera. In order to overcome the difficulties of isolating high-purity exosomes from biological fluid, we recently established EV-Second column (Extracellular Vesicle isolation by Size Exclusion Chromatography ON Drip, commercially available from GL Sciences International Inc.) which allows rapid isolation of high-purity exosomes by simple procedure. Using EV-Second columns, we isolated serum exosomes from 58 individuals (normal donors; n = 10, early stage GC patients; n = 16, advanced stage GC patients; n = 18, and scirrhous type GC patients; n = 14). Following quantitative LC/MS analysis identified 832 exosomal proteins in which 13 proteins showed significant up-regulation in GC-derived exosomes (t-test, $p < 0.05$, fold change > 2.0, and valid value > 80%). Especially, PN-1 was detected as a highly-enriched protein cargo in GC exosomes ($p = 5.30\times10^{-6}$, fold change = 8.37). In multiple tissue array analysis, PN-1 strongly expressed in cancer tissues whereas almost no expression was observed in normal mucosa. To examine the role of the Exo-PN-1 in the tumor microenvironment, Exo-PN-1 was isolated from human gastric cancer cells. Flow cytometry and immunofluorescent analysis indicated potential contribution of PN-1^{+} exosomes to apoptosis resistance in gastric cancer cells via deregulation of intracellular pH homeostasis, resulting in induction of anoikis resistance and metastatic potential to gastric cancer cells. These results provide the first evidence of the Exo-PN-1 delivers apoptosis resistance signals to tumor microenvironment during gastric cancer progression.

POSTER 022
Proteomic study on advanced glycation end-products treatment in kidney of mice of mice

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Advanced glycation end products (AGEs) are heterogeneous cross-linked sugar-derived proteins which could accumulate in

patient of diabetic nephropathy (DN). AGEs are thought to be involved in the pathogenesis of DN via multifactorial mechanisms such as oxidative stress generation and overproduction of various growth factors and cytokines. Nε-(carboxymethyl) lysine-conjugated bovine serum albumin (CML-BSA) is a major component of AGEs. To investigate a role of CML-BSA in the regulation of diabetic nephropathy, we performed in vivo animal experiment and proteomic analysis. The experimental conditions were as followed: for mouse model, control mouse, Streptozotocin treatment (STZ), STZ + CML-BSA. To confirm the effect of CML on mitochondrial dysfunction, HK-2 cells, which are human renal tubular cells, were treated with CML-BSA and examined by electron microscopy to directly observe the mitochondrial dysfunction morphology. This study aims to analyse CML-mediated protein network in mice kidneys. Chronic consumption of CML induced grade 1 necrosis of renal tubules. In addition, from the proteomic analysis, we identified 981, 1025, and 911 proteins in the control, STZ, STZ+CML mouse kidney, respectively. Among these proteins, only 75 protein s expressed in AGEs-treated mouse kidney were identified. As a result of IPA analysis, CML-mediated differential proteins were involved in mitochondrial dysfunction. To confirm the effect of CML-BSA on mitochondrial dysfunction, HK-2 cells, which are human renal tubular cells, were treated with CML-BSA and examined by electron microscopy to directly observe the mitochondrial dysfunction morphology. In addition, damage to mitochondrial DNA and mitochondrial membrane potential was induced by CML-BSA in HK-2 cells. Many of proteins are functionally associated with kidney toxicity and specific mitochondrial dysfunction related proteins were identified in CML-treated mice kidney. We found grade 1 metastatic necrosis of renal tubules without inflammation in CML-treated mice kidney. CML are likely to induce DN by inducing chronic mitochondrial dysfunction in the mice kidney tubules.

POSTER 023

Proline-rich protein 4 (PRR4) as a potential tear biomarker for personalized diagnosis of dry eye disease and glaucoma

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Dry eye syndrome (DES) and glaucoma are two most common ocular diseases with high global prevalence. Despite the widespread studies of these pathologies, the fundamental etiology underlying these diseases and inter-individual variations in disease diagnosis remain elusive. Therefore, this study was undertaken to identify potential tear protein biomarkers for both ocular pathologies in individuals utilizing the targeted proteomics strategy.

Tear samples were collected with Schirmer's strips from 140 individuals. Subjects were categorized into aqueous-deficient DES (DRYaq: n=20), evaporative DES (DRYlip: n=20), a combination of DES (DRYaqlip: n=20), glaucoma (GLM; n=20), a combination of DES and glaucoma (GLM_DRY; n=20) and healthy subjects (CTRL; n=40). Samples were analyzed individually utilizing a targeted MS strategy called accurate inclusion mass screening. This strategy has been optimized for rapid and robust identification of 128 signature peptides representing various tear proteoforms. The acquired continuum MS spectra were analyzed by MaxQuant, followed by statistical analyses utilizing Perseus and functional annotation analysis with Ingenuity Pathway Analysis.

As many as 39 significantly differentially abundant peptides ($P < 0.05$) were identified in the designated groups, namely S100A8, S100A9, ANXA2, ANXA1, CLU, PRR4, MSLN, ZG16B, IGHA1 and ALDH1A1. Functional annotation analysis of these proteins demonstrated significant regulation of the metabolic, apoptotic, immune and inflammatory pathways. Particularly, PRR4 was found to be increased in the glaucoma compared to DES and CTRL groups for the first time, which might be related to heightened neurological processes. In addition, this study has identified six major variations in the expression profiles of PRR4 isoforms in different individuals.

This is the first study demonstrating the significant discriminant proteome correlation between glaucoma and DES patients and inter-individual expression variations of PRR4 isoforms in a large cohort. Multiplexed approach utilizing this targeted proteomics strategy was instrumental to unravel novel molecular processes involving these pathologies in a personalized manner.

POSTER 024

A fast, simple, and robust sample preparation workflow enables high-throughput plasma protein profiling.

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Plasma is the most predominant resource for biomarker discovery. Mass spectrometry based proteomics has been used in plasma biomarker discovery as it can provide quantitative information of hundreds proteins in just several hours. But due to several technical challenges, the proteins identified in plasma are limited. To solve these challenges in biomarker discovery from plasma samples, we combined different strategies to build a fast, simple and highly robust workflow on an Orbitrap Fusion LumosTM mass spectrometer. For the sample preparation, we minimized sample preparation steps by depleting top-12 abundant protein in plasma and denaturing with guanidine. With tandem digestion by LysC and trypsin, we got excellent recovery and reduced missed cleavages in plasma digestion. This sample preparation workflow could also be done in 96-well format to provide much higher throughput. To get precise and robust quantification, we used data-independent acquisition (DIA) for single-shot analysis. We first fractionated top-12 abundant protein depleted plasma to build a plasma spectral library. With excellent sensitivity and scan speed of the Orbitrap Fusion LumosTM, we got a spectral library of 4,061 proteins and 23,720 peptides, which is the comprehensive plasma dataset data we knew. For single shot analysis, we quantified more than 1,000 proteins in a single run from top-12 abundant protein depleted plasma. The median CV of quantified proteins is less than 20%.

POSTER 025

Distinguishing pancreatic cancer from benign diseases and healthy individuals by mass spectrometry-based metabolomic pipeline

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Objectives: By investigating the overall serum metabolic differences among pancreatic cancer (PC)、benign diseases (BD) and healthy individuals (normal control, NC), in this study

we aim to discover the particular metabolites as potential diagnostic markers for early diagnosis of pancreatic cancer.

Design and methods: We combined both reversed-phase (C18) and hydrophilic interaction (HILIC) liquid chromatography separation and a high-resolution quadrupole time-of-flight mass spectrometer detection which was operated in negative(ESI–) and positive(ESI+) electrospray ionization mode together , in order to comprehensively analyze serum metabolites obtained from 20 patients with PC, 10 patients with BD and 10 NC. SWATH analysis was further utilized to validate metabolites which we have found in the discovery stage.

Results: On the basis of our study, we identified 122 metabolites in total corresponding to various important pathways, such as ***,***,** pathways in PC vs Control(BD and NC) Besides ,according to the PCA analysis based on the amount of the metabolites, the three groups can be distinguished well from each other. Students' T-test were used to find the altered metabolites, and 10 metabolites (hippuric acid, L-Aspartyl-L-phenylalanine, 3-Hydroxybutyric acid, trigonelline, 1,9-Dimethyluric acid, L-Glutamic acid, hexadecanedioic acid, erucic acid, indoxyl and 3-Indolepropionic acid)were more than 3 times different between BD and NC, while 14 metabolites can distinguish PC and NC.

Conclusions: The newly found metabolites may be potential biomarkers for PC that can differentiate PC and BD patients and healthy individuals. More serum samples is now being collected and used for the validation and verification study.

POSTER 026

Plasma proteomics in children diagnosted with acute lymphoblastic leukemia: a pilot study

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In Latin American countries, the incidence and mortality rates of Acute Lymphoblastic Leukemia (ALL) are high. In Colombia, during the last decade, ALL has been the most prevalent cancer among children between 0-14 years of age. According to the National Institute of Health for 2014, 43% of deaths in children under 18 years were attributed to ALL. In the face of this public health problem and coupled with the fact that the knowledge of the proteome of the child population is little, we proposed the study of the plasma proteome of Colombian children diagnosed with B-ALL in search of proteins that could have potential as markers of disease and may reflect processes altered by the presence of the disease or as a response to this.

A proteomic study by LC-MS/MS and quantification by label-free using Progenesis QI software was performed in search of proteins differentially expressed between healthy children and diagnosed with B-ALL. We quantified a total of 460 proteins in blood plasma depleted. Statistical analysis was performed using MSstats, and Reproducibility Optimized Test Statistics (ROTS) in R package, 25 differentially expressed proteins in plasma of patients diagnosed with B-ALL compared with healthy controls with fold change >2 and *P*-values <0.05. These proteins are mainly associated with acute-phase processes, alterations of the extracellular matrix, adhesion and cellular signaling. Among them, we can highlight the Fetuin, Butyrylcholinesterase, Coagulation Factor 13 Chain A, Gelsolin, Platelet Factor 4, Peptidase Inhibitor 16 and 1-Phosphatidylinositol 3-phosphate 5-kinase proteins. The results provide new information about the plasma proteome of Colombian children and allow to propose a prioritized list of proteins with potential as biomarkers, as well as to contribute to the understanding of the leukemia.

POSTER 027

Quantitative proteomic signature of first-episode psychosis patients' PBMCs – preliminary results

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Schizophrenia is a complex and chronic psychiatric disorder for which there is still no biomarker. The diagnosis of the disease is mainly based on clinical interview with no biomolecular support which can be used to increase diagnosis confidence or to guide prognosis. Moreover, medication resistant patients need to be subjected to long and unhelpful therapy trials before initiating clozapine, which is far from the goal of a more personalized and preventive medicine.

In the present project we aim at analyzing the proteic content of the peripheral blood mononuclear cells (PBMCs) of minimally medicated first-episode psychosis patients, comparing with healthy controls, in order to depict proteins or major pathways that may play a role at the onset of the disease.

To achieve this goal a state of the art quantitative proteomics approach, SWATH-MS, was used in a preliminary cohort of 6 samples. In total, 1413 proteins were quantified allowing for a good representation of the numerous major pathways. From these, several proteins met the statistical and quality filters and were considered as altered. Multivariate analysis using the differentially expressed proteins demonstrated that these were enough to distinguish between the study groups, and after functional analysis of these same proteins some metabolic pathways were highlighted, with special emphasis for the innate immune system, in particular the complement cascade.

As future perspectives, this line of research will be pursued with a projected increase of the cohort analyzed, as well as the comparison of the proteomic alterations through the course of disease and with other major psychiatric disorders.

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POSTER 028

Searching for new blood biomarkers of Wilson's disease using translational proteomics.

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Background

Wilson's disease is a rare genetic disorder triggered by mutations in the ATP7B gene. These mutations lead to the dysfunction of a transport protein (ATP7B) involved in copper excretion. This disease is characterized by defects in copper transport and by toxic copper overloads, primarily in the liver and the brain. Copper toxicity leads to liver injury and neuropsychiatric disorders which manifest either as acute (fulminant hepatitis) or chronic (cirrhosis) episodes. We engaged a translational study of Wilson's disease pathophysiology using the preclinical ATP7B-/- murine model and human clinical samples.

Methods

Firstly, we explored plasma proteome modifications induced by disease progression in the ATP7B-/- murine model for Wilson's disease. Plasma sample from ATP7B-/- and wild type (WT) mice were collected at defined stages of disease and were characterized using mass-spectrometry based proteomic analysis (LC-MS/MS). Then, we verified these proteome modifications in a larger sample cohort using isotope dilution standards and targeted mass spectrometry analysis (LC-SRM). This panel of protein was finally evaluated using LC-SRM in plasma samples from Wilson's disease patients.

Results

Plasma proteome investigations led to the identification of 14 plasma proteins differentially abundant between ATP7B-/- and WT mice. Six proteins were validated in an independent series of murine plasma samples by quantitative LC- SRM. Most of these proteins were involved in metal homeostasis regulation, lipid metabolism, inflammation, liver development and regeneration.

Conclusion

Using discovery and targeted proteomics, we could identify plasma proteins that may be useful for Wilson's disease diagnosis and monitoring of disease progression.

POSTER 029
Identification of diagnostic biomarkers for lung cancer by quantitative proteomic analysis
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Lung cancer is the most common malignant tumors with approximately 27% of all cancer deaths per year worldwide. Current diagnosis and therapies are not sufficient to reduce the mortality of lung cancer. Therefore, early detection and systemic therapy are urgently needed. To date, quantitative proteomics is widely used to reveal the differentially expressed proteins in normal and cancer tissues. Combining bioinformatics analysis, proteomic data provides information on molecular interactions, signal pathways, and biomarker recognition. In this study, we compared the protein expression profiles in lung cancer tissues and adjacent normal lung tissues from 7 patients through high-resolution label-free mass spectrometry. The total 3005 proteins were identified, out of which 159 proteins were differently expressed between lung cancer tissues and normal tissues. Next, through bioinformatics analysis, six proteins overexpressed significantly will be select to verify by western blot. Then, the six proteins will be identified in the 70 patients through Quantitative Dot Blot technique, which is a high-throughput immunoassay method. This research will provide candidate biomarkers for the diagnosis of lung cancer. Our research provides a new method for identifying cancer biomarkers by combining quantitative proteomic with QDB.

POSTER 030
Identification of differential expression proteins in esophageal cancer by label-free quantitative proteomics analysis
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Background

Esophageal cancer (EC) is one of the most aggressive malignant tumors worldwide and also a common cause of cancer-related death. There are two histological subtypes of EC: one is esophageal squamous cell carcinoma (ESCC) and the other is esophageal adenocarcinoma (EAC). ESCC has a very high incidence in developing countries, especially in China. Clearly, the discovery of potential biomarkers for early diagnosis of EC are urgently needed. Quantitative proteomic methods have now been applied across many cancer tissues, such as breast cancer, lung cancer, pancreatic cancer, melanoma and ESCC. To date, no specific markers of EAC have been identified. we used proteomics techniques to study the differential expression proteins between esophageal tumor tissues and adjacent normal tissues.

Method

Six Pairs of esophageal cancer and adjacent normal esophageal tissue were obtained from surgical resection and analyzed with QExactive Plus coupled with nanoLC system with a label-free quantitative approach. We identified differently expressed proteins among the total of 2080 identified proteins, 57 were differentially expressed between esophageal cancer and normal esophageal tissues. Among the 87 differential proteins,

PTMA, NID2, RBM3, PPP1CA, PAK2 proteins with very significant differences were screened out($p<0.001$). PTMA, NID2, RBM3, PPP1CA, PAK2 were validated by Western blotting. The results were further validated in another cohort including 60 patients with Quantitative Dot Blot, which was recently developed for high throughput immunoblot validation.

Results

The results of WB showed that the expression of five factors in esophageal cancer was higher than that in normal esophageal tissue. QDB results showed that the expression of PTMA and NID2 in esophageal cancer is greater than normal esophageal tissue in the vast majority of patients, and this result has statistical significance.

POSTER 031
Investigating the proteases/peptidases implicated in the Urinary normal peptidome generation; towards new trends in Biomarker discovery.
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Investigating the profile of normal urine peptidome in relation to proteases/peptidases involved in its generation is extremely important as it may reflect the normal physiology of the body, and their changes may indicate disorders occurred in vivo. In this study, we analyzed low molecular proteins/peptides separated from urine samples of 24 healthy volunteers by LC-MS. Our results suggested that 229 precursor native peptides were generated from their precursor intracellular or extracellular proteins by the activity of endopeptidases. Among them, 167 peptides were identified as predicted intact forms without degradation/cleavage, whereas amino acid residues were trimmed in the remaining 62 peptides at N or C-terminus or both by exopeptidases to generate 216 fragment peptides. Interestingly, these 62 precursor peptides were predicted to have short half-life and high entropy compared to those remained intact without degradation/cleavage. Additionally, ~95% of the generated precursor native peptides require being cleaved at N and C-terminus cleavage sites to set free from their precursor proteins. Moreover, ~ 59% of these cleavage sites exist in structured (helix, sheet) buried regions, at which peptidases are not easy to access, indicating that the precursor proteins have to be linearized before such cleavage. Peptidases prediction showed that metallopeptidases, such as MMP-9 and MMP12, serine peptidases, neutrophil elastase (ELAN) and cathepsin G (CTSG) peptidases were highly predicted peptidases to generate urine native peptides from precursor proteins. Interestingly, investigating the trimming profile of urinary precursor native peptides showed that amino and carboxypeptidases, which hydrolyze the first or last peptide bond, were assumed to be involved in the generation of the urine native peptides rather than other di or tri exopeptidases with high contribution of exopeptidases which cleave preferentially hydrophobic amino acids.

POSTER 032

Strategy to establish new clinical biomarkers: From proteomics selection of biomarker candidates to validation for clinical use

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MS-based proteomics has been employed for biomarker discovery in biofluid, such as plasma and urine. However, low throughput and quantitativity of the MS-based proteomics were the serious bottleneck to provide new biomarkers useful to clinical medicine. Especially, validation of biomarkers with a large number of samples is not easy by the MS-based proteomics. To solve this bottleneck, we have set up a high throughput antibody-based validation platform after biomarker discovery in urine by label-free quantitative proteomics, SWATH with a small number of samples. ELISA is one of the most frequently tools to measure the amounts of antigens in a large number of samples, however, its establishment is not easy since two different antibodies against a candidate protein are necessary. Therefore, we employed a surface plasmon resonance (SPR)-based measurement by using ProteOn XPR36 (BioRad) and a single monoclonal antibody for each biomarker candidate, which allowed a label-free measurement of proteins with a large number of samples since the antigen-antibody interactions was detected on a 6 × 6 interaction array sensor chip. On a tip, interaction of 6 samples with 6 different antibodies was measured in a short time.

In this presentation, we will show our antibody-based SPR platform for validation of biomarker candidates for kidney site-unique injuries in urine with more than 500 samples. Several different monoclonal antibodies against one biomarker candidate were examined to select a single antibody suitable for the SPR measurement. The amounts of proteins in urine were calculated after adjustment by a decline curve with sequential measurements and by a dose-response curve with a standard control sample.

Urine biomarkers for kidney site-unique injuries were successfully validated by our platform and the detail data will be presented.

POSTER 033

Early candidate biomarkers in urine of Walker-256 lung metastasis rat model.

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Detection of cancer metastasis at its early stage is important for the management and prediction of cancer progression. Urine, which is not regulated by homeostatic mechanisms, reflects systemic changes in the whole body and can potentially be used for the early detection of cancer metastasis. In this study, a lung metastasis of a rat model was established by tail-vein injection of Walker-256 cells. On the fourth day, lung metastasis nodules appeared. On the sixth day, the body weight decreased. Urine samples were collected at days 2, 4, 6 and 9 after injection, and the urinary proteomes were profiled. On days 2, 4, 6 and 9, 11, 25, 34 and 44 differential proteins were identified in 7 lung metastatic rats. Seventeen and 18 differential proteins were identified on the second day and the fourth day respectively, which indicated that the differential urinary proteins changed significantly two days before lung metastasis nodules appeared. All these 17 and 18 differential proteins had been reported to associate with both lung cancer and breast cancer. Differential urinary proteins differed in Walker-256 lung metastasis rat models and its subcutaneous rat models. A total of 9 differential proteins (NHRF1, CLIC1, EZRI, AMPN, ACY1A, HSP7C, BTD, NID2, and CFAD) were identified in all 7 lung metastatic rats at more than one time point but none of them were identified in the subcutaneous rat model. Our results indicate that (1) the urine proteome changed significantly, even on the second day after tail-vein injection of Walker-256 cells and that (2) the urinary differential proteins were different in Walker-256 lung metastatic and subcutaneous models.

Keywords: cancer biomarkers, urine proteome, cancer metastasis, early detection

POSTER 034

A diagnostic panel of urine protein biomarkers predicts lung cancer from healthy controls and other tumors

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Purpose: Development of noninvasive, tumor-specific urinary biomarkers that is able to differentiate lung cancer patients from healthy individuals and other cancer patients.

Methods

High-throughput, nano LC-MS/MS proteomic technique was used to measure urinary proteins in healthy controls (CTL, n=33), benign pulmonary diseases (Benign, n = 40), lung cancer (LC, n=33), bladder cancer (BC, n=17), cervical cancer (CCA, n=25), colorectal cancer (CRC, n=22), esophageal cancer (EC, n=14), and gastric cancer (GC, n=47) patients. Urine proteins were collected by ultracentrifugation, separated by an SDS-PAGE gel. After in-gel trypsin digestion, peptides were analyzed by nano LC-MS/MS. A panel of five markers was selected from the training set by a random forest model with feature selection algorithm. Test sets (CTL vs LC, CTL vs Benign, BC vs LC, CCA vs LC, CRC vs LC, EC vs LC, GC vs LC) were used to validate prediction accuracy and tumor specificity.

Results

A list of candidate biomarkers was nominated and evaluated. A panel of five protein markers (FTL: Ferritin light chain; MAPK1IP1L: Mitogen-Activated Protein Kinase 1 Interacting Protein 1 Like; FGB: Fibrinogen Beta Chain; RAB33B: RAB33B, Member RAS Oncogene Family; RAB15: RAB15, Member RAS Oncogene Family) in the predictive model has correctly classified majority of lung cancer cases both in the training set and the test sets, which contain healthy controls, benign lung diseases, lung cancer patients, and other tumors.

Conclusions

A combination of five urinary biomarkers can discriminate lung cancer patients from control groups as well as from other common tumors. The biomarker panel and the predictive model may be considered as an auxiliary diagnostic tool along with imaging technology for lung cancer diagnosis.

Keywords

Lung cancer, machine learning, urinary biomarkers

POSTER 035

Analysis of toxicologically relevant proteins in pesticide-treated HepaRG cells by MS-based immunoassays

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Due to the use of pesticide mixtures in agriculture, humans are exposed to multiple pesticides via food. Up to date, there is not much data available on mixture effects. The European Union regulations (Reg No 1107/2009; Reg No 528/2012) require the analysis of potential cumulative or synergistic effects of multiple pesticide and/or biocide exposure. With traditional toxicological approaches, animal testing would increase due to the rising number of available substances. That means it is important to develop *in vitro* methods for the assessment of pesticide combinations to reduce animal testing.

Based on mRNA expression experiments, we selected a number of proteins and analyzed them with mass spectrometry-(MS) based immunoassays to investigate the influence of pesticides. This methodology includes a tryptic digestion of proteins followed by an antibody-based immune enrichment of proteotypic target peptides. As readout UHPLC-MS was used. The analysis of toxicologically important biomarkers focused on cytochrome P450 enzymes (CYPs, phase I enzymes), UDP-glucuronosyltransferases (UGTs, phase II enzymes), transporters (Phase 0 and III) and others.

We used HepaRG cells, a well-established human hepatocyte system, to test the effects of single pesticides and combinations thereof. Rifampicin and CITCO served as prototypical inducers. So far, we analyzed 24 proteins quantitatively in cells treated with 27 different pesticides and 3 combinations. For TNFRSF12A a 3.5-fold induction effect was observed after high dosage treatment with prochloraz and S100P protein level was increased by a factor of 7.5 after treatment with flusilazole suggesting two independent induction pathways.

In sum, we investigated protein induction and repression effects of 27 different pesticides on 24 proteins in HepaRG cells. We observed that the protein induction of CYP1A1, CYP1A2, CYP3A4, MDR1, TNFRSF12A and S100P increased depending on the chemical structure of pesticides.

POSTER 036

Identification of tumor specific peptides using HLA peptidome

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Tumor-reactive T cell therapy has been considered a promising immunotherapy for cancer patients. Nevertheless, the reality is that there is a lack of technology platforms for identifying tumor-specific antigen and antigen response-T cells. First, we have tried to identify effective peptides by separating HLA peptides from membrane proteins. We found more HLA bound peptides from cellular membran proteins than from whole cell proteins using breast cancer cell lines. Applying this method, we identified HLA presented peptides from primary breast cancer cell and PDX-derived cancer cells. The following step, we have been establishing stable cell lines expressing patient HLA types in order to isolate T cells responding on identified peptides. This study will perform the identification of the tumor antigen response TCR sequence following identification of a patient's specific HLA peptide and the separation of peptide response-T cell. Ultimately, this study could provide a useful strategy for separating tumor-response T cells and for identifying tumor-response TCR sequences for TCR engineering T-cell production.

POSTER 037

Proteomic profiling of proteolytic processing events in plasma samples from melanoma patients

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Melanoma is the most agressive form of skin cancer, responsible for more than 80% of skin cancer-related mortality

due to poor prognosis in advanced stages of the disease.The imbalance in cellular homeostasis due to oncogenesis has important effect on protein synthesis and degradation, therefore on the repertoire of proteins secreted in cancer patients.Liquid biopsies supply tumor progression information from biological fluids such as plasma, saliva and urine, providing a significant source of circulating DNA, RNA, and proteins implicated in oncogenesis.In this context, the N-terminal end of proteins, as well as the post-translational modifications undergone by the α-amino group or side chain of the N-terminal residue have several implications in maintaining the homeostasis of various biological processes such as cell localization and turnover of most proteins.Plasma samples of 16 melanoma patients with distinct stages were provided by the Bio bank from the São Paulo Cancer Institute . Plasma samples were depleted from albumin and IgG and subjected to in solution trypsin digestion followed by LC-MS/MS or, alternatively, subjected to Terminal Amine Isotopic Labeling of Substrates (TAILS) protocol in order to allow the profiling of proteolytic events. Qualitative analysis of plasma samples (evaluated by SDS-PAGE) revealed similar patterns in the proteome diversity, regardless of the stage of the disease. Proteins such as Vitronectin, Vimentin, Transthyretin, Proteglycan 4 and Arylsulfatase A were found by shotgun proteomics analysis in all 16 plasma samples. Moreover, TAILS analyses resulted in a number of processed proteins in the plasma of melanoma patients, including Melanoma-associated antigen B16, Galectin-3-binding protein, Leucine-rich repeat-containing protein 9, Secreted phosphoprotein 24kD, Gelsolin and Ceruloplasmin, some of them may represent novel processed forms with yet unknown biological roles. Our results may represent potential (new) melanoma markers, providing new translational alternatives for monitoring the progression of the disease.

POSTER 038

Quantitative shotgun proteomics unveils candidate novel cervical cancer-specific proteins.

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Cervical cancer is one of most common female cancer worldwide. We aimed to identify the proteins implicated in the development of cervical cancer. Multidimensional LC-MS/MS was performed for the analysis of biological samples labeled with isobaric mass tags for relative and absolute quantitation (iTRAQ) to identify proteins differentially expressed in cervical cancer in relation to non-cancerous cervix tissues. We identified 211 common proteins in two experimental replicates in cervical cancer tissues, signal transduction components, epidermal cell proliferation proteins, antiapoptotic proteins and structural cell components. Of these, 43 proteins were differentially expressed. To investigate the role of RAB14 and RCN3 in cervical cancer, we inoculated separately overexpressing RAB14 HeLa Cells, RCN3 overexpressing cells, and together RAB14-RCN3 HeLa cells in nu/nu mice. The experiments were followed for 7 weeks and tumor size was evaluated. RAB14 protein expression was positively correlated with tumor size increasing in nu/nu mice inoculated with over-expressing RAB14HeLa cells only more than in RCN3 HeLa cells and RAB14-RCN3 together in HeLa cells. Our results indicate that Reticulocalbin 3 and RAB14 expressions may be used as a novel prognostic biomarker in cervical cancer.

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POSTER 039

The proteome of OSCC-derived extracellular vesicles reflects tumor aggressiveness and clinical staging

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More than 300,000 new cases of head and neck cancer occur per year worldwide, and despite the improvement of treatment modalities in the last years, still, there is a high morbidity rate (37.8%) after five years of diagnosis. Oral Squamous Cell Carcinoma (OSCC) account for 90% of all malignancies in the oral cavity, and there are limitations in the standard multimodal management of OSCC, since patients in the same TNM (tumor-node-metastasis classification) can have a distinct clinical outcome. Therefore, robust prognostic markers or therapeutic targets are awaited. Extracellular vesicles (EVs) have recently emerged as significant physiological and pathological roles in cell-cell communication through interactions mediated by cell receptors or by transferring bioactive molecules changing the phenotype of the recipient cells. The tumor cell-derived EVs can modify the characteristics of the tumor microenvironment, supporting metastatic progression in several tumors. The detection of alterations in the EVs proteome may, therefore, contribute to the understanding of the cellular mechanisms modulated in OSCC and helping to improve the clinical outcome in OSCC. This study analyzed the cargo proteins of different EVs from less and high aggressive oral cancer cells and OSCC biopsies at different tumor staging to understand their possible role in microenvironment modulation and oral cancer progression using nanoparticle tracking, immunolabeling, and mass spectrometry analyses. Our results indicate that EVs from aggressive cells have exclusive proteins overrepresented by distinct biological processes as tumor microenvironment modulation and immune system regulation. Furthermore, the protein content of OSCC-derived EVs correlated with tumor clinical staging and risk of metastasis. In conclusion, the proteomic profiling of EVs reveals potential markers of tumor aggressiveness and clinical stage.

POSTER 040

Unravelling the impact of ASPP-PP1 interactions using phosphoproteomics studies in KI mouse and cancer cell lines.

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Phosphatases play an important role in regulating a wide range of fundamental cellular processes by mediating dephosphorylation of majority of proteins. They derive their specificity from from interacting with numerous regulatory subunits, for e.g., Serine/threonine protein phosphatase 1 (PP1) and the apoptosis-stimulating proteins of p53 (ASPP) family members (ASPP2 and the inhibitory iASPP), which bind to PP1 via defined interaction motifs. To understand the impact

of ASPP-PP1 interactions on different cellular/metabolic pathways, phosphoproteomic investigations were carried out using mass spectrometry.

Studies were undertaken using U2OS and AGS cell lines expressing mutant ASPP2 (PP1 binding deficient), and cardiomyocytes (isolated from the mouse hearts derived from either iASPP-null mice or mice expressing a mutant iASPP (PP1 binding deficient). The proteome and phosphoproteome of ASPP-WT/ ASPP-KO/ PP1 binding deficient KI mutants were compared to understand the impact of the ASPP-PP1 interaction. These investigations were aimed at identifying ASPP-PP1 substrates by identifying a subset of proteins whose expression levels are not affected by ASPP status but whose phosphorylation status are clearly altered in the KI and KO compared to that seen in WT.

For the phosphoproteomics, the protein extracts were digested using routine in solution digestion protocol followed by C18 desalting. Quantitative comparisons were carried out using label free and dimethyl labeling approaches. For a deep proteome coverage, the digested peptides were fractionated using high pH reverse phase pre-fractionation. The phosphopeptides were enriched using TiO_2 enrichment and both the non-enriched and enriched samples were analyzed using HCD fragmentation on Fusion Lumos. The MS data was analyzed with MaxQuant and the statistical analysis were done using Perseus and R.

Our approach validated some of the known targets of ASPP-PP1 interactions reported in the literature, in addition to identifying some new candidates which can further be validated by IPs and histological studies.

POSTER 041

Bioenergetic Reprogramming profoundly changes the Mitochondrial Proteome resulting in marked changes to Morphology and Susceptibility to Induced Cell Death

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Aerobic glycolysis is a characteristic of many tumor cells and can be targeted with the anti-glycolytic 2DG, which sensitises cells to apoptosis. However, increasing evidence suggests that some tumors are glycolysis independent and can switch to oxidative phosphorylation (ox-phos) to survive. In order to study how cellular energy generation and programmed cell death is mediated at the protein level we have developed protocols to characterise and quantitate the mitoproteome of leukemic cells under differing bioenergetic conditions using a model to switch from glycolysis to ox-phos.

We have then investigated the interplay between the mitochondrial proteome and ultra-structural dynamics, cell death, and bioenergetics. Leukaemia-derived (Z138) cells maintained in galactose-supplemented media grow normally with unaltered cellular ATP levels, which are maintained by up-regulation of mitochondrial ox-phos. However, galactose-maintained cells are exquisitely sensitive to canonical mitotoxins. In contrast, galactose cells exhibit decreased sensitivity to canonical inducers of caspase-dependent apoptotic cell death. To investigate the underlying mechanisms we have used label-free LC-MS/MS to compare the mitochondrial proteome in glucose and galactose-maintained cells. Our studies show bioenergetic rewiring results in profound to changes in the mitochondrial proteome, which modulate both the sensitivity and the mode of cell death in response to mitotoxins or classical inducers of cell death. Our detailed analysis shows differentials in key apoptotic and bioenergetic proteins, in addition to proteins involved in mitochondrial dynamics.

The LC-MS/MS results lead us to use electron microscopy to reconstruct the 3D structure of the cells which show significant changes in mitochondrial structure and other cellular organelles.

POSTER 042

Characterization of Malignant Pleural Mesothelioma by comprehensive proteomics study

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MPM is a rare pleural malignancy with an annual incidence of 2500 persons in the United States and is mainly caused by asbestos exposure. In this study, we did a comprehensive proteomic analysis of 12 MPM patients sample. Up to 20ug of the peptide was fractionated by orthogonal pH reverse phase column fractionation method and ran on Orbitrap Fusion with 75 min gradient for 6 fractions per sample. Global proteome profiling was calculated using NCBI Human RefSeq database. Next-generation sequencing data from the BWH cohort, the MSKCC cohort, and the COSMIC database were used to generate a reference database containing 2,299 missense mutation sites in 1,885 proteins from 640 MPM tumors where a detected mutation alters the amino acid sequence. A peptide database containing a 29–amino acid length sequence, which includes point mutation site in the middle of the sequence, was generated.

By comprehensive proteomics study, we identified 11,047 gene products and also identified 140 mutated peptides including MHC class I and II neoantigens. Together with current MS-based proteome profiling CyTOF, and mRNA transcriptome profiling was also performed using identical samples. Data-driven analyses of these three independent studies identified 2 distinct immunologic subtypes of MPM with vastly different cellular composition, activation states, and immunologic function; mass spectrometry demonstrated the differential abundance of MHC-I and -II neopeptides directly identified between these subtypes. Proteomics characterization showed the high correlation with the response to programmed cell death 1 (PD-1) inhibition treatment.

Our data demonstrated that high abundance of a neoantigen, in the presence of high expression of its corresponding MHC protein, underlies the strongest clinical responses to checkpoint blockade and highlights the importance of both neo- antigen abundance and neo-antigen presentation in clinical responses to these agents.

POSTER 043

Investigating novel mutant p53 interacting proteins in cancer cells

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p53 is a transcription factor that is mutated in over 50% of cancers. Missense mutations in the DNA binding domain of p53 can result in a gain-of-function (GOF) phenotype, leading to increased cell proliferation and tumor formation. Our lab previously showed that prevalent mutant p53 (mtp53) forms modify chromatin through their interaction with ETS2 and activation of non-canonical transcriptional targets (MOZ, MLL1, and MLL2). Aside from ETS2, other mtp53 partners have been identified, including Sp1, NF-Y, and PML. However, whether specific proteins are critical for the stability and the GOF effect of mtp53 remains to be seen. To this end, we developed a quantitative mass spectrometry-based strategy, combined with molecular and genomic approaches, to identify and validate novel mtp53 binding partners from cancer cell lines with varying GOF p53 mutations. Our preliminary data identified the transcriptional corepressor TRIM28 as a candidate mtp53 interacting partner, as it was identified in all 4 GOF cell lines tested (VU1365, HUPT3, MDA468, and PANC1). TRIM28 has been shown to interact with MDM2 to promote wild type p53 ubiquitylation and degradation; however, its role in regulating mtp53 has not been determined. Ultimately, our studies will identify and validate further novel proteins critical for the GOF activity of mtp53. Characterizing these novel interacting partners of mtp53 will shed light into the molecular mechanisms underlying cancer and thus will provide new therapeutic targets to destabilize mutant p53 interactions in cancer cells.

POSTER 044
Mass spectrometry-based proteomic profiling of pediatric neuroblastic tumors

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Neuroblastic tumors are a heterogeneous group of embryonal neoplasms arising from the sympathetic nervous system in children. Histopathologic categories are defined by variable combinations of immature and maturing neuroblasts, ganglion cells and Schwannian stroma. These categories have major prognostic significance. In this study, we compared quantitative proteomic profiles of the three major subtypes of neuroblastic tumors via Sequential Window Acquisition of all Theoretical Mass Spectra-Mass Spectrometry (SWATH-MS) to examine the impact of tumor phenotype on global tissue protein expression patterns. The cohort comprised 43 samples from 42 patients, consisting of 35 neuroblastomas (Schwannian stroma poor) (NB), three ganglioneuroblastomas (Schwannian stroma rich) (GNB) and five ganglioneuromas (Schwannian stroma dominant) (GN). Tissues had been stored at -80°C for up to 27 years. Frozen sections were taken for histopathologic review, and peptides were extracted using barocyclers. Samples were analysed by SWATH-MS with technical duplicates, and the data were processed using OpenSWATH with PyProphet and TRIC. We identified a total of 55,793 unique peptides and 5,706 proteins across all SWATH-MS runs with a 1% false discovery rate. Dimensionality reduction via principal component analysis demonstrated a clear separation of NBs from the Schwannian stroma rich / Schwannian stroma dominant tumor types GNB and GN. A sparse partial least squares discriminant analysis identified important proteins that contributed to this distinction, including the protein encoded by the neurofilament light polypeptide (*NEFL*) gene. Interestingly, NEFL has been associated with NB differentiation, and higher relative expression in GNB / GN cases has previously been reported at the mRNA level. Taken together, our results demonstrate that proteomic profiles obtained via SWATH-MS analysis can discriminate phenotypically distinct neuroblastic tumor types. This method has potential to provide a novel diagnostic approach.

POSTER 045
Generation of the CanPath prototype - a platform for predictive cancer pathway modelling

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CanPathPro is funded by the European Union's Horizon 2020 programme. The overall objective is to build and validate a combined experimental and systems biology platform, which will be used in testing cancer signaling hypotheses. It combines highly defined mouse and organotypic experimental systems, high-dimensional data including next generation sequencing and quantitative proteomics, and computational models for data integration, visualization and modelling.

Mouse cancer models are characterized by quantitative transcriptome; quantitative mass spectrometry including phospho-proteome; histopathology and histochemistry. Proteomics data is obtained using data independent acquisition (DIA) in parallel to mRNA expression data, allowing for combination and direct correlation of the omics datasets.

Deep protein and phospho-peptide inventories for DIA data analysis were generated, containing until now more than 8'000 proteins and 15'000 phosphorylated peptides for analysis of mammary cell models and more than 10'000 proteins for mammary tumor tissues. Absolute label-free protein abundance estimation Top3 method[1] based on reference peptides spiked into the samples is applied to allow for comparison across sample batches and quantitative modelling.

Here we present results for mammary gland cell line models (Cdh1-fl/AKT1[E17K] and Cdh1-fl + PTEN-fl) treated with wortmannin (primary target: Pik3ca) and MK-2206 (Akt inhibitor). Overall, low correlation of fold-changes in transcriptome vs. proteome after drug treatments was observed, which confirms previous findings that following perturbation, RNA and protein level effects tend to become un-correlated. Furthermore, pathway analyses of transcriptome, proteome and phospho-proteome data were performed and used for the development and refinement of a mouse-specific systems biology model. Differential expression analysis identified relevant genes, proteins and phospho-proteins for the extension and refinement of the model topology. Currently the model integrates modules of the signal transduction pathways Egfr/Erbb2, Fgfr, insulin, Akt, Mtor, Myc, Ras and cell cycle regulation integrating in total about 180 different genes as well as related proteins and phospho-proteins.

POSTER 046

Pitchfork approach for membrane proteome profiling of human pheochromocytoma and paraganglioma

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Roughly a quarter of human genes encode integral membrane proteins (IMPs). Their specific functions and localizations make IMPs attractive drug targets. However, their amphipathy, lack of trypsin cleavage sites and their relatively low expression levels complicate proteomic analyses of IMPs.

Among the most effective approaches for proteomic analysis of low-abundant IMPs are “divide and conquer” methods that selectively target either soluble (extra-membrane) or hydrophobic (transmembrane) segments of IMPs. To maximize the membrane proteome coverage, we combined these both strategies with a standard detergent-based tryptic digest into a multi-pronged “Pitchfork approach”.

Specifically, we employed two well-established methods of glycopeptide enrichment (lectin-FASP and SPEG) along with our recently introduced hPTC method (high pH, trypsin, CNBr) which allows identification of IMPs based on their hydrophobic alpha-helices. This multi-pronged approach targets different features of IMPs and allows us to identify broader range of IMPs than the classical proteomic strategies. We apply the “Pitchfork approach” to membrane proteome profiling of human pheochromocytoma and paraganglioma (PHEO & PGL). These neuroendocrine tumors are very rare, and there are no effective therapies available. Our goal is to gather data for a detailed description of membrane proteome of human PHEO & PGL that could help identify new drug targets and diagnostic markers. On average, we identify 800-1300 IMPs in each patient sample. It represents nearly 2000 unique IMPs in all PHEO & PGL samples analyzed to date. Among the identified proteins, we routinely observe numerous so-called “missing proteins”.

POSTER 047

Proteomic Analysis of Nasopharyngeal Carcinoma Cells with Activated NLRP3 Inflammsome Specks by iTRAQ Technology

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NLRP3 inflammasomes play a key role in tumour control, and their expression levels are favourable prognostic markers and promising therapeutic targets in nasopharyngeal carcinoma patients. NLRP3 inflammasome activation often forms specks in cells upon activation by ATP or nigercin. In this study, we analyzed the differentially expressed proteins in cells with or without specks after NLRP3 activation by proteomic (iTRAQ) analysis. We discovered that proteins differentially over-represented in speck(+) cells enriched into two GeneGO pathways, oxidative phosphorylation and ubiquinone metabolism, suggesting that mitochondria is a major organelle participated in speck(+) cells. Physiologically, speck(+) cells showed stronger mitochondrial dysfunctions in mitochondrial ROS (mtROS) production. Biochemical analyses further confirmed that several electron transport chain (ETC) components are highly enriched in speck(+) cells and are likely participated in speck formation, caspase 1 activation and IL-1b secretion. These findings suggested that mitochondrial ETC components may play crucial roles in regulation of NLRP3 inflammasome activation. Future study will focus on understanding the relationship among ETC components, inflammsome activation and NPC pathogenesis.

POSTER 048

Quantitative Phosphoproteomics Indicates Altered Cell Migration in Prolonged Cabozantinib-Treated Renal Cell Carcinoma Cell Lines

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Patients with recurrent or metastatic renal cell carcinoma (mRCC) usually develop resistance to targeted tyrosine kinase inhibitors (TKI). Recently, a new FDA-approved TKI cabozantinib can override the resistance by inhibiting pro-metastatic MET and AXL signaling pathways, which have been linked to resistance to TKI, *e.g.* sunitinib. Although cabozantinib is believed to be a new gold standard in managing TKI-resistant mRCC, it is still possible that mRCC can develop resistance to chronic cabozantinib treatment. Since cabozantinib inhibits receptor tyrosine kinases, it is highly possible that prolonged cabozantinib treatment changes intracellular phosphorylation dynamics.

In this study, two RCC cell lines, Caki-1 and 786-O, were used for the long-term cabozantinib treatment for more than 4 months. Although cabozantinib slowed down cell growth and inhibited MET phosphorylation, an increase of ERK phosphorylation were observed in long-term cabozantinib-treated cells, indicating that cabozantinib may also perturb other cellular signaling pathways. Using quantitative phosphoproteomic approach using dimethyl labeling and sequential immobilized metal affinity chromatography, potential signaling pathways associated with prolonged cabozantinib treatment were identified, including MAPK-associated pathways and cell-cell adhesions. The followed-on validation by transwell assay also showed an increased cell migration in prolonged cabozantinib treatment. Herein our preliminary data indicated the potential risk of cabozantinib in long-term treatment of mRCC.

POSTER 049

Biomarker discovery in triple negative breast cancer using iTRAQ-based quantitative proteomic analysis

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Triple Negative Breast Cancer (TNBC) is often be more aggressive than other types of breast cancer which is treated with surgery, radiation therapy, and/or chemotherapy. Researchers are paying a great deal of attention to TNBC and working to find new and better ways to treat it. Here, iTRAQ-based quantitative proteomic analysis has been applied to reveal candidate biomarkers for diagnosis and treatment of TNBC. Totally, 4570 proteins were identified in different stage of TNBC tissues and paired para-cancerous tissues. 652 proteins were changed in patients with Grade I or II, among which 254 were up-regulated and 398 were down-regulated. In addition, for patients with Grade III, 330 proteins were increased and 478 proteins were decreased. For instance, candidate protein S100-A7, interferon-induced GTP-binding

protein and Metallothionein-4 were elevated in tumor tissues compared with adjacent nontumor tissues, while mucin-like protein 1, zinc-alpha-2-glycoprotein and lactotransferrin reduced. Differentially expressed proteins were further analyzed by bioinformatic analyses, including GO function classification annotation, ingenuity pathway analysis and KEGG signaling pathway analysis. Comparing to para-cancerous tissues, various signaling pathways and metabolic processes, including PPAR pathways, PI3K-Akt pathway, one-carbon metabolism, amino acid synthesis, and lipid metabolism were activated in TNBC cancer tissues. LXR/RXR axis was also significantly activated in TNCB tumor tissues. We further explored the regulatory effects of LXR/RXR axis on cell proliferation and invasion ability *in vitro*. Thus, our study provides a new direction for the diagnosis and treatment of TNBC through the biomarker discovery using iTRAQ labeling quantitative proteomic approach.

POSTER 050
Comprehensive proteomic mapping of chronic myelogenous leukemia

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Abstract:

Chronic myelogenous leukemia is considered one of the common leukemia types. Here, we deeply investigated the nearly full proteome of cell derived from 53- year old female chronic myelogenous leukemia patient in blast crisis representing K562. Proteins were extracted to denaturated status, reduced and alkylated and subjected to endopeptidase trypsin digestion with modification. Tryptic peptides were then stage tipped and analyzed using long gradient in 7 replicates using TripleTOF 5600+. Merged results identified over 4700 highly confident proteins. Our finding identified several potential modification such as phosphorylation, acetylation and oxidation that accounts in the biological activities of proteins. Additionally, several cell differentiation and apoptosis component were reported which controls the growth of these leukemic cells.

Conclusion: We present her a comprehensive protein catalogue to Chronic myelogenous leukemia that would add a value for better understanding the pathological processes of leukemia.

KEYWORDS: Chronic myelogenous leukemia, proteomics, high throughput, proteome

POSTER 051
Systems-wide profiling of proteolytic events in murine melanoma secretome using Terminal Amine Isotopic Labeling of Substrates

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Melanoma is an aggressive skin cancer and a lethal melanocytic neoplasm with increasing annual number of cases, faster than any other solid tumor. The imbalance of cellular homeostasis during oncogenesis has a marked effect on the repertoire of the proteins secreted by malignant cells (the secretome). Proteases are key effectors of irreversible signaling events, with important roles in homeostasis as well as in disease processes such as cancer. In this context, we used Terminal Amine Isotopic Labeling of Substrates (TAILS) to perform a system-wide investigation on the N-terminome of the secretomes derived from a paired set of mouse melanocyte cell lines: melan-a (normal melanocyte) and Tm1 (its tumoral counterpart). TAILS analysis allowed the profiling of co-translational modifications such as acetylated N-termini as well as proteolytic events in both secretomes. Although no significant difference has been found in the proportion of acetylated N-termini in both cell line secretomes, when evaluating amino acid identities at the scissile bond in internal peptides it was possible to detect significant differences, suggesting distinct proteolytic processes acting in the normal and tumoral secretomes. The mapping of cleavage sites in the tumoral secretome suggested functional roles of active proteases in important biological processes related to oncogenesis, such as processing growth factors, cleaving extracellular matrix (ECM) proteins and shedding of ectopic domains from the cell surface. In the context of the tumor microenvironment, these results suggest important biological roles of proteolytic processing in murine melanoma secreted proteins as well as shed light into the N-terminal modifications of secreted proteins from both cell lines.

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POSTER 052
Proteomic Profiles of Glioma Subtypes and Glioblastoma Stem Cells Reveal Conserved Profiles According to IDH Mutation Status

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Diffuse gliomas, as a group, represent the most common brain tumor in adults and carry a remarkably variable clinical course. Histologically, low and intermediate grade lesions (WHO grade II-III) show nuclear atypia and mitotic activity, respectively, while higher grade tumors (WHO grade IV) show necrosis and/or microvascular proliferation. Molecular profiling of brain tumors has provided significant insights into pathogenesis, classification and prognostication of diffuse gliomas. However, previous molecular studies of glioma have largely focused on genomic readouts and targeted proteomic profiles. Here, we utilize liquid chromatography tandem mass spectrometry (LC-MS/MS) to profile genomically-defined cohorts of gliomas. Together, we identified over 4,897 unique proteins using shotgun MS in 30 clinical samples spanning 2 cohorts that included molecular subclasses based on IDH and 1p19q co-deletion status and all four WHO grades. By profiling frozen, macrodissected formalin-fixed paraffin-embedded tumors and established glioma stem-like cells cultured in vitro, we identified proteomic profiles that are confirmed in multiple models of glioma pathogenesis. Although bulk frozen tumors were accurately classified according to lower and higher grade status based on MS proteomic profiles, distinction of WHO grade III vs. IV and IDH mutation status in tumors was only achieved in our FFPE tumor analysis. As higher-grade gliomas are

infiltrative, our approach of FFPE tissue macrodissection highlights the usefulness of purifying brain tumor sections prior to molecular profiling. Given the downstream and central position proteins occupy in driving biological processes, our analysis complements recent genomic glioma profiling efforts and highlights how proteomics can help define more personalized prognostic and predictive biomarkers for precision care.

POSTER 053

Proteomics confirms lower cancer cell-surface uPAR superimposed on KRAS mutation carrying cells can negate many of the hallmarks of cancer

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Cancer metastasis is the primary cause of mortality. To date molecular mechanisms underpinning it remain elusive. Metastasis is propagated by driver mutations in oncogenes and suppressors genes (e.g., KRAS, BRAF, APC, PTEN, SMAD4, PIK3CA, AKT1 and TP53), accompanied by passenger mutations and underlying genomic instability. To understand the biological processes involved, a unifying framework developed by Hanahan and Weinberg, called the 'hallmarks of cancer' (HoC) organizes unique complexities of cancer. HoCs include; (1) sustaining proliferative signaling, (2) activating invasion/metastasis, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) evading growth suppression, (7) deregulating cellular energetics metabolism, (8) avoiding immune destruction, (9) tumour-promoting inflammation and (10) genome instability/mutation. Underlying these HoCs, genome instability generates mutational genomic diversity, with inflammation amplifying the HoCs. Recognizing how critical interacting node protein expressions changes impact HoCs will accelerate cancer therapeutic development.

In this study, we asked if decreased expression (↓~44%) of the lynchpin protein urokinase plasminogen activator receptor (uPAR) in HCT116ASuPAR cells negates HoCs - reversing changes driven by a KRAS and PIK3CA mutant background. Comprehensive proteome coverage (whole cell lysis combined with 2 membrane extraction methods) encompassed a broad and significant number of HoC - driven biochemical pathways. Coupling Ingenuity pathway analysis with in-house bioinformatics, demonstrated that ↓uPAR expression negates some pathway in each of the HoCs, that the majority are associated with invasion/metastasis, resisting cell death or sustaining proliferation. This result closely parallels a uPAR search in a PubMed text mining engine called CHAT (Cancer Hallmarks Analytics Tool). uPAR expression changed expression of known metastasis-related and uPAR interacting membrane proteins like EGFR, caveolin, vitronectin and integrins. Collectively, we show uPAR is a critical lynchpin capable of altering signaling pathways that regulating the HoC in a 'classical' colorectal cancer mutational background cell model.

POSTER 054

A quantitative analysis of colon adenocarcinoma using MS-based proteomics

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Introduction and objectives:

Adenocarcinomas are the cancers originating from the gland forming cells of the colon and rectal lining and are known to be the most common type of colorectal cancer. In this study, we used proteomics approach with an aim to identify protein biomarkers which can aid in early detection of colon adenocarcinomas to be precise.

Methods: Proteins from tumor tissue of colon adenocarcinoma subjects and their matched controls were subjected to 4-plex iTRAQ labelling followed by off-gel fractionation prior to LC-MS/MS run. The proteins identified using either Spectrum Mill and/or Trans Proteome Pipeline were subjected to DAVID and the proteins common between the two analyses were compared with the data from CPTAC portal. The expression level of few of the shortlisted panel of proteins was validated using MRM approach.

Results and Discussion: A list of 285 unique proteins was identified to be significantly dysregulated in colon adenocarcinoma as compared to its matched controls using either SM or TPP. These proteins were found to be involved in glycolysis, pentose phosphate pathway, biosynthesis of amino acids, protein processing, spliceosome, proteosome, focal adhesion and proteoglycans in cancer. 94 of the 285 proteins were identified by both- SM and TPP. 34 of these 94 proteins were found to be dysregulated with same trend as that in data reported on CPTAC portal and 17 of these 34 proteins were not identified/not altered with same trend or not significantly altered in either grades of glioma and meningioma. 7 of these 17 proteins were validated using MRM approach. The proteins identified from this study could be investigated further to unravel the potential of these proteins to be considered as biomarkers for early detection of colon adenocarcinoma.

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POSTER 055

Proteomic and integrated omic analyses reveal drivers in a subset of aggressive primary lung cancers

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Non-small cell lung carcinoma (NSCLC) is the leading cause of cancer death in humans worldwide, illustrating the lack of understanding of molecular mechanisms governing the aggressive phenotypes associated with this disease. The proteome is the ultimate effector of the cancer phenotype. Our integrated omics analyses of NSCLC showed that lung cancer proteomes differ significantly from the proteomes of normal lung, which cannot be predicted based on measures of DNA and mRNA. This suggests that analysis of proteome

remodelling in lung tumors has the potential to reveal new insights into cancer biology. Indeed, we found that signatures of co-regulated primary lung cancer proteins accurately identify histology subtypes, and metabolism proteome signatures shared between primary and patient-derived xenograft (PDX) tumors have prognostic impact. The ability of primary NSCLC to engraft and form a PDX is prognostic of poor outcome. We hypothesized that primary NSCLC tumors that can engraft may possess proteome signatures responsible for their more aggressive phenotype. LC-MS/MS was used to compare primary engrafting and non-engrafting NSCLC tumors, which revealed signatures of metabolism proteins associated with engraftment. These data suggest that altered metabolic states may be a feature of particularly aggressive lung tumors associated with poor patient survival. Our findings illustrate the clinical utility of proteomics to stratify patient tumors according to their distinctively remodelled proteomes, and which may suggest new therapeutic modalities.

POSTER 056

Integrated Proteogenomic Data Analysis Pipeline and Its Applications to the Analysis of CPTAC Ovarian Cancer Data

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Proteogenomic analysis of tumors provides comprehensive and integrated characterization of tumor specimens using multiple genomic and proteomic data generation pipelines. Investigation of the genomic and proteomic data using proteogenomic data analysis pipeline would be critical to understand the regulations in the alterations of genes, proteins, and post-translational modifications. There are existing software tools for genomic and mass spectrometry based proteomic data analysis. However, there are needs for data analysis pipeline to integrate both genomic and proteomic data. Here, we describe an proteogenomic pipeline with the purpose of standardizing quality control assessment for both genomic and proteomic data sets as well as integrated genomic and proteomic data analyses such differential expression analysis, subtypes classification of tumors and non-tumors, and crossing-omics correlation analysis for exploring and maximizing meaningful findings from proteomic and genomic data. The pipeline has been applied in the studies of the ovarian tumor specimens from the project of Clinical Proteomic Tumor Analysis Consortium (CPTAC). In practice, the pipeline significantly shortened the quality assessment period to validate the quality of data generation. Moreover, with the multiple data analysis templates implemented in the pipeline, it provides a series of routine data analysis outputs for further investigation. In ovarian cancer study, the integrated proteogenomic analysis of the expression of glycosylated peptides and glycosylation enzymes reveals the differential glycosylation mechanism in ovarian tumor and normal tissues. To conclude, our results show that the pipeline provides standard quality control assessment for each omic data set and the integrated proteogenomic analysis to multi-omic data with reduced data analysis time, reducing redundant coding to enable efficient data analysis.

POSTER 057

Integrative mass spectrometry and RNA-sequencing identifies candidate immunotherapeutic targets in neuroblastoma

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BACKGROUND: Neuroblastoma (NB) is an embryonal tumor of the sympathetic nervous system that accounts for 12% of childhood cancer deaths. Despite multimodal therapy, survival probability for high-risk NB patients remains below 50% and relapsed NB is incurable. The cell surface landscape of NB is unknown and an unbiased survey of these proteins will facilitate the identification of immunotherapeutic targets.

METHODS. To identify cell surface proteins in NB, we performed plasma membrane protein extraction utilizing an optimized sucrose density gradient methodology followed by nano-liquid chromatography coupled to mass spectrometry (nLC-MS/MS) in nine NB cell lines and ten patient derived xenografts (PDX). Proteomic data was integrated with RNA-sequencing data to determine differential expression between NB (n=2242) and normal tissues (n=7859). Proteins were validated by tissue microarray (TMA) and *in-vitro* functional studies were performed following genetic manipulation of candidate targets to assess cell proliferation, differentiation and viability.

RESULTS. Using mass spectrometry, we have identified 4826 membrane proteins and yielded on average 66% (range:60-68%) membrane protein enrichment with high reproducibility between biological replicates (80%; range:78-84%). We detected 1010 proteins with an extracellular domain and filtered based on abundance to identify 343 NB-specific plasma membrane proteins. Our analyses confirmed known cell surface proteins in development as immunotherapeutic targets in NB and prioritized DLL3 and DLK1. DLL3 silencing with two shRNAs (range:48-78% knockdown) resulted in reduced viability ($P=3.8x10^{-3}$) and proliferation ($P=1.08x10^{-3}$) compared to control in two high expressing NB cell lines. We have identified an enhancer driving high expression of DLK1 in a subset of NB and in-vitro functional studies are ongoing to assess biological relevance.

CONCLUSION. We have developed the first MS-based surfaceome of NB. These data were integrated with RNA and DNA approaches to prioritize candidate immunotherapeutic targets. Several cell surface proteins, including DLL3 and DLK1, are undergoing further functional characterization and drug development.

POSTER 058

Bothrops jararaca snake venom increases level of several cancer-related proteins on different tumor cell lines.

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Venoms of animals such as snakes, scorpions and spiders have been studied for their therapeutic potential and they have already been used for the development of drugs for the treatment of several types of diseases such hypertension and cancer. Although numerous works describes the biochemical and physiological characterization of snake venom action on cell lines or tissues, none has mapped the proteome of cells under snake venoms treatment. In this work, we have characterized the proteome of MDA-MB231, MCF-7 and HeLa tumor cell lines, and HEK293 normal cell line, treated with *Bothrops jararaca* snake venom at different concentrations. Based on cytotoxic assays results of *B. jararaca* treatment on these cells, we submitted these cell lines to low (0.63 µg/mL) and high/sub-lethal (2.5 µg/mL) doses of *B. jararaca* venom for 24h. Cells were lysed with cold 8M urea followed by reduction and alkylation, trypsin digestion, and desalting followed by LC-MS/MS analysis. Label-free semi-quantitative proteomic analysis of the MDA-MB231 cell line showed three proteins (CUL4A, CLTB and HIST2H3A) whose abundancies increased 5.5, 8.1 and 9.1x, respectively, when compared to no treatment control; MCF-7 cell line showed 13 proteins whose abundancies increased >5x from which the MSH2, that plays an essential role in DNA repair, increases 24x; and HeLa cell line showed four proteins whose abundancies increased >5x where the histone H2B1K presented an >100x increase when incubated with high dose of venom. Interestingly enough, none of these proteins presented increased abundancy when normal HEK293 cells were treated with venom. Several of these proteins play important roles related to cancer such as cell proliferation, invasion, metastasis, apoptosis, and stress response. This data shows that this snake venom or some of its components, may have a potential for cancer therapy by inducing imbalance in the robustness of cancer cells.

POSTER 059
Neoadjuvant chemotherapy-treated ovarian cancer patients have unique tumor proteome alterations associated with volume of residual disease.

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Objective: High-grade serous ovarian cancer (HGSOC) is treated by cytoreductive surgery followed by platinum and taxol chemotherapy. Patients deemed unlikely to have complete cytoreduction are prioritized for neoadjuvant chemotherapy (NACT) followed by surgery. The objective of this study was to determine proteomic alterations in matched chemotherapy naïve and NACT-treated HGSOC.

Methods: Matched formalin-fixed, paraffin-embedded tumor tissues from chemotherapy naïve (pre) and post-NACT treatment were identified for n=26 HGSOC patients. Tumor cells were collected by laser microdissection and subjected to heat-induced antigen retrieval followed by tryptic digestion. Peptides were labeled with tandem-mass tag (TMT) isobaric labeling reagents and analyzed by multiplexed quantitative proteomics on a Fusion Lumos MS (Thermo). Functional inference was performed using IPA (Qiagen).

Results: Differential analyses of 5,681 total proteins quantified by at least two peptide spectral matches revealed 151 proteins as significantly altered between post and pre-NACT tissues (LIMMA, adjusted $p \leq 0.05$). Functional inference revealed activation of pathways regulating cell survival, fatty-acid metabolism, and leukocyte movement and inhibition of pathways regulating migration and metastasis in post vs. pre-NACT tissues. We further identified 200 protein alterations in pre-NACT treated tissues from patients with no residual disease (R0) or with macroscopic disease (R1, < 1 cm) following interval debulking surgery. Functional analyses revealed activation of viral infection signaling as well as binding/ adhesion of cells to connective tissues and inhibition of cellular apoptosis and death signaling pathways. We further validated abundance alterations for 9 residual disease protein candidates in an independent cohort of R1 (n=71) and R0 (n=28) HGSOC tissues (LIMMA, $p \leq 0.05$, Zhang H, 2016).

Conclusion: Quantitative proteomic analyses of matched NACT-treated tissues from HGSOC patients revealed alterations in cellular survival and cell movement signaling following NACT treatment. We further validate proteome alterations in tumors harvested from patients exhibiting different residual disease burdens.

POSTER 060
Inhibition of cell proliferation and altered proteome analysis after GnRH agonist treatment in glioblastoma cell line

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Introduction: Gonadotropin-Releasing Hormone (GnRH) receptor, belonging to the rhodopsin-like G-protein coupled receptor (GPCR) family and involved in GnRH signaling, has been reported to be expressed in several tumors including glioblastoma multiforme (GBM), one of the most malignant and aggressive forms of primary brain tumors. However, the molecular targets associated with GnRH receptor are not clearly understood in GBM and other cancers. The present study aims at investigating the effect of GnRH agonist (Gosarelin acetate) on cell proliferation and associated signaling pathways in GBM cell line, LN229.

Material and Methods: LN229 cells were treated with different concentrations of GnRH agonist (10^{-5} M to 10^{-10} M) and the effect on cell proliferation was analyzed by cell counting and MTT assay. Further, protein isolated from control and GnRH agonist treated cells were trypsin digested, labeled with iTRAQ reagents and analyzed using LC-MS/MS technique to identify differentially expressed proteins.

Results: The treatment with different concentrations of GnRH agonist showed reduction in proliferation potential and maximum reduction was observed to be 48.73% at 10^{-6} M

concentration. Quantitative proteomic analysis after GnRH agonist treatment led to identification of a total of 16 proteins to be deregulated, including downregulation of adenylyl cyclase-associated protein 1 (CAP1) and upregulation of cofilin-2 (CFL2). CAP1 is involved in regulation of actin filaments by promoting cofilin-induced actin filament depolymerization, mediating cell morphogenesis and cell motility, and its overexpression has been reported to increase cell proliferation, migration and invasion of neural glioma cells.

Conclusion: These results show that GnRH receptor is expressed in GBM cell line, LN229, and treatment with GnRH agonist (10^{-6} M) leads to significant reduction in cell proliferation. Quantitative proteomic analysis suggests a possible role of CFL2 and CAP1 in GnRH signaling mediated cell proliferation and may be further investigated in GnRH receptor-positive GBM clinical samples.

POSTER 061

HER2 proteomic signature in gastric cancer

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Background

The exact molecular mechanisms underlying unique clinicopathological phenotypes of HER2-positive gastric cancer remain to be defined.

Methods

We performed a global proteome profiling analysis of frozen HER2-positive gastric cancer endoscopic biopsies and adjacent normal tissue counterparts, using nanoACQUITY UPLC online-coupled to a Q Exactive Orbitrap mass spectrometer.

Results

Our global proteome profiling analysis quantified more than 8,000 protein groups with frozen tissue specimen obtained using endoscopic biopsy forceps. Reporter ion intensities of selected proteins well correlated with immunostaining results. We identified 45 proteins whose iTRAQ reporter ratio highly (R > 0.8) correlated with that of ERBB2 (*HER2 proteomic signature*). Tumors with weak HER2 proteomic signature had significantly worse prognosis following trastuzumab-based treatment. The median overall survival (OS) and progression-free survival (PFS) for patients with a strong HER2 proteomic signature were 26.1 and 14.9 months, respectively, whereas the median OS and PFS for patients with a weak HER2 proteomic signature were 3.9 and 3.6 months, respectively (log rank P values, 0.02 and 0.007, respectively). In contrast, neither PFS nor OS significantly differed according to the status of HER2 immunostaining grade in this patient population.

Conclusions

HER2 proteomic signature in gastric cancer well correlated with clinical response to trastuzumab-based treatment. Endoscopic biopsy tissue samples can be used for global proteome profiling analysis of predictive markers.

POSTER 062

Post-translational crosstalk networks identify strategies to overcome EMT-mediated resistance to EGFR inhibitors

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Epithelial-mesenchymal transition (EMT) mediates intrinsic and developmental resistance to epidermal growth factor receptor (EGFR) inhibitors. This constitutes a major hurdle in lung cancer treatment. Despite EMT-TFs (transcription factors) having been identified as the master regulators of EMT, multilayer control of EMT regulators orchestrated by EMT-PTM (post-translational modification) mediated signaling networks are not fully understood. Here, we integrated proteomics, pathway, network, and cell viability analyses to characterize EMT-PTM signaling. We sequentially enriched four proteomes (expression, phosphorylation, ubiquitination, and acetylation) in erlotinib-sensitive HCC4006 and matched EMT resistant cells (HCC4006ER). We identified 6,641 proteins, 2,418 unique pSTY-sites, 784 unique UbK-sites and 713 unique AcK-sites, respectively. We obtained EMT-associated proteins (377 increased and 1377 decreased; $p<0.05$, fold>2) and PTMs, including pSTY (141 increased, 191 decreased), UbK (29 increased, 32 decreased) and AcK (14 increased, 46 decreased). Pathway and TF binding site analyses of differentially expressed and PTM-changed proteins revealed enrichment of 163/284 activated pathways and 8/15 TFs, respectively. Strikingly, Wnt signaling was found in both enrichment results. We demonstrated that inhibiting Casein Kinase 1δ/1ε, a positive regulator of Wnt signaling decreases the viability of H4006ER more than HCC4006. We constructed an EMT signaling network composed of 206 proteins with PTM changes. Integrative informatics revealed cross-talk among PTMs within the network. We further functionally interrogated the EMT network by assaying cell viability and migration with siRNA and small molecule inhibitors. We identified 12 EMT-associated proteins. Of, those, knocking-down a TF promyelocytic leukemia (PML) protein resulted in the largest decrease in HCC4006ER cell viability. Cross-talk analysis revealed that PML is cross-regulated by 21 enzymes through phosphorylation, ubiquitination, and acetylation. Cell viability analysis confirmed the combination effect of co-targeting PML and EGFR with siRNA and inhibitor, respectively. Collectively, our multiple-proteomics strategy enabled decoding the complex interplay in PTM modulation and identified potential strategies for overcoming EMT-mediated resistance.

POSTER 063

Breast cancer quantitative proteome and proteogenomic landscape

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We present a proteome-centric multi-omics examination of the breast cancer (BC) molecular landscape. Unbiased analyses of deep tumor proteomes recapitulate PAM50 BC subtypes while further distinguishing poor-prognosis basal-like and luminal B tumors by immune component infiltration, suggesting the current classification is incomplete. Proteome-based networks distinguishes BC subtype-specific functional protein modules with co-expression of known drug targets marking ductal carcinoma in situ regions of normal-like tumors, lending to a more accurate classification of this poorly defined subtype. Additionally, we find the effects of copy number alterations to be dampened dependent on protein-level gene regulation, and transcripts within prognostic mRNA panels to be reliable protein surrogates, underscoring the value of proteome quantification for prognostication and phenotypic classification. Furthermore, proteogenomic analyses identify protein products mapping to "non-coding" genomic regions or corresponding to un-described alternative gene translations; suggesting proteome profiling and downstream proteogenomic analyses can be applied to identify candidate tumor-specific immunotherapeutic targets arising from instability of the cancer genome.

POSTER 064
Polycomb loss enhances oncogenesis but leads to therapeutic vulnerabilities in malignant peripheral nerve sheath tumors

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Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas in which loss of function mutations in the polycomb repressive complex 2 (PRC2) promote tumor progression. To better understand how PRC2 loss contributes to pathogenesis, we conducted parallel proteomic and epigenomic analysis of human MPNSTs with and without PRC2 loss ($MPNST_{LOSS}$ vs. $MPNST_{RET}$).

$MPNST_{LOSS}$ showed decreased H3K27me3 and increased of H3K27 acetylation. This was accompanied by hyperacetylation of H4, a marker of open chromatin, and increased H3K36me2 and DNA hypermethylation. At the level of the proteome, $MPNST_{LOSS}$ had an increased abundance of chromatin remodelers and markers of cell growth and division and decreased interferon signaling and antigen presentation.

To assess whether PRC2 function was directly linked to the proteome changes seen in human tumors, we restored PRC2 function in $MPNST_{LOSS}$ cell lines and measured the proteome response. PRC2 reconstitution downregulated pathways that were upregulated in $MPNST_{LOSS}$ and increased interferon pathway expression and MHC presentation. The same effects were observed in $MPNST_{LOSS}$ cell lines with knockdown of NSD2, the methyltransferase responsible for H3K36me2, suggesting functional antagonism between H3K36me2 and H3K27me3. NSD2 knockdown also decreased proliferation and DNA methylation and activated transcription of endogenous retroviral elements (ERV), thereby linking H3K36me2 loss with interferon activation, which occurs as a consequence of ERV expression. $MPNST_{LOSS}$ were found to be highly sensitive to both DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), both of which similarly activate interferons through induction of ERVs.

Together, these results demonstrate that PRC2 loss promotes global increases in open chromatin that enhance oncogenic pathway expression but promote genomic instability and render MPNST heavily reliant on DNA methylation to prevent spurious transcription initiation, including of ERV. Consequently, MPNST are highly sensitive to therapeutics promoting further destabilization through increased acetylation (HDACi) or decreased DNA methylation (DNMTi).

POSTER 065
Epigenetic dysregulation drives altered chromatin-reader interactions in diffuse intrinsic pontine glioma

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Despite representing only 10% of pediatric brain cancers, diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain cancer death in children. It was recently determined that nearly 80% of DIPGs harbor a mutation in one of the sixteen genes encoding histone H3. Interestingly, the mutation always causes a K-to-M substitution at position 27 (K27M). Subsequently, our group helped show that the mutant histone proteins function via a dominant negative mechanism, stably associating with a subunit of the polycomb repressive complex (PRC2) and thereby reducing H3 K27 di- and trimethylation genome-wide (K27me2 and K27me3, respectively). It has since been shown that H3 K27M expression in neural progenitor cells promotes growth, impairs differentiation, and facilitates transformation but its mechanism is still incompletely understood. We reasoned that by using modern proteomics approaches to (1) better define how this histone mutation affects the global epigenome, and (2) leveraging this information to rationally screen for protein-chromatin interactions that it might dysregulate, we might begin to elucidate its oncogenic mechanism.

Characterization of histone post-translational modifications by nanoLC-MS in various H3 K27M-expressing cell models and in DIPG cells demonstrated that mutant histone expression is associated with aberrant patterns of H3 K36 dimethylation (K36me2). We then sought to identify putative “readers” of H3 K36me2 using a synthetic peptide pulldown/global proteomics approach. Top hits from this reader screen included a striking number of proteins associated with rRNA transcription and processing, suggesting that this mutation may promote cell transformation by altering rRNA expression dynamics, and perhaps ribosome biogenesis. Here we will describe our approach and early functional validation efforts.

POSTER 066
Rapid plasma biomarker validation by reverse phase protein array

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The context of circulating blood proteins may represent underlying physiological and pathogenic conditions. The blood

proteome therefore is considered an ample source of biomarker discovery. However, any plasma/serum biomarker candidates identified by proteomic approaches must be validated in a statistically sufficient number of cases and controls using a different quantitative method before being considered for clinical application. Conventionally, Enzyme-Linked ImmunoSorbent Assay (ELISA) has been used for such validation, but the standard sandwich ELISA assay requires two antibodies that do not interfere with each other. As a result the development of ELISA usually takes several months for every biomarker candidate protein. And more importantly, ELISA requires a relative large volume (~100 µL) of samples. Because the supply of clinical materials is often limited, it may be unfavorable to use hundreds of microlitters of precise samples for preliminary experiments. For the rapid selection and validation of plasma biomarker candidates, we report the establishment of a high-density fluorescence reverse phase protein array (fRPPA) platform. Plasma samples were serially diluted and printed in quadruplicate onto a hydrophobic glass surface in a format of 6144 spots within an area of 17.65 ´ 34.57 mm. The location of each spot was visualized by staining human IgG (green), and the relative amounts of candidate biomarker proteins were quantified by hybridization with specific antibodies (red). Fluorescent signal intensity showed linearity in the plasma dilution range of ´32 to ´4096 in a quality control experiment and was highly reproducible among independent 4 experiments. Over 78% of spots showed coefficients of variation (CV) values of less than 0.1. Here we report the identification and validation of a novel biomarker for pancreatic cancer.

POSTER 067

Analysis of the epidermal growth factor-induced phosphorylation of actinin-4involved in cancer metastasis

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Actinin-4 (ACTN4), an actin-bundling protein previously identified by our laboratory, is closely associated with cell motility, cancer metastasis, and various cellular processes. Previous studies have reported three ACTN4 mutations related to familial focal segmental glomerulosclerosis,and an alternative splicing variant (ACTN4-Va) related to high-grade neuroendocrine carcinoma of the lung.Actin filament reconfigurationisnecessary forchanges incellular structure,including for cellsurface protrusion andfocal adhesion. These processes are reported to involve epidermal growth factor-induced tyrosine phosphorylation of ACTN4, and calpain-dependent cleavage of the phosphorylated ACTN4. Wepostulatedthatthe tyrosinekinase responsible forphosphorylatingACTN4 isan epidermal growth factor receptor (EGFR). Thus, in this study, we aimedtovalidate the enzyme responsible for phosphorylating ACTN4,and to identify the phosphorylatedtyrosineresidues.

All of the mutations among the three familial mutations and ACTN4-Va were restricted to the actin-binding domain (ABD). Increased actin binding was previously observed upon phosphorylation at residue Y265 in the ABD. We found that the mutations and the phosphorylated residue were located on the side of the helix, facing the helix-helix interface of two subdomains in the ABD. Additionally, A549 cell lines overexpressing ACTN4 Y265 mutations showed decreased cell motility, and the tyrosine O-sulfated mimic of ACTN4 Y265 increased binding to actin.

Usingan invitrokinaseassay, weconfirmedthe tyrosinephosphorylationofACTN4 byEGFR. We determined the phosphorylation sites of trypsin digestion on the phosphorylated ACTN4 using TripleTOF5600 (AB SCIEX, Framingham, MA) and two-dimensional image converted analysis of LC-MS (2DICAL).The mass spectroscopy sequence coverage of the ACTN4 peptide was77.6%, andsix newphosphorylationsiteswereidentified.Our findings suggest that the three-dimensional structures of the mutated peptides and ACTN4-Va differed from that of ACTN4, and that these changes were responsible for the different biological characteristics of cancer metastasis.

POSTER 068

BRK mediated phosphorylation regulates SMAD4 control of the TGF-b/SMAD4 signaling pathway to control SLUG, SNAIL and metastatic potential.

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The tumor-suppressing function of SMAD4 is frequently subverted during mammary tumorigenesis, leading to cancer growth, invasion and metastasis. A long-standing concept is that SMAD4 is not regulated by phosphorylation but ubiquitination. Interestingly, our search for signaling pathways regulated by BRK, a non-receptor protein tyrosine kinase that is up-regulated in ~80% of invasive ductal breast tumors, led us to discover that BRK competitively binds and phosphorylates SMAD4, and regulates components of the TGF-β/ SMAD4 signaling pathway. Additionally, tyrosine phosphorylated SMAD4 suppresses cell homeostasis and apoptotic signaling pathways and upregulates several oncogenic signaling cascades. A constitutively active BRK mutant, BRK-Y447F, phosphorylates SMAD4 resulting in its recognition by ubiquitin-proteasome system, which accelerates SMAD4 degradation. In accordance with this, we also observed an inverse protein expression pattern of BRK and SMAD4 in a panel of breast cancer cell lines and breast tumors. Beside phosphorylation of SMAD4, activated BRK reorganizes SMAD4 protein-protein interactions. Activated BRK rewriting of SMAD4 protein-protein interactions might cause a wider genome accessibility resulting in repression of tumor suppressor FRK, decreased cell adhesion ability, and induction of epithelial-mesenchymal transition (EMT). Thus, our findings suggest that BRK is a potential therapeutic target in SMAD4-associated cancers.

POSTER 069

Screening of novel molecular therapeutic targets for tongue cancer using a kinase antibody library

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Background

Enzymes of protein phosphorylation (protein kinases) are associated with the invasion, proliferation, and metabolism of cancer cells. The inhibition of kinases has recently garnered great attention as a molecular-targeted therapy for cancer. There are only two molecular-targeted drugs approved for treating head and neck cancer in Japan, and further development of molecular-targeted therapy and diagnostic technology is needed. Antibody-based proteomics approaches have been applied to tissue microarrays (TMAs) for screening novel therapeutic targets and predictive biomarkers of cancer. To identify novel molecular targets for patients with stage I/II tongue cancer, we screened 430 kinase antibodies using antibody-based proteomics.

Methods

In total, 41 patients who underwent surgical resection for stage I/II tongue cancer were enrolled into this study. TMAs were prepared from formalin-fixed, paraffin-embedded pathological blocks. Fluorescent immunostaining was performed to identify antigens on the TMAs. Protein kinase expression was profiled using a platform for high-throughput digital pathology imaging of protein expression and a library of 430 protein kinase antibodies. We used in-house Automated Quantitative Virtual Immunofluorescence Pathology software, which was developed at the National Cancer Center Research Institute, for the quantification of protein expression. Hierarchical cluster analysis was performed using quantitative data.

Results

Two kinases showed statistically significant differences in pathological differentiation, mode of invasion (Jakobsson classification), and late metastasis to the cervical lymph nodes. One of them was associated with significantly poorer disease-free survival and overall survival in comparison to patients without expression of the kinase.

Conclusions

Using antibody-based proteomics, we identified a kinase as a novel candidate therapeutic target for patients with stage I/II tongue cancer. Expression of the kinase was significantly associated with late metastasis to the cervical lymph nodes and poor prognosis.

POSTER 070
Identification of Aggressive Prostate Cancers: In-depth Proteomics of Tissues and post-DRE urines

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Current prostate cancer (PCa) prognostic factors stratify patients into risk groups, but are inaccurate in predicting outcome, resulting in over-treatment of many men with indolent disease. In addition, men on active surveillance are required to undergo repeated needle biopsies, subjecting them to associated risks. A pressing need in PCa management is the development of improved prognostic factors that enable follow-up of men with low-risk disease in a non-invasive manner. Our team has applied proteomics analyses of direct expressed prostatic secretions (dEPS), post-DRE-urines and frozen tissues to identify novel proteomics and proteogenomics signatures of aggressive disease. Combining comprehensive proteomics profiling of stratified dEPS fluids with targeted proteomics and computational biology we discovered robust proteomic signatures for extracapsular prostate cancer (Kim et al. *Nat Commun*. 2016). We are extending on these discoveries by developing novel approaches for in-depth proteomics profiling of prostate fluids stratified into low, intermediate and high-risk prostate cancer (Gleason Score 3+3, 3+4, 4+3, 4+4). Our goals are to develop liquid biopsy signatures to follow patients on active surveillance. In parallel, we are performing proteomics analyses of frozen prostate tissues that have already been extensively profiled by the Canadian Prostate Cancer Genome Network. Briefly, we have integrated genomic, epigenomic, transcriptomic, and proteomic data generated from 55 intermediate-risk prostate cancer patients. We discovered that the prostate cancer proteome yields four subgroups that differ from previously published DNA-based subgroups, and are associated with differential BCR. Furthermore, machine learning approaches were used to determine which biomolecule is best at predicting BCR in these patients. Our data indicated that integration of complementary biomolecules led to the best predictive accuracy (*i.e.* proteins and methylation). Our results show that proteomics complements other -omics data in stratifying prostate cancer patients, and is an underutilized repository of clinically useful features for precision medicine.

POSTER 071
High resolution protein mapping of *ROS1*-rearranged NSCLC cell lines: defining mechanisms of acquired crizotinib resistance

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Lung cancer is one of the most common and lethal malignancies globally, with non-small cell lung cancer (NSCLC) accounting for 85% of all lung cancer cases. The identification of oncogenic mutations in receptor tyrosine kinases such as EGFR and, less commonly, rearrangement of ROS1 , ALK, and RET, has influenced treatment strategies through use of tyrosine kinase inhibitors (TKI) directed against these targets. Although use of these TKIs often leads to dramatic and prolonged responses in those patients with sensitising mutations, acquired resistance eventually ensues.

To identify new resistance mechanisms of a first-line ROS1 inhibitor, crizotinib, we used latest-generation mass spectrometry. We mapped the proteomes of a parental NSCLC cell line sensitive to crizotinib (HCC78, harbouring the SLC34A2-ROS1 fusion gene) and its matched crizotinib-resistant subline (HCC78_CR) established by long term exposure to crizotinib. Protein profiling was performed using

the SWATH-MS 2.0 algorithm, conducted on the Sciex 6600 TripleTOF. In total, LC-MS/MS data was extracted for 2543 proteins.

Overall, 266 proteins were differentially expressed between cell lines (FC>1.5, p<0.05). Ingenuity Pathway Analysis listed "Cell Death and Survival" and "Nucleic Acid Metabolism" as the top Molecular and Cellular Functions between the sublines, with key proteins also involved in Cellular Development, Assembly and Organisation, Growth and Proliferation.

This is the first time that these lung cancer cell lines have been comprehensively profiled by SWATH-MS. Protein mapping will increase the understanding of the mechanisms involved in the acquisition of TKI resistance, which is crucial for the development of rational strategies to overcome resistance in the clinic.

POSTER 072
Differences in plasma fibrin clot composition in patients with thrombotic antiphospholipid syndrome compared with venous thromboembolism

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Objectives: The prothrombotic fibrin clot phenotype has been reported in patients with thrombotic antiphospholipid syndrome (APS) and venous thromboembolism (VTE). Protein composition of plasma fibrin clots in APS has not been studied.

Methods: We evaluated 23 patients with thrombotic APS, 19 with VTE alone, and 20 well-matched controls. A proteomic analysis of fibrin clots generated from citrated plasma was based on liquid chromatography-mass spectrometry.

Results: Plasma levels of thrombospondin-1 (TSP1), apolipoprotein(a), A-I, and B-100, complement components (C)3a, C5b-C9, histidine-rich glycoprotein (HRG), and prothrombin were evaluated using immunoenzymatic tests. In plasma fibrin clots of APS patients, compared with VTE subjects and controls, we identified decreased amounts of (pro)thrombin (1.8-2.0-fold difference [FD]), antithrombin-III (1.5-FD), apolipoprotein A-I (1.5-FD), and HRG (1.4-FD) with no differences in plasma levels of antithrombin, prothrombin, along with lower plasma HRG and apolipoprotein AI. In APS patients, plasma HRG positively correlated with amounts of clot-bound HRG, while apolipoprotein A-I was inversely associated with clot-bound levels of this protein. The most predominant proteins within the clots of APS patients compared to VTE subjects and controls were bone marrow proteoglycan (23-39-FD), C5-C9 (1.6-5.5-FD), immunoglobulins (1.6-4.8-FD), apolipoprotein B-100 (2.0-FD), platelet-derived proteins (5.6-6.3-FD), and TSP1 (3.3-FD). Plasma C5b-C9 positively correlated with clot-bound C5b-C9 amounts.

Conclusion: Our study is the first to demonstrate differences in the protein composition of fibrin clots generated from plasma of thrombotic APS patients versus those with VTE alone. Our clot proteomic approach could be useful to identify plasma proteins with potential clinical utility as biomarkers in thrombotic diseases.

POSTER 073
Integrated Dissection of the Cysteine Oxidative Modification Proteome During Cardiac Hypertrophy

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Cysteine oxidative modification of cellular proteins is crucial for many aspects of cardiac hypertrophy development. However, integrated dissection of multiple types of cysteine oxidation proteome in cardiac hypertrophy is currently missing. Here we developed a novel discovery platform encompassing a customized biotin switch-based quantitative proteomics pipeline and an advanced analytic workflow to comprehensively profile the landscape of cysteine oxidation in ISO-induced cardiac hypertrophy mouse model. Specifically, we identified a total of 1,655 proteins containing 3,324 oxidized cysteine sites by at least one of the following three modifications: reversible cysteine oxidation, cysteine sulfinylation (CysSO2H), and cysteine sulfonylation (CysSO3H). Analyzing the hypertrophy signatures that are reproducibly discovered from computational workflow highlighted a group of fatty acid beta-oxidation enzymes with a continual decreased temporal pattern and a significant decreased abundance in reversible oxidation with no temporal or abundance change in total cysteine, revealing the oxidative regulatory map of fatty acid metabolism in cardiac hypertrophy, which is featured by an overall reduced oxidative metabolism. Our cysteine oxidation platform depicts a dynamic and integrated landscape of the cysteine oxidative proteome, through the extracted molecular signatures, and provides critical mechanistic insights in cardiac hypertrophy.

POSTER 074
Phosphopeptide enrichment and analysis of human ischemic cardiomyopathic tissues reveal infarct versus non-infarct unique signaling pathways

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Ischemic cardiomyopathy (ICM) is the most common cause of heart failure yet its underlying signaling mechanisms remain poorly understood. Specific enrichment and profiling of phosphorylated proteins have been shown to reveal previously underappreciated yet key signaling pathways in models of cardiovascular diseases. Here, we performed LC-MS/MS (liquid chromatography-tandem mass spectrometry)-based phosphoproteomics on a Q-Exactive HF to identify differentially regulated signaling pathways that underlie the progression and development of ICM. Patient cardiac tissue samples from left ventricle infarct and non-infarct regions of ICM patients (n=4) were labeled with 10-plex tandem mass tags for relative quantification and fractionated using high performance-hydrophilic interaction chromatography. Fractions were enriched for phosphorylated peptides using TiO_2 immobilized to magnetic beads and subjected to LC-MS/MS. Initial analysis

of infarct and non-infarct samples identified 12,242 phosphorylation sites which corresponded to 2715 phosphoproteins. From a total of 2877 significantly altered phosphorylation sites (FDR q-value<0.05), 1404 and 1473 sites were found to be upregulated and downregulated, respectively, in the infarct samples compared to non-infarct. Furthermore, hierarchical clustering and 2D principal component analysis demonstrated distinct clustering of samples according to their regions. Pathways key to cardiac remodeling including *$a_V b_3$ integrin pathway* and *cellular response to TGFb stimulus* were significantly enriched in proteins upregulated in infarct samples. Functional enrichment analysis also revealed previously poorly-characterized associations between ICM and pathways such as *cell morphogenesis* and *post-translational modifications* which warrant follow-up investigations on the proteins involved and their functional roles in ICM progression. Currently ongoing studies involve overlaying our extensive ICM dataset with publicly-available datasets of ICM and other cardiomyopathies. This large-scale study of human ICM at both protein and pathway levels add indispensable knowledge and advances our current understanding of ICM progression and development with the central aim of discovering novel therapeutic targets.

POSTER 075

Phospholamban targeted proximity labeling for proteomic mapping of sarcoendoplasmic reticulum subdomains

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Introduction: Phospholamban (PLN) is essential for normal stress-adaptation of heart function. While PLN is tail-anchored in the sarcoendoplasmic reticulum (SER), its dephosphorylated form binds to the Ca^{2+} ATPase SERCA2a and potentially to other important SER proteins. To elucidate the PLN associated signalosome in endogenous SER domains, we developed a PLN-specific proximity assay.

Methods: For proteomic labeling, APEX2, a genetically engineered peroxidase was fused N-terminally to generate APEX2-PLN. Lentiviral expression, bicistronically expressed with eGFP, and biotin phenol treatment were used to label proteins in nanometric proximity of PLN by biotinylation in living neonatal rat cardiomyocytes (NRCM). APEX2-biotinylated proteins were enriched by pulldown, processed and analyzed by mass spectrometry (LC-MS/MS). Additionally, for ratiometric analysis, NRCM were cultivated with stable isotope labeled amino acids (SILAC). A truncated APEX2-PLN(Δ1-31) construct was used as SER-targeted, cytosolic domain-incompetent control and enhanced green fluorescent protein (eGFP) as negative control.

Results: APEX2-PLN expression in NRCM was confirmed by Western blot (WB) and fluorescence microscopy through the bicistronically expressed eGFP. Confocal analysis showed that APEX2-PLN co-localizes with endogenous SERCA2a similar to endogenous PLN. APEX2-biotinylation of endogenous proteins was confirmed by streptavidin-RD680 WB. The medium and heavy labeled amino acids (SILAC) were incorporated at a rate of 97.4 % (SD 0.024 %; n=10), thus enabling global quantitative proteomic analysis. GO-term analysis showed an enrichment of proteins involved in cardiac muscle tissue development and regulation of ATPase activity.

Discussion: A PLN-specific strategy for proximity labeling was successfully developed in live NRCM and verified for known interaction partners. Next, proteomic proximity analysis can be employed to identify previously unknown PLN protein-protein interactions and their signalosome subdomains in the neonatal and adult heart. Furthermore, by combining the proximity data with high-resolution microscopy, PLN interactions in distinct, previously unknown SER membrane domains can be identified.

POSTER 076

Profiling the Proteomic and Lipidomic Dysregulations of Mouse Aorta during Atherosclerotic Plaque Formation

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As the main cause of coronary heart disease and other cardiovascular diseases, atherosclerosis (AS) is a chronic and progressive multifocal arterial disease associated with lipid deposition, inflammatory response and abnormal remodeling of the arterial wall1). Current treatment strategies of atherogenic hyperlipidaemia prevent only 30% of all cardiovascular events, thus novel treatment strategies driven by more comprehensive knowledge of AS are highly warranted1). In this study, we adopted quantitative proteomics and lipidomics to systematically depict the lipid deposition and vascular remodeling processes during the plaque formation. Lipidomic analysis were performed on serum and aorta of the ApoE-/- versus wild type (WT) mice with high fat (HFD) or Normal (ND) diet. Over 1000 lipid species were identified in total. Increased lipidome complexity of the aorta as well as declined lipidome complexity of the serum were noticed, and accumulated PE, LPC, and CE were observed in the aorta of ApoE-/- mice with HFD. The results demonstrated that certain lipid classes and species were increased in aorta and declined in serum during the pathological process, implying that these lipids accumulated and deposited in aorta from peripheral blood. Quantitative proteomics of the aorta during atherogenesis comprised more than 6, 200 proteins, 219 of which differential expressed in WT mice and 229 of which differential expressed in ApoE-/- mice comparing HFD with ND. The variations of protein expression represented the remodeling of the vascular smooth muscle cells (VSMC) during AS progression. Notably, there are 11 proteins reversely changed their expression status in response to high fat stimulation in ApoE-/- versus WT mice. These proteins were associated with MYOCD inhibition and necrosis activation. Further data mining and functional study are ongoing, and this research would accelerate progress toward the comprehensive understanding of AS pathophysiology and pave the way for discovering novel risk factors and treatment strategies.

POSTER 077

GLOBAL HUMAN AND MOUSE PHOSPHOPROTEOMIC PROFILING OF SIGNALING PATHWAY ABERRATIONS IN HYPERTROPHIC CARDIOMYOPATHY

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Cardiovascular diseases (CVDs) account for 17.7 million deaths worldwide annually and are predicted to remain one of the leading causes of mortality in the developed world. Hypertrophic cardiomyopathy (HCM) resulting from the abnormal thickening of the heart muscle is the most commonly inherited heart condition often caused by mutations in genes coding for sarcomere proteins and where causative gene mutations are absent, the process of hypertrophic cardiac remodeling also occurs secondary to pathologic stimuli such as hypertension and valve disease. However, the molecular signaling mechanisms of pathological initiation and progression of this diseases are multifaceted and largely unknown. This greatly complicates diagnosis and direction of treatment. Therefore, we have conducted a study to identify the signaling changes associated with HCM in patient samples and the transverse aortic constriction (TAC) mouse model of cardiac hypertrophy utilizing isobaric tag (TMT) based and label free quantitative (phospho)proteomics, respectively. For this purpose, a 2 dimensional LC-MS pipeline was developed utilizing hydrophilic interaction chromatography (HILIC) and TiO2-based phosphopeptide enrichment. This resulted in the identification of 8359 unique phosphorylation sites on 3118 mouse cardiac phosphoproteins, and 6827 phosphorylation sites on 1778 human cardiac proteins. Functional enrichment analysis was performed to identify aberrant signaling pathways and other annotated biological processes from a compendium of publically available databases (ie. Gene Ontology, KEGG, MSigDB, NCI Nature PID, NetPath, Panther, Reactome) resulting in the identification of 1064 mouse and 441 human pathways affected as a result of cardiac muscle hypertrophy. This study has allowed for an unprecedented overview into protein, phosphorylation, and pathway level changes occurring in HCM patient-derived cardiac muscle together with an equally comprehensive comparison to the pressure-overload mouse model of cardiac hypertrophy.

POSTER 078
Development of the Human Proteome Peptide Catalog – A comprehensive Repository of Reference Peptides for the Human Proteome

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Background

Synthetic reference peptides are an integral part of current MS based proteomics. While in discovery/shotgun proteomics reference peptides aid in assuring the correct assignment of spectra to peptide sequences, in targeted proteomics heavily labelled peptides are essential as internal standards. Our project ProteomeTools[1] aims to provide data for reference peptides covering almost all proteins of the human proteome. We describe here the development of a repository of >400,000 reference peptides with multiple options for searching suitable peptides, links to reference spectra, as well as access to the physical reagents.

Methods

Peptides were selected based on known proteotypicity, or - when this was not known - by synthesizing all suitable peptides of a protein. The peptides were prepared by SPOT synthesis, combined in pools of 1000 peptides per pool, and analyzed by LC-MS/MS (using five different fragmentation methods). Data were analyzed with Maxquant.

Results

Based on the obtained data, 408,131 peptides with an Andromeda score >50 were selected. The peptides represent 19,903 human proteins and 41,636 isoforms of these proteins. For 19,274 proteins (97% of the total number of proteins) at least three peptides per protein fulfilled the selection criteria. A publicly available database was set up that applies fast and easy usable filter functions to select suitable peptides, provide links to reference spectra, as well as a means to order user-defined subsets of peptides.

Conclusions

A repository of validated reference peptides for proteomics is presented which includes >400,000 peptides, covering essentially all human proteins. For each peptide CID, HCD, ETD and EThcD spectra are available through ProteomicsDB [2]. Ongoing efforts focus on the expansion of the peptide list, including peptides carrying important PTMs.

[1] D. P. Zolg et al. *Nat. Meth.* **2017**, *14(3)*, 259-262.

[2] T. Schmidt et al. *Nucl. Acids Res.* **2018**, *46*, D1271-D1281.

POSTER 079
Analysis of receptor tyrosine kinase inhibitor distribution in tumor xenograft mouse using MALDI mass spectrometry imaging

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Receptor tyrosine kinase inhibitor (RTKi) has been widely used to treat non-small-cell lung carcinoma (NSCLC) patients. RTKi has been known to acquire resistance in the patients treated resulting in low efficacy on NSCLC. Many studies have aimed to unveil the cause of resistance by exploring the molecular mechanism of the drug resistancy in vitro. However, in vivo studies have not fully addressed for low efficacy of RTKi in tumor growth. Here, we propose the novel evidence of RTKi's low efficacy in vivo through analysis of its distribution image

using MALDI MSI (matrix-assisted laser desorption ionization imaging) and IHC (Immunohistochemistry) of a protein target of RTKi. We found that H1299, human lung cancer cells, show weak growth inhibition upon RTKi treatment leading to low efficacy in xenograft model. From the H1299 xenograft model mouse, we identified that RTKi was distributed on RTKi-treated tumor, liver and kidney tissue co-localizing with its target protein. Notably, RTKi was localized higher in liver and kidney than tumor tissue via the analysis of quantified intensity. Collectively, these results demonstrated that low efficacy of RTKi in H1299 xenograft mouse model may come from its nonspecific distribution in the number of organs.

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POSTER 080

Elucidating off-target proteins of RTKi by combinatory method of label-free DARTS and LC-MS/MS

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Identifying small molecule's target proteins and demonstrating its mode of action provide the fundamental basis for pharmaceutical applications. Currently, receptor tyrosine kinase inhibitors (RTKi) are used to treat non-small-cell lung carcinoma (NSCLC) patients mainly targetting EGFR with activating mutations to suppress tumorogenesis utilizing EGFR canonical pathway. However, the acquired resistances including 'gatekeeper' mutation and undesirable off-target related effects have been reported; these effects include a variety of phenomena ranging from genomic instability to skin rash. Hence, we propose combinatory method utilizing drug affinity responsive target stability (DARTS) along with LC-MS/MS methods to identify target proteins inducing resistance of Tyrosine Kinase Inhibitor (TKI) and off-target effects. Combinatory method of DARTS and LC-MS/MS has the strength in elucidating a pool of potential target proteins of small molecules *in vitro*. DARTS method delineates the chemical-protein interactions and these interactions can be identified by conventional LC-MS/MS SWATH analysis. These identified potential protein targets are then subsequently analyzed bioinformatically. Thus, by performing DARTS and LC-MS/MS on currently available TKIs in clinical settings, we attempt to demonstrate TKI's mode of action and explain the undesirable off-target effects and acquired resistances. By far, we have elucidated 3 potential new targets of TKI, and the modes of action studies on these potential target proteins will be presented.

POSTER 081

Identification of the missing protein Hyaluronan Synthase 1 in mesenchymal stem cells derived from adipose tissue or umbilical cord

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14% of the human proteome still holds by proteins whose existence is not confirmed by mass spectrometry. We performed a proteomic profiling of mesenchymal stem cells (MSCs) derived from fat tissue or umbilical cord and identified peptides derived from of 13 of such missing proteins. Remarkably, we found compelling evidence for the expression of hyaluronan synthase 1 (NX_Q92839-1) and confirmed its identification by the fragmentation of 4 heavy-labeled peptides that coeluted with their endogenous light counterparts. Our data also suggest that MSCs constitute a promising source for the detection of missing proteins.

POSTER 082

Missing protein detection in human embryonic tissues using a proteogenomics approach

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Bakground: The reliable detection of all the human proteins using mass spectrometry-based technologies is one of the Human Proteome Project (HPP) main goals. Bioinformatics pipelines have been developed making use of publicly available transcriptomics and proteomics experiments in order to select the biological samples where the missing proteins, proteins without MS experimental evidence in the neXtProt database, are highly expressed. Methods: In a proteogenomics approach we analyzed transcriptomic data of both, mature tissues from the Genotype-Tissue Expression (GTEx) project and embryonic tissues from public datasets. Proteomics experiments of embryonic cell lines downloaded from PRIDE database are also being analyzed using a bioinformatics pipeline based on the integration of different search engine identifications. This strategy benefits from the complementarity of these search engines to increase the proteome coverage. The same parameters will be used for all the search engines and the results will be compared following the HPP guidelines compliant procedure. Results: We identified genes highly expressed in all the embryonic tissues that are not expressed in mature tissues, and their functional analysis confirmed the enrichment of missing protein coding genes. We have already identified 3 missing proteins in the proteomics experiments based on only one unique peptide. The functional roles of these proteins support their detection in embryonic tissues. Conclusions: Although further validation of these missing protein observations is required, these preliminary results are promising considering that we have only analyzed 5 of the 40 proteomics experiments downloaded from PRIDE.

POSTER 083

Chromosome 19: Progress in hunting missing proteins and future strategies.

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The HPP Human Proteome Project (HPP) is a collaborative effort of different groups from around the world to understand the human proteome. The Chromosome-centric Human Proteome Project (C-HPP) is an initiative to detect and characterize neXtProt PE 2, 3, 4 missing proteins (MPs). The main effort of the Mexican Consortium in 2017−2018 was intended to investigate the proteome of chromosome 19 (Chr19), in different types of biological material; umbilical cord (vein, artery and Wharton jelly) cervical cancer cell lines (lysine acetylation stoichiometry in cervical cancer cells, using chemical inhibition of epigenetic enzymes such as lysine deacetylases to search for appropriate expression conditions of MPs, and treating cells with proteosome inhibitors to momentarily increase the stability of short-lived proteins, it could be the case to detect missing proteins with evidence at the transcript level), breast cancer cell lines (acetylation in mitochondria) and cancer cell glioblastoma (vesicles), additionally, the next stage involved, large-scale proteomics analysis to identify and validate the presence of the MPs using synthetic peptides. On the other hand, some bioinformatics analyzes to characterize the MP in the Chr19 according to their feasibility to be identified in proteomics experiments were conducted and another ones are actually in process. We report progress toward the next-50 MP Challenge with 19 newly-validated Chr19 PE1 proteins, of which 17 were based on mass spectrometry analysis from acetylation in cervical cancer, one in umbilical cord studies, and another one in vesicles of glioblastoma cancer. Notable among these new PE1 proteins 10 of the 11 missing proteins identified are Zinc-finger proteins, and also 10 of 11 were detected expressed in cell treated with EX527 inhibitor. Additionally, we observed how "in silico" studies can be used to capture individual peptides from major data repositories. This work was supported by a DGAPA-PAPIIT grant IN213216 and CONACYT grant 220790.

POSTER 084

Creating a complete human full-length plasmid collection for C-HPP and proteomics studies

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According to the neXtProt database, around 14% of human proteins deduced from the genome sequences have no evidence of protein existence and thus are considered as “missing proteins”. The main goals of C-HPP (Chromosome-based Human Proteome Project) consortium are to identify the missing proteins in each chromosome and test their functions. One of the key reagents for achieving the goals is the cDNA plasmid for the missing proteins, which can be used, for example, for developing targeted mass spectrometry such as SRM based on *in vitro* transcription and translation (IVTT), while mammalian expression-compatible plasmids can be utilized to study their cellular functions. For more than 10 years, our group has been cloning the cDNAs by both traditional library-based cloning and, more recently, gene synthesis towards completion of the full-length human plasmid collection, based on highly flexible Gateway cloning system. Recently, we have assembled a comprehensive and one of the world’s largest collections of full-length plasmids representing more than 75% of all human protein-coding genes and are distributing the collection through our repository and distribution web portal DNASU (dnasu.org). As a member of C-HPP working on the missing proteins in the chromosome 10, we have been providing the IVTT-compatible plasmids for missing proteins to other C-HPP groups for IVTT-assisted SRM and continue to generate more plasmids. Currently, we have full-length IVTT plasmids for around 50% of ~2,780 missing proteins and aim to cover >90% within the next year. The unique resource of missing protein plasmid set will be distributed to the entire C-HPP team for protein identification and functional studies. In addition, the complete human full-length plasmid collection will allow true proteome-level studies including antibody screens, protein-protein interaction studies, and cell-based screening.

POSTER 085

Chromosome 17 Missing Proteins: Recent Progress and Future Directions as Part of the Next-50MP Challenge

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The Chromosome-centric Human Proteome Project (C-HPP) announced in September 2016 an initiative to accelerate progress on detection and characterization of neXtProt PE2,3,4 “missing proteins” (MPs) with a mandate to each chromosome team to find about 50 MPs over 2 years. Here we report major progress toward the next-50 MP Challenge with 43 newly-validated Chr 17 PE1 proteins, of which 25 were based on mass spectrometry (MS), 12 on protein-protein interactions, 3 on a combination of MS and PPI, and 3 with other types of data. Notable among these new PE1 proteins were 5 keratin-associated proteins, a single olfactory receptor, and five additional membrane-embedded proteins. We evaluate the prospects of finding the remaining 105 MPs coded for on Chr 17, focusing on MS and protein-protein interaction approaches. We present a list of 35 prioritized MPs with specific approaches that may be used in further MS and PPI experimental studies. Among the 35 prioritized MPs, 25 are considered suitable for MS detection, 10 of which have a proteotypic peptide already detected. The other 10 prioritized MPs are prioritized for protein-protein interaction assays, similarly to keratin-associated proteins and a single olfactory receptors detected in the past 18 months. Additionally, we demonstrate how in silico studies can be used to capture individual peptides from major data repositories, documenting 1MP that appears to be a strong candidate for PE1. We are close to our goal of finding 50 MPs for Chr 17.

In addition, we commend C-HPP colleagues this strategy of examining families of proteins across the entire proteome for those most amenable to focused detection with MS, with protein-protein interaction assays, with enrichment methods, with guidance from tissue-specific transcript expression, and with additional approaches pioneered by various C-HPP teams.

POSTER 086

Multi-omics data analysis pipeline for identifying, functional annotating of single amino-acid variants.

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With the advent of high-throughput genome sequencing analysis, numerous phenotype associated non-synonymous single nucleotide variants (nsSNVs) have been characterized. However, only a few nsSNVs could be confirmed in protein level that named single amino acid variants (SAAVs). Some

studies tried to characterize and pinpoint pathogenic SAAVs. Unfortunately, most of the related phenotype and biological function of identified SAAVs have still remained unknown. Here, we developed the multi-omics data analysis pipeline to identify, annotate and retrieve pathogenic SAAVs from proteomic and genomic data. The pipeline provides a reference protein sequence database which contained more than five million SAAVs. The annotation database contained various kinds of comprehensive biological, clinical and pharmacological information. It enables ranged queries to search for condition-specific SAAVs that related to post-translational modifications (PTM) such as phosphorylation, glycosylation, acetylation, etc. It can also retrieve the SAAVs that are associated with disease, drug, genes, genomic location, and reported information on the 1000 Genome, dbSNP, and COSMIC.

POSTER 087
CONGENITAL ZIKA SYNDROME (MICROCEPHALY) SIGNATURE IN AMNIOTIC FLUID

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Background: In January 2016, ASOM suggested that congenital Zika syndrome (CZS) was due to ZIKV infection during pregnancy. Real-time PCR confirmed Zika virus in brain tissue and amniotic fluid (AF). However, it is not known which biological processes and metabolic pathways are affected by this infection. We used discovery-driven techniques to investigate ZIKV signature found in amniotic fluid of pregnant women bearing microcephalic fetuses

Methods: Methods: AF from pregnant woman were collected by puncture, submitted to real-time PCR and grouped in AF ZIKV positive (n=4, two with microcephaly) and AF ZIKV negative (n=3). Proteins were precipitated by acetone, solubilized with 7M urea / 2M thiourea and 2 % sodium dodecyl cholate, DTT reduced and iodoacetamide alkylated, and trypsin digested (1:50). Each sample was analyzed 4x in an EASY 1000 – nLC/Q-Exactive Plus (Thermo). Raw data were processed using Proteome Discoverer 2.1, and the SuperQuant strategy. Data analysis used Perseus and String software

Results: Data from ZIKV-positive microcephalic brains compared to ZIKV-negative resulted in the detection of 38 up-regulated proteins that negatively affected the regulation of proteolysis and the protein activation cascade whereas the 22 down-regulated were efficient in disturbing immune response to infection. Comparison between ZIKV-positive microcephalic versus ZIKV-positively-infected brains without microcephaly evidenced up-regulation of protein processing, response to wounding and peroxide catabolic processes (17 proteins) and down-regulation of extracellular matrix organization as well as collagen fibril organization (22 proteins). Finally, ZIKV-positive against ZIKV-negative protein brain data disclosed 39 up-regulated proteins that also negatively regulated endopeptidase activity, proteolysis, heparin binding and 21 down-regulated proteins affecting peptidyl-cysteine S-nitrosylation, glycolysis, retina homeostasis, and response to bacterium.

Conclusions: Our data indicate that the ZIKV signature in the amniotic fluid of fetuses bearing microcephaly up-regulates proteolysis and the protein activation cascade and down-regulates the immune response, extracellular matrix organization, and disassembly.

Keywords: proteomics, ZIKV, microcephaly

POSTER 088
ASV-ID, a Proteogenomic Analysis Method for Identifying Alternative Splice Variants of the Human Proteome

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One of the goals of the Chromosome-centric Human Proteome Project (C-HPP) is to map protein isoforms produced by alternative splicing of genes. However, identification of alternative splice variants (ASVs) by mass spectrometry remains a major challenge, because ASVs usually contain highly homologous peptide sequences. A routine protein sequence analysis suggests that more than half of those proteins investigated do not generate two or more uniquely mapping peptides that would enable their isoforms to be distinguished. Here we developed a new proteogenomics method, termed “ASV-ID” (*alternative splicing var*iants *id*entifier), that enables identification of ASVs by using a cell type–specific protein sequence database that is supported by RNA-Seq data. We were able to identify 1,935 distinct proteins under highly stringent conditions. Furthermore, 841 of those 1,935 proteins were verified with supporting peptides that would not have been otherwise identified without transcriptional expression information. We also demonstrate that ASV-ID enables detection of 19 differently expressed isoforms present in several cell lines. Thus, a new workflow employing ASV-ID will have the potential to map those difficult protein isoforms yet to be identified in a simple and robust way.

POSTER 089
Deciphering the dark proteome: use of the testis and characterization of two dark proteins

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The “neXt-CP50 Challenge” aims at functionally characterizing the PE1 proteins with completely unknown functions (uPE1). Additionally, the C-HPP consortium will now focus on “dark proteins” which include uPE1, missing proteins (PE 2-4), smORFs and proteins from lncRNAs or any uncharacterized transcripts.

As previously described, the testis is probably the most promising organ to search for missing proteins. The aim of the present work was to study dark proteins in the testis with a priority given to uncharacterized proteins. We analyzed the mRNA expression of “uncharacterized” proteins extracted from the Human Protein Atlas (HPA) RNA database and demonstrated that most display higher expression in the testis than in the other human tissues. We then performed a LC-MS/MS analysis of a human testis protein extract and identified unambiguously 5,578 proteins among which 30 were annotated as “uncharacterized”. Two uncharacterized proteins were

further selected based on their testis specificity. Combining publicly available expression data and immunohistochemistry, we showed that A0A0U1RQG5 is a putative cancer/testis antigen, specifically expressed in the testis, where it accumulates in the cytoplasm of elongated spermatids. We also demonstrated that PNMA6E, which has been referenced as a uPE1 since the beginning of our study, is enriched in the testis, where it is found in the germ cell nuclei during spermatogenesis.

Finally, we looked for the gene and protein expression of other dark proteins, uPE1 and missing proteins (PE2-4), in a series of human tissues. Most of the uPE1 and missing proteins are highly expressed in the testis, at both gene and protein levels. We conclude that the testis may be a relevant organ to study the dark proteome, as the function of most dark proteins may be related to spermatogenesis and germ cell differentiation.

POSTER 090

Improvement of Peptide Separation for Exploring the Missing Proteins Localized on Membranes

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With an enormous effort from the society of Human Proteome Project (HPP), the list of missing proteins (MPs) has been greatly shrunk. A bioinformatic analysis reveals higher occupation of MPs with stronger hydrophobicity. Considering detection of membrane proteins not only benefited from effective extraction from the intact proteins, improvement of peptide separation is another approach to assist identification of hydrophobic peptides, especially for those derived from MPs. Herein, we proposed the peptides prepared from the membrane fractions of human cell lines and placenta would be well separated by organic solvent at high concentration from other hydrophilic peptides. Taking a combination strategy of peptide separation with assistant of 98% acetonitrile, more hydrophobic peptides and proteins were detected from the supernatants of organic solvent, as compared with that gained from the pellets. A total of 30 MPs with 114 unique peptides, at least 2 non-nested unique peptides with at least 9 amino acids, were identified at protein FDR<1%, including 7, 12 and 13 MPs obtained from the membrane preparations of K562, Hela and human placenta, respectively. Of the 30 MPs identified in this study, 19 were theoretically categorized as membrane proteins or extracellular proteins. Furthermore, 20 MPs were verified with at least 2 non-nested unique peptide through parallel reaction monitoring with the corresponding peptides chemically synthesized. Organic solvent at high concentration, therefore, was proven as an efficient way to improve exploration of the hydrophobic MP.

POSTER 091

Measuring Histone Protein Dynamics by Hydrogen-Deuterium Exchange Mass Spectrometry

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Genetic information in eukaryotic cells is physiologically interpreted as chromatin, the fundamental repeating unit of which is the nucleosome. Individual nucleosomes are composed of a histone octamer containing two copies each of core histones H2A, H2B, H3, and H4 which are wrapped by approximately 147 base pairs of DNA in a left-handed superhelix. Nucleosome formation, as well as the folding of nucleosomes into higher order chromatin structures, naturally precludes DNA regulatory proteins from accessing target recognition sequences. Thus, histone, nucleosome, and chromatin structural dynamics play a critical role in genomic processes such as gene transcription by governing access to the underlying DNA template. We have employed hydrogen-deuterium exchange followed by mass spectrometry (HDX-MS) to study structural changes undergone by histones as a result from nucleosome formation. Critically, we are able to obtain unprecedented high-resolution structural information spanning histone N-terminal 'tails' using electron-transfer dissociation to fragment corresponding peptide species. These regions of the nucleosome are known to play crucial roles in the regulation of chromatin structure as well as the recruitment of chromatin effector proteins, and, due to their unstructured and highly dynamic nature, have remained elusive to more classical structural approaches such as cryo-electron microscopy or X-ray crystallography. We will also discuss ongoing work exploring structural dynamics of other members of the histone protein family.

POSTER 092

Clinical proteomics analysis using data independent acquisition (DIA) identified classifiers for molecular characterization of Lymphoma

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Lymphoma is the 7th-most common human cancer in the world, leading to 56.6 thousand new cases and 30.5 thousand deaths worldwide in 2012. As a highly heterogeneous cancer group with various lymphoproliferative disorders, lymphoma is often categorized as two major types: Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). NHL, further consists of B-cell neoplasms (B-NHL) and T/NK-cell neoplasms (T-NHL) as two subtypes, accounts for almost 90% of all lymphoma cases. Accurate characterization of lymphoma categories is essential for efficient clinical treatment. Besides the traditional lymphoma classification mainly based on the types of the original lymphocytes, morphological, phenotypic and genetic characteristics, molecular characterization of lymphoma, particularly at the protein level, is highly demanded in clinical practice. In this study, we conducted a comprehensive proteomics analysis on 109 lymph node samples from clinical patients of various types including B-NHL, T-NHL, HL, Lymphadenitis, Tumor metastatic lymph node (TLN), and Non-neoplastic lymph node (TNM: N0). Mass spectrometry analysis of these clinical samples was conducted on Orbitrap Fusion using data independent acquisition (DIA) method and the acquired data were processed using the Spectronaut software. A total of 2674 proteins (13031 peptides) were consistently quantified among the analyzed samples. Statistics analysis using Lasso and T-test generated sets of NHL-specific protein classifiers that can significantly distinguish it from Lymphadenitis, Non-neoplastic lymph node, or Tumor metastatic lymph node, respectively. In addition, two proteins were identified that can distinguish B-NHL from T-NHL.

Furthermore, by investigation of the clinical information of our lymphoma patients, we discovered several proteins correlated with the aggressive and prognostic status of the lymphoma. Discussion about these proteins as potential biomarkers on lymphoma classification, prognosis, and therapeutic decision will be presented.

POSTER 093

Development of robust and reproducible renal proteome assays from kidney biopsies

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Currently, the treatment of renal diseases is held back because of the lack of good biomarkers. The main biochemical indicators currently used to diagnose kidney disease are only general markers for renal dysfunction, which are not specific to particular diseases. More in depth knowledge of proteome changes specific to diseases phenotypes would aid diagnostics and overall treatment. The gold standard currently used in diagnostics is renal biopsy followed by histochemical staining. However, it's know that diagnostics based on tissue morphology is subjective as inter-observer variability among expert pathologists can lead to differential diagnoses and treatment choice.

We set out to develop standardized protocols for analysis of fresh frozen FF and FFPE tissues that could be used diagnostically in the clinic. We demonstrate that proteome analysis of kidney tissues of 'real life' biopsy material of only 15µm thick and approximately 10 mm2. With the developed method rapid analysis of both FF and FFPE tissue is achieved with comparable numbers of identifications averaging 800 and 724, respectively. The method shows that overall good overlap of the identified proteins is achieved between FF and FFPE tissue ranging from 52.3-69.3% for individual samples. The developed SOP is easily transferable to the clinic and does not require expensive instrumentation, new skills or the use of chemicals and detergents for the de-crosslinking FFPE samples, and it is MS compatible.

We will discuss SWATH-MS analysis of FFPE tissue sections using the and show the quantitative analysis large numbers of proteins tissue sections, from both FF and FFPE. Interestingly we recorded quantitative analysis of 951 proteins across our minute FFPE samples. Additional work will be updated at the meeting, including steps towards collecting a large cohort of renal samples.

POSTER 095

Biomarker candidate discovery in blood and cerebral spinal fluid from patients with neurodegenerative diseases

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Backgroud: Parkinson's disease (PD), progressive supranuclear palsy (PSP) and multiple system atrophy (MSA) have overlapping and distinct molecular deficits leading to disease and cell-type specific protein aggregation. We utilized a mass spectrometry (MS)-based proteomic analysis of plasma and cerebral spinal fluid (CSF) to identify candidate biomarkers for each disease and molecular pathways distinguishing between diseases. Targeted multiple reaction monitoring (MRM) MS methods were used to more precisely quantify plasma and CSF protein levels.

Methods: High abundant proteins (HAPs) were first removed from plasma (top14, MARS, Hu-14, Agilent) or CSF (Top2, Pierce). Flowthrough digests from each sample was analyzed by LC-IMS-TOF (SynaptG2-Si, Waters). Progenesis QI for proteomics was used for peptide and protein identification and quantification across all samples. Both univariate and multivariate data analysis were performed for candidate discovery, as well as multiple normalization methods and characterization of candidate biomarker ratios. Orthogonal partial least squares discriminant analysis (OPLS-DA) models were developed to detect peptide patterns that best discriminate between the pre-defined sample groups.

Results: We developed panels of candidates that can discriminate neurodegenerative diseases from normal control patients or between diseases. Some candidates were also further validated by LC-MRM with excellent precision and accuracy.

POSTER 096

Investigation of targetable Biomarkers for Non-small-cell lung carcinoma (NSCLC) in human blood plasma

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Non-small-cell lung cancer (NSCLC) constitutes the majority of all diagnosed cases of lung cancer. Regardless of recent advances in distinct therapies, NSCLC still presents a relatively higher mortality when compared with other cancer types. From a clinical perspective, the early detection is the key factor lowering its mortality rate; therefore the identification of targetable biomarkers involved in the molecular mechanisms underlying genesis and progression of NSCLC is crucial. In this direction, blood plasma samples were obtained from 18 NSCLC patients (adenocarcinoma stages I and IV) and from 9 controls (e.g. benign lung diseases). The ultra-sensitive magnetic beads-based SP3 protocol was here adapted and applied to plasma preparation in order to facilitated scalability, speed, and throughput of our analysis. Therefore, only 20µg (~2µl) of total protein was necessary for profiling the proteomes of NSCLC patients and controls. Subsequently, SP3 recovered peptides were isobaric labeled with TMT 9-plex and individual samples were mixed according to groups (stage I, stage IV and control). Samples were further off-line fractionated by high-pH reverse phase chromatography into 16 fractions and analyzed on a Q Exactive HF-X. Using a distinct approach, quantitative information was extracted on both MS1 and MS2 levels, making it possible to access at the same time (i) major differences among study groups (LFQ-like comparison) and (ii) particular individual variations among individuals patients (TMT ratios). Finally, proteins presenting the most striking differential expression in our experiment were further selected for parallel reaction monitoring (PRM) validation. In summary, we here

present the application of the SP3 protocol to blood plasma preparation as well as the novel utilization of two levels of quantitative information extracted from a single MS run. By integrating different state-of-the-art proteomic approaches, we expect to uncover and establishment proteomic-based biomarkers for NSCLC in human blood plasma.

POSTER 097
Differential Plasma Proteomic Analysis of B-thalassemia patients in response to Hydroxyurea treatment

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B-thalassemia is the most common genetic disorder caused by mutations in β-globin gene results in absence and reduced production of Adult Hemoglobin (HbA). Regular blood transfusions and effective iron chelation is necessary for survival of β-thalassemia patients. An emerging therapeutic approach to handle β thalassaemia is production of fetal hemoglobin (HbF). Hydroxyurea (HU), a potent HbF inducing agent, has shown to be an effective drug for β-thalassemia management with variable responses from transfusion independence to null outcome. Diversified response of β-thalassemia patients to HU therapy remains ambiguous for hematologist. Clinical proteomics has revolutionized the study of differential protein expression associated with disease and offers a unique technique to monitor the response to treatment .In current study, we focused on comparative analysis of plasma proteome in pre- and post- HU-treated β-thalassemia patients, as well as responders and non-responders to HU treatment. Plasma was collected from β-thalassemia patients before and after 6 months of HU treatment, and the treated group were sub-categorized on the basis of response to HU. Label-free LC-MS/MS analysis was performed on nano-HPLC coupled Q-Exactive Orbitrap Plus mass spectrometer. Quantitative data and statistical analysis was carried out using MaxQuant and Perseus respectively. Proteomics analysis revealed identification of 400 proteins in all groups, among them the abundances of twenty eight proteins were significantly different in pre- vs post- HU treated groups, with, twenty six proteins being differentially expressed in responder vs non-responder groups. Transferrin receptor protein 1 (TfR) was the most down-regulated and hemopexin and haptoglobin were the most up-regulated proteins in plasma after HU treatment whereas proteins with most significant difference between responder and non-responder was carbonic anhydrase 1, hemoglobin subunit gamma-1 and peroxiredoxin-2.

POSTER 098
Peripheral Immunophenoprofiles in Non-small-cell Lung Cancer Patients Carrying Different *EGFR* Genotypes

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Immunotherapy emerges as a new paradigm for the treatment of non-small-cell lung cancer (NSCLC), yet the histopathologic diagnosis is insufficient to predict responsive patients. Additional detection assays are required for better classification of the responders. To develop potential molecular indicators, we conducted personalized membrane proteome quantitation on peripheral blood mononuclear cells (PBMC) from NSCLC patients with *EGFR* wildtype (WT), L858R or exon 19 deletion (Del_19) to unravel the correlation of membrane proteome changes, *EGFR* genotypes, and immunotherapy responses. 2,072 proteins were confidently identified of which 1,600 proteins were quantified. To investigate the status of PBMC, we constructed the immune cell marker database based on well-established surface markers and the transcriptomics profiles and examined their expression levels in different patients. Our data suggested that the *EGFR* mutant patients exhibited decreased levels of both MHC class II antigen presenting pathways and activated CD8 T cells compared to WT patient. In addition, we discovered proteins, including ANXA1, SPN, STAT1 and STAT2, that could differentiate patients with different *EGFR* genotypes. With further validations in a larger cohort of patient samples, we expect that our discovered PBMC membrane proteome signatures will yield additional parameters to gauge patient immune status and to predict responders for immunotherapeutic intervention.

POSTER 099
Immunodepletion using MARS-14 column enables higher coverage of CSF proteome than "equalization" by ProteoMiner hexapeptide ligand library.

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Its direct contact with central nervous system makes cerebrospinal fluid (CSF) a potential source of disease biomarkers. CSF is formed by ultrafiltration of blood plasma and by active secretion by choroid plexus of the brain. Similar to plasma, presence of highly abundant (plasma) proteins limits the depth of CSF analysis.

Since there is no dedicated method for depletion of the most abundant proteins in CSF, we evaluated the effect of two methods developed for blood plasma in CSF. We compared immunodepletion by MARS 14 column with “equalization” by ProteoMiner hexapaptide ligand library.

Triplicate LC-MS/MS analysis of crude (non-depleted) CSF sample resulted in identification of 475 protein groups. Depletion of the most abundant proteins by MARS 14 column increased the number of identified protein groups by 63% to total 773. The “equalisation” of protein concentrations using ProteoMiner increased the number of identified protein groups only by 29% (611 protein groups) compared to crude CSF.

Since there is a concern about a loss of potential biomarkers during the depletion, we analysed also the “waste” fractions (i.e. the proteins retained by MARS 14 column and proteins not bound to the ProteoMiner beads). MARS 14 cartridge retained 214 unique protein groups, while the “waste” fraction of ProteoMiner contained 272 protein groups. Significant number of these molecules were unique for the “waste” i.e. not detected in the depleted CSFs. Among those, we found several proteins relevant for physiology or pathology of central nervous system.

In summary, both methods significantly improve CSF proteome coverage, MARS 14 immunodepletion being more efficient, increasing the number of identified proteins by more than 60% compared to crude CSF. We also demonstrate that it is beneficial to analyze also the “waste” CSF fractions, in order to

maximize the CSF proteome coverage in the quest for neurological biomarkers.

POSTER 100

Identification and overexpression of proteins during Human Corneal Epithelial Wound Healing in Vitro Model

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Integrity of the corneal epithelium depends the health of corneal which are maintained by different factors like corneal architecture, tear quantity and quality and corneal sensitivity. Altering any of these elements can lead to a corneal epithelial defect. We aimed to investigate the alterations in protein expressions during corneal epithelial migration to demonstrate the networks of the total identified proteins with potential dual functions. In this study, human corneal epithelial cells lines (HCEC) have been used for wound healing model. Mechanical wound was made in HCEC lines and rate of healing was monitored at 6, 12, 18, 24, 30 and 36 hours of post wounding using TScratch software tool. Epithelium was scrapped at each hours, followed by protein extraction. Protein quantification was done using BCA kit. The proteins extracted from wounded and unwounded model at each hours were subjected to SDS-PAGE and two -dimensional electrophoresis (2DE). Mass Spectrometry (MALDI TOF) was done to identify the proteins through protein Matrix Science database searches. The differentially expressed and identified proteins were further validated by western blot analysis. To further elucidate the potential biological mechanisms and functional association involving the identified proteins in wound healing, we used IPA (http://www.ingenuity.com) and STRING. A significant finding of the present study is the identification of Cdk10,ETS2, EFNB3,RAB 34, RRAS, HSP22 and HSP90 in healing corneal epithelium at active phase of migration. This study describes for the first time a direct link between CDK10 and ETS2 and this interaction association network analysis confirms the close interacting relationship between CDK10 and ETS2 proteins.
Conclusion: The present communication initially provides new evidence for the potential role of identified proteins in migrating epithelial cells as well as it increases the understanding of normal and abnormal corneal function with likely relevance to corneal disease and transplants.

POSTER 101

Distinction between molecular subtypes Group 3 and Group 4 of medulloblastoma using quantitative proteomics

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Medulloblastoma, an embryonal neuroectodermal tumor of the cerebellum, is the most common malignant brain tumor in children. Biological studies revealed consensus molecular subgroups of medulloblastoma, namely WNT, SHH, Group 3 and Group 4 that exhibit distinctive transcriptional and epigenetic signatures, but divergent epidemiological and prognostic features. Personalized therapies for medulloblastomas warrant investigation of underlying molecular driver events, especially in heterogenous Group 3 and Group 4 disease. Emerging technologies as quantitative proteomics may allow more refined description of the medulloblastoma landscape.

The fresh frozen medulloblastoma tissues designated into Group 3 (n=7) or Group 4 (n=10) according to the transcriptomic profiling were lyzed, proteins were digested and tryptic peptides were labeled using TMT 10plex kit (Thermo Fisher Scientific). Mass spectrometry LC-MS/MS analysis was employed with high pH reverse peptide fractionation resulted in higher number of quantified proteins. Mass spectra were evaluated and statistically processed with Proteome Discoverer 2.2.

We identified biological markers that could be potentially used for distinction between Group 3 and Group 4 medulloblastomas in two independent sets of samples. We detected 38 and 35 proteins with significantly upregulated or downregulated level in Group 3 versus Group 4, respectively. We compared our proteomic data with gene expression data from publicly available datasets using “R2: Genomics Analysis and Visualization Platform” (http://r2.amc.nl) and observed a high concordance between protein levels and gene expression that confirmed robustness of our results obtained from a relatively small set of medulloblastoma tissue samples available for proteomic analyses. Some of these proteins/genes were already described as useful for distinction between medulloblastoma molecular subgroups or important in medulloblastoma pathogenesis (netrin receptors, Ras-related protein Rab-3C, Insulin-like growth factor 2, mRNA-binding protein 3, and cellular retinoic acid-binding protein 2). In next step, the selected proteins will be validated as distinctive biomarkers between molecular subgroups of medulloblastoma.

POSTER 102

Differential analysis of the proteome of ovarian endometriosis

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Background:

Endometriosis is a chronic, estrogen-dependent gynaecological disorder characterized by the presence of endometrial glands and stroma at extra-uterine sites. Its aetiology is poorly understood. It is a heterogeneous disease which has lesions of different phenotypes and anatomical locations. Ovarian endometriosis is a phenotype commonly associated with “revised American Society for Reproductive Medicine” (rASRM) stages III and IV of endometriosis. The aim of our study is to identify changes in the ovarian endometriosis proteome that may aid in our understanding of the pathophysiology of this disease.

Methods:

Age-matched (29.4±4.7yrs) infertile women with regular menstrual history, in their proliferative phase of menstrual cycle, were included in the study. Proteins extracted from paired ectopic and eutopic endometrium samples of patients suffering from endometriosis (n=8) and control endometrium samples (n=6) of the disease free infertile patients were used. The proteins were separated using 2D-DIGE and differentially

expressed spots were picked, trypsin digested and subjected to MALDI-TOF/MS. Data was analyzed using Mascot software for identification and KEGG tool was used for pathway analysis.

Results:

A total of 53 significantly dysregulated proteins could be successfully identified. These proteins are involved in metabolic pathways like glycolysis/gluconeogenesis, amino acid metabolism and oxidative phosphorylation, cell signaling, apoptosis, cell cycle, structural and stress response proteins. Some of the downregulated proteins in ectopic tissue are SIRPA, CCNI, GULP1, ANX4, EEFSEC and GDI2. Proteins like DNM1L, TUBAL3, HSPA2, TPI1 and PRDX6 were upregulated, whereas TST protein was solely present in ectopic samples.

Conclusion:

In our study, the proteomic analysis of well characterized ectopic and eutopic tissue samples has identified novel proteins associated with ovarian endometriosis.

POSTER 103

Translating serologic response to the *Candida albicans* cell wall-associated proteome during dimorphic transition into a molecular discriminator for invasive candidiasis

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Invasive candidiasis (IC) is a leading infectious cause of mobility and mortality in cancer, post-surgical and intensive care patients if not diagnosed and treated early. However, IC is difficult to diagnose at an early stage, resulting in delayed therapy and ensuing fatal outcomes. We examined whether profiling of the serologic response to the *Candida albicans* yeast and hyphal cell surface-associated proteomes in IC might uncover a molecular discriminator for IC. We assessed serum IgG antibody-reactivity profiles to the *C. albicans* cell surface-associated proteomes from yeast and hyphal growth forms in IC and non-IC patients by immunoproteomic and bioinformatic analyses. Capture ELISA assays were used to validate the obtained results. Unsupervised two-way hierarchical clustering and principal component analyses of these IgG antibody-reactivity patterns discriminated IC patients from non-IC patients. Pairwise correlation mapping across serum specimens further revealed that distinct biologic changes underlay commensal-to-pathogen and yeast-to-hypha transitions. Supervised classification analyses with leave-one-out cross-validation identified a 3-IgG antibody-reactivity signature as the best predictor for IC. Multivariate logistic-regression models unveiled a positive association between this signature and IC risk. This association was unbiased by classical clinical IC-risk factors and other patients-related variables. Receiver-operating-characteristic curve analysis highlighted that this signature showed a good ability to differentiate between IC and non-IC patients. Capture ELISA assays confirmed its discriminatory power in an independent group of IC and non-IC patients. We conclude that a 3-IgG antibody-reactivity signature may be valuable for early and accurate diagnosis of IC. Our findings further offer new insight into pathogenic processes triggered during dimorphic transition and IC pathogenesis. This work was supported by BIO-2015-65147-R, PRB3 (IPT17/0019-ISCIII-SGEFI/ERDF) and RD16/0016/0011.

POSTER 104

Proteomics of Laser-captured Microdissected Glomeruli and Tubulointerstitium Reveals Compartment-Specific Altered Extracellular Matrix of Kidney Allografts with Antibody-Mediated Rejection

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BACKGROUND: Kidney transplantation is the optimal treatment for end-stage kidney disease, but most grafts fail prematurely. Antibody-mediated rejection (AMR) accounts for >50% of graft loss. AMR is caused by antibodies against HLA and non-HLA antigens. These antibodies are directed against proteins in two main compartments of the kidney: glomeruli and tubulointerstitium. **We hypothesized that kidney injury in AMR is associated with compartment-specific proteome alterations that may uncover the mechanisms of early antibody-mediated injury.**

METHODS: We performed laser-capture-microdissection to isolate glomeruli and tubulointerstitium from formalin-fixed-paraffin-embedded kidney biopsies, and subjected unfractionated samples to label-free proteome analysis by LC-MS/MS on Q-Exactive-Plus mass spectrometer. Analyses were performed using MaxQuant, Perseus and pathDIP. We compared 8 biopsies with AMR with 23 matched 'non-AMR' biopsies with T-cell rejection or acute tubular necrosis.

RESULTS: We identified 2026 proteins in glomeruli and 2426 in tubulointerstitium. Podocyte-specific proteins were exclusively found in the glomeruli (NPHS1, NPHS2, PTPRO), while tubular proteins were only in tubulointerstitium (LRP2, CUBN, UMOD), indicating enrichment in compartment-specific proteins. 141 proteins were differentially expressed ($p<0.05$) in AMR vs. non-AMR glomeruli (73 upregulated, 68 downregulated), and 123 in the tubulointerstitium (15 upregulated, 108 downregulated). Eleven proteins were significantly differentially expressed in AMR vs non-AMR($q<0.05$). Proteins involved in HLA-mediated antigen presentation were increased in AMR, in both compartments. Interestingly, proteins significantly decreased in both compartments in AMR (e.g.LAMC1, COL1A1, NID1) were components of the basement membranes, and belonged to processes such as integrin signaling, collagen, extracellular matrix (ECM) and cytoskeleton. Levels of collagens, laminins, and other ECM proteins correlated directly and significantly, suggesting co-regulation in AMR.

CONCLUSION: Basement membranes are often remodeled in late chronic AMR and are the targets of non-HLA antibodies, suggesting that **these proteomic changes may represent early, important alterations in AMR**.

POSTER 105

The investigation of drug resistance of Acinetobacter baumannii based on DIA quantitative proteomics approach

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Acinetobacter baumannii (Ab) has emerged as one of successful pathogens that responsible for a majority of nosocomial infections, whose pan-drug resistance together with hypermutable property has been recognized as research focus. As changes of protein expression level would more straightforwardly show the functional changes of drug resistant Ab other than genomics or transcriptomic approaches, thus we steered our research emphasis toward large scale quantification proteomic investigation of Ab clinical isolates. Totally, we collected 58 highly resistant (HR), 54 low resistant (LR) and 12 intermediate resistant (IR) isolates according to drug susceptibility test of 20 kinds of 10 categories of antibiotic drugs. Based on data independent acquisition (DIA) quantification strategy, each isolate had an identification of approximately 4000 protein groups in average that was comparable to its genome scale. Ab isolates were classified into 6 majority groups with 90 steadily classified isolates according to 1534 reliably quantified protein entries. Proteome difference between HR to LR or IR isolates revealed the drug resistance might be correlated with DNA synthesis and repair, translation regulation, redox homeostasis, metabolic process and porin activity. A thymidylate synthase (THYA) together with a dihydrofolate reductase (J715_0112) were found to be significantly positively correlated with drug susceptibility, suggesting DNA synthesis was increased along with enhancement of drug susceptibility. The unprecedented scale of proteome data provides functional context to interpret mechanism of drug resistance of Ab.

POSTER 106

Cross-omics analysis of proteome and transcriptome dynamics during clinical peritoneal dialysis therapy

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Peritoneal dialysis (PD) is not only an important renal replacement therapy, it also represents a modality of scheduled repeated exposure to PD fluids (PDF) which disrupt peritoneal immune defenses and cause increased sterile inflammation. Alanyl-glutamine (AG) has recently been shown to restore cytoprotective stress responses in experimental PD and improve patients' survival and immune function of leucocytes in critically-ill patients. Addition of AG may therefore restore adequate responses of peritoneal cell populations to stress and infectious stimuli.

Samples from a randomized clinical pilot-trial were analyzed in a cross-omics approach of peritoneal effluent cells (transcriptomics using RNAseq) and soluble proteins (proteomics using LC-MS) to investigate the effect of AG on the interplay of peritoneal cell populations and fluid transport in PD.

In the clinical trial stable patients on chronic PD were treated in a cross-over design with standard PDF or with AG-supplemented PDF. Peritoneal immune-competence was analyzed by functional ex-vivo stimulated cytokine release of effluent cells. From each PD dwell unstimulated cells and cell-free PD-effluent was collected at multiple time-points of the dwell. Cellular responses were assessed on the RNA level and soluble proteins in cell-free PD-effluent were analyzed by a recently established depletion and enrichment workflow based on combinatorial peptide ligand library (CPLL) beads, filter-aided sample preparation (FASP) and tandem mass tag (TMT) based LC/MS.

We were able to quantify approximately 10,000 transcripts in PD effluent cells and 2,500 proteins in the PD effluent proteome. Interestingly, differential changes in the proteome could in part be explained by co-regulated biological processes observed on the transcript level. The remaining effects on the proteome are likely due to changes in transport characteristics, supported by clinical findings in patients treated with AG added to PDF. These results correlated with increased ex-vivo stimulated cytokine release reflecting restoration of suppressed peritoneal immune responses by AG.

POSTER 107

Diagnosis of malignant pleural mesothelioma cancer relying on targeted proteomics in blood

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Introduction: Blood based diagnosis of malignant pleural mesothelioma (MPM) cancer is not yet part of the clinical routine. Single biomarkers based on antibody assays in blood are clinically approved, but they have only limited sensitivity for MPM detection or monitoring of people at risk, like asbestos exposed individuals. We investigated a previously proposed multiplexed targeted proteomic signature for the detection of MPM in blood.

Methods: We investigated a multicenter collected cohort of serum samples from 214 MPM patients and 191 asbestos exposed donors. We performed N-linked glycoprotein enrichment strategies in 96-well plates. We applied selected reaction monitoring (SRM) for the quantitative investigation of a 6 candidate biomarker peptide signature in a training set of 106 MPM and 106 asbestos exposed donors and in a validation set of 108 MPM and 85 asbestos exposed. We compared the predictive accuracy of the multiplexed proteomic signature with the FDA approved ELISA assay for the currently best MPM blood biomarker soluble-mesothelin related peptides (SMRP) from the mesothelin protein. Also, we assessed prognostic value of the proteomic signature for survival of MPM patients after treatment.

Results: The multiplexed signature had higher accuracy compared to single biomarkers assessed by mass spectrometry. The proteomic signature had an area under the receiver-operating characteristic curve (AUC) of 0.742 for the discrimination between MPM and asbestos exposed donors of the validation set and of 0.735 for early stages MPM patients (stage I/II). Predictive accuracy of the ELISA assay for SMRP was not significantly different and had AUC of 0.795 for discriminating MPM and asbestos exposed (P = 0.248) and

AUC of 0.7444 for early stages MPM (P = 0.890). The two diagnostic strategies correlated poorly. The proteomic signature separated MPM patients with significantly different survival.

Conclusion: Targeted proteomics has clinical potential for the diagnosis of MPM cancer in blood.

POSTER 108
Automated Workflow Composition in Mass Spectrometry-Based Proteomics

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A large number of software tool for mass spectrometry data analysis, especially in the proteomics domain, are designed to perform discrete operations as building blocks for assembly in larger workflows. We recently published the results from a renewed effort in automatic workflow composition of software tools for mass spectrometry-based proteomics, demonstrating in a number of use cases derived from common proteomics experimental designs and research questions the feasibility of assembling functionally annotated tools in analysis workflows from raw data all the way through to statistics and visualization.

In this presentation, we will review the current state-of-the-art of automatic workflow composition and the next logical steps, including how to build executable workflows from generic specifications. The presentation will also survey the landscape of data formats, environments, operations and software for mass spectrometry data analysis, with particular focus on the functional software annotation in the bio.tools registry.

Results will include reuse of data in public repositories and assembly of executable workflows in common platforms, from low-level scripts to high-level workflow managers. A major driver behind automatic workflow composition is to compare multiple different but semantically equivalent workflows on the same data, to verify that the biological conclusions drawn are robust and take a step toward more reproducible science. We will also discuss how this could be done in practise.

POSTER 109
Comprehensive computational pipeline for conventional MS/MS data processing and open search-based PTM characterization

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Here we present a comprehensive suite of tools assisting with application of the new search engine MSFragger in a wide range of data analysis scenarios. As an ultrafast tool, MSFragger is valuable when conventional tools are too slow, e.g. for identification of endogenous peptides that requires non-specific enzyme searches. It is particularly useful for open (wide mass tolerance) searching which has become an effective strategy to look for novel post-translational modifications (PTMs) or, more generally, some recurring structural differences in observed molecules. By integrating multiple computational components, our pipeline facilitates the entire process from searching raw data, validation of the search results, visualization of observed mass shifts and subsequent PTM annotation to normalized TMT quantification of multiple data sets. The whole software suite is implemented for both Windows and Linux platforms. The pipeline includes running data conversion, search through MSFragger, post processing using Philosopher (peptide and protein validation using PeptideProphet and ProteinProphet), mass calibration and open search result refinement using Crystal-C, and detection of commonly occurring mass differences with DeltaMass. It also supports downstream PTM characterization with PTM-Shepherd, an automated tool that characterizes PTMs based on attributes such as amino acid localization, fragmentation spectra similarity, and relative modification rates. The pipeline has default configurations to run almost without any user interaction in both conventional (Closed) and Open search modes. Quantitative analysis is provided via two different label-free methods; spectral counting and spectral intensities. MS1 spectra intensities are computed using a re-implementation of the moFF algorithm, providing a simple but robust method of estimating peak apex intensities. Robust TMT isobaric-labeling quantification is also provided via Philosopher, different normalization methods in PSM and protein levels has been implemented for convenient usage and interpretation.

POSTER 110
Functional analysis of protein lists using popular proteins across human diseases

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Identifying the popular proteins within a biomedical topic (e.g., a disease, cell type, or organ) can yield insights into research trends and important pathways within a research area of interest. We previously described a workflow to calculate the semantic similarity between a protein and literature publications within a topic, using the search function on PubMed and a Gene2PubMed reference dataset from NCBI. Here we queried a collection of >10,000 disease phenotype terms in standardized vocabularies. We show that disease terms are associated with specific popular protein lists that inform on the protein-disease relationships. We further implemented a reverse protein search strategy over the precompiled terms, which associates an input protein with the terms in which it is intensively investigated (e.g., the query input “troponin” returns the disease term cardiomyopathy and the phenotype chest pain). We evaluated whether the curated popular protein lists could be used as an annotation source for functional analysis, e.g., given a list of differentially expressed genes/proteins from an omics experiment, return whether the up-regulated entities are enriched in genes/proteins that are intensively researched in a particular disease. To do so, we retrieved public transcriptomics data on a rodent transverse aortic constriction model of heart failure. Reverse popular protein queries provided complementary annotations to Gene Ontology (GO), e.g., we found significant enrichment of regulated genes to be intensively researched in “collagen disease" and “cartilage disease" terms (hypergeometric Padj < 0.05), corresponding to enrichment of GO “extracellular matrix organization" term; as well as enrichment of ”mitochondrial disease" genes which corresponds to GO “mitochondrial electron transport, NADH to

ubiquinone". Moreover, reverse popular protein queries suggest an involvement of genes in “atrial fibrillation" which was not apparent from enriched GO terms, highlighting the potential utility of additional annotation sources in functional analysis.

POSTER 111
Charge Deconvolution of Crowded Spectra

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Mass measurement of an intact protein is often a simple experiment yielding limited information, for example, a quick check that the correct gene was cloned and expressed. Intact mass analysis, however, can also be applied to many more challenging experiments, including antibody-drug conjugates, bispecific antibodies, stoichiometry of complexes, proteoform profiling, phosphorylation and glycosylation profiling, and protein/ligand and protein/protein binding assays. These applications, however, can strain or break the standard charge deconvolution algorithms (MaxEnt and ReSpect), which were developed for simpler target molecules and have not changed substantially over 20 years. Here we describe a new charge deconvolution algorithm that uses both isotope spacing and multiple charge states, and show how it performs for challenging applications. We validate charge deconvolution and monoisotopic mass setting on well-studied data sets with both MS1 and MS2. We validate quantitation on synthetic and semi-synthetic MS1 data, for which we have quantitative ground truth.

POSTER 112
A novel computational strategy for top down proteomics, based on all ion fragmentation and capillary electrophoresis

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Analysis of phospho-proteomes by enrichment can give us insights into how and when proteins are modified, but it cannot easily tell us about coordinated action of modifications on protein molecules. For true analysis of the biological entities as they function in cells, improvements are needed in our ability to separate proteoforms analytically and to interpret intact protein spectra. We are developing software for deconvolution of top-down spectra generated on a Thermo Fusion Orbitrap, coupled to capillary electrophoresis (CE), which is capable of time resolving near-isobaric proteoforms.

seaMass-TD is used to deconvolute MS1 and MS2 spectra, even in the absence of isotopic resolution of proteoform ions. We also tested DeconTools for deconvolution of MS2 spectra, where isotopic resolution could be achieved due to the generally lower charge states of fragment ions. Due the wide isolation window, a correlation scheme was used to assign product ions to likely precursors. Fragment ions were assigned to proteoforms using a new search engine we have prototyped.

Using alpha-casein, we demonstrate the ability to time resolve differentially modified forms of the protein, which cannot usually be achieved by LC. To increase signal-to-noise on precursors, we performed a low-resolution MS1 scan, followed by a wide isolation window (1000Da), and high-resolution MS2 fragmentation by HCD/ETD. Software was developed for detecting features in the raw data, along the time axis even in the absence of isotopic resolution, typically required for decharging spectra. The pipeline was able to correlate fragment ions to precursor ions, demonstrating improved specificity and sensitivity than commercial software currently available for top down data. A search engine was created to assign fragments to proteins, in this case known from bottom-up analysis of the same samples. Further development of the software is planned for analysis of samples with higher complexity, and quantitative analysis of proteoforms.

POSTER 113
An online service for proteomics data mining using clustered spectra

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Background

We have clustered ~190 million spectra and demonstrated that spectrum clustering can be used to enhance proteomics data analysis: originally unidentified spectra can be identified and individual Peptide-Spectrum-Matches (PSMs) can also be evaluated by using clusters of identified spectra. However, online services able to integrate the clustered spectra and provide this functionality for individual datasets are currently not available.

Methods

Here we present our newly developed *phoenix-enhancer* online web service(http://enhancer.ncpsb.org). Based on our released PRIDE Cluster data and a newly developed pipeline (https://github.com/phoenix-cluster/analysis-pipeline), four functionalities are provided: 1) Evaluate the original identifications in an individual dataset, to find low confident PSMs which could correspond to wrong identifications; 2) Provide confidence scores for all matched originally identified PSMs, to help users to evaluate the quality of each PSM (complementary to getting a global FDR); 3) Propose new PSMs to originally unidentified spectra; and 4) Provide a collection of browsing and visualization tools to analyse and save the results.

Results

We applied the *phoenix-enhancer* pipeline to 69 proteomics datasets and demonstrated that it could detect 0.5% questionable PSMs in the original identifications, at the same time identifying 12.4% more spectra. Additionally, the pipeline provided individual confidence scores for 33.6% of the original PSMs. On *phoenix-enhancer* website, users can upload their own MS files or choose a public ProteomeXchange dataset ID the pipeline is then run on our server, and the results are provided to the users. The visualization tools indicate how the confidence scores are distributed and allow users to compare interactively the target spectrum to the consensus spectrum from matched clusters.

Conclusions

The *phoenix-enhancer* analysis pipeline and web service can help users to get more PSMs from their data and validate existing PSMs provided by standard search tools. The code is open-source, enabling its integration into existing proteomics analysis workflows.

POSTER 114
OpenProt unveils yet unseen depths of eukaryotic proteomes

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Eukaryotic coding transcripts have traditionally been described as organized by one coding sequence (CDS), encoding a canonical protein (refProt), flanked by two untranslated regions. Hence, eukaryotic genes are believed to code for one refProt and its splicing derived isoforms.

However, recent studies showing the expression and function of proteins from alternative open reading frames (altORFs) force us to reconsider this architecture. AltORFs are found upstream and downstream of the canonical CDS as well as overlapping it in a shifted frame, or in transcripts currently annotated as non-coding. These data underline the polycistronic potential of eukaryotic genes.

Here we present a new method of transcript annotation which incorporates this increased coding potential by allowing a more comprehensive description of stored proteomic information. We performed genome wide in silico translation where currently known proteins, called refProt, are annotated alongside their isoforms and alternative proteins encoded by altORFs.

These predictions are challenged and validated using high throughput search of Ribo-seq, proteomics, and conservation data to identify expressed alternative proteins. Functional annotations for several species are displayed on a user-friendly web platform: OpenProt.

We used OpenProt to reprocess large-scale affinity purification mass spectrometry data of human proteins. After extensive quality control, integration of alternative proteins revealed a more complex interactome, hinting at their role in pivotal cellular pathways. In parallel, we re-analysed sequencing data for pathology associated variants from 2 studies: one on breast cancer, and one on neurodegenerative diseases. We highlighted mutational hotspots on specific genomic loci corresponding to altORFs, and identified pathological variants synonymous in the reference ORF, but leading to a stop codon in the altORF.

With the aim of igniting discoveries and revealing the real depth of proteomes, novel annotations and associated evidence are made public at www.OpenProt.org

POSTER 115
Using Sub-Ranked Database Matching Scores for Improving the Peptide and Protein Identification Performance

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In MS-base proteomics and peptidomics, identification of the peptide is mainly based on data-dependent acquisition (DDA) approach. However, in the peptidomics and proteomics study, a large portion of the MS/MS spectra acquired by the DDA cannot confidently match to a specific peptide sequence. In additional to the detection of the tryptic peptide in the proteomics study, it is especially difficult to identify endogenous peptides. This is because enzymes for hydrolyzing precursor proteins are mostly unknown. With the use of matched random/decoy sequences to estimate the false-positive discovery rate (FDR), the number and average score of estimated false-positive hits for NES search was higher than the semi-tryptic peptide specific (sTPS) search. For improving the identification performance of endogenous peptide using NES search, the Delta-Score (DS) were employed in our previous study. To understand how DS improves the identification performance, the score distributions of the 1st and 2nd ranked hits were studied. We further developed a new scoring method named confidence scoring (CS), which considered the 1st to 10th matching scores of each spectrum. For the identification of tryptic peptide using NES search, the peptide identification sensitivity of CS was 12% and 2% higher than the Mascot score and DS, respectively. In the sTPS search, the number of the identified peptides of CS and DS was 12% and 25% higher than the Mascot score, respectively. This method does not require complex computational steps and can significantly improve the sensitivity for novel peptide hormone discovery.

POSTER 116
TACO, a database integrating transcriptome alterations, pathway and prognosis in cancers

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While numerous cancer sequencing data is already publicly available, to systematically dig out meaningful correlations from those data is still challenging for cancer biologists who do not possess related computer skills. Here, we downloaded all the expression levels of miRNA and mRNAs from firehose and build a database, Transcriptome Alteration in Cancer Omnibus (TACO), which aims to link the transcription alterations and transcriptome regulatory network with downstream pathway alterations and clinical outcomes in 22 cancer types. TACO provides: 1. Volcano plots for selection of significantly differentially expressed miRNA or mRNA (DEGs) 2. Correlations between the expression levels of miRNA and their targets 3. Gene Set Enrichment Analysis for significantly altered pathways 4. KEGG pathway enrichment analysis for selected or uploaded gene list 5. Gene Ontology (GO) enrichment analysis for selected or uploaded gene list 6. Survival-related signature selection from transcriptome. DEGs selected on TACO or a gene list uploaded by user can be used for the pathway analysis and survival prediction model construction. These signatures have great potentialities for patient stratification and treatment decisions in later clinical applications. We believe that TACO, as an integrated transcriptome database, comes with a user-friendly query interface will be invaluable for cancer biologist to investigate the transcriptome regulatory networks alterations and the following clinical outcome in cancers systematically.

POSTER 117
Development and Validation of The multi-marker panel for Diagnosis of Pancreatic Cancer Using Deep Learning Algorithm

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Proteomics has emerged as a new technology to diagnose diseases. Conventionally, proteomics data analysis for diagnosis is done using machine learning based classification methods. However, it remains unknown whether deep-learning, a class of increasingly popular statistical method, is suitable for classifying proteomics data. Here we use a cohort of 401 pancreatic cancer (also known as pancreatic ductal adenocarcinoma, PDAC) patients, and 458 healthy controls to test the accuracies of a deep-learning (DL) algorithm, as well as five other widely used machine learning methods: random forest (RF), support vector machines (SVM), logistic regression (LR), k-nearest neighbors (KNN) and naïve bayes (NB). Our experiment found that DL had the highest predictive accuracy in classifying PDAC patients from control compared to other machine learning algorithms. DL also performed best when analyzing sample sets from three different institutions. These results corroborate that implementation of deep learning algorithms can improve the performance of diagnostic assays in clinical settings.

POSTER 118
Impact of Different Quantitation and Normalization Algorithms on Proteomics-based Biomarker Discovery – A Case Study on Lung Cancer Tissue Data

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Computational analysis of mass spectrometry data generated from clinical tissue proteomics experiments can help identify potential biomarkers for cancer detection, prognosis, and diagnosis. Despite a great deal of effort being made in data analysis for biomarker discovery, there is rather limited endeavor on analyzing the impact of applying different quantitation algorithms, algorithmic parameters, and normalization approaches. To address the issue, we used 8 batches of TMT-10 labeling data sets acquired from tumor and adjacent normal tissue samples of 32 early-stage lung cancer patients. These data were subject to Mascot and SEQUEST search through Proteome Discoverer. Identified proteins were then quantified with Multi-Q 2 using different normalization approaches and ratio calculation algorithms. Furthermore, the effects of purity correction and ratio compression, a common phenomenon caused by co-eluting peptide ions in the precursor isolation window, were also analyzed.

Potential biomarkers are usually chosen from proteins having significant fold changes. Our analyses reveal that the list of potential biomarkers can vary drastically when employing different normalization methods for quantitation of large-scale proteomics experiments. Furthermore, the selection of different ratio calculation algorithms can also lead to generally moderate changes of protein ratios but possibly critical changes of low-abundance proteins of interest. Therefore, both are two major factors affecting the output of biomarker discovery. We also note that isotope purity correction on reporter ions can sometimes produce extremely large fold changes which seem unrealistic. The situation also occurs when performing a correction algorithm for ratio compression. For both cases, it is crucial to select an appropriate protein ratio calculation algorithm for remedying the effect of possibly generating overestimated fold changes.

POSTER 119
Improved survival prognostication of node-positive malignant melanoma patients utilizing shotgun proteomics guided by histopathological characterization and genomic data

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Metastatic melanoma is one of the most common deadly cancers, and robust biomarkers are still needed. Here we combine the protein expression of 111 melanoma lymph node metastases with in-depth histopathological analysis, clinical data, and genomic profiles to discover novel protein biomarkers. Some of the prognostic proteins have not previously been linked to melanoma while others exhibit unexpected relationship to survival, which exemplifies complexity of melanoma and the need for further large scale multi-omics studies.

We show that the protein expression of the metastases is related to the survival of the patient using a PLS-Cox

model. The expression of 1306 proteins from each sample is projected onto a latent variable which is used as input

to our Cox model. Furthermore, we cluster our samples both in a fully unsupervised way as well as in a supervised way

that encourages clustering with respect to survival. As expected, the supervised clusters clearly separate the

samples in terms of survival (p=0.000066). The unsupervised clusters show weaker but still significant differences in survival (p=0.0028).

Finally, we compare our protein-derived clusters to previously published melanoma subtypes derived from genes profiles and find that while there is some overlap they are largely complementary suggesting that proteomics adds valuable information on top of genomics for metastatic melanoma.

POSTER 120
Improved peptide identification in shotgun proteomics data using an efficient open search engine

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Shotgun proteomics has grown rapidly in recent decades, especially for peptide and protein identification. However, more than 50% of MS/MS data acquired in shotgun proteomics have not been successfully identified. As shown in a number of

studies, unexpected modifications is a major reason underlying the low identification rate, and several other factors also hinder precise peptide identification, *e.g.*, semi- and non-specific digestion, in-source fragmentation and co-eluting peptides in mixed spectra.

We have developed a novel database search algorithm, Open-pFind, to efficiently identify peptides even in an ultra-large search space which takes into account unexpected modifications, amino acid mutations, semi- or non-specific digestion and co-eluting peptides. We re-analyzed an entire human proteome dataset consisting of ~25 million spectra. It took Open-pFind ~5 hours on a 64-core workstation to search all of these spectra. More than one million peptides were identified, which were 86.7% more than those reported previously. The results obtained with Open-pFind demonstrated that the characteristics of MS/MS data vary according to different methods for sample preparation and LC-MS/MS. For example, ~10–20% of cysteines in all identified peptides were not modified by carbamidomethylation as usually expected. Furthermore, cysteine propionamidation was detected at very low levels in the bRPLC fractionation samples but was one of the most abundant cysteine modifications in the in-gel digested samples, which demonstrates the ability of Open-pFind to detect the diverse modification types in different datasets. In addition, 14,064 proteins, corresponding to 12,723 genes, were supported by at least two protein-unique peptides. Only two olfactory receptors were reported by Open-pFind and each was supported by only two protein-unique peptides derived from two PSMs These results demonstrated that open search strategies, as made practical by Open-pFind, will most likely be the preferred tools for large-scale MS/MS data analyses in the future.

POSTER 121
ProteinExplorer: a repository-scale resource for exploration of protein detection in public mass spectrometry datasets

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High throughput tandem mass spectrometry has enabled the detection and identification of over 75% of all proteins predicted to result in translated gene products in the human genome. In fact, the galloping rate of data acquisition and drive for sharing of mass spectrometry data in the proteomics community has led to the current availability of many tens of terabytes of public data in thousands of human datasets. Building on the systematic reanalysis of public datasets, we have shown how this data can be used to build a community-scale spectral library of 2.1 million precursors for over 1 million unique sequences from over 19,000 proteins (including spectra of synthetic peptides). However, it has remained challenging to find and inspect spectra of peptides covering functional protein regions or matching to novel proteins (in which case it is also important to compare with spectra of synthetic peptides). ProteinExplorer addresses these challenges with an intuitive interface mapping tens of millions of identifications to functional sites on nearly all human proteins. In addition, ProteinExplorer facilitates the confirmation of detection of novel proteins by tracking each identification back to the original dataset and mass spectrometry file, and by greatly facilitating the selection and inspection of HPP-compliant peptides whose spectra can be matched to spectra of synthetic peptides. Illustrating this functionality, ProteinExplorer already includes HPP-compliant evidence for 140 novel proteins (with 225 more having only one supporting peptide), 66% of which with peptides matching to synthetic peptide spectra from either the ProteomeTools or Bioplex datasets. Finally, ProteinExplorer further facilitates the progression towards comprehensive, community-driven detection of novel proteins by allowing users to rate spectra and to contribute spectra to a community library of peptides mapping to novel proteins but whose preliminary identities have not yet been fully established with community-scale false discovery rates and synthetic peptide spectra.

POSTER 122
Fast and Efficient Mapping of Peptide Sequences and their Variants to Proteome Databases Using Full Inverted Indices

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Peptide sequences derived from MS/MS spectra, whether via database searching, spectral library matching, or de novo sequence analysis, need to be mapped to the reference proteome in order to determine the protein content of the sample being analyzed. Proteotypic peptides are of special interest in confidently identifying proteoforms as well as for generating assays for targeted experiments such as SRM. Naturally occurring variants in protein sequences exacerbate this mapping issue.

While the nascent PEFF format allows for the representation of such variants, software is needed to efficiently map observed sequences to all possible variants. Here we present an approach and software tools to perform this mapping.

One common approach used by search platforms is to create a full inverted index data structure, where a search term is indexed along with a position into the document that contains it. We have taken advantage of this method and modified it for use with protein sequence databases.

An index was generated for a database of 42,164 Homo-Sapiens proteins exported from neXtProt in PEFF format, using sequence segments of five amino acids in length. The index is written as flat text files that contains: a header section with information about the file and parameters used in the indexing; a list of proteins with aliases to save disk space; a meta-index of in-file byte offsets to landmark positions in order to speed up finding of desired segments; the segments index.

The mapping tool takes peptides from a list as input, and maps them to all matching protein sequences (considering all PEFF variants) using the segments index. Hundreds of peptide sequences can be mapped in a fraction of a second, and all 1.4 million sequences from PeptideAtlas in a few minutes. The tool also supports wildcards and fuzzy matching.

POSTER 123
ProDiGy^{KDS}: towards the omics datasets analyses of precision medicine based on the PMap

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ProDiGy^{KDS} is a professional omics datasets knowledge discovery gateway, which is designed for exploring, visualizing,

and analyzing omics profile datasets from organ, tissue or cell lines based on PMap (precision medicine knowledge map).

A friendly interface enables researchers to interactively search, filter and download datasets across samples. And several tools are integrated to provide data visualization, including CAPER, UbiBrowser, Pathview, SuperHeatmap, Network-view and Hierarchical network. And a galaxy system is used to integrate the database, data analyses and data presentation seamlessly. ProDiGy^{KDS} also established a MeSH ontology-based gene annotation and enRichment analysis systEm (MORE). For the gene list submitted by users, MORE will search co-occurrence gene-MeSH term relations in PubMed, and further utilize hypergeometric distribution to identify significantly enriched MeSH terms.

The intuitive Web interface of the portal not only makes omics datasets analyses accessible to researchers and clinicians freely and handily, and also thus facilitate medical and biological discoveries in precision medicine.

POSTER 124
Evaluation of protein-protein interaction detection methods as a source of capturing domain-motif interactions

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One of the main pursuits in proteomics is to understand the complex network of protein-protein Interactions (PPI) that underpin biological processes. Two major classes of PPI are domain-domain interactions (DDI) between globular proteins, and domain-motif interactions (DMI) between a globular domain and a short linear motif (SLiM) in its partner. Advances in high-throughput experimental techniques have been applied at large-scale in an attempt to characterise the interactome of various organisms. However, PPI networks being identified by these high-throughput experiments have low resolution as compared to low-throughput technologies, such as protein co-crystallization. Furthermore, large-scale approaches may be poor at capturing low affinity or transient interactions, which includes the majority of known DMI. To date, several studies have been conducted to identify how well these PPI data can capture protein complexes, but the ability of high-throughput PPI-detection methods to capture DMI remains a largely unanswered question.

To help system biologists choose appropriate methods for predicting different types of interactions, we conducted a comprehensive comparison study on existing high-throughput PPI datasets. We have integrated PPI data, SLiM predictions, domain compositions and known SLiM-domain binding partnerships to identify possible DMI and DDI within interactomes. We identify PPI data that are enriched for DMI or DDI versus a background expectation generated by randomising the PPI within the network. Despite returning relatively few experimentally validated DMI when compare to interaction databases, we present evidence that high-throughput PPI data is enriched for DMI and thus potentially useful for the prediction of novel SLiMs. We discuss the relative merits of co-fractionation followed by mass spectrometry (CoFrac-MS), affinity purification coupled mass spectrometry (AP-MS), and yeast two hybrid (Y2H) for capturing DMI and DDI, as well as potential quality versus quantity trade-offs in DMI prediction.

POSTER 125
Reactome Multi-Scale Pathway Visualisation

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Reactome (https://reactome.org) is a free, open-source, open-data, curated and peer-reviewed knowledge base of biomolecular pathways, currently covering 2,106 Pathways; 10,712 protein coding genes; 11,302 reactions; 27,452 literature references and 1,800 small molecules. Pathways are arranged in a hierarchical structure, allowing the user to navigate from high level concepts like immune system to detailed pathway diagrams showing biomolecular events like membrane transport or phosphorylation.

The Reactome curation strategy focuses on the annotation of “normal” pathways in human. However, we increasingly annotate disease-specific pathway modifications, grouped in three major classes: loss of function (typically metabolic disease phenotypes), gain of function (typically cancer phenotypes), and host-pathogen interactions.

Here, we present new developments in the multi-scale Reactome visualization system that facilitate navigation through the pathway hierarchy and enable efficient reuse of Reactome visualizations for users' own research presentations and publications.

For the higher levels of the hierarchy, Reactome now provides scalable, interactive textbook-style diagrams in SVG format, which are also freely downloadable and editable. Repeated diagram elements like 'mitochondrion' or 'receptor' are freely available as a library of graphic elements at https://reactome.org/icon-lib. Detailed lower-level diagrams are now downloadable in editable PPTX format as sets of interconnected objects, as well as in standard png format.

POSTER 126
Proteostasis Network in NAFLD Mice from heavy water metabolic labeling and LC-MS.

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Technological advances in MS combined with metabolic labeling are enabling researchers to profile *in vivo* proteome dynamics of a large number of proteins. These experiments provide opportunities for interrogating the networks and pathways controlling cellular proteostasis.The inferences from such models and networks will be crucial for designing specific interventions and perturbations of dysfunctional networks.

However, the analysis of the proteome time course data of stable isotope labeling poses computational challenges in bioinformatics, statistical data processing and modeling. No methods exist for modeling networks and pathways that are involved in proteostasis based on the data sets from high-throughput proteomics experiments.

We will discuss the dysregulation of proteostasis networks of the murine liver proteome in a model of Non-Alcoholic Fatty Liver Disease (NAFLD). Our studies show that the changes in

the proteome dynamics NAFLD are complementary to those mediated by of unfolded protein response. Our data shows that the attenuation of protein synthesis is modulated via decreased stability of ribosomal proteins in 40S ribosomal subunit.

POSTER 127

The Human Proteome as of 2018, from the HUPO Human Proteome Project

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The global proteomics community has made significant progress on the two overall goals of the HUPO Human Proteome Project (HPP): (1) completing the protein parts list, with annual updates of a Draft Human Proteome; and (2) making proteomics a well-integrated complement to genomics, transcriptomics, epigenomics, and metabolomics throughout life sciences research. Here we report updated metrics for the human proteome based on neXtProt 2018-01-17, PeptideAtlas 2018-01, and the Human Protein Atlas (v6, 2017). There are now 17,470 neXtProt PE1 confirmed proteins (89% of predicted PE1-4 proteins), up from 13,664 in 2012-12 and 17,008 in 2017-01. Conversely, the number of PE2,3,4 "missing proteins" has decreased to 2186 from 6400 in 2012 and 2579 in 2017, using the stringent HPP Guidelines v2 (2016): see

https://www.nextprot.org/proteins/search?mode=advanced&queryId=NXQ_00204. In the fifth annual *Journal of Proteome Research* HPP Special Issue (2017-12), 73 additional missing proteins were identified by: further focus on testis and sperm-specific expression; enrichment of less abundant proteins with ProteoMiner beads plus Triton-X solubilization of membrane proteins; use of less studied tissues, like bladder and kidney; and matching of spectra attributed to pairs of "stranded proteotypic peptides" in GPMdb and/or PeptideAtlas to spectra available in SRM Atlas. We are stimulating progress on functional annotation of the Human Proteome, including 1260 unannotated PE1 proteins (uPE1): **https://tinyurl.com/upe1proteins**, as well as structural, splice, and PTM variants of all proteins. HPP-related developments useful for broad life science research include the SRMAtlas with spectra for proteotypic peptides of nearly all predicted human proteins, lists of the most published proteins in organ-specific and biofluid research detectable and quantifiable with targeted proteomics, and the emergence of DIA-SWATH-MS. Manuscripts for the HPP 2018 JPR special issue that are on-line by HUPO2018 will be highlighted.

POSTER 128

Clustering Tandem Mass Spectra using Locality Sensitive Hashing

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Projects such as CPTC generate large amount of tandem mass (MS/MS) spectra that are often highly redundant (i.e., containing many spectra from same peptides). It is computing intensive to identify massive MS/MS spectra using peptide search engines such as Mascot, SEQUEST and MSGF+. To save the computation time, a fine-curated database of proteins is provided for database search, in which usually peptides from *in silico* digestion of proteins are used, and hence spectra from peptides containing mutations or post-translational modifications (PTMs) are not identified.

Clustering MS/MS spectra can significantly reduce the redundancy in large spectra datasets by replacing similar spectra (e.g., from same peptides), with the *consensus* spectrum built from these similar spectra. As a result, much fewer consensus spectra are retained for database searching, leading to acceleration of the peptide identification process. Furthermore, the consensus spectra with improved signal-to-noise ratio can improve peptide identification results.

We here present an open-source software msCRUSH, which was implemented based on locality sensitive hashing (LSH) to speed up spectra clustering. msCRUSH first bins potential similar spectra using LSH then merges similar spectra, whereas conventional mass spectra clustering algorithms compare spectra against each other, which is very time consuming for large mass spectra datasets. When tested on a massive spectra dataset containing 18.4 million spectra (including 11.5 million spectra of charge 2+), msCRUSH runs 7.6-12.1x faster than the state-of-the-art PRIDE Cluster, achieving higher sensitivity and comparable accuracy. Using the consensus spectra reported by msCRUSH, 5% and 4% more unique peptides, respectively, can be identified compared to the identification results from the raw spectra at the same false discovery rate (1% FDR), using common spectra search engines MSGF+ and Mascot.

POSTER 129

The UniProt Knowledgebase – from genome to proteome to function

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The UniProt knowledgebase (www.uniprot.org) is the key reference resource used by proteomics scientists. UniProtKB provides the high-quality, non-redundant sequences which underpin the mapping of peptides to proteins by database search engines and delivers Reference protein datasets for a broad taxonomic range of species. Protein identifications can be made at the isoform or post-processed chain level whilst observed sequence features can be mapped to post-translational modifications (PTM) sites, functional domains and conserved regions of sequence manually captured by UniProtKB biocurators. Direct links between protein and genomic sequence data allows researchers to explore a wealth of information, including identifying variations relevant to disease, and predicting their functional consequence by aligning, for example, with catalytic sites or binding regions.

For many years UniProt has been working with the proteomics community to integrate proteomics data into the UniProtKB database, thus providing experimental supporting evidences for the existence of specific isoforms, variants and PTMs

predicted from genomic sequence. The database works closely with the HUPO Proteomics Standards Initiative workgroups and supports collaborations which have grown out of this work, such as ProteomeXchange (http://www.proteomexchange.org/) and the IMEx Consortium (www.imexconsortium.org), cross-referencing data from these repositories within UniProtKB. The ProtVista visualization tool enables the graphical representation of experimental proteomic and genomic variation public datasets aligned with manually curated or computationally predicted protein sequence features in the UniProt Knowledgebase (e.g. www.uniprot.org/uniprot/Q8NI35#showFeaturesViewer). Additionally, high-confidence binary protein interactions are imported from the IMEx databases and displayed in an adjacency viewer (e.g www.uniprot.org/uniprot/P10074#interaction).

UniProt provides mappings to its Reference proteomes via the website, the FTP site (ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/knowledgebase/proteomics_mapping/) and programmatically by means of a new RESTful API (www.ebi.ac.uk/proteins/api/doc/) which also provides many additional types of data such as genomic coordinates.

POSTER 130
Global detection and quantification of modified ribonucleosides from RNA using a HPLC coupled mass spectrometry approach

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Epitranscriptomics has emerged in recent years to investigate the role of RNA modifications and might provide a crucial link between epigenomics and proteomics in understanding many fundamental cellular processes. Using a Lumos™ Tribrid™ Mass Spectrometer coupled with a HPLC system, this study demonstrates a robust workflow to detect/quantify ribonucleosides (RNs) in E. coli tRNA and mammalian total RNA using standard RNs.

Hydrolyzed RNA and standard RNs were resolved on a Hypersil Gold aQ column using a linear 30-minute HPLC gradient with 80% acetonitrile as mobile phase. Lumos™ was operated in positive ion mode with a scan range of 50-400 m/z and a 40% HCD collision energy along with a 60% HCD collision energy in MS^3 scan mode. Raw data were imported into Skyline 3.7 along with a transition list for interpretation and quantification. Calibration curves were generated using standard RNs with serial dilutions in quadruplicates. Calculated R-squared for individual calibration curve was >0.95 with analyte concentration ranging from 5-100 ng/ul. Quantification using the generated calibration curves was validated (when available) by analyzing a nucleoside test mix (Sigma-Aldrich) containing 12 nucleosides with known concentration.

The detection level ranged from undetectable to 0.7ng and 4.7ng out of 60ng of total mammalian RNA for the four 2-O'-methylated and canonical RNs, respectively. These ranges were from 0 to 0.7ng and 7.1ng out of 25ng E. coli tRNA suggesting a >20-fold increase in detection limit when total RNA was fractionated. Using this approach, coeluting positional isomers might also be distinguished using an "in-house" algorithm to interpret extracted MS^3 data provided that the pair of MS^3 fragment ions were different in mass. Further method development would be required to expand on the number of detectable and quantifiable modified ribonucleosides in hope of providing a comprehensive epitranscriptomic profile of RNA modifications in different cellular states.

POSTER 131
Batch effects in large-scale proteomic studies: diagnostics and correction

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Recent technical advances in mass spectrometry based proteomics have significantly increased robustness, sample throughput, and sample to sample reproducibility to a degree that large-scale studies consisting of hundreds of samples are becoming routine. Whereas increased sample numbers facilitate statistical association of proteome states with phenotypes, they generally come at the price of introducing batch effects, that decrease the power to identify the underlying biological variance. We will show how batch effects can be diagnosed and what approaches can be taken to correct for them.

Bottom-up proteomics is a multistep process, consisting of biosample collection, protein digestion, and mass-spectra acquisition. Using four large proteomics datasets, we show how to diagnose the contribution of each of these steps to the overall experimental batch effect. We find that proteome level effects can be efficiently visualized/diagnosed with tools such as boxplots, hierarchical clustering, and Principal Variance Component Analysis. Furthermore, we also identify a subset of technical artifacts that can only be diagnosed at the single peptide level.

We then move on to show how to guide batch effect correction using information gathered at the diagnostic stage. Here, we find that batch effects originating from discrete factors, such as instrument or protein digestion batches, can be efficiently corrected using standard approaches borrowed from the genomic field. Additionally, we report the presence of mass-spectrometry specific non-linear trends associated with sample running order. To address these, we propose a new correction procedure based on LOESS trend fitting.

Finally, we demonstrate, how the same diagnostic tools can be used to assess the performance of the applied corrections and how these approaches improve signal in the samples under investigation.

POSTER 132
ProteomeTools: Update of the world's largest synthetic peptide and data resource for human proteome research

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The ProteomeTools project (www.proteometools.org) addresses the growing need for synthetic peptide standards and “ground truth” datasets. LC-MS data, spectral-libraries, developed tools and – within reason – reagents generated in this project are freely available to the community. Here we report on the latest progress, availability and usefulness of the acquired data and showcase new tools of interest for proteome scientists.

The physical ProteomeTools Peptide Libraries (PROPEL) now comprise >1 million peptides, covering essentially all (98.5%) human proteins and protein isoforms as well as more than 10 focused libraries for e.g. HLA Class I peptides, single amino acid variant and post-translationally modified peptides (e.g. phosphorylation, acetylation, ubiquitination). In addition to measurements in 11 fragmentation modes on an Orbitrap Lumos, data was also acquired on QTOF and ion mobility instruments and we recently added measurements of peptides derivatized by tandem mass tags (TMT) and dimethyl labels to the roster.

We highlight the use of the PTM libraries by systematically characterizing 21 different PTMs for their LC-MS properties, discovering novel diagnostic ions for lysine PTMs. We point out, that site localization for phosphorylated peptides can be improved when utilizing fragment ion intensity information. Our large HLA peptide library can be used to validate the growing publicly available data in this area. The expanded ProteomeTools Spectrum Compendium (PROSPEC) was used to train PROSIT, a generic artificial neural network, for the prediction of retention time indices and fragment ion intensities (R>0.98 for both) for both unmodified and modified peptides. We demonstrate the use of *in-silico* generated spectral libraries for DDA and DIA/SWATH data analysis for multiple organisms and proteases. We implemented PROSIT in ProteomicsDB to enable the generation of custom spectral libraries on-demand from deposited experimental, synthetic peptide or predicted fragment spectra. All data, spectral-libraries and tools are available via PRIDE, ProteomeTools.org and ProteomicsDB, respectively.

POSTER 133
Mutated Nucleotide and Amino-acid sequence Generator (MuNAGe): novel proteogenomics software to generate sample-specific database

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In proteomics, the mass spectrometric protein identification relies on the search against public proteome database, such as Swiss-Prot. Thus, the mutated proteins arose from sample-specific genomic aberrations cannot be identified, even when mass spectrometry detect the ion peaks derived from them. The proteome data reflecting genomic aberrations of tumor tissues should be useful resources for biomarker development and drug target identification, because of their high specificity. Genome and transcriptome data of samples and software to generate sample-specific database are required to address this problem. With this notion, we developed a novel proteogenomic software; Mutated Nucleotide and Amino-acid sequence Generator (MuNAGe). MuNAGe allows us to construct sample-specific database for mass spectrometric protein identification. The performance of MuNAGe was evaluated using the data of whole-genome sequencing, RNA sequencing, and LC-MS/MS of three lung cancer cell lines. We found that thousands of proteins including those from Vogelstein's cancer-related genes were identified when we used the database made by MuNAGe. The identified proteins included those which were not detected with conventional mass spectrometric protein identification using Swiss-Prot. We conclude that MuNAGe is a powerful took to investigate the cancer-associated proteomic alterations. MuNAGe has potentials to integrate the multi-omics data such as those of miRNA expression, and posttranslational modifications. Thus, by developing MuNAGe and integrating more relevant data, we will be able to identify the proteomic features which can be used for biomarker development and drug target discovery.

POSTER 134
Identification of cross-linked peptides and oxidation products in CRP exposed to UV and Rose Bengal-mediated oxidation

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Background:

The cyclic AMP receptor protein (CRP) is a global regulator of gene expression in *E. coli* and other Gram negative bacteria. It binds as a dimer with DNA sites and with RNA polymerase to activate transcription.

The hypothesis of this work is that CRP, and its complexes with cAMP and DNA, may be a critical target for singlet oxygen and UV light induced damage, and that light absorption by the protein may give rise to both site-specific DNA damage and protein cross-links.

Methods:

CRP protein was exposed to either Rose Bengal or UV light. Bruker Impact II ESI-QTOF (Bruker Daltonics) and Orbitrap Fusion (Thermo Scientific) mass spectrometers were used to analyze oxidation products. An 18O-labeling strategy was used for identification of cross-linked peptides. Proteins were digested with trypsin in the presence of either 16O water or 18O water, mixed and analyzed using LC-MS/MS. Data analysis was carried out both by manual spectral interpretation and by using different software including MaxQuant, GPMAW and MassAI.

Results:

The data obtained in this work indicate that UV light and Rose Bengal-mediated oxidation markedly affect CRP structure.

Three different types of cross-linked peptides have been identified, including di-tyrosine, histidine-arginine and lysine-tyrosine cross-links. The presence of these cross-links is indicated by the 8 Da shift in the MS spectra due to the 18O labeling and also by the shift in the cross-linked peptide mass due to the nature of these particular cross-links.

Conclusions:

The CRP system is highly susceptible to UV light and Rose Bengal-mediate oxidation, resulting in protein cross-linking and CRP-mediated site-selective damage to the DNA to which the complex is bound. These exciting observations provide a new paradigm for modulating gene expression and growth of bacteria and also potentially human tumor cells.

Keywords:

LC-MS/MS, 18O labeling, free radical oxidation, cross-linking

POSTER 135
MOLECULAR ARCHITECTURE OF THE ANTIOPHIDIC PROTEIN DM64 AND ITS COMPLEX WITH MYOTOXIN II FROM BOTHROPS ASPER VENOM

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The natural resistance to Viperidae envenomation observed in the South American marsupial *Didelphis aurita* can be partially explained by the presence of circulating DM64, a specialized toxin scavenger glycoprotein of 64 kDa. It specifically targets myotoxic phospholipases A2, which account for most local tissue damage associated with snake bites. To be useful as a potential new lead structure for drug development, the understanding of its structure-function relationship is paramount. This paper investigated the noncovalent complex formed between native DM64 and the 14 kDa myotoxin II isolated from *Bothrops asper* venom (PDB code 1CLP). Analytical ultracentrifugation, size exclusion chromatography and/or small-angle X-ray scattering data indicated that both proteins are monomeric in solution and form a 1:1 stoichiometric complex. Each of the five immunoglobulin-like domains of DM64 was individually modeled using the I-Tasser software suite. Then, distance constraints generated by chemical cross-linking of the complex, coupled to high-resolution mass spectrometry, were used to dock the DM64 domains (Rosetta software suite), using the crystal structure of myotoxin II as a reference point. Based on this hybrid strategy, a refined three-dimensional model of the inhibitor is proposed, in which the relative position of the domains is in good agreement with the experimental data. Additionally, inter-protein cross-links provide a first glimpse of the inhibitor´s regions that may be implicated in toxin-antitoxin interaction, showing the involvement of the third and fifth domains of DM64 and the C-terminus of the toxin. This study highlights the potential of integrative structural biology for furthering our understanding of the structural features that govern important protein-protein interactions.

POSTER 136
Comprehensive phosphoproteomics analysis of drug-treated cardiomyocytes using versatile DIA workflows

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Introduction:

Data-Independent Acquisition (DIA) has recently emerged as a powerful technique for the analysis of large-scale proteomics data. Here, we would like to explore the potential of different database searching approaches recently introduced in the DIA community for the investigation of phosphoproteomics data sets. Therefore, we challenged cardiomyocytes with doxorubicin (DOX) and DMSO as a control in five biological replicates for 20 min and 120 min before lysis and investigated the perturbed phosphoproteome by DIA.

Methods:

Untreated, DOX and DMSO-treated phosphopeptides extracts were analysed by Liquid Chromatography-Mass Spectrometry (LC-MS) on a Q Exactive™ HF instrument (Thermofisher Scientific) in DIA mode. Raw MS data were imported into Spectronaut™ Pulsar X software for phosphopeptide identification/quantification, where sample specific library (directDIA) and hybrid library (project specific library combined with directDIA)-based workflows were evaluated.

Results:

In total, 25 phosphoproteome samples were measured using a DIA acquisition method that allowed optimal records of data points per peaks at MS2 level. For directDIA, raw files were directly searched against the fasta human protein database and led to the identification of 15189 phosphopeptides and 2279 phosphoprotein groups (Qvalue « 0.01), resulting in a data completeness of 81.5% at the precursor level. For the hybrid library-based workflow, 20777 phosphopeptides and 3002 phosphoprotein groups were identified and quantified with a data completeness of 75.6%. Overall, the overlap between both workflows was 56.8% and 75.2% at the phosphopetide level and the phosphoprotein level, respectively. When analysing enriched gene ontology (GO)-terms among the significantly regulated phosphoproteins, both DIA workflows identified similar cellular functions, however for the hybrid library-based workflow more pathways could be identified.

Conclusion:

Versatile DIA workflows have shown to provide complementary biological insights for unravelling the phosphoproteome of drug-treated cardiomyocytes and can be easily applied to any other human cell systems.

POSTER 137
DISCO: Exploration of DIA Data Using Data-Driven Analysis

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Data Independent Acquisition (DIA) is a mass-spectrometry technique that promises to become viable for clinical biomarker discovery and quantitation. DIA generates a mass-spectrometry profile of each sample by fragmenting and measuring all ions in isolation windows spanning the effective m/z range of the instrument. Fragmentation is applied to all eluting peptides in the effective range, whether observed as a

signal in an MS^1 scan or not. Software tools for analysis of DIA data are categorized as either assay-driven or data-driven, with DISCO falling into the latter category. We apply both categories of tools demonstrating the utility of the approaches to DIA analysis.

DISCO implements *in silico* a DDA-like method to DIA data post-acquisition. DISCO extracts correlating signals in RT space from the MS^1 and MS^2 spectra. Signals are extracted based on peptide features that are detected by the internal use of the Hardklör API in MS^1 and MS^2 scans. Separately, DISCO is able to extract signals for features identified with other tools, e.g. DIAUmpire. Given Hardklör or DIAUmpire peptide feature targets, DISCO identifies the fragments that correlate with target signals and reports results as mzML data.

Generally, assay-driven analysis tools look for evidence of specific signals within DIA data, usually based on peptide spectral libraries. In contrast, data-driven analysis tools, such as DISCO and DIAUmpire, create processed spectra based on correlating signals in DIA data. The generated spectra are then be searched using standard search engine algorithms like DDA data.

Furthermore, we've applied DISCO to DIA data in combination with other TPP validation and quantitation tools to identify and quantify peptides, without the need to generate DIA spectral libraries, and inferred differential peptides and proteins within each of the analyzed samples. This approach can be used to confidently identify features of significance and clinical relevance in large biobank studies.

POSTER 138
Strategies and challenges for big clinical SWATH-MS dataset analysis

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Diabetes is a metabolic syndrome which has become a global burden. Around 8% of the population gets affected by this complex disease, of which 90% suffers from type-2 diabetes (T2D) that is caused by several risk factors such as diet, genetic and environment. Early diagnosis with predictive and preventive measures can provide insight for the development of potential candidates for the disease. However, current strategies are not robust enough to handle big clinical data to infer biological relevant information. SWATH-MS or data independent acquisition (DIA) provides high accuracy quantitation and deeper depth of proteome coverage in label free proteomics, when coupled with stable and robust analytical chromatographic methods. Although, SWATH-MS based software tools encounter restriction in handling, processing and analyzing such large clinical datasets. Here we implemented both library based and spectrum based approaches to identify significant candidates in large clinical studies. The workflows enable processing of hundreds of samples in an automated unattended operation with superior high performance characteristics at both acquisition and data analyzing stages. Steps to achieve these types of results and operational specifics will be discussed.

POSTER 139
Accelerating DIA Studies to Extend Workflow Utility, Using Fast Microflow LC Gradients

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Background: Microflow LC has been used increasing in quantitative proteomics in combination with SWATH® Acquisition, to provide better robustness and higher throughput when measuring larger sample cohorts. With higher flow rates, sample loading, trap/column washing and equilibration and gradient formation are all accelerated, allowing much faster run times to be achieved. Here, the impact of gradient length on protein quantitation results with DIA was explored.

Methods: Microflow LC was performed on the TripleTOF® 6600 System using the nanoLC™ 425 system plumbed in microflow mode. Trap-elute workflow was used and a range of gradient lengths from 5 – 45 mins was explored, and key acquisition parameters for SWATH® Acquisition were varied to optimize for the much faster run times. Data was processed with SWATH 2.0 microapp in PeakView® Software 2.2 and results were analyzed using the SWATH Replicates Template.

Conclusions: Using complex digested cell lysates, SWATH experiments were performed using gradient lengths ranging from 5-45 mins and protein quantitation results were assessed. Fast MS/MS acquisition rates were found to be critical because this enabled more smaller variable Q1 windows to improve S/N for quantitation. Even with the fastest gradients, methods with 60-100 windows with very fast accumulation times of 15 msec improved results. As expected, total # of protein quantified decreased when shortening the gradient from 45 to 5mins. For example, with the 10min gradient, the peptide and protein ID rates dropped by about 60 and 70% respectively relative to the 45min gradient for the three matrices tested (on 2 instruments). That said, the 5min gradient method still enabled the quantitation of >1600 proteins and >4000 peptides in a total run time of 18 mins.

The full optimization results will be presented.

POSTER 140
Simplifying the Use of Ion Libraries During Data Processing of Data Independent Acquisition Proteomics Data

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As the use of data independent acquisition (DIA) grows in proteomics research, the need for improved data processing workflows increases. The most common data processing workflow is to use spectral ion libraries to drive targeted extraction of peptide / fragment areas from the data, using the m/z and retention time information contained in the library. Increasing the size and quality of the ion library has been shown to increase the proteins reliably quantified from a dataset. Retention time (RT) correlation between ion library and

the dataset is another key factor that determines quality of data extraction. Two algorithms were explored to simplify the data extraction workflow for SWATH® Acquisition data within the OneOmics™ Project cloud processing pipeline: the automatic merging of related ion libraries followed by auto RT calibration.

POSTER 141

Accurate benchmarking of acquisition parameters and processing softwares for Data Independent Acquisition analyses of proteomic samples

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In the past few years, proteomic workflows using Data-Independent Acquisition (DIA) have emerged as a promising technology to allow a deep coverage and a highly reproducible quantification of protein samples, and overcoming the stochastic effect of Data-Dependent Acquisition (DDA). Nevertheless, no consensus can be found in the literature neither for data acquisitions nor for data processing: acquisition parameters have to compromise between the window size (sensitivity), cycle time (chromatographic resolution) and mass range (coverage), while softwares have to deconvolute complex and chimeric spectra. Data-Independent Acquisition appears as a powerful tool but still challenging.

In order to accurately benchmark acquisition parameters as well as processing softwares for DIA analyses, we have used triplicates of a complex proteomic standard consists of a *E.coli* whole protein extract background spiked with five known concentrations of 48 human proteins (UPS1 -Sigma) ranging from 10 amol to 50 fmol/ug of *E.coli*. The corresponding protein digests were analyzed on an Orbitrap Tribrid instrument (Thermo Scientific) using four different types of DIA schemes (narrow, wide, overlapped or variable size windows) and the signals from the resulting spectra were extracted by five different softwares (Skyline, Specter, DIA-Umpire, Spectronaut Pulsar and Scaffold-DIA).

The use of known concentrations of human UPS1 proteins in a *E.coli* background allowed us to assess the linearity, sensitivity and reproducibility of each method but also, by ratio comparison between different UPS1 concentrations, to define their specificity (False Positive Rates) and sensitivity (True Positive Rates) for the detection of variant proteins.

Finally, by optimizing acquisition parameters allowing a deep proteome coverage and selecting processing workflows for an accurate and reproducible quantification, we have been able to define the best suitable pipelines for the DIA analysis of complex proteomic samples.

POSTER 142

MS2 chromatograms alignment for improved protein quantification in large-scale targeted proteomics studies

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Liquid chromatography coupled to tandem mass-spectrometry (LC-MS/MS) is widely employed in molecular biology for proteome quantitation. The SWATH-MS (Sequential Window Acquisition of all THeoretical mass spectra) strategy allows researchers to obtain a quantitative proteomics data matrix in a high-throughput manner. However, current approaches suffer from missing quantitative values because of chromatogram misalignment across multiple runs. We have previously described TRIC, the first software for retention time alignment of targeted proteomics data which increases data matrix completeness from 69% to 87% while simultaneously reducing error rates. However, current approaches do not properly account for nonlinear, localized dependencies which make alignment computationally challenging. Hence, the chromatogram alignment has been the major limiting factor in the use of data independent acquisition for high-throughput and quantitative proteomics. Here, we develop an approach which uses a pairwise local alignment of MS/MS-based chromatograms across multiple runs. A dynamic programming based approach is used to provide non-linear retention time alignment for chromatogram pairs of complex mixtures and can also account for localized dependency in the LC-elution output.

We benchmark our algorithm on a set of over 437 manually validated chromatographic peaks; our algorithm typically aligns 94% peaks compared to 87% by global alignment method used in TRIC. Moreover, we have tested our algorithm on a more heterogeneous SWATH data from 24 human-plasma runs. The runs were acquired over a period of six months which introduces an additional complexity in the dataset. Some peptides have their LC-elution order switched across these runs which make peak alignment theoretically impossible for monotonic global methods, while chromatogram alignment is able to align them precisely. However, preferring alignment within a time window of global alignment further improves accuracy and could also be used to correct peak annotations obtained from OpenSWATH. We are also building an R-package for chromatogram alignment for the community.

POSTER 143

Identification, Quantification and Monitoring of Low-Abundance Host Cell Proteins During Monoclonal Antibody Purification

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In recent years, LC/MS-based assays have been adopted as orthogonal techniques to ELISA for HCP analysis due to their flexibility and sensitivity. Here we explored the capabilities of a single dimension chromatographic assay, coupled with mass spectrometry using two different data independent acquisition modes for detection of peptide precursors and their fragments. While in MSE mode all peptide ions produced by the electrospray source are transmitted by the quadrupole analyzer, in SONAR mode the quadrupole slides over the mass range of interest during the time required for recording a single MS-spectrum by the TOF-analyzer. Co-eluting precursor ions with different m/z are separated during the rapid quadrupole scan and their corresponding fragmentation spectra are acquired using an identical quadrupole separation. Mass spectra are recorded with high-resolution (~ 25,000) for precursors/fragments.

The first step of the HCP identification and quantification workflow is the HCP Discovery Assay performed in SONAR mode using extensive (90 min) peptide separations. Following data processing with Progenesis QI for proteomics 4.0, the HCPs are identified by a proteome-wide database search. In addition, SONAR MS/MS fragmentation spectra can be assembled into spectral libraries, containing peptide

precursors, charge states and retention times. In the second step of the HCP workflow, additional HCP samples resulted from the purification of the same biopharmaceutical, are analyzed by higher–throughput HCP Monitoring Assays employing data-independent MSE acquisition with 30 min peptide separations. The LC/MSE dataset is then searched against the spectral library for HCP identification and quantification at every step during biopharmaceutical purification. An antibody product, initially purified by Protein A affinity chromatography was further purified by SCX (strong-cation exchange) chromatography using different elution conditions. Two HCPs and 4 spiked protein standards were identified across 5 mAb preparations (one Protein A and 4 SCX fractions). The sensitivity of both assays (Discovery and Monitoring) was 10 ppm.

POSTER 144

Comparison of Quantitative Reproducibility Between DDA Precursor and DIA Fragment Quantification Techniques

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A common LC-MS/MS workflow for unlabeled proteomics experiments relies on data-dependent acquisition (DDA) and precursor intensity values for quantification. However, inaccurate quantitative results due to interference from closely-eluting peptides is a fundamental concern. Data-independent acquisition (DIA) coupled with fragment-based quantification has the potential to alleviate this problem. To compare the performance of these two methods, we analyzed multiple replicate injections of a HeLa digest using precursor quantification from DDA data and fragment-based quantification from DIA data.

Replicates of a commercially available tryptic HeLa cell digest were analyzed by HPLC-ESI-MS on a Thermo Fisher Orbitrap Fusion Lumos mass spectrometer. DDA data were collected using the “top speed” scan strategy. For DIA, separate injections over six limited precursor m/z ranges and 4-m/z MS2 windows were used to build a DIA chromatogram library. Experimental DIA data were obtained for precursors from m/z 390 - 1015 and sets of 12-m/z MS2 windows. DDA data were searched against the UniProt Human FASTA database using Mascot version 2.6.0. Precursor intensity values were calculated using Mascot Distiller version 2.6.3.0. Mascot search results were loaded into Scaffold 4.8.4 for visualization and probability assignments, and Scaffold perSPECtives version 2.1.0 for quantification. Scaffold DIA version 1.0.1 was used to create a narrow-window DIA chromatogram library, to search the wide-window HeLa experimental data and perform fragment-based quantification.

This study compared the coefficients of variation across technical replicates at both the peptide and protein levels based on DDA precursor intensity values and DIA fragment intensities. Our results indicated that quantitative variability among replicate injections was markedly reduced for fragment-based quantification of DIA data compared to precursor intensity quantification for DDA data.

POSTER 145

Pros and cons of isobaric labelling quantification and label free single shot DIA

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Label free quantification (LFQ) and isobaric labelling quantification (ILQ) are the two most popular quantification workflows in discovery proteomics. Here, we wanted to compare the two workflows with various analysis pipelines. For ILQ, we chose TMT 10-plex, for LFQ we chose single shot data-independent acquisition (DIA). Data acquisition was performed in two laboratories: at Leibniz Institute of Ageing (FLI) and at Biognosys (BGS).

We generated a gold standard set of ten samples composed of a complex mouse cerebellum background spiked with the UPS2 protein standard (Sigma) in five different concentrations. LC-MS data was acquired on an Orbitrap Fusion Lumos (Thermo) in both laboratories and with both workflows. For TMT an MS3 method was used, for DIA a method as described in (Bruderer et al. 2017) was chosen. Data were analyzed with Proteome Discoverer (Thermo) and Spectronaut (Biognosys).

Generally, protein identifications were higher with TMT and protein sequence coverage higher with DIA. The quantitative performance in specific detection of differential abundance was measured using the partial area under the curve or the known true positives in the top 200 candidates. The quantitative performance of DIA depended heavily on whether both, MS1 and MS2 information, was used for quantification. Further, we compared various of ways of generating a library. We had very good results when including the data from directly searching the DIA runs.

Overall quantitative performance of both methods was similar in both laboratories. It turned out that for the analysis of the DIA data it was beneficial to use both quantitative information levels available in DIA, at MS1 and MS2 level. We see the strength in LFQ DIA particularly in a single shot workflow for dozens to thousands of samples and for ILQ TMT particularly for very deep coverage of a few samples.

POSTER 146

Machine learning of 1566 prostate proteomes generated by PCT-SWATH uncovers a protein signature predicting survival that outperforms Gleason score

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Patients diagnosed with prostate cancer at an intermediate Gleason score can harbor aggressive or non-aggressive disease that can presently not be accurately distinguished, resulting in under– or over treatment of a significant number of patients. In this study, we performed quantitative proteomic analysis of 1566 prostate tissue samples from 278 patients procured from the ProCOC (Prostate Cancer Outcomes Cohort). The samples were processed via pressure-cycling technology and the data acquired by SWATH mass spectrometry. We analyzed tumor tissues classified by a pathologist alongside matched benign tissue samples for each patient. The entire sample cohort was then divided into 31 batches, with each containing 17 to 29 samples. Each batch comprised of two control samples to evaluate technical reproducibility, and was analyzed twice in ProCan, Sydney. Altogether we quantified 2800 SwissProt proteins (FDR<1%) in all the samples, with an average missing value of 30%, while 73% of proteins were quantified in over 50% of samples. We corrected for batch effects and imputed missing values based on technical and biological replicates. This comprehensive data set allowed us to apply machine learning methods to find protein signatures that associate proteotypes with clinical outcomes. Random forest enabled us to achieve an area under the curve of 0.92 when separating tumor from benign samples. Moreover, we used a random survival forest model to identify the 50 most important proteins, constructed a risk score based on Cox regression, and created high and low risk groups by median dichotomization. Our risk groups predicted time to recurrence better than Gleason score. In conclusion, we demonstrate the feasibility to perform quantitative proteomics analysis for the largest SWATH data set to-date (>1500 proteomes) and present a protein signature that outperforms the current gold standard for prostate cancer prognosis.

POSTER 147

Investigating the plasma proteome using a novel data independent (DIA) approach for determining the mechanistic processes involved in respiratory conditions

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Chronic obstructive pulmonary disease (COPD) is the name for a group of lung conditions that cause breathing difficulties. It includes emphysema, which is damage to the air sacs in the lungs, and chronic bronchitis, which is the long-term inflammation of the airways. COPD is a common condition that mainly affects older members of the population, with many having a history of smoking. Asthma is a common lung condition, causing occasional breathing difficulties. It affects people of all ages and often starts in childhood, although it can also develop for the first time in adults.

A NanoAcquity LC equipped with a 300micron x 100mm column and delivering a reversed phase gradient over 45minutes at 7uL/min was coupled to a Xevo G2XS QTof mass spectrometer operating in the SONAR mode of acquisition. SONAR is a DIA method whereby a low-resolution quadrupole mass filter was scanned repetitively and both precursor and MS-MS data can be acquired at spectral rates approaching 2000 spectra per second. The method produced a high duty-cycle, highly specific and unbiased two-dimensional data set. Data was processed using Progenesis QIP, ProteinLynx Global Server (Waters Corp) and Scaffold (Proteome Software).

For initial studies, 6 each of human COPD, Asthma and control plasma samples were obtained. Samples were reduced, alkylated and digested with trypsin overnight to give a concentration of 7.4ug/uL and diluted further such that 5ug was loaded for each chromatographic run. The 18 samples were analysed in three sets in a random order, with pooled quality control samples injected in between. Data analysis through Progenesis QIP generated a total of 816 protein identifications, with 302 quantifiable when an Anova p-value of <= 0.05 was applied. Mutlivariate analysis showed separation of the different groups with further refinement based upon the sample metadata. Curated data was then subjected to pathway analysis.

POSTER 149

Protein Profiling of Drought Tolerance and –Susceptible Soybean Cultivars Showed Major Shift in Antioxidant and Defense Proteins' Abundance

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Production of Soybean (Glycine max), an important oil seed crop is severely influenced by several abiotic factors, such as flooding, and drought. Water stress is one of the ruminating factors of soybean crop. Plants adapt to water and temperature stresses via modulating signal molecules associating the protein changes thus altering several metabolic pathways. The present research is to understand the synergistic effects of water and heat stress on altered metabolic pathways associated with the stresses in two contrast cultivars. Relative protein abundance in leaf tissue of tolerant and susceptible cultivars was studied by 2-DE Gel electrophoresis followed by characterizing the proteins in mass spectrometry. Drought tolerant and susceptible cultivars were exposed to different heat and water stress conditions independently and in synergistic. The leaf samples were collected at regular intervals and proteins were extracted from these samples in replicates. These samples were characterized in LC MS/MS after trypsin digestion, indicated over sixty proteins were relatively abundant in response to both stresses. Functional annotation of the identified proteins reveal majority of photosynthesis, metabolism, defense and transport. Heat responsive-proteins were highly abundant in response to heat stress and combined stress in tolerant cultivar, while these proteins were in low abundance in susceptible cultivar. In both cultivars, proteins involved in folding and biosynthesis were either highly abundant or remains unchanged to heat stress. Thus, in two contrast cultivars, relative abundance of proteins was observed, and the drought tolerance is cultivar specific. Furthermore, a pattern of cross-tolerance phenomenon was detected in both cultivars for two different stresses when subjected independently. Proteins involved in antioxidant defense were most relatively abundant, whereas proteins associated with photosynthesis, secondary metabolism, and amino acid and protein biosynthesis were detected in low abundance to heat stress.

POSTER 150

Differentiation of α2,3 and α2,6 Sialic Acid-Linked Glycan Isomers using Differential Mobility Spectrometry

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Background: Glycosylation is among the most complex post-translational modifications, as many isomeric forms can result from only a few glycan units linked to a protein. In particular, sialic acid groups on glycans can present a critical isomer issue, as their linkage configurations have important consequences for biological function. Here, we employ differential mobility spectrometry (DMS) to separate isomeric glycans containing α2,3- and α2,6-linked sialic acid to avoid long complex chromatographic separations.

Methods: Several different isomeric glycan mixtures were analyzed, sialyated disaccharides, trisaccharides as well as more complex sialylated biantennary complex glycans. Experiments were performed using a QTRAP® 6500 System equipped with a DMS cell. Various method parameters were optimized to maximize resolution including chemical modifier, resolving gas flow, separation and compensation voltages.

Conclusions: Here the ability to separate glycans based on linkage was demonstrated with small and large glycans. DMS-MS was employed for the analysis of deprotonated molecules of α2,3 and α2,6 sialic acid-linked isomeric glycan pairs, in the presence of methanol chemical modifier which induces different shifts in CoV space. Initially, DMS separation was shown for two sets of trisaccharide pairs, H2S1, and H1N1S1 (glycan compositions given in terms of hexose (H), N-acetylhexosamine (N), fucose (F), and N-acetylneuraminic acid (S)). Baseline separation in CoV ramps of the α2,3 from the α2,6 isomer was observed for both glycan pairs when infusing. Next, the separation of a pair of doubly deprotonated complex fucosylated di-sialylated biantennary glycans, (H5N4F1S2) was demostrated. For all three glycan pairs, a more negative CoV was observed for the α2,3 than for the α2,6 isomer, suggesting the α2,3 isomers bind more strongly with methanol vapour than the α2,6 isomers. Also, corresponding CID MS/MS data allowed differentiation of the α2,3 from the α2,6 isomer based on diagnostic fragment ions.

POSTER 151

Breast Cancer Tumor Necrosis Associated Peptide and Glycan Co-localizations in FFPE Tissues by MALDI Imaging Mass Spectrometry

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Tissue necrosis is a form of cell death common in advanced and aggressive solid tumors, and is associated with areas of intratumoral chronic ischemia. The histopathology of necrotic regions appears as a scaffold of cellular membrane remnants, reflective of the hypoxia and cell degradation events associated with this cellular death pathway. Depending on the context in which it is found, tissue necrosis within tumors can be a positive or a negative indicator of the disease state. In patients receiving anti-cancer therapies, the presence of necrosis is generally viewed as an encouraging sign that the patient is reacting to the therapy and an immune response is being elicited. However, in tumors from individuals who have not been treated, the presence of necrosis is viewed as a negative prognostic indicator because it is often associated with highly aggressive breast, colon and lung cancers. Changes in the glycosylation of cell surface proteins is another common feature of cancer progression and aggressive disease states. Using a recently developed mass spectrometry imaging approach to evaluate N-linked glycan distributions in human formalin-fixed clinical cancer tissues, differences in the glycan structures of regions of tumor, stroma and necrosis were evaluated in breast cancer tissues. As these glycans were released from glycoprotein carriers, different proteomic mass spectrometry approaches were used to identify these glycoproteins. In this study, we use imaging mass spectrometry to query the tryptic peptide and N-glycan content of the tissue microenvironment. Serial sections were used to evaluate signatures from single or combined enzymatic digests. Off-slide peptide sequencing of extracted peptides was also done, as well as correlative immunohistochemistry staining. The long term goal will be to assess the novel necrosis-associated glycoproteins as new diagnostic or therapeutic targets.

POSTER 152

Automatic Identification and Quantitation of Site-Specific N- and O-Glycoproteins in Human Serum with IQ-GPA and Database Search

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Human serum has been intensively studied to discover biomarkers with various N- and O-glycoproteins associated with human pathological state including cancers. Because of the micro-heterogeneity and macro-heterogeneity of N- and O-glycoproteins, site-specific N- and O-glycosylation analysis in human serum is still challenging. Here, we have developed GlycoProteome Analyzer (IQ-GPA 2.0) for high throughput analysis of N- and O-glycoproteome, which combines methods for tandem mass spectrometry with a database search and algorithmic suite. All protein samples in human serum were digested with trypsin. Then, hydrophobic interaction liquid chromatography (HILIC)-enriched site-specific N- and O-glycopeptides were analyzed by nano-reversed-phase liquid chromatography (nRPLC) coupled to Orbitrap MS with HCD, CID and EThcD-MS/MS fragmentation. The resultant MS/MS data were then computationally analyzed using specific algorithms within the IQ-GPA 2.0 suite: automatic identification of the glycan compositions and glycopeptide sequences by using four different scoring systems such as M-score for glycopeptide selection from oxonium-ions, S-score for theoretical isotope pattern match for molecular ion, Y-score for glycopeptide identification, and P-score for pinpointing O- and N-glycosylation sites from MS/MS fragmentation methods. It has been designed to easily handle high-throughput glycoproteomic data with graphical user interfaces. Microsoft Azure Cloud provides more than thousands of CPU to increase throughput for data analysis. Therefore, users can perform glycoproteome analysis easily through IQ-GPA 2.0 without any high-powered computer. The IQ-GPA 2.0 is demonstrated on the website, https://www.igpa.kr/cloud/. The efficiency of IQ-GPA 2.0 was demonstrated by automatically identifying unique N- and O-glycopeptides with estimated false discovery rate ≤ 1% from over 100 glycoproteins in human serum sample that are known to contain highly glycosylated proteins. IQ-GPA 2.0 is capable for precise identification of tryptic N- and O-glycopeptides without manual validation and automated label-free quantitation of large capacity of N- and O-glycoproteins.

POSTER 153

Development of biomarker for biliary tract cancer and cholelithiasis using serum haptoglobin glycan

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Background:

Biliary tract means pathway of bile secreted from the liver, composed of bile ducts and gall bladder. Since these organs are located in the deep part of the body, it is difficult to find out genesis of cancer and is associated with bad prognosis. In addition, since clinical symptoms such as obstructive jaundice are very similar to cholelithiasis, it is difficult to distinguish cancer and inflammatory diseases. We reported that fucosylated glycans of serum haptoglobin (Hpt) were significantly increased in serum samples of digestive system cancers. In this study, in order to investigate whether this fucosylated glycan is a useful biomarker for bile tract cancer, we analyzed glycan structures of serum Hpt derived from healthy volunteers, patients with cholelithiasis, bile duct cancer and gall bladder cancer.

Methods:

Hpt was immunoprecipitated from serum and subjected to SDS-PAGE and transferred onto PVDF membrane. *N*-Glycans were released from Hpt by PNGaseF and were reduced to form alditol *N*-glycans. Structural analysis of glycans and the linkage analysis of fucosylation were performed by LC-ESI MS.

Results:

Lewis-fucosylated glycans were significantly increased in samples of gall bladder cancer and bile duct cancer. On the other hand, core-fucosylated glycans were significantly increased in samples of cholelithiasis and bile duct cancer.

Conclusions:

Hpt is a glycoprotein produced in the liver and binds to hemoglobin. We speculate that increase in Lewis-fucosylated glycans is due to binding with hemoglobin in the bloodstream which is increased by genesis of cancer. On the other hand, increase in core-fucosylated glycans is due to choking of cancer cells and calculi in the bile duct, and bile flowing normally into duodenum flows back into bloodstream. Fucosylated glycans of serum Hpt could be a useful biomarker for early diagnosis of biliary tract cancer, identification of the primary tumor location and discrimination diagnosis with cholelithiasis.

POSTER 154

ABO antigens on the epithelial cell membrane decrease in cancerous parts

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Background:

Glycosylation is a critical post-translational modification of proteins. Glycan structures change by diseases such as inflammation and tumor. Therefore, structural changed glycan on proteins could be useful targets for diagnosis and treatment. In this study, we comprehensively analyzed glycan structures on cancer tissues to investigate cytoscreening marker and therapeutic target for especially pancreatic cancer.

Methods:

Pieces of pancreatic-, liver- and breast-cancer tissue removed at the time of the surgical procedure were used in this study. Cell membrane proteins were purified from cancerous part and the surrounding non-cancerous part in each cancer tissue, and *O*-glycans were released by b-elimination from cell membrane proteins as alditol glycans. The alditol *O*-glycans were analyzed by LC-ESI MS. Based on these results by MS, quantitation of mRNA of fucosyltransferase 2 (FUT2) which synthesizes H (O) antigen on epithelial cells was performed using qRT-PCR.

Results:

In almost pancreatic-cancer tissue samples, ABO antigens (H, -Galα1-2Fuc; A, H+α1-3GalNAc; B, H+α1-3Gal) were observed in non-cancerous part, whereas they were barely observed in the cancerous part. However, in a few pancreatic-cancer tissue samples, ABO antigens were hardly detected even in the non-cancerous part. Moreover, in liver-cancer tissue samples and breast-cancer tissue samples, ABO antigens were not detected in both non-cancerous parts and cancerous parts.

Conclusion:

It is already reported that activities of various fucosyltransferases change in cancerous cells. Although amount of glycans of ABO antigens was not clearly correlated with amount of mRNA of FUT2 in this study, we suggest that the reduced activity of FUT2 results in decrease of ABO antigens in cancerous part of pancreatic-cancer tissues. Currently, we investigate the causes of difference in ABO antigen expression among organs. ABO antigens would be a useful cytoscreening marker that can distinguish between cancerous and noncancerous parts in pancreatic-cancer tissue.

Keywords:

ABO antigens, glycan, pancreatic cancer, cytoscreening marker

POSTER 155

Influence of glucan on DNA repair in human lymphotes after radiation exposure.

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Influence of glucan on DNA repair in human lymphotes after radiation exposure.

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Macro fungi are distinguished as important natural resources with therapeutic potential. Glucan was isolated from the

mushroom Ganoderma lucidum, a basidiomycete white rot macro fungus that has been used extensively for therapeutic use in China and Japan for years. The Characterisation of the compound was done by IR, NMR, Gel filtration chromatography and paper chromatography. The molecular weight was found to be 1.6 x 106 Daltons. In vivo and ex vivo experiments were carried out to study the rate of DNA repair in the presence and absence of the compound. Comet assay was performed using the method of Singh with minor modifications in human leukocytes. Chromosomal aberration studies were carried out in mouse bone marrow chromosomes. The comet parameters, percent DNA, tail length, tail moment and olive tail moment were reduced to nearly normal levels in the presence of glucan after radiation exposure. Chromosomal aberrations and individual aberrations were also reduced by glucan when administered to mice after radiation exposure. The result of present investigation reveals the potentials of glucan from G. lucidum in increasing the rate of DNA repair which makes it useful in medical scenario. The ability of bioactive polysaccharides and polysaccharide-bound proteins to modulate immune cells can be due to the structural diversity and variability of these macromolecules. The bioactive glucanes and proteoglucans isolated from medicinal mushrooms are the most promising class of immunoceutics. Glucan appear to be beneficial to humans with impaired immune systems, and those suffering from infectious diseases and cancer, as well as in helping patient recovery from chemotherapy and radiotherapy.

POSTER 156

Hydrophilic multilayer mesoporous magnetic probe for endogenous glycopeptides analysis from complex biosample

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Endogenous glycopeptides, which is one of the most important post-translational modifications (PTMs) of peptidomes, are consanguineously relative to the biomarkers discovery of serious disease. Therefore, high efficiency separation and enrichment of endogenous glycopeptides from complex biosamples is of great importance for glycopeptidome analysis. In this work, a hydrophilic magnetic mesoporous nanocomposites with core-shell Fe3O4@TiO2 structure was synthesized by grafting glucose-6-phosphate (G6P) on the anatase shell. Due to the high hydrophilicity of G6P, size-exclusive ability of mesoporous silica and strong magnetic responsiveness of magnetic nanocomposites. The Fe3O4@TiO2@mSiO2@G6P was applied to enrich glycopeptides from horseradish peroxidase (HRP) and immunoglobulin (IgG) digests efficiently. Furthermore, a total of 36 endogenous glycopeptides were identified from human saliva.

POSTER 157

Glycopeptide Fragmentation Optimization and Quantitation by Multi Collision Energy Ramp Scanning Quadrupole Data Independent Acquisition

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Data-independent acquisition (DIA) approaches have gained increasing popularity due to their high reproducibility. Both targeted and discovery workflows are now routinely used as they can provide both qualitative and quantitative information in a single experiment. Here, we describe and evaluate a novel DIA acquisition method, termed scanning quadrupole DIA, whereby the resolving quadrupole of a high-resolution benchtop Q-TOF instrument is repetitively scanned using overlapping precursor m/z windows to enable both high sensitivity and reproducible acquisition of all fragment ions.

LC-MS data were collected in a modified data independent mode of acquisition using continuous quadrupole scanning between *m/z* 800 to 1600. oa-TOF mass spectra were recorded for each quadrupole position and stored into 200 discrete bins. Three alternating data functions (modes) were acquired: in the low energy MS1 mode, data were collected at 6eV collision energy without quadrupole scanning. In two separate elevated energy MS2 modes, the collision energy was ramped from 12 to 23 eV and from 27 to 50 eV. The spectral acquisition times were between 0.1 s and 0.5 s for each mode.

Assessment of the raw data shows that implementation of the hybrid mode of acquisition results in improved fragmentation when compared with data collected using a single collision energy ramp. For each raw data file, in case of untargeted, library-independent searches, two processed peak list files were generated from the individual high-energy CID MS2 data streams. . In-solution digest from tryptic haptoglobulin (HP), a defense response regulator, was used as a model system for method development. Immunoaffinity and HILIC glycopeptide HP enriched plasma sample from three human lung cancer cell lines were contrasted in terms of their relative quantitative glycopeptide profiles. Over 30 potential different glycan structures were characterized, with a significant number showing over expression for cell lines resulting from patients with non-small cell carcinomas.

POSTER 158

In-depth structural characterization of Erythropoietin

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Recombinant erythropoietins (EPOs) are an important class of biotherapeutics that stimulate red blood cell production. Pharmacokinetic parameters of EPOs are known to be importantly affected by their glycosylation including sialic acid and its O-acetylation. Therefore, in-depth characterization of glycosylation is critical step in development and manufacturing processes to ensure efficacy, safety, and pharmacokinetic properties of EPOs. Recently, mass spectrometry (MS) has been emerged as premier tool for glycomic analysis. Recently, it provides rapid and sensitive detection of sample preparation and analysis can be used as a precise tool for structural elucidation. Therefore, Mass has contributed significantly to recent progress towards understanding the role of the glycome in biotherapeutics glycoproteins including mAbs, recombinant proteins, therapeutic enzymes, and fusion proteins. In this study, we provide of glycan analysis platform for glycomic characterization of EPO biotherapeutics.

POSTER 159

Proteomics and intact glycoproteomics analysis of exosomes from macrophage cell lines

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Exosomes as intercellular messengers have played an important role in intercellular communication and transfering cytosolic proteins, lipids, and RNA between cells of membrane. Plenty of exosome proteins have their specific potential biological functions in cancer disease including their contribution to tumorigenesis and metastasis. However, the landscape of exosome protein, particularly the glycoprotein is still insufficient. This work used ultracentrifugation which is deemed to a traditional and stable exosome enrichment method to get exosomes of macrophage cell lines. Then made a profiling of proteins and glycoproteins by the method of bottom up strategy and zichilic enrincment, and got the result from LC-MS analysis. The pepetides and glycopepetides which were identified in exosomes of macrophage cell line can integrate the data of macrophage proteins and secretory proteins that may give more indication of tumorigenesis and metastasis.

POSTER 160
The nature of phosphatidylinositol mannosidases (PIMs) interaction with the PPE68 protein – revealing novel insights in its immunogenicity and virulence

Nagender Rao Rameshwaram[1]; Kristina Thomsson Hulthe[2]; Rahila Qureshi[1]; Chunsheng Jin[2]; Niclas G Karlsson[2]; Sangita Mukhopadhyay[1]

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Background: Mycobacterial protein glycosylation is understudied and for only very few glycoproteins glycosylation sites have been described. Mycobacteria have been suggested to possess O-linked glycosylation pathways that display many commonalities with their eukaryotic and archaeal counterparts as well as some unexpected variations. Studies have demonstrated that PPE68 is a glycoprotein and plays an important role in *M. tuberculosis* pathogenicity and represent as potential vaccine candidate and diagnostic tool. Our preliminary results and shreds of evidence have shown that *M. tuberculosis* PPE68 protein stained positive for glycosylation *in vitro* (PAS staining) and glycosylated PPE68 is highly immunogenic in contrast to the deglycosylated PPE68. However, it is still unclear why does PPE68 wears an attire of such a modification? If PPE68 has a potential of being a therapeutic target it seems interesting to understand the protein glycosylation pattern to identify attributes connected to its immunogenicity using advanced strategies of glycomics and glycoproteomics.

Methods: Dot-blotting/electroblotting, Release of *N*-linked glycans, Release/reduction of *O*-linked glycans, Desalting of reduced *N*- and *O*-linked glycans, PGC-LC-ESI MS/MS of glycans

Results: Using glycomics and glycoproteomics, we demonstrated that the glycosylation of the PPE68 protein is due to its attachment to PIMs. The preliminary finding suggests that this is a covalent bond, rather than just non-covalent interaction. The bond is labile against acidic and alkaline hydrolysis and the glycosylation can partly be removed from the protein core by nucleophiles. Results also suggested the participation of aspartic and glutamic acid carboxylic side chain in the binding of the PIMs to PPE68.

Conclusions: Our study hints to a novel role of PPE68 as a PIM anchored protein that implicates its role in immune signaling in *M. tuberculosis* survival inside the host. This would also improve our understanding of the intricacies of mycobacterial protein glycosylation systems.

Keywords: PGC-LC-ESI MS/MS of glycans, glycoproteomics, glycomics

POSTER 161
Glycoproteomics-based Signatures for Tumor Subtyping and Clinical Outcome in Human High-Grade Serous Ovarian Cancer

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Tumor heterogeneity could exhibit significant differences at the molecular level due to an array of co-occurring genomic, transcriptional, translational, and post-translational regulations. As one of the most abundant and complex protein-translational modifications, protein glycosylation is known to be associated with tumor progression, metastasis and survival. To investigate the role of protein glycosylation in tumor heterogeneity of high-grade serous ovarian carcinoma (HGSOC), we performed mass-spectrometry (MS)-based quantitative proteomic analyses on 122 TCGA ovarian tumorous tissues for global proteomics and glycoproteomics on both glycosite-containing peptides and intact glycopeptides. Bi-cluster analysis of intact glycoproteomics showed a strong relationship between N-glycan structures and molecular subtypes, for example, sialylation and differentiated subtype, fucosylation and mesenchymal subtype. Further survival analysis displayed that intact glycopeptide signature of mesenchymal subtype indicated a poor clinical outcome in HGSOC. In addition, through integrated analyses of genomics, transcriptomics and proteomics with glycoproteomics, we studied the expression correlation between different types of glycoprotein product (i.e. mRNAs, proteins, glycosite-containing peptides and intact glycopeptides). The results showed that while glycoprotein expression was major regulated by substrate glycoprotein in this study, certain glycosylation enzymes also played major rule to the production of different types of glycans conjugated to different glycoproteins at specific glycosylation sites and the glycosylation enzymes further coordinated the tumor heterogeneity. This indicates that glycoprotein synthesis involves glycoprotein substrates as well as a series of glycosylation biosynthesis enzymes that are regulated by several factors including gene and protein expression, and protein functions. Glycoproteomics-based signatures identified in different subtypes of HGSOC and their association with clinical outcome may provide clues for precision medicine and targeted therapy on glycosylation genes.

POSTER 162
GlycoStore: A Bioinformatics Platform for LC and CE Glycomics Data

Matthew Campbell[1]; Sophie Zhao[2]; Jodie Abrahams[1]; Ian Walsh[2]; Louise Royle[3]; Pauline Rudd[2]

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GlycoStore (http://www.glycostore.org) is a chromatographic and electrophoretic retention database of N-,O- and GSL

glycans characterised from a range of glycoproteins, glycolipids and biotherapeutics.It is a continuation of the GlycoBase project, which addresses many of the technological limitations of GlycoBase, in particular, improvements to the bioinformatics architecture, enhancing data annotations and connectivity with external resources.

The database has four levels. The first brings together annotated glycomics data sourced from a number of analytical platforms including ultra-high performance liquid chromatography, porous graphitized carbon chromatography with MS detection, and capillary electrophoresis with laser induced fluorescence detection. The second level provides access to a growing, curated database of published literature, with a focus on data that has become available over the past five years, filling an information gap between GlycoBase and GlycoStore. This content includes a number of features such as a detailed comparison of serum glycosylation profiles derived from healthy individuals with different diseases, providing insights into how glycosylation changes are associated with health and disease. The third level is a new search tool that allows users to efficiently filter structure entries based on annotated features (e.g. epitopes and mass), by category type, and glycoprotein. The last level is the provision of dedicated Semantic resources that provides a platform for developers to query and mine the available content as part of the international GlyGen project.

Metadata is critical for connecting, finding and reusing glycomics data. Our aim is to create a metadata specification and glycomic collections registry to facilitate data discovery. We are developing a web-based interface and visualisation tools enabling users to explore GlycoStore, compare data sets, and filter by structural and analytical features. It also provides the functionality to access/download data, as well as to visualise regions of interest and connections with data available in other UniCarb resources.

POSTER 163
Genomic, proteomic, and glycoproteomic characterization of human high-grade serous ovarian carcinoma

Yingwei Hu; Jianbo Pan; Punit Shah; Minghui Ao; Lijun Chen; Michael Schnaubelt; Jiang Qian; Zhen Zhang; Daniel W. Chan; Hui Zhang

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Many gene products exhibit extensive structural micro-heterogeneity due to an array of co-occurring post-translational modifications. These protein modifications are not synthesized with genomic template and often affect the functionality of the proteins and therefore need to be characterized in detail in order to determine their structural and functional relationships and their potential linkage with genome and proteome. Here, we performed genomic and proteomic analysis of human high-grade serous ovarian carcinoma (HGSOC) and non-cancerous tissues. The correlation of different protein modifications with genomic and proteomic data was investigated in this study. Taking glycoprotein expression, glycosylation occupancy, and glycosite-specific glycosylation as a model system, we found that abundance at glycosites is regulated by the overall glycoprotein expression, while glycosylation at each individual glycosylation site contains glycosylation-site-specific heterogeneity and it is regulated by the protein abundance of the glycoproteins as well as the levels of glycosylation enzymes that are involved in the glycosylation biosynthesis pathway. This study bridges the gaps among protein glycosylation, protein expression, and gene expression by providing the most complete landscape of glycoproteome in related to proteome and genome, which would be beneficial for stratifying other protein modifications for changes of cancer gene products based on genetic alterations of cancer. Furthermore, using the data from glycoproteomics, proteomics, and genomics, we defined and demonstrated the possibility of classifying the pathological outcome of cancer from normal tissues of HGSOC using glycans on the glycoproteins from tissues.

POSTER 164
Exploring the Cell-, Protein- and Tumour-Grade-Specific *N*-Glycosylation Forming the Prostate Cancer Tumour-Microenvironment

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Prostate cancer (PCa) is the second most common male cancer. Our knowledge of the aberrant mechanisms underpinning the disease including the involvement of the glycoproteome remains incomplete. Advances in mass spectrometry-based glycomics and glycoproteomics have opened up exciting avenues for deep characterisation of the immensely complex glycoproteome and for monitoring changes with PCa development and progression. We have used integrated glycomics and glycoproteomics to explore the cell-, protein- and tumour grade-specific *N*-glycosylation signatures associated with the PCa tumour-microenvironment. Surgically-removed fresh PCa tissues grouped into five PCa grades (n = 10/group) and tissues from benign hyperplasia patients (n = 5) were investigated. Quantitative *N*-glycomics using PGC-LC-MS/MS detailed the structure and abundance of 183 unique *N*-glycans across the tissue cohort and indicated significant regulation of the *N*-glycome during PCa including increased paucimannosylation and decreased high mannosylation in high-grade tumours. Quantitative glycoproteomics using TMT labelling, high pH-RP-SPE fractionation and HILIC-SPE glycopeptide enrichment prior to Orbitrap-based detection was employed to identify and quantify 4,648 unique intact *N*-glycopeptides from 349 glycoproteins and 1,350 de-*N*-glycosylated peptides from 520 glycoproteins across the tissues. After normalisation for changes in protein abundance and site occupancy, clear site-specific glycoform regulation mirroring the glycome data was observed with PCa onset and development. Several highly cell-specific glycoproteins of prostate epithelial cell, neutrophil, macrophage and lymphocyte origins were identified reflecting the cellular heterogeneity within the PCa tumour-microenvironment. Excitingly, the site-specific glycosylation of these cell-specific glycoproteins indicated, for the first time, that the malignant prostatic epithelial cells are undergoing a profoundly different glycoproteome regulation than the innate and adaptive immune cells in PCa. In conclusion, this study shows that quantitative glycoproteomics can provide unprecedented insight into key molecular features contributing to the highly diverse tumour-microenvironment allowing us to deconstruct the regulation of the cell-, protein- and tumour-grade-specific *N*-glycosylation associated with PCa onset and development.

POSTER 165
Automated annotation of glycoproteomics mass spectrometry studies enabled by the integration of DrawGlycan with GlycoPAT

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Glycoproteomics experiments have adopted the traditional high-throughput LC-MS^n proteomics workflow to analyze site-specific glycosylation. While a few computational tools are available for the analysis of such studies, they typically lack facilities for high-quality, visual annotation of MS/MS fragmentation spectra, and quantitative glycoproteomics. To address these limitations, we have added new modules for data analysis and also integrated two different, open-source glycoinformatics tools developed in our laboratory: i. DrawGlycan-SNFG, for the sketching of glycans in Symbol Nomenclature for Glycans (SNFG) format (*Glycobiology.* 27(3): 200-205); and ii. GlycoPAT, for the scoring of high-throughput glycoproteomics MS data (*Mol Cell Proteomics.* 16(11): 2032-2047). The resulting platform independent software (GlycoPAT2.0) includes a three-tabbed visual interface for i. Viewing a summary of the spectrum scoring results, including cross-correlation and probability based analysis. Annotated MS^n spectrum are also presented, with all identified glycan and peptide bond fragmentations rendered using DrawGlycan-SNFG sketches. ii. Label-free quantitative glycoproteomics analysis and validation of precursor monoisotopic peak assignment based on the molecular isotopic distribution profile. iii. Comprehensive spectrum annotation with full DrawGlycan-SNFG rendering and detailed analysis of all matched peaks. Besides the enhanced visualization of results, the presentation will also cover key features of GlycoPAT2.0 that enable false discovery rate calculations, increased computational speed, parallel computing on local PCs, and efficient XML-based result storage. Examples will be presented using high throughput glycoproteomics datasets that utilize either cancer cells or human blood plasma glycoproteins. Overall, GlycoPAT is an easy to use, modular computational program for high quality analysis of shotgun glycoproteomics studies. Efforts have been made to develop an intiuitive, visual, user-friendly interface and also advanced scoring algorithms.

POSTER 166
mOGP 1.0-Making O-glycoproteomics More Convenient and Meaningful

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Mucin-type O-glycosylation is involved in many physical and pathological processes. With recent advances in mass spectrometry technologies, the scale of mucin-type O-glycoprotein identification is increasing. However, without efficient data integration and mining, the identification results can be hardly used effectively. Bioinformatics tools for mucin-type O-glycosylation remains poor and inadequate. Thus, it is need sorely to develop bioinformatics tools for better promoting O-glycosylation study.

Herein, we constructed a database-mOGP1.0. It is currently the largest O-glycoprotein database. A total of 9375 glycosylation sites and 6662 site-specific glycans mapping to 1481 glycoproteins from various species were recorded with unified inclusion and documentation standard. The content of this database includes proteins, peptide sequences, glycosylation sites, site-specific glycan structures, sample sources, experimental methods and the references. Based on the database, the mOGP website (http://www.oglyp.org/) was established and equipped with a user-friendly graphical interface. It comprises four main functional parts: statistic analysis, database search, O-glycosylation site prediction and data submit. In the statistic analysis part, users can have a general overview of the mOGP knowledge base, including the number of glycans, glycosylation sites, peptides and proteins recorded in the database, frequency distribution of mucin-type O-glycoproteins in major taxonomic categories and the comparison of database scale between the mOGP and the other two main databases. In the database search part, users can get all the recorded information in the database of an O-glycoprotein that they care about. An O-glycosylation site prediction tool was developed and equipped on the website. With this tool, users can predict the possibility of an S/T site that could be O-glycosylated. Users can also submit their published experimental results on the website. We will recorded in the database after carefully check.

mOGP is the largest O-glycoprotein database and provides a web-based interface that greatly facilitate the study on mucin-type O-glycoproteins.

POSTER 167
Towards universal glycoproteome analysis using pGlycoNovo: intact N-glycopeptide profiling across seven model species

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Large-scale profiling of intact glycopeptide is an essential part of glycosylation study. Although we have witnessed many developments of glycopeptide analysis over the past years, most large-scale glycopeptide profiling was performed on either human or mouse samples. Universal glycoproteome analysis, or intact N-glycopeptide analysis on different species, is not possible using existing methods and is urgently needed for glycoproteome study.

A database-free algorithm for intact glycopeptide analysis, named pGlycoNovo was developed for universal glycoproteome analysis. Using dedicated search engine pGlycoNovo and optimized one-step tandem MS workflow (pGlyco2.0, ref 1), we performed large-scale intact N-glycopeptide profiling on seven model species, including A.thaliana, C.elegans, D.melanogaster, D.rerio, M.musculus, S.cerevisiae and S.pombe.

We have identified more than 40,000 unique N-glycopeptides at less than 2% FDR. Data quality was monitored using decoy protein database and strict glycan score threshold. Isotope labelled C.elegans and S.pombe were used as validation (pGlyco 2.0, ref 1). We found many intact-glycopeptides with atypical glycans not found in human or mouse, such as intact-glycopeptides with more than 3 core-fucoses in C.elegans and intact-glycopeptides with Xly in A.thaliana. These atypical glycans were validated using glycomic analysis. Our large-scale study of intact glycopeptides across seven model species provided important resource for the glycoproteome community.

1. Liu, M. Q.; Zeng, W. F.; Fang, P.; et al. Nat. Commun. 2017, 8, 438.

POSTER 168

Protein glycosylation in pancreatic ductal adenocarcinoma and its implication in chemoresistance

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Glycosylation plays an important role in epithelial cancers, including pancreatic ductal adenocarcinoma. However, little is known about the glycoproteome of the human pancreas or its alterations associated with pancreatic tumorigenesis. Using quantitative glycoproteomics approach, we investigated protein N-glycosylation in pancreatic tumor tissue in comparison to normal pancreas and chronic pancreatitis tissue. The study lead to the discovery of a roster of glycoproteins with aberrant N-glycosylation level associated with pancreatic cancer, including mucin-5AC (MUC5AC), carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), insulin-like growth factor binding protein (IGFBP3) and galectin-3-binding protein (LGALS3BP). Pathway analysis of cancer associated aberrant glycoproteins revealed that increased activity of N-glycosylation was implicated in several pancreatic cancer pathways, including TGF-β, TNF, NF-kappa-B and TFEB related lysosomal changes. Studies from others and our lab have evidenced that protein glycosylation pathway plays an important role in chemoresistance of pancreatic cancer. We demonstrated that disruption of glutamine metabolic pathways effectively improved the efficacy of gemcitabine treatment on drug-resistant pancreatic cancer cells. Such a disruption induces a cascade of events which impacts glycan biosynthesis through Hexosamine Biosynthesis Pathway (HBP), as well as cellular redox homeostasis, resulting in global changes in protein glycosylation, expression and functional effects. The induced proteome alterations in the resistant cancer cells and the secreted exosomes are intricately associated with the reduction in cell proliferation and the enhancement of cancer cell chemosensitivity. Proteins associated with EGFR signaling, including downstream AKT-mTOR pathways, MAPK pathway, as well as redox enzymes were down regulated in response to disruption of glutamine metabolic pathways. Our study may help to elucidate glycosylation events underlying tumorigenesis and drug resistance, and facilitate the development of novel adjuvant treatment to enhance the cytotoxicity of gemcitabine towards chemoresistant pancreatic cancer.

POSTER 169

1st Human Glycoproteomics Initiative (HGI) Study: Community Evaluation of Software for Automated Intact Glycopeptide Identification by Mass Spectrometry

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It is increasingly apparent that protein glycosylation is involved in many biological functions. Analytical advances in mass spectrometry have now facilitated LC-MS/MS-based glycoproteomics studies that are identifying hundreds and even thousands of unique intact glycopeptides from a single experiment. However, significant bottlenecks still exist in the accurate annotation of the large volumes of MS/MS spectral data and in the correct identification of the corresponding intact glycopeptides. Efficient software for automated glycopeptide identification has become essential. Glycoproteomics has recently seen the development, by both commercial and academic developers, of software tools that show great promise for this automated or semi-automated annotation and identification of intact glycopeptides from MS/MS spectral evidence. However, their relative performance and utility remains unknown.

This is the first interlaboratory study since the Human Glycoproteomics Initiative (HGI) was formed as part of the Human Proteome Project (HPP) and follows on from the work done by the original HUPO HGPI. This study evaluates the performance of the currently available glycoproteomics informatics capabilities for intact glycopeptide identification in complex human specimens. The study participants span both expert users and software developers across both industry and research in the glycoproteomics community. All participants are supplied the same two high resolution LC-MS/MS datasets of intact *N*- and *O*-glycopeptides acquired from analysis of digested human serum glycoproteins using complementary fragmentation modes (HCD, EThcD, ETciD and CID). The participants have provided reports of identified intact *N*- and *O*-glycopeptides, annotated spectra and details of the informatics approaches they used.

Preliminary study results provide interesting insights into the diversity of community approaches for glycopeptide analysis, the group-to-group (vari)ability to accurately identify intact glycopeptides and the performance of the available glycoproteomics software. Documenting the current status of glycoproteomics software is vital to improve further analytical development and stimulate the application of system-wide glycopeptide analysis by more researchers.

POSTER 170

Rapid profiling of prostate cancer-specific PSA glycoforms as a specificity-enhanced secondary biomarker

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Prostate cancer (PCa) is one of the most commonly diagnosed cancer in men worldwide. Serum prostate specific antigen (PSA) is a powerful biomarker widely used for diagnosing PCa. However, high false positive rate of PSA screening is a great issue to be resolved.

In this study, we performed comprehensive and quantitative profiling of glycan structures on serum PSA using energy resolved oxonium ion monitoring (Erexim) technology (Anal Chem, 2012, 84:9655). In order to improve the specificity to

preclude a false positive diagnosis in PSA test, we identified cancer-associated glycoforms of PSA which would be specifically generated in PCa cells.

The Erexim parameters of LCMS-8060 triple quadrupole mass spectrometer (Shimadzu) were optimized for quantify multiple glycan structures on PSA. In total 67 glycan structures on PSA, even from 0.1% content structures, were quantitatively monitored in 25 minutes run without enzymatic glycan release or chemical labeling.

PSA glycoforms were then quantitatively evaluated for sera from 15 PCa patients or 15 benign prostatic hyperplasia (BPH) patients whose PSA levels were at “gray zone” (4.0-10.0 ng/ml). PSA in sera was immunoprecipitated and analyzed by Erexim technology. As the result, a couple of PCa-specific glycoforms were statistically extracted, and subjected to establishment of a novel PCa-specific diagnostics based on logistic regression (PSA G-index). When diagnostic power was evaluated using a validation sample set (15 BPH and 15 PCa patients), the AUC of PSA G-index was 1.00, while that of total PSA or PSA f/T ratio were 0.50 and 0.60, respectively. Moreover, both PSA glycoforms showed significant correlation with Gleason scores.

Thus PSA G-index could serve as not only the effective secondary screening method to preclude false positive diagnosis in PSA screening, but also the potential prostate cancer grading marker.

POSTER 172
Quantitative Phosphoproteomics Reveals in vivo Boron Deficiency Induced Signaling Dynamics in Arabidopsis Roots

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Mass spectrometry has been instrumental in enabling the study of molecular signaling on a cellular scale by way of site-specific quantification of protein post translational modification, in particular phosphorylation. Here we describe an improved tandem MOAC combined phosphoprotein / phosphopeptide enrichment strategy, a scalable phosphoproteomics approach that allows rapid identification of thousands of phophopeptides in plant material. We apply this technology to generate time-resolved maps of boron signaling in Arabidopsis roots. We show that the successive enrichment of phosphoproteins in a first and phosphopeptide extraction in a second step using our optimized procedure strongly enriched the root phosphoproteome. Our results reveal that boron deficiency affects over 20% of the measured root phosphoproteome and that many phosphorylation sites with known biological function, and an even larger number of previously undescribed sites, are modified during the time course of boron deficiency. We identify transcription factors as key regulators of hormone signaling pathways that control gene expression in boron deprived plants. Phosphorylation increased in response to boron deficiency at previously unreported sites on boron efflux transporters BOR2 and BOR3. Furthermore, our phosphorylation kinetics data demonstrate that MAPK cascades mediate polarized transport of boron in Arabidopsis roots.

POSTER 173
De Novo Sequencing of Tandem Mass Spectra Reveals Dark Matter of Cyclopeptidomics

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Introduction. Cyclopeptides represent an important class of natural products with an unparalleled track record in pharmacology. Although the Global Natural Products Social[1] (GNPS) molecular network already contains over a billion of mass spectra of natural products, less than one percent of them have been identified[1] and it remains unclear how many spectra originating from still unknown bioactive cyclopeptides have been already deposited into this network. To address this bottleneck, we developed CycloNovo algorithm for de novo cyclopeptide sequencing based on the concept of de Bruijn graphs, the workhorse of modern genome sequencing algorithms. CycloNovo identified many new cyclopeptides that were further validated using transcriptome sequencing and genome mining. Our analysis suggests that the currently known cyclopeptides represent the tip of the iceberg of all cyclopeptides with spectra already present in the GNPS.

Methods. CycloNovo pipeline includes (i) computing the spectral convolution[2] of each spectrum, and extracting the set putative amino acids in the unknown cyclopeptide, (ii) computing compositions of masses that matches the precursor mass, (iii) finding potential 5-mers for each composition, (iv)constructing de Bruijn graphs with those 5-mers, (v) traversing the de Bruijn graph and generating candidate sequences, and (vi) computing the Peptide-Spectrum-Match score and P-value for each candidate sequence.

Results. We applied CycloNovo to a high-resolution spectral dataset generated from human stool. Our analysis revealed 703 cyclopeptide spectra corresponding to 79 unique putative cyclopeptides forming 61 spectral families (identified by MS-Cluster[3] and molecular networking[4], respectively). Dereplicator[5] and CycloNovo analysis revealed only eight matches against known cyclopeptides with P-value<10^{-15} that originated from antimicrobial cyclopeptides from Flax seed and citrus plants. This is the first demonstration that shows anti-microbial cyclopeptides from consumed plants remaining stable in a proteolytic environment of the human gut, raising a question of how these bioactive cyclopeptides affect the bacterial composition of the human microbiome.

[1]doi:10.1038/nbt.3597, [2]doi:10.1038/nmeth.1350, [3]doi:10.1038/nmeth.1609, [4]doi:10.1073/pnas.1203689109, [5]doi:10.1038/nchembio.2219

POSTER 174
Mass Spectrometry Imaging of synovium reveals molecular profiles with diagnostic potential in Arthritis

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Rheumatoid (RA) and Psoriatic (PsA) arthritis are immune-mediated chronic inflammatory diseases. Synovium is the initial site of inflammation in PsA and RA joints and can be used for diagnostic purposes, since pathophysiological events occurring in this tissue are likely to reflect the clinical status and outcome in patients. The purpose of this study is to create a new method for patient classification based on the spatially resolved molecular profiles of their synovia obtained by mass spectrometry imaging (MSI).

PsA (n=25) and RA (n=21) synovium biopsies were cut and deposited on conductive slides. Lipid and peptide profiles were acquired on a RapifleX MALDI Tissuetyper™ TOF/TOF in positive reflector mode, and metabolites using a 9.4 T solariX FT-ICR. Spatial resolution was set up at 50 µm for all analyses. Multivariate data analysis was used to search for molecular species showing the highest differences between groups.

MALDI-MSI revealed differential protein and metabolite profiles between groups. Discriminant analysis performed on the data allowed the separation of PsA from RA samples. PsA showed higher levels of 30 lipid species, compared to RA. Among them, several phosphatidylethanolamines were significantly more abundant in PsA, whereas phosphatidic acids displayed a stronger intensity in RA. Phosphatidylcholines showed a specific localization in high inflammation and blood vessel-containing areas of RA synovium, according histological images. Also, the levels of 14 metabolites were altered in PsA versus RA. Interestingly, RA tissue showed a higher abundance of sugars, including N-acetylgalactosamine 6-sulfate and glucuronic acid 1-phosphate. PsA and RA cohorts were also distinguished based on synovium proteomic signatures. In total, 57 peptides were differentially abundant between both groups.

For the first time, RA and PsA synovium have been discriminated on the basis of their proteomic and metabolomic signatures revealed by MALDI-MSI. These results could support clinical decision-making in the diagnosis of arthritic patients.

POSTER 175
Optimised Desorption Electrospray Ionisation Mass Spectrometry Imaging (DESI-MSI) method for the analysis of proteins/peptides directly from tissue sections

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Desorption electrospray ionisation mass spectrometry imaging (DESI-MSI) is typically known for the mapping of small molecules such as lipids, directly from tissue sections. Whilst the detection of spotted protein standards from target plates has previously been shown, the detection of large biomolecules directly from tissue sections has presented difficulty. Here we describe a newly developed method combining a series of optimized parameters and conditions which allow the extraction of large biomolecules from the tissue in droplet form.

All experiments were carried out on a SYNAPT HDMS G2-S*i* Q-ToF with a Prosolia2D DESI stage. A modified spray head assembly and inlet capillary with a heated sheath connected to an adjustable power supply were used to generate variable heating. Optimisations were performed assessing tissue pre-treatment, spray solvent flow rate, nebulising gas pressure and solvent composition.

The heated inlet capillary provided enhanced ionisation of proteins and peptides from tissue. The heated inlet allowed further optimisation in terms of sprayer inlet geometry, solvent composition, nebulising gas pressure and solvent flow rate. The additional orthogonal separation of ion mobility was essential to observe the most abundant charge series in the highly complex data set. Within the rat liver DESI MSI data, the most abundant charge series observed were those belonging to Haemoglobin with charge states from 9+ to 21+ observed. Extraction of the trendlines via the Driftscope software package and subsequent use of the maximum entropy 1 algorithm allowed a putative assignment of four Haemoglobin subunits: Alpha -1 and Alpha-2 (with an aspartic acid substituted with alanine), Beta 1 and Beta 1 with serine substituted with threonine. For the other tissue types, additional charge series putatively assigned to fatty acid binding protein, heat shock protein and cytochrome c oxidase subunit for human liver tissue section and myelin basic protein for brain tissue were observed.

POSTER 176
A Chemical Derivatization Strategy for Extending the Identification of MHC Class I Immunopeptides

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Neoantigen-based therapeutic vaccines have foreseeable impacts on tumor eradication and patient survival. Mass spectrometry (MS)-based immunopeptidomics has the capacity to identify tumor-associated epitopes, pinpointing mutation-bearing major histocompatibility complex (MHC)-binding peptides. This approach nevertheless presents several challenges, including the identification of low-abundance peptides. In addition, MHC peptides have much lower MS/MS identification rates than tryptic peptides due to their shorter sequence and lack of basic amino acid at C-termini. In this study, we report the development and application of a novel chemical derivatization strategy that combines the analysis of native, dimethylated and alkylamidated peptides by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to expand the coverage of the MHC peptidome. The results revealed that dimethylation could increase hydrophobicity and ionization efficiency of MHC class I peptides while alkylamidation could significantly improve the fragmentation by producing more y- ions during MS/MS processing. Thus the combination of dimethylation and alkylamidation enabled the identification of peptides that could not be identified from the analysis of their native form. Using this strategy, we identified 3148 unique MHC I peptides from HCT 116 cell lines, compared to only 1388 peptides identified in their native form. Among these, 10 mutation-bearing peptides were identified with high confidence, indicating that this chemical derivatization strategy is a promising approach for neoantigen discovery in clinical applications.

POSTER 177
A new algorithm for identification of immunopeptides from LC-MS data with high sensitivity

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Personalized immunotherapy in ideal case should depend on the neoantigens present on the cancer cell surface, of one person, one tumor, and one time. A few research groups reported direct identification of mutated peptides isolated from human leucocyte antigens (HLA) by LC-MS. Until recently, MS technologies were not sensitive enough to do this. The key challenge is to deal with the low abundance of these peptides. DIA technology promises to capture the low abundance data. However, there is no working algorithm/system to de novo sequence DIA data. Most database search method for DIA spectra also depend on spectrum library that significantly limits their abilities to find mutated peptides. For the first time, we have introduced deep learning into peptide de novo sequencing with DIA data.

Our deep learning system combines two convolutional neural networks and a long short-term memory (LSTM) recurrent neural network for de novo peptide sequencing. In this model, the first convolutional neural network, called ion-CNN, learns local alternative peak features of all possible next amino acid candidates given the currently predicted partial peptide. The second convolutional neural network, called spectrum-CNN, learns general features of the spectrum and passes them to the LSTM network. The LSTM learns sequence patterns of the currently predicted partial peptide in association with the spectrum features from the spectrum-CNN. The information learned by the ion-CNN and LSTM networks is then integrated to make the final prediction.

This talk will present the testing results with seven LC-MS data sets with DIA approach. The results showed that the deep learning system could handles DIA data, and de novo sequence several peptides from each DIA spectrum, with comparable accuracy to DDA data. Further, the system solves the problem of peptide identification for DIA spectra, combining database search and de novo sequencing to identify low abundance peptides.

POSTER 178
Detection of citrullinated residues in glucose-regulated protein 78 in human islets of Langerhans by LC-MS/MS using data dependent acquisition.

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Citrullination is a calcium-dependent process, induced by enzymatic conversion of arginine into citrulline residues on proteins, mediated by peptidylarginine deiminases, resulting in a loss of a positive charge and an increase of 0.98402 Da. Although citrullination is observed in normal physiological processes, abnormal citrullination, often associated with inflammation, can provoke autoimmunity by generating altered self-epitopes that are specifically targeted by autoantibodies or autoreactive T cells. We have previously shown in NOD mice, a mouse model for type 1 diabetes, that citrullinated glucose-regulated protein 78 (GRP78) is an autoantigen, generating autoreactive CD4+ T-cells and circulating autoantibodies. The aim of this study was to optimize the detection of citrullinated residues in human islet proteins by LC-MS/MS. Islets were exposed in vitro to cytokines (IL-1β, TNF-α and IFN-γ) and 10 µg of protein lysate was subjected to LC-MS/MS (Orbitrap Q Exactive), focusing on citrullination of GRP78. First, taking into account the minor difference between the monoisotopic 13C peaks corresponding to the native, non-citrullinated peptide and the peaks of citrullinated or deamidated (also resulting in an increase of 0.98402 Da) peptides, unbiased LC-MS/MS analysis did not reveal any citrullinations. To distinguish, synthetic IDVNGILRVTAE (native), IDVDGILRVTAE (deamidated) and IDVNGILXVTAE (citrullinated) GRP78 Glu-C peptides were generated and analyzed by LC-MS/MS. We measured clear differences in retention times: 182.53 ± 1.87 min, 189.18 ± 0.66 min and 209.92 ± 1.91 min, respectively. By subsequent targeted LC-MS/MS (data dependent acquisition with inclusion list) on human islets, fragmentation data were obtained which enabled us to unambiguously assign the precursor ions with correct mass to the native and citrullinated peptides and to distinguish them from the deamidated peptide, the latter based on the diagnostic b+-ion of m/z 612.34. A combination of correct m/z, retention times and diagnostic fragmentation data led us to conclude that GRP78 is citrullinated in human islets.

POSTER 179
Immunoproteomic approach for identification of allergenic proteins in pecan nut and read oak pollen grains

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Common aeroallergens responsible for allergic rhinitis include, mold, dust mites, spores and pollen(1). Pollen is a relevant aeroallergen in areas where ornamental and orchard trees are present, it is also capable of traveling long distances as well as its atmospheric concentration fluctuates as seasons do(2). Allergenicity is mainly attributed to pollen protein and glycoprotein composition. Moreover, a lot of pollen allergens include structurally similar proteins that can be cross-reactive in triggering allergenic reactions as well as in diagnostic tests. Therefore, identification of allergenic proteins in pollen is essential to provide a set of proteins and glycoproteins useful for specific allergy diagnosis(3). Pecan nut (Carya illinoinensis) and red oak (Quercus rubra) belong to one of the main order of trees implicated in hypersensitivity reactions triggered by pollen(4).

This study aims to expand the repertoire of allergenic proteins and glycoproteins from this two allergenic species employing immunoproteomic tools. Total soluble pollen proteins were extracted and separated by SDS-PAGE and 2-DE, stained to identify glycosylated proteins using the periodic acid-Schiff method prior Coomassie blue stain, and analyzed by western blot using sera from allergic patients.

Red oak pollen proteins shown to be poorly glycosylated in comparison to those of pecan nut. Western blot revealed that pecan nut pollen protein extract contains allergens of molecular weights around 6-180 kDa (6, 20-40 and 180 kDa), whereas red oak pollen allergens ranged from 10-115 kDa. We are currently analyzing IgE recognized proteins by tandem mass spectrometry and bioinformatics analysis in order to reveal

allergens identities. Characterization of these allergenic proteins may contribute to developing of new therapeutic approaches in allergic disease.

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POSTER 180
Characterisation of flucloxacillin-modified proteins leading to the presentation of flucloxacillin-modified MHC peptides, and their importance in iDILI.

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Introduction

Flucloxacillin is a β-lactam antibiotic associated with idiosyncratic drug-induced liver injury (iDILI). Although expression of the Major Histocompatibility complex (MHC) class I allele HLA-B*57:01, responsible for presenting peptides to the immune system, increases susceptibility, little is known of the pathological mechanisms involved in disease induction. Off target protein interactions are suspected to drive the reaction, either through non-immune mediated pathways, through the modification of peptides that are presented by HLA-B*57:01 or both. In this study, we aimed to characterise the proteins haptenated by flucloxacillin using a range of proteomic techniques. As protein haptenation, followed by antigen processing and presentation of the drug-derived antigenic determinants may drive the adverse event, we also investigated the precise nature of the peptides presented by the risk allele (the immunopeptidome).

Methods

Anti-flucloxacillin antibody was used to identify flucloxacillin modified proteins in multiple cell lines. Western blot, immunocytochemistry and immunoprecipitation were utilised for their identification, and characterised by mass spectrometry. To investigate the HLA-B*57:01 immunopeptidome, C1R-B*57:01, B-lymphoblastoid cells transfected with HLA-B*57:01, were incubated with flucloxacillin. HLA-B*57:01-peptide complexes were immunoaffinity purified, the peptides eluted and analysed by mass spectrometry.

Results

Haptenated proteins that could play a role in non-immune mediated pathways were discovered. Intracellular protein modification was identified, with localisation appearing in bile canaliculi. Further investigation to understand the effect of flucloxacillin on membrane transporters is ongoing. Drug-modified peptides were identified within the HLA-B*57:01 immunopeptidome. Direct modification of the HLA-B*57:01 was also observed and may lead to presentation of neo-antigens. As such, in depth analysis of the global impact of flucloxacillin on the immunopeptidome is ongoing.

Conclusions

Further investigation into the identification and role of specific haptenated proteins in the onset of iDILI is ongoing. However, for the first time we have demonstrated that drug-modified peptides can be naturally presented by MHC molecules.

POSTER 181
Constrained De Novo Sequencing of neo-Epitope Peptides using Tandem Mass Spectrometry

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We present a novel constrained de novo sequencing algorithm for neoepitope identification. It is a hybrid approach of de novo sequencing and database searching algorithms: it explores the entire space of peptide sequences 9-12 residues, but assigns a different prior probability to each putative peptide according to MHC-I specific PSSMs, such that the peptide with a motif with high immunogenicity incorporates a high prior probability into the posterior probability score of the peptide-spectrum matches. We extended dynamic programming (DP) algorithm for de novo to determine the peptide sequences with the optimal posterior matching scores for each given MS/MS spectrum.

We tested our algorithm in a LC-MS/MS dataset for detecting the neoepitope peptides bound by the HLA-C*0501 molecules. Our method could detect about 19,017 neoepitope peptides of lengths between 9 to 12 residues with estimated false discovery rate below 1%. In contrast, the database search approach (using MSGF+ against the human protein database) identified about 4,415 PSMs (1,804 unique peptides), in which 2,104 PSMs (764 unique peptides) have the length between 9 to 12 residues as putative neoepitope peptides. Out of the 2,104 PSMs, 1,269 were also identified by our method. A majority (791 out of 1,269) of the PSMs were exact matches, while most (360 out of 478) remaining PSMs contain only a swap of consecutive residues in peptide sequences. Finally, we tested a conventional de novo sequencing algorithm uniNovo on the same dataset. It reported sequence tags on 1,863 MS/MS spectra, but with low sequence coverage (on average three amino acid residues per peptide), and thus cannot be used in neoepitope peptide sequencing. These results imply that the constrained de novo sequencing algorithm benefit from the prior probabilities (provided by the PSSMs) to distinguish the most likely neoepitope peptides from other peptides sharing similar sequences.

POSTER 182
CCSPredict: Using a machine learning approach for higher confidence in Lipid identification

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The use of ion mobility-featured mass spectrometers offers new options for higher confidence in annotations of target molecules. First, with the additional separation dimension compounds co-eluting from LC columns can be separated. The benefit is that a subsequent fragmentation will result in cleaner MS/MS spectra – crucial for any ID in lipidomics or other small

molecule workflows. Moreover, ion mobility enables the determination of the collisional cross sections of ions. These values are specific properties for any ion species under given conditions (type of gas, pressure, temperature). Therefore, acquired values can be used for identification if they are compared to *in-silico* generated data or used in a library-based approach.

We present a new tool for the prediction of lipid CCS values. It is fully integrated in MetaboScape 4.0 and is based on a machine learning approach[1] that was extended significantly to cover a wider range of lipid structures. Predicted values can be compared with the ones measured on a Bruker timsTOF Pro instrument. The Trapped Ion Mobility Spectrometry technology enables the exact measurement of CCS values at a very high reproducibility and TIMS resolving power. Both are critical pre-requisites to make full use of the increased confidence by CCSPredict. This workflow helps to assign the structural classes of lipids.

POSTER 183
High Throughput Targeted Workflows for Metabolomics / Lipidomics Studies

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Background: Targeted quantitation using LC-MRM is robust, high-throughput strategy for the identification and quantitation of large numbers of metabolites and lipids in complex samples, providing high quality identification and quantitation and simplifying the downstream informatics. Availability of methods that cover a comprehensive list of key biologically relevant metabolites or lipids are an essential tool for a biomarker researcher.

Methods: Two methods each ~25 mins in duration, have been optimized for the QTRAP® 6500+ system with IonDrive™ Turbo V Source. Method 1 targets a total of 312 polar metabolites across the key metabolic pathways (187 positive mode and 176 negative mode MRM's) using HILIC separation at pH9 using Luna- NH2 columns (Phenomenex). Method 2, using the *Scheduled* MRM™ Algorithm, targets over 1200 lipid molecular species using a XBridge Amide 3.5 µm column (Waters). Data was processed using MultiQuant™ Software 3.0.

Conclusions: For Method 1, the Luna-NH2 HILIC chromatography provided excellent chromatographic separation of polar metabolites, and with method adjustments to loading conditions very good retention time reproducibility for polar metabolites was achieved. Both high flow and microflow chromatography was explored, with microflow providing a significant improvement in sensitivity and therefore metabolite coverage.

Due to the challenge of isomer interference, the chromatography for method 2 was optimized to provide separation between lipid classes, focusing on CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS. An internal standard strategy was developed to simplify retention time determination and quantification. The target list is customizable and expandable to include new lipid classes or the list can be shortened for a class-specific study.

Further details on the methods and their utility will be provided.

POSTER 184
Metabolomics profiling of parapneumonic effusion reveals regulatory roles of dipeptides for neutrophils

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Pleural effusion (PE) is one of common complications for various diseases. Malignancy and pneumonia account for the two leading causes of exudative PE generation. Approximately 40% patients with bacterial pneumonia accompany generation of parapneumonic effusion (PPE). Based on the severity of inflammation, PPEs could be categorized as the early-stage uncomplicated PPE (UPPE), the advanced-stage complicated PPE (CPPE), and the most serious end, empyema. Antibiotic treatment at early stage of PPE can prevent the PPE progression and reduce the mortality. Metabolomics analysis may assist in understanding causes of PPE and developing treatment strategy of PPE. To this end, the metabolome of PPE and malignant PE (MPE) has been quantitatively detected with the differential 12C-/13C-isotope dansylation labeling-based mass spectrometry. Compared to the MPE, the levels of 9 dipeptides are elevated in the PPE samples. Furthermore, the levels of 13 dipeptides in CPPE were higher than that in UPPE, suggesting that the dipeptides may involve in the PPE progression and regulation of inflammation. We further determined the effect of dipeptide treatment on neutrophils, the major cell type in PPE. The treatment of dipeptides indeed can increase the production of interleukin 8 in the HL-60-derived neutrophilic cells. In sum, we profiled the metabolome of PE and show for the first time that the dipeptides may involve in the activation of neutrophils that represents PPE progression.

POSTER 185
Analysis of RNA Mononucleosides by DIA nanoLC-MS/MS Can Reveal Inducible tRNA Wobble Position Modification Abundance Changes

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RNA has been shown to be modified on both the base and ribose moieties to create over 140 distinct nucleotides. Both the identification of undiscovered RNA species and the understanding of functions of known modifications remain far from complete. These modifications are highly variable between organisms, and their presence on different subtypes of RNA has only been partially characterized. Some of the modifications are known to be dynamic due to previously identified writer and eraser proteins, and others have been shown to be inducible, and can directly affect protein translation efficacy. In order to study these the functions of these modifications, we have developed a nanoLC-MS/MS method that uses a porous graphitic carbon stationary phase, is compatible with conventional formic acid based proteomics mobile phases, and employs data independent acquisition (DIA) fragmentation.

This method has been applied to salt stressed *Arabidopsis thaliana* to investigate changes in the modifications of mRNA, rRNA, and tRNA. Prior work has shown that ribosomal pausing can occur in salt stressed conditions, and translation efficiency decreases on some mRNA transcripts. Preliminary results from our MS method identified a

modification, 5-aminomethyluridine (nm5), which, although understudied in *Arabidopsis thaliana*, has been shown to be critical in the wobble position (U34) of multiple tRNAs in multiple species. This work suggests a possible role of a tRNA modification in translation efficacy in salt stress conditions.

POSTER 186

Developments of Liquid Chromatography-Mass Spectrometry Methods for Endogenous Metabolites in Glycolysis and Endogenous Nucleotides Used in Cancer Therapy

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High performance liquid chromatography-mass spectrometry (HPLC-MS) is a widely used technique in analytical science, which combines the robust separation capability of HPLC with the sensitivity and selectivity of MS. In the field of bio-analytical chemistry, HPLC-MS is the most important technique in proteomics and metabolomics.

Endogenous metabolites in glycolysis as well as endogenous nucleotides play an important role in both substance metabolism and energy metabolism, and both types of metabolites can be applied in anticancer therapies. Analysis of endogenous nucleotides and endogenous metabolites in glycolysis is of interest for investigation in many biological studies. Among these multiple metabolites, separation of sugar phosphates and nucleotides with phosphate groups by LC–MS is the most challenging task due to their high polarity nature, similar structure including isomers and large difference in concentrations of those phosphorylated metabolites. Here, we described a HPLC-MS method, achieving the good separation of phosphorylated metabolites (PEP, DHAP, 3GAP, 2PG, 3PG, G1P, G6P, F6P, FBP, PA) in glycolysis and a general HPLC-MS method for simultaneous quantification of ten endogenous nucleotides with phosphate groups (ATP, CTP, GTP, UTP, dATP, dCTP, dGTP, TTP, CDP, dCDP), which both utilized a porous graphitic carbon column. Different organic mobile phases, modifiers were evaluated and hydrophilic interaction chromatography columns were tested compared with results obtained with the Hypercarb column.

In HPLC-MS analysis, analytes with different properties require different types of liquid chromatography. In this study, high pH reversed-phase liquid chromatography was selected for the analysis of anthraquinone derivatives, and hydrophilic interaction chromatography (HILIC) was chosen for S-adenosyl methionine, S-adenosyl homocysteine, homocysteine and methionine.

POSTER 187

Metaproteomic analysis of the infant fecal microbiome.

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A metaproteomics analysis was conducted on the infant fecal microbiome to characterize global protein expression in 8 samples obtained from infants with a range of early-life experiences. Samples included breast-, formula- or mixed-fed, mode of delivery, and antibiotic treatment and one set of monozygotic twins. Although label-free mass spectrometry-based proteomics is routinely used for the identification and quantification of thousands of proteins in complex samples, the metaproteomic analysis of the gut microbiome presents particular technical challenges. Among them: the extreme complexity and dynamic range of member taxa/species, the need for matched, well-annotated metagenomics databases, and the high inter-protein sequence redundancy/similarity between related members. In this study, a metaproteomic approach was developed for assessment of the biological phenotype and functioning, as a complement to 16S rRNA sequencing analysis to identify constituent taxa. A sample preparation method was developed for recovery and lysis of bacterial cells, followed by trypsin digestion, and pre-fractionation using Strong Cation Exchange chromatography. Samples were then subjected to high performance LC-MS/MS. Data was searched against the Human Microbiome Project database, and a homology-based meta-clustering strategy was used to combine peptides from multiple species into representative proteins. Bacterial taxonomies were also identified, based on species-specific protein sequences, and protein metaclusters were assigned to pathways and functional groups. The results obtained demonstrate the applicability of this approach for performing qualitative comparisons of human fecal microbiome composition, physiology and metabolism, and also provided a more detailed assessment of microbial composition in comparison to 16S rRNA.

POSTER 188

Prediction-based reduction of the search space leads to increased identifications in metaproteomics without impacting sensitivity.

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Metaproteomics search databases typically take on enormous sizes since the *a priori* unknown composition of metaproteomics samples requires the inclusion of proteomes of hundreds to thousands of species that could potentially be found in the samples (Blackburn *et al.*, 2016). A major consequence is that the identification rate in metaproteomics experiments remains drastically below the identification rate in single-species proteomics. Therefore, reducing the database size will not only decrease computation time, but can simultaneously increase identification rate.

To reduce database size, I used predictions from the machine learning algorithm CP-DT (Fannes *et al.*, 2013). This algorithm, originally intended to predict likely tryptic cleavage sites based on an ensemble of decision trees, has been shown to also be a useful predictor of the likelihood of observing a given peptide in a proteomics experiment. Indeed, if a large database (1.85 million protein sequences) is *in silico* digested using CP-DT, most peptides are marked as highly unlikely to be observed by the mass spectrometer. Moreover, if the peptide search space is reduced to only the top-35% scoring peptides according to CP-DT, more than 95% of the peptides that were actually observed by the mass spectrometer, are recovered.

From preliminary results I can conclude that a CP-DT-based reduction of the search space indeed leads to an increased identification rate, while the false discovery rate (FDR) remains under control.

POSTER 189

Influence of the gut microbiota on histone acetylation through butyrate oxidation

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The gut microbiota is a diverse microbial community that inhabits the human intestinal tract and accomplishes functions related to host defense, digestion, and immunoregulation. Given that numerous conditions, such as inflammatory bowel disease (IBD), are associated with a disrupted microbiota, investigating the molecular interactions that occur between the microbiota and host is important for understanding the pathogenesis of disease and devising effective therapeutics. One mode of interaction involves small molecule metabolites, such as butyrate. Butyrate, a product of bacterial fermentation, has long been known to inhibit histone deacetylases, which represent one of the many chromatin-modifying enzymes that regulate gene expression by controlling the post-translational modifications on histones. Thus, the microbiota has the potential to broadly modulate host cell epigenetics and gene expression. Accordingly, using quantitative mass spectrometry, we have observed that mice lacking a microbiota have reduced levels of histone acetylation in the colon, possibly because of unopposed HDAC activity in the absence of butyrate. However, since butyrate is metabolized by colonocytes, the loss of acetylation may also stem from less oxidation of butyrate to acetyl-CoA, which is the necessary cofactor for histone acetylation. Indeed, through isotope tracing analyses performed in cell culture and in mice, we have demonstrated unequivocally that butyrate and inulin, a fermentable plant polysaccharide, provide carbon for histone acetylation reactions. Ongoing work is focused on mapping the precise genomic locations of the reduced acetylation in germ-free mice and the consequent effect on gene expression, determining the relative contribution of butyrate to histone acetylation as an HDAC inhibitor versus a source of acetyl-CoA, and analyzing acetylation levels in IBD. Overall, our findings will advance insight into how the microbiota influences host cell epigenetics and gene expression programs, which may be relevant to the pathogenesis of inflammatory disorders, such as IBD.

POSTER 190
Multi-omic profiling of the liver in a rat model of type 2 diabetes

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Altered glucose metabolism via insulin resistance is a hallmark of type 2 diabetes (T2D), clinically observed as the inability to maintain postprandial blood glucose levels (BGL). Associated with energetic excess arising from caloric overload, T2D is linked to excess non-esterified fatty acid production and rising nutrient levels, which influence metabolic processes. The liver plays a pivotal role in the pathogenesis of T2D, via elevated gluconeogenesis, whereby glycogen stores are liberated, further elevating BGL. It is important to understand the molecular adaptations of the liver to the metabolic flux and insulin resistance arising from T2D. To achieve this we performed a multi-omic analysis including proteomics, lipidomics and metabolomics in a rat model of T2D combining the effects of high fat diet feeding (calorie overload) and streptozotocin (elevated BGL). To quantify alterations in protein abundance, samples were isobarically tagged prior to mass spectrometry (MS). Discovery lipidomics was achieved with relative quantitation by comparison with synthetic standards. Targeted metabolomics was performed using multiple reaction monitoring, in the presence of deuterated metabolite standards. We quantified close to 7,000 proteins, 300 lipid species and 100 metabolites in the course of this study. Proteomics revealed increased levels of proteins regulating phospholipid biosynthesis and fatty acid metabolism. A concurrent decrease in proteins involved in steroid biosynthesis was observed. Lipid analysis show increased sphingomyelin levels and decreased levels of phosphatidylcholines in T2D, both of which are components of cell membranes and can play a role in metabolic and apoptotic signalling. Elevated levels of branched chain amino acids as well as changes in metabolites indicative of altered energy and amino acid metabolism were detected by metabolomics. The current study has identified changes in protein, lipid composition and metabolite levels indicative of dysregulated energy utilisation and molecular adaptations that contribute to the pathogenesis of T2D.

POSTER 191
An Integrated Analysis of Proteomics, Peptidomics, Metabolomics, and Inflammation Markers for Assessment of Pre-analytical Variability of Human Plasma

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Introduction: Deviations in blood and plasma processing prior to analysis are potential confounders in biomarker research. Thus, the aim of this study was to investigate the impact of pre-analytical variables on proteins, peptides, metabolites and inflammation markers in human plasma.

Methods: Human EDTA blood samples were collected from 20 self-reported healthy volunteers and processed to plasma under the following pre-analytical conditions: immediate blood processing at a centrifugal force of 2,500×*g* (Control) or 1,300×*g* (Lowxg), prolonged blood incubation for 6 hours at 0°C (B6h0C) or room temperature (RT, B6hRT) before processing to plasma, and prolonged plasma incubation for 24 hours at 4°C (P24h4C) or RT (P24hRT) before freezing. All plasma samples were stored at -80°C prior to analysis. Proteins were measured using the SOMAscan assay, peptides and metabolites were analyzed using mass spectrometry-based peptidomics and metabolomics, and inflammation markers were measured by multiplex immunoassay.

Results: Plasma proteins were most affected by Lowxg and B6h0C, resulting in a significant change of 15% and 11% of 1305 tested proteins. Metabolites were most affected by B6hRT and P24hRT, resulting in a significant change of 20% and 11% of 237 quantified metabolites. Peptides were most affected by P24hRT and P24h4C, resulting in a significant change of 40% and 23% of 43 quantified peptides. Inflammation markers were most affected by P24hRT and B6h0C, resulting in a significant change of 41% and 14% of 22 quantified inflammation markers.

Conclusion: Delays in EDTA blood and plasma processing have a major impact on the short-term stability of proteins, peptides, metabolites and inflammation markers. To achieve reliable results and ensure reproducibility, we generally recommend performing sample processing at 0-4°C and reducing the incubation times of blood and plasma to a minimum. In addition, for proteomics analysis, blood processing to plasma should be done at RT with consistent centrifugal force.

POSTER 192

Proteogenomic approach reveals translation from untranslated regions in gastric cancer

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Translation initiation and termination are key steps in translation and expression of gene products and contribute to the diversity of protein forms from a single gene. In molecular genetics, translation usually initiates at a START codon (usually AUG) and terminates at a STOP codon (usually UAA, UAG or UGA). However, this mechanism has been overlooked in terms of protein expression, regulation, and interaction.

In this work, we found the alternative translation initiation and termination at untranslated regions (UTRs) by assuming the exceptional mechanisms, such as leaky scanning or translational read-through. To identify alternative selection of translation initiation and termination, we constructed a novel protein sequence database, "tUTRDB (translated UTR database)," which included in silico translation of sequences from UTR. In doing so, we assumed that the START codons applied with single nucleotide variants (SNVs) in 5'-UTR can serve as an alternative START codon. Similarly, we assumed that SNVs to the STOP codon can possibly give a new signal to adopt an alternative STOP codon. Over 100 million mass spectra obtained from proteome in cancer patients were analyzed using tUTRDB. A multi-stage method was used to effectively identify novel peptides while distinguishing them from noise and artefacts in mass spectrometry and controlling their error rate.

In the analysis of 80 tissue samples from patients with early onset gastric cancer, we could identify 454 unique peptides (290 in 5'-UTR and 164 in 3'-UTR regions) as potential translated UTR peptides. Further bioinformatics analysis showed that these newly found 5'-UTR peptides were likely to be expressed due to alternative translation initiation.

POSTER 193

The mechanisms for the formation of proteome complexity revealed by multi-omic analyses

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During evolution, proteome complexity increased substantially due to the emergence of new genes, the increasing of gene/protein complexity, the expansion of isoforms produced by the same gene and diverse structural conformations from the same protein sequence. Here, we report the new understanding of the mechanisms for the formation of proteome complexity from the analyses based on the high-throughput genomic and gene expression data of representative species.

Firstly, from the viewpoint of gene complexity and age grade, we found that complex genes tend to be utilized preferentially at each developmental stage and each adult OTCs (organ, tissue or cell types). In contrast, young genes tend to be expressed in specific spatiotemporal states. These results indicate that complex and young genes contribute to organismal complexity at two different levels: complex genes contribute to the complexity of individual proteomes in certain states, whereas young genes contribute to the diversity of proteomes in different spatiotemporal states.

Secondly, at the isoform level, for more than 90 percent of the ubiquitously- expressed genes, there is at least one isoform which is expressed ubiquitously across different spatiotemporal states. There are complementary relationships among the isoforms which are not expressed widely. For most of genes, there are positive correlations between isoform length and expression width, indicating the complex isoforms are required widely.

Thirdly, the contribution of disordered proteins/domains to the complex protein structure and interaction network was explored. We found that younger protein domains tend to be highly disordered, in particular in higher eukaryotes. The disordered proteins/domains prefer to participate in complex regulatory functions, and tend to have higher PPI connectivity. The increasing requirement of complex functions during evolution may facilitate the emergence of disordered proteins/domains.

Our work provides new insights into the mechanisms of the formation of proteome complexity at three levels: gene, isoform and protein structure.

POSTER 194

A High Throughput Single Platform For High Throughput Quantitative MultiOmic Studies

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A high throughput targeted UPLC-MS/MS single platform, employing a reversed-phase gradient separation, has been developed for the quantification/monitoring of small molecule metabolites, lipids and peptides. The platform employs a single LC column and mobile phase combination which allows the analysis of multiple analyte classes with either positive or negative ion MRM detection.

The use of metabolic profiling (metabonomics/metabolomics) to discover biomarkers of organismal response to environmental and physiological change is now widespread. In biomedical applications metabolic profiling is being deployed as a method for finding novel, mechanistic, biomarkers of disease with obvious potential for improving diagnosis, and patient stratification. Hypothesis driven metabolomics delivers detailed qualitative and quantitative analysis on specific pathways or classes of metabolites, allowing researchers to analyse the effects of disease or treatments in greater detail.

These targeted assays usually employ "bespoke" methods which are optimized for each pathway or metabolic class making multiplexing assays difficult. We have developed a single analytical LC-MS/MS platform which is rapid, simple and reliable. The methodology employs a single LC column / mobile-phase combination which facilitate bile acids, biogenic

amine, free fatty acids, acyl carnitines, lipids and 100 protein panel. This single platform approach has been employed for the analysis of plasma from a liver cancer study, showing excellent throughput and sensitivity.

POSTER 195

A comprehensive integrative multiomics investigation of malaria and Dengue

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Malaria and dengue are life-threatening infectious diseases, which manifest similar febrile symptoms. The severe forms of vivax malaria and complicated dengue hemorrhagic fever (DHF) are posing serious health issues. There is urgent need to identify prognostic markers for severity as well as understand disease pathogenesis to save large population from the febrile infectious disease mortality in India and other parts of the world.

In our study we have used comprehensive integrative multiomics approach including proteomics and metabolomics of the host serum and proteomics of the parasite (i) to understand the underlying pathobiology, (ii) to identify signatures of disease severity and (iii) to discover biomarkers for rapid disease diagnosis. A quantitative serum proteomics analysis of severe and non-severe vivax malaria patients as compared to healthy controls provided evidences for the modulation of diverse physiological pathways including oxidative stress, cytoskeletal regulation, lipid metabolism and complement cascades in severe vivax malaria. Metabolomics analysis confirmed modulation of pathways similar to those identified in our proteomics studies. Metabolites such as proline, 3-nitrotyrosine, dihydropteroic acid, harderoporphyrin, and others like homogentisic acid, indoleacetaldehyde, phenylacetic acid and alicyluric acid were up and down regulated in severe cases respectively suggesting their role as makers for severity.

A study of the proteome of P. vivax clinical isolates revealed highly abundant parasite proteins. Few proteins such as such as tryptophan-rich antigen, Pv-fam-d protein, Plasmodium exported protein, Pvstp1 and a hypothetical protein were detected in more than 80% of the patients. Simultaneously, we also identified a few merozoite surface proteins, metabolic enzymes and a putative uncharacterized protein amongst others in the sera of vivax patients for evaluation as diagnostic antigens. Our findings provide new avenues to tackle the challenges of febrile infectious disease diagnosis and enhances our understanding of disease pathogenesis and severity.

POSTER 196

Proteogenomics landscape of dehydration-afflicted grasspea: new insights into stress tolerance

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Grasspea is a legume known as an excellent source of protein and antioxidants, besides other nutritional traits. It is notable for its water-use efficiency as a stress tolerant species. Despite superior morpho-physiological attributes, grasspea has remained outside the realm of systematic molecular profiling. Three-week-old grasspea seedlings were subjected to gradual dehydration over a period of 144 h by withholding water. The physiological responses of grasspea were construed by an increase in ROS, disruption in membrane integrity and osmotic imbalance during 72-96 h of dehydration. We evaluated the temporal effects of dehydration at the proteomic, transcriptome and metabolome levels using 2-DE, RNA-seq and GC-MS. The RNA-seq data was used to generate an *in-house* database for grasspea (LSDB), which aided in proteomic identifications. Next, we compared the differentially expressed mRNAs and their translated products so as to obtain insights into post-transcriptional regulation. The dehydration-induced differential proteomic, transcriptomic and metabolomic analyses, revealed 120 proteins, 5201 genes and 59 metabolites, of various functional classes. The proteogenomics analyses highlight crucial metabolic pathways intimately associated with dehydration-responsive signal transduction, presumably orchestrated by proteins belonging to an array of functional classes. The dehydration tolerance and/or avoidance mechanism of grasspea appeared to be enforced through remodulation of dehydration-responsive proteins belonging to protein biosynthesis, protein folding, photosynthesis and cell defense. We report, for the first time, the dehydration-induced proteogenomics landscape of grasspea, whose genome is yet to be sequenced. The cross-species comparison of the proteomes, transcriptomes and metabolomes provides evidence for potential biomarkers in grasspea, which may be useful in crop improvement program.

POSTER 197

Proteomic and Proteogenomic Heterogeneity of HeLa Cells across Laboratories: Implications for Research Reproducibility

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Reproducibility is a cornerstone of scientific research. In this study, we aim to thoroughly explore the molecular variations in a given cell line with the "*same name*" across different laboratories.

We comprehensively assayed the genome, transcriptome, and proteome dosages, as well as proteostasis and phenotypic responses for 14 HeLa cell variants across 13 laboratories worldwide. Pervasive copy number variation (CNV) differences

were discovered between Hela cells, which are organized by domains, large chromosomal segments, and even whole chromosomes. Collectively, we quantified transcripts for 11,365 genes (average RPKM>1) by mRNA-seq. Using the SWATH mass spectrometry, we confidently quantified 5,030 proteins (1% FDR by PyProphet), spanning a range of 100 to >10E6 copies per cell. We further determined the proteome-wide protein turnover rate by pulse-chase SILAC (pSILAC) labeling followed by SWATH-MS for 2,084 proteins in each HeLa cell line. Based on these aggregate multi-omic datasets, we discovered significant heterogeneity between HeLa variants, especially between lines of the CCL2 and Kyoto variety. Importantly we observed progressive divergence (i.e., 6-7% of genes expressed) within a specific cell line over 50 successive passages. We discovered organelle-specific proteome remodeling and buffering of protein abundance by protein complex stoichiometry, mediated by the adaptation of protein turnover rates. By associating quantitative proteotype and phenotypes we identified protein patterns that explained the varying response of the different HeLa lines to Salmonella infection. Finally we generated a HeLa Proteome Website to navigate and contemplate the data.

Altogether, we show that HeLa cells from different providers can yield diverse gene expressions and varied biological experiment results even under identical culturing conditions, and thus provided a new angle for understanding the broad reproducibility crisis. As a further study, we are assessing the protein turnover diversity of different splice variants by the matched mRNA-seq and protein turnover data of the same samples.

POSTER 198
Personal Proteogenomic Analysis Using Haploid Genome Assemblies

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Mass spectrometry approaches are widely used for the analysis of proteomes, providing insight into protein changes across individuals, tissues, and diseases. However, these analyses often do not take into account the genetic makeup of the individuals from which the sample was obtained. The ENTEx project (part of the GTEx project), is focused on deep profiling of selected overlapping human tissues across individuals using shared technologies. This initiative has generated RNAseq and full haploid genome sequencing of multiple tissues from multiple individuals. Using these same paired samples we have generated protein mass spectrometry data, conducting both TMT quantification experiments to accurately compare protein expression across the tissues and individuals, and deep proteome experiments to maximise the proteome coverage achieved. We have developed an approach to search proteomics data using transcript assemblies built from the personal haploid genomes of individuals and their corresponding RNAseq data. Our personal proteogenomics pipeline aims to identify variant peptides, detect alternative splicing events, and discover allele specific expression across individuals and tissues. We demonstrate that a large number of tryptic peptides are specific to each individual and are missed in proteomics searches against reference protein sequence databases. Our identifications and pipeline also form the basis for a workflow using proteomics data in the annotation of personal genomes as part of the GENCODE project.

POSTER 199
An integrated atlas of protein expression in human cancer derived from publicly available datasets

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DNA and RNA-based omics technologies have been successfully applied to profile primary tumours and the corresponding cancer cell line models. These studies often include hundreds or thousands of samples. In contrast, while measuring the de facto functional units of a cancer cell, and a large proportion of potential cancer therapeutic targets, proteomics studies are usually much smaller in scale.

Here we have mined the literature and public proteomics resources to discover individual cancer-related quantitative proteomics datasets and integrated them in a consistent manner. Most of them were obtained from the PRIDE database. A joint reanalysis of over 7.5k raw mass spectrometry (MS) files (using the MaxQuant computational platform) allowed identification of 14964 and 11990 genes across 148 popular cancer cell lines, and 231 individual clinical tumour samples (originating from 11 cancer types), respectively. After extensive benchmarking, a robust normalisation strategy was applied to remove systematic biases and ensure high quality of the quantification.

Unsupervised hierarchical clustering of the resulting proteomics profiles allowed us to recover some broad groups related to the cancer lineage and tissue of origin. Integrating proteomics with RNA-seq measurements showed relatively high correlation between RNA and protein abundances within cell lines (Spearman = 0.46-0.68). Furthermore, protein expression in cell lines generally mirrored that of primary tumours. However, we were able to identify some cell lines as potentially poor models of tumours at the proteomics level, as was reflected by the low correlations of protein abundance among them.

Finally, we applied machine learning approaches to determine whether cell line sensitivity to anti-cancer compounds can be predicted directly from protein expression and whether proteomics features are more informative than other omics data types.

Overall, this work provides a unique reference dataset of protein expression in multiple cancer types and can be used to advance our understanding of cancer biology.

POSTER 200
Multi-Omic Characterisation of Bladder and Lung Carcinomas using a Novel Scanning Quadrupole DIA Acquisition Method

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Cancer is one of the most complex, life threatening diseases, existing in many forms which have unknown pathogenesis. A

combination of genetic and lifestyle factors are known to contribute towards increasing the probability of encountering cancer. Plasma samples were prepared for proteomic, metabolomic and lipidomic analyses. Label-free LC-MS data were acquired using a oa-QTof platform utilizing a broadband acquisition technique (SONAR) data independent acquisition workflow.

Small molecule analysis consisted of using Progenesis QI for data processing to provide normalised values prior to statistical analysis. Unsupervised MVA of the data showed clear distinction between cohorts. OPLS-DA was used to filter for features of significant correlation and covariance prior to identification using HMDB (metabolites) and LipidMaps (lipids). Identifications matching the following criteria, mass accuracy <5 ppm, ANOVA p <0.05, %CV <30 and fold change >2 were considered for further interrogation. SONAR-based analysis indicates that the scanning quadrupole DIA enables over an order of magnitude more specificity than a static quadrupole operated with the same resolution and it was found that a quadrupole transmission window of approximately 10 Da provided optimum identifications.

Proteomic data were processed using Progenesis QI for Proteomics and searched against a Uniprot *Homo sapien* database, containing reviewed entries and limited to 1% FDR. Additionally, we also searched the data against a spectral library using Spectronaut and comparatively analysed the results from both workflows. A number of significant proteins with differential regulation were exhibited for a number of protein groups involved in antigen and lipid binding. Proteins occurring in a minimum of two out of three replicates and with ANOVA p <0.05 were considered. Biological significance of the results was established by merging data from all three omic experiments and performing pathway analysis. A number of significant pathways including complement activation, B cell mediated immunity and receptor signalling were identified as key pathways.

POSTER 201
Phenome Study on the Tissues of Esophageal Squamous Cell Carcinoma

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Esophageal carcinoma is a life threating disease, while importantly, most cases of esophageal squamous cell carcinoma (ESCC) are found in China. Even with great effort, the molecular mechanism and clinical diagnosis of ESCC has remained to be resolved yet. The new approach thus becomes an urgent issue in this field. In this study, for the first time we are pursuing the molecular subtypes of ESCC and the dysregulated pathways relevant to the ESCC tissues based upon phenomic strategy.

Fourth paired tissue samples (ESCC and its adjacent regions) derived from strict diagnosis criteria were collected, and the phenomics, including transcriptome, proteome and metabolome, was employed to analyze these samples. At transcriptome level, among the 18,263 genes quantified in all the samples, 4,670 were defined as differential ones between ESCC and adjacent tissues, including 271 participating in lipid metabolism. The information related with alternative splicing and mutation in each sample was also acquired upon RNA-seq data. At proteome level, a new dataset generated from Swissprot database and the alternative splicing and mutation reference database from RNA-seq data was adopted for protein, and DIA mode was taken for quantifying protein abundance. The abundance correlation between mRNA and protein was found in a relatively poor relationship. The impact of genetic variation onto the abundance of proteins was further assessed, indicating that the effect of LoF (loss of function) mutations on the respective protein abundances prone was down regulated. At metabolome level, 980 differential lipid features were perceived between ESCC and its adjacent tissues. The integration of the data from lipid-related mRNAs, proteins and lipids suggested that the abundance responses at both transcriptome and proteome levels to ESCC were in well agreement with the abnormal lipid metabolism in ESCC, especially for the dysregulated pathways involved in metabolism of sphingolipids, arachidonic acid and fatty acids.

POSTER 202
Proteomic profile of the hippocampus from patients with mesial temporal lobe epilepsy reveals the molecular mechanisms related to disease progression

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Mesial temporal lobe epilepsy (MTLE) is the most common form of epilepsy in adults, leading to refractory seizures in about 40% of patients, some of whom can benefit from epilepsy surgery. In most patients with MTLE hippocampal sclerosis (HS) is identified in pathological examination, which is usually resected during surgery. Here we performed laser-microdissection to isolate the neurons from the hippocampal Dentate gyrus (DG) of two groups of patients: G1 with less than 20 years of seizures onset (N=5), G2 with more than 20 years (N=5) and compared them with controls from autopsy (N=5). We used label-free proteomics, and the analysis was performed using an LTQ-Orbitrap Elite. For the bioinformatics analysis, we used ProteomeDiscoverer and Perseus software. Overall, we identified 5140 protein groups, after filtering these to all valid values, we obtained an average of 2100 proteins. In the G1 we identified 208 differentially expressed proteins, and in the G2 we identified 171. The mainly differentially expressed proteins in G1 were upregulated, and in the G2 they were predominantly downregulated. Interesting, we have identified the TAU protein downregulated in the G1 but not in the G2. In addition, we identified the b-amyloid protein downregulated in the G2 patients but not in the G1. These results were also found when we look deeper into the enriched pathways and biological processes of the two groups of patients, revealing that there are remarkable differences between patients according to disease duration.

POSTER 203

Development of the CSF-PR 2.0 tool for exploring cerebrospinal fluid mass spectrometry biomarker datasets - updated with new data

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The growing amount of mass spectrometry generated datasets require user-friendly tools for storing and exploring of the published data. We developed CSF-PR 2.0 (http://probe.uib.no/csf-pr-2.0) as an online data repository and interactive tool for exploration of mass spectrometry generated datasets from quantitative cerebrospinal fluid biomarker datasets, focusing on multiple sclerosis, Parkinson's disease and Alzheimer's disease. Using stringent criteria, we mined scientific databases (PubMed and Web of Science) and extracted recent high-quality mass spectrometry experimental data for inclusion in CSF-PR 2.0. The freely available online tool can be used to browse, filter, visualize and compare these published datasets, from various research groups, in a user-friendly environment.

Since the release, several new papers fulfilling our stringent criteria have been published, and we have continuously updated the tool with new datasets, now presenting quantitative data for thousands of cerebrospinal fluid proteins. Keeping CSF-PR 2.0 up-to-date with the latest research is important to keep the resource relevant and useful for the community. As the resource grows with more and more datasets, it will become easier to obtain a more comprehensive overview of how protein abundances change between these disease groups and piece by piece a larger picture can be drawn.

Further updates, such as the addition of absolute protein measurements in patient samples, are currently in development.

POSTER 204

Network based integration of proteomic and genomic data unravels new key astrocytic players in ALS

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Amyotrophic Lateral Sclerosis (ALS) is an untreatable and fatal disorder characterized by progressive loss neurons in the spinal cord and in the brain, leading to muscle paralysis and to a variable degree of dementia. A number of ALS-causing genetic mutations have been suggested to trigger neuronal pathology both in familial forms representing about 10% of all cases and in sporadic cases. Superoxide dismutase-1 (SOD1) mutation is one of the commonest familial form, which have been extensively studied and is known to share neuronal pathomechanistic pathways with other disease forms. However, in addition to neurons, now glial cells, such as astrocytes, emerge as important contributors to pathology, yet the precise manifestation of mutations remain elusive. In order to identify key disrupted molecular pathways in astrocytes, we integrated ALS-related genome wide association data with published proteomic and transcriptomic datasets deriving from human astrocytes affected by a SOD1 mutation. We used a network propagation approach with community detection via short random-walks, to define gene modules enriched in genetic drivers and SOD1 mutation related molecular changes. We have found that the concordant defect in KIF5, ACADS, RPS4Y1 protein and transcript levels in SOD1 astrocytes overlaps with disease-driving gene modules in a broad spectrum of mutations. This highlights potentially targetable common astrocytic pathways in ALS, which is a subject of our current biological investigation.

POSTER 205

The role of circulating extracellular vesicles in systemic response to ischemic stroke

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Background

Ischemic stroke is the result of vessel blockage, impairing blood supply within a brain region, inducing neuron depolarization and necrotic cell death in the ischemic core, surrounded by an area of apoptotic cells. Besides local events, a systemic reaction is also triggered by the activation of acute-phase response, which is mediated by the release of acute-phase proteins (APPs) in the blood. APPs can be detected in the circulation before signs of inflammatory response in the brain.

Extracellular vesicles (EVs) are recognized messengers of intercellular communication and have been focus of attention due to potential function in prognosis. Since EVs protein cargo is disease-dependent, this work used mass spectrometry-based proteomics to investigate the role of circulating microvesicles and exosomes after brain injury as the promotor of a systemic response.

Methods

Plasma from mice subjected or not to permanent middle cerebral artery occlusion (p-MCAo) was collected after 12h. Microvesicles (MVs) and exosomes (Exs) were isolated by ultracentrifugation. After protein extraction and tryptic digestion, chemical labeling (iTRAQ) was adopted, followed by HILIC fractionation and nLC-MS/MS analysis.

Results

Using this strategy, we were able to identify a total of 940 MVs and 794 Exs proteins. Relative quantification of proteins identified with two or more peptides showed 131 MVs and 128 Exs regulated proteins between mice subjected to p-MCAo and sham (Limma test q-value<0,05). Among those, 96 and 103 were up-regulated while 35 and 24 were downregulated in microvesicles and exosomes, respectively.

Immune system process, oxidoreductase activity and cellular response to IL-4 were among the overrepresented biological processes while pathway prediction analysis showed "Inflammatory response" among overrepresented annotations (FDR5%).

Conclusions

Induction of ischemic stroke in mice can modify in the protein cargo of blood stream circulating EVs after 12h. Microvesicles and exosomes bear different protein content.

Keywords

Ischemic stroke; extracellular vesicles; systemic response; proteomics

POSTER 206

Phosphoproteomic analysis of the dorsal Dentate Gyrus laser-microdissected from the hippocampus of an animal model of mesial temporal lobe epilepsy

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Mesial temporal lobe epilepsy (MTLE) is the most frequent form of epilepsy in adults, present in 40% of patients, many of whom do not respond to clinical treatment. In most patients with MTLE, there is a distinct lesion in the mesial temporal structures, including the hippocampal formation. The dentate gyrus (DG) is an integral part of the hippocampus and may be subdivided into dorsal and ventral regions in a longitudinal disposition in rodents. Despite its similar neuronal composition, the dorsal and ventral portions of the DG have different connections with cortical and subcortical areas. We performed a phosphopeptide enrichment of the dorsal DG laser-microdissected from an animal model of MTLE (N=5). First, we labeled the samples using TMT10-plex, to increase the amount of proteins whiting the pool. After pooling the samples, we performed a phosphopeptide enrichment using the High-select TiO2 kit from ThermoFisher, following the manufacturer's instruction. The samples were analyzed using an LTQ-Orbitrap, and for the bioinformatics, we used MaxQuant and Perseus software. Our preliminary analysis showed 163 phosphorylated protein groups (400 peptides), 20 of which were differentially expressed. Compared with the non-enriched fraction, this number represents 33% of the total proteins identified. Because the yield of microdissection is minimal, only 25ug of protein for each sample, the enrichment process posed a significant challenge. Therefore, additional procedures are being applied to improve the current protocol used. Techniques that take advantage of small amounts of proteins are essential when performing isolation of specific cell populations to enhance the specificity of the information obtained using high-throughput technologies.

POSTER 207

Sex-dependent differences in hippocampal proteome from organotypic slice cultures.

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Organotypic brain slice cultures are an important tool for studying biochemical and physiological properties of neuronal circuits since it preserves the cytoarchitecture and synaptic circuits of the intact brain structure. The hippocampal organotypic culture serves as a model for studying neurodegeneration, neurotoxicity, neuroinflammation and neuroprotection. Many central nervous system functions and pathological conditions are sexually dimorphic, a characteristic determined in mammalian brain by cell architecture, neurochemical composition and susceptibility to several disorders even in the neonatal period. Our goal was to investigate whether sex differences can be also attributable to the hippocampal proteome per se in the organotypic culture. Using LC-MS/MS, a total of 4615 proteins were identified, with at least two unique peptides and protein FDR of less than 1%. Analysis showed that 1291 proteins presented differences in relative abundance between male and female hippocampus. KOBAS pathway analysis indicated that metabolism was the most significantly enriched term (corrected p-value = 0.024). Regarding carbohydrate metabolism, proteins involved in glycolysis (19 annotated) and pentose phosphate pathways (8 annotated) presented higher abundance in females (17 and 7 from the total annotated for each pathway, respectively). In amino- and nucleotide sugar (8 annotated) and pyruvate metabolisms (13 annotated), the same pattern of abundance in females was observed (7 and 9, respectively). Surprisingly, from the 26 proteins annotated to oxidative phosphorylation, 25 were increased in males. Concerning lipid metabolism, proteins of fatty acid (FA) biosynthesis and elongation (12 proteins) were more abundant in females (10 proteins), though FA synthase were increased in males. In FA degradation pathway, all the 20 proteins annotated were in higher concentration in females. This study highlights sex differences in hippocampus proteome as an important criterion to understand the mechanism of certain diseases and to develop efficacious therapeutic tools to treat diseases that present a predilection for one sex.

POSTER 208

Early changes in the human hippocampal proteome at the onset of tau burden

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Background: Alzheimer's disease (AD) is the most common neurodegenerative disorder being characterized by progressive impairment of memory and cognition, resulting in dementia. Deposition of extracellular amyloid β peptide (Aβ) and intracellular hyperphosphorylated protein tau are among the major pathological hallmarks. Deposition of Aβ and tau aggregates follows predictable patterns where tau burden consists of 6 stages (I-VI) according to Braak/Braak (B/B) histopathological system. Nevertheless, it remains to be fully elucidated which processes are dysregulated by tau early in disease course. In this work, we investigated alterations that occur in the proteome of human hippocampus (CA1) at the onset of tau burden (B/B III and IV), using a mass spectrometry based approach.

Methods: Post mortem human brain samples (n=11) were obtained from the Human Brain Tissue Bank (Semmelweis University). Samples were classified into disease stages according to tau pathology (B/B stages) and prepared with a modified filter-aided sample preparation method. The nLC-MS/MS analysis was performed on a Q Exactive mass spectrometer equipped with an EASY- nLC 1000 system. Database search was done with Proteome Discoverer v2.1. Data analysis was performed with Perseus, DAVID and String.

Results: In total, 3156 protein groups were identified (protein FDR< 0.01 and at least 2 peptides/protein). In total, 156

proteins were differentially expressed (ANOVA, p< 0.05, Fold Change≥ 1.5). Hierarchical clustering segregated dysregulated proteins into two major expression profiles, clusters up- and down-regulated in AD. Calcineurin complex, protein kinase C and synaptic pathways were significantly enriched in AD B/B IV samples. Fatty acid metabolism, gene expression and splicing pathways were enriched in controls.

Conclusions: This work identified changes in the hippocampal proteome at the onset of tau burden in humans (B/B III/IV). Altered pathways might represent early triggers of neurodegeneration in this brain region helping to unravel mechanisms underlying AD.

POSTER 209

Intron-mediated enhancement boosts Rtn4 circRNA expression: A robust method for exploring circRNA function

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CircRNAs are expressed in many important biological processes. Studying their function

requires an effective expression method. When we used intron-mediated enhancement (IME)

to improve circRNA expression of the mouse Rtn4 (Nogo, a key protein in Nogo-Rho

pathways) circRNA as a test case, we achieved a 4-6-fold improvement compared to an

existing method. We further developed this approach into a general circRNA expression

vector pCircRNA-DMo. An unexpected feature of our approach is its ability to promote

translation of circRNA into detectable amounts of proteins. Intriguingly, both monomer and

multimer peptides can be observed as a result of rolling circle translation of RTN4 circRNA.

We also confirmed the presence of both peptide forms in human and mouse brains,

highlighting the significance of circRNA translation in vivo. In summary, we demonstrate the

significant advantage of IME in enhancing circRNA biogenesis and hence our vector offers a

robust platform for exploring potential circRNA peptide-encoding functions.

POSTER 210

Histone β-hydroxybutyrylation in neuroblastoma cells

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Extensive studies over the past few decades have shown that post-translational modifications (PTMs) of the histone proteins are critical to the regulation of chromatin activities, such as transcription. Histone lysine acylations are derived from diverse metabolite intermediates, indicating their potential roles linking gene transcription with cellular metabolism. We recently identified lysine β-hydroxybutyrylation (Kbhb) as a new type of histone mark, which is induced under ketogenic conditions such as prolonged starvation and type I diabetes mouse model. After prolonged (48h) starvation, induced histone Kbhb in mouse liver is associated with increased expression of genes that are enriched in several neurodegenerative disease pathways as well as starvation responsive metabolic pathways. Interestingly, prolonged starvation also induced histone Kbhb levels in mouse brain, although to a lesser extent. Ketogenic diet has long been used to treat epilepsy, and numerous studies have suggested a neuroprotective role of β-hydroxybutyrate in mouse models of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, etc. Therefore, it may be of great importance in studying the function of histone Kbhb in the human neural systems. In the present study, we set out to examine the role of histone Kbhb in gene regulation in two neuroblastoma cell lines, human Shep and SH-SY5Y cells. Mass spectrometric analysis identified 30 histone Kbhb sites in Shep and SH-SY5Y cells. Both Western blotting and SILAC based quantification demonstrated that β-hydroxybutyrate significantly increased histone Kbhb levels but not histone acetylation.

We are currently looking for regulatory enzymes that can modulate levels of histone Kbhb marks both in vitro and in vivo.

POSTER 211

'Silver nanoparticle-induced expression of proteins related to oxidative stress and neurodegeneration in an in vitro human blood-brain barrier model'

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Silver nanoparticles (AgNPs) have been reported to penetrate the central nervous system (CNS) and induce neurotoxicity. However, there is a paucity of understanding of the toxicity of AgNPs and their effect on the blood-brain barrier (BBB) including the underlying molecular mechanism(s) of action. Using an in vitro BBB model and mass spectrometry-based proteomics, we investigated alterations in the proteomes of brain endothelial cells and astrocytes at different time points after AgNPs exposure (24 and 48 hours). Our data showed that several proteins involved in neuro-disorders and neuro-degeneration were significantly upregulated in endothelial cells while proteins responsible for maintaining brain homeostasis were significantly downregulated. Pro-inflammatory pathways were significantly upregulated at 24 hours post-AgNPs exposure, while at 48 hours proteins involved in BBB damage and anti-inflammation were significantly upregulated, suggesting that by the later time point, protection pathways had been activated to rescue the cells from AgNPs-induced toxicity. Our study suggests that in the initial stage of exposure, AgNPs exerted direct cellular stress on the endothelial cells by triggering a pro-inflammatory cascade. This study provides detailed insight into the toxic potency of AgNPs on BBB model

and adds to the understanding of the adaptive role of BBB with regards to metallic toxicity.

POSTER 212

Proteomic analysis of rat hippocampus exposed to 10-day morphine treatment and subsequent 20-day drug withdrawal

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Opioid addiction is recognized as a chronic relapsing brain disease resulting from repeated exposure to opioid drugs. Cellular and molecular mechanisms underlying the ability of organism return back to the physiological norm after cessation of drug supply are not fully understood. Proteomic analysis was performed in post-nuclear supernatant fraction (PNS) prepared from hippocampus of rats exposed to increasing doses of morphine (10-40 mg/kg) for 10 days and sacrificed 24 h (groups ±M10) or 20 days (groups ±M10/−M20) after the last dose of morphine. We utilized 2D-DIGE and MALDI-TOF MS/MS analysis. The 8 altered proteins were identified in group (+M10) when compared with group (−M10). Surprisingly, this number was increased to 15 after 20 days of abstinence (groups ±M10/−M20). Among those 15 proteins, the highly altered level of α-synuclein, β-synuclein, α-enolase, and glyceraldehyde-3-phosphate dehydrogenase persisted for 20 days since the drug withdrawal. Immunoblot analysis of 2D gels by specific antibodies oriented against α/β-synucleins and GAPDH confirmed data obtained by 2D-DIGE and MALDI-TOF MS/MS. This result is just the opposite when compared with that observed in forebrain cortex, where the number of altered proteins was decreased from 28 (groups ±M10) to 14 (groups ±M10/−M20) when determined by gel-based analysis or from 113 (groups ±M10) to 19 (groups ±M10/−M20) when determined by label-free quantification. Our work indicates that morphine-induced change of protein composition of rat hippocampus is different from that of forebrain cortex. The two functionally distinct parts of CNS respond to disturbance of the homeostatic balance of organism by drug addiction in a different manner with the aim to restore the physiological norm.

POSTER 213

Unveiling the mice cerebellum proteomic changes under the effect of rattle snake *Crotalus durissus terrificus* venom

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Snake venoms have been used in traditional medicine for the treatment of several diseases. Despite the inherent difficulty in the development of new drugs from natural products, the advancement of technologies such as MS-based proteomics has allowed researchers to find key molecules for the diagnostic and treatment of several diseases. Although several works has described the biochemical and physiological characterization of the snake venoms effects on tissues or cells, there are few studies characterizing the proteomic profile of the effect of snake venom on organs and tissues aiming at the evaluating the affected molecular targets. In this work we analyzed the proteomic profile of cerebellum of mice inoculated with the neurotoxic venom of the rattle snake *Crotalus durissus terrificus* in order to detect key proteins and/or pathways affected by this venom. Swiss mice were inoculated with the venom at 0.5 LD50 (0.5 µg/ mouse) for 2h, 12h, 24h and, 48h. Cerebellum label-free semi-quantitative LC-MS/MS analysis of protein abundancy at these time points revealed that among the 8,113 different proteins identified, 1,095 proteins were up (>2.0) or down (< 0.5) –regulated at some time point when compared to 0h control. Fifty nine, 36, 36 and 49 proteins increase abundancy after 2h, 12h, 24h, and 48h, respectively, most of which related to binding and catalytic activity. Interestingly enough, proteins such as UPF0449, succinate CoA ligase, and Ankyrin repeat domain presented fold changes over 10x when compared to 0h. Moreover, proteins such as ubiquitin-conjugated enzyme E2D3 and PTC7 protein phosphatase homolog, down-regulate after 2h or 12h after venom administration and up-regulate again after 48h. This analysis shows us the molecular complexity of the envenoming and highlights the potential for finding target proteins that are modulated in some neurological diseases, turning snake venom or some of its components a potential therapeutic agent.

POSTER 214

A Label-free Quantification Approach to Identify Differentially Expressed Proteins between Wild Type and Transgenic Alzheimer Rat Brains

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Alzheimer's disease (AD) is the most common form of dementia with age being the largest risk factor. Amyloid-beta (Aβ) plaque deposition in the brain is a hallmark of AD pathogenesis. A transgenic (Tg) rat model was recently developed expressing mutant human amyloid precursor protein (*APP_{sw}*) and presenilin 1 (*PS1ΔE9*) genes, which are known to cause early-onset familial AD. These rats manifest age-dependent AD pathologies as well as cognitive dysfunction. In order to identify proteome-wide network alterations in this transgenic AD (Tg-AD) rat model at different ages, we performed proteomic analyses of the frontal cortex of 23 wild-type and Tg-AD rats (6, 12, and 20-month old). Briefly, the tissues were homogenized followed by trypsin digestion and peptides were resolved by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) on an Orbitrap Fusion platform. Protein identification and quantification was performed using the label-free quantification (LFQ) algorithm in MaxQuant. Our proteomics data verify presence of the human isoform of amyloid beta precursor protein (APP) in Tg-AD rats, the expression of which is positively correlated with age. Preliminary weighted gene co-expression network analysis (WGCNA) identifies additional proteins (e.g., CLU, LAMP1, APOE) that also show strong positive correlation with age. These proteins are mainly associated with microglial and astrocytic cell types and are upregulated in aged Tg-AD rats compared to wild-type controls. Conversely, some proteins, which are enriched with astrocytic markers (e.g., KBTBD11, SNTB1, SLC1A2), show reduced expression of proteins with age; however, most of them are not significantly different between WT and Tg-AD rats. There is another category of proteins (e.g., ACAT2, ASNA1, MLYCD) that are upregulated in Tg-AD rats but do not show any age dependence. In conclusion, we identified genotype and age-dependent differential expression of proteins between wild-type and transgenic Alzheimer rats that have relevance to the development of Aβ plaque pathology.

POSTER 215

Quantitation of Intact Proteins in Human Plasma Using Top-Down Parallel Reaction Monitoring-MS

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Direct quantitation of proteins in complex biological matrices by mass spectrometry remains a challenge. Here, we describe a novel top-down parallel reaction monitoring-mass spectrometry (PRM-MS) assay, enabled by microflow LC-nanospray MS using a silicon microfluidic LC-MS chip. We demonstrated direct analysis of intact proteins such as somatropin in human plasma, achieving sensitivity (0.1−1.0 fmole) and speed (1−5 min) on par with enzyme-linked immunosorbent assay (ELISA).

POSTER 216

Micro Pillar Array Columns: A novel robust chromatography platform for deep and reproducible proteome coverage

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Bottom-up proteomics relies on the use of 50 cm long packed columns, coupled to mass spectrometry to analyze digested protein samples. Micrograms of samples are injected and separated using nanoliter flowrates and longer solvent gradients. However, gradient times above 240 minutes using packed column have resulted only in marginal gains in identification. Both robustness and reproducibility do not yet fulfil the promise of routine use of such workflows.

PharmaFluidics' µPAC™ technology (micro Pillar Array Column) utilizes a unique and novel approach, taking a chromatographic support structure that builds upon micromachining chromatographic separation beds into silicon. This results in exceptional properties in terms of chromatographic performance, repeatability and reproducibility, flexibility and robustness.

Chromatographic performance of the µPAC™ is demonstrated in nanoflow (300 nL/min) and capillary flow (1 µL/min), as well as for short and longer gradients using commercially available retention standards.

Column efficiencies are demonstrated using 1 µl direct injection runs from a dilution series of HeLa-digests (ranging from 0.01 to 1 µg/µL), coupled to high resolution mass spectrometry. Highest efficiencies are shown using nano flowrates and increased throughput capabilities using capillary flowrates.

Long term stability and repeatability is illustrated using a 6 months continuous column evaluation, running sequences of Cytochrome C standard, HeLa-digest and blanks (1 hour runs, 3526 injections in total, 1000 HeLa-digest injections) showing less than 1% retention time variation for the Cytochrome C peptides over the whole period.

Further robustness is demonstrated using three sets of samples that are either contaminated with detergents routinely used in the sample preparation (NP-40; Triton X-114) or contain precipitating compounds. Whereas these samples consistently prove to be problematic for packed bed columns in terms of retention time deterioration or column clogging, the µPAC™ columns show stable pressure profiles and a retention time variation below 2%.

POSTER 217

Comprehensive Proteome Mapping of a human cancer cell line using LC-FAIMS Pro-MS/MS

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Data-dependent LC−MS/MS strategies applied to complex biological samples aim to maximize the number of identified peptides. Limitations in LC separation and MS dynamic range presently restrict identifications in single shot proteomics experiments to only ~50,000 unique peptides and 6,000 protein groups with a 2 hour gradient. To circumvent these limitations, samples are often pre-fractionated prior to LC-MS/MS analysis. However, pre-fractionation is laborious and can considerably increase the sample preparation and data acquisition time.

We analyzed human cancer cell line samples (HeLa) with an Orbitrap Fusion Lumos Tribrid MS coupled with FAIMS (High Field Asymmetric Waveform Ion Mobility Spectrometry) Pro and an Easy-nLC 1000. FAIMS separation is carried out using a 5000 V_{p-p} bi-sinusoidal waveform operating at a frequency of 3MHz. The ion separation gap between the inner and outer electrode was 1.5 mm producing a high field strength dispersing high abundance multiply charged ions between -40V to -100V. Compensation voltages (CV) between -50 V and -85 V were exploited with a 1.0 ms CV switch time to characterize FAIMS gas-phase fractionation. Peptide and proteins were identified with Proteome Discoverer 2.3 and filtered with 1% FDR.

The Orbitrap Fusion Lumos Tribrid MS typically collects ~220,000 MS/MS spectra during a single two hour data-dependent LC-MS/MS analysis of HeLa sample. This relatively large pool of MS/MS spectra converts to ~70,000 PSMs, ~50,000 unique peptides, and ~6,000 protein groups. Identical analysis with FAIMS Pro produces ~270,000 MS/MS, ~100,000 PSMs, ~ 70,000 unique peptides, and ~8,000 protein groups. This translates into 25% more MS/MS, 40% more PSMs, 40% more unique peptide sequences, and 33% more proteins. We demonstrate that FAIMS coupled with an Orbitrap Fusion Lumos Tribrid MS can drastically increase peptide identifications from a single-shot experiment and can mitigate the need for sample pre-fractionation.

POSTER 218

Fast Microflow Chromatography for Accelerating Protein Identification Experiments

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Background: Proteomics has typically been done using nanoflow LC for sensitivity but the time to results slow. With higher flow rates, sample can be loaded faster, trap/column can be washed and equilibrated faster, and gradients are formed faster, allowing much faster run times to be achieved. When sample run times are important, microflow LC offers a great

alternative for higher-throughput proteomics. Here, the impact on protein identification results due to faster gradients was explored.

Methods: Microflow LC was performed on the TripleTOF® 6600 System using the nanoLC™ 425 system plumbed in microflow mode. Trap-elute workflow was used and a range of gradient lengths from 5 – 45 mins was explored, and key acquisition parameters were varied to optimize for the much faster run times. Data was processed with ProteinPilot™ Software 5.0 as well as a prototype version of ProteinPilot running in the Cloud environment.

Conclusions: Using digested cell lysates, gradient lengths ranging from 5-45 mins were compared and protein identification results were assessed. Fast MS/MS acquisition rates were found to be critical for highest peptide IDs; in multiple tests, the use of 90 MS/MS per cycle with 15msec accumulation times was found to be optimal. As expected, total # of protein identifications decreased with shorter gradients, however the drop was not as large as expected. Only a small drop was observed when shortening the gradient from 45 to 20mins. With the 10min gradient, peptide/protein ID rates dropped by about 60 and 75% respectively relative to 45mins for the three matrices tested (2 instruments), but still provided >1500 protein and >8500 peptide IDs. The full optimization results will be presented.

As data can be collected so quickly, use of ProteinPilot in the cloud was also explored. Initial observations indicate time to results will be significantly improved, further exploration is underway.

POSTER 219
EASYpep - A New Simplified and Optimized Workflow for MS Sample Preparation

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Advances in mass spectrometry (MS) instrumentation has enabled routine analysis of complex protein samples. However, sample preparation methods are not standardized with many protocols taking 8-24hrs in addition to suffering from low peptide yields, poor digestion efficiency and low reproducibility. Here, we describe a simplified sample prep kit containing pre-formulated reagents and a standardized protocol that can be used to efficiently process 10µg to 100µg protein samples in less than 2 hours. In this study, we evaluated the scalability, compatibility, and reproducibility of this sample preparation kit compared to previous published methods.

Protein extraction from cells and tissues was evaluated using cell lysis buffer and universal nuclease for nucleic acid disruption compared to standard sonication methods. A rapid (<10min.) reduction/alkylation solution was developed as well as a combined trypsin/LysC protease mixture for protein digestion. A mixed mode peptide clean-up procedure using a novel spin column format was used for detergent removal. Peptides were quantified and normalized using the Pierce™ Quantitative Colorimetric Peptide Assay prior to LC-MS analysis using a Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap™ mass spectrometer.

Our new standardized workflow yielded 10-20% higher number of peptides and proteins with lower missed cleavages (<90%) compared to other commercial kits or homebrew methods. We demonstrate that our optimized protocol reduces hands on time to less than 30 minutes with total sample processing time from intact cells to cleaned-up peptides under 1.5 hours. Finally, we show this procedure is compatible with isobaric labeling reagents such as Tandem Mass Tags (TMT) and Label Free Quantitative (LFQ) methods to reproducibly quantify protein abundances. The protocol has been successfully tested with several sample types such cell lines (HeLa, CHO, A549, 293), purified proteins and various mouse tissues (heart, liver, lung). Overall, our new kit greatly simplifies proteomic sample preparation for protein identification and quantitation.

POSTER 220
Universal Sample Processing for Highly Reproducible Proteomic Sample Preparation of Diverse Sample Types

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Irreproducibility in initial sample preparation is typically the highest source of variability in bottom-up proteomics. Protein extraction and solubilization remain challenging due to the extremely diverse solubilities of proteins, the very different physical-chemical properties of different biological samples and, especially for tissues, the difficulty in reproducibly accessing and thus extracting proteins.

Here, we present a unique sample preparation system for reproducible protein extraction applicable without change to a wide range of sample types. This approach is enabled by combining the Covaris Adaptive Focused Acoustics (AFA) technology; the solubilizing power of 5% SDS; and ProtiFi S-Trap technology. AFA imparts strong controlled acoustic forces to homogenize samples and force proteins into solution (especially with 5% SDS), thus fully extracting them. S-Trap sample processing rapidly concentrates proteins, cleans them of contaminants (including 5% SDS), and quickly digests them in-column. The combined system is designed for maximum ease-of-use, throughput, yield and reproducibility. It consists of: 1) sample dry pulverization (as needed) by Covaris cryoPREP at –196 ºC; 2) thermally controlled AFA lysis and protein extraction in 5% SDS; and 3) ProtiFi S-Trap processing to concentrate, clean and digest proteins. Our system is highly scalable. All steps can be performed in a 96-well plate format.

We apply this system without change to multiple tissues, including extremely labile samples like pancreatic tissue and tough samples like compact bone. We compare these results to the use of a bead beater. We demonstrate that this unique Covaris/ProtiFi system: 1) has the highest reproducibility; 2) produces the highest protein yield; and 3) reproducibly extracts all cellular compartments. We also demonstrate this combined proteomic workflow on Formalin-Fixed Paraffin-Embedded tissues with reverse crosslinking.

We anticipate the combined workflow of Covaris AFA and ProtiFi S-Trap sample processing will enable reproducibility in bottom-up proteomics and support the translation of proteomics into clinical applications.

POSTER 221
Toward the ideal mass analyzer with data-independent acquisition and parallel accumulation – serial fragmentation (diaPASEF)

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In LC-MS/MS based bottom up proteomics, state-of-the-art mass spectrometers efficiently transfer ions into the vacuum, but mass analyze only a small fraction of the ion beam. Data-independent acquisition (DIA) methods often employ small mass isolation windows to increase specificity and thus utilize only about 1-3% of all available ions. In principle, a 100% duty cycle could be achieved by parallel ion storage and sequential release from a trapped ion mobility (TIMS) device into a quadrupole time-of-flight mass analyzer. Synchronizing the ion release from the TIMS device with the quadrupole (PASEF) increases the MS/MS sequencing speed by more than 10-fold without any loss in sensitivity in online DDA experiments (Meier et al., doi:10.1101/336743). Here, we asked if the PASEF principle can be transferred to DIA, combining the advantages of both.

We adapted the firmware of a prototype timsTOF Pro instrument (Bruker) with a customized scan mode that employs the correlation of mass and ion mobility to full advantage. Analysis of the four-dimensional data space (intensity, retention time, ion mobility and mass) has been incorporated into the OpenSWATH software. For a single TIMS scan, we defined up to 24 DIA windows as a function of the TIMS scan time. Depending on the isolation window width, the majority of the multiply charged ion species could be sampled in every single TIMS scan - approaching a 100% duty cycle. Conversely, narrower isolation windows increase specificity, however, at the loss of duty cycle and thus sensitivity. We will show results obtained from multiple schemes for precursor selection window size and placement to balance specificity and duty cycle for different amounts of whole-cell proteome digests.

In summary, the 'diaPASEF' method presented here captures and utilizes a very large proportion of the available ion current – going a long way towards the ideal of a mass analyzer.

POSTER 222
Magnetic HILIC microparticles enabling automated offi-line MS sample preparation

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In proteomics research an increased sample cohort is required to detect small, yet significant, modifications in the proteome related to onset and/or progression of a disease. However, sample preparation is a bottle-neck and remains the *Achilles Heel* of mass spectrometry analysis, with current methods lacking throughput, transferability, and reproducibility required to deal with large sample numbers. To address these limitations, we are currently developing workflows consisting of modular, robust, automatable, versatile steps for routine Mass Spec sample preparation in Proteomics. This research outlines several workflows for robust clean-up of proteins and peptides utilizing magnetic HILIC microparticles. The aim is to provide a variety of methods suitable for the vast array of possible samples that require MS analysis. We have developed 2 HILIC-based workflows for protein (with on-bead reduction) as well as peptide preparation. These workflows showed excellent recovery as well as performance against currently used methods. Compatibility of the protein clean-up workflow has been illustrated using a wide range of routine detergents, salts, and chaotropes. In this work we further evaluate the effect of different wash buffers for contaminant removal as well as the compatibility of HILIC clean-up of peptides against common contaminants encountered in Proteomics experiments. We also investigate the effect of buffer composition for on-bead digestion and peptide recovery.

POSTER 223
Short LC-gradients for high throughput and deep shotgun proteomics using PASEF on a TIMS equipped QTOF

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High sample throughput in proteomics, similar to that in genomics, is highly desirable. Moreover, the highest analytical depth in proteomics is only achieved on fractionated samples, requiring subsequent analysis with short gradients to achieve reasonable overall measurement times per sample. The timsTOF Pro with trapped ion mobility spectrometry (TIMS) offers additional separation power and increased peak capacity. The powerful Parallel Accumulation Serial Fragmentation (PASEF) method (Meier et al., JPR 2015) for very high sequencing speed is perfectly suited for proteome analysis on short gradients. We have optimized MS conditions, column lengths and LC overhead times to obtain runs of 28.8 min injection to injection (50 samples/day) on the nanoElute (Bruker Daltonics). TIMS gas phase separation for high peak capacity and fast acquisition allows identifications of more than 4200 protein groups from 250 ng of a proteolytic digest of a human cancer cell line (HeLa) in 28.8 min injection to injection, enabling the analysis of large sample cohorts within a reasonable depth and measurement time. To demonstrate analytical depth achievable with short columns, offline LC-fractionation was performed on a HeLa digest and digests of murine cerebellum. Samples were fractionated on high pH reversed-phase columns into 24 concatenated samples. Subsequent measurement allowed the identification of more than 100.000 unique peptides and more than 9.000 protein groups in less than 12 h of measurement time. We conclude that analysis on short gradients with the timsTOF Pro provides both, high throughput for a large number of samples without sacrificing proteome depth. Running short gradients on the timsTOF Pro with pre-fractionated samples provides an optimized strategy for proteome wide identifications of peptide collisional cross sections (CCS) and enables fast generation of libraries for DDA and DIA.

POSTER 224
Cutting-edge nanoLC column technology and its capabilities in advanced mass spectrometry proteomics

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Nanoscale liquid chromatography-mass spectrometry (nanoLC-MS) has been proven to be the most powerful tool for proteomics research. Cutting-edge high-resolution nanoLC-tandem MS (MS/MS) enables characterizing >10,000 proteoforms (1%FDR) for >1,500 proteins from top-down approach and measuring >6,500 proteins and >55,000 peptides (1%FDR) from bottom-up approach by using a simple single run of a single-dimension experiment. The state-of-the-art of ultra-sensitive nanoLC-MS/MS provides a sensitivity that allows for performing proteomics characterization of a single cell (single cell proteomics) with promising proteome coverage broadness of >2000 proteins. Characterization of protein phosphorylation modifications can also be carried out with ultra-sensitive mode for small proteomic targets; for example, identifications of >5,000 phosphopeptides (1%FDR) and >2,000 phosphorylated proteins from samples containing a total protein content of <1 microgram. Proteomics data quality commonly quantitated by the number of peptides/proteoforms/proteins assigned is controlled by nanoLC performance when a specific mass spectrometer is applied for data acquisition, while different nanoLC formats are typically required to achieve best datasets for different proteomic tasks. This presentation presents the proteomic analysis coverage and sensitivity achievable with use of cutting-edge nanoLC- MS/MS platforms for situations of most bottom-up and top-down proteomics applications.

POSTER 225
Comparison of two solid-phase extraction (SPE) methods for the identification and quantification of porcine retinal protein markers by LC-MS/MS

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Proper sample preparation protocols represent a critical step for liquid chromatography-mass spectrometry (LC-MS)-based proteomic study designs and are influencing the speed, performance and automation of high-throughput data acquisition. Main objective of this study was to compare two SPE-based sample preparation protocols (SOLAµTM HRP SPE spin plates from Thermo Fisher Scientific and ZIPTIP® C18 pipette tips from Merck Millipore) regarding analytical performance, protein recovery and analysis speed. The house swine (Sus scrofa domestica) represents a promising animal model for studying human eye diseases including glaucoma and provides excellent requirements for the qualitative and quantitative MS-based comparison in terms of ocular proteomics. In total 6 technical replicates of two protein fractions (extracted with 0.1% DDM or 1% TFA) from porcine retinal tissues were subjected for in-gel trypsin digestion and purified with both SPE-based workflows (N=3) prior LC-MS/MS analysis. On average both protein fractions (DDM and TFA) provided the identification of 550±70/305±48 proteins after ZIPTIP® purification protocol and SOLAµTM workflow resulted in the detection of 513±55/300±33 proteins (FDR<1%). Venn diagram analysis revealed an average overlap of 65±2 % (DDM fraction) and 69±4 % (TFA fraction) regarding protein identifications between both SPE-based methods. Quantitative analysis of 24 glaucoma-related marker proteins also showed no significant differences (P>0.05) regarding protein recovery between both SPE methods. Solely marker protein MECP2 showed a significant (P=0.02) higher abundance in ZIPTIP®-purified replicates in comparison to SOLAµTM-treated study samples, but was not confirmed by verification experiment (P=0.24). In conclusion, both SPE-based purification methods worked equally well regarding analytical performance and protein recovery, whereas the analysis speed and the semi-automatic properties of the SOLAµTM spin plates workflow is much more convenient in comparison to the manual ZIPTIP® C18 pipette tip protocol.

POSTER 226
Maximum MS utilization in high-throughput and deep dive low-flow LC-MS proteomics

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Introduction

Common caveats of low-flow LC-MS methodology such as long column washing, equilibration and sample loading times have hitherto prevented its adoption in application areas where high throughput and maximum MS usage combined with sensitivity are paramount. Using novel, intelligent single and tandem LC-MS methods, we show how low-flow LC techniques can be used to deliver efficient, high throughput robust analytics with almost constant MS data acquisition for both fast, screening, as well as deep-dive applications, thus shattering the common myths associated with such workflows.

Methods

The UltiMate 3000 RSLCnano system in nano-flow pre-concentration configuration was combined with the Q Exactive HF-X to separate peptides from HeLa cell lysate protein digests on either an ES800 EASY-Spray column for single LC-MS or a pair of Acclaim PepMapC18 columns for tandem LC-MS applications. DDA data were processed with Proteome Discoverer™ 2.2 software. The false discovery rate for peptides and proteins was set at 1%.

Results

We developed fast low-flow LC methods which permit the analysis of 180 samples in 24 hours yielding robust peak performance with consistent reproducible DDA data. Excellent peak characteristics were achieved with peptide retention time S.D.s below 0.1 min peak area variation not exceeding 10 %, and PWHM below three seconds.

Furthermore, we conceived a novel tandem low-flow LC setup capable of near 100% MS utilization and high-throughput analysis which eliminates MS instrument idle time and boosts LC-MS productivity to > 200 samples per 24 hours whilst generating minimal carryover.

We then show how the novel intelligent method attributes can be implemented into a tandem nanoLC for a deep dive into the proteome that enables the identification of > 5000 protein groups and > 40K peptide groups per 90 min gradient whilst still achieving a throughput of 16 samples per 24 hours.

POSTER 227
Proteome level de novo sequencing with a pair of newly developed mirror proteases of super-LysargiNase and Ac-Trypsin

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De novo peptide sequencing for an individual pure protein has improved remarkably with the progress of mass spectrometry but there still exists incomplete peptide fragmentation and indistinguishable series of fragmented ions, which interrupted the interpretation of amino acid sequence of MS2. This is even worse at proteome level. Here, we developed a series of high efficiency proteases, including LysargiNase with super activity and acetylated trypsin with high specificity. Taking advantage of these paired mirror proteinases, we developed a novel algorithm, pNovoM for automated de novo sequencing. The combination of paired mirror image spectra results nearly complete series of product ions, which facilitate the de novo sequencing on both of purified protein and proteome samples.

POSTER 228
Results As Soon As Possible (rASAP): 2 hours from lysis to label free quantification of cells and tissues using subtilisin

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We have previously demonstrated the use of the broad specificity protease subtilisin for efficient, reproducible and fast digestion of lysates. Subtilisin enables quantitative (phospho)proteomics and provides access to novel, particularly Pro-rich areas of the (phospho)proteome. Here, we developed an optimized protocol rASAP, spanning a total of two hours from homogenization/lysis to database search, including carbamidomethylation, BCA assay, digestion and LC-MS/MS analysis. rASAP is compatible with label free quantification (LFQ) of organelles, cells and tissues: We could identify 753 proteins from yeast mitochondria, 1752 from HeLa and 777 from mouse heart with at least one unique peptide at 1% FDR. To evaluate whether rASAP can be used for rapid LFQ, we analyzed human fibroblasts from healthy individuals and patients with a mutation in VCP (c.464G>A), in biological triplicates. VCP encodes for the transitional ER ATPase and is required for the fragmentation of Golgi stacks and the formation of the transitional ER. The mutation causes an inclusion body myopathy, characterized by disabling muscle weakness, osteolytic bone lesions consistent with Paget disease, and premature frontotemporal dementia (IBMPFD1; MIM: # 167320). Using PeptideShaker and Progenesis we quantified 1423 proteins at 1% protein FDR, 1140 with >2 unique peptides, with high reproducibility across replicates. We detected significant proteomic changes in line with the neuropathological phenotype, in good correlation with complementary trypsin-based data. A network of secreted proteins of the extracellular matrix showed was significantly reduced, including alpha-collagens, thrombospondin-1 and 2, tenascin-X, gremlin-1, serine protease HTRA1 and the procollagen C-endopeptidase enhancer 1. This is in agreement with the demonstrated role of VCP in secretion via modulation of ER-Golgi trafficking. Neuronal vulnerability is indicated by reduced levels of LSAMP, and proteins involved in neuronal/axonal regeneration such as tenascin-X, adducins and DCLK1 might be of potential therapeutic relevance.

Additional patient samples are being analyzed using subtilisin-based MRM assays.

POSTER 229
Snapshots of the EGFR signaling pathway acquired with high temporal resolution using a microfluidic device

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Quantitative phosphoproteomics is routinely applied for the in-depth analysis of protein phosphorylation events in cells and tissues. A precise understanding of cellular signaling, however, requires individual snaphots of the phosphoproteome that also reflect its dynamics, ideally with the potential to provide temporal resolution down to the second range. Manual sample preparation is not suited for studying early time points of signaling (< 10 s) and lacks reproducibility (errors > 1 s). Therefore, we developed a microfluidic micromixing device (M2D) that allows automated stimulation and lysis of cells with temporal resolution of 100+/-80 ms. We combined M2D with quantitative phosphoproteomics to study early stages of EGFR signaling. We demonstrate that cell and ligand-containing solutions are completely mixed after 100 ms, which is in good agreement with computational modeling. Using HEK293 cells we demonstrate, that passage through M2D does not induce cellular signaling and that technical replicates for 0.5, 1.0, 3.0 and 5.0 seconds of EGFR stimulation lead to consistent results. Next, we quantified around 19,000 phosphopeptides (phosphoRS >99%) across different time points of EGFR stimulation. Our data allow following signal propagation over time. The EGFR pathway shows regulation already after 3 s, whereas MAPK and mTOR signaling pathways showed no significant regulation even after 10 s of EGF stimulation. We developed targeted PRM assays using stable isotope labeled reference standards (SIS) for more than 40 phosphorylated and non-phosphorylated peptides of the EGFR pathway and followed their absolute changes between 0.1 s and 10 s of EGF stimulation. Significant changes on EGFR pTyr sites could be detected already after 0.5 and 1.0 s of stimulation.

In summary, our data demonstrate that large-scale and targeted quantitative phosphoproteomics can be used to generate comprehensive and precise dynamic maps of cellular signaling with high temporal resolution to follow phosphorylation dynamics on the molecular level.

POSTER 230
High Resolution Peptide Separation for Illuminating Human Proteome

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Modern mass spectrometric instruments allow identifying approximately ten thousand proteins from human cultured cell lines with pre-fractionation at peptide level. Further improvement could be achieved by increasing the resolution both in m/z and retention time in LC/MS separation and/or by adding another dimension such as ion mobility to expand the separation space. We have employed meter-long monolithic

silica C18 columns to improve LC separation, Together with high-resolution and accurate-mass (HR/AM) mass spectrometers, ultrahigh resolution LC based on meter-long monolithic silica capillary columns have been employed to unveil the complex human proteomes, resulting in higher sensitivity and specificity in quantitative proteomics. We explored this approach to develop other types of columns such as HILIC, zwitterionic HILIC and anionic HILIC to provide complementary separation selectivity to the C18 silica monolith columns. Using tryptic peptides from human HeLa cell lysate proteins, we identified comparable numbers of peptides and proteins in HILIC with those in RPLC using a C18-modified monolithic silica column when shallow gradients were applied. Since HILIC mode LC/MS shows orthogonal selectivity to RPLC mode LC/MS, it is powerful to use two-dimensional LC modes to increase proteome coverage, although the total measurement time is increased. To solve this issue, we introduced another approach called 'slice-shot proteomics', where only a single representative fraction in the first dimension separation is analyzed by the second dimension LC. Using meter-long nanoHILIC/nanoRPLC/MS/MS with silica monolithic formats, we successfully identified more proteins in slice-shot mode than one-shot mode due to the reduction of the sample complexity. In this presentation, we will also show other examples of high resolution separation-driven proteomics to target particular groups of peptides of interests to unveil the human proteome.

POSTER 231

Developing tools to facilitate blood-based protein biomarker discovery in a non-model organism

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State-of-the-art biomolecular analysis is no longer limited to model organisms and is becoming routine in non-model organisms. Major drivers of this emerging bioanalytical capacity include increasing accessibility and quality of sequenced genomes as well as high-resolution fast-duty cycle mass spectrometers for proteomic analysis. Blood is an easily acquired biofluid, proximate to many tissues and rich in predictors or indicators of disease state. However, it is challenging to identify and measure low abundance proteins due to the large dynamic range of proteins. Data-independent acquisition (DIA) should be able to overcome many of these issues by avoiding the stochastic nature of data-dependent acquisition (DDA) and by providing relative quantification at the MS/MS level. We evaluated this concept in a non-model organism, the Atlantic bottlenose dolphin (*Tursiops truncatus*), and performed both DDA and DIA analysis of plasma digests from 10 individuals using nano-LC-MS/MS with a Thermo Orbitrap Fusion Lumos. Publicly available DDA analysis of digests from six different dolphin tissues and a depleted pool of plasmas were used to enhance the DIA analysis. The DDA analysis identified 189 protein groups (108 to 153 per sample) while DIA identified 403 protein groups experiment wide (269 to 380 per sample). In an experimental replicate the DDA analysis provided count data for 101 protein groups, 59 with a relative standard deviation (RSD) < 20 %. Similarly the DIA analysis generated relative quantification for 260 protein groups, 216 with an RSD<20 %. Ongoing studies are using companion RNA-seq data to identify individual specific transcript variants and will attempt to detect these proteins in plasma. Overall, these results demonstrate that given the right tools it is possible to perform high-quality bioanalytical measurements in a non-model organism. Moreover, these methods exemplify good analytical workflows for plasma analysis, such as utilizing tissue/disease specific spectral libraries to improve specificity and detection.

POSTER 232

Screening and epitope mapping of antibodies for immuno-mass spectrometric assays using a novel immuno-MALDI (iMALDI) approach

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Introduction Immuno-mass spectrometric (MS) technologies improve assay sensitivity by enriching target proteins or peptides before MS analysis. However, those assays rely on the availability and functionality of antibodies, and the screening and epitope mapping of these antibodies is often time-consuming and expensive. In this work, we present a rapid and high-throughput approach for antibody screening and epitope mapping using post immuno-enrichment on-bead digestion immuno-MALDI (PIOD-iMALDI), which will accelerate the assay development process for highly sensitive protein quantification in biological samples.

Methods Antibodies were conjugated onto Protein G functionalized Dynabeads and MagPlex microspheres. The antibody-beads were incubated with PBS buffer containing recombinant antigens for immuno-enrichment and then washed. Next, post-capture digestion was performed by re-suspending the beads in trypsin/LysC solutions. After washing, the beads were transferred to a MALDI plate, matrix solutions were spotted onto the beads, and the epitope-containing peptides were eluted from the beads and analyzed by MALDI-TOF/TOF or LC-MS/MS.

Results As a proof-of-concept, 8 antibodies targeting 7 recombinant-protein fragments were tested. For protein digestion, the ratio of protein:trypsin/LysC (weight:weight) that gave the highest signal-to-noise ratio was found to be 12.5:1. Compared to Protein G Dyna beads, MagPlex beads provided more epitope-containing peptides and higher S/N ratios. We also found that 3 mg/mL of α-cyano-4-hydroxycinnamic acid in the MALDI matrix solution performed the best. After optimization, 7 out of 8 antibodies were found to be suitable for immuno-MS assays and their epitopes were revealed. The sequences of some target peptides were further confirmed by MS/MS using either a MALDI-TOF/TOF or a LC-MS instrument, while others need further optimization to obtain optimal signals. Interestingly, these epitopes were also found while epitope mapping the antibodies on peptide arrays. Future automation of the assay procedure will allow high-throughput screening of antibodies for immuno-MS methods, including iMALDI.

POSTER 233

A genetic approach toward mass spectrometry-based comprehensive and sensitive quantification of yeast proteome

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Recent advances in quantitative proteomics rely on improvement of mass spectrometry performance and analytical platform, and large-scale construction of internal standards derived from endogenous protein sequences to achieve proteome-wide quantification with high coverage and sensitivity. Successful quantification of individual proteins depends on the presence of proteolytic peptides suited for detection by mass spectrometry. However, a subset of proteins, especially of small molecules, have no or limited number of peptides that can be detected with high sensitivity. Furthermore, huge complexity and dynamic range of proteome make it difficult to quantify very low-abundance proteins.

As a strategy to overcome these problems, we present a genetic approach using the budding yeast in which unique artificial peptide-tags suited for mass spectrometric detection are introduced into genomic locus encoding individual proteins. Peptide-tags fused with endogenous proteins are purified and then only peptide-tags that represent the individual protein abundances are analyzed by mass spectrometry, allowing reduction of peptide complexity and sensitive detection and quantification even of proteins with low copy-number. The use of a gene editing method, CRISPR/Cas9 system, makes it feasible to generate yeast cells where unique peptide-tags are fused to a multitude of proteins. Here, we would show the progress in selection of peptide-tag sequences, throughput of tag integration into multiple open reading frames in single yeast strain, and improvement of sensitivity for low-abundance proteins.

POSTER 234
Quantifying the fetal tissue translatome using a novel isotopic labeling approach reveals temporal and tissue-specific regulatory networks during development

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During development, multicellular, complex organisms differentiate from a single zygote into different cell types in a highly ordered and reproducible manner. Precise spatial and temporal regulation of the gene expression program is crucial for normal development. Therefore, determining the precise timing of protein expression in tissues is necessary for understanding the complex regulatory networks involved during development. In this study, we developed a method for quantifying the embryonic tissue translatome, i.e., protein translation rates of various tissues at different stages of mouse fetal development. Using this technique, we relate changes in tissue specific translation rates to activation of various signaling pathways. To determine developmental stage-specific protein translation rates, fetal mice were administered a single pulse of isotopic amino acids via the vitelline vein. This minimally invasive procedure bypasses the need to administer labeled amino acids in the diet of the pregnant mouse and allows for the precise timing of developmental stage-specific protein labeling. Isotopic amino acids circulate throughout the fetus and are transported to developing organs where they are used by the translational machinery for protein synthesis. At specific time points after amino acid introduction, fetal tissue is harvested and subsequently analyzed using quantitative mass spectrometry. Analyzing multiple time points allows us to quantify protein synthesis rates in various tissues including the brain, heart, kidney, liver, and lung. We find that the developing fetus reprograms the proteome in a tissue and stage specific manner.

POSTER 235
Advance Analysis of Proteomics and Peptidomics by Ion Mobility Mass Spectrometry for Biomarker Discovery

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Introduction and Objectives

For biomarker discovery, many trials have been going on by mass spectrometry (MS) using several samples of plasma, tissue, urine or others worldwide. Now a day, technologies of MS are still developing to provide novel systems. In fact, a number of proteins identified with high confidence by MS have increased for decades. However, there are still limitations on the analysis in such as separation and/or fragmentation of proteins/peptides. Here we show the results of proteomics and peptidomics for human urine analyzed by the new technology of Ion Mobility system named PASEF, which can separate peptides by ion mobility prior to MS.

Methods

Human urine samples were obtained from healthy volunteers and the protein was precipitated by methanol/chloroform to digest with trypsin for proteomics. For peptidomics, urine peptides were separated by Molecular Weight Cut Off (MWCO) filtration. Both tryptic peptides and native peptides were purified by C18 spin columns and analyzed by timsTOF Pro MS (Bruker Daltonics) using PASEF. All MS and MS/MS data were analyzed by MASCOT for protein and peptide identification.

Results and Discussion

Proteomics: ≈more than 2,000 proteins were identified by of a single run of one urine sample. This result indicated that roughly 1.5~2 times higher number of identification than that obtained by other MS instruments in our laboratory. Peptide spectrum match (PSMs) and distinct peptide identification also increased compared with previous work.

Peptidomics: In this study, we identified more than 5,000 PSMs and 3,000 distinct peptides. From our result, C-peptide, which absolutely emit to the urine, was identified with intact form.

Conclusion

Identification of proteins and peptides are still improving in this field. Sample preparation, MS analysis method and database search reflect to identification. This new system of MS spectrometry will promote more identification with high confidence and it can be helpful to discover the biomarker in the future.

POSTER 236
Development of a Functional Proteomics Technology for Biomarker and Drug target Discovery

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Proteins play essential roles in numerous biological processes and being able to pinpoint functional differences between cell or tissue samples can greatly aid in understanding disease processes and metabolic changes. The PEP technology allows systematic analysis of protein functions within a proteome. Hundreds of functional proteins can be separated and functionally assayed to generate a comprehensive three-dimensional landscape of protein families such as protein kinases, phosphatases, proteinases and oxido-reductases. This information can then be integrated into other genomic and proteomic knowledge bases to provide further insight of important biological processes such as cancer development and aging.

In the PEP technology, complex protein mixtures are first separated by a modified two-dimensional gel electrophoresis process, giving enhanced resolution while still maintaining protein function. This is followed by an efficient protein transfer to a specially designed 1536-well Protein Elution Plate. After further transfer of the samples from the PEP plate to multiple 384-well microplates, functional assays can be performed on each well to generate an enzyme activity profile displayed in 3-D. Protein components of each well can be further characterized by mass spectrometry if desired.

Using the PEP technology, functional biomarker candidates have been identified from lung and breast cancer patient serum. Both qualitative and quantitative differences of metabolic enzymes and proteases were observed when comparing the cancer patient serum and normal serum. Some of the active enzymes were identified by mass spectrometry and validated in selected bioassays. It is believed that this functional proteomics technology provides a unique approach in the discovery of potential cancer biomarkers for diagnostic and prognostic applications.

POSTER 237
Improved iST workflows for the streamlined analysis of tissues and high-throughput preparation of samples using isobaric labelling

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Recently, the straightforward and robust in-StageTip (iST) method for streamlined sample processing of various sample types including cell lines and body fluids (e.g. plasma, serum, CSF, urine) was described (Kulak et al., Nat Meth, 2014). Here, we present further developments based on the iST technology.

First, we present the iST-NHS adaptation that allows chemical labelling of peptides in the very same reaction device used for cell lysis, protein denaturation, reduction, alkylation and digestion, thus minimising sample loss, overall hands-on time and the amount of required chemical labels.

Using yeast cells, we employed this iST-NHS approach and compared different ratios of tandem mass tag (TMT) reagents per µg of peptides input material. While most labelling workflows utilise a TMT to peptide ratio of 8:1, we found that a ratio of 4:1 results in the highest number of identified peptides while still achieving labelling efficiencies of more than 99.5% using the iST-NHS method. In addition, the iST-NHS method is fully compatible with 96well plate formats and automated robotic systems enabling high-throughput isobaric labelling experiments.

Second, we present an improved iST workflow for the lysis of tissue (fresh brain, heart and liver samples, as well as FFPE samples) directly in the reaction device used for the downstream sample preparation, thus avoiding lysate transferring steps and minimising sample loss. We present identification of 6,000-10,000 proteins for the aforementioned tissue types demonstrating in-depth proteome analysis with minimal sample preparation efforts. With this improved iST workflow, up to 24 tissue samples can be prepared in parallel and in less than 3 hours from wet tissue to ready-to-measure peptides.

POSTER 238
Cell Lysate Microarray for Mapping the Network of Genetic Regulators for Histone Marks

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Proteins, as the major executer for cell progresses and functions, its abundance and the level of post-translational modifications, are tightly monitored by regulators. Genetic perturbation could help us to understand the relationships between genes and protein functions. Herein, to explore the impact of the genome-wide interruption on certain protein, we developed a cell lysate microarray on kilo-conditions (CLICK) with 4,837 knockout (YKO) and 322 temperature-sensitive (ts) mutant strains of yeast (Saccharomyces cerevisiae). Taking histone marks as examples, a general workflow was established for the global identification of upstream regulators. Through a single CLICK array test, we obtained a series of regulators for H3K4me3, which covers most of the known regulators in S.accharomyces. We also noted that several group of proteins that are involved in negatively regulation of H3K4me3. Further, we discovered that Cab4p and Cab5p, two key enzymes of CoA biosynthesis, play central roles in histone acylation. Because of its general applicability, CLICK array could be easily adopted to rapid and global identification of upstream protein/enzyme(s) that regulate/modify the level of a protein or the posttranslational modification of a non-histone protein.

POSTER 239
Development of an efficient proteomics sample preparation method for human gut proteomics

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Human gut harbors trillions of microbes which constitute a very complex ecological community that has recently been implicated in multiple human health and disease conditions. Thousands of microbial species have been identified in human gut, primarily based on metagenomics work. These microbes through a very complex network of proteins and metabolites influence the host physiology. To evaluate their contribution toward host physiology, meta-proteomic and meta-metabolomic studies are critical. Gut microbial proteomics currently has a major challenge, which is underrepresentation of a significant proportion of microbial flora due to lack of optimal cell lysis protocols. We evaluated multiple published protocols for extracting microbial proteins from fecal samples and determined that they leave out major portions of microbiota

un-lysed. This leads to underrepresentation of hard to lyse microbial populations as well as less abundant taxa. We have evaluated multiple lysis methods and evaluated the lysis efficiency as well as peptide pool identified by LC-MS/MS on Q-Exactive HF platform. We will present comparative data about three different lysis methods i.e., glass beads based, sonication based and freezer mill based in combination with chemical and enzymatic lysis. We observed significantly improved lysis efficiency and greater representation of hitherto elusive, hard to lyse taxa, when we combined enzyme treatment followed by freezing the sample in liquid nitrogen and crushing it in freezer mill under liquid nitrogen. This improved protocol of proteomics sample preparation from human fecal material has the potential to rapidly advance the human gut proteomics field.

POSTER 240

Plasma proteome signature of sepsis: a functionally connected protein network.

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Sepsis is an extreme host response to infection that leads to loss of organ function and cardiovascular integrity. Mortality from sepsis is on the rise. Despite more than three decades of research and clinical trials, specific diagnostic and therapeutic strategies for sepsis are still absent. We report here the use of LFQ- and TMT-based quantitative proteomics to study the plasma proteome in five mouse models of sepsis. A knowledge-based interpretation of the data revealed a protein network with extensive connectivity through documented functional or physical interactions. The individual proteins in the network all have a documented role in sepsis and are known to be extracellular. The changes in protein abundance observed in the mouse models of sepsis have for the most part the same directionality (increased or decreased abundance) as reported in the literature for human sepsis. We have named this network the Plasma Proteome Signature of Sepsis (PPSS). The PPSS is a quantifiable molecular readout that could supplant the current symptom-based approach used to diagnose sepsis. This type of molecular interpretation of sepsis, its progression, and its response to therapeutic intervention is an important step in advancing our understanding of sepsis, and for discovering and evaluating new therapeutic strategies.

POSTER 241

Quantitative proteomics and phosphoproteomic profiling of THP1 cells after dengue infection

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A piece of the puzzle to understand dengue virus-host cell response involves the identification of proteins expressed in the infected cells as well as their post-translational modifications and the dynamics of the biological processes implicated during virus replication. However, only few large-scale proteomic studies have been focused on dengue virus infection. In this work, isobaric tag for relative and absolute quantification (iTRAQ) and mass spectrometry were used to determine changes in the expression of proteins and phosphoproteins of human macrophage THP-1 cell line infected with dengue virus, at 24 and 48 hours post-infection (hpi). We describe changes in site-specific phosphorylation dynamics of 206 and 208 phosphoproteins at 24 and 48 hpi, respectively. One hundred forty three phosphoproteins were altered in both conditions. Similarly, MS-iTRAQ analysis allowed determining changes in the expression of nonphosphorylated proteins, 10 at 24 hpi and 12 at 48 hpi, respectively. Analysis by gene ontology enrichment showed that most of the proteins identified are phosphoproteins involved in Fc gamma R mediated phagocytosis, endocytosis, cGMP-PKG signaling, cytoskeleton, splicing, MAPK signaling, among others. Our results will help to increase knowledge about dengue biology and pathogenesis.

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POSTER 242

Insights into the Human Pathodegradome of the *Staphylococcus aureus* V8 Protease

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The opportunistic pathogen *Staphylococcus aureus* can cause a wealth of human infections, many of which are systemic in nature as a result of host tissue invasion. Virulence factors, such as secreted proteases, are key to this invasive lifestyle, but efforts to study their targets are limited by the low-throughput nature of classical gene-knockout/tagging or immuno-based approaches. Developments in the field of degradomics, however, means that studying pathogen protease-host interactions on a global scale is within reach. Herein, we investigated the cleavage of human serum proteins by *S. aureus* V8 protease (GluC or SspA) using two different N-terminomics approaches (Terminal Amine Biotinylation, TAB, and Terminal Amine Isotopic Labelling of Substrates, TAILS), followed by LC-MS/MS and peptide assignment using MaxQuant with its integrated Andromeda search engine. TAB resulted in detection of 642 peptides corresponding to biotinylated N-termini in 203 proteins, 53 peptides of which were unique to the V8 protease treated condition, representing putative V8 cleavage sites in 22 human serum proteins. TAILS permitted detection of >1000 peptides with blocked N-termini (i.e. isotopic or native modification of amino groups) across all conditions, with putative cleavage sites in >100 proteins. Targets of V8 included previously established complement system proteins (C1, C3, C4, C6, C8, CFB, CFH) and immunoglobulin chains. Novel targets likely involved in pathogenesis were also uncovered, such as host protease inhibitors (antithrombins, antitrypsins), iron sequestration proteins (transferrin, hemopexin), clotting (thrombin, clotting factors), and inhibitors of *S. aureus* quorum sensing (apolipoprotein B, other apolipoproteins). A shortlist of candidate targets underwent immunoblotting to confirm degradation by V8 protease (including C3, C4, A2M, PrtH, and IGHG1). Ultimately, this work sheds light on the multifarious roles of the V8 protease during *S. aureus* infection and is, to our knowledge, the first global study of host targets for a bacterial protease.

POSTER 243

Systems-Wide Hijacking of Host Cells During Herpes Simplex Virus (HSV-1) Infection

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Herpes simplex virus (HSV-1) successful infection is the result of global modulation of the host cell proteome. This includes harnessing host protein complexes for viral replication and manipulating host chromatin to prevent cell apoptosis and antiviral responses. These fine-tuned events are still mostly unknown, especially when considering how they synergistically act together to trick a eukaryotic cell into producing abundant viral progeny. We present a system level characterization of proteome dynamics during the HSV-1 lytic infection of human foreskin fibroblast cells. We specifically employ an integrative perspective that links cell signaling with changes in chromatin state during virus invasion. Our study includes identification and quantification of the host and viral proteomes, phosphoproteomes, chromatin bound proteomes and post-translational modifications (PTMs) on cellular histones during the time course of infection. Globally, we accurately quantified more than 4,000 proteins, 200 differently modified histone peptides and 9,000 phosphorylation sites on cellular proteins. In addition, we identified 67 viral proteins and quantified 571 phosphorylation events on viral proteins, which is currently the most comprehensive map of the HSV-1 phosphoproteome. This multi-omics dataset was combined by assessing trends in protein/PTM abundance resolved over a time-course, and networking the chromatin associated proteome with modifications on histones. Results highlighted that stress-activated protein kinase activity and histone acetyltransferase complexes were globally upregulated during viral infection. These observations were validated by quantifying a steady increase of histone H3 acetylation and phosphorylation marks that are indicators of open chromatin. For example, the relative increase in abundance of the histone mark H3K9acS10ph highlighted signaling activities activated in cells during infection. Together, our epi-phospho-proteomics data provide an unprecedented systems overview to investigate cross-talking events of the human and viral proteome dynamics during infection. These data reveal potential candidates for biomarker targets that can be inhibited to arrest viral infection and spread.

POSTER 244

Tc-STAMS2: a novel *Trypanosoma cruzi* strain typing assay using MS2 peptide spectral libraries

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Chagas disease also known as American trypanosomiasis is caused by the *Trypanosoma cruzi* parasite. Over the last 30 years, Chagas disease has expanded from a neglected tropical disease to an emergent global health problem worldwide. *T. cruzi* strains were classified to seven genetic groups, named discrete typing units (DTUs) based on genetic, virulence and immunological features. Each DTU has been associated to diverse clinical manifestations related to *T. cruzi* genetic variability. Due to that, several DTU typing methods have been introduced based on genetic signatures. However, limitations exist on the genome sequence knowledge. Here, we aimed to develop a *Trypanosoma cruzi* Strain Typing Assay using MS/MS peptide spectral libraries (Tc-STAMS2).

The Tc-STAMS2 method uses shotgun proteomics combined with spectral library search to assign and discriminate *T. cruzi* strains independently on the genome knowledge. The method is based on the construction of a library of MS/MS peptide spectra built using *T. cruzi* reference strains. For identification, the MS/MS peptide spectra of unknown *T. cruzi* strains are matched against the library using the SpectraST algorithm. The Tc-STAMS2 method allowed correct identification of all DTUs with high confidence discriminating intra-DTU strains. The method was robust towards different sample preparations, length of chromatographic gradients and fragmentation techniques. Moreover, a pilot inter-laboratory study showed the applicability to different MS platforms.

This is the first study that develops a MS-based platform for *T. cruzi* strain typing. Its robustness towards different experimental and biological variables makes it a valuable complementary strategy to the current *T. cruzi* genotyping assays. Moreover, this method can be used to identify DTU-specific features correlated with the strain phenotype and has the potential to be applied to other infectious diseases.

POSTER 245

The Impact of Mutations on Protein Expression Pattern in Intracellular Bacteria *Ehrlichia chaffeensis*

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The rickettsial pathogenic bacteria *Ehrlichia chaffeensis* causes human monocytic ehrlichiosis tick-borne disease in vertebrate hosts and humans. Mutation in *E. chaffeensis* genome aid in better understanding of infection and persistence in host cells and in development of attenuated vaccines. We document the impact of mutations on the pathogen's global protein expression and the influence of protein abundance on mutant's attenuation and protection of vertebrate host against infection. iTRAQ labelling and mass spectrometry analysis of *E. chaffeensis* wildtype and mutants identified 564 proteins covering about 63% of the genome. Mutation in ECH_0379 antiporter gene, causing attenuated growth in vertebrate hosts, led to overexpression of p28 outer membrane proteins, molecular chaperons and metabolic enzymes while a mutation downstream to the ECH_0490 gene, caused minimal impact on the pathogen's *in vivo* growth, impacted major changes in outer membrane proteins, transcriptional regulators and effector proteins expression. ECH_0660 gene mutation, caused the pathogen's rapid clearance and offered protection against infection in vertebrate hosts, had a minimal impact on proteome. The proteomics data offer novel insights in global proteome alterations induced by mutations and how proteomic changes influence pathogen's ability of resistance and protection from the host.

POSTER 246

Culture independent label free method for milk metaproteome and resistome analysis

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Dairy cow mastitis (CM) is the cause of a great financial loss for animal production industry. The average cost of a single case of CM is around $ 179. Late and imprecise diagnose lead to costs increase and to a more difficult therapeutic intervention.

Milk microbiota and, more widely, milk cellular fraction that includes as well somatic cells of mammary gland, represent a great source of biological information.

The main aim of this work is to analyze through proteomics both the bacterial and cellular compartment of raw milk in order to gain information about the whole bacterial composition and about the index of antibiotic resistance.

The cellular fraction isolated from raw milk has been collected and subjected to bead beating to achieve bacterial lysis. After quantitation, precipitated proteins have been digested using Filter-aided sample preparation (FASP) and analyzed through Label-free in ion mobility–enhanced data-independent acquisition (DIA) proteomics analysis by Expression configuration mode (HDMSe). Raw data were analyzed with ProteinLynx Global Server v. 3.0.2 (PLGS, Waters Corp.) against two different protein databases.

Results highlighted the identification of 400 proteins in total, of this ones, 285 proteins were identified as Bos taurus proteins, 115 as bacterial proteins and, 11 of the identified proteins were related to the CARD database and therefore actively involved in antibiotic resistant processes.

This method allowed the culture free analysis of raw milk bacterial consortia and provided an index of intrinsic antimicrobial resistance that could be consistently related to antibiotic contamination in the environment

POSTER 247
Identification of Serine 119 as an effective inhibitor binding site of *M. tuberculosis* ubiquitin-like protein ligase PafA

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Owing to the spread of multidrug resistance (MDR) and extensive drug resistance (XDR), there is a pressing need to identify potential targets for the development of more-effective anti-*M. tuberculosis* (*Mtb*) drugs. PafA, as the sole Prokaryotic Ubiquitin-like Protein ligase in the Pup-proteasome System (PPS) of *Mtb*, is an attractive drug target. Here, we show that the activity of purified *Mtb* PafA is significantly inhibited upon the association of AEBSF (4-(2-aminoethyl) benzenesulfonyl fluoride) to PafA residue Serine 119 (S119). Mutation of S119 to amino acids that resemble AEBSF has similar inhibitory effects on the activity of purified *Mtb* PafA. Structural analysis reveals that although S119 is distant from the PafA catalytic site, it is located at a critical position in the groove where PafA binds the C-terminal region of Pup. Phenotypic studies demonstrate that S119 plays critical roles in the function of *Mtb* PafA when tested in *M. smegmatis*. Our study suggests that targeting S119 is a promising direction for developing an inhibitor of *M. tuberculosis* PafA.

POSTER 248
Ion Mobility High Resolution QTOF MS - Impact of PASEF on detection of post-translational modifications

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Background

Post-translational modifications (PTMs) of proteins are implicated all key biological processes. Signal transduction mechanisms and enzymatic modifications in autoimmune diseases generate dynamic and sub- stoichiometric levels of PTMs. Most strategies aimed at detecting PTMs necessitate efficient enrichment for deep coverage of the proteoforms, however, limitits an unbiased detection of PTMs. In this study we have investigated the impact of chromatographic conditions and ability of PASEF to dig deeper into the PTMed proteome. Furthermore, we have addressed the ability to map PTMs without any known enrichment strategy.

Methods

From tissue extracts obtained from human, mouse and pig origin we isolated the proteome by filter-assisted- sample preparation. Fractions of samples were separated with or without high pI fractionation and analyzed without PTM enrichment by UPLC-PASEF-MS (Bruker timsTOF PRO). The proteome and protein modifications were characterized by database searching using BSI PEAKS PRO as well as MS-GF+ using high-performance supercomputing (HPC).

Results & Conclusion

Initial investigations evaluated the proteome coverage by varying chromatographic strategies ID numbers of 5700-6300 proteins from 200ng material at 1%FDR. Ultimately, a combined analysis of pH fractionated resulted in 12.000+ proteins (>8000 protein groups at high sequence coverage). An unbiased PTM detection strategy including de-novo based sequencing of high quality fragment ion spectra resulted in a higher degree of PTMs per sample load. In conclusion, application of PASEF increases the proteome coverage compared to standard DDA allowing deeper coverage of PTM´s. A drawback compared to enrichment based strategies remain a wide detection of non-modified peptides.

POSTER 249
Multiplexed quantitative analysis of APC/C-specific ubiquitin substrates

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Biological repositories, such as BioGRID currently list more than 13,000 post-translational modifications with ubiquitin and ubiquitin-like molecules (UBLs) on proteins encoded by almost 10,000 genes, indicating that the majority of the proteins encoded by the human genome are modified. Protein ubiquitination plays an important role in the regulation of the proteome in many cellular processes including cell cycle, therefore the deep quantitative characterization of protein ubiquitination can reveal critical ubiquitin ligase-substrate relationships in a biological system. Here we report an optimized isobaric labelling-based quantitative workflow for the identification of target ubiquitin ligase substrates in WT and E3-inactivated cell lines. As a proof of concept, we performed

ubiquitinome analysis of mitotic WT and HeLa cells, where the APC/C has been inactivated by depleting its essential ANAPC4 subunit using an auxin-dependent nanobody. K-ε-GG motif enrichment of ubiquitin peptides by immunoprecipitation was performed using two different antibodies, and the diGG-peptides were labelled by TMT followed by high pH reverse phase fractionation and LC-MS/MS analysis. We quantified over 14,000 TMT labelled diGG-modified unique peptides from ~4,700 proteins. These included 199 ubiquitinated peptides from 38 proteins that are known mitotic substrates of APC/C. Statistical analysis showed decreased ubiquitination levels for a curated list of mitotic APC/C substrates in the absence of the APC/C confirming the utility of the approach in identifying substrates of ubiquitin ligases. Overall we propose a streamline workflow for the deep characterization of ubiquitin ligase-substrate associations.

POSTER 250

Novel antibody reagents for the characterization of protein ADP-ribosylation

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Poly-ADP-ribose polymerases (PARPs) catalyze the transfer of ADP-ribose from β-NAD+ and release nicotinamide in the process. ADP-ribosylation predominantly occurs on amino acid side chains of proteins (such as lysine, arginine, glutamate, aspartate, cysteine, serine), but it has also been described to occur on protein amino termini as well as on DNA and tRNA.1 The most widely studied PARPs (PARP1, 2, 5a and 5b) can synthesize linear or branched chains of up to ~200 ADP-ribose units (PARylation).2 However, there are 13 additional PARPs which transfer only a single ADP-ribose unit to their target residue (MARylation). The best-known function of poly-ADP-ribose chains is to serve as a scaffold for the recruitment of DNA repair proteins that contain PAR-binding modules to sites of DNA damage. ADP-ribosylation is also involved in a variety of additional cellular processes, including cell stress responses, mitotic spindle formation, chromatin decondensation, retroviral silencing, RNA biology, and transcription.3 Even though MAR/PARylation is of central importance to cellular function, there are no commercially available antibodies that recognize both MARylated and PARylated proteins. Therefore, novel rabbit monoclonal antibodies have been produced and characterized against this modification on proteins, and their utility for the detection of ADP-ribosylated proteins by ELISA, western blot, dot blot, and immunofluorescence assays has been validated.

POSTER 251

High Sensitivity Phosphoproteomics using PASEF on a TIMS-QTOF mass spectrometer

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High resolution mass spectrometry-based proteomics has become a powerful tool to study signal transduction pathways. Sensitivity, sequencing speed and peak capacity are prerequisites for deep identification and quantification into the phosphoproteome. Here, we evaluate trapped ion mobility spectrometry (TIMS) in combination with QTOF technology using parallel accumulation-serial fragmentation (PASEF) acquisition mode for deep phosphoproteomic analysis at record acquisition speeds.

Phosphopeptides enriched from proteolytic digests of HeLa cells or primary neurons were nano-HPLC separated (nanoElute, Bruker Daltonics) on a 250 mm pulled column (IonOpticks, Australia) and analyzed on a high-resolution timsTOF Pro mass spectrometer (Bruker Daltonics) using the PASEF acquisition method. Data were analyzed using PEAKS studio (Bioinformatics solutions Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry).

Increased peak capacity provided by the TIMS separation, PASEF sequencing speed (> 120 Hz) and sensitivity using low sample amounts (200 µg of total protein before IMAC enrichment) enabled a deep phosphoproteome analysis where > 7,400 unique phosphopeptides were identified in a 15 min gradient. Increasing gradient length to 90 min and keeping the same load we identified > 17,400 unique phosphopeptides. TIMS also enables mobility separation of co-eluting positional isomeric phosphopeptides, differing only by phosphorylation localization. The mobility separation results in non-chimeric MS/MS spectra allowing both qualitative and quantitative site localization, fundamentally unique to this technology platform. Concatenated phosphopeptide enrichment strategies (Fe-NTA and TiO2) using primary hippocampal neurons (1.25 mg starting material) demonstrated more than 32,800 unique phosphopeptides identified using PEAKS studio in a single injection in a DDA workflow. The results show that TIMS and PASEF provide fast and comprehensive phosphoproteome analysis.

POSTER 252

An approach for the site-specific quantitation of protein core fucosylation in a large scale

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Core fucosylation (CF) represents a special form of N-glycan modification of proteins, and its dysregulation displays important roles in many pathological and biological processes. Studies in the CF glycoproteome most focused on the occurrence of CF on N-glycosites. Whereas increasing evidence showed that not only the occurrence of CF, but also the proportion of CF glycan relative to the total glycan content on a glycosites, may link to the regulation of proteins functions. Here, a rapid, large-scale, accurate approach was developed for the identification and quantitation of site-specific core fucosylation. After HILIC enrichment, Endo H and Endo F3 were utilized to obtain simplified glycopeptide structure, i.e. peptiede-GlcNAc and peptide-GlcNAc-Fucose. Peak area extraction of the corresponding two forms were performed, thus the occupancy of core fucosylation compared to whole N-glycosylation were calculated using label free method. We demonstrated the feasibility of this strategy using standard glycoproteins and applied this strategy to two hepatocellular carcinoma cell lines, HepG2 and LM3. In total, more than 2000 high confidence core fucosylation sites and their occupancy were determined, among which more than 500 N-glycosites have obvious changes in core fucosylation occupancy.

POSTER 253

Quantitative Proteomics and Phosphoproteomics Analysis Revealed Different Regulatory Mechanisms of Halothane and Rendement Napole Genes in Porcine Muscle Metabolism

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Pigs with the Halothane (HAL) or Rendement Napole (RN) gene mutations demonstrate abnormal muscle energy metabolism patterns and produce meat with poor quality, classified as pale, soft, and exudative (PSE) meat, but it is not well understood how HAL and RN mutations regulate glucose and energy metabolism in porcine muscle. To investigate the potential signaling pathways and phosphorylation events related to these mutations, muscle samples were collected from four genotypes of pigs, wild type, RN, HAL, and RN-HAL double mutations, and subjected to quantitative proteomic and phosphoproteomic analysis using the TiO2 enrichment strategy. The study led to the identification of 932 proteins from the nonmodified peptide fractions and 1885 phosphoproteins with 9619 phosphorylation sites from the enriched fractions. Among them, 128 proteins at total protein level and 323 phosphosites from 91 phosphoproteins were significantly regulated in mutant genotypes. The quantitative analysis revealed that the RN mutation mainly affected the protein expression abundance in muscle. Specifically, high expression was observed for proteins related to mitochondrial respiratory chain and energy metabolism, thereby enhancing the muscle oxidative capacity. The high content of UDP-glucose pyrophosphorylase 2 (UGP2) in RN mutant animals may contribute to high glycogen storage. However, the HAL mutation mainly contributes to the up-regulation of phosphorylation in proteins related to calcium signaling, muscle contraction, glycogen, glucose, and energy metabolism, and cellular stress. The increased phosphorylation of Ca2+/calmodulin-dependent protein kinase II (CAMK2) in HAL mutation may act as a key regulator in these processes of muscle. Our findings indicate the different regulatory mechanisms of RN and HAL mutations in relation to porcine muscle energy metabolism and meat quality.

POSTER 254

Posttranslational Modifications and Data-Independent Acquisitions – Challenges and Opportunities

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Background

Identification and quantification of posttranslational modifications (PTM) presents a unique challenge to proteomic studies. Often PTMs feature low abundances and many possible functionally-distinct locations within each protein. Although label-free data independent acquisitions (DIA or SWATHAcquisition) have been extensively used for global proteomics workflows and quantification of proteins, so far there are only few studies applying DIA to study post-translational modifications. Here, we present several DIA-PTM studies, specifically of phosphorylated as well as acylated proteins using different affinity enrichment approaches.

Methods

Samples were obtained from human and mouse tissues (liver, brain) as well as from a human MKN45 gastric cancer cell line treated +/− Met inhibitor SU11274 and the general kinase inhibitor staurosporine. Post-translationally modified peptides (phospho-Tyr and phospho-(Ser/Thr)-containing peptides, as well as acetylated/succinylated peptides) were enriched using a variety of different PTMScan affinity enrichment kits (CST). Samples were subsequently subjected to label-free quantification. Data-independent acquisitions (SWATH) were performed on a TripleTOF® 6600 system. Sets of isotopically labeled PTM-peptides were acquired using both DIA and parallel reaction monitoring (PRM) for library generation.

Results

We performed various enrichment strategies using PTMScan affinity enrichment kits, such as the PTMScan Multipathway Enrichment Kit and PTMScan® Direct Tyrosine and Serine/Threonine Kinases Reagents monitoring130 Ser/Thr kinases, 385 unique phosphorylation sites, and 671 unique phosphorylation sites regulated by 120 Tyr kinases. Quantification in Skyline allowed for assessment of PTM site localization and specificity. In addition, we have used acetyl-lysine and succinyl-lysine PTMScan® kits to investigate different types of acylation and specifically developing simultaneous affinity enrichment workflows by combining different PTM antibodies for 'one-pot enrichment' approaches. This study emphasizes the importance of assessing protein PTM isoforms based on PTM site localization, and DIA offers comprehensive data acquisition tailored to monitor the complexity of PTM workflows.

Keywords

Post-translational modifications, data-independent acquisition, affinity enrichment

POSTER 255

Expanding the citrullinome of synovial fibrinogen from rheumatoid arthritis using MS: identification of putative sites of pathogenic and prognostic relevance

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Citrullination is an arginine-dependent post-translational protein modification, which is not disease specific but inflammation-dependent in general and is speculated to play a significant pathogenic role in rheumatoid arthritis. In our study, we have applied a mass spectrometry-based proteomics approach for identification of citrullination sites in fibrinogen and for estimation of citrulline occupancy at individual sites. In

fibrinogen contained in synovial fluid from four RA patients, 38 citrullinated sites were identified, 20 of which have not been previously reported to be citrullinated *in vivo*. Citrullination at site α84, α123, α129, α547, α573, α591, β334 and γ134 was identified in more than one patient and were therefore regarded as hotspots. Patient samples with high disease activity were found to have more citrullination sites and higher citrulline occupancy than in samples from patients with low disease activity. Following citrullination *in vitro* using human recombinant peptidylarginine deiminase (PAD) 2, a total of 54 citrullination sites were identified, including 14 hitherto unreported *in vitro* citrullination sites. Uncovering and quantification of certain citrullination sites may prove to have diagnostic or prognostic value in RA and may improve and strengthen our understanding of the immune pathogenesis.

POSTER 256

Radio-sensitizing effects of VE-821: phosphoproteomic and metabolomic changes after ATR inhibition in irradiated MOLT-4 cells

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Current anti-cancer strategies take advantage of tumor specific abnormalities in DNA damage response to radio- or chemo-therapy. Inhibition of the ATR/Chk1 pathway has been shown to be synthetically lethal in p53- or ATM- deficient cells and in cells with high levels of oncogene-induced replication stress. In the presented data, we aimed to elucidate molecular mechanisms underlying radiosensitization of T-lymphocyte leukemic MOLT-4 cells by VE-821, a highly specific and potent inhibitor of ATR. To do so, we combined quantitative proteomics, phosphoproteomics, and metabolomics. We quantified 623 differentially regulated phosphorylation sites and detected changes not only in DDR-related pathways and kinases, but also in pathways and kinases maintaining cellular metabolism. Importantly, we found downregulation of mTOR, the main regulator of cellular metabolism, which was most likely induced by an off-target effect of VE-821, and we propose that mTOR inhibition could be one of the factors responsible for the phenotype observed after treating MOLT-4 cells with 10 μM VE-821. More than two hundred intermediary metabolites were detected in the metabolomic analysis. Metabolomic data analysis indicated that VE-821 potentiated metabolic disruption induced by irradiation and influenced the response to irradiation-induced oxidative stress. Upon irradiation, recovery of damaged deoxynucleotides might be negatively affected by the inhibitor, slowing down DNA repair by their deficiency. Taken together, this is the first study describing a complex network of cellular events that are either ATR-dependent or triggered by ATR inhibition in irradiated MOLT-4 cells.

POSTER 257

Method development for phosphorylation and glycosylation detection using Orbitrap Fusion Lumos

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Nearly all proteins undergo post-translational modifications (PTMs) which are crucial for function, structure, activity, expression of the proteins and protein interactions. Particularly glycosylation and phosphorylation are involved in pathways critical for signaling, and are often altered in disease states. Mass spectrometry (MS) is a powerful tool for the identification of protein glycosylation and phosphorylation due to its sensitivity of detection and its ability to analyze complex mixtures. Combining multiple MS fragmentation techniques (i.e. HCD, ETD, etc) allows for a comprehensive structural characterization of modified proteins.

Phosphorylation occurs on serine, threonine, and tyrosine residues. Typical methods for enrichment include immobilized metal affinity chromatography (IMAC) or titanium dioxide (TiO_2) beads, which have selective affinity and interact with phosphopeptides. Comparing both methods we identified more unique phosphosites with IMAC compared to TiO_2 enrichment. MS fragmentation techniques are also crucial for successful identification of PTMs and the most phosphosites were identified with IT-(ion trap)-HCD fragmentation using a top speed method (scan cycle with a maximum of 3 seconds).

Glycosylation can occur on asparagines (N-linked) or on serine or threonine residues (O-linked). Enrichment strategies such as TiO_2, which is selective for sialic acid-containing glycopeptides, and lectins, with their ability to bind glycoconjugates, are often used. In our comparison the lectin enrichment exceeded the TiO_2 strategy. In addition lectin enrichment on Filter Aided Sample Prep (FASP) filters is preferable for O-linked glycopeptides, lectin enrichment on agarose beads is superior for N-linked glycosylation. Similarly to phosphorylation, we compared different MS fragmentation techniques and identified the IT-ETD_OT (orbitrap)-HCD as the method that provides the most complete protein glycosylation coverage.

POSTER 258

(Phospho)proteomics quantification strategies: A systematic comparison of SILAC, TMT and label-free techniques to study EGFR signal transduction networks in CRC

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Proteomics techniques have been shown to be a valuable tool for discovering disease-related molecular mechanisms in cellular signal transduction networks. In particular, post-translational modifications (PTM) such as phosphorylations can be assessed at large scale. The Epithelial growth factor receptor (EGFR) signaling network is known to play an essential role in proliferation and survival of colorectal cancer. Therefore, EGFR is currently a target of therapeutic antibodies blocking EGFR signaling (e.g. Cetuximab). In this study, we performed a systematic comparison of proteomics quantification strategies and investigated the EGFR signaling network on the proteome and phosphoproteome level.

Therefore, the colorectal cancer cell line DiFi was selected as a model to investigate the dynamics of the EGFR signaling network on (phospho)proteome level upon treatment with Cetuximab after 0, 3 and 24 h. Mass spectrometry-based proteomics were applied to systematically compare the labeling

approaches SILAC, TMT and label-free quantification. We assessed their coverage, reproducibility, technical variability, complementarity and statistical power. Almost no regulation or complementary findings between the individual approaches were detected on the proteome level, whereas a high fraction of phospho-sites was significantly regulated. Moreover, the three labeling approaches showed a pronounced heterogeneity of the quantified phosphoproteome fractions compared to the proteome. The label-free approach resulted in the deepest (phospho)proteome coverage compared to SILAC and TMT, but the superior precision of the latter techniques allowed for the detection of more differentially abundant observations and greater statistical power. Phosphorylation dynamics and the analysis of differentially abundant phospho-sites showed a strong downregulation of the MAPK signaling pathway and other EGFR-related signaling modules in the first three hours, followed by an adaptive response represented by recovered abundances after 24 h antibody treatment.

Our results suggest that in the studied setup SILAC is the most advantageous method to investigate cellular signaling and dynamics on proteome and phosphoproteome level.

POSTER 259
Adenoviral proteins E1B55K and E4orf6 use non-degradative ubiquitination to regulate viral late protein expression

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During the early phase of infection, viruses co-opt host signaling processes and counter host antiviral defenses in order to establish favorable conditions for the late phase infection. Adenovirus is a DNA virus important both for its role in human diseases and for its archetypal insights in understanding virus-host interactions. Two early adenoviral proteins, E1B55K and E4orf6, associate into a host Cullin-5 ubiquitin ligase complex and redirect substrate recognition to promote an environment conducive for viral replication. Mutating these viral genes impairs production of late viral proteins and reduces viral progeny. Previous work has identified a limited number of substrates of the E1B55K/E4orf6 complex which are ubiquitinated and degraded upon infection. However, these known targets do not account for the decrease in late viral protein production. Here we identify new targets of the viral ubiquitin ligase that may account for the late phase defects in the E1B55K-deficient virus. We transduced HeLa cells with E1B55K and E4orf6, enriched for ubiquitinated proteins over a timecourse of E1B55K/E4orf6 expression, and applied mass spectrometry to identify potential substrates. We combined these results with proteomic analysis to normalize ubiquitin levels against protein abundance. In total, we identified >6000 proteins and >4000 modified peptides. Our integrated analysis reveals that ubiquitination occurs on proteins that decrease over time, characteristic of proteasome degradation. We also detected ubiquitination on proteins unchanged in abundance, suggesting non-degradative signaling. Our potential non-degradative ubiquitin targets are enriched in RNA-binding proteins, with hnRNP-C and RALY among the most significantly modified. We show that these homologous proteins are specifically ubiquitinated by the E1B55K/E4orf6 complex and that they play a functional role in viral late protein production in an E1B55K-dependent context. Overall, we provide the first known example of viral-mediated non-degradative ubiquitin signaling to affect protein production.

POSTER 260
A Specific Dual Functional Probe-Hydrophilic Mercaptosuccinic Acid Coupled Magnetic Mesoporous Titania for Simultaneous Capture of Glycopeptides and Phosphopeptides

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Nowadays, the research importance of both glycosylation and phosphorylation has been widely recognized, since they are two kinds of most common and significant post-translational modifications and have close relation to many serious diseases. Also, studies show that glycosylation and phosphorylation in organism do not function independently but mutually most of the time. Thus, simultaneous analysis of glycosylation and phosphorylation would be a real asset for understanding the disease pathology or investigation of novel treatment strategies, etc. Herein, a novel specific dual functional probe-hydrophilic mercaptosuccinic acid coupled magnetic mesoporous titania (denoted as Fe3O4@mTiO2-MSA) has been developed for simultaneous capture of glycopeptides and phosphopeptides for further LC-MS/MS analysis. The dual functional probe combines all the merits of metal oxide affinity chromatography (MOAC) and hydrophilic interaction liquid chromatography (HILIC), as well as the advantage of strong magnetic response of magnetic microsphere, besides, those of mesoporous titania. Eventually, Fe3O4@mTiO2-MSA exhibit outstanding enrichment capacity not only in digestion of standard proteins but also in human saliva. 327 phosphopeptides and 65 glycopeptides were identified simultaneously from three isolated replicates of merely 5 μL human saliva samples, and among them, the phosphorylation sites and glycosylation sites coexisted in 20 peptide segments, indicating the great potential of the novel dual functional probe in simultaneous profiling of glycoproteomics and phosphoproteomics.

POSTER 261
Quantitative Proteomic Approaches Identify Regulatory Enzymes and Substrates for Lysine 2-Hydroxyisobutyrylation Pathway

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Lysine 2-hydroxyisobutyrylation (Khib) is a recently reported, evolutionarily conserved protein posttranslational modification. Histone Khib marks correlate with active gene expression, and exhibit distinct features from the widely studied histone lysine acetylation, suggesting its critical roles in epigenetics and transcriptional control. Nevertheless, key elements for regulating this pathway remain unknown, hindering functional studies of this modification in diverse biological systems and disease settings.

Here we report the systematic investigation of key elements for the Khib pathway. We identified 6548 Khib sites on 1725 substrate proteins. Bioinformatics analysis of the substrate proteins reveals that Khib is closely related to transcription, translation, protein degradation, and energy metabolism. In

addition, we identified more than 300 proteins and 19 domains that can bind to histone Khib marks, directly or indirectly. These proteins may mediate transducing signals of histone Khib and therefore offer clues about the novel functions of histone Khib marks in chromatin regulation. Finally, we demonstrated that Tip60, a member of MYST family acetyltransferase, could catalyze lysine 2-hydroxyisobutyrylation both *in vitro* and *in vivo*, thus providing new opportunities to investigate the physiological functions of this enzyme in Khib pathway. Quantitative Khib proteomic analysis of wild-type and Tip60 overexpressing cells identified 497 potential Tip60-regulated Khib substrate sites. Further analysis revealed potential impacts of Tip60 on tuning protein functions through the regulation of Khib.

Our study thus discovered both the “writer” and “erasers” for histone Khib marks, and major Khib protein substrates. These results not only illustrate the landscape of this new lysine acylation pathway, but also open new avenues for studying diverse functions of cellular metabolites associated with this pathway.

POSTER 262
Assessing an Automated Phosphopeptide Enrichment for high-throughput Malignant Melanoma phosphoproteomics from patient tissues

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The Cancer Moonshot Initiative represents an effort to drive cancer research and focus of treatment to each patient`s unique case. As a strategic partner, we focus in Malignant Melanoma (MM) research. Due the MM complexity, aggressiveness, high incidence and the lack of well-defined biomarkers, clinical proteomics studies consider large MM patient cohorts with properly stored, collected and documented samples in order to cover deeply several aspects of MM. One of the focus of MM proteomics is phosphoproteomics due the relevance of phosphorylation in many biological processes (e.g. RAS/RAF/MAPK pathway in MM proliferation). Analyzing phosphopeptides from patient tissues is challenging because the low amount of starting material, susceptibility to enrichment variation and sample losses. With the aim to overcome this, we explored the Agilent AssayMAP Bravo platform to conduct Fe(III)-IMAC-based phosphopeptide enrichment in MM samples in an automatic workflow. Sensitivity analysis allowed identifying more than 1000 phosphopeptides injecting 12 ug of starting material; enrichment performance showed good linearity with increasing amounts of input material up to 100 ug. On the other hand, enrichment using individual MM tissues identified up to 12000 phosphopeptides and displayed a very high selectivity (85-98%) for all peptide input materials tested, as well as high correlation between experimental replicates. Deeper coverage of MM phosphoproteome was evaluated using high pH fractionation, more than 14000 phosphopeptides were identified in seven fractions obtained from 60 ug of starting material. MM phosphoproteome reflected pathways related with MM development, vitamin D and melanin processing; compared with other studies, we detected phosphoproteins exclusively in our data set involving immunological resistance against MM. The high sensitivity achieved with this technology allowed obtaining comparable results to studies using 16-fold more starting material from melanoma cultured cells, in this way, MM phosphopeptide analysis progresses towards a high degree of sensitivity and robustness achievable with automatic technologies.

POSTER 263
FDR Estimation for Hybrid Mass Spectral Library Search Identifications in Bottom-up Proteomics

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We present a method for FDR estimation of hybrid mass spectral library search identifications. Briefly, the hybrid search can identify peptides that contain unanticipated modifications by matching both ion m/z and mass losses (Burke et al, JPR, 2016). The DeltaMass, or mass difference between the query and reference spectral library peptide, is expected to correspond to a modification. We further analyze the relationship between DeltaMass and FDR by searching against 100 concatenated randomized decoy spectral libraries. Resulting high scoring decoy identifications provide insight into the likelihood of a given DeltaMass to be identified in a decoy search and enable more accurate FDR estimation. DeltaMass values that were found to be confidently identified in multiple proteomic studies were further analyzed.

The hybrid mass spectral library search was performed using NIST MSPepSearch with raw tandem mass spectra from four datasets (Tissue-iTRAQ, Tissue-TMT, Unlabeled-Cells, Unlabeled-Plasma) with a product ion tolerance of 40 ppm. Each dataset was searched against the publicly available human Orbitrap HCD target spectral library (peptide.nist.gov), with the appropriate isobaric mass tag when applicable, as well as either a reverse or randomized decoy spectral library constructed from the reference peptide spectral library. DeltaMass values obtained from the hybrid search output were centroided with a precursor mass tolerance of 5 ppm. The probability of a given DeltaMass to be identified in a decoy spectral library search was evaluated by concatenating 100 randomized decoy spectral libraries (112,797,000 total decoy spectra). Results show that a majority of decoy DeltaMass values are uniformly distributed with the exception of < 10% of DeltaMass values that have a higher probability of being identified in a decoy library search. The number of high scoring decoy identifications from 100 decoy spectral libraries allows for more accurate FDR estimation and shows that FDR may be corrected in a modification-specific manner.

POSTER 264
Proteomic-scale approaches for quantifying irreversible cysteine redox post-translational modifications using parallel reaction monitoring mass spectrometry in myocardial ischemia / reperfusion

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Redox post-translational modification (PTM) is an important defence against, and marker of, pathogenesis. Cysteine (Cys) is the most redox active amino acid and Cys-redox PTMs are considered either biologically reversible (e.g. S-glutathionylation) or irreversible (sulfinic [Cys-SO2H] and sulfonic [Cys-SO3H] acids), the latter of which ‘tags’ proteins for degradation. Irreversible Cys redox PTMs are considered amongst the very least abundant PTMs under physiological conditions. We developed an enrichment method to examine these PTMs on a proteome-scale that employs electrostatic

repulsion of Cys-SO2H/SO3H-containing peptides from cationic resins ('negative' selection) followed by 'positive' selection using hydrophilic interaction chromatography (HILIC). We identified >300 Cys-SO2H/SO3H sites from rat myocardial tissue subjected to physiologically relevant concentrations of H2O2 (<100μM) or to ischemia/reperfusion (I/R) injury via Langendorff perfusion. I/R significantly increased Cys-SO2H/SO3H-modified peptides from proteins involved in mitochondrial fatty acid biosynthesis and the tricarboxylic acid cycle. A multiplexed quantitative analysis of both reversible and irreversible Cys PTMs identified redox targets in response to I/R and in the presence of a thiol-based antioxidant (N-2-mercaptopropionylglycine). Relative quantification of irreversibly oxidized Cys peptides was performed by parallel reaction monitoring (PRM-MS). Our approach enabled quantitative profiling of reversible/irreversible Cys PTMs in response to oxidants/antioxidants during I/R injury.

POSTER 265
Developing Workflow for Simultaneous Analyses of Phosphoproteomics and Glycoproteomics
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Enrichment of modified peptides from global peptides is inevitable in mass spectrometric analysis protein modifications because of their importance in the study of cellular functions and low stoichiometry in the global proteomic analysis. Recent advances in enrichment methods for modified peptides such as phosphopeptides and intact glycopeptides (IGPs) show that the methods for proteomic analyses of both protein modifications are robust. We have recently observed and reported a large number of IGPs from phosphoproteomic analysis using IMAC-based phosphopeptides enrichment procedure. To determine whether phosphorylated peptides could be specifically isolated from co-enriched IGPs in IMAC experiments with different pH, IMAC procedures were performed at different pH conditions and we found that the enrichment of phosphopeptides at pH 2.0 was the optimal condition for having highest number of phosphopeptide identifications, but co-enrichment of phosphopeptides and glycopeptides was in inevitable in all pH range. The hydrophilic enrichments of IGPs performed before or after IMAC enrichment subsequently were evaluated to determine the optimal workflow for simultaneous analyses of phosphoproteomics and glycoproteomics and IMAC enrichment followed by hydrophilic enrichment was chosen as the optimized workflow. Applying the workflow to the TMT-labeled peptides from luminal and basal-like type of breast cancer patient-derived xenograft (PDX) models allowed quantitative analyses of phospho- and glycoproteomics with17,582 phosphopeptides and 3,468 glycopeptides identified, and 1,237 phosphopeptides and 236 glycopeptides showed significant expression differences between luminal and basal-like respectively. This method allows simultaneously analyze phosphoprotein and glycoprotein modifications, extending our understanding of roles of glycosylation and phosphorylation in biology and diseases.

POSTER 266
Dehydration-induced alterations phosphorylation status in the nuclear proteomic landscape of chickpea
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Non-availability of water or dehydration remains recurring climatic disorder greatly affecting yield of major food crops, legumes in particular. Dehydration response in plants utilizes a signal transduction pathway where molecular signals are transmitted to the cell interior and dictate cell fate decision. To circumvent the restrictions imposed by fixed number of genomic sequences, an array of well-designed co- and post-translational layers of regulations and modifications are implemented. Phosphorylation, the most abundant posttranslational modifications (PTMs), intimately regulate almost every signalling cascades and acts as precise molecular triggers. Nucleus, designated as the cell's control centre, hosts genetic information and regulates gene expression. We aimed directed investigation of the nuclear proteome and phosphoproteome, and focused onto proteome-wide analysis of dehydration-response. Four-week-old chickpea seedlings were subjected to gradual dehydration and nuclear proteins were extracted from unstressed control as well as 72 and 144 h of stressed tissues. Proteomic analyses identified 4832 proteins and 478 phosphosites, corresponding to 299 unique phosphoproteins involved in an array of cellular processes including protein modification and gene expression regulation, among others. Only 174 proteins were common among the identified nuclear proteins (NPs) and phosphoproteins (NPPs). The identified set dehydration-responsive NPs and NPPs, demonstrated mutually exclusive stress responses. In addition to several novel kinases, phosphatases, transcription factors and 660 uncharacterised proteins, notably, several spliceosome complex related proteins and splicing factors displayed dehydration induced alterations in their phosphorylation status. Association mapping among the dehydration responsive NPPs, revealed strong interaction between the splicing-related proteins and proteins associated with circadian rhythm, flowering time and miRNA biogenesis. Further, phospho-motif analysis revealed preferential stress-induced dephosphorylation of proline directed pSP motif. These results would not only provide regulatory snapshots of dehydration-responsive crucial cellular pathways, but also serve as a basis for future studies on stress adaptation in plants.

POSTER 267
A Novel Method of Quantifying Protein Methylation Utilizing SWATH-MS
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Protein methylation of arginine and lysine is an important post-translational modification in disease but little is known about its effects. Global protein methylation analysis has recently been enabled by the development of enrichment techniques utilizing peptide immunoprecipitations of methylated lysine/arginine and subsequent mass spectrometry. However, enrichment techniques require large amounts of antibody and sample to maximize coverage. We hypothesize that protein methylation can be studied without the need to enrich each sample through application of a data independent acquisition (DIA) approach, creating a hyper-methylated peptide library to which each experimental sample is compared. We anticipate this would increase coverage of the methylome and provide an

opportunity to study global protein methylation, as well as total protein quantification from the same sample.

We have developed an informatics based approach to distinguish methylated peptides from their un-methylated forms, enabling quantification of methylated peptides in complex protein lysate. We used two mouse models of Non-alcoholic steatohepatitis (NASH) where protein methylation states are altered in constructing this approach, both with altered levels of the primary methyl donor S-Adenosyl Methionine (SAMe) due to knockdown of interacting proteins. SAMe+ has constitutively elevated methylation levels, due to its high levels of SAMe. SAMe- has constitutively lower levels of methylation due to its decrease in SAMe.

To create a hyper-methylated library, SCX peptide fractionation of mouse livers from the SAMe+ model was utilized, resulting in a library that contains 551 methylated peptides. The SAMe- model was utilized for generation of DIA files to compare to the hyper-methylated library. The individual liver samples (n=6/condition) were run by DIA against the methyl-library. We found 143 methylated peptides corresponding to 95 proteins and saw a significant decrease in methylation in the SAMe- mice compared to wild-type controls. Furthermore, treatment of SAMe- mice with exogenous SAMe led to a normalization of methylation.

POSTER 268

Hybrid insulin peptides (HIPs) are detectable in human islets by mass spectrometry

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We recently discovered that hybrid peptides containing an insulin C-peptide fragment linked by a peptide bond to sequences derived from other proteins are present in the insulin-producing beta cells of mouse pancreatic islets. These hybrid insulin peptides (HIPs) are antigens for pathogenic CD4 T cells in the non-obese diabetic (NOD) mouse model of autoimmune type 1 diabetes (T1D). Furthermore, HIP-reactive CD4 T cell clones have been isolated from the pancreata of human donors with T1D. Here, we demonstrate by mass spectrometry that HIPs are indeed present in human islets. Because of technical challenges associated with confidently identifying mass spectra corresponding to HIPs, we also present a set of criteria to be considered when analyzing mass spectra to reduce the likelihood of incorrectly assigning HIP sequences as interpretations. Our findings provide a foundation for future studies investigating the role of HIPs as autoantigens in T1D and highlight the potential complexity of the beta cell proteome.

POSTER 269

oxSWATH: an integrative method for a comprehensive redox-centered analysis combined with a generic differential proteomics screening

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Most of the redox proteomics strategies are focused on the identification and relative quantification of cysteine oxidation without considering the variation in the total levels of the proteins. However, protein synthesis and protein degradation belong also to the regulatory mechanisms of cells, being therefore important to consider the changes in total protein levels in PTMs' focused analyses. Therefore, a novel method called oxSWATH was developed. The proposed method combines the SWATH-MS acquisition with differential alkylation using non-isotopically labeled alkylating reagents, therefore allowing the integration of information regarding relative cysteine oxidation with the analysis of the total protein levels in a cost-effective high-throughput approach.

The method was tested using a redox-regulated protein and further applied to a comparative analysis of secretomes obtained under control or oxidative stress conditions to strengthen the importance of considering the overall proteome changes. Using oxSWATH it was possible to determine both the relative proportion of reduced and reversible oxidized oxoforms, as well as the total levels of each fraction. Moreover, since samples are acquired in SWATH-MS mode, besides the redox centered analysis, a generic differential protein expression analysis can be also performed, allowing a truly comprehensive evaluation of proteomics changes upon the oxidative stimulus.

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POSTER 270

Ub^{KEKS} : a novel ubiquitin variant expressed from a pseudogene.

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Post-translational modification by ubiquitin and ubiquitin-like modifier proteins regulates cellular processes at almost every level. The cell produces ubiquitin from 4 genes which contain all the same sequence. Through analysis of large scale proteomics looking for alternative open reading frames using OpenProt database (www.openprot.com), we identified a novel ubiquitin variant expressed from the UBBP4 pseudogene. This new ubiquitin gene contains 4 different amino acids compared to canonical ubiquitin (Q2**K**, K33**E**, Q49**K** and N60**S**) and is named Ub^{KEKS}. Evidences in ribosome profiling, mRNA expression and proteomics experiments confirmed the

expression of this new ubiquitin through several different tissues. Biochemical experiments revealed that this new ubiquitin isoform can modify various proteins in the cell. Proteasomal inhibition with MG132 further demonstrated that the proteins modified by Ub^{KEKS} are not targeted to degradation. This result was confirmed by AP-MS where several proteasomal subunits interact with ubiquitin, but not with Ub^{KEKS}. Moreover, immunofluorescence revealed that ubiquitylated proteins aggregated in the cytoplasm following proteasomal inhibition, but not proteins modified with Ub^{KEKS}. The structures of both ubiquitin and Ub^{KEKS} were resolved by NMR studies and showed no significant differences between them. Furthermore, mass spectrometry analysis revealed that the lysine at the position 49 on Ub^{KEKS} can be used to initiate a new poly-ubiquitin chain. In addition, when the lysine 49 is modified, the lysine 48 is always modified at the same time on Ub^{KEKS}. Modeling these two linkages on Ub^{KEKS} highlights a steric hindrance with the proteasomal receptor. Finally, we identified that PCNA can be modified by both ubiquitin and Ub^{KEKS} on the same lysine which is involved in the choice of DNA repair pathway following replication block. In conclusion, we identified a new post-translational modification related to ubiquitin with different functions.

POSTER 271
Quantitative proteomics identifies novel PIAS1 protein substrates involved in cell migration and motility

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SUMOylation is a covalent and reversible modification of a target protein by small ubiquitin-like modifier (SUMO). Conjugation of SUMO to target proteins requires the dimeric SUMO E1 SAE1/SAE2 to activate SUMO and the single E2 Ubc9 that, in combination with a few known E3 ligases, directs conjugation and ensures target specificity. PIAS1 (Protein Inhibitor of Activated STAT, 1) was originally discovered as a transcriptional regulator. It plays a crucial role in various cellular pathways, including the STAT pathway, the p53 pathway and the steroid hormone signaling pathway. PIAS1 can sumoylate PML (at Lys-65 and Lys-160) and PML-RAR and promotes their ubiquitin-mediated degradation. Increasing evidence shows that PIAS1 is overexpressed in various human cancers, such as prostate and lung cancers, and PIAS1 overexpression is associated with tumorigenesis. To comprehensively understand the mechanism of action of PIAS1, we developed a quantitative SUMO proteomic approach to systematically identify potential substrates of PIAS1. In this study, 983 SUMO sites on 544 proteins were quantified across 6 biological replicates using a workflow comprising the use of PIAS1 overexpression, Ni-NTA purification, peptide level immunoenrichment and LC-MS/MS analysis. Among them, 177 SUMO sites on 124 proteins were significantly regulated by PIAS1, and represent putative PIAS1 substrates. Analysis of this data at the systems level revealed PIAS1 substrates involved in diverse cellular processes, including the transcriptional regulation, cytoskeleton dynamics and protein SUMOylation. Further functional studies on vimentin (VIM), a key protein involved in the cytoskeleton, revealed that PIAS1 exerts its effects on migration and invasion of HeLa cells, through the SUMOylation of Lys-439 and Lys-445 residues. Our results provide not only a new strategy for identification of SUMO E3 ligase substrates but also novel insights into the mechanisms underlying the function of PIAS1 in cancer cells.

POSTER 272
Proteoform Atlas Of Extracellular Matrix Predicts Clade Specific Functionality Of Wall-associated Signaling Components In Plant

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Evolution of extracellular matrix (ECM) and transition to multicellularity reflects diversity of proteoforms and modular domain structures across taxonomic species. Wall associated signaling components in ECM regulate mechano-sensing and turgor driven deformation. This modulation is known to be mediated by post-translational modification such as phosphorylation and glycosylation. To determine the complexity in wall-associated signaling components, we have developed the ECM proteoform atlas of a food legume, chickpea (*Cicer arietinum*). Mass spectrometric analysis led to the identification of 2111 proteins and 411 unique phosphopeptides spanning 369 phosphoproteins, presumably involved in a variety of biological functions viz. cell wall remodeling, mechanical signaling, innate defense, protein folding and degradation. Data analyses revealed ECM proteoforms of unknown functions and highlight components restricted and/or expanded in taxon-specific manner leading to neofunctionalization of paralogous ECM proteoforms. Analyses of domain conservation/divergence patterns in ECM proteoforms highlights dynamic attribute of protein repertoire across plant kingdom. *In silico* prediction and mass spectrometric identification of site-specific phosphorylation of amino acid residues indicated their possible regulatory and functional effect on ECM signaling network. Further, we interrogated the dataset using cluster and network analyses that identified key protein modules and small correlation groups. Our study highlights complexity of ECM dynamics and elucidated the proteoform network that branches to several signaling pathways. To our knowledge, this is the first report on the comprehensive understanding of the complex proteoform signaling network operating in plant extracellular matrix.

POSTER 273
Redox regulation of fetal and adult hematopoiesis

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Increasing evidence point to a distinct regulation of redox homeostasis in fetal and adult hematopoietic stem cells (HSCs). Fetal HSCs are characterized by higher levels of reactive oxygen species (ROS) and weaker equipment with proteins crucial for antioxidant defense of the cell compared to adult HSCs. Whether the distinct regulation of redox homeostasis plays a role in the initiation and progression of hematological malignancies remains unclear. In this work, we used a quantitative mass spectrometry-based proteomics approach to characterize proteins susceptible to protein thiol oxidative modifications in fetal liver hematopoietic stem and progenitor cell (HSPCs) with the purpose to reveal target

proteins with a key role in redox regulation of children leukemia development.

In this study, we applied a sequential iodoTMT labeling strategy and nanoLC-MS3 method to characterize cellular redoxome and identify protein thiol targets of ROS in three replicates of mouse fetal liver (FL) and adult bone marrow (ABM) FACS-sorted HSPCs. We will discuss adaptation of the method to low starting material (400'000 sorted HSPCs ~20ug per condition), optimization of anti-TMT enrichment and accurate quantification on peptide level.

In total, we identified 4184 Cys peptides corresponding to 2216 protein groups with on average 87% enrichment-efficiency in three replicates of FL and ABM mouse HSPCs. We found 110 cysteine peptides corresponding to 103 proteins to be significantly higher oxidized in FL compared to ABM. 124 proteins were found to be oxidized only in FL HSPCs. Significantly differentially oxidized proteins were enriched for biological processes related to protein translation, especially initiation and elongation of translation and formation of translational preinitiation complex.

Overall, our work characterized cellular redoxome and protein targets of ROS undergoing reversible modifications in fetal and adult HSPCs. Our study has provided deep insights into redox regulation of fetal and adult hematopoiesis.

POSTER 274
MS search parameter refinement avoids ADPr-acceptor site localization bias and identifies tyrosine as novel ADPr-acceptor site with significant functional consequences

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Protein ADP-ribosylation is a physiologically and pathologically important post-translational modification (PTM) that regulates numerous cellular activities. Despite recent biochemical and mass spectrometry (MS)-based breakthroughs, comprehensive ADP-ribosylome identification and accurate ADP-ribose (ADPr) acceptor-site localization remains challenging. Specifically, the recent identification of serine (S) an as the main genotoxic stress-induced ADPr-acceptor amino acid highlighted the fact that accurate ADPr-acceptor site localization remains challenging due to the fact MS-search engines introduce bias by requiring the user to predefine all possible ADPr-acceptor amino acids. Following this revelation, we set out to develop an unbiased method to define all ADPr-acceptor amino acids. Develppoment of the resulting multistep ADP-ribosylome data analysis workflow led to the generation of an ADPr-site Localization Spectra Database (ADPr-LSD) that contains 288 high-quality spectra where the ADPr-acceptor site is confidently localized within each ADP-ribosylated peptide. Interestingly, construction of the ADPr-LSD led to the identification of tyrosine (Y) as a novel *in vivo* ADPr-acceptor amino acid. shRNA-based knockdown studies provide proteome-wide evidence demonstrating that oxidative stress strongly and specifically induces ARTD1-dependent serine and tyrosine ADP-ribosylation *in vivo*. *In vitro* ADP-ribosylation assays and further MS analyses confirmed that tyrosine functions as an ADP-ribose-acceptor amino acid in RPS3A (Y155) and HPF1 (Y238), while site-directed mutagenesis demonstrated that RPS3A-Y155 is the main ADPr-acceptor site for this *trans*-ADP-ribosylation target protein. Moreover, we provide solid evidence that ARTD1-mediated tyrosine ADPr modifications are dependent on HPF1 and HPF1 ADP-ribosylation at Y238. Taken together, our findings validate the functional importance of tyrosine as novel ADPr-acceptor amino acid and indicated the broad and far-reaching influences low abundant ADPr modifications can have on cellular functions. Finally, the ADPr-LSD resource provides important ADPr spectral references that can be used by the research community as a training tool to ensure high-quality ADPr-spectra identifications and ADPr-site localizations.

POSTER 275
Global age-specific changes in protein post translational modifications (PTMs) in neonatal, paediatric and adult plasma

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Developmental proteomics describes age-specific differences in the proteome of healthy individuals. We have recently utilised SWATH-MS to establish age-specific differences in the plasma proteome of neonates, children, and adults [1]; and have established age-specific differences in PTM of individual proteins (e.g. fibrinogen [2]). This study exploited the data re-mining facility of SWATH-MS to detect global age-specific changes in PTMs of plasma proteins amongst various age groups of healthy donors, without enrichment for specifically targeted modifications.

The plasma 2D LC ESI MS/MS IDA data were searched with ID focus allowing Biological Modifications using ProteinPilot V5.0 (SCIEX). This search contained matching PTM peptides without emphasising any particular type and was built as a library to extract SWATH-MS data generated during our previous study [1]. We categorized the extracted peptide PTMs, determined their differential expression across groups, compared this with the un-modified peptides where possible, and summarized and clustered the different categories of proteins undergoing modification.

We mined our previously published SWATH-MS plasma data [1] with the new in-house plasma library. 458 proteins were found and globally matched PTM peptides were included. There were significant age-specific differences in protein PTMs including methylation, acetylation, formylation, phosphorylation and glycosylation in the plasma samples of neonates compared to children and adults. Methylation was mostly associated with peptides/proteins that are associated with adaptive immunity. Differences in phosphorylation were observed for a small number of peptides, one of which was associated with Fibrinogen alpha chain.

This study demonstrates the re-use of SWATH data to find novel information and forms the foundation for future studies characterising of the global, age-specific changes in PTMs in the setting of healthy development. This knowledge will be

critical for early detection of disease and/or design of novel therapeutic approaches.

1. Bjelosevic S, et al. Molecular and Cellular Proteomics. 2017;16:924-35.

2. Ignjatovic V et al. Blood Coagulation and Fibrinolysis. 2011;22: 110–7.

POSTER 276

An automated and reproducible workflow for human cancer cell line phosphopeptide analysis

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Background

Immobilized metal affinity chromatography (IMAC) using a nitrilotriacetic acid (NTA) chelating ligand functionalized with Fe(III) is one of the most widely adopted phosphopeptide enrichment techniques for LC/MS applications. Agilent AssayMAP Bravo platform is able to provide a fully automated and highly selective phosphopeptide enrichment workflow using high capacity Fe(III)-NTA cartridges. In this study, we evaluated how the ratio of total peptide sample amount to affinity resin affects the performance of the enrichment results.

Methods

The Agilent AssayMAP was used for automated phosphopeptide enrichment with Fe(III)-NTA cartridge. Human MCF7 breast cancer cell line tryptic digest was loaded onto the cartridges with four different sample to resin ratios. With a 90-min nanoLC gradient, 6550 iFunnel Q-TOF was used for phosphopeptide discovery. MRM analysis of 20 light and heavy pre-spiked phosphopeptide standards was performed on 6495B QQQ to measure their overall recovery from the enrichment procedure.

Results

Prior to enrichment, about 1.5% of peptides were identified as phosphopeptides. After enrichment, phosphopeptide selectivity was routinely above 90% across all 12 samples. Injecting 1/5 of the final enriched sample, Spectrum Mill was able to identify about 1,200 to 1,500 total number of phosphopeptides from the 4 ratios of sample. About 55% of all phosphorylation sites were assigned. The recovery rates were consistent for all the standards across the 4 ratios of sample.

Conclusions

AssayMAP Bravo provides fully automated, highly selective and reproducible enrichment for phophopeptides. Nanodapter effectively converts UHPLC to nanoflow UHPLC which allows users to have both standard flow and nanoflow LC in one system. Together with nanoESI source, 6550 Q-TOF offers the ultimate sensitivity for maximum number of phosphopeptide identification. 6495B Triple Quadrupole offers fast, accurate and robust MRM based peptide quantitation.

POSTER 277

Do not dry TMT-labeled phosphopeptide samples in presence of NHS-quenching reagent

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One of the most common chemistries used to label primary amines utilizes N-hydroxysuccinimide (NHS). TMT, an isobaric tag used for quantitative proteomics also incorporates NHS. During the labeling reaction of TMT, excess reagent is quenched by a nucleophile, such as hydroxylamine. Although the NHS-ester conjugation is most commonly used in quantitative proteomics, no such study has been reported yet pinpointing the actual effect of isobaric labeling procedures on phosphopeptide identification. We report here a detrimental effect of NHS-quenching reagent on phosphopeptides. We found an impairment in the degree of phosphopeptide identification when hydroxylamine-quenched TMT-labeled samples were vacuum-dried prior to phosphoenrichment. Vacuum-drying in presence of hydroxylamine promoted β-elimination of phosphate groups from phosphoserine and phosphothreonine while having a minimalistic effect on phosphotyrosine. This negative impact of hydroxylamine was reduced by direct desalting after appropriate dilution of quenched samples. Based on the results, we suggest not to dry TMT (iTRAQ as well)-labeled phosphopeptide samples in the presence of NHS-quenching reagent.

POSTER 278

Effective Mass Spectrometry-Based Methods to Globally and Site-Specifically Analyze Glycoproteins

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Protein glycosylation is ubiquitous in biological systems and essential for cell survival. It regulates many cellular events and aberrant protein glycosylation is directly related to human disease, including cancer and infectious diseases. Glycoproteins contain a wealth of valuable information regarding cellular developmental and diseased statuses. However, due to the low abundance of many glycoproteins and the heterogeneity of glycans, it is extraordinarily challenging to globally analyze glycoproteins in complex biological samples. Based on the common features of glycans, we have developed effective chemical and enzymatic methods to globally analyze protein glycosylation by mass spectrometry. Glycoproteins located on the cell surface are especially interesting because they frequently regulate extracellular events. Novel methods have been developed to specifically tag surface glycoproteins for global and site-specific analysis. In combination with multiplexed proteomics, we quantified the dynamics of surface glycoproteins. Global analysis of protein glycosylation will lead to a better understanding of glycoprotein functions, and the molecular mechanisms of diseases, and the identification of glycoproteins as disease biomarkers and drug targets.

POSTER 279

Revealing the regulation of growth and the fatty acid metabolism of the mandibular gland of honeybee based on phosphoproteomic analysis

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The mandibular glands (MGs) of honeybee (*Apis mellifera*) is of great importance regarding its role in the secretion of fatty acids and highly volatile pheromones. Previous proteomic studies revealed that the MG proteome dynamically changes with their age-dependent tasks. However, how protein phosphorylation regulates the physiological events in MGs, has not been investigated yet.

In this study we characterized the phosphorylations of the MG proteins of both Italian bee (ITB) and Royal jelly bee (RJB) — a line that selected for RJ production. We identified 2103 phosphoproteins with 5126 phosphosites in the MG of ITB and RJB with 3 different ages. The 1194 phosphoproteins that shared by both bees are involved in “RNA processing”, “cytoskeleton organization” and “protein phosphorylation”. More than 180 phosphoproteins were identified as kinases that participate in various signaling pathways of autophagy, phosphatidylinositol, Wnt, etc. PCA analysis revealed that the MG phosphorylations of the bees were genetically distant at nurse stage, with the contribution of the differentiated phosphorylations related to “regulation of Rho protein signal transduction”, “positive regulation of transcription”, and “phosphorus metabolic process”. We also found that multiple enzymes that related with the metabolism of fatty acids were phosphorylated, including Pyruvate dehydrogenase E1 component subunit alpha, fatty acid synthase (FAS), 3-ketoacyl-CoA thiolase, acetyl-CoA carboxylase, medium-chain specific acyl-CoA dehydrogenase and aldehyde dehydrogenase. It is striking that the dephosphorylation of FAS resulted in significantly decreased enzymatic activity.

In summary, this study is the first In-depth analysis of the phosphoproteome of honeybee mandibular gland. We conclude that protein phosphorylations in MG are universally happened and dynamically changed with the ages/tasks; Phosphoprotein regulate many critical biological processes including cytoskeleton organization and multiple signaling pathways in MG; protein phosphorylation of fatty acid synthase activates its enzymatic activity.

POSTER 280
Peripheral blood proteins associated with long-term response to lithium in bipolar disorder

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Lithium is the gold standard mood stabilizing medication for the treatment of bipolar disorder (BD). Lithium is superior to other medications in protecting against manic and depressive mood episodes and has unique anti-suicidal properties. However, only about 30% of BD patients respond optimally to the drug, while 30% are intermediate responders and 30% respond poorly. Recent genetic and molecular studies suggest a detectable biological profile for lithium responsiveness, however no clinical biomarker has been developed that could guide targeted treatment.

We report on the first proteomic study assessing lithium response in peripheral blood. We determined lithium effectiveness retrospectively in a cohort of 22 BD patients, using the ALDA lithium response scale (9 responders, 13 non-responders). Whole blood samples were analysed by LC-MS/MS, using an Ultimate 3000 RSLC system (Thermo-Fisher Scientific) coupled to an Impact II™ QTOF mass spectrometer (Bruker Daltonics) via an Advance CaptiveSpray source (Bruker Daltonics). MS scans were acquired in a mass range of 300 to 2200 m/z in a data-dependent fashion, using the Bruker AutoMSMS method, singly charged precursor ions were excluded, and relative collision energy determined by precursor m/z and ranged from 23% to 65%. Data analysed in MaxQuant using standard LFQ settings

Samples were run in triplicates. Overall, 668 proteins were identified. Data filtering, quality control, and differential group comparisons were carried out in Perseus. 213 proteins remained for final analysis, of which 9 were significantly different between the responders and non-responders at $p<0.05$. The most robust finding ($p<0.005$) represented a protein that has previously been implicated in psychiatric disorders. If replicated in larger and prospectively assessed cohorts, our findings might have the potential to generate novel insights into lithium’s mechanism of action, and might be candidates for prognostic biomarker development for lithium therapy in BD.

POSTER 281
Immunoproteomic Identification of IgE binding proteins from Ligustrum Pollen: Monosensitized- vs Polysensitized Allergic Patients

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Introduction and Objectives.

Introduction and Objectives.

The prevalence of IgE-mediated pollen allergic diseases is increasing worldwide with some atopic subjects being sensitized to only one allergen (monosensitization) while other patients are sensitized to more than one allergen (polysensitization) as determined by skin prick test or in vitro diagnostics. Mass spectrometry (MS) may entirely replace these classic methods, as it allows sequence-specific protein identification including sequence variants. The aim of the present study is to investigate novel Ligustrum allergens from the tree Ligustrum lucidum implicated in both monosensitized- and polysensitized allergic patients.

Methods. Ligustrum pollen proteins were extracted by a modified phenolic extraction and protein separation was performed using two-dimensional electrophoresis and blotted onto PVDF membranes (Immun-Blot, Bio-Rad). After electro-transference, membranes were incubated overnight with pooled será from Ligustrum-pollen allergic subjects (monosensitized n=4 and polysensitized n=6). Analisis of immunoreactive protein spots were performed with an ACQUITY UPLC System (Waters, Milford, MA, USA) coupled to a Mass Spectrometer (SYNAPT-HDMS,Waters).

Results. The 2DE immunoblots obtained using pooled sera from Ligustrum sensitive patients revealed two profile patterns:

polysensitized patients showed six unique IgE binding allergens including Olea 12.01, Fra e 12.01, Fructokinase, Fra e 11.01, Populus nigra and actin. Both, monosensitized- and polysensitized subjects showed in common six IgE binding proteins spots, corresponding to Profilin, Enolase, Pollen-specific Polygalacturonase, Fra e 9.01 (β-1,3-glucanase), Alanine aminotransferase, and ATP synthase beta subunit. Individual análisis showed that 70% of polysensitized Ligustrum sensitive patients exhibited the six unique IgE binding allergen described above.

Conclusion. The present study reports six novel IgE-binding protein allergens from Ligustrum l. Interestingly, these IgE-binding allergens were a feature of polysensitized- but not monosensitized patients. These findings may lead to establishing biomarkers for allergy diagnosis and therapeutics from the framework of precision medicine.

POSTER 283
A mass spectrometric proteome profiling workflow from heart tissue to accelerate cardiac research and diagnostics

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Introduction: The success of proteomics in precision medicine rests on the availability of medium- to high-throughput analytical protocols that provide sufficient analytical depth. Cardiac tissue biopsies promise to be a highly valuable source of information for a molecular understanding and, potentially, detailed diagnosis of severe heart disease. We propose and demonstrate a rapid analytical workflow consisting of pressure-assisted tissue lysis, magnetic bead-assisted processing, and label-free mass spectrometry that enables medium throughput analysis of clinical samples.

Methods: 1-3 mm3 samples of human heart tissue were lysed and digested using Pressure Cycling Technology (PCT) in a Barocycler 2320 (Pressure Biosciences). Samples were processed and desalted on hydrophilically coated magnetic beads following the SP3 protocol (Hughes et al., 2014), and analyzed by SWATH mass spectrometry on hybrid quadrupole/time-of-flight mass spectrometer (TripleTOF 5600+, Sciex). Different pre-separation strategies for building project-specific spectral libraries including direct analysis, SDS-PAGE and neutral pH Reversed Phase (nPH-RP) separations were evaluated.

Results: Sample processing of cardiac biopsy samples was evaluated and optimized using SDS-PAGE. Processed samples were analyzed by LC/MS/MS in data dependent acquisition (DDA) mode using different pre-fractionation techniques. nPH-RP separation of digested peptide samples provided the most comprehensive peptide and protein identification results (1372 proteins @ 1% FDR) followed by SDS-PAGE separation of proteins (964) and replicate direct analysis (663). Data-independent analysis (DIA) by SWATH mass spectrometry showed that miniature biopsy samples could be profiled at high reproducibility and a sample throughput of 8-10 samples/day.

Discussion: We demonstrate an integrated workflow for rapid proteome profiling from human cardiac tissue. While offering moderate analytical depth, the proposed workflow is easily parallelized and allows for the analysis of up to 12 samples/day, at a turnaround time of 1-2 days. The use of parallelized pressure cycling technology and magnetic bead processing allows for straightforward upscaling of sample handling.

POSTER 284
An Oncoproteogenomic Strategy for Multiplexed Screening of EGFR Mutations in Non-small-cell Lung Cancer

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High-throughput and systematic sequencing of cancer genome has enabled the comprehensive characterization of somatic mutations. However, the expression level of mutated proteins encoded from mutation bearing genes is not always quantitatively correlated. The oncoproteogenomics analysis of mutated proteins, which integrates cancer proteomics with genomics data, could facilitate identification of cancer-specific protein mutations in clinical samples. Thus, we aimed to develop MS-based oncoproteogenomics strategies for screening of tumor-specific mutations on oncoproteins. For proof-of-concept of our new methodology, non-small cell lung cancer (NSCLC), the most common type of lung cancer cases, and the current clinically used targeted therapy target, *EGFR* mutations, will be used as a model. *EGFR* mutations were discovered in patients with lung adenocarcinoma to associate with response to EGFR tyrosine kinase inhibitors. Up to now, the *EGFR* mutation induced alterations in EGFR protein expression to modulate function activity is still unclear.

We developed a mass spectrometry (MS)-based oncoproteogenomics strategy which integrated bioinformatics analysis of mutant protein sequences for protease prediction, affinity purification of EGFR, parallel enzymatic gel-assisted digestions, LC-MS/MS analysis, and customized database searching using multiple engines, for identification of 34 mutated versus 33 wild-type EGFR proteins. To quantify cellular wild-type and mutant EGFR proteins, targeted LC-PRM-MS approach was established with synthetic peptides. We successfully identified and quantified EGFR del_746-750 peptide with high confidence and precision in PC9 cells by fragment ions covering the deletion of 5 amino acids (ELREA). In addition, the corresponding wild-type peptide was identified in digested sample. The unambiguous identification of EGFR L858R and G719A also revealed concomitant and heterogeneous expressions of mutant and wild-type EGFR proteins in NSCLC. These results indicated that our strategies can precisely determine the status of mutations at protein level and will be a promising platform to assess the expression level of oncoproteins with multiple mutations.

Keywords

Oncoproteogenomics, Mass-spectrometry, EGFR

POSTER 285
Development of an assay for monitoring plasma protein variants for clinical use to assess novel therapies for acute liver dysfunction

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Acute Liver Disorder is a fast decline of liver functionality, resulting in rapid multi organ failure and fatal without medical intervention. Only available treatment is transplantation of whole or partial donor liver. Liver transplantation has several drawbacks despite its routine practice. Liver is the only organ that can regenerate itself. Novel treatments that allow time for regeneration include transplantation of hepatocytes embedded in material with low immunogenicity. In order for such therapy to be evaluated there is a need to monitor the embedded hepatocytes/host liver viability *in-vivo.* Inherently metabolites cannot distinguish individuals. Proteins however have natural single nucleotide polymorphisms (SNPs). If the SNP profiles of the donor hepatocytes and the host are significantly different from each other a distinction and hence an ability to monitor donor hepatocyte and recipient regenerated liver function becomes feasible.

We developed a multiplex MRM-LC-MS/MS method to distinguish and eventually measure consensus and SNP containing tryptic peptides from, a selection of hepatocyte secreted proteins.

A SNP database search was performed and tryptic peptides for 19 pairs of consensus and variant sequences were chosen for 10 proteins. Peptides were evaluated for their LC-MS/MS performance after which 8 pairs performed satisfactory. We evaluated the assay in a test cohort of 23 anonymous plasma samples. Expression of variants was variable in heterozygotes between SNPs ie it would range from 15%- 50% of protein expression depending on SNP. Although the level would remain consistent for each specific SNP allowing us to create a threshold for each variant expression. We observed that 3 subjects had no variants, 8 subjects had 1 variant, 8 had 2 variants, 3 had 3 variants, and 1 had 4 variants. Overall 47% of the test cohort had a unique plasma variant profile. Further evaluation on quantitative use of the assay is to be performed.

POSTER 286
The yeast interactome approaching completeness – combining a robust high-throughput pull-down workflow with a fast and sensitive Evosep/timsTOF setting

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Mapping protein-protein interactions (PPIs) provides fundamental information on the regulation and structure of biological systems and is crucial for the global assessment of the function of a cell. With the onset of proteomics, the use of affinity purification coupled to mass spectrometry (AP-MS) has thus been established as a major tool for the mapping of PPIs in several organisms. Previous large-scale interaction studies in *Saccharomyces cerevisiae*, in which about only one-third of all proteins were successfully purified, made use of non-quantitative MS and tandem-affinity purification strategies. In order to identify only true interactors in single gel bands, those approaches needed very stringent washing steps and therefore huge amounts of cell lysate as input material.

Recent improvements in sensitivity and speed in MS technology and the establishment of affinity enrichment coupled to MS (AE-MS), let us develop a novel, robust and highly reproducible workflow for the large-scale identification of PPIs in *S. cerevisiae*. The need of only about 500 µg of proteins per pull-down, not only allows us to fully grow the endogenously tagged strains, but also to perform lysis and the pull-down itself in a 96-well format. To minimize overall measurement time and to avoid long HPLC duty cycles we made use of the novel EvosepOne system to process 60 pull-downs per day. In combination with the timsTOF analyzer (Bruker), this setting provides one of the highest sequencing-speed, sensitivity, and robustness available. Analysis of a selection of nuclear baits representing a broad range of cellular protein abundances shows their efficient and reproducible enrichment together with known complex members.

The underlying work will allow us to generate the first accurate and comprehensive ‘interactome’ of any species.

POSTER 287
EGFR Interactome Reveals Multiple Pathways and Regulatory Mechanism of Drug-resistance in Non-Small Cell Lung Cancer

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Epidermal growth factor receptor (EGFR) is one of the most popular onco-protein for cancer targeted therapy. The acquired secondary mutation of EGFR induced by tyrosine kinase inhibitor (TKI) therapy has been reported as the major factor of drug resistance in non-small cell lung cancer (NSCLC) patients. EGFR regulates cancer pathogenesis by homo-/hetero-dimerization with EGFR family members, followed by complex interactions to recruit downstream associated proteins to subsequently activate its signaling pathways. Thus, it is intriguing to study whether the mutant EGFR (mtEGFR) may recruit different interacting partners and alter downstream signaling. Two groups of NSCLC cell lines with primary secondary EGFR mutation, H3255 (L858R)/H1975 (L858R-T790M) and PC9 (Del19)/CL68 (Del19-T790M), were used as drug sensitive and resistant NSCLC models. Affinity purification mass spectrometry (AP-MS) was applied to map the different mtEGFR protein-protein interactions (PPIs). The candidate EGFR-binding proteins were stringently filtered by quantitative comparison with control group and elimination of non-specific binding in CRAPome database. The results revealed that around 100 proteins were confidently identified to represent potential mtEGFR-binding partners. Bioinformatics analysis indicated endocytosis pathway which is responsible for vesicle formation and protein degradation potentially crucial for drug resistance caused by T790M mutation. We suggest that the

regulation of EGFR trafficking involve with protein ubiquitination and degradation, and the mechanism may provide survival advantage and resistance of therapy for cancer cell. Next, we will clarify the biological function of targeted interactor and pathway involved in TKI-resistant mechanism in the near future. The knowledge of these results may provide insight on the design of novel therapeutic strategy to overcome drug resistance and tumor relapse in NSCLC

Keywords: Interactome, Affinity purification mass spectrometry, EGFR mutation, TKI resistance

POSTER 288
Probing novel Immunoglobulin Super Family receptor interactions on the cell surface

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Receptor-ligand interactions on the extracellular surface are responsible for key signaling pathways both during homeostasis and under diseased states. While intracellular protein networks have been relatively well studied, the extracellular proteome remains considerably underrepresented in any databases. One of the key hurdles is the detection of low-affinity transient interactions that are characteristic of extracellular protein networks. We have developed a workflow for robust detection of protein interactions in high throughput that allows us to interrogate extracellular targets for binding to most single transmembrane receptors. We have used this technology to study the Immunoglobulin Superfamily (IgSF), one of the largest gene families in the human genome and main therapeutic targets that remain largely uncharacterized. Our lab has successfully detected over 1,000 predicted interactions by probing more than 600,000 pairwise IgSF-STM receptor interactions. In this study, using a multimerization-based method for detection of low affinity interactions on the cell surface, we validate several of the new IgSF receptor interactions identified. Using this strategy, we have revealed previously unknown links between families of receptors that suggest completely unrecognized functionalities in the extracellular environment.

POSTER 289
A Sequential Affinity Purification and Mass Spectrometry Approach for Identifying Shared Interactions of Associated Protein Pairs

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Mass Spectrometry has been a powerful tool in identifying protein interactions. Previously, Gilmore and coworkers have applied Affinity Purification followed by Mass Spectrometry analysis (AP-MS) approach in search for interactions of WD40 repeats containing protein 76 (WDR76, also named CMR1 or Ydl156w), which is a largely uncharacterized chromatin associated protein. [1] The result indicates that WDR76 is possibly a multi-functional protein in combination with different partners. Here we focused on deciphering the function of WDR76 when binding to Spindlin1 (SPIN1, also named OCR), a WDR76 associated protein identified from AP-MS analysis. We used different fluorescent spectroscopy methods to validate the interaction of WDR76 and SPIN1 in live cells. To identify shared interactions of both proteins, we developed a sequential affinity purification followed by mass spectrometry analysis approach (AP-Re-AP-MS). By adding an extra dimension of purification, shared interacting proteins are not only distinguished from the interactions of each single bait, but also enriched for easier detection. Using this method, we have obtained a reasonable list of WDR76-SPIN1 co-binding candidates. The new method shows its great potential in providing more valuable insights when studying two proteins of interests that are not robustly co-purified with each other.

[1]Gilmore, J.M., et al., WDR76 Co-Localizes with Heterochromatin Related Proteins and Rapidly Responds to DNA Damage. PLoS One, 2016. 11(6): p. e0155492.

POSTER 290
Proteomics uncovers lipid raft protein NTAL as a regulator of leukemia cells proliferation and death.

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Lipid rafts are highly ordered membrane domains enriched in cholesterol and sphingolipids that provide a scaffold for signal transduction proteins. Altered raft structure has been implicated in cancer progression. These domains can be disrupted by alkylphospholipids (APLs), which induces apoptosis in leukemia cells but spares normal hematopoietic and epithelial cells. Using quantitative SILAC-based proteomics, we identified targets of APL treatment in a lipid raft-enriched fraction of leukemic cells. Among the regulated proteins, we uncovered NTAL (non-T-cell activation linker), which is an adaptor protein associated with lipid rafts in its palmitoylated form and expressed in B lymphocytes and myeloid cells. Loss of NTAL was observed as early as the 3 hours after treatment with APL and followed by degradation of NTAL by proteassome. NTAL knockdown decreased cell proliferation and increased cell sensitivity to APLs. Furthermore, NTAL-knockdown in NB4 and U937 cells decreased engrafted tumor masses in NSG mouse, suggesting the participation of NTAL in tumor growth. Using affinity purification in combination with label-free proteomics, we identified and validated new interactions of NTAL with protein members of the LAMTOR family in leukemic cell lines. NTAL-knockdown inhibited the activation of Akt and mTOR pathways, which was associated with the activation of Ras and increased expression of apoptotic markers. Development of autophagy molecular markers, induced mitochondrial dysfunction and excessive reactive oxygen species (ROS) formation was also observed in NTAL knockdown NB4 cells. Taken together, we demonstrate the participation of NTAL together with LAMTOR proteins in a dynamic macromolecular complex that regulates fundamental cell processes such as cell proliferation, apoptosis and autophagy, highlighting the importance of these lipid raft adaptor proteins in acute myeloid leukemia cell signaling.

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POSTER 291

Proximity-based proteomic profiling of DNA double-strand break repair proteins identifies Shieldin complex as novel regulator of NHEJ

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DNA double-strand breaks (DSBs) are the most deleterious lesions which, if left unrepaired, compromise the genomic integrity of cells and lead to several diseases such as cancer. Repair of DSBs involves a signaling cascade which leads to focal accumulation of many proteins on chromatin. Proteomic analyses of these chromatin-bound protein complexes have remained a challenge owing to requisite of harsh biochemical methods for extraction purposes, which disrupts protein-protein interactions. Therefore, to circumvent this issue, we have used proximity-based proteomics approach to provide for the first time a high-resolution neighborhood interaction map of key DNA damage proteins namely 53BP1, BRCA1 and MDC1. Our findings reveal rich network intricacies and implicate a new protein complex (which we named Shieldin) in the DSBs repair pathway.

POSTER 292

Multilayered proteomic analysis of cancer-related mutations in the Dyrk2 kinase complex

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A central issue of current biology is the functional interpretation of the huge amount of genomic data derived from large-scale DNA sequence analysis and their translation into disease phenotypes. The cellular modules that link genotypes to specific phenotypes are protein complexes. In order to relate cancer-associated genomic mutations to structural and functional changes of protein complexes we applied a multilayered proteomic workflow combining advanced quantitative and structural proteomic methods to the poorly characterized Dyrk2 kinase complex. Dyrk2 is suggested to be involved in regulating key cellular processes such as cell proliferation, cytokinesis and cellular differentiation. A dysregulation and mutation of Dyrk2 have been found in various cancer types classifying Dyrk2 as potential oncogene.

We performed a proteomic interaction analysis of the Dyrk2 kinase network using affinity purification mass spectrometry (AP-MS) and proximity-dependent biotin identification (BioID-MS) and identified about 80 highly confident interactors, in particular proteins involved in cell cycle regulation, apoptosis and nuclear transport. The integration of cancer-related point mutations into Dyrk2 affected the interaction network and caused a disassembly of the Dyrk2 kinase complex. Phosphoproteomic profiling by label-free quantitative mass spectrometry (DIA-SWATH-MS) revealed that Dyrk2 mutations affecting the interaction network also lead to significant changes in the cellular phosphoproteome. Many of the regulated phosphosites belong to proteins acting in cell proliferation in agreement with an increased proliferation rate of Dyrk2 KO cells. Furthermore, phosphosites that were significantly downregulated in Dyrk2 deleted cells contain the conserved Dyrk2 phosphorylation motif thus representing potential new substrates of the kinase. Finally, in order to elucidate the structural basis of the observed functional phenotypes we performed a topological analysis of Dyrk2 and its mutant variants by cross-linking coupled mass spectrometry (XL-MS).

In summary, the integrated proteomic workflow provided novel biological insights into a cancer-associated kinase complex.

POSTER 293

Assembling active histone deacetylases into chromatin remodelers: a complex task illuminated using well-placed affinity tags to probe protein interaction networks.

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As members of several histone deacetylase complexes (NuRD, Sin3, CoREST and MiDAC), HDAC1 and HDAC2 control important cellular processes by deacetylating histones. Histone deacetylation results in a more compact chromatin environment, limiting access of the transcriptional machinery to genes and silencing transcription. Aberrant operation of HDAC1/2 containing complexes can result in uncontrolled cell growth and HDAC1/2 are therefore the targets of inhibitors such as suberanilohydroxamic acid (SAHA) for cancer treatment. Although affinity-purification mass spectrometry (AP-MS) approaches have helped define the composition of completed HDAC1/2 containing histone deacetylase complexes, it is less clear how the HDAC1/2 enzymes are folded and assembled into such functional complexes.

While using an AP-MS approach to map HDAC1/2 protein interaction networks, we noticed that N-terminally tagged versions of HDAC1 and HDAC2 did not assemble into HDAC complexes as expected, but instead appeared to be stalled with components of the prefoldin-CCT chaperonin pathway. These N-terminally tagged HDACs were also catalytically inactive. Like HDACs, the CCT complex is also misregulated in disease and has recently been proposed as a druggable target for breast cancer treatment. In contrast to the N-terminally tagged HDACs, C-terminally tagged HDAC1 and HDAC2 captured complete histone deacetylase complexes and the purified proteins were enzymatically active. This tag-mediated reprogramming of the HDAC1/2 protein interaction network suggests a mechanism whereby HDAC1 is first loaded into the CCT complex by prefoldin to complete folding, and then assembled into active, functional HDAC complexes. This process appears to happen in the nucleus. Such a mechanism is similar to one first proposed by Guenther et al. for HSC70/CCT-mediated assembly of HDAC3 into the SMRT complex. The sequential association of HDAC1/2, first with prefoldin-CCT components and then with HDAC complex components, provides a rationale for combining drugs inhibiting multiple complexes for the treatment of disease.

POSTER 294

Interactome Analysis of ERα and AP-2γ in breast cancer cells

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ERα is a member of the nuclear receptor superfamily of transcription factors known to be important in promoting the growth and progression of breast cancer. It does this mainly by acting in concert with a host of associated factors to regulate transcription. Because of the central role of the receptor, ERα breast cancer patients undergo anti-estrogen therapy, but many patients will relapse and become resistant to the therapy. Therefore, identifying all of the interacting protein partners of ERα and understanding how they function in ERα-mediated transcription is important for developing new strategies for breast cancer treatment. To achieve this, we performed chromatin immunoprecipitation coupled to mass spectrometry in MCF-7 cells for ERα and AP-2γ, a DNA binding transcription factor that we previously characterized as a collaborative factor of ERα. Overall, we discovered 175 and 316 protein interactors of ERα and AP-2γ, respectively. We validated these interactions independently using the BioID assay. Overlapping of the ERα and AP-2γ interacting proteins revealed these two transcription factors share a large number of common protein partners, many of which are novel interactors. In knock-down experiments, many of the common protein interactors showed global effects on estrogen-dependent transcription. Currently, we are investigating their genome-wide roles on ERa- and AP-2γ-dependent transcription as well as their functional importance in breast cancer biology. In summary, using a proteomics approach we have identified novel protein interactors of ERα and AP-2γ that may become novel therapeutic targets in breast cancer.

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POSTER 295
Systematic Analysis of the Mitochondrial Protein Synthesis Network

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Mitochondria are linked to numerous human diseases including cardiovascular and neurodegenerative diseases and cancers. Mitochondria are unique in that 13 proteins of the electron transport chain (ETC) are encoded by the mitochondrial genome while the majority of ~1000 proteins of this organelle are nuclear-encoded. The 13 proteins are synthesized by mitochondrial ribosomes within the matrix, but assemble into the ETC complex within the inner mitochondrial membrane through a poorly understood mechanism. One model posits that these ETC membrane proteins are co-translationally inserted, potentially with the assistance of protein factors located in proximity with the membrane (i.e. “insertases”). Here, we explored the use of proximity biotinylation via APEX2, combined with quantitative Tandem mass tagging (TMT) proteomics, to search for proteins near the protein exit channel of the mitoribosome that may facilitate co-translational insertion of ETC subunits into the membrane. HEK-293 cells were engineered to expressing either MRPL22-APEX2 or MRPL4-APEX2, both of which place APEX2 near the exit channel where it may transfer biotinylation of proximal proteins involved in membrane insertion. As a control, MRPL40-APEX2 located distal to the exit channel was employed. Cells were treated with H2O2 (1 min) in the presence of biotin-phenol, samples were enriched for biotinylated proteins and proteins quantified by TMT proteomics. Comparative enrichment analysis led to the identification of 39 candidate that are selectively enriched with MRPL4-APEX2 and MRPL22-APEX2. Among these was LETM1, a human ortholog of Mdm38p, one of the three insertases proposed in yeast. Studies currently underway seek to further characterize LETM1 as a potential insertase in mammals and to evaluate additional candidate proteins identified through the proximity biotinylation strategy. Our study constitutes the first systematic search for the candidate ETC insertases in the mammalian system, and suggests that this approach may provide a general method for uncovering proteins associated with nascent chains on ribosomes.

POSTER 296
Evaluating the timsTOFPRO bottom-up proteomics platform potential for Proteoform Profiling and Top-Down approaches

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As the bottom up proteomics approaches are now well established as protein detection, quantitation and characterization tools, the complementarity of proteoform profiling and Top-Down approaches is also becoming clearer : they provide information on the distribution of protein isoforms and degradation products which cannot be distinguished after digestion

Ultra-high resolution Q-TOFs are well suited for the analysis of complex protein mixtures as dynamic range, mass resolution and accuracy can be obtained without compromising one to increase the other. We are now evaluating the influence of recently introduced trapped ion mobility (TIMS) when processing the data with top-down software suites.

Undigested protein mixtures of E.Coli (Bruker Daltonics), Yeast (Promega) , and non-depleted plasma (Sigma), are separated on a 150X2.1 mm Aeris Widepore C4 column (Phenomenex), or on a 50 cm X 100 µm monolithic ProSwift column (ThermoFisher) HPLC’s are coupled to a timsTOF Pro UHR-Q-Tof (Bruker) operating in TIMS-off LC-MS or auto LC-MS/MS

Data have been automatically processed in Data Analysis 5.1 (Bruker Daltonics) using the Proteoform Profiling 1.0 routine (Bruker Daltonics).

Identifications were performed using the MASH software (Ying Ge’s group).

TIMS-off LC-MS analyses obtained from an 50µg injection of an E.Coli cell Lysate on the 2,1mm C4 column allowed detection of over 800 proteoforms using a 15 min gradient, mimicking results that had been previously obtained using an Impact II UHR-Q-TOF. Isotopic resolution could be obtained for 30Kda proteins in complex mixtures and the mass accuracy of deconvoluted proteins was well within 5ppm, while the isotopic ratio accuracy was preserved. The intra-spectral dynamic range for intact proteins was well over 3.5 orders of magnitude, and identification was demonstrated for parent ions with intensity 20 times lower than the BPC Base peak intensity. The possible use of complementary TIMS separation will be discussed.

POSTER 297
H2AV lysine crotonylation: an epigenetic switch during human myogenic differentiation

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Variable abundance of histone forms and their post-translational modifications (PTMs) modulate gene expression and thereby confer tissue specificity. Previously, crotonylation of histone lysine (Kcr) was reported to enrich in active promoters or enhancers in human somatic and mouse male germ cells. Based on this, we attempted to establish if crotonylation of histones regulated human myogenesis.

We extracted histones from matched cultures of immortalized human myoblast cells grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS and differentiated myotubes and quantitated PTMs using nanoliquid chromatography-tandem mass spectrometry (nLC-MS) on an LTQ-Orbitrap by bottom-up approach in a data-dependent acquisition mode. Both states had comparable protein abundance even though H2A variant transcripts were differentially expressed. Further analysis of PTMs demonstrated myotube H2A.V to be reproducibly crotonylated at K13. We confirmed the MS/MS using synthetic H2A.V and H2AZ peptides. Using site-specific antibodies, we validated the increased abundance of H2AVK13Cr in myotubes by western blotting, flow cytometry and confocal microscopy. Next, we established that p300 catalyzed crotonylation of H2AVK4, K7, K11 and K15 of H2AV 1-19 peptide in an in vitro assay with crotonyl CoA cofactor. We determined the enrichment of H2AVK13Cr on myogenic marker genes: *myoD*, *myoG* and *myf5*and also checked the specificity of p300 as the “crotonyl transferase” by chromatin immunoprecipitation (ChIP)-qPCR in cells treated with C464, a specific inhibitor of p300. H2AVK13Cr was enriched in the *myoG* and *myf5* and this enrichment was abolished by C464. Immunofluorescence demonstrated that C464 inhibited myoblast differentiation, as observed by cellular morphology and myosin heavy chain expression. ChIP-Seq was unsuccessful because of the substoichiometric levels of the mark. Presently, we are characterizing the protein-protein interactome of H2AVK13Cr using peptide pulldown assays and mass spectrometry. With this, we hope to characterize the epigenetic features and functional importance of H2AVK13Cr during myogenic differentiation.

POSTER 298
Metabolic labeling and quantitative proteomics for interrogation of proteome-wide acetylation dynamics

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Protein acetylation is critical in various biological processes, and aberrant acetylation can lead to a myriad of disease states. Thus, acetylated proteins and acetyltransferases are therapeutic targets, and understanding acetylation rate dynamics is of utmost importance. Here we performed a comprehensive characterization of acetylation rates on non-histone proteins using mass spectrometry based proteomics and metabolomics. Protein acetylation rates were determined by incorporating an isotopically labeled donor (13C-glucose or D3-acetate), which is metabolically converted into heavy acetyl-CoA and subsequently incorporated into proteins. Labeled HeLa sample time points were collected and analyzed by nanoLC-MS/MS to monitor the rates and trends of heavy acetyl incorporation. Results showed that, out of 1821 acetylated peptides detected by 13C-glucose labeling, 900 peptides had a significantly increasing acetylation trend. Predictably, as the acetate pathway is less utilized by HeLa cells during growth, only 126 out of 2120 peptides from the D3-acetate experiment had a significantly increasing acetylation trend. The quantitative values of acetylation sites were grouped based on their turnover speed; faster rates were enriched on proteins associated with chromatin and RNA metabolism, while slower rates were more typical on lipid metabolism related proteins. Interestingly, we did not observe any enriched sequence motif for any turnover group (fast, medium, or slow rates). However, we could estimate a significant depletion of histidine residues on all acetylated peptides detected. Next, we mapped whether detected acetyls were within known protein domains. We identified over 300 sites that mapped to specific protein domains. For example, several acetylation sites with fast turnover mapped within the activation loop of the histone acetyltransferase p300, indicating the importance of these sites in its regulatory feedback mechanism. Our workflow defines the most significant dynamics of protein acetylation using unsupervised clustering and statistics, which can be used to indicate potential acetylation candidates for active enzyme regulation.

POSTER 299
Cellular Senescence; A Driver Of The Pro-aging Side Effects Of Antiretroviral Therapies

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Aging generates a myriad of phenotypes and pathologies that impair tissue function. One approach to gaining deeper insights into the causes and consequences of aging is to study genetic and environmental conditions that accelerate the process. In humans, this approach may be afforded by the unexpected side effects of therapies used to treat HIV-AIDS. Antiretroviral Therapy (ART) has dramatically improved the prognosis of HIV-infected patients. However, long-term use is now associated with multiple signs of premature aging, including lipodystrophy, osteoporosis, type 2 diabetes, cardiovascular disease and cancer.

Two drug classes are implicated in these progeroid effects: nucleotide reverse transcriptase inhibitors (NRTIs), which can inhibit the mammalian mitochondrial DNA polymerase gamma (PolG) resulting in mitochondrial dysfunction, and protease inhibitors (PIs), which can inhibit the mammalian protease ZMPSTE24. ZMPSTE24 processes the nuclear protein lamin A (LMNA), defects in which cause Hutchinson-Gilford progeria syndrome (HGPS) in humans. Both mitochondrial dysfunction and ZMPSTE24 inhibition induce cellular senescence, a state of essentially permanent growth arrest. Senescent cells accumulate with age and are thought to contribute to a multitude of aging phenotypes, primarily through the

senescence-associated secretory phenotype (SASP), which includes numerous pro-inflammatory molecules.

To determine whether ART might promote age-related phenotypes by inducing cellular senescence, we cultured human fibroblasts with NRTI's or PIs. Treated cells arrested growth and expressed several markers of cellular senescence and the SASP. We are testing the hypothesis that ART causes the accumulation of senescent cells in vivo, which contribute to aging phenotypes through the SASP. Using unbiased mass spectrometry and data-independent acquisitions, we will characterize the full complement of -- and potentially unique -- SASP factors associated with ART administration. These studies will help identify biomarkers for aging phenotypes linked to senescent cells and determine whether patients receiving ART might benefit from senolytic drugs, that reduce senescence burden.

POSTER 300
Use of Nucleic Acid Programmable Protein Array (NAPPA) to Study Autoantibodies in Alzheimer's Disease

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Autoimmunity plays an important role in Alzheimer's disease (AD) onset and/or progression as evidenced by the presence of AD-specific autoantibodies (AAbs). Immunoproteomics has shown promise in transforming research on disease-specific AAbs, but its potential in identification of AD-specific AAbs has not been fully realized. We have developed an AAb biomarker discovery pipeline built on an innovative immunoproteomics technology, namely Nucleic Acid Programmable Protein Array (NAPPA). Our goal is to test the feasibility of identification of AD-specific AAbs using our NAPPA platform and generate preliminary data to support expanded statistically powered AAb biomarker discovery studies in AD.

We have adopted a three-stage strategy, including discovery, verification, and validation, to identify AAb biomarkers of AD. We have obtained paired serum/cerebrospinal fluid (CSF) samples of 60 AD patients and 60 non-dementia controls (NDC) from the Banner Health. Subjects were randomly split into Discovery and Validation Sets so that both sets included 3 AD groups and 3 NDC groups with 10 serum/CSF samples in each. The Discovery Set was used to screen against thousands of human proteins displayed on NAPPA, to identify candidate biomarkers based on their immune responses. Immuno-reactivity of the identified candidates to the Discovery Set was confirmed by ELISA. Further, candidates that passed confirmation were validated with ELISA using the independent Validation Set. Top performers will be assessed by classification performance, as well as by bioinformatics analysis of biological functions and possible pathway enrichment to reveal their potential biological relevance and significance in the onset and/or progression of AD.

POSTER 301
Analysis of Differentially Expressed Hippocampal Proteins: Predicted role of Nuclear HIST4H4 and HIST1H2BB protein in AD pathology

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Extensive research into the genomics of neurodegenerative disorders such as AD-a complex brain disorder and affects over 35 million people worldwide has revealed the importance of nuclear protein like histone family proteins in AD pathology. Brain hippocampus is critical for learning and memory and is particularly vulnerable to ageing, implied by decrease hippocampal volume and neuronal, impaired synaptic integrity, signaling, decreased trophic factors, diminished neurogenesis, and increased neuroinflammation. The underlying mechanisms for this structural and functional decline still needs further elucidation

Here, we present the computational analysis of, hippocampus nuclear proteins, HIST4H4 and HIST1H2BB protein exploring molecular networks of interacting targets by STRING database, IPA and KEGG Pathway analysis –, that may provide insights predicting role and insight into the biological processes underlying AD pathology.

We performed a detailed analysis through merge network tools along with experimental validation of expression patterns. Nuclear proteins from Human AD brain hippocampus and age matched controls were compared using 2DE followed by ESI-Q-TOF mass spectrometry. Data reveals 106 unique proteins in 162 spots with a significant abundance difference between the experimental groups. The differentially expressed selected proteins were mapped to existing biological networks to elucidate the disease-related pathways. Our results demonstrate downregulation of histone H4 and H2B1B and SETDB1 in AD hippocampus; increasing the possibility of arresting cell cycle progression in S phase playing a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability The expression perturbation and interaction network of these proteins predicts a decline in cellular function and integrity leading to chromosomal instability. SETDB1 a histone methyltransferase which specifically di- and tri-methylate lysine 9 of histone H3, effectively repressing gene transcription.

The interaction of APP, NFkB and AKT with the identified proteins requires further validation which could open a new window for understanding the pathogenesis of AD

POSTER 302
A Recombinant Asp-Specific Protease for Bottom-up and Multi-Enzyme LC-MS/MS Workflows

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Bottom-up mass spectrometry workflows typically utilize trypsin to digest proteins into peptides suitable for LC-MS/MS analysis. While trypsin is an excellent protease, alternative proteases are useful for numerous applications including increasing protein sequence coverage and identifying post-translational modifications. Here we report the expression, purification and characterization of a recombinant protease which displays both high cleavage efficiency and a strong preference for cleavage N-terminal to aspartic acid.

Recombinant Asp-N (rAsp-N) was expressed and purified from *E. coli*. FRET-based peptide assays indicated an optimal pH range of 7-9 and activity that was inhibited by EDTA as expected for a zinc-metalloprotease. Digestion performance

was monitored using LC-MS/MS which identified over 1000 proteins and 6000 unique peptides from a yeast digest confirming the utility of rAsp-N for mass spec applications. rAsp-N is highly efficient with a missed cleavage rate below 15% and shows a strong preference for cleavage N-terminal to aspartate residues. Moderate specificity for glutamic acid was observed particularly during longer digestions. Lack of cleavage specificity at isoaspartic acid was determined using synthetic “PENNY” peptides derived from the IgG constant region followed by HPLC.

We also demonstrated the utility of rAsp-N for characterization of therapeutic mAbs. High sequence coverage of both heavy and light chains of the NISTmAb reference IgG was achieved by LC-MS/MS. Furthermore, we show that combining peptides derived from two orthogonal enzyme digests (rAsp-N and Trypsin) can improve sequence coverage of NISTmAb to 100% from a single injection, even without optimization of the chromatography gradient. Combining peptides derived from digestion with orthogonal proteases prior to LC-MS/MS analysis could be a general strategy to ensure high sequence coverage while simultaneously reducing demand on instrument time. In summary, rAsp-N should be a valuable tool for bottom-up workflows including conventional proteomics and peptide mapping of therapeutic proteins.

POSTER 303
Clarification of the Signaling Network Affected by the TNIK Inhibitor, NCB-0846, Using Reverse-phase Protein Array

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Introduction

Aberrant activation of Wnt signaling is a hallmark of colorectal cancers (CRC), and is believed to contribute to the maintenance of cancer stem cells (CSCs). CSCs have self-renewal ability, thus potentially giving rise to a new tumor cell population. Therefore, blocking the Wnt signaling pathway could be an effective approach for eliminating CSCs. We have previously identified Traf2- and Nck-interacting kinase (TNIK) as an essential regulator of the T-cell factor-4 and β-catenin transcriptional complex, and develped a TNIK inhibitor, NCB-0846, with potent anti-Wnt signaling activity, thus attenuating certain CSC traits such as expression of cancer stem cell markers, sphere forming activity, and tumorigenicity. The present study was conducted to clarify in detail the mechanism of action of NCB-0846, including its off-target effects, in CRC cells.

Methods

We examined the phosphorylation status of 180 key signaling nodes in CRC cell lines treated with NCB-0846 and its diastereomer, NCB-0970, which has 13-fold weaker TNIK-inhibitory activity than NCB-0846, using our in-house high-density fluorescence reverse-phase protein array (RPPA) platform. Phosphoproteins significantly upregulated and downregulated by treatment with NCB-0846 were validated by immunoblot analyses.

Results and Discussion

RPPA analysis revealed that phosphorylated histone H2A (γ-H2AX), p53 and chk2, well-known substrates of ataxia telangiectasia–mutated (ATM), were significantly upregulated by NCB-0846, indicating that NCB-0846 elicited activation of the ATM-chk2 DNA damage response pathway. In addition, in NCB-0846-treated cells, expression of phosphorylated histone H3, a marker of mitosis, was completely blocked, whereas expression of an apoptosis marker, cleaved PRAP, became detectable. These findings suggest that G2 arrest led to apoptosis through activation of the ATM-chk2 DNA damage response pathway.

Conclusion

Clarification of the mechanism of action of NCB-0846 in CRC cells will help to confirm its clinical safety, thereby expediting its clinical application.

POSTER 304
Assessment of awareness about immunization among parents in population

Hira Sabir Malik
Bahria university, Karachi, Pakistan

Title:

Assessment of awareness about immunization among parents in population

Introduction and Objectives

Introduction:

Immunization is safe and effective-measure against avertable-diseases in all-over-the-world that prevents children from life threatening infectious diseases.

Awareness and attitude regarding importance of vaccines among parents play significant role to vaccinate their children.

However, Pakistan has some of high rates of deaths among children due to lack of awareness and poor health management, mainly; Improper-vaccination.

Objectives:

To evaluate the status of awareness and attitude about importance of immunization in parents of different status (Upper, middle, lower class) in population of Karachi.

Methods:

A survey-based Cross-sectional-study was conducted in different towns of Karachi. Total 600 Parents were included who have at least one child under-the-age of 10. Out of 600-Questionnaire,

200 were filled by parents in different areas by visiting them door to door, 200 were filled by parents in various schools while the remaining 200-Questionnaire were filled by the parents visiting hospitals. Informed-consent with regards to

confidentiality was provided. Epi-info and SPSS software were used for data management.

Results and Discussion:

Data collected from all three status. Majority of High-class respondents 79% were aware of children immunization, Middleclass 64%, Whereas, Lower-class 49% respectively.

68% respondents of all three classes agreed that child immunization is important for children's healthy future.

70% of the respondents were of opinion that immunization is more beneficial than harmful, While only 42% respondents answered correctly about vaccination schedule.

Generally, Parents have good knowledge about importance of immunization with average of 70.5% responding correctly.

Although, Lower class needs to have more awareness about Importance of Immunization and knowledge for proper scheduling to vaccinate their children.

Conclusion:

Data shows that Parents have positive-attitude and awareness about importance of immunization in general but lacking proper knowledge and guidance. Therefore, The study-results reinforce recommendations for use of educational-programs to improve the immunization knowledge and practice.

POSTER 305

Intracellular Tandem Mass Tag (TMT) Proteomic Analyses of HIV-1 Infected Macrophages after Cocaine and Sig1R Antagonist (BD1047) Treatments

Omar Vélez López[1]; Loyda Meléndez[2]; Abiel Roche Lima[2]; Kelvin Carrasquillo Carrión[2]; Carla Salgado Ramírez[2]; Yadira Cantres Rosario[2]; Eraysy Machín Martínez[2]; Manuel Alvarez Ríos[3]

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Purpose: After human immunodeficiency virus (HIV-1) infection, macrophages (MDM) cross the brain blood barrier (BBB), and secrete several factors that contribute to neuropathology and HIV associated neurocognitive disorders (HAND). One secreted factor is cathepsin B (CATB), a lysosomal cysteine protease whose secretion is exacerbated by cocaine. We have found that pretreating MDM with a sigma-1 receptor antagonist (BD1047) prior to cocaine reduces HIV-1, CATB and neuronal apoptosis levels *in vitro*. We hypothesized that BD1047 treatment will reverse intracellular pathways related to reduction of CATB secretion and neurotoxicity that could be targets for therapy.

Experimental Design: Uninfected and infected MDM lysates from three donors treated with or without cocaine and BD1047 were collected, separated by an SDS-PAGE gel, trypsin digested and tagged using a ten (10) plex tandem mass tags (TMT) per treatment. Proteins were quantitated by LC-MS/MS using Thermo Q-Exactive and analyzed by Proteome Discoverer, R-Limma Statistics, Ingenuity Pathways and validated by Western Blots.

Results: A total of 850 MDM deregulated proteins had a significant fold change (FC≥|2|) in the uninfected vs. infected group, while 802 proteins were observed in the infected+ cocaine vs. infected group. For the infected + BD1047/cocaine vs. infected +cocaine group 912 deregulated proteins had a FC≥|2|. Main functions of abundant proteins involve homeostasis (CA2, HSPA4, and ARF1) cellular stress (CSTB, STIP1, and PDXK), lipid metabolism (APOE) and cellular transport (ANXA2, TRFC, and CD44).

Conclusions and clinical relevance: Intracellular proteome differences might explain mechanisms of HIV infection, CATB secretion, and the potential targets for therapeutics in cocaine users.

Acknowledgments: This research is supported in part by: R25-GM061838 (OV), G12RR03051 (LMM), R25-GM082406, SC1GM11369–01(LMM), RCMI-U54MD007600, PRINBRE-5P20GM103475 and University of Puerto Rico School of Medicine and Biomedical Sciences Deanships.

POSTER 306

Development of data-independent MS platform to quantify phenol soluble modulins isoforms in culture media of *Staphylococcus aureus* from bacteremia patients

Jiyoung Yu[1]; Eun Sil Kim[2, 3]; Yumi Oh[1, 2]; Hwangkyo Jeong[1, 2]; Jeonghun Yeom[1]; Yong Pil Chong[3]; Yang Soo Kim[3]; Kyung-Kon Kim[1, 2]

[1]Asan Medical Center, Seoul, South Korea; [2]University of Ulsan, College of Medicine, Seoul, South Korea; [3]Department of Infectious Diseases, Asan Medical Ce, Seoul, South Korea

Phenol-soluble modulins (PSMs), a group of toxic proteins that are produced by *Staphylococcus* bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA) are an important virulence factor. PSMs from MRSA have been thought to be a one of cause of severe infections. Therfore, in-time detection of PSM in MRSA-infected patients is important clinical unmet needs. So far, only qualitative detection of PSM isoforms have been performed based on UV-HPLC analysis system and requires a lot of cell culture media, which takes time. Recently, MS platform have been applied to detect PSMs but it is not quantitiave and time-effective, also. In this study, SWATH (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra) - MS was developed to quantify several isoforms of PSMs in small volume of culture media (50ul) of *Staphylococcus aureus* isolated from sepsis patients. As a result, PSM alpha1, alpha2, alpha3, alpha4, beta1 and delta toxin were quantifiled in a 20min-single LC-MS run with 30min sample preparation. In addition, several proteoforms including N-termianl and/or C-terminal cleavage forms of each PSM were also identified and quantified. Now this PSM platform are developed to apply liquid biopsy samples (serum and/or plasma) from bateremia patients to detect and quantify several isoforms of PSMs. At the same time, correlation between clnical symptoms and cleavagee forms of PSMs will be investigated further. This research was suppoorted by a grant of the Korea Health technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea. (Grant Number: HI15C2918)

POSTER 307

Comparative proteome analysis for Korean specific *Staphylococcus aureus*

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A gram-positive and common bacterium causing skin, respiratory infection, Staphylococcus aureus can get multi-resistance to beta-lactam antibiotics (methicillin, dicloxacillin, nafcillin, oxacillin and the cephalosporins) via horizontal gene transfer of mecA gene and natural selection. One of them is methicillin-resistant Staphylococcus aureus (MRSA). This resistance makes MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous. To diagnose outbreaks of MRSA, the bacterium should be cultured from body-fluid samples of “unknown fever” patients including blood, urine or sputum to perform confirmatory tests early-on. However, there has been no protein biomarker for quick and easy diagnosis of MRSA from patint specimen. So far, initial treatment of the infection is often based upon suspicion and techniques by the treating physician using quantitative PCR procedures, which takes long time as confirmatory tests early-on. Herein, proteome of 2 kinds genotype of two kinds of S. aureus (MRSA and MSSA) was analyzed using high resolutiuon mass spectrometry coupled with nanoflow liquid chromatography (Nano LC). Among identified proteins, tagatose 1,6-diphosphate alsolase and nodulation efficiency protein D are expressed as MRSA specific. These MRSA specific proteins can be applied as a valuable resource to develop rapid diagnosis of MRSA using patient’s blood or urine, enabling easy and quick diagniosis. This research was suppoorted by a grant of the Korea Health technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea. (Grant Number: HI15C2918)

POSTER 308
Proteomic analysis of bacterial peptide products from stress-modified mRNAs using a synthetic biology approach

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RNase activation is a key component of bacterial stress responses, affecting the stability of mRNA targets. Current methods for collecting mRNA for transcriptomics focus on eliminating fragmented and small RNAs, resulting in the loss of information about any RNase-modified mRNAs and their incomplete peptide products. We hypothesize that characterizing programmed changes to mRNA stability will provide new fundamental insights into the dynamic translational landscape of bacterial stress responses. To address this, we have developed a synthetic RNA platform that allows for co-translational labeling of non-stop mRNA peptide products in live bacteria. Our platform leverages naturally-evolved features of transfer-messenger RNAs (tmRNA), tRNA-like RNAs found in all sequenced bacteria. tmRNA naturally serves as a ribosome rescue system that alleviates ribosomal stalling on non-stop mRNAs. A unique, short tag reading frame (TRF) encoded on tmRNAs facilitates the addition of a peptidyl degradation tag to the nascent, incomplete polypeptide, which targets the aberrant peptide product for directed proteolysis. For our synthetic platform, the TRF offers an attractive modular domain to introduce synthetic polypeptide tag sequences of interest. We have thus far developed an inducible platform in E. coli by recoding the native TRF sequence to introduce a 6x-HIS tag, which allows us to stabilize, isolate, and measure tagged aberrant peptide abundance. We have successfully isolated 6xHIS-tagged proteins using affinity purification, verified controlled tagging using western blots, and assessed broad-spectrum tag introduction using mass spectrometry. Excitingly, our preliminary work shows that tagged protein profiles are markedly different under stress conditions. In response to oxidative stress, for example, we have identified an increased diversity of both unique and overrepresented peptides in our tagged subproteome, indicating wide-spread mRNA damage. We anticipate our synthetic platform will improve upon studies using current techniques in bacterial models by providing new information to a previously underappreciated aspect of bacterial stress responses.

POSTER 309
Comparative Proteomic Profiling Reveals New Insight between Different Growth Phase of Biofilm Extractomes from *Staphylococcus aureus* Using TMT-based Quantitative MS

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Background

Staphylococcus aureus and coagulase-negative staphylococci comprises approximately 65% of infections associated with medical devices and are well known for their biofilm formatting ability. Currently, there is no efficient method for early biofilm detection. Therefore, we aimed to construct a comprehensive reference map followed by identifying marker proteins between the different growth phase of biofilm, and then perform pathway analysis, subcellular localisation and protein-protein interaction (PPI) network mapping.

Methods

S. aureus reference strain (ATCC 25923) was grown in tryptic soy broth to produce a 24-hour planktonic, 3-day wet biofilm (3dwb), and 12-day wet biofilm (12dwb). The Centres for Disease Control biofilm reactor was used to grow biofilms. Protein extraction, fractionation, reduction, alkylation and digestion steps were performed prior to Multiplex labelling using Tandem Mass Tag (TMT) 10-plex reagent, respectively. TMT-based mass spectrometry (MS) was performed, and protein identification and relative quantitation of protein levels were performed using Proteome Discoverer (version 1.3). Statistical analysis was done using the TMTPrePro R package.

Results

We identified 1636 total biofilm extractomes, of which 20 and 34 proteins, involved in recognised protein pathways, were significantly (>2-fold) up-regulated in 3dwb and 12dwb respectively. In particular, hyaluronidase encoded by hysA is significantly up-regulated in 3dwb. HysA is involved in dispersing established biofilms by degradation of hyaluronic acid. In contrast, 31 and 14 proteins, in recognised pathways, were significantly (>2-fold) down-regulated in 3dwb and 12dwb respectively. In addition, we showed a significant range of quantitative proteomic shifts and changes in metabolic process

in 3dwb and 12dwb. Moreover, PPI network showed significantly more interactions for 3dwb and 12dwb.

Conclusions

This is the first report using high throughput TMT-based MS from *S. aureus*. The proteins identified may be potential candidates for vaccines, anti-biofilm agents, and diagnostic biomarkers for *S. aureus* biofilm-related infections associated with implantable medical devices.

POSTER 310

Systematic analysis revealed a subset of heat shock response genes are required for optimal growth of *Halobacterium salinarum*

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Halobacterium salinarum, an extremely halophilic archaeon, thrives in high salt environments such as the Great Salt Lake, Dead Sea, and solar salterns. To explore the importance of ten of the conserved heat shock protein (*hsp*) genes and four other genes induced by temperature upshift, 14 double gene deletion mutants (i.e., *Δura3Δhsp2, Δura3Δhsp3, Δura3Δhsp4, Δura3Δhsp5, Δura3ΔcctA, Δura3ΔdnaJ, Δura3ΔdpsA, Δura3ΔftsJ, Δura3ΔgrpE, Δura3Δlon, Δura3Δpan1, Δura3Δ0016h, Δura3Δ1843c,* and *Δura3Δvng2008h*) were constructed and compared their growth patterns with Δ*ura3* host at 37oC, 42oC, and 49oC. Distinct growth reduction patterns in one or more temperatures were observed among some but not all of the mutants. Spectral counting based label-free quantitative proteomics analysis of five and three mutants with reduced growth at 37oC and 49oC, respectively, found unshared expression changes among these mutants. Our preliminary data suggested some of the *hsp* genes may play a collaborative role for optimal growth at environmental temperatures.

POSTER 311

Probing protein state in bacteria by thermal proteome profiling

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New technologies for studying microbes and antimicrobial mechanism of action are currently warranted due to the rise of antibiotic resistance. Thermal proteome profiling (TPP) is based on the principle that proteins become more resistant to heat-induced unfolding when bound to a ligand. Here, we used TPP in *Escherichia coli* to study the *in vivo* thermostability of its proteome, and to assess how genetic and chemical perturbations affect it.

We found that the *E. coli* proteome was more thermostable than the human one, which is consistent with the ability of this organism to grow at higher temperatures. Interestingly, protein thermal stability depended on subcellular location—forming a high-to-low gradient from the cell surface to the cytoplasm. Further, the subunits of protein complexes located in a single subcellular compartment (e.g., cytosol, inner membrane, or periplasm) melted in a similar manner, while protein complexes spanning multiple compartments had their subunits melting in a location-wise manner. This indicates that proteins thermally stabilize each other, but that the intrinsic stabilization conferred by protein localization (coded in the sequence) overrides this behavior. In agreement, knocking-out one of the members of a complex led to the destabilization of the remainder of the complex. Finally, by combining TPP *in vitro* and *in vivo*, we correctly identified targets of known antimicrobial drugs, the downstream effects of their inhibition, and possible resistance mechanisms.

In conclusion, TPP provides a novel way of systematically phenotyping the cell. This platform can be used to improve our understanding of basic bacterial biology by gaining insights into protein structure, protein complex formation, metabolic activity, and drug-protein interactions.

POSTER 312

Multi-omics analysis of a nutrient transport protein required for full virulence in *Campylobacter jejuni*

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Campylobacter jejuni is the leading cause of bacterial gastroenteritis in the developed world. Infection is predominantly caused by the consumption of undercooked or poorly prepared poultry. *C. jejuni* exists mainly as a commensal within the intestines of chickens, but is pathogenic in humans. While the mechanism of this difference is unknown, factors such as motility and nutrient uptake are significant in the host-pathogen nexus. *C. jejuni* is considered assaccharolytic and primarily utilizes amino and organic acids as carbon sources, with only some strains able to utilize fucose. We conducted label-based LC-MS/MS proteomics of *C. jejuni* NCTC11168O to identify proteins associated with growth in environments that mimic host conditions (e.g. deoxycholate, iron limitation, presence of mucin). We quantified 1561 proteins, equating to ~93% of the predicted *C. jejuni* proteome. The most significantly induced protein (mean 4.6-fold induction) was the product of the *cj0025* gene, which has been annotated as a 'putative C4-dicarboxylate transporter'. Deletion of *cj0025* resulted in reduced *C. jejuni* motility, increased susceptibility to polymyxin B, and reduced biofilm formation. Human epithelial cell infection assays confirmed *Δcj0025 C. jejuni* demonstrated significantly reduced adherence and invasion. To determine the function of Cj0025, metabolomic profiles of media inoculated with *C. jejuni* wild-type or *Δcj0025* were compared, with focus on the uptake of amino acids and Krebs cycle intermediates. These assays showed that *Δcj0025* was capable of utilizing all amino and organic acids commensurate with the wild-type. Sequence similarity to a family of bacterial cystine (Cys-Cys) transporters was determined and medium cystine levels were significantly maintained in *Δcj0025* mutants compared with wild-type. Growth supplemented with a toxic mimic of cystine, selenocystine, significantly inhibited wild-type growth, but did not affect *Δcj0025*. We confirm that Cj0025 is a cystine transporter, which we have named TcyP consistent with the nomenclature of homologous proteins.

POSTER 313

Automated TMT Labeling Using Solid Phase Micro Extraction Cartridges

Greg Foster; Aaron Robitaille; Daniel Lopez-Ferrer

Thermo Fisher Scientific, San Jose, CA

Standardizing sample preparation and isobaric labeling with TMT reagents is a necessary step in providing proteomics

users with high confidence quantitative data. Here we combine one-pot chemistry with TMT reagents, micro sample preparation cartridges, and a liquid handler to automate sample preparation and TMT labeling. We have developed an automated standardized end-to-end solution which will digest, clean up and TMT label within two hours to increase sample throughput. The system developed here integrates sample preparation in proteomics including cell lysis, digestion, TMT labeling and desalting. The system is a modified CTC PAL 3 autosampler with a solid phase micro-extraction unit and a temperature controlled agitator. Samples and reagents are loaded into their respective trays. Users are guided through a GUI to select a TMT method (0, 2, 6, 10, 11) according to the number of samples that are going to be prepared. While the samples go through the digestion process, the SPE cartridges are activated and equilibrated. Once the digestion process is finished, the peptides are TMT labeled, loaded, washed, and eluted autonomously within 30 minutes. Peptide mixtures are then UV quantified, normalized and pooled before LC-MS analysis. The TMT labeling instrument successfully labeled HeLa cells utilizing the isobaric chemical tag kits tested. Ten samples (labeled with Thermo Scientific TMT 10plex reagents) were prepared in 1.5 hours, reducing hands-on time, sample loss and user variability seen in traditional methods. When compared to traditional methods, a 50% increase recovered peptide was observed. We have determined this workflow solution is reproducible (CVs<10%), scales from 5ug-100ug of peptide input, is applicable to purified proteins, lysates, and intact mammalian cells.

POSTER 314
Standardization of Sample Preparation for Proteomics Applications

Aaron Robitaille[1]; Greg Foster[1]; Ryan Bomgarden[2]; Sergei Snovida[2]; Daniel Lopez-Ferrer[1]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Rockford, IL

Mass spectrometry based proteomics is the preferred method for in-depth characterization of the protein components of biological systems. However, there is no standardized method for proteomic sample preparation, contributing to a lack of reproducible results. Current proteomic sample preparation methods may consist of some or all of the following steps: cell lysis, protein quantification, reduction, alkylation, digestion, desalting, and peptide quantification. These steps are performed sequentially, requiring significant user involvement and long incubation times. Therefore sample preparation can be a significant bottleneck in proteomic studies. Thus we developed a standardized workflow that overcomes the above-mentioned disadvantages and/or limitations. We show the scalability, versatility, and reproducibility of a single step protocol that combines the cell lysis, protein denaturation, reduction, alkylation, and digestion while reducing the sample preparation time to approximately one hour. Samples are directly added to a reagent mixture that includes pre-measured quantities of a buffering salt, a detergent, a reducing agent, an alkylating agent, and an immobilized proteolytic enzyme. Next, the rapid digestion of proteins is facilitated by agitated heating. Peptides are then desalted and detergent is removed through a mixed mode clean-up procedure. The resulting peptides are UV quantified and normalized prior to LC-MS analysis. We demonstrate that the one-step protocol reduces hands on time to 10 min, and total sample processing time from intact cells to cleaned-up peptides in 75 min. We determined this workflow scales from 10ug-100ug of protein input, and the procedure is versatile and applicable to purified proteins, lysates, and intact mammalian cells. Finally, we show this procedure is compatible with isobaric labeling reagents such as Tandem Mass Tags (TMT) and Label Free Quantitative (LFQ) methods to reproducibly quantify protein abundances. We present a standardized workflow for proteomic sample preparation that is scalable, versatile, and reproducible, while being compatible with automation.

POSTER 315
Efficient Plasma Sample Preparation for MS-Based Quantitative Profiling

Sergei Snovida; Amarjeet Flora; Ryan Bomgarden; John Rogers
Thermo Fisher Scientific, Rockford, IL

The large dynamic range in protein abundance of plasma samples is a major problem associated with plasma/serum-based biomarker discovery experiments, and depletion of abundant proteins is required in order to identify and measure changes in prognostic or diagnostic plasma proteins. We have optimized the production and immobilization of immunoaffinity ligands to develop new top2 and top12 abundant protein depletion resins. In addition, we comprehensively evaluated specificity, efficiency, and reproducibility of abundant protein depletion from human plasma samples. Finally, we have optimized this procedure for the downstream applications with the Tandem Mass Tag (TMT) reagent-based workflows, with a particular emphasis on minimizing sample handling steps and reducing the overall preparation time.

Commercially obtained pooled human plasma samples were used to assess selectivity, efficiency, and binding capacity of the depletion resins by SDS gel, ELSIA, and liquid chromatography mass spectrometry experiments (LC-MS). Replicate samples were used to assess these parameters for reproducibility. Both label-free approaches and tandem mass tag (TMT) reagents were used for relative quantitation of human plasma proteins by LC-MS. High-pH reversed-phase fractionation was performed on the TMT-labeled samples to enable deep and comprehensive proteome coverage. All samples were analyzed on Thermo Scientific™ Orbitrap FusionTM TribridTM mass spectrometer and processed using Thermo Scientific™ Proteome Discoverer™ 2.2 software. TMT-labeled samples were analyzed using synchronous precursor selection mode (SPS) for accurate, interference-free quantitative comparison.

We have evaluated several sample preparation workflows, including those with protein-level desalting by solvent precipitation and peptide-level only desalting, to assess the approaches on the basis of efficiency, ease of use, reproducibility, and yield, and have optimized the workflow for depleted and undepleted plasma samples to attain quick and efficient sample preparation. The workflow is compatible with TMT-reagent labeling and downstream peptide-level high pH reversed phase fractionation.

POSTER 316
Evaluation of timsTOF Pro in multiplexed workflows

Henry Shwe; Joel Federspiel; Xinlei Sheng; Ileana Cristea; Tharan Srikumar
Princeton University, Princeton, NJ

Trapped ion mobility spectrometry (TIMS) consists of a compact ion tunnel, in which nitrogen gas pushes ions against

an electric field gradient until equilibrium. Ions can then be scanned out of the device by stepwise lowering of the electric field. By synchronizing the sequential release of ions with the precursor isolation in the quadrupole, the speed and sensitivity of MS/MS scans are increased in a method dubbed PASEF (Meier et al., J Proteome Res. 2015). Here we utilize PASEF data-dependent acquisition on a Bruker timsTOF Pro to explore temporal proteome changes in human cells during viral infection. We compared the depth of coverage and quantification accuracy with other MS platforms.

First, we aimed to assess the accuracy of the quantification. Ratio distortion has been reported for multiplexed sample analysis when using traditional MS2 based workflows due to interfering ions co-isolated with the precursor ions. The application of ion-mobility separation in PASEF mode could improve the quantitation accuracy and recover the dynamic ratios in the samples. Therefore, we determined the effect of ratio compression using a mixture of human and E. coli samples. HEK293 cells were labeled with tandem mass tags (TMT6plex) and mixed at ratio of 1:0:1:1:1:1. E. coli cells were mixed at ratio of 0:1:1:2:10:4. The human and E. coli peptides were then mixed at a ratio of 25:9. Next, we further optimized the acquisition system parameters for the timsTOF to improve the quantification and sensitivity. Lastly, we applied our optimized parameters and TMT labeling to analyze proteome changes in primary human fibroblasts during infection with two major human pathogens, herpes simplex virus 1 (HSV-1) and human cytomegalovirus (HCMV). The temporal acquisition during infection showed changes associated with immune response early in infection, as well as alterations linked to the assembly of new viral particles late in infection.

POSTER 317
Development of a Quantitative Proteomic Standard for Tandem Mass Tags (TMT)

Jae Choi[1]; Aaron Robitaille[2]; Tabiwang Arrey[3]; Rosa Viner[2]; Andreas Huhmer[2]; John Rogers[1]

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Introduction: Quantitative proteomics strategies using Tandem Mass Tags (TMT) enablable sample multiplexing and precise measurement of protein abundance. However, co-isolated ion interference can suppress accurate ratio quantification. Employing MS3 methods with synchronous precursor selection (SPS) on Orbitrap Tribrid mass spectrometers can minimize ion interference. Therefore, we developed a standardized commercially available TMT11plex yeast digest standard to detect co-isolation interference and enable MS method optimization,

Methods: Here, we utilized TMT11plex to label peptides from four strains of Saccharomyces cerevisiae; a parental line and three lines respectively lacking the non-essential protein Met6, His4, or Ura2. Tryptic peptides from the strains lacking gene MET6, HIS4, OR URA2 were labeled in triplicate, while the parental line was labeled in duplicate.

Preliminary Data: We demonstrate that a TMT11plex yeast digest standard can be used as a proteomic reference standard to measure protein/peptides identification and optimize acquisition and data analysis methods to limit co-isolation interference, as well as diagnosis MS instrument status by monitoring mass accuracy, ion injection time, and reporter ion signal to noise. We then used the standard to establish a standardized workflow including two LC methods (50min or 120min gradients) for a variety of nano-spray liquid chromatography setups including Easy-nLC 1200 and 1000, and Dionex U3000, optimized MS acquisition settings for Hybrid or Tribrid Orbitrap mass spectrometers, and data analysis in Proteome Discoverer 2.2. The TMT11plex yeast digest standard provides mass spectrometry users a tool to measure the accuracy, precision, and dynamic range assessments for different mass spectrometry approaches, and is an excellent quality control assay to the assessment of the LC and MS instrument status when combined with a standardized workflow.

Novel Aspects: We present development PierceTM TMT11plex yeast digest standard that can be used to detect co-isolation interference for Tandem Mass Tags (TMT) as well as diagnosis MS instrument status.

POSTER 318
Method Development for Quantification of Vitamin D-Binding Protein in Prenatal Serum using LC-MRM

Lisa Kilpatrick[1]; Ashley Boggs[2]; Stephen Long[2]; Karen Phinney[1]

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Introduction:

Vitamin D-binding protein (VDBP) is the primary transporter of vitamin D metabolites in serum to target tissues. There is an interest in calculating the unbound or bioavailable fractions of 25-hydroxyvitamin D [25(OH)D] which requires an accurate measurement of the VDBP concentration. Measuring the concentration of bioavailable 25(OH)D may be important during pregnancy since deficiency has been associated with adverse outcomes for both the mother and baby. Studies show that immunoassays for VDBP may not give comparable results and there are no standards currently available to assess their accuracy. Therefore, a method using liquid chromatography-multiple reaction monitoring (LC-MRM) was developed to quantify VDBP in candidate SRM (cSRM) 1949 Frozen Prenatal Serum, a 4-level pooled material collected from women before and during each trimester of pregnancy.

Methods:

Three vials were of each level of cSRM 1949 (NIST) were analyzed in triplicate. An aliquot was removed from each and denatured with trifluoroethanol, reduced with TCEP, alkylated with iodoacetamide and digested overnight with trypsin. Isotopically labeled peptides were used as internal standards during analysis by LC-MRM. Protein concentrations were calculated from the average concentrations of selected peptides monitored during MRM.

Conclusions:

In this study, a method was developed for the quantification of VDBP in prenatal serum to determine if an increase in

concentration could be detected by LC-MRM. To ensure the accuracy of the VDBP concentrations measured, method parameters were tested that might affect peptide concentrations such as trypsin amount and interferences during MRM. Using the optimized method, the concentration of VDBP in the samples was found to increase approximately 1.8-fold during the progression of pregnancy from about 4.0 to 7.3 micromol/kg (210 to 387 mg/L). These results may be useful in other laboratories as a reference for assay calibration of VDBP in serum.

POSTER 319

Highly reproducible and accurate label free quantification using the PASEF method on a TIMS-QTOF mass spectrometer

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The quality of quantification is mainly dependent on three different parameters: performance of the instrument, optimized acquisition methods, and sophisticated data analysis software. Here we show highly reproducible and accurate quantification using a TIMS-QTOF mass spectrometer with the PASEF acquisition method (providing a sequencing speed of > 120 Hz). We used a nanoElute (Bruker Daltonics) nano-flow HPLC on-line coupled to a high-resolution TIMS QTOF (timsTOF Pro, Bruker Daltonics). The peptide mixtures (< 200 ng) were loaded onto a 25 cm pulled emitter column (IonOpticks, Australia). Data were acquired using the PASEF method. Data analysis was performed using the software packages of PEAKS studio (Bioinformatics Solutions Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry) which have optimized several parameters of their algorithm for the processing of 4-dimensional PASEF data. To evaluate the reproducibility of PASEF, we first analyzed 200 ng of a complex peptide mixture derived from a mammalian cell line in the single-run format using a 90 min gradient and optimized the PASEF parameters accordingly. A deep proteome coverage could be achieved with more than 5300 proteins families identified in each run. Comparing label free intensities between technical replicates shows excellent reproducibility with a R2 = 0.98. We also determined the coefficient of variation of the label free intensities, also with good reproducibility. While good reproducibility is a pre-requisite for good quantitative performance, accurate quantification of differentially expressed proteins remains challenging over a wide concentration range and requires a robust analytical platform. For evaluation of the accuracy of quantitation we spiked E. coli and yeast digests in different ratios (1:4 and 2:1) into HeLa digest and injected 150 ng sample material and measured each ratio in triplicate analyses. Peptides identified from the different species could be clearly separated by their expected ratios using LFQ analysis.

POSTER 320

Landscape of Deubiquitinating enzymes (DUBs) in KRAS mutants using activity based protein profiling (ABPP) and bioinformatics analysis

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Dysregulation of ubiquitin signaling has been linked to KRAS mutations. Hence, designing cancer therapies that manipulate protein ubiquitination in context to KRAS mutations is very crucial. One of the potential ways is to inhibit DUBs. Unfortunately, due to the absence of a better detection system for DUBs based on their activity, target discovery is lacking. We aim to profile DUBs and their activity in lung cancer cell lines and tumor tissues.

We used ABPP to profile DUBs in 25 different lung cancer cell lines harboring KRAS mutations. ABPP uses chemical probes that are active-site directed and covalently bind to a class of enzymes in complex proteome. Whole cell lysates were incubated with HA-UB-VME and HA-UB-PA probes and ABPP pull-down was performed. Enriched proteins were trypsin digested and peptides were analyzed using LC-MS/MS. DUBs were quantified by MAXQUANT software.

A total of 50 DUBs were identified ranging from USP, OTU, JAMM, UCH and MJD families. 22 DUBs were present in all cell lines tested, some of which include ATXN3, EIF3H, OTUB1, PRPF8, PSMD7, USP14, UCHL5 and VCPIP1. Functional analysis using various databases and protein-protein interaction network analysis reveal that most of the active DUBs are involved in cell cycle, DNA damage response, DNA repair and RNA splicing. For example, multiple databases point out that VCPIP1, CPOS5, CYLD and USP9X are involved in cell cycle regulation, USP10, USP3, USP28 and UFD1L are involved in DNA damage response and PRPF8 is involved in RNA splicing.

These profiles of active DUB in lung cancer cell lines can be used in future experiments to assign functions of these DUB and define targets for chemical interventions. Additional studies are planned to examine DUB activity using similar methodology in lung cancer tumor tissues, including adenocarcinoma (KRAS mutant and wildtype) and squamous cell lung cancer.

POSTER 321

In-depth secretome analysis in stage-specific colon cancer cell lines

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Despite major improvements in knowledge, therapies and clinical management, cancer generally remains a deadly disease. Globally colorectal cancer (CRC) is the third most common cancer in women and the fourth most common cancer in men. Cancer cells acquire several capabilities to fully develop their malignant phenotypes. For many of these acquired abilities, arrays of tumor cell secretory factors are necessary to initiate and maintain these processes. These factors are collectively referred to as the 'cancer secretome', which includes all proteins secreted, shed or leaking from a cancer cell or tissue under certain conditions and at a certain time. The cancer secretome can thus be considered to represent the tumor microenvironment that plays a key role in tumor-promoting processes, such as angiogenesis, migration, and invasion. Here using a Mass spectrometry approach to characterize the secretome of different subtypes of CRC cell lines, we have identified 1507 and 2466 proteins from the HT29 and DLD1 cellular secretome, respectively. SignalP analysis

disclosed 113 (HT29) and 263 proteins (DLD1) with a signal peptide that were secreted via the classical pathway; SecretomeP analysis predicted a total of 397 (HT29) and 709 (DLD1) proteins that could be secreted via the non-classical pathway. Gene Ontology (GO) analysis revealed that most of the proteins were related to Protein Metabolism, Signal Transduction, and Cell Communication. In using SWATH-MS quantitative analysis, 121 proteins were down-regulated and 7 were up-regulated in the HT29 secretome, in comparison with DLD1. Further, pathway analysis revealed that most of the identified proteins were associated with the platelet-derived growth factor receptor (PDGFR) and the epithelial to mesenchymal transition (EMT) signaling pathways. In conclusion, this study characterizes the secretome signature of the HT29 (Stage 2) and DLD1 (Stage 3) CRC cell lines, providing a list of putative CRC biomarkers related to a specific tumor stage.

POSTER 322
Fully validated SRM-MS-based method for absolute quantification of PIVKA-II in human serum: Clinical applications for patients with HCC

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Protein induced by vitamin K absence or antagonist-II (PIVKA-II), an abnormal form of prothrombin, is used as a serological biomarker that aids in the diagnosis of hepatocellular carcinoma (HCC). PIVKA-II is typically measured by liquid binding assay (LiBA). However, without an internal standard, it is difficult to obtain accurate results. Thus, we aimed to develop a selected reaction monitoring-mass spectrometry (SRM-MS)-based assay to quantify PIVKA-II in serum. Our SRM-MS assay entailed the addition of a protein analog as an internal standard, the enrichment of PIVKA-II using a monoclonal antibody, chymotrypsin digestion, online desalting, and SRM-MS analysis. The performance of the SRM-MS assay was compared with that of LiBA in 400 human serum samples (100 chronic hepatitis, 100 liver cirrhosis, and 200 HCC). Integrated multinational guidelines were followed to validate the assay for clinical implementation. The linearity ranged from 1.28 to 100,000 ng/mL, and the use of a labeled protein analog as an internal standard allowed the error from the sample preparation to be corrected, improving the precision and accuracy. The SRM-MS assay was validated to meet all of the criteria of the compliance with guidelines per the US Food and Drug Administration (FDA), European medicines agency (EMA), Korea FDA (KFDA), and Clinical & Laboratory Standards Institute (CLSI). We have developed and validated a robust and reproducible SRM-MS assay that is superior to the conventional method of distinguishing HCC from noncancer patients, based on PIVKA-II levels, and satisfies clinical standards. This method has potential applications in quantifying other protein biomarkers.

POSTER 323
Clinical Assay for AFP-L3 Using Multiple Reaction Monitoring-Mass Spectrometry for Diagnosing Hepatocellular Carcinoma.

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BACKGROUND: Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) is a serum biomarker for hepatocellular carcinoma (HCC). AFP-L3 is typically measured by liquid-phase binding assay (LiBA). However, LiBA does not always reflect AFP-L3 concentrations, due to its low analytical sensitivity. Thus, we aimed to develop an analytically sensitive multiple reaction monitoring-mass spectrometry (MRM-MS) assay to quantify AFP-L3 in serum.

METHODS: The assay entailed the addition of a stable isotope-labeled internal standard protein analog, the enrichment of AFP using a monoclonal antibody, the fractionation of AFP-L3 using *lens culinaris agglutinin* lectin, deglycosylation, trypsin digestion, online desalting, and MRM-MS analysis. The performance of the MRM-MS assay was compared with that of LiBA in 400 human serum samples (100 chronic hepatitis, 100 liver cirrhosis, and 200 HCC). Integrated multinational guidelines were followed to validate the assay for clinical implementation.

RESULTS: The lower limit of quantification of the MRM-MS assay (0.051 ng/mL) for AFP-L3 was below that of LiBA (0.300 ng/mL). Thus, AFP-L3, which was not observed by LiBA in HCC samples (n = 39), was detected by the MRM-MS assay, improving the clinical value of AFP-L3 as a biomarker by switching to a more analytical sensitive platform. The method was validated, meeting all of the criteria in integrated multinational guidelines.

CONCLUSIONS: Due to the lower incidence of false negatives, the MRM-MS assay is more suitable than LiBA for early detection of HCC.

POSTER 324
A novel UHPLC-MRM-MS methodology for reproducible and fast quantification of histone PTMs

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Histones are structural proteins that modulate chromatin structure and gene expression. Their post translational modifications (PTMs) recruit transcription factors and directly affect chromatin state through chemical interactions. Aberrant levels of these modifications are found in many diseases such as over methylation of Histone 3 Lysine 4 (H3K4) and Histone 3 Lysine 27 (H3K27) in acute myeloid leukemia (AML) and multiple lineage leukemia (MLL) (Chi et al, Nature Reviews Cancer 2010). The ability to quantify these changes reliably, at low costs, and in high throughput experiments will allow for large scale studies and has the potential to revolutionize clinical diagnostics. Mass spectrometry (MS) is the method of choice for this analysis; however, it requires expensive instrumentation, each sample requires one hour of run time, and the use of nano-chromatography is susceptible to batch effects. To address these limitations, we coupled standard flow ultra-high-pressure liquid chromatography (UHPLC) to a triple quadrupole mass spectrometer using multiple reaction monitoring (MRM) acquisition mode to quantify 57 peptides and

65 known PTMs. Our method obtained highly reproducible results with a median coefficient of variation 8.5% in 1 hour with comparable results in as low as 20 minutes. For quality control and correction for batch effects, we included a synthetic histone-like peptide to indicate efficient digestion and chemical derivatization in each analysis. Furthermore, we corrected for ionization biases by including a synthetic histone peptide library. Together, our new UHPLC-MRM-MS method has reduced the analysis time and increased reproducibility in quantification opening new opportunities for translational medicine and clinical diagnostics. We hope to increase the number of targets for a more comprehensive histone PTM analysis and to add a computational platform within our existing EpiProfile 2.0 software with future efforts.

POSTER 325

Development of a multiplexed assay for oral cancer candidate biomarkers using peptide immunoaffinity enrichment and targeted mass spectrometry

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Oral cancer is one of the most common cancers worldwide, and there are currently no biomarkers approved for aiding its management. Although many potential oral cancer biomarkers have been discovered, very few have been verified in body fluid specimens in parallel to evaluate their clinical utility. The lack of appropriate multiplexed assays for chosen targets represents one of the bottlenecks to achieving this goal. In the present study, we develop a peptide immunoaffinity enrichment-coupled multiple reaction monitoring-mass spectrometry (SISCAPA-MRM) assay for verifying multiple reported oral cancer biomarkers in saliva. We successfully produced 363 clones of mouse anti-peptide monoclonal antibodies (mAbs) against 36 of 49 selected targets, and characterized useful mAbs against 24 targets in terms of their binding affinity for peptide antigens and immuno-capture ability. Comparative analyses revealed that an equilibrium dissociation constant (KD) cut-off value <2.82 x 10-9 M could identify most clones with an immuno-capture recovery rate >5%. Using these mAbs, we assembled a 24-plex SISCAPA-MRM assay and optimized assay conditions in a 25-microgram saliva matrix background. This multiplexed assay showed reasonable precision (median coefficient of variation, 7.16 to 32.09%), with lower limits of quantitation (LLOQ) of <10, 10–50, and >50 ng/ml for 14, 7 and 3 targets, respectively. When applied to a model saliva sample pooled from oral cancer patients, this assay could detect 19 targets at higher salivary levels than their LLOQs. Finally, we demonstrated the utility of this assay for quantification of multiple targets in individual saliva samples (20 healthy donors and 21 oral cancer patients), showing that levels of six targets were significantly altered in cancer compared with the control group. We propose that this assay could be used in future studies to compare the clinical utility of multiple oral cancer biomarker candidates in a large cohort of saliva samples.

POSTER 326

Proteomic Comparison of 11 Human Tissues using 11-plex Tandem Mass Tags and Synchronous Precursor Selection MS3 Analysis.

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The 'druggable genome' has been published and although this list gives some insight into possible drug targets, it doesn't include information on tissue specificity, and therefore likely favorable safety profile. Using 11-plex tandem mass tags (TMT), we have created a protein expression map enabling insight into the tissue-specific human proteome. 11 Human tissues (Brain, Colon, Heart, Ileum, Jejunum, Kidney, Lung, Liver, Skeletal Muscle, Pancreas, and Spleen) from multiple donors were sent to IQ Proteomics (Cambridge, MA) for analysis. We chose TMT as simultaneous analysis of the samples prevents variation in MS performance and missing values amongst the 11 tissues. MS3 was used to minimize reporter ion interference to better enable determination of specificity amongst tissues. An early pilot study comparing 10 human livers showed significant donor-to-donor variability, raising concerns that a comparison of tissues using a single donor may not truly be reflective of general human tissue biology. We therefore chose a pooled approach, pooling on average samples from 7 donors for each tissue. A heat map of all 8,500 quantified proteins showed the brain as the most distinct tissue. However, gene family specific heat maps looked quite different. For example, in the serine hydrolase gene family heat map, the most distinct tissue by far was the pancreas. The quantified proteins that are part of the druggable genome were ranked for feasibility by: presence of chemical matter, crystal structure (aids structure-based drug design), disease relevance, and tissue specificity, with a maximum score of 4. One protein that met all of those criteria was the serine hydrolase Kallikrein-6 (KLK6). KLK6 can be inhibited by benzamidine and activated by glycosaminoglycans, has four PDB entries, and is a brain-specific protein shown to exacerbate disease in an autoimmune model of multiple sclerosis. Other brain-specific proteins with a score of 4 will be discussed.

POSTER 327

TARGETED PROTEOMICS APPLIED TO THE RESEARCH IN PUBLIC HEALTH: ANALYSIS OF ENERGY METABOLISM OF ISOLATED MONOCYTES FROM SEPSIS PATIENTS PLASMA

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Targeted proteomics is an approach based on mass spectrometry that accurately detects and quantifies pre-selected analytes in complex biological mixtures and is the actual gold standard technique to verify/validate shotgun results. Ions with specific m/z values are monitored in a fast, selective, sensitive, and reproducible manner across a wide dynamic range. Therefore, it represents a robust analytical technique (complementary to immunoassays) which is crucial to advancing clinical proteomic research. Previous shotgun results indicated altered energy metabolism in monocytes isolated from patients with acute sepsis compared to the control

group. To better characterize these alterations, 85 proteins from glycolysis, tricarboxylic acid cycle, β-oxidation, and oxidative phosphorylation were monitored by Selected Reaction Monitoring (SRM) in extracts of monocytes isolated from blood of septic patients (in acute and recovery phases) and from the blood of controls (infection without sepsis). Proteotypic peptides generated by in silico trypsin digestion that attended the criteria of quantotypic peptides were selected. The spectral library included both MS/MS spectra obtained by shotgun proteomics on a QExactive Plus mass spectrometer and fragmentation spectra retrieved from the PeptideAtlas database (NIST_human_QTOF_2012-04-20). After collision energy optimization, the final method consisted of 330 optimized transitions, corresponding to 63 proteins monitored. The quantitative data from five samples of control individuals were compared to nine samples of acute phase patients and nine samples of recovery phase patients, in technical duplicates. In the comparison between control and acute phase groups, ten proteins were found differentially abundant (p-value ≤ 0.05): two were down-regulated (2-oxoglutarate dehydrogenase and electron transfer flavoprotein-ubiquinone oxidoreductase) while eight were up-regulated (e.g.: hexokinase-2 and 2,4-dienoyl-CoA reductase) in sepsis samples. The targeted method was successfully employed for the analysis of 63 proteins, shedding light on the modulation of the energy metabolic pathways in clinical samples of patients at critical state of sepsis.

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POSTER 328

Discrepancy in stably expressed proteins within and across human tissue types

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In recent years, large-scale omics analyses have mapped human gene and protein expression to characterize and compare different cells and tissues. It has been shown that some proteins are mostly expressed in a limited number of tissues, while others are widely and uniformly distributed throughout the human body. Such uniform proteins have been proposed as references for quality control and normalization of omics data, as well as loading controls for blotting techniques. However, these studies did not specifically consider within-tissue variability across human subjects and its impact on normalization in the analysis of the same tissue type from many individuals. Here, we therefore analyze inter-sample variability in proteomic datasets from human liver. We describe the characteristics of the least and most variable proteins, and provide a set of proteins with uniform expression across tissue samples.

Surprisingly, many previously described proteins with low variability across tissue types showed large within-tissue variability. We observed a wide distribution in variabilities, with highly abundant proteins showing less variable expression across individuals. The least variable proteins within tissues were generally confined to the cytosol or mitochondria, while the most variable proteins were more spread across different subcellular compartments. Further, proteins essential for cell survival were overrepresented in the low variability range, suggesting that survival requires expression of these proteins at a certain level in all individuals. Supporting this, inter-sample variability showed an inverse correlation with previously established protein turnover rates.

In conclusion, the differences in variability within and across tissues highlight the difficulties in selecting single proteins for use as references in all tissue types.

POSTER 329

Analysis of Grape Berry Proteome to Determine their Relative Abundance During Berry development and Ripening

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Muscadine grape (Vitis rotundifolia) cultivars are widely grown in southeast US and are popular for unique nutraceutical and enological traits. Studies demonstrated their potential anticancer activity on human cancer cell lines. The grape berry extracts contained variety of phenolics associated with anticancer activity. The goal of this research is to determine the molecular events associated with the berry development and pathways regulating the accumulation of metabolites. The objectives were to: investigate the proteome profiles from berry at different developmental stages; 2. Determine protein-protein interactions across the developmental stages; 3. Determine the metabolic pathways associated with berry development and ripening. Berries were classified into four categories based on brix value. Total proteins were extracted from 4 different stages of berry development and ripening. Mass spectrometry assisted with LC-MS/MS spectra were acquired on an LTQ XL mass spectrometer (Thermo). Spectrum-peptide matching was performed with X! Tandem. A protein database was compiled from reviewed V. vinifera protein entries in UniProt. Normalized spectral abundance factors (NSAF) when calculated, detected 2796 total proteins of which 515 were differentially expressed among the berry stages of developing and ripening. Based on our previous results with iTRAQ, we obtained more protein identifications using present label free method of proteins in grape berry. Gene ontology studies revealed, pathways associated with defense, secondary metabolites and anticancer activity were shown changes in relative abundance across the stages. Berries at brix 15, stage 3 has a major shift in the regulation of several proteins including photosynthesis, stress, PR proteins, flavor. Protein-interaction studies of these differentially expressed proteins revealed several orthologous proteins showing interactions in Arabidopsis interactome database.

POSTER 330

Rapid Qualitative and Absolute Quantification of Plasma based proteins using a Novel Scanning Quadrupole DIA Acquisition Method

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Quantitative proteomics often incorporates the use of stable isotope labels (SILs) to provide absolute quantification. Recent advancements have seen the introduction of peptide panels allowing the quantification of over 500 proteins in plasma

sample sets. However, this is technically challenging when attempting to acquire the data using more traditional MS acquisition modes such as multiple reaction monitoring (MRM), since the duty cycle of the instrument is compromised and therefore results in under sampling. An alternative approach is to apply a data independent analysis (DIA) methodology, allowing for high throughput whilst also ensuring high rates of data acquisition and specificity. Here we describe the use of a kit consisting of SIL's capable of quantifying >500 plasma-based proteins in conjunction with a novel scanning quadrupole DIA acquisition schema in order to quantify proteins of interest for patient cohorts diagnosed with respiratory disorders.

Undepleted human plasma originating from controls and patients diagnosed with chronic obstructive pulmonary disorder (COPD) and asthma were reduced, alkylated and tryptically digested overnight. Prior to LC-MS analysis, samples for their individual groups were pooled and spiked with PQ500 SIL peptides (Biognosys). In all cases, samples were separated using various LC gradients (15, 30 and 60 minutes). MS data were acquired using SONAR, whereby the quadrupole (MS1) was continuously scanned between *m/z* 400-900 using a quadrupole transmission width of approximately 20 Da, whilst the TOF scanned between m/z 50-2000. In all cases, precursor and product ion information were collected. The LC-MS data were processed with Spectronaut Pulsar X (Biognosys AG and Progenesis QI for Proteomics (Non-linear Dynamics). Multivariate statistical analysis showed distinct differences between all three cohorts and proteins corresponding to 86% of the spectral library were quantified. CV's for each group were all found to be <5% in all cases and was maintained across all gradient lengths.

POSTER 331
Absolute Quantification of Apolipoproteins Using a High Precision QPrEST-Based SRM Assay

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Cardiovascular disease (CVD) is the most frequent cause of death globally, but many of the incidences are preventable by simple means, such as alteration in life style or medication. The overall aim is to decrease the plasma levels of triglycerides and cholesterol, which are deposited as coronary artery plaques causing hypertension. The deposited lipid droplets are transported by lipoprotein complexes and an integral part of evaluating the efficiency of the treatment is to accurately and robustly monitor the levels of this protein class. Different compositions of apolipoproteins are involved in the formation of high-density lipoproteins (HDL) and low-density lipoproteins (LDL). An efficient, cost effective and relatively fast way of monitoring the apolipoprotein plasma levels is to utilize a multiplex mass spectrometry approach. In order to achieve robust and reproducible results across many samples an advantage is to use internal standards for quantifying the endogenous protein levels. A common problem with many stable isotope labeled standards is that they are added after digestion or not processed by the proteolytic enzyme in the same way as the endogenous proteins would. By adding an internal standard in the very beginning of the sample preparation that is digested by the proteolytic enzyme to release the standard peptides, a more robust and reliable quantification strategy can be achieved. In this study, a high precision targeted proteomics assay for monitoring twelve different apolipoproteins using stable isotope labeled protein fragments (QPrESTs) together with selective reaction monitoring (SRM) is presented. The QPrEST-based multiplex assay delivers protein measurements with high reproducibility (coefficient of variation range 4.5-8.8 %) across samples digested in triplicate over five days. The assay was subsequently used to assess the apolipoprotein levels in clinical samples (EFFECT1, placebo vs. EpanovaTM vs. Fenofibrate) confirming known effects of fenofibrate on apolipoproteins associated with HDL and VDL.

POSTER 332
The Proteomic and Biochemical Studies demonstrate that 300-Mediated Lysine 2-Hydroxyisobutyrylation Regulates Glycolysis

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Lysine 2-hydroxyisobutyrylation (Khib) is an evolutionarily conserved and widespread histone mark like lysine acetylation (Kac). Here we identified p300 as a "writer" for Khib in mammalian cells. Using a SILAC-based quantitative proteomics approach, we identified 4239 unique Khib sites on 1459 proteins and 3682 unique Kac sites on 1887 proteins, respectively, with varied numbers of modification sites on each substrate. The analysis also identified 149 Khib sites and 693 Kac sites whose normalized abundance was decreased by more than 50% in p300 KO cells compared to WT cells (quantified in at least two of three replicates). The proteins containing these lysine sites were therefore considered as potential p300-targeted substrates. In addition, although some protein substrates bear both Kac and Khib sites, we found about 82% of the global Khib sites did not overlap with Kac sites. Strikingly, only 6 out of the 149 p300-targeted Khib sites overlapped with the p300-targeted Kac sites, suggesting that p300 has different substrate selectivity toward Khib and Kac. In support of this notion, the p300-targeted Khib and Kac sites show distinct flanking sequence motifs. Interestingly, we found that diverse cellular proteins, particularly glycolytic enzymes, are targeted by p300 for Khib but not for Kac. Deletion of p300 significantly reduces Khib levels on several p300-dependent, Khib-specific sites on key glycolytic enzymes, decreasing their catalytic activities. Therefore, we revealed a p300-catalyzed, Khib-specific molecular mechanism that regulates cellular glucose metabolism. Our findings further indicate that p300 has an intrinsic ability to select short-chain acyl-CoA-dependent protein substrates, addressing a long-standing issue regarding the specificity of diverse lysine acylations.

POSTER 333
Global proteomic profiling of dehydration-modulated mitochondrial dynamics and defense response in rice

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Water-deficit or dehydration greatly limits crop productivity worldwide. More than 50% of average crop yield losses experienced worldwide is due to exposure to abiotic stress, especially dehydration. Therefore, to improve water-use efficiency in crops as well as to make climate resilient varieties, it is imperative to investigate the molecular mechanism of stress tolerance. Rice (*Oryza sativa*, L.), being the most important food crop in the world, is an integral part of human diet and nutrition. It is the world's second most important cereal crop with annual production of more than 480 million metric tons. The role of mitochondria generated ROS is well known in regulation of cellular signalling; however, much of its role in stress adaptation remains largely unexplored. We dissected the dehydration-responsive mitochondrial proteome of rice through iTRAQ-based proteomics approach and identified 101 putative regulators involved in stress adaptation. A critical screening of the mitochondrial proteome revealed an unknown sensor protein, harboring a domain of unknown function putatively involved in dehydration response. Analysis of its transcript abundance under dehydration, salinity and oxidative stress showed consistent stress-induced upregulation. Mitochondrial localization of the censor was confirmed by transient expression assay in Nicotiana leaves as well as by immunoblot analysis. Proteome-scale interactome networks coupled with yeast two-hybrid screening and co-immunoprecipitation analysis confirmed its interaction with peroxiredoxin (Prx). Co-transformation of the censor and Prx in yeast showed increased ROS scavenging ability. To further characterize its function *in vivo*, we generated transgenic rice overexpressing the sensor under the control of *rd-29* promoter. The overexpressing seedlings displayed increased ROS catabolism with a strikingly higher survivability, suggesting that the sensor protein might function as a positive regulator of dehydration response in rice in specific and plants in general.

POSTER 334
IonStar enables high-precision, low-missing-data proteomics quantification in large biological cohorts

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Reproducible quantification of large biological cohorts is critical for clinical/pharmaceutical proteomics, yet remains challenging because most prevalent methods suffer from drastically declined commonly-quantified proteins and substantially deteriorated quantitative quality as cohort size expands. MS2-based Data-independent Acquisition approaches represent tremendous advancements in reproducible protein measurement, but often with limited depth. We developed IonStar, an MS1-based quantitative approach enabling in-depth, high-quality quantification of large cohorts by combining efficient/reproducible experimental procedures with unique data processing components such as an efficient 3D chromatographic alignment, a sensitive and selective direct ion-current extraction, and stringent post-feature-generation quality control. Compared with several popular label-free methods, IonStar exhibited far lower missing data(0.1%), superior quantitative accuracy/precision(~5% intra-group CV), the widest protein-abundance range and highest sensitivity/specificity for identifying protein changes(<5% False Altered-protein Discovery) in a N=20 benchmark sample set. We demonstrated the usage of IonStar by a large-scale investigation of traumatic injuries and pharmacological treatments in rat brains(N=100), quantifying >7,000 unique protein groups (>99.8% without missing data across the 100 samples) with low FDR, ≥2 unique peptide/protein and high quantitative precision. IonStar represents a reliable and robust solution for precise and reproducible protein measurement in large cohorts.

POSTER 335
Proteome-wide analysis of the NSm protein effect in primary macrophages following Rift Valley fever virus infection

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Rift valley fever virus (RVFV, Phenuiviridae, Phlebovirus) is endemic in Africa and recently entered the Arabian Peninsula from which it may spread into Europe. RVFV affects primarily ruminants with a high mortality rate in fetuses and newborns, highly impacting local economy and agriculture [1]. In humans, symptoms include hepatitis and hemorrhagic fever, with a fatality rate varying from 1 to 10% during epidemics. RVFV encodes several nonstructural proteins known to act as virulence factors in mammalian hosts (NSs and NSm) or to promote viral dissemination in arthropod vectors (NSm-GN) [2, 3]. The present study focuses on the functional characterization of the viral NSm protein. NSm is a 14 kDa protein localized at the outer mitochondrial membrane in infected cells [3]. Our collaborators previously showed that a mutant virus lacking NSm expression (del-NSm) is attenuated in mice [2] and produced at low titers in murine macrophage-like cell lines [2]. In order to identify the underlying mechanisms of pathogenicity, herein we compared the proteomes and phosphoproteomes of primary macrophages infected by either wild-type (WT) or viruses deficient for NSm or NSs expression using quantitative mass spectrometry strategies. We found that NSm acts cooperatively with the NSs virulence factor and modulates the expression level of cellular proteins involved in cell-mediated immune response, oxidative phosphorylation and fatty acid metabolism. This points to a complex regulation of the mitochondrial platform by the viral NSm protein.

[1] Ikegami and al., Immunology and Pathogenesis of Viral Hemorrhagic Fevers, 2009

[2] Kreher and al., Emerging Microbes and Infection, 2014

[3] Terasaki and al., Journal of Virology, 2013

POSTER 336
Accurate, Sensitive, and Precise Multiplexed Proteomics Using the Complement Reporter Ion Cluster

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Quantitative analysis of proteomes across multiple time points, organelles, and perturbations is essential for understanding both fundamental biology and disease states. The development of isobaric tags (e.g., TMT) has enabled the simultaneous measurement of peptide abundances across several different

conditions. These multiplexed approaches are promising in principle because of advantages in throughput and measurement quality. However, in practice, existing multiplexing approaches suffer from key limitations. In its simple implementation (TMT-MS2), measurements are distorted by chemical noise leading to poor measurement accuracy. The current state-of-the-art (TMT-MS3) addresses this but requires specialized quadrupole-iontrap-Orbitrap instrumentation. The complement reporter ion approach (TMTc) produces high accuracy measurements and is compatible with many more instruments, like quadrupole-Orbitraps. However, the required deconvolution of the TMTc cluster leads to poor measurement precision. Here, we introduce TMTc+, which adds the modeling of the MS2-isolation step into the deconvolution algorithm. The resulting measurements are comparable in precision to TMT-MS3/MS2. The improved duty cycle and lower filtering requirements make TMTc+ more sensitive than TMT-MS3 and comparable with TMT-MS2. At the same time, unlike TMT-MS2, TMTc+ is exquisitely able to distinguish signal from chemical noise even outperforming TMT-MS3. Lastly, we compare TMTc+ to quantitative label-free proteomics of total HeLa lysate and find that TMTc+ quantifies 7.8k versus 3.9k proteins in a 5-plex sample. At the same time, the median coefficient of variation improves from 13% to 4%. Thus, TMTc+ advances quantitative proteomics by enabling accurate, sensitive, and precise multiplexed experiments on more commonly used instruments.

POSTER 337
Quantitative proteomics uncovers a novel USP9X substrate TTK for tumorigenesis in non-small cell lung cancer

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Lung cancer is one of the leading causes of cancer death worldwide. The X-linked deubiquitinase, USP9X, has been reported to be an oncogene in non-small cell lung cancer (NSCLC) and is correlated with poor prognosis. However, the molecular mechanism of USP9X regulating tumorigenesis is less defined. In our study, we used TMT-based quantitative proteomic approach to analyze A549 cells stably expressing shGFP or shUSP9X, resulting in a total of 7471 proteins identified and quantified, including 22 proteins remarkably down regulated in USP9X knockdown cells (fold change > 1.5, students' t test P value < 0.05). Of which, TTK was selected as a potential substrate of USP9X. We further demonstrated that depletion of USP9X increased TTK ubiquitination. USP9X stabilized TTK via interaction and deubiquitination of TTK. Knockdown of USP9X or TTK suppressed proliferation, migration, invasion of A549 cells and tumor growth in nude mouse. We also observed significant correlation between USP9X and TTK expression levels in human NSCLC tissues, where they were over-expressed compared with matched adjacent normal tissues. Taken together, our data demonstrated that the USP9X-TTK axis may play a critical role in NSCLC, and could be considered as the potential therapeutic target.

POSTER 338
Applications of SureQuant™ Pathway Panels for Quantitative Analysis of Cancer Signaling Proteins

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Introduction:

The AKT/mTOR and RAS/ERK pathways represent mechanisms for cells to regulate survival, proliferation, and motility. These signaling pathways play a central role in tumor progression and drug resistance. Highly accurate monitoring of these pathway proteins has not been achieved, due to poor reproducibility, unreliable quantitation, and lack of standardized methods and reagents. To overcome these challenges, the novel SureQuant™ pathway panels have been applied, which utilize an optimized multiplex immunoprecipitation to targeted mass spectrometry (mIP-tMS) workflow. mIP-tMS assays can quantitate multiple proteins, PTMs and interacting partners, which creates new possibilities for a broad range of applications, including cancer diagnosis and prognosis, drug development, and precision medicine.

Method:

The SureQuant™ AKT pathway (total or phospho), RAS, or TP53 panels include a multiplex IP module (antibodies and lysate), MS sample prep, absolute or relative quantitation modules (AQUA Ultimate peptides standards), and software. Serum-starved, inhibitor-treated (LY294002/NVP-BEZ235/Rapamycin) HCT116, A549, and MCF7 cells were stimulated with hIGF-1. SureQuant™ AKT pathway or RAS panels were used to determine the absolute concentration of target peptides using targeted MS analysis. The panels were benchmarked against Western blotting (WB) using cell lysates, as well as several tissue lysates.

Results:

Previously, we showed the feasibility of optimized mIP-tMS assays to quantitate AKT and RAS pathway proteins across 2 cancer cell lines ± LY294002. The SureQuant™ multiplex pathway panels allowed absolute quantitation of multiple total and phosphorylated targets in low to sub-nanogram concentrations across unstimulated, hIGF-1 stimulated and inhibited cell lysates as well as tissue/xenograft lysates. Benchmarking of mIP-tMS assays demonstrated moderate correlation relative to WB. These results may be due to differences in the specificity of antibodies used for each assay technique.

Conclusion

SureQuant™ pathway panels allowed simultaneous absolute quantitation of AKT pathway, RAS proteins and PTMs in a streamlined, standardized workflow.

POSTER 339

Targeted Mass Spectrometry Assay Kits for Absolute Quantitation of Signaling Pathway Proteins

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Introduction:

Many genetic alterations in cancer cells modify the protein expression from AKT, RAS and TP53 pathways. Quantitative measurement of alterations in the expression of pathway proteins and post-translational modifications (PTM) is necessary for classifying disease states, monitoring cancer progression and determining treatment response. Major bottlenecks for quantitation of these proteins are the lack of rigorously verified methods and reagents, and reliance on Western blotting. We have optimized a multiplex immunoprecipitation to targeted mass spectrometry (mIP-tMS) workflow to develop the SureQuant™ pathway panels, achieving simultaneous enrichment and absolute quantitation of multiple total and phosphorylated proteins from the AKT pathway, RAS, and TP53.

Method:

The SureQuant™ total and phospho pathway panels contain two modules: 1) The IP-MS Sample Prep Module includes reagents necessary to immunoenrich AKT pathway, RAS, or TP53 proteins, and perform MS sample preparation in one day 2) The Absolute or Relative Quantitation Modules include a Pierce™ LC-MS/MS System Suitability Standard, AQUA UltimateHeavy and/or AQUA UltimateLight Peptides, and verified MS instrument and data analysis methods. A calibration curve is generated using constant amounts of heavy peptide and variable amounts of light peptide (0.03-200fmol). IP-enriched, digested samples spiked with heavy peptides are analyzed using targeted MS (nanoLC-PRM/MS) and Skyline software.

Results:

Previously, we verified antibodies and target peptides to AKT and RAS pathways using an optimized mIP-tMS workflow. From the standard curve, all target peptides were monitored with <20% CV, 3 orders of magnitude dynamic range, linearity (R^2) >0.97, and accuracy of 80-120% in a complex matrix. Using the SureQuant™ pathway panels, absolute quantitation of 37 target peptides in unknown samples was achieved with <20% CV across multiple cancer cell lines.

Conclusion:

SureQuant™ pathway kit panels enable multiplexed enrichment, detection, and absolute quantitation of proteins and PTMs in the AKT pathway, as well as RAS and TP53 at low-fmol concentrations.

POSTER 340

Systems toxicology assessment of potential modified risk tobacco products: effects on lung, liver, and heart in ApoE-/- mice using iTRAQ

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Modified risk tobacco products (MRTPs) were developed to reduce smoking-related health risks. In a 6-month inhalation toxicity study with Apoe$^{-/-}$ mice, two potential MRTPs, namely Tobacco Heating System (THS) 2.2 and Carbon Heating Tobacco System (CHTP) 1.2, were assessed. The MRTPs were compared to cigarette smoke (CS) at matching aerosol/CS nicotine concentrations (28 µg nicotine/L, 3 hours per day). Fresh air exposure served as a control and the effects of smoking cessation or switching to CHTP 1.2 after 3 months of CS exposure were also evaluated. Within this systems toxicology study, effects on classical toxicology, as well as omics endpoints were assessed.

Here, we present the proteomics results on lung, liver, and heart analyzed using isobaric Tag for Relative and Absolute Quantitation (iTRAQ). Eight replicates per group were analyzed at 3 time points (3, 4, 6 months) for a total of 128 samples per organ. Across the whole dataset, a total of 2508, 2008 and 1173, proteins were quantified for lung, liver and heart. CS elicited an extensive exposure response in the lung, including an immune and oxidative stress response (up to 500 differentially expressed proteins). Both THS2.2 and CHTP1.2 aerosol exposure were associated with much more limited molecular effects than CS on these processes in the lung. CS exposure also induced significant protein alterations in liver, including xenobiotic metabolism, oxidative stress, and iron metabolism related proteins. In contrast, upon THS2.2 and CHTP1.2 aerosols exposure no differential protein expression was observed. Finally, no differentially expressed proteins were detected in the heart proteome among the test groups.

Overall, this work supports reduced biological effects of THS2.2 and CHTP1.2 aerosols, compared with CS, in the Apoe-/- mouse model. Within this systems toxicology assessment study, proteomics complemented and confirmed the results from classical toxicological endpoints as well as transcriptomics, lipidomics and metabolomics endpoints.

POSTER 341

High Throughput Signaling Pathway Analysis Using Multiplex-Immunoprecipitation and Fast LC-PRM

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Background

Signaling pathways play a central role in development and disease. Precise measurements of total form and post-translational modifications (PTM) of signaling proteins (e.g., phosphorylation) are vital to gain insights into mechanisms of

diseases as well as to monitor therapeutic responses. Currently, monitoring of signaling pathways still mainly rely on immunoassays, which provide exquisite sensitivity and analytical throughput. However, LC-MS based workflows have significant advantages in terms of specificity, quantification accuracy, and multiplexing capability. Here, we propose to combine multiplex immuno-precipitation of proteins from the Akt/mTOR pathway with fast LC-PRM analyses to measure aberrant activation of this critical signaling pathway in cancer cell line.

Methods

Thirteen “total” and “phosphorylated” proteins of AKT/mTOR pathway were targeted in multiplex immuno-precipitation assay. PRM-based analyses were performed on a Thermo Scientific™ Q-Exactive™ HF and HF-X. Chromatographic separations were carried out using Evosep-One system.

Results

The optimal LC method was developed to allow conventional PRM analyses of the 32 pairs of stable isotopically labeled (SIL) and endogenous peptides corresponding to AKT/mTOR protein targets with high sensitivity and analytical throughput, while maintaining acceptable chromatographic sampling rate. This fast LC-PRM method, providing a throughput of 60 sample analyses per day, was applied to examine Akt/mTOR signaling in hIGF-1 stimulated HCT116 cells. Analyses of immuno-enriched samples benefited from a significant increase in the signals of endogenous peptides as well as a significant background reduction. Specific measurements of phosphorylated proteins revealed a clear activation of the pathway in hIGF-1 stimulated cells. Advanced PRM acquisition methods further improved the analytical throughput (up to 200 samples/day) without compromising the sensitivity and selectivity of measurements. This assay will be commercially available with IP antibodies, quantification standards, and SIL peptides. It can be coupled with a transcriptomic panel to provide additional insights into the regulatory mechanism of the Akt/mTOR signaling pathway.

POSTER 342

Proteomic analysis with inguinal white adipose tissue of CXCL5 KO mice revealed increased energy consumption activity

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Obesity belongs to a low degree of inflammation. Chemokines released from macrophages in adipose tissue affect obesity related diseases. CXC ligand 5 (CXCL5) is a member of CXC chemokine family and has been shown to be highly expressed in macrophages in white adipose tissue. To clarify the role of CXCL5 in adipose tissue, we performed a proteomic analysis with inguinal white adipose tissue (iWAT). iWAT was dissected from wild type and Ccl5-KO mice and total protein was extracted. The proteins were digested into peptides using the Filter Aided Sample Prep (FASP) protocol, a method of tryptic digestion of proteins into purified peptides in a filter. Digested peptides were labeled with Isobaric tags for relative and absolute quantitation (iTRAQ) to normalize protein amount quantitatively. Total 2558 proteins were identified and Uncoupled protein 1 (UCP1) was found to be a top differentially expressed protein in Cxcl5-KO compared with Wild type mouse. Interestingly, Gene Ontology analysis (GO) identified that most of proteins are enriched in the process of fatty acid beta-oxidation and mitochondrial localization. Moreover, Chip enriched assay revealed that PPARg is the top transcriptional factor for the proteins upregulated in Cxcl5-KO. These results strongly suggest that Cxcl5 is highly involved in energy metabolism in adipose tissue and targeting Cxcl5 could be therapeutic target for the people with disorder of energy metabolism.

POSTER 343

Profiling the Kinome and Phosphoproteome of mutant KRAS-driven Pancreatic Ductal Adenocarcinoma

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Using multiplexed inhibitor bead/ mass spectrometry (MIB/MS), global kinome profiling was performed in multiple mutant K-Ras pancreatic cancer cell lines and the specific effects of K-Ras knockdown examined. At the same time, a parallel analysis of the phosphoproteome was performed in these cells and the data was compared to the effects of select Erk inhibition with the small molecule SCH772984. The results of these studies demonstrated significant kinome remodeling in response to both early (24 hr) and late (72hr) siRNA knockdown of K-Ras. A subset of kinases increased or decreased relative to control cells, however of particular interest were the cell cycle kinases (Wee1, CDK6, PLK) and an increase in the potentially compensatory MEK5-ERK5 kinases. MIB/MS kinase competition assays demonstrated that SCH772984 was a highly selective Erk inhibitor. Consistent with this, phosphoproteomics analysis revealed that SCH772984 incubation reduced the phosphorylation of a number of known Erk substrates (c-Myc, EIF4EBP, IRS1) as well as potentially novel ones. The results of these studies demonstrate the utility of the combined kinome and phosphoproteome analysis to elucidate key cell signaling events linked to K-Ras.

POSTER 344

Global proteome landscape during antigent dependent differentiation reveals dynamic cell signaling profiles accross distinct B-cells subpopulations

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Human B-cell diferentiation has been extensively investigated on genomic and transcriptomic grounds; however, no studies have accomplised so far detailed analysis of antigen-dependent maturation-associated human B cells subpopulations from a proteomics perspective. Here, we investigate for the first time the quantitative proteomic profiles of B-cells undergoing antigen-dependent maturation using a label-free LC-MS/MS approach applied on 5 purified B-cell subpopulations (näive, centroblasts, centrocytes, memory and plasma B cells) from five reactive tonsils. Our results revealed that the actual differences among these B-cell subpopulations are a combination of expression of a few maturation stage-specific protein within each B-cell subset and maturation-

associated changes in relative protein expressin levels. The considerable overlap of the proteome of the 5 studied B-cell subsets strenghts the key role of the regulation of the stoichiometry of molecules associated with antigen recognition and presentation, cell survival and other signaling cascades crucial for the transition between each B-cell maturation stage.

POSTER 345
Multi-faceted chemical and genetic knock-down approaches to mapping functional protein networks of DNA methyltransferase I (DNMT1)

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Patterns of DNA methylation, a key epigenetic mark across the eukaryotic genome, are established and maintained by the DNA methyltransferase proteins (DNMTs). DNA Methyltransferase I (DNMT1) is the principal DNA methyltransferase in eukaryotic cells, preferentially methylating hemi-methylated CpG sites to replicate patterns of methylation after DNA replication. A key question, given the important role of DNMTs, is how their function and activity is regulated. DNMT1 abundance and stability is primarily regulated through post-translational level gain or loss of protein-protein interactions and addition or removal of post-translational modifications. DNMT1 protein-protein interactions include proteins that function in the DNA replication machinery and chromatin modifying proteins as well as proteins that directly regulate the stability and abundance of DNMT1 through modification of PTMs. We previously showed that DNMT1 is regulated by the activity of the Wnt signalling pathway via a protein-protein interaction between DNMT1 and β-catenin. In this work, we are using multiple approaches to characterize the DNMT1 protein functional network in colorectal cancer cells. First, chemical knock-down of DNMT1 using the nucleoside analog 5-azacytidine was used to induce selective proteasomal degradation of DNMT1. Second, colorectal cancer cells expressing DNMT1 hypermorphic alleles were compared to cells expressing wild-type DNMT1. We performed quantitative label-free LC-MS/MS in both studies using Nano-LC separation and a Waters Synapt G2-S mass-spectrometer. These studies identified multiple perturbations of pathways and processes induced by DNMT1 knock-down. We identified multiple chromatin-structure, metabolic and epigenetic-related proteins that are sensitive to 5-azacytidine including the epigenetic regulator UHRF1. Analysis of the proteome of DNMT1 hypermorphic cells indicates an important, potentially non-methylation dependent role for DNMT1 in the regulation of epithelial-mesenchymal transition. In summary, deep, functional analysis of cells with perturbed DNMT1 protein expression identifies novel functional DNMT1-related protein networks in cancer cells.

POSTER 346
The Functional Role of Mitochondrial Sirtuin Signalling Underlying Acute Angiotensin II-Mediated Oxidative Stress in Mouse Ophthalmic Artery

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Angiotensin II (Ang II)-induced vascular oxidative stress represents one of the aetiologies underlying several ocular disorders. However, the molecular mechanisms of Ang II-mediated oxidative damage in the retrobulbar vasculature remain to be elucidated. Therefore, this study investigated the acute vascular proteome changes associated with Ang II-elicited oxidative insult in the ophthalmic artery (OA).

OA was isolated from male C57Bl/6J mice (n=15) and incubated overnight in Dulbecco's modified Eagle's medium without phenol red containing either vehicle or Ang II (0.1 µM) at physiological conditions. Artery samples were pooled to obtain three biological replicates (n=5 mice/ pool/ group). Vascular proteins were extracted using a new in-house method specifically catered for rapid and robust protein extraction from small amounts of microvascular samples with tissue protein extraction reagent and homogenization with zirconium oxide beads in a bullet blender homogenizer. Label-free quantitative proteomics based on 1DE combined with the LC-ESI-LTQ-Orbitrap MS system was employed for proteome characterization. The acquired MS spectra were analysed by MaxQuant, followed by statistical analyses employing Perseus and, functional annotation and pathway analyses with Ingenuity Pathway Analysis.

A total of 389 proteins were identified in the mouse OA with less than 1 % false discovery rate. Among these, 87 proteins were differentially expressed in the Ang II group compared to control. A significant number of differentially expressed protein clusters were involved in mitochondrial dysfunction, sirtuin signalling and oxidative phosphorylation. Correspondingly, histones (HIST1H3A, HIST2H3A), several mitochondrial proteins (ACADVL, ALDH4A1 and SDHA), S100-family proteins (S100A11, S100A9 and S100B) and neuronal nitric oxide synthase (NOS1) were observed to be exclusively expressed in the Ang II group.

Conclusively, this is the first in-depth study that characterized the proteome changes in the murine OA following acute Ang II-induced oxidative stress, which demonstrated a significant regulation of the mitochondrial sirtuin signalling as an acute phase reactant.

POSTER 347
Assessment of MFG-E8 protein using Label Free and iTRAQ Proteomics for an Intrinsic Component of the Cell Growth Regulator

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The glycoprotein lactadherin also known as MFG-E8 mediates phagocytic clearance of apoptotic cells and influences the physiological cyclic changes taking place in mammary gland. Recent study shows that MFGE-8 is the part of MFGM and plays a major role in mammary gland development. In our current work we have shown that lactadherin isoform MFGE8 controls the cell growth process of epithelial cells by indirectly regulating transcription factors. We have successfully downregulated the MFGE8 protein through stable transfection of MFGE8 shRNA. Mass spectrometry-based (Q-TOF) proteome analysis by iTRAQ approach after fractionation (30 fractions) identified total of ~12500 proteins in stably transfected silenced MFGE8 Buffalo mammary epithelial cell line through Transproteomics Pipe Line (TPP) using multiple search engines such as Comet, Tandem, SpectraST, and Mascot. Bioinformatics analysis connected with Cytoscape showed that MFGE 8 makes direct protein-protein interaction

with GSN, RALGAPA2, CD9, ITGB5 and ALB. DAVID analysis shows that these proteins are involved in diverse biological processes, cellular functions, molecular functions and pathways. Furthermore, with the help of Reactome and KEGG pathway database we identified that MFGE 8 and GSN together responsible for regulation of proliferation. These *in silico* results were later confirmed by qRT-PCR and western blot. Furthermore, BrdU, MTT, Neutral red, Caspase 7/9, Cell grow confluence, Wound healing, and Trans-well migration assay validated that stably transfected cells has more lifespan compared to normal counterpart. Overall this study supports that MFGE8 together with GSN regulates the proliferation of cells. Till date to the best of our knowledge, this is the first study that shows the direct interaction of MFGE8 in regulation of cell growth.

POSTER 348
Quantitation of AKT 1+2, PTEN and PI3K p110α by immuno-MALDI

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Introduction

To improve cancer patient stratification and overcome the drawbacks of currently used approaches to assess signaling pathway proteins, we set out to develop immuno-matrix assisted laser ionization/desorption (iMALDI) assays combined with a phosphatase-based phosphopeptide quantitation (PPQ) approach to measure PI3K/AKT/mTOR pathway activity. Specifically, we targeted the C-terminal tryptic peptides of AKT1 and AKT2 due to their involvement in full kinase activation.

Methods

Following trypsin digestion of cell lysate, stable isotope-labeled standard (SIS) peptides are added. After splitting the solution, one aliquot is treated with phosphatase. Bead-coupled antibodies enrich the non-phosphorylated target peptides, which are washed and spotted onto a MALDI plate, and acidic MALDI matrix elutes the peptides. The resulting light/heavy ratios of both aliquots allow the calculation of protein expression levels and peptide phosphorylation stoichiometry.

Results

The iMALDI assays were validated for linear range and accuracy, as well as interference screening, requiring only ~50 µg of total cell or tissue lysate protein to quantify both AKT1 and AKT2 expression levels and phosphorylation stoichiometry. We were able to quantify AKT1 and AKT2 from various cell lines and fresh frozen tumor samples. A direct comparison of four pairs of normal and adjacent tumor tissues showed elevated AKT1 phosphorylation stoichiometry of ~40% in a colorectal cancer liver metastasis, and significantly elevated AKT1 (3.6-fold) and AKT2 (2.2-fold) expression levels in a surgical breast tumor sample. Additionally, PTEN and PI3K p110α iMALDI assays were developed and combined into a single multiplexed assay with a LOD of approximately 0.5 fmol using 10 ug total lysate protein per replicate. The linear range spans two orders of magnitude. Endogenous protein levels were quantified in cancer cell lines and fresh frozen tumor samples.

The next steps include measuring patient-derived mouse xenograft tissue and patient samples, as well as correlating the results to the clinical treatment.

POSTER 349
Quantitative Phosphoproteomics Reveals the Downstream Kinase-Substrate Regulation Network Upon Kappa-Opioid Receptor Activation

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Kappa opioid receptor (KOR) is a member of G-protein coupled receptor (GPCR) expressed in serotonergic neurons and neuronal terminals. The involvements of KOR ligands in nociception, diuresis, emotion, cognition, and immune system have been extensively studied. KOR agonists possess a strong analgesic effect, but can also cause side effects e.g. dysphoria. It has been demonstrated that the study on G-protein-mediated signaling upon KOR activation can help develop therapeutics to reduce the side effects. In this study, quantitative phosphoproteomics based on dimethyl labeling and TiO2 beads-coupled phosphopeptides enrichment, was applied to analyzing KOR-mediated dynamic protein phosphorylation changes upon KOR agonists (LPK-26 or U50,488) treatments in three KOR over-expressed monoclonal human embryonic kidney 293T (HEK 293T) cell lines. A total of 23,155 phosphosites from 4,578 proteins were identified. Of which, 7,490 Class I sites were quantified across all the three cell lines under two agonists treatments. And 305 phosphosites were consistently changed, including 212 up-regulated and 93 down-regulated phosphosites. The subsequent substrate-kinase prediction analysis revealed that 18 kinases were potentially activated under the agonists stimulation, and the further Western Blot experiments confirmed the up-regulated activity of two kinases AKT1 and p70S6K. In summary, a specific kinase-substrate regulation network upon KOR activation was generated, which may shed light on the investigation of KOR signaling pathways and the discovery of targeted therapy for KOR-related diseases.

POSTER 350
Dissection of single-cell gene regulation by simultaneous digital mRNA and protein quantification

Gretchen Lam; Christina Chang; Nidhanjali Bansal; Devon Jensen; James Ghadiali; David Craft; Jody C Martin; Janice Lai; Mirko Corselli; Regina Lam; David Rosenfeld; H. Christina Fan; Eleen Y. Shum

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Cell-surface marker analysis using flow cytometry or single cell RNA-seq has allowed characterization of immune subpopulations and a greater understanding of the complexity of immune cells. However, restrictions on protein-only or RNA-only analysis can greatly limit the understanding of how genes are regulated in cells. For example, many cell surface markers – such as CD4 in T cells – has thousands of protein copies per

cell using antigen density calculations, yet is fueled by a small number of mRNA transcripts per CD4+ T cell.

To bridge the understanding of protein and mRNA expression differences, we used BD™ AbSeq on the BD RhapsodyTM platform to provide digital quantification of both protein and mRNA expression levels in single cells. An oligo-conjugated antibody panel against immune-relevant cell-surface markers was created and used for this multi-omic gene expression profiling effort. This approach is coupled with mRNA analysis using the BD Immune Response Panel, Hs, a method of RNA-Seq with higher sensitivity of detection of immune markers.

We studied the dynamics of early immune cell activation in vitro to understand this response on transcriptional, post-transcriptional, translational, and post-translational levels. Different time points following phorbol myristate acetate (PMA) and ionomycin treatment were collected and multiplexed onto BD Rhapsody cartridge for single cell capture and analysis. Using BD AbSeq on BD Rhapsody, we were able to detect the difference in mRNA and protein levels of crucial markers, allowing us to dissect the intricate gene regulatory pathways during an immune response in a single cell level.

POSTER 351
In-Depth Proteome Profiling of Single Cells Including Circulating Tumor Cells by Nanodroplet Sample Processing and Ultrasensitive LC-MS

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Human tissues contain a variety of cell types and subtypes with distinct functions, and understanding heterogeneity at the single cell level is of great interest for biomedical research. Although MS-based proteomic analyses are capable of quantifying thousands of proteins, the extension to single cell studies has been largely ineffective. This is primarily due to protein and peptide losses during sample processing. To address this, we have developed nanoPOTS (Nanodroplet Processing in One-pot for Trace Samples) to efficiently process and analyze single mammalian cells. Cultured HeLa cells, primary lung cells, and spiked circulating tumor cells (CTCs) isolated from whole blood were FACS-sorted or isolated by laser microdissection into nanoPOTS chips based on cell morphology or immunohistochemistry staining. All processing steps were carried out in nanowells having a total processing volume <200 nL to reduce protein losses to surfaces. A custom robotic nanopipetting system was used to dispense reagents and collect prepared samples. The processed samples were analyzed by nanoLC/MS. We first used FACS-sorted HeLa cells to evaluate the sensitivity of present system. We are able to identify an average of 669 protein groups (n=3) per HeLa cell, with 332 quantifiable protein groups having valid LFQ values in at least 2 samples. We also tested whether the single cell proteomic platform could discriminate cell types isolated from human tissues. Antibody-labeled epithelial and fibroblast cells from the lung tissue of a healthy donor were used for the evaluation, and the two cell types were well clustered without overlap. Statistical analysis revealed a panel of proteins that were enriched for each cell type, which could serve as protein signatures. Finally, we analyzed spiked CTCs isolated from whole blood and could differentiate the single CTCs from white blood cells based on their proteomic signatures.

POSTER 352
Dissection of cell cycle dependent variations of the human proteome

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The Cell Atlas, part of the Human Protein Atlas HPA, focuses on localizing the human proteins on a subcellular level using immunofluorescence and confocal microscopy. Using the image collection in the Cell Atlas, my research aims to characterize variability of protein expression at a single cell level, investigate the cell-cycle dependency of the human proteome and quantify changes both in expression levels and spatial distribution in non-perturbed asynchronous cells with single cell resolution.

Cells derived from the same clonal population display variability in protein expression patterns. This variability is noted within immunofluorescence images and we denote it single cell variation (SCV). This project aims to identify the human proteins that show such a dynamic property both in terms of expression levels and spatial distribution. By re-analyzing all images in the Cell Atlas, we have classified around 1,500, corresponding to 13% of studied proteins, to display such variability across different cell lines. We hypothesize this variation to mainly be linked to parameters such as cell cycle position, stress and cell confluence. It is reasonable to expect a big fraction of these variations to be related to cell cycle progression, as cells are grown under asynchronous conditions. To investigate the correlation to cell cycle, the SCV classified proteins were stained in the U-2 OS FUCCI cell line. The FUCCI cells are tagged with two different fluorescent proteins fused to cell cycle regulators that allow cell cycle monitoring; Cdt1, expressed in G1 phase, and Geminin, expressed in S and G2 phases. In this manner we could identify 545 proteins with expression variation correlated to cell cycle progression, corresponding to 41% of the tested proteins.

Here, we present an overview of the single cell variations observed for the human proteome and the path ahead to define the spatiotemporal proteome organization of the human cell.

POSTER 353
HDX at single residue resolution reveals principles of aggregation of functional amyloids

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Functional amyloids create structures similar to disease-associated amyloids, but their aggregates fulfill significant biological functions without incurring toxicity. Their self-assembly stems not from the protein misfolding, but is carefully governed by sophisticated mechanisms, including the unique structural features.

We employ the Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) to study CsgA, a functional amyloid produced by enteric bacteria. CsgA is a proteinaceous scaffold of biofilms, extracellular communities protecting bacteria from the unfavourable environmental conditions. The aggregation

propensity of CsgA is enhanced by the presence of five imperfectly repeated regions. A removal of specific repeats results in a radical decrease of amyloid properties. Aside from various chaperon proteins, the self-assembly of CsgA is also modulated through the presence of gatekeeper residues (asparagine and glycine).

Protein dynamics and structure stability studied with HDX-MS is routinely limited to a peptide resolution of 5 to 20 amino acid residues length regions. Our study based on single amino-acid residue resolution approach shows participation of individual residues in the self-assembly of CsgA and get deeper insight into nature of aggregation phenomena. We focus especially on regions experimentally confirmed to influence the aggregation propensity of the protein. We evaluate the role of gatekeeper residues and point that their role depends on their localization and surrounding amino acids.

POSTER 354

Advances in Orbitrap Instrumentation for Native Top-Down Analysis of Non-Covalent Protein Complexes

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Native MS is a powerful technique to study protein-ligand interactions and macromolecular assemblies. Top-down of intact protein complexes using MS3 have only recently been reported. However, poor fragmentation into subunits and stripped complexes in the front end of MS limits the use for native top-down analysis using a pseudo-MS^3. In this work we examine this limitation and look for extending native top-down to allow interrogation of heteromeric protein assemblies like proteasome. Experiments were performed on a modified Q Exactive Plus in which the ability to perform pseudo-MS^3 and transmission of high m/z ions were improved by several hardware and software modifications. The most important being pulsed trapping of ions in the injection flatapole region ('in-source trapping') and reduction of frequency of RF voltages applied to bent flatapole, quadrupole, transfer multipole, C-trap and HCD cell. Other modifications include gas pressure control, adjustment of voltage ramp rate of Orbitrap and increase of the maximum HCD energy.The modified instruments performance was tested for several homomeric and heteromeric soluble protein complexes and membrane proteins. During analysis intact protein complex is initially transferred through the MS without fragmentation using only moderate desolvation energy, yielding MS^1 spectrum. Dissociation of protein complex into its subunits in the inject flatapole region gives the MS^2 spectrum, and it is followed by quadrupole selection and fragmentation of individual subunits in HCD cell enabling sequence analysis via MS^3.Using in-source trapping, very efficient desolvation and fragmentation of GroEL 14-mer complex into monomer and stripped complexes has been observed. This was followed by quadrupole selection of the monomer and fragmentation by HCD which resulted in identification of 112 b and y ions which represent 21% residue cleavages. Furthermore, in-source trapping allowed very efficient removal of detergent micelles and pseudo-MS^3 analysis of the LmrP membrane protein from Bacillus subtilis.

POSTER 355

Structural analysis of the natural snake venom metalloendopeptidase inhibitor BJ46a and its target toxin, jararhagin, based on XL-MS and HDX-MS

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Snakebite envenoming is a growing public health problem, affecting millions worldwide. The intravenous administration of antivenom is the only available treatment and several studies have shown that local effects are only partially neutralized. A current trend in antivenom research is the study of natural and synthetic inhibitors that could be used soon after the envenomation event to restrain local tissue degradation. The inhibitor BJ46a is a glycoprotein isolated from Bothrops jararaca serum that inhibits the catalytic activity of snake venom metalloendopeptidases (SVMPs) through the formation of a stable high-affinity non-covalent complex with the toxin. However, the structural features that govern this interaction are largely unknown. In this work, the regions of interaction between BJ46a and the SVMP jararhagin have been analyzed using the techniques of cross-linking-mass spectrometry (XL-MS) and hydrogen-deuterium exchange-MS (HDX-MS). The complex BJ46a-jararhagin was stabilized with the crosslinker BS3 and the new XPLEX strategy targeting acidic residues. The stabilized complex was digested with trypsin and analyzed by nLC-MS/MS on a Q-Exactive Plus. Data analysis used the SIM-XL software, followed by manual validation of all spectra. For HDX-MS, the complex was exposed to D2O for 10, 100, 1000, and 10.000 s; at the end of each time-point, the reaction was quenched with an 8M Urea/1M TCEP solution. The samples were rapidly digested with pepsin and the resulting peptides were separated on a C18 column at 0 °C and analyzed on an LTQ-Orbitrap XL MS. The results, analyzed using the HDExaminer software, indicate the involvement of the two cystatin domains of BJ46a in the interaction; regions of protection/deprotection have been mapped on the metalloendopeptidase and cysteine-rich domains of jararhagin, suggesting two interaction sites for BJ46a upon complex formation. The understanding of BJ46a inhibitory mechanism may lead to the development of new effective targeted therapeutics to prevent local tissue damage.

POSTER 356

Top Down Mass Spectrometry With Multiple MS/MS Strategies to Identify Age-related Proteoform Changes in Tear Fluid.

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Tear fluid functions as lubrication of the eye surface, contains a cocktail of antimicrobials to prevent bacterial infections, washes away foreign matter that is deposited in the eye during our daytime activities, and keeps the eye surface clean and smooth. Tears have the potential to be a source of biomarkers for diagnosing eye-related diseases. First, it is a fluid that can easily be obtained in a non-invasive manner. Second, it is localized to the organ of interest to the ophthalmologist (the eye) and not prone to the same dilution problems associated with biomarker fluids like plasma. Third, tear fluid composition changes with the health of the eye (as well as with age).

In this work, we have coupled high sensitivity top-down mass spectrometry to enable the characterization of tear proteoforms in less than 1 h with an elegant combination of bioinformatics algorithms. Our approach consists of a simplified two-step workflow. First, samples are then analyzed over a 30 min LC gradient by MS and MS/MS using various fragmentation mechanisms. And then, MSMS are searched against a protein database. Label free quantitation is perform by aligning the raw files in the chromatographic dimension and extracting the areas under the curve for each of the identified molecular species. Intensities were then normalized and a t-test was performed to identify differential events. PCA analysis shows that samples within the age groups are more similar. And among the significant proteins in the dataset, lacritin is the one that changes most with age. This protein is involve in the promotion of basal tearing and which low levels are associated with dry eye syndrome. Overall, this strategy offers a powerful option for discovery and characterization of potential tear biomarkers that could be used for the screening of diseases, both eye related or other.

POSTER 357

Improved Intact Protein Sequence Analysis by 21 Tesla FT-ICR MS/MS Drives Development of New Data Interpretation Strategies

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A common strategy for dealing with complex isotope distributions inherent to intact protein analysis is to use “averagine” distributions to determine monoisotopic mass. However, when the monoisotopic peak is of low S:N, or is not observed, averagine fits are often shifted by 1-2 isotopologues. Additionally, mass measurement accuracy is limited to the difference between the averagine and true elemental compositions. Fragments visible within unprocessed MS/MS spectra are often unassigned because, following deconvolution, they fall outside the narrow mass tolerance used for fragment matching. As this approach was designed prior to the high resolution and high mass accuracy data now readily achievable at 21 T, algorithms must be revised or replaced by new methodologies to reap the full benefit of such state-of-the-art mass spectrometers. Here, we discuss a single, fully annotated, ETD MS/MS spectrum of intact bovine CAII (29 kDa). The manually curated results were compared to results obtained by use of two different methodologies: 1.) Xtract deconvolution of the spectrum and fragments matched by Prosight Lite and 2.) Comparison of the observed isotope distributions to distributions calculated from the elemental composition of fragment ions. A total of 1203 fragment ions were assigned by manual examination of the data. These account for ~95% of the total ion current (TIC) observed in the spectrum and 91% sequence coverage is derived. Discounting false positive matches, use of Xtract and most abundant isotope search strategies obtained 78% and 88% sequence coverage, respectively. Comparison of c/z• ion TIC contribution revealed that, in this case, c ions were identified consistently across both search strategies, however Xtract was less able to accurately deconvolve z• ions of high charge state and m/z. We hope that the work described will spark further dialogue, and help guide the design of future data analysis software for intact protein sequence analysis.

POSTER 358

High-speed recovery workflow for electrophoretically separated proteins in polyacrylamide gels and its applications to mass spectrometry

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Polyacrylamide gel electrophoresis (PAGE) is a powerful, widely used technique for separating proteins extracted from complex biological samples; however, difficulties withdrawing intact proteins from gel matrices hinders further analysis. We discovered that protein staining with Coomassie brilliant blue (CBB) immediately after electrophoresis improved the extraction efficiency. In an established extraction procedure using CBB, protein recovery in a wide molecular weight range was achieved within 10 minutes without a surfactant, and high recoveries were obtained from dried, archived gels. On the other hand, by using the mild detergent octylglucoside instead of CBB for proteins separated by native PAGE, protein complexes larger than 400 kDa were rapidly recovered. Recovered proteins retained their native structure and were suitable for structural analysis by native mass spectrometry. Ultrafast protein recovery after PAGE enabled us to develop a novel extraction workflow with sample pretreatment for analyzing intact proteins from biological samples. The established workflow would facilitate in-depth proteomics and protein structure analyses as well as sample purification, storage, and transport.

POSTER 359

Elucidation of dasatinib action in gastric cancer cells harnessing MS-based activity-based protein profiling

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Dasatinib is a multi-target tyrosine kinase inhibitor whose main targets include BCR-ABL, Src family kinase. Src activity is known to be elevated in gastric cancer. Here, we investigated molecular mechanism of Dasatinib action to provide novel insights into target-based therapies for gastric cancer (GC). To provide a landscape of Dasatinib targets in gastric cancer cells, we performed activity-based protein profiling (ABPP) harnessing Desthiobiotin-ATP probe combined with LC-MS/MS. siRNA screening was conducted to prioritize relevant targets for further analysis. 22 and 17 kinases were identified as potential Dasatinib targets in Dasatinib sensitive (SNU-216, MKN-1) and resistant GC cells (SNU-484, SNU-601), respectively. siRNA screening for 10 kinases, which were identified only in Dasatinib sensitive GC cells, revealed that ablation of RPS6KA3 expression reduced cell viability most significantly. Our results suggest RPS6KA3 is one of the targets of Dasatinib, which could be important for maintaining GC cell survival. Further, combination of SRC and RPS6KA3 inhibitors could be a novel therapeutic approach for the treatment of GC.