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HUMAN PROTEOME ORGANIZATION

HUPO Brain Proteome Project
www.HBPP.org

25th HUPO Human Brain Proteome Project Workshop

Program

May 3-4, 2016

Stockholm, Sweden

This workshop will provide a forum for researchers to meet and discuss the latest developments in neuroproteomic initiatives to better understand brain disorders

The HUPO Human Brain Proteome Project (HBPP):

Scientific board
Lea Grinberg, *UCSF, USA & Brazilian Brain Bank, São Paulo, Brazil*
Katrin Marcus, *Medizinisches Proteom-Center, Bochum, Germany*
Oliver Schubert, *University of Adelaide, Australia*
Charlotte Teunissen, *VU University Medical Center Amsterdam, Netherlands*
Daniel Martins-de-Souza, *University of Campinas, Brazil*
Peter Nilsson, *SciLifeLab, KTH, Stockholm, Sweden*

Local organisers
Peter Nilsson and Anna Häggmark
SciLifeLab, KTH, Stockholm, Sweden

HBPP mission:

"Create and harbour a broad and global network of neuroproteomic researchers with a focus of attracting young researchers"

www.hupo.org/hbpp

Karolinska Institutet

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THE HUMAN PROTEIN ATLAS

KTH VETENSKAP OCH KONST

HBPP

In collaboration with

ATLAS ANTIBODIES

25th HUPO Human Brain Proteome Project Workshop

	Tuesday May 3	Wednesday May 4
8.30		
9.00		Session 4: **Alzheimer's disease**
9.30		
10.00	Coffee and registration	
	Welcome	Coffee
10.30	HBPP history	
11.00	Session 1: **Tissue-based proteomic profiling**	Session 5: **PD and other dementias**
11.30		
12.00		
12.30	Lunch	Lunch
13.00		
13.30	Session 2: **Technical advancements in neuroproteomics**	Session 6: **Autoimmunity in brain disorders**
14.00		
14.30		Coffee break
15.00		Session 7: **Other neurological disorders**
15.30	Coffee break	
16.00	Session 3: **Psychiatric disorders**	General discussion and concluding remarks
16.30		
17.00		
17.30		
19.00	Pub and mingle	
22.00	Dinner	

25th HUPO Human Brain Proteome Project Workshop

Tuesday May 3

09.30-10.00 **Coffee and registration**

10.00-10.15 **Welcome**

Peter Nilsson, KTH - Royal Institute of Technology, Stockholm, Sweden
Anna Häggmark, KTH - Royal Institute of Technology, Stockholm, Sweden

10.15-10.30 **HBPP history**

Helmut E Meyer, ISAS - Leibniz-Institut für Analytische Wissenschaften, Dortmund, Germany
Young Mok Park, Korean Basic Research Institute, Ochang, Korea

10.30-12.00 **Session 1: Tissue-based proteomic profiling**

Chair: Lea T. Grinberg

10.30-10.45 **Beyond the horizons in neuropathology**

Lea T. Grinberg, UCSF, USA & Brazilian Brain Bank, São Paulo, Brazil

Neuropathology is a relatively old discipline known for still using methods developed over a century ago. Difficulties in procurement and examination of clinical specimens, the advent of high-throughput –omics, and advances in animal models with their ability to provide insight into the mechanisms of a disease process, led to a waning of interest in neuropathology that has been rendered obsolete. My talk will focus on the transformation of neuropathology into a 21st-century science on the forefront of advances in neurosciences. By incorporating elements of molecular biology, improved microscopy, advancing graphics, and technology to deal with big data in the study of human brain specimens, neuropathology is transforming biomarker discovery and treatment development in several areas of neurology and rapidly reinstating itself as a valuable player in the contemporary neurological research.

10.45-11.00 **Addressing the challenges of quantitative myelin proteomics**

Olaf Jahn, Proteomics Group, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany

Rapid signal propagation along vertebrate axons is facilitated by their insulation with myelin, a plasma membrane specialization of glial cells. Recent insights from 'omics' approaches indicate an active role of myelinating glial cells as essential supporters of axonal functions. Once myelinated, the long-term integrity of axons depends on glial supply of metabolites and neurotrophic factors, and its failure is involved in many neurological disorders. Technical advances in myelin proteomics form the basis for a better understanding of these axon-glial interactions and pave the way for quantitative comparison of myelin proteomes from mutant mice or patient material.
Keywords: myelin proteomics, synaptic protein complexes, biomarker for neurological diseases

11.00-11.15 **Brainstem: a promising target for new discoveries in neurodegenerative diseases**

Renata Leite, Physiopathology in Aging Lab/Brazilian Aging Brain Study Group—LIM22, University of Sao Paulo Medical School, Sao Paulo, Brazil; Discipline of Geriatrics, University of Sao Paulo Medical School, Sao Paulo, Brazil

Many open questions remain about neurodegenerative diseases (NDs). In the case of Parkinson's disease, it has been demonstrated that neuromelanin-harboring neurons in the substantia nigra are vulnerable to abnormal protein deposition and substantial neuronal loss, but the correlation between these two types of lesions has yet to be established. In both Parkinson's and Alzheimer's disease, studies have shown that pathological processes begin in the brainstem. Therefore, proteomic analysis of postmortem human brainstems holds the potential to unveil important information about this correlation and other important issues related to the initial stages and progression of NDs.
Keywords: human brain, aging, neurodegenerative diseases

11.15-11.30 **Antibody based profiling of protein distribution in the brain, focus on normal physiology and disease**

Jan Mulder, Science for Life Laboratory, Department of Neuroscience, Karolinska Institutet, Stockholm , Sweden

Completion of the genome projects and recent developments in transcriptomics have revealed the blueprint of the human body. We now have a near complete overview of all genes and proteins and are able to link genes to organs and tissues. The challenge for the future is to increase the resolution of this body-map by linking proteins to cells, cellular compartments and cellular functions. Human Protein Atlas project aims to provide this essential layer of information using an antibody based approach. Mapping protein distribution in the brain with its complex cellular organisation, broad spectrum of physiological functions, expressing more than 70% of all genes, is among the specific aims within the HPA project.

11.30-11.45 **How to explore brain proteins in the normal tissue atlas**

Evelina Sjöstedt, Science for Life Laboratory, Department of proteomics, KTH, Stockholm, Sweden and Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

In the Human Protein Atlas so far, a few selected brain regions is selected as representation of the human normal brain. This is gradually changing by adding more regions and specialized tissue sections, thereby providing a better overview of the protein location in the normal human brain.
Keywords: Normal brain, protein localization, FFPE tissue

11.45-12.00 **Atlas Antibodies: Advanced research reagents for studying human proteins**

Eugenia Kuteeva, Atlas Antibodies AB

Atlas Antibodies, a company founded by researches from the Human Protein Atlas project, is manufacturing and commercialising the affinity reagents for studying the human proteome. Our product portfolio includes a large number of highly specific and selective polyclonal antibodies, covering more than 15000 gene products. In addition, a growing number of monoclonal antibodies for selected targets is being developed in-house. Our latest QPrEST product line represents a new category of isotope-labelled internal standards for mass spectrometry (MS)-based protein quantification offering distinct advantages to existing products. This presentation will focus on our products of relevance for nervous system studies.
Keywords: Antibody development, Immunohistochemistry, Histology

12.00-13.00 Lunch

Restaurang Königs, Nobels väg 18

25th HUPO Human Brain Proteome Project Workshop

13.00-15.00 **Session 2: Technical advancements in neuroproteomics**
Chair: Oliver Schubert

13.00-13.15 **Revealing proteomics of cerebral organoids from human pluripotent stem cells: aiming to understand the mechanisms of brain diseases**
Juliana Minardi Nascimento, Laboratory of Neuroproteomics, Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Sao Paulo, Brazil

Pluripotent stem cells can differentiate into any cell type of an organism. Moreover, they have a remarkable capacity to self-organize and develop into three-dimensional structures resembling miniature organs. Cerebral organoids recreate early steps of the human cerebral cortex development, showing great potential for human modeling studies, particularly those with a developmental component. Analyzing global protein expression of cerebral organoids we found proteins broadly distributed on functional activities such as cell growth, energy metabolism and cell communication and signaling, which are correlated to cortical brain tissue. Proteomics add knowledge about information and connections being formed, atop architecture and self-organization already described
Keywords: neuroproteomics, pluripotent stem cells, cerebral organoids

13.15-13.30 **MRM as proteomic tool for the analysis of cerebrospinal fluid in neurodegenerative diseases**
Patrick Oeckl ,Department of Neurology, Ulm University Hospital, Ulm, Germany

The discovery of new biomarkers in cerebrospinal fluid (CSF) is highly appreciated to facilitate and improve diagnosis of neurodegenerative diseases. To date, immunoassays are the first choice for biomarker measurements in CSF due to their convenience and speed, but their applicability is hampered for new biomarker candidates by unknown specificity and limited availability of antibodies. The highly selective multiple reaction monitoring (MRM) is an attractive alternative which in principal allows the analysis of any biomarker and also multiplexing but studies using MRM for the analysis of CSF biomarkers are sparse. In this context, I will present successful applications of MRM for CSF biomarkers such as ubiquitin and also discuss considerations and experiences regarding the unique properties of CSF.
Keywords: Neurochemical Biomarker, Proteomics, Multiple Reaction Monitoring

13.30-13.45 **Brain up your analysis methodology - Proteome Bioinformatics and Biostatistics in Clinical Research**
Martin Eisenacher, Medizinisches Proteom-Center, Ruhr-University Bochum, Bochum, Germany

This presentation presents facets of the work in the research area Medical Bioinformatics of the Medizinisches Proteom-Center, which are linked to the Proteomics performed with mass spectrometry and the practical analysis of clinical research data. One topic is the protein inference / protein ambiguity, which takes place in “bottom-up Proteomics”, when reporting the proteins explained by the actually identified peptides. Furthermore the standards for the description and storage of Proteomics data and conversion of data and results into these formats will be sketched. Also a method for identifying sample subgroups will be summarized, which can be used to detect yet unknown “molecular subgroups” of heterogeneous diseases. The activities are available for the Proteomics community in a BMBF-funded “German Network for Bioinformatics Infrastructure (de.NBI, www.denbi.de)”.
Keywords: Bioinformatics of Proteomics, Proteomics Standard Formats, Biostatistics in Clinical Research

25th HUPO Human Brain Proteome Project Workshop

13.45-14.00 **Diagnostic and therapeutic potential of extracellular vesicles**

Fouzi El Magraoui, Biomedical Research , Human Brain Proteomics, Leibniz-Institut für Analytische Wissenschaften –ISAS e.V., Dortmund, Germany

Extracellular vesicles have emerged as important mediators of various cellular functions. They are secreted by every cell type and are detectable in every body fluid e.g. blood (serum and plasma), urine, breast milk, sweat, saliva, ascites fluid, and cerebral spinal fluid. Recent research demonstrate their (patho)physiological roles in various diseases, including cancer, infectious diseases and neurodegenerative disorders. In cooperation with our partners we performed different multi- omics approaches to investigate the potential of the EVs. Thus we have been able to detect various AD related biomarkers in platelet derived EVs. In other projects we have been able to identify surrogate markers of Stroke and Graft-versus-Host-Disease related therapeutic EVs.

Keywords: Extracellular Vesicles, Multi-Omics, Neurodegeneration

14.00-14.15 **Clinical proteomics and peptidomics for identification of cerebrospinal fluid biomarkers of neurodegenerative disorders**

Johan Gobom, Institute of Neuroscience and Physiology, Section of Psychiatry and Neurochemistry, University of Gothenburg

Clinical proteomics is an emerging field that has the potential to contribute greatly to research into our most common neurodegenerative diseases by uncovering biomarkers for diagnosis and monitoring treatment effects, and stimulating the formulation of new hypotheses on disease mechanisms. We present methods developed for proteomic and peptidomic analysis in cerebrospinal fluid and their application to biomarker discovery in Alzheimer's disease and Parkinsonian syndromes.

Keywords: CSF proteomics; neurodegeneration; biomarkers

14.15-14.30 **Antibody-based CSF profiling within multiple sclerosis reveals disease-asociated profiles of GAP43 and SERPINA3**

Anna Häggmark, Affinity Proteomics, Scilifelab, KTH - Royal Institute of Technology, Stockholm, Sweden

Antibody suspension bead arrays enable multiplexed and high-throughput protein profiling in unfractionated body fluids through a direct labeling approach. A CSF profiling protocol was established and applied to profile 43 selected proteins by 101 antibodies in almost 600 CSF samples from a multiple sclerosis cohort. Two proteins, GAP43 and SERPINA3 were found with altered levels between sample groups. The developed assay procedure offers new possibilities for broad-scale protein profiling of CSF within neurological disorders. This technology, with its ability to screen up to 384 samples and 384 analytes in parallel, combined with the unique antibody resources from the Human Protein Atlas creates great potential for future research in the quest of finding disease related patterns.

Keywords: Affinity Proteomics, CSF, multiple sclerosis

14.30-14.45 **Effects of hypertension on the brain: an initial top-down proteomic analysis.**

Jens R. Coorssen, School of Medicine, Nanoscale Organisation & Dynamics Group, School of Science & Health, Western Sydney University, Australia

Keywords: 2DE, mouse model, disease initiation and progression

14.45-15.00 **Quantifying Dementia**

Judith Steen, Harvard Medical School, Boston Children's Hospital, F.M. Kirby Center for Neurobiology, Boston, USA

Our laboratory uses computational methods to interface and mine proteomics and genomic data in an effort to learn about regeneration and degeneration of neurons. This approach allows us to understand how neurons are born and what makes them die. Our most recent efforts have focused on dementia including Alzheimer's disease, where neurons degenerate and eventually die causing the loss of memory and function in patients. A new technology that we developed called FLEXITau allowed us to stratify different types of patients suffering from dementia connected with a protein called Tau. This technology allowed us to identify specific molecular changes in tau from patients who have dementia but not in tau from healthy people.

Keywords: Quantitative-Proteomics, Bioinformatics, Neurodegeneration

15.00-15.30 Coffee break

15.30-17.30 Session 3: Psychiatric disorders

Chair: Daniel Martins-de-Souza

15.30-15.45 **Biological pathways modulated by antipsychotics in the blood plasma of schizophrenia patients and their association to a clinical response**

Daniel Martins-de-Souza, Laboratory of Neuroproteomics, Dept of Biochemistry, University of Campinas (UNICAMP), Campinas, São Paulo, Brazil

Our main goals are unraveling biochemical pathways modulated byantipsychotic medication as well as revealing proteins that can act aspotential biomarkers indicating the likelihood of a successfultreatment. Our latest results showed that all patients analysedpresent essentially the same biochemical pathways triggeredindependent of the antipsychotic response outcome. However, weobserved that these pathways were regulated in different directions inblood samples from those who responded well to antipsychotics,compared with those who had a poorer outcome.

Keywords: psychiatry, schizophrenia, translational psychiatry

15.45-16.00 **Peripheral blood gene expression and proteomic analysis implicates B-cell development and ribosomal proteins in cognitive dysfunction in people with remitted Major Depression**

K. Oliver Schubert, Northern Adelaide Local Health Network - Mental Health Services, Discipline of Psychiatry, School of Medicine, University of Adelaide, Adelaide, Australia

Cognitive impairments are observed in a substantial proportion of patients suffering from Major Depressive Disorder (MDD), significantly impacting on patients' psychosocial functioning and quality of life. We utilized whole-blood transcriptomic data from remitted MDD patients for weighted gene coexpression network analysis (WGCNA), and identified16 transcriptomic modules. One module was significantly correlated with poor versus better cognitive performance, containing ribosomal genes and modulators of B cell biology. On the plasma protein level, SWATH-MS detected 43% of module gene products, and group differences were confirmed. The experimental workflow may represent an effective approach to blood biomarker discovery for psychiatric phenotypes.

Keywords: Psychiatric Proteomics, Psychotic Disorders, Youth Mental Health

16.00-16.15 **Proteomic approaches to understanding the postsynaptic density in schizophrenia and bipolar disorder**

David Cotter, Royal College of Surgeons in Ireland, Dublin, Ireland

The postsynaptic density (PSD) contains a complex set of proteins of known relevance to neuropsychiatric disorders such as schizophrenia and bipolar disorder through its roles in synaptic plasticity and cognitive function. Genomic and clinical support for the involvement of the PSD in neuropsychiatric disorders is increasingly robust. We utilized PSD enrichment methods and label-free proteomic methods to characterize the disease-associated PSD proteome in schizophrenia and bipolar disorder for the first time. We enriched for this anatomical structure in the anterior cingulate cortex of 16 bipolar disorder, 20 schizophrenia cases and 20 controls from the Stanley Medical Research Institute and used unbiased shotgun proteomics incorporating label-free quantitation to identify differentially expressed proteins. Quantitative investigation of the PSD revealed more than 288 differentially expressed proteins in bipolar disorder and pathway analysis of the differentially expressed proteins implicated mitochondrial associated proteins, oxidative phosphorylation and the tricarboxylic acid cycle and also calcium signaling, and endocytosis in both disorders. Protein translation was implicated in bipolar disorder alone and long-term potentiation was implicated in schizophrenia alone. Our data provide robust evidence implicating PSD associated proteins, and specifically mitochondrial function with the PSD in bipolar disorder and schizophrenia. Calcium signaling, long term potentiation, endocytosis and protein translation were also implicated and together the findings converge to highlight a role for the regulation of synaptic plasticity in the major psychosis.

Keywords: neuroproteomics, plasma biomarkers, schizophrenia

16.15-16.30 **Investigating protein pathology in schizophrenia by identifying the insoluble proteome**

Carsten Korth, Institut für Neuropathologie, Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany

Chronic brain diseases are characterized by a failure in proteostasis as seen in the classical neurodegenerative diseases. Since chronic mental illnesses like schizophrenia or the recurrent affective disorders are also characterized by a chronic course, we hypothesized that to biologically characterize these conditions, the identification of a proteostasis phenotype would be revealing. We purified the insoluble proteome, i.e. those proteins that pellet after ultracentifugation and a biochemical multi-step fraction. This fraction was analyzed for 1. Candidate proteins, 2. epitope discovery, and 3. By LC-MS/MS. We find DISC1, dysbindin, CRMP1, TRIOBP1, NKCC1 as novel candidate proteins for schizophrenia and confirm their protein pathology in novel animal models.

Keywords: molecular psychiatry, insoluble proteome, schizophrenia

16.30-16.45 **Proteome-wide study of cerebrospinal fluid biomarkers for bipolar disorder - multiplexed semi-quantification by mass spectrometry**

Jessica Holmén Larsson ,Institute of Neuroscience and Physiology, Neurochemical pathophysiology and diagnostics research unit, Sahlgrenska Academy at Goteborg University, Mölndal, Sweden

Our aim is to explore pathophysiological mechanisms for Bipolar disorder (BD) and to identify novel biological CSF markers for diagnostic purposes. The digested CSF proteins of 15 BD patients and 15 matched controls, were labeled with isobaric Tandem Mass Tags (TMT) for multiplexed semi-quantification and analyzed by nano-liquid chromatography-mass spectrometry. 676 proteins were semi-quantified and 36 showed significantly ($p<0.05$) altered levels in the BD patients as compared to controls. A majority of the altered proteins are brain-specific and involved in e.g. cell growth/communication/adhesion, immune response and protein metabolism. The results will now be validated in two larger independent BD cohorts.

Keywords: Neuropsychiatry, Cerebrospinal fluid, Biomarker

25th HUPO Human Brain Proteome Project Workshop

16.45-17.00 **Advancing Biomarker Discovery in Age 12 Children with Psychotic Disorder: Results from the ALSPAC Cohort**

Jane A English, Department of Psychiatry, Royal College of Surgeons in Ireland, Dublin, Ireland

Biomarkers of early psychosis are urgently needed to allow the early identification and treatment of those at the highest risk of developing psychosis. We developed a simultaneous discovery and targeted proteomic workflow to profile and verify biomarker candidates of psychotic disorder in age 12 plasma samples from the Avon Longitudinal Study of Parents and Children (ALSPAC). Using Data Dependent Analysis (DDA) on the Thermo Q-Exactive, we profiled 37 plasma samples from age 12 children with an outcome of psychotic disorder, and 38 age matched controls. We identified 61 proteins ($p<0.05$) as potential biomarker candidates, 35 of which remained significant following FDR ($q=0.05$). ROC analysis was used to evaluate the top 8 discriminatory Variables of Importance (VIP), resulting in an AUC of 0.89. Targeted proteomic analysis of these proteins using Data Independent Analysis (DIA) on the Thermo Q-Exactive is underway.

Keywords: Biomarker Discovery, Psychosis, ALSPAC Cohort

17.00-17.15 **Uppsala Psychiatric Patient Samples - UPP**

Janet Cunningham, Department of Neuroscience, Psychiatry, Uppsala University, Uppsala, Sweden

Uppsala Psychiatric Patient samples (UPP) is a biobank with material and data from well over 600 individuals. Patients have given informed consent to research endocrine, immunological and genetic contributions to psychiatric symptoms. The collection has three patient subgroups: i) a small scale level where individuals with extreme and atypical phenotypes are included using exploratory methods and examined on a case-rapport basis ii) a large scale population based level which is important for establishing generalizability of theories developed in the small scale work and proposed by others and finally iii) collections in conjunction with other specific treatment studies.

Keywords: Psychiatry, inflammation, autoimmunity

17.15-17.30 **Gene expression alterations of complement factors in schizophrenia**

Eva Lindholm Carlström, Uppsala University, Uppsala, Sweden

In general, the main focus of my research includes genetic analyses and gene expression studies to identify genetic causes of schizophrenia. We have performed transcriptome analyses of 112 brain tissue samples derived from 65 individuals with schizophrenia and 47 age and sex matched control samples. Analysis revealed 74 significant differentially expressed genes (adjusted p-value < 0.05). There was an enrichment of immune system genes, with a striking up-regulation of the complement system ($p=49 \times 10\text{-}7$). Our findings support the previous hypothesis that hyper-activation of the complement cascade is involved in schizophrenia pathology.

Keywords: Schizophrenia, genetics, gene expression

17.30-19.00 Pub and mingle

SciLifelab, Tomtebodavägen 23

18.00-18.30 An update on the Human Protein Atlas

Mathias Uhlen, SciLifeLab, KTH - Royal Institute of Technology, Stockholm, Sweden

19.00-22.00 Dinner

SciLifelab, Tomtebodavägen 23

25th HUPO Human Brain Proteome Project Workshop

Wednesday May 4

08.30-10.00 **Session 4: Alzheimer's disease**
Chair: Helmut E Meyer

08.30-08.45 **Biomarker for early AD diagnostics**
Helmut E Meyer, Leibniz-Institut für Analytische Wissenschaften –ISAS e.V., Dortmund, Germany

Alzheimer dementia (AD) is the most prevalent neurodegenerative disorder of the elderly and about 6% of the general population over 65 will be affected. Besides all efforts targeting these protein aggregates over the last 20 years to develop effective AD-therapies no success could be reported so far. We started a joint effort in finding early onset biomarkers by analyzing extracellular vesicles (EVs) in the blood which are produced by senescent platelets and other cells in the circulation. Subfractions of these EVs are enriched in AD-related proteins and lipids and may be the causative agent of the onset and progression of AD.
Keywords: Biomarker, Alzheimer, Diagnostic

08.45-09.00 **Brain enriched proteins in CSF – GAP43 and friends**
Julia Remnestål, Affinity Proteomics, Scilifelab, KTH - Royal Institute of Technology, Stockholm, Sweden

Advancements in transcriptomic technologies have enabled comparative studies allowing identification of genes with tissue-enriched expression. Using antibodies and direct labeling of proteins we have profiled 280 brain-enriched proteins in cerebrospinal fluid from patients with Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB) in order to investigate potential associations to neurodegenerative diseases. Several proteins displayed differences in expression levels between diseased patients and controls with the two synaptic proteins GAP43 and NRGN specifically demonstrating a significant increase in CSF from patients with AD in two independent cohorts.
Keywords: protein profiling, affinity proteomics, neurodegenerative disorders

09.00-09.15 **Characterization of the Abeta and Neurogranin peptide patterns – from blood to the brain**
Erik Portelius, Institute of Neuroscience and Physiology, Sahlgrenska Academy at Goteborg University, Mölndal, Sweden

Alzheimer's disease (AD) is the most common neuropsychiatric disorder in the aging population and is characterized by plaques and tangles in the brain as well as synaptic loss. The last decades have witnessed an explosion in studies of the role of amyloid-β in the progression to AD. Although we now have a deep knowledge about the disease, we still do not understand how these different processes leads to the symptoms of AD or what it is that triggers the disease. Thus, a deeper characterization of key proteins in blood, cerebrospinal fluid and brain tissue is needed.
Keywords: Targeted proteomics; Alzheimer´s disease; Mass spectrometry

09.15-09.30 **Molecular endophenotypes in Alzheimer's disease**
Pieter Jelle Visser, Alzheimer Centre, VU University Medical Centre, Amsterdam, The Netherlands

Objective: Alzheimer's disease (AD) is a genetically and clinically heterogeneous disorder. We studied whether molecular subtypes can be defined with a dual clustering approach of proteins in cerebrospinal fluid (CSF). Methods: Data was analyzed of 220 CSF proteins of 273 subjects (79 controls; 130 mild cognitive impairment (MCI); 64 AD) from the ADNI study. Data were normalized and clustered with non-negative matrix factorization. Results: Three clusters were identified, which showed a similar distribution of diagnosis and APOEe4 genotype. Clusters differed in the proportion of people with abnormal amyloid (Cluster 1: 83%; Cluster 2: 56%; Cluster 3: 74%), and abnormal tau (Cluster 1: 12%; Cluster 2: 30%; Cluster 3: 57%). Conclusion: Endophenotypes based on CSF were associated with heterogeneity in AD.
Keywords: Alzheimer's disease, Cerebrospinal fluid, endophenotypes

25th HUPO Human Brain Proteome Project Workshop

09.30-09.45 **Proteomics in Alzheimer's disease**

Kim Kultima, Department of Medical Sciences, CARAMBA, Cancer Pharmacology and Computational Medicine, Uppsala University, Uppsala, Sweden

Alzheimer's disease (AD) is a chronic neurodegenerative disorder accounting for more than 50% of all dementia cases. The AD neuropathology is characterized by formation of extracellular plaques and intracellular neurofibrillary tangles consisting of aggregated amyloid-β and tau, respectively. The disease mechanism has only been partially elucidated and is believed to involve many other proteins. We have used mass spectrometry based proteomic methods to analyze the Alzheimer's disease brain and CSF in combination with antibody based verification to increasing our knowledge in the AD neuropathology.

Keywords: Proteomics, Alzheimer's, Dementia

09.45-10.00 **Thinking outside of the brain in Alzheimer's disease using proteomics technology**

Renã A. S. Robinson, Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

The periphery is implicated in the pathogenesis of Alzheimer's disease however a full understanding of the role of the periphery in Alzheimer's does not exist. Recently it was demonstrated that activation of the peripheral immune response helps to lower brain inflammation and remove amyloid-beta. Also amyloid-beta production and clearance in the brain is likely influenced by peripheral organs such as the liver. Investigating changes in human peripheral tissues can be challenging making animal models of Alzheimer's disease extremely useful. The Robinson group develops high throughput proteomics methods to establish multiple hypotheses about the role of peripheral tissues in Alzheimer's disease.

Keywords: proteomics, Alzheimer's disease, mass spectrometry

10.00-10.30 Coffee break

10.30-12.00 Session 5: PD and other dementias

Chair: Katrin Marcus

10.30-10.45 **Analysis of cerebrospinal fluid using high resolution mass spectrometry**

Katrin Marcus, Medizinisches Proteom-Center, Ruhr-University Bochum, Bochum, Germany

Nowadays, the most common neurodegenerative disorders are Alzheimer's and Parkinson's disease (AD, PD). Although these disorders are extensively investigated, an exact diagnosis can only be confirmed post-mortem. To facilitate therapeutic intervention, an early diagnosis as well as the possibility to monitor disease progression is important. Since cerebrospinal fluid (CSF) is the body fluid that surrounds the brain it is a promising source for biomarker discovery of neurodegenerative disorders. For this reason multiple proteomic studies of CSF were performed to investigate differences between healthy individuals and patients suffering from neurodegenerative diseases. Here we show a label-free mass spectrometry (MS) approach to analyze CSF in a cross-sectional and longitudinal de novo PD-cohort, which provides the great advantage of fast and cheap sample preparation as well as interesting protein biomarker candidates.

Keywords: proteomics, mass spectrometry, neurodegeneration, biomarker research,

10.45-11.00 **Robust metabolic biomarkers for Parkinson's disease**

Karsten Hiller, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

The diagnosis of early-stage Parkinson's disease (PD) is based on clinical assessment of motor and non-motor symptoms, without considering metabolic biomarkers. Additional tests are required to significantly improve diagnosis and patient care. By using a non-targeted gas chromatography coupled to mass spectrometry (GC-MS) approach, we investigated metabolic changes in cerebrospinal fluid (CSF) from early-stage PD patients, displaying the first motor symptoms of the disease. We compared these metabolite levels with age-, gender- and education-matched healthy controls. We found that dehydroascorbic acid levels were significantly lower (FDR < 0.001) and fructose, mannose and threonic acid levels were significantly higher (FDR = 0.007, FDR < 0.001, FDR = 0.004, respectively) in early-stage PD compared to healthy controls. These changes reflect pathological oxidative stress responses, as well as protein glycation/glycosylation reactions in PD. Using a machine learning approach based on logistic regression, we successfully predicted the origin (PD vs. healthy controls) of independent CSF samples, based on a biosignature composed of mannose, threonic acid and fructose. Our results enhance the understanding about the PD related CSF metabolome and could be useful for further evaluation of the observed changes in other "omics" approaches and early-stage diagnosis of PD.

Keywords: metabolism, metabolomics, mass spectrometry

11.00-11.15 **RTP801/REDD1 interactome in a cellular model of PD**

Cristina Malagelada Grau, Unit of Biochemistry, Department of Biomedicine, University of Barcelona, Barcelona, Spain

mTOR pathway deregulation has become a hallmark in neurodegenerative disorders, since a fine-tuned regulation of mTOR activities is crucial for neuron function and survival. RTP801/REDD1 has become one of the most puzzling regulators of mTOR. Although the mechanism is not completely understood, RTP801 inactivates mTOR and Akt via the tuberous sclerosis complex (TSC1/TSC2) in many cellular contexts. Understanding the mechanisms by which RTP801 induces cell death is crucial to design any effective therapeutic approaches. Here, we performed a proteomic analysis to identify potential new RTP801 protein interactors, to better comprehend its regulatory mechanisms over mTOR.

Keywords: neurodegeneration Parkinson's disease mTOR signaling

11.15-11.30 **Proteomics Profiling of the Substantia Nigra Reveals Association of RNA Metabolism Pathways and Arp2/3-Mediated Actin Nucleation with Lewy Body Presence**

Vladislav Petyuk, Pacific Northwest National Laboratory, Richland, USA

Proteinaceous aggregates containing a-synuclein protein known as Lewy bodies is a hallmark of majority cases of Parkinson's and a number of other diverse neurodegenerative diseases. To gain insights on proteins and associated pathways associated with the presence of Lewy bodies we performed a quantitative proteomic profiling. We analyzed substantia nigra tissue from 51 subjects arranged into three groups: cases with Lewy bodies, controls with matching neuronal loss and controls with normal neuronal density. The key findings are downregulation of mRNA processing pathways and upregulation of Arp2/3 complex-mediated actin nucleation in the cases of Lewy body-dependent neuronal loss.

Keywords: Parkinsons's disease, Lewy bodies, neurodegeneration

11.30-11.45 **Identification of CSF biomarkers for specific dementia's by Mass spectrometry proteomics**

Charlotte Teunissen, VU University Medical Center Amsterdam – department of Clinical Chemistry, Amsterdam, Netherlands

Neurodegenerative diseases leading to dementia are a clinically and pathologically heterogeneous group of disorders, for which there is currently no cure. Diagnosis of different subtypes of dementia relies largely on clinical examination, and there is a strong need for cerebrospinal fluid (CSF) biomarkers to aid diagnosis. Our overall aim is to develop CSF biomarkers for dementia subtypes with a proven relation to pathology. For this, we have mapped changes in the proteome of ante-mortem cerebrospinal fluid (CSF) and post-mortem brain tissue of patients at different stages of Alzheimer's and frontotemporal dementia (FTD). We will integrate tissue and CSF proteomics results to select the most promising biomarker proteins for each specific dementia type.

Keywords: biomarkers for neurological diseases, dementia disorders, immunoassays

11.45-12.00 **Integrated Biomarker Discovery in Parkinsonian Disorders**

Miles Trupp, Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden

Development of effective therapeutics for Parkinson's disease (PD) has been hindered by the late stage and imprecision of initial diagnosis. PD shares similar clinical presentations with molecularly distinct neurodegenerative diseases, such as multiple system atrophy (MSA) and progressive supranuclear palsy (PSP). We are analyzing CSF and plasma from the extensive biobanks at Umeå University Hospital towards identifying biomarkers for Parkinson's disease prediction and progression. We are utilizing mass-spectrometry based metabolomics and proteomics for discovery and MRM validation studies. Results indicate that precise, parallel quantification of biomarkers from multiple pathways has the potential to define subgroups of Parkinson's disease with different molecular etiologies.

Keywords: Mass-spectrometry, Biomarkers, Neurodegeneration

12.00-13.00 Lunch

Restaurang Jöns Jacob, Retzius väg 22

25th HUPO Human Brain Proteome Project Workshop

13.00-14.15 **Session 6: Autoimmunity in brain disorders**
Chair: Peter Nilsson

13.00-13.15 **Anoctamin 2 as a novel autoimmune target in multiple sclerosis**
Peter Nilsson, Affinity Proteomics, Scilifelab, KTH - Royal Institute of Technology, Stockholm, Sweden

*We profiled the autoantibody repertoire in 2,169 plasma samples within multiple sclerosis using suspension bead arrays, built with 384 human protein fragments selected from an initial screening with 11,520 antigens. Increased autoantibody reactivity against the chloride channel protein anoctamin 2 (ANO2) in MS cases was identified. This finding was validated in independent assays with alternative protein constructs and by epitope mapping with peptides. We also found a strong interaction between the presence of ANO2 autoantibodies and the HLA complex MS-associated DRB1*15 allele. The findings presented here demonstrate that an ANO2 autoimmune sub-phenotype may exist in MS.*
Keywords: affinity proteomics, autoantibody profiling, protein microarrays

13.15-13.30 **New insights into brain disorders**
Caroline May, Medizinisches Proteom-Center, Ruhr-Universität Bochum, Bochum, Germany

The brain can be subdivided into different regions, fields, and cellular layers. Moreover, different neuronal and glial cells can be distinguished. The separation of brain-derived cells is challenging, because isolating intact neurons is not feasible with traditional methods. Therefore, in the past most of the studies were performed with whole brain lysate. Within these studies dominant cell types, like e.g. glial cells, can mask neuron-specific information. We present a new strategy to enrich neurons using laser microdissection and subsequent mass spectrometric analysis. This analysis will deepen our understanding of brain function and how certain neurons are altered in brain diseases.

13.30-13.45 **High-density Antibody and Phage Microarray screening of prefrontal cortex brain tissue and sera of Alzheimer Disease Patients for the identification of specific AD markers**
Rodrigo Barderas, Functional Proteomics of Chronic Diseases, Biochemistry and Molecular Biology I Department, Chemistry Faculty, Complutense University of Madrid, Madrid, Spain

Alzheimer's Disease (AD) is a severe neurodegenerative disorder with a high socioeconomic impact. It is estimated to affect 80 million people worldwide in 2050. An accurate and early diagnosis of AD is difficult and definitive diagnosis requires post-mortem verification. We are currently using high-density antibody and phage microarrays for the study of changes in protein expression in the prefrontal cortex and the humoral response of AD patients, respectively. The identification of deregulated proteins and target proteins of autoantibodies should provide new information for the elucidation of altered pathways and cellular processes and for the discovery of new AD-specific biomarkers.
Keywords: Quantitative neuroproteomics, mass spectrometry, protein, phage and antibody microarrays

13.45-14.00 **TBA**
Dolores Cahill, University College Dublin, Dublin, Ireland

14.00-14.15 **Profiling autoantibody repertoires within psychiatric disorders**

David Just, Affinity Proteomics, Scilifelab, KTH - Royal Institute of Technology, Stockholm, Sweden

In this project we aimed to investigate the autoantibody repertoire in patients with psychiatric disorders. Therefore we screened more than 500 serum samples in a first discovery phase using different clinical cohorts. We utilized protein fragments generated within the Human Protein Atlas for a targeted screening using the suspension bead array technology. Here we selected approx. 300 protein fragments with a length of roughly 100 amino acids that have a known disease background. Additionally we used the in house generated planar arrays for an untargeted screening of 1152 protein fragments on a subset of our cohort collection. Our findings clearly indicate altered immune response in patients with psychiatric disorders and by further validating these putative autoimmune targets we could gain insights into the autoantigens associated to psychiatric disorders.

Keywords: Psychiatry, Autoimmunity, Microarray

14.15-14.30 Coffee break

14.30-15.30 Session 7: Other neurological disorders

Chair: Charlotte Teunissen

14.30-14.45 **Somalogic-based proteomics to identify and validate biomarkers in blood of Multiple Sclerosis**

Arjan Malekzadeh, VU University Medical Center Amsterdam, Netherlands

14.45-15.00 **Vascular biomarkers in ALS neurodegeneration**

Sebastian Lewandowski, Vascular Biology Group, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Human brain is irreversibly dependent on constant blood flow to meet its high demand for energy. As a consequence, interruptions in blood flow efficiency often cause or contribute to neurodegenerative diseases. Our aim is to study the cerebral vessel injury in mouse models and patients with amyotrophic lateral sclerosis (ALS) neurodegeneration where decreased cerebral blood flow indicates rapid disease progression. With the help of immune bead proteomics platform we have identified several vascular protein factors in plasma of 360 ALS patients with the potential to serve as biomarkers for cerebral injury.

Keywords: Neurodegeneration, cerebral blood flow, ALS

25th HUPO Human Brain Proteome Project Workshop

15.00-15.15 **Multiplexed MRM Quantitation of Candidate Protein Biomarkers in Human CSF**

Christoph Borchers, University of Victoria - Genome British Columbia Proteomics Centre, Victoria, BC, Canada

Multiplexed quantitation can expedite the verification and validation stages of the protein biomarker pipeline leading to enhanced personalized medicine. The most promising strategy for protein biomarker verification centers on the MRM technology with stable isotope-labeled standards (SIS) employed within a bottom-up proteomic workflow. Since cerebrospinal fluid (CSF) is an important biofluid for studying central nervous system (CNS)-related diseases, we have developed a rapid and robust, MRM-based method to quantify the largest panel of candidate CSF protein biomarkers in a single analytical experiment. The method is antibody-/fractionation-free and utilizes a complex mixture of in-house synthesized internal standards, as well as standard-flow UHPLC and dynamic MRM, for enhanced robustness and peptide multiplexing. Our final method enabled the reproducible quantitation of 130 proteins (inferred from 311 interference-free peptides) in a 43 min LC-MRM/MS run. Several of these have putative association to neurodegenerative disease, such as the potentially diagnostic Alzheimer's disease markers osteopontin and heart fatty acid binding protein. Overall, the 130 proteins span a 5 order of magnitude concentration range (from 118 µg/mL to 550 pg/mL; as revealed by standard curves), and can be readily interrogated as a complete or subset panel in subsequent verification studies of patient CSF samples.

15.15-15.30 **Proteomics in multiple sclerosis**

Claire Bridel, VU University Medical Center Amsterdam, Netherlands

15.30-16.00 General discussion and concluding remarks

25th HUPO Human Brain Proteome Project Workshop

General information

Venue
The meeting will be held in Samuelssonsalen, Tomtebodavägen 6, Karolinska Institutet, Solna (#1 on the map).

Registration
All participants should register outside the lecture hall Samuelssonsalen before the meeting starts to receive a name badge and printed program.

Uploading of presentations
All presenters should upload their presentations on a common computer before the session starts.

Food and drinks
Coffee will be served during breaks outside the lecture hall, Tomtebodavägen 6

Lunch on Tuesday will be served at Restaurang Königs (Nobels väg 18, #2 on the map)

Both the pub and dinner will be held at SciLifeLab (Tomtebodavägen 23, #3 on the map). Enter the main entrance and take right after the reception for the pub and left for the dinner.

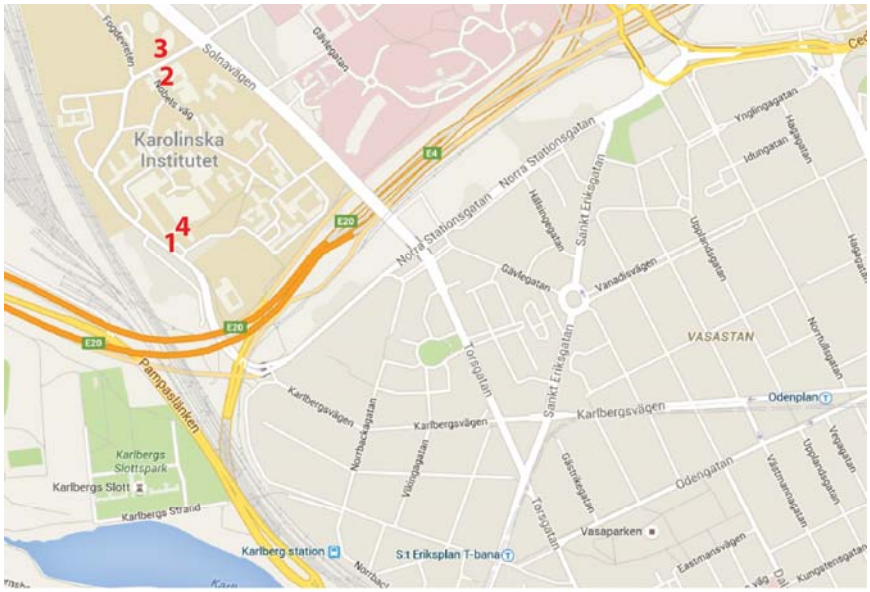
On Wednesday we will have lunch at Restaurang Jöns Jacob (Retzius väg 22, #4 on the map)

Transportation
The Karolinska Institutet campus in Solna is situated within approximately 15 min walking distance from subway station St Eriksplan. Alternatively, buss 3 and 77 travel from St Eriksplan to Karolinska Sjukhuset (get of at bus stop Thorax) and bus 69 leave from S:t Eriksgatan and stop at Karolinska Institutet västra. Tickets can **not** be bought on the bus, see www.sl.se/en for more information.

The two major taxi companies in Stockholm are Taxi Stockholm
(+46 8 150 000) and Taxi 020 (+46 20 202020)

Internet
The wireless network Eduroam is available in the Karolinska campus area. A guest network is also available named KI-Guest, password NobelVT2016

25th HUPO Human Brain Proteome Project Workshop



1. Lecture hall, Samuelssonsalen, Tomtebodavägen 6
2. Restaurang Königs, Nobels väg 18
3. SciLifeLab, Tomtebodavägen 23
4. Restaurang Jöns Jacob, Retzius väg 22

Bus stop Karolinska Institutet västra (*) and Thorax (**)

25th HUPO Human Brain Proteome Project Workshop

List of participants

Australia		
Jens R. Coorssen	University of Western Sydney, Campbelltown, Australia	J.Coorssen@westernsydney.edu.au
K. Oliver Schubert	University of Adelaide, Adelaide, Australia	oliver.schubert@adelaide.edu.au
Brazil		
Daniel Martins-de-Souza	University of Campinas (UNICAMP), Campinas, São Paulo, Brazil	danms90@gmail.com
Juliana Minardi Nascimento	University of Campinas (UNICAMP), Campinas, São Paulo, Brazil	juminardi@gmail.com
Lea T. Grinberg	USA & Brazilian Brain Bank, São Paulo, Brazil	Lea.Grinberg@ucsf.edu
Renata Leite	University of Sao Paulo Medical School, São Paulo, Brazil	renataleite@usp.br
Canada		
Christoph Borchers	University of Victoria, Victoria, Canada	christoph@proteincentre.com
Germany		
Caroline May	Ruhr University, Bochum, Germany	caroline.may@rub.de
Carsten Korth	Heinrich Heine Universität Düsseldorf, Germany	ckorth@uni-duesseldorf.de
Fouzi El Magraoui	ISAS - Leibniz-Institut für Analytische Wissenschaften, Dortmund, Germany	fouzi.elmagraoui@isas.de
Helmut E. Meyer	ISAS - Leibniz-Institut für Analytische Wissenschaften, Dortmund, Germany	helmut.meyer@isas.de
Katrin Marcus	Ruhr University, Bochum, Germany	katrin.marcus@rub.de
Martin Eisenacher	Ruhr University, Bochum, Germany	martin.eisenacher@rub.de
Nirmal Raman Kannaiyan	Ludwig Maximilian University of Munich, Germany	nirmal.kannaiyan@med.uni-muenchen.de
Olaf Jahn	Max-Planck-Institute of Experimental Medicine, Goettingen, Germany	jahn@em.mpg.de
Patrick Oeckl	Ulm University Hospital, Ulm, Germany	patrick.oeckl@uni-ulm.de
Ireland		
David Cotter	Royal College of Surgeons in Ireland, Dublin, Ireland	drcotter@rcsi.ie
Dolores Cahill	University College Dublin, Dublin, Ireland	dolores.cahill@ucd.ie
Jane A English	Royal College of Surgeons in Ireland, Dublin, Ireland	janeenglish@rcsi.ie
Korea		
Young Mok Park	Institute for Basic Science, Daejeon, Korea	youngmok.park@gmail.com
Luxembourg		
Karsten Hiller	University of Luxembourg, Luxembourg	karsten.hiller@uni.lu
Netherlands		
Arjan Malekzadeh	VU University Medical Center Amsterdam, Amsterdam, Netherlands	a.malekzadeh@vumc.nl
Charlotte Teunissen	VU University Medical Center Amsterdam, Amsterdam, Netherlands	c.teunissen@vumc.nl
Claire Bridel	VU University Medical Center Amsterdam,Amsterdam, Netherlands	c.bridel@vumc.nl
Pieter Jelle Visser	VU University Medical Center Amsterdam, Amsterdam, Netherlands	pj.visser@maastrichtuniversity.nl
Spain		
Cristina Malagelada Grau	University of Barcelona, Barcelona, Spain	cristina.malagelada@ub.edu
Rodrigo Barderas	Complutense University of Madrid, Madrid, Spain	rbarderas@quim.ucm.es

25th HUPO Human Brain Proteome Project Workshop

Sweden

Anna Häggmark	KTH - Royal Institute of Technology, Stockholm, Sweden	anna.haggmark@scilifelab.se
Annica Rasmusson	Uppsala University, Uppsala, Sweden	annica.rasmusson@medsci.uu.se
Claudia Fredolini	KTH - Royal Institute of Technology, Stockholm, Sweden	claudia.fredolini@scilifelab.se
David Just	KTH - Royal Institute of Technology, Stockholm, Sweden	david.just@scilifelab.se
Elisa Pin	KTH - Royal Institute of Technology, Stockholm, Sweden	elisa.pin@scilifelab.se
Eni Andersson	KTH - Royal Institute of Technology, Stockholm, Sweden	eni.andersson@scilifelab.se
Erik Portelius	Sahlgrenska Academy at Goteborg University, Mölndal, Sweden	Erik.Portelius@neuro.gu.se
Eugenia Kuteeva	Atlas Antibodies AB	eugenia.kuteeva@atlasantibodies.com
Eva Lindholm Carlström	Uppsala University, Uppsala, Sweden	eva.lindholm@igp.uu.se
Evelina Sjöstedt	Uppsala University, Uppsala, Sweden	evelina.sjostedt@igp.uu.se
Jan Mulder	Karolinska Institutet, Stockholm, Sweden	jan.mulder@ki.se
Janet Cunningham	Uppsala University, Uppsala, Sweden	janet.cunningham@neuro.uu.se
Jessica Holmén Larsson	Sahlgrenska Academy at Goteborg University, Mölndal, Sweden	Jessica.Holmen-Larsson@neuro.gu.se
Johan Gobom	University of Gothenburg, Göteborg, Sweden	Johan.Gobom@neuro.gu.se
Julia Remnestål	KTH - Royal Institute of Technology, Stockholm, Sweden	julia.remnestal@scilifelab.se
Kim Kultima	Uppsala University, Uppsala, Sweden	kim.kultima@medsci.uu.se
Maria Mikus	KTH - Royal Institute of Technology, Stockholm, Sweden	maria.mikus@scilifeleb.se
Mathias Uhlén	KTH - Royal Institute of Technology, Stockholm, Sweden	mathias.uhlen@scilifelab.se
Miles Trupp	Umeå University, Umeå, Sweden	miles.trupp@umu.se
Payam Emami	Uppsala University, Uppsala, Sweden	payam.emami@medsci.uu.se
Peter Nilsson	KTH - Royal Institute of Technology, Stockholm, Sweden	peter.nilsson@scilifelab.se
Sebastian Lewandowski	Karolinska Institutet, Stockholm, Sweden	Sebastian.Lewandowski@ki.se

USA

Judith Steen	Boston Children's Hospital, Boston, USA	Judith.Steen@childrens.harvard.edu
Renã A. S. Robinson	University of Pittsburgh, Pittsburgh, USA	rena@pitt.edu
Vladislav Petyuk	Pacific Northwest National Laboratory, Richland, USA	vladislav.petyuk@pnnl.gov