



The Human Glycoproteomics Initiative Workshop on Glycoproteomics and Glycoinformatics

US HUPO March 7, 2023

- Nicholas M. Riley, Ph.D.**
Community Evaluation of Glycoproteomics Software
and HGI Update
- Carlito B. Lebrilla, Ph.D.**
Translating glycomic and glycoproteomic discoveries to
products for human health
- Peggi M. Angel, Ph.D.**
Glycomics Imaging for Analysis of Tissues, Cells, Biofluids,
and Target Glycoproteins
- Yixuan (Axe) Xie, Ph.D.**
GlycanDIA Empowers Deep Profiling in Glycomic Analysis



COMMUNITY EVALUATION

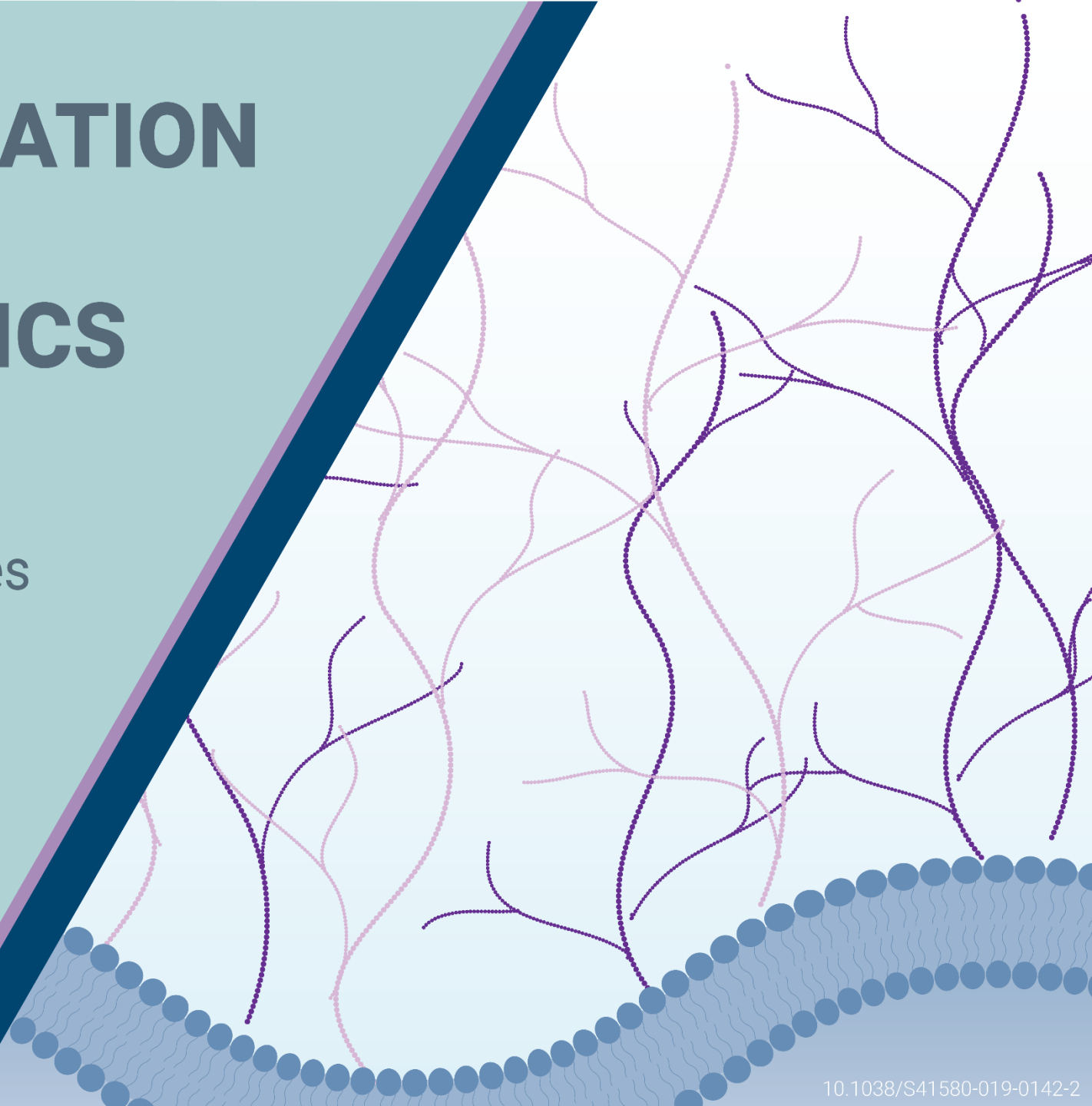
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GLYCOPROTEOMICS SOFTWARE

Observations and outcomes
from the 1st HUPO-HGI
Community Study

Nicholas M. Riley, Ph.D.

US HUPO Workshop
March 7, 2023





Studying **genomics** reveals what a cell is capable of;
transcriptomics gives a view of what the
cell is planning to do.

To find out what the cell is actually doing,
however, requires
proteomics and **metabolomics**.

Seydel, Nature Methods,
2021, 18: 1452-1456



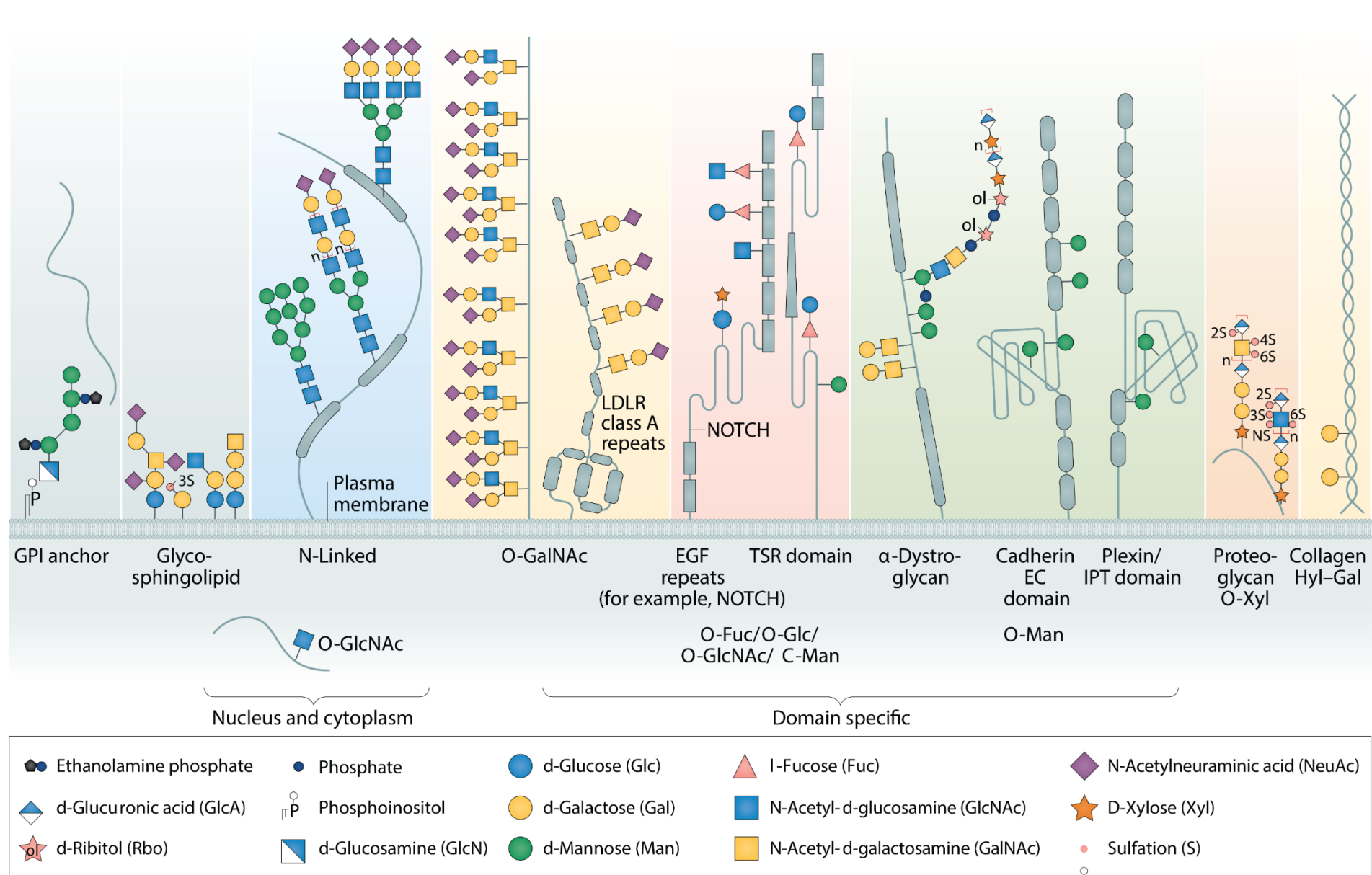
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To find out what the cell is actually doing,
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proteomics and **metabolomics... and**

Seydel, Nature Methods, **glyco(proteo)mics**
2021, 18: 1452-1456

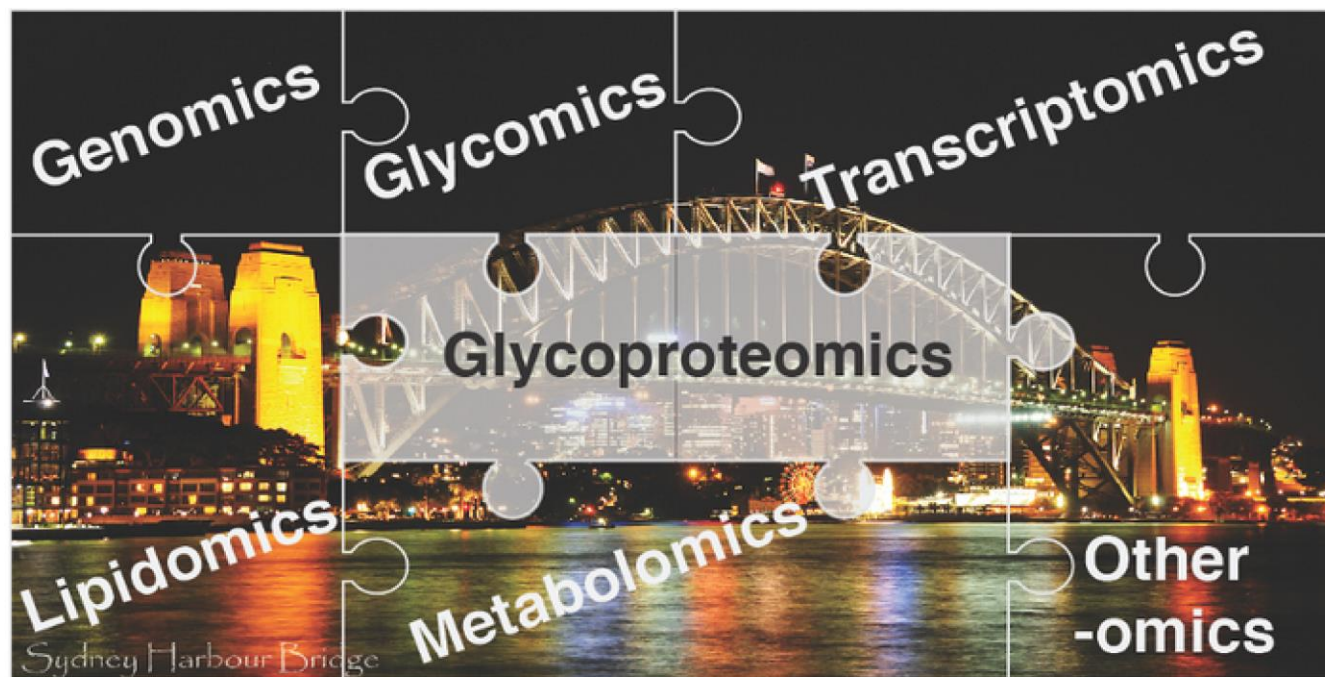
Glycocalyx





HUMAN GLYCOPROTEOMICS INITIATIVE

"Our overall aim is to help the community to create the toolboxes required to address unexplored glycobiology-focused fundamental and applied research questions in human health and disease."





HUMAN PROTEOME ORGANIZATION

translating the code of life

JOIN / PROTEOMICS



ABOUT ▶

HUMAN PROTEOME PROJECT (HPP) ▼

EVENTS AND EDUCATION ▶

PUBLICATIONS ▶

MEMBERSHIP ▶

MISSION

THE GRAND PROJECT

ABOUT THE HPP

HPP PROGRESS TO DATE

LEADERSHIP ▶

C-HPP

HUMAN PROTEOME REFERENCE LIBRARY

B/D-HPP ▶

RESOURCES

HPP RESOURCE PILLARS ▶

NEWS

HPP Q & A

HPP PUBLICATIONS ▶

SAVE THE DATE
SEPTEMBER 17-21, 2023

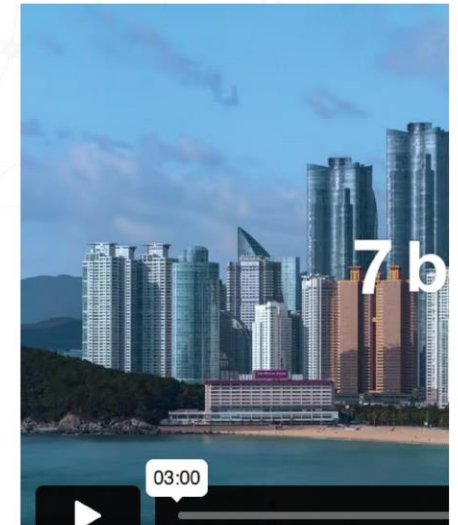


IMMUNO PEPTIDOME

HUMAN GLYCOPROTEOMICS INITIATIVE ▶

HUMAN PLASMA PROTEOME PROJECT

BRAIN PROTEOME PROJECT



7b

03:00

1ST HGI 2017-2021

HGI FURTHER READING

2ND HGI STUDY

POST-STUDY BENCHMARKING

HU
BUS
202

See you
BEXCO, B

2023.h



TR

The Hur

THE CODE

an international scientific

Past, current and future glyco-centric initiatives under B/D-HPP (HUPO)



Naoyuki Taniguchi Director
Hisashi Narimatsu Director

Martin Frank Co-director
James Paulson Co-director
Kazuyuki Nakamura Co-director

2004-16: Glycomics-centric community studies

1st HGPI Study: Yoshinao Wada¹
2nd HGPI Study: Anne Dell²
3rd HGPI Study: Pauline Rudd³



HUMAN GLYCOPROTEOMICS INITIATIVE

Morten Thaysen-Andersen Chair
Daniel Kolarich Co-chair
Nicolle H. Packer Co-chair

Rebeca Kawahara ECR representative
Tiago Oliveira ECR representative

2017- : Glycoproteomics-centric community studies

1st HGI Study: Nicki Packer & Morten Andersen⁴
2nd HGI Study: Stacy Malaker & Nick Riley (launched, early phase)
3rd HGI Study: Daniel Kolarich (scheduled, stay tuned)

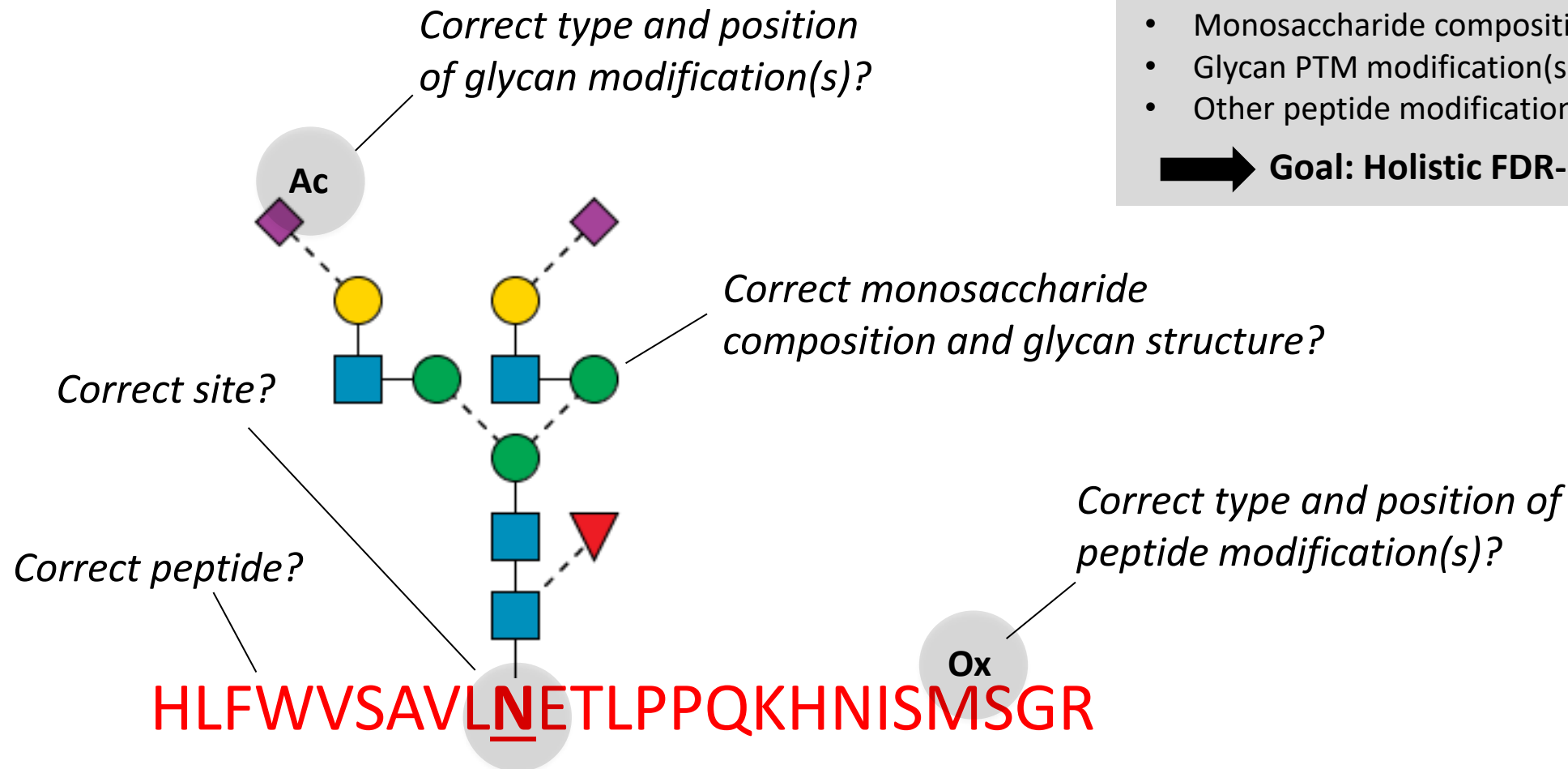
¹Wada et al., *Glycobiology*. 17(4)411. 2007.

²Wada et al., *Mol Cell Proteomics*. 9(4)719. 2010.

³Ito et al., *Glycoconj J*. 33(3)405, 2016.

⁴Kawahara et al., *Nat Methods*. 18, 1304, 2021.

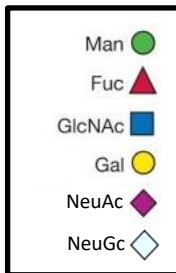
Glycopeptide identification is challenging!



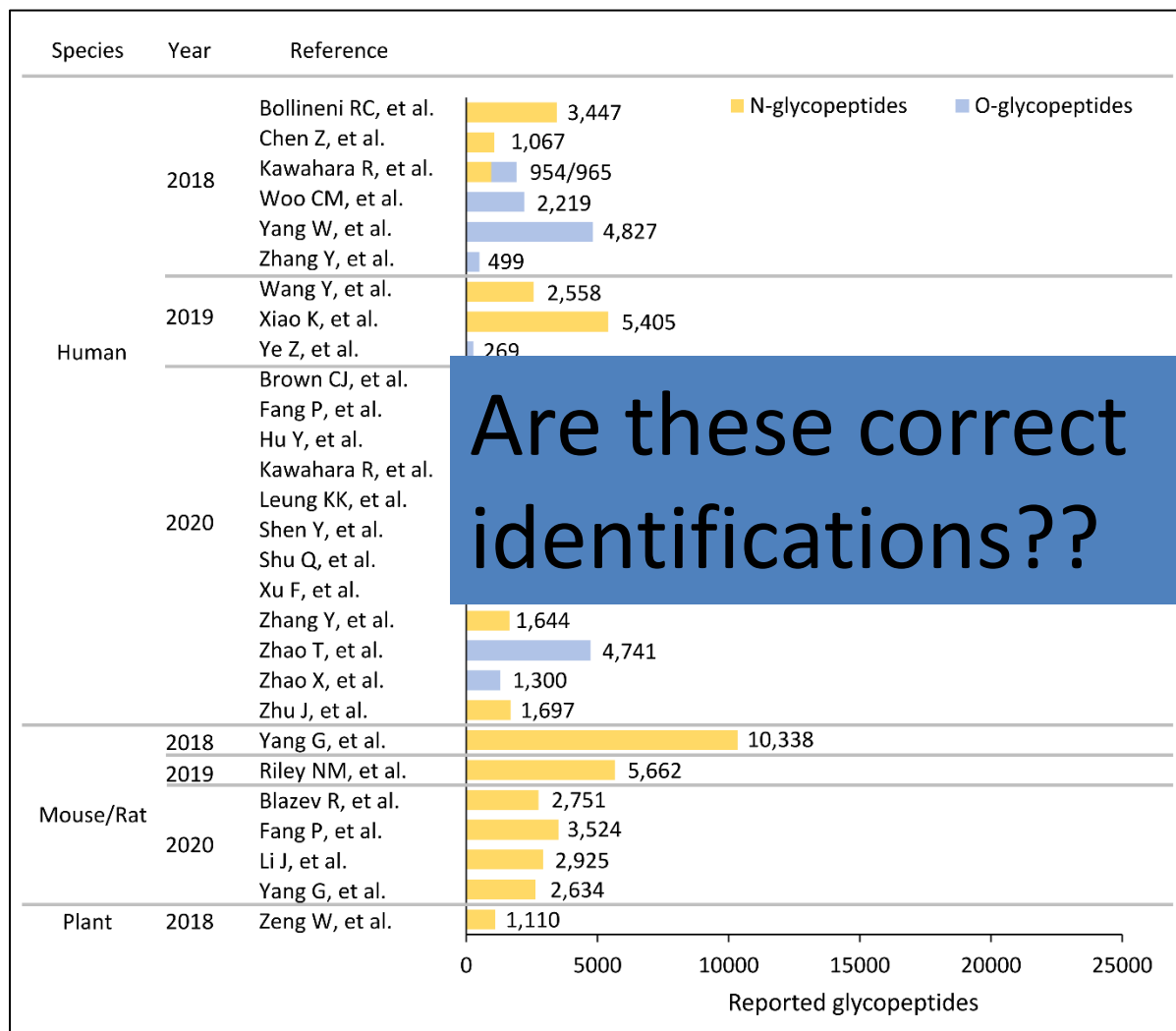
- Peptide identity
- Site localization
- Monosaccharide composition
- Glycan PTM modification(s)
- Other peptide modification(s)

Each level can be correctly or incorrectly identified

➔ Goal: Holistic FDR-based identification



The rise of glycoproteomics...



Are these correct identifications??

Biochemical Society Transactions (2021)
<https://doi.org/10.1042/BST20200222>

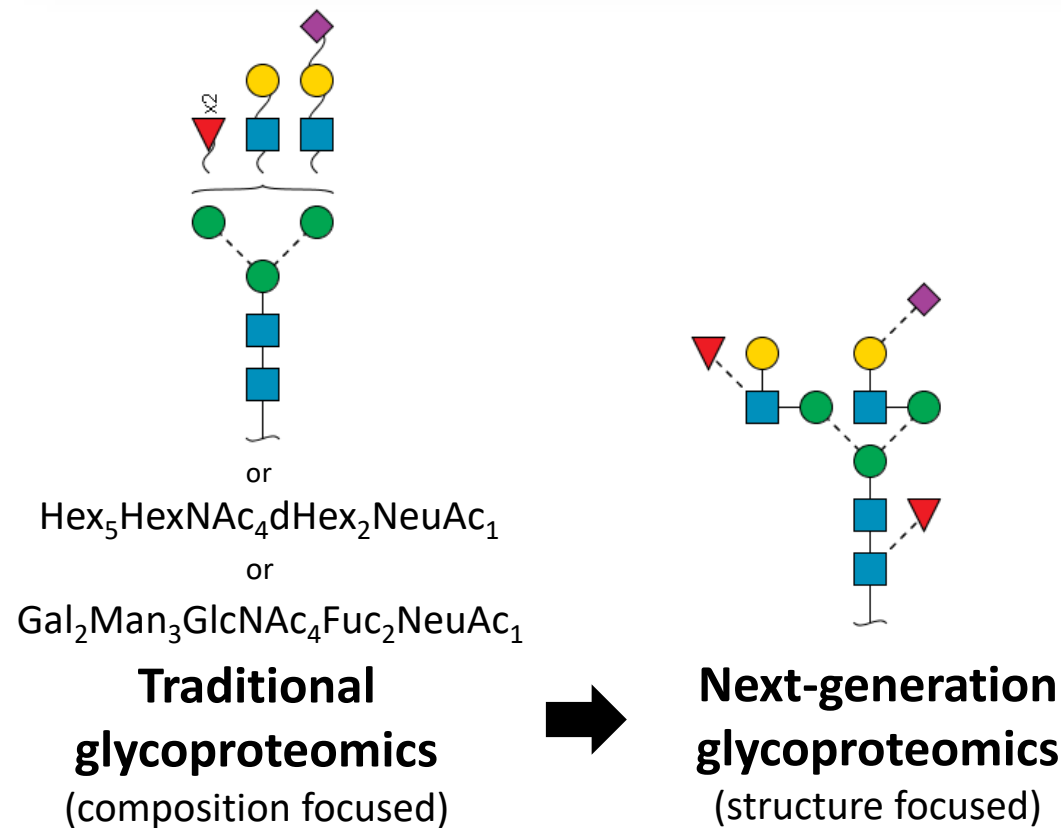


Review Article

Towards structure-focused glycoproteomics

Anastasia Chernykh¹, Rebeca Kawahara¹ and Morten Thaysen-Andersen^{1,2}

¹Department of Molecular Sciences, Macquarie University, Sydney, NSW, Australia; ²Biomolecular Discovery Research Centre, Macquarie University, Sydney, NSW, Australia

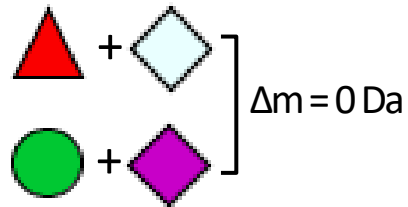


Thaysen-Andersen, Packer, Schulz. *Mol Cell Proteomics*. 15(6):1773-90. 2016.

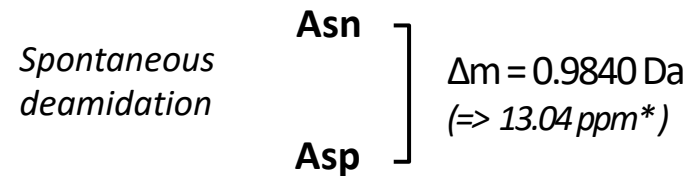
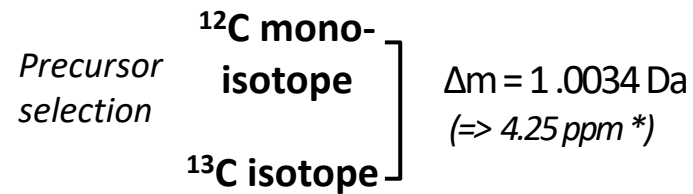
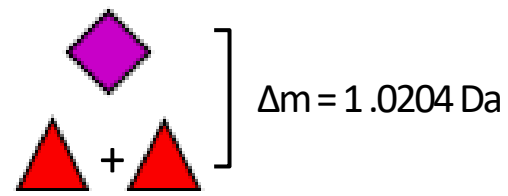
Chernykh, Kawahara, Thaysen-Andersen. *Biochem Soc Trans*. 49(1):161-186. 2021.

Difficult-to-identify glycopeptide features....

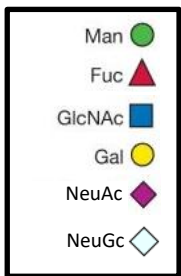
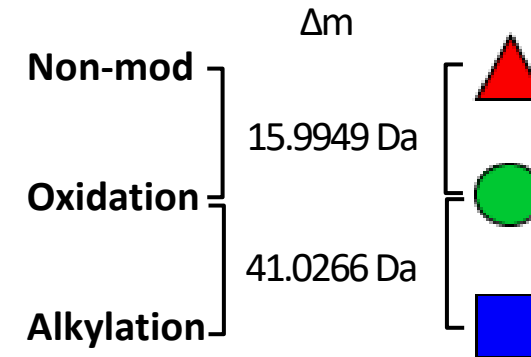
Isobaric monosaccharide combinations



Near-isobaric monosaccharide combinations



Ambiguous methionine modifications



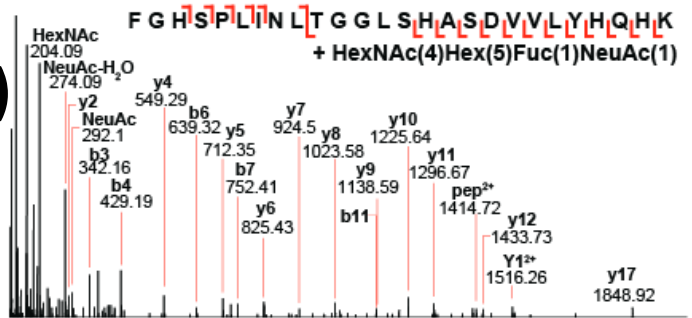
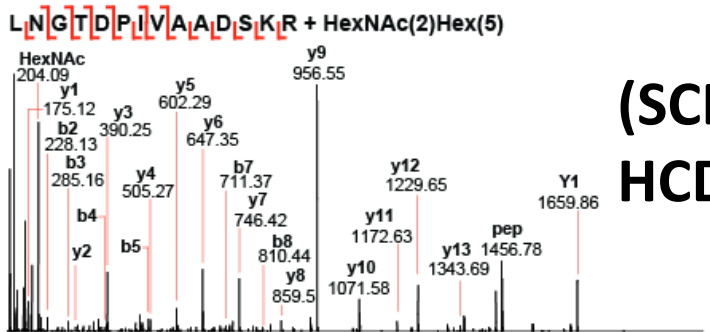
Modified from:

- Lee et al. *J Proteome Res.* 15(10):3904-3915. **2016**.
- Darula et al., *Anal Chem.* 87(12):6297-302. **2015**.

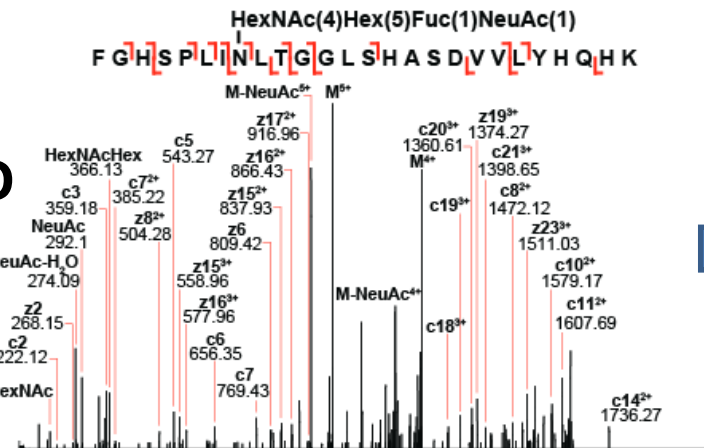
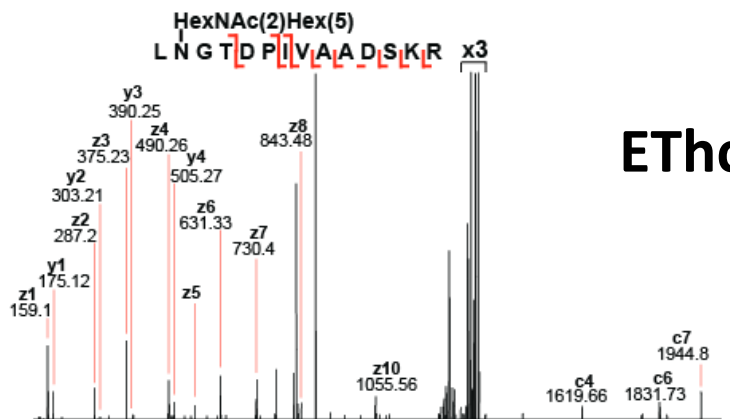
* Calculated for a standard size tryptic N-glycopeptide of 4 kDa

Fragmentation modes for intact glycopeptides

Peptide identification

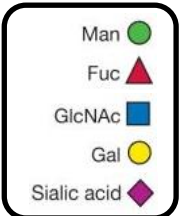
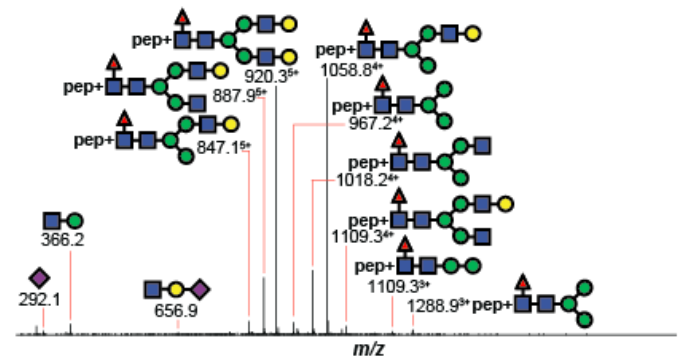
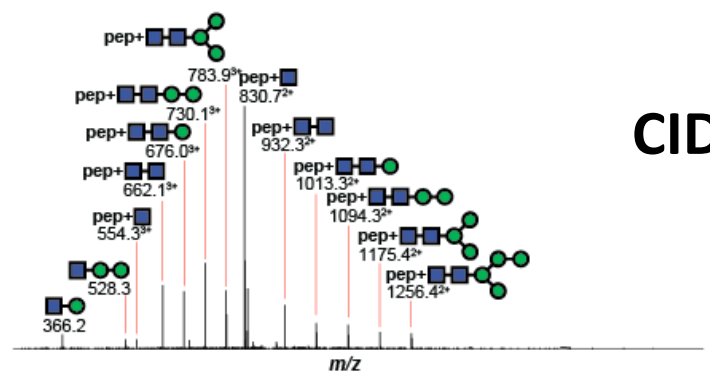


Site identification



10,000s-100,000s glycopeptide MS/MS per run

Glycan identification



Parker, Thaysen-Andersen, Fazakerley, Holliday, Packer, James. Mol Cell Proteomics. 15(1):141-53. 2016.

Informatics developments in glycoproteomics

How do these software compare??

Latest developments and applications of the key components in N-glycoproteomics

Component of glycoproteomics workflow	References
Simple preparation of N-glycopeptides	
Enrichment	
HILIC SPE	(21, 89, 94, 95, 105-107, 110, 135, 159, 161, 162, 165, 181-184)
Synthetic polymer mixed-mode HILIC SPE	(98, 99, 101, 102, 108)
Lectin SPE/chromatography	(7, 9, 13, 40, 60, 88, 104, 107, 135, 138, 140, 165, 182)
Hydrazide chemistry*	(48, 52, 140, 185)
Boronic acid magnetic beads	(80-82)
Acetone precipitation	(86, 103)
Porous graphitized carbon (PGC)-RP SPE	(89, 138)
Size exclusion chromatography	(87, 88)
Metabolic labelling and enrichment	(84, 85)
Neutral/high pH RP-LC	(7, 13, 46, 105, 106, 108, 138, 152)
2D (SCX/RP) -LC (online)	(159)
2D (HILIC/RP) -LC (online)	(186)
2D (PGC/RP) -LC (online)	(135)
	(110)
	(138)
Separation/fractionation	
	(105, 107, 108, 110, 159, 161, 162, 165, 26, 131, 138, 157, 26, 157, 159, 187)
	(94, 103, 106-108, 152, 183, 184, 186)
Identification strategies	
Protein Prospector	(21, 46, 100, 101, 102, 103, 104, 105, 150)
Byonic	(46, 108, 152, 162)
GPQuest	(87, 88, 104)
GlycopeptideID	(126, 154, 155)
Sweet-Heart	(157, 165)
GlycoFragWork	(40, 138)
GlycoMod	(158-161)
Less used programs (i. e. GlycoMaster DB, GRIP, MAGIC, GlycoPep Evaluator)	(86, 94, 103, 107, 134, 138, 162, 184, 186)
Other (e.g. in-house program/script, manual annotation etc)	(21, 88, 105-108, 162)
N-Glycome-assisted identification	(21, 46, 88, 94, 103, 105-108, 135, 159, 162)
Parallel proteome- and/or	

Advances in glycoproteomics software:

- **IQ-GPA** (Park et al., 2016)
- **pGlyco** (Zeng et al., 2016)
- **Sweet-NET** (Nasir et al., 2016)
- **Byonic** (Bern et al., 2012)
- **Protein Prospector** (Chalkley et al., 2008)
- **GPQuest** (Toghi Eshghi et al., 2015)
- **GlycopeptideID** (Joenväärä et al., 2013)
- **SweetHeart** (Wu et al., 2013)
- **GlycoFragWork** (Mayampurath et al., 2014)
- **GlycoMaster DB** (He et al., 2014)
- **GP Finder** (Strum et al., 2013)
- **MAGIC** (Lynn et al., 2015)
- **OMSSA** (Geer et al., 2004)
- **MS Amanda** (Dorfer et al., 2014)
- **Glycopep Evaluator** (Zhu et al., 2014)
- etc.....

Recent tools (2019-21):
MSFragger-glyco, pGlyco3,
O-Pair Search, StrucGP...

Recent glyco-informatic reviews:

Hu H, Khatri K, Zaia J. *Mass Spectrom Rev.* 36(4):475-498. 2017.
Abrahams J, Taherzadeh G, Jarvas G, Guttman A, Zhou Y, Campbell MP. *Curr Opin Struct Biol.* 62:56-69. 2020.



nature | methods

ANALYSIS

<https://doi.org/10.1038/s41592-021-01309-x>

Check for updates

OPEN

Community evaluation of glycoproteomics informatics solutions reveals high-performance search strategies for serum glycopeptide analysis

Rebeca Kawahara¹, Anastasia Chernykh¹, Kathirvel Alagesan², Marshall Bern³, Weiqian Cao⁴, Robert J. Chalkley⁵, Kai Cheng⁶, Matthew S. Choo⁷, Nathan Edwards^{8,9}, Radoslav Goldman^{8,9,10}, Marcus Hoffmann¹¹, Yingwei Hu¹², Yifan Huang¹³, Jin Young Kim¹⁴, Doron Kletter³, Benoit Liquet^{15,16}, Mingqi Liu⁴, Yehia Mechref¹³, Bo Meng¹⁷, Sriram Neelamegham⁶, Terry Nguyen-Khuong⁷, Jonas Nilsson¹⁸, Adam Pap^{19,20}, Gun Wook Park¹⁴, Benjamin L. Parker²¹, Cassandra L. Pegg²², Josef M. Penninger^{23,24}, Toan K. Phung²², Markus Pioch¹¹, Erdmann Rapp^{11,25}, Enes Sakalli²³, Miloslav Sanda^{8,10}, Benjamin L. Schulz²², Nichollas E. Scott²⁶, Georgy Sofronov¹⁵, Johannes Stadlmann²³, Sergey Y. Vakhrushev²⁷, Christina M. Woo²⁸, Hung-Yi Wu²⁸, Pengyuan Yang⁴, Wantao Ying¹⁷, Hui Zhang¹², Yong Zhang¹⁷, Jingfu Zhao¹³, Joseph Zaia²⁹, Stuart M. Haslam³⁰, Giuseppe Palmisano³¹, Jong Shin Yoo^{14,32}, Göran Larson³³, Kai-Hooi Khoo³⁴, Katalin F. Medzihradzsky^{5,19}, Daniel Kolarich², Nicolle H. Packer^{1,2,35} and Morten Thaysen-Andersen^{1,35} ✉

The 1st HGI Study (2017-2021)

(Continuation of former HGPI headed by Prof Taniguchi and Prof Narimatsu)



(2004-2016)

Objective

Evaluate the performance of the current glycoproteomics software and strategies for glycopeptide identification from LC-MS/MS data

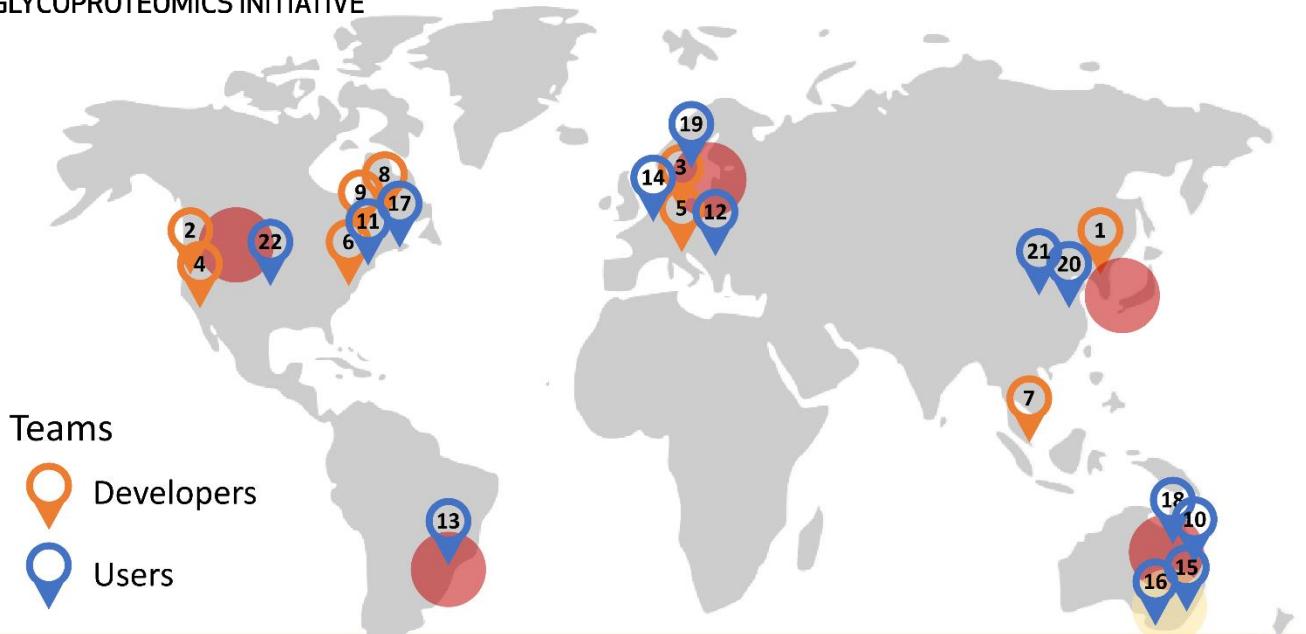
1st HGI Study Committee:

Morten Thaysen-Andersen, Nicolle H. Packer, Daniel Kolarich, Kay-Hooi Khoo, Katalin Medzihradzsky, Joe Zaia, Goran Larson, Stuart Haslam, Giuseppe Palmisano, Jong Shin Yoo



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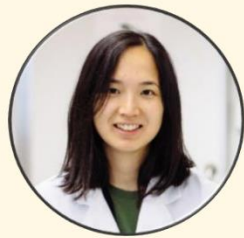
The 1st HGI Study



Objective

Evaluate the performance of the current glycoproteomics software and strategies for glycopeptide identification from LC-MS/MS data

Early Career Researchers



Dr Rebeca Kawahara (first author)



Mrs Anastasia Chernykh (second author)

HGI Leadership



Dr Morten Thaysen-Andersen (HGI Director)



A/Prof. Daniel Kolarich (HGI Deputy-Directors)



Prof. Nicolle H. Packer (HGI Deputy-Directors)

1st HGI Study Committee:

Morten Thaysen-Andersen, Nicolle H. Packer, Daniel Kolarich, Kay-Hooi Khoo, Katalin Medzihradzky, Joe Zaia, Goran Larson, Stuart Haslam, Giuseppe Palmisano, Jong Shin Yoo

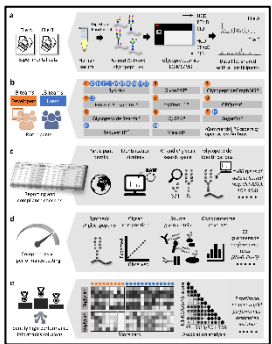
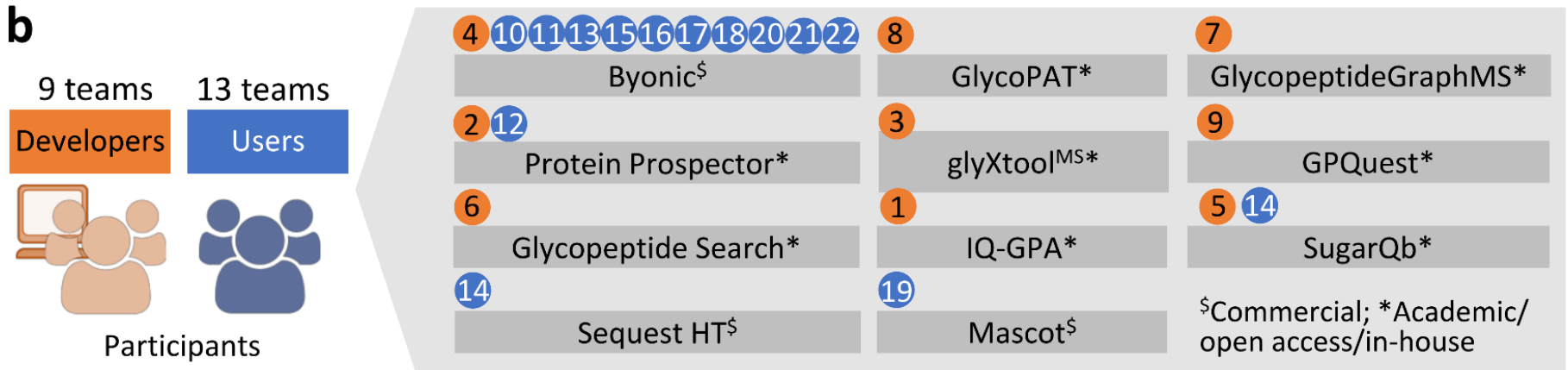
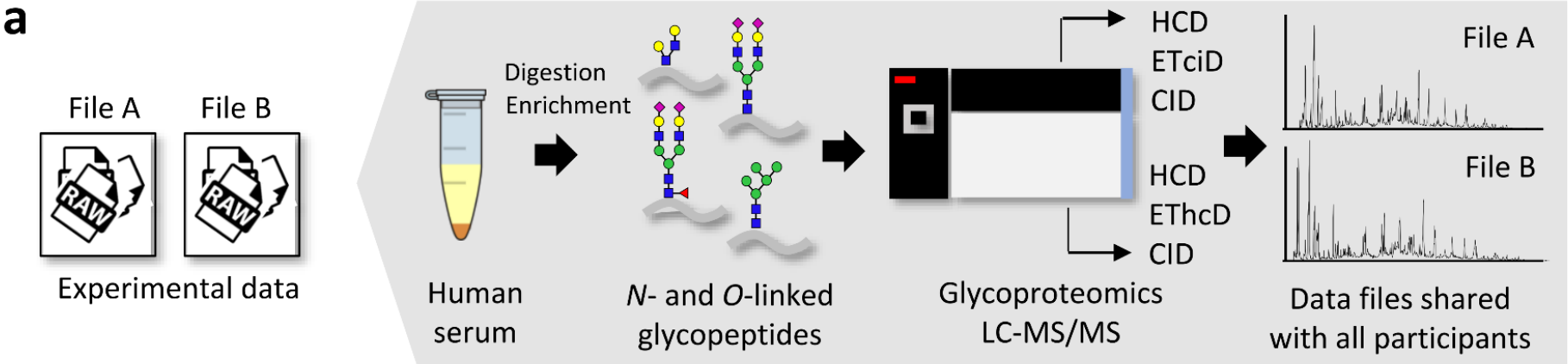


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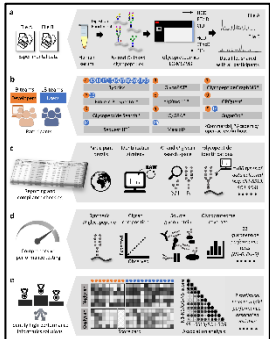
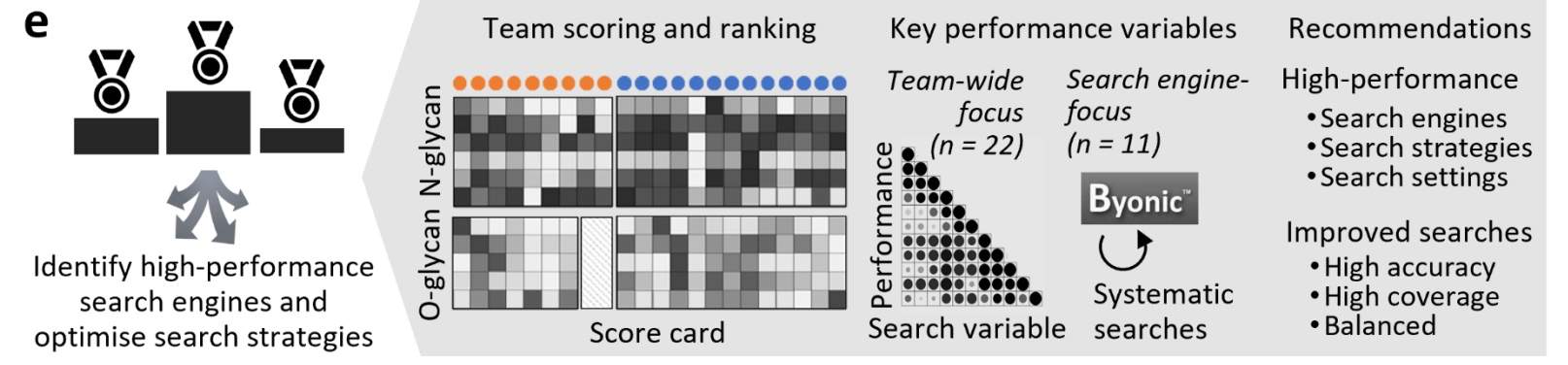
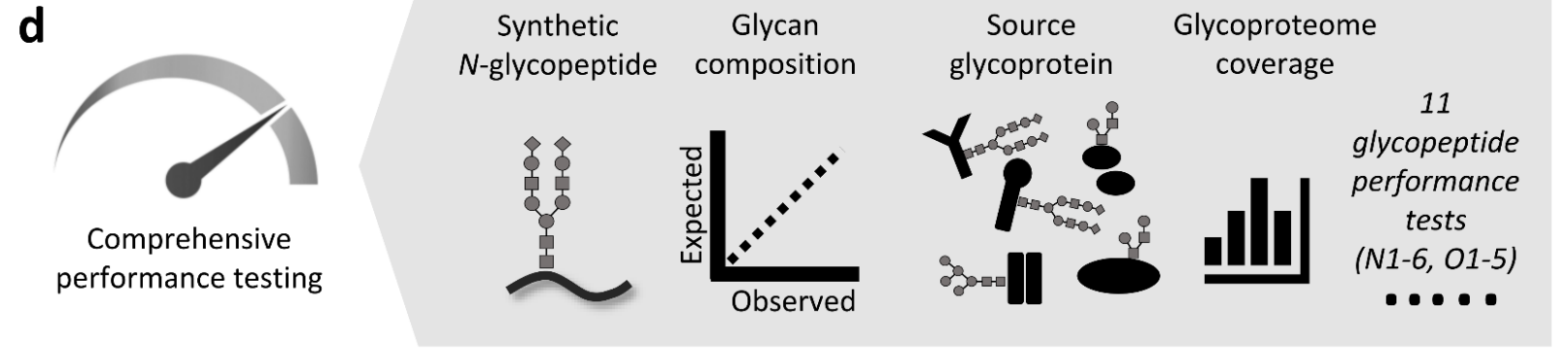
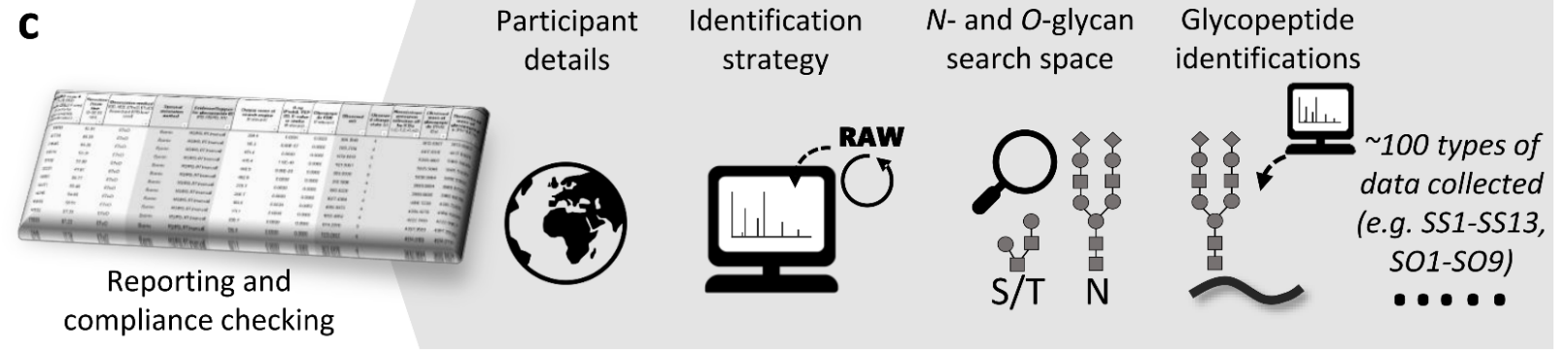
Approach





HUMAN GLYCOPROTEOMICS INITIATIVE

Approach

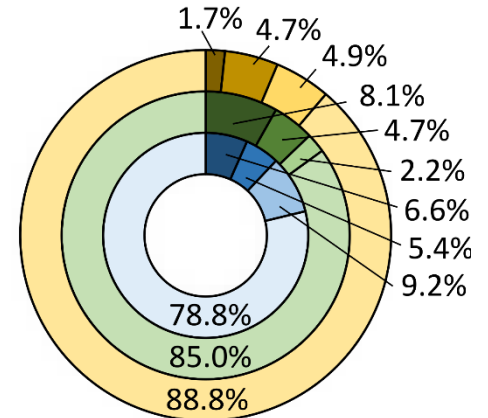
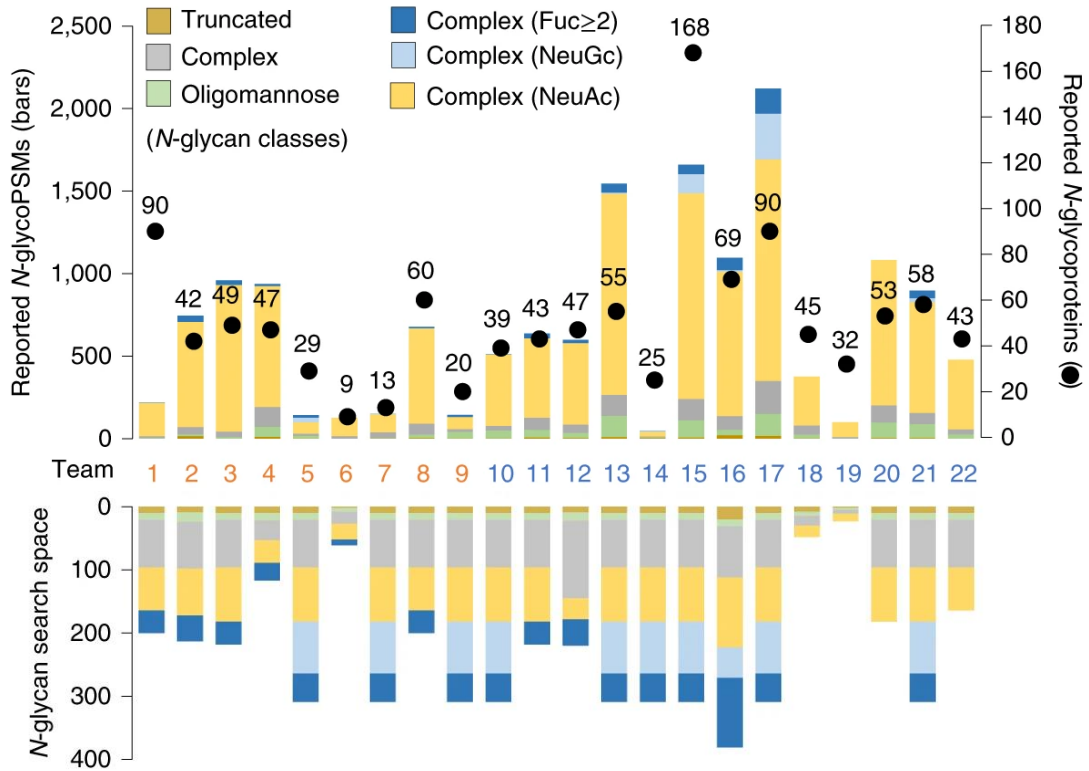




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Dramatic variation in the glycopeptide identification

Identified glycopeptides / glycoproteins



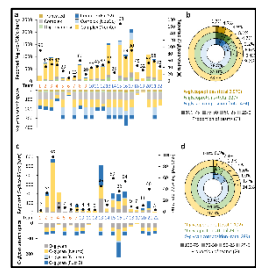
N-glycopeptides (total 2,576)
 N-glycoproteins (total 320)
 N-glycan composition (total 424)

■ 100-75 ■ 75-50 ■ 50-25 ■ 25-0
 Proportion of teams (%)

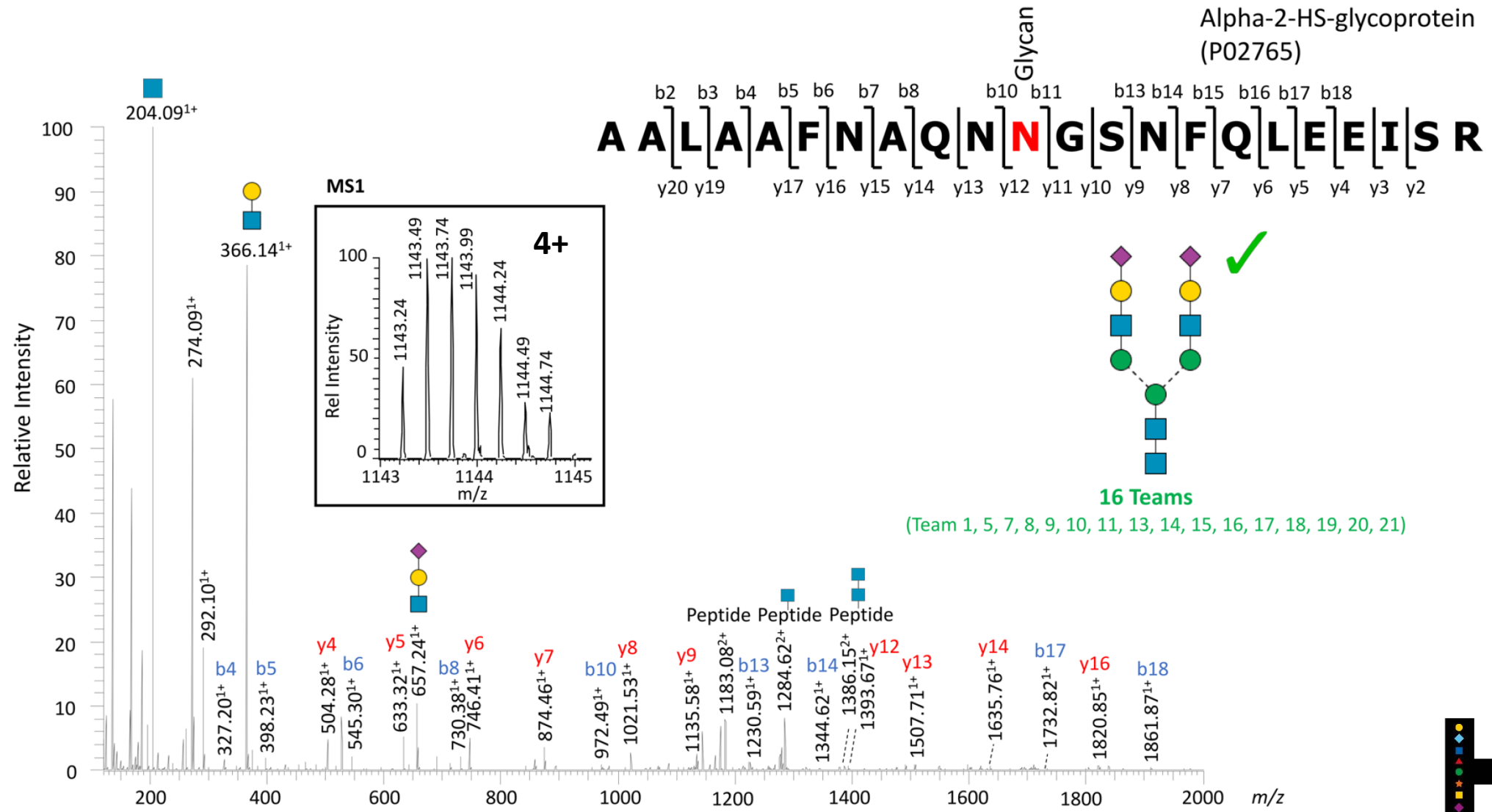


163 consensus N-glycopeptides
 (>50% of teams)
 (GlyConnect ID 2943)

Glycan search space

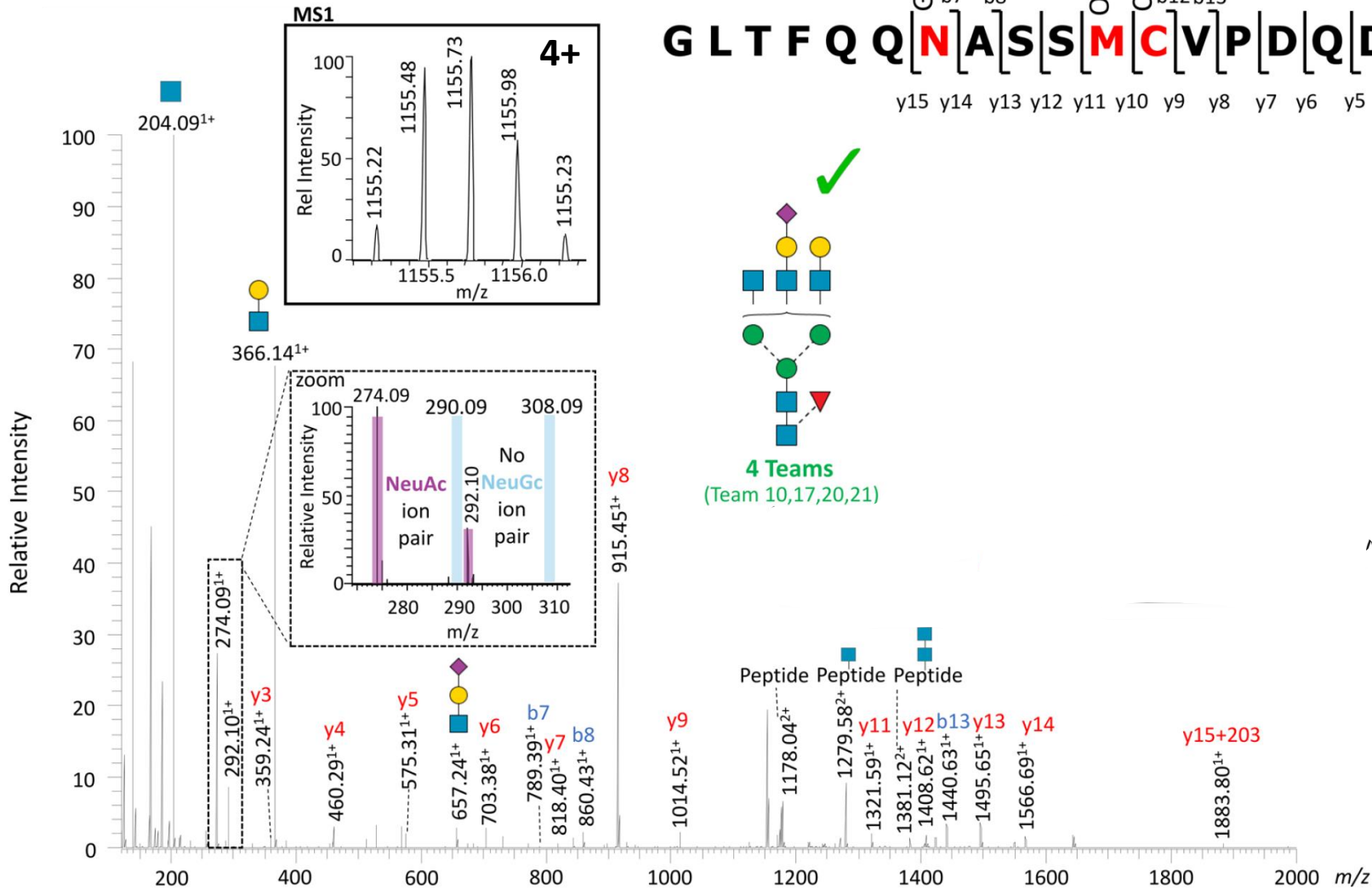


Example of an “easy” HCD-MS/MS spectrum (consensus N-glycopeptide)



Example of a challenging HCD-MS/MS spectrum

Immunoglobulin heavy
constant mu (P01871)

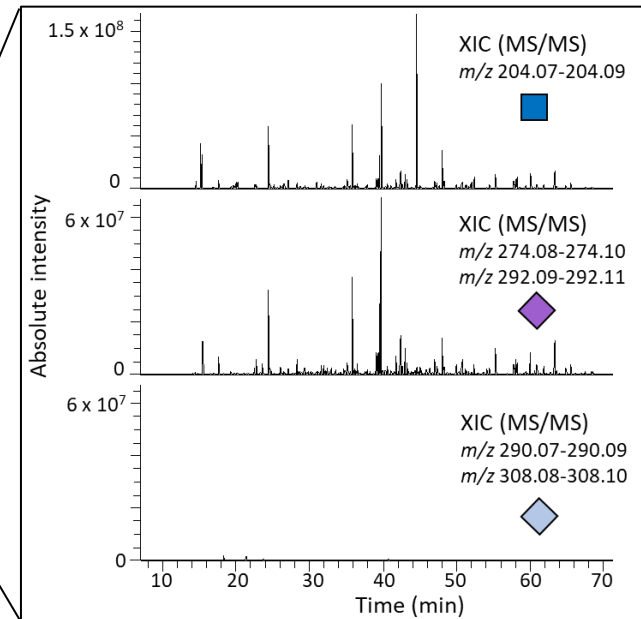
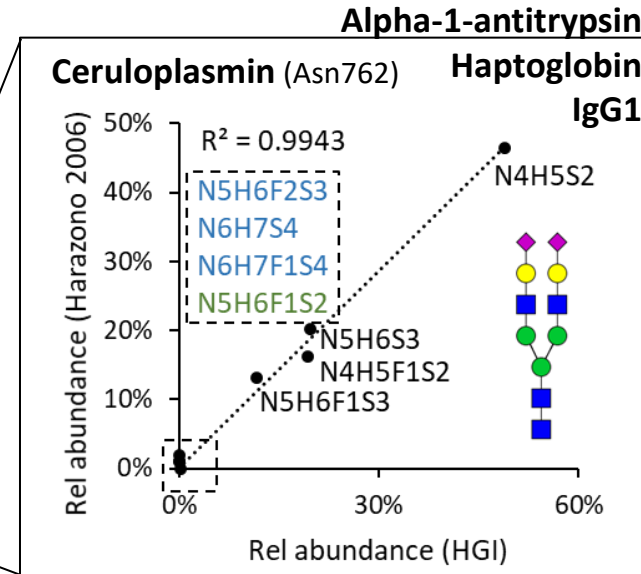


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How was performance assessed?

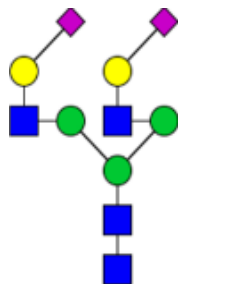
N-glycopeptide data

Performance tests		Description of scoring
N1	Synthetic N-glycopeptide	Accuracy (specificity, %) and coverage (sensitivity, %) of the identification of the synthetic glycopeptide
N2	N-glycan composition*	Match of expected (Clerc et al., 2016 ²⁸) and reported glycan distribution in serum
N3	Source N-glycoprotein*	Match of reported source glycoproteins to expected serum glycoproteins (Clerc et al., 2016 ²⁸ , Sun et al., 2018 ⁴⁴)
N4	N-glycoproteome coverage	Number of unique glycopeptides
N5	Commonly reported 'consensus' N-glycopeptides	Proportion of reported glycopeptides out of the consensus glycopeptides reported by most teams
N6	NeuGc and multi-Fuc N-glycopeptides	Reported non-NeuGc and non-Fuc ≥ 2 containing glyco-PSMs out of total PSMs



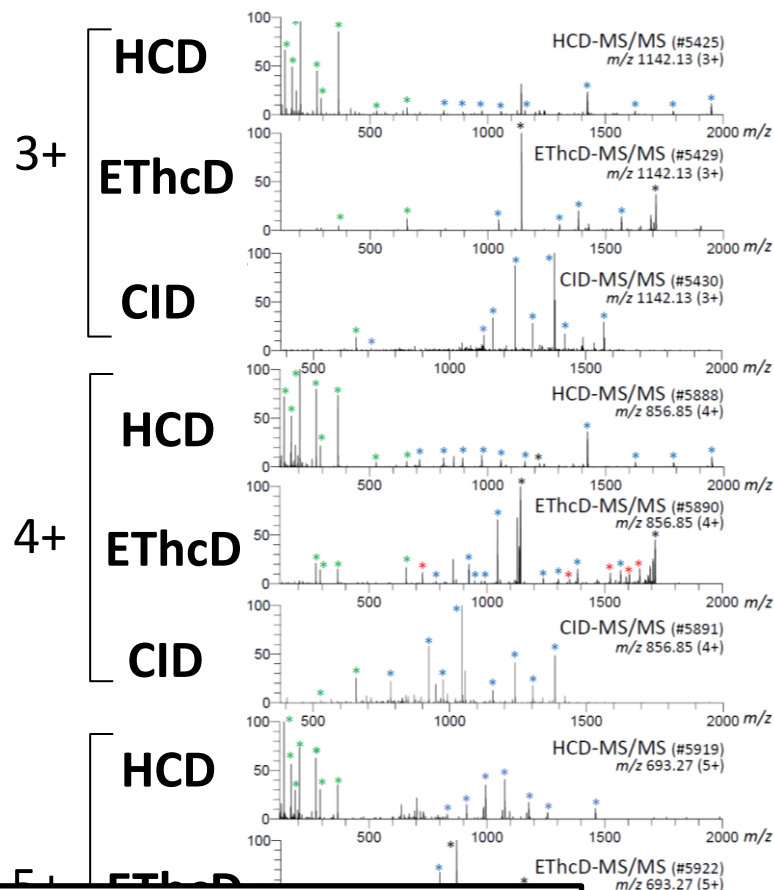


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EVFVHPNYSK
(P04070)

- * Oxonium ions and B-ions
- * Y-ions (peptide + glycan fragment)
- * Peptide backbone fragments (b-/y-/c-/z-ions)
- * Unfragmented precursor



Synthetic N-glycopeptide (performance test N1)

Team																					
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22

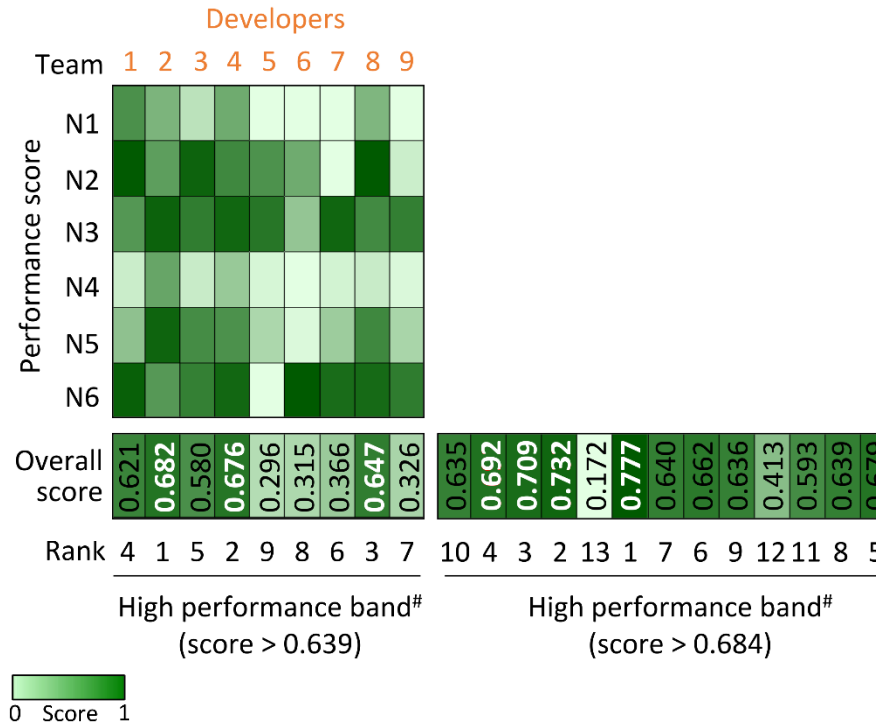
Team	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Sensitivity (%)	50	67	13	38	0	0	0	33	0	25	29	33	33	0	75	27	18	38	0	25	17	40
Specificity (%)	100	50	100	100	0	0	0	100	0	100	100	100	100	0	100	100	100	100	0	100	100	100
Score (0-100)	50	34	13	38	0	0	0	33	0	25	29	33	33	0	75	27	18	38	0	25	17	40

- Correct identification
- Incorrect identification
- MS/MS type not included in search
- Not reported by team



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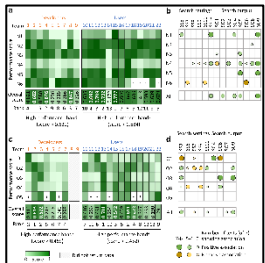
N-glycoproteomics performance



Top performers:

1. Protein Prospector (Team 2)
2. Byonic™ (Team 4)
3. GlycoPAT (Team 8)

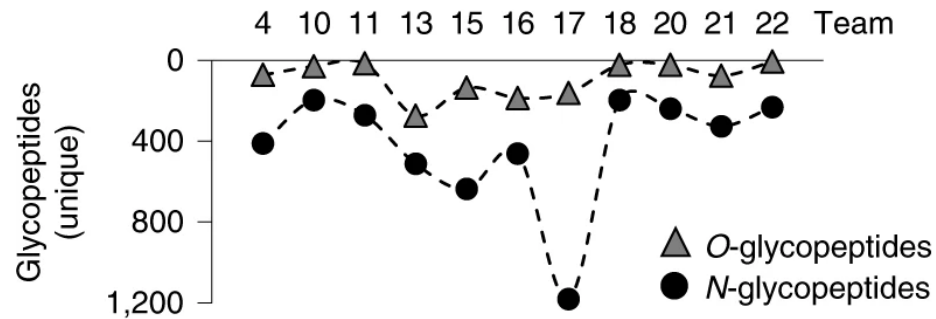
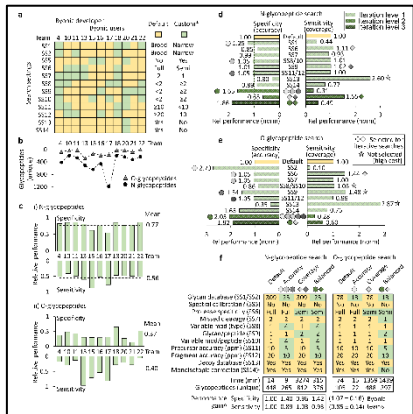
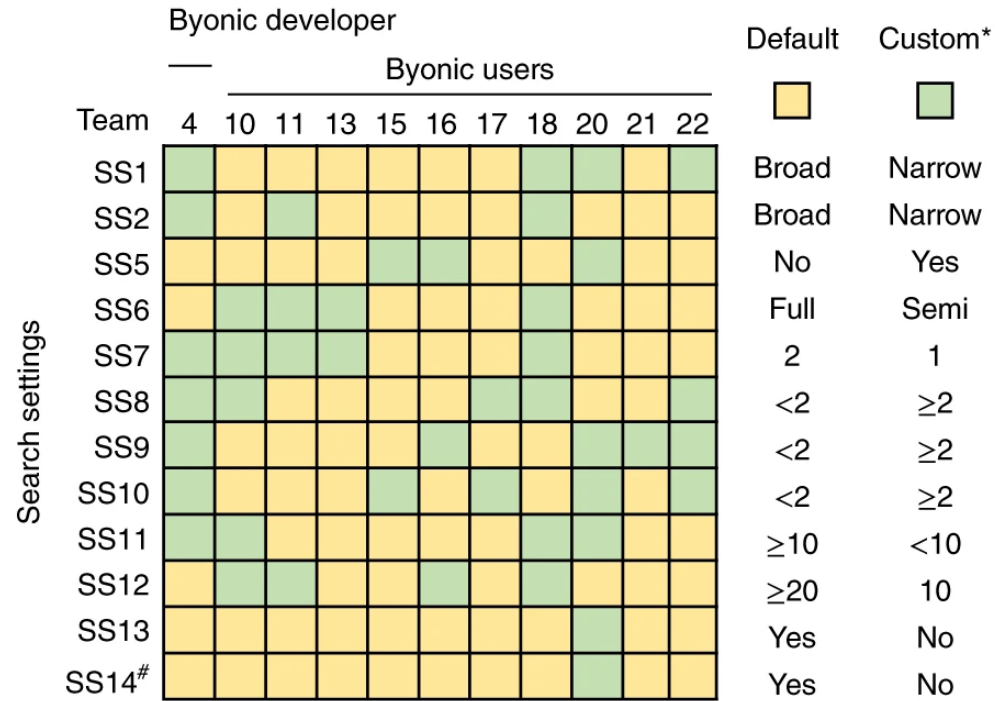
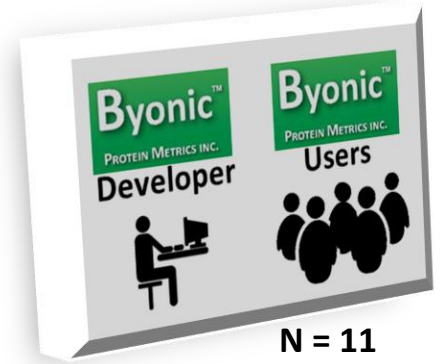
Performance tasks:
 N1. Synthetic N-glycopeptide
 N2. N-glycan composition
 N3. N-glycoprotein identity
 N4. N-glycopeptide coverage
 N5. Common "consensus" N-glycopeptides
 N6. NeuGc and multi-Fuc N-glycopeptides



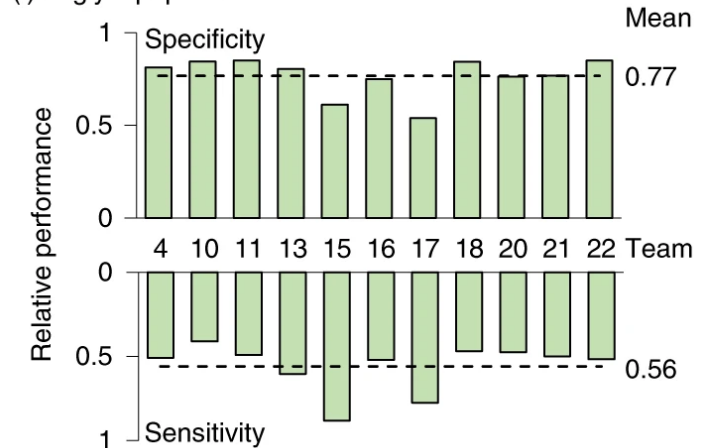


HUMAN GLYCOPROTEOMICS INITIATIVE

Search engine-centric analysis



(i) N-glycopeptides



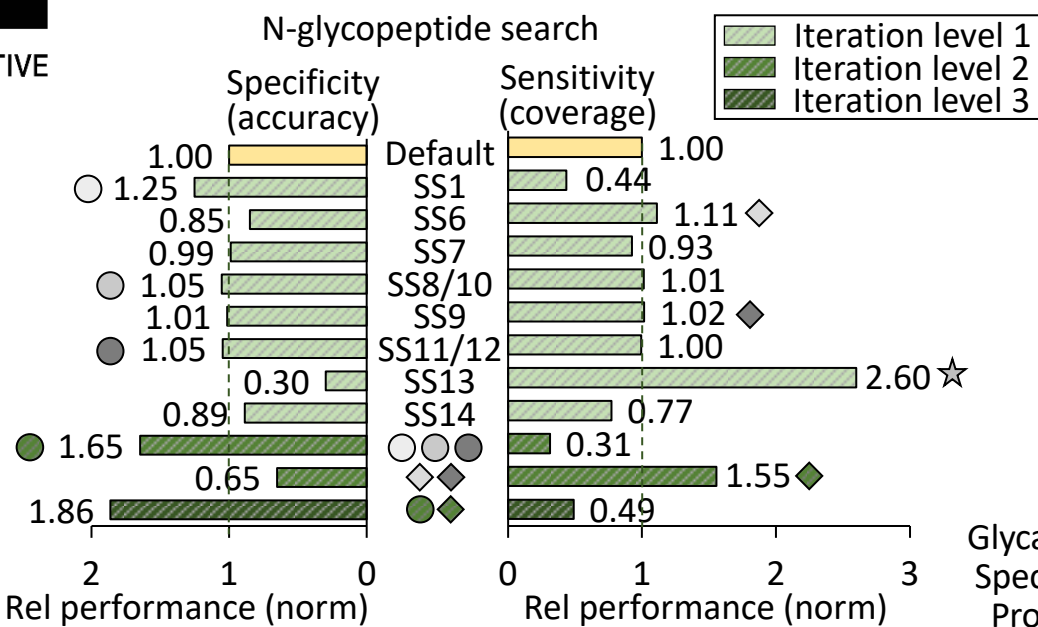


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Byonic-centric analysis – improving performance



Evaluating the impact of each search setting on performance



◇ Selected for iterative searches
★ Not selected (high cost)

N-glycopeptide search

Default Accuracy Coverage Balanced

- Glycan database (SS1/SS2)
- Spectral calibration? (SS5)
- Protease specificity (SS6)
- Missed cleavage (SS7)
- Variable mod (type) (SS8)
- Glycan/peptide (SS9)
- Variable mod/peptide (SS10)
- Precursor accuracy (ppm) (SS11)
- Fragment accuracy (ppm) (SS12)
- Decoy database (SS13)
- Monoisotopic correction (SS14)#

	Default	Accuracy	Coverage	Balanced
Glycan database (SS1/SS2)	309	25	309	25
Spectral calibration? (SS5)	No	No	No	No
Protease specificity (SS6)	Full	Full	Semi	Semi
Missed cleavage (SS7)	2	2	2	2
Variable mod (type) (SS8)	1	4	1	4
Glycan/peptide (SS9)	1	1	2	2
Variable mod/peptide (SS10)	1	4	1	4
Precursor accuracy (ppm) (SS11)	10	5	10	5
Fragment accuracy (ppm) (SS12)	20	10	20	10
Decoy database (SS13)	Yes	Yes	Yes	Yes
Monoisotopic correction (SS14)#	Yes	Yes	Yes	Yes

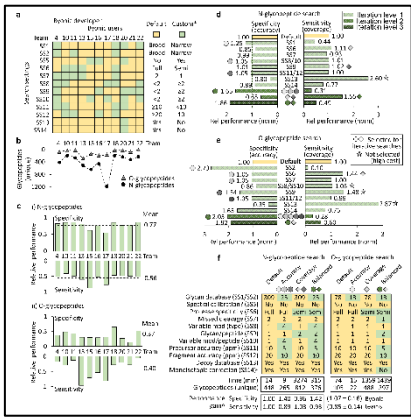
Recommended settings for improved glycoproteomics data analysis



Time (min)
Glycopeptides (unique)
Performance gain^
Specificity
Sensitivity

Time (min)	14	9	3274	315
Glycopeptides (unique)	448	265	812	376
Specificity	1.00	1.40	0.96	1.42
Sensitivity	1.00	0.89	1.03	0.93

(1.07 ± 0.16) Byonic
(0.85 ± 0.14) teams





HUMAN GLYCOPROTEOMICS INITIATIVE

Key conclusions from 1st HGI study

- Diverse software exist for comprehensive glycopeptide data analysis
- Dramatic variance of glycopeptides identified across teams (high FDRs)
- Systematic and fair evaluation of relative software and team performance:
 - High-performance software (Protein Prospector, Byonic, IQ-GPA, GlycoPAT, glyXtool^{MS})
 - High-performance search strategies identified => improved search strategies recommended
- All team reports and data publicly available to encourage re-interrogation
- Follow-up study to test latest software (MSFragger-glyco, O-Pair Search, pGlyco3, StrucGP...)

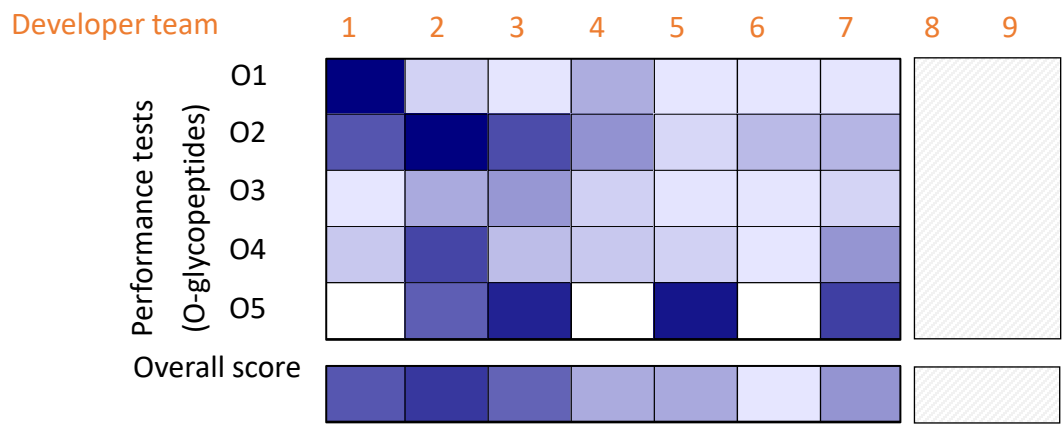
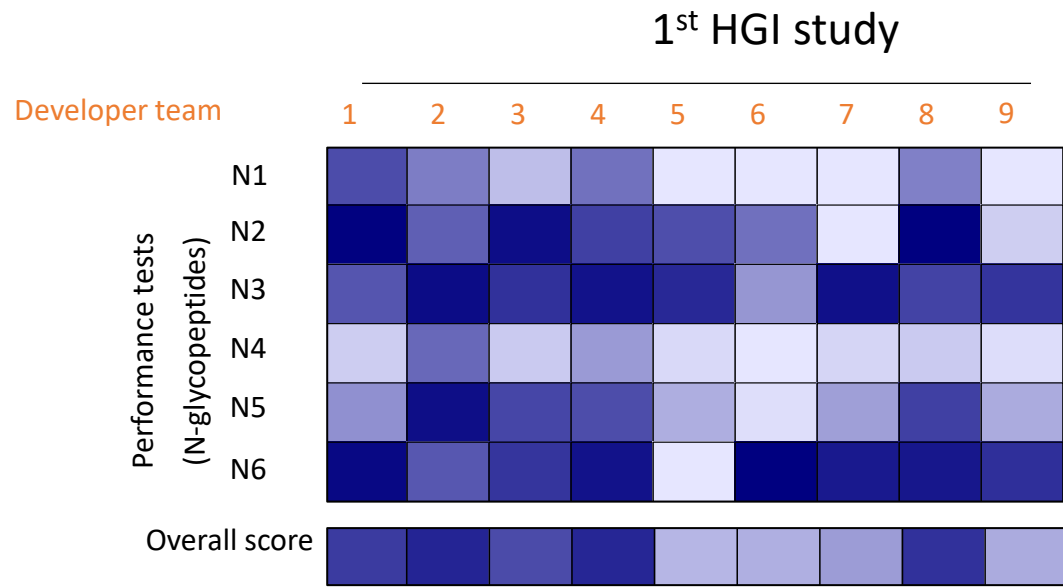
“Want to do glycoproteomics well?”

Use a powerful search engine, tailored search settings and post-search filtering criteria, and know your glycobiology!”

Benjamin Schulz, UQ

- Kawahara et al., Nat Methods, 2021 Community evaluation of glycoproteomics informatics solutions reveals high-performance search strategies for serum glycopeptide analysis. Nat Methods. 2021.
- Chau, Chernykh, Ugonotti, Parker, Kawahara, Thaysen-Andersen, Methods in Mol Biology, 2022 Glycomics-assisted Glycoproteomics Enables Deep and Unbiased N-Glycoproteome Profiling of Complex Biological Specimens

Post-study benchmarking of recent glycopeptide software following 1st HGI study



Post-study benchmarking →

Open for benchmarking of new glycoproteomics software or updated versions of existing tools...

1st HGI developer software

- 1: IQ-GPA
- 2: Protein Prospector
- 3: glyXtool^{MS}
- 4: Byonic
- 5: SugarQb
- 6: Glycopeptide Search
- 7: GlycopeptideGraphMS
- 8: GlycoPAT
- 9: GPQuest

Post-study team software

- 23: MS-Fragger-Glyco

To make best use of methodology and outcomes of the 1st HGI study and to bridge to the 2nd HGI study, the Human Glycoproteomics Initiative are offering a post-study benchmarking opportunity for new and updated software against the performance of the nine developer teams that completed the 1st HGI study ([Kawahara et al., Nat Methods, 2021](#)).

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Study Participants:

Jong Shin Yoo, Jin Young Kim, Gun Wook Park, Robert J Chalkley, Erdmann Rapp, Markus Pioch, Marcus Hoffmann, Marshall Bern, Doron Kletter, Johannes Stadlmann, Josef M. Penninger, Enes Sakalli, Nathan Edwards, Terry Nguyen-Khuong, Matthew S. Choo, Sriram Neelamegham, Kai Cheng, Hui Zhang, Yingwei Hu, Daniel Kolarich, Kathirvel Alagesan, Miloslav Sanda, Radoslav Goldman, Katalin Medzihradsky, Adam Pap, Guiseppe Palmisano, Sergey Vakhrushev, Benjamin L. Parker, Nichollas Scott, Christina Woo, Hung-Yi Wu, Benjamin L. Schulz, Cassandra L. Pegg, Toan K. Phung, Jonas Nilsson, Göran Larson, Pengyuan Yang, Weiqian Cao, Wantao Ying, Zhang Yong, Meng Bo, Yehia Mechref, Jingfu Zhao, Yifan Huang

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Glycoinformatics:

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Data acquisition:

Rosa Viner, Sergei Snovida (Thermo Fisher Scientific)



Australian Government

Australian Research Council
(FT210100455)



MACQUARIE
University
SYDNEY · AUSTRALIA

HGI Community Wide Study 2



HUMAN GLYCOPROTEOMICS INITIATIVE

Organized under HUPO

**Second Phase
launched 11/2021**

organizers:



Dr. Stacy
Malaker

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Dr. Nick
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The major goal of this second study is to identify strengths and weaknesses of the very latest glycoproteomics software for glycopeptide **identification** and **quantitation**

The first study incorporated both users and developers, but this study is targeting only the **software developers** themselves.

This means that we are specifically recruiting those community members who actively develop glycoproteomic-capable software tools



HGI Study 2 Advisory Committee



Co-Chairs



Nicholas M. Riley, PhD
Stanford University, Palo Alto, CA, USA



Stacy Malaker, PhD
Yale University, New Haven, CT, USA



Devon Kohler
Northeastern University, Boston, USA



Frédérique Lisacek, PhD
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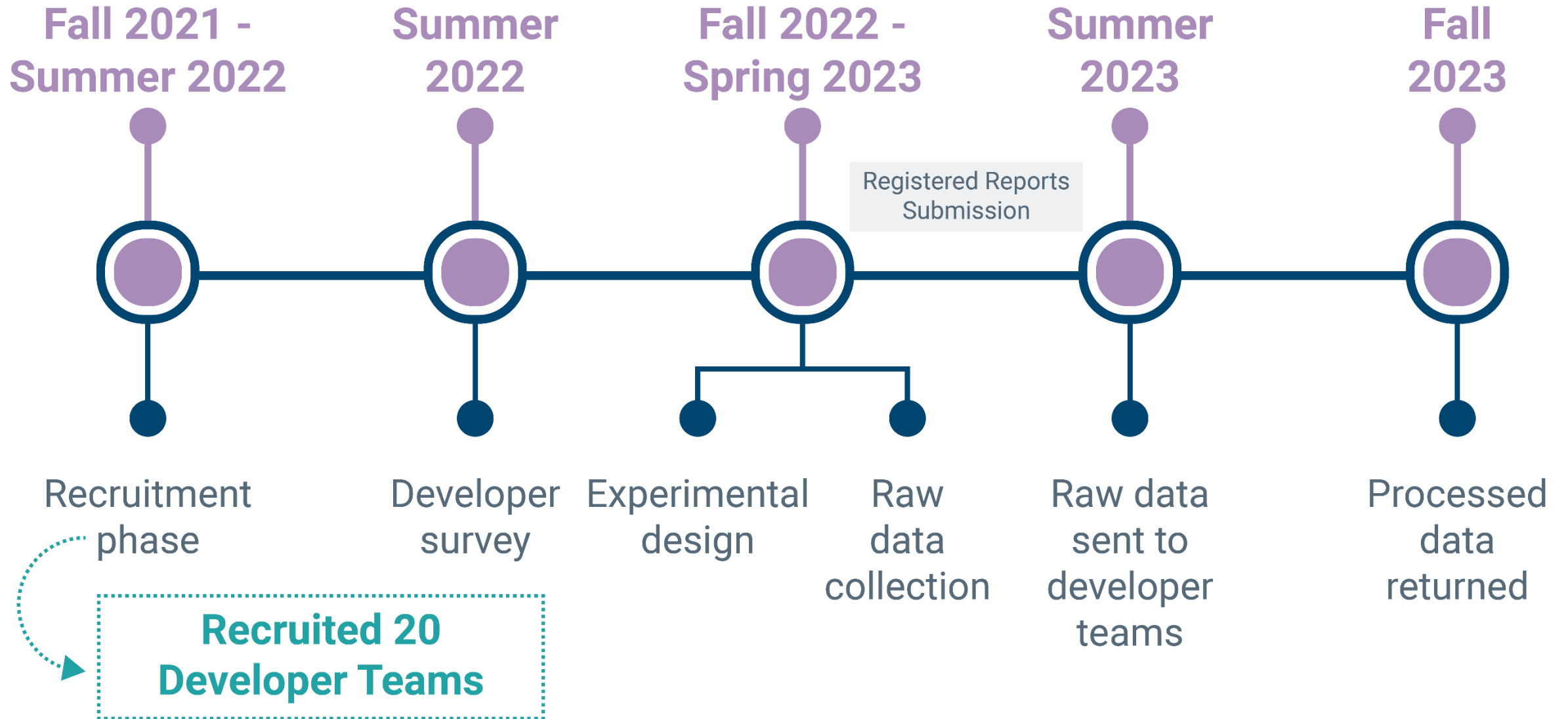
Kiyoko Aoki-Kinoshita, PhD
Soka University, Tokyo, Japan



Nichollas E. Scott, PhD
University of Melbourne, VIC, Australia



HGI Community Wide Study 2





HGI Community Wide Study 2

Module A

Module B

Module C

teams can choose which modules to participate in (one, two, or all three)
design based on input from developers of what data types their software could process
data collected on Orbitrap Tribrid

HGI Community Wide Study 2

Module A

N-glyco-focused
glycoprotein standards

3 raw files

Data Acquisition:

DDA,
steppedHCD

Quantitation:

LFQ (possible, not req'd)

Module B

Module C

teams can choose which modules to participate in (one, two, or all three)
design based on input from developers of what data types their software could process
data collected on Orbitrap Tribrid

HGI Community Wide Study 2

Module A

N-glyco-focused
glycoprotein standards

3 raw files

Data Acquisition:
DDA,
steppedHCD

Quantitation:
LFQ (possible, not req'd)

Module B

O-glyco-focused
glycoprotein standards

3 raw files

Data Acquisition:
DDA,
HCDpdETHcD

Quantitation:
LFQ (possible, not req'd)

Module C

teams can choose which modules to participate in (one, two, or all three)
design based on input from developers of what data types their software could process
data collected on Orbitrap Tribrid

HGI Community Wide Study 2

Module A

N-glyco-focused
glycoprotein standards

3 raw files

Data Acquisition:
DDA,
steppedHCD

Quantitation:
LFQ (possible, not req'd)

Module B

O-glyco-focused
glycoprotein standards

3 raw files

Data Acquisition:
DDA,
HCDpdETHcD

Quantitation:
LFQ (possible, not req'd)

Module C

N-glyco-focused
enriched lysate

6 raw files

Data Acquisition:
DDA, steppedHCD
and HCDpdETHcD

Quantitation:
LFQ (possible, not req'd)

teams can choose which modules to participate in (one, two, or all three)
design based on input from developers of what data types their software could process
data collected on Orbitrap Tribrid

Want to know more and contribute.....?



HUMAN GLYCOPROTEOMICS INITIATIVE

<https://www.hupo.org/Human-Glycoproteomics-Initiative>

**Second Phase
launched 11/2021**

organizers:



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**Dr. Nick
Riley**

nmriley@uw.edu

2nd HGI Study: Stacy Malaker & Nick Riley (launched, early phase)

3rd HGI Study: Daniel Kolarich (scheduled, stay tuned)



The Human Glycoproteomics Initiative Workshop on Glycoproteomics and Glycoinformatics

US HUPO March 7, 2023

Nicholas M. Riley, Ph.D.

Community Evaluation of Glycoproteomics Software
and HGI Update

Carlito B. Lebrilla, Ph.D.

Translating glycomic and glycoproteomic discoveries to
products for human health

Peggi M. Angel, Ph.D.

Glycomics Imaging for Analysis of Tissues, Cells, Biofluids,
and Target Glycoproteins

Yixuan (Axe) Xie, Ph.D.

GlycanDIA Empowers Deep Profiling in Glycomic Analysis

