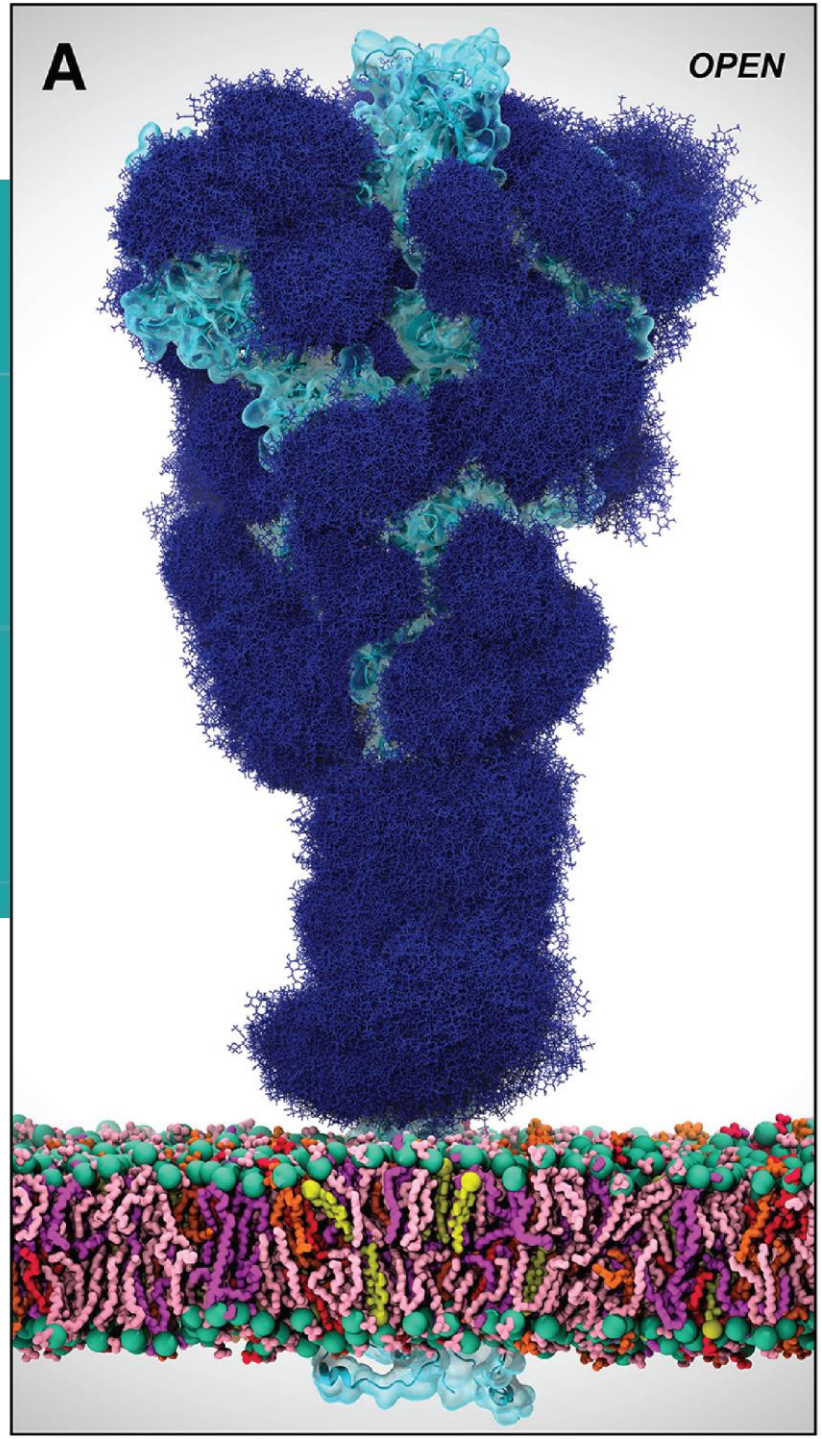
Reasons to be excited about current efforts in glycoproteomics

Nicholas M. Riley

SUMS Seminar Series, October 1, 2020

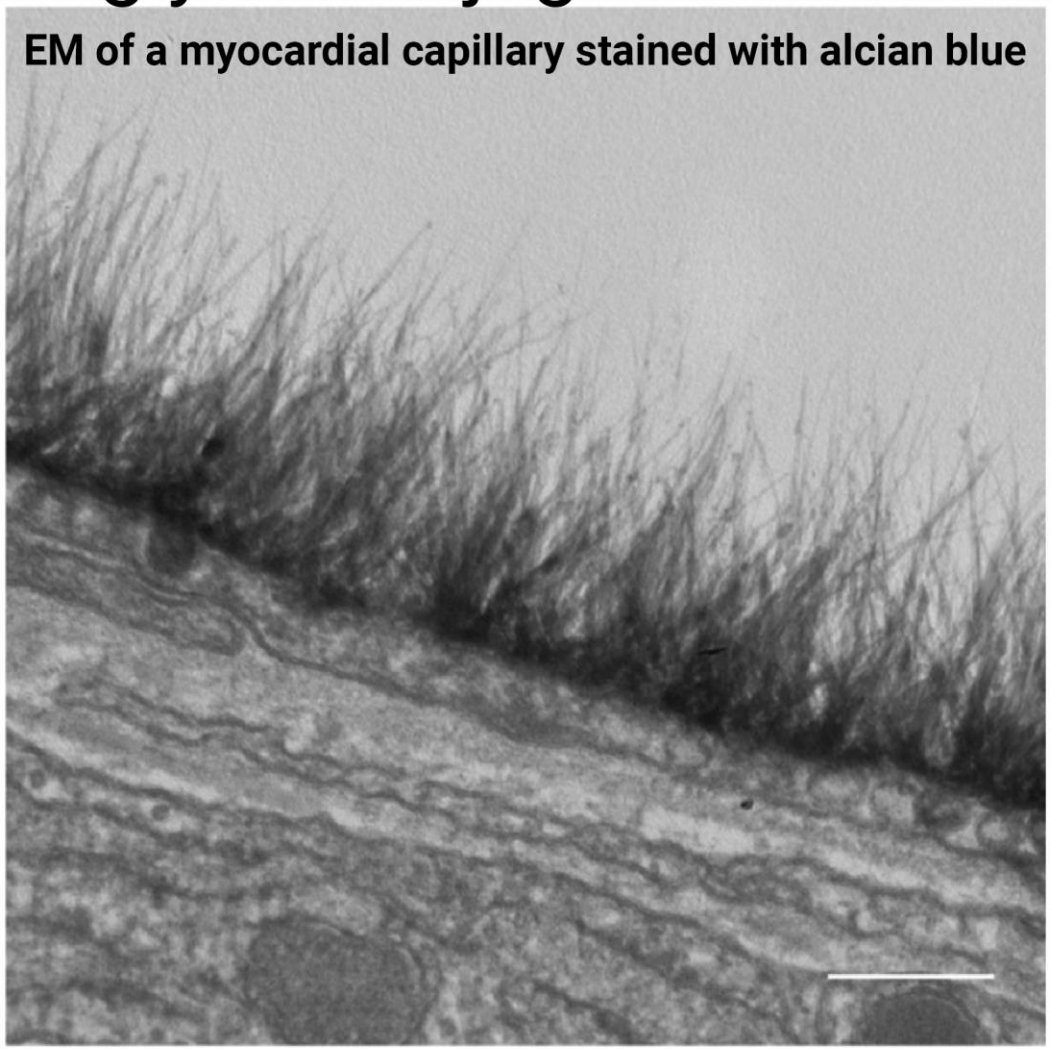
@riley_nm1



Casalino et al., ACS Central Science, 2020, 10.1021/acscentsci.0c01056

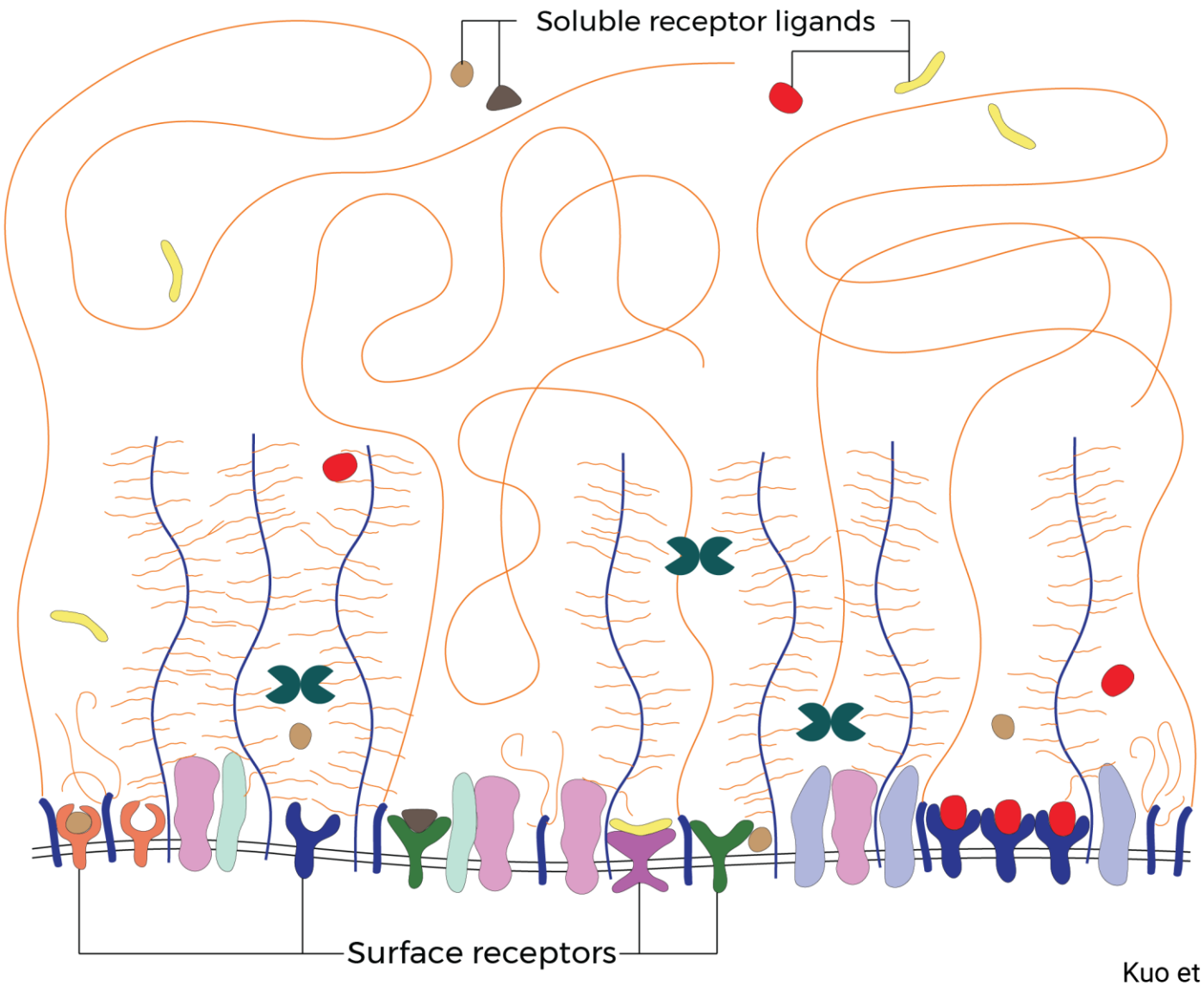
EVERY CELL HAS A GLYCOCALYX

collection of glyco-conjugates at the cell surface



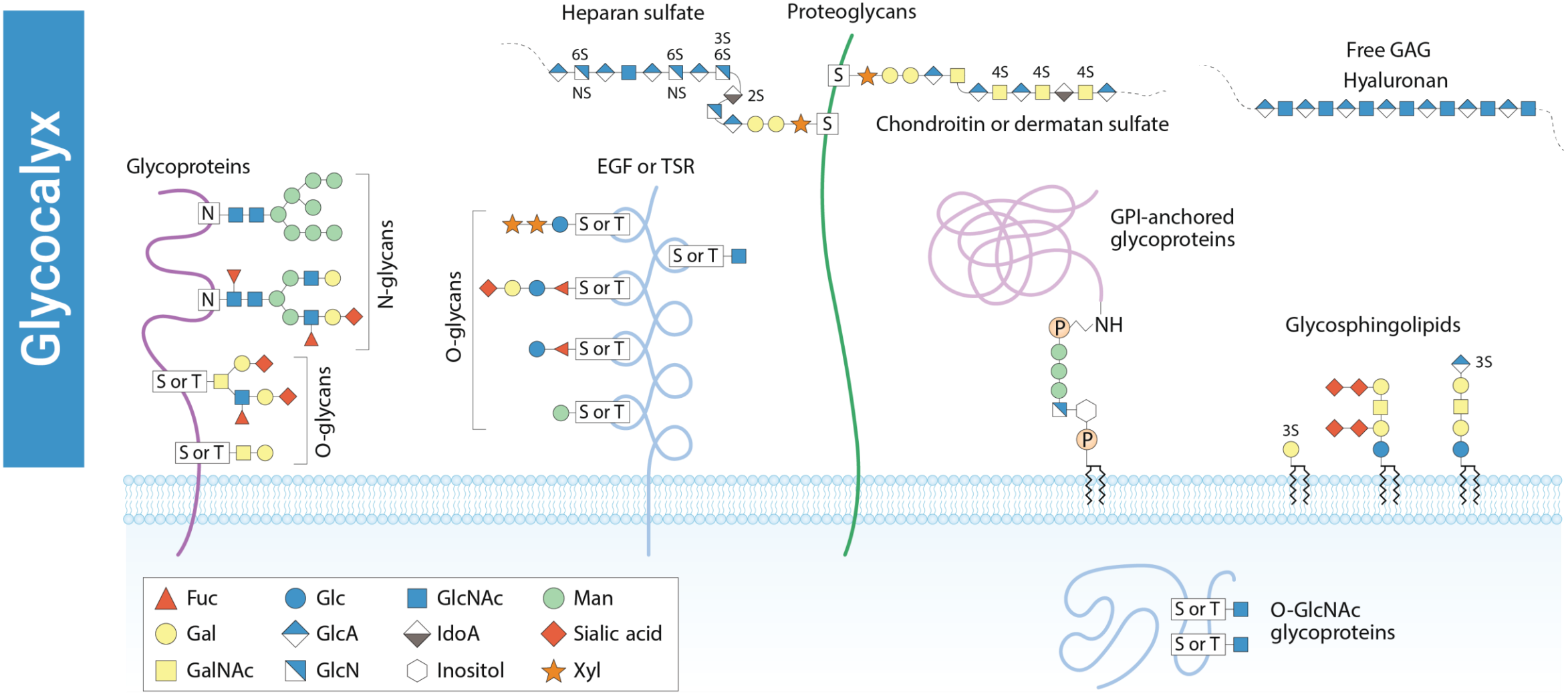
Dane, M. J. C. et al. American Journal of Physiology - Renal Physiology 308, F956–F966 (2015).

(GLYCO)BIOLOGY AT THE CELL SURFACE



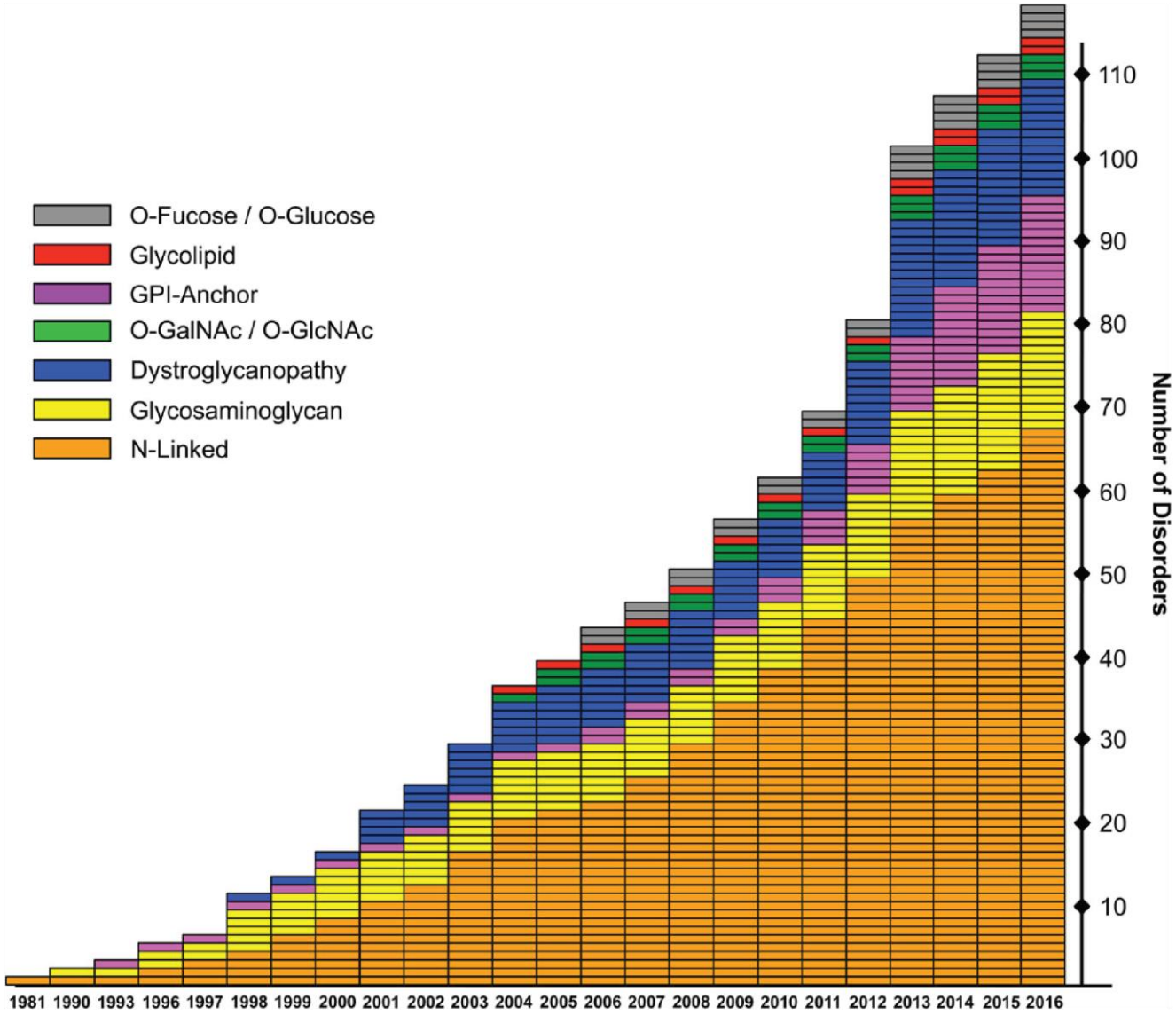
Kuo et al., Nature Physics, 2018, 14: 658–669

HUMAN GLYCOSYLATION

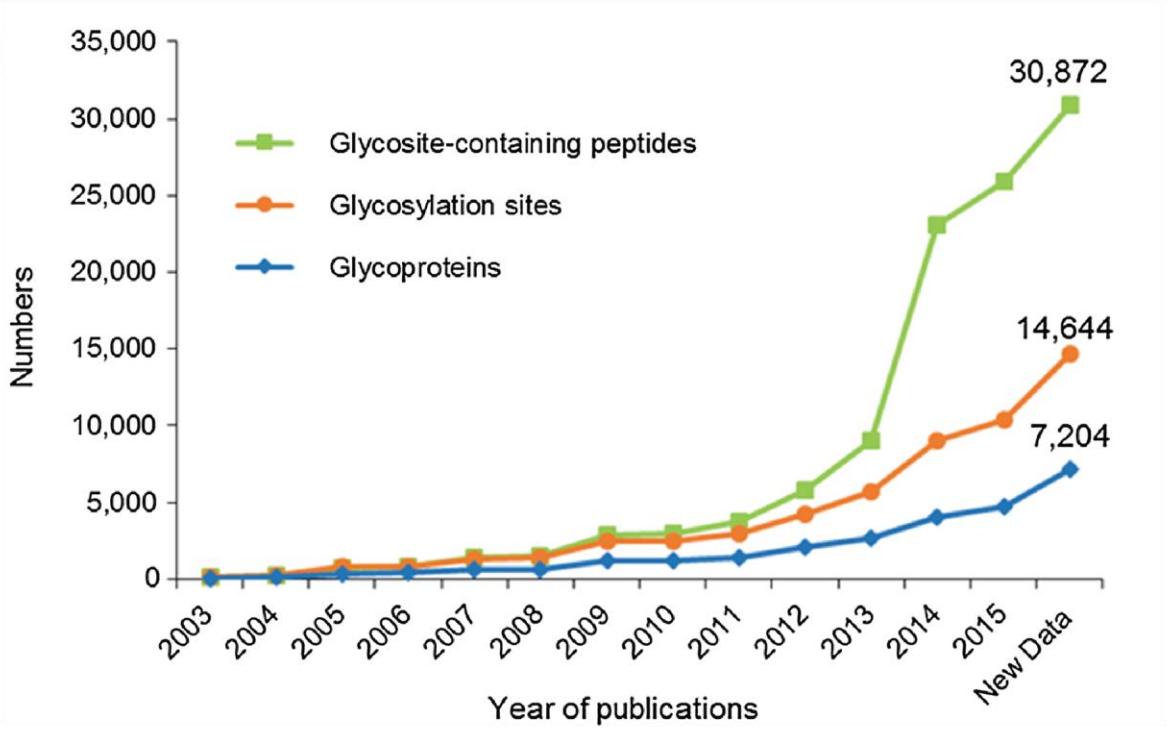


Reily, Novak, et al., Nature Reviews Nephrology, 2019, 15: 346-366

GLYCOSYLATION IN HEALTH AND DISEASE

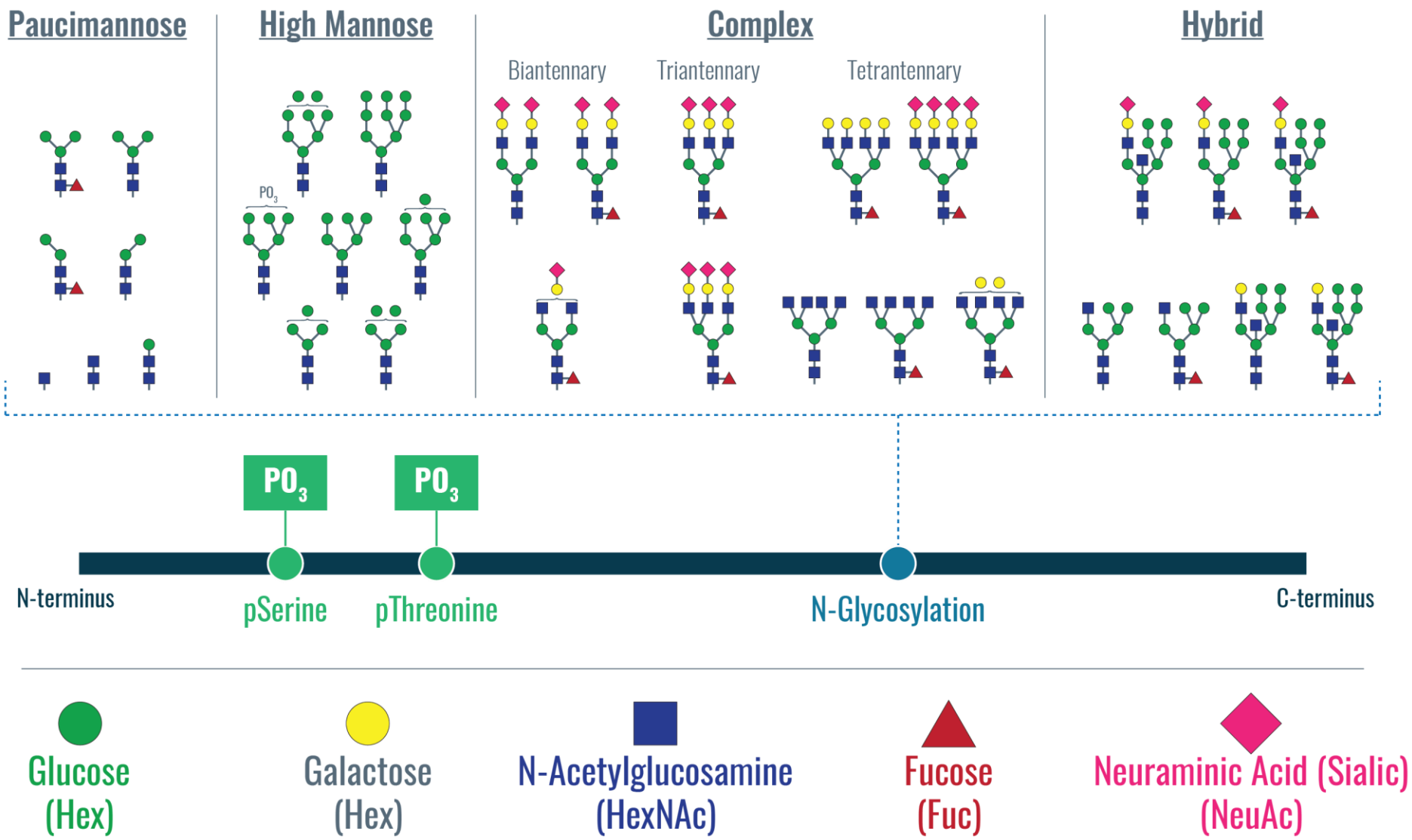


Freeze, et al., Am Journal of Hum. Genetics, 2014, 94: 161-175; updated by Varki 2017

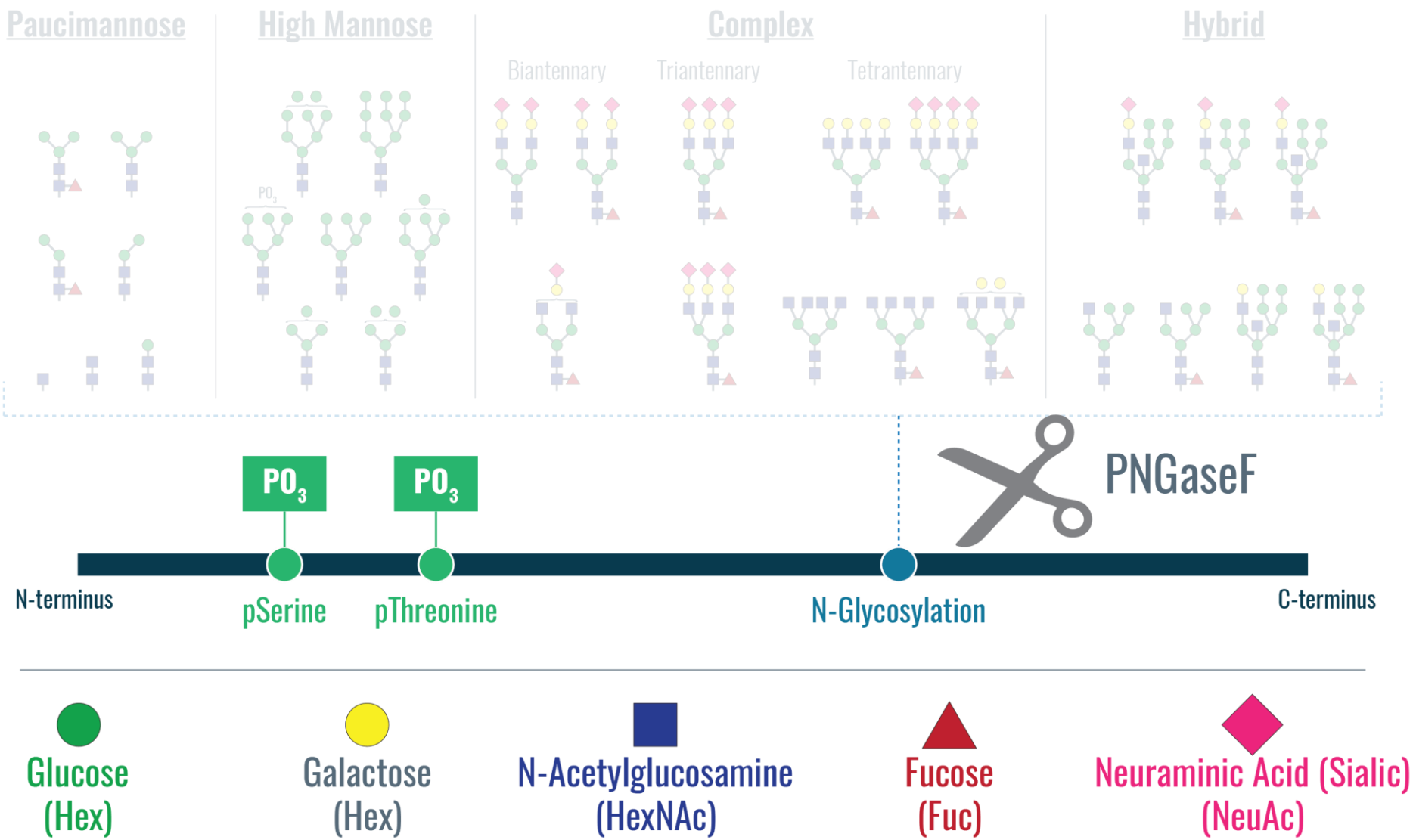


Sun, Zhang, et al., Clinical Proteomics, 2019, 16: 35

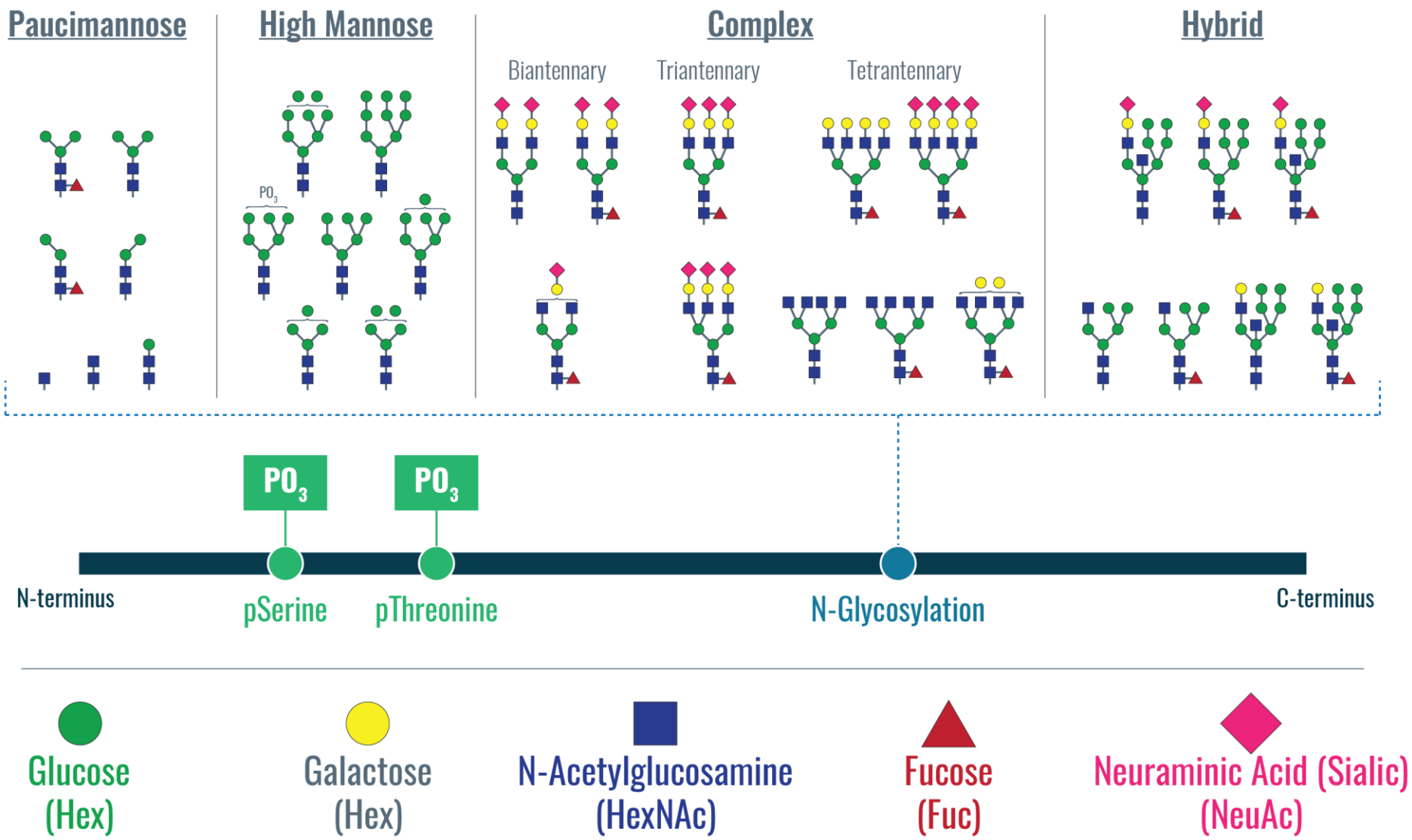
SITE-SPECIFIC MICROHETEROGENEITY



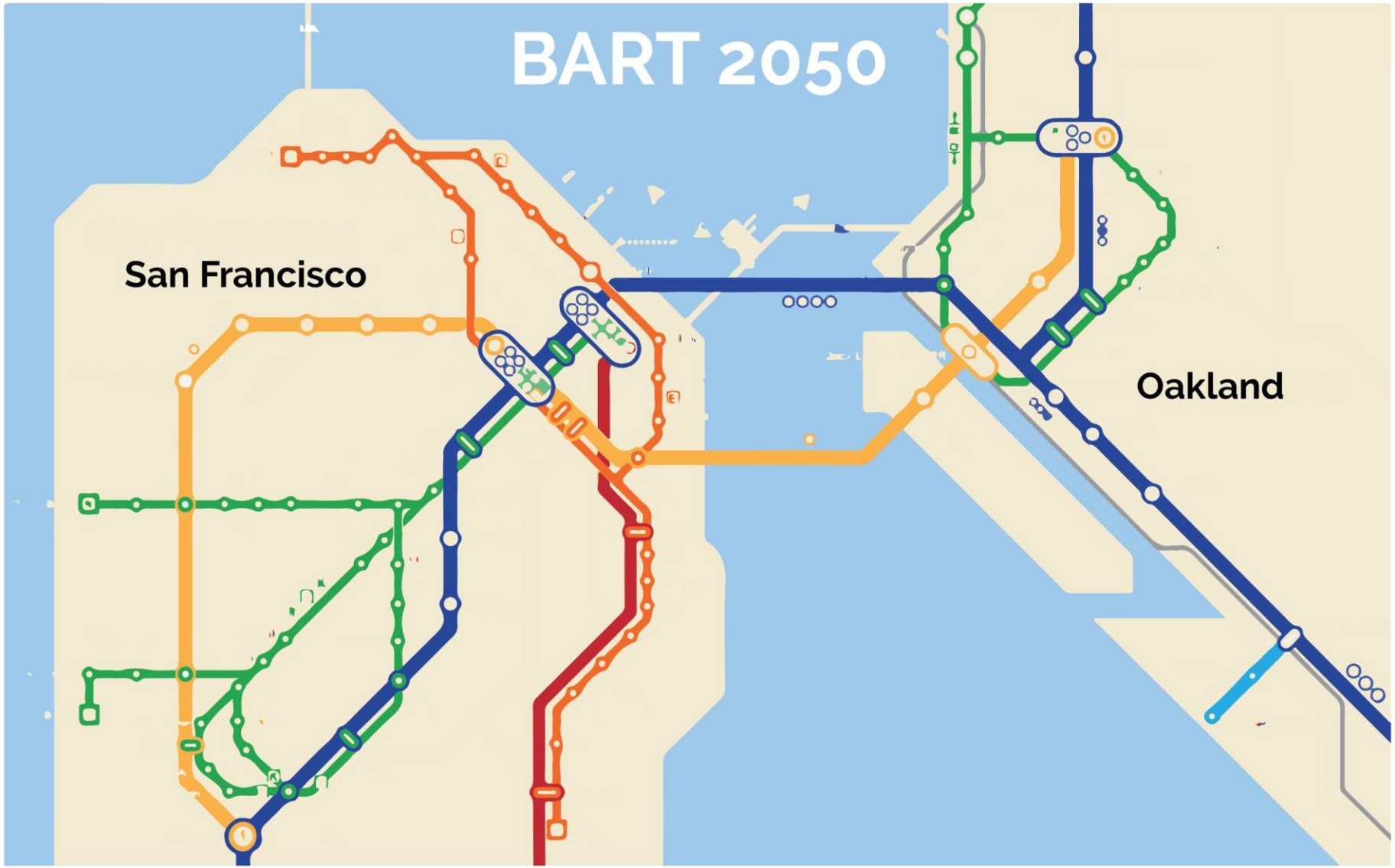
DE-GLYCOPEPTIDES: EASIER, BUT AT A PRICE



INTACT GLYCOPEPTIDES: MICROHETEROGENEITY

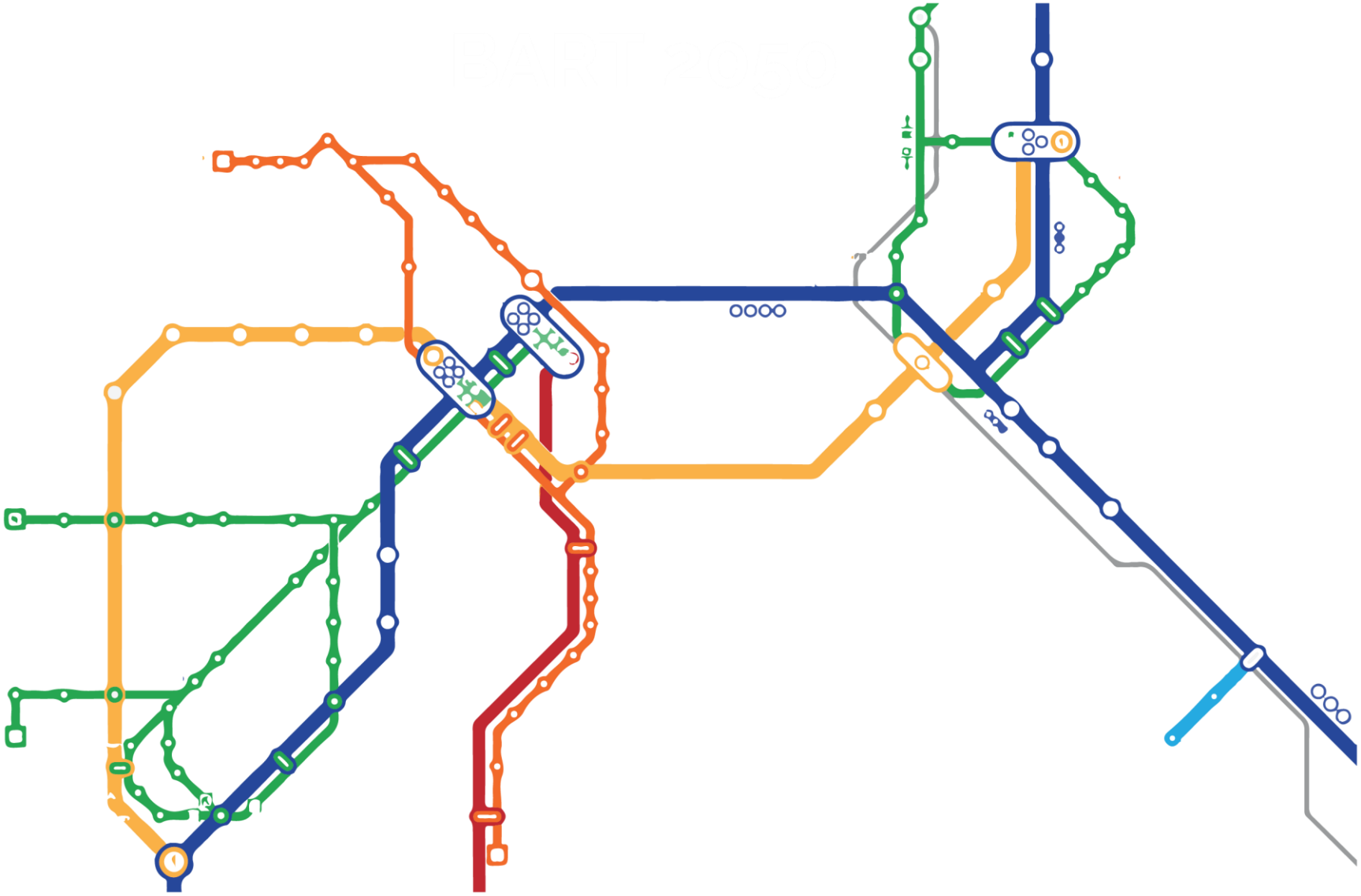


MAPPING THE GLYCOPROTEOME



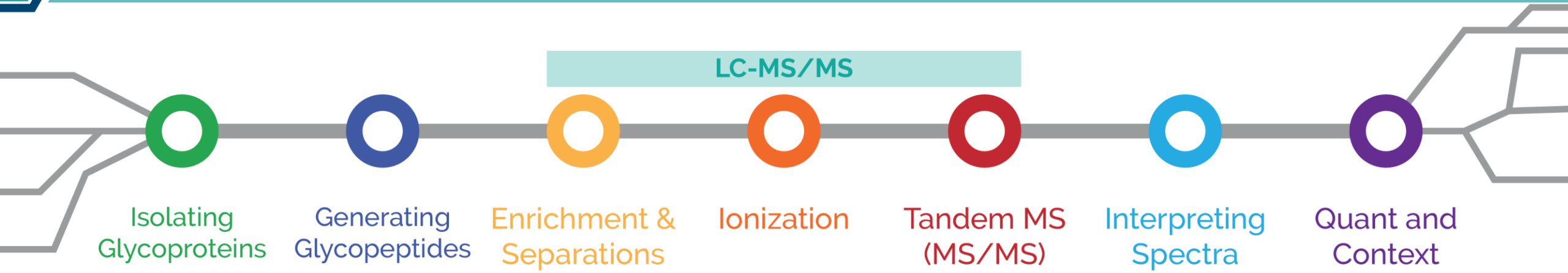
https://futuretravel.today/bay-area-2050-the-bart-metro-map-ab83b22d3d8b

MAPPING THE GLYCOPROTEOME

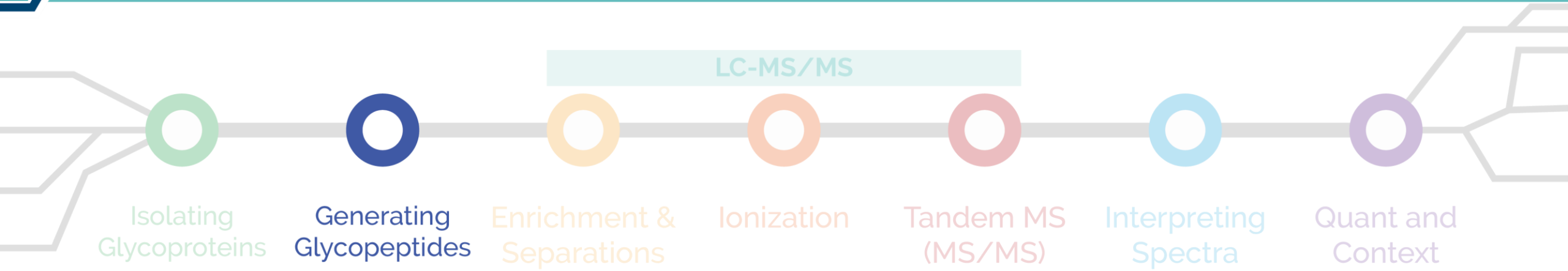


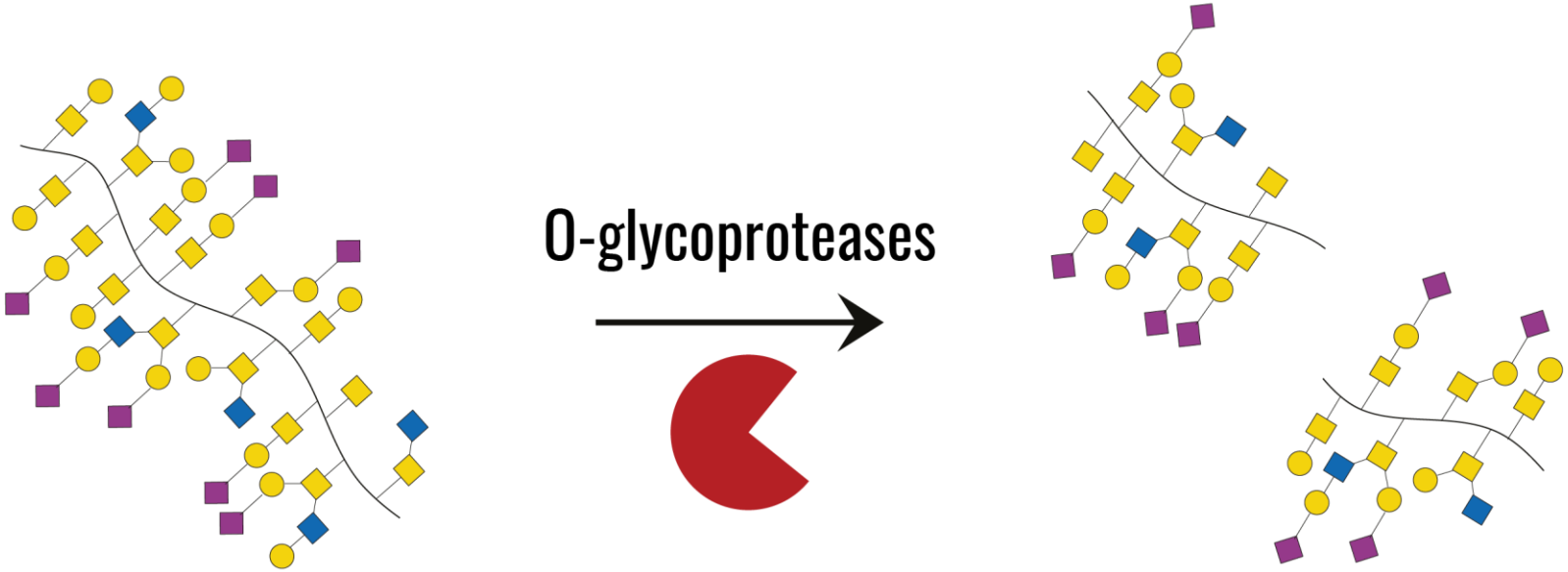
https://futuretravel.today/bay-area-2050-the-bart-metro-map-ab83b22d3d8b

MAPPING THE GLYCOPROTEOME



THE RISE OF O-GLYCOPROTEASES





THE RISE OF O-GLYCOPROTEASES

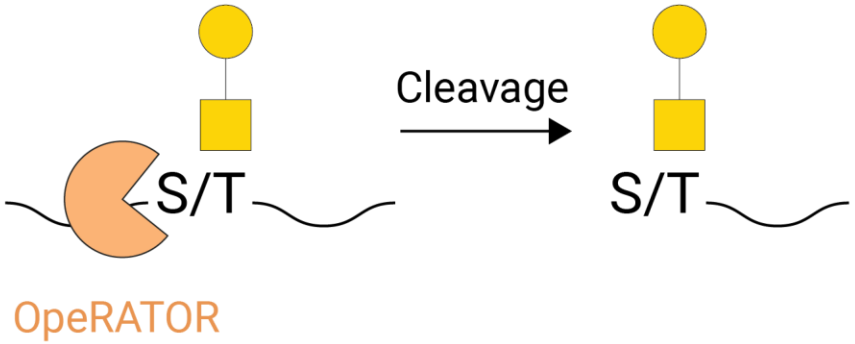
OpeRATOR:
broadly active on glyco-S and -T

Mapping the O-glycoproteome using site-specific extraction of O-linked glycopeptides (EXoO)

Weiming Yang, Minghui Ao, Yingwei Hu, Qing Kay Li & Hui Zhang*

Deciphering Protein O-Glycosylation: Solid-Phase Chemoenzymatic Cleavage and Enrichment

Shuang Yang, Philip Onigman, Wells W. Wu, Jonathan Sjogren, Helen Nyhlen, Rong-Fong Shen, and John Cipollo



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New Results
Comment on this paper

The glycoprotease CpaA secreted by medically relevant *Acinetobacter* species targets multiple O-linked host glycoproteins

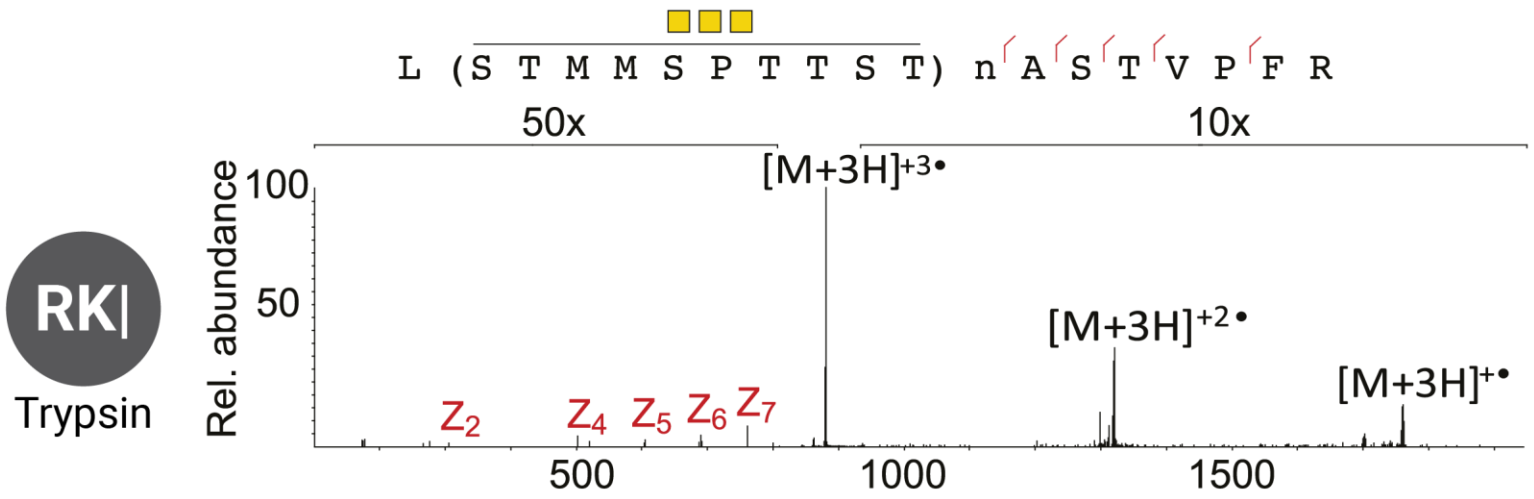
M. Florencia Haurat, Nichollas E. Scott, Gisela Di Venanzio, Juvenal Lopez, Benajmin Pluvinage, Alisdair B. Boraston, Michael J. Ferracane, Mario F. Feldman

doi: https://doi.org/10.1101/2020.07.22.216978

CpaA

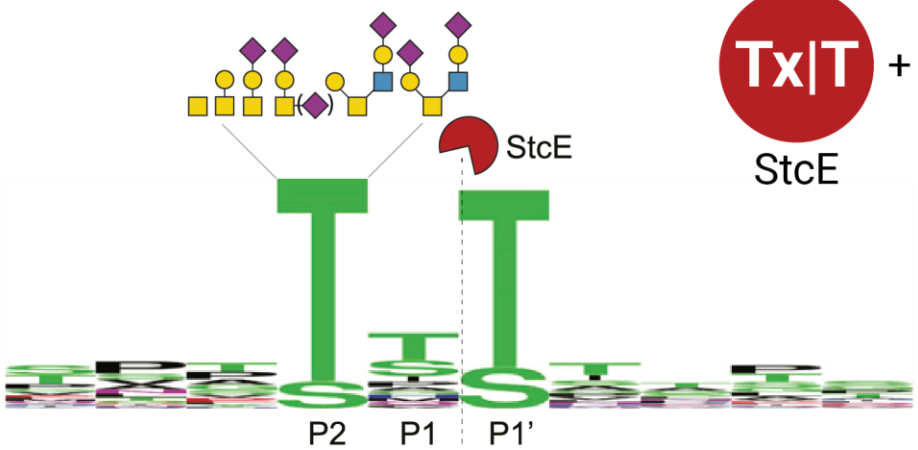
	P1	P1'
Factor XII	AQCQTP	TQAAPP
Factor XII	PPKPQP	TTRTPP
Fetuin	AEAEAP	SAVPDA
AFt	AEAEAP	SAVPDA
CD55	TSKVPP	TVQKPT
CD55	PTVQKP	TTVNVP
CD46	ASGPRP	TYKPPV
TIM1	PAETHP	TTLQGA
Etanercept	SQHTQP	TPEPST
Etanercept	EPSTAP	STSFLL
Etanercept	LLPMGP	SPPAEG

MS-AMENABLE O-GLYCOPEPTIDES

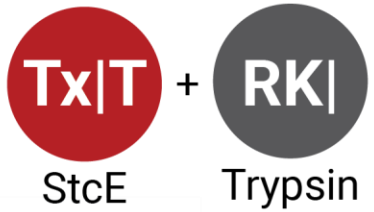


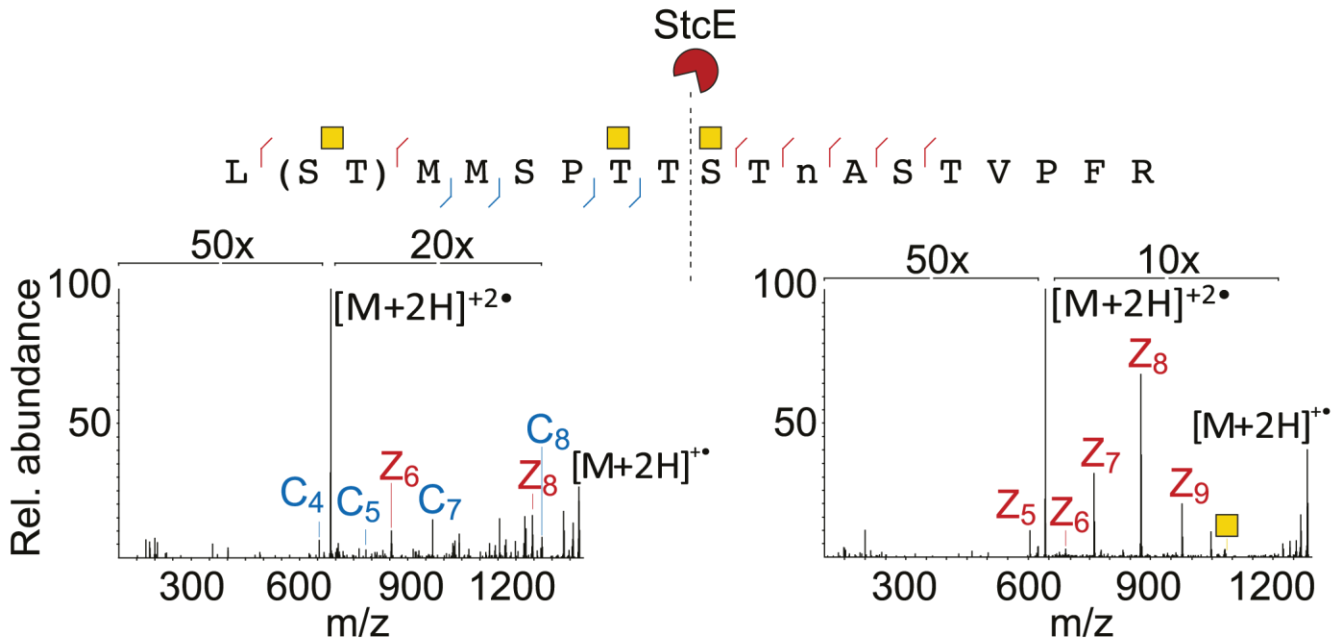
Malaker, Pedram, Bertozzi et al., PNAS 116(15) 7278-7287 (2019)

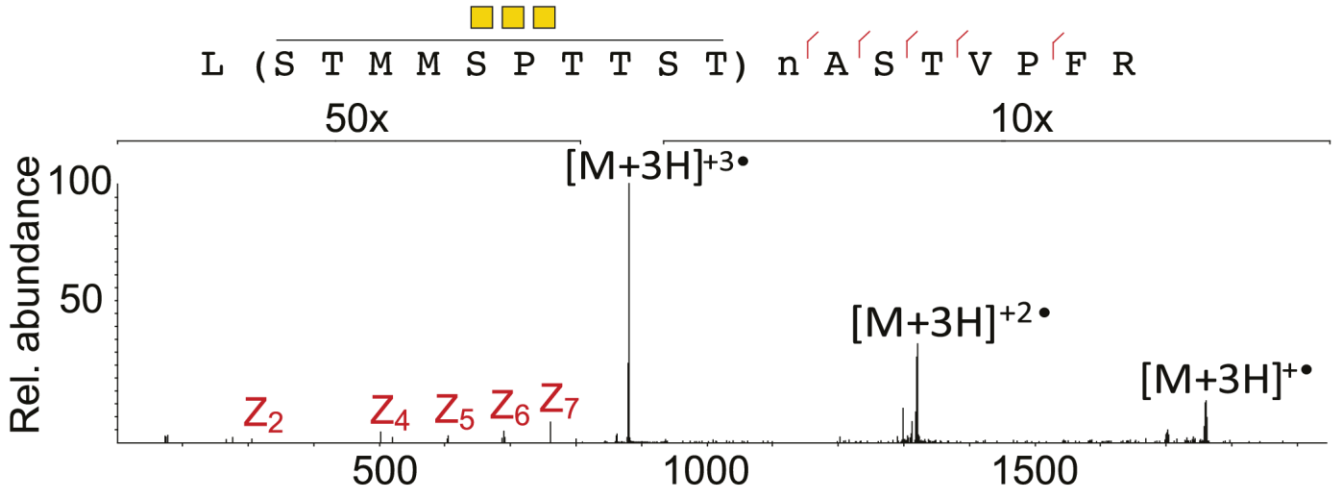
MS-AMENABLE O-GLYCOPEPTIDES



Mininum Cleavage Motif







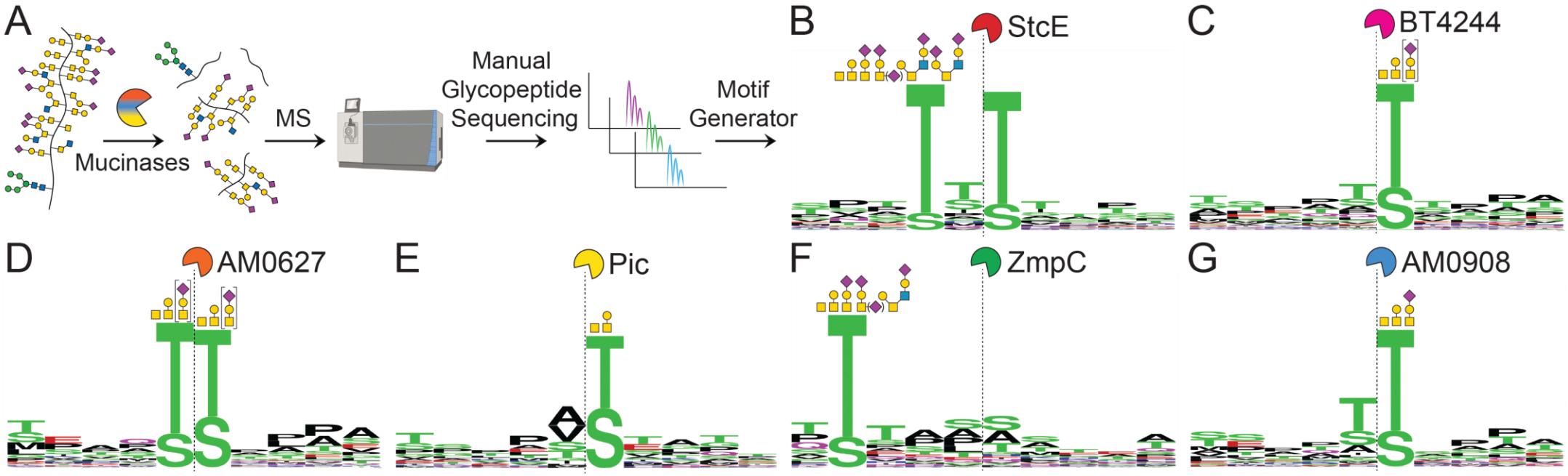
Malaker, Pedram, Bertozzi et al., PNAS 116(15) 7278-7287 (2019)

O-GLYCOPROTEASE SUBSETS: MUCINASES

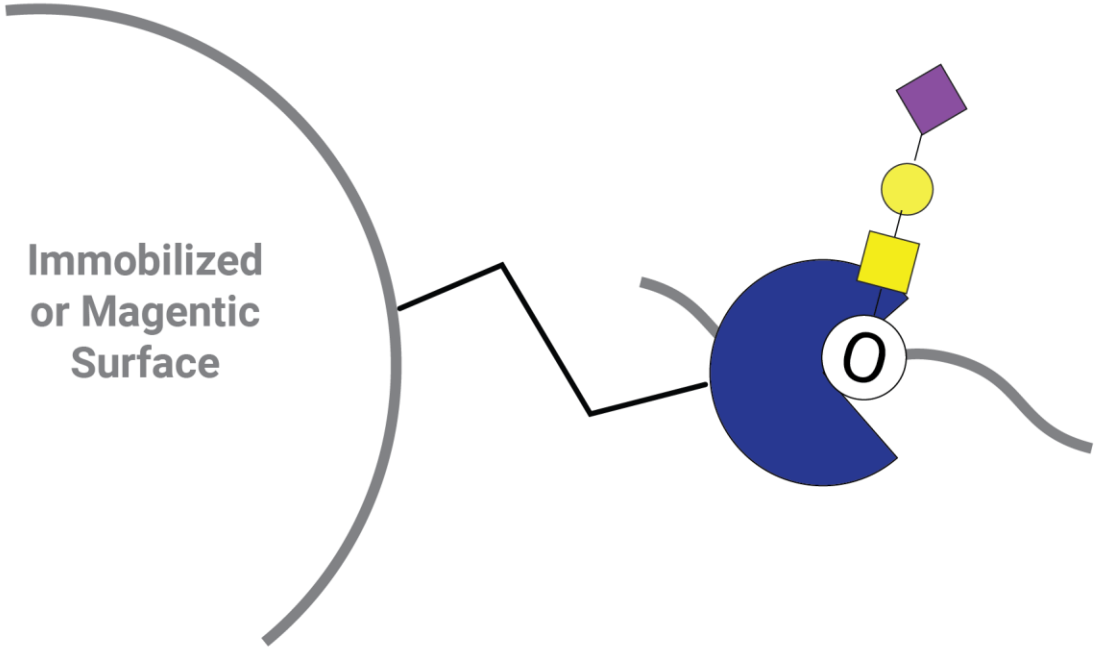
An enzymatic toolkit for selective proteolysis, detection, and visualization of mucin-domain glycoproteins

D. Judy Shon[a], Stacy A. Malaker[a], Kayvon Pedram[a], Emily Yang[a], Venkatesh Krishnan[b], Oliver Dorigo[b], and Carolyn R. Bertozzi[a,c,1]

PNAS September 1, 2020 117 (35) 21299-21307

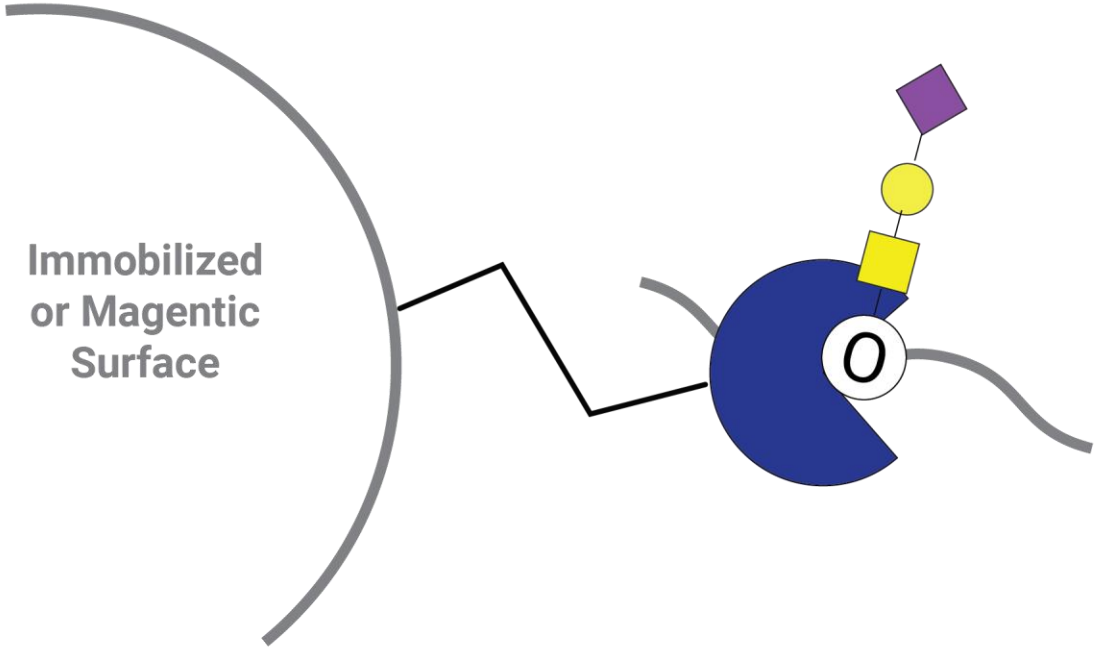


O-GLYCOPROTEASE ENRICHMENTS



O-GLYCOPROTEASE ENRICHMENTS

GlycoCatch: Genovis (OpeRATOR)

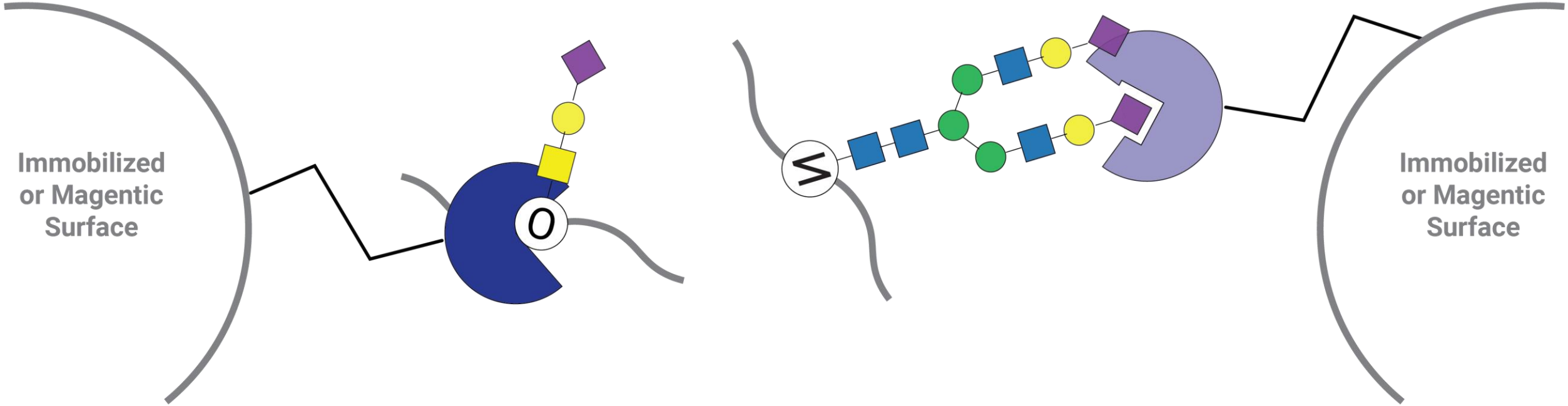


ENZYMES FOR ENRICHMENTS IN GLYCO

GlycoCatch: Genovis (OpeRATOR)

SiaFind Lectenz Kits (Engineered Sialidases)

Lectenz Bio
Glycoscience Made Simple



CHEMICAL BIOLOGY AND GLYCOPROTEOMICS

LC-MS/MS

Isolating Glycoproteins | Generating Glycopeptides | Enrichment & Separations | Ionization | Tandem MS (MS/MS) | Interpreting Spectra | Quant and Context

CHEMICAL REVIEWS

Review

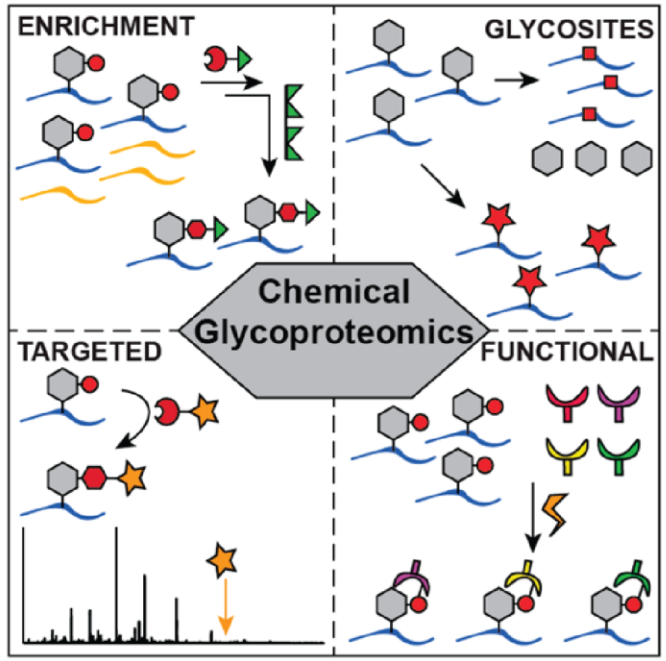
pubs.acs.org/CR

Chemical Glycoproteomics

Krishnan K. Palaniappan[†] and Carolyn R. Bertozzi[*,‡,§]

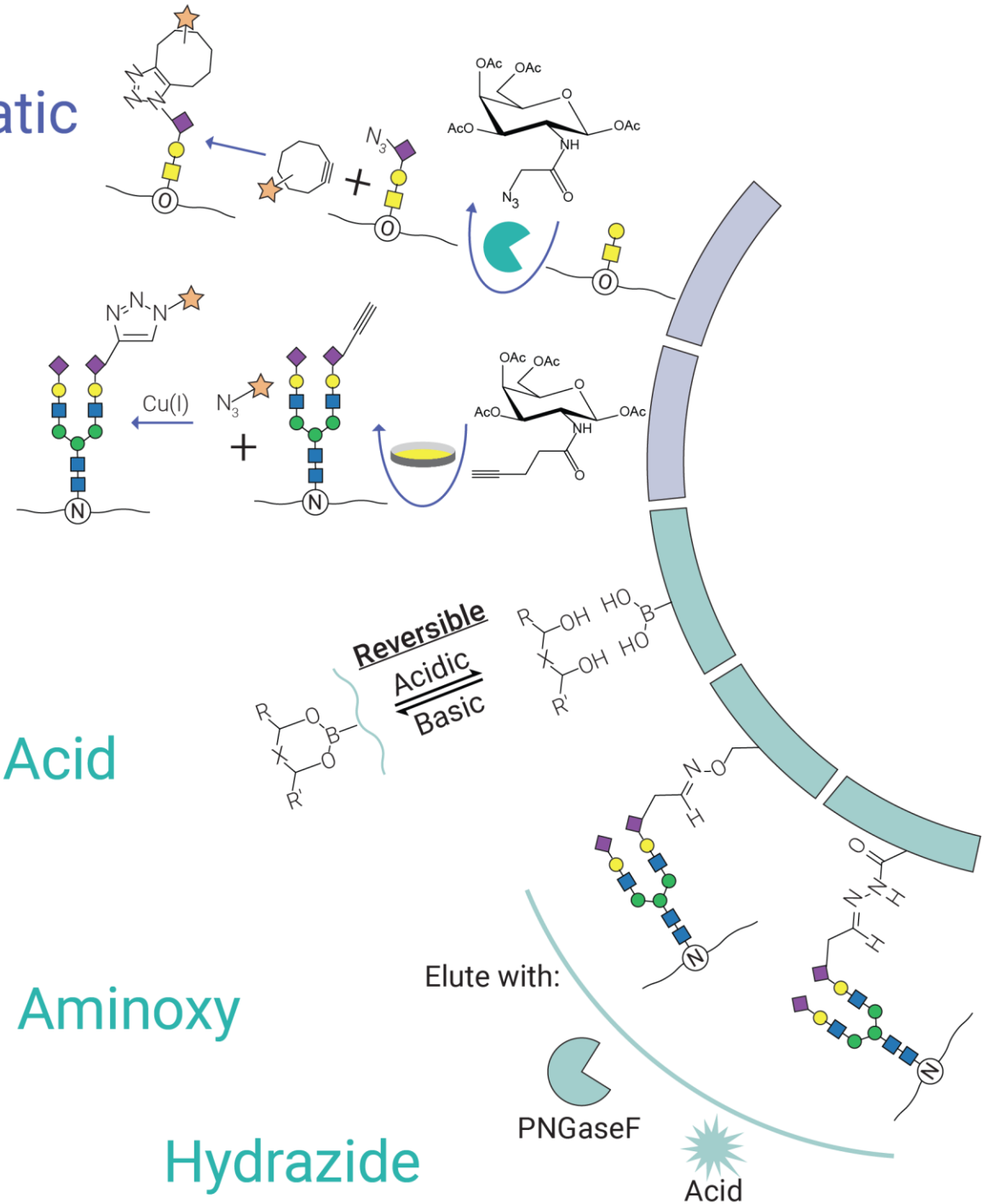
[†]Verily Life Sciences, 269 East Grand Ave., South San Francisco, California 94080, United States

[‡]Department of Chemistry and [§]Howard Hughes Medical Institute, Stanford University, Stanford, California 94305, United States



Bioorthogonal Chemoenzymatic

Bioorthogonal Metabolic



Boronic Acid

Aminoxy

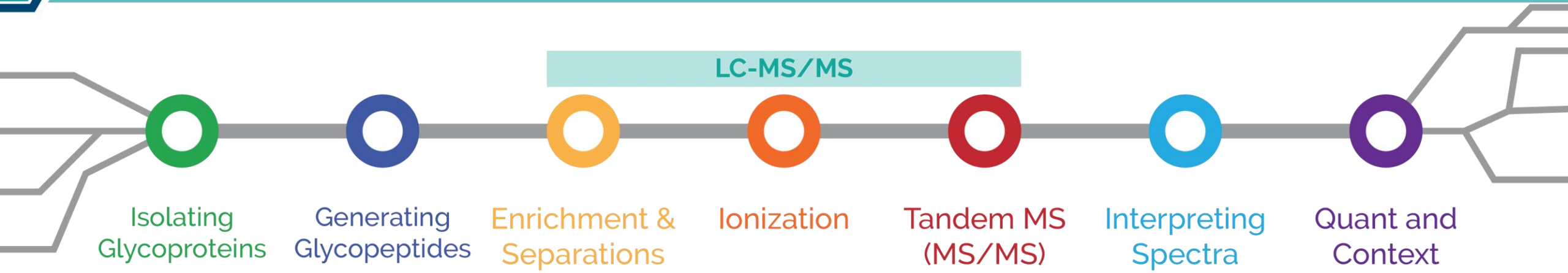
Hydrazide

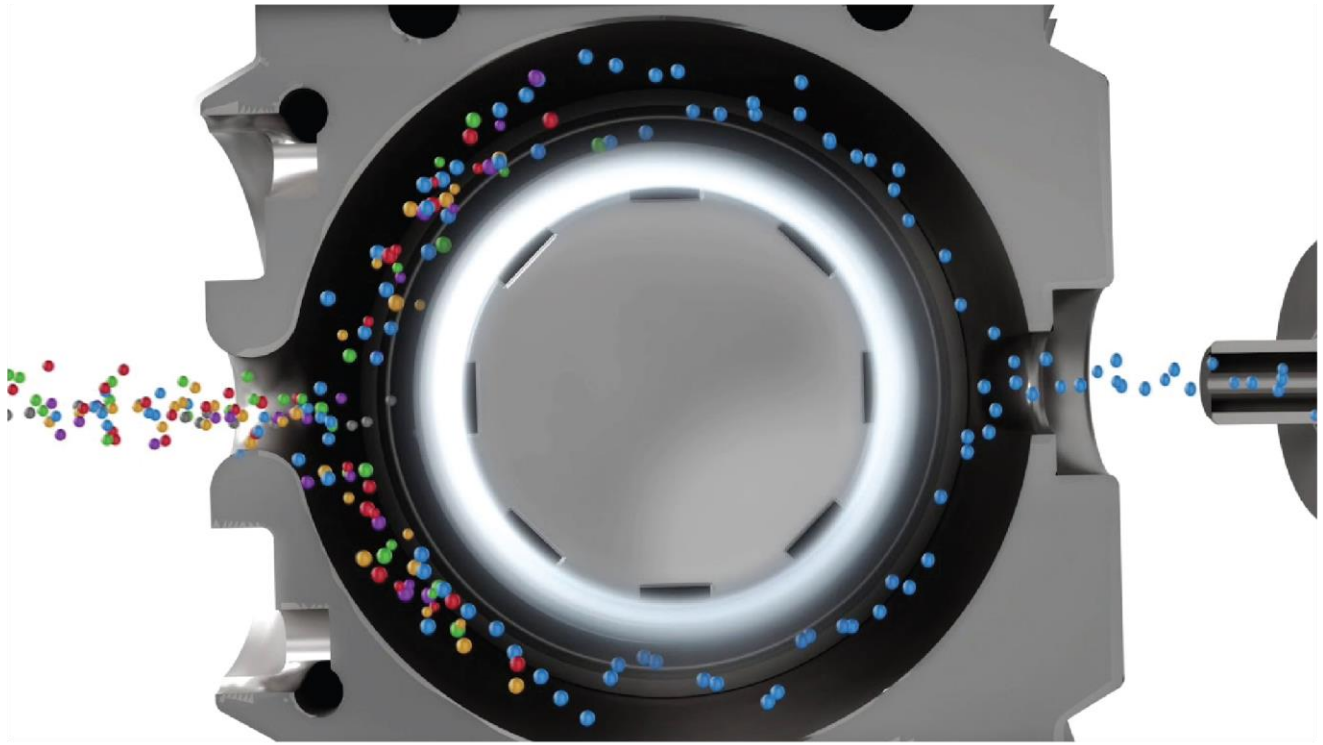
MCP MOLECULAR & CELLULAR PROTEOMICS

A Pragmatic Guide to Enrichment Strategies for Mass Spectrometry-based Glycoproteomics

Riley, Bertozzi, Pitteri, Mol. & Cell. Prot, 2020

GAS-PHASE ENRICHMENT





Field Asymmetric Ion Mobility Spectrometry (FAIMS)

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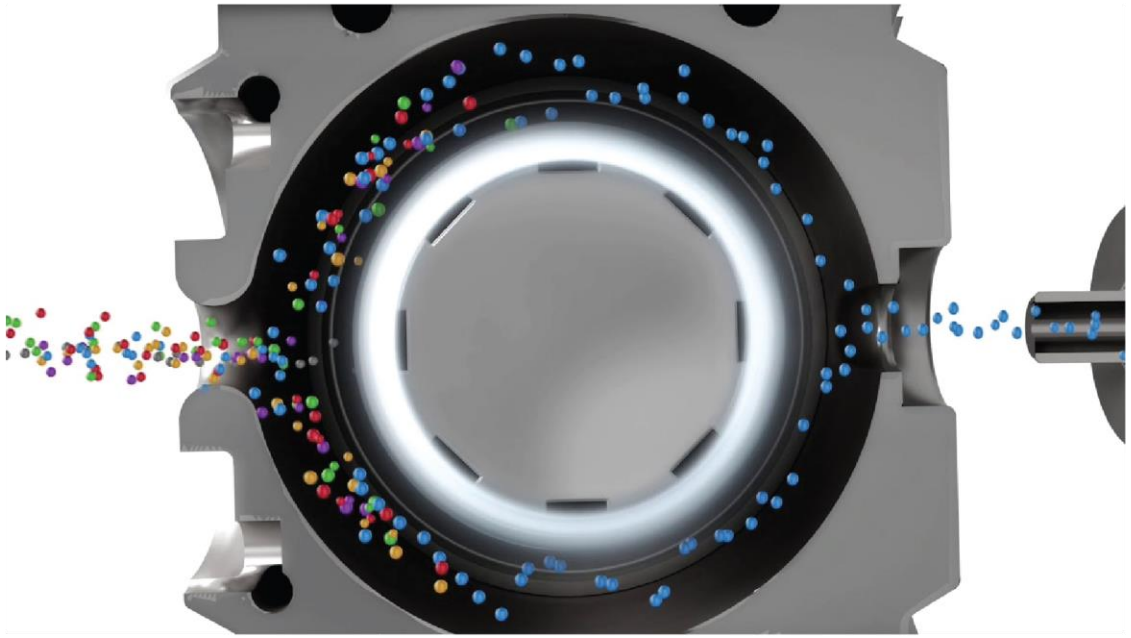
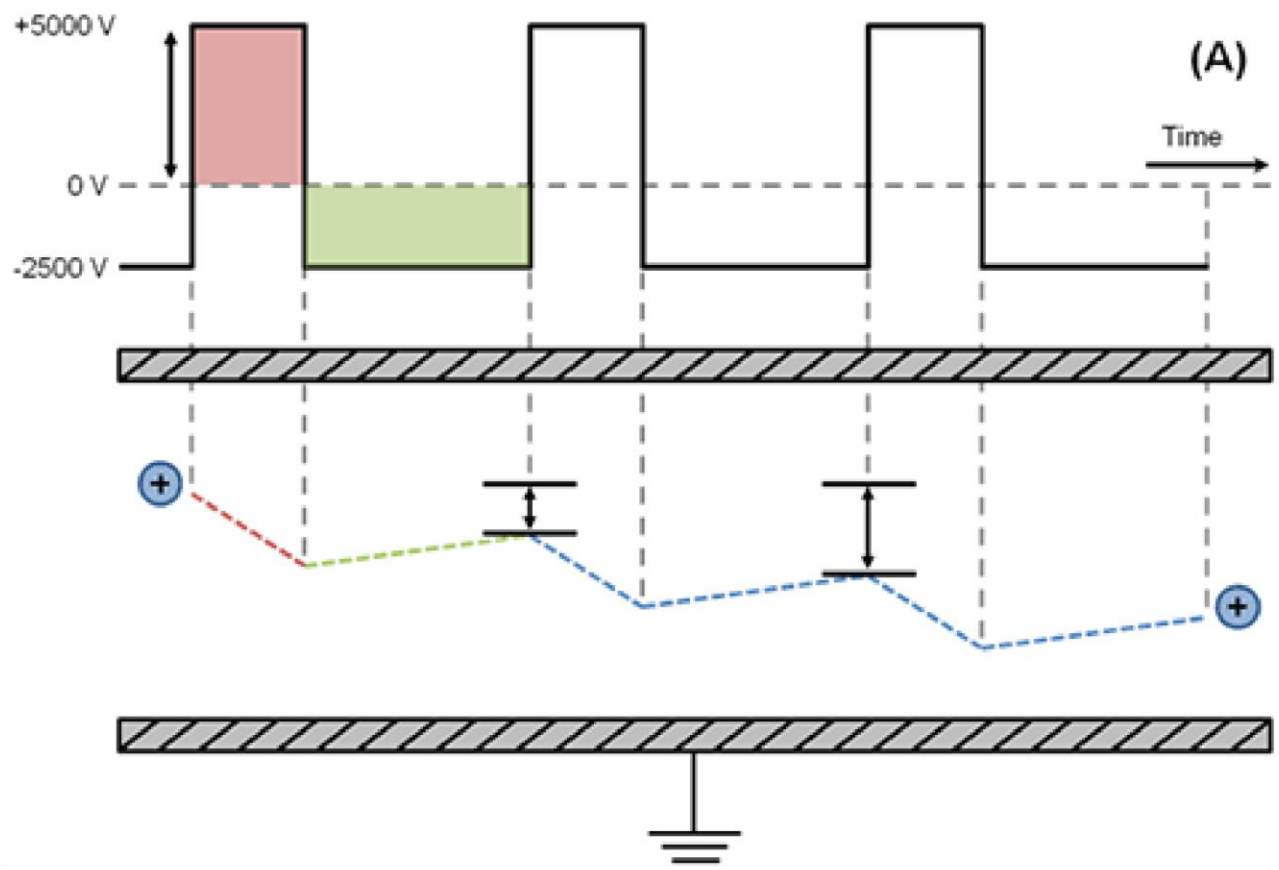
What are we missing by using hydrophilic enrichment? Improving bacterial glycoproteome coverage using total proteome and FAIMS analysis.

Ameera Raudah Ahmad Izaham[1] Ching-Seng Ang[2], Shuai Nie[2], Lauren E. Bird[1], Nicholas A. Williamson[2] and Nichollas E. Scott[1#]

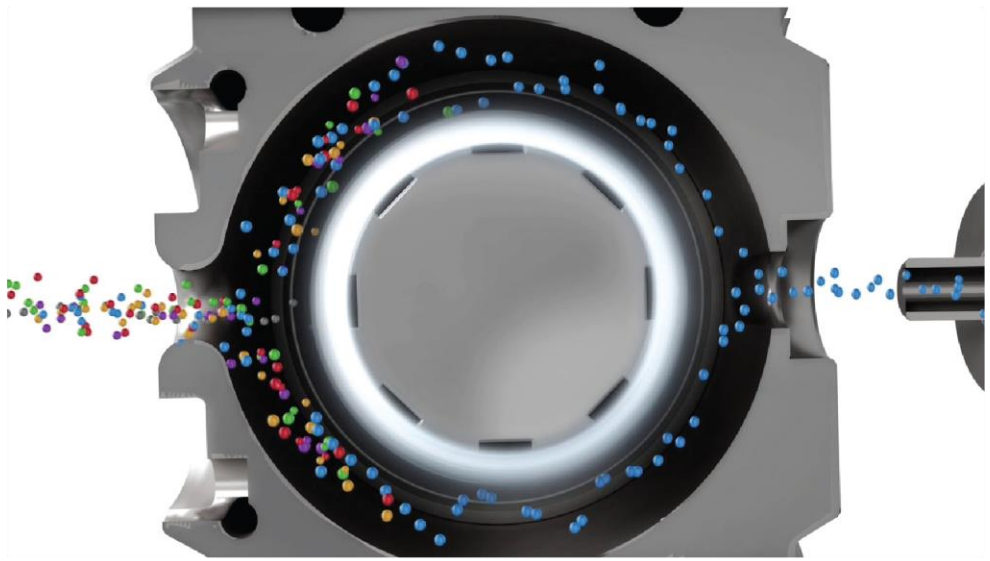
FAIMS

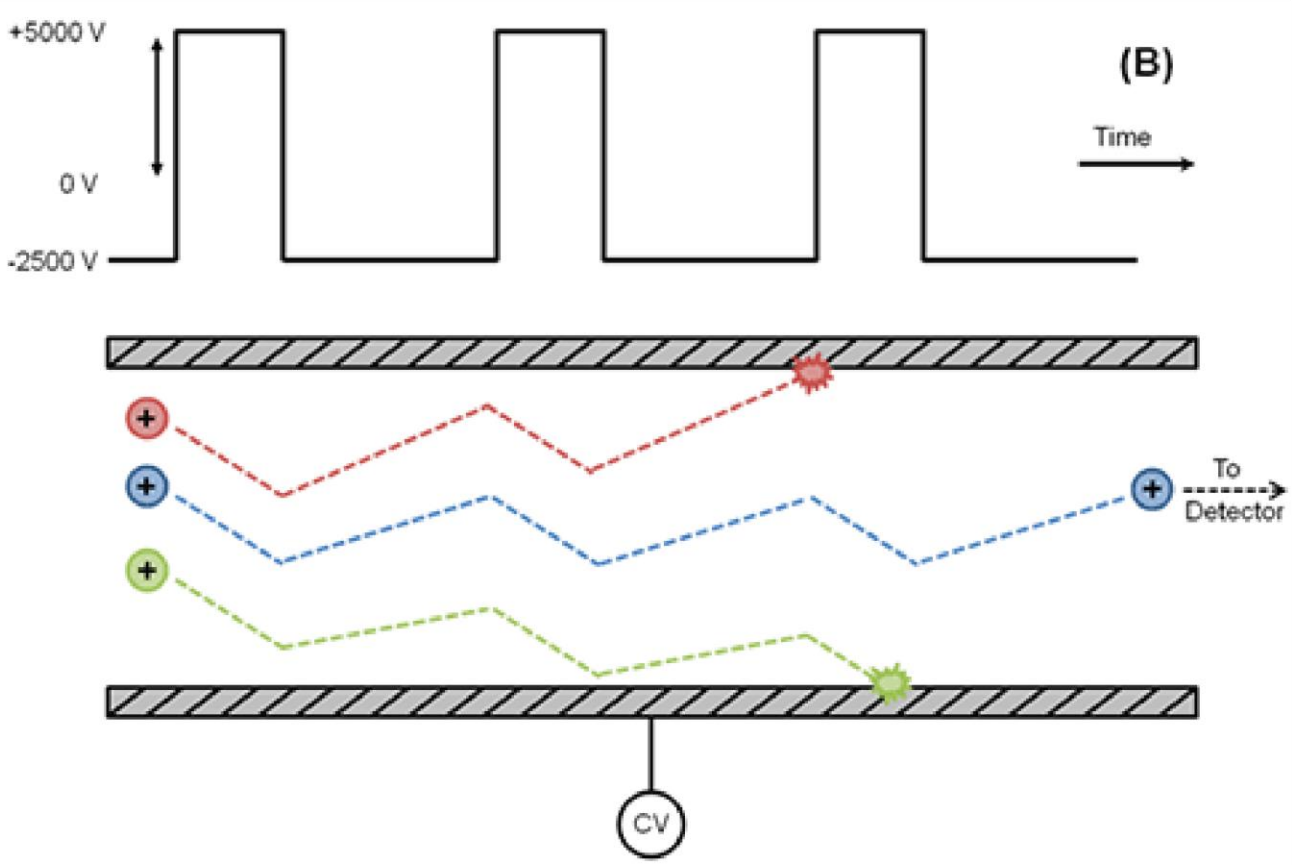




https://yost.chem.ufl.edu/research/faims/

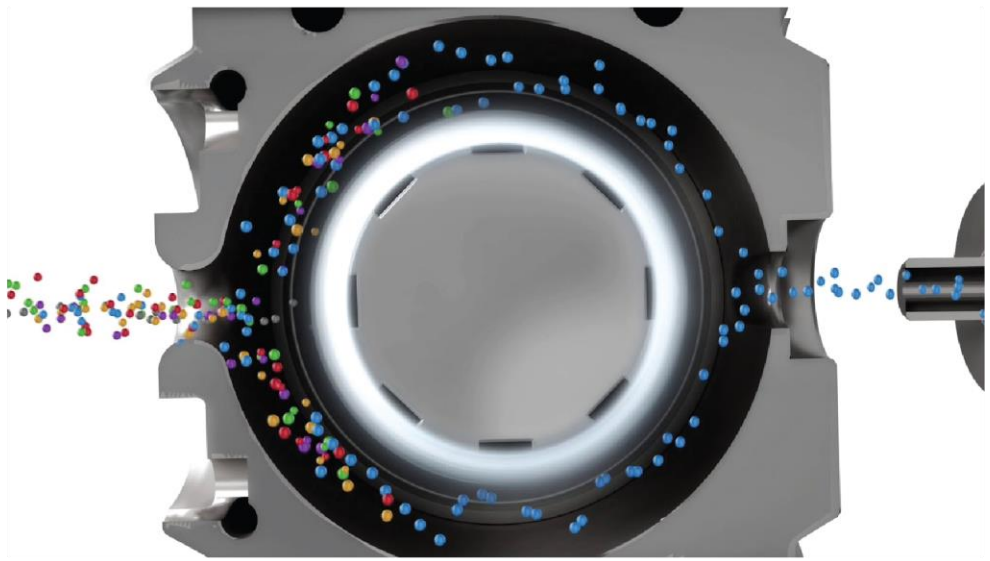
FAIMS

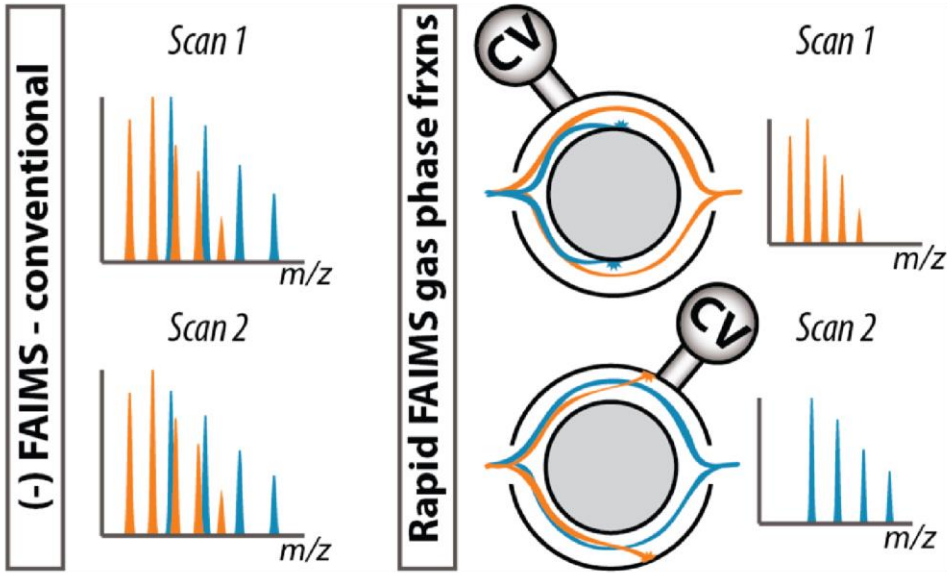


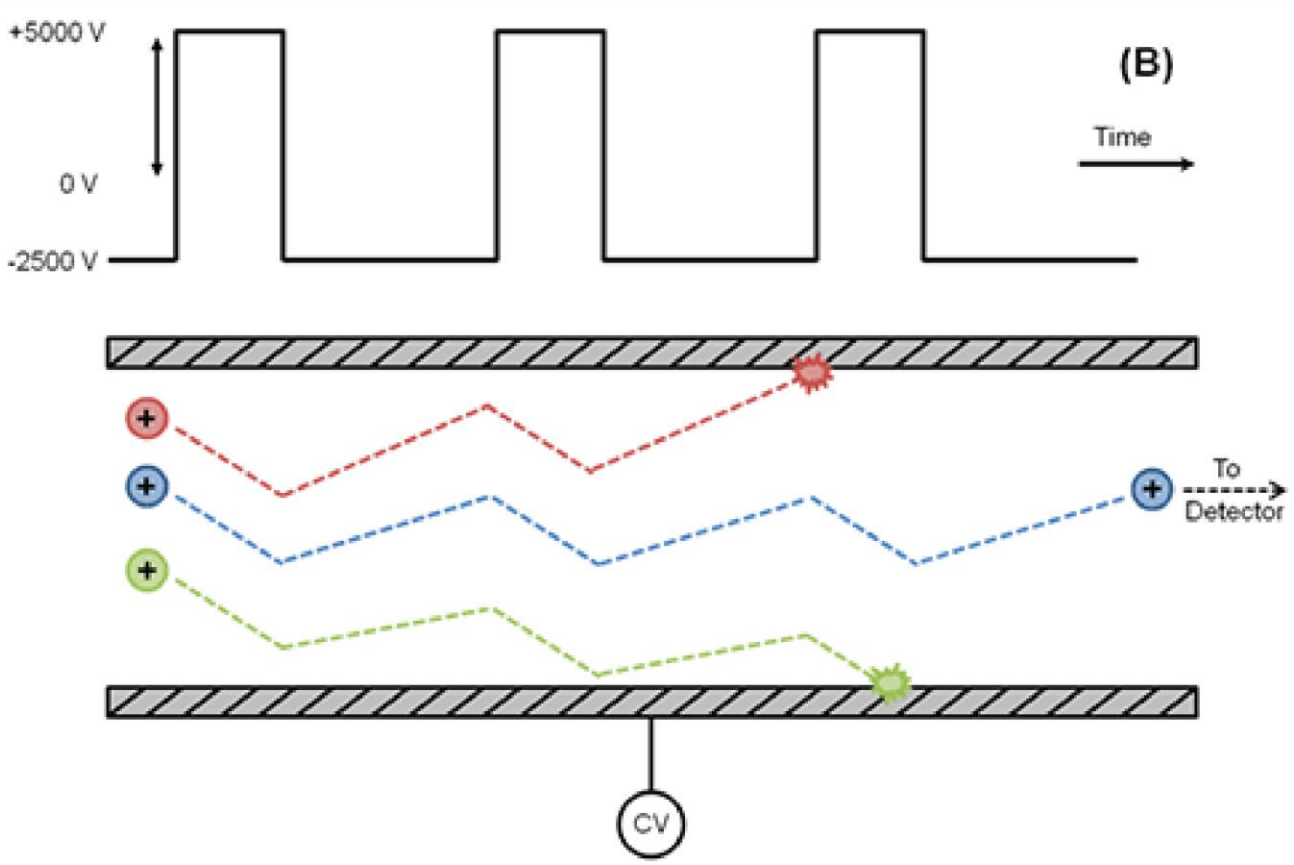


https://yost.chem.ufl.edu/research/faims/

FAIMS







Hebert et al., Analytical Chem, 201, 90: 9529-9537
https://yost.chem.ufl.edu/research/faims/

FAIMS FOR GLYCOPEPTIDES

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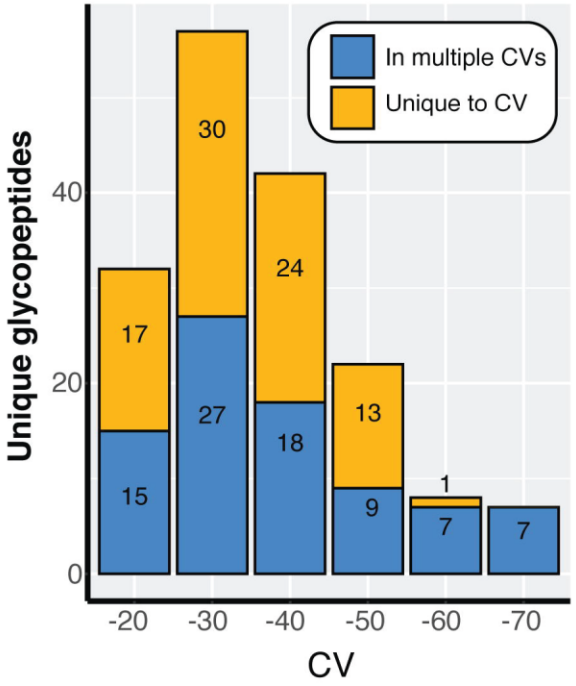
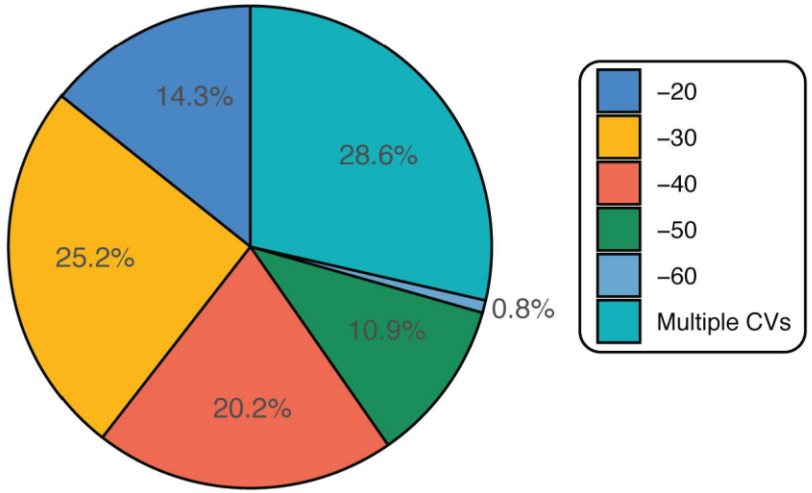
What are we missing by using hydrophilic enrichment? Improving bacterial glycoproteome coverage using total proteome and FAIMS analysis.

Ameera Raudah Ahmad Izaham[1] Ching-Seng Ang[2], Shuai Nie[2], Lauren E. Bird[1], Nicholas A. Williamson[2] and Nichollas E. Scott[1#]

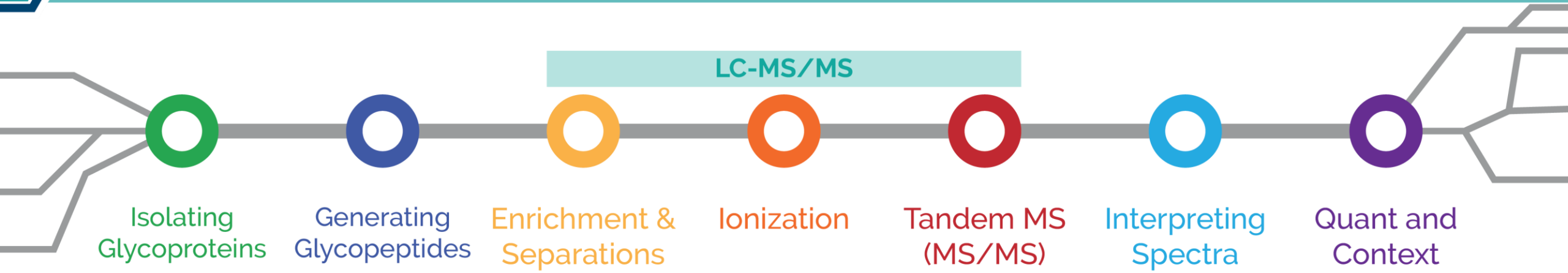
A) Unique to CVs



B) Unique glycopeptides observed across all CVs

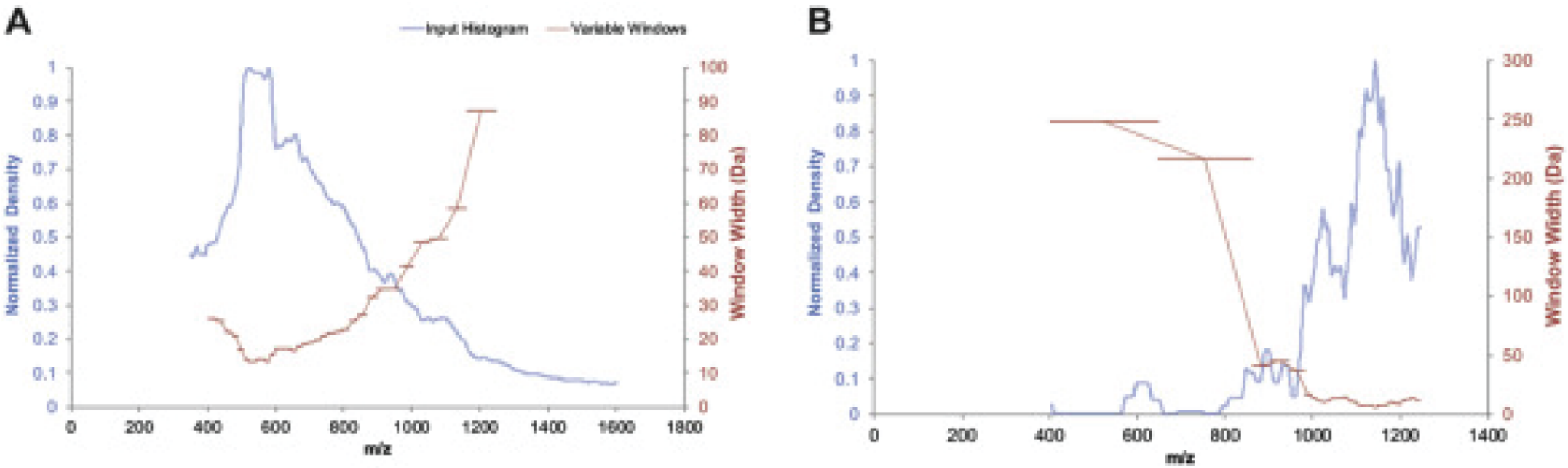


GAS-PHASE ENRICHMENT: DIA

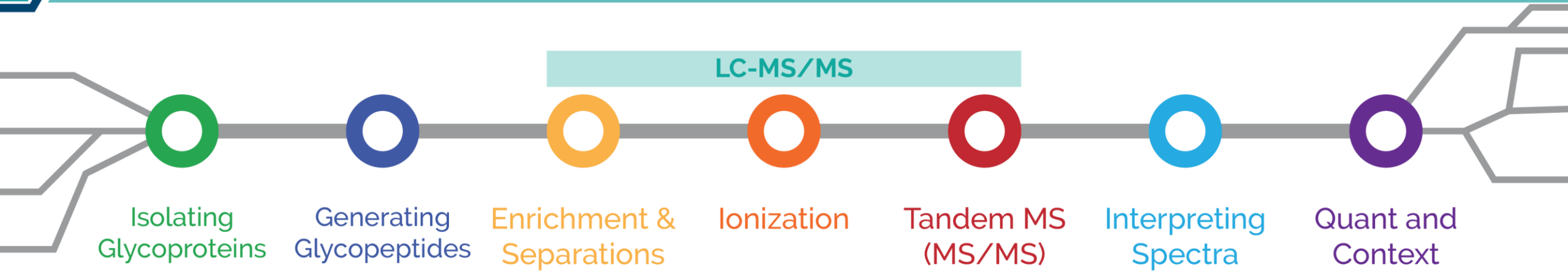


Glycopeptide variable window SWATH for improved data independent acquisition glycoprotein analysis

Chun Zhou[a], Benjamin L. Schulz[a,b,c,**]



GAS-PHASE ENRICHMENT: DIA

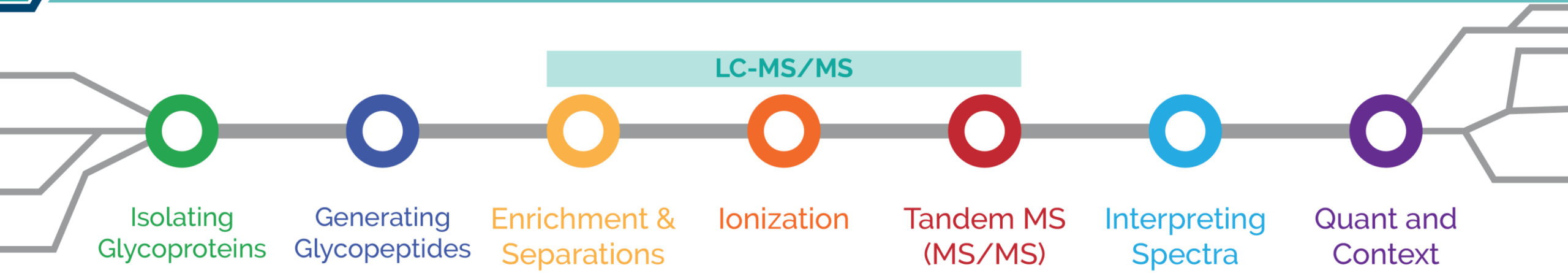


ARTICLES
https://doi.org/10.1038/s41592-019-0504-x
nature methods

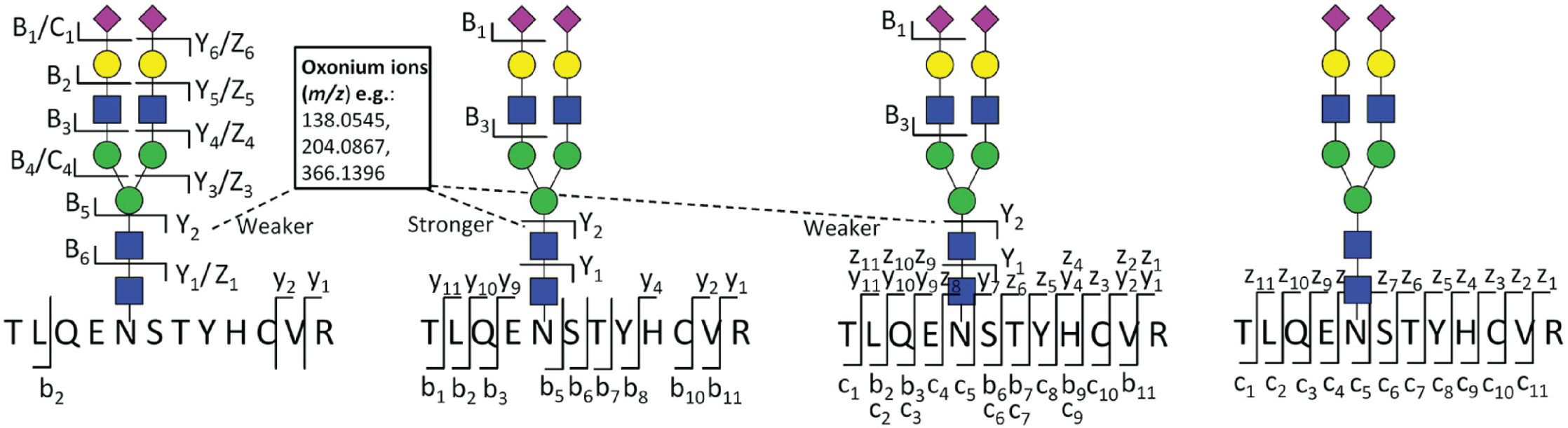
Glyco-DIA: a method for quantitative *O*-glycoproteomics with in silico-boosted glycopeptide libraries

Zilu Ye[1], Yang Mao[1,2], Henrik Clausen[1] and Sergey Y. Vakhrushev[1*]

GLYCOPEPTIDE TANDEM MS



GLYCOPEPTIDE FRAGMENTATION

Fragmentation type	Resonance activation CID	Beam-type CID / HCD	Electron transfer / higher-energy dissociation (EThcD)	Conventional ETD / ECD
Typical instrument platform	Ion trap (linear, 3D)	Q-TOFs, Orbitraps	Orbitrap Fusion/Lumos	Ion traps, Orbitraps, FT-ICR
Typical bond cleavages and fragment formation				
Glycopeptide information	▪ Glycan identification* ▪ Peptide mass identification	▪ Peptide identification** ▪ Partial glycan identification	▪ Peptide identification** ▪ Site identification ▪ Partial glycan identification	▪ Peptide identification ▪ Site identification ▪ Glycan mass identification

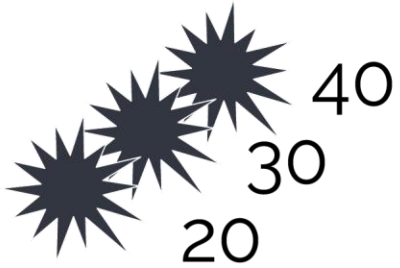
Thaysen-Andersen et al., MCP, 2016, 15: 10.1074/mcp.O115.057638, 1773–1790

GLYCOPEPTIDE FRAGMENTATION

Fragmentation methods affect instrumentation decisions

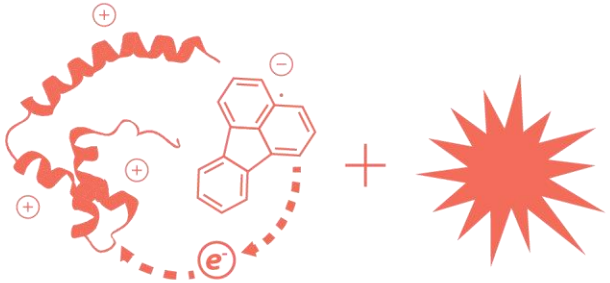
HCD

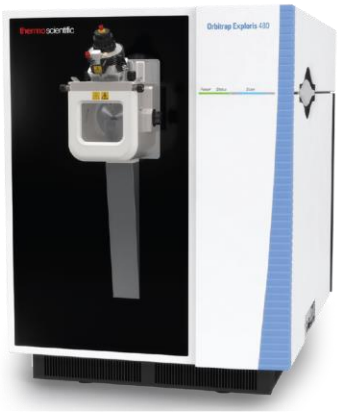
sceHCD



ETD

EThcD





Orbitrap Exploris 480 (Thermo)

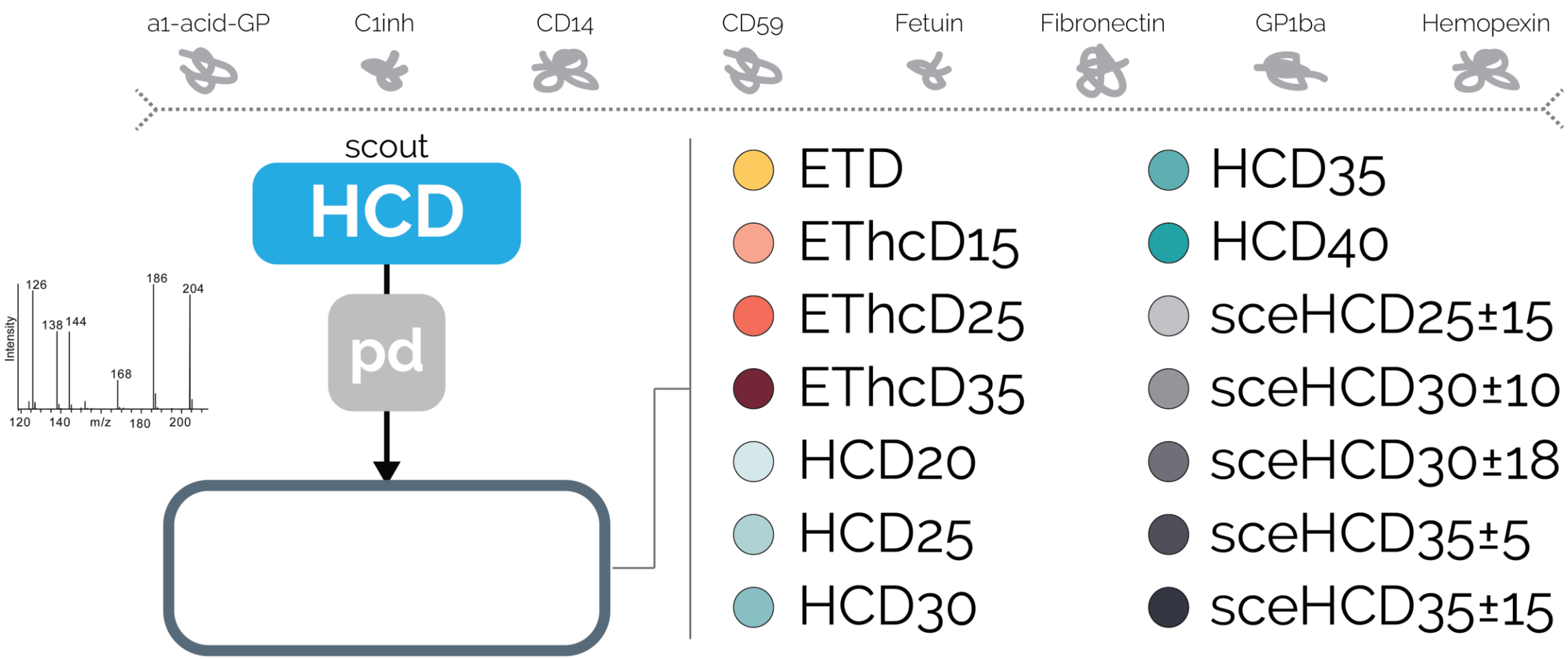
timsTOF Pro (Bruker)

Orbitrap Eclipse (Thermo)

solariX XR (Bruker)

Synapt G2-Si (Waters)

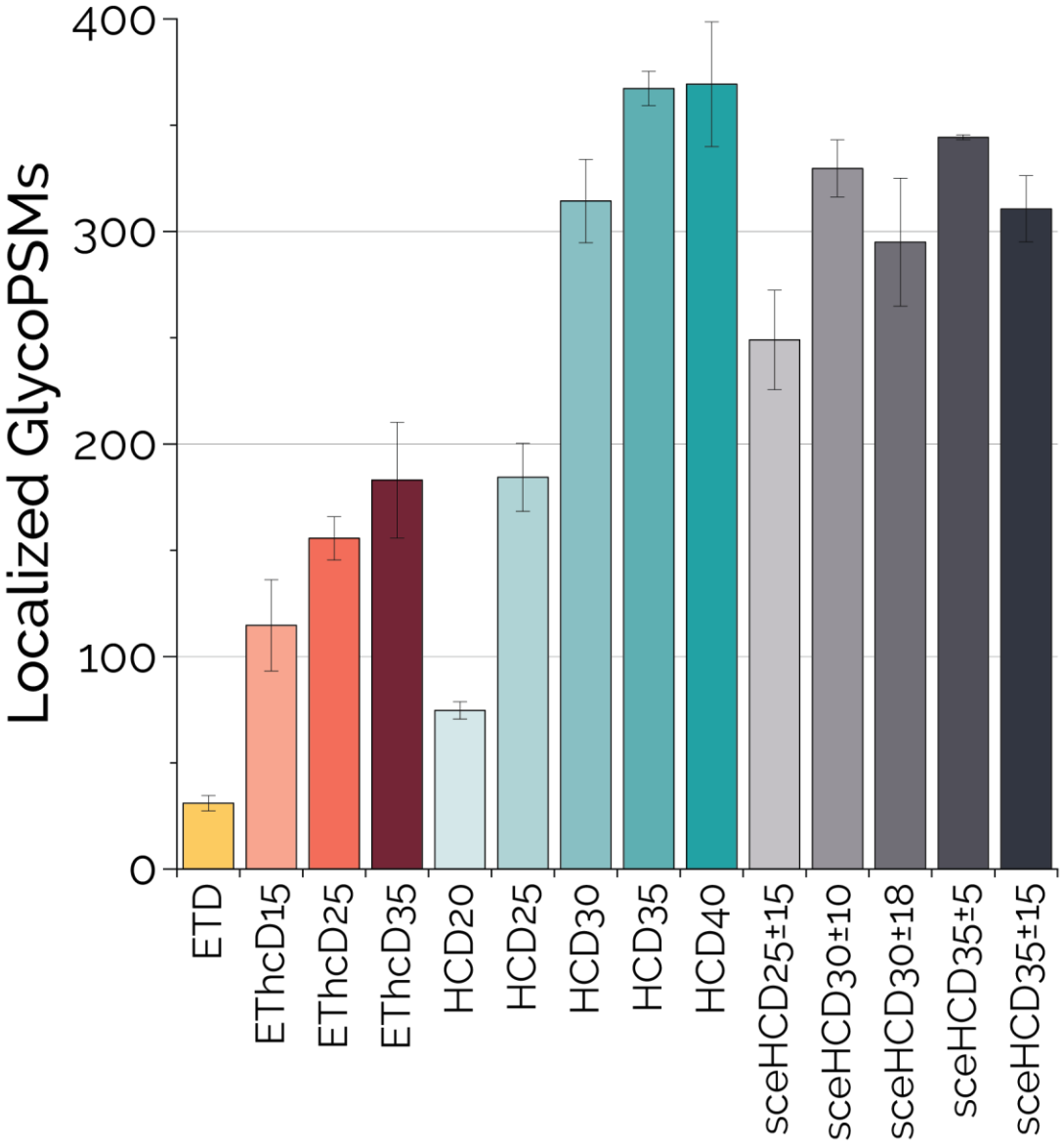
GLYCOPEPTIDE FRAGMENTATION



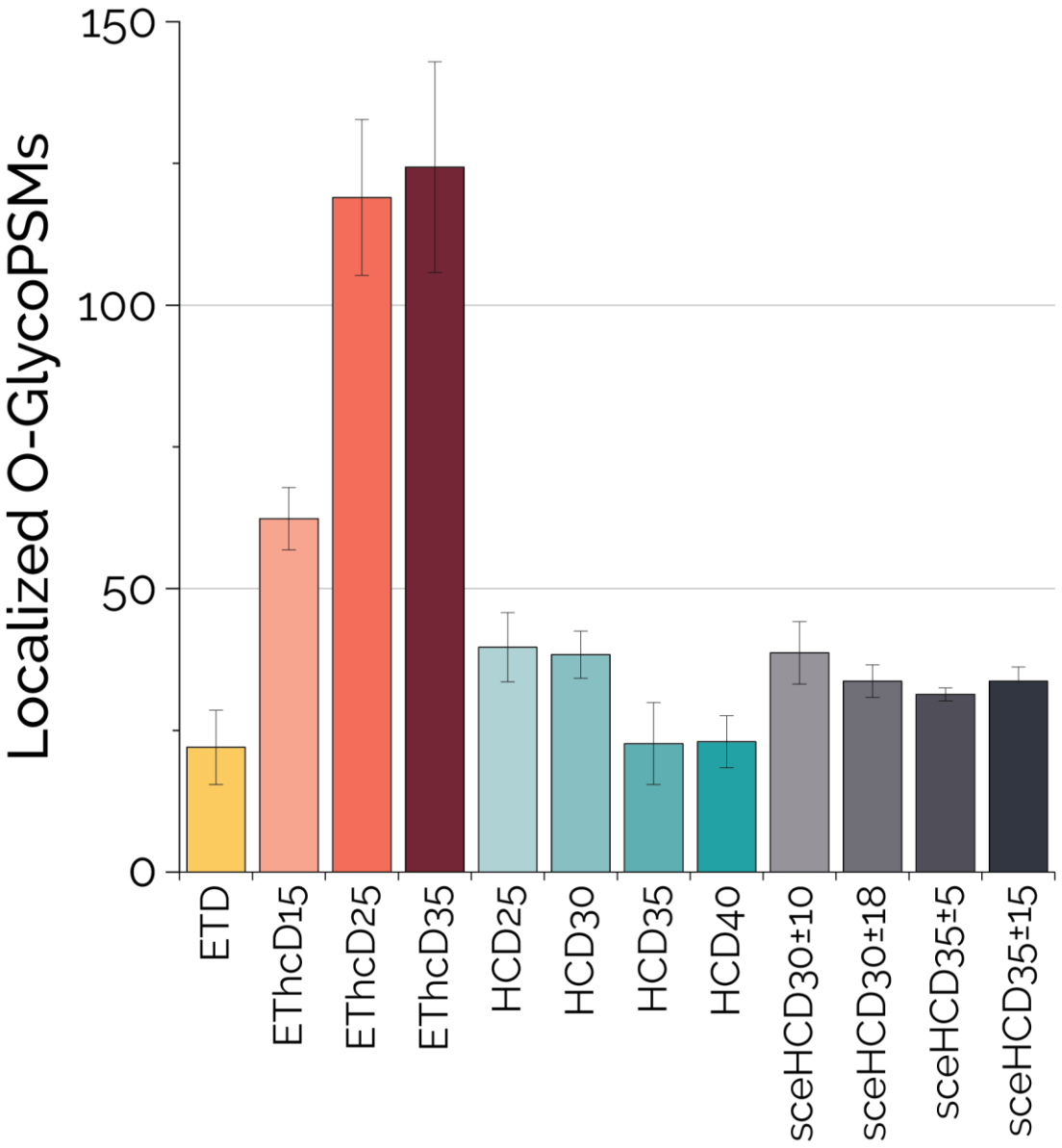
FRAGMENTATION SCORECARD

	ETD	EThcD15	EThcD25	EThcD35	HCD20	HCD25	HCD30	HCD35	HCD40	sceHCD25±15	sceHCD30±10	sceHCD30±18	sceHCD35±5	sceHCD35±15
Acquisition Speed														
Peptide Fragmentation														
Glycan Fragmentation														
Localization														
Use for N-glycopeptides														
Use for O-glycopeptides														

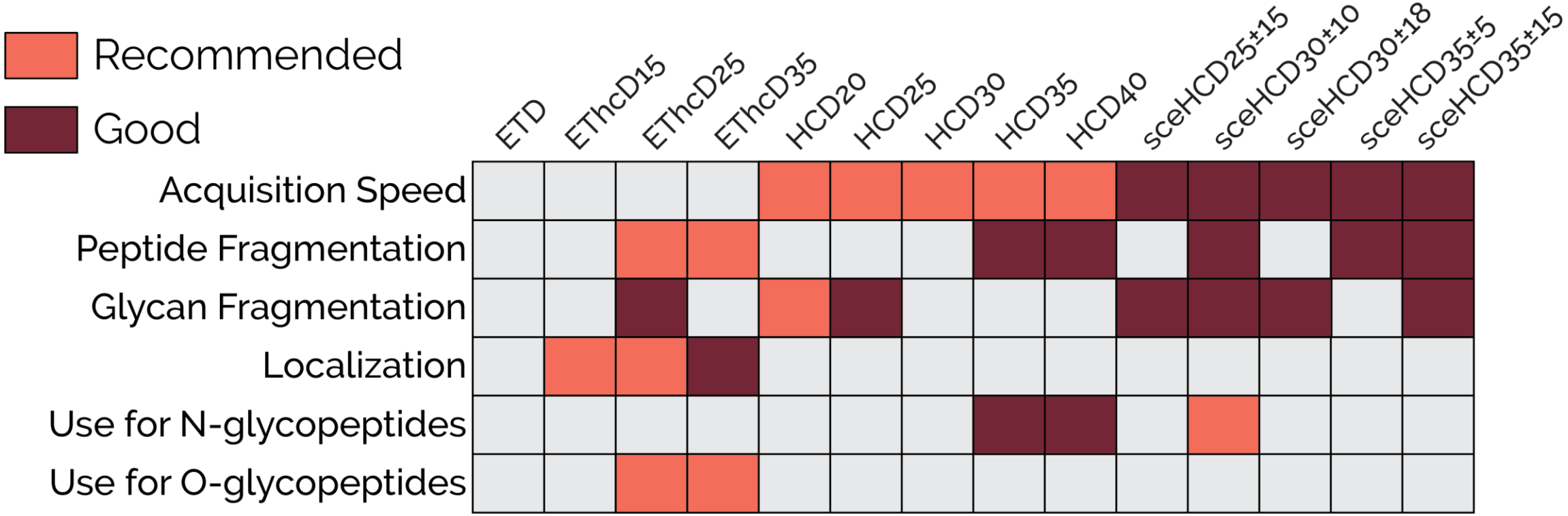
N-GLYCOPEPTIDE FRAGMENTATION



O-GLYCOPEPTIDE FRAGMENTATION



FRAGMENTATION SCORECARD



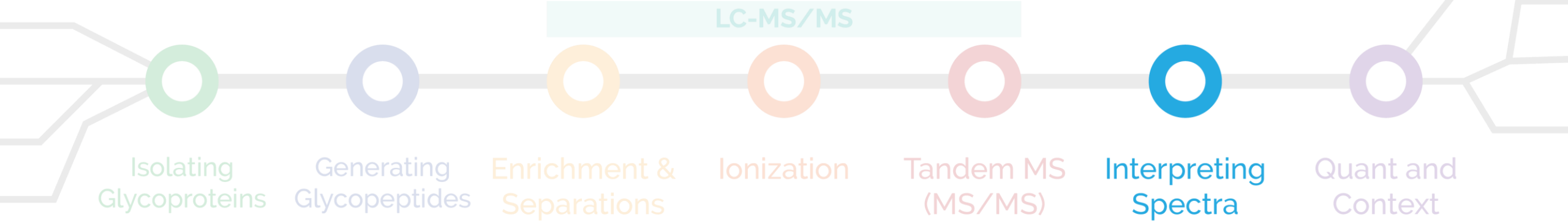
pubs.acs.org/jpr Article

Optimal Dissociation Methods Differ for *N*- and *O*-Glycopeptides

Nicholas M. Riley, Stacy A. Malaker, Marc D. Driessen, and Carolyn R. Bertozzi*

Cite This: https://dx.doi.org/10.1021/acs.jproteome.0c00218 Read Online

GLYCOINFORMATICS



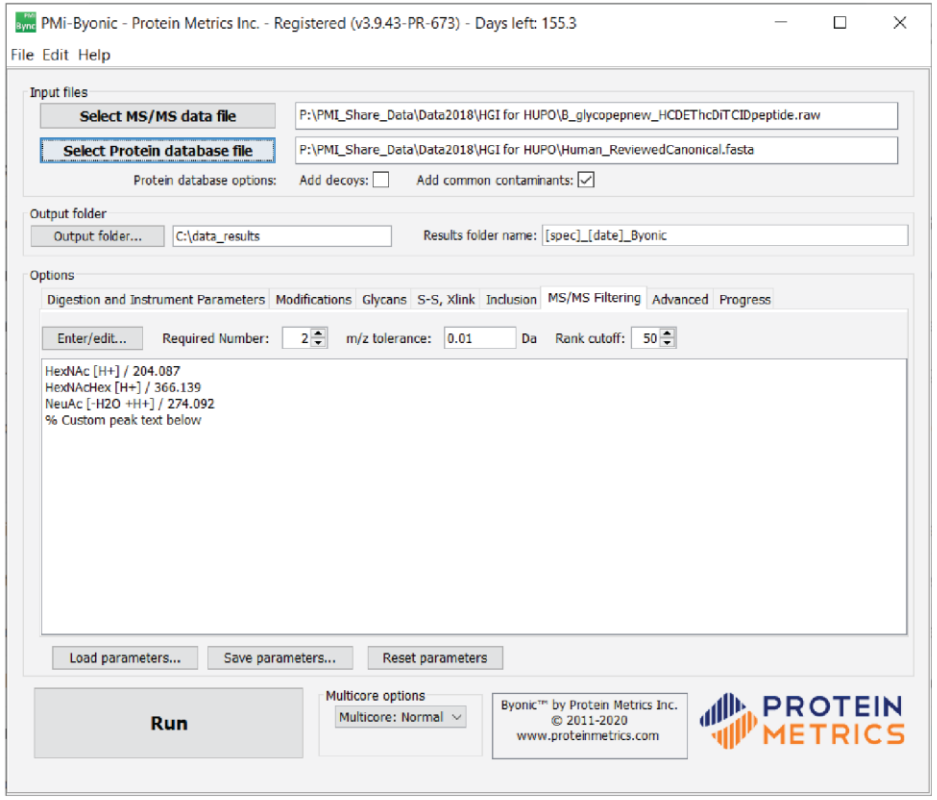
Byonic	pGlyco2.0
Available through Stanford (Protein Metrics)	Free (Academic software)
GUI with viewing options	GUI with limited (but still usable) options
Flexible with fragmentation methods	Designed for sceHCD (more coming soon)
Compatible with N- and O-glycopeptides	Generally only works for N-glycopeptides

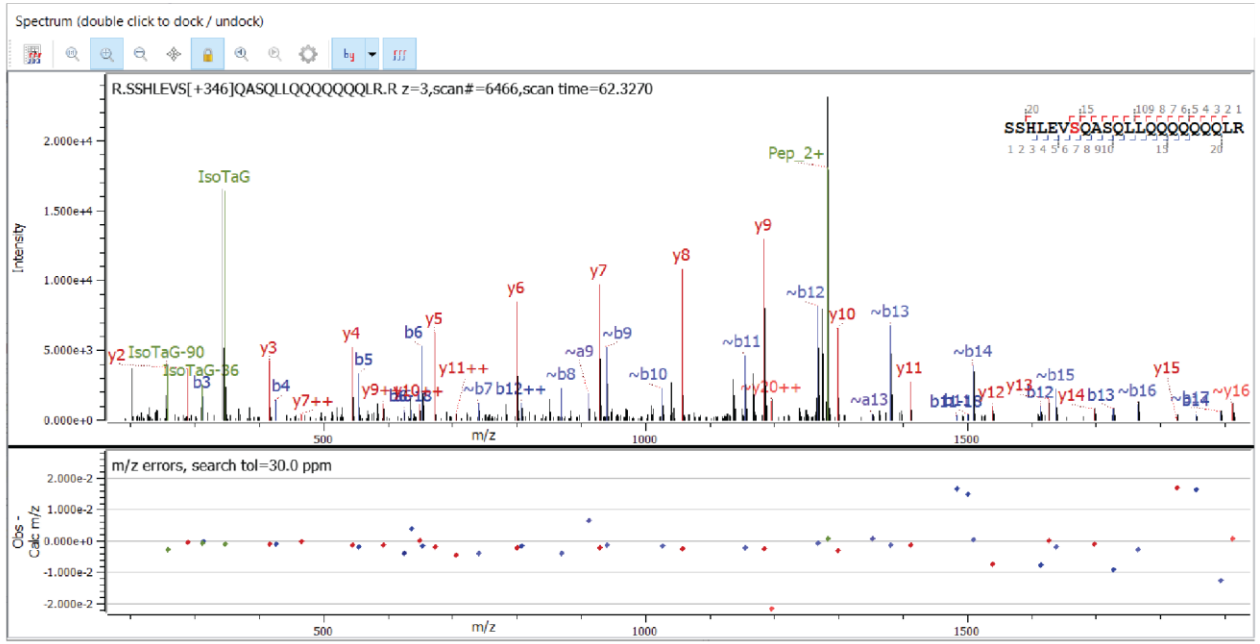
RECENT BYONIC UPGRADES

MCP | MOLECULAR & CELLULAR PROTEOMICS

Peak Filtering, Peak Annotation, and Wildcard Search for Glycoproteomics

Abhishek Roushan, Gary M. Wilson, Doron Kletter, K. Ilker Sen, Wilfred Tang,
Yong J. Kil, Eric Carlson, Marshall Bern*





VARIABLE MOD SEARCHING

Current Canon:

Peptide-centric
DB generation

different theoretical spectra
for each PTM considered

O-glycopeptide from CD43 (leukosialin)

TGSLEPSSGASGPQVSSVK

7 potential O-glycosites,
allow up to 3 to be modified:

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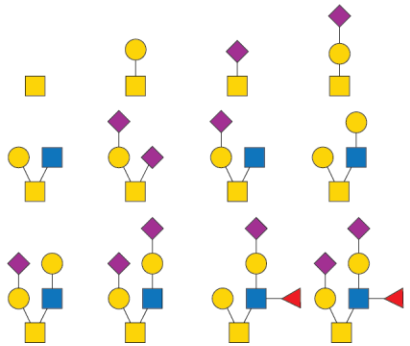
+



12 common
O-glycans

>60,000
combinations to consider

...for one peptide

OPEN MOD SEARCHES

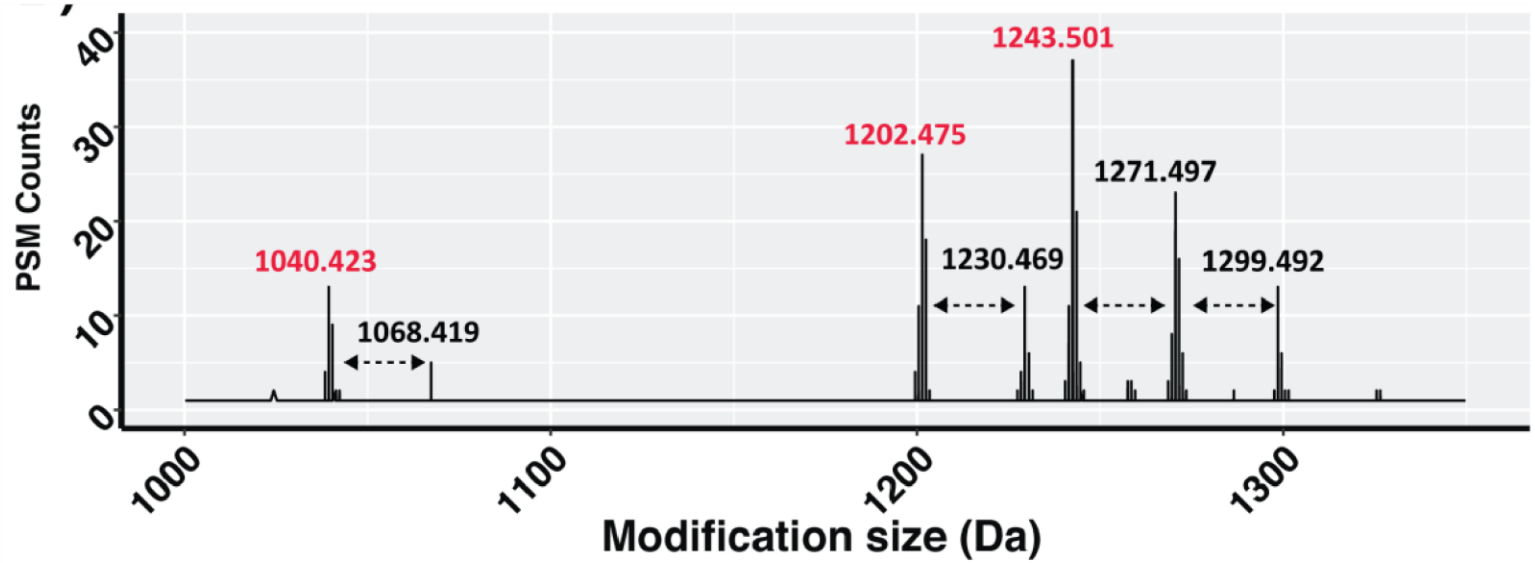
Identify peptide candidates without pre-determined modifications

OPEN MOD SEARCHES WITH BYONIC

MCP MOLECULAR & CELLULAR PROTEOMICS

Open database searching enables the identification and comparison of bacterial glycoproteomes without defining glycan compositions prior to searching

Ameera Raudah Ahmad Izaham[1] and Nichollas E. Scott[1#]



OPEN MOD SEARCHES WITH OTHER PLATFORMS

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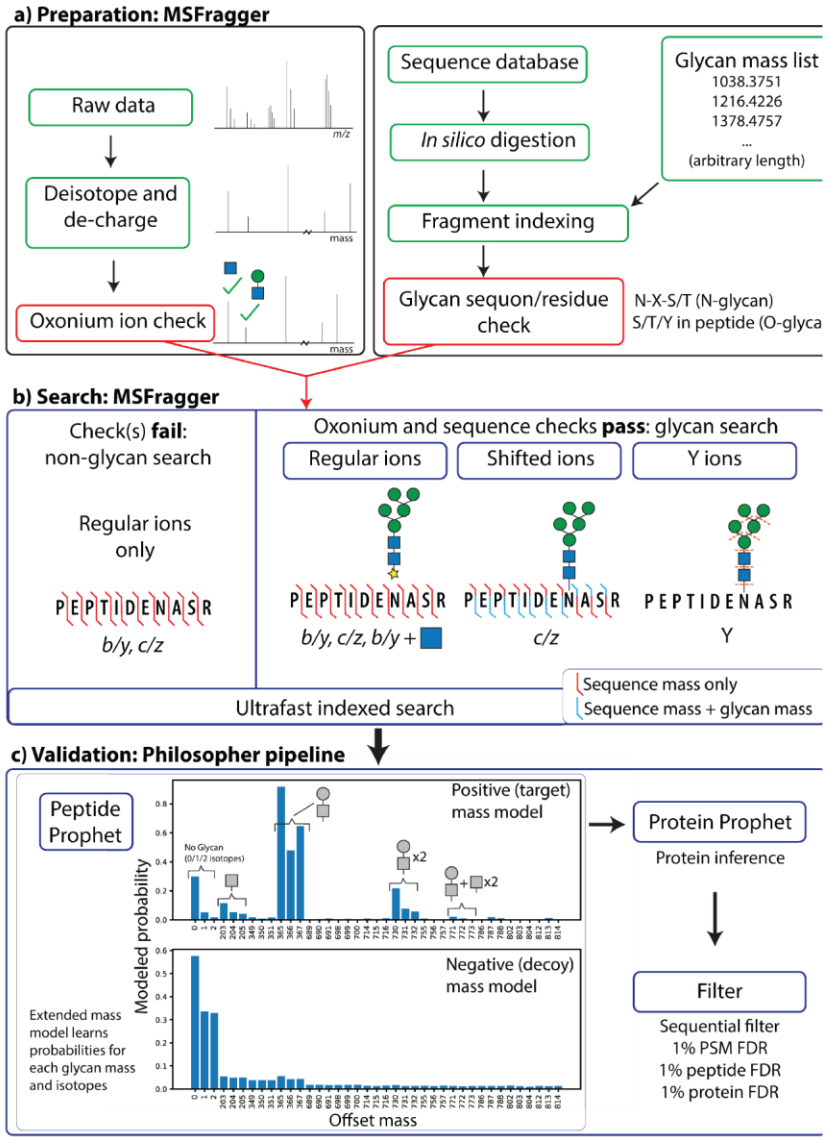
THE PREPRINT SERVER FOR BIOLOGY

Fast and Comprehensive N- and O-glycoproteomics analysis with MSFragger-Glyco

Daniel A. Polasky[1], Fengchao Yu[1], Guo Ci Teo[1], Alexey I. Nesvizhskii*[1,2]



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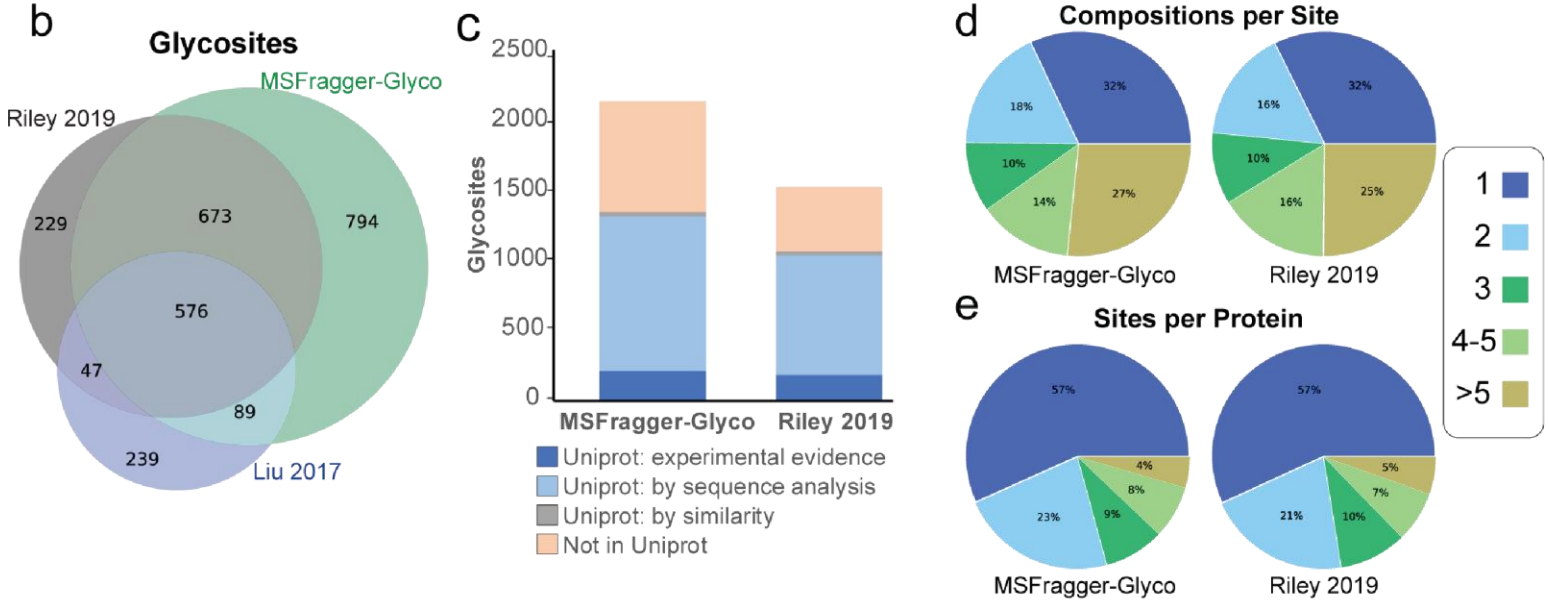
a

Search	Compositions searched	GlycoPSMs	Unique glycopeptides	Unique glycoproteins	Unique glycosites
MSFragger	182	44,187	2,822	1,070	2,133
Riley 2019	182	24,099	1,803	771	1,545

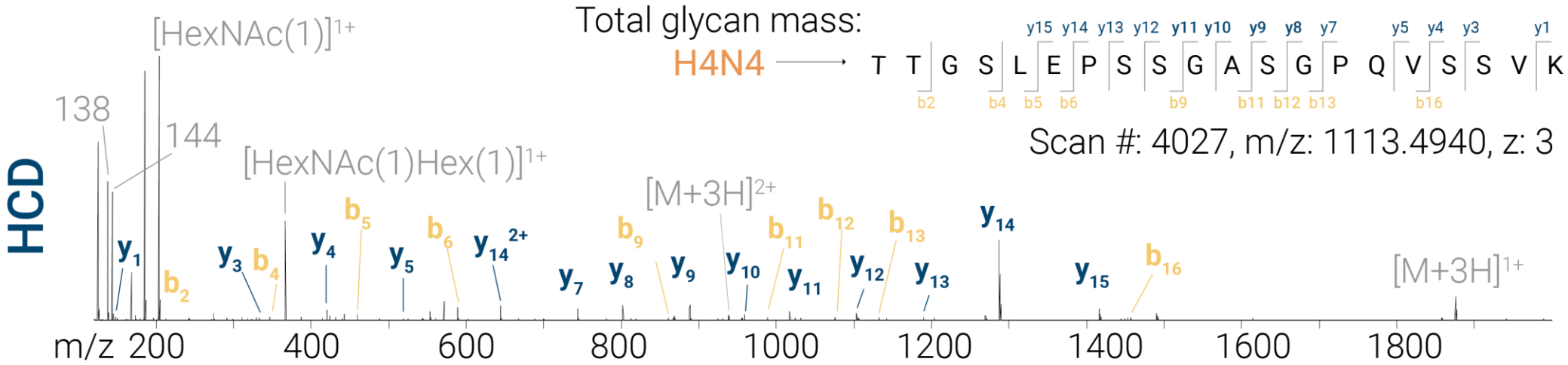


O-PAIR SEARCH FOR O-GLYCOPEPTIDES

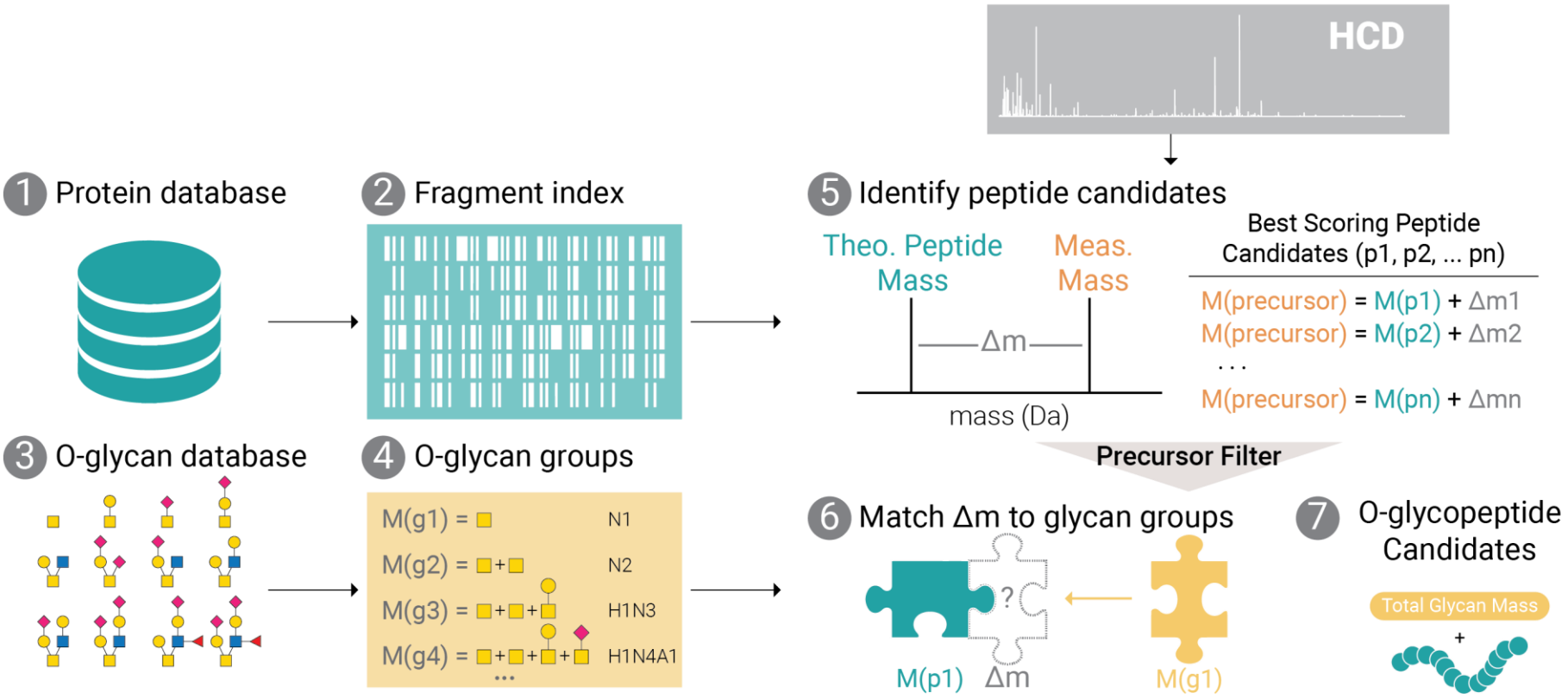
Identify peptide candidates without pre-determined modifications

Collision-based dissociation

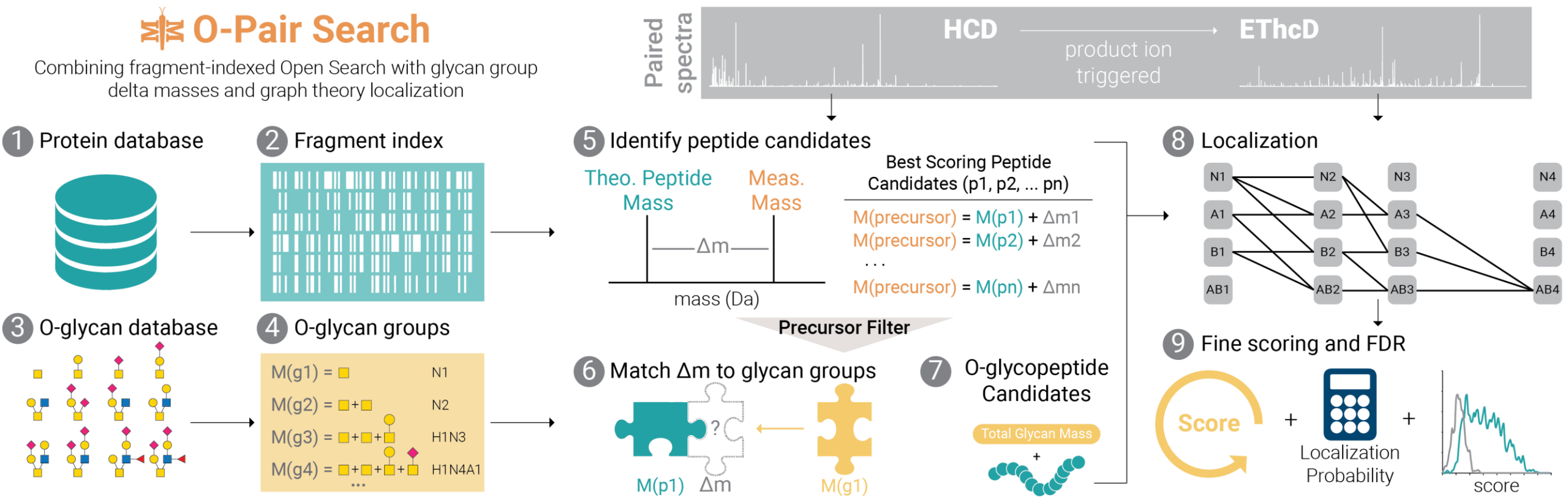
Higher-energy collisional dissociation (HCD)



O-PAIR SEARCH FOR O-GLYCOPEPTIDES



O-PAIR SEARCH FOR O-GLYCOPEPTIDES

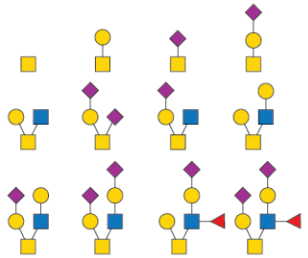


More information:

bioRxiv Pre-Print: https://www.biorxiv.org/content/10.1101/2020.05.18.102327v1: provisionally accepted at Nature Methods

O-PAIR SEARCH PERFORMANCE

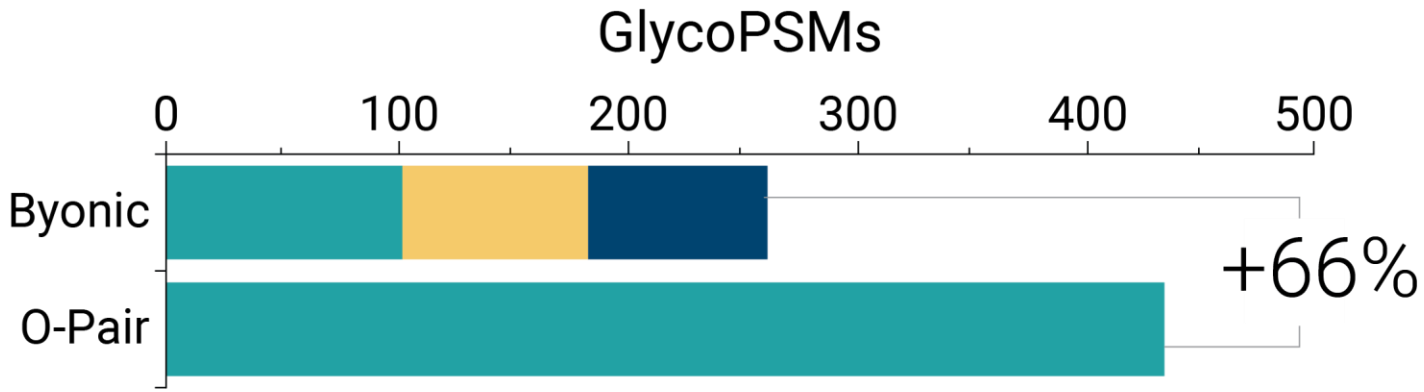
12 O-glycan DB



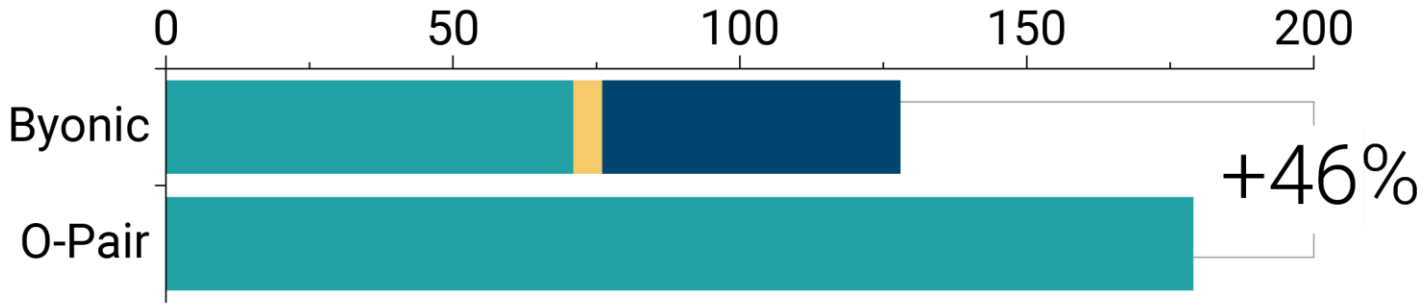
Mucins
MUC16
CD43
Podocalyxin
PSGL-1

Riley et al., JPR, 19 (8): 3286–3301 (2020)

Glycans per peptide	Byonic Time (min)	O-Pair Time (min)	Speed Improvement	Localized Glycosites (Byonic / O-Pair)
2glycans	16.26	0.36	**45.24**	33 / 76
3glycans	862.76	0.40	**2,161.09**	41 / 120
4glycans	DNF	0.52	NA	NA / 134



All



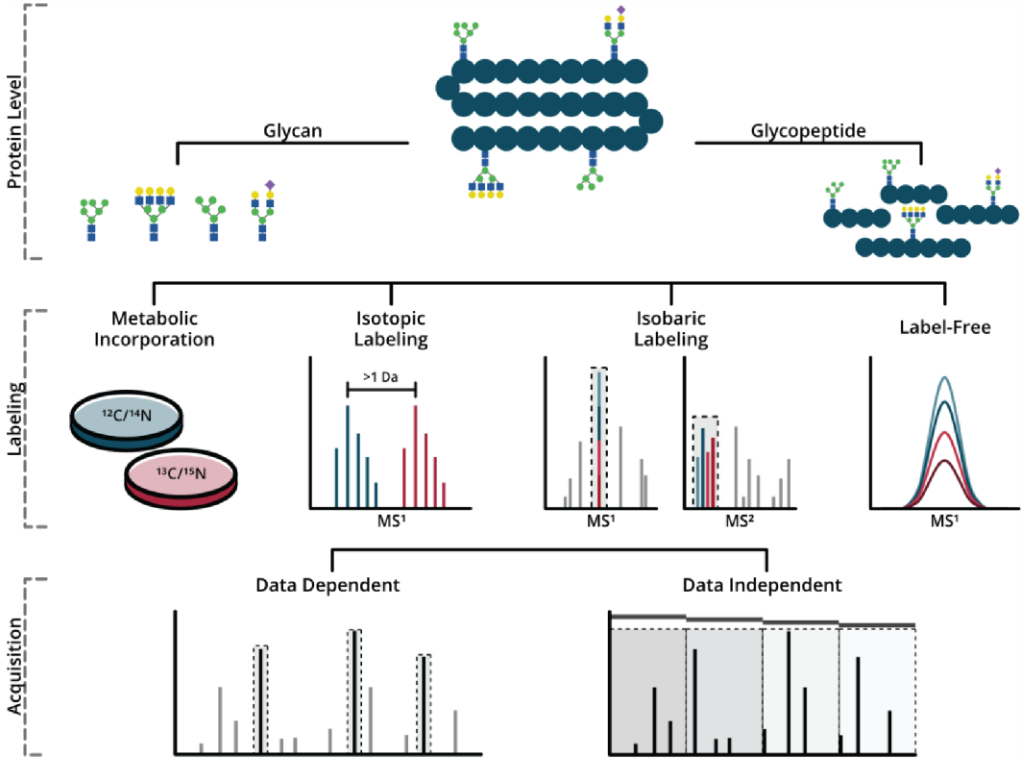
Localized
(Level 1 and 1b)

HCD-EThcD pair
HCD alone
EThcD alone

GLYCOPEPTIDE QUANT

LC-MS/MS

Isolating Glycoproteins | Generating Glycopeptides | Enrichment & Separations | Ionization | Tandem MS (MS/MS) | Interpreting Spectra | Quant and Context



MCP MOLECULAR & CELLULAR PROTEOMICS

Recent Advances in Analytical Approaches for Glycan and Glycopeptide Quantitation

Daniel G. Delafield[1] and Lingjun Li[1,2,*]

GLYCOPEPTIDE QUANT: NOT STRAIGHTFORWARD

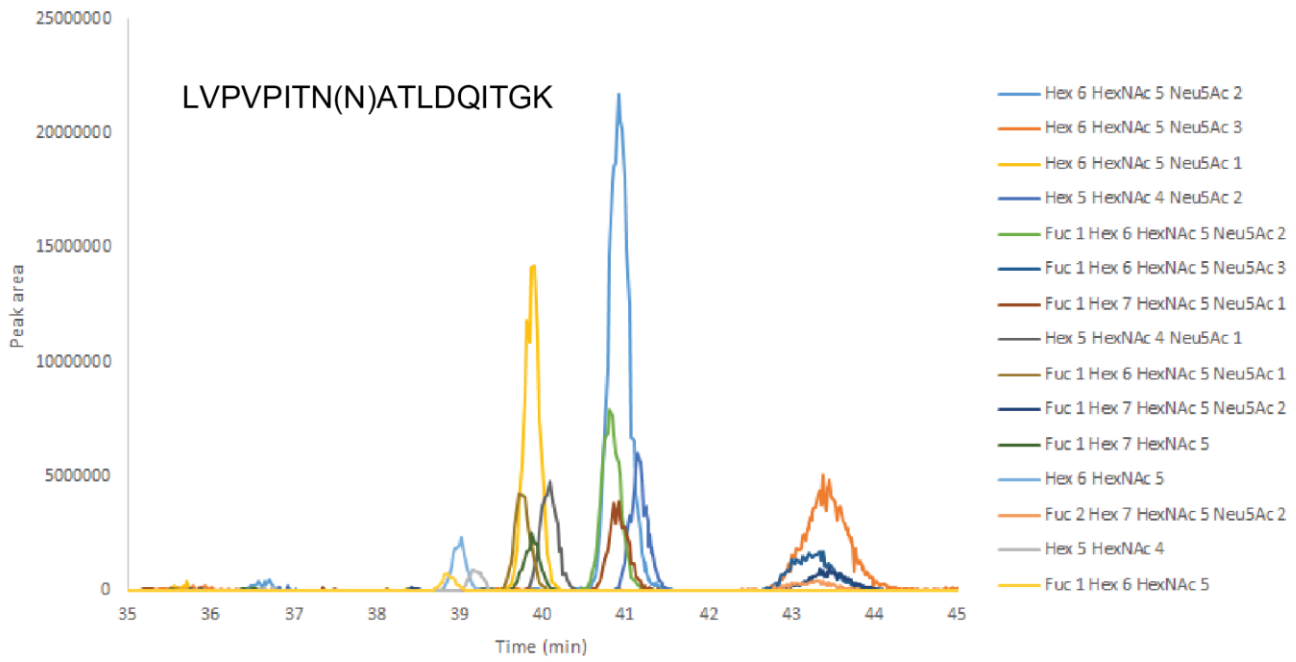
LC-MS/MS

Isolating Glycoproteins | Generating Glycopeptides | Enrichment & Separations | Ionization | Tandem MS (MS/MS) | Interpreting Spectra | Quant and Context

MCP MOLECULAR & CELLULAR PROTEOMICS

Calculating glycoprotein similarities from mass spectrometric data

William Hackett[1] and Joseph Zaia[1,2]



ISOBARIC LABELING ALSO HAS CHALLENGES

Multiplexed Comparative Analysis of Intact Glycopeptides Using Electron-Transfer Dissociation and Synchronous Precursor Selection Based Triple-Stage Mass Spectrometry

Hongbin Zhu,* Chen Qiu, Connie M. Gryniewicz-Ruzicka, David A. Keire, and Hongping Ye

Cite This: *Anal. Chem.* 2020, 92, 7547–7555

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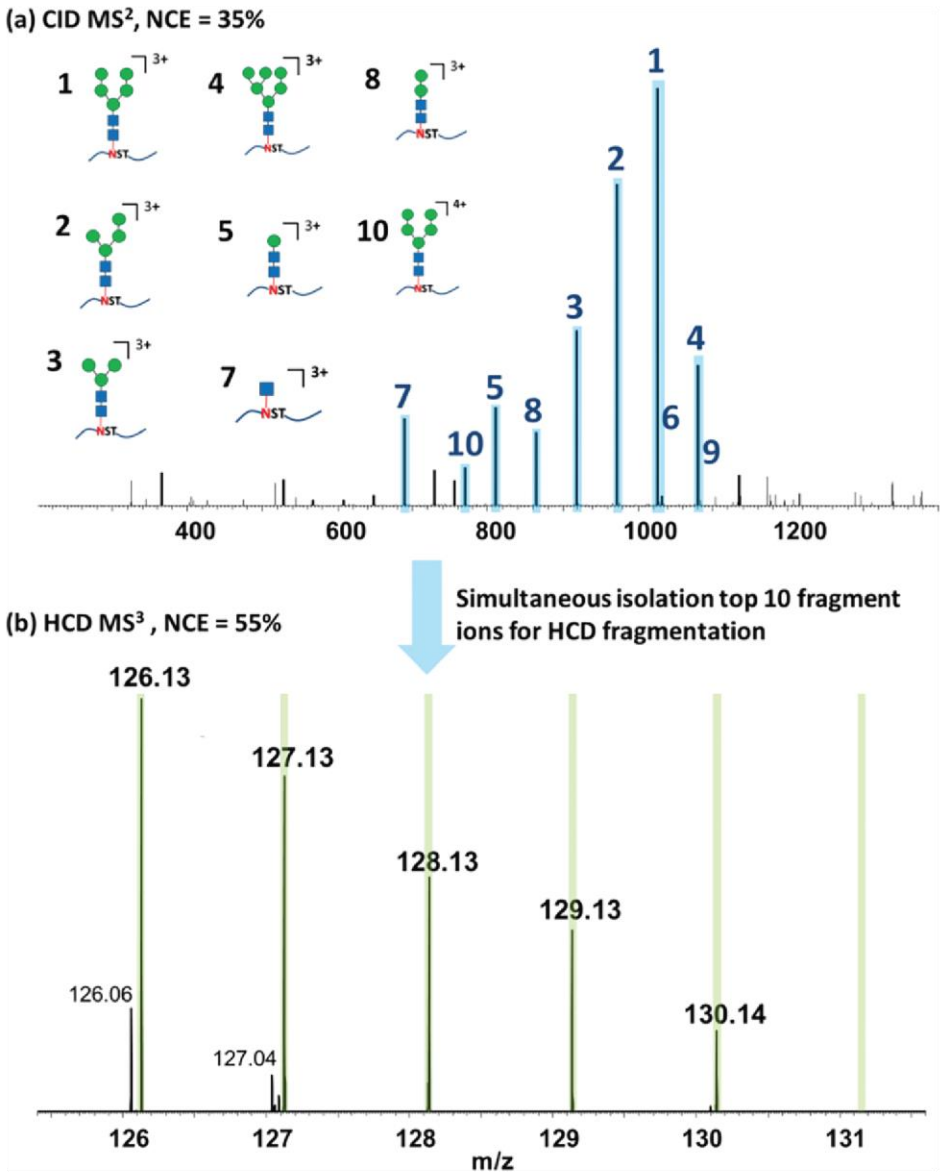

ISOBARIC LABELING ALSO HAS CHALLENGES

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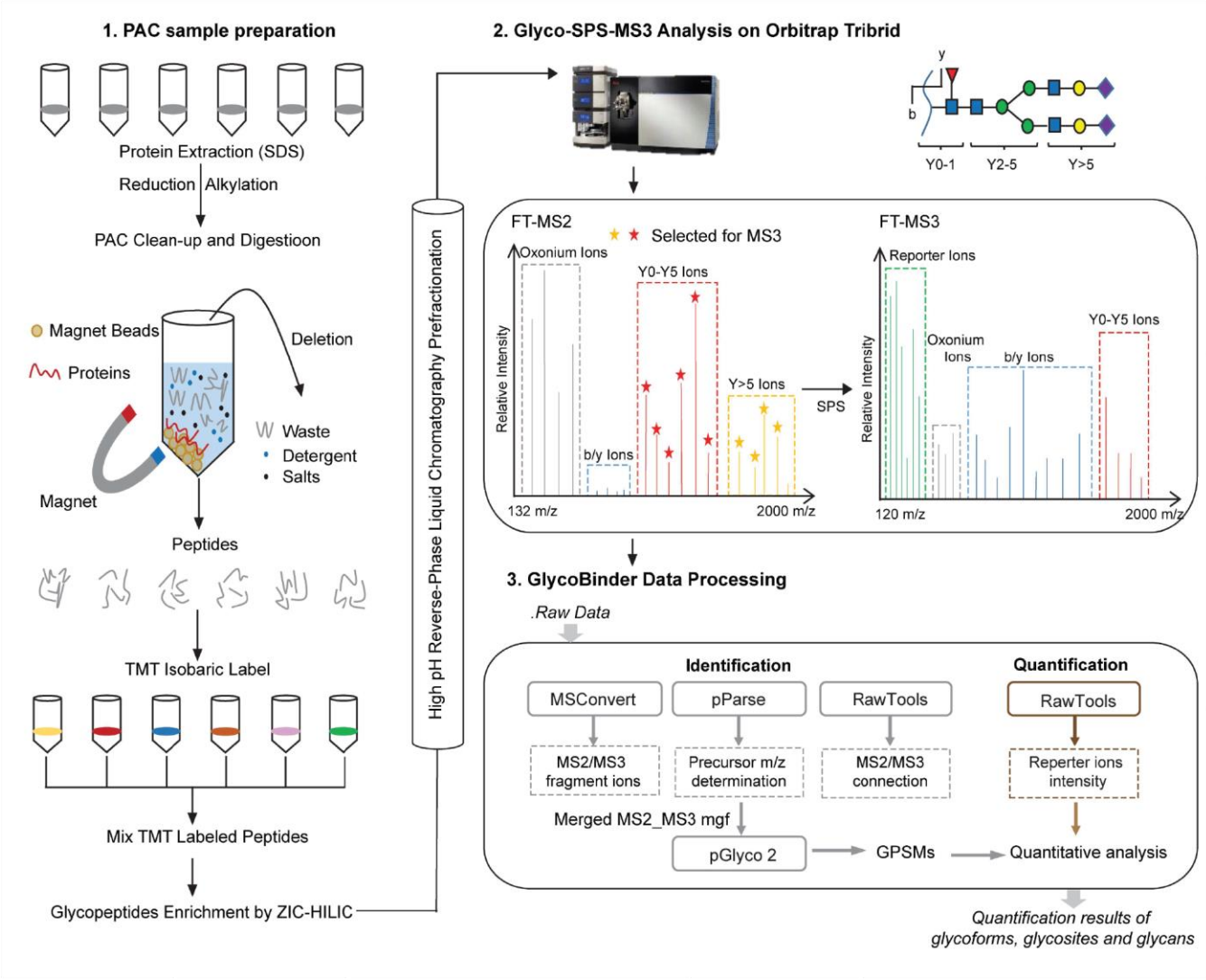
SugarQuant: a streamlined pipeline for multiplexed quantitative site-specific N-glycoproteomics

Pan Fang[1*], Yanlong Ji[1, 2, §*], Ivan Silbern[1, 3], Carmen Doebele[2], Momchil Ninov[1], Christof Lenz[1,3], Thomas Oellerich[2, 4, 5], Kuan-Ting Pan[1, §, ¶], Henning Urlaub[1, 3, ¶]



HOW TO REPRESENT DIMENSIONALITY?

Quantitative Longitudinal Inventory of the *N*-Glycoproteome of Human Milk from a Single Donor Reveals the Highly Variable Repertoire and Dynamic Site-Specific Changes

Jing Zhu,[⊥] Yu-Hsien Lin,[⊥] Kelly A. Dingess, Marko Mank, Bernd Stahl, and Albert J. R. Heck*

Cite This: *J. Proteome Res.* 2020, 19, 1941–1952

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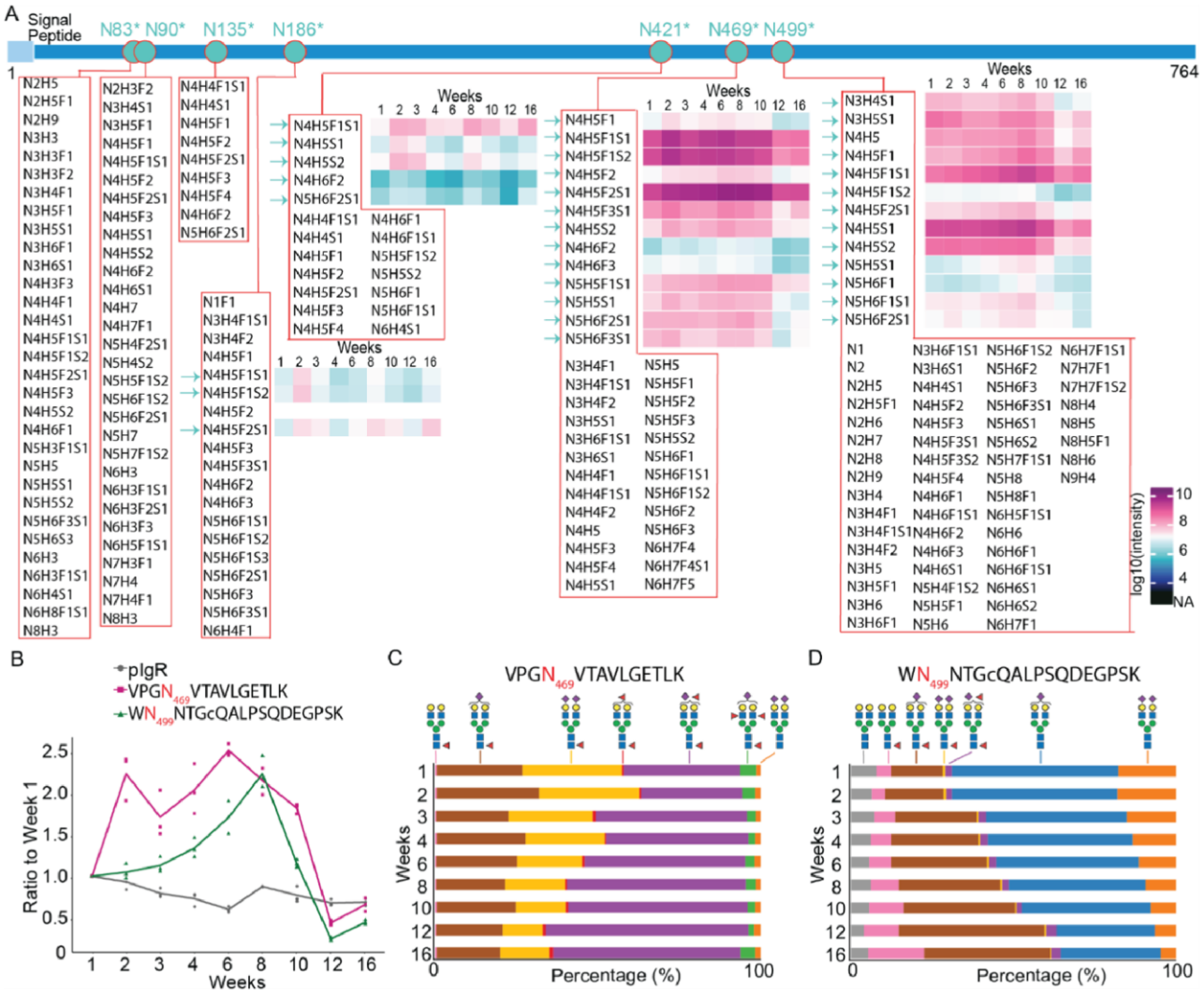
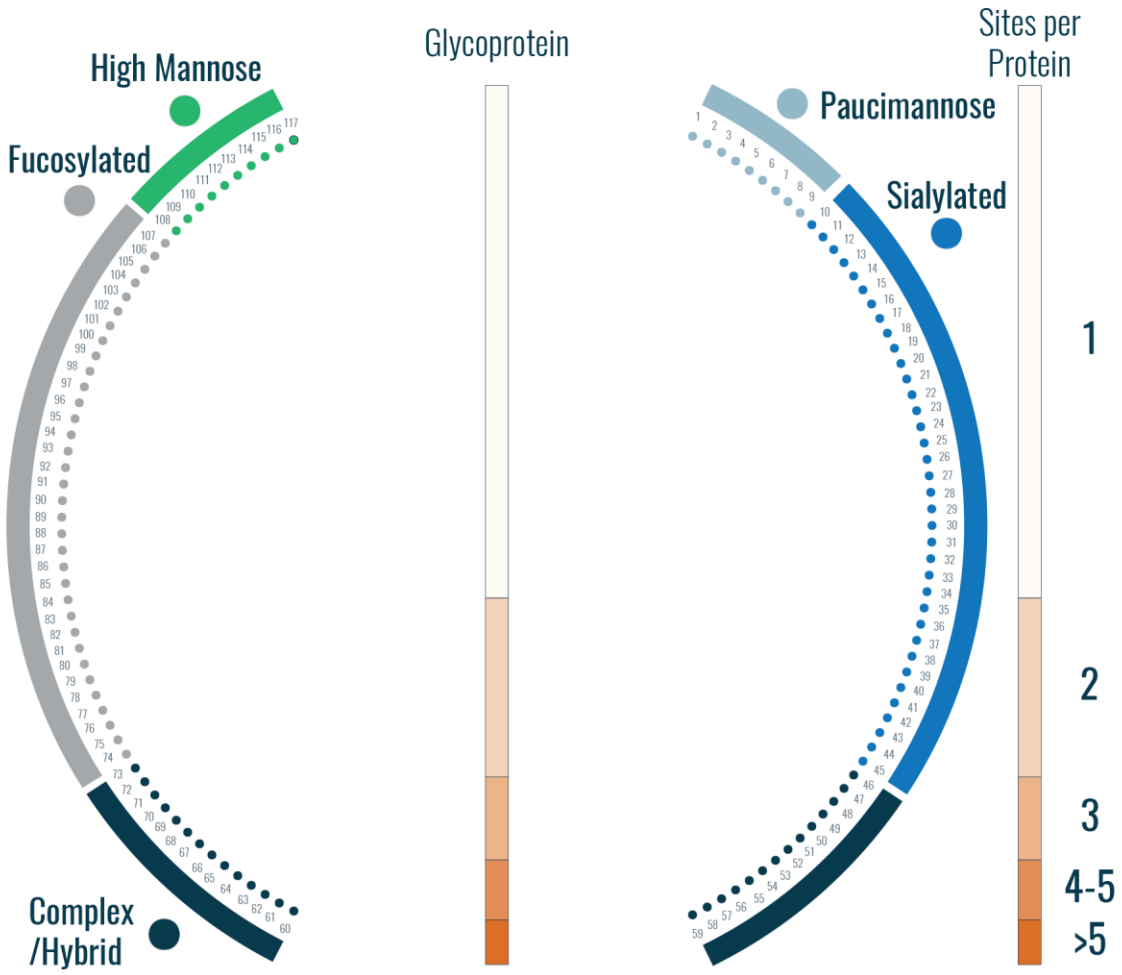


HOW TO REPRESENT DIMENSIONALITY?

Capturing site-specific heterogeneity with large-scale N-glycoproteome analysis

Nicholas M. Riley[1,2,5], Alexander S. Hebert[1], Michael S. Westphall[1] & Joshua J. Coon[1,2,3,4]

Glycoprotein-
Glycan Networks



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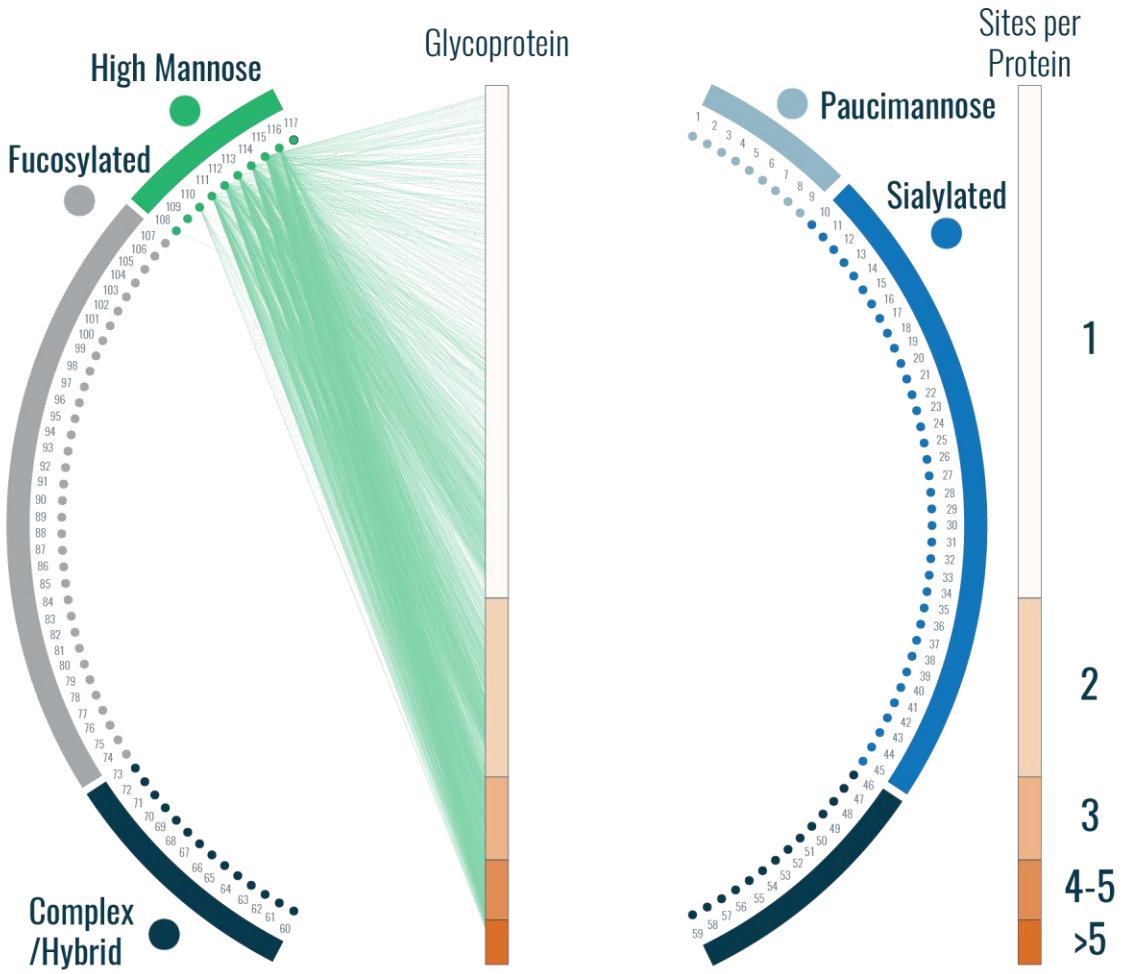

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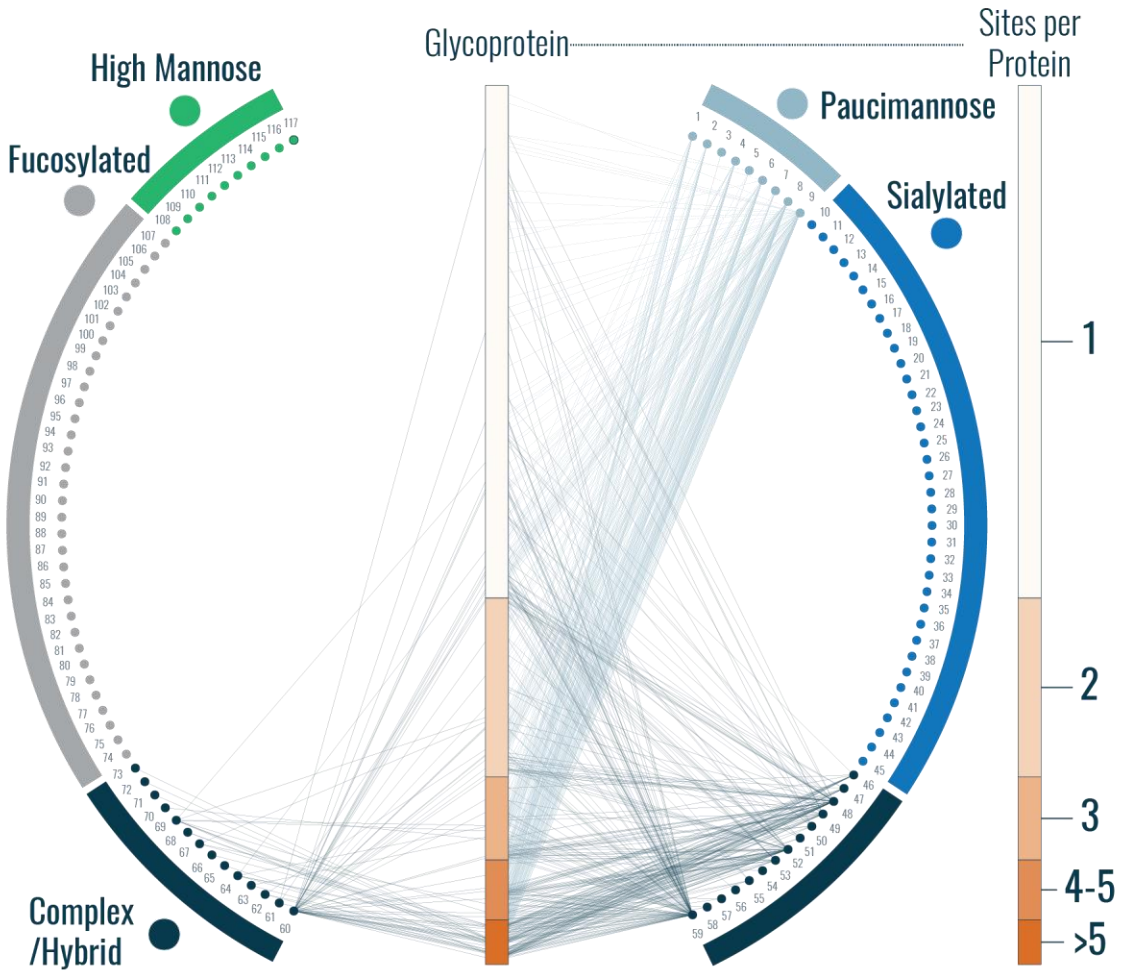

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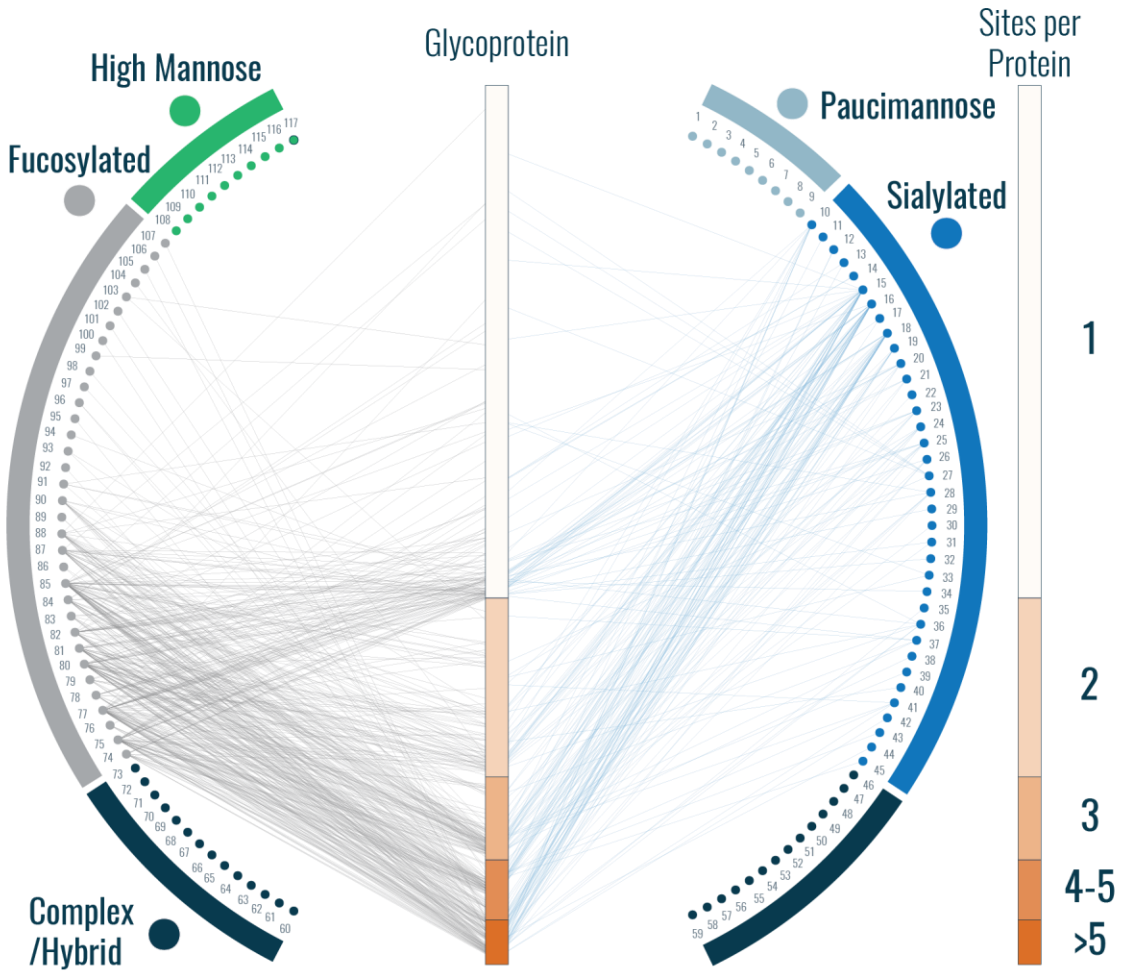

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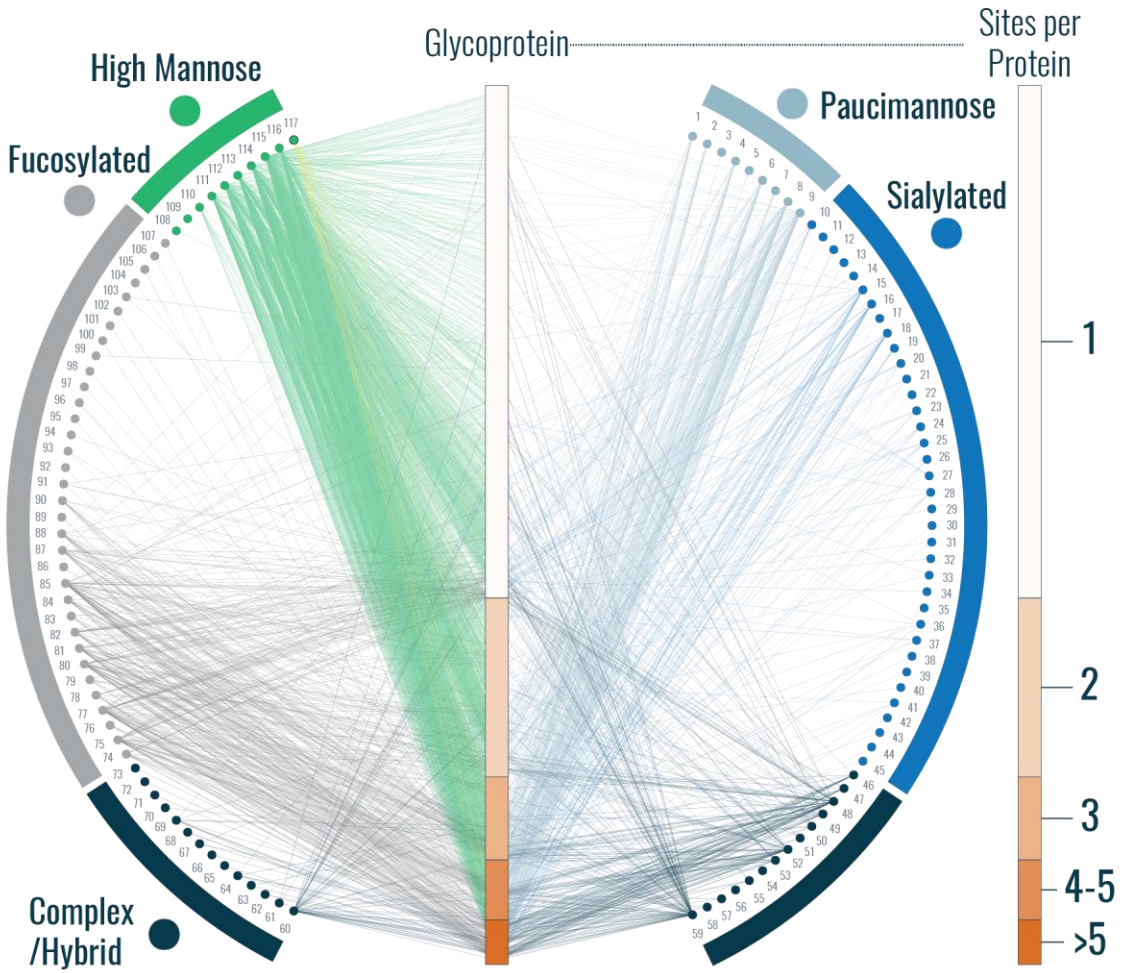

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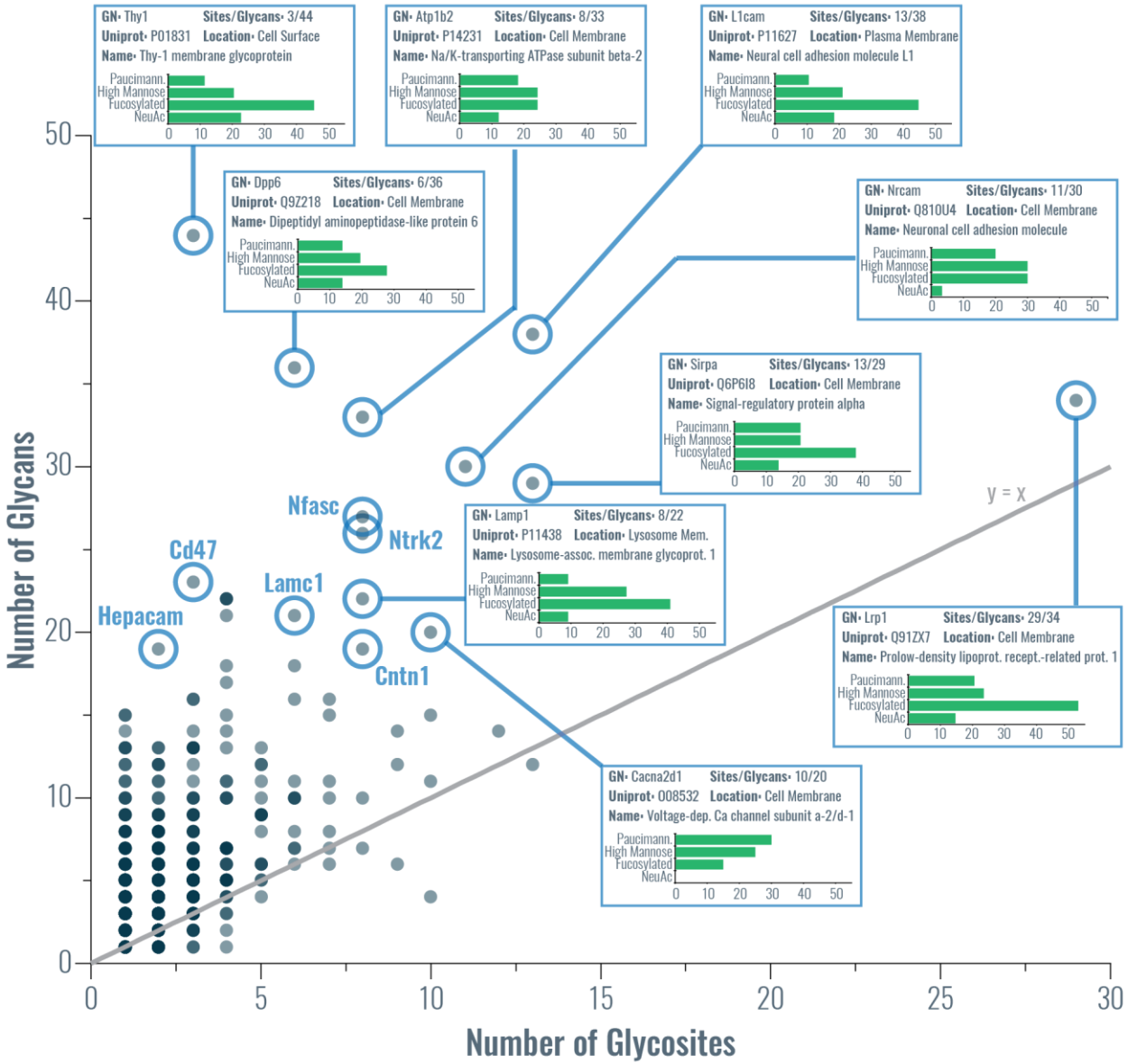

HOW TO REPRESENT DIMENSIONALITY?

Journal of proteome research
Cite This: J. Proteome Res. 2019, 18, 664−677
Article
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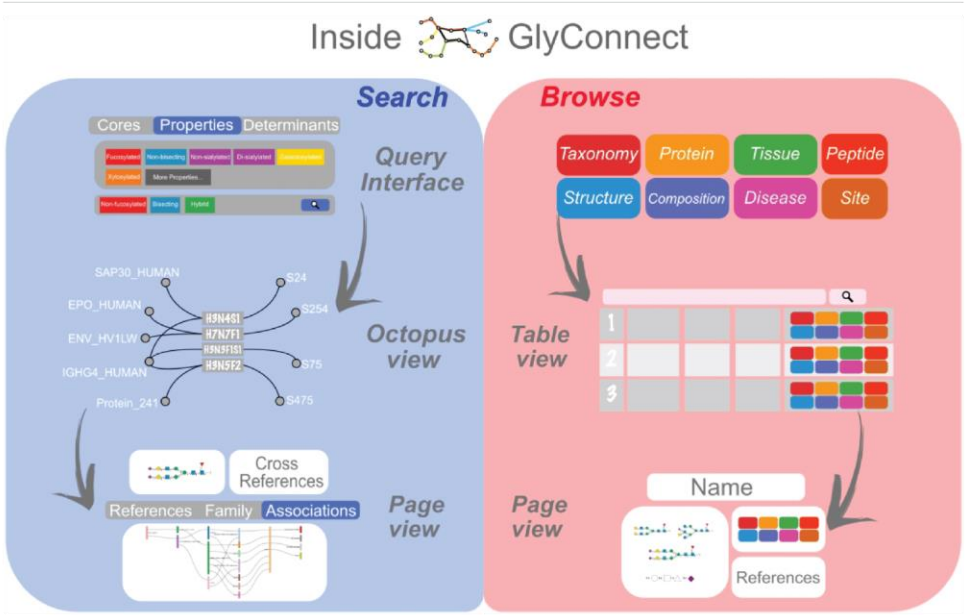
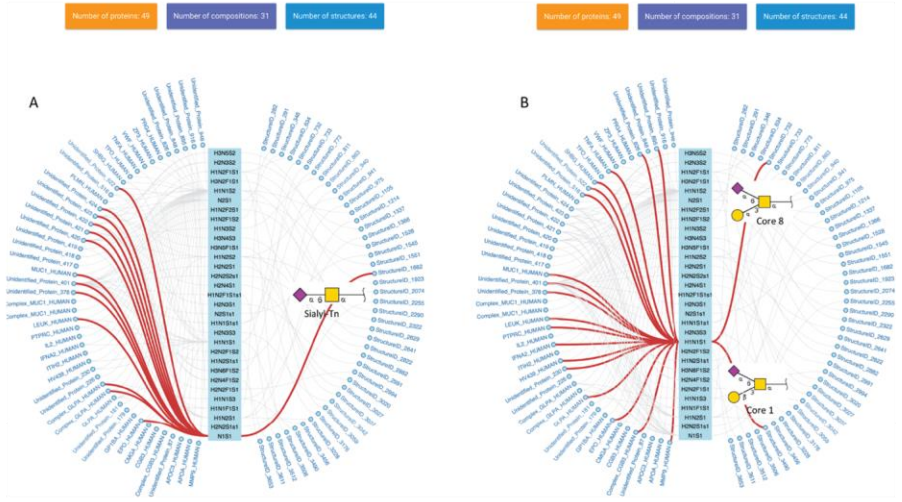
GlyConnect: Glycoproteomics Goes Visual, Interactive, and Analytical

Davide Alocci,[†,‡] Julien Mariethoz,[†,‡] Alessandra Gastaldello,[†,‡] Elisabeth Gasteiger,[§] Niclas G. Karlsson,[∥] Daniel Kolarich,[⊥,#] Nicolle H. Packer,[⊥,#,▽] and Frédérique Lisacek*[,†,‡,○]

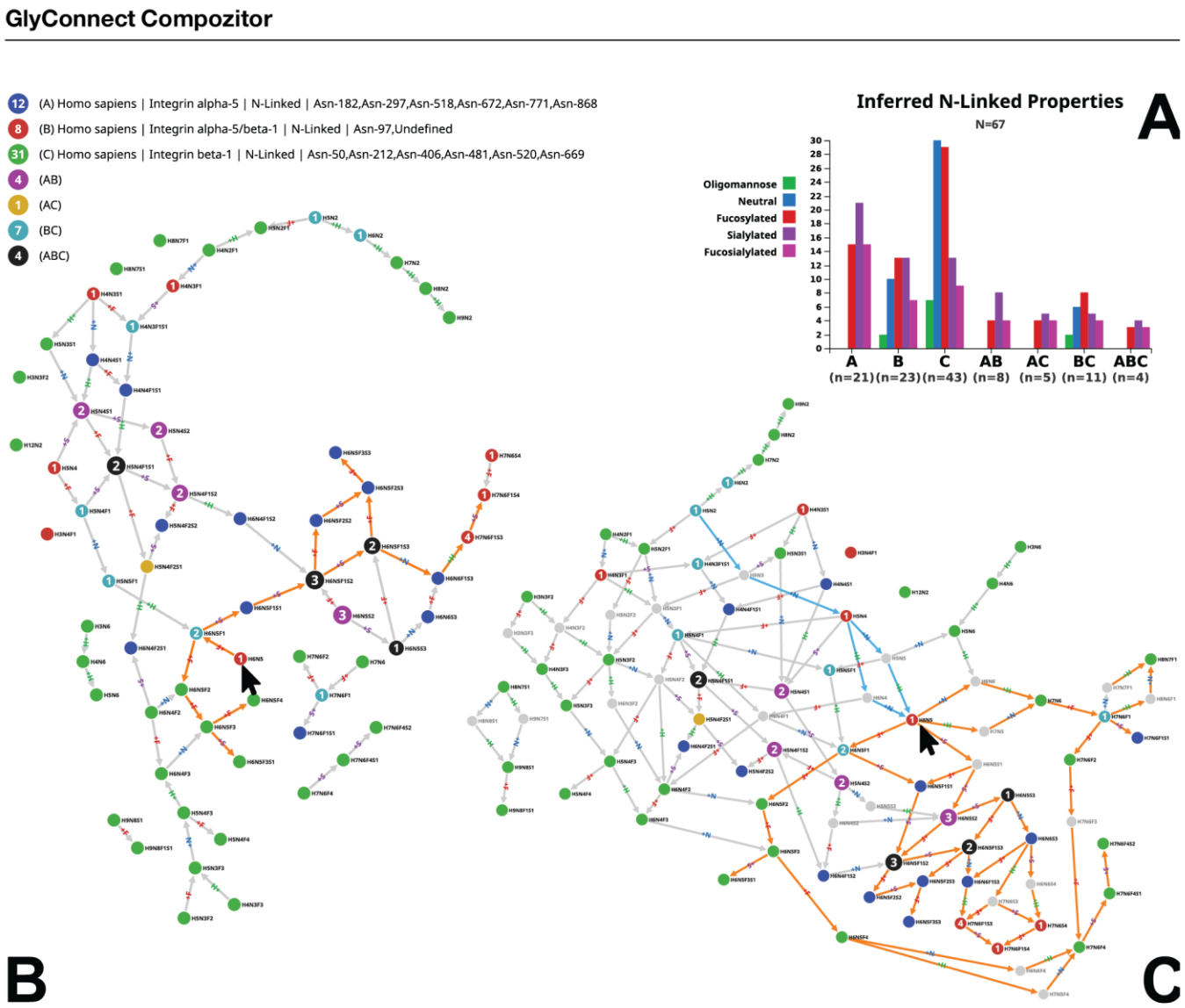




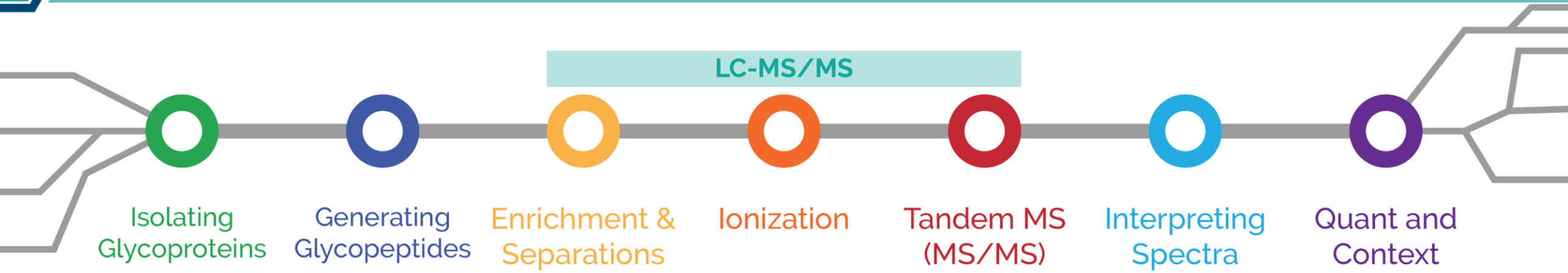
HOW TO REPRESENT DIMENSIONALITY?

Examining and Fine-tuning the Selection of Glycan Compositions with GlyConnect Compozitor

Thibault Robin[1,2,3,4], Julien Mariethoz[1,2], and Frédérique Lisacek[1,2,5,*]



CONCERTED EFFORT FOR INFORMATICS



Letter to Glyco-Forum

The GlySpace Alliance: toward a collaborative global glycoinformatics community

Kiyoko F Aoki-Kinoshita[1], Frederique Lisacek[2], Raja Mazumder[3], William S York[4] and Nicolle H Packer[5]

[1]Glycan & Life Science Integration Center (GaLSIC), Faculty of Science and Engineering, Soka University, 1-236 Tangi-machi, Hachioji, Tokyo, Japan, 192-8577, [2]Proteome Informatics Group, SIB Swiss Institute of Bioinformatics, Computer Science Department, University of Geneva, route de Drize 7, CH - 1227 Geneva Switzerland, and also Section of Biology, University of Geneva, Geneva, Switzerland, [3]Department of Biochemistry & Molecular Medicine, and Department of Medicine, School of Medicine and Health Sciences, George Washington University, Ross Hall, 2300 Eye St., NW, Washington, DC 20037, USA, [4]Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens, GA 30602, USA, and [5]Department of Molecular Sciences, Faculty of Science & Engineering, Rm 307, Building E8C, Macquarie University, Sydney, NSW 2109, Australia

[1]To whom correspondence should be addressed: Tel/Fax: +81-42-691-4116; e-mail: kkiyoko@soka.ac.jp

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Bertozzi Group

Allis Chien

Ryan Leib

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Wed-Thurs October 28-29, 2020
SUMS Research Application Symposium

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