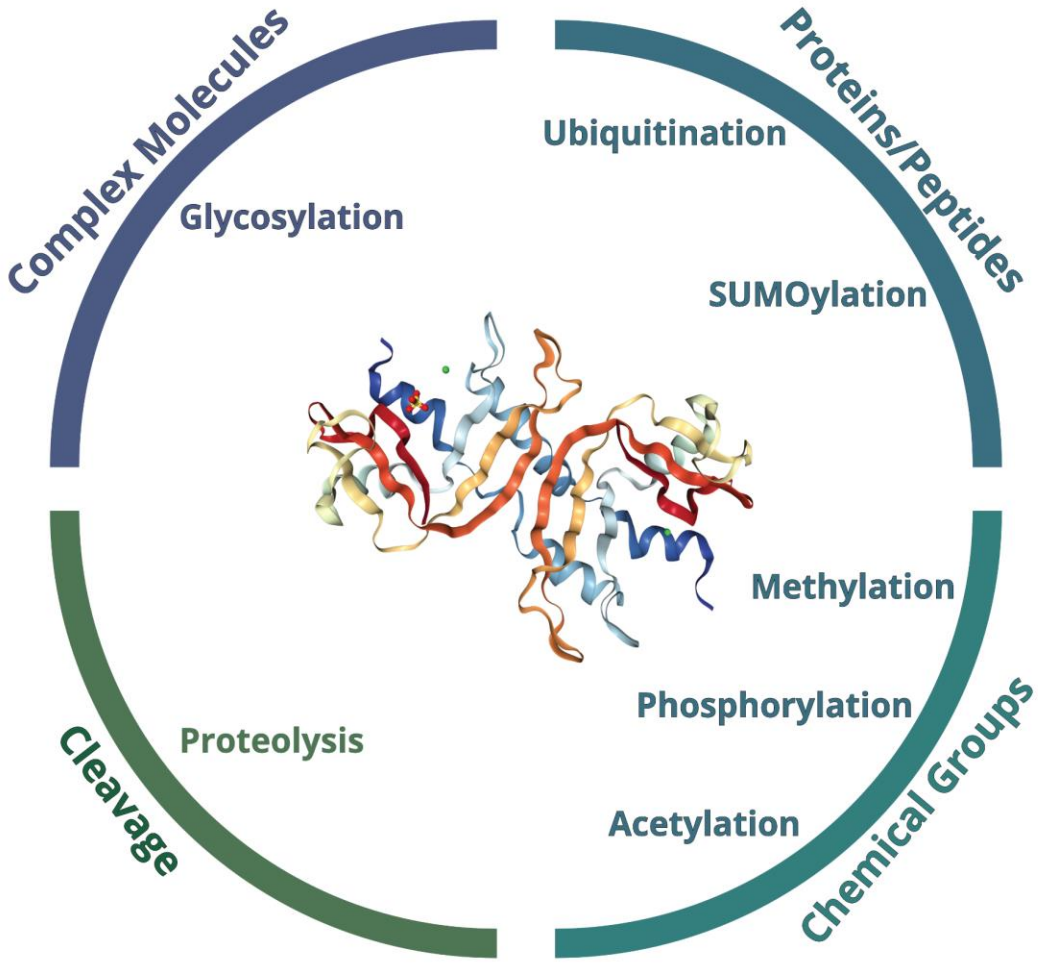
Uncovering Glycoprotein Compositional and Structural Heterogeneity Through Capillary Electrophoresis-Ion Mobility Mass Spectrometry

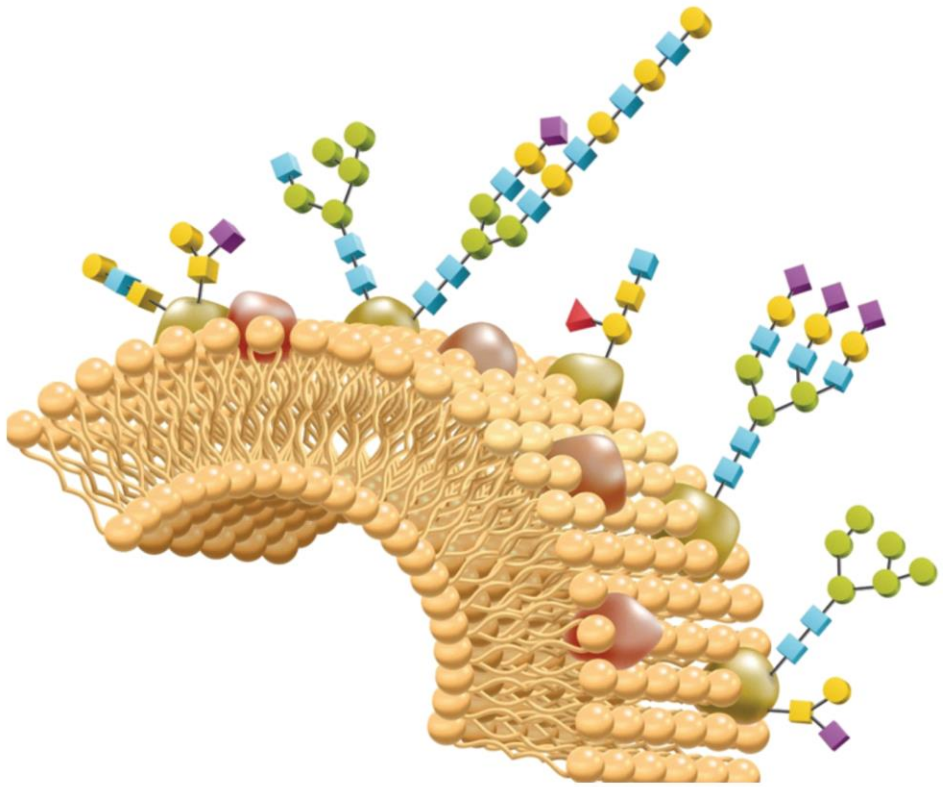
Graham Delafield
University of Wisconsin-Madison

Proteome Diversity



Wang, Y.-C.; Peterson, S. E.; Loring, J. F., Protein post-translational modifications and regulation of pluripotency in human stem cells. Cell Research 2014, 24 (2), 143-160.

Glycosylation



Function

Cellular communication and immune response

Intrinsic/extrinsic signaling pathways

Protein folding and viability

Disease

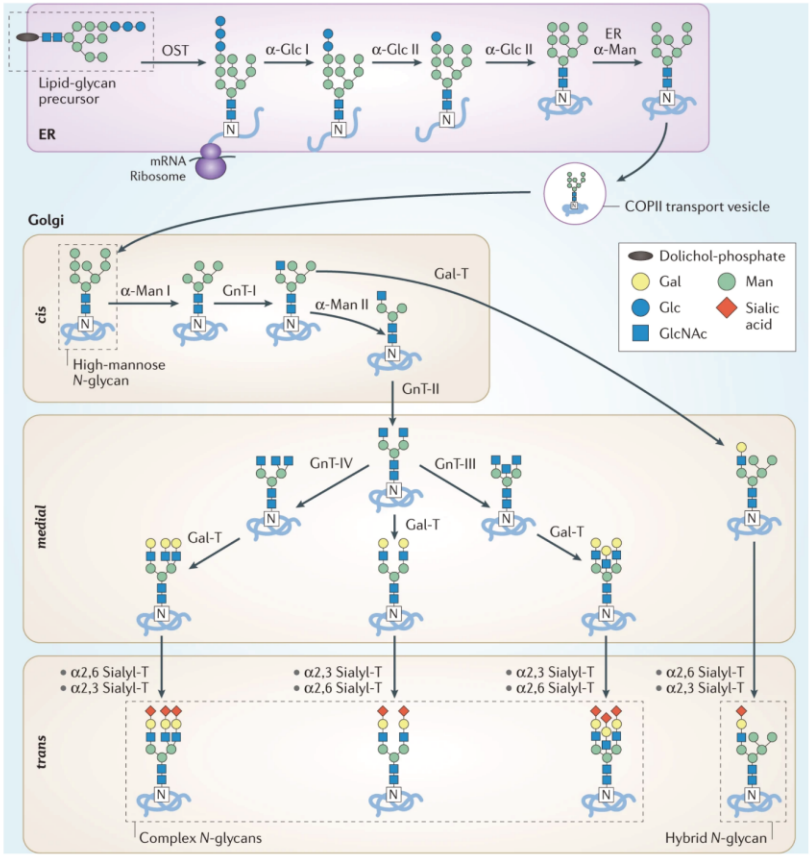
Target for pathogen invasion

Altered expression during disease propagation

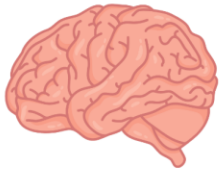
Aberrant profiles across numerous disease

1. Varki, A., Essentials of glycobiology. 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y., 2009; p xxix, 784 p.
2. Arnold, J. N.; Wormald, M. R.; Sim, R. B.; Rudd, P. M.; Dwek, R. A., Annu Rev Immunol 2007, 25, 21-50.
3. Lemmers, R. F. H.; Vilaj, M.; Urda, D.; Agakov, F.; Šimurina, M.; Klaric, L.; Rudan, I.; Campbell, H.; Hayward, C.; Wilson, J. F.; Lieverse, A. G.; Gornik, O.; Sijbrands, E. J. G.; Lauc, G.; van Hoek, M., Biochimica et Biophysica Acta (BBA) - General Subjects 2017, 1861 (9), 2240-2249.

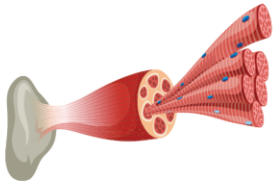
Relevance of Glycan Structure



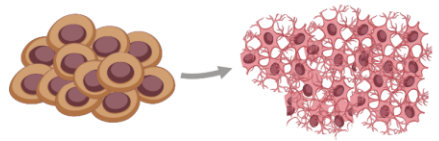
Blood Donor Groups



Neurological Disorders



Myopathy



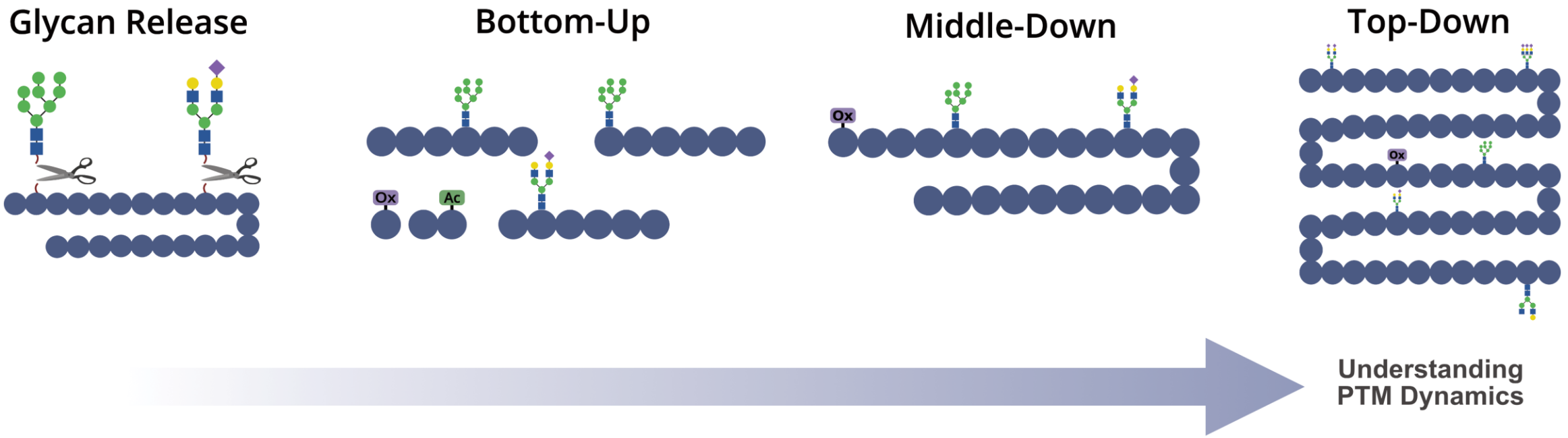
Cancer

Comprehensive evaluation of glycosylation microheterogeneity has revealed numerous connections to human disease

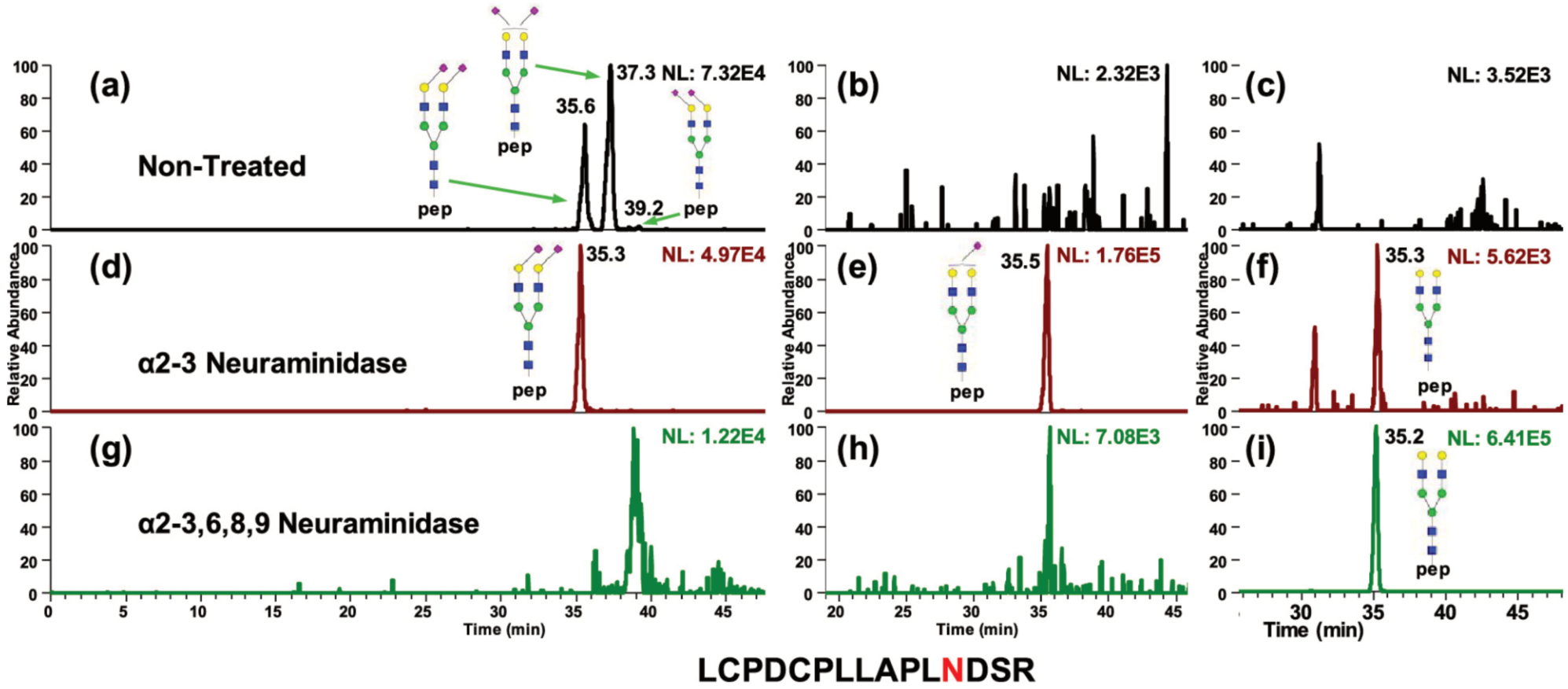
Structural diversity in presented glycans may be used as potential biomarkers and are uniquely implicated in biological processes

Reily, C., Stewart, T.J., Renfrow, M.B. et al. Glycosylation in health and disease. Nat Rev Nephrol 15, 346–366 (2019).

Glycosylation Analysis

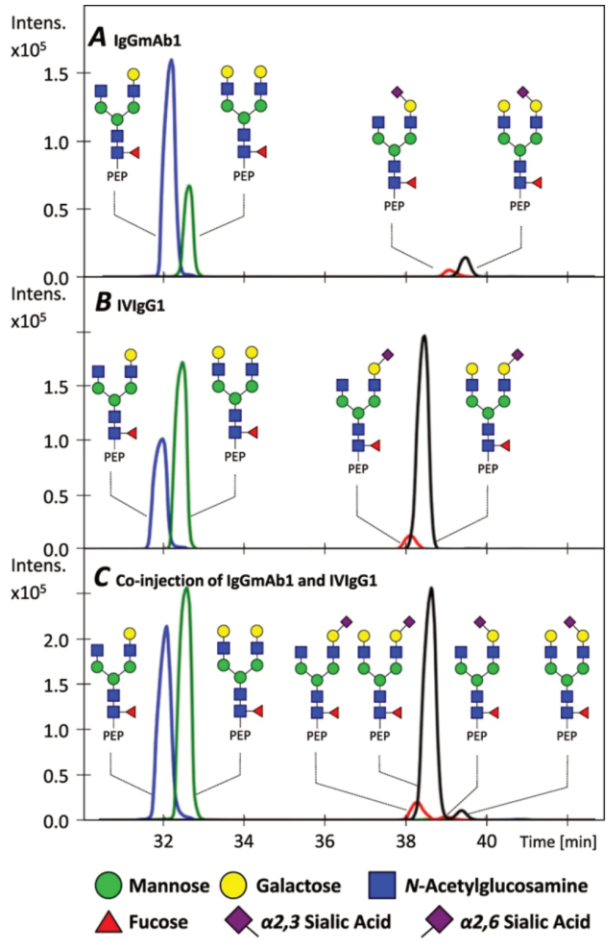


Recent Developments



Zhu, R.; Huang, Y.; Zhao, J.; Zhong, J.; Mechref, Y., Analytical Chemistry 2020, 92 (14), 9556-9565.
Zhou, S.; Huang, Y.; Dong, X.; Peng, W.; et al. Analytical Chemistry 2017, 89 (12), 6590-6597.

Can CE Compete?



Kammeijer, G.S.M., Jansen, B.C., Kohler, I. et al. Sci Rep 7, 3733 (2017).
Kshitij Khatri, et al. Analytical Chemistry 2017 89 (12), 6645-6655

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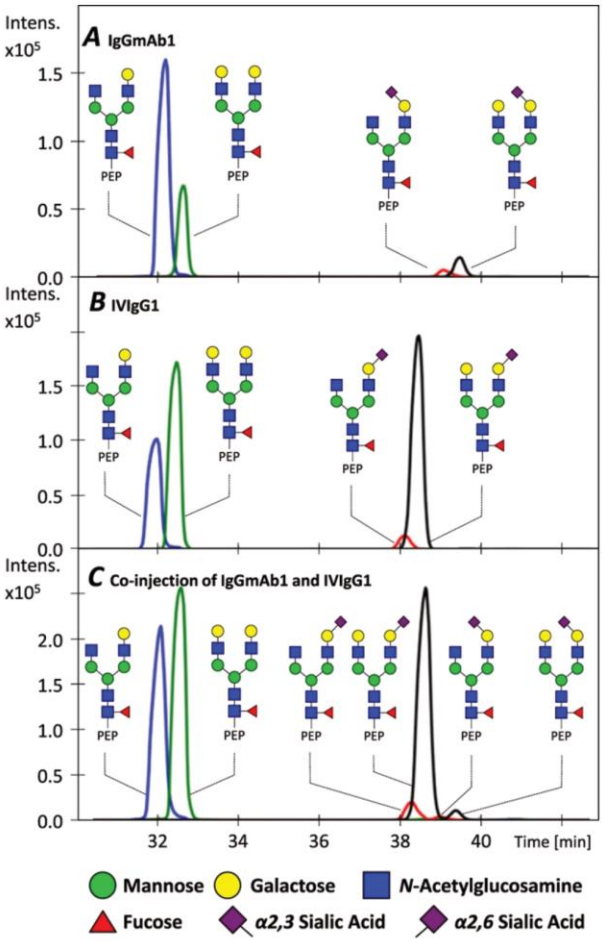
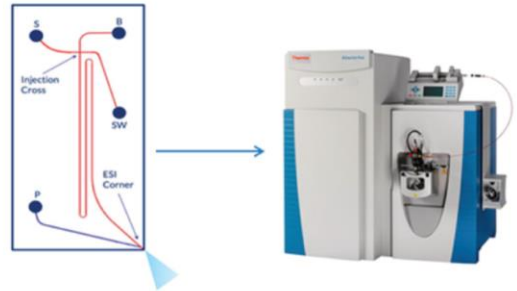
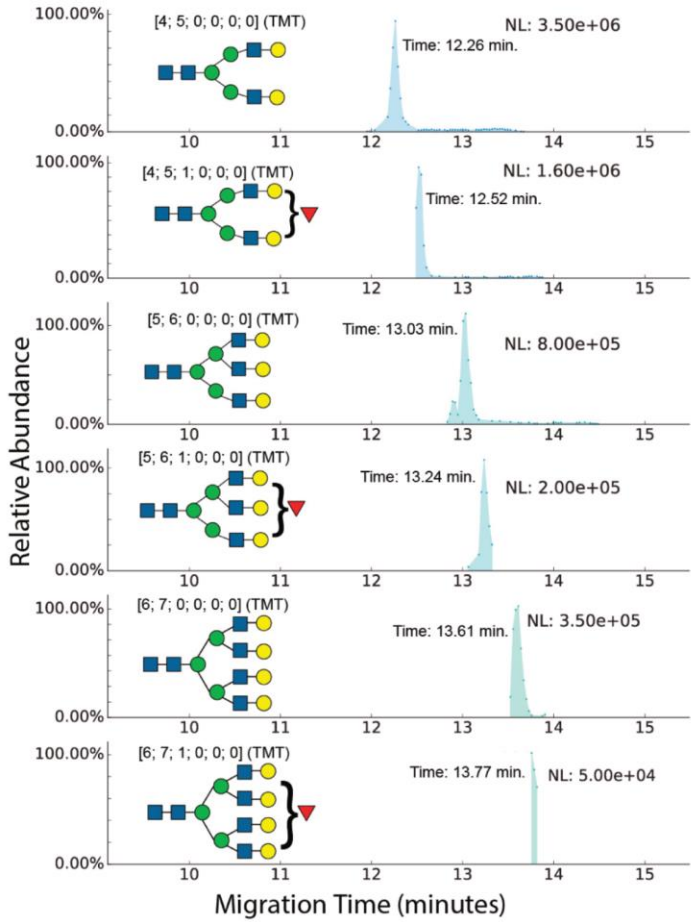
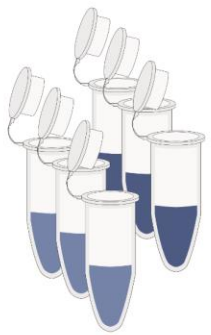




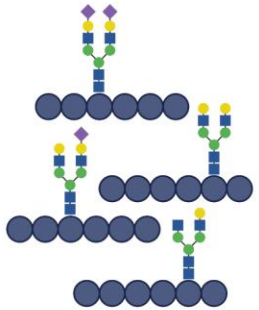


Kammeijer, G.S.M., Jansen, B.C., Kohler, I. et al. Sci Rep 7, 3733 (2017).
Kshitij Khatri, et al. Analytical Chemistry 2017 89 (12), 6645-6655

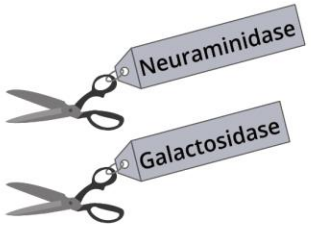
Bridging the Gap



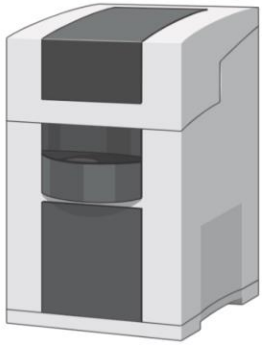
Sample Preparation



Enrichment

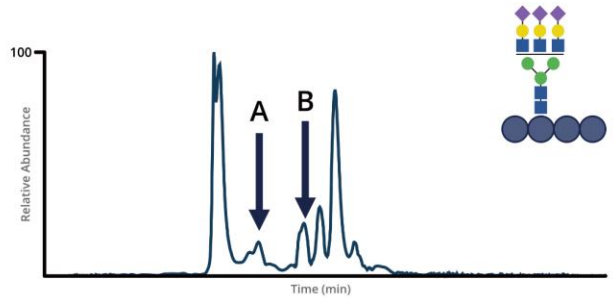


Enzymatic Treatment



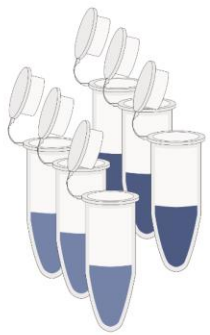
Capillary Electrophoresis

Mass Spectrometry



Migration Discrimination

Bridging the Gap



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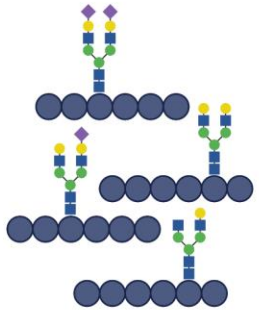
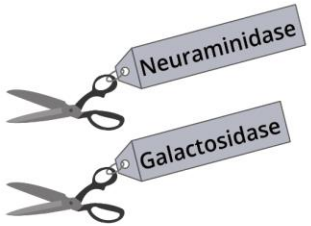


Enrichment



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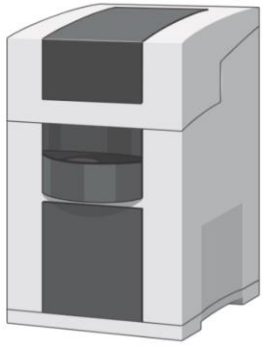
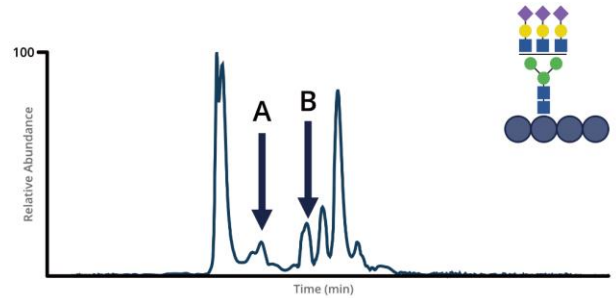


Capillary Electrophoresis

Ion Mobility



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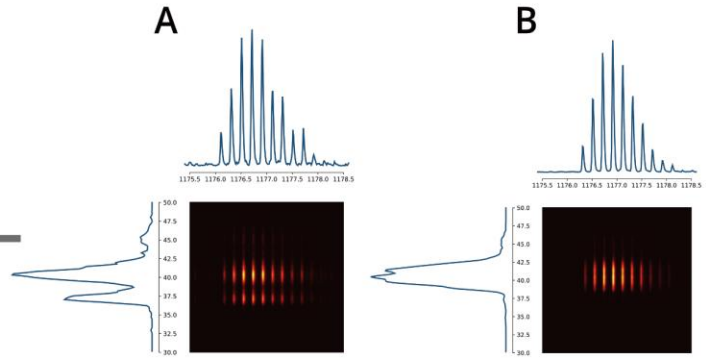
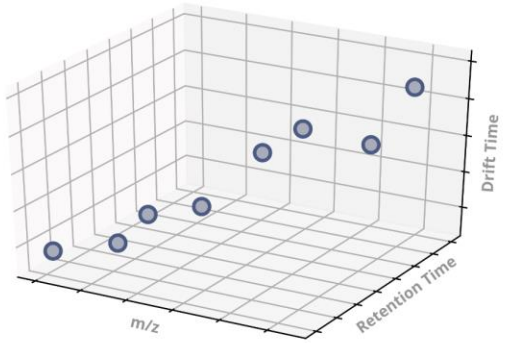
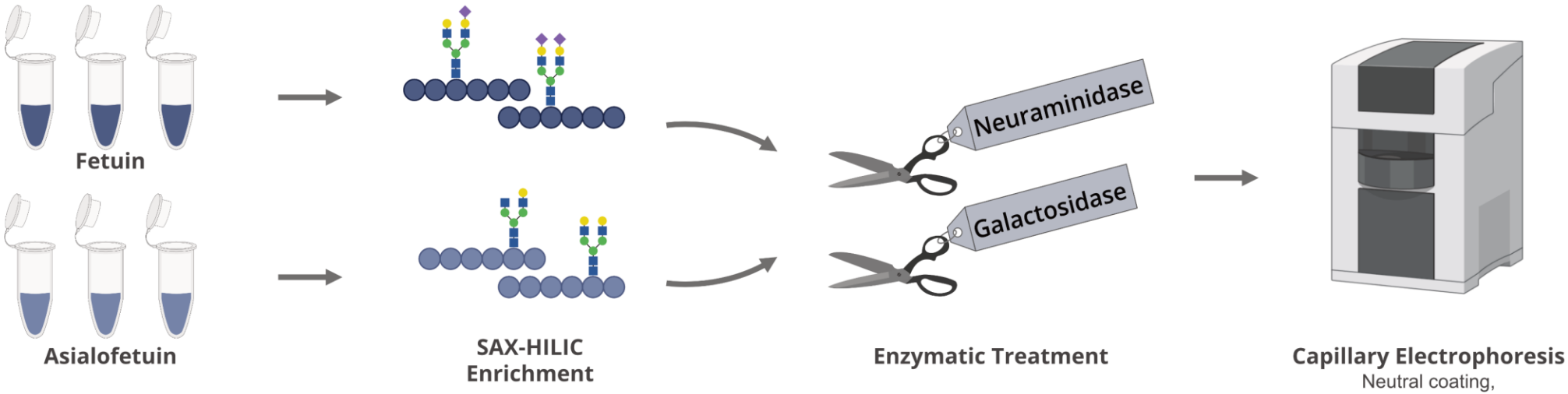


Gas Phase Discrimination



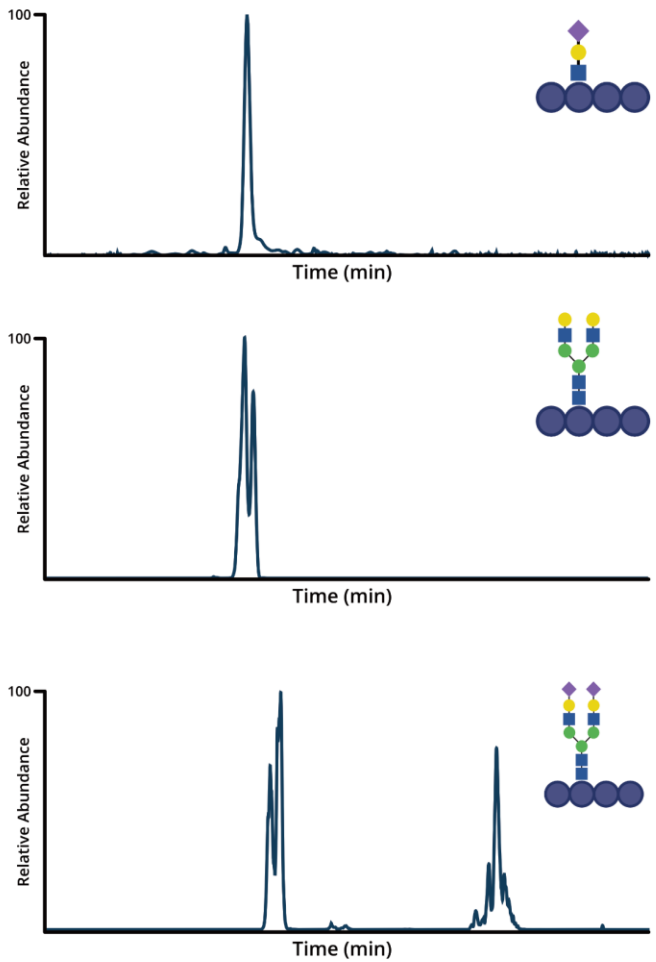
Database-like Assignment

Experimental Workflow



Klein, J., Carvalho, L., & Zaia, J. (2018). Application of Network Smoothing to Glycan LC-MS Profiling.

Separation Capacity



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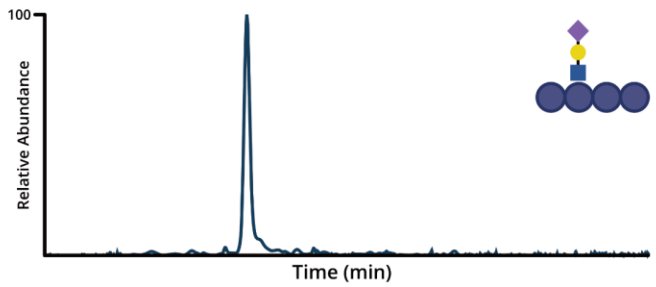

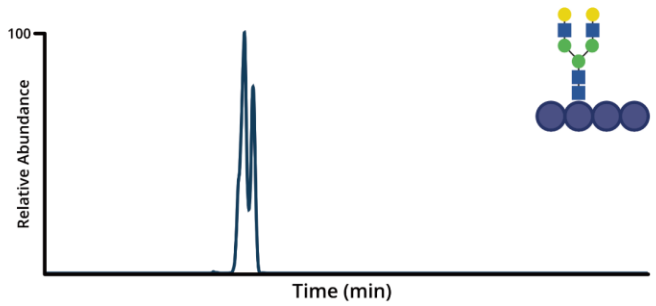

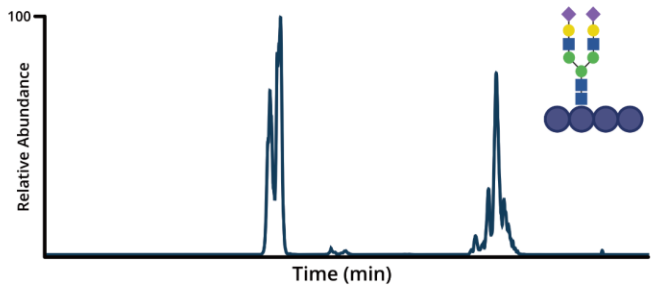

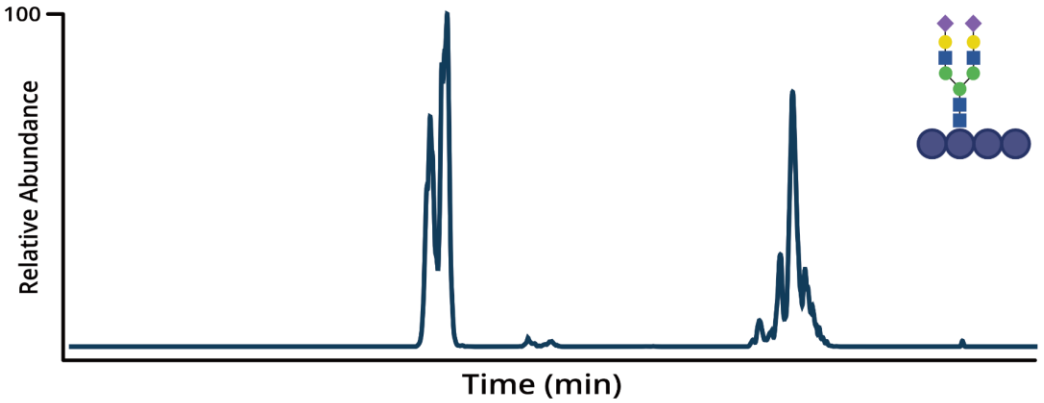

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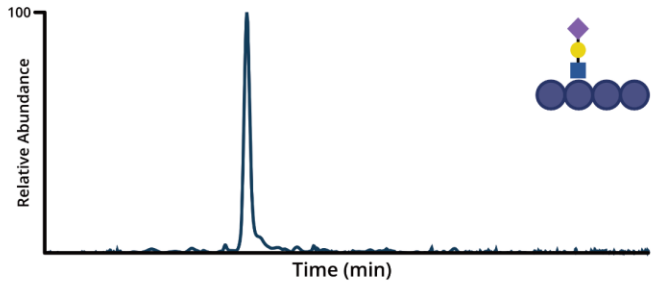

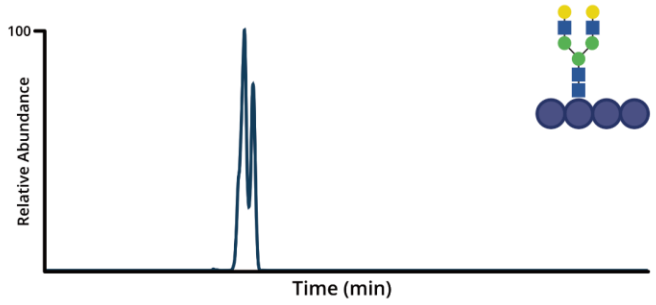

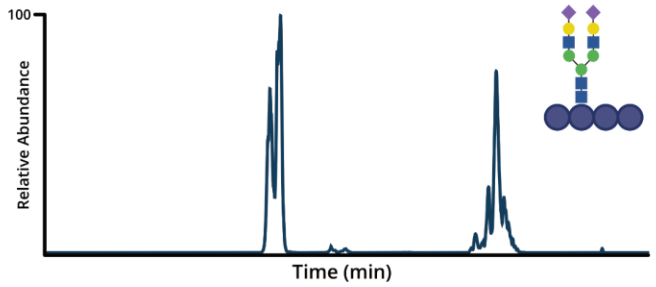

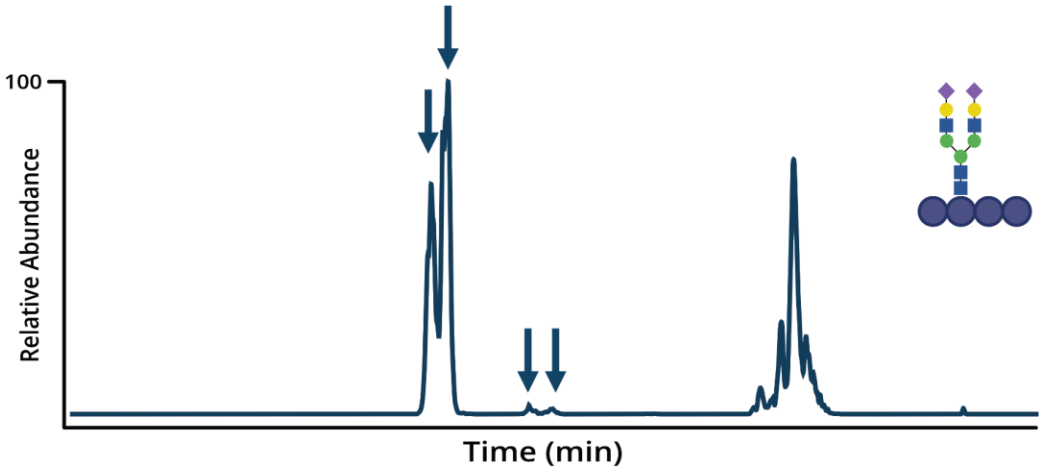

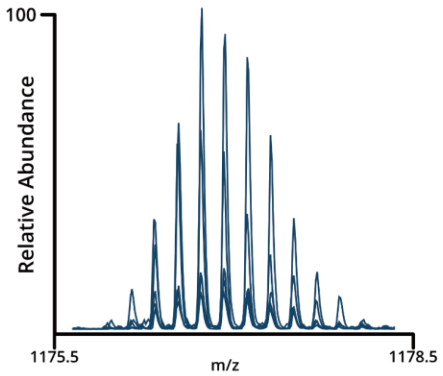

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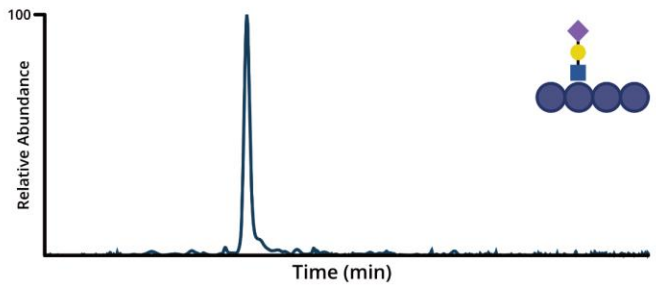

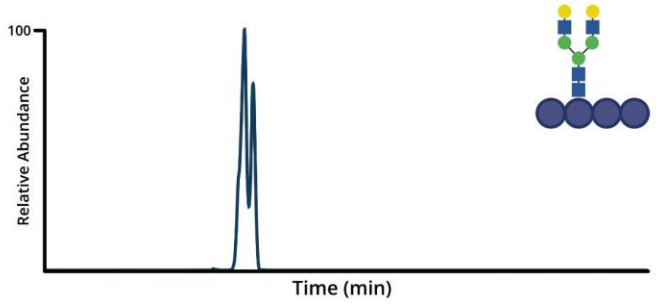

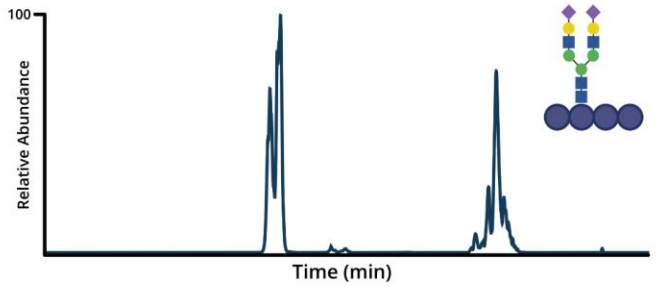

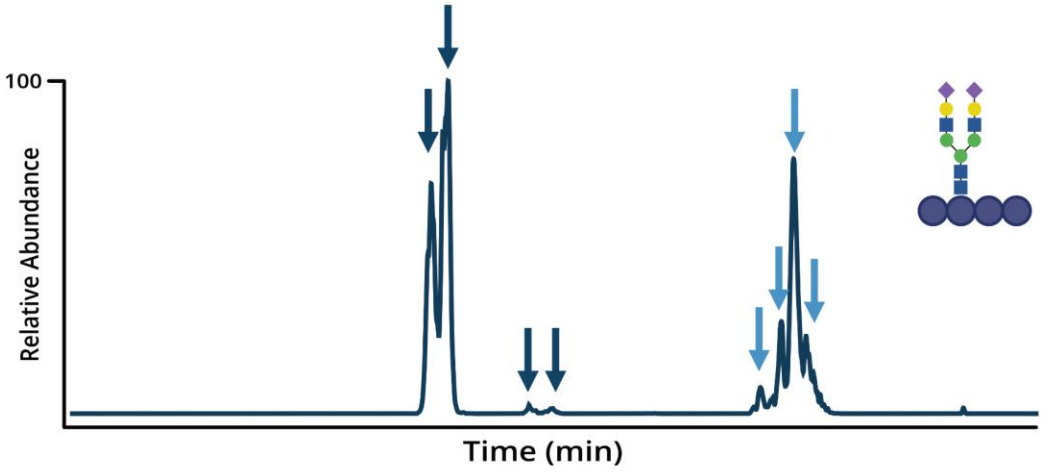

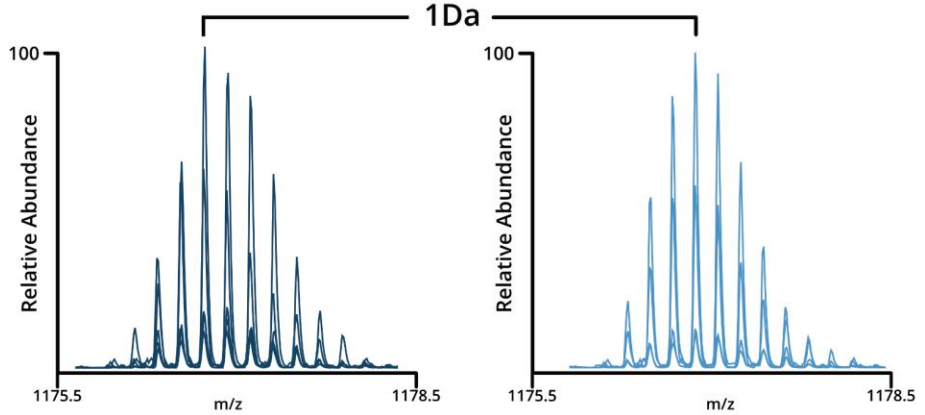

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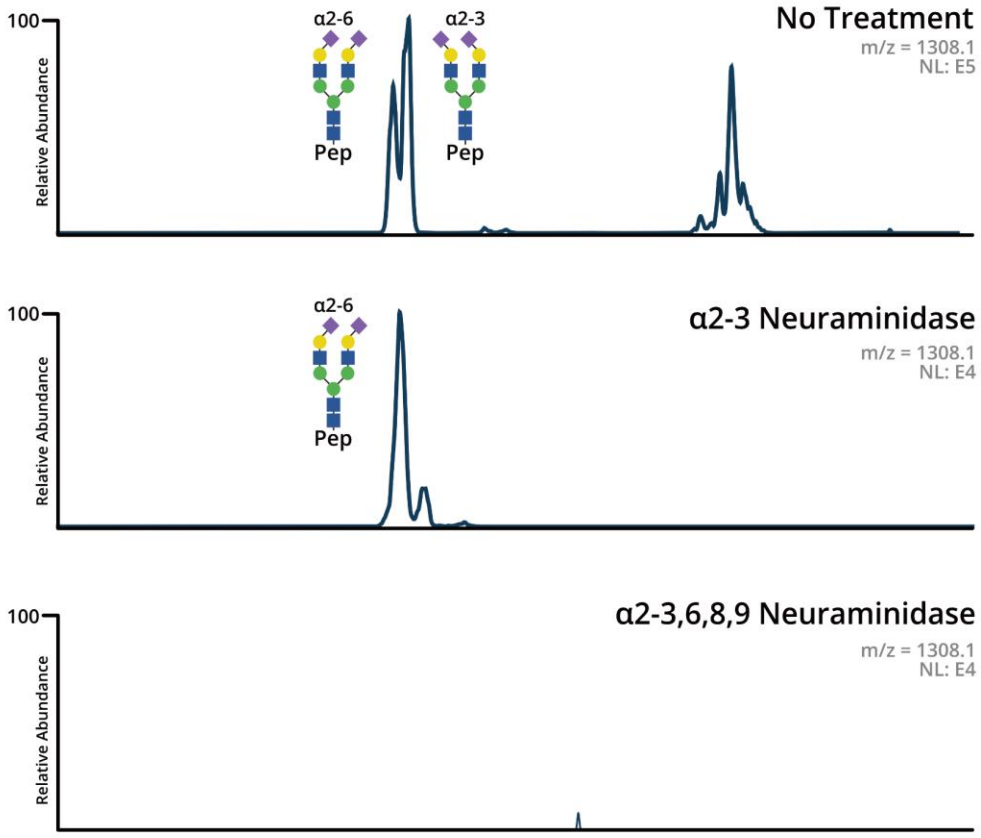

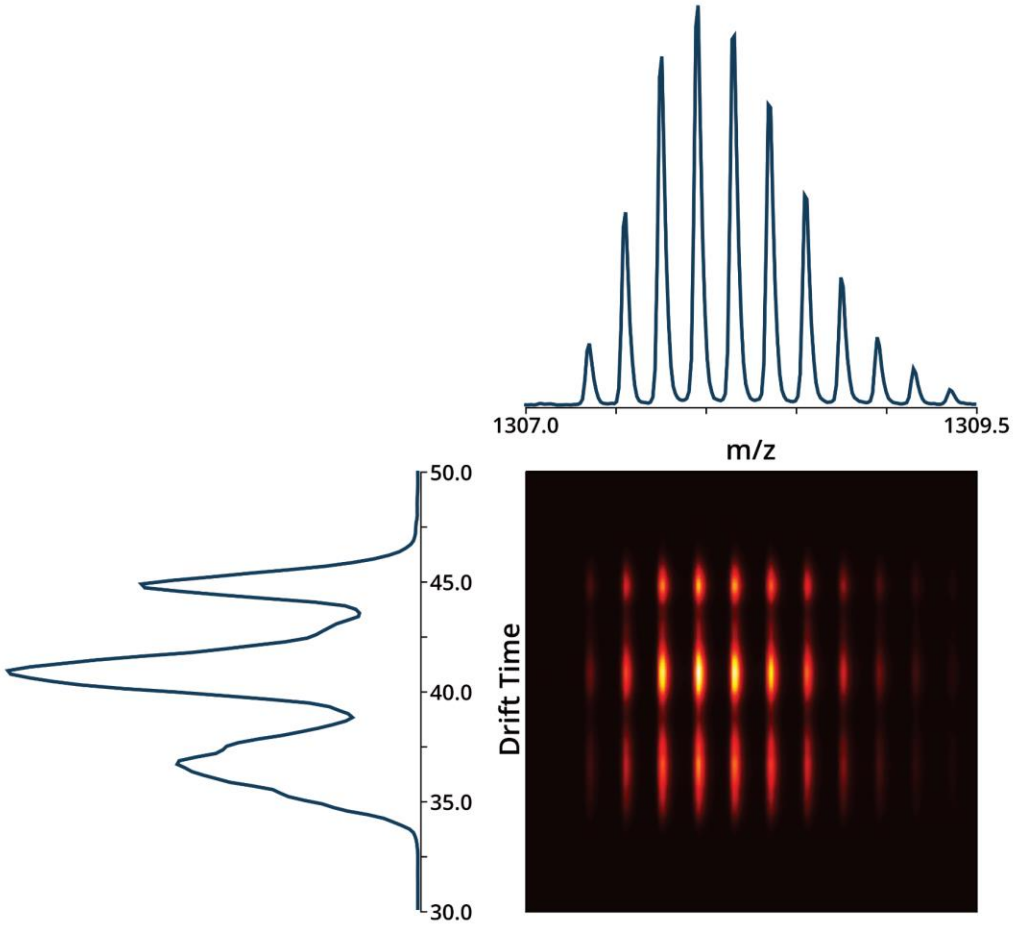

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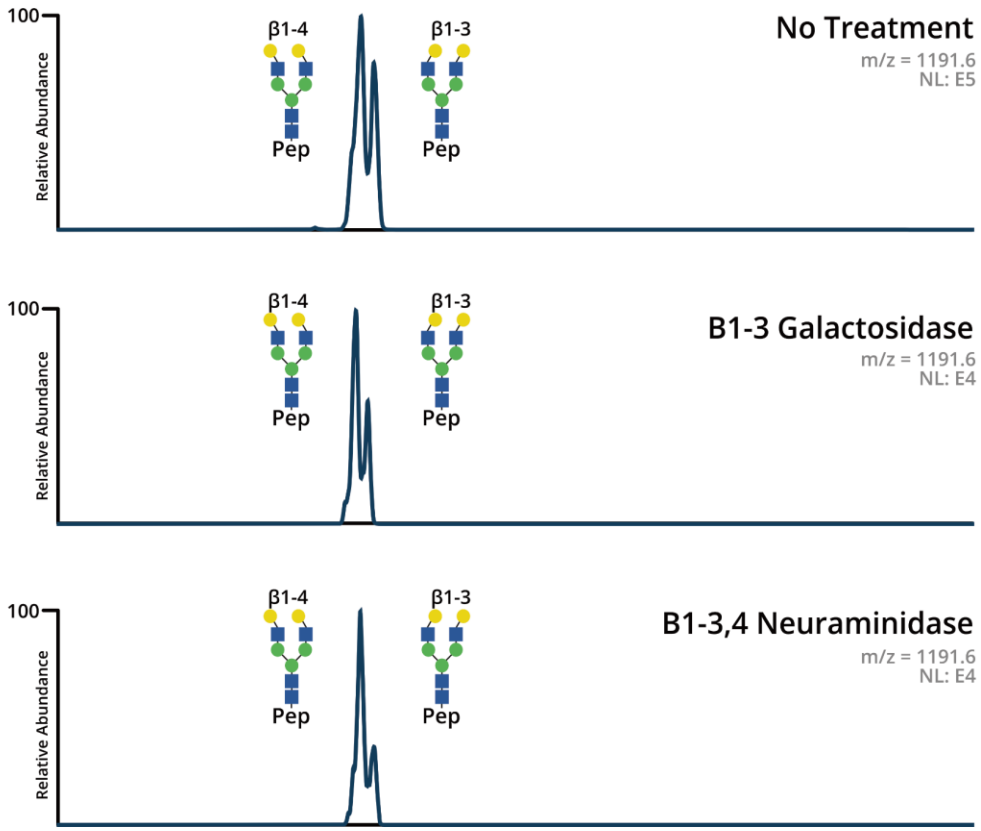
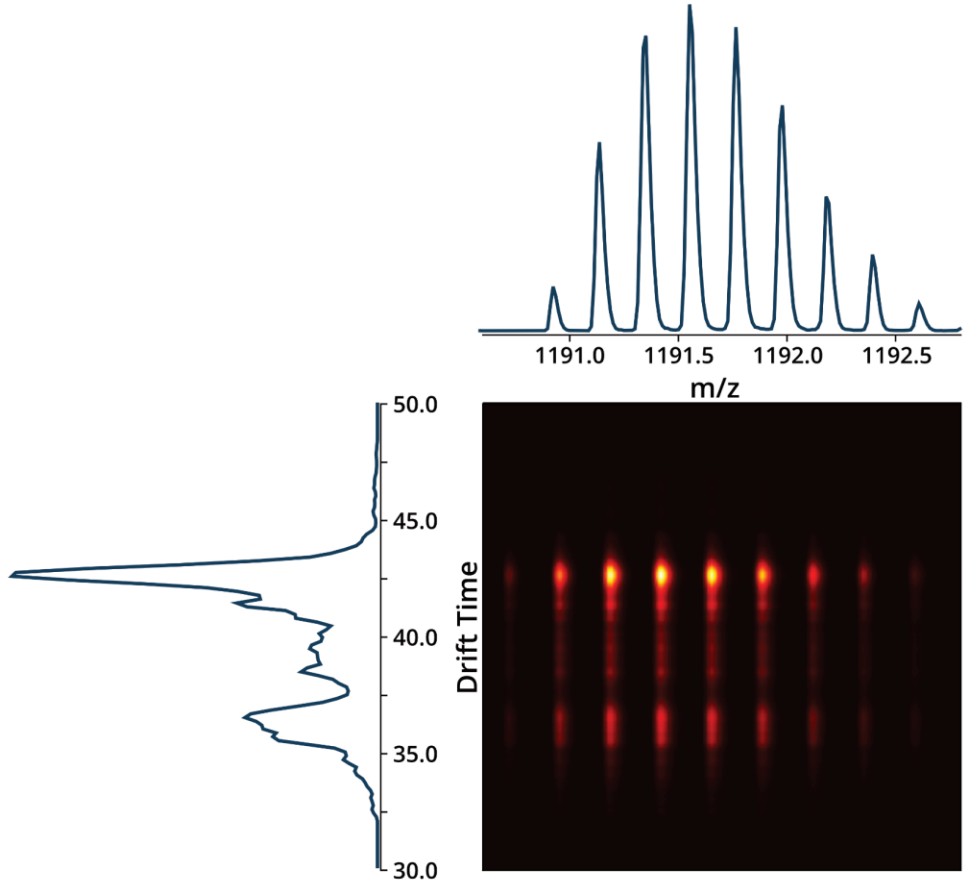
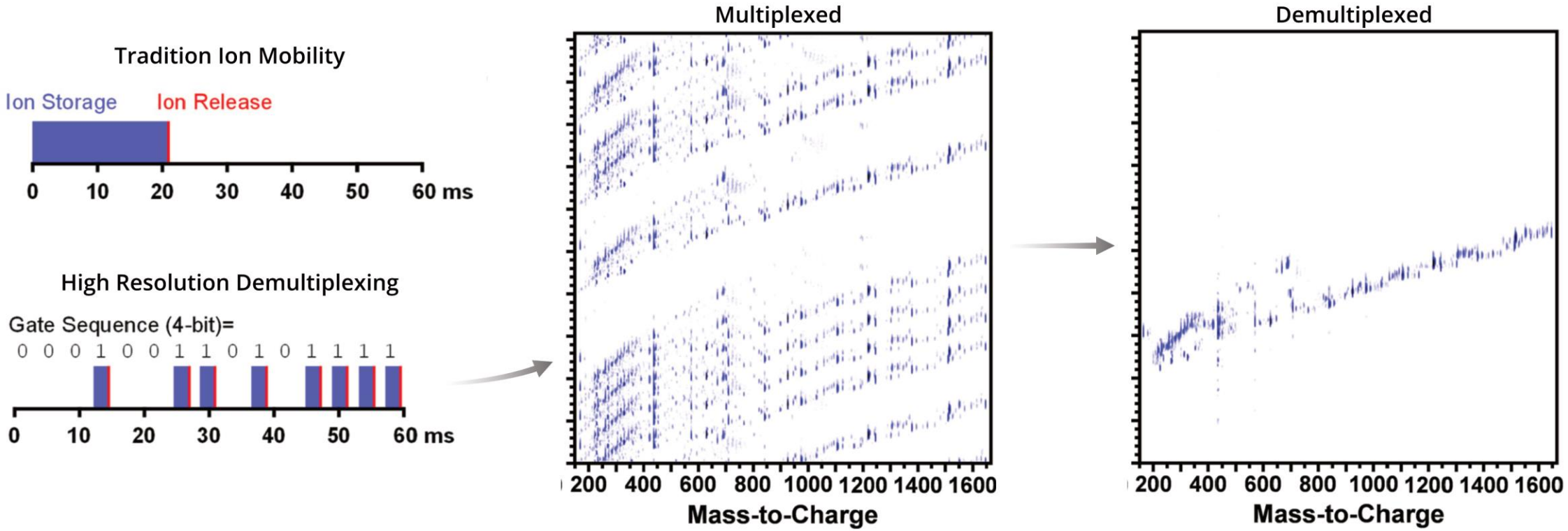




Pursuing Higher Resolution



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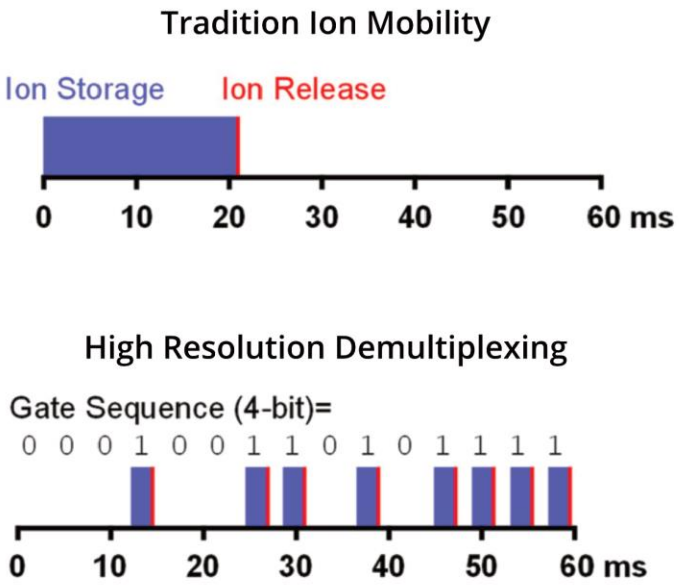
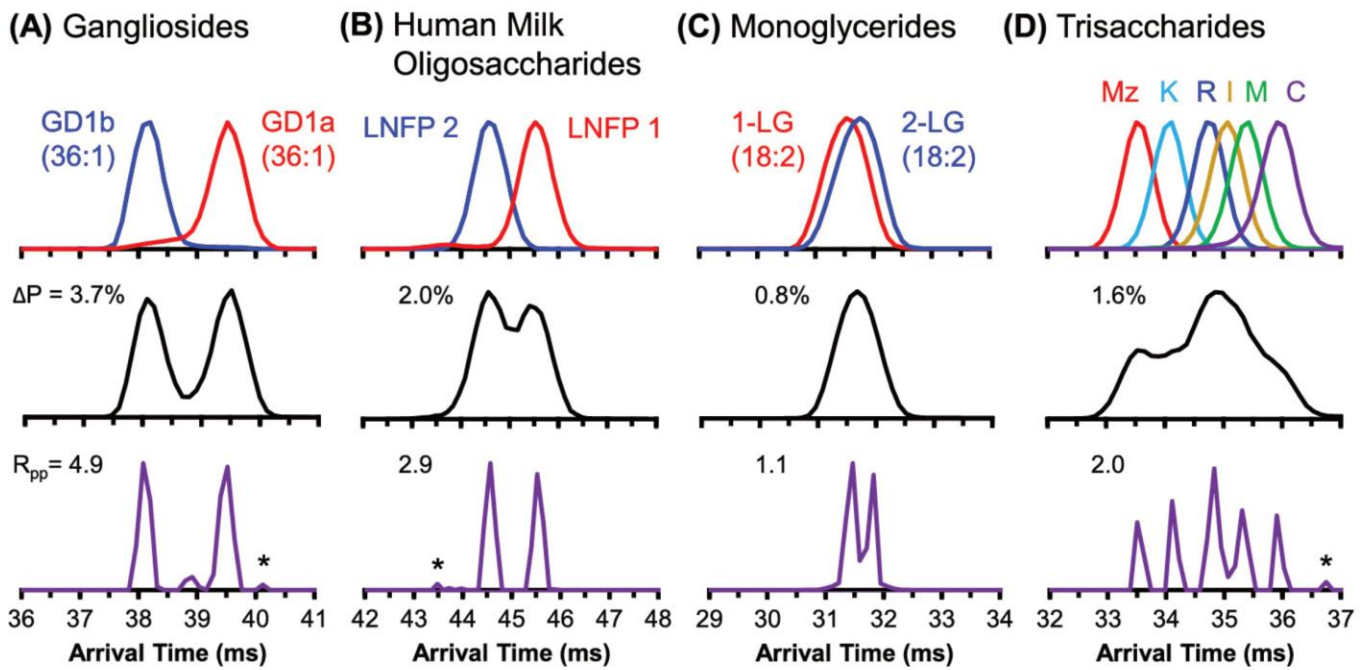
Analytical Chemistry 2020 92 (14), 9482-9492

Pursuing Higher Resolution





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Conclusions

- CE demonstrates excellent capacity to separate glycopeptide isomers
- Enzymatic digestion of glycopeptides can facilitate assignment of glycosidic bonds
- High-resolution demultiplexing may provide unique insight into glycopeptide structural identification
- Feature detection and mass matching poses limitations in profiling depth

Acknowledgments

Li Research Group

Dr. Lingjun Li

Dr. Yusi Cui

CMP Scientific

Dr. James Xia

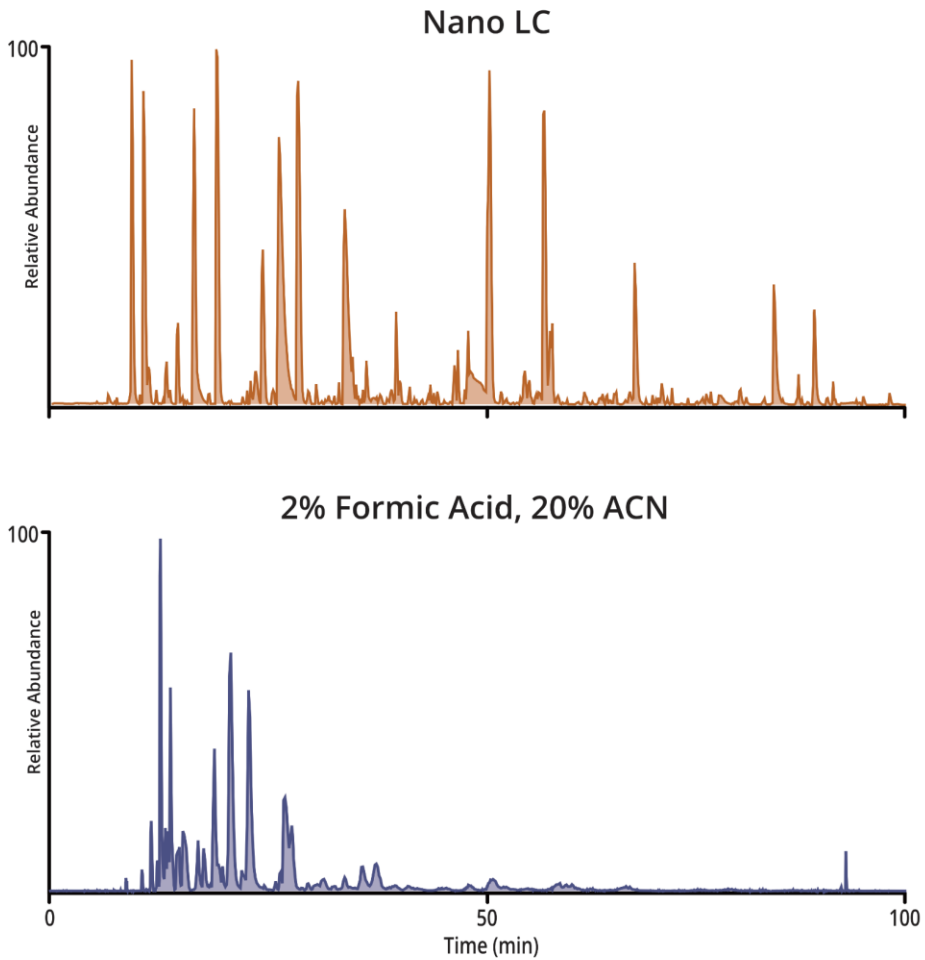
Agilent Technologies

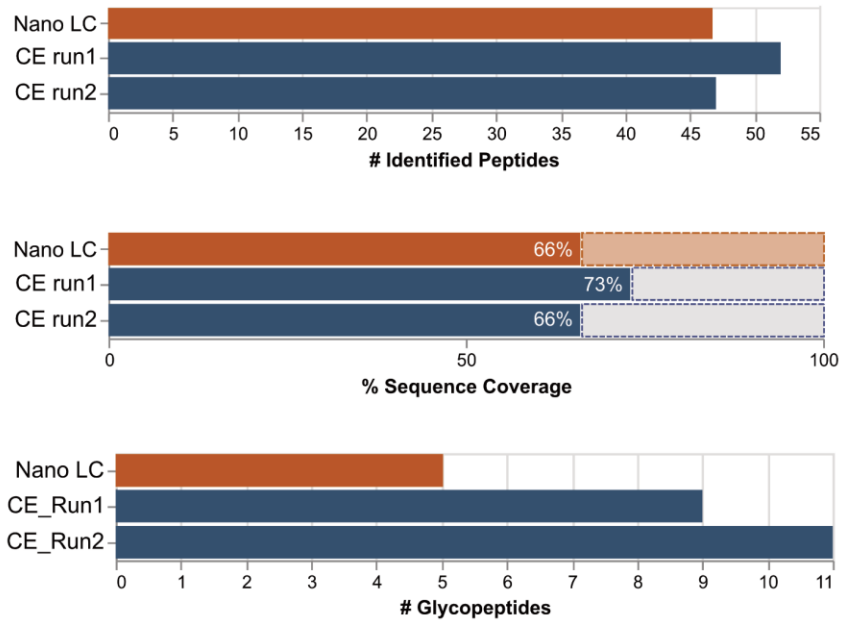
John Sausen
John Fjeldsted
Rebecca Glaskin

Limitations

- Capillary electrophoresis demonstrates high separation capacity at cost of concentration requirements
- Highly sialylated species subjected to greatest peak broadening
- Profiling depth of IM-QTOF can provide limitations when analyzing complex, unknown samples
- High resolution demultiplexing is computationally expensive

LC vs CE





- Capillary electrophoresis demonstrates comparable and improved performance against traditional LC
- However, the separation regime is not the limiting factor in proteomicv analyses