N-Glycopeptide Feature Identification by Revealing Trends Between Analyte Composition and Compensation Field Through FAIMS-Coupled MS Platform

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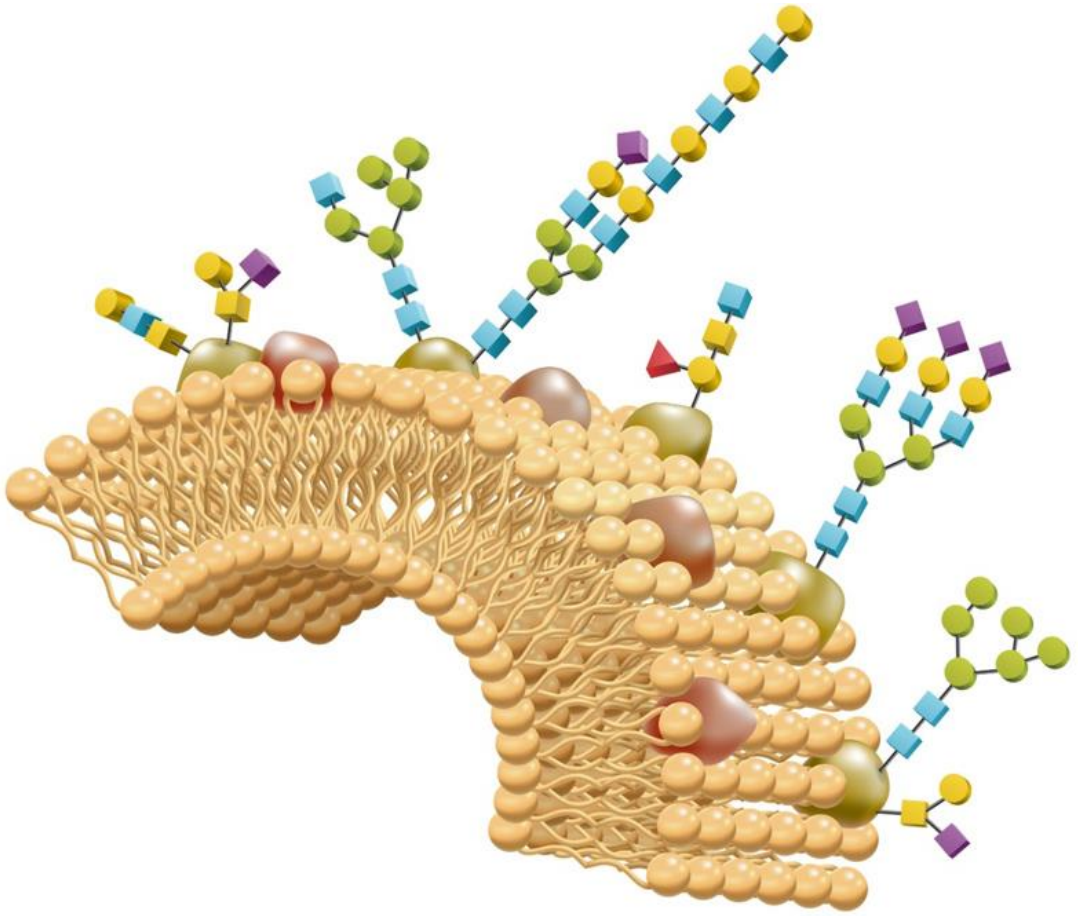
Implications of Glycosylation



Function

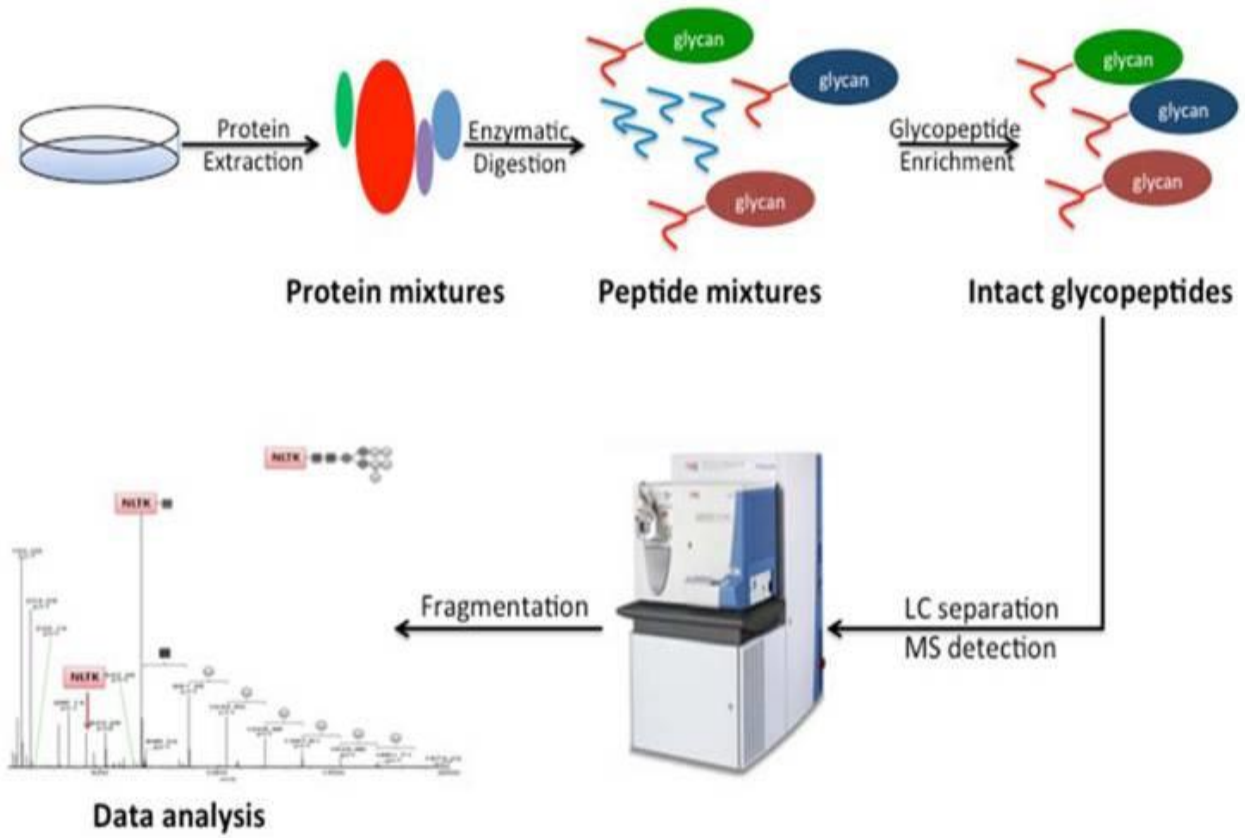
- Cellular communication and immune response
- Extrinsic and intrinsic signaling pathways
- Impact protein folding

Disease

- Target of foreign invasion and tolerance
- Indicators of autoimmune diseases such as RA
- Glycan patterns associated with type 2 diabetes

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.

Understanding Intact Glycopeptides



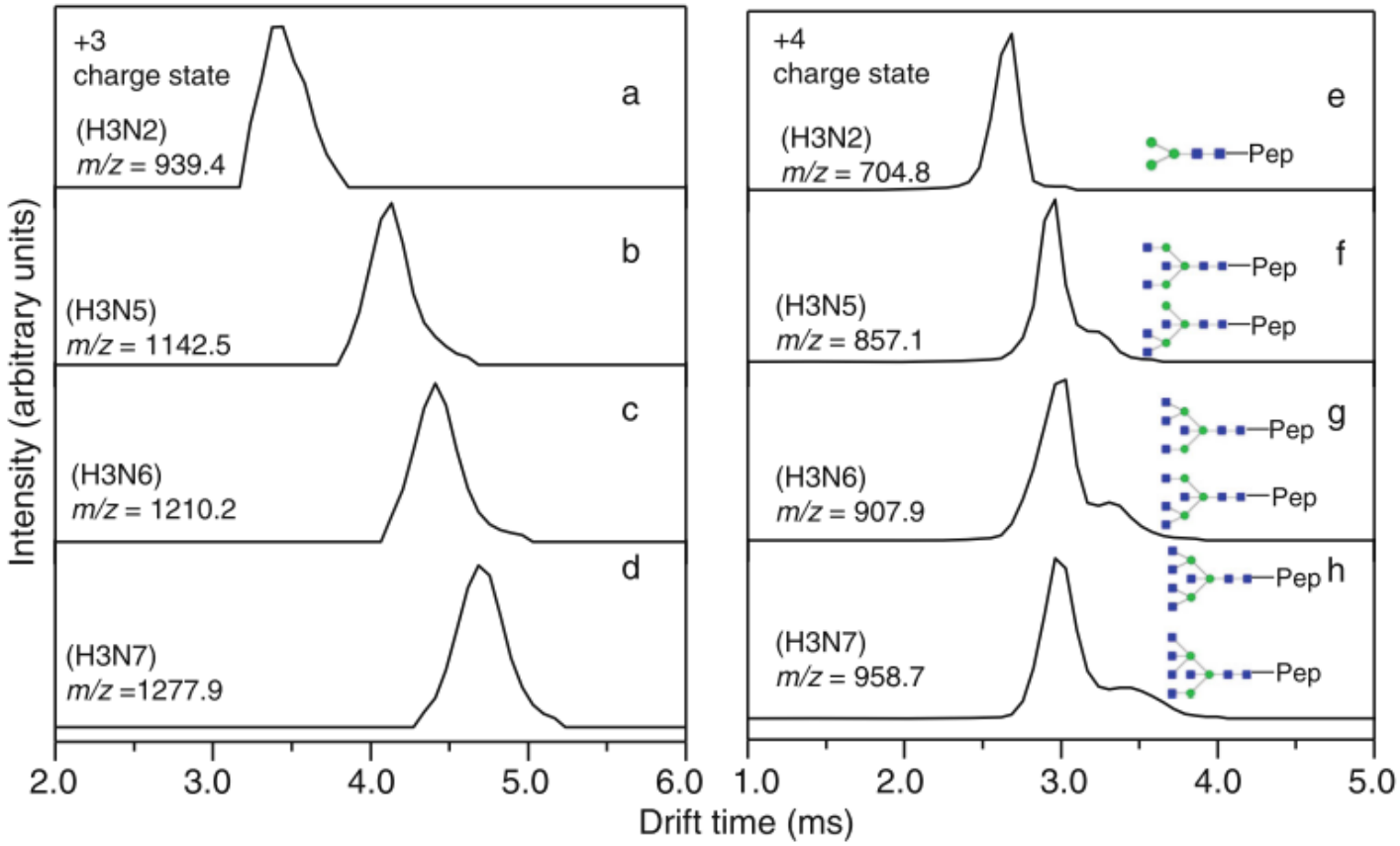
Limitations of Convention

- No universal separation approach
- Glycopeptides are often low in abundance and need enrichment
- Heterogeneity makes glycoform separation difficult

Additional dimension of gas-phase separation could provide complementary information

Cao, L.; Qu, Y.; Zhang, Z.; Wang, Z.; Prytkova, I.; Wu, S., Intact glycopeptide characterization using mass spectrometry. *Expert Rev Proteomics* **2016,** *13* (5), 513-22.

Leveraging Ion Mobility

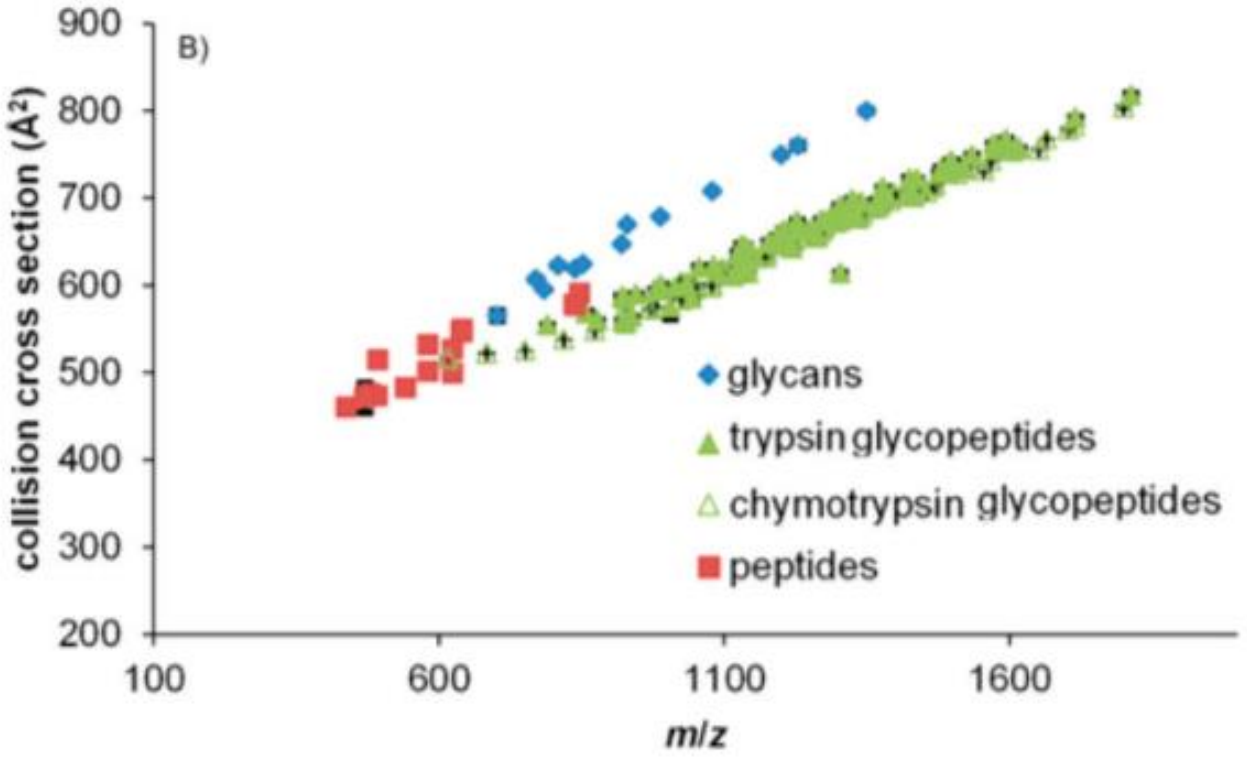


Glycoform Separation

- IMS proven useful for glycoform analysis
- Systematic drift time changes indicate glycosylation patterns
- Demonstrated improved resolution with increase charge state

Zhu, F.; Trinidad, J. C.; Clemmer, D. E., Glycopeptide Site Heterogeneity and Structural Diversity Determined by Combined Lectin Affinity Chromatography/IMS/CID/MS Techniques. *Journal of The American Society for Mass Spectrometry* **2015,** *26* (7), 1092-1102.

Leveraging Ion Mobility

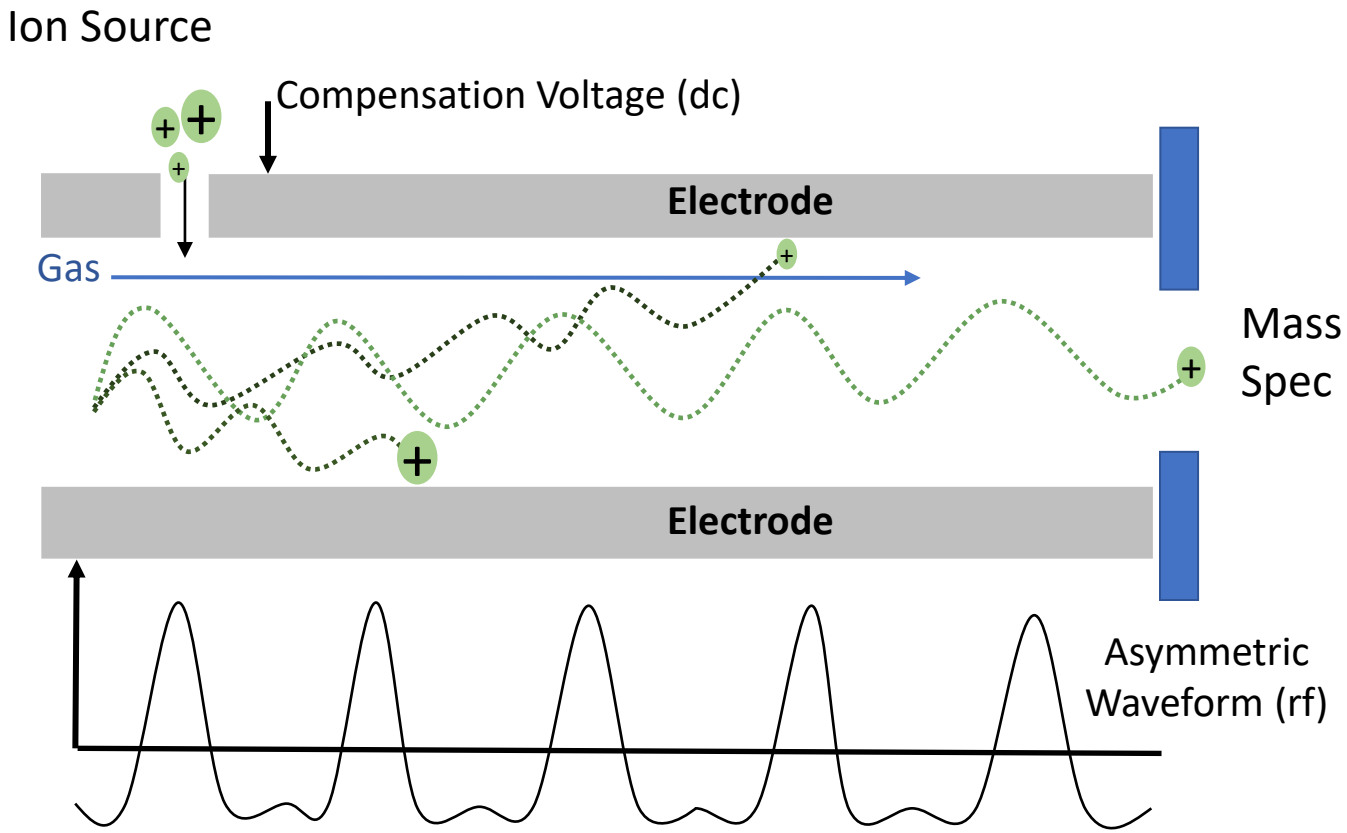


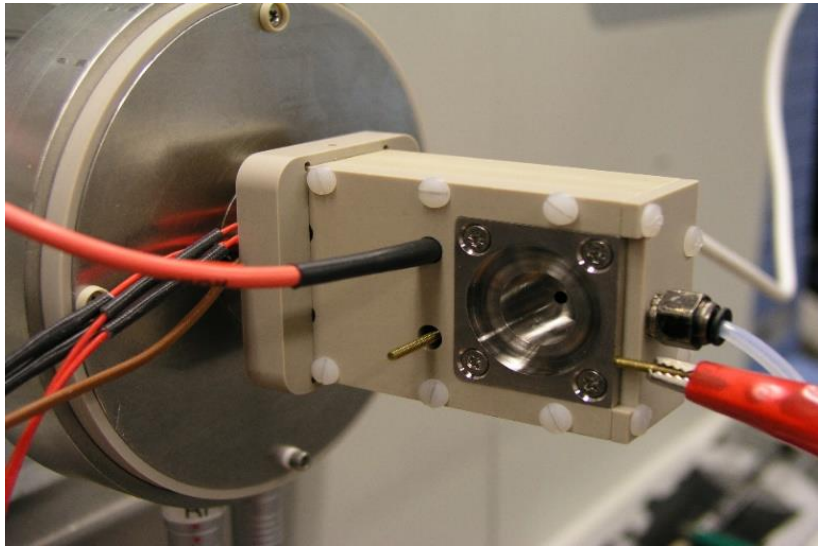
Database Compilation

- Demonstrated unique trends for:
  - Permethylated Glycans
  - Glycopeptides
  - Non-glycosylated peptides
- Collection of CCS values makes feature identification possible
- Trends are clear, but would benefit from clearer separation

Glaskin, R. S.; Khatri, K.; Wang, Q.; Zaia, J.; Costello, C. E., Construction of a Database of Collision Cross Section Values for Glycopeptides, Glycans, and Peptides Determined by IM-MS. *Anal Chem* **2017,** *89* (8), 4452-4460.

Differential Ion Mobility - FAIMS

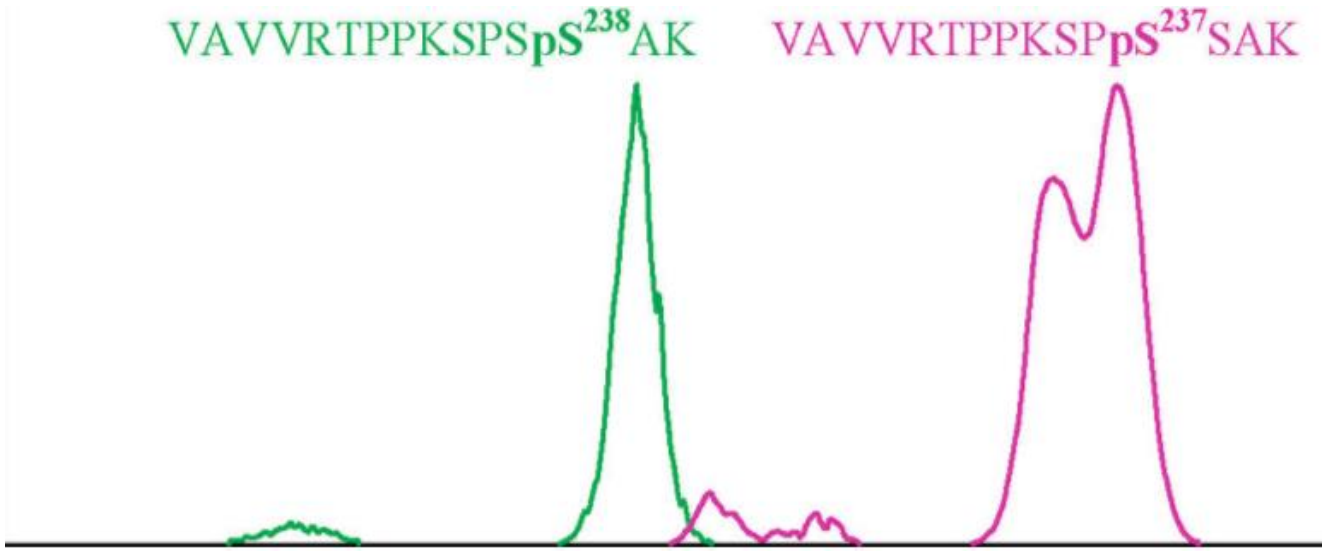




Planar FAIMS courtesy of Heartland Mobility

Shvartsburg, A. A.; Smith, R. D., Fundamentals of Traveling Wave Ion Mobility Spectrometry. *Analytical chemistry* **2008,** *80* (24), 9689-9699.

Differential Ion Mobility - FAIMS



Demonstrated success in separating:
- Site specific modifications
- Peptide Sequence Isomers
- Cis/Trans isomers
- Proteoforms (middle down)

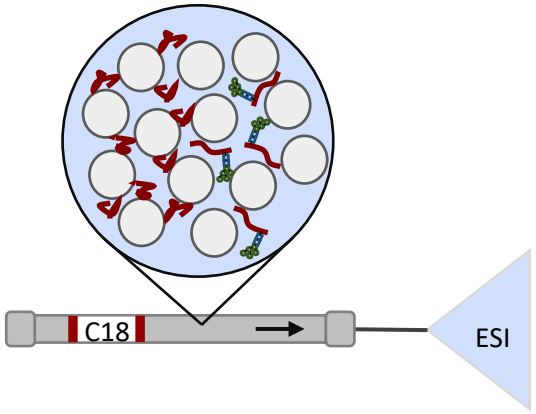
Advantages
- Separation based on dipole allignment
- Good orthogonality to MS

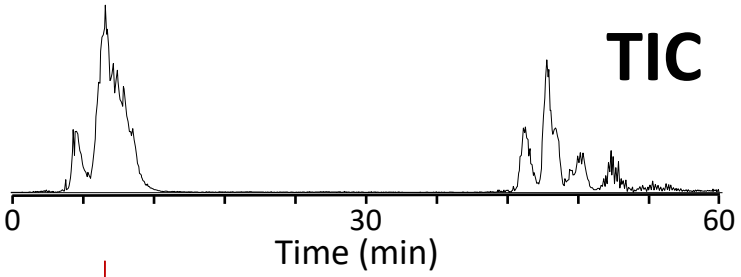
Utilizing FAIMS could provide discrimination of modified and unmodified peptides and offer possible separation of isobaric species.

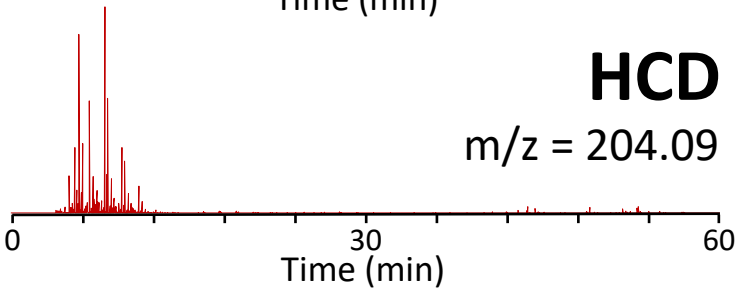
1. Shvartsburg, A. A.; Singer, D.; Smith, R. D.; Hoffmann, R., *Analytical Chemistry* **2011,** *83* (13), 5078-5085.
2. Shvartsburg, A. A.; Creese, A. J.; Smith, R. D.; Cooper, H. J., *Analytical chemistry* **2011,** *83* (18), 6918-6923.
3. Shliaha, P. V.; Baird, M. A.; Nielsen, M. M.; Gorshkov, V.; Bowman, A. P.; Kaszycki, J. L.; Jensen, O. N.; Shvartsburg, A. A., *Anal. Chem. (Washington, DC, U. S.)* **2017**, Ahead of Print.
4. Ulasi, G. N.; Creese, A. J.; Hui, S. X.; Penn, C. W.; Cooper, H. J., *Proteomics* **2015,** *15* (16), 2733-45.

Proposed Workflow

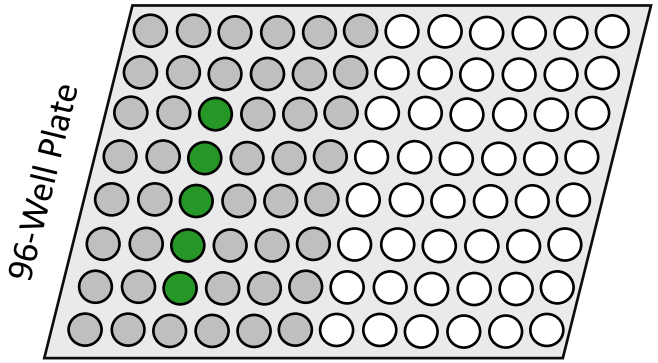
1 Online Monitoring



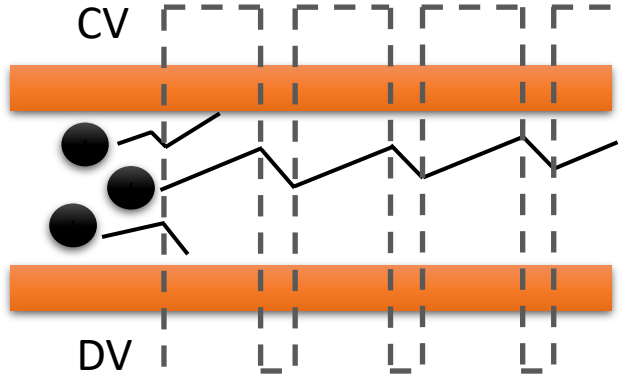


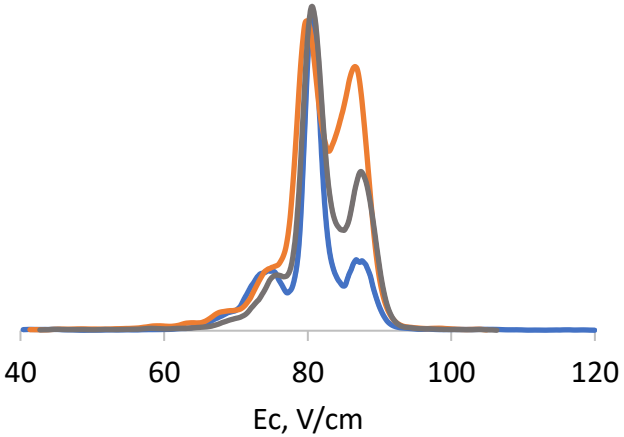


2 Concurrent Fractionation Using Triversa Nanomate

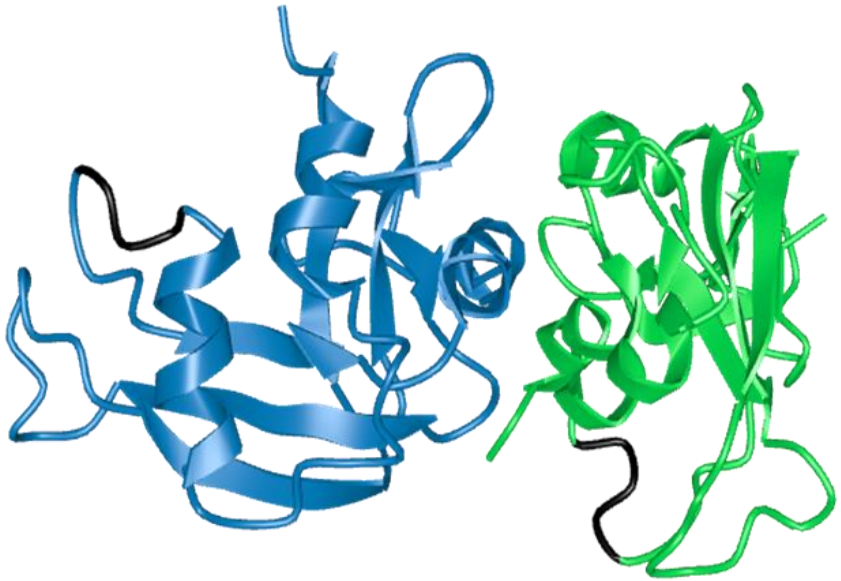


3 FAIMS Filtering

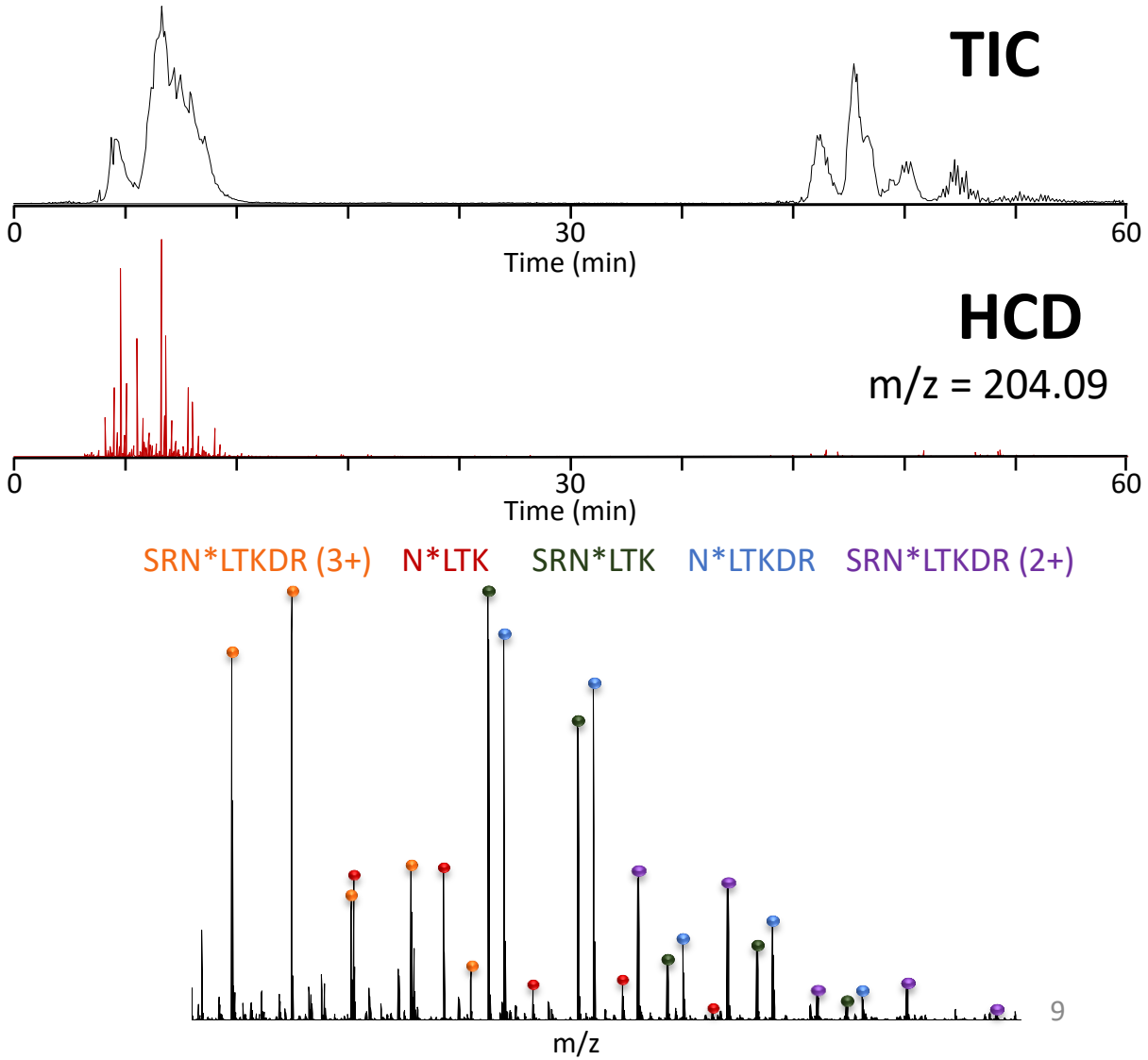




Online Oxonium Ion Monitoring



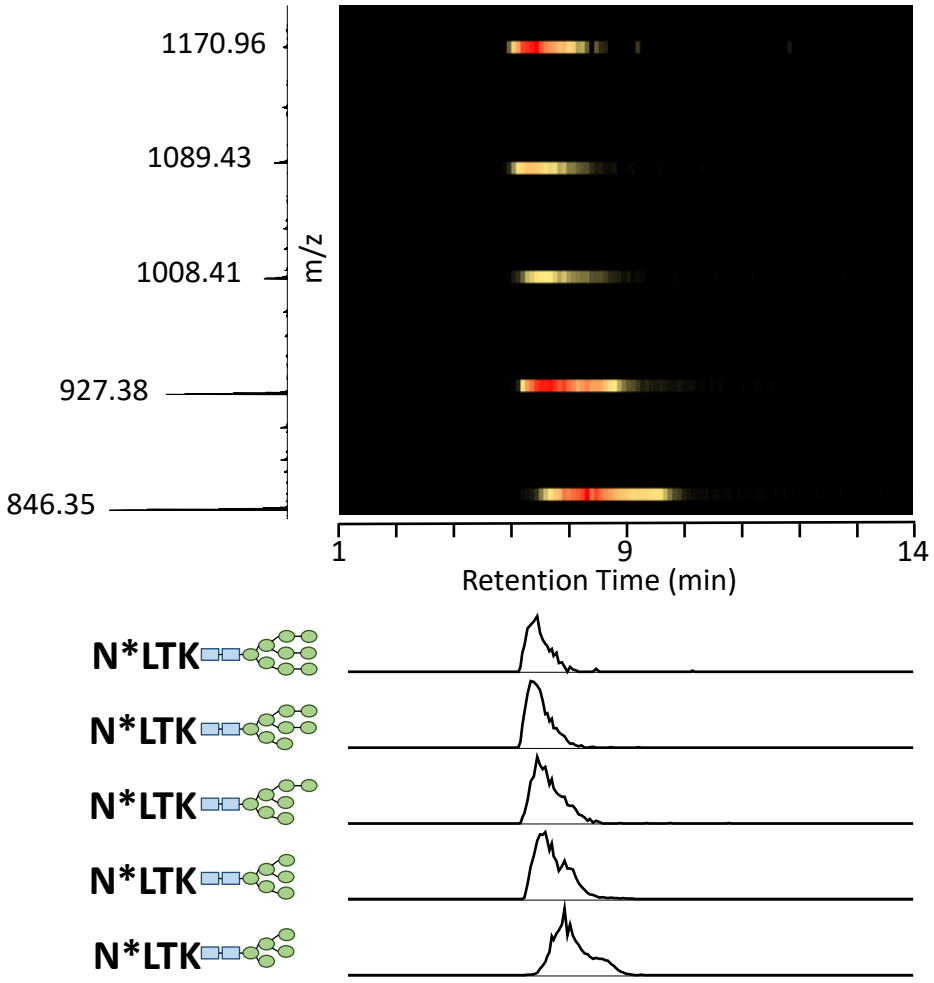
Ribonuclease B



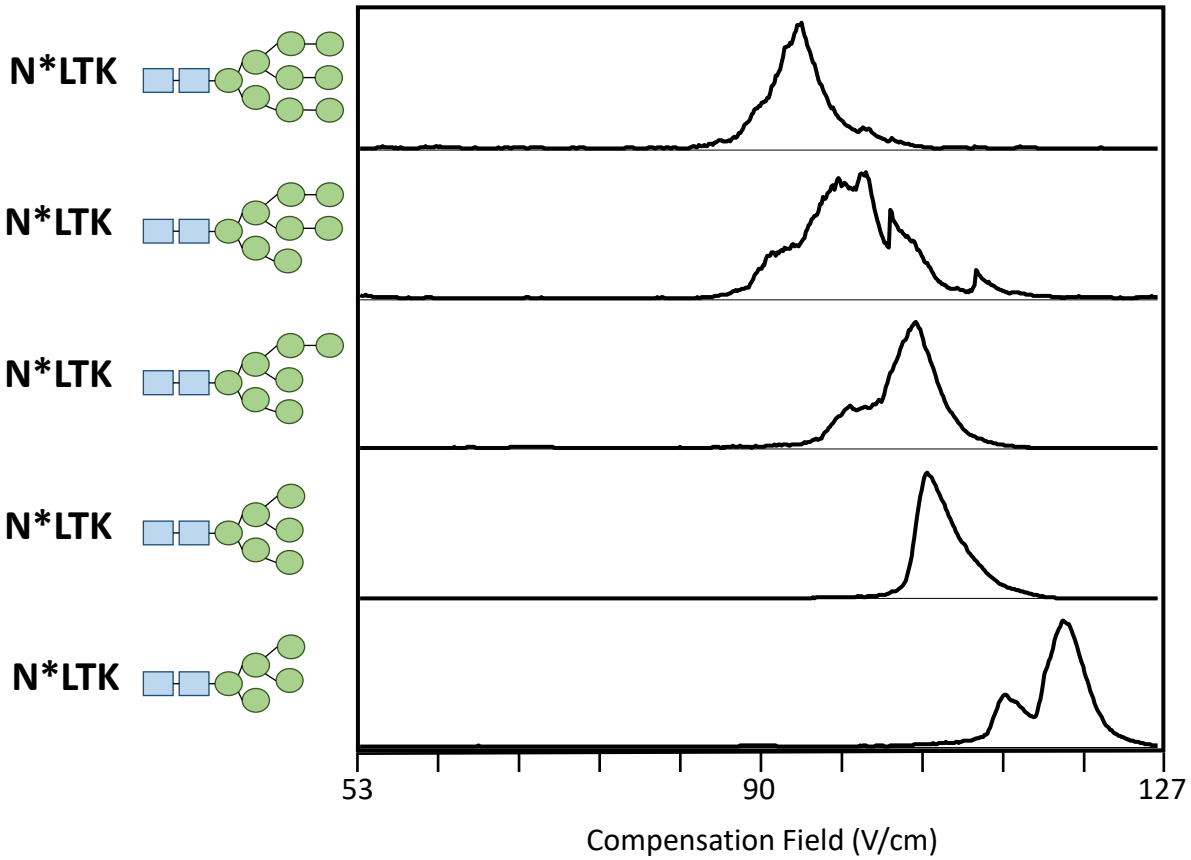
- 20 confidently assigned glycopeptides
- Targeted intact glycopeptide enrichment through online oxonium ion monitoring

FAIMS Analysis

RPLC separation

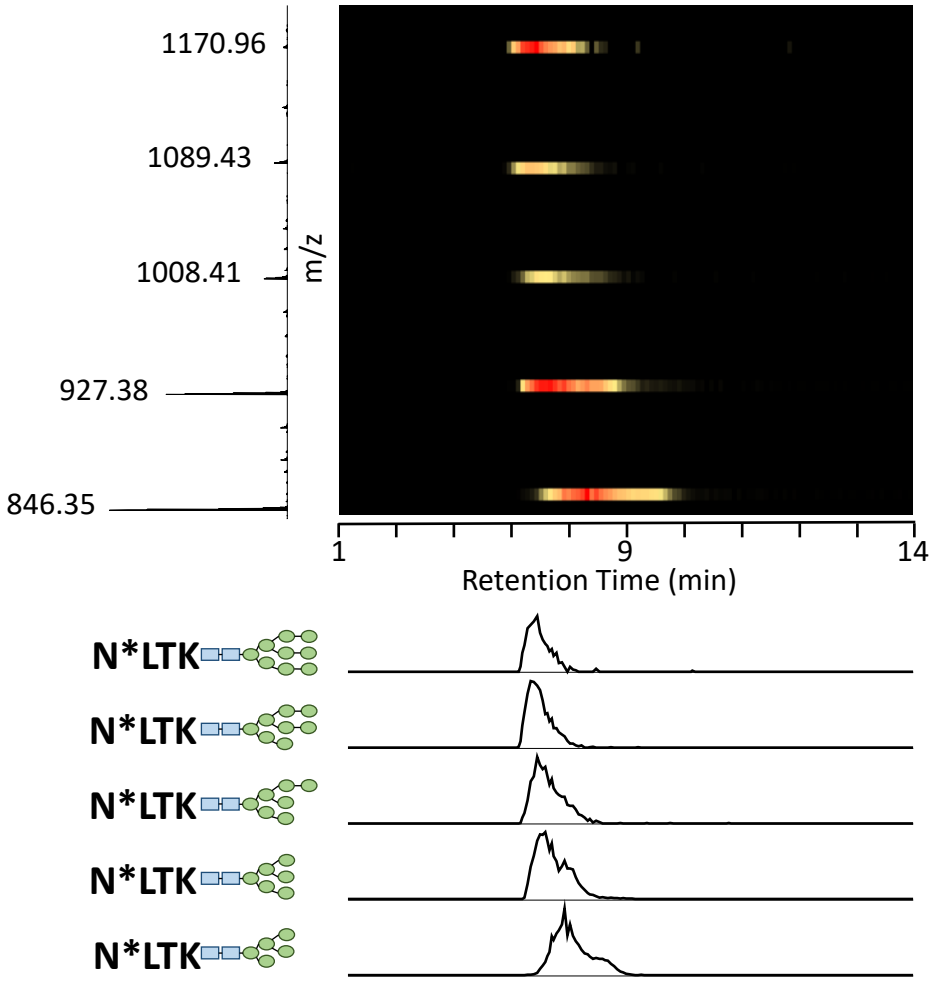


FAIMS separation (60% He)



FAIMS Analysis

RPLC separation



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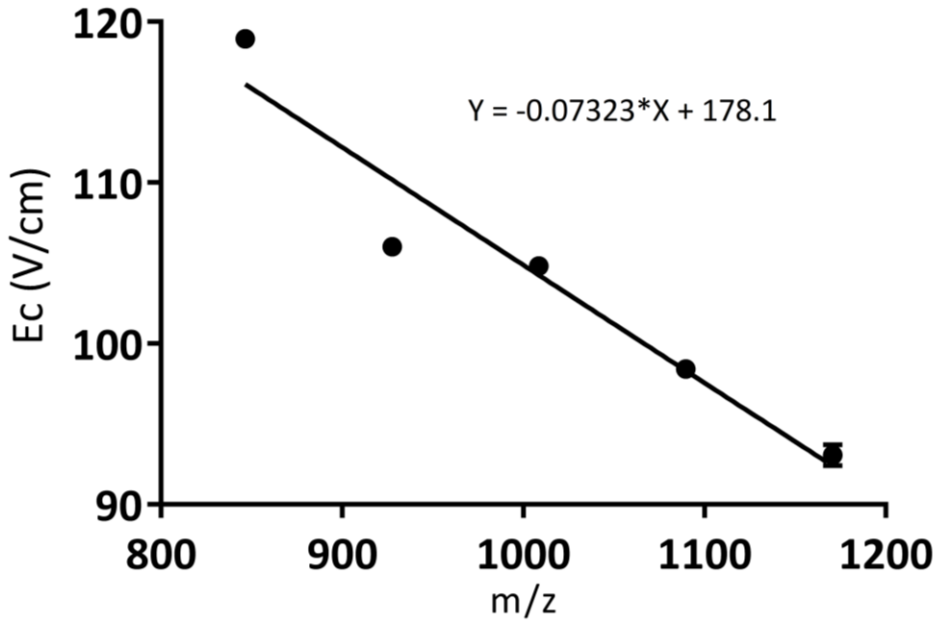
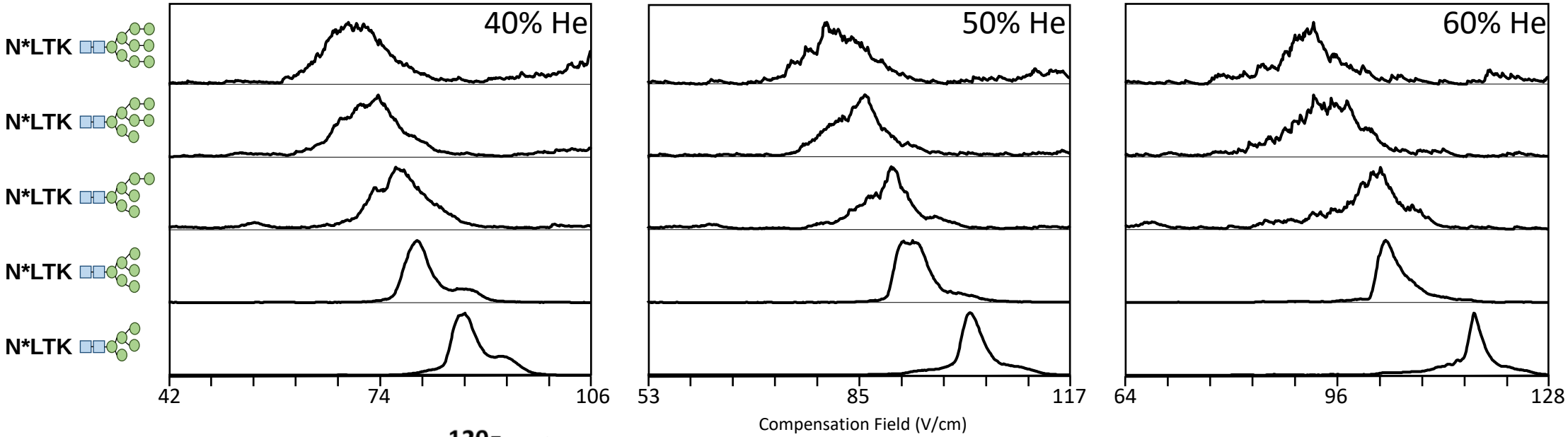
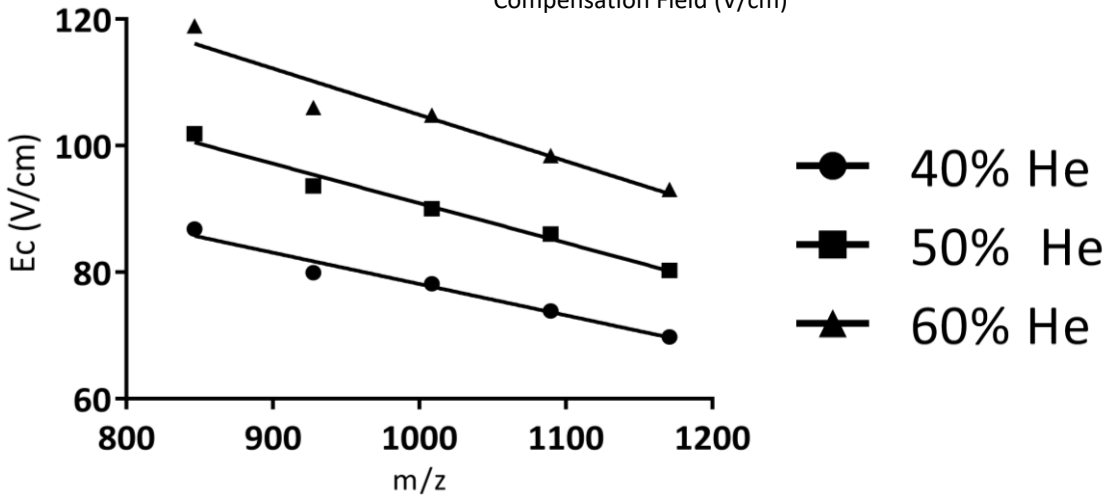


Separation of heterogenous intact glycoforms with the same peptide backbone

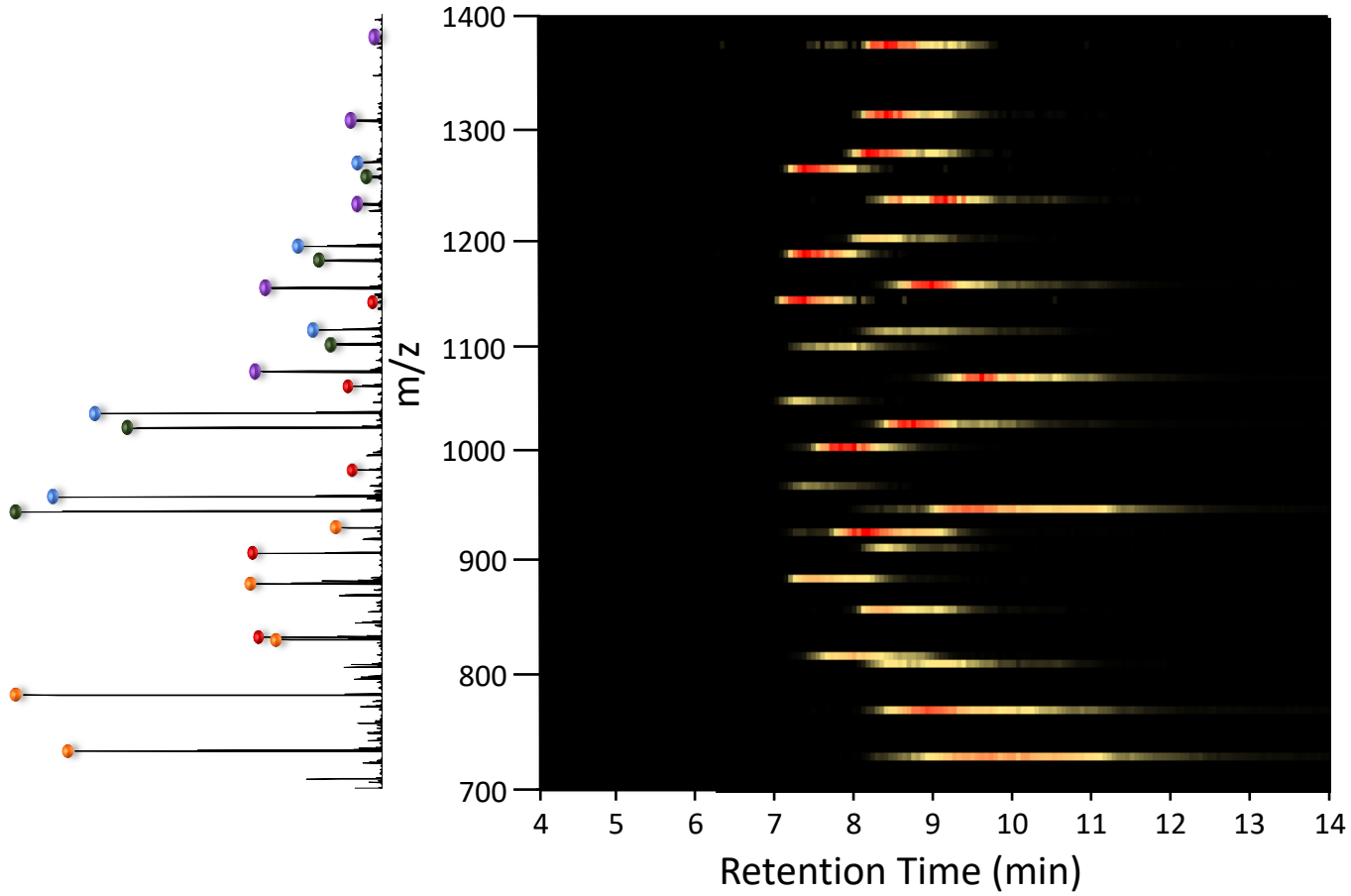
FAIMS under different helium conditions



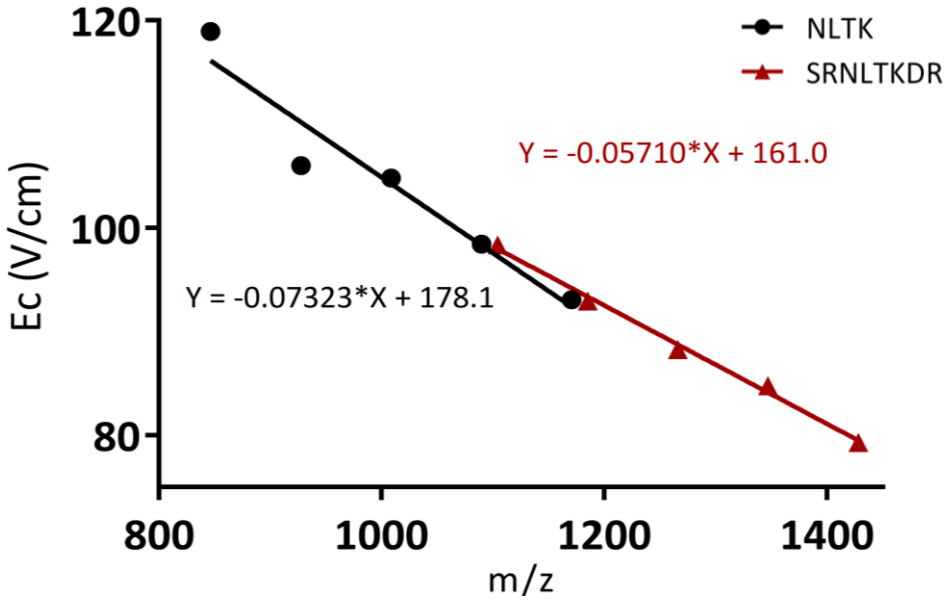


FAIMS Analysis

RPLC separation



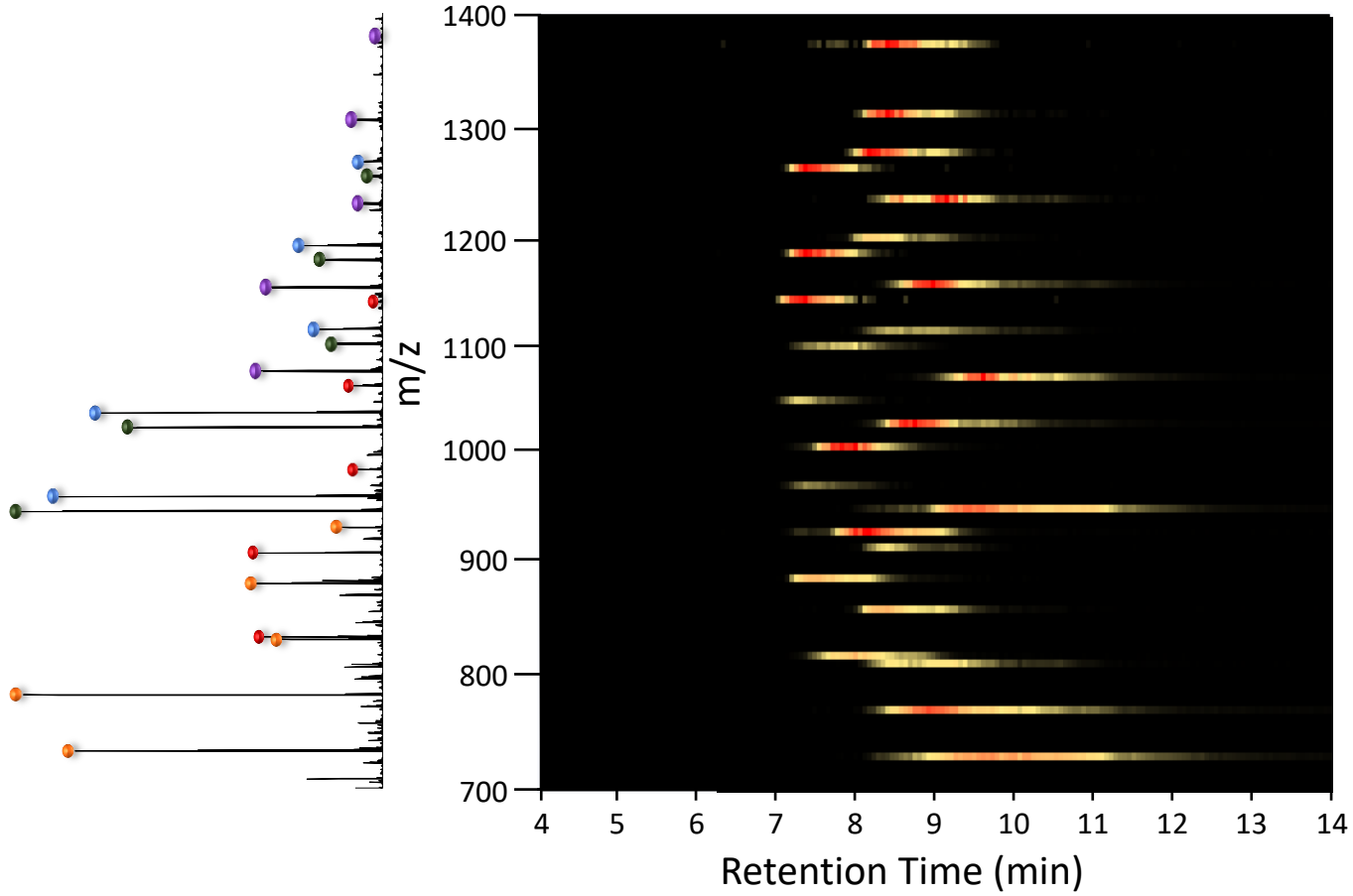
Differentiation in Trend



Glycoforms of varying peptide backbone are greatly differentiated

FAIMS Analysis

RPLC separation



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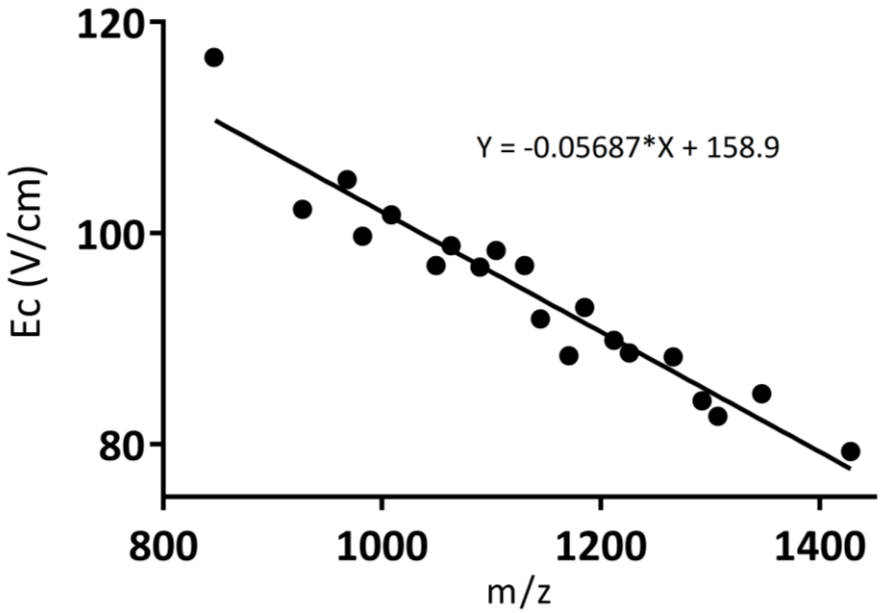
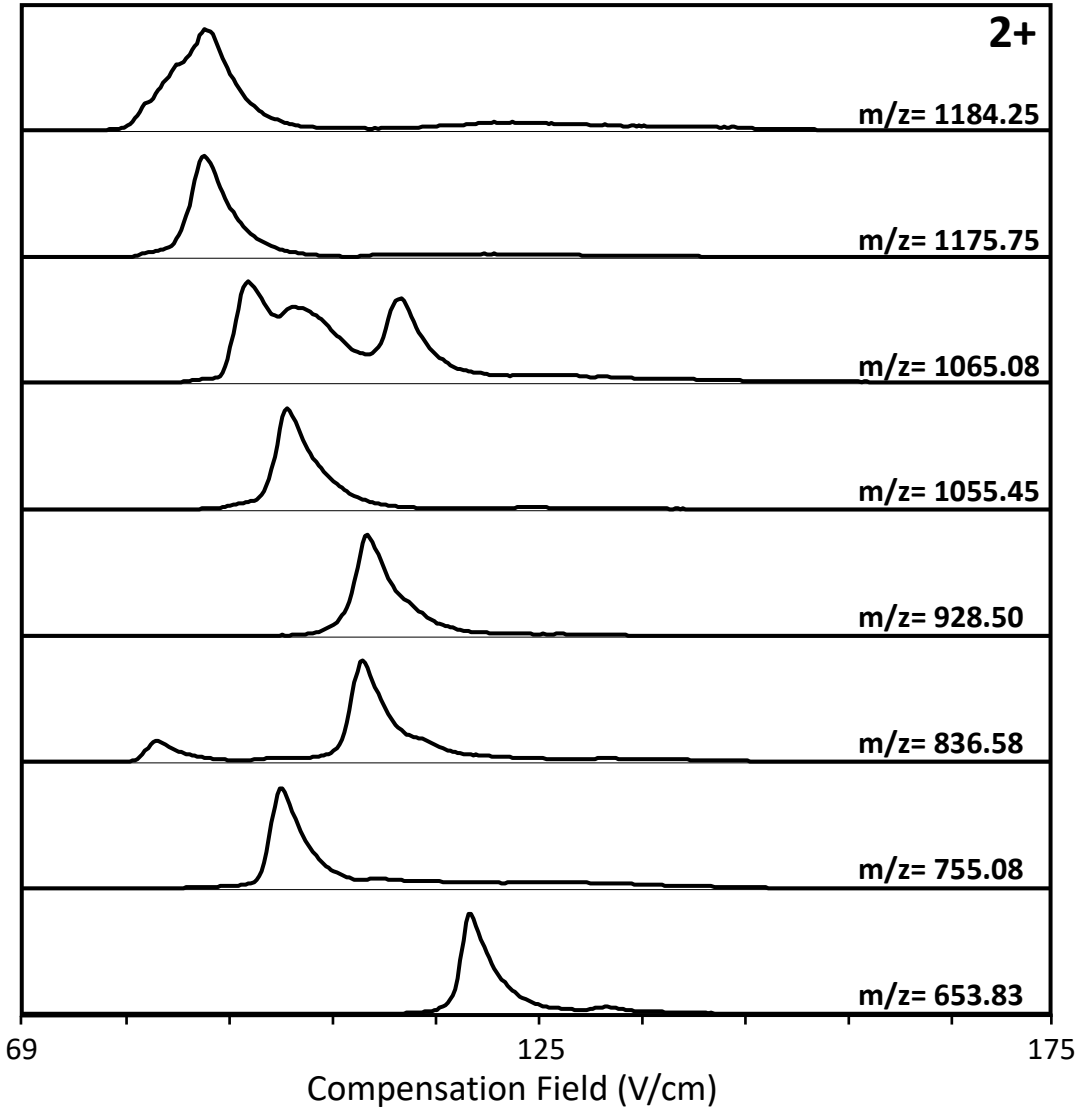
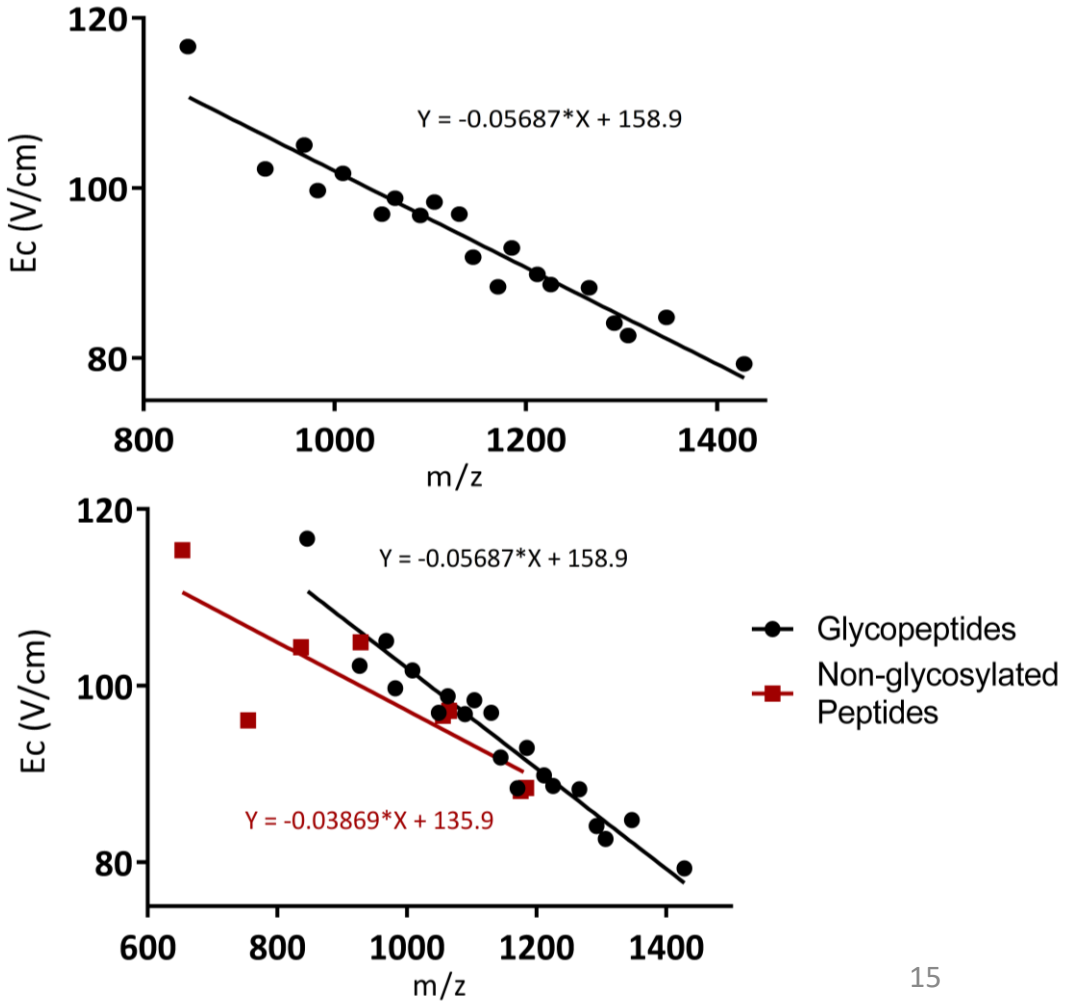


Glycopeptides demonstrate strong correlation, regardless of composition

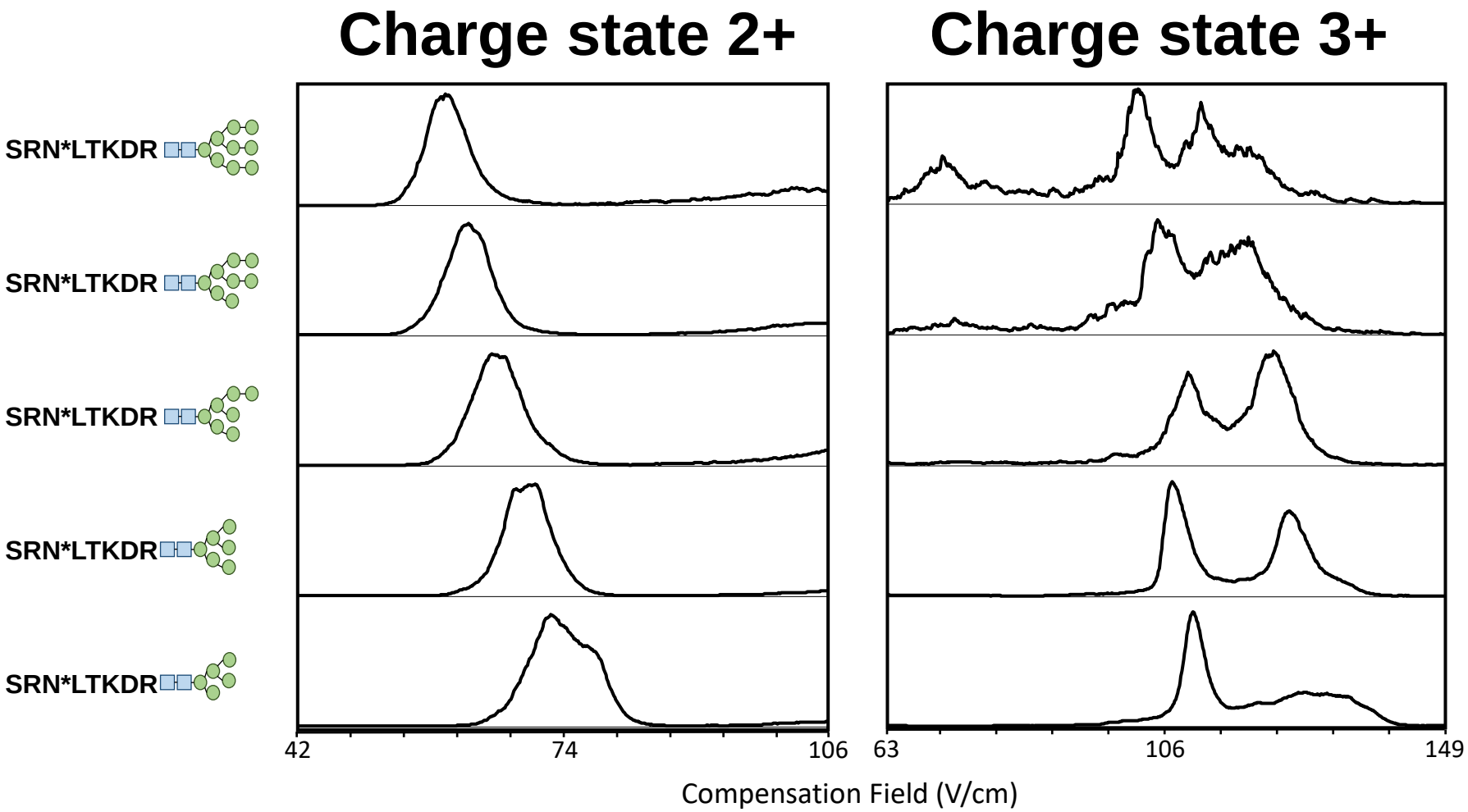
Differentiating Non-Glycopeptides



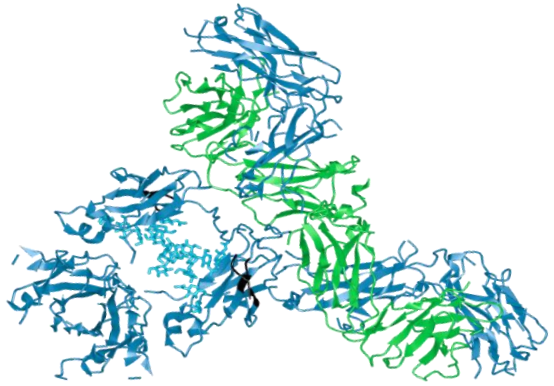
FAIMS separation (60% He)



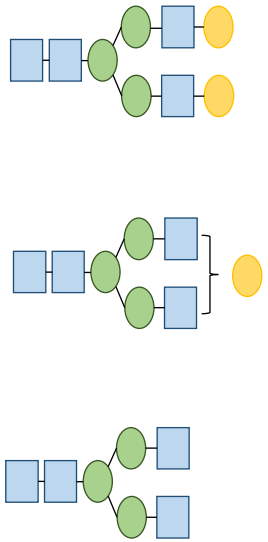
Charge State Evaluation

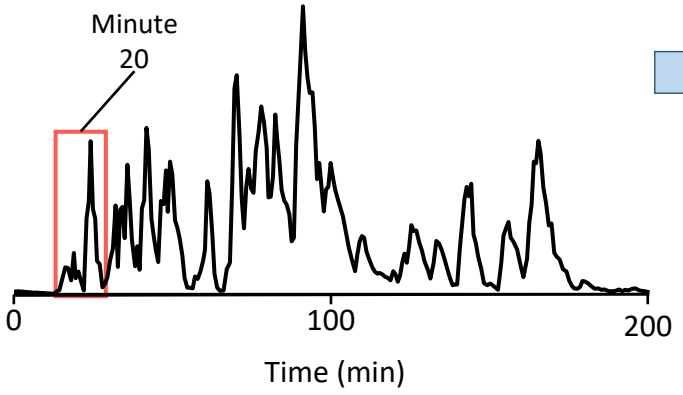


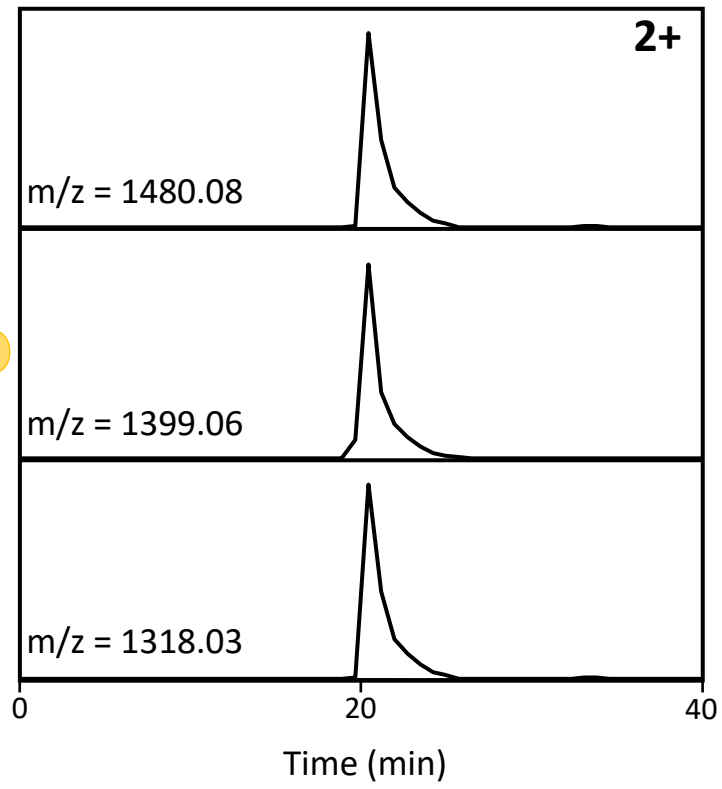
Antibody Application

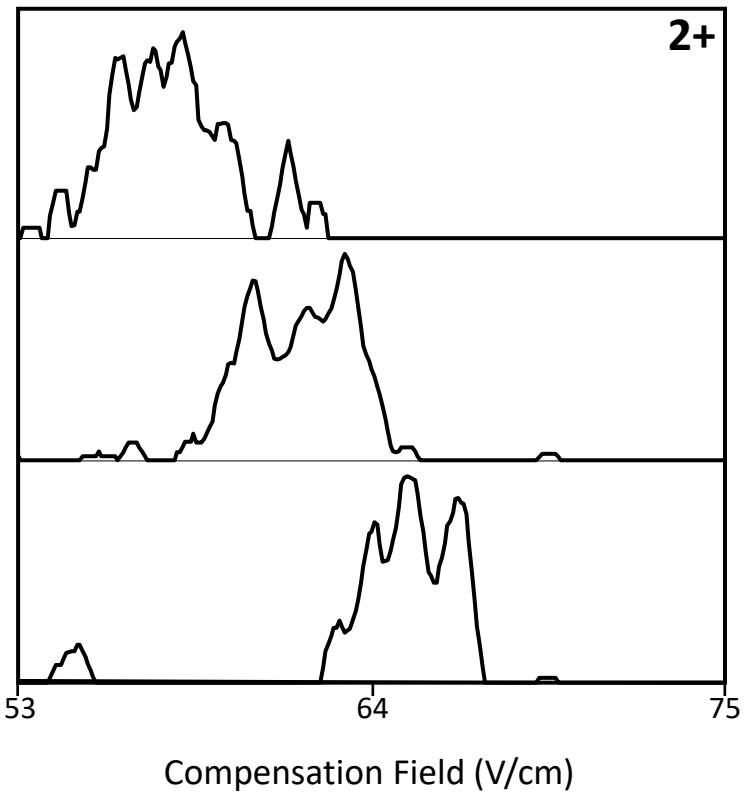


Immunoglobin G1









Conclusion

- Concurrent fractionation method can be universally applied for glycopeptide enrichment

- FAIMS demonstrates resolving power for:
  - Variable backbone glycoforms
  - Unique peptides with different glycans
  - Optimized resolution for given gas composition
  - Increased separation with change in charge state

- Multifaceted, tunable method that may provide additional information for gas-phase glycopeptide studies

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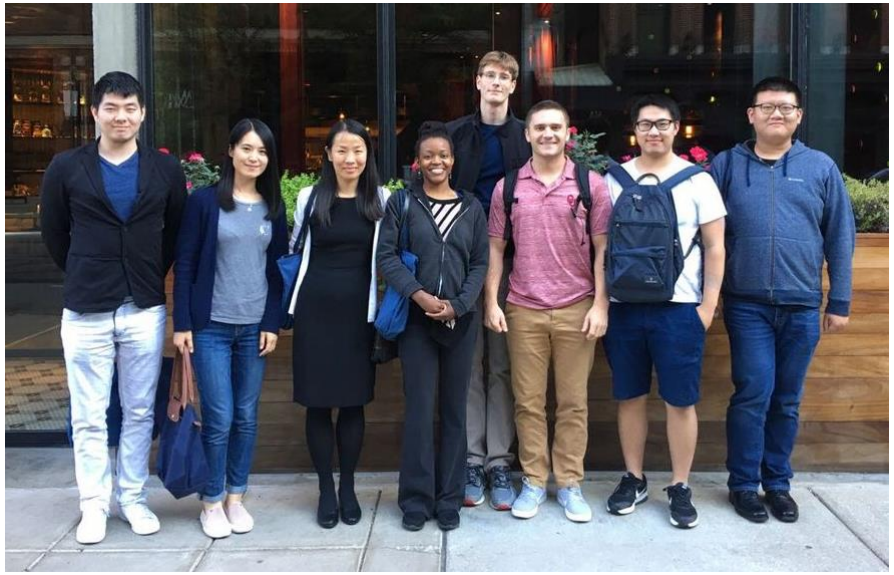
DEPARTMENT OF CHEMISTRY & BIOCHEMISTRY
The UNIVERSITY of OKLAHOMA



Wichita State University

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Matt Baird

WICHITA STATE UNIVERSITY

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OCAST
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