Differential Photofragmentation Patterns for Mobility Selected Glycans

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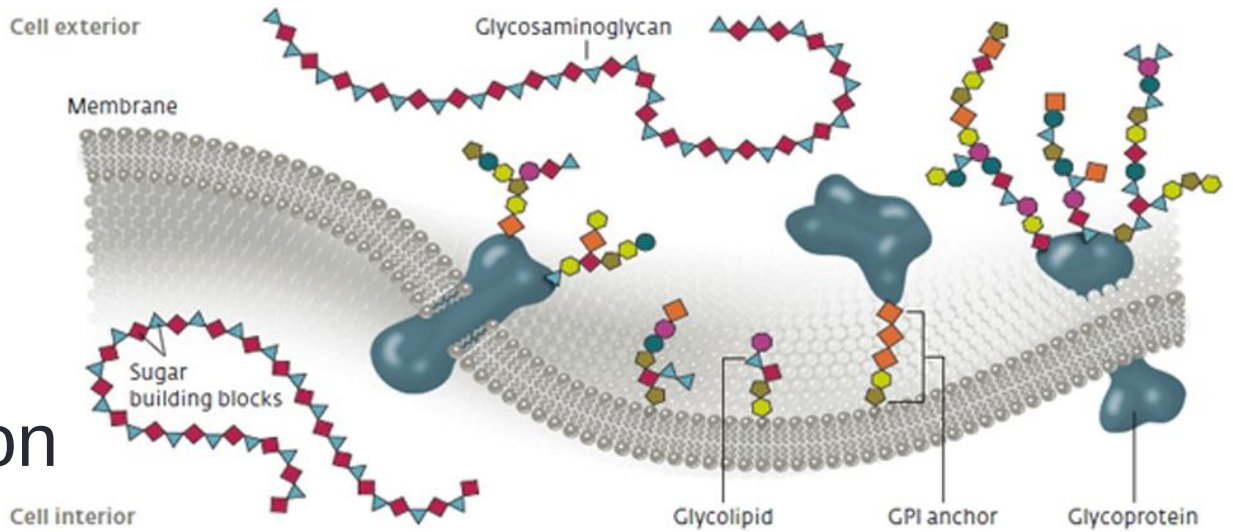
Why Care About Glycans?

- Occupy vital roles in biological systems
- Examples:
  - Immune response
  - Blood type determination
  - Glycoprotein structure
  - Cell recognition



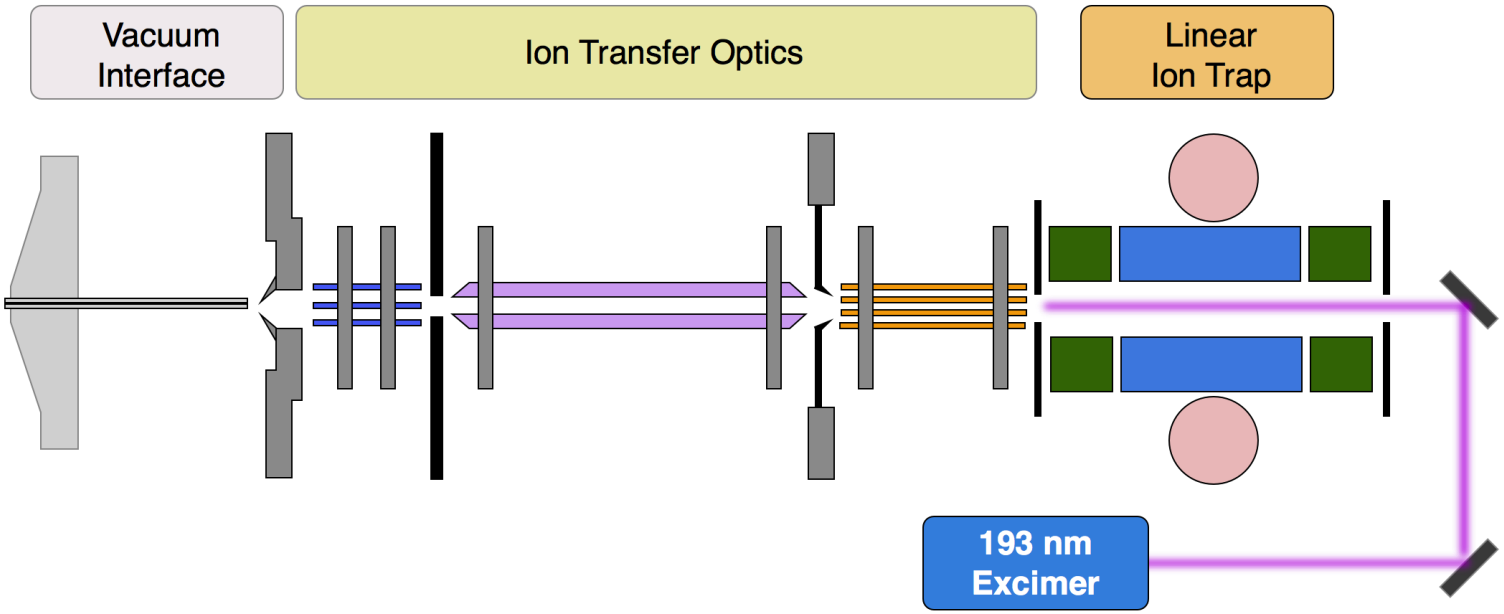
http://www.mpikg.mpg.de/3420137/glycans.jpg

Challenges in Glycan Analysis

- Branching of carbohydrates further complicates glycan identification via MS
  - Isomer distinction particularly difficult
- Greater difficulty in ionization via ESI of glycans compared to other biomolecules (e.g. proteins)
  - Adduction with cations and anions
  - Solutions: Labeling and derivatization

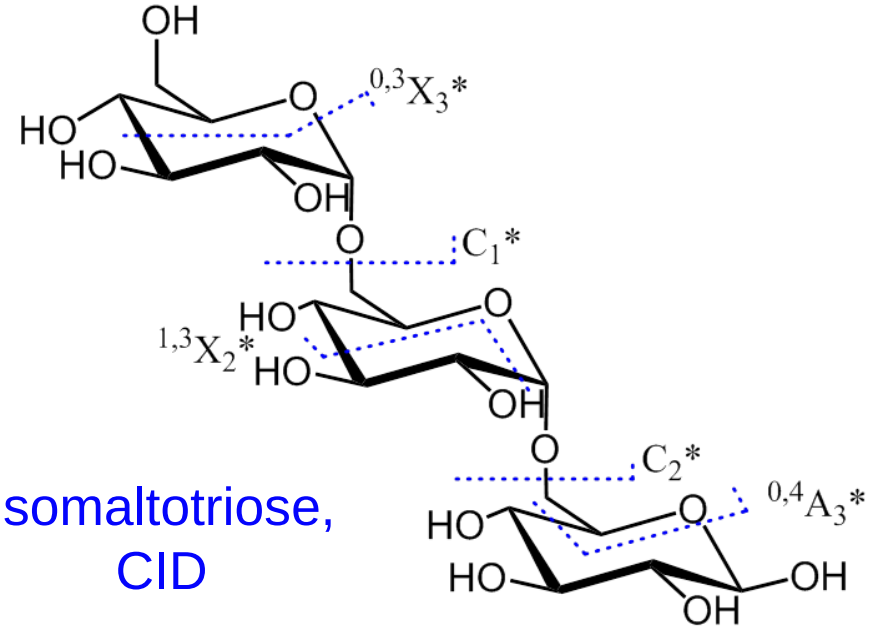
MS/MS of Glycans

- Tandem MS required for determining glycan composition and structure
  - CID, ECD, ETD, HCD
- Often require *a priori* knowledge of system to yield useful information
- What about other fragmentation methods?
  - UVPD

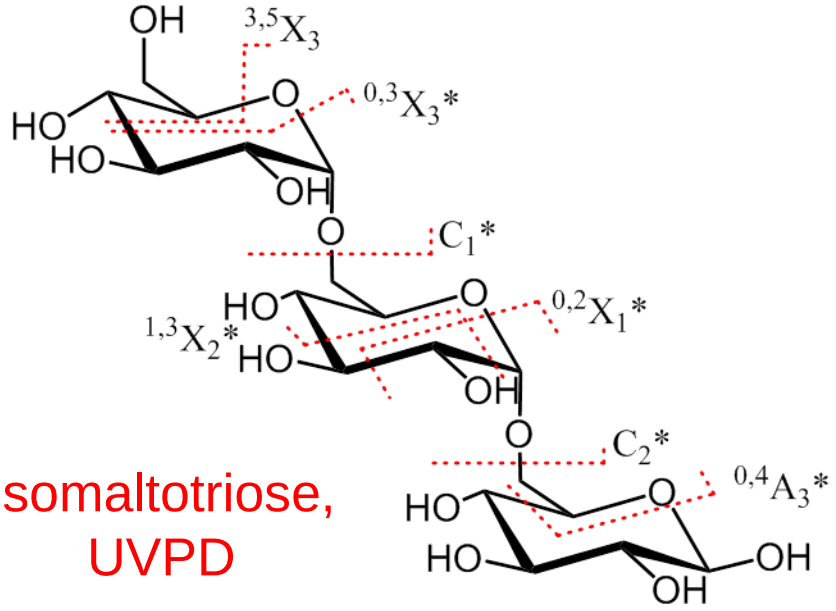


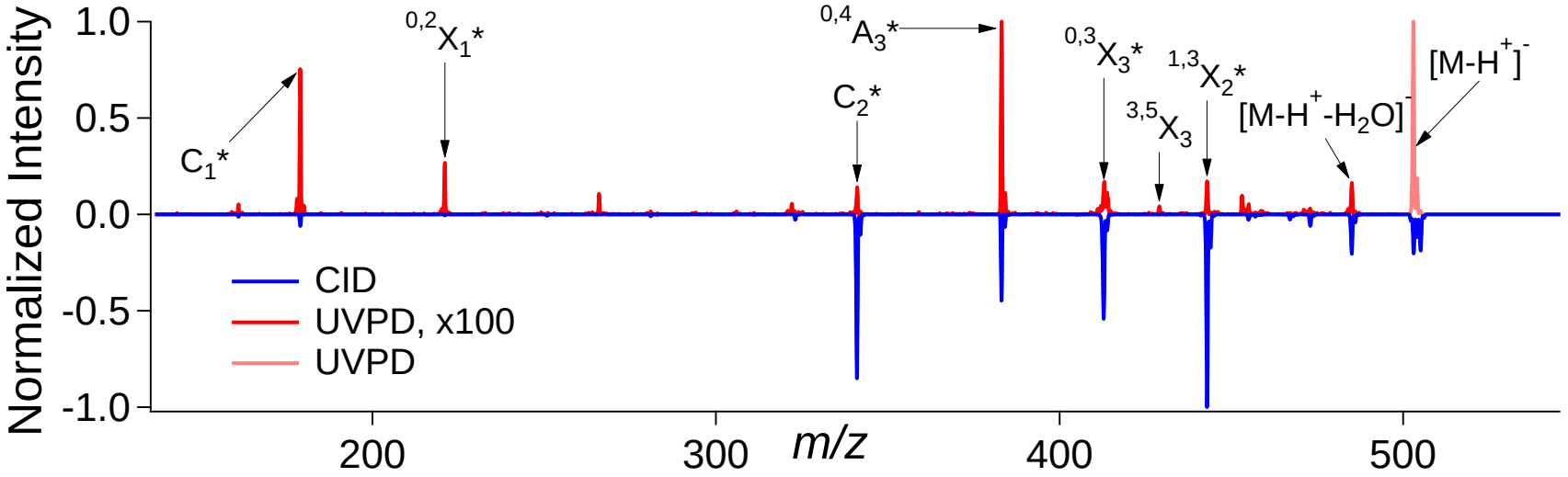
MS/MS of Glycans: UVPD and CID

Isomaltotriose, CID

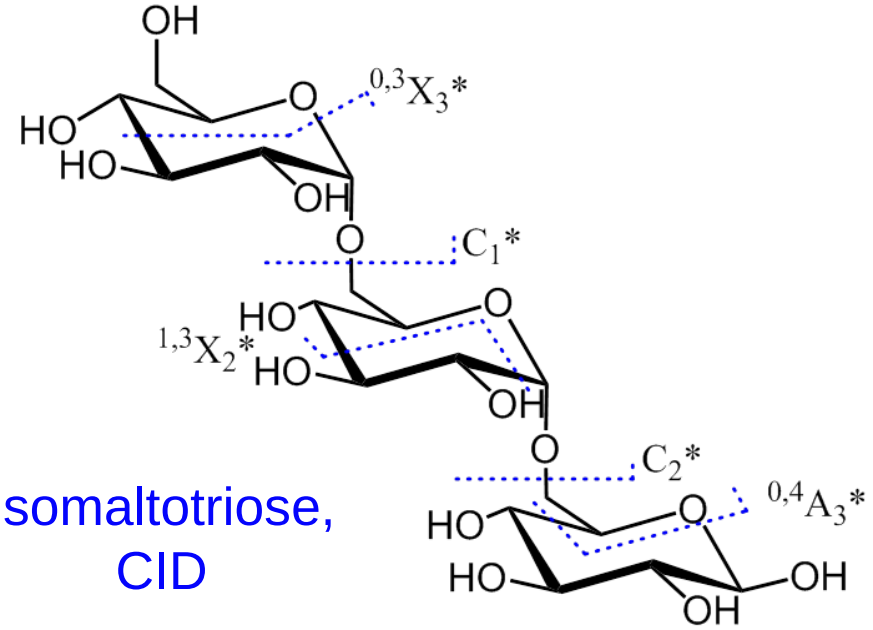


Isomaltotriose, UVPD

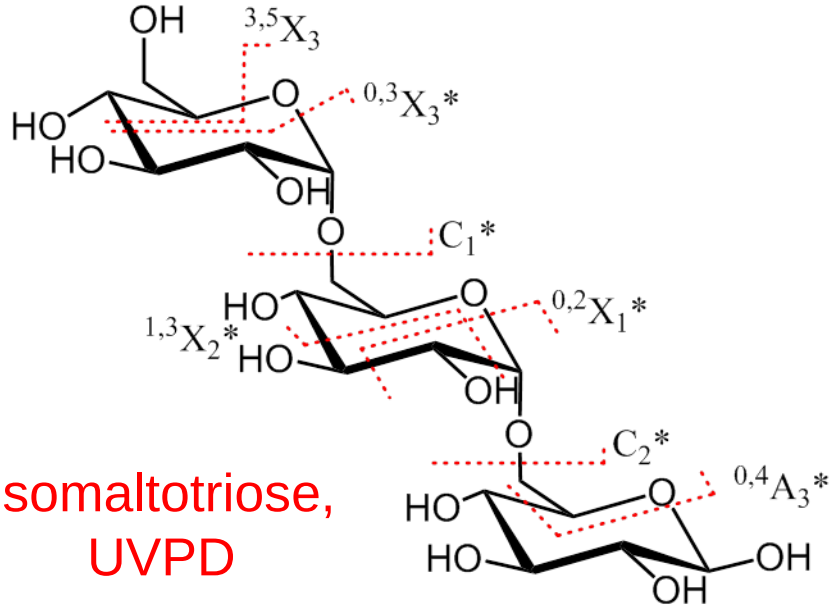




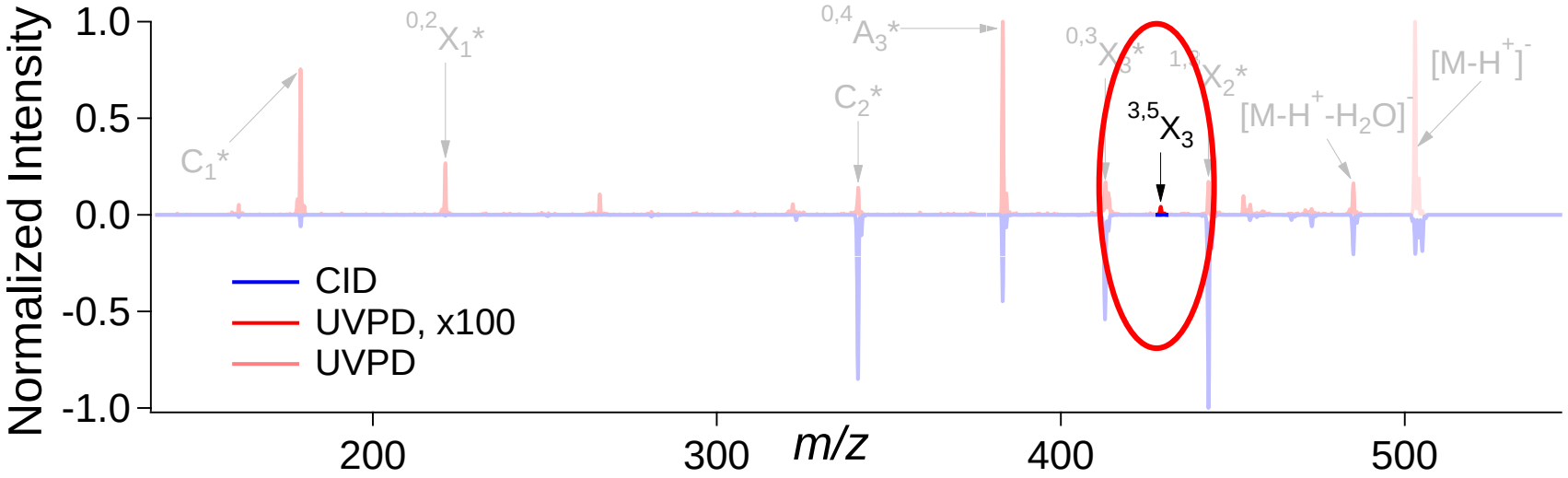
MS/MS of Glycans: UVPD and CID



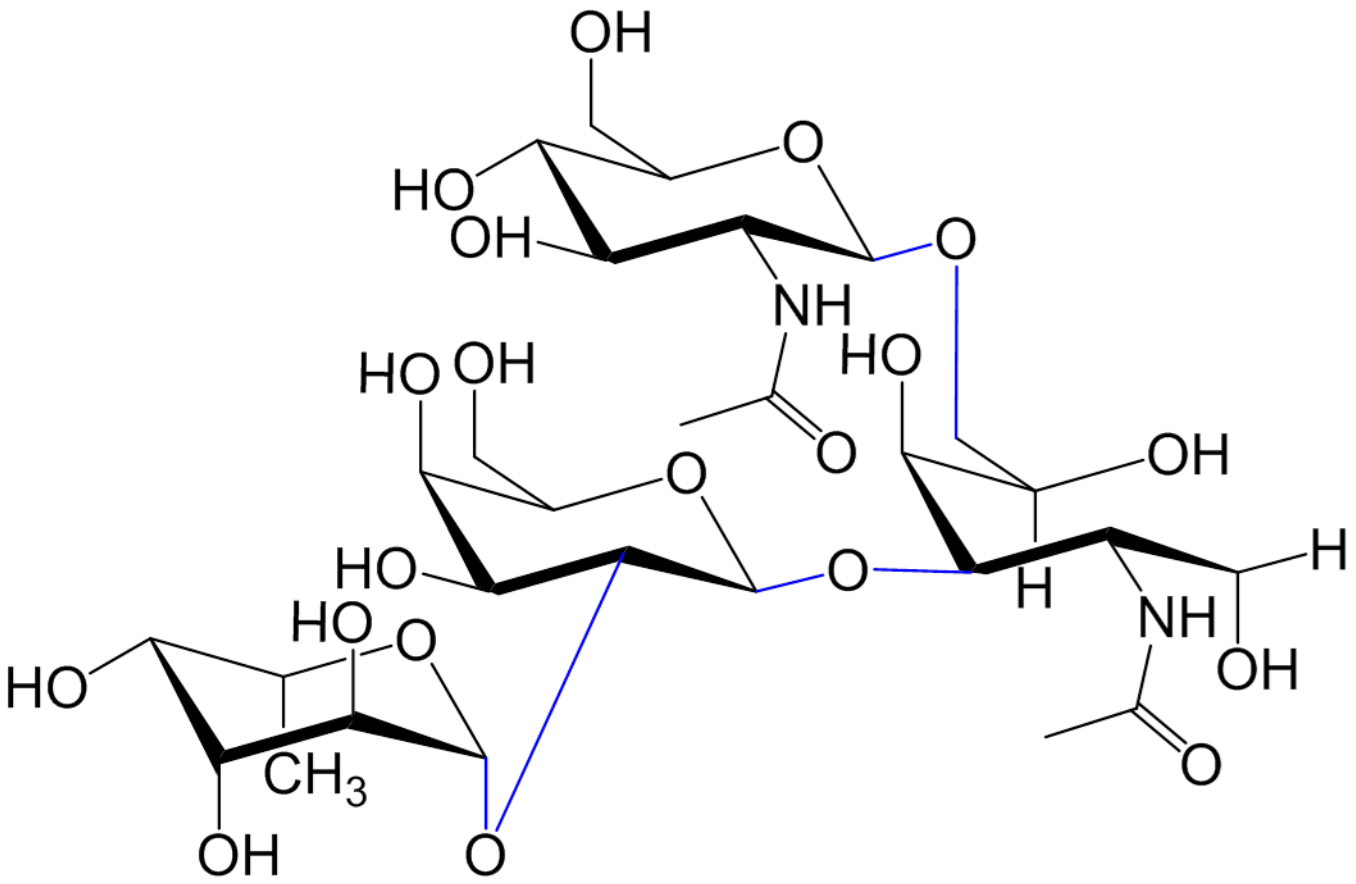
Isomaltotriose, CID



Isomaltotriose, UVPD

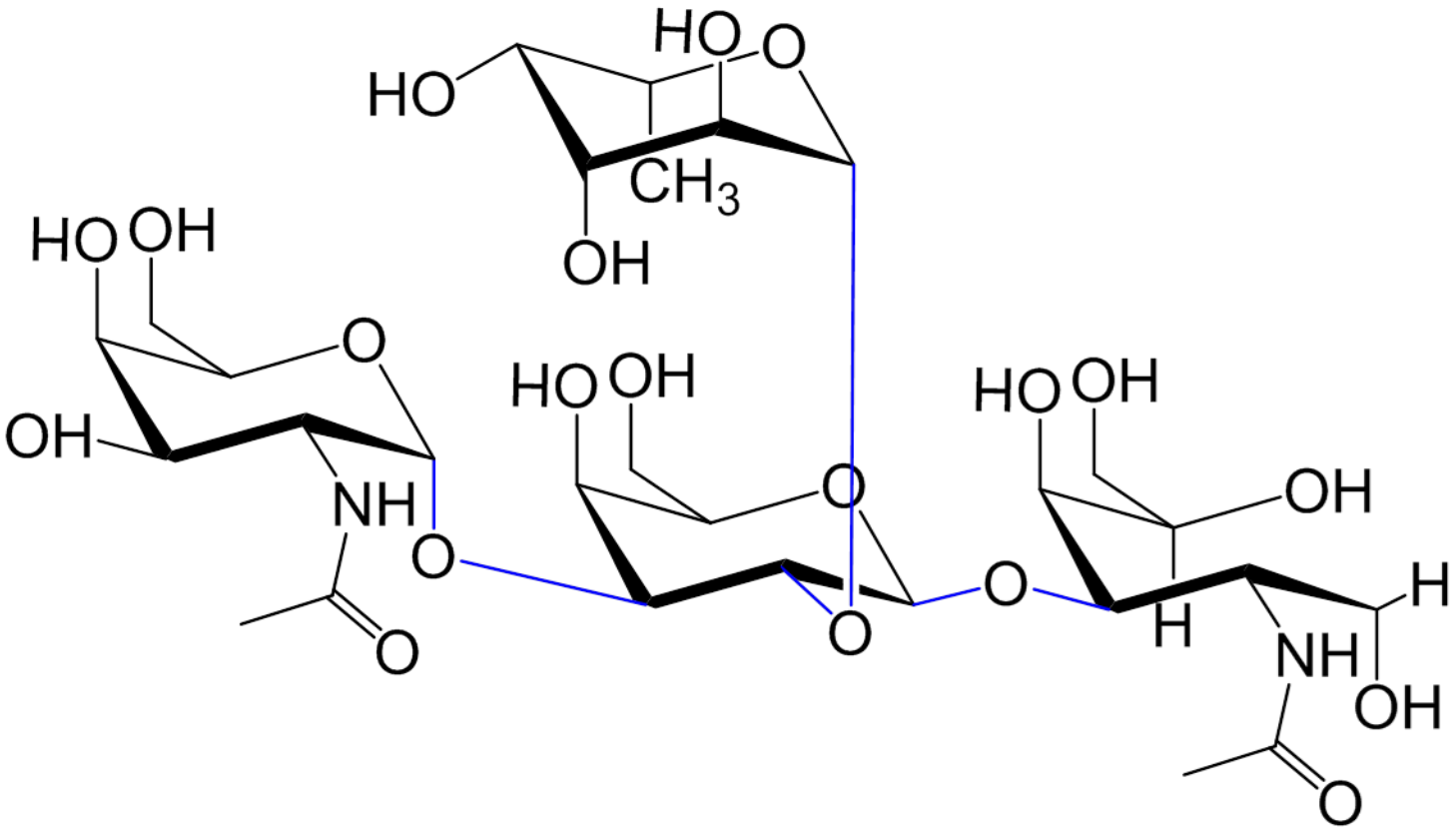


Isomeric Glycans Analyzed



(GlcNAc-β-1,6)(Fuc-α-1,2-Gal-β-1,3)GalNAc-ol,
a.k.a. “Tetra L”

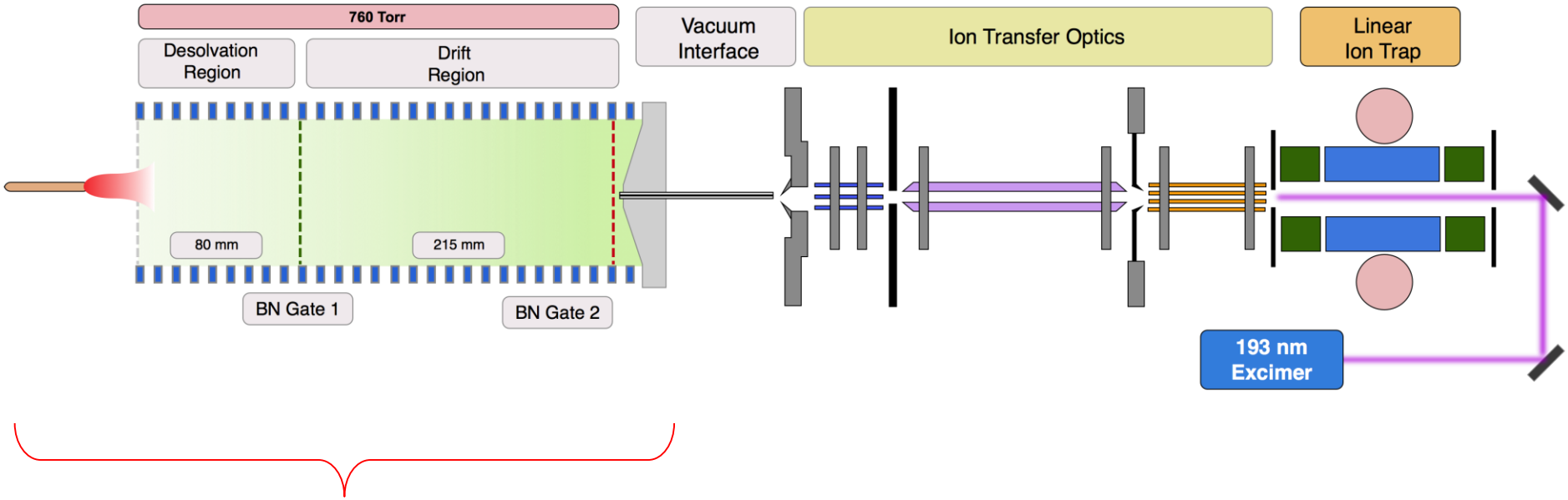
Isomeric Glycans Analyzed



(GalN-α-1,3)(Fuc-α-1,2)Gal-β-1,3-GalNAc-ol,
a.k.a. “Tetra B”

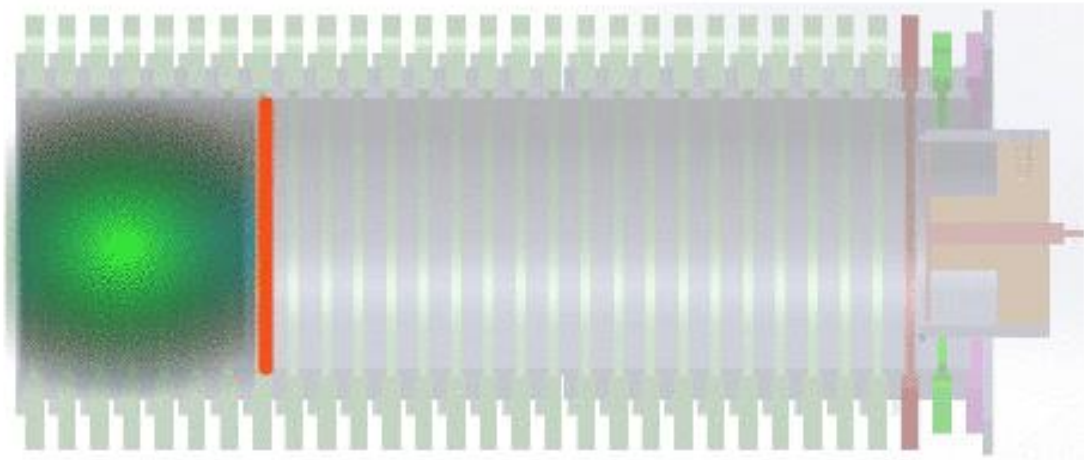
GAS PHASE SEPARATION: DUAL-GATE IMS

- ExcellIMS MA3100 dual-gate IMS
- Dual-gate IMS technology developed at Washington State University



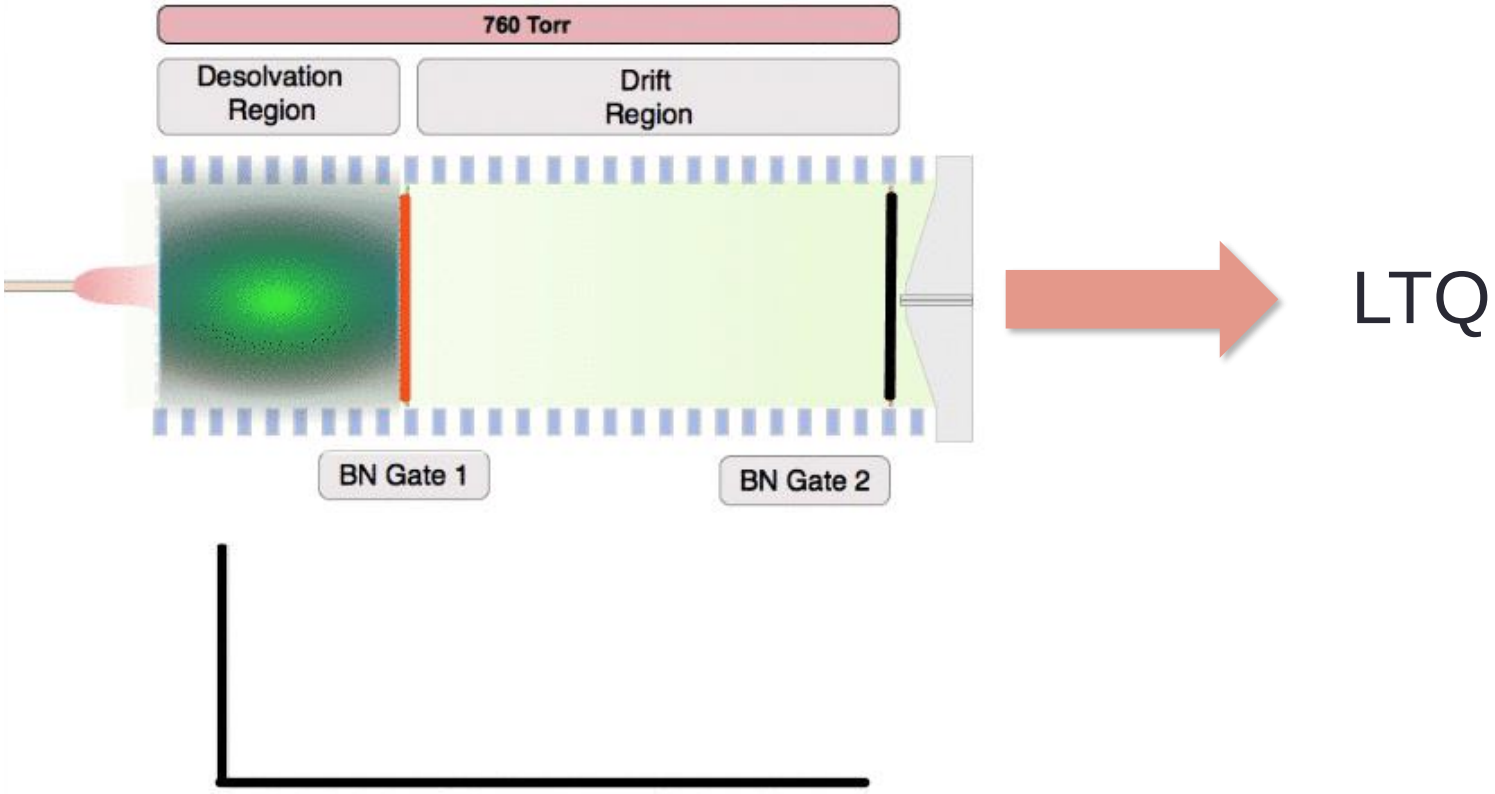
Gas Phase Separation: Dual-Gate IMS

- Faraday Mode



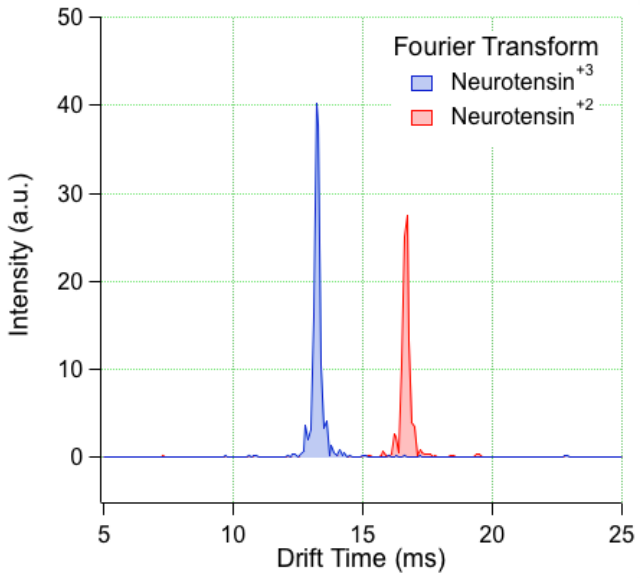
Gas Phase Separation: Dual-Gate IMS

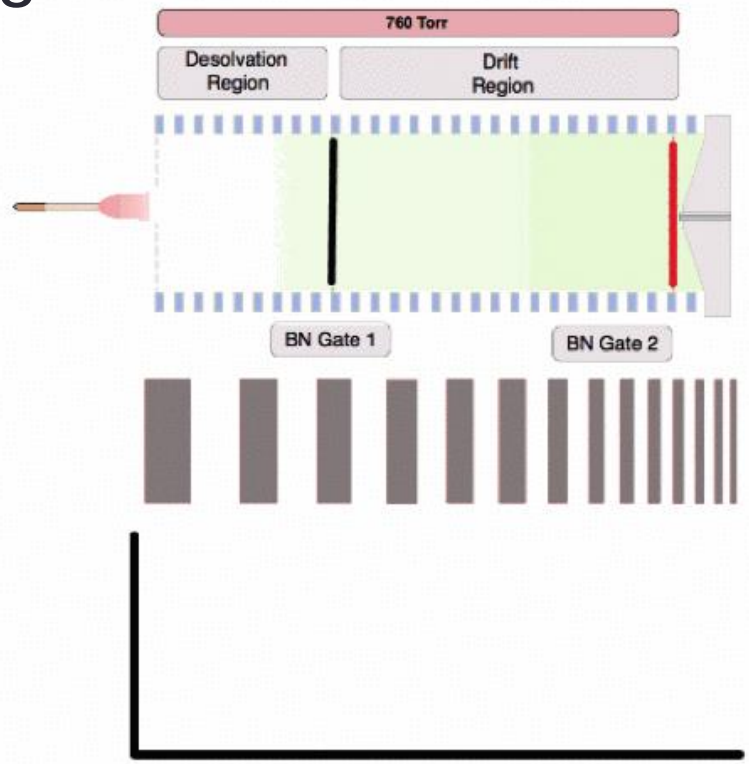
- Gated Mode
  - Drift time window selection



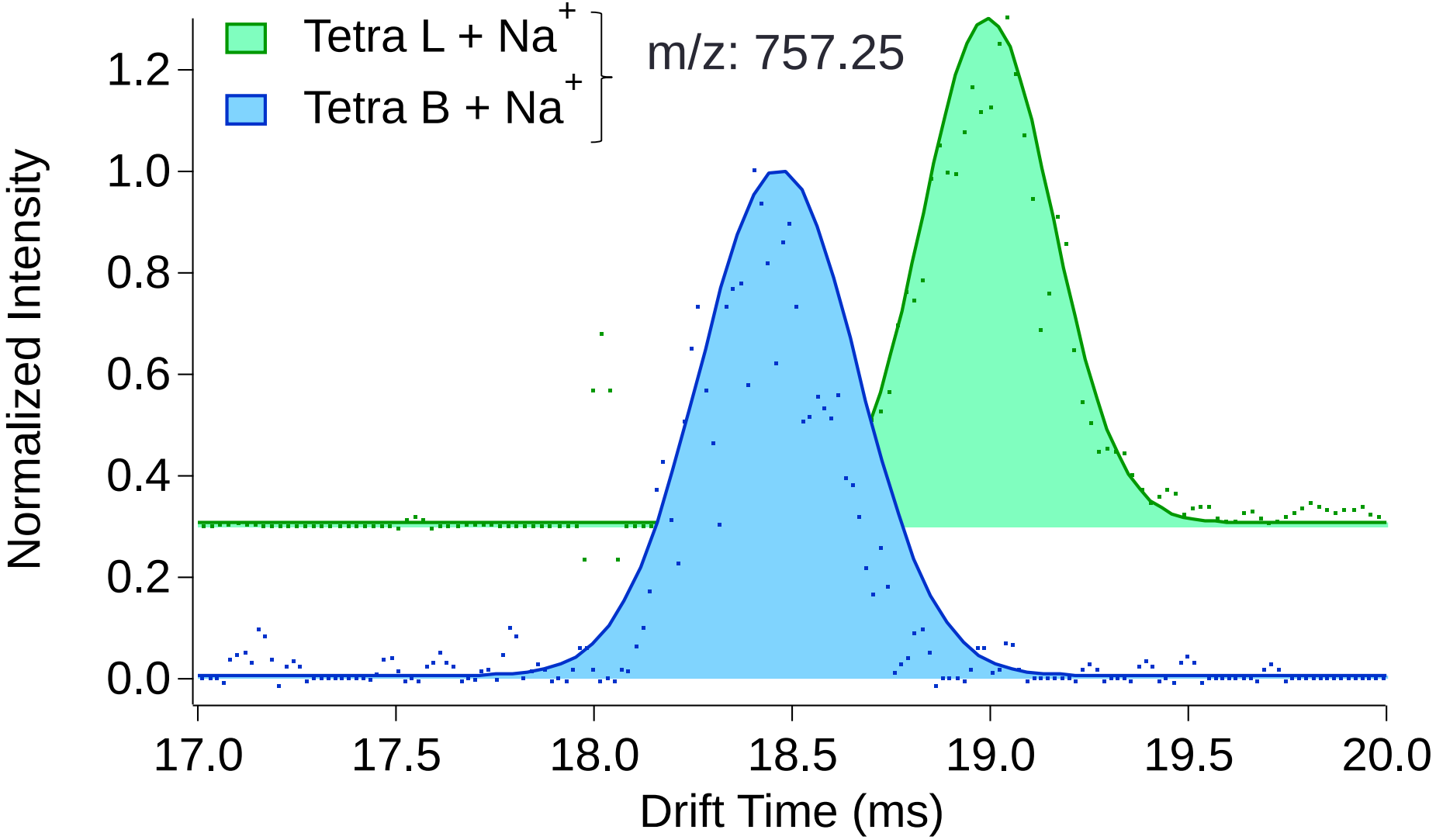
Gas Phase Separation: Dual-Gate IMS

- Fourier Transform Operation
  - Enhanced Duty Cycle
  - Effective LTQ Coupling
    - Full mobility spectrum

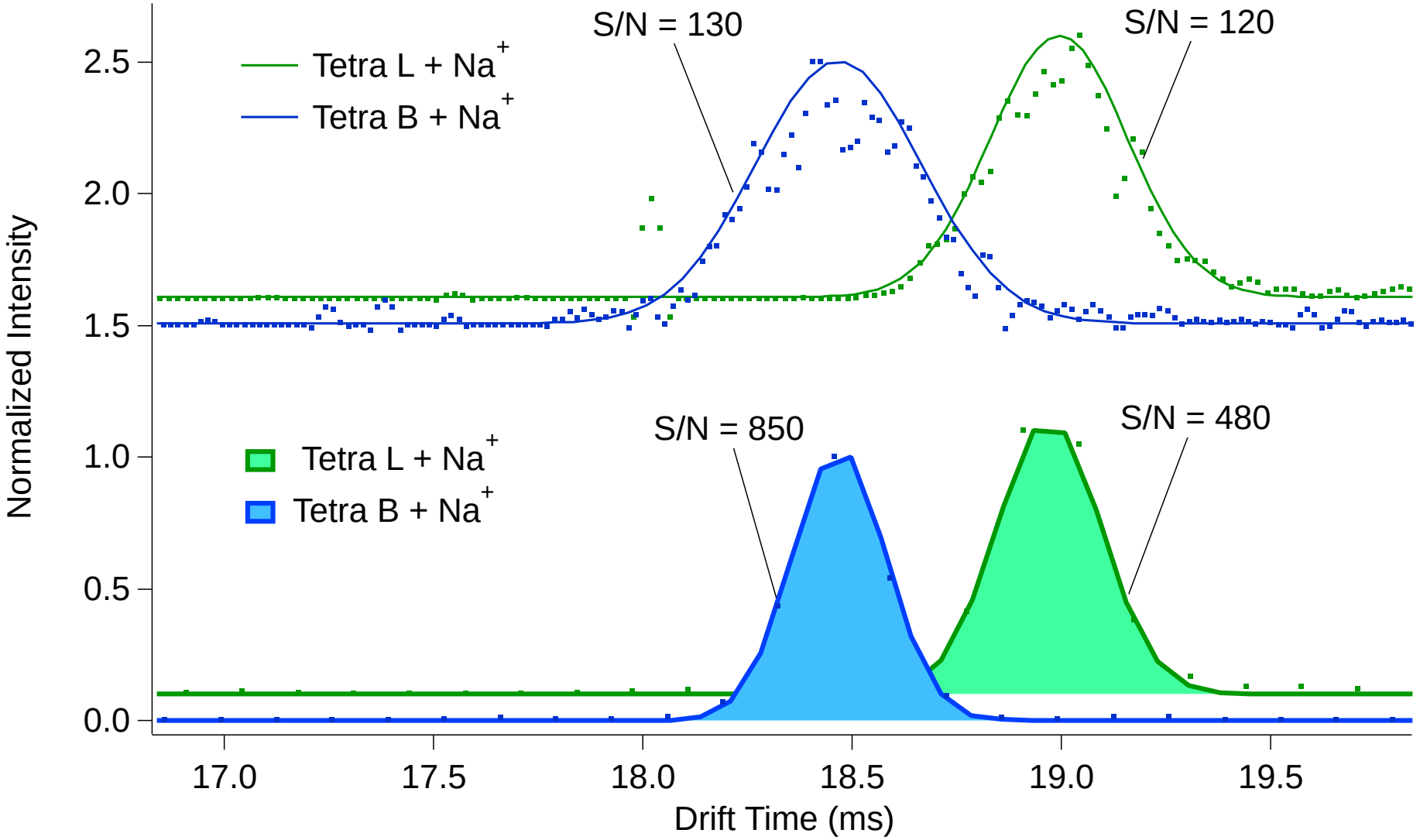




SCANNED IMS OF ISOMERIC GLYCANS

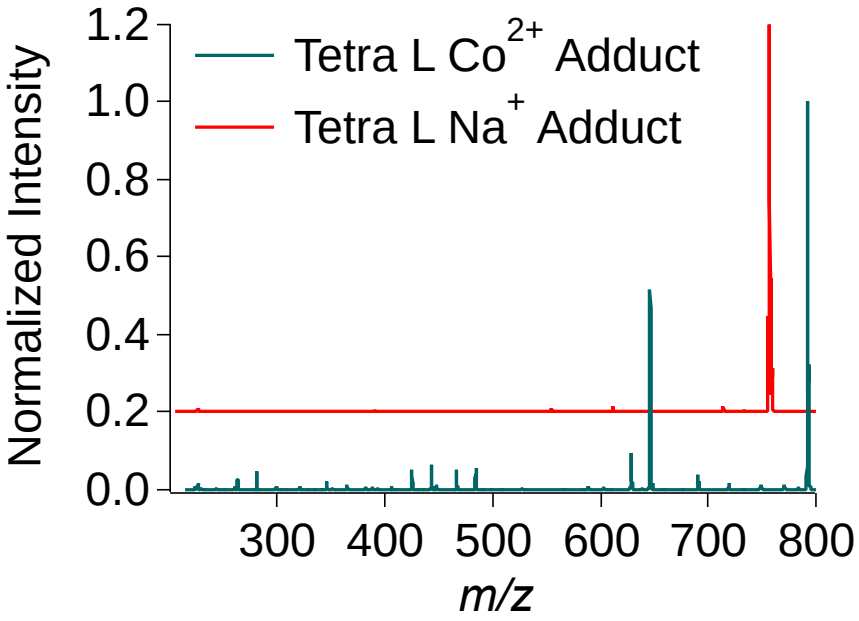


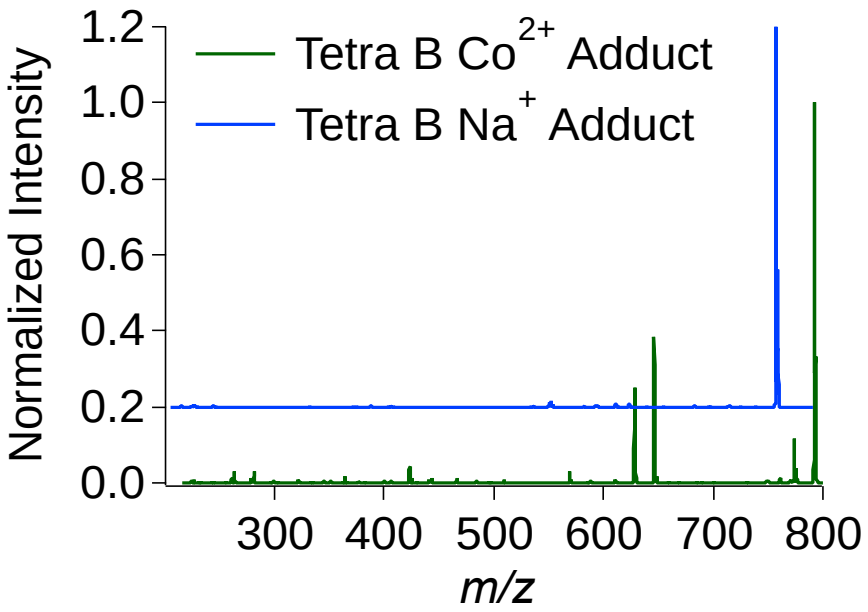
Fourier Transform and Dual-Gate IMS



UVPD of Cation-Glycan Adducts

- However, UVPD of sodiated glycans yields poor relative fragment intensities
- Lebrilla, Harvey groups have shown the impact of different cations on fragmentation



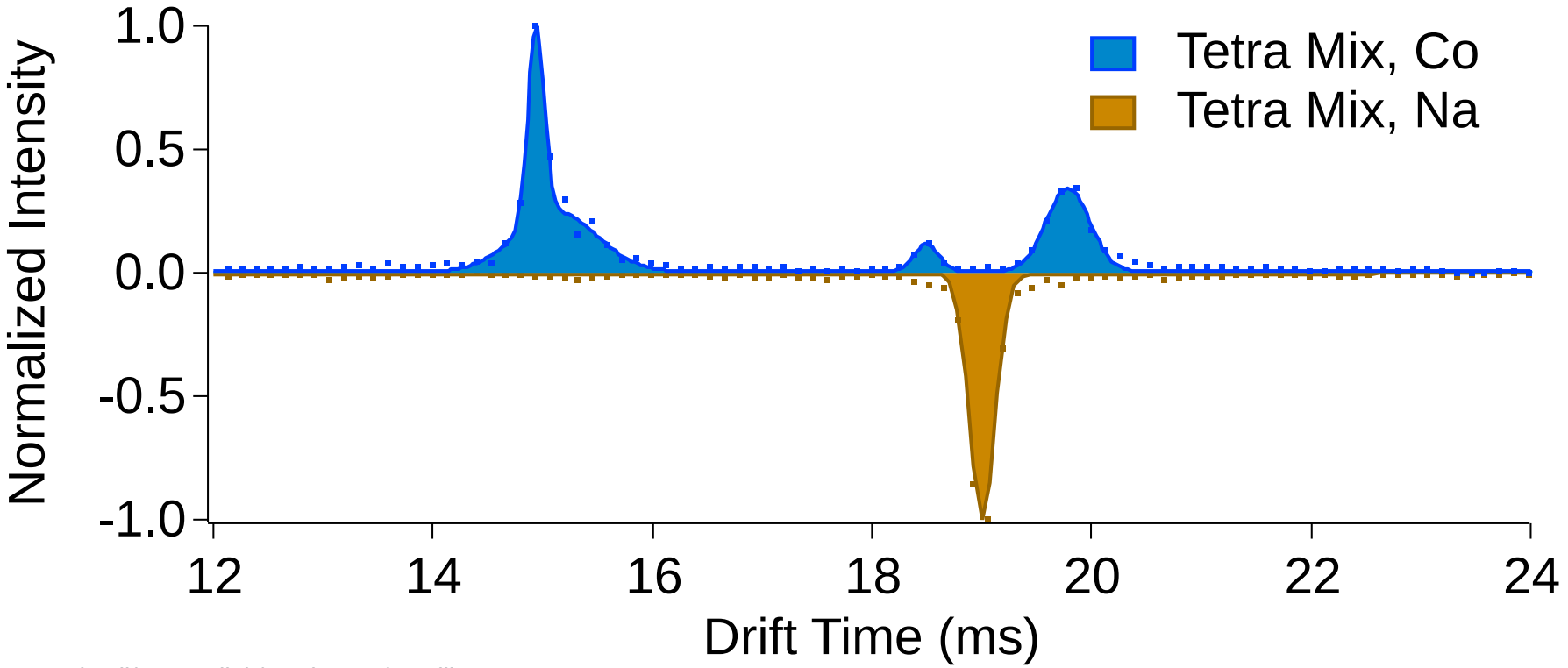


M. Cancilla/S. Penn/J. Carroll/C. Lebrilla. *J. Am. Chem. Soc.* **1996**, *118*, 6736-6745.

D. Harvey. *J. Am. Soc Mass Spectrom.* 2001, 12 926-937.

Co^{2+}-Glycan Adduct Separation

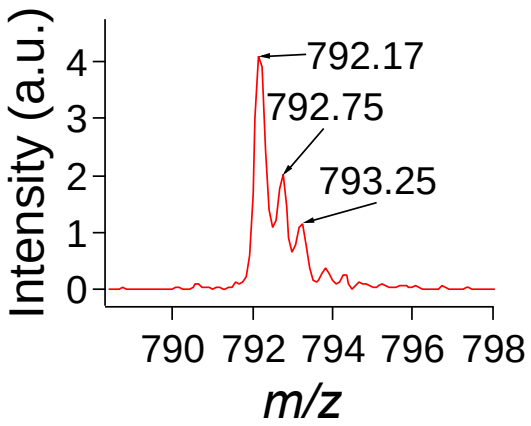
- Also, addition of Co(II) Acetate allows for greater separation of a mixture of the glycans

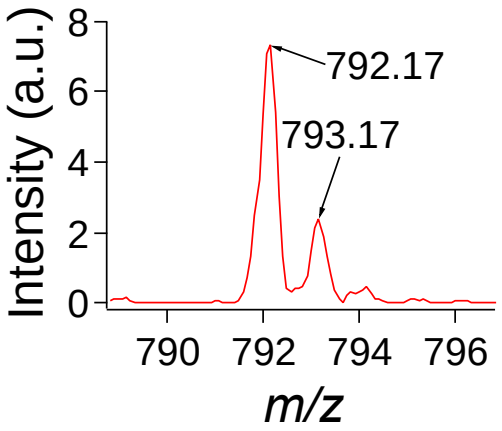


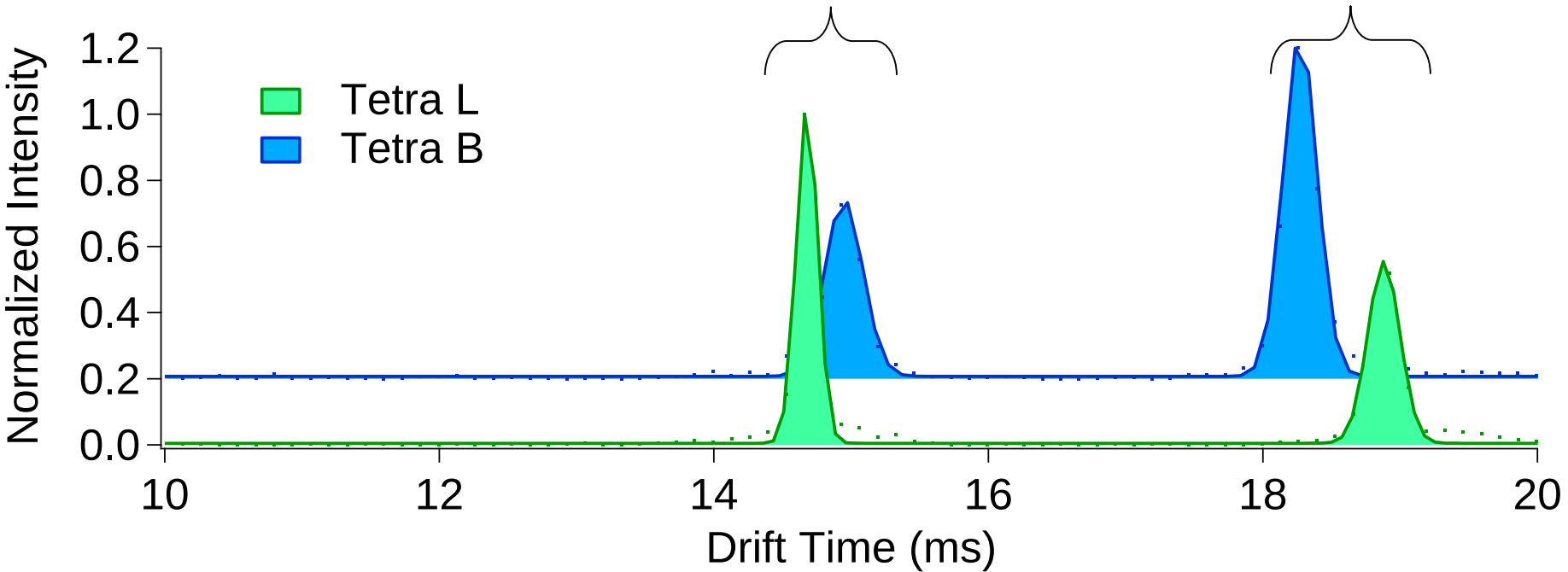
P. Dwivedi/B. Bendiak/B. Clowers/H. Hill. *J. Am. Soc. Mass Spectrom.* 2007, *18*, 1163-1175.

Co^{2+}-Glycan Adducts

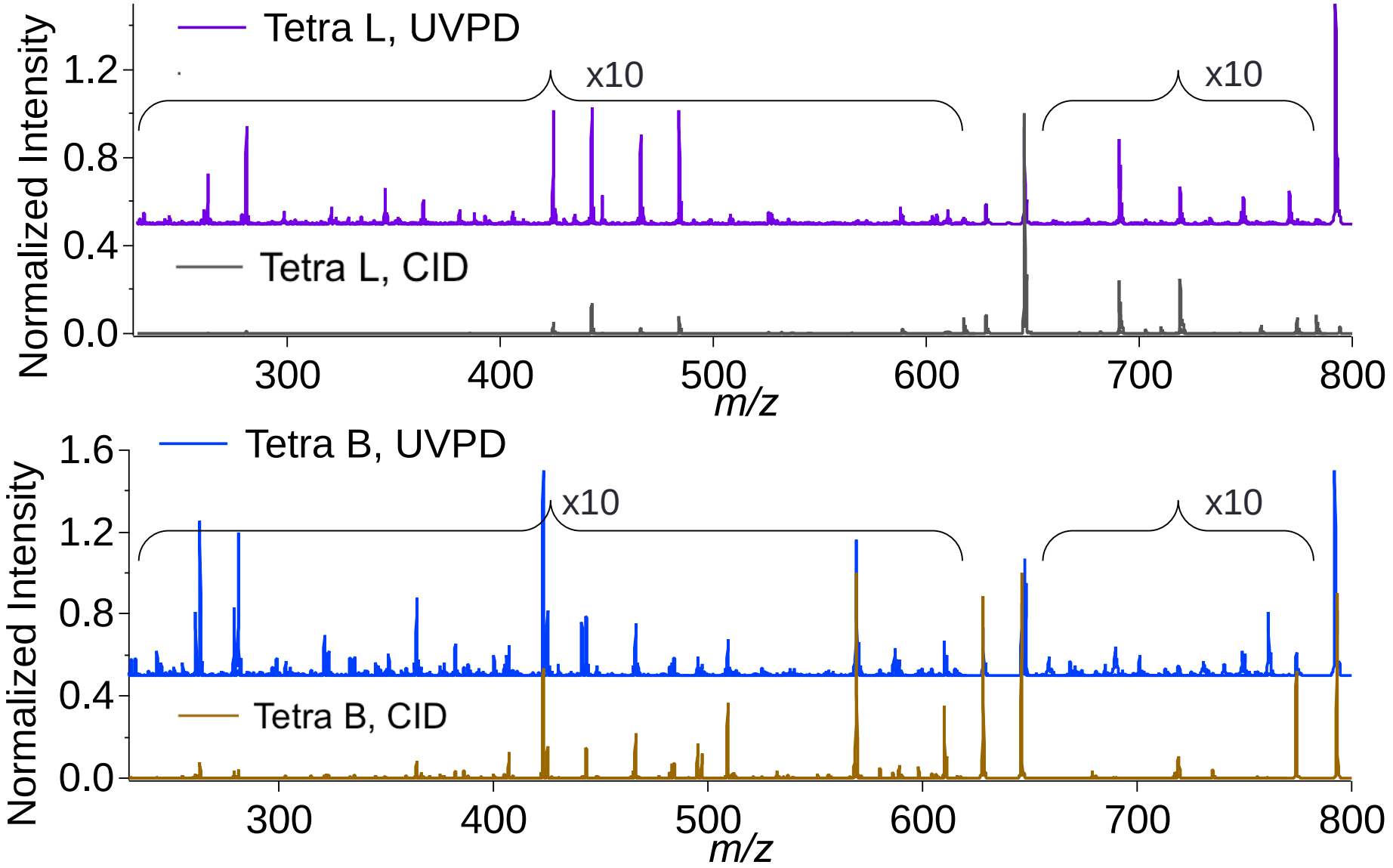
- Two drift time peaks for m/z 792, corresponding to $[2M-2H+2Co^{2+}]^{2+}$ and $[M-H+Co^{2+}]^{+}$



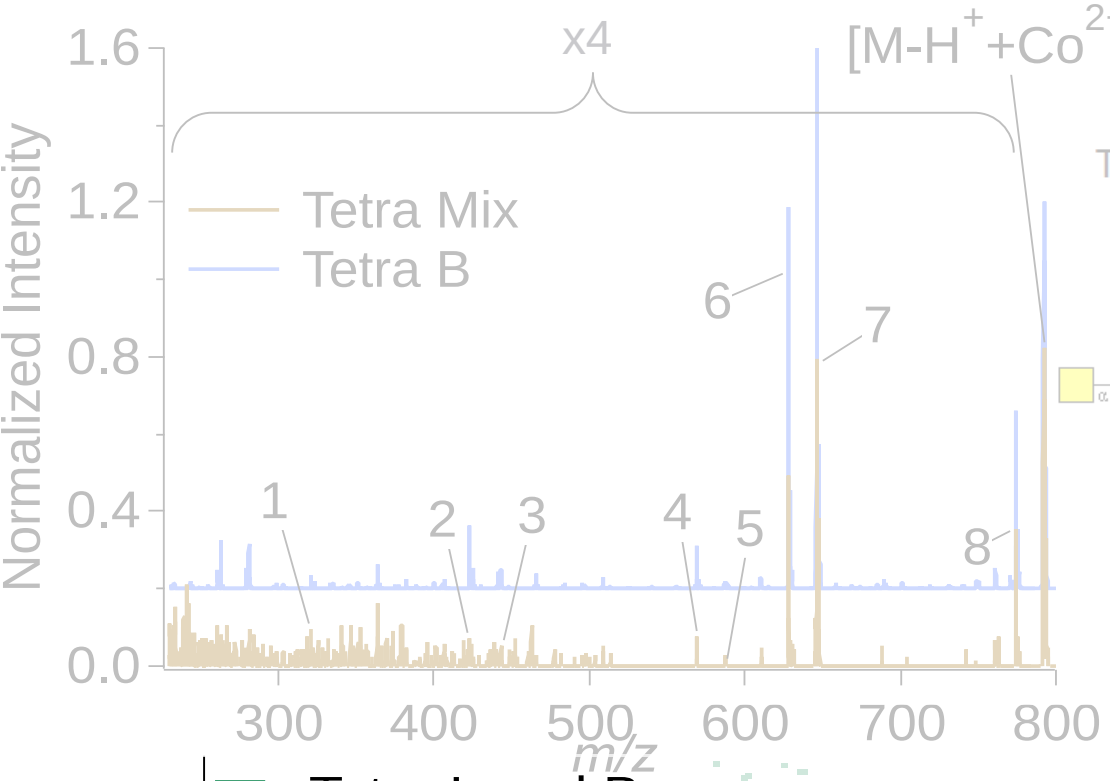


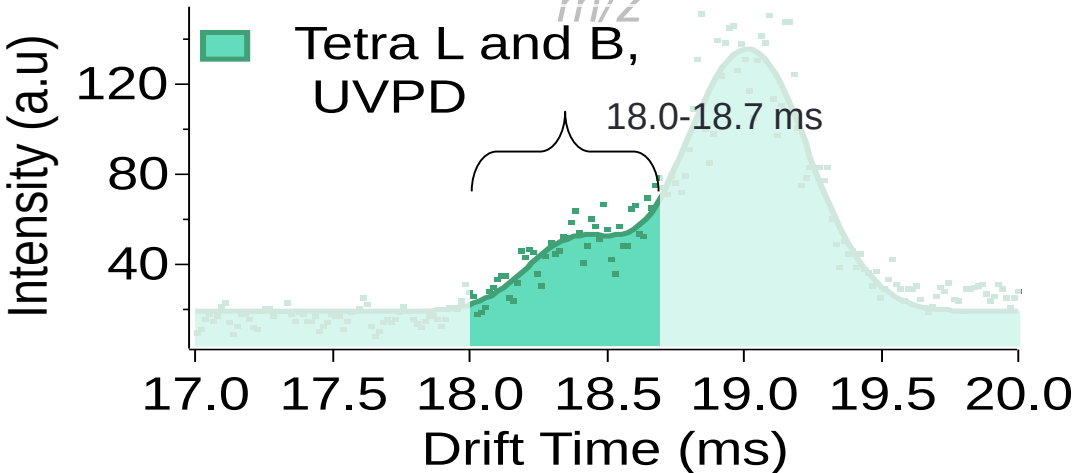


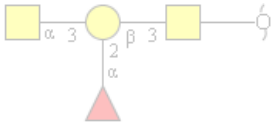
MS/MS of Co^{2+}-Glycan Adducts



Drift Time Separation and UVPD

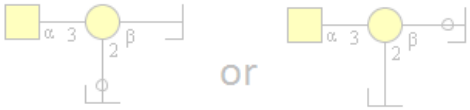




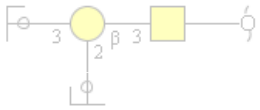


Tetra B Precursor: 792.21

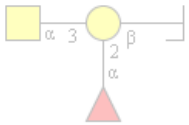
1) 320.92



2) 423.14



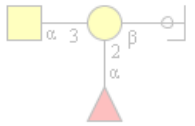
3) 443.17



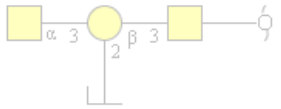
4) 569.05

= GalNac

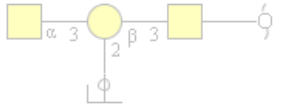
= Gal



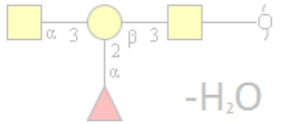
5) 587.17



6) 628.24



7) 646.21

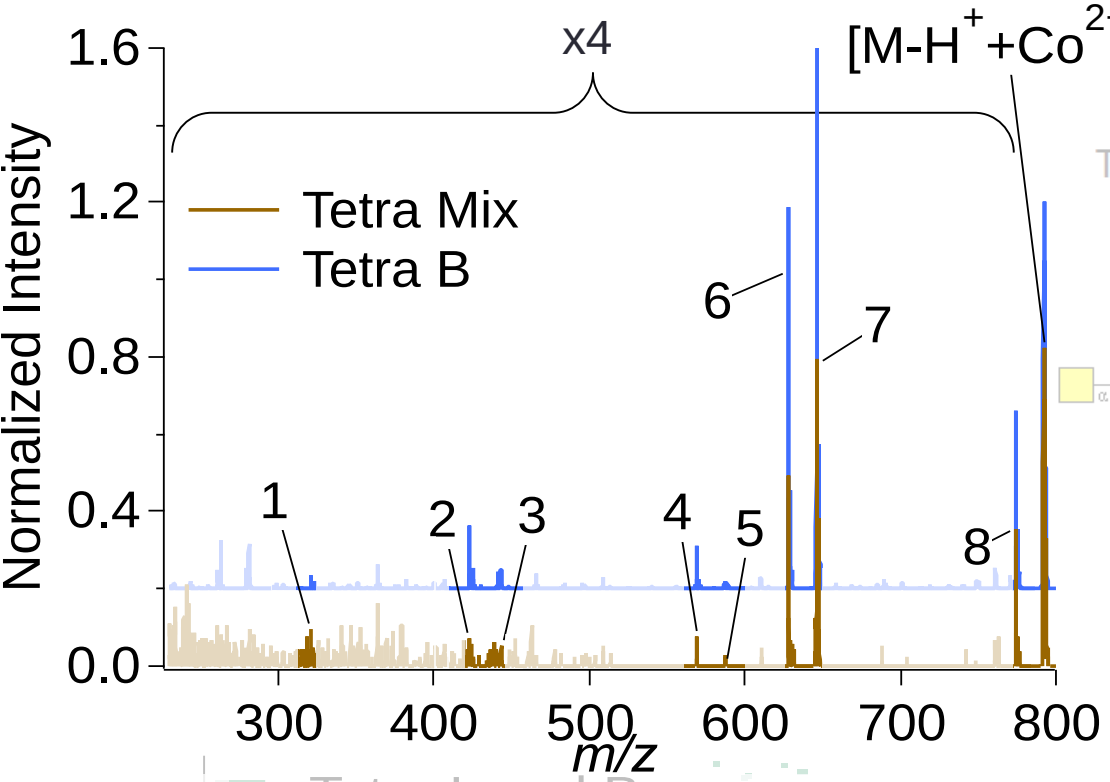


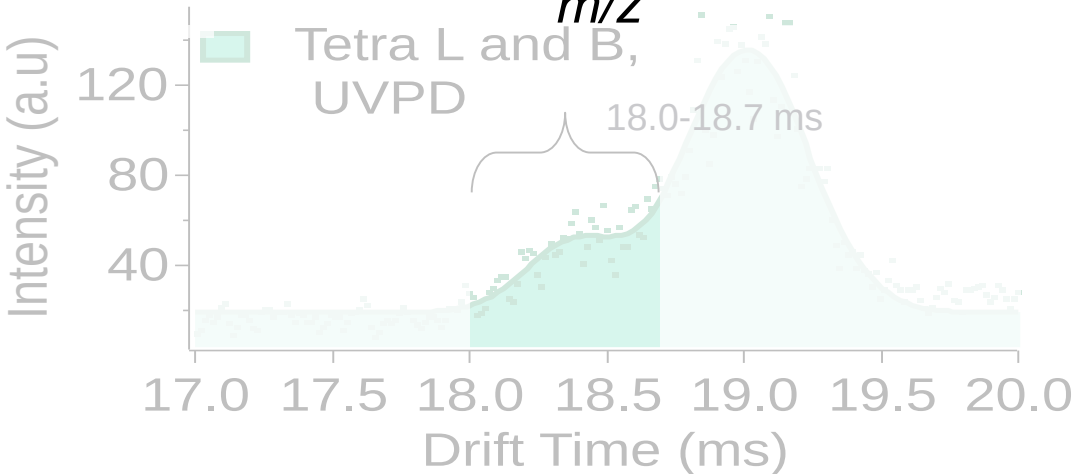
8) 774.28

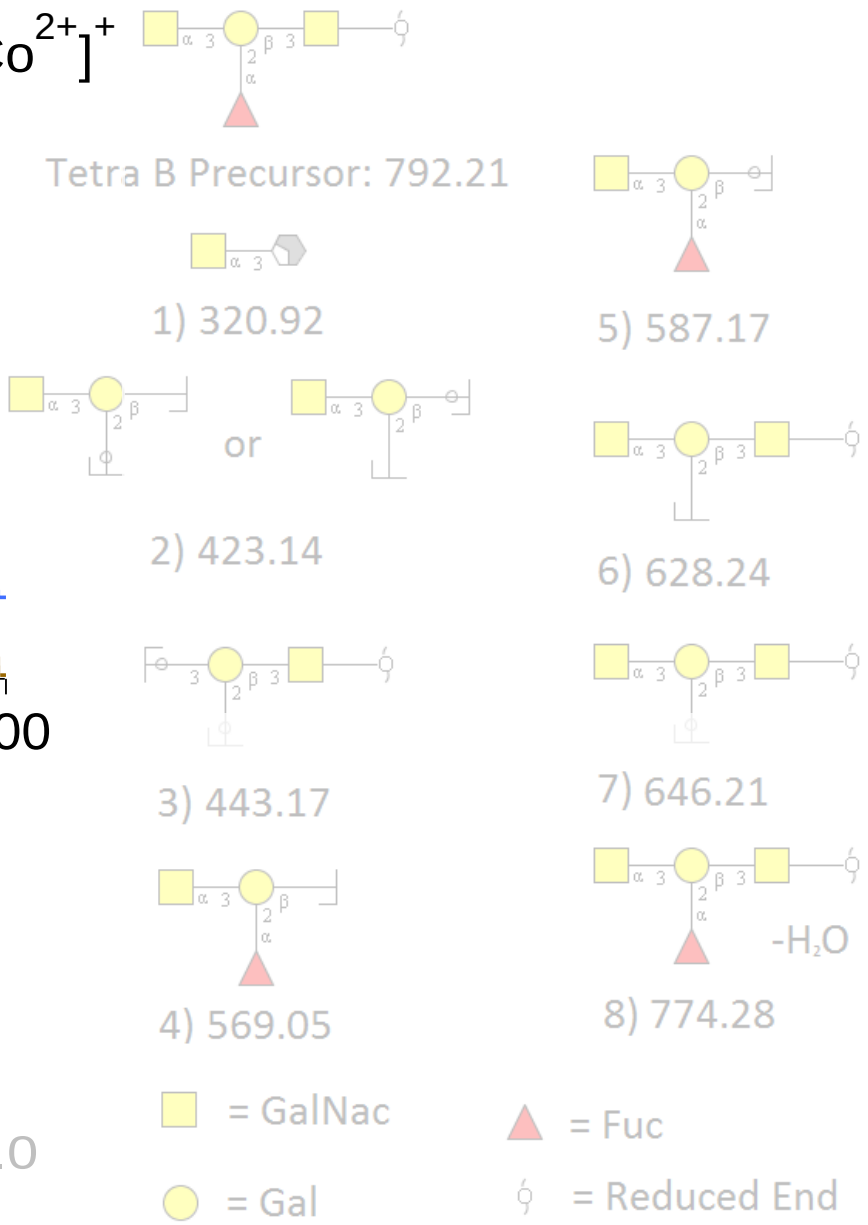
= Fuc

= Reduced End

Drift Time Separation and UVPD







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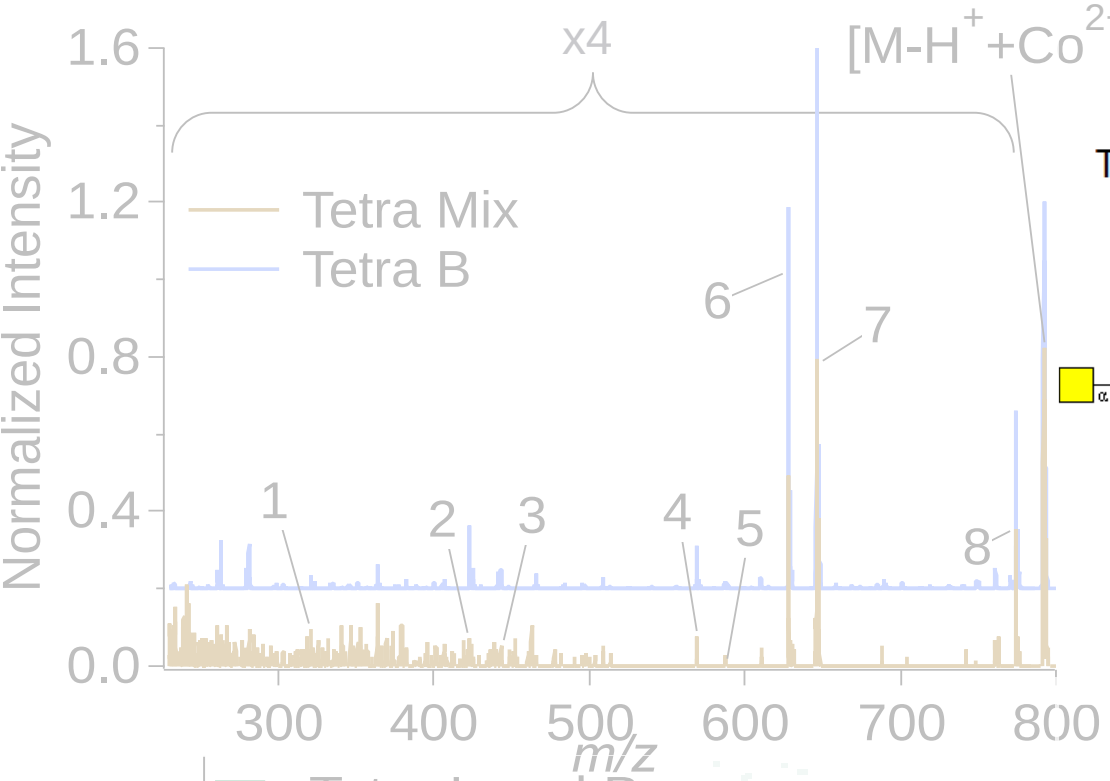

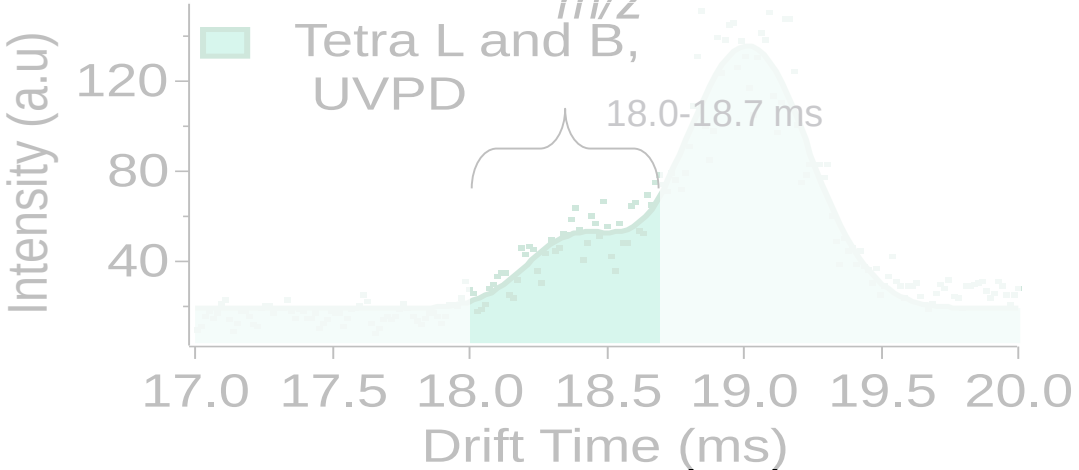

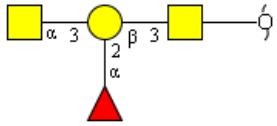

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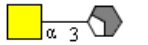

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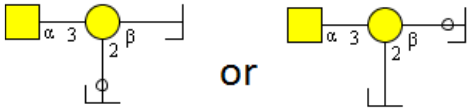
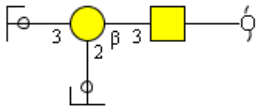


or

2) 423.14



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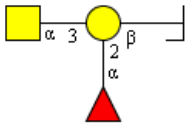

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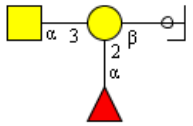

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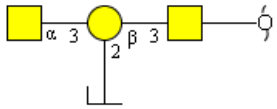

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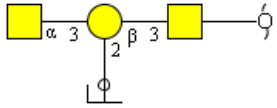

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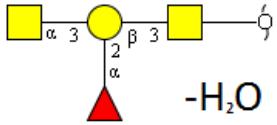

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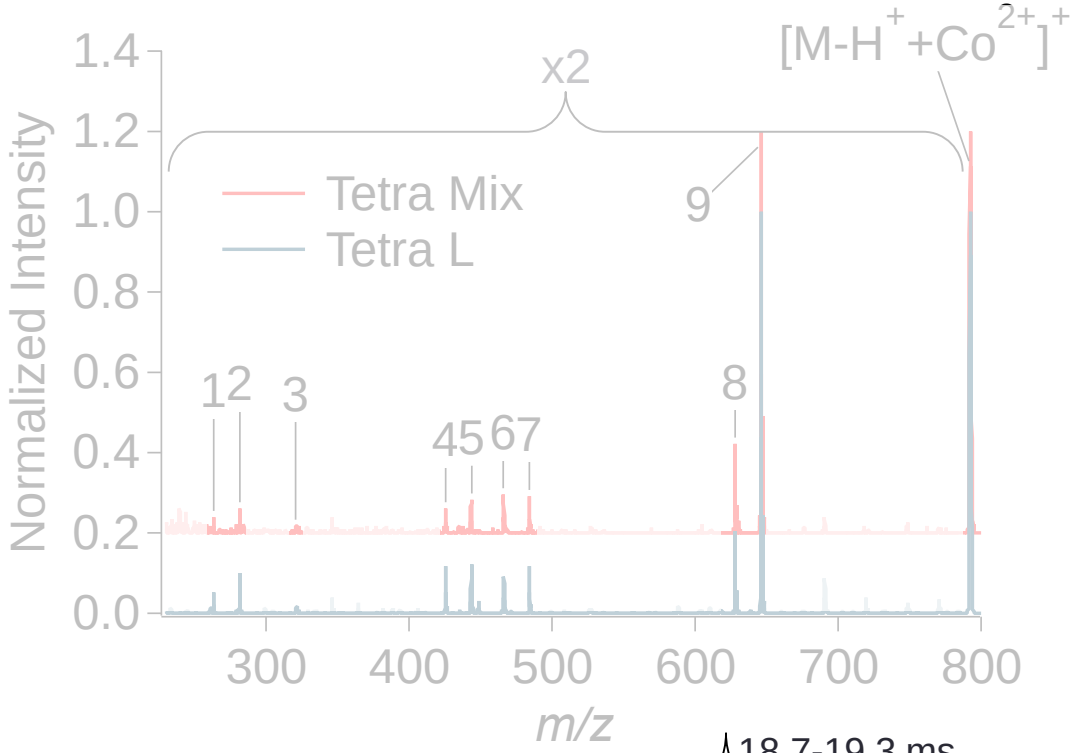
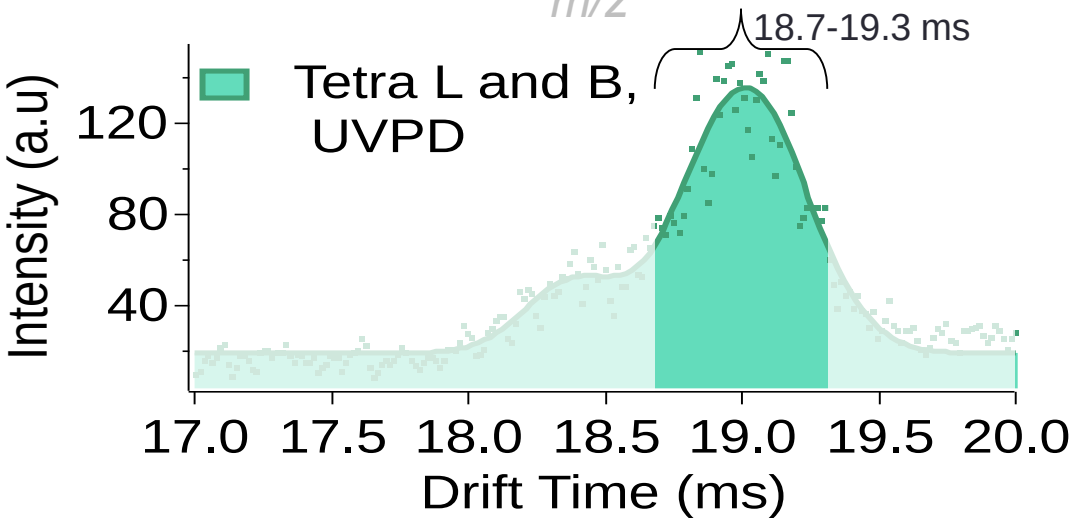
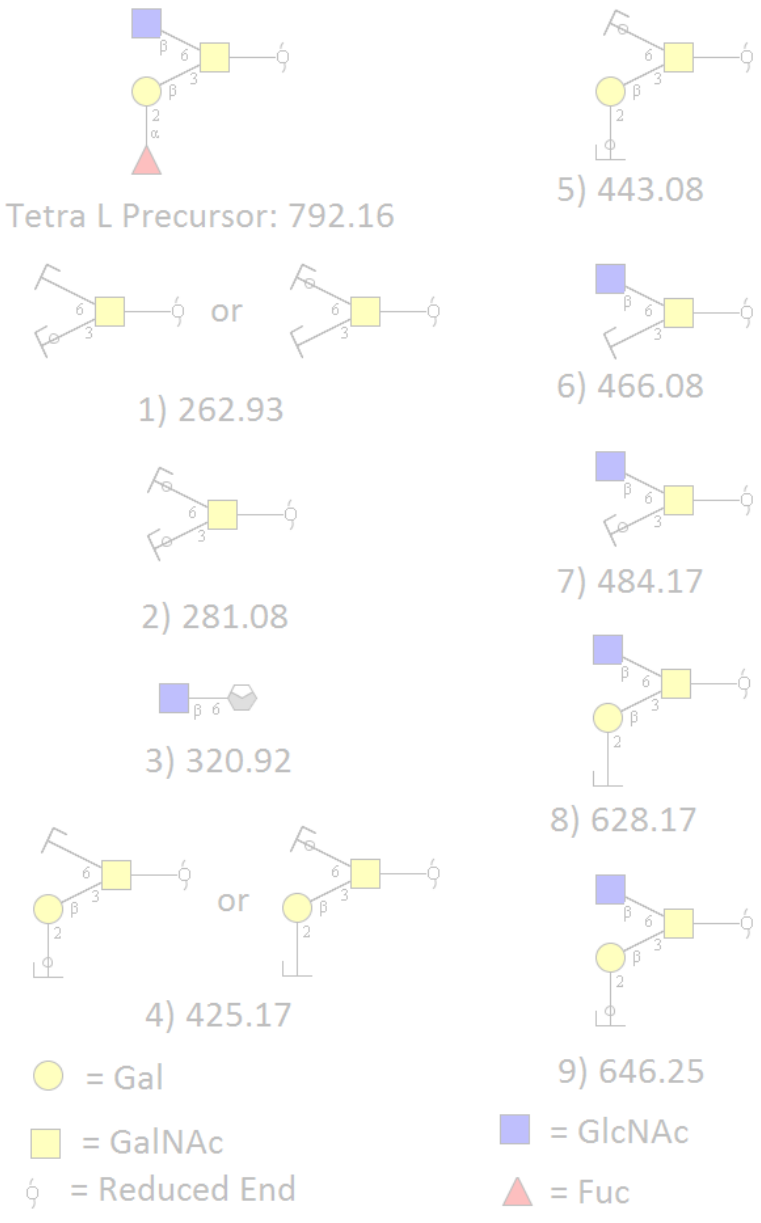
Drift Time Separation and UVPD







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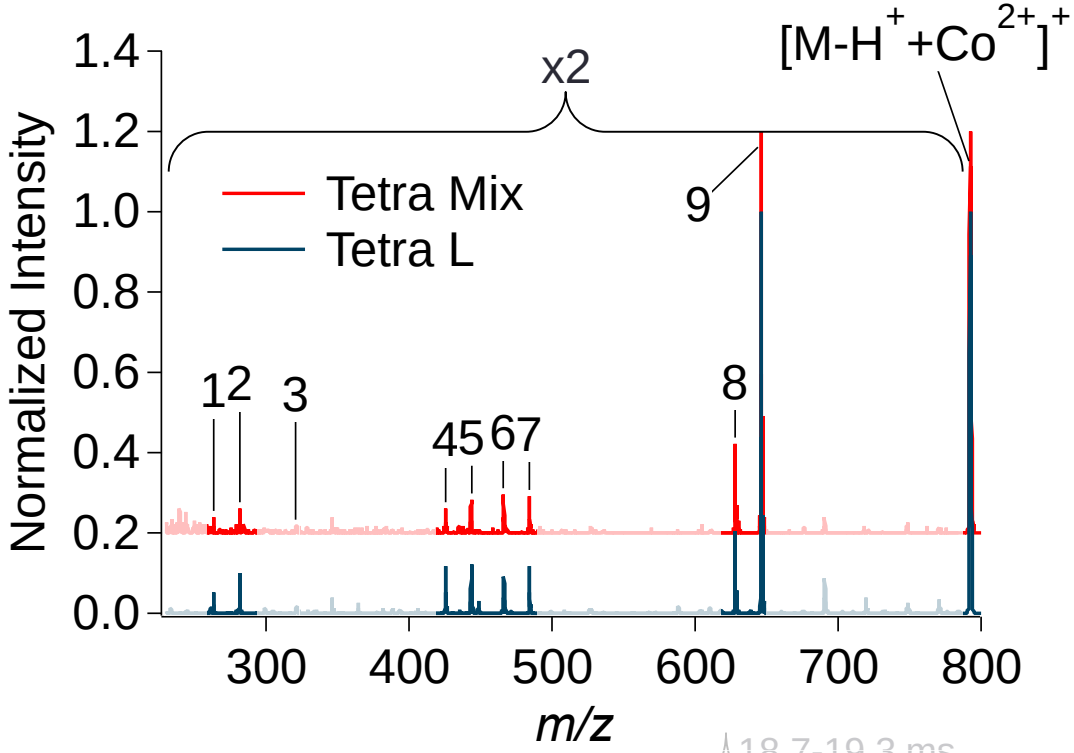
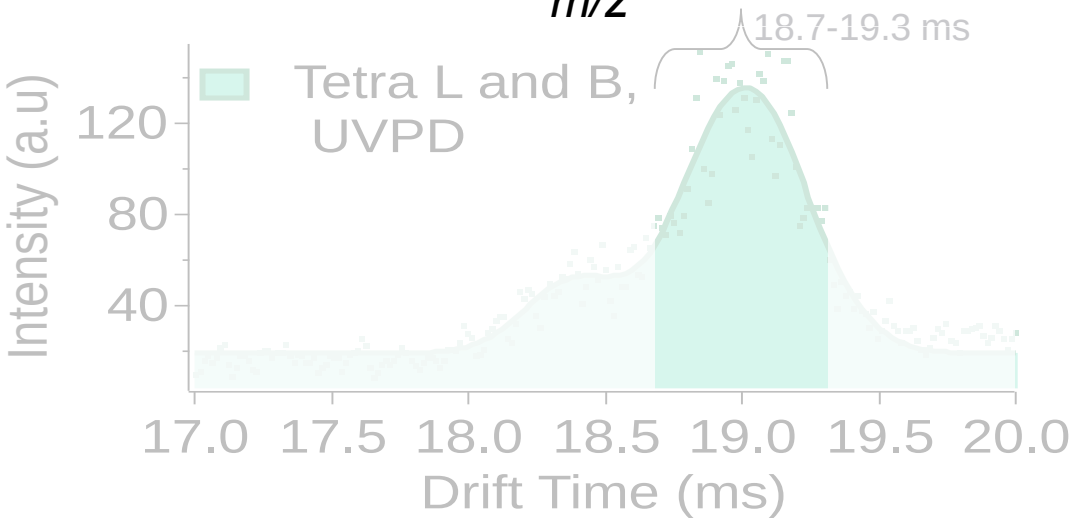
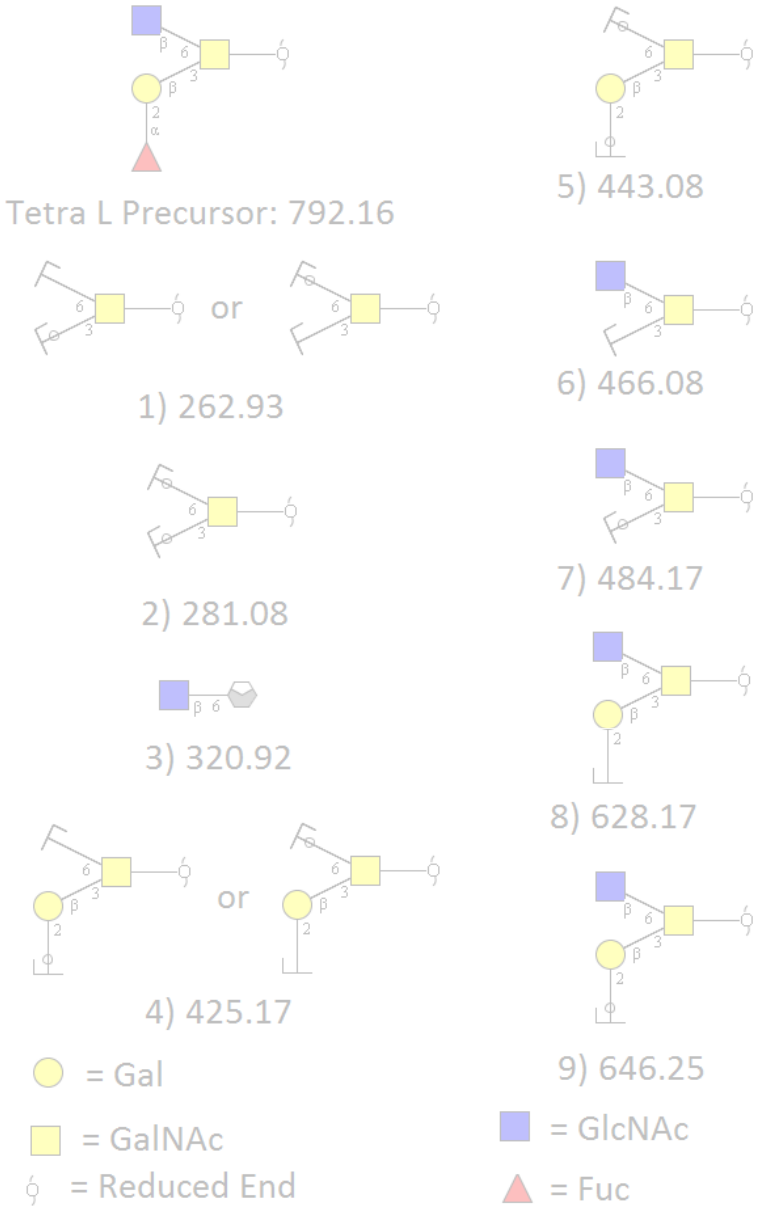
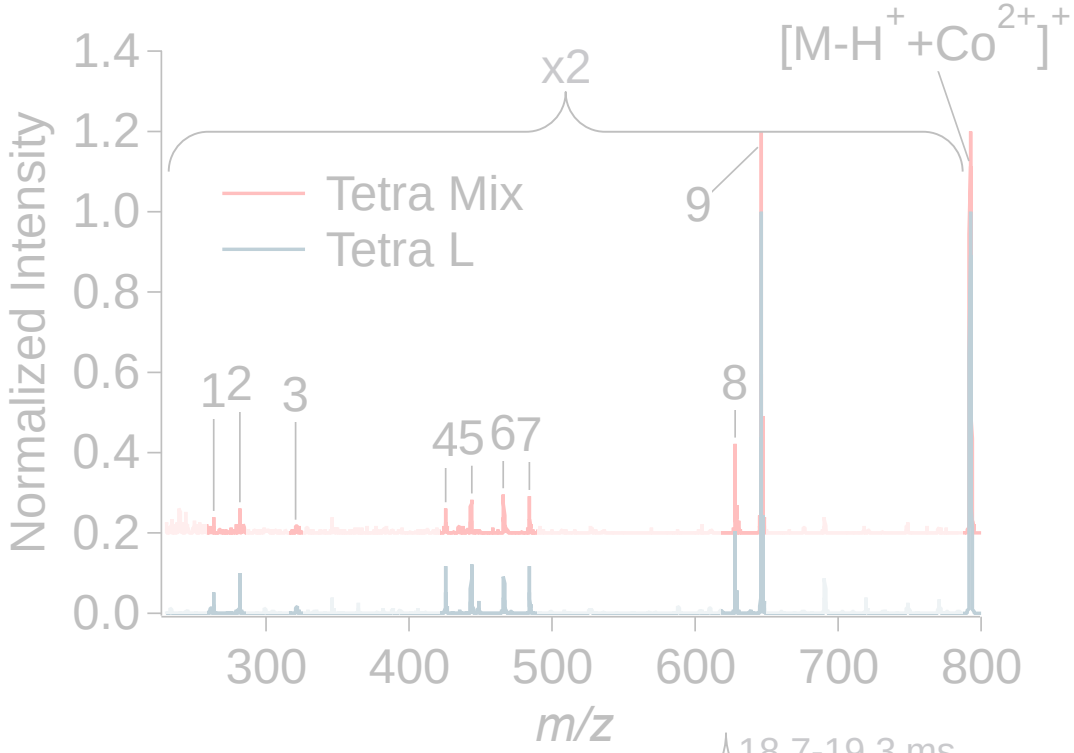
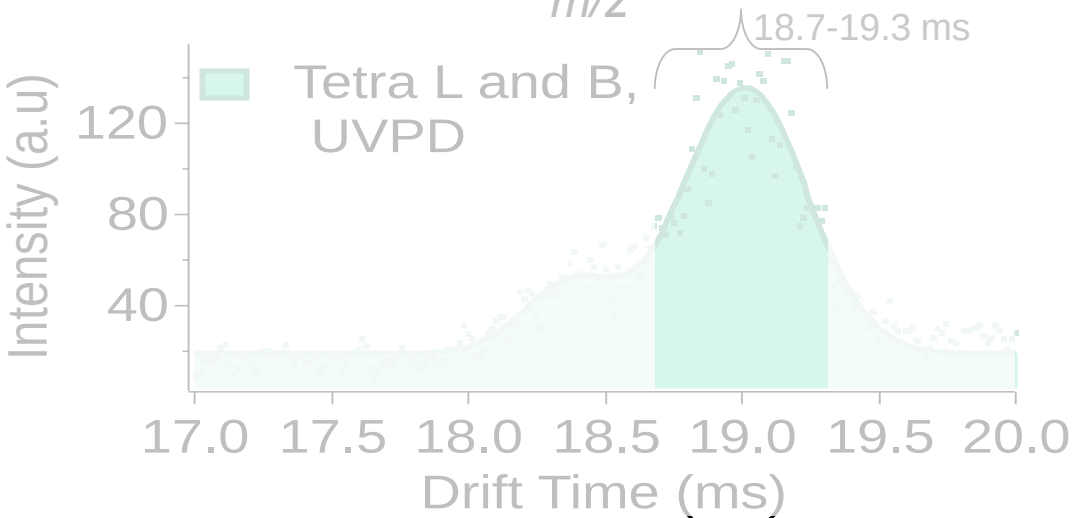


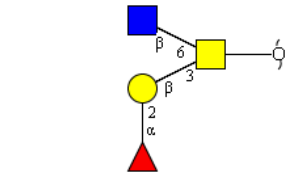




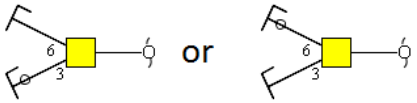
Drift Time Separation and UVPD



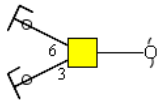




Tetra L Precursor: 792.16



1) 262.93



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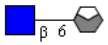

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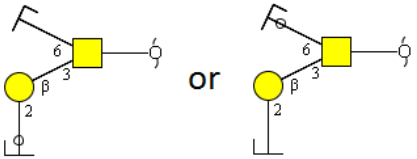
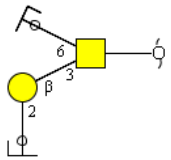


4) 425.17

= Gal

= GalNAc

= Reduced End



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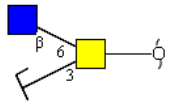
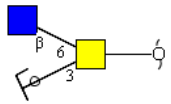
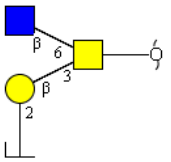


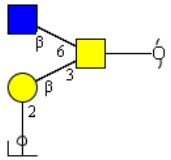
6) 466.08



7) 484.17



8) 628.17



9) 646.25

= GlcNAc

= Fuc

SUMMARY

- Effective isomeric separation of NMR-identified O-glycans and identification of diagnostic UVPD fragments.
- Influence of Co^{2+} Metal Adduction
  - Enhanced UVPD fragmentation yields
  - Resolved mobility distributions
- Resurrection of the FT-IMS technique
  - Enhanced throughput
  - *Reasonable* levels of sensitivity

Future Directions

- UVPD-IM-MS
  - Isomeric glycans, glycoconjugates
  - Influence of multiply charged cations on biomolecule fragmentation via UVPD
    - Impact of charge location
- FT-IMS via Dual-Gate IMS
  - Sensitivity, selectivity, and speed
  - Hybrid multiplexing modes
    - Enhance ion throughput

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