# 2-Dimensional Electron Capture Dissociation Fourier Transform Ion Cyclotron Resonance Mass Spectrometry of N-Linked Glycopeptides

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## Introduction

- Glycosylation is an important and complex post-translational modification that acts in wide variety of functions ranging from forming diffusion barriers to recognition and binding. These functions are derived from their structure and aspects such as site occupancy, variation in composition and linkage type, and location of the glycan. Improving the range of tools available to study these components will afford a greater understanding of the functions of glycans and how they operate.

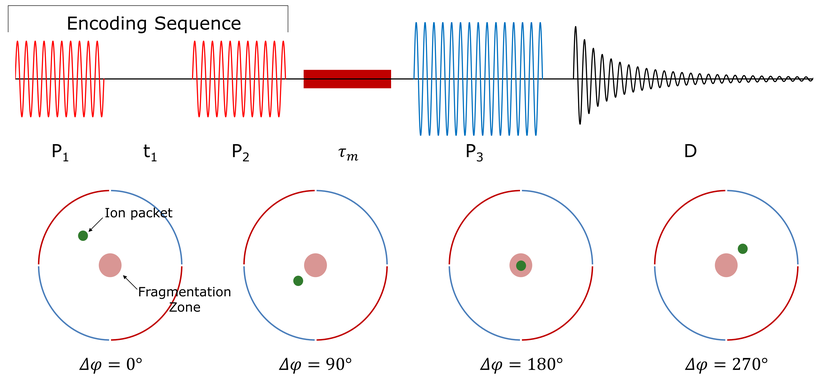


***Figure 1.*** *Pulse sequence for 2D FTICR experiments comprised of encoding pulses ($P_1$ and $P_2$), a variable encoding delay ($t_1$), a fragmentation period ($\tau_m$) an excitation pulse ($P_3$) and a detection period (D)* ***(top)****. The effects of phase ($\Delta\varphi$) after $P_2$ on the cyclotron radius of ion packet relative to a fragmentation zone within an FTICR cell* ***(bottom)****. Adapted from review by van Agthoven et. al.* [1]

- 2-dimensional Fourier Transform ion cyclotron resonance mass spectrometry (2D FTICR) is a form of data independent analysis that generates spectra containing both precursor ions and their correspondent fragment ions. This is performed using a pulse sequence (Fig 1) that is iterated over some number of times, varying an encoding delay between two encoding pulses. This encoding period modulates precursor ions into and away from a fragmentation zone at a periodicity dependent upon its cyclotron frequency. By performing two sequential Fourier Transforms over the data, a spectrum relating fragments to their precursors is generated.

## Methods and Instrumentation

- Bovine Serum Fetuin and Coral Tree Lectin are reduced, alkylated, and enzymatically digested using trypsin. Glycopeptides are then enriched utilizing zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) tips.

- Enriched fractions are diluted by a factor of between 2 and 15 to 50:50 acetonitrile: water and 0.1% formic acid. Fractions are then electrosprayed using an Advion Nanomate paired with a Bruker 15 T SolariX XR Fourier Transform ion cyclotron resonance mass spectrometer. Electron capture dissociation (ECD) spectra are collected either in 1 dimension using quadrupole selection or in 2-dimensions utilizing pulse programs provided by the Peter O'Connor Group. 2D data is processed and viewed using Spectrometry Processing Innovative Kernel (SPIKE).

## Preliminary: 2-Dimensional ECD Spectra of Glycopeptides

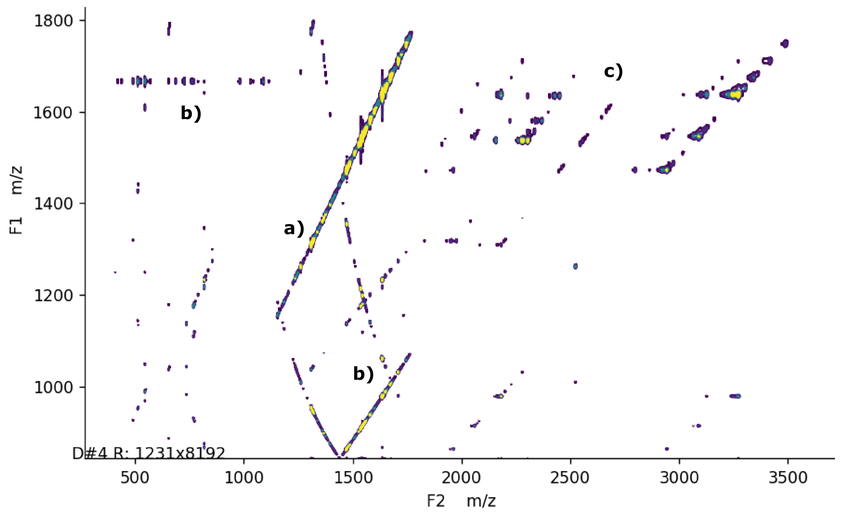


***Figure 2.*** *2D ECD spectrum of tryptically digested bovine serum fetuin. Major features include the autocorrelation line* ***(a)****, harmonics* ***(b)****, and a region containing mostly charge reduced precursor and fragments of glycopeptides* ***(c)****.*

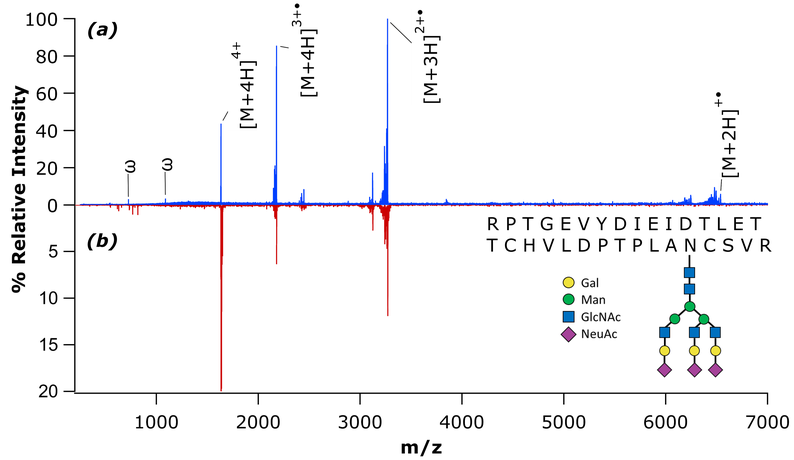


***Figure 3.*** *1D ECD spectrum of glycopeptide from bovine serum fetuin* ***(a)*** *overlaid with a 1D horizontal extract from the 2D spectrum in Fig. 2* ***(b)****. Predominant features include the 4+ precursor, charge reduced species, and side chain losses. The scale for the bottom spectrum is zoomed in to show features.*

## Preliminary 1-Dimensional Spectra

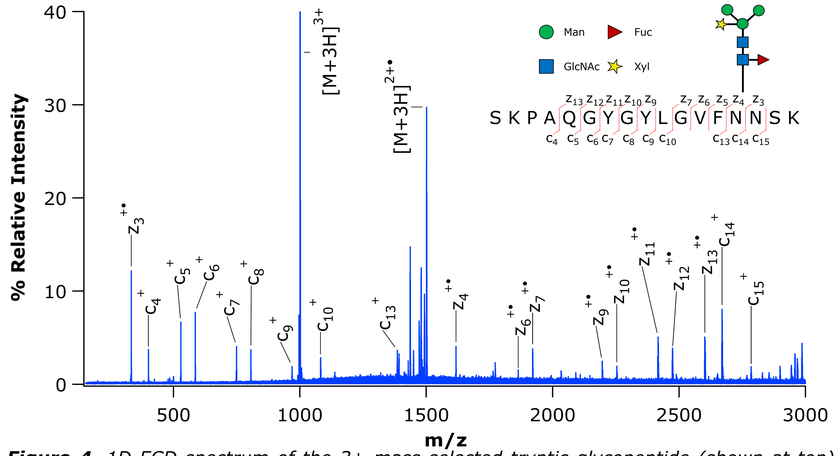


***Figure 4.*** *1D ECD spectrum of the 3+ mass selected tryptic glycopeptide (shown at top) from coral tree lectin.*

## Preliminary Conclusions and Directions

- Preliminary findings suggest the potential of 2D FTICR to determine the site of glycosylation for protease digested N-linked glycopeptides.
- Future directions will focus on improving the practicality of 2D FTICR application to glycopeptides.
  - Inclusion of IRMPD for fragmentation of the glycan
  - Expansion of standards analyzed
  - Modification of enrichment for increased signal/noise and spray stability

## References

1. van Agthoven, M. A.; Lam, Y. P. Y.; O'Connor, P. B.; Rolando, C.; Delsuc, M.-A., Two-dimensional mass spectrometry: new perspectives for tandem mass spectrometry. *European Biophysics Journal* **2019,** *48* (3), 213-229
2. van Agthoven, M. A.; Chiron, L.; Coutouly, M.-A.; Delsuc, M.-A.; Rolando, C., Two-Dimensional ECD FT-ICR Mass Spectrometry of Peptides and Glycopeptides. *Analytical Chemistry* **2012,** *84* (13), 5589-5595.

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