Site-Specific Characterizations of Glycosylation by Electrospray Quadrupole Ion-Mobility Time-of-Flight Mass Spectrometry

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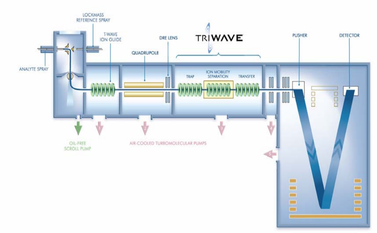
OVERVIEW

- A new class of mass spectrometer has been developed by Waters (Synapt™ HDMS™) with the unique capability to fractionate biomolecules in the gas phase by size, shape, and charge prior to mass spectrometric detection.
- The unique design of Synapt HDMS system enables researchers to simplify complex samples and permit the in-depth characterization of hybrid structures (e.g. glycopeptides) within a single analysis.
- This poster shows specific applications to the analysis of glycopeptides by use of ion-mobility separations and Time Aligned Parallel (TAP) fragmentation.

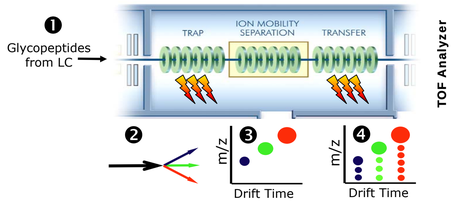
- The LC/MS system was configured with a Waters nanoACUITY UPLC™ chromatography system and a Waters Synapt™ HDMS™ quadrupole ion-mobility time-of-flight mass spectrometer.
- Synapt HDMS was operated in mobility-TOF mode for all analyses. MassLynx 4.1 software was used for instrument control and data processing.
- LC separations were accomplished on a 75µmx100mm nanoACQUITY BEH C18 column. Peptides were resolved using a linear acetonitrile gradient in 0.1% formic acid.

ADDING ION MOBILITY TO A Q-TOF MS



- The ion-mobility section is comprised by three Traveling Wave-enabled Stacked Ring Ion Guides (SRIG).
- The TRAP ion guide is used to accumulate ions and release them as packets for ion mobility separation.
- The TRANSFER ion guide conveys the mobility separated ions to the oa-TOF for mass analysis.
- Fragmentation can take place either in the TRAP or in the TRANSFER ion guide or both.

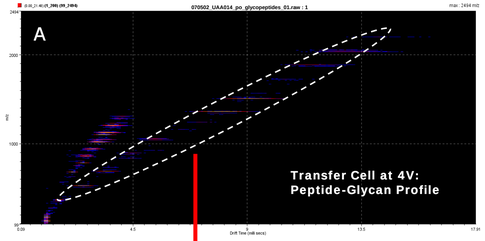
TIME ALIGNED PARALLEL(TAP) FRAGMENTATION



1. Select glycopeptides using quadrupole
2. Randomly fragment glycan on peptide at medium energy in TRAP cell
3. Separate cleaved glycans from glycopeptides by ion mobility
4. Alternate TRANSFER CELL between low and elevated energy modes to:
   Low: Obtain glycan sequence
   Elevated: Fragment and sequence peptides

N-LINKED GLYCOPEPTIDE ANALYSIS

Figure 1. Determining glycan sequence and glycopeptide amino acid sequence directly from N-linked glycopeptides using Time Aligned Parallel (TAP) fragmentation. (A) Driftscope heat map showing the separation of the product ions by the IMS cell. The fragment ions were from a triply charged precursor ion of a glycopeptide from IgG1 (*m/z* 922.08). (B) The fragment ions produced by the CID process in the trap cell at a medium-level collision energy yielded information on the glycan sequence. (C) Driftscope heat map showing the fragmentation of the peptide ion with a single sugar moiety attached (*m/z* 1306.30). The fragmentation took place in the transfer cell after the IMS separation. (D) MS/MS spectrum of the product ion at *m/z* 1306.30 from the second-stage fragmentation process in the transfer cell, showing the peptide backbone fragmentation. Symbols: (■) HexNAc (●) Manose (○) Hex (∇) Fucose



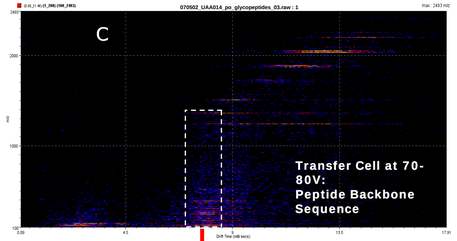
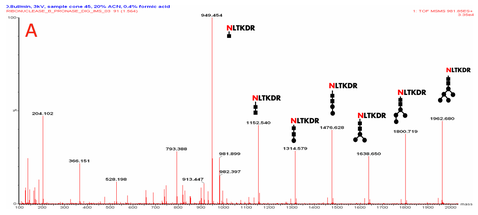
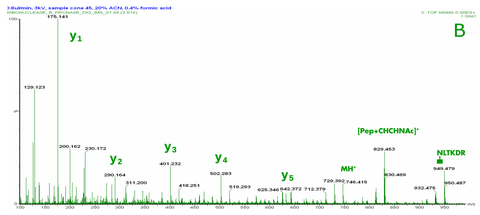


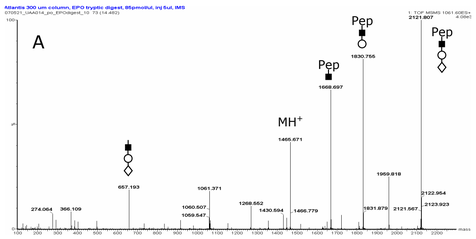
Figure 2. Another example of TAP fragmentation application in determining glycan sequence and glycopeptide amino acid sequence directly from N-linked glycopeptides. (A). MS/MS spectrum of a glycopeptide from Ribonuclease B (*m/z* 981.85) to yield the glycan sequence. (B). Second-stage fragmentation of the ion with a single sugar moiety attached (*m/z* 949.45) in the transfer cell, showing the peptide





O-linked Glycopeptide Analysis

Figure 3. Analyzing O-linked glycopeptide from recombinant Human Erythropoietin (EPO) using TAP fragmentation. (A) Glycan sequence information obtained via low energy fragmentation in the trap cell; (B) Determining the glycosylation site by fragmenting the product ion with a single sugar moiety (MH_2^{2+}, *m/z*: 834.92) in the transfer cell.



CONCLUSIONS

- A novel approach that utilizes the ion-mobility separation capability and the unique two-collision design of Synapt is presented to perform detailed characterizations of glycopeptides.
- The approach is demonstrated by analyses of both N-linked and O-linked peptides from several model proteins, providing both the glycan sequence information as well as glycosylation site.
- The ability to resolve molecules by mass, size, shape and charge has enabled researchers to distinguish products of multiple parallel dissociation stages, and resolve different populations of biomolecules in the gas phase.