Expanding the capabilities for biotherapeutic molecule analysis using electrospray ion-mobility quadrupole time of flight mass spectrometry (ESI-Q-IM-TOFMS)

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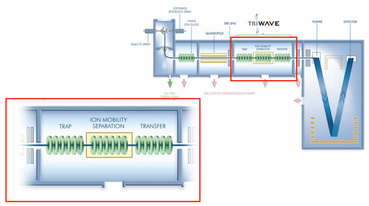
OVERVIEW

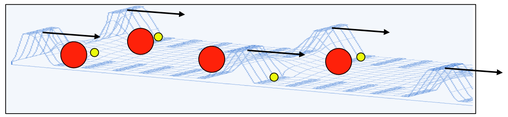
- A new class of mass spectrometer has been developed by Waters (Synapt HDMS™) with the capability to fractionate biomolecules in the gas phase by size, shape, and charge prior to mass spectrometric detection.
- This capability can be exploited by researchers to simplify complex samples, simplify or eliminate chromatographic separations, or permit the full characterization of hybrid structures (e.g. glycopeptides) within a single analysis.
- This poster will discuss the theory behind coupling ion mobility separations with traditional QTOF based biomolecular mass analysis, and show specific application to the analysis of monoclonal immunoglobulins, glycoproteins, and the complex peptide and glycan mixtures resulting from these biomolecules.

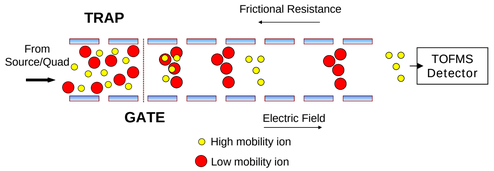
A Synapt HDMS™ LC/MS system (Waters) was configured with a nanoACUITY UPLC™ nanoscale chromatography system and a Synapt HDMS quadrupole ion mobility time of flight mass spectrometer. The system was operated in mobility-TOF mode for all analyses. MassLynx 4.1 software was used for instrument control and data processing. LC separations were accomplished on a 300µ x 100 mm 1.7µ NanoEase™ BEH C18 column (Peptides) or a prototype 1.0 x 50 mm desalting cartridge (Proteins). Peptides were resolved using a linear acetonitrile gradient in 0.1% formic acid, while a step gradient was used for bolus elution of the desalted reduced antibody.

ADDING ION MOBILITY TO A QTOF MS



A three element Traveling Wave Ion Guide (TWIG) based mobility cell is placed where the traditional collision cell would be found in a QTOF mass spectrometer. The TRAP ion guide can collect ions and deliver them as packets for ion mobility separation in the central ion guide. The TRANSFER cell permits efficient transfer of ions to the TOFMS detector. By adjusting voltages in the TRAP and TRANSFER ion guides, molecules can be dissociated before and/or after the mobility separation in the central ion guide.

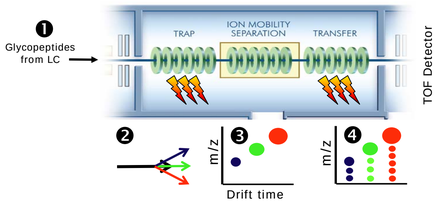




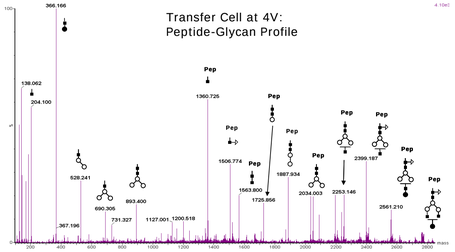
Ion mobility separations in the central ion guide were accomplished by propelling ions forward with series of traveling waves of electrical potential against the frictional resistance of neutral gas molecules in the cell. Molecules with higher cross-sectional areas (larger or more extended structures) advance less efficiently, and have lower mobility through the cell. Overall mobility is determined by the charge, size, and shape of an ion.

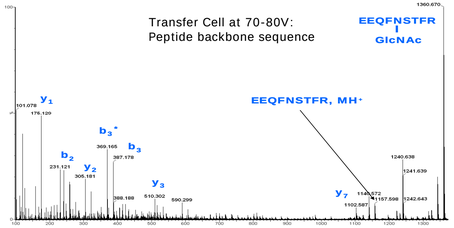
GLYCOPEPTIDE ANALYSIS

Time Aligned Parallel Fragmentation

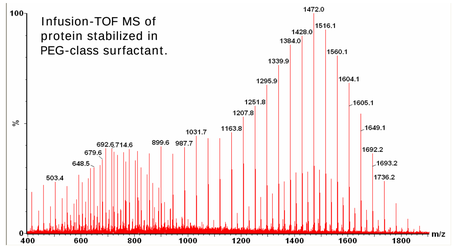


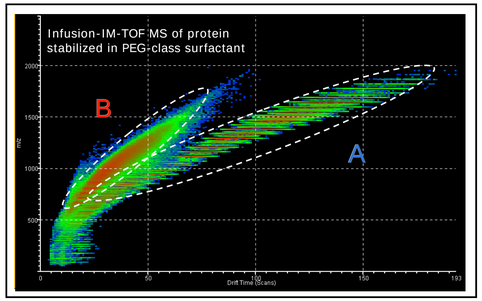
1. Select glycopeptides using quadrupole
2. Randomly fragment glycan on peptide at medium energy (15-25 V) in TRAP region
3. Separate cleaved glycans from glycopeptides by ion mobility
4. Alternate TRANSFER CELL between low (4 V) and elevated energy (70-80 V) modes to:
   - Low: Obtain glycan sequence
   - Elevated: Fragment and sequence peptide



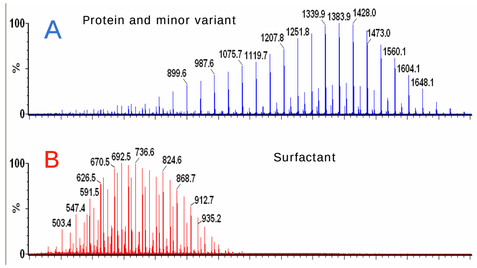


PROTEIN-SURFACTANT MIXTURE



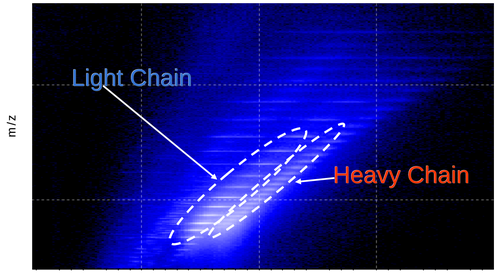


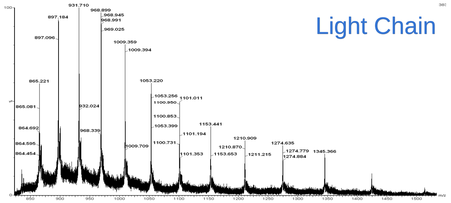
Summed spectra for protein (A) and surfactant (B) obtained by combining ions with drift profiles shown above.

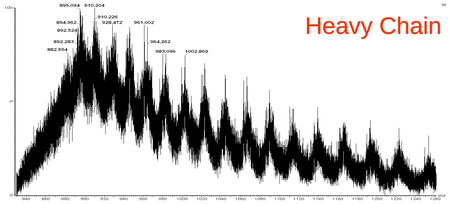


REDUCED IgG1

Coeluting heavy and light chains from a reduced IgG1 desalting run could be resolved in the gas phase by ion mobility.







CONCLUSIONS

- We have presented the analysis of model therapeutic proteins by multiple modes of ion mobility time-of-flight mass spectrometry.
- The capability to resolve molecules by size, shape and charge has permitted researchers to distinguish products of multiple parallel dissociation stages, and resolve different populations of biomolecules in the gas phase.

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