Structural Characterization of a Recombinant Monoclonal Antibody by Electrospray Ion-Mobility Time-of-Flight Mass Spectrometry

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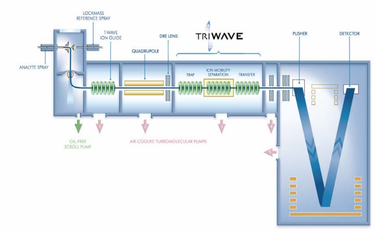
OVERVIEW

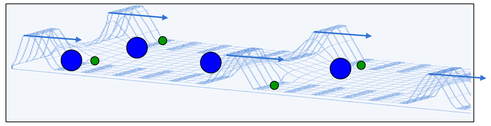
- A new class of mass spectrometer has been developed by Waters (Synapt™ HDMS™) with the unique capability to fractionate biomolecules in the gas phase by size, shape, and charge prior to mass spectrometric detection.
- The Synapt HDMS system enables researchers to simplify complex samples, simplify or eliminate chromatographic separations, or permit the in-depth characterization of hybrid structures (e.g. glycopeptides) within a single analysis.
- This poster presents the design and theory behind Synapt HDMS, and shows specific applications to the analysis of subunits of monoclonal immunoglobulins, glycoproteins, and the complex peptide and glycan mixtures resulting from these biomolecules.

- 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) or a prototype 2.1 x 5 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.

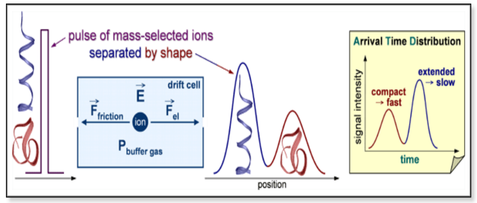
ADDING ION MOBILITY TO A Q-TOF MS



- 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.



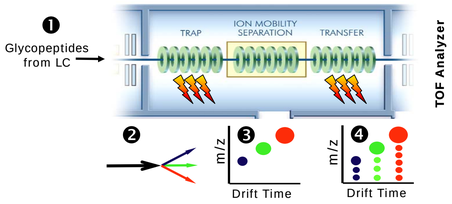
- IM separations is achieved by propelling ions forward through a series of traveling waves of electrical pulses against the frictional resistance of neutral gas molecules in the cell.
- Ions with higher cross-sectional areas (larger or more extended structures) advance less efficiently, and have lower mobility through the cell.



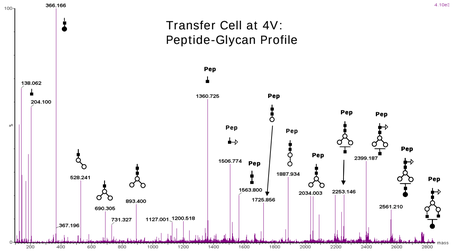
http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml

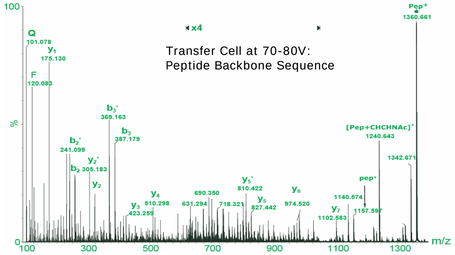
GLYCOPEPTIDE ANALYSIS

Time Aligned Parallel Fragmentation



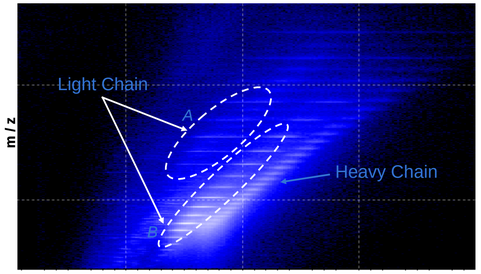
❶ Select glycopeptides using quadrupole
❷ Randomly fragment glycan on peptide at medium energy (15-25 V) in TRAP cell
❸ Separate cleaved glycans from glycopeptides by ion mobility
❹ Alternate TRANSFER CELL between low (4 V) and elevated energy (70-80 V) modes to:
Low: Obtain glycan sequence
Elevated: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

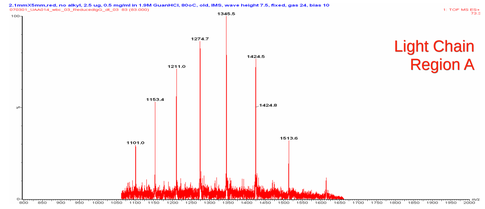


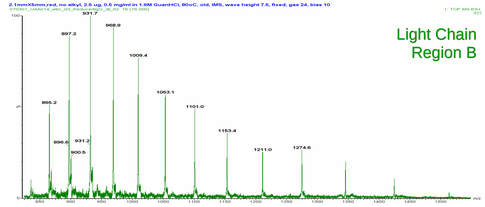


REDUCED IgG1

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





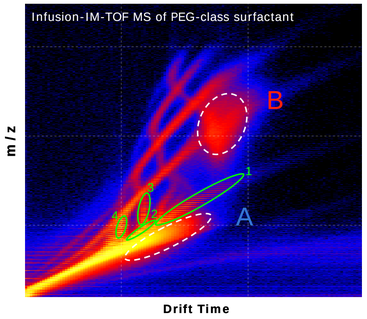


CONCLUSIONS

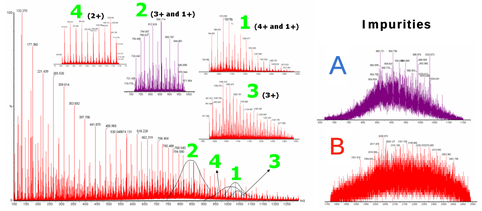
- We have presented the analysis of model therapeutic proteins by multiple modes of ion mobility time-of-flight mass spectrometry.
- The ability to resolve molecules by mass, size, shape and charge has enabled researchers to distinguish products of multiple parallel dissociation stages, and resolve different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

PEG classification by IM-TOF MS

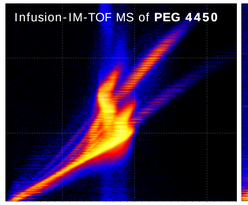
The chemical nature of PEG makes characterisation by conventional methods extremely challenging (determining the extent, and site of peGylation; characterisation of low level components). IM-TOF MS is capable of characterizing and differentiating PEG by their sizes; also quick determining of impurities.

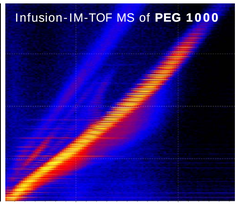


Summed spectra obtained by combining ions with drift profiles shown above.



IM-TOF MS Differences between different PEG-class surfactant





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