RAPID STRUCTURAL ANALYSIS OF MONOCLONAL ANTIBODY AND SYNTHETIC POLYMERS USING 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 within a single analysis.
- This poster presents the design and theory behind Synapt HDMS, and shows specific applications.

INTRODUCTION

Structural characterizations of intact MAb molecules are challenging due to their high molecular mass, hydrophobic nature, and presence of sugar moieties. Conversely, the analysis of intact protein reduces dramatically the time for sample preparation and data interpretation, and it also minimizes the chance of introducing putative modifications, which are often observed during peptide mapping.

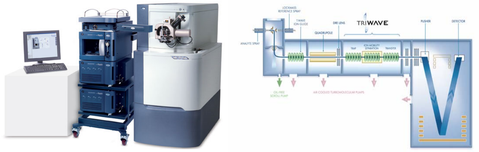
The aim of this study is to perform structural characterization on a monoclonal antibody (IgG1, κ), and synthetic polymers. The presented study covers the results in the following areas:

- ♦ analyzing intact antibodies to map the glycosylation profile of the antibody
- ♦ separating the light and heavy chains of the reduced antibody by ion mobility to yield further characterizations
- ♦ fragmenting the intact light chain subunit to obtain partial amino acid sequence of the light chain
- ♦ characterizing and differentiating PEGs by their sizes and conformations in a quick infusion experiment

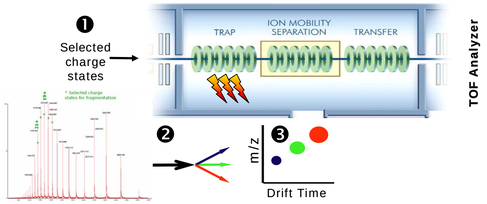
EXPERIMENTAL METHODS

- 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.
- On-line desalting was accomplished on a prototype 2.1 x 5 mm desalting cartridge. A step gradient was used for bolus elution of the desalted intact and reduced antibody.

THEORY



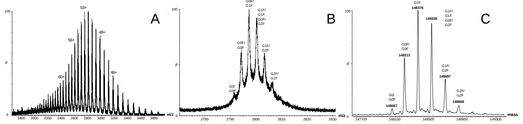
- The ion-mobility section is comprised by three Traveling Wave-enabled Stacked Ring Ion Guides (SRIG).
- The TRAP ion guide is used to accumulate ions and release them as packets for ion mobility separation.
- The TRANSFER ion guide conveys the mobility separated ions to the oa-TOF for mass analysis.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



❶ Protein introduced into the ion-source. Select charge states for fragmentation using quadrupole.
❷ Intact protein subjected to high energy in the TRAP portion of triwave.
❸ Separation of +1, +2, +3, and multi- charged peptide fragments.

RESULTS
Intact IgG1

Figure 1. (**A**) Positive ion ESI mass spectrum of an intact monoclonal antibody acquired in V mode with resolution 10,000. (**B**) Positive ion ESI mass spectrum for 53+ charged ion of the intact monoclonal antibody. Peaks represent various glycoforms. (**C**) Deconvoluted ESI mass spectra of the intact monoclonal antibody IgG1 containing multiple peaks due to the sugar heterogeneity.



REDUCED IgG1

Figure 2. (**A**) Ion mobility DriftScope showing the separation of heavy chains and light chains from the reduced IgG1. (**B**) The summed ESI mass spectrum of light chain (**C**) The summed ESI mass spectrum of heavy chain. (**D, E**) Deconvoluted ESI mass spectra of light chain and heavy chain.

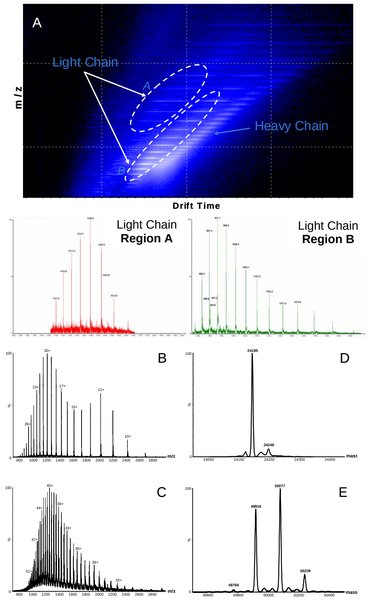
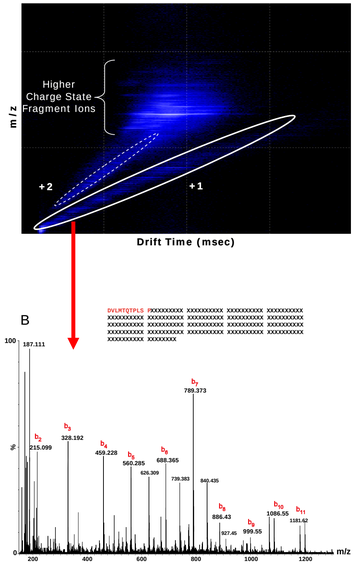


Figure 3. (**A**) Ion mobility DriftScope of the fragment ions from fragmentations of the intact light chain. Singly charged species are clearly separated from the other multiply charged fragments by ion mobility. These fragments contain partial amino acid sequence of the light chain (**B**) The summed mass spectrum of all the singly charged ions generated from the intact light chain fragmentation process.

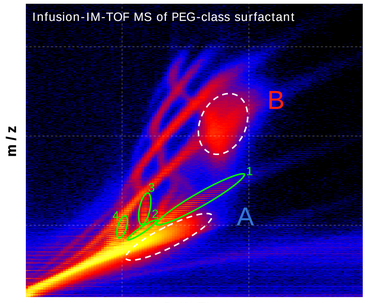


CONCLUSIONS

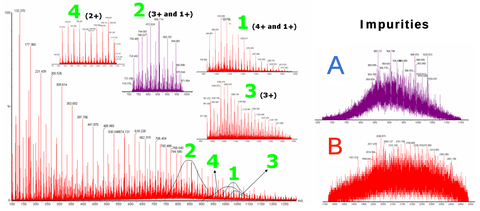
- ♦ Synapt HDMS (ESI-IMS-TOF MS) has been successfully used for the structural characterization of a mouse monoclonal antibody.
- ♦ Synapt offers unique, enabling functionalities for characterization and differentiating PEGs and its derivatives by their sizes and conformations in a quick infusion experiment. In addition, the impurities and low molecular mass species in PEG material can be promptly observed from 3D heat maps by simple eye-view.
- ♦ The system combines high-efficiency, ion-mobility based measurements and separations with high-performance quadrupole, time-of-flight mass spectrometry to deliver enhanced specificity and sample definition beyond that achievable by conventional mass spectrometers.
- ♦ All of the results demonstrate that Synapt HDMS mass spectrometer is a superior tool to characterize MAb and other complex protein pharmaceuticals.

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