High Throughput Determination of Protein Conformational Changes

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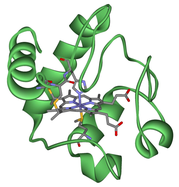
OVERVIEW

- Conformational changes in biopharmaceutical proteins can alter potency and pharmacological properties of the final drug product.
- 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 (via an ion mobility separation) prior to MS detection.
- This capability can be exploited by researchers to simplify analysis of complex samples, or profile structural heterogeneities within a protein preparation.
- This poster will review theory behind coupling biomolecule ion mobility separations with traditional QTOF mass analysis, and show direct application to the analysis of protein structural heterogeneity.

METHODS

A Synapt HDMS™ system (Waters) was operated in mobility-TOF mode for all analyses. MassLynx 4.1 was used for instrument control and data processing.



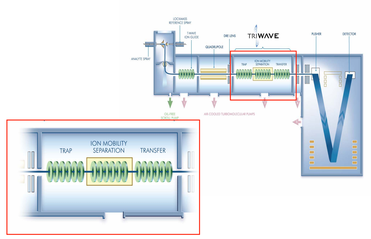
Bovine Cytochrome c
MW (+ Heme) = 12384

Cytochrome c (Bovine heart, Sigma, 2 pmol/µL) was prepared in 2.5 mM ammonium acetate (pH 6.6 or 3.0) or in 50% MeOH/50% 5.0 mM ammonium acetate (pH 6.6). Samples were introduced to MS by infusion at a flow rate of 10 µL/min, and data were acquired over several minutes.

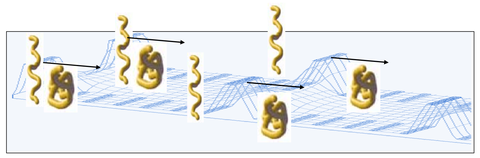
MS Conditions

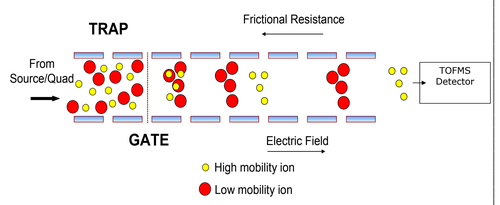
MS System:	Waters Synapt™ HDMS™
Ionization Mode:	ESI Positive
Capillary Voltage:	2.8 kV
Cone Voltage:	40 V
Desolvation Temp:	50 ˚C
Desolvation Gas:	250 L/Hr
Source Temp:	50 ˚C
Acquisition Range:	500—3000 m/z
Trap Collision Energies:	6 V
IMS Gas:	N_2 gas
IMS gas pressure:	0.7mbar
Pulse Height:	Variable, 9-12 v

ADDING ION MOBILITY TO A QTOF MS



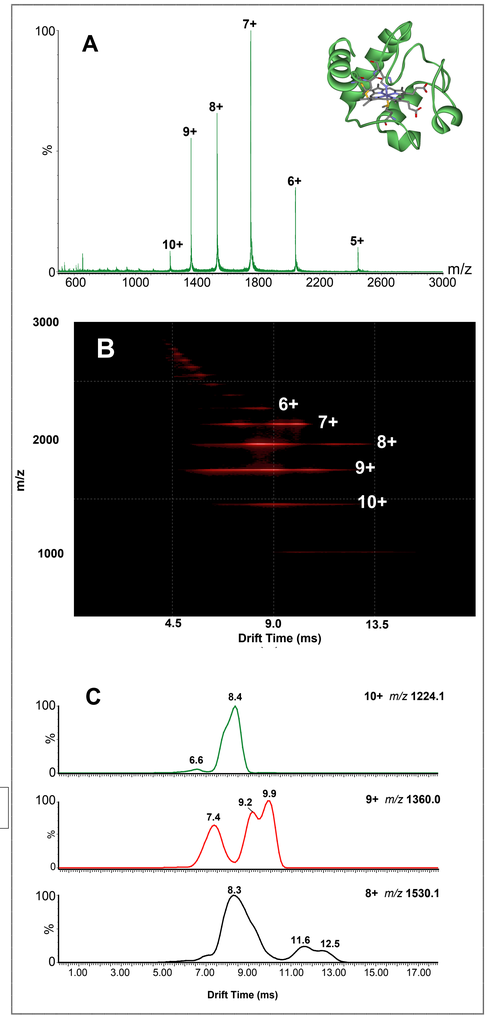
A three element traveling wave ion guide mobility cell is placed where a traditional collision cell would be found in a QTOF mass spectrometer. The TRAP ion guide can collect ions and deliver them as packets for ion mobility separation in the central ion guide. The TRANSFER cell permits efficient transfer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





Ion mobility separations in the central ion guide are 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.

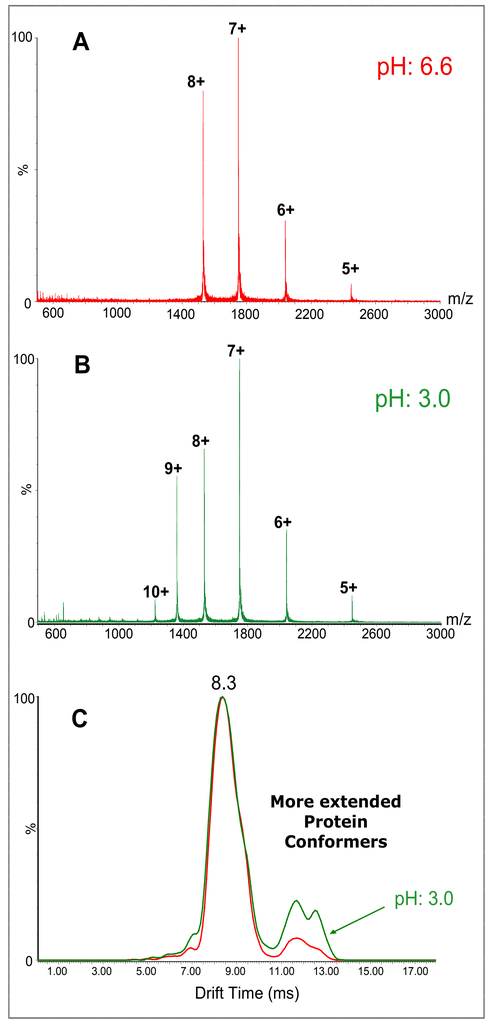
ION MOBILITY REVEALS THAT AN ESI CHARGE STATE CAN CONTAIN MULTIPLE PROTEIN CONFORMERS



(A) ESI-IMS-MS spectrum of Cytochrome c in ammonium acetate (pH 3.0)
(B) DriftScope plot showing ion mobility drift time vs. m/z for the same analysis
(C) Ion mobility drift time profiles for the 10+, 9+, and 8+ ions

A single charge state can contain multiple protein conformers that can be resolved by an ion mobility separation as drift time peaks.

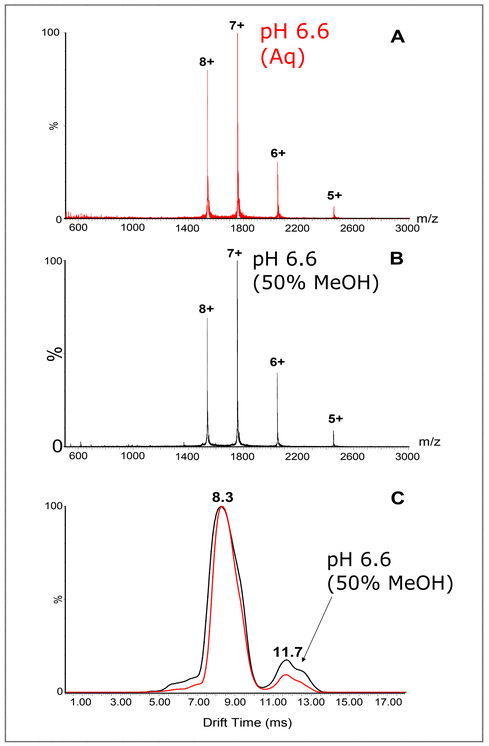
CYTOCHROME C (8+) INFUSED AT pH 6.6 AND 3.0 SHOWS NATIVE AND EXTENDED STRUCTURES



(A) ESI-IMS-MS spectrum of Cytochrome c in ammonium acetate (pH 6.6)
(B) ESI-IMS-MS spectrum of Cytochrome c in ammonium acetate (pH 3.0)
(C) Ion mobility drift time profiles for the 8+ ions in each condition

As the infused protein solution is acidified, the mobility plot of the 8+ ion reveals greater prominence of extended protein conformers.

CYTOCHROME C (8+) INFUSED IN 0%(A) AND 50% BUFFERED METHANOL



(A) ESI-IMS-MS spectrum of Cytochrome c in ammonium acetate (pH 6.6)
(B) ESI-IMS-MS spectrum of Cytochrome c in AA/50% MeOH (pH 6.6)
(C) Ion mobility drift time profiles for the 8+ ions in each condition

More subtle denaturing conditions reveal differences in ion mobility profiles that cannot be inferred from protein charge state distributions.

CONCLUSIONS

- Ion mobility separations provide an additional separation dimension that permits resolution of underlying protein structural distributions within individual charges states of a protein electrospray mass spectrum.
- Protein structural differences in the liquid phase can translate into measurable conformational differences apparent in the gas phase mobility of protein ions.
- Scientists can use this capability to study the stability, refolding, and underlying structural heterogeneity of biotherapeutic proteins.
- Such structural experiments can be accomplished by MS infusion studies requiring only minutes of data acquisition.

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