CHARACTERIZING HIGH-ORDER STRUCTURES OF THERAPEUTIC PROTEINS BY ELECTROSPRAY ION-MOBILITY MASS SPECTROMETRY

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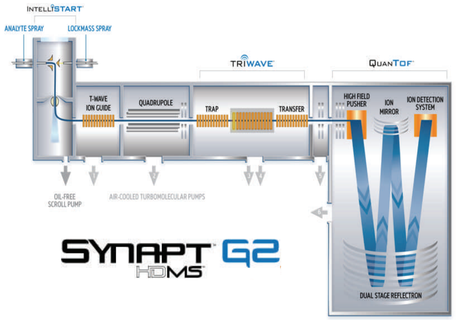
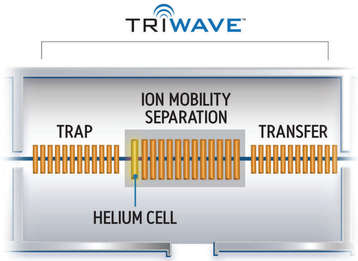
OVERVIEW

- Reproducible safety and efficacy profiles for biotherapeutic proteins require manufacturers to produce products with consistent tertiary structures
- Methods for determining changes in higher order structure are invaluable for the assessment of product quality
- In this work, Waters SYNAPT® G2 HDMS™ is used to characterize biotherapeutic protein tertiary structures and data from orthogonal analytical techniques is applied to corroborate the IMS results
- Cross-sectional areas measured by IMS method are in good agreement with those calculated using the MOBCAL computations

- The system was configured with a Waters SYNAPT G2 HDMS quadrupole ion-mobility time-of-flight mass spectrometer and a Triversa nanomate from Advion Bioscience.
- The SYNAPT G2 HDMS was operated in the mobility-TOF mode (resolution mode) for all analyses. MassLynx™ 4.1 software was used for instrument control and data processing. DriftScope™ 2.1 was used to display the ion-mobility data.
- Recombinant therapeutic proteins (insulin, interferon alpha-2a, GCSF, rhGH) from multiple commercial manufacturers were analyzed under varying formulated conditions (10.0 pmol/µL in 100.0 mM ammonium acetate, pH 7.0 or 3.0).

WATERS SYNAPT G2 HDMS



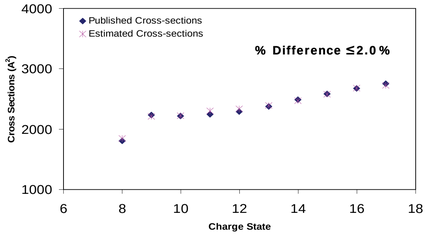


DETERNIMING THE CCS OF PROTEINS BY SYNAPT G2 HDMS

1. Protein standard, whale sperm myoglobin, was prepared in 50% acetonitrile (v/v), 0.1% formic acid at 1.0 pmol/µL solution
2. The protein solution was infused into Synapt G2 HDMS and analyzed under the exactly same ion mobility conditions as that for therapeutic proteins
3. IMS calibration was carried out using multiply charged ions (17^+ to 6^+) of known collisional cross-section areas* of myoglobin
4. The IMS calibration was validated using the multiply charged ions of *Cytochrome c*, whose CCSs have previously been determined on a standard IMS drift tube*
5. Determine the CCS values for each of charge states of therapeutic proteins using the calibration plot

* All published CCS data from http://www.indiana.edu/~clemmer/

Good matches are found between T-Wave™ derived CCS values and the literature data



Experimentally determined cross-section values for different charge states of human insulin analogs under near physiological conditions

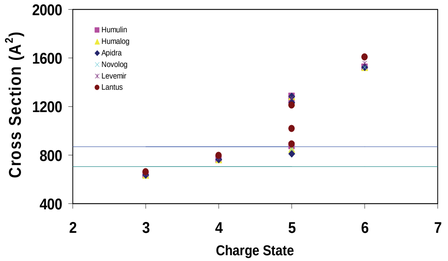


Table 1. MOBCAL and DriftScope calculation based on the PDB files for the therapeutic proteins. Collisional cross-section values displayed are derived from the Projection Approximation (PA) and the Exact hard Sphere Scattering calculation.

Proteins				Calc. Cross-section (Å²)		
				DriftScope	MOBCAL	
Description	Swiss-Prot ID	Experimental Method	PDB File	PA	PA	EHSS
Human Growth Hormone (hGH)	SOMA_HUMAN	X-Ray	1AXI	1636	1618	2062
Human Colony-Stimulating Factor (G-CSF)	CSF3_HUMAN	X-Ray	2DPQ	1504	1474	1862
Interferon alpha-2b	IFNA2_HUMAN	NMR	1ITF	1595	2104	2615
Human Insulin	INS_HUMAN	X-Ray	1MSO	1113	1121	1393
				706	711	869

Analysis of Insulin Analogs by IM Mass Spectrometry

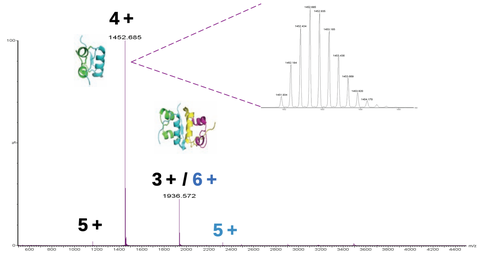


Figure 1. Mass spectra from a non-denatured solution of human insulin.

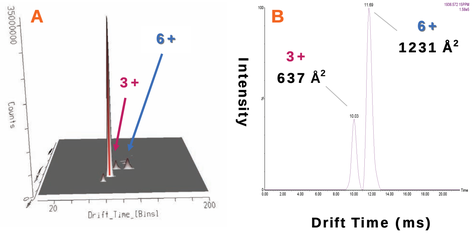


Figure 2. IMS separation of monomeric insulin from dimeric insulin. (A) 3-D plot showing the IMS separation of insulin ions at different charge state (B). Arrival time distribution for the selected charge (3+) of insulin monomer. The determined CCS values are also labeled at the peak top.

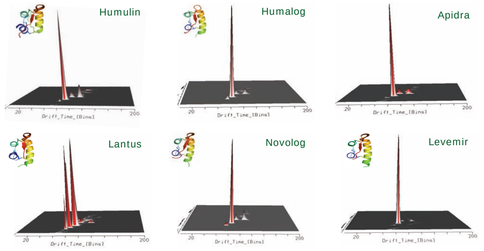


Figure 3. 3-D plot showing the IMS separation of insulin ions at different charge states for selected insulin analogs. A quick comparison on the distribution of protein conformation is obtained for the selected samples.

Analysis of Conformation Changes of Interferon alpha 2b by Ion-Mobility MS

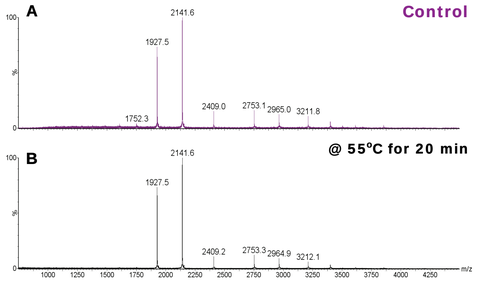


Figure 4. ESI spectra of Interferon alfa2b from a pH 7.0 solution before (A) and after (B) high-temperature stress.

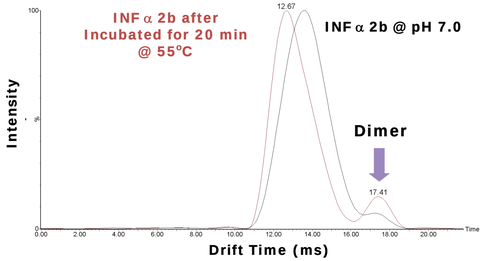


Figure 5. Arrival time distribution for the selected charge (4+) of Interferon alfa2b from a pH7.0 solution before (black trace) and after (orange trace) high-temperature stress. The data clearly shows the increase of protein dimmer in the sample after high-temperature incubation.

CONCLUSIONS

- Ion mobility mass spectrometry, based on travelling wave technology, provides a rapid, sensitive, reproducible method for measuring mass selected arrival time distributions
- Estimates of orientationally averaged cross section values provide good agreement with both drift cell values and those calculated from X-ray and NMR data
- Insight into biologically relevant processes is feasible using this approach

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