# INVESTIGATING PRIMARY AND HIGHER-ORDER PROTEIN STRUCTURE ANALYSIS USING A NOVEL CYCLIC ION MOBILITY ENABLED QTOF PLATFORM

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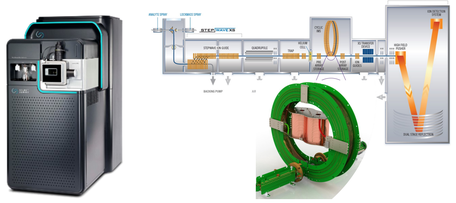
SELECT SERIES
Waters Advanced Mass Spectrometry

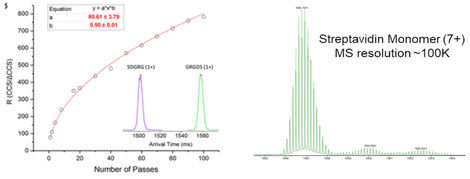
## OVERVIEW

- Biotherapeutic analysis challenges are not always addressable by high resolution LC and MS separations.
- Gas phase ion mobility-based separations have provided an additional dimension capable of resolving ions based on properties of charge and collisional cross-section (CCS).
- These IMS separations can resolve many isomeric species that are both isobaric and co-eluting, or reveal insights into the folded structures of proteins and complexes.
- A novel ion mobility MS platform, the SELECT SERIES Cyclic IMS (in a Q-cIMS-oaTOF configuration), has been developed to address such complex challenges.
- Higher (and selectable) ion mobility resolution is enabled by the cyclic geometry of the IMS cell, and capabilities for IMS-IMS and IMS$^n$ of selected ions and their fragments.
- This poster highlights early peptide and protein studies that have been conducted with this novel platform to address questions of primary and higher order structural analysis.

## SELECT SERIES CYCLIC IMS



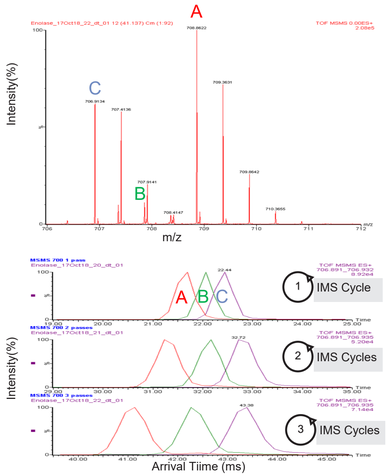
The Waters SELECT SERIES Cyclic Ion Mobility QTOF MS. The cyclic ion mobility module is between the quadrupole and TOF detector. Fragmentation is possible prior to, after, or within the Cyclic IMS module. IMS/IMS & IMS$^n$ (w/ion selection) are novel supported modes of operation.




Streptavidin Monomer (7+)
MS resolution ~100K

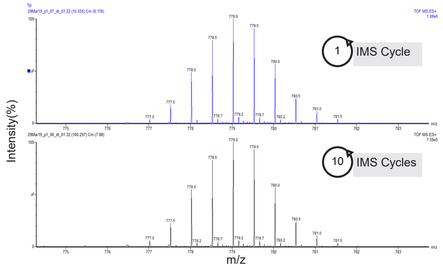
Ion Mobility resolution is scalable by selecting the number of cycles of IMS separation, with IMS resolution ($\Omega/\Delta\Omega$) increasing as the square root of the number of cycles . 100 IMS cycles generates ~800 IMS resolution, with an ion signal loss of only ~1% per cycle. TOF Detection can be acquired with selectable MS resolution of 60,000 (V-Optics) to 100,000 (W-Optics).

## PEPTIDE ION MOBILITY



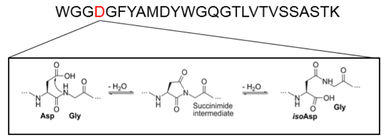

Three coeluting peptides within an LC/MS peptide map (TOP Panel) of yeast enolase, were resolved to a 10% valley using 3 cycles of IMS separation (BOTTOM Panel) imparting greater effective peak capacity to the analysis.

## DEUTERATED PEPTIDES

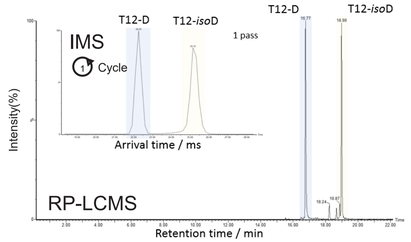



The standard HDX P1 Peptide (HHHHHHIIKIIK) was deuterated in solution, acidified in 0.2% cold formic acid, and Infused to the system via chilled syringe. No back-exchange of deuterium was observed after 10 IMS cycles (~110 msec) prior to MS detection. Deuterium scrambling studies are ongoing in support of potential HDX-MS applications.

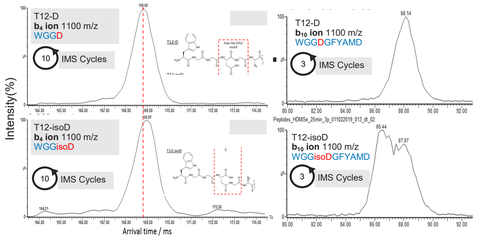
## STUDY OF PEPTIDE ISOMERIZATION




Trastuzumab (Tmab) HC T12 peptide was synthesized in both Asp and isoAsp forms, then mixed 1:1 as a model system for studying of Asp isomerization.

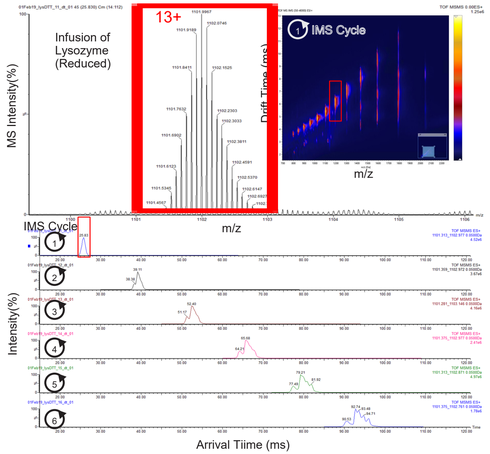



Tmab HC peptide T12 (above) in a 1:1 mixture of the Asp and isoAsp forms can be baseline resolved by UPLC chromatography or by a single pass through the Cyclic IMS module prior to MS detection. This enables reduced chromatographic burdens for deamidated peptide resolution.



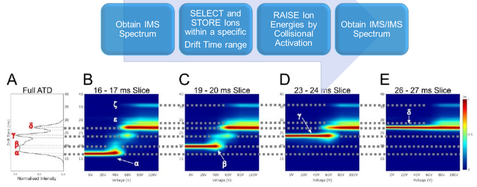

Surprisingly, if we fragment the Tmab HC T12 peptide ions, the first potentially diagnostic ion (b4+) does not show the ion mobility resolution of the Asp and isoAsp forms that were evident in the full length tryptic peptide. This observation (ABOVE LEFT) is maintained even following 10 cycles of IMS separation. Larger b-ions such as the B10 ion are diagnostic of the isomeric form (ABOVE RIGHT).

## PROTEIN IMS



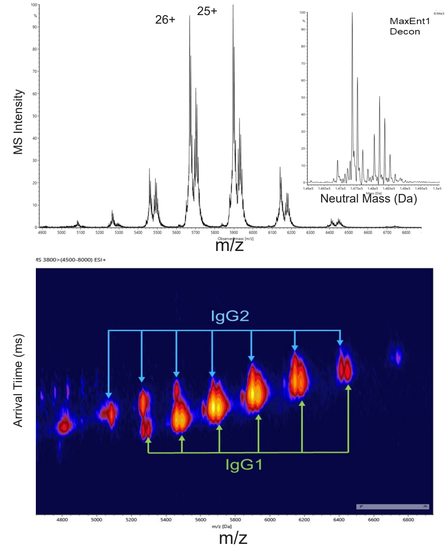

Reduced lysozyme yields a simple mass spectrum (Top Panel) where the dominant charge state (13+) has a single drift time distribution (Red Box) using a single IMS Cycle. Additional IMS Cycles (Bottom Panel) resolve greater structural heterogeneity in this molecule.

## COLLISION INDUCED UNFOLDING




Cytochrome c was infused under native MS conditions. The drift plot (Panel A) of the 7+ ion exposed to 20V in the Trap region reveals a population of conformers. If we select the *Alpha* form in the IMS region and expose to elevated energy (60-80V) and reanalyse by IMS-MS, we observe (Panel B) conformers *Beta* thru *Delta* and two new minor forms (*Epsilon and Zeta*). Repeating this isolation, excitation, reanalysis cycle for the other 7+ conformers *Beta* (Panel C), *Gamma* (Panel D), and *Delta* (Panel E) we see the directionality of gas phase unfolding for this molecule.

## IgG$_1$ AND IgG$_2$ COINFUSION




Co-infusion of an IgG1 and IgG2 molecule generates a mass spectrum and deconvoluted mass spectrum (TOP Panel) with largely resolved component signals. One IMS Cycle revealed (BOTTOM Panel) a single structural form for the IgG$_1$ and two major structural forms for the IgG$_2$ molecules.

## CONCLUSIONS

- A cyclic IMS enabled QTOF MS with a novel geometry (Q-cIMS-oaTOF) was described with unique capabilities for selectable high resolution ion mobility and high resolution mass analysis.
- The ability to go beyond chromatographic and m/z based separations was demonstrated. The cyclic IMS provided increased peptide separation capacity for peptide mapping and unique selectivity for targeted study of peptide variants and their fragments.
- Use of the cyclic IMS functionality enabled recognition of protein conformers for the native molecule, and studying corresponding unfolding pathways generated by collisional activation.
- This Cyclic IMS QTOF platform represents a novel tool capable of addressing the increasing complexity of modern and emerging biotherapeutic molecules.

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