DEVELOPMENT OF A MODIFIED CYCLIC IMS PLATFORM FOR ENHANCED BIOMOLECULE CHARACTERIZATION

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INTRODUCTION

Cyclic ion mobility-mass spectrometry is a high-performance tool for a wide range of applications. In particular, the capability to perform native ion mobility, electron-based fragmentation, surface-induced dissociation, and high sensitivity bottom-up and intact-level studies make it a valuable tool in biomolecule characterization. To extend the capabilities in these areas, we have developed a number of hardware and software modifications two of which we describe here:

- **Dynamic Field Declustering**: a modified StepWave™ Ion Guide designed to improve declustering and desolvation.
- **Wideband Enhancement**: an acquisition mode which provides increased sensitivity across a broad mass range
- **CIU Workflow Improvements:** a simplified workflow to rapidly generate CIU experiments

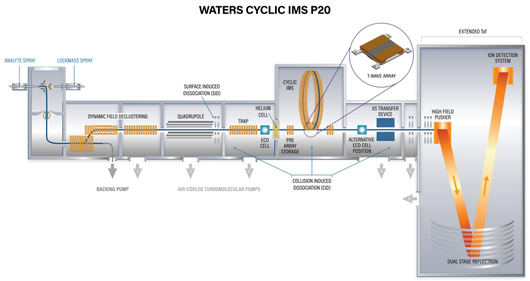


Figure 2. A schematic of the Cyclic™ IMS P20 Mass Spectrometer configured with ECD and DFD. The system has an upper mass range of more than m/z 100,000 and can be configured with a high mass quadrupole capable of resolving to m/z 32,000.

DYNAMIC FIELD DECLUSTERING

A modified version of the StepWave Ion Guide has been developed containing a declustering region in the second stage of the device comprising two parallel plates electrodes above and below the ion beam and a series ring electrodes either side of the beam.

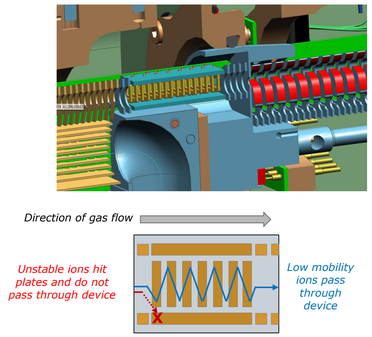


Figure 1: The declustering region of the of the enhanced declustering StepWave Ion Guide used in DFD. In ordinary operation the device functions as a normal ion guide focusing and transmitting the ions into the next region of ion optics; in DFD mode the device acts as a low-pass mobility filter by applying a tunable square wave, RF potential between the plate electrodes (shown in turquoise). This potential causes the ions to oscillate between the plates increasing their path length through the device. The increase in path length increases the number of collisions experienced by the ions leading to improved desolvation and removal of non-specific adducts such as buffer molecules.

Figure 2 shows mass spectra obtained from Streptavidin sprayed from 200 mM ammonium acetate at increasing declustering potential using a fixed frequency of 60 kHz.

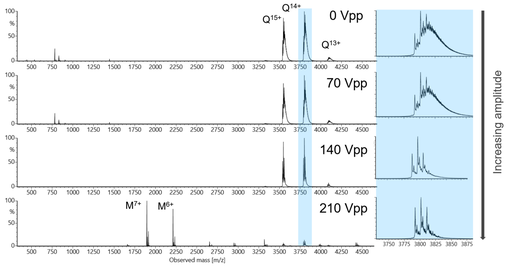


Figure 3: Native Mass Spectra of Streptavidin at different DFD potentials. Labels identify the monomer (M) and tertramer (Q) species. The box to the right shows a zoomed region of the spectrum for the 14+ tetramer ion. Individual peaks in the cluster are different N-terminal methionine clip variants of the protein.

With the declustering potential off the observed species can be seen to be heavily adducted with no resolved structure. As the potential increases more of the adduct molecules are removed cleaning up the spectra and enhancing the effective resolution of the data. At the highest declustering potential the tetramer has been dissociated raising the possibility of using this device as part of a pseudo-MS^3 workflow in combination with downstream fragmentation using CID, ECD or SID.

WIDEBAND ENHANCEMENT

Tof duty cycle is inherently limited by the rate at which the frequency at which the ions can be sampled. The duty cycle of an ion of interest can be greatly increased by synchronizing the pusher with the arrival of those ions in the pusher. By further synchronizing the pusher with the exit of ions from the IMS it is possible to selectively increase sensitivity across a wide mass range; we refer to this as Wideband Enhancement (WBE)

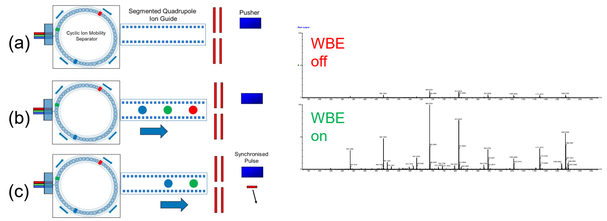


Figure 4: Wideband Enchancement: (a) ions are separated in the cIMS device; (b) as ions exit the cIMS they are gated into the transfer optics while maintaining the mobility separation; (c) the pusher is synchronized with the gating to increase the duty cycle of the ions of interest as they exit the IMS. The spectra to the right show ms/ms spectra generated for [glu]-fibrinopeptide b with WBE on and off demonstrating a signal boost of up to 10x across the mass range.

WBE uses a user-defined look-up table containing start and end masses for the enhancement region and corresponding start and end drift times. By adjusting these settings it is possible to selectively enhance different regions of the mass/drift space (see figure 6). WBE can be used in tandem with any HD acquisition mode and any pre-IMS fragmentation technique (figs 6 & 7).

CIU WORKFLOW IMPROVEMENTS

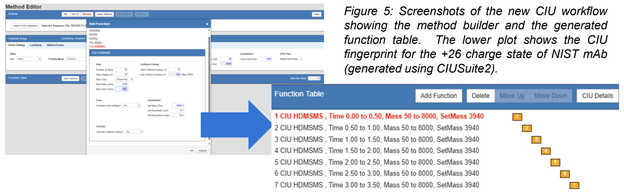
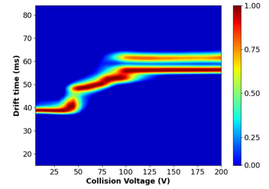


Figure 5: Screenshots of the new CIU workflow showing the method builder and the generated function table. The lower plot shows the CIU fingerprint for the +26 charge state of NIST mAb (generated using CIUSuite2).

A new CIU acquisition type has been included in Cyclic IMS P20 Mass Spectrometer. Users can easily create CIU methods by defining the desired start collision voltage, voltage step, step time and number of steps. The instrument then automatically creates the acquisition method greatly reducing the amount of time required to generate CIU data.



WBE RESULTS & APPLICATIONS

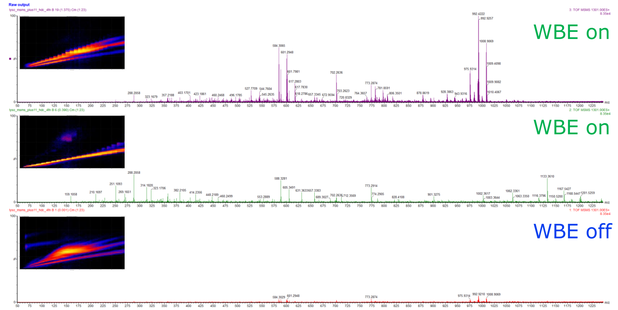


Figure 6: CID spectra of +11 lysozyme with different WBE settings. The top spectrum used settings designed to enhance +2 and +3 fragment ions while the middle spectrum used settings designed to enhance +1 species. Such data can be generated in a single acquisition using multiple functions. Driftscope™ images illustrate the enhancement.

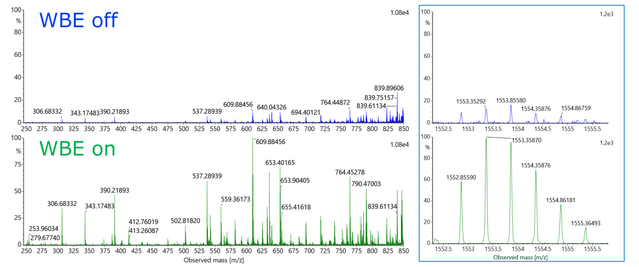


Figure 7: ECD fragmentation data for ubiquitin obtained using a Waters ECD cell in the pre-IMS position. The spectra on the left show the low mass fragments with WBE on and off demonstrating increased intensity of up to 10-fold for c- and z-ions; the spectra on the left shows a similar increase in intensity for a higher *m/z* fragment. Combining WBE with ECD fragmentation has been demonstrated to improve sequence coverage (see WP660).

CONCLUSIONS

- DFD using the Enhanced Declustering StepWave Ion Guide is a powerful tool for the analysis of proteins under native conditions
- DFD allows a user to selectively clean-up and simplify complex mass spectra by removing matrix ion cluster and non-specific adducts revealing hidden features in their data
- Wideband Enhancement boosts sensitivity across a wide mass range by up to 10 x, enabling high-sensitivity tandem-MS without compromising resolution. Due to its lower fragmentation efficiency ECD can particularly benefit from the application of WBE.
- These techniques can be used in combination with other recent advances in the cIMS platform such as increased mass range and CIU workflow improvements making Cyclic IMS P20 Mass Spectrometer a powerful tool for large biomolecule analysis

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