EXPANDING NATIVE MASS SPECTROMETRY CAPABILITIES FOR SOLUBLE AND MEMBRANE PROTEINS USING A QUADRUPOLE-ION MOBILITY-TIME-OF-FLIGHT MASS SPECTROMETRY SYSTEM

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OVERVIEW

- We present a powerful, flexible mass spectrometry system for native mass spectrometry
- The SELECT SERIES™ Cyclic™ IMS instrument is available with a wide range of technologies to enhance native MS workflows
- The cyclic ion mobility device enables high resolution mobility separation of conformers and the determination of $^{TW}CCS_{N2}$ values
- Membrane protein species can be efficiently released from their micellar complexes by activation in the source and collision cells
- The electron capture dissociation device can be placed pre– OR post–IMS increasing experimental options for top-down sequencing
- A newly available software enhancement enables automated surface induced dissociation experiments

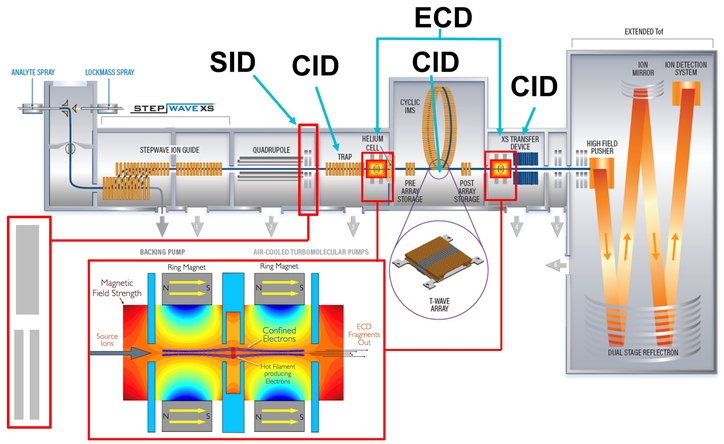


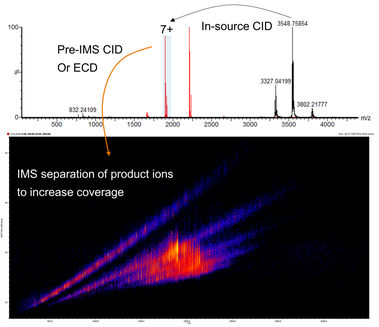
Figure 1. Schematic of the SELECT SERIES Cyclic IMS showing the suite of fragmentation options. The ECD cell can be placed pre– or post-IMS. CID can be performed pre-, post-, or intra-IMS. SID is performed pre-IMS.

METHODS

All experiments were conducted on a SELECT SERIES Cyclic IMS mass spectrometer equipped with a pre-mobility SID device and an ExD cell (eMSion, Corvallis, OR, USA). All proteins were electrosprayed from 2 µm I.D. borosilicate glass nanocapillaries (World Precision Instruments, FL, USA). Electrospray voltage was applied using a piece of platinum wire inserted into the rear opening of the capillary. Streptavidin was purchased from Pierce, and made up to a final concentration of 1 µM tetramer complex in 200 mM ammonium acetate. For in-source dissociation, a cone voltage of 120 V was used. Trap CID was performed at 100 V. For GroEL, a 1.2 µM concentration solution was prepared in 200 mM ammonium acetate. A single pass of ion mobility was performed and maximum sensitivity was obtained with a racetrack bias of 55 V and a corresponding array offset (separate and inject steps) of 55 V). All CCS values were determined by calibration with IMScal19[1] using polyalanine and bovine serum albumin as calibrants. The membrane proteins OmpF and pfMATE were electrosprayed at concentration of 4 µM in 200 mM ammonium acetate in the detergents β-OG and C8E4, respectively. For automated SID, streptavidin was electrosprayed as above, with the sample list running a SID voltage ramp from 10 to 100 V.

COMPLEX-DOWN PROTEIN SEQUENCING

The suite of fragmentation modes on the SELECT SERIES Cyclic IMS instrument enables a range of experiments to be performed on native proteins. Non-covalently bound protein ions can be dissociated by activating in the source region with the cone voltage and then ejected subunits can be interrogated by subsequent CID or ECD to generate second generation product ions in so-called 'complex-down' experiments. Here we applied this methodology to the streptavidin tetramer, exploiting the capability to perform mobility separation on the second generation product ions to increase coverage. We performed dissociation on the streptavidin tetramer to release monomer subunits, followed by quadrupole isolation of the monomer 7+ charge state. The monomer ions were then subjected to CID or ECD. The top-down data for the streptavidin monomer was deconvoluted with the BayesSpray algorithm in waters_connect™ and matched using ProSight Lite[2]. 78 % coverage was obtained using ECD and 89 % with CID. The combined coverage was **98 %.**



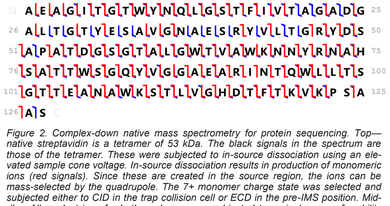


Figure 2. Complex-down native mass spectrometry for protein sequencing. Top—native streptavidin is a tetramer of 53 kDa. The black signals in the spectrum are those of the tetramer. These were subjected to in-source dissociation using an elevated sample cone voltage. In-source dissociation results in production of monomeric ions (red signals). Since these are created in the source region, the ions can be mass-selected by the quadrupole. The 7+ monomer charge state was selected and subjected either to CID in the trap collision cell or ECD in the pre-IMS position. Middle—All product ions for both analyses were subjected to a single pass of mobility separation. The mobility separation is visible in the ion mobility-mass spectrum shown. The data were separated into 5 different arrival time-m/z regions and each one deconvolved separately. The ion lists were then submitted to Prosight Lite for identification. The coverage obtained for ECD was 78 %, CID was 89 %, with a combined coverage of 98 %.

STATE-OF-THE-ART CCS CALIBRATION

As a result of the non-linear and time-variant behaviour of the voltages in TWIMS, calibration of CCS values is required. In recent years the method of calibration has been vastly improved in both precision and accuracy as well as useability by the introduction of IMScal19[1]. This tool is freely available and consists of a simple GUI which enables CCS determination of a wide range of analytes, even with both small molecules and large proteins present in the same datafile.

The $^{TW}CCS_{N2}$ of GroEL was determine to be 220 nm^2 after calibration with polyalanine and bovine serum albumin ions, in excellent agreement with reference values and those from other instrument platforms[3].

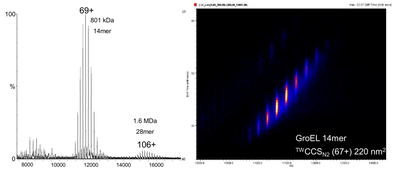


Figure 3. CCS determination of protein complexes with IMScal19. The CCS of the GroEL 14mer was measured after a single pass of cyclic ion mobility separation (220 nm^2). The value was obtained through calibration with polyalanine and BSA ions.

MEMBRANE PROTEIN NATIVE MS

Membrane proteins are an important class of protein as they make up 30 % of the human proteome and 60 % of all approved drug targets. Characterisation of these proteins is an attractive capability in labs performing native MS in both academia and industry. Key to the analysis of membrane proteins is the ability to remove bound detergent micelles that surround the proteins at the point of ionisation. This is typically done by collisional activation either in the source region or the collision cells of the mass spectrometer.

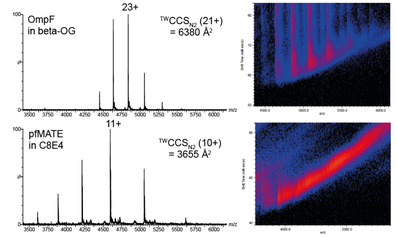


Figure 4. Membrane protein native MS on the SELECT SERIES Cyclic IMS. Top—Spectrum of OmpF released from a β-OG micelle using the cone voltage and trap collision voltage. The CCS of the 21+ charge state was measured as 6380 Å^2. Bottom—Spectrum of pfMATE released from a C8E4 micelle using the cone voltage only. The CCS of the 10+ charge state was measured as 3655 Å^2. For both these proteins the declustering voltages had to be reduced to minimise any gas phase unfolding for the purpose of native-like, folded, CCS determination.

Figure 4 shows native mass spectra of two membrane proteins. Firstly, we electrosprayed the bacterial protein OmpF, a trimeric protein complex of 110 kDa. It was electrosprayed in the detergent β-OG. Collisional activation in both the source and the trap collision cell enabled removal of the detergent micelle to obtain a high quality native mass spectrum centred on the 23+ charge state. By tuning the collisional activation to a minimum, the $^{TW}CCS_{N2}$ of the lower energy folded protein complex could be measured (6380 Å^2). Next we performed MS analysis of the multidrug and toxin extrusion protein from *Pyrococcus furiosus*, *pf*MATE at 50 kDa in mass. This protein was electrosprayed in the detergent C8E4 and could be adequately removed from the detergent micelle by using the sampling cone voltage in the source region. The $^{TW}CCS_{N2}$ was measured as 3655 Å^2.

AUTOMATED SID ACQUISITION

Surface induced dissociation is a powerful activation technique in native mass spectrometry used to interrogate the structures of, and construct interaction maps of, biomolecular complexes. Up until its launch as an option on the SELECT SERIES Cyclic IMS instrument SID has not been available on a commercial MS platform. Since then the implementation of the technology has developed and recently a software enhancement has been made available to enable automated SID control and data acquisition.

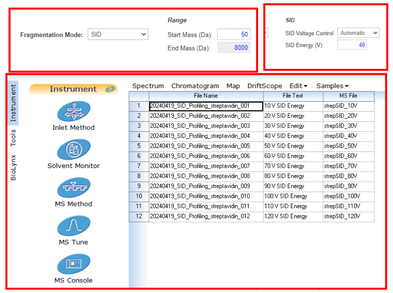


Figure 5. Automated SID control on the SELECT SERIES Cyclic IMS. Top—method parameters include a fragmentation mode selection and single SID voltage selection. Bottom—The method can be browsed in to the Masslynx sample list for automated data acquisition.

The benefit of automated SID is that data can be acquired faster and with less user intervention. Moreover, it is simple to set-up so-called 'SID Profiles' (Figure 7) scanning the SID voltage ranges to find optimum dissociation parameters.

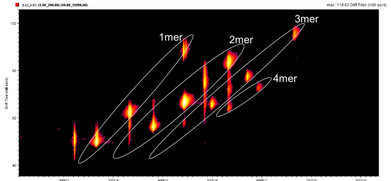


Figure 6. Automated SID control on the SELECT SERIES Cyclic IMS. Top—method parameters include a fragmentation mode selection and single SID voltage selection. Bottom—The method can be browsed in to the Masslynx sample list for automated data acquisition.

The combination of ion mobility and SID has been proven to be beneficial. The positioning of the SID device prior to ion mobility means that the product ions are separated from each other by virtue of their shape and charge (Figure 6). This results in separation between SID product ions with the same *m/z* but different mass, which simplifies spectrum assignment, particularly when studying protein oligomers. Figure 6 shows an ion mobility mass spectrum of streptavidin at 40 V SID voltage and demonstrates the advantage of mobility separation. At 4400 m/z, for example, monomers (3+), dimers (6+) and trimers (9+) are all separated by mobility.

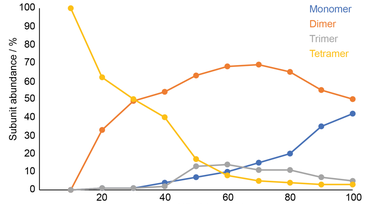


Figure 7. SID profile of the 11+ charge state of streptavidin. As the tetramer is consumed with increasing SID voltage, the dimer becomes the dominant product ion. At higher energies the monomer increases in intensity with relatively small amounts of trimer being detected.

CONCLUSION

- **The SELECT SERIES Cyclic IMS mass spectrometer is a powerful platform for native MS.**
- **An unrivalled suite of fragmentation options enables a wide range of experiments to interrogate structure and sequence.**
- **CID and ECD enabled near complete coverage of streptavidin in complex-down sequencing experiments.**
- **High quality spectra were obtained from the 800 kDa GroEL complex.**
- **Efficient micelle removal using the source and trap collision voltages enabled accurate mass measurement of intact membrane protein ions.**
- **Highly precise CCS values were measured using the IMSCal19 tool.**
- **For the first time an automated SID implementation is reported, increasing the useability of the SELECT SERIES Cyclic IMS system.**

References

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