Structural Characterisation of Intact Proteins using Electron Capture Dissociation within an Ion Mobility enabled TOF

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OVERVIEW

An electron capture dissociation (ECD) cell that entraps low-energy electrons from a heated filament using magnets and DC electrostatic lens elements was installed within a modified Synapt G2-Si .

The cell enables ECD before and after the travelling wave ion mobility device of the Synapt G2-Si.

This research study will focus on results generated following implementation of the ECD cell post ion mobility.

INTRODUCTION

Collision Induced Dissociation (CID) is the preferred dissociation technique for tandem MS given its simplicity, ease of implementation as well as reliable and robust performance for many analytes. However, CID of large proteins, in particular non-covalent complexes offers very little structurally informative sequence ions. The product ions generated are typically formed through sub-unit ejection of the intact complex. Electron Transfer Dissociation (ETD) and Electron Capture Dissociation (ECD) are known to provide sequence-specific information following dissociation with little disruption of non-covalent interactions. Unlike CID labile PTM's are preserved during fragmentation and sequence information is obtained for large proteins.

METHODS

The e-MSion ECD cell is comprised of 7 electrostatic lenses, electrons are emitted from a hot loop rhenium filament coated with ytrrium oxide. They are constrained by two samarium alloy magnets to the central axis which is aligned with the flight path of the ions exiting the ion mobility cell. The standard transfer cell was shortened to accommodate the ECD cell.

For collision induced unfolding (CIU), ions are activated as a function of either the source cone voltage or trap cell collision energy within the Synapt G2-S*i*. CIU conformational changes can be observed through ion mobility drift time changes as unfolding occurs. Information relating to the higher order structure of the protein may be obtained. In addition, by recording the sequence ions generated by ECD the location of unfolding can be mapped upon an X-ray crystal structure (if available). Top-down and middle-down ECD data is complex requiring accurate deconvolution. This is being investigated using software BayeSpray/Unifi (Waters), BayeSpray/ProSight and IMTBX + Grppr/Unifi/ProSight and MassTodon. The opportunity to perform ECD pre-IMS will clearly improve product ion identification for this analysis.

UPLC: H-Class
Column: Acquity UPLC Protein BEH SEC Column (200Å, 1.7µM, 2.1mm x 150mm); Column Temperature: 30°C; Sample Temperature: 8°C; Injection volume: 5µL
Mobile Phase: 100mM Ammonium Acetate with a flow rate of 100µL/min provided Isocratic Native Protein Elution

MS: Synapt G2-S*i* with ECD modification; Ionisation: ESI (+VE)
Capillary voltage: 2.1kV; Cone voltage: 75-150V; Source Temperature: 50-110°C; Desolvation Gas: 800L/hr

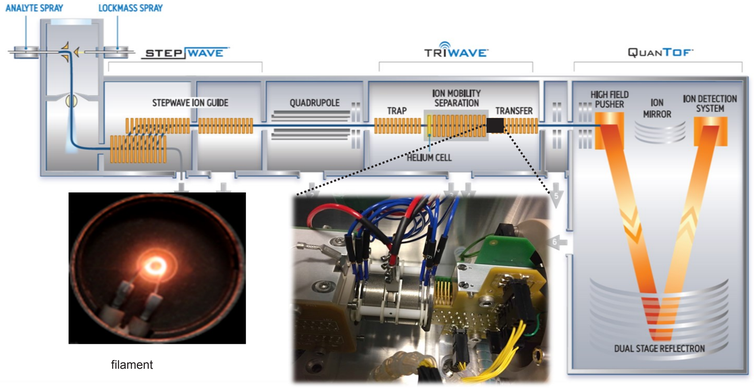

Figure 1: Schematic of the Synapt G2-Si and photograph following inclusion of the ECD cell post-ion mobility

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RESULTS

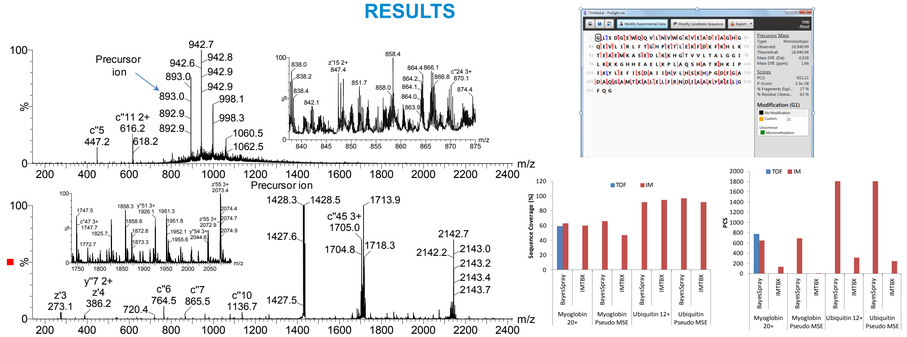

Figure 2: Denatured Infusion ECD MS/MS of myoglobin (top) and ubiquitin (bottom). Following spectral deconvolution of the ECD data, a sequence coverage map of the product ions obtained from myoglobin 20+ was provided from ProSight software. Inset tables represent sequence coverage and PC scores.

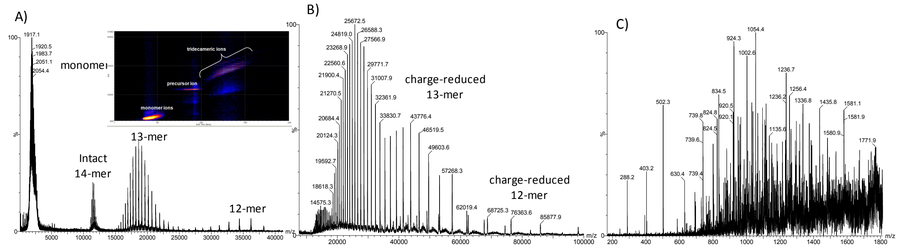

Figure 3: A) MS spectrum of intact native GroEL (800kDa) with trap energy 100V, showing ion transmission with ECD cell in place (filament off). B) ECnoD (filament on) of quadrupole selected GroEL m/z 10,992 $[M+73H]^{73+}$ highlighting extreme charge-reduction and bimodal distribution C) ECD of quadrupole selected m/z 1909 $[M+30H]^{30+}$ from denatured GroEL monomer.

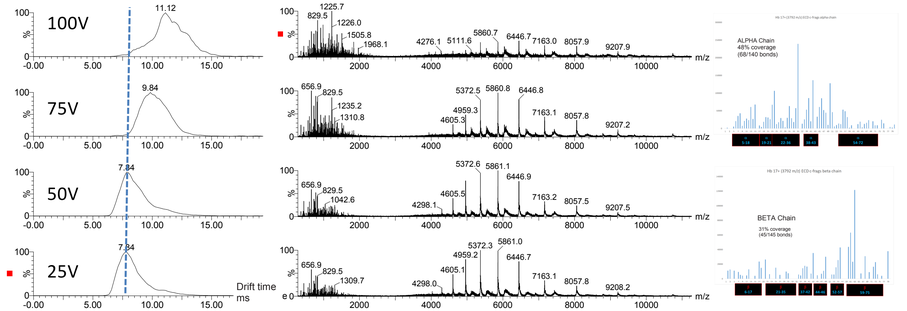

Figure 4: Native hemoglobin tetramer (64kDa) (SEC-CIU IMS-ECD) with varying cone voltages. *Spectra show lower m/z ECD product ions together with charge-reduced product ions at higher m/z. Note the quadrupole was operated in RF-only mode. Sequence coverage of both chains was determined using Masstodon software.*

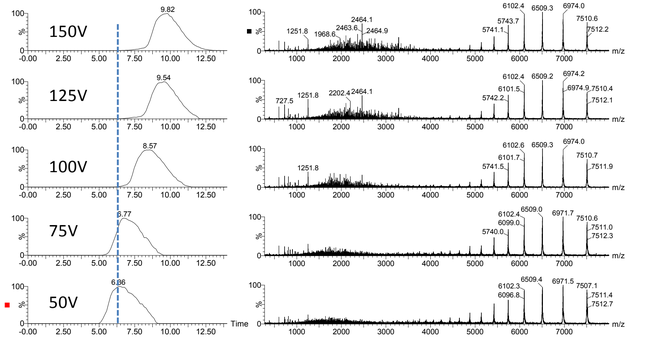

Figure 5: SEC-CIU IMS-ECD, Native NIST mAb *(Fab* 97kDa, *SEC and IMS selected) following reaction with ideS. Low m/z ECD product ions together with high m/z charge-reduced product ions are observed in the spectrum. More low m/z ECD product ions are observed as unfolding occurs. Note the quadrupole was operated in RF-only mode.*

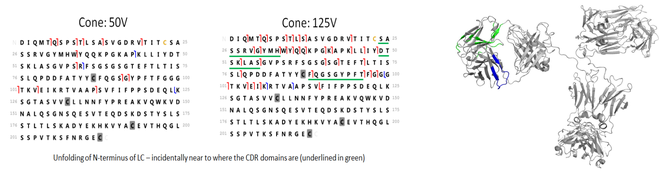

Figure 6: Sequence coverage for the ECD Fab LC portion was 27%, and the Fab Fd' portion was 16% with CV 125V. This does not include fragments that would include portions of both domains (which is the C-terminus of both because of a disulfide bond) For an analogous crystal structure, Green—LC sequence with increased fragmentation upon cone activation and Blue HC sequence fragments at 50V and 125V cone activation.

CONCLUSIONS

- An electrostatic ECD was fitted to a modified Synapt for post ion mobility fragmentation.
- Intact proteins and non-covalent protein complexes were analyzed using size exclusion chromatography with ECD from eluting peaks of 10-20s (FWHM).
- Collision Induced Unfolding (CIU) combined with IMS and ECD allows surface mapping for location of protein unfolding.
- ECD of peptides is yet to be investigated.
- Improvements in overall protein sequence coverage is expected with the ECD cell positioned pre-IMS since ion mobility will improve the peak capacity.
- The choice of the location of the ECD cell presents new experimental research possibilities.