Advances in hardware design and function of the new timsOmni MS platform

D. Papanastasiou[1]; A. Smyrnakis[1]; M. Kosmopoulou[1]; A. Grigoriadis[1]; I. Orfanopoulos[1]; N. Manolis[1]; I. Panagiotopoulos[1]; R. Gioves1; A. Lekkas[1]; F. Busch[2]; J.F. Greisch[2]; S. Pengelley[3]; C. Gebhardt[3]; A. Apalategui[3]; J. Koehling[3]; M. Krause[3]; J. Decker[3]; N. Goedecke[3]; E. Carrascosa[3]; O. Raether[3]

[1]Fasnatech Science & Technology, Athens, Greece;
[2]Bruker Switzerland AG, Fällanden, Switzerland
[3]Bruker Daltonics, Bremen, Germany

Introduction

Innovations in MS instrumentation are driven by the growing demand for comprehensive characterization of proteins and a wide array of molecular compounds. Central to meeting this demand is the advancement of complementary fragmentation techniques, which are critical for generating high-resolution structural and sequence information required for precise analyte identification. Here, we detail recent innovations in the design and method implementation on the timsOmni™ MS platform, highlighting its outstanding versatility and broad applicability.

Instrumentation

Essential components of the timsOmni platform shown in Fig. 1 include:

- An array of ESI sources covering the entire range of flow rates, from analytical and low micro-flow ESI (VIP-HESI), to online nano-flow ESI (CaptiveSpray), and offline nano-flow ESI (NEOS) for native MS.
- 1.3 mm i.d. inlet capillary delivering nanoamperes of ion current to an RF ion funnel operated at 10 mbar.
- A stacked-ring RF ion guide configured for collisional activation enabling desolvation, Collision Induced Unfolding (CIU) and in-source Collision Induced Dissociation (isCID) upstream of the Trapped Ion Mobility Spectrometer (TIMS).
- A high-capacity dual TIMS analyzer with ion accumulation and ion mobility separation regions, further configured with a gate to allow only ions of a selected mobility to be transmitted.
- An additional collisional activation region at the exit of the TIMS operated at 0.5 mbar pressure.
- A quadrupole mass filter with isolation capabilities extended to 4500 m/z.
- The Omnitrap™ platform facilitating MS^n functionality with ion enrichment and trapped electron-based fragmentation (eXd) with fine electron energy control.
- A new collision cell design supplied with a ramped AC signal for mass selective ejection of ions to improve the duty cycle of the OA TOF analyzer.

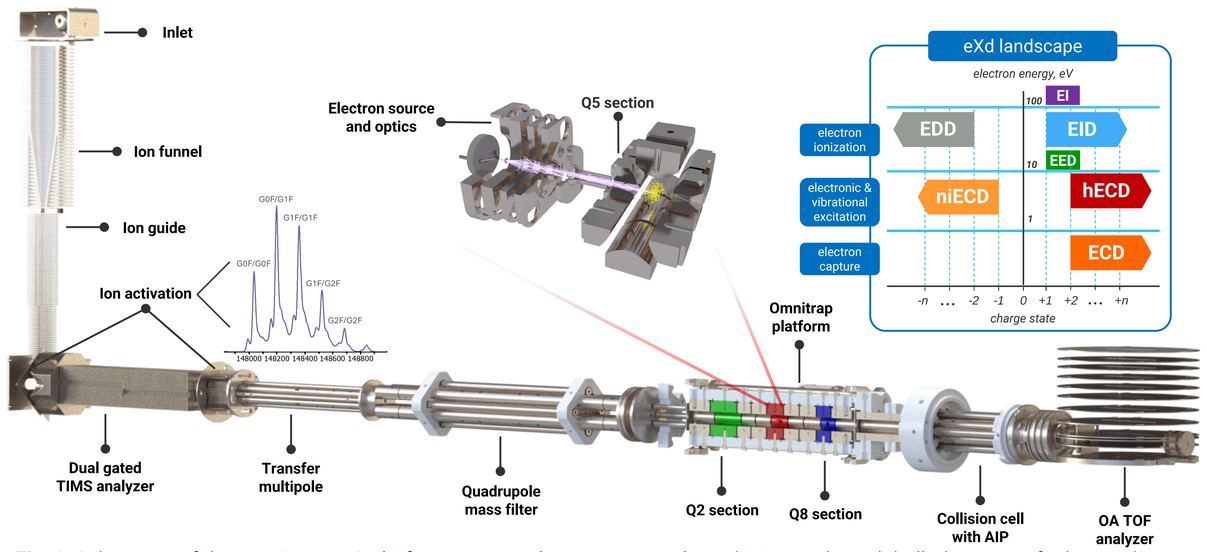

Fig. 1 Schematic of the timsOmni MS platform. Essential components along the ion path are labelled. A magnified view shows the external electron source coupled to Q5. The eXd reaction landscape enabled by fine electron energy control is portrayed. Desolvation of native NIST mAb T ~3 mbar pressure is demonstrated upstream of the TIMS analyzer.

CIU – TIMS – ECD

Electron capture dissociation (ECD) is a soft fragmentation technique and can be used as a probe for protein structure in the gas phase. Combining ECD with CIU allows monitoring the dynamics of protein unfolding. Fig. 2 shows a CIU-TIMS-ECD experiment performed with native carbonic anhydrase where conformational changes for charge state 11+ are monitored as a function of activation energy and regions of structural flexibility or rigidity along the backbone are observed by ECD fragmentation.

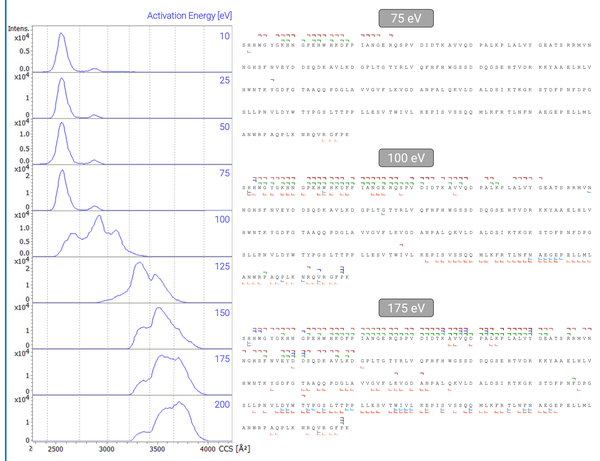

Fig. 2 CIU-TIMS-ECD experiments performed with native carbonic anhydrase charge state 11+ and ECD sequence maps for different activation levels providing a unique unfolding fingerprint for this protein.

Targeted middle-down LC-MS/MS

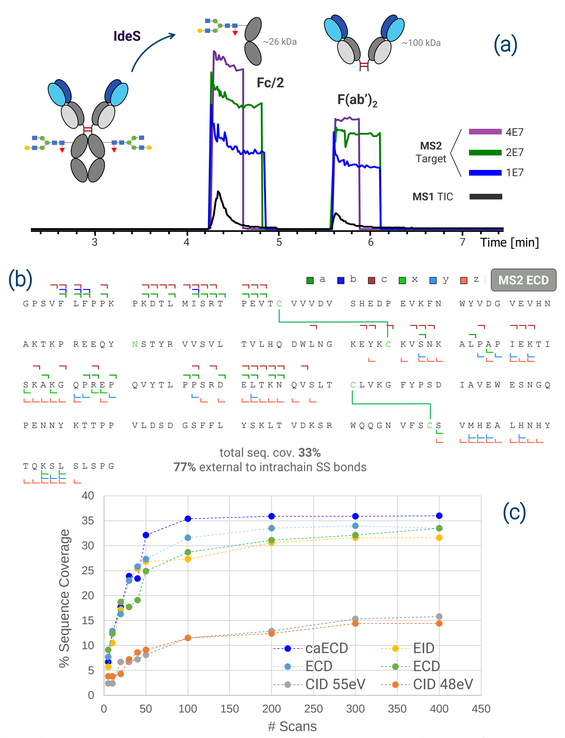

Fig. 3 Dynamic precursor accumulation actively regulates ion flow in MS/MS scans. (a) LC separation of Adalimumab subunits with the MS2 signal controlled dynamically, (b) Fc/2 sequence map for a 2e7 target, and (c) sequence coverage *vs* number of scans for Fc/2 in offline experiments comparing different dissociation methods (~300ms/scan).

Glycopeptide characterization with eXd

LC-MS analysis of a transferrin digest was performed, and O-/N-glycopeptides were targeted for fragmentation. Fig. 4 shows the LC trace (100ng/µL, 0.2µL) and traces corresponding to selected N-glycopeptides accumulated in the Omnitrap and subjected to eXd. Fig. 5 shows the annotated glycopeptide ECD and EID mass spectra.

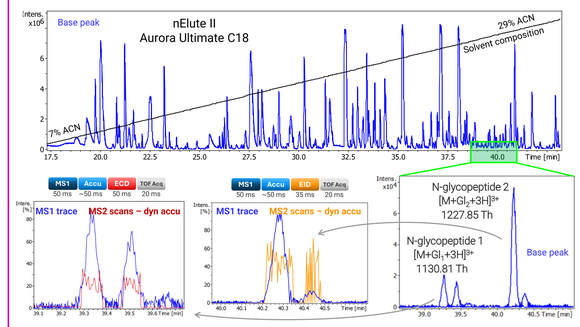

Fig. 4 LCMS analysis of transferrin digest and details highlighting the selection of the two low abundance N-glycopeptides and the corresponding MS1 and MS2 traces.

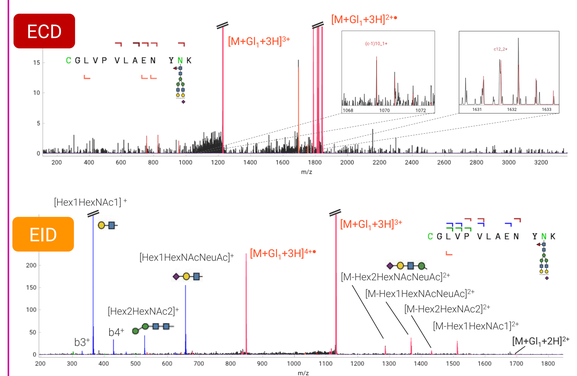

Fig. 5 (a) ECD of C(Carbamidomethyl)GLVPVLAENYN(HexNAc4Hex5NeuAc)K highlighting the c_{10}^+ and c_{12}^{2+} fragments verifying the position of the glycan and (b) the corresponding EID mass spectrum with abundant fragments related to the glycan.

Conclusions

- The timsOmni combines TIMS and omnitrap MS^n eXd technologies for structural characterization of biomolecules.
- Dynamic accumulation of precursor ions in MS/MS scans provides high s/n mass spectra for proteins and peptides.

New Instrumentation