

Protein Characterization with CIU and MSⁿ eXd on the timsOmni platform

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Introduction

Recent innovations in mass spectrometry have expanded the capabilities available for protein characterization. Here we demonstrate the analytical flexibility provided in the new timsOmni™ platform, which combines trapped ion mobility spectrometry (TIMS) with the Omnitrap® multidimensional MSⁿ ion processor.

Instrumentation and Methods

The new timsOmni platform incorporates a stacked-ring RF ion guide which enables desolvation, Collision Induced Unfolding (CIU) and in-source Collision Induced Dissociation (isCID) upstream of a TIMS analyzer and Omnitrap MSⁿ ion processor with electron activated dissociation (eXd) capabilities (Fig. 1).

For CIU-TIMS-ECD experiments, 5 μM carbonic anhydrase (Merck) was dissolved in 150 mM ammonium acetate, infused at 3 μL/min, activated by 10 – 200 eV for CIU and fragmented by Electron Capture Dissociation (ECD) (Fig. 2).

For isCID-MSⁿ, intact, non-reduced NIST mAb (Merck) was diluted to 3.3 μM in 50 % acetonitrile/50 % water with 0.1 % formic acid, loaded into coated, open type offline emitters (Humanix Cellomics) and infused using the new NEOS source (Bruker). isCID was applied to release

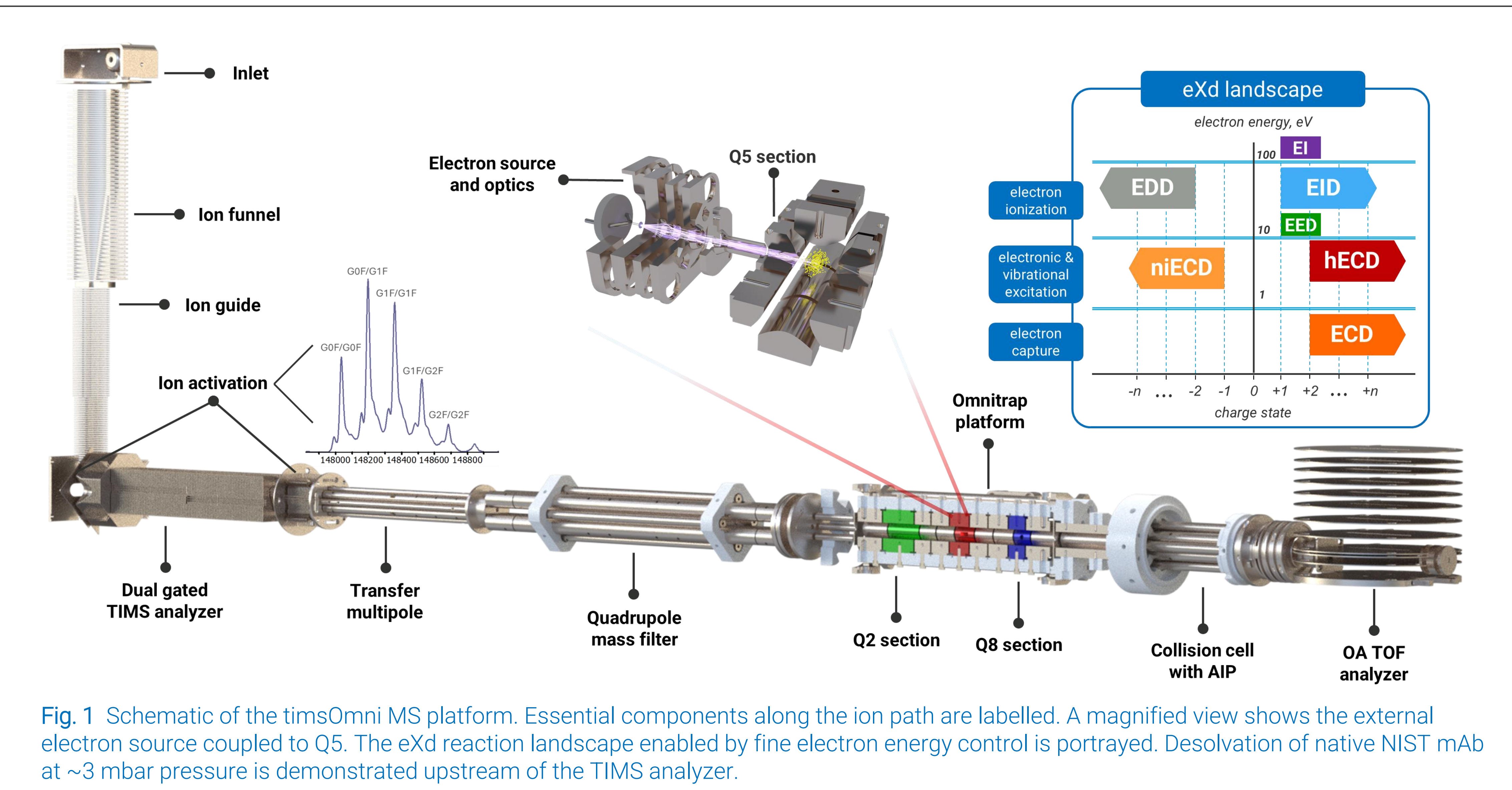


Fig. 1 Schematic of the timsOmni MS platform. Essential components along the ion path are labelled. A magnified view shows the external electron source coupled to Q5. The eXd reaction landscape enabled by fine electron energy control is portrayed. Desolvation of native NIST mAb at ~3 mbar pressure is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

light chain (LC) subunits and N-terminal LC-b117 and HC-b109 fragments, which were isolated in the quadrupole prior to MSⁿ fragmentation (Fig. 3).

All spectra were processed using OmniScope™ software (Bruker).

Results and Discussion

CIU of native carbonic anhydrase charge state 11+ revealed 6 distinct Collisional Cross Section (CCS) profiles (Fig. 2a), which were subsequently fragmented by ECD. ECD is a soft fragmentation technique and can be used as a probe for protein structure in the gas phase. Combining both techniques allows monitoring the dynamics of protein unfolding, in this case conformational changes by CIU correlated with the observation of regions of structural flexibility detected by ECD (Fig. 2b).

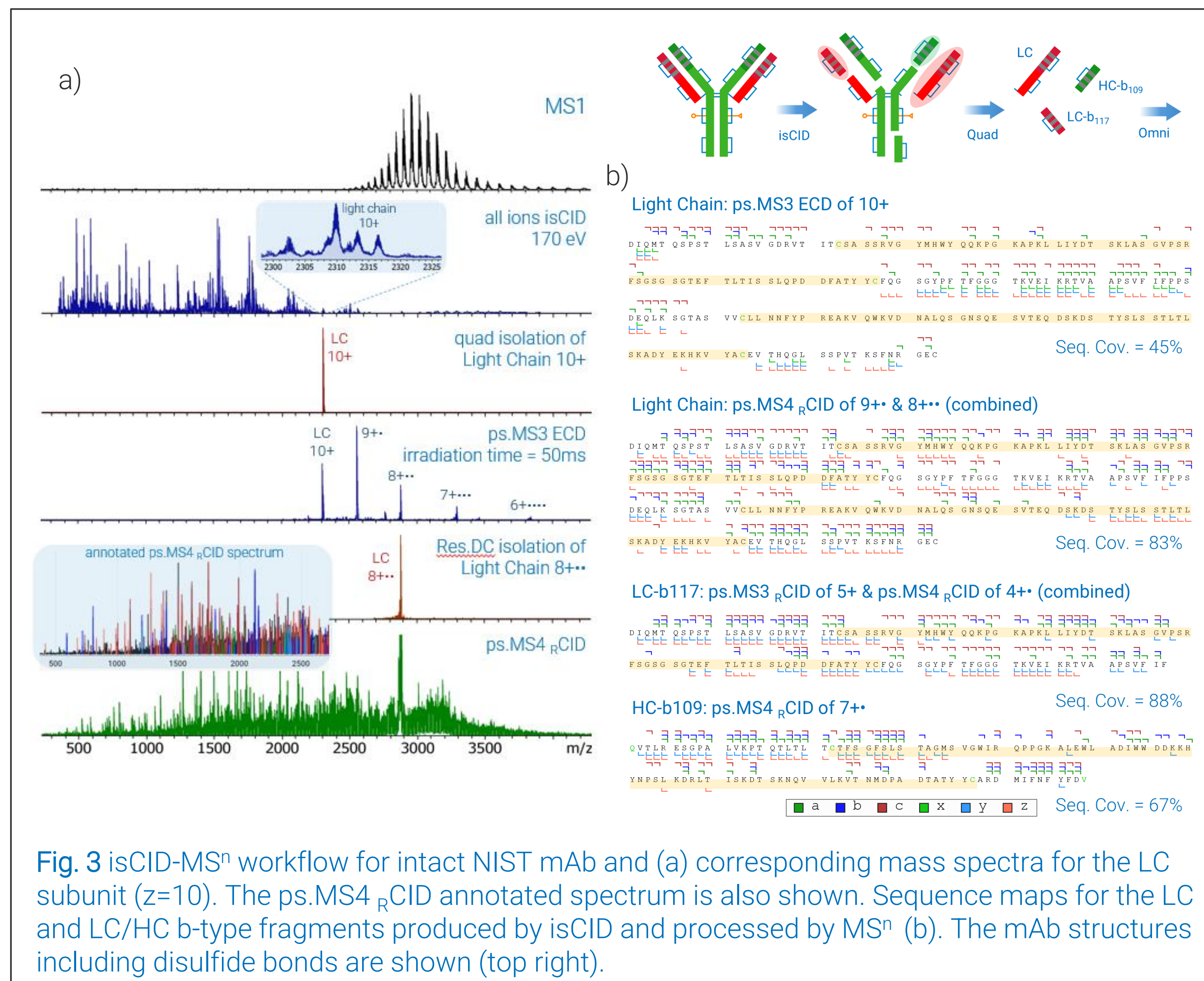


Fig. 3 isCID-MSⁿ workflow for intact NIST mAb and (a) corresponding mass spectra for the LC subunit (z=10). The ps.MS4_rCID annotated spectrum is also shown. Sequence maps for the LC and LC/HC b-type fragments produced by isCID and processed by MSⁿ (b). The mAb structures including disulfide bonds are shown (top right).

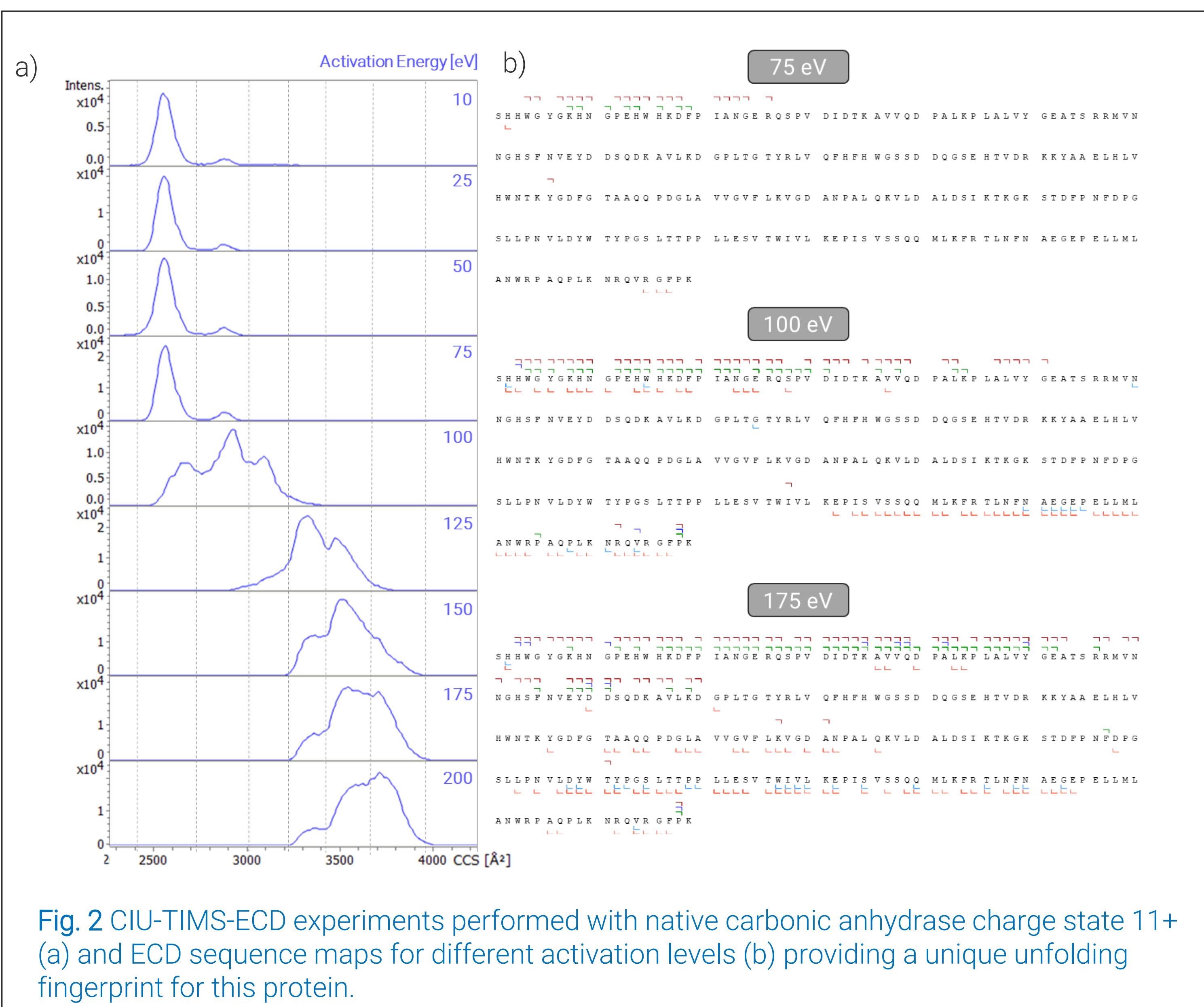


Fig. 2 CIU-TIMS-ECD experiments performed with native carbonic anhydrase charge state 11+ (a) and ECD sequence maps for different activation levels (b) providing a unique unfolding fingerprint for this protein.

The isCID-MSⁿ workflow is very flexible and can combine any of the CID and eXd fragmentation techniques that are offered in the timsOmni platform (Fig. 1). Fig. 3a shows a series of mass spectra in each step of the isCID-MSⁿ workflow as applied to intact NIST mAb. MS2 CID produces abundant b-type fragments, and in this example the LC subunit (10⁺) was produced by isCID and subsequently mass selected and subjected to MS3 ECD, followed by MS4_rCID. Pseudo MS3 (ps.MS3) ECD of the LC charge state 10⁺ gives near complete sequence coverage in regions external to the intrachain SS bonds (Fig. 3b). Additional collisional activation by ps.MS4_rCID of the charge-reduced LC ions (ECnD products) shows complete reduction of the first intrachain bond and partial reduction of the second, resulting in 83% sequence coverage. Interestingly, the reduction of the first intrachain bond in the LC subunit appears to be more efficient compared to the reduction observed for the b117 LC

fragment ions when processed by ps.MS3 ECD and ps.MS4_rCID. The same workflow is applied to the b109 HC fragment where only partial reduction of the first intrachain SS bond is observed.

Conclusions

- The timsOmni combines TIMS and omnitrap MSⁿ eXd technologies
- CIU and ECD monitor the dynamics of protein unfolding
- isCID-MSⁿ workflow enables deep sequencing of biomolecules
- Flexible MSⁿ platform combining CIU with CID and eXd to provide comprehensive fragmentation toolkit

Protein Characterization