

Offline tandem MSⁿ workflows on the timsOmni platform for deep sequencing of intact proteins and mAbs

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Introduction

• The broader use of top-down mass spectrometry (TDMS) is hindered, in part, by signal dilution into multiple charge states, numerous dissociation pathways and a plethora of isotopic peaks observed per fragment ion, resulting in low signal-to-noise (s/n) mass spectra and poor sequence coverage.

• The diverse multiple-stage activation workflows available in the Omnitrap™ platform show great promise for generating complementary information to enhance sequence coverage, however, their effectiveness in the context of TDMS is dependent upon improving the charge capacity of the overall instrument design, necessary for improving the s/n in TD mass spectra.

• Here we describe offline MSⁿ modes realized on the new timsOmni™ MS instrument comprising a high re-rate orthogonal acceleration time-of-flight (OA-TOF) analyzer capable of accommodating a flux of >5M charges/ms. Exceptional sequence coverage is demonstrated for proteins sprayed offline and through extended averaging across multiple MSⁿ scans to enhance the fidelity of fragment-ion isotopic-envelopes.

Methods

Intact, non-reduced NISTmAb was diluted to 3.3 μM in H₂O:ACN in 50:50 ratio (v/v) with 0.1% formic acid. Electrospray ionization (ESI) was performed using the new NEOS nanoESI source (Bruker). Samples were loaded into coated, open type nanospray tips (Humanix Cellomics) biased at ~1.2kV. Collisional activation was applied for desolvation and in-source collision induced dissociation (isCID) for releasing light chain subunits and N-terminal fragments. Fig. 1 shows the MSⁿ experiments performed in this study. OmniScape™ software was used for data analysis.

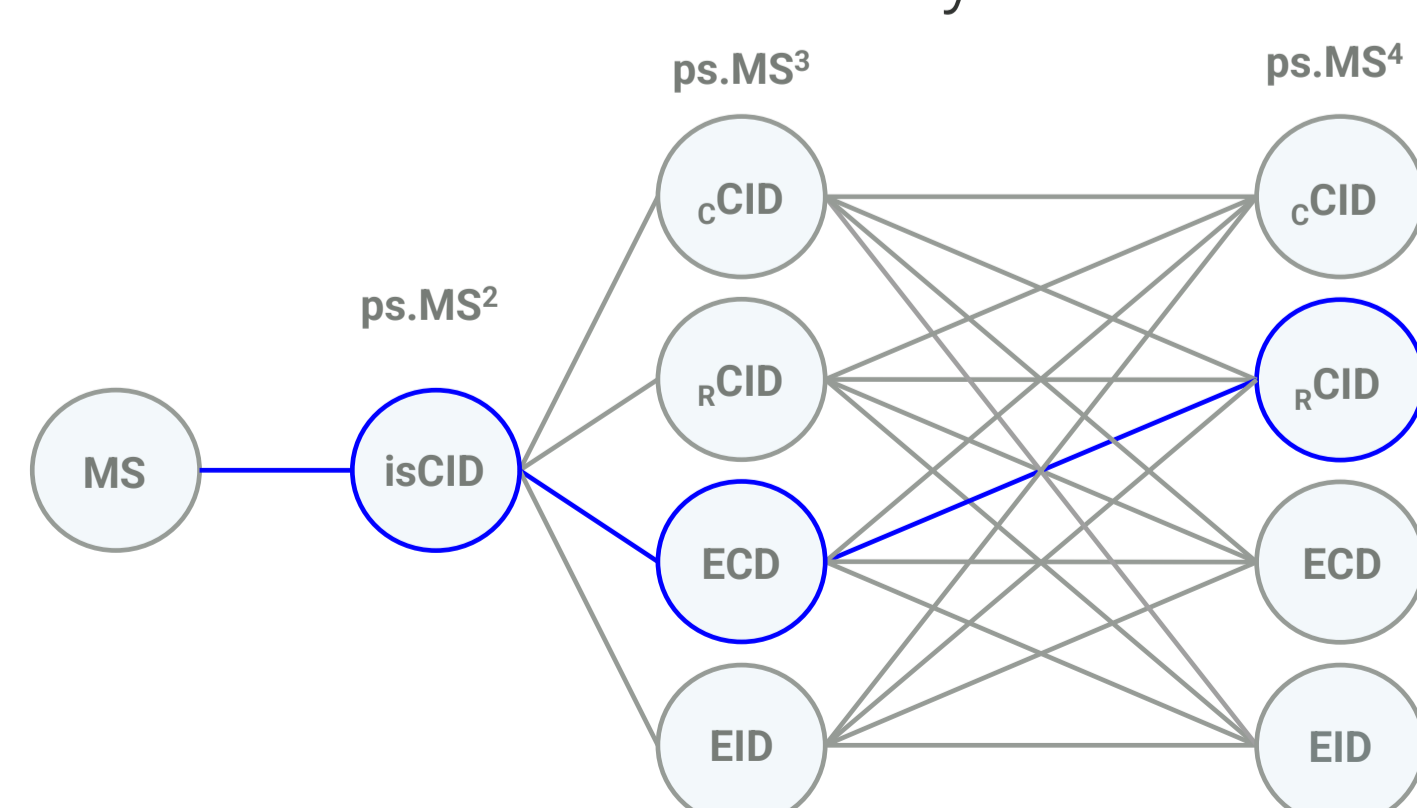


Fig. 1 Ion activation network and the MSⁿ pathway deployed for the analysis of intact non-reduced mAbs including isCID, ECD and rCID.

Results

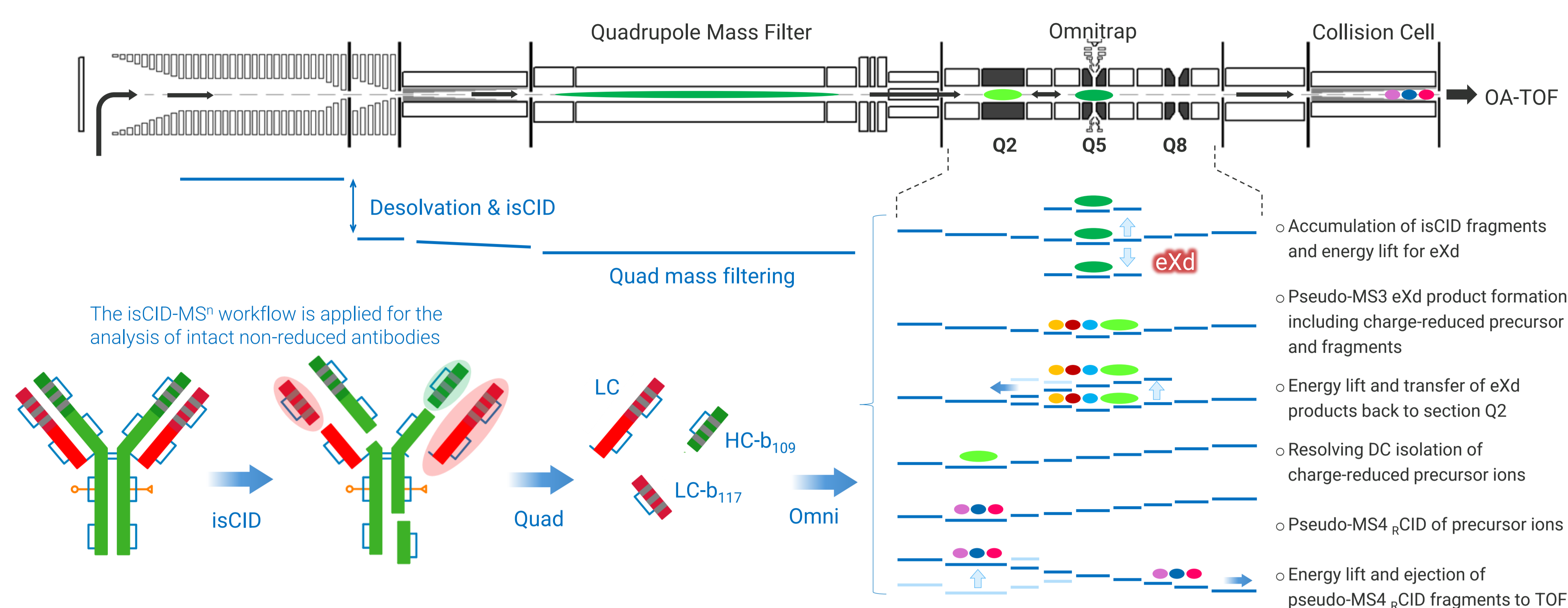


Fig. 3 Instrument schematic highlighting desolvation and isCID at 0.5 mbar pressure inside an ion funnel, followed by quadrupole mass selection of subunits and isCID fragments accumulated in the Omnitrap platform for MSⁿ processing.

Multimodal fragmentation results are shown in Fig. 2. MS2 ECD and EID provide complete sequence coverage across CDR3 of the LC and HC subunits via the formation of c- and a-type fragments. Reaction times are adjusted to 50 ms in both methods and the accumulation time for selected isCID product ions is set to ~1s. ECD and EID also provide partial reduction of the first intrachain disulfide bond of the LC and in the Fc/2 part of the HC. MS2 rCID produces abundant b-type fragments.

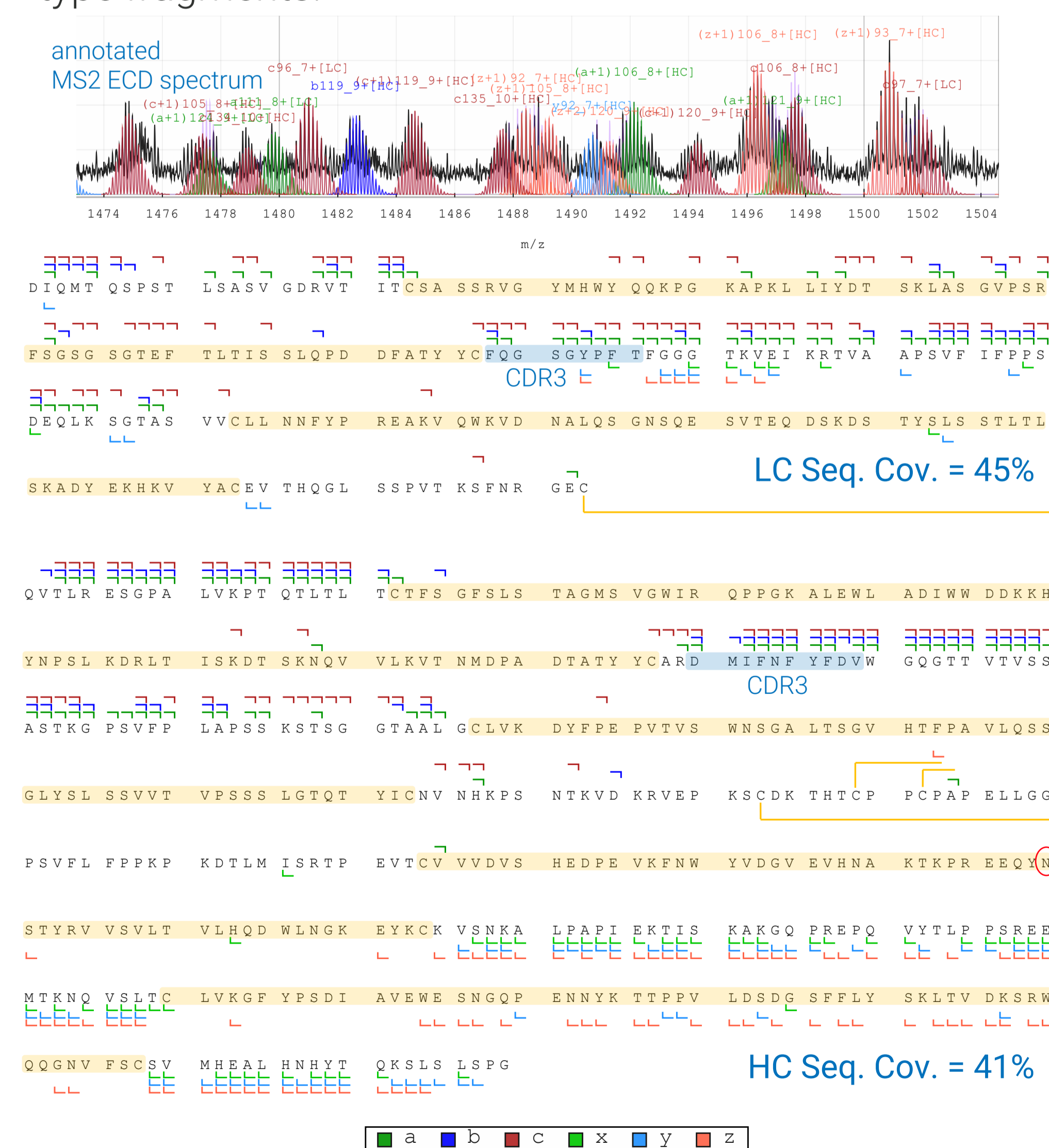


Fig. 2 Combined sequence maps of LC and HC using rCID, ECD and EID data generated from 49+, 52+, and 55+ ions.

MS2 CID produces abundant b-type fragments, which are subsequently mass selected and subjected to MS3 ECD, followed by MS4 rCID. Fig. 4 shows a series of mass spectra in each step of the isCID-MSⁿ workflow.

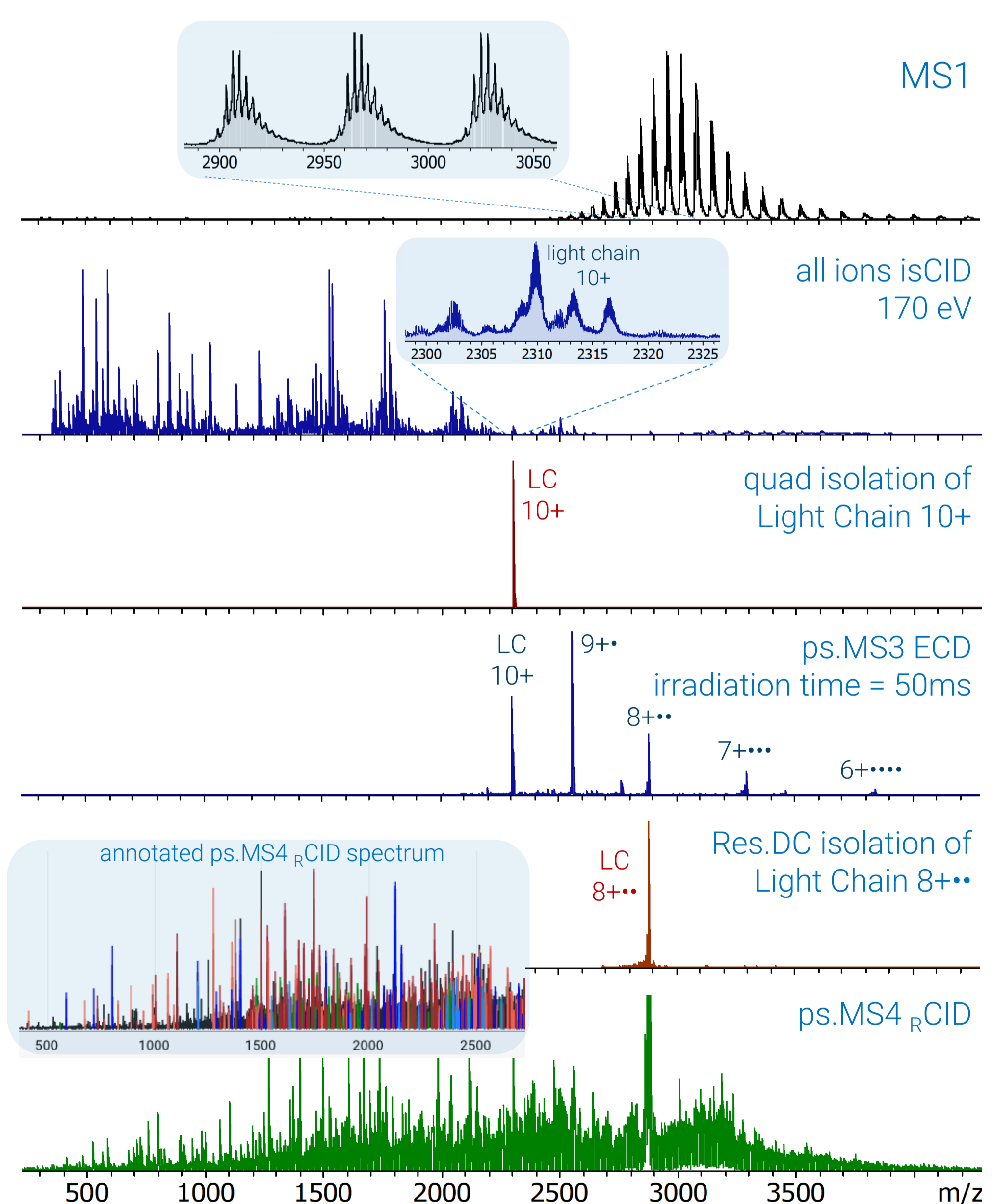
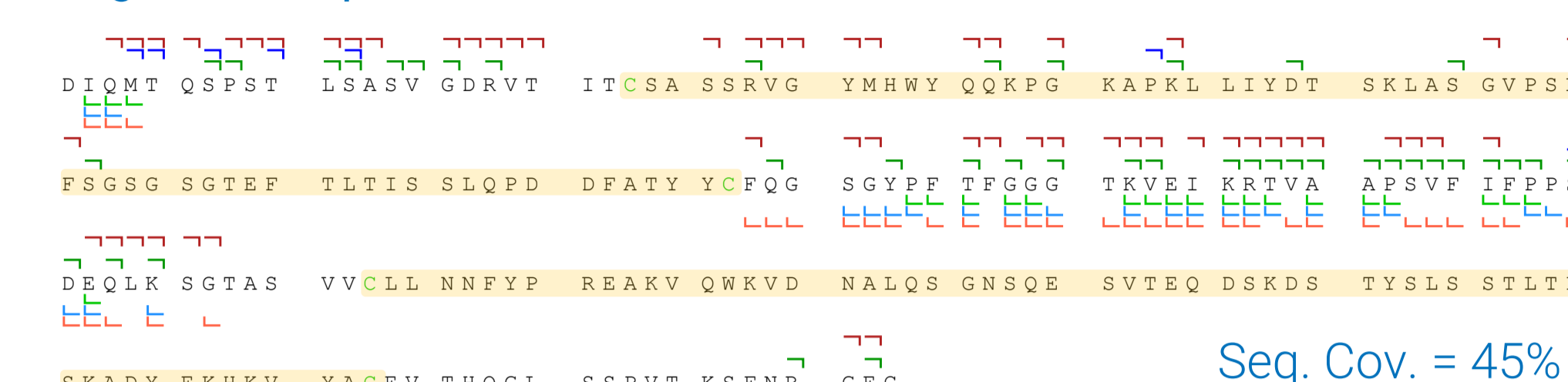
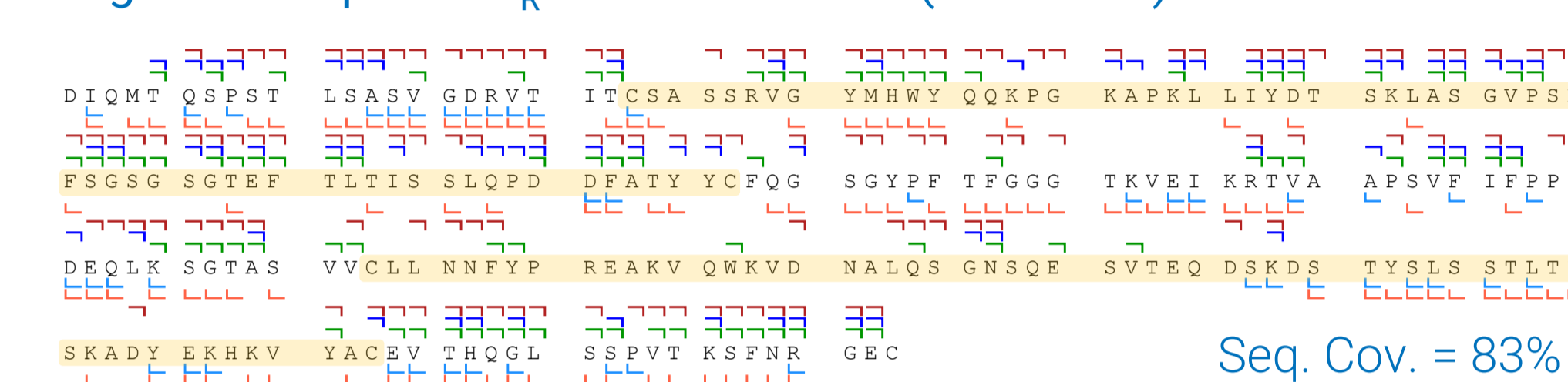


Fig. 4 isCID-MSⁿ workflow for intact NISTmAb and corresponding mass spectra for the LC subunit (z=10), as described in Fig. 3. The ps.MS4 rCID annotated spectrum is also shown (data analysis using OmniScape™).

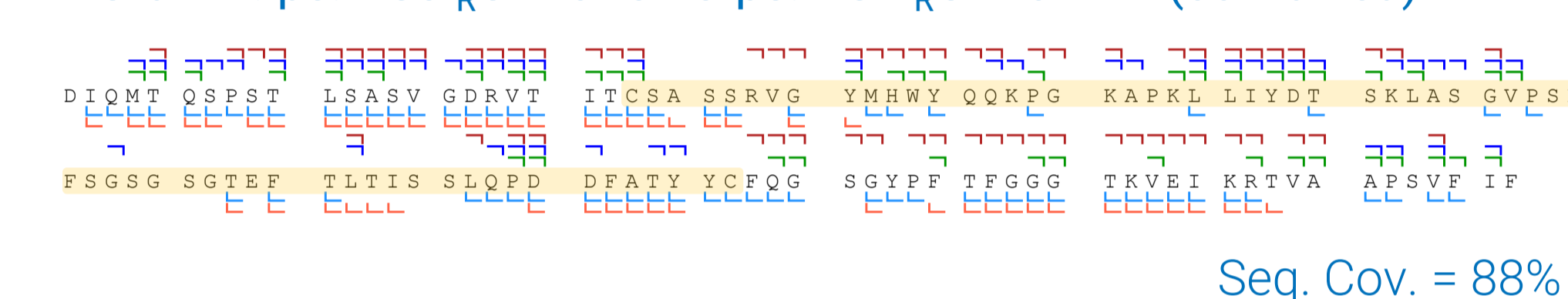
Light Chain: ps.MS3 ECD of 10+



Light Chain: ps.MS4 rCID of 9+• & 8+•• (combined)



LC-b117: ps.MS3 rCID of 5+• & ps.MS4 rCID of 4+•• (combined)



HC-b109: ps.MS4 rCID of 7+••

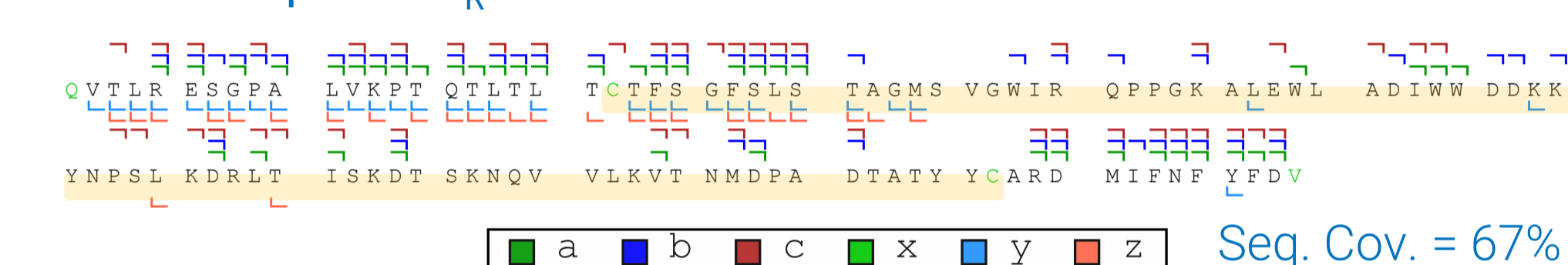


Fig. 5 Sequence maps for the LC and LC/HC b-type fragments produced by isCID and processed by MSⁿ.

ps.MS3 ECD of the LC charge state 10+ gives near complete sequence coverage in regions externally to the intrachain SS bonds. Additional collisional activation by ps.MS4 rCID of the charge-reduced LC ions (ECNoD products) shows complete reduction of the first intrachain bond and partial reduction of the second, resulting in 83% sequence coverage. Surprisingly, the reduction of the first intrachain SS 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

- ▶ Offline isCID-MSⁿ workflows are implemented in the new timsOmni platform.
- ▶ Deep sequencing is demonstrated for non-reduced denatured intact mAbs.
- ▶ Reduction of intrachain disulfide bonds is accomplished by ps.MS4 rCID of charge-reduced ECNoD product ions, while MS3 ECD can be used for disulfide mapping.

TDMS of intact mAbs