Multidimensional Multiple-Stage Top-Down Analysis of Intact non-reduced Antibodies in the Omnitrap Platform coupled to Orbitrap Mass Spectrometry

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INTRODUCTION

- Multidimensional multiple-stage top-down experiments are performed in the Omnitrap™ platform for in-depth characterization of intact non-reduced monoclonal antibodies (mAbs).
- Intact mAbs are activated and dissociated in MSn workflows involving resonance excitation in argon pulsed gas and via reactions with externally injected near-zero energy electrons.
- High quality information-rich top-down mass spectra are generated in MS3 ECD and MS4 CID experiments enabled by operating the Omnitrap platform in ion-accumulation mode.
- Dissociation of intramolecular disulfide bonds and superior sequence coverage are observed in MS4 CID experiments of radical ions.

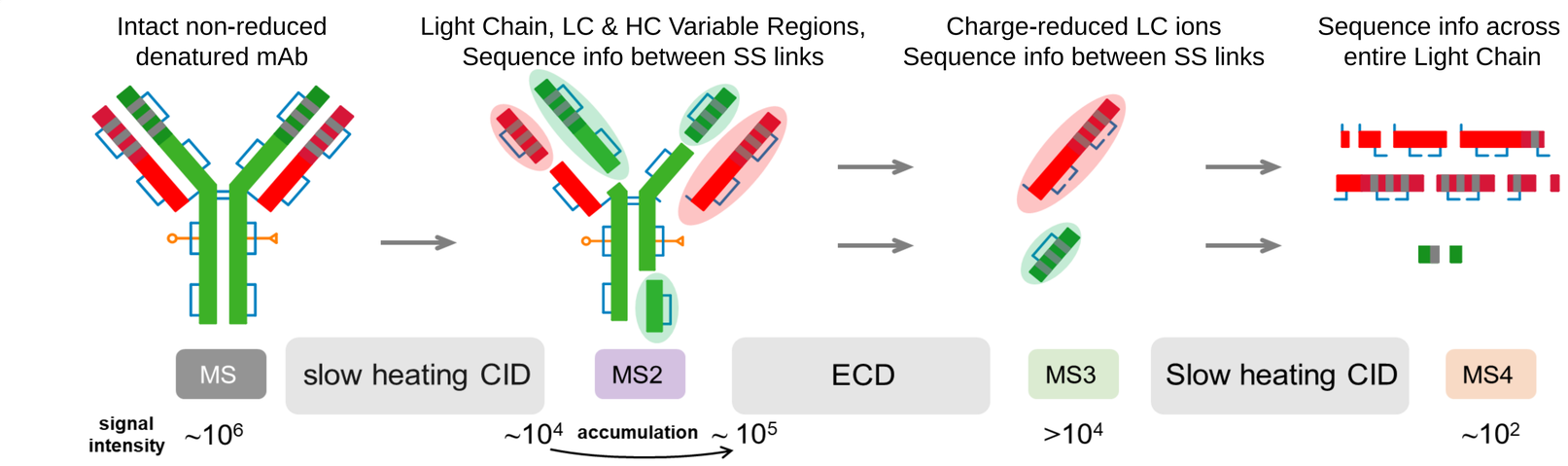

Figure 1: *Dissociation pathways of intact mAbs in a multiple-stage MS4 experimental workflow involving collisional activation and electron capture reactions.*

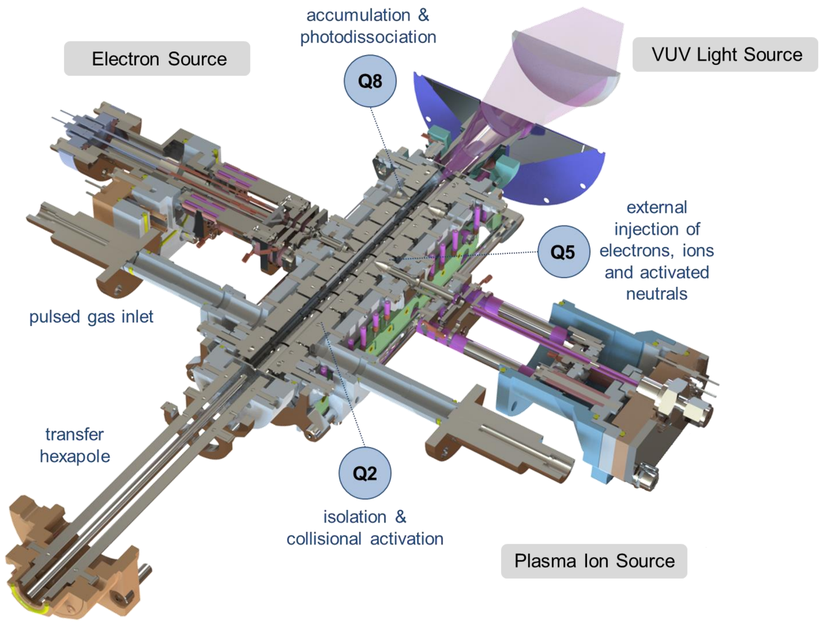

Figure 2: *The Omnitrap platform is a segmented RF linear ion trap enabling multidimensional multiple-stage tandem mass spectrometry.*

INSTRUMENTATION & METHODS

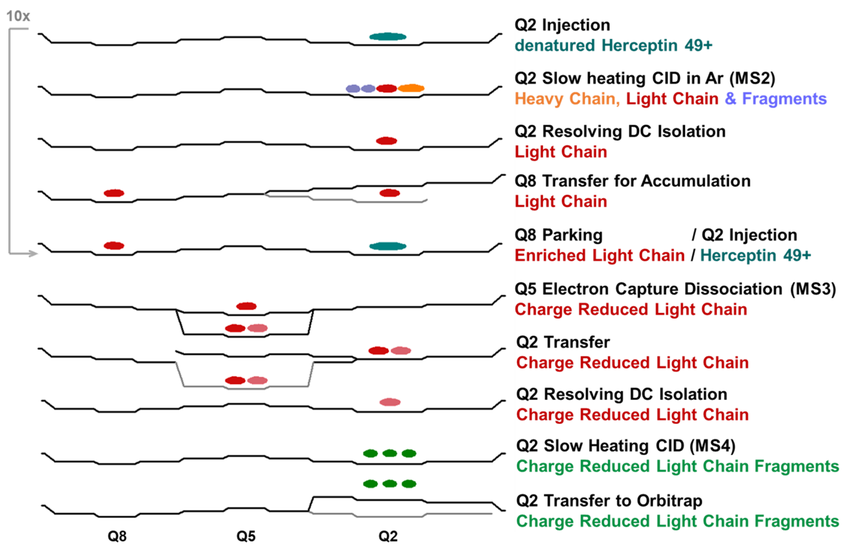

Figure 3: *Ion processing steps in an MS4 top-down workflow for the analysis of intact mAbs involving slow-Heating CID of charge reduced ECD precursor ions.*

The Omnitrap platform is coupled to a Q Exactive™ Plus instrument upgraded with the BioPharma option. All glycosylated forms of Herceptin® (charge state 49+) were isolated in the quadrupole mass filter of the Q Exactive and subjected to a two-step collisional activation process in segment Q2 of the Omnitrap platform involving slow-heating CID of the precursor, followed by broadband-excitation CID of all isotopically unresolved, high-mass dissociation product ions.

Light chain (LC) ions were isolated in Q2 and transferred in segment Q8 for storage. The accumulated LC ions were subsequently transferred in segment Q5 for reacting with low energy electrons. Charge-reduced ECD precursor ions and low intensity fragments were formed within 100 ms. The charge reduced LC ions were finally isolated in Q2 and subjected to MS4 CID. Mass analysis was performed with the Orbitrap™ mass analyzer.

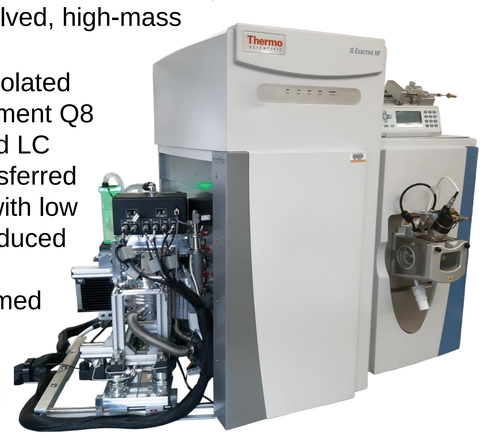

Figure 4: *The Omnitrap platform coupled to a Q Exactive Orbitrap Mass Spectrometer.*

MS2 CID & Broadband Excitation of intact mAb

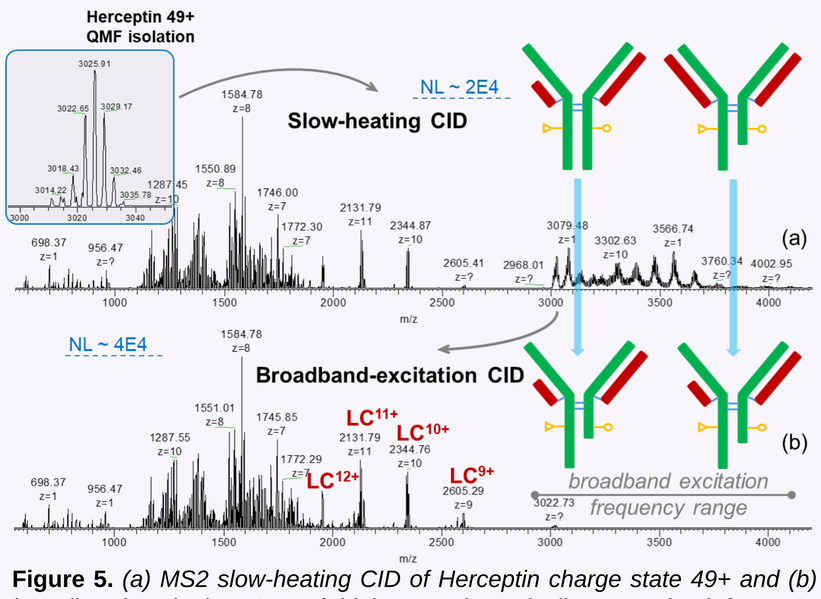

Figure 5. *(a) MS2 slow-heating CID of Herceptin charge state 49+ and (b) broadband-excitation CID of high-mass isotopically unresolved fragment ions*

Figure 6. *Sequence coverage map of the 49+ charge state of intact Herceptin generated in PeakFinder and corresponding to the mass spectrum of Figure 5(b).*

Second generation fragments are produced in the second step of the MS2 CID experiment by collisional activation of the isotopically-unresolved higher m/z fragments by the application of a broadband excitation signal (Figure 5). The second-generation fragments enhance the intensity of the first-generation species by a factor of 2x, while no new fragments are generated in the process. Dissociation of the intermolecular bond connecting the light and heavy chains produces an heterogeneous population of fragment ions with variations on the cysteine side chains (Figure 6).

Ion Accumulation & MS3 ECD of the Light Chain

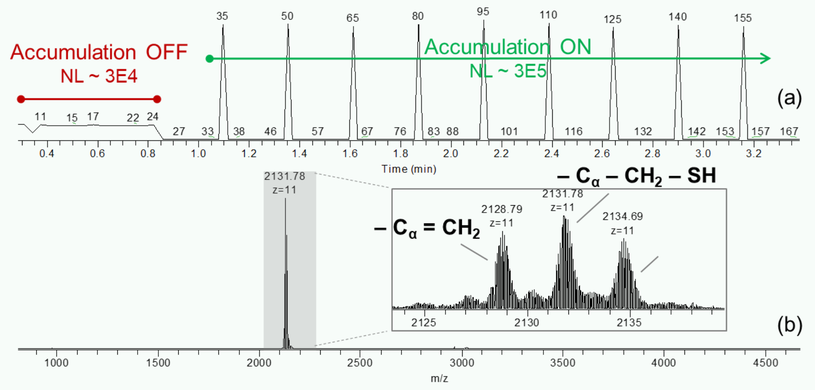

Figure 7. (a) *TIC plot showing enrichment of the LC ion population in accumulation mode and (b) the isolation window highlighting variations on the cysteine side chain generated upon intermolecular disulfide bond cleavage by CID.*

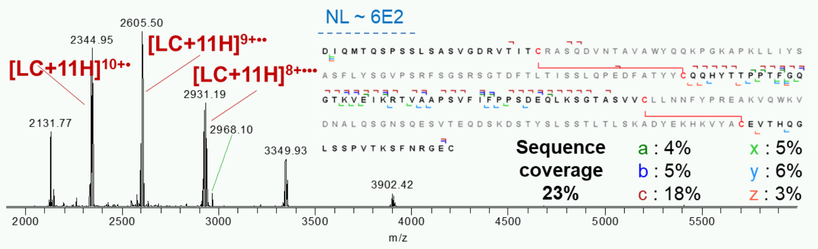

Figure 8. *MS3 ECD mass spectrum showing charge-reduced LC ions serving as the precursor species in the MS4 step. Also shown is the sequence coverage obtained by extending ECD reaction time to enhance dissociation of the LC.*

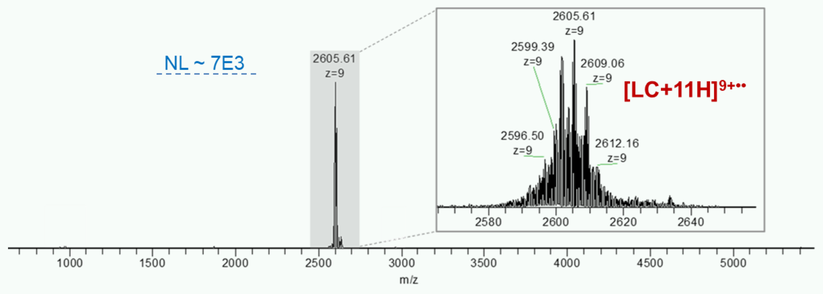

Figure 9. *Mass spectrum showing the isolated charge-reduced LC ions formed by electron capture at MS3 level.*

MS4 CID of the charged reduced LC ions

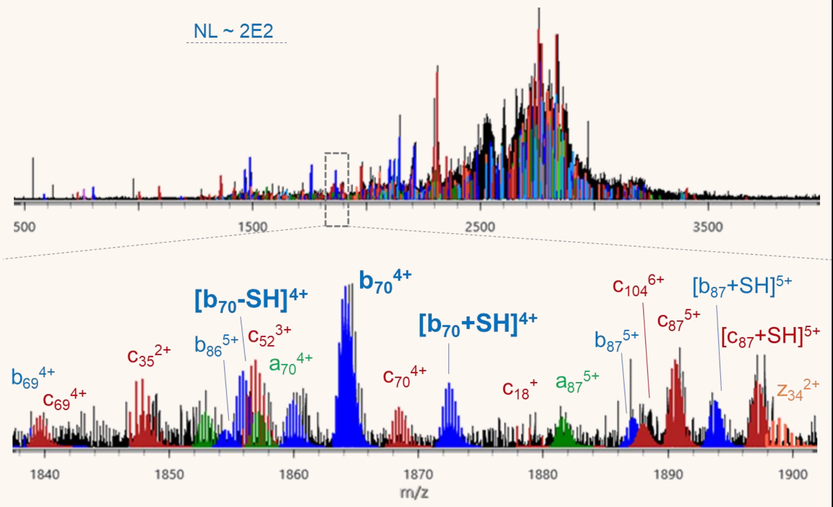

Figure 10. *Annotated MS4 CID mass spectrum highlighting the triplet fragment ion configuration arising from the dissociation of the first intramolecular SS bond.*

The spectrum complexity is attributed to variations in the cleavage position of the intramolecular SS linkages, as observed for example for b and c fragments (Figure 10). The variability observed in the cysteine side chain for C-terminal fragments may arise from the reduction of either the intra- or inter-molecular SS linkages. The MS4 workflow presented here for the LC charge state 9+•• ions was extended to other charge states of the LC (10+•) as well as to the heavy chain (HC) fragment $b_{110}^{6+\bullet}$. The corresponding sequence maps demonstrating enhanced coverage are shown in Figures 11 and 12, respectively.

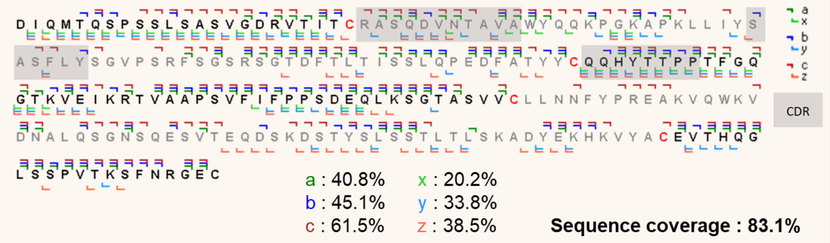

Figure 11. *Sequence map produced by MS4 CID and combining information from selected charge states 10+• and 9+•• of the LC.*

FIGURE 12. *HC fragment b110+• sequence map produced by MS4 CID.*

CONCLUSIONS & OUTLOOK

- The first top down MS4 experiment performed in the Omnitrap platform for the analysis of intact mAbs shows great promise for achieving complete sequence coverage.
- The ion accumulation functionality is a powerful method for increasing the depth of analysis in top-down MS. The ion concentration in these experiments ranges across five orders of magnitude.
- Novel multi-dimensional multiple-stage workflows incorporating the entire fragmentation toolbox are currently being explored to improve results.

ACKNOWLEDGEMENTS

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