Top-down Analysis of Intact Antibodies under Denatured and Native Conditions on the Omnitrap Platform Coupled to an Orbitrap Mass Spectrometer

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INTRODUCTION

The Omnitrap was coupled to a Thermo Scientific™ Q Exactive™ Plus instrument with Biopharma option. The analysis of intact monoclonal antibodies was performed using a combination of fragmentation techniques.

INSTRUMENTION & METHODS

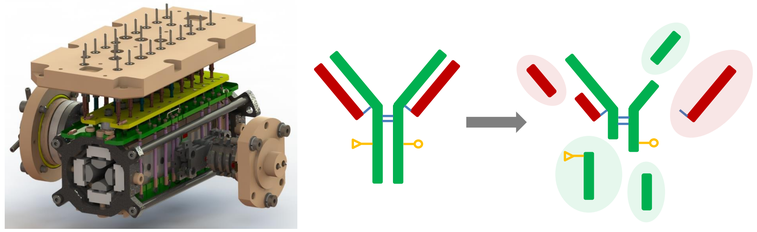

FIGURE 1. The omnitrap platform and mAb fragmentation pattern.

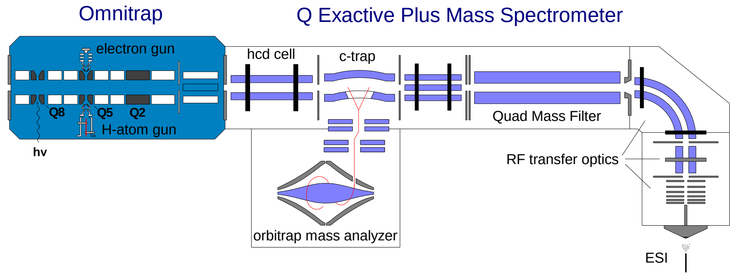

FIGURE 2. Schematic diagram of a Q Exactive Plus mass spectrometer equipped with the omnitrap platform.

Herceptin® (Genentech) was buffer exchanged into 100mM ammonium acetate and diluted to a working concentration of 5uM. For denatured analysis, buffer exchanged Herceptin was diluted to 5uM in water: acetonitrile in 50:50 ratio (v/v) with 0.1% formic acid. Static nanoelectrospray experiments were performed using pulled borosilicate glass coated emitters.

RESULTS

Slow heating CID with additional broadband excitation of the higher m/z first generation fragments, ECD and collisionally activated ECD of charge state 49+ precursor ions were performed and the data analyzed.

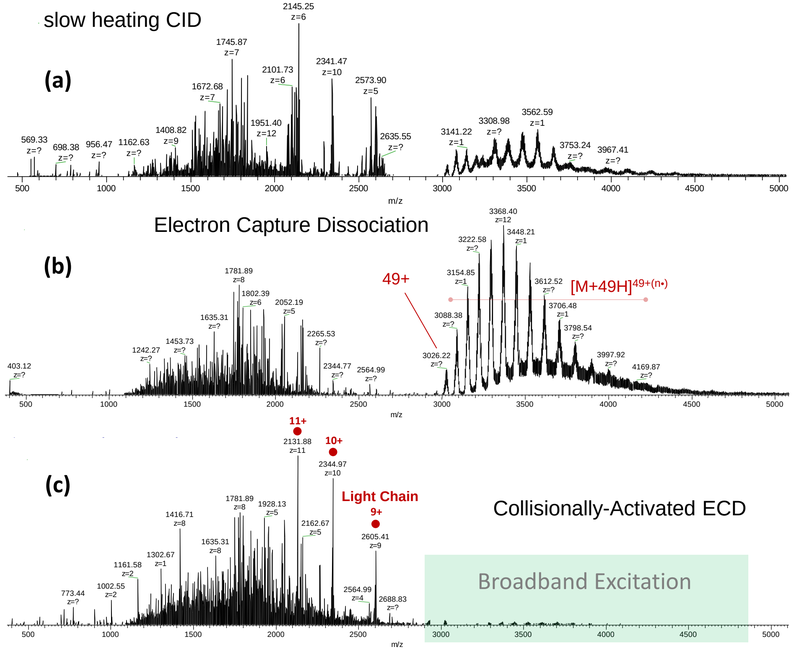

FIGURE 3. MS2 and Pseudo MS3 spectra of denatured 49+ charge state.

The signal-to-noise ratio of isotopically resolved fragment ions in MS2 CID was enhanced by broadband excitation of the higher-m/z first-generation fragments. A new series of fragment ions was produced by collisional activation of ECD charged-reduced radical species, increasing the number of b and c N-terminal fragments and enhancing sequence coverage of the ECD experiment further.

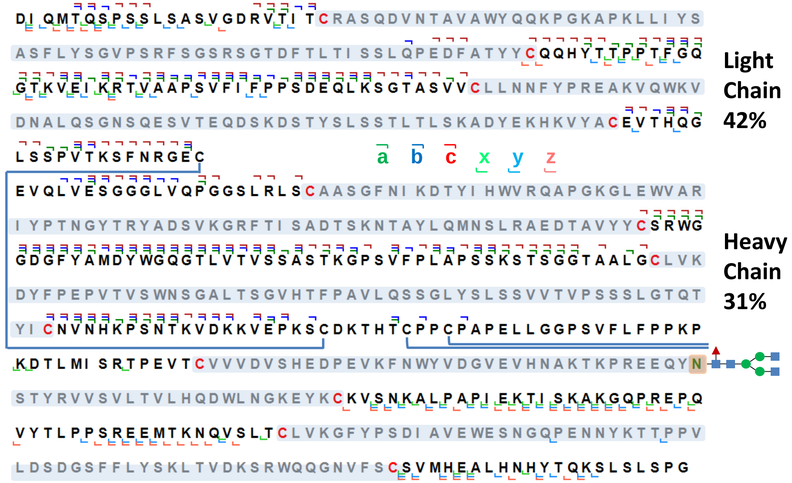

FIGURE 4. Sequence coverage map (±6ppm) obtained for Herceptin 49+ charge state ions subjected to collisionally-activated ECD.

In MS2 CID experiments the overall assigned signal was increased from 48% to 73% by considering internal fragments. In CA-ECD the overall signal assigned corresponding to primary fragments is 63%. A series of C-terminal fragment ions containing the G0F and G1F modifications have also been identified in both MS2 CID and pseudo MS3 CA-ECD experiments.

CONCLUSIONS

Efficient top-down analysis of a monoclonal antibody is performed successfully in the omnitrap platform. Spectra were processed manually increasing the confidence in the assignments of primary and internal fragment ions. Data processing was greatly facilitated by a new software tool “PeakFinder” currently under development by Fasmatech.