Native Top-Down Analysis of Intact Antibodies using Multiple Dissociation Techniques on a Tribrid Quadrupole Orbitrap Linear Ion Trap Mass Spectrometer

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ABSTRACT

Purpose: Evaluate the performance of the new Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer for native top-down analysis of intact antibodies

Methods: Native top-down analysis using High Mass Range MS^n (HMR^n) operation mode and multiple dissociation methods available on the new Orbitrap Eclipse Tribrid mass spectrometer

Results: Demonstrated excellent performance of the new Orbitrap Eclipse Tribrid mass spectrometer for top-down structural characterization of intact antibodies

INTRODUCTION

Therapeutic monoclonal antibodies (mAbs) have gained considerable importance over the past years due to their use to treat cancer and autoimmune diseases. Mass spectrometry plays a significant role among the analytical tools used for the analysis of therapeutic mAbs, being able to provide valuable information on antibody properties such as intact mass, amino acid sequence, disulfide bridges and post-translational modifications including glycosylation. Usually MS analysis is performed at the peptide level which requires several sample preparation steps prior to analysis, including denaturation, reduction, alkylation, digestion, and release of glycan chains. Here we present a more straightforward, top-down approach which combines multiple dissociation techniques on an Orbitrap Eclipse Tribrid mass spectrometer for the analysis of intact mAbs in native form.

MATERIALS AND METHODS

Sample Preparation

Herceptin® (trastuzumab) IgG mAb (Genentech) was buffer exchanged prior to top-down analysis into 200mM ammonium acetate using Micro Bio-Spin® 6 columns (Bio-Rad). The antibody solution was analyzed at a concentration of 2 ug/ul in either 20mM aqueous ammonium acetate or in a mixture containing acetonitrile:water, 1:1 with 0.1% formic acid.

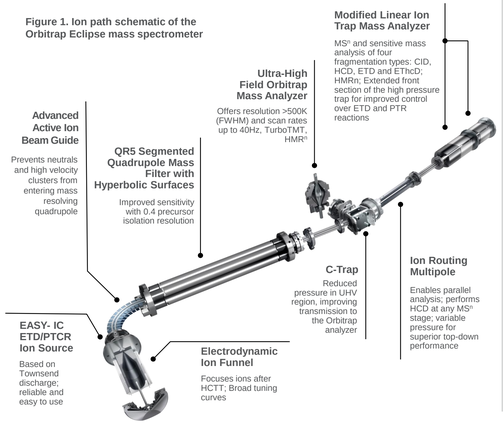
Test Method(s)

Top-down analysis of the intact antibody was carried out on an Orbitrap Eclipse mass spectrometer in HMR^n mode using direct infusion electrospray.

Data Analysis

Data analysis was performed using either Thermo Scientific™ BioPharma Finder™ 3.1 software, or TD Validator, or Xtract and ProSight Lite software.

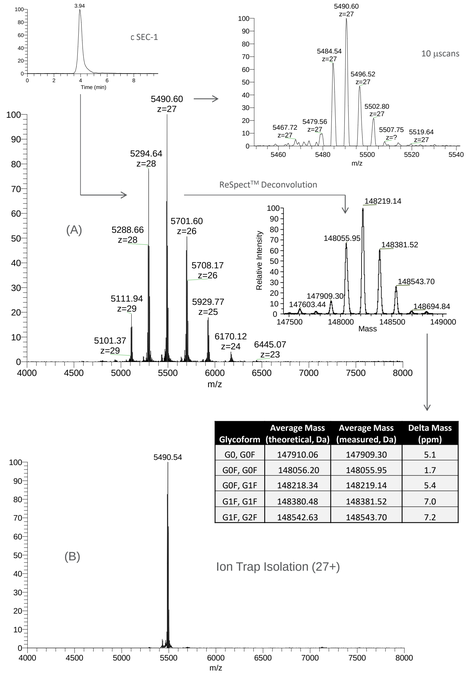
Figure 1. Ion path schematic of the Orbitrap Eclipse mass spectrometer



RESULTS

The instrument used for all experiments was the new Orbitrap Eclipse Tribrid quadrupole Orbitrap linear ion trap mass spectrometer with improved high mass transmission, Orbitrap mass detection up to 8,000 m/z and high mass selection in the linear ion trap up to 8,000 m/z. These improvements are necessary for the analysis of antibody samples on the intact level under native conditions (Figure 2) requiring the transmission and detection of masses beyond the standard Orbitrap mass range of Tribrid instruments of up to 6,000 m/z.

Figure 2. (A) SEC-LC/MS analysis of intact Herceptin antibody under native conditions, (B) Linear ion trap isolation of the intact Herceptin 27+ ion at m/z 5490.6



Glycoform	Average Mass (theoretical, Da)	Average Mass (measured, Da)	Delta Mass (ppm)
G0, G0F	147910.06	147909.30	5.1
G0F, G0F	148056.20	148055.95	1.7
G0F, G1F	148218.34	148219.14	5.4
G1F, G1F	148380.48	148381.52	7.0
G1F, G2F	148542.63	148543.70	7.2

TOP-DOWN ANALYSIS OF INTACT HERCEPTIN IN NATIVE CONDITIONS

Native top-down analysis of intact Herceptin antibody using a combination of HCD, ETD EThcD, and UVPD produced a sequence coverage of 43% (see Figure 4). Examples of tandem mass spectra are shown in Figure 3.

Figure 3. HCD, ETD, EThcD and UVPD spectra of the 27+ charged intact Herceptin

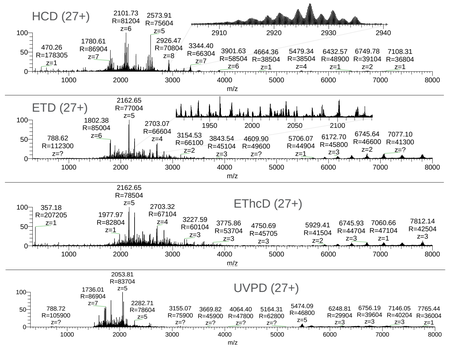
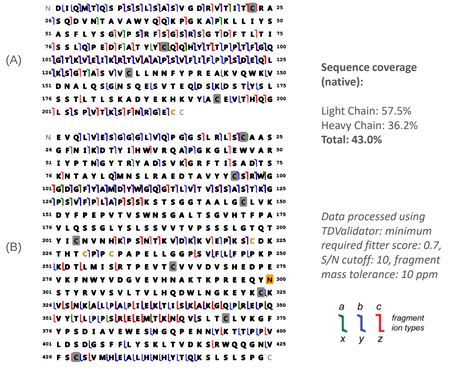


Figure 4. Graphical fragmentation maps for intact Herceptin resulting from the combination of HCD, ETD, EThcD and UVPD matched fragment ions. (A) shows the light chain while (B) the heavy chain.



Sequence coverage (native):

Light Chain: 57.5%
Heavy Chain: 36.2%
Total: 43.0%

Data processed using TDValidator: minimum required fitter score: 0.7, S/N cutoff: 10, fragment mass tolerance: 10 ppm

a b c
x y z fragment ion types

TOP-DOWN ANALYSIS OF INTACT HERCEPTIN IN DENATURING CONDITIONS

Similar top-down experiments were performed under denaturing conditions (see Figure 5). The sequence coverage obtained using the combination of the same fragmentation methods was slightly lower (39.7%, see Figure 7). The Venn diagrams in Figure 6 show the residue cleavages overlap for the light and heavy chains of the Herceptin antibody.

Figure 5. (A) Full MS spectrum of intact Herceptin under denaturing conditions, (B) Isolation in linear ion trap of the 49+ ion, (C-F) HCD, ETD, EThcD, and UVPD spectra of the 49+ charged intact Herceptin

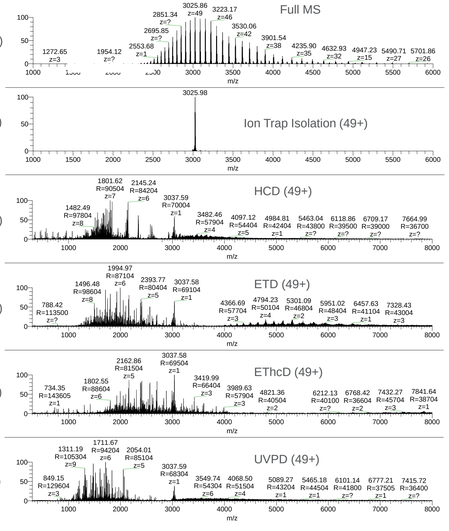


Figure 6. Venn diagrams depicting overlap of residue cleavages in native and denaturing conditions for both, light and heavy chains

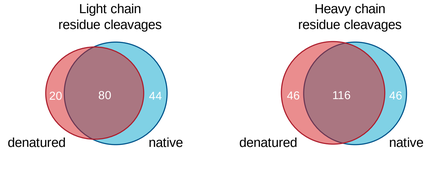
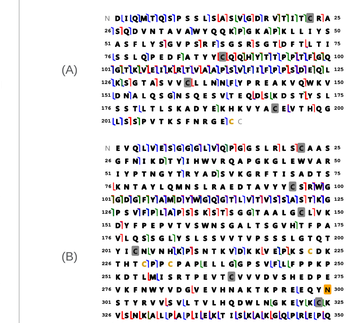


Figure 7. Graphical fragmentation maps for intact Herceptin under denaturing conditions resulting from the combination of HCD, ETD, EThcD and UVPD matched fragment ions. (A) shows the light chain while (B) the heavy chain.



Sequence coverage (denatured):

Light Chain: 47.2%
Heavy Chain: 36.2%
Total: 39.7%

Data processed using TDValidator: minimum required fitter score: 0.7, S/N cutoff: 10, fragment mass tolerance: 10 ppm

By combining the results obtained from top-down analysis of intact Herceptin antibody in both, native and denaturing conditions, the **total sequence coverage** increased to **53.3%**. The **light chain sequence coverage** increased to **67.9%**, the **heavy chain sequence coverage** increased to **46.4%**.

CONCLUSIONS

- In this work we demonstrated outstanding performance of the new Orbitrap Eclipse Tribrid mass spectrometer for native top-down structural characterization of intact antibodies using multiple dissociation methods.

TRADEMARKS/LICENSING

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