Improving Intact Antibody Characterization by Orbitrap Mass Spectrometry

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Introduction
Over the last decade, mass spectrometric techniques have gained significant importance in diagnostic and therapeutic applications over the past years. In order to verify the purity of the isolated molecule, to provide a reproducible, safe, and effective biological drug compound, the correct protein sequence as well as the presence and relative abundance of different glycoforms should be confirmed. We present an approach to analyze an intact monoclonal antibody in a highly sensitive and reproducible condition by using the Orbitrap Elite hybrid mass spectrometer. The intact antibody respectively the separated light and heavy chains were analyzed in full MS experiments as well as with top-down experiments using in-source CID (SID), CID, HCD and ETD fragmentation techniques making use of the high resolution of the mass spectrometer. For data evaluation Prodigy PC 2.0 and Protein Deconvolution 1.0 software packages were used.

Methods
Sample Preparation
Humira® (adalimumab, Figure 2 [1]) The intact antibody (144 kDa) was dissolved in 0.1% FA at 1 mg/ml, 3 µg Humira® were loaded onto the column, eluted with solvent A: 0.1% FA, 2% ACN in H2O, 1258+ (C) and 1258+ (Thermo Fisher Scientific), solvent A: 0.1 % FA, 2 % ACN in H2O, eluted R=124.100 1258+ (C).

Results
The analysis of large proteins of the size of intact antibodies (>150 kDa) is challenging due to their relatively large mass and low sensitivity. This method of choice for intact antibodies is to use the shortest transient duration (4 ms) available on the Orbitrap-Elite (Figure 6).

Conclusion
The identification and relative intensities of intact antibodies on the Orbitrap Elite heavy chain acquired in SIM scan mode (z=43). 60 µscans were averaged. Deconvoluted mass 50,891.04317 Da. The internal on the light, double charge-state resolution of the charge state detected at mz 1185 and masses obtained after deconvolution using Xtract.

Abbreviations
ACN, acetonitrile; CID, collision-induced dissociation; C-Trap, curved trapezoidal trap; DDA, data-dependent acquisition; FA, formic acid; HCD, high energy collisional-induced dissociation; m/z, molecular ion; MS, mass spectrometry; microLC, micro-scale LC; nanoLC, nano-scale LC; SIM, selected ion monitoring.

References

Acknowledgements
We would like to thank Paul Thomas from the North-Western Uni. (USA) for submitting the Humira® ETD data.

Figure captions
FIGURE 1: General structure of mAbs and their biological and chemical characteristics.

FIGURE 2: 3D structure of Humira® highlighting the attached glycans and cysteine residues forming inter- and intra-disulfide bridges.

For analyzing Humira® light chain (24 kDa) and heavy chain (51 kDa) separately, 30 µg Humira® was reduced with DTT (20-fold molar excess, 37°C) and 11-fold cross-linked with viscosilacylation (20-fold molar excess, room temperature for 30 min in the dark).

FIGURE 3: Schematics of the Orbitrap Elite hybrid mass spectrometer equipped with an ETD source.

FIGURE 4: A Surveyor HPLC Purify was coupled to an Orbitrap Elite™ ETD mass spectrometer (all Thermo Fisher Scientific) (Figure 3) [2].

FIGURE 5: General structure of mAbs and their biological and chemical characteristics.

FIGURE 6: Humira® heavy chain acquired in SIM scan mode (m/z=43). 60 µscans were averaged. Deconvoluted mass 50,891.04317 Da. The internal on the light, double charge-state resolution of the charge state detected at m/z 1185 and masses obtained after deconvolution using Xtract.