HIGH RESOLUTION ORBITRAP NATIVE MS FOR MABS AND RELATED PRODUCTS CHARACTERIZATION

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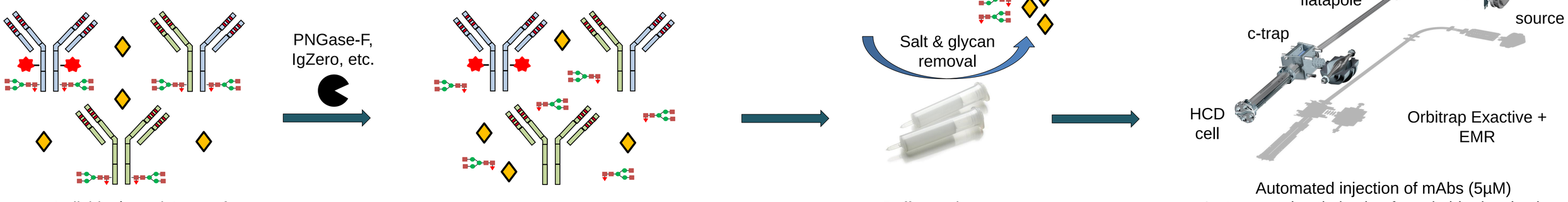
Introduction

- With more than 30 candidates clinically approved since 1985, monoclonal antibodies (mAbs) have become the fastest growing class of biotherapeutic agents.
- High target specificity as effector functions of these immunoglobulins, recently potentialized by drug conjugation, remain tightly bound to their structural features.
- This source of heterogeneity, stemming from both mAbs production (encoding sequences, post-translational modifications) and processing (engineered alterations, storage, administration) is now further enhanced by biosimilars arrival, involving a thorough characterization of these mosaic mixtures.
- In this context, native ESI-MS combined to high resolution appears as a challenging approach to depict an exhaustive snapshot of in-solution species while preserving non covalent complexes. Brought together, these informations allow to concomitantly evidence mAbs heterogeneity and specificity.

Instrumentation : Exactive Plus EMR Orbitrap

- 140,000 resolution (@ m/z 200) for isotopic resolution of small to mid size proteins.
- Unmatched desolvation using in-source CID and HCD cell.
- Extended mass range up to m/z 20,000 for large protein assemblies.
- Mass accuracy (external calibration) : <5ppm RMS for m/z 6,000-10,000.

Native MS on mAbs and related products



Individual or mixtures of mAbs/bAbs/ADCs in storage buffer (4°C)

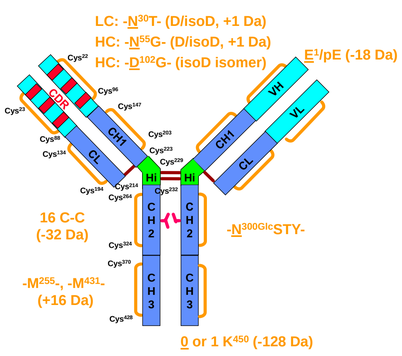
Sample deglycosylation

Buffer exchange by gel filtration ($AcONH_4$ 150 mM, pH7.5)

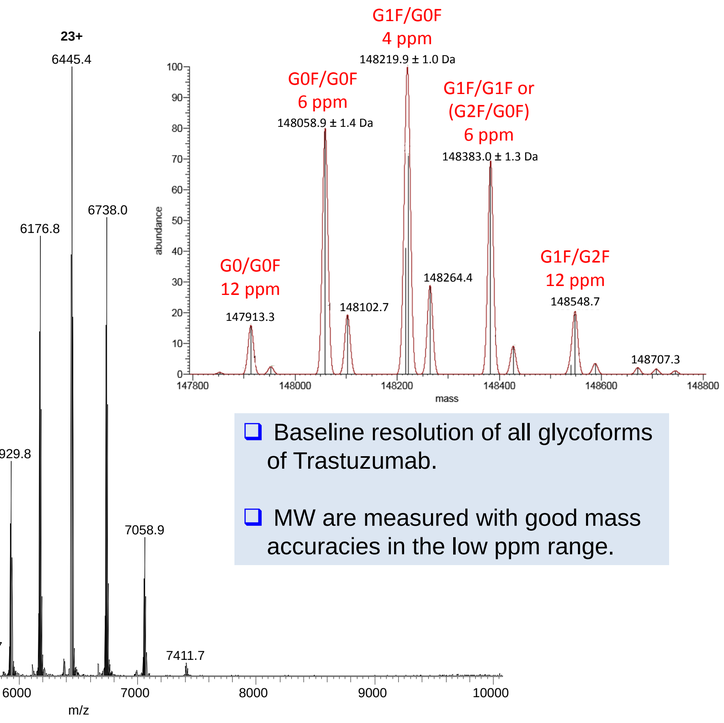
Automated injection of mAbs (5µM)
Instrumental optimisation for suitable desolvation, high m/z transmission and native complex preservation

RESULTS

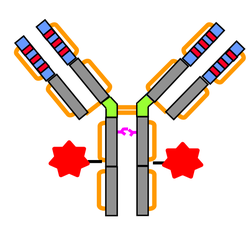
High resolution native MS analysis of the intact monoclonal antibody Trastuzumab



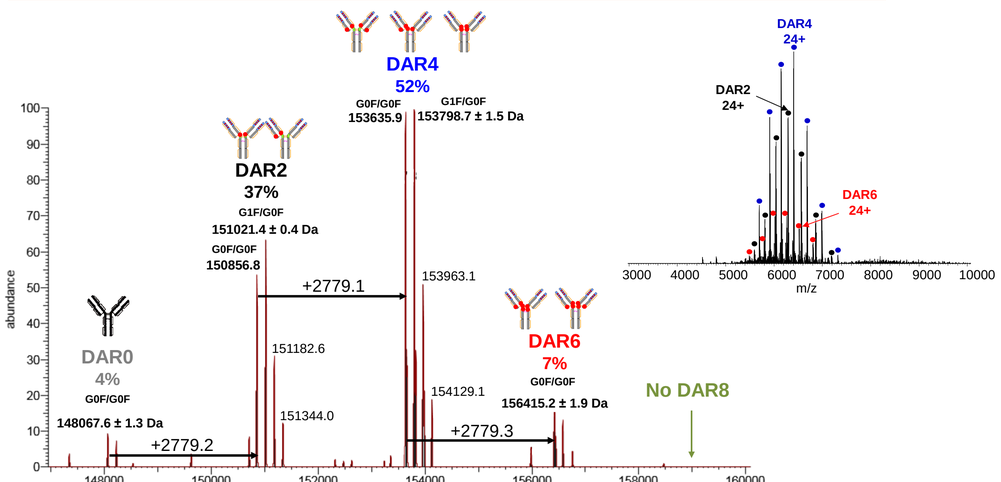
$C_{6560}H_{10132}O_{2090}N_{1728}S_{44}$



- Baseline resolution of all glycoforms of Trastuzumab.
- MW are measured with good mass accuracies in the low ppm range.

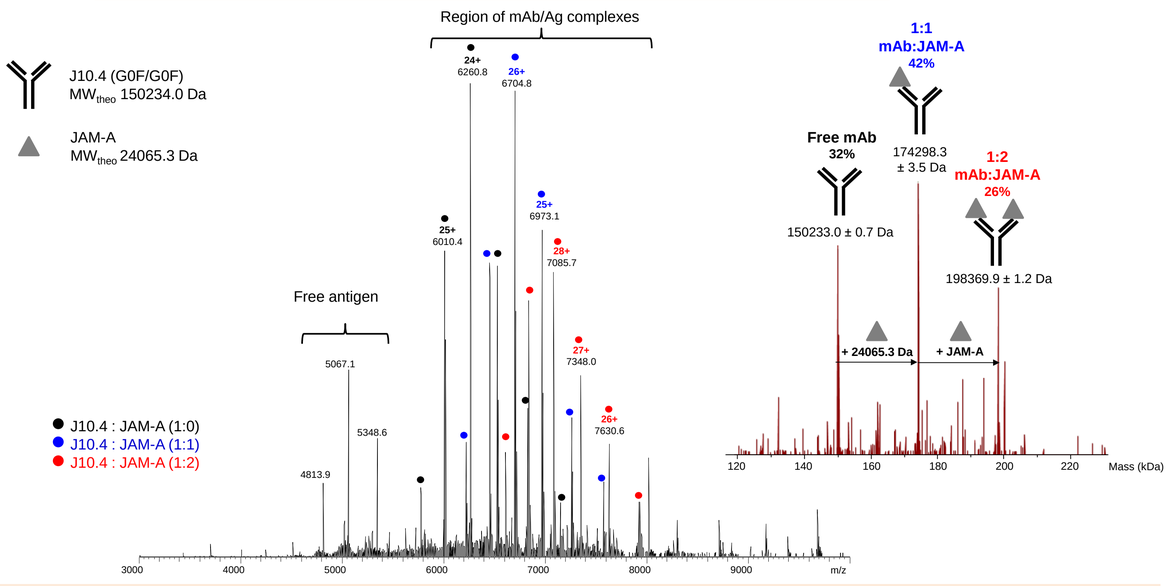


Native MS analysis of a monoclonal Antibody-Drug-Conjugate (ADC) model



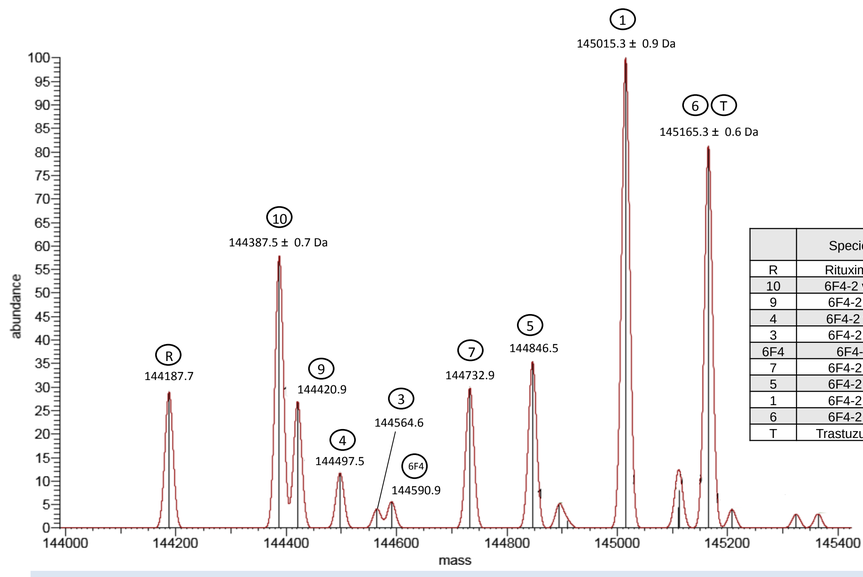
- Populations with 2 (black, 37%), 4 (blue, 52%) and 6 (red, 7%) payloads were detected. Minor ion species corresponding to unconjugated DAR0 mAb (grey, 4%) and no DAR8 were detected.
- The drug loading clearly increases in steps of two (+2779 Da), which corresponds to binding of one payload to the two accessible cystein amino acids after disulfide bridge reduction.
- For each set of peaks, the drug-to-antibody ratio (DAR) can be determined. Relative ratios of each detected compound were determined using MS peak intensities.

Native MS analysis of immune mAb/antigen complexes



- Direct observation of non-covalent immune complexes without any chemical modification.
- Detection of three main ion species: the intact free mAb (MW 150233.0 ± 0.7 Da), 1:1 (MW 174298.3 ± 3.5 Da) and 1:2 (MW 198369.9 ± 1.2 Da) mAb:JAM-A complexes.
- Relative abundances were estimated from MS peak intensities and proportions of mAb:Ag complexes at 1:1 and 1:2 stoichiometries were observed to be 42% and 26% respectively, while free mAb represents 32%.

Native MS analysis of a mixture of 11 N-deglycosylated humanized antibodies



	Species	Theoretical masses (Da)	Measured masses (Da)	Mass accuracy (ppm)
R	Rituximab	144186.3	144187.7	9.7
10	6F4-2 v10	144388.3	144387.5	5.5
9	6F4-2 v9	144420.5	144420.9	2.8
4	6F4-2 v 4	144498.4	144497.5	6.2
3	6F4-2 v3	144564.4	144564.6	1.4
6F4	6F4-2	144585.5	144590.9	37.3
7	6F4-2 v7	144732.5	144732.9	2.8
5	6F4-2 v5	144846.9	144846.5	2.8
1	6F4-2 v1	145015.3	145015.3	0
6	6F4-2 v6	145163.3	N.D	N.D
T	Trastuzumab	145165.5	145165.3	1.4

- Well-resolved ion signals @ R=140,000 + accurate MW measurements = unambiguous assignment of 10 out of the 11 compounds.
- Trastuzumab and Hz6F4-2v6 could not be differentiated due to very close molecular weights (2 Da).
- Peaks corresponding to Hz6F4-2 and Hz6F4-2v3 differ by only 21 Da in mass: they are clearly distinguished on the mass spectrum, however, are not baseline resolved from each other.

Conclusions

- MW measurements of mAbs and related products in the low ppm mass deviation range allow to identify all species simultaneously present in solution and to assess the number of DAR and relative abundance of mAb/Ag complexes while peaks intensities serve for relative quantification of the detected species.
- The Orbitrap high resolving power can baseline resolve mAbs glycoforms, ensuring an excellent mass accuracy in low ppm range.
- ADC compounds can be accurately analyzed, with a mass difference between peaks corresponding to different additional numbers of payloads/drugs. For each set of peaks, the drug-to-antibody ratio (DAR) can be determined, as well as the relative ratio of each detected compound.
- Native Orbitrap MS can reveal the number of antigens bound to mAbs. Relative abundances of mAb/Ag complexes at different stoichiometries can be achieved from MS peak intensities.
- Native MS Orbitrap system enables the high throughput screening of mAb mixtures, ensuring a excellent mass accuracy for each individual mAb.

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