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Capillary Electrophoresis – Mass Spectrometry for Intact Mass Analysis of Antibodies and ADCs

Aran Paulus[1], Chien-Hsun Chen[1], Andreas Krupke[2], Stephane Houel[1], Daniel Lopez-Ferrer[1] and Michael Wenz[2]
[1]Thermo Fisher Scientific, Chromatography and Mass Spectrometry Division, San Jose, CA 95134 and [2]Thermo Fisher Scientific, Life Sciences Solutions, South San Francisco, CA 94080 USA

ABSTRACT

Correct identification of heterogeneity of monoclonal antibodies (mAbs) and antibody-drug-conjugates (ADCs) is an essential analytical tool in the biopharmaceutical industry. High resolution accurate mass spectrometry (HRAM) has established itself as a powerful tool for the characterization of such compounds; however some proteoforms might not be observed due to potential sample complexity. Here, we used the ZipChip™ system from 908 Devices made of micro-fabricated glass chips, coupled to a Thermo Scientific™ Q Exactive™ Plus or Q Exactive™ HF mass spectrometer with the Biopharma option to separate and characterize charge variants of the NIST mAb standard and an ADC derived from it. We were able to identify the three published charge variants with 0, 1 or 2 lysine residues. Using the Thermo Scientific™ BioPharma Finder™ 2.0 software package, accurate intact mass for at least 15 glyco-forms were obtained. The NIST mAb has only one glycan site per heavy chain, which was modified using click chemistry to generate an ADC. The resulting model ADC also had charge variants, which were successfully separated and annotated with the correct MS1 by CEMS.

INTRODUCTION

The main goal of this work was to develop a method to characterize heterogeneous mAb and ADCs in a top-down approach. This was achieved by combining the powerful combination of efficient separation by capillary electrophoresis (CE) and HRAM together with data analysis software. The separation of proteoform charge variants is a challenge using chromatography, but CE provides an ideal separation technique for this problem. Microfluidic CE is advantageous for the mAb analysis given its good separation efficiency and short migration times because of high field strength and short separation channel length. The Orbitrap™ mass spectrometer family is also a good option for this application, and the Q Exactive HF MS with Biopharma option, with its extended m/z 8000 range, is particularly well-suited to the analysis of very large biomolecules. The BioPharma Finder software package is specifically designed to characterize complex proteins using dedicated deconvolution of mass spectra for individual time windows in combination with a thorough comparison to a reference database with all variants. In this study, the combination of microfluidic CE separation, MS detection, and data analysis is presented to solve one of the most challenging workflows in the biopharma industry.

MATERIALS AND METHODS

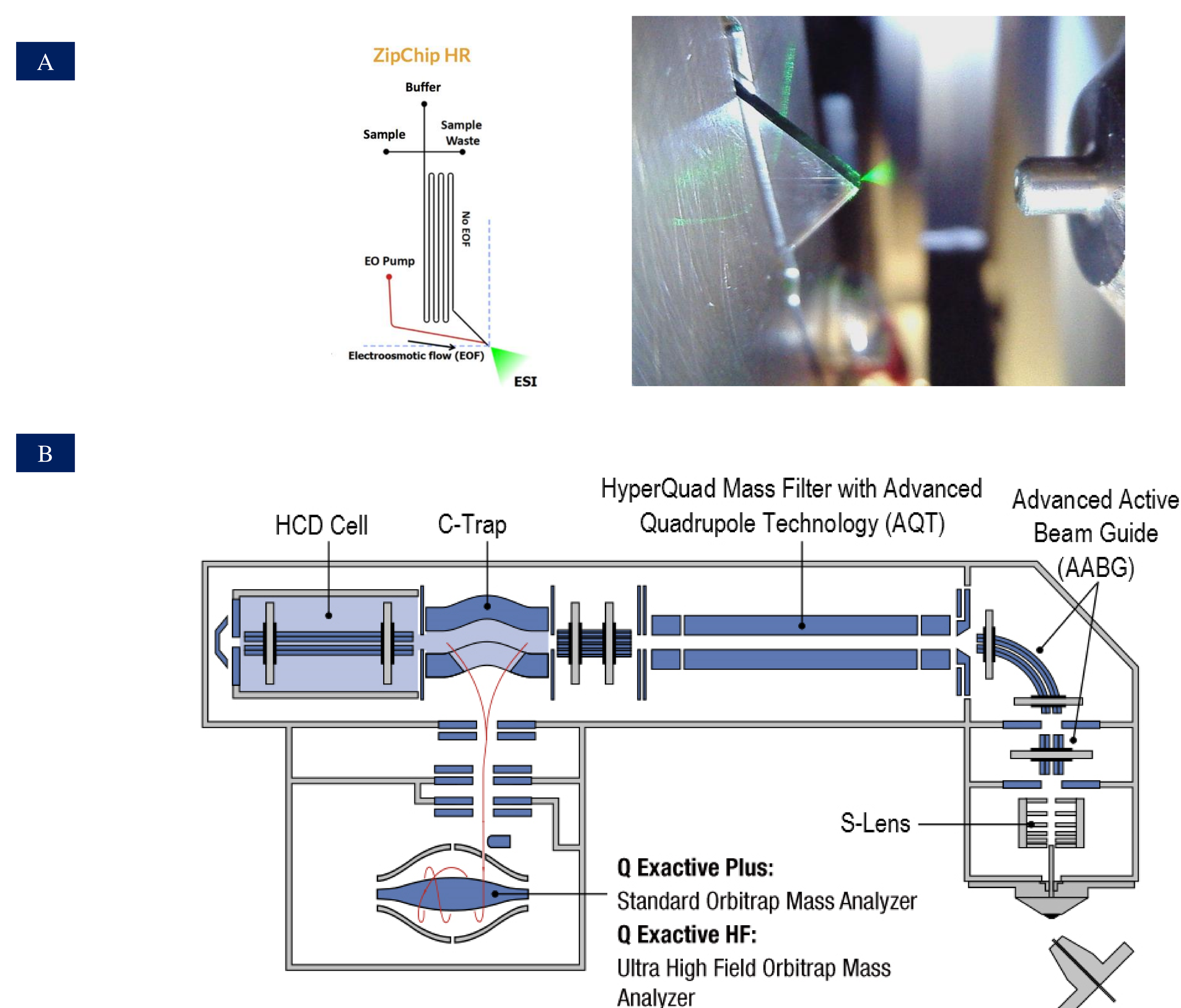
The NIST reference material (NIST mAb standard RM 8671) used in this study has a published sequence including post translational modifications (PTMs). It is a recombinant humanized IgG1κ antibody with any process-related impurities removed through various purification steps. The heavy chain of this mAb is known to have a high abundance of PTMs such as N-terminal pyroglutamination, c-terminal lysine clipping, and glycosylation. 10µg/µL of raw sample was diluted with deionized water to 0.5µg/µL prior to injection. No desalting procedure is necessary for this series of CEMS experiments. The ZipChip HR chip was primed with background electrolyte solution (BGE) consisting of 0.2% acetic acid and 10% IPA (pH=3.17). Once a stable electrospray was observed, 10µL of 0.5µg/µL mAb was pipetted into the sample reservoir. Pressure injection with 2 psi for 4s was used for sample loading, introducing 0.4nL corresponding to 0.2ng of mAb into the separation channel. 20kV was applied for CE separation within 22 cm-length of channel, and ~2kV was used for nano-ESI. Each run was finished in less than 3 minutes. The NIST mAb standard was labeled using click chemistry with a negatively charged fluoresceine derivative or biotin, which were attached on both N-linked glyco units on the heavy chain, adding either two times1145.37Da for AF488 or two times 857.03Da for the biotin label to the MW of the antibody. The Q Exactive HF was set to the optimized conditions for ultra-high mass biomolecule, and to maintain a scan rate of 6 scans/sec. In-source CID (100V) was applied to assist desolvation. m/z range (2500~6000) was selected for this mildly denaturing condition. BioPharma Finder 2.0 was used throughout for data analysis. The sequence and PTM information of the NIST mAb was first set up in protein sequence manager. The identification of different variants can be accomplished with the intact mass of the variants were deconvoluted to the intact mass with the ReSpect algorithm.

Workflow

Figure 1. Characterization of mAb/ADC is accomplished by separation with the ZipChip device, on-line analysis with high resolution Q Exactive Plus or Q Exactive HF with BioPharma option, and identification with BioPharma Finder 2.0 software.

INSTRUMENTATION

Figure 2. A) Schematic of high-resolution (HR) microfluidic CE chip with a 22 cm channel and integrated nano-ESI emitter. The nano-ESI spray can be monitored with the integrated red laser and camera.
B) Schematic of Q Exactive mass spectrometer family including the high-performance quadrupole precursor selection and high-resolution, accurate-mass orbitrap detection. The BioPharma Finder software package is optimized for 3 protein workflows: high-mass-range (HMR) mode for intact analysis, intact protein mode for subunit analysis, and normal mode for peptide mapping.



SAMPLES

Figure 3: NIST mAb 8671 including amino-acid sequence, disulfide-0-bond linkage, and post-translational modifications

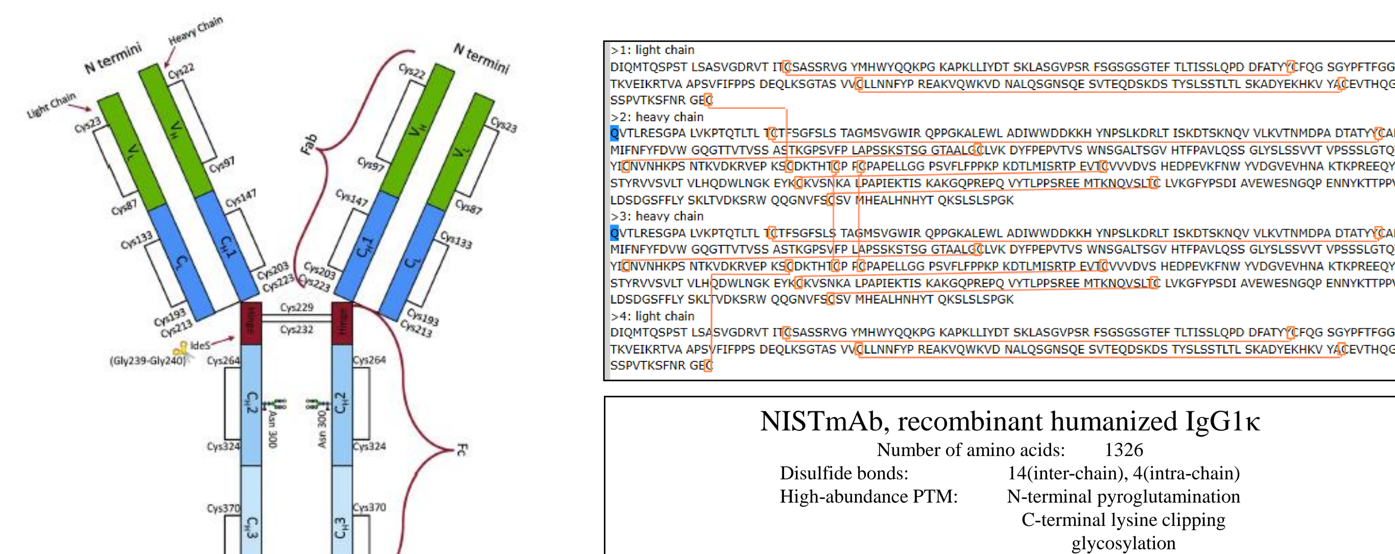


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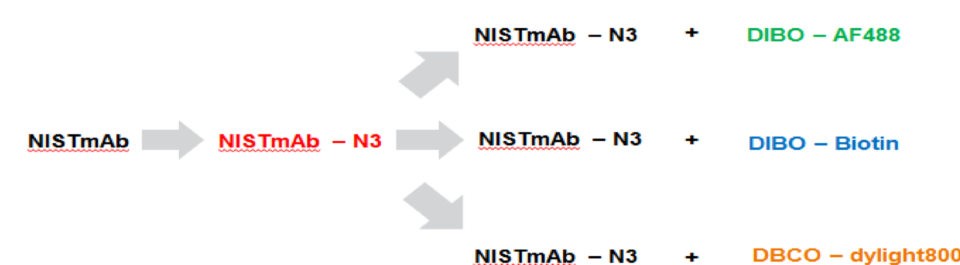
Figure 4: . Reaction scheme to obtain ADCs from the NIST mAb standard using click chemistry. Three different payloads were generated, using AF488, Biotin and dylight800. CEMS analysis in Figure 8



RESULTS

Figure 5. A) Electropherogram of the NIST mAb in a 3 min run on a HR microfluidic chip hyphenated to Q Exactive HF BioPharma mass spectrometer. Three different lysine variants were separated. B) Three mass spectra were extracted from the three peaks of the electropherogram. Lysine variant (128Da) can be identified after the comparison of deconvoluted mass spectra. Higher number of lysine on the C-terminal of heavy chain provides higher mobility due to the additional positive charges from lysine

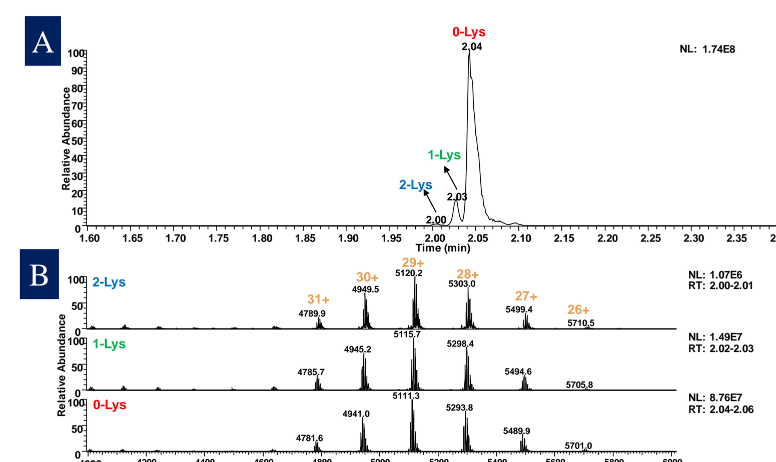


Figure 6. Three deconvoluted mass spectra from different migration time intervals, corresponding to different lysine variants. A) mAb with 0 lysine adduct including 5 glycosylated variants B) mAb with 1 lysine adduct including 4 glycosylated variants C) mAb with 2 lysine adducts including 5 glycosylated variants

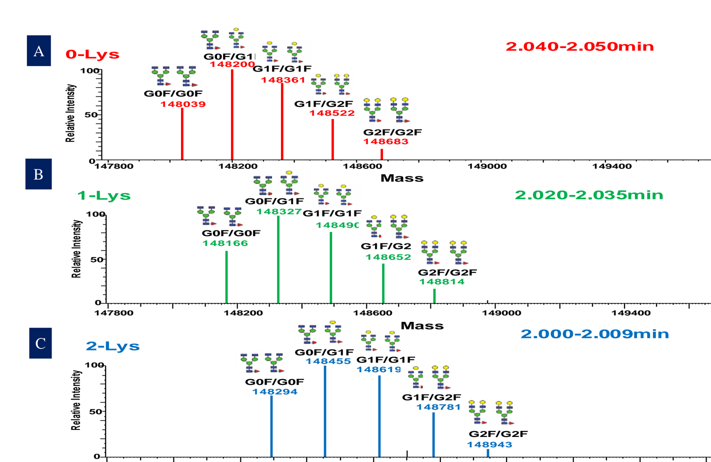
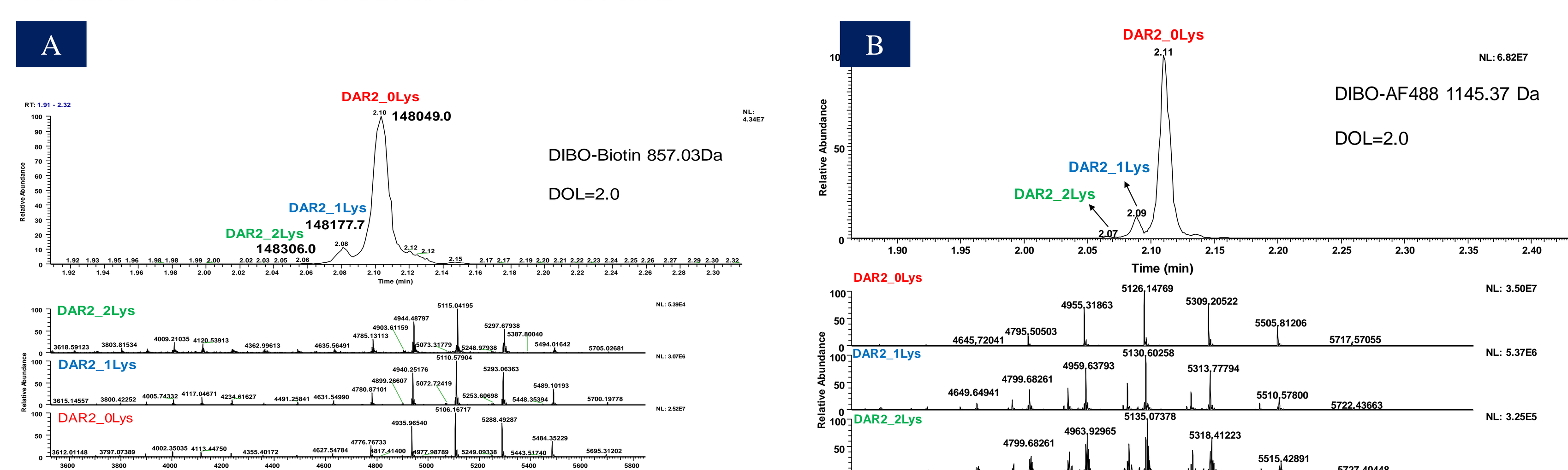


Figure 7: Identified proteoforms of the NIST mAb through the BioPharma Finder 2.0 software

	Protein Name	Modification	Average Mass	Theoretical Mass	Mass Error (ppm)	Relative Abundance	RT Range
1	NIST mAb	1xG0FG1F	148199.98	148199.21	5.3	100.00	2.035 - 2.078
2	NIST mAb	1xG1FG1F	148361.07	148361.35	1.9	86.33	2.035 - 2.078
3	NIST mAb	1xG0FG0F	148038.73	148037.07	11.2	60.90	2.035 - 2.078
4	NIST mAb	1xG1FG2F	148522.19	148523.49	8.7	44.32	2.036 - 2.078
5	NIST mAb	1xG2FG2F	148682.56	148685.63	20.6	10.78	2.037 - 2.051
6	NIST mAb plus 1K	1xG0FG1F	148327.28	148327.41	0.9	10.67	2.012 - 2.035
7	NIST mAb plus 1K	1xG1FG1F	148490.08	148489.55	3.6	8.32	2.013 - 2.035
8	NIST mAb plus 1K	1xG0FG0F	148165.99	148165.27	4.9	6.65	2.021 - 2.034
9	NIST mAb plus 1K	1xG1FG2F	148651.79	148651.69	0.7	4.26	2.017 - 2.036
10	NIST mAb plus 1K	1xG2FG2F	148811.51	148813.83	15.5	0.80	2.017 - 2.026
11	NIST mAb plus 2K	1xG1FG1F	148619.07	148617.75	8.9	0.67	1.995 - 2.013
12	NIST mAb plus 2K	1xG0FG1F	148455.13	148455.61	3.2	0.53	2.001 - 2.012
13	NIST mAb plus 2K	1xG0FG0F	148294.46	148293.47	6.7	0.46	1.995 - 2.012
14	NIST mAb plus 2K	1xG1FG2F	148780.80	148779.89	6.1	0.33	1.999 - 2.012
15	NIST mAb plus 2K	1xG2FG2F	148942.99	148942.03	6.4	0.07	2.003 - 2.010

Figure 8: CEMS of NIST derived ADCs with A) AFF88, B) Biotin as payload on both N-glycan chains. CEMS separation conditions identical to conditions used for NIST mAb.



CONCLUSIONS

- NISTmAb and NISTmAb derived ADC samples with charge variants were separated and characterized using microfabricated glass chips in a CEMS experiment in less than 3 minutes by coupling the 908 ZipChip to a Q Exactive HF MS with Biopharma option. No sample preparation and desalting was necessary.
- Most proteoforms listed in the NIST mAb Reference Material were identified via CEMS: 5 glycosylated variants including G0F/GOF, G0F/G1F, G1F/G1F, G1F/G2F, and G2F/G2F from 2-lys, 1-lys, and 0-lys migration time frames.
- The relative sample amounts of the detected charge variants covers 3 orders of dynamic range.
- Despite the acidic separation conditions, charge state envelope of 26-31 is very close to native conditions for the mAb.
- The NISTmAb derived ADCs exhibit, as expected, the same charge heterogeneity.
- For the AF488 and Biotin as payloads, only the variant with 2 payloads were observed confirming the high yield of the click chemistry.

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TRADEMARKS/LICENSING

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