thermo scientific

POSTER NOTE 65000

Characterization of intact monoclonal antibody with microfluidic chip electrophoresis mass spectrometry

Authors

Chien-Hsun Chen,[1] Andreas Krupke,[2] Stephane Houel,[1] Aaron Bailey,[1] Aran Paulus,[1] Daniel Lopez-Ferrer,[1] Andreas FR Huhmer,[1] Shiaw-Min Chen,[2] Achim Karger,[2] Steve Williams,[2] Michael Wenz,[2] Scott Mellors,[3] Chris Petty[3]

[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, South San Francisco, CA; 908 Devices, Boston, MA

ABSTRACT

Heterogeneity of monoclonal antibodies (mAbs), including glycosylation variants, carboxyl terminal truncation, and deamidation products, is an important analytical problem in the biopharmaceutical industry. High resolution mass spectrometry is a powerful tool for the characterization of mAbs, however, some proteoforms might not be observed due to sample complexity. In this study, a microfluidic chip electrophoresis system (ZipChip™ from 908 Devices), coupled to the high resolution Thermo Scientific™ Q Exactive™ Biopharma platform, was used to separate and characterize the NIST mAb (RM 8671), capturing distinct lysine variants and respective proteoforms. Preliminary data provides identification of most proteoforms listed in the NIST mAb Referenced Material 8671.

INTRODUCTION

The main goal of this work was to develop a method to characterize heterogeneous mAb on an intact protein level. In order to resolve the complexity of mAb, a powerful combination of an efficient separation system, coupled to high-performance mass spectrometer (MS), and data analysis software is necessary. The separation of charge variants is still a challenging task, especially on an intact protein level. Capillary electrophoresis (CE) provides an ideal separation technique for charge variants of mAb. Microfluidic CE provides advantages in characterizing mAb given its good separation efficiency and short migration time based on high field strength and short separation channel. High-performance MS, including high mass accuracy and resolution, is also important for antibody characterization. An Orbitrap™ mass spectrometer is a good option for this application, and the Q Exactive BioPharma platform is suitable for the analysis of very large biomolecules. The challenge of protein MS data analysis usually increases with its molecular weight. The Thermo Scientific™ BioPharma Finder™ software is specifically designed to characterize the complex protein via delicate deconvolution of mass spectra for individual time windows in combination with a thorough comparison to a reference database with all variants. In this study, a powerful combination of microfluidic CE separation, MS detection, and data analysis is presented to solve one of the most challenging workflows in the biopharma industry.

ThermoFisher SCIENTIFIC

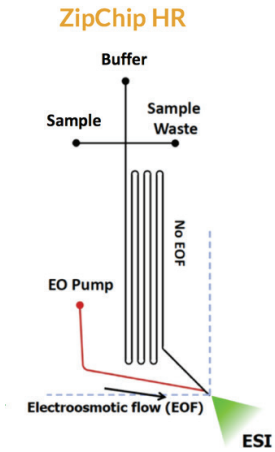
Reference material from the NIST mAb standard (8671) with the known sequence and post translational modifications (PTMs) was used in this study. The NIST mAb is a recombinant humanized IgG1κ antibody, and process-related impurities have been removed through various purification steps. The heavy chain 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 into 0.5 µg/µL for the experiments. No desalting procedure is necessary for this series of measurement. The 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, 10 µL of 0.5 µg/µL mAb was pipetted into the sample reservoir. Pressure injection with 2 psi and 4 s was used for sample loading, introducing 0.4 nL with 0. 2ng of mAb to the separation channel. High voltage of ~20kV was applied for CE separation within 22-cm-length of channel, and ~2kV was used for nano-ESI. Each run was finished in 4 min.

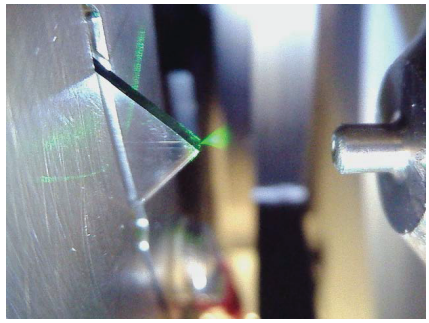
The Q Exactive mass spectrometer was set to the optimized conditions for an ultra-high mass biomolecule, and to maintain a scan rate of 6 scans/sec. In-source CID (100 e.v.) was applied to assist desolvation. An m/z range (2500-6000) was selected for this mild-denatured condition. BioPharma Finder 2.0 software was used in this study 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 after the mass spectra were deconvoluted to the intact mass using the ReSpect™ algorithm.

Figure 1. Characterization of mAb is accomplished by separation with the ZipChip device, online analysis using the high resolution Q Exactive BioPharma platform, and identification with BioPharma Finder software.

Instrumentation

A





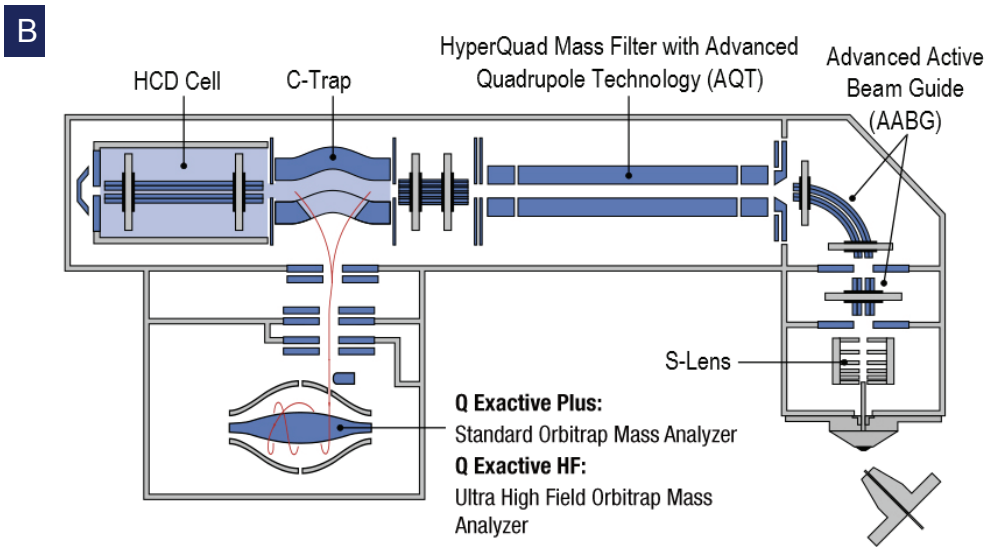
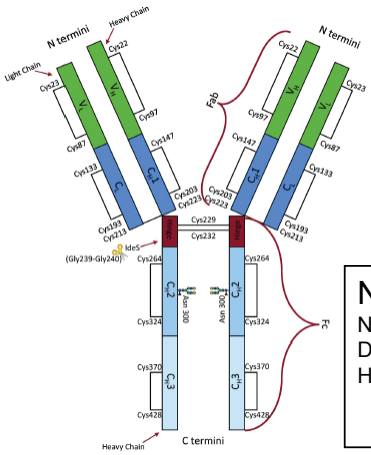


Figure 2. A) Schematic of high-resolution (HR) microfluidic CE chip with 22 cm channel and integrated nano-ESI emitter. The nano-ESI spray can be monitored with the integrated green laser and camera. B) Schematic of the Q Exactive mass spectrometer, including the high-performance quadrupole precursor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

Sample



>1: light chain
DIQMTQSPST LSASVGDRVT ITCSASSRVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

>2: heavy chain
AVTLRESGPA LVKPTQTLTL TCTFSGFSLS TAGMSVGWIR QPPGKALEWL ADIWWDDKKH YNPSLKDRLT ISKDTSKNQV VLKVTNMDPA DTATYYCARD MIFNFYFDVW GQGTTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

>3: heavy chain
AVTLRESGPA LVKPTQTLTL TCTFSGFSLS TAGMSVGWIR QPPGKALEWL ADIWWDDKKH YNPSLKDRLT ISKDTSKNQV VLKVTNMDPA DTATYYCARD MIFNFYFDVW GQGTTVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

>4: light chain
DIQMTQSPST LSASVGDRVT ITCSASSRVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKVEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFYP REAKVQWKVD NALQSGNSQE SVTEQDSKDS TYSLSSTLTL SKADYEKHKV YACEVTHQGL SSPVTKSFNR GEC

NISTmAb, recombinant humanized IgG1κ

Number of amino acids:	1326
Disulfide bonds:	14(inter-chain), 2(intra-chain)
High-abundance PTM:	N-terminal pyroglutamination C-terminal lysine clipping glycosylation

Figure 3. Information on NISTmAb 8671 including amino-acid sequence, disulfide-bond linkage, and post-translational modification.

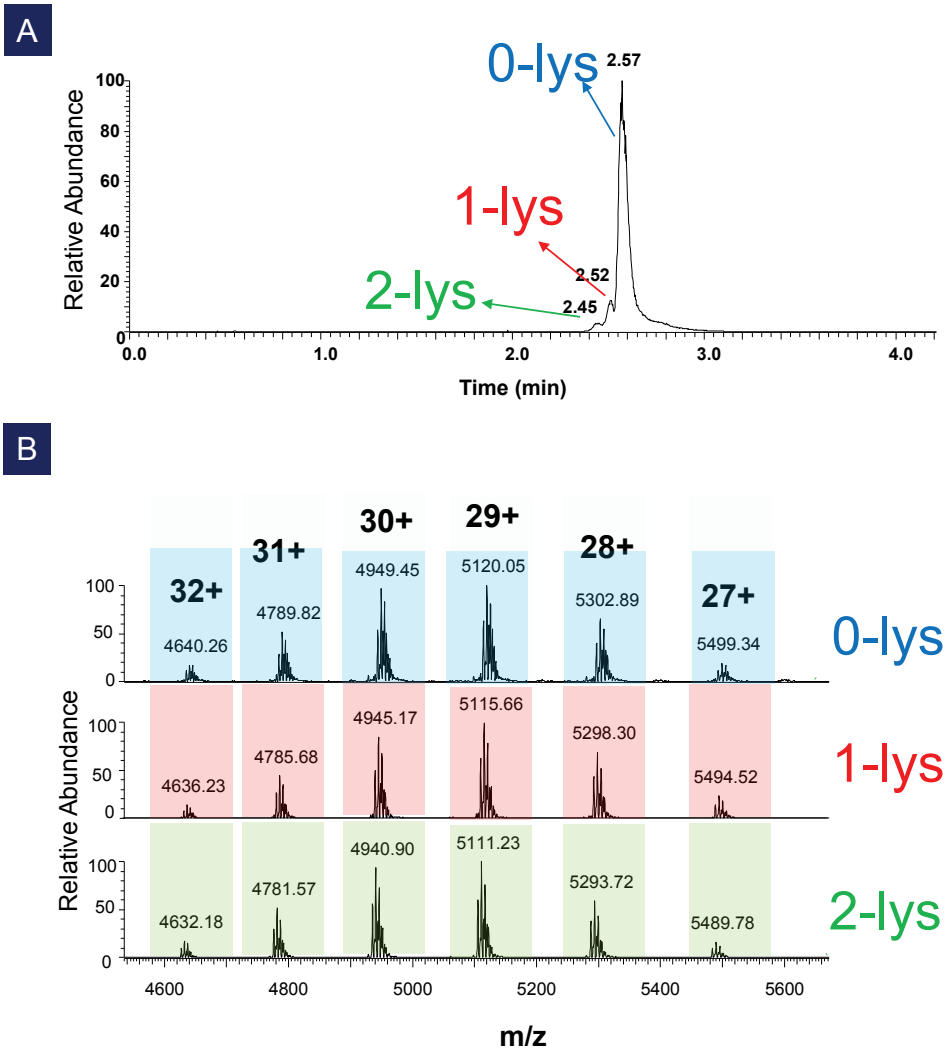


Figure 4. A) Electropherograms of NIST mAb in a 4 min run on a HR microfluidic chip hyphenated to the Q Exactive BioPharma platform. Three different forms of lysine variants were separated under a field strength of ~1000 V/cm. B) Three mass spectra were extracted from the three peaks on the electropherogram. Lysine variant (128 Da) can be identified after the comparison of deconvoluted mass spectra. The higher number of lysines on the C-terminal of heavy chain provides higher mobility due to the additional positive charges from lysine.

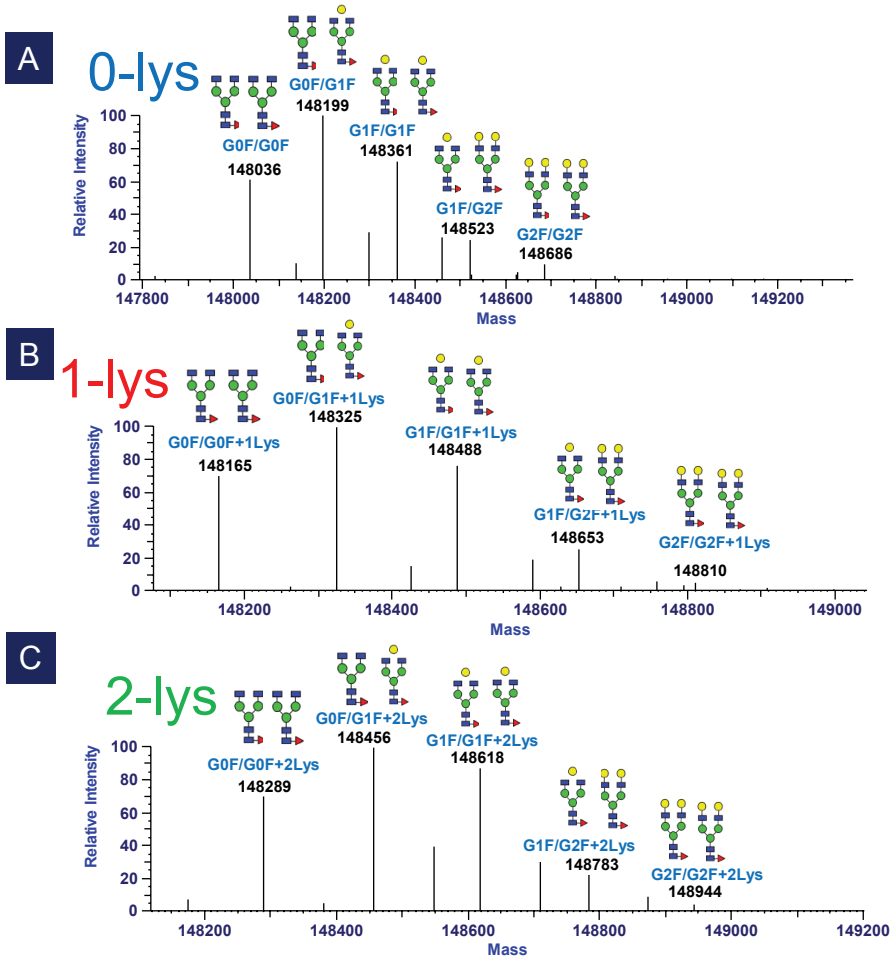


Figure 5. Three deconvoluted mass spectra from different migration time intervals corresponding to different lysine variants. A) mAb with 2 lysine adduct, including 5 glycosylated variant was detected B) mAb with 1 lysine adduct including 4 glycosylated variant was detected C) mAb without lysine adduct including 5 glycosylated variant was detected.

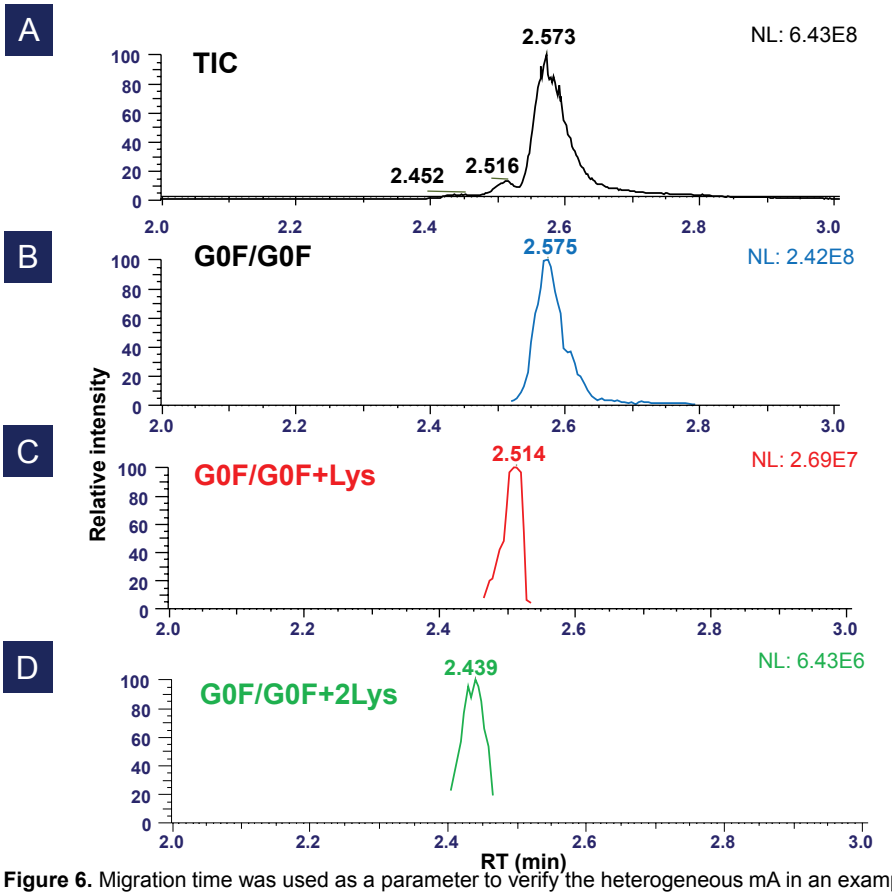


Figure 6. Migration time was used as a parameter to verify the heterogeneous mA in an example of specific glycosylation form G0F/G0F. A) Total Ion Chromatograph (TIC) in a 4 min run of NIST mAb. B) TIC from the specific form of G0F/G0F. C) TIC from the specific form of G0F/G0F+Lys. D) TIC from the specific form of G0F/G0F+2Lys

Protein Name	Average Mass (Da)	Theoretical Mass (Da)	Mass Error (ppm)	Relative Abundance	Migration Time (min)
G0F/G1F	148198.57	148199.21	4.3	100.00	2.525 - 2.796
G1F/G1F	148361.47	148361.35	0.9	72.91	2.529 - 2.796
G0F/G0F	148035.72	148037.07	9.1	61.98	2.516 - 2.796
G1F/G2F	148523.22	148523.49	1.8	26.25	2.555 - 2.796
G2F/G2F	148685.71	148685.63	0.6	12.21	2.525 - 2.796
G0F/G1F+1Lys	148324.56	148327.41	19.2	7.13	2.465 - 2.543
G1F/G1F+1Lys	148488.20	148489.55	9.1	5.05	2.465 - 2.532
G0F/G0F+1Lys	148164.61	148165.27	4.4	4.45	2.460 - 2.538
G1F/G2F+1Lys	148652.87	148651.69	7.9	1.90	2.474 - 2.538
G2F/G2F+1Lys	148810.62	148813.83	21.5	0.49	2.495 - 2.538
G0F/G1F+2Lys	148456.27	148455.61	4.5	1.54	2.416 - 2.463
G0F/G0F+2Lys	148289.32	148293.47	28.0	1.17	2.399 - 2.468
G1F/G1F+2Lys	148617.97	148617.75	1.5	1.15	2.399 - 2.463
G1F/G2F+2Lys	148782.93	148779.89	20.5	0.38	2.405 - 2.457
G2F/G2F+2Lys	148944.03	148942.03	13.4	0.10	2.416 - 2.457

Table 1. Identified proteoforms of NIST mAb through the BioPharma Finder 2.0 software.

CONCLUSIONS

- Clear mAb signal was detected within four minutes coupling 908 Devices' ZipChip system with the Q Exactive BioPharma platform. No sample preparation or desalting was necessary.
- Lysine variants of NIST mAb can be separated on a microfluidic chip electrophoresis device. The mobility was increased with additional positive charges added to the c-terminal of heavy chain from lysine.
- Five glycosylated variants including G0F/GOF, G0F/G1F, G1F/G1F, G1F/G2F, and G2F/G2F were observed from 2-lys, 1-lys, and 0-lys migration timeframes.
- Peaks with different lysine variants can be distinguished from the electropherogram.
- Three orders of dynamic range for detection was achieved.
- Most of the proteoforms listed in the NIST mAb Reference Material can be identified in this study.

REFERENCES

1. John E. Schiel, Darryl L. Davis, and Oleg V. Borisov, "State-of-the-Art and Emerging technologies for Therapeutic Monoclonal Antibody Characterization: The NISTmAb Case Study", American Chemical Society (2015)

2. Erin A. Redman, J. Scott Mellors, Jason A. Starkey, and Michael Ramsey, "Characterization of Intact Antibody Drug Conjugate Variants Using Microfluidic Capillary Electrophoresis – Mass Spectrometry" Anal. Chem. 88, 2220-2226 (2016)

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help and support of Jennifer N. Sutton and Michael A. Blank from Thermo Fisher Scientific.

Find out more at **www.thermofisher.com**

PN65000-EN 0917S

ThermoFisher SCIENTIFIC