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Peptide Mapping of Tryptic Digests for mAbs using a novel ECD cell on the 6545XT AdvanceBio LC/Q-TOF Mass Spectrometer

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Introduction

Post-translation modifications (PTMs) on monoclonal antibodies (mAb) play an important role in the safety, efficacy, and binding of a therapeutic to its target [1]. Common examples of PTMs that seek to be identified include glycosylation and phosphorylation. One challenge in the identification of these PTMs is that dissociation techniques such as collision-induced dissociation (CID) can break apart these fragile modifications. Here, we describe the use of an electron-based dissociation technique (ExD) that supplies low-energy electrons for the fragmentation of tryptic digests that contain glycosylation at asparagine consensus sites (NXS/T). The fragmentation of trastuzumab tryptic digest on a 6545XT AdvanceBio LC/Q-TOF using CID is compared to illustrate that CID alone will fragment these labile PTMs.

Experimental

Tryptic digests for mAbs were generated using the Agilent AssayMAP Bravo system using either an in-solution digestion or single-pot (SP3) protocol. Following digestion, peptides were reconstituted in 0.1% formic acid in water at a concentration of ~0.5 µg/µL. Peptides were added to a G7167B multisampler at 6°C inside a 1290 Infinity II LC system. LC/MS data was collected on a 6545XT equipped with an ExD cell and searched using a pre-released version of MassHunter BioConfirm 12.1 software. For tuning, the Extended Dynamic Range (2 GHz) mode was used with the 100 – 3000 *m/z* range.

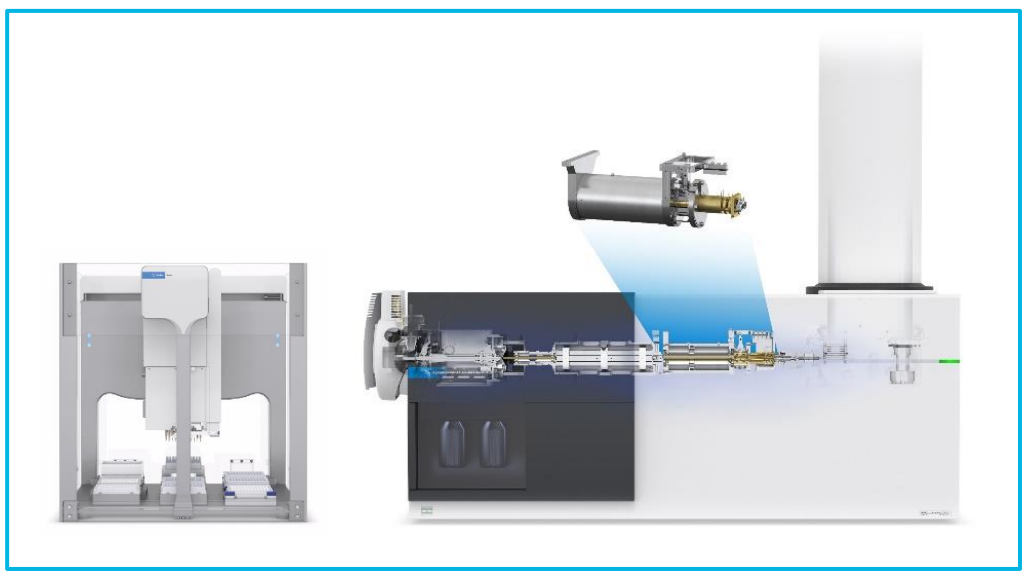

Figure 1. AssayMAP Bravo (left) and Agilent 6545XT AdvanceBio LC/Q-TOF with ExD cell (right).

Experimental

LC Conditions	
Solvent A	Water with 0.1% FA
Solvent B	ACN with 0.1% FA
Gradient	0-90 min, 2-40% B 90.5-94 min, 80% B 94.5-100 min, 2-40% B 100-104 min, 80% B
Injection volume	6 µL
Flow rate	0.3 mL/min
Column temperature	40°C
MS Conditions	
Gas temperature	250 °C
Drying gas	10 L/min
Nebulizer	25 psi
Sheath gas temperature	250 °C
Sheath gas flow	12 L/min
Vcap	3500 V
Nozzle voltage	0 V
Fragmentor	170 V
Skimmer	65 V
Reference mass	922.0098
MS^1 range	200 – 3000 *m/z*
Acquisition rate	3 spectra/sec
MS/MS range	100 – 3000 *m/z*
Acquisition rate	3 spectra/sec
Isolation width	Medium (~4 *m/z*)
Precursors/cycle	Top 5
Threshold for MS/MS	3000 counts and 0.001%
Precursor charge	2+, 3+, >3+
Target	50,000 counts/spectrum

Glycopeptide signal in base peak chromatogram

Glycopeptides for NIST and trastuzumab typically contribute <10% relative contribution to the base peak chromatogram on the Agilent 6545XT AdvanceBio LC/Q-TOF with ExD cell. However, these signals still allow for excellent sequence coverage along the peptide backbone for these labile molecules (see Figure 3 and 4).

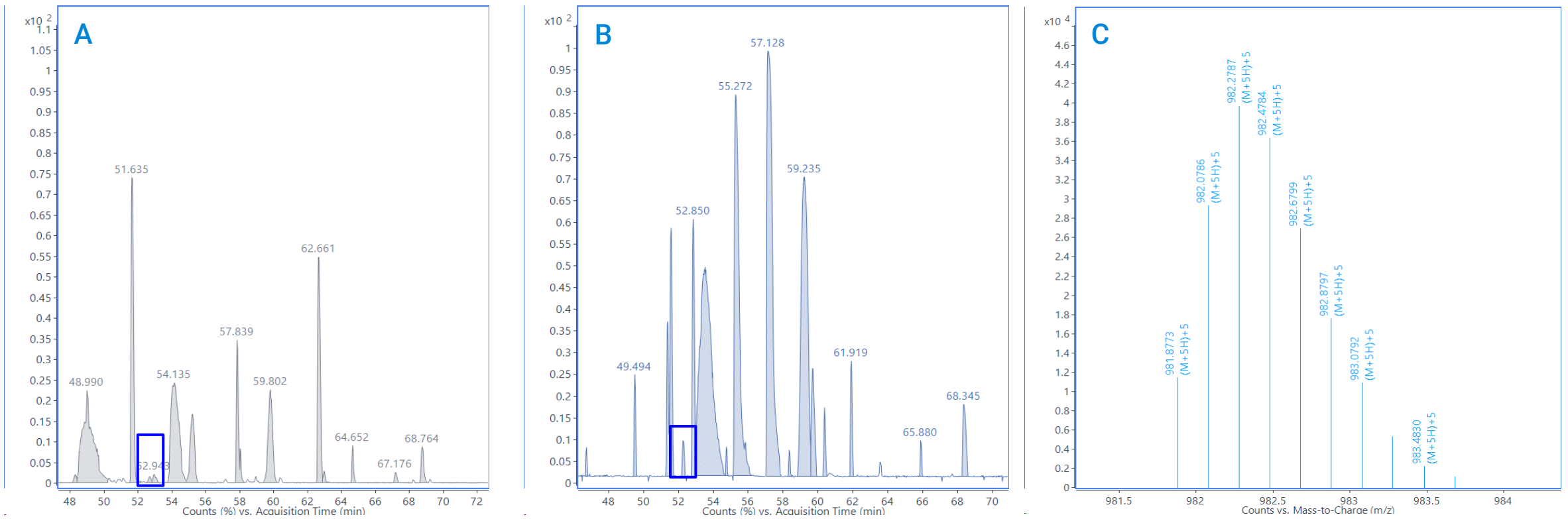

Figure 2. Zoomed-in region of the base peak chromatogram illustrating the relative abundance of the glycopeptide signal (blue box) for (A) NIST and (B) Trastuzumab. A representative MS^1 spectrum is displayed for Trastuzumab in (C) in centroid mode. MS/MS spectra for these glycopeptides are measured using ExD or CID mode with the MS criteria displayed in Table 1.

NIST glycopeptide MS/MS on 6545XT with ExD cell

Tryptic digests of NIST mAb in ExD mode resulted in a sequence coverage of ~99%. In addition, glycopeptide MS/MS illustrated excellent sequence coverage along the peptide backbone (Figure 3). Noteworthy is the appearance of *c*-type peptide fragments that contain G1F (solid blue box) and those without G1F (dashed blue box).

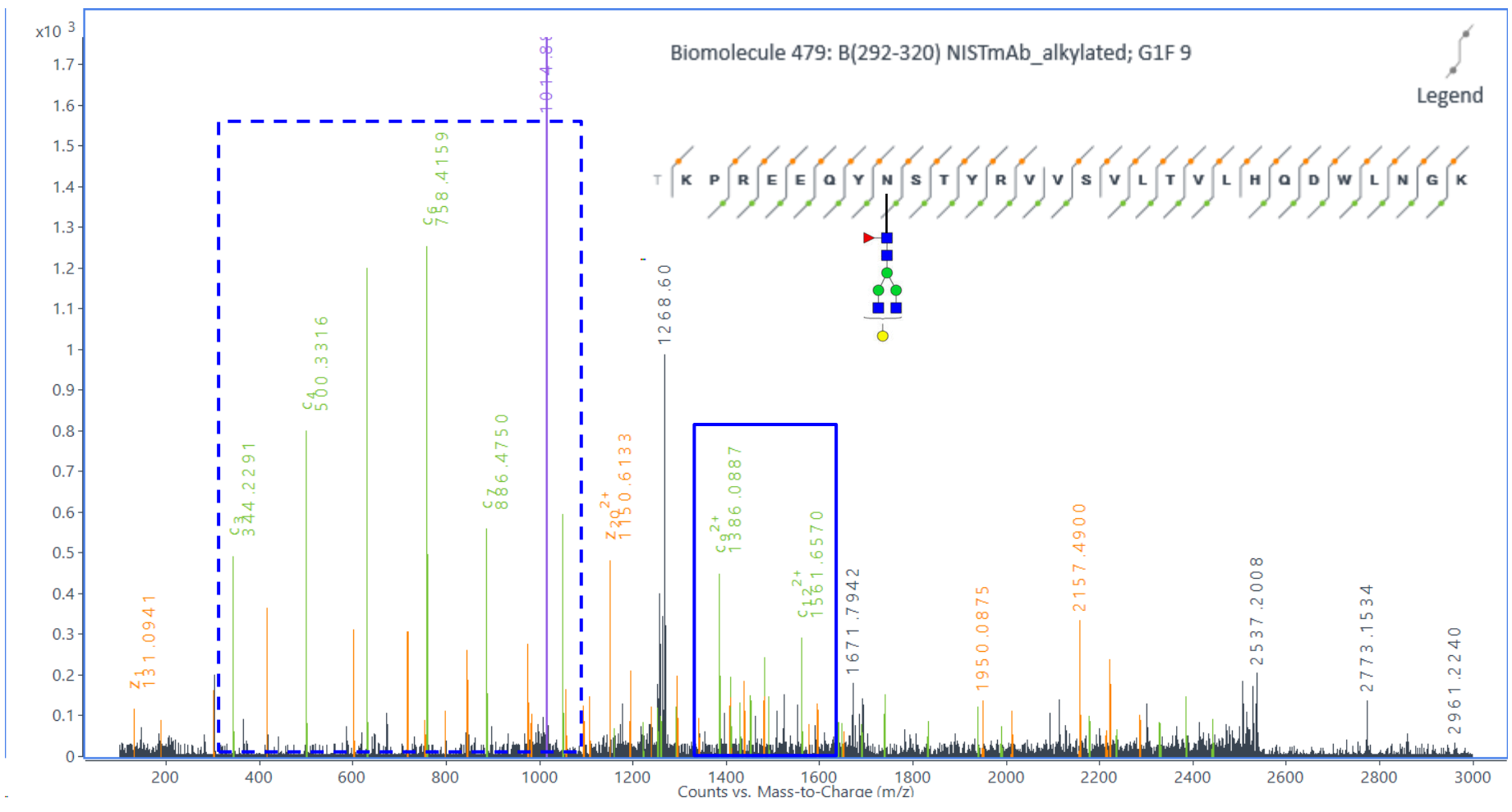

Figure 3. MS/MS spectrum and peptide ladder for TKPREEQYNSTYRVVSVLTVLHQDWLNGK with G1F modification.

Results and Discussion

Trastuzumab glycopeptide MS/MS on 6545XT with ExD cell

Tryptic digests of trastuzumab in ExD mode resulted in a sequence coverage of ~99%. Glycopeptide MS/MS illustrated excellent sequence coverage along the peptide backbone (Figure 4A), though CID generated the HexNAc glycan fragment at *m/z* 204.086 (Figure 4B). Like NIST, there is an abundance of *c*-type fragment ions that contain the glycopeptide. In addition, there are *z*-type fragment ions with (blue box) and without the glycopeptide (blue dashed box). A zoomed-in region also shows the mass difference between z_{21}^{2+} (with G0F) and z_{20}^{2+} (without G0F) fragment ions.

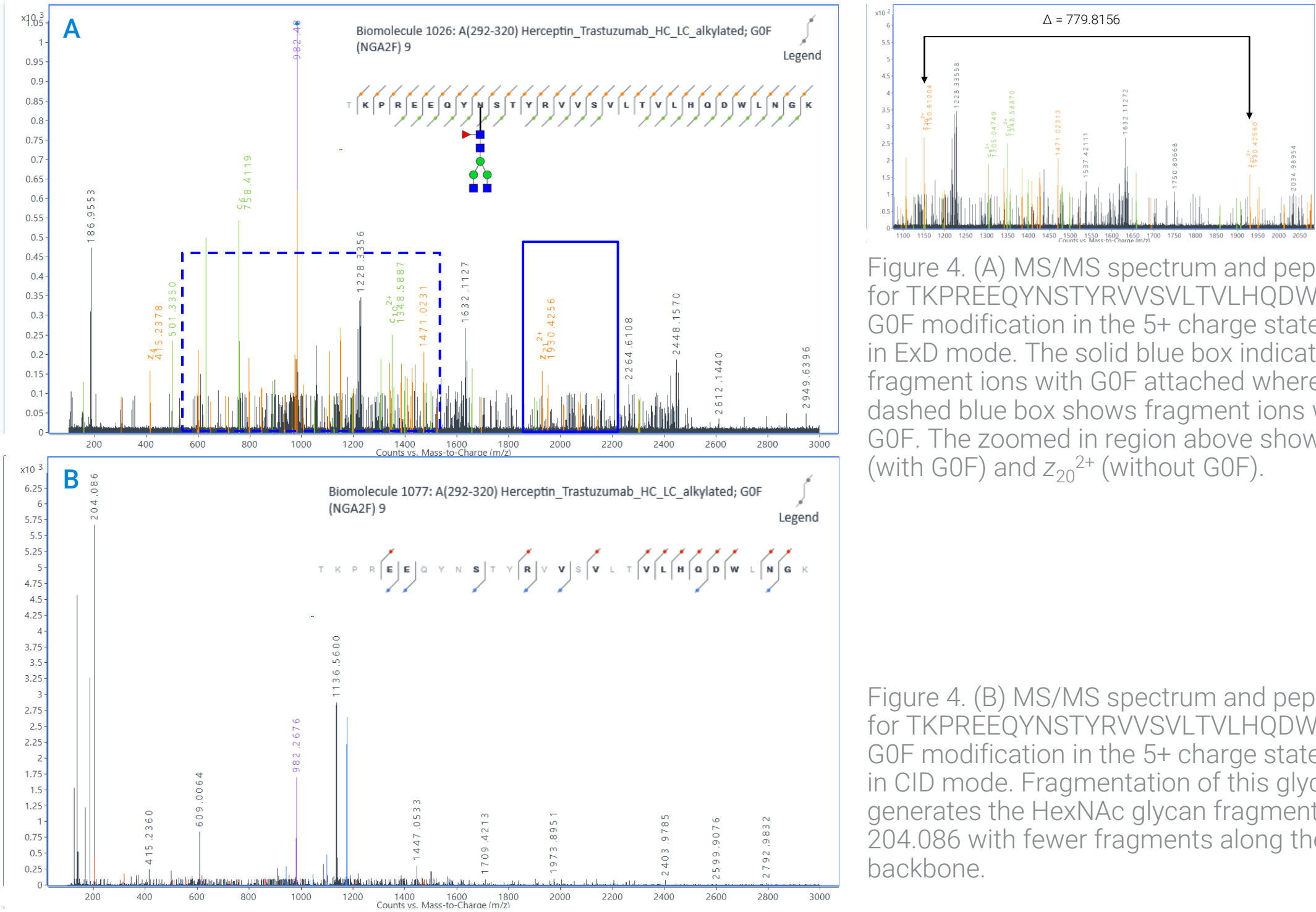

Figure 4. (A) MS/MS spectrum and peptide ladder for TKPREEQYNSTYRVVSVLTVLHQDWLNGK with G0F modification in the 5+ charge state operated in ExD mode. The solid blue box indicates fragment ions with G0F attached whereas the dashed blue box shows fragment ions without G0F. The zoomed in region above shows z_{21}^{2+} (with G0F) and z_{20}^{2+} (without G0F).

Figure 4. (B) MS/MS spectrum and peptide ladder for TKPREEQYNSTYRVVSVLTVLHQDWLNGK with G0F modification in the 5+ charge state operated in CID mode. Fragmentation of this glycopeptide generates the HexNAc glycan fragment at *m/z* 204.086 with fewer fragments along the peptide backbone.

Conclusions

The Agilent 6545XT AdvanceBio LC/Q-TOF with ExD cell enables glycopeptide analysis with excellent sequence coverage assessed using MassHunter BioConfirm 12.1

- LC/MS conditions were optimized to detect low-abundant glycopeptide species from NIST and trastuzumab
- MS/MS spectra for glycopeptides using ExD show peptide fragments with intact glycans and excellent sequence coverage along the peptide backbone
- For CID mode, no intact glycan fragments are observed. Instead, HexNAc glycan fragment at *m/z* 204.086 is observed

References

[1] Zheng K, Bantog C, Bayer R. The impact of glycosylation on monoclonal antibody conformation and stability. MAbs. 2011 Nov-Dec;3(6):568-76. doi: 10.4161/mabs.3.6.17922. Epub 2011 Nov 1. PMID: 22123061; PMCID: PMC3242843.

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