

# Enhanced Glycopeptide Identification Using the SYNAPT XS Q-ToF with Ion Mobility Enabled Wideband Enhancement

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This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

In this application brief, we examine the utility of ion mobility enabled Wideband Enhancement to boost signal, increase coverage, and improve the identification confidence of glycopeptides.

## Benefits

Utilization of Wideband Enhancement increases glycopeptide identification confidence when using SYNAPT XS.

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## Introduction

In collision induced dissociation (CID) of glycopeptides, the tandem mass spectrum consists of characteristic glycan oxonium fragment ions, multiply charged sugar loss ions, and peptide backbone fragment ions. Due to them being the least favored route of fragmentation, these singly charged backbone ions tend to be of very low intensity and thus have a detrimental effect on peptide identification. However, there is a mode of operation available on the Waters SYNAPT XS Mass Spectrometer that can be programmed to use the ion mobility

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characteristics of certain ions and synchronize them with the time of flight (ToF) pusher; this mode is termed Wideband Enhancement (WE).<sup>1,2</sup> Utilizing this mode of acquisition results in improved ToF duty cycle from a routine 15% to 85% for ions of the desired charge state. In order to account for all charge states related to the glycopeptide ions, multiple injections of the same sample are performed, with WE enabled alternatively for the different charge states.

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## Results and Discussion

Tryptic digest samples of several proteins (transferrin, fetuin, alpha antitrypsin, glyco alpha 1, IgM, and IgG) were purified using size exclusion chromatography (SEC).

Purified glycopeptides were then separated on an ACQUITY UPLC M-Class System. This was configured in trapping mode, comprised of trap (ACQUITY UPLC Symmetry C<sub>18</sub> Column, 180 µm x 20 mm, p/n: 186007496) and analytical (nanoEase HSS T3 Column, 75 µm x 250mm, p/n: 186008818) columns. The analytical column eluent was coupled with a PicoTip emitter attached to a nanoFlow ESI source housed upon a SYNAPT XS Mass 2 Spectrometer. A reversed-phase gradient was performed, whereby acetonitrile containing 0.1% formic acid was raised from 5–40% over a 30-minute period. Database searches of the data were performed using GlycopeptideID (Applied Numerics, Helsinki, Finland).

In order to perform Wideband Enhancement calibration, a glycopeptide ion was isolated by the quadrupole and, by raising the collision energy, a typical fragment ion spectrum was produced. When visualizing the data using DriftScope Software as an *m/z* vs. drift time plot, the fragment ions of different charge state and class exhibit different trends (Figure 1). Two Wideband Enhancement calibrations for singly and doubly charged ions were created and used in subsequent experiments.

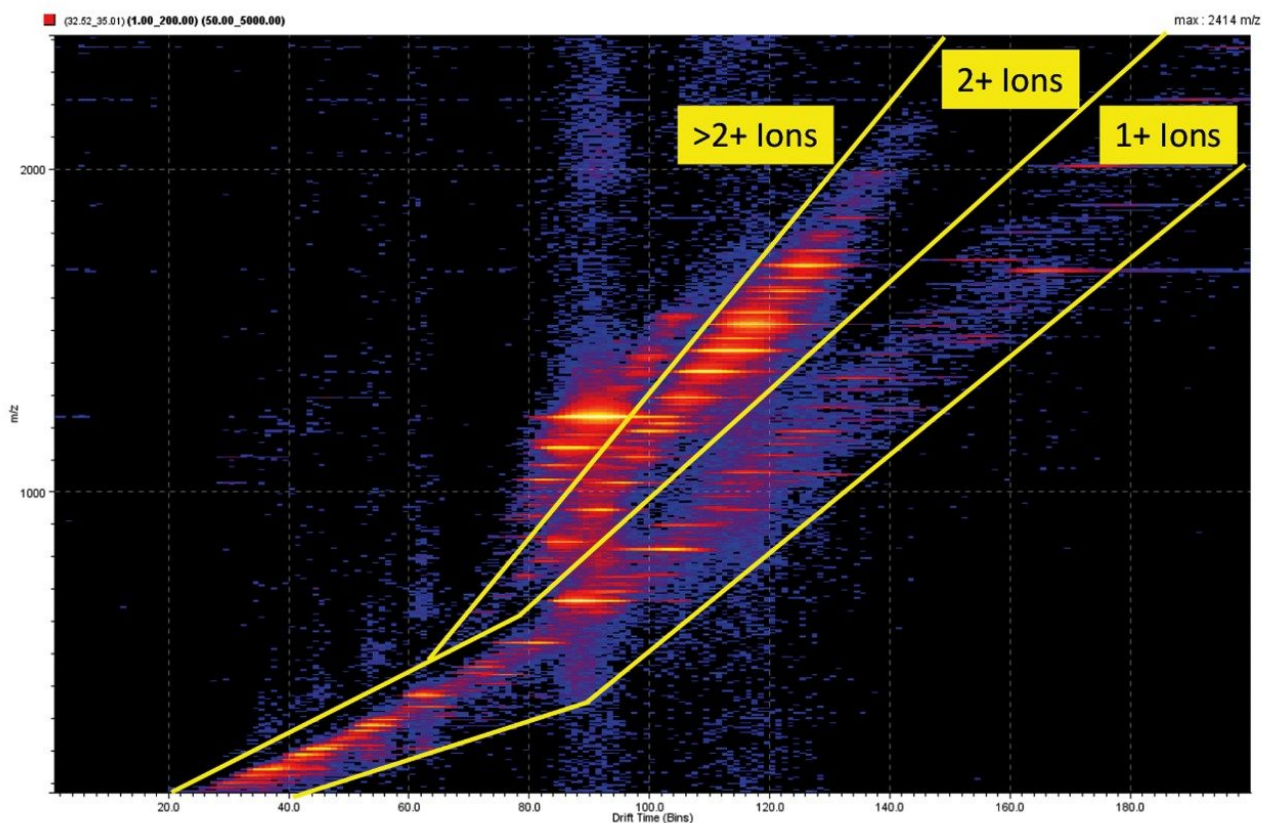


Figure 1. *m/z* vs drift time plot for MS/MS fragmentation of a triply charged glycopeptide. Observed are regions of different charge state from which Wideband Enhancement (WE) calibrations can be created.

Figure 2 shows the effect of using a singly charged WE calibration on a small section of the MS/MS spectrum. The axes are linked and show large signal increase between the two. Figure 3 shows the power of ion mobility in separating species with different charge states but whose isotope patterns overlap on the *m/z* scale. The spectrum with no WE (i.e., the spectrum that is equivalent to that observed with no ion mobility separation), shows two different overlapping species. By applying different WE calibrations, the glycopeptide related ions are separated and mass measured without interference from each other. In order to produce datafiles that can be searched against glycopeptide databases, the mass measured spectrum data is compiled into a peak list and submitted for identification. Figure 4 shows the GlycopeptideID (Applied Numerics, Helsinki, Finland) search result from a glycopeptide, CGLVPVLAENYNK, from human transferrin. Figure 5 compares peptide sequence coverage with no WE and singly charged WE applied for two glycopeptides, CGLVPVLAENYNK from transferrin and EEQYNSTYR from IgG. In both cases, increases in coverage and peptide score are observed when utilizing

the Wideband Enhancement feature.

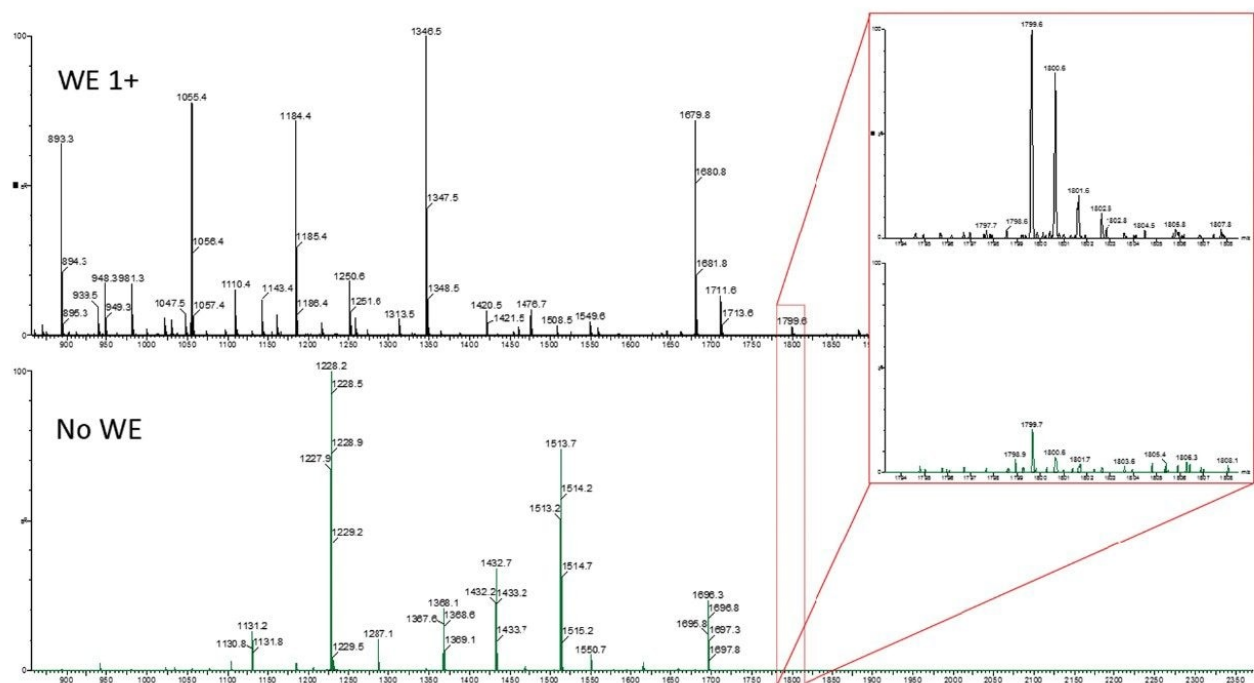


Figure 2. Raw spectra with no Wideband Enhancement (bottom trace) and singly charged WE applied (top trace). Bottom trace is dominated by multiply charged fragments and top dominated by singly charged fragments, including peptide backbone sequence ions. Inset is the signal increase for a singly charged fragment in a small  $m/z$  region (axes are linked). Signal increases of 5–10x are typically observed.

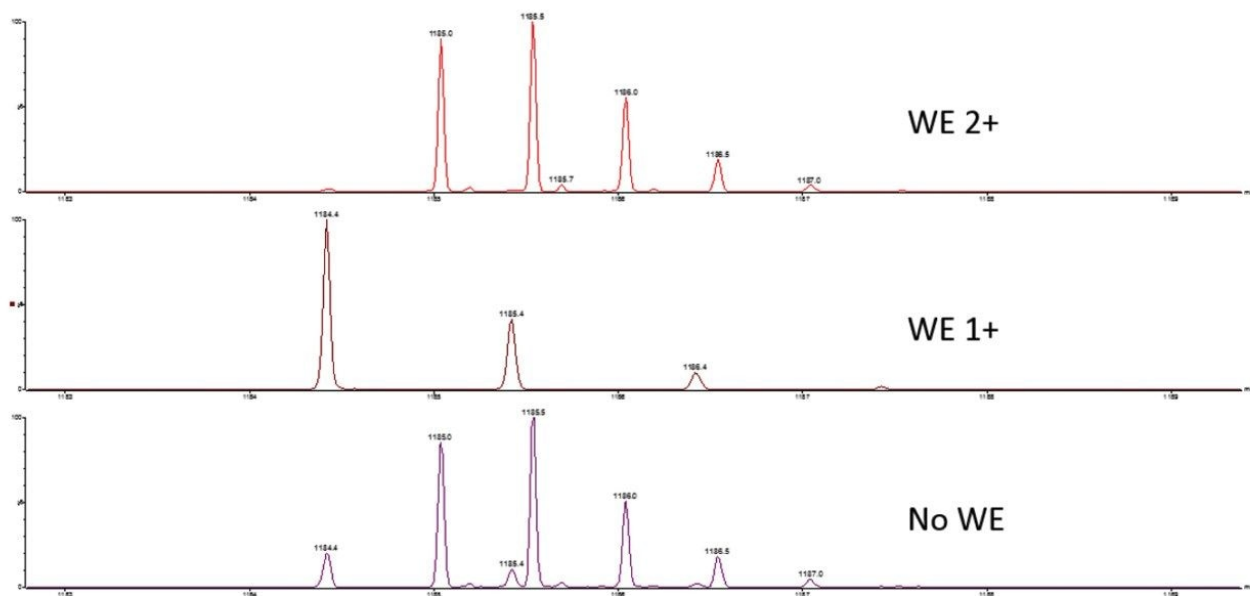


Figure 3. Fragment ions overlapping on the  $m/z$  scale but which exhibit different charge states (1+ and 2+) can be separated by IMS. As can be seen, the singly charged ion is much smaller in the no WE spectrum. When using WE, ions of different charge states can be extracted, effectively removing interferences, and then be identified with more confidence in subsequent database searches.

**m/z 1227.8465 charge 3 scan 0-0**

Score= 186.57 , Hits= 70 , Explained Intensity= 0.56  
 Peptide: TRFE\_HUMAN[421,433]:CGLVPLAENYK  
 Glycan: S2H5N4  
 Charge: 3H

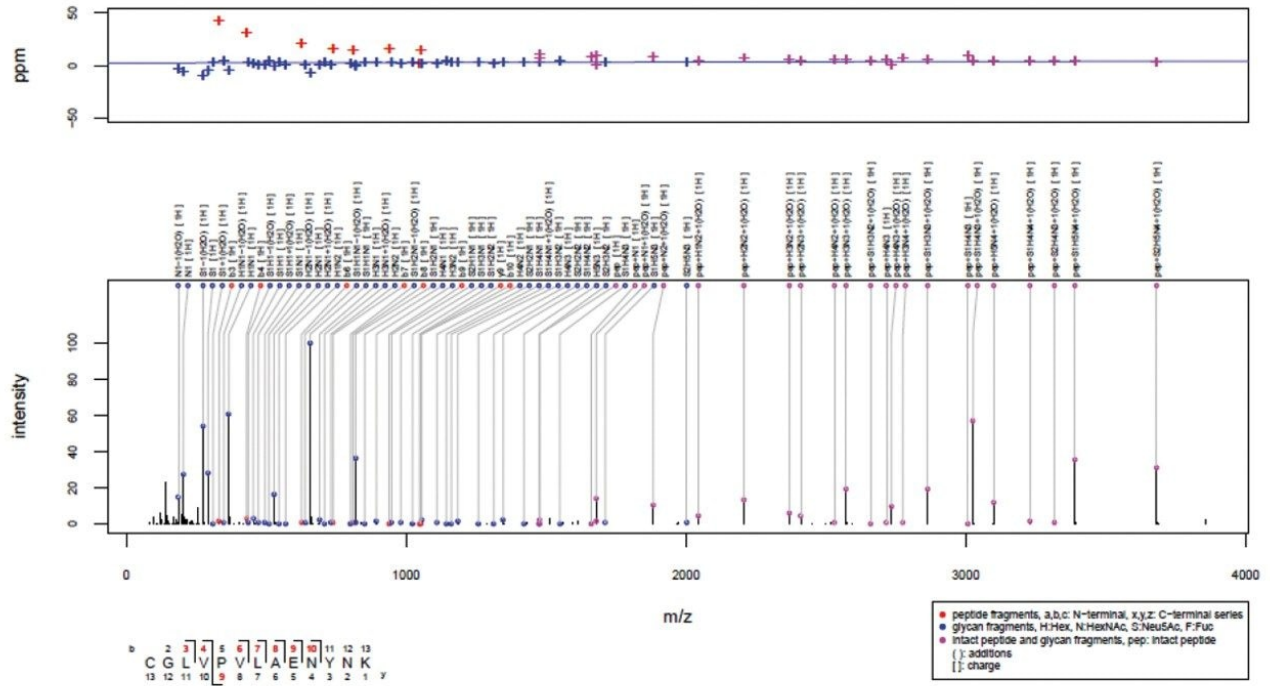


Figure 4. Example GlycopeptideID search result for 'CGLVPLAENYK' from human transferrin.

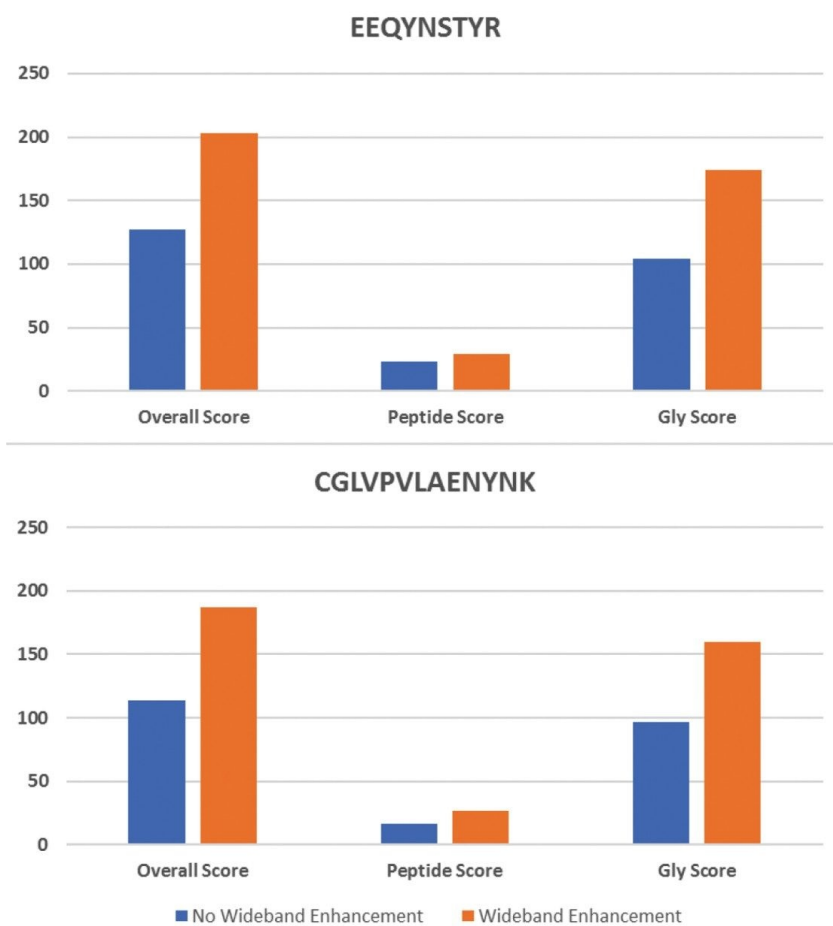


Figure 5. Increases in peptide score, glyco score, and overall score observed for two glycopeptides, CGLVPVLAENYNK (transferrin) and EQYNSTYR (IgG).

## Conclusion

This application brief has demonstrated the use and associated technical benefits of ion mobility enabled Wideband Enhancement using the SYNAPT XS Mass Spectrometer for the study of glycopeptides. Applying WE is shown to overcome the common problem often encountered with glycopeptide analyses, whereby achieving good sequence information, especially for singly charged peptide backbone ions, can be challenging. By

ensuring that the ion mobility characteristics of singly charged ions are synchronized with the instrument's ToF pusher, peptide sequence score increases are observed for glycopeptides from different proteins, thereby increasing their identification confidence. Glycopeptide identifications are ascertained by searching peak lists (.pkl) using GlycopeptideID software.

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## References

1. Richardson, K. et al. High Definition Data Directed Analysis: The Application of Quadrupole Ion Mobility Time-of-Flight Mass Spectrometry for Untargeted Proteomics Studies. Waters Application Note, 720004729EN, 2013.
  2. Helm, D. et al. Ion Mobility Tandem Mass Spectrometry Enhances Performance of Bottom-Up Proteomics, *MCP*, 2014, 13 (12), 3709-15.
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