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응용 자료

Enhanced Performance of the SYNAPT XS and Its Impact on Hydrogen Deuterium Exchange Mass Spectrometry (HDX MS) Data Quality

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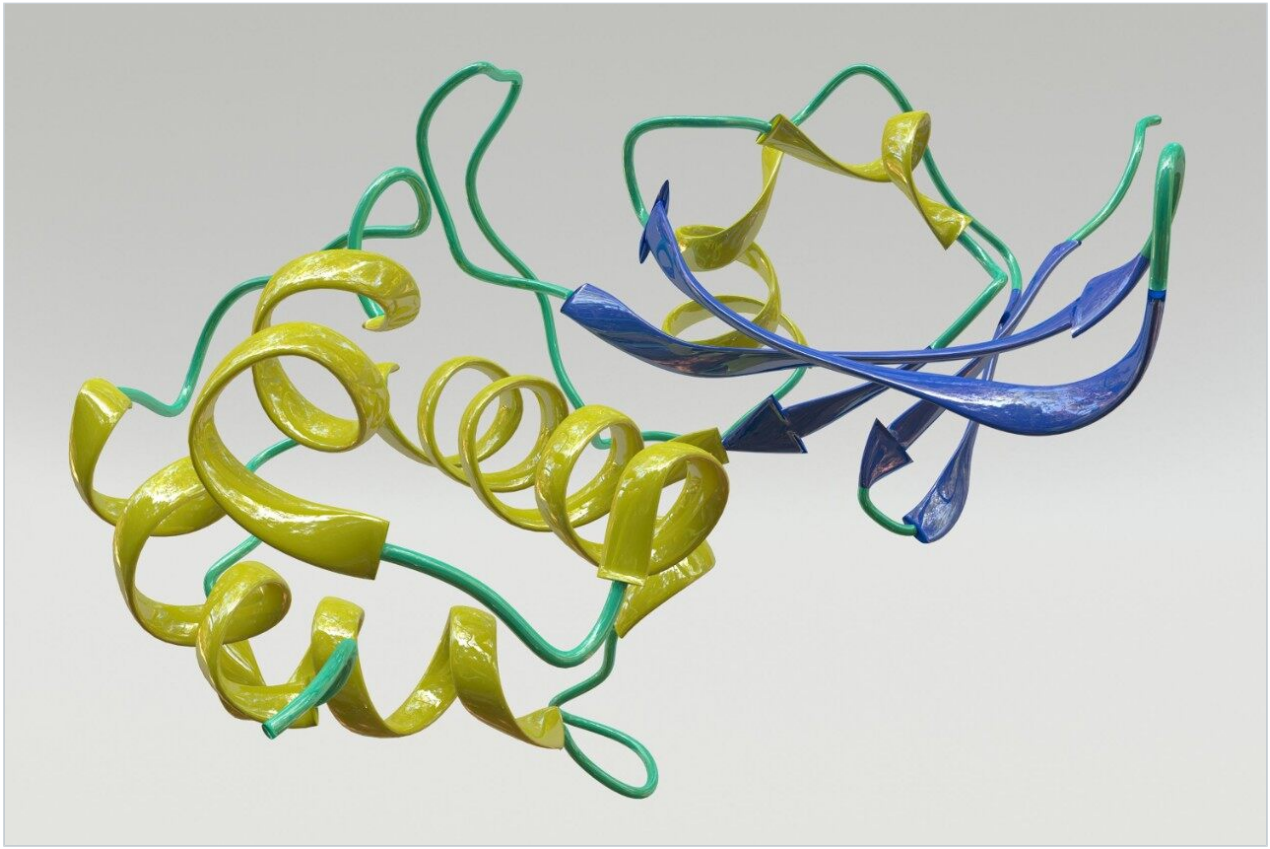

This is an Application Brief and does not contain a detailed

Experimental section.

Abstract

This application brief exhibits and explains why the StepWave XS ion guide and longer flight tube path length in the SYNAPT XS High Resolution Mass Spectrometer facilitate improved data quality and confidence of HDX MS experimental results.

Benefits

The SYNAPT XS provides enhanced sensitivity and improved data quality for HDX MS experiments.

Introduction

Hydrogen deuterium exchange mass spectrometry (HDX MS) is a technique that enables investigation of local conformation and structural dynamics within a protein, including mAb-based biotherapeutics and vaccines. This approach has many applications, ranging from elucidation of disease mechanism, protein folding, binding site identification, and epitope/ paratope mapping to biopharmaceutical comparability and stability studies.

The rate of hydrogen-deuterium exchange is governed by the degree of structural bonding enabling it to function as a structural probe.The combination of rapid on-line proteolysis with pepsin, chromatographic separation of peptides, and MS data acquisition enable the protein's localized deuterium uptake to be measured along its residue sequence.

The Waters HDX MS workflow enables acquisition of data with reduced back-exchange by shortening acquisition time. This is achieved via rapid, high-pressure online digestion (Enzymate BEH Pepsin Column) and high-pressure analytical chromatography (ACQUITY UPLC M-Class System). Further, the ion mobility separation of the SYNAPT XS improves resolution of complex mixtures, allowing more peptides to be characterized during analysis. Incorporation of the optional LEAP automation system vastly simplifies data acquisition and ensures consistency in sample preparation.

Figure 1. HDX MS workflow solution and the SYNAPT XS High Resolution Mass Spectrometer.

Results and Discussion

The SYNAPT XS includes improvements that can address and minimize the main challenges in HDX data collection: sensitivity and H/D back-exchange. The addition of a third-generation design StepWave XS ion guide provides more efficient and lower energy ion transfer through the high-pressure source region. In this design, a voltage gradient draws ions into the upper ion guide, which ends in a segmented quadrupole that maximizes ion focusing and reduces energy deposition, and the uncharged solvent molecules exit through the lower ion guide. In addition, the flight tube path length has been extended by 45 cm, leading to better mass resolution. This, paired with the ion mobility separation available on the SYNAPT XS, enhances the peak capacity of the separation and increases the number of peptide identifications, hence improved sequence coverages. The specific benefit to HDX data is better mass resolution of overlapping isotopic clusters and improved sensitivity due to better ion focusing by the segmented quadrupole.

Sequence coverage improvements were validated using the Waters HDX Phos B Check Standard, analyzed on both the SYNAPT XS and SYNAPT G2-Si mass spectrometers. Conditions for the sample preparation and

instrument set-up can be found in Tables 1 and 2.

Sample	
Waters HDX Phos B Check Standard	Stock concentration of 16.0 µM
Solutions	
Equilibration buffer	10 mM potassium phosphate, pH 7.0
Quench buffer	100 mM potassium phosphate, pH 2.5
Pepsin column wash solution	1.5 M guanidine hydrochloride, 4.0% acetonitrile, 0.8% formic acid, pH 2.5
Incubation/quench volumes	
Protein sample volume	3.0 µL
Equilibration buffer volume	57 µL
Transfer volume	50.0 µL
Quench volume (kept at 0.5 °C)	50.0 µL
Loop volume	50.0 µL
Injection volume	95.0 µL

Table 1. Sample conditions.

MS methods	
Modes	Sensitivity and resolution, HDMS^E 50–2000 *m/z*, 0.4 s scan times
Mass range collision energy gradient	20–45 V ramp
Lockmass	Glu-fibrinopeptide B
ESI source settings	
Capillary/cone voltages	2.80 kV/25 V
Source/desolvation temp.	80/175 °C
Gas flow	400 L/hr

Table 2. Instrument conditions.

Four replicates were processed in ProteinLynx Global SERVER (PLGS 3.0.3), and peptide ion accounting files were imported into DynamX HDX Analysis Software (v3.0). Peptides identified by PLGS were filtered for both quality of identification and reproducibility by applying restrictive parameters addressed previously.[1] The resulting data (Figures 2 and 3) shows increases in both peptide identification and sequence coverage.

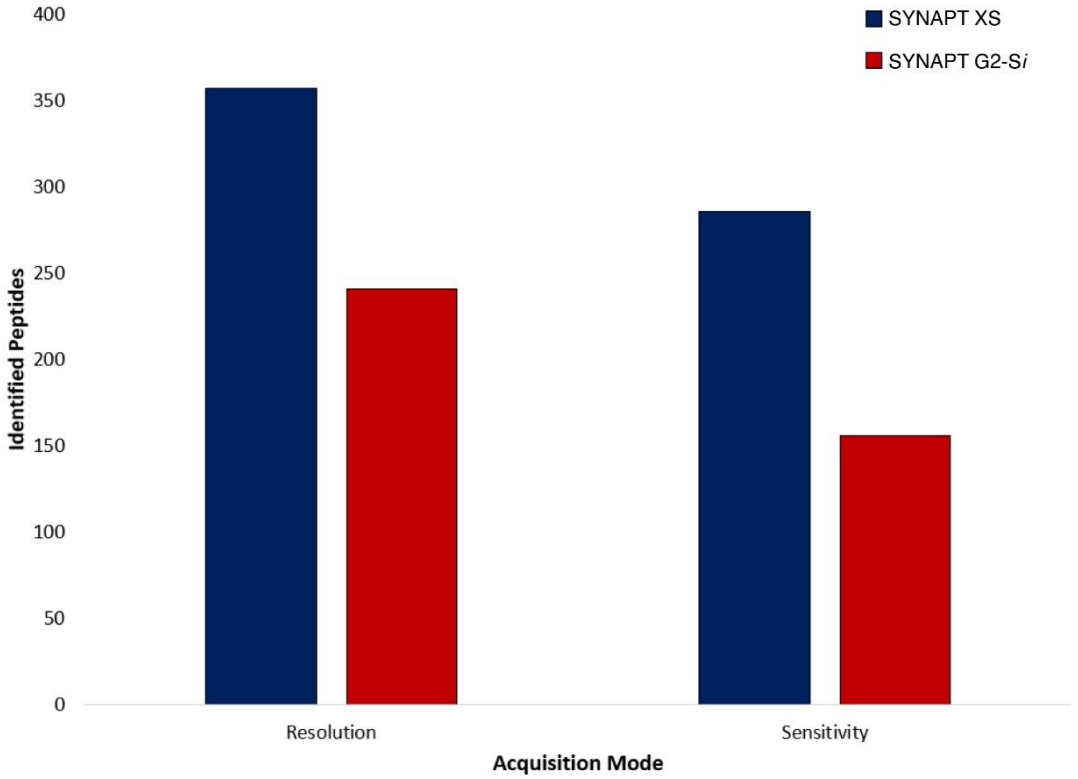

Figure 2. Numbers of identified peptides in both resolution and sensitivity modes.

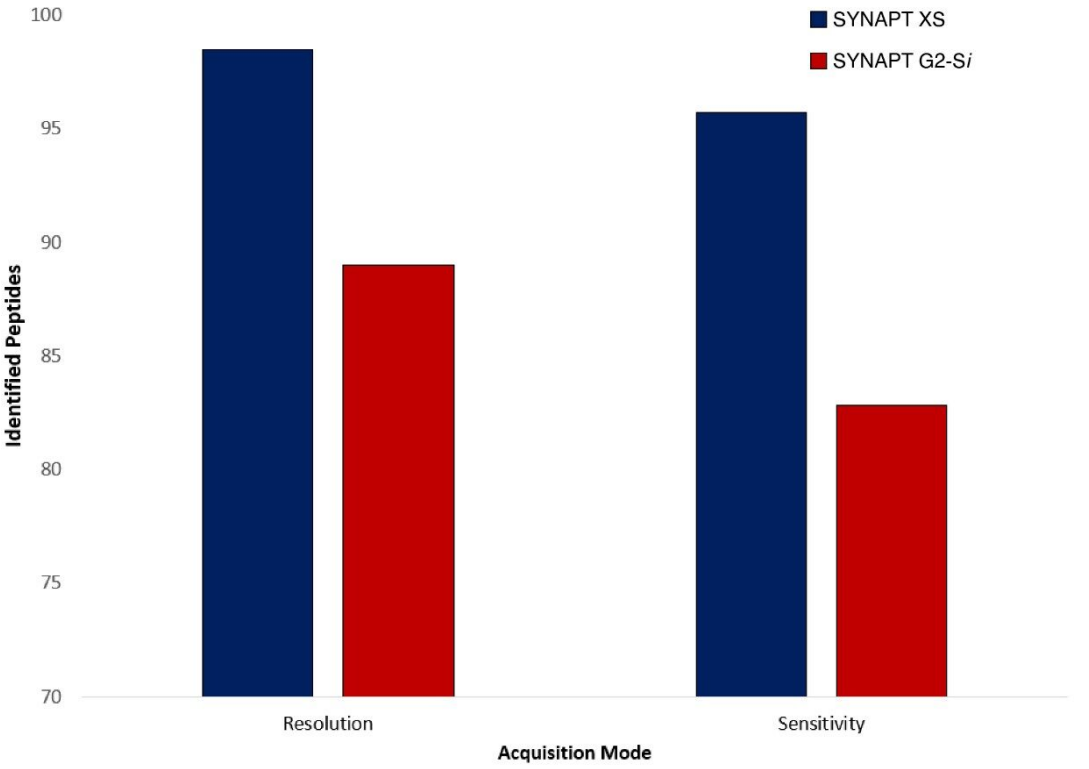

Figure 3. Sequence coverage results in both resolution and sensitivity modes.

In-source H/D back-exchange was investigated through a comparative study of a trypsin digestion of yeast enolase, following the dilution and quench parameters listed in Table 1. After a five-minute incubation in labeling buffer and injection via the automated sample handling system, a decrease in back-exchange was demonstrated on the SYNAPT XS relative to the previous generation instrument, the SYNAPT G2-*Si*. Optimal voltage gradient and RF parameters were experimentally selected for the StepWave XS based on average deuterium uptake and isotopic bimodality of specific peptides known to be more susceptible to back-exchange. The increase in observed deuteration for this example clearly demonstrates the improved performance of the SYNAPT XS (Figure 4).

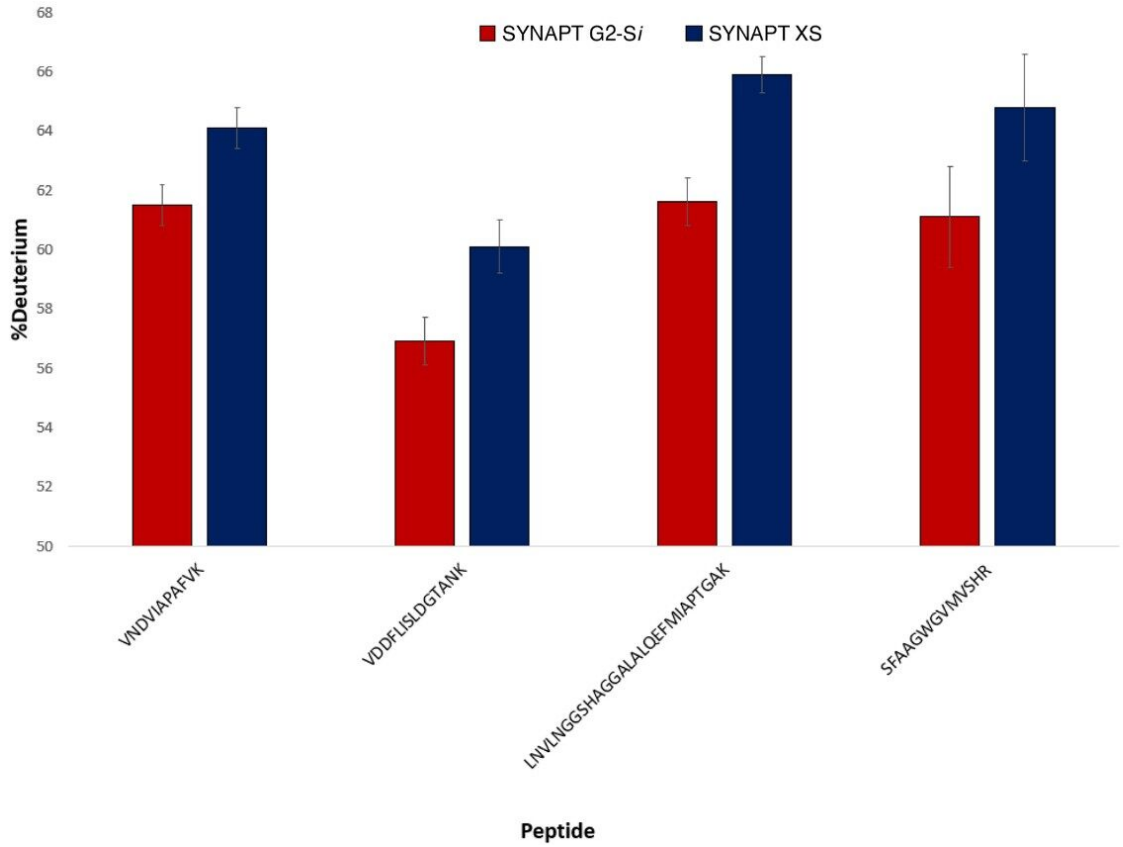

Figure 4. Deuterium retention for four peptides from yeast enolase tryptic peptides.

Conclusion

The SYNAPT XS High Resolution Mass Spectrometer improves sensitivity and resolution of HDX data, while significantly decreasing back-exchange; all three are key attributes for HDX experiments. The StepWave XS and longer ToF flight path builds upon the foundation of an orthogonal approach of HDMS^E and ion mobility spectrometry that combines separation of precursor peptide ions with data independent collision induced fragmentations for superior peptide identification and sequence coverage. The improvements in performance of the SYNAPT XS will provide benefits in terms of protein sequence coverage and peptide redundancy and will enable researchers to study proteins in increasingly complex systems.

References

1. Sorensen, L. *et al.* Optimized Workflow for Selecting Peptides for HDX-MS Data Analyses. *J. Am. Soc. Mass Spectrom*. 2018, 29, 2278–2281.

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ACQUITY UPLC M-Class System <https://www.waters.com/134776759>

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StepWave <https://www.waters.com/134673601>

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