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Applikationsbericht

Hydrogen Deuterium Exchange Coupled with SELECT SERIES Cyclic IMS Mass Spectrometer for Higher Workflow Efficiency

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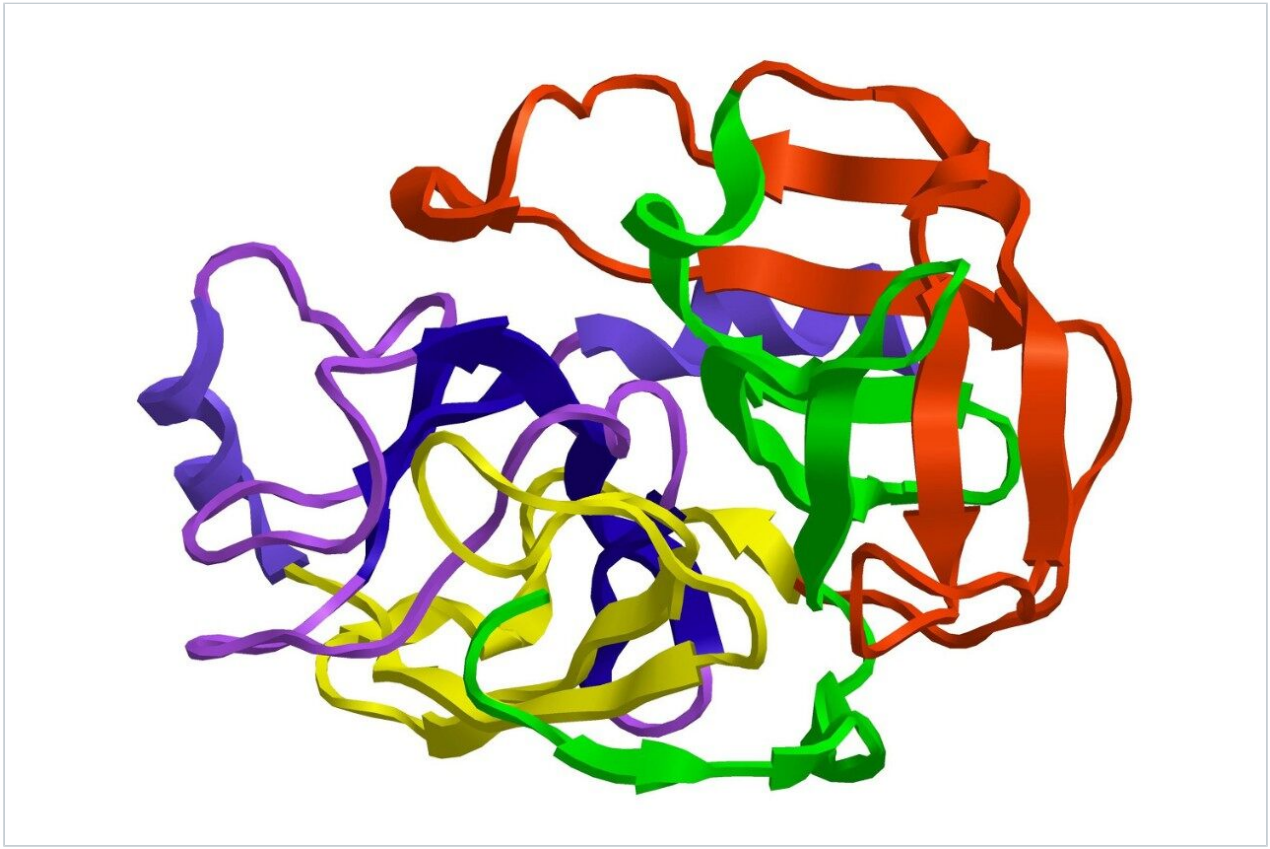

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Abstract

Hydrogen deuterium exchange is increasingly employed in the study of larger proteins and protein complexes, and in drug candidate screening applications. These more challenging experiments drive a need for improved performance in the capacity of the LC-MS System. The new SELECT SERIES Cyclic IMS, with higher ion mobility and mass resolution offers improved peak capacity to address these challenges. Here, the performance of the Cyclic IMS System for hydrogen deuterium exchange is demonstrated relative to the SYNAPT G2-Si and SYNAPT XS and for sample limited applications.

Benefits

- The improved sensitivity, detector dynamic range, mass resolution, and ion mobility performance of the SELECT SERIES Cyclic IMS System yields improved peptide identifications and reduced peak saturation for highly abundant peptides
- The improved sensitivity and peak capacity of the Cyclic IMS System is expected to permit use of truncated chromatographic gradients, allowing higher daily throughput and reduced amide-exchange

Introduction

Characterization of higher order structure in proteins and protein complexes is integral for understanding the function and mechanisms of protein action and is essential to the evaluation and development of therapeutic biomolecules. Hydrogen deuterium exchange (HDX) facilitates localization of binding sites and regions of conformational change and provides information regarding flexibility and protein dynamics. HDX complements high spatial resolution techniques such as NMR, cryo-EM, and X-ray crystallography and results can typically be obtained with significantly higher throughput. Hence, HDX has been used as a cost-effective approach for epitope mapping screening and continued development regarding the throughput of the approach is expected to be widely beneficial.[1]

Amide hydrogen exchange occurs spontaneously in solution and can be exploited to monitor the hydrogen bonding protection that occurs along the backbone of proteins. Typical experimental protocols involve dilution of a stock protein into a deuterated buffer. Incubation in the deuterated buffer for variable time periods enables measurement of protein backbone kinetics in the seconds to hours timeframe. Measurement

of deuterium uptake involves a low temperature, low pH quench followed by rapid pepsin digestion and LC-MS. Utilization of shorter chromatographic gradients yields higher deuterium retention but results in higher spectral complexity, which can be a challenge to in-silico database searches. Consequently, the quality of experimental data is typically a balance between protein size and chromatographic length. As the trend over recent years has been the study of larger protein complexes, often with tens of subunits, spectral complexity is increasingly a challenge.

Experimental

Sample Description

Phosphorylase B (p/n 186006930 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/186006930-hdx-phos-b-check-standard.html>) was diluted to stock concentrations of 8, 16, and 32 µM in 10 mM phosphate buffer, pH 7.0.

Method Conditions

Intact protein was mixed at a 1:20 ratio with 10 mM, pH 7.0 phosphate buffer, and quenched with a 1:1 dilution into 100 mM, pH 2.4 phosphate buffer at 0 °C prior to injection into the LC System. Intact protein was passed over a Waters Enzymate Pepsin Column and collected on a VanGuard C_{18} trap Column. LC-MS was performed at 0 °C using a 1 mm x 100 mm BEH C_{18} Column. Gradients, trap times, and flow rates are shown in the table below. A 7-minute effective gradient was used and peptides were analyzed using an HDMS^E method in which either a single or double pass of the ion mobility cell was used. HDMS^E is a data-independent acquisition mode in which low energy and elevated energy data is alternately collected in a rapid cycle for all peptides. Alignment of retention and ion mobility drift times are used in data processing to associate precursor peptides and their corresponding fragment ions. Traveling wave and other relevant instrument parameters for the Cyclic IMS are listed below; instrument parameters for the SYNAPT G2-S*i* and SYNAPT XS have been reported previously.[2]

LC Conditions

LC system:	ACQUITY UPLC M-Class with HDX-2 automation

Detection:	SELECT SERIES Cyclic IMS
Vials:	Total Recovery
Column(s):	ACQUITY UPLC BEH C_{18} VanGuard Pre-column 2.1 x 5 mm p/n 186003975; ACQUITY UPLC 1 x 100 mm BEH C_{18} Column, 1.7 µm particles p/n 186002346; Enzymate BEH Pepsin Column p/n 186007233
Column temp.:	0 °C
Sample temp.:	0 °C
Injection volume:	50 µL
Flow rate:	40 µL/min
Mobile phase A:	H_2O, 0.1% FA
Mobile phase B:	ACN, 0.1% FA

Gradient Table 1

Time (min)	Flow (µL/min)	%A	%B	Curve
Trapping: 0.0	75	100	NA	6
Trapping: 0.2	100	100	NA	6
Trapping: 1.0	100	100	NA	6
Trapping: 1.2	200	100	NA	6
Trapping: 2.0	200	100	NA	6
Trapping: 2.9	75	100	NA	6
0.0	40	95	5	6
7.0	40	65	35	6
7.5	40	15	85	6
8.5	40	15	85	6
9.0	40	95	5	6
12.0	40	95	5	6

MS Conditions

MS system:	SELECT SERIES Cyclic IMS
Ionization mode:	Positive
Acquisition range:	50–2000
Capillary voltage:	2.8kV
Collision energy:	20V–29V
Cone voltage:	30V
StepWave1 offset	8V
StepWave2 offset	12V
Number of passes	1–2

Racetrack TW height	25V
Racetrack TW velocity	375 m/s
ADC pushes per bin	1

Data Management

Chromatography software:	MassLynx SCN 1016
MS software:	Quartz 2.4.1
Informatics:	PLGS 3.0.3, DynamX 3.0

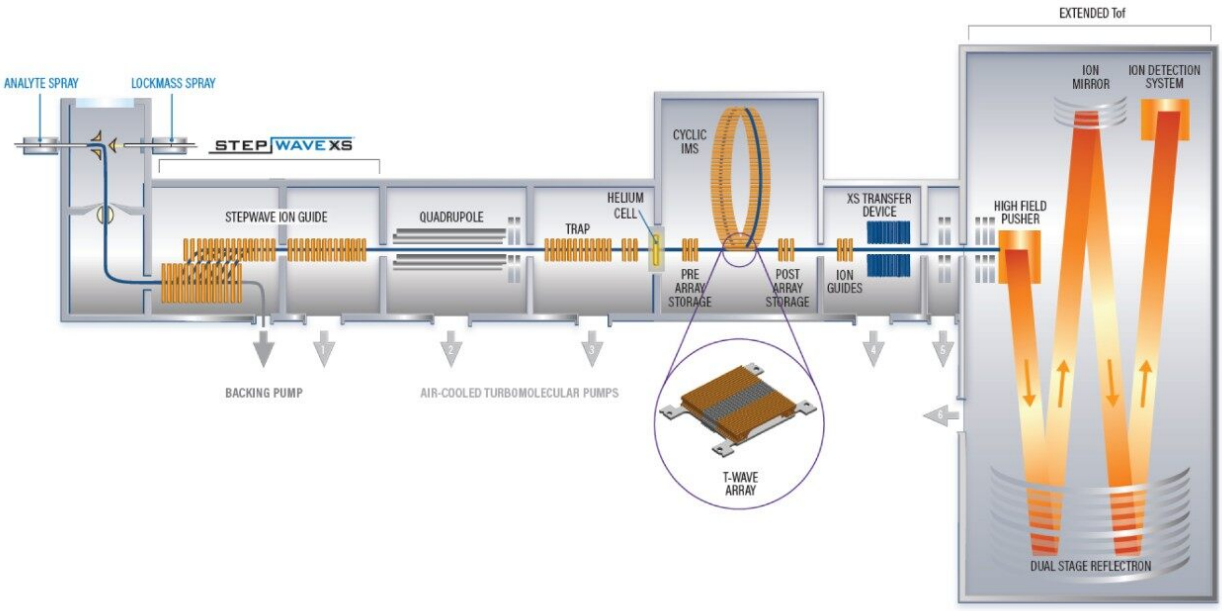

Figure 1. Instrument diagram of the SELECT SERIES Cyclic IMS.

Results and Discussion

Initial experiments were undertaken to benchmark the performance of the Cyclic IMS System relative to the SYNAPT G2-S*i* and the recently released SYNAPT XS Mass Spectrometer. Data was collected using HDMS^E with 32 µM stock protein solution and with identical StepWave settings between the SYNAPT XS and Cyclic IMS. Peptide map experiments of Phosphorylase B were used to evaluate the performance of the system in terms of peptide identifications in complex mixtures. Triplicate injections were independently processed in PLGS 3.0.3 using standard processing parameters and the resulting peptide files imported into DynamX and filtered as described previously.[3] The number of identified peptides and resultant sequence coverage obtained on the three instruments is reported in Figure 2. Improved performance was observed on the SYNAPT XS and Cyclic IMS relative to the SYNAPT G2-S*i* in terms of the number of identified peptides and sequence coverage. Approximately 240, 360, and 560 peptides were identified using the SYNAPT G2-S*i*, SYNAPT XS, and Cyclic IMS, respectively. The addition of the StepWave XS device in the SYNAPT XS and Cyclic IMS yields improved sensitivity, which likely contributes to the improvement to the number of identifications. The Cyclic IMS System has several other attributes, including the scalable IMS resolution, XS transfer device, and the dual gain ADC detection system that likely contribute to its improved performance over the SYNAPT XS. Significantly, the dual gain ADC provides improved dynamic range for highly abundant ions. Sequence coverages for the three instruments were 89, 98.5, and 100%. The small difference in coverage for the SYNAPT XS and Cyclic IMS is a direct result of the coverage already being exceptionally high – proteins with longer sequences are likely to experience a greater gain in coverage. Consequently, loading and gradient truncation experiments were undertaken for the Cyclic IMS to evaluate HDX peptide mapping performance for lower loadings and with shorter chromatographic separations.

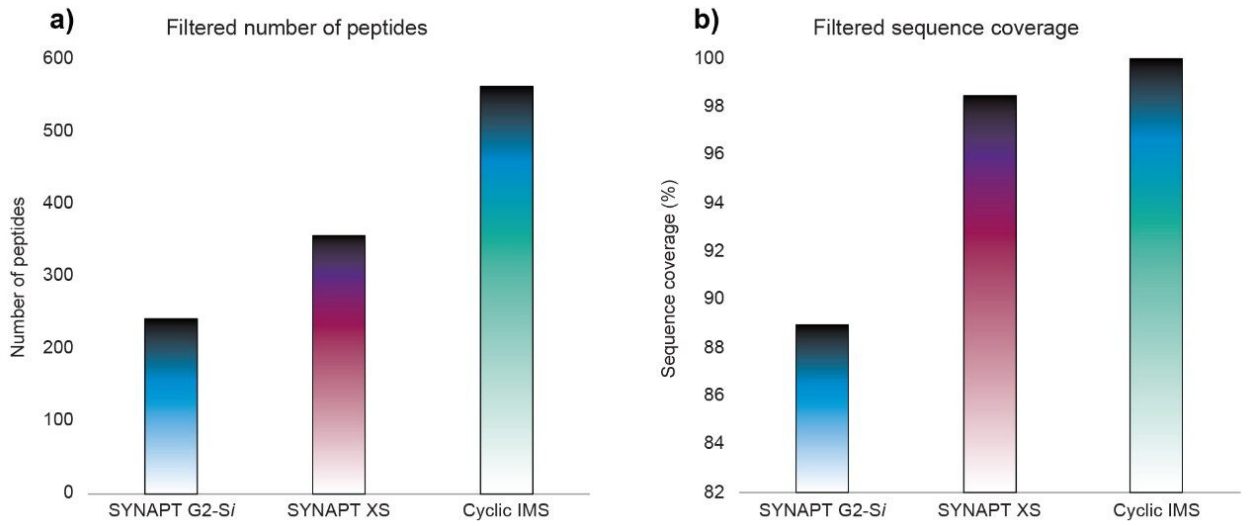

Figure 2. Number of identified peptic peptides and sequence coverage following filtering for phosphorylase B using SYNAPT G2-Si, SYNAPT XS, and Cyclic IMS Mass Spectrometers.

A challenge of HDX experiments is that peptides resulting from low frequency pepsin cleavage often need to be monitored in order to access specific regions of the protein sequence. Consequently, high sample loadings are often used experimentally to obtain better signal for these peptides. As a result, highly abundant peptides often appear with saturated and distorted isotope ratios and often contain artifactual peaks resulting from overloading the detector. The Cyclic IMS System features a dual gain ADC detector that improves the dynamic range of detection for highly abundant ions and the ADC improves the linear response of the detector at high ion currents. It was hypothesized that this feature would ameliorate the ion saturation often seen in HDX experiments and the data obtained supports this. In Figure 3, isotope distributions are shown for two peptide ions on the SYNAPT XS (bottom) and Cyclic IMS (middle), demonstrating the improved accuracy of the isotope measurements for high intensity ions. The theoretical isotope distribution for both ions is shown on the top.

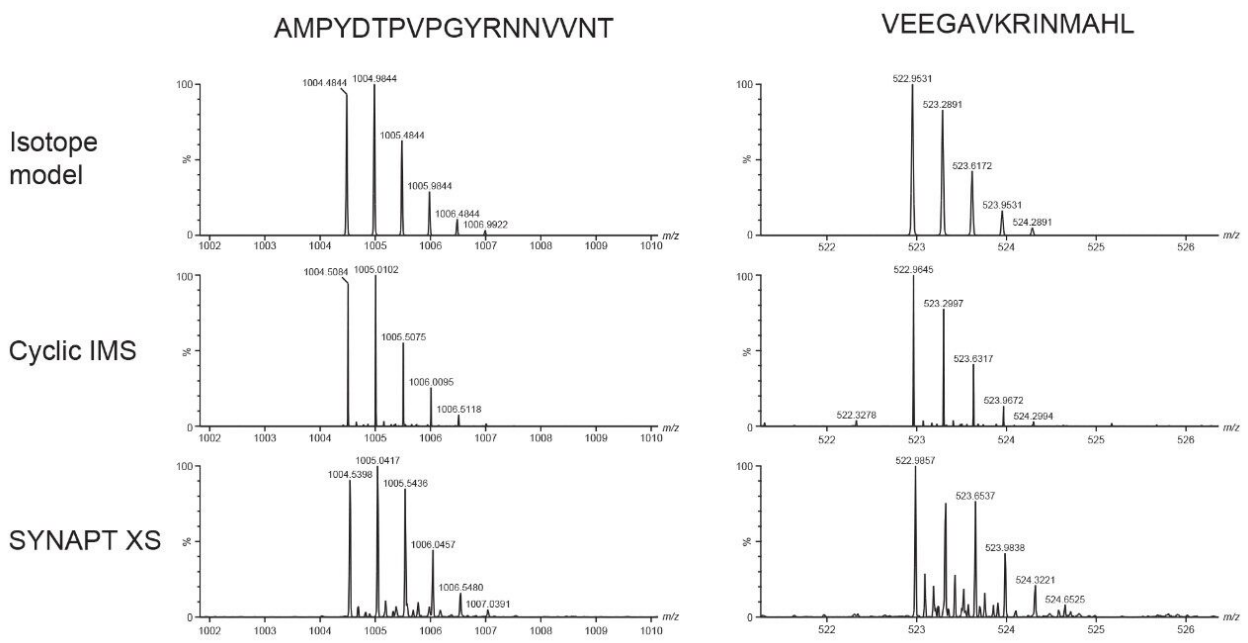

Figure 3. Isotope distributions for peptides AMPYDTPVPGYRNNVVNT and VEEGAVKRINMAHL obtained on the SYNAPT XS (bottom) and Cyclic IMS (middle). The theoretical isotope distribution for each peptide is shown on the top for reference.

Peptide identifications and sequence coverage for phosphorylase B were examined using 8, 16, and 32 µM stock solutions to evaluate HDX performance on the Cyclic IMS under more sample-limited situations. Using the labeling (1:20) and quench dilutions (1:1) and a 50 µL loop, this corresponds to 10, 20, and 40 pmol injections. Data was collected in HDMS^E mode using the same conditions described above. The data was processed using PLGS 3.0.3 but with modified Peptide 3D parameters used to specifically account for the slightly wider arrival time distributions observed with longer mobility separations. This had the effect of increasing the number of peptides in the 32 µM stock solution injections to 588. Figure 4 summarizes the results of this experiment. As expected, lower loads yielded fewer peptide identifications; however, it should be noted that the same thresholds were used for all three conditions. It may be possible to recover some peptide identifications by lowering the thresholds for the lower load data. Nevertheless, better than 90% filtered sequence coverage was obtained for the 16 and 8 µM solutions, 93%, and 95%, respectively, indicating that good performance HDX peptide mapping can be achieved with lower concentration stock solutions, allowing for study of more sample limited proteins.

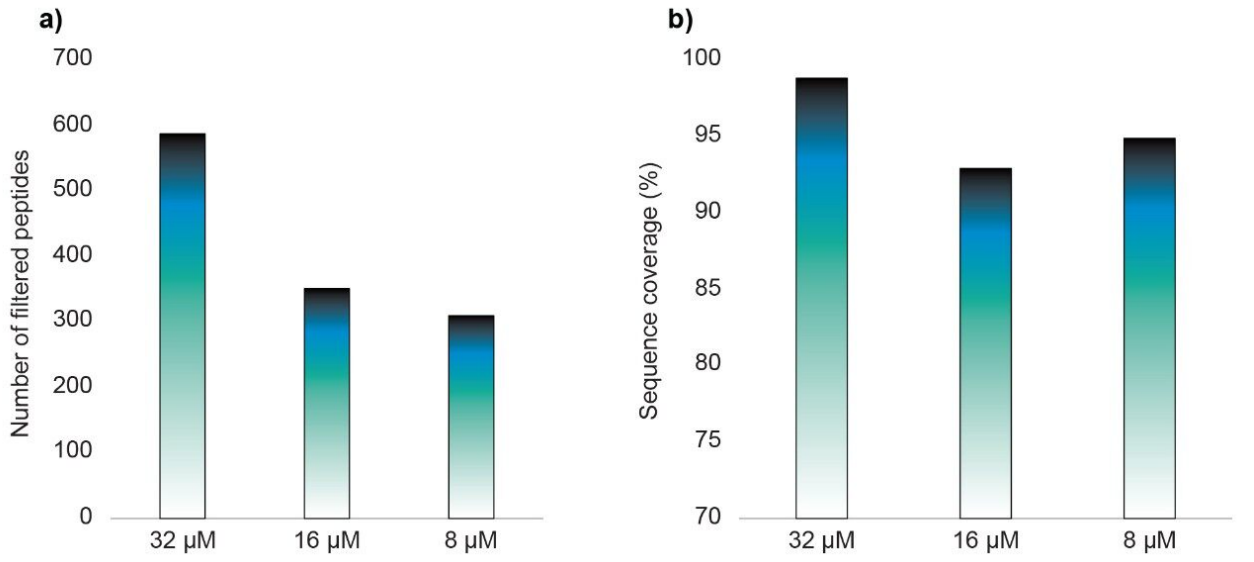

Figure 4. Number of identified peptic peptides and sequence coverage following filtering for phosphorylase B using 32, 16, and 8 µM stock solutions for online HDX peptide mapping with the Cyclic IMS.

Conclusion

We have previously shown a significant improvement in the quality of HDX data obtained from the SYNAPT XS compared to the older generation system, SYNAPT G2-*Si*. Here, the new SELECT SERIES Cyclic IMS is demonstrated to offer the highest level of performance and has a profound impact on the quality of data obtainable for hydrogen-deuterium exchange experiments. Improved mass and ion mobility resolution and sensitivity result in 30% more peptide identifications than on the SYNAPT XS while the dynamic range afforded by the dual gain detector allows for more accurate measurement of isotope distributions for highly abundant peptides. The improved sensitivity of the system permits a 4x load reduction while retaining better than 95% sequence coverage. This demonstrable improvement to peak capacity and sensitivity is expected to afford use of truncated chromatographic gradients, enhancing daily throughput, and reducing the per injection cost of the system.

References

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3. Sørensen, L., Salbo, R. Optimized Workflow for Selecting Peptides for HDX-MS Data Analyses. *J. Am. Soc. Mass Spectrom.* 29, 2278–2281 (2018). https://doi.org/10.1007/s13361-018-2056-1 < https://doi.org/10.1007/s13361-018-2056-1> .

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