AUTOMATED ANALYSIS OF HYDROGEN / DEUTERIUM EXCHANGE MASS SPECTROMETRY DATA USING DYNAMX

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INTRODUCTION

Hydrogen/deuterium exchange mass spectrometry (HDX MS) is a useful analytical method for the study of protein dynamics and changes to protein conformation. Recent improvements in LC-MS systems have made HDX MS an indispensible tool for the discovery and development of protein drugs.

Conventionally, HDX data are interpreted manually or - at best - processed with semi-automated tools to determine the deuterium uptake at peptide level. This is time-consuming because of the need to track hundreds of peptides across multiple time-courses in comparative analyses. In order to improve the efficiency of data processing, an innovative HDX software tool, DynamX was deployed in this study.

The automated software extracts information on peptic peptides using retention time, intensity, fragment ions, drift time, and mass accuracy. DynamX tracks all peptides that are reproducibly found in replicates, ensuring consistency in monitoring the deuterium exchange. The software also calculates the amount of deuteration and displays the results in convenient comparative views: uptake curves, a 'butterfly' chart, and a difference chart. The data processing time is significantly reduced from months for manual processing to hours for automated processing. Ion mobility separation (IMS) is used in the HDX LC-MS workflow, providing additional, orthogonal separations to chromatography and mass dimensions. Overlapping interfering ions are successfully resolved by IMS and displayed in DynamX.

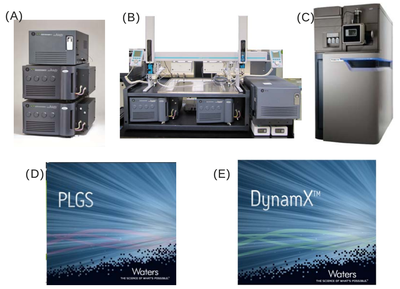

Figure 1. Waters HDX system solution includes a nanoACQUITY UPLC with HDX technology (A), A Leap automation manager (B), Synapt G2 HDMS (C). Waters software package for automated HDX data processing. Both ProteinLynx Global Server 2.5 (D) and DynamX software (E) were deployed for peptide identification and deuteration calculation, respectively.

METHODS

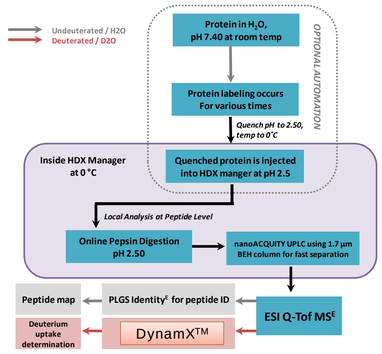

Figure 2. HDX workflow at peptide level. For undeuterated peptides, the digest was labeled with H2O, quenched, and separated. LC-MS^E data were processed in PLGS in order to construct the peptide map. The protein was labeled with D2O, and deuterated peptides were processed in DynamX software to calculate the deuterium uptake.

LC / MS system

Waters nanoACQUITY UPLC® with HDX Technology
- Binary Solvent Manager (BSM)
- Auxillary Solvent Manger (ASM)
- HDX Manager

Waters Synapt™ G2 HDMS™
- ESI positive mode
- Capillary / Cone : 3.0 kV / 37 V
- Source / Desolvation : 80 °C / 175 °C
- Desolvation gas : 800 L/h

Chromatography

Analytical column was an ACQUITY UPLC® BEH C18 1.7 µm 1.0 x 50 mm. The trap column was an ACQUITY VanGuard® Pre-column, BEH C18 1.7 µm 2.1 x 5 mm. Online pepsin digestion was performed using a 2.1 x 30 mm immobilized pepsin column (Applied Biosystems).

MS Data Collection and Processing

MS^E data were collected for all analyses. Undeuterated analyses were processed using ProteinLynx Global Server (PLGS) 2.5 with Identity^E informatics. DynamX was used to measure the deuterium uptake of each peptide as a function of deuterium exposure time.

RESULTS AND DISCUSSION

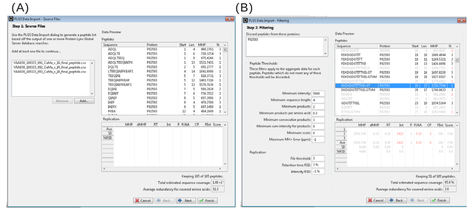

Figure 3. Importing PLGS output files (A) and peptide filtering (B). In these steps the reproducible peptic peptides are confidently selected from the PLGS database search in replicated digests.

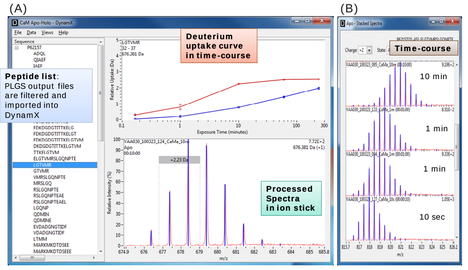

Figure 4. Processed results displayed in DynamX main window: a peptide list, uptake curves, and spectra were shown in left, top right, and bottom right panels, respectively (A). The stacked spectra in time-course were shown (B).

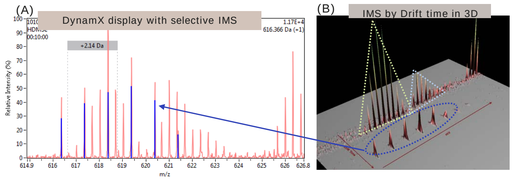

Figure 5. Spectra obtained for a peptide, ATLGLAA (+1, residue 149-155) from a phosphorylase b digest after a ten minute deuterium exposure. The two dimensional plot (A) shows a complex spectra resulting from the superimposition the target +1 isotope cluster (indicated by blue overlay) with two other +3 clusters. A three dimensional plot (B) reveals that each of the three clusters are baseline resolved in the mobility dimension. The ion mobility separation allows to resolve the interfering ions that belong to other peptides. This is helpful to be able to make reliable calculation of deuterium uptake of the peptide of interest.

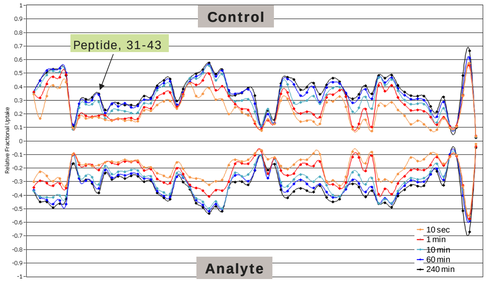

Figure 6. Butterfly chart displayed in DynamX. Two different batches of samples were compared in a relative fractional uptake. Each point represents a peptide that had a uptake in 0.17, 1, 10, 60, and 240 min of labeling time (orange, red, light blue, dark blue, and black, respectively). The y-axis is the fractional deuterium uptakes that the mass was not corrected for back-exchange. The plotting algorithm was published in elsewhere[1].

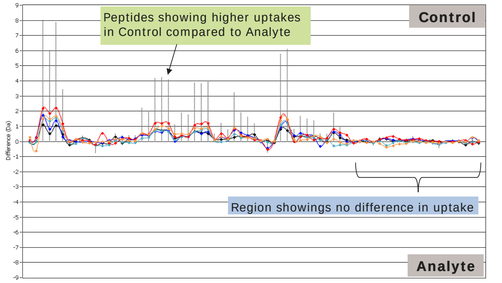

Figure 7. Difference chart displayed in DynamX. This chart takes the average relative uptake measured and subtracts the value measured for each peptide and exposure time. The horizontal coordinate and plot colors are the same as in Figure 6. The measured differences are near zero for most peptides, indicating there is little measureable difference in deuterium uptake between Control and Analyte. Positive values in vertical bars indicate greater deuterium uptake in Control. These peptides from Control had significantly different uptakes, which mean that this region is where the conformational change occurred in corresponding peptides. This format of comparability chart helps to interpret the HDX results efficiently.

Figure 8. Reduced data analysis time using DynamX software. Conventional data processing was done manually, however with this new HDX software the data can be processed automatically. A large number of spectra were reviewed in typical HDX experiments for each uptake calculation at multiple time-points, replication, and multiple batches. For example, approximately 8280 spectra were collected for this IgG study as described below.

- *# of peptides generated per IgG = 230*
- *# of labeling time-points (0, 0.17, 1, 10, 60, 240 min) = 6*
- *# of batches (control, 2 mutated batches) = 3*
- *# of replication = 2*
- *230 x 6 x 3 x 2 = 8280 spectra to process*

A fully automated data processing utilizing DynamX software significantly reduces the data analysis time compared to manual processing. The completed data analyses can be done within 1-4 days.

CONCLUSION

- **Waters offers a complete HDX system solution including an informatics package for protein conformation analyses.**
- **The data analysis time was significantly reduced from a full month of manual processing to days of automation using DynamX.**
- **Ion Mobility MS data processed using DynamX offers higher degree of data mining capability.**
- **Effective data visualization tools help to understand the complex HDX data sets qualitatively and quantitatively.**

References

1. Houde et.al. (2011) J. Pharm. Sci., 100, (6) 2071-2086

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