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 indispensable 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-points in comparative studies. With new HDX software, the data can be processed automatically. An innovative HDX software tool, DynamX, was deployed in this study.

Figure 1. Waters HDX system solution includes a nanoACQUITY UPLC LC / MS system (A), a Leap automation manager (B), Synapt G2 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 0.15 x 5 mm immobilized pepsin column (Applied Biosystems).

The completed data analyses can be done within 1-4 days for automated processing. An 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 2. HDX workflow at peptide level. For undeuterated peptides, the signal size and shape (C) will differ from that of deuterated peptides (D). In both cases, the protein is labeled with D2O (O) and separated. LC-MS E MSE data were collected for all analyses. Underdeuterated analyses were processed using ProteomeX Global Server (PLGS) 3.5 with identity information. DynamX was used to measure the deuterium uptake of each peptide at a function of deuterium exposure time.

Figure 3. Importing PLGS output file (A) and peptide filtering (B). In these steps the non-native peptide peaks are completely removed 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 frames, respectively (A). The stacked spectra in time-course were shown above (B).

Figure 5. Spectra obtained for a peptide, APGLAG (+1), residue 144-150 from a phosphatase 2a digest after a two minute deuterium exposure. The two dimensional plot (A), where a contour plot is relating from the intensification of +3 deuterium uptake to the relative intensification of the +1 deuterium uptake, can be used to resolve interfering ions that belong to other peptides. This is helpful to be able to resolve interfering ions that belong to other peptides. This is helpful to be able to resolve interfering ions that belong to other peptides.

Figure 6. Results of data analysis times using DynamX software. Deuterium uptake measurements were made for each peptide at the indicated time points. In Figure 6, the measured differences are near zero for most peptides, indicating that there is little measureable difference in deuterium uptake between Control and Analyte. Positive values in vertical bars indicate greater deuterium uptake in Control. These peptide from Control had significantly different uptake, which means that this region is where the conformational change occurred. The format of comparison chart helps to view the data as shown below.

CONCLUSION

- Waters offers a complete HDX system solution including an informatics package for protein conformation analysis.
- 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 HDX MS data set qualitatively and quantitatively.

References


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