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The Development of a Fully Customized HDX-MS Platform with Exceptional Performance on Back-Exchange, Robustness and Reproducibility

Introduction

As an analytical technique, HDX-MS has been widely applied for studying protein folding, protein structural alteration due to ligand binding, protein-protein interaction and protein modifications in academic research groups. Recently, industry application of HDX-MS has been rapidly growing, including applications on epitope mapping and biosimilar developments. Here we present data generated from a customized CTC-PAL platform for automated HDX-MS experiments. The system was designed to allow versatile control of each HDX experimental steps and to minimize the back-exchange level.

Methods

A dual-valve set-up was designed to enable online protease column digestion. A three-component cooling system was designed to control temperature for HD exchange, digestion and LC-injection separately. We selected MeCour to manufacture the cooling system to allow rapid and precise control of temperatures during HDX reactions and quenching. The Cycle composer software was used to accurately control the timing for HDX and deanaturation/quench duration.

An antibody purchased from Waters was used to perform HDX experiments. Three different protease columns were tested to maximize the number of overlapping peptides. Proteolytic peptides were separated using a customized C8 column and monitored by Q Exactive MS. The MS/MS raw data were searched using PepFinder. The deuterium uptake values were analyzed by HDX workbench.

Preliminary Data

We have tested the deuterium back exchange of our customized HDX-MS platform using a fully deuterated glufib peptide. All the parameters and HDX-MS platform configuration remain unchanged except for putting on a connection union instead of an enzyme column during the deuterium back-exchange test. Δm=11.0 Da was obtained (the theoretical maximum Δm for glufib is 12 Da), which demonstrates the exceptional performance of this system on deuterium retainability. Incorporating our in-house made enzyme columns (pepsin, protease type XVIII and protease XIII), we consistently obtained 100% sequence coverage with significant increased sequence redundancy for a commercially available antibody and most of other proteins being tested in this customized HDX-MS platform. The retention time shift and the relative standard deviation of measured deuterium uptake for almost all the detected peptides in triplicated runs are within 0.2 min and less than 5%, respectively, which demonstrates the excellent reproducibility of the developed HDX MS platform.

Novel Aspect

A fully customized HDX-MS platform was developed with exceptional reproducibility, robustness, and deuterium retainability.