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

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Novel Aspect

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

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

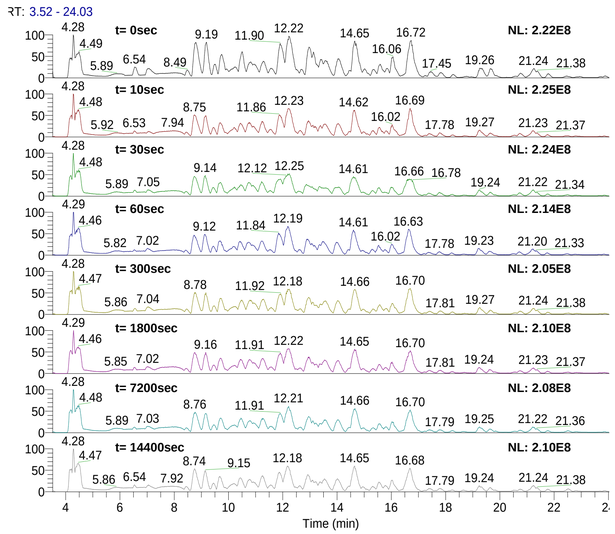
A dual-valve set-up was designed to enable online protease column digestion. A three component cooling system was designed to control temperature for HD exchange, digestion and LC-injection separately. We selected MeCour to manufacture the cooling system to allow rapid and precise control of temperatures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

HDX automation: CTC-PAL coupled with MeCour's temperature control systems
Enzyme columns: NBA_pepsin, NBA_pepsin-XIII dual(w/w, 1:1)
Quench condition: 2M guanidine HCl/0.425M TCEP (final pH2.5).
MS: Q Exactive MS plus.
Database search software: Mascot.
HDX raw data analysis: HDX workbench.

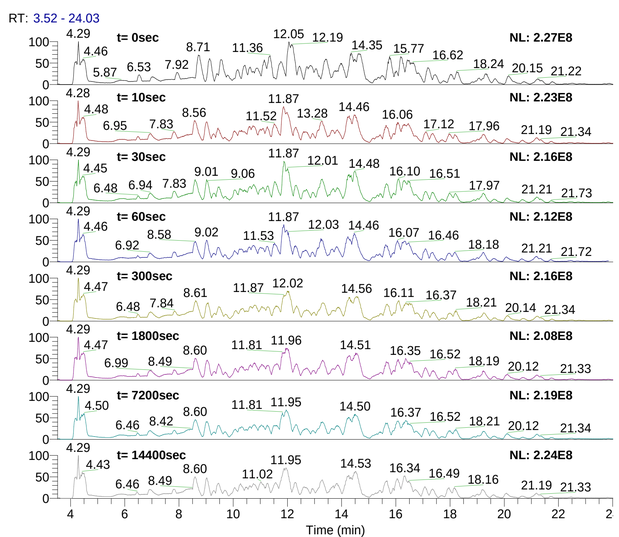
Results

Figure 1. Robustness evaluated by performing epitope mapping for Herceptin (an anti-human Her-2 mAb).

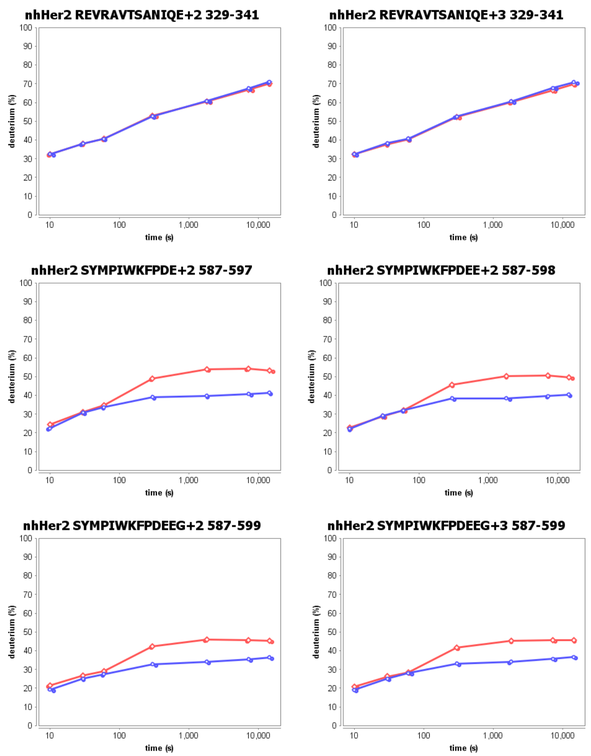
(a) BPC for Her-2 alone.



(b) BPC for Her-2+Herceptin.



(c) Representative deuterium uptake plots.



(c) Differential heat map for Her-2 and Her-2+Herceptin.

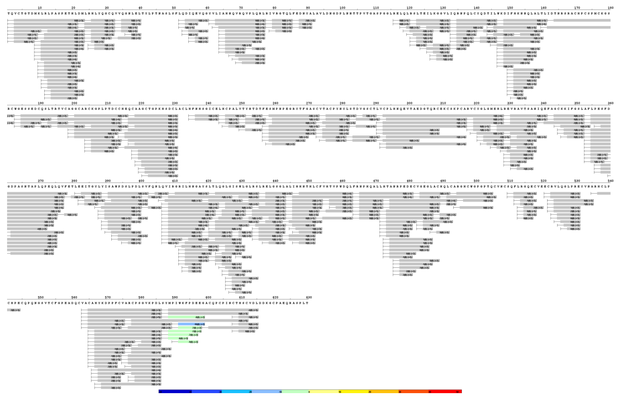


Figure 2. High deuterium retainability (low deuterium back exchange) evaluated using fully deuterated Glufib.

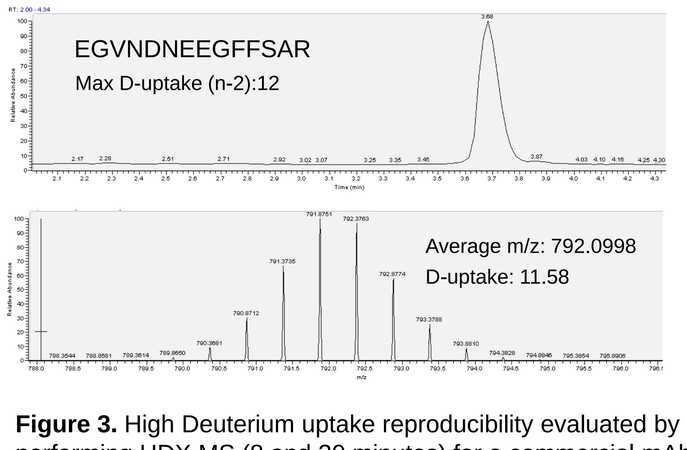
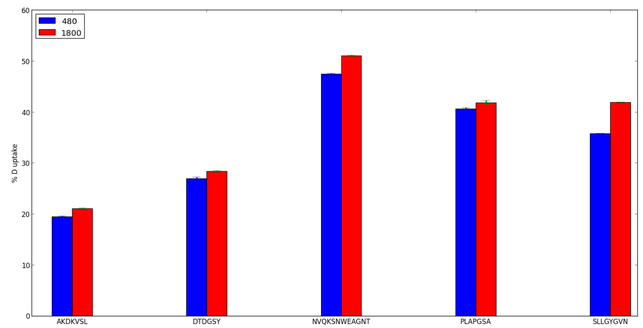


Figure 3. High Deuterium uptake reproducibility evaluated by performing HDX MS (8 and 30 minutes) for a commercial mAb in triplicates. All standard deviations are less than 0.6%.



Conclusions

- ✓ **The performance of the system has been optimized so that the retention time variation is less than 0.2 min in a relative long 20 minute gradient.**
- ✓ **The fact that the fully deuterated peptide standard showed minimum back-exchange level ensures the system to reveal true conformation information without suffering from back-exchange.**
- ✓ **The excellent reproducibility of the system allows standard deviation of D-uptake levels to be less than 0.05-0.1 Da or 1%, which is essential to confidently monitor subtle differences (2%) for protein solution conformation.**