NEW FEATURES IN BIOINFORMATICS SOFTWARE FOR AUTOMATED PROCESSING OF HDX MS DATA

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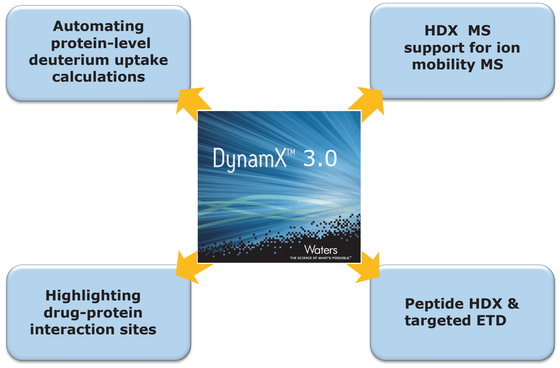
INTRODUCTION

DynamX is an HDX data analysis software, which is designed to systematically select spectra and measure the mass change of the deuterated form. The most recent version (DynamX 3.0) with new features allows researchers to assess protein conformational changes quickly in a fully automated fashion:

- **Processes HDX data at the global, peptide, and fragment levels**
- **Displays the results in comparative views**
  - **Uptake Curves and Charts**
  - **Butterfly and Difference Plots**
  - **Coverage maps with enhanced visualization options**
  - **Heat map (automatically transfers HDX data to the 3D structure in PyMOL)**

To highlight improvements made in DynamX 3.0, the structural stability of human IgG2 was studied under denatured conditions using HDX MS. The conformational changes of IgG2 caused by addition of Guanidine·HCl are displayed here. This study demonstrates the susceptibility of IgG2 to denaturation and ranks its structural stability. Several regions in CH2, CH1, CL domains are disturbed in different degrees by denaturation and display more solvent accessibility. The most stable domain is CH3.



METHODS

The antibody IgG2 samples (Denosumab, Amgen) at 1 mg/ml in 200 mM phosphate buffer, pH 6.8 with 1.0 M guanidine hydrochloride were incubated at 25 °C for 18 h. The samples were then labeled with D2O buffer at 25 °C for various time. The labeling reactions were then quenched by reducing temperature to 0 °C and pH to 2.5.

Intact Protein Analysis

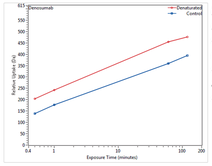


Figure 1. *The intact deuterium uptake plot. The native (control) and denatured forms of IgG2 were compared in blue and red, respectively. The uptake curves were plotted in the relative deuterium level in y-axis as function of time in x-axis. They are relative uptake values and no back-exchange correction was applied.*

Peptide Analysis

Uptake, Butterfly and Difference Plots

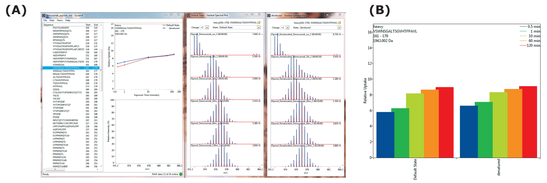


Figure 2. (A) *Peptide list is displayed in the left panel of the main window. The relative uptake curve of representative peptide (HC 161-179) is shown in the top right panel. The raw spectra of the peptide in the +2 charge for both states are displayed in stacked spectral plot for 5 labeling time points (0, 30 sec, 1 min, 10 min, 60 min, and 120 min).* ***(B)*** *The uptake chart.*

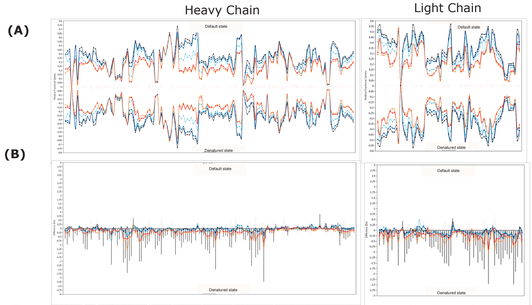


Figure 3. (A) *Butterfly plot: the relative deuterium uptake is plotted for both native IgG2 (top) and denatured IgG2 (bottom). Each point along the x-axis corresponds to an individual peptide. The yellow, red, cyan, blue and black lines correspond to data acquired at 0.5, 1, 10, 60, 120 min of deuterium labeling. Each data point is an average of three experiments and the standard deviation is shown in gray.* ***(B)*** *Difference plot. The differential uptake (ΔD) is plotted for comparison. The gray line is the cumulative differential uptake from all 5 time points.*

RESULTS AND DISCUSSION

Heat Map

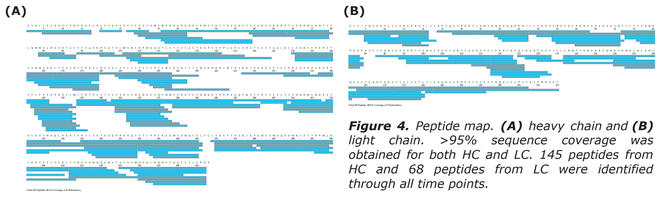


Figure 4. *Peptide map.* ***(A)*** *heavy chain and* ***(B)*** *light chain. >95% sequence coverage was obtained for both HC and LC. 145 peptides from HC and 68 peptides from LC were identified through all time points.*

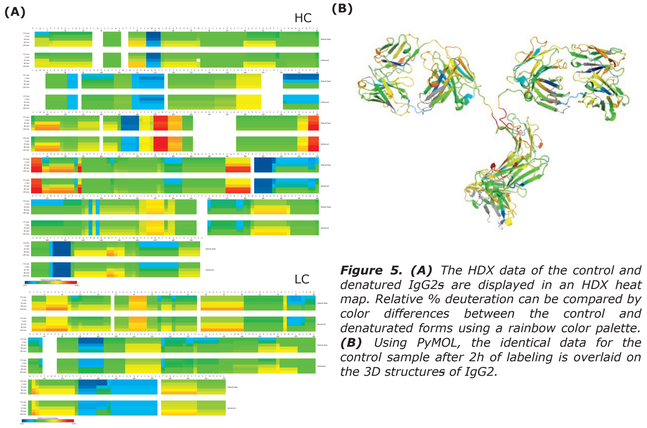


Figure 5. (A) *The HDX data of the control and denatured IgG2s are displayed in an HDX heat map. Relative % deuteration can be compared by color differences between the control and denatured forms using a rainbow color palette.* ***(B)*** *Using PyMOL, the identical data for the control sample after 2h of labeling is overlaid on the 3D structures of IgG2.*

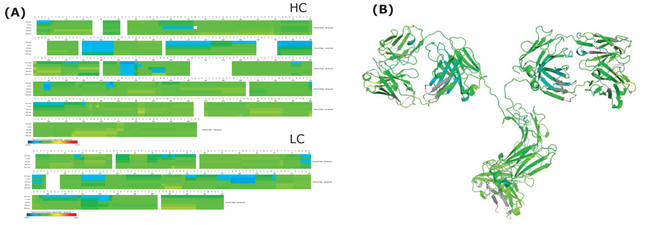


Figure 6. (A) The comparison of the relative fractional uptake of the two states is displayed on the heat map of IgG2. Using a rainbow color palette, the % difference is shown. **(B)** Using PyMOL, an example of the differential fractional uptake of 2 hr labeling is shown on the 3D structure of IgG2.

Coverage Map

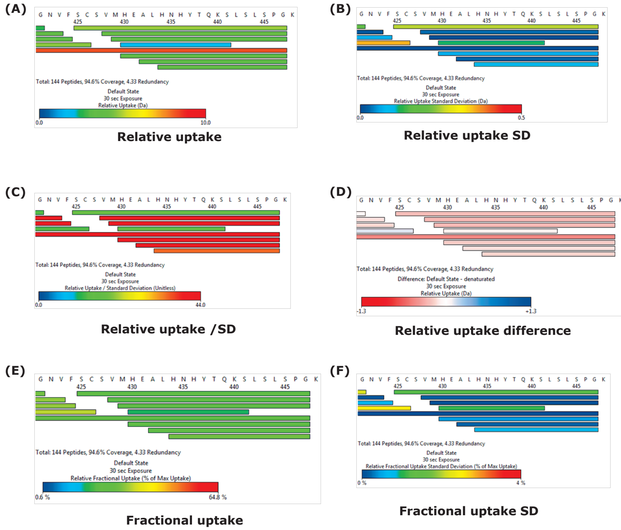


Figure 7. New features of coverage map.

CONCLUSION

- **New features of DynamX:**
  - Automates processing of intact protein, peptide digest, and electron transfer dissociation (ETD) HDX data.
  - Communicates HDX uptake and sample differences through versatile coverage map and heat map displays.
  - Facilitates localization of structural differences between samples, conditions, states, and time courses.
  - Exports to PyMOL (Schrödinger) for structural modeling of HDX-MS data.
- **The case study of IgG2 indicates:**
  - Most regions of IgG2, except the CH3 domain, lost their native confirmation under denatured conditions
  - The data demonstrates the susceptibility of IgG2 to denaturation and ranks its structural stability.

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