[PRODUCT SOLUTION]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

DynamX HDX Data Analysis Software 3.0

Simplifying Hydrogen Deuterium Exchange Interpretation

PROTEIN CONFORMATION ANALYSIS MADE SIMPLE

In HDX MS studies, replicate data are collected across multiple time points and varied species (native vs. mutant, innovator vs. biosimilar, bound vs. unbound). Manually curating this data is not time-efficient and requires expert interpretation.

DynamX™ HDX Data Analysis Software 3.0 is designed to systematically select spectra and measure the mass change of the deuterated form. Taking advantage of the sharper peaks and better separations available with UPLC,® along with comprehensive MS^E detection, the software is able to automate data sorting and display – an important improvement for HDX MS.

Analysis time is significantly reduced with the automated capabilities of DynamX Software. Proteins, peptides, and fragments are tracked across replicates, ensuring consistent monitoring of deuterium uptake. The software conveniently displays the results in comparative views: uptake curves, butterfly charts, coverage maps, and difference charts.

INDUSTRY LEADING HDX MS INFORMATICS

DynamX Software helps researchers assess possible conformational changes in their proteins quickly, as well as:

- Automates processing of intact protein, peptide digest, and electron transfer dissociation (ETD) fragment level HDX data.
- Supports ETD fragment analysis for residue-specific structural information.
- Communicates HDX uptake and sample differences through enhanced coverage map and heat map displays.
- Processes and displays ion mobility separation (HD MS^E) data for more in-depth protein coverage.
- Facilitates localization of structural differences between samples, conditions, states, and time courses.
- Supports interpretation of protein-ligand interactions and binding dynamics.
- Exports to PyMOL (Schrödinger) for structural modeling of HDX MS data.

PROTEIN CONFORMATION ANALYSIS MADE SIMPLE

The ACQUITY UPLC® M-Class System with HDX Technology provides a robust platform to study changes in higher order protein structure.

When used with Waters SYNAPT® and Xevo® MS technologies, MassLynx® Software, innovative DynamX HDX Data Analysis Software 3.0, and ProteinLynx Global SERVER™ (PLGS) Software, the ACQUITY UPLC M-Class System with HDX Technology enables automated determination of changes in protein conformation through confident identification and best-in-class reproducibility.

DISCOVER MORE INFORMATION ABOUT YOUR PROTEIN

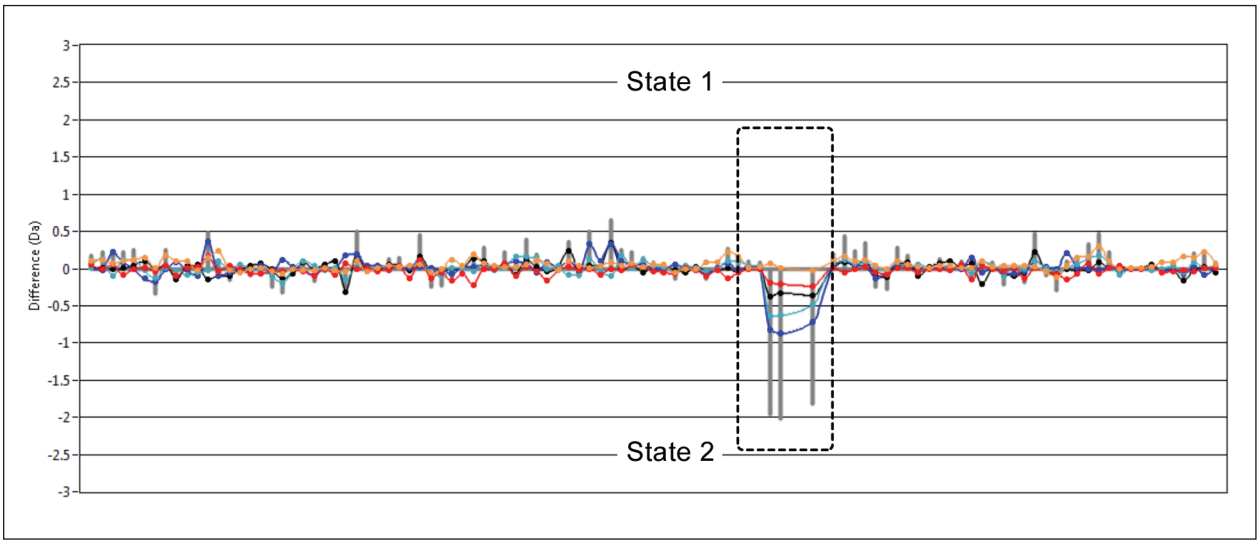
Waters HDX Technology enables in-depth data mining, such as:

- **Drug candidate binding to a protein target molecule**
- **Protein-protein interactions**
- **Biopharmaceutical product development**

LOCALIZE STRUCTURAL DIFFERENCES BETWEEN SAMPLES, CONDITIONS, STATES, TIME COURSES

When trying to localize structural differences, the user wants to quickly interpret results and accurately identify the sites of modification. Enhanced visualization options in DynamX Software provide the user with the tools necessary to complete these activities with confidence.

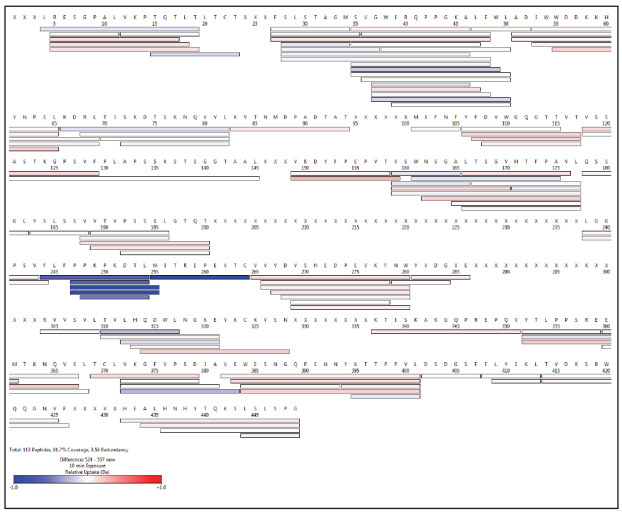
The Butterfly Difference Plot clearly identifies local structural effects limited to a site of mutation.



Butterfly Difference Plot in DynamX Software.

The Coverage Map is also included, highlighting newly enhanced visualization options in DynamX Software that simplify the process of defining structural differences.

Coverage map showing fractional uptake data in difference mode.

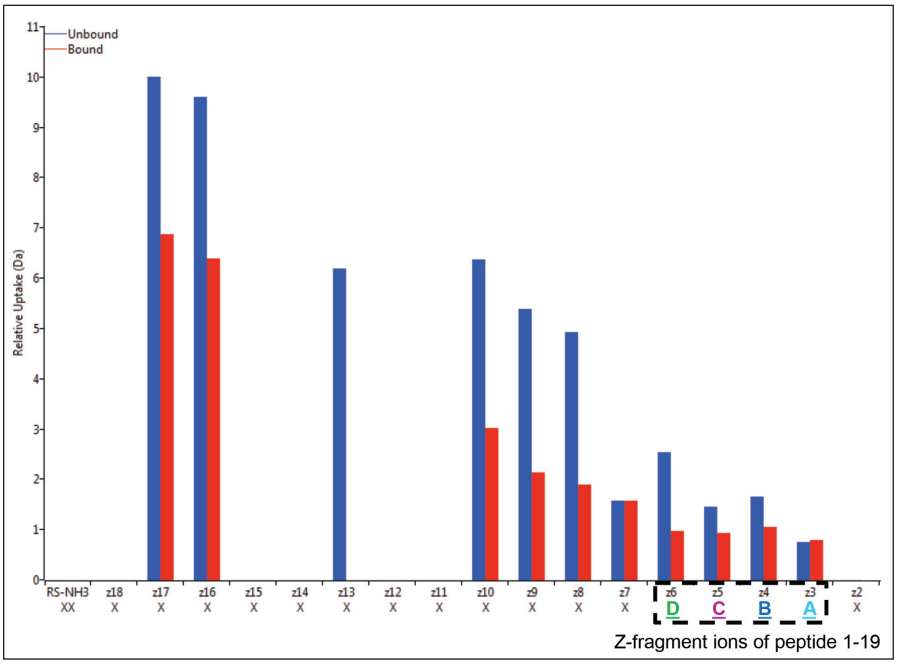


Left, using a rainbow palette; right, using a blue-white-red palette.

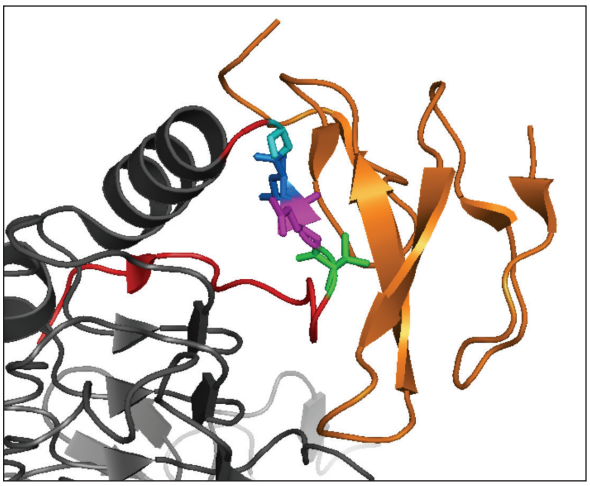
ETD FRAGMENTATION FOR RESIDUE–SPECIFIC STRUCTURAL INFORMATION

DynamX Software can now automatically process ETD data. Based on the ETD fragmentation data of the unbound and bound peptide, the user is able to locate the exact binding site down to the amino acid residue level.

The D C B A in the chart below correspond to the colored amino acids in the structural model. Significant uptake difference was observed between the bound and unbound forms evidenced by the z-ion fragment series. The unbound protein had more deuterium uptake at the binding site, while the bound protein had less as a result of protection from exchange by the interaction.



Significant uptake difference was observed between the bound and unbound forms in the z-ion series. Lower uptake in the bound form was caused from protection from exchange.



Bound (EGFR+Adnectin): colored residues15-18 are a known region of interaction (Ramamurthy, et. al., Structure, 2012, 20, 259–269).

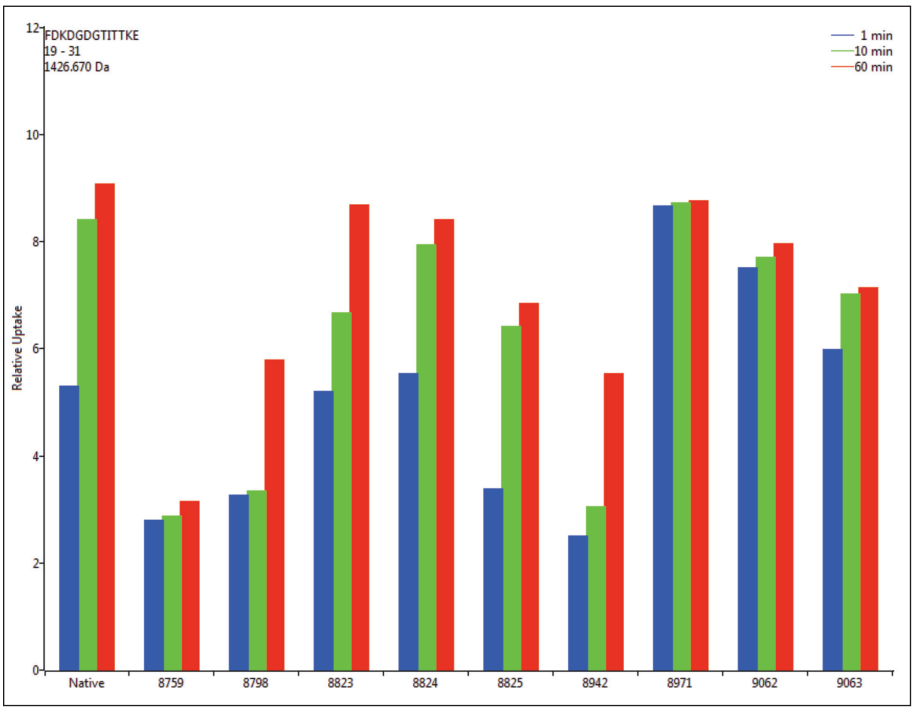
DYNAMX SOFTWARE SIMPLIFIES HDX INTERPRETATION BY PERFORMING THESE TASKS:

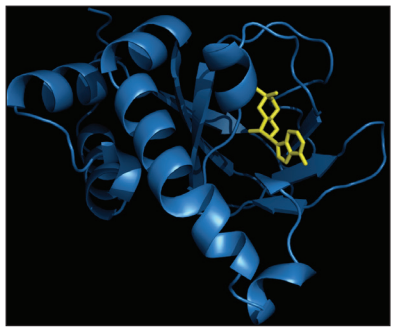
- Aggregates search results from ProteinLynx Global SERVER (PLGS) to generate a list of peptides for interrogation during the experiment.
- Processes mass spectrometer data files to search for ions belonging to each peptide.
- Determines deuterium uptake automatically.
- Summarizes data as a function of replication and experimental state.
- Visualizes data for easier interpretation.
- Allows interaction with data; easily navigate data and modify results if necessary.

INTERPRET PROTEIN-LIGAND BINDING SITE DYNAMICS

HDX MS answers the question, "Where does a molecule interact with the target protein?" This powerful method is extensively used to map protein-ligand and protein-protein interactions. Recently it has also been applied to monitor drug-protein interactions and the mapping of conformational dynamics of the transient protein complexes.

DynamX Software provides enhanced visualization for protein-ligand binding studies. In a small molecule binding experiment, the native state and several small molecule candidates and their binding (altered exchange) results over three time points (1, 10, and 60 min) are charted. DynamX Software also supports such protein-ligand binding studies at the intact level.





Native state and several small molecule candidates are compared over three time points.

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