[PRODUCT SOLUTION]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

ACQUITY UPLC M-Class System with HDX Technology

Integration of Hydrogen Deuterium Exchange with High Resolution MS

QUANTIFY CHANGES IN PROTEIN CONFORMATION WITH CONFIDENCE

The Waters® ACQUITY UPLC® M-Class System with HDX Technology provides high resolution MS and intuitive informatics enabling confident measurement of changes in protein conformation by controlling important experimental parameters.

Hydrogen Deuterium Exchange combined with mass spectrometry (HDX-MS)

Since the first use,[1] Hydrogen Deuterium Exchange (HDX) has relied upon the mass difference between Hydrogen (H) and Deuterium (D) to determine changes in higher order structure. Over the ensuing 50 years, high resolution MS has provided HDX with increased accuracy, higher precision, while UPLC enabled fast separations of peptides and, in turn, the ability to analyze larger proteins.

Today scientists use HDX routinely to determine:

- Conformation in biopharma product development
- Lot-to-lot comparability
- Process changes
- Biosimilar development
- Protein dynamics
- *In vitro* stability
- Folding kinetics
- Protein ligand interactions
- Drug candidate selection
- Epitope mapping
- Membrane receptor interfaces

HDX is among the least invasive probes to study protein conformation. Proteins in physiological buffers will exchange acidic protons in relation to their solvent accessibility and Hydrogen bonding. A change in the rate or number of exchanges of H for D indicates a change in protein conformation. Once H exchanges for D at a given time point, the reaction must be frozen in time for analysis. Years ago, researchers used homemade LC set ups with various components held at 0 °C that may have yielded poor separation efficiency. Results management was complicated because of vast quantities of high resolution data. These analytical hurdles were severe challenges to routine use and adoption of HDX – until now.

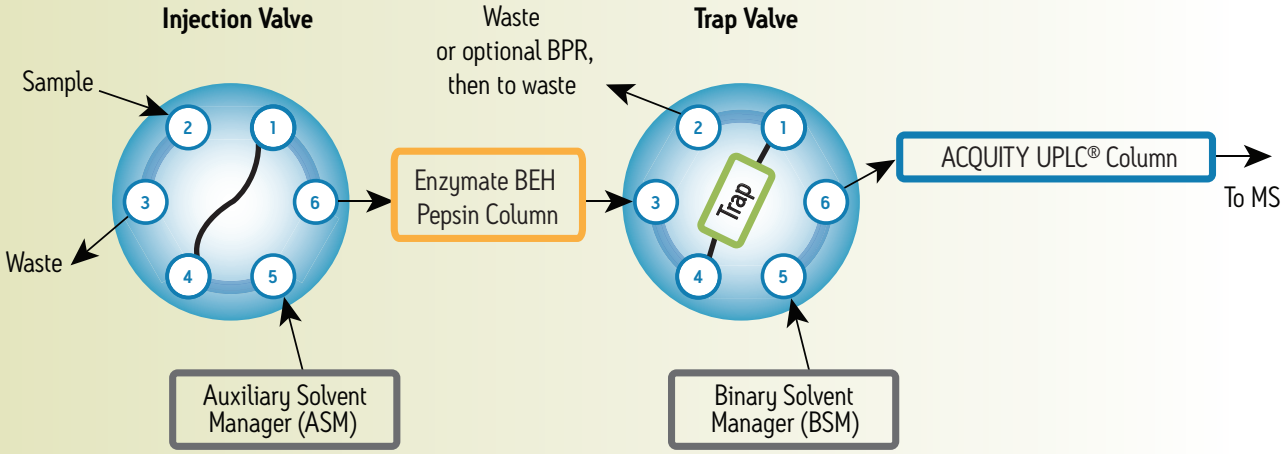
For the past 10 years, Waters has collaborated with HDX thought leaders worldwide to develop and refine an integrated HDX-MS system based on UPLC® and Waters high resolution MS.[2] The ACQUITY UPLC M-Class System with HDX Technology enables reproducible sample preparation, UPLC separation, and results management – that does not require weeks (and weeks) of manual data analysis.

ACQUITY UPLC M-Class System with HDX Technology provides:

- Rapid chromatographic resolution at 0 °C with UPLC Technology
- Intuitive, interactive DynamX™ HDX Data Analysis Software, shortening the time between experiment and answers
- Repeatable peptic digestion using Waters Enzymate™ BEH Pepsin Column, with option to digest at 15,000 PSI
- Superior reproducibility and reliability
- Integrated with Waters high resolution MS for precise determination of Deuterium label
- Added dimension of separation with IMS Technology

ACQUITY UPLC M-Class System with HDX Technology.

UNPRECEDENTED HDX SYSTEM TECHNOLOGY



Fluidic model of the ACQUITY UPLC M-Class HDX Manager.

HDX fluidics – inside the module

The ACQUITY UPLC M-Class HDX Manager is more than just a cold box. It contains two (2) software-driven 6-position valves, an injection valve and trapping valve, maintained at 0 °C. These valves service a trapping and analytical column for UPLC resolution. A pepsin column resides (yellow box) within a separate, temperature controlled compartment.

- The injection valve accepts sample into the sample loop. For local analysis at the peptide level, the sample elutes into the pepsin column using eluent from the Auxiliary Solvent Manager (ASM).
- The on-line digestion typically occurs within 30 seconds. The Back Pressure Regulator (BPR) modulates the pressure on the pepsin column.
- Following digestion, the micro Binary Solvent Manager (µBSM) elutes and traps the peptides on to a trapping column, washing unwanted solutes to waste.
- Once the peptides are retained, the trapping valve state changes, and gradient elution begins.
- Peptides elute from the trapping column and are refocused on the ACQUITY UPLC M-Class Column for analysis by high resolution MS.
- If intact analysis is required, the sample bypasses the pepsin column and proceeds directly to the trapping column and analytical column as previously described.

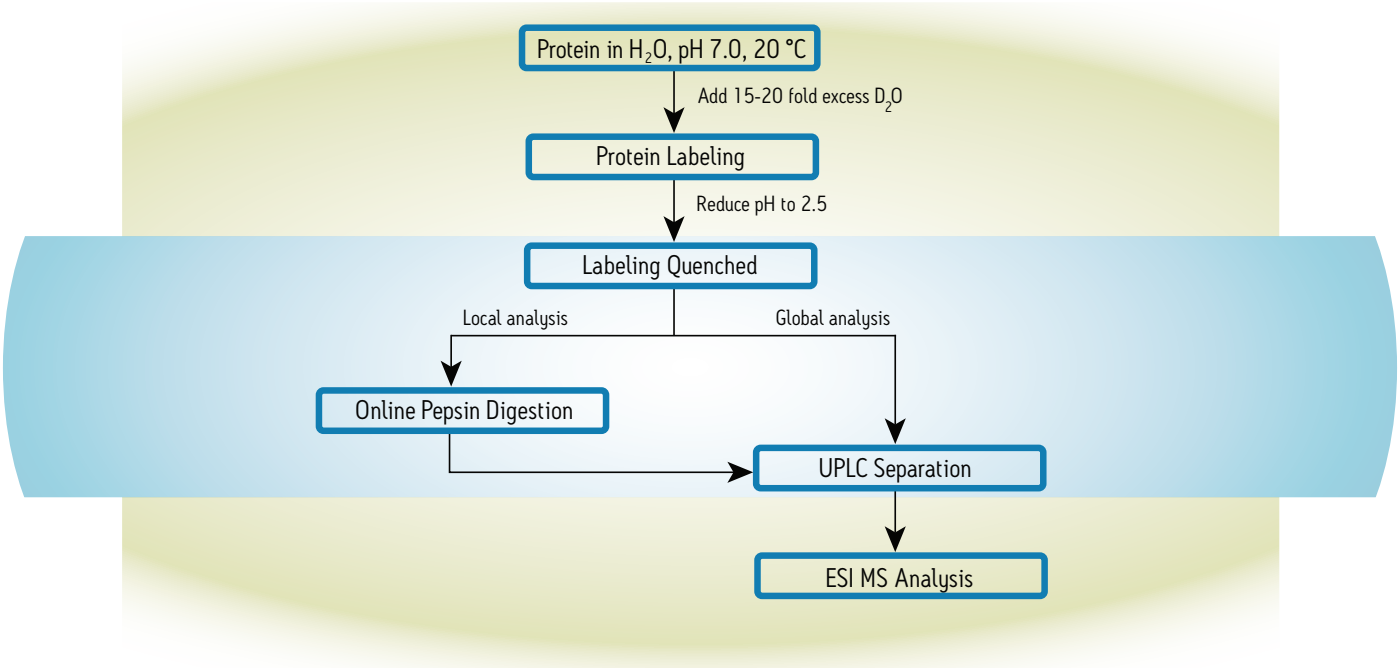
UNATTENDED OPERATION FOR THE MOST COMPLEX HDX EXPERIMENTS

The LEAP HDX-2 Automation Manager schedules the experiments and automates the steps in an HDX protocol leading to increased throughput, precision, and accuracy.

The LEAP HDX-2 Automation Manager uses the Chronos scheduling application to automate labeling, quenching, and injection into the ACQUITY UPLC M-Class HDX Manager for global or local analysis, providing unattended operation for a wide variety of HDX experiments.

ACQUITY UPLC M-Class System with HDX-2 Automation.

HOW HDX WORKS



ACQUITY UPLC M-Class System with HDX Technology workflow.

- Deuterium oxide (D_2O) is added to the protein sample, solubilized in a physiological buffer.
- The exchange reaction proceeds until a defined end point or multiple time points for rate determinations.
- The exchange reaction is stopped by the addition of a quench buffer to reduce the pH and to add chaotrophes and reductants, if required.
- The sample is injected into the injection port of the HDX Manager. Within the HDX Manager (indicated in the blue box above) the sample may be analyzed as an intact protein or exposed to on-line pepsin digestion.
- If the LEAP HDX-2 Automation Manager is used, the addition of D_2O, quenching reaction, and injection into the HDX Manager, are done automatically.
- The Enzymate digestion column has a separate temperature controlled compartment for controlled digestion. The optional BPR exerts user selectable pressure on the peptic digestion.
- For either global or local analysis, the sample is trapped and resolved with sub-2-µm UPLC particles for increased chromatographic resolution at 0 °C.

HDX RESULTS MADE SIMPLE

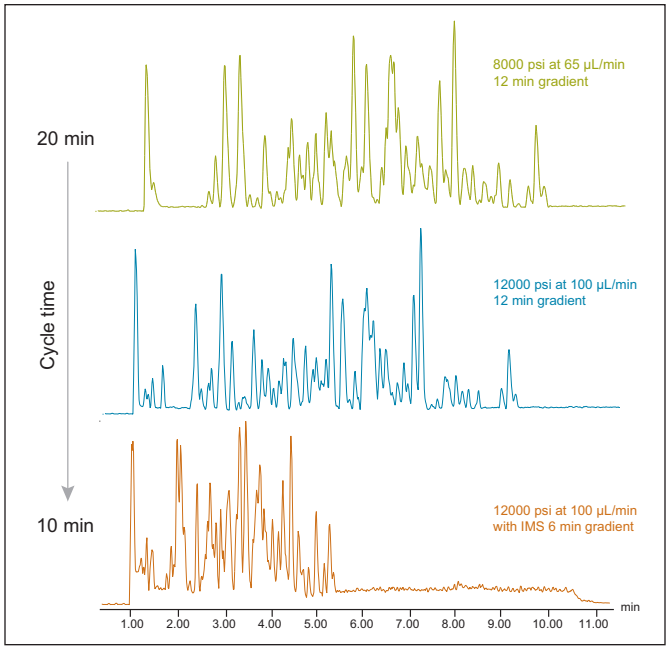
HDX-MS experiments collected for several time points with varied conditions can generate a significant number of high resolution MS spectra. In the past, researchers would spend days manually interrogating each spectrum. Today, using the new DynamX HDX Data Analysis Software, meaningful results can be obtained in minutes. The data are presented interactively, allowing the user to review and modify results if needed.

EXTRA SEPARATION DIMENSIONS FOR THE BEST HDX DATA

HDX-MS enables investigations of wide-ranging protein populations that can yield complex peptic peptide separations. An ideal separation would present each peptide to the electrospray source sequentially for ionization and MS detection. Increasing the temperature of the separation or extending the run-times can yield higher peak capacity separations. However, the quench conditions used to maintain the deuterium label, cold temperatures and rapid separations, prohibit this approach and generally result in deceased peak capacity. A poor separation may present multiple peaks to the MS, leading to complex mass spectra and possible data loss.

Improving mass transfer through use of smaller particles in UltraPerformance Liquid Chromatography (UPLC) increases HDX data quality.[3] UPLC enables faster separations while maintaining increased peak capacity as compared to traditional HPLC separations under quench conditions. As HDX reaches towards the analysis of the megaDalton protein complex and other complex protein systems, UPLC and high resolution MS benefit from another orthogonal separation technology. Triwave® ion mobility separations (IMS) bridge the gap between MS and UPLC to dig deeper so you can find structural changes that you've never seen before.

Increased confidence with increased speed



Using UPLC, high resolution MS and IMS can dramatically decrease run times while delivering high peak capacity separations. This orthogonal approach yields faster separation times, higher throughput, and unequivocal spectral information.

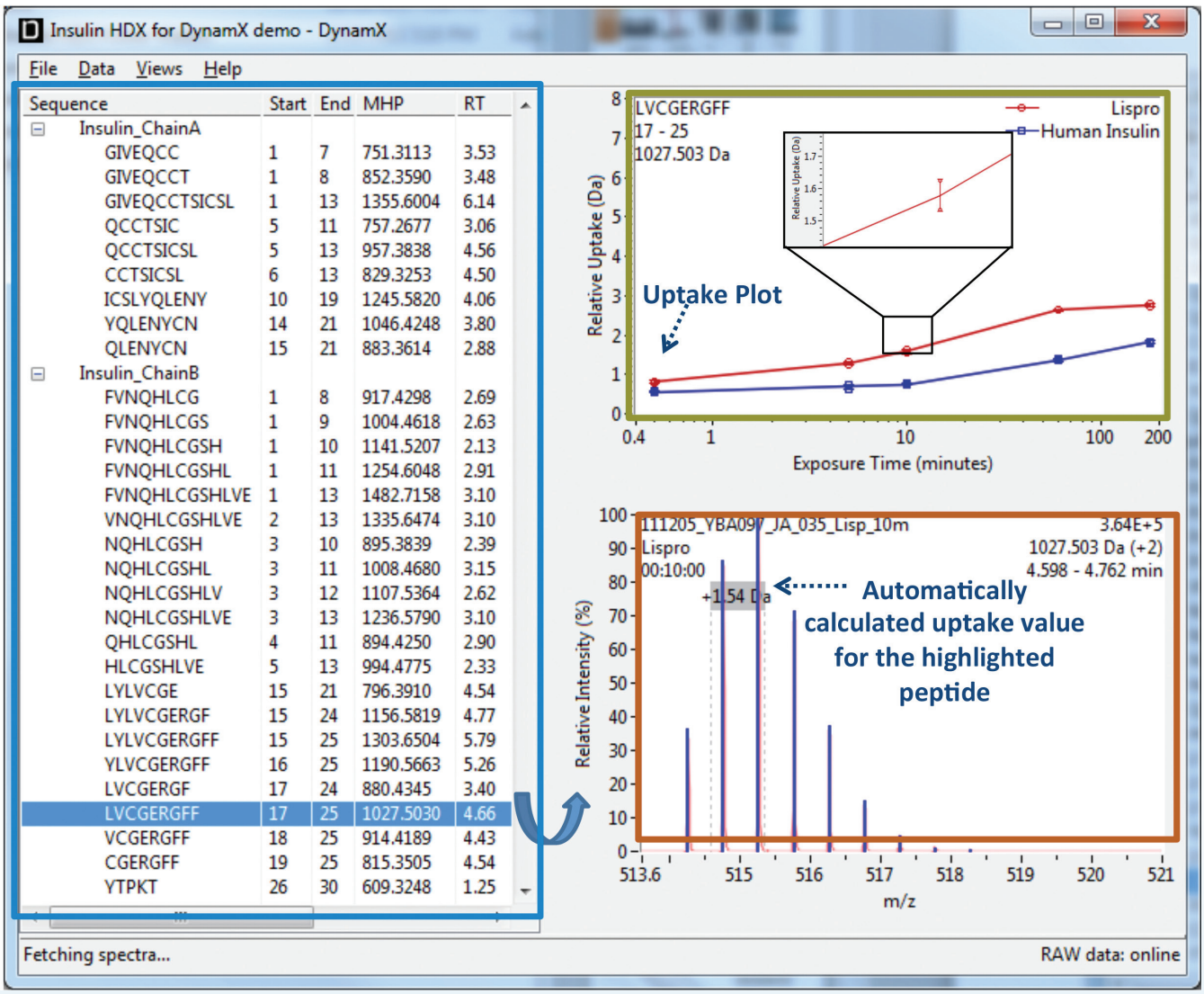
INDUSTRY LEADING HDX MS INFORMATICS

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.

What DynamX HDX Data Analysis Software tells you:

- The peptide list (blue pane) is the master list of peptides the experiment is tracking through the analysis.
- The uptake curve (green pane) is shown for the selected peptide. The extent of deuterium uptake is the core metric tracked in any HDX experiment. Replicate runs are visualized, demonstrating the reproducible data obtained with the HDX platform.
- The spectrum for a selected point in the uptake curve (orange pane). The raw spectrum is shown in red. Overlaid in blue are the centroid data that have been identified and matched to the peptide. The view shows a graphical representation of the calculated uptake.

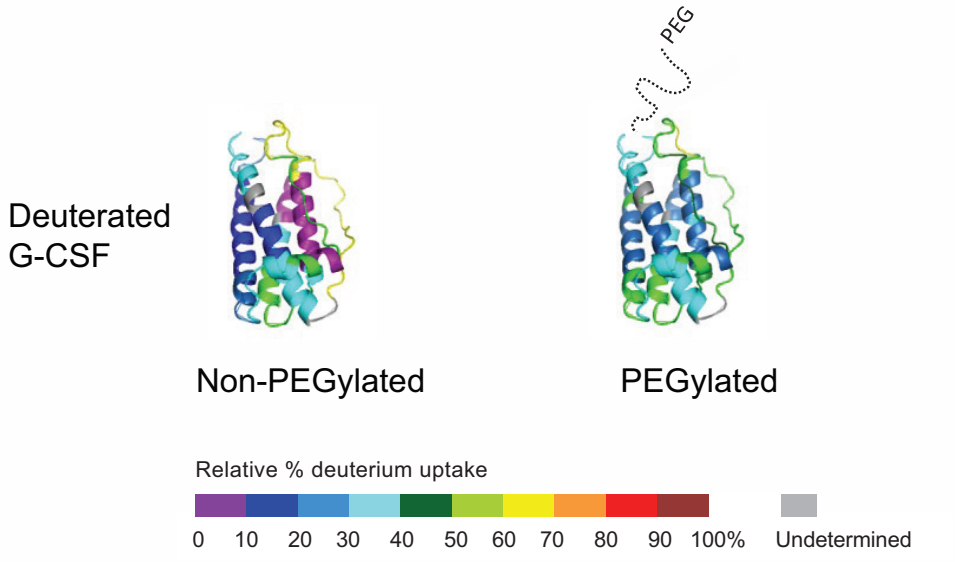


DynamX HDX Data Analysis Software automates HDX-MS data processing, displaying the deuterium uptake results in a simple and interactive software interface.

WHAT DOES HDX TELL YOU?

An HDX experiment measures changes in protein conformation. Differences in deuterium uptake are directly related to changes in solvent accessibility and hydrogen bonding of a protein. The more open or relaxed the conformation, the more uptake. Similarly, the more closed the conformation, the less deuterium will be able to exchange with the protein molecule.

HDX pinpoints changes in protein conformation with PEGylation. The HDX heat map of the 3D structure of granulocyte colony-stimulating factor (GCSF) provides easy, rapid data interpretation to understand the effect of ligand binding upon protein dynamics.



TOTAL SYSTEM SOLUTIONS FOR CONFORMATIONAL ANALYSES

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, ProteinLynx Global SERVER,™ and powerful application managers, 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.

References

1. Hvidt and Linderstrøm-Lang (1954). Biochim. Biophys. Acta 14, 574
2. Jacob, Roxana E. and Engen, J. Hydrogen Exchange Mass Spectrometry: Are We Out of the Quicksand? J. *Am. Soc. Mass Spectrom.* (2012) 23:1003Y1010.
3. Wu Y, Engen JR, Hobbins WB, 2006 Ultra performance liquid chromatography (UPLC) further improves hydrogen/deuterium exchange mass spectrometry *J. Am. Soc Mass Spectrom.* 17:163–67.

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