

DeutEx: A new software solution for Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) data analysis

Abstract

DeutEx [1] is a versatile software package designed for the analysis of Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) data. It supports the processing of high-resolution, high mass accuracy MS data from Bruker MRMS (FT-ICR MS) as well as data from timsTOF, q-TOF, and even MALDI-TOF instruments. DeutEx operates on peak-picked data stored in an open format (simple text file), which can be easily exported from Bruker's DataAnalysis software.

Keywords:
DeutEx Software,
Hydrogen/Deuterium Exchange
Mass Spectrometry,
Protein Structure Analysis,
Protein Dynamics,
Protein-Ligand Interactions,
Membrane Proteins,
Structural Biology

Introduction

Hydrogen/Deuterium Exchange Mass Spectrometry (HDX-MS) is a powerful technique used to understand the structure and dynamics of proteins [2]. It is widely used in biopharmaceutical research, structural biology and beyond to study:

- **Protein Structure Analysis:** HDX-MS aids in monitoring the folding and unfolding processes of proteins by probing the exchange of hydrogen atoms with deuterium in the protein's backbone. This provides insights into the stability and flexibility of different protein regions.
- **Protein Dynamics:** It allows researchers to study the dynamic behavior of proteins in solution, revealing how proteins change their conformation over time and under different conditions.
- **Protein-Ligand Interactions:** HDX-MS is crucial for identifying binding sites and uncovering how ligands (such as drugs) interact with proteins. This information is vital for drug design and development.
- **Membrane Proteins:** The technique has been successfully adapted to study membrane proteins, which are often challenging to analyze using other methods. HDX-MS can provide detailed information about their structure and function.

Applicability

- **Drug Design and Development:** HDX-MS helps identify binding sites and understand how drug candidates and antibodies interact with target proteins. This information is crucial for optimizing drug efficacy and specificity.
- **Biotechnology:** It is employed in the development of biopharmaceuticals, such as therapeutic antibodies, to ensure their stability and efficacy for drug safety and regulatory purposes.
- **Structural Biology:** HDX-MS complements other structural biology techniques like X-ray crystallography, (cryo-)electron microscopy and NMR spectroscopy providing a more comprehensive knowledge of protein structures.
- **Disease Research:** The technique is used to study proteins involved in diseases helping to elucidate disease mechanisms and identify therapeutic targets.

HDX-MS plays a crucial role in both biochemical and pharmaceutical research. Its ability to provide detailed insights into protein behavior is invaluable for:

- **Advancing Biological Understanding:** By revealing the structural and dynamic properties of proteins, HDX-MS helps scientists gain deeper insight into biological processes.
- **Developing New Therapies:** The detailed information on protein-ligand interactions and protein dynamics aids in the design and development of new drugs and therapies, making it a key tool in the pharmaceutical industry.

The typical continuous labelling HDX-MS workflow is illustrated in Figure 1.

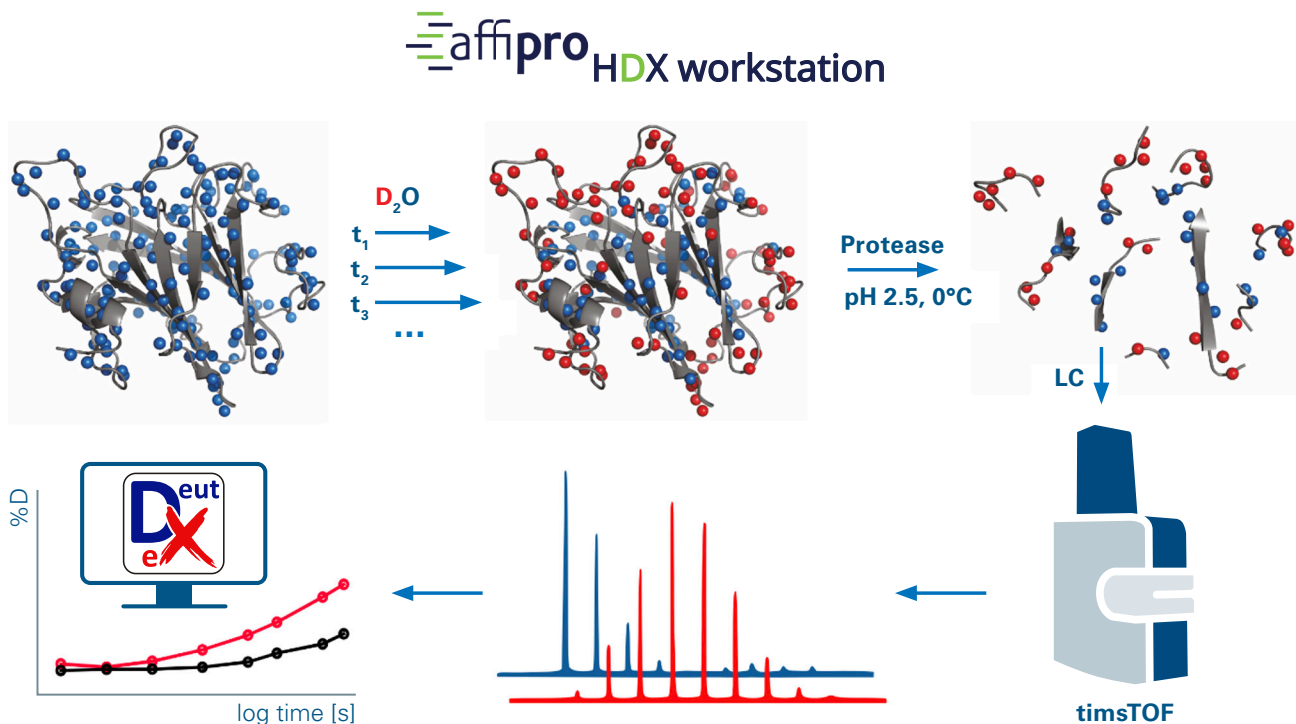


Figure 1. Overview of an HDX-MS workflow (continuous labelling).

H/D exchange starts by 5- to 20-fold dilution of protein samples into a deuterated buffer. This is handled automatically by a CTC/PAL robot (manual injection is possible as well). Labelling is done for seconds to hours, while acidification to pH 2.5 with optional reduction and denaturation is done at the end to stop the reaction. Immediate proteolysis and (if needed) deglycosylation is done online using AffiPro immobilized columns. Resulting peptides are online desalted, separated by a UPLC (C18, pH 2.5, 0°C to minimize deuterium label loss) and analyzed by ESI-MS.

Experimental

To illustrate the DeutEx workflow, apo- and holo-myoglobin were investigated in a simple HDX experiment, with deuteration times ranging from 20 seconds to 2 hours. Online digestion was performed on a co-immobilized nepenthesin-2/pepsin column (Affipro, Czech Republic). Prior to the HDX measurements utilizing MS scans with TIMS off, DDA-PASEF data was acquired on non-deuterated apo-myoglobin for MS/MS peptide matching, using Mascot as the protein search engine. Data analysis, schematically outlined in Figure 2, was performed in DeutEx with MSTools [3] (<https://peterslab.org/MSTools/>) used for heat map plotting and open-source PyMol for protein 3D structure visualization.

NOTE - While the focus here is on Bruker timsTOF series mass spectrometers, DeutEx and HDX-MS workflow are supported on all Bruker QTOF and MRMS instruments.

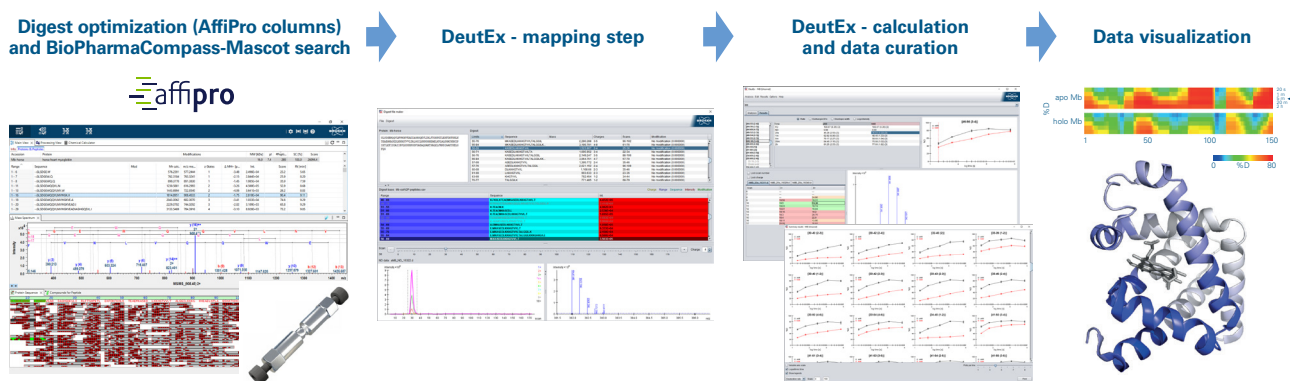


Figure 2. Overview of an HDX-MS workflow in DeutEx.

The processing starts by digest optimization using various protease columns and digestion conditions. LC-MS/MS including PASEF is used in combination with BioPharma Compass® for a MASCOT database search. The resulting peptide lists are loaded into DeutEx mapping interface together with protein sequence and non-deuterated LC-MS file exported from DataAnalysis. Data are then compiled into folders according to experimental conditions and mass calculations for all peptides and conditions are performed in just a few minutes without user input. Data can then be reviewed, manually curated if needed and exported for visualization using MSTools and PyMol.

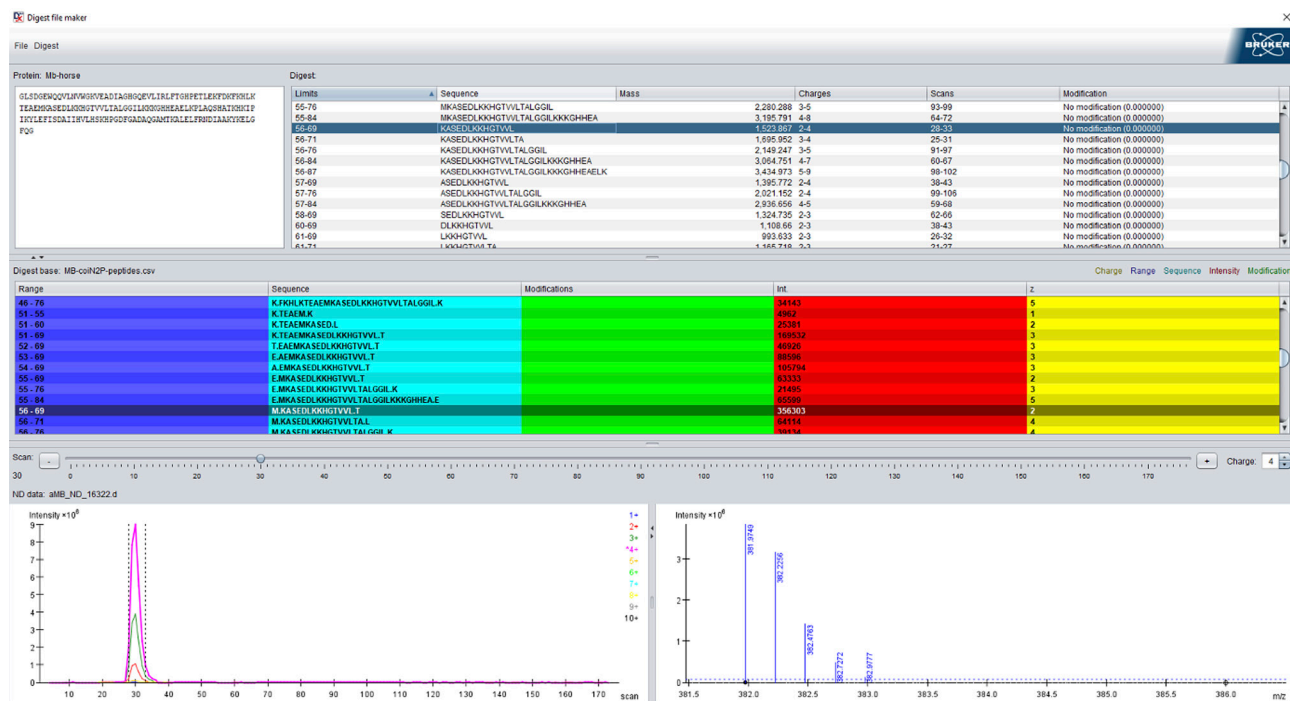


Figure 3. DeutEx mapping interface.

Mapping is used to review the peptides identified by MS/MS database searching. Chromatographic elution profile is evaluated and additional charge states of each peptide, not necessarily selected for MS/MS are detected and included.

Data Analysis and Visualization

DeutEx offers essential functionality for both in-depth and quick assessment of acquired HDX-MS data. The software streamlines all steps of the HDX-MS workflow, including the calculation of deuteration for all investigated peptides within the dataset and the relevant visualization of the results. This is depicted in Figure 3 and 4, where the peptide roadmap generation and HDX data review are shown. It offers largely automated processing and is capable to process numerous experimental conditions simultaneously, while preserving the ability of the user to interact with the data and provide manual curation. For downstream analysis and visualization (Figure 5), data export is available in accordance with the common recommendations for performing, interpreting, and reporting HDX-MS experiments [4], as required by many journals and standard operating procedures.

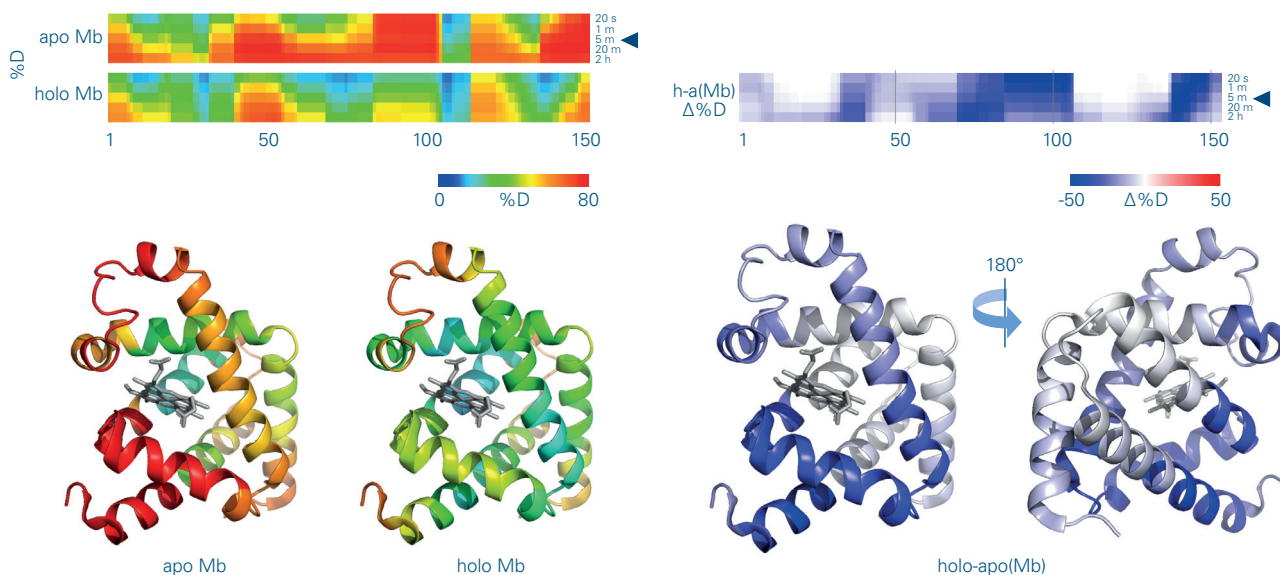


Figure 5. Final data visualization examples using MSTools and PyMol.

Data can be visualized in many ways – for example a rainbow heatmap showing the time-resolved deuteration pattern in apo- and holo-myoglobin and the corresponding structure coloring for the 5 min exchange time. The right panel shows a differential display of the stabilization and protection of myoglobin upon heme binding, using a (red)-blue-white color gradient.

Conclusions

DeutEx is your ultimate solution for processing HDX-MS data. Designed for seamless compatibility with Bruker instruments, DeutEx ensures precise and highly efficient data analysis with the user in full control at every step of the process, if desired. As a Java-based, standalone program, it offers platform independence and ease of use, making it an essential tool for researchers and professionals in the HDX-MS field. Experience the reliability and efficiency of DeutEx for your complex datasets and elevate your MS data processing to the next level.

References

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