



deMix

User manual



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1. Introduction

1.1. About deMix

One of the most popular methods for examining protein conformational changes and dynamics is hydrogen/deuterium exchange (HDX) with mass spectrometry (MS). We previously developed a fully automated algorithm to analyze deuterated isotopic distributions in-depth called deMix (Na et al. 2019 [1]). Here, we introduce the graphical user interface(GUI) version of deMix. The software automatically analyzes HDX data and facilitates the interrogation of data and results. deMix offers visualization of isotopic cluster distributions and sequence coverage maps in heat map form to compare deuteration rates over time.

What deMix provides:

Peptide centric view (Deuterated Distributions)

- Allows users to compare a natural isotope distribution and the corresponding deuterated isotope distribution in one of HDX experiments across D₂O labeling with theoretical, aggregated (over elution time spans), and manually annotated distributions for the chosen peptide.
- Offers a deuteration rate (or deuterium uptake) plot across D₂O labeling time for the selected peptide.

Protein centric view (Dynamics)

- Allows users to view the dynamics of the protein.
- Offers sequence coverage maps for HDX-MS data. Visually represents the HDX rate of each D₂O labeling times within the HDX-MS dataset using colors (= Heat Map).

User-friendly environment

- Includes intuitive and interactive GUI or features.
- Allows users to focus more on analysis rather than spend time adapting to the software.

2. Installation

2.1 Requirements

java version > 17.05

To check (type this in the command prompt) = **java -version**

Download java here: <https://www.oracle.com/java/technologies/downloads/>

2. Installation

2.2

Windows/MacOs/Linux

deMix Downloads:

Windows

- Download Windows version. & Extract the compressed file.
- Double Click the bat file → Run anyway/Run

MacOs

- Download Windows version. & Extract the compressed file.
- Right-click the deMix → Open with Terminal → Open.

Linux

- Download the linux version. & Extract the compressed file.
- Double Click the .sh

* Remove visited_directories file if exists (when first downloaded).

3. Peptide Centric View

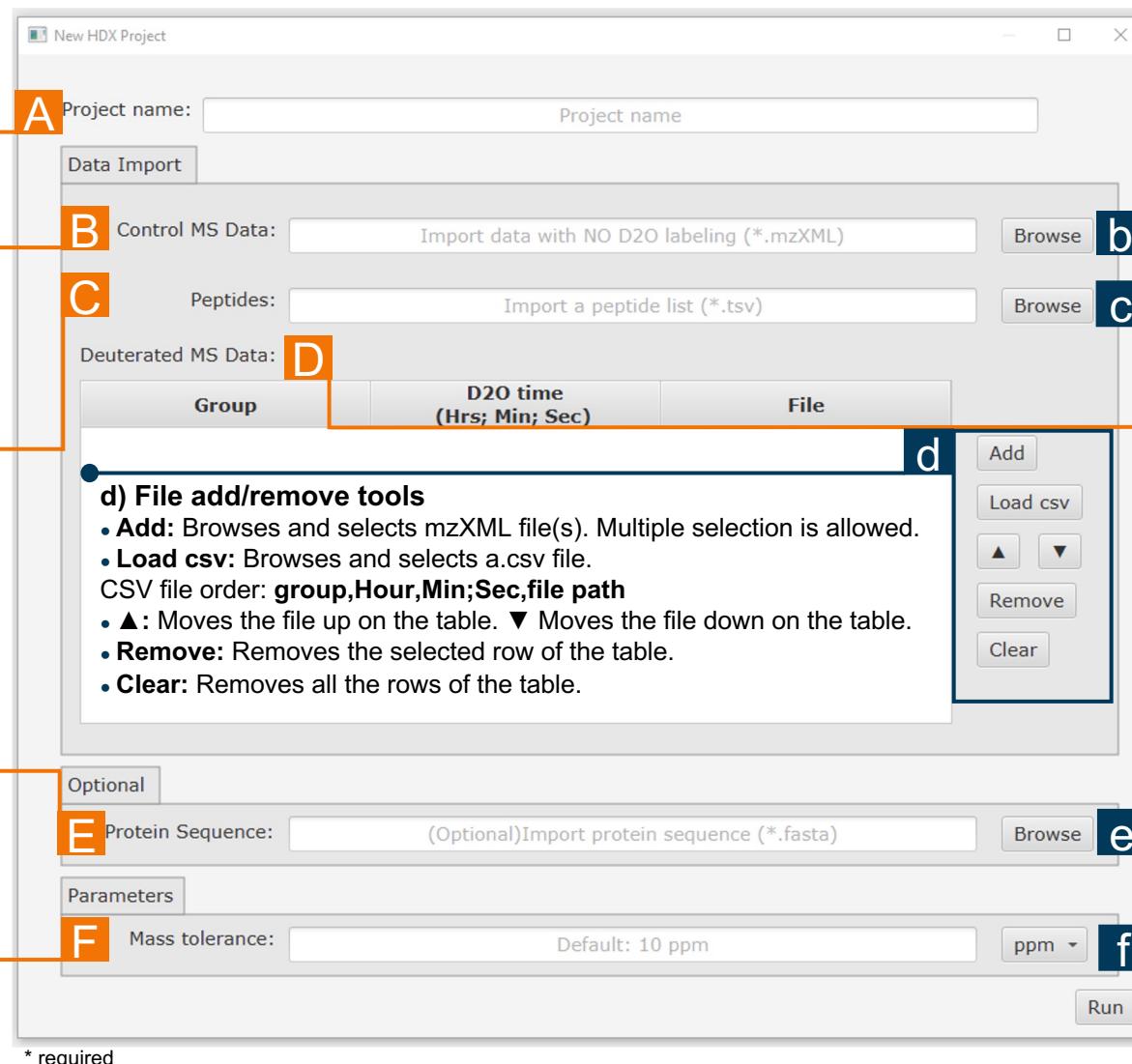
3. Protein Centric View

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3.1. File Control

3.1.1. Creating a New deMix Project

Click Path: File > New Project



A) Project name *

- Specify Project Name

B) Control MS Data *

- Import control data which does not have D2O labeling.
- File Format: mzXML

C) Peptides *

- Import a file containing peptides to be examined.
- Make sure that the file contains two columns named 'peptide' and 'charge' for peptides and charge states, respectively.
- File Format: tsv

E) Protein Sequence

- Import a protein sequence data
- File Format: fasta

F) Mass Tolerance

- Specify the mass tolerance (ppm or da).
- default = 100ppm

b) Browse button

- Browses and selects .mzXML files. Multiple selection is allowed.

c) Browse button

- Browses and selects a .tsv file.

D) Deuterated MS Data *

- Import D2O labeled data set(s) (With Group and D2O Time). User must provide D2O labeling.
- File Format: MSXML

e) Browse button

- Browses and selects a .fasta file.

f) ppm/da menu

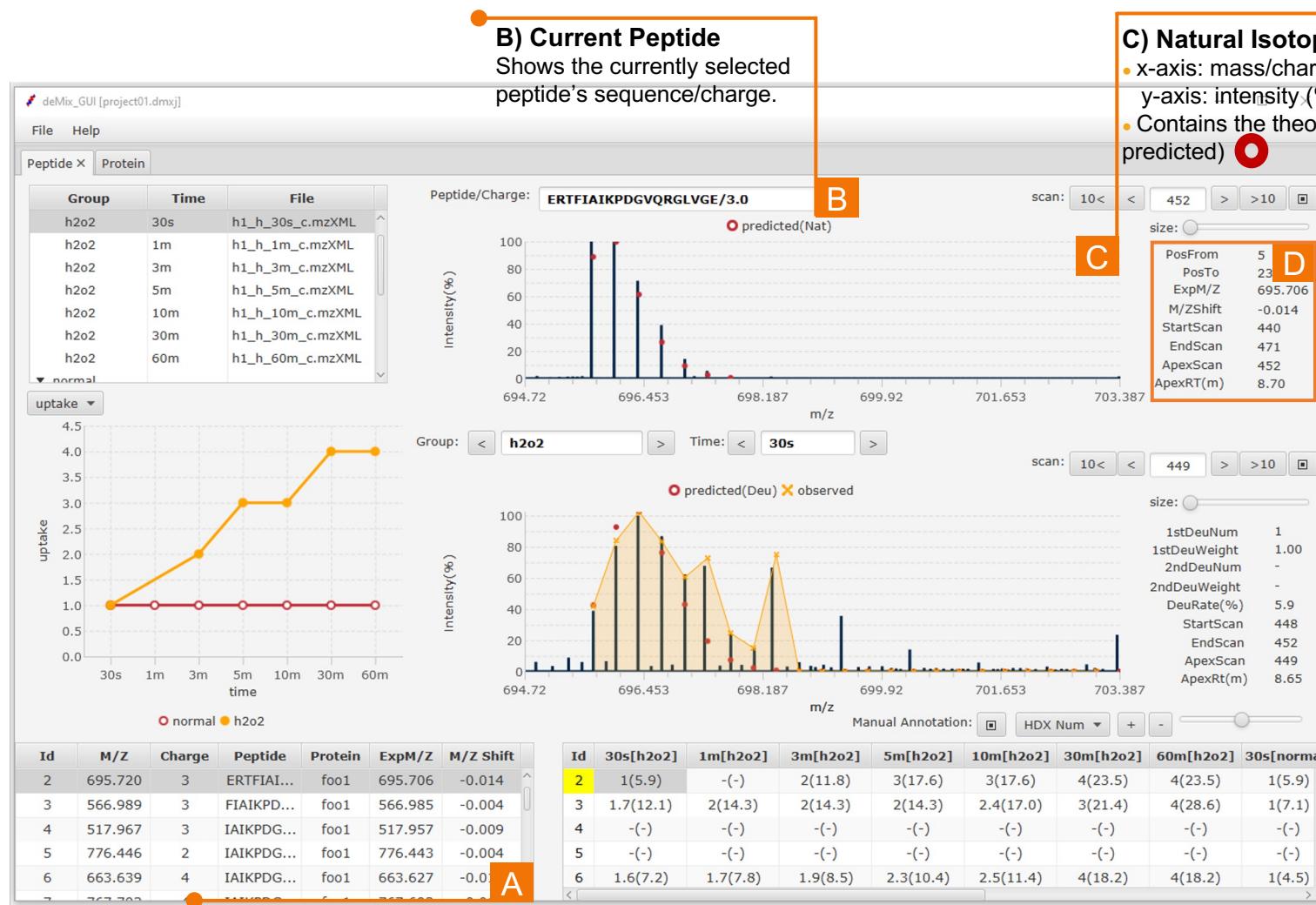
- Sets the unit of the mass tolerance. (Default: ppm)

3.1.2. Opening an deMix project

Click Path: File > New Project > Open (selected file)

3.2. Natural Isotope Distribution

3.2.1. Distribution Explanations



A) PeptideTable

- Contains peptide's information.

3.2. Natural Isotope Distribution

3.2.2. Distribution Functions

Distribution Control: A → B → C

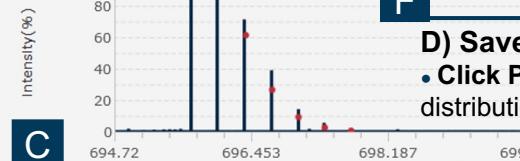
deMix_GUI [project01.dmxj]		
File	Help	
Peptide X	Protein	
Group	Time	File
h2o2	30s	h1_h_30s_c.mzXML
h2o2	1m	h1_h_1m_c.mzXML
h2o2	3m	h1_h_3m_c.mzXML
h2o2	5m	h1_h_5m_c.mzXML
h2o2	10m	h1_h_10m_c.mzXML
h2o2	30m	h1_h_30m_c.mzXML
h2o2	60m	h1_h_60m_c.mzXML

B) Current Peptide

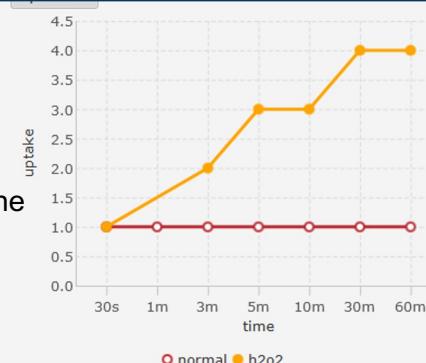
- The selected peptide's sequence & charge appears.

B

Peptide/Charge: ERTFIAIKPDGVQRGLVGE/3.0



C



A) Peptide Selection

- Select a peptide by clicking a row in the table.
- Keyboard(up & down arrow keys) support available

A

ID	M/Z	Charge	Peptide	Protein	ExpM/Z	M/Z Shift
2	695.720	3	ERTFIAI...	foo1	695.706	-0.013
3	566.989	3	FIAIKPD...	foo1	566.985	-0.004
4	517.967	3	IAIKPDG...	foo1	517.957	-0.009
5	776.446	2	IAIKPDG...	foo1	776.443	-0.004
6	663.639	4	IAIKPDG...	foo1	663.627	-0.013

D) Scan Mover

- 10<: Jump 10 scans backward.
- <: Go to previous scan.
- >: Go to the next scan.
- >10: Jump 10 scans forward.
- : Go to the apex scan.

D

scan: 10< < 452 > >10 □

size: PosFrom 5 PosTo 23 ExpM/Z 695.706 M/ZShift -0.014 StartScan 440 EndScan 471 ApexScan 452 ApexRT(m) 8.70

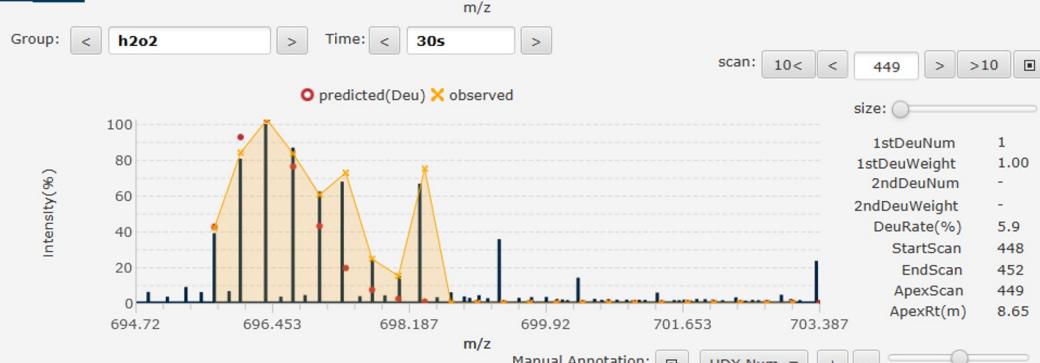
E

Text box: current scan & type the desired scan number to go to that scan.

F) D Save the Distribution

- Click Path:** Right-click the distribution> Browse> Save > Finish

C

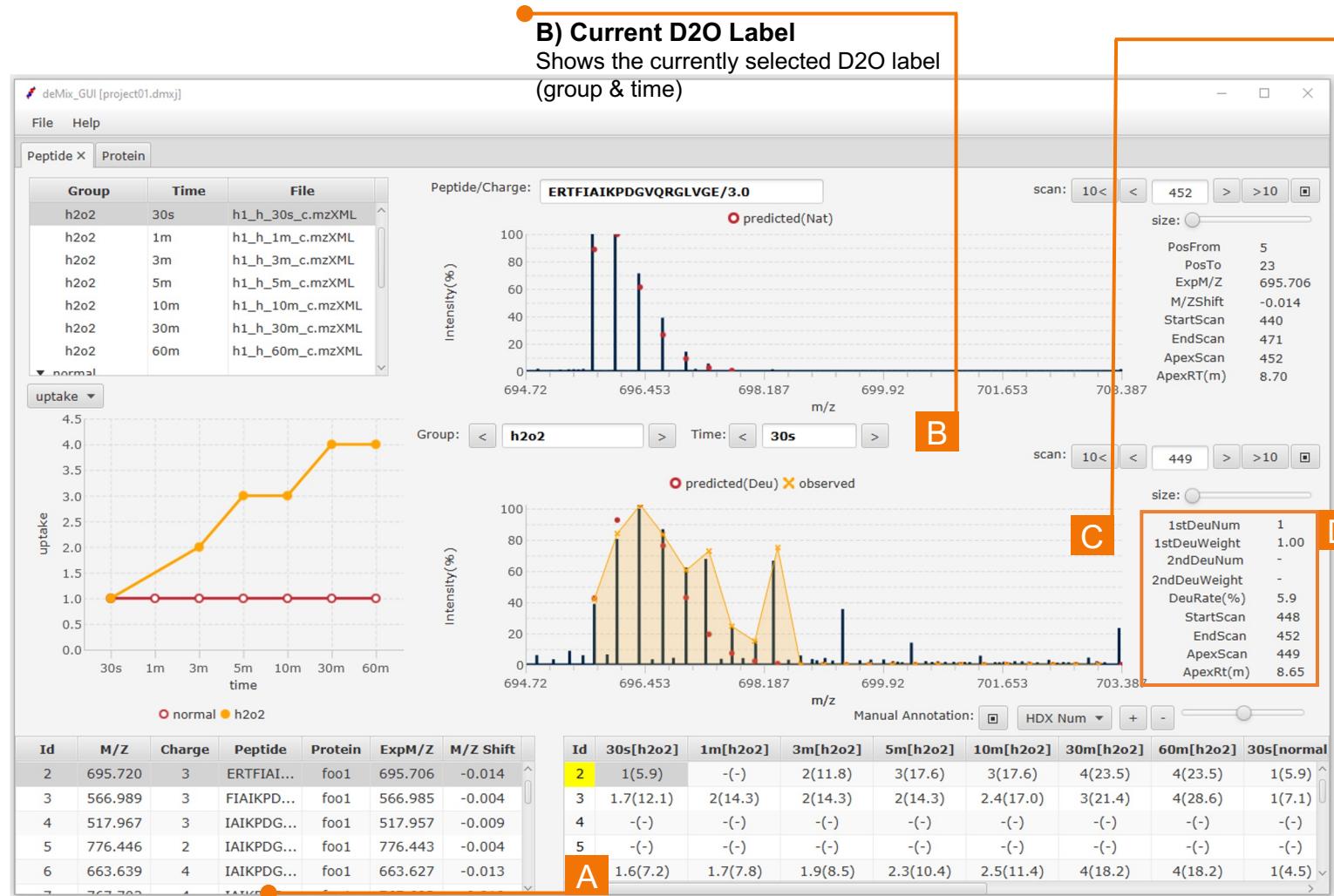


A

ID	30s[h2o2]	1m[h2o2]	3m[h2o2]	5m[h2o2]	10m[h2o2]	30m[h2o2]	60m[h2o2]	30s[normal]
2	1(5.9)	-(-)	2(11.8)	3(17.6)	3(17.6)	4(23.5)	4(23.5)	1(5.9) ^
3	1.7(12.1)	2(14.3)	2(14.3)	2(14.3)	2.4(17.0)	3(21.4)	4(28.6)	1(7.1)
4	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)
5	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)
6	1.6(7.2)	1.7(7.8)	1.9(8.5)	2.3(10.4)	2.5(11.4)	4(18.2)	4(18.2)	1(4.5) ^

3.3. Deuterated Isotope Distribution

3.3.1. Distribution Explanations



C) Deuterated Isotope Distribution (Deu.)

- x-axis: mass/charge(m/z)
- y-axis: intensity (%)
- Contains the theoretical distribution of the Deu. (=predicted)
- Contains the distribution of the aggregated scans. (=observed)

D) Deu. Information

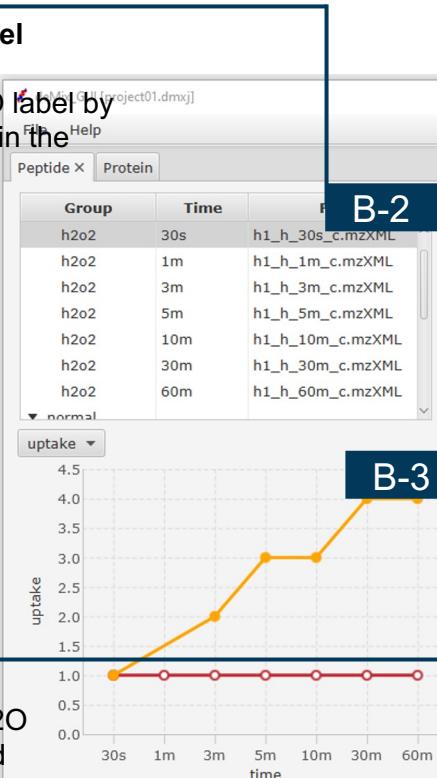
- 1stDeuNum:** Deuterated number (Deuterium Uptake) assuming a unimodal distribution (a single deuterium value).
- 1stDeuWeight:** Proportion of the 1stDeuNum form in the distribution. The weight factor for more abundant num is over 90%, only the abundant one is reported (not accepted as a bimodal distribution).
- 2ndDeuNum:** Second deuterated number assuming the bimodal distribution (i.e., two deuterated forms are simultaneously observed).
- 2ndDeuWeight:** Proportion of the 2ndDeuNum form in the distribution.
- StartScan:** Scan number where the peptide elution begins.
- EndScan:** Scan number where the peptide elution ends.
- ApexScan:** Scan number where the peptide elution is at the peak.
- ApexRT(m):** Retention time in minutes when the peptide elution is at the peak.

3.3. Deuterated Isotope Distribution

3.3.2. Distribution Functions

B-2) D2O Label Selection

- Select a D2O label by clicking a row in the sample view.



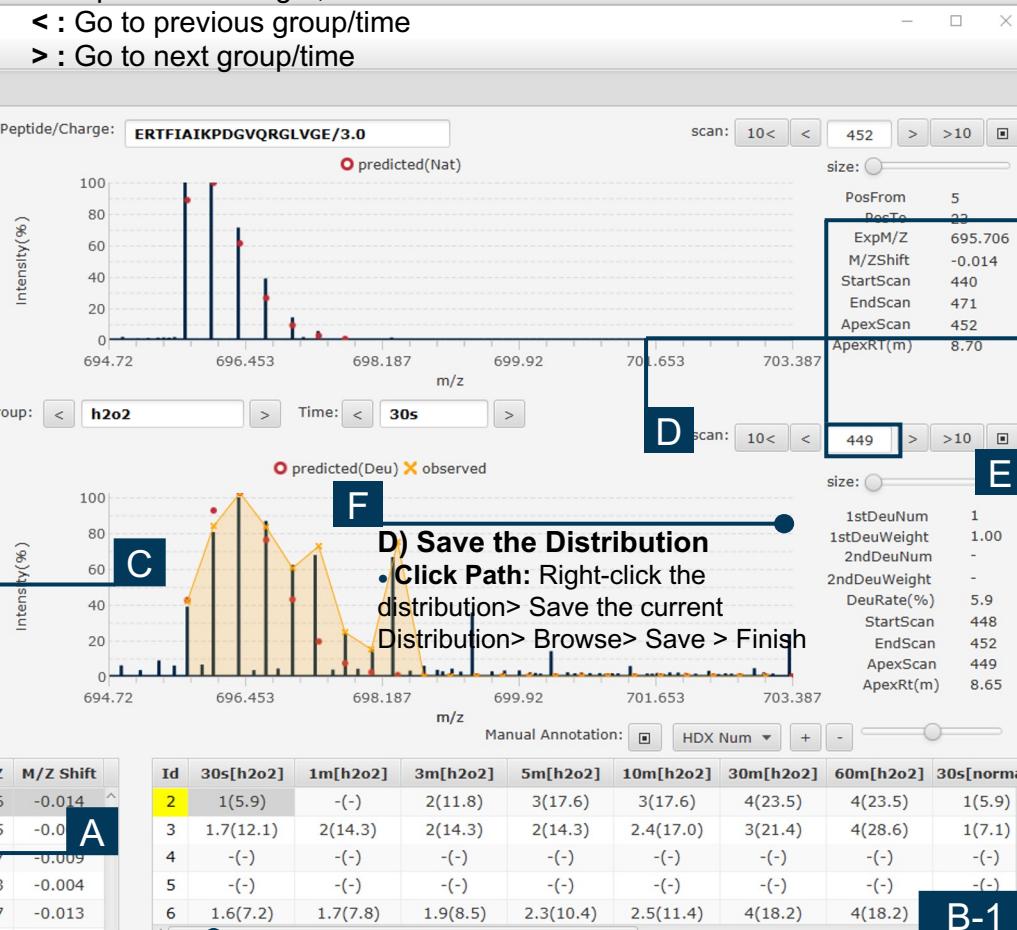
B-2) D2O Label Selection

- Select a D2O label by clicking a row in the sample view.

B-3) D2O Label Selection

- Select a D2O label by selecting Group & Time using <, > buttons.
< : Go to previous group/time
> : Go to next group/time

Distribution Control: A → B-1/B-2/B-3 → C



B-1) D2O Label Selection

- Select a D2O label by clicking a cell in the table.
- The cell must be in the same row as the highlighted id.

C) Distribution

- The selected D2O label's deuterated isotope distribution & information (on the right) appears.

A) Peptide Selection

- Select a peptide by clicking a row in the table.
- Keyboard(up & down arrow keys) support available

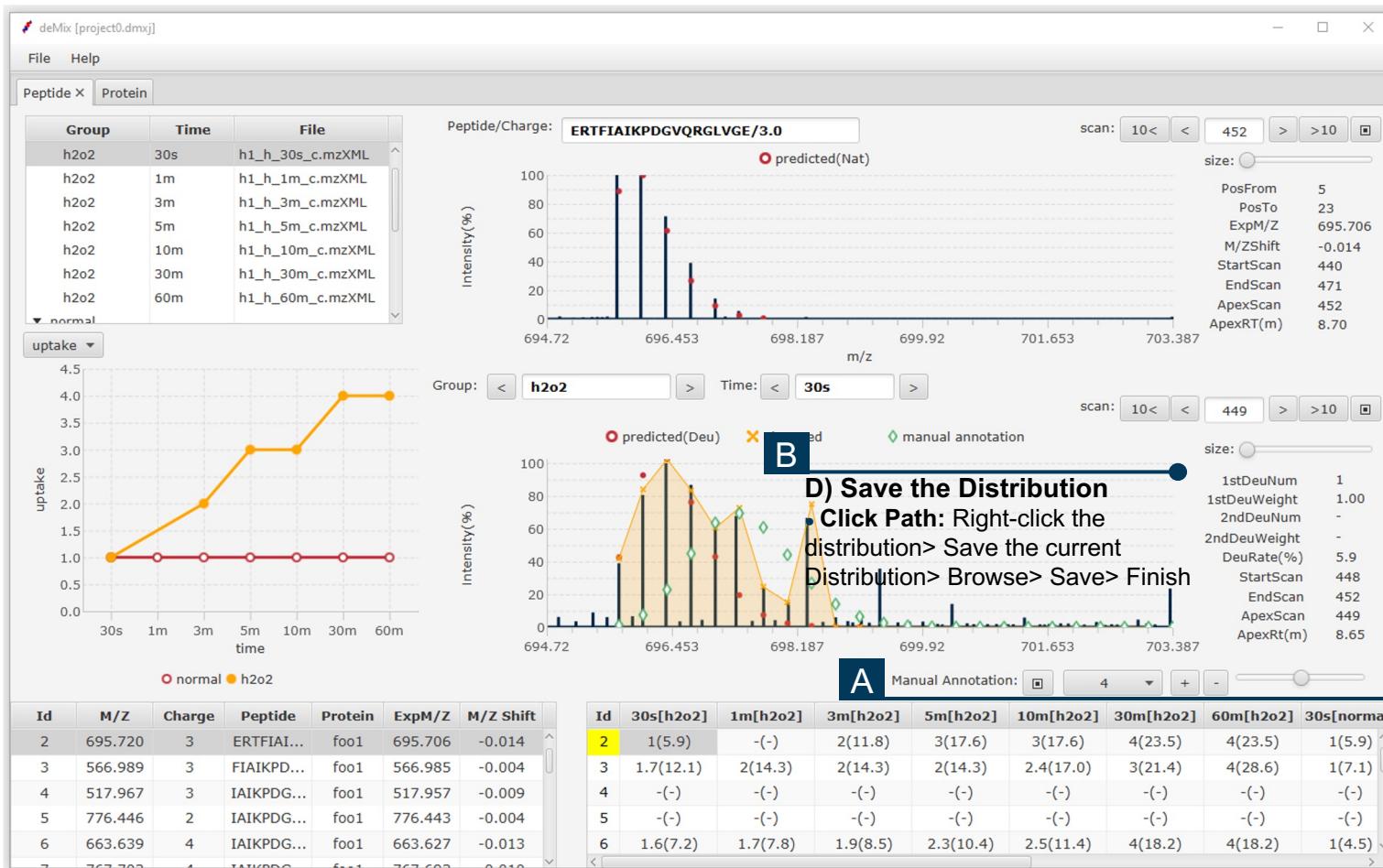
ID	M/Z	Charge	Peptide	Protein	ExpM/Z	M/Z Shift
2	695.720	3	ERTFIAI...	foo1	695.706	-0.014
3	566.989	3	FIAIKPD...	foo1	566.985	-0.0
4	517.957	3	IAIKPDG...	foo1	517.957	-0.009
5	776.446	2	IAIKPDG...	foo1	776.443	-0.004
6	663.639	4	IAIKPDG...	foo1	663.627	-0.013
7	767.700	1	IAIKPDG...	foo1	767.700	-0.000

ID	30s[h2o2]	1m[h2o2]	3m[h2o2]	5m[h2o2]	10m[h2o2]	30m[h2o2]	60m[h2o2]	30s[normal]
2	1(5.9)	-(-)	2(11.8)	3(17.6)	3(17.6)	4(23.5)	4(23.5)	1(5.9)
3	1.7(12.1)	2(14.3)	2(14.3)	2(14.3)	2.4(17.0)	3(21.4)	4(28.6)	1(7.1)
4	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)
5	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)
6	1.6(7.2)	1.7(7.8)	1.9(8.5)	2.3(10.4)	2.5(11.4)	4(18.2)	4(18.2)	

B-1

3.3. Deuterated Isotope Distribution

3.3.3. Manual Annotation

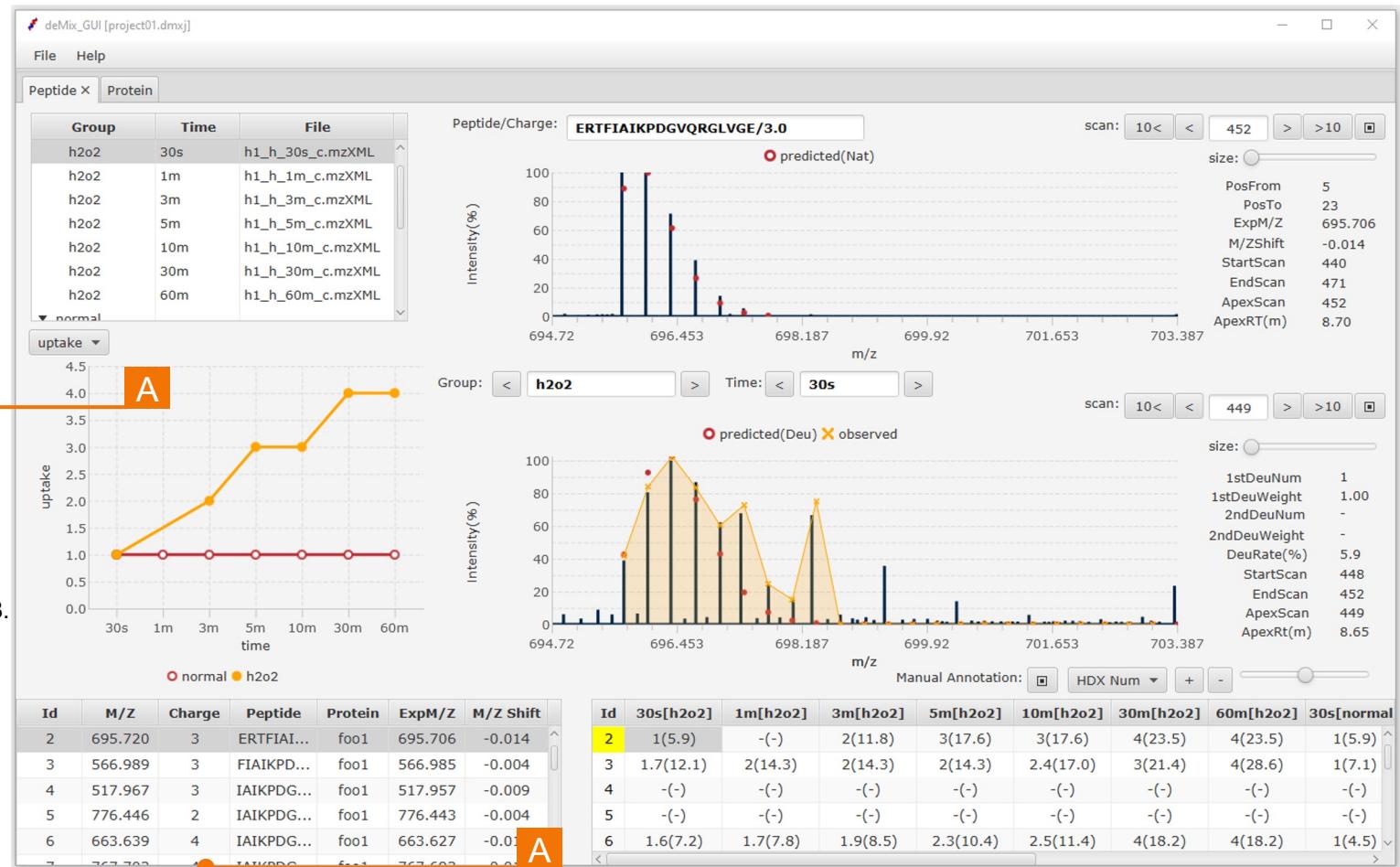


A) Manual Annotation

- : Set manual annotation to system's theoretical distribution (predicted)
- **HDX Num Menu:** A menu with all the possible HDX numbers, which are from 1 to length of the peptide -1. The menu box shows the currently selected HDX Number.
- + : Increases the y-axis value of the distribution for the set amount.
Calculation (each data point): Current y-axis value * 1.05
- - : Decrease the y-axis value of the distribution for the set amount.
Calculation (each data point): Current y-axis value / 1.05
- : Increases/decreases the y-axis value of the distribution
- The slider ranges from 0 to 10 (positive real numbers). The default value is 5 (original).
- Increase: drag the slider to the right (dragged value > 5).
- Decrease: drag the slider to the left (dragged value < 5)
- Dragged Amount: |5- dragged value|
- Decrease Calculation (each data point): y-axis value / (1 + Dragged Amount)
- Increase Calculation (each data point): y-axis value * (1 + Dragged Amount)

3.4. HDX Plot

3.4.1. Plot Explanations



A) PeptideTable

- Contains peptide's information.

3.4. HDX Plot

3.4.2. Plot Functions

Plot Control: A → B → C

B) Current Peptide

- The selected peptide's sequence & charge appears.

D) Rate/Uptake Button

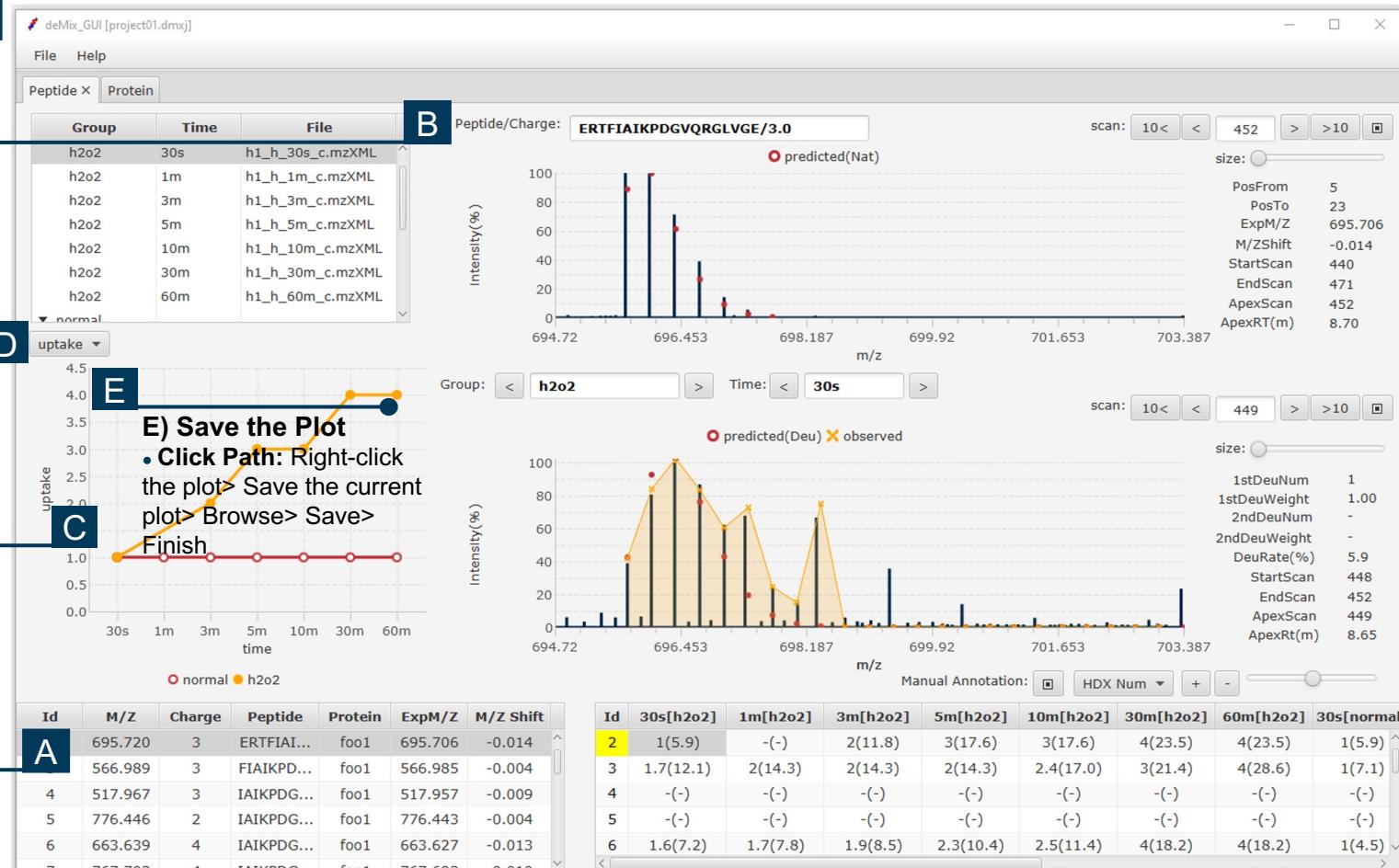
- Changes the metric of the HDX measurement.
- The metric for the y-axis is set to "uptake" by default.
- For rate(%), the range of the y-axis is set to 0 to 100 (does not vary).

C) HDX Plot

- The HDX plot appears across all the D₂O labeling time.
- The range of the y-axis varies for each peptide selection.
- Order Control:** Right-click the plot> Ascend/Descend

A) Peptide Selection

- Select a peptide by clicking a row in the table.
- Keyboard(up & down arrow keys) support available



3.5. Sequence Coverage Map

3.5.1. Coverage Map Explanations

A) D2O time

- Shows all the D2O times across the data.
- The deuteration rate at the only checked times are shown in the heat map.

C) Deuteration

- Shows all the D2O labeling groups across the data.
- The checked group's deuteration over time across the peptides is shown as a heat map.

D) Differential analysis

- Performs the differential analysis between two groups. (control = First Group, treatment = Second Group)
- The rate differences between the two groups are shown as a heat map



3.5. Sequence Coverage Map

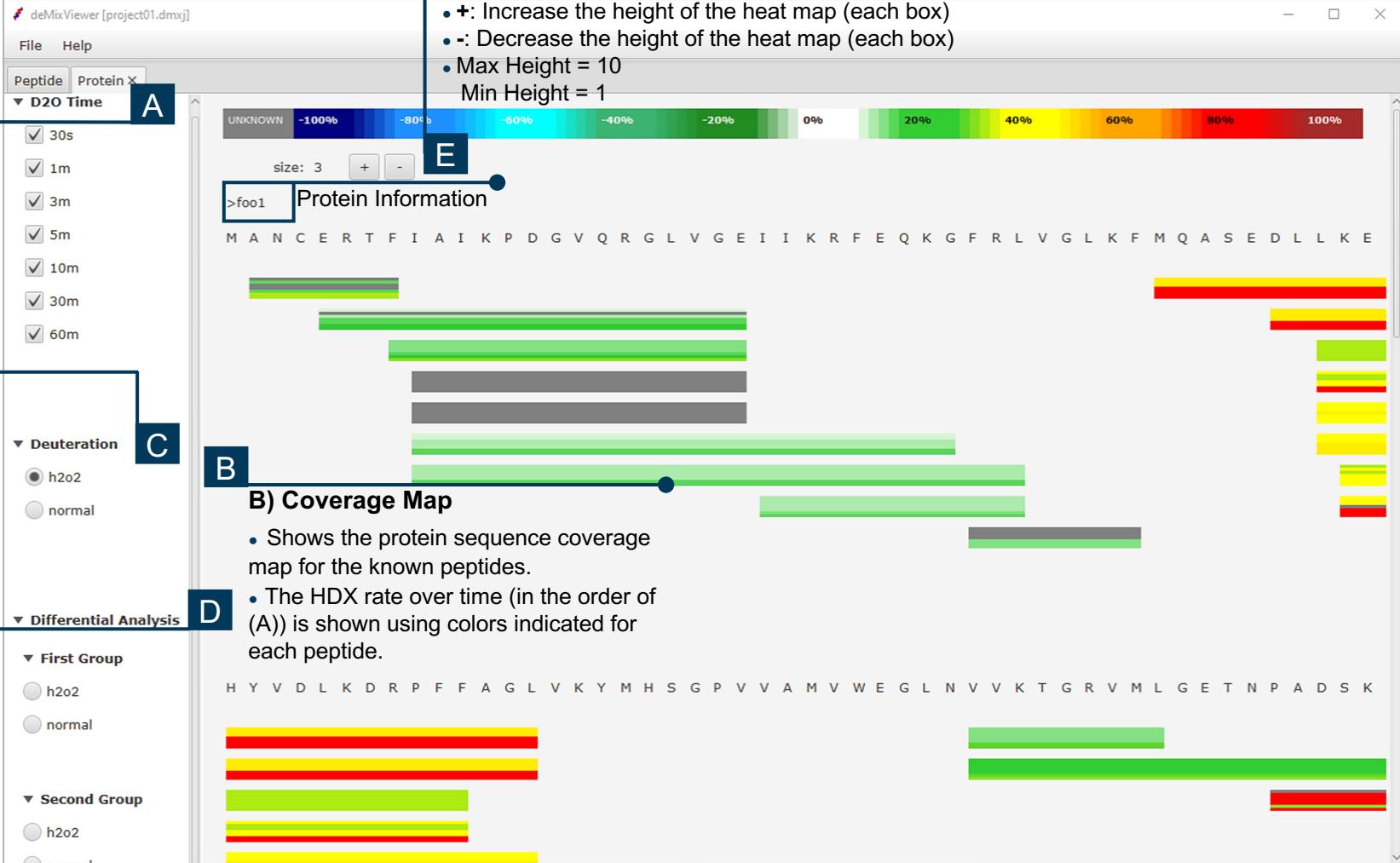
3.5.1. Coverage Map Functions

Map Control: A → C/D → B

A) D2O time

- Select D2O labeling times to include.

A

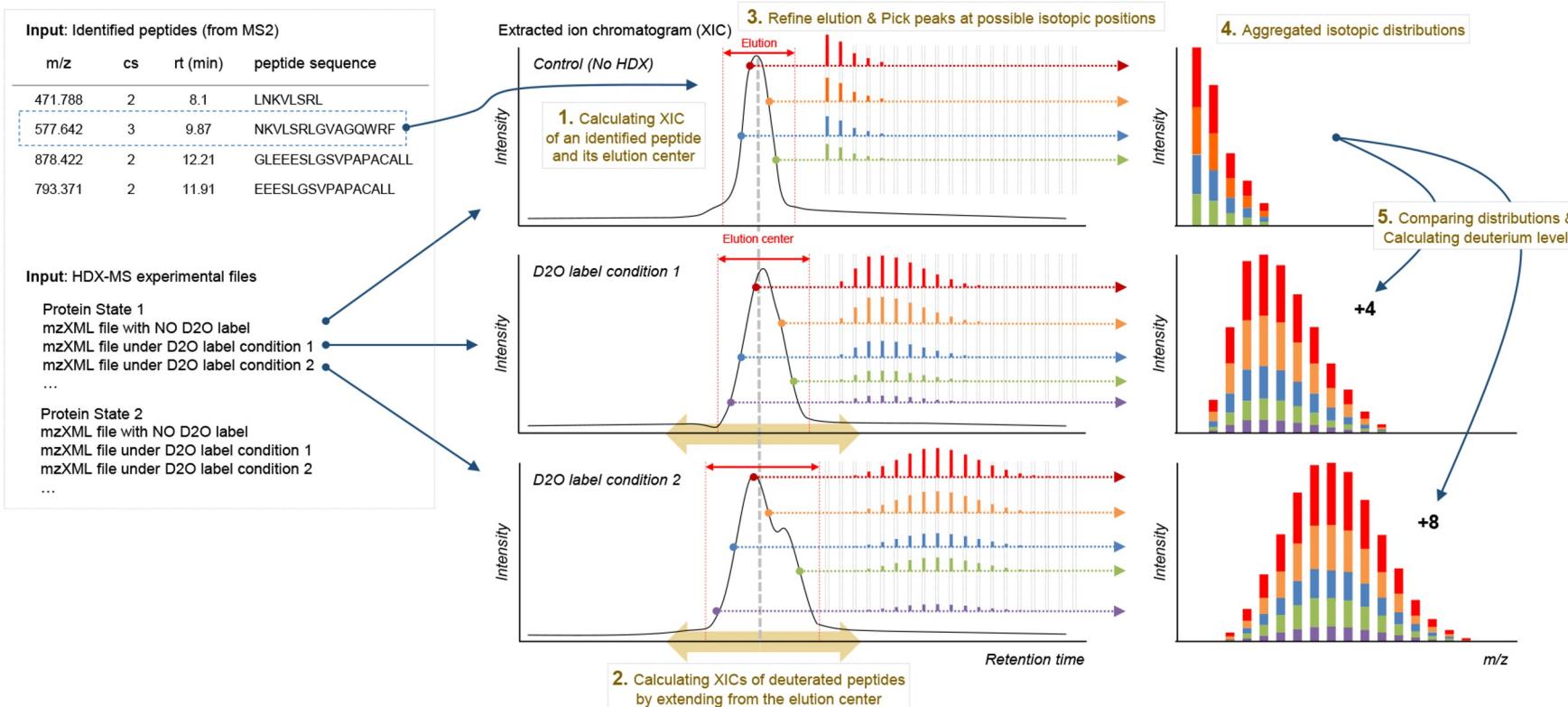


E) Size Controller

- +: Increase the height of the heat map (each box)
- : Decrease the height of the heat map (each box)
- Max Height = 10
- Min Height = 1

4. Appendix

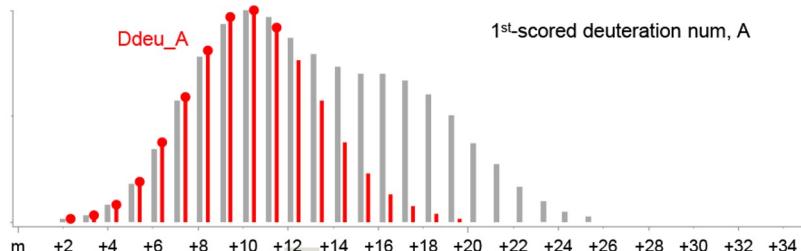
4.1. deMix Workflow



- 1) given peptides identified from MS2, deMix generates a theoretical isotopic distribution for each peptide and compares observed distributions in MS1 spectra (non-deuterated sample), constructing its extracted ion chromatogram (XIC).
- 2) based on the XIC in the non-deuterated sample, XICs of peptides in deuterated samples are constructed, where our assumption is that the related XICs across samples partially overlap or are shifted within a certain range (e.g., ± 40 scans) although they may not totally overlap.
- 3) deMix refines each XIC and selects isotopic distribution peaks corresponding to presumably the same peptide ion within a determined elution time span.
- 4) deMix aggregates all detected isotopic peaks into a single isotopic distribution. The aggregated isotopic distributions are regarded more robust than individual distributions; and 5) based on the aggregated isotopic distributions, deuterium numbers are determined.

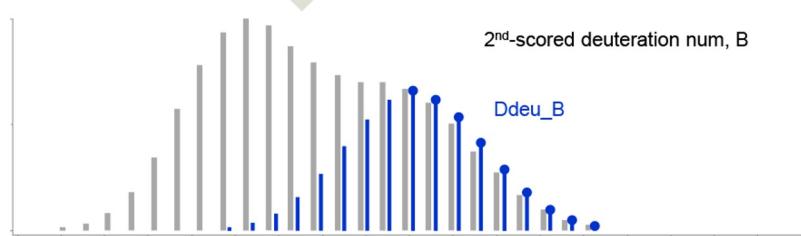
4. Appendix

4.2. Bimodal Analysis



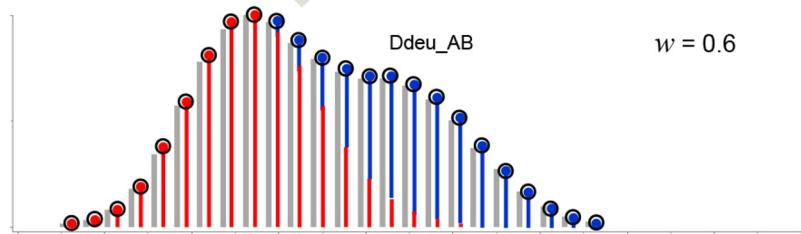
1) $\frac{\text{unexplained area}}{\text{explained area}} > \alpha$ (e.g. 0.2)

YES → NO Report a single value, A



$$Ddeu_{AB} = w \cdot Ddeu_A + (1-w) \cdot Ddeu_B \quad (0 < w < 1)$$

Find w to minimize the error($Ddeu_{AB}$)



2) $\text{error}(Ddeu_{AB}) < \beta \cdot \text{error}(Ddeu_A)$

$0.1 \leq w \leq 0.9$

YES → NO Report a single value, A

Report two values, A and B

Bimodal distribution analysis.

- a) After the initial unimodal distribution analysis, it is assessed how well the observed distribution is explained.
- b) If a significant portion is not explained, the next bimodal distribution analysis is performed, where weights of the two distributions are optimized.
- c) After bimodal distribution analysis, it is assessed how much the error is improved and whether both distributions are all fairly abundant. Only if all criteria are satisfied, two deuterium numbers are reported.

$$\text{DeuRate}(\%) = (1\text{stDeuNum} \cdot 1\text{stDeuWeight} + 2\text{ndDeuNum} \cdot 2\text{ndDeuWeight}) \cdot 100$$

4. Appendix

4.3.

Referencces

[1] Na, S., Lee, JJ., Joo, J.W.J. *et al.* deMix: Decoding Deuterated Distributions from Heterogeneous Protein States via HDX-MS. *Sci Rep* **9**, 3176 (2019).

<https://doi.org/10.1038/s41598-019-39512-8>