deMix Manual

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Introduction

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

1. Peptide centric view (Deuterated Distributions)

1.1. 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

1.2. Offers a deuteration rate (or deuterium uptake) plot across D2O labeling time for the selected peptide.

2. Protein centric view (Dynamics)

2.1. Allows users to view the dynamics of the protein.

2.2. Offers sequence coverage maps for HDX-MS data. Visually represents the HDX rate of each D2O labeling times within the HDX-MS datasetusing colors (= Heat Map).

Installation

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/

deMix Downloads

1. Windows

1.1. Download Windows version. & Extract the compressed file.

1.2. Double Click the bat file → Run anyway/Run

2. MacOS

2.1. Download MacOS version. & Extract the compressed file.

2.2. Right-click the deMix → Open with Terminal → Open.

3. Linux

3.1. Download the linux version. & Extract the compressed file.

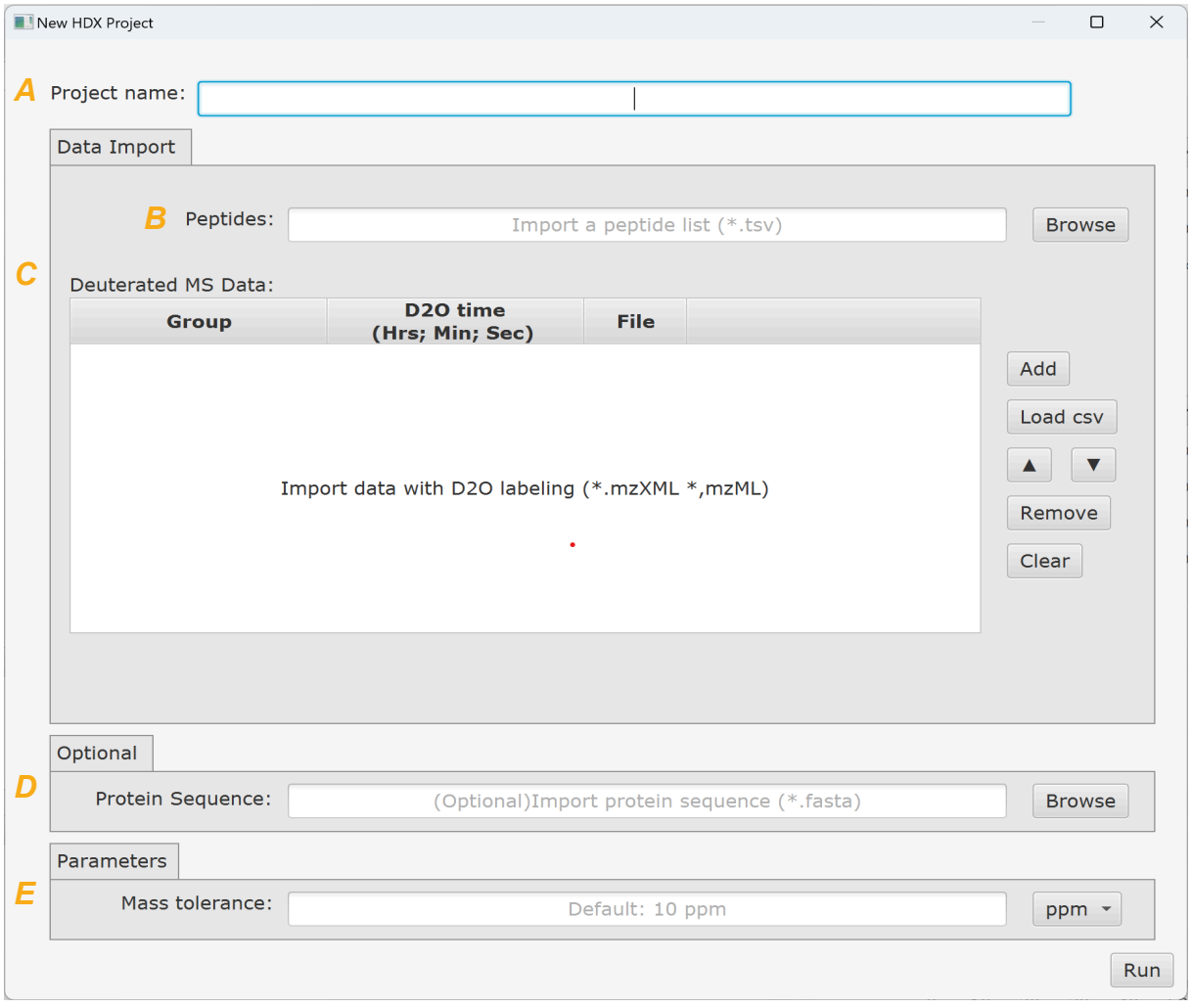
3.2. Double Click the .sh

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

How to Begin

Create new deMix Project

Click File → New Project



A. Project Name

- Specify the project name.

B. Peptides

- Import a file containing peptides to be examined using Browse button.
- Make sure that the file contains two columns named ‘peptide’ and ‘charge’ for peptides and charge states respectively.
- **File Format**: tsv

C. Deuterated MS Data

- Import D2O labeled data set(s) (With Group and D2O Time).
- **File Format**: MSXML, MZML
- **Buttons**: Add: Browse and selects both control data file and deuterated MS data file. User must provide D2O labeling. (view page 7 for more information)
- ▲: Moves the file up on the table.
- ▼ Moves the file down on the table.
- **Remove**: Removes the selected row of the table.
- **Clear**: Removes all the rows of the table.

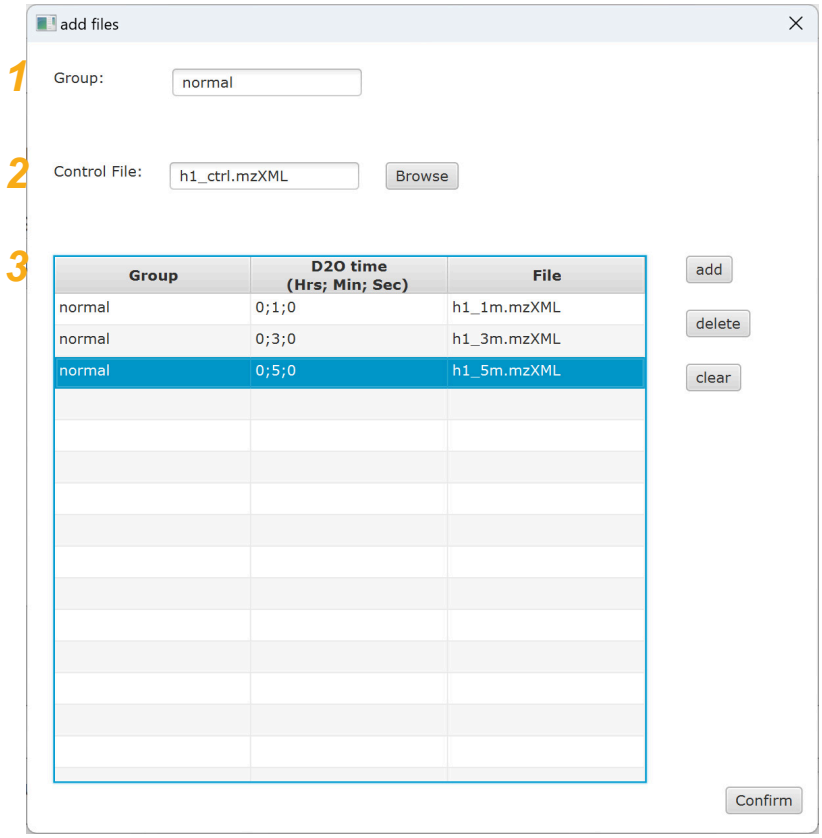
D. Protein Sequence

- Import a protein sequence data Browse button.
- File Format: fasta

E. Mass Tolerance

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

Deuterated MS Data Info



1. Group Name

1.1. Specify the name of the group (ex: normal, treatment..etc..)

2. Control File

2.1. Import control file for the group using Browse button

(ex: control file for the normal condition/ control file for the h2o2 solution condition)

3. Deuterated MS Data

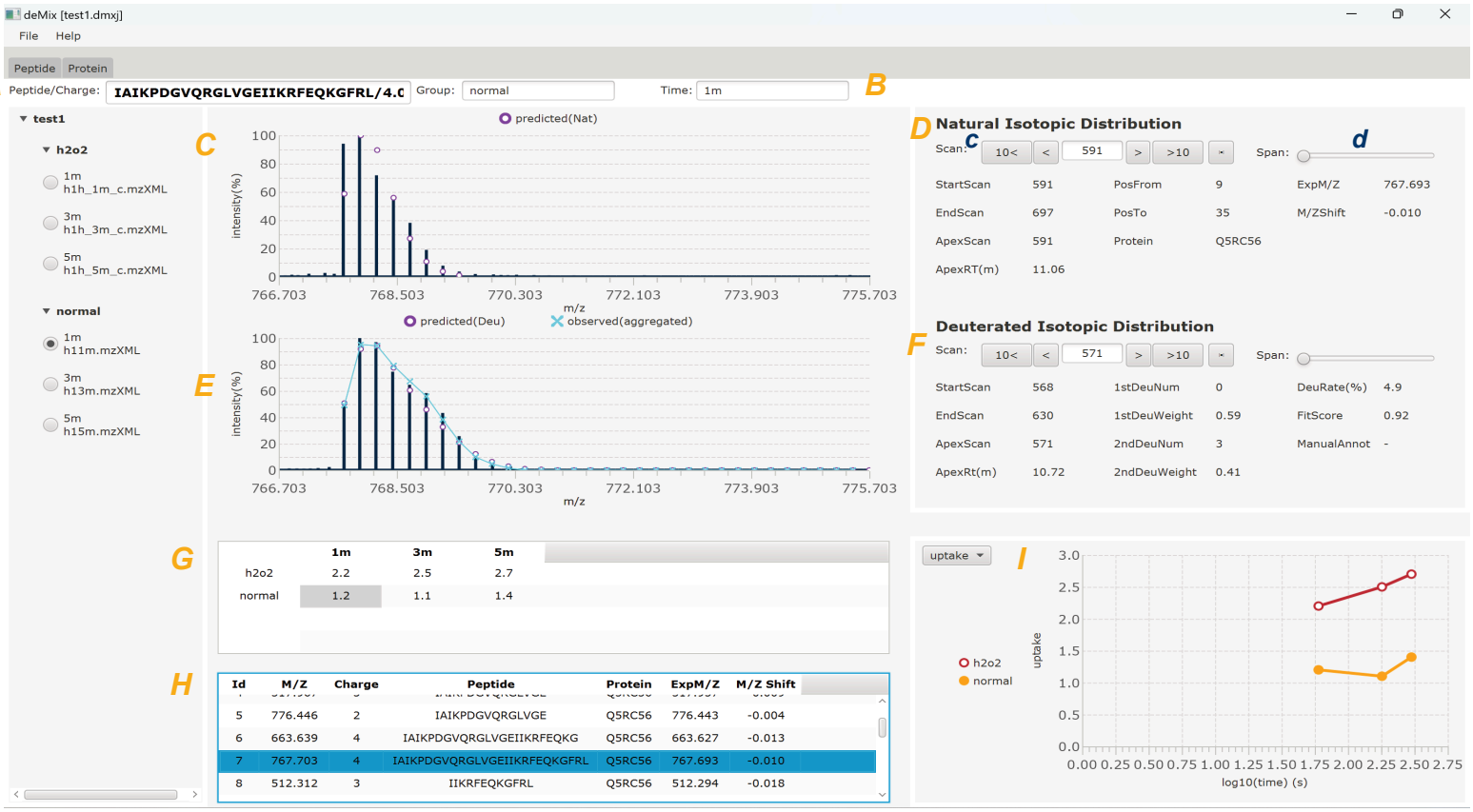
3.1. Add deuterated MS data for the group.

3.2. **Group**: name of the group (default is the name of the group specified in the Group section)

3.3. **D2O time**: enter the D2O labeling time in the order of (Hrs;Min;Sec) ex) 1 minute 30 seconds 0;1;30

3.4. **File**: name of the file selected

Peptide Centric View



A. Sample View

a. What it does

- Describes current file name, experiment groups, and files.

B. Current Status

a. What it does

- Shows currently selected peptide's sequence/charge and D2O label.

C. Natural Isotope Distribution (Nat.)

a. What it does

- Describes natural isotope distribution of selected peptide & D2O label

b. Plot Description

- x-axis: mass/charge(m/z)
- y-axis: intensity (%)
- Contains the theoretical distribution of the Nat. (= predicted ○)

c. Plot Function

- **Save as png**

  Right-click the distribution> save the current distribution> Browse > Finish

D. Natural Isotope Distribution Controller

a. What it does

- Shows information of current natural isotope distribution

b. Natural Isotope Distribution Information

- **StartScan**: Scan number where the peptide elution begins.
- **EndScan**: Scan number where the peptide elution ends.
- **ApexScan**: Scan number where the peptide elution is peaks.
- **ApexRT**(m): Retention time in minutes when the peptide elution peaks.
- **PosFrom**: Where the selected peptide starts in the given protein.
- **PosTo**: Where the selected peptide ends in the given protein.
- **ExpM/Z**: The expected starting m/z value of the distribution.
- **M/Zshift**: Observed m/z -Theoretical m/z

c. 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. *Span Controller*

- Adjusts the size (zoom out / zoom in) of the distribution.
- **Zoom out**: drag the slider to the right.
- **Zoom in**: drag the slider to the left.
- The slider ranges from 0 to 10 (positive real numbers).
- The zoomed in/out amount calculation: 5 * size (dragged position).
- The minimal m/z calculation: original m/z -size * 5.
- The maximum m/z calculation: original m/z + size * 5.

E. Deuterated Isotope Distribution (Deu)

a. *What it does*

- *Describes natural isotope distribution of selected peptide & D2O label*

b. *Plot Description*

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

c. *Plot Function*

- **Save as png**

  Right-click the distribution> save the current distribution> Browse > Finish

- **Manual annotation** of **predicted:** Set manual annotation to system's theoretical distribution (predicted)

  Generate: Right-click the distribution> Select HDX Num

  Remove: Right-click the distribution> Remove Manual Annotation

F. Deuterated Isotope Distribution Controller

a. *What it does*

- Shows information of current deuterated isotope distribution

b. *Deuterated Isotope Distribution Information*

- **StartScan**: Scan number where the peptide elution begins.
- **EndScan**: Scan number where the peptide elution ends.
- **ApexScan**: Scan number where the peptide elution peaks.
- **ApexRT**(m): Retention time in minutes when the peptide elution peaks.
-

- **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.

G. HDX Table

a. *What it does*

- Contains each D2O label's HDX Number of each D2O label.

b. *Table Function*

- Select Current Condition → Change Distribution(s)

  Select a D2O label by clicking a cell in the table

- **Change Unit**

  Right-click the table> convert to rate (%) /convert to uptake (HDX Num)

H. Peptide Table

a. *What it does*

- Contains currently selected D2O label group's entire peptide information.

b. *Table Function*

- Select Peptide → Change Distribution(s)

  Click a row to choose a peptide

I. HDX Plot

a. *What it does*

- Shows HDX uptake/rate across all D2O labeling time

b. *Plot Description*

- x-axis: log time (D2O labeling time)
- y-axis: uptake or HDX rate(%)

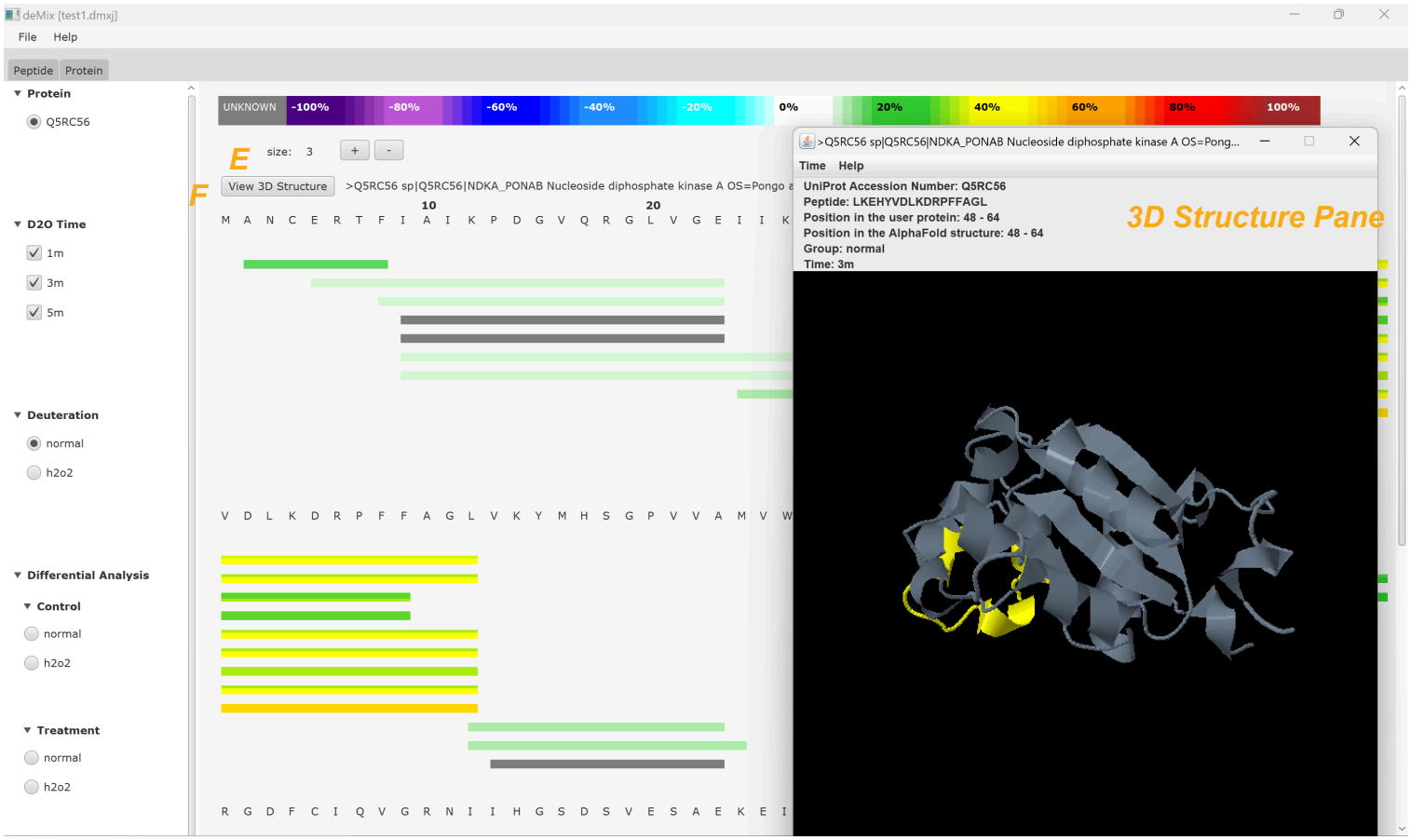
  use uptake/rate menu to convert

c. *Plot Function*

- **Save as png**

  Right-click the plot > save the current plot > Browse > Finish

Protein Centric View



A. Protein Menu

a. ***What it does***

- Shows a list of proteins & currently selected protein
- The selected protein's HDX rates over time across the peptides are drawn as a heat map.

B. D2O Time Selector

a. ***What it does***

- Shows a list of D2O time available & currently selected times.
- Controls which D2O times are included in heat map.
  - Default is set to include all times. The unselected time's HDX rates will be excluded from the heat map.

C. D2O Group Selector

a. ***What it does***

- The selected D2O group's HDX rates over time across the peptides are drawn as a heat map

D. Differential Analysis

a. ***What it does***

- Does the differential analysis between two D2O-labeled groups (control and treatment) by comparing their hydrogen-deuterium exchange (HDX) rates. It generates a heat map to visualize the percentage differences in HDX rates between the groups.

E. Size controller

a. ***What it does***

- Controls the size of the heat map.

F. 3D Structure Viewer

a. ***What it does***

- The 3D Structure Viewer button maps the HDX rates onto a 3D model of the protein structure, using color to represent varying exchange rates. This provides a visual representation of the HDX data in the context of the protein's spatial arrangement.