HDX Match User Guide 20150423.

The HDX Match program is designed for determining the deuteration level of the c- and z-ion protein fragments in top-down ECD-FTMS HDX protein analysis. The level of deuteration is determined by fitting theoretically predicted deuterated isotopic envelopes to the experimental data.

The program operates using protein sequence data, a non-HDX mass-intensity list and one or two HDX mass-intensity lists of ECD-FTMS/MS spectra as input.

The workflow of the software includes:
1) identifying the fragment ions in the non-HDX spectrum;
2) calculating the maximum number of exchangeable protons for the fragment;
3) locating the corresponding fragment's isotopic cluster in the HDX spectrum;
4) calculating and plotting of the theoretical deuterated isotopic distribution for all of the exchangeable protons exchanged;
5) gradually lowering the number of exchanged protons in the fragment and re-calculating/re-plotting the deuterated isotopic distribution until the best fit to the experimental data (as given by highest R^2 value) is found;
6) adding the deuteration value with best fit to the list of deuterated fragments found;
7) repeating steps 1-6 for the next fragment until all the fragments are analyzed;
8) saving the results to a file.

The program's graphical interface includes protein sequence text box, three spectrum panels, fragment ion list boxes, mass list boxes, control parameters input boxes, result output boxes and action buttons.

Following is a step-by-step example of how to operate the program:
1. Load the protein sequence by clicking the "Load" button in the top left corner next to protein sequence box.
2. Load the non-HDX mass list by clicking the "Load" button in the top right corner next to the Mass list 1 label. (Mass-intensity lists should be stored as tab-separated text files.)
3. Load the HDX mass list by clicking the "Load" button next to the Mass list 2 label.
4. Load the second HDX mass list by clicking the "Load" button next to the Mass list 3 label. (optional)
5. Select the ion type, minimum and maximum charge, and the mass range for fragments to be predicted, optionally check the "Current m/z range" check box if you prefer that for a fragment m/z range to be automatically selected according to a zoomed-in region of the spectrum. Click the "Predict" button. The theoretically predicted average fragment masses will be listed in the "Fragment ions" box next to the non-HDX spectrum panel.
6. Select a region of the non-HDX mass spectrum to analyze by clicking-and-dragging the left mouse button over the top spectrum panel (Note: to zoom out to the full spectrum, single click the left-mouse-button over the spectrum panel; to shift the mass range of the spectrum, click-and-drag the left mouse button in the space below the x-axis; to stretch the spectrum horizontally or vertically, click-and-drag the right mouse button in the space below the x-axis or in the space to the left of the y-axis, respectively). Change the ion type, minimum and maximum charge, and the mass range for which fragment ions to be predicted, if necessary. Click the "Predict" button.
7. Select the fragment ion of interest from the list by clicking on the fragment line in the "Fragment ions" box. Click the "Locate" button. The theoretical isotopic distribution will be plotted in red. The experimental masses taken into consideration for the calculation of R^2 will be highlighted in the "Mass list 1" box, and the maximum number of exchangeable protons will be displayed in the "Number of H_{exch}" box. Optionally, change the "% Maximum theoretical intensity cutoff" value and "Match m/z tolerance value" and click the "Locate" button again, if necessary. Note: it is preferable to recalibrate the spectra before loading the mass lists into the program to allow the use of the lowest possible "Match m/z tolerance" values.
8. Click the "Locate" button next to the HDX spectrum panel. The theoretical deuterated isotopic distribution will be plotted in red; the experimental masses used for the calculation of R^2 will be highlighted in the "Mass list 1" box, and the R^2 value with the number of exchanged protons used for the calculation will be posted in the "R^2" box. Lower the number of exchanged protons in the "Number of H_{exch}" box, and click the "Locate" button again. To refresh the R^2 list and to start over, click the non-HDX "Locate" button again. Choose the best fit (i.e., the one with the highest R^2 value) and click the "Add" button. A line with the fragment ion designation, charge state, average mass, R^2 value of the fit, and the number of exchanged and protected protons will be displayed in the "HDX Fragment ions" box. Note: to adjust the fit, you can change the "% D_2O" value and "% of maximum theoretical distribution intensity cut-off" value.
9. Repeat steps 5-8 until the entire non-HDX spectrum is processed.
10. Click the "Save" button. Fragments list will be saved to the tab-separated text output file in the same directory as the program's and can be directly opened in Excel.