Frequently Asked Questions (FAQ)

Last updated 8 August 2006
During the Beta Test period, this document may be frequently updated.
Make sure that you have the latest version.

General

1. What does HX-Express do?

   HX-Express automates part of the process of analyzing HXMS data. You will still need to identify the MS features of interest by inspecting your spectra/chromatograms. HX-Express processes a single spectral feature (*i.e.*, peptide or protein charge state) over a series of H/D exchange time intervals. HX-Express determines the centroid of the feature and the neutral mass. If undeuterated controls are included, relative deuterium levels can be computed:

   $$\text{Relative deuterium level} = m - m_{0\%}$$

   Where m is the mass after H/D exchange and $m_{0\%}$ is the unexchanged mass.

   If a totally deuterated control is also included, the % deuteration can also be determined:

   $$\% \text{ deuteration} = \frac{\left(m - m_{0\%}\right)}{\left(m - m_{100\%}\right)} \times 100\%$$

   where $m_{100\%}$ is the totally deuterated mass.

   In addition, the width of the isotopic distribution, a useful diagnostic for EX1 exchange, can also be measured.

   HX-Express generates a report showing how the deuterium level and peak width vary with exchange time.

   Since most HXMS data is used to compare different states of the same protein (*e.g.* wild-type *vs.* mutant, *etc.*), several sets of data for the same peptide or protein can be combined into a single analysis.

2. Where can I learn more about HX-Express.?

   Please see the paper Weis, D. D., Kass, I. J., and Engen, J. R. "Semi-automated data processing of hydrogen exchange mass spectra using HX-Express" in *J. Amer. Soc. Mass Spectrom.*, in press.

Installation

3. What are the system/software requirements for HX-Express?

   HX-Express is a series of Visual Basic for Applications (VBA) macros that run inside a Microsoft Excel workbook. To use HX-Express, you will need Microsoft Excel version 97 or later. HX-Express has been tested on Excel 2000 and Excel 2003 running on PCs with the Windows 2000 and Windows XP Professional operating systems. It has not been tested on a Macintosh. A minimum of 512 Mb of system memory is recommended.

4. How do I install HX-Express?

   Simply download the Excel workbook named *HX-Express Beta.xls*. Make a backup copy of HX-Express and keep it in a safe location in case your working copy becomes corrupted. It is also a good idea to make *HX-Express Beta.xls* a read-only file.

5. How do I run HX-Express?

   Opening *HX-Express Beta.xls* will start the HX-Express program as long as Excel is configured to run macros.

6. How do I configure Excel to run macros?

   To protect your computer from viruses that can be encoded into Excel macros, Excel, by default, will not execute them. You will need to lower your macro security level from Very High or High to Medium or Low.

   Under Tools | Macro | Security, set the Security Level to Medium or Low and Click OK. Close this workbook and reopen it.

   At the Medium security level, you will be prompted to Enable macros every time any workbook containing macros is opened. At the Low security level, macros will always be enabled without a prompt which leaves your computer vulnerable to macro viruses.

   If your security level is too high, you should find these instructions repeated on the worksheet that appears when you open *HX-Express Beta.xls*.

7. I got an error message that said the macros in this worksheet were disabled because the security was high. What should I do?

    Your macro security level is too high, see the answer to the previous question.

8. How can I make sure that HX-Express is working correctly?

    Download a copy of *test_data.xls* and *test_results.xls*. Open the test_data.xls file and analyze it using the parameters listed in the text box. Compare your results to those shown in the *test_results.xls* file.

Using HX-Express

9. I opened HX-Express, now what do I do?

    You will find system help available within the program. Go to the HX-Express pull down menu and select Help.

10. How does HX-Express access my mass spectrometry data?

    You will need to import data from your mass spectrometry software. How you do this depends on the software that you have. The most direct method is simply to paste data into a new workbook (you can not paste data directly into the HX-Express worksheets). See the Sample Data worksheet (look at the tabs along the bottom) in HX-Express for examples of how the data should be formatted. The data is inserted into the worksheet in two column (m/z, intensity) format. The first row of the worksheet is used to designate the H/D exchange interval. Microsoft Excel can currently handle 65,536 rows of data in a worksheet, so each individual spectrum must contain no more than 65,536 m/z / intensity pairs. Typically, spectra processed by HX-Express will contain 500-2000 pairs.

    Use a new worksheet in a single workbook for each data series. For example if you were comparing a peptide from a wild-type protein with the same peptide from three mutant forms (A, B, and C), use four worksheets. The name of the worksheet indicates from which member of the series the data was obtained. So in this example, the worksheets could be named wildtype, A, B, and C. You need to create a new workbook for each spectral feature that you wish to analyze.

    For spectra displayed in the Waters MassLynx software, the data can be directly imported from the MassLynx. Use the Get Spectra From MassLynx item in the HX-Express menu. You will be prompted to for a destination and worksheet name for the data. See the HX-Express system help for more information.

11. What is the difference between a worksheet and a workbook?

Each Excel file is a workbook. A workbook usually contains one or more worksheets. Different worksheets within a workbook may be accessed by clicking on the appropriate tab on the tabpane at the bottom of the Excel window.

12. How do I rename a worksheet?

You can rename a worksheet by: double clicking on the worksheet tab, right click on the tab and choose Rename, or select the worksheet and use Format | Sheet | Rename.

13. What are the requirements on the imported data?

To minimize analysis time, the m/z range should be narrow, encompassing just a small region on either side of the isotopic distribution. Adjacent spectral features should be deleted from the data. Each spectrum is analyzed individually, so it is not necessary for the m/z range to be the same for each spectrum

It is important that the spectra be well-smoothed. The peak detection algorithm in HX-Express is mislead by noisy peak tops. For peptide spectra with ~10,000 resolution, 2×4 point smoothing is generally adequate.

It is also important that the spectra contain a sufficient number of data points. At a minimum, each spectral feature (peptide isotopic peak or protein charge state envelope) should contain at least 20 data points. It may be necessary to parse high resolution data (*e.g.*, FTMS) to reduce the number of data points between the spectral features.

Make sure that data is imported with enough precision that the m/z values are distinct. For example 1036.472 *vs.* 1036.474 are distinct, but to two decimals places (1036.47 *vs.* 1036.47) they are the same.

14. What is the format for annotating the H/D exchange times to the spectra?

The easiest way is to use the Add Exchange Time Labels menu item in the HX-Express menu. Exchange times can also be entered manually. Any combination of units seconds, minutes, and hours can be used. HX-Express recognizes any time unit starting with "s" as seconds, any time unit starting with "m" as minutes, and any time unit starting with "h" as hours. The time unit 0 or anything starting with "un" will be identified as undeuterated and anything starting with "t" or "d" will be identified as totally deuterated.

15. How do the different parameters affect the analysis?

The most important parameter is the charge state (z), you will need to determine this manually by inspection. The charge state affects both the peak detection (see below) and also the determination of the neutral mass. If the charge state is set incorrectly for $z > 1$, the analysis will fail.

The width of the isotopic distribution is measured at a fraction of the intensity of the most intense peak (the base peak). This quantity is expressed a a percentage of base peak intensity (% BPI). It can be set between 10 and 90% BPI. Both the centroid and the isotopic distribution width will be measured over the portion of the spectrum defined by this quantity. If this value is set too high, erroneous results will be obtained for asymmetric isotopic distributions.

The Use Isotopic Peak Detection option determines how HX-Express analyzes the data. For spectra with well-resolved isotopic peaks, this feature should be left ON. The width of the isotopic distribution will obtained from a measurement of an empirical peak-to-peak envelope. This feature can be turned off for spectra that lack isotopic peaks (such as for highly charged features or transformed protein spectra) or where peak detection is problematic. HX-Express will bypass peak detection where necessary, but it may not be consistent across a spectrum series.

The intensity tolerance is used to discriminate against noise in the spectrum during peak identification. It is expressed as a percentage of the maximum peak in the spectrum (% BPI). The m/z tolerance specifies how close an isotopic peak needs to its predicted m/z value. This quantity is expressed in terms of number of data points since the m/z data is discrete. The actual m/z tolernace will depend on the spacing of the m/z data.

To experiment with the effects of changes in the parameters, use the Analyze Single Spectrum menu item. Only the spectrum in the active window will be analyzed.

16. I analyzed the data and saw whole bunch of Excel worksheets fly by. What next?

Take a look at the Summary page first and see if the results appear reasonable. Then you can use the Tabpane in the lower left-hand corner of the Excel workbook to move through the individual spectra one at a time to see if the analysis looks good. An entire set of 10-20 processed spectra can be visually scrutinized in about one minute.

17. I do not like the results of my analysis, can I reprocess the data?

Yes. Simply choose Analyze Spectra from any worksheet in the output workbook (the one that contains your Summary worksheet). Or you can carry out a new analysis on the worksheet that contains your original imported data.

18. Can I use different parameters for different spectra in a collection?

HX-Express does not have this feature. You can, however, analyze spectra individually using the Analyze Single Spectrum… menu item. You will then need to manually collect all of the data and make your own summary and plots.

Troubleshooting

19. I think that I have found a bug in HX-Express. What should I do?

    Please email the author, David Weis (dweis@unm.edu). Describe the problem that you are having and also include the text of any error messages that you receive. Indicate which version of HX-Express you are using and also the version of Excel and your operating system. Finally attach an Excel file containing the data that is causing the trouble, if applicable.

20. I'm having trouble with "garbage" data that is adjacent to the spectral feature of interest. What can I do?

    There are a number of things to try. If the feature is small, it may be possible to discriminate against it by raising the % BPI of the distribution width and/or the intensity peak tolerance. Lowering the m/z peak tolerance may also help. If none of these work, you may need to remove the offending region of the spectrum by manually deleting it from the Excel file. Use Excel's Edit | Delete | Shift Cells Up feature to remove the data so that there will not be gap in the data that is left. If you are working with data that has already been processed, you will need to turn off the worksheet protection in order to edit the data, go to Tools | Protection | Unprotect Sheet… Alternatively, you can simply set the intensity values to zero or to the baseline value.

21. I keep getting a peak detection error that asks me to verify the charge state. I am sure that the charge state is correct. What should I do?

    There are a number of factors that could cause this problem. First, try raising the m/z peak tolerance. If this does not help, the problem may be that the data is too noisy or not smoothed enough. If the peak tops appear, peak detection errors can result. Either try using additional smoothing or turn off the Use Isotopic Peak Detection option.

22. I got a "centroid divide by zero" error. What does this mean?

    Typically, this is seen with bad data. Either the precision was not set high enough that there are duplicated m/z values, or there are not enough data points in each spectral feature. Review the answer to question 10 (What are the requirements on the imported data?).

23. Some of the processed spectra have "ERROR" in the title and do not appear to have been processed. What happened?

    When HX-Express is unable to process a particular spectrum, this is the format it uses to indicate that there was a problem. The raw spectrum is displayed in the plot. Often a quick inspection will reveal the nature of the problem. Also, review the answers to the last few questions to see if any apply.

24. I tried to save the HX-Express workbook and got a warning about corrupting HX-Express. What should I do?

There is generally no need to modify or save the HX-Express workbook. If for some reason you do need to save a modified version, use File | Save As and save it under a different file name.

25. I tried to edit an output worksheet but got an error message that the sheet was read-only or protected. What happened?

To protect output data from being damaged by modifications by the user all of the output worksheets are protected. You can disable the protection by going to Tools | Protection | Unprotect Sheet…. Protection can be restored by using Tools | Protection | Protect Sheet… Also, if you reprocess the data, HX-Express will reset the protection automatically.

Other

26. There is a feature that I would like to see added to HX-Express. How do I request it?

Please contact the author, David Weis (dweis@unm.edu) with your suggestions. Please be aware that HX-Express is free software, and the author's time is limited.

27. I would like to add a feature to HX-Express. How do I go about doing this?

Your contribution would be welcome! Please review the terms of the GNU General Public License (GPL). You can distribute your own version of HX-Express with its own name subject to the terms of the GNU GPL. Alternatively, if you would like your contribution to be part of HX-Express it may be possible to incorporate your additions to a new version of HX-Express. Please contact David Weis (dweis@unm.edu) for more information.

28. Are there plans for any new features?

Hopefully, version 1.0 or 1.1 will include (1) a utility to edit the spectra (see question 14, I'm having trouble with "garbage" data that is adjacent to the spectral feature of interest. What can I do?) and (2) the ability to carry out a back-exchange correction.