Day 2: Pep3D Tutorial

Visualize data using Pep3D

Introduction

Pep3D is a tool for visualizing LC MS data. This brief exercise will introduce you to the

1. Log on to PETUNIA go to Browse Files
   1.1. Open a web browser and go to http://localhost/tpp-bin/tpp_gui.pl. Log on using user name *guest* and password *guest*. Go to the Browse Files tab under the Tools menu. Using the links under *DIRECTORIES:*, browse to `c:\Inetpub\wwwroot\ISB\data\class\QualScore\RaftFlow\`
2. Visualizing mzXML files directly using Pep3D
   2.1. When Petunia detects an mzXML file in a file listing, it allows the user to open it directly using Pep3D. Scroll down the file listing until you see raftflow31.mzXML. Click on the [Pep3D] link to launch Pep3D. A new browser window will open showing the Pep3D interface. This interface allows the user to adjust various parameters that effect the way that the Pep3D image is drawn. For now, accept the default parameters and click the Generate Pep3D Image button. After a short time, an image representing the LC MS data will appear.
   2.2. Note the large amount of 'empty space' at the bottom of the image. This area is blank because the mass spectrometer did not acquire any data in that mass range. Adjust the range that Pep3D renders by selecting Selected in the M/Z Range drop-down box and entering a range of 400 to 1600. Click the Generate Pep3D Image button. After a short time, an adjusted image will appear.
   2.3. Note how the elution appears compressed between 25 to 65 minutes. Stretch this section of the image by choosing Selected from the Elution time range drop-down box and entering a range of 25 to 65 minutes. Click the Generate Pep3D Image button and wait for the adjusted image to appear. You will notice that it is considerably narrower that the original. To increase the width or height of an image you simply adjust the values of the Resolution and Size parameters. It helps to think of these parameters as 'units per pixel'. Set the Size parameter of the Elution time range to 5 and click the Generate Pep3D Image. What was the effect on the image?
   2.4. Pep3D can also overlay on the image the co-ordinates of MS/MS events. Select CID from the Display peptides drop-down box and click the Generate Pep3D Image. The blue squares represent CID events. Click on a blue square CID. A new browser window will open showing an image of the selected CID spectrum.

3. Visualizing LC MS data with peptide identifications

3.1. Pep3D can also be opened from within PepXMLViewer. Return to Petunia and use the Browse Files tab locate the interact-qualscore.xml file in the QualScore\RaftFlow directory. Click on the [PepXML] link to open the PepXMLViewer. Click on the PEP3D link between the Summary and Display Options sections to launch Pep3D. A new browser window will open containing the Pep3D interface. Accept the default parameters and click the Generate Pep3D Image. When Pep3D is given a pepXML file as input, it will generate an image for each mzXML that is represented in that pepXML.

3.2. Unlike the previous example, CID attempts are now colour coded to reflect the PeptideProphet probability of their corresponding peptide assignments. Below each Pep3D image there is a coloured scale ranging from probability 1 to 0.5, red to light green respectively. Click on a red squre. As before, a new browser window will open and display an image of the MS/MS spectrum however now the assigned peptide sequence and its search scores are also shown.

3.3. Look at the peptide/CID ratio at the top of each Pep3D image. Which run has the highest ratio? Compare it's Pep3D image to that of the run with the lowest ratio. Are there any major differences?