PROCESSING 1D SPECTRA USING VNMRJ

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PROCESSING 1D NMR SPECTRA USING VNMRJ

1. INTRODUCTION

This worksheet describes the procedures needed to manipulate process and analyze spectra that have been completed on any of the spectrometers. In addition, the steps needed to save a processed spectrum will be described.

2. OVERVIEW

This section provides an overview of the VNMRJ processing panel and graphics control buttons used for processing 1D NMR spectra. The VNMRJ screen is displayed in Figure 1.
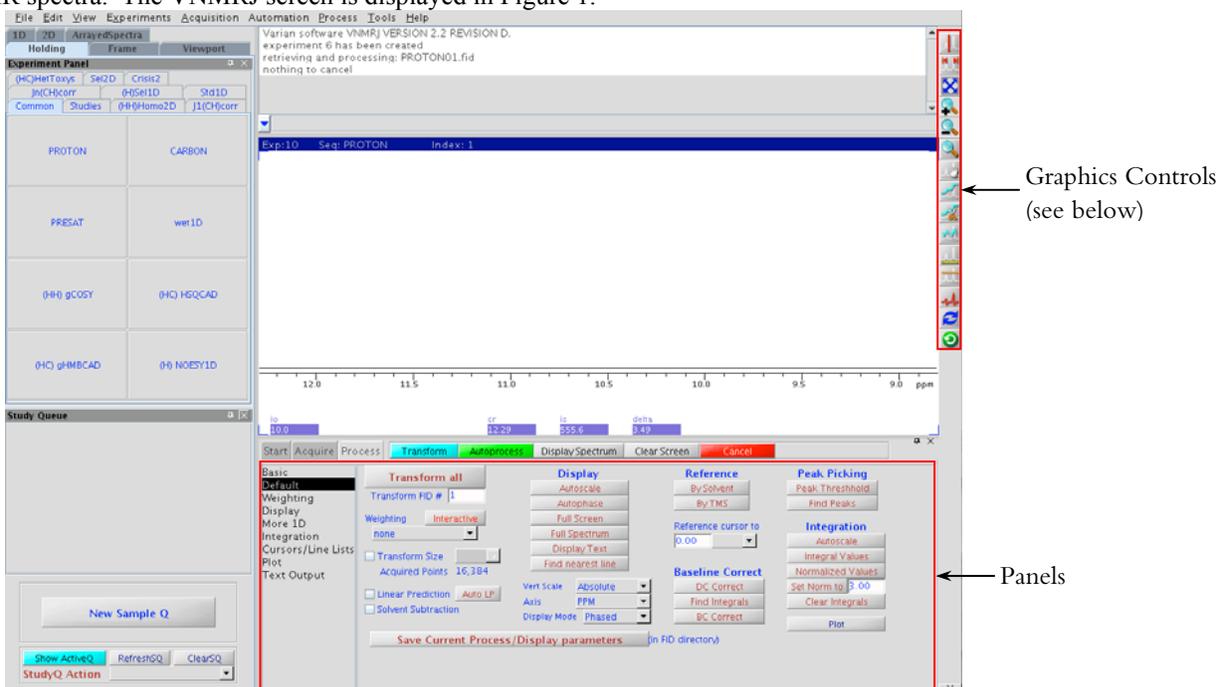

Figure 1. The **Process** panel, D**efault** page and Graphics Controls. Together, these are used to manipulate and process 1D NMR spectra.

The graphics controls buttons are displayed in Figure 2. The text below describes the various functions of the graphics controls buttons.

a) One/Two Cursors: *Toggles between one cursor and two cursor mode. One cursor is used to select a specific peak. Two cursors are used to select a specific region in the spectrum. (See section 3.3.2)*

b) Show Full Spectrum: *Zooms out to show the entire spectrum. (See section 3.3.4)*

c) Reset To Full Display: *Returns to original full spectrum. (See section 3.3.4)*

d) Zoom In: *Used to zoom into a selected region of the spectrum. (See section 3.3.2)*

e) Zoom Out: *Used to zoom one level out and see a broader view of the spectrum. (See section 3.3.2)*

f) Zoom Mode: *Used to highlight and zoom in to a specific area of the spectrum. (See section 3.3.1)*

g) Pan & Stretch Mode: *Used to move the spectrum upfield or downfield, and zoom in and out. (See section 3.3.3)*

h) Show Integral Regions: *Shows integrals on all peaks within the spectrum. (See section 5.3)*

i) Show/Hide Axis: *Used to choose whether or not the chemical shift scale is displayed.*

j) Show/Hide Threshold: *Used to set the threshold when peak picking. Any peaks below the threshold will not be picked, while those the threshold will be picked. (See section 5.1)*

k) Phase Mode: *Used to adjust the peaks to pure absorption. (See section 3.4.1)*

l) Redraw: *Hides any peak picks or integrals. .*

m) Return: *Returns to previous graphics control menu.*

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a) Two Cursors
b) Show Full Spectrum
c) Reset To Full Display
d) Zoom In
e) Zoom Out
f) Zoom Mode
g) Pan & Stretch Mode
h) Show Integral Regions
i) Show/Hide Axis
j) Show/Hide Threshold
k) Phase Mode
l) Redraw
m) Return

n) Show Full Integrals
o) Define Integral Regions
p) Integral Lvl/Tlt

q) Hide Integrals
r) Delete All Integrals
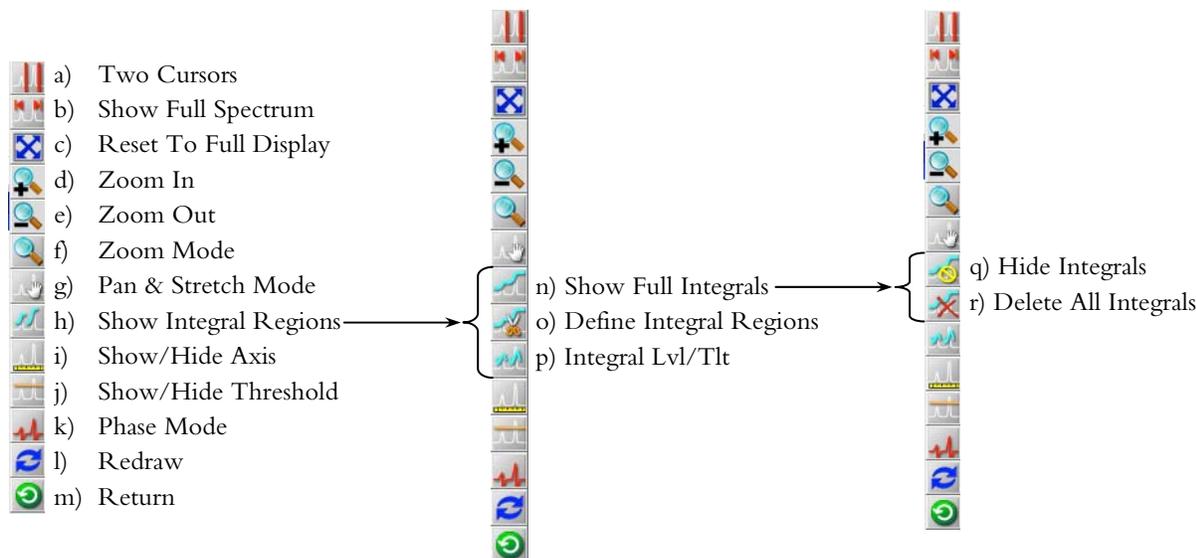
Figure 2. Graphics controls used to expand, phase, peak–pick and integrate NMR spectra.

n) Show Full Integral: *Shows integrals across all peaks in the spectrum.*

o) Define Integral Regions: *Used to "cut" the integrals. Defines what is a peak and the areas that are baseline. (See section 5.3)*

p) Integral Lvl/Tlt: *Adjusts the angle of the integrals in order to to obtain more accurate integral values. (See section 5.3)*

q) Hide Integral: *Hides integrals from spectrum, but does not delete them.*

r) Delete All Integral Regions: *Deletes all integral regions, but integrals remain displayed.*

3. OPENING YOUR SPECTRUM

1) From the VNMRJ main screen go to **File → Open…**.
2) Locate the directory where the spectrum is saved; your data should be in /home/data/group_name/your_name/.
3) Double click on the .fid file of the 1D NMR spectrum you would like to process.

4. SPECTRUM MANIPULATION AND REFERENCING

4.1. Adjusting the Vertical Scale

There are two methods in which to adjust the vertical scale of the spectrum.

4.1.1. Using the middle mouse button
The middle mouse button (the scroll button) can be used in two different ways to modify the vertical scale:
1) Click the middle mouse button above the baseline of the NMR spectrum to increase the vertical scale or click below the baseline of the spectrum to decrease the vertical scale.
OR
2) Click and hold the middle mouse button and drag the mouse up or down to increase or decrease the vertical scale, respectively.

4.1.2. Automatic adjustment using autoscale button
1) Go to the **Process** panel, **Default** Page.
2) Under the Display subheading click on   Autoscale .
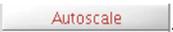
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4.2. Zooming In and Out

There are several ways in which to zoom into and out of a specific chemical shift region of interest.

4.2.1. Using the zoom mode button

1) Click on 🔍 located on the right hand side of the screen.
2) Click and hold the left mouse button and move it upfield or downfield to select the region of interest. The selected region will turn light purple.
3) Release the left mouse button once the region of interest is selected.

4.2.2. Using the double cursors, zoom-in and zoom-out button

1) Click on ▯▯.
2) Left click on the left-hand edge of the area you are interested in looking at (a red vertical line should appear indicating the boundary of the area you want to look at).
3) Right click on the right-hand edge of the area you are interested in (another red vertical line should appear).
4) Click on 🔍 to zoom in on this area of the spectrum.
5) Click on 🔍 to zoom out of this area of the spectrum.

4.2.3. Using the pan & stretch mode button

1) Click on ✋.
2) Click and hold the right mouse button
3) Move the mouse up to zoom in, or move the mouse down to zoom out.

4.2.4. Using the show full spectrum or reset to full display buttons

1) At any point you are able to return to the original full spectrum by clicking on either 🔀 or ✖.

4.3. Moving the Spectrum Upfield or Downfield

When zoomed in to a particular region, you may move the spectrum downfield or upfield using the pan & stretch mode button.

1) Click on ✋.
2) Place the cursor in the middle of the spectrum.
3) Click and hold the left mouse button.
4) Move the mouse left to move the spectrum left and view a downfield region or move the mouse right to move the spectrum right and view an upfield region.
5) Repeat steps 2 and 3 to move more upfield or more downfield.

4.4. Phasing

4.4.1. Manual phasing

The spectrum will be automatically phased using the VNMRJ software, which should be sufficient. If peaks are not phased to pure absorption, then manual phasing is necessary. If manual phasing is required, then:

1) Click on ⌁ located on the right hand side of the screen.
2) Left click and hold on the largest peak. A set of red vertical lines will appear on either side of the peak.
3) Drag the mouse up and down until the peak is in phase.
4) Exit phase mode by clicking on ⌁.

4.4.2. Automatic phasing

To re-apply VNMRJ's automatic phasing:
1) Go to the **Process** panel, **Default** Page.
2) Under the Display subheading click on [Autophase].

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4.5. Chemical Shift Referencing

VNMRJ automatically references the spectrum based on the lock signal of the chosen solvent. Solvent chemical shifts can be found in the binders located at each NMR spectrometer or from the CIL webpage, http://www.isotope.com/cil/products/images/nmrchart.pdf.

Periodically the referencing is incorrect and manual referencing can be accomplished in two ways.

4.5.1.   Using the By Solvent Button
1)   Go to the **Process** panel, **Default** page.
2)   Under the Reference sub-heading choose [By Solvent] or [By TMS].

4.5.2.   Manual chemical shift referencing
1)   Go to the **Process** panel, **Default** Page.
2)   Place the single cursor on the solvent or reference peak.
3)   Under the Display sub-heading click [Find nearest line].
4)   Under "Reference cursor to", select ppm from the drop down menu
5)   Enter the chemical shift value for the peak in the window and hit enter.

5. ANALYZING YOUR NMR SPECTRUM

5.1. Peak Picking

1)   Go to the **Process** panel, **Default** page.
2)   Click on [ ] to set the peak threshold.
3)   Left-click on the spectrum at a desired height to set the peak threshold. Peaks under the orange horizontal line will not be peak-picked, but peaks above the line will be peak-picked.
4)   Under the Peak Picking heading click on [Find Peaks]. The chemical shift values of the peaks will be displayed above each peak in ppm.

5.2. Determining J–Coupling Constants

5.2.1.   Method 1: For a single J-coupling value
1)   Click on [ ].
2)   Left-click on the downfield peak (left peak), then right-click on the upfield peak (right peak).
3)   In the command line type "delta?". The frequency difference between the two peaks is displayed in Hertz.

5.2.2.   Method 2: For multiple J-couplings
1)   Go to the **Process** panel, **Default** page.
2)   Under the Display heading, find the drop down menu next to Axis, select Hz.
3)   Under the Peak Picking heading click on [Peak Threshhold].
4)   Set the peak threshold by clicking on the spectrum below the height of the peaks of interest.
5)   Click on [Find Peaks]. Peak frequencies in Hz will then be displayed above each of the peaks. The J-coupling value is the difference between the two values.

5.3. Integrating Peaks

1)   Go to the **Process** panel, **Default** page.
2)   Zoom-in on the peaks of interest as described in section 4.2.
3)   Click on [ ] to display default integral lines. The integral lines are displayed in green.
4)   Clear the default integrals by clicking on [Clear Integrals].
5)   Increase or decrease the vertical scale of the integrals using the middle mouse button; clicking above the integrals will increase the vertical scale while clicking below the integrals will decrease the vertical scale.
6)   Click on [ ] to adjust the tilt of the integrals and achieve a flat baseline between peaks.
7)   Left-click on the spectrum and hold the mouse button. Drag up to increase the right-hand side height of the integrals and drag down to decrease the right-hand side height of the integrals until a flat baseline is achieved.
8)   Release the mouse button.
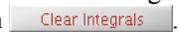
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9) Click on . "Cut" the integrals by left-clicking on the left and right-side of all peaks you want integrated. Selected integrals will have a solid green line; the baseline integral line will be dotted.
10) To remove a cut, right-click on the integral to be removed.
11) The integrals must be normalized, which can be done in two ways:
    a) To set integral value of a single peak and normalize all integrals with respect to that:
        i)   click on to enter single-cursor mode.
        ii) place the cursor on the peak of interest.
        iii) input the number of protons that comprise the peak in the `Set Norm to` field, followed by enter.
        iv) click on `Set Norm to`.
        v)   click on `Integral Values`; the integral values will be displayed.
    b) To set the integrals so the sum of all integrals equals a number:
        i)   input the total number of protons in the molecule into the `Set Norm to` field, followed by enter.
        ii) click on `Set Norm to`.
        iii) click on `Normalized Values`; the integral values will be displayed.

6. PLOTTING

6.1. Automatic Plotting

1) Go to the **Process** panel, **Plot** page. Before sending the spectrum to the printer, you may choose how you would like the NMR parameters, integrals and peak frequencies printed.
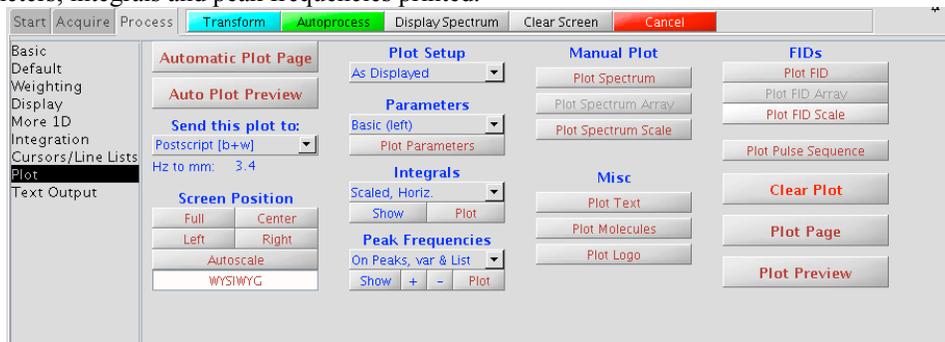

Figure 3. The **Process** panel, **Plot** Page. This page is used to select plot settings, print NMR spectra and save graphics files.

2) Under the Send this plot to: section, select NMR_HPplot.
3) Under the Parameters section, choose which parameters you want and how you want them listed on the printed spectra. The options given include:
    Basic (left)   prints only the major parameters on the left side of the page (recommended).
    Basic (right)   prints only the major parameters on the right side of the page.
    Full (left)   prints all parameters on the left side of the page
    Full (right)   prints all parameters on the right side of the page
    None   prints no parameters
4) Under the Peak Frequencies section choose how you want the peak frequencies to appear on your spectra from the drop down menu and then click on `Show`. The options given include:
    On Peaks, var, ppm   prints the shift values in ppm directly on top of each peak
    On Peaks, var, Hz   prints the shift values in Hz directly on top of each peak
    On Peaks, top, ppm   prints the shift values in ppm at the top of the page (recommended)
    On Peaks, top, Hz   prints the shift values in Hz at the top of the page
    As A list   prints the shift values as a list on the page
    On Peaks, var & List   prints the shift values on top of each peak and as a list
    On Peaks top & List   prints the shift values at the top of the page and as a list
    None   prints no peak values

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5) Under the Integrals section choose how you want the integration values to appear below the axis from the drop down menu and then click on Show . NOTE: If one of the two vertical options were chosen, you must type `vp=12` in the command line.

Scaled, Horiz.	prints the scaled values horizontally below the axis (recommended)
Scaled, Vert.	prints the scaled values vertically below the axis
Normalized, Horiz.	prints the normalized values horizontally below the axis
Normalized, Vert.	prints the normalized values vertically below the axis
None	prints no integral values

6) Once plot settings have been adjusted click on Automatic Plot Page .

6.2. Manual Plotting

More control of what is plotted is achieved if you manually plot the data. You may print the FID, pulse sequence, etc. Simply click the buttons labeled Plot *** to plot the desired item, then Plot Page to send it to the printer. To print the same spectrum as the automatic plotting, follow the procedure below.

1) Under the Send this plot to: section, select NMR_HPplot.
2) Select the plot parameters under the Parameters section as described in 6.1, click on Plot Parameters .
3) Select the peak pick appearance under the Peak Frequencies section as described in 6.1; click on Plot .
4) Select the integration appearance under the Integrals section as described in 6.1; click on Plot .
5) Under the Manual Plot section click on Plot Spectrum and Plot Spectrum Scale .
6) Click on Plot Page to send the plot to the printer.

7. SAVING THE PROCESSED SPECTRUM

VNMRJ automatically saves the FID, but doesn't save the processed spectrum. The processed spectrum can be saved in a universal NMR format known as JCAMP, which can then be read into any NMR software. Unfortunately only 1D NMR spectra can be saved in this manner; there is currently no way in which to save a processed 2D spectrum.

1) In the command line, type `svsj`. A message will pop-up displaying the directory in which the processed spectrum was saved. The file will have a .dx extension.

8. PREPARING GRAPHICS/POSTSCRIPT FILES

VNMRJ saves pictures (or graphics) as a postscript file, which is a high-quality LINUX graphics file. Once saved, you may FTP the postscript file to your own computer and edit the file directly in graphics programs such as CorelDraw, Adobe Illustrator or Adobe Photoshop. Alternately, if you have Adobe Distiller installed or use a Mac, if you double-click on the postscript file it will be automatically converted to a PDF file.

1) Go to the **Process** panel, **Plot** page.

2) Under the Send this plot to: section select Postscript [b+w] Send this plot to: Postscript [b+w] ▼ .
3) Select all spectra parameters as described in section 6.2.
4) In the command line, change the directory by typing `cd('/home/data/group_name/username')`, where *groupname* and *username* are replaced with your particular values. The plot will be saved in this directory.
5) In the command line, type `page('filename.ps')` to create a postscript file, where *filename* is replaced by whatever you would like to call the plot.