PROCESSING & PLOTTING gHSQC or gHMQC

There are so many issues that are involved in processing, displaying, and plotting data from these experiments, that a separate manual was warranted. Even this "bare bones" guide to processing & plotting can't cover everything that you might need to know and do. Please ask for help the first time you perform this, to minimize your frustration (if for no other reason).

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Conventions in this manual:

Boldface text indicates commands to be typed at the computer

<angle brackets> are used to designate a key to be pressed (i.e. **<Ret>** for Return/Enter)

[square brackets] designate an icon/button in the VNMR menu to be *clicked*

Mouse Conventions: *click*, by default, refers to the Left Mouse Button.

- **LMB** will be used to designate the Left Mouse Button
- **MMB** will be used to designate the Middle Mouse Button
- **RMB** will be used to designate the Right Mouse Button
- Sometimes you will need to *hold*, rather than *click* the mouse button. This means that you should press and hold the button down throughout the operation.

Note: All **commands** in boldface assume that you press **<Return>** afterwards.

Processing and Plotting the 2D-HSQC spectrum: Please note, there are many options and many different ways to optimize the data processing for 2D NMR spectra. One can not cover all possible situations in a "Bare Bones" manual. You should expect that you will have to learn much more than is in this manual about how to properly process your data to get the best results.

1). Select your printer/plotter and paper size first.

2). Type **jexp1** to join Exp:1, and load the 1D-^{1}H spectrum from step 6 on the previous page.

3). If you have a 1D-^{13}C spectrum acquired on the same instrument as the gHSQC/HMQC spectrum, type **jexp2** (or any other valid Exp:#), and load the 2D-HSQC spectrum inot EXP2.

4). Type **jexp3** (or any other valid experiment number) to join EXP3, and load the 2D-gHSQC or gHMQC spectrum.

5). Type **setLP1** to activate forward linear prediction (improves resolution and quality).

6). type **gaussian** or **sqcosine** to set the window-function parameters.

   a) The two most common *window functions* used for these experiments is either a COS^2 or Gaussian window. Generally gaussian improves S:N, and sqcosine generally improves resolution (less S:N). Choose the one that best fits your data.

7). type **pgHSQC** or **gHMQC** to process the data.

8). If the contour plot is not centered, type **fullt** followed by **dconi**.

9). NOTE: the command **dconi** is analogous to "ds" for 1D spectra. If you lose your 2D image, and you want to view it, type **dconi** to re-display the 2D spectrum. You don't need to re-type pgHSQC or pgHMQC unless you want to try different processing parameters. In fact, re-processing every time will waste a lot of your time!

10). Adjusting the contour plot:

   a). *click* the **MMB** on any spot in the 2d-display, to scale the spectrum so that spot just touches the *floor* of the contour plot. *Clicking* **MMB** on a taller peak will reduce the *vs* to make smaller peaks disappear.

b). Alternatively, you can *click* **MMB** on the colored scale (on the right) to reduce the minimum level displayed. I recommend using (a), rather than (b).

c). You can fine-tune the peaks displayed peaks by *clicking* on **[vs+20%]** or **[vs-20%]**. *Be careful NOT to click on [Autoplot] by accident. This is an easy mistake, and if you have lots of noise on your display it can shutdown the computer you are using for a long time.*

11). Setting the Chemical Shift Reference:

a). Expand your plot on a peak of known chemical shift (in either the ^{1}H or ^{13}C dimension)

b). Set the cursor (cross-hairs) on the peak.

1). To set the shift in f1(^{13}C), type: **rl1(#.##d)**. The "d" is important because the ^{13}C dimension is the "decoupler" dimension.

2). To set the shift in f2(^{1}H), type: **rl(#.##p)**.

12). Changing the orientation of the 2D plot (i.e. F1 mode vs. F2 mode):

a). The X-axis defines the working "mode", so if the ^{13}C axis is on the bottom, you are working in F1-mode, if the ^{1}H axis is on the bottom you are working in F2-mode. To change modes, do the following:

1). *Click* on [Return], *click* on [More], then *click* on [F1mode] or [F2mode] to switch modes.

13). Phasing the 2D-NMR spectrum: if your 2D peaks are not phased, they will appear partially negative and partially positive. To fix this, perform the following steps in order…bear in mind that this takes some practice to get it right.:

a). *Click* on **[Full]** to be sure you are looking at the full spectrum. A bug in the software will not allow you to phase on an expanded region.

b). *Click* on the **[phase2d]** button, and use the left button to select a trace through a peak near the center of the contour plot. You should see the peak in the 1D trace at the top.

c). *Click* on the **[spec1]** button, then click on **[phase]**.

d). Use the **LMB** to phase this peak, then *click* on **[Done]**, then **[Return]**

e). If the peaks away from center are still not phased, you will need to repeat the operation as follows (this takes practice to get it right, ask for help if necessary):

1). *Click* on **[phase2d]**, and select a trace through a peak near the left-edge of the spectrum.

2). *Click* on **[spec1]**, then select a trace through a peak near the right-edge of the spectrum, and *click* on **[spec2]**.

3). *Click* on **[phase]**, and you will see the trace for "spec1". Phase this peak using the LMB. Note the position of the peak in "spec1"…if you have an axis displayed, it helps, or use your finger or something to note the position of the peak in "spec1".

4). *Click* on **[spectrum 2]**, and *click* the **LMB** once where the peak was previously in "spec1". There will probably be nothing there, but click once there anyway. Move your pointer to the other peak ("spec2"), then use the **RMB** to phase this peak (up or down, whichever is appropriate). You shouldn't have to change this peak very much.

5). When the peak on the right is phased as closely as possible, *click* on **[Done]**.

14). Plotting: there are three options…select your plotter before proceeding. Note: if you are using large (11x17) paper, you need to substitute "plhxcorbig" for "plhxcor". Otherwise, a small plot will be printed on big paper.

a). If you have a high-resolution ^{13}C spectrum of your sample loaded in EXP2, you can plot with your high-resolution 1D spectra along the sides.

1). Type: **plhxcor(15,1.4,1,2)**…. ,where "15" is the number of contours, "1.4" is the spacing between the contours (should be between 1.1 and 2.0), "1" is the EXP# containing the high-resolution proton spectrum, and "2" is the EXP# containing the carbon spectrum. Note: if you use a 1D ^{13}C spectrum acquired at a different frequency, your ^{13}C spectrum will not line-up with your 2D peaks.

b). If you want to plot your high-resolution, 1D proton spectrum along the ^{1}H axis, but you don't have a high-resolution ^{13}C spectrum acquired on the same instrument, you can do the following:

1). You need to perform a "dummy plot" to resize the window on the screen. To do this type the following: **plhxcor_nopg(10,2,1,-1)** …wait a few seconds until activity stops…type **pgcl** (this dumps the plot into nowhere).

2). What you do next depends upon whether your x-axis is ^{13}C or ^{1}H. We will assume that the X-axis is the ^{13}C axis for these instructions. See step 11 above to switch the X-axis display mode.

3). Zoom-in on the region you wish to plot.

a) ***click*** **[Proj]**, then ***click*** **[Hproj(max)]**, then set the vertical scale for the 1D projection with **MMB** (caution: don't click in the 2D window, or you will mess-up the scaling of the 2D window).

b) ***Click*** **[Plot]**, to add the projection trace to the plot.

c) Type **plhxcor(15,1.4,1,-1)** to complete the plot. {The numbers mean: 15 contours, separated by 1.4 intensity units, the 1D-^{1}H spectrum is in EXP1, and the "-1" means to suppress the plotting of the ^{13}C axis (however, we plotted the ^{13}C trace in the previous step)}.

d) Type **dconi** to re-display the plot, *click* **[Full]**, and repeat the process to plot expansions of all of the regions that you wish to plot.

c). Plotting with projections (low-resolution) on both sides. Advantage is that the plot is bigger on the paper.

1). from *dconi* , ***click*** **[Proj]**, then ***click*** **[Hproj(max)]**, then set the vertical scale for the 1D projection with **MMB** (caution: don't click in the 2D window, or you will mess-up the scaling of the 2D window).

2). ***Click*** **[Plot]**,

3). ***Click*** **[Vproj(max)]** then **[Plot]**.

4). ***Click*** **[Cancel], [Return], [Plot], [All Contours], [All Parameters],** & **[Page]**.

d). Dealing with phase-edited gHSQC data (where CH_2 signals have the opposite phase of CH & CH_3 signals in the 2D plot): The 1D-projections in steps (b) and (c) above will only show positive peaks; therefore, only the positive signals will show up on the projections. By default, the CH_2 signals are negative (blue), and the CH/CH_3 signals are positive (red/orange).

1). Follow the instructions above (b) or (c) to generate your plots, showing the projections from the positive (red/orange) peaks. To make a plot that shows the negative(blue) peaks, you have to invert the phase. This can be done as follows:

2). Type **phase(180)** , then type **dconi** to re-display the plot. The colors should be reversed.

3). Repeat the plotting steps above, but now you have inverted the phase so that the peaks not previously shown on the projections will now appear. With phase-edited gHSQC, you basically need to generate one set of plots for the CH/CH_3 signals, and separate plots for the CH_2 signals.

15). Generating 1D-projections from 2D gHSQC/gHMQC spectra for plotting as 1D ^{13}C spectra:

a). You will need an unused experiment, in which to place the 1D-projection. For example, if you are using EXP1 for your 1D-1H spectrum and EXP2 for your 2D spectrum, you could use EXP3 for your 1D-projection. So, in the following example, we will use (3) as the experiment number for your projections.

b). You must have the ^{13}C axis on the bottom. As described earlier, you can click [Return], [More], then [F1mode] to change the orientation.

c). With the 2D plot on the screen (dconi), type **proj(3)** then **jexp3** to join EXP3.

d). Type **f <space> full <space> ds** to be sure you are seeing the full ^{13}C spectral window. You can use the **MMB** as usual to adjust the vertical scale of the plot. You will notice that the carbon peaks are broader than a normal ^{13}C spectrum. This is normal, and characteristic of the lower resolution in the 2D experiment.

e). Use the threshold (**[th]**) and **dpf** command to display the frequencies of all ^{13}C signals.

f). You can use the normal plotting commands to create 1D plots of the ^{13}C projections.

g). <u>NOTE</u>: for phase-edited gHSQC spectra, you will have to do separate projections for the CH/CH_3 and CH_2 signals because only positive signals will appear in the projection. The methods described in part 14(d) above apply here as well.

1). go back to your 2D-gHSQC spectrum (if it is in EXP2, type **jexp2**, then **dconi**).
2). With the 2D plot on the screen, type **phase(180)** then **dconi**, and the spectrum should be inverted (colors are swapped).
3). Repeat the steps above {section 15(a-f)} to plot the peaks that were previously inverted.
4). In this way, you can make separate plots, one containing CH & CH_3 signals, and a separate plot containing only CH_2 resonances.

16). <u>Plotting to a PostScript file, instead of printing on paper</u>:

a). Make sure you select the 8x11 LaserJet 4MV as your plotter before proceeding.

b). Follow the plotting instructions above; however, substitute "**plhxcor_nopg**" for "**plhxcor**". For example, if you follow step 14(b) above, you would do the following:

1). Perform a "dummy plot" to resize the window on the screen. To do this type the following: **plhxcor_nopg(10,2,1,-1)** …wait a few seconds until activity stops…type **pgcl** (this dumps the plot into nowhere).
2). What you do next depends upon whether your x-axis is ^{13}C or ^{1}H. We will assume that the X-axis is the ^{13}C axis for these instructions. See step 11 above to switch the X-axis display mode.
3). Zoom-in on the region you wish to plot.
   a) ***click*** **[Proj]**, then ***click*** **[Hproj(max)]**, then set the vertical scale for the 1D projection with **MMB** (caution: don't click in the 2D window, or you will mess-up the scaling of the 2D window).
   b) ***Click*** **[Plot]**, to add the projection trace to the plot.
   c) Type **plhxcor_nopg(15,1.4,1,-1)** to complete the plot. {The numbers mean: 15 contours, separated by 1.4 intensity units, the 1D-^{1}H spectrum is in EXP1, and the "-1" means to suppress the plotting of the ^{13}C axis (however, we plotted the ^{13}C trace in the previous step)}.
4). Notice the "PS" in the upper-left corner of the screen. This means that all of the plot elements have been created, and the program is awaiting the "page" command to send to the printer. If you are observant, you should have noticed this before.
5). Type **page('*filename*.ps')**, substituting your own filename for *filename*. This will write the plot to the file "*filename*.ps" instead of to the printer. If you wanted to, you could type page, and the plot would be sent to the printer.

c). You can transfer this file to one of the PCs in the lab (using secure-shell sftp), and save it for editing in a program like Adobe Illustrator. This is a great way to make figures of your 2D NMR spectra.