

PROCESSING & PLOTTING gHMBC

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).

January 18, 2005

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.

- 1). Select your printer/plotter and paper size first.
- 2). Type **jexp1** to join Exp:1, and load the 1D-¹H spectrum from step 6 on the previous page.
- 3). If you have a 1D-¹³C 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 **sinebell** or **sinesq** to set the window-function parameters.
 - a) The two most common *window functions* used for this experiments is sinebell; however, sine² (sinesq) will sometimes improve the Signal:Noise ratio for gHMBC.
- 7). type **wft2d** 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 wft2d 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 ^1H or ^{13}C dimension)
 - b). Set the cursor (cross-hairs) on the peak.
 - 1). To set the shift in $f1(^{13}\text{C})$, type: **rl1(##d)**. The “d” is important because the ^{13}C dimension is the “decoupler” dimension.
 - 2). To set the shift in $f2(^1\text{H})$, 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 ^1H 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). Plotting: there are three options...select your plotter before proceeding. Note: if you are using large (11x17) paper, you need to substitute “plhxcorb_{ig}” 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 ^1H 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 ^1H . 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- ^1H 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.
 - 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**].

14). Generating 1D-projections from 2D gHMBC 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.

15). Plotting to a PostScript file, instead of printing on paper:

- 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 ^1H . 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- ^1H 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.