

BARE BONES GUIDE TO ROESY

on the Inova-400&500

This guide is written assuming proficiency in basic operation of the Varian NMR instrument. You should be experienced in performing basic 1-dimensional NMR experiments before attempting to perform 2D experiments on your own. 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.

Important Considerations before starting:

- You can perform this experiment in either the 4-nucleus (n4n) probe, or the triple-inverse (ntr) probe; however, the sensitivity is MUCH better with the ntr probe. It is not a big deal (~5 minutes) to change probes, so unless you have a concentrated sample, it is suggested that the ntr probe be used. ROESY cross-peaks can be very weak, so good S:N is important.
- ROESY, when done properly, is a time-consuming experiment (due to relaxation considerations). Plan for at least 2-hours, even for relatively concentrated samples..

- 1) Make sure you are working in Exp:1 or Exp:2 (**jexp1**, or **jexp2**).
- 2) Acquire a normal ¹H NMR spectrum, setting **nt=4**.
- 3) Zoom-In on the region containing peaks of interest (Note: if you zoom-in on a region with peaks outside that region, you will have *folded* peaks in your spectrum...this can be OK, or it can be a problem; depending upon where the folded peaks land). *Note: it is a good idea to leave a little baseline on between the end peaks and the edge of the narrowed spectral window.*
- 4) Type **movesw**, and this will set the spectral width and offset to match your selected window.
- 5) Type **pw(90)**, to set the pulse angle to 90°.
- 6) With **nt=4**, type **ga** to acquire a new spectrum, then phase it.
- 7) Save this spectrum (if you wish) in your directory, as a 1-D trace for plotting beside your 2D contour plot.
- 8) Type **mp(1,100) <space> jexp100** (assuming you are working in Exp: 1, substitute "2" if you are starting in Exp:2). You should now be in Exp:100
- 9) Type **ROESY** (all caps) to load the 2D-ROESY parameters.
 - a) The ROESY parameters should load, and you should see the pulse sequence on the screen.
- 10) Set nt (# transients):
 - a) Type **nt=8** (ROESY requires at least 8 scans for a complete phase-cycle).
 - b) Use **nt=16** for more dilute samples (will take 2x as long).
 - c) The default relaxation delay ("d1") should work fine because this pulse sequence uses pulsed field gradients to suppress artifacts from short recycle delays. For a more optimized spectrum, and if you aren't time limited, you can increase the recycle delay. Type **d1=3** to set the relaxation delay to 3 sec., *d1* should (theoretically) be > 3xT1. Be aware that increasing *d1* will significantly increase the total experiment time.

- 11) Setting the *mixing time*: the mixing time is the time allowed for cross-relaxation to occur. ROESY is a transient NOE experiment, in which the *rate* of cross-relaxation is distance dependent. Therefore, the rate of *buildup* of cross-peak intensities provides distance information. If “mix” is too long, other relaxation mechanisms can interfere with inter-proton distance information. If “mix” is too small, weak NOEs might not be observed.
 - a) Type **mix?**, and note the value displayed (probably 0.2)...this is 200msec, which is a good starting value. Increasing *mix* will emphasize weaker NOEs; however, it is not usually recommended to set *mix* > 0.5 sec.
 - b) Type **mix=#**, where # is in sec (so “mix=0.2” would set mix to 200msec, usually a good first guess.).
- 12) Set the number of increments in the *t1* dimension:
 - a) Type **ni?**, and note the current value for ni.
 - b) If the value of ni is < 128, type **ni=128**. This improves resolution.
 - i) For optimum resolution in the *t1* dimension, ni=256 is recommended, but the total experiment time will increase by 2x.
- 13) Type **time** to see how long the experiment will take, adjust parameters as necessary.
- 14) Type **go** to start the experiment.
 - a) After a block is completed, type **gaussian** or **sqcosine**, followed by **wft(1)**, and phase this first slice, using *aph* (may or may not work), or use manual phasing if necessary. Good phasing of the first slice is very important in good phasing in 2-dimensions.
 - i) After ~40 or more blocks in *t1*, you can type **setLP1** then **gaussian** then **wft2da** to see if your spectrum is coming along nicely.
 - b) If you must stop the acquisition before it is done (i.e., you run out of time), always stop the 2D experiment by typing: **sa('nt')**. This will stop the experiment at the end of the current FID.
 - c) When the acquisition is done, you should process the spectrum to be sure the results are acceptable.
 - i) The two most common *window functions* used for ROESYs is either a COS² or Gaussian window. Generally gaussian improves S:N, and sqcosine improves resolution (less S:N). Choose the one that best fits your data, and gives the best result.
 - ii) Type **setLP1**, then **gaussian** or **sqcosine** to set the apodization window function then type **wftwda**. Click the **[Full]** button if necessary. Note, the *diagonal* will be negative, and (for small molecules ... mw < 1,000) cross-peaks should be positive. You might need to touch-up the phasing (see *processing* below).
- 15) Correcting the baseline in 2-dimensions: Often, baseline roll is a problem with ROESY. Correct as follows:
 - a) Type **gaussian** then **wft(1)**, and phase as necessary.
 - b) Use the integral-resets (click **[PartInteg]**, then **[resets]**), to define integral regions containing all peaks. The gaps between integral regions will be used for baseline correction. Type **bc** to see if the integral regions give a reasonable baseline after bc.
 - c) After typing **setLP1**, then **gaussian** (or **sqcosine**), & **wft2da**, type **bc2d('f2')**. (bc2d('f2')) only works if proper integral regions have been defined on a 1D-trace).
- 16) Type **svf**, followed by your filename, to save the data for processing.
- 17) Quitting:
 - a) Type **jexp1**, eject your sample, and re-insert the reference.
 - b) Be sure to turn the spinner back on in **[Acqi]**.
 - c) Lock and shim on the reference, and sign-out in the logbook.

Processing and Plotting the 2D-ROESY spectrum:

- 1). Select your printer/plotter and paper size first.
- 2). Type **jexp1** to join Exp:1, and load the 1D-¹H spectrum from step 8 on the previous page.
- 3). Type **jexp2** (or any other valid Exp:#), and load the 2D-ROESY spectrum
- 4). Type **setLP1** to activate forward linear prediction (improves resolution and quality).
- 5). type **gaussian** or **sqcosine** to set the window-function parameters.
- 6). type **wft2da** to process the data.
- 7). If integral regions for baseline correction were defined before saving (step 15 in the previous section), then:
 - a). type **bc2d('f2')** to correct the baseline.
 - b). you can follow the instructions in step 15 above (in their entirety) if you didn't save the integral regions prior to saving the 2D spectrum.
- 8). If the contour plot is not centered, type **center** followed by **dconi**.
- 9). NOTE: the command **dconi** is analogous to "ds" for 1D spectra. If you lose your 2D image on the screen, and you want to view it, type **dconi** to re-display the 2D spectrum. You don't need to re-type wft2da 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 on the diagonal.
 - b). Set the cursor (cross-hairs) on the peak, and type **rl(###p)**, followed by **rl(###p)**, where ### is the chemical shift of the selected peak on the diagonal.
- 12). Phasing the 2D-NMR spectrum: if your 2D peaks are not phased, they will appear partially negative and partially positive. The phasing in the F2 dimension is set by phasing the first trace (step 14a and 15a above). You might still need to phase the 2D spectrum in the F1 dimension, but you must have the F1 axis on the bottom.
 - a). If the F2 dimension is currently on the bottom, you **MUST** change to "F1-Mode". *Click [Return], then click [More], then click [F1mode].* This should put the F1 axis on the bottom of the 2D plot.
 - 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**].

13). Plotting: there are three options...You should have already selected your plotter, but double-check before proceeding.

- a). Plotting with your high-resolution 1D spectrum (in Exp: 1) displayed along the sides.
 - 1). If using BIG (11"x17") paper, substitute "**plcosybig**" for "plcosy" in the following instructions.
 - 2). Type **plcosy(15,1.5,1)** . This means: draw 15 contours, separated by 1.5 level units, and the 1D spectrum that is in Exp: 1 on the sides.
 - a) Note: if your 1D spectrum is very small due to intense singlets, you can multiply the 1D trace by a factor of "n" by typing: **plcosy(15,1.5,1,n)** (i.e. plcosy(15,1.5,1,5) will multiply the 1D trace intensity by 5x).
- b). If you want to print to a PostScript file instead of to the printer, do the following:
 - 1). Select the 8x11 LaserJet 4MV as your printer.
 - 2). Substitute "**plcosy_nopg**" for "plcosy" above
 - 3). After executing **plcosy_nopg(15,1.5,1,n)**, type **page('filename.ps')** and your plot will be saved in your directory as a PostScript file under the name "filename.ps". Of course, use your own *filename* instead of "filename".
- c). Plotting with projections (low-resolution) on the sides.
 - 1). from *dcon1* , **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]**.

-R.Shoemaker