

**Quick Guide for VnmrJ 2D NMR**  
IU NMR Facility - September, 2010

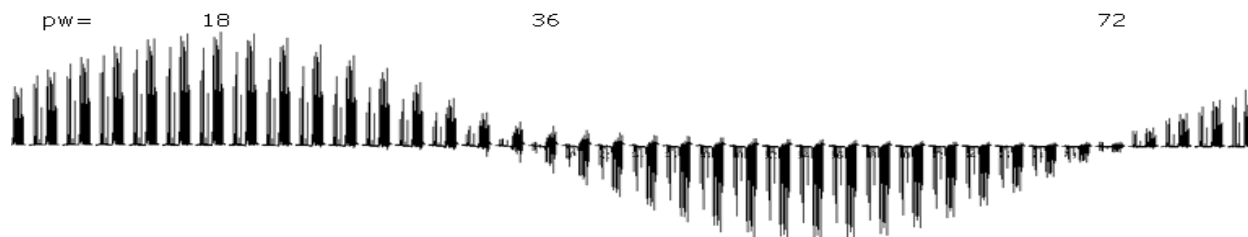
- 1) Start with sample locked, shimmed and spinning off.
- 2) To setup a 2D experiment type one of the following: **iuocosy, iudqcosy, iunoesy, iuroesy, iuhmqc, iuhsqc, iuhmbc** or **iucigar**. The actual pulse sequence used will vary between instruments as the I400 has gradients and the I500 has both gradients and shaped pulses available. See individual guides for more information. Other 2D experiments can be setup from the Experiments menu but you will often need NMR staff help to get them to work well. *Recommendations*: COSY, NOESY, HMQC and even HMBC work well on the VXR400 but most other 2D experiments should be run on the I400 and I500 to take advantage of gradients.
- 3) To adjust experiment time and sensitivity, change **d1** (relaxation delay), **nt** (number of scans) or **ni** (number of 2D scans). Typically: a) Lower **d1** for fast gCOSY on I400 or I500 (0.1s) or fast gHMQC (0.2s) b) Increase **nt** for more scans per increment to increase signal to noise. c) Increase **ni** for more 2D scans to increase resolution and S/N in second dimension, typically for COSY, DQCOSY, NOESY.
- 4) Make sure the **gain** is adjusted correctly as it cannot be automatically set in 2D experiments. If you get the receiver overflow error and the rcvr ovfl light on the remote monitor blinks, gain is too high and needs to be lowered ("**gain=42**", maximum is 60). With low concentration samples you can usually increase gain.
- 5) From the **Acquisition** tab you can confirm the experiment time with "**Show Time**", start acquisition with "**Acquire**" and stop acquisition with "**Stop**" buttons. You can stop 2D experiments anytime and the data will be saved up until the last 2D increment acquired. The commands: "**time**", "**ga**" and "**sa**" also perform these functions.
- 6) Use the **Process -> Basic** template for basic processing functions. The typical FT data size is 2k x 2k. You can turn Linear Prediction (linear algebraic extension of 2D data) on or off here and try different weighting functions. Linear Prediction is very useful but is turned off by default with the IU experiments so that intermediate results can be checked. To easily turn off linear prediction you can also type "**proc1='ft'**". After setting these values use the "**Process**" button to perform the 2D Fast Fourier Transform. The "**Display**" button will reload the display if needed. The command string "**wft2da f full dconi**" can also be used to process and display a 2D spectrum after acquisition.
- 7) Use the toolbar on the right side of the display to specify expansions and increase or decrease the vertical scale in 20% increments. Vertical scale is controlled with the parameter **vs2d** so "**vs2d=200**" works too.
- 8) Phasing 2D data can take a few iterations. For fully phase sensitive data you must have "**pmode='full'**". In general, typing **wft(1)** and phasing this first 2D increment is a good way to start. Do this and type **phase(180)** for NOESY spectra. In f2 mode ("**trace=f2**") the phase parameters are **rp** and **lp**, the same as for 1D spectra. In f1 mode ("**trace=f1**") they are **rp1** and **lp1**. If in doubt, set them all to zero. Start in f2 mode, select horizontal traces by clicking on them and typing "**ds**". Phase as you would with 1D spectra. Check with "**dconi**". Repeat in f1 mode, then repeat both again as needed.
- 9) VnmrJ 2D referencing is on the **Plot -> Default** template. To use commands, with homonuclear experiments (COSY, NOESY, etc.) put the cursor at a reference position and type "**rl(7.26p)**" for the f2 dimension and "**rl1(7.26p)**" for the f1 dimension. For heteronuclear experiments (HMQC, HMBC, etc.) f2 is the same but for f1 use the format "**rl1(77d)**".
- 10) There is no good way to display and plot 2D peak picking information with VnmrJ. The Peak Picking toolbars (click circle icon upper right, bottom submenu is Peak Picking) can be used for onscreen assignments that can be saved and plotted with the data, but this process is difficult for casual use.

11) The same **Process -> Basic** menu we have been using also contains simple plot options. The **Process -> Plot** template is overly complicated but has more options. Another alternative is to use our custom "**plot2d**" command and follow the prompts. **plot2d** also allows you to plot the actual 1D spectra stored in other experiments along the edges of the 2D spectrum.

All our 2D experiments use **pw** for  $^1\text{H}$  pulses and **pw<sub>x</sub>** for  $^{13}\text{C}$  pulses. The defaults should give good results but for best performance you can calibrate them directly on your sample.

To determine  $^1\text{H}$  90 degree pulse width (**pw**):

- 1) Setup a Proton experiment, then set "**nt=1 gain=20**".
- 2) Type "**array('pw',40,2,2) ga**" to array pw from 2us to 82us in increments of 2us.
- 3) Type "**ds(1) f full**" and phase the first increment, can use aph.
- 4) Type "**dssh dssl('value')**" to display the series of spectra like below.
- 5) The 90 degree pulse width will give maximum signal but this is usually not as obvious as in this example, so the nulls at 360 (divided by 4) or 180 degrees (divided by 2) are usually used. In this example the exact null at **pw(360)=72** can be used to set **pw(90)=18**. **pw** is typically 12 to 20us with our default probes.
- 6) You can also vary pw one step at a time once you have the phase set to find the best null signal. 0.5us accuracy is good enough.



To determine  $^{13}\text{C}$  90 degree pulse width (**pw<sub>x</sub>**):

- 1) Setup an HMQC or HSQC experiment with **iuhmqc** or **iuhsqc**. HMQC gives more signal but HSQC is more sensitive to inaccurate **pw<sub>x</sub>** values.
- 2) Type "**ni=1 phase=1**" then "**array('pw<sub>x</sub>',7,11,1) ga**" to array **pw<sub>x</sub>** from 11us to 17us in 1us steps. **pw<sub>x</sub>** is typically 13-15us on our spectrometers. You may also need to increase **nt** to give more signal.
- 3) Phase a spectrum with a lot of signal using for example "**ds(3) f full**", then type "**dssh dssl('value')**" to display the whole series as below. From this data the optimal **pw<sub>x</sub>** can be visually interpolated as 13.5 us.
- 4) Sometimes it helps to use absolute value mode, which removes the need for phasing, for example "**av dssh dssl('value')**". The command "**ph**" will restore phase sensitive mode.

