Setting up 2D NMR experiments on UVM's Varian 500 spectrometer

written by Monika Ivancic (4-10-2020)

This is a brief guide to help you set up some 2D experiments on the Varian. These fairly simple instructions assume that you have a **small organic molecule in CDCl$_3$ solvent**. If your solvent is different, you should ask for Monika's help to tune the probe. Or see probe tuning instructions in this guide. We will not optimize the 90° pulse for these experiments, typically the calibrated value works well for a small molecule in CDCl$_3$.

You will first need to acquire a ^{1}H 1D of your sample, the standard parameters in UVMproton are sufficient. There are several good reasons for acquiring this spectrum. The ^{1}H 1D is best for judging shim quality, using a singlet or the TMS peak. If your ^{1}H 1D spectrum works out, you can be assured that the spectrometer is working. You also *MUST* optimize the sweep width (SW) of your spectrum, so you're not collecting a lot of noise during your 2D acquisition. Optimizing the SW can be done interactively, and the new SW will be carried through to your 2D experiments.

Preparation instructions:

1. Acquire a ^{1}H 1D spectrum (should be stored as PROTON_01) under the folder you created for your sample.
2. On the processed spectrum, optimize the SW with the red thread lines, by selecting a point about 1ppm to the left of your left most peak and using a L-click. Then moving to about 1ppm to the right of your right most peak and using a R-click. This must be followed by typing 'movesw' in the command line or clicking on the "moveSW" icon.
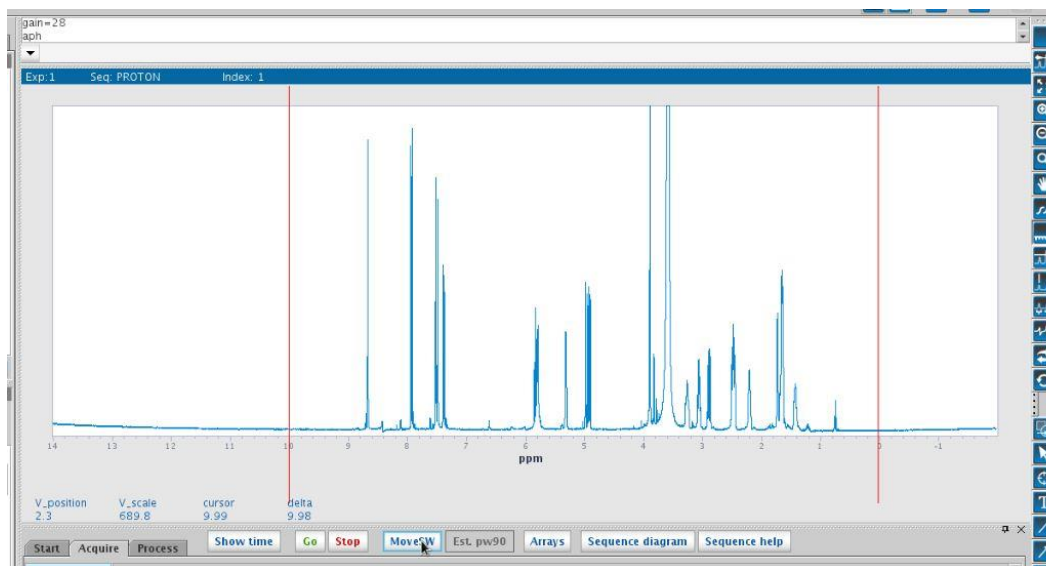
3. Then re-acquire the spectrum, but also change the pulse width, so that a 90 pulse is used. This dataset will now be stored as PROTON_02 in the same folder.
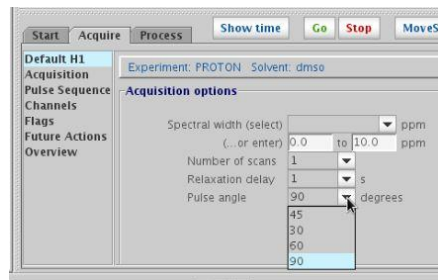
Setting up a ¹H,¹H - COSY experiment:

4. Your ¹H 1D spectrum is likely located in 'exp 1' (or a higher number if you've used the multiple expts windows). Let's leave this spectrum in 'exp 1' and set up the 2D COSY in 'exp 2'. You will need to type 'mf(1,2) jexp2 wft', but if you've never used 'exp 2' before, you will likely get an error, so you will need to create the experiment with 'cexp 2' first. Then retype the previous command (you can just hit the up arrow to get to what you entered before).

5. Now go to the pulldown menu under 'Experiments' and choose 'Homonuclear correlations', then hover over 'Gradient COSY' and select it. The experiment parameters get entered here, however the macro copies the 'sw' and 'tof' numbers from your ¹H 1D that had just been sitting here in 'exp 2'. The same numbers will be used for the 2nd dimension (sw1 and tof1), since this is a ¹H to ¹H correlation experiment.
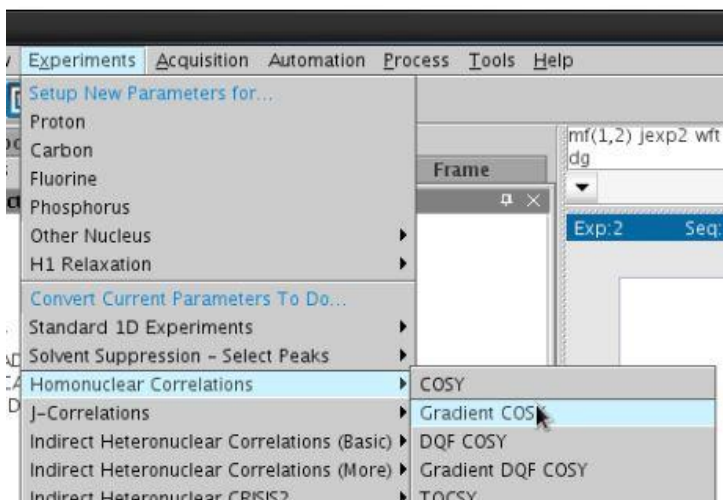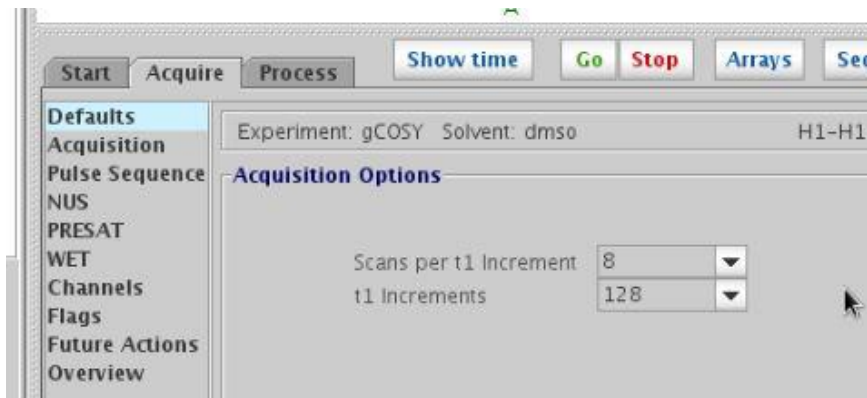
6. **saving expt**: Now go to the 'Future Actions' tab, and make sure that process/save is selected as the option. This way your experiment will be automatically saved when you use the 'Go' button to start the experiment.
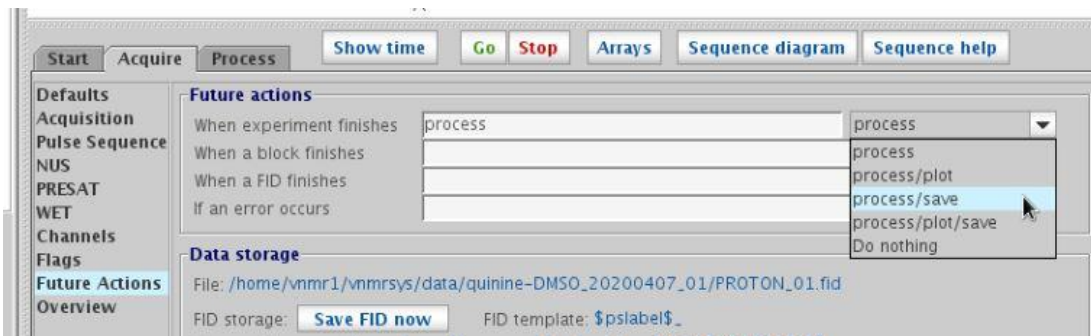
7. **nt and d1**: In the 'Defaults' tab check all pertinent parameters. You may want to make the d1 relaxation delay longer, say 2 sec ('d1=2' in command line). You will likely need more scans, so change the number of scans to something higher, like 4 or 8 (this will affect your total experiment time). There is a 'show time' button you can click to get the time estimate or type 'time' in the command line.
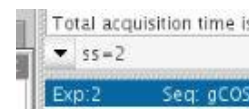
8. **ni**: A 2D experiment is composed of a bunch of 1D experiments and the parameter that commands this is 'ni' or number of t1 increments. In the basic COSY, ni is set to 128, and should be sufficient for you to observe through bond correlations in your molecule. If you think you need more resolution in the 2nd dimension (or need to resolve small couplings) you may change ni to 256 (type 'ni=256' or use the drop down arrow under 't1 increments'). This should double the length of time your experiment will take. The 'ni' parameter doesn't need to be a 2^n number, you can use ni=200 or any number.

9. **gain**: let's make sure the receiver gain (gain) is properly set and that you won't overflow the receiver with signal. Start with changing the number of steady state scans to a small number, like 2 ('ss=2'). Set 'gain' to a high number, such as 58 (gain=58). Then type 'go' (in the command line) and let the experiment start acquiring. Watch for a 'receiver overflow' or 'ADC overflow' error at the bottom of the screen. If it

comes up, you need to stop the experiment using the 'aa' command, then lower gain (say use 'gain=52') and re-acquire with 'go'. If the error creeps up again, you will need to lower gain and repeat. Once the error doesn't come up anymore, you've reached a good value for gain, but still 'aa' the experiment. The higher number you can use for gain, the better the sensitivity of the experiment will be. (highest gain = 60, lowest gain = 1)

10. **Checking signal to noise ratio**: You may use the scans acquired in part 9, just type 'wft (1)' or 'wft (2)' and make sure you see peaks (you are processing the 1st or 2nd 1D fid in the 2D experiment). A COSY spectrum is quite sensitive, so you should see fairly large peaks. If your peaks are too small, increase the 'nt' parameter (number of scans). You may double it, though do use an even number.
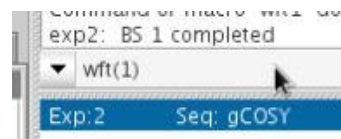
11. **Start your experiment**: When you're satisfied with all the above parameters, your experiment is ready to be started. Be sure to set ss back to the original value (type 'ss=32'). If a COSY is all you desire, you may now click on the green "Go" button and let the experiment acquire. If you need to set up more 2D experiments, follow the instructions below before starting acquisition.

Setting up a ^1H,^{13}C HSQC (1-bond) experiment:

12. Create a new experiment to acquire this data in. Use 'cexp 3' to create experiment 3, then move your ^1H 1D into this experiment (type 'mf(1,3) jexp3 wft'). Now go to the pulldown menu under 'Experiments' and choose 'Heteronuclear correlations', then hover over HSQC and select it. The experiment parameters get entered here, however the macro copies the 'sw' and 'tof' numbers from your ^1H 1D that had just been sitting here in 'exp 3'.
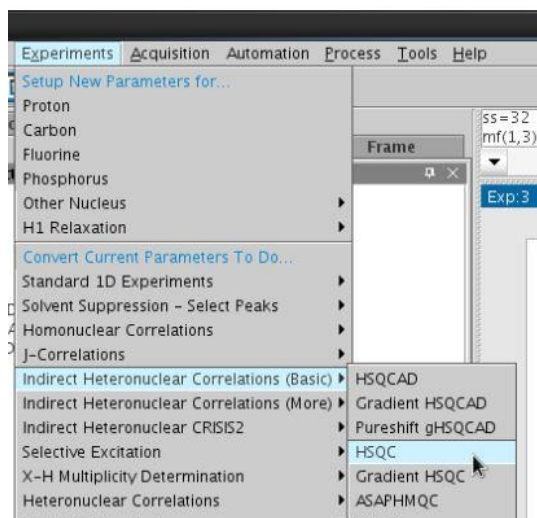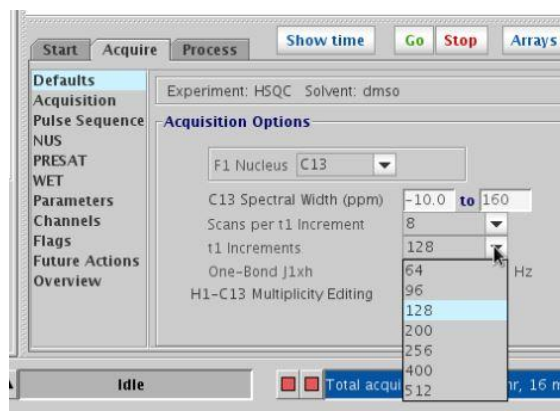
13. **Choosing ^{13}C SW**: Under 'Defaults' you should see sweep width for ^{13}C only. By default the ^{13}C SW is set to go from -10ppm to 160ppm. Remember that this is a 1-bond experiment and typically only 4°Cs lie above 160ppm, so expanding the SW might not be necessary. However, if you would like to make SW larger, enter new numbers in the high and or low ppm entries.
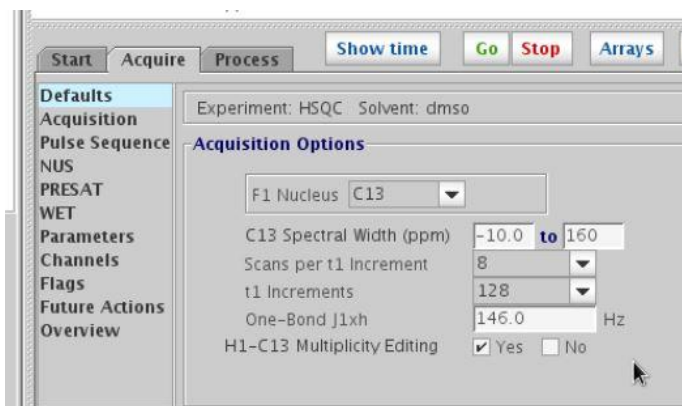
14. **saving expt**: As in the gCOSY, go to the 'Future Actions' tab, and make sure that process/save is selected as the option. This way your experiment will be automatically saved when you use the green 'Go' button to start the experiment.

15. **nt and d1**: In the 'Defaults' tab check all pertinent parameters. You may want to make the d1 relaxation delay longer, say 2 sec ('d1=2' in command line). You will likely need more scans, so change the number of scans to something higher, like 4 or 8 or possibly even 16 if your sample is dilute (this will affect your total experiment time). There is a 'show time' button you can click to get the time estimate or type 'time' in the command line.

16. **ni**: As mentioned for the gCOSY above, a 2D experiment is composed of a bunch of 1D experiments and the parameter that commands this is 'ni' or number of increments. In the basic HSQC, ni is set to 128, and should be sufficient for you to observe through bond correlations in your molecule (^1H - ^{13}C). If you think you need more resolution in the 2nd dimension (or have ^{13}C signals that aren't dispersed) you may change ni to 256 (type 'ni=256'). This should double the length of time your experiment will take. The 'ni' parameter doesn't need to be a 2^n number, you can use ni=200 or any number.

17. **gain**: let's make sure the receiver gain (gain) is properly set and that you won't overflow the receiver with signal. Start with changing the number of steady state scans to a small number, like 2 ('ss=2'). Set 'gain' to a high number, such as 58 (gain=58). Then type go (in the command line) and let the experiment start acquiring. Watch for a 'receiver overflow' or 'ADC overflow' error at the bottom of the screen. If it comes up, you need to stop the experiment using the 'aa' command, then lower gain (say use 'gain=52') and re-acquire with 'go'. If the error creeps up again, you will need to lower gain and repeat. Once the error doesn't come up anymore, you've reached a good value for gain, but still 'aa' the experiment. The higher number you can use for gain, the better the sensitivity of the experiment. (highest gain = 60, lowest gain = 1) The HSQC is much less sensitive than the gCOSY, so your gain here should be larger than in the gCOSY experiment.
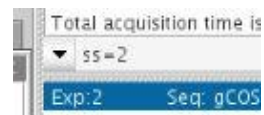
18. **Checking signal to noise ratio**: You may use the scans acquired in part 9, just type 'wft (1)' or 'wft (2)' and make sure you see peaks (you are processing the 1st or 2nd 1D fid in the 2D experiment). Or type 'go' and wait until a few t1 slices (nis) have acquired before halting acquisition with 'aa'. A HSQC experiment is less sensitive than the gCOSY, just make sure you see some peaks. The peaks may not be phased properly, which is normal, just make sure the peaks are there (peaks in the example below are sufficient).   If your peaks are too small or non-existent, increase the 'nt' parameter (number of scans). You may double it, and make sure to use an even number.
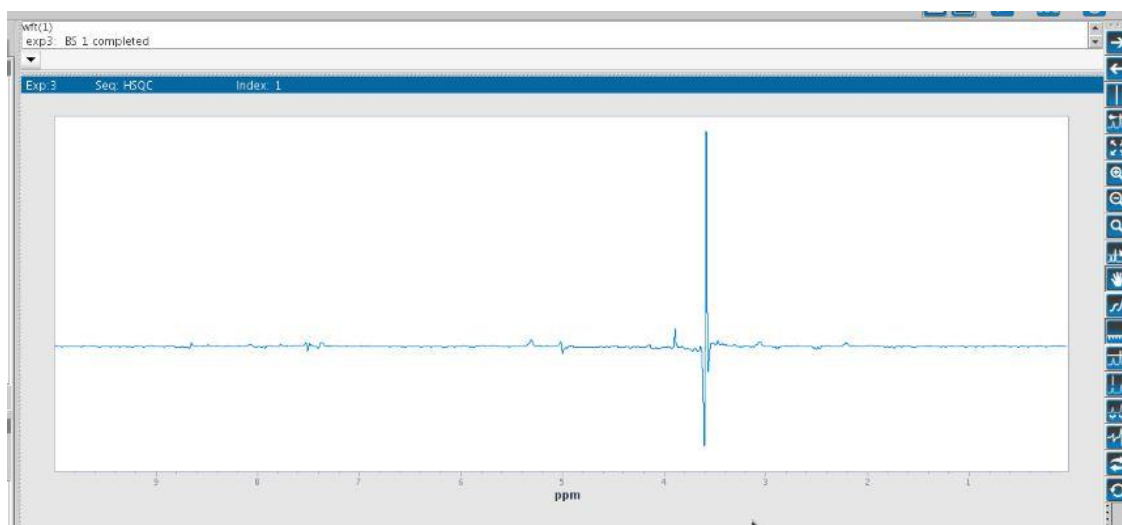

19. **Start your experiment**: When you're satisfied with all the above parameters, your experiment is ready to be started. Be sure to set ss back to the original value (type 'ss=32'). If a HSQC is all you desire, you may now click on the green "Go"

button and let the experiment acquire. If you need to set up more 2D experiments, follow the instructions below (or above) before starting acquisition.

Setting up a $^1H,^{13}C$ HMBC (2,3+-bond) experiment:

20. Create a new experiment to acquire this data in. Use 'cexp 4' to create experiment 4, then move your ^1H 1D into this experiment (type 'mf(1,4) jexp4 wft'). Now go to the pulldown menu under 'Experiments' and choose 'Heteronuclear correlations', then hover over gHMBCAD and select it. The experiment parameters get entered here, however the macro copies the 'sw' and 'tof' numbers from your ^1H 1D that had just been sitting here in 'exp 4'.
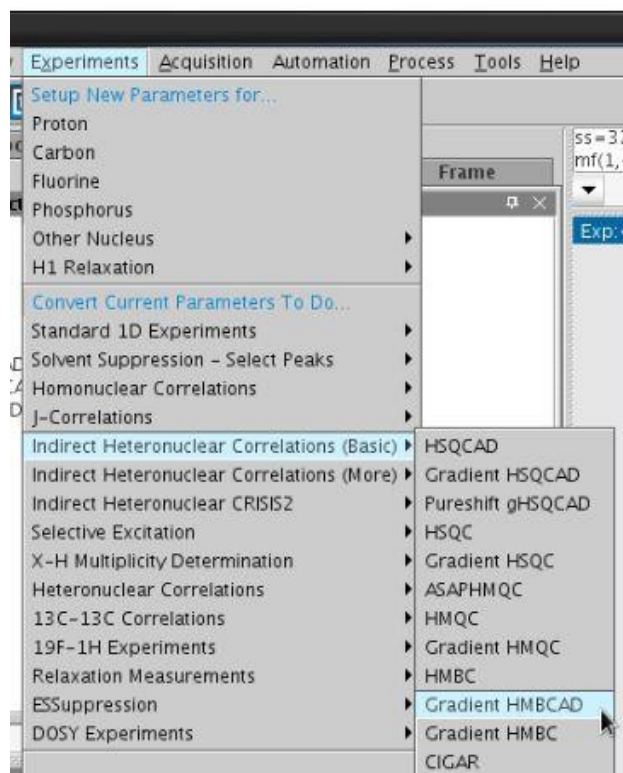

21. **Choosing ^{13}C SW**: Under 'Defaults' you should see sweep width for the ^{13}C dimension only. By default the ^{13}C SW is set to go from -15ppm to 225ppm. This is a multi-bond experiment and ALL Cs should appear in this spectrum.   You may change the default settings to suit those of your molecule. Enter new numbers in the high and or low ppm entry boxes. (If TMS is in your sample, make sure the low ppm number is below 0ppm).
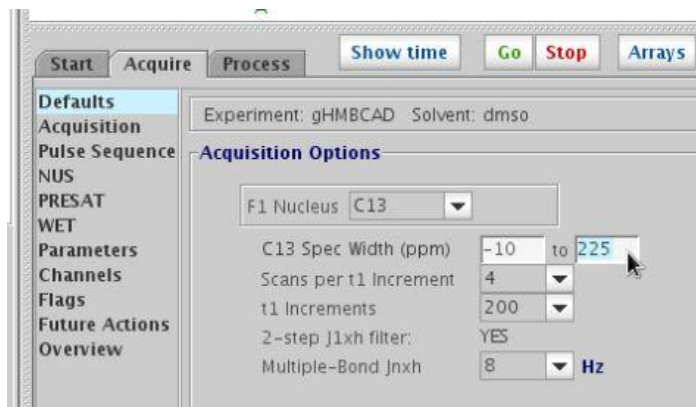
22. **saving expt**: Go to the 'Future Actions' tab, and make sure that process/save is selected as the option. This way your experiment will be automatically saved when you use the green 'Go' button to start the experiment later.

23. **nt and d1**: In the 'Defaults' tab check all pertinent parameters. You may want to make the d1 relaxation delay longer, say 2 sec ('d1=2' in command line). You will likely need more scans, so change the number of scans to something higher, like 8 or 16 or possibly even 32 if your sample is dilute (this will affect your total experiment time). There is a 'show time' button you can click to get the time estimate or type 'time' in the command line. The HMBC is the least sensitive experiment here, so you may need more scans than in the HSQC.

24. **long range coupling constant**:
The HMBC uses a 'mixing time' during which the long range correlations develop. This time is optimized based on the long range coupling values in your molecule (multiple-bond J_{nxh}). A 'catch all' long range coupling constant that should give most all $2,3^+$-bond peaks is 8 Hz. If an expected correlation does not appear in your spectrum, you may re-run your HMBC with either a smaller value (3 Hz) or larger value (12 Hz). Sometimes peaks don't appear because of the geometry between bonds in the molecule.
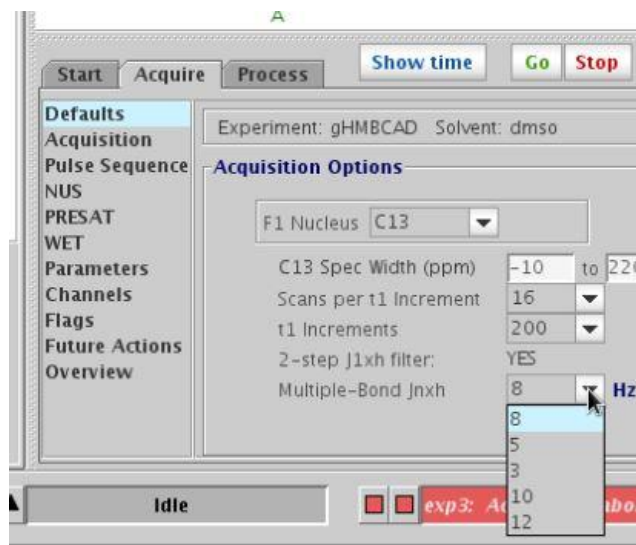
25. **ni**: As mentioned for the gCOSY above, a 2D experiment is composed of a bunch of 1D experiments and the parameter that commands this is 'ni' or number of increments. For the gHMBCAD, ni is set to 200, and should be sufficient for you to observe through bond correlations in your molecule (^1H - ^{13}C). If you think you need more resolution in the 2nd dimension (or have ^{13}C signals that aren't dispersed) you may change ni to 256 (type 'ni=256') or larger. This should increase the length of time your experiment will take.

26. **gain**: let's make sure the receiver gain (gain) is properly set and that you won't overflow the receiver with signal. Start with changing the number of steady state scans to a small number, like 2 ('ss=2'). Set 'gain' to a high number, such as 58 (gain=58). Then **type** go (in the command line) and let the experiment start acquiring. Watch for a 'receiver overflow' or 'ADC overflow' error at the bottom of the screen. If it comes up, you need to stop the experiment using the 'aa' command, then lower gain (say use 'gain=52') and re-acquire with 'go'. If the error creeps up again, you will need to lower gain and repeat. Once the error doesn't come up anymore, you've reached a good value for gain, but still 'aa' the experiment. The higher number you can use for gain, the better the sensitivity of the experiment (highest gain = 60, lowest gain = 1). The gHMBCAD is less sensitive than the HSQC, so your gain may be larger than in the HSQC experiment.

27. **Checking signal to noise ratio**: You may use the scans acquired in part 9, just type 'wft (1)' or 'wft (2)' and make sure you see peaks (you are processing the 1st or 2nd 1D fid in the 2D experiment). Or type 'go' and wait until a few slices (nis) have acquired before halting acquisition with 'aa'. Check the status at the bottom, it will tell you which FID you are on and how many scans have been acquired. (In this example, it has acquired 14 scans of the 2nd FID, and still has 3h, 58min and 17sec to go.)   A gHMBCAD experiment is less sensitive than the gCOSY, just make sure you see some peaks. The first ni ('wft 1') will have positively phased peaks, while the higher ni's will have both positive and negative peaks. If your peaks are too small or non-existent, increase the 'nt' parameter (number of scans) and repeat this s/n test. Shown is the 1st slice of a gHMBCAD with nt=16, and the peaks look sufficient to get a good 2D spectrum.
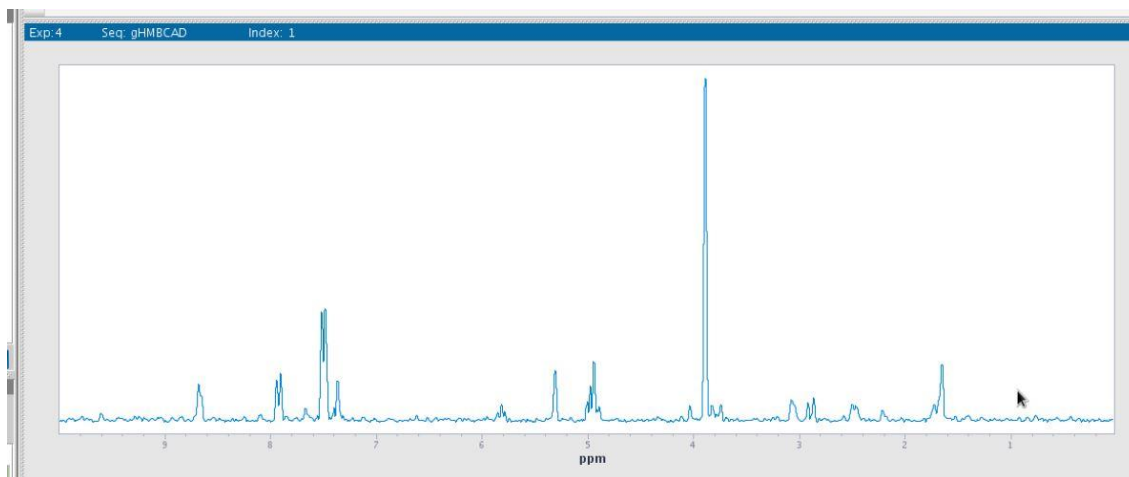
28. **Start your experiment**: When you're satisfied with all the above parameters, your experiment is ready to be started. Be sure to set ss back to the original value (type 'ss=32'). If a gHMBCAD is all you desire, you may now click on the green "Go" button and let the experiment acquire. If you need to set up more 2D experiments, follow the instructions above before starting acquisition.

29. **Starting ALL 3 experiments**: If you are acquiring a gCOSY, HSQC and gHMBCAD all on the same sample, you should start them after setting up all 3 experiments. You should know how long each experiment will take and when all 3 experiments will be done acquiring. To start acquisition, go to experiment 2 ('jexp 2') where you have the gCOSY set up and click on the green 'GO' icon to start the experiment. You should see the experiment starting by looking at the indicator at the bottom of the screen. Also, the "Study Queue" will show the already acquired experiments (PROTON_01) in green and the queued up one (gCOSY) in turquoise.
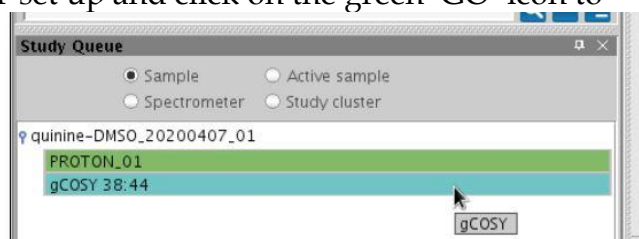
Once you're convinced this has started, go to experiment 3 ('jexp3') where you have the HSQC set up. Click on the green 'GO' icon, which will queue up the experiment to run after the gCOSY is done. The "Study Queue" will show the currently acquiring experiment (gCOSY) in red, and the queued up one (HSQC) in turquoise.
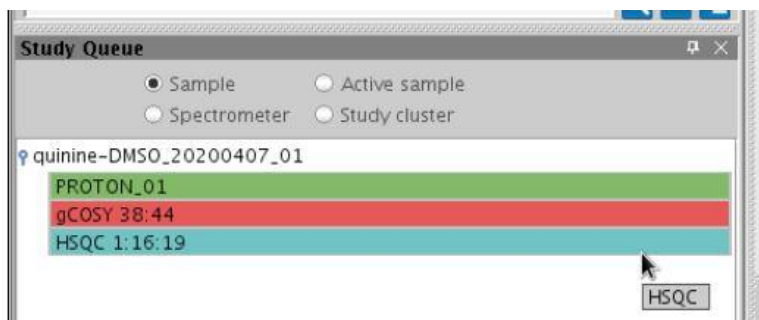
Last, go to experiment 4 ('jexp 4') where you have the gHMBCAD set up. Click the green 'GO' button to queue up this experiment to acquire after the HSQC. The "Study Queue" on the left will show the newly queued up experiment (gHMBCAD) in turquoise.
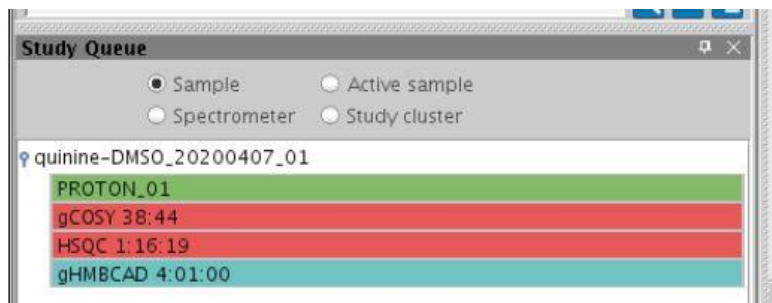
Don't be too concerned about a 'waveform timing error' that pops up when you start the gHMBCAD experiment. It should still acquire normally despite this error.

waveform timing error of 3.44 percent for shape of duration 465.4 usec

MAKE SURE TO PUT UP AN 'EXPERIMENT IN PROGRESS' SIGN OR A NOTE! So other users will know that you're occupying the spectrometer. The time that it will take to complete your experiments should also be blocked on the spectrometer calendar.