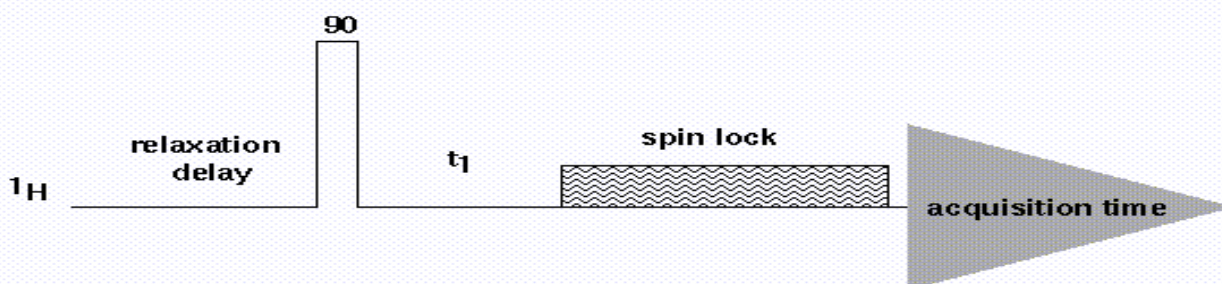


Wender Group Literature Review



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The Lit Review's Guide to NMR Usage Special Issue

By the Wender group NMR team

Nicole Deschamps

Vassil Elitzin

Orion Jankowski

Tad Jessop

John Kowalski

Steven Lynch

Travis Williams



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Section 1: Getting Started

Where is the NMR?

There are 2 NMR centers. One is on the third floor of Mudd (gem300.stanford.edu, mudd300@yahoo). The remainder of the NMR instruments is in the basement of Stauffer I. These are the venerable 200 (gem200.stanford.edu), the 10 mm 300, the mercury 400 (merc400.stanford.edu, stauffer400@yahoo), the XL 400, the 500 (ui500.stanford.edu, ui500@yahoo), and the 600 (ui600.stanford.edu).

How to get an account

To get an account on any NMR instrument, you must first learn to acquire a proton spectrum on that instrument and then go to Steven Lynch and ask to be checked out. He'll take care of you. Accounts on the NMR1 workstation can be obtained by request from Steven.

Section 2: Setting Up

Shimming the Instrument:

The magnet can be shimmed manually by loading a standard proton pulse sequence, opening the acquisition window and adjusting the shims. Opening the acquisition window is a little different on each instrument, so have someone show you the first time you do it. TIP: if the acquisition window won't come up (a problem on the 300, type "su acqproc" twice in a terminal window. TIP#2: don't accidentally hit the FID button.

Tuning the Instrument:

Tuning is the process of finding the proper center frequency for the RF filter for the instrument—like tuning your radio. Fundamentally, tuning is as important as shimming in the spectrum acquisition process. Typically, one would want to tune the probe if you want to detect a nucleus other than proton or ^{13}C , or if the probe is not tuned to your sample. (The pulse width for full excitation will be longer than standard. The sensitivity will thus be less. Even if the pulse width for full excitation was calibrated, the sensitivity will be less.)

The probe might not be well tuned when you login if the instrument was not left in the standard setup, tuned to proton on one channel and carbon on the other on an ethylbenzene sample in chloroform. Even assuming that the instrument was in its standard setup, there are reasons that the probe would not be tuned for your sample. Tuning is very dependent upon temperature and solvent/ionic strength. If your solvent is something other than chloroform, the probe will not be tuned for your sample. Some solvents are close to enough to chloroform that you may not see an effect (toluene); some solvents are so far from chloroform that you may not see anything in your spectra (D_2O with high salt). Also, if you have a lot of sample ($\sim > 20$ mgs), the tuning will not be the same as for a weakly concentrated sample.

Tuning should not usually be necessary, if you are working with sample similar to the standard and acquiring proton or carbon spectra. If the spectra do not look good, then checking the tuning is a good idea.

Most NMR instruments are tuned with external hardware and the NMR directors do this. The ui500, however, is user tunable. It is the only thing that a casual user will ever do that has the potential

to destroy the probe, however, do have someone look over your shoulder the first few times you do it. Here's what to do for the proton channel.

1. The software needs to tell the hardware which channel is which, so a pulse sequence must be setup (su) with the proper channels - Transmitter/channel 1- ^1H , decoupler/ channel 2- ^{13}C , decoupler/ channel 3- ^{15}N .
2. The cables to the probe must be inserted into the tuning interface/preamp box. For safety, unplug the cable to the XMTR on the right (^1H) side of the tuning box, then unplug the cable that connects the box with the air flow meters and the ^1H channel of the probe and plug that into the tuning interface.
3. Plug the output cable of the tuning/preamp box to the tuning output below the tune meter. Turn on the tuning meter to channel 1, sensitivity scale 9.
4. Now its time to tune. Adjust the two gold (inner and outer) knobs on the probe that say proton to minimize the number on the digital tune meter. You should be able to tune down to either 000 or 001 (dB). TIP: If you are having trouble getting below a certain number, deliberately make one of the knobs worse and adjust the other one to minimize, if you cannot get to the number you were previously at, turn that knob back the other way, and minimize the second knob.
5. After tuning to 001, then turn off tune meter by turning it to channel 0, disconnect/ reconnect the cables and return them to their original position.

^{13}C should also be tuned (and ^{15}N if there are ^{15}N pulses). Either ^{13}C or ^{15}N are done similarly except now the broadband side of the tune/ preamp box is used, disconnect the XMTR on the left/broadband side of the tune/preamp box, plug the ^{13}C cable from the probe into the tune interface and the cable going to the broadband output hope into the tune output. ^{13}C should be tuned on channel 2, ^{15}N on channel 3; like ^1H , they should be tunable to 001 on the sensitivity scale of 9.

How to Gradient Shim:

The Unity 600, Unity 500, and Mercury 400 instruments are enabled with gradient autoshimming. It's easy to use once it's set up on your account. This is how to use it.

1. Load and lock on your sample. You may wish to make some crude adjustments in Z1C and Z1C.
2. Create an experiment. On the main menu hit workspace then create. Change into this experiment. If you just created experiment 2, type `jexp2`.
3. Setup a normal ^1H spectrum.
4. Type `gmapsys`.
5. Load or create a shim map. This needs to be done once every time the probe is changed. Get Steven or one of the NMR gurus to help you with it, or just follow the instructions below. If you already have a shimmap, skip to step 6.
6. Set `nt=8` if you are using chloroform.
7. Hit "`autoshim on z`". Watch the pretty pictures go by. The program will autoshim Z1C, Z2C, Z3 and Z4 on the 400. Z5 will also be shimmed on the 500.
8. When the pretty pictures are done, open the acqui window and turn the spin back on. If desired, tweak the fine Z1 and Z2.

Change experiments to whatever you were working on and go about your life. (Type `jexp1`)
We don't recommend trying to do anything besides gradient shim in your gradient shimming experiment.

These instructions cover usage of the gradient shimming function once you have loaded a shimmap. There have been some shimmap issues (particularly on the ui500) lately, so (assuming that the issues don't effect your account) here's how to load the system shimmap.

1. In your gradient shimming experiment type `gmapsys`.
2. Hit the buttons "Shim Maps", "Shimmap Files", "Cd to Userdir", and "Cd to Systemdir".
3. Click on the most recent shimmap file then hit "Load Shimmap".
4. Type `cd` to change the directory back to your home directory.
5. Verify that `pw=800` and that `nt=8`. (type "`dg`" to view parameters).
6. Autoshim as usual. You will have to type "`gmapsys`" again.

Don't forget to lock on the standard when you finish using the instrument!

Section 3: Direct Detect Experiments

How to acquire a ^1H spectrum:

You will be instructed on how to take a proton spectrum when you get your first NMR account.

How to acquire a ^{13}C spectrum:

The ^{13}C spectrum is identical to the ^1H spectrum, except you will need to load the standard 2 pulse ^{13}C experiment instead of the the standard 2 pulse ^1H experiment. Data is processed in the same way.

How to acquire a ^{19}F or ^{31}P spectrum:

Detection of ^{19}F or ^{31}P can be accomplished without tuning on the Mercury 400. Simply, load in parameter set with ^{19}F or ^{31}P as nucleus being detected and type `su` to setup parameters. The `su` command tells the console to setup detection of fluorine or phosphorus. Detection of ^{19}F or ^{31}P cannot be accomplished without tuning the probe on the Inova 500. On the Unitys, do the following.

1. First a parameter set must be loaded with correct nucleus being detected. Do this with the menus. Select main menu, setup, nucleus/ solvent, ^{19}F , whatever.
2. Type `su` to setup parameters.
3. Next, the probe must be tuned. ^{19}F does not require a tuning stick, and is the only nucleus that must be tuned on the high-band channel (the proton channel). All other nuclei are tuned on the broadband (X) channel. Follow the tuning instructions above.
4. Take an ordinary 1D spectrum.

*IMPORTANT: Be sure to retune to the standard sample when you are finished!
Failure to do so will result in a whuppin'.*

Section 4: Coupling and Decoupling | ¹H-¹H Correlation

How to Acquire 1D Decoupling Spectrum on the ui500:

Decoupling is very powerful technique when you need to assign all the protons in your spectrum. It is similar to COSY in that it will allow you to identify “spin systems”, or parts of your molecule where two or more protons are coupled to one another. Therefore, 1D decoupling and COSY can in most cases be used interchangeably. Below is a set of parameters that works for most molecules we deal with.

1. Load or acquire a 1D proton. (This will be the reference spectrum.) It is advisable that you use the same number of scans (n_t) for the reference and all other subsequent spectra.
2. Pick line to be decoupled “n1”, then type “sd”. In other words, we will beat the crap out of that proton and not allow it to relax (literally). What that will do is cause a change in the shape and splitting patterns of all protons that are normally coupled to the one we are irradiating. In some cases you can use this information to even calculate the coupling constant between the irradiated proton and the ones showing a change.
3. For the first decoupling experiment type the following, no thinking necessary. They really do work well. If you desire more info, refer to the manuals in the NMR room.
 - a) dn='H1'
 - b) dm='nny'
 - c) dmm='c'
 - d) dmf=10k
 - e) dpwr=12
 - f) homo='y'
 - g) go
 - h) Transform your spectrum. Type wft aph.
 - i) Print your spectrum. Type pl pscale page.
4. For subsequent decouplings, it is only necessary to repeat steps 2 and 3g-i. Walk your way thru the spectrum to see all couplings.
5. Don't forget to save your spectra with the svf ('my_decoupled_spectrum') command.

If the decoupling is too broad (i.e. the signals are too close together), it is possible to reduce this by changing the “dpwr” setting. If you have enough sample to take a quick proton (let's say 3-5 mgs for most small molecules), this whole experience takes about 10-15 min.

How to Acquire 2D-COSY Data:

The COSY experiments, as most of you know, reveals two and three bond proton couplings. Four bond couplings may appear, but are typically faint, and can generally not be expected or counted on. The COSY macro on our Varian systems uses phase cycling, and cannot reasonably be performed in less than one hour. However, the 400, 500, and 600 MHz instruments have pulse-field gradient generators installed (PFG), allowing you to run the gCOSY experiment (gradient COSY), which does not require phase cycling. Most samples can be run with one pulse per increment, making this experiment barely over five minutes long. Using a 15 minute time-slot, you should be able to easily fit a gCOSY experiment into your routine. gCOSY spectra can be quite useful for crude samples as well, and we recommend taking them whenever you have time.

The gCOSY experiment is very forgiving. You can almost just run the macro directly and get good results. I follow roughly the following procedure, but you are welcome to mess around with it to suit your style.

1. Load or acquire a normal 1D-proton experiment. Note which experiment number you are in (listed at the top of the VNMR window). Because the digital resolution of the COSY

experiment is low, it is useful to move the cursors to just bracket the region of the spectrum with signal in it (all signals, not just the ones you want—you'll need something on which to reference) and type `movesw`. Then reacquire the proton spectrum and save it using `svf('my_proton_spectrum')`.

2. While your proton is acquiring, you can set up the gCOSY parameters. For this discussion, we will assume you are running the proton acquisition in experiment one (`exp1`) and the gCOSY in experiment two (`exp2`). Enter `mp(1,2)` to transfer useful information such as sweep-width, gain, etc. to the new workspace. Then type `jexp2` to join experiment two, followed by `gCOSY` to load the macro. Text case matters!! The all lower case macro exists, but does not work. Sometimes you have to type it twice on the 400 if it gives you an error.
3. You should now see the new macro entitling the upper window, indicating that the experiment has been loaded successfully. You could run the macro directly, but if you adjust a few parameters, it will come out much nicer. First, type `ni=256`. This will increase your resolution in T1. Then, enter `d1=1` and `nt=1` or `nt=2`. Finally, type `time`. The total predicted experiment time will appear, it should be 5 to 10 minutes, depending on the number of transients and increments you selected.
4. You are ready to go! Bring up the acquisition window or type `aqci`. Go to the lock menu and turn off the spinner. Watch the lock level! It should not fall by more than about 25%. If it does, you should shim the non-spinning shims. (Even if it falls more than this, you can run the experiment anyway.)
5. Close the acquisition window and enter `go`. Now is a good time to set integration resets and print your 1D-proton data. When the gCOSY is complete, join the experiment in which it is running (type `jexp2` if you are not already there), and type `wft2d` to transform the data. Use the command `foldt` to symmetrize it if you wish. Be sure to save it using the `svf('my_cosy_spectrum')` command! One of the bugs in VNMR is that if you print your data before the acquisition is complete for a 2D experiment, the data-set is erased!
6. Now you need to reference the gCOSY to chloroform (or solvent/signal of choice). The COSY looks much better when displayed in contour mode: type `dconi('dpcon',15,1.5)` and wait as VNMR uses all of its algorithmic knowledge to render your data. Locate the reference signal with your cursor, and enter `r1(7.26p)`, `r11(7.26p)` to reference both axes. The middle mouse button sets the `vs2d` variable roughly so that where you click is at the edge of the last rendered contour. You can also type `vs2d=####`, if you know about where you want to be.
7. If you have a lot of t1 noise, you can reduce it by symmetrizing the spectrum using `foldt`. Redisplay the contours after this operation with `dconi('dpcon',15,1.5)`. Beware: a folded spectrum can make real signals disappear, while placing false ones where you least expect them. You should always compare with the unfolded spectrum to be sure you are not fooled!
8. Once the on-screen rendering looks good, you can print the spectrum by typing `plcosy(15,1.5,1)`. The last number in this macro is the experiment number that contains your 1D proton spectrum. If the sweep-widths are not the same for the two experiments, your printout will look stupid.
9. Navigating the data-set using VNMR is non-intuitive, and takes about as long to explain as it does to learn by trial and error. Just try out the interface to see what everything does. Once you're saved the spectrum, you can come back to the shop and one of the NMR gurus will help you play with it.

10. Enjoy your data!

How to Acquire a TOCSY:

The TOCSY experiment reveals multiple bond proton couplings. The TOCSY macro on our Varian systems does not use pulse-field gradients, and can be run in ~30 min depending on sample size. I've tested this on the UI500, but not on the other instruments. On the merc400 or ui600, your results may vary.

1. Load or acquire a normal 1D-proton experiment. Note which experiment number you are in (listed at the top of the VNMR window). Because the digital resolution of the TOCSY experiment is low, it is useful to move the cursors to just bracket the region of the spectrum with signal in it (all signals, not just the ones you want—you'll need something on which to reference) and type `movesw`. Then reacquire the proton spectrum and save it using `svf('my_proton_spectrum')`.
2. While your proton is acquiring, you can set up the TOCSY parameters. For this discussion, we will assume you are running the proton acquisition in experiment one (`exp1`) and the TOCSY in experiment two (`exp2`). Enter `mp(1,2)` to transfer useful information such as sweep-width, gain, etc. to the new workspace. Then type `jexp2` to join experiment two, followed by `TOCSY` to load the macro. Text case matters!!
3. You should now see the new macro entitling the upper window, indicating that the experiment has been loaded successfully. You could run the macro directly, but if you adjust a few parameters, it will come out much nicer. First, type `ni=256`. This will increase your resolution in T1. Then, enter `d1=1` and `nt=2` or `nt=4`.
4. You are ready to go! Bring up the acquisition window or type `aqci`. Go to the lock menu and turn off the spinner (if it's on). Watch the lock level! It should not fall by more than about 25%. If it does, you should shim the non-spinning shims.
5. Close the acquisition window and enter `go`. Now is a good time to set integration resets and print your 1D-proton data. When the TOCSY is complete, join the experiment in which it is running (type `jexp2` if you are not already there), and type `wft2da` to transform the data. Use the command `foldt` to symmetrize it if you wish. Be sure to save it using the `svf('my_tocsy_spectrum')` command!
6. Now you need to reference the TOCSY to chloroform (or solvent/signal of choice). The TOCSY looks much better when displayed in contour mode: type `dconi('dpcon',15,1.5)`. Locate the reference signal with your cursor, and enter `r1(7.26p), r11(7.26p)` to reference both axes. The middle mouse button sets the `vs2d` variable roughly so that where you click is at the edge of the last rendered contour. You can also type `vs2d=#####`, if you know about where you want to be.
7. Once the on-screen rendering looks good, you can print the spectrum by typing `pcon(15,1.5) page`.
8. Navigating the data-set using VNMR is non-intuitive, and takes about as long to explain as it does to learn by trial and error. Just try out the interface to see what everything does. Once you're saved the spectrum, you can come back to the shop and one of the NMR gurus will help you play with it.
9. Enjoy your data (but not *too* much)!

Parameters in the TOCSY:

mix = mixing time. As in NOESY and ROESY, the longer the mixing time, the further the magnetization will transfer and as in NOESY and ROESY, the intensity of the cross peak is proportional to the distance (in this case the number of bonds) between the two protons, but also as in NOESY, other factors affect intensity of the cross peak. A good mixing time to see most correlations is 80 ms; mix = 0.08 seconds. A good mixing time to see only close correlations is 20 ms; mix = 0.02 seconds

□

pw = pulse width of high power 90° pulse

tpwr = high power proton power, correlates with pw

□

p1 = low power 90° pulse should be ~35 ms at p1lv1=46 on PFG probe (50.5 microseconds on broadband probe at p1lv1=49)

□

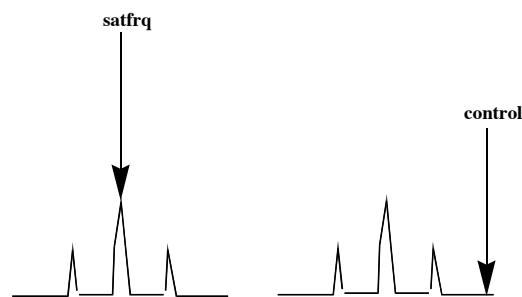
p1lv1 = low power, correlates with p1; should be power of 90* pulse of ~35 ms, p1lv1 = 46 on PFG probe

Section 5: Nuclear Overhauser Effect Experiments for ¹H

How to acquire 1D NOE spectrum on the ui500:

A Nuclear Overhauser Effect (NOE) spectrum can provide information about the relative positions of atoms in space. Typically, it's best to degas your sample before taking an NOE, because ³O₂ will allow for relaxation of the NOE buildup. NOEs can be acquired on the new Mercury 400 (see below) or the Unity 500. The mercury console does not support the cyclenoe command, so the procedures are different for the two instruments. Here's how to get NOEs on the ui500.

1. Acquire or load a 1D ¹H spectrum in exp1. (Type jexp1 to go experiment 1 if necessary.)
2. Select signal you want to irradiate and place cursor at the center of the signal. Type "sd" and note the corresponding frequency value. This will be the value that you enter as the satfrq for the NOE experiment.
3. Note the coupling pattern and the smallest coupling constant (in Hertz).
4. Now select a zero point near your signal, type "sd" and note the corresponding frequency value. This will be the value that you enter as the control for the NOE experiment.
5. Join experiment 2 (type "jexp2") and acquire (or load) a regular proton spectrum.
6. Now you are ready for cyclenoe! Type in the following commands:



cyclenoe

dg – this displays the parameters for you (dg = display goods?)

control=1234 - enter value you obtained from your proton spectrum

satfrq=1239 - enter value you obtained from your proton spectrum

nt=128 (or some multiple of 16. This is the number of pulses it acquires.)

bs=16

ss=1

d1=0.2 or longer if you want. d1 (that's "dee one") is the pulse delay.

spacing=7.6 or smallest coupling constant in Hz

pattern=3 (triplet or more)

=2 (doublet)

=1 (singlet)

go

7. Use `wft` to transform the data. Integrate the peaks and then calculate NOE... NOE=positive area/negative area!
8. If the negative peaks fall below the bottom of the screen/ page you can type `”vp=60”` or whatever number to lift up the baseline. 12 is the minimum, ~200 is a good ceiling.
9. Don't forget to save your spectrum with the `svf('my_noe_spectrum')` command.

How to acquire 1D-NOEs on the Mercury 400:

The Mercury 400 does not have the cycleNOE macro, which is available on the ui500, so the procedure for 1D-NOE acquisition is a bit different. It goes like this:

1. Acquire or load a normal 1D ¹H spectrum in the usual fashion.
2. Type `noedif` to load the pulse sequence.
3. Set the 1, 2, or 4 frequencies that you want to irradiate (`fn=<Hz>`). The `sd` command will help you identify the exact numbers for the `fn`s. Put the cursor on the target frequency and type `sd`. The program will spit out a number like 4383.5. Now type `f0=4383.5`. Repeat this process for `f1`, `f2`, `f3` as needed.
4. Specify an area of the spectrum where there is not signal with `dofff=<Hz>`. This works just like step 3; type `dofff=3456.4`, or whatever.
5. Set the decoupler mode `dm='ynn'`.
6. Set the decoupler to a reasonable (10) power. Type `dpwr=10`.
7. Set the block size to 32. 32 is the only acceptable block size. Type `bs=32`.
8. Set the number of transients to any even number of blocks. Type `nt=32`, for example.
9. Increase the line broadening. Type `lb=2`.
10. Type `go`. Workup the data as usual.

Save your spectrum by typing `svf` and following the on-screen instructions. One of the NMR gurus can help you analyze it back at the shop.

How to Acquire 2D-ROESY Data:

The ROESY experiment is relatively unforgiving. While you do not need much sample to run one, you do need to be sure your pulse widths are set properly. Do not just tune the probe and expect them to be fine, you really should check them (using arrayed `pw`). Also, the ROESY experiment will take at least overnight so be prepared. In most other ways, the ROESY is much like the `gCOSY` both in terms of the parameters you will set and the appearance of the result. There is no sign change in the ROE dependence on molecular weight, so you shouldn't have to worry about the fluke zero ROE.

1. Set up a 1D-proton parameter set and carefully tune the probe with the spinner off. Observe the `tpwr` setting and check to be sure you know what your `pw90` is.
2. Load or acquire a normal 1D-proton experiment. Turn the spinner back on if you like. Note which experiment number you are in (listed at the top of the VNMR window). Because the digital resolution of the ROESY experiment is low, it is useful to move the cursors to just bracket the region of the spectrum with signal in it (all signals, not just the ones you want—you'll need something on which to reference) and type `movesw`. Then reacquire the proton spectrum and save it using `svf('my_proton_spectrum')`.

3. While your proton is acquiring, you can set up the ROESY parameters. For this discussion, we will assume you are running the proton acquisition in experiment one (exp1) and the ROESY in experiment two (exp2). Enter `mp(1,2)` to transfer useful information such as sweep-width, gain, etc. to the new workspace. Then type `jexp2` to join experiment two, followed by `ROESY` to load the macro. The all lower case macro exists, but does not work.
4. You should now see the new macro entitling the upper window, indicating that the experiment has been loaded successfully. You could run the macro directly, but if you adjust a few parameters, it will come out much nicer. First, type `ni=256`. This will increase your resolution in T1. Then, enter `d1=1` and `nt=16` or greater. Finally, type `time`. The total predicted experiment time will appear, it should be around six hours or more, depending on the number of transients and increments you selected.
5. You are ready to go! Bring up the acquisition window or type `aqci`. Go to the lock menu and turn off the spinner. Watch the lock level! It should not fall by more than about 25%. If it does, you should shim the non-spinning shims. (Even if it falls more than this, you can run the experiment anyway.)
6. Close the acquisition window and enter `go`. Now is a good time to go home because you won't see much for a while. You might want to transform after the second increment just to be sure your spectral width is as you desire. When the ROESY is complete, and a new day has started, join the experiment in which it is running (type `jexp2` if you are not already there), and type `wft2da` to transform the data. You cannot symmetrize the data because it is phase sensitive. Be sure to save it using the `svf('my_rosey_spectrum')` command!
7. Display, reference, and print your ROESY just like you did with your COSY.
8. Enjoy your data! You will find that exchanging protons have a sign opposite to normal ROESY correlations. Furthermore, the correlate to the solvent protons. Now you can finally figure out that all those unidentified doublets are actually hydroxyl groups.

Other Parameters in the ROESY

- `np` = number of acquired points (t2, F2, detect dimension) ~ 2048 is good
- `sw1` = sweep width in t1 dimension; `sw=sw1` in 1H/1H experiment. This is set by the `movesw` command.
- `pw` = 90° pulse width = 8.25 (tpwr = 60 on gradient probe; `pw` = 16.25 tpwr = 58 on broadband probe)
- `mix` – mixing time, the longer the mixing time the more NOE transfer. The correlation time of the molecule which is inversely proportional to the size of the molecule determines the appropriate mixing time. suggested to be ~ 300 ms for 2000 MW molecules. Multiple mixing times are often acquired on the same molecule to distinguish protons very close (< 3 angstroms) from those that are < 6 angstroms. At short (~40-100 ms mixing times) very little to no transfer will occur to protons > 3 angstroms. Although NOE is proportional to the distance between the protons, other factors affect the intensity at the longer mixing times.
- `slpw` = spin lock pulse width; ROESY has a spin-lock period which uses lower power pulses, ideal 90° pulse width for spin lock is about 40 ms. This pulse should be calibrated to the appropriate power. `slpw=35` microseconds at `slpwr=46` on PFG probe (`slpw=50.5` at `slpwr=49` on broadband probe)
- `slpwr` = spin lock power, correlated with `slpw`. Again this power should be the power for an approximately 40 ms 90° pulse on ¹H. `slpwr=46` on PFG probe

Section 6: Carbon Correlation ^1H - ^{13}C

How to acquire an HSQC spectrum:

The HSQC experiment allows you to assign one-bond hetero-nuclear correlations, typically from proton to carbon or proton to nitrogen. It is an indirect detect experiment, which means that the instrument observed proton signals which provide information about the attached carbons. It is considerably more sensitive than the analogous HETCOR, which provides the same information. It is also more sensitive than a 1D- ^{13}C . Often you can collect a very nice HSQC on a sample that is too dilute to take a 1D- ^{13}C in reasonable time.

Like the COSY experiment outlined last month, the HSQC uses field gradients. You can run this experiment on the Mercury 400, Unity 500 or Unity 600 instruments. It is fairly sensitive to probe tuning on the carbon channel, so if you are not using chloroform, you will need to tune the probe. (Probe tuning is an activity that is better demonstrated than written about. See Steven or Orion for more information.)

1. Run a normal proton spectrum.
2. As with the COSY, adjust the sweep width(s) to accommodate all your signals because you will want to take advantage of all the resolution you can get. Set the cursors around the proton signals and type "movesw" to get the proton sweep width (sw).
3. Move the proton parameters to a new experiment. For the purposes of these instructions, we will assume the 1D-proton spectrum is in experiment 1 and the HSQC is in experiment 2. Move parameters with mp(1,2), then join experiment 2 with jexp2. Run the HSQC macro by typing gHSQC.
4. Turn the spinner off in the aqci window if it is not off already. The lock signal should not drop by more than 10% or so. If it does, you might want to shim the non-spinning shims.
5. The carbon sweep width should be fine for most compounds (unless you have an aldehyde). If you need to adjust it, set sw1 and dof to appropriate values (take a carbon, set the cursors around the peaks, type dof and sw to get dof and sw1).
6. The pulse delay should be 1 second (d1 = 1).
7. The default number of increments is 128. I find this to be inadequate, and I recommend setting ni = 256.
8. The number of transients per increment should be set according to your sample concentration. If you can take a carbon spectrum in 10 minutes, nt=1 is fine. If a carbon spectrum takes you an hour, try nt=4.
9. Type go, and surf the web for a few minutes on the iMac behind the 500.
10. After about 30 increments, you should see most of your signals, but they will be broad. Turn off the linear prediction (type proc1='ft'), and transform with wft2da. If you see nothing, or your sweep widths are not what you expected, abort the experiment and try again.
11. When the experiment is done (although you can stop it any time you think the data you need is there), transform again with wft2da. You can draw contours, as with the COSY, using dconi('dpcon',15,1.5) or a variation thereof.
12. Finally, print your spectrum with either a menu command, or by entering plcosy(15,1.5). Other 2D processing techniques are the same as with the COSY.
13. Don't forget to save your spectrum with the svf('my_hsqc_spectrum') command.
14. Enjoy your data!

If your spectrum looks like crap, ask one of the NMR gurus to help you optimize the processing parameters back at the lab.

Other Parameters in HSQC:

- `tof`= center of proton spectrum.
- `sw`= sweep width in proton dimension.
- `solvent`= set solvent to appropriate solvent.
- `pw`= 90° ¹H pulse at `tpwr` (high power) = 8.25 microseconds (at `tpwr` = 60)
- `tpwr` = high power ¹H = 60
- `pwxc`= 90° Carbon pulse = 12.5 microseconds (at `pwxc` = 60)
- `pwxc` = high power of Carbon pulse = 60
- `j1xh`= coupling constant of ¹³C-¹H. Slightly less than the real coupling constant should be entered, aliphatic `j1xh` = ~110-160 Hz, aromatic ~160-230 Hz. `1/2j1xh` is the delay time to transfer magnetization from ¹H to ¹³C. If this is set wrong, there will be incomplete magnetization transfer; and less signal, and possibly phasing difficulties in `t1`. However it is often impossible to set `j1xh` correctly for all carbons in the molecule so there are two compromises, by setting `1/2` way between the two extremes or run 2 separate spectra.
- `d1`= relaxation delay, time between scans (~1s-2s); it might be necessary to lengthen `d1` to 2-4s for protons with long T1 times (methyls, aromatics, vinyls)
- `np`= # of points in `t2`, correlated with `sw` and `at`; usually set to ~2K = 2048, if the data will be processed with Felix software, `np` must be a power of 2 (512, 1024, 2048, 4096...)
- `at`= acquisition time, correlated with `sw` and `np`, usually you set `np` and `sw` and let the computer set `at`, but it is important that `at` not be too long (~>10% of the `d1`) because there is decoupling during acquisition, and decoupling puts heat into the probe, the longer the `at`, the greater the heat; `d1` allows the probe to cool.
- `sw1`= sweep width in `t1`, should be wide enough to include all Carbon resonances with ~2 ppm of space on the spectral edges. If your protonated carbon spectrum (non protonated carbons do not matter) covers a large range (>100 ppm), more than 1 spectrum are suggested, one centered on upfield carbon resonances, one on downfield, for better sensitivity and resolution.
- `dof`= decoupler offset; center of the carbon spectrum in Hz. `dof`=0 is approximately = 94 ppm for Carbon (`dof` 2 is the equivalent for the 2nd decoupler = ¹⁵N, `dof` = 0 is approximately = 97 ppm). The `dof` along with the `sw1` should be optimized for your sample. Ideally you want all your resonances within the sweepwidth, and then you set `sw1` and `dof` accordingly. Note that resonances that are far away from the center (~100 ppm) will be offresonance and have weaker signals than those on resonance.
- `ni`= number of increments in `t1` (literally the number of `t1` points, sort of the equivalent of `np` in `t1`). Similar to `nt`, this should be minimized because, the greater the `ni`, the longer the experiment. 32 points is often sufficient for `ni`, for better resolution, more `t1` points might be acquired (up to ~256), but note that relaxation of Carbon is faster than proton, so signal disappears at less `t1` points- macromolecules often have no signal in less than 128 points in Carbon (and ~48 of ¹⁵N). `ni` is normally set to an even number, but odd numbers should be acceptable.
- `phase` = phase is the parameter for acquiring quadrature detection in `t1`, `phase` = 1,2 when you run the 2D.

The following parameters are relevant but should not need to be changed:

- `dm`= decoupler modulation; normally set to 'nny', to allow for decoupling during acquisition, check this by displaying the pulse sequence (`dps`).
- `dmm`= decoupler modulation mode; normally set to 'ccg' for GARP decoupling of ^{13}C during acquisition.
- `dpwr`= decoupler power during acquisition, should never be set to >49 ; $\sim 42-45$ should be sufficient to fully decouple spectrum, the greater the power, the greater the range of decoupling.

How to Acquire an HMBC Spectrum:

The HMBC experiment allows you to assign multiple-bond hetero-nuclear correlations, typically from proton to carbon or proton to nitrogen. It is an indirect detect experiment, which means that the instrument observed proton signals which provide information about the attached carbons. Readers with a good memory will find these instructions disturbingly similar to those for the HSQC. In fact, much of it is identical. (Read: if you can run an HSQC, you can also run an HMBC.) HMBC is very useful to show the location of things like methyl-ethers and other connectivity issues where there is not a contiguous proton spin system. This should tell you the answer to everyone's favorite HMBC question, do you observe multiple bond correlations through carbon-oxygen bonds. The answer is YES! Let me say this again...

You will see HMBC correlations through carbon-oxygen bonds!!!

Another nice thing about the HMBC experiment is the fact that it is more forgiving to NMR hacks. You do not need to really tune the probe or set pulse widths as accurately as you do with HSQC. On the other hand, you will usually need to run the experiment longer than for an HSQC.

Like the COSY experiment outlined in Part I, the HMBC uses field gradients. You can run this experiment on the Mercury 400, Unity 500 or Unity 600 instruments. It is somewhat sensitive to probe tuning on the carbon channel, so if you are not using chloroform, you should tune the probe. (Probe tuning is an activity that is better demonstrated than written about. See Steven or Orion for more information.)

1. Run a normal proton spectrum.
2. As with the COSY, adjust the sweep width(s) to accommodate all your signals because you will want to take advantage of all the resolution you can get. Set the cursors around the proton signals and type "movesw" to get the proton sweep width (`sw`).
3. Move the proton parameters to a new experiment. For the purposes of these instructions, we will assume the 1D-proton spectrum is in experiment 1 and the HMBC is in experiment 2. Move parameters with `mp (1 , 2)`, then join experiment 2 with `jexp2`. Run the HMBC macro by typing `gHMBC`.
4. Turn the spinner off in the `aqci` window if it is not off already. The lock signal should not drop by more than 10% or so. If it does, you might want to shim the non-spinning shims.
5. The carbon sweep width should be fine for most compounds (unless you have an aldehyde). If you need to adjust it, set `sw1` and `dof` to appropriate values (take a carbon, set the cursors around the peaks, type `t of` and `sw` to get `dof` and `sw1`).
6. The pulse delay should be 1 second (`d1=1`).
7. The default number of increments is 128. I find this to be inadequate, and I recommend setting `ni = 256`.

8. The number of transients per increment should be set according to your sample concentration. If you can take a carbon spectrum in 10 minutes, `nt=1` is fine. If a carbon spectrum takes you an hour, try `nt=4`.
9. Type `go`, and surf the web for a few minutes on the iMac behind the 500.
10. After about 30 increments, you should see most of your signals, but they will be broad. Turn off the linear prediction (type `proc1='ft'`), and transform with `wft2da`. If you see nothing, or your sweep widths are not what you expected, abort the experiment and try again.
11. When the experiment is done (although you can stop it any time you think the data you need is there), transform again with `wft2da`. You can draw contours, as with the COSY, using `dconi('dpcon',15,1.5)` or a variation thereof.
12. Finally, print your spectrum with either a menu command, or by entering `plcosy(15,1.5)`. Other 2D processing techniques are the same as with the COSY.
13. Don't forget to save your spectrum with the `svf('my_hmhc_spectrum')` command.
14. Enjoy your data!

If your spectrum looks like crap, ask one of the NMR gurus to help you optimize the processing parameters back at the lab. As always, Steven is more than happy to help and instruct.

Other Parameters in HMBC: (Most parameters are similar to HSQC)

- `jnxh`= coupling constant of multiple bond correlations; generally is empirically determined, a value of 8Hz seems to work well for 2000 mw molecules. Probably should be increased for larger molecules, possibly decreased for smaller ones. The smaller the `jnxh`, the greater the transfer, but the longer the time to transfer leading to loss of signal due to T2 relaxation. This parameter is empirically determined.
- `nt`= number of scans; HMBC is a little less sensitive than HSQC so more scans might be necessary.
- `ni`= number of increments in second dimension; since this is magnitude spectrum (phase=1) and `sw1` is probably a lot larger, `ni` should be at least twice as much as in HSQC.
- `phase`= phase should be set to 1, this spectrum is run as a magnitude spectrum (phase=1), not phase sensitive.

□

Section 7: Dynamic Behavior|VT| Solvent Suppression

How to do Variable Temperature Experiments:

The Inova 600, Inova 500, and Mercury 400 can do variable temperature experiments. Before you do any VT work, however, be sure that the temperature range you wish to use will not damage the probe in the instrument. Steven is the only person who can reliably tell you about this. Typically, any temperature from -20 °C to 80 °C is OK with any probe.

1. Lock and load your sample.
2. Type `"vttype=2"` then `"su"`
3. Type `"temp=60 su"` to set the temperature to 60 °C, or whatever temperature you want. Watch the temp in the `acqui` window equilibrate to wherever you put it.
4. Type `"vttype=0"` then `"su"` to prevent instrument macros from resetting your temperature mid-experiment.
5. Reshim.

6. Run your experiment.
7. If you want to change temperatures, repeat steps 1- 6.
8. Go about your life.

Poor man's D₂O/water suppression:

If you are taking a spectrum in D₂O and the large water peak is bothering you, the following little trick will reduce the height of the solvent peak.

1. Set up and acquire a quick proton (ie 4 scans).
2. Place the cursor on the solvent peak and type nl to move to the peak of the nearest line.
3. Type movetof to move the transmission frequency. There is decreased detection sensitivity at the frequency of transmission and this is the source of the water suppression.
4. You are now ready to acquire your spectra and work it up as usual.

Section 8: Post-Acquisition Processing

How to Flatten the Baseline in your Spectrum from any Instrument:

Are you tired of having spectra with rolling baselines? There's something you can do. It's the "bc" command.

1. Acquire or load any 1D NMR spectrum.
2. Define integrals in the usual fashion. This step is required, and you have to get all of the non-trivial peaks, including water, NMR solvents, impurities, etc.
3. Type "bc". You will have to re-type "bc" every time you ft or wft your spectrum.
4. Go about your life.

This trick works well for ¹H, ¹H-NOE, ¹⁹F, and ¹³C spectra. It may seem stupid to integrate your carbon spectrum, but it's what you have to do to make the bc command work. You don't have to display or print the integrals.

How to process and view NMR data with VNMR on your desk in the lab:

Are you tired of slaving away over paper spectra when writing reports? Would you rather have a copy of VNMR running at your desk? Here's how to do it.

What you will need:

1. An *NMRI account*. Steven will get this for you. You need not be checked out or anything.
2. A *macintosh* running system 10 or higher. The bookstore can furnish this for a reasonable price.
3. *X-Darwin*. This can be found in the software updates section of the wender server.
4. *NMR data*. This can be acquired on any of the department's varian NMR instruments. If you have none, you can probably borrow from a friend. You should also ask yourself, "Self, how is it that you've been in graduate school all this time and don't have any NMR data? Have you really been being excellent?"

What you do:

1. Start *X-Darwin* on your *macintosh*.
2. In an Xterm window, establish a secure connection to your *NMR1 account*. Type “`xhost nmr1`” then “`ssh nmr1`”. It will ask you what display type you want. The correct answer is “`x`” for (X)windows. It will then ask you what display server you’re using. Look at the prompt in your xterm and it will show your local host name. Type that (type “`DNab704df1`” or whatever).
3. At the `nmr1` prompt type “`xdir&`” to FTP your *NMR data* over from whatever instrument you acquired it on. The software interface for XDIR is intuitive.
4. Quit XDIR.
5. Type “`vnmr&`” at your `nmr1` prompt.
6. Load and process your spectra. Ask for help if you need it.

Don’t forget: if you tell it to print, your print job will show up on the printer next to NMR1, right behind the ui500 terminal.

How to print digitally from VNMR:

If you’re using VNMR remotely from your desk and you want to print a spectrum to your local printer, you can have `vnmr` export the file as a postscript file. Here’s how.

1. Display your spectrum in whatever way you want in printed.
2. Type whatever plotting command you need followed by `page('filename.eps')`. For example, type `pl pll pir pscale page('spectrum.eps')` or `pcon('pos',15,1.5) page('spectrum.eps')`, whichever meets your needs.
3. Open an ftp session to whatever machine you’re running VNMR on. Retrieve the eps file. You can now play with this file like any other ordinary postscript file. Acrobat can open it and save it as a PDF if you wish.
4. If using MacOS X you can print your eps file by opening terminal and typing the following command in the directory that contains your spectrum.eps file: `lp spectrum.eps`.