Basics scheme for the running of Biomolecular NMR experiments

This manual is written for NMR samples dissolved in water containing 5-20% D2O. Prior to starting ensure that you have a sample containing the adequate labeling, D2O concentration, sample strength and volume. The sample requirements are strongly dependent on the size of the molecule and the experiments you need to aquire. Generally the using standard NMR tubes (Wilmad 535p-7) sample volume should be about 500 micro liter (more than 400!), have a concentration of more than 0.5 mM sample and less than 250mM mono valent salt concentration.
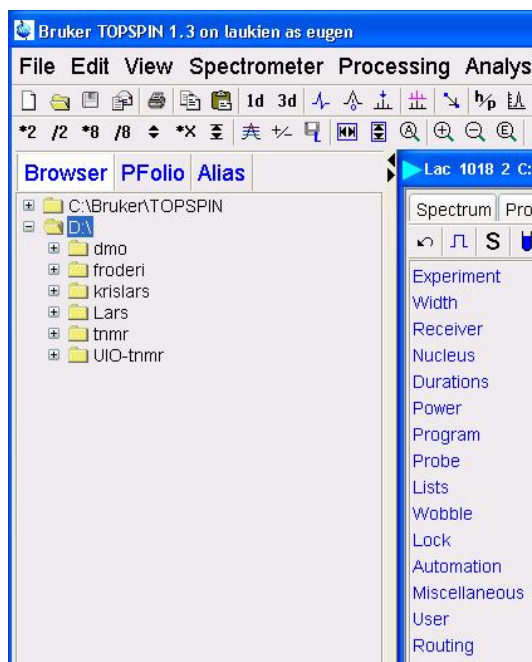
Step by step description of what to do

Starting Topspin and a new experiment

1. Log on to the NMR instrument (standard windows, UoO password and username).

2. Start Topspin by clicking the icon on the desktop.

3. Start a new experiment setup by first opening an existing experiment. These are found on the left hand side of TOPSPIN.

4. Type **edc** ↵ on the command line. Edit the NAME, EXPNO, PROCNO, DIR, USER according to your own requirements. Click on the OK button. The software will copy all setup files into the new experiment and bring you there.
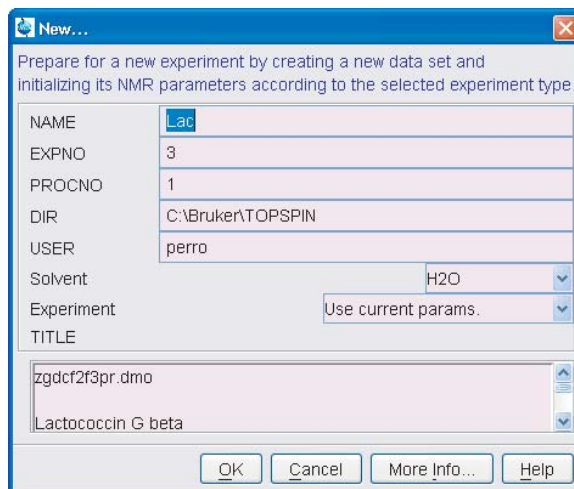
***Introducing a new sample to the magnet, lock, tune, match and shimming***

5. Eject the sample currently in the magnet, by first pressing the lock on/off on the BSMS panel followed by lift on/off. (BSMS panel is to the right of the keyboard). There should always be a sample locked in the magnet, the sample taken out will either be a $CDCl_3$ sample or the sample from the last user. Remove the old sample from the top of the magnet. Check that the height of the sample in the sample spinner is correctly positioned applying the squared plastic measuring device. Insert the new sample by pressing the lift on/off on the BSMS once.

6. Lock the sample by writing **lock** ↵ on the command line, and chose H_2O+D_2O as solvent. Watch the lock level rise. By clicking on the small lock display you may get a larger version.
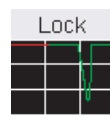

7. Read in a parameter set by typing **rpar PROTON.pek all** ↵ (or other needed parameter set if more than one channel is to be used).

8. Perform an automated or manual tuning and matching by typing respectively **atma** ↵ or **atmm** ↵ and pressing enter. For this to work as wanted ensure that you have read in a parameter set with all the channels (nuclei) configured so that all channels may be tuned (if the last user was using a standard water sample it is typically sufficient to tune and match the proton channel.)
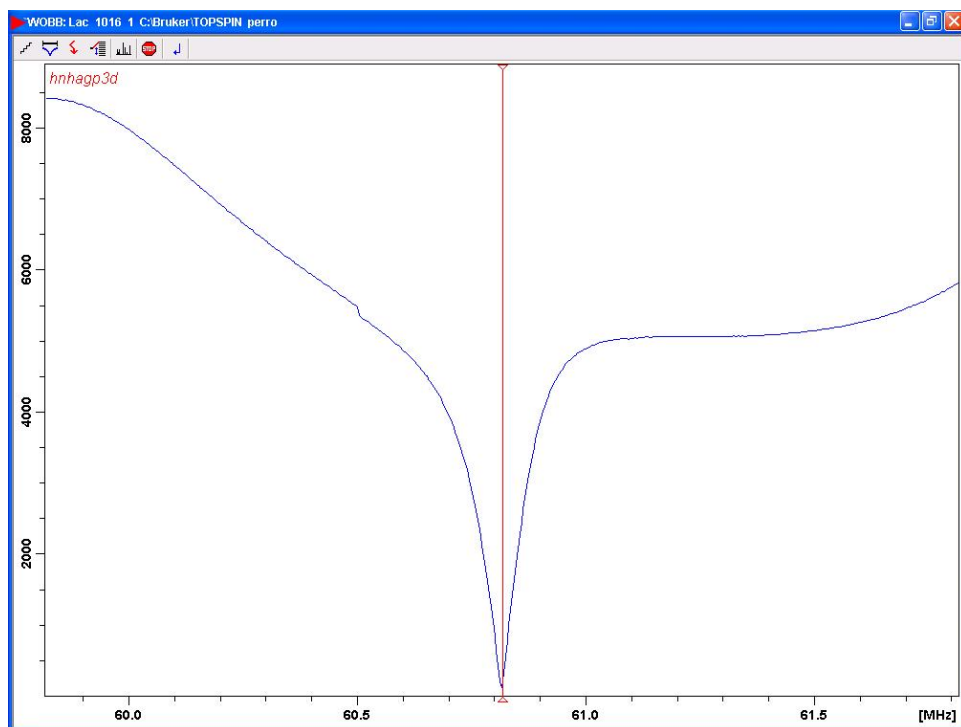
9. Shim the instrument by typing **gradshim** ↵. Check that the wanted shim setup is correctly set and click on Start Gradient Shimming. If the sample is containing water it is suggested that the Shimming method is 1D and FILENAME is Z1-Z5_20 as shown in the figure to the right. Wait until the shimming is finished and check the shim result. The shim typically takes 3 minutes to perform to sets of shimming.
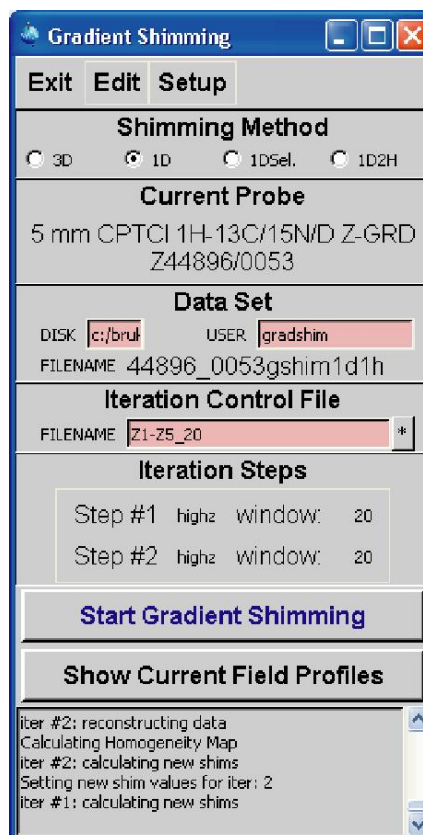
10. When observing the result and make sure that the curve is flat within +-.4 units for the vertical window of +- 20 units. Large deviations may indicate that the sample is improperly inserted, the wrong shim parameters where used or the lock solvent is incorrect.
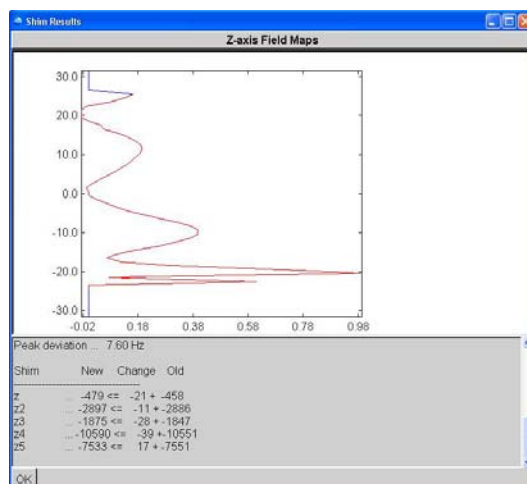
If the last user did not shim on water or the shims are not good, try to obtain an other shim set by typing **rsh** ↵ and chose an appropriate shimfile from the list. Start from start of this point again. It is customary to shim Z-by applying the

gradient shimming and if needed shim the X and Y manually on the shim consol (BSMS consol).

Measuring the 90 degree proton pulse, optimizing the O1 and writing a macro.

11. a.) Start the optimization of the experiment setup. First make sure that you are running a pulse program giving hard 90 degree proton pulses by **rpar PROTON.pek all** ↵ followed by typing **getprosol** ↵ on the command line. Type **pl1** ↵ on the command line. A box appears check that the number is 5.6db or larger (I usually apply 6db, remember the number). **pl1** is the hard pulse power on the proton channel. Type **p1 1** followed by enter. Type **zg** ↵. When the spectrum is aquired type **efp** ↵, **apk0** ↵. Check the spectrum. The large central peak (if not central this indicates that the lock was incorrectly set, repeat the lock procedure) should be perfectly phased.
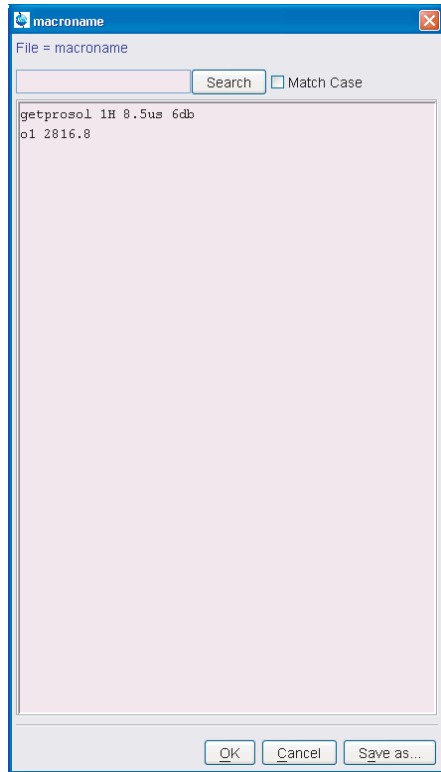
b.)   Increase the **p1** by typing **p1 36** ↵ on the command line. Write **zgefp** ↵. **p1** is typically used for the 90 degreee proton pulse.

c.)   Check when the new fourier transformed spectrum pops up if the phased peak is positive or negative. If the peak is negative increase p1 if positive decrease p1 followed by **zgefp** ↵. If the peak is both phased positive and negative you have a value for p1. Repeat this point until it is.
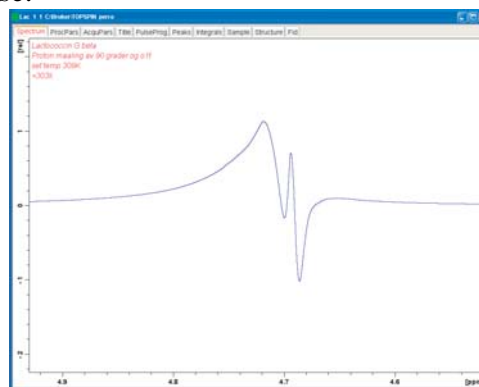

d.)   Take the p1 value and divide it by 4. This is your 90 degree proton pulse length at this power level (the number you remembered in 11.a)).

12. If the shimming was done correctly you should have a sharp peak of opposite phase to the major peak in the middle of the water hump (As shown in the figure in 11). Click the cursor and check what the frequency value of the sharp component is write down the number and remember it as the o1 value. (The value is shown at the upper right corner of the spectrum window.)

```
macroname
File = macroname
[          ] [Search] ☐ Match Case

getprosol 1H 8.5us 6db
o1 2816.8




                        [OK] [Cancel] [Save as...]
```

13. Write a macro for the following experiments. Type **edmac macroname** ↵ (your choice of name) on the command line. A new screen pops up. In this new screen you write the following commands on the first line: **getprosol 1H 6db 8us**. 6db is changed for whatever the value you are remembering from 11.a) and 8us is the value obtain for p1 value in 11d.) on the second line you write the following: **o1 2816.8**. Exchange the value for whatever value you obtained for o1 in 12.). Click on ok.

Running an experiment and phasing the resulting spectrum
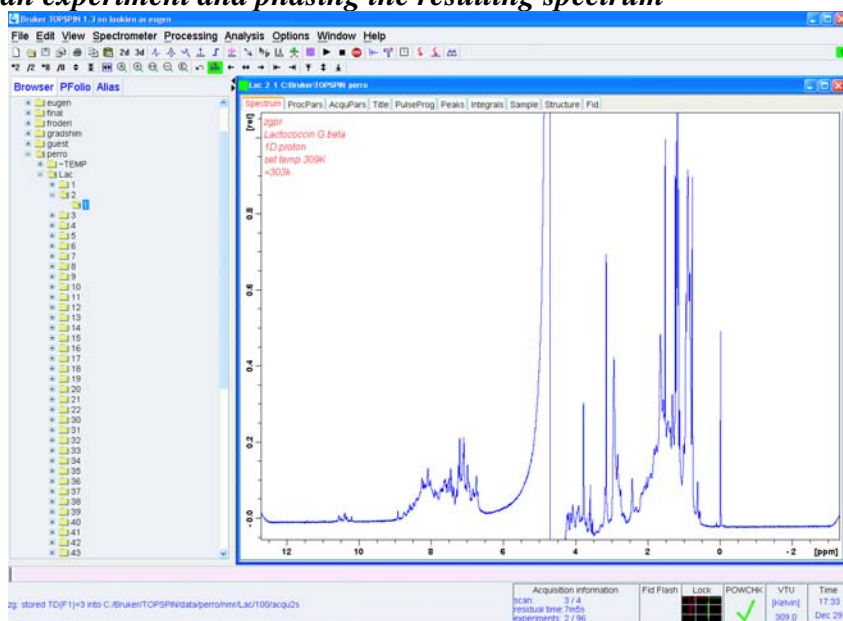
14. You are now ready to run the first real experiments but first you need to make a new experiment number type **iexpno** on the command line followed by **rpar ZGGPPR.pek all** ↵, *macroname* ↵, **rga** ↵ and **zgefp** ↵. When the new spectrum pops up you should look be able to see other peaks in addition to the hump from the water peak. Phase the spectrum by clicking on the phase icon ⟋ and then adjusting
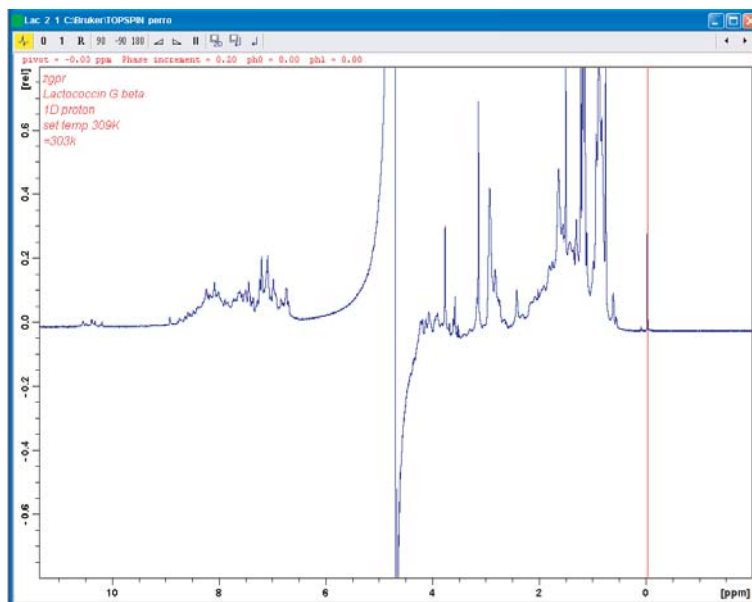
phc0 clicking on 0 in the newly opened window (0 and 1 in the upper left corner of the window) ensure that you get a nicely phased peak before you adjust the phc1 (If the phase pivot was on the wrong peak adjust the pivot by left and right clicking on a sharp peak in the spectrum (red vertical line indicates the pivot line).

It is often best to chose a strong peak on the left or the right side of the spectrum.) Click with the mouse on save and return ⊟.

15. Read in a parameter set by typing **rpar ZGGPPR.pek all** ↵ (or other needed parameter set if more than one channel is to be used). Look at the spectrum if the water peak is strong the **rg** is low (check by typing **rg** on the command line followed by enter) and either o1 is wrong or the powerlevel **pl9** used for water suppression is to low and should be changed. To change this value first type **pulse 75Hz** ↵. Read the db needed for this effect from the popup window (later refered to as **value**) and press ok. You have a value for a new presat pulse, type **pl9 value** ↵ (from the popup window, value should always be larger than 50db on the cryoprobe!). Continue by typing **rga** ↵ and wait until it finishes followed by **zgefp** ↵. If the water suppression is adequate you are done, if the water peak still is large repeat 11.b.) with increased decupling power (**pulse 100Hz** ↵). Remember the **pl9** setting it may be needed in multi dimensional experiments. It may be practical to add a line to the macro macroname containing **pl9 value**. This may be done by repeating 13. Some of you may want to run an alternative method for optimizing the o1 at this point using the command **gs** ↵ and looking at the size of the fid adjusting o1. Using **gs** ↵ will not be explained further here.

16. You should now have a relatively nice 1D proton spectrum. However, other water suppression methods may work better, and it is suggested that you try one of pulse sequences using excitation sculpting to obtain an improved spectrum. If your sample is labeled you should use one of the techniques using decoupling pulses on the X and or Y channels (F2 and F3). Decide on the experiment to perform. Type **iexpno** ↵ followed by enter.

17. Go to the spectrum window manager and chose click on the acqpar. Change the top line to the correct pulse sequences (zgesgp if the pulse sequence you wish to run is standard proton no decoupling). Alternatively you read in the parameter file for excitation sculpting, **rpar ZGESGP.pek all** ↵. For other options look in attached document. If you are not using more channels go directly to point 21. A parameter file may be generated for all proton observe techniques in the future.
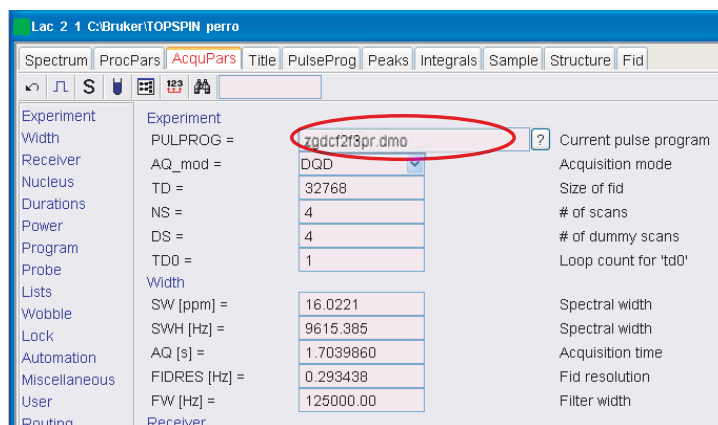
18. **edasp** ↵ check that the needed channels for the experiment you are going to use are set correctly by clicking on default followed by Save. The screen below shows what typically is correct settings.
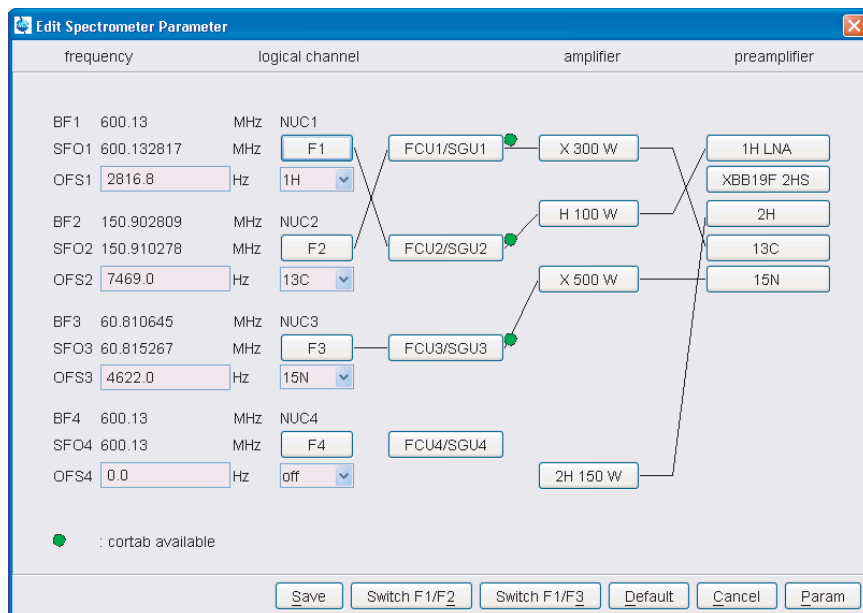
19. Check that the settings are correct (they should be if you used one of the tested parameter files). First check that the **aq** is less than 140ms for decoupled spectra (longer acquisition times will ruin the cryoprobe). Reduce the **aq** by reducing the **TD** (**TD 2048**?) or increasing **SW** so that a value smaller than maximum is obtained. If there is no decoupling pulses used the **TD** may be chosen so that it no sampling is done after the signal have died.
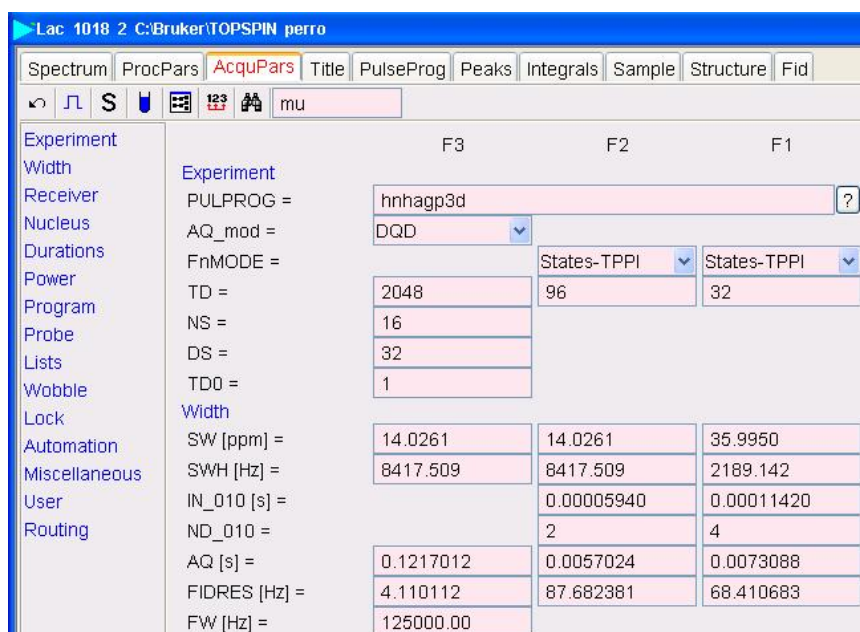
20. If two decoupling channels are running during observation also increase the decoupling pulse lengths and decrease the power level for the decoupling pulses by increasing the pl by 6 db and the double the pulse length on both channels!. Then press on the square symbol ⊓ in the AcquPars and check that the gradients are set correctly, that there is a shaped pulse for the exitation sculpting experiment, and finally that the gradient shapes are those that you wish to use (I always use the gradient shape SMSQ10.100 for all gradients). Sometimes the setting of pulse lengths and sw may give negative timings for incremented delays d0 or other calculated delays. Make sure that all your timings are "positive", non positive timings will usually give the value 5000000s as the length of the delay and an error message when the experiment is started. Try changing sw or the length of gradient pulses for other delays to ensure that all delays are "positive".

21. You should now be ready to perform the experiments, first we adjust the reciver gain by typing **rga** ↵. Check what **rg** becomes, **rg** should not be larger than 256 (not needed since the preamp is linear from 256 and upwards, therefore no improvements in noise levels). Small value for **rg** indicates that the water suppression is not functioning as it is supposed to. Finally type **zgefp** ↵, when the new experiment is done go to the spectrum window in the window manager and look. You may need to phase the spectrum as described in 11a.). You should now have a perfect 1d spectrum of your sample.

Obtaining the multidimensional for structure analysis
Running a multiple of experiments

22. Many of you would no like to obtain a 2D or 3D spectra. Which spectrum you wish to run will depend on the type of use you have for the resulting NMR spectra and the labeling of your sample. A list of possible **rpar** files and experiments are described in the attached document. The chosen experiment is not going to be explained her but generally we do the following. First we increase the experiment number (**iexpno**, **i** or **edc** (See point 3).

23. Running 2D or 3D spectra or a series of such is time consuming, it is therefore always a good idea to set up several spectra in a row and run them in a series. The series of experiments may be started when you are sure everything is ok by the application of **multizg** ↵. First you go to the first of the experiments you want to start by **re expno** ↵ (expno is the experiment number for the first of the experiments), followed by **multizg** ↵ then enter the number of experiments to be run in the popup window and hit enter. If you are running for several days decide on some experimental technique that you will apply as a test to ensure that the sample is stable and did not break down during your experiments. Run this experiment before and after each time consuming experiment.

24. Read in the parameter set by typing **rpar EXPNAME all** ↵. Then you will for 2d or 3d have a popup screen telling you that you are removing 1d… you just click ok. This is typically done by doing as described in 18 changing the rpar name and pulse sequence to the appropriate values. Several experiments require that you set a mixing time, the length of this will depend on the type of experiment (If you are uncertain you should ask some one that knows), usually an appropriate value was inserted when you ran the macro *macroname* ↵ (in 12). TOCSY type experiments on proton have mixing times typically of 60ms (the magnetization travels about one 3J in 20ms) while NOESY 150ms.

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|		F3	F2	F1
Experiment				
	PULPROG =	hnhagp3d		
	AQ_mod =	DQD		
	FnMODE =		States-TPPI	States-TPPI
	TD =	2048	96	32
	NS =	16		
	DS =	32		
	TD0 =	1		
Width				
	SW [ppm] =	14.0261	14.0261	35.9950
	SWH [Hz] =	8417.509	8417.509	2189.142
	IN_010 [s] =		0.00005940	0.00011420
	ND_010 =		2	4
	AQ [s] =	0.1217012	0.0057024	0.0073088
	FIDRES [Hz] =	4.110112	87.682381	68.410683
	FW [Hz] =	125000.00		

25. Make sure you have the right, spectrometer setup (**edasp** as in **18**), spectrum setup; sweep width, o2p.. and number of increments in the direct and indirect dimensions. The correct values should have been loaded when you used **rpar EXPNAME↵,** but you may want to change them to increase resolution or decrease experiment time. It may be needed to confront the text in the pulse program to ensure that every thing is correct (hints will usually be written, if you used the rpar files in the table with the suggested pulse sequences this is done automatically).

26. Since you may have decoupling and always make sure to check on point 18-21 before testing or starting the experiments.

27. If a preset is needed in the sequence it is beneficiary to reset the pl9 to an appropriate value such as the one decided on in the presat experiment. Do determine the receiver gain by typing **rga**↵ return, and check the value with **rg**↵ return.

28. If you are running 3d spectra it is always smart to run 2D versions of the experiment first to ensure that you know the projections, and that everything is done correctly (some thing wrong and several days on the spectrometer may be wasted!) This is easily done with the setting the number of points in one of the dimensions to 1 and run the experiment. (always check **rga**↵ prior to running an experiment).
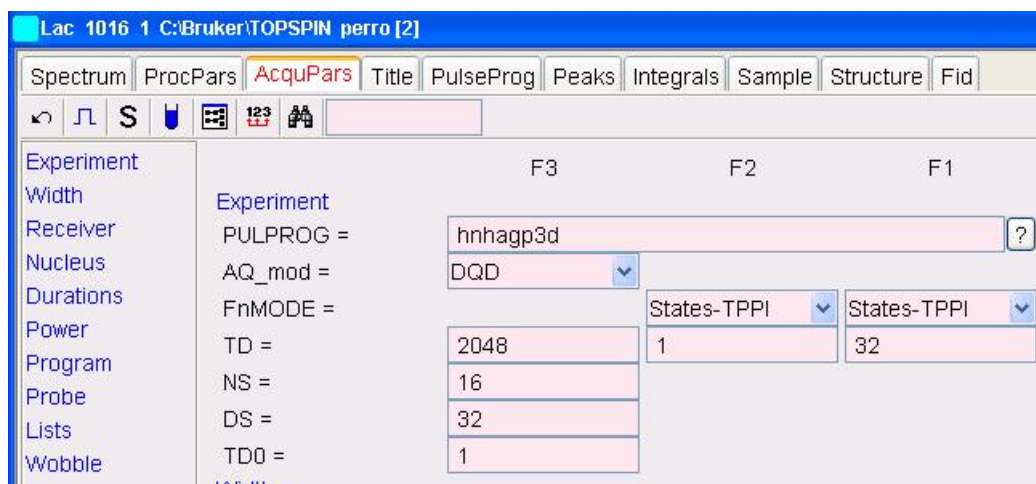
29. The obtained plane of a 2D may be Fourier transformed by **xfb** ↵. Investigate the result. 2D phasing is done as described for 1d but you now have both rows and columns (the red lines indicates the rows and columns along which the phasing is done). Typically the indirect dimension should not need to be phased, however, phase adjustment parameters are needed for several sequences these are found in the pulse sequence file, adjust them and do **xfb** ↵.
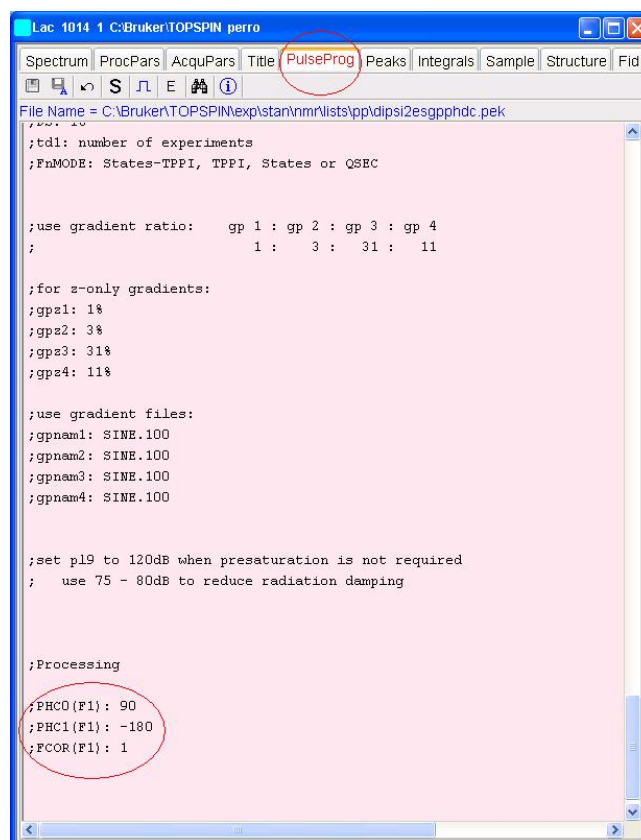
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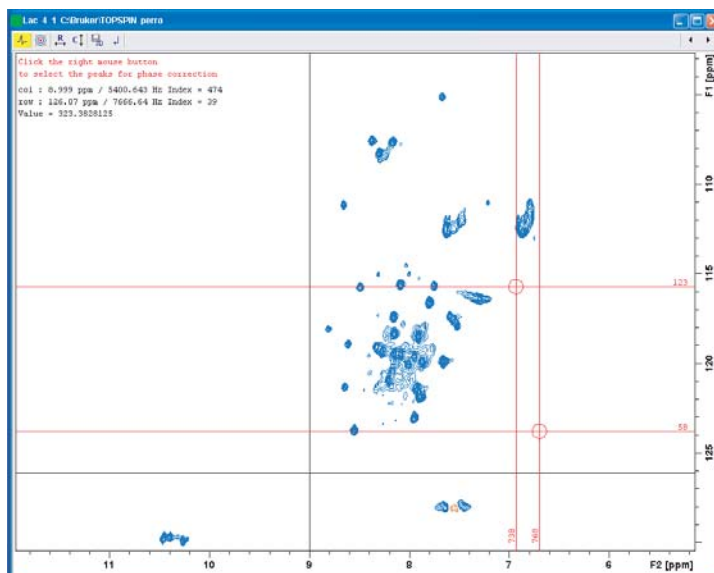
Click on 〜 and go with the cursor to the phasing window. Right and left click to add lines along witch you wish to phase, at least two lines so that you can se how peaks at opposite sides of the spectrum are all correctly phased. Click on the row icon ↕ ↕ in the phase window and click save and return 🖳, return ↵. A nice 2D should now show up on your screen.

31. Repeat with points 16 to 18 until you have set up the number of experiments you wish to do. Go back to the first experiment by using **re EXPNO** ↵, write **multizg** ↵ on the command line and type in the number of experiments to be started followed by enter. The instrument will tell you for how long the instrument will be running.

32. It is usually smart to check inn at the spectrometer to ensure that every thing is working ok at least once a day if you are running experiments or series of such that will run for several days.

Before you finish

33. If you will be finishing early always inform the next user in good time. When finished you are yourself responsible for your samples and should take them out of the instrument and insert a lock sample. Samples left in the instrument at the end of your instrument time will be removed by the next user, and may be lost.