

# Frequently Asked NMR related Questions

Last updated 2008.03.21 by Huaping Mo

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## ARX300

### 1. arx300 (or other spectrometer) cannot spin or cannot reach steady rate

Check the following: 1. take out the sample and check the sample depth (should be less than 20 mm). 2. change to the lock sample and see if it spins. 3. put the cap on the magnet may help. 4. change the spinning air flow (ask PINMRF staff). 5. the shim stack might be dirty and need cleaning (ask PINMRF staff).

### 2. arx300 (or other arx spectrometer) cannot lock

Check 1. if you have loaded a good shim set (bad shims may give lousy lock signal) by "rpar shims.qnp" and then "lock\_solvent" with "solvent" as cdcl, meth, dmsd or acet. 2. update the solvent in eda to the correct one. Then do lopo, and lock. 3. you may need to adjust the lock phase. For CDCl<sub>3</sub>, the lock phase should be around 210. 4. you can manually change the lock field drift to see if you can get the lock signal. 5. try some more reasonable lock power (20) and lock gain (120). 6. consider to reset scm if both 1 and 2 fail to fix the problem (see PINMRF staff help; see appendix for procedures).

### 3. there's no 13C, 31P or 19F signal on arx300

1. check if you have enough compound and have accumulated enough scans. If the solvent peak (or some other sample with known signal) shows up nicely after 1 scan, and you have not observed any compound signal after 1k scan, it's likely that you do not have insufficient amount of compound.

2. conformational exchange (any chance of tautomerism?) may make peaks broad and disappear. Check proton spectrum and see if there's any sign of broadening. In case of conformational exchange, raise or lower temperature may make the peaks re-appear.

3. arx300 has a qnp probe, which selects 13C/31P/19F detection by a motor. Normally you would hear a tick sound when you switch detection to 13C or 31P or 19F. If the motor fails to move the stick on the probe (it happened several times before, and the probe is fairly old) to the right position, then you will not hear the click sound. As a result, you may not get any meaningful signal at all. Make sure you see the motor moving position 1 for 13C detection, 2 for 31P and 3 for 19F. If you see motor at position 2 while you try to observe 13C, you need do ii first. Hopefully the motor will move to the right place. If not, you may try to read a parameter set for 19F or 31P, ii, then read in 13C parameter set and ii gain. Consult with PINMRF staff if you have difficulties.

The light on the HPPR box should be left off for automatic switching. After a power down, make sure the light is off.

### 4. the spectrometer is pulsing (or running some experiments) while nobody is actively logged in

One possibility is people log off without exiting from xwinnmr, potentially leaving some active process (e.g. data acquisition) running in the background. Ask PINMRF staff to kill the active process for you. PLEASE exit from the "File" menu of xwinnmr or iconnmr, before logging out.

The other possibility is that somebody (PINMRF staff) is running experiments remotely. Type “who” in any unix shell to find out who’s logged in remotely. Call the person and ask about the problem.

## **5. I cannot run any experiment with xwinnmr or iconnmr**

1. check above to see if there’s any leftover process from the last user.
2. check any error message when you try to start data acquisition.

## **6. How to tune x-nucleus for (19F/31P/13C) qnp probe**

For normal applications, qnp probe need not be tuned. In case needed, first check which channel needs tuned. The shorter thin rod is for 13C. The longer thin rod is for 19F. the red “normal” rods affect all 19F/31P/13C.

If only 19F or 13C tune is bad, only adjust the corresponding thin rod

If 31P is bad, then 1. tune 31P: adjust the “normal” red rods labeled with “T” and “M”. 2. tune 13C, by adjusting the short thin rod. 3. Lastly tune 19F, by the long thin rod. 4. check all tune again, to make sure all tuning is good (may not be perfect though).

Make sure the motor position is at where it should be when tuning or observing: 13C <-> position 1; 31P <-> position 2; 19F <-> position 3.

## **DRX500-2**

### **1. on drx500-2, the lock or shim (lineshape) is poor**

Make sure 1. you recognize the right probe type (we typically have either TXI or BBO installed: you will see “TXI” or BB sign on the probe). 2. Load a good starting shim set (rsh shims.txi or rsh shims.bbo). 3. type lock and then select the right solvent from the list. Optimize shim after lock is found.

If you have a short sample, or the sample is not aligned to the center, you will observe poor lock signal especially at the beginning. You may observe quite some z/z2 adjust necessary for the shim.

### **2. lineshape is still poor on drx500-2**

Try harder with shim. If you have a short sample, you may have to spend more time in shimming. If the volume is less than 500 ul, then you have to make sure the center of the sample align with the center of the probe coil (check with PINMRF staff on how). If the volume is less than 450 ul, you will typically observe worse line-shape even with center aligned. With 450ul or less, you may find spinning the sample is not helpful, since it would generate some turbulence within the detected region. When that happens, you may observe some fluctuation of the lock. If you have a short sample, and are not happy with line-shape, my advice is to add more solvent to 500ul.

Examine all proton peaks. Are they all broad or just some of them are broad. Are all peaks distorted in the same way (flat on top, asymmetric on sides). If yes, it is likely that the shim is bad. Work on shim more: 1. load a good starting shim set (e.g. rsh shims.txi or shims.bbo) 2. shim on lock signal for z1, z2 and z3. 3. if peaks have tails on the right, change z4 to lower

values (e.g. -4000 to -45000). If tails on the left, change z4 to higher values (e.g. -4000 to -3500). Re-adjust lower z1, z2 and z3 shims.

Consult with PINMRF on how to use gradient auto shim.

### **3. some peaks are split while I believe shim is fine**

Split peaks might be seen for some resonances when you have non-standard nmr tube assembly such as an insert in the NMR tube.

In such a case, make sure shim is really fine. The inner tube components will most likely show peaks with good line-shapes. You may have to work on non-spinning shims (x and y) to see if you can improve.

The fact is, there's a boundary between insert and tube, and it might be difficult to get very good line-shape for the components in the out tube

### **4. sensitivity on drx500-2 is poor or there is no signal**

Check 1. if you have recognized the right probe and loaded parameters/shims for it. 2. tune and match probe, especially for the channel of interest. You will have to see a dip for the tuning curve; no red light flashes on the pre-amp box. 3. check if the right filter is used. 4. check receiver gain and pulselength. 5. for 2d's, it is very important to have right pulselength, and gradients.

### **5. cannot tune the probe, or there's no tuning response**

First make sure cables are connected right, and appropriate filters are used. For the x-channel on BBO, you will use the stick to dial tune / match numbers to the pre-set values (hanging off from the probe).

Make sure that you are tuning/matching the right channel, which is being wobbled. Double check the wobble acqu curve for the nucleus / frequency.

If you have tuned for too long, the tuning program may time out and stop giving any signal. The tuning curve may appear flat (and high) when that happens. Do it and wait at least a few minutes (so that some components used in tuning can cool down), you should be able to confirm or continue you tuning with wobb again.

### **6. some abnormal temperature is observed in edte window.**

First, check if you have heater or cooling gas turned on. If you are using heater and temperature is too high, you may wish to turn off the heater. If you use a cooling gas, and temperature is too low, you may wish to turn off the cooling.

When the thermo-couple cable gets loose, you may observe strange temperature. check the cable if you know how. If not, consult with PINMRF staff.

Absurd temperature, such 0k or >500K, might be observed. In such a case, try existing edte and type edte within xwinnmr again and see if it helps.

Check if the right temperature sensor is selected. Drx500-2 may have TXI or BBO probe installed at any given time. TXI and BBO use different sensors, which can be selected from edte menu. Consult with PINMRF staff if further information is needed.

### **7. temperature is not stable**

Make sure the right sensor is chosen, and proper gas flow rate is used.

If you just turned on or off the cooling unit, you may have to wait a little while to see temperature stabilizing. You may need to conduct a self-tune if you changed the air flow, or cooling unit (on / off).

Consider read a probe's configuration file within edte.

## 8. there's no gradient

BBO probe is not equipment with gradient thus when it is installed, there should be no pulsed gradient.

If TXI is installed and you do not see the expected gradient, do

1. make sure gradient amplifier is on.
2. make sure a gradient probe is installed (a BBO probe is not a gradient probe and you cannot run any gradient experiment with that probe!).
3. make sure the probe is set to the right type in edhead.
3. check gradient cable and amplifier lights (make sure no overload or overheat/red lights)

## 9. how do I make a shim map

In order for gradshim to work, a shim map (for a probe) needs to be obtained. A proton gradient shim will require a proton gradient map. A deuterium shim will need a deuterium map too. A 3D proton gradient shim will also need a 3d proton map. Normally PINMRF will make the shim map for everybody to use.

For PINMRF reference, to make a 1D deuterium shim map,

1. make sample with single dominant peak. Log in as nmrsu and launch xwinnmr
2. create a dataset (2H, 1, 1)
3. rpar gradshim1d2dccl3.35 (or parameter similar. Change o1p from 7.27ppm to 2.5ppm so that the 2H pulse is right on resonance. Update title, or other parameters (TE1, TE2 or pulse length and level) if needed. Do "zg" and then "fmc". You should see a gradient image. Save the parameter set as gradshim1d2hdms0.35
4. type gradshim -> shim map. Set parameter set as gradshim, and dataset u user mo. Keep TE1 as 5us and TE2 as 125 us. Set shim group for mapping: highz.
5. make shim map. This process will take tens of minutes.

After completion, a 1D 2H shim map is made. This map will be okay for 1D 2H gradient shim in DMSO. Though the 2H shim map will likely to be the same for DMSO and CDCl3 samples, the controlling parameters should be different (o1p are different). Thus a 2H gradshim would tend to require gradsim1d2dccl3.35 to work best.

A 3D 1H shim map will take a number of hours to complete, thus it is not recommended to do often.

## 10. how do I use gradient shim

First make sure that you have a gradient probe (TXI) installed.

Make sure that you are not actively acquiring data.

You have to understand that gradient shim will work easily if you have a single dominate proton or deuterium peak in NMR (e.g. a sample in undeuterated water, or a sample in D2O, or a sample in CDCl3). Consult with PINMRF staff if you have multiple solvent 1H or 2H peaks (e.g. deuterated CD3OD).

Find some reasonable good starting shim values (you can do rsh shims.txi).

Within xwinnmr, launch gradshim. First time user may have to wait a couple of minutes for automatic setup. In the select 1d1h or 1d2h. change user to your username. Define the region (typically around 20; if you have a long sample, this number can be as big as 24 or 26. if you have a short sample, then that number has to be smaller).

Click start gradient shim. While deuterium gradient shim is in progress, you may lose lock signal. This is fine. The lock will recover upon completion. 1D 2H gradshim may take several minutes. If proton gradient is in progress, you will see lock signal going up and down, which is normal. It will become steady after completion. 1D 1H gradshim should be faster than 1D 2H.

After gradient shim is completed, you should expect improved lock signal. If starting shim is not very good, you may need to repeat gradshim several times. After that, you no clear lock gain can be obtained by hand shim, gradshim can be considered to be a success / final.

Also, after completion, you will see a z gradient shim map. You should not see big variations in the colored region (horizontal variation within 0.5; the smaller the variation, the better the z shim).

## **11. what are the probe choices on drx500-2**

There are two frequently used probes for drx500-2: TXI and BBO. TXI is gradient equipped and good for proton detection and normal homonuclear 2D's or proton – carbon/nitrogen 2Ds.

BBO is not gradient equipped and is good for direct x-nucleus observe (e.g.  $^{13}\text{C}$ ). In theory, we can observe nucleus from 6MHz to 202MHz (31P) on drx500-2.

A third probe is called TBI, which has a z-gradient, and the second channel is set to carbon and third one set to x-nucleus.

## **12. How do I recognize the probe type**

TXI probe has a "TXI" label on it. BBO has "BB" label for the x channel.

## **13. why do I see a bump around 120 ppm in carbon spectrum**

This happens when you use TXI probe for direct carbon observation. The bump around 100 ppm comes from the building material for the probe. Thus it is real, though it's not from the sample.

You can do a baseline correction in the affected region if you wish to make it disappear. The following is one of the ways: 1. by "edp", set absf1 and absf2 to cover the whole region (max and min. chemical shifts. as 200 and -10ppm, for example). Then do "abs". 2. do "edp" again, and set absf1 and absf2 around the bump region (say, 150ppm and 90ppm). 3. Do "absf" again, and the bump should go away. If you are not happy with the result, you can do "efp" to start over, and repeat steps 1, 2 and 3.

The best way to get rid of the bump is to use BBO probe, which has higher carbon sensitivity, and does not have the building material that gives rise to carbon broad peak ~100 ppm.

## **14. why do I observe strange line-shape in NOESY while other 2D's (COSY, HMQC etc) seem to be fine.**

On drx500-2, COSY and HMQC for routine users are set up as magnitude mode. They do not have "phase" problems. NOESY or TOCSY is set up as phase sensitive (has to be, since diagonals have different signs from crosspeaks). Thus you do have to worry about phases.

There are also times when 2D phase is not properly done. Refer to the next question on how to phase 2D.

There appear also times that a NOESY or TOCSY spectrum cannot be phased. While I have not encountered the problem myself, I did see people get spectra with that problem. Here are my suggestions: 1. do try to phase both dimensions 2. check 90 degree pulses (which may root from imperfect tuning). If there are significantly off from the "real", it's likely that the overall signal would be attenuated, and the sequence's phase cycling wouldn't be efficient: undesirable pathways (cosy types) will seep in. cosy type crosspeaks cannot be phased into pure absorptive mode. 3. make sure that there's no aliasing (or fold over). 4. even then all pulses have been properly calibrated, cosy type of peaks can still appear. The appearance of cosy type peaks suggests the affected protons are J-coupled, and thus should be in close proximity, which most of the time warrants no further action taken to get rid of cosy peaks.

## 15. how to phase a 2D (noesy or tocsy) spectrum

First, magnitude spectrum does not require phasing. You only worry about phase when the 2D is in phase-sensitive mode. At PINMRF, phase sensitive 2D's include NOESY and TOCSY. COSY, HMQC and HMBC are set up as magnitude mode. However you can set up a phase sensitive COSY or HMQC if you know how.

Set the xwinnmr to extended mode (by "setres"; you only have to do this once). In extended mode, you will have more icons within xwinnmr window, which make your life much easier.

To phase a phase-sensitive spectrum, you will set the display for both positive and negatives: click +/- icon till you see both negative and positive peaks are shown. Change the display level so that there is not significant noise on display and you have clear signals. When you are happy with the display, click "DefPlot" icon, and confirm suggested entries. The new display levels should be updated.

Open up the current pulsesequence (edcpul or by any other means). Read to the end, which may suggest phase correction values for the indirect dimension. In the case of noesy (sequence: noesygp), the suggested values for F1(indirect) dimension are PHC0=90, PHC1=-180 and FCOR=1. for tocsy (sequence: mlevgp), PHC0 is 180, PHC1=-180 and FCOR=1 for F1 (indirect) dimension. open up "edp" and type in those numbers to the right place. Also make sure that phase modes in both dimensions are selected as "pk" (typically they are set right for you from the parameter set: noesy and tocsy requires phase sensitive mode; for magnitude mode (e.g. cosy/hmqc/hmbc, select "MC";). Reset F2 (direct dimension) phases (PHC0 and PHC1) to 0 (you should not have very large PHC1 value in F2 dimension; PHC0 should also be within -360 to 360; if exceeds, add 360 or -360 to bring down). type xfb to have all update processed data with the new phases.

Click on "phase" button on the left. Then window will change to the phase mode, with the miniature spectrum moved to the upper left. You will start phase on the direct detection dimension first. Click "row" and click with middle mouse button on a horizontal trace of the 2D spectrum. Click "move to 1" so that the selected horizontal 1D trace will show up in window 1. click "row" again and put another trace into ("move to 2") window 2. You can choose to select still one more row to move into window 3. It is recommended that you include peaks from both high and low ppm's.

Press left mouse button (without releasing) on ph0. Move the mouse (keep pressed) up or down, to phase one end of spectrum to absorptive appearance. Press left mouse button (without

releasing) on ph1. Move the mouse (cursor) up or down, to phase the other end of spectrum to absorptive appearance as well. Repeat ph0 and ph1 as most peaks (or major peaks) are absorptive (either positive or negative). This is what you should expect: diagonal peaks, which tend to be much more significant in noesy, will be phase to positive; off diagonal peaks, can appear as positive or negative: large molecule noesy is positive; small molecule noesy is negative; tocsy should always be positive).

Click on "return". Then you will be asked if you just want to return (no phase action taken to the current data set) or apply the phase. You will typically apply the phase. Then a new FT will be conducted, with additional phase corrections. Repeat this till you are satisfied. In the end, you can start another round of xfb to be sure that all phases have been correctly set.

Sometimes you will see streaks running vertically or horizontally. You can apply abs2 or abs1 to make corrections for baseline.

There are also times other artifacts appear: no all peaks can be phase into expected positive or negative. However, to achieve best results, you will have most peaks phased in pure phase. If you have trouble with phasings, you may have to go back to the beginning and redo the whole thing. Consult with PINMRF if you further questions.

## **16. my 2D spectrum has either positive or negative vertical(F1) streaks**

There are two sources for streaks in F1 dimension: 1. unwanted magnetization which is not completely canceled by phase-cycling or gradients. Those streaks may appear as randomly positive or negative. 2. digital sampling for the first point, which can be over or under fourier transformed. This streak may appear all positive or all negative. 3. truncation artifacts, which result from the truncation of FID at the end of the (pseudo) FID

Increase the number of phase cycles, increase instrument stability, decrease temperature variation, and increase (with jurisdiction) gradients may help reduce those artifacts caused in 1. Uniform positive or negative streaks in 2. are processing artifacts and can be easily taken care of by changing the FCOR number of F1 dimension in "edp". Use a smooth window function(e.g. cosine bell), which gradual force FID to zero at the end of the acquisition, will reduce the streaks caused by 3.

If the first increment in F1 starts with time zero, then the FCOR for F1 should be set to 0.5. If first increment starts with half dwell or full dwell time, then FCOR should be set to 1. Half dwell time has been very frequently used in pulseprograms, which has the advantage of predictable phase, flatter baseline and favorable phases for aliased peaks. In F1 dimension, phc0 frequently is 90 and phc1 -180, if half dwell time is used (noesy has phc0/phc1 as 90/-180, while tocsy has 180/-180).

FCOR has a range of 0 to 2. In general, larger FCOR will have positive streaks in F1 (assuming the main peak along F1 is phased positive). Small FCOR leads to negative streaks. You may empirically change the size of FCOR so that the streaks can be minimized.

For magnitude mode 2D's (COSY or HMQC or HMBC), it seems that setting FCOR matters little.

After you have played with FCOR, you can further reduce the streaks by "abs1", which applies a polynomial baseline correction function along the F1 dimension.



# AV800

## 1. where are my files located

All interesting files are most likely located under /u/exp/stan/nmr/lists/. Pulse programs are under /u/exp/stan/nmr/lists/pp (for system pulse programs) and /u/exp/stan/nmr/lists/pp/user (for user pulse programs). Data are normally under /u/data/username/nmr

## 2. bugs found topspin (2.1)

1. after a shaped pulse, the power level might be left in a undefined state (while in xwin3.5, the power will resume to the last defined or default power level). An explicit power definition might be required before another pulse can be executed. e.g.

Bruker pulse program (in topspin 2.1):

```
(p14:sp3 ph21):f2
```

```
10u
```

```
(p3 ph5):f2
```

Should be changed to

```
(p14:sp3 ph21):f2
```

```
10u p12:f2
```

```
(p3 ph5):f2
```

2. adjusting shaped pulse power level (fine power tune mode) may lead to significant spectrometer misbehaviors, including abnormal temperature fluctuation (5 degrees from set temperature, leading to lock signal fluctuation) and acquisition failure. In one case, receiver would hang up and stops sending acquisition completion signal. Error message may appear as “Error setting Receiver frequency, or “Error 141 in RX\_1” or “parcomprg\_rx22(0) failed”

In one case, I found that ii would restore spectrometer to the normal state.

In another occasion, ii failed and the message appeared as “waiting for IPSO to start” (which takes forever). I tried a number of remedies, including “ha” and reset the IPSO. In the end, the spectrometer reverts to the normal state.

## 3. shim is not as good as expected

It is much more difficult to manually shim on higher fields. On the other hand, high field instrument tend to have newer / better consoles (especially with gradients) to great assisting shimming.

Topshim, by default, would use the solvent signal (set by last lock command; either deuterium for deuterated solvent or proton for H<sub>2</sub>O) and perform 1D z shim. Thus it will not attempt to adjust any off-z shims.

I have found different solvents require at least different off-z shims. In particular, you can easily find some x,y shim changes if you change the sample from one type of solvent (chloroform) to another (water). Of course, for water sample, you can use 3D shim most of the time, if you do not mind spending some extra time (~10 minutes) in doing so.

Alternatively, it is highly recommended that you initialize shim with some pre-determined solvent specific values. As such, you only need 1D z shim for reasonably good result.

#### **4. probe tuning or 90 degree pulse becomes off, after I set up a new experiment.**

The cause might be that topspin remembers the probe tune/match position for the locked solvent. Your sample may contain different amount of salt even for the same solvent, thus the proton tune may needs adjustment.

What happened might be that in running a 1D proton acquisition, you have tuned the proton channel and calibrated the pulse length, however you may not have saved the position. Then in setting up a heteronuclear experiment, you try to tune again for  $^{13}\text{C}$  or  $^{15}\text{N}$  (which is not tuned in you previous 1D acquisition). The tuning program (atmm) may automatically revert to the last saved position and gives the wrong position (tuning) for proton.

Remedies: save tuning positions after satisfactory results are found; re-examine all nuclei's tuning once atmm is launched.

#### **5. 90 degree pulse is off or the spectrometer appears to have low sensitivity in 2D/3D or have strange phases**

Make sure the probe is tuned it best. Then recalibrate 90 degree pulse length. For salty samples, the pulse length can increase from about 8us (@0.9db) fro a salt free sample to 15us for a sample containing about 200 mM of salt.

Poor spectrometer tuning is a frequent reason for poor sensitivity.

Wrong 90 degree pulse also often causes poor sensitivity and spectral artifacts, including strange phases.

#### **6. misc. issues**

When atmm in executed, the program recalls the last tuning/matching positions (for all nuclei?). Thus it is highly recommend that you go through all nuclei and make sure they are all tuned well. If significant changes are made, 90 degree pulse should be recalibrated.

Autolock should be kept long for experiments that run for longer times. I do notice that the lock level may drop significantly after about 1 hour if it is not turned on.

## Any spectrometer

### 1. the lock signal is unstable

Check 1. if you have too much lock power (saturating the  $^2\text{H}$  signal). Lower that power should actually get the lock level even higher. 2. make sure that you have a decent shim. 3. Do not try to change shim too fast and too much: read in a good shim set, and activate "fine" button on the shim panel.

### 2. lock signal is poor / noisy

Check 1. if you have enough deuterium in the sample 2. are you locking the right deuterium signal: if you have multiple deuterium signal, lock the strongest one (e.g. lock  $\text{CD}_3$  part for MeOD). 3. have a decent starting shim. 4. avoid too high or too low lock power. 5. deuterium channel may have some problem.

Drx500-1's deuterium channel might get inadvertently changed when somebody is trying to tuning proton. If the change on lock is small, it can be changed back, just based on lock signal and the operator's memory of how much has been done. To do a thorough work, the deuterium channel on drx500-1 can be tuned. 1. set up a deuterium obs. Experiment, for example you can rpar gradshim1d2h.cryo. 2. start cwobb and you be able to tune deuterium like you would tune for proton.

### 3. can I use a short sample

Typically we recommend 600  $\mu\text{l}$  for bruker and 700  $\mu\text{l}$  for varian. However, you can get by with 400-500  $\mu\text{l}$  volume. In that case, make sure the center of the sample aligns with the center of the detector.

With a short sample, you may have to spend more time in shimming, and the lineshape wouldn't be as good.

4. what do I do if I have very small amount of sample  
use less solvent (~300  $\mu\text{l}$  instead of 600  $\mu\text{l}$  or more), and try Shigemii tube

### 5. can I run variable temperature experiment

You can safely run experiment from RT to 50C. For higher or lower temps and for extended time, consult with PINMRF staff.

### 6. my spectrum has rolling baseline

Most likely the receiver gain is set too high, resulting in first FID points clipping. Click on the fid icon, and you may see the beginning part of the FID is flat (clipped).

Lower the receiver again, or do auto receiver gain adjustment (rga on bruker, or turn auto gain on for varian).

### 7. peaks are found in unexpected regions (say -1pm)

In some cases, protons/carbons several bonds way from Si atom can have small or negative chemical shifts. In other cases, the peaks are aliased: they exceed the highest chemical shift that

sw is set up to observe (typically come out well above 10 ppm), and come back to the low chemical shift region.

To avoid aliasing, you can increase spectral width and see if chemical shift changes or not. If all peaks are exactly the same chemical shift position, then there is no aliasing.

## 8. How do I find out if a proton is attached to carbon or nitrogen or anything else

Solutions: 1. run a 2D HSQC type of experiment 2. check if peaks of interest are sharp. If so, you can examine their satellite peaks, with some estimated coupling constant. For example, for CH proton, expect 1% of main peak intensity satellites with a distance of 130 Hz apart. For NH proton, expect 0.4% main peak intensity (satellites arising from  $^{15}\text{N}$ ;  $\sim 90\text{Hz}$  apart). Decoupling  $^{14}\text{N}$  could sharpen the NH proton in some cases (when NH exchange rate is slow).

OH should not have any visible satellites.

## 9. I cannot operate the spectrometer

Make sure that you know how to operate spectrometer. Check if the computer is dead, and spectrometer looks “alive”.

It's possible that somebody is controlling/operating the spectrometer remotely. Use “who” in unix shell to find out who's logged in. Call them and ask about your problem.

## 10. what are the sensitivities for PINMRF spectrometers.

For most users, they can access 300 (bruker or varian), 400 (bruker) and 500 (bruker).

	300	400	drx500-2 with TXI	drx500-2 with BBO	drx500-1
$^1\text{H}$ sensitivity	100	200	300	800	3000
$^{13}\text{C}$ sen.	100	200	100	300	200

The best proton sensitivity is offered by drx500-1 with cryoprobe. The best carbon sensitivity is offered by drx500-2 with BBO set-up (which also has the best sensitivity for non-H/F x nucleus).

## Offline computers: pathfinder (sgi), atlantis (linux box) and PC's

### 1. pathfinder does not allow me to log in

Check 1. do you have a account on pathfinder or are you using the right password. Try to find out if anybody else can log in. 2. if nobody can log in, then the computer may need a reset (reboot).

### 2. pathfinder does not print (from xwinplot)

Check 1. change printer set up to postscript, or "hp laserjet 4V". sometimes one will allow you to printer while the other one errors out. 2. log out and try to log back in. if cannot log in. then the computer may need a reset.

We suggest that you use shigemi tube for low volume. Ask PINMRF staff on how to get / borrow /use one.

### 3. pathfinder freezes

Sometimes when you click too many times too fast in xwinnmr session, the computer may freeze. Killing the xwinnmr job (by PINMRF staff) should solve the problem. If not, then consider to force logging user out (kill xdm process) remotely from another computer. The user or superuser can do that.

The user can also reset sgi computer by pressing these four keys simultaneously:

Left-shift

Left-control

F12

(keypad) /

Reboot or reset is the last resort. Unplug the computer if everything else has failed.

### 4. I couldn't find my files on pathfinder (when I log in, my home directory is /, instead of /mnt/d1/myusername). And I cannot find my desktop icons.

That's because /mnt/d1 is not mounted. A super user should mount manually. (/mnt/d1 should mount automatically upon rebooting but it can somehow get unmounted manually or by itself(malfunction?!))

### 5. where I can find the spectrometer data

On the spectrometer, it is under /u/data/username/nmr

On the offline computer, the local data is under /mount/d2/data/username/nmr (eq. of /u/data/username/nmr). The spectrometer data is also mounted on offline computer as /nfs/spectrometername/d2/data/username/nmr (this is located on the spectrometer).

From your (unix) desktop, you should see a folder icon. Double click it, and type in /nfs in the path(directory) box and hit return. You should see a bunch of spectrometers/computers in the

folder. Then you can type in the path, one at a time (and hit return) to move to your destination. Alternatively you can drag and drop folders to the top left corner folder box.

## 6. I can see my data in my data folder, but xwinnmr cannot find it

First try “search” option from the xwinnmr file menu. After you select disk u and username (make sure it is not guest!), you should see all data directory.

If you still cannot get to the data in xwinnmr, make sure that the data is in the right place. Your data should have such a structure: /u/data/username/nmr/expt\_name/expt\_number, with expt\_name as the name of your data, and expt\_number as a natural integer.

## 7. how to copy or retrieve spectrometer data

In general, (remote, or mounted) nmr data is stored under /nfs/spectrometername/d2/data/username/nmr/

If the data is mounted to the local computer, you can press and drag the data. If the data is not mounted locally, you can do secure copy: `scp -r username@computername:path/filename ./`

The local computer data is located under /u/data/username/nmr (or /mnt/d2/data/username/nmr)

For example, if you wish to transfer files from arx300 to pathfinder

1. on pathfinder, open a shell (the kind of window you normally type “xwinnmr”; you will do that in step 4). Type

**cd /mnt/d2/data/your\_username/nmr**

2. type

**scp -r arx300:/mnt/d2/data/your\_username/nmr/file\_name ./**

The directory “file\_name” along with its contents will be transferred from arx300 to pathfinder (Warning: if pathfinder has a file with the exact name as the “file\_name” above, it will be overwritten!)

3. you will be asked for your **password** on arx300. Type that in.

4. after done, you can type **xwinnmr** and look at the newly transferred data.

## 8. how to back up individual spectrometer data

The easiest way might be backing up data on a CD. To do that, you can

1. transfer the data that reside on any PINMRF network computer to your workstation PC through secure FTP (part of the package of secure shell client; see below). Once you connect to the remote site through secure shell, you can open up a file transfer window, which allows you drag and drop files from remote site to local disk. The remote data is typically saved under /u/data/username/nmr.

2. burn CD on the local PC. Do double check file integrity and do the backward transfer to make sure you can recover the data.

## 9. how to open remote display on a local computer

Case 1, if you wish to display remote unix box application(xwinmr) on a local unix box you will do: 1. on local unix box, xhost + remote host (to allow remote control). 2. while you remote log in to the other unix box, do: setenv DISPLAY localcomputer:0.0 (fail to do so may have graphics showing on the remote unix host, even though another user is actively logged-in; the other user can quite the graphics, though). 3. launch the application on the remote unix host, then its application graphics should display locally.

Case 2, for graphics display from a remote unix application (xwinmr) on a local PC you can use secure shell client software and exceed (for X-window). Both of them can be downloaded free from Purdue web site (as the university has licenses for them). 1. launch secure shell client from the pc. Connect to the remote computer (you need the computer name or ip address; login and password). 2. launch exceed 3. in the secure shell, type who to find out your computer's name (such as challenger as a partial name or dhcp180-176.ipph.purdue.edu as the full name); type setenv DISPLAY local\_PC\_name:0.0 and return. 3. in the secure shell, launch the graphical application. Remote application should now have a local (PC) display.

Certain applications will not work. e.g. jot will only display properly on a sgi computer. Instead, you should use other more general editors such as nedit, xedit or even vi. There are also times that two computers' color display does not match and graphics may no display. In color mismatch case, either the remote computer's color display or local PC's changed. To change the local PC's color display, for windows xp, you can go to start -> application (secure shell client) -> right hand mouse click to property -> select compatibility tab -> check or select 256 colors. (for windows 2000, you may change to true color 32bit or 256 color for display /settings). Repeat procedures in previous paragraph, and hopefully the remote display will come out nicely.

Exceed here is to allow xwindow display (communicate between remote and local host on graphics). If it is not running, or you do not have other ways of graphics communication, you will see some msg like: "NEdit: Can't open display" in the shell when you try to use nedit. In such a case, you should type "setenv DISPLAY localpcIP:0.0" (or a command similar to this) to set local display.

Case 3, for graphics display from a remote unix application (xwinmr) on a local PC, and you do not have exceed. As of 8/31/2006, Purdue no longer has a valid license for exceed package (many existing ones, if not paid separately for, including mine, have expired) The alternative is xcygwin, which can be downloaded from <http://x.cygwin.com/>. (you need download and install both X11 and utils package). cygen is a GNU project, thus I expect it to be freely available and should work for most PCs in the foreseeable future. The following is how I get it to work (I have a windows XP and windows 2000 to display a remote redhat 9 linux box or sgi).

1. launch cywin
2. in the new xwindow (linux looking; bash shell), type startx, or /usr/X11R6/bin/startx
3. Still another new xwin will pop up. In this xwin, type xhost + atlantis.pinmrf.purdue.edu (the remote host you plan to run graphics on; or you can use aliases defined in the /etc/hosts files); then ssh to atlantis (either ssh username@atlantis, or ssh atlantis -l username)
4. once you log into atlantis, type "setenv DISPLAY dhcp180-176.ipph.purdue.edu:0.0"
5. nedit, nmrsim, or nmrDraw will display locally. xwinmr typically will not, due to some graphical mis-matches (see next to find out how to take care of that)
6. xwinmr(3.1 or 3.5) may complain about setting display depth to 8 planes. For the two PCs I have, I was able to set windows display settings to 256 colors (8 bits, I suppose) on one computer, and thus display xwinmr properly. For the other PC (windows xp), I

did not have such a choice to set to 256 colors from windows/display/settings (maybe because it's a new LCD monitor and does not even bother to give me the choice of displaying obsolete 256 colors). However, on my desktop or on the Start menu, I can right-click the program(Cygwin) and then click Properties, then I click the Compatibility tab, and select the Run in 256 colors check box. In the end, I can have xwinmr to display on my desktops again.

Case 4, graphics display from a remote unix application on a MAC.  
Since I do not have a mac, I cannot elaborate details. But I would expect that a similar procedure described in case 2 would work.

## **Xwinmr processing / Xwinplot printing related**

### **1. I cannot start xwinmr when I type in the command**

The likely reasons include 1. somebody else may have left his/her xwinmr still running and logged out (left the xwinmr running in the background). In this case, you can type `uxproc` or `ps -ef` to figure out the offending processes and kill them. 2. a license conflict (may result from a wrong system time). In such a case, you type `date` to see if the time is right. You need to become a superuser to set the computer time. Several times that I have found atlantis somehow got a wrong time (now it's year 2006 while it may have shown as 2000). I suppose it may get that from forced reboot. To take care of the time problem once and for all, I have a set up a `ntpd` on atlantis so the time will not be wrong again.

### **2. I cannot find my data in xwinmr**

The first time when you use xwinmr, you may find a blank screen, with the user as guest. If you have just removed the dataset that you quit xwinmr from last time, you will also see that screen. You need to find your dataset. This can be done by either "search" or browse command.

In addition, the bruker data structure is `/u/data/username/expermentname/exptnumber/`. The `fid` or `ser` file etc. should be directly under `exptnumber`. For example, you should find `/u/data/mo/test/1/fid`. There are times that when people transfer their data by drag and drop, the whole directory is put into a wrong place, resulting in something like `fid` is found under `/u/data/mo/test/1/1/fid`. If that happens, you will not be able to see the data through xwinmr.

### **3. how do I print 1D plot**

We recommend xwinplot. If have any problem, use `edo` command within xwinmr, prior to xwinplot. Select the layout that you like.

### **4. when I print from xwinplot, I get a stack of text prints instead of my plot**

That's because the printer is not interpreting the post-script properly. Do the following

1. take out all paper supply from the printer
2. turn off the printer

3. make sure that there is not any pending printing job in the queue (you may ask a superuser to clean it up)
4. after a couple of minutes, turn the printer back on, and put back paper supply: make sure it does not print garbage text gain.
5. quit xwinplot (do not save any .xwp file) and restart it from xwinnmr. If you have saved .xwp file, you need xwinnmr/edo/layout->+/layoutname.xwp and then do xwinplot. the default layoutname.xwp should have everything set up for you.
6. print from winplot. The printer should be hp laserjet (not post-script).

## 5. how do I overlay two or more 1D spectra

In xwinplot, set up the first 1d (width, height and everything in the final form). Select the spectrum and click right hand mouse. Make two or more copies. Then click on data icon => edit => select dataset that you wish to overlay or compare, and append, and apply and okay  
Right mouse click on any spectrum to select. Click edit icon and apply the dataset to the spectrum. The spectrum will get updated. Repeat this to all spectra.

## 6. how to print a 2D spectrum with projections

First make sure that you have selected a proper layout. For 2d homonuclear data, you will select the layout like 2D\_homo.xwp, and for 2d heteronuclear data, you may wish to choose 2D\_inv.xwp.

Use xwp command within xwinnmr. 2d projections should be created (if not available through individual 1D's). To create projections, you can use the command rhpp (horizontal positive projection) and rvpp (vertical). Those data will be stored in process defined in edc2. if not defined, they will be in proc 98 and 99.

Within xwinplot, select the plot, and you can click on edit or 1d/2d edit icon. You can further manipulate the output appearance. If you wish, you force the projection to be taken from any processed 1d data: 1. select the 2D plot. 2. enter "edit" mode. 3. after the edit window appears, scroll down to the projection part, and check left and top projections. You can also change the size here. 4. click projection data set. You might be asked to select data. Go ahead and select/append 1D data sets for projections, and the current 2D dataset. 4. select the 2D dataset and apply. 5. now all datasets are in; go back to edit mode if necessary and change the projection to the right data set.

Alternatively, you can create projections through "edo" from the 2d dataset. In the pop-up window, you will select projection "yes" and then "edit projections" (they are all click icons). Then you will be asked for the 1d projection data set.

Note: projections can be built from the 2D itself, or from separate 1D's. Spectra will be aligned by chemical shifts.

## 7. how to pick peaks

Define (pscal)region as global (or any region of interest) through edp command first, or you can click on dp1 icon which will then prompt you chemical shift regions. Then you Click on utilities icon(if unavailable, use setres to change the xwinnmr to extended mode). Change 1D plot vertical axis unit to cm (YU icon). Normalize some medium sized peak to 1cm through CU icon.

Click on MI to define the lower threshold of peak picking. Click on MAXI icon to define the max peak intensity (peaks higher than that will not be picked).

Command pps should show you the list of peaks.

## **8. my xwinplot does not have peaks or integrals**

First make sure that peaks are picked and integrals are defined and integrated. Refer to above procedure for peak picking (xwp\_pp and xwp\_lp before xwinplot may help). For integration, abs command does automatic baseline correction and integration. Alternatively you can manually integrate peaks through xwinnmr analysis / manual integration (integ command). Re-launch xwinnmr. If peaks or integrals still do not show up, select the plot. Click on 1d/2d edit icon. Check "show peaks" and/ or "show integrals".

## **9. how to change peak picking bar / integration line appearance in xwinplot**

Select the plot. Click the edit icon. Scroll down and check or uncheck "show peak marks" or "show integrals". There you can also easily change other attributes of the peaks and integrals.

Further, click the 1d/2d edit icon. On the top for the "Scope", uncheck the spectrum and check mark "integral". Use \*8 \*2 /8 /2 other icons to change the size / vertical position of the integration.

## **10. how to make xwinplot output for presentation or publication**

From the xwinplot option menu, select printer set up. In the printer type box, you can select postscript, or scroll toward the end select any of the graphics type there (e.g. tiff or png or jpeg).

Then go to file => print => to file, and give a file name, with an extension that suggest the output file type. Remember the path of the saved file. You need that when you transfer it to your desktop pc.

Refer to "back up individual spectrometer data" section for file transfer.

## **11. how do I compare two 1d spectra (without printing)**

Use "dual" command. If second data set is not found, xwinnmr will ask you to define it. Or you can use edc2 to define the second data set. The third data set is used for saving any manipulated data (sum or different etc. see below)

## **12. how do I add or subtract two data sets**

It is highly recommended that you do not acquire two sets of data and save FIDs together in one file. Instead, you should save them as two different FIDs. Because, if either FID is bad, or there is slight frequency shift, the FID sum will not be good.

You can process two fids separately, sum the resulting spectra up and save in a new file. To do that, you will do edc2. Define the third data set by edc2 command (second data set is used for comparison by "dual"). Then do "dual" and sum or difference the two data. "return" will prompt you if wish to save changed data in the third data set (defined by edc2).

Please note, manipulation on spectra has no impact on raw FID data.

### **13. how to calculate/calibrate a shaped pulse**

Type `stdisp` within `xwinnmr` to launch shape tool. Define the shape under "Shapes", or open a shape file from "File" menu. Click Analyze => integrate shape. Fill in the values for shaped pulse length and rotation angle, and hard 90 degree pulse length. Click "OK" (NOT "Apply!"). The change of power level (in dB) from hard pulse will be returned.

Calibration of shaped pulse can be further verified or adjusted by experimentally. But the calculated values should be close enough for normal application.

### **14. how to run a shaped pulse simulation**

From above, click Analyze => simulate. Fill in the pulse length and total rotation (you do not need power level), and "OK" (not "Apply!"). Excitation profile of this shaped pulse will show in a new window. Click on x, y or other icons to change the property. It is assumed that the initial magnization is Z. Alternatively you can change the initial state and other simulation parameters through "Calculate" => "Time evolution" or "Excitation Profile".

### **15. how to calculate/simulate a adiabatic pulse**

In shape tool (launched by `stdisp`), create the adiabatic shaped pulse through "Shapes" menu or open an existing adiabatic pulse file.

For example, to create a `wurst` pulse, click "Shapes" => "`wurst`". You will be asked to supply shape parameters, important ones of which are total sweep-width (40000 Hz), length of pulse (which is smaller than  $1/J$ . use 1500us), and amplitude power index(20). Then "Ok". Results page will pop up and you click "seen". Then click "Analyze" => "integrate adiabatic shape". You will input length of 1500us and length of hard 90 pulse (e.g. 12us). Click "OK" and results page will pop up again. Import number is "change of power from hard pulse in db: 13". So you can set this adiabatic pulse power level as 13.

Since the calculation assumed adiabaticity of 5. you can safely decrease the power level by several dB's without significantly affecting the effective decoupling width.

Click "Analyze" and "OK" pulse length of 1500us and total rotation of 180, the excitation profile will appear in the new "block module".

Again you can always change the initial states for the simulation or switch to observe other component (x or y).

# Appendix

## Arx300 SCM reset

The SCM reset (red) button is located in the lower left inside the spectrometer console. The following is taken from Bruker's web site

1a) If you look at the top SCM INTERFACE board, the 2 white slide switches, should be in the down position and the green connector with the 2 white wires should be connected to J8 (located below the 2 white slide switches and to the left of the SCM KEYBOARD Connector). On the front of the scm chassis, below the red reset button (SCM RESET) you will see a blue slide switch (SCM INIT ON/OFF). You will need to slide this switch (using a jeweler's screwdriver) up to the SCM ON position.

2a) Next push and hold the red SCM RESET button above the blue slide switch for 5 seconds and then let go.

3a) Now slide the blue SCM INIT switch (mentioned in step #1a) to the OFF position as you found in step #1a.

NOTE: By doing either of these reset procedures, your shims and field values, will be zeroed out and your sweep amplitude and rate, lock phase, power and gain will go to default values. So you will need to read in a shim file and move the field value to properly look for a lock signal and adjust your lock phase, power and gain, sweep amplitude and rate accordingly to get your lock signal back to normal.

The above procedure is a "soft" reset. If you continue to have problems, a "hard" reset can be performed.

This procedure MUST BE FOLLOWED IN THIS ORDER or damage could occur to your scm boards.

### How To Reinitialize SCM - (HARD RESET)

1) Remove burndy connector SCM1 (this connector is located on lower left corner of the top board called the scm interface).

2) Remove burndy connector SCM2 (this connector is located on the left hand corner of the SCM motherboard). If you have the rack style ARX/AMX2/ASX, this connector is located in the back of the scm chassis. You will have to take rear panel off of console and you will see it in back of this SCM chassis.

3) You will need to remove the 4 ribbon cables that are located on the front of the scm boards, that tie 2 boards together. You will also have to remove the ribbon cable on the upper left corner of the scm interface (top board) which goes to the SCM motherboard and the scm keyboard cable, which is located on the top board to the

right of the 2 white slide switches to reset scm. NOTE: If you have a rack style ARX/AMX2/ASX console, you will not need to remove these 2 cables only! The 4 front ribbon cables and SCM1 and SCM2 burndy connectors only will need to be removed.

4) You will to pull out the top scm board ( called the SCM Interface ) and the bottom board ( called the SCM CPU ) slightly a couple of inches.

5) You then need to push the SCM CPU board, then the SCM Interface boards back into the motherboard.

6) Reconnect the 4 ribbon cables on the front of the 2 scm boards, then the scm keyboard cable, then the scm motherboard to scm interface cable.

7) Reconnect burndy connector SCM2 first, the reconnect SCM1 burndy connector 2nd. NOTE: This step is very critical or could damage scm boards!

8) Now that all burndy and ribbon cables are connected, you follow the same procedure, mentioned above, for the "Soft" Reset. Now your error and buzzing noise should be gone.

### **QNP probe**

The QNP switch light on the HPPR box should be in "off" state for automatic switch. After a power down, the light may become on and needs to be recycled to off.