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AVIII-600 Training Manual

09/17/2012 S.D.C.

Location: **1232 Hach Hall**
Contact: **Shu Xu or Sarah Cady, 1234 Hach Hall**

Safety

All researchers working in 1232 Hach Hall must complete the EH&S Course "*Fire Safety and Extinguisher Training."* Please do not prepare samples directly in 1232 Hach Hall. Aprons, safety glasses, and rubber gloves are available in 1238A Hach Hall if you wish to conduct your sample preparation in CIF facilities. Researchers may wear lab coats and safety glasses in the 1232 Hach Hall, but please remove all gloves before handling NMR equipment or computers. Please do not bring any large ferromagnetic objects into the lab without permission. This includes certain chairs, bicycles, gas cylinders and tools.

Properly dispose of waste solvents and glass pipettes in the containers provided in 1238A. There is a broken glass container located in 1232 Hach in case of broken NMR tubes or other glassware. All of the computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

Some safety concerns specific to high-field cryogenic superconducting magnets include:

- Users should remove credit cards, cell phones, mp3 players, keys and other ferromagnetic objects from pockets before approaching a magnet.
- Users with pacemakers or joint replacements should have a staff member assist them with the insertion of samples into the magnet as to prevent serious harm or injury.

In case of magnet quench, the room will be filled with gaseous helium, which will be evident from a white cloud and an alarm sounding on the oxygen sensor. If you are in the room during a magnet quench, please exit as soon as possible – crawling on the floor if need be to reduce helium inhalation. If you are outside the room during a magnet quench, do not enter the room without proper breathing apparatus until the oxygen sensor alarm has stopped sounding.

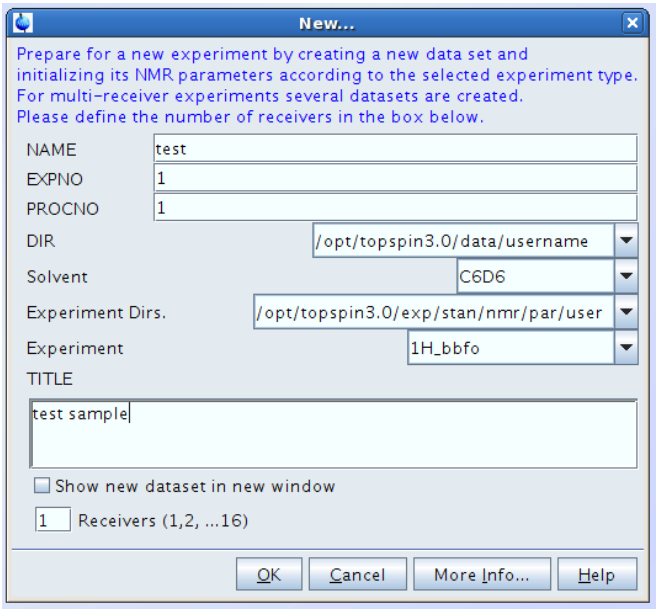
Introduction

You must receive training before using this piece of equipment. The Bruker AVIII-600 features a narrow bore 14.1 tesla/600 MHz magnet equipped with two probes: a Normal geometry $^{2}H/^{1}H$/BB BBFO SmartProbe capable of tuning to nuclei of ^{109}Ag-^{19}F on the broadband channel, and an inverse geometry $^{2}H/^{1}H/^{13}C$/BB inverse probe with a dedicated 13C channel and ^{109}Ag - ^{19}F range on the broadband channel. Topspin 3.0 is used for data acquisition, and either the MNova software or Topspin 3.0 is used for data processing. This instrument is used for routine $^{1}H/^{13}C$ characterization experiments, routine X nucleus detection, variable temperature and kinetics experiments, 1D selective experiments (APT, DEPT, NOESY1D) and 2D and 3D experiments.

Overview

The AVIII-600 is controlled by a CentOS Linux PC communicating via two Ethernet ports – one to the instrument and one to the internet. The computer is part of a local area network in 1232 Hach Hall and is safeguarded behind a firewall. In order to minimize instrument time when users are waiting, the data acquired is immediately accessible on one of four data stations in 1232 Hach. The data is also accessible via the CIF Research Files Cloud Storage. When you log out, your data is instantly uploaded to the storage cloud. More information regarding Remote Data Access is available on the CIF website: http://dev.cif.iastate.edu/remote-data-access

1D Experiment Quick Setup



1. **edc<enter>** to create a new file.
    a. Choose the Experiment – 1H_bbfo or 13C_bbfo for basic 1D $^{1}H/^{13}C$ experiments.
2. **lock<enter>** and choose solvent from pop-up window.
3. **atma<enter>** to tune probe.
    a. Typically not required for ^{1}H experiment, but almost always required for any X experiment.
    b. Sometimes ^{31}P is difficult to tune automatically, and may require the use of the interactive tuning through **atmm.**
    c. **ONLY USE ATMA FOR "NORMAL" NUCLEI.** ^{1}H, ^{2}H, ^{13}C, ^{11}B, ^{15}N, ^{29}Si, ^{19}F, ^{17}O, ^{77}Se have all been calibrated through **atma.** Consult Sarah or Shu if you are trying to tune to a new or unusual nucleus.
4. **ro<enter>** controls the rotation speed and on/off, if needed.
5. **topshim<enter>** will start the autoshimming routine. When Topshim is finished, there will be a message in the bottom left-hand corner in the screen. Make sure the sample re-locks after shimming by checking the **lockdisp** screen.
6. Check parameters : **ns, td, o1p, sw** before starting acquisition (see explanation below)
7. **rga; zg<enter>** performs auto receiver gain and starts the experiment.
8. **tr<enter>** to transfer the data during acquisition
9. **ef<enter>** to Fourier transform the data with exponential line broadening
10. **apk<enter>** to automatically adjust the spectral phase (**ef; apk<enter>** in a string to do these actions simultaneously)
11. **halt<enter>** will stop the experiment
12. Data is automatically saved after an experiment ends, **tr<enter>** is typed or **halt<enter>** is typed. There's no additional saving step in Topspin.
13. After your experiment has finished, type **ej<enter>** to eject the sample, and type **ij<enter>** to turn off the eject air. Exit the software and log out.

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Detailed Experimental Setup

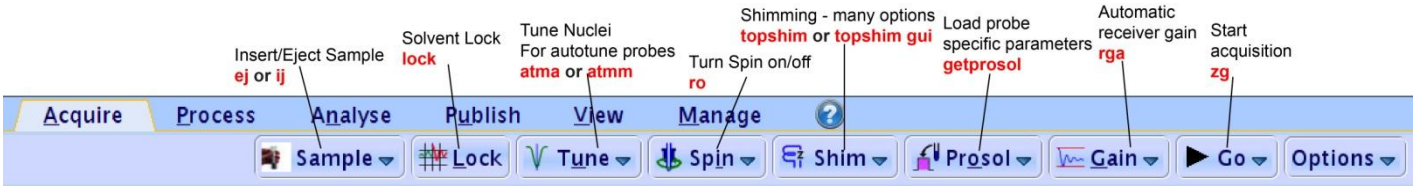
After logging in, start Topspin by double-clicking on the icon on the desktop.

The AVIII-600 does not have an external mechanism for sample ejection/insertion, so users must be logged into the computer with Topspin running in order to turn on the eject air.

1. Be sure the instrument is set up for the desired nucleus.
    a. For all nuclei except proton, the probe tune values must be the same as those written on the sheet taped to the front of the magnet.
2. Slide the sample into the spinner and adjust the depth using the depth gauge.
    a. Sample height must be 5 cm (0.7ml). This is required to make shimming faster and improve spectra.
3. Wipe both the sample and the spinner with a KimWipe.
4. Type **ej** into the command line to turn on the eject air. Type **ij** to lower the sample into the magnet.

Set up a 1D Experiment

1. In the Topspin 3 Flow Interface, the easiest way to run experiments is through the **Acquire** tab, shown below. From the **Acquire** tab you can insert/eject samples, lock, tune, turn spin on/off, shim, autogain and start the experiment. The image below shows what each button does in addition to showing the corresponding typed command in **red**.

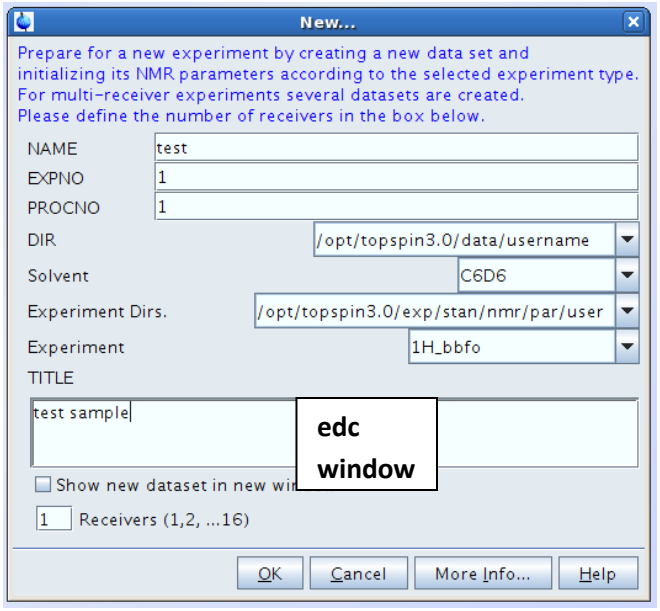


2. Create a new data file by typing **edc <enter>**
    a. Enter the desired **NAME** (typically the name of your sample or however you name your files) and **EXPNO** (1, 2, 3….) in the window that appears.
    b. Select your **SOLVENT**
    c. Make sure the **Experiment Dirs.** Is set to /opt/topspin3.0/exp/stan/nmr/par/user and select your **Experiment** from the drop down menu below. **1H_BBFO** is a standard ^{1}H 1D **13C_BBFO** is a standard ^{13}C 1D. **BBFO** indicates the type of probe. The **BBFO probe** will be used most often. The other probe is the **TBI probe** is the other probe, and does not feature autotune.
    d. Click **OK** to create the new experiment.

e. OPTIONAL: After the experiment has been created, you may type **ased <enter>** to modify the parameters. Number of scans may be modified by typing **ns <enter>** and enter the number and hit ok.

f. OPTIONAL: You may also modify parameters such as the center frequency: **o1p<enter>** and sweep width (window size): **sw<enter>**

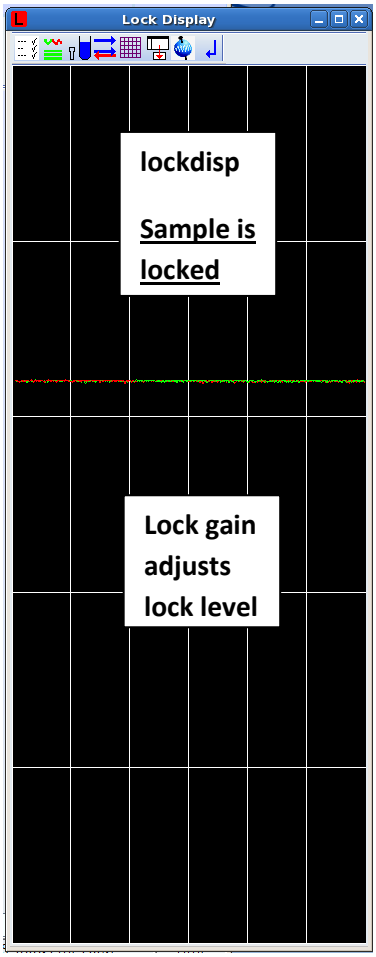
g. OPTIONAL: If you accidentally choose the wrong experiment, you can type **rpar<enter>** and choose a different experiment



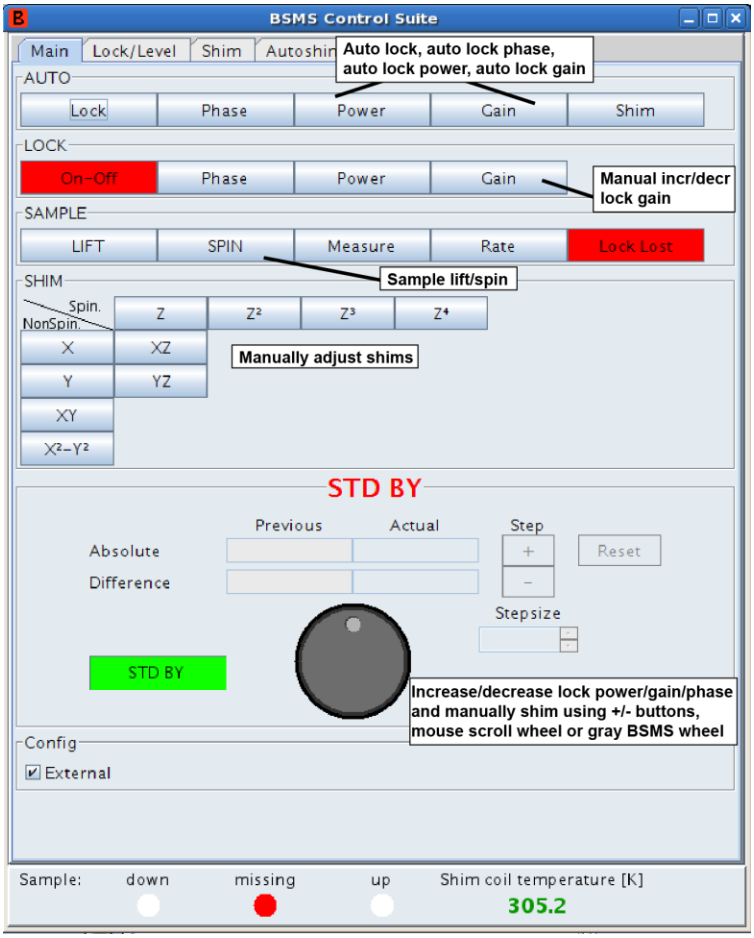
Locking

1. If you do not see a lock window type **lockdisp<enter>** in the command line or click the **lockdisp** button:  . The lock signal is a green/red line moving across the lock display.
2. Type **lock<enter>** or click the 

button in the **Acquire** tab and choose your solvent to lock. When the sample is unlocked, the red/green line will appear at the bottom of the **lockdisp** window as shown in the first window below. When the spectrometer is locking, an FID will appear when the locking finds the correct field setting, and then it will gradually increase to find the correct **LOCK GAIN** setting, as shown in the BSMS panel below.
3. Once it is locked, the red/green line should be stable in the upper half of the **lockdisp** window. You can adjust the position of the red/green line (the lock level, essentially) by increasing or decreasing **LOCK GAIN** and **LOCK POWER** on the BSMS window (type **bsmsdisp<enter>** or click  to see the window). Sometimes the **LOCK GAIN** is too high, and it can be adjusted with **AUTO GAIN** or by reducing **LOCK GAIN** so that the lock signal is approximately 2/3rds of the way up the lock display screen.



4. The **BSMS Display (bsmsdisp)** pictured below has several tabs. The **Main** tab contains most of the basic controls including **Auto Lock/Gain** (in the first row) and **Manual Lock On/Off and Lock Gain** (second row). The sample **Lift** and **Spin** can also be controlled through the **BSMS Display,** in addition to manual shimming of the lower order shims. All of the levels can be controlled using the **Step +/-** buttons, the **Gray Wheel,** or the **Scroll Wheel** on the mouse. The step size can be controlled to determine how fast the parameter is changed.



Tuning

1. Tuning is not generally necessary for ^{1}H experiments, but is generally necessary for all X experiments.
2. Make sure the correct experiment has been loaded before tuning the probe. This ensures the computer sees the correct channels once the tuning routine has been started.
3. Tuning is achieved through the "Tune" button 

or by typing **amta<enter>**
    a. **ONLY USE ATMA FOR "NORMAL" NUCLEI.** ^{1}H, ^{2}H, ^{13}C, ^{11}B, ^{15}N, ^{29}Si, ^{19}F, ^{17}O, ^{77}Se have all been calibrated through **atma.** Consult Sarah or Shu if you are trying to tune to a new or unusual nucleus.
4. The tuning routine will automatically end once all channels have been tuned. You do not need to re-tune the probe for subsequent experiments if the channels remain the same (all ^{1}H, ^{13}C, for example).

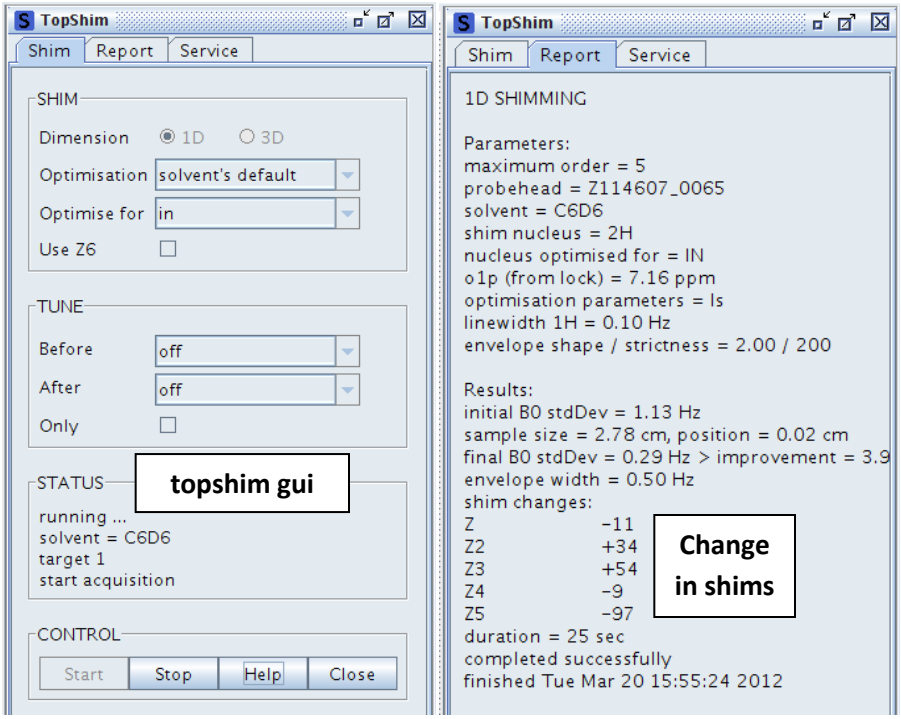
Shimming

1. The new Bruker systems feature an automatic gradient shimming routine called **Topshim. Topshim** has many different options to assist in shimming different NMR tubes and different temperature scenarios. After locking your sample as above, to access the basic **Topshim** shimming routine, either type **topshim<enter>** in the command line or press the 

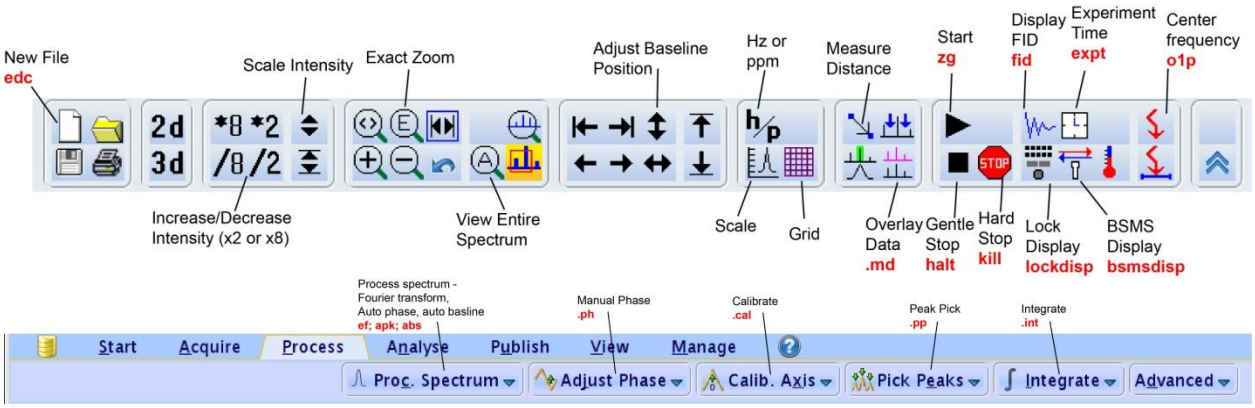
button in the acquire tab. The **Topshim** routine will start running, and the text **topshim: finished** will appear in the bottom left corner.

Topshim Options & Troubleshooting:

1. Typing **topshim gui<enter>** in the command line will pop up the **Topshim window** as pictured below. In this window, additional options are available and a shimming **Report** will be available after the shimming is complete (as shown in the second picture). The most common option to select here would be "**Use Z6**" which will shim **Z1-Z6**. (This may take a little longer.) The **Report** window will show how much the shims have changed for your particular sample. This is interesting in cases where your sample is difficult to shim – sometimes **Z4** or **Z5** will change by a large value and give strange lineshape. If this happens, type **rsh standard.shim<enter>** in the command line and start over.



2. To shim up to **Z8,** type **topshim ordmax=8<enter>** in the command line. Typically doing one initial **Topshim** (**Z1-Z5**) followed by a second **Topshim** up to **Z8** will give better and faster results.
    a. **IN GENERAL IT IS NOT RECOMMENDED TO SHIM UP TO Z8.** In general experience, the shimming for the higher order shims will change significantly and can affect your lineshape.
3. If you are using variable temperature and **NOT** rotating your sample, use convection compensation by typing **topshim convcomp<enter>**



Prosol 

1. During installation, a standard set of power levels and pulse lengths have been saved to a chip in the NMR probe. **Prosol** is a feature that allows the software to communicate with the probe to determine which probe is installed and the standard power/pulses for that specific probe.
    a. If you load a pulse sequence from the /user directory as shown above, we have already loaded the **prosol** parameters for those parameter sets. Clicking the **prosol** button will reload the standard parameters and may change any parameters you have already modified (**ns, o1p, sw,** etc.)
    b. If you load a pulse sequence from the /par directory, these are **standard Bruker parameter sets and do not contain any probe-specific parameters.** In this case**, you must click prosol before proceeding in order to load the probe parameters.** Otherwise, the power levels are all set to 0, and no signal will be generated.

Parameter Consideration

1. Before starting your experiment, consider a variety of parameters and make sure they are appropriately set for your particular experiment.
    a. **ns<enter>** number of scans.
    b. **td<enter>** number of acquired points. If the signal is being truncated (i.e. the FID is not done decaying), then **td** should be increased. Default **td = 64*1024 = 65536**
    c. **o1p<enter>** the center of the spectrum in ppm.
    d. **sw<enter>** the width of the spectrum in ppm. (Span of spectrum = **o1p ± ½*sw**)

Gain 

1. Gain is essentially the “volume” of the spectrometer receiver. If you have a very concentrated sample, the spectrometer needs less “volume” in order to “hear” the signal and vice versa for a low concentration sample.
2. Clicking **Gain** or typing **rga<enter>** will automatically set the receiver gain based on how much signal is in your specific sample.
    a. Gain can also be set manually by typing **rg <enter>** and entering a value in the pop-up box.
    b. Gain = 0 will result in no signal, Gain = 203 is the maximum receiver gain.

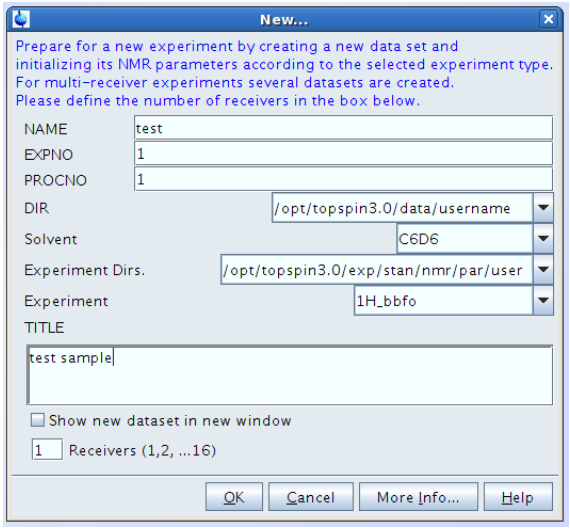
Go 

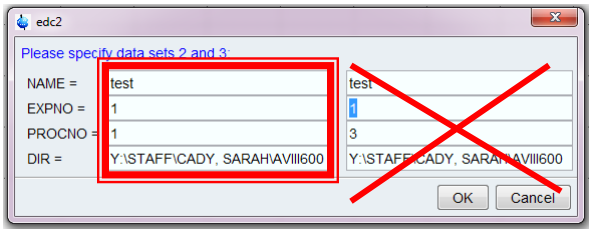
14. Before clicking **Go,** double check the parameters listed above: **ns, td, o1p, sw**
15. Type **expt<enter>** or hit the clock button:  if you want to calculate the length of the experiment.
16. To start the acquisition, **rga; zg<enter>** can be typed as one string to complete auto receiver gain and start the acquisition in one keystroke.
    a. To look at your data during acquisition type **tr<enter>** and then **ef; apk<enter>**
    b. If, after the experiment has finished, you decide you need more scans, the **go<enter>** command will add the number of scans in **ns** to the experiment. Typing **go** will cause the spectrometer to warn you that you're going to overwrite your data, but this does not actually happen.
    c. If you decide that you experiment has good enough signal-to-noise, typing **halt** will stop the experiment at that particular scan. You can also type **halt 64<enter>** and the expt will halt after 64 scans (or any number of scans that you choose).

Set up a 2D Experiment

1. Lock and shim your sample and run a 1D ^{1}H as you normally would. The 1D ^{1}H will be used to set the frequency range in the 2D experiment.
2. Enter the **Integration** 

menu in the **Process** tab.
    a. Select the region of the 1D ^{1}H that you'd like to include in the 2D acquisition.
    b. Note that if the region you select does not contain all peaks, peaks outside the integration region may "fold" back in and appear at weird places in the resultant spectrum.
3. Type **edc <enter>** to create the 2D file. If a 1D ^{1}H is in EXPNO 1, typically a 2D is in EXPNO 2 in the same folder. Click **OK.**
4. After the new 2D file has been created, type **edc2 <enter>**. Enter **NAME** and **EXPNO** of your ORIGINAL proton file under data set 2 (The one you just acquired). You don't need to change anything for data set 3. Click **Save**. (See second image below.)
5. At this point if you are running HSQC, HMBC, or some other heteronuclear experiment it is necessary to tune the probe using **atma<enter>**. If you ran **atma** during the 1D ^{1}H, it would not have tuned the X channel.
6. Type **xaua <enter>** to start the 2D acquisition. The command **xaua** is a macro that will stop sample rotation, check the 1D ^{1}H to detect the appropriate region, do rga and zg.
    a. If you have set up several 2D's in series, the command **multixaua<enter>** also works to run a series of experiments.





7. To look at the spectrum during acquisition, type **xfb <enter>**. The 2D spectrum can be viewed after at least 2 slices have finished acquiring. The **tr** command **<u>does not work</u>** during 2D acquisitions.

Variable Temperature Experiments

This section is meant to be a reference for those running variable temperature experiments. **You should be trained by Shu or Sarah before attempting variable temperature experiments independently.**

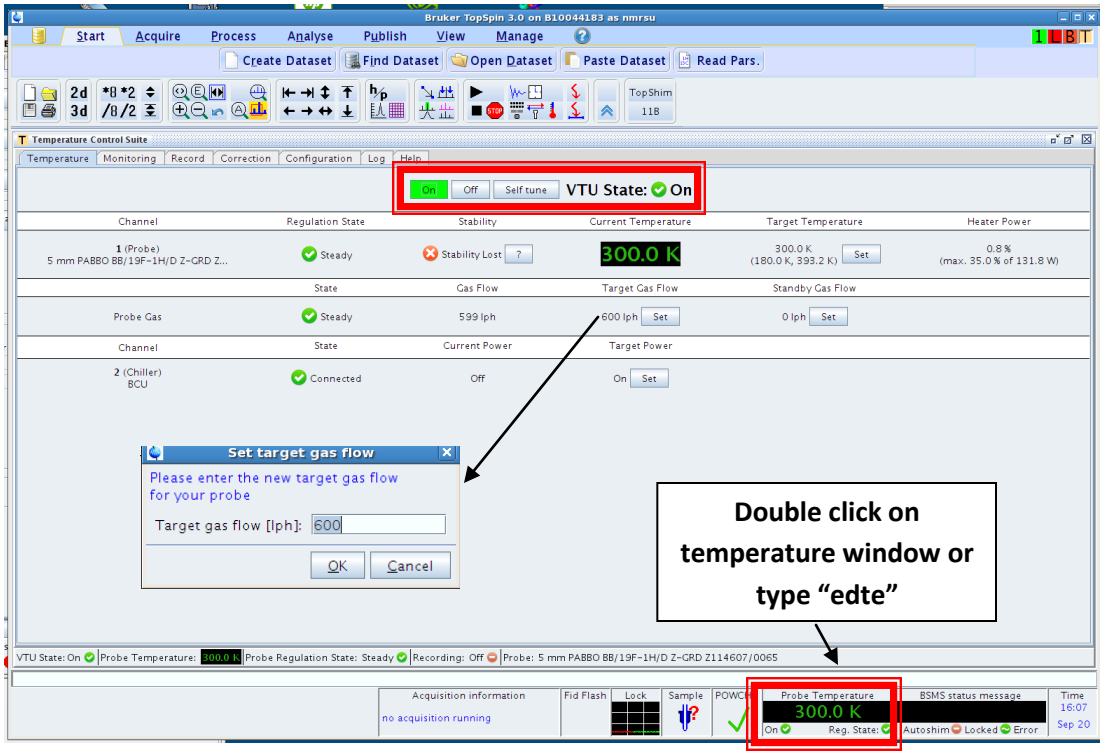
There are several initial considerations before starting a variable temperature experiment, depending on the desired temperature for the experiment:

1. The AVIII-600 probe temperature limits range from -150 °C to +150 °C. (Both probes.)
2. The desired temperature will determine which solvents are appropriate for your experiment. Consider this first before proceeding. Solvent freezing/boiling points are listed on the chemical shift calibrations tables at each spectrometer station.
    a. If your tube freezes inside the probe it could result in a broken tube.
    b. If your sample boils inside the probe it could result in solvent spilling. It will definitely result in bad spectra due to an instable lock signal.
3. The desired temperature also determines which spinner should be used.
    a. Ceramic spinner (white): -150 °C to **+150 °C**
        i. **The ceramic spinner must be HEATED before inserting the tube.**
    b. Kel-F spinner (opaque plastic): -150 °C to **+120 °C**
    c. POM spinner (blue plastic): -100 °C to **+80 °C**

4. The desired temperature determines whether you can use the chiller (**BCU-I**) or the **liquid nitrogen exchanger.**
    a. **BCU-I** can reach temperatures down to approximately +5 °C. The chiller is also on when heating the sample to compensate for fluctuations in the heater power.
    b. The **liquid nitrogen exchanger** is required for temperatures lower than +5 °C

Variable Temperature Controls and Chiller

1. The **Temperature Control Suite** window (shown below) can be accessed by double-clicking on the temperature at the bottom right-hand corner, or by typing **edte<enter>**



2. Once inside the Temperature Control Suite, users can set the Target Temperature and Target Gas Flow.
3. Make sure the **VTU State** is set to **On** as shown at the top of the **Temperature Control Suite** window. You will also see a little green check mark: ✅ in the **Probe Temperature** display on the bottom right corner of the screen.
   a. In general the **VTU State** should always be set to **On** to ensure there is air flow through the probe.
4. Click **Set** under Target Temperature after determining the desired temperature. The Current Temperature will appear **Green** when it has stabilized at the Target Temperature. .
   a. If the Current Temperature is lower than the Target Temperature, the temperature display will appear in **Blue**: Current Temperature 299.3 K
   b. If the Current Temperature is higher than the Target Temperature, the temperature display will appear in **Red**: Current Temperature 299.7 K
5. The Target Gas Flow should remain at **600 lph** or higher. The **maximum** recommended gas flow is **~1000 lph**. Click **Set** under **Target Gas Flow** to change flow to the desired level.
   a. If the Target Gas Flow is set to 0, there will be no VT air, and heavier J-Young tubes or heavier Kel-F/Ceramic spinners may not eject properly.
   b. If the Target Gas Flow is set much higher than 1000 lph, the spinner may float above the probe, and the sample won't lock/shim properly.

6. The BCU-I is located next to the magnet, adjacent to the console. The heater is located **inside the probe. <u>The BCU-I supplies chilled air that flows over the heater, and the heater switches on and off to regulate temperature.</u>**

7. If you are heating or cooling using the BCU-I, change the BCU-I status switch to **Remote**. The Switch should be in the **Flush** position when not heating or cooling.
    a. If you are using the **liquid nitrogen exchanger,** the status switch should also be set to **Remote.**
8. Once the BCU-I is set to **Remote** and the temperature is set in the **Temperature Control Suite,** the software will automatically set the temperature.
9. Once the desired temperature has been reached, wait 1-2 minutes for the temperature to equilibrate in the sample before proceeding with **Topshim** and **Acquisition.**
    a. It is advised that you have **Spinning** on, set to at least 20 Hz when using the variable temperature controls. This ensures the solvent is "mixed" by the spinning and no temperature gradient exists across the sample.
    b. It is also advised that you wait until the temperature is equilibrated until running **Topshim.** Shimming can change significantly depending on the sample temperature (and having VT air can also affect the state of the sample coil and shim coil).
    c. If you choose not to spin, use the command **topshim convcomp<enter>** to turn on convection compensation. This can compensate for a temperature gradient across the sample that can arise when not spinning.
10. When you are finished with the experiment, return the set temperature in the **Temperature Control Suite** to 298 K, and return the switch on the BCU-I to **Flush. <u>Do NOT turn the VTU State to OFF.</u>** Eject the sample and log out.

Liquid Nitrogen Exchanger

The **liquid nitrogen exchanger** is used for experiments where the desired temperature is lower than ~0 °C. **EXTREME CARE** must be exercised when using the **liquid nitrogen exchanger** for several reasons:

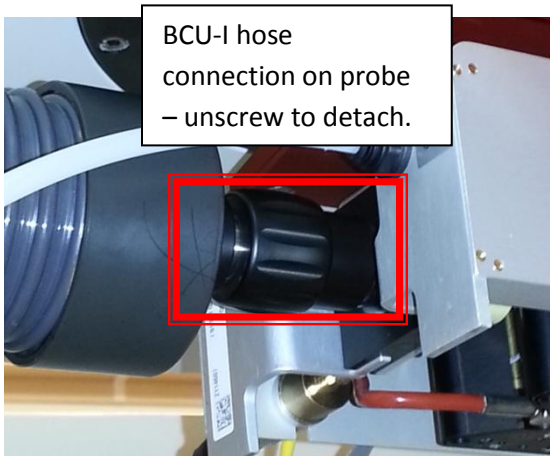
1. Running very cold air through the probe can result in condensation on the shim coil, which can result in **MAGNET QUENCH.** This is probably the worst possible thing you can do, so make sure you fully understand the **liquid nitrogen exchanger** before proceeding.
2. Running very cold air through the probe can result in condensation on the probe, which can result in **DAMAGE TO THE PROBE AND ARCING.** Be sure to flush dry, room temperature air through the probe after a low temperature experiment.
3. Occasionally, for long experiments, the hose can **FREEZE TO THE PROBE.** If this happens, be sure to follow the instructions to free the hose from the probe at the end of this section.

Attaching the Liquid Nitrogen Exchanger

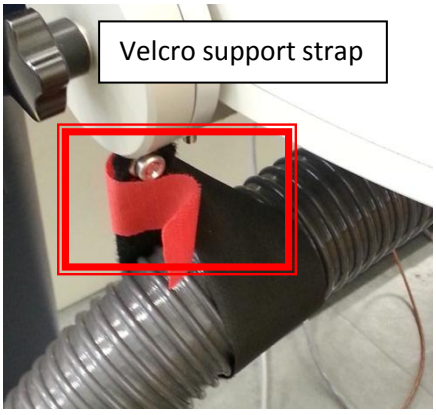
LN2 exchanger coil

1. The **liquid nitrogen exchanger** works by blowing air from the **BCU-I unit** through a coil which is submerged in liquid nitrogen. The air is pre-cooled by the **BCU-I** and then further cooled by exchanging heat with the liquid nitrogen bath before being blown into the probe.
2. Take the **liquid nitrogen exchanger** from its spot on top of the console, and slowly lower it into a full liquid nitrogen dewar. There may be some splash, so watch your feet!
3. Before disconnecting the **BCU-I** hose from the probe, be sure to switch the **VTU State** to **OFF** as described above. **Switching the VTU state to OFF at the computer ensures the probe heater does not stay on for an extended period to compensate for lack of VT air flow.**
4. Detach the **BCU-I hose** from the probe by unscrewing the hose from the VT port. The connection is shown at the photo below on the left.
5. Once the hose connection has been freed, detach the Velcro support strap shown below on the right and remove the hose from the side of the magnet.

BCU-I hose connection on probe – unscrew to detach.



Velcro support strap

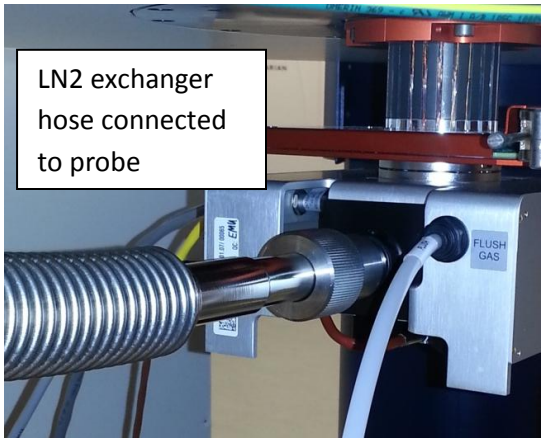


6. The screw-type probe connection of the BCU-I hose (shown at left) must use an adapter in order to connect to the **liquid nitrogen exchanger.** The adapter is shown to the right. The adapted hose connects to the exchanger with a clip.

Normal probe connection

LN2 exchanger adapter

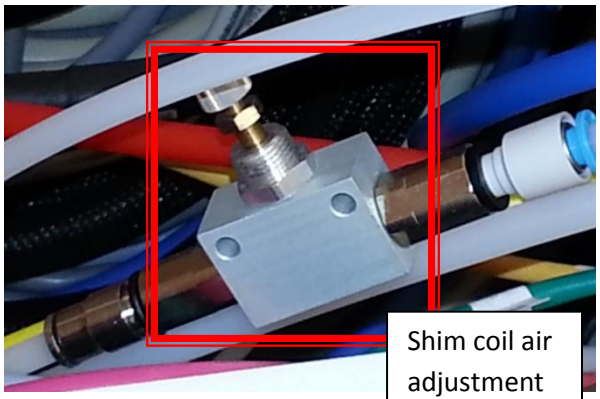
7. Once the BCU-I hose has been connected to the exchanger with the clip, make sure the hose is positioned such that the connector piece doesn't get crimped from the weight of the hose. Sometimes this involves wedging the BCU-I hose in between the dewar and the spectrometer console.



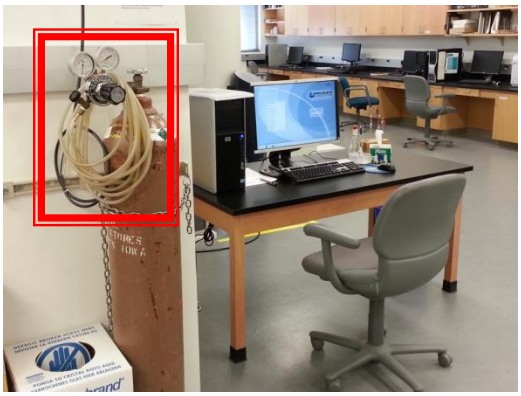
8. Attach the **liquid nitrogen exchanger** hose to the probe as shown in the photo. It connects in the same manner as the BCU-I hose. Also re-attach the **Velcro support strap** as for the BCU-I hose (shown on previous page).

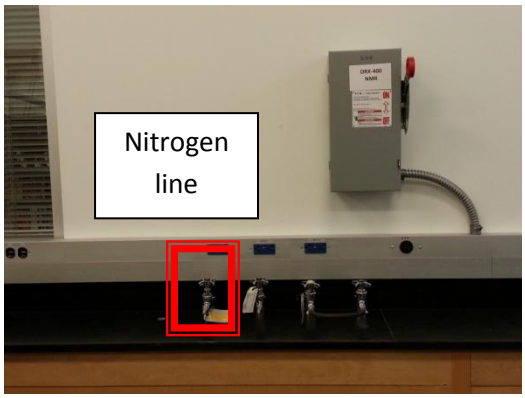
9. Switch the BCU-I on to **Remote** mode (step 7 in the previous section). Back at the computer, switch the **VTU State** back to **ON** ✅, and set the desired temperature and air flow. Higher air flow will be required for low temperatures (~1000 lph) and lower air flow will be required for temperatures close to 0 °C (~200 lph).

10. If you are doing very low temperature experiments (-50 °C and below), the computer may warn you that the shim coil temperature is too low, and that you need to make sure the **Flush Gas** and the **Shim Coil Air** is flowing.
    a. The **Flush Gas** should be flowing by default, and does not require adjustment.
    b. The **Shim Coil Air** can be increased by adjusting the knob on the small metal box just off the back of the console near the BCU-I box**. <u>There is no gauge on this valve, but for very low temperature experiments I just make sure to adjust the air flow until I can discern an audible hissing coming from inside the magnet.</u>**



11. Once you are done with the low temperature experiment, turn the **VTU State** back to **OFF** before disconnecting the **liquid nitrogen exchanger,** and disconnect the **BCU-I hose** from the **liquid nitrogen exchanger.** This ensures two things:
    a. No more cold air is flowing into the probe.
    b. The heater inside the probe will not stay on for an extended period of time to compensate for lack of airflow.
12. Disconnect the **liquid nitrogen exchanger hose** from the probe. Sometimes the connection is frozen on to the probe. If this is the case, get Sarah or Shu for assistance or proceed as follows:
    a. There is a length of Tygon tubing attached to a gas cylinder next to the spectrometer computer. Disconnect the tubing from the tank and take it over to the available nitrogen line on the far side of the AVIII600, below the circuit breaker for the DRX400.





    b. Attach the tubing to the nitrogen line and turn the nitrogen gas on. Blow the nitrogen gas on to the metal connection of the **liquid nitrogen exchanger** right where it connects to the probe. Continue streaming nitrogen gas on to the connection until it thaws sufficiently, and you are able to unscrew the connection. Continue blowing nitrogen gas over the base of the probe for a minute or two to evaporate some condensation.
    c. Turn the nitrogen off, disconnect the tubing, and return the tubing to the gas cylinder by the spectrometer computer.

13. Once the **liquid nitrogen exchanger** has been freed from the probe, remove the **liquid nitrogen exchanger** from the dewar, and place it back in its storage spot draped over the console. (The coil usually rests on the back of the console to ensure no one accidentally touches it while it is still cold.)
14. Reconnect the BCU-I air hose to the probe, and turn the switch on the BCU-I unit to **Flush.**
15. Back at the computer switch the **VTU State** back to **ON** ✅, and change the set temperature to **298 K.**
16. In summary:
    a. Liquid nitrogen exchanger has been removed from dewar.
    b. BCU-I hose has been reconnected to probe.
    c. BCU-I unit has been changed to **Flush.**
    d. At the spectrometer computer, the **VTU State** is switched back to **ON** ✅, and the set temperature is **298 K.**
17. Eject your sample and log out.

Data Processing

After data acquisition has finished, Topspin is an excellent tool for data processing, and can be more powerful than MNova in certain situations, especially for 2D, kinetics and DOSY data. Some images below are from University of Ottawa NMR blog: http://u-of-o-nmr-facility.blogspot.com/

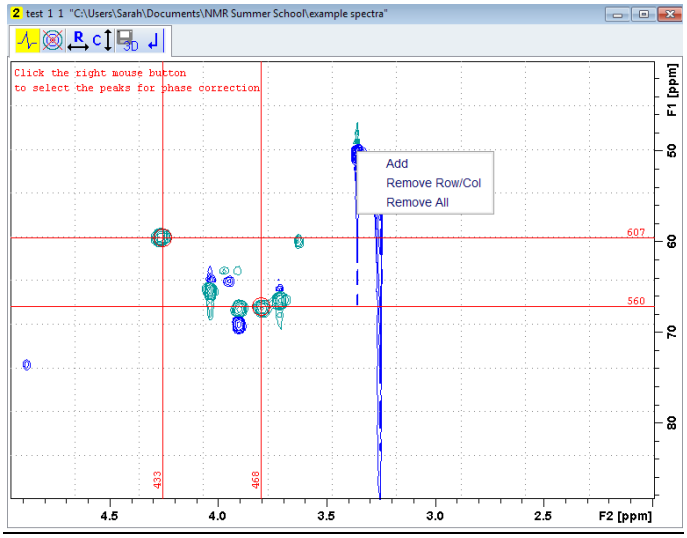
1. Fourier transform – In order to take the data from time domain to frequency domain, we must first Fourier transform the data. In Topspin this is accomplished with the command **ef<enter>.**

2. Phasing – When data is acquired, the resultant phase of the signal is arbitrary relative to the receiver phase. Thus, a phase correction must be applied to the data in order to get the signal to be flat along the baseline. In Topspin, the command for automatic phase is **apk<enter>.** This typically works well for most ^{1}H spectra. To enter the manual phase correction dialog, type **.ph<enter>** or click on the 

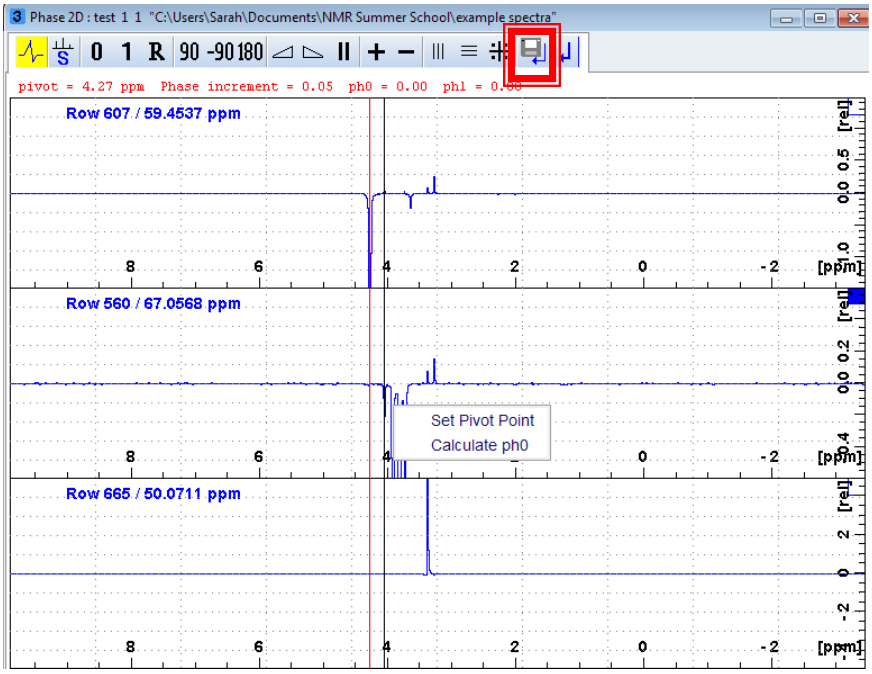
button in the **Process** tab. Click and drag the "0" button in order to adjust the phase of the tallest peak. After that is phased, click and drag the "1" to adjust the phase of peaks far from the tallest peak (selected automatically by Topspin). You can also right click on the spectrum and click "Set Pivot Point" in order to make a different peak the "center" peak. Click the save and return button (in red square below) to save the change in phase.

3. Phasing 2D Data – When phasing a 2D, the process is slightly more complicated. Instead of just phasing one spectrum, the 2D phase dialog requires that you phase rows and columns. Enter the phase correction dialog by typing **.ph<enter>** or click on the 

button in the **Process** tab. Right click on 3-4 picks spread out throughout the spectrum to select them for phasing. In the example HSQC below, the experiment is phase sensitive which means CH_3 and CH peaks are phased opposite from CH_2 peaks (C peaks will not appear). The teal color is negative phase, the bright blue is positive. These are default Topspin colors and can be changed in the preferences.

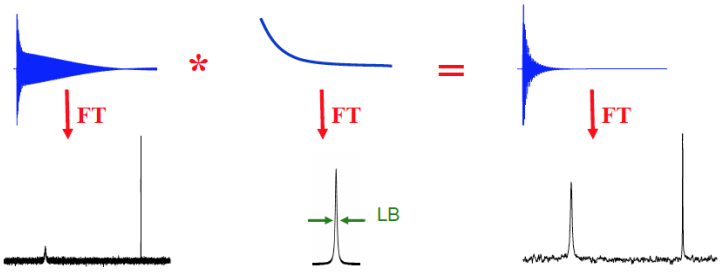


Once the peaks have been selected click either the row or column button:  . Once inside the row or column phase window, you will see 1D "slices" of the peaks that you selected in the previous window. Phase these slices as you see fit. You can also change the pivot point by right clicking on the spectrum, just as for 1D phasing. When finished, click the save and return button just as for 1D phasing. This will return you to the peak selection screen above.

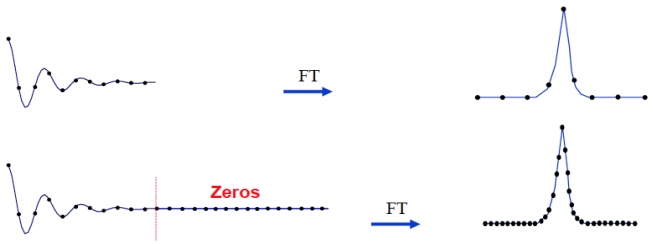


After you've phase the rows, proceed to phase the columns using the same procedure. Hit the return button in the peak selection window  after you've finished phasing rows and columns.

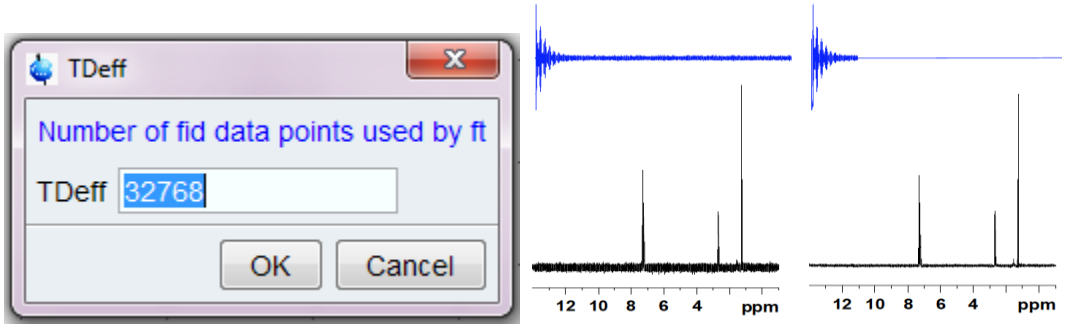
4. <u>Line broadening</u> – Line broadening assists in improving the signal to noise of a spectrum. When we perform the command **ef,** the "e" stands for exponential and the "f" stands for Fourier transform. So we are doing a FT with exponential line broadening. The amount of line broadening you apply to your spectrum is controlled by the command **lb<enter>.** For a typical ^{1}H, less than 0.5 Hz is necessary, and for a typical ^{13}C, 1-3 Hz is a good value. If too much line broadening is applied, obviously the lines will become too broad and unresolved. To observe the spectrum without <u>any</u> line broadening, use the command **ft** (just a Fourier transform) or **fp** (Fourier transform plus phase).



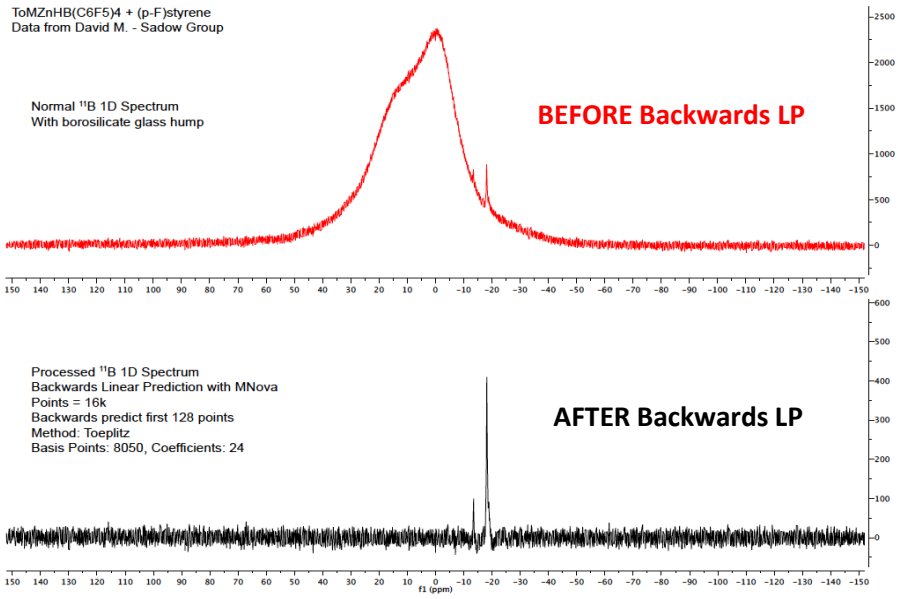
5. Zero filling – This technique enhances the digital resolution of your spectrum by adding zeroes to the end of the FID, as shown below. In Topspin, the command for zero filling is **si<enter>.** In general, **si=2*td** will give good digital resolution.



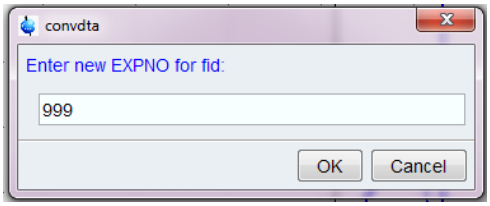
6. Reducing acquisition time in processing – If the acquisition time is much longer than the time it takes for the signal to fully decay, then the spectrometer is just acquiring noise for much longer than necessary. In Topspin, the way to alter this is through the command **tdeff<enter>.** For example, if in the spectrum below the **td = 65536 (64*1024)**, and we want **td = 32678,** then we would use the command **tdeff<enter>** and put the number **32678** in the dialog box that opens. Then **efp<enter>** to process only the number of points entered in the **tdeff** dialog box.

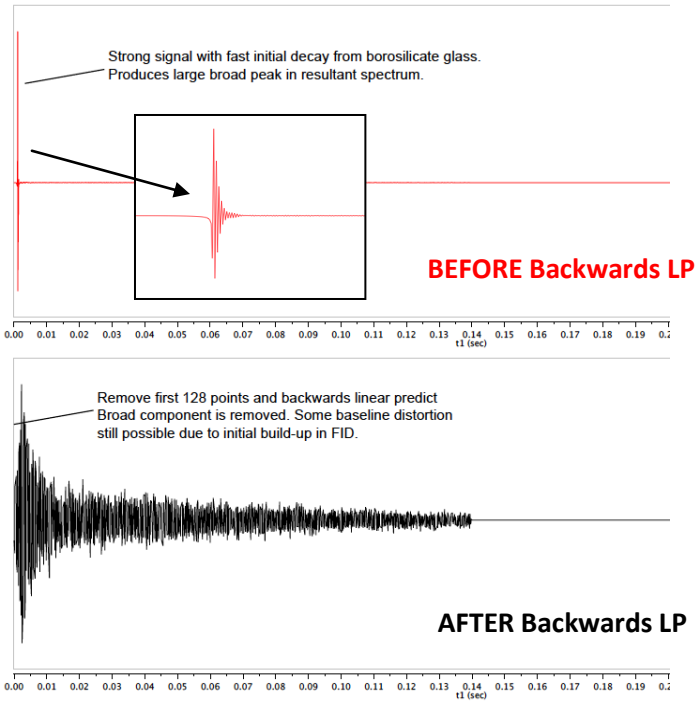


7. Backwards linear prediction – For individuals that collect ^{11}B data, the appearance of a glass peak from the NMR tube can be an unwanted addition to spectra. This broad peak is a result of the very fast initial decay from the solid glass. This peak can be "deleted" through processing techniques in Topspin (and MNova) using backward linear prediction.



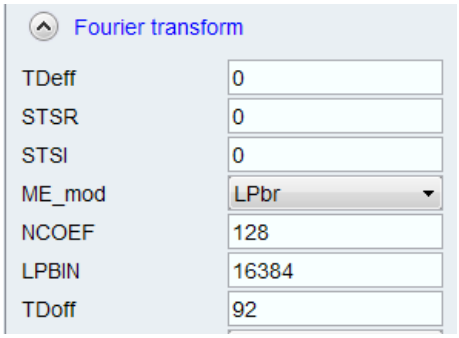
a. After acquiring an ^{11}B spectrum and processing it using **ef**, type the command **convdta<enter>** to create a new data set where the backwards linear prediction will be performed. The **convdta** dialog will ask for an expt # and I usually type "999".





b. Once the new data set has been created, type **edp<enter>** or click the **Proc Pars** tab to access the processing parameters. Scroll down to the **Fourier Transform** section, where a few parameters must be modified.

c. **ME_mod** should be LPbr (Linear predict backwards real).
NCOEFF should be 128 or more.
LPBIN is the number of predicted points, typically 16k (16*1024) is a good value.
TDoff is the number of points that are "removed" and backwards linear predicted by the software. This number needs the most adjusting experimentally. If too few points are removed, the glass peak will remain. If too many points are removed, there will be significant aberrations in the baseline.



d. After all of the parameters have been set, click back on the **Spectrum** tab, and type **ef** to process the backwards linear predicted data. It should result in a spectrum with a fairly clean baseline. If the baseline is not very flat or has aberrations, adjust TDoff until you find an appropriate value.

Bruker AVIII600 Experiment Guide

Common Topspin Commands

absn – auto baseline correction
apk – auto phase correction
ased – all experimental parameters (pulse lengths, number of scans, delays, etc.)
atma – auto tune and match
atmm – manual tune and match with interface (use for ^{31}P)
aq – acquisition time
bsmsdisp – display window with lift/lock/shim tools
.cal – interactive chemical shift calibration window
convdta – create data set for backwards linear prediction
d1 – recycle delay, time in between scans
ds – dummy scans before acquisition, steady state magnetization is reached
edc – create new experiment from a parameter set
ef – fourier transform and apply exponential broadening, controlled by **lb**
efp – fourier transform, exponential broadening and phase (either phased with **apk** or **.ph**)
ej/ij – eject air/insert air
go – continue acquisition after it has ended (adds more scans, can change scans in **ns**)
halt – abort acquisition
.int – interactive integration window
humpcal – peak width calculation
lb – line broadening, typically <1.0 Hz for ^{1}H, 3-5 Hz for ^{13}C
lock – lock on a solvent, choose from a popup window, can also type **lock cdcl3** and so on
lockdisp – display lock window
ns – number of scans
o1p – center of spectrum in ppm (**sw** controls spectral window size)
.ph – interactive phase corregion
.pp – interactive peak picking window
rg – receiver gain, set automatically with **rga**
rga – automatic receiver gain setting
ro – turn sample rotation on or off
rpar or **read** – read parameter set
rsh – read shim file, **rsh standard.shim** to load standard shim set
.sino – signal to noise interactive window
.sret – save and return from peak pick/integration/phase window
sw – spectral window size in ppm
topshim – start gradient shimming
topshim gui – enter topshim interface
topshim convcomp – topshim with convection compensation for VT experiments
td – number of data points in acquisition
tr – transfer data during acquisition, follow with **ef; apk**
wsh – write shim set specifically for your samples – save with your initials
wrpa – copy expt parameters & acquisition to another file
xaua – start a 2D and call the spectral window from the **edc2** setting, **multixaua** also works for starting a string of 2D experiments
xfb – fourier transform a 2D such as COSY or HMBC
xf2 – fourier transform an arrayed 2D such as kinetics or T1
zg – zero, go – deletes any old data and starts acquisition. Acquisition can be continued with **go**

To create and run a new experiment:

ej – turns on the eject air to float your sample, ij will insert your sample

bsmsdisp – display BSMS panel (for lock, shim, insert/eject, lock gain, etc.)

lockdisp – display the lock signal panel

edc – create new file:
Name: folder name of your choice.
Directory: /home/data/yourusername
Choose parameters from drop down menu (1H_bbfo, 13C_bbfo, etc.)

atma – auto tune and match. Correct parameters must be pre-selected (i.e. ^{13}C or ^{31}P experiment directed above) for the probe to know which nuclei it needs to tune. If atma fails, type atma again.

lock – auto lock on solvent of your choice. Choose solvent from popup window.

topshim gui – interface for Topshim gradient shimming. Click "Start" to being shimming.

ns – number of scans

rga – automatic receiver gain

zg – zero go - deletes any old data and starts the experiment

tr – transfer the data to the disk so you can fourier transform (can be done at any time)

halt – stop the experiment – can also type "halt 64", etc. and experiment will halt at specified scan

ef – fourier transforms the data. You can also use efp after you have done apk. (efp uses the phase calculated in apk)

apk – auto phase the data

Peak picking, calibration & integration can be done through the interactive buttons in the "Process" tab

Other commands:

atmm – manual interface for tuning the probe (click left/right buttons)

xaua – for starting a 2D experiment