

Transitioning to VNMRJ 2.3A and the Cold Probe

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Introduction

Welcome to the redesigned 800 facility, things are a little different here than they were before the cold probe arrived. This document is meant to get you up to speed with the changes and hopefully prepare you for what awaits when you first run experiments. This document will be available at the facility, so please follow through all of the steps outlined here the first few times you run. If you make these things a habit, the new system/software will not seem as foreign after a while. I have split this document up into a main section that deals with starting an experiment from scratch, and several appendices. If there is something that you do

not understand it is possible that it has been dealt with under the appendices. I have also tried to include explanations of why each of these steps is important, so that no one needs to perform these tasks based on blind faith. If there is a step that you do not understand, or you disagree with, please do not hesitate to ask me about it. We are all here to learn from each other. Now let's start at the beginning!

Task 1: Start and/or connect to the VNC server

Instructions:

A VNC server may be started that points to any display number 1-99. Pick a number for yourself that you will remember and that is unlikely to be used by anyone else. I use display 99 (and will use it as an example in the following commands, replace it with your number when you follow these instructions) and I usually reserve 44 for vnmr1. I discourage using numbers 1-10 as those are the ones most likely to be used if someone forgets to specify a display number. Whatever number you pick, check to make sure that it is not used already by someone else, or if you started it previously, check to make sure it is still running. In a terminal window (Applications:Accessories:Terminal if you are not connected via ssh) on the 800 host type:

```
listvnc
```

(Check /usr/local/bin/listvnc to see what this script is actually doing). This will spit out a series of lines listing the currently running vnc servers like this:

```
User username is running vncserver :99
```

If there is no output, no servers are running. If you do not already have a server running you need to start one using the command:

```
vncserver :99 -geometry 1280x1024
```

This should echo the line:

```
New 'nmr800:99 (username)' desktop is nmr800:99
```

You can use any geometry you like here, but VNMRJ does not look very good at display resolutions less than this.

Now you can connect to the server via a VNC viewer. How you do this will depend on how you are logged into the 800 host. If you are physically at the 800 facility, open a terminal window (Applications:Accessories:Terminal) and type:

```
vncviewer :99 -Shared
```

It will ask for your VNC password, which may be different than your login password. If you forgot your password you can reset it by first killing the server, then changing the password:

```
vncserver -kill :99
```

```
vncpasswd
```

```
vncserver :99 -geometry 1280x1024
```

If you are connecting via ssh the procedure is slightly different as you must tunnel the display port through the ssh connection. If you did not connect this way initially, exit the ssh and reconnect using the following command:

```
ssh -p portnumber username@cunmr800.colorado.edu -L  
5901:127.0.0.1:5999
```

Here I've excluded the port number and user name for security reasons. The numbers following the "-L" specify the port tunneling for ssh. In this case the last number specifies the port that you wish to forward from the remote machine (in this case the VNC server port, 59xx, with xx being your server number), and the first number specifying the local port that you wish to tunnel to (in this case 5901, which is the VNC server display :1). After successfully connecting, you can connect the VNC viewer (i.e. Chicken of the VNC or TightVNC) for your local machine to display :1. You should now be connected to the VNC session from the 800.

Reason:

I recommend that everyone run VNMRJ and the spectrometer from a VNC session regardless of whether you plan to physically be at the facility or run remotely. The reason for this is that VNMRJ should be left running for the entire time that your experiment runs. There are several log files that are recording information about your experiment and the spectrometer, and if you exit VNMRJ after starting an experiment, these logs will not be written correctly. Also, even if you start a run from the 800 facility, if you wish to check on the status of the experiment as it continues you can do so easily. As soon as you access the VNC session remotely it will be in exactly the state in which you left it last. Finally, using the VNC session makes it much easier to save your data both during and after the experiment is complete. The save data function for VNMRJ saves a lot of files to your fid directory that make troubleshooting in the future much easier. If you merely copy files from the acqfil directory, rather than doing a full save, these files will not be saved and may complicate things later on.

Task 2: Check pulse widths and powers on website or login screen

Instructions:

When you login via ssh you will notice that a message is broadcast to your terminal before your login prompt appears. This message contains information about the current status of the spectrometer, including pulse widths and the locations of

relevant files. If you are not logging in via ssh, this same information can be obtained by typing the command:

```
cat /etc/motd
```

in a terminal window. The pulse widths can also be obtained from the website (<http://cunmr800.colorado.edu>) under “NMR Resources>User Resources:800MHz Pulse and Shape Calibrations”. Make a note of these values for your reference.

Reason:

When you set up your experiment you should always check the relevant pulse parameters before running. If the values you obtain are significantly different from what is published online, it indicates a possible problem. Having the full set of calibrations can also help when you are setting up sequences with pulse shapes from scratch.

Task 3: Connect to the cryobay and check probe temperature

Instructions:

You are now ready to start VNMRJ, which can be done either by typing “vnmrj” in a terminal window, or by double-clicking the VNMRJ icon on your desktop. The first thing you should do after VNMRJ is open is connect to the cryobay to view the status of the cold probe. On the left side of the screen you will see the “holding panel” with a series of tabs at the top. Click on the “Cryo” tab, and it will open a tab with fields for all the relevant cold probe information. Note that these are all blank initially. Click on the “Connect” button and it will populate the fields with the information from the cryobay. Check that the probe temperature is at 20K and that the Heater is showing a value that is around 40%.

Reason:

Apart from the importance of checking the probe temperature/heater current during the setup of your experiment, connecting to the cryobay begins logging information about the status of the cold probe for the duration of your experiment. If something goes wrong during your acquisition and you have not connected to the cryobay, you will not be able to determine when the problem occurred. Get into the habit of connecting every time you start VNMRJ.

Task 4: Activate BioPack with HCN probefile

Instructions:

This is especially important the first time you use your account at the 800, but is a good habit to get into so that you know you are getting the most accurate parameters for your experiment setup.

Check that the BioPack “application” is enabled in your account. Do this by going to the “Experiments” menu and checking that there is an entry for “Protein Triple-Resonance Experiments” either active or greyed-out. If these entries are there it means that BioPack is enabled, proceed with step 2.

*If they are not there enable the BioPack application by choosing “Applications...” from the “Edit” menu. Under the entry “USERDIR” type “/vnmr/biopack” and choose “Enabled” from the dropdown on the left. Under the field for “Application Label” type “BioPack”. Click the “OK” button, the item above should now be available in the “Experiments” menu.

*If the BioPack entries under the “Experiments” menu are greyed out, choose “Experiments:Activate BioPack:Activate BioPack and Use Current User Probefile”. Ignore the instructions on the screen, exit and restart VNMRJ, and make sure to reconnect to the cryobay.

Now copy the system probefile to your user probefile by typing in a terminal window:

```
cp -r /vnmr/probes/HCN ~/vnmrsys/probes/.
```

Return to VNMRJ and type “ghn_co” in the VNMRJ command line. Choose the “Globals and Probefile” parameter page from the “Setup” tab, and check that the pulse parameters for proton, carbon and nitrogen agree with those that you noted upon login. Also check that the “Automatic Gradient (WURST) Shaping” checkbox is checked, and that “BioPack Power Limits” are activated and set as follows:

1H Observe Power: 59

13C Decoupling Power: 42

1H/13C Spinlock Field (Hz):7994, 8000

15N Decoupling Power: 44

2H Decoupling Power: 48

Click the “Update Probefile with Parameter Values in this Parameter Set” button.

Reason:

BioPack experiments can be quite a useful tool in setting up and/or troubleshooting sequences. However, BioPack is incredibly dependent on an accurate probefile for successful function. I maintain a good copy of this probefile using calibrations that have been done correctly under the system probefile directory. Copying this probefile to your directory ensures that you have the most up-to-date calibrations. In addition, under the new software, the correct values for dof and dof2 change with each change of the lock frequency. By loading up the latest probefile you will ensure that these values are correct for the current state of the spectrometer. Finally, if for some reason the cold probe is out for service and the room temperature probe is

back in use, this will procedure will ensure that you have parameters for the currently installed probe.

Task 5: Ensure pfgon='nny'

Instructions:

Type “pfgon?” in the VNMRJ command line and make sure that “ pfgon='nny' ”. This can also be done under “Edit:System Settings”, by setting the “Gradient Amplifier” for “Z” to “On” in the dropdown. In addition check that gradientshaping='y', if the parameter does not exist it may be created via a button on the “Setup:Globals and Probefile” parameter page.

Reason:

Under new accounts pfgon is set to 'nnn' by default. You should only have to do this once, but checking it eliminates any confusion you may experience if your gradient shimming profile has only a single narrow peak.

Task 6: Ensure that sample is correct volume and centered correctly

Instructions:

H2O/D2O samples should be in Shigemi tubes, and the plunger should be set so that the sample region is between 16mm and 18mm long. The volume of sample required for this varies depending on the tube, but will usually be less than 300 µL. 100% D2O samples may be in regular 5mm tubes if you have enough volume. It will also help if you use a Shigemi tube that you know works well at high field. We have seen several tubes that are fine at 600 MHz and below, but terrible at 800 and 900 MHz.

Reason:

Water suppression is of utmost importance for cold probes. Most suppression techniques for BioPack experiments employ flipback pulses that selectively move water magnetization back to Z either pre- or post-hard pulse. Inside the homogeneous region of the RF coil (16mm) these pulses are very accurate, however outside of the coil region the pulses do not behave as expected. As a result you will not gain any protein signal from regions outside of the coil; only water will contribute. For this reason it is beneficial to have your sample entirely constrained within the RF coil.

Task 7: Insert your sample into the magnet

Instructions:

Climb the platform to the top of the magnet and eject the sample that is already inside. There is a silver switch attached to a circuit board next to the top of the barrel that can be used to activate the sample eject air. This is easier to use with the sample catcher than activating the air from VNMRJ. When the sample ejects, remove the sample from the spinner and place your sample in the spinner using the depth gauge. There is only one spinner for the cold probe, which looks like a regular spinner except for a groove that is cut in the bottom. ****Do not attempt to use a spinner without this groove, damage to your sample and/or the probe may occur!**** Make sure that your sample is centered in the dotted box marked on the depth gauge and that it does not extend more than 1mm outside of the box on either side. Make sure the eject air is on (switch is flipped up) and place the sample/spinner into the sample catcher. The spinner should sit on top of the sample catcher in the "caught" position. Press the bronze lever on the side of the catcher and the sample should drop slightly and begin bobbing on the eject air. You should now be able to let go of the lever and the sample should not pop up high enough to be caught by the catcher. If it does get caught, adjust the eject air pressure slightly by turning the silver needle valve next to the upper barrel clockwise, press the bronze lever again and check that the sample floats properly. You should now be able to insert the sample using the switch. ****If you turn off the eject air and the sample does not drop, do not touch the sample catcher or the sample!!! Immediately turn the eject air back on, or the sample may fall into the probe unassisted and cause significant damage!****

Reason:

The sample catcher is an integral safety mechanism for the cold probe. Inside the probe the RF coil is maintained at 20 degrees Kelvin by the helium compressor/probe heater. That region is separated from the room temperature region of the coil by just a few millimeters of vacuum. If that vacuum fails, or if sample VT air is disrupted, the temperature in the sample region can drop very quickly, freezing the sample. If a water sample freezes, it can expand very rapidly and cause damage to the nearby probe components. For this reason, if any error is detected with the cold probe, the system will activate the emergency eject air (which is separate from the sample eject air) pushing the sample out of the cold probe before any damage can occur. However, if the eject air were to shut off after the sample was ejected and there was nothing to catch the sample, the sample could drop back into the cold probe. For this reason, the sample catcher is designed to hold the sample at the top of the magnet even if the emergency eject air loses pressure.

Task 8: Lock the sample

Instructions:

This is not really different from locking on any of the other spectrometers. Choose the “Lock” parameter page from the “Setup” tab. Make sure the “Activate Lock” checkbox is deselected and then click on the “Lock Scan” button to optimize Z0. For a 10% D2O sample, the lock power will usually be around 38 with a Lock Gain of 46, but it is possible to use a higher Lock Power with a sample containing less D2O. Values for Z0 and Lock Phase vary significantly over time, but should not change much from day-to-day. At this point it is not necessary to fully optimize the lock parameters as you will have to re-optimize after tuning and shimming.

Reason:

While the magnet that provides the main NMR field (B0) is superconducting, it does lose a very small amount of current over time. This means that the Larmour frequencies for the nuclei in your sample are slowly drifting. If you were to collect a long experiment, averaging the signal of these drifting frequencies, the spectrum would look broad and spread out. Fortunately, while the Larmour frequencies are drifting, they are drifting at the same rate for all of the nuclei in your sample, and the difference between them is static. When you “lock” your sample, you are finding the Larmour frequency for the deuterium in your sample (in most cases from D2O). Once you “activate the lock” the spectrometer tracks how this frequency changes during the course of your experiments and adjusts the value of Z0 to counteract the drift of the main field. If you do not provide a good starting point for the spectrometer it is possible for the system to lose the lock signal during the experiment, ruining the data that you are collecting.

Task 9: Tune the probe

Instructions:

No re-cabling is required for tuning with the new console. Under the magnet is a small box that has the tune interface on it. Push the channel button to select the channel that you want to tune (1 – proton, 2 – carbon, 3 – nitrogen). Attenuation should be set to 9 for normal tuning. Attached to the bottom of the cold probe is an amber plastic tuning/VT interface. There is an amber knurled ring that can be turned so that the appropriate tuning selection appears in the small window (choices are 1HT, 1HM, 2H, 13C, 15N). Once the proper selection appears in the window, the brass knob can be pushed up into the probe, engaging the tune adjustment mechanism (you will feel it seat into the probe properly). Turn the brass knob to adjust the tune, and make sure the knob disengages the probe before turning to the next selection. For proton, you may have to switch back and forth between tune and match to optimize the signal. For each channel minimize the number on the tune box as much as possible. Ideally each value should be around 90 when fully tuned, but in practice this value will depend on the room temperature

and can change from day-to-day. Also carbon does not seem to tune below 100 for the cold probe.

Reason:

The coils in the probe transmit effectively only certain frequency ranges. Frequencies outside of that range are reflected back to the amplifiers that broadcast the RF. During your experiment you need to ensure that all of the RF that you broadcast ends up exciting your sample, and not returning to the amplifier. By tuning the probe you are adjusting the frequency range over which the coil transmits, minimizing the power that is reflected. The number on the tune display is a representation of the amount of reflected power, so you need to minimize this number. The tuning for each coil depends on the temperature of the coil, and the composition of the sample that is contained within the coils. In the case of the cold probe, the temperature of the proton coil is constant (20K) regardless of what VT temperature is chosen, so the probe tune will not change significantly with differences in VT temperature. However, changes in sample buffers, and in particular the amount of salt in a buffer, will significantly affect the tuning of the proton channel. For these reasons you should check the tuning every time you change samples or temperatures.

Task 10: Shim the sample

Instructions:

It is important to optimize the homogeneity of the magnetic field around the sample. This is done by adjusting current in small coils outside the probe, and is called shimming. Shimming can be done manually, or automatically using the gradient system. Usually it is best to start by shimming Z1 and Z2 manually to optimize the lock level before starting a gradient shimming experiment. Go to the "Setup:Shim" parameter panel and adjust Z1 and Z2 using the left and right mouse buttons. The left button decreases the value for the shim, the right button increases the value. The middle mouse button can be used to switch the "coarseness" of the adjustment. By default the options are 1, 10, or 100, but each of these can be switched to any value by holding the shift key and middle clicking the button. Switch back and forth between Z1 and Z2 until the lock level is maximized. If you wish you may adjust Z3 in the same fashion, returning to reoptimize Z1 and Z2 afterwards.

Once you have adjusted the shims by hand, you can gradient map and shim the field automatically. Type "gmapsys" or select Setup Gradient Shimming from the "Setup:Lock" parameter panel. If you have a shimmap that you have used before you may select it from the dropdown menu to load the parameters. If not you can click the "PFG H1" button to load parameters for shimming on H₂O/D₂O samples (PFG H2 would be used for 100% D₂O samples). Click the Acquire Trial Spectra button to acquire two gradient images of the sample. Type "full ds" to view the first spectrum, and then set the threshold by typing "th" (or by clicking the threshold button). Adjust the threshold to ~20% of the maximum, but make sure that it is not

below the discontinuities from the sample tube if you are using a shigemi tube. Set the left and right cursors where the threshold crosses the profile and then click "Set window from cursors". You can now enter a shimmap name and click "Make shimmap using current settings" to start the mapping process. It is important to make a new map every time you remove a sample from the magnet as there is no guarantee the sample is in the same place every time. Once the shimmap is complete you can select the number of shims you would like to use for shimming (usually 4-5 for shigemi tubes) and click "Gradient Autoshim on Z". You can monitor the progress of the shimming via a text window (Setup:Text Out) to make sure that the shimming has not set any shim out of range. Once you are satisfied with your shimset you can quit the experiment by clicking the "Quit Gradient Autoshim" button. Once you have completed shimming you should return to Task 8 to reoptimize the lock.

Reason:

This may be something that you do just out of habit, but you may have not thought of why it is important. In the case of small organic molecules the lineshape is very narrow, and usually limited by the homogeneity of the magnetic field. For this reason organic chemists are very thorough when they shim their samples, and often run with the sample spinning to obtain the best possible lineshape in their spectra. But for biochemists, protein samples routinely have very broad lineshapes due only to the relaxation of the magnetization in the sample. In these cases the quality of the homogeneity of the magnetic field is a relatively small contribution to the overall lineshape. The water lineshape, however, is affected by homogeneity. This means that a better shimset will give you a narrower water resonance, and improve the performance of any of the water suppression techniques that are employed. So it is still important to shim biological samples, however the reason behind the shimming is different.

Task 11: Check pulse widths, setup parameter sets, and run experiments

Instructions:

Many users have different techniques and procedures that they use when setting up experiments on the spectrometers. It is best to follow whatever procedure you are used to when setting up experiments, but if you find that you are unclear on why you are performing a specific task in a specific way please don't hesitate to ask. Here is a brief checklist of things to check/confirm before starting an experiment regardless of the procedure you are using.

1. Calibrate proton pulse width and power
2. Load parameter set into experiment
3. Check pulse widths and powers for all pulses
4. Check shape calibrations for shapes used in sequences
5. Set offsets/spectral widths (see appendix)

6. Set decoupling parameters
7. Set flags (f1180, c180_flg, etc.)
8. Check first trace for signal
9. Optimize selection gradients
10. Optimize tof for water suppression
11. Optimize flipback pulse fine power
12. Optimize gradients for water suppression
13. Run 2D test planes if it is a 3D or pseudo-3D
14. Setup full experiment

Appendix 1: VNMRJ Primer

This version of VNMRJ (2.3A) has a few changes from the last version, most of them are good, but some are difficult to get used to. Here is a rundown of some of the interfaces for VNMRJ that may be useful to know as well as potential pitfalls.

1. Applications

As we saw in the instructions above VNMRJ keeps some of the functionality of the system separate from the base VNMRJ installation in “Applications”. Each application is kept in its own separate directory in /vnmr, so for example the files for the BioPack application are now contained within /vnmr/biopack. If you have not activated the application you will not have access to the sequences and macros within that directory. This may seem confusing at first, but it is really an advantage as if you are experiencing a problem that may be related to a certain pulse sequence you can actually disable all of the applications to see if the problem still exists in a base VNMRJ installation. This was very difficult to accomplish prior to VNMRJ 2.3A.

The Applications are activated via the “Edit:Applications” menu, but by default your account should be set to have BioPack activated. If you need to use any sequences in the “AutoTest” application (usually sequences like ATgecho) you will have to activate that application by hand. It is preferable to activate the application rather than just to copy the sequence to your psglib as some sequences depend on other files within the application and may cause problems compiling and running without the full application present.

The main application that you will need to concern yourself with is BioPack. In the past BioPack was installed by default over the base VNMRJ installation and you may not have even known that you were using sequences provided by a standalone package. If you have done any protein NMR chances are you have run a BioPack sequence even if it was just a standard water suppression experiment (water). The BioPack application depends on a set of calibrations present in a probe file (HCN), and it will set up all of the sequences based on pulse width and power calibrations that it finds there. As you can imagine if these calibrations are incorrect it will drastically affect the data that you obtain. For this reason a good set of calibrations is maintained in the probefile /vnmr/probes/HCN, which will contain all of the most recent calibrations for the probe that is installed in the system (whether it is the

cold probe or the room temperature probe). Make sure before you set up any experiments that you have copied this probefile from the system directory to your user directory (`~/vnmrsys/probes/HCN`) ensuring you have the correct set of calibrations. If you start all of your experiments from the good probefile, rather than pulling up old parameter sets which may have been run on different probes/systems, you are less likely to miss variables that might have changed and cause damage to the probe.

2. File Manipulation

If you do wish to pull up parameter sets that have been used before it is preferable that you load them into an experiment that is not going to be used to run anything (like `exp999`) and transfer the parameters to a new version of the sequence. If you do setup from an old parameter set there are many possible issues that can arise, for example some experiments have caused the current shimset to be corrupted (if you find this has happened you may need to run the macro “`limitshims`” to fix the problem). There are three dialogs used for manipulating files: Open, Save and Browser. Use the File:Open dialog to open `.fid` files or `.par` files (not all of these may be displayed until you change the “File Type” at the bottom of the dialog). Use the File:Save interface to save your experiments, this will save the `fid` files and other files that can be useful in diagnosing problems down the road. If you do not save using the Save dialog, some of these files will not be preserved. Finally the Tools:Browser dialog is used to manipulate things such as shimsets. Files can be dragged from the Browser dialog to the “Shim” panel to load shims from a previous experiment.

3. Offsets under VNMRJ 2.3A

Another problem that can be encountered with the new software has to do with the frequency offsets used by all sequences (in particular `dof` and `dof2`). In this version of VNMRJ (VNMRJ 2.3A) the frequency offsets are all adjusted based on the lock frequency. This is so that the offsets can be specified in ppm. Unfortunately, to allow the functionality of previous experiments to be preserved, this new scaling occurs after the `dof` value has already been specified. What this means is that every time that the parameter `lockfreq` is changed (about every 3-6 months on the 800), the correct values of `dof` and `dof2` change as well. So if you try to run an experiment that was run with a different value for `lockfreq` without updating `dof` and `dof2`, your spectrum will not be centered correctly, and may be folded at the edges. If you follow the instructions above and setup experiments from BioPack using a fresh copy of the probefile then your `dof` and `dof2` values should be updated correctly. If you are setting up experiments on your own, however, you will need to reset the offsets before running the experiment. The macro “`setoffset`” allows you to specify any frequency offset in ppm using the syntax:

```
setoffset('NUC',ppmval):var
```

where NUC is the nucleus of interest (i.e. C13, N15), ppmval is the ppm for your offset (i.e. 43, 119) and var is the variable you wish to set (i.e. dof, dof2). So to set dof2 for the center of a protein spectrum you would type:

```
setoffset('N15',119):dof2
```

In addition to this macro, this functionality has been built into the VNMRJ interface as well. You can set the dof and dof2 values for most experiments by going to the “Acquire:Channels” parameter page and using the dropdown menu “Set C13 Carrier at” or “Set N15 Carrier at” to specify a value in ppm. If you get into the habit of doing this every time you set up an experiment you will find you will not have a problem with the new procedure. It also outlines the importance of setting up and running 2D test planes before starting experiments, this way if you forget the offsets, you will see the problem right away.

4. The Locator/Holding Panel

One final piece of useful functionality in VNMRJ exists in the “Holding” panel. You can switch to the holding panel using the tabs under the main menus in the top left corner of the screen. Almost anything that you use frequently can be placed in the holding panel to allow for quick access. The most useful thing I have found is to place your list of experiments (or workspaces) in the holding panel so that you can switch experiments merely by double clicking on the one you want to switch to. To do this you need to open the locator (Tools:Locator) and click on the magnifying glass to “Sort Workspaces, All”. Once your experiment list appears drag each experiment from the locator into the holding panel. If you add new experiments, or if you exit VNMRJ you may need to reopen the locator to refresh the list.

Appendix 2: Cold Probe Primer

1. Introduction

The cold probe system for the 800 is a little different than systems on other spectrometers within the CU system. You should familiarize yourself a little with how the cold probe works so that you can more easily recognize problems when they arise. The cold probe system consists of three main systems, the compressor, the cryobay and the cold probe itself.

2. Compressor

The helium compressor is located in the corner of the lab near the magnet monitors. You will hear it whistling as soon as you enter the lab, as it is not sound-proofed at all. If you do not hear the whistle as you enter this means that either the cold probe has warmed up for some reason, or the room temperature probe is installed. In either case it is good to note the presence (or lack of) the squeaking. The helium compressor consists of a closed loop of helium gas that is compressed to high pressure, and sent through the supply line to the cryobay. The return line from the cryobay contains “low pressure” helium coming from the cold head.

3. Cryobay

The cryobay is the large bay that is placed near the magnet. It is responsible for all of the controls of the cryogenic system, as well as the vacuum system and the cold head. The controls interface with the VNMR console, but are autonomous, meaning that if the console/host computer are shut down the cold probe will continue to function.

The vacuum system is used to maintain a high vacuum in the cold probe while it is running, this maintains the thermal isolation of the sample region of the probe from the cold coil region, and is why you can have a sample at 295 Kelvin just a few millimeters from 20 Kelvin. If the vacuum is compromised, the probe will be automatically warm up and the sample will be ejected from the probe and be caught by the sample catcher at the top of the magnet. If the sample remains in the probe when the vacuum is lost it will freeze and expand, possibly damaging the cold probe.

The cold head is the section responsible for cooling the probe, and consists of two cooling stages. The cooling stages allow high-pressure helium gas from the compressor to expand into a low pressure region. If you remember back to undergrad P-Chem, or if you have ever held a CO₂ cartridge after use, you know that rapid gas expansion causes a cooling effect. The first cooling stage cools the helium to approximately 30 Kelvin and serves to pre-cool the second stage, which further cools the helium to 13 Kelvin. After the second cooling stage the cold helium travels to the probe to cool it.

4. The Cold Probe

The cold probe consists of two sections, one at 20K and the other (the sample region) at approximately 295K. The temperature of the sample region is maintained by the VT heater/FTS system as it was in the room temperature probe. Unlike the room temperature probe, however, if the VT gas flow is interrupted, the temperature of the sample region can drop precipitously due to the proximity of the sample region to the cold region. VT air must never be disconnected from the probe for this reason. In addition you may need to use a higher VT flow rate, which can be adjusted under VNMRJ's "Setup:Spin & Temp" parameter panel (make sure it is set to 15 L/min).

The cold region of the probe contains the RF coils for the probe as well as the preamps (both high-band and broad-band). The cold helium from the cryobay cools the probe to 13K, and the probe contains a probe heater, which warms the probe from 13K to 20K. The current supplied to the probe heater is variable and is adjusted to maintain a constant probe temperature. This is due to the fact that any change in the temperature of the coils results in a significant change in the phase of the RF coming from the coils. This is why the heater current is such a significant parameter, it is a reflection of how much power (heating) is being put into the probe at any given time. The lower the probe heater current, the more power that is being supplied to the probe by pulses. In past cryobays, the probe heater current was specified in Watts (usually around 4-5W) and was visible in the cryobay window.

For the new cryobay, this value is reported as a percentage (usually around 40%) of full power, and can be monitored through the VNMRJ software directly (see instructions above). The important parameters that need to be monitored through the software are probe temperature (20K, but will change slightly after experiments are started and the probe heater is not yet compensating, use lots of “ss” scans) and the heater current (~40% under idle conditions, lower if decoupling is being applied).

Appendix 3: Tuning the probe using “mtune”

Occasionally it may be useful to view the entire frequency sweep when tuning the probe, rather than just looking at the number on the tune interface. To do this, use the “mtune” macro to setup an experiment to sweep frequency.

Once the mtune experiment has been setup you will be left in the “Setup:Probe Tune” parameter panel, which looks similar to the Gradient Shim panel. Start by selecting the RF channel you wish to tune (usually 1H-channel 1, 13C-channel 2, 15N-channel 3, 2H-channel 4). Now that you have chosen a channel, set the “Center Frequency” using the dropdown, to the nucleus you wish to tune. This should set the frequency in the field to the right, however this can also be set by hand if need be.

Start the frequency sweep by clicking “Start Probe Tune”, you should see a line across the display with a dip near the center. If you cannot see the dip you may need to press the “Autoscale” button, or even set a larger range for the frequency sweep (default is 3 MHz). When tuning 2H on channel 4, it is also common for spikes to be present throughout the spectrum.

Adjust the dip so that it is centered on the line in the middle of the display, which corresponds to the “true” frequency for the nuclei in question. For proton adjust “1H-T” to change the position of the dip with respect to the marker, and adjust “1H-M” to maximize the size of the dip. You may need to go back and forth between these two adjustments to fully optimize the tune. Ideally the dip should be centered and the base of the dip should be all the way to the bottom of the display. For C13, N15 and H2, you will only be able to adjust the position of the dip.

You should also bear in mind that for certain samples you may not be able to fully tune proton without affecting the 2H tune (and the lock level). In these cases after tuning proton, you will not be able to see the dip when tuning channel 4 (2H). If this occurs, try to detune “1H-M” while watching the mtune display for channel 4. A small change in the proton match should be sufficient to restore the tuning dip for 2H and allow you to lock the sample.