

Using the Cold Probe on the Varian Inova-600 NMR Spectrometer

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***** WARNING *****

Read through the whole manual before using the cold probe

Failure to follow the guidelines in this handout could result in severe damage to the Cold Probe and to your sample.

All NMR experiments must be modified to account for the substantial differences in the technology of cold probes.

If you are using the cold probe for the first time, make sure that you check with the manager that your experiments are acceptable for use on the cold probe. Failure to do so will require that your group pay for any repair costs that are incurred due to damage caused by your experiment.

If you have any doubts about how to run the cold probe, ASK, it is better to spend 10 minutes figuring it out, than 3 months waiting for the probe to come back from the repair shop.

1. Overview

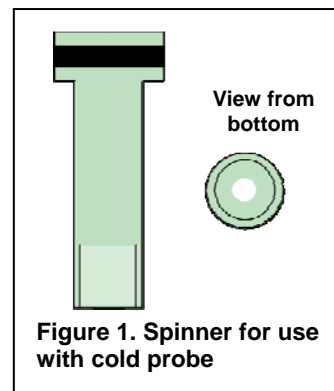
This manual is intended for users who are experienced at operating Varian NMR spectrometers at a relatively high level of proficiency. The intent is to point out the issues pertaining to the cold-probes, how the use of the cold-probe differs from a conventional probe, and the issues that directly affect how one does spectroscopy with these probes and how to avoid damage to the probe are highlighted.

(Note: Some of the figures have been used with permission from Varian .)

Doing NMR spectroscopy with a cold-probe is essentially the same as with a conventional probe; however, certain properties of the cold-probe must be understood to obtain optimal results. Each of these will be address in further detail later in this document. The primary issues that should be understood are:

Sample Spinners

The salt tolerant cold probe can accept square tubes as well as round tubes. To allow this to happen there are new spinners for use on the cold probe. The spinner for square tubes is obvious, the spinner for round tubes has a slot cut into the bottom (See Figure 1). Use of other spinners will mean that your sample will not get into the probe.



Rf Pulse Ringdown Times:

Due to the high “Q” (Quality-factor) of these probes, the ring-down time of the RF coil after a pulse is significantly longer. This means that the delay between the end of the pulse, and start of signal detection is longer. Since pre-acquisition delays can adversely effect the appearance of the spectrum (mostly in frequency-dependent phase errors, and other baseline issues), there are new parameters that deal with this problem. The parameter “**qcomp**” (for Q-compensation) is used, and is dependent upon the settings of the “**dsp**” parameter.

Rf Power Handling:

The coil in the probe is maintained at a precise temperature by using an Intelligent Temperature Controller (ITC) to heat super-cooled helium gas to a stable temperature of 25.0 Kelvin under normal operation. When RF power is inserted into the probe, especially when using broadband decoupling, i.e. ^{15}N and ^{13}C , this causes heating of the probe and this is compensated by changing the power in the heater controlled by the ITC. The heater power (in Watts) is displayed in the CryoBay Monitor window. As the net RF power in an experiment increases, the heater power will drop to maintain a steady probe-head temperature of 25.0 Kelvin. If too much power is used, such that lowering the heater power cannot maintain the probe-head temperature, then the tuning will be lost, and ultimately damage to the probe (or the receiver pre-amplifier in the probe) could occur!

Pulsed Field Gradient Performance:

The inductance of the gradient coil in these probes is much higher. Adjustments have been made to the gradient amplifier to compensate for this; in addition, the software has built-in, rudimentary shaping of the rise/fall times of the gradient pulses to further improve the gradient performance (esp. the amplitude stability). A new parameter called “**gradientshaping**” (a global “flag” parameter) is used to activate the shaping of the gradient pulses.

WARNING Gradient shaping only works for pulse-sequences that use the “**zgradpulse**” statement to control gradients. Many early experimental pulse-sequences, particularly those obtained from Lewis Kay in Toronto do not use this parameter. Damage to the probe may occur if you use one of these experiments without any modification

Instrument Cabling:

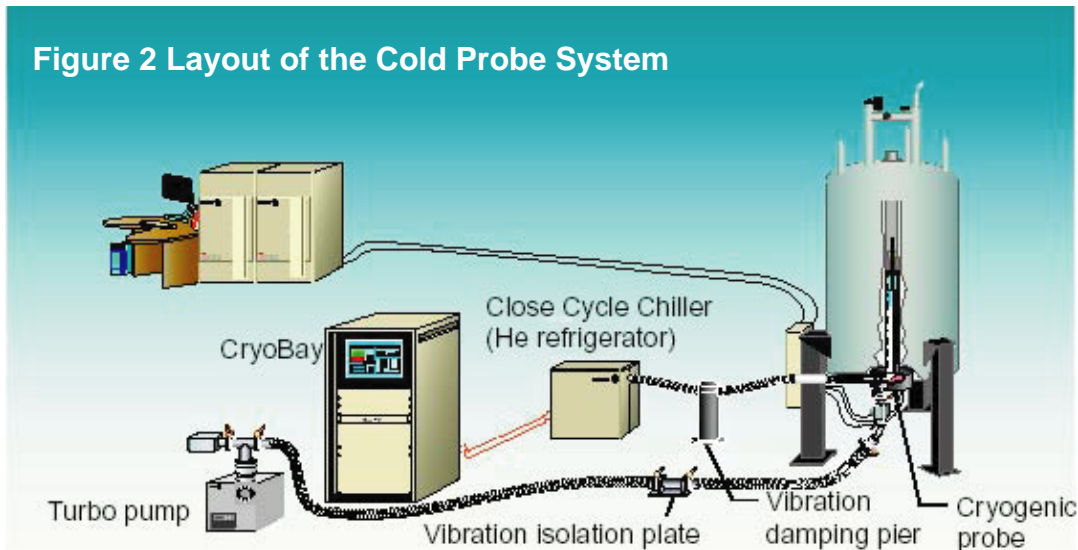
The preamplifier for the proton observe channel is now located inside the cryogenically cooled region of the probe. Consequently a different receiver/pre-amplifier module (called a “Cryopreamp Driver”) has replaced the original preamp. The way this system is setup for operation and probe tuning is different.

Probe Tuning:

In addition to cabling issues, the cold probe has a different user-interface for probe tuning. A single brass knob is used to tune all channels. A larger knob is used to position the tuning stick to engage the tuning element for each channel. Tuning channels are available for ^1H Tune, ^1H Match, ^{13}C Tune, ^{15}N Tune, and ^2H Tune.

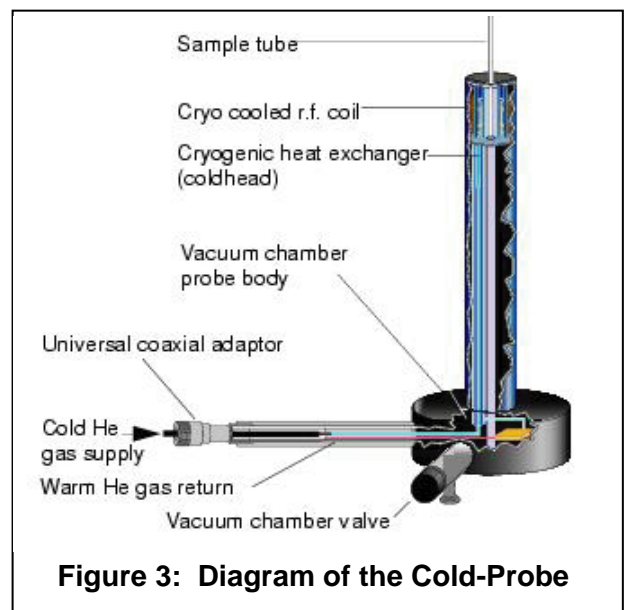
2. Description of the Cold Probe System

The layout of the cold-probe system and the individual components are shown in Figure 2. The basic theory of how this works and what each component does is as follows:



- **Cryobay**
 - Helium gas is compressed by a compressor inside the CryoBay and this is fed into the Close Cycle Chiller (CCC), (which is the box located in the pit of the 600) where it undergoes thermal expansion.
- **Closed Cycle Chiller**
 - Inside the CCC, thermal expansion of the He gas is used to cool the cold-head to below 25 Kelvin. A small amount of the Helium gas is routed through the cold-head in the CCC where it is cooled to below 25 Kelvin, and then flows through the flexible stainless steel line to the side-arm of the probe, and up through the probe.
- **Probe**
 - Within the probe, the cold He gas is used to cool the tuning elements, the coils, and the receiver pre-amplifier to 25 K (Figure 3) The probe contains a vacuum chamber at $\sim 10^{-8}$ - 10^{-9} Torr to thermally isolate the cryogenic circuit from the outside world. As a result the sample and the magnet are kept at or near room temperature, while the probe is at 25 K.

WARNING: Even though the sample is relatively well insulated from the cryogenic temperatures inside the probe, it is imperative that dry air or nitrogen is kept flowing through the VT-port in the probe. Without VT air-flow, the sample will cool at a rate of $\sim 1^{\circ}\text{C}$. per minute (until it freezes).



- **The Intelligent Temperature Controller**
 - The ITC is located inside the cryobay, and can be seen at the back of the cryobay. It uses a variable heater inside the probe to maintain the desired temperature at the coldhead.

3. Preparing for NMR Experiments on the Cold Probe:

Doing NMR is not much different than a normal probe. The main issues are associated with how much power you can use in your experiments for decoupling and spin locking etc.

A. Monitoring the Status of the Cold Probe.

Before starting an experiment check the status of the probe in the “CryoBay monitor window” (Figure 4). Note the value of “Temp” and “Heater” readings in the box outlined in red. You need to know the values of these settings when the probe is not in use, so that you can judge how much power you are using in your experiments.

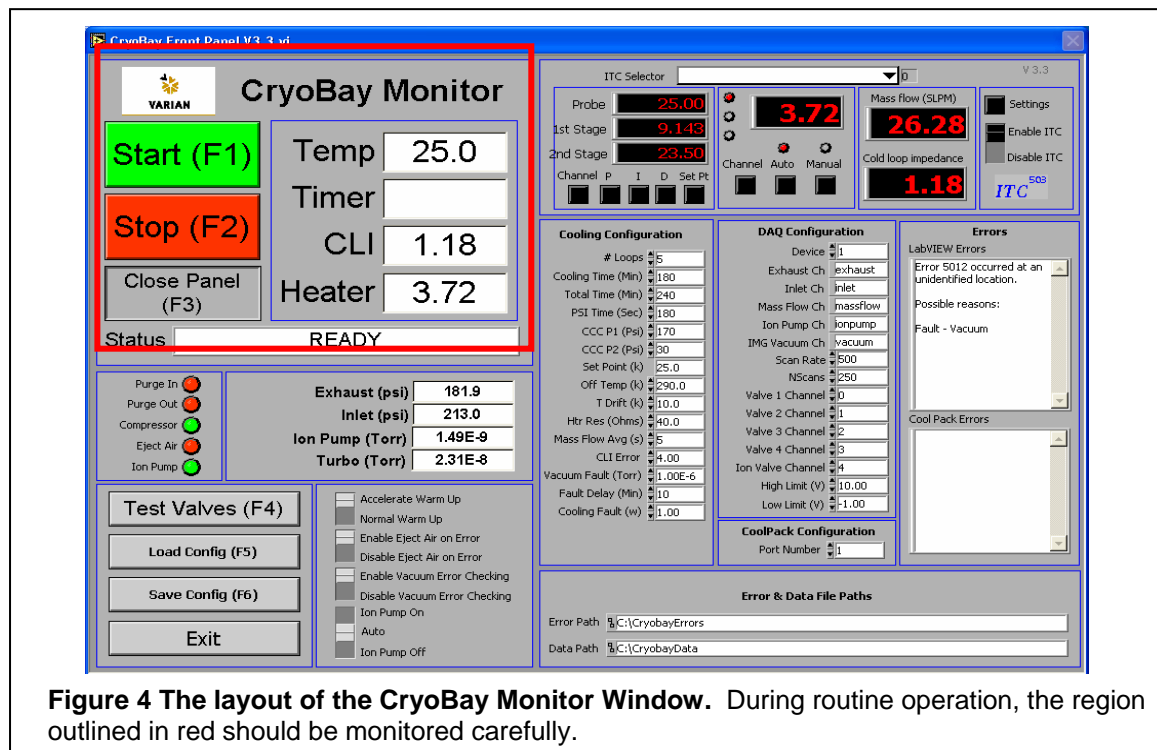


Figure 4 The layout of the CryoBay Monitor Window. During routine operation, the region outlined in red should be monitored carefully.

Temp: is the temperature of the cold-head inside the probe, and is essentially the temperature of the RF coil. This value should not change by more than +/- 0.5 K when an experiment starts. Once steady state has been reached this value should be stable at 25 K.

Heater: is the amount of power, in Watts, required to heat the super-cooled He gas to 25 K. This value should not change by more than 1 Watt compared to its starting value. If too much power is used during an experiment then the probe will not be able to maintain a steady temperature and damage may occur to the probe and your sample.

Timer is the amount of time left until the probe will be ready for us, if it is in cool down mode.

B. Sample Insertion and Ejection

The cold probe can accommodate both round and rectangular NMR tubes. Rectangular tubes are used to reduce noise when the sample contains high concentrations of salt. There are two different spinners for these probes. The spinner for the rectangular tubes has a rectangular slot as you might expect. The spinner for the round tubes has a slot cut into the bottom of the spinner (See Figure 1).

There is a danger of your sample freezing and causing damage to the probe if there is a loss of temperature regulation if the VT gas flow is turned off. Therefore the probe is equipped with an emergency sample eject system. As part of this system, the top of the bore tube on magnet has been modified to include a "Sample Catcher". This consists of a small brass toggle switch that will prevent the sample going into the magnet. In an emergency, the sample is very forcefully ejected from the probe so that it pops up above the catcher, and as it drops back down is caught on the lip of the catcher.

To insert your sample, place the spinner into the bore tube so that it sits on the lip of the sample catcher. Slide the brass nut on the front of the catcher to the "Off" position (Figure 5), your sample should slip down over the catcher and float freely on the stream of eject air. Then slide the switch back to the "On" position. You can then proceed as normal.



Figure 5: Sample Catcher for Emergency Eject System

WARNING * The sample catcher must be in the "On" position if a sample is in the magnet. Serious damage to your sample and the probe may result if the catcher is left in the "Off" position.**

C. Instrument/Probe Cabling

Cabling for ^{13}C , ^{15}N , and ^2H is the same as a conventional probe, including the location of the bandpass filters. ^1H cabling is different. Refer to Figure 6, and the following instructions to ensure that the system is cabled properly.

- The cable from the High-Band Transmitter plugs into the top port "HB XMTR (J5303)"
- HBPROBE XMTR (J5331) goes to the "1H" port on the probe.
- HB PROBE/PREAMP (J5335) connects to the "1H RCVR" port on the probe.
- OUTPUT (J5302) is connected to the same cable as a normal pre-amplifier (Output cable connects to the mixer in the magnet leg interface).

Signal path:

The transmitter pulse is routed to the probe from port J5331 on the cryopreamp driver to the probe. This cable (a "fat" cable) also carries the DC voltage for gating the Transmit/Receive Switch.

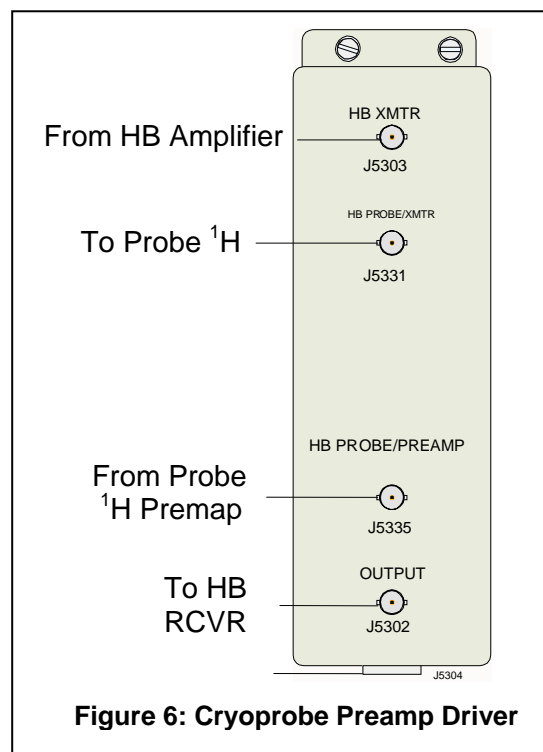


Figure 6: Cryoprobe Preamp Driver

After pulsing, the NMR signal is amplified in the pre-amplifier located inside the probe, and this signal comes back to J5335 on the cryopreamp driver. The signal is further amplified, and sent to the mixer/RCVR via the OUTPUT (J5302) port

D. Probe Tuning:

For ^{13}C , ^{15}N , and ^2H channels, the cabling to tune the probe is the same as with a normal probe. The “Probe” connector on the tune interface connects to the appropriate channel on the probe, and the cable connected to the “Output” port of the BROADBAND Pre-Amplifier is connected to the “Output” port of the tune-interface.

For the ^1H channel, THE CABLING SETUP FOR TUNING IS DIFFERENT. (Figure 7)

- Disconnect the thick cable connected to the top port of the cryopreamp driver (J5303). (Figure 7 A and B)
- Using a short BNC cable, connect the now empty port J5303 (Figure 7B) to the PROBE port of the tune-interface module (Figure 7C)
- Move the cable from J5202 (OUTPUT) to the OUTPUT port of the tune-interface.
- **DO NOT REMOVE** the cables going to the ^1H channel of the probe (J5331).
- The system is now ready to tune the ^1H channel of the probe.

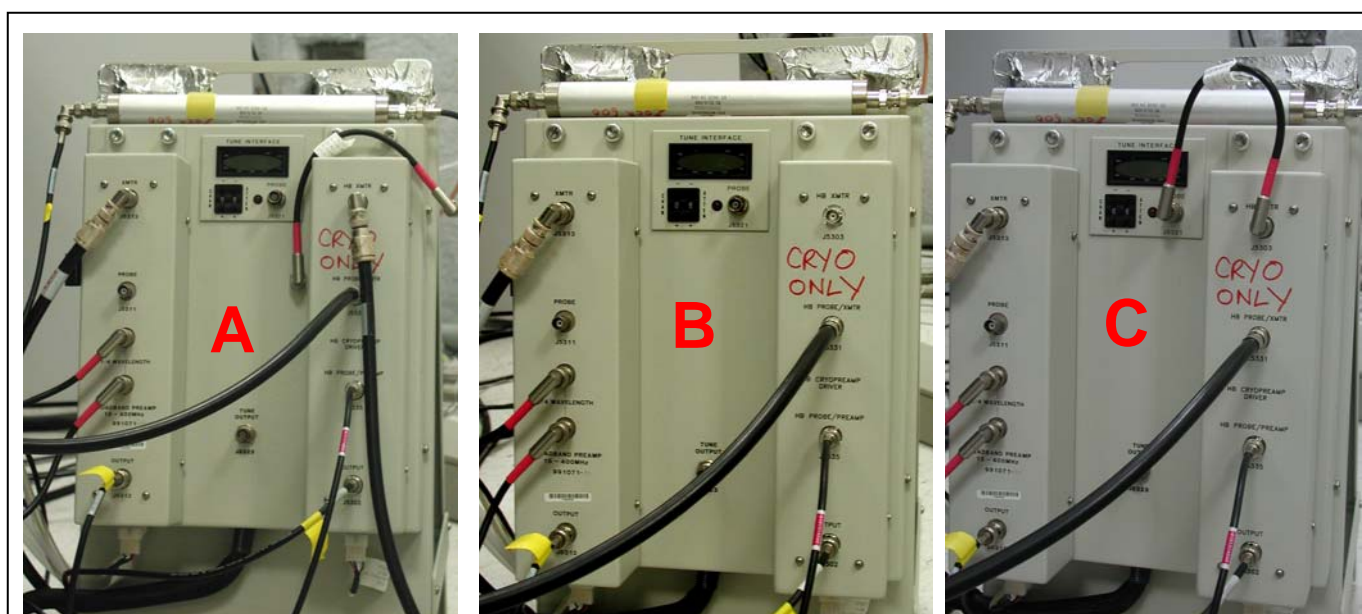


Figure 7 Cabling of 1H Preamp for normal operation and tuning. (A) Normal operation (B) After removing HB transmitter cable and (C) After bridging HB transmitter and “Probe” connector on Tune interface

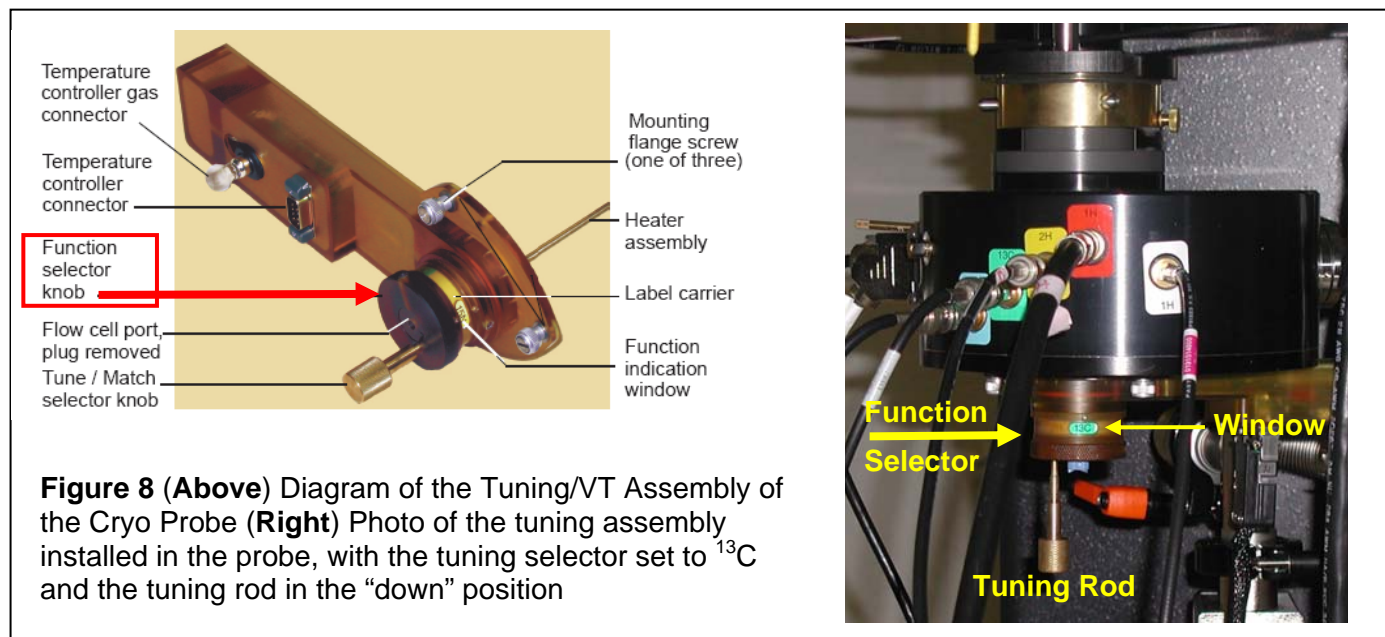
CAUTION: Extreme care must be exercised when working under the magnet. Be very careful not to touch the side-arm or any of the vacuum connections on the probe. Do not lean on, pull or otherwise press on any part of the cold-probe assembly to support or steady yourself while working under the probe. The probe is under a high vacuum, and any disturbance to this vacuum will cause the probe to shut down and warm up to room temperature.

- At the probe, the process of tuning is significantly different. Refer to Figure 8 below for the following description which shows the tuning assembly at the bottom of the probe.
- The tuning assembly consists of two parts, the first part is a small brass “tuning knob” which is used to tune the selected channel.

IMPORTANT, the tuning knob slides up and down into the probe to engage the actual tuning rods inside the probe. The tuning knob **MUST BE PULLED DOWN** before a different channel can be selected.

- Before starting tuning, make sure that the tuning knob is pulled all the way down (Figure 8).
- Use the “**function selector knob**” to select which part of the probe circuit is being tuned. The window in the side of the function selector shows which part is being tuned. The options are:

1H –M	Proton Match	1H-T	Proton Tune	
2H	Deuterium Tune	13C	Carbon Tune	15N Nitrogen Tune



- Rotate the “**Function Selector**” until the desired channel is visible in the Indicator Window
- Cable the Preamplifier/Tune Interface as required (described above)
- Selected the appropriate channel number on the reflected display meter as usual
- Push the brass Tune/Match knob up until it seats fully into the slot
- Turn the brass knob to tune and minimize the reflected power on the tuning meter

Proton Channel Tuning:

- Rotate the Function Selector to 1H-T (1H Tune),
- Push the brass Tune/Match knob up until it is fully seated.
- Adjust the 1H-Tune for minimum reflected power.
- LOWER the Brass Tune/Match knob (pull-down),
- Rotate the Function Selector so that 1H-M is in the window,
- Push the brass knob up to engage, and adjust the 1H-Match for minimum reflected power.
- Repeat until the probe is optimally tuned and matched.

Note that the tuning dip is extremely sharp with these probes; therefore, the sensitivity of the tuning is very high. In contrast, the match is very broad and may need to be turned some distance. Typically proton will tune to a reflected power of ~ 2 , and C and N to values $\sim 4-10$. It takes a patience and a careful touch to properly tune these probes.

- After the probe is tuned, be sure to re-cable the system for normal operation,
- For tuning the ^{13}C and ^{15}N channels (tune-only, no match adjustment), the cabling for probe tuning is the same as for a conventional probe.

4. Setting Up Experiments:

Variable Temperature (Sample Temperature):

- The cold probe has a VT range of 0 to +50 degrees Celsius. DO NOT EXCEED THESE LIMITS!
- Do not set the FTS below -15 degrees C.
- **ALWAYS** ensure that there is VT gas flow to the probe (20 L/min). This is very important to prevent the sample from freezing.

Radiofrequency Power Handling Issues:

As with any probe, using too much Rf power for too long a time can damage the probe. The cold-probe is more efficient (in terms of B1 field vs. Rf power); therefore, the user must be cognizant of the correct calibrations for this probe. The power levels for the cold probe must be adjusted from those values used in experiments performed on the regular probe. The values below represent safe operating limits for ^{13}C and ^{15}N Decoupling, and ^1H Spin-Locks. If multiple spin-locks and multi-nucleus decoupling is used, you must adjust your pulse sequence parameters so that you do not put too much power into the probe.

- **Standard Pulse Widths**

1H	8.2 μs at 55 dB
13C	14 μs at 59 dB
15N	40 μs at 60 dB
- **Safe levels for ^{13}C Decoupling:**
 - dpwr = 43 (pw - 80.4 μs , 3.1 KHz field) for a maximum of 12 0msec
 - dpwr = 45 (pw - 63.9 μs , 3.9 KHz field) for a maximum of 60 msec
- **Safe levels for ^{13}C Spinlock**
 - pwr = 52dB (8.7 KHz) for a maximum of 25 msec
- **Safe levels for ^{15}N Decoupling:**
 - dpwr(2) = 42 (pw = 306 μs , 0.8 KHz field) for a maximum of 120msec
- **Safe Levels for ^1H Spin-Locks:**
 - TOCSY, spin-lock pwr = 44 (pw - 29.4 μs , 8.5 KHz field) for a maximum of 80 msec
 - ROESY, spin-lock pwr = 34 (2.6 KHz field) for a maximum of 450msec
- **Safe Levels for ^2H Decoupling:**
 - pw and dpwr(3) 48 dB (pw = 400 μs , field-strength 0.6 KHz) for a maximum of 60 msec

NOTE “ For ^{13}C decoupling, you should use WURST-40 decoupling schemes instead of GARP, wherever possible. This will reduce the amount of probe heating.

On the **CryoBay Monitor**, there is a value for “HEATER”. This represents the number of watts being used in the heater to maintain the probe temperature of 25 Kelvin. As more RF power is delivered to the probe, the HEATER wattage will drop. **PAY ATTENTION** to this heater power when an experiment is started. If the heater power drops by more than **1.2 Watts**, **STOP YOUR ACQUISITION** (aa), Adjust your power levels, spin lock times, acquisition times and relaxation delays to reduce this.

Using Pulsed Field Gradients:

To improve the stability of gradient pulses with the cold-probe, all gradients are implemented as shaped gradients so that the gradient is turned on and off in a smoothed fashion, albeit somewhat crude. This shaping is turned on for all experiments by setting the parameter **gradientshaping='y'**.

It is recommended that all gradient amplitudes used are less than 18,000. Larger gradient amplitudes can lead to a loss in gradient reproducibility, and a reduction in signal to noise.

WARNING, Only pulse sequences that use the “zgradpulse” statement to apply gradients will use gradient shaping. A number of earlier pulse sequences, particularly those obtained from **Dr. Lewis Kay's** group in Toronto use the “rgradient” parameter to apply gradients. **DO NOT USE THESE experiments on the cold probe.** They must be edited to have all the rgradient statements replaced with the “zgradpulse” statement

Data Acquisition Issues:

Your data acquisition with the cold-probe is affected by the long ring-down times of the Rf pulses. To deal with this, a new global, flag parameter called **qcomp** is used in conjunction with **oversampling (dsp='i')**.

- Make sure that **dsp='i'** (dsp?).
- The parameter **qcomp** should be set to 'y' by typing **qcomp='y'**.
- The parameter **rof2** should be set to 2-4 usec
- If digital signal processing is turned off at any time (dsp='n'), then **qcomp** will also be set to 'n'.
- It must be reset to 'y' manually, after dsp='i' is executed.
- The software will reset the oversampling any time the spectral window (**sw**) is adjusted!
- The parameter **oversamp** indicates the amount of oversampling being used. If **oversamp** is too large, acquisition errors can occur!
- BEFORE STARTING YOUR ACQUISITION type oversamp? to check the amount of oversampling being used. There is no real reason for it to be greater than 16.

Summary:

Before starting your acquisition with the cold probe, you should do the following:

- **dsp='i'** (or dsp? to make sure that it is set to 'i')
- **qcomp='y'** (or qcomp? to check)
- **oversamp=16** (or oversamp? to check)
- **rof2=2-4** (not > 5)
- **gradientshaping='y'**
- **CHECK** your decoupling and spin-lock powers, decoupling and spin-lock times, and your acquisition time to be sure that you do not exceed the limits specified for the probe (see previous page).
- After starting your acquisition, **CLOSELY** monitor the CryoBay Monitor window, paying close attention to the Heater power, and the Temperature! You should monitor this for at least 5 minutes after the acquisition begins.
- If the heater drops more than 1.2 Watts from the baseline (i.e. if “Heater” drops from 3.9 to 2.7), **ABORT** the acquisition (aa), and check your decoupler powers, spin-lock powers, decoupling times, and spin-lock times (see above). Remember that in most cases, the acquisition time (at) also represents a decoupling time!
- For long (multi-day) experiments, check the information in the CryoBay Monitor Window each time you check on your acquisition.