

Variable Temperature NMR Experiments

Why Low temperature NMR?

- Chemical instability
- Following a low temperature reaction
- Following exchange processes/conformational changes.

Why high temperature NMR?

- Solubility issues
- Broad peaks
- Following an elevated temperature reaction
- Following exchange processes/conformational changes

Initial Considerations:

- Solvent melting/boiling point.
- Solubility (solubility can be reduced at lower temps)
- Do you need to know exact temperature? If so, calibration is necessary.
- Experiment time: Allow ~ 10 minutes between each change of 10 degrees, both cooling down, and coming back to room temperature.
- If you want to start at -78°C , you need to have a blank nmr tube with your solvent in the NMR as it cools down...shim on the blank, then exchange with your sample (which you already have cooled to -78°C externally) when the blank has reached the desired temperature.

Special Rules and Limits:

USE THE CERAMIC SPINNERS FOR ALL VARIABLE TEMPERARATURE EXPERIMENTS!

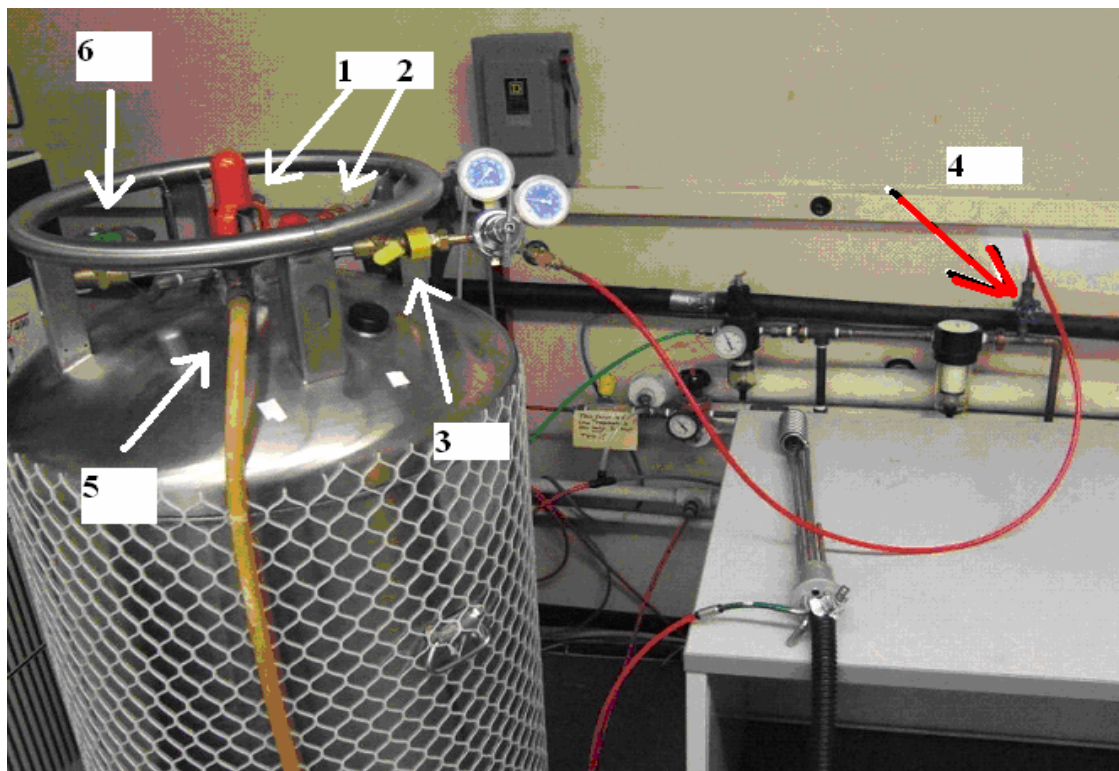
- DO NOT CONDUCT ANY VARIABLE TEMPERATURE EXPERIMENTS UNLESS YOU HAVE RECEIVED PERMISSION FROM RUSS HOPSON.
- The upper limit on heating is 160°C. Nitrogen should be used instead of air for temperatures exceeding ~90°C.
- The lower limit for cooling is -150°C. Nitrogen should always be used instead of air for any low temperature experiments. Contact Russ @33069 if you need to conduct experiments at temperatures below -78°C.
- Always double check that you have turned off the heater when you are finished!!! Just logging out does not turn off the heater.
- It is better to ask questions if you are unsure about something. You can do serious damage to the probe if you do not follow instructions.
- Never conduct high temperature experiments in a sealed tube or near the boiling point of the solvent.
- Never leave the NMR room for more than 20 minute intervals while conducting low temperature NMR experiments, or high temperature NMR experiments above 80°C.
- **Always double check that you have turned off the heater when you are finished!!! Just logging out does not turn off the heater.**

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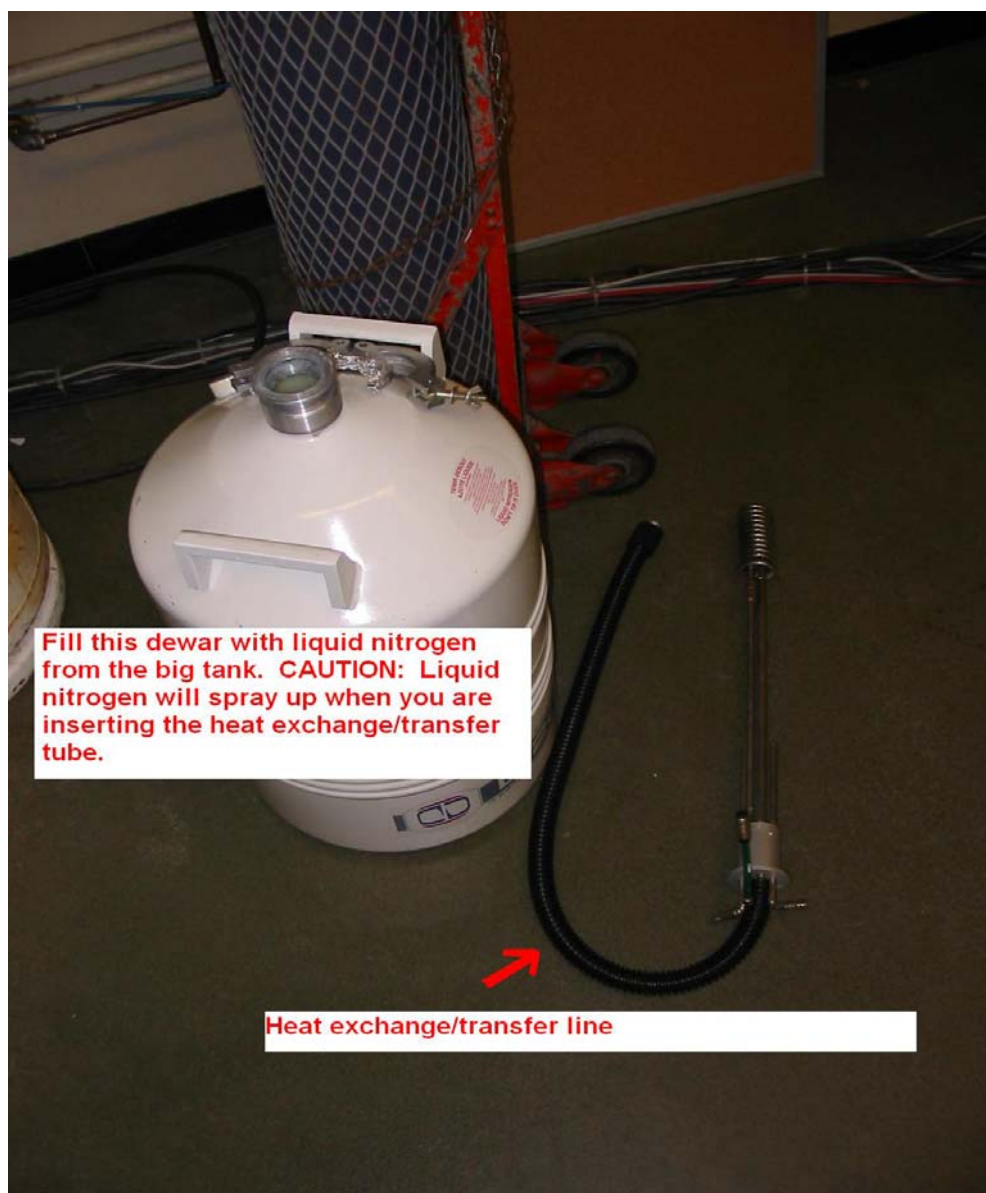
How to set up a low temperature experiment:

NOTE: It is advisable to fill the small liquid nitrogen dewar from the big one the day before you plan to run the experiment (See step 2). Be sure to use safety goggles and gloves when handling liquid nitrogen. Check the internal pressure before attempting a liquid transfer (it should be less than equal to 100psi for liquid nitrogen transfers (if it is greater than this, exercise extreme caution when opening the valve to fill the small dewar. After filling the dewar, the tank pressure should be significantly diminished. At this point it is advisable to open the green valve (~1/4 turn) #6 in figure below and leave it that way overnite. Do not forget to close the valve in the morning.

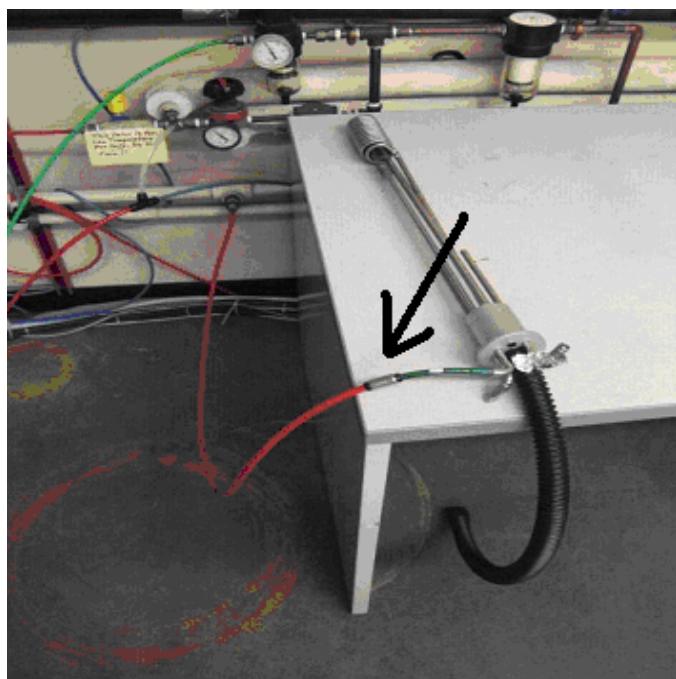
1. Make sure large silver N₂(l) tank in GC410 is at least ½ full (see Figure). Label 1 points to the gauge for checking the tank level. Label #2 points to the pressure gauge for the tank which should be between 100 < 200 psi to provide the desired pressure for spinning the ejecting the sample during the experiment. If the pressure is less than 100 PSI, turn the green knob labeled #6 all the way open and come back and check it in an hour. It is best to check this the night before and if it needs some pressure building, open the green knob just slightly and leave it overnite. PLEASE DO NOT FORGET TO CLOSE THIS VALVE WHEN YOU HAVE BUILT SUFFICIENT PRESSURE!



2. Filling the low temperature NMR Dewar. **CAUTION!! LIQUID NITROGEN SHOULD ONLY BE HANDLED WITH THE APPROPRIATE PERSONAL PROTECTIVE EQUIPMENT (CRYO-GLOVES AND EYE PROTECTION)!** The small dewar is kind of heavy when full so it is a good idea to fill it near the magnet where it will be used as the large dewar has wheels. Insert the yellow tubing connected to the liquid valve ~ 6" into the small Dewar (#5 in figure) and slowly turn the liquid control valve while holding the hose. Once the yellow hose freezes, you can open the valve more to increase the rate. When liquid spills out of the top, the Dewar is full. Turn off the liquid valve and wait for the yellow hose to thaw before trying to remove it from the Dewar.

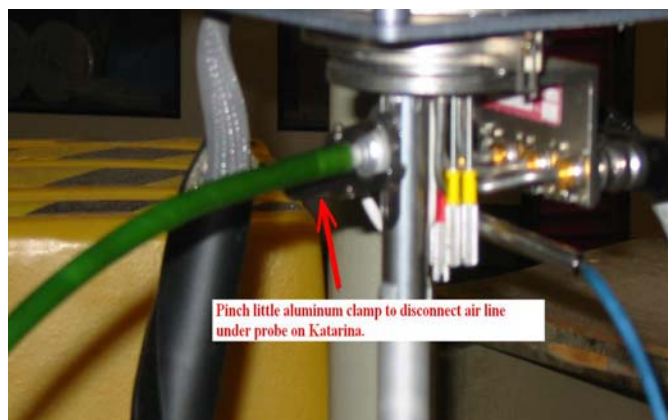


3. Make sure the regulator is on the large tank (first figure - #3) on the 'gas use' port. Make sure the red hose is running from this regulator to the nipple above the blue handled knob (first figure - #4). Make sure the inside pressure gauge is between 100-200psi (first figure - #2). Turn off the house air – behind Katarina – pull the handle up toward the ceiling to turn off. Open the 'gas use' valve on the large dewar and then open the blue handle knob. You have just exchanged 'house air' for nitrogen from the large tank.
4. Be sure to flush the Heat exchanger/transfer line with house nitrogen before you insert it into the small dewar to ensure that there is no condensed water present. Insert the red tube into the coupling joint on the exchanger and turn the house nitrogen valve on to about the same angle shown in the picture. If you open it all the way up the hose will fly off out of the coupling. Allow the nitrogen gas to flow for at least 5 minutes.



You can feel with your hand to see if any moisture is coming out. If you have not done so already, launch Topspin and type 'edte' [enter]. Turn the gas flow down to 130 L/H.

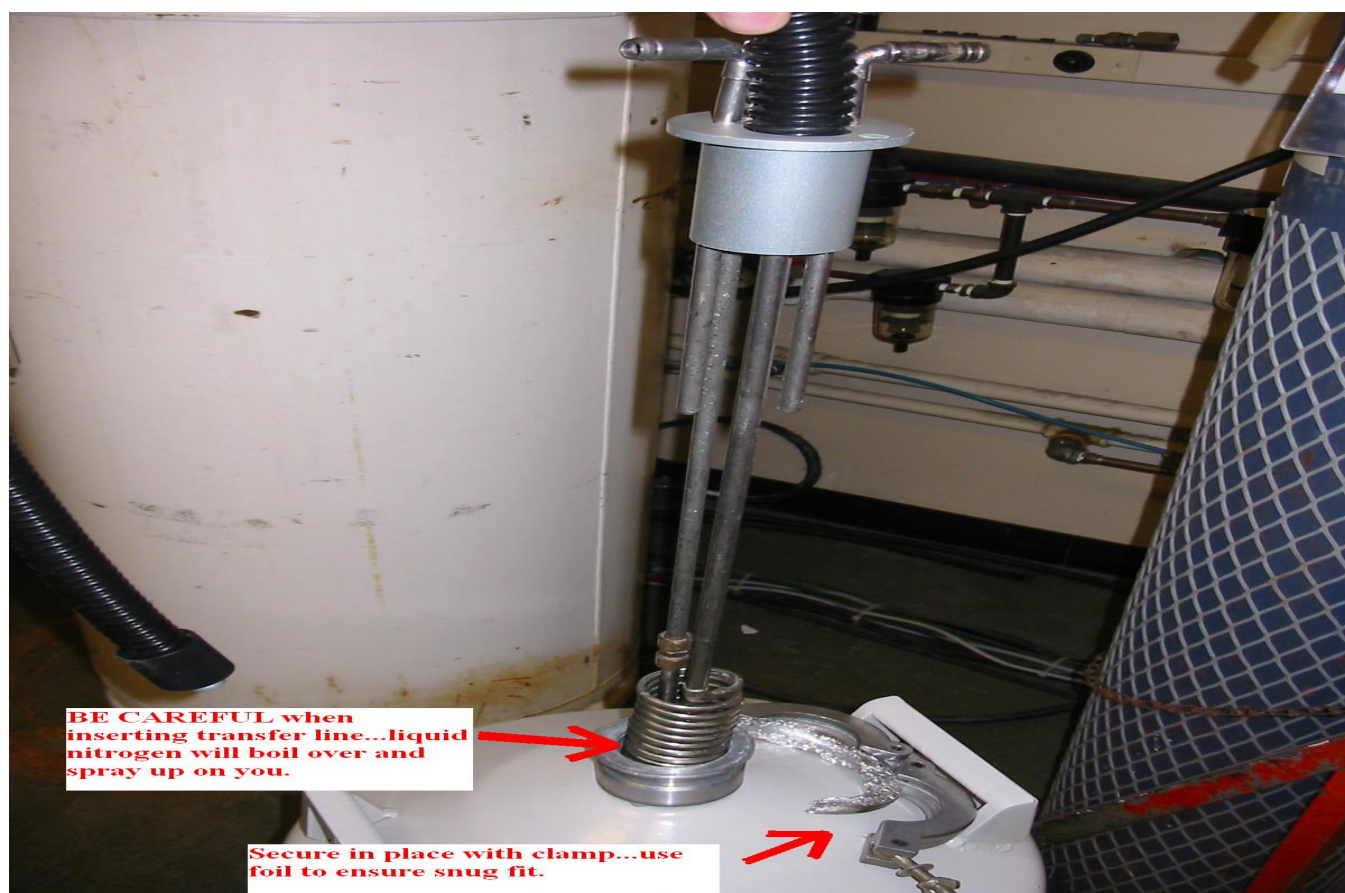
Remove the gas line from the probe (see figure below) by pinching the little aluminum clamp. Remove the nipple from the end of the line and place it on the rim of the magnet base. Insert this line into the coupler on the heat exchanger. You are now ready to lower the heat exchanger into the Dewar. Feel



with your hand at the end of the black insulated transfer line to make sure you feel nitrogen gas coming out.

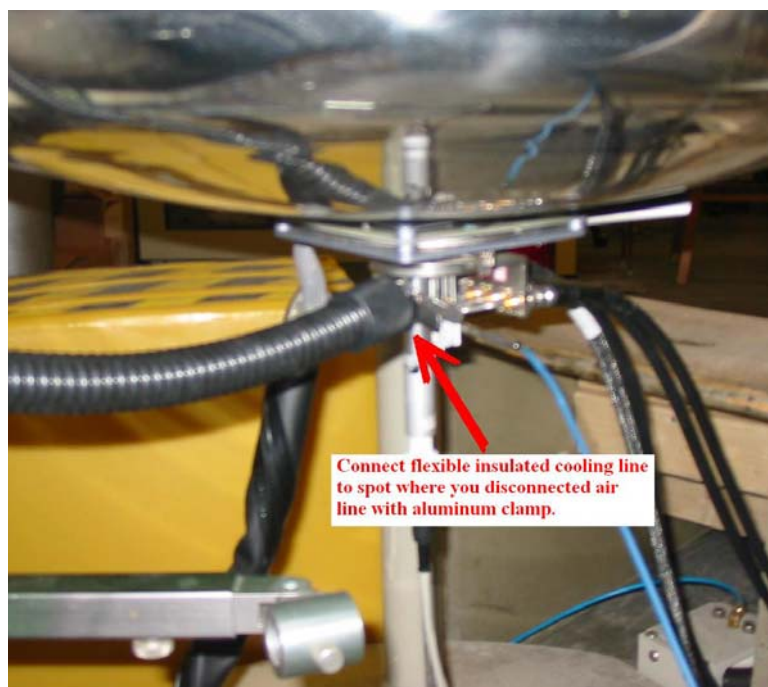
CAUTION!! LIQUID NITROGEN WILL BOIL OVER VIOLENTLY DURING THE PROCESS OF INSERTING THE HEAT EXCHANGER INTO THE DEWAR. WEAR APPROPRIATE GLOVES, FOOTWEAR AND EYE PROTECTION!

Insert heat exchange/transfer line SLOWLY into small dewar and secure in place with the clamp and aluminum foil. When finished, move small dewar over behind spectrometer.



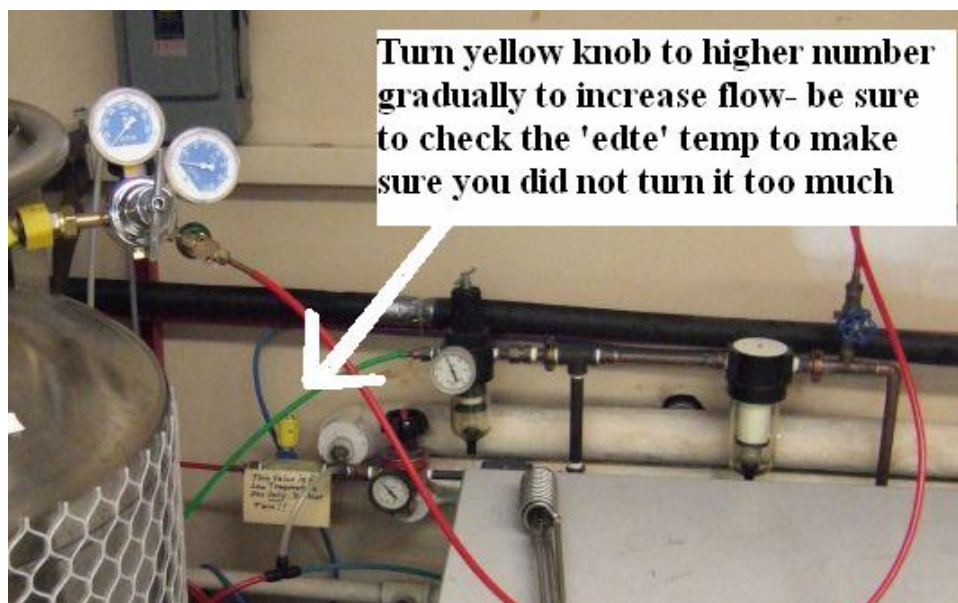
Attach the insulated black cooling line to the probe with the aluminum clamp. Go back to the workstation and click the 'off' button next to 'heater' in the edte window to turn on the heater (the button should now read 'on'). Set the temperature to 15°C and increase the flow rate back to 530L/H. Click the 'set max' button next to heater in the 'edte' window and make sure this is ~10%. The goal is to maintain the desired temperature with the least amount of heating (lower % value here). Do not cool or heat

at faster than 10°C/10 minutes. It is easier to lock and shim if you start at room temperature locked and shim as you cool or heat. Remember to do 'atma' on Katarina at each temperature to tune. When you go below 50C, it is no longer possible to tune properly on the 1H channel. See Russ for the proper procedure for optimizing tuning/matching at lower temperatures.



Connect flexible insulated cooling line to spot where you disconnected air line with aluminum clamp.

NOTE: For temperatures below 0°C, you will need to adjust the little yellow knob shown in the figure below. Turn the knob slightly toward a higher number when you begin to have trouble reaching your temperature (if you turn it too much you will overshoot your temperature so it is best to turn a little and go back to observe the effect).



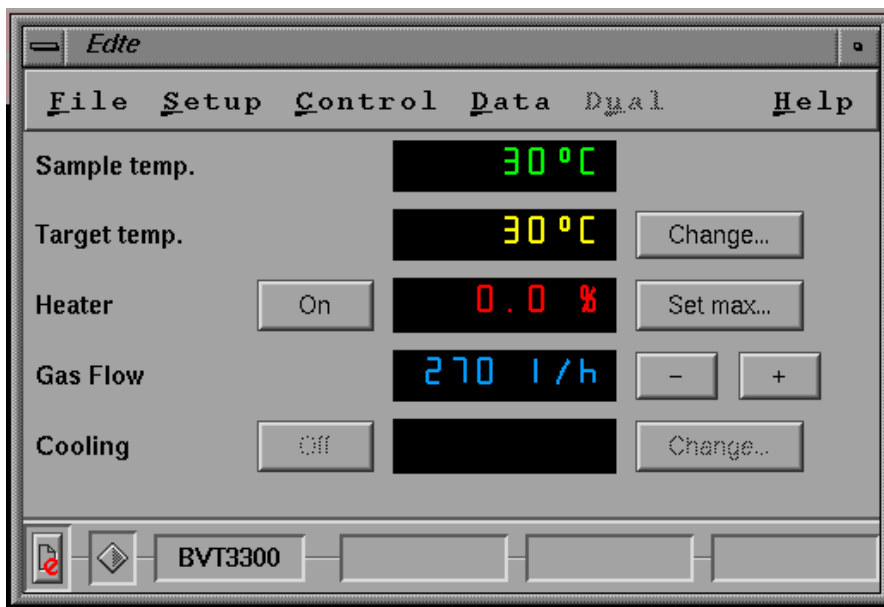
Turn yellow knob to higher number gradually to increase flow- be sure to check the 'edte' temp to make sure you did not turn it too much

- Do not forget to return the temperature to room temperature in 10°/10 minute increments. Warming up from low temperature experiments – when you reach 0°C, remove the green line from the coupler on the heat exchanger apparatus, put the aluminum nipple back in the line and exchange this with the black insulated line such that you are bypassing the Dewar with the nitrogen. This aids in the thawing out process.
- Return system to the state it was in before you switched over to nitrogen if applicable.

How to setup a high temperature experiment below 90°C:

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- Setup and normal proton experiment (ie. Lock and Shim your sample).
- Type 'edte' [enter]. Click the 'Change' button in the 'Target temp.' category and change the temperature in 10°C increments (allow 10 minutes between each change of 10°C) to achieve your target temperature. Click the 'Off' button in the 'Heater' category to turn on the heater. You will need to reshim the magnet at each new temperature. You should tune and match the probe when you have reached your desired temperature (type atma [enter] for Katarina; See procedure on back of COSY instructions for Tara or Oksana).



3. When you are finished with your experiment, return the system to room temperature by lowering the target temperature in 10°C increments (every 10 minutes). **TURN OFF THE HEATER BEFORE EXITING FROM THE EDTE UTILITY WINDOW.**

How to setup a high temperature experiment >90°C.

1. Follow steps 3 of the low temperature instructions to exchange the house air for nitrogen gas. Then follow the instructions for how to setup a high temperature experiment <90°C. If you need further assistance, contact Russ Hopson @33069.

How to calibrate the temperature:

Low temperature – use a solution of 4% CH₃OH in CD₃OD.

Source: Bruker Instruments, Inc. VT-Calibration Manual

4% Methanol in Methanol-d₄:

180-300K, $T = (3.86 - \Delta)/0.00782$ (approximate)

for more accurate values, use the following, depending on T:

180-230K, $T = (3.72 - \Delta)/0.007143$

230-270K, $T = (3.92 - \Delta)/0.008$

270-300K, $T = (4.109 - \Delta)/0.008708$

(Δ is the shift difference (ppm) between the CH₃ and OH peaks)

High Temperature – use a solution of 80% ethylene glycol in DMSO-d₆.

Source: Bruker Instruments, Inc. VT-Calibration Manual

80% Ethylene Glycol (DMSO-d₆): $T = (4.218 - \Delta)/0.009132$

100% Ethylene Glycol: $T = (4.637 - \Delta)/0.009967$

(Δ is the shift difference (ppm) between CH₂ & OH peaks)