

1D Proton NMR on Bruker NMRs using Topspin

What is included in this Handout?

- Website, Data Server, Web-based time reservation, NMR prediction
- Quick start guide
- Spectrometer locking and shimming
- Understanding data sets
- Parameters and acquisition
- Phasing, display, integration,
- Plotting, writing spectra as graphic files
- Fixing problems
- Variable temperature NMR
- Automated processing and integration
- List of NMR Techniques for structure determination

Website, Data Server, NMR time reservation, NMR Prediction

Website There is an NMR web site accessible from the main chemistry web page: <http://www.columbia.edu/cu/chemistry/groups/nmr>. On it you can find copies of many handouts as well as descriptions of some experiments and an instructional video for variable temperature NMR.

Data Server There is a server, nmr14.chem.columbia.edu, from which one can easily download NMR data from all NMR spectrometers to Windows PCs located anywhere. The freeware program WinSep can be used to download data easily to your PC. The freeware program SpinWorks can be used for PC processing. See separate handout.

Time reservation Time on the NMR instruments is reserved via a web-based system. Go to <http://nmr01.chem.columbia.edu>. Access to this site is restricted to Columbia affiliated computers (for example, if at home you have AOL internet, you will not have access). Each instrument has different sign-up rules. In room 208 Havemeyer, there is a Windows PC that is convenient for checking who is currently signed up. Your password for this system is NOT the password for the NMR instruments.













Spectral Prediction On a Windows PC in room 208, an NMR spectral prediction program runs through the MestreNova software. The predicted chemical shifts are superior to those in simple programs such as ChemDraw. Ask for a quick demo.

Magnet Safety

The magnetic fields within about one meter of the magnetic are extremely strong. **Do NOT bring any iron containing materials (tools, gas cylinders, etc.) near the magnet!** Magnetic media such as floppy disks, tapes, and credit cards can be erased if brought close to the magnet. Mechanical watches (those with hands) can be damaged.

Quick Start Guide

1. Insert sample. **ALWAYS SET DEPTH with depth gauge!! Failure to set depth will break your sample and/or the instrument.** Clean spinner with ethanol.
2. Open shell. Type **Topspin** to start program. Or click on Topspin icon.
3. Type **new** - define the data set. Be sure the user name is set to your login id. Set DIR to either /300nb, /300wb, /400, or /500a.

4. Type **lock**, choose correct solvent. Type **lockdisp** to generate lock display.
5. Type **rsh today** If necessary. This resets the shims to the standard best values.
6. Maximize lock level using Z and Z2.
7. Type **rpar protonstd** - reads in default proton parameters
8. Type **start** (macro that includes rga and zg commands) automatically sets receiver gain (rga) and starts acquisition (zg).
9. Type **halt** - stop FID collection before ns scans are completed, or type **tr** - transfer data to processing computer without interrupting acquisition (useful for processing and phasing while acquisition is still in progress).
10. **Type ef or efp**- exponential multiplication (em) and fouier transformation (ft) and/or phase correction (pk).
11. Click phase . Using left button, scroll **0** and **1** to phase. Click save+return .
12. Click calibrate . Place cursor on peak and click left button.
13. Click integrate . Click  to define beginning and end of integral; press left button and drag to define. Right click under integral to select. Adjust selected integral tails to be parallel with baseline by scrolling bias and then slope  . To calibrate, right click under integral and select calibrate. Click save+return .
14. Click peak peak . Make sure define region is selected . With left button drag the box to include the top of all peaks to be picked. To add a peak, click  and select peak with cursor and left click. Click save+return .
15. To print what is on the screen on 8.5x11 paper, select File, Print (Ctrl P). Select print active window and click OK. Select printer room208_letter or room208_11x17. This print method doesn't work properly in room 119 (it takes TOO much time). Set margins properly: 0.5 in. on all sides is good. Click print.
16. To print using the plot editor, select File, Print. Select "Print with layout – start Plot Editor (plot)". You can print on either 11x17 or 8.5x11 with this method. Choose appropriate LAYOUT (see printing section). Click OK.
17. To save to disk as a graphic file, select File, Export. The type of file is determined by its extension.
18. To read in existing data, e.g. on datastation computers, drag data from the browser window into the main window. If your directory is not listed, right click and select "Add New Data Dir..." and add either /300nb, /300wb, /400, or /500a.
19. Exit the Topspin program BEFORE logging off the computer.
20. Questions? READ the rest of this guide BEFORE ASKING!

Sample Preparation

The tube should be filled with a deuterated solvent to a minimum depth of 5.0cm (about 0.60ml). Lesser depths will make shimming the magnet homogeneity difficult. Greater depths are O.K., except for variable temperature experiments. The amount of sample required for a proton spectrum ranges from less than 1mg/ml to about 20mg/ml (mw=400). Too much sample can result in a loss of resolution or a distorted spectrum. This includes not just the sample of interest, but any proton source such as protonated buffers, residual protonated solvents, and water. About 5mg/ml is sufficient. The solution should be free from any solid, such as undissolved solute, or dust. Filter the solution, if necessary.

Starting up and exiting

When the instrument is free, the login screen will be shown. Never use the instrument under a different persons login. To start the NMR program, open a LINUX shell and type **Topspin**. Or click on the icon.

It is very important to exit the program in the correct manner. **Exit the program BEFORE logging off the computer!** (Logging off the computer first leaves processes running and causes problems.) To exit Topspin, type **exit**, and then click on *OK*. To exit from the Linux computer, hold down the right mouse key and select *logout* and then *OK*. (Simply typing *exit* at the Linux prompt does not log you off the computer.)

Loading the sample and locking

All the lock and shim controls are located on buttons on the BSMS keypad (Bruker Shim Microprocessor System).

Push *lift on/off* to eject sample (typing **ej** will also do it, **ij** for insert).

- **Always use the depth gauge to set the position of the tube within the spinner.** Failure to use the depth gauge will result in your tube being broken inside the NMR **and/or the NMR instrument being broken.**
- **Clean the spinner with ethanol.** Failure to clean the spinner will result in spinning problems and the NMR staff getting angry.

Type **lock**, choose the solvent and wait several seconds. Picking the wrong solvent may result in your peaks appearing in the wrong place or not at all. Type **lockdisp** to generate the lock window. Adjust lock gain such that the horizontal line is approximately two-thirds of the way up the screen. You may adjust lock phase for a maximum.

Shimming

If necessary, reset the shims to the standard best values, by typing **rsh today**. Optimize Z and Z². Be sure the *fine* button is illuminated. The goal is to maximize the lock level by adjusting the shim values (lock gain is not a shim value; use lock gain to keep the lock display in the middle of the display range). Adjust the shim values slowly since there is a delay in the lock response. The procedure for shimming is as follows:

1. Adjust Z1 for maximum lock level.
2. Maximize Z2,
3. Repeat until no further improvement is seen. Reduce lock gain if the lock signal goes to the top of the screen. Always end with Z1.
4. Press standby when finished.

Do not adjust Z3 or Z4. The proper value of Z4 requires hours of shimming. If you have followed the above procedure, any change in Z4 should reduce the lock level. If and only if, the sample is not spinning, you should adjust x and y.

Data Sets

All data is automatically written to disk. If you do not change the data set name before acquiring data, the new data will over-write existing data. Thus defining a data set is one of the first steps and should be done prior to reading in parameters. Type **new**:

Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type.

NAME	COURSE
EXPNO	1
PROCNO	1
DIR	/400
USER	decatur
Solvent	CDC13
Experiment	Use current params.
TITLE	

The five parameters must follow these guidelines:

- ◆ *NAME* - name of data set - usually describing your sample. The use of several special symbols in the name, such as /, !, \$, * and . are not permitted and will cause errors.
- ◆ *EXPNO* - experiment number (can only be numbers)
- ◆ *PROCNO* - process number (can only be numbers)
- ◆ *USER* - must be set to your exact login ID.
- ◆ *Dir* - name of the physical disk where your data is stored

Each instrument has a different disk unit and their names are given in the following table:

	NMR Instrument			
	300nb (rm 119)	300wb (rm208)	400	500
Disk Name	/300nb	/300wb	/400	/500a

The parameters defining the current data set are found at the top of the Topspin data window. The parameters are in the order Name Expno Procno Dir User. The parameters represent directory names on the computer's disk and are arranged in a tree structure with the disk unit at the top. For example, under a user name (yours) there may be many names of data sets. Under one name, there may be many experiment numbers, and under a given experiment number, there may be many process numbers. The hierarchy of data set nomenclature is shown in figure 1.

Raw data, that is, FIDs, and parameters are identified at the experiment number level. Completely independent data can be identified by different names or experiment numbers. Spectra are identified at the process number level. Different process numbers are only used for different processing (window functions, FT, difference spectra) of the same raw FID.

File Commands

To read in existing data, e.g. on datastation computers, drag data from the browser window into the main window. If your directory is not listed, right click and select "Add New Data Dir..." and add either /300nb, /300wb, /400, or /500a.

Or, select File, Open and choose "Open NMR data stored in standard Bruker format".

Data sets have UNIX permissions. You only have permission to operate on (phase, delete, etc...) data sets that you have created.

Set-up and Acquisition

Type **rp_{ar} protonstd** (and click on copy all) to read in the standard proton parameters. This overwrites existing parameters. If you wish to change parameter values, such as for **ns**, you must do so after you have given this command.

At this point it may be necessary to tune the probe. Tuning the probe refers to adjusting capacitor rods on the probe in order to make the resonance frequency of the probe circuit equal to the NMR frequency.

Usually this is not necessary for routine proton. It is necessary for more advanced proton experiments (COSY, NOE) and **all carbon experiments**. To tune the probe, type **wobb**. The tune window should open, if it doesn't type **acqu**. In a few seconds a tuning curve with a "dip" will be displayed. The tuning and matching rods are now adjusted to make the "dip" both centered and as deep as possible. Type or click **stop** to end this tuning procedure.

Type **rga** and wait several seconds to automatically set the receiver gain, rg. This step is necessary for proton NMR. Values of rg range from 1 to 32,000 with 1 being the minimum. *(Do not use the start or rga command for carbon spectra.)* Type **zg** to start an acquisition and it will continue until NS scans are acquired. The command **start** which does both **rga** and **zg** may be given instead. Typing **halt** stops an acquisition while retaining the data. Typing **stop** aborts an acquisition and discards the data. Typing **expt** calculates the length of time the experiment will take. After the acquisition is finished or halted, type **ef** to perform exponential multiplication and transform your data or **efp**, which also performs the last-saved phase correction.

During long acquisitions, such as for dilute samples, it is desirable to look at the transformed spectrum without stopping the acquisition. Although this is automatically done with Varian software, an extra command is required on Bruker systems. Type **tr**, followed by **ef** (or other suitable command) to transform the FID.

Parameters

The parameters can be accessed from the tabs in the data window or one can type **eda**(acquisition) or **edp**(processing). Alternatively, each parameter may be changed by simply typing its name, and then changing the value. Some important parameters are as follows:

- sw - spectral width
- o1p - transmitter center (center of spectrum)
- ns - number of scans
- rg - receiver gain
- d1 - relaxation delay
- p1 - pulse width (for simple 1D acquisition)
- aq - FID acquisition time
- td - number of points used to define FID
- si - number of points used during FT (can be different from td)

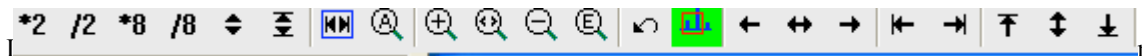
Processing

The following commands are useful for 1D NMR:


- **em** - exponential multiplication on the FID, uses the parameter LB. This improves signal to noise at the expense of resolution.
- **lb** - this controls the degree of broadening added and affects your signal-to-noise. To see its effect, simply change its value and re-Fourier Transform with **ef**.
- **gm** - gaussian multiplication on the fid, uses the parameters LB and GB.
- **ft** - fouier transform
- **ef** - combines **em** and **ft**
- **gf** - combines **gm** and **ft**
- **pk** - phase correct, applies the last phase correction to the spectrum. Useful when you have phased a primlinary spectrum, (with only a few scans) and wish to apply the same phase correction to the final spectrum.
- **efp** - combines **em**, **ft**, and **pk**.
- **abs**- automatic baseline correction
- **apk** – automatic phase correction

To investigate different window functions, click on *process* and select *manual window adjust*.





Display



The above shows the buttons for changing the display. Useful buttons include:

-  This resets the horizontal display which is useful when the spectrum is outside the normal window or to return to the full spectrum after an expansion.

PHASING

Click phase . The largest peak has a vertical, red, line going through it. Place the cursor on , and depressing (keeping it depressed) the left mouse button while moving the mouse, phase the largest peak. Place the cursor on , phase the rest of the spectrum. When done, click return and then select *save & return* . To exit without memorizing the new phase, select *return*.





EXPANSIONS


The easiest way to do horizontal expansions on the screen is to click the left mouse button and drag.


CALIBRATION

Click calibrate . Place cursor on peak and click left button.





INTEGRATION

Click integrate . Click  to define beginning and end of integral; press left button and drag to define. To phase or reference an integral, they must first be selected. Right click under integral to select. Adjust selected integral tails to be parallel with baseline by scrolling bias and then slope  . A properly defined integral should extend beyond the apparent ends of the peak (if there is no other adjacent peak). A properly phased integral should be horizontal before and after the peak.

If peaks are very close together, phasing the integrals separately may not be possible. The best procedure is then to define one large integral that covers the entire spectrum, adjust bias and slope, and then “chop” it into smaller integrals. To do this “chopping” click .

To calibrate, right click under integral and select calibrate. To leave integration, click save+return .




PEAK PICKING

Peak picking refers to creating a list of peak shifts to be printed. Click peak pick . Make sure define region is selected . With left button drag the box to include the top of all peaks to be picked. To add a peak manually to the already listed ones, click  and select peak with cursor and left click. Click save+return .

TITLE

Click on the Title tab in the data window.

MULTISPECTRA MODE

Click  and drag spectra into the data window. There seems to be no limit to the number of spectra that can be displayed. To space them uniformly, click . To give them a horizontal offset, scroll . One can print directly from this window using the Ctrl P print method but one *cannot* start the Plot Editor from multispectra mode and have more than one spectrum displayed.

Plotting using print command (in room 208 ONLY)

To print what is on the screen on 8.5x11 paper, select File, Print (Ctrl P). Select print active window and click OK. Select printer room208_letter, room208_11x17. In room 119, this method doesn't work properly (it takes too long to print). Set margins properly: 0.5 in. on all sides is good. Click print.

This print output can be saved to disk as a graphic file such as JPEG. Select File, Export. The type of file is determined by its extension.

Plotting using Topspin Plot Editor (formally Xwinplot)





To print using the plot editor, select File, Print. Select "Print with layout – start Plot Editor (plot)". You can print on either 11x17 or 8.5x11 with this method. The paper size, printer location, and whether the data is 1D or 2D depends on the LAYOUT chosen. Choose a LAYOUT from the table below. Click OK.

Valid LAYOUT files for Plot Editor	
1D119_11x17	1D119_8.5x11
2Dhomo119_11x17	2Dhomo119_8.5x11
1D208_11x17	1D208_8.5x11
2Dhomo208_11x17	2Dhomo208_8.5x11
2Dinv208_11x17	2Dinv208_8.5x11

The Plot Editor is a very versatile graphic object editor. It allows several different spectra to be plotted on the same page, expansions to be plotted on the same page, and many other features. An important idea to remember about this editor is that it is only a display editor. All objects, i.e., integrals, peak lists, etc, must be defined from within the main Topspin program before you enter the Plot Editor.



A picture of the left side of the interface is shown. The current “mode” is in the lower left corner. The mode controls the type of actions performed by the mouse. The mode is set by clicking on its icon. The most useful modes are:

- 
 Spectrum mode. This is used to draw spectra and is always the first mode used,
- 
 Object Selector mode. This is used to edit already drawn spectra. Objects can be moved and resized and the spectra within them can be moved and expanded.
- 
 Expand mode. This is just one way of making an expansion. By mousing down with the left button you draw out a rectangle which defines the region to fill the object.
- 
 Multi-spectra mode. This is one way to bring in several spectra and allows control of their alignment.
- Other useful modes include Title and Annotate (ABC), which is found by clicking on the Basic button.

To bring in a spectrum, set the mode to spectrum. Move the cursor into the main drawing area, hold down the left mouse button and, as you move the mouse, it draws out the spectrum of the current data set. Hold the mouse down until the spectrum covers most of the main space.

Object selection and editing

Each spectrum brought into the editor is considered an object. The object as well as the spectrum within it can be moved, resized, deleted, and edited. Set the mode to object selector. Then click within the drawn spectrum to edit it. Green dots should now surround this object. Using the left mouse button, the object can be resized. Using the middle mouse button, the object can be moved. These tasks are accomplished by placing the pointer on a green dot “handle”, depressing the mouse and dragging. Selected objects can be deleted by clicking on *delete*.

To edit the spectrum within the object, while the green dots surround a spectrum, click on *ID-Edit*. From here you can expand, or move the spectrum within the object. Expansions can be made in this way. This is also where integrals and pick peaking are turned on.

Insets and expansions

An inset is an expansion of part of the spectrum. To make an inset, you must bring in second complete spectrum and then edit it so that only the region of interest is displayed. To do so, set the mode to spectrum, bring in a spectrum, change the mode to object selector, select the new spectrum, and then resize it by grabbing its green dot “handles”. Then click on *ID-edit* and expand and shift the spectrum to display what you want.

Alternatively, once the second spectrum is brought in, it can be expanded by using the *EXPAND* mode. Click the left mouse button and while keeping it depressed, draw out a box around the expansion you want.

Integrals and peak picking

Integrals and peak peaking may automatically be displayed on start-up but if they are not, follow this procedure:

To display integrals or peak peaks for a given spectrum, they are turned on within the 1D-EDIT editor. For peak peaking, however, an additional step is required before this step. Click on *Topspin*, then *Topspin interface*, and then *create peak list*.

Integrals and the threshold for peak peaking must be defined from within the main Topspin program prior to displaying them here. If you have forgotten to do these steps you do not need to exit from Plot Editor. You simply need to go back to the Topspin window.

If your integrals or peak listings are not what you want, you must go back to Topspin to change them. For peak-peaking, you must reset the threshold, and then type pps. Then within Plot Editor, under the Topspin tab at the top, select *update data*.

Spectrum Line width too thick?


The spectrum line width is often by default too thick, resulting in a low resolution plot. You can fix this. Click attributes and select super-thin for line width. Then under file (within the attributes box), select save as default. The default line width will now be super thin.

More than one spectrum and aligning them

One can also bring in several different spectra. For this to work, you must follow this procedure: click on *data*, and then *edit*. This brings up the portfolio editor. Select a data set and then click on *append* and then *apply*. The name of the data set is now in the data set selector window. Repeat this for each data set to be brought in. Now within the data set selector, highlight a data set and click on *apply*. Draw out the spectrum. Now, highlight a different data set in the data set selector, click on *apply* and draw out the next spectrum. Repeat for each remaining spectrum. The only problem with this method is that they are not necessarily aligned.

An easy way to get two spectra to have the same displayed dimensions (and thus aligning them) is to do the following: Copy the first spectrum and then replace the copy with the second spectrum. To do this, follow the above procedure to load the data sets names into the data set selector. Draw out the first spectrum. Switch to green-dot-mode and select the spectrum and then click on *copy*. Move this spectrum by placing the cursor on a green dot and using the middle button, drag the spectrum. Then, in the data set selector, highlight the second spectrum and click on *SET*. The second spectrum should now appear.



A third method utilizes the multi-spectra mode, . This is useful for displaying spectra with consecutive experiment numbers. Follow this procedure: First, add all desired spectra into the Data Set Selector using the method described above. Then, select multi-spectrum mode, and draw out your spectrum. Only one spectrum will be drawn out initially. Then, select object selector mode, select the spectrum, and then click *edit*. Towards the bottom of this edit list, are the parameters Number of Stacked Spectra and Spectra Offset. Enter the number of spectra and click on *Apply*. Multiple spectra should now be displayed. Spectra Offset adjust both the vertical and horizontal offset.

Title and annotation

To display the title assigned in Topspin, set the mode to Title, and then click on the left mouse. Place the title and click again on the left mouse.

To annotate, set the mode to ABC. Place the pointer, click on the left mouse, type your comment, and click the left mouse again. This is an easy way to add a title.

Plotting

There is a dotted black box around the screen. Only things within this box will actually be plotted. To plot, click on *file*, then *print...* Select *print*.

If you are interested in importing spectra into Word or other programs, the Plot Editor can write the spectrum in the TIFF or JPEG, or PDF format which can be transferred to PCs and Macs. To print to a file, under Options, Printer setup, scroll down through the printer types. At the bottom there are JPEG, PDF, TIFF, EPSI, and PCX. Select your preferred format and click OK at the bottom. To print, under File, select Print, To File and use your home directory (/home/yourloginname/). Since files in your home directory are not automatically deleted, please delete these yourself when you are finished.

Data Stations, off-line processing

To increase the throughput of the routine 300 and the 400 spectrometers, use the data station whenever someone is waiting for the instrument. Do not sign up for NMR time if you are only processing or plotting data. When using the data stations, your data is not “transferred” or copied to the other computer but is only accessed over the network.

Problems

The following are common problems on Bruker spectrometers and their solutions:

1. The commands rga, start, or zg do not respond or are “hung”. The solution: reboot the spectrometer's computer (not the Linux box). Open up the console and push the red button labeled "reset" on the CCU board located in the upper left of the console. Wait for 1 minute. Exit the Topspin program and then restart it. Type ii. Proceed.

2. The Linux computer is frozen. Solution 1. Try to kill the process responsible. Hold Ctrl-Alt-F1 and then log in. Type killtopspin to kill all Topspin processes. Hold alt-F7 to return to the X graphical interface. Solution 2: Reboot the Linux computer. **Do not push the power button on the Linux boxes.** To reboot, hold Ctrl-Alt-F1 and then, after a few moments, Ctrl-Alt-Delete.

Here is a list of computers and their names:

<u>NMR</u>	<u>Computer Name</u>
500	nmr15
400	nmr11
300wb	nmr12
300nb	nmr16
datastation in 208(by 400)	nmr08
datastation in 208(by itself)	nmr10
datastation in 119	nmr09
SGI datastation	nmrsgi04

Archiving Data – Deletion Policy

Data is currently not being deleted but this is subject to change. Data may be backed up using CD, DVD, USB memory sticks, or downloaded to PC from the NMR data server. See NMR data server handout for details.

Variable Temperature

Each of the instruments has a different variable temperature range. **The variable temperature range of the 400 and 500 is limited to -80°C (193K) to $+80^{\circ}\text{C}$ (353K). The range of the 300nb and 300wb are -100°C (173K) to $+100^{\circ}\text{C}$ (373K). Do not exceed these ranges. For temperatures above about 30°C , the ceramic spinner should be used. The plastic spinner will expand, deform and become stuck when heated above room temperature. The ceramic spinner should only be for VT work since it is fragile and will break if dropped (cost \$500). **Ask for assistance the first time you set up a VT experiment.****

Operating the Variable Temperature Controller

There is now a video which describes the operation of the VT controller. See below for details. There are two types of VT controllers: one for the 500 and one for the 300wb, 300nb, and 400. The difference is that the 500 has manual buttons that enable the heater currents for the sample and the liquid-nitrogen evaporator, that are located on the controller. On the other instruments, these functions are software buttons, found within **edte**, the VT editor. The procedure described below assumes software buttons as for the 300wb, 400, and 300nb. The 400 and 300nb share the liquid nitrogen apparatus (there is only one for the two instruments). Make arrangements for either John or Paul to set up the apparatus for you on these instruments.

Low Temperature VT

This system work as follows: a heater within a liquid nitrogen dewar boils off cold gaseous nitrogen which then exits through an insulated hose that is attached to the probe by a ball-and-socket joint. This cold gas is then heated, by the sample heater, to the desired temperature. A sensor, located near the sample, sends a signal to the controller, which then increases or decreases the sample heater current so as to maintain the desired temperature.

- **On the 400 and 500, the MINIMUM Temperature is -80°C .**
- Fill the liquid nitrogen dewar and insert the nitrogen boil-off assembly and hose into the dewar. Be sure an O-ring is in place. Clamp it using the metal compression fitting. A full dewar lasts at least 10 hours.
- Remove the green air hose from the probe and connect the nitrogen boil-off hose to the probe with the ball-socket joint. This ball and socket joint is very fragile and can easily be broken. The dewar inside the probe costs \$600. To reduce the chances of breakage, be sure to not move the dewar after the hose is attached. That is, move the dewar into place and THEN attach the hose.
- Type **edte** to invoke the VT editor. Click on change next to target temperature and enter the desired temperature in Kelvin. There are two buttons within **edte**: **Heater** which enables the sample heater, and **Cooling** which enables the nitrogen boil-off heater. Turn these both on.
- You may need to adjust the cooling power which controls the current to the nitrogen boil-off heater. To do so, click on change next to the Cooling window. More current generates more cold gas. More gas allows a lower temperature to be reached but too much gas will lift the sample out of the probe. A value of 30% is the maximum usable value and allows a temperature of 200 K to be reached (on the 300wb). You may also need to change the maximum sample heater current. A higher value allows a higher temperature to be reached. Do not set this above 50%. If a high temperature cannot be reached, try reducing the cooling current.
- Once the target temperature has been reached, allow the temperature to stabilize for at least 10 minutes before taking an NMR spectrum. Although the controller may indicate the sample temperature is at the target value, the sample will lag behind the sensor because of its greater thermal mass.

- Once you are at the target temperature, the probe must be tuned and the sample shimmed. Both shimming and probe tuning depend on sample temperature.
- To shut down and return to room temperature, follow this procedure:
- Turn off both the sample heater and nitrogen boil-off heater. This is very important since the next step is to disconnect the gas source and the sample heater **MUST BE OFF** when no gas is flowing or it could burn out.
- Warm the ball-and-socket joint with the heat-gun. Often there is frost at the hose/probe connection and if the two are frozen together, forcing them apart can break the probe dewar (cost \$600). Allow them to melt naturally, if necessary. Caution: the heat gun is slightly magnetic.
- Disconnect the nitrogen boil-off hose and reconnect the green air hose.
- Turn back on the sample heater and set the sample temperature to a value between the current temperature and room temperature. When this new target is reached, set the target temperature to 300 K, room temperature.
- Put away the liquid nitrogen assembly.
- Allow enough time (at least 15 minutes) for the probe to warm to room temperature before the next person's time begins. Until the temperature is stabilized, the shim settings drift and a poor spectrum will result. Be courteous!

High Temperature VT (above room temperature)

High temperature VT is much easier. Read the above procedure but the following is all that is necessary. One does not need liquid nitrogen for high temperature work.

- **On 400 and 500, the MAXIMUM Temperature is 80C.**
- Use the ceramic spinner. The plastic spinner expands, deforms and becomes stuck at high temperature. **Caution! The ceramic spinner will break if dropped and costs \$500.**
- Type **edte** to invoke the VT editor. Click on change next to target temperature and enter the desired temperature in Kelvin. Check the above limits on maximum sample temperature. Click on the **Heater** button which enables the sample heater. (it may already be on)
- When finished, simply enter a new target temperature of 300 K.
- Allow sufficient time for the probe to cool before the next person's time.

Caution: Note the boiling point of your sample solvent and keep the temperature 10 K, below this value.

Near Room Temperature VT (slightly below)

For temperatures between 273 K and 300 K it is best to not use liquid nitrogen as the cooling gas. This is because the sample heater must be used at a very high current and provides poor temperature stability as well as increased risk of heater burn-out. It is better (although not absolutely necessary) to use dry-ice ethanol as the coolant. In this set-up air passes through a coil of copper tubing which is immersed in dry ice/ethanol. This acts as a heat exchanger and cools the air that then goes to the probe. Ask for a quick demonstration.

Temperature Accuracy

The controller does not necessarily provide an accurate temperature. To know the actual temperature it is necessary to use an NMR thermometer - the temperature dependent shifts of methanol or ethylene glycol. Over the range 250-320K the difference in shift between the methyl and hydroxyl resonances of 100% methanol, δ in ppm, is given by

$$T(K) = 403.0 - 29.53 d - 23.87 d^2$$

Over the range 300-370K, the difference in shift between the methylene and hydroxyl resonances of 100% ethylene glycol, d in ppm, is given by

$$T(K) = 466.0 - 101.6 d$$

both of these equations are taken from Cavanagh, Fairbrother, Palmer, and Skelton, "Protein Spectroscopy", Academic Press, New York, 1996.

Videos

There is a training video on how to do variable temperature NMR, with emphasis on low temperature NMR. It can be viewed from NMR web site (see first page).

Multi-Spectra Processing

There are several programs that allow multiple spectra to be processed automatically.

multiefp – this does efp on successive experiment numbers using the phase correction determined in the first experiment number. To use this, go to the first experiment number, phase correct your spectrum, save it, and type multiefp and enter the number of spectra to be processed.

multi_integ – this integrates of series of spectra in successive experiment numbers using the same integration regions. The data must be in successive experiment or process numbers. To use this, go to any experiment number, phase correct, and integrate your spectrum (exit integration by saving it as intrng). Then type wmisc, select intrng and give or select a file name. This saves your integral regions as a file. Then type edo and give a file name for CURPRIN (any name will do). Type multi_integ, enter 0 if your data is in sequential experiment numbers (the usual case), enter the first experiment number, the number of experiments to be integrated, and then the name of the file (above) containing integral regions. The results are written to a file int.all in the directory listed in the completion message.

NMR Techniques for Structure Determination

The following techniques are implemented on the NMR instruments in Chemistry. Please ask John Decatur for assistance. Others are available upon request.

1. H-H connectivity -through J coupling

COSY
COSY-DQF
TOCSY-2D
selective TOCSY-1D

2. J measurement (H-H)

H-H Homonuclear decoupling
Spectral simulation (Xsim, gNMR program)
COSY-DQF
ECOSY

3. H-H spatial proximity

NOESY-2D
ROESY-2D
1D-gradient selected NOESY

4. Carbon-proton connectivity - through J coupling

HMQC - direct correlation - one bond
HSQC - direct correlation - one bond
HMBC - long range correlation - 2 to 3 bond
INADEQUATE - proton detected - long range correlation

5. J measurement (C-H long range)

Exside

6. Water suppression

watgate
presaturation

7. Exchange - Relaxation

2D EXESY (NOESY)
selective magnetization transfer 1D
T1 measurement

8. Heteronuclear NMR

Inorganic nuclei, DEPT spectra, etc...

9. Diffusion

diffusion measurements, size determination

10. Solid-State NMR

Carbon, nitrogen, silicon CP-MAS