BRUKER NMR TRAINING

Magnetic Resonance Research Center of the University of Notre Dame

8/18/2022

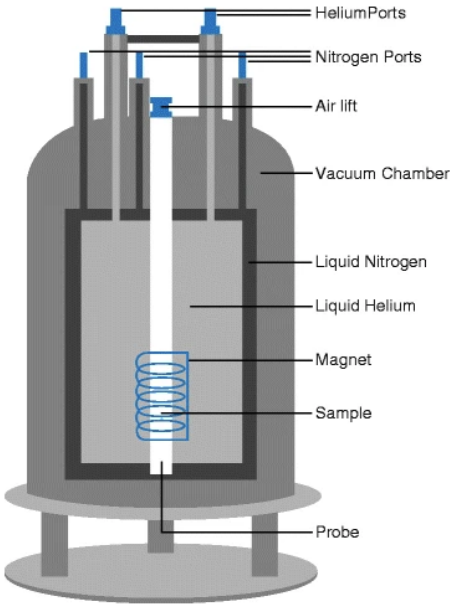
This is a beginner user guide for collecting basic proton and carbon 1D NMR experiments on Bruker instruments at MRRC. If you have any questions, please, do not hesitate to email NMR staff at nmr@nd.edu

Safety

People with cardiac pacemakers are not allowed in NMR rooms!

The magnet is potentially hazardous due to:

- The large attractive force it exerts on ferromagnetic objects.
- The large content of liquid nitrogen and helium.



Magnetic Safety

A magnetic field surrounds the magnet in all directions. This field (known as the stray field) is invisible. Objects made of ferromagnetic materials, e.g. iron, steel etc. will be attracted to the magnet. If a ferromagnetic object is brought too close, it may suddenly be drawn into the magnet with surprising force. This may damage the magnet, or cause personal injury to anybody in the way!

Do not allow small steel objects (screwdrivers, bolts etc.) near the magnet. These could cause serious damage if drawn into the magnet bore.

Cryogenic Safety

The magnet contains relatively large quantities of liquid helium and nitrogen. These liquids, referred to as cryogens, serve to keep the magnet core at a very low temperature. Direct contact with these liquids can cause frostbite.

Exit the space immediately in the event of a magnet quench, whereupon the room may suddenly fill with evaporated gas reducing the level of oxygen needed to for breathing.

Low-Temperature Experiments Safety

Users may need to use a portable liquid nitrogen dewar for variable temperature experiments. Always handle cryogenic liquids carefully. Their extremely low temperatures can produce cryogenic burns of the skin and freeze underlying tissue. The required personal protective equipment (PPE) for handling cryogens includes **safety glasses**, **thermal insulated gloves**, **long-sleeved shirts**, **trousers without cuffs,** and **closed shoes**. An optional PPE is a **full face shield** over safety glasses.

A special note on insulated gloves: Gloves should be loose-fitting so they are able to be quickly removed if cryogenic liquid is spilled on them. Insulated gloves are not made to permit the hands to be put into a cryogenic liquid. They will only provide short-term protection from accidental contact with the liquid. The NMR room SCH164 has a pair of cryogenic gloves for you to use.

Chemical Safety

Users should be fully aware of any hazards associated with the samples they are working with. Organic compounds may be highly flammable, corrosive, carcinogenic etc. **No open chemicals are allowed in NMR rooms.** All samples must be prepared and sealed in the user lab. To carry the samples to the NMR room you must use an enclosed sample tube carrier.

Users must follow all regulations found in the Notre Dame Laboratory Safety Manual and Chemical Hygiene Plan. This can be found at:
https://riskmanagement.nd.edu/laboratory-safety/chemical-safety/

NMR Tube Breakages

We are all human, and sometimes things go wrong. If you break a tube in an NMR lab, please try and clean up as much as possible, collecting the broken glass and disposing of it in a safe manner (glass box). We have provided spill kits, brooms, paper towels and glass disposal boxes for you in each lab. Normally, these items will be found by the door or a specific marked location. If a tube breaks **inside the magnet**, you should **stop work immediately** and **inform NMR facility personnel**. If this occurs outside normal working hours, then you should leave a large note on the instrument to warn others not to use it. Please email us and leave a note.

Contact Info:

NOTE: An email sent to **nmr@nd.edu** automatically reaches all NMR facility personnel.

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Standard NMR Workflow

Step 1. Prepare your sample

(for solution-state NMR instruments):

1. In your laboratory (wear gloves and goggles!): dissolve your compound in a deuterated solvent to achieve, optimally, 10-50 mM for proton detection, and 50-200 mM for carbon. Lower concentrations will increase necessary acquisition time (quadratically).
2. Place your sample solution in a 5 mm NMR tube and tightly close it with a cap. Please, make sure to use a tube that is **no longer than 9"**.
3. **IMPORTANT**: Clean up outside of the NMR tube to remove any residue of the chemicals.
4. **IMPORTANT**: Remove your gloves and **leave them in your lab**. You are **not allowed** to enter the NMR room wearing your gloves. This is a violation punishable by revoking NMR access!
5. Place the NMR tube in a secondary container and tightly close it. NMR facility provides some number of sample tube carriers you can use.
6. Remove your goggles and carry the container to the NMR lab.
7. In the NMR lab, **put on your goggles** to place the NMR tube in a spinner and in the NMR magnet.
8. Once the sample is in a sample changer, you may remove your goggles and proceed with operating the NMR instrument.
9. When you have finished your experiments and ejected your sample from the magnet, **put on your goggles**.
10. Place your NMR tube into a secondary container and tightly close it.
11. Now you may remove your goggles and transport the sample back to your laboratory.

NOTE: If you have multiple samples, every time you intend to hold the NMR tube in your hand, your goggles must be on!

NMR time booking

Prior to using any NMR instrument, you must **book time in iLab** (except for the automatic 400). After you came into the NMR room:

1. start your session in Kiosk;
2. log into the computer workstation using your ND credentials.

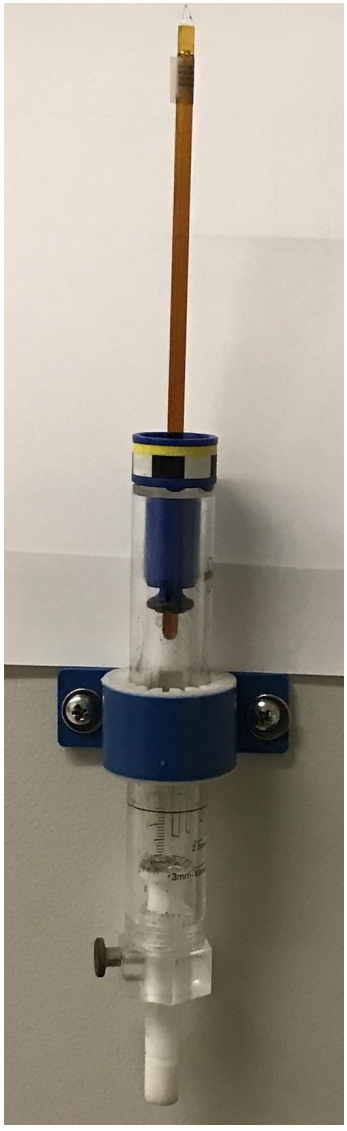
Step 2. Insert your sample into a spinner

The NMR tube with the sample is inserted in a plastic spinner before loading into the NMR instrument. A sample depth gauge controls the depth of the sample tube in the spinner to ensure that the sample is correctly aligned with the coils inside the probe.

Please, note that the minimum sample height (solution column) is 40 mm.

1. Holding the sample by the top, insert the sample tube into the spinner directly in the spinner rack. **Do not touch the spinner!**
2. Place the spinner in the depth gauge.
3. Gently push the sample tube down so it touches the white plastic base.
4. Move the sample into the sample changer carousel holding it by the sample tube.

NOTE: To remove the tube from the spinner, use a paper napkin to hold the spinner.

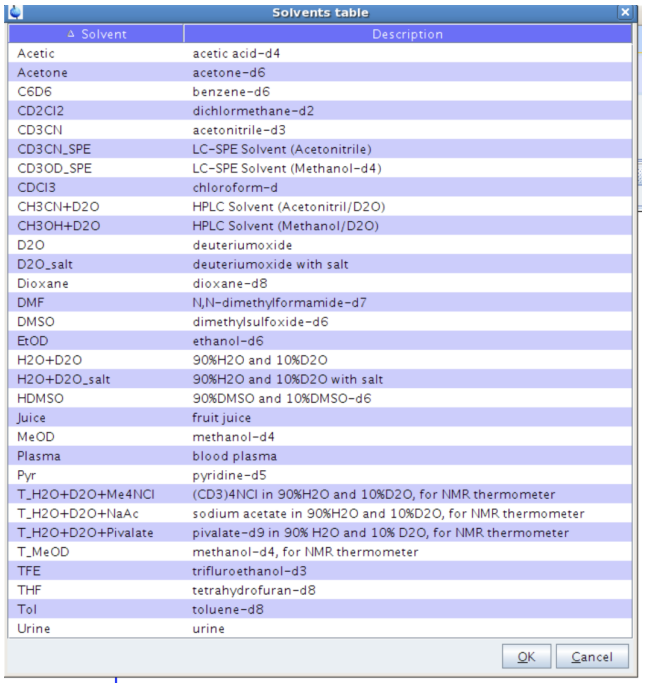


Step 3. Insert your sample into the magnet

On Stepan 400, McCourtney 500, and Bruker 800, **do not move** samples that are already in the carousel. Pick any free position for your sample.

To insert the sample with the spinner into the magnet use the following procedure:

1. Place your sample in any free slot of the sample changer. Note what slot it is in.
2. Type **`lock off`** and then **`ro off`** to unlock and stop spinning the current sample.
3. Type the command ***`sx N`***. Where **'N'** is the slot your sample is in, i.e., ***`sx 6`***. This will eject the current sample and load the sample from the slot 6.



NOTE: The standard $CDCl_3$sample must be put back into the magnet and locked after you finished your experiments and before you log out.

Step 4. Load a standard shim set

Type **rsh** and find **bbfo-latest** in the list. Click **Read.** This is required to "erase memory" of the previous user's sample from the instrument.

Step 5. Lock your sample

Your sample **must** contain a deuterated solvent!

1. Type the command **`lock`**. This will display the solvent selection menu (right).
2. Select your solvent and click OK.
3. The system will begin to lock automatically.

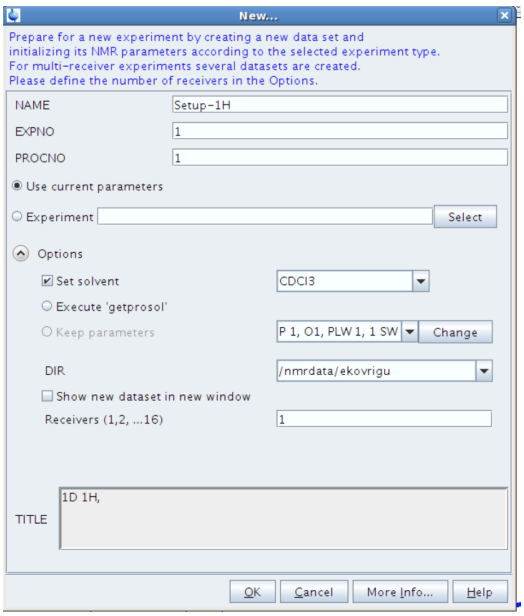
NOTE: **lock off** unlocks the sample.

Step 6. Create a new data set for proton 1D

In a home directory of each user, we set up two standard experiments for acquisition of 1D 1H and 13C spectra, respectively. They can be used to create new folders for your measurements.

First, **you always create and record a proton spectrum**:

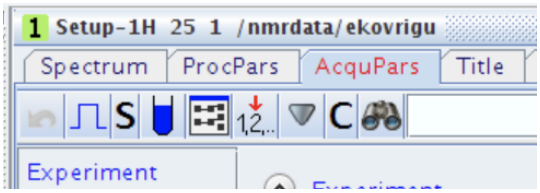
1. Drag the *Setup_H1* file from your folder into the spectrum display area
2. Click Start → Create Dataset (or type **new**)
3. Specify the dataset **NAME**. Set **EXPNO** to 1
4. Check the **Use current parameters** radio button



5. Type your sample name and description in the **TITLE** box.
6. Click **OK**. The dataset is created and now displayed.
7. On the command line, type **getprosol**.

You may check acquisition parameters by clicking **AcquPars** tab. The blue "square wave" button will display a shortened list of most important parameters.

Likewise, processing parameters may be viewed in **ProcPars** tab.



Step 7. Tune the probe

NOTE: It is critical that you created the dataset and have it displayed **before** you start tuning. The tuning routine will look **into your dataset** to know what nucleus you want to work with, therefore, which channel to tune.

Type **atma** to automatically tune. Watch the screen for a tuning curve: it must end with centered resonance and give a message "tuning … OK" in the status bar.

IMPORTANT: If you intend to record experiments on the **same sample** with the **same nucleus** (all made from the same Setup-... file) then one tuning is enough. However, if you continue to a different nucleus (like from Setup-1H to Setup-13C) you must **create a new experiment** and **issue atma again.**

Step 8. Start spinning

Spinning improves resolution and sensitivity as well as shimming performance.

Type the command `ro on` to start sample rotation.
NOTE: `ro off` stops sample rotation.

Step 9. Shim the probe

Shimming adjusts magnetic field to make signals narrow and symmetrical. **Every time** you ejected and inserted a sample, **you must shim** again. For shimming to work, the sample must be locked (in previous steps). Type the command `topshim` to start automatic shimming.

If you see errors and shimming fails, verify that:

1. You are using a **deuterated** solvent;
2. Your sample height is 40 mm or more;
3. You do **not** see a significant precipitation, bubbles, or phase separation in the sample volume;
4. Locking step was successful.

If your answer is **yes** to **all** of these checks, please, contact NMR staff.

Step 10. Acquire NMR data

1. In **AcqPars** tab, set the desired number of scans, **NS**. The value of NS should be **even** or divisible by **4** or **8** (better). How long the experiment will take can be checked by typing **expt.**
2. Type **rga** (this sets receiver gain)
3. Type **zg** to start the acquisition.
4. Checking data while the experiment is running: type **tr**, wait till you see a message in the status bar "... saved" and then type **efp; apk**
5. Enter **halt** if data is good.

Step 11. More NMR experiments

If you need to record a carbon 1D on **the same sample:**

1. Go to **Step 6**: create a new experiment starting with **Setup-13C**
2. Skip **Step 8** (it is already spinning)
3. Skip **Step 9** (no need to shim again)

If you need to record spectra on **a different sample**: Go to **Step 2**

Acquisition Rules of Thumb

1. If you switch a sample you must re-lock, re-tune, re-shim;

2. If you keep the sample in the probe and switch a nucleus, you must re-tune (atma).
   NOTE: If you did a carbon experiment, no need to re-tune for proton because the carbon experiment tunes both proton and carbon channels.

3. If you repeat experiments on the same nucleus, the locking, tuning, and shimming remain valid.

Access to NMR Data

Your data is automatically saved to our NMR Data server at nmr.nd.edu/nmr-data-server/.
NOTE: Only **newly created experiments** are copied to the data server. If you decided to re-run acquisition in the existing experimental folder, the data will **not** be updated on a server!

Processing

We advise everyone to perform final processing of your data on your own computers**.** You may download Topspin on your own workstation free of charge for academic use from our homepage. Additionally, MNova Lite CDE is also available free to all users on ND campus. For all details see nmr.nd.edu/software/. Below are instructions on how to reference the spectral axis, integrate and pick peaks in Topspin.

Referencing

It is conventional in NMR spectroscopy to calibrate the spectrum by setting the TMS peak to 0 ppm. Prior to calibration it may be useful to expand the spectrum horizontally in the region on either side of the TMS signal itself, as this will aid in pinpointing the exact position of the peak. If the sample does not contain TMS use a residual solvent signal for referencing. Chemical shift values for common solvents are found in the standard solvent tables.

Expand the Spectrum Horizontally

1. Using the mouse position the cursor within the spectral window and click the left mouse button. It will automatically be tied to the spectrum.
2. Using the mouse position the cursor on one side of the region of interest and click and hold the left mouse button.
3. Using the mouse position the cursor on the other side of the region of interest and release the left mouse button. The defined region will be automatically expanded to fill the entire screen.

Calibration Procedure

1. Open the "Processing" tab.
2. Click on "calibrate". The cursor will be automatically tied to the spectrum. Move the mouse until the cursor is positioned on the TMS or solvent peak.
3. Click the left mouse button, and when prompted for the cursor frequency in ppm, enter zero for TMS or solvent chemical shift value for the solvent.

An automatic calibration of the spectrum may also be achieved by entering the command ***sref*** if your sample has TMS. This procedure searches for a single peak around 0ppm, which may cause an error if your sample doesn't contain TMS. This starts a procedure in which the software searches for a signal in the region of 0 ppm and automatically sets its value to exactly 0 ppm. For the ***sref*** procedure to work, the appropriate solvent must be chosen in the lock routine.

Integrating the spectrum

1. Click on the '**Process**' tab, the '**Integrate**' button in the TopSpin Menu bar.
2. Make sure the "Define new integral range" button is highlighted.

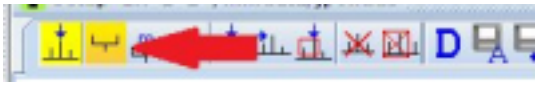
3. Place the cursor downfield of the first peak of interest.
4. Press the left mouse button and drag the mouse up-field of the peak of interest.
5. Release the left mouse button to return.
6. Repeat for each additional peak/region of interest.

1. Place the cursor within the integral label of any peak you wish to calibrate and press the right mouse button.
2. Select '***calibrate'***.
3. Enter the desired value of the selected integral.
4. Click on the floppy disk icon to save the integrals.

Peak Picking

Peak picking can be performed interactively in the peak picking mode.

1. Click on the '**Process**' tab, the '**Peak Pick**' button in the TopSpin Menu bar. This puts you in a "Interactive Peak Pick" mode.



2. Make sure the "Define new peak picking range" button is highlighted.
3. Press and hold the left mouse button over the peaks of interest. A green highlight will form. 4. Release the left mouse button.
5. Click on the floppy disk icon to save the peak pick.

Plotting

To Plot, or Print your spectra, enter the command ***prnt***. This will automatically print your spectra as displayed (what you see is what you get).

You may also use the commands below if you want to change the look of your plot. ● ***plot*** : will start the Plot Editor, the interactive program for viewing or designing plot layouts. ● ***autoplot*** : will plot data according to Plot Editor layout (1D,2D), with the current limits and scaling.
● ***prnt*** : will plot exactly what is in the spectra window of TopSpin (what you see is what you get).