

Instruction for New Users of Bruker 400 and 500

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Introduction

The 400 MHz and 500 MHz NMR are equipped with Prodigy® cryoprobe (cooled by liquid N₂) which substantially improves the signal sensitivity. For a 50mM sample, 64 scans are enough to generate a good quality 13C spectrum. For this sample, running a proton (ns=16) and a 13C (ns=64), including auto lock and shim, and atma, takes a total of 10 minutes.

Table 1 lists typical number of scans (NS) and experimental time required on 400 and 500 for several concentrations. Since you will use IconNMR for automated runs, and IconNMR does not allow you to halt experiment in the middle, you should have an estimate of how many scans you will need. Number of scans needed is usually determined by your sample's molar concentration.

Table 1. typical NS and experimental time required on the 500 for various concentrations

Concentration	Expt (name in IconNMR)	NS	Expt time
>=40mM	Regular 13C (C13CPD)	64	4 m
	COSY (COSYGPSW)	1	4 m
	HMQC (HMQCGP)	1	4 m
	DEPT (C13DEPT135)	64	4 m
10mM	Regular 13C, DEPT	1024	1 hr
	COSY, HMQC	16	1 hr
3 mM	Regular 13C, DEPT	10240	overnight
	COSY, HMQC	128	overnight

All other common nuclei (19F, 31P, 29Si, etc) can be run on both 400 and 500.

Both the 400 and 500 also have 24-sample autosamplers so that multiple samples and multiple experiments can be run with several simple mouse clicks with Topspin's automation module, IconNMR. Since IconNMR only allows limited customization of experiments (ns, d1, etc.) and data processing, we design the user interface in such a way that users can run standard experiments using IconNMR and can also exit IconNMR to do more free roaming in Topspin.

IconNMR is especially convenient for overnight runs. You can easily set up multiple samples and/or multiple experiments for each sample for overnight. Running in IconNMR is generally quicker than in Topspin for running multiple samples or multiple experiments for a sample. The opposite is true for running a simple proton experiment for a single sample.

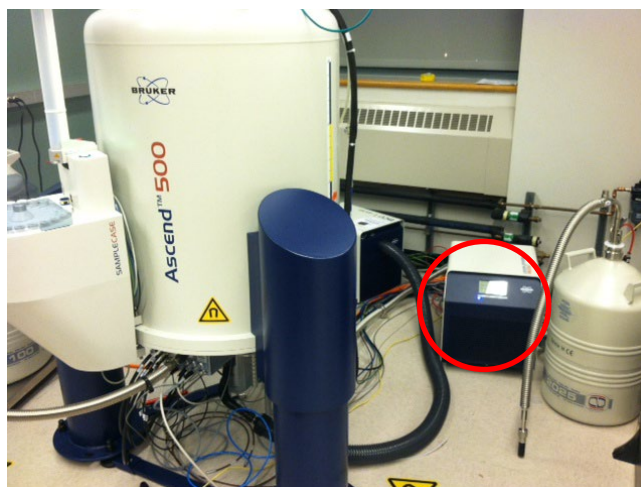


Figure 1. Cryo platform (circled). You should see a blue LED steadily lit before you submit an expt.

You will have two sets of usernames/pswds. The first set is a group username/pswd (username is your advisor's name. passwd to notify separately), for logging onto Linux. The second set is for IconNMR.

Safety and Sample Preparation

The big magnet has strong magnetic field around it that can destroy watches, credit cards, and cell phone memories. These should be left on the table when you change samples. Never bring large steel objects (heat guns, wrenches, other large tools) close to the magnet!

Put spinners on your samples and carefully measure sample depth.

Before you log in, check if the blue light on the cryo platform (circled in Figure 1) is on. If the blue light is not on or is blinking, the cryoprobe is not ready or is malfunctioning. Do not run anything and report to me immediately.

Running Experiments in IconNMR



Figure 2. The autosampler and the stopper (circled)

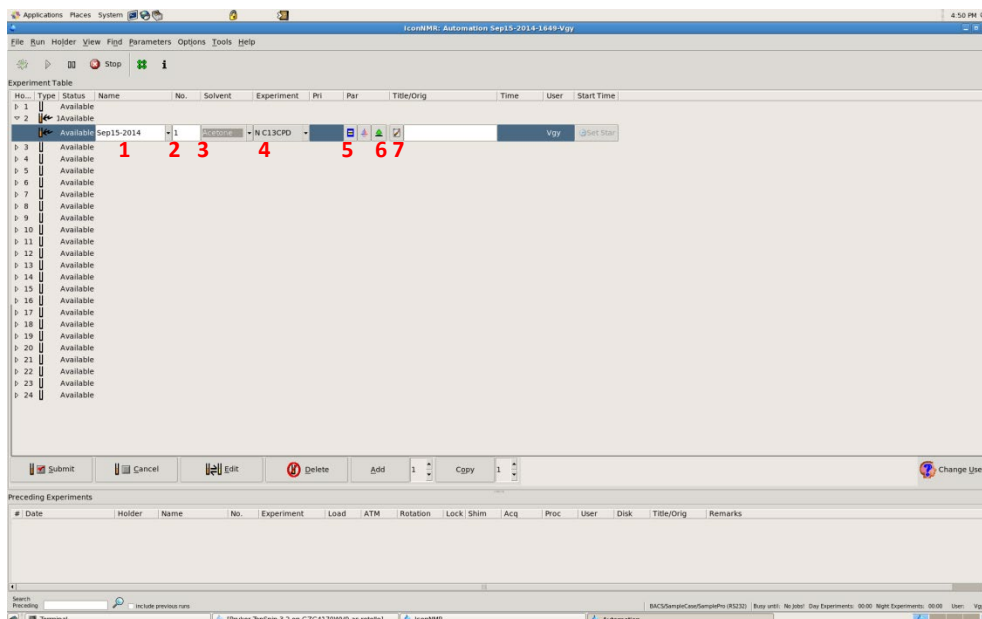


Figure 3. The automation interface. The numbered icons are explained in the text.

- Log onto the computer using your group username/pswd. Double click Topspin 3.2
- The IconNMR automation interface will appear. Type in your username/pswd (you will have an individual username if you are from a big NMR-using group, or just use your group name if you are from a smaller group).

- If the IconNMR interface does not appear, you can go into the IconNMR Interface by typing *icona* at the Topspin command line.
- Put spinner(s) onto your sample(s), carefully measure their depth using the depth gauge. Put the sample/spinner assembly onto the autosampler and note which position is for which sample. It saves you some time to put them on the left side of the current position (see Figure 2) as the autosampler rotates clockwise. The sample should not have peeled-off labels or other sticking-out things that are wider than the spinner or taller than the red stopper (circled in Figure 2). These will obstruct the sample transport.
- For each sample, double click on their corresponding holder number and put in experiment information. Click each icon one by one to from left to right to input/modify information (see Figure 3 to see which number corresponds to which function):
 1. Sample name. You can either type in a name that you like or choose a standard-format one from the drop-down menu.
 2. Experiment number. You can leave it alone. It will start with 1 and increment for additional experiments for the same sample.
 3. Solvent
 4. Experiment that you want to run. Scroll down the list to see all the experiments that are available to you at a mouse click! The most popular ones are near the top: PROTON, regular 13C (C13CPD), DEPT (C13DEPT135), COSY (COSYGPSW), HMQC (HMQCGP). 2D spectra contain many 1D slices so that the experiment seems longer than your prescribed *ns* (for example, for *ns* = 1, expt time = ca. 4 min). However, a 4-min 2D experiment is often more than enough to generate a high-quality spectrum (see Table 1). Note that some 2D experiments would automatically require running a 1D proton as a preparative step for parameter optimization.
 5. Acquisition parameters. Change as needed. To determine a suitable *ns* for your sample, please refer to Table 1. For proton experiments of common sample concentration, 16 scans are more than enough. Once you change some parameters, the icon will become an exclamation mark, which is normal.
 6. Toggle Printing icon, which toggles between a tree, which indicates no auto printout, and a printer, which indicates an auto printout. Auto printout is often not very useful as you need to zoom in regions before printing anyway.
 7. Title. Input additional info (optional) for your sample.
- Click “Add” (in the bar below all the sample holders) to add more experiments (e.g., 13C, DEPT etc.) to the sample. For regular 13C (name C13CPD) or DEPT (name C13DEPT135), the default *ns* is 1024, so you need to modify it if you have a high sample concentration.
- When all the information is completed, highlight all the sample holder lines, then click Submit. The runs will start. If you only highlight a single experiment or a single holder, only that experiment or that holder will be submitted to the queue. But you can continue to submit additional experiments when other expts are running. You can also delete or edit experiment entries as desired. If you have several samples and want each sample to run with the same parameters, use the Copy button.

- As the experiments are being run, you can double click the entry in the bottom pane of the automation window (“Preceding experiments” window, see Figure 3) to view the progress of your experiments. This will be displayed in the Topspin window. In this window the command line and many menu options are grayed out – it is designed this way – but most view and print buttons work.

When your experiments are all done, click the “Stop Automation” button in the IconNMR Auto Online Controls window or click the Stop button in the Automation Interface (on top left in Figure 3). Then quit icon-nmr (there are several IconNMR windows to close!) Now you are in Topspin. Note that you have to stop the automation before you quit IconNMR, and you have to quit IconNMR to fully use Topspin. If you try to close the automation window without clicking Stop, you will confuse Topspin and get yourself stuck.

When you are done, you can quit Topspin, transfer your data, then log out. If you happen to have the longer kind of NMR tube, you might find it difficult to remove from the autosampler as it is directly underneath the vertical transport channel. You can press the blue button on the left side of the carousel (it is illuminated and has a rotation sign on top) to advance the carousel position. Then you should be able to remove the sample easily.

If you wish to do some customized experiments in Topspin, please read the next section.

Data transfer works the same way as before. Your data are stored in /opt/topspin/data/[your id]/nmr.

Please remove your samples from the autosampler when done. Any samples left on the autosampler more than one day can be taken by other users who are willing to clean and reuse the tubes.

Running samples in Topspin

If you need to deviate from the acquisition or processing parameters set by Icon-NMR, you can quit Icon-NMR and run your experiments in Topspin. In the following, the words in *italics* are the commands that you will need to type on the Topspin command line. The words in the squared brackets [] are parameters which you will need to substitute with your own parameters.

- *sx* [*sample holder #*] – this inserts your sample. *sx* stands for sample exchanger.
- *edc* – This creates your data file. In the window that pops out:
 - Give a new name to your file.
 - Expand the Options tab if nothing is shown below it.
 - Click Experiment, then click Select. In the pop-out window, find the small “Source” window on the top-right and select /opt/.../user. Choose the experiment that you want to run:
 - Proton: PROTON1
 - Carbon: CARBON1

- Dept135: DEPT135-1
 - COSY: COSY1
 - HMQC: HMQC1
 - 19F: F19NEW (this one has a rolling baseline. A better version with a better baseline is F19ECHO)
 - For any other experiments that are not listed in this window, select the other directory in Source window (the one that does not end with */user*; has a lot more choices but is also confusing to new users) and find it.
 - Select your solvent. Check “Execute getprosol”.
 - Update the data path in DIR (e.g., */opt/topspin3.2/data/[your data folder name]/nmr*).
 - Keep everything else unchanged.
 - Click OK at the bottom of the *edc* window.
- Change experiment parameters as needed (*ns*, *d1*, etc). Now your new file has all the proper parameters.
- *atma*. NOTE: you **cannot** skip this for proton. If the previous user was running 19F, then if you run 1H without doing *atma*, you will see no signal. If you run 1H in *IconNMR*, *atma* is automatically done for you.
- *rsh shims.best*. This loads in a good starting shimming parameter set.
- *lock*, then select your solvent.
- *lockdisp*. This opens the lock window. Make sure that the green/red scanning line is on the upper half of the window when lock is finished. If the line is near the bottom when lock is finished, locking has failed. Notify your czar or myself.
- *topshim convcomp*. (*convcomp* is a parameter for *topshim* which stands for Convection Compensation). Wait to see the “*topshim: completed*” message displayed below the command line
- *rga* (alternatively, you can type *rg* and manually input a number. For 13C, you can simply always use 203, which is the maximum.)
- *zg*
- for longer experiments, type *tr* then *efp* to check the first few scans (1D). For 2D experiments, directly type *xfb* to examine the preliminary 2D spectrum.
- *halt* when you see that your spectrum is good enough
- process your data – *efp* (1D); *apk*; *abs*; etc. For 2D, the FT command is *xfb*, which stands for Execute FT in Both Dimensions. For pseudo-2D (T1, T2, kinetics, diffusion, etc), the FT command is *xf2*, which stands for Execute FT in 2nd Dimension.
- *sx ej* or simply *ej*. This ejects your sample. If you happen to have the longer kind of NMR tube, you might find it difficult to remove from the autosampler as it is directly underneath the vertical transport channel. You can press the blue button on the left side of the carousel (it is illuminated and has a rotation sign on top) to advance the carousel position. Then you should be able to remove the sample easily.

Note for 19F users

Currently 19F cannot be done in automation. To run 19F, please refer to “Running samples in Topspin” section above.