

NUCLEAR MAGNETIC RESONANCE RESEARCH RESOURCE

AVANCE-500 Reactive Sample Slots

Dr. Mike Lumsden
Coordinator, NMR-3
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The Bruker AVANCE-500 (AV-500) spectrometer operates by default in automation using Bruker's B-ACS 60 autosampler and the software program ICON-NMR. Approximately 90% of all NMR experimentation on the 500 is performed in this manner. The remaining 10% is performed via walk-up access during the so-called "reactive sample slots" or via special request reservations. Reactive sample slots are designed for those researchers who need to perform NMR experiments on reactive or unstable samples as well as for experiments in protio solvents. NMR-3 training courses are available for both of these modes of access; this guide covers details concerning reactive sample slot usage. A separate document is available on the NMR-3 website covering the details of acquiring NMR data in automation.

**** CAUTION ****

The wearing of safety glasses is mandatory whereas lab coats & protective gloves (latex, nitrile, etc) are prohibited at all times while working in NMR-3!

Preparing for a Reactive Sample Slot...

1. Reactive Sample slots are available Monday through Friday, starting at 4 pm. No other start time is possible. These slots must be reserved in FACES in an analogous manner to making a reservation on the AV-300 or AC-250. You may reserve up to a maximum of 5 hours of time.
2. At 4 pm (not before) login to ICON-NMR using the "Icon" account and click the pause icon (**Figure 1**).
3. The queue will be suspended (and your experiments can begin) **AFTER** the current automation experiment completes. This could take minutes or up to an hour.

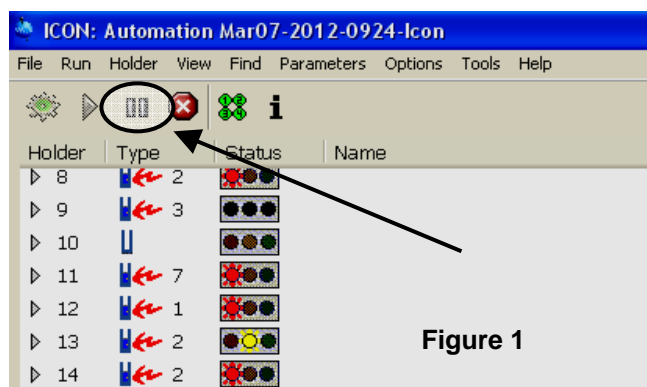


Figure 1

Starting & Preparing your NMR Sample...

4. Once the current automation experiment completes and the queue is suspended, your reactive sample slot booking can begin. You can have up to 5 hours of time beginning at this point. For example, suppose you have reserved a slot in FACES from 4 - 6 pm and the queue suspends at 4:25 pm. You are permitted to work on the spectrometer from 4:25 - 6:25 pm even though the FACES reservation is offset by 25 minutes.
5. Clean your entire NMR tube using a kimwipe. Then insert your NMR tube into one of the available blue spinners, making sure that you do not touch the spinner with your hands (avoids transferring grease from your fingers). If your tube is loose (moves easily) in the selected spinner, please choose a different one.
6. Adjust the position of the NMR tube in the spinner using the sample depth gauge attached to the desk.
7. Using isopropanol and a kimwipe, thoroughly clean your NMR tube in the area below the spinner where the sample resides. Do not get isopropanol on the blue spinner!

Running NMR Experiments (In Deuterated Solvents)...

- Log back into ICON-NMR using the "Icon" account (no password). Minimize the ICON-NMR automation windows or drag them to an area that is unobtrusive. Make sure TopSpin is displayed; if not it may be minimized on the taskbar.

** ATTENTION **

You must not exit ICON-NMR during your reactive sample slot booking!

- You will perform tasks on the spectrometer during your booking using a home-made TopSpin button panel (bpan) called "EZ". If EZ is open, the TopSpin interface will look similar to **Figure 2**. The bpan is along the right-hand column of the screen. If EZ is not open, click the EZ icon at the extreme right of the upper TopSpin toolbar (see **Figure 2**). Make sure a dataset window is displayed before starting (see tips & tricks).
- Perform the following steps to get your sample (prepared in a deuterated solvent) inserted into the magnet and ready for NMR:
 - Click the AIR ON button and wait until you hear a beep. This takes ~ 15 seconds!
 - Place your sample at the top of the magnet bore and click the AIR OFF button. Wait until you hear the beep.
 - Click the LOCK button and select your deuterated solvent from the drop-down list. Click OK and wait until you hear the beep.
 - Click SHIM to perform an automatic Topshim on your sample. This could take 30 seconds or up to a couple of minutes depending upon the starting field homogeneity. Wait until you hear the beep.

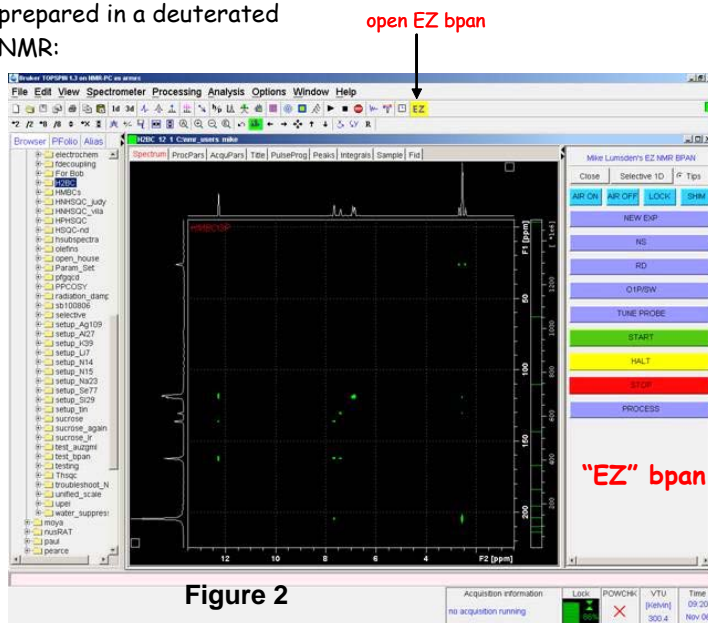


Figure 2

- Perform the following steps to run any NMR experiment on your sample:
 - Click NEW EXPERIMENT. The interface that opens is shown in **Figure 3**. Edit the "NAME" and "EXPNO" fields as appropriate and ensure the "USER" field matches your ICON-NMR login (normally your supervisor's last name). The "DIR" field should never change and should be C:\nmr_users. Choose your deuterated solvent from the "Solvent" drop-down list. Check the "Experiment Dirs" field and ensure there is a "/user" at the end of the directory specified. Click the "Experiment" drop down list and choose your experiment, ensuring you select only experiments named 1d_* or 2d_*. Lastly, you can enter a title in the "TITLE" field. Click OK when finished.
 - If you wish, click the NS button to change the number of scans and/or the RD button to change the relaxation delay. If you make changes to either, the new (modified) experiment time is displayed for convenience.
 - Click the O1P/SW button to change the middle of your spectrum (O1P) and the spectral window (SW). Both of these variables are in ppm units. For example, when O1P = 5 ppm and SW = 20 ppm, you will obtain a spectrum that ranges from 15 ppm to -5 ppm (+/- 10 ppm on either side of 5 ppm for a total SW of 20 ppm). If you click O1P/SW for a 2D experiment like HSQC, you will be asked to specify the information in both the F1 and F2 dimensions.

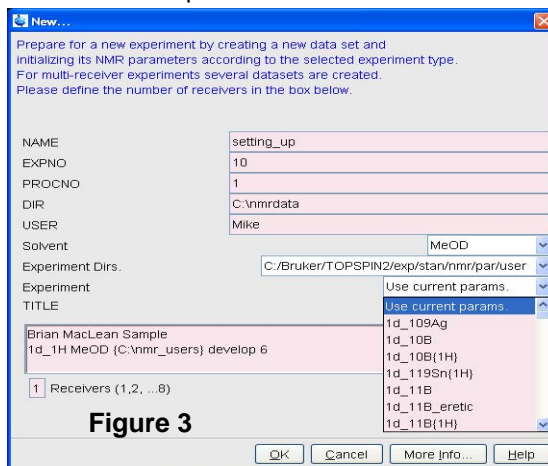


Figure 3

- d. Click "TUNE PROBE" and wait for the beep. (Note that this step is skipped if you are setting up a queue of experiments to run unattended).
- e. Start the experiment by clicking the green START button.

Stopping an Experiment early or Running it longer...

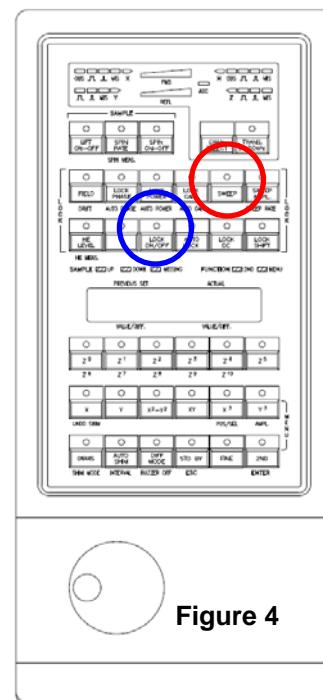
12. If you wish to stop your experiment early (i.e. you have more signal than expected and so NS was set too high), press the yellow HALT button. The experiment will be stopped after the next scan completes.
13. If you wish to stop your experiment early because something appears wrong (i.e. there is no signal or a very weak one), press the red STOP button. Your experiment will be stopped immediately and the data will not be written to disk.
14. If the number of scans completes and you decide you would like to acquire additional scans, do NOT use the green bpan START button. Instead, set NS to the number of additional scans you would like to obtain and then type "GO" in the TopSpin command line. By using "GO" instead of the start button, you add on to the existing signal rather than "throwing it away" and starting with no signal.

Running Additional Experiments &/or Samples...

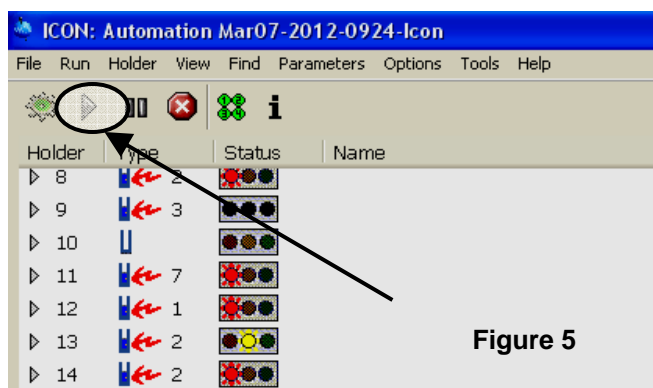
15. If you wish to perform additional experiments on the same sample, go back to Step 11.
16. If you wish to study another sample, click the AIR ON to remove your first sample from the magnet and then return to Step 10b.

When You Are Finished...

17. Turn off the lock by pressing the "LOCK ON/OFF button on the keypad beside the computer keyboard (see Figure 4 - button circled in blue).



18. Eject the sample from the magnet by clicking the AIR ON button.
19. Once you have your sample (remember to retrieve it by grabbing the NMR tube, not the blue plastic spinner), click the AIR OFF button.
20. If another user will be working in the reactive sample slot after you, you are done. However, if you are the last user for the evening, resume the ICON-NMR queue by clicking the play button in ICON-NMR (Figure 5).
21. Return the steps to the front of the magnet and the plastic fencing back in place along the yellow floor line.



Retrieving Your NMR Data...

22. When your experiments finish, NMR data is automatically archived to a NMR-3 networked computer in Room 426B. This computer is running an ftp server (ip address provided during training). You can download your data using ftp, visit 426B with a USB flash drive to retrieve your data, or process your data directly on this computer. Please refer to the document on our web-site: <http://nmr3.chemistry.dal.ca/docs/fileservers%20guide.pdf> for further instructions on using the ftp option.

Tips & Tricks...

- Make sure that a dataset window is open within TopSpin before locking and shimming your sample. It doesn't matter which dataset but it's important something is open for the lock/shim operations.
- When creating a new experiment (Step 11a; Figure 3), the top option in the list of experiments is always "Use current params". Selecting this allows you to copy all the parameters from the current dataset into your new dataset instead of a standard set of parameters. This option will be useful whenever you run a given experiment

and consistently make changes to the default parameters. For example, let's suppose you have a protocol where you always run your carbon experiments with 256 scans

- You can easily check the progress of your experiment while it is still running. If the experiment is one dimensional, type "tr" in the command line to transfer the data to disk. After the data is written, you can click the PROCESS button in the bpan to process the data automatically. If the experiment is two dimensional or higher, you can click the PROCESS button at any time without typing "tr" first.
- When you hover your mouse over any button in the bpan, an information window will open for ~5 seconds explaining the function of the button. Once you become proficient at using the EZ bpan to acquire spectra, you may wish to disable these info windows by deselecting the "Tips" radio button near the top of the bpan.

Concerning NMR Samples in Non-Deuterated Solvents

- Both locking and shimming using the EZ button panel rely on your sample being made up in a deuterated solvent. For NMR samples where this is not the case, steps 10c (locking) and 10d (shimming) must be skipped. Note that shimming can still be performed but a manual procedure must be followed. Please talk to me about this if you'd like to learn how to manually shim.
- The lock sweep must be disabled. It is controlled by a button on the keypad beside the computer monitor called SWEEP (see **Figure 4 - button circled in red**). Find this button and if the green light is lit, press it once to turn the light (and the sweep) off.

Chaining Together Experiments to Run Unattended

- Make sure all items in step 10 above are completed (your sample is in the magnet locked and shimmed).
- There is no need to ever click the TUNE PROBE button as the queue program does this automatically!
- Click on New Exp and setup the first experiment in your queue, changing any parameters you wish.
- Repeat the above item for as many experiments as you wish to queue up, incrementing the experiment number each time by 1.
- Return to the first experiment in your queue by using the "re #" TopSpin command where # corresponds to your first experiment number.
- Issue the command "multizg2.ml". This program will prompt you for the number of experiments you have queued up and will also give you an estimate of the amount of time your queue will take.
- If you halt an experiment in a queue before NS scans have been reached, the multizg2.ml program will move to the next experiment.