NUCLEAR MAGNETIC RESONANCE RESEARCH RESOURCE

AUTOMATION on the AVANCE 500

Dr. Mike Lumsden
Coordinator, NMR-3
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The Bruker AVANCE 500 (AV500) 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. NMR-3 training courses are available for both modes of access. This guide covers the details concerning automation and so is for those submitting samples to the B-ACS 60 autosampler. A separate document is available on the NMR-3 website covering the details of acquiring NMR data outside of automation (i.e. reactive sample slot reservations).

Introduction…

The B-ACS 60 is an automatic sample changer with holders for up to a maximum of 60 NMR samples. It is software driven by Bruker's ICON-NMR program. This guide covers ICON-NMR version 4.2.4 Build 10 (TopSpin 2.1 patchlevel 4). Starting in February, 2012; ICON-NMR accounts are no longer created for each individual trained researcher. Trained researchers within a given research group now login to a single ICONNMR account in their research supervisor's name. The password for this account can be obtained from trained colleagues in your lab. In the case of a new account needing setup, please consult with NMR-3 staff.

** 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 your NMR sample…

1. Prepare your NMR sample as usual, ensuring that:
   a. You use a high quality NMR tube that is not chipped or cracked at the top.
   b. Your NMR tube **is at least 7" long**.
   c. Any parafilm or Teflon wrapping does not extend below the allowed level.
   d. Your tube does not contain a paper or flag label of any kind. All tubes are to be labelled in permanent marker either directly on the cap or on the glass directly below the cap. Labels must always include your initials.
   If in doubt about items b &/or c, use the gauges posted near the spectrometer to check your sample.
2. 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.
3. Adjust the position of the NMR tube in the spinner using the sample depth gauge attached to the desk.
4. 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!

**Placing your NMR sample in the sample changer…**

5. Under normal circumstances, you will submit your sample to a queue that has already been started and is running experiments. To determine the best position to place your sample, first take note of the current sample position by checking the small display screen on the front of the B-ACS (**Figure 1**). Your sample should then be placed in the next highest numbered holder that is vacant. Holder numbers increment in a counter-clockwise direction around the carousel (**Figure 2**). Make note of the holder number and if you have more than one sample, be sure to note which sample is in which holder.

** EXCEPTION ** When you are submitting the first sample to a new queue, you should submit to holder 1, regardless of the carousel's positioning.

Figure 1

> **** CAUTION ****
>
> Be very careful not to nudge or place any weight on the magnet when submitting your samples!
>
> Never try to place or remove a sample from the carousel when the robotic arm is in motion!
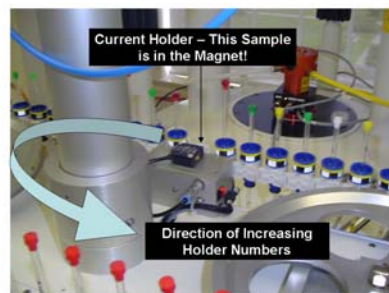
Logging into ICON-NMR and opening a new experiment line…

6. Login using the "ICON-NMR: Identify User" window which contains a list of all ICON-NMR accounts (**Figure 3**). If this window is not visible, there will be a different window open called "Automation Locked". To unlock automation and open the list of accounts, you click the CHANGE USER button 🔵 on the right hand side of the display. Select your research supervisor's account or your corporate account by double clicking on it and entering your password (passwords are case-sensitive). You are now logged in.
7. Double click or hit enter on the holder number that corresponds with your sample holder. This opens an empty experiment line below the holder line where you enter information for the first experiment on that sample.

Entering experiment details and submitting to the queue…

8. With reference to **Figure 4** and moving through the columns from left to right:
    a. *Holder* contains a triangular symbol and a number (1 through 60) corresponding to the holder numbers on the carousel. The triangle orientation depends on whether the individual experiments are displayed or not. In **Figure 4**, the only holder with experiments displayed is holder 20 - notice the different triangle orientation for this holder as compared to the others.
    b. *Type* contains a picture of a NMR tube which is either empty (holder is empty or no experiment line opened) or partly filled (holder contains a sample with at least 1 experiment line opened). In the latter case, a curly arrow and an integer show the number of experiment lines opened. **In Figure 4**, notice the empty tube displayed for holder 10 (indicating that holder 10 at the carousel is empty).
    c. *Status* uses a color coded traffic light system to indicate the status of the given experiment as follows (Holder numbers are with reference to **Figure 4**):
        i.   Right Light Green: experiment is running (holder 20)
        ii.   Middle Light Yellow: experiment queued & waiting (holder 13)
        iii. Left Light Red: experiment completed successfully
        iv.   All Lights Out: experiment info not yet submitted (holder 10)
        v.   All Lights Black: experiment failed (holder 9)
    d: *Name* is where you enter a sample name. There are some default choices in the drop-down list or you can enter your own custom sample name. What you enter here is ultimately a folder on the disk so please
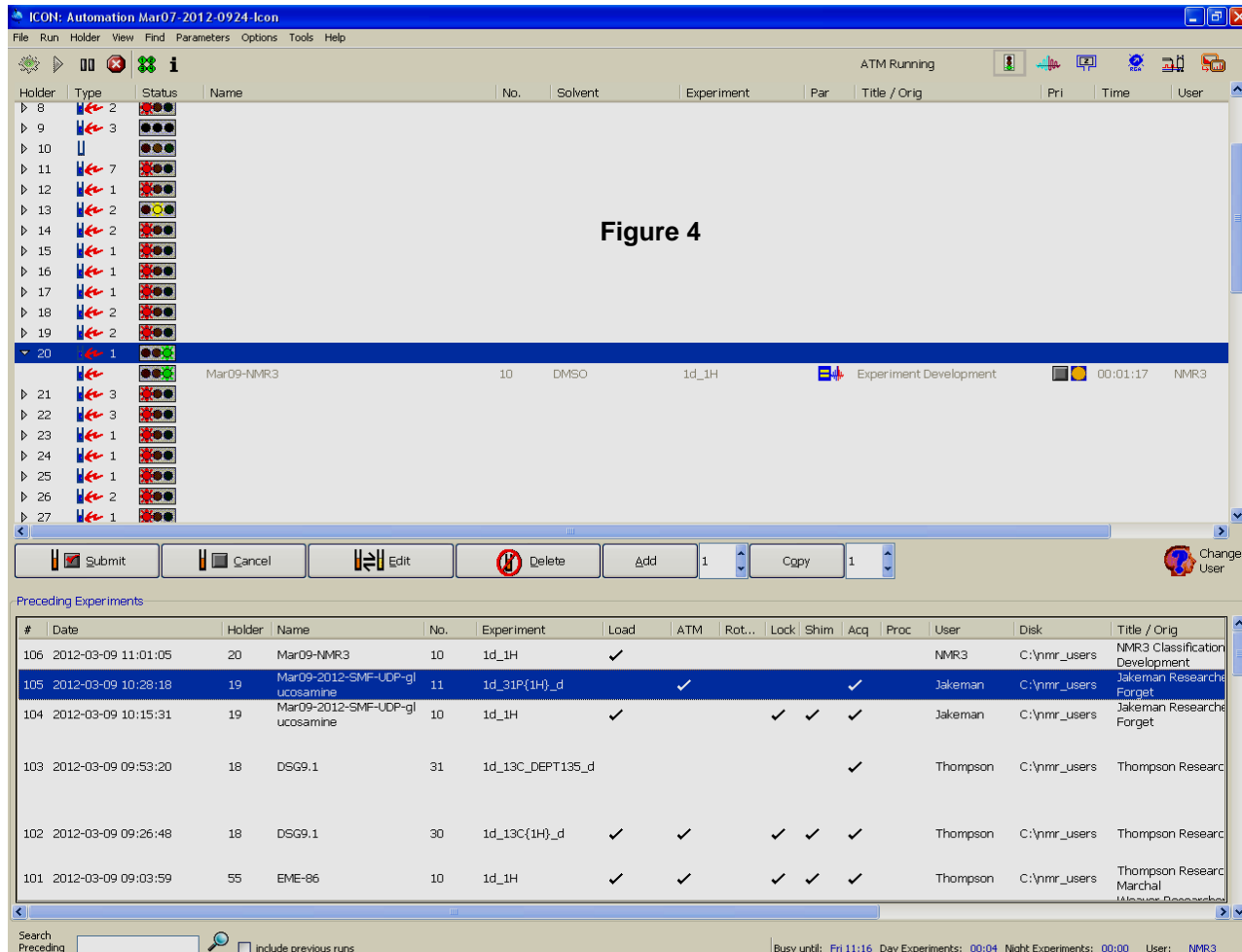

Figure 3

Figure 4

restrict your name to numbers/letters as well as dashes/underscores please!

e. *No.* is the experiment number for a given sample. By default, the experiment number will begin at "10" and increment by "1" for each new experiment added to a holder. If you submit additional samples and use the same "*Name*", the experiment number will start at the next increment of 10. You may modify the experiment numbers if you wish, keeping in mind different experiments on a given sample must never have the same experiment number or else the data will be overwritten.

f. *Solvent* contains a drop-down list of all deuterated solvents available for use. Choose yours from the list. Note that if you change the solvent for any experiment line on a given holder, all other experiment lines for that holder will be updated to the new solvent automatically.

g. *Experiment*: Choose an NMR experiment that you wish to perform from the drop-down list (**Figure 5**). All experiments have an associated mode that can be one of two values: "day" or "night". The mode of an experiment influences the order in which it gets executed during peak time (day time). More information is provided on this topic in the final section on page 6. Note that the upper half of the list contains experiments that a number of different research

Figure 5

groups use. Because they are of general utility, separate day and night modes exist and you select the proper one based upon the "20-minute rule" (discussed below). The bottom half of the list contains more specialized experiments, often requested by a single research group. For these experiments, only a lower priority night mode is available (regardless of the experiment length). With reference to **Figure 5**, users can determine the mode of an experiment easily as most are appended with a "_d" for day and "_n" for night. In addition, a comment is typically provided for each experiment indicating (among other things) the mode.

Experiments have another associated parameter called "Type" which can also be one of two values: "normal" or "composite". Normal experiments have an "n" or "N" beside them and in the left hand column of the experiment list whereas composites have a "c" or "C" (see **Figure 5**). The case of this letter is yet a third way to tell the mode of an experiment – lowercase is night mode. Composite experiments are dependent upon
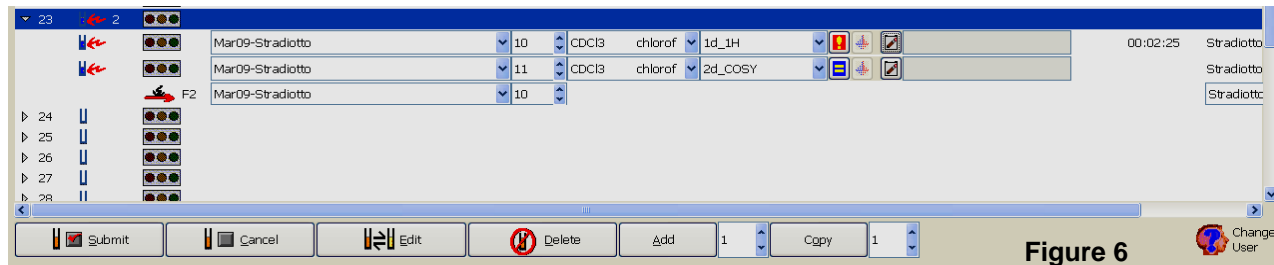

Figure 6

information from a previously run experiment (the so-called reference experiment) whereas normal experiments are independent and will run stand-alone. If you select a composite experiment from your list, the reference experiment(s) will open automatically. Shown in **Figure 6** is the result after selecting a COSY experiment in holder 23. Since COSY is composite, the reference experiment (1d_1H) opens on a line above the COSY. Also notice a line below the COSY with a red "F2" arrow. This line in unique to composites and must "point" to the reference experiment. In other words, the information specified for "Name" and "No." on this pointer line must correspond to the "Name" and "No." for the proton reference experiment. At the time of this writing, the only composite experiments available are 2D sweep width optimized experiments in the F2 dimension which all rely on a 1D proton experiment as reference.

h. *Par*: This column contains two icons, the first resembling a blue equal (=) sign. Clicking on this equal sign opens a window that allows you to change the value of certain parameters. If you change the default value for any parameter, the blue equal sign will change to a red exclamation point. An example is shown in Figure 6, where NS has been changed for the proton experiment whereas the COSY parameters are still the defaults. Once your changes have been made, click on the OK button. The 4 most common parameters users modify are NS (number scans), D1 (relaxation delay), O1P (middle of spectrum in ppm), and SW (sweep width in ppm).

The second icon is a deuterium lock signal and allows users to control the default lock, shim, and tune/match settings. It was available in the past to run experiments unlocked (for samples in protio solvent). However, as of February, 2012, this capability has been disabled (icon is greyed out); users wishing to run NMR experiments unlocked cannot use automation and so must reserve the spectrometer or use a different one.

i. *Title/Orig*: Click to open a text entry box similar to that shown in **Figure 7** and enter a title for the experiment. Additionally, at the top of the text box is a drop down list of trained researchers from your

Figure 7

group – select your name from the list. Note that your experiments cannot be submitted until you select your name from this list. Also note that submitting experiments using another researcher's name is strictly prohibited! When finished, click the "Set Title" button to set the title for only that experiment. Click "Set and Copy Title" to copy the title to all experiments for a given sample.

j. *Pri*: The ability to prioritize experiments in ICON-NMR as well as convert between day/night experiments has been disabled for all researchers.

k. *Time*: The estimated time it will take to complete your experiment is shown here after you submit the experiment. However, if you change any of the parameters in the parameter editor, the time will automatically be calculated even before the experiment is submitted.

l. *User*: The user who submitted the experiment. Users can only make changes to experiments submitted under his/her account – experiments submitted from different accounts can only be modified upon entering the super user password.

9. Once all information has been entered, make sure the experiment is highlighted and click the "SUBMIT" button (see **Figure 4**). The status column should then change from an unlit traffic light to a yellow light.

Setting up additional experiments and/or additional samples...

10a. To perform additional experiments on a sample, first type the number of additional experiments in the field beside the "Add" button (**Figure 4**). Highlight the holder number and then click "Add". New experiment line(s) will be created depending upon the number in the Add field. The majority of the information from the first experiment will automatically be carried forward and the experiment numbers will automatically be incremented by one. All that remains is to select the individual experiments and change any parameters for each experiment line.

b. If you have more than one sample, you can either go back to step 7 _or_ make use of the "Copy" button (beside the "Add" button in **Figure 4**). Type the number of copies you wish to make in the "Copy" field, highlight the holder number containing your first sample, and click "Copy". This will copy the information from your first sample into the next available holder(s), incrementing the experiment to the next highest multiple of 10.

Finishing Up...

11. Once you have entered all of the experiments you wish to perform on a given sample, make sure that they have _all been submitted_. You can do this individually or you can use a shortcut - highlight the holder number and either click "Submit" or use the right mouse button. All experiments associated with that sample/holder will then be submitted with one click.

12. Once all experiments are submitted, log-out of ICON-NMR by clicking the "Change User" icon which opens the "ICON-NMR: Identify User" window. Click the "Lock Icon-NMR" button which locks and prevents other users from using your account.

Retrieving Your NMR Samples...

13. Please be sure to return to the facility in a timely fashion to retrieve your NMR samples. You will find your samples in one of the following places:
    a. **Still in the B-ACS carousel**. If your sample is still in the carousel, be extremely careful removing it (lift straight up very gently). Once you retrieve your sample, login to ICON-NMR and delete the finished experiments associated with that holder. This frees up the holder for another user to submit a sample.
    b. **In a numbered position in the NMR tube rack**. There is a tube rack on the desk devoted to NMR samples that have been analyzed. The rack is labelled "Finished" and there are 60 numbered positions in the rack. Look for your sample in the same position in the rack as it was in the carousel. There is no need to delete your experiments from ICON-NMR in this case as an NMR-3 staff member will have already done this.
    c. **In the fume hood in Room 429.** If your position in the tube rack was needed for a subsequent sample, your sample will be moved to a beaker in a hood in Room 429 – a small wet lab near the AV-500 spectrometer. Note that you will often end up searching for your sample(s) from a large number of tubes belonging to other researchers so please ensure your tubes are clearly labelled and the label includes your initials.

Retrieving Your NMR Data...

14. Retrieving and/or processing NMR data is not permitted using the AV-500 workstation. When ICON-NMR experiments complete, 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/fileserver%20guide.pdf for further instructions on using the ftp option.

Tips & Tricks...

- You can monitor the status of the queue from the NMR-3 facility website using an icon available below the main menu – the icon is labelled "Queue Status".
- When submitting a mixture of day and night mode experiments, order the experiments such that day experiments are listed at the top and night experiments at the bottom.

- If you are submitting multiple samples in differing solvents, group your samples together according to the solvent. For example, if you submit 4 samples and 3 are in $CDCl_3$ and the other in DMSO, make sure the 3 in $CDCl_3$ are ordered consecutively.
- You can use the "Busy Until" section in the bottom right hand corner of the ICONNMR interface to determine the length of both the day and night experiments that are ahead of you. For example, in **Figure 8**, this section shows

Figure 8

Search Preceding [ ] ☐ include previous runs   Busy until: Wed 14:17 Day Experiments: 00:00 Night Experiments: 01:09 User: Schepp

that there are no day modes left but 1 hour and 9 minutes of night mode experiments queued. Obviously, you must utilize this timing information before you click submit on your own experiments - once you do that, your individual experiment lengths will be added to these total durations.

- You can make changes to submitted experiments provided they have not yet started. To do that, you must first click the Cancel ▮▊ button to cancel the submission and then click Edit ▐₌▌. Make any changes required and then resubmit your experiments.

DAY vs. NIGHT Automation Modes

The automation setup on the AV-500 has a sample control system implemented that gives preferential treatment during peak time to some experiments that are kept below a maximum duration. This duration was referred to in Section 8(g) and is known as the 20 minute rule. In this same section you learned that every experiment in your list has a designation of either day mode or night mode. Experiments that are of general use to most researchers have both modes available and you select the proper one according to the 20 minute rule (20 minutes or below – select day mode / > 20 minutes – select night mode).

It is important to understand how the order in which samples go in the magnet is determined and experiments get run. Towards this end, please note that the sample control system operates in two different modes depending upon the time of day. These modes are referred to as day mode and night mode:
- **Day Mode:** operational between the hours of 9:00 am and 5:00 pm, Monday through Friday. While in day mode, the control system goes incrementally from holder to holder and will run day mode experiments only. Since day mode experiments are of general use to most researchers and less than 20 minutes in duration, this operational mode eliminates the possibility for long experiments as well as special request experiments to get executed. This is desirable during the day Monday through Friday when the spectrometer is typically the busiest.
- **Night Mode:** operational between the hours of 5:00 pm and 9:00 am the next day, Monday through Friday, as well as all day Saturday and Sunday (i.e. Saturday at 10:00 am is still night mode). While in night mode, the control system runs all experiments, regardless of their length/mode, in the order that they were submitted. No preferential treatment is given to shorter experiments.

By way of example, let's suppose the system has just ejected a sample in holder #48 and holders #49 and #50 both have samples with a single experiment submitted. There is a night mode carbon submitted to holder #49 and a day mode phosphorus submitted to holder #50. The sample in holder #49 was submitted before the one in holder #50. The order in which these two submitted experiments get executed depends upon the time of day. If the system is in day mode, holder #49 will be skipped and the system will insert the sample in holder #50 and run the day phosphorus. If the system is in night mode, the sample in holder #49 will be placed in the magnet and the night carbon will be executed before going to holder #50.

Additional Notes
- If the system is in day mode and it retrieves a sample that has both day and night mode experiments submitted, the day mode experiments will get executed but not the nights. Once the day experiments are completed, the sample is ejected from the magnet and it will go back in once the system reverts to night mode.
- If the system is in day mode and all day experiments are completed, night time experiments can run during this "idle daytime" provided the experiment duration is less than 1 hour.