AN600 Operating Procedures

Department of Biochemistry NMR Core
(https://www.utsouthwestern.edu/labs/nmr/users/)

1. Log in to IconNMR by double-clicking on your group's name on the user list, entering its password, and pressing Enter. If you don't see the user list when you start, click on **[Change User]** to bring it back.
2. Go to IconNMR's top menu, click on **{Holder}** -> **{Delete Completed}** to clear all the completed samples. Samples in the queue or currently running will remain.
3. Insert your sample (must be 8" or shorter) in a Bruker spinner and adjust its position in the spinner with the Bruker sample depth gauge.
4. Place your sample in an available (green or gray) and optimal position as follows:
   a. If the instrument is idle, put your sample 2 positions up from the center, *e.g.*, if the center position is 5, put your sample in 7. Do not put your sample in the center position or the one before it.
   b. If there are samples running and waiting, put yours after the last one in the same queue (day or night) you are running. Note that samples should be arranged counter-clockwise in the tray starting on the left side of the center position and placed one after another, *i.e.*, do not skip positions. The tray rotates clockwise.
   c. Stay clear of the liquid nitrogen transfer line on the left side of the sample tray and make sure you don't accidentally bump into it.
   d. Note that if you have more than one samples to run, put them all in the tray first before going to the next step.
5. In IconNMR, double-click on your sample's position number which will create the first experiment, then
   a. If there are samples already in the queue, check the "Busy until" time (see Additional Information <10.k> below) and make sure there is enough time left for your sample.
   b. Under **Disk**, select where to store your data from the drop-down list. Contact the manager if your directory is not on that drop-down list.
   c. Under **Name**, enter your sample name as you would on the other instruments. Use only letters, numbers, ".", "-", and "_".
   d. **No.** is the experiment number. It will start at 1 and be incremented when you add more experiments for the same sample. If the sample name already exists, it will increment the experiment number based on that sample instead of starting from 1.
   e. Under **Solvent**, select your sample's solvent from the drop-down list, default is CDCl3.
   f. Under **Experiment**, select one experiment from the drop-down list, default is PROTON.
   g. Under **Pri**, the Sun/Moon icon stands for DayQ/NightQ. Default is DayQ. The star icon stands for priority and is disabled for everyone. If you are setting up a NightQ sample during DayQ time, you must select NightQ for each experiment in the sample.

h. Under **Par**, click on the [=] sign and change the parameters based on your sample and experiment. See Additional Information <10.n> for parameter setting recommendations. Note that you must change at least one parameter (even with the same value) and press Enter or OK in order to show the experiment time under **Time**.

i. Under **Title**, you can enter something more descriptive for the sample and click on **[Set Title]**

j. **Start Time** shows the experiment's start time which is not user changeable

k. To add more experiments for the same sample, click on **[Add]** and fill in the required information as above.

6. Make sure the sample position is selected, not an individual experiment, and click on **[Submit]**. If an experiment is selected when you click on submit for a multi experiments sample, only the selected experiment is submitted, and you will have to submit the remaining experiments for that sample.

7. When done, click on **[Change User]** which is located on the opposite side of the **[Submit]** button. Do not close IconNMR or TopSpin, and do not log out of the iconsu Linux user account!!!

8. Sign the logbook – PRINT your name (not just your initials) and fill in the rest of the required information. You are required to sign the logbook every time you use the instrument. Remember to add 3 minutes per sample to the total time you use on the logbook.

9. ***Additional Information:***

a. No broken, chipped, or cracked NMR tubes are allowed on any instrument.

b. Make sure the cap on the NMR tube is tight and secure.

c. Never hold a sample by the cap.

d. Make sure any marking/label on the tube is above where the top of the spinner would be.

e. Both 7" and 8" NMR tubes can be used on this instrument, but tubes that are longer than 8" cannot be used. Please be reminded that 7" tubes cannot be used on the MR400. So to keep things simple, I recommend phasing out 7" tubes and only order 8" tubes in the future.

f. Shigemi tubes for Agilent/Varian instruments cannot be used on Bruker instruments due to the differences in the lengths of the bottom filling, which is 15mm for Agilent/Varian and 8mm for Bruker. However, Shigemi tubes for Bruker should be usable on Agilent/Varian instruments.

g. Sample position color coding: When IconNMR starts, all positions are green and available, grey is deleted finished samples and available, blue is currently running, pink is sample in queue for both DayQ and NightQ samples, and red is sample with error.

h. Do not put samples in or remove samples from the center position or the position before it.

i. To remove the sample in the center position, you must either wait for another sample to start, or if yours is the last sample in the queue and the manual tray advance button lights up in **blue**, push that blue button once to advance the tray by one position. Do not touch any other buttons!

j. Do not put samples in or remove samples from the tray while the instrument is changing samples.

k. The following information is shown at the bottom of the screen from left to right:
   - *Sample: 15* – Current center sample position
   - *300.2K* – Sample temperature, normally set at 27C
   - *Busy until: Sun 14:59* – This would be the start time for the next sample submitted and allow you to figure out if there is enough time left for your sample. It would show "No Jobs!" if the instrument is idle.
   - *Day: ##:##* – This is the total time for all the samples in DayQ including the currently running sample
   - *Night: ##:##* – This is the total time for all the samples in NightQ including the currently running sample
   - *User: iconsu* – Currently logged in user

l. On Bruker instruments, 1D spectral window is defined by SW (spectral width in ppm) and O1P (center of spectral window in ppm). For example, to have a -2ppm to 14ppm spectral window, you would set SW=16 and O1P=6. For 2D HSQC and HMBC, the C13 spectral window is defined by 1SW and 1O1P.

m. Acquiring 1D data in blocks and checking its progress REMOTELY (not locally): set NS to be the block size and TD0 the number of blocks. Total number of scans = NS x TD0. For example, to run a 13C with 1600 scans in blocks of 16, set NS=16 and TD0=100. This is the only way to check your long 13C experiment before it finishes.

n. List of experiments and recommended setting for the most relevant parameters. Note that all 2D experiments are set up to optimize the 1H spectral window automatically and acquire with nonuniform sampling which will cut the experiment time by half.
   - PROTON – SW (default 16ppm; if changed, change AQ back to its default value), O1P (default 6), NS (default 4, 12 seconds)
   - C13CPD45 (normal 13C) – SW (default 240ppm; if changed, change AQ back to its default value), O1P (default 110ppm), NS (default 24, 1 minute; a 10mg sample with MW < 500 will need about 20 scans, 50 seconds)
   - C13DEPTQ-135 – SW (default 240ppm; if changed, change AQ back to default value), O1P (default 110ppm), NS (same as a normal 13C)
   - F19 – SW (default 240ppm; if changed, change AQ back to its default value), O1P (default -85ppm), NS (default 8, 15 seconds; needs about 25% more scans than 1H)
   - P31CPD45 – SW (default 300ppm; if changed, change AQ back to its default value), O1P (default 50ppm), NS (default 16, 20 seconds; it's about 380 times more sensitive than 13C)
   - B11CPD45 – SW (default 410ppm; if changed, change AQ back to its default value), O1P (default 0ppm), NS (default 32, 50 seconds; it's about 150 times more sensitive than 13C, but with much broader linewidths)
   - gCOSY60-NUS – NS (≥1, depends on concentration and size of coupling constants), 1TD (200 – 400, depends on degree of 1H signal overlapping and/or spectral width). Default NS=1, 1TD=200, 3 minutes.
   - gHSQC-NUS – NS (depends on concentration, NS=1 for samples with more than 5-10mg), 1TD (200 – 400, depends on degree of 13C signal overlapping and/or spectral width), 1SW, 1O1P (1SW and 1O1P define the 13C spectral window and should be

optimized to include only 13C's with directly attached 1H). Default NS=1, 1TD=256, 1SW=160, 1O1P=75 (spectral window 5 to 145ppm), 6 minutes.

- gHMBC-NUS – NS (4 to 6 times the NS for HSQC), 1TD (400 – 800, depends on degree of 13C signal overlapping and/or spectral width), 1SW, 1O1P (1SW and 1O1P define the 13C spectral window and should include ALL 13C's). Default NS=4, 1TD=512, 1SW=240, 1O1P=110 (spectral window -10 to 230ppm), 21 minutes.
- NOESY-NUS – NS (10 to 50 times the NS for a normal 1H, depends on size of NOE), 1TD (200 – 500, depends on the degree of 1H signal overlapping, spectral width, and 1H – 1H spacial distance), D8 (0.5 to 1 sec for small molecules). Default NS=4, 1TD=256, D8=0.6, 25 minutes.
- ROESY-NUS – NS (10 to 50 times the NS for a normal 1H, depends on size of NOE), 1TD (200 – 500, depends on degree of 1H signal overlapping, spectral width, and 1H – 1H spacial distance). Default NS=4, 1TD=256, 22 minutes.
- TOCSY-NUS – NS (similar to COSY), 1TD (200 – 400, depends on degree of 1H signal overlapping and/or spectral width), D9 (With the default of 80ms, you may see cross peaks from 1H up to 5 or 6 bonds away in the same spin system. A setting of 20 - 30ms will limit the cross peaks to mainly from geminal and vicinal 1H's). Default NS=1, 1TD=256, 4 minutes.
- C13IG45 – C13 with inverse H1 decoupling and a 20sec D1 for accurate C13 integration. SW (default 240ppm; if changed, change AQ back to default value), O1P (default 110ppm), NS (default 16, 6 minutes).
- N15IG45 – N15 with inverse gated H1 decoupling. SW (default 500ppm; if changed, change AQ back to default value), O1P (default 200ppm), NS (need about 45 times the NS for 13C; default 512, 48 minutes).
- N15INEPT – For N15 with directly attached H1's. SW (default 500ppm; if changed, change AQ back to default value), O1P (default 200ppm), NS (need about 20 times the NS for 13C; default 512, 32 minutes).
- PROB11DEC (1H with B11 broadband decoupling) – similar to a normal 1H
- PROP31DEC (1H with P31 broadband decoupling) – similar to a normal 1H

o. Double-clicking on a finished experiment inside the "Preceding Experiments" window will display the spectrum in a pop-up TopSpin window where you will only be able to zoom in and out and change vertical scale and nothing else. Don't attempt to do anything else in this restricted TopSpin window. Click anywhere inside IconNMR to return to IconNMR.

p. Partially completed 1D (set up with TD0) and 2D data can not be displayed in TopSpin during their acquisition, but can be viewed remotely in Mnova. Remote viewing is the only way to check your data before the acquisition is complete.

q. Submitted samples can be deleted before they start. Just select the sample position and click on **[Delete]**.

r. Once a sample is running, it can not be deleted.

s. Experiments within a submitted sample can be deleted individually before they start. Just select the experiments, instead of the sample, and click on **[Delete]**.

t. Submitted experiments in the queue cannot be edited/changed. The only way to change a submitted experiment is to delete that experiment (not the sample), add it back and make

the needed changes, and resubmit that experiment (not the sample). Note that the resubmitted experiment will be put at the end of the queue if there are samples queue up after yours.

u. Experiments can be added to a submitted sample as long as it has not finished. Just select the sample and click on **[Add]**, then submit the added experiment (not the sample). Note that the added experiment will be put at the end of the queue if there are samples queue up after yours.

v. Stopping an experiment: Due to potential data loss and concern with automation queue not being restarted properly, users will not be allowed to stop an experiment.

w. Troubleshooting: Occasionally, shimming may fail and your sample might end up staying inside the magnet. Your sample will be ejected when the next sample starts running. If there is no sample queues up after yours and you need your sample back, you may push the green button next to the blue manual tray advance button to manually eject your sample.

x. **Schedule and Rules**:

DayQ 8:00AM – 6:00PM daily, maximum 30 min or 8 samples whichever is less (see the *one-hour rule* below)

NightQ 6:00PM – 8:00AM daily, maximum 2 hours on weekdays and 4 hours on weekends. Submission starts at 3:00PM.

- The *one-hour-rule*: One hour *after the finish* of your previous block of 30 minutes of sample(s), you may submit another block of sample(s). There is no limit on how many blocks one can have in a day as long as the one-hour-rule is followed.
- Prior permission is required if you need more time than the limit.
- You must check and make sure there is enough time left in DayQ or NightQ for your experiments before submitting your samples (see <10.g> on how).
- The total number of NightQ samples submitted by all users before NightQ starts should be no more than 12, so that DayQ users will have at least 12 sample positions available.
- Please observe the NightQ submission start time. I intend to check, and early submissions will be removed without notice.
- Use of this instrument is on a first-come, first-served basis. No reservation is required or allowed. If you have a need that cannot be met because of the above policy, please let me know so that alternative arrangement or a one-time exception to this policy can be made.