

# Instruction for Operating the **Bruker Avance III 800** MHz NMR Spectrometers in UTMB

Written by Tianzhi Wang, date: February 8, 2013.

No food, no drink in NMR room and no internet in NMR host computer except for transferring data. **No new experiments can be set up without permission from Tianzhi Wang.** Do not move any unit without permission from Tianzhi Wang

**PLEASE MAKE A NOTE OF ANY PROBLEMS YOU ENCOUNTER-NO MATTER HOW TRIVIAL THEY MAY SEE.**

1. Type your user name (e.g. tzwang) followed by [return].
2. Type your password then [return].
3. Double left-click on the 'topspin3.0' icon. The topspin program starts.
4. Type 'edte' on the command line from topspin. A pop-up window (VTU) will appear after a few seconds. Set the sample temperature ( $^{\circ}\text{C}$ ) whatever you want to run your experiment. **CAUTION!!!** Change the temperature step by step, typically  $5^{\circ}\text{C}$  each time. **For protecting probe, never change the temperature faster than  $10^{\circ}\text{C}$  every 10 minutes.** Once getting stable temperature, you can change the target temperature again). **Important!!! NEVER CHANGE GAS FLOW RATE AND TURN OFF HEATER ON THE VTU UNIT.** The power is off when the temperature is at or higher than  $25^{\circ}\text{C}$ , otherwise, you should set the chiller target power is weak or on, depending on which the temperature you need. For the temperature range  $2-25^{\circ}\text{C}$ , select 'cp\_low' calibration under the 'correction', select 'cp\_High' for the temperature range  $26-70^{\circ}\text{C}$ .
5. Prepare to put your sample in the magnet. Leave your personal stuff on the desk, because the magnet will wipe out you credit card and affect your wristwatches. Take off the black cap or the small piece of paper on the magnet. Place your sample tube into the spinner and the spinner in the depth gauge. (MAXIMUM SAMPLE DEPTH 20.00 mm, for shigemi tube, the minimum sample depth is 16.0 mm). Wipe the tube and spinner turbine with a Kimwipe. To insert your sample press the key 'LIFT ON-OFF' on the BSMS keyboard or type 'ej' on the command line. WAIT about 10 seconds until air is hissing. Make sure the air flow is enough to suspend your sample tube. Press the LIFT key or type 'ij'. The sample will gently drop into the magnet. Never put the black cap back on the magnet when you have a sample in the magnet in cryoprobe (ONLY for regular probe you must do so). If you do so, you have a risk to freeze the NMR tube and break it in the magnet when power outage occurs.

6. **Spinner selection:** The blue spinner is for RT probe and white color spinner is for Cryoprobe and can be used at the range of temperature 0-70<sup>0</sup>C. If your sample temperature is out of this range, the ceramic spinner should be used. **No ceramic spinner for Cryoprobe.**
7. Double left-click on a small black square region at the lower right of the screen, marked 'lock', lock window will appear pop-up.
8. To setup an experiment by selecting the experiment form 'tzwang' directory and then type 'edc', change the user name from 'tzwang' to your user name and choose a name for your experiment (These experiments are for Cryoprobe). Or, you can type 'rpar' to copy the desired experiment from the pop-up window. For example, selecting 'PROTON' for 1D proton NMR, selecting 'ZGPR' for 1D proton with presaturation to suppress water, selecting 'P3919gp' for 1D proton with water gate to suppress water, selecting 'HSQCETF3GPSI' for <sup>15</sup>N-<sup>1</sup>H HSQC experiment and so on. Please contact Tianzhi Wang for your first time to setup new experiment, because protein NMR is more complicated than organic NMR, The former includes shaped pulse which the pulse length is much longer than hard pulse. So, setup a new experiment carefully. Any Question? Talk to Tianzhi Wang.
9. Type 'getprosol'.
10. Type 'lock' choose the solvent you using, type 'loopadj' to get best lock parameters for the long time experiments and stabilize your lock.
11. Type 'atmm' for tuning and matching or 'atma' for automatically tuning. **For water based solvent sample, only "ATMM" can be used for tuning.**
12. Type 'topshim 1d' to use 1d shimming. Type 'topshim 3d' for 3D gradient shimming, if necessary. If you have small amount of sample, you MUST shim it manually. Type 'wsh' and select the proper name to save your current shimming data in case you need it latter. **For the better shimming on Cryoprobe, the option of "convcomp" is necessary (e.g. topshim convcomp).**
13. Check the [plw1=12W@-10.79 dB](#), [plw2=155w@-21.90 dB](#) and [plw3=260w@-24.15 dB](#), p1 is about 8~13 us, p2 is about 12us and p21 about 35us for CRYOPROBE before when you run any experiment. There is no more p11 or p12... available to change the power level on command line. However, you can change your power level on dB unit under 'ased' window. You also can calculate your power level by using  $p2/p1 = (plw1/plw2)^{0.5}$ .
14. Optimize your acquisition parameters and choose the best spectral width for each dimension. You may type '**pulsecal**' to calculate your p1 and plw1 for single peak (e.g. H<sub>2</sub>O). You also can use the 'gs' mode to optimize the 'o1' and the power level of the soft pulse for the water flip back, and thus optimizing the water suppression.
15. You may type '**pulsecal**' to calculate your p1 and plw1 for single peak (e.g. H<sub>2</sub>O). You also can use the 'gs' mode to optimize the 'o1' and the power level of the soft pulse for the water suppression experiments.
16. To keep the same scale when measuring the 90<sup>0</sup> pulse width of water through 360<sup>0</sup> by typing ".keep".
17. Type 'paropt' to optimize 'p 1, p 21, cnst4' on 1 D mode, typically 16 1d experiments to be run.

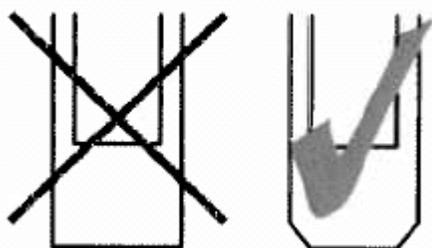
18. You can update the proton channel parameters by using 'getprosol 1H (90-degree pulse length (power in DB for the 90-degree pulse) (for example, getprosol 1H 10 -10.79) (-10.79 is in dB) on the command line. **This cannot be used for the strong power e.g. spinlock experiments.**
19. Type 'rga' to get 'rg' value automatically.
20. As for 3D experiments, you must run 2 2D projections first to optimize your spectral width and carries on each nucleus, and make sure you have good 2D projections, because 3D experiments need to be run for several days.
21. Never set d1 SHORTER THAN **1s**.
22. Once you finish the optimization, go back to your desired experiment, type 'expt' to check your experiment time, type 'zg' to run your experiment. You **MUST** wait for a couple of minutes to watch the first a few fids to make sure NO overflow and also look at cryopanel whether the power reservation drop too fast or not (e.g. NMR coil heater never drops 6 unit). If something happens, **please stop your experiment immediately (type 'aa' on the command line to stop your experiment)** and write down it on the notebook and logbook then see Tianzhi wang (409-747-6821).
23. Once your experiment is done, delock by press 'LOCK ON\_OFF' and then press the key 'LIFT ON-OFF' on the BSMS keyboard. Take off your sample from magnet. **(Please put the dummy sample 99.98% methanol inside the magnet, lock to methanol and never put the black cap on the top of the magnet for cryoprobe).**

If you have any questions or problems when you are running experiments please contact Tianzhi Wang (409)747-6821.

## Important notes for 800 MHz TCI CryoProbe:

1. **Do not TUNE or Match or apply any RF power to the Cryoprobe when it is not cold.**
2. **Don't run high salt sample on CryoProbe by yourselves.**
3. **Never beyond the temperature range 5-70 °C.**
4. **Never set the following power levels beyond the following values for each channels:  $^1\text{H} \leq 12 \text{ W} = -10.79 \text{ dB}$ ,  $^2\text{H} \leq 38 \text{ W} = -15.80 \text{ dB}$ ,  $^{13}\text{C} \leq 155 \text{ W} = -21.90$  and  $^{15}\text{N} \leq 260 \text{ W} = -24.15 \text{ dB}$ .**

5. The all pulse levels were calculated based on Non-lossy samples, such as organic solvents or water without salt. For your actual used sample, the pulse width should be changed instead of change power level.
6. Never set d1 SHORTER THAN 1s and the acquisition time AQ for 2D experiments never longer than 100 ms.
7. When hard pulses are applied simultaneously on two or three of the following nuclei channels- $^{13}\text{C}$ ,  $^{15}\text{N}$ , then the power on the used channels has to be reduced by 3dB each.
8. Simultaneous decoupling of  $^{13}\text{C}$ ,  $^{15}\text{N}$  requires reduced power on each of the used channels: 3dB less power should be used for both  $^{13}\text{C}$ ,  $^{15}\text{N}$ . For simultaneous decoupling it is recommended to use optimized conditions (e.g. adiabatic decoupling on  $^{13}\text{C}$  with a Q-factor of 2.5).
9.  $^{15}\text{N}$  CPMG  $T_2$  experiment. The total length of the CPMG element should not exceed 250 ms, and the  $180^\circ$  pulse should not be shorter than 90  $\mu\text{s}$  (corresponding to a  $90^\circ$  pulse of 45  $\mu\text{s}$ ). The recycle delay must be greater than 2.5s with an inter-pulse delay of 900 $\mu\text{s}$  (between two  $180^\circ$   $^{15}\text{N}$  pulses).
10. Shigemi tube. Make sure the following Bruker Shigemi tube will be used. Otherwise, the cryoprobe could be damaged.



Only Shigemi tubes with  
TAPERED edges on the  
bottom are allowed!

