Tips for Acquiring NMR Data on the Agilent 600

1. Sample volume should be at least 500 µl (normal) or 300 µl (shigemi tubes).
2. Insert sample in sample holder and position it appropriately (record sample depth in your lab notebook).
3. Record the inlet air flow rate.
4. Record the probe temperature of the air going into the probe; change the temperature if necessary using the **temp** command. When you do so, make sure the selected temperature is ~10 °C above the temperature of the FTS water bath, otherwise, change the temperature of the FTS unit accordingly.
5. After temperature has equilibrated (the green light next to the display is steady), you can tune the probe. ***However, tune the probe only if necessary (e.g. if your sample has >150 mM salt) or if you measure long pulse-widths (>9 µs; step 9).***
6. Optimize the lock signal.
7. Before shimming (be sure there's D_2O in the sample!), record the values for z^0, z^1, z^2, z^3, z^4, z^5, z^6, x, y, xz^2, yz^2, xz, and yz – this can be done by typing **dgs**. Manually adjust $z^1$and z^2 before shimming with gradients.
8. Acquire a 1D ^{1}H experiment to check quality of shims. For water samples, **tpwr** = 54, **pw** = 1, **gain** = 0; **tof** should be centered on the water resonance. Process the data (using **ft** not **wft**) and pay attention to the lineshape (symmetrical) and linewidth (<20 Hz) of the water resonance. Record optimized **tof** and the lineshape/linewidth in your notebook.
9. Measure the 90° ^{1}H pulsewidth (record **pw** and **tpwr** values) and acquire a 1D ^{1}H spectrum with water suppression to check the status of your sample! This is straightforward and you will be saving a lot of time and effort. Analyze the spectrum to see if there are any problems (e.g. with sensitivity, extra peaks, aggregation, artifacts).
10. Acquire an appropriate 2D spectrum (^{1}H-^{15}N HSQC, ^{1}H-^{13}C HSQC). You could use standard parameters – ask Yongbo for some of the critical parameters. Again, analyze the spectrum to see if there are any problems (e.g. with sensitivity, extra peaks, aggregation, artifacts). Stop and consult Ishwar or Yongbo if you see potential problems.
11. Record 3D spectra. Before you start, always record the orthogonal first planes (2D) of the 3D experiment. Ideally, all the spectra described in steps 10-12 (1D, 2D, first planes of 3D experiments) should go into your notebook. Important parameters for 3D experiments include, **np, ni, ni2, sw, sw1, sw2, tof, dof, dof2, phase, phase2, pw** (variable), spin lock power and spin lock pulse widths for TOCSY (variable), mixing times for NOESY (variable). Some of the key parameters may vary depending on experiment – check with Yongbo/Ishwar.