**Bruker Avance I 800 MHz Protocol**

Protocol by Taylor Arhar 042319, based on verbal instructions from Mark Kelly

**Sample Preparation:**

• Combine labeled protein, unlabeled protein, buffer, and D2O. Final volume should be at least 500 μL.

• Filter samples (I use 0.1 μm spin filters).

• Carefully pipette samples into NMR tubes, making sure not to introduce bubbles.

• Load NMR tubes into blue sample holders (stored in a case near the computer) and center the sample using the tool kept on the computer desk.

• Walk up the stairs (after removing things that you don’t want to get demagnetized—ex ID badge, watch, cell phone) and place sample in a numbered position in the sample changer.

**Instrument Preparation:**

• After logging onto the computer, open the Bruker software, Topspin 4.0.6.

• Check the temperature and set it to the desired temperature (285K for K18 HSQC).

• To move a sample in position # into the magnet, type “sx #”.

**Preparing for an Experiment:**

1. rsh

• Type “rsh”.

• Select 0\_CURRENT shim file.

• Click “read”.

1. lock

• Type “lock”.

• Select H2O + D2O (90%, 10%).

1. Tuning

• Under data tab, choose 0\_TUNE\_ALL\_NEO, drag and drop to the right window.

• Click Wobb All button. Three windows should open.

• Adjust N, C, and H tuning using the adjustment tool stored under the magnet. The goal is to achieve a sharp peak that hits the bottom of the window and to center the peak around the vertical line.

• Type “stop”.

• Tuning only needs to be performed once for a set of successive experiments, but should always be done before the first experiment.

1. Shimming

• Click TopShim Gui button. A panel should open.

• Under the shim tab, select 3D dimension.

• Check the parameters box and type “3dfast”.

• Click start.

• When shimming is complete, check the final B0 standard deviation under the report tab (1-3 is ok, <1 is great).

• Under the shim tab, click on the after dropdown menu. Select the final option (Z-XY-…etc).

• Check the box that says only.

• Click start. This step lowers the lock level, making it less likely to amplify noise.

• When shimming is complete, select 1D dimension under the shim tab.

• Uncheck the parameters box.

• Click start.

• When shimming is complete, check the final B0 standard deviation under the report tab.

• If final B0 standard deviation is <0.2, shimming is complete. If it is >0.2, check the Z6 box and repeat 1D shimming (2000-10000 for Z6 are ok).

1. Proton Pulse Calibration

• Under data tab, choose 0\_1H\_CAL\_NEO, drag and drop to the right window.

• Type “p1”.

• Set p1 to 1.0 usec.

• Type “zg”.

• Type “qfp”.

• Type “.ph”.

• Phase the spectrum by holding down the 0 button and moving mouse up and down to get a completely positive, centered signal.

• Return and save the phased spectrum by clicking on the return and save button (it has a left arrow and a disc figure).

• Type “p1”.

• Set p1 to increasing pulse times (likely 33-35 for my samples).

• Type “qfp”.

• Increase the pulse time until the negative signal has diminished to a minimum.

• Divide this p1 by 4 and record it for future use.

1. Pre-saturation

• Under data tab, choose 0\_1H\_PS\_NEO, drag and drop to the right window.

• Type “p1”.

• Set p1 to the value that was determined by the proton pulse calibration.

• Type “gs” (ghost scan). A panel should open.

• Under the offset tab, toggle to reduce the FIDarea. This reduces the H2O signal.

• When finished, click save all button and then click stop button.

• Type “o1p”.

• Record this value (the water frequency of the sample). This value is expected to be close to 4.7.

**Starting an Experiment:**

• A new experiment can be started by manually setting parameters or by copying the parameterset of a previous experiment.

• To start a new experiment and manually set up the parameters:

1. New
2. Read parameterset (choose the correct one for your type of experiment; for K18 HSQC, select etfpf3gpsi).
3. Select “do nothing”.
4. Write a title for the experiment.
5. Under the acquisition parameters tab (Acqu pars), other parameters can be manually set (for K18 HSQC, perform 4 scans, 64 dummy scans, set delay time to 1.5).

• To start a new experiment using parameters used by a previous experiment:

1. Select a previous experiment acquired using the desired parameters (I drag and drop the spectrum to make sure it’s selected).
2. Type “edc”. A window should appear.
3. Adjust the title, experiment number, and description to reflect the new/current experiment.

• Once acquisition parameters have been set, type “getprosol 1H # -10.76”, where # is the p1 value determined during the proton pulse calibration.

• Type “o1p”.

• Set o1p to the value determined during pre-saturation.

• Type “zg”.

• Once dummy scans have been completed and the spectrum is being acquired, you can look at the 2D spectrum by typing “xfb”.