**Nuclear Magnetic Resonance Spectroscopy (NMR) SOP**

NOTE: This is all based on my memory of my work with Julien, it is very important to do this with someone who has run an experiment before when you first do it.

NOTE: You will need ~300uL of at least 100uM sample for the 3D NMR. You can go lower in concentration for the 2D experiments, it will require doubling the time (to ~4hours). The 3D experiments take a week to run and so the time cannot be doubled.

NOTE: pH and salt concentration are critical. You cannot go above pH 7.5 (the higher the pH, the worse your sample resolution will be due to more exchange between protein and water) and you should aim for ~50mM NaCl.

1. Measure the concentration of your N15 labeled protein
2. Add 10% D20 by volume to your protein
   * 1. NOTE: This is by volume, you will dilute your protein
     2. NOTE: This serves as the “lock” for when you are trying to align the magnet
3. Load your sample into the NMR tube using an extra long glass pipet tip
   1. Put your sample in the tube. Hold the tube with your thumb underneath it so that you have a firm base for c
   2. Put the plunger in and pull it out quickly several times to remove excess air
   3. Put the plunger in until it sits right on top of the sample and sharply press down with your thumb. The goal is to have no bubbles under the plunger and to have the meniscus of the sample be very slightly above the top of the plunger
      1. NOTE: The type of NMR tube matters when you set up the instrument, Julien uses glass tubes
      2. NOTE: Julien has a box of extra-long pipets in his lab, Amy’s lab makes them by pulling the glass pipet tips over flame but I broke the tips and couldn’t figure it out
      3. **NOTE:** It is very easy to get bubbles in your sample and lose sample, so if you are not sure you can practice with water.
   4. Wrap the top of the tube, where it connects with the plunger, with saran wrap to keep it from moving
   5. Label the tube using a small piece of paper at the top of the tube
4. Bring your sample to the NMR instrument
5. Put a ring on the top of your tube
   1. This is a ring of plastic that keeps the tube in one position
6. Using the depth gauge, align your tube so that the sample will sit in the magnet
   1. The depth gauge is a small piece of plastic that your tube will sit in. Depending on the thickness of your tube you will need to move it up or down within the ring
7. Type EDTE into the Topspin program
   1. This stands for Edit Temp. Most experiments will be done at room temperature, changing the temperature can give you different results based on how your protein behaves.
8. Wait for the spectrometer to reach the correct temperature
9. Type EJ into the Topspin program
   1. This stands for eject, you will hear air flow start and you are OK to put your sample in the machine
      1. **NOTE:** If you do not hear air flow, DO NOT insert your tube. It will fall into the machine and could break the tube or the machine.
10. Using the step ladder, climb to the top of the machine and sit your sample on the opening
11. Type IJ into the Topspin program
    1. This stands for inject, air flow will cut off and your sample will lower.
12. Type lock into the Topspin program
13. Select 10% D20 and 90% H20
    1. This sets the magnet lock for your buffer sample, it should be green
14. Type topshimgui; into the Topspin program
    1. Select Before: Z-X-Y, After: Z, Parameters: Shigemi
       1. NOTE: This shims the tube, or aligns it to be centered in the magnet. Setting it to Z means the tube will be moved up and down repeatedly until it finds a good spot. Shigemi is the glass tube used by Julien.
       2. NOTE: Depending on the previous use, it may not shim. You will have to do so manually and it is best to get someone else to help with that
       3. NOTE: You will see something on the right that looks like a heart monitor. There will be a line that crosses that window left to right and back again. After locking, the line should stabilize at a certain height. After shimming, the line will move but it should again stabilize. If it does not, your sample was not shimmed properly.
15. Type wobb into the Topspin program
    1. Moving under the spectrometer, use the small wrench to turn two dials to tune the magnet. There should be an LED display nearby with blinking lights in the X and Y directions
    2. One dial moves things in the x-plane, one moves them in the y-plane
    3. Move the x-plane until only the center square is lit, then move the y-plane until only the center square is lit. Repeat until it is finished, adjusting the y can change the x.
       1. **NOTE:** One dial is closer to you and one is farther away, I do not remember which is which. ASK whoever you are working with and watch them do it first.
16. Your sample is now ready to run
17. Select a previous experiment and type edc into the Topspin program
18. Change the name of the experiment and enter your sample details
    * 1. NOTE: edc copies the experimental conditions of the previous run, but changes the name and so allows you to keep everything consistent
19. Type zg into Topspin
    1. This runs the experiment. After the first scan you can check the progress and adjust any parameters.
20. Type rser 1 into the Topspin program
21. Type sinm;fp into the Topspin program
    1. This generates a 1D spectra. You can overlay this with previous spectra to gauge the protein concentration and if you need to stop it and run a new experiment with different parameters.
22. Type xfb into the Topspin program
    1. This generates the 2D spectra
23. If you need to change parameters, type stop into Topspin
24. You can change two parameters to enhance signal
    1. ns is number of scans, increasing this increases your signal
       1. NOTE: after entering your new number of scans (2, 4, 8, 16, 32), type expt and hit enter to see how long the experiment will take
    2. rga is receiver gain analysis and will pick a receiver gain to optimize your signal. Type rg to enter the number from the analysis for your experiment
25. To visualize data, open your old runs and enter “rser1, sinm;fp, xfb” to see a 2D run. The spectra can then be manipulated and printed as an image.
26. To get a high quality image for publications, the topspin NMR file must be modified in some way to change it to a ucsf file to open in SPARKY.
    * 1. NOTE: I do not know how to do this and I don’t know what it changes exactly, but Julien offered to do it for me if I need help.
27. Once opened in Sparky, the spectra can be modified again to make it look nice.
28. Open Sparky
    1. This requires download of Sparky and of XQuartz
29. Hit “file -> open” and open the file
    * 1. NOTE: Be careful of where you save the file, it can be difficult to find
30. Type zo to zoom out and zi to zoom in
31. If peaks look like dots and not rings of circles, or if you have too much background, you can adjust the contour levels. Go to View -> more -> contour levels and select new values for positive and negative
    1. Can save positive lowest as 6.5e^6 to remove background peaks
    2. From this window, you can also change the color of the peaks.
32. Hit apply to the contour level changes you made
33. Type ci to invert the background to white
34. Go to file-> print and save it to your folder, it will save as a ps file (like jpeg)
    * 1. NOTE: In the print window you can select the axes range you want to print