From FID to 2D: Processing HSQC Data Using NMRPipe

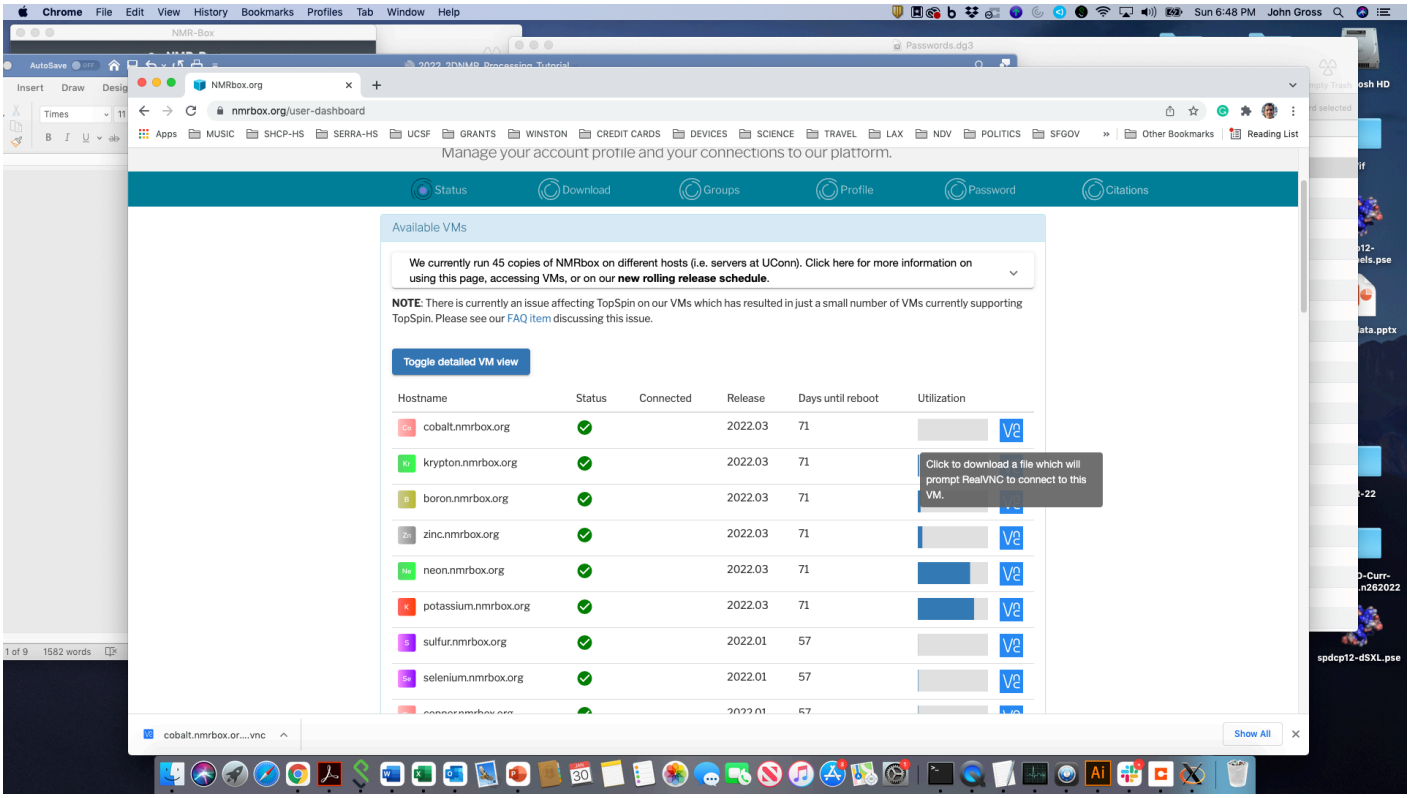
Macro Methods

The data recorded during a NMR experiment are stored in the form of a digitized free induction decay (FID), which is in the time domain. In order for us to gain information regarding the chemical environment of individual nuclei that contribute to the observed FID, we need to convert the data into the frequency domain using a Fourier transformation. To do this we will utilize the NMRPipe software package within the NMRBox virtual desktop environment. NMRPipe is a powerful program for converting and processing multi-dimensional NMR data. With that in mind, the purpose of this tutorial is to walk you through the various steps involved in processing 2D nitrogen and carbon HSQC datasets recorded for Nb6 and mNb6 alone and in the presence of Spike RBD.

In general, the steps for processing data are: (1) download data to your computer;however, this is not nessary since all the data are available in NMRBox (2) Convert the data to NMRPipe format (3) Fourier transform and phase the first FID and (4) iterate NMRPipe functions through the entire series of 1D experiments.

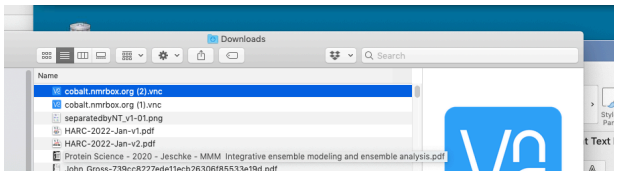
Part I: Launch the NMRBox Virtual Environment

1- Login to NMRBox by going to : nmrbox.org. Once you are logged in click on 'My Account' at the top right hand side of the web page. There you see a list of virtual machines that you may use. I typically use one with the least utilization. Click on the blue VNC icon to download the virtual machine. NB, you'll



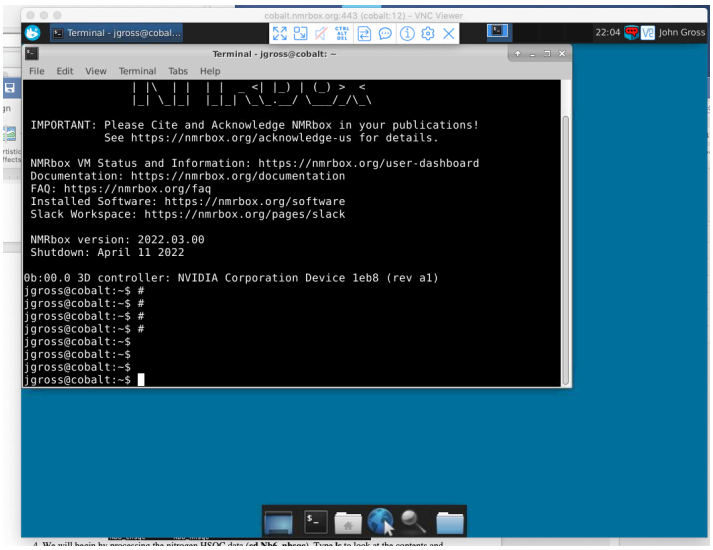
need to have VNC Viewer installed on your computer , as recommended first day of class , for this step to work.

2-Go to downloads folder and double click on the virtual machine that was downloaded to start your session.



You'll need to enter your credentials for RealVNC that you used to download VNCviewer.

2. After starting NMRBox , open a Terminal by clicking on the terminal icon at the bottom of the virtual desktop.



We are going to process the HSQC data for Nb6 together and you will then be responsible for processing the remaining datasets in your group.

3. Descend into the directory for Nb6 (**cd ~/EVENTS/ 2022-UCSF-204B/Nb6)**. Type **ls** again and you should see directories for the carbon (chsqc) and nitrogen (nhsqc) HSQC experiments.

```
[Ryans-MacBook-Pro:~/nmr/2021_Macro_Methods_NMR] rwtibble% cd Nb6
[Ryans-MacBook-Pro:~/nmr/2021_Macro_Methods_NMR/Nb6] rwtibble% ls
Nb6_chsqc       Nb6_nhsqc
```

4. We will begin by processing the nitrogen HSQC data (**cd Nb6_nhsqc**). Type **ls** to look at the contents and ensure the data has copied over correctly:

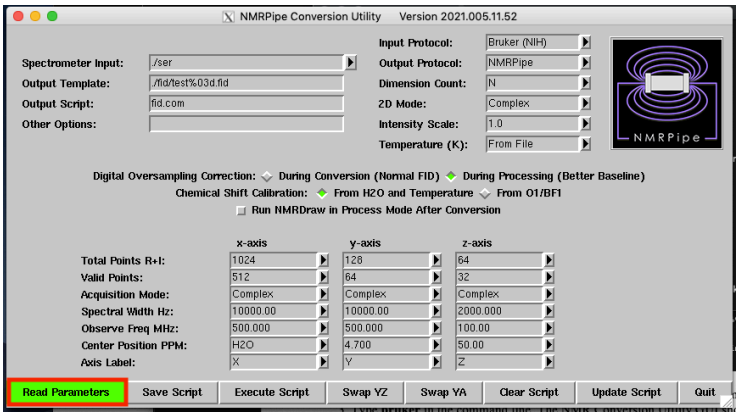
```
[Ryans-MacBook-Pro:~/nmr/2021_Macro_Methods_NMR/Nb6] rwtibble% cd Nb6_nhsqc/
[Ryans-MacBook-Pro:2021_Macro_Methods_NMR/Nb6/Nb6_nhsqc] rwtibble% ls
EA                      format.temp             specpar
acqu                    gpnam1                  spnam1
acqu2                   gpnam2                  spnam13
acqu2s                  gpnam3                  stanprogram2495
acqus                   pdata                   uxnmr.info
adcInfo_TRX1.xml        pulseprogram            uxnmr.par
audita.txt              scon2                   vtc_pid_settings
cpdprg3                 ser
format.ased             shimvalues
```

Part II: Convert from Bruker to NMRPipe format

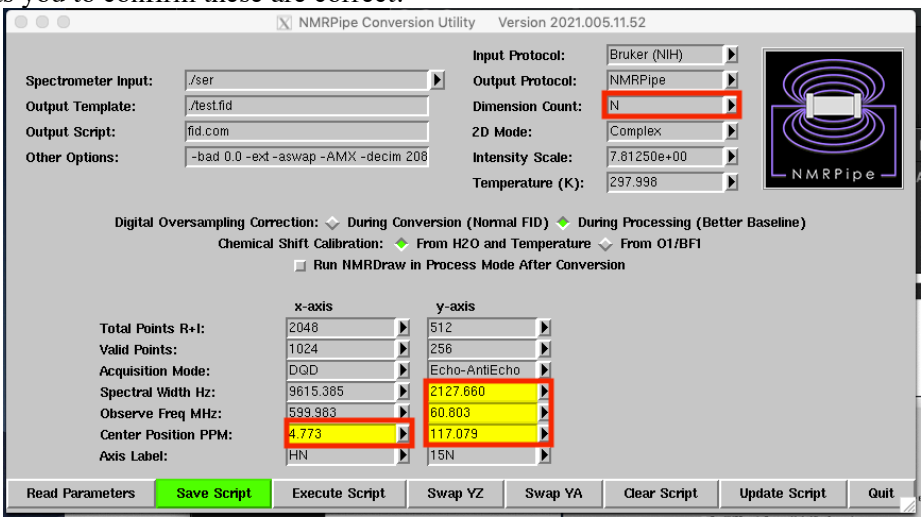
Next, we need to convert the data from Bruker serial files to NMRPipe format.

5. Type **bruker** in the command line. The NMR Conversion Utility GUI should open.

6. The **Read Parameters** button should be highlighted. Click this to read in the raw data. You should have two columns after clicking this button (x- and y- axis).

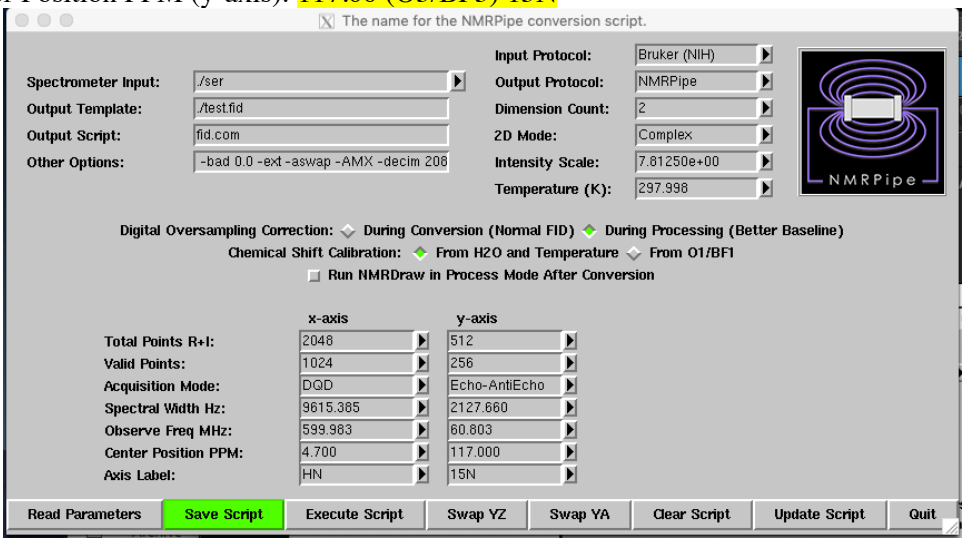


7. The parameters marked in the screenshot below, many of which are highlighted in yellow, need to be updated: *Note:* highlighted parameters are obtained from the experimental acquisition parameters and NMRPipe wants you to confirm these are correct.

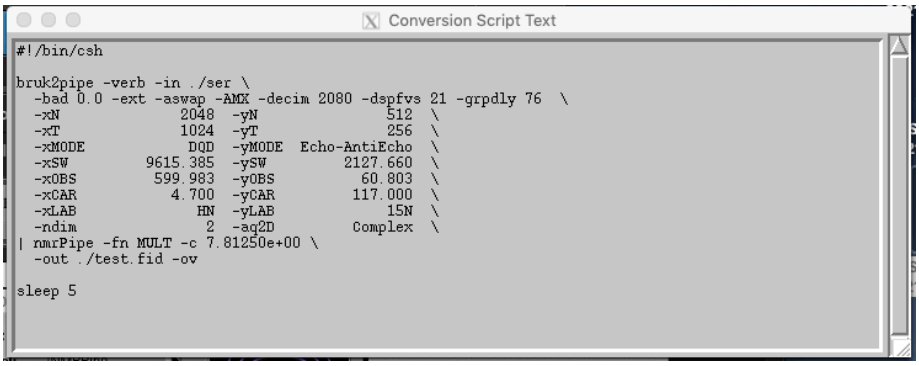


Click on the arrows by each value highlighted above and select the following options:

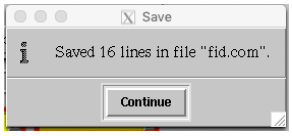
Dimension Count: 2D
Center Position PPM (x-axis): 4.700 (O1/BF1) 1H
Spectral Width Hz (y-axis): 2127.660
Observe Freq MHz (y-axis): 60.803 (SFO3) 15N
Center Position PPM (y-axis): 117.00 (O3/BF3) 15N



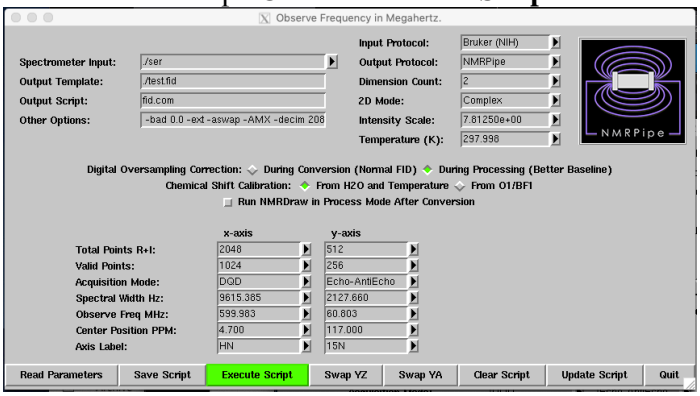
In addition, an output script we will use for converting the data (titled fid.com) is shown in a second window. Any edits you make in the selection window should also be changed in this script. Make sure the values are correct.



8. Click the **Save Script** button (highlighted in green). Click **Continue** on the window that appears to confirm the output file, fid.com, has been written.



9. Next, we will execute the conversion script. Click the **Execute Script** button.



A pop-up window should then appear:

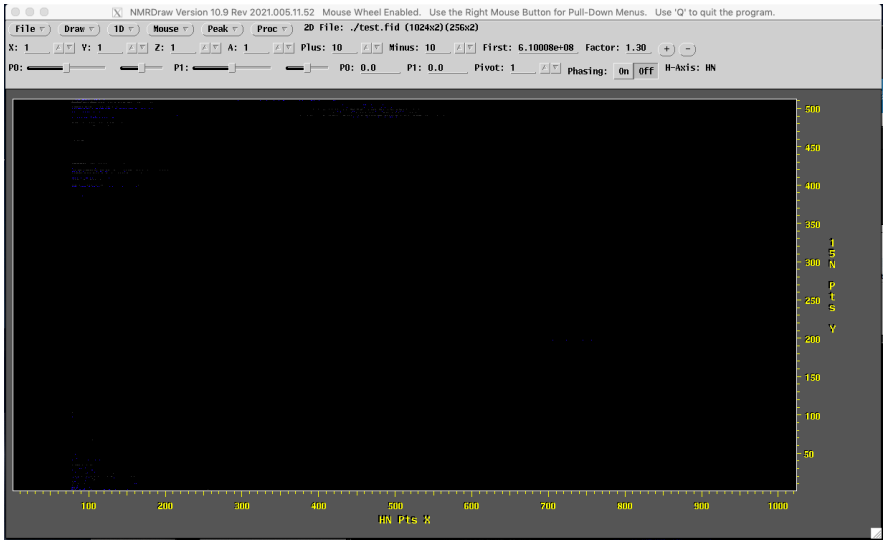
Alternatively, you can run the conversion script by quitting the Conversion Utility and executing the script in the terminal (**./fid.com**). Note you may have to type >chmod u+x fid.com to gain permission to execute this script.

10. NMRPipe should have created a file with the converted data called **test.fid**. Type **ls** and confirm it is present.

```
[Ryans-MacBook-Pro:2021_Macro_Methods_NMR/Nb6/Nb6_nhsqc] rwtibble% ls
EA                  format.ased         shimvalues
acqu                format.temp         specpar
acqu2               gpnam1              spnam1
acqu2s              gpnam2              spnam13
acqus               gpnam3              stapprogram2495
adcInfo_TRX1.xml    pdata               test.fid
audita.txt          pulseprogram        uxnmr.info
cpdprg3             scon2               uxnmr.par
fid.com             ser                 vtc_pid_settings
```

Part III: Manually phase the FID using NMRDraw.

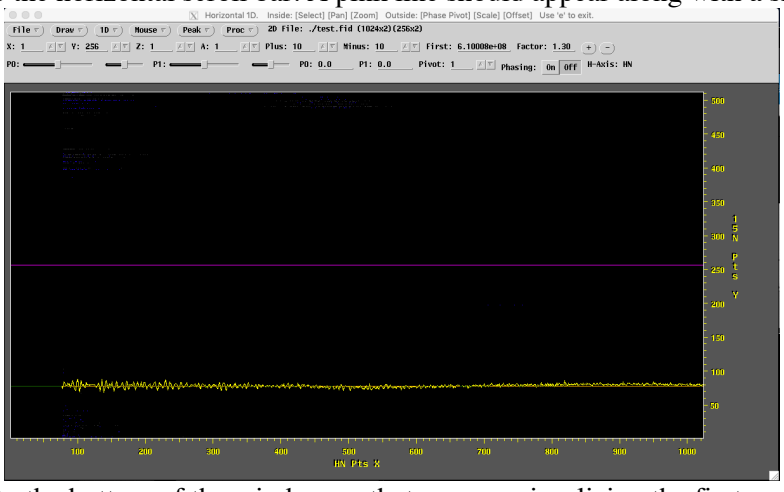
11. Open the NMRDraw suite by typing **nmrDraw** in the command line. You should see the following window appear:



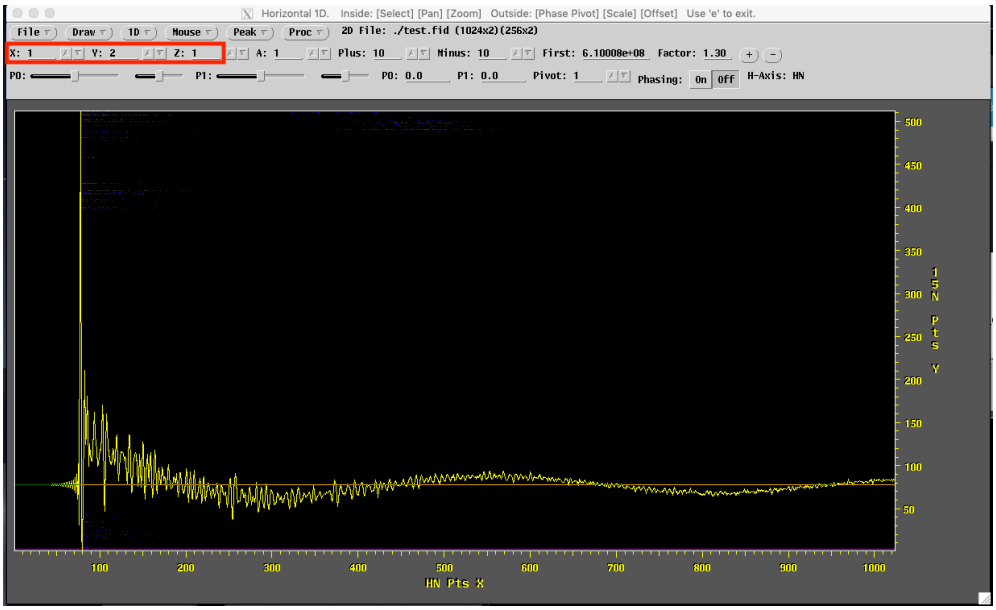
A quick note on navigating NMRDraw: Use right-click to access the pull-down menu for each of the options in the menu bar. Using left-click automatically performs the first action in each pull down menu. Alternatively, there are letter codes listed to the left of each menu option that can be used to perform a given action.

At this point, NMRDraw automatically read in the test.fid file and displayed the FIDs. You'll notice the scale of the x- and y-axes correspond to the number of real data points we collected for both the hydrogen (direct) and nitrogen (indirect) dimensions.

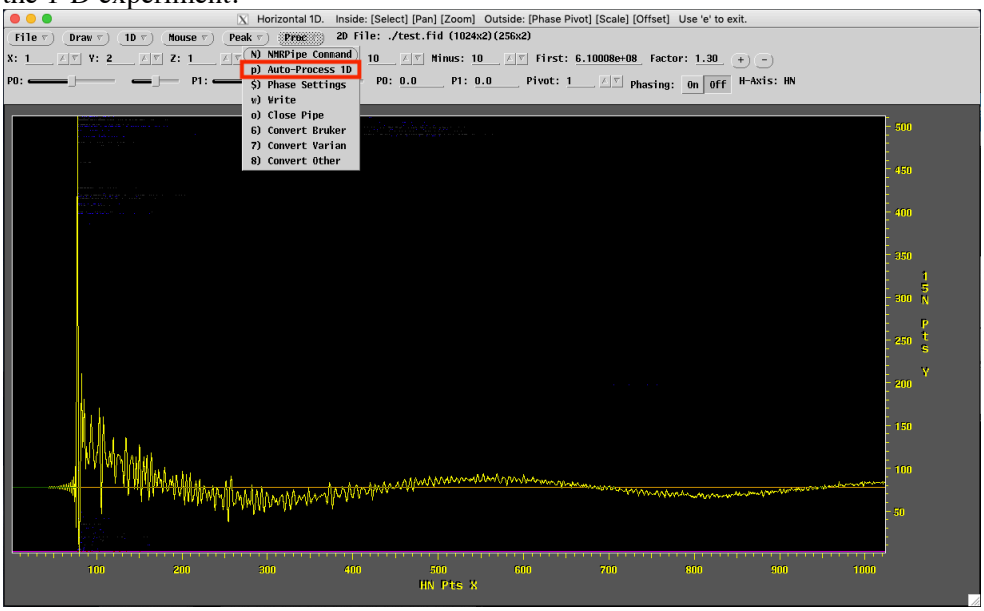
12. Type **h** to activate the horizontal scroll bar. A pink line should appear along with a single FID.



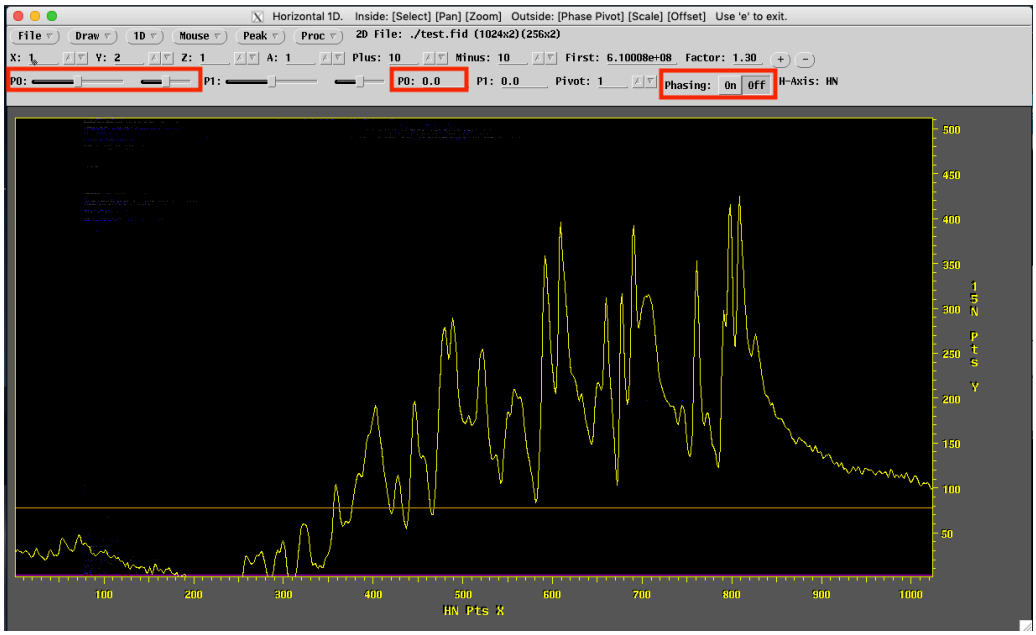
13. Pull the pink bar to the bottom of the window so that you are visualizing the first recorded FID. The coordinates near the top of the GUI should read **X: 2; Y:2; Z: 1**.



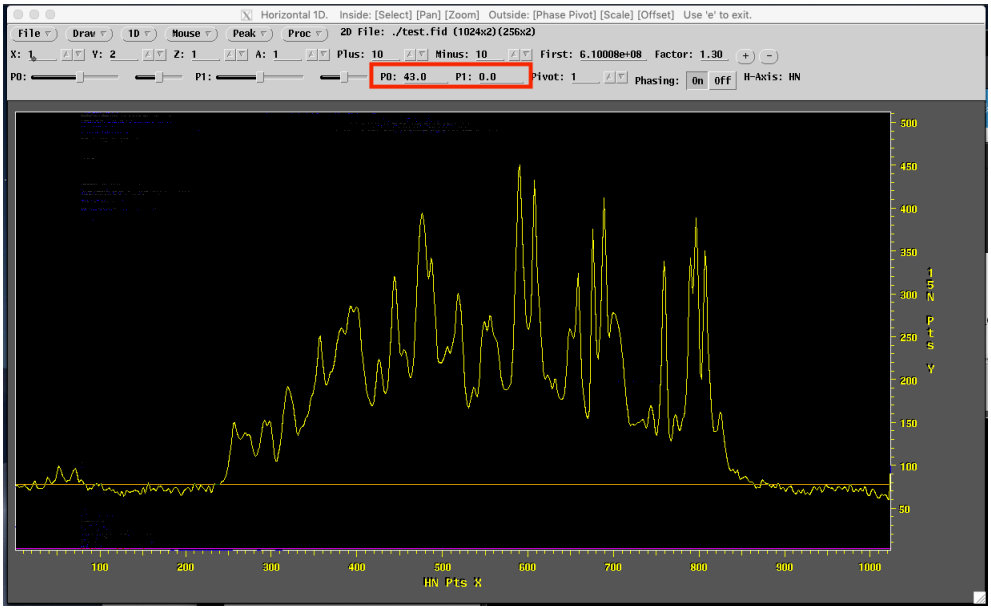
14. Right click the **Proc** button and select **Auto-Process 1D** (or type **p**). This will perform a Fourier transform of the 1-D experiment.



15. Manually phase the spectrum by clicking the phasing **ON** button. Manipulate the zero-order phasing (**P0**) by sliding the left scroll bar back and forth to correct the phasing and make the baseline as flat as possible. The right scroll bar can be used to finely change the phasing.



16. Once you have corrected the phasing, note the number next to **P0** (i.e. 43). Also, make sure **P1** has not been changed from zero.



17. After recording the value for P0, you can exit NMRDraw.

While we just determined the phase correction for a single FID, we need to apply this to all the FIDs we recorded. Luckily, we can run a processing script that will apply the same set of corrections to each FID in order to generate our 2D spectrum.

Part IV: Implement nhsqc.com file through the data

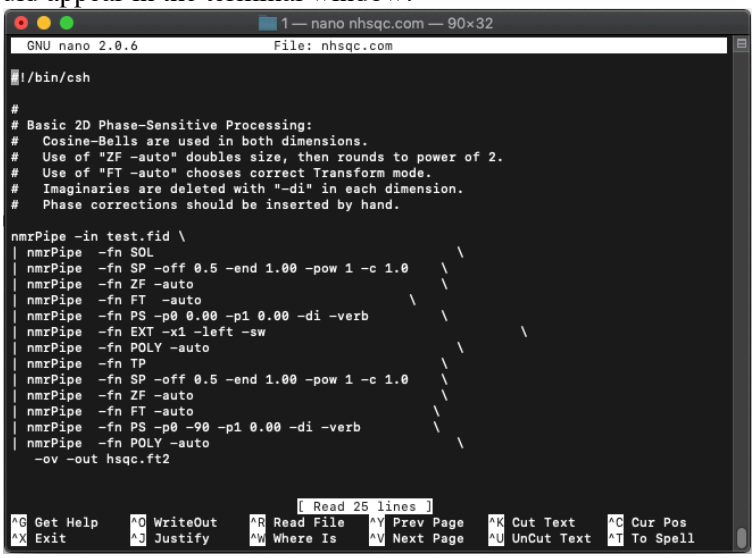
18. In the Processing_Scripts located in ~/EVENTS/2022-UCSF-204B, there is a file called **nhsqc.com**. I recommend keeping 'originals' of this file and other processing scripts in this directory and copy them to your working directory:

cp ~/EVENTS/2022-UCSF-204B/Processing_Scripts/nhsqc.com .

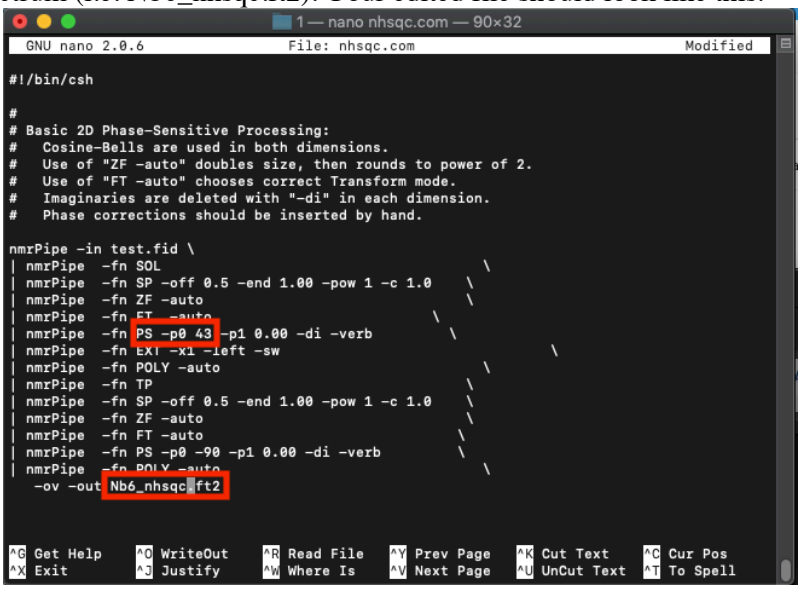
19. Type **ls** to make sure it correctly copied over.

```
[Ryans-MacBook-Pro:2021_Macro_Methods_NMR/Nb6/Nb6_nhsqc] rwtibble% ls
EA                      format.temp             specpar
acqu                    gpnam1                  spnam1
acqu2                   gpnam2                  spnam13
acqu2s                  gpnam3                  stanprogram2495
acqus                   nhsqc.com               test.fid
adcInfo_TRX1.xml        pdata                   uxnmr.info
audita.txt              pulseprogram            uxnmr.par
cpdprg3                 scon2                   vtc_pid_settings
fid.com                 ser
format.ased             shimvalues
```

20. Edit the nhsqc.com file using vi or nano (I will use nano here). To do this, type **nano nhsqc.com** and the contents of the file should appear in the terminal window.



21. Use the arrows to navigate to the PS command and edit the value next to P0 with the one you determined in NMRDraw (i.e. 43)
22. Navigate to the end of the file where it shows the output file (in this case the default is hsqc.ft2) and edit it to describe the spectrum (i.e. Nb6_nhsqc.ft2). Your edited file should look like this:

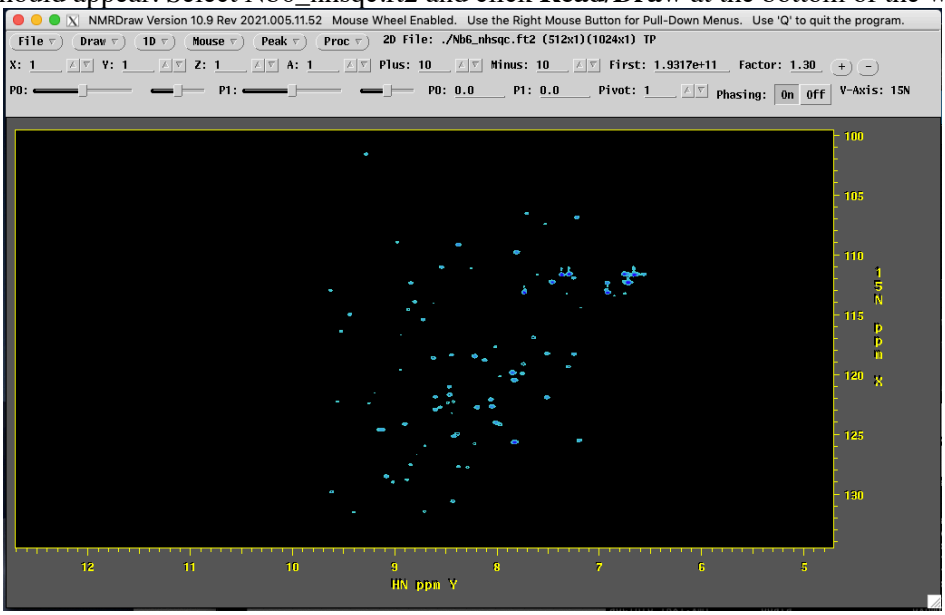


23. Save this file by pressing **control^X**. Type **Y** to save the modified file and press ENTER to overwrite the original nhsqc.com. Alternatively, you can provide a new name and create a new file with this name (becomes more useful as you make more specific changes in the file). If you do choose to create a new file, you will likely have to make it executable by typing **chmod +x nhsqc.com** in the terminal.
24. Run the processing file by typing ./nhsqc.com in the command line. The file will iterate through all of the experiments and output the ft2 file. If typing this produces an error, you likely need to make the file

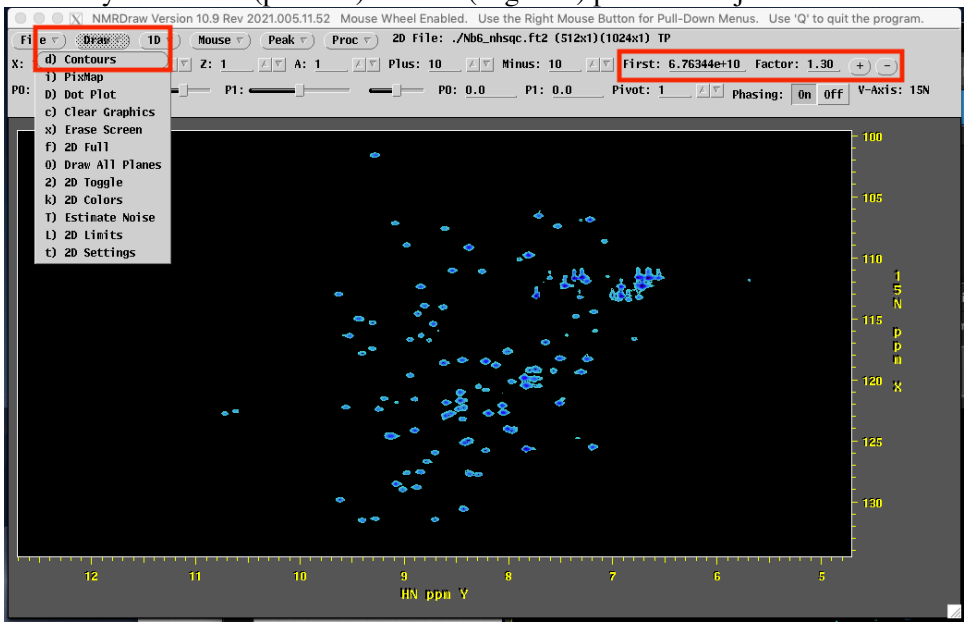
executable. Do so by typing **chmod +x nhsqc.com** in the terminal and try again. Type **ls** and you should now see the processed 2D file (**Nb6_nhsqc.ft2**).

```
[Ryans-MacBook-Pro:2021_Macro_Methods_NMR/Nb6/Nb6_nhsqc] rwtibble% ./nhsqc.com
PS       512 of 512    HN
PS      1024 of 1024   15N
[Ryans-MacBook-Pro:2021_Macro_Methods_NMR/Nb6/Nb6_nhsqc] rwtibble% ls
FA                      format.ased             shimvalues
Nb6_nhsqc.ft2           format.temp             specpar
acqu                    gpnam1                  spnam1
acqu2                   gpnam2                  spnam13
acqu2s                  gpnam3                  stanprogram2495
acqus                   nhsqc.com               test.fid
adcInfo_TRX1.xml        pdata                   uxnmr.info
audita.txt              pulseprogram            uxnmr.par
cpdprg3                 scon2                   vtc_pid_settings
fid.com                 ser
```

25. Type **nmrDraw** and you should now see the processed 2D spectrum! If NMRDraw did not automatically open the 2D spectrum, right-click the **File** button and click Select File (or type 'S' from the main screen). A new window should appear. Select Nb6_nhsqc.ft2 and click **Read/Draw** at the bottom of the window.



26. Use the + and – button in the upper right corner of the window to adjust the display threshold (First: xxxe+xxx) so the peaks more clearly shown. After each click of the + or – button, you'll need to left-click on the Draw button, which resets the contour level (alternatively, type **d**) If you set the contour too low, then you'll start to see many small blue (positive) and red (negative) peaks. Readjust the contour level



Macro Methods

Look for any indications of a poorly phased spectrum by noticing if peaks appear to be abruptly cut-off and there is a large amount of red, which indicates negative peak height. If this is the case, you can further refine the phasing by typing **h** or **v** to look at individual slices along the x- or y-axes, respectively, and use the scroll bars to improve phasing as we did previously. If you do change the phasing, note this new value, edit, and rerun the processing file. Iterate through this process until you have a well-phased spectrum.

Part IV: Exiting NMR Box

1-After terminating NMR Box desktop environment make sure you disconnect from the virtual session by going to 'My Account' in NMRBox and looking at your virtual machine status. In the example below , the user is still connected to cobalt.nmrbox.org. Click on the Icon under connected to terminate the session. NB, NMRBox will only allow 3-4 connections from a given user so it is important that you disconnect your sessions.