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 that is maintained by the NIST (https://www.ibbr.umd.edu/nmrpipe/index.html). NMRPipe is a powerful tool for converting and processing multi-dimensional biomolecular. With that in mind, the purpose of this tutorial is to walk you through the various steps involved in processing 2D NHSQC data recorded for Hsp90-NTD in the presence of small molecules in the second week of the class.

In general, the steps for processing data are: (1) download data to your computer (2) Convert the data to NMRPipe format (3) Transform and phase the first FID and (4) iterate NMRPipe functions through the entire series of 1D experiments.

In order to execute the NMRPipe GUI and commands you will need to either change your default shell to C-shell (csh) or launch a C-shell session in terminal by typing **csh** in a terminal window. In addition, please be sure NMRPipe is installed properly and can be accessed from the terminal—(tutorial for NMRPipe install on MacOS: http://fraserlab.com/static/pdf/methods/NMRPipe_Install_MacOS.pdf). To test if things are working, type **nmrPipe** in terminal and it should return the version you have installed.

Part I: Download data to local computer

1. Folders for both the small molecule screen and titrations are located in a UCSF Box folder that will be shared with the class. Download the data into the nmr directory you should have created when installing NMRPipe.
2. Once the data has been copied over, open terminal (or XQuartz) and descend into the directory for the screen. Make sure you have the correct number of data folders (should be 26) and enter the experiment that contains the data for the NTD alone (subdirectory 260):

    cd Hsp90_NTD_Method_screen/
    ls (should now see 26 experiments: 10 – 260)
    cd 260/

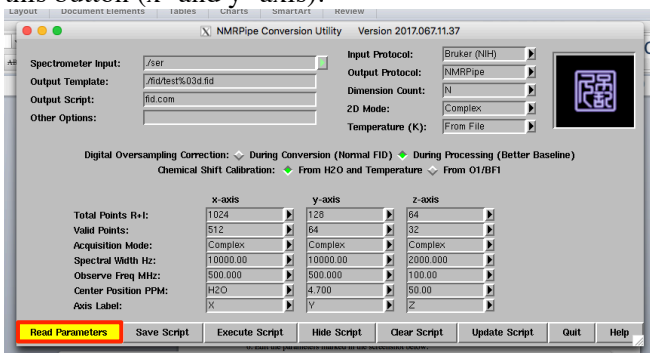
Type **ls** to look at the contents. If everything copied over correctly you should see this:

```
[[iPad-010060105007:~/nmr/nmrdata/Hsp90_auto_test] student% cd 10
[[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ls
acqu            audita.txt      cpdprg8         prosol_History  spnam9
acqu2           cag_par         format.ased     pulseprogram    uxnmr.par
acqu2s          cag_pars        format.temp     scon
acqus           cpdprg3         pdata           ser
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student%
```

Part II: Convert from Bruker to NMRPipe format

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

3. Type **bruker** in the command line. The NMR Conversion Utility GUI should open.
4. 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).



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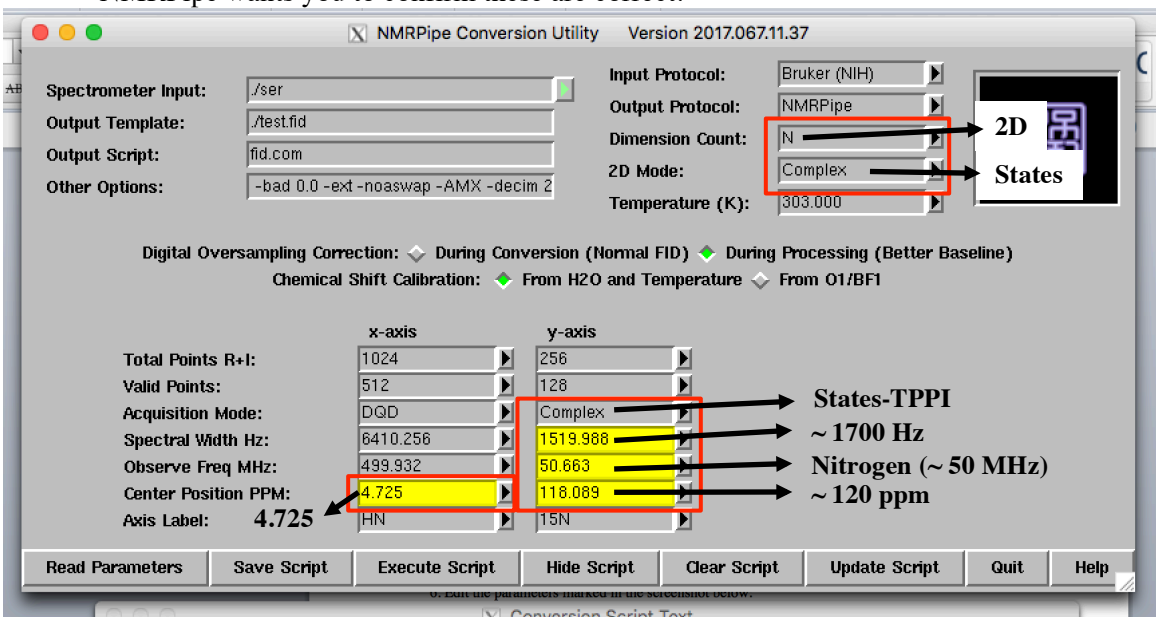
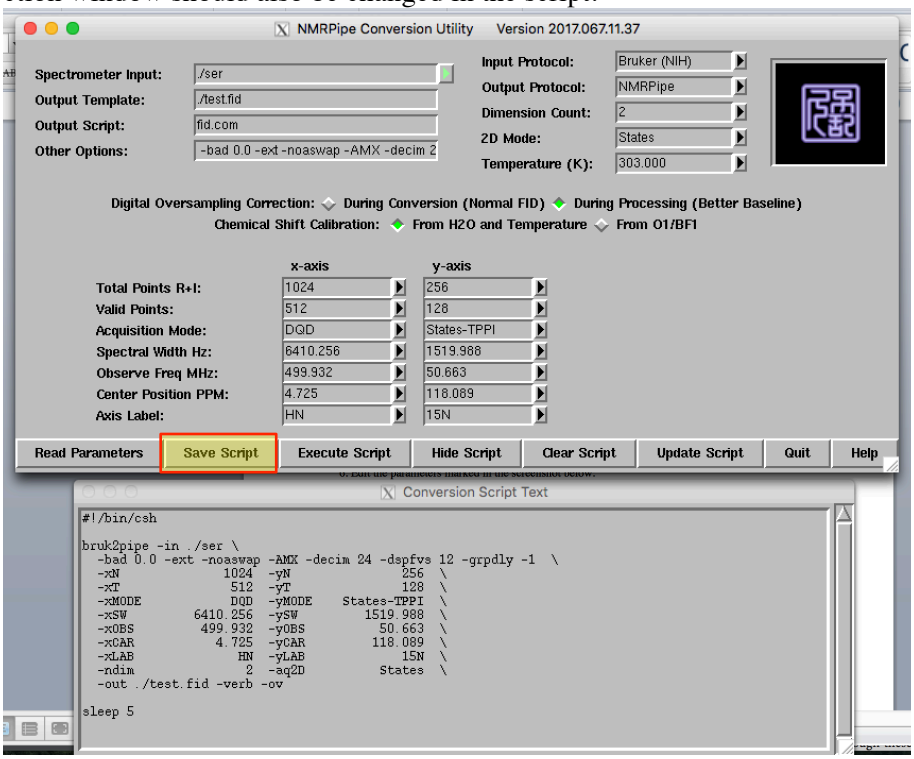
5. Edit the parameters marked in the screenshot below:

Note: highlighted parameters are obtained from the experimental acquisition parameters and NMRPipe wants you to confirm these are correct.



A copy of the output script that will be written (titled fid.com) is shown in a second window. Any edits you make in the selection window should also be changed in the script.



6. Click the **Save Script** button. Click **Continue** on the window that appears to write the output file. Close the Conversion Utility.

7. Type **ls** in the command line. The file **fid.com** should have been created.

```
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ls
acqu            audita.txt      cpdprg8         pdata           ser
acqu2           cag_par         fid.com         prosol_History  spnam9
acqu2s          cag_pars        format.ased     pulseprogram    uxnmr.par
acqus           cpdprg3         format.temp     scon
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student%
```

8. Run the conversion script by typing **./fid.com** and then look for the output file **test.fid**.

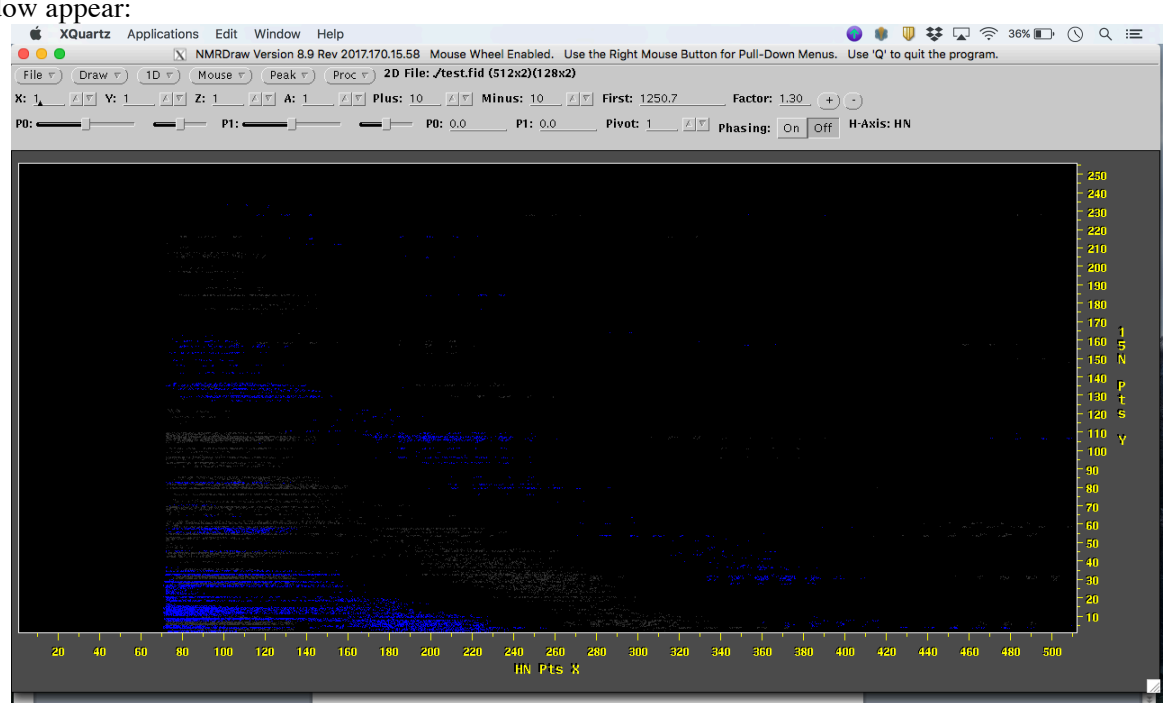
```
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ./fid.com

Bruker AMX --> NMRPipe Conversion.
Input File: ./ser
Output File: ./test.fid
2D Sizes: (1024 Real+Imag)(256 Real+Imag)
Byte Swap Mode: ON

Slice 256 of 256
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ls
acqu            audita.txt      cpdprg8         pdata           ser
acqu2           cag_par         fid.com         prosol_History  spnam9
acqu2s          cag_pars        format.ased     pulseprogram    test.fid
acqus           cpdprg3         format.temp     scon            uxnmr.par
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student%
```

Part III: Manually phase the FID using NMRDraw.

9. 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.

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

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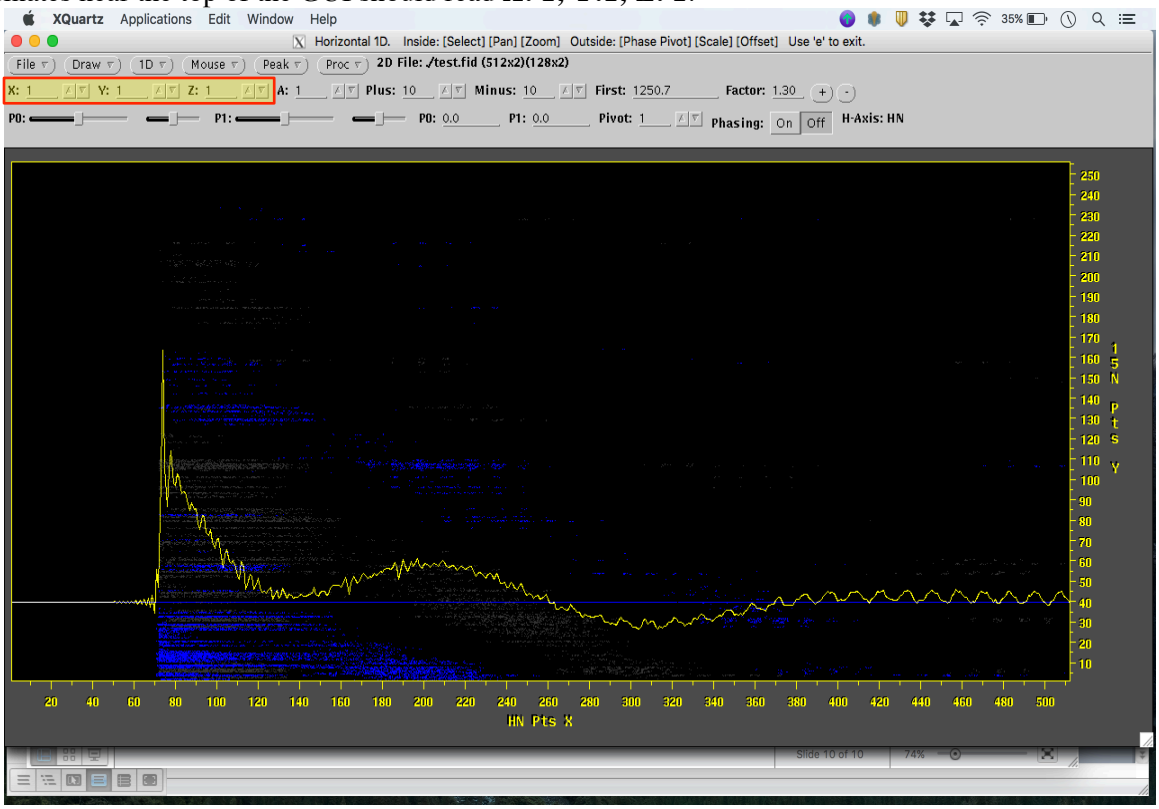
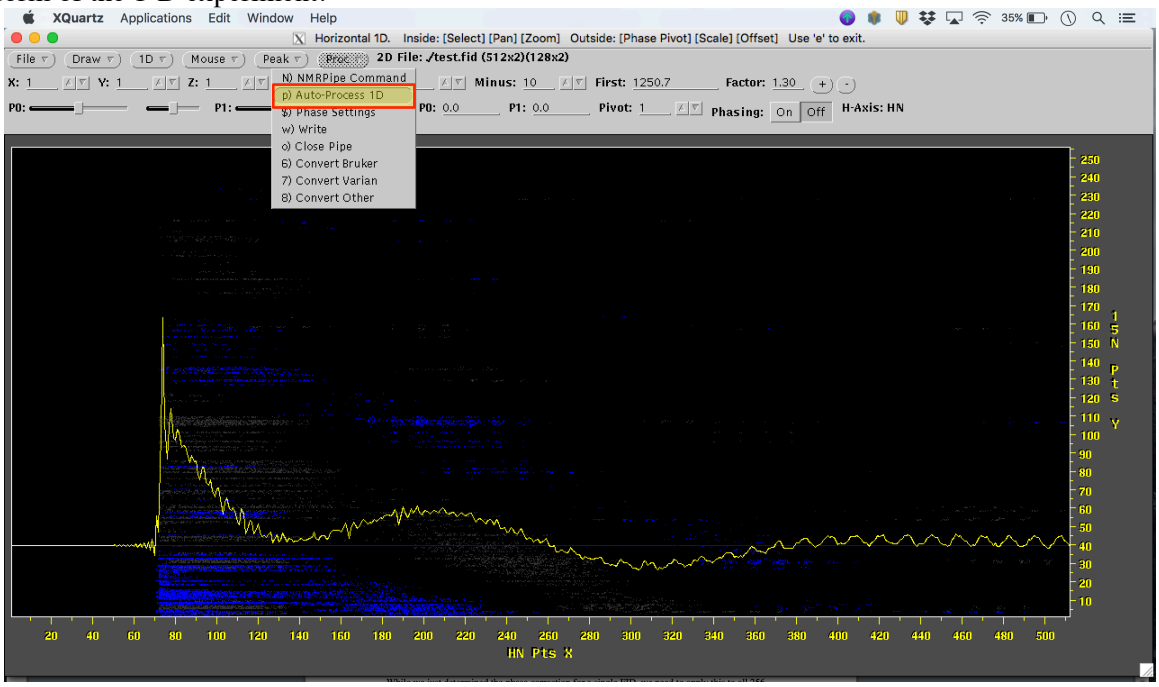
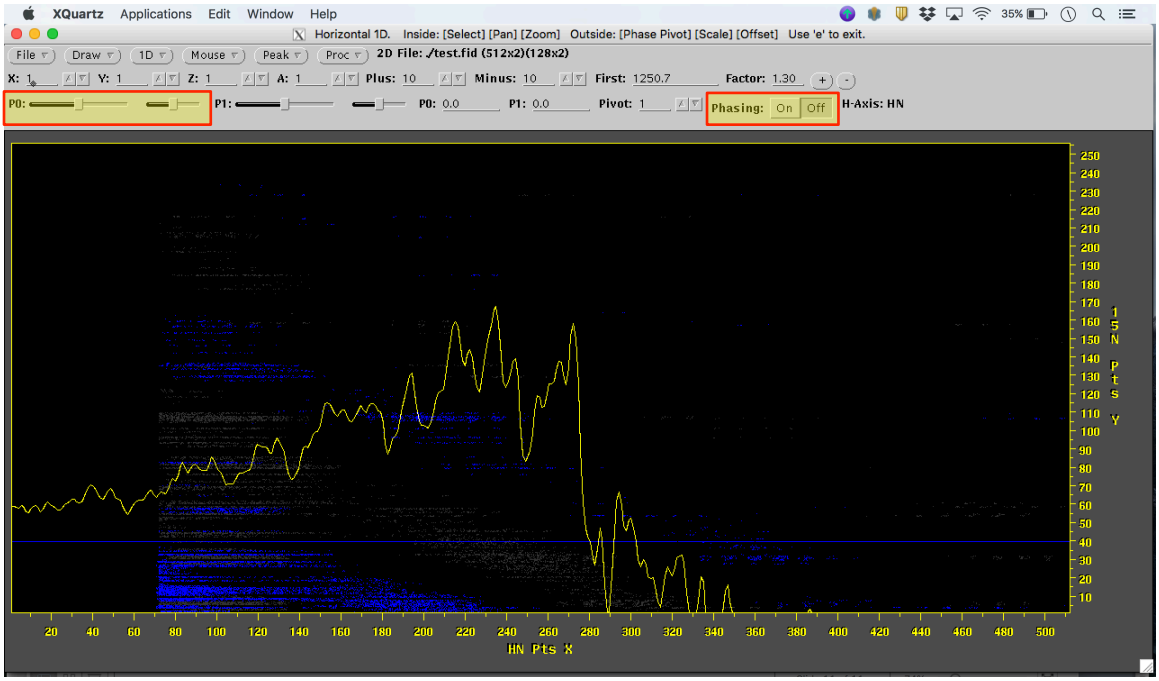
11. Pull the pink bar to the bottom of the window so that you are visualizing the first recorded FID. The coordinates near the top of the GUI should read **X: 1; Y:1; Z: 1**.



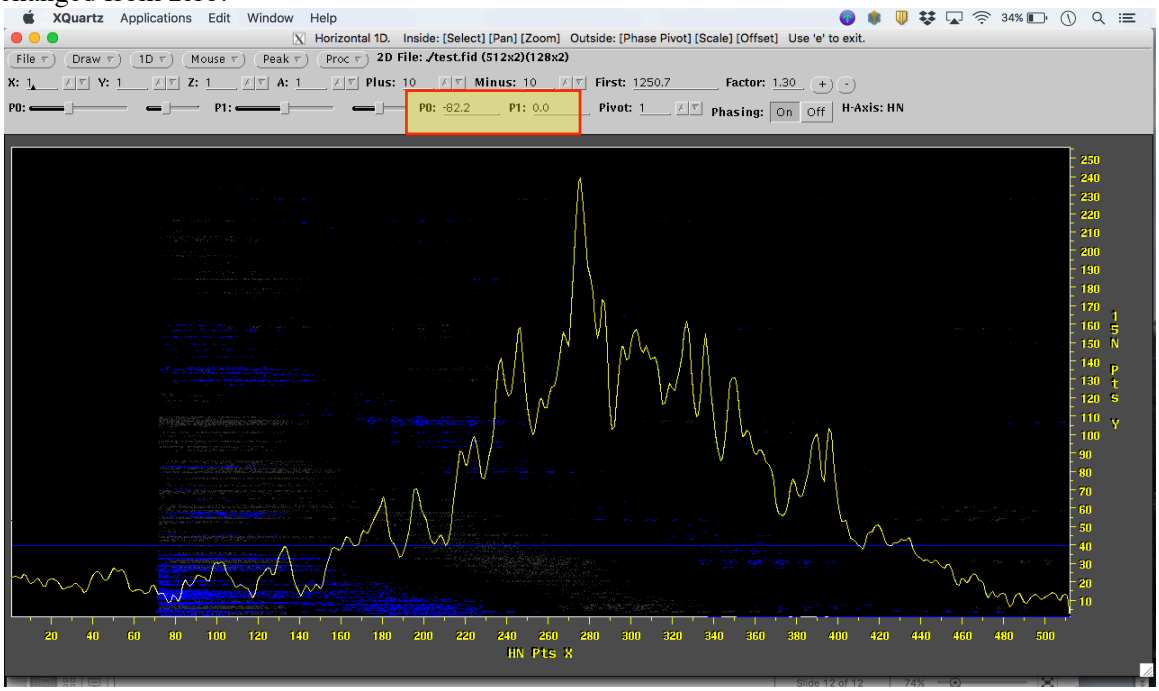
12. Right click the **Proc** button and select **Auto-Process 1-D** (or type **p**). This will perform a Fourier transform of the 1-D experiment.



13. 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.



14. Once you have corrected the phasing, note the number next to **P0** (i.e. -50). Also, make sure **P1** has not been changed from zero.



15. 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_500.com file through the data

16. Download the nhsqc_500.com file from the Macro Methods website and move it into your NMR directory (I like to keep the original in my "home" NMR directory and make copies for each experiment since I sometimes need to change additional parameters for a specific experiment). Once downloaded, you will likely need to make this file an executable by typing **chmod +x nhsqc_500.com**.

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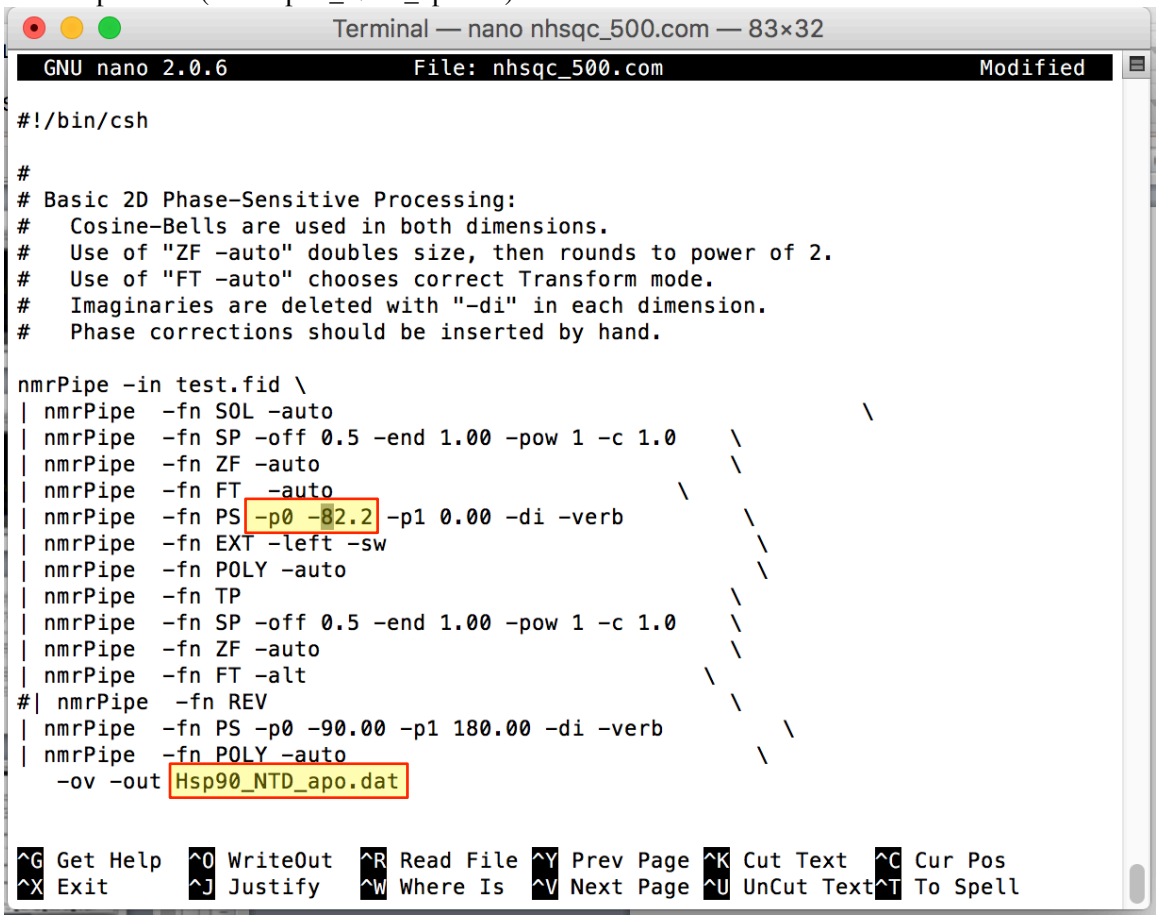
17. Copy the nhsqc_500.com file to the current directory using **cp**:
    e.g. cp /Users/student/nmr/nhsqc_500.com /Users/student/nmr/nmrdata/Hsp90_NTD_screen/10/
18. Again type **ls** to make sure it correctly copied over.

```
[[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ls                ]
acqu            cag_par         format.ased     pulseprogram    uxnmr.par
acqu2           cag_pars        format.temp     scon
acqu2s          cpdprg3         nhsqc_500.com   ser
acqus           cpdprg8         pdata           spnam9
audita.txt      fid.com         prosol_History  test.fid
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student%
```

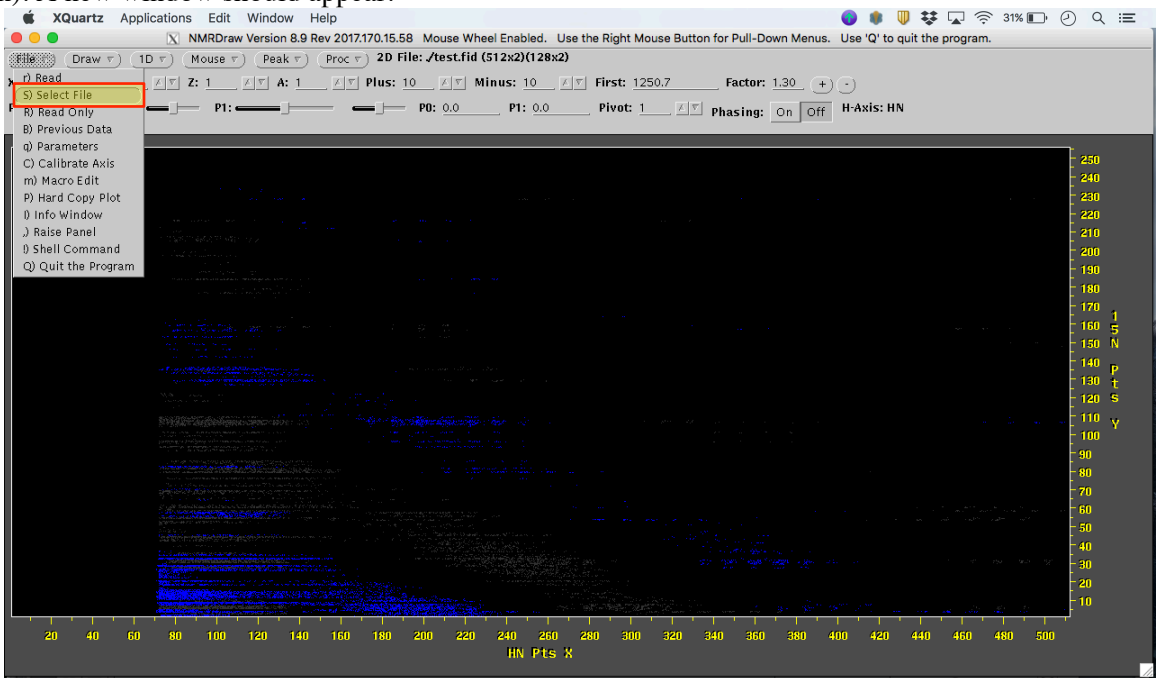
19. Edit the nhsqc.com file using vi or nano (I will use nano here). To do this, type **nano nhsqc.com** and the contents of the file should appear in the terminal window.
20. Use the arrows to navigate to the first command PS command and edit the value next to P0 with the one you determined in NMRDraw.
21. Navigate to the end of the file where is shows the output file (in this case it is hsqc.dat) and change this to describe the spectrum (i.e. Hsp90_NTD_apo.dat).



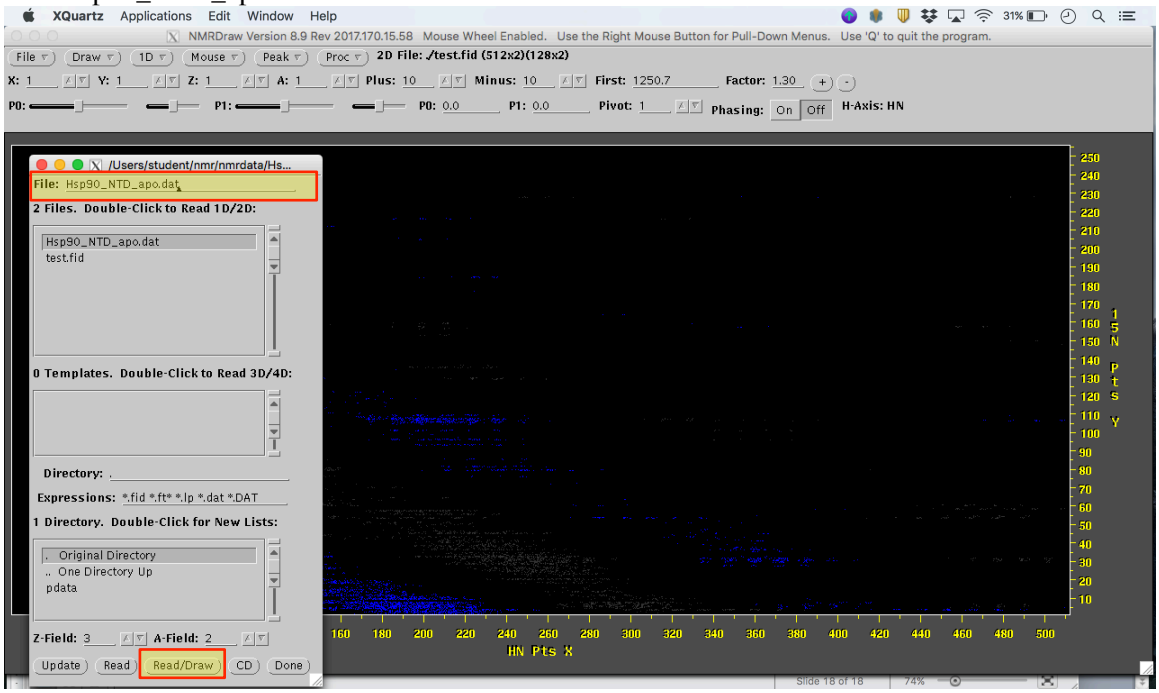
22. Save this file by pressing **control+X**. Type **Y** to save the modified file and press ENTER to overwrite the original nhsqc.com. Alternatively, you can provide a new name and create a new file with this name (becomes more useful as you make more specific changes in the file). If you do choose to create a new file, you will likely have to make it executable using the chmod command mentioned above.
23. 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.

```
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ./nhsqc_500.com
PS        256 of 256
PS        512 of 512
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student% ls
Hsp90_NTD_apo.dat       cpdprg3                 pulseprogram
acqu                    cpdprg8                 scon
acqu2                   fid.com                 ser
acqu2s                  format.ased             spnam9
acqus                   format.temp             test.fid
audita.txt              nhsqc_500.com           uxnmr.par
cag_par                 pdata
cag_pars                prosol_History
[iPad-010060105007:nmrdata/Hsp90_auto_test/10] student%
```

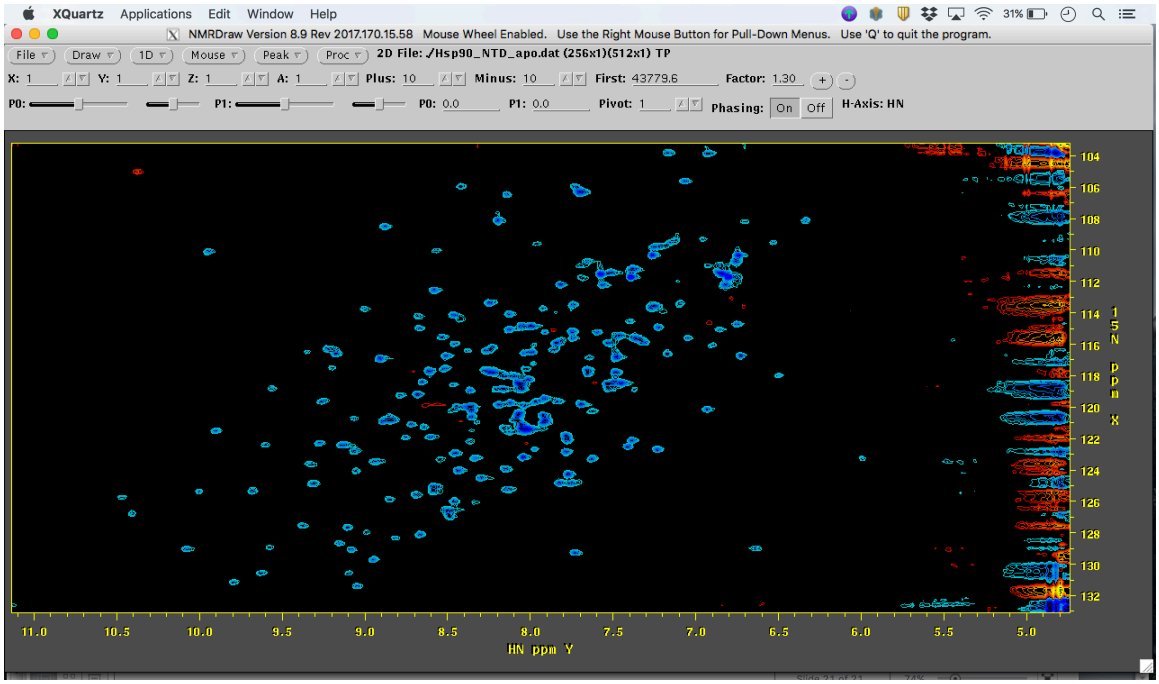
24. Open NMRDraw again and right-click the **File** button and click Select File (or type ‘S’ from the main screen). A new window should appear.



25. Select Hsp90_NTD_apo.dat and click **Read/Draw** at the bottom of the window.



You should now see a 2D spectrum appear!



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.

Once you have a reasonable spectrum, it's time to do one last conversion so we can visualize it in Sparky.

Part V: Convert to Sparky (.ucsf) format

While NMRPipe is great for processing data, it is less straightforward for manipulating spectra to generate overlays and display assignments. With this in mind, we will use Sparky for the majority of our data analysis and you should have downloaded it from the NMRFAM website (http://www.nmrfam.wisc.edu/nmrfam-sparky-distribution.htm)

NMRFAM-Sparky includes a GUI that will allow you to convert NMRPipe files to sparky (.ucsf) format, but it is cumbersome to use. Luckily, a command-line executable can be implemented to help streamline the process. To do this, we will need to modify the c-shell start-up file so it contains a link to the conversion file.

26. In terminal, type **nano ~/.cshrc** and the start-up file will appear in the window. Navigate to the bottom and enter the following:

```
alias pipe2ucsf '/Applications/nmrfam-sparky-mac/easy_pipe2ucsf.app/Contents/MacOS/pipe2ucsf'
```

Note: This link should work if you installed Sparky in your applications folder. If you did not, it will be necessary to enter the correct path.
27. Save the file (**control+X** followed by **Y**). Type **source ~/.cshrc** to rerun the start-up script. You'll only need to do this the first time since the .cshrc file is executed each time c-shell is started.
28. Type **pipe2ucsf** and if everything went well, you should see the following:

```
[CCB-2093:nmrdata/Hsp90_auto_test/10] student% pipe2ucsf
Syntax: pipe2ucsf [-<axis-order>] <pipe-file> <ucsf-file>
  where <axis-order> is a string of digits (eg. 312)

Example:

        % pipe2ucsf noesy150.pipe noesy150.ucsf

The nmrPipe file can be 2D, 3D, or 4D data.  The nmrPipe data must
be in a single file.  See documentation for the xyz2pipe command that
comes with nmrPipe for how to combine planes of a 3D or 4D spectrum
into a single file. For more information see the Sparky documentation
manual/files.html.
```

29. Convert the .dat file to .ucsf by typing **pipe2ucsf Hsp90_NTD_apo.dat Hsp90_NTD_apo.ucsf** and press **enter**. Type **ls** and make sure the .ucsf file has been created.