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 (<u>https://www.ibbr.umd.edu/nmrpipe/index.html</u>). 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 (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.

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: <u>http://fraserlab.com/static/pdf/methods/NMRPipe_Install_MacOS.pdf</u>). 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. A Box folder containing all of the data has been shared with you. Download the data into the nmr directory you should have created when installing NMRPipe. For ease of locating the data, I would recommend creating a NMR data directory (**mkdir nmrdata**) in your NMR folder.

2. Once the data has been copied over, open terminal (or XQuartz) and descend into the directory:

cd ~/nmr/nmrdata/2021_Macro_Methods_NMR

ls (should now see 5 directories)

[[Ryans-MacBook-Pro:~/nm	r/2021_Macro_Methods_N	MR] rwtibble% ls
Nb6	Processing Scripts	mNb6_RBD_complex
Nb6_RBD_complex	mNb6	_

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 Nb6). Type ls again and you should see directories for the carbon (chsqc) and nitrogen (nhsqc) HSQC experiments.

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:

.0	copied over conteeling	•	
	[[Ryans-MacBook-Pro:~/n	mr/2021_Macro_Methods_NMF	R/Nb6] rwtibble% cd Nb6_nhsqc/
	[[Ryans-MacBook-Pro:202	1_Macro_Methods_NMR/Nb6/M	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).

•••		X NMRPipe Co	nversio	on Utility \	ersion 20	021.00	5.11.52		
				Input	Protocol		Bruker (NIH)	_
Spectrometer Input:	./ser			Outp	ut Protoc	ol:	NMRPipe		3)
Output Template:	./fid/test%03d.	fid		Dime	nsion Cou	int:	N		
Output Script:	fid.com			2D M	ode:		Complex		シ)
Other Options:				Inten	sity Scal	e:	1.0		
				Temp	erature (к):	From File		ipe 🚽
		x-axis		y-axis		z-ax	is		
Total Point	o D. h	1024	Þ	128			38		
Valid Point	3 1171.				N	64		N	
	e•	512				64 32) N	
		512 Complex		64	Þ	32	lex	Þ	
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Acquisition	Mode: Idth Hz:	Complex	Þ	64 Complex) 	32 Comp	.000))	
Acquisition Spectral W	Mode: idth Hz: req MHz:	Complex 10000.00 500.000 H2O))))	64 Complex 10000.00))))	32 Comp 2000 100.0 50.00	.000 IO		
Acquisition Spectral W Observe F	Mode: lidth Hz: req MHz: ition PPM:	Complex 10000.00 500.000	Þ	64 Complex 10000.00 500.000))))	32 Comp 2000 100.0	.000 IO		

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.

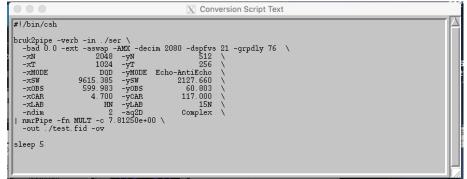
		X NMRPipe Conver	sion Utili	ty Version 2021.0	05.11.52		
				Input Protocol:	Bruker (NIH)		
Spectrometer Input:	./ser			Output Protocol:	NMRPipe		$ \rightarrow $
Output Template:	./test.fid			Dimension Count:	N		
Output Script:	fid.com			2D Mode:	Complex		2)
Other Options:	-bad 0.0 -ext	-aswap -AMX -decim	208	Intensity Scale:	7.81250e+00		
				Temperature (K):	297.998		oe ─
	Chemica	I Shift Calibration: I Shift Calibration:		ss Mode After Conve	*		
Total Po	ints R+I:	2048					
Valid Poi	nts:	1024		Þ			
Acquisiti	on Mode:	DQD	Echo-	AntiEcho			
Spectral	Width Hz:	9615.385		A			-
	Freq MHz:	599.983					
	osition PPM:	<mark>4.773 🔰 🕨</mark>		79			
Axis Lab	el:	HN	15N				
Read Parameters	Save Script	Execute Script	Swap	YZ Swap YA	Clear Script	Update Script	Quit

Click on the arrows by each value highlighted above and select the following options: Dimension Count: 2D

Cente	r Position PP	M (x-axis)	: <mark>4.700 (O</mark>	<mark>1/B</mark>	F1) 11	ł			
Spect	ral Width Hz	(y-axis): <mark>2</mark>	127.660						
	ve Freq MHz			<mark>03</mark>) 15N				
	r Position PPI					.5N			
_						e conversion scr	ipt.		
					Inp	ut Protocol:	Bruker (NIH)		
	Spectrometer Input:	./ser			Du Out	put Protocol:	NMRPipe		
	Output Template:	./test.fid			Din	nension Count:	2		
	Output Script:	fid.com			2D	Mode:	Complex		
	Other Options:	-bad 0.0 -ext	-aswap -AMX -dec	im 20	8 Inte	ensity Scale:	7.81250e+00		
					Ter	nperature (K):	297.998		pe 🗕
	Digital	Oversempling Cor	raction: A During	Cons	version (No	mal EID) 🔶 Dur	ing Processing (Be	ttor Posolino)	
	Digital		Shift Calibration:				• • • •	tter baseline)	
			🔲 Run NMRDr	aw in	Process M	ode After Convei	sion		
	T-t-t D-1		x-axis	TN	y-axis 512	N			
	Total Point		1024		256				
	Valid Poir		DQD	F.	Echo-Antil	icho N			
	Acquisitio	Width Hz:	9615.385	Ĕ.	2127.660				
		Freq MHz:	599.983	ĥ	60.803				
		nition PPM:	4.700	ĥ	117.000				
	Axis Labe		HN	ĥ	15N				
	T MIS ECON		1		1.014				
	Read Parameters	Save Script	Execute Script		Swap YZ	Swap YA	Clear Script	Update Script	Quit

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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.

Saved 16 lines in file "fid.com".			X Save
Continue	i	Saved 1	6 lines in file "fid.com".
			Continue

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

Read Parameters	Save Script	Execute Script	S	Swap YZ	Swap YA	Clear Script	Update Script	Q
Axis Labe	l:	HN		15N	Þ			
	sition PPM:			117.000				
Observe l			- 1	80.803				
Spectral \	Midth Hz:	9615.385		2127.660				
Acquisitio	n Mode:	DQD		Echo-AntiEcho	Þ			
Valid Poin	ts:	1024		256	Þ			
Total Poin	ts R+I:	2048		512	Þ			
		x-axis		v-axis	and conve			
Digital		rection: 🔷 During C I Shift Calibration: < 🔲 Run NMRDray	Fro	om H2O and Te	emperature	From 01/BF1	etter Baseline)	
					ature (K):	297.998		ipe
Other Options:	-bad 0.0 -ext	-aswap -AMX -decim	208	Intensit	y Scale:	7.81250e+00		
Output Script:	fid.com			2D Mod	ə:	Complex		2)
Output Template:	./test.fid			Dimensi	on Count:	2		
Spectrometer Input:	./ser				Protocol:	NMRPipe		3
			_	Input Pr Output	0100001.	Bruker (NIH)		

A pop-up window should then appear

	X NMRPipe	
Bruker AMX> NMRPipe (Input File: ./ser Output Macro: /usr/loca 2D Sizes: (2048 Real+Ima Byte Swap Mode: OFF	/bin/nmrtxt/bruk_ranceY.M	
Slice 512 of 512		

Alternatively, you can run the conversion script by quitting the Conversion Utility and executing the script in the terminal (**/fid.com**).

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

[[Ryans-MacBook-Pro:2	021_Macro_Methods_NMR/	/Nb6/Nb6_nhsqc] rwtibble% ls
EA	format.ased	shimvalues
acqu	format.temp	specpar
acqu2	gpnam1	spnam1
acqu2s	gpnam2	spnam13
acqus	gpnam3	stanprogram2495
adcInfo_TRX1.xml	pdata	test.fid
audita.txt	pulseprogram	uxnmr.info
cpdprg3	scon2	uxnmr.par
fid.com	ser	vtc_pid_settings

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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:



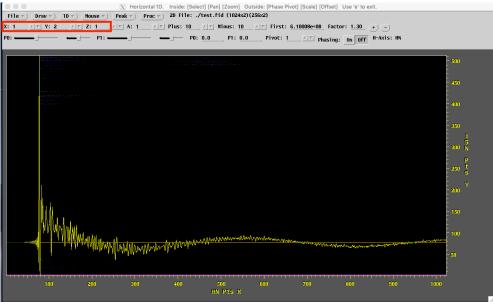
A quick note on navigating NMRDraw: Use right-click to access the pull-down menu for each of the options in the menu bar. Using left-click automatically performs the first action in each pull down menu. Alternatively, there are letter codes listed to the left of each menu option that can be used to perform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

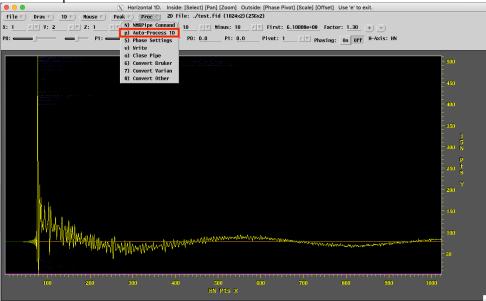
12. Type h to activate the horizontal scroll bar. A pink line should appear along with a single FID.

		an an Capita	h felger se			500
						- 450
						400
						-
						- 350
						- 300
						250
						200
						- 150
man management	warman production and		o only goon allow the	 and the state of the	 	- 100

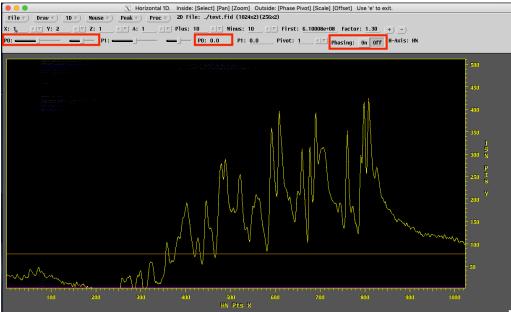
13. Pull the pink bar to the bottom of the window so that you are visualizing the first recorded FID. The coordinates near the top of the GUI should read X: 2; Y:1; Z: 1.



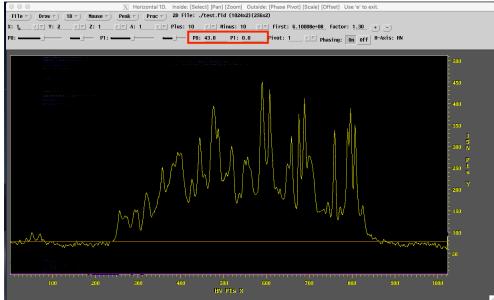
14. Right click the **Proc** button and select **Auto-Process 1D** (or type **p**). This will perform a Fourier transform of the 1-D experiment.



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 directory you downloaded from Box, 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 ~/nmr/nmrdata/2021_Macro_Methods_NMR/Processing_Scripts/nhsqc.com .
19. Type Is to make sure it correctly copied over.

[[Ryans-MacBook-Pro:2 EA	/021_Macro_Methods_NMR/ format.temp	Nb6/Nb6_nhsqc] rwtibble% ls/ specpar
acqu	gpnam1	spram1
acqu2	gpnam2	spnam13
acqu2s	dpnam3	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.

0 🔴 🔵	🚞 1 — nano nhsqc.co	m — 90×32			
GNU nano 2.0.6	File: nhsqc.com				E
#!/bin/csh					
# Basic 2D Phase-Sensi					
	ed in both dimensions.				
	oubles size, then rounds to				
	chooses correct Transform mo				
	leted with "-di" in each dim	ension.			
# Phase corrections	should be inserted by hand.				
nmrPipe -in test.fid \					
nmrPipe -fn SOL		λ.			
	0.5 -end 1.00 -pow 1 -c 1.0	X.			
nmrPipe -fn ZF -aut		Ň			
nmrPipe -fn FT -au	:o				
nmrPipe -fn PS -p0	0.00 -p1 0.00 -di -verb				
nmrPipe -fn EXT -x1					
nmrPipe -fn POLY -a	ito				
nmrPipe —fn TP		Λ.			
	0.5 -end 1.00 -pow 1 -c 1.0	N			
nmrPipe _fn ZF _aut		\ \			
nmrPipe -fn FT -aut		N N			
	-90 —p1 0.00 —di —verb	``,			
nmrPipe -fn POLY -a	to	`			
-ov -out hsqc.ft2					
	[Read 25 line:	•]			
AG Get Help AO Writ			Cut Text	^C Cur Pos	
^X Exit ^J Just	ify <mark>^W</mark> Where Is <mark>^V</mark> Ne:	kt Page ^U	UnCut Text	^T To Spell	

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:

0 🕘 🔵	🚞 1 — nano nhsqc	.com — 90×32		
GNU nano 2.0.6	File: nhsqc.com			Modified
#!/bin/csh				
#!/bin/csn				
#				
	Sensitive Processing:			
	are used in both dimensions.			
	uto" doubles size, then rounds			
	to" chooses correct Transform			
	re deleted with "-di" in each d			
# Phase correct	ions should be inserted by hand			
nmrPipe -in test.	Fid \			
nmrPipe -fn SO		١.		
nmrPipe -fn SP		.0 \		
nmrPipe -fn ZF		Ň		
nmrPipe -fn ET	-auto			
	-p0 43 -p1 0.00 -di -verb	١		
nmrPipe -fn EX			۱	
nmrPipe -fn PO	Y -auto	· `		
nmrPipe -fn TP	-off 0.5 -end 1.00 -pow 1 -c 1	<u>^</u>		
nmrPipe -fn SP				
nmrPipe -fn FT		``		
	-p0 -90 -p1 0.00 -di -verb	Ň		
nmrPipe _fp_P0		· \		
-ov -out Nb6_n				
			.	a
				Cur Pos To Spell
"X EXIT ^J	Justity where is AV	Next Page AU	UNCUT Text *1	to spell

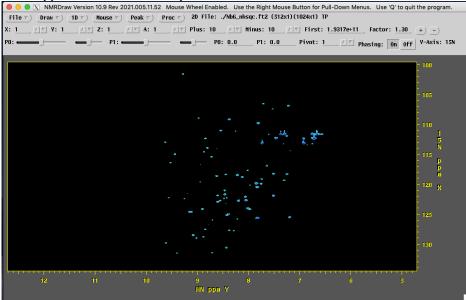
23. Save this file by pressing control^X. Type Y to save the modified file and press ENTER to overwrite the original nhsqc.com. Alternatively, you can provide a new name and create a new file with this name (becomes more useful as you make more specific changes in the file). If you do choose to create 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

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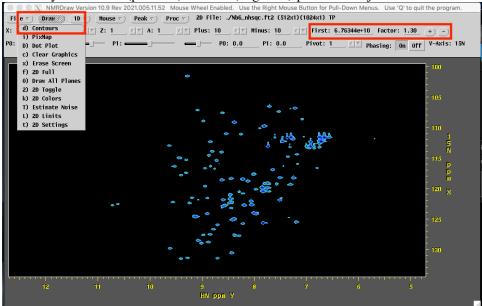
executable. Do so by typing **chmod +x nhsqc.com** in the terminal and try again. Type **ls** and you should now see the processed 2D file (**Nb6 nhsqc.ft2**).

[[Ryans-MacBook-Pr	o:2021_Macro_Methods_M	NMR/Nb6/Nb6_nhsqc] rwtibble%	./nhsqc.com
PS 512 of 5	12 HN		
PS 1024 of 1	.024 15N		
[[Ryans-MacBook-Pr	o:2021_Macro_Methods_M	MR/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



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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 \mathbf{h} or \mathbf{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.