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.

	cobalt.nmrbox.org:443 (cobalt:12) - VNC Viewer
-	🕒 🖬 Terminal - jgross@cobal 🔀 🖸 🧭 👔 😥 💬 🚯 🗙 🔛 💶 🛛 22:04 🦉 🕅 John Gross
	Terminal - jgross@cobalt: ~ + _ □ ×
	File Edit View Terminal Tabs Help
н In	\ _ < _ > < _ \ _ _ _ \/ \/ \/
tistic	IMPORTANT: Please Cite and Acknowledge NMRbox in your publications! See https://nmrbox.org/acknowledge-us for details.
fects	NMBbox VM Status and Information: https://nmrbox.org/user-dashboard Documentation: https://nmrbox.org/documentation FAG: https://nmrbox.org/faq Installed Software: https://nmrbox.org/pages/slack
_	NMRbox version: 2022.03.00 Shutdown: April 11 2022
	0b:00.0 3D controller: NVIDIA Corporation Device leb8 (rev al) jgrossgeobalt:-5 # jgrossgeobalt:-5 # jgrossgeobalt:-5 # jgrossgeobalt:-5 # jgrossgeobalt:-5 jgrossgeobalt:-5 jgrossgeobalt:-5

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.



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:

·	eopiea over concerny		
	[[Ryans-MacBook-Pro:~/nm	r/2021_Macro_Methods_NMR,	/Nb6] rwtibble% cd Nb6_nhsqc/
	[[Ryans-MacBook-Pro:2021	_Macro_Methods_NMR/Nb6/Ni	b6_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 Daint	o D. h	x-axis		y-axis		Z-93	38		
Volid Deint	3 1171.	11067			N	64		N	
VALUE PURCH	e•	512	- 1	64		64 32) N	
Acquisition	s: Mode:	512 Complex	- È	64 Complex)))	64 32 Comr	lex		
Acquisition Spectral W	s: Mode: idth Hz:	512 Complex 10000.00	Þ	64 Complex 10000.00))))	64 32 Comp 2000	olex .000		
Acquisition Spectral W Observe F	s: Mode: Idth Hz: req MHz:	512 Complex 10000.00 500.000)))	64 Complex 10000.00 500.000))))	64 32 Comp 2000 100.0	olex .000		
Acquisition Spectral W Observe Fi Center Pos	s: Mode: Ndth Hz: req MHz: NHa:	512 Complex 10000.00 500.000 H2O))))	64 Complex 10000.00 500.000 4.700))))	64 32 2000 100.0	olex .000 IO		
Acquisition Spectral W Observe F Center Pos Axis Label:	s: Mode: Ndth Hz: req MHz: NHD:	512 Complex 10000.00 500.000 H2O X		64 Complex 10000.00 500.000 4.700 Y)))))	64 32 2000 100.0 50.00 Z	olex .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 Utilit	y 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		o e 🗕
	Chemica	I Shift Calibration: I Shift Calibration:	From H2 in Proces	20 and Temperature ss Mode After Conve is	◇ From O1/BF1 rsion		
Total Po	ints R+I:	2048	512	Þ			
Valid Poi	nts:	1024	256	Þ			
Acquisiti	on Mode:	DQD	Echo-	AntiEcho 🕨			
Spectral	Width Hz:	9615.385	2127.6	560 <u>)</u>			-
Observe	Freq MHz:	599.983	60.80	3			
Center F	osition PPM:	<mark>4.773 🔰</mark>	117.0	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 PPI	M (x-axis)	: <mark>4.700 (O1</mark>	/B	F1) 1F	I				
Spect	tral Width Hz (y-axis): 2127.660									
Obser	ve Frea MHz	(v-axis): (50.803 (SFC	<u>)</u> 3) 15N					
Cente	r Position PPI	M (y-axis)	: <mark>117.00 (O</mark>	3/]	BF3) 1	<mark>5N</mark>				
	The name for the NMRPipe conversion script.									
					Inpu	it Protocol:	Bruker (NIH)			
	Spectrometer Input:	./ser			▶ Out	put Protocol:	NMRPipe			
	Output Template:	./test.fid			Dim	ension Count:	2			
	Output Script:		2D Mode:		Mode:	Complex)		
	Other Options:	-aswap -AMX -decir	n 208	8 Inte	nsity Scale:	7.81250e+00				
					Ten	perature (K):	297.998		ре 🗕	
	Divital Avancampling Connection: 🔶 During Conversion (Normal EID) 📥 During Processing (Datter Pasalina)									
		Chemica	Shift Calibration:	F	rom H2O ar	d Temperature	From O1/BF1	tion busioning)		
			🔲 Run NMRDra	w in	Process Mo	ode After Conver	sion			
			v-avis		v-avis					
	Total Poi	nts R+I:	2048	Þ	512					
	Valid Poir	its:	1024	Þ	256					
	Acquisitio	n Mode:	DQD	Þ	Echo-AntiE	icho 🕨				
	Spectral	Width Hz:	9615.385	Þ	2127.660	Þ				
	Observe	Freq MHz:	599.983	Þ	60.803	Þ				
	Center Po	osition PPM:	4.700		117.000					
	Axis Labe	91:	HN		15N	Þ				
	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".	00		X Save
Continue	ů	Saved	16 lines in file "fid.com".
			Continue

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

			A 00301	ve i requ	iency in meguneric.			
	Spectrometer Input: Output Template: Output Script: Other Options:	/ser /test.fid fid.com -bad 0.0 -ext	-aswap -AMX -decim	208	Input Protocol: Output Protocol: Dimension Count: 2D Mode: Intensity Scale: Temperature (K):	Bruker (NIH) NMRPipe 2 Complex 7.81250e+00 297.998		ip e
	Digital	Oversampling Cor Chemica	rection: 🔶 During Co I Shift Calibration: 🔹	nversion From H	n (Normal FID) 🔶 Dur 120 and Temperature	ring Processing (Be	tter Baseline)	
			🔄 Run NMRDraw	in Proce	ess Mode After Conver	rsion		
	Total Poir Valid Poin Acquisitio Spectral Observe Center Pc Axis Labe	nts R+I: nts: m Mode: Width Hz: Freq MHz: usition PPM: N: Save Scrint	x-axis 2048 1024 DQD 9615.385 593.983 4.700 HN Execute Scelet	y-a 512 256 Echo 2127 60.8 117.1 15N	xis	Goor Seciet	Indate Sesint	Quit
	Read Parameters	Save Script	Execute Script	swa	p yz Swap yA	Clear Script	Update Script	Quit
A pop-up window should	then appe	ear:						
	,	Bruker AMX> Input File: ./s Output Macro: /	NMRPipe Conversion ver vsr/local/bin/nmrt	X NI	MRPipe		e	



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	stanprogram2495
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+2	021 Macro Methods NMR	Nh6/Nh6 phsgcl 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.

📀 🕒 📄 1 — nano nhsqc.com — 90×32	
GNU nano 2.0.6 File: nhsqc.com	₿
!/bin/csh	
<pre># # Basic 2D Phase-Sensitive Processing: # Cosine-Bells are used in both dimensions. # Use of "ZF -auto" doubles size, then rounds to power of 2. # Use of "FT -auto" chooses correct Transform mode. # Imaginaries are deleted with "-di" in each dimension. # Phase corrections should be inserted by hand.</pre>	
nmrPipe -in test.fid \ \ nmrPipe -fn SOL \ nmrPipe -fn SD - off 0.5 -end 1.00 -pow 1 -c 1.0 \ \ nmrPipe -fn FT -auto \ nmrPipe -fn FT -suto \ nmrPipe -fn FT -auto \	
[Read 25 lines] ^G Get Help ^O WriteOut ^R Read File ^Y Prev Page ^K Cut Text ^C Cur Pos ^X Exit ^J Justify ^W Where Is ^V Next 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:

. 😐 🕒 😐	📰 1 — nano nh	sqc.com — 90×32			
GNU nano 2.0.6	File: nhsqc.	.com		Modified	E
#!/bin/csh					
#					
# Basic 2D Phase-Sensit	ive Processing:				
# Use of "ZF -auto" of	oubles size, then rour	nds to power of 2			
# Use of "FT -auto" of	hooses correct Transfo	orm mode.			
# Imaginaries are del	eted with "-di" in eac	ch dimension.			
# Phase corrections s	hould be inserted by h	hand.			
nmrPipe -in test.fid \					
nmrPipe -fn SOL		۸			
nmrPipe -fn SP -off	0.5 -end 1.00 -pow 1 -	-c 1.0 \			
nmrPipe -fn ZF -auto		, \ ,			
nmrPipe -fn PS -p0 (3 -p1 0.00 -di -verb	``			
nmrPipe -fn EXI -x1	-left -sw		1		
nmrPipe -fn POLY -au	ito				
nmrPipe -fn TP	0.5	\			
nmrPipe -Th SP -off	0.5 -end 1.00 -pow 1 -	-01.0 \			
nmrPipe -fn FT -auto	(``			
nmrPipe _fn PS _p0 -	90 -p1 0.00 -di -verb				
nmrPipe _fp_POLY_a	10				
-ov -out Nb6_nhsqc.1	t2				
^G Get Help ^O Write	Out ^R Read File	^Y Prev Page ^	K Cut Text	^C Cur Pos	
^X Exit ^J Justi	fy <u>^W</u> Where Is	^V Next Page ^	U UnCut Text	^⊤ 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-M	acBoo	k-P	ro:202	21_Macro_Method:	s_NMR/Nb6/N	b6_nhsqc]	rwtibble%	./nhsqc.com
PS	512	of	512	HN				
PS	1024	of	1024	15N				
[[Ryans-M	acBoo	k–P	ro:202	21_Macro_Method:	s_NMR/Nb6/N	b6_nhsqc]	rwtibble%	ls
EA				format.ased		shimvalue	s	
Nb6_nhsq	c.ft2			format.temp		specpar		
acqu				gpnam1		spnam1		
acqu2				gpnam2		spnam13		
acqu2s				gpnam3		stanprogr	am2495	
acqus				nhsqc.com		test.fid		
adcInfo_	TRX1.	xml		pdata		uxnmr.inf	0	
audita.t	xt			pulseprogram		uxnmr.par		
cpdprg3				scon2		vtc_pid_s	ettings	
fid com				SAT				

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.

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.

