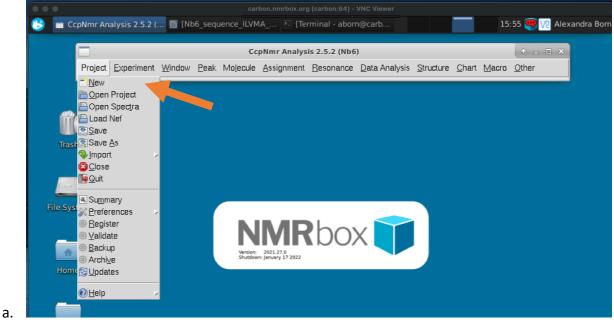
# ccpNMR Tutorial

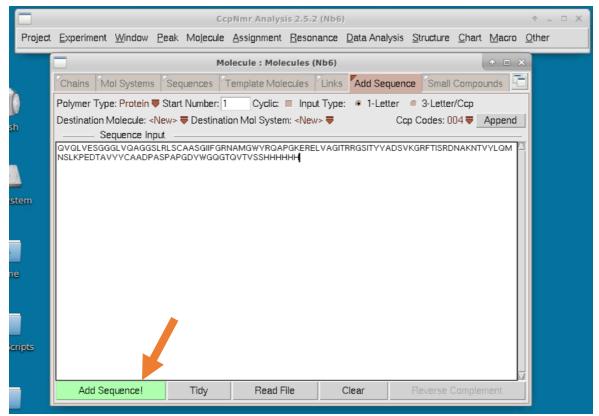
- 1. In the NMRbox terminal, type "analysis" to open ccpNMR analysis
- 2. Create new Project



- b. Project>New>Name:Nb6\_MM
- 3. To add Nb6 amino acid sequence:
  - a. In ccpNMR, go to Molecule>Molecules>Add Sequence

		_	CcpNn	nr Analysis 2.5.	2 (Nb6)			x
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	Chains Mo	ol Systems	quences Temp	plate Molecules	Links Add Seq	uence Small Co	ompounds 🛅	
h	Mol System	? <sup> ≓</sup> Chain <sup>? =</sup> Code	Molecule Template	Residues	Chain ? Fragments	Molecular Types	?⊫? Details	
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ripts								

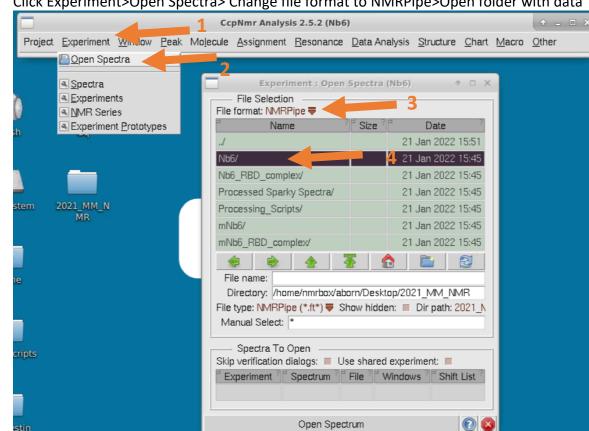
- b. Open "Nb6\_sequence\_ILVMA\_resonances.docx" just by double clicking the file in the folder on NMRbox (not in ccpNMR). Copy Nb6 amino acid sequence (CTRL C for everyone, regardless if your computer is Mac/Windows)
- c. Paste sequence into ccpNMR (CTRL V)



## d. Add Sequence> hit OK 3 times when prompted

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5																		

4. Load Nb6 <sup>15</sup>N-HSQC



b. Open spectra: Find file to upload>Give name>Open experiment

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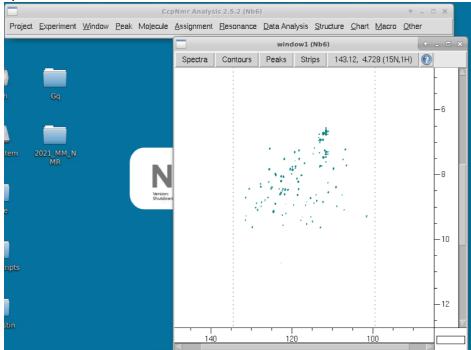
c. Referencing box will appear> click commit in top right hand corner

# a. Click Experiment>Open Spectra> Change file format to NMRPipe>Open folder with data

# ?	Experiment Name	t ? <sup>#</sup> External ? Source	External ? <sup>a</sup> Name	Category	5 H		Type Synonym		Full ? Type	Alt Types
1	Nb6_Nhsq	C E	E	through-bo	nd	15N	HSQC/H	MQC	<sub>E</sub> H[N]	<none></none>
	Propa	agate Experimen	nt Type			Ed	it Experin	nent Pr	ototypes	
_	Experiment D	)im-Dim Transfer	rs				Dimensio	on Map	ping —	
Ē		Transfer Type Between Dims	Second Dim		p Ref n Dim		Isotope	Re	ef Measure	ment
E	1 (15N)	onebond	<sub>E</sub> 2 (1H)	1	E	2	15N	2 Shi	ft(N) ∨ariab	de timing
				2	E	1	1H	3,1 Sh	ift(H) varia	ble timing

d. Choose "Type Synonym" (should auto-pick 15N-HSQC/HMQC> Close-All done

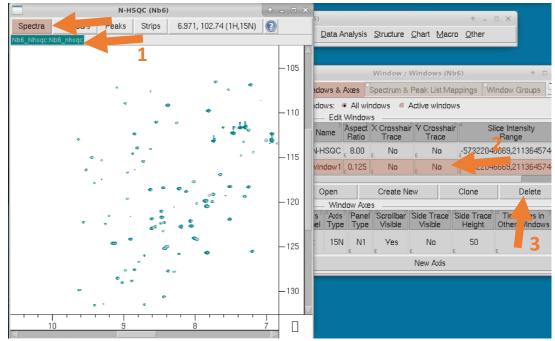
e. Spectra loaded



- 5. <u>Change Spectra Window</u>: Unfortunately, the spectra's dimensions are flipped from what we typically want, so we just need to switch the 1H and 15N Dimensions
  - a. Window>Windows>Create New

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New window na —— Axes x 1H ♥ y 15N —— Viev 9 Space	ame: N-HSQC ■ z1 None ■ d Spectra trum	<sup>⊨</sup> Visibl	₹ z3	None 🗮	z4 None olbar?					

- d. New window will appear with the spectra with correct axes (may need choose spectra in window.
  - i. You may also want to delete the old "window 1" to help declutter



6. Adjust spectra contour levels: Contours>More>Choose 20 levels and multiplier of 1.2

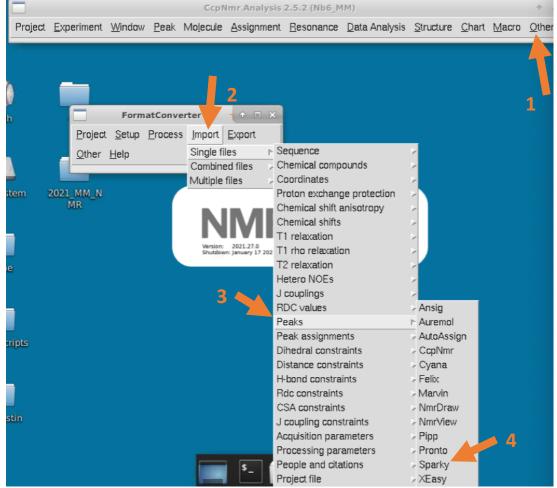
	N-	H5QC (Nb6)		↑ _ □ X				$\uparrow$	_ 0	×	
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	- e - 1	`	• •	Negative lev	/els						
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	-		•								

- 7. <u>Save experiment:</u> can do anytime and should be saved frequently afterwards. Project>Save As.
- 8. Adjust spectra parameters like color, font size, etc...
  - a. (This is optional now, but will definitely be useful to know how to do once you add other spectra) Experiment> Spectra> Display Options

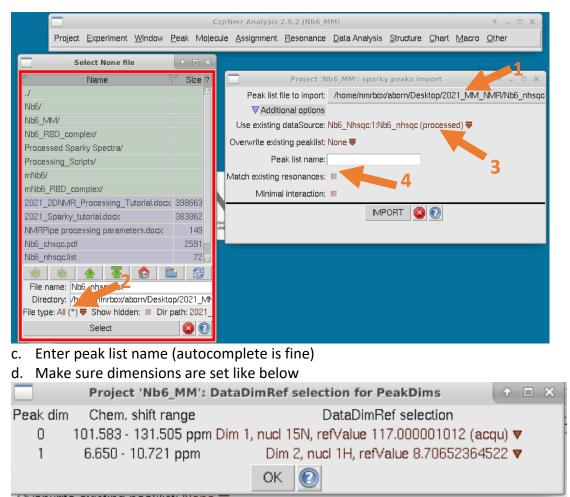
		CcpNmr Analysis 2	.5.2 (Nb6_MM)	<ul> <li>.</li> </ul>	×
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E <u>O</u> pen	Spectra		N-HSQC (Nb6_MM)	↑ _ □ X	
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Contour Levels		Contour Files	Propagate Contours	Set Ranks From Orde	
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#### 9. Load assignment file

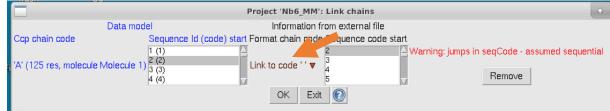
### a. Other> Format Converter> Import > Single Files> Peaks> Sparky



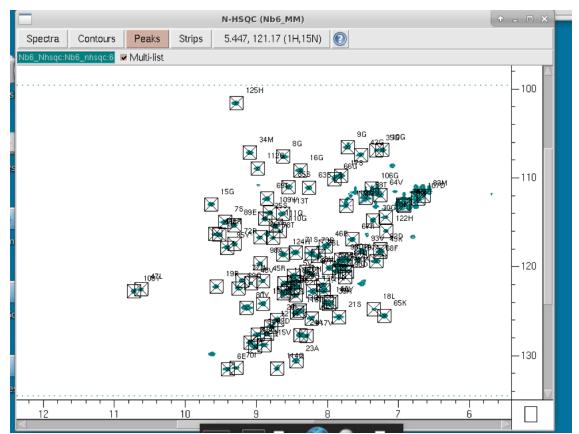
b. Fill in info like below. To find your peak list, click "All" in File Type> Nb6\_nhsqc.list> select> Use existing data source> Unclick match existing resonance> Import



- e. Popup says you were successful in importing file, hit next/ok
- f. Run linkResonances- since we previously defined the molecular system by loading the amino acid sequence, we will now link the amino acid sequence to the important peak list/resonances. Just hit "yes". Link with default settings.
- g. Link chains as below: most important to select "link to code ' ' "" in middle column> Ok



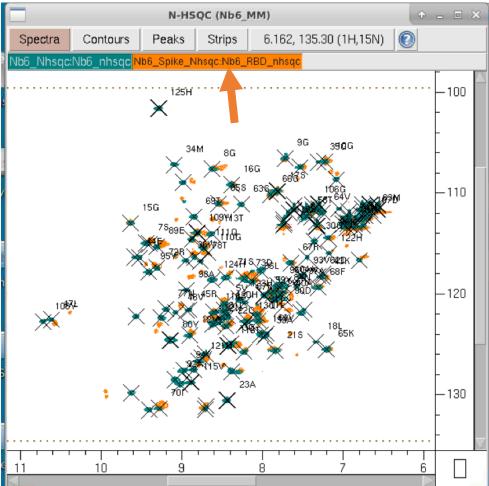
- h. Should get popup that linkResonances ran successfully> Ok
- i. SAVE PROJECT!!!
- 10. Center peaks
  - a. When you look at your spectra, you will notice that the markers do not line up precisely with the top of the peak. We want these markers to line up perfectly to accurately determine peak position and height/intensity. Left click anywhere on the spectra while holding down and span over all the peaks so all peaks are selected (shown as boxes)



- b. Then click "shift p" (holding down shift when push p). The markers should have moved to the max of each peak. Scan the spectra to make sure this occurred. You can zoom into the spectra using the wheel of your mouse (if have 3 button mouse). Move around spectra using the vertical and horizontal scroll bars
- c. Delete old peak list. Peak> Peak list> Select old Nb6 NHSQC peak list (should have 0 peaks)> Delete
- 11. <u>Load <sup>13</sup>C-HSQC of Nb6</u> exactly as we previously loaded the <sup>15</sup>N-HSQC. When you choose the experiment, pick "13C HSQC/HMQC". Adjust contour levels as well.
  - a. You will need to flip the H and C dimensions as done previously: Window> Windows> Create New. After new H-C window created, delete C-H window for less clutter!

Win	dow : New Wind	low (Nb6_MM)	+ = ×
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New window name: C-H	ISQC		10w3. 1 🗸
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/x1H♥ y13C♥ z1 No		🔻 z3 None 🗮 z4 N	one 🗮
Viewed Spectra	a		
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Nb6_Chsqc:Nb6_chs	sqc <sub>F</sub> No	F Yes	1
C			
		( <u> </u>	
Create Window!	Selected Visible	Selected Not Visible	Selected Not In Toolbar

- 12. <u>Load <sup>15</sup>N-HSQC of Nb6+Spike</u> as previous. You should not need to make a new window this time. Change the color of the spectra if you'd like
- 13. Overlay the spectra
  - a. In the spectra tab, toggled between which spectra are shown so both apo Nb6 and Nb6+Spike are overlaid.



b. Adjust referencing values in Nb6\_Spike: Experiment> Spectra> Referencing> Choose correct Nb6\_spike spectrum> Adjust reference ppm as listed

			Exp	eriment : Sp	ectra (Nb6_M	M)			• • • •				
Spectr	a Display	Options Reference	ing Tolerance	es File Deta	ils Data Loc	ations		Delete Sp	ectrum 📑 💽 🄇				
pectru	ectrum: Nb6_Spike_Nhsqc:Nb6_RBD_nhsqc = Use reduced dimensionality options: Reference changes keep constant peak: point ppm												
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2	1H	<sub>E</sub> 800.134	7.811	e 6250.000	<sub>e</sub> 8.611	<sub>E</sub> 513.000	<sub>e</sub> 1024	<sub>E</sub> O					
		Add Sub-dimens	ion Copy			F	Remove Sub-dir	mension					
_													

- 14. Transfer peak assignments from Nb6 (apo) to Nb6+Spike
  - a. Peak>Peak Lists> Select Nb6\_Nhsqc> Copy Peaks> Choose destination list of Nb6\_Spike\_NHSQC

Experiment ?	Spectrum	? T ist	Active?	Color	Symbol	No. Peaks	% Assigned	Synthetic?	3
Nb6_Nhsqc	Nb6_nhsqc		Yes	<sub>E</sub> black	- X	123	69.1	No	<sub>e</sub> sparky format,
Nb6_Chsqc	Nb6_chs	1	Yes	<sub>E</sub> black	E X	0	0.0	No	E-1
Nb6_Spike_Nhsqc		qc 1	Yes	<sub>e</sub> black	E X	123	69.1	No	E
	1. Sour	ce							
							3. De	stinati	on
					2.				

b. As there are many peaks that are unassigned, I would delete the unassigned peaks from the list. Peak> Peak lists> Peak table> Select Peak list "Nb6\_Spike"> Sort list by clicking "assign F1"> Select with cursor and holding shift key to select all unassigned peaks> Delete

					Peak : Pe	ak Lists (Nb	6_MM)			•	
Pe	eak Lists	Peak -	Table	Synthet	ic Lists					(	0
Pea	ak List: Nb	6_Spike	_Nhsqc:I	Nb6_RBD	_nhsqc:1 🛡	Position Uni	t: ppm 🗮 🛛 S	Strip Selected	📕 🔲 Find	Peak Wir	ndow: I
Sta	atus: Any 🖣	🔻 Structi	ure: <no< td=""><td>ne&gt; 🗮</td><td></td><td>1</td><td>St</td><td>trip Locations</td><td>📕 🔲 Go To</td><td>Position <no< td=""><td>ne&gt; 🗮</td></no<></td></no<>	ne> 🗮		1	St	trip Locations	📕 🔲 Go To	Position <no< td=""><td>ne&gt; 🗮</td></no<>	ne> 🗮
<b>#</b> ₽?	Position F1	Position F2	Assign F1	Assign F2	Height	Volume ?	Line Width F1 (Hz)	Line Width F2 (Hz)	Merit	Details	? <sup>⊨</sup> Fi?
52	<sub>E</sub> 113.240	<sub>e</sub> 6.752	E		-6.165e+10	<sub>E</sub> 6.004e+11	<sub>e</sub> 167.086	<sub>E</sub> 236.532	1.000 <sub>E</sub> No (	original numbe	er <sub>e</sub> 🛆
53	<sub>E</sub> 111.649	<sub>e</sub> 7.607	E	E	<sub>E</sub> 330 <b>e</b> +10	<sub>E</sub> 2.305 <b>e</b> +11	<sub>e</sub> 72.554	<sub>E</sub> 27.901 <sub>E</sub>	1.000 <sub>E</sub> No (	original numbe	er <sub>E</sub>
54	<sub>E</sub> 113.240	<sub>e</sub> 7.319	E	E	<sub>e</sub> 4.799e+10	<sub>e</sub> 4.152 <mark>e+</mark> 11	<sub>e</sub> 70.112	<sub>e</sub> 47.527 <sub>e</sub>	1.000 <sub>e</sub> No (	original numbe	er <sub>E</sub>
56	<sub>E</sub> 113.428	<sub>e</sub> 6.852	E	E	<sub>e</sub> 8.122 <b>e</b> +10	<sub>e</sub> 7.706 <b>e</b> +11	<sub>e</sub> 61.374	<sub>E</sub> 217.301 <sub>E</sub>	1.000 <sub>e</sub> No (	original numbe	er <sub>E</sub>
59	<sub>E</sub> 112.660	<sub>e</sub> 7.738	E		<sub>e</sub> 4.139e+10	<sub>e</sub> 3.344e+11	<sub>e</sub> 86.434	<sub>e</sub> 32.632 <sub>e</sub>	1.000 <sub>e</sub> No (	original numbe	er <sub>E</sub>
65	<sub>E</sub> 113.071	<sub>e</sub> 7.002	E	E	<sub>e</sub> 4.734 <b>e</b> +10	<sub>e</sub> 3.564 <b>e</b> +11	<sub>e</sub> 50.683	<sub>E</sub> 104.622	1.000 <sub>e</sub> No (	original numbe	er <sub>e</sub>
74	<sub>E</sub> 111.618	<sub>e</sub> 7.460	E	E	<sub>€</sub> 1.097e+10	<sub>€</sub> 7.575 <b>e</b> +10	<sub>e</sub> 29.696			original numbe	
77	<sub>E</sub> 113.428	<sub>e</sub> 6.949	E	E	L	<sub>E</sub> 1.217e+12	<sub>E</sub> 53.885			original numbe	
79	<sub>E</sub> 111.786		E	E		<sub>€</sub> 1.007e+11	<sub>E</sub> 11.035			original numbe	
80	<sub>E</sub> 116.368	<sub>E</sub> 8.842	E	E		<sub>E</sub> 1.529e+11	<sub>e</sub> 244.426			original numbe	
82	<u>_</u>		E		-	E1.006e+11	<sub>E</sub> 30.414		·	priginal numbe	
84		E 7.083	E	E	<sub>E</sub> 8.5⊿1e+09	€4.766e+10	E 17.042			priginal numbe	
89	<sub>E</sub> 111.837	<sub>E</sub> 6.923	E	E	362e+09	<sub>E</sub> 6.637e+10	<sub>E</sub> 9.637	<sub>E</sub> 345.989 <sub>E</sub>	1.000 ENO (	original numbe	er <sub>E</sub>
Δ	dd Ec	lit [] In	nalias	Delete	Assign	Deassign	n Set De	etails Set	As Current	Resonar	ices
_								Assign Propa			

15. <u>Adjust peak assignments on Nb6+Spike spectra</u>: this will be difficult as many peaks move/disappear. Do your best to assign shift perturbations typically by assigning peaks to nearest shift. Consider changing the contour levels to aid in the assignment. If a peak has completely disappeared and/or hard to

assign, DON'T move or delete it. A peak that moves so much it can't be assigned is also useful information.

- a. To adjust peaks, just click on the assignment you want to move with left cursor, then move your cursor to where the peak has moved, and click "p". Then, select your peak again, and click "shift+p" for the program to center it at the maximum intensity.
- b. Disclaimer: this will be difficult, as most peaks move/disappear. Typically, the binding partner would be added in smaller amounts too so we can see smaller shifts making the spectra easier to assign.
- 16. Plot chemical shift perturbations vs amino acid number
  - a. Data Analysis> Shift Differences> Peak list A "Nb6\_Nhsqc"> Peal list B "Nb6\_Spike"
  - b. Sort by residue (click)

	,		· · ·		CcpNm	<sup>-</sup> Analysi	s 2.5.2 (I	Npe_WW	)				
Proje	ct <u>E</u> xperimer	nt <u>W</u> ind	low <u>P</u>	eak N	1o <u>l</u> ecule <u>A</u> s	ssignmer	nt <u>R</u> esor	nance <u>D</u>	ata Analysis	<u>S</u> tructur	re <u>C</u> hart	Macro	<u>0</u>
		_	_	_	N-HSC	C (Nb6	MM)		Measuremen				
	s	pectra	Cont	tours	Peaks	Strips	7.404,	, 114.4	<u>N</u> MR Series			_	
1									Shift Differe				
9									<u>H</u> eteronucle 3J H-Hα <u>C</u> ou				
י ו	<u> </u>		Dat	a Anal	ysis : Shift	Differe	nces (Nbi	SIMMO				_	
	Peak List C	omnoria							Follow <u>Inten</u> Follow <u>S</u> hift	-	-		
		ompans otions -		niit Lisi	. Companso	in j seqi	uence Allų		PALES: Aligi				
	Peak List		b6_Nh:	sqc:Nb6	6_nhsqc:4 🖣	Peak L	ist B:		MODULE: A			3	
tem	Atom Nar	mes 1: H	I,H1			Atom N	lames 2: [	N					
	Scale fac	tor 1: 1	.0000			Scale fa	actor 2: 🛛	0.15000					
	Residue(s)	Reson.	<sup>≡</sup> Shift <sup>?</sup>	=Shift?	<sup>⊨</sup> _∆1 <sup>?</sup>	Reson?	<sup>≓</sup> Shift ?	<sup>≓</sup> Shift ?	<sup>⊨</sup> _∆2 ?		hift <sup>?</sup> 2		
e	2Val	1 H	1A	1B	(ppm) 7.878e-03	2 N	2A	2B 120.276	(ppm) -0.195	Sum [ 0.037 0	Dist '		
e	3Gln	H		7.842 8.359	-0.025	N		124.899		0.037 0			
	5Val	Н		8.475		N		120.780		0.034 0			
	7Ser	Н		9.411	-0.031	N		114.755		0.065 0			
rinte	8Gly	Н	8.622	8.608	-0.014	N	107.589	107.384	-0.205	0.045 0	.034		
ripts	9Gly	Н	7.711	7.726	0.015	N	106.545	106.540	-5.246 <b>e</b> -03	0.016 0	.015		
	10Gly	Н	7.223	7.222	-8.355 <b>e</b> -04	N	106.871	106.737	-0.134	0.021 0	.020		
	11Leu	Н	8.054	8.058	3.876 <b>e</b> -03	N	122.672	122.600	-0.072	0.015 0	.011 🗸		
	Show Pe	eaks	U U	pdate	Ma	ak <b>e</b> Shift	Difference	e List	Show (	On Struct	ure		
stin		_		_		_							

c. Anywhere in the graph: right click> Graph> Seq Num> Shift Dist

Peak List O Peak List Atom Na Scale fac	otions – t A: N mes 1: H	b6_Nh: I,H1		t Compariso 6_nhsqc:4₹	P	eak L tom N	ist B: lames 2: 1 actor 2: [	Vb6_ V	Spike	_Nhsqc:Nbf	6_RBD	_nhsqc	×1
∃ Residue(s)	Reson: 1	<sup>≓</sup> Shift <sup>?</sup> 1A	<sup>≓</sup> Shift <sup>?</sup> 1B	<sup>⊨</sup> Δ1 ? (ppm)	R	eson? 2	<sup>≓</sup> Shift ? 2A	<sup>∃</sup> Sh 2l	nift ? B	<sup>≝</sup> ∆2 ? (ppm)	<sup>≓</sup> Shift? Sum	Shift? Dist	?
2Val	Н	7.834	7.842	7.878 <b>e</b> -03		Ν	120.471	120	120.276	-0.195	0.037	0.030	Δ
3Gln	Н	8.384	8.359	Filter		N	124.958	124	.899	-0.059	0.034	0.027	$\square$
5∀al	Н	8.468	8,475	Export		N	121.009	120		-0.229	0.041	0.035	
7Ser	Н	9.442	9-411-	Graph	1		w Number			-0.226	0.065	0.046	
8Gly	Н	8.622	8.608	Print Table info			sidue(s)		384	-0.205	0.045	0.034	
9Gly	Н		7.728			X:Shi X:Shi	ft 1A ft 1P		540	-5.246 <b>e</b> -03 w Number	0.016	0.015	
10Gly	Н			-8.355 <b>e</b> -04			(ppm)				0.021	0.020	
11Leu	Н			3.876e-03			ft 2A			sidue(s) ift 1A	0.015	0.011	
Show Peaks		Update Ma			ke	X:Shi	ft 2B ! (ppm) ft Sum ft Dist		Y:Sh Y:∆ Y:Sh Y:Sh	ift1B I(ppm) ift2B	On Structure		
					\$	X:Sec	q Num	r N	Y:Sh	2 (ppm) ift Sum ift Dist			

d. Can adjust the type of graph (line/bar)