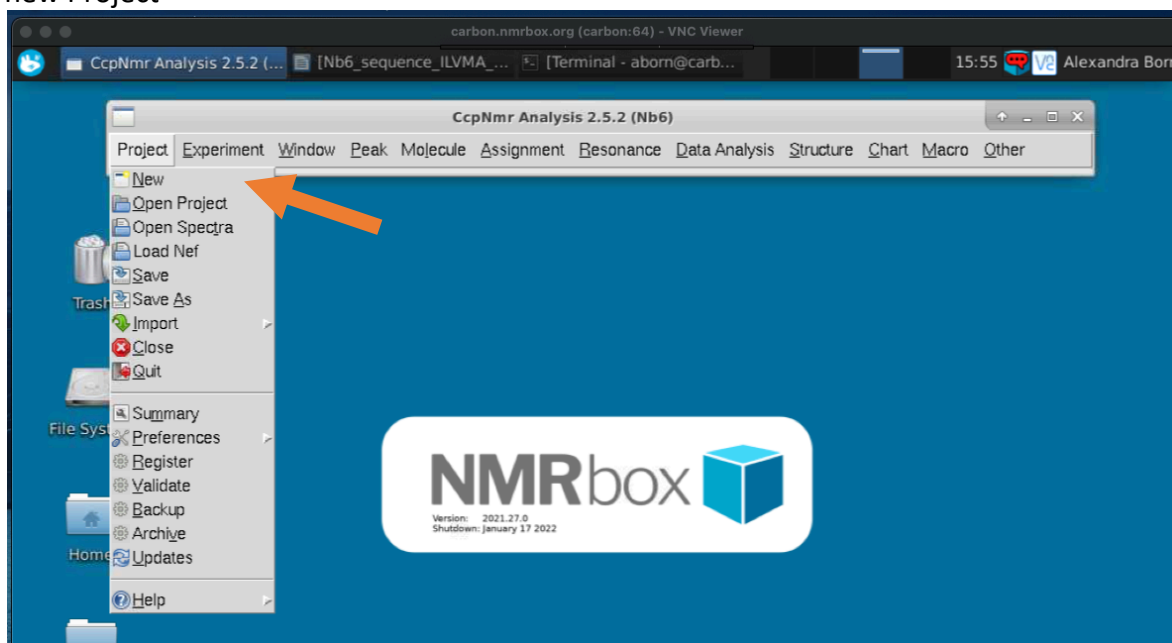
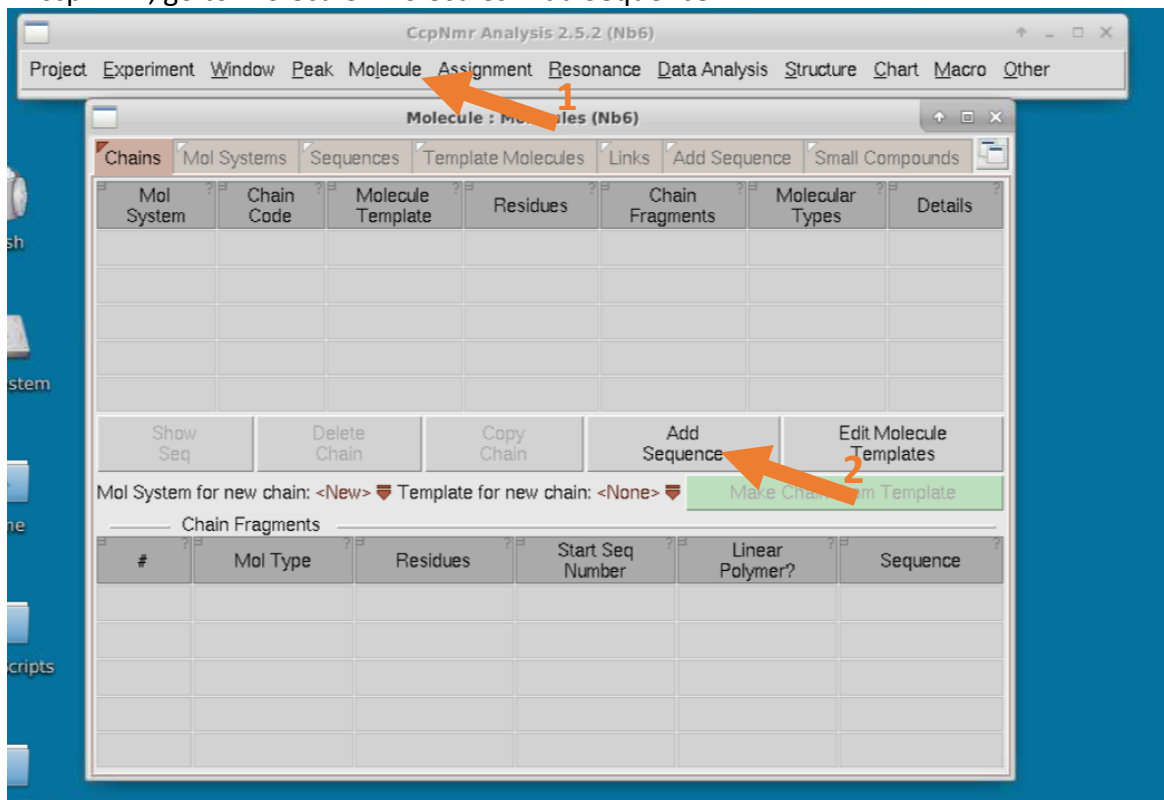


ccpNMR Tutorial

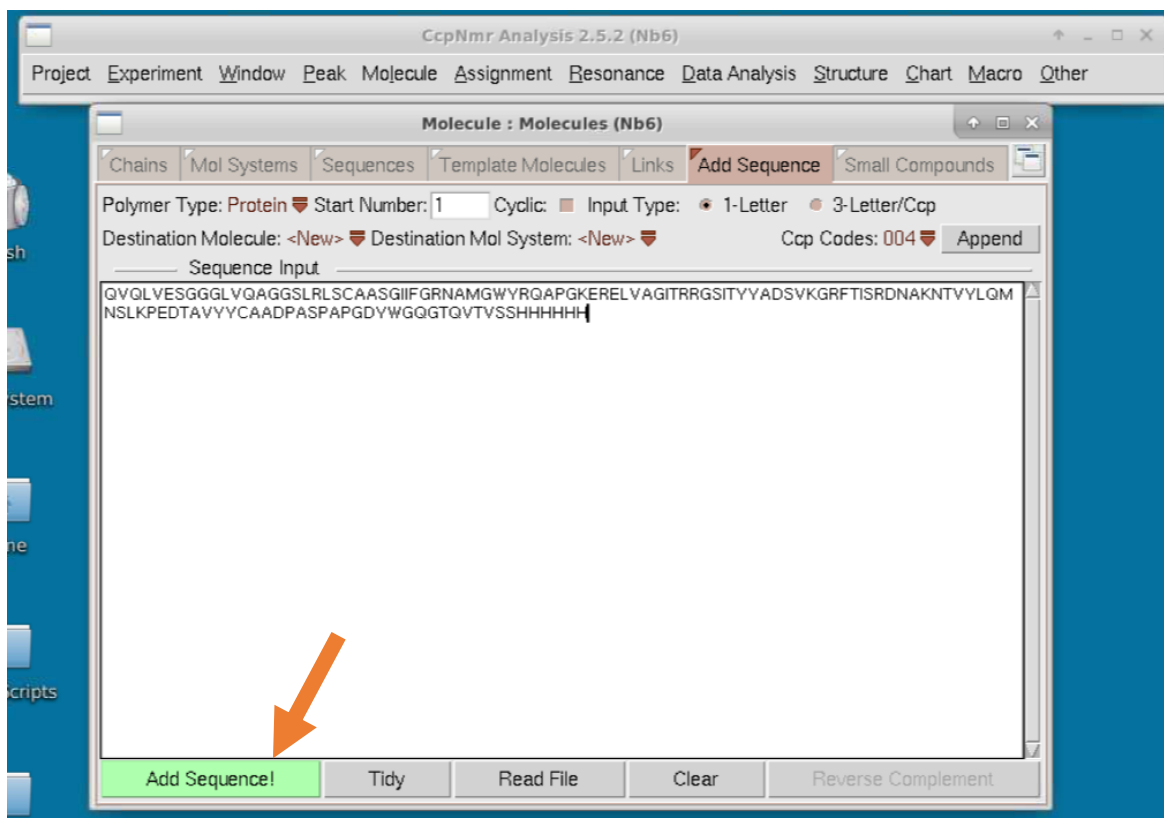
1. In the NMRbox terminal, type “analysis” to open ccpNMR analysis
2. Create new Project



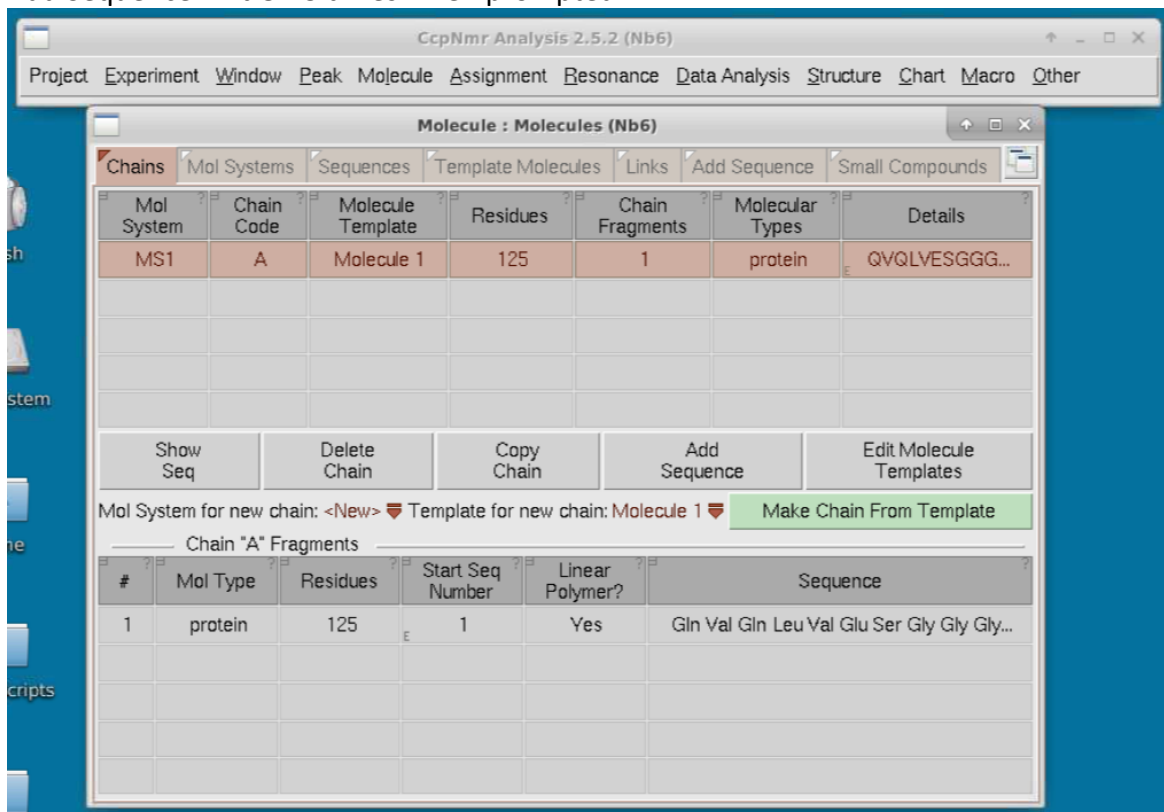
- a.
 - b. Project>New>Name:Nb6_MM
3. To add Nb6 amino acid sequence:
 - a. In ccpNMR, go to Molecule>Molecules>Add Sequence



- 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)

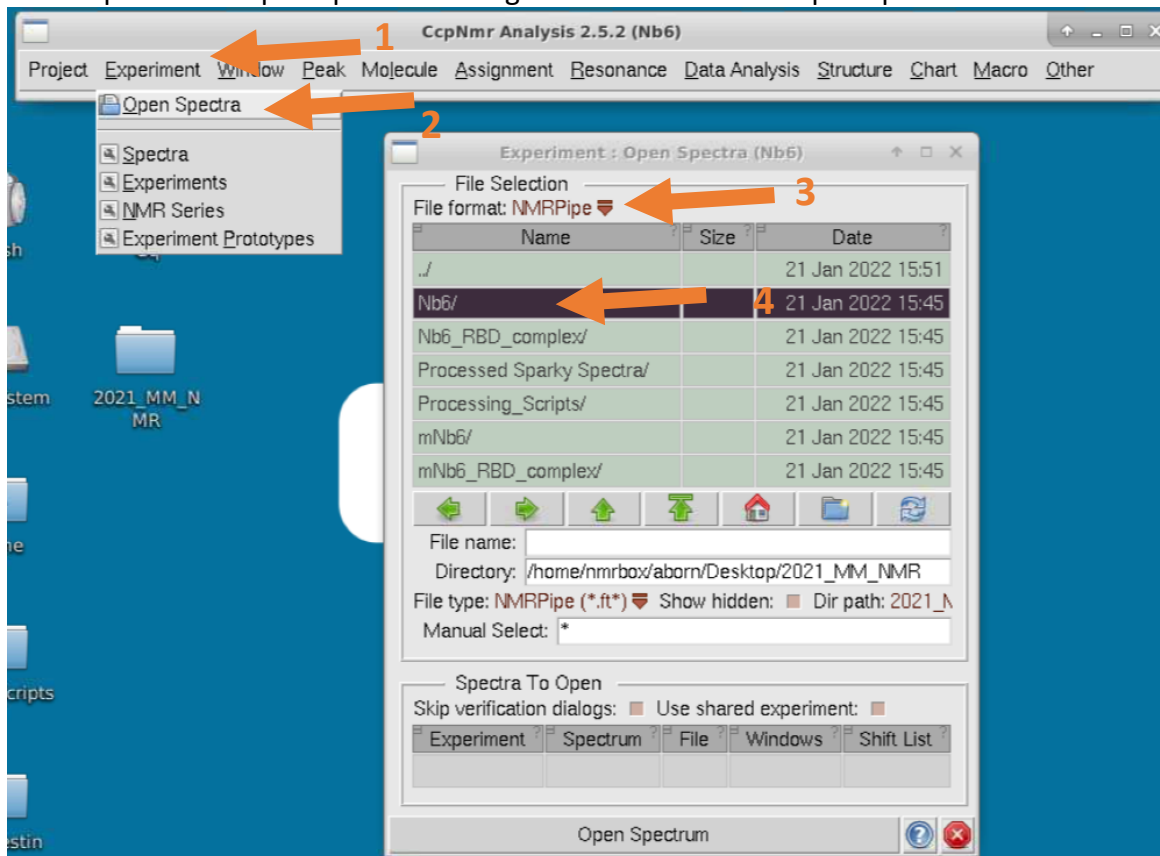


- d. Add Sequence> hit OK 3 times when prompted

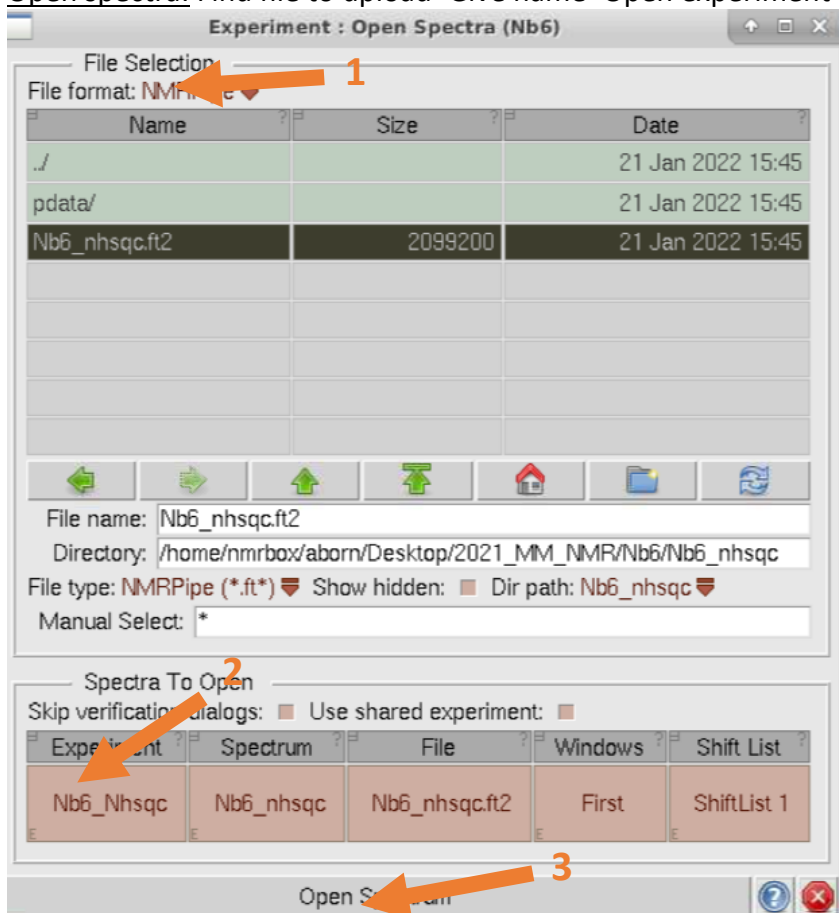


4. Load Nb6 ^{15}N -HSQC

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

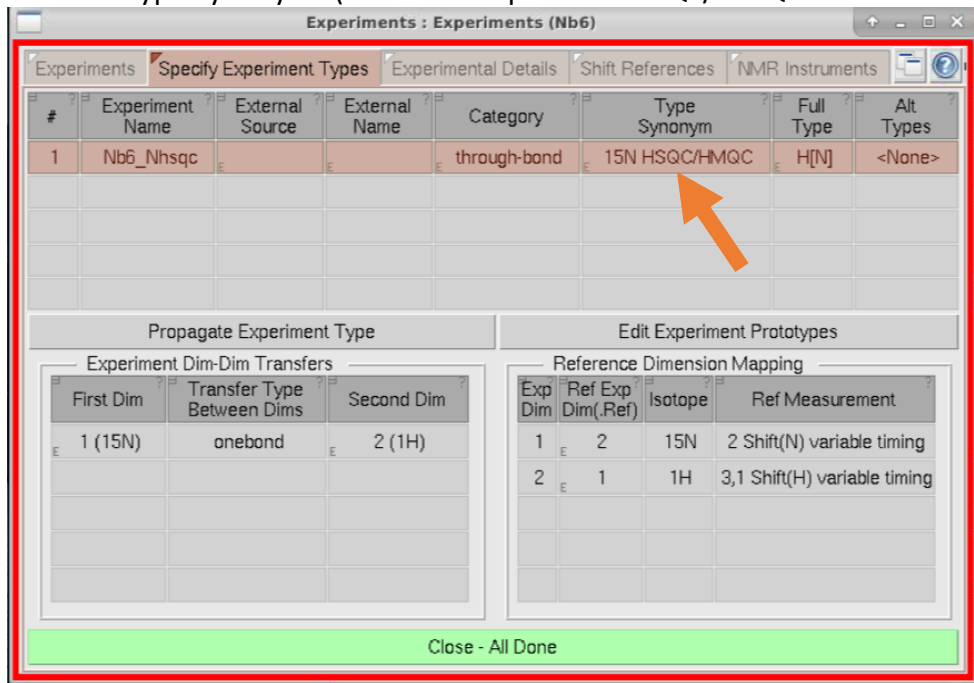


- b. Open spectra: Find file to upload>Give name>Open experiment

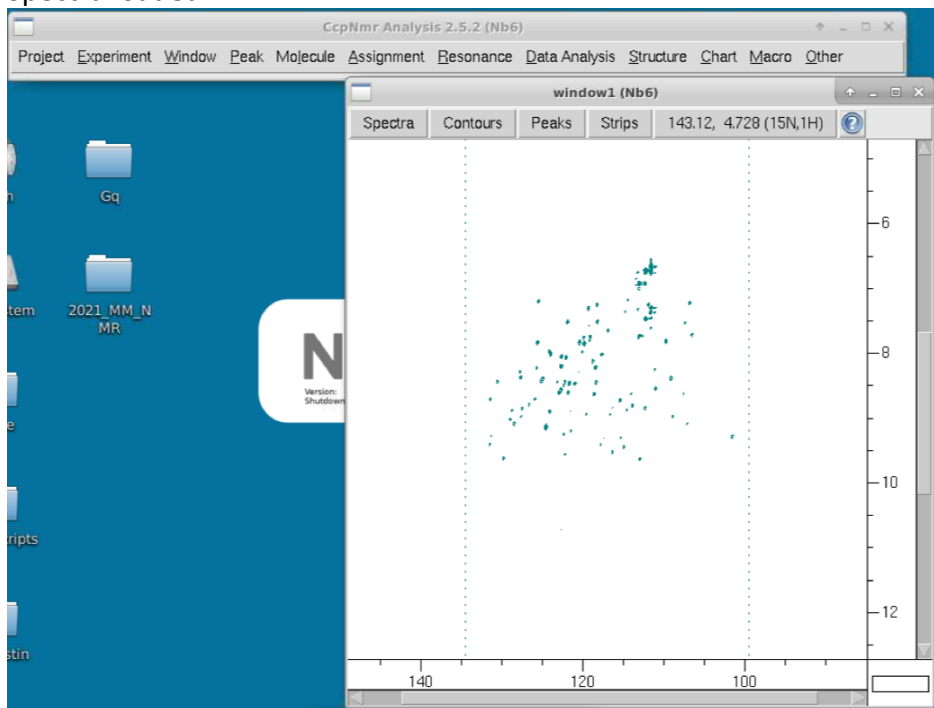


- c. Referencing box will appear> click commit in top right hand corner

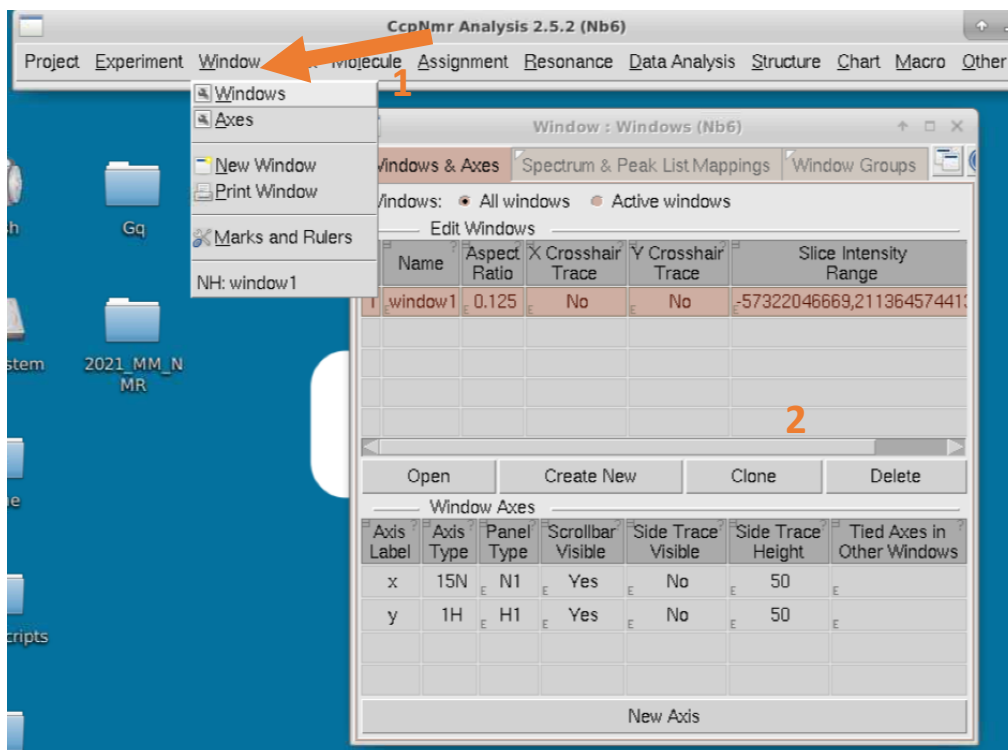
- d. Choose "Type Synonym" (should auto-pick 15N-HSQC/HMQC> Close-All done



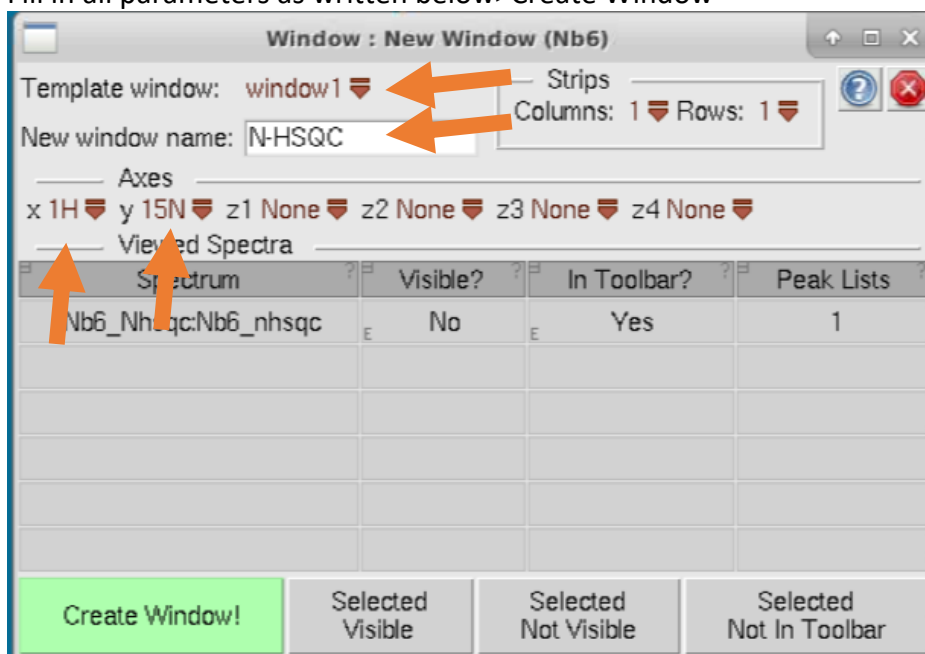
- e. Spectra loaded



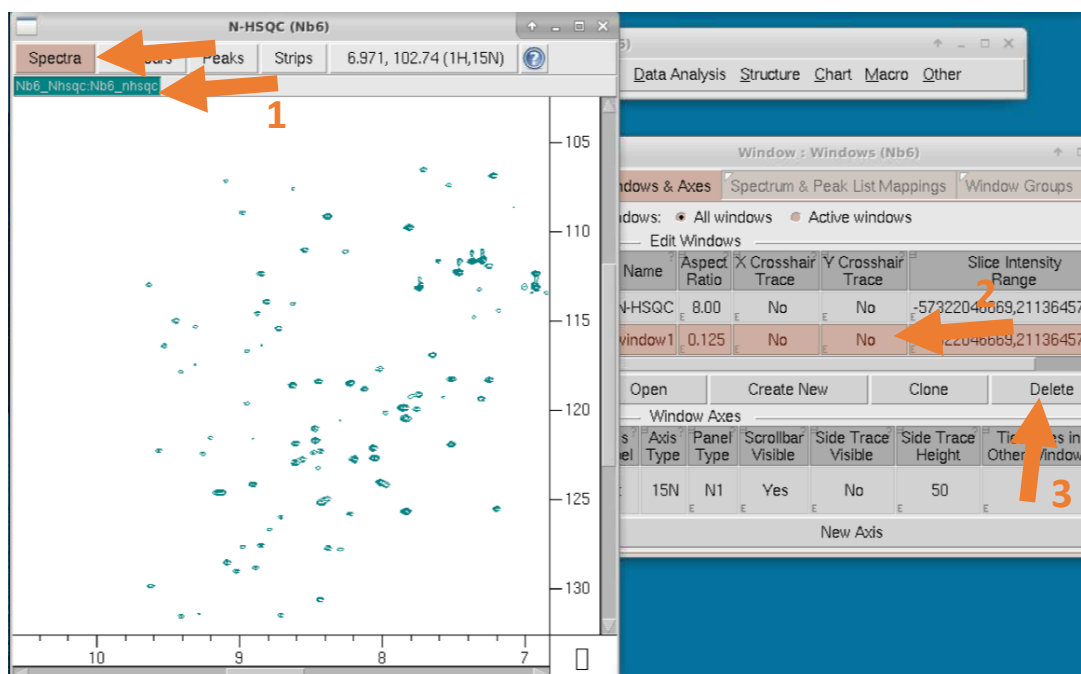
5. Change Spectra Window: Unfortunately, the spectra's dimensions are flipped from what we typically want, so we just need to switch the 1H and 15N Dimensions
- Window>Windows>Create New



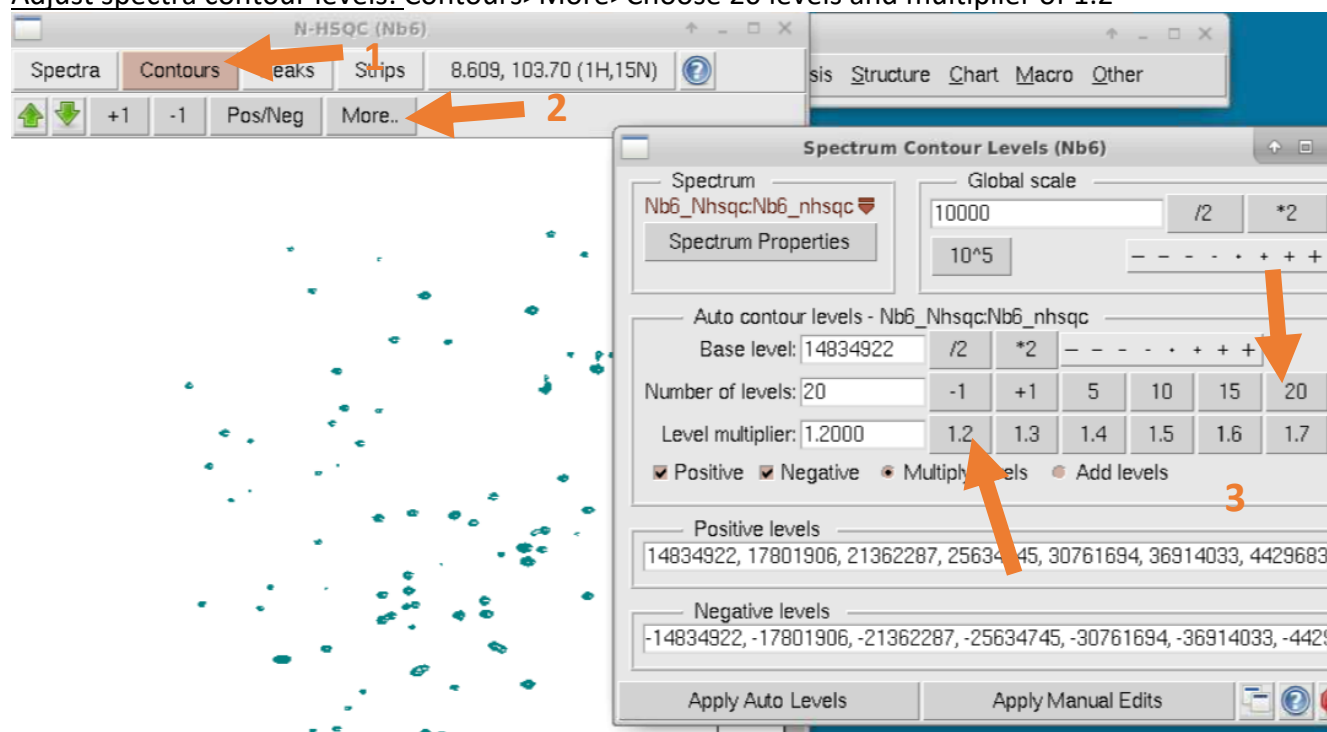
- b.
- c. Fill in all parameters as written below>Create Window



- 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



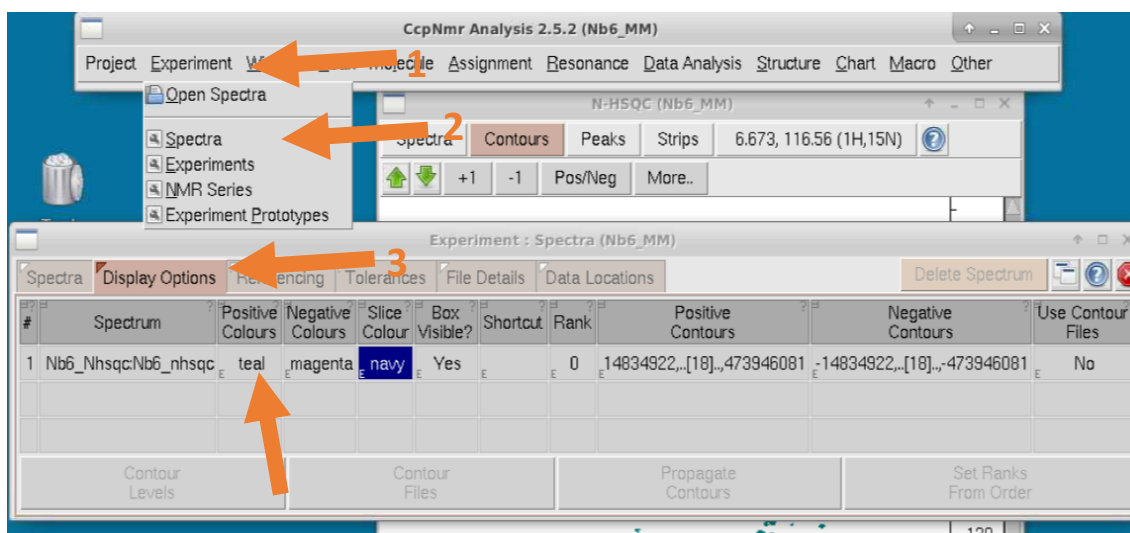
6. Adjust spectra contour levels: Contours>More>Choose 20 levels and multiplier of 1.2



7. Save experiment: can do anytime and should be saved frequently afterwards. Project>Save As.

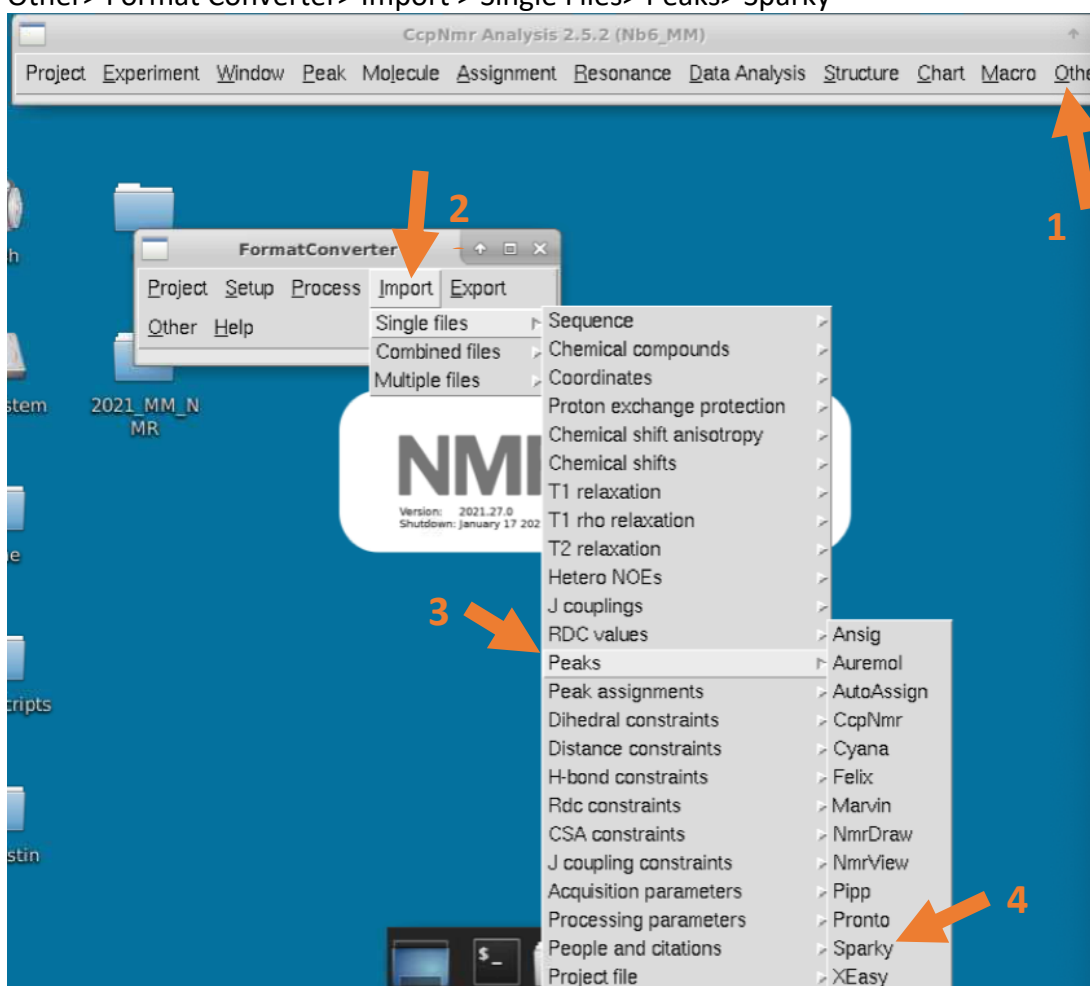
8. Adjust spectra parameters like color, font size, etc...

- a. (This is optional now, but will definitely be useful to know how to do once you add other spectra) Experiment> Spectra> Display Options



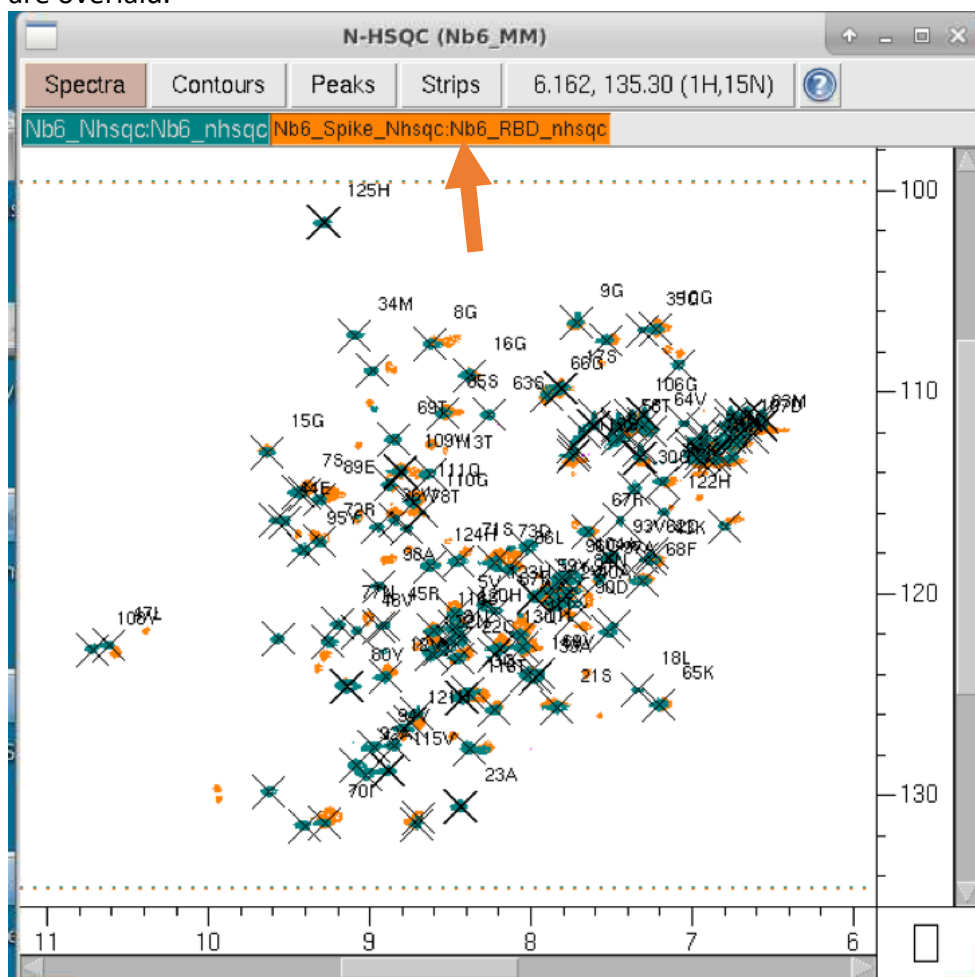
9. Load assignment file

- Other> Format Converter> Import > Single Files> Peaks> Sparky



- 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

12. Load ¹⁵N-HSQC of Nb6+Spike 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

Experiment : Spectra (Nb6_MM)

Spectra Display Options Referencing Tolerances File Details Data Locations Delete Spectrum

Spectrum: Nb6_Spike_Nhsqc:Nb6_RBD_nhsqc Use reduced dimensionality options: Reference changes keep constant peak: point ppm

Dim	Isotope	Spectrometer frequency (MHz)	Spectral width (ppm)	Spectral width (Hz)	Reference ppm	Reference point	Orig. number of points	Point offset	Minimum aliased frequency (ppm)
1	15N	81.086	35.000	2838.008	117.000	257.000	512	0	
2	1H	800.134	7.811	6250.000	8.611	513.000	1024	0	

Add Sub-dimension Copy Remove Sub-dimension

14. Transfer peak assignments from Nb6 (apo) to Nb6+Spike
a. Peak>Peak Lists> Select Nb6 Nhsgc> Copy Peaks> Choose destination list of Nb6 Spike NHSQC

Peak : Peak Lists (Nb6_MM)

Peak Lists Peak Table Synthetic Lists

Experiment	Spectrum	List	Active?	Color	Symbol	No. Peaks	% Assigned	Synthetic?	
Nb6_Nhsqc	Nb6_nhsqc	4	Yes	black	x	123	69.1	No	sparky format, f
Nb6_Chscq	Nb6_chscq	1	Yes	black	x	0	0.0	No	
Nb6_Spike_Nhsqc	Nb6_RBD_nhsqc	1	Yes	black	x	123	69.1	No	

1. Source

3. Destination

2.

Edit Peaks Delete Add Sister List Copy Peaks Subtract Peaks Shift Whole Peak List

- 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 : Peak Lists (Nb6_MM)

Peak Lists Peak Table Synthetic Lists

Peak List: Nb6_Spike_Nhsqc:Nb6_RBD_nhsqc:1 Position Unit: ppm Strip Selected Find Peak Window: P

Status: Any Structure: <None> Strip Locations Go To Position <None>

#	Position F1	Position F2	Assign F1	Assign F2	Height	Volume	Line Width F1 (Hz)	Line Width F2 (Hz)	Merit	Details	FI ?
52	113.240	6.752			6.165e+10	6.004e+11	167.086	236.532	1.000	No original number	
53	111.649	7.607			3.280e+10	2.305e+11	72.554	27.901	1.000	No original number	
54	113.240	7.319			4.799e+10	4.152e+11	70.112	47.527	1.000	No original number	
56	113.428	6.852			8.122e+10	7.706e+11	61.374	217.301	1.000	No original number	
59	112.660	7.738			4.139e+10	3.344e+11	86.434	32.632	1.000	No original number	
65	113.071	7.002			4.734e+10	3.564e+11	50.683	104.622	1.000	No original number	
74	111.618	7.460			1.097e+10	7.575e+10	29.696	87.830	1.000	No original number	
77	113.428	6.949			1.015e+11	1.217e+12	53.885	86.004	1.000	No original number	
79	111.786	7.464			2.360e+10	1.007e+11	11.035	103.435	1.000	No original number	
80	116.368	8.842			1.846e+10	1.529e+11	244.426	49.082	1.000	No original number	
82	111.156	6.759			8.216e+09	1.006e+11	30.414	278.137	1.000	No original number	
84	108.661	7.083			8.551e+09	4.766e+10	17.042	15.846	1.000	No original number	
89	111.837	6.923			7.362e+09	6.637e+10	9.637	345.989	1.000	No original number	

1

2

3

Add Edit Unalias Delete Assign Deassign Set Details Set As Current Resonances

Deassign Dim Recalc Fit Recalc Volume Show On Structure Propagate Assign Propagate Merit Propagate Details

15. Adjust peak assignments on Nb6+Spike spectra: 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.

- 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.
- 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

- Data Analysis> Shift Differences> Peak list A "Nb6_Nhsqc"> Peak list B "Nb6_Spike"
- Sort by residue (click)

Data Analysis : Shift Differences (Nb6_MM)

Options

Peak List A: Nb6_Nhsqc:Nb6_nhsqc:4 Peak List B: Nb6_Spike

Atom Names 1: H,H1 Atom Names 2: N

Scale factor 1: 1.0000 Scale factor 2: 0.15000

Residue(s)	Reson. 1	Shift 1A	Shift 1B	$\Delta 1$ (ppm)	Reson. 2	Shift 2A	Shift 2B	$\Delta 2$ (ppm)	Shift Sum	Shift Dist
2Val	H	7.834	7.842	7.878e-03	N	120.471	120.276	-0.195	0.037	0.030
3Gln	H	8.384	8.359	-0.025	N	124.958	124.899	-0.059	0.034	0.027
5Val	H	8.468	8.475	6.609e-03	N	121.009	120.780	-0.229	0.041	0.035
7Ser	H	9.442	9.411	-0.031	N	114.981	114.755	-0.226	0.065	0.046
8Gly	H	8.622	8.608	-0.014	N	107.589	107.384	-0.205	0.045	0.034
9Gly	H	7.711	7.726	0.015	N	106.545	106.540	-5.246e-03	0.016	0.015
10Gly	H	7.223	7.222	-8.355e-04	N	106.871	106.737	-0.134	0.021	0.020
11Leu	H	8.054	8.058	3.876e-03	N	122.672	122.600	-0.072	0.015	0.011

Show Peaks Update Make Shift Difference List Show On Structure

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

Data Analysis : Shift Differences (Nb6_MM)

Peak List Comparison Shift List Comparison Sequence Alignment

Options

Peak List A: Nb6_Nhsqc:Nb6_nhsqc:4 Peak List B: Nb6_Spike_Nhsqc:Nb6_RBD_nhsqc:1

Atom Names 1: H,H1 Atom Names 2: N

Scale factor 1: 1.0000 Scale factor 2: 0.15000

Residue(s)	Reson. 1	Shift 1A	Shift 1B	$\Delta 1$ (ppm)	Reson. 2	Shift 2A	Shift 2B	$\Delta 2$ (ppm)	Shift Sum	Shift Dist
2Val	H	7.834	7.842	7.878e-03	N	120.471	120.276	-0.195	0.037	0.030
3Gln	H	8.384	8.358		N	124.958	124.899	-0.059	0.034	0.027
5Val	H	8.468	8.475		N	121.009	120.780	-0.229	0.041	0.035
7Ser	H	9.442	9.411							
8Gly	H	8.622	8.606							
9Gly	H	7.711	7.726							
10Gly	H	7.223	7.222	-8.355e-04						
11Leu	H	8.054	8.058	3.876e-03						

Show Peaks Update Make

Filter
Export
Graph
Print
Table info
Font

X:Row Number
X:Residue(s)
X:Shift 1A
X:Shift 1B
X: $\Delta 1$ (ppm)
X:Shift 2A
X:Shift 2B
X: $\Delta 2$ (ppm)
X:Shift Sum
X:Shift Dist
X:Seq Num

Y:Row Number
Y:Residue(s)
Y:Shift 1A
Y:Shift 1B
Y: $\Delta 1$ (ppm)
Y:Shift 2A
Y:Shift 2B
Y: $\Delta 2$ (ppm)
Y:Shift Sum
Y:Shift Dist

On Structure

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