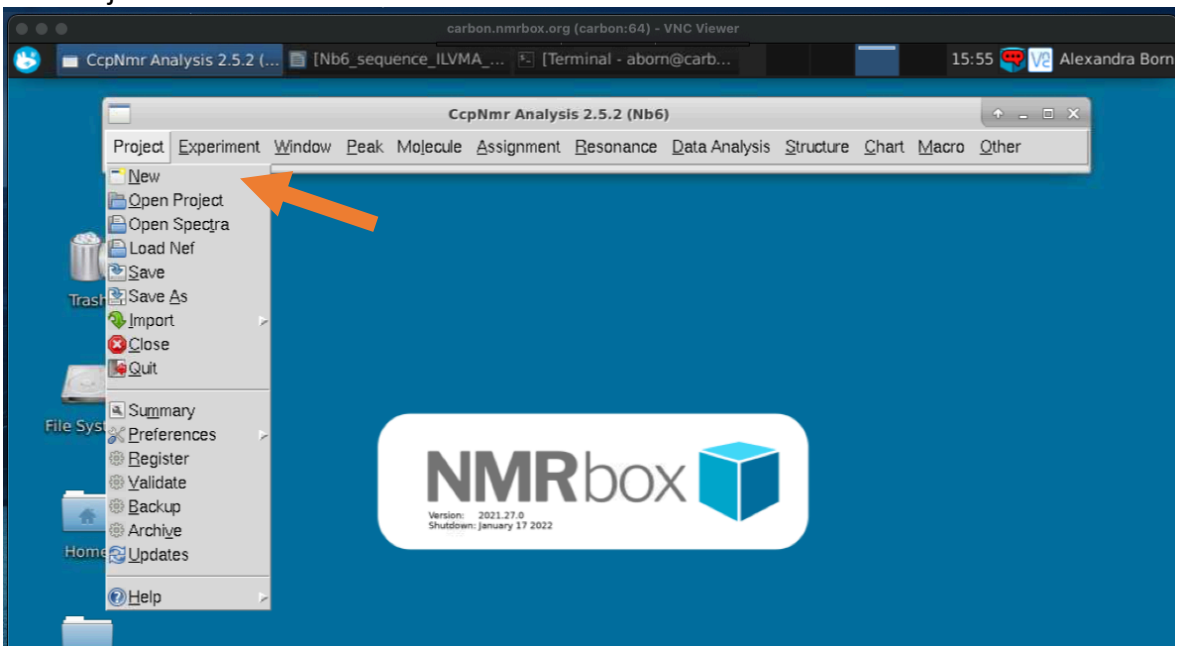
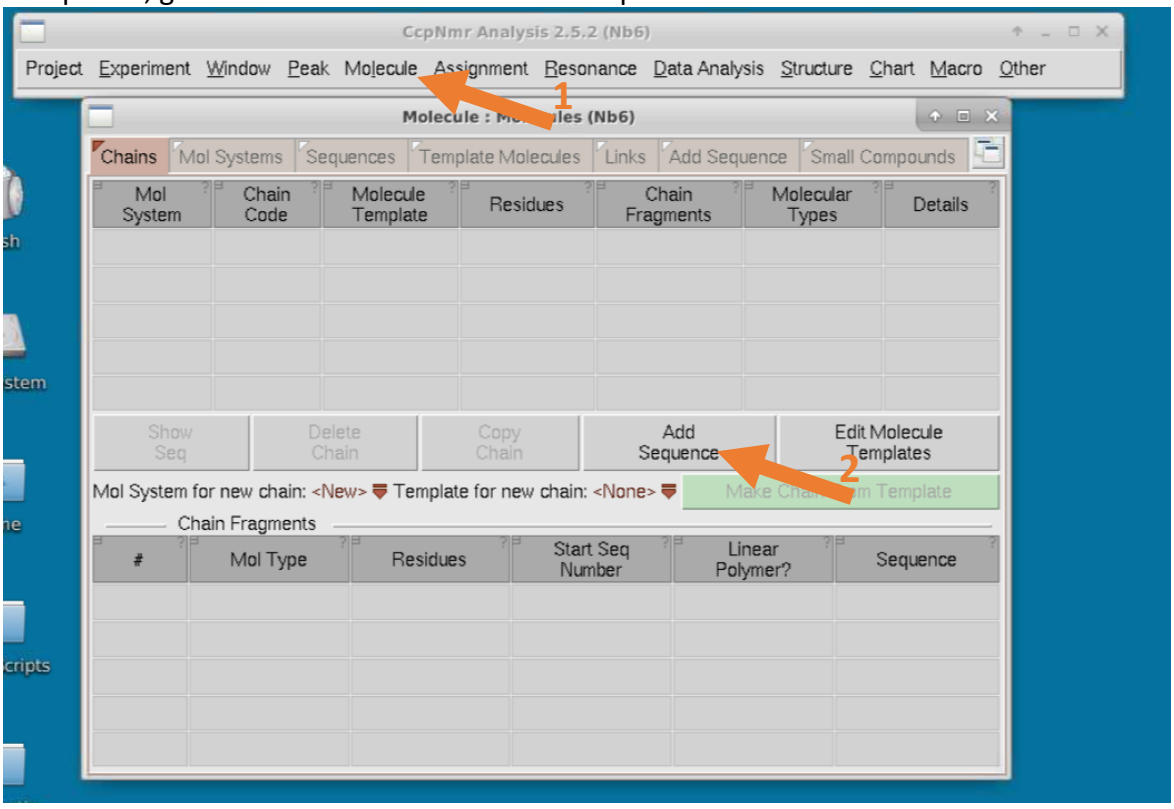
ccpNMR Tutorial

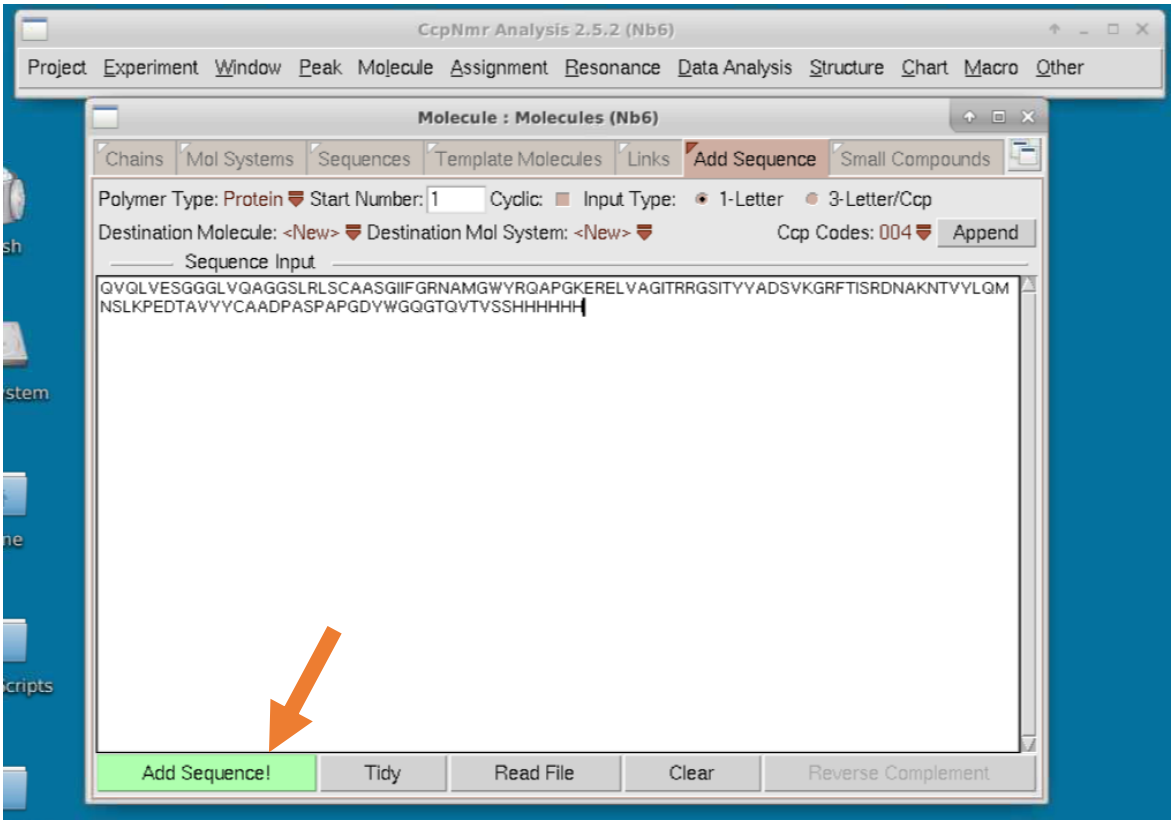
1. In the NMRbox terminal, type “analysis” to open ccpNMR analysis
2. Create new Project



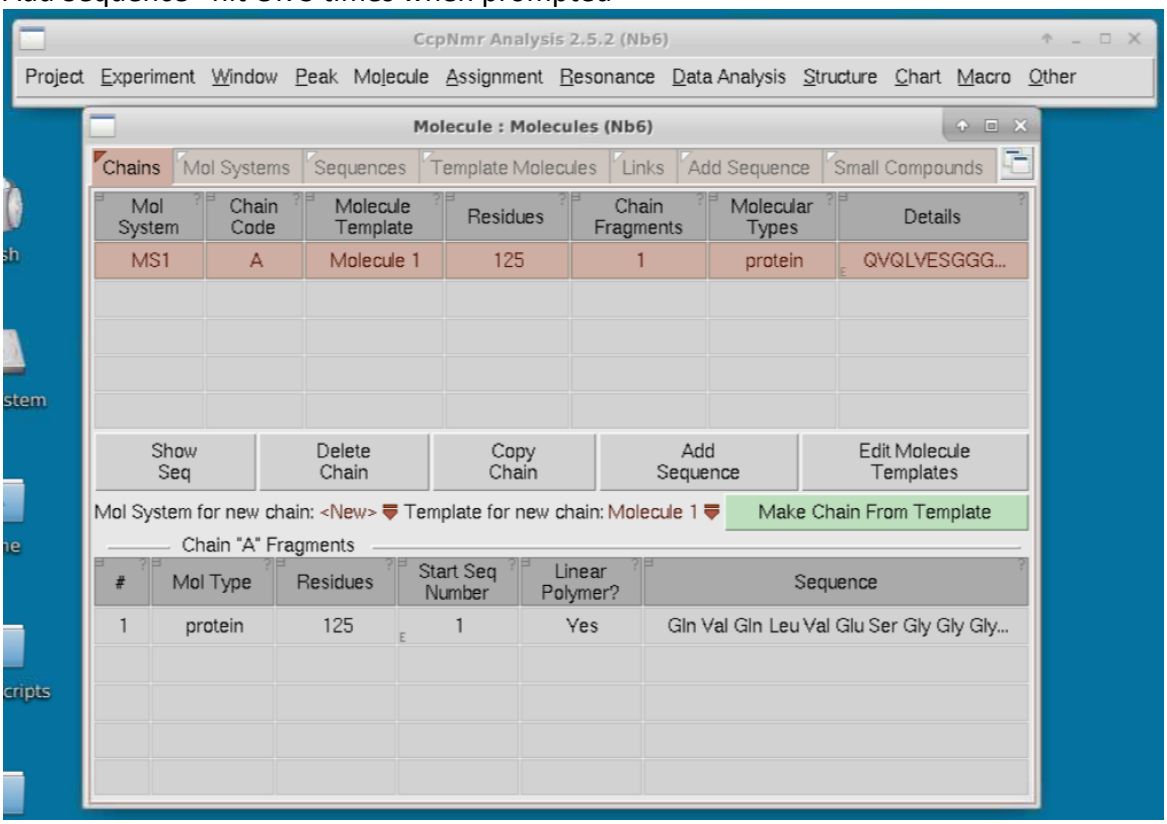
   a.
   b. Project>New>Name:Nb6_MM
3. <u>To add Nb6 amino acid sequence</u>:
   a. In ccpNMR, go to Molecule>Molecules>Add Sequence



   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)

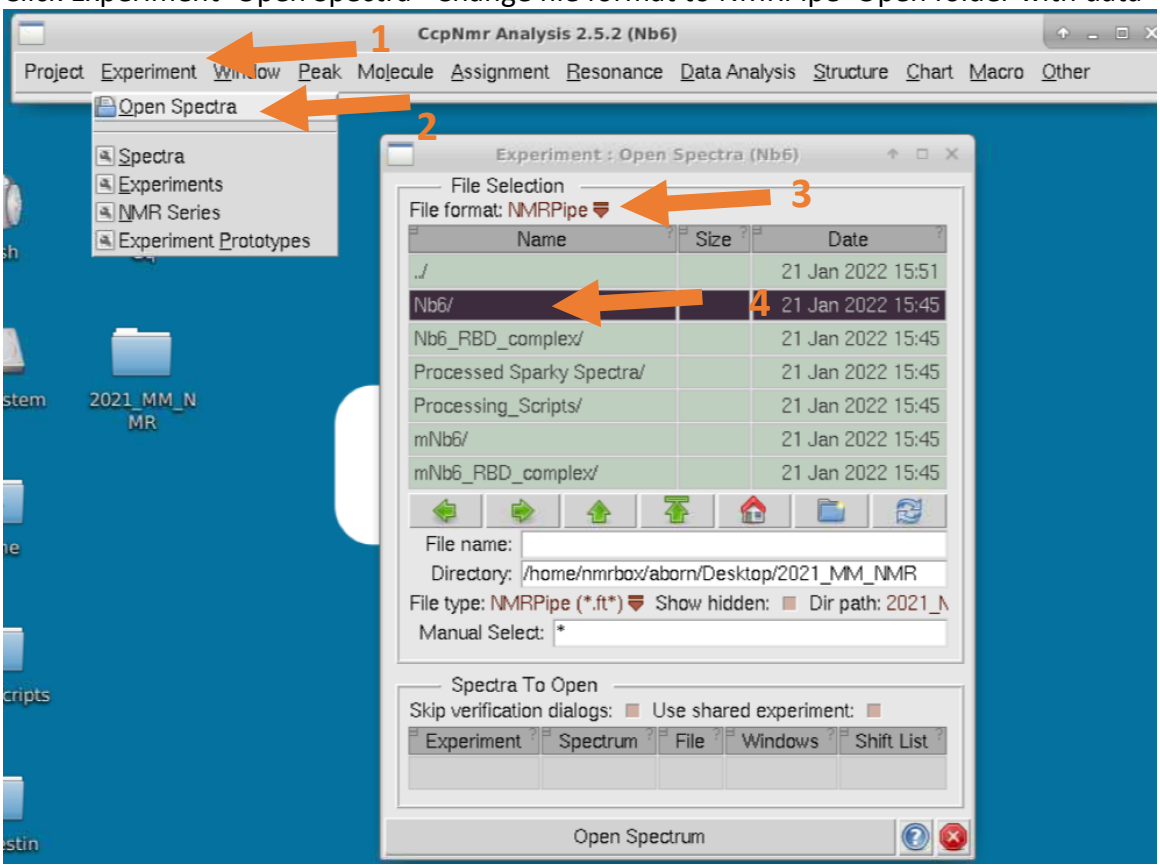


d. Add Sequence> hit OK 3 times when prompted

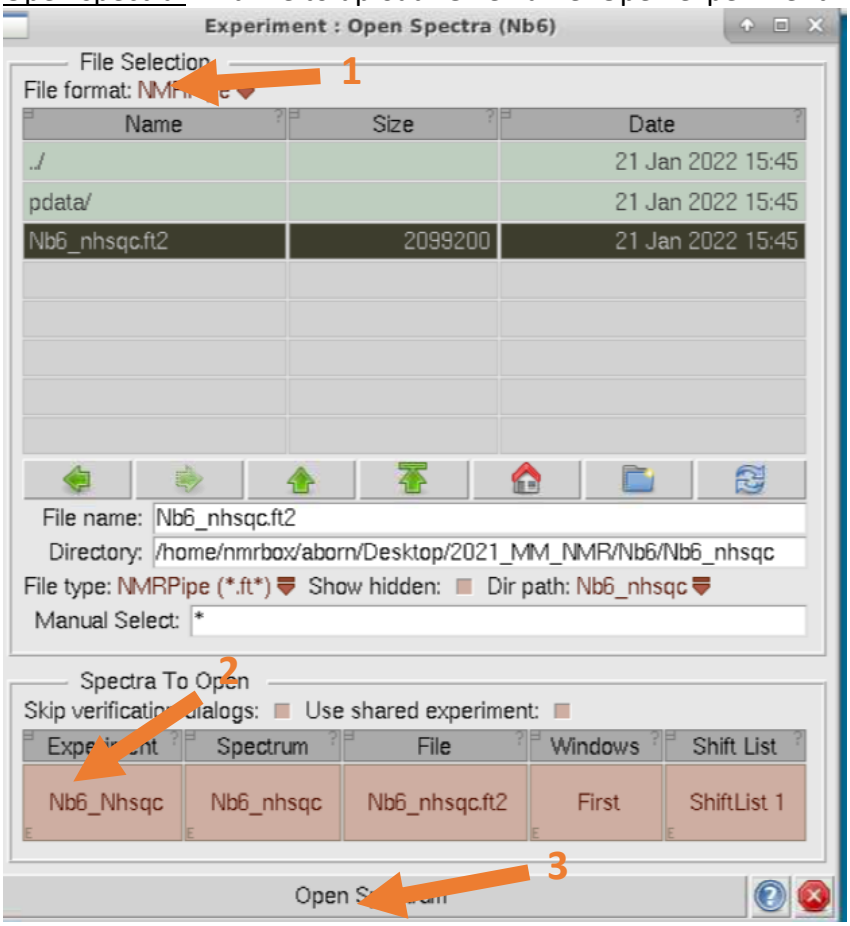


4. Load Nb6 ^{15}N-HSQC

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

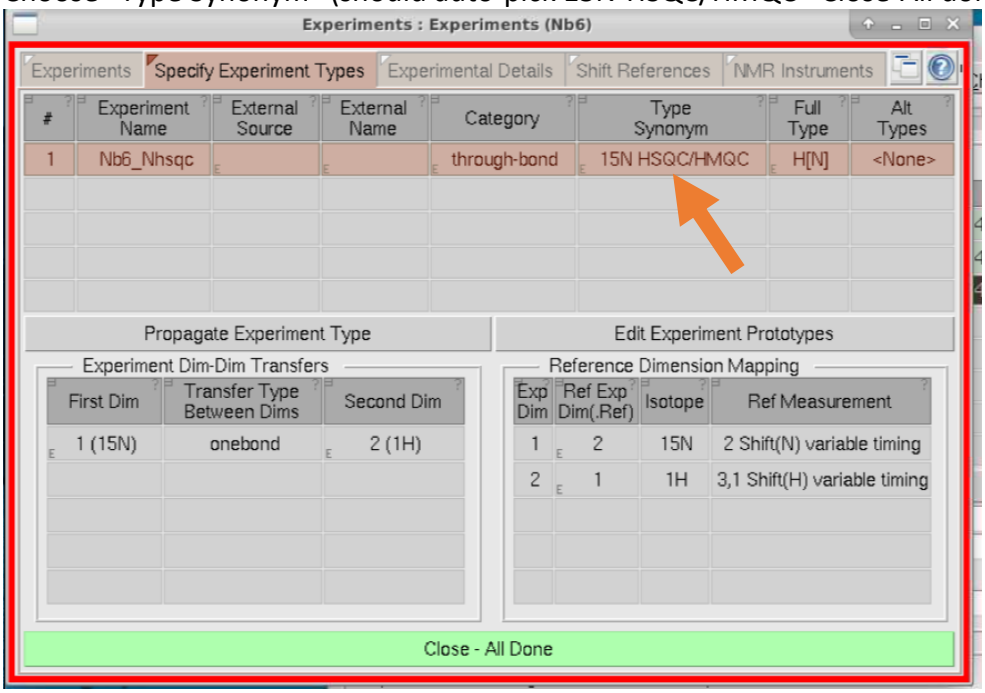


b. Open spectra: Find file to upload>Give name>Open experiment

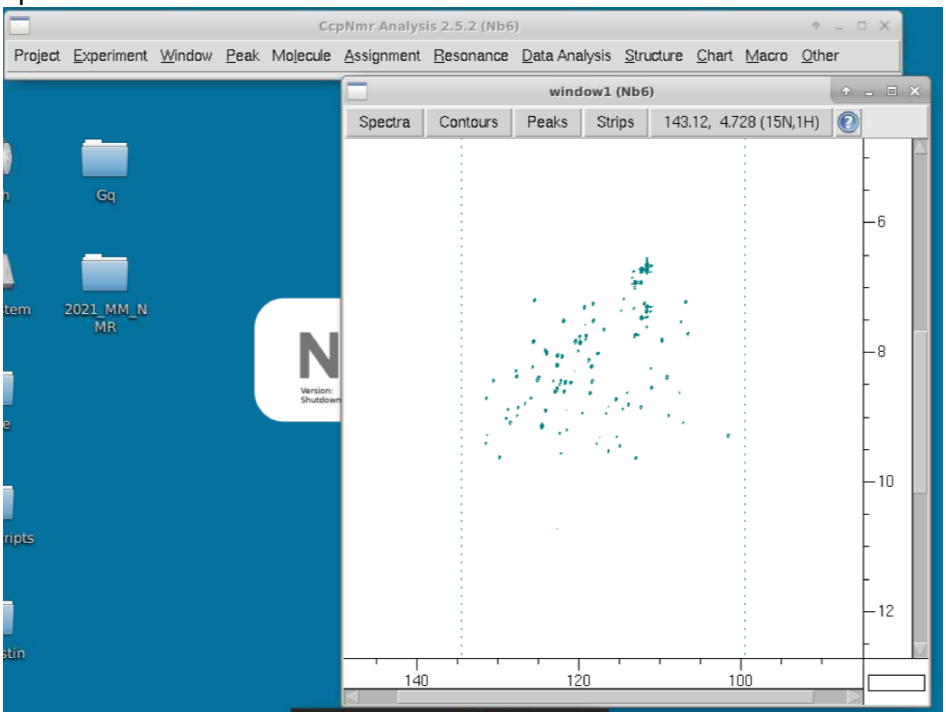


c. Referencing box will appear> click commit in top right hand corner

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

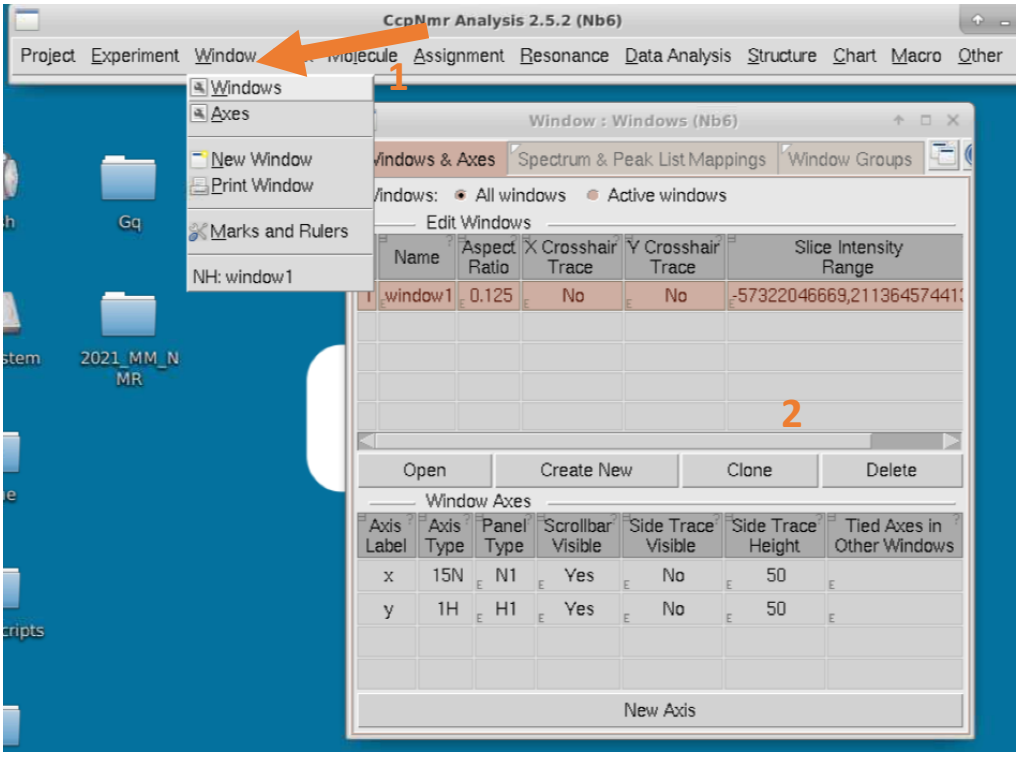


e. Spectra loaded

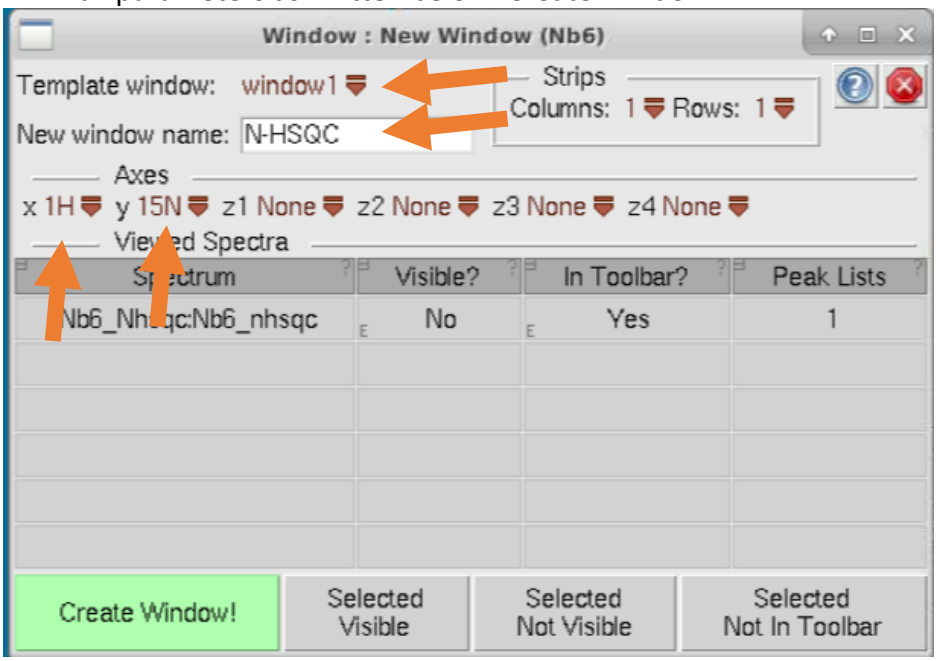


5. <u>Change Spectra Window</u>: Unfortunately, the spectra’s dimensions are flipped from what we typically want, so we just need to switch the 1H and 15N Dimensions
   a. Window>Windows>Create New

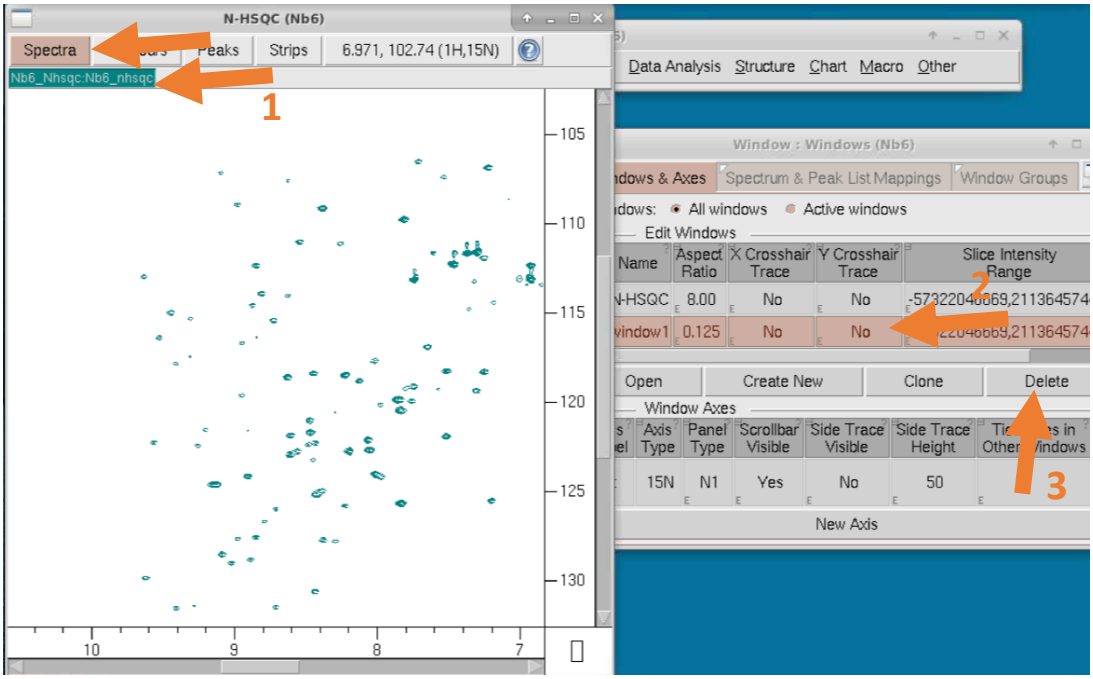
b.



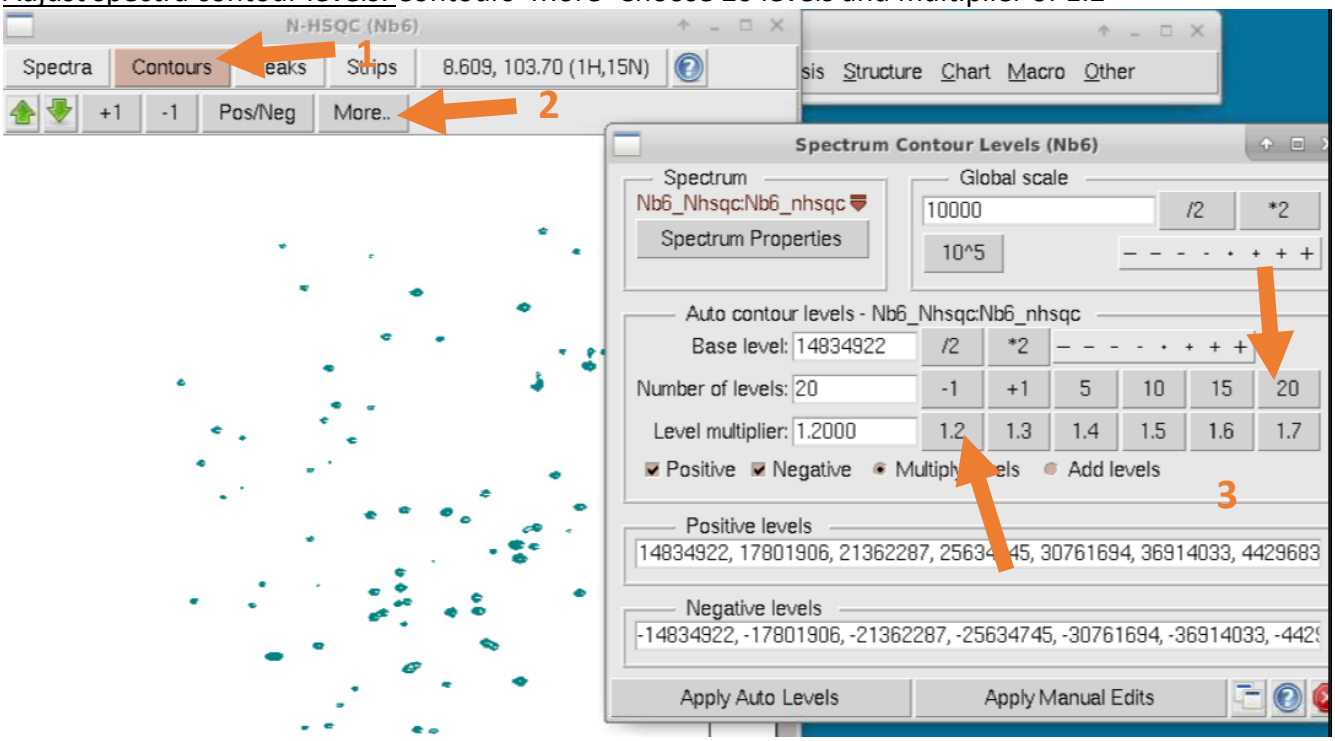
c. Fill in all parameters as written below>Create Window



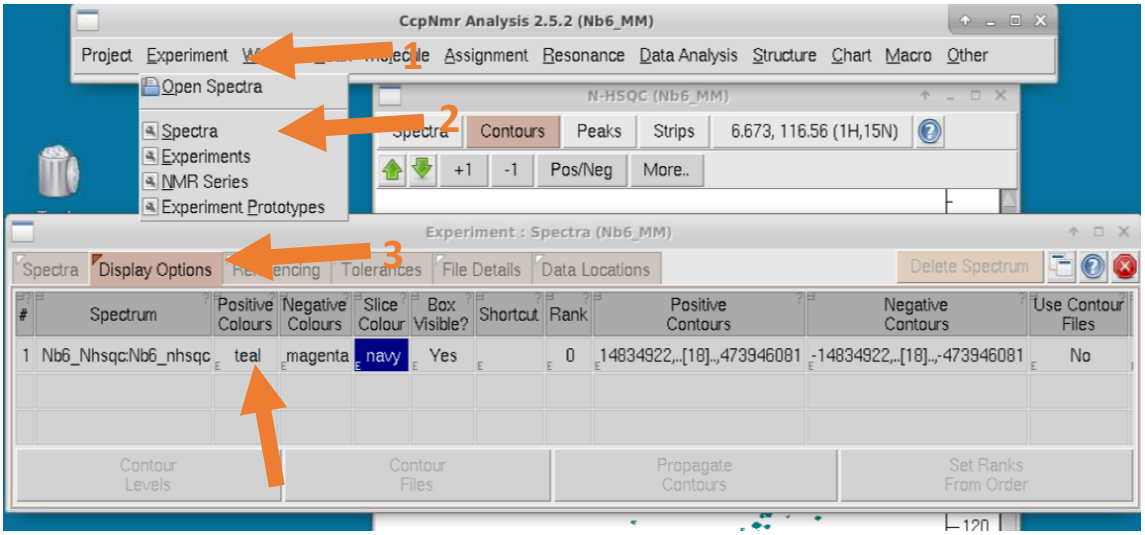
d. New window will appear with the spectra with correct axes (may need choose spectra in window.
    i. You may also want to delete the old “window 1” to help declutter



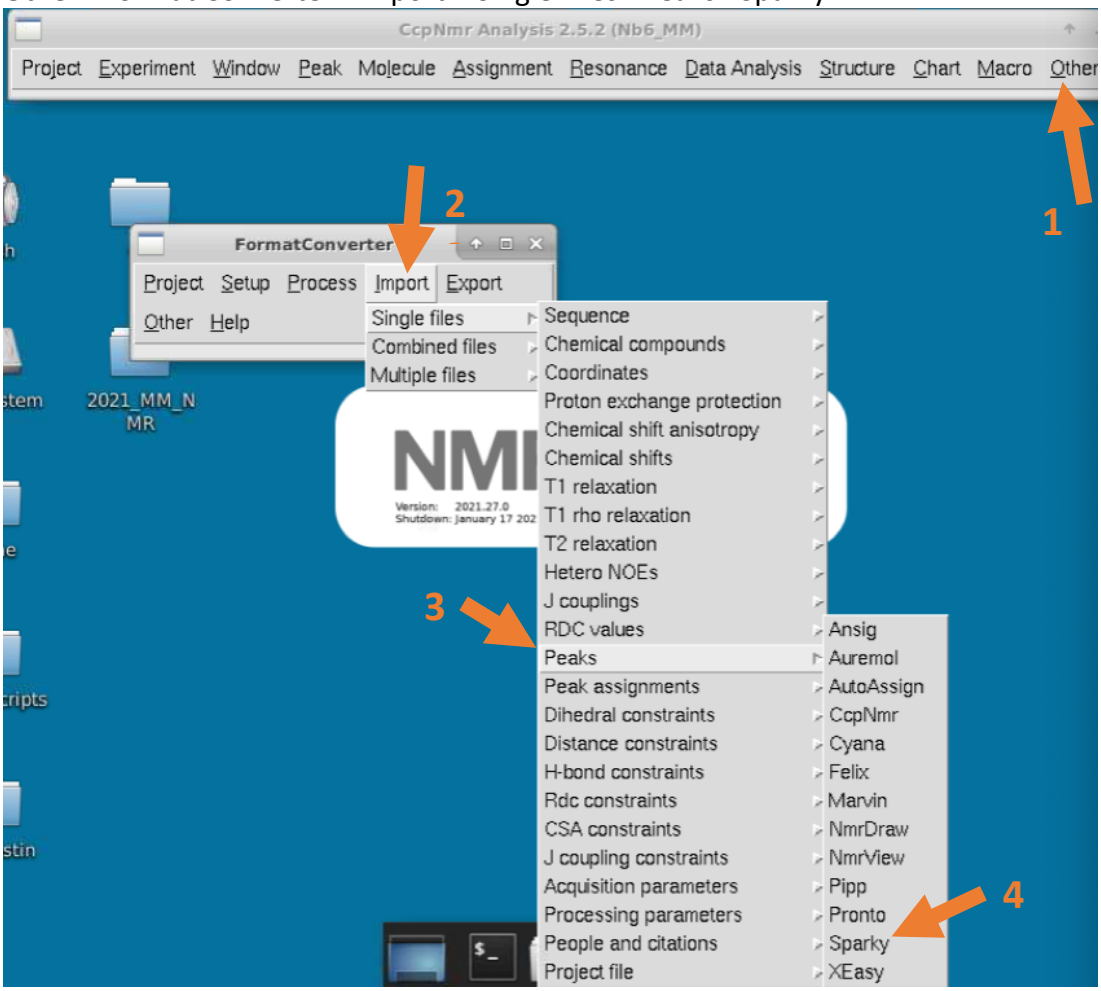
6. Adjust spectra contour levels: Contours>More>Choose 20 levels and multiplier of 1.2



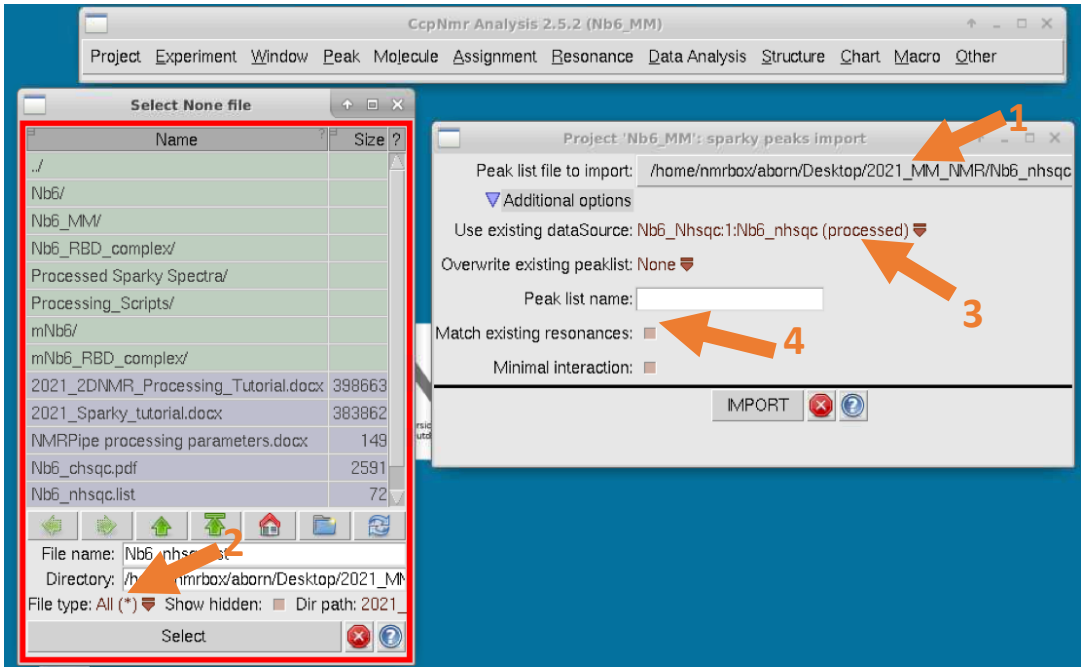
7. Save experiment: can do anytime and should be saved frequently afterwards. Project>Save As.
8. Adjust spectra parameters like color, font size, etc…
    a. (This is optional now, but will definitely be useful to know how to do once you add other spectra) Experiment> Spectra> Display Options



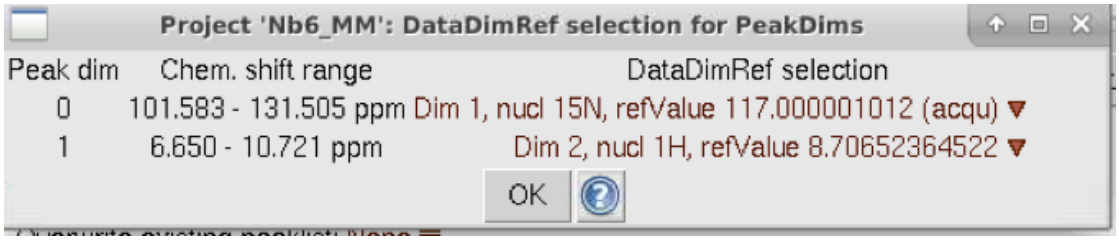
9. Load assignment file
    a. Other> Format Converter> Import > Single Files> Peaks> Sparky



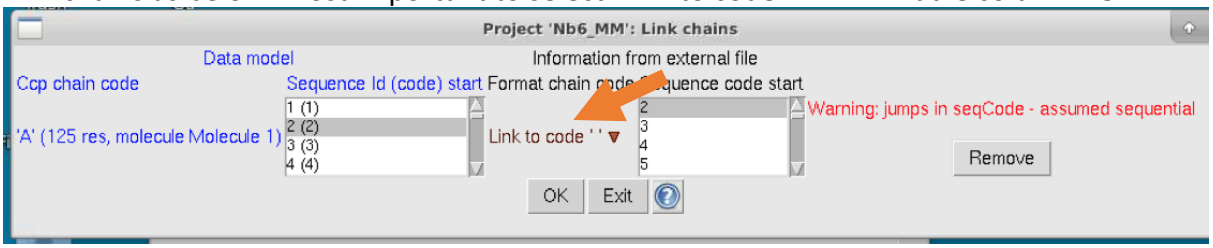
    b. Fill in info like below. To find your peak list, click “All” in File Type> Nb6_nhsqc.list> select> Use existing data source> Unclick match existing resonance> Import



c. Enter peak list name (autocomplete is fine)
d. Make sure dimensions are set like below

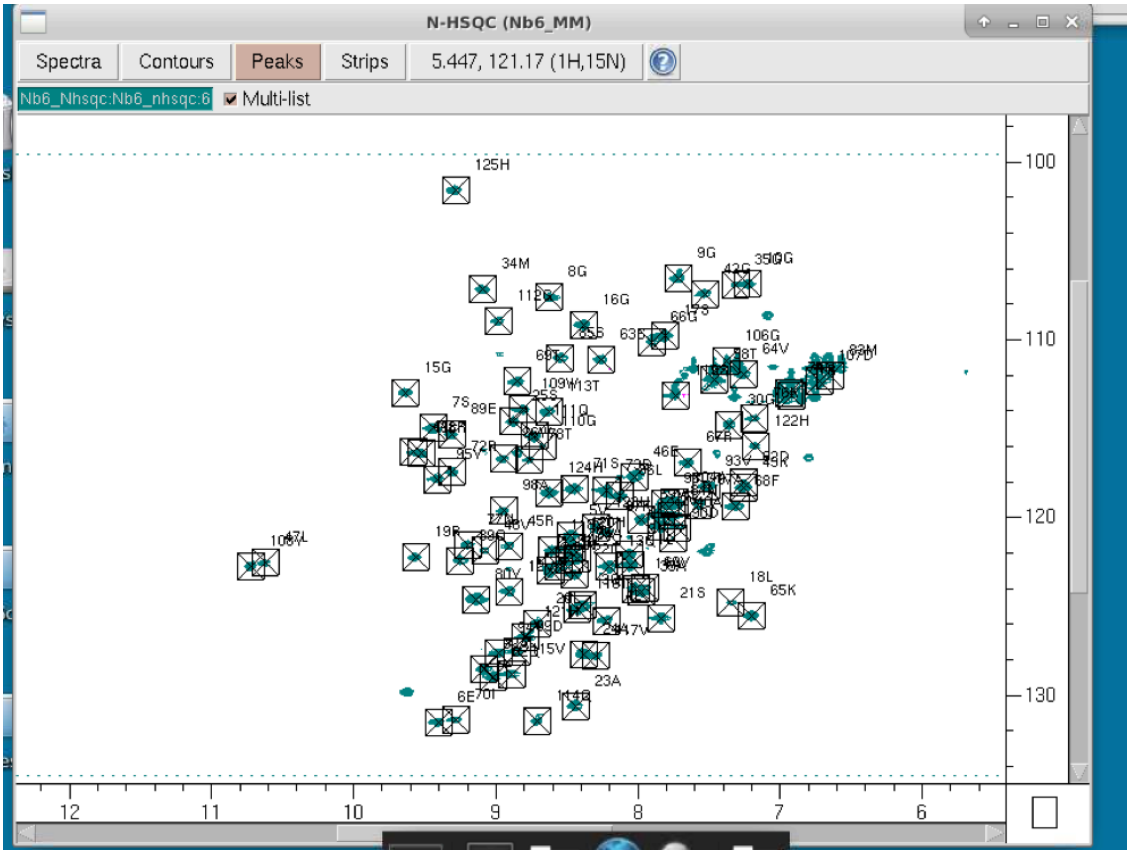


e. Popup says you were successful in importing file, hit next/ok
f. Run linkResonances- since we previously defined the molecular system by loading the amino acid sequence, we will now link the amino acid sequence to the important peak list/resonances. Just hit “yes”. Link with default settings.
g. Link 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)

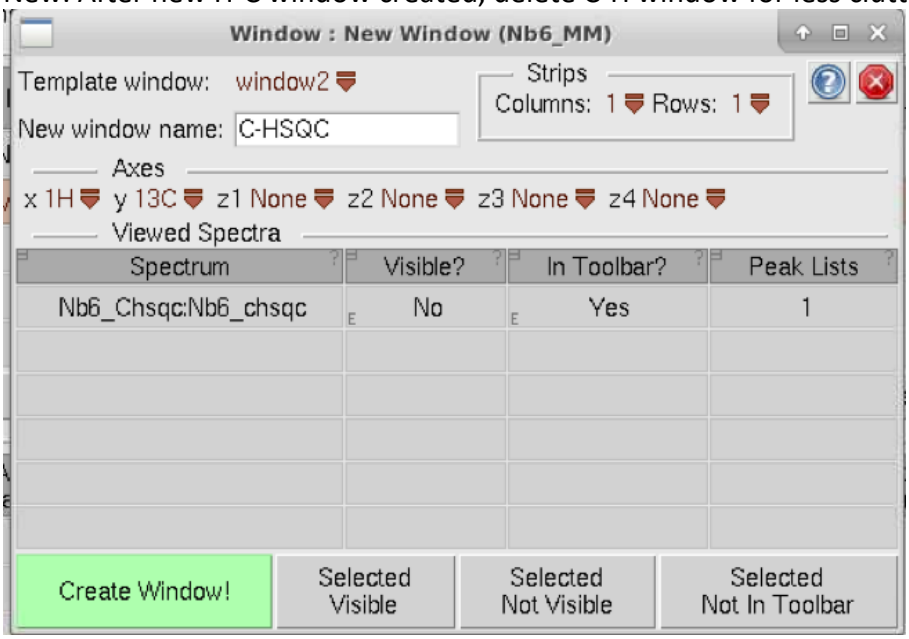


b. Then click “shift p” (holding down shift when push p). The markers should have moved to the max of each peak. Scan the spectra to make sure this occurred. You can zoom into the spectra using the wheel of your mouse (if have 3 button mouse). Move around spectra using the vertical and horizontal scroll bars

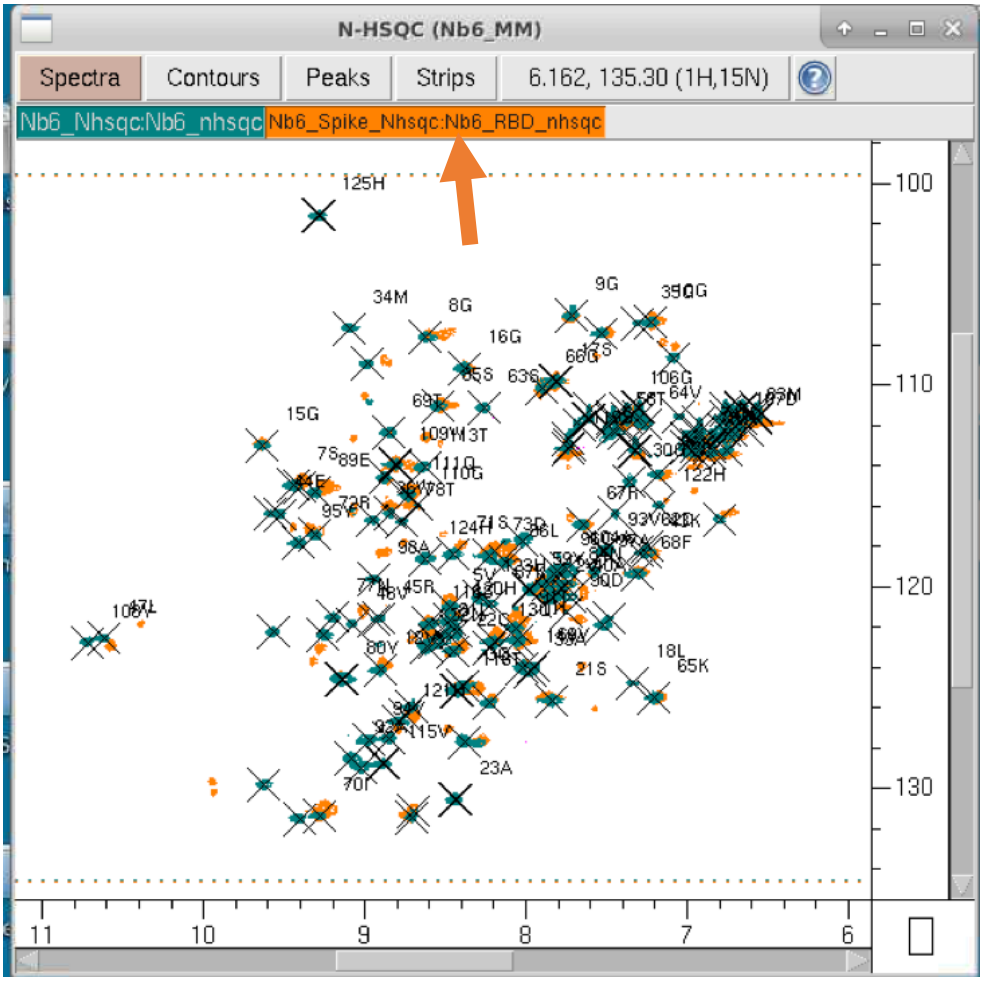
c. Delete old peak list. Peak> Peak list> Select old Nb6 NHSQC peak list (should have 0 peaks)> Delete

11. Load ^{13}C-HSQC of Nb6 exactly as we previously loaded the ^{15}N-HSQC. When you choose the experiment, pick “13C HSQC/HMQC”. Adjust contour levels as well.

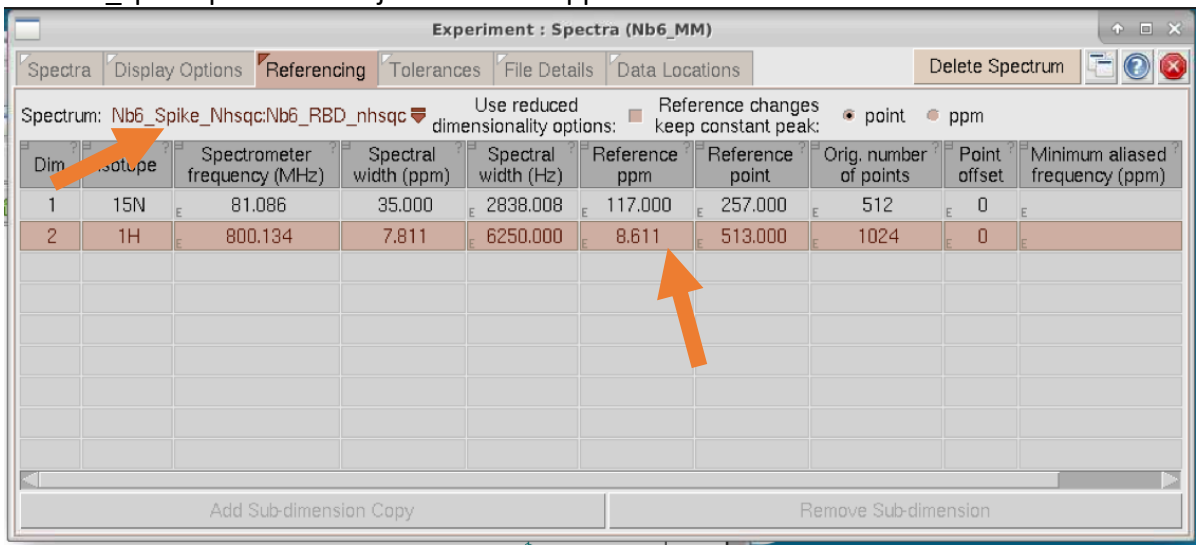
a. You will need to flip the H and C dimensions as done previously: Window> Windows> Create New. After new H-C window created, delete C-H window for less clutter!



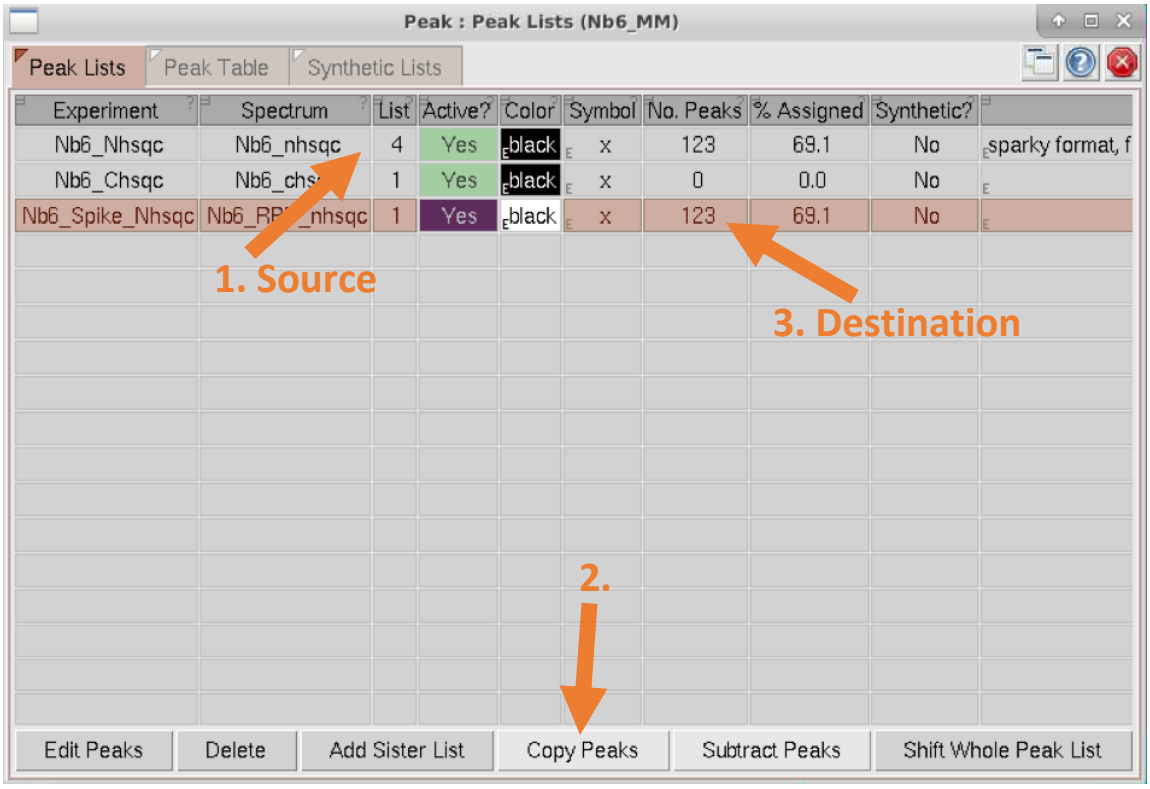
12. Load ^{15}N-HSQC of Nb6+Spike as previous. You should not need to make a new window this time. Change the color of the spectra if you'd like
13. Overlay the spectra
    a. In the spectra tab, toggled between which spectra are shown so both apo Nb6 and Nb6+Spike are overlaid.



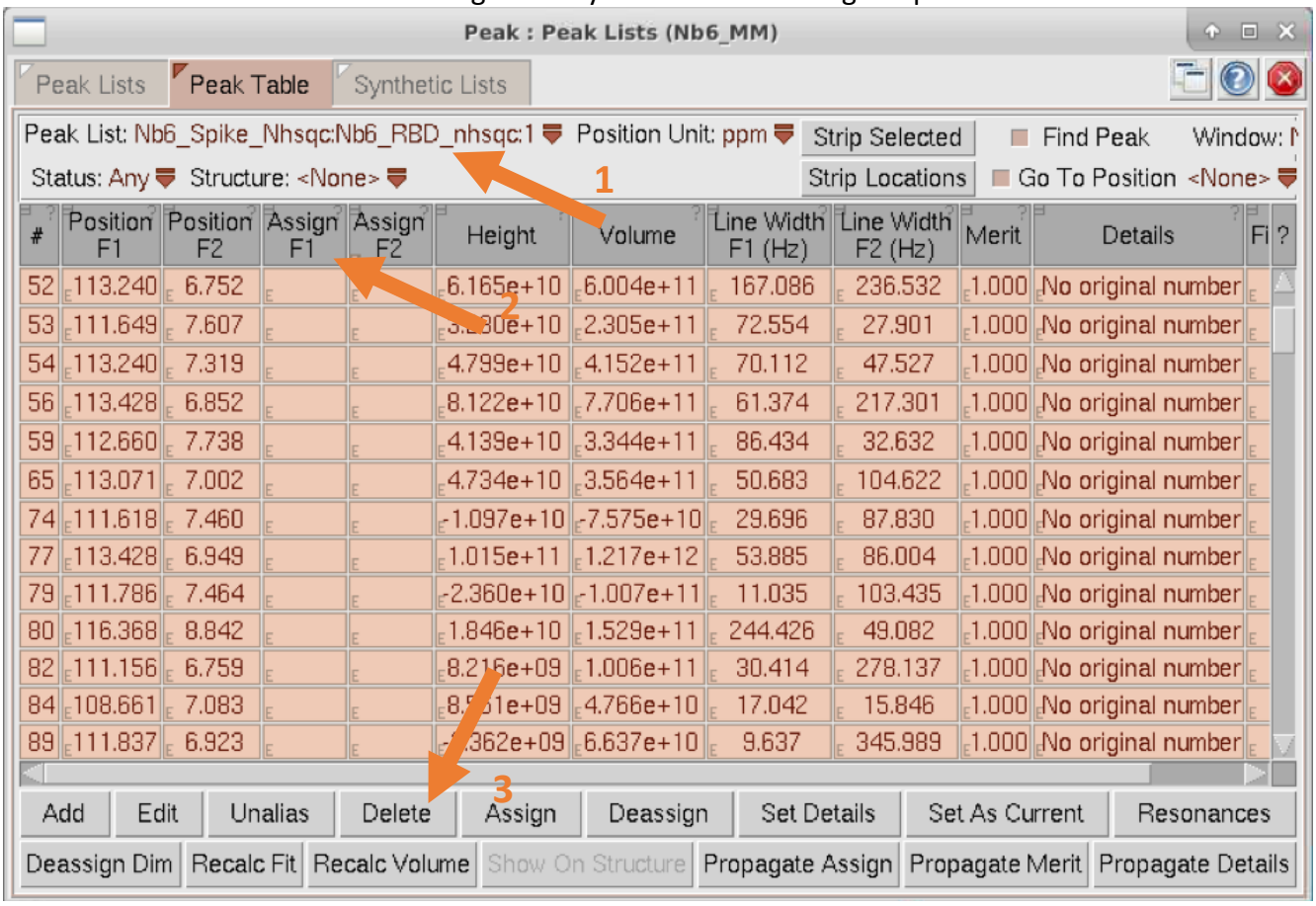
    b. Adjust referencing values in Nb6_Spike: Experiment> Spectra> Referencing> Choose correct Nb6_spike spectrum> Adjust reference ppm as listed



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



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

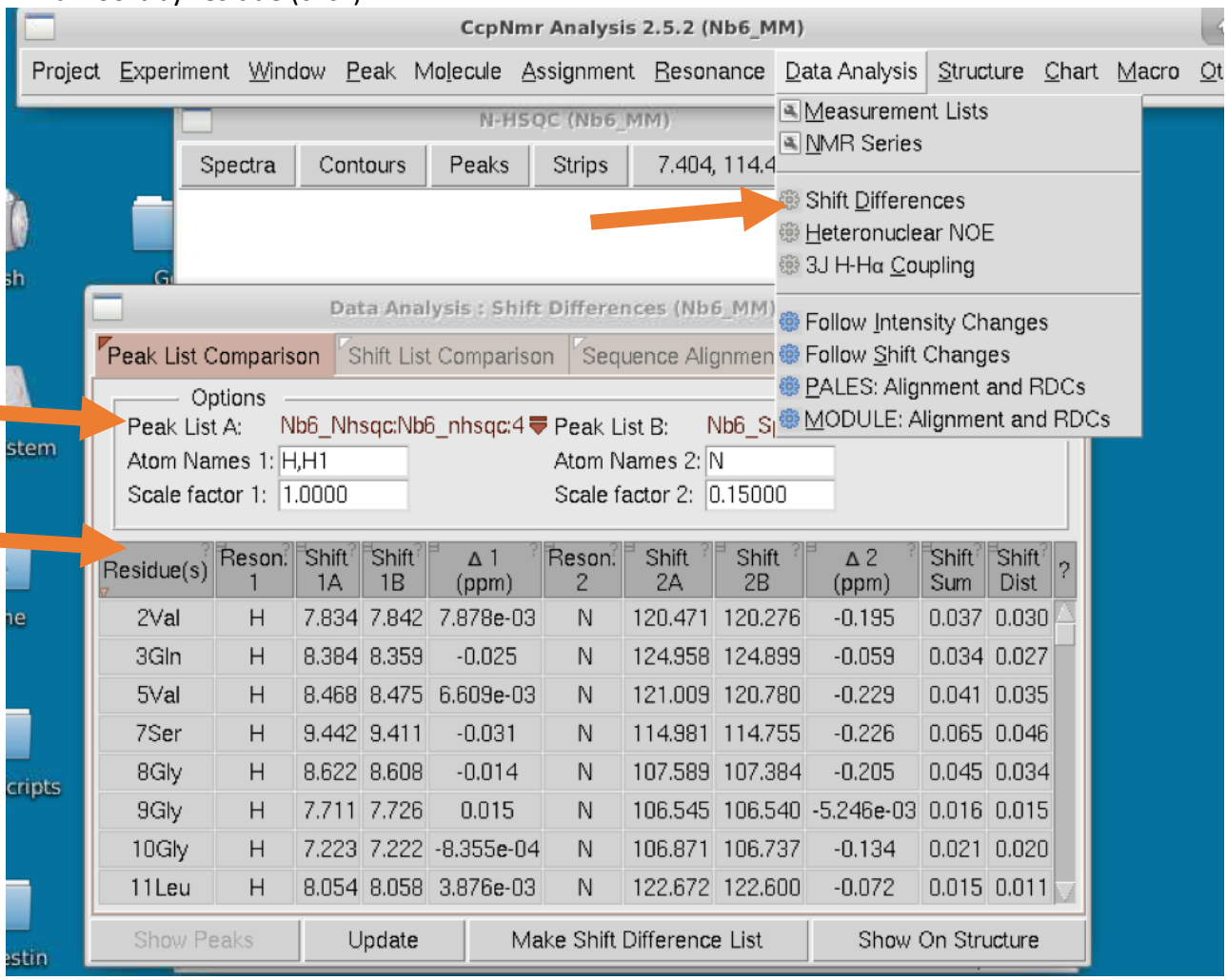


15. Adjust peak assignments on Nb6+Spike spectra: this will be difficult as many peaks move/disappear. Do your best to assign shift perturbations typically by assigning peaks to nearest shift. Consider changing the contour levels to aid in the assignment. If a peak has completely disappeared and/or hard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

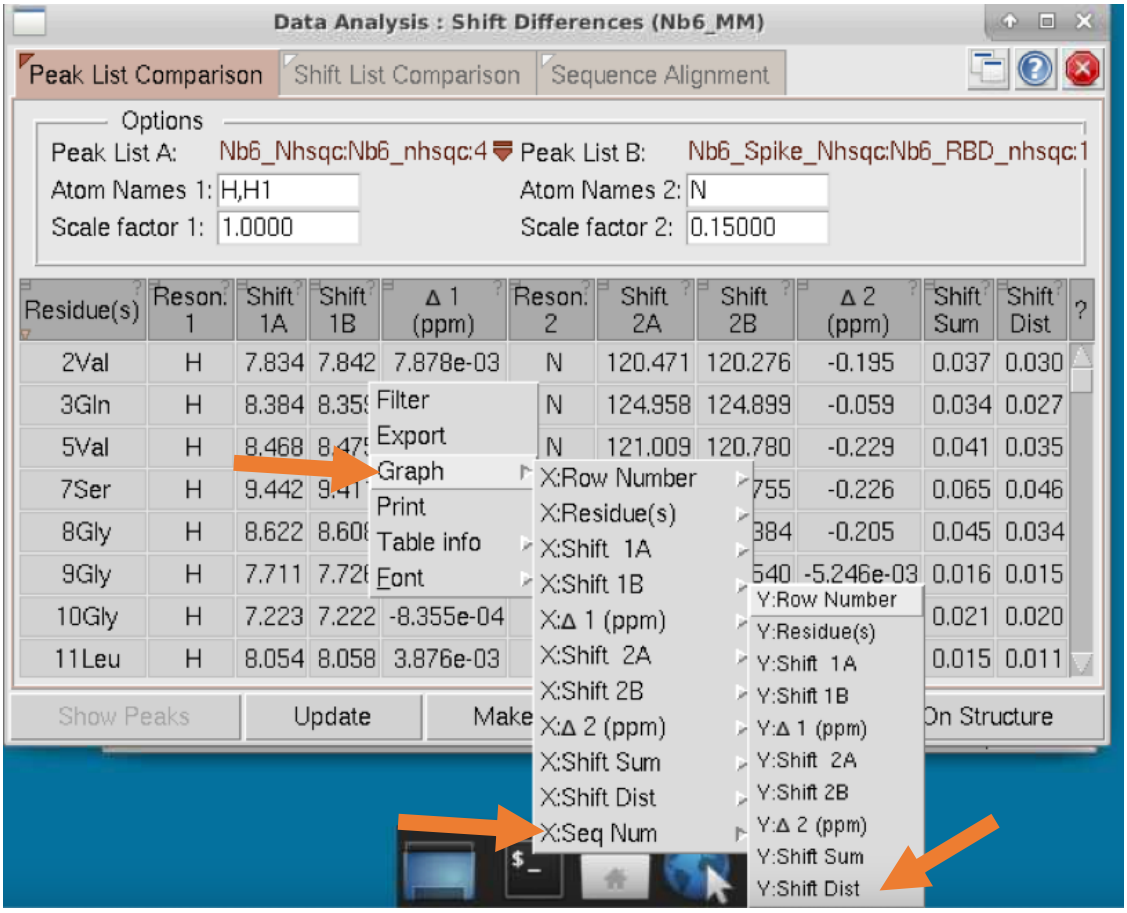
assign, DON'T move or delete it. A peak that moves so much it can't be assigned is also useful information.

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)



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



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