NMRFAM-SPARKY Quick Tutorial
Macro Methods

Now that you have processed the 2D spectra for both Nb6 and mNb6 in complex with Spike RBD, it's time to begin comparing spectra and gain insights into the dynamic behavior of the nanobodies how they interact with the RBD. We will use Sparky for the data analysis since it has a multitude of useful functions and is generally more convenient to use than NMRPipe. Sparky was originally created by Tom Goddard and Tom Ferrin at UCSF and is currently maintained by the NMRFAM at the University of Wisconsin. Sparky is under constant development and this tutorial is by no means exhaustive of its capabilities. The aim of this tutorial is to familiarize you with some of the basic features you need to examine spectra and begin generating useful information for determining chemical shift perturbations.

In addition to buttons and menus, Sparky utilizes a number of two-letter commands that allow you to easily manipulate spectra and a complete list of these commands can be accessed by typing **hl**. The Sparky manual itself can be accessed by typing **he** and provides information about some of the program's features. In the instructions that follow, I will frequently refer to the two-letter codes since they are easier to implement but I have also included instructions for accessing some of the features from the drop-down menu.

1. Open NMRFAM-SPARKY from the applications folder. If this is the first time opening Sparky, you will be prompted to allow the program to create data directories (Save, Lists, Projects).
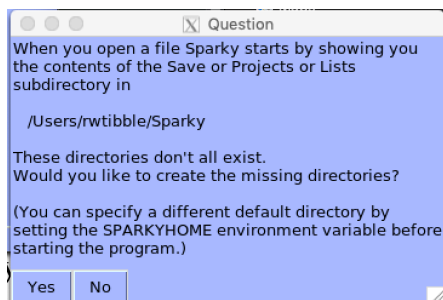
I recommend setting SPARKYHOME to be in your nmrdata directory, which is likely not the default location, so click **NO** on the pop-up window. You can change the location of these folders later by selecting NMRFAM -> Utilities -> Change SPARKYHOME. After setting SPARKYHOME, you'll need to restart Sparky.
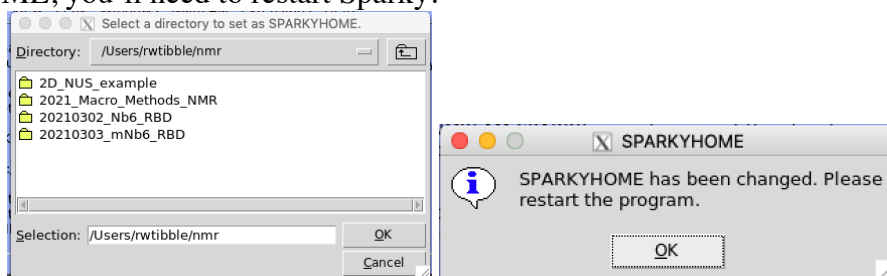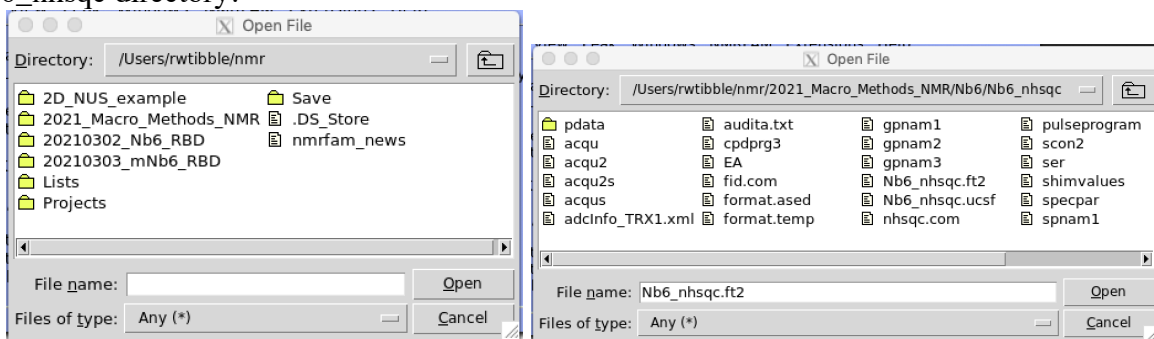

2. Now that Sparky has created the necessary directories, we need to provide a list of NH assignments we can read onto the spectrum later. This file, **Nb6_nhsqc.list**, is also in the Box Folder you downloaded. Move it into the Lists folder.
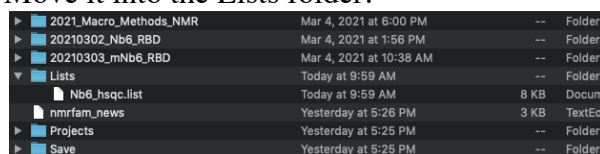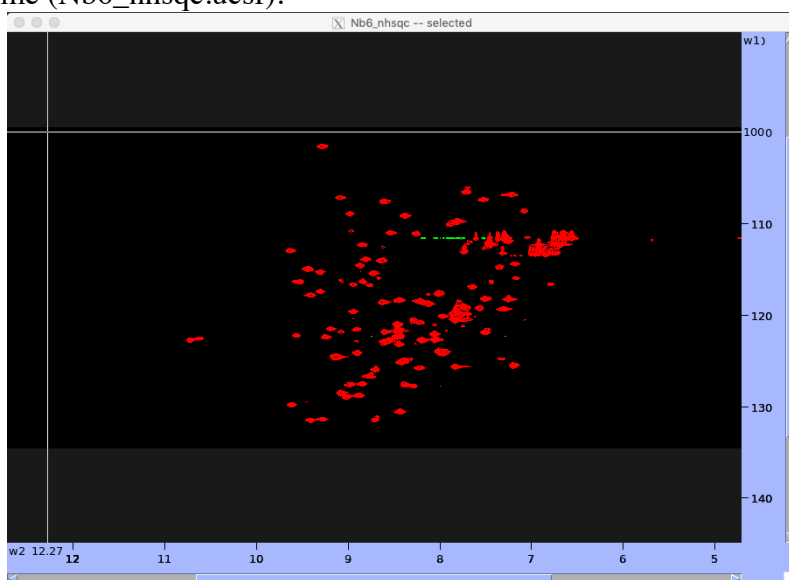
3. Open the NHSQC spectrum for Nb6: **fo** or File -> Open and select the processed .ft2 file in the Nb6_nhsqc directory.

Note: The default Sparky format is .ucsf, which is automatically generated upon opening the .ft2 file for the first time (Nb6_nhsqc.ucsf).

4. Type **ct** (View->More->Contour Levels) to open the contour window. Change the values to those shown at right and click **Apply**. You may also change the color of the spectrum from the drop-down menu in this window.
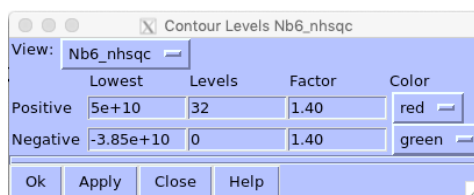
A few words for navigating around a spectrum:
Take a moment to look at the Pointer Mode (**pm**) window. It should currently be in Zoom Mode. Click and form a box to zoom on the region from ~8.5-9.5 ppm in the hydrogen dimension and ~120-125 ppm in the nitrogen dimension. Use the arrow keys to move around the spectrum while maintaining the same window view. You can type **zi** or **zo** to zoom in or out, respectively. A couple of particularly useful commands are **zp**, which restores the previous view, and **zf**, which will return to the full spectrum view.
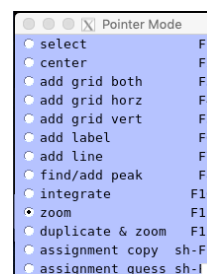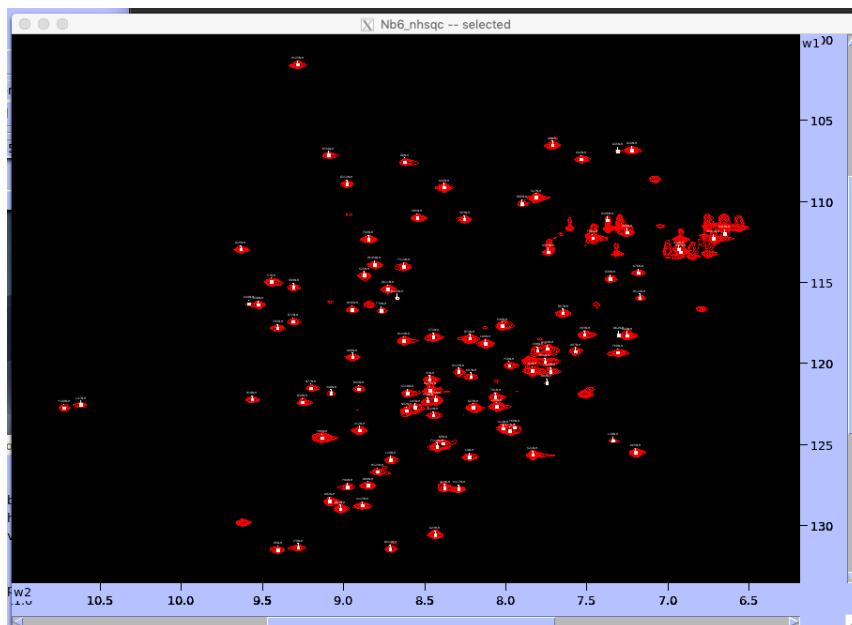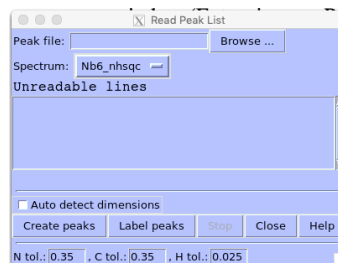
Now we will read in the resonance assignments and display them on the spectrum.

5. Return to a view of the full spectrum by typing **zf** and type **rp** to open the Read Peak List window (Extensions -> Peak -> Read peak list.

6. Click **Browse** and navigate to your List directory and click **Nb6_nhsqc.list**

7. Click **Create Peaks** and the spectrum should be populated with assignments for Nb6.

8. Type **lc** and then **lu** to center the labels and then uncover them from one another. The label sizes is likely quite small and hard to read. You can change the properties by typing **oz** and changing the values in the pop-up window.
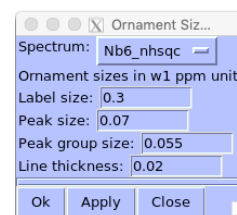
9. Display the peak list by typing **lt** (Peak -> Peak List…). It has three columns: (1) Assignment (2) Nitrogen chemical shift and (3) Hydrogen chemical shift. Double-clicking on an entry centers the spectrum on that peak.
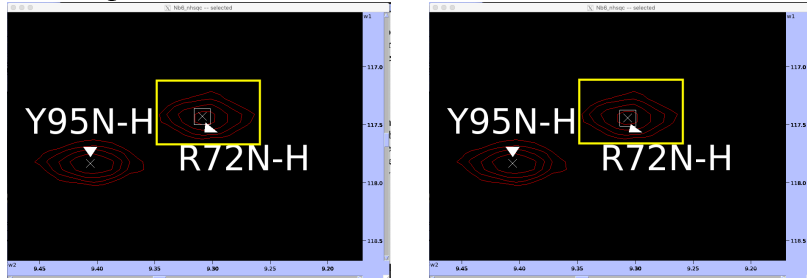
10. Navigate around the spectrum (or click on each entry in the peak list) and note if there are any peak labels that appear to be off-centered from the resonance. Enter Select mode (click the
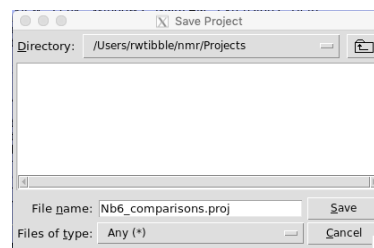
radio button or type **fn+F1**) and click on the resonance. A box will appear around the X. Type **pc** and the peak should move to the center of the resonance.


Before continuing to adjust other peaks, save your session as a project (File -> Project/Save As or **ja**). I highly recommend saving the project file regularly so you don't lose progress (File -> Project/Save or **js**).


In addition to saving the spectrum, we need to save the adjusted peak list. Between adjusting peaks, click **Update** in the peak list window and the corrected peaks positions will be recorded. Then, click **Save** and overwrite the initial peak list. Now you will have an accurate table of peak positions, which will be needed for calculating chemical shift perturbations later in the course.

Now, we'll open the mNb6 spectrum and overlay it onto the Nb6 spectrum to see how the two mutations affect the spectrum.

11. Open (**fo,** or File -> Open) the mNb6 NHSQC spectrum (**mNb6_nhsqc.ft2**) and adjust the contour level (**ct**) as you did for the Nb6 spectrum in (4). Also, change the color of the spectrum to something that will contrast with the Nb6 spectrum (cyan in this tutorial).
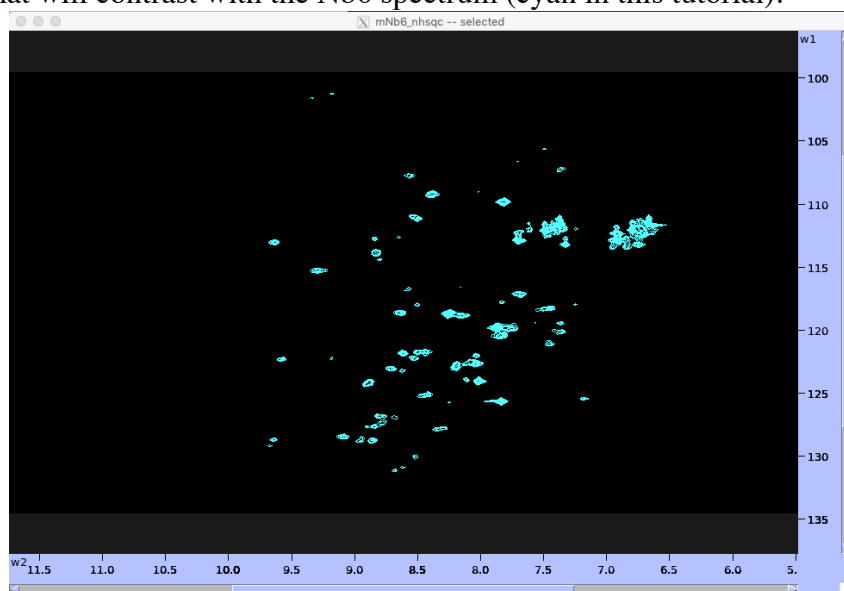
12. Type **ol** and the spectra overlay window will appear. Change the **From View** to mNb6_nhsqc spectrum and the **Onto View** to Nb6_nhsqc. Click **add**.
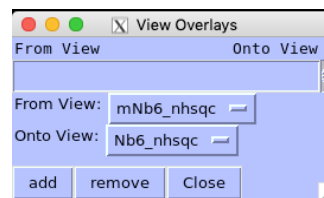
13. Navigate to the Nb6 spectrum and you should see the spectrum for mNb6 overlaid. You will notice there are significant changes and they do not overlay well. There are numerous perturbations and resonances have disappeared.
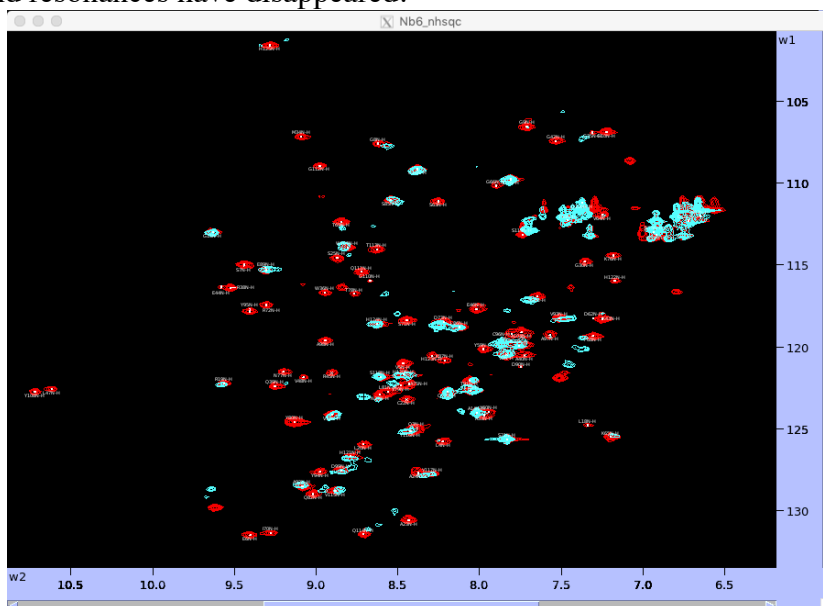
What we'll want to do is try and note the peaks that have changed. One way of doing this is to determine chemical shift perturbations (CSPs). For example, zoom in on the resonance for R67. We can see a noticeable perturbation of between Nb6 and mNb6. In select mode, we can move the peak from the red to the cyan peak. Note: **pc** will not work for centering since there is not a peak at the cyan location in the Nb6 spectrum. If you want to be able to use pc, you'll need to reverse the overlay we initially did.
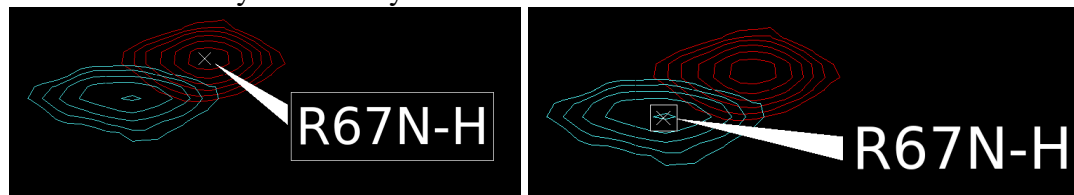
14. Once you've moved this peak, update the peak list and save it as mNb6_nhsqc.list.
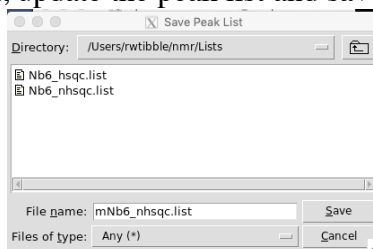
You can continue to move around the spectrum and adjust the peak positions.

In addition to peaks that have moved, many peaks have broadened beyond detection. Take note of these peaks because the ratio of peak intensities between two spectra is another means of quantitating changes. Once you have finished creating a peak list for mNb6, you can integrate the respective spectra. This part of the tutorial will walk you through integrating the Nb6 spectrum.

15. Navigate to the Nb6 spectrum and remove any overlays. Be sure the peak labels are displayed and the contour level has been set to an appropriate cutoff so the resonances are clearly observed.

16. Type **it** and a peak integration window will appear (shown at right). Change the check boxes and values in the window so it matches those shown:

> *Note:* Be sure to select the proper spectrum each time since you can apply different settings to individual spectra.

17. Click **Apply**.
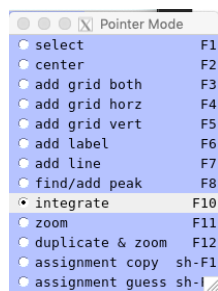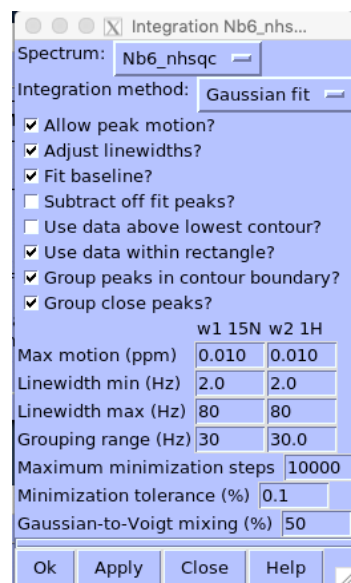
18. Navigate to the Pointer Mode window and switch to *integrate* (hold the function key and type F10).

19. Click and draw a box around the peaks you would like to integrate. Once you release the mouse, Sparky will begin calculating the volume. This may take a few minutes to complete but you can tell when the integration is done when a circle replaces the sqaure the peak (as shown in the screenshot for S63N-H). I have found trying to integrate many peaks at one time can cause Sparky to crash, so I suggest integrating subsets of peaks and routinely saving your project.
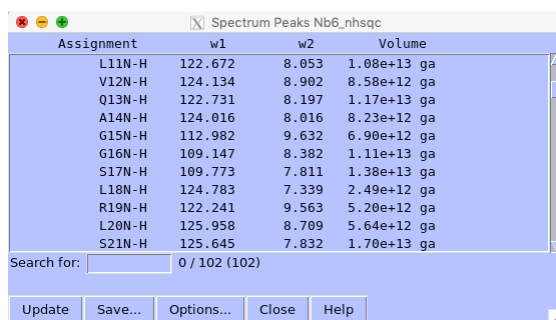
20. In order to record the peak volumes, display the peak list by typing **lt** and click **Options**. A separate window will appear with a variety of variables to include in the peak list. Check the box next to Volume and click **Apply**.
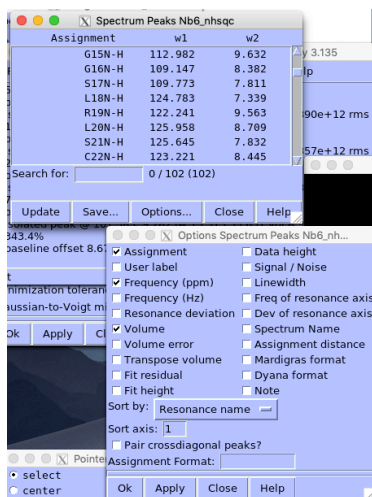
21. The peak list should now be populated with an additional column containing the calculated volume for a given peak:

Click **Save** to write the peak list. As is the case for moving peaks, any time you re-integrate a peak, be sure to click **Update** and re-save the list.

At this point, you can closely examine the spectra for Nb6 and mNb6 in complex with the Spike RBD.