

NMRFAM-SPARKY Quick Tutorial

Macro Methods

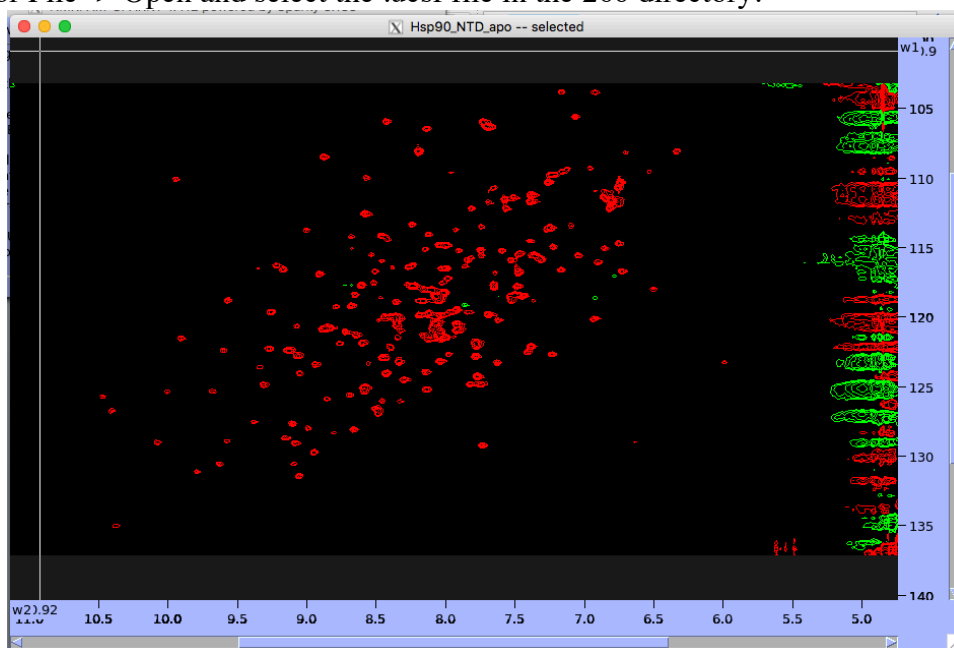
Now that you have processed the 2D spectra for Hsp90 mixed with various small molecules from the screen, it's time to begin comparing spectra and gain insights into if/how these fragments interact with the NTD. We will use Sparky for the data analysis since it has a multitude of useful functions and 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 will need to examine spectra and begin generating useful information you will need later for determining chemical shift perturbations.

In addition to buttons, 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.

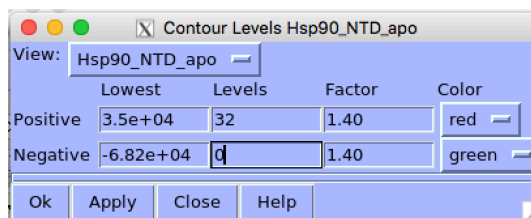
1. Open NMRFAM-SPARKY from the applications folder. If this is the first time opening Sparky, you will be prompted to allow the program to create data directories (Save, Lists, Projects). You can change the location of these folders later by selecting NMRFAM -> Utilities -> Change SPARKYHOME.

2. Open the spectrum for the NTD alone:

fo or File -> Open and select the .ucsf file in the 260 directory.



3. 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.



NMRFAM-SPARKY Quick Tutorial

Macro Methods

A few words for navigating around a spectrum:

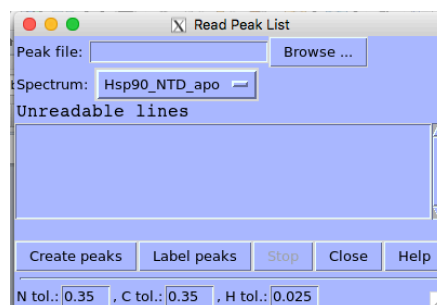
Take a moment to look at the Pointer Mode window. It should currently be in Zoom Mode. Click and form a box to zoom on the region from ~9.5-10.5 ppm in the hydrogen dimension and ~124-126 ppm in the nitrogen dimension. Use the arrow keys to move around the spectrum while maintaining the same window view. 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.

Before continuing, download the peak list from the Box folder (hsp90_ntd.list) and save it in your List folder.

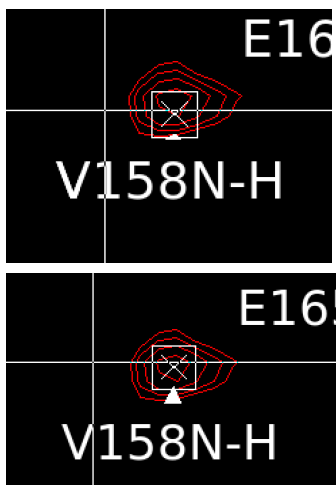
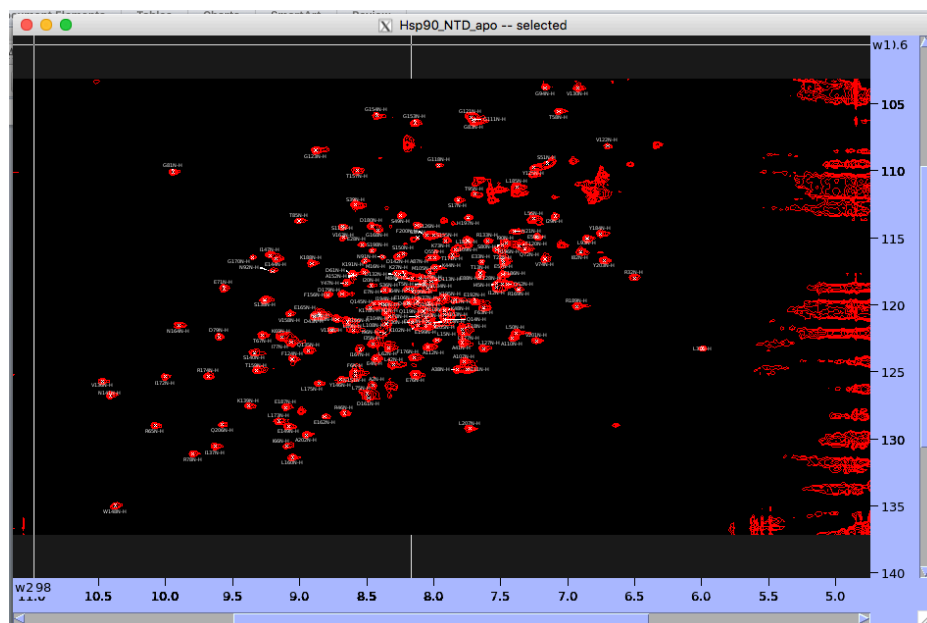
4. Return to a view of the full spectrum by typing **zf** and type **rp** to open the Read Peak List window.

5. Click **Browse** and navigate to your List directory and click hsp90_ntd.list

6. Click **Create Peaks** and the spectrum should be populated with assignments for the NTD.



7. Type **lc** and then **lu** to center the labels and then uncover them from one another.

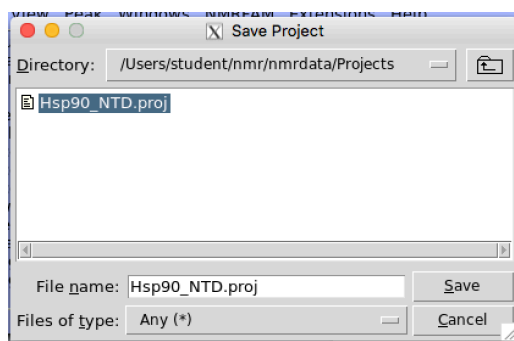


8. Navigate around the spectrum and note if there are any peak labels that appear to be off-centered from the resonance. Enter Select mode (click the radio button or type **fn+F1**) and click on the resonance. A box will appear around the X. Type **pc** and the peak should move to the center of the resonance. Before continuing to adjust other peaks, save

NMRFAM-SPARKY Quick Tutorial

Macro Methods

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**).



9. Once you've re-centered peak markers, type **lt** and the spectrum list will appear. It has three columns: (1) Assignment (2) Nitrogen chemical shift and (3) Hydrogen chemical shift. These values have been updated for any peaks you have adjusted but they have not been saved to a peak list you can upload onto another spectrum as we did initially. Click **Save** and save this list under a new name (e.g. hsp90_ntd_v2.list). Now you will have an accurate table of peak positions, which will be needed for calculating chemical shift perturbations later in the course.

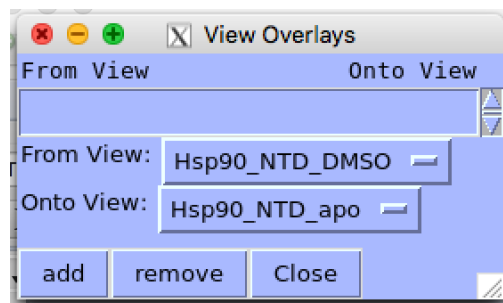
Assignment	w1	w2
M1N-H	120.606	8.357
A2N-H	125.993	8.456
S3N-H	114.990	8.120
E4N-H	124.010	8.435
T5N-H	118.481	8.074
F6N-H	124.968	8.586
E7N-H	118.894	8.364
F8N-H	120.884	8.120

Search for: 0 / 190 (190)

Update Save... Options... Close Help

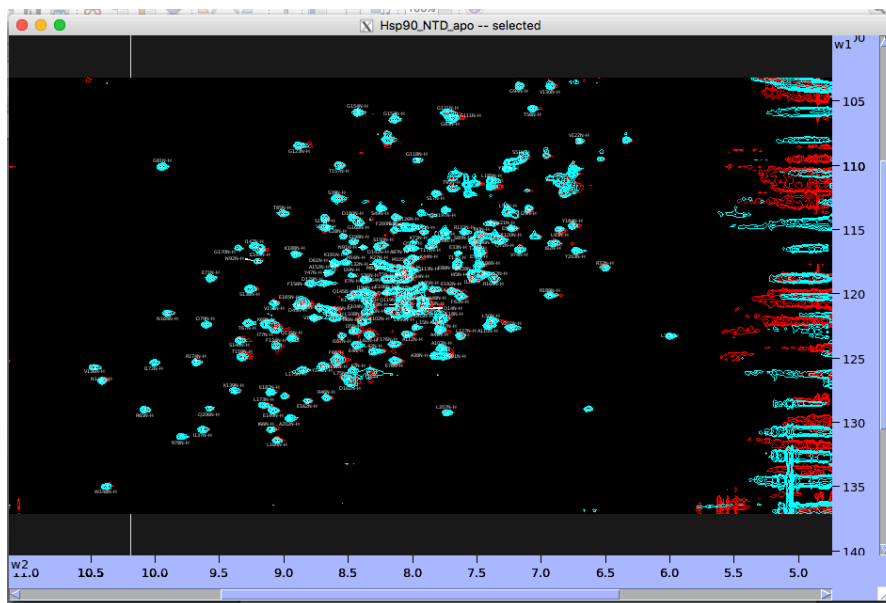
Now, let's overlay the DMSO control spectrum to determine if there are chemical shift perturbations.

10. Open (**fo**) the DMSO control spectrum and adjust the contour level (**ct**) as you did for the NTD alone spectrum in (3). Also, change the color of the spectrum to something that will contrast with the apo spectrum (cyan in this tutorial).



11. Type **ol** and the spectra overlay window will appear. Change the **From View** to the DMSO spectrum and the **Onto View** to the apo. Click **add**.

12. Navigate to the apo spectrum and you should see both spectra. Take a moment to look for any chemical shift changes. There shouldn't be any large perturbations but there will likely be a few small changes. Since most of the compounds were



NMRFAM-SPARKY Quick Tutorial

Macro Methods

dissolved in DMSO, we want to monitor for changes that are more significant than DMSO alone. With that in mind, return to the DMSO spectrum and read in the peak list you saved earlier (**rp**). As you did for the wild-type spectrum, take some time to select and center peaks (**pc**) that are clearly off center.

13. Once you are finished, type **lt** and the peak table for the DMSO spectrum will appear. Save it as a new peak list and you can use this file as a reference when observing spectra that contain small molecules in DMSO from the screen.

At this point, you can examine the other spectra that contain small molecules and begin assessing where they may bind on the NTD. In the coming weeks, you will quantitate changes in the spectra and be able to display these changes on the crystal structure of the NTD.