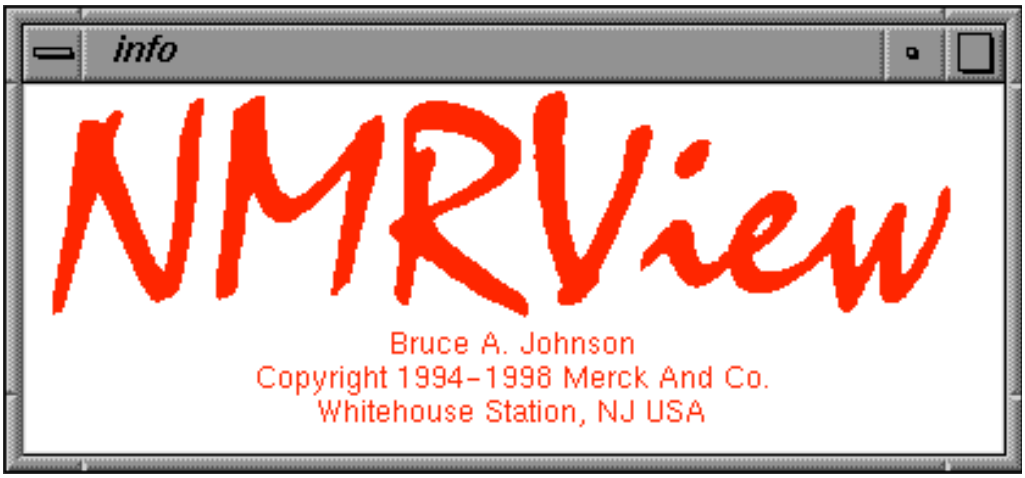



# NMR Assignments using NMRView I: Introduction to NMRView

In your home directory, you should have a subdirectory called "nmrview". The contents of that directory should be as shown below (subdirectories are followed by a / ):

```
8190.dba            jump.tcl          nmrview1-mac.pdf   nvtcl/      pl198t.nv
acc_c_average.pdb   keys.tcl          nmrview_2labs.tar  pl181t.nv   pl206t.nv
hsqc.tcl            MYDatabase5.str   nmrview2-mac.pdf   pl185t.nv
```

THE FOLLOWING ARE VERY IMPORTANT!

-in the file **hsqc.tcl** in the nmrview directory, there should be several (4 or 5) pointers to the nmrview subdirectory in your home directory. You need to change these to point to YOUR nmrview subdirectory in YOUR home directory. For instance, on the LINUX computers, this will be something like "/home/your-user-name/nmrview" where "your-user-name" is your user name.

-do the same for the single (1) occurrence in the file **MYDatabase5.str** in the nmrview directory (the variable _Template_filename should be "/home/your-user-name/nmrview")

-finally, you should "cd" into your nmrview directory

-you are now ready to begin

## Start the Program

-start the program

-on Mac computers, simply click on the *NMRView* icon in the Dock

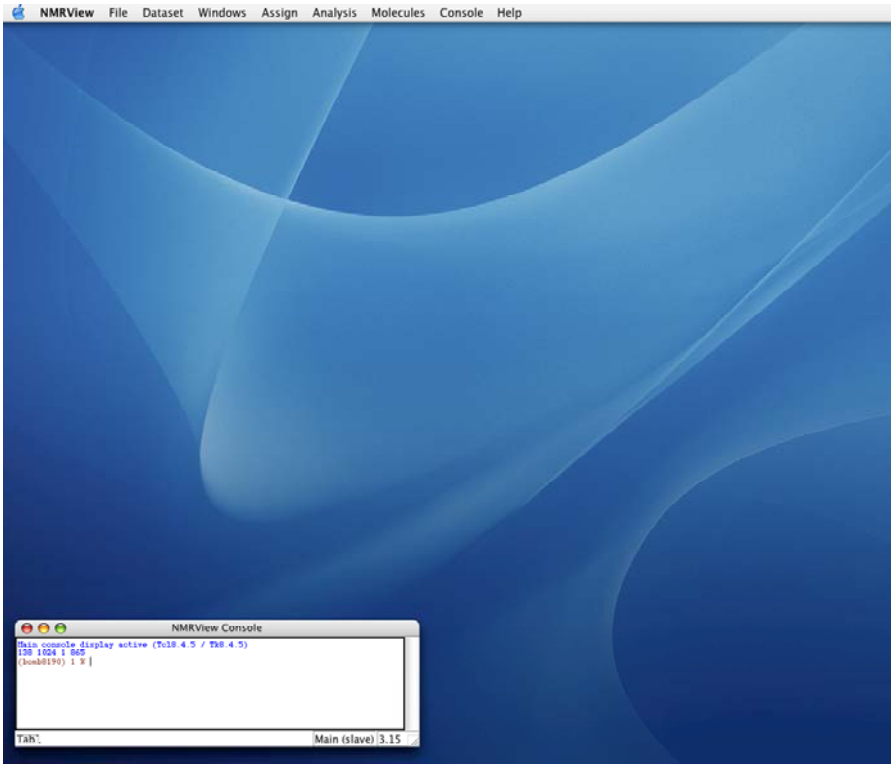
-on LINUX computers, open a terminal window, cd into your nmrview directory, and type "nv5" (no quotes, followed by return of course)

You should see 3 windows/menus appear:

1). The first is the **"Info" window**, and says *NMRView* in big red letters. This window will disappear in a few seconds

2). The second is the **"main NMRView" menu**, and will appear at the top of your screen.
-on Mac computers, this is as shown below
-on LINUX, there will be a separate, movable window that appears at the top of the screen

3). The third is the **"NMRView Console" window**, and will appear at the bottom of your screen. You can enter commands in the console window command line.




## Help

-in the **"main NMRView" menu**, choose **Help**, then **User's Manual (Internal Viewer)**

-go ahead and browse through some of the options - you should find lots of information in the manual that will be of use to you later on as you become more familiar with the software

-choose **Close** to exit the User's Manual

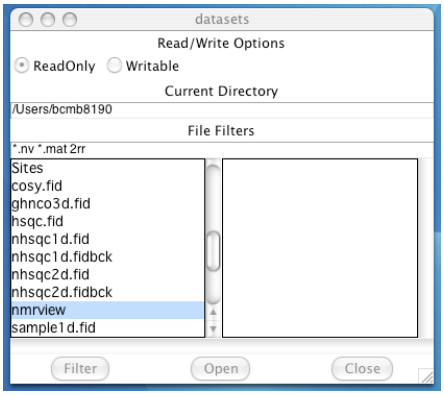
## Exercise 1: 2D $^{1}H$, $^{15}N$-HSQC

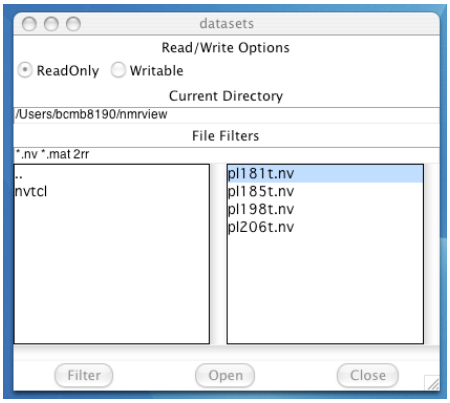
The spectra of a short peptide (24 residues) bound to RNA will serve to demonstrate NMRView. The sequence of the peptide is:

```
-2 -1  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22
 g  s  M  D  A  Q  T  R  R  R  E  R  R  A  E  K  Q  A  Q  W  K  A  A  N
```

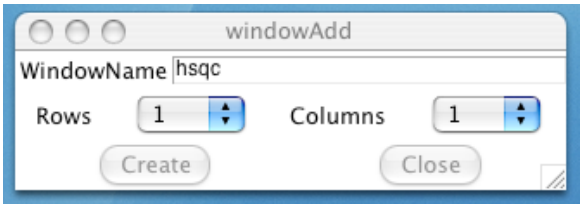
First, we will simply look at the $^{1}H$, $^{15}N$-HSQC spectrum, and get used to some of the features of NMRView.

-in the **"main NMRView" menu**, choose **Dataset**, then **Open Datasets**. A new window will appear called **"datasets"**. Select the file named pl181t.nv (this file is in your nmrview directory), then click **Open**.




-in the **"main NMRView" menu**, choose **Windows**, then **Add**. A new small window will appear, in which you are asked to name the new window. Type **hsqc** (lower case) in the box. Then click the **Create** button.

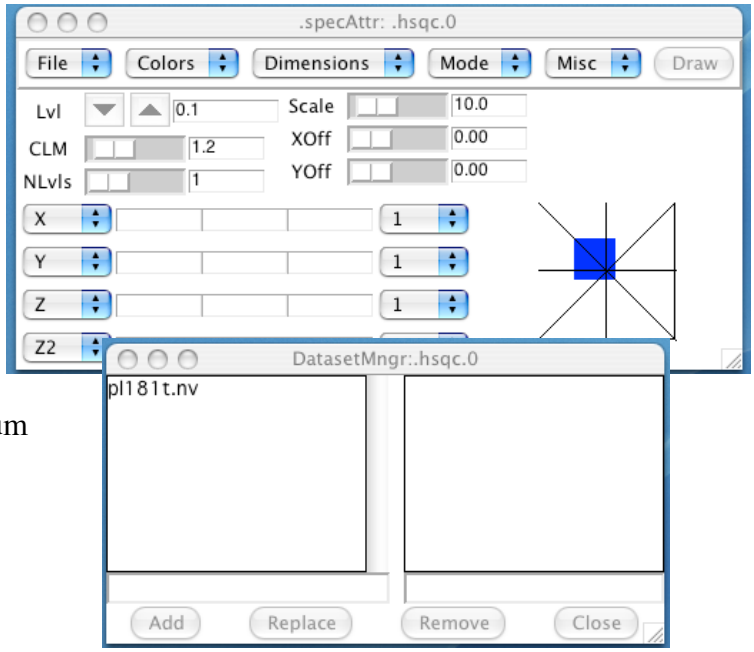


-a new window will appear called "**hsqc"**
-put the cursor in this window and click the **RIGHT** mouse button. A new menu should appear called the **Attributes menu**. *-on Mac computers, if this does not work, click the left mouse button while holding down the command (apple) key.*

Use the **LEFT** mouse button and click on **Attributes**. A new window will appear called **".specAttr: .hsqc.0"**.


***NOTE: For Mac computers, in the rest of the tutorial, commands using the RIGHT mouse button may not work. Instead, use the left mouse button while holding down the command (apple) key.***

-in **".specAttr" window**, choose **File**, then **Dataset**. Yet another new window will appear (**"DatasetMngr"**) that will show you what datasets are open. You should only see one entry in the window (pl181t.nv). Click on this filename to highlight it (select it), and then click the **Add** button. Click **Close** to close this window.
-in the **".specAttr" window** click the **Draw** button. You should see the spectrum appear in the **"hsqc" window**.



***Spectrum attributes***

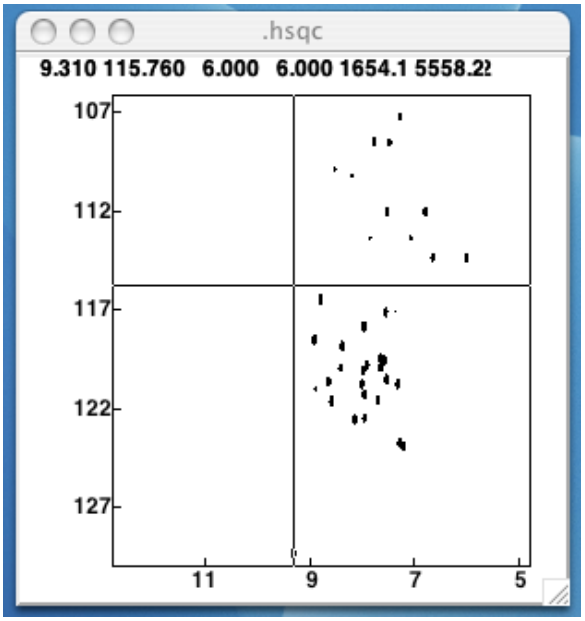
-you can adjust many aspects of the spectral display using the **".specAttr" window**. After you make an adjustment using one of the menu items, one of the slider bars, or one of the arrows, you have to click **Draw** for the change to take affect

-from the **"specAttr" window** menu, choose **Colors**, then **Positive** then **Blue**, and the peaks in the spectrum turn blue

-change the contour level using the **Lvl** arrows (click the down arrow 6 or 7 times and then click **Draw**)

-change **NLvls** to 1 using the sliding bar, then click **Draw**

-**CLM** (Contour Level Multiplier) changes the distance between the contours that make a peak. You can try changing that to change the appearance of a peak composed of more than 1 contour (remember to click **Draw** after you make changes)




-you can adjust what region of the spectrum is displayed by changing the values in the X and Y boxes corresponding to the displayed chemical shift ranges. A better way is to use the LEFT mouse button to adjust a pair of black lines in the display to form the lower left-hand corner of a box, and the MIDDLE mouse button to form the upper-right corner of the box. Then, with the cursor in the **"hsqc" window**, click the RIGHT mouse button to show the **Attributes menu**, then click **View** and then **Expand**. This will zoom to the selected region. RIGHT click, then **View**, and then **Full** will return to the full spectrum.

***Note: Again, the middle and right mouse button commands may not work on the Mac. In order to zoom on a region, you may have to enter the values in the X and Y boxes.***

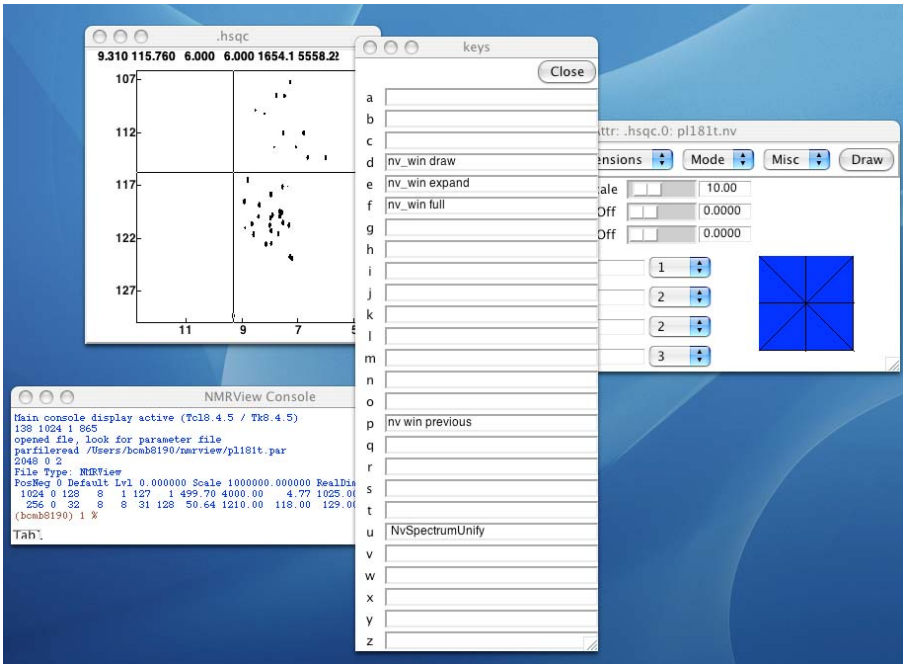
-you can scroll through 1D "slices" of the spectrum by placing the cursor in the **"hsqc" window**, RIGHT click, then **Slice** and then **X**. Using the LEFT mouse button, grab the horizontal black line and drag it vertically on the spectrum. A 1D slice (spectrum) through the data at the position of the black line will show up on the screen. You can also do this with **Y**. You can adjust how and where the 1D spectrum is displayed using **Scale**, **XOff**, and **YOff**.

***Defining Keyboard Shortcut Keys***

-you can define keyboard keys to perform some tasks such as zooming in (expanding) and drawing, rather than using the menus and mouse clicks

-put the cursor in the **"hsqc" window**, RIGHT click to show the **Attributes menu**, then click **Misc** and then **Key Binding**

-a new window called **keys** will appear. There are 26 lines in the window, each with a corresponding letter of the alphabet (keyboard) labeling it. In lines e, f, d, and p in the window, enter the following (do not include the quotes, and ***make sure press ENTER at the end of each line:***




e line **nv_win expand**
f line **nv_win full**
d line **nv_win draw**
p line **nv_win previous**

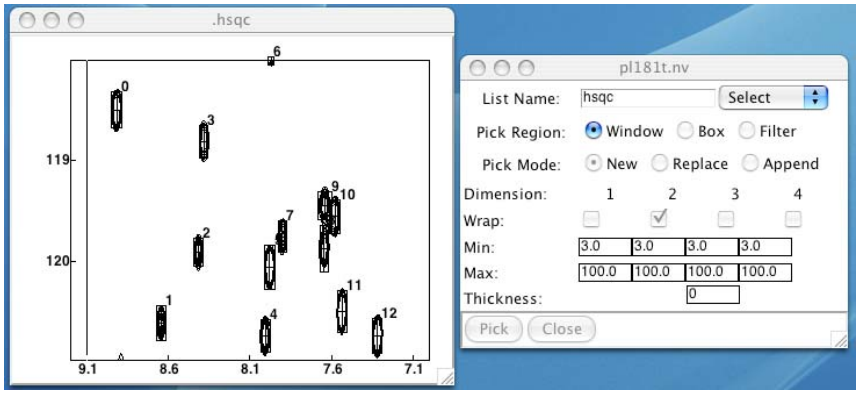
-close the **"keys" window**

-now, with the cursor in the **"hsqc" window**, typing **e** will zoom in on a selected area, typing **f** will display the full spectrum, typing **d** will draw the spectrum (no need to click **Draw** anymore!), etc. You can do this with most any command.

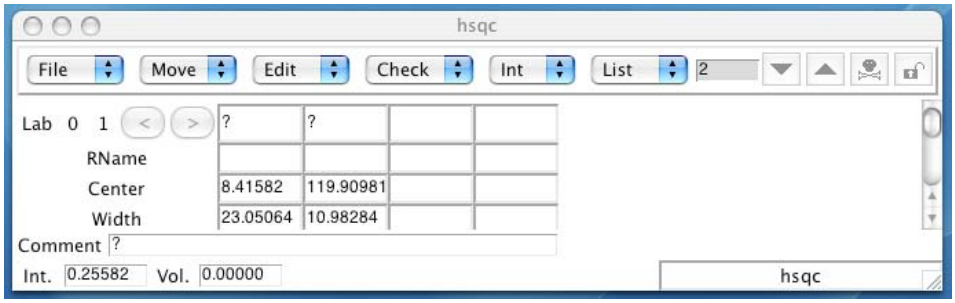
***Picking Peaks***

-select a region of the spectrum where there are 8 or 9 peaks and expand that region

-once you have expanded the region and have 8 or 9 peaks displayed, put the cursor in the **"hsqc" window**, RIGHT click to show the **Attributes menu,** then **Peak**, then **Pick**. Yet another new window will appear. In this new window, in the **List Name** box, enter **hsqc** (this will be the name of our list of peaks). In this window, make sure that **Window** is selected for **Pick Region**, make sure that **New** is selected for **Pick Mode**, and for **Wrap**, make sure that **2** is selected. Then click **Pick**. In the **"hsqc" window**, you'll see small boxes with crosshairs placed on the peaks, one for each peak (hopefully).




-from the **"main NMRView" menu**, select **Assign** then **Peaks**. Yet another new window will appear called **"peakPanel"**. From the **List** menu in this window, select **hsqc**. ***Note: if you try to resize this window, some of the functionality of the window MIGHT be lost. If so, close the window and begin again and do not resize.***




-you can look at the data for the different picks by clicking the up and down arrows. This will scroll through the data for all of the picks one at a time

-you can name (assign) a peak by replacing the question marks in the first line with appropriate names (like 20[H], 20[HN] for the amide H and amide N of residue 20). We don't need to do this now because we aren't ready to assign any of our data.

-you can delete a pick as follows: If you have, for instance, 9 picks, and you want to delete pick #3, using the arrows scroll through to number 3. Then click (firmly) the **skull and crossbones icon**. When you move the cursor away from the skull and crossbones, it should turn red. If you click **Draw**, the boxes on the peaks will turn red also. To permanently remove the pick from the list, in this window under **Edit** select **Compress**.

***Scripts***

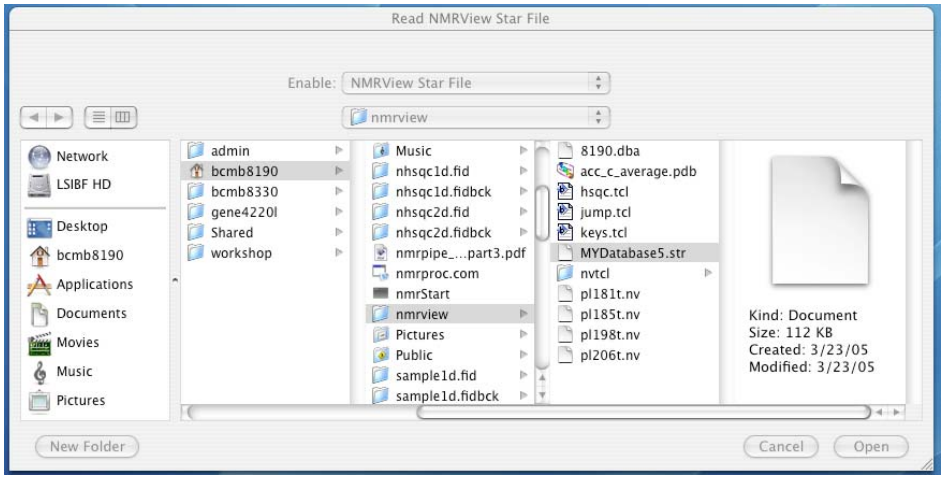
-scripts offer an alternative route for performing repetitive tasks or automating certain tasks.
-exit the program by typing **exit** in the **"NMRView Console" window**
-start the program again
-in the **"NMRView Console" window**, type **cd nmrview** (return), **source hsqc.tcl** (return), and then type **hsqc** (return)
-the hsqc spectrum should appear in a new window, etc. If you look at the file "hsqc.tcl" in your directory, you'll see that the first part of the file ("proc hsqc") is a set of commands to open the display window, display the hsqc spectrum, etc. You'll also notice that several commands to define keyboard shortcuts are also in there. This type of script or macro can be very useful (we'll see more later)

**Exercise 2: 3D datasets for sequential resonance assignments**

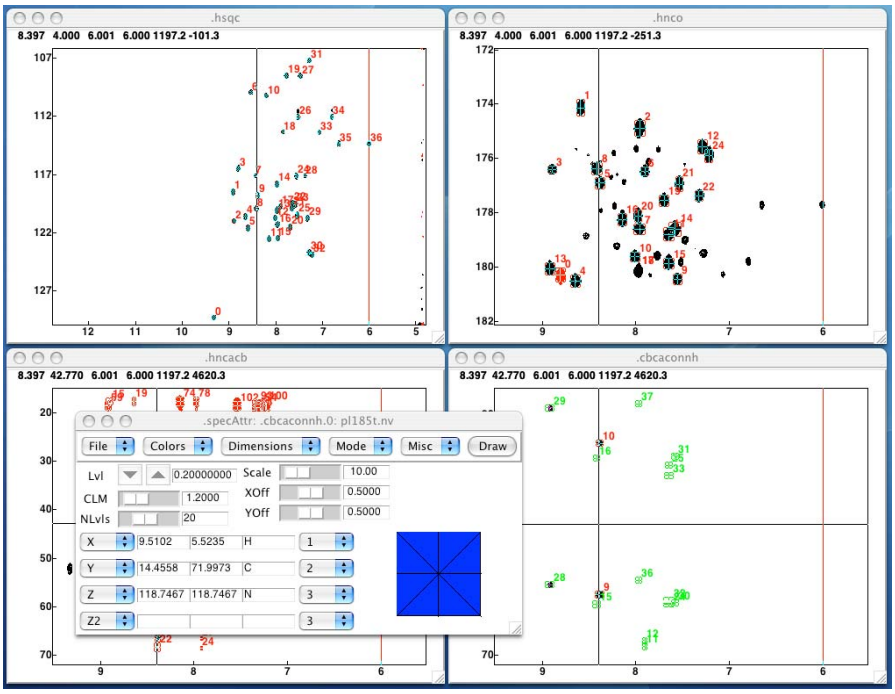
We will take a quick look at some 3D datasets that we will use next time
for demonstrating resonance assignments

-exit the program by typing exit in the **"NMRView Console" window**
-start the program again
-in the **"main NMRView" menu**, select **File**, then **Read Star File**. A new window will appear called **"ReadNMRView Star File" window**. In this window, select the star file (database) called **MYDatabase5.str** and then click **Open**
-another new window will appear. Close this window.

-the window shown below only applies to Mac computers



-in the **"NMRView Console" window**, type **cd nmrview** (return) **source hsqc.tcl** (return), then **seqass** (return), then **source jump.tcl** (return)
-4 new windows should appear: **"hsqc"**, **"hnco"**, **"hncacb"**, and **"cbcaconh"**




-put the cursor in the **"cbcaconh"** window. Right click to get the **"Attributes" menu**, then click on **Attributes** to get the **".specAttr" menu**. Put the cursor in the **"cbcaconh" window** and use the up arrow (↑) *on the keyboard* to scroll through the planes ($^{15}$N) of the cbcaconh spectrum. ***If this doesn't work, click on the 'Z' button and try again.*** You can monitor the $^{15}$N chemical shift of the plane by looking at "**Z**" in the **".specAttr" window**. Change "**Z**" to 118.74. Remember, you'll have to click **Draw** for this to take effect.
-using the down **Lvl** arrow on the **".specAttr" window**, change the contour level (remember to click **Draw** to see the changes) so that you can see the data (peaks)
-in the **"main NMRView" menu**, select **Analysis** (or **Assign**, depending on the program version) then **Peaks** then **List** then **cbcaconh**. Scroll through the list to peak 10. Do the chemical shifts in the list correspond to those shown in the spectrum?
-you can put the cursor in the **"hncacb" window** to change the display of the hncacb spectrum and the peaks in it (click inside the window), and you can do the same for the hnco spectrum in the **"hnco" window**
-in the cbcaconh spectrum, the $^{1}$H and $^{15}$N chemical shifts of peaks/picks 9 and 10 correspond to Threonine 5 in the peptide. The $^{13}$C chemical shifts (57.4, 25.9) correspond to what nuclei in what amino acid? What is the chemical shift for the carbonyl carbon of Glutamine 4?