

NMR Assignments using NMRView II: Sequential Assignments

DO THE FOLLOWING, IF YOU HAVE NOT ALREADY DONE SO:

Check 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	

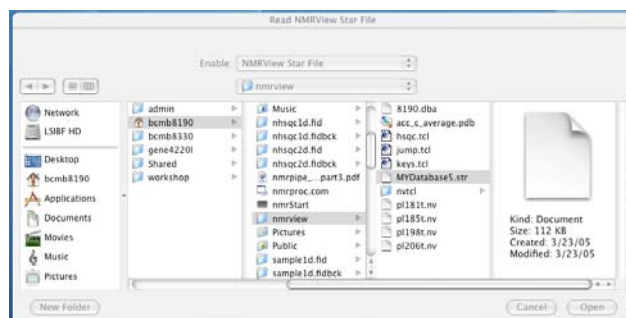
THE FOLLOWING ARE VERY IMPORTANT!

- 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

Getting Started / Review

- 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)

The window below is for Mac OS X

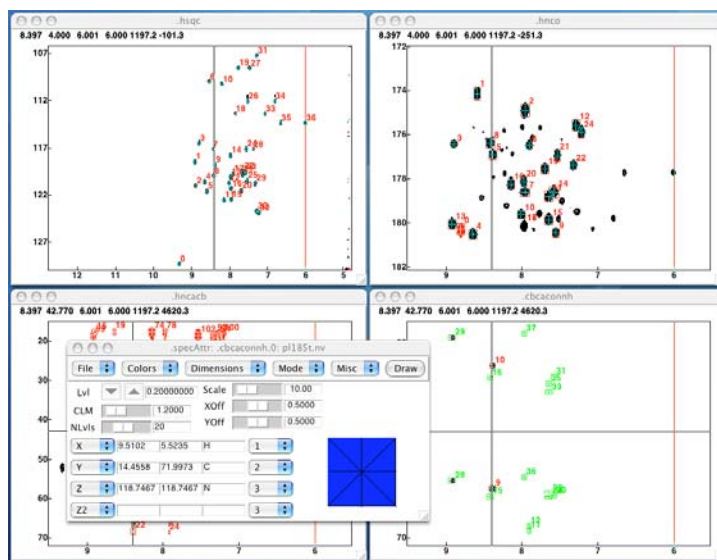


- in the “**main NMRView**” menu, select **File**, then **Read Star File**. A new window will appear called “**ReadNMRView Star File**” window. In this window, in the **nmrview** directory, select the star file (database) called **MYDatabase5.str** and then click **Open**
- another new window will appear. Close this window.

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 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 (¹⁵N) of the cbcaconh spectrum. You can monitor the ¹⁵N chemical shift of the plane by looking at “**Z**” in the “**specAttr**” window. Change “**Z**” to 118.74.



- 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 **Assign** 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

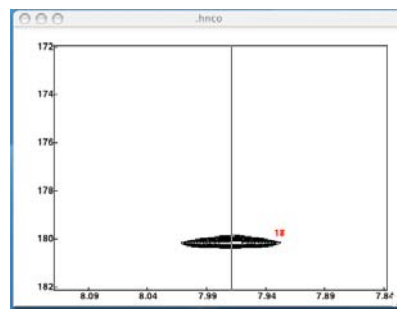
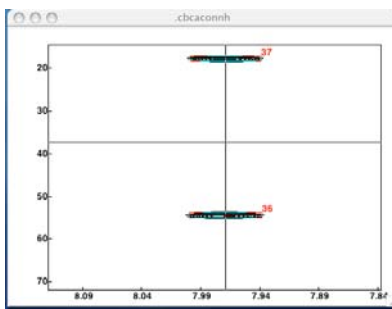
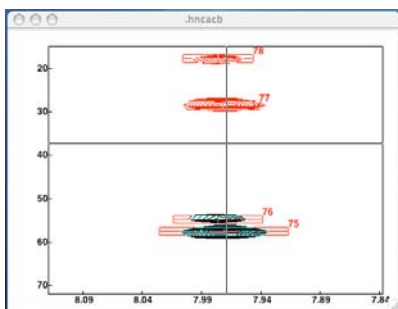
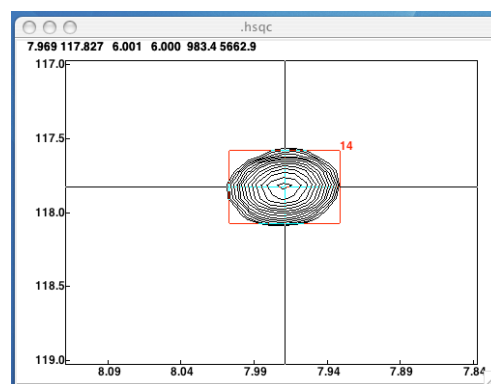
Exercise 1: Sequential assignments of a short peptide (bound to RNA)

The spectra of a short peptide (24 residues) bound to RNA will serve to demonstrate the process of sequential assignments using NMRView. The peptide sequence 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

The spectra in the 4 windows, the **“hsqc” window**, the **“hnco” window**, the **“hncacb” window**, and the **“cbcaconh” window** are of this peptide, and correspond to the 2D ^1H , ^{15}N -HSQC spectrum, the 3D HNCO spectrum, the 3D HNCACB spectrum, and the 3D CBCA(CO)NH spectrum, respectively.

-during the last lab you learned how to use the cursor to make a box around a small region in a spectrum and expand that region. In the hsqc spectrum, expand the region around peak #14. Now, place the crosshair cursor directly on the center of the peak. The center is about 7.97 ppm (^1H) and 117.8 ppm (^{15}N). Now press **j** on the keyboard (stands for ‘jump’: look at the macro ‘jump.tcl’). This command will select the ^{15}N plane in each of the 3D spectra closest to 117.8 ppm (as it turns out, this is exactly 118.00 ppm), and it will place the vertical cursor at exactly 7.97 ppm.



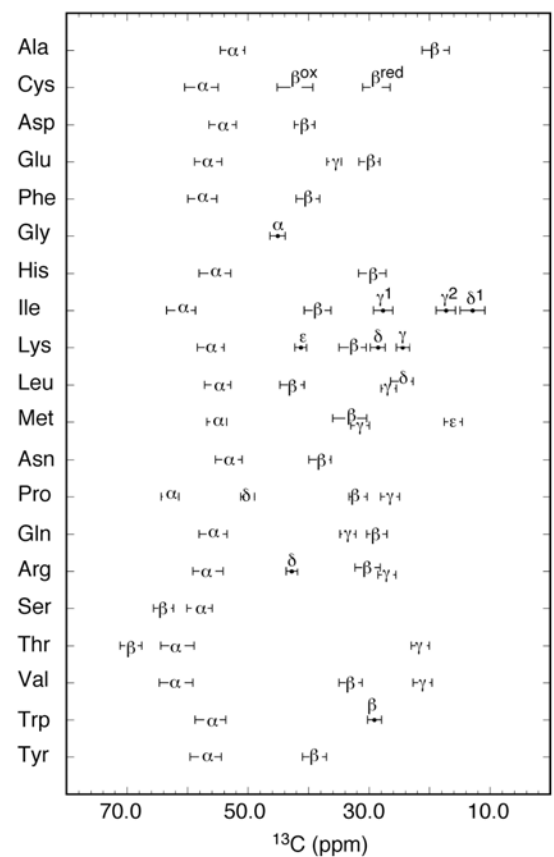
Look at the CBCA(CO)NH spectrum. With the ^{15}N plane set to 118.00 ppm (you can see this in the **“specAttr” window**), the boxes around the peaks should be red in color. Using the \uparrow and \downarrow arrows on the keyboard, change the ^{15}N plane. You’ll see the red color change to a different color. When the boxes are red, it indicates that the center of the box in that dimension (in this case, in the ^{15}N dimension) is nearest the center of the box. If the box is properly centered on the peak, the red color should indicate that the peak has its maximum intensity in that dimension on that plane (i.e. it is approximately centered at the chemical shift of the plane).

From the CBCA(CO)NH spectrum, one finds that the chemical shifts for $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ of the previous residue ($^{13}\text{C}^\alpha_{i-1}$ and $^{13}\text{C}^\beta_{i-1}$) in the sequence are 54.43 and 17.70 ppm respectively. From the HNCACB spectrum, one then finds that the chemical shifts for $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ of the same residue are 57.50 and 28.51 ppm. The HNCO spectrum tells us that the chemical shift of the carbonyl carbon of the previous residue ($^{13}\text{C}'_{i-1}$) is 180.16 ppm.

	#14
$^1\text{H}^{\text{N}}$	= 7.97
^{15}N	= 117.83
$^{13}\text{C}^\alpha$	= 57.50
$^{13}\text{C}^\beta$	= 28.51
$^{13}\text{C}^\alpha_{i-1}$	= 54.43
$^{13}\text{C}^\beta_{i-1}$	= 17.70
$^{13}\text{C}'_{i-1}$	= 180.16

Considering chemical shift information

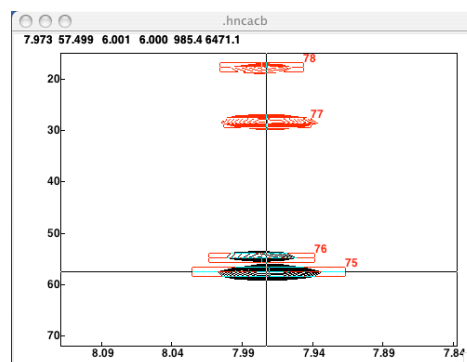
Chemical shift information can be useful at all stages of most all assignment protocols. We know from the CBCA(CO)NH spectrum that the chemical shift of the beta carbon ($^{13}\text{C}^\beta$) of the previous residue in our sequence ($^{13}\text{C}^\beta_{i-1}$) is 17.70 ppm. From a chart of ^{13}C chemical shifts in proteins (right), we see that this residue must be an alanine (Ala). From the sequence of our protein (peptide), we see that there are several Ala residues (Ala3, Ala12, Ala16, Ala20, and Ala21). The beta carbon chemical shift of residue i ($^{13}\text{C}^\beta_i$) is 28.51, indicating this residue is either Cys, Glu, His, Lys, Met, Pro, Gln, Arg, or Trp. There are no Cys, His or Pro residues in our peptide, and of the remaining possibilities, only Gln and Glu follow Ala in the sequence. So, residues $i-1$ and i in our sequence are either Ala3-Gln4, Ala12-Glu13, or Ala16-Gln17.



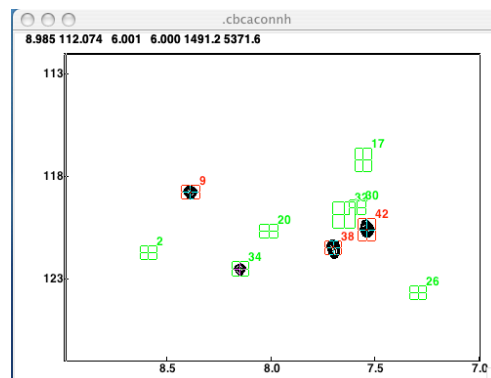
Connecting to residue $i+1$

From the HNCACB spectrum, we know that the ^{13}C chemical shifts for the alpha and beta carbons of residue i ($^{13}\text{C}^\alpha_i$ and $^{13}\text{C}^\beta_i$) are 57.50 and 28.51 ppm respectively. We know, therefore, that in the CBCA(CO)NH spectrum (and in the HNCACB spectrum) residue $i+1$ will show two peaks with ^{13}C chemical shifts of 57.50 and 28.51 ppm, corresponding to the chemical shifts of residue i . In order to find residue $i+1$, because our protein (peptide) is short, we could search through all of our data (using the HSQC spectrum and the procedure outlined above using the 'jump' command) to find these peaks in the CBCA(CO)NH (and HNCACB) spectrum. Alternatively, we can look specifically for peaks in the 3D CBCA(CO)NH (and HNCACB) spectrum with the correct chemical shifts directly. We'll use this latter method.

-in the **"hncacb"** window, place the black crosshairs directly on the center of the peak with the ^{13}C chemical shift of 57.50 ppm ($^{13}\text{C}^\alpha_i$). Press **n** on the keyboard (for 'next'). This command will cause the display in the **"cbcaconh"** window to change: displayed will be a slice through the 3D spectrum at a ^{13}C chemical shift of ~57.50 ppm (the program will pick the plane with the chemical shift closest to this: in this case, it is 57.4986 ppm). The x axis will remain $^1\text{H}^\text{N}$, but the y axis now will be ^{15}N .



-in the **"cbcaconh"** window you'll see three peaks with red boxes, indicating that for these peaks their maximum intensities are closer to the chemical shift of this plane than to any other (peaks #9, #38, and #42). We should also check for red boxes on adjacent planes (to allow for digital resolution issues and poor placement of boxes around peaks). So, using the \uparrow and \downarrow arrows on the keyboard, first move one plane \downarrow (57.9517 ppm). You'll find one peak with a red box here (#34). Then move one plane \uparrow (57.0455 ppm). You'll find no additional peaks here with red boxes on this plane. In the **"main NMRView"** menu, select **Assign**, then **Peaks** then **List**, and then select **cbcaconh**. You can scroll through the list until you find peaks #9, #38, #42, and #34 and then you can write down a table of the chemical shifts of these peaks:



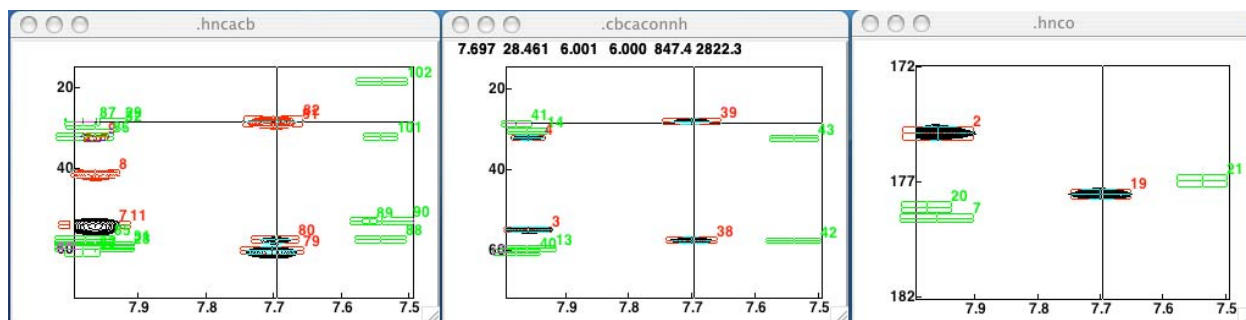
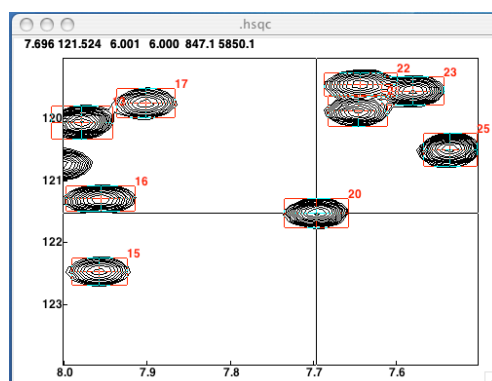
	$^1\text{H}^\text{N}$	^{15}N	$^{13}\text{C}^\alpha$
#9	8.39	118.76	57.41
#38	7.70	121.46	57.61
#42	7.54	120.59	57.63
#34	8.15	122.49	57.97

-now, we want to repeat the above procedure with the ^{13}C chemical shift of the beta carbon of residue i . So, in the **“hncacb” window**, place the black crosshairs directly on the center of the peak with the ^{13}C chemical shift of 28.51 ppm ($^{13}\text{C}^\beta$). Then, as before, press **n** on the keyboard. Continue as before and find the peaks in the CBCA(CO)NH spectrum with chemical shifts near to 28.51. You should find 5 of them as follows:

	$^1\text{H}^\text{N}$	^{15}N	$^{13}\text{C}^\beta$
#31	7.58	119.50	28.57
#41	7.98	120.12	28.54
#39	7.70	121.46	28.06
#16	8.42	119.88	28.98
#21	8.01	120.66	28.83

-you’ll notice that peak #38 and peak #39 have the same $^1\text{H}^\text{N}$ and ^{15}N shifts. This indicates that for this single $^1\text{H}^\text{N}$, ^{15}N pair, there are two ^{13}C correlations with $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ chemical shifts that match those that we are looking for. Therefore, peaks #38 and #39 tell us what the ^1H and ^{15}N shifts of residue $i+1$ are.

-so, now go back to the **“hsqc” window**. Find the peak with $^1\text{H}^\text{N}$ =7.70 ppm, and ^{15}N =121.46 ppm (peak #20). Expand the region around this peak, place the black crosshairs directly on the center of this peak, and press **j** on the keyboard as before. In the **“cbcaconh” window** you’ll see peaks #38 and #39, and the corresponding peaks in the HNCACB spectrum in the **“hncacb” window**.



-from the HNCACB spectrum, the ^{13}C chemical shifts of residue $i+1$ are found to be ~ 60.2 ppm ($^{13}\text{C}^\alpha$) and ~ 28.7 ppm ($^{13}\text{C}^\beta$). Based on these values and the table of ^{13}C chemical shifts shown previously, and ignoring amino acid residues not in our peptide, the $^{13}\text{C}^\alpha$ shifts suggest that residue $i+1$ could be Tyr, Trp, Val, Thr, Ser, Ile or Arg, whereas the $^{13}\text{C}^\beta$ shifts suggest that residue $i+1$ could be Glu, Lys, Met, Gln, Arg, or Trp. Based on this, what do you think is the sequence of amino acids that correspond to residues $i-1$, i , and $i+1$?

	#14	#20
$^1\text{H}^\text{N}$	= 7.97	7.70
^{15}N	= 117.83	121.52
$^{13}\text{C}^\alpha$	= 57.50	60.22
$^{13}\text{C}^\beta$	= 28.51	28.92
$^{13}\text{C}^\alpha_{i-1}$	= 54.43	57.61
$^{13}\text{C}^\beta_{i-1}$	= 17.70	28.06
$^{13}\text{C}'_{i-1}$	= 180.16	177.55

-from the table above, you see that we have quite good agreement (± 0.11 ppm) between the value predicted for $^{13}\text{C}^\alpha$ for residue i from the HNCACB spectrum (corresponding to peak #14 in the HSQC spectrum) and from the CBCA(CO)NH spectrum (57.50 vs 57.61 ppm). However, this is not the case for $^{13}\text{C}^\beta$ (± 0.45 ppm). Why do you suppose this is?

-try to link as many amino acids in the sequence as you can, and try to decide what the identities of these amino acids are

Finale

Once the identities of the amino acids are determined, once can give the peaks names (“**main NMRView**” **menu**, select **Assign**, then **Peaks** then **List**, and then select **hsqc** or **hncacb** or whatever, then replace the ? in each column with the appropriate name). The **Assign** option also permits the user to display the “**seq**” **window** (select **Sequence**), the “**Assign Panel**” (select **Atoms**), and other useful tools for assigning and cataloguing information. The dataset for the small peptide that you have is a good one for exploring some of these options, especially if you plan on using this software in the future for more challenging assignments.