

Lab #8 Problem Set

This problem set is due on Lab #9. As usual, if you have any problem getting time on DRX300, logging-in, finding the sample, collecting data, processing data, analyzing data, or plotting data, please see either Mark Girvin or Sean Cahill. *Late reports will be marked 50% off.*

1. Turn in the 1D proton spectrum and the 2D ^1H - ^{13}C HSQC spectrum that was obtained on the peptide on DRX300 and identify each cross peak of the 2D spectrum (you do not need to assign the phenylalanine aromatic ring protons, just identify the region they appear in).
2. Turn in a Strips2 plot of the 3D HNCA & HN(CO)CA with the strips plotted in sequence order (G-F-L) and identify all of the the crosspeaks observed in each strip.
3. Turn in a Strips2 plot of the 3D CBCANH & CBCA(CO)NH with the strips plotted in sequence order (G-F-L) and identify all of the the crosspeaks observed in each strip.

Data Collection:

put the labeled peptide in the DRX300 at 25°C and tune and shim sample. (note: you will be running a 2D ^1H - ^{13}C HSQC on the sample so tune probe accordingly).

create a new dataset and run a 1D proton spectrum to make sure you see a reasonable spectrum

create a new dataset and run a 2D ^1H - ^{13}C HSQC spectrum using the macro “2DhcHSQC” (note: you will get optimal performance if you calibrate the ^1H 90 degree pulse on the sample and adjust the parameters accordingly). This macro will set up the experiment for you, but you must run (using “zg”) and process (using “xfb”) using separate commands.

Data Analysis:

The following NMRView datasets should be present in the GFL directory that you created in Lab#7:

2DhnHSQC_298K	a 2D ^1H - ^{15}N HSQC
2DhcHSQC_300K	a 2D ^1H - ^{13}C HSQC
3DhnNOESYHSQC_298K	a 300ms 3D ^{15}N NOESY-HSQC
3DhnTOCSYHSQC_298K	an 80ms 3D ^{15}N TOCSY-HSQC
3DHNCA_300K	a 3D HNCA spectrum
3DHNcoCA_300K	a 3D HN(CO)CA spectrum
3DCBCANH_300K	a 3D CBCANH spectrum
3DCBCAcoNH_300K	a 3D CBCA(CO)NH spectrum
3DHcCHCOSY_300K	a 3D H(C)CH-COSY spectrum
3DHcCHTOCSY_300K	a 3D H(C)CH-TOCSY spectrum

Analysis of HNCA and HN(CO)CA: Peak pick the 2D ^1H - ^{15}N HSQC and use the Strips2 function in NMRView (see Lab#7) to line up strips of the HN(CO)CA and HNCA for each crosspeak in the HSQC. The *C α* /*C α* -1 signals in the HNCA strip and the *C α* -1 signals in the HN(CO)CA strip can be used to sequentially assign each spin system.

Analysis of CBCANH and CBCA(CO)NH: Analysis can be done using the same procedure as used for HNCA/HN(CO)CA datasets except that both the *C α* and *C β* crosspeaks can be used to make sequential assignments – this is especially useful when sequential assignments cannot be made because of degeneracy of the *C α* chemical shifts. Furthermore, the chemical shifts of the *C α* and *C β* can be used to help “type” or make residue specific assignments for each crosspeak in the HSQC (see table of ^{13}C chemical shifts of each amino acid).

Analysis of HCCH-COSY and HCCH-TOCSY: Go to the *C α* and *C β* planes of a given spin system using the HCCH-COSY/HCCH-TOCSY datasets. The ^1H - ^1H correlations in these planes should enable you to assign all of the ^1H signals within the spin system. The 2D ^1H - ^{13}C HSQC or the “hi-

c” planes of the HCCH-COSY/HCCH-TOCSY can then be used to assign the ^{13}C signals based on the previously made ^1H assignments.

Plotting a Strips2 window: To make a plot of Strips2 window, do the following:

- Activate Strips2 window by clicking left mouse button over desired window.
- Click right mouse button within window and pull down to “Misc” -> “Plot”
- Within the plot window, setup with the following parameters:
 - X Pos 2
 - Y Pos 1
 - Width 8
 - Height 6
- Click on PlotAll button and a postscript file will be created in your current working directory on nmr0 (by default, it will be named “ps.dat”). NOTE:
- The file can be sent to the printer using the following command within a shell on nmr0 “lp ps.dat” (make sure you are in the directory containing the file to be plotted).