

Lab #7 Problem Set

This problem set is due on Lab #8. 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 - ^{15}N HSQC spectrum that was obtained on the peptide on DRX300 and label each cross peak of the 2D spectrum with residue type, sequence position and ^1H and ^{15}N chemical shifts.
2. Turn in plots of ^1H - ^1H planes from both the 3D ^{15}N TOCSY-HSQC and 3D ^{15}N NOESY-HSQC for each of the crosspeaks in the 2D ^1H - ^{15}N HSQC and identify the crosspeaks arising from HN protons and H α protons observed in each plot.

Experimental:

put the labeled peptide in the DRX300 at 25°C and tune and shim sample. (note: you will be running a 2D ^1H - ^{15}N 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 - ^{15}N HSQC spectrum using the macro “2DhnHSQC” (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:

Use the NMRView software to make assignments of the crosspeaks in the HSQC of the peptide. NMRView datasets are located in `nmr0:/disks/unmr0/data/class/GFL` (dataset naming is based on the experiment that was used). To view and analyze the datasets from your account, do the following (also see NMRView instructions in handout):

- login to `nmr0`
- make sure you are in your home directory by typing “`cd`”
- make a directory for the GFL NMRView files by typing “`mkdir GFL`”
- change into the new GFL directory by typing “`cd GFL`”
- copy the files from the class directory into your new GFL directory by typing “`cp ~class/GFL/* .`”
- start the NMRView software by typing “`nmrview`”

Opening Datasets:

The following NMRView datasets (in addition to several others that will be used in Lab#8) should be present in your directory:

2DhnHSQC_298K	a 2D ^1H - ^{15}N HSQC
3DhnNOESYHSQC_298K	a 300ms 3D ^{15}N NOESY-HSQC
3DhnTOCSYHSQC_298K	an 80ms 3D ^{15}N TOCSY-HSQC

- open up a dataset by clicking under the main menu on “Dataset”, click on “Open and Draw Dataset” then double click with left mouse button on the desired dataset name. Multiple files can be opened by double clicking on each dataset to be opened. Click on “Close” in the File menu when all datasets have been opened.
- Activate a spectral window by clicking on left hand mouse button within the desired window.

Activate the 2D ^1H - ^{15}N HSQC spectrum window by clicking on left hand mouse button within window. Use the black cursor line to determine the ^{15}N chemical shift of each crosspeak – this information will be used to view planes in the 3D datasets.

Examine ^1H - ^1H planes from the 3D ^{15}N TOCSY-HSQC at the appropriate ^{15}N chemical shift to characterize the spin system type of each crosspeak in the 2D ^1H - ^{15}N HSQC. Activate the 3D ^{15}N TOCSY-HSQC spectrum window by clicking on left hand mouse button within window. Click on the right hand mouse button and pull down to “Attributes”. Type in the desired ^{15}N chemical shift for the Z-dimension (n) fields then click on the “Draw” button to draw the TOCSY plane for that ^{15}N chemical shift position.

Examine ^1H - ^1H planes from the 3D ^{15}N NOESY-HSQC to identify the sequence position of each spin system characterized from the 3D ^{15}N TOCSY-HSQC (note: because the peptide is small and random coil, the $\text{HN}_i\text{-H}\alpha_i$ NOEs will be much smaller than the $\text{HN}_i\text{-H}\alpha_{i-1}$ NOEs for each residue and the $\text{HN}_i\text{-HN}_{i-1}$ NOEs are not observed). Activate the 3D ^{15}N NOESY-HSQC spectrum window by clicking on left hand mouse button within window. Click on the right hand mouse button and pull down to “Attributes”. Type in the desired ^{15}N chemical shift for the Z-dimension (n) fields then click on the “Draw” button to draw the NOESY plane for that ^{15}N chemical shift position.

Use the “Strips2” function (to be discussed in class and see below) to line up TOCSY and NOESY strips for each crosspeak in the 2D ^1H - ^{15}N HSQC.

Peak Picking: before using Strips2, the 2D ^1H - ^{15}N HSQC spectrum must be peak-picked as follows:

- Activate the 2D HSQC spectrum window by clicking on left hand mouse button within window. Now, click on right hand mouse button and pull down to “Peak” -> “Pick”
- A new peak window will appear. Type in a name for peak list (e.g. – hnhsqc).
- Click on “Pick” button to pick peaks within window (note: can click on Pick Region = Box button to pick peaks within region defined by black and red cursors).
- Picked peaks will be shown on spectrum – the attributes for peaks can be viewed and changed by holding down the right mouse button within spectral window and pull down to “Peak” -> “Peak Attributes”.

Using Dual Strips (Strips2):

- From main menu bar, click on “Windows” -> “Strip2”
 - Enter a window name (e.g. noesytoesy) and a new graphical Strips2 window will appear
 - Click on “Pars” button within Strips2 window and fill in information within table (click on down arrows for each entry to get a pulldown of choices). Dataset1 should be 3D NOESY-HSQC filename, Dataset2 should be 3D TOCSY-HSQC filename and Read1 and Read2 should contain HSQC peaklist name. Fill out bottom of table with following information:
 - MaxWindows 6
 - Dim X1 hn
 - Dim Z1 n
 - Dim X2 hn
 - Dim Z2 n
- Click on the “Accept” button and then click on “Close” button.
- Click on “All” button within Strips2 window to generate strips
 - Attributes of the strips can be changed using the “Attrib” button within the Strips2 window.

Plotting Data:

To make a plot of a plane in a 3D spectrum or of the dual strips (Strips2) window, do the following:

- Activate desired window by clicking left mouse button over 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 Plot button (for strips, 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”)
- 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).