

# Structure Calculation Lab 03/14/07

Lab Problem Set #6: Due at next lab 03/21/07

1. Identify all NOEs from the amide protons of Lys4 and Met21, record their maximal intensity, and estimate upper limits for distance restraints.
2. Complete the distance constraint file for the structure calculations using your new constraints.
3. Calculate initial structures for the peptide using DYANA.
4. Examine and evaluate the initial structures using MOLMOL.
5. Calculate complete structures using additional restraints
6. Evaluate the structures
7. *OPTIONAL* Compare to the structure of the peptide in a detergent micelle (from the PDB).

## 1. Identify NOEs from the NH protons of Lys4 and Met21 to all H<sup>N</sup>, H<sup>α</sup>, and H<sup>β</sup>

A number of files will be needed and will be created during this lab. Make a new directory to hold them. Open a UNIX shell. Login to "nmr0". Make a new directory by typing: "mkdir lab6". Change to that directory by typing: "cd lab6". Copy all the necessary files to your new directory by typing: "cp ~class/lab6/\* ." - don't forget the final period.

Use the NOESY with the 300msec mixing time in nmrvview. Within nmrvview, use the two cursors to isolate the NOE cross-peak that you are interested in, and type "getval" in the NMRView "Console" window. The cursor limits, and peak statistics will be displayed there:

```
center  2.1402 jitter   3.3179 min    -0.1002 max   3.3179 <== this is what you want
extreme 3.3179 volume 46.3839 evolume 0.0000 mean  0.2811  sdev    0.5862
```

For each of the two NH protons use the assignment table (sorted by chemical shifts) and:

- Examine the H<sup>N</sup>-H<sup>N</sup> region (7.6-8.8ppm x 7.6-8.8ppm) for all H<sup>N</sup>-H<sup>N</sup> NOEs involving that proton - on both sides of the diagonal.
- Examine the H<sup>N</sup>-H<sup>α</sup> region (7.6-8.8ppm x 3.5-5.0ppm AND 3.5-5.0ppm x 7.6-8.8ppm, *i.e.* on both sides of the diagonal, especially if you are unsure of a cross peak) for all H<sup>N</sup>-H<sup>α</sup> NOEs involving that proton.
- Examine the H<sup>N</sup>-H<sup>β</sup> region (7.6-8.8ppm x 1.2-4.2ppm AND 1.2-4.2ppm x 7.6-8.8ppm, *i.e.* on both sides of the diagonal, especially if you are unsure of a cross peak) for all H<sup>N</sup>-H<sup>β</sup> NOEs involving that proton.
- Measure and record the *intensity* of each of these NOE cross peaks.
- Estimate an upper distance limit (in Å) for each NOE:

Intensity > 6, set the upper limit to 2.8

Intensity < 6 and > 1.5 set the upper limit to 3.5

Intensity < 1.5, set the upper limit to 5.0

Create a table of your NOEs, as shown below (using Leu 6 as an example):

Res#	AA	ATOM	Res#	AA	ATOM	NOE Intensity	Upper Distance Limit
6	LEU	HN	7	HIS	HN	4.3	3.5
6	LEU	HN	3	GLY	HA1	0.62	5.0
... etc							

Hand-written is fine. Use the atom names from the assignment table. The letter "Q" means a pseudo atom representing more than one proton, when chemical shifts are degenerate. For example, if the two H<sup>β</sup> of a Leu have the same chemical shift, one would use "QB" as the atom name. If you cannot say which of two or more protons is giving rise to the NOE, note both down in a separate table, as possible assignments.

## **2. Use your NOEs to complete the distance restraint list for the structure calculations.**

In your "lab6" directory, edit the file called "mag.upl" to add your new distance restraints for the H<sup>N</sup> of Lys4 and Met21. You can use the *vi* editor if you know it ("vi mag.upl"), or the program "jot" if you don't (type "jot", then use the menu command to open the mag.upl file).

When you've got the file open, go down to the entries for Lys4; they'll look like:

```
"4 LYS HA 5 PHE HN 3.58".
```

This is the same as your table, leaving out the intensity.

Enter your data for 4 LYS, trying to maintain the same spacing between entries, capitalization, and number formats that are used for the other entries.

Then scroll down to 21 MET, and enter your restraints for that HN as well.

Save the file under the same name (mag.upl). Now you're ready to:

## **3. Calculate initial structures of the peptide using DYANA.**

Make sure you are in your "lab6" directory. Start DYANA by typing "dyana1.5". You should see a message and the dyana command prompt "dyana>". Execute the following commands in dyana - not the comments in the second column.

<b>Command</b>	<b>What it does</b>
dyanalib	loads the library describing amino acids and nucleic acids
read seq mag	loads the peptide sequence, from the file "mag.seq"
read upl mag	reads the upper distance limit constraint file
seed=163742	or your favorite 6-digit number, as a random number seed
calc_all 15	Calculates 30 structures from random starting conformation
struct sort	sorts the structures according to target function value (fit to data)
struct list	lists statistics on all 30 structures
struct sel 1..10	select the best 10 structures
write_all pdb	saves the best structures as pdb files
name=init_mag	
struct viol	prints the distance and angle restraint violations => note and report NOE violations > 0.2 Å
quit	exit the program

Congratulations, you've just calculated your NMR structures. Now, take a look at them.

#### 4. Examine the initial structures.

You'll use the molecular graphics program MolMol. Start the program with the structures loaded by typing "molmol init\_mag\*.pdb".

Align the 10 models by selecting the backbone atoms, and superimposing them to minimize the RMS deviations between the selected atoms by typing:

```
SelectAtom "bb"
```

```
Fit to_first
```

(*type slowly and watch the command line as you type*; MOLMOL fills in parts of the commands as soon as it recognizes them)

- Note *and report* the RMSD value reported on the status line.

Display side chains with the following commands:

```
SelectBond "heavysc"
```

```
StyleBond line
```

```
ColorBond 0.0 1.0 0.0
```

 (or your favorite combo of red, green, and blue (0.0 - 1.0 ea.)

Now find the best fit and RMSDs for all non-hydrogens by typing:

```
SelectAtom "heavy"
```

```
Fit to_first
```

- Also note *and report* the RMSD value reported on the status line.
- Finally, make a plot of the models. Arrange the model to get a view you like, using the mouse to rotate the models by clicking and dragging in a blank region of the background. Then type:

PlotPS mag\_initial.ps

Exit the program from the File->Quit menu option.

Print out your figure by typing "lp mag\_initial.ps" at the UNIX command prompt.

*Note:* much can be done in MolMol with the menus and the buttons, and you can write your own macros. For example, to superimpose the structures initially you could first click on the "bb" button on the side panel, then use the Edit->Fit menu sequence to do the alignment. Help info is available from the help menu, and via an HTML manual.

## **5. Calculate complete structures using additional constraints**

Repeat the structure calculation using additional constraints.

If you can now identify any of your previously ambiguous NOEs (only one of the protons at the given chemical shift is within 5Å of the HN (of K4 or M21) in the initial models, add those distance constraints to the mag.upl file as you did earlier.

To measure a distance in MOLMOL:

1. Read in just one structure ("molmol init\_mag001.pdb")
2. Select the two atoms you'd like the distance between ("SelectAtom ":6@HN, :9@HN" for the distance between the HN's of residues 6 & 9), then 3) type AddDist.

We also have some hydrogen bond and dihedral angle constraints to use as additional data. Once you've added any NOE constraints to the mag.upl file, start dyana again by typing "dyana1.5". Then enter the following commands:

Command	What it does
dyanalib	loads the library describing amino acids and nucleic acids
read seq mag	loads the peptide sequence, from the file "mag.seq"
read upl mag	reads the upper distance limit constraint file
read upl mag_hb append	adds the hydrogen bond constraint file
read aco mag	reads the angle constraint file (from coupling constants)
seed=194274	or your favorite 6-digit number, as a random number seed
calc_all 30	calculates 30 structures from random starting conformation
struct sort	sorts the structures according to target function value (fit to data)
struct sel 1..10	select the best 10 structures
write_all pdb name=mag	saves the best structures as pdb files
struct list	lists statistics on all 30 structures
struct viol	prints restraint violations
distance stat	prints # of intra-residue, short, medium & long range distance restraints.
seqplot mag_seq.ps	make a PostScript plot of sequential NOE constraints
ramachandran mag_rama.ps	make a Ramachandran plot for all selected structures, and reports statistics
quit	exit the program

- Note and report all NOE violations  $> 0.2 \text{ \AA}$ , and angle violation  $> 5$  degrees.
- Note the numbers of intra-residues, and short, medium, and long-range inter-residue constraints
- Note how many phi-psi angle pairs are within the favored regions of the Ramachandran plot
- Print out the plots by typing "lp mag\_seq.ps" and "lp mag\_rama.ps"

## 6. Evaluate the structures

Load the structures into MolMol as before, by typing "molmol mag\*.pdb".

- Obtain the RMSDs for backbone and all non-H atoms as in section 5.
- Make a final plot of the ensemble, aligned using the backbone atoms, and print it out.
- Prepare a summary table describing the structures. Include numbers of constraints, numbers of constraint violations, and the size of the largest violation. Also include RMSDs for backbone and all non-H atoms.
- Evaluate how well the backbone dihedral angles fall into favored regions of the Ramachandran plot.
- Finally, how much did the additional NOEs and H-bond restraints improve the structure?

## 7. **OPTIONAL** Compare to the structure of the peptide in a detergent micelle (from the PDB).

The structure of magainin bound to a detergent micelle has been solved: Gesell et al, *J. Biomol. NMR* **9**, 127-35 (1997). Download the coordinates from the PDB:

<http://www.rcsb.org/pdb/>

- Type 2MAG into the search entry field, and hit enter
- Click on "Display File" -> PDB File (in left-hand panel)
- Click on "OK" to save as 2MAG.pdb

Read both structures into molmol (For simplicity, just the first one of each: to do this, within MolMol type "DialMol on", check the "select" property box, "Control-click" all the structural models that you want to delete, then type "RemoveMol" at the MolMol command prompt). Superimpose them. There's a slight trick here – the PDB entry has an extra "residue" at the end (a capping NH<sub>2</sub> as residue 24) and your models only have 23 residues. MolMol will complain that the number of residues doesn't match if you just type "SelectAtom "bb"". So instead, select the residue range: SelectAtom ":1-23 & bb" then Fit to\_first, etc.

- Report RMSDs, and plot the superimposed structures. How similar are they?

### Turn in at next lab:

1. Your list of NOE assignments, intensities and upper limits from step 1.
2. Your evaluation of the initial structures, and your additional NOE assignments
3. Your plot of the final ensemble of structures
4. Your evaluation of the structure (step 6)
5. *Optionally* a plot, RMSDs, and description of the differences between the structures of magainin in H<sub>2</sub>O/TFE solution, vs on a detergent micelle.

## Magainin Assignments, sorted by residue

## Assignments, sorted by chemical shift

RES#	A.A.	ATOM	ppm	RES#	A.A.	ATOM	ppm	RES#	A.A.	ATOM	ppm	RES#	A.A.	ATOM	ppm
1	Gly	HN	8.47	12	Phe	HA	4.59	20	Ile	QD1	0.71	20	Ile	HA	3.85
1	Gly	HA1	3.97	12	Phe	HB2	3.01	20	Ile	QG2	0.84	13	Gly	HA1	3.95
1	Gly	HA2	4.27	12	Phe	HB3	3.23	6	Leu	QQD	0.92	1	Gly	HA1	3.97
2	Ile	HN	8.68	13	Gly	HN	8.14	17	Val	QG1	0.97	3	Gly	HA2	3.98
2	Ile	HA	4.31	13	Gly	HA1	3.95	2	Ile	QG1	1.03	8	Ser	HB3	3.98
2	Ile	HB	2.00	13	Gly	HA2	4.08	17	Val	QG2	1.08	23	Ser	QB	4.00
2	Ile	QG1	1.03	14	Lys	HN	8.24	11	Lys	HG2	1.08	6	Leu	HA	4.07
3	Gly	HN	8.65	14	Lys	HA	4.14	11	Lys	HG3	1.22	9	Ala	HA	4.07
3	Gly	HA1	3.84	14	Lys	QB	1.89	9	Ala	QB	1.29	13	Gly	HA2	4.08
3	Gly	HA2	3.98	14	Lys	HG2	1.50	4	Lys	QG	1.41	10	Lys	HA	4.12
4	Lys	HN	8.04	14	Lys	HG3	1.58	10	Lys	HG2	1.44	11	Lys	HA	4.12
4	Lys	HA	4.17	14	Lys	QD	1.75	15	Ala	QB	1.46	14	Lys	HA	4.14
4	Lys	QB	1.87	14	Lys	QE	3.01	14	Lys	HG2	1.50	4	Lys	HA	4.17
4	Lys	QG	1.41	15	Ala	HN	8.13	6	Leu	HG	1.50	19	Glu	HA	4.23
4	Lys	QD	1.72	15	Ala	HA	4.24	10	Lys	HG3	1.53	15	Ala	HA	4.24
5	Phe	HN	7.89	15	Ala	QB	1.46	14	Lys	HG3	1.58	1	Gly	HA2	4.27
5	Phe	HA	4.45	16	Phe	HN	7.99	11	Lys	QD	1.58	2	Ile	HA	4.31
5	Phe	HB2	3.22	16	Phe	HA	4.43	11	Lys	QB	1.64	21	Met	HA	4.34
5	Phe	HB3	3.31	16	Phe	HB2	3.11	10	Lys	QD	1.71	8	Ser	HA	4.35
6	Leu	HN	8.34	16	Phe	HB3	3.16	4	Lys	QD	1.72	23	Ser	HA	4.42
6	Leu	HA	4.07	17	Val	HN	7.88	14	Lys	QD	1.75	16	Phe	HA	4.43
6	Leu	QB	1.82	17	Val	HA	3.65	6	Leu	QB	1.82	7	His	HA	4.44
6	Leu	HG	1.50	17	Val	HB	2.13	10	Lys	QB	1.85	5	Phe	HA	4.45
6	Leu	QQD	0.92	17	Val	QG1	0.97	4	Lys	QB	1.87	12	Phe	HA	4.59
7	His	HN	8.25	17	Val	QG2	1.08	14	Lys	QB	1.89	22	Asn	HA	4.78
7	His	HA	4.44	18	Gly	HN	8.06	20	Ile	HB	1.91	11	Lys	HN	7.77
7	His	QB	3.33	18	Gly	QA	3.83	2	Ile	HB	2.00	19	Glu	HN	7.83
8	Ser	HN	7.95	19	Glu	HN	7.83	21	Met	QB	2.12	17	Val	HN	7.88
8	Ser	HA	4.35	19	Glu	HA	4.23	17	Val	HB	2.13	5	Phe	HN	7.89
8	Ser	HB2	3.83	19	Glu	QB	2.14	19	Glu	QB	2.14	20	Ile	HN	7.89
8	Ser	HB3	3.98	19	Glu	QG	2.45	19	Glu	QG	2.45	23	Ser	HN	7.92
9	Ala	HN	8.26	20	Ile	HN	7.89	21	Met	HG2	2.57	22	Asn	HN	7.95
9	Ala	HA	4.07	20	Ile	HA	3.85	21	Met	HG3	2.69	8	Ser	HN	7.95
9	Ala	QB	1.29	20	Ile	HB	1.91	22	Asn	HB2	2.82	10	Lys	HN	7.99
10	Lys	HN	7.99	20	Ile	QG2	0.84	11	Lys	QE	2.90	16	Phe	HN	7.99
10	Lys	HA	4.12	20	Ile	QD1	0.71	22	Asn	HB3	2.93	4	Lys	HN	8.04
10	Lys	QB	1.85	21	Met	HN	8.31	12	Phe	HB2	3.01	12	Phe	HN	8.06
10	Lys	HG2	1.44	21	Met	HA	4.34	14	Lys	QE	3.01	18	Gly	HN	8.06
10	Lys	HG3	1.53	21	Met	QB	2.12	16	Phe	HB2	3.11	15	Ala	HN	8.13
10	Lys	QD	1.71	21	Met	HG2	2.57	16	Phe	HB3	3.16	13	Gly	HN	8.14
11	Lys	HN	7.77	21	Met	HG3	2.69	5	Phe	HB2	3.22	14	Lys	HN	8.24
11	Lys	HA	4.12	22	Asn	HN	7.95	12	Phe	HB3	3.23	7	His	HN	8.25
11	Lys	QB	1.64	22	Asn	HA	4.78	5	Phe	HB3	3.31	9	Ala	HN	8.26
11	Lys	HG2	1.08	22	Asn	HB2	2.82	7	His	QB	3.33	21	Met	HN	8.31
11	Lys	HG3	1.22	22	Asn	HB3	2.93	17	Val	HA	3.65	6	Leu	HN	8.34
11	Lys	QD	1.58	23	Ser	HN	7.92	18	Gly	QA	3.83	1	Gly	HN	8.47
11	Lys	QE	2.90	23	Ser	HA	4.42	8	Ser	HB2	3.83	3	Gly	HN	8.65
12	Phe	HN	8.06	23	Ser	QB	4.00	3	Gly	HA1	3.84	2	Ile	HN	8.68