Problem Set #4 – Chemistry 391 **Name**_____

Due September 27th in class

1. Does the NMR model of the switch mutant describe an alpha helix or 3_{10} helix? Explain briefly. A drawing would be permitted.

2. On the NMR data from Cordes et al. (2003). See Table I.

a. Was enough data collected to prepare a high-resolution model for this 53-residue protein? Explain briefly.

b. Do the calculated models fit the data that were collected? What data are you using to reach that conclusion?

c. Is there good structural agreement between the models? Again, indicate the relevant data.

d. Do the models have appropriate stereochemistry? That is, do they possess structural parameters consistent with generally understood principles of protein structure? Once again, what entries in the table support your answer?

Modeling: Part II

Not all models are created equal. Skepticism should reign. Consider the crystallographic model of a Zn^{2+} containing enzyme with a bound tripeptide of leucine. Download the file **LLL.pse** from the Moodle page. The paper that reports this model focuses on the conformation of the bound peptide. To view it more carefully, select **zoom** under the **A** button next to peptide.

3. There are four "obvious" stereochemical problems with the conformation of the tripeptide modeled here. Your mission, should you choose to accept it is to:

a. Find two inappropriate dihedral angles in the backbone (there are three that I found).

b. Find one bond angle in the backbone that is clearly not appropriate for the hybridization state (I found two).

If you aren't having luck with your eyeballs, you can type in the **get_angle** command. The LLL tripeptide is numbered 301-303. Thus, to get the bond angle formed by N, CA and C of the first leucine, type "**get_angle 301/N,301/CA,301/C".** The value of the bond angle will appear above the command line. Also, if you'd like to see whether the electron density calculated from the x-ray data support this odd conformation, click on the **density** button and it will show the density specific to the tripeptide.

4**.** In a ground-breaking paper, Kleywegt and Jones (*Structure*, **3**, 535-540, 1995) published two refinements of models using crystallographic data from the cellular retinoic acid binding protein. In one case, they reversed the protein sequence (yes, they did something so wild no one in their right mind would ever repeat it) and placed the resulting unnatural peptide chain in the electron density map. In separate trial, they took the correct sequence and placed it in the map and refined it. The following statistics resulted (I'm not specifying which refinement effort led to model X or Y). Questions next page.

Model	**X**	**Y**
Resolution range (Å)	8.0–3.0	8.0–2.9
R-factor	0.214	0.251
Rmsd bond lengths (Å)	0.009	0.009
Rmsd bond angles (°)	2.1	1.6
Ramachandran plot, most favored areas (%)	42.7	81.6
Additional allowed areas (%)	36.3	16.0
Generously allowed areas (%)	12.1	1.6
Disallowed areas (%)	8.9	0.8

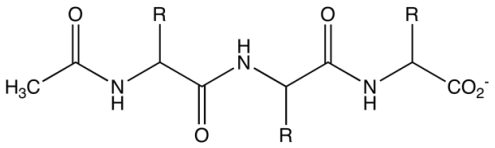
4. Kleywegt question.

a. Which model is in better agreement with the data? Why?

b. Which model has better stereochemical and conformational parameters? Explain your answer explicitly.

c. Which is the wrong structure? Explain why you think so.

5. Solve and NMR problem! Consider an N-acetylated tripeptide that contains a alanine, glycine and serine residue, but in unknown sequence. On the next page are two mock NMR spectra. Spectrum (A) is a 2D COSY spectrum for the tripeptide and spectrum (B) is a 2D NOESY spectrum. For simplicity I have made the peaks that are in the COSY spectrum circles and those that are unique to the NOESY spectrum squares.



a. Reproduce the following table and identify the chemical shifts of each set of protons belonging to each type of residue (you only need to use the COSY spectrum for this). Note that the "α ^{1}H" for the acetyl group is the methyl group.

	Chemical shifts (ppm)		
	Amide ^{1}H	α ^{1}H	β ^{1}H
Ala			
Gly			-
Ser			
Acetyl	-		-

b. Use the NOESY spectrum to determine the sequence of the tripeptide. State your logic briefly. If you are curious which cross-peaks you would expect to see (< 5 Å), you can download a simple model of a tripeptide (one that has the right bond and dihedral angles!): **tripeptide.pse**

Reading of the week:
Cordes et al. (1999) Evolution of a Protein Fold in Vitro. *Science* **284**, 325-327.

1. What is the guiding question/hypothesis of this work?

2. Write the initial and mutated sequence spanning residues 9-14. How can these sequences be described as amphiphilic?

3. Look at Figure 1.

a. What valuable information do panels A and B provide? Specify what each panel reports on.

b. Similarly what is the import of panels C and D?

4. In Figure 2, left, what is being plotted on the y-axis and how does it support the arguments made by the authors?

5. Figure 2, right, is a NOESY spectrum of switch Arc.

a. Note the regions of the spectrum plotted on the y-axis and the x-axis. What atom types contribute resonances in each region?

b. Residues 10, 11, 14, 21 and 45 contribute to this spectrum (the atom types are given as Greek letters – the ID's can be seen in my amino acid handout of several weeks ago). Indicate the cross-peaks that are particularly relevant to this study and how they support the claims of the authors.

6. Footnote 9 includes much of relevance to the NMR work reported here. Originally 28 models of the switch mutant were produced, but only 14 of those were employed in structural analysis of the protein. Provide two reasons, with brief explanations, for the exclusion of the other 14.

a.

b.

7. They comment on features associated with the β strand of Arc that make it amenable to structural variation. Note two of them, with a brief explanation.

a.

b.

8. Summarize the driving force behind the structural change observed in this work.