This is a sampler of problems from previous midterm Exam 2. Obviously, the problems on the upcoming exam will be *different*.

Problem 1.

a. A protein has binding affinity for its ligand (a peptide) of $K_a = 2\ 10^5\ M^{-1}$ at pH 5.0 and 25^{o}C. At what concentration of the ligand is half of the protein bound?

b. What fraction of the protein is bound at ligand concentration of 1.25 μM (a reminder: 1 μM $=10^{-6}$ M)?

c. At what ligand concentration will be 80% of the protein bound?

d. When the pH was raised to 6.5, the K_d increased to 20 μM. Is the binding tighter or weaker at this pH compared to pH5.0? Explain why.

e. What functional groups/residues are most likely responsible for this change in the binding affinity with pH?

Problem 2.

Answer the following questions about protein structure (use back of the page if you need more space):

(1) What does the Ramachandran plot represent? What are the parameters shown on the Ramachandran plot and what do they characterize?

(2) What groups are connected by the hydrogen bonds in the α-helix and in the β-sheet? Compare the characteristic hydrogen bonding patterns (i.e. what residues are connected) and the orientation of the hydrogen bonds in these two elements of secondary structure.

(3) Explain the meaning of terms “primary”, “secondary”, ”supersecondary”, “tertiary”, and “quarternary” structure.

Problem 3.

A 14-residue peptide, **LFILYKDGEALRTI**, in the context of a protein forms a β-hairpin, consisting of two antiparallel β-strands connected by a β-turn. You have found that the amide nitrogen of phenylalanine forms a hydrogen bond with the carbonyl oxygen of threonine. Based on your finding, characterize the structure of the β-hairpin:

(a) In the peptide sequence below clearly indicate, which residues belong to the two β-strands and which to the β-turn. Explain your reasoning.

(b) Draw *schematically* the hydrogen bonding pattern for this β-hairpin. Do not draw the side chains – just indicate what residues are connected by H-bonds. How many hydrogen bonds between the backbone atoms are present in the structure? Explain your reasoning.

Problem 4.

Both myoglobin and hemoglobin utilize heme to bind oxygen, the tertiary structure of myoglobin is very similar to that of the individual subunits in hemoglobin. And yet, the biological functions of the two molecules are very different.

a. What is the principal difference in the character of oxygen binding to hemoglobin and to myoglobin?

b. What are the principal structural differences between these two molecules that are responsible for the difference in their function?

c. Describe in structural terms the mechanism of allosteric effect of oxygen binding to hemoglobin, i.e. what changes (if any) in the secondary, tertiary, and/or quarternary structure occur upon O2 binding.

d. What could be thermodynamic explanation of the nature of positive cooperativity of O2 binding to hemoglobin, i.e. why the binding affinity increases when one or more O2 molecules are already bound.

e. All following molecules when present in the blood impede the ability of hemoglobin to bind oxygen: CO, CO2, H+, and 2,3-bisphosphoglycerate (BPG). What is the principal difference in the mechanism of how they affect oxygen binding?

Problem 5. You study ligand binding to two proteins, A and B. You measured the concentration, [PL], of the ligand-bound form of the protein at various ligand concentrations, [L]. The data are summarized in the two tables below. Note that you do not know the total concentration of the protein, but you know that adding more ligand did not noticeably change the bound-protein concentration.

Protein	A
[L], μM	[PL], μM
0	0.000
5	0.040
10	0.067
50	0.143
200	0.182
500	0.192
2000	0.198

Protein	B
[L], μM	[PL], μM
0	0.00
15	0.21
30	0.33
100	0.55
300	0.68
1000	0.74
3000	0.76

Based on these data, answer the following questions:
(1) Determine the K_d values for each of the proteins. Explain your assumptions.

(2) Which of the two proteins binds the ligand tighter? Explain your reasoning.

(3) Is this binding cooperative or non-cooperative? How would you check it?

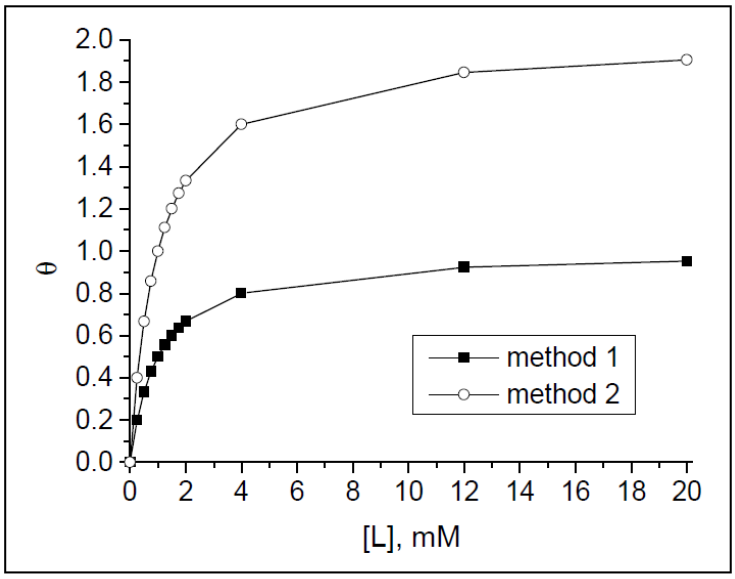
Problem 6.
Indicate which of the following statements are true and which are false.

true	false	
		α-helix is held by hydrogen bonds between amino acid side chains in the positions ***i*** and ***i+4***
		Ramachandran plot is a graphical representation of the sterically allowed conformations of peptide planes
		The extended, β-conformation, is characterized by a zigzag backbone geometry, where the backbone NH bonds belonging to residues in the positions ***i*** and ***i+2*** are located on the same side of the β-strand and are almost parallel to each other
		Hydrophobic effect is the main driving force in protein folding
		The backbone NH bonds of the neighboring residues in the β-strand are located on the opposite sides of the strand and are almost orthogonal (perpendicular) to the direction of the strand.
		The *cis* isomer is the highly favorable isomer for most peptide bonds except those preceding proline.
		The geometry of the β-sheet formation requires that the participating residues be nearby on the polypeptide chain
		The term secondary structure refers to local conformation of the protein backbone, disregarding the conformation of side chains
		The dihedral angle ϕ characterizes the orientation of the peptide plane at the amino end of the residue
		Protein tertiary structure is the three-dimensional arrangement of its 2^{o} structural elements, α-helices and β-sheets, held together by hydrogen bonds between them.
		Nuclear magnetic resonance (NMR) provides structural information in terms of interatomic distances and bond orientations, but not the actual atom coordinates.
		Quarternary structure refers to the three-dimensional arrangement of polypeptide subunits in a protein consisting of two or more polypeptide chains.

Problem 7.

Ligand binding studies were performed by adding ligand to a certain amount of protein X. The fraction θ of protein bound to ligand was assessed using two different methods: (1) by measuring the concentration of the protein bound to ligand and (2) by measuring the concentration of ligand bound to protein. In both cases θ was derived as $\theta = [PL]/[P]_{total}$, where [PL] represents the results of these measurements and $[P]_{total}$ is the total concentration of the protein present in solution. The data are shown in the table below and in the Figure (the data points are connected by lines just to guide the eye, these lines do not represent any fitting curves).

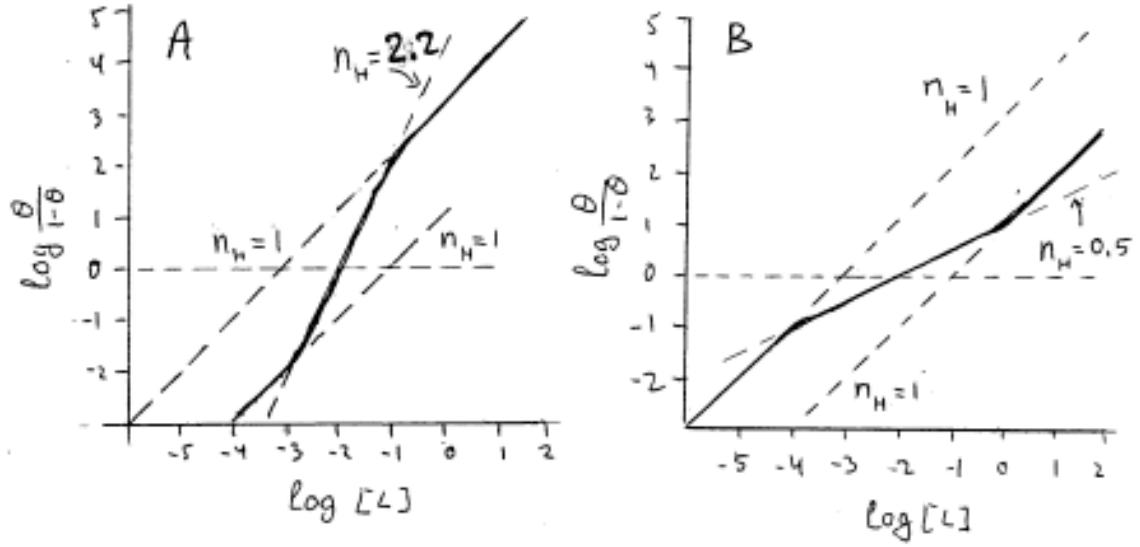
1	2	3
[L] in mM	θ method 1	θ method 2
0	0	0
0.25	0.200	0.400
0.50	0.333	0.667
0.75	0.429	0.857
1.00	0.500	1.000
1.25	0.556	1.111
1.50	0.600	1.200
1.75	0.636	1.273
2.00	0.667	1.333
4.00	0.800	1.600
12.00	0.923	1.846
20.00	0.952	1.905



1. Explain the difference between the results of these two measurements.
2. How many ligand binding sites are on the protein molecule?
3. What is the affinity of protein X for the ligand? Explain your reasoning
4. What could you assume about possible cooperativity of the ligand binding from these data? Suggest additional data analysis that could help verify your assumption.

Problem 8.

Ligand binding to proteins A and B is characterized by Hill plots shown below.



a. What conclusions about possible cooperativity of the binding can you draw from these plots? Explain the different shape of the binding curves.
b. Based on these plots, what is the minimal number of ligand binding sites on protein molecule for each of the proteins? Explain.
c. Which of the two proteins binds ligand tighter at the midpoint of the binding curve (i.e. where $\theta = 0.5$)? Explain.
d. For each of the proteins determine and compare their binding affinities in their high- and low-affinity states.
e. For each of the proteins draw schematically their ligand binding curves in the coordinates θ versus [L].