Biochemistry 461 Your Name:_____

March 30, 1995

Exam #2 Your SS#:_____

Prof. Jason Kahn

Your Signature:_____

Please have photo ID available.

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Some information which may be useful is provided on the bottom half of the next page.

Explanations should be concise, no more than three or four sentences.

You will probably not need a calculator for this exam.

No other study aids or materials are permitted.

Do not write anything on the top half of the next page.

Look over the entire exam before starting — some questions are easier than others, and don't get hung up on problems 1 and 2.

READ THIS: Each problem has a total of 24 points, but you can receive no more than 20 of these points. This means you can skip an occasional part of a question without penalty.

Score: Question 1: _____ out of 20

Question 2: _____ out of 20

Question 3: _____ out of 20

Question 4: _____ out of 20

Question 5: _____ out of 20

Total: _____ out of 100

Do Not Write Above This Line

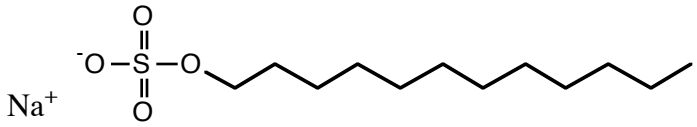
Possibly Useful Information:

O_2 binding equation for myoglobin and hemoglobin:

$$Y_{O_2} = \frac{pO_2{}^n}{p_{50}{}^n + pO_2{}^n}$$

$n \equiv$ Hill coefficient, $Y_{O_2} \equiv$ fractional O_2 saturation

Structure of SDS (sodium dodecyl sulfate):

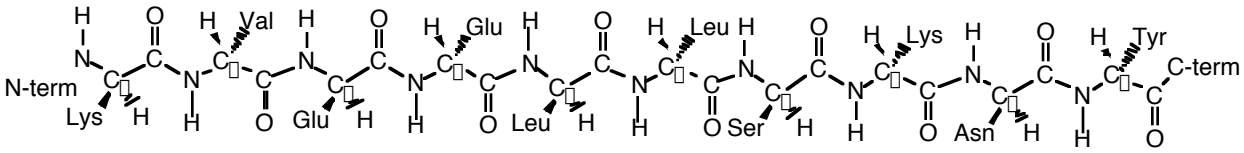


1. Secondary structure: α-helices and β-sheets (maximum of 20/24 points).

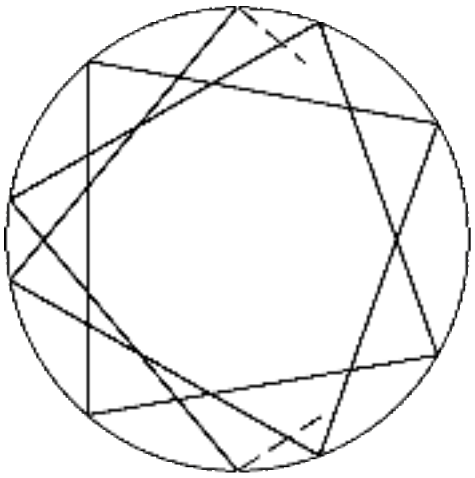
(a) On the diagram of a part of an extended polypeptide chain given below...

i. (5 pts) Draw the six backbone hydrogen bonds which would be formed among the residues shown if the peptide were part of an α-helix.

ii. (2 pts) Indicate the direction of the helix dipole moment with an arrow drawn from positive to negative.



(b) (3 pts) On the helical wheel representation to the right, enter the amino acid from the sequence in (a) which would be at each position. The N-terminal Lysine is entered for you, and the helix runs from N-term to C-term away from you, into the page.



(c) (2 pts) Does this α-helix have an amphipathic moment (i.e. one hydrophobic side and one hydrophilic side)? If so, indicate which side is which on the helical wheel.

Circle one: Yes No

(question 1 is continued on the next page)

(d) The chain shown below is part of an anti-parallel β-pleated sheet.

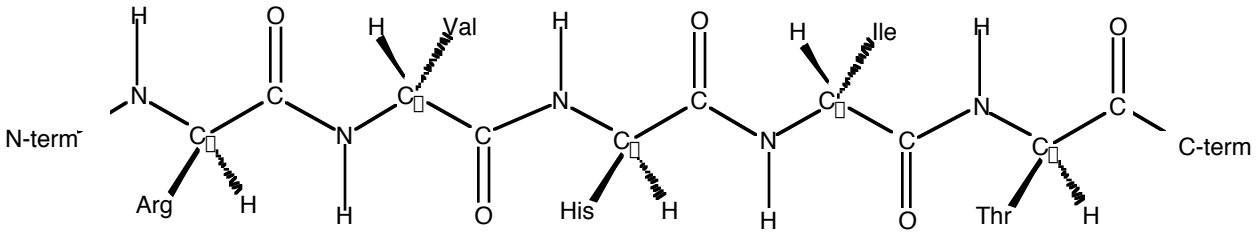
i. (5 pts) Draw in the backbone of <u>three residues</u> of the next strand of the sheet below the one given, indicating the hydrogen bonds which are formed. You need not indicate side-chain stereochemistry.

ii. (1 pt) Indicate the N-terminal and C-terminal ends of the strand you drew.

iii. (1 pt) Does the β-sheet have a dipole moment from backbone hydrogen bonding? If so, indicate its direction on your diagram.

Circle one: Yes No

iv. (2 pts) Can a β-sheet have an amphipathic moment ? Circle one: Yes No



(e) (3 pts) Short peptides can form stable α-helices, but there are no peptide β-sheets. Why not? (One or two sentences).

2. Tertiary structure: protein structural characteristics, protein folding. These are not trick questions, and each can be answered in no more than three or four sentences (maximum of 20/24 points).

(a) (6 points) What is the mechanism of protein denaturation due to changes in pH? List two of the forces which stabilize tertiary structure in proteins that are affected when the pH is changed (e.g. hydrophobic effect, London forces...). Describe why this causes the protein to unfold.

(b) (6 points) Briefly and qualitatively describe the Levinthal paradox concerning the kinetics of protein folding. What does this tell us about ordered vs. random mechanisms of folding?

(c) (6 points) In lecture, we emphasized that only thermodynamic differences between folded and unfolded forms are relevant to protein stability. Based on this idea, and recalling that glycine is the most flexible and proline the most rigid of amino acids, explain the following two observations with one fundamental mechanism:

i. Glycine does not tend to be in α-helical or β-sheet regions of proteins, instead appearing in less structured regions. Substitution of Gly for Ala in an α-helix destabilizes the helix.

ii. When the Ramachandran angles for an alanine at a particular site correspond to those allowed for proline, substituting a proline for the alanine at that site can stabilize the folded protein.

(d) (6 points) What are the functions of peptidyl prolyl cis-trans isomerases (a.k.a. PPI's, rotamases) and of the GroEL chaperonins in the process of protein folding *in vivo* ?

3. Allostery: Hemoglobin and myoglobin (maximum of 20/24 points).

The graph below shows the dependence of the fractional oxygen saturation (Y_{O_2}) for hemoglobin vs. the partial pressure of O_2, for p_{50} = 30 torr, Hill coefficient n = 2.8, at pH 7.2. On this same graph, draw the following four binding curves, paying special attention to the shape of the curve. You do not need to do any quantitative calculations. The definition of the Hill coefficient is given on page 2.

(a) (4 pts) The binding curve for myoglobin, n = 1 by definition, with p_{50} = 3 torr.

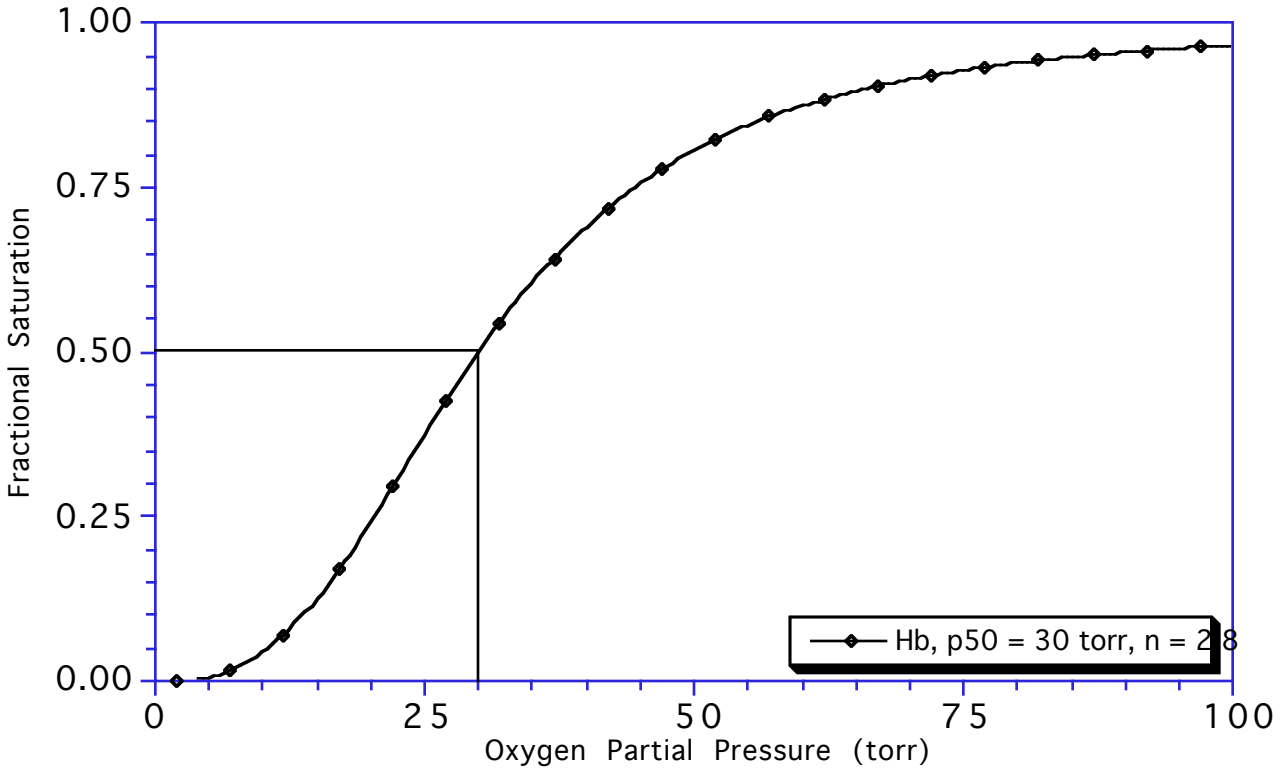
(b) (6 pts) The binding curve for a hemoglobin mutant with n = 3.8 and p_{50} = 30 torr.

(c) (6 pts) The binding curve for hemoglobin at pH 8, recalling that hemoglobin releases protons upon binding oxygen. Assume that n does not change.

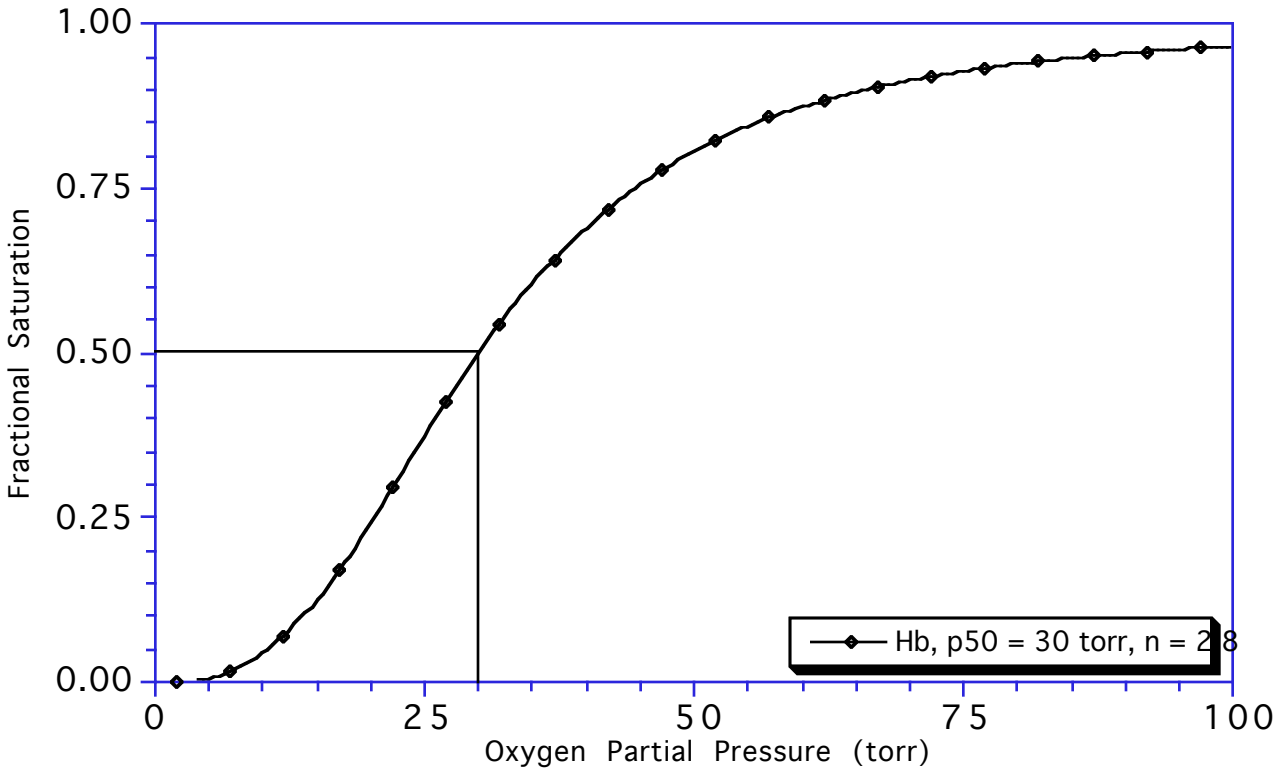
(d) (8 pts) The binding curve for O_2 binding to hemoglobin, imagining that one heme of each Hb tetramer has a tightly-bound CO, recalling that CO converts Hb to the R form.

Scratch space:

Draw final answers here. Label your curves (a)-(d) clearly: If you mess up, this graph is repeated on the next page, but if you draw on both you must indicate clearly which one we are to grade!



Question 3 graph repeated: ONLY ONE will be graded



4. Methods: Electrophoresis, ion exchange, reverse-phase chromatography (maximum of 20/24 points).

(a) In SDS-polyacrylamide gel electrophoresis gels, proteins are denatured and given a uniform negative charge density upon interacting with the detergent SDS (p. 2). They are then separated by migration through an electric field. The force on the protein (and therefore its velocity) is proportional to its charge, but it becomes more difficult to get through the gel pores as the protein's size increases, and therefore the mobility decreases with size in a regular way.

i. (6 pts) It has been observed that the molecular weights determined for membrane proteins by this method are often wrong. Recall the mechanism of detergent-induced denaturation and the fact that the insides of membranes are hydrophobic. What then are the physical causes for the aberrant behavior of membrane proteins in SDS-PAGE?

(continued...)

ii. (2 pts) Would you expect that the actual mobility (speed of migration through the gel) would be greater or less for a membrane protein than for a normal protein of the same molecular weight?

Circle one: Increased mobility Decreased mobility

(b) (8 pts) Define affinity chromatography and give an example.

(c) Cation-exchange chromatography is based on the reversible binding of molecules in solution to a negatively-charged immobile resin (e.g. column-linked sulfate groups $R\text{-}SO_3^-$). Reverse-phase chromatography is based on binding to a hydrophobic column (e.g. a C_{18} alkane). You have a solution containing four dipeptides: Lys-Lys, Asp-Ala, Val-Ile, and Gly-His.

i. (4 pts) Give the order in which these four dipeptides will elute from a cation-exchange column at pH 6 and a one-sentence explanation of your reasoning.

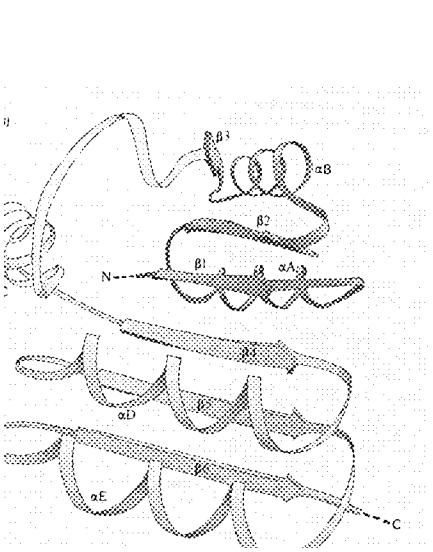
First off the column_____ Second_____ Third_____ Last_____

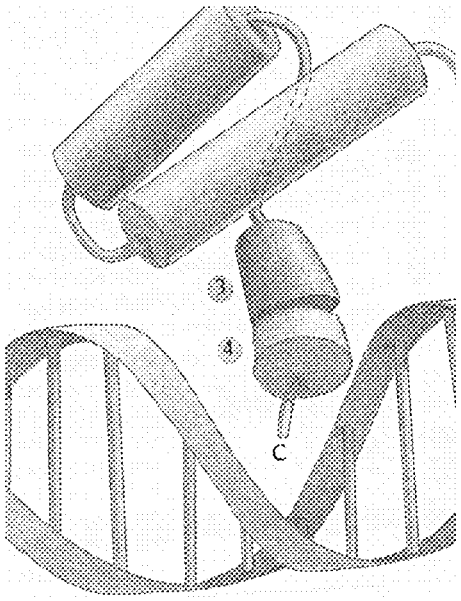
ii. (2 pts) What would happen to your separation process if it were done at pH 8?

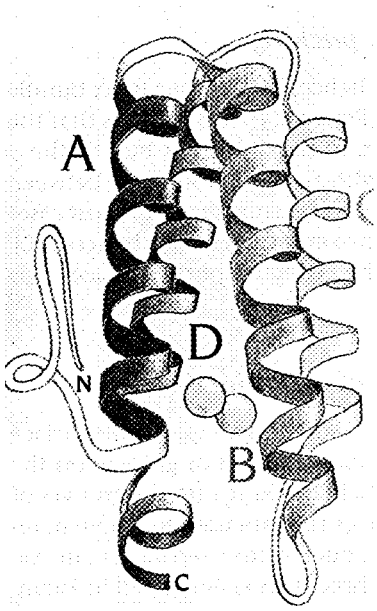
ii. (2 pts) Which dipeptide would elute <u>last</u> from a C_{18} column? Give a one-sentence explanation:

5. Domain structure and evolution (maximum of 20/24 points).

(a) (9 points) Three common supersecondary structures are shown below. Choose from this list to identify them: α/β barrel, helix-turn-helix, Rossman fold, 4-helix bundle, β-meander, SH2 domain.







Identify each:

_____ _____ _____

_____

(b) (3 pts) What does the Rossman fold do?

(c) The sequences below are from related eukaryotic transcription factors which bind zinc in order to fold.

Residue #:	1	2	3	4	5	...	16	17	18	19	20	21
A:	Phe	Gln	Cys	Asp	Val	...	Leu	Ser	Arg	His	Ile	Lys
B:	Phe	Gln	Cys	Asp	Ile	...	Leu	Ser	Arg	His	Ile	Lys
C:	Tyr	Asn	Cys	Glu	Ala	...	Leu	Thr	Lys	His	Val	Gln
D:	Tyr	Asn	Cys	Lys	Asp	...	Ile	Arg	Gly	His	Asp	Glu
Evolution:	___	___	___	___	___		___	___	___	_I_	___	___

i. (6 points) On the line below each amino acid, write "I" if it is invariant, "C" for conserved, and "V" for variable.

ii. (2 points) What might the invariant His19 be doing? (hint — what does His do in Hb?).

iii. (4 points) The above proteins come from human, plant, chimpanzee, and frog. We know that sequence (A) is from the human protein. Guess at which sequences correspond to the other three and give your reasoning (one sentence):

A:__human___ B:_____C_____ D_____