Biochemistry 461, Section I Your Printed Name:_____

April 10, 1997

Exam #2 Your SS#:_____

Prof. Jason D. Kahn

Your Signature:_____

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 this page.

Explanations should be concise and answer the specific question asked. You have more space on the exam than is necessary—don't think you need to fill the page.

You will not need a calculator for this exam, and no other study aids or materials are permitted.

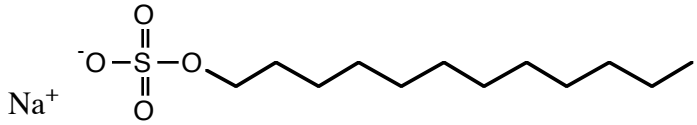
<u>Possibly Useful Information:</u>

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):



George Washington was born in 1732, which is 1000 □ √3

1. (20 pts) Protein Folding Mechanisms.

(a; 6 pts) What is the Anfinsen experiment and what does it tell us about protein structure?

(b; 6 pts) In general, what do chaperonins do to aid protein folding (what would happen to chaperonin-requiring misfolded proteins if there were no chaperonins)?

(c; 8 pts) Sketch and describe the rugged energy landscape paradigm for protein folding. How does it explain, for example, the observation that some proteins can partition among fast and slow pathways during folding? Please don't feel you need to cover the whole page.

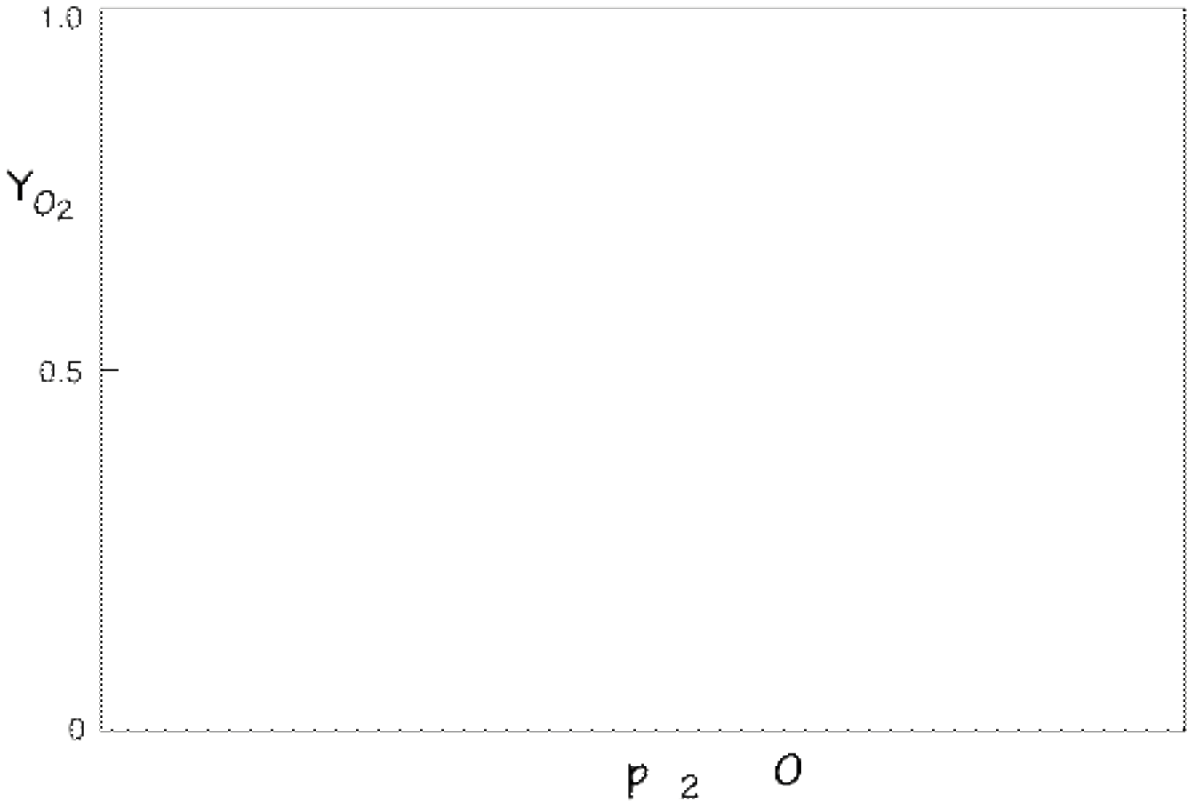
2. (20 points) Hemoglobin and Allostery.

(a; 5 pts) Speculate on why, when allosteric regulation is needed, proteins with interesting quaternary structures rather than single-domain proteins are often evolved to fill the need. Give a second common example (i.e. other than allostery) of a type of function for 4° structure.

(b; 4 pts) Describe one structural difference between the r and t or R and T states of hemoglobin.

(c; 5 pts) This problem should look familiar: An anemic individual whose blood has only half the normal Hb content may appear to be in good health. Yet, a normal individual is incapacitated by exposure to sufficient carbon monoxide to occupy half of his/her heme sites (CO binds to Hb with 200 times greater affinity than does O_2). Explain.

(d; 6 pts) Sketch the oxygen binding curves (Y_{O_2} vs. pO_2) you would expect for an imaginary hemoglobin locked into the R form, one locked into the T form, and real hemoglobin. Indicate $p50$ for the latter. You do not need the actual numbers; the shapes of the curves are what's important.



3. (20 points) Peptide Chemistry and Mapping.

(a; 13 pts) You are analyzing a peptide and make the folowing observations.

1. Amino acid composition is: (A, S, K, F, V, P, G, R, L, T, Q, M)
2. Edman degradation gives the following products for the first five cycles:
   1: Pro, Gly 2: Ser, Ala 3: Arg, something weird 4: Phe 5: Leu
3. Chymotrypsin digestion gives a fragment LMTQ and one other product.
4. Trypsin digestion gives GSR and one other product.

What is the original peptide? (Hint: lysine has an ϵ-amino group which is forming a peptide bond with another amino acid, and is therefore trypsin-resistant.)

(b; 3 pts) What is the common analytical-chemistry basis for the high sensitivity of amino acid analysis and the Edman degradation?

(c; 4 pts) Give one reason why scientists still use peptide sequencing/mapping methods, even though it's so much easier to determine protein sequences from DNA sequences rather than directly. Masochism is not an acceptable answer.

4. (20 points) Protein Purification and Analysis.

(a; 7 pts) Draw a picture and give a brief description of ion-exchange chromatography (IEC). Which type of IEC would you use for a positively charged protein and why?

(b; 6 pts) Phosphorylated proteins, which have added negative charge, often run more slowly on SDS-PAGE gels than the same proteins in the non-phosphorylated form. Suggest a possible explanation for this curious observation.

(c; 7 pts) Comparison to the known structure of a homologous protein is the best way to predict the structure of a newly-sequenced but otherwise uncharacterized protein. If this is impossible, people often apply the empirical Chou-Fasman rules (secondary structure predilection rules). Briefly, what are these (in general)?

5. (20 points) Domain Structure, Folding, and Evolution.

(a; 12 pts) In a set of four wild type homologous proteins, presumably with similar 3° structures, you notice a pattern of amino acid substitutions at four positions, as tabulated below. The mutant protein 5 (= protein 1: Asp84 -> Val) fails to fold. Identify two residues which are likely to be in the core of the protein, one surface residue, and one active site residue, and give a rationale for your guesses. Explain why the mutant fails to fold.

Position (irrelevant):	57	65	72	84
Protein 1	Arg	His	Ser	Asp
Protein 2	Glu	His	Thr	Lys
Protein 3	Ile	His	Ala	Val
Protein 4	Val	His	Asp	Leu
Mutant protein 5	Arg	His	Ser	Val
Probable location (core, surface, etc.)	_____	_____	_____	_____

(b; 3 pts) Name three super-secondary structure elements.

(d; 5 pts) Why do proteins tend to denature when organic solvents are added to the solution? Base your answer on a comparison of folded vs. unfolded states ± denaturant.

Do Not Write Below This Line

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**