Biochemistry 461, Section I **Your Printed Name:**_____

May 21, 1998

Final Exam **Your SS#:**_____

Prof. Jason D. Kahn

Your Signature:_____

You have 120 minutes for this exam.

The exam has 7 questions, worth 200 points. Do all 7 questions. In many cases, you do not need to answer earlier parts of questions to answer the later ones.

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

Explanations should be concise and answer the specific question asked.

You will need a calculator for this exam. No other study aids or materials are permitted.

There will be a viewing at a time and place to be announced on the class web page. Final grades will be available only through MARS.

Possibly Useful Information:

Michaelis-Menten equation: $v_0 = V_{max}[S]/(K_M + [S])$, where $V_{max} = k_{cat}[E]_t$

Type of inhibition	Apparent K_M	Apparent V_{max}	Apparent V_{max}/K_M
Competitive	αK_M	V_{max}	$(1/\alpha)\ V_{max}/K_M$
Uncompetitive	$(1/\alpha')\ K_M$	$(1/\alpha')\ V_{max}$	V_{max}/K_M
Mixed	$(\alpha/\alpha')K_M$	$(1/\alpha')\ V_{max}$	$(1/\alpha)\ V_{max}/K_M$
Noncompetitive ($\alpha=\alpha'$)	K_M	$(1/\alpha)\ V_{max}$	$(1/\alpha)\ V_{max}/K_M$

$\alpha = 1 + [I]/K_I$ $\alpha' = 1 + [I]/K_{I'}$

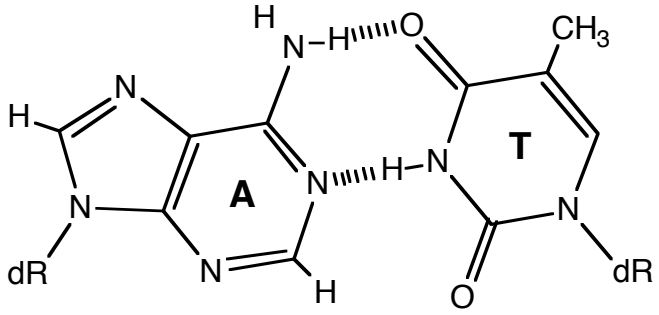
Henderson-Hasselbach equation: $pH = pK_a + \log([A^-]/[HA])$

$\Delta G = \Delta H - T\Delta S = \Delta G^\circ + RT\ln Q$, where Q has the form of an equilibrium constant

RT = 2500 J/mole today

1. (30 points) Nucleic Acids, Amino Acids, Hydrogen Bonding

Adenine and thymine are shown below, H-bonded as they are in a W-C base pair.



(a; 10 pts) On the structure above, identify the major and minor groove sides of the base pair, and label the hydrogen bond donors and acceptors in each groove.

(b; 10 pts) Asparagine residues in DNA binding domains are often found to recognize adenine in duplex DNA. Draw a plausible structure for the interaction of an Asn side chain with the major groove edge of adenine.

(c;10 pts) The Watson-Crick arrangement is not the only way that A and T can base pair. Draw an alternative arrangement of A and T that might be observed if the sugar-phosphate backbone didn't hold the bases in place in a helix (in other words, a different way to hydrogen-bond them). There is space on the next page for your answer if you need it.

2. (15 pts) Hemoglobin and Allostery.

Hemoglobin binds CO_2, although not at the heme iron. The T state binds CO_2 much better than the R state (via a reversible covalent linkage).

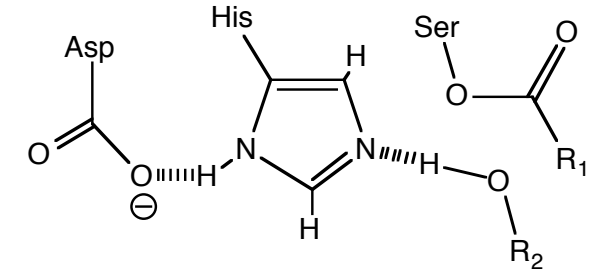
(a; 7 pts) What effect would increased levels of CO_2 have on the p50 for O_2 binding to Hb? Explain your reasoning.

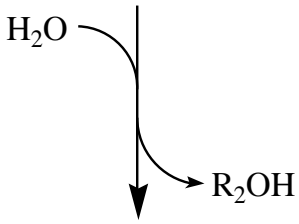
(b; 8 pts) Where is CO_2 high in the body and where is it low? Thus, what are two likely physiological roles for CO_2 binding to Hb?

3. (35 points) Serine Protease Mechanism, pH

The structure below is the acyl-enzyme intermediate in the hydrolysis of an ester by a serine protease, which has a mechanism similar to the amide hydrolysis we have studied.

(a; 16 pts) Draw the structures for the next two steps, paying attention to protonation states. The first step is just the replacement of the alcohol leaving group with water. There is no need to draw the Asp side chain.





(b; 6 pts) How does this example illustrate covalent catalysis?

As pH is varied, the k_{cat} for chymotrypsin is found to be directly proportional to the amount of the His_{57} in the catalytic triad which is in the His as opposed to $HisH^+$ form, while K_{diss} for substrate is constant. The pK_a for His_{57} is 7.2.

(c; 5 pts) Is the histidine acting as a general acid or as a general base? How do you know?

(d; 8 pts) Calculate the ratio of k_{cat} at pH 6.7 to k_{cat} at pH 7.7; in other words, calculate $[His]/([His] + [HisH^+])$ at the two pH's and take the ratio of the answers.

4. (30 pts) Biomembranes, Protein Structure

Many membrane proteins are attached in the hydrophobic interior of the lipid bilayer by one or more α helices which span the membrane.

(a; 8 pts) How can we sometimes identify the trans-membrane α helices by examination of the protein sequence, even for a new protein with no homology to any others?

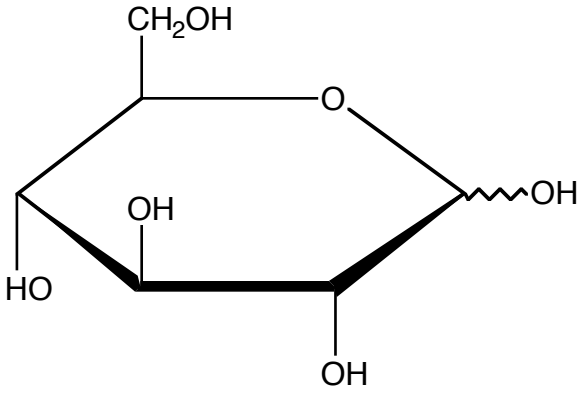
(b; 8 pts) When β sheets are found in membranes, they are usually complete barrels, not one or two strands. Why (contrast with α helices in membranes)?

(c; 6 pts) Soluble proteins which interact with membrane receptors are often anchored to the membrane by hydrophobic lipid tails. How does this improve the efficiency of signal transduction?

(d; 8pts) Why are biomembranes made of two-tailed phospholipids rather than single-tailed detergents?

5. (30 points) Carbohydrates, Bioenergetics

The Haworth projection for glucose is shown at the right.



(a; 6 pts) Draw the Haworth projection for galactose, the C-4 epimer of glucose.

(b; 8 pts) Draw the Haworth projection of lactose, which is β-D-Galactopyranosyl(1→4)Glucopyranose. Circle the two anomeric carbons.

(c; 6 pts) Why are we careful to specify which anomer of galactose is present on lactose, but find it much less important to specify the anomer of the glucose unit?

(d; 10 pts) The enzyme β-galactosidase hydrolyzes the β(1→4) linkage in lactose in the cell. This enzyme has no cofactor requirements and does not use ATP, just like proteases. What does this simple observation tell us about the thermodynamic stability of the disaccharide relative to its constituent monosaccharides? Thus, without being able to say anything about the detailed pathway, what must the cell do in order to synthesize polysaccharides?

6. (35 points) Michaelis-Menten Kinetics and Inhibition

Irreversible affinity labels or mechanism based inhibitors react in an enzyme active site and thereby inactivate the enzyme.

(a; 6 pts) Give one example of an irreversible inhibitor and the enzyme it inhibits (if you don't know the names, a description or structure will do).

(b; 5 pts) Give one reason why irreversible enzyme inhibitors are important in contemporary society.

(c; 8 pts) Why do irreversible inhibitors usually resemble substrates or transition states?

(d; 8 pts) Based on your answer to (c), explain why a high concentration of true substrate can protect an enzyme from inactivation by an irreversible inhibitor. What kind of inhibition kinetics (non-, un-, or just plain competitive) does this remind you of?

(e; 8 pts) In fact, if an enzyme is partially inactivated by treatment with an irreversible inhibitor (without substrate) for a short period of time followed by removal of the inhibitor, subsequent characterization of the Michaelis-Menten kinetics of the treated enzyme preparation shows a pattern characteristic of non-competitive inhibition. Explain why apparent non-competitive inhibition is observed. (Hint: what is the kinetic signature of non-competitive inhibition, and what is the definition of V_{max}?)

7. (35 points) Protein (and Course) Structure and Folding

The GroEL/GroES chaperonin complex in *Escherichia coli* has a large cavity in which small proteins are believed to fold.

(a; 5 pts) What undesirable protein folding side reaction have chaperones evolved to prevent?

(b; 10 pts) The folding cavity is too small to hold more than one protein substrate. The cavity has been called an "Anfinsen cage", after the scientist who did the original ribonuclease refolding studies. Why? (Anfinsen is dead, so it's not just flattery.)

(c; 10 pts) Draw the structure of proline. Why does proline break the α helix? Why does it break the β sheet? (Different answers)

(d; 10 pts) What topics in this course would you have liked to have seen

(1) folded into another course, one you didn't have to take?

(2) unfolded to show more detail of their inner workings?

Thank you very much for your exceptional attention and interest this semester. I hope you found the course useful and stimulating.

Do Not Write Below This Line

Score:

Question 1: _____ out of 30

Question 2: _____ out of 15

Question 3: _____ out of 35

Question 4: _____ out of 30

Question 5: _____ out of 30

Question 6: _____ out of 35

Question 7: _____ out of 35

Total: _____ out of 210