Biochemistry 461 **Your Printed Name:**_____

Section I

February 24, 1998 **Your SS#:**_____

Exam #1

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.

Explanations should be concise, a couple of sentences.

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

Possibly Useful Information:

$K_a = [H^+][A^-]/[HA]$ for the dissociation of HA, so $pH = pK_a + \log([A^-]/[HA])$

$K_w = 10^{-14} = [HO^-][H^+]$

RT = 2476 J/mole today

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

$\Delta G = -nF\Delta E$, where F = 96500 J/(V•mole), *n* = number of electrons transferred

$\Delta G^\circ = -30.5$ kJ/mole for $ATP^{4-} + H_2O \rightleftharpoons ADP^{3-} + HPO_4^{2-} + H^+$

Redox half-reactions:

$NAD^+ + 2H^+ + 2\,e^- \rightleftharpoons NADH + H^+$ $\quad E^\circ = -0.315$ V

SO_4^{2-} (sulfate) $+ 2H^+ + 2\,e^- \rightleftharpoons SO_3^{2-} + H_2O$ $\quad E^\circ = 0.48$ V

NO_3^- (nitrate) $+ 2H^+ + 2\,e^- \rightleftharpoons NO_2^{2-} + H_2O$ $\quad E^\circ = 0.42$ V

$^1/_2\,O_2 + 2H^+ + 2\,e^- \rightleftharpoons H_2O$ $\quad E^\circ = 0.815$ V

1. (23 pts) Redox reactions and bioenergetics.

We make most of our ATP from the redox reaction below, in a process called oxidative phosphorylation:

$$NADH + H^+ + {}^1/_2\, O_2 \rightleftharpoons NAD^+ + H_2O$$

(a; 8 pts) Calculate $\Delta E°'$, $\Delta G°'$, and K_{eq} for this reaction. How many ATPs could be synthesized from ADP and P_i by coupling to this reaction (per NADH oxidized)?

(b; 3 pts) Oxidative phosphorylation provide examples of three ways in which biological systems store energy. List them (in general).

(c; 8 pts) In the sediments at the bottoms of lakes, there is very little oxygen. Anaerobic bacteria in the mud can use sulfate or nitrate as electron acceptors instead of oxygen. Write the balanced chemical reaction and calculate ΔE° and ΔG° for the reduction of sulfate by NADH. How many ATPs per NADH could be obtained from this reaction? Would nitrate reduction provide more ATP or less ATP than sulfate reduction?

(d; 4 pts) It is found that oxygen-reducing, sulfate-reducing, and nitrate-reducing bacteria are stratified, i.e. they occupy different sediments. Based on the ATP calculations above, suggest one reason for stratification.

2. (18 points) Thermodynamics and bioenergetics.

(a; 7 pts) Some uphill reactions can be effectively driven by addition of reactant or removal of product, but some cannot. If the cellular concentration of ATP must be maintained at 2 mM, and the P_i concentration is 5 mM, what concentration of ADP would be necessary in order to provide net synthesis of ATP?

(b; 5 pts) Aspartyl phosphate (Asp-phosphate) residues are high-energy intermediates in several biochemical reactions (e.g. the sodium-potassium pump). The $\Delta G°$ for hydrolysis to give Asp + phosphate is about -42 kJ/mole. Draw aspartyl phosphate and the products of hydrolysis, all at pH 7 (assume phosphate is HPO_4^{2-}).

(c; 6 pts) In contrast to Asp-phosphate, the $\Delta G°'$ for the hydrolysis of Ser-phosphate is only –12 kJ/mole. Why is the $\Delta G°'$ of hydrolysis so much more negative for Asp-phosphate than for Ser-phosphate?—give two reasons based on the structures of reactants and products.

3. (21 points) Amino acids, peptides, and hydrogen bonding.

(a; 10 pts) Draw the covalent structure of the tripeptide Tyr-Pro-Val, with a *trans* peptide bonds, as it would exist at pH 7. Indicate the two protons which would be lost at pH 12. Indicate two hydrogen bond donors and two acceptors on your structure. Write the 1-letter code for each of the three amino acids below the structure. Indicate which peptide bond might be found in the *cis* conformation in a protein.

(b; 5 pts) Draw a plausible hydrogen-bonding structure between the side chains of threonine and histidine. Identify donor and acceptor (more than one answer possible).

(c; 6 pts) Draw the Asn side chain. Why isn't the NH_2 group of the side chain protonated? How is your answer related to the geometry of the peptide bond?

4. (16 points) Thermodynamics and philosophy

(a; 4 pts) What does TANSTAAFL stand for, and what is the more formal statement for this first law of thermodynamics?

(b; 8 pts) Consider a growing baby mouse in a box (the system) exchanging whatever is necessary with the surroundings to maintain life. The mouse can only survive if the entropy of the universe increases. Give two ways in which the mouse increases the entropy of the surroundings.

(c; 4 pts) We have agreed that the absolute stereochemistry of the amino acids is not critical to this course. However, it is important that there is an absolute (L = S) stereochemistry. Why (how many diastereomers could a 100-aa protein form if $C_{\square}$ stereochemistry were random)?

5. (22 points) Acid-base chemistry.

(a; 4 pts) Which buffer from the table at the right would you choose for an experiment to be done at pH 6 and which at pH 8? Explain your reasoning briefly.

Buffer	pK_a□
Acetate	4.75
MES	6.1
HEPES	7.5
CAPSO	9.6

(b; 8 pts) A cysteine residue in a protein active site has a pK_a of 7.6 (referring to deprotonation of the -SH group as below). What fraction of the cysteines are deprotonated ($-S^-$) at pH 6 and at pH 8?

$$Cys\text{-}SH \rightleftharpoons Cys\text{-}S^- + H^+ \qquad pK_a = 7.6$$

[For c-d] Usually biochemists are careful to perform all experiments in buffered solution. However, in doing NMR experiments, which require high protein concentration, one can sometimes get away without buffering once the pH is adjusted to the desired value.

(c; 3 pts) What is the pH of 0.3 mM HCl?

(d; 7 pts) Assuming that a protein NMR sample, at pH 6.5, is 1 ml of a 1 mM solution of a protein with 10 histidines (all with $pK_a = 6.5$), what would the pH become upon addition of HCl to a final concentration of 0.3 mM? Thus, explain why we don't need additional buffering:

Do Not Write Below This Line

Score:

Question 1: _____ out of 23: Redox reactions and bioenergetics

Question 2: _____ out of 18: Thermodynamics and bioenergetics

Question 3: _____ out of 21: Amino acids, peptides, and hydrogen bonding

Question 4: _____ out of 16: Thermodynamics and philosophy

Question 5: _____ out of 22: Acid-base chemistry

Total: _____ **out of 100**