Biochemistry 461, Section I

February 27, 1997

Exam #1

Prof. Jason D. Kahn

Your Printed Name:________________________________

Your SS#:________________________________

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 the next page.

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.

<u>Useful Information:</u>

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

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

RT = 2476 J/mole today

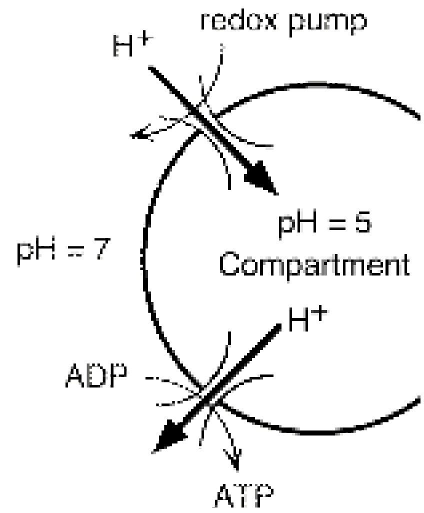
$\Delta G = \Delta G°' + RT\ln Q$, where Q has the form of an equilibrium constant

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

$N_A = 6.02 \times 10^{23}$ particles per mole

1. (18 pts) Redox reactions and the Nernst equation.

The oxidation of NADH by sulfate can be used to create a proton gradient, which ATP synthase then taps to make ATP, as in the sketch to the right. The relevant standard reduction potentials at pH 7 are given below.



$NAD^+ + H^+ + 2\,e^- \rightleftharpoons NADH \qquad E°' = -0.315\text{ V}$

$SO_4^{2-} + 2H^+ + 2\,e^- \rightleftharpoons SO_3^{2-} + H_2O \qquad E°' = 0.48\text{ V}$

(a; 8 pts) Write the <u>balanced equation</u> for this redox reaction, calculate <u>$\Delta E°'$ and $\Delta G°'$</u>, and calculate the <u>equilibrium constant</u> for the reaction.

(b; 6 pts) If the pH is 5 on the inside of the compartment and 7 on the outside, what is <u>ΔG for moving a proton in</u>to the compartment? (Ignore any membrane potentials.) <u>How many protons</u> can be pumped per NADH oxidized?

(c; 4 pts) If the volume of the compartment is 10^{-17} liters, how many protons are in it at pH 5? If the single ATP synthase in the membrane can allow 10 protons per second to exit the compartment, how long can ATP synthesis continue once NADH oxidation is shut off?

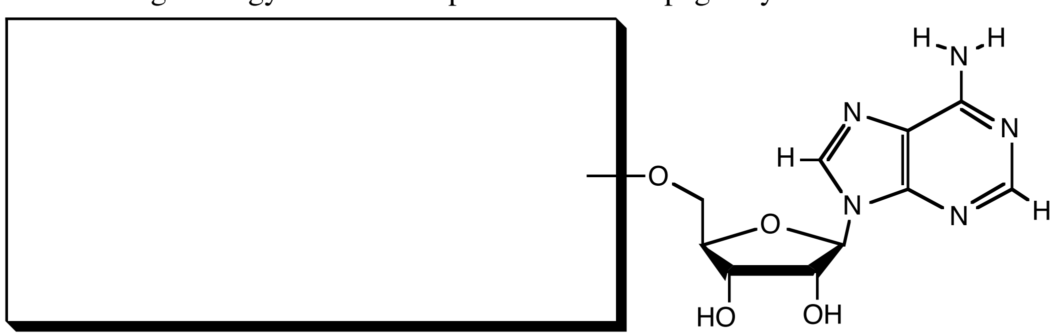
2. (20 points) Bioenergetics.

(a; 4 pts) List <u>two ways that cells store energy</u>.

The hydrolysis of the □-□phosphoanhydride bond of adenosine triphosphate (ATP) is accompanied by a large negative free energy change:

$$\text{ATP} \rightleftharpoons \text{ADP} + \text{P}_i \quad \Delta G°' = -30.5 \text{ kJ/mole}$$

(b; 7 pts) <u>Draw the triphosphate moiety</u> of ATP in its predominant ionic form at pH 7, adding onto the structure for adenosine given below. <u>Indicate the "high-energy" phosphoanhydride bonds</u>. Give <u>two reasons for the large negative $\Delta G°'$</u> for ATP hydrolysis. In other words, why are phosphoanhydride bonds "high-energy"? There is space on the next page if you need it.



(c; 5 pts) Calculate ΔG for ATP hydrolysis at [ADP] = 2 mM, [ATP] = 8 mM, [P_i] = 5 mM. Assume the biochemical standard state.

(d; 4 pts) The ATP hydrolysis reaction actually liberates a proton, as below. Normally we simply ignore this as part of working at the biochemical standard state (pH 7).

$$ATP^{-4} + H_2O \rightleftharpoons ADP^{-3} + HPO_4^{-2} + H^+ \qquad \Delta G°' = -30.5 \text{ kJ/mole}$$

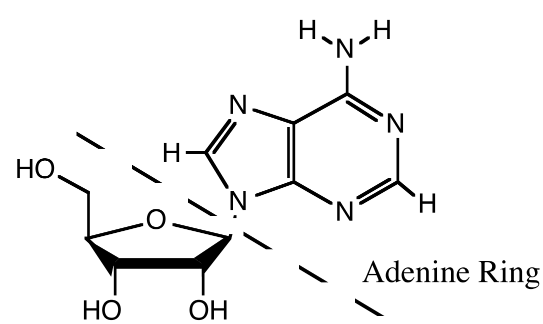
The equation shows that decreasing the pH reduces the driving force for hydrolysis. In spite of this fact, it is found that decreasing the pH increases the rate of hydrolysis of ATP. How can you explain this apparent paradox?

3. (20 points) Amino acids, peptides, and hydrogen bonding.

(a; 10 pts) Draw the covalent structure of the tripeptide Tyr-Lys-Leu, with all functional groups in their predominant ionic forms at pH 7. Indicate which protons would be removed at pH 12, which is above the pK_a of all ionizable side chains except Arg. Write the 1-letter code for each of the three amino acids below the structure.

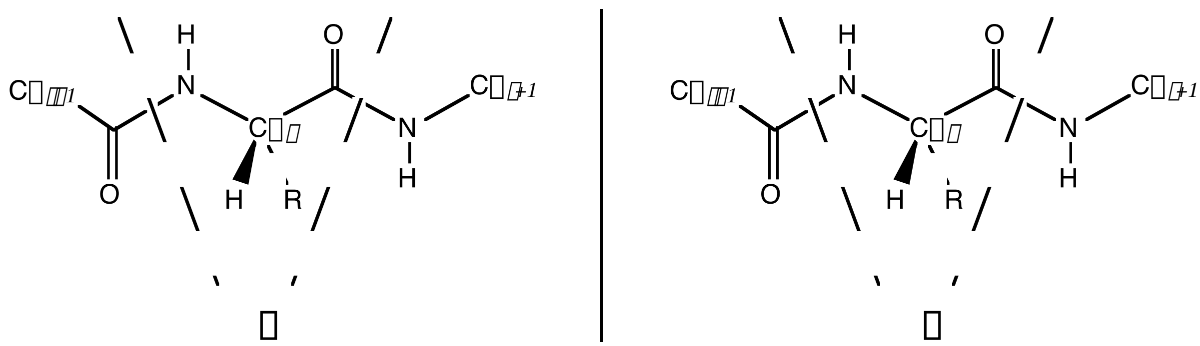
(b; 3 pts) Which amino acid provides biological buffering capacity? Why? Name the two sulfur-containing amino acids (full names for all three, please).

(c; 7 pts) On the picture of adenosine at the right, circle the H's which can be donated in hydrogen bonds. Draw in a plausible structure for the asparagine side chain making two hydrogen bonds to the adenine ring.

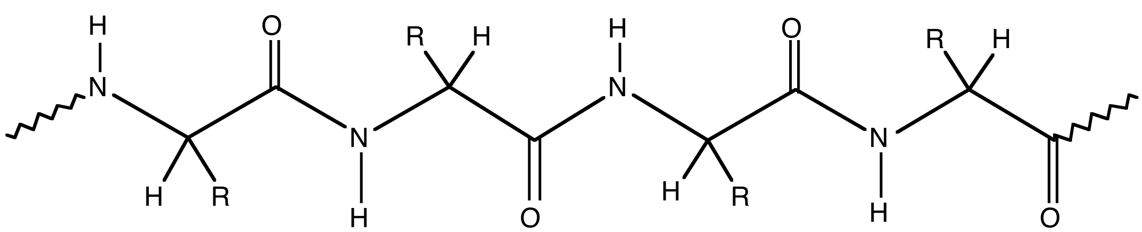


4. (22 points) Secondary structure and the Ramachandran plot.

(a; 4 pts) On the left extended polypeptide below, indicate which four atoms define the φ angle for amino acid *i*, which is bracketed by the dashed lines. On the right side indicate which four atoms define the ψ angle. On the left-hand diagram, draw two parallelograms indicating which sets of atoms are constrained to lie in a plane.



(b; 8 pts) Draw in three residues of the <u>antiparallel β-sheet partner</u> below the extended polypeptide chain drawn here. Include backbone hydrogen bonds and indicate where the next strand (i.e. if you were to draw a third one) would H-bond. Indicate side chains with R. Indicate the N terminus and the C terminus of the strand you draw.



(c; 8 pts) Discuss how α-helices and β-sheets can exhibit "sidedness", *i.e*. asymmetric chemical properties, in different ways. Draw sketches to illustrate.

(d; 2 pts) Name the 3-D molecule viewer program used in the in class computer demonstrations of secondary structure.

5. (20 points) Acid-Base Chemistry.

Tris(hydroxymethyl)aminomethane or "Tris", $(HOCH_2)_3CNH_2$, is a commonly used buffer in biochemical experiments. The pK_a for the reaction below is 8.06 at 25 °C.

$$\text{TrisH}^+ \rightleftharpoons \text{Tris} + \text{H}^+$$

(a; 7 pts) Using the information above and the equation for water self-dissociation (K_w), calculate the pH of a 0.1 M solution of Tris (*i.e.* $c_0 = 0.1$ M), assuming that what you spoon out of the bottle is the unprotonated form ("Tris base").

(b; 6 pts) How much of a 10 M stock of the strong acid HCl would you have to add to 100 ml of the above solution to bring the pH down to 7.5? Ignore the volume change of the solution.

(c; 4 pts) Tris has the unfortunate property that its pK_a is quite temperature dependent, decreasing to 6.66 at 75 °C. What would the pH of the solution you made in (b) become at 75 °C, ignoring any change in K_w? (Hint: imagine you had added the acid at 75 °C.) If you could not answer (b), assume 1 ml of HCl was added.

(d; 3 pts) The pK_a's of many weak acids decrease with increasing temperature, i.e. they become stronger acids. Speculate as to why this might be.

Do Not Write Below This Line

Score:	Question 1:	______	out of 18: Nernst equation
	Question 2:	______	out of 20: Thermodynamics and bioenergetics
	Question 3:	______	out of 20: Amino acids and peptides
	Question 4:	______	out of 22: Secondary structure
	Question 5:	______	out of 20: Acid-Base
	Total:	______	**out of 100**