UNIVERSITY OF CALIFORNIA, BERKELEY
CHEM C130/MCB C100A
MIDTERM EXAMINATION #3
November 17th, 2016

INSTRUCTORS: John Kuriyan and David Savage

THE TIME LIMIT FOR THIS EXAMINATION: 1 HOUR 50 MINUTES

SIGNATURE : _____

YOUR NAME:_____

PLEASE PRINT AND SIGN your name (**IN INDELIBLE INK**) on the line above (& on the top right hand corner of every page).

PLEASE CIRCLE the name of your GSI:

Alec Heckert	Thomas Laughlin
Pei Liu	Mark O'Dair
Kersh Thevasundaram	Carl Ward

PLEASE WRITE all of your answers **AS LEGIBLY AS POSSIBLE**.

SCORING. The exam consists of 5 questions totaling 100 pts as broken down in this table:

Question	Part A	Part B	Part C	Part D	Your Total	Max Score
1.	10	10				20
2.	6	4	10			20
3.	10	4	6			20
4.	10	10				20
5	-	-	-	-		20
				TOTAL		100

Q1 (20 points)
Q1A. (10 points) You are studying the binding of a ligand to a receptor protein and believe that a van't Hoff-type analysis could be a good way to analyze the system.

(i) (2 points) Briefly explain what must be true about system to reach this conclusion.

ANSWER: The enthalpy and entropy changes of the system must be constant over the temperature ranges of interest. This can be true for ligand binding studies.

(ii) (6 points) A careful experimental analysis applying the van't Hoff equation to the binding equilibrium (under biochemical standard conditions) reveals this hypothesis is justified and you measure the following five data points. Dash line in plot represents a linear fit to these five points, which are also shown in the table on the right.

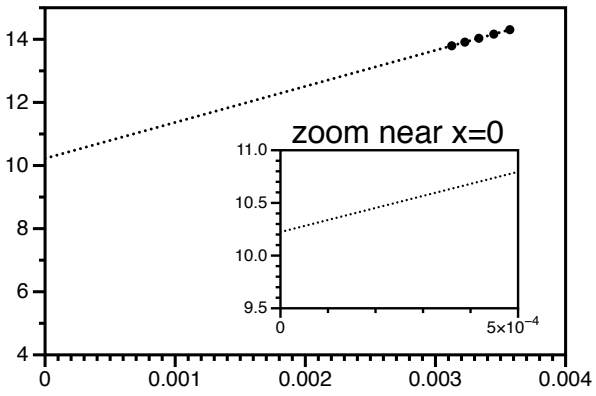


Table of data

1/T	ln(K)
0.003571	14.305
0.003448	14.164
0.003333	14.033
0.003225	13.910
0.003125	13.795

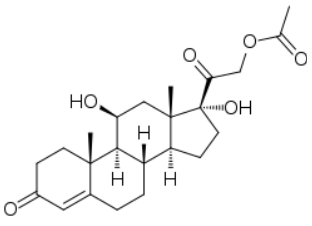
What are the values for ΔS°' and ΔH°' for the binding reaction?

ANSWER:

Fit to van't Hoff plot is

ΔS°' = 85 J/ (mol*K)
and ΔH°' = -9.5 kJ/mol

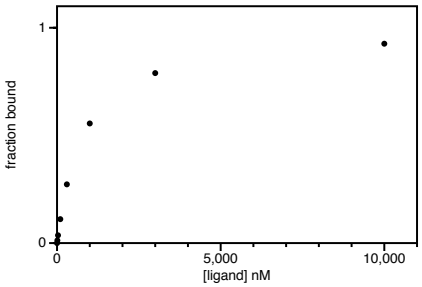
Q1A cont.
(iii) (2 points)
The ligand under study is the molecule cortisol shown below. Comment on the values obtained in the van't Hoff analysis and discuss in terms of a mechanism of binding.



ANSWER: The interaction has a moderately strong enthalpy component and a larger entropy component. Cortisol is a relatively hydrophobic molecule, which can contribute to the affinity through hydrophobic interactions. Water liberated from this binding may also contribute to a favorable entropy, as would the fact that the sterol ring is relatively rigid and would not suffer a large entropic cost by binding to the protein.

Q1B (10 points).

(i) (6 points) Imagine that you had carried out a binding analysis of the system using an alternative technique and had measured the binding isotherm as a function of [cortisol] concentration at 300K. Using the data from Q1A, calculate and annotate the plot with the K_D and concentration of ligand when the receptor is 90% occupied with cortisol.



ANSWER: At 300K for $\Delta G°' = \Delta H°' - T\Delta S°'$
$= -9.5$ kJ/mol - 300K * 85 J/ (mol*K) = -35 kJ/mol
$\Rightarrow \Delta G°' = RT \ln K_D = -35$ kJ/mol $\Rightarrow K_D = 800$ nM
$f_{bound} = [L] / ([L] + K_D) \Rightarrow 0.9 = [L] / ([L] + 800\ nM) \Rightarrow [L] = 7200\ nM$

(ii). (4 points). Suppose that the typical cortisol concentration found in the cell is 400 nM. Comment on the occupancy of receptor binding if cortisol level were to change dramatically and how this could affect the responsiveness of a signaling pathway to input.

ANSWER: At 400 nM the receptor would have a fractional occupancy < 50%. If levels were to change, such as to increase, this would dramatically alter the occupancy. This might allow the receptor to be sensitive to changes in ligand concentration and output a signal accordingly.

Q2 (20 points)
In the table of standard reduction potentials in the TMOL textbook, you may have noticed that two values are given for the reduction of the flavin-containing molecule FAD (an important electron carrier in the cell) as reproduced below. Answer the questions below, assuming the biochemical standard state unless noted.

For free FAD:	$FAD + 2H^+ + 2e^- \rightarrow FADH_2$	$E^{\circ\prime} = -0.219$ V
For protein bound FAD: (a 'flavoprotein')	$(FAD)_P + 2H^+ + 2e^- \rightarrow (FADH_2)_P$	$E^{\circ\prime} = -0.040$ V

Q2A. (6 points) An electrochemical cell is set up using electrodes under biochemical standard conditions. One half cell contains the free FAD solution and the other half cell contains a protein-bound FAD solution. What is the standard voltage and free energy associated with the cell? Specify which form of flavin is oxidized and which is reduced as a consequence of the reaction.

ANSWER: Side with more negative E°' will be the reducing agent (i.e. it oxidized), so :

$(FAD)_P + 2H^+ + 2e^- \rightarrow (FADH_2)_P$	***$E^{\circ\prime} = -0.040$ V is reduced***
$FADH_2 \rightarrow FAD + 2H^+ + 2e^-$	***$E^{\circ\prime} = +0.219$ V is oxidized (note direction is rev.)***
The net reaction is:	
$(FAD)_P + FADH_2 \rightarrow (FADH_2)_P + FAD$	***$\Delta E^{\circ\prime} = -0.040 + .219 = 0.179$ V.***

$\Delta G^\circ = - v \mathcal{F} \Delta E^\circ = -$ (2 electrons) $\times$ (96,500 C) $\times$ 0.179 V
$= -34.5$ kJ / mol

Q2B. (4 points) Suppose a new cell is created such that one half cell contains 10 μM of FAD and 100 μM $FADH_2$ and the other half cell contains 10 μM each of $(FAD)_P$ and $(FADH_2)_P$. Both half cells are at pH=7 and 300K. If these are connected with wires to a voltmeter, what would the cell potential be? What is the reaction free energy of under these conditions?

ANSWER: From Eq. 11.50
$\Delta E = \Delta E^\circ - RT / (v \mathbb{F}) \ln Q$
$= 0.179$ V $- 2500$ J / (2 electrons) $\times$ (96,500 C) * ln ($10^{-5} * 10^{-5} / (10^{-4} * 10^{-5})$)
$= 0.179$ V + 0.030 V = .209 V

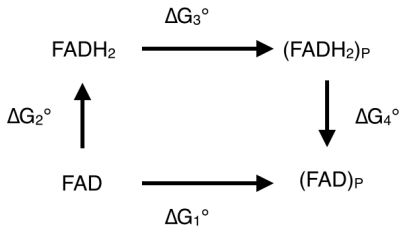
$\Delta G = - v \mathcal{F} \Delta E = -$ 2 electrons $\times$ 96,500 C *.209 V $= -$ 40.4 kJ/mol

Q2C. (10 points).

(i) (2 points) Flavin-based electron carriers like FAD are critical to the cell. Name an intermediate in their biosynthesis.

ANSWER: FAD stands for flavin adenine dinucleotide. Adenine, adenosine (AMP, ADP, ATP OK too), ribose, and riboflavin would all be acceptable.

(ii) (8 points)
Now consider the process of FAD and $FADH_2$ binding to the apoflavoprotein under biochemical standard conditions. Use the idea of a thermodynamic cycle to determine whether FAD or $FADH_2$ binds more tightly to the flavoprotein and what the ratio of binding constants must be. Note that apoflavoprotein is just the flavin binding protein before any form of flavin binds.



$\Delta G_1° = - RT \ln K_1$
$\Delta G_2° = - v \mathcal{F} E°' = - 2 \times 96{,}500\ C \times - 0.219\ V = 42.3\ kJ/mol$
$\Delta G_3° = - RT \ln K_3$
$\Delta G_4° = - v \mathcal{F} E°' = - 2 \times 96{,}500\ C \times + 0.04\ V = - 7.7\ kJ/mol$
$\Rightarrow \Delta G_1° = \Delta G_2° + \Delta G_3° + \Delta G_4° = 42.3\ kJ/mol + \Delta G_3° + - 7.7\ kJ/mol$
$\Rightarrow \Delta G_1° - \Delta G_3° = 34.6\ kJ/mol$
$= -RT \ln (K_1 / K_3)$ (from above)
$\Rightarrow$ combine terms. $K_1 / K_3 = \sim 9 \times 10^{-7}$. $\Rightarrow K_3 >> K_1$ and $FADH_2$ binds more tightly.

Q3. (20 points)

Q3A (10 points)
You are studying the first reaction in glycolysis (at 300 K) catalyzed by the enzyme hexokinase. The hexokinase reaction is the following:
ATP + Glucose $\rightleftharpoons$ ADP + Glucose 6-phosphate

(i) (1 point) The absolute value of the $\Delta G^{\circ\prime}$ is estimated to be of magnitude 17.3 kJ/mol. Is the sign of this value likely to be positive or negative. Briefly justify.

Answer: This reaction is coupled to the action of ATP. We know that there is significant amounts of free energy available in ATP hydrolysis resulting from the negative repulsion of the phosphoryl groups. Glucose 6-phosphate is a monophosphate and would not have this problem so it is likely this is of lower energy. Thus, the reaction is probable favorably and the sign is negative.

(ii) (3 points)
Suppose that the measured concentrations in E. coli are ATP = 10 mM, Pi = 10 μM, ADP = 0.5 mM, glucose is 10 mM, glucose 6-phosphate is 10 mM as well. Calculate the reaction free energy for the hexokinase reaction.

$\Delta G = \Delta G^{\circ\prime} + RT \ln Q$
$\Delta G = -17.3 + RT \ln ([10^{-2}] [5 \times 10^{-4}] / ([10^{-2}] [10^{-2}]))$
$= -17.3 - 7.5 = -24.8$ kJ/mol

(iii) (4 points)
When the cell is switched to a different carbon source, such as glycerol, the intracellular concentration of glucose will fall dramatically as it is no longer available for uptake, but the remaining reactants/products will stay at their original concentration. The cell needs a large pool of glucose (i.e. ~mM concentration) for other biochemical reactions. Solve for the concentration of glucose when this reaction is at equilibrium, assuming the concentrations of the other reactants and products are the same. What does this mean for other reactions in the cell that rely on glucose?

Answer:
This is a large problem for the cell because the reaction is coupled to the ATP pool directly and so any glucose is thermodynamically favored to be phosphorylated.

For example, the reaction will be $\Delta G = 0$ at roughly $Q = 10^3$ (i.e. e^(17.3/RT). This would only be possible if $Q = [10^{-2}] [5 \times 10^{-4}] / ([10^{-2}] [\text{glucose}]) = 10^3$
Solving for [glucose] = 5×10^{-7} M which is an extremely small concentration. This is about 100 molecules per the volume of a typical E. coli cell and would be unsuitable for facilitating the requisite biochemical pathways.

(iv) (2 points)
Propose a simple thermodynamically-favorable solution to this problem involving glucose-6-phosphate.

Answer: One possible solution is to simply hydrolyze the phosphoryl moiety of glucose-6-phosphate and produce glucose and Pi without a reaction directly involving ATP. This reaction is extremely thermodynamically favorable and is often used in biochemical pathways.

Q3B (4 points)
Suppose that when a cell is fed galactose (another sugar it can readily catabolize) at an external concentration of 100 mM, its internal concentration is 1 mM. Calculate the difference in the chemical potential of galactose for the intracellular and extracellular environments. Comment on the implication of this value for metabolic reactions that are coupled to the cellular galactose pool.

Answer:
The difference in chemical potential related to transfer of molecules from high potential to low potential is:
$\Delta\mu$ = RT ln (C2/C1)
= 2.5 x ln 0.01 = -11.5 kJ/mol

Thus, the entry of galactose into the cell is a favorable process that would readily happen. Likewise, since galactose is coupled with other biochemical reactions this favorable free energy may be coupled to and drive them forward.

Q3C. (6 points)

(i) (2 points)
The pKa of the commonly used buffer Tris is 8.1. Justify whether Tris is a good buffering agent by calculating the final pH of a solution of 1 mM Tris acid and 1 mM of conjugate Tris base in water (at 298 K) after the addition of 1 μM HCl.

Answer: before HCl, note that Tris is the dominant ion in solution (as opposed to H+ coming from water) so, pH = pKa + log ([A-]/[HA])
= 8.1 + log ([.001]/[.001] = 8.1

after HCl, a fully dissociated strong acid that titrates away conjugate base
pH = pKa + log (([A-] - HCl protonation effect)/([HA] + HCl protonation effect))
= 8.1 + log (([.001] - [0.00001]) / ([.001] + [0.00001]) = 8.0913

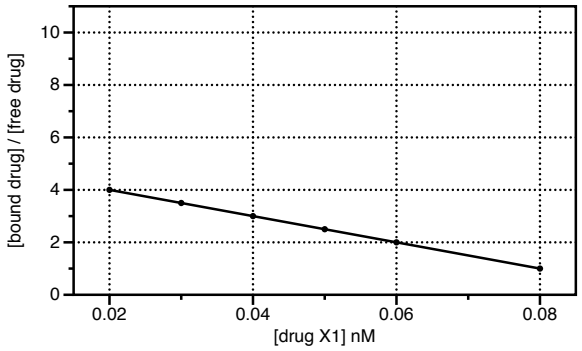
(ii) (4 points)
Calculate the concentrations of protons after the same addition of HCl to the water without the Tris buffer component.

Answer:
The concentration of H+ in water results from both the normal ion product dissociation of water (e.g. 10^{-7} M H+) and from the dissociation of HCl.

K_w = [H+] * [OH-] = 10^{-14} where [H+] = [H+(wat)] + [H+ (HCl)] and H+(wat) = [OH-]
by substitution and solving the quadratic, H+(wat) = [OH-] = 10^{-8} M

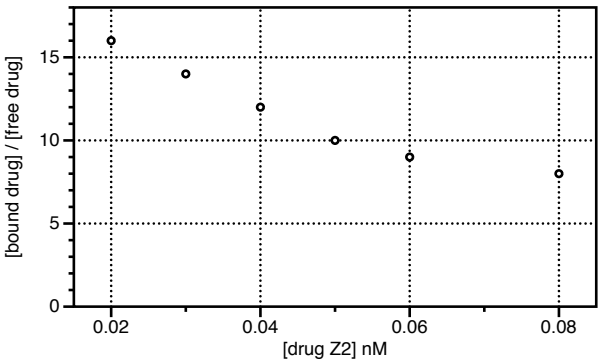
[H+] = [H+(wat)] + [H+ (HCl)] = 10^{-8} M + 10^{-6} M = 1.01 x 10^{-6} M

Q4 (20 points)
Q4A (10 points)
(i) (5 points)
A scientist working at a biotech company screens a series of potential new drugs for targeting a receptor on cancer cells that is known to be oncogenic. Drug X1 is thought to be particularly promising and a Scatchard analysis is performed with it using lysates from cancer cells. The curve below is obtained. Calculate the K_D for drug X1 and the concentration of the oncogenic receptor protein.



Answer: The fit of the plot to a Scatchard equation is that: K_D = 0.02 nM and $[P]_{tot}$ = 0.1 nM

(ii) (5 points)
During screening, a second potential drug target, Z2, is also identified. A pilot Scatchard analysis is performed at low [Z2] and suggests the molecule is promising. A larger screen of concentrations is performed and the plot below is obtained. Describe the argument for and against pursuing Z2 as a new drug lead, including a specific mechanism against it.



Answer: Z2 is apparently promising but has significant issues. The initial slope of the curve at low [Z2] suggest the molecule is particularly potent. This slope is 4x steeper than X1 above and the K_D is 0.005 nM. However, at higher concentrations the curve becomes non-linear. An interpretation of this curve is that there is a higher amount of bound drug than would be expected for a given concentration. In the absence of cooperativity (which could be an answer), the most like scenario is that there is some non-specific binding to a different, non-target protein. This would likely reduce the chance of producing a 'clean' targeting drug specific to the receptor of interest.

Q4B. (10 points)
The following experiments are carried out at 300K and pH 7.

(i) (2 points) Structural analysis of the active site of the WT receptor protein in Q3A above reveals there is a critical Asp residue that is directly adjacent to a Glu. What is the qualitative effect of the latter on the protonation state of the former?

Answer: Placing two like-charged residues nearby one another will have the opposite effect of the stabilizing interaction discussed in class. For this reason, it is likely that the pK_a of the residue will become less acidic, i.e. rise.

(ii) (4 points)
In the mutated oncogenic form of the receptor, the Glu residue is mutated to Lys. The original Asp pK_a was 5. The Lys interaction stabilizes the deprotonated state by a difference of 8 kJ/mol. What is the pK_a of this Asp in the mutated receptor?

Answer:
For the WT protein we can determine a ΔG of deprotonation as follows:
$\log ([A^-]/[HA] = pH - pK_a = 2 = \log K$ and thus $K = 100$
$\Rightarrow \Delta G\ (WT) = - RT \ln K = - 11.5$ kJ/mol

$\Delta G = \Delta G\ (WT) + \Delta G\ (mut)$
$= -11.5 + -8 = - 19.5$ kJ/mol $\Rightarrow$ $K = 2441$

$pK_a = pH - \log ([A^-]/[HA] = 7 - \log (2441) = 7 - 3.4 = 3.6$

(iii) (3 points)
Binding of the X1 drug in Q4A above disrupts the Asp-Lys interaction such that the Lys is free to adopt other conformations. Structural analysis by the laboratories of Jane and David Richardson at Duke U. has shown there are 27 relatively common rotamers for Lys. Use this information to make a simple estimate of the upper bound for the contribution of the entropy of Lys to the binding energy of X1.

$\Delta S\ (Lys) = S_{bound} - S_{unbound}$
Assuming an increase in multiplicity from 1 to 27
$\Delta S\ (Lys) = R \ln 27 - R \ln 1 = R * (3.3 - 0) = 27.4$ J / (mol*K)
at 300K this would be $- \Delta S * T = -8.2$ kJ/mol

(iv) (1 points)
What assumption about Lys rotamers does this estimate require?

Answer:
This assumes that the rotamers are all equally likely, i.e. they are isoenergetic.

Q5 (20 points).
Multiple choice, fill in the blank, and True/False questions. **Circle** the ***best*** option or write in blank.
+2 points for each correct answer and -1 point for each wrong answer. To get the maximum score you do not need to answer all of the questions so be careful not to answer questions incorrectly. Maximum points: 20. Minimum points: 0.

(i) The correct units for K_D are:
a. M^1
b. M^{-1}
c. $M^{-1} \cdot sec^{-1}$
d. it depends on the rate constant
e. none of the above

(ii) If the concentration of a reactant for a reaction is increased then the free energy of the reaction will be decreased.
True / False

(iii) The lowest value of pH possible is 0.
True / **False**

(iv) Chemical reactions with a large negative ΔG° value occur rapidly.
True / **False**

(v) The value of an equilibrium constant will increase with increasing temperature.
True / **False**

(vi) Protein stability curves are well described by using the van't Hoff relationship.
True / **False**

(vii) The idea that a negative Gibbs free energy for a reaction means it is spontaneous process applies only for a system isolated from the surroundings.
True / **False**

(viii) A rough estimate for the work required by a cell to synthesize ATP is:
a. 60 kJ/mol
b. 30 kJ/mol
c. -30 kJ/mol
d. -60 kJ/mol
e. none of the above

(ix) A negative ΔG° means the reaction will go to completion.
True / **False**

(x) The chemical potential for a reactant is proportional to the logarithm of concentration of the reactant.
True / False

(xi) An adjacent positive charge is likely to raise the pK_a of a lysine side chain.
True / **False**

(xii) Potential is best described as the force per charge.
True / **False**

(xiii)
The thermodynamics of protein folding is usually the same, regardless of the protein.
True / False

(xiv) A buffer is always most robust to pH changes near its pK_a.
True / False