UNIVERSITY OF CALIFORNIA, BERKELEY
CHEM C130/MCB C100A
MIDTERM EXAMINATION #3
November 19th, 2014

INSTRUCTORS: John Kuriyan and David Savage

THE TIME LIMIT FOR THIS EXAMINATION: 1 HOUR 50 MINUTES

SIGNATURE:

_____

Please **SIGN** your name on the line above in **INDELIBLE INK**.

YOUR NAME:

_____

PLEASE PRINT 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:

Pradeep Bandaru Caroline Cypranowska

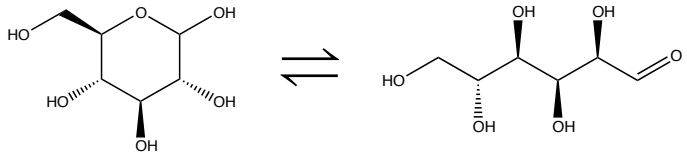
Julian Hassinger Madeleine Jensen

Laura Nocka

PLEASE WRITE all of your answers **AS LEGIBLY AS POSSIBLE**.
Note that any exam submitted for a regrade should be written in indelible ink.
SCORING. The exam consists of 6 questions totaling 100 pts as broken down in this table:

Question	Part A	Part B	Part C	Part D	Your Total	Max Score
1.	4	5	4	5		18
2.	3	4	10	5		22
3.	5	11	4			20
4.	4	4	4			12
5	6	7	5			18
6	10					10
				TOTAL		100

Q1. (18 points). Sugars exist in an equilibria between linear and cyclized forms, as shown for the hexose molecule below. Answer the following questions regarding equilibria.



Q1A (4 points)

A researcher is investigating the relative energetics of these two forms. She finds that at 1 mM concentration, the hexose is 99.98% in the cyclic form at 25° C. Calculate the standard free energy change for forming the cyclic form from linear at this temperature.

B. (5 points).

In trying to conceptualize what could be driving the equilibrium, the researcher wanted to develop a model for the entropy change upon cyclization. Her model assumes that there are approximately three equal energy rotational conformations about each linear C-C bond. Using this assumption, estimate the standard entropy change due to the loss of conformational freedom for hexose in the cyclic state. State any additional assumptions.

Q1C (4points)
It turns out that although this calculation does a good job of estimating the entropy changes due to freedom of rotation, it does not do a good job of predicting the actual entropy for the reaction. What is missing from the calculation that may explain the error?

Q1D (5 points)
The researcher now repeats the experiment Q1A at a temperature of 70° C and finds the sugar is 98% cyclic. Calculate the standard state enthalpy change for forming the cyclic hexose form.

Q2. (22 points) Many of the enzymes in central carbon metabolism are crucial to a tumor's ability to grow and are potential cancer drug targets. While working at a biotech company on the peninsula, you perform a screen of natural product inhibitors against Enzyme X in this pathway, which happens to be a dimer, and isolate two potential drug candidates: A2 and G9. Carefully answer the questions regarding A2 and G9 binding Enzyme X below.

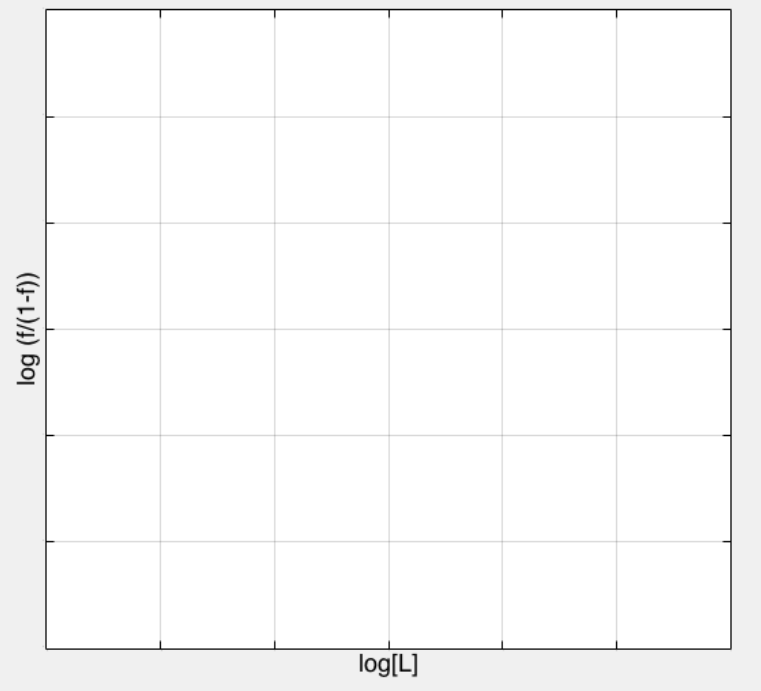
Q2A (3 points)
In binding assays, A2 appears to bind only once to X and has a measured K_D of 50 nM for X. What is the fraction of protein with ligand bound when [A2] = 100 nM?

Q2B (4 points)
The binding of G9 to X appears to involve an allosteric mechanism and two different K_Ds are measured: K_{D1} = 10 nM and K_{D2} = 1 nM. What is the fraction of protein with ligand bound when [G9] = 100 nM?

Q2C (10 points)

i. (5 points). Using the equation for ***f***, the fraction of bound protein in a single-site binding model, derive an expression for log (f/(f-1)) as a function of log([L]).

ii. (5 points) Use your knowledge of the expression above to graph the binding isotherm for each single binding site as well as the cooperative binding isotherm for G9 binding Enzyme X. Note on the plot where one can determine binding parameters including the Hill Coefficient and whether this specific binding mechanism displays positive or negative cooperativity.



Q2D (5 points)
G9 is extremely effective in chemotherapy trials and is a blockbuster drug. Unfortunately, in some patients with aggressive cancers, mutations arise in Enzyme X that reduces the potency of G9. These mutations are found on the dimer interface and reduce the Hill Coefficient to n_H = 0.25. K_{D1} does not change. What is the new K_{D2} and what is the fraction of protein with bound drug at 100 nM now?

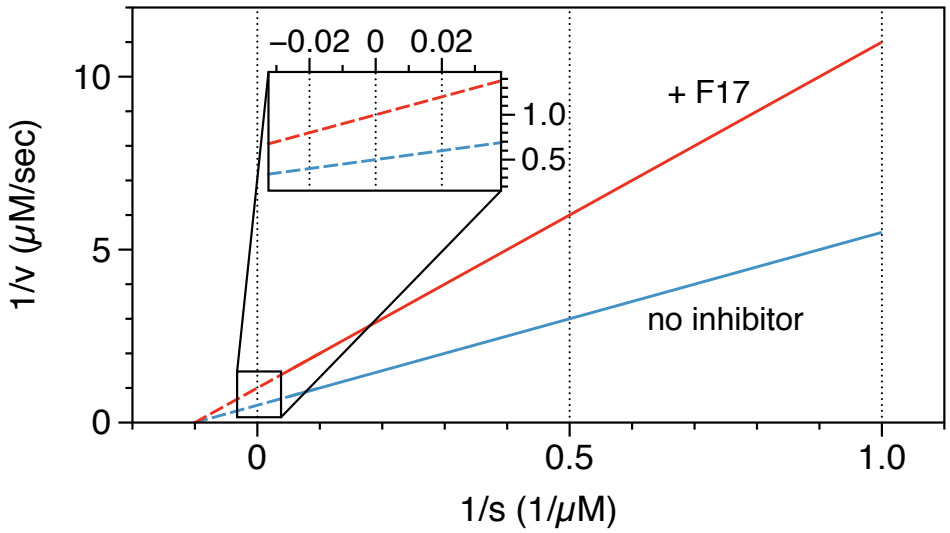
Q3. (20 points) Let's continue with the same system as above, but now focus on kinetics and enzyme mechanism.

3A (5 points)
Enzyme X catalyzes the reaction: A + B $\rightleftharpoons$ C. However, B is at high concentrations inside the cell and can effectively be ignored in the kinetic analysis.

i. (2 points) This reaction is therefore _____ order but can be treated as _____ order in a kinetic analysis.

ii). (3 points). Ignoring reversibility and any specific enzyme mechanism model for the moment, you perform a kinetic analysis of the rate of this reaction in the presence of enzyme catalyst and measure an effective rate constant of k' = 200 sec^{-1} for the decrease in [A]. Suppose that you initiate a reaction and follow the decrease of [A] in time. After 0.01 sec, what percent of the initial amount of [A] is left?

Q3B (11 points)
Unfortunately, the drug resistance mutations noted in Question 2D continue to plague G9 and eventually the drug is scrapped as a therapeutic. Your medicinal chemistry team restarts the project and isolates a third molecule, F17. You perform a detailed enzyme kinetic analysis in the presence and absence of the F17 inhibitor. The data is shown below. Answer the following questions.



Q3B cont.

i. (5 points). Using the plot above, solve for the V_{max} and K_M of the enzyme without inhibitor and the apparent V^*_{max} and K^*_M upon addition of inhibitor.

ii. (3 points). What mode of inhibition does F17 have? Justify.

iii. (3 points). What does this mechanism mean about drug binding with respect to the natural substrates?

Q3C (4 points)
Unfortunately, your new molecule F17 is not as potent as A2 and G9 and your team needs to increase the affinity of F17 for Enzyme X. What is a general strategy you could take? Include a discussion of entropy.

Q4.(12 points). Let's continue with enzyme mechanism but now focus on pH and equilibria.

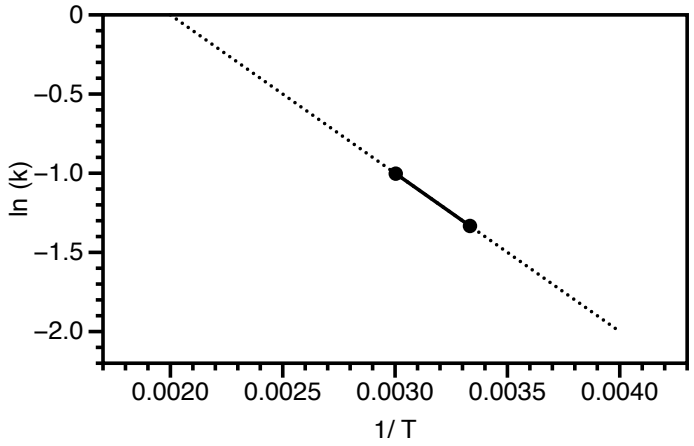
Q4A (4 points)
Histidine side chains are commonly employed for catalysis in the active sites of enzymes. Suppose the pKa of His (for the 2nd proton) in a typical protein is 7.0. Calculate the fraction of side chain which exists in the protonated state at pH 7.4.

Q4B (4 points)
In many proteases, the hydroxyl side chain of an active site Ser is deprotonated and therefore activated for a nucleophilic attack on the polypeptide substrate. The initial deprotonation is accomplished by an adjacent His. Suppose there is a 3rd amino acid in the active site, which serves to adjust the pKa of His. Hypothesize what the identity of this amino acid could be and justify your answer. Is the pKa higher or lower?

Q4C (4 points)
Due to this unique orientation of the active site, the histidine is 99.99% protonated at pH 7.4. What is its pKa?

Q5 (18 points)

Q5A. (6 points) Imagine that your are studying an uncatalyzed first order reaction, using an Arrhenius Plot analysis. You generate the following linear data (focus on points noted, for simplicity).



The values for the data points drawn as circles are:
(0.00300, -1.003)
and (0.00333, -1.3333)

What is the activation energy and preexponential factor for this reaction? You must have correct units.

Q5B. (7 points)

i. (4 points) An enzyme accelerates the rate 10x over the uncatalyzed reaction by perturbing the activation energy. Calculate the difference in the activation energy.

Q5B cont.

ii. (3 points). What happens to the rate of this reaction when the amount of catalyst is doubled? Assume that the [substrate] > [enzyme]. Justify.

Q5C. (5 points).

i. (2 points). The E_A (enzyme) is less than E_A (uncatalyzed). How does this change the spontaneity of the reaction?

ii. (3 points). Describe the general molecular mechanism the enzyme is using to facilitate this rate increase.

Q6 (10 points). Multiple choice, fill in the blank, and True/False questions. Circle the correct option (or circle either TRUE or FALSE). +1 points for each correct answer, -0.5 point for each wrong answer. Be careful not to answer questions incorrectly. Unanswered questions do not change the score. Minimum points is 0.

(i) The correct units for the dissociation constant, 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) The specificity constant or catalytic efficiency is the ratio between _____ and _____.

(iii) A typical drug has a dissociation constant for its target in the range of:

a. mM - μM

b. μM - nM

c. pM - nM

d. pM - fM

(iv) The half-life of a zero order reaction depends on the concentration of the reactant. True / False.

(v) In our simple model of protein folding the entropy of the unfolded state is proportional to the _____ of the number of conformations.

(vi) The diffusion limit sets a maximum on the rate an enzyme can catalyze a reaction. True / False.

(vii) For a multi-step reaction, the overall rate is best described by:

a. the slowest rate constant

b. the fastest rate constant

c. the sum of the rate constants

(viii) In a differential scanning calorimeter, what parameter is actually scanned: _____.

(ix) Reversible reactions, by definition, have similar forward and reverse rates constants. True / False.

(x) A hyperbolic binding curve is indicative of a mechanism involving allostery. True / False.