UNIVERSITY OF CALIFORNIA, BERKELEY
CHEM C130/MCB C100A
MIDTERM EXAMINATION #3
November 18th, 2015

INSTRUCTORS: John Kuriyan and David Savage

THE TIME LIMIT FOR THIS EXAMINATION: 1 HOUR 50 MINUTES

SIGNATURE: _____

YOUR NAME:_____

YOUR STUDENT ID NUMBER:_____

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:

Eric Greene Helen Hobbs

Madeleine Jensen Robert Louder

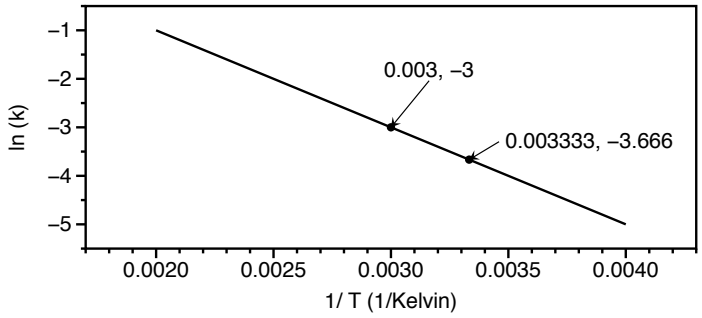
Piere Rodriquez

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 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.	6	10	4			20
2.	10	6	4			20
3.	6	4	5	5		20
4.	12	8				20
5						20
				TOTAL		100

Q1 (20 points)

Q1A. (6 points) Imagine that your are studying an uncatalyzed, reversible, first order reaction of the form **A** ⇌ **B** using an Arrhenius Plot analysis. You generate the following data:



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

ln (k) = (-E_A / R) * 1/T + ln (A)
Slope = (-3.666 + 3) / (.003333 - 0.003) = -2000 = -E_A / R. E_A = 16.6 kJ/mol

Substitute to solve for A. ⇒ -3 = -2000 * 0.003 + ln(A).

ln(A) = 3.
A = 20.1
Units for A must match the rate constant, which for a first order reaction is sec^{-1}

Q1B. (10 points)

i. (3 points) Suppose that an enzyme catalyzes this reaction only by stabilizing the transition state and has an E_A = 5 kJ/mol. How much does the enzyme accelerate the rate of this reaction at 300K?

$k_{cat} / k_{uncat} = e^{(-(E(cat) - E(uncat)))/RT} = e^{-(5-16)/ 2.5)} \approx 104$ fold

Q1B. continued.

ii. (3 points) Your colleague down the hall works on an enzyme that is not homologous to your enzyme but can catalyze the same reaction. You test it and find that it yields an even greater rate acceleration than your enzyme. However, the Arrhenius analysis says the E_A is still 5 kJ/mol. Give two reasons for how this is possible.

There are three possible mechanisms used by enzymes to catalyze a reaction: i. lower the activation energy, increase the pre-exponential constant by structural orientation (electrostatic steering) of the reactants and catalyzing the reaction by a different mechanism altogether. Since this enzyme is not homologous (i.e. it's a different protein altogether) the latter are reasonable explanations.

iii. (2 points) Consider the uncatalyzed reaction along a simple reaction coordinate in which the products are of lower energy than the reactants. Ignoring the effect of concentration, comment on the relative rates of the forward and reverse reactions.

If ΔG is < 0, the forward reaction would have a smaller activation energy and would be faster than the reverse.

iv. (2 points) Now consider your enzyme from (i) above. How does it affect the forward and reverse reaction rates?

T**he enzyme lowers E_A but does so for both directions. If you calculate the rate enhancement for both the forward and the reverse, you will find that the enzyme affects both rates equally.**

Q1C. (4 points) You perform a Michaelis-Menten analysis of your enzyme and measure the k_{cat} and K_M. You can use these parameters to calculate a second order rate constant for the reaction. Describe what a second order rate constant means in our Michaelis-Menten model and state the units. Under what experimental conditions is it valid?

A second order rate constant is the proportionality constant describing the reaction rate for two interacting species. In this case, the enzyme and the reactant A. A second order rate constant has units M^{-1} s^{-1}.

Recall that $v_0 = V_{max} / (1 + K_m / [S])$. At very low substrate concentrations, this simplifies to $v_0 \approx V_{max} / (K_m / [S])$. Since $V_{max} = k_2 * [E]_0$, this can be rewritten to $v_0 \approx (k_2 / K_M) * [E]_0 * [S]$, which has the expected form of a second order reaction with rate constant (k_2 / K_M).

Q2. (20 points)

Q2A. (10 points)

Consider a protein 200 residues in length at 300K. We wish to understand the energetics behind its folding by developing a simple model in a vacuum. First, consider the entropy changes upon folding.

i. (2 points) Suppose that you assume each residue has 5 possible conformations. Ignoring differences in amino acid side chains, what does the assumption require?

The underlying assumption is that the residues are independent of one another and that the polymer can cross over itself. We know this is not true (i.e. the Ramachandran diagram) but is a reasonable assumption for an order of magnitude calculation.

ii. (6 points) Using the assumption of 5 conformers above, calculate the ΔS° for the folding reaction in a vacuum.

Use the statistical entropy definition, S = k ln W, to carry out this calculation.

Since each residue can adopt 5 conformations, the entropy of the unfolded state is:
$S^\circ_{unfolded}$ = R ln (5^200) = R * 200 ln (5) = 2675 J/(K mol).

S°_{folded} = 0 as an assumption that there is only 1 native state.

Thus, ΔS° = -2675 J/(K mol).

iii. (2 points). This calculation leaves out one important component of the entropic forces which shapes the protein folding problem. What is that?

The effect of water is completely ignored here. Water is liberated from interacting with hydrophobic groups in the unfolded protein (aka the hydrophobic effect) resulting in a favorable entropic gain.

Q2B. (6 points) Suppose that there is no interaction between residues in the unfolded state. What would the per residue enthalpic contribution have to be for the protein to be 10% folded at 300 K.

First, calculate the $\Delta G°$ for K_{fold} = [folded] / [unfolded]
$\Delta G° = - RT \ln K_{fold} = -RT \ln (.1 / .9) = +5.5$ kJ/mol

Second, $\Delta G° = \Delta H° - T\Delta S°$
and thus, $\Delta H° = \Delta G° + T\Delta S° = 5.5$ kJ/mol + 300 K * -2675 J/(K mol) = 802.5 kJ/mol

On a per residue basis this is roughly 800 / 200 ≈ 4 kJ/mol, which is actually a reasonable number, given our knowledge of energetics.

Q2C. (4 points). Consider protein folding as a chemical reaction, that is a reaction of the form U ⇌ F. It turns out that some expressed proteins remain folded and functional despite the folded state being thermodynamically unfavorable. Propose a mechanism for this observation using only the concept of the reaction coordinate.

There are some proteins that are kinetically 'trapped' in a folded state. That is, although the free energy favors the unfolded state, the kinetic barrier (i.e. E_A) is so large that they are trapped in the active form and do not unfold for long periods ($t_{1/2}$ can be on the order of years) of time.

Q3. (20 points)
A chemist analyzing the reaction of Compound X being converted to a single product (P) makes the following observations. Answer the questions below.

Q3A. (6 points) She starts the reaction with 50 mM substrate and measures an initial rate of consumption of 100 mM/min. Curious, she sets up another reaction with 25 mM substrate and measures an initial rate of 12.5 mM/min. What is the differential rate law describing the normalized reaction rate of X consumption as a function of time?

The reaction order is unknown.

rate = -k [X]^y

rate1/rate2 = 8 = -k[0.05]^y / -k[0.025]^y

solving for y yields y = 3

The reaction is third order and the rate equation is proportional to d[X]/dt = - k [X] ^3

To properly account for the stoichiometry, i.e. that 3 molecules of reactant are consumed to make only 1 molecule of product, the corrected rate equation is thus:

1/3 * d[X]/dt = - k [X] ^3

Q3B. (4 points) What is the rate constant for the reaction, making sure to have proper units?

The rate constant can be determined by substitution into the equation rate = -k [X]^y.
I.e. (1/3) * -100 M/min = -k [0.05M]^3

Solve for k = 267 <u>M^-2*min^-1</u>

Q3C. (5 points) The original reaction was carried out at 20 °C. She then heats the reaction to 50 °C and determines it goes 10 times faster. At what temperature would the reaction go 100 times faster?

The ratio of the Arrhenius equation k = A*e^-(E_A / RT) can be solved:

1/10 = A*e^-(E_A / R*293) / A*e^-(E_A / R* 323)

To calculate E_A = 61.4 kJ/mol

This value can be plugged back into the equation to determine at what T, k is 100x

1/100 = A*e^-(61,400 / R*293) / A*e^-(61,400 / R* T)

Solving, yields T = 358.5 K or 85.5 °C

Q3D. (5 points) The reaction at 20 °C (and 50 mM initial reactant) is allowed to reach equilibrium and the chemist determines that only 2 mM of reactant remains in the flask. What is the ∆G° for the reaction?

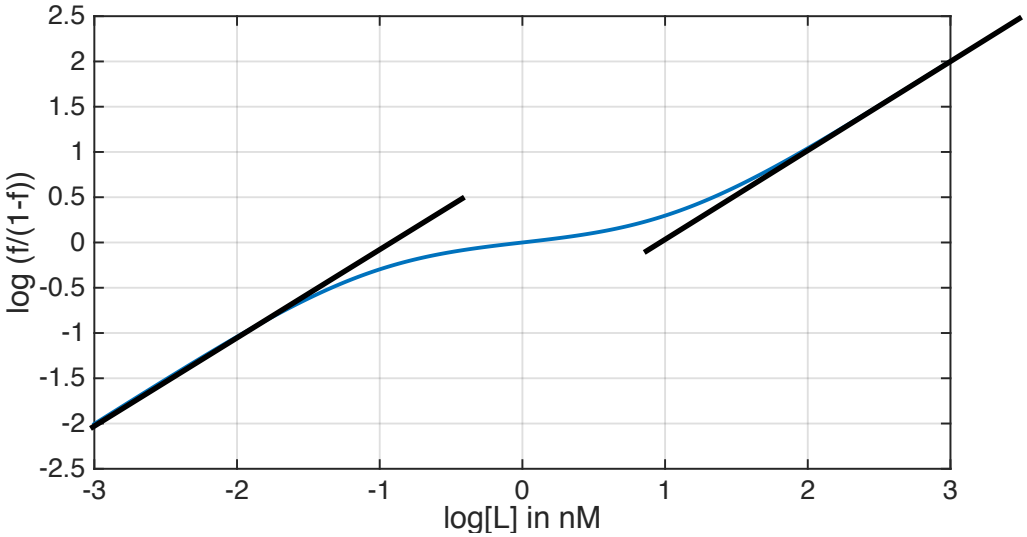
If 2 mM reactant remains, then 48 mM was consumed producing 48 / 3 = 16 mM of product.

Solve for K_{eq} = [product] / [reactant]^3 = [0.016 M] / [0.002]^3 = 2 x 10^6

∆G° = - RT ln K_{eq} = - * 8.314 * 293 * ln (2 x 10^6) = -35.3 kJ/mol

Q4: (20 points).
Q4A. (12 points) Shown below is a binding isotherm for a ligand binding to a protein.

(i). (4 points) The protein has two binding sites for the ligand. What are the K_D values for each binding site? Mark on the plot how this is determined.



K_{D1} = 10^{-1} = 0.1 nM
K_{D2} = 10^{1} = 10 nM

(ii) Calculate the Hill Coefficient. What type of cooperativity does the binding display? (4 points)

From Eq14.6 n_H = 2 / (1 + (K_{D2}/K_{D1})^.5) = 2 / (1 + (10/.1)^.5) = 0.18

K_{D1} < K_{D2} (or n_H < 1) so the protein exhibits negative cooperativity.
This could also be estimated from slope at y = 0.

(iii) What fraction of protein is bound with ligand when [L] = 1 μM? (4 points)

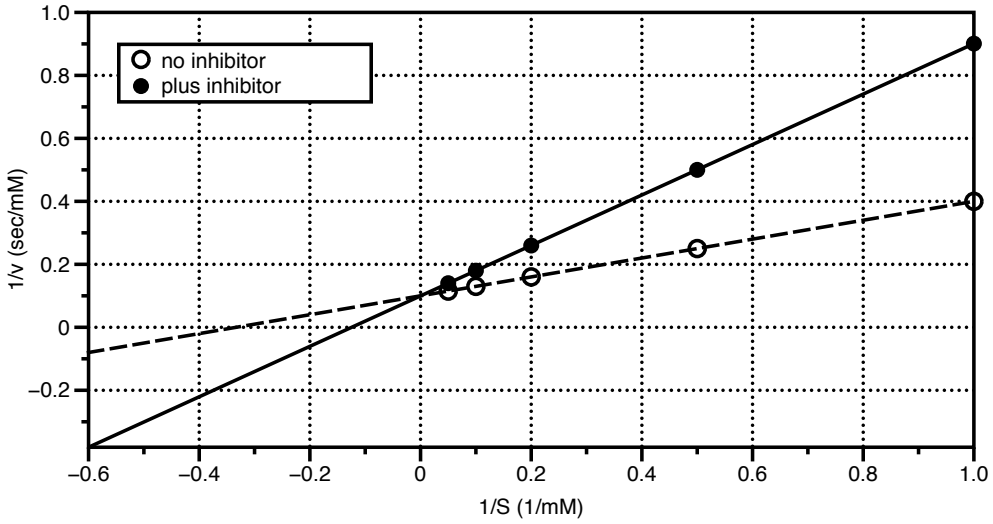
Eq. 14.5 is derived under the assumption of positive cooperativity and is therefore not appropriate.

However, from the plot above, f/(1-f) = 10^2 = 100 when [L] = 1 μM. Solving, yields f ≈ 99%.

Q4B. (8 points)

You have analyzed an enzyme with Michaelis-Menten kinetics in the absence and presence of an inhibitor. Answer the following questions:

(i). (4 points) The data are displayed below. What are the apparent values for V_{max} and K_M in each case? Show your work or mark on the plot how you accomplished this.



No treatment:

Slope = K_M / V_{max} = 0.3

Substitute slope into 1/v = slope × 1 / [S] + 1/V_{max} @ one data point

E.g. .25 = 0.3 × 0.5 + 1/V_{max} ⇒ V_{max} = 10 mM/sec

K_M / V_{max} = 0.3 ⇒ K_M = 3 mM

+ Inhibitor:

Slope = K_M / V_{max} = 0.8

Substitute slope into 1/v = slope × 1 / [S] + 1/V_{max} @ one data point

E.g. .5 = 0.8 × 0.5 + 1/V_{max} ⇒ V_{max} = 10 mM/sec

K_M / V_{max} = 0.8 ⇒ K^*_M = 8 mM

Or by y intercept = -1/Km and x intercept = 1/Vmax

(ii). (2 points) What type of inhibitor is the molecule?

The inhibitor changes the slope of the line (i.e. the y intercept or K_M) and is therefore a competitive inhibitor.

(iii). (2 points) The inhibition experiment was carried out at 100 μM inhibitor. Calculate the K_I for the molecule.

K^*_M = K_M / (1 + [I]/K_I)

K^*_M = 8 = 3* (1+ [0.1] / K_I) ⇒ K_I = 0.060 mM

Q5 (20 points).
Multiple choice, fill in the blank, and True/False questions. **Circle** the correct 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. Unanswered questions do not change the score. 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) The rate of substrate binding to an enzyme is a first order kinetic process.
True / **False**

(iii) If a solution is made by adding 10 mM (final concentration) of weak acid to water, the pH will equal the pK_A of the acid.
True / **False**

(iv) Positional entropy suggests that is it favorable for a hydrophobic molecule and water to mix at low concentrations.
True / False

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

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

(vii) 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

(viii) The van't Hoff equation can be used to describe the stability of a protein as a function of temperature.
True / **False**

(ix) In a differential scanning calorimeter, the parameter ___**temperature**____ is scanned.

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

a. <u>the slowest rate constant</u>
b. the fastest rate constant
c. the sum of the rate constants

(xi) A catalyzed reaction is more likely to be limited by how fast the molecules can diffuse in the solution than an uncatalyzed one.
<u>True</u> / False

(xii) A negative $\Delta G°$ means the reaction will go to completion.
True / **<u>False</u>**

(xiii) The chemical potential for a reactant is linearly proportional to the concentration of the reactant.
True / **<u>False</u>**

(xiv) The rate of substrate binding typically limits the overall rate of product formation for an enzyme.
True / **<u>False</u>**

(xv) An adjacent negative charge is likely to raise the pK_A of a lysine side chain.
<u>True</u> / False