Quantitative Physiology I / Molecular and Cellular Systems; BMEN E4001x
HW1: Chemical Kinetics & Equilibria
Due September 25, 2025, 11:00PM US Eastern Time

1) Enzyme kinetics (10 points total)

An Enzyme (E), that you are working with in the lab acts on a Substrate (S) to yield a Product (P). Preliminary experiments suggest that in the presence of an Activator (A) molecule, the reaction rate speeds up. You hypothesize that this activator is allosteric, interacting with a site on the enzyme that is separate from the substrate binding site. Your goal is to predict how the reaction rate changes as a function of Substrate and Activator concentration in this scenario, which will be tested in subsequent experiments. Assume:

- The Enzyme (E) has two binding sites, one for Substrate (S) and a separate one for Activator (A).
- Binding of E to S and A is independent and reversible, meaning that the presence of S or A in the respective binding site does not affect the binding of the other molecule to the enzyme.
- An intermediate complex of E bound to S, with no A concurrently bound to the enzyme, can irreversibly create Product (P) at a fixed, basal rate. E is regenerated in this step. This is the basic enzymatic pathway discussed in class.
- An intermediate complex of E bound to both S and A can irreversibly create P at a rate higher than that of the ES intermediate. Upon creation of the product, the enzyme-activator complex (EA) is regenerated.
- Binding of S, A, and E are considered biomolecular reactions. That is, trimolecular interactions of A and S binding to E in a single step are rare and can be ignored for this problem. Unbinding of S and A from a complex of E, A, and S in a single step can similarly be ignored.

1.1) Draw a diagram of the reaction system. Include all fundamental rate constants. (2 pts)

1.2) List the set of differential equations that describes the time evolution of E, S, A, P, and all intermediates. Also provide a conservation equation that includes all forms of the enzyme. (2 pts)

1.3) List the simplified equations that result from applying the Equilibrium approximation to this system. Simply list, do not solve the system of equations. (2 pts)

1.4) List the simplified equations that result from applying the Quasi-State approximation to this system. Simply list but do not solve the system of equations. (2 pts)

1.5) Derive an expression for reaction rate (V) as a function of the concentrations of substrate (S) and activator (A), as well as total enzyme concentration E_0. (2 pts)

2&3) Enzyme inhibition (10 points)

2) You are investigating the efficacy of a new antibiotic agent that inhibits the activity of an enzyme, E, which converts a substrate, S, into a product, P, which is essential for bacterial function.

Your task this week is to compare two strains of bacteria, X and Y, which express different versions of the enzyme. Some details

- Substrate, S, is present at 15 mM inside the bacteria
- In the absence of inhibitor, both strains of bacteria produce product at 5 mM/min.
- The inhibitor, I, acts as an uncompetitive inhibitor.
- At [I] = 200 μM, strain X bacteria are killed.

	strain X	strain Y
K_M, binding of S to E	15 mM	30 mM
K_I, binding of I to E	100 μM	60 μM

2.1) Determine the maximum reaction rate of strain X compared to strain Y.
That is, calculate $V_{max,,X}/V_{max,Y}$. (1 pt)

2.2) For strain X, determine the reaction rate below which cells are killed. (1 pt)

2.3) What concentration of Inhibitor is needed to kill strain B? Assume strain Y dies at the same reaction rate as for strain X, which you calculated in 2.2. (1 pt)

2.4) For both strains at [I] = 100 μM, calculate the fraction of enzymatic activity remaining relative to the uninhibited condition.
That is, calculate V([I]=100 μM)/V([I]=0 μM) for strains X and Y (1 pt)

2.5) Sketch V vs. [I] for both strains on the same axes. Which strain has a steeper slope? (1 pt)

For your reflection (not graded):

- The targeted enzymes in strains X and Y have different values of K_M and K_I. Which of these parameters (K_M or K_I) is more important in determining the effect of the inhibitor on enzyme function? What information is needed to make this choice?
- Molecules that act through uncompetitive inhibition are rare in bacterial systems. Explore examples of how uncompetitive inhibition are used in other systems.
- Your lab just developed a variant of this inhibitor that acts as a noncompetitive counterpart, with the same K_M and K_I values for each strain. Is this a better inhibitor than the original, uncompetitive version?

3) Data of an enzyme inhibition experiment are presented in the table below, consisting of the rate of reaction (in mM /s) as a function of substrate concentration [S] (in mM) and inhibitor concentration [I] (also in mM). Assuming that enzyme is always present at a concentration of 100 nM:

Reaction rates (mM / s)	***Inhibitor concentration [I] (mM)***		
	0	**0.2**	**0.4**
Substrate concentration [S] (mM)			
0.1	1.031	0.374	0.279
0.2	1.515	0.422	0.305
0.4	2.000	0.451	0.319
0.8	2.299	0.467	0.327
1.5	2.500	0.475	0.331
2	2.500	0.477	0.332

Please note, question 3 is separate from 2. These are different experiments.

3.1) Estimate V_{max}, K_m, and K_i; remember, V_{max} and K_m are based on just the interactions between substrate and enzyme, and are independent of the effect of inhibitor. (4 pts)

3.2) Identify the mode of inhibition (competitive, noncompetitive, or uncompetitive), and explain this choice. (1 pt)

4) Hemoglobin (10 points)

In class, it was calculated that the capacity of blood to contain dissolved oxygen is not enough to supply the body's oxygen needs. Specifically:

- A 70kg body at rest needs 1.2e-2 mol/min O_2
- $[O_2]_{dissolved}=s_{O2}*P_{O2}$. For O_2 into aqueous solutions, s_{O2}=1.4e-6 M/mmHg
- In the lungs, P_{O2}=100 mmHg, in tissues, P_{O2}=40 mmHg.
- Cardiac output is 5 L/min.

Thus, the rate of delivery of O_2 by dissolved gas is:

$[P_{O2,lungs}* s_{O2}- P_{O2,tissues}* s_{O2}]$*cardiac output =

[(100 mmHg)*(1.4e-6M/mmHg)-(40 mmHg)*(1.4e-6M/mmHg)*(5 L/min) = 4.2e-4 mol/min

Hemoglobin (Hb) helps to meet this demand:

- Hb is present at 2.3 mM
- Binding constant of Hb with O_2: K_M = 25.85 mmHg
- Each Hb molecule can bind 4 O_2 molecules
- The behavior of Hb can be estimated with good success by the Hill equation, with n = 2.45

4.1) With these assumptions, what is the oxygen delivery capacity (how fast oxygen can be transported from lungs to tissue) of blood (in mol/min) with this hemoglobin? (2 pts)

4.2) Suppose we could control the Hill coefficient for Hemoglobin. What is the oxygen delivery capacity for n=1? for n=4? (2 pts)

4.3) What is the relative increase in delivery capacity afforded by cooperativity between the Hb subunits? That is, how much does capacity increase when n=2.45 relative to n=1? Explain the results for n=4. (2 pts)

4.4) Under conditions of increased oxygen demand, the P_{O2} of tissues drops to 20 mmHg. What is the relative increase in oxygen delivery capacity at n=2.45 versus n=1? (2 pts)

4.5) As a result of an unexpected, global change, oxygen in the atmosphere drops, resulting in a change of P_{O2} in the lungs from 100 mmHg to 80 mmHg. Identify a single adaptation in the parameters used in this problem (such as K_M or n) that would restore oxygen delivery to the level you identified in part 4.1. Provide a quantitative change, such as K_M increases/decreases to ??? mmHg. (2 pts)

For your reflection (not graded): Cooperative binding is often posed as a way for amplifying oxygen transport. Can you define the conditions for which this is true?