

Quantitative Physiology I / Molecular and Cellular Systems; BMEN E4001x

HW1: Chemical Kinetics & Equilibria

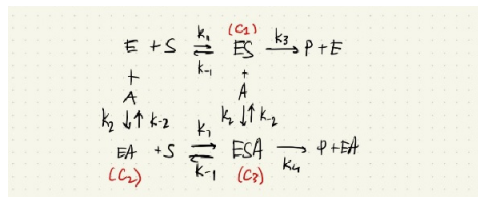
Due September 25, 2025, 11:00PM US Eastern Time

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 bimolecular 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.



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

$$\text{let } \left\{ \begin{array}{ll} [E] = e & [ES] = c_1 \\ [S] = s & [EA] = c_2 \\ [A] = a & [ESA] = c_3 \end{array} \right\}$$

$$\frac{de}{dt} = -k_1 es + k_{-1} c_1 - k_2 ea + k_{-2} c_2 + k_3 c_1$$

$$\frac{ds}{dt} = -k_1 es + k_{-1} c_1 - k_{-1} c_2 s + k_{-1} c_3$$

$$\frac{da}{dt} = -k_2 ea + k_{-2} c_2 - k_{-2} c_1 a + k_{-2} c_3$$

$$\frac{dc_1}{dt} = -k_{-1} c_1 + k_1 es - k_{-2} c_1 a + k_{-2} c_3 - k_3 c_1$$

$$\frac{dc_2}{dt} = -k_{-2} c_2 + k_2 ea - k_{-1} c_2 s + k_{-1} c_3 + k_4 c_3$$

$$\frac{dc_3}{dt} = -k_{-1} c_3 + k_{-1} c_2 s - k_{-2} c_3 + k_2 c_1 a - k_4 c_3$$

$$\frac{dp}{dt} = k_3 c_1 + k_4 c_3$$

$$e_0 = e + c_1 + c_2 + c_3$$

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

Equilibrium (forward = backward rate)

$$k_1 es = k_{-1} c_1$$

$$k_2 ea = k_{-2} c_2$$

$$k_{-2} c_1 a = k_{-2} c_3$$

$$k_{-1} c_2 s = k_{-1} c_3$$

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.

Quasi Steady State ($\frac{dc_i}{dt} \rightarrow 0$)

$$\frac{dc_1}{dt} = 0$$

$$\frac{dc_2}{dt} = 0$$

$$\frac{dc_3}{dt} = 0$$

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 .

$$K_A = \frac{k_{-2}}{k_2}$$

$$k_1 e_s = k_{-1} c_1$$

$$e_s = \frac{k_{-1}}{k_1} c_1$$

$$e = \frac{K_s}{s} c_1 \quad \left(K_s = \frac{k_{-1}}{k_s} \right)$$

$$k_2 e a = k_{-2} c_2$$

$$c_2 = \frac{1}{K_A} e a$$

$$c_3 = \frac{1}{K_A} c_1 a$$

$$k_1 c_2 s = k_{-1} c_3$$

$$c_2 = \frac{K_s}{s} c_3 = \frac{K_s}{K_A} \frac{c_1 a}{s}$$

$$e_0 = e + c_1 + c_2 + c_3$$

$$e + c_1 = e_0 - c_2 - c_3$$

$$c_1 \left(\frac{K_s}{s} + 1 \right) = e_0 - c_2 - c_3$$

$$c_1 \left(\frac{K_s}{s} + 1 \right) = e_0 - \frac{K_s}{K_A} \frac{c_1 a}{s} - \frac{1}{K_A} c_1 a$$

$$= e_0 - \frac{c_1 a}{K_A} \left(\frac{K_s}{s} + 1 \right)$$

$$c_1 (s + K_s) = e_0 s - \frac{c_1 a}{K_A} (s + K_s)$$

$$c_1 (s + K_s) + c_1 \frac{a}{K_A} (s + K_s) = e_0 s$$

$$c_1 (s + K_s) \left[1 + \frac{a}{K_A} \right] = e_0 s$$

$$c_1 = \frac{e_0 s}{\left[1 + \frac{a}{K_A} \right] [s + K_s]}$$

$$V = \frac{dp}{dt} = k_3 c_1 + k_4 c_3$$

$$= k_3 \frac{e_0 s}{\left(1 + \frac{a}{K_A} \right) (s + K_s)} + k_4 \frac{c_1 a}{K_A}$$

$$= k_3 \left(\frac{1}{1 + \frac{a}{K_A}} \right) \left(\frac{s}{s + K_s} \right) + k_4 \frac{a}{K_A} \left(\frac{1}{1 + \frac{a}{K_A}} \right) \left(\frac{s}{s + K_s} \right)$$

$$V = e_0 \left[\left(k_3 + k_4 \frac{a}{K_A} \right) \left(\frac{1}{1 + \frac{a}{K_A}} \right) \right] \frac{s}{s + K_s}$$

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 $[I] = 200 \mu\text{M}$, 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 $V_{\max,X}/V_{\max,Y}$.

$$V = V_{\max} * (1/(1+[I]/K_I)) * [S]/(K_m/(1+[I]/K_I) + [S]).$$

Reaction is maximum when assuming $I=0$. Invert.

Invert.

$$V_{\max,X} = 10 \text{ mM/min}, V_{\max,Y} = 15 \text{ mM/min};$$

$$V_{\max,X}/V_{\max,Y} = 0.67$$

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

$$V = V_{\max} * (1/(1+[I]/K_I)) * [S]/(K_m/(1+[I]/K_I) + [S]);$$

$$\text{evaluate at } I = 200 \mu\text{M}, S = 15 \text{ mM}, K_{m,X} = 15 \text{ mM}, K_{I,X} = 100 \mu\text{M}, V_{\max,X} = 10 \text{ mM/min}$$

$$V = 2.5 \text{ mM/min}$$

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

$$V = V_{\max} * (1/(1+[I]/K_I)) * [S]/(K_m/(1+[I]/K_I) + [S]);$$

$$\text{evaluate at } S = 15 \text{ mM}, K_{m,Y} = 30 \text{ mM}, K_{I,Y} = 60 \mu\text{M}, V_{\max,Y} = 15 \text{ mM/min}, V = 2.5 \text{ mM/min}$$

$$[I] = 180 \mu\text{M}$$

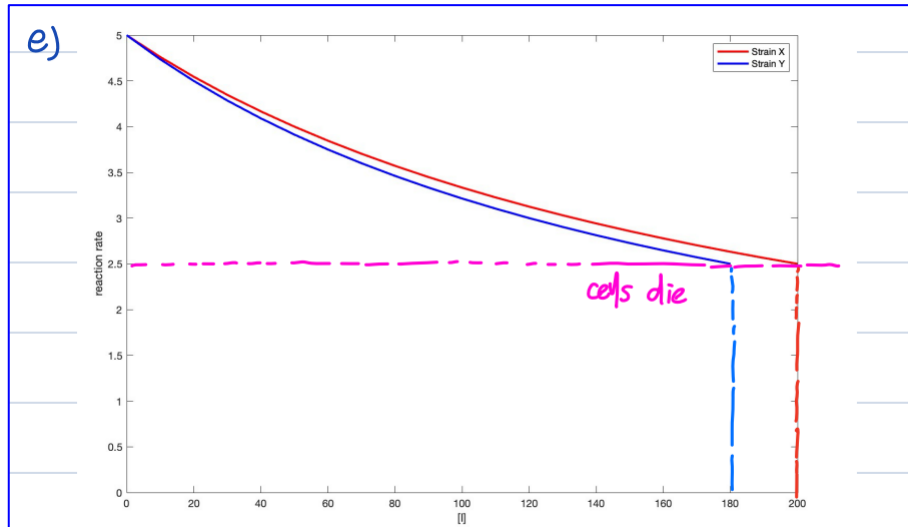
2.4) For both strains at $[I] = 100 \mu\text{M}$, calculate the fraction of enzymatic activity remaining relative to the uninhibited condition.

That is, calculate $V([I]=100 \mu\text{M})/V([I]=0 \mu\text{M})$ for strains X and Y

$$V = V_{\max} * (1/(1+[I]/K_I)) * [S]/(K_m/(1+[I]/K_I) + [S]);$$

Remaining activity
For strain X, 66.7%
For strain Y, 64.3%

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



X axis in μM
Y axis in mM/min .
Strain Y has a steeper slope.

For your reflection (not graded):

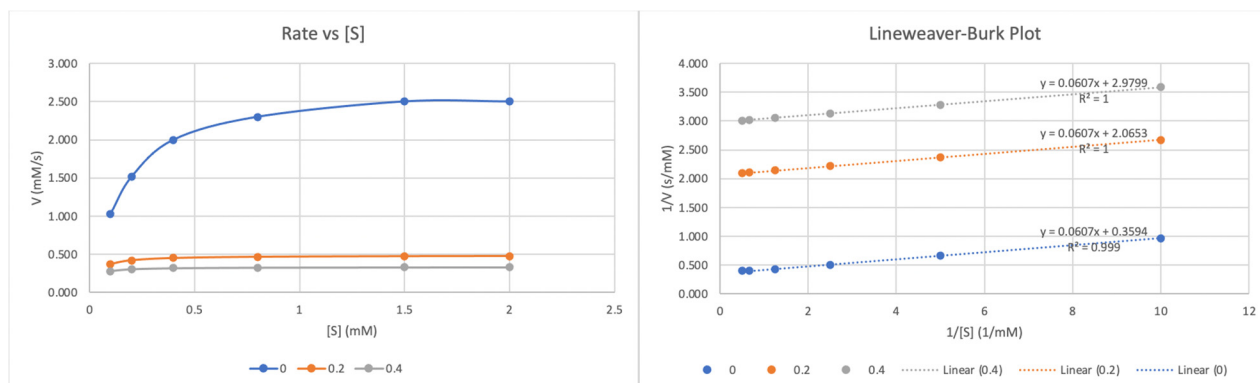
- The targeted enzymes in strains X and Y have different values of K_M and K_I . Which of these parameters (K_M or K_I) is more important in determining the effect of the inhibitor on enzyme function? What information is needed to make this choice?
- Molecules that act through uncompetitive inhibition 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:
- 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.
- 3.2) Identify the mode of inhibition (competitive, noncompetitive, or uncompetitive), and explain this choice.

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

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

The approach is to examine how the presence of inhibitor changes the *apparent* values of V_{\max} , K_m , and K_i . These can be estimated from a graph of V vs. $[S]$ using contemporary curve fitting approaches. However, the classic method of getting the reaction characteristics is the Lineweaver-Burk plot, comparing $1/V$ against $1/[S]$. From here, a range of methods can be used to estimate V_{\max} , K_m , and K_i . The strongest is to use all the data to estimate each variable, but other methods, such as estimating V_{\max} from a subset of data then using this to inform the rest of the calculations, are acceptable.



$$V_{\max,app} = \frac{1}{Y - int}$$

$$K_{m,app} = -\frac{1}{X - int}$$

$$\frac{K_{m,app}}{V_{max,app}} = slope$$

The slopes of the three lines are the same → uncompetitive inhibition.

$$V = V_{max} \frac{1}{1 + [I]/K_i} \frac{[S]}{\frac{K_s}{1 + [I]/K_i} + [S]}$$

$$\frac{1}{V} = \frac{1 + [I]/K_i}{V_{max}} + \frac{K_s}{V_{max}[S]}$$

[I]	Equations	X-intercept	$K_{m,app} (s^{-1})$	$V_{max,app} (\frac{mM}{s})$	$K_i (mM)$
0 mM	Y = 0.0607x + 0.3594	-5.921	0.169	2.782	
0.2 mM	y = 0.0607x + 2.0653	-34.02	0.029	0.484	0.042
0.4 mM	y = 0.0607x + 2.9799	-49.09	0.020	0.336	0.055

- Values used to generate data:
 - $V_{max} = 2.7 \text{ mM/s}$
 - $K_m = 0.15 \text{ mM}$
 - $K_i = 0.05 \text{ mM}$

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 1.2×10^{-2} mol/min O_2
- $[O_2]_{\text{dissolved}} = \alpha_{O_2} \cdot P_{O_2}$. For O_2 into aqueous solutions, $\alpha_{O_2} = 1.4 \times 10^{-6}$ M/mmHg
- In the lungs, $P_{O_2} = 100$ mmHg, in tissues, $P_{O_2} = 40$ mmHg.
- Cardiac output is 5 L/min.

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

$$[P_{O_2, \text{lungs}} \cdot \alpha_{O_2} - P_{O_2, \text{tissues}} \cdot \alpha_{O_2}] \cdot \text{cardiac output} =$$

$$[(100 \text{ mmHg}) \cdot (1.4 \times 10^{-6} \text{ M/mmHg}) - (40 \text{ mmHg}) \cdot (1.4 \times 10^{-6} \text{ M/mmHg})] \cdot (5 \text{ L/min}) = 4.2 \times 10^{-4} \text{ mol/min}$$

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?

oxygen delivery, Hb = (cardiac output) * [Hb] * 4 * (Saturation($P=100$ mmHg) - Saturation($P=40$ mmHg)) = $5 \text{ L/min} \cdot 2.3 \text{ mM} \cdot 4 \cdot (.965 - .7445) = 1.014 \times 10^{-2} \text{ mol/min}$;
Total delivery, add dissolved gas = $1.06 \times 10^{-2} \text{ mol/min}$
a little low, but closer to the O_2 needs of the body

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

$n=1$; delivery = $5 \cdot 2.3 \times 10^{-3} \cdot 4 \cdot (.795 - .607) = 9.0 \times 10^{-3} \text{ mol/min}$
 $n=4$; delivery = $5 \cdot 2.3 \times 10^{-3} \cdot 4 \cdot (.995 - .851) = 7.0 \times 10^{-3} \text{ mol/min}$

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 $n=4$.

Cooperativity with $n=2.45$ affords a 16% increase ($\sim 1 \times 10^{-3} \text{ mol/min}$) in the oxygen delivery capacity of blood
For $n=4$, the oxygen delivery capacity is diminished as the O_2 remains bound to the Hb at the partial pressure of O_2 in tissues

4.4) Under conditions of increased oxygen demand, the P_{O_2} of tissues drops to 20 mmHg. What is the relative increase in oxygen delivery capacity at $n=2.45$ versus $n=1$?

Oxygen delivery capacity for $n=2.45$ is 1.70 times that at $n=1$.

4.5) As a result of an unexpected, global change, oxygen in the atmosphere drops, resulting in a change of P_{O_2} 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.

Some solutions: conc of Hb increases to 2.6 mM, K_m increases to 27.99 mmHg, solubility increases to 7.63×10^{-6} M/mmHg (not likely), cardiac output increases to 5.67 L/min. No change in n will compensate.

Moment for 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?