10.1: General Principles of Catalysis

Q10.1a
One day in class about enzyme kinetics, Jack comes over to you and asks, "I know enzymes are biological catalysts, but I do not understand how it works. Can you explain how enzymes make reactions go faster? And is it only faster in one direction?"

S10.1a
Because the activation energy is the energy hill between reactants and products, enzymes decreasing the size of the hill also decreases the amount of energy needed for reactions to go in either direction. A smaller energy hill allows reactants and products to overcome the barrier quicker, resulting a faster reaction rate.

Q10.1b
If your student colleagues argues that a catalysts affects only the rate of only one direction of a reaction. Explain why he is correct or not.

S10.1b
False. Catalysts affect rate by providing an alternative mechanism which has a lower transition state energy. It's impossible to lower transition state energy for only one direction of a reaction. (It'd be like making a hill shorter from the north, but keeping it the same height from the south.)

Q10.1c
How does the enzyme catalysis affect both forward and reverse reaction?

S10.1c
The enzyme catalyst lowers the Gibb energy of transition state, which reduces the activation energy of both reactions. Therefore, it makes reactions occur faster.

Q10.2a
Given enzyme-catalyzed reaction $k_1 = 4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-1} = 6 \times 10^4 \text{ s}^{-1}$ and $k_2 = 2.0 \times 10^3 \text{ s}^{-1}$. Determine if the enzyme–substrate binding follow the equilibrium or not?

S10.2a
The dissociation constant $k_s = k_{-1} / k_1 = 6 \times 10^4 \text{ s}^{-1} / 4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} = 0.15 \text{ M}$
The Michaelis constant $k_M = k_1 + k_2$ / $k_1 = 6 \times 10^4$ s$^{-1}$ + 2.0 $\times$ 10$^3$ s$^{-1}$ / 4 $\times$ 10$^6$ M$^{-1}$ s$^{-1} = 0.155$ M

The two constants are not equal. Therefore, the binding does not follow the equilibrium scheme.

Q10.2b

Is it appropriate to use the rapid equilibrium scheme to model the kinetics of a catalyzed reaction with the following rate constants?

\[ k_1 = 7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1} \]
\[ k_{-1} = 8 \times 10^5 \text{ s}^{-1} \]
\[ k_2 = 5 \times 10^4 \text{ s}^{-1} \]

S10.2b

Assuming rapid equilibrium is appropriate when $k_{-1} \gg k_2$, so that $k_M = k_S$. In this case, the values of $k_1$ and $k_2$ are not dramatically different, so we can calculate $K_M$ and $K_S$ and compare.

1. Calculate $K_M$:

\[ K_M = \frac{k_{-1} + k_2}{k_1} \]
\[ K_M = \frac{8 \times 10^5 \text{ s}^{-1} + 5 \times 10^4 \text{ s}^{-1}}{7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}} \]
\[ K_M = 0.01 \text{ M} \]

2. Calculate $K_S$:

\[ K_S = \frac{k_{-1}}{k_1} \]
\[ K_S = \frac{8 \times 10^5 \text{ s}^{-1}}{7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}} \]
\[ K_S = 0.01 \text{ M} \]

Since $K_M = K_S$, it is appropriate to assume rapid equilibrium.

Q10.2c

Given $k_1 = 7 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, $k_{-1} = 6 \times 10^4 \text{ s}^{-1}$, $k_3 = 2 \times 10^3 \text{ s}^{-1}$, determine if the enzyme substance binding follow the equilibrium or steady state scheme?

\[ K_s = \frac{k_{-1}}{k_1} \]
\[ K_s = \frac{6 \times 10^4 \text{ s}^{-1}}{7 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}} \]
\[ K_s = 8.8 \times 10^{-3} \text{ M} \]

\[ K_M = \frac{k_{-1} + k_2}{k_1} \]
\[ K_M = \frac{6 \times 10^4 \text{ s}^{-1} + 2 \times 10^3 \text{ s}^{-1}}{7 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}} \]
\[ K_M = 8.8 \times 10^{-3} \text{ M} \]

\[ k_{-1} + k_2 = 7 \times 10^4 \text{ s}^{-1} \]
\[ k_{-1} + k_2 = 8 \times 10^3 \text{ s}^{-1} \]
\[ k_{-1} + k_2 = 5 \times 10^4 \text{ s}^{-1} \]
Q10.3a

The substrate N-acetylglycine ethyl ester can be catalyzed by the enzyme carbonic anhydrase. This enzyme has a turnover rate of 30,000 s\(^{-1}\). Determine how long it will take carbonic anhydrase to cleave the substrate.

\[
\begin{align*}
\text{\(t\)} &= \frac{1}{k_{\text{cat}}} = \frac{1}{30,000 \text{ s}^{-1}} = 3.33 \times 10^{-5} \\
\text{\([5 \text{ minutes}] (7.1 \times 10^{-6}) = 3.5 \times 10^{-5}\)}
\end{align*}
\]

Q10.3b

RuBisCO is an enzyme in the Calvin cycle that fixes atmospheric carbon and has a turnover rate of 3.3 s\(^{-1}\). How long does it take RuBisCO to fix one molecule of carbon dioxide?

Spacefilling structure of RuBisCO created using Rasmol and the 8RUC file from the Protein Data Bank.

\[
\begin{align*}
\text{\(t\)} &= \frac{1}{k_{\text{cat}}} = \frac{1}{30,000 \text{ s}^{-1}} = 3.33 \times 10^{-5} \\
\text{\([5 \text{ minutes}] (7.1 \times 10^{-6}) = 3.5 \times 10^{-5}\)}
\end{align*}
\]

S10.3b

The turnover number is the number of molecules of substrate per unit time (when the enzyme is fully saturated). So simply take the reciprocal to find the time per molecule of substrate.
\[
\frac{1}{3.3 \text{ s}^{-1}} = 0.30 \text{ s}
\]

Note: RuBisCO is a notoriously slow enzyme.

**Q10.3c**
The catalyze of acetylcholine has a rate 50000 s\(^{-1}\). Calculate the time for the enzyme to cleave one Ach molecule.

**S10.3c**
\[ t = \frac{1}{k_2} = \frac{1}{50000} = 2.0 \times 10^{-5} \text{s} \]

**Q10.3d**
Carbonic anhydrase, an enzyme that catalyzes the dehydration of carbonic acid to form carbonic acid, has the turnover rate of \(k_{\text{cat}}\) 4.0 \times 10^5 \text{ s}^{-1}. Calculate how long does it take for the enzyme to cleave one molecule carbonic acid?

**S10.3d**
The time required for the enzyme to cleave one molecule carbonic acid:
\[ t = \frac{1}{k_{\text{cat}}} = \frac{1}{4 \times 10^5 \text{s}^{-1}} = 2.5 \times 10^{-6} \text{s} = 2.5\mu\text{s} \]

**Q10.3e**
p-nitrophenyl acetate (PNPA) is catalyzed by chymotrypsin to yield p-nitrophenolate ion and acetate ion. The turnover rate of that enzyme is 40,000 s\(^{-1}\). How long will it take for the enzyme to produce 1 mole of Nitrophenyl acetate?

**S10.3e**
It takes 1 second to convert all 40,000 molecule substrate into the product, so: \[ t = \frac{40,000}{9.5 \times 10^5} \] = 3.8 sec.

### 10.2: The Equations of Enzyme Kinetics

**Q10.4**
What is plotted on the x and y axes on a Lineweaver-Burk plot? Show how to derive the equation for the plot from the equation
\[
\frac{v_0}{V_{\text{max}}} = \frac{[S]}{K_M + [S]}
\]
and explain how \(V_{\text{max}}\) and \(K_M\) can be found from the graph's intercepts. *Hint: A Lineweaver-Burk plot is also sometimes called a double reciprocal plot.*
The x-axis is $1/v_0$, and the x-axis is $1/V_{\text{max}}$.

1. Take the reciprocal of both sides of the equation.

   \[
   \frac{1}{v_0} = \frac{K_M + [S]}{V_{\text{max}} [S]} \]

   \[
   \frac{1}{v_0} = \frac{K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} \]

2. Set $1/[S] = 0$ to find the y-intercept, and show that it relates to $V_{\text{max}}$.

   \[
   Y_{\text{int}} = \frac{1}{V_{\text{max}}} \]

   \[
   V_{\text{max}} = \frac{1}{Y_{\text{int}}} \]

3. Set $1/v_0 = 0$ to find the x-intercept, and show that it relates to $K_M$.

   \[
   0 = \frac{K_M}{V_{\text{max}}} X_{\text{int}} + \frac{1}{V_{\text{max}}} \]

   \[
   \frac{K_M}{V_{\text{max}}} X_{\text{int}} = -\frac{1}{V_{\text{max}}} \]

   \[
   X_{\text{int}} = -\frac{1}{K_M} \]

   \[
   K_M = -\frac{1}{X_{\text{int}}} \]

Q10.4a

Derive the Michaelis-Menten equation by assuming rapid equilibrium.

\[
E + S \overset{K_{-1}}{\rightleftharpoons} ES \overset{K_2}{\rightarrow} E + P
\]

\[
v_0 = \frac{V_{\text{max}} [S]}{K_M + [S]}\]

S10.4a

\[
\frac{[E][S]}{dt} = K_{-1}[ES]
\]

\[
\frac{[ES]}{dt} = K_1[E][S]
\]

\[
\frac{[E][S]}{dt} = \frac{[ES]}{dt}
\]

\[
K_{-1}[ES] = K_1[E][S]
\]

\[
K_M = \frac{k_{-1}}{k_1}
\]

\[
K_M = \frac{[E_0][S] - [ES][S]}{[ES]}
\]

\[
K_M[ES] = [E_0][S]
\]

\[
[ES] = \frac{[E_0][S]}{K_M + [S]}
\]

\[
v_0 = \frac{K_2[E_0][S]}{K_M + [S]}
\]
since \(v_{\text{max}} = k_2[E]_0\)
\(v(0) = \frac{V_{\text{max}}[S]}{K_M+[S]}\)

Q10.4b

We know that the Michaelis Menten derivation for the following reaction:

\[\text{E + S } \rightleftharpoons \text{ES } \rightarrow \text{E + P}\]

However, what if the reaction took place in a different scenario whereby:

\[\text{E + S } \rightleftharpoons \text{ES}_1 \rightarrow \text{ES}_2 \rightarrow \text{E + P}\]

What would be the corresponding Michaelis-Menten equation now?

S10.4b

This is the an outline for determining an expression for the rate of substrate conversion in the given case:

1. Set up the reaction with rate constants, assuming \(k_2 \approx k_3 \approx 0\):
   \[\text{E + S } \rightleftharpoons [k_1][k_{-1}]\]
   \[\text{ES}_1 \rightarrow [k_2] \text{ES}_2 \rightarrow [k_3] \text{E + P}\]

2. Set up the differential equations describing the reaction, i.e., the rate of change for each component with time. The rate of substrate change, for example, will be:
   \[\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES_1]\]

3. Choose initial conditions and set up two equations for conservation of mass. For example, the initial concentration of enzyme must equal the sum of the concentrations of E, ES, and ES_2.

4. Make the steady-state assumption: assume that the concentrations of the intermediate complexes do not change on the time-scale of product formation, i.e.,
   \[\frac{d[ES_1]}{dt} \approx \frac{d[ES_2]}{dt} \approx 0\]

5. Solve for \(-r_S\), the negative rate of substrate conversion, obtaining the Michaelis-Menten expression describing the kinetics of the given situation.

Q10.4c

Prove that \(K_s\) equals the concentration S when the initial rate is half its maximum value.

S10.4c

We have:

\[v_o = \frac{V_{\text{max}}}[S]}{K_M + [S]}\]

Divided both said by 1

\[\frac{1}{v_o} = \frac{K_M + [S]}{V_{\text{max}}}[S]\]
Q10.5a
An enzyme that has a $K_m$ value of $4.6 \times 10^{-5} \text{ M}$ is studied at an initial substrate concentration of 0.041 M. After a minute, it is found that 7.3 µM of product has been produced. Calculate the value of $V_{max}$ and the amount of product formed after 4.5 minutes.

Q10.5b
An solution initially contains a catalytic amount of an enzyme with $K_m = 1.5 \text{ mM}$, 0.25 M of substrate, and no product. After 45 seconds, the solution contains 25 µM of product. Find $V_{max}$ and the concentration of product after 2.0 minutes.

**Hint:** $[S] >> K_m$

S10.5b
1. Find the initial velocity.

$$ v_0 = \frac{25 \text{ µM}}{0.75 \text{ min}} $$

$$ v_0 = 33.3 \text{ µM/min} $$
2. Use $v_0$, $[S]$, and $K_M$ to solve for $V_{\text{max}}$.

$$ v_0 = \frac{V_{\text{max}} [S]}{K_M + [S]} $$

$$ V_{\text{max}} = v_0 \left( \frac{K_M}{[S]} + 1 \right) $$

$$ V_{\text{max}} = 33.3 \mu M/min \left( \frac{1.5\ mM}{0.25\ M} \times \frac{M}{1000\ mM} + 1 \right) $$

$$ V_{\text{max}} = 33.3 \mu M/min $$

Notice that $v_0 = V_{\text{max}}$. Since $[S] >> K_M$, the reaction will continue with a velocity of $V_{\text{max}}$ for the remainder of the two minutes.

3. Predict $[P]$ after 2.0 minutes at the rate $V_{\text{max}}$.

$$ [P] = V_{\text{max}} \times t $$

$$ [P] = 33.3 \mu M/min \times 2.0\ min $$

$$ [P] = 66.6 \mu M $$

Q10.5c

A particular enzyme at a research facility is being studied by a group of graduate students. This enzyme has a $K_m$ value of $5.0 \times 10^{-6}$ M. The students study this enzyme with an initial substrate concentration of 0.055 M. At one minute, 7 µM of product was made. What is the amount of product produced after 5 minutes. What is the $V_{\text{max}}$?

S10.5c

$$ V_o = \frac{V_{\text{max}} [\text{substrate}]}{K_m + [\text{substrate}]} $$

$7.0 \times 10^{-6} M = \frac{V_{\text{max}} (0.055 M)}{(5.0 \times 10^{-6} M + 0.055 M)}$

$V_{\text{max}} = 7.1 \times 10^{-6} \text{ M/min}$

At 5 minutes the amount of product formed is:

Q10.5d

Calculate the value

$$ v_o $$

if an enzyme has

$$ K_M = 5.4 \times 10^{-4} $$

value

$$ V_{\text{max}} = 48\mu M/min^{-1} $$

and

$$ [S] = 0.001 M $$
S10.5d

We have:

\[ v_o = \frac{V_{\text{max}} [S]}{K_M + [S]} \]

\[ v_o = \frac{48 \times 0.001}{5.4 \times 10^{-4} + 0.001} = 31 \mu M \text{min}^{-1} \]

Q10.6a

Given the values, \([S]/10^{-4} \text{ M} \) 3.0 4.6 10.5 16.5

\([v_o]/10^{-6} \text{ M} \text{ min}^{-1} \) 2.64 3.5 6.2 7.8

construct a Lineweaver-Burk plot, and assuming Michaelis-Menten kinetics, calculate the values of \(V_{\text{max}}, K_M, \) and \(k_2\) using the constructed plot.

Q10.6b

The data below represents the data recorded after the hydrolysis of a substrate by an enzyme.

<table>
<thead>
<tr>
<th>[S]/10^{-4} \text{ M}</th>
<th>2.1</th>
<th>4.2</th>
<th>9.3</th>
<th>14.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>[v_o] / 10^{-6} \text{ M} \text{ min}^{-1}</td>
<td>1.2</td>
<td>3.1</td>
<td>6.3</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Calculate \(V_{\text{max}}, K_M\) and \(K_2\) using a Lineweaver-Burk plot. Assume Michaelis. Given \([E]_0\) is 5.0 \(X\) \(10^{-6} \) \text{ M}

S10.6b

<table>
<thead>
<tr>
<th>((1/[S])/10^{-3} \text{ M}^{-1})</th>
<th>((1/[v_o])/10^{-5} \text{ M}^{-1} \text{ min}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>8.3</td>
</tr>
<tr>
<td>2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>0.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Figure: Lineweaver-Burk Plot
The linear equation for the graph above is:
y = 176x + 4.23 \times 10^4
To solve for \( V_{\text{max}} \) use:
Intercept = \( \frac{1}{V_{\text{max}}} \)
\( V_{\text{max}} = \frac{1}{\left(4.23 \times 10^4\right)} = 2.36 \times 10^{-5} \)
To solve for \( K_m \) use
Slope = \( \frac{K_m}{V_{\text{max}}} \)
\( (176)(2.36 \times 10^{-5}) = 4.2 \times 10^{-5} \)
To solve for \( K_2 \) use
\( K_2 = \frac{V_{\text{max}}}{[E]_0} = (2.36 \times 10^{-5})(5.0 \times 10^{-6} \text{ M}) = 4.72 \text{ min}^{-1} \)

Q10.6c
Using the table below, calculate the \( K_M \), \( V_{\text{max}} \), and slope.

<table>
<thead>
<tr>
<th>( \frac{1}{V_{\text{max}}} ) (10^{-3} \text{ sec/M}^{-1})</th>
<th>( \frac{[S]}(10^{2} \text{ M}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.9</td>
<td>0.3</td>
</tr>
<tr>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>4.4</td>
<td>1.8</td>
</tr>
<tr>
<td>5.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>

S10.6c
\[
\text{slope}=\left\langle \frac{dfrac{5.1times10^3-2.9times10^3}}{3.3times10^2-0.3times10^2}\right\rangle=6.73times10^{-6}
\]
\[
\left\langle dfrac{5.1times10^3}{0.3times10^2}+dfrac{1}{V_{\text{max}}}\right\rangle=2.9times10^{-3}
\]
\[
\left\langle \frac{1}{V_{\text{max}}}\right\rangle=370.62\text{ Ms}^{-1}
\]
\[
\left\langle \frac{10^{-6}-6.73times10^{-6}}{0.3times10^2}\right\rangle=0.00270
\]
\[
\left\langle \frac{1}{V_{\text{max}}}\right\rangle=0.00943
\]
\[
\left\langle K_{\text{(M)}}\right\rangle=106.044
\]
Q10.6d

Given the value

\([S]\) = 0.00032 and

\(K_M = 3.5 \times 10^{-5}\).

Find the ratio between \(v_o\) and \(V_{max}\).

S10.6d

\[ v_o = \frac{V_{max} [S]}{K_M + [S]} \]

Divided both side by 1:

\[ \frac{1}{v_o} = \frac{K_M}{V_{max} [S]} + \frac{1}{V_{max}} \]

Divided both side by 1 again:

\[ v_o = \frac{V_{max} [S]}{K_{max}} + V_{max} \]

\[ v_o = V_{max} \left( \frac{[S]}{K_{max}} + 1 \right) \]

\[ \frac{v_o}{V_{max}} = \frac{[S]}{K_M} + 1 \]

\[ \frac{v_o}{V_{max}} = \frac{0.00032}{3.5 \times 10^{-5}} + 1 = \frac{71}{7} = 10.14 \]

Q10.7a

From this graph determine the \(K_M\) and \(V_{max}\)?
S10.7b

From his graph we can see that the value $K_M$ is 2. Then we look to see where $K_M$ is half. At that point, we see that $K_M/2$ is 1 and the x-value for that coordinate is 1. This means $V_{max}$ is 1.

Q10.7b

Neutral sphingomyelinase 2 converts sphingomyelin into ceramide and phosphocholine. Assume its $V_{max}$ is 35 $\mu$M min$^{-1}$. When you provide $3 \times 10^{-5}$ M of sphingomyelin, you observe an initial velocity of 6.0 $\mu$M min$^{-1}$. Calculate the $K_M$ for this reaction, rounding to 3 significant figures.

$$\frac{1}{V_o} = \frac{K_M}{V_{max}[S]} + \frac{1}{V_{max}}$$

$$\frac{1}{6.0} = \frac{K_M}{(35)(30)} + \frac{1}{35}$$

$$\frac{1}{6.0} - \frac{1}{35} = \frac{K_M}{1050}$$

$$K_M = 145 \mu M$$

Q10.8a

We are given a second order equation: $r=k[A][B]$. The concentration of A is 0.05g and the concentration of B is 2.5g. We decide this difference is great enough to treat this as a pseudo first reaction? What concentration is held constant and why? Write the new equation.

S10.8a

We hold B constant because the concentration is so much larger, so it should be close to constant for the reaction. The new equation would be $r=k'[a]$

Q10.9a

Name and briefly describe two types of reactions that do not follow Michaelis-Menten kinetics.

S10.9a

Irreversible inhibition - the inhibitor binds covalently and irreversibly to the enzyme
Allosteric interactions - the binding of effectors at allosteric sites (away from the active site) influence substrate binding.

**Q10.9b**

For the reaction mechanism below, how does the concentration of C affect the concentration of B?

\[ A \xrightleftharpoons{} B \rightarrow C \]

**S10.9b**

Because the second state is irreversible (i.e., since arrow), it does not matter if you have a large or little concentration of C, it would not affect \( \Delta \) and hence the kinetics of the reaction. However, the concentration of \( A \) would clearly affect the concentration of \( B \).

**10.3: Chymotrypsin: A Case Study**

**Q1**

1. Speculate on how the catalytic rate constant can be determined from the spectrophotogram.
2. How can product be consistently produced if the rate of change of the ES complex is 0?
3. How would the rate of product formation change if:
   a. the substrate concentration were doubled?
   b. the enzyme concentration were doubled?
   c. The reaction was carried out in mono-deuterated water instead of \( \text{H}_2\text{O} \) (comment qualitatively)?
4. Explain the role of hydrogen bonding in protein hydrolysis catalyzed by chymotrypsin.
5. What would the spectrophotogram look like if the reaction proceeded via a steady-state mechanism instead of pre-equilibrium.

**S2**

1. The catalytic rate constant can be deduced from the graph by simply determining the slope of the line where the reaction demonstrates 0-order kinetics (the linear part).
2. This is pre-equilibrium kinetics in action. The ES complex is formed from E and S at a faster rate than any other step in the reaction. As soon as ES is converted to *ES, another mole of ES is produced from an infinite supply of E + S. This means that the amount of ES and E + S is constantly at equilibrium, and thus the change of either with respect to time is 0.
   a. No change.
   b. Two-fold increase.
   c. Because water is involved in the final, slowest step of the mechanism, deuterating the water would decrease the rate of the overall reaction from 5- to 30-fold.
3. Initially, hydrogen bonding between the enzymes histidine and serine side chains weakens the bond of serine’s O-H. This allows a facilitated nucleophilic attack of the hydroxyl oxygen on the substrates carbonyl group. Conversely, in the final step of the reaction, the bound serine oxygen forms a hydrogen bond with a protonated histidine, which allows for easier cleavage from the substrate.
4. The graph would show similar 0-order kinetics, but the line would intercept the Y-axis at an absorbance of 0 instead of the 1:1 mole ratio of nitrophenolate to enzyme.

10.4: Multisubstrate Systems

10.5: Enzyme Inhibition

10.6: Allosteric Interactions

10.7: The Effect of pH on Enzyme Kinetics