Section 2.6a:
Chemical Kinetics

“Gotta take another data point real quick!”
Chemical kinetics (or reaction kinetics) is the study of rates of chemical reactions—enzyme kinetics is concerned with reactions catalyzed by enzymes!

Michaelis-Menten kinetics describes a reaction scheme that best models the action of simple enzymes—eg that convert a single substrate into a product.

Mastering enzyme kinetics is all about befriending algebra and calculus coupled with mastering the units of various parameters—treat each parameter in terms of its SI units!

The [] brackets enclosing a parameter are used to indicate either its concentration or units—the context provides the distinction!

The >> or << sign usually implies greater than or less than at least an order of magnitude!

Y is n orders of magnitude greater than X—what does this mean?:

- if n=1 => Y = 10^1.X => One order of magnitude
- if n=2 => Y = 10^2.X => Two orders of magnitude
- if n=3 => Y = 10^3.X => Three orders of magnitude

The “order of magnitude” is expressed in terms of the logarithmic scale!
- Consider the following first-order reaction:
  \[ A \rightarrow P \]

- The reaction rate, \( \frac{d[A]}{dt} \), associated with the decay of A into P at time t is given by:
  \[ \frac{d[A]}{dt} = -k[A] \]  \[1.1\]
  \[ \Rightarrow \frac{d[P]}{dt} = k[A] = k([A]_0 - [P]) \]  \[1.2\]

  where  
  \([A]_0 = \text{Concentration @ time } t=0 \text{ of reactant } A \ [M]\)  
  \([A] = \text{Concentration @ time } t \text{ of reactant } A \ [M]\)  
  \([P] = \text{Concentration @ time } t \text{ of reactant } P \ [M]\)  
  \(k = \text{First-order rate constant (s}^{-1})\)

- Integrating Eq[1.1] with respect to t yields:
  \[ \int \frac{d[A]}{[A]} = -k \int dt \]
  \[ \Rightarrow \ln[A] - \ln[A]_0 = -kt \]
  \[ \Rightarrow \ln[A] = -kt + \ln[A]_0 \]  \[1.3\]

  where \([A]_0\) is the (initial) concentration of reactant A @ time zero \((t_0)\)—recall from calculus that \(\int (1/x)dx = \ln x\)

- Thus, according to Eq[1.3], a plot of \(\ln[A]\) versus t will generate a straight line with slope \(-k\) and y-intercept equal to \(\ln[A]_0\) for a first-order reaction!

- Why the units of \(k\) are \(s^{-1}\)?—from Eq[1.1], we have:
  \[ [k] = \{[d[A]]/[dt]\}.\{1/[[A]]\} \]
  \[ [k] = \{M/s\}.\{1/M\} \]
  \[ [k] = s^{-1} \]
Elementary Kinetics: Second-Order Reaction

- Consider the following second-order reaction:
  \[ A + L \rightarrow P \]

- The reaction rate, \( \frac{d[A]}{dt} \), associated with the decay of \( A \) into \( P \) at time \( t \) is given by:
  \[
  \frac{d[A]}{dt} = -k[A][L] \tag{2.1}
  \]
  
  where  
  \[ [A] = \text{Concentration @ time } t \text{ of reactant } A \text{ (M)} \]
  \[ [L] = \text{Concentration @ time } t \text{ of reactant } L \text{ (M)} \]
  \[ k = \text{Second-order rate constant (M}^{-1}\text{s}^{-1}) \]

- Assuming \([A]=[L]\), Eq\[2.1\] becomes:
  \[
  \frac{d[A]}{dt} = -k[A]^2 \tag{2.2}
  \]
  
  \[
  \Rightarrow \quad \frac{d[P]}{dt} = k[A]^2 = k([A]_0-P)^2 \tag{2.3}
  \]

- Integrating Eq\[2.2\] with respect to \( t \) yields:
  \[
  \int -\frac{d[A]}{[A]^2} = -k \int dt
  \]
  
  \[
  \Rightarrow \quad -1/[A] + 1/[A]_0 = -kt
  \]
  
  \[
  \Rightarrow \quad 1/[A] = kt + 1/[A]_0 \tag{2.4}
  \]
  
  where \([A]_0\) is the (initial) concentration of reactant \( A \) @ time zero \((t_0)\)—recall from calculus that \( \int (1/x^2)dx = -(1/x) \)

- Thus, according to Eq\[2.4\], a plot of \( 1/[A] \) versus \( t \) will generate a straight line with slope \( k \) and \( y \)-intercept equal to \( 1/[A]_0 \) for a second-order reaction!

- Why the units of \( k \) are \( M^{-1}s^{-1} \)?—from Eq\[2.2\], we have:
  \[
  [k] = \{[d[A]]/[dt]\}\{1/[A]^2\}
  \]
  \[
  [k] = \{M/s\}\{1/M^2\}
  \]
  \[
  [k] = M^{-1}s^{-1}
  \]
Elementary Kinetics: Pseudo-First-Order Reaction

- Consider the following second-order reaction governed by the second-order rate constant \( k \) (M\(^{-1}\)s\(^{-1}\)):

\[
A + L \xrightarrow{k} P
\]

- Now, let us assume that \([L] \gg [A]\), then the change in \([L]\) is negligible over the course of reaction relative to change in \([A]\)—in other words, the rate of the above reaction is primarily determined by \([A]\)—or simply put, the above reaction will essentially behave like a first-order reaction:

\[
A \xrightarrow{k[L]} P
\]

- Kineticists refer to the above scenario as a “pseudo-first order reaction” with a pseudo-first-order rate constant \((k')\) given by the following relationship:

\[
k' = k[L] \quad [3.1]
\]

with the units of \(k'\) being s\(^{-1}\)! Why?!

- The rate equations for the pseudo-first order reaction are analogous to the bona fide first-order reaction:

\[
\frac{d[A]}{dt} = -k'[A] \quad [3.2]
\]

\[
\ln[A] = -k't + \ln[A]_0 \quad [3.3]
\]

- Now, taking anti-ln of both sides of Eq[3.3] generates the corresponding exponential equation:

\[
[A] = [A]_0 \exp(-k't) \quad [3.4]
\]

- Thus, according to Eq[3.4], a plot of \([A]\) vs \(t\) will generate an exponential decay curve—such plots are referred to as “exponential kinetics” and form the basis of enzyme kinetics and protein-ligand kinetics.
Michaelis-Menten Kinetics: Reaction Scheme

- In Michaelis-Menten kinetics, the conversion of a substrate (S) into product (P) by an enzyme (E) is proposed to proceed via an enzyme-substrate (ES) complex intermediate as embodied in the above reaction scheme.

- Let us define the concentrations—all expressed in the units of \( M \) (mol/L)—for the various reactants, intermediates and products as follows:
  \[
  \begin{align*}
  [E]_0 &= \text{Free (total) enzyme concentration @ time } t_0 \\
  [E] &= \text{Free (available) enzyme concentration @ time } t \\
  [S] &= \text{Substrate concentration @ time } t_0 \\
  [ES] &= \text{Enzyme-substrate complex concentration @ time } t \\
  [P] &= \text{Product concentration @ time } t
  \end{align*}
  \]

- With elementary kinetics under our belt, we can easily infer what the various rate constants are:
  \[
  \begin{align*}
  k_{+1} &= \text{Second-order rate constant (M}^{-1}\text{s}^{-1}) \\
  k_{-1} &= \text{First-order rate constant (s}^{-1}) \\
  k_{+2} &= \text{First-order rate constant (s}^{-1}) \\
  k_{-2} &= \text{Second-order rate constant (M}^{-1}\text{s}^{-1})
  \end{align*}
  \]

- Since the reversible conversion of P to ES is negligible for the forward reaction, it is assumed that \( k_{+2} \gg k_{-2} \) (contribution of \( k_{-2} \) is ignored!)
Given that the total enzyme \([E_0]\) only exists in two states—either as free enzyme \((E)\) or that bound to the substrate \((ES)\)—we have the following relationship at time \(t\) in the reaction:

\[
[E]_0 = [E] + [ES]
\]  

[4.1]

Next, assuming that \(k_{+2} \gg k_{-2}\) (\(k_{-2}\) is negligible!), the rate of formation of \(ES\) at time \(t\) is given by:

\[
\frac{d[ES]}{dt} = k_{+1}[E][S] - k_{-1}[ES] - k_{+2}[ES]
\]  

[4.2]

Given that \([S]\) is not limiting or simply put \([S] \gg [E]\), \([ES]\) remains more or less constant over the steady-state phase of the reaction (excluding the initial and final stages)—this is called the “steady-state assumption”—ie the rate of formation of \([ES]\) equals its decay!

- In calculus terms, we can express the steady-state assumption as:

\[
\frac{d[ES]}{dt} = 0
\]  

[4.3]

- Such steady-state assumption is a hallmark of Michaelis-Menten kinetics!
Michaelis-Menten Kinetics: Initial Velocity

- Now, combining Eq[4.2] and Eq[4.3], we have:
\[ k_{+1}[E][S] - k_{-1}[ES] - k_{+2}[ES] = 0 \]
\[ \Rightarrow k_{+1}[E][S] = k_{-1}[ES] + k_{+2}[ES] \quad [4.4] \]

- Next, we combine Eq[4.1] with Eq[4.4] so as to eliminate the variable [E]:
\[ k_{+1}[S].([E]_0 - [ES]) = k_{-1}[ES] + k_{+2}[ES] \]
\[ \Rightarrow ([S]/[ES]).([E]_0 - [ES]) = (k_{-1} + k_{+2})/k_{+1} \quad [4.5] \]

- For simplicity, the various rate constants on the right side of Eq[4.5] can be treated as a single composite steady-state constant—designated \( K_M \)—this is the Michaelis constant defined as:
\[ K_M = (k_{-1} + k_{+2})/k_{+1} \quad [4.6] \]

- Now, let us combine Eq[4.5] and Eq[4.6]:
\[ K_M = ([S]/[ES]).([E]_0 - [ES]) \]
\[ \Rightarrow K_M[ES] = [S].([E]_0 - [ES]) \quad [4.7] \]

- Solving Eq[4.7] for [ES] and multiplying both sides with \( k_{+2} \) yields:
\[ K_M[ES] = [S][E]_0 - [S][ES] \]
\[ \Rightarrow K_M = ([S][E]_0)/[ES] - [S] \]
\[ \Rightarrow K_M + [S] = ([S][E]_0)/[ES] \]
\[ \Rightarrow [ES] = ([S][E]_0)/(K_M + [S]) \]
\[ \Rightarrow k_{+2}[ES] = k_{+2}([S][E]_0)/(K_M + [S]) \quad [4.8] \]

- Now, the initial reaction rate or velocity \( (v) \) of the formation of product P can be expressed as (with the units of \( v \) being \( Ms^{-1} \)):
\[ v = d[P]/dt = k_{+2}[ES] \quad [4.9] \]
Combining Eq[4.8] and Eq[4.9], we have:
\[ v = k_{+2}([S][E]_0)/(K_M + [S]) \] [4.10]

The maximum reaction velocity (\( V_{\text{max}} \)) occurs when the enzyme is fully saturated with substrate—ie when [ES] is no longer-rate-limiting and approaches [E]₀:
\[ [ES] \rightarrow [E]_0 \]

Thus, under such saturating conditions, the reaction reaches \( V_{\text{max}} \) solely determined by the product of \( k_{+2} \) and [E]₀:
\[ V_{\text{max}} = k_{+2}[E]_0 \] [4.11]

Now, combining Eq[4.10] and Eq[4.11], we obtain:
\[ v = \frac{V_{\text{max}}[S]}{K_M + [S]} \] [4.12]

Eq[4.12] is the Michaelis-Menten equation that describes a hyperbola characteristic of substrates catalyzed by simple enzymes—ie a plot of \( v \) versus [S]—or the Michaelis-Menten Plot!

Can we determine the values of \( K_M \) and \( V_{\text{max}} \) from the Michaelis-Menten plot? If so, should we adopt this approach?
- Assuming that \( v = \frac{V_{\text{max}}}{2} \), Eq[4.12] reduces to:

\[
\frac{V_{\text{max}}}{2} = \frac{V_{\text{max}}[S]}{K_M + [S]}
\]

\[
\Rightarrow K_M + [S] = 2[S]
\]

\[
\Rightarrow K_M = [S]
\]  \[4.13\]

- Simply put, \( K_M \) is the value of \( [S] \) @ \( v = \frac{V_{\text{max}}}{2} \)—ie \( K_M \) is indicative of the affinity of an enzyme for a substrate—the lower the \( K_M \), the greater the affinity!

- Accordingly, from a Michaelis-Menten plot, one COULD directly determine the values of \( K_M \) and \( V_{\text{max}} \) using a least-squares fit of the experimental data points to Eq[4.12]

- Thus, \( K_M \) (M) would be the value of \( [S] \) @ \( V_{\text{max}}/2 \), and \( V_{\text{max}} \) (Ms\(^{-1}\)) the value of \( v \) when the curve plateaus out—but, where exactly does the curve plateau out?!!!

- Although the curve asymptotically approaches \( V_{\text{max}} \) @ very high values of \( [S] \), accurate determination of \( V_{\text{max}} \) is still far from certain

- Because of such uncertainty, we LINEARIZE Eq[4.12] to determine the values of \( K_M \) and \( V_{\text{max}} \)—enter the Lineweaver-Burk plot!
Michaelis-Menten Kinetics: Lineweaver-Burk Plot

- Reciprocating both sides of Eq[4.12], we obtain:
  \[
  \frac{1}{v} = \frac{K_M + [S]}{V_{max}[S]}
  \]
  \[
  \Rightarrow \quad \frac{1}{v} = \frac{K_M}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}
  \]
  \[
  \Rightarrow \quad \frac{1}{v} = \left(\frac{K_M}{V_{max}}\right)\cdot\left(\frac{1}{[S]}\right) + \left(\frac{1}{V_{max}}\right) \quad [4.14]
  \]
  \[
  \Rightarrow \quad y = (m)\cdot(x) + (b)
  \]

- Eq[4.14] is the equation of a straight line—it is called the Lineweaver-Burk equation

- Thus, a plot of 1/v versus 1/[S] generates a straight line from which the various kinetic parameters can be accurately determined using a linear fit with:
  
  slope = \(\frac{K_M}{V_{max}}\)
  
  y-intercept = \(\frac{1}{V_{max}}\)
  
  x-intercept = -1/\(K_M\)

- When 1/v=0 (in order to determine the x-intercept), Eq[4.14] reduces to:
  \[
  0 = \left(\frac{K_M}{V_{max}}\right)\cdot\left(\frac{1}{[S]}\right) + \left(\frac{1}{V_{max}}\right)
  \]
  \[
  \Rightarrow \quad \left(\frac{K_M}{V_{max}}\right)\cdot\left(\frac{1}{[S]}\right) = \left(-\frac{1}{V_{max}}\right)
  \]
  \[
  \Rightarrow \quad K_M\cdot(1/[S]) = -1
  \]
  \[
  \Rightarrow \quad 1/[S] = -1/K_M \quad [4.15]
  \]

- The double-reciprocal plot of 1/v versus 1/[S] is called the Lineweaver-Burk plot
Michaelis-Menten Kinetics: Catalytic Constant

- In order to assess the catalytic efficiency of an enzyme, a parameter called the catalytic constant \((k_{\text{cat}})\) is defined as follows (with the units of \(k_{\text{cat}}\) being \(s^{-1}\)):
\[
k_{\text{cat}} = \frac{V_{\text{max}}}{[E]_0}
\]  
[4.16]

- Now, combining Eq[4.11] and Eq[4.16], tells us that \(k_{\text{cat}}\) is the first-order rate constant \((k_{+2})\) associated with the decay of ES into P and E—an inherent consequence of steady-state assumption:
\[
k_{\text{cat}}[E]_0 = k_{+2}[E]_0
\Rightarrow
k_{\text{cat}} = k_{+2}
\]  
[4.17]

- Thus, \(k_{\text{cat}}\) is essentially the number of reaction turnovers or catalytic cycles per unit time accomplished by a particular enzyme—hence, \(k_{\text{cat}}\) is also referred to as the “turnover number”

- But, \(k_{\text{cat}}\) only tells us how good an enzyme is @ turning over the substrate after it is already bound to the active site—it tells us nothing about how good an enzyme is @ binding the substrate for if this cannot occur efficiently, substrate binding would become the rate-limiting step and diminish the overall performance of the enzyme even if \(k_{\text{cat}}\) is working @ supersonic speed!

- So what else do we need beside \(k_{\text{cat}}\) to accurately determine the catalytic efficiency of an enzyme? What kinetic parameter determines how good an enzyme is @ binding the substrate? Would \(K_M\) suffice? If so, what do we do with it?
Michaelis-Menten Kinetics: Catalytic Efficiency

- During the initial “burst” phase of the reaction when \([S] \ll K_M\), we can say that:
  \[K_M + [S] \approx K_M\] [4.18]

- Additionally, during the initial “burst” phase of the reaction, the amount of free enzyme available is virtually the same as the amount of enzyme initially added, or simply put:
  \[[E] \approx [E]_0\] [4.19]

- Thus, combining Eq[4.18] and Eq[4.19] with Eq[4.10], we have:
  \[v = \frac{k_{+2}[S][E]}{K_M}\]
  \[v = \frac{k_{cat}/K_M}{[S][E]}\] [4.20]

- Eq[4.20] is a second-order reaction with \(k_{cat}/K_M\) quotient being the second-order rate constant with units of \(M^{-1}s^{-1}\)

- The \(k_{cat}/K_M\) parameter is an indicator of the overall catalytic efficiency—ie how efficiently an enzyme converts a substrate into product

- Given that the catalytic efficiency will eventually reach an upper limit due to the limitation of substrate and product molecules to diffuse in and out of the enzyme active site (particularly within the highly viscous milieu of the living cell), the \(k_{cat}/K_M\) parameter unsurprisingly displays the diffusion-controlled limit of about \(10^9 M^{-1}s^{-1}\)—some enzymes can however operate in excess of this limit via the so-called quantum tunneling!
### Michaelis-Menten Kinetics: Comparison of $k_{\text{cat}}/K_M$

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>$K_M$ (M)</th>
<th>$k_{\text{cat}}$ (s$^{-1}$)</th>
<th>$k_{\text{cat}}/K_M$ (M$^{-1}$ · s$^{-1}$)</th>
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<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td>Acetylcholine</td>
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<td>$1.4 \times 10^4$</td>
<td>$1.5 \times 10^8$</td>
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<td>Carbonic anhydrase</td>
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<td>$1.0 \times 10^6$</td>
<td>$8.3 \times 10^7$</td>
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<tr>
<td></td>
<td>$\text{HCO}_3^-$</td>
<td>$2.6 \times 10^{-2}$</td>
<td>$4.0 \times 10^5$</td>
<td>$1.5 \times 10^7$</td>
</tr>
<tr>
<td>Catalase</td>
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<td>$2.5 \times 10^{-2}$</td>
<td>$1.0 \times 10^7$</td>
<td>$4.0 \times 10^8$</td>
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<td>$4.4 \times 10^{-1}$</td>
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<td>$1.2 \times 10^{-1}$</td>
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<td>$1.0 \times 10^4$</td>
<td>$4.0 \times 10^5$</td>
</tr>
</tbody>
</table>
Exercise 2.6a

- Write the rate equations for a first-order and a second-order reaction

- What are the differences between initial velocity, and maximum velocity for an enzymatic reaction?

- Derive the Michaelis–Menten equation. Show the workout and reasoning behind each assumption.

- What do the values of $K_M$ and $k_{cat}/K_M$ reveal about an enzyme?

- Why an enzyme cannot have a $k_{cat}/K_M$ value greater than $10^9 \text{ M}^{-1}\text{s}^{-1}$?

- Write the Lineweaver–Burk equation and describe the features of a Lineweaver–Burk plot
Section 2.6b: Enzyme Inhibition
**Synopsis 2.6b**

- **Enzyme inhibition** is central to understanding the action of drugs on enzymes.

- Enzyme inhibitors (e.g., drugs) interact reversibly or irreversibly with an enzyme to alter its $K_M$ and/or $V_{max}$ values—such inhibitors can be classified into three types: competitive, uncompetitive, and mixed (or noncompetitive).

- A **competitive inhibitor** competes with the substrate (S) for binding to the free enzyme (E)—it increases the $K_M$ but has no effect on the $V_{max}$.

- An **uncompetitive inhibitor** binds only to enzyme-substrate (ES) complex and does NOT compete with the substrate (S) for binding to the free enzyme (E)—it decreases both the $K_M$ and $V_{max}$.

- A **mixed inhibitor** competes with the substrate (S) for binding to the free enzyme (E) but uncompetitively binds to the enzyme-substrate (ES) complex—it decreases the $V_{max}$ but $K_M$ may increase or decrease.

- A **noncompetitive inhibitor** is a mixed inhibitor that indiscriminately or noncompetitively binds to both the free enzyme (E) and the enzyme-substrate (ES) complex with an equal affinity—it decreases the $V_{max}$ but has no effect on $K_M$.

- In short, enzyme inhibition can be divided into THREE categories:
  1. Competitive Inhibition
  2. Uncompetitive Inhibition
  3. Mixed Inhibition
In competitive inhibition, an inhibitor usually resembles the substrate—it is a chemical analogue of the substrate and exerts its action by binding to the active site.

Thus, a competitive inhibitor (I) directly competes with the substrate (S) for the active site of the free enzyme (E)—but cannot be converted to a product (P)—thereby essentially acting as a ligand to sterically block substrate binding.

In the generalized model shown, El is the catalytically-inactive enzyme-inhibitor complex—the equilibrium dissociation constant (K_I) associated with the formation of EI is given by:

\[ K_I = \frac{[E][I]}{[EI]} \]

where
- \([E] = \text{Concentration (M) of freely available enzyme @ equilibrium}
- \([I] = \text{Concentration (M) of freely available inhibitor @ equilibrium}
- \([EI] = \text{Concentration (M) of enzyme-inhibitor complex @ equilibrium} \]
Since a competitive inhibitor reduces the concentration of available enzyme for substrate binding, $K_M$ is INCREASED by a factor $\alpha$ ($\geq 1$) but $V_{\text{max}}$ is unaffected in the Michaelis-Menten equation:

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

[1.2]

where $\alpha = 1 + \frac{[EI]}{[E]} = 1 + \frac{[I]}{K_I}$

$[I]$ = Total concentration of inhibitor added

- Simply put, Eq[1.2] tells us that the presence of $I$ makes $[S]$ appear to be less than it really is—accordingly, increasing $[S]$ by a factor of $\alpha$ or more will override the effect of competitive inhibitor:

$\text{when } [S] \to \infty, \ v \to V_{\text{max}}$
For competitive inhibition, the Lineweaver-Burk equation adopts the form:

\[ \frac{1}{v} = \left( \frac{\alpha K_M}{V_{\text{max}}} \right) \left( \frac{1}{[S]} \right) + \left( \frac{1}{V_{\text{max}}} \right) \]  \hspace{1cm} [1.3]

Thus, a plot of \( 1/v \) versus \( 1/[S] \) generates a straight line from which the various kinetic parameters can be accurately determined using a linear fit with:

- slope = \( \frac{\alpha K_M}{V_{\text{max}}} \)
- y-intercept = \( \frac{1}{V_{\text{max}}} \)
- x-intercept = -\( 1/\alpha K_M \)

Because a competitive inhibitor only affects \( K_M \) but not \( V_{\text{max}} \), multiple Lineweaver-Burk plots generated as a function of [I] intersect on the ordinate (y-axis) at \( 1/V_{\text{max}} \).

For a given set of [I] and \( \alpha \), the extent of competitive inhibition in terms of \( K_I \) can be directly computed from the relationship:

\[ \alpha = 1 + \left\{ \frac{[I]}{K_i} \right\} \]  \hspace{1cm} [1.4]

Since Eq[1.4] is an equation of a straight line with respect to \( \alpha \) and [I], one can also calculate \( K_i \) from the slope of a linear plot of \( \alpha \) versus [I]—a more accurate procedure.
(2) Uncompetitive Inhibition: General Model

In uncompetitive (NOT noncompetitive!) inhibition, an uncompetitive inhibitor (I) directly binds to the enzyme-substrate (ES) complex but NOT to the free enzyme (E).

While it may or may not resemble the substrate, an uncompetitive inhibitor exerts its effect by virtue of its ability to distort the enzyme active site so as to render it catalytically-inactive—the inhibitor must bind at an allosteric site located away from the active site!

In the generalized model shown, ESI is the catalytically-inactive enzyme-substrate-inhibitor complex—the equilibrium dissociation constant ($K'_I$) associated with the formation of ESI is given by:

$$K'_I = \frac{[ES][I]}{[ESI]}$$

where

- $[ES] =$ Concentration (M) of freely available ES complex @ equilibrium
- $[I] =$ Concentration (M) of freely available inhibitor @ equilibrium
- $[ESI] =$ Concentration (M) of ESI complex @ equilibrium
Since an uncompetitive inhibitor reduces the concentration of ES complex available for product generation, both $K_M$ and $V_{max}$ are DECREASED by a factor $\alpha'$ (>= 1) in the Michaelis-Menten equation:

$$v = \frac{(V_{max}/\alpha')[S]}{(K_M/\alpha')+ [S]}$$  \[2.2\]

where  

$$\alpha' = 1 + \frac{[ESI]/[ES]}{[I]/K'_I} = 1 + \frac{[I]}{K'_I}$$

$[I] = \text{Total concentration of inhibitor added!}$

- Eq[2.2] tells us that while uncompetitive inhibition reduces both $K_M$ and $V_{max}$ by a factor $\alpha'$, it does not affect the overall $K_M/V_{max}$ quotient!

- Unlike competitive inhibition, increasing $[S]$ does NOT reverse the effect of an uncompetitive inhibitor!
(2) Uncompetitive Inhibition: Lineweaver-Burk Plot

- For uncompetitive inhibition, the Lineweaver-Burk equation adopts the form:
  \[ \frac{1}{v} = \left( \frac{K_M}{V_{\text{max}}} \right) \left( \frac{1}{[S]} \right) + \left( \frac{\alpha'}{V_{\text{max}}} \right) \]  \[ 2.3 \]

- Thus, a plot of \( \frac{1}{v} \) versus \( \frac{1}{[S]} \) generates a straight line from which the various kinetic parameters can be accurately determined using a linear fit with:
  - \( \text{slope} = \frac{K_M}{V_{\text{max}}} \)
  - \( \text{y-intercept} = \frac{\alpha'}{V_{\text{max}}} \)
  - \( \text{x-intercept} = -\frac{\alpha'}{K_M} \)

- Because an uncompetitive inhibitor does NOT affect the \( \frac{K_M}{V_{\text{max}}} \) quotient, multiple Lineweaver-Burk plots generated as a function of \([I]\) harbor the same slope—they never intersect either @ abscissa (x-axis) or ordinate (y-axis)

- For a given set of \([I]\) and \(\alpha'\), the extent of uncompetitive inhibition in terms of \(K'_I\) can be directly computed from the relationship:
  \[ \alpha' = 1 + \frac{[I]}{K'_I} \]  \[ 2.4 \]

- Since Eq[2.4] is an equation of a straight line with respect to \(\alpha'\) and \([I]\), one can also calculate \(K'_I\) from the slope of a linear plot of \(\alpha'\) versus \([I]\)—a more accurate procedure
Mixed Inhibition: General Model

- In mixed (or noncompetitive) inhibition, a mixed inhibitor \( I \) binds to both free enzyme \( E \) and enzyme-substrate (ES) complex—it has characteristics of both the competitive and uncompetitive inhibition!

- While it may or may not resemble the substrate, a mixed inhibitor exerts its effect by virtue of its ability to not only block substrate binding but also distorting the enzyme active site so as to render it catalytically-inactive—the inhibitor must bind at an allosteric site located away from the active site!

- In the generalized model shown, \( EI \) is the catalytically-inactive \( EI \) complex, and \( ESI \) is the catalytically-inactive enzyme-substrate-inhibitor complex—the equilibrium dissociation constants \( (K_I \text{ and } K'_I) \) respectively associated with the formation of \( EI \) and \( ESI \) are given by:

\[
K_I = \frac{[E][I]}{[EI]} \quad [3.1]
\]

\[
K'_I = \frac{[ES][I]}{[ESI]} \quad [3.2]
\]

where
- \([E]\) = Concentration (M) of freely available enzyme @ equilibrium
- \([ES]\) = Concentration (M) of freely available ES complex @ equilibrium
- \([I]\) = Concentration (M) of freely available inhibitor @ equilibrium
- \([EI]\) = Concentration (M) of \( EI \) complex @ equilibrium
- \([ESI]\) = Concentration (M) of \( ESI \) complex @ equilibrium
(3) Mixed Inhibition: Michaelis-Menten Plot

- Since a mixed inhibitor harbors the characteristics of both the competitive and uncompetitive inhibition, both $K_M$ and $V_{max}$ are MODULATED—$K_M$ increases by a factor $\alpha$ ($\geq 1$) due to competitive inhibition, and both $K_M$ & $V_{max}$ decrease by a factor $\alpha'$ ($\geq 1$) due to uncompetitive inhibition—in the Michaelis-Menten equation:

$$v = \frac{(V_{max}/\alpha')[S]}{((\alpha/\alpha')K_M)+ [S]}$$

where 
$\alpha = 1 + [{[EI]/[E]} = 1 + {[I]/K_I}$
$\alpha' = 1 + [{[ESI]/[ES]} = 1 + {[I]/K_I'}$

$[I]$ = Total concentration of inhibitor added

- Eq[3.3] tells us that while mixed inhibition must always reduce $V_{max}$ by a factor $\alpha'$, it has a subtle effect on $K_M$—simply put, $K_M$ may increase ($\alpha > \alpha'$) or decrease ($\alpha < \alpha'$) depending on the values of factors $\alpha$ and $\alpha'$, or it may even remain unaffected ($\alpha=\alpha'$)—inhibitor binds to both E and ES with an equal affinity (a special case of mixed inhibition referred to as noncompetitive inhibition)
(3) Mixed Inhibition: Lineweaver-Burk Plot

- For mixed inhibition, the Lineweaver-Burk equation adopts the form:
  \[ \frac{1}{v} = \left( \frac{\alpha K_M}{V_{\text{max}}} \right) \left( \frac{1}{[S]} \right) + \left( \frac{\alpha' V_{\text{max}}}{V_{\text{max}}} \right) \]  
  \[ [3.4] \]

- Thus, a plot of \( \frac{1}{v} \) versus \( \frac{1}{[S]} \) generates a straight line from which the various kinetic parameters can be accurately determined using a linear fit with:
  - slope = \( \frac{\alpha K_M}{V_{\text{max}}} \)
  - y-intercept = \( \frac{\alpha' V_{\text{max}}}{V_{\text{max}}} \)
  - x-intercept = - \( \frac{\alpha' \alpha K_M}{\alpha} \)

- For mixed inhibition, multiple Lineweaver-Burk plots generated as a function of \([I]\) can never intersect at the ordinate but they intersect on its left provided that \( \alpha \neq \alpha' \), or intersect at the abscissa when \( \alpha = \alpha' \)

- For a given set of \([I]\) and \( \alpha' \), the extent of uncompetitive inhibition in terms of \( K'_I \) can be directly computed from the relationship:
  \[ \alpha = 1 + \left\{ \frac{[I]}{K_I} \right\} \]  
  \[ [3.5] \]
  \[ \alpha' = 1 + \left\{ \frac{[I]}{K'_I} \right\} \]  
  \[ [3.6] \]

- Since Eq[3.5] is an equation of a straight line with respect to \( \alpha \) and \([I]\), one can also calculate \( K_I \) from the slope of a linear plot of \( \alpha \) versus \([I]\)—ditto for \( K'_I \)
# Enzyme Inhibition: Comparison of Mechanisms

<table>
<thead>
<tr>
<th>Type of Inhibition</th>
<th>Michaelis–Menten Equation</th>
<th>Lineweaver–Burk Equation</th>
<th>Effect of Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>( v_o = \frac{V_{\text{max}}[S]}{K_M + [S]} )</td>
<td>( \frac{1}{\frac{1}{v_o}} = \frac{K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} )</td>
<td>None</td>
</tr>
<tr>
<td>Competitive</td>
<td>( v_o = \frac{V_{\text{max}}[S]}{\alpha K_M + [S]} )</td>
<td>( \frac{1}{\frac{1}{v_o}} = \frac{\alpha K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{1}{V_{\text{max}}} )</td>
<td>Increases ( K_{M}^{\text{app}} )</td>
</tr>
<tr>
<td>Uncompetitive</td>
<td>( v_o = \frac{V_{\text{max}}[S]}{K_M + \alpha'[S]} = \frac{(V_{\text{max}}/\alpha')[S]}{K_M/\alpha' + [S]} )</td>
<td>( \frac{1}{\frac{1}{v_o}} = \frac{K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{\alpha'}{V_{\text{max}}} )</td>
<td>Decreases ( K_{M}^{\text{app}} ) and ( V_{\text{max}}^{\text{app}} )</td>
</tr>
<tr>
<td>Mixed (noncompetitive)</td>
<td>( v_o = \frac{V_{\text{max}}[S]}{\alpha K_M + \alpha'[S]} = \frac{(V_{\text{max}}/\alpha')[S]}{(\alpha/\alpha')K_M + [S]} )</td>
<td>( \frac{1}{\frac{1}{v_o}} = \frac{\alpha K_M}{V_{\text{max}}} \frac{1}{[S]} + \frac{\alpha'}{V_{\text{max}}} )</td>
<td>Decreases ( V_{\text{max}}^{\text{app}} ); may increase or decrease ( K_{M}^{\text{app}} )</td>
</tr>
</tbody>
</table>

\( ^\alpha \alpha = 1 + \frac{[I]}{K_i} \) and \( \alpha' = 1 + \frac{[I]}{K'_i} \).
Exercise 2.6b

- Why might an enzyme’s substrate, transition state, and product all serve as starting points for the design of a competitive inhibitor?

- Describe the effects of competitive, uncompetitive, and mixed inhibitors on $K_M$ and $V_{max}$

- How can inhibitor binding to an enzyme be quantified?