Reaction Rates (reaction velocities): To measure a reaction rate we monitor the disappearance of reactants or appearance of products.

\[ 2\text{NO}_2 + \text{F}_2 \rightarrow 2\text{NO}_2\text{F} \]

- Protein Folding: 1st order reaction
- DNA annealing: 2nd order reaction

Rates of enzyme reactions are affected by:
- Enzymes/catalysts
- Substrates
- Temperature
- Concentrations
Why study enzyme kinetics?

- Quantitative description of biocatalysis
- Understand catalytic mechanism
- Find effective inhibitors
- Understand regulation of activity

General Observations

- Enzymes are able to exert their influence at very low concentrations ~ [enzyme] = nM
- The initial rate (velocity) is linear with [enzyme].
- The initial velocity increases with [substrate] at low [substrate].
- The initial velocity approaches a maximum at high [substrate].

The initial velocity increases with [S] at low [S].

[Graph showing initial velocity vs. product concentration over time with different substrate concentrations (S).]

The initial velocity increases with [S] at low [S].

[Graph showing initial velocity vs. substrate concentration with a curve reaching a maximum at high substrate concentration.]
• Start with a mechanistic model
• Identify constraints and assumptions
• Solve for velocity (d[Product]/dt)

Simplest enzyme mechanism

- One reactant (S)
- One intermediate (ES)
- One product (P)

1. First step: The enzyme (E) and the substrate (S) reversibly and quickly form a non-covalent ES complex.
2. Second step: The ES complex undergoes a chemical transformation and dissociates to give product (P) and enzyme (E).
3. \( v = k_{cat}[ES] \)
4. Many enzymatic reactions follow Michaelis–Menten kinetics, even though enzyme mechanisms are always more complicated than the Michaelis–Menten model.
5. For real enzymatic reactions use \( k_{cat} \) instead of \( k_2 \).

The Enzyme-Substrate Complex (ES)

- The enzyme binds non-covalently to the substrate to form a non-covalent ES complex
  - the ES complex is known as the Michaelis complex.
  - A Michaelis complex is stabilized by molecular interactions (non-covalent interactions).
  - Michaelis complexes form quickly and dissociate quickly.
The enzyme is either free ([E]) or bound ([ES]): [E] = [ES] + [E].

At sufficiently high [S] all of the enzyme is tied up as ES (i.e., [E] = [ES], according to Le Chatelier’s Principle)

At high [S] the enzyme is working at full capacity (v = v_{max}).

The full capacity velocity is determined only by k_{cat} and [E_0].

k_{cat} = turnover #: number of moles of substrate produced per time per enzyme active site.

For any enzyme it is possible (pretty easy) to determine k_{cat}.

To understand and compare enzymes we need to know how well the enzyme binds to S (i.e., what happens in the first part of the reaction.) k_{cat} does not tell us anything about how well the enzyme binds to the substrate.

**Assumptions**

1. k_1, k_{-1} >> k_2 (i.e., the first step is fast and is always at equilibrium).
2. d[ES]/dt = 0 (i.e., the system is at steady state.)
   \[
   \frac{d[ES]}{dt} = \text{rate of formation of ES} - \text{rate of breakdown of ES} \\
   \approx 0 \text{ (at steady state)}
   \]
3. There is a single reaction/dissociation step (i.e., k_2 = k_{cat}).
4. S_{tot} = [S] + [ES] = [S]
5. There is no back reaction of P to ES (i.e. [P] = 0). This assumption allows us to ignore k_2. We measure initial velocities, when [P] = 0.

The time dependence of everything (in a Michaelis-Menten reaction)
Now: we derive the Michaelis-Menten Equation

\[
d[ES]/dt = k_1[E][S] - k_1[ES] - k_2[ES] = 0 \quad \text{(steady state assumption, see previous graph)}
\]

solve for [ES] (do the algebra)

\[
[ES] = [E][S] k_1/(k_1 + k_2)
\]

Define \( K_M \) (Michaelis Constant)

\[
K_M = (k_1 + k_2)/k_1 \quad \Rightarrow \quad [ES] = [E][S]/K_M
\]

rearrange to give \( K_M = [E][S]/[ES] \)

\[
v = \frac{v_{\text{max}}[S]}{K_M + [S]} \quad \text{Michaelis Menten Equation}
\]

When \([S] = K_M\) then,

\[
v = \frac{v_{\text{max}}[S]}{[S] + [S]} = \frac{v_{\text{max}}}{2}
\]

This is saying that when \( K_M = [S] \), the reaction runs at half maximum velocity.
Significance of $K_M$

- $K_M = \frac{[E][S]}{[ES]}$ and $K_M = \frac{k_{-1} + k_2}{k_1}$.
- $K_M$ is the apparent dissociation constant of the ES complex. A dissociation constant ($K_D$) is the reciprocal of the equilibrium constant ($K_D = K_A^{-1}$). $K_M$ is a measure of a substrate’s affinity for the enzyme (but it is the reciprocal of the affinity).
- If $k_1, k_{-1} >> k_2$, then $K_M = k_{-1}$.
- $K_M$ is the substrate concentration required to reach half-maximal velocity ($v_{max}/2$). A small $K_M$ means the substrate binds tightly to the enzyme and saturates (max out) the enzyme.
- $K_M$ is the “turnover number” indicates the rate at which the enzyme converts to substrate per second.
- If there are multiple catalytic steps (see trypsin), then each of those rate constants contributes to $K_M$.
- The microscopic meaning of $K_M$ depends on the details of the mechanism.

Significance of $k_{cat}$

- $v_{max} = k_{cat}[E]_{tot}$
- $k_{cat}$: For the simplest possible mechanism, where ES is the only intermediate, and dissociation is fast, then $k_{cat} = k_2$.
- If dissociation is slow then $k_{cat}$ is the dissociation rate constant also contributes to $k_{cat}$.
- If one catalytic step is much slower than all the others (and than the dissociation step), then the rate constant for that step is approximately equal to $k_{cat}$.
- $k_{cat}$ is the “turnover number” indicates the rate at which the enzyme turns over, i.e., how many substrate molecules one catalytic site converts to substrate per second.
- If there are multiple catalytic steps (see trypsin), then each of those rate constants contributes to $k_{cat}$.
- The microscopic meaning of $k_{cat}$ depends on the details of the mechanism.

Significance of $k_{cat}/K_M$

- $k_{cat}/K_M$ is the catalytic efficiency. It is used to rank enzymes. A big $k_{cat}/K_M$ means that an enzyme binds tightly to a substrate (small $K_M$).
- $k_{cat}/K_M$ can be used to estimate the reaction velocity from the total enzyme concentration ($[E]_0$). $k_{cat}/K_M = 10^9$ => diffusion control.
- $k_{cat}/K_M$ is the specificity constant. It is used to distinguish and describe various substrates.

Data analysis

- It would be useful to have a linear plot of the MM equation.
- Lineweaver and Burk (1934) proposed the following: take the reciprocal of both sides and rearrange.
- Collect data at a fixed $[E]_0$. 

\[ \frac{1}{v} = \frac{1}{k_{cat}[E]_{tot}[S]} \]
Michaelis-Menten Kinetics

\[ v = \frac{v_{\text{max}} [S]}{K_M + [S]} \]  

Michaelis Menten Equation

take the reciprocal

\[ \frac{1}{v} \frac{K_M + [S]}{v_{\text{max}} [S]} = \frac{K_M}{v_{\text{max}} [S]} + \frac{1}{v_{\text{max}}} \]

Graph \( \frac{1}{v} \) versus \( \frac{1}{[S]} \)

the y \((1/v)\) intercept \((1/[S] = 0)\) is \(1/v_{\text{max}}\)
the x \((1/[S])\) intercept \((1/v = 0)\) is \(-1/K_M\)
the slope is \(K_M/v_{\text{max}}\)

Lineweaver-Burk-Plot

the y \((1/v)\) intercept \((1/[S] = 0)\) is \(1/v_{\text{max}}\)
the x \((1/[S])\) intercept \((1/v = 0)\) is \(-1/K_M\)
the slope is \(K_M/v_{\text{max}}\)

Enzyme Inhibition

Competitive Inhibition

\[ \begin{align*}
E + S & \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \\
\text{+} & \\
I & \\
\text{E}I & \underset{k_2}{\rightarrow} P + E \\
\text{+} & \\
S & \rightarrow \text{NO REACTION}
\end{align*} \]
Competitive Inhibition

\[
\frac{[E][I]}{[EI]} = K_i \quad \text{inhibitor dissociation constant}
\]

\[
[E]_0 = [E] - [ES] - [EI] \quad \text{total enzyme concentration}
\]

\[
\alpha = 1 + \frac{[I]}{K_i}
\]

\[
\frac{1}{v} = \frac{\alpha K_M + [S]}{v_{\max} [S]} = \frac{\alpha K_M}{v_{\max} [S]} + \frac{1}{v_{\max}}
\]

Uncompetitive Inhibition

\[
E + S \overset{k_1}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} P + E
\]

\[
E + S + [I] \overset{k_{-1}}{\rightarrow} ESI \overset{K'_{1}}{\rightarrow} \text{NO REACTION}
\]
Uncompetitive Inhibition

\[
\frac{[ES][I]}{[ESI]} = K_I \quad \text{inhibitor dissociation constant}
\]

\[
[E]_0 = [E] - [ES] - [ESI] \quad \text{total enzyme concentration}
\]

\[
\frac{1}{v} = \frac{K_M}{v_{max}[S]} + \frac{\alpha}{v_{max}}
\]

Mixed (competitive and uncompetitive) Inhibition

\[
E + S \xleftrightarrow[k_1]{k_{-1}} ES \xrightarrow[k_2]{\text{I}} P + E + \text{I}
\]

\[
K_I \quad \text{I} \quad K'_I \quad \text{EI} \quad \text{ESI} \quad \text{NO REACTION}
\]

Increasing

[II]

\[
\alpha' = 2
\]

\[
\alpha' = 1.5
\]

\[
\alpha' = 1 (\text{no inhibitor})
\]

Slope = \(\frac{K_M}{v_{max}}\)

\[
\alpha' = 1 + \frac{[I]}{K_1}
\]

\[
\alpha' = 1 + \frac{[I]}{K_1}
\]