Kinetics - The study of the rates of chemical processes

Thermodynamics - Pertains to the different energies associated with different chemical states
Enzymes

**Catalyst**: A substance which increases the rate (velocity) of a chemical reaction without itself being changed in the overall process.

**Enzyme**: Protein catalyst.

\[ \text{Substrate} + E \rightleftharpoons \text{Product} + E \]

- Enzymes accelerate the rates of reactions in both directions.
- Enzymes do not affect the state of equilibrium, only the rate.
Figure 11.22
The cascade process in blood clotting. Each factor in the pathway can exist in an inactive form (red) or an active form (green). The cascade of proteolytic activations can start from exposure of blood at damaged tissue surfaces (intrinsic pathway) or from internal trauma to blood vessels (extrinsic pathway). The common result is activation of fibrinogen to clotting fibrin. Auxiliary factors that aid some steps are also shown. An asterisk (*) denotes serine proteases.
Table 5.4
Some proteolytic enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Preferred Site</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>$R_1 = \text{Lys, Arg}$</td>
<td>From digestive systems of animals, many other sources</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>$R_1 = \text{Tyr, Phe, Leu, Ileu, Val, Trp, and His at high pH}$</td>
<td>Same as trypsin</td>
</tr>
<tr>
<td>Pepsin</td>
<td>$R_1 = \text{Phe, Leu, many others}$</td>
<td>Same as trypsin but confined to stomach, where pH is low</td>
</tr>
<tr>
<td>Thrombin</td>
<td>$R_1 = \text{Arg}$</td>
<td>From blood; involved in coagulation</td>
</tr>
<tr>
<td>Papain</td>
<td>$R_1 = \text{Arg, Lys, Phe-X (CO side of residue next to Phe)}$</td>
<td>From papaya latex</td>
</tr>
<tr>
<td>Bromelain</td>
<td>$R_1 = \text{Lys, Ala, Tyr, Gly}$</td>
<td>From pineapple</td>
</tr>
<tr>
<td>Thermolysin</td>
<td>$R_2 = \text{same residues as chymotrypsin}$</td>
<td>From <em>Bacillus thermoproteolyticus</em></td>
</tr>
<tr>
<td>Subtilisin</td>
<td>Very little specificity</td>
<td>From various bacilli</td>
</tr>
<tr>
<td>Carboxypeptidase A</td>
<td>$R_2 = \text{C-terminal amino acid}$</td>
<td>From digestive systems of animals</td>
</tr>
</tbody>
</table>

Figure 11.19
Schematic view of pancreatic zymogen activation. Zymogens are in red, active proteases in yellow or green.
transient state.

no enzyme.

activation energy.

\[ \Delta G^* \]

w/ enzyme.

R \rightarrow P

\[ \xi \]

Extent of Reaction.

Reaction Coordinate.

\[ k = Q \exp(-\Delta G^*/RT) \]  
Arrhenius Eqn.

\[ \Delta G^* = \Delta H^* - T \Delta S^* \]
Enzymes can catalyze reactions by stabilizing the transition state.

Peptide Bond Hydrolysis:

\[ \text{C-N} \rightarrow [\text{C-N}] \rightarrow \text{C-OH} + \text{H-N-R} \]

Induced Fit: Show overhead.

Serine Protease: Show overhead.
Figure 10.7
Enzyme and endothermic factors in catalysis. Two reactants are bound to an enzyme, which causes their correct mutual orientation and propinquity and binds them most strongly in the transition state.

Figure 10.10
The conformational change in hexokinase induced by glucose binding. (a) Before glucose binding. (b) After glucose binding. The two major domains of the enzyme are shaded differently to distinguish them.
Figure 10.11
Triose-phosphate isomerase. (a) Structure of one of the two subunits in the enzymatically active dimer. The β-barrel structure is shown in gray, and the active site region in green. (b) Detail of the active site in the yeast enzyme, with DHAP bound. Three residues critical to the reaction are Glu 165, His 95, and Lys 12.

Figure 10.24
Active site of the protease carboxypeptidase A. The zinc atom serves as a metal catalyst to promote hydrolysis. The bond cleaved is indicated with a wedge.
Transient Kinetics Vs Steady-state Kinetics
Transient Kinetics

Case I: Irreversible 1st Order Reaction

\[ \text{A} \xrightarrow{k} \text{B} \]

\[ V = \text{reaction rate} \quad k = \text{rate constant} \]

\[ V = -\frac{d[A]}{dt} = \frac{d[B]}{dt} \]

\[ V = k [A] \]

the more A that is consumed, the faster the formation of B.

Sec \[ \text{sec}^{-1} \quad \text{M} \]

1st Order.
\[ k[A] = -\frac{d[A]}{dt} \]

\[-\int_{0}^{t} k \, dt = \int_{A_0}^{A(t)} \frac{d[A]}{[A]} \quad \text{dye IJ} \]

\[-k \left|_{t_0}^{t} \right. = \ln \left( \frac{A(t)}{A_0} \right) \]

\[-k t = \ln \left( \frac{A(t)}{A_0} \right) \]

\[ A(t) = A_0 e^{-kt} \]

\[ m = -k \]

Graph showing \( \ln \left( \frac{A(t)}{A_0} \right) \) vs. \( t \) with a slope of \( -k \).
Case II: Irreversible $2^{nd}$ Order Reaction

$$2A \xrightarrow{k} C$$

$$A + B \xrightarrow{k} D.$$  

$2^{nd}$ Rate Constant.  

$$V = \frac{d[C]}{dt} = k [A]^2 - N^2 \quad \text{let;}$$

integrate this curve.

$$\frac{d[A]}{dt} = k [A][B]$$

$$\frac{dc}{dt} = -\frac{1}{2} \frac{dA}{dt} \quad \text{Since 2 moles of A are consumed for each mole of C produced.}$$

$$-\frac{1}{2} \frac{dA}{dt} = k A^2$$

$$\int_{A_0}^{A(t)} \frac{dA}{A^2} = \int_0^t 2k \, dt$$

$$-\frac{1}{A} \bigg|_{A_0}^{A(t)} = -2k t \bigg|_0^t$$
\[ \frac{1}{A_o} - \frac{1}{A(t)} = -2kt \]

\[ \frac{1}{A(t)} = \frac{1}{A_o} + 2kt. \]
Case III Steady-State Enzyme Catalysis
(Michaelis-Menten kinetics)

Simple one-substrate, one-product reaction.

\[ E + S \xrightarrow{k_i} ES \xrightarrow{k_{cat}} E + P. \]

\[ \frac{k_i}{k_i} \]

Assumption:

1) \( E + P \rightleftharpoons ES \)

(Reverse reaction)

Step

fast, rate-limiting

\[ V = \frac{d[P]}{dx} = k_{cat} [ES] \]

1st Order, Im. React.

Don't Erase.

Need to derive expression for \([ES]\)
III A. Michaelis - Menten Analysis. (1913)

**M.M. Assumption**

Assume \( k_{cat} \ll k_1, k_{-1} \)

Then \( E + S \) always in equilibrium with \( ES \).

\[ K_s = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} \]

Dissociation constant: \( K_s \)

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

We know \( [E]_t \) free enzyme

\[ [E] = [E]_t - [ES] \]

Substitute (3) into (2).

\[ [ES] = \frac{[E]_t [S]}{K_s} - \frac{[ES][S]}{K_s} \]

Rearranging.

\[ [ES] \left[ 1 + \frac{[S]}{K_s} \right] = \frac{[E]_t [S]}{K_s} \]
Dividing through by \([1 + \frac{[E]}{K_s}]\)

\([ES] = \frac{[E] \cdot [S]}{K_s \left[1 + \frac{[E]}{K_s}\right]}\)

Simplifying

\([ES] = \frac{[E][S]}{K_s + [E][S]}\)

And substituting \([E]_d\) to \([E]\).

\[V = \frac{k_{cat} [E]_d [S]}{K_s + [ES]}\]

M.M. Eqn.
III. Briggs-Haldane Analysis (1925)

\[ E + S \xrightarrow{K_{cat}} ES \xrightarrow{K_{-1}} E + P \]

we assumed these are always in equilibrium but this cannot be exactly correct since some ES is leaking out to produce.

In general,

\[ \frac{K_{cat}}{K_{-1}} \] is not always true.

Steady State Assumption: The intermediate ES will first build up, but then it will reach steady state - at which its concentration remains almost constant.

Concl.

Steady State.
(1) \[ \frac{d [ES]}{dt} = k_1 [E] [S] - k_{-1} [ES] - k_{cat} [ES] \]

\underline{formation} \quad \underline{breakdown}

At steady state

(2) \[ \frac{d [ES]}{dt} \approx 0 \]

Set Eqn (1) = 0, solve for [ES].

\[ (k_{-1} + k_{cat}) [ES] \equiv k_1 [E] [S] \]

(3) \[ [ES] \equiv \frac{k_1}{k_{-1} + k_{cat}} [E] [S] \]

(4) \[ [ES] \equiv \gamma [E] [S] \]
\[
[E]_T = [E] + [ES]
\]
(5)
\[
[E] = [E]_T - [ES]
\]

Substituting (5) into (4).

(6) \[
[ES] \simeq \gamma [E]_T [S] - \gamma [ES][ES]
\]

Rearranging.

(7) \[
[ES][1 + \gamma [S]] \simeq \gamma [E]_T [S]
\]

Solve for \( [ES] \).

(8) \[
[ES] = \frac{\gamma [E]_T [S]}{1 + \gamma [S]}
\]

or

(9) \[
[ES] = \frac{[E]_T [S]}{1/\gamma + [S]}
\]
Dehar \( \frac{1}{b} = K_m = \frac{k_{-1} + k_{cat}}{k_1} \)

Subst. (9) into \( \star \):

\[
V = \frac{k_{cat} [E]^\dagger [S]}{K_m + [S]} \quad \text{(mm)}
\]

Eqn. (20)

\[\text{devide by } k_m, k_{cat}\]

\[V_{max} = k_{cat} [E]^\dagger\]

\[\frac{V_{max}}{2} \]

\[V_{max} = k_{cat} [E]^\dagger \]

\[\frac{V_{max}}{2}\]

\[V \rightarrow V_{max} \quad \frac{1}{2} V_{max} \quad V_{max} \]

\[\lim_{S \rightarrow 0} V = 0\]

\[\lim_{S \rightarrow \infty} V = \frac{k_{cat} [E]^\dagger [S]}{[S]} = k_{cat} [E]^\dagger = V_{max}\]

\[(L'\text{Hospital's Rule)}\]

\[\text{at } [E]^\dagger = [E]_{0}\]
At $\frac{V_{\text{max}}}{2}$,

$$V = \frac{V_{\text{max}}}{2} = \frac{k_{\text{cat}} [E]_0}{2} = \frac{k_{\text{cat}} [E]_0 [S]}{K_m + [S]}$$

$$K_m [S] = 2 [S]$$

$$K_m = [S]$$

**Significance of $K_m$ and $k_{\text{cat}}$:**

$$K_m = \frac{k_{-1} + k_{\text{cat}}}{k_1} \quad \text{(Not a true Equil. Const.)}$$

$$K_S = \frac{k_{-1}}{k_1} \quad \text{(True Equilibrium)}$$

If $k_{\text{cat}} \ll k_{-1}$, $K_m \approx K_S$. Then $K_m$ reflects the strength of binding.

However, if $k_{\text{cat}}$ is large, it will make $K_m$ large even if binding is strong.
The interpretation of $K_m$ as an effective dissociation constant must be used with caution.

$k_{cat}$ - rate of formation of product from $E$S. Units: $s^{-1}$

($\text{turn over number}$).

$\frac{1}{k_{cat}}$ - time required for enzyme to "turn over" one substrate molecule.

$\text{e.g. } k_{cat} = 10 \text{ sec}^{-1}$

$\frac{1}{k_{cat}} = 0.1 \text{ sec per substrate molecule}$.
\[ \frac{K_{\text{cat}}}{K_m} \]

Consider kinetics at very low substrate concentration.

\[ V = \frac{K_{\text{cat}}[E][S]}{K_m + [S]} = \frac{K_{\text{cat}}}{K_m} \cdot [E][S] \]

Since \([E] \approx [E]_I\)

Recall that for a 2° Reaction

\[ A + B \rightarrow C \]

\[ V = k_2 [A][B] \]

Hence \( \frac{K_{\text{cat}}}{K_m} \) is analogous to the 2° rate constant for combining \( E + S \).

\[ E + S \xrightarrow{K_{\text{cat}}/K_m} ES \]

When comparing substrates or mutant enzymes.

Hence - provides a direct measure of enzyme efficiency and specificity.
Optimal Enzyme

\[ k_{\text{cat}} > k_{-1} \]

\[ E + S \xrightarrow{k_{-1}} ES \xrightarrow{k_{\text{cat}}} E + P. \]

Then

\[ K_m = \frac{k_{-1} + k_{\text{cat}}}{k_{-1}} \approx \frac{k_{\text{cat}}}{k_{-1}} \]

and

\[ \frac{k_{\text{cat}}}{K_m} \approx \frac{k_{\text{cat}}}{k_{-1}} \approx k_{-1} \]

Every collision results in an enzyme-substrate complex.

If every collision results in an enzyme-substrate complex, \( k_{-1} = 10^{-8} - 10^{-9} \text{ M}^{-1}\text{s}^{-1} \).

For Tissue Phosphatase Isomerase (TIM),

\[ \frac{k_{\text{cat}}}{K_m} = 2.4 \times 10^8 \text{ M}^{-1}\text{s}^{-1} \]

Almost perfect enzyme.

Yeast \rightarrow Vertebrates - very little change.
Concept of Initial Rates

Initial rate \[ \frac{\Delta A}{\Delta t} \]

\[ A_0 = 10 \]
\[ A_0 = 5 \]
\[ A_0 = 2 \]

\[ V = k[A] \]

\[ 2 \text{ init Conc. of } A \]
Measuring $K_m$ and $k_{cat}$

Measure initial rates of \( \frac{\Delta [S]}{\Delta t} = V \)

at different values of \([S]\)

\[
V = \frac{k_{cat} [E]_0 [S]}{K_m + [S]} = \frac{V_{max} [S]}{K_m + [S]}
\]

\[
\frac{1}{V} = \frac{K_m}{V_{max} [S]} + \frac{1}{V_{max}}
\]

\[
k_{cat} = \frac{V_{max} [E]_0}{[S]}
\]

Get $K_m$

then $k_{cat} = \frac{V_{max}}{[E]_0}$
Alternatively,

\[ V = \frac{k_{cat} [E]_0 [S]}{[S] + K_m} = \frac{V_{max} [S]}{[S] + K_m} \]

Rearranging,

\[ V = \frac{V [S] + V K_m}{[S]} \]

\[ V_{max} = V + \frac{V K_m}{[S]} \]

\[ V = V_{max} - \frac{V K_m}{[S]} \]

\[ V \approx V_{max} \]

\[ m = -\frac{K_m}{V_{max}} \]

Eadie-Hofstee Plot
Enzyme Regulation

1. Reversible Inhibition
   (non-covalent binding of inhibitor)

1A. Competitive Inhibition - Inhibitor binds at active site

\[ E + S \overset{k_m}{\underset{E}\rightleftarrows} ES \overset{k_{cat}}{\longrightarrow} E + P \]

\[ + I \]

\[ \uparrow K_i \]

[ES] + [EI]

Use

\[ [E]_t = [E] + [ES] + [EI] \]

Can show

\[ \frac{V}{[S]} = \frac{k_{cat} [E]_t [S]}{[E]_t [S] + k_m (1 + [I]/K_i)} \]
Can be rewritten:

\[ V = \frac{k_{cat} [E]_t [S]}{[S] + K_m^{app}} \]

\[ V_{max} \]

\[ \frac{V}{V_{max}} \]

\[ \frac{V}{V_{max}} = \frac{1}{K_m + \frac{K_m^{app}}{[S]}} \]

\[ -\frac{1}{V_{max}} = \frac{1}{I[S]} \]

\[ \frac{1}{V} = \frac{1}{K_m^{app}} - \frac{1}{K_m} \]

\[ \Rightarrow V_{max} \text{ not changed by } I \]

\[ \text{Competitive Inhibition} \]

\[ \text{Change } K_m \text{, not } V_{max}. \]
1B. Non-Competitive Inhibition

\[
E + S \underset{K_m}{\stackrel{K_m}{\rightleftharpoons}} ES \xrightarrow{K_{cat}+I} E + P
\]

\[
I + E \underset{K_i}{\xrightarrow{+I}} E + I
\]

\[
EI + S \underset{K_m}{\xrightarrow{+I}} EIS
\]

\[
V = \frac{\{k_{cat}/(1 + I/K_i)\}[E][S]}{[S] + K_m}
\]

\[
\frac{V}{V_{max}(+I)} = \frac{1}{V_{max}(+I) \frac{K_m}{[S]}} + \frac{1}{V_{max}(-I)} \frac{1}{K_m} \frac{1}{[S]}
\]

Inhibitor binds to second site, independent of enzyme.
1c. Uncompetitive Inhibition (inhibitor binds only to \( E\) complex)

\[
E + S \xrightarrow{k_m} ES \xrightarrow{k_{cat}} E + P
\]

\[+\quad \downarrow \quad E_S I\]

\[V = \frac{k_{cat} [E][S]}{[S](1 + [I]/K_i) + K_m}\]

\[\frac{1}{V_{max}}\quad \downarrow \quad +I\]

\[\frac{1}{V}\quad \frac{1}{V_{max}}\quad \frac{1}{[S]}\quad \frac{1}{K_m}\quad \frac{1}{K_{cat}}\quad \frac{1}{[S]}\quad \frac{1}{K_m}\quad \text{Both } k_{cat} \text{ and } K_m \text{ affected.}\]
10. Allostereic Effects.

Hemoglobin.
Aspartate Carbamoyl transferase