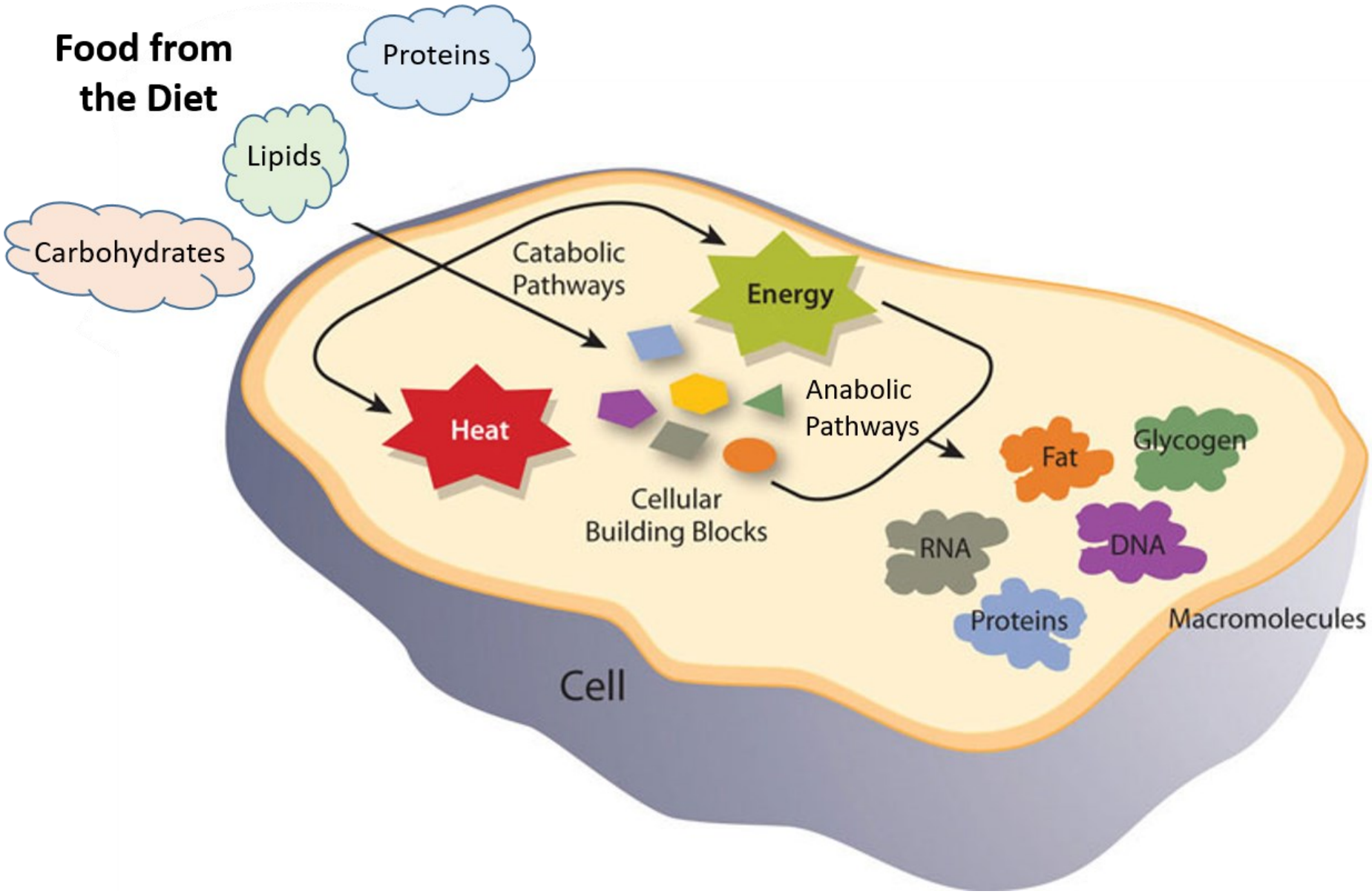
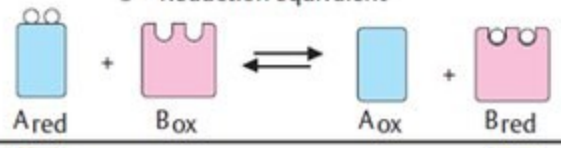
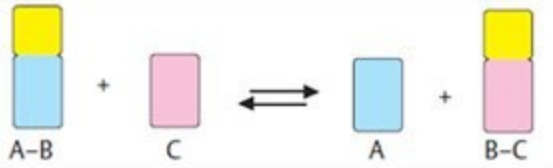
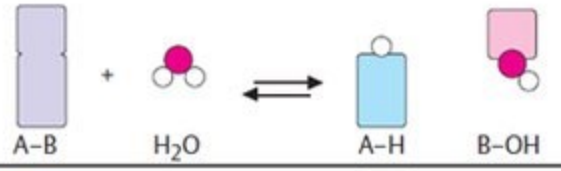
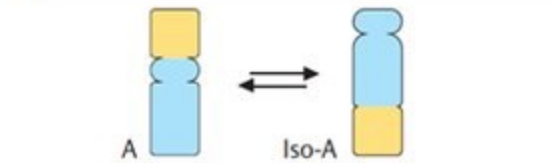
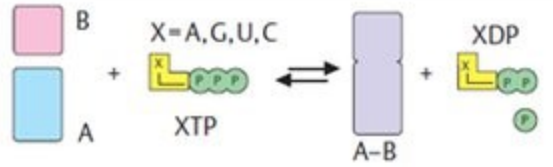
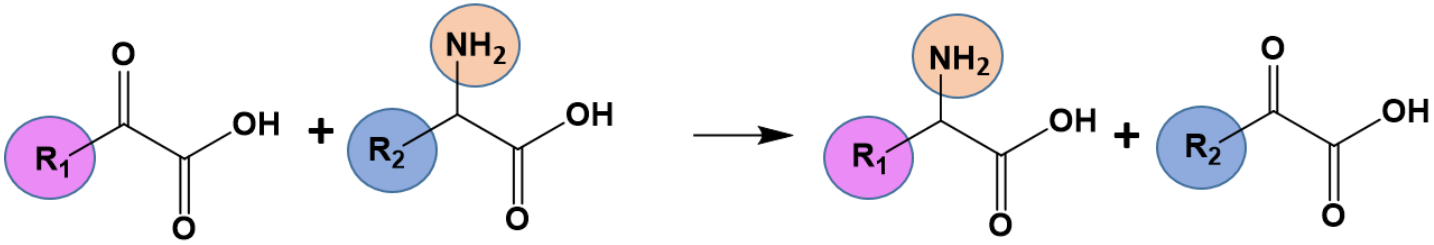
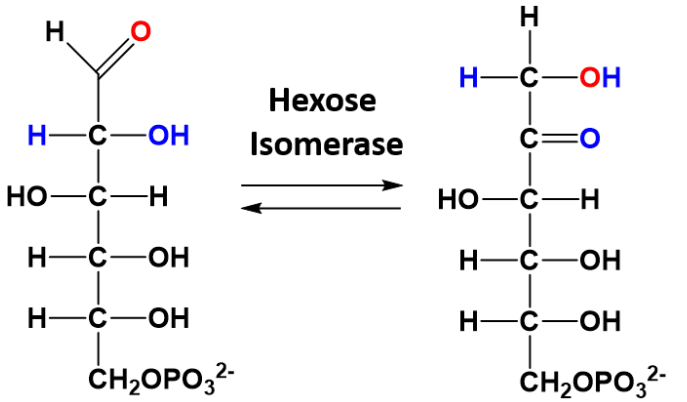

Food from the Diet
Proteins
Lipids
Carbohydrates
Catabolic Pathways
Energy
Heat
Anabolic Pathways
Cellular Building Blocks
Fat
Glycogen
RNA
DNA
Proteins
Macromolecules
Cell

Naming of enzymes

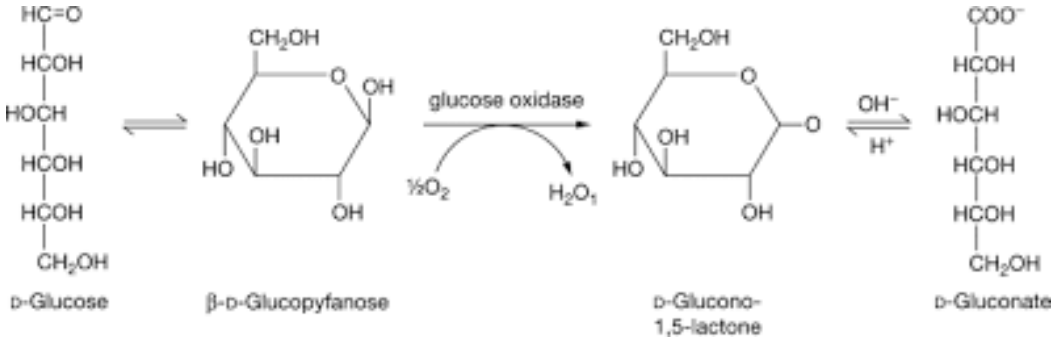
Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent  	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	 	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	 	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	 	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	 	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	 	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases



α-keto acid α-amino acid NEW α-amino acid NEW α-keto acid



Glucose 6-phosphate Fructose 6-phosphate

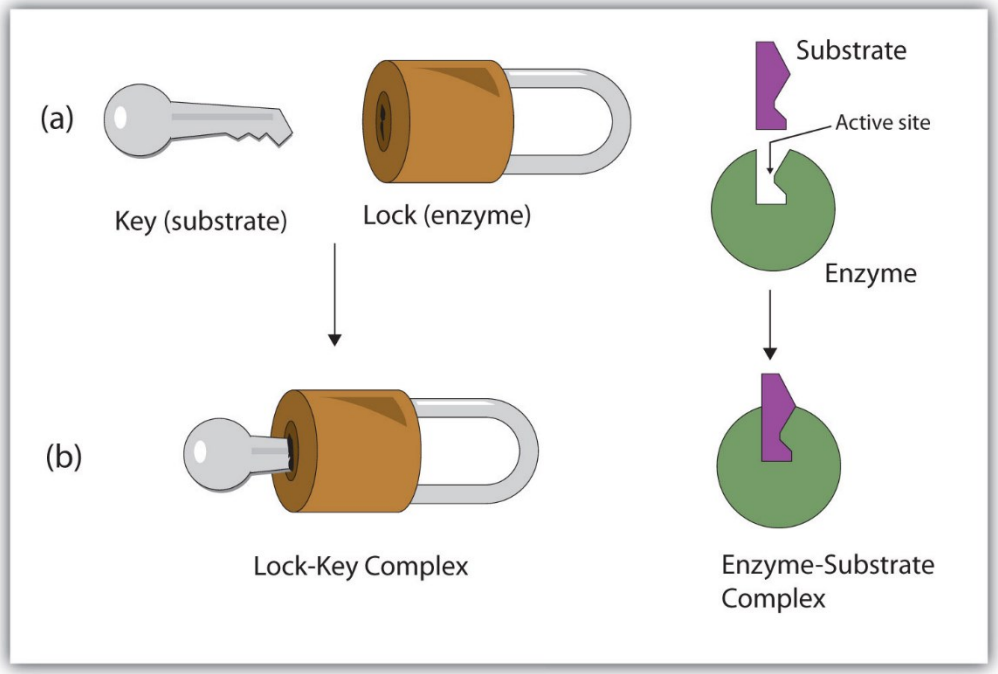


Structure and specificity

Enzymes are usually specific to the **reactions** they catalyze and the **substrates** that are involved in these reactions. **Shape complementarity, charge complementarity, and hydrophilic/hydrophobic** characters of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, chemoselectivity.

Structure and specificity

Lock and Key Mechanism



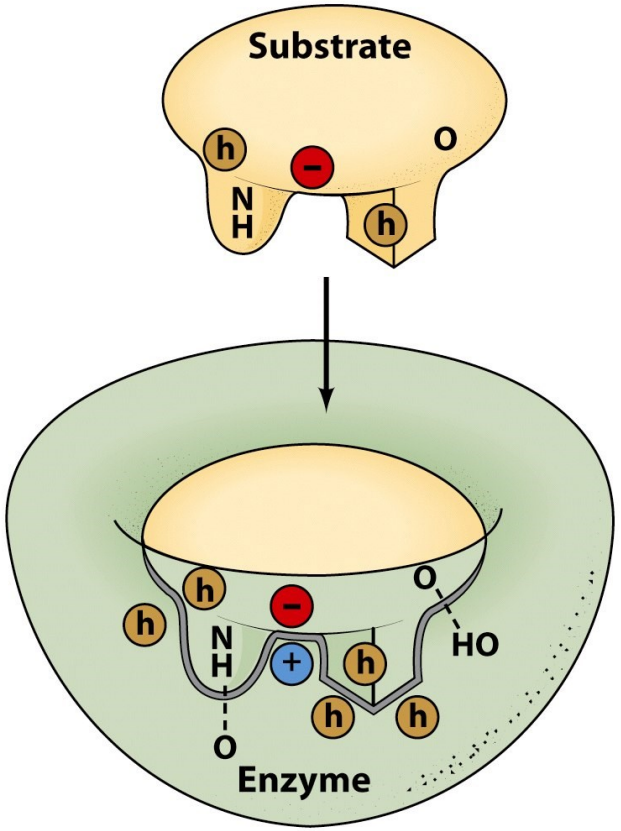
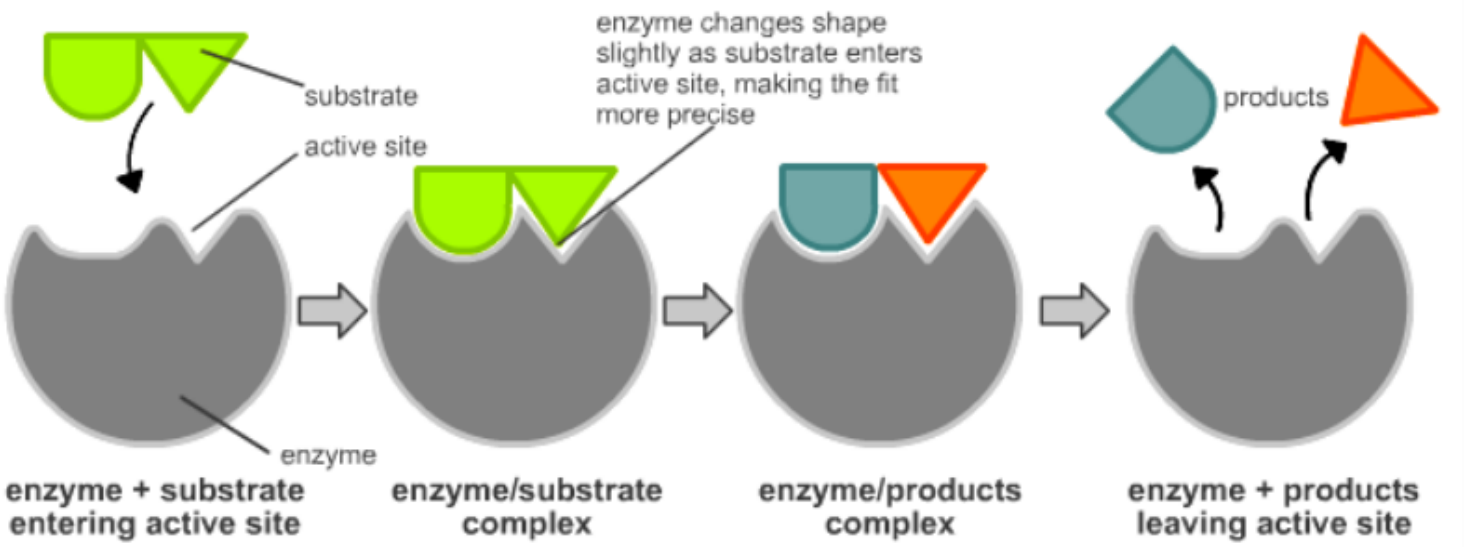


Figure 11-1 Fundamentals of Biochemistry, 2/e

Structure and specificity

Induced fit model

In 1958 Daniel Koshland suggested a modification to the "lock and key" model. Since **enzymes are rather flexible structures**, the active site can be modified as the substrate interacts with the enzyme. As a result, **the amino acid side chains making up the active site are molded into a precise shape which enables the enzyme to perform its catalytic function.** In some cases the substrate molecule also changes shape slightly as it enters the active site. This model explains both **enzyme specificity** and the **stabilization of the transition state**.



How enzymes enhance reaction rate?

Activation energy and reaction coordinate

Much of our understanding of how enzyme catalyze chemical reactions comes from **transition state theory.** (developed by Henry Eyring, in 1930s)

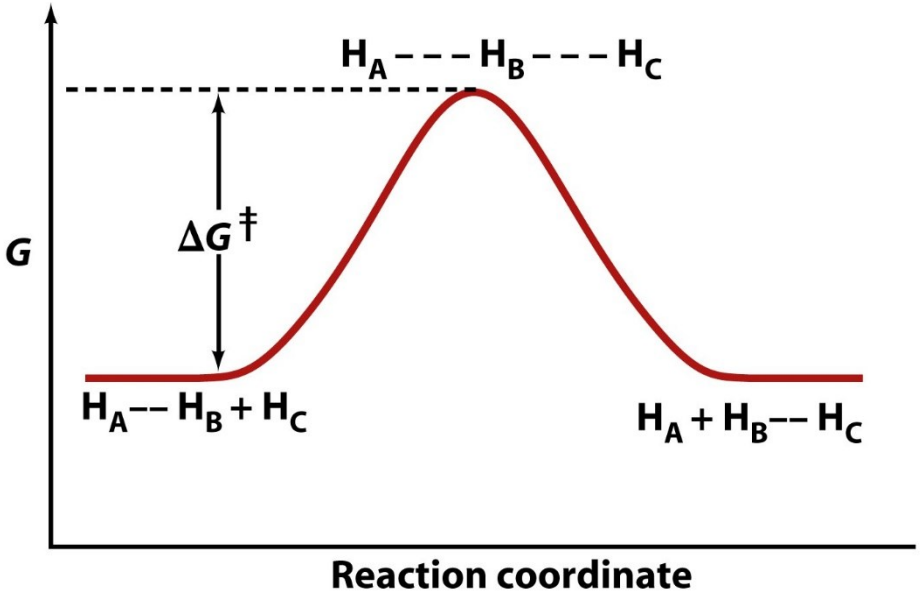


Figure 11-5a Fundamentals of Biochemistry, 2/e
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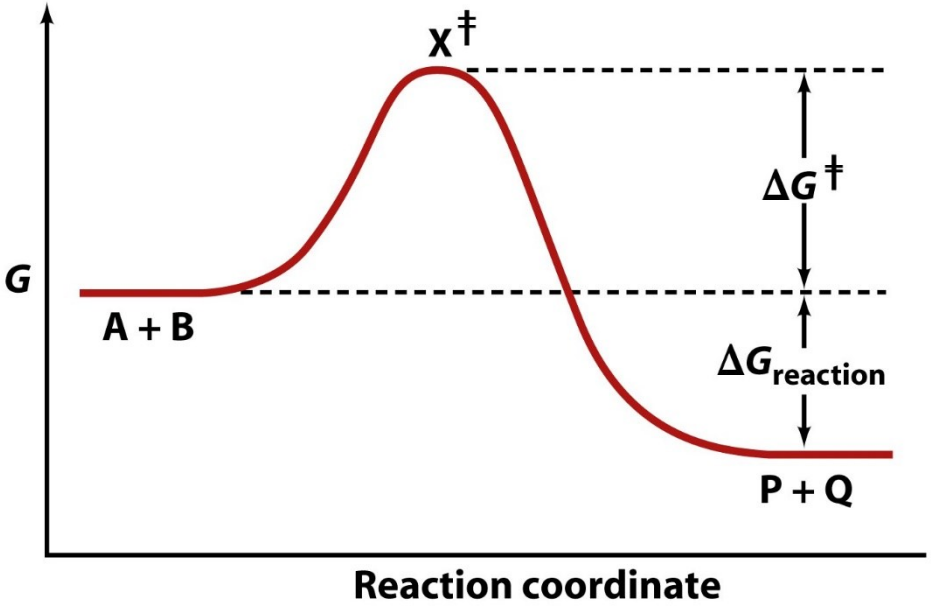

Figure 11-5b Fundamentals of Biochemistry, 2/e
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Reaction coordinate: reactants generally approach one and another **along the path of minimum free energy**.

$\Delta G^{\ddagger}$: the free energy of the transition state less than that of the reactants is known as the activation energy.

2. Activation energy and reaction coordinate

Much of our understanding of how enzyme catalyze chemical reactions comes from **transition state theory**. (developed by Henry Eyring, in 1930s)

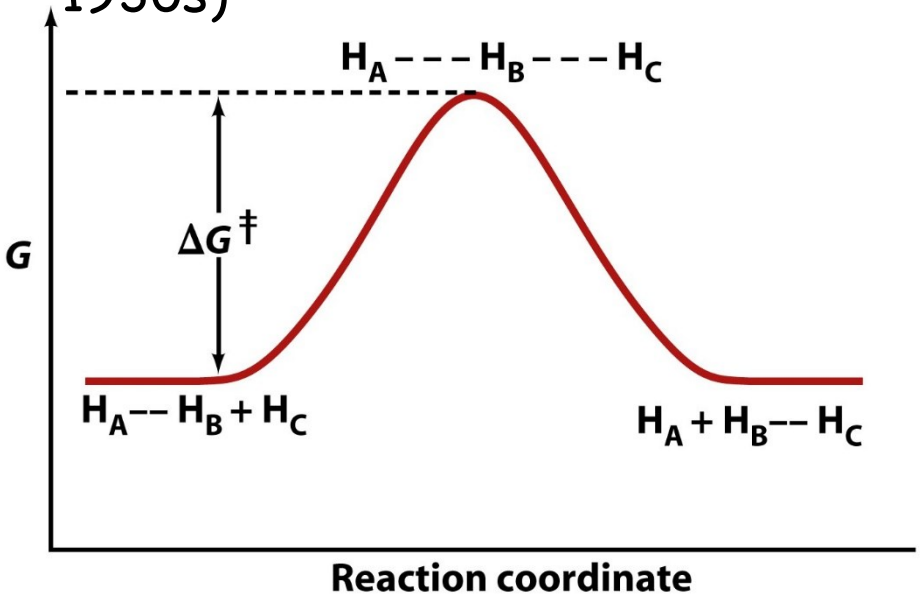

Figure 11-5a Fundamentals of Biochemistry, 2/e
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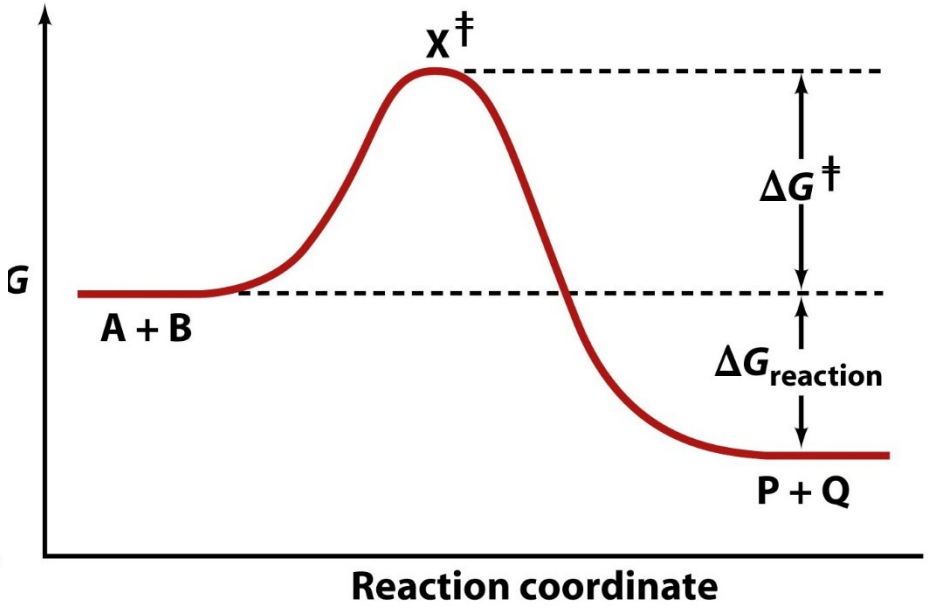

igure 11-5b Fundamentals of Biochemistry, 2/e
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$$rate = Ae^{-\Delta G^{\pm}/RT}$$

$$\ln rate = \ln A - \frac{\Delta G}{RT}$$

Arrhenius plot

Enzyme speed up reactions by lowering activation energy

As with all catalysts, all reactions catalyzed by enzymes must be "spontaneous" (containing a net negative Gibbs free energy). In the presence of an enzyme, a reaction runs in the same direction as it would without the enzyme, just more quickly.

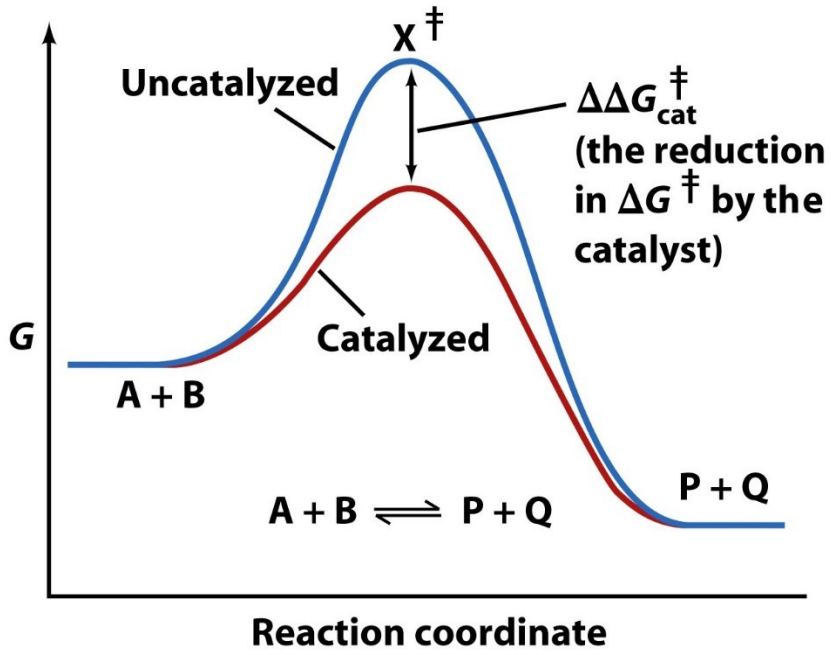


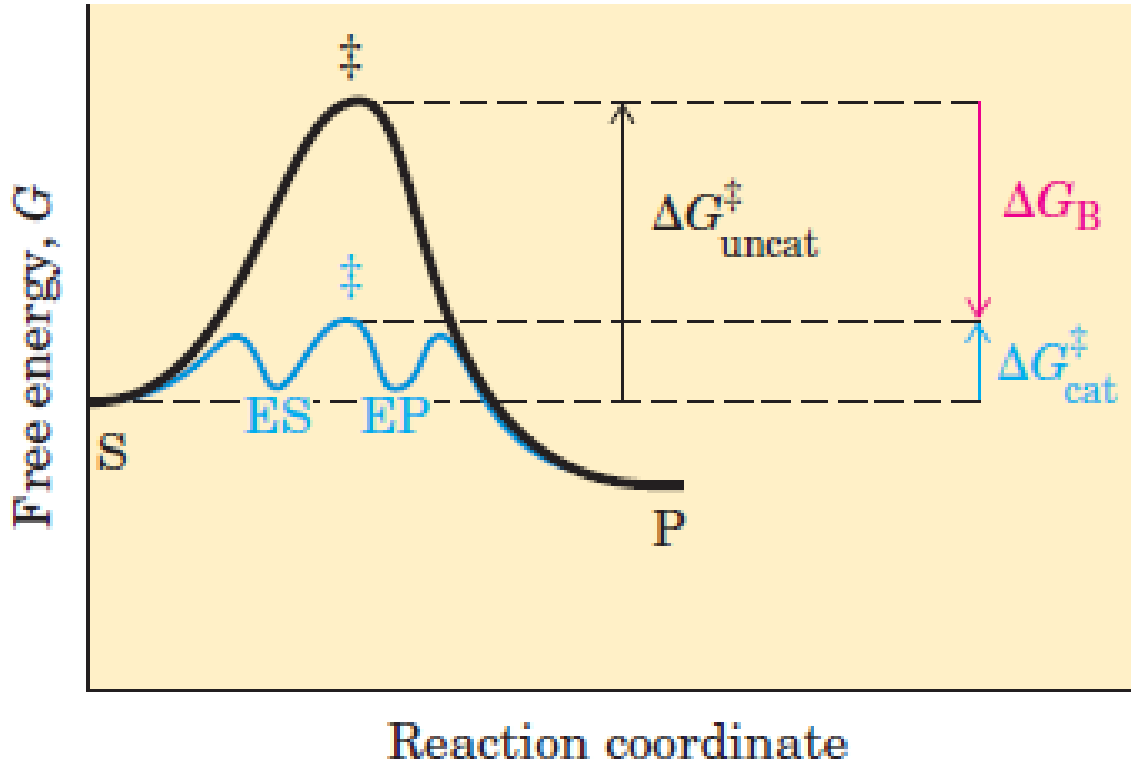
Figure 11-7 Fundamentals of Biochemistry, 2/e

$$\frac{rate_{catalyzed}}{rate_{uncatalyzed}} = e^{\Delta\Delta G_{cat}/RT}$$

$$K_{eq} = \frac{k_{forward}}{k_{backward}}$$

1RT=~2.5 KJ/mol
25 KJ/mol, 2×10^4 fold faster
40 KJ/mol, ~ 10^7fold faster.

Enzymes catalyze the forward and backward reactions equally. They do not alter the equilibrium itself, but only the speed at which it is reached.



Role of binding energy in catalysis. To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which $\Delta G^{\ddagger}$ is lowered. Much of this energy comes from binding energy (ΔG_B) contributed by formation of weak noncovalent interactions between substrate and enzyme in the transition state.

Reaction Kinetics

$A \rightarrow P$ May occur through a sequence of **elementary reactions**

$$A \rightarrow I_1 \rightarrow I_2 \rightarrow P$$

Where I_1 and I_2 symbolize **intermediates.**

Reaction order indicates the number of molecules participating in an **elementary reaction**.

First order reaction: $A \rightarrow P$ (unimolecular reaction)

Second order reaction (must be bimolecular reaction)

Termolecular reaction are unusual. Any higher order of reaction is unknown

Rate equation indicates the progress of a reaction as a function of time.

First order reaction

$$A \xrightarrow{k} P$$ k: the rate constant

v: the reaction rate or reaction velocity can be expressed by the rate of appearance of product or disappearance of reactant.

$$v = \frac{d[P]}{dt} = -\frac{d[A]}{dt} = k[A]$$

$$\frac{d[A]}{[A]} = d\ln[A] = -kt$$

$$\int_{[A]_0}^{[A]} d\ln[A] = -k\int_0^t dt$$

$$at\ [A] = \frac{[A]_0}{2}, t = t_{1/2}$$

$$-\ln 2 = -kt_{1/2}$$

$$t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}$$

$$[A] = [A]_0 e^{-kt}$$

$$\ln\frac{[A]}{[A]_0} = -kt$$

First order reaction

$$A \xrightarrow{k} P$$

k: the rate constant

v: the reaction rate or reaction velocity can be expressed by the rate of appearance of product or disappearance of reactant.

In a first order reaction:
(1) Reaction rate constant has a unit of s^{-1}
(2) Plot ln[A] vs t gives a straight line with slope = -k
(3) $t_{1/2}$ is a constant, independent of the initial reaction concentration.

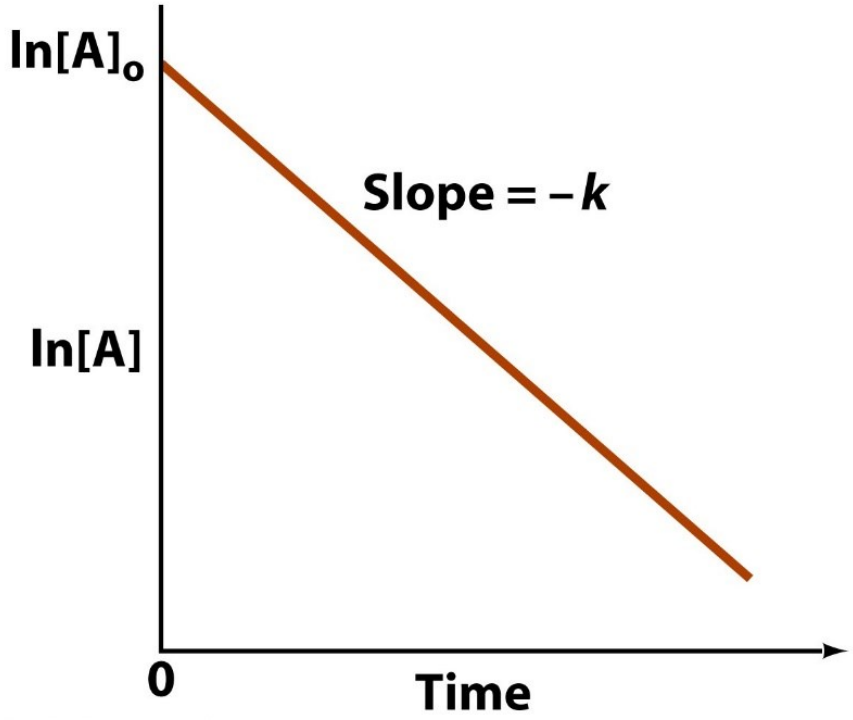


Figure 12-1 Fundamentals of Biochemistry, 2/e

Radioactive decay is first order reaction

Radionuclide	Half-life	Type of Radiation[a]
^{3}H	12 years	β
^{14}C	5715 years	β
^{24}Na	15 hours	β
^{32}P	14 days	β
^{35}S	87 days	β
^{40}K	1.25×10^{9} years	β
^{45}Ca	163 days	β
^{125}I	59 days	γ
^{131}I	8 days	β, γ

[a] β particles are emitted electrons, and γ rays are emitted photons.

Box 12-1 Table 1 Fundamentals of Biochemistry, 2/e

Second order reaction

$2A \rightarrow P$

$$v = -\frac{d[A]}{dt} = k[A]^2$$

$$-\frac{d[A]}{[A]^2} = kdt$$

$$\int_{[A]_0}^{[A]} -\frac{d[A]}{[A]^2} = k\int_0^t dt$$

$$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$$

$$at [A] = \frac{[A]_0}{2}, t = t_{1/2}$$

$$\frac{2}{[A]_0} = \frac{1}{[A]_0} + kt_{1/2}$$

$$t_{1/2} = \frac{1}{k[A]_0}$$

Second order reaction

$2A \rightarrow P$

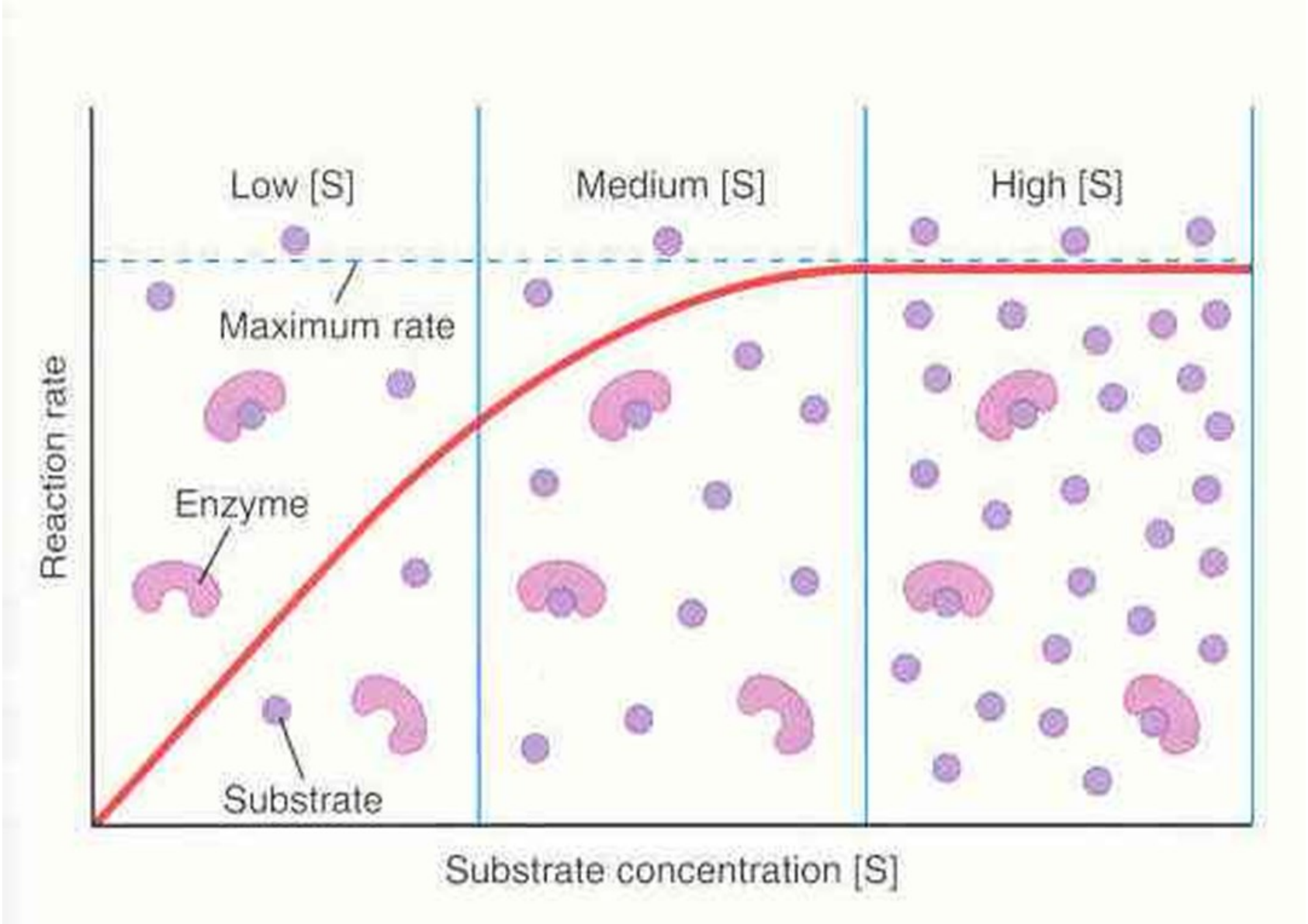
- **In a Second order reaction**
- (1) k has a unit of $M^{-1} S^{-1}$
- (2) plot 1/[A] vs t gives a straight line, with slope = k
- (3) $t_{1/2}$ depends on the initial reactant concentration
- $[A]_0 \uparrow t_{1/2}\downarrow$

(2) $A+B \rightarrow P$

It is often convenient to increase one reaction relative to the other, e.g [B]>>[A], so [B] does not change much through the reaction. k'=k[B]

The second order reaction is reduced to a

pseudo-first order reaction.


Low [S]
Medium [S]
High [S]
Maximum rate
Enzyme
Substrate
Reaction rate
Substrate concentration [S]

Enzyme Kinetics (single substrate reactions)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E$$

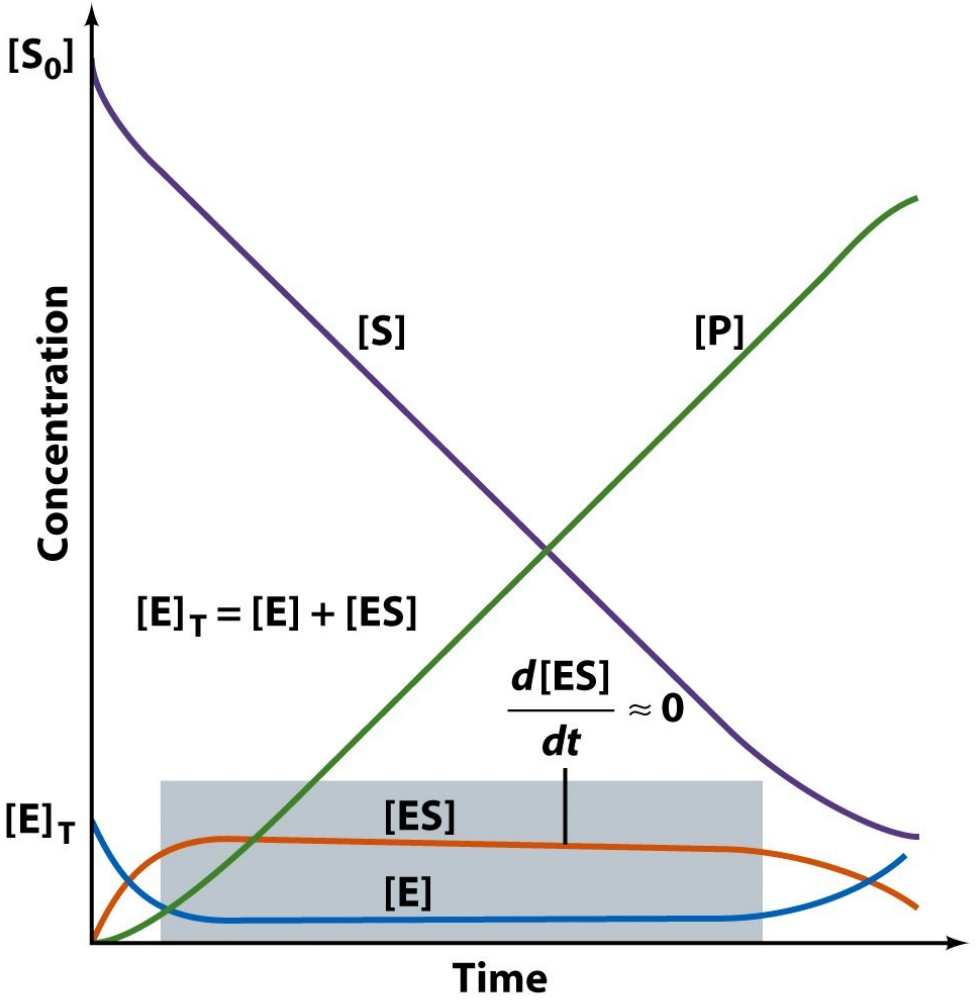


Figure 12-2 Fundamentals of Biochemistry, 2/e

Enzyme Kinetics (single substrate reactions)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P + E$$

$$v_0 = (\frac{d[P]}{dt})_{t\to 0} = k_2[ES]$$

A steady state assumption:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0$$

$$[E] = [E]_T - [ES]$$

$$k_1([E]_T - [ES])[S] = (k_{-1} + k_2)[ES]$$

$$\frac{([E]_T - [ES])[S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_M$$

$$K_M[ES] = ([E]_T - [ES])[S]$$

$$[ES] = \frac{[E]_T[S]}{K_M + [S]}$$

$$v_0 = (\frac{d[P]}{dt})_{t\to 0} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$

Michaelis-Menten equation (initial velocity)

$$v_0 = \left(\frac{d[P]}{dt}\right)_{t\to 0} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$

(1) As [S]>>K_M, when enzyme is saturated $v_0 \to v_{\max} = k_2[E]_T$ (pseudo-first order reaction)

(2) K_M =[S], at which v_0=v_{max}/2,

Michaelis constant has a simple operational definition: the substrate concentration at which the reaction velocity is half-maximal, unit of concentration.

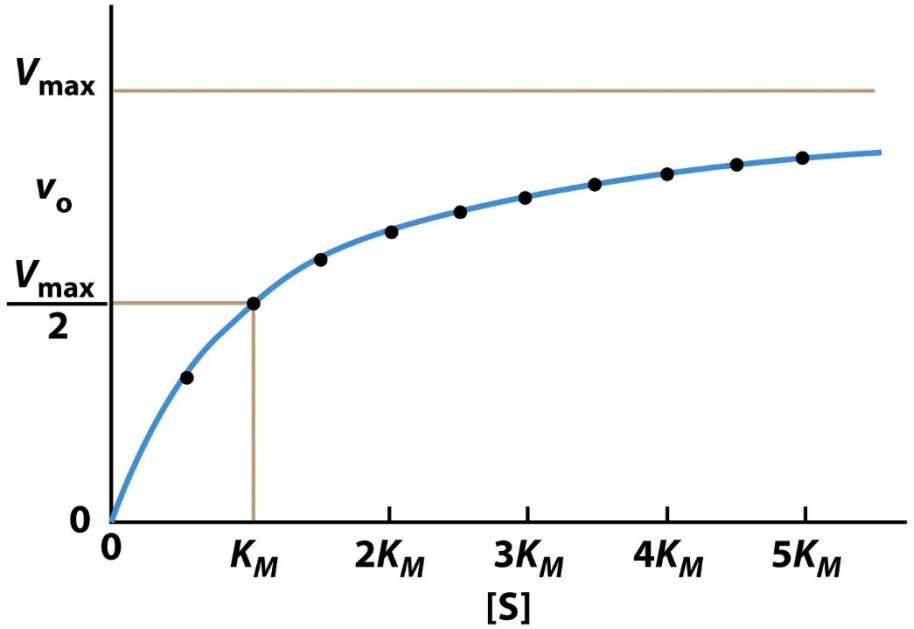


Figure 12-3 Fundamentals of Biochemistry, 2/e
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$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

K_M is unique for each enzyme-substrate pair

Table 12-1 The Values of K_M, k_{cat}, and k_{cat}/K_M for Some Enzymes and Substrates

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^{4}	1.5×10^{8}
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^{6}	8.3×10^{7}
	HCO_3^-	2.6×10^{-2}	4.0×10^{5}	1.5×10^{7}
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^{7}	4.0×10^{8}
Chymotrypsin	*N*-Accetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^{2}	2.9×10^{5}
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^{2}	1.6×10^{8}
	Malate	2.5×10^{-5}	9.0×10^{2}	3.6×10^{7}
Urease	Urea	2.5×10^{-2}	1.0×10^{4}	4.0×10^{5}

Table 12-1 Fundamentals of Biochemistry, 2/e

Analysis of kinetic data: Lineweaver-Burk or double reciprocal plot

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]} \rightarrow \boxed{\frac{1}{v_o} = \left(\frac{K_M}{V_{\max}}\right)\frac{1}{[S]} + \frac{1}{V_{\max}}}$$

Unnumbered figure pg 367 Fundamentals of Biochemistry, 2/e

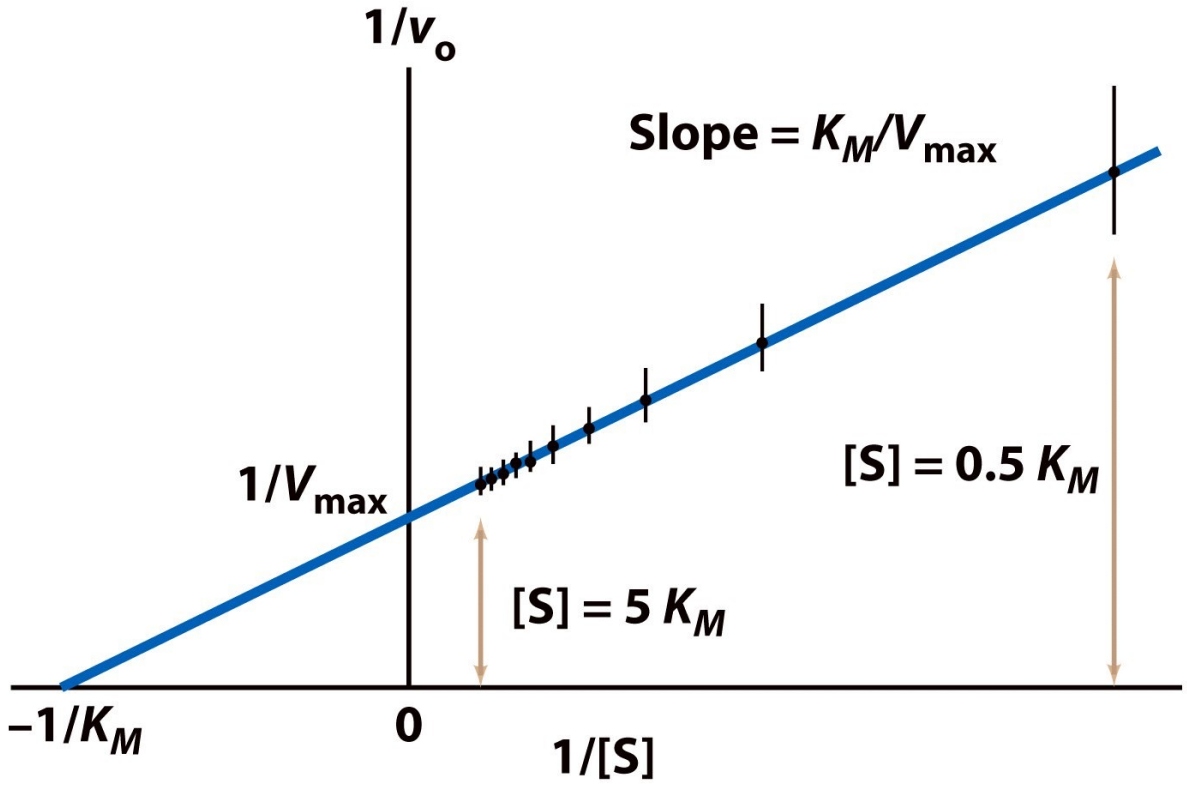


Figure 12-4 Fundamentals of Biochemistry, 2/e

Enzyme inhibition

- Substances that reduce an enzyme's activity are known as **inhibitor**.

- **Inhibition mechanism**:
  - **Irreversible**: **inactivator**

  - **Reversible**:
    - **Competitive inhibition**: compete directly with the normal substrate for an enzyme's substrate binding site, inhibitor **structurally resembles the substrate**. **Product inhibition**. **Transition state analog** may be even better inhibitor.

    - **Uncompetitive inhibition:** the inhibitor binds directly to the enzyme-substrate complex, ES, but not to the free enzyme. Inhibitor need not resemble the substrate.

    - **Mixed inhibition:** inhibitor affects the enzyme in both substrate binding and catalytic activity.

(a) Competitive inhibition

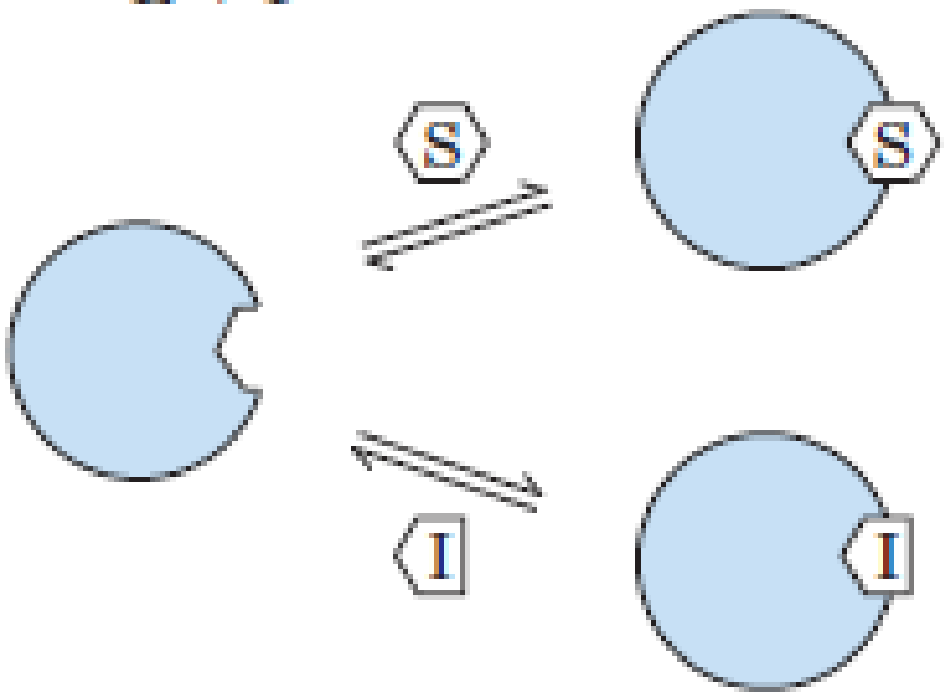
$$E + S \rightleftharpoons ES \longrightarrow E + P$$

$$+$$

$$I$$

$$\Big\downarrow K_I$$

$$EI$$



Competitive inhibition

A competitive inhibitor reduces the concentration of free enzyme available for substrate binding, but does not affect the enzyme's turnover number. The apparent K_M is increased by a factor of α.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$+$$

$$I$$

$$K_I \Downarrow$$

$$EI + S \longrightarrow \text{NO REACTION}$$

K_I: inhibition constant, the dissociation constant of the enzyme-inhibitor binding

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

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

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

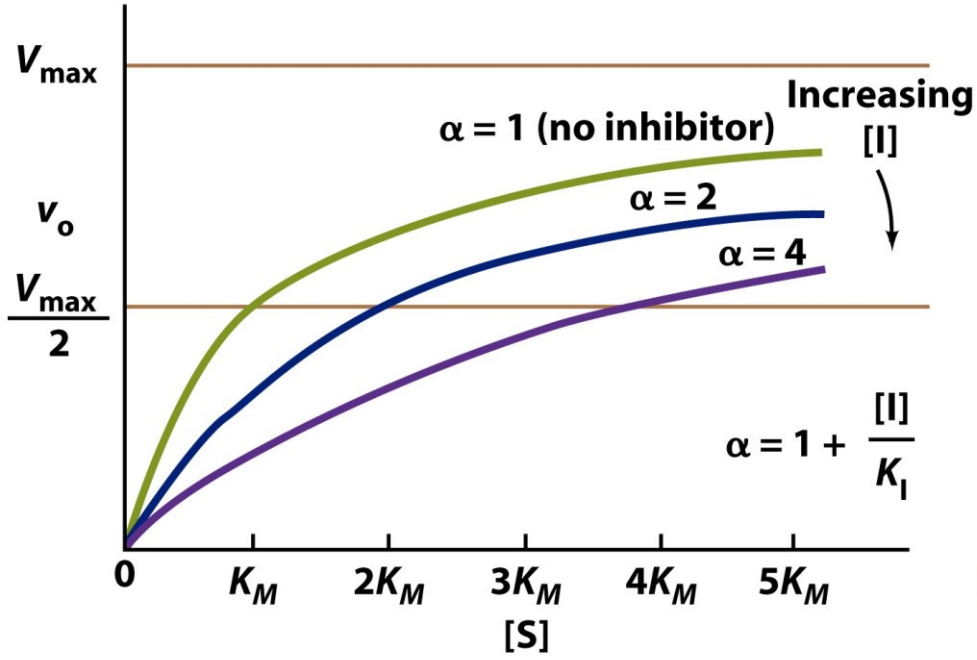


Figure 12-6 Fundamentals of Biochemistry, 2/e
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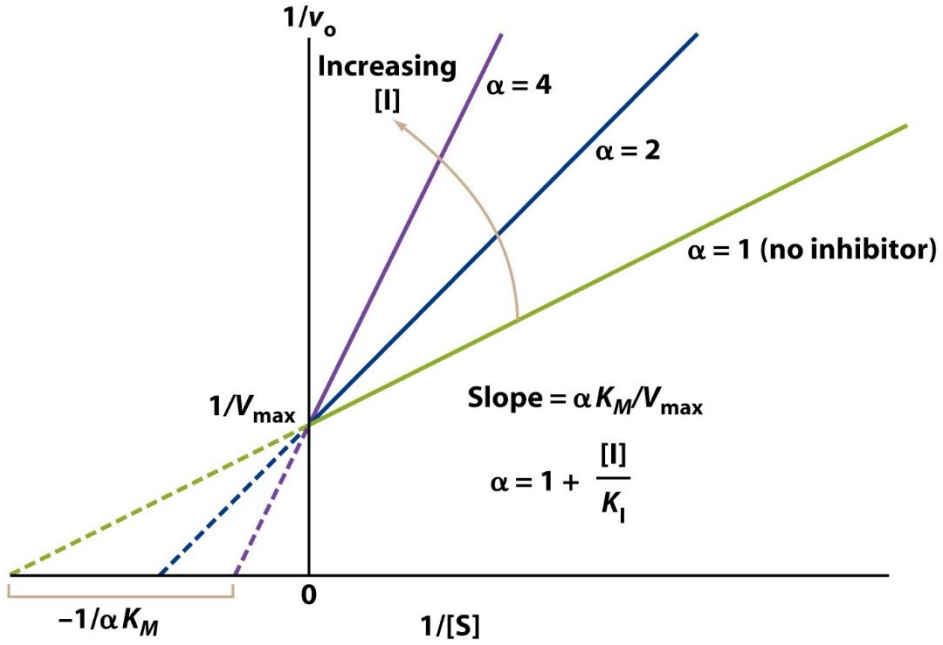

Figure 12-7 Fundamentals of Biochemistry, 2/e
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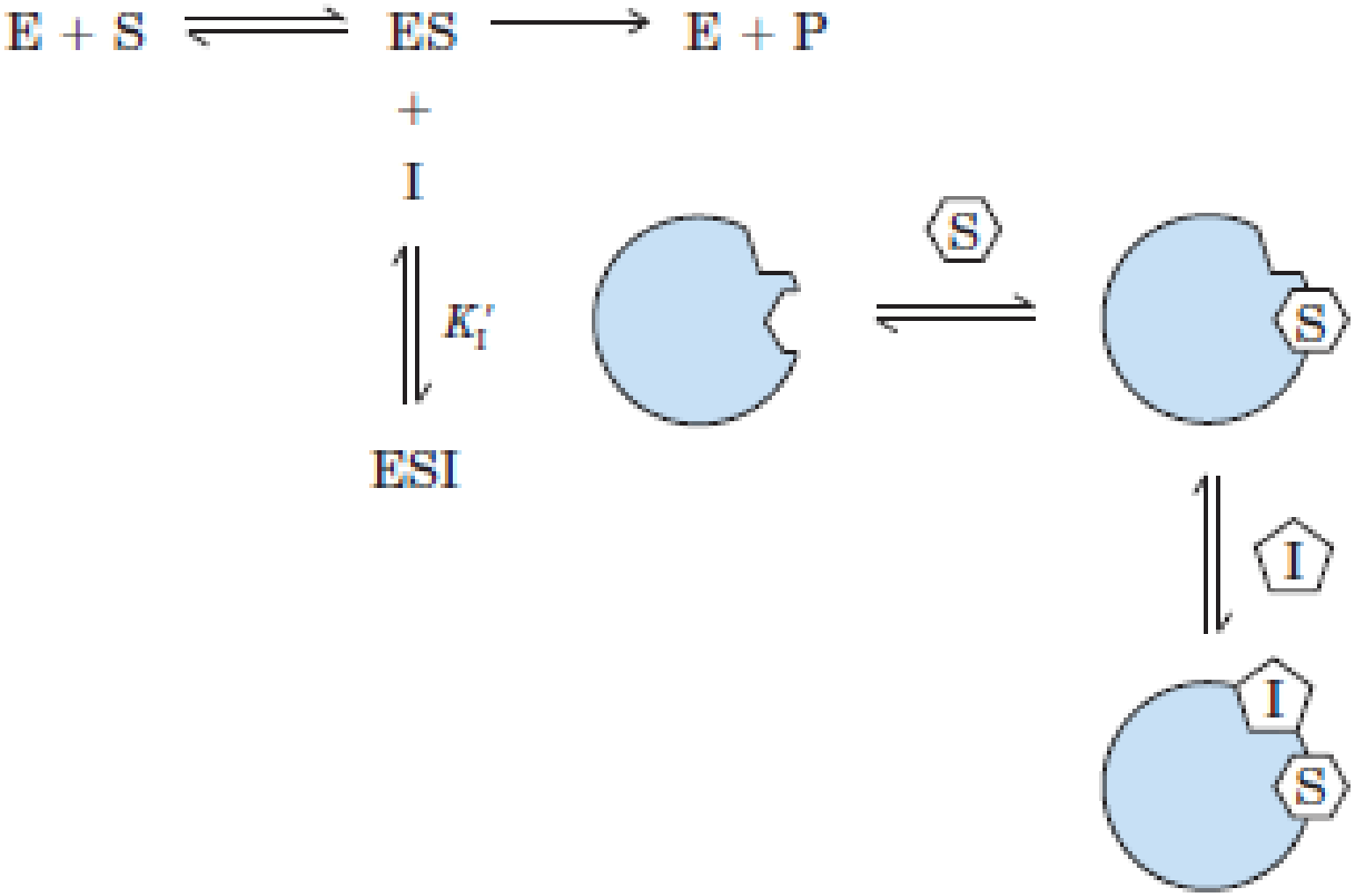
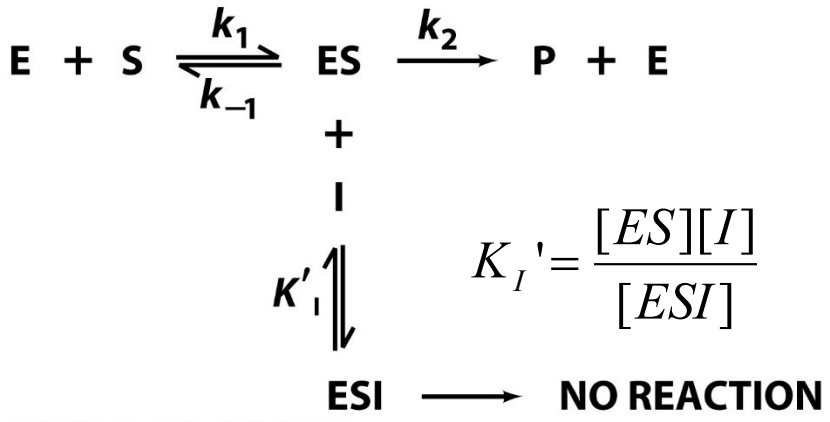
(b) Uncompetitive inhibition



Uncompetitive inhibition



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

Unnumbered figure pg 375 Fundamentals of Biochemistry, 2/e

$$\frac{1}{v_0} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

decrease both apparent K_M and V_{max} by a factor of α'.

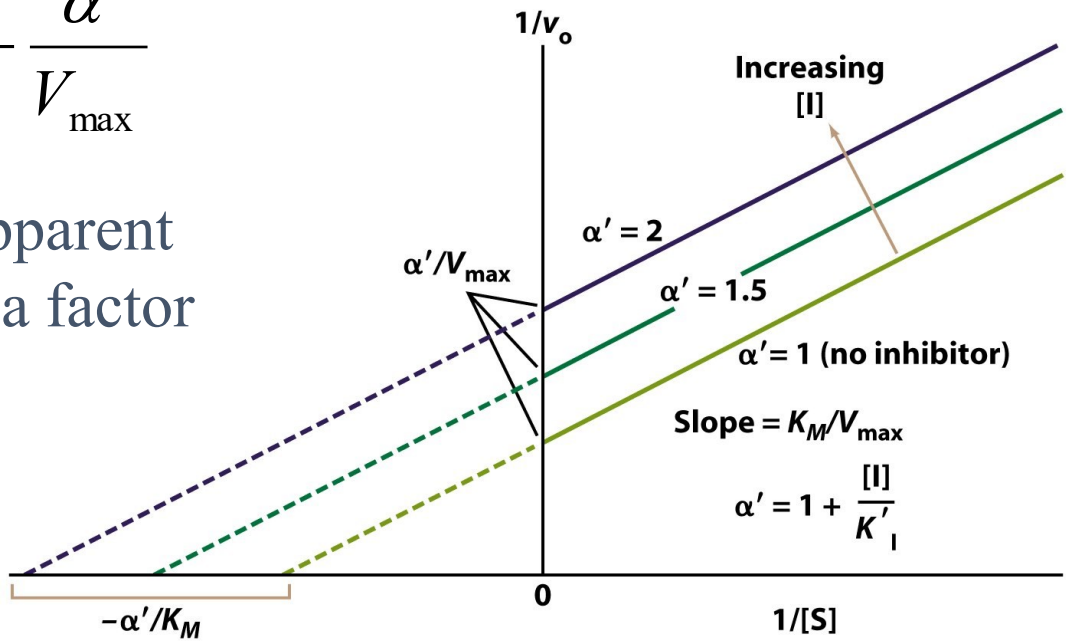
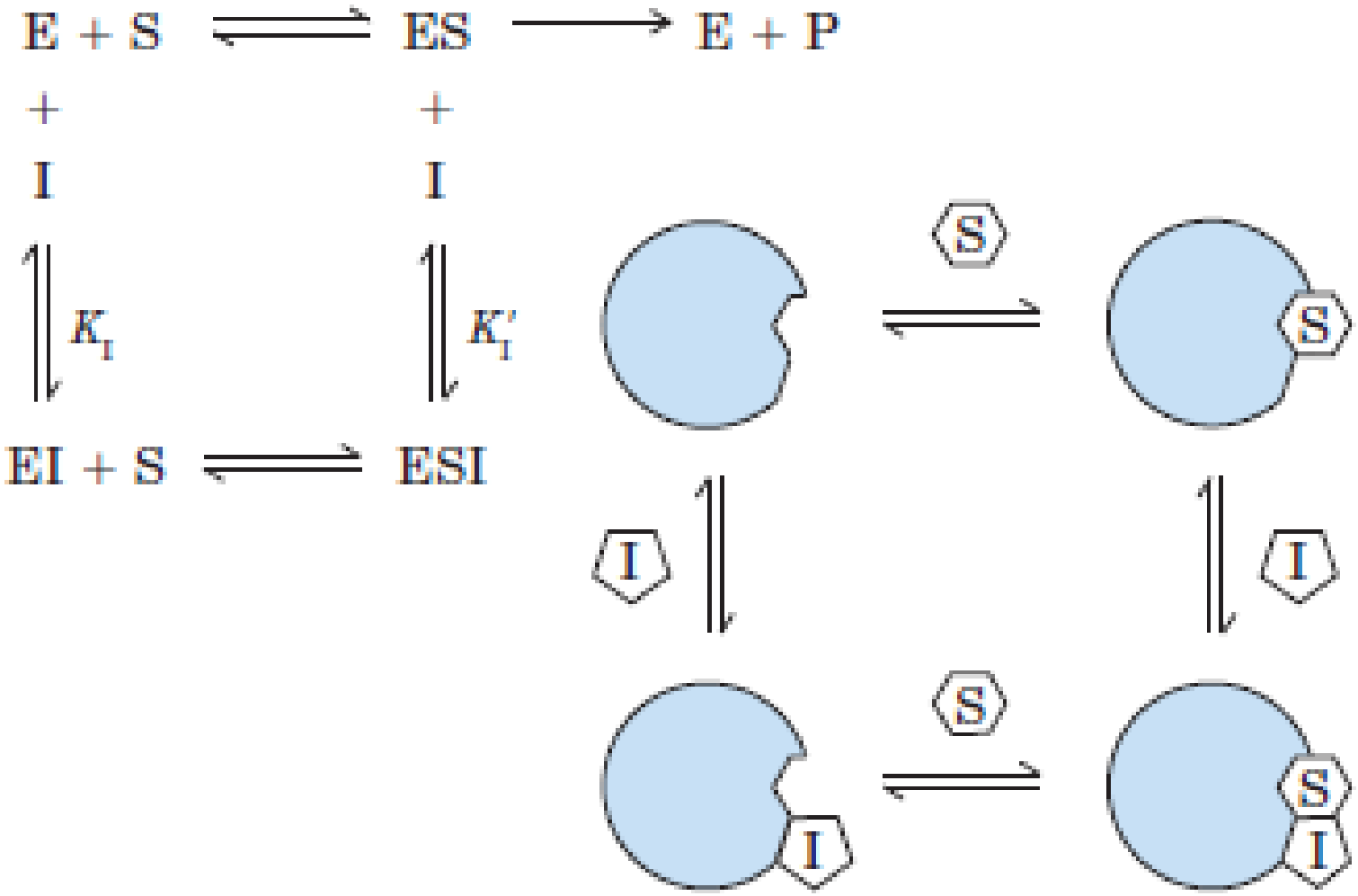
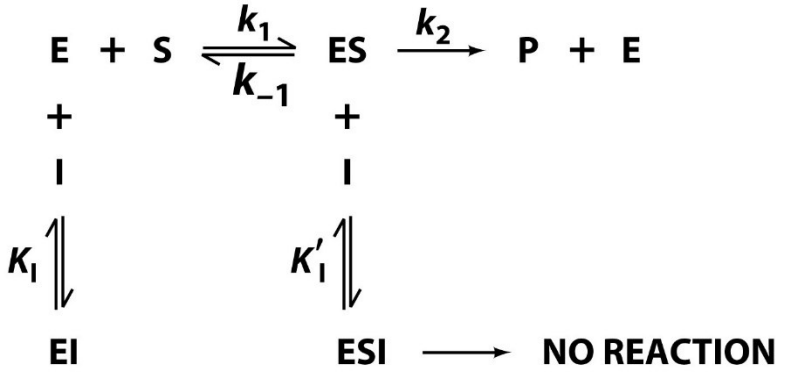


Figure 12-8 Fundamentals of Biochemistry, 2/e

(c) Mixed inhibition



Mixed inhibition



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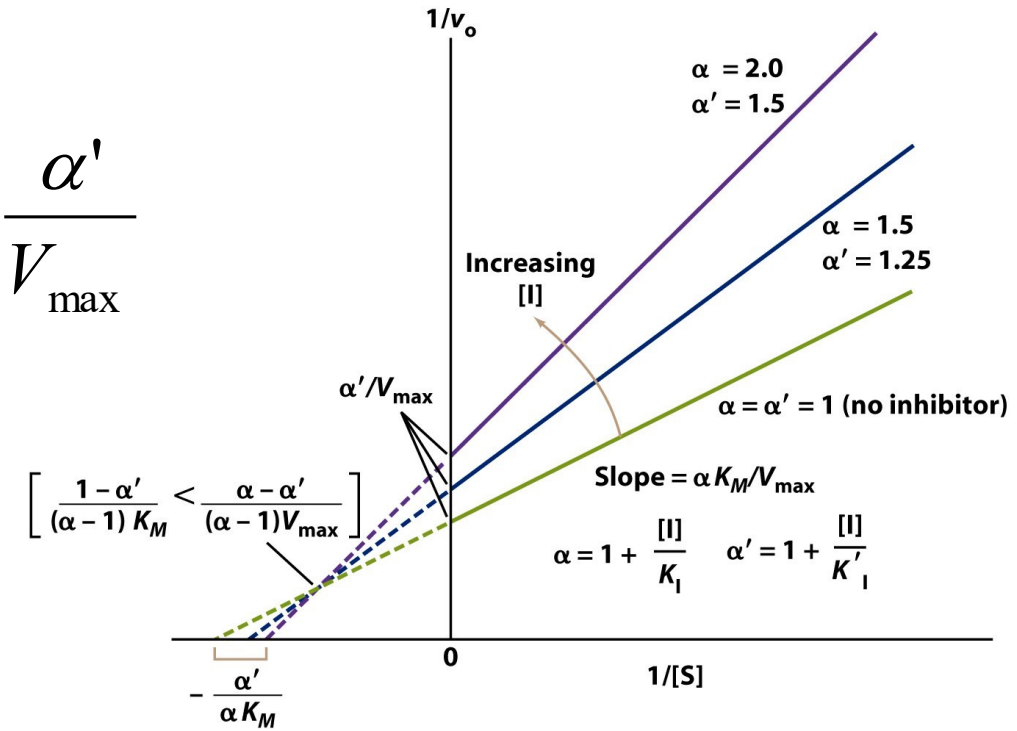
$$\frac{1}{v_0} = \frac{\alpha K_M}{V_{\max}} \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$



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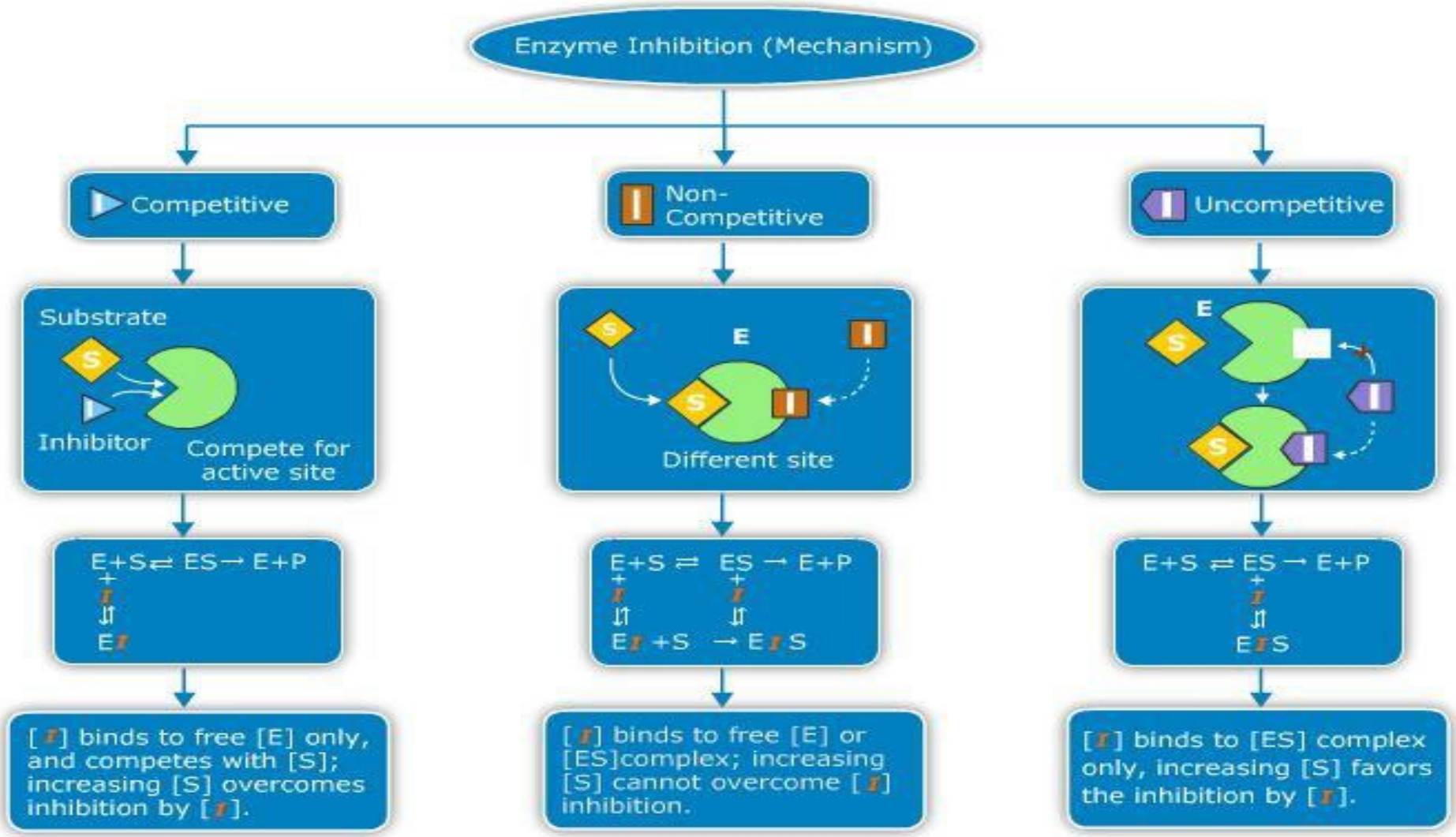
Enzyme Inhibition (Mechanism)
Competitive
Non-Competitive
Uncompetitive
Substrate
S
Inhibitor
Compete for active site
E
S
I
Different site
E
S
I
E+S⇌ ES→ E+P
+
I
⇵
EI
E+S ⇌ ES → E+P
+ +
I I
⇵ ⇵
EI +S → EIS
E+S ⇌ ES → E+P
+
I
⇵
EIS
[I] binds to free [E] only, and competes with [S]; increasing [S] overcomes inhibition by [I].
[I] binds to free [E] or [ES]complex; increasing [S] cannot overcome [I] inhibition.
[I] binds to [ES] complex only, increasing [S] favors the inhibition by [I].

Summary of enzyme inhibition kinetics analysis

Table 12-2 Effects of Inhibitors on Michaelis–Menten Reactions[a]

Type of Inhibition	Michaelis–Menten Equation	Lineweaver–Burk Equation	Effect of Inhibitor
None	$v_o = \frac{V_{max}[S]}{K_M + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	None
Competitive	$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$	Increases K_M^{app}
Uncompetitive	$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{K_M/\alpha' + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases K_M^{app} and V_{max}^{app}
Mixed (noncompetitive)	$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{(\alpha/\alpha')K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}}\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases V_{max}^{app}; may increase or decrease K_M^{app}

[a] $\alpha = 1 + \frac{[I]}{K_I}$ and $\alpha' = 1 + \frac{[I]}{K_I'}$

Table 12-2 Fundamentals of Biochemistry, 2/e

Control of enzyme activity

- **1. control of enzyme availability**: through change of the rate of synthesis and the rate of degradation of the enzyme in the cell.

- **2. control of enzyme activity**: through structural alteration that influence the enzyme's substrate-binding affinity or turnover number.

  - A. "allosteric regulation". an enzyme's substrate-binding affinity may likewise vary with the binding of small molecules, called **allosteric effectors**.

  - B. covalent modification through phosphorylation or dephosphorylation of specific residues.

Drug design

- Structure-based drug design, also known as rational drug design
- Combinatorial chemistry and high-throughput screening, back to "make many compounds and see what they do" approach.
- Many drugs target to inhibit enzymes or signaling proteins (receptors).