Chapter 6

Enzymes

1. Introduction to enzymes

- **Enzymes are catalytically active biological macromolecules**
- **Enzymes are catalysts of biological systems**
- **Almost every biochemical reaction is catalyzed by an enzyme**
- **Study of enzymatic processes is the oldest field of biochemistry, dating back to late 1700s**
- **Study of enzymes has dominated biochemistry in the past and continues to do so**

Introduction to enzymes

- **Most enzymes are globular proteins, however, some catalytic RNAs (ribozymes) also catalyze reactions**
- **Many enzymes require nonprotein coenzymes or cofactors for their catalytic activity**
- **Enzymes are classified according to the type of reactions they catalyze**
- **All enzymes have formal E.C. numbers and names and most have trivial names**

TABLE 6–1 Some Inorganic Ions That Serve as Cofactors for Enzymes

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Table 6-1
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TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

Table 6-2
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Subdivision of cofactors

- **Metals**
- **Small organic molecules (coenzymes)**
- **A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group**
- **A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme**
- **The protein part of such an enzyme is called the apoenzyme or apoprotein**

Classification of enzymes

- **Enzymes are classified according to the type of reactions they catalyze**
- **Enzymes are divided into six classes, each with subclasses**
- **Each enzyme is assigned a four-part classification number and a systematic name, which identifies the reaction it catalyzes**
- **Most enzyme have trivial names**

TABLE 6–3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

Table 6-3
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Enzymes are classified by the reactions they catalyze (www.chem.qmul.ac.uk/iubmb/enzyme)

Example of enzyme naming

ATP + D-glucose ⟶ ADP+glucose-6-phosphate

- **The formal systematic name of the enzyme catalyzing the reaction is ATP:glucose phosphotransferase**
- **Its Enzyme Commission number (E.C. number) is 2.7.1.1.**
- **The first number (2) denotes the class name (transferase)**
- **The second number (7) denotes the subclass (phosphotransferase)**
- **The third number (1) denotes a phosphotransferase with a hydroxyl group as acceptor**
- **The fourth number (1) denotes D-glucose as the phosphoryl group acceptor**
- **The trivial name is hexokinase**

Characteristics of enzymes

- **Higher reaction rates**
- **Greater reaction specificity**
- **Milder reaction conditions**
- **Capacity for regulation**
- **Enzymes affect the rate of a reaction, not equilibrium**
- **Enzymes lower the activation energy**
- **Enzymes use binding energy to lower the activation energy**
- **Enzymes are not used up in the reaction**

The two most striking characteristics of enzymes

1. **Catalytic capacity---Enzymes accelerate reactions by a factor of 10^5 to 10^{17}. Most reactions in biological systems do not take place at perceptible rates in the absence of enzymes.**
2. **Specificity---Enzymes are specific both in the reactions that they catalyze and in their choice of reactants.**

TABLE 6–5 Some Rate Enhancements Produced by Enzymes

Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

Table 6-5
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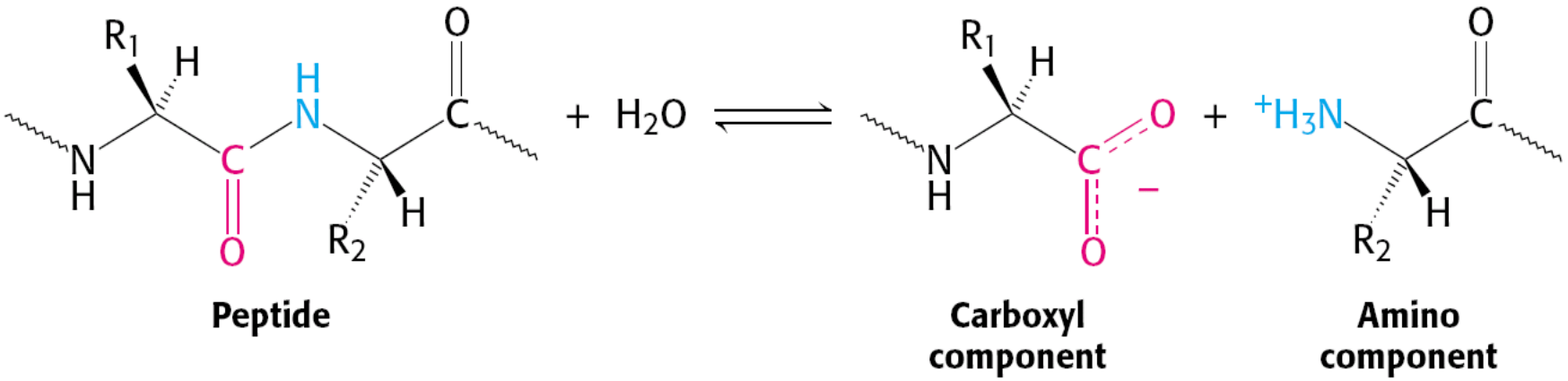
Specificity of enzymes

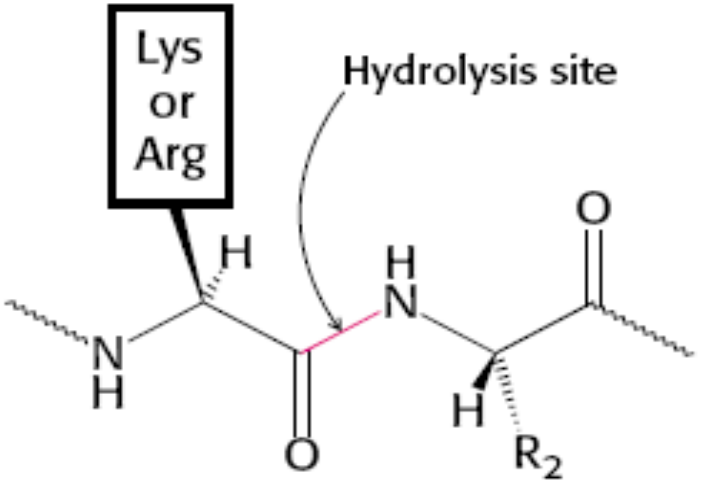
- **Enzymes are highly specific both in the reactions that they catalyze and in their choice of reactants, which are called substrates.**
- **An enzyme usually catalyzes a single chemical reaction or a set of closely related reactions.**
- **Side reactions leading to the wasteful formation of by-products are rare in enzyme-catalyzed reactions.**
- **The specificity of an enzyme is due to the precise interaction of the substrate with the enzyme. This precision is a result of the intricate three-dimensional structure of the enzyme protein.**

Examples demonstrating the specificity of enzymes



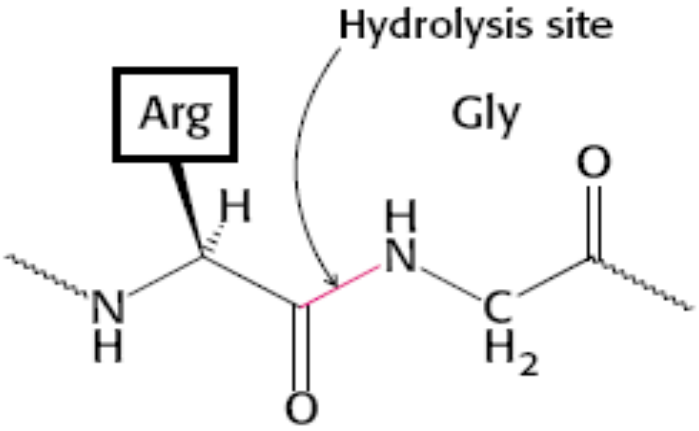
Proteolytic enzymes catalyze proteolysis, the hydrolysis of a peptide bond.

Enzyme specificity



(A)

Trypsin: cleaves on the carboxyl side of arginine and lysine residues



(B)

Thrombin: cleaves Arg-Gly bonds in particular sequences

2. Free energy is a useful thermodynamic function for understanding enzymes

- **The free energy difference between the products and reactants. (It determines whether the reaction will be spontaneous.)**
- **The energy required to initiate the conversion of reactants to products. (It determines the rate of the reaction.)**

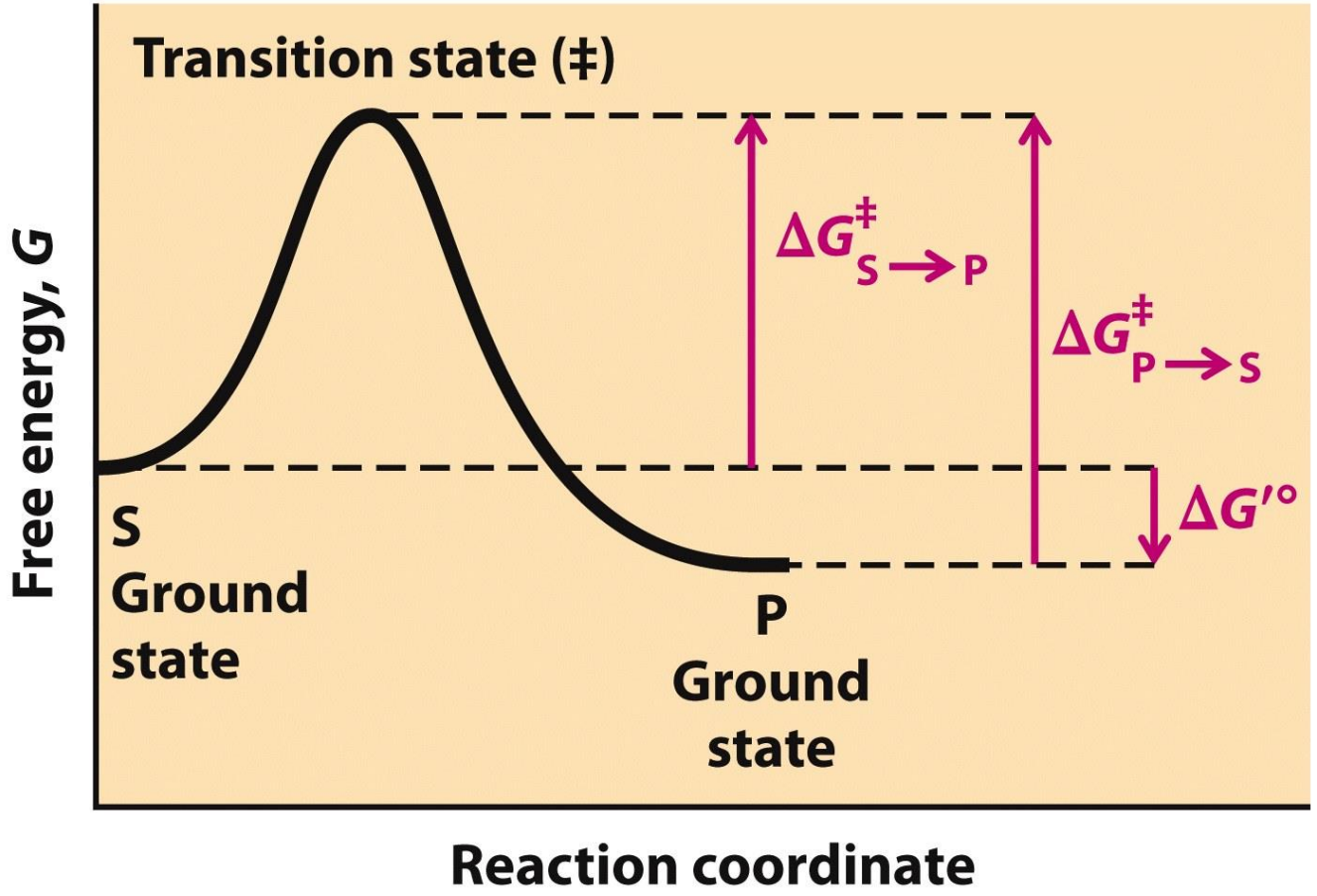


Figure 6-2
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Reaction coordinate diagram

The free energy of the system is plotted against the progress of the reaction S → P . A diagram of this kind is a description of the energy changes during the reaction, and the horizontal axis (reaction coordinate) reflects the progressive chemical changes (e.g., bond breakage or formation) as S is converted to P.

The activation energies, ΔG°, for the S → P and P → S reactions are indicated.

$\Delta G'^{\circ}$ is the overall standard free-energy change in the direction S → P.

The free-energy change provides information about the spontaneity but not the rate of a reaction

ΔG = G (products) – G (substrates)

- **ΔG depends only on the free energy of the products and the free energy of the reactants**
- **ΔG is independent of the molecular mechanism of the transformation**
- **ΔG provides no information about the rate of a reaction**

$\Delta G < 0$ --- the reaction can occur spontaneously

$\Delta G = 0$ --- the reaction is in equilibrium

$\Delta G > 0$ --- the reaction cannot occur spontaneously

Enzymes alter only the reaction rate not the reaction equilibrium

- **An enzyme cannot alter the laws of thermodynamics and consequently cannot alter the equilibrium of a chemical reaction (ΔG)**
- **This inability means that an enzyme accelerates the forward and reverse reactions by precisely the same factor**

Enzymes function to lower the activation energy

- **Enzymes accelerate reactions by decreasing $G^{\ddagger}$, the free energy of activation.**
- **More molecules have the required energy to reach the transition state.**
- **Reaction is speeded up.**
- **Essence of catalysis: specific binding of the transition state**

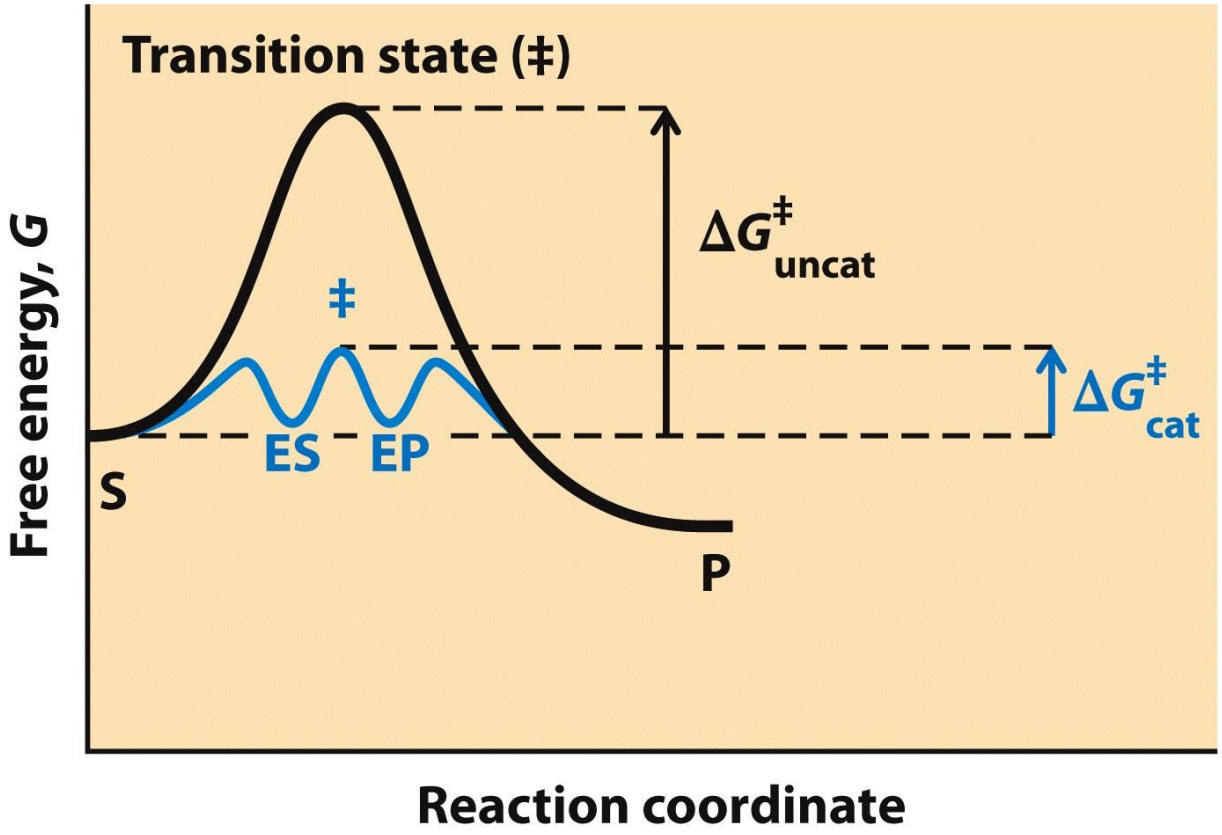

Figure 6-3
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Reaction coordinate diagram comparing enzyme-catalyzed and uncatalyzed reactions

The terms $\Delta G^{\ddagger}_{uncat}$ and $\Delta G^{\ddagger}_{cat}$ correspond to the activation energy for the uncatalyzed reaction and the overall activation energy for the catalyzed reaction.

The activation energy is lower when the enzyme catalyzes the reaction.

The formation of an enzyme-substrate complex is the first step in enzymatic catalysis

- **Enzymes act by binding substrates**

  ---the non-covalent enzyme-substrate complex is central to the action of enzymes

  ---allows thinking in terms of chemical interactions

  ---allows development of kinetic equations
- **Active site: a specific region that binds substrates**
- **Catalytic group: the residues that directly participate in the reaction**
- **Enzyme accelerate reactions by facilitating the formation of transition state**

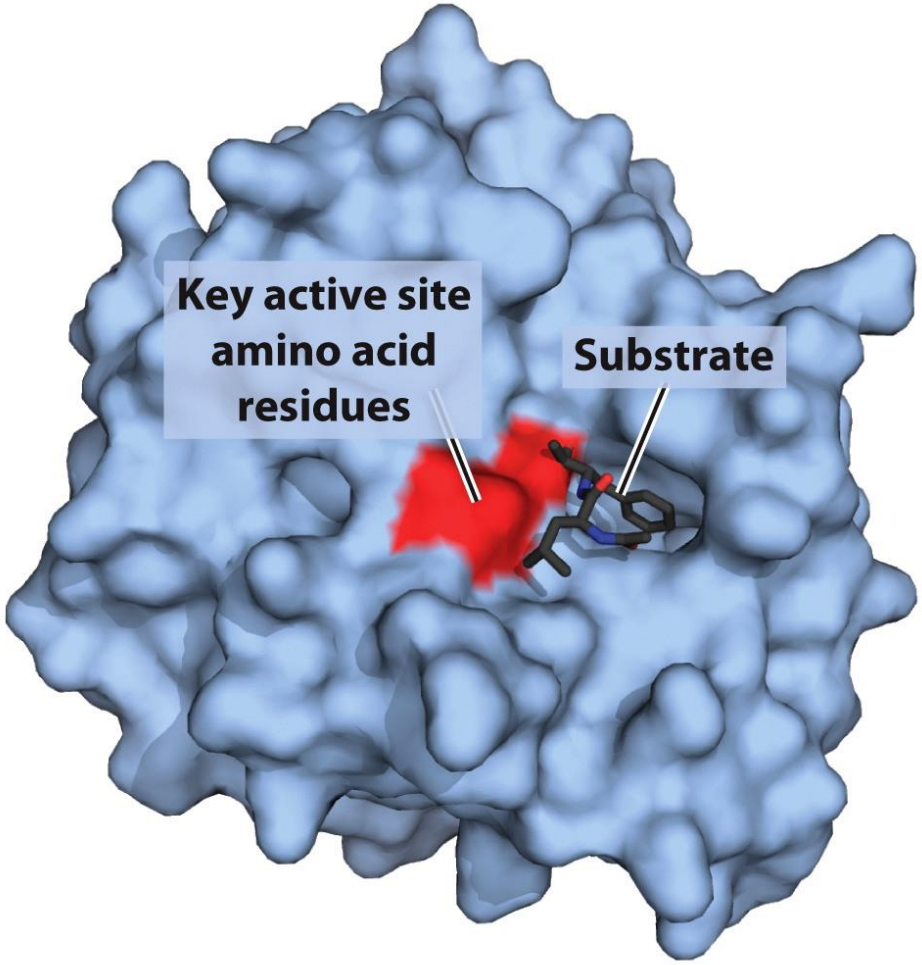

Figure 6-1
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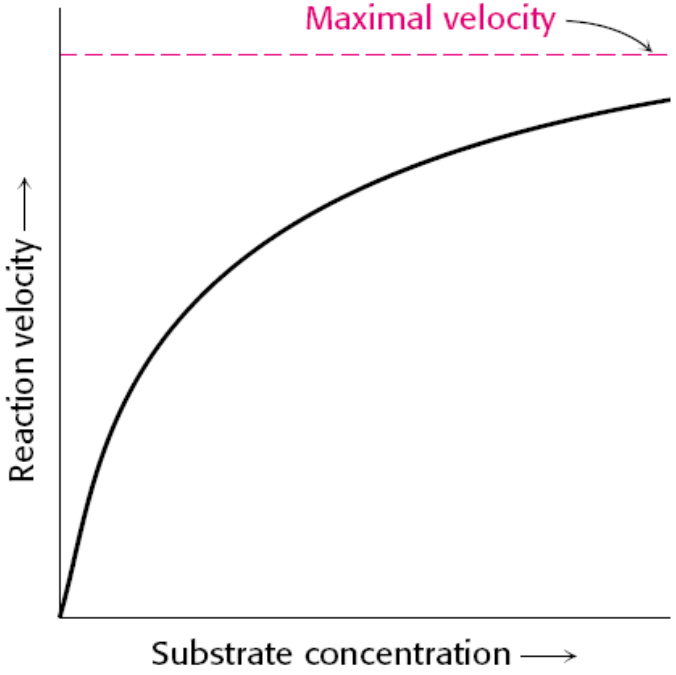
Binding of a substrate to an enzyme at the active site

The enzyme chymotrypsin with bound substrate. Some key active-site amino acid residues appear as a red splotch on the enzyme surface.

Evidence for the existence of an enzyme - substrate complex (I)

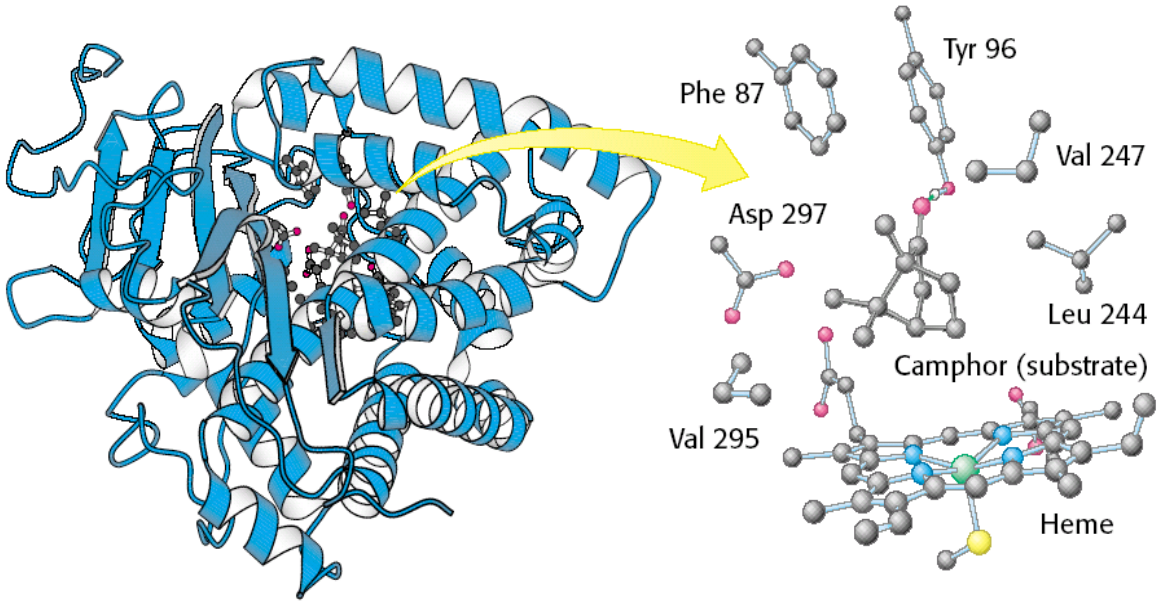
An enzyme-catalyzed reaction has a maximal velocity. At a sufficiently high substrate concentration, all the catalytic sites are filled and so the reaction rate cannot increase.



An enzyme-catalyzed reaction reaches a maximal velocity.

Evidence for the existence of an enzyme - substrate complex (II)

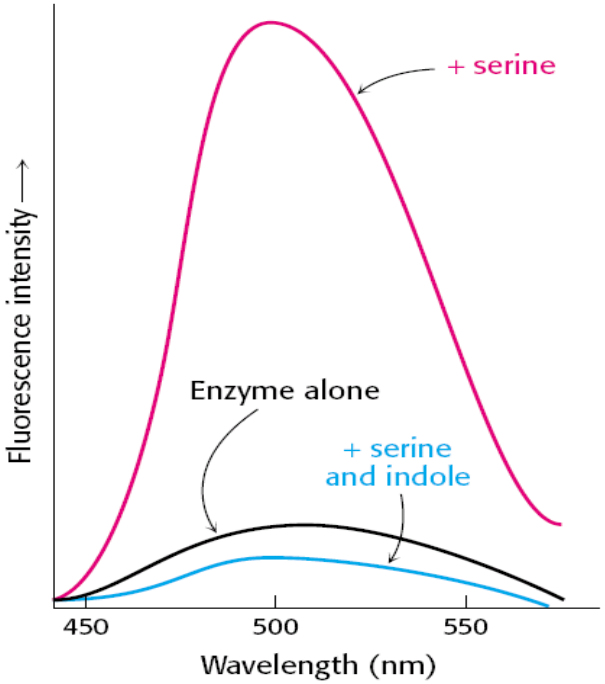
X-ray crystallography provides images of enzyme-substrate complexes.



Structure of an enzyme-substrate complex (cytochrome P-450 - camphor)

Evidence for the existence of an enzyme - substrate complex (III)

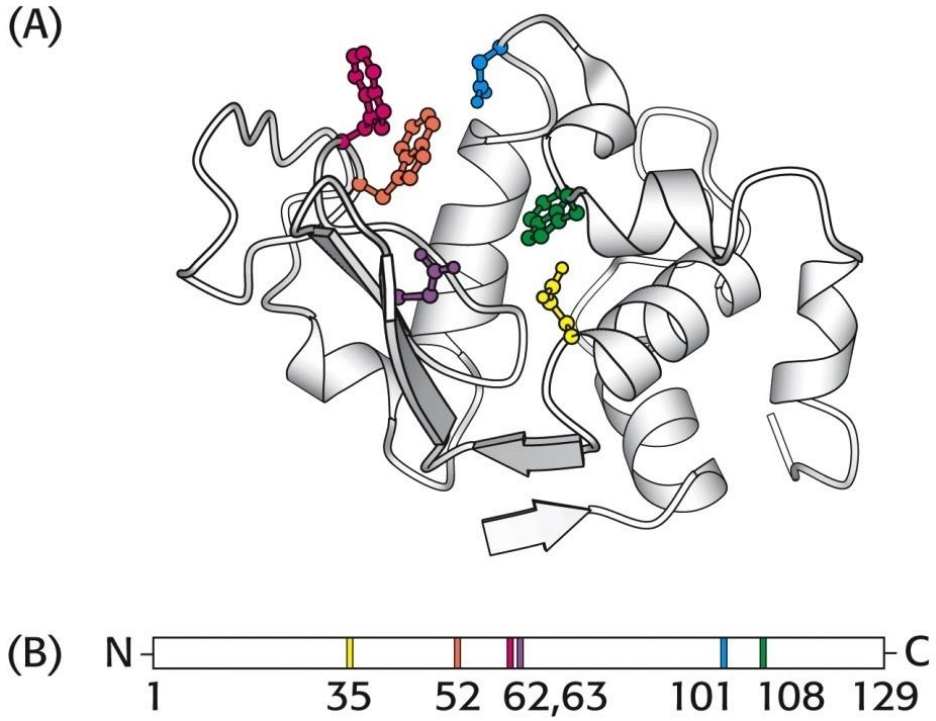
The spectroscopic characteristics of many enzymes and substrates change on formation of an enzyme-substrate complex.



Fluorescence intensity of the pyridoxal phosphate prosthetic group at the active site of tryptophan synthetase changes on addition of serine and indole, the substrates.

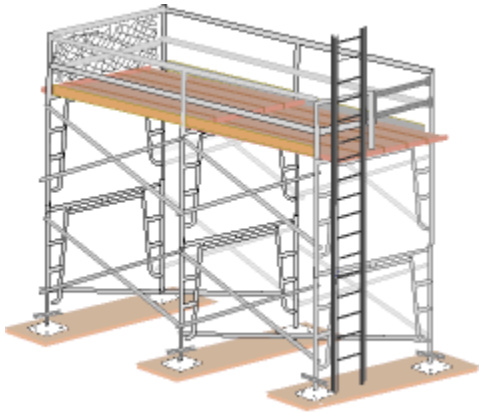
The active sites of enzymes have some common features

1. **The active site is a 3D cleft formed by groups that come from different parts of the amino acid sequence.**



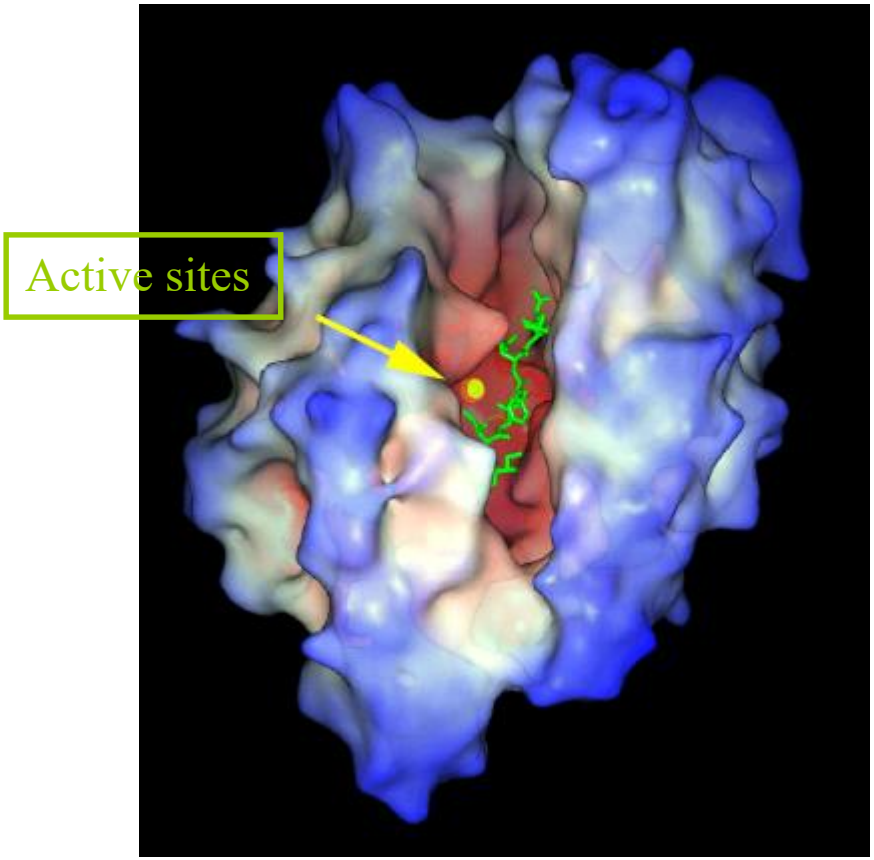
The active site of lysozyme is composed of residues that come from different parts of the polypeptide chain.

2. **The active site takes up a relatively small part of the total volume of an enzyme. The 'extra' amino acids serve as a scaffold to create the 3D active site from amino acids that are far apart in the primary structure.**

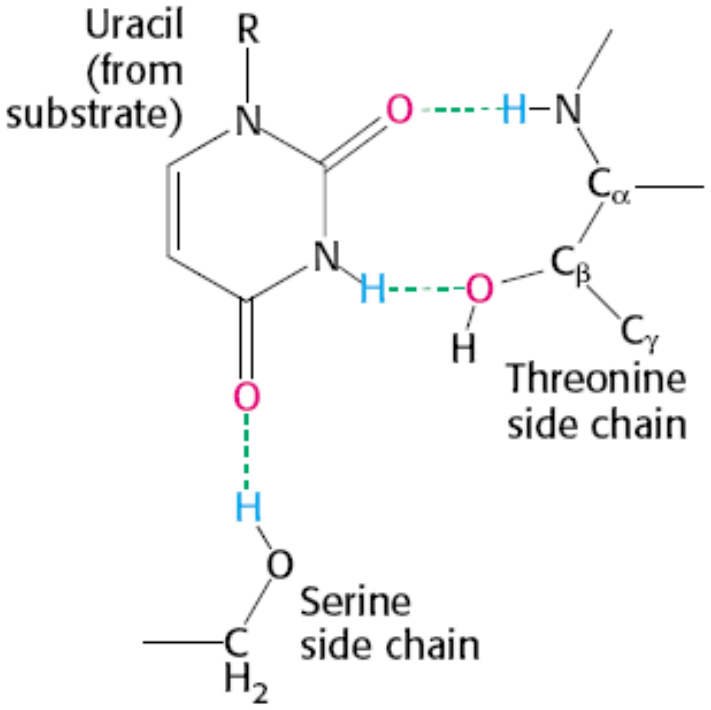


(scaffold)

3. Active sites are clefts (lined by mostly nonpolar residues).



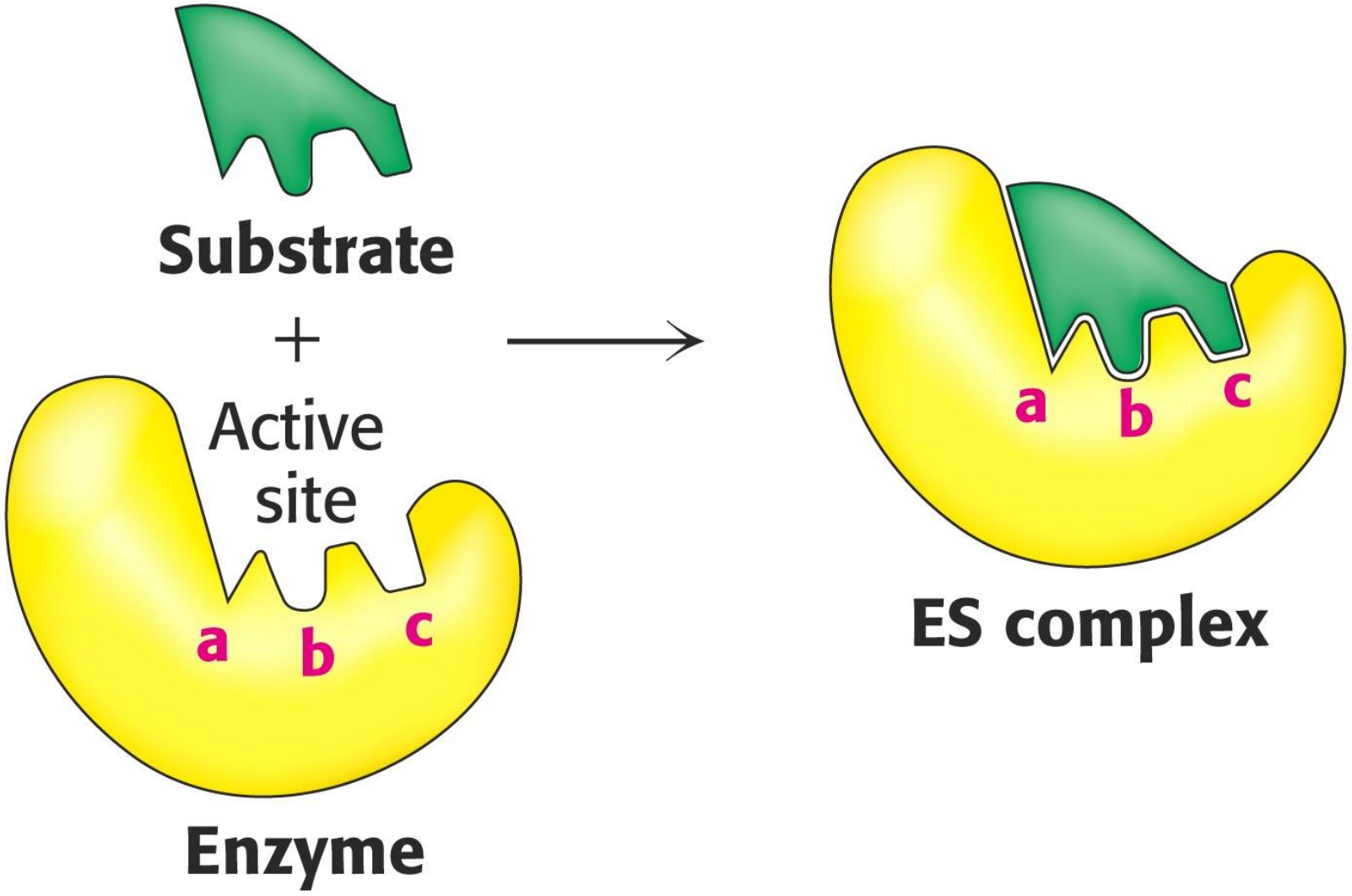
4. Substrates are bound to enzymes by multiple weak attractions.



Hydrogen bonds between an enzyme and substrate

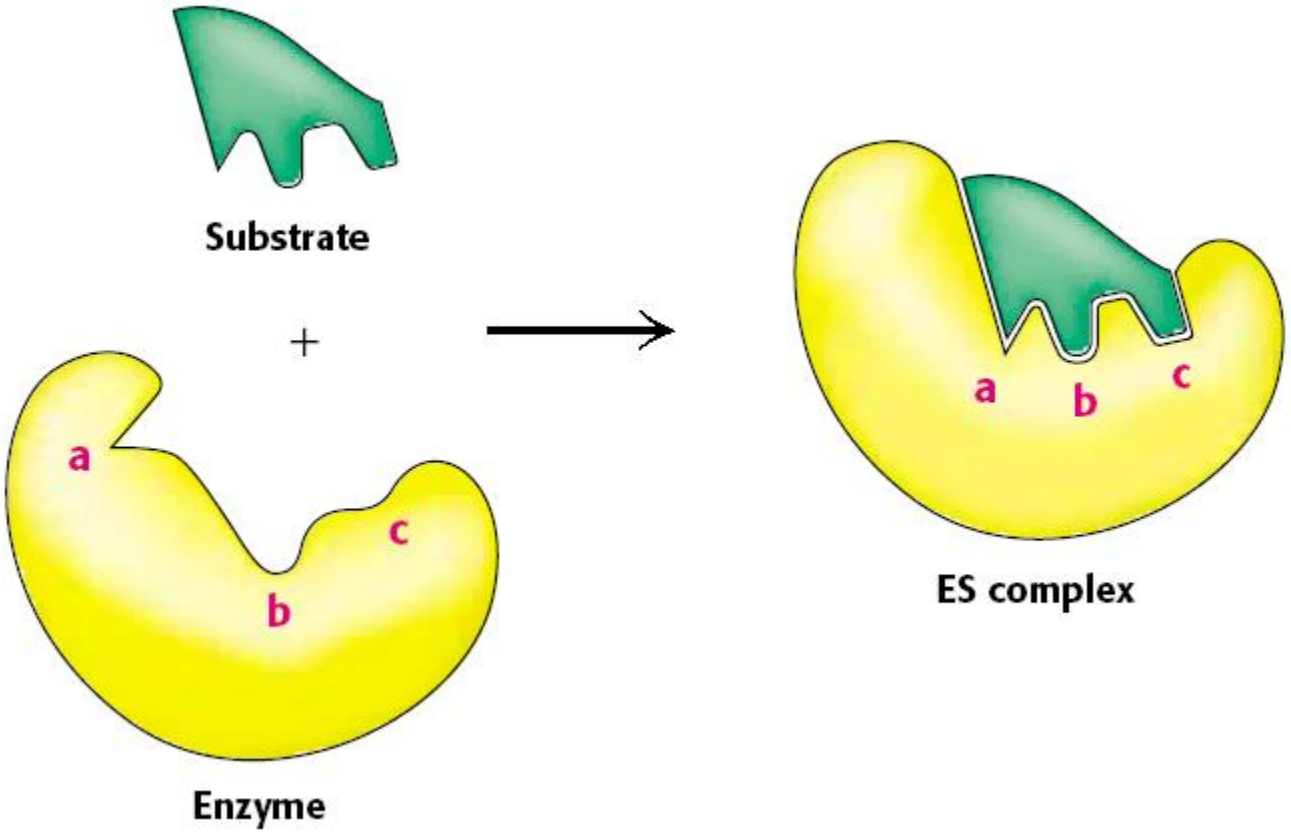
5. The specificity of binding depends on the precisely defined arrangement of atoms in an active site. Substrates are bound to enzymes by multiple weak interactions. Because the enzyme and the substrate interact by means of short-range forces that require close contact, a substrate must have a matching shape to fit into the site.

- **"Lock and Key" model (Emil Fischer, 1890)**
- **"Induced-Fit" model (Daniel E. Koshland, Jr., 1958): The active site of enzymes assume a shape that is complementary to that of the transition state only after the substrate is bound.**



Lock-and-Key model of enzyme-substrate binding

In this model, the active site of the unbound enzyme is complementary in shape to the substrate.



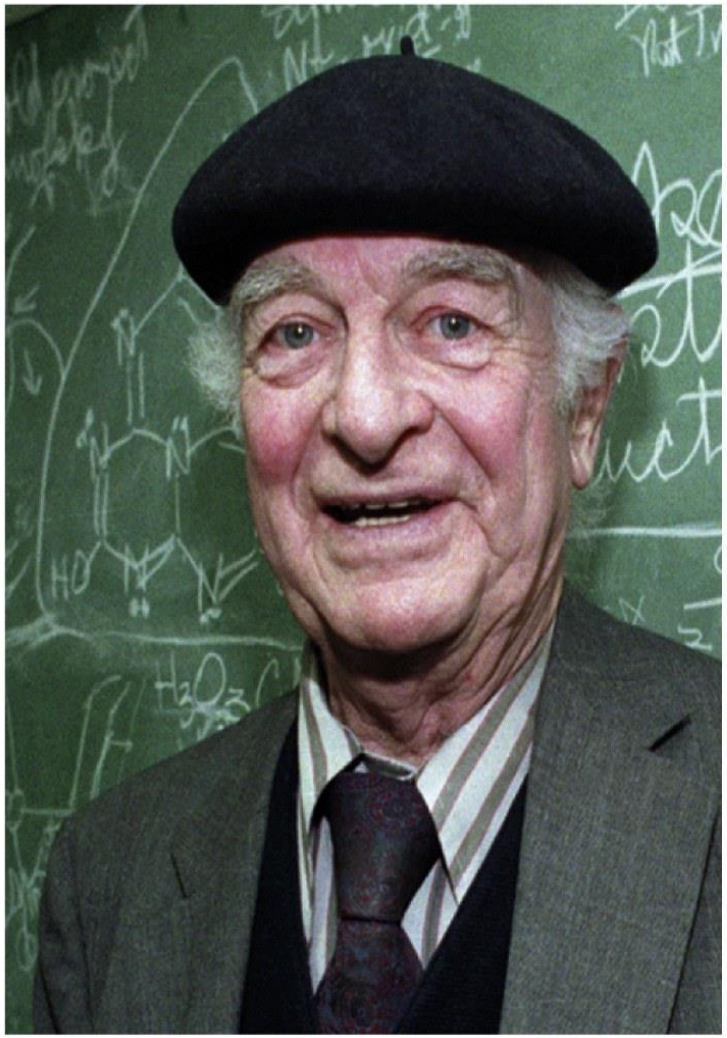
Induced-Fit model of enzyme-substrate binding

In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound.

"I think that enzymes are molecules that are complementary in structure to the activated complexes of the reactions that they catalyze, that is, to the molecular configuration that is intermediate between the reacting substances and the products of reaction for these catalyzed processes. The attraction of the enzyme molecule for the activated complex would thus lead to a decrease in its energy and hence to a decrease in the energy of activation of the reaction and to an increase in the rate of reaction."

—LINUS PAULING

Nature 161(1948):707



Linus Pauling, 1901–1994

Unnumbered 4 p115a

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Enzymes are complementary to the reaction transition state

The idea was proposed by Linus Pauling in 1946:

- **Enzyme's active sites are complimentary to the transition state of the reaction**
- **Optimal interactions between substrate and enzyme occur only in the transition state**
- **stronger interactions with the transition state as compared to the ground state lower the activation energy**

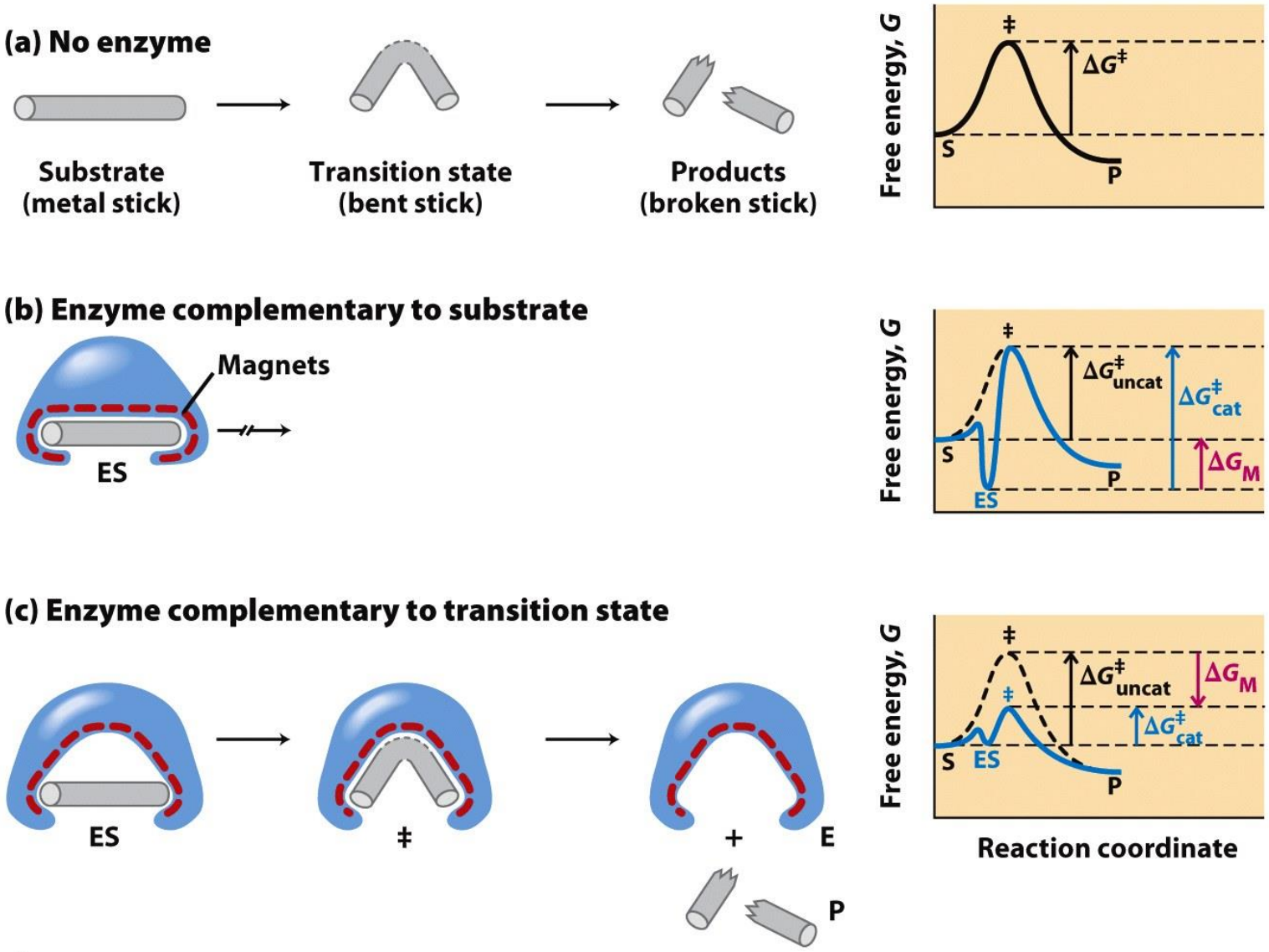

Figure 6-5
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An imaginary enzyme (stickase) designed to catalyze breakage of a metal stick

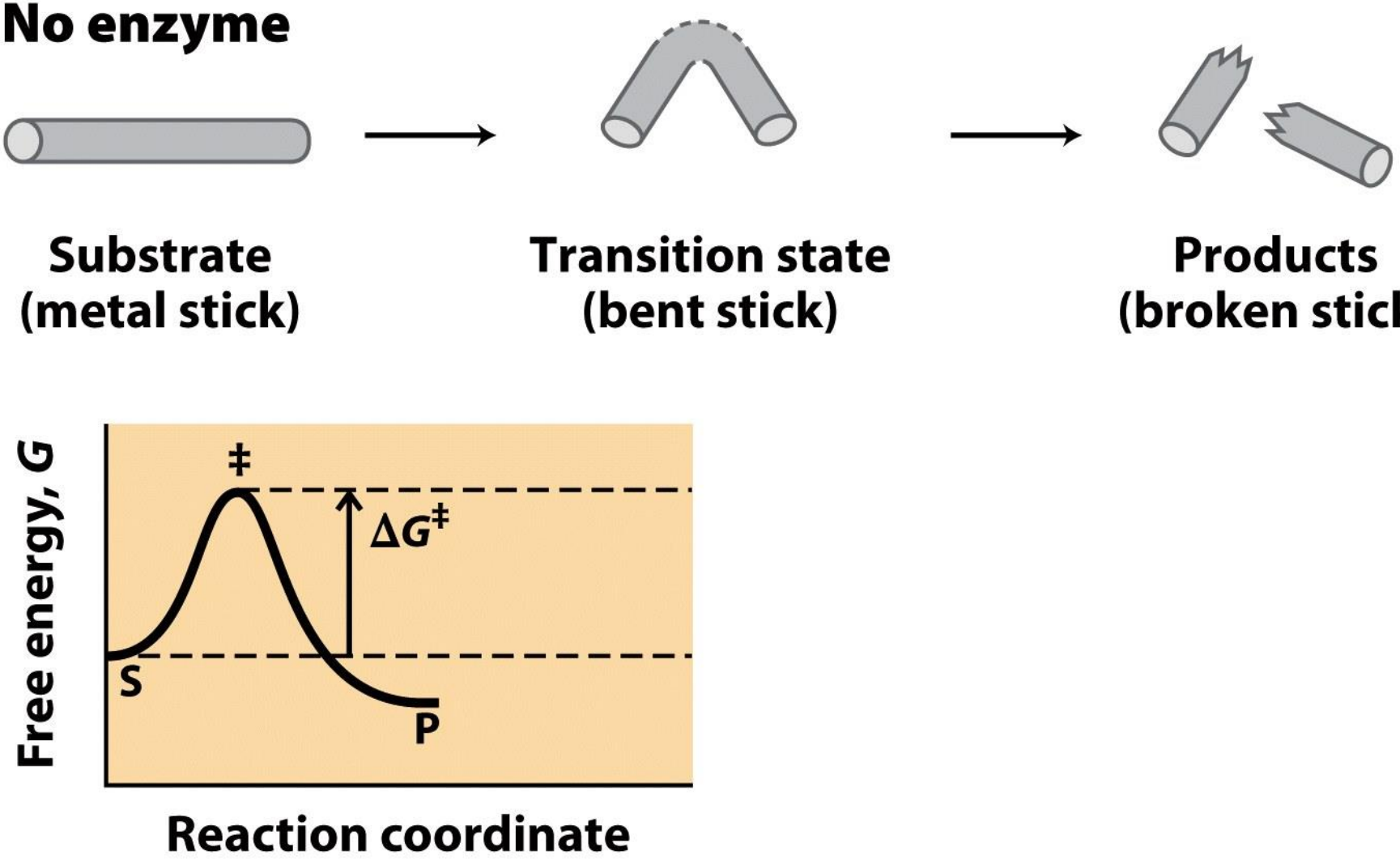

Figure 6-5a
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Enzyme complementary to substrate

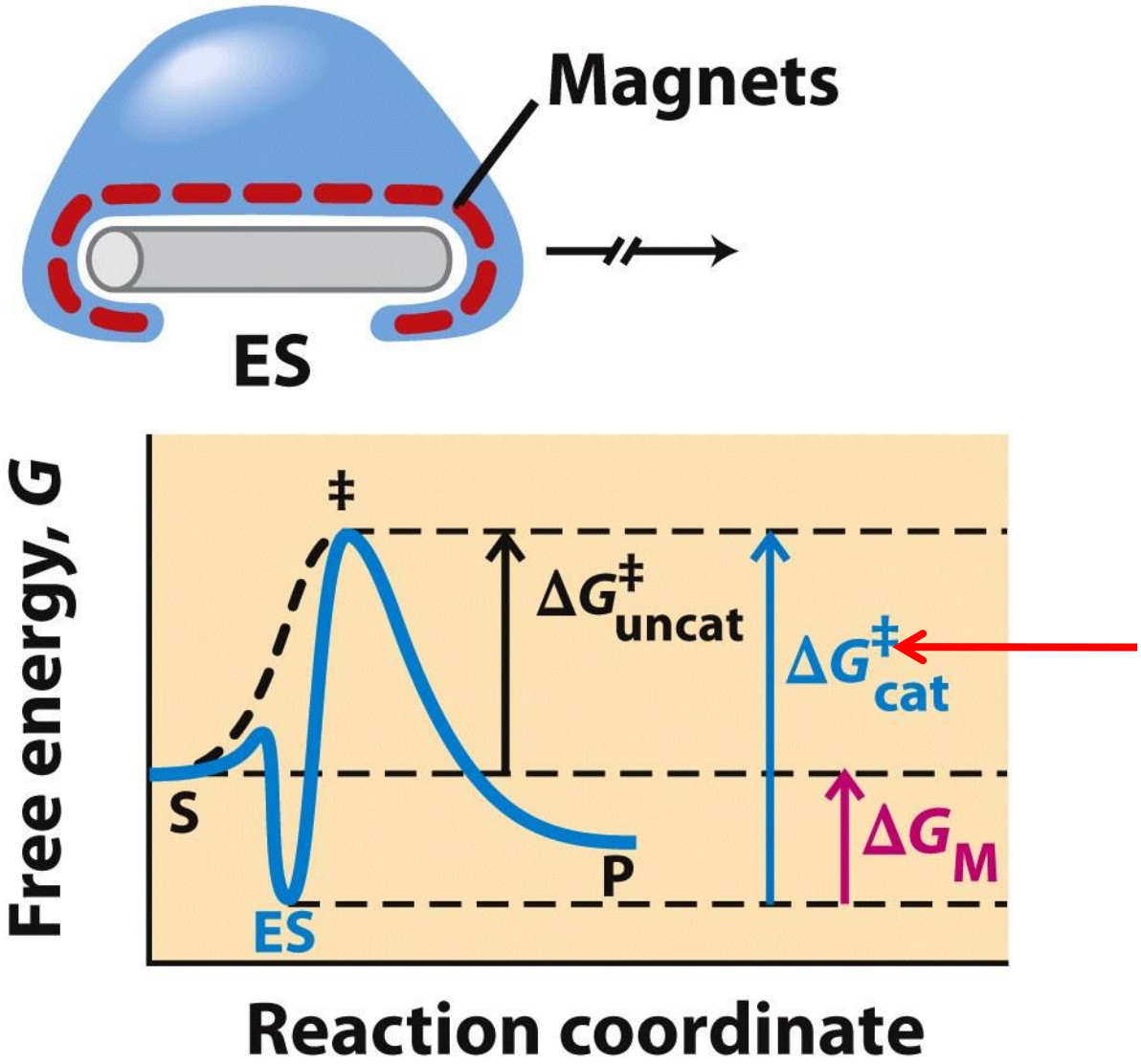

Figure 6-5b
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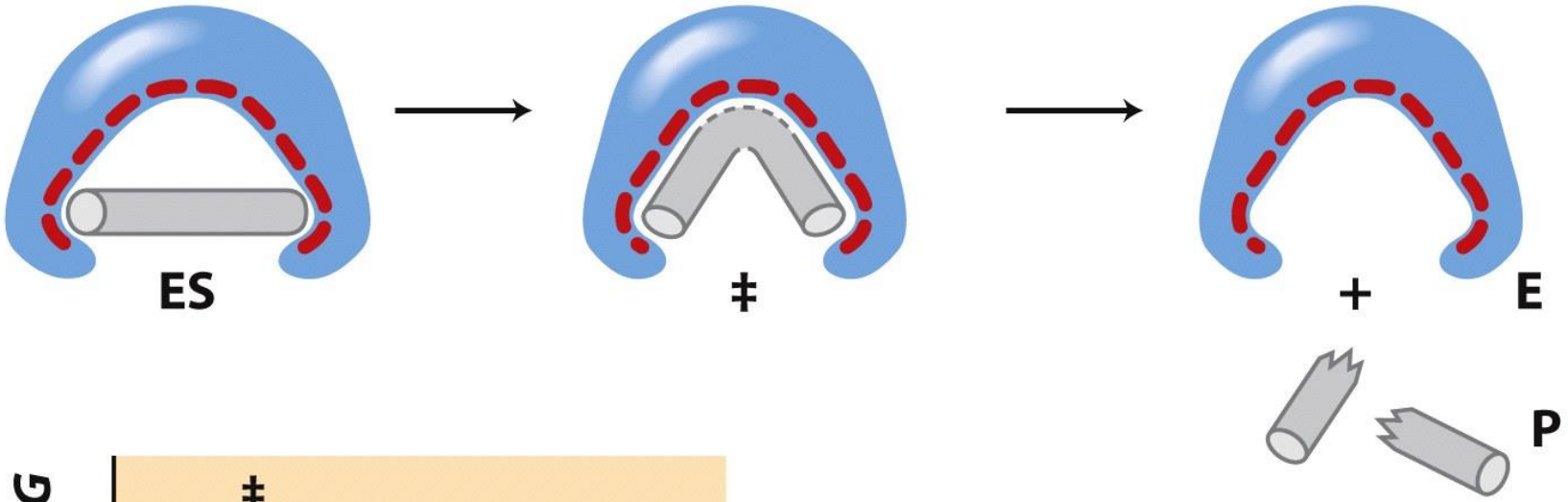
Enzyme complementary to transition state



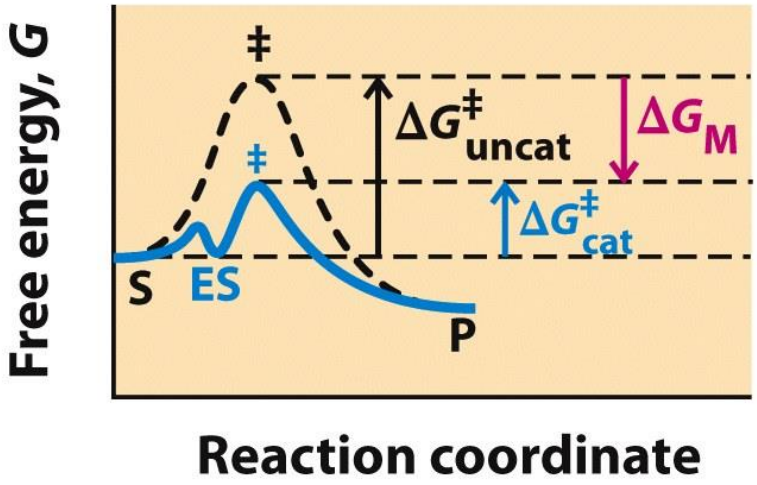


Figure 6-5c
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ΔG_M, the difference between the transition-state energies of the uncatalyzed and catalyzed reactions, is contributed by the magnetic interactions between the stick and stickase.

Weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis.

Binding energy contributes to reaction specificity and catalysis

- **Enzyme - catalyzed reactions are characterized by the formation of a complex between substrate and enzyme (ES complex)**
- **Weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis**
- **The same binding energy also gives an enzyme its specificity, which is derived from the formation of many weak binding interactions between the enzyme and the substrate**
- **The need for multiple interactions is one reason for the large size of enzymes**

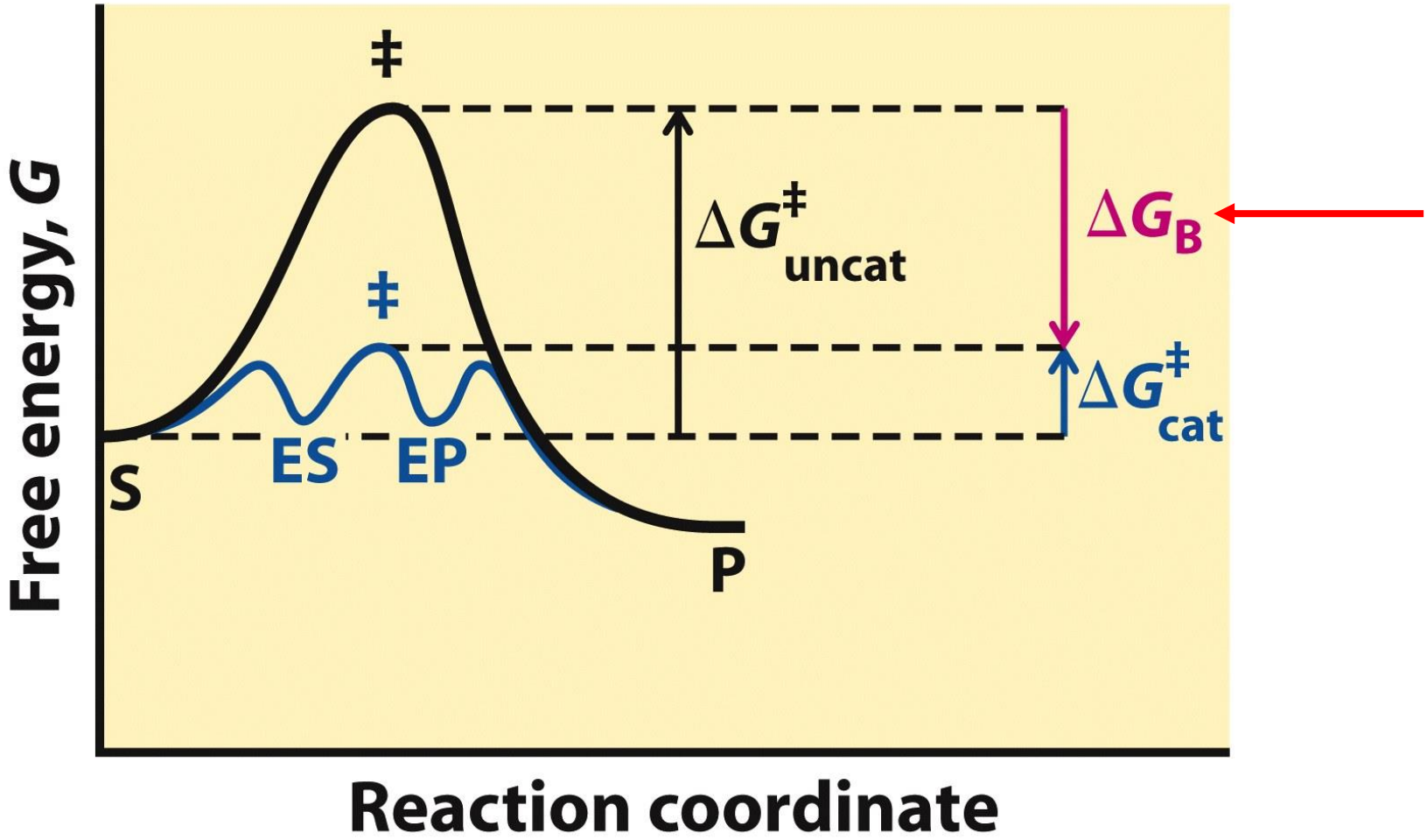

Figure 6-6
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Role of binding energy in catalysis

To lower the activation energy for a reaction, the system must acquire an amount of energy equivalent to the amount by which $\Delta G‡$ 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.

3. Enzyme kinetics

- **Kinetics is the study of the rate of a reaction and how it changes in response to changes in experimental parameters**
- **Rate of enzymatic reaction is affected by**
  - **Enzyme**
  - **Substrate**
  - **Effectors**
  - **Temperature**

Why study enzyme kinetics?

- **Quantitative description of biocatalysis**
- **Determine the order of binding of substrates**
- **Understand catalytic mechanism**
- **Find effective inhibitors**
- **Understand regulation of activity**

Leonor Michaelis
1875–1949

Maud Menten
1879–1960

Unnumbered 6 p203
Lehninger Principles of Biochemistry, Fifth Edition

The Michaelis-Menten Model accounts for the kinetic properties of many enzymes

Derivation of enzyme kinetics equations

- **Start with a model mechanism**
- **Identify constraints and assumptions**
- **Carry out algebra ...**
  - **... or graph theory for complex reactions**

Simplest Model Mechanism: $E + S \rightleftarrows ES \rightarrow E + P$

- One reactant, one product, no inhibitors

Kinetic description of enzymatic activity

Consider an enzyme that catalyzes the S to P by the following pathway:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Enzyme kinetics are more easily approached if we can ignore the reverse reaction. We define V_o as the rate of catalysis (the rate of increase in product with time) when [P] is low; that is, at times close to zero (V_o: initial velocity or initial rate)

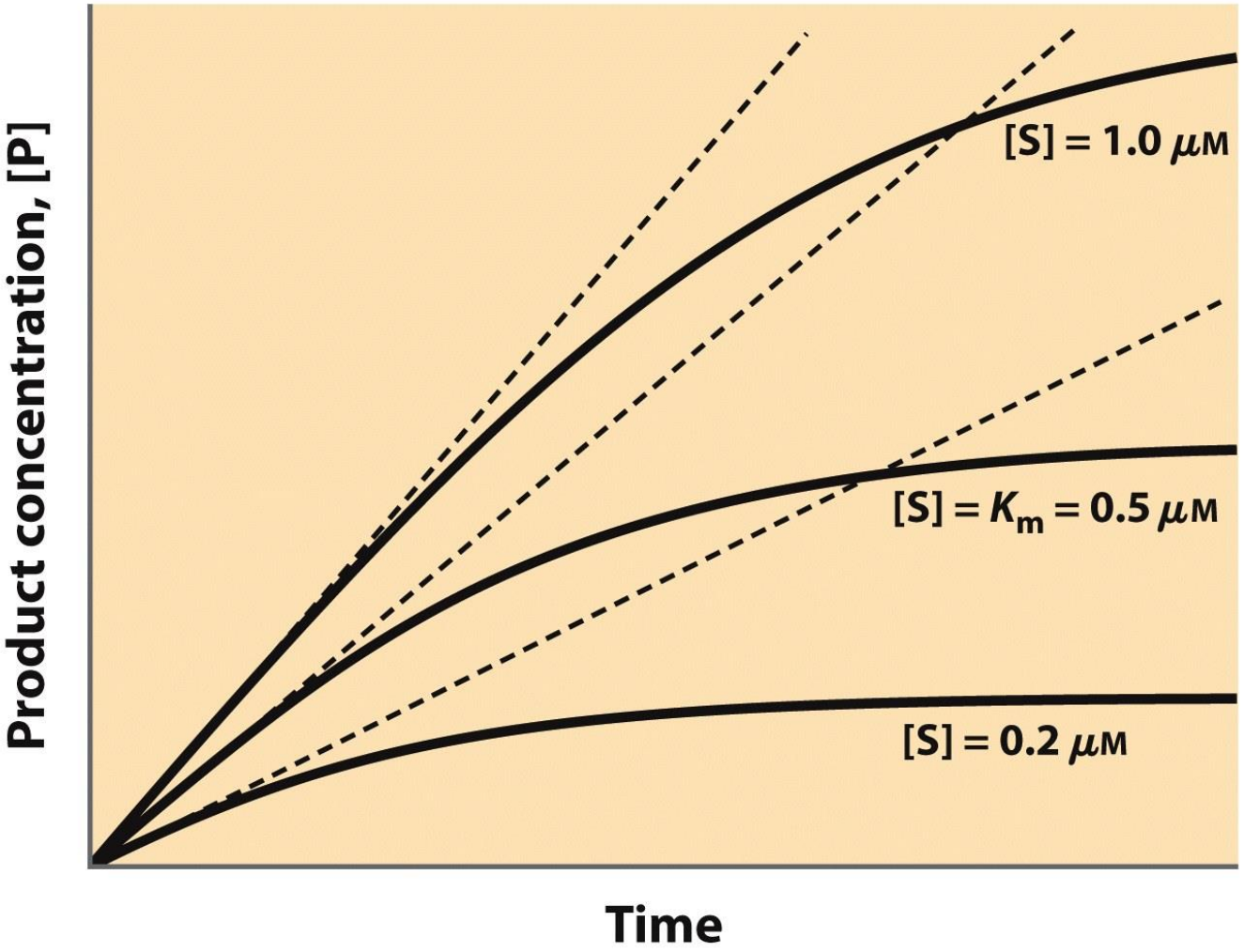

Figure 6-10
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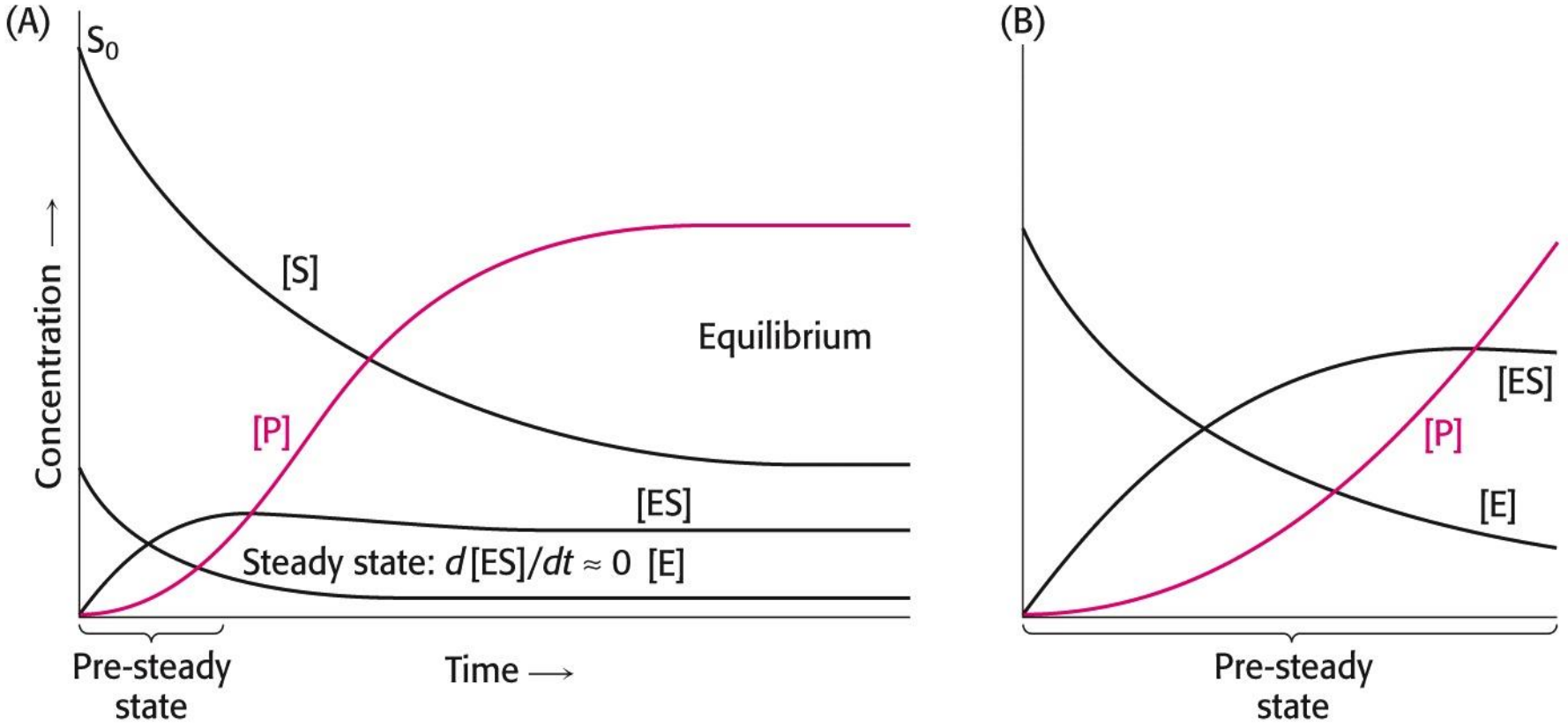
Initial velocities of enzyme-catalyzed reactions

A tangent to each curve taken at time = 0 defines the initial velocity, V_0, of each reaction.



Changes in the concentration of reaction participants of an enzyme-catalyzed reaction with time

Concentration changes under (A) steady-state condition in which the rate at which the ES complex forms balances the rate at which it breaks down and (B) the pre-steady-state condition

How to get the Michaelis-Menten Equation

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \qquad (6\text{–}10)$$

$$V_0 = k_2[\mathrm{ES}] \qquad (6\text{–}11)$$

We want to express [ES] in terms of known quantities

Total enzyme concentration,
the sum of free and substrate-bound enzyme

$$\text{Rate of ES formation} = k_1([\mathrm{E_t}] - [\mathrm{ES}])[\mathrm{S}] \qquad (6\text{–}12)$$

$$\text{Rate of ES breakdown} = k_{-1}[\mathrm{ES}] + k_2[\mathrm{ES}] \qquad (6\text{–}13)$$

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

At steady-state:

rate of formation (ES) = rate of breakdown (ES)

$$k_1([E_t] - [ES])[S] = k_{-1}[ES] + k_2[ES] \qquad (6\text{–}14)$$

$$k_1[E_t][S] - k_1[ES][S] = (k_{-1} + k_2)[ES] \qquad (6\text{–}15)$$

$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES] \qquad (6\text{–}16)$$

$$[ES] = \frac{k_1[E_t][S]}{k_1[S] + k_{-1} + k_2} \qquad (6\text{–}17)$$

$$[ES] = \frac{[E_t][S]}{[S] + (k_2 + k_{-1})/k_1} \qquad (6\text{–}18)$$

$(k_2 + k_{-1})/k_1$ **defined as Michaelis constant (K_m), has the unit of concentration**

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{[\mathrm{S}] + (k_2 + k_{-1})/k_1} \qquad (6\text{–}18)$$

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]} \qquad (6\text{–}19)$$

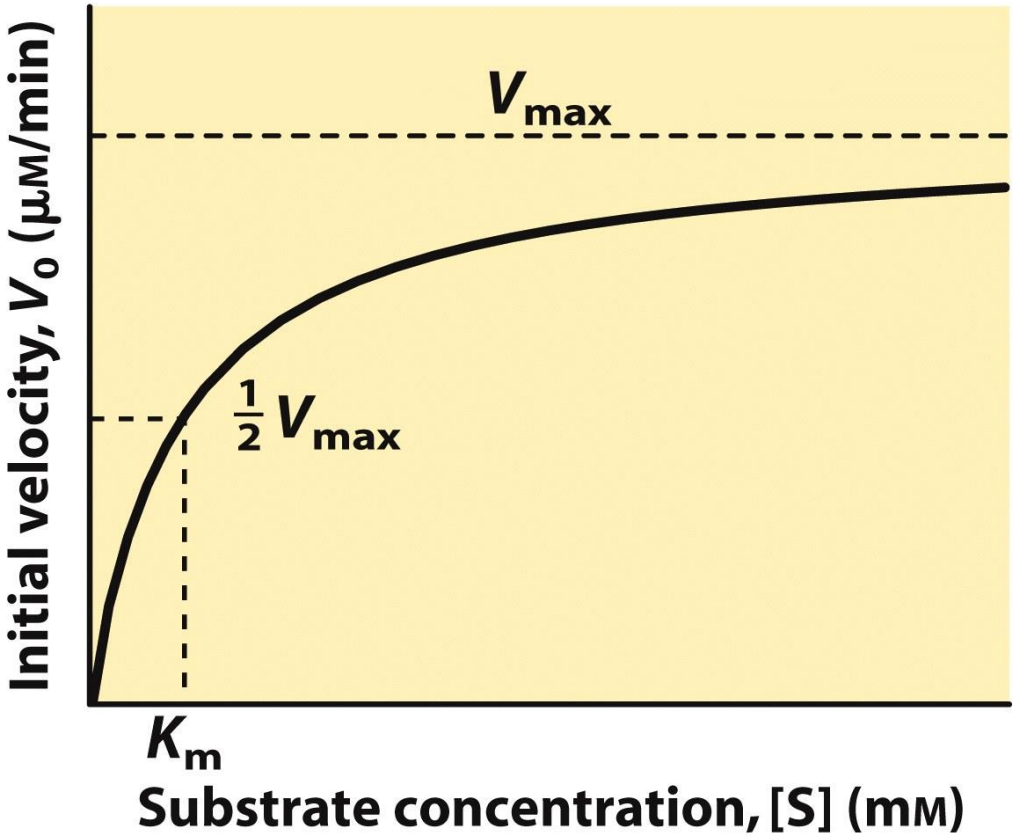
$$V_0 = k_2[\mathrm{ES}] \qquad (6\text{–}11)$$

$$V_0 = \frac{k_2[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]} \qquad (6\text{–}20)$$

The maximal rate, V_{max}, is attained when the catalytic sites on the enzyme are saturated with substrate --- that is, when $[ES] = [E]_T$

Michaelis-Menten equation:

$$V_0 = \frac{V_\mathrm{max}\,[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$



$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

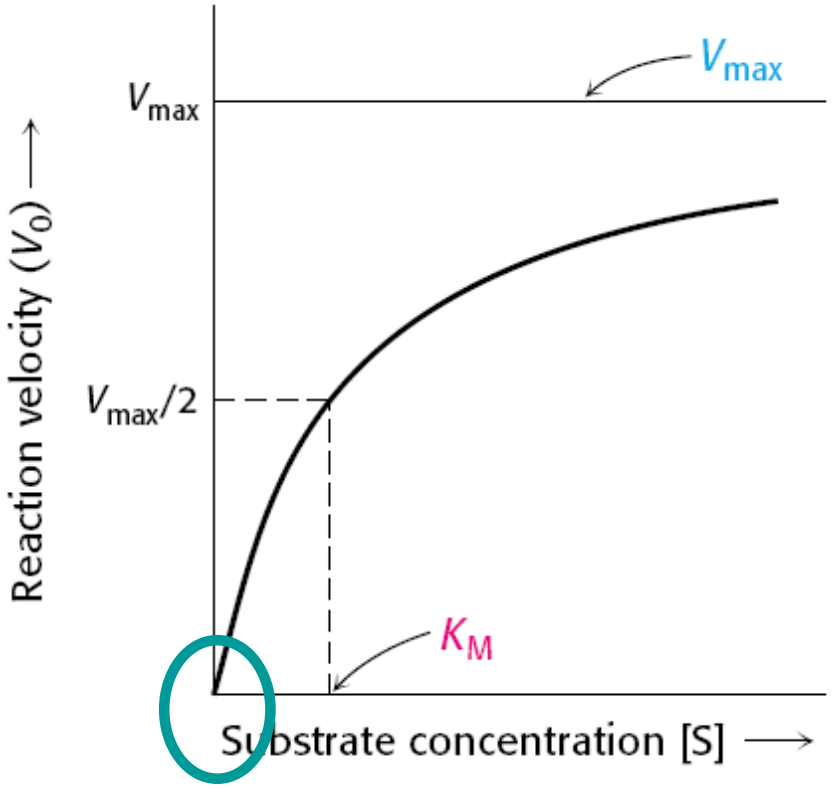
Figure 6-11
Lehninger Principles of Biochemistry, Fifth Edition

Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction

The Michaelis constant (K_m) is the substrate concentration yielding a velocity of $V_{max}/2$.

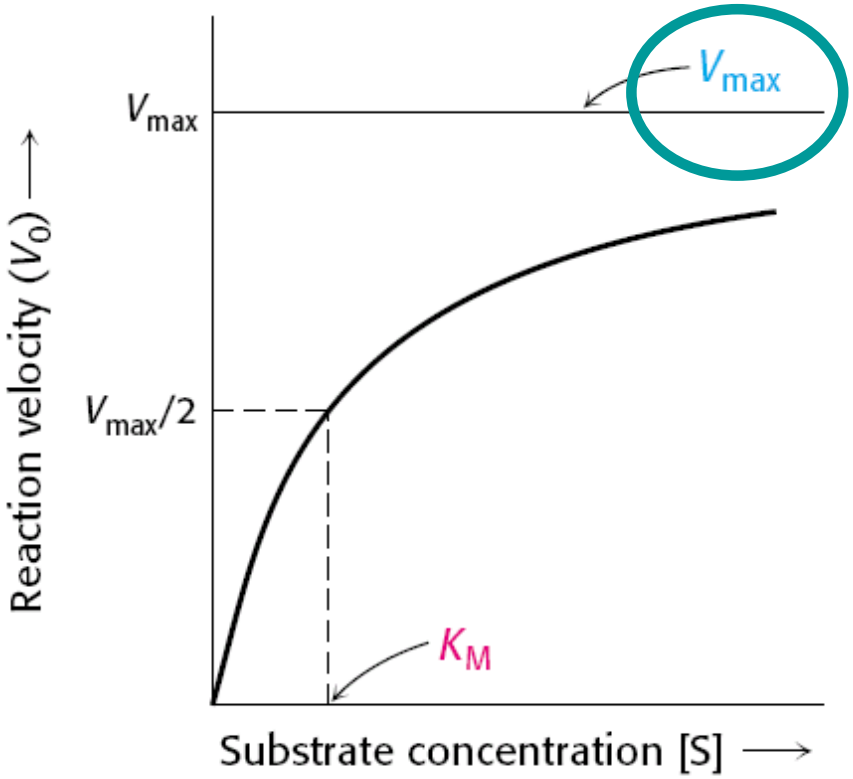
Dissection of the Michaelis-Menten Equation

$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$



At low [S], [S] << K_m: $V_o = V_{max}$ [S]/K_m ; that is, $V_o \propto$ [S]

$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$



At high [S], [S] >> K_m: $V_o = V_{max}$

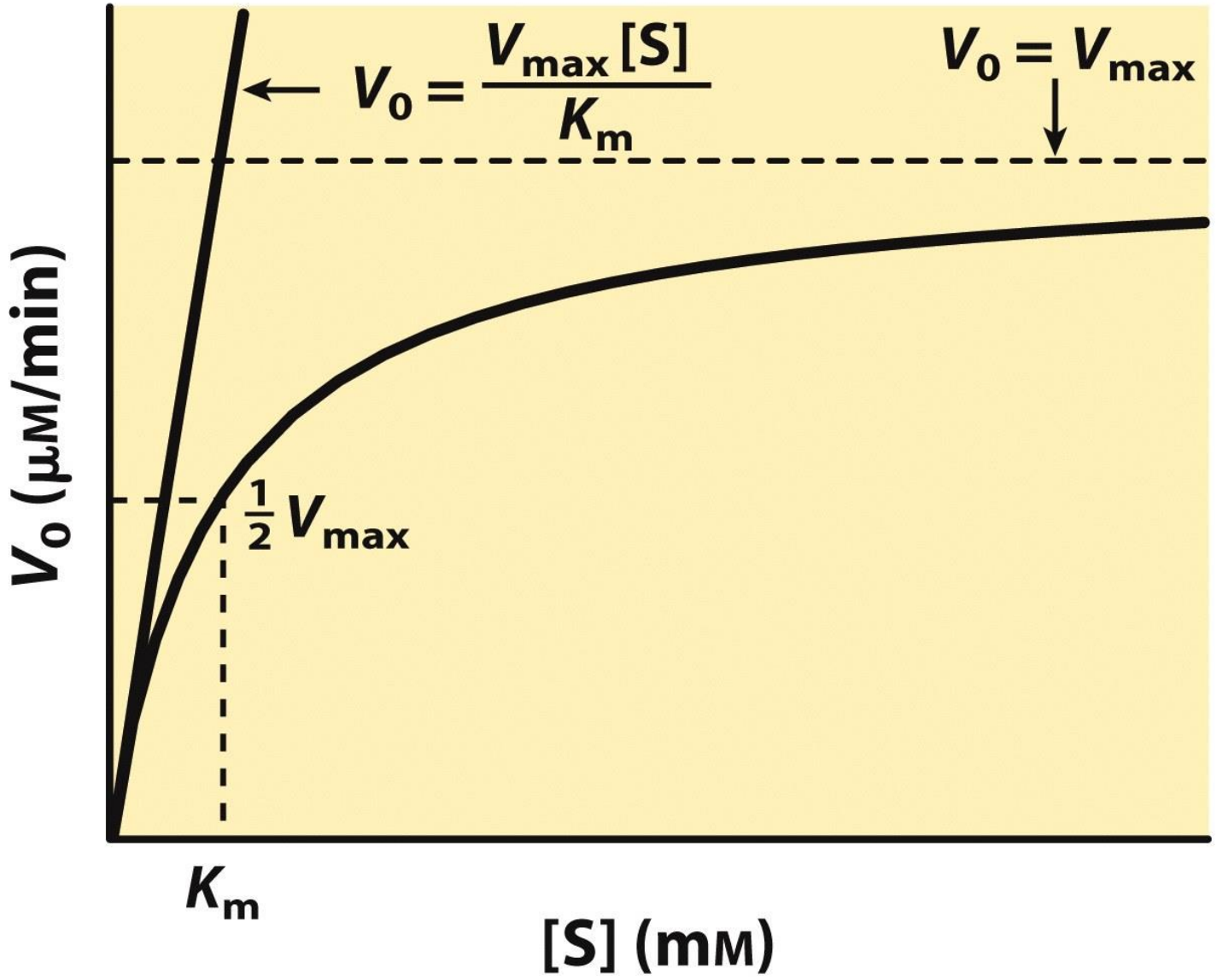


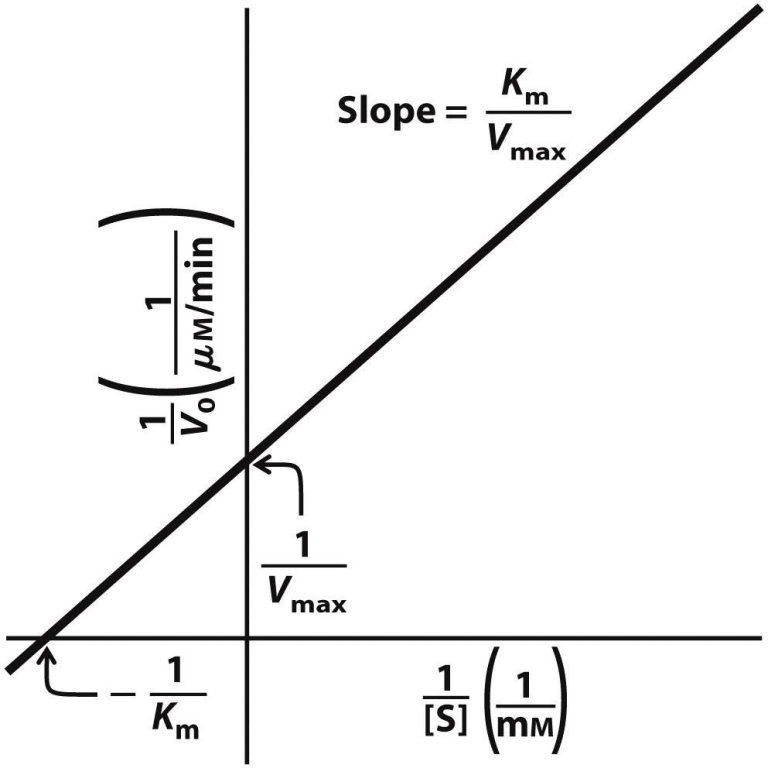
Figure 6-12
Lehninger Principles of Biochemistry, Fifth Edition

Dependence of initial velocity on substrate concentration

Determine K_m and V_{max} by double-reciprocal plots

Lineweaver-Burk equation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \longrightarrow \frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



Box 6-1 figure 1
Lehninger Principles of Biochemistry, Fifth Edition

Double-reciprocal plot of enzyme kinetics is generated by plotting $1/V_0$ as a function 1/[S]. The slope is the K_m/V_{max}, the intercept on the vertical axis is $1/V_{max}$, and the intercept on the horizontal axis is $-1/K_m$.

Significance of K_m value

- K_m provides a measure of the substrate concentration required for significant catalysis to occur
- K_m can be used as a measure of the enzyme's binding affinity when $k_2 << k_{-1}$ ($K_m = k_{-1}/k_1$)

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

- K_m is characteristic for each enzyme acting on a given substrate
- When K_m is known, the fraction of sites filled, f_{ES}, at any substrate concentration can be calculated from

$$f_{ES} = \frac{V}{V_{max}} = \frac{[S]}{[S] + K_M}$$

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Table 6-6
Lehninger Principles of Biochemistry, Fifth Edition

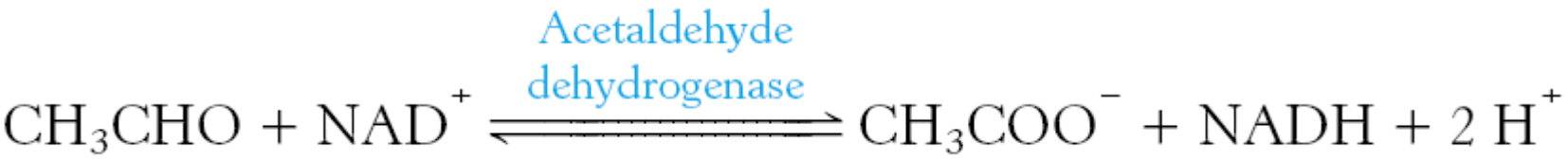
The K_m value for an enzyme depends on the particular substrate and on environmental conditions such as pH, temperature, and ionic strength.

Physical consequence of K_m (Sensitivity of individuals to ethanol)

$$CH_3CH_2OH + NAD^+ \xrightleftharpoons{\text{Alchohol dehydrogenase}} CH_3CHO + H^+ + NADH$$

Excess CH_3CHO → **Facial flushing, rapid heart rate …**

Normally, the acetaldehyde, which is the cause of the symptoms when present at high concentrations, is processed to acetate by acetaldehyde dehydrogenase.



Two forms of acetaldehyde dehydrogenase:

1. **Cytosolic: high K_m → high concentration of substrate is needed for the enzyme to achieve (V_{max} /2)**
2. **Mitochondrial: low K_m → low concentration of substrate is needed for the enzyme to achieve (V_{max} /2)**

For a certain amount of CH_3CHO, conversion into CH_3COO^- by the mitochondrial form is faster. (In certain time, less CH_3CHO is converted by the cytosolic form.)

In some individuals, the mitochondrial form is mutated and thus is less active. Since the cytosolic form has higher K_m, which means that more CH_3CHO is accumulated, therefore, these people are more susceptible to facial flushing and rapid heart rate after alcohol intake.

Significance of V_{max} value

V_{max} reveals the turnover number of an enzyme (k_2, also called k_{cat}), which is the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.

V_{max} is achieved when $[ES]=[E]_T$. Thus

$$V_{max} = k_2[E]_T \longrightarrow k_2 = V_{max}/[E]_T$$

TABLE 6–7 Turnover Numbers, k_{cat}, of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

Table 6-7
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Kinetic perfection in enzymatic catalysis: the k_{cat}/K_m criterion

Under physiological conditions, the [S]/K_m ratio is typically between 0.01 and 1.0, V_{max} is not achieved.

$$V_0 = k_2[\mathrm{ES}] \qquad [\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{M}}$$

$$V_0 = \frac{k_{\mathrm{cat}}}{K_\mathrm{M}}[\mathrm{E}][\mathrm{S}]$$

When [S] << K_m, [E]=[E]$_T$. Thus

$$V_0 = \frac{k_{\mathrm{cat}}}{K_\mathrm{M}}[\mathrm{S}][\mathrm{E}]_\mathrm{T}$$

$$V_0 = \frac{k_{cat}}{K_M}[S][E]_T$$

$$S + E \xrightarrow{k_{cat}/K_m} E + P$$

- **When [S] << K_m, k_{cat}/K_m is the rate constant for the interaction of E and S**
- **k_{cat}/K_m can be used as a measure of catalytic efficiency**

Chymotrypsin has a preference for cleaving next to bulky, hydrophobic side chains.

TABLE 8.7 Substrate preferences of chymotrypsin

Amino acid in ester	Amino acid side chain	k_{cat}/K_M ($s^{-1}\,M^{-1}$)
Glycine	—H	1.3×10^{-1}
Valine	—CH(CH$_3$)$_2$	2.0
Norvaline	—$CH_2CH_2CH_3$	3.6×10^{2}
Norleucine	—$CH_2CH_2CH_2CH_3$	3.0×10^{3}
Phenylalanine	—CH_2—C$_6$H$_5$	1.0×10^{5}

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 7.3.

How efficient can an enzyme be? (How high can k_{cat}/K_m be?)

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

Substituting for K_m gives

$$\frac{k_{cat}}{K_M} = \frac{k_{cat}}{k_{-1} + k_{cat}/k_1} = \frac{k_{cat}}{k_{cat} + k_{-1}} k_1 < k_1 \qquad (30)$$

The ultimate limit on the value of k_{cat}/K_m is set by k_1, the rate constant of formation of the ES complex. This rate constant cannot be faster than the diffusion-controlled encounter of an enzyme and its substrate.

The ratio k_{cat}/K_m provides a good measure of catalytic efficiency

Kinetic perfection

Enzymes such as these that have k_{cat}/K_m ratios at the upper limits have attained kinetic perfection. Their catalytic velocity is restricted only by the rate at which they encounter substrate in the solution.

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science,* p. 166, W. H. Freeman and Company, New York.

Table 6-8

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Determination of kinetic parameters

Nonlinear Michaelis-Menten plot should be used to calculate parameters K_m and V_{max}

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Linearized double-reciprocal plot is good for analysis of two-substrate data or inhibition

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

Effect of substrate concentration

- **Ideal Rate:**

$$V_0 = \frac{V_{\max}[\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

- **Deviations due to:**
  - **–Limitation of measurements**
  - **–Substrate inhibition**
  - **–Substrate prep contains inhibitors**
  - **–Enzyme prep contains inhibitors**

Two-substrate reactions

- **Kinetic mechanism: the order of binding of substrates and release of products**

- **When two or more reactants are involved, enzyme kinetics allows to distinguish between different kinetic mechanisms**
- **Sequential mechanism (ternary complex)**
- **Ping-Pong mechanism (double-displacement)**

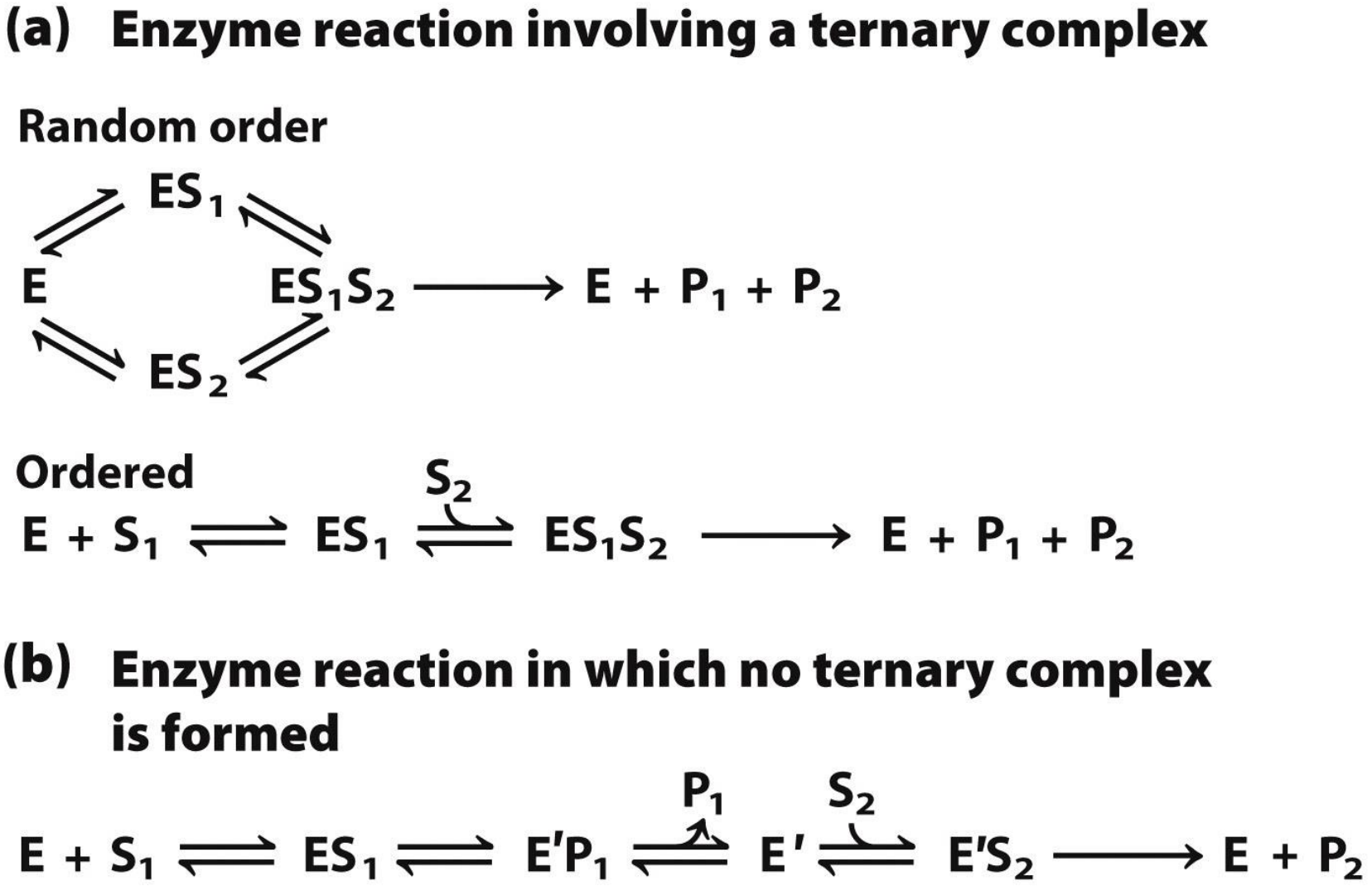

Figure 6-13
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Common mechanisms for enzyme-catalyzed bisubstrate reactions

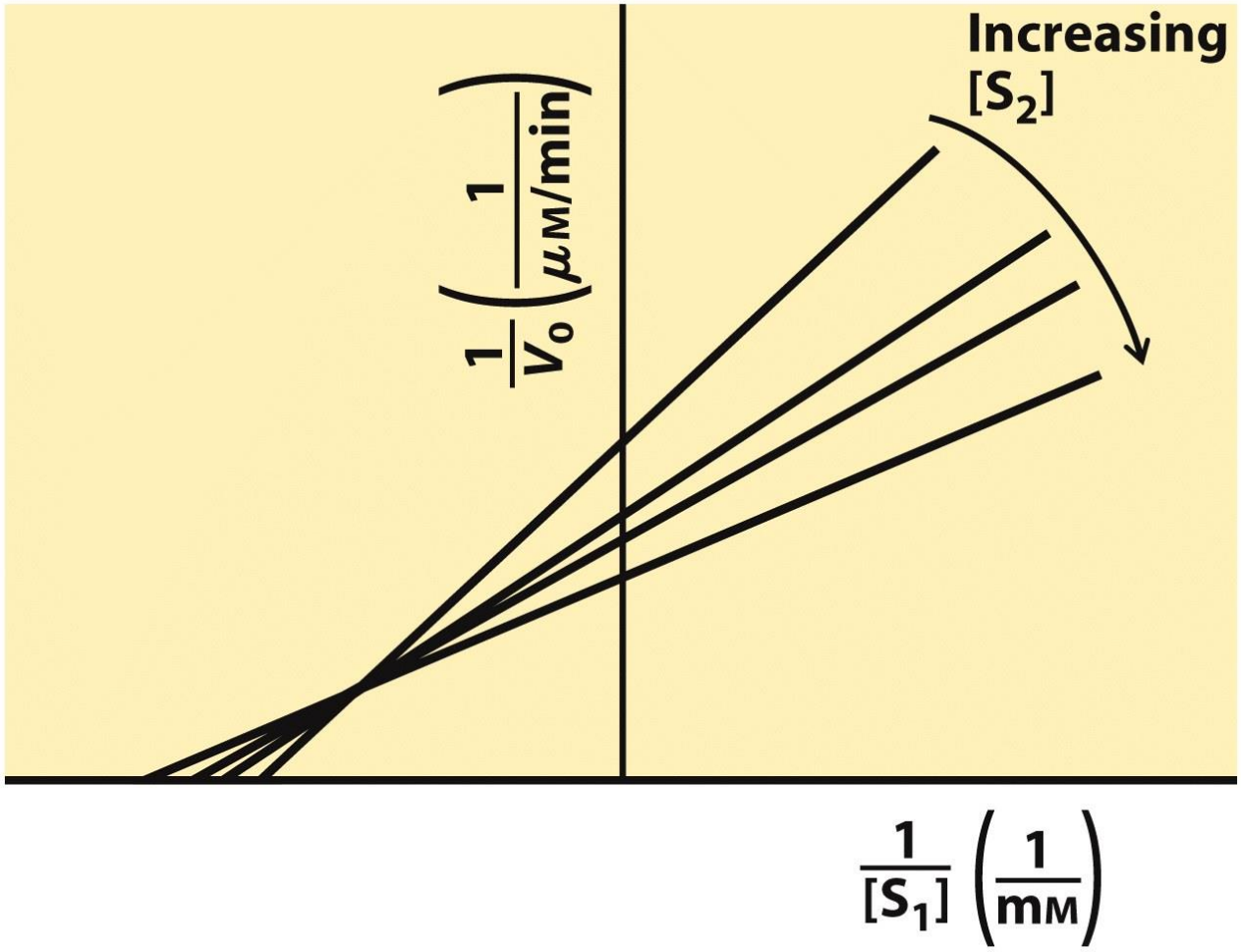

Figure 6-14a
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Steady-state kinetic analysis of bisubstrate reactions

Intersecting lines indicate that a ternary complex is formed in the reaction

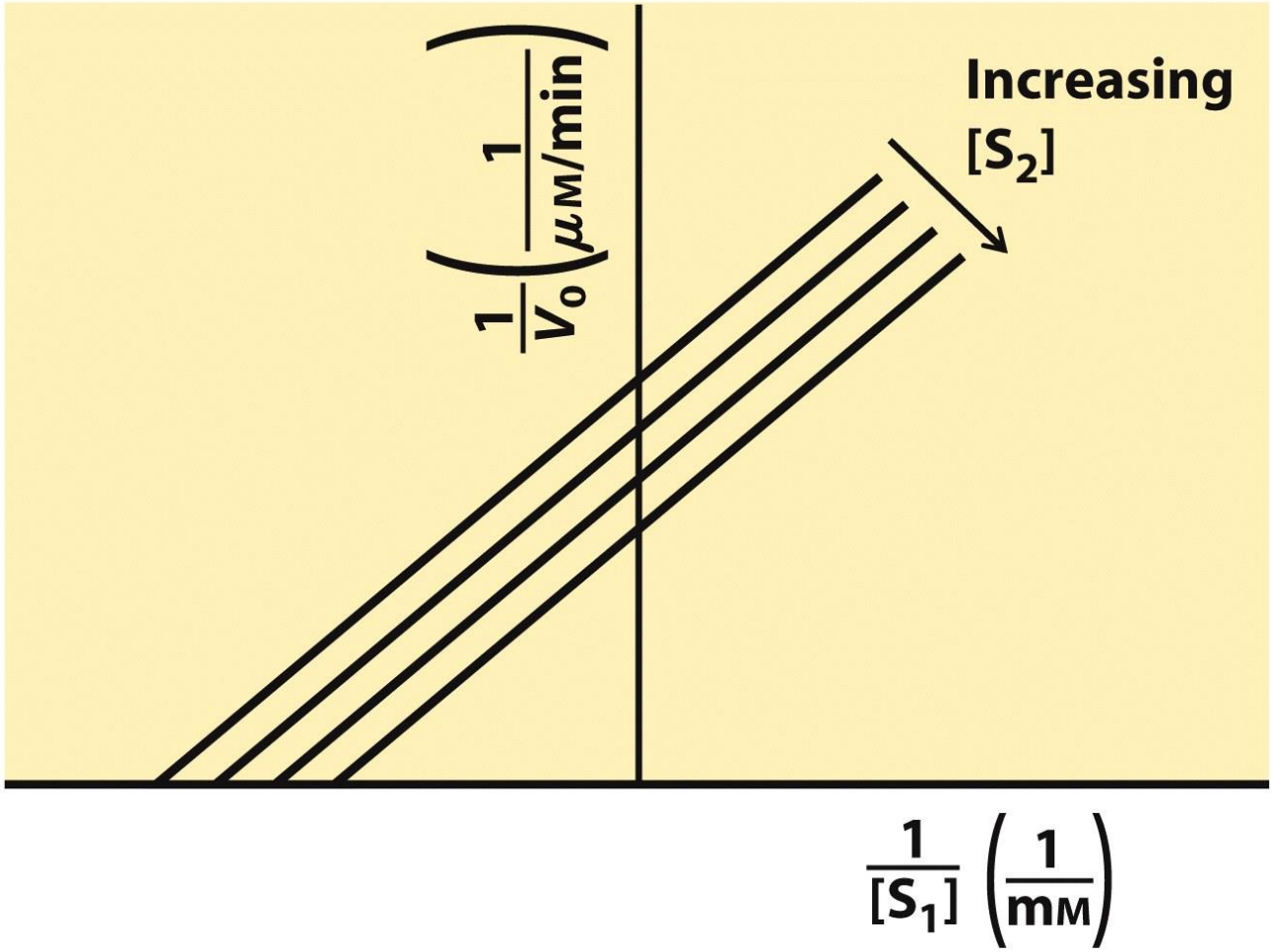

Figure 6-14b
Lehninger Principles of Biochemistry, Fifth Edition

Steady-state kinetic analysis of bisubstrate reactions

Parallel lines indicate a Ping-Pong (double-displacement) pathway

Enzyme inhibitors are compounds that decrease enzyme's activity

- **Irreversible inhibitors (inactivators) react with the enzyme**
  - **one inhibitor molecule can permanently shut off one enzyme molecule**
  - **they are often powerful toxins but also may be used as drugs**

- **Reversible inhibitors bind to, and can dissociate from the enzyme**
  - **they are often structural analogs of substrates or products**
  - **they are often used as drugs to slow down a specific enzyme**
  - **may be competitive, uncompetitive, or mixed**

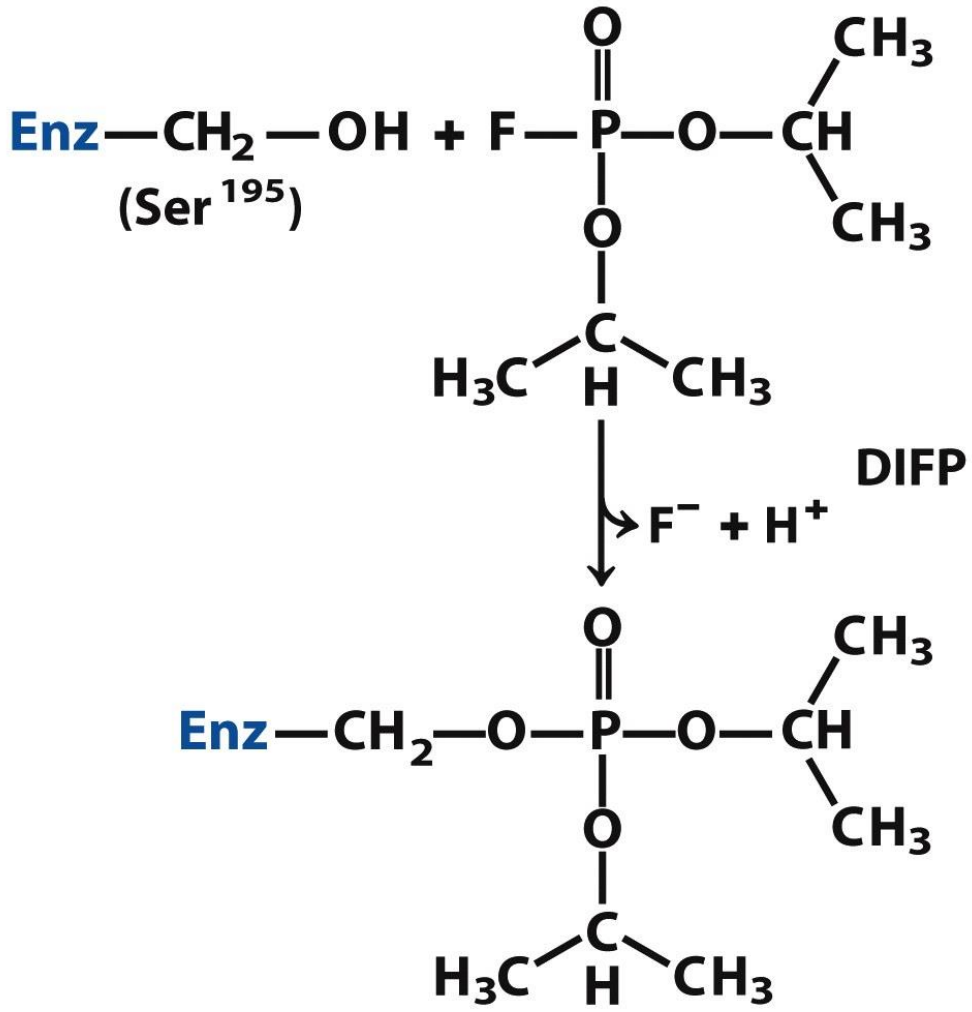

Figure 6-16
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Irreversible inhibitors bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or form a particularly stable noncovalent association.

Irreversible inhibition

Reaction of chymotrypsin with diisopropylfluorophosphate (DIFP), which modifies Ser^{195}, irreversibly inhibits the enzyme. This has led to the conclusion that Ser^{195} is the key active-site Ser residue in chymotrypsin.

Classification of Reversible inhibitors

Reversible inhibitor can bind:

- **To the free enzyme and prevent the binding of the substrate**
- **To the enzyme-substrate complex and prevent the reaction**

Competitive inhibition

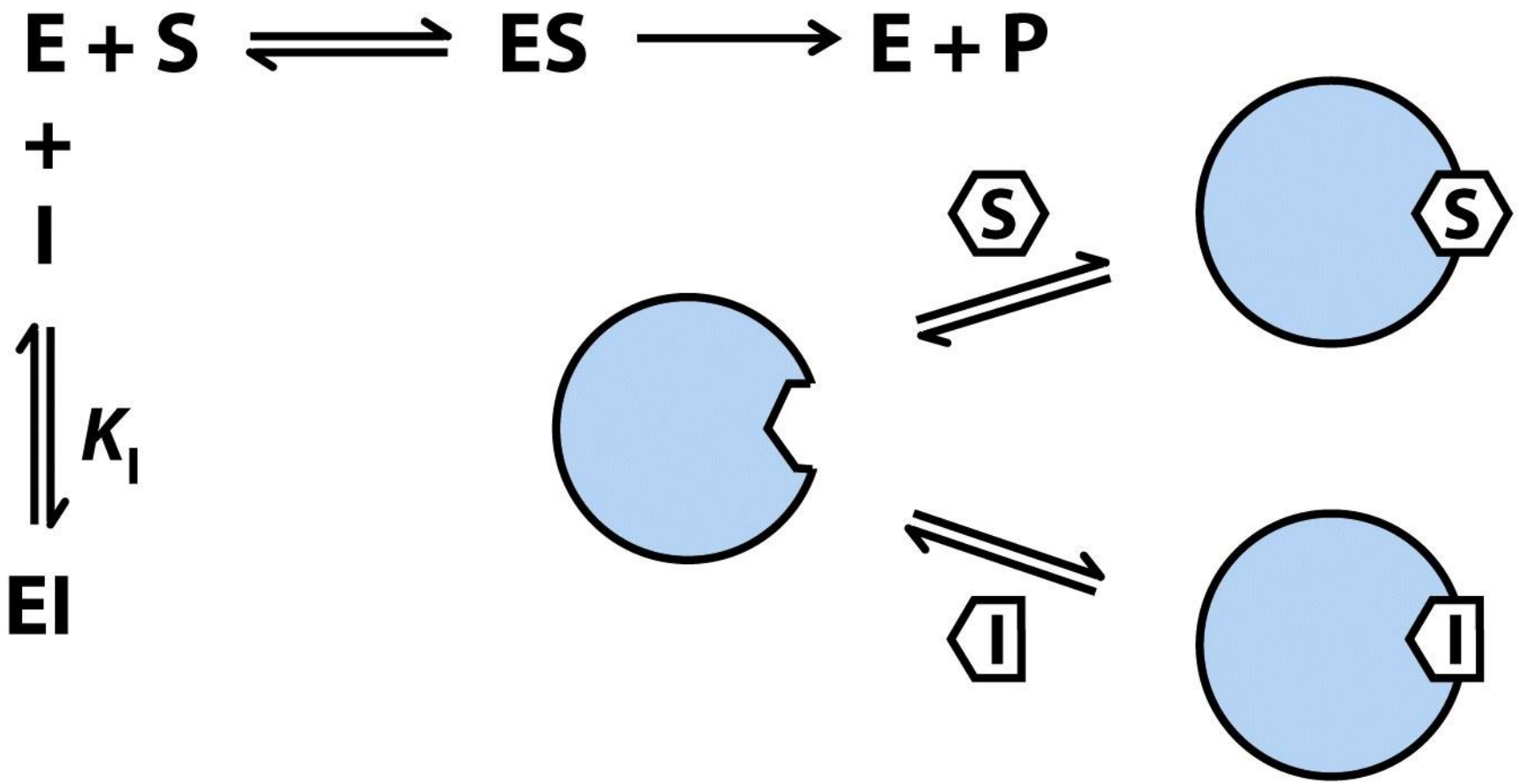
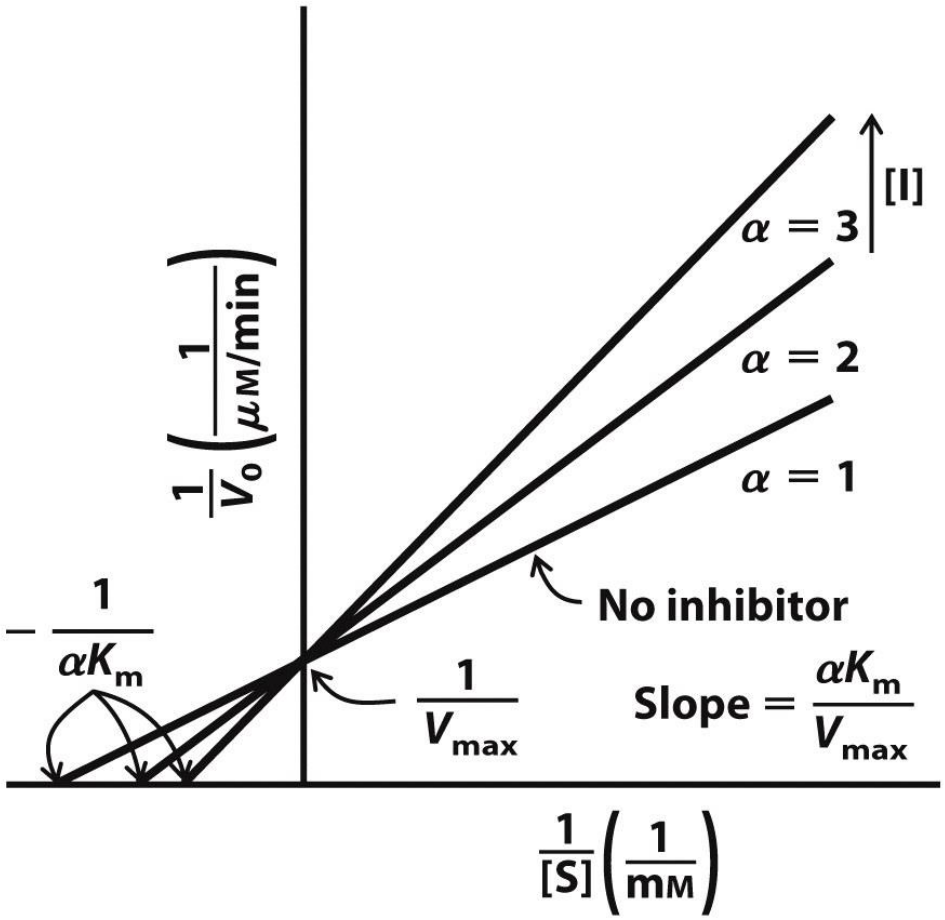


Figure 6-15a
Lehninger Principles of Biochemistry, Fifth Edition

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

$$V_0 = \frac{V_{max}\,[S]}{\alpha K_m + [S]}$$



Box 6-2 figure 1
Lehninger Principles of Biochemistry, Fifth Edition

The term α is greater than 1.0 when a competitive inhibitor is present

Competitive inhibitors bind to the enzyme's active site; K_I is the equilibrium constant for inhibitor binding to E.

Lines intersect at the y-axis

Uncompetitive inhibition

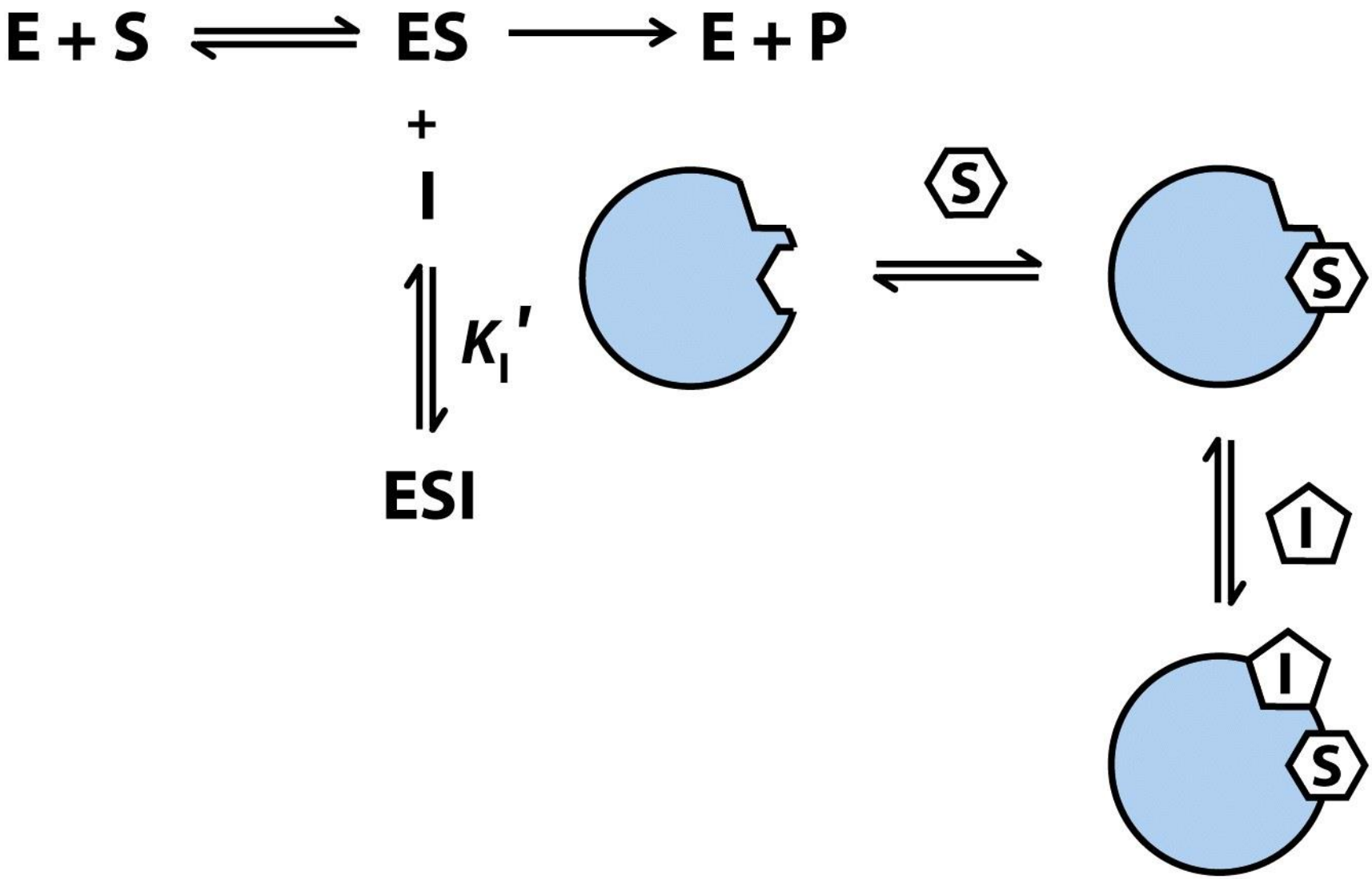


Figure 6-15b
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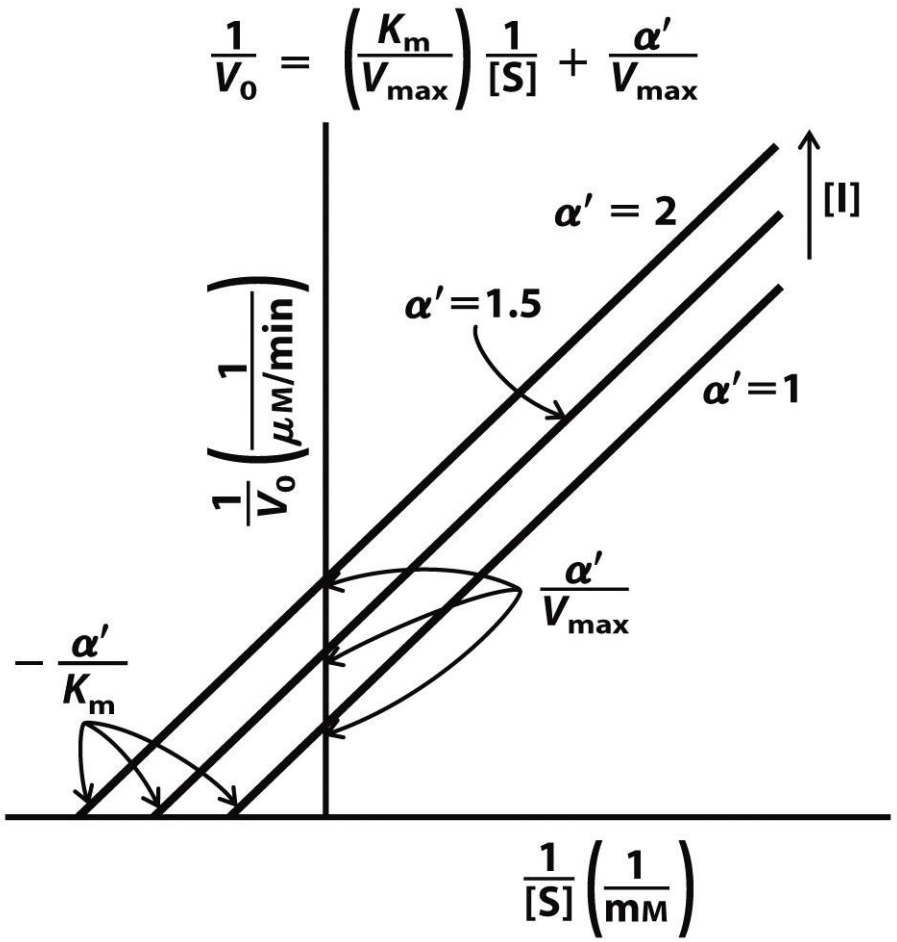


Box 6-2 figure 2
Lehninger Principles of Biochemistry, Fifth Edition

$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]}$$

Uncompetitive inhibitors bind at a separate site, but bind only to the ES complex; K_I' is the equilibrium constant for inhibitor binding to ES.

Lines are parallel

Mixed inhibition

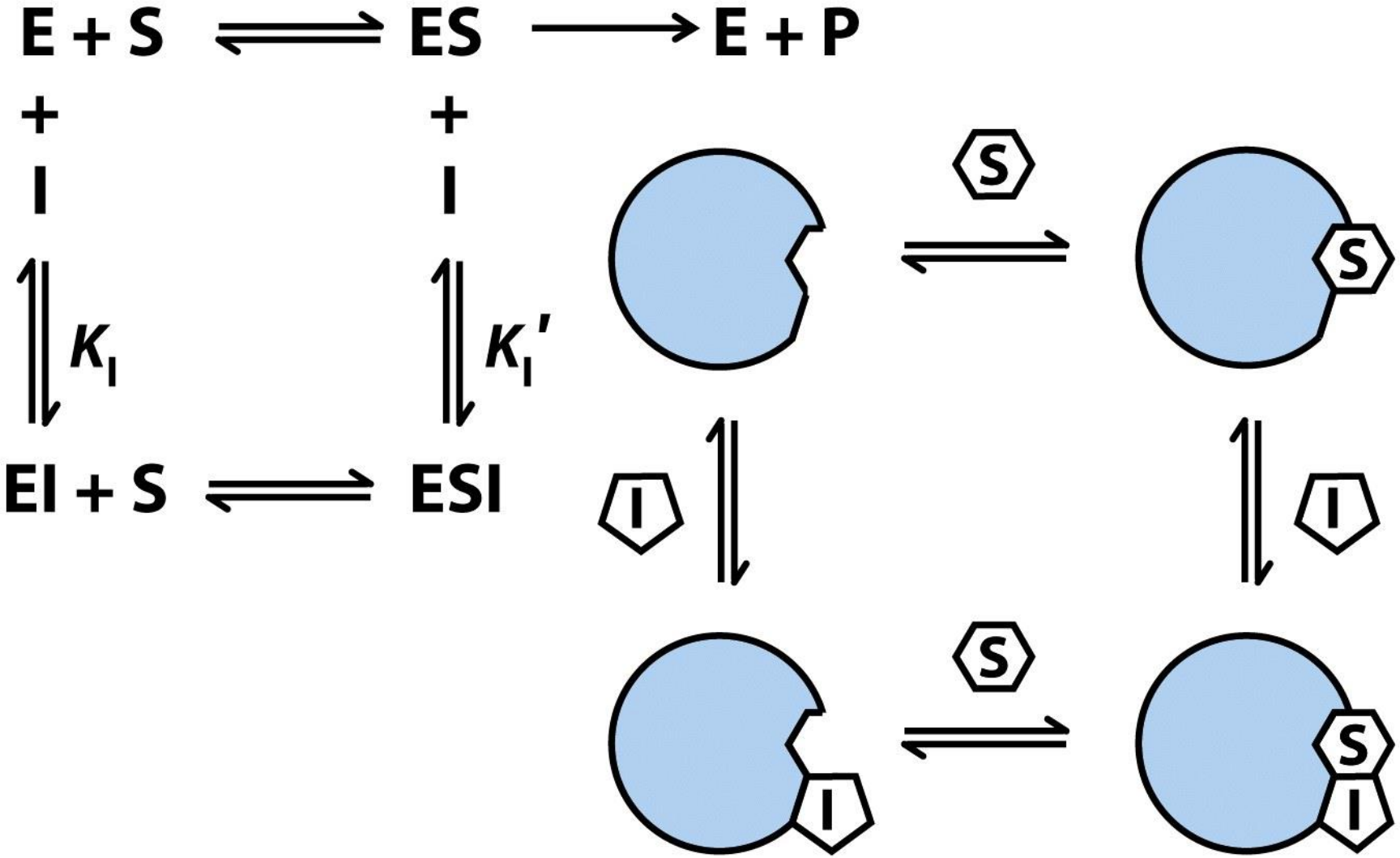
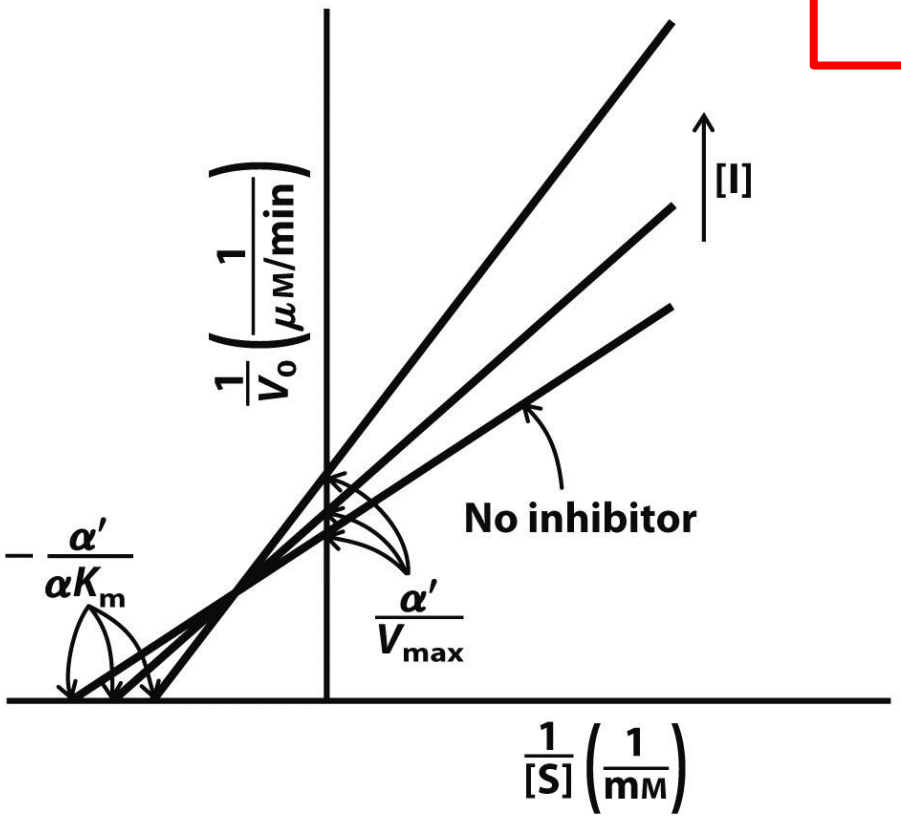


Figure 6-15c
Lehninger Principles of Biochemistry, Fifth Edition

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

$$V_0 = \frac{V_{max}\,[S]}{\alpha K_m + \alpha'[S]}$$



Box 6-2 figure 3
Lehninger Principles of Biochemistry, Fifth Edition

Mixed inhibitors bind at a separate site, but may bind to either E or ES.

Lines intersect left from the y-axis

*****

TABLE 6–9 Effects of Reversible Inhibitors on Apparent V_{max} and Apparent K_m

Inhibitor type	Apparent V_{max}	Apparent K_m
None	V_{max}	K_m
Competitive	V_{max}	αK_m ↑
Uncompetitive	V_{max}/α' ↓	K_m/α' ↓
Mixed	V_{max}/α' ↓	$\alpha K_m/\alpha'$

Table 6-9
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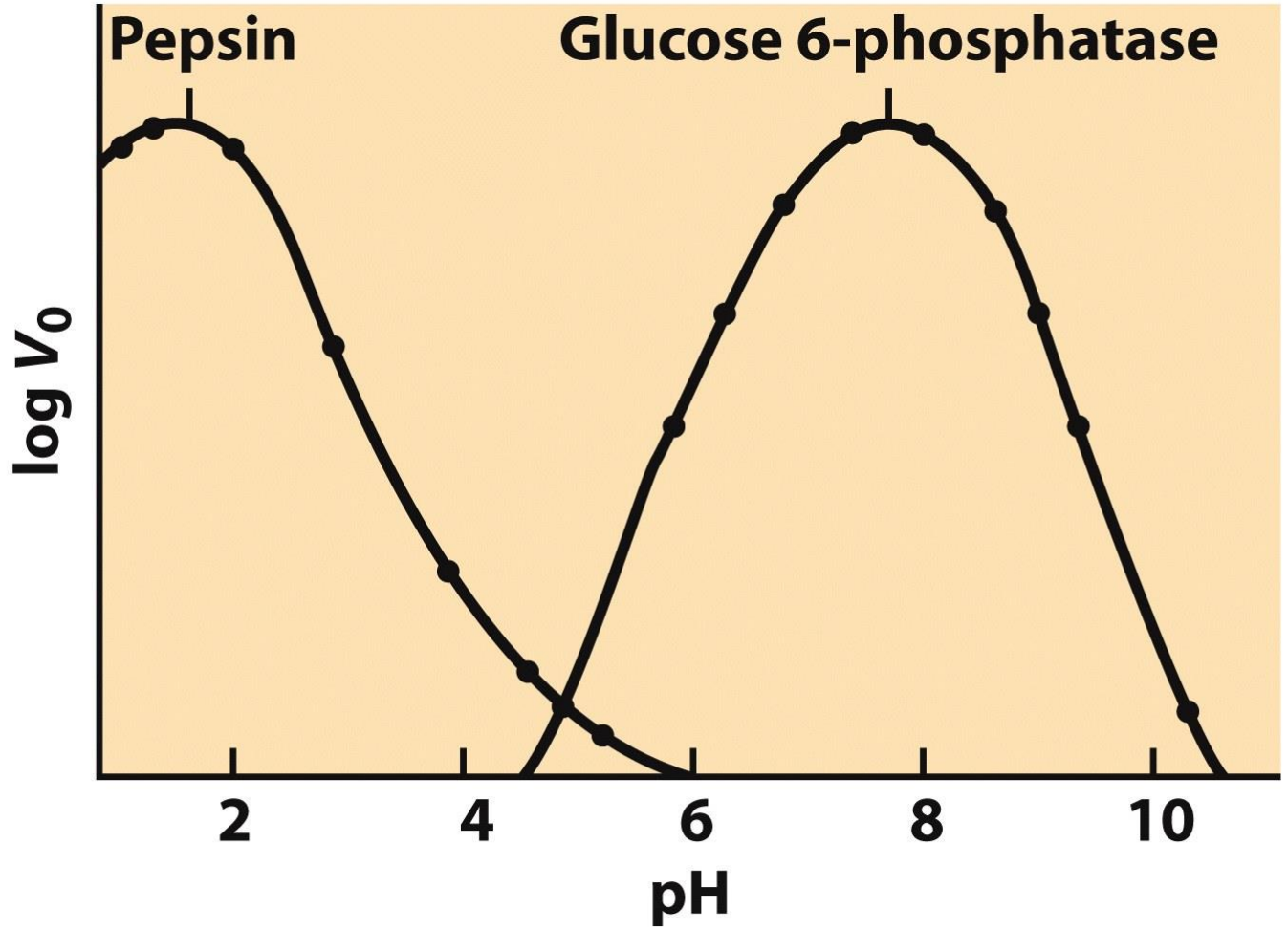


Figure 6-17
Lehninger Principles of Biochemistry, Fifth Edition

The pH-activity profiles of two enzymes

The pH optimum for the activity of an enzyme is generally close to the pH of the environment in which the enzyme is normally found.

4. Examples of enzymatic reactions

Specific catalytic groups contribute to catalysis

- **acid-base catalysis: give and take protons**
- **covalent catalysis: a transient covalent bond is formed between the enzyme and the substrate**
- **metal ion catalysis: metal ion participate in the catalysis**

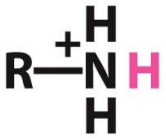
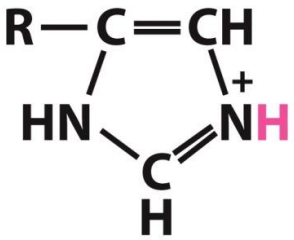
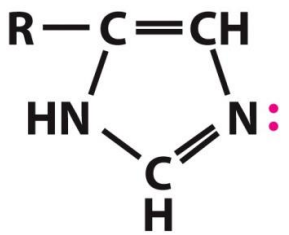
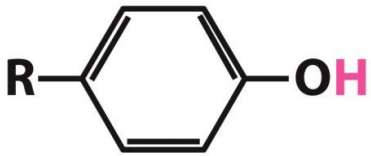
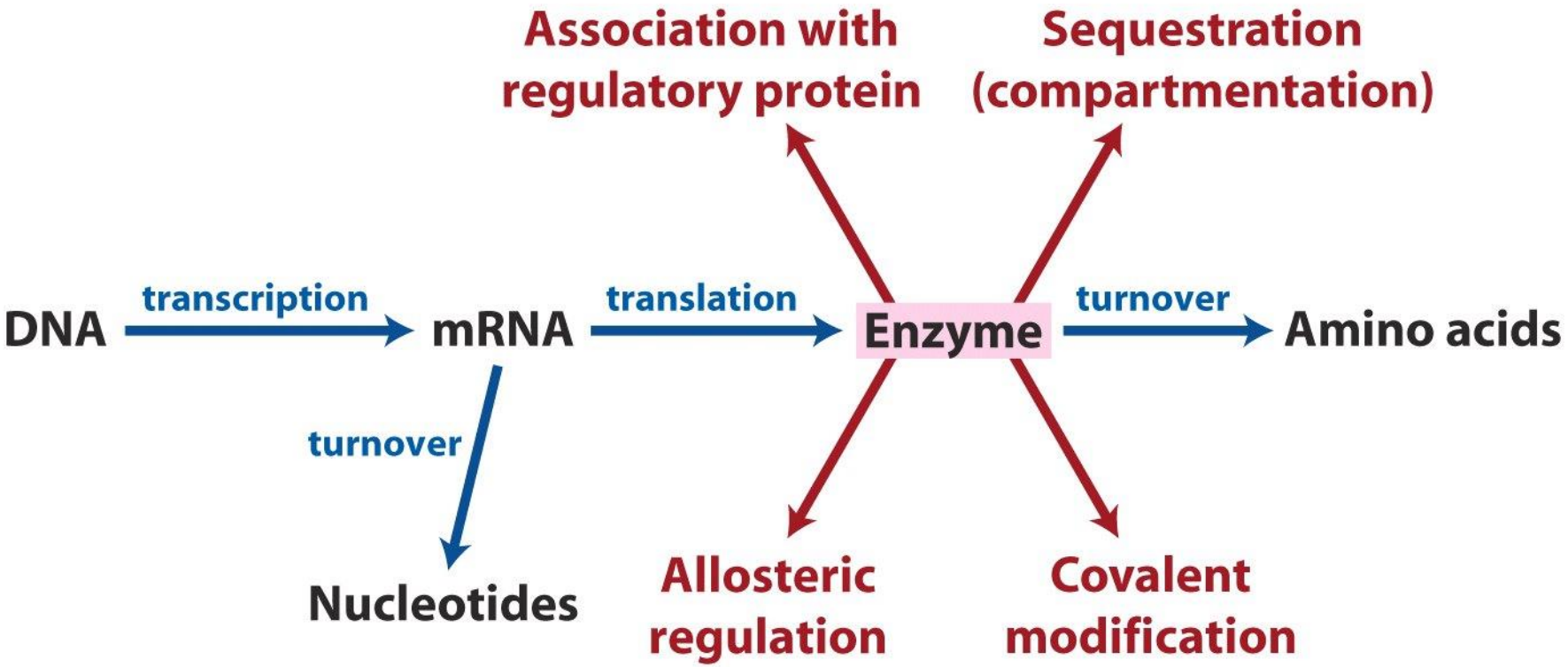
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp		
Lys, Arg		
Cys		
His		
Ser		
Tyr		

Figure 6-9
Lehninger Principles of Biochemistry, Sixth Edition

Amino acids in general acid-base catalysis

5. Regulatory enzymes

- **Exhibit increased or decreased catalytic activity in response to certain signals**
- **The first enzyme in most multienzyme systems**
- **Multisubunit proteins, with active site and regulatory sites on separate subunits**
- **Allosteric regulation by reversible, noncovalent binding of regulatory compounds called allosteric modulators or allosteric effectors**
- **Covalent regulation: reversible, covalent modification of one or more amino acids in enzyme**
- **Regulated steps are catalyzed by allosteric enzymes in many pathways**



Factors that determine the activity of an enzyme

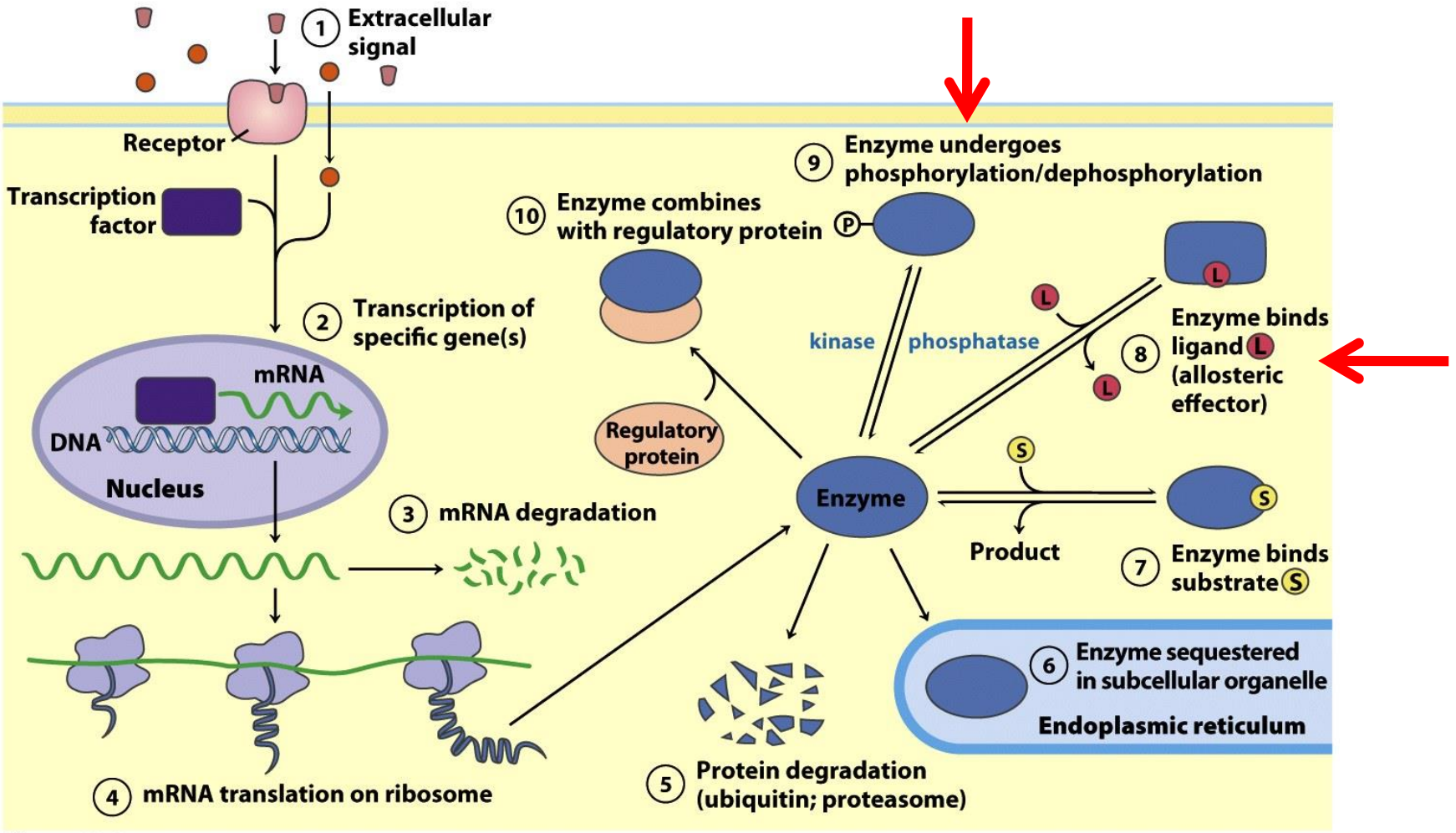


Figure 15-2
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Factors that determine the activity of an enzyme

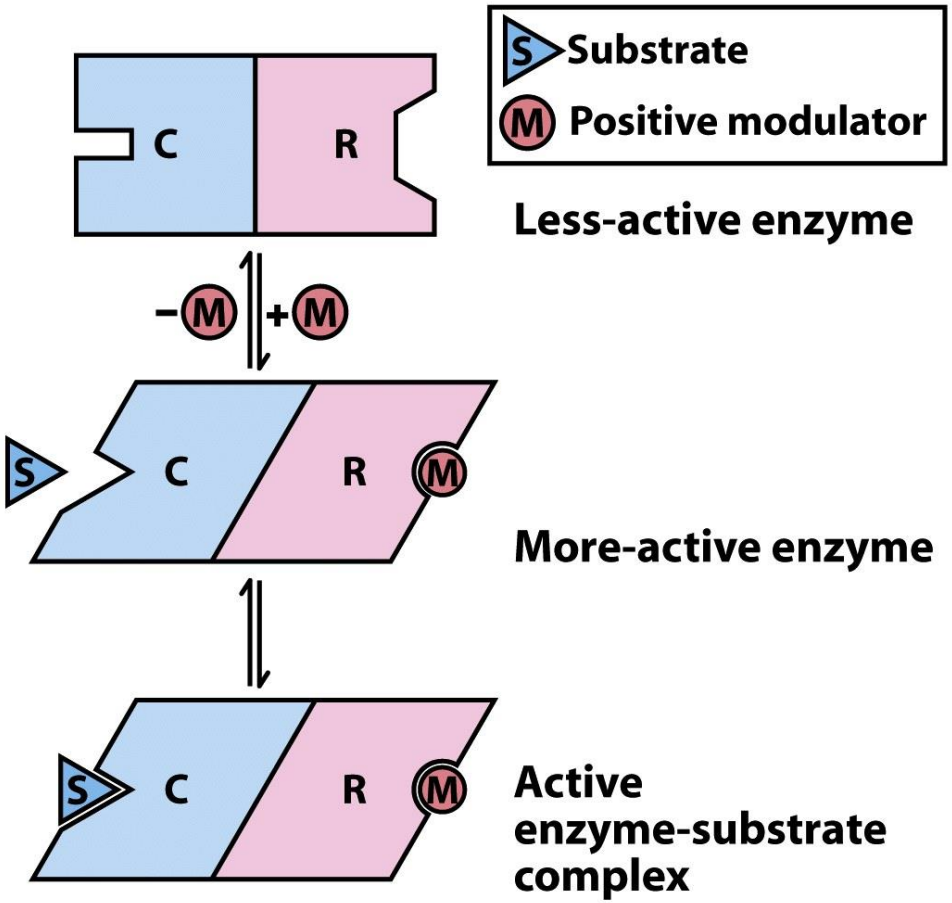

Figure 6-31
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Subunit interactions in an allosteric enzyme, and interactions with inhibitors and activators

In many allosteric enzymes the substrate binding site and the modulator binding site(s) are on different subunits, the catalytic (C) and regulatory (R) subunits, respectively. Binding of the positive (stimulatory) modulator (M) to its specific site on the regulatory subunit is communicated to the catalytic subunit through a conformational change. This change renders the catalytic subunit active and capable of binding the substrate (S) with higher affinity. On dissociation of the modulator from the regulatory subunit, the enzyme reverts to its inactive or less active form.

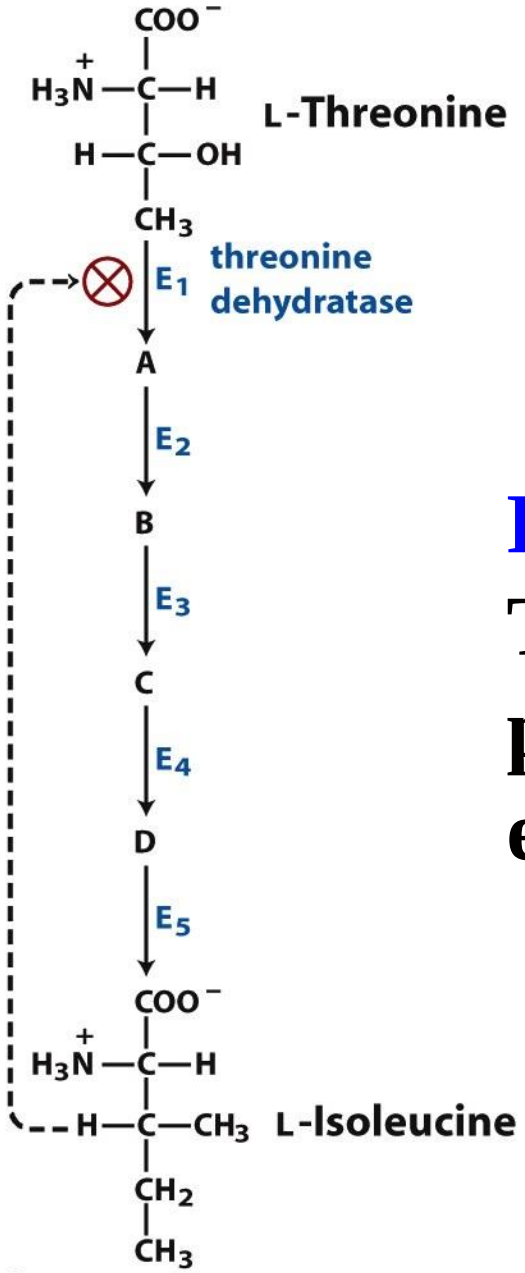

Figure 6-33
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Feedback inhibition:

The end product of a pathway inhibits the first enzyme of the pathway

Allosteric enzymes do not obey Michaelis-Menten kinetics

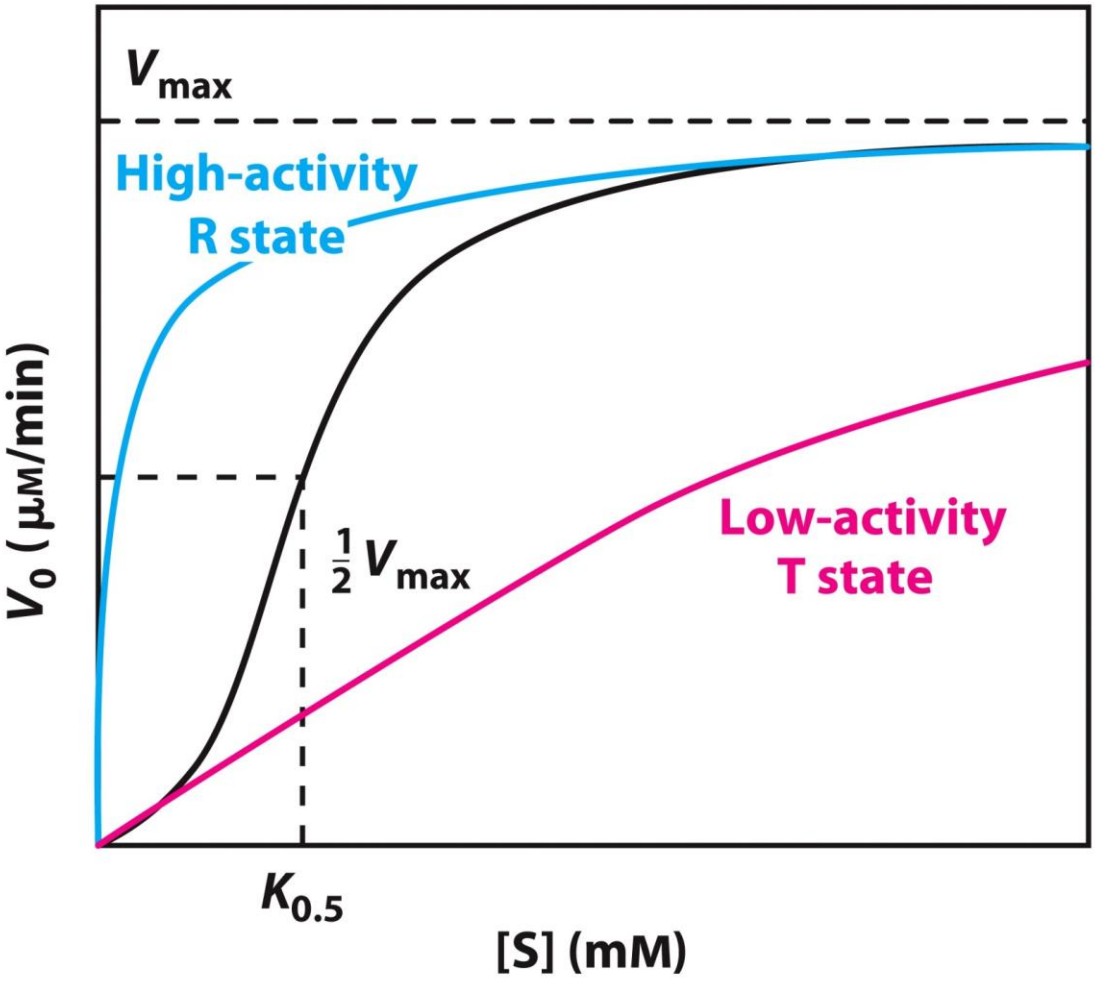

Figure 6-34a
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A homotropic enzyme: the substrate also serves as a positive (stimulatory) modulator, or activator

Substrate-activity curves for representative allosteric enzymes

Allosteric enzymes display a sigmoidal dependence of reaction velocity on substrate concentration.

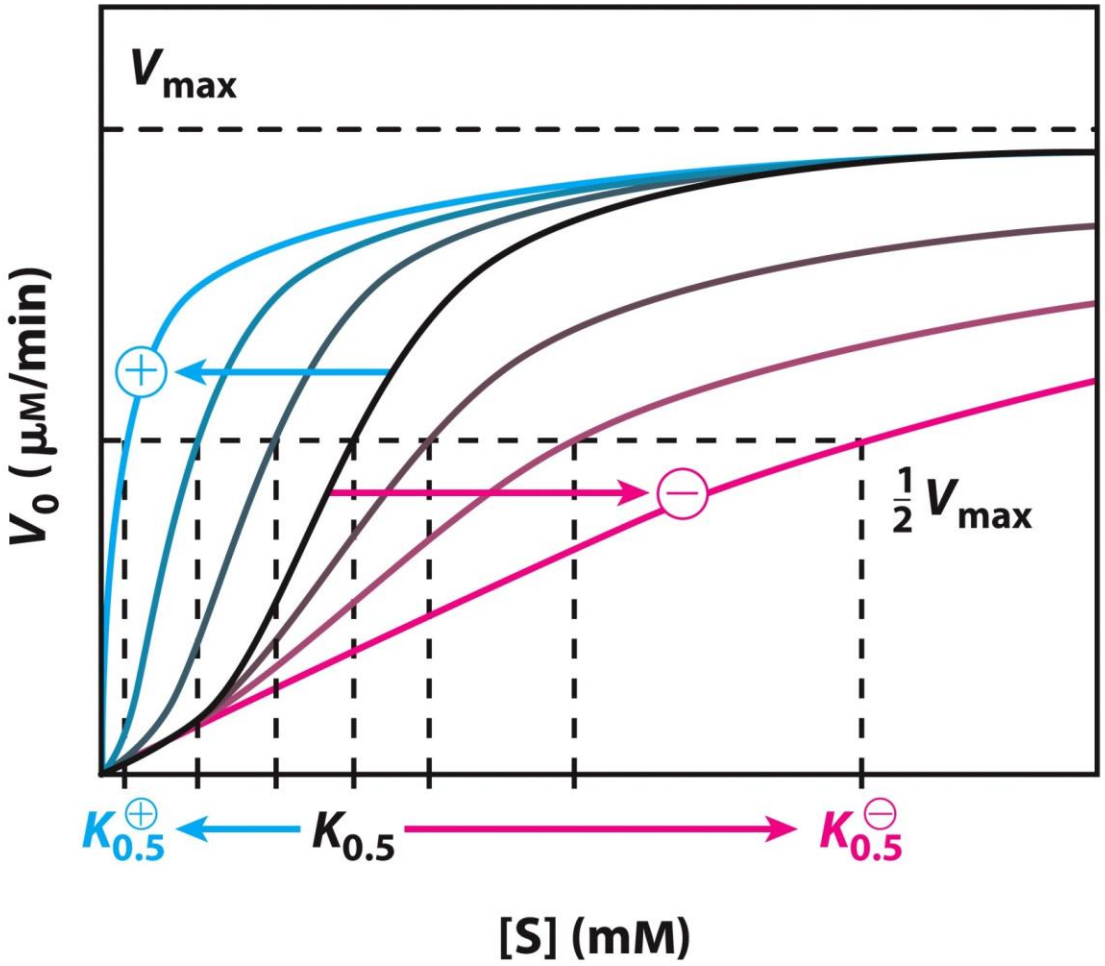

Figure 6-34b
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The kinetic behavior of allosteric enzymes reflects cooperative interactions among enzyme subunits.

Substrate-activity curves for representative allosteric enzymes

The effects of a positive modulator (+) and a negative modulator (–) on an allosteric enzyme in which $K_{0.5}$ is altered without a change in V_{max}. The central curve shows the substrate-activity relationship without a modulator.

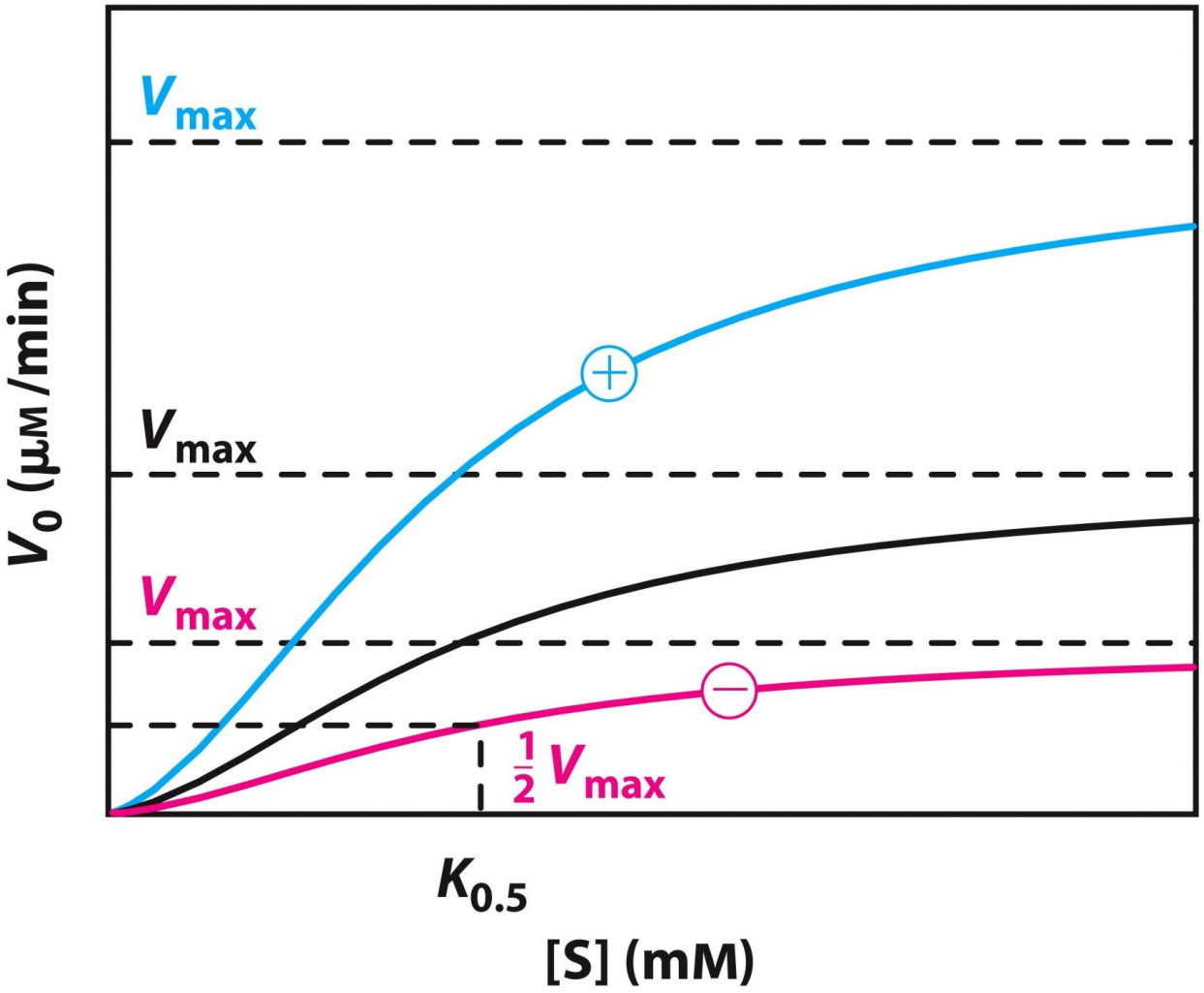

Figure 6-34c
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Substrate-activity curves for representative allosteric enzymes

A less common type of modulation, in which V_{max} is altered and $K_{0.5}$ is nearly constant

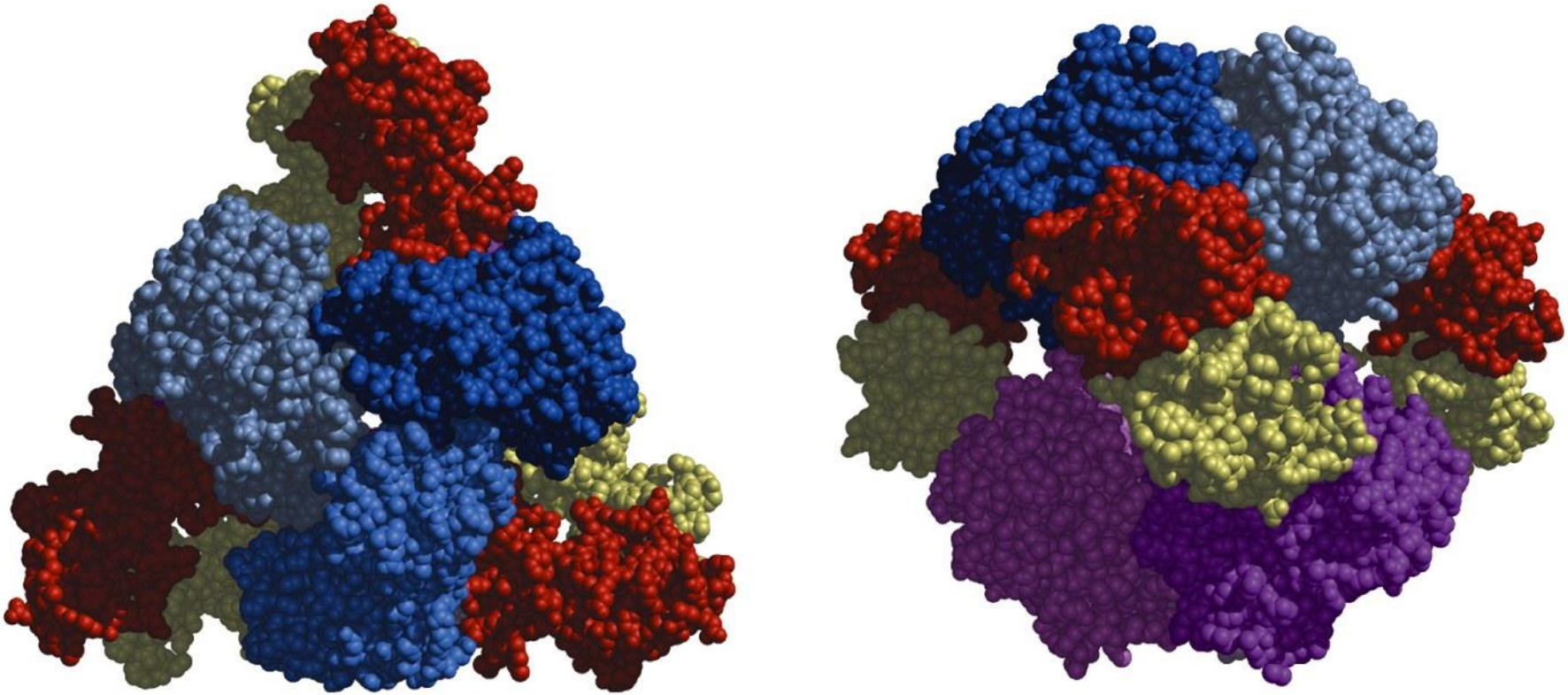

Figure 6-32
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Two views of the regulatory enzyme aspartate transcarbamoylase

This allosteric regulatory enzyme has two stacked catalytic clusters, each with three catalytic polypeptide chains (in shades of blue and purple), and three regulatory clusters, each with two regulatory polypeptide chains (in red and yellow). The regulatory clusters form the points of a triangle surrounding the catalytic subunits. Binding sites for allosteric modulators are on the regulatory subunits. Modulator binding produces large changes in enzyme conformation and activity.

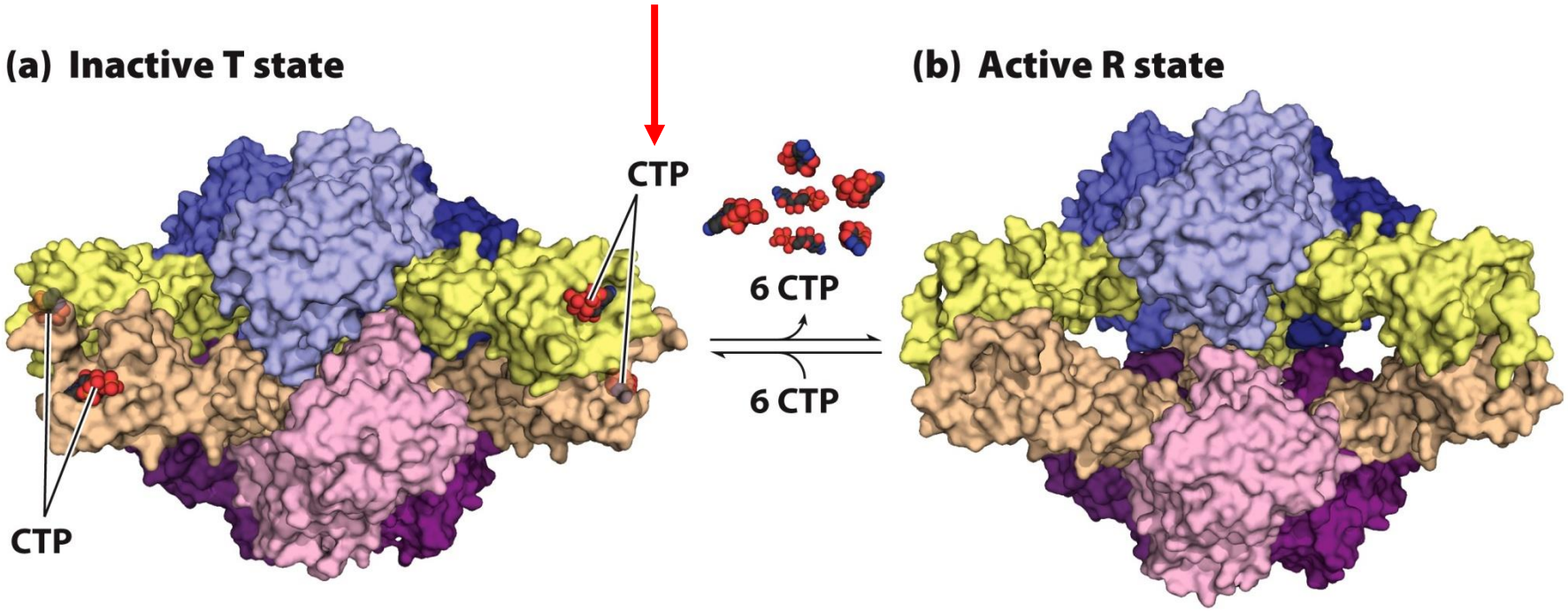

Figure 6-33
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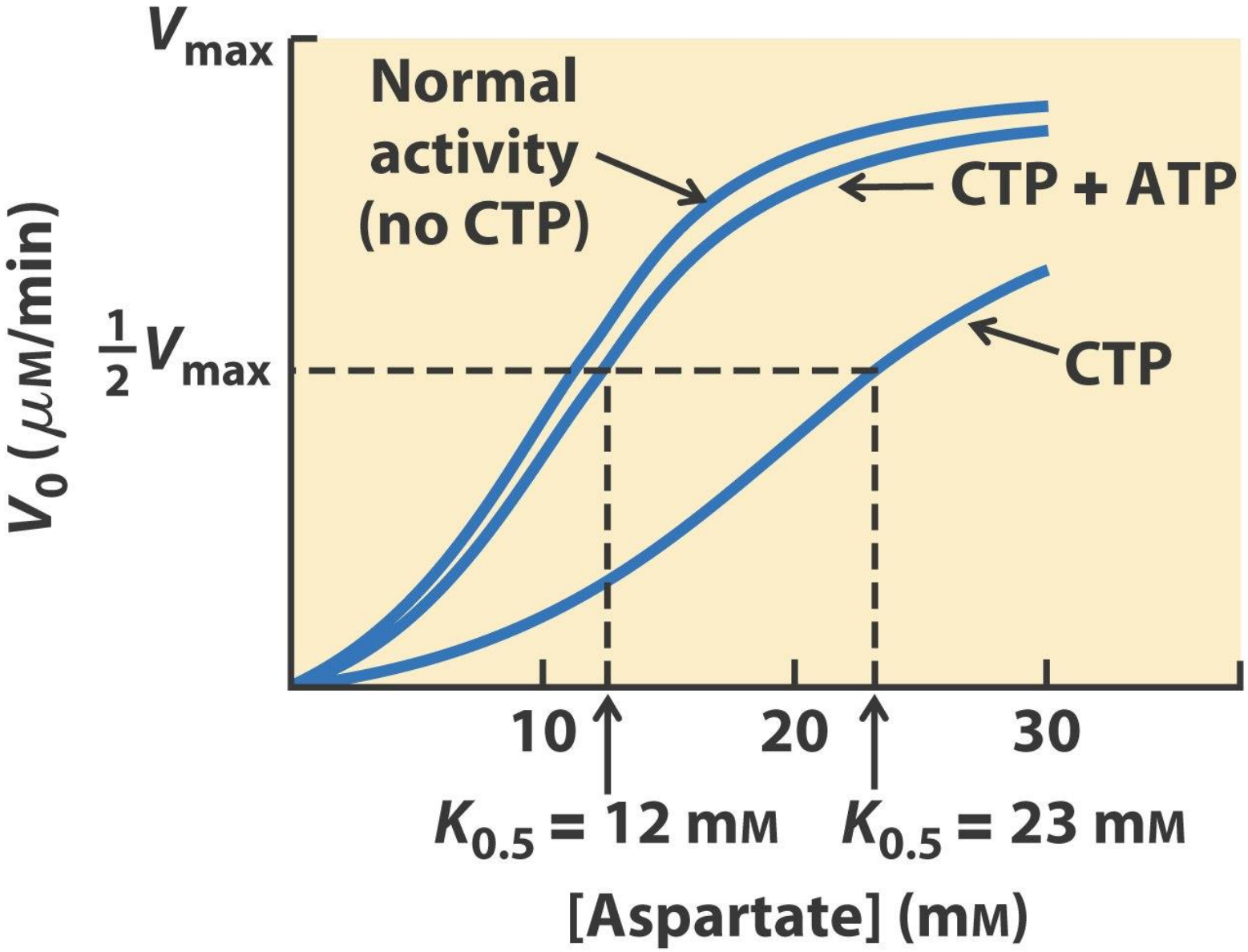
Allosteric regulation of aspartate transcarbamoylase by CTP



Allosteric regulation of aspartate transcarbamoylase by CTP and ATP

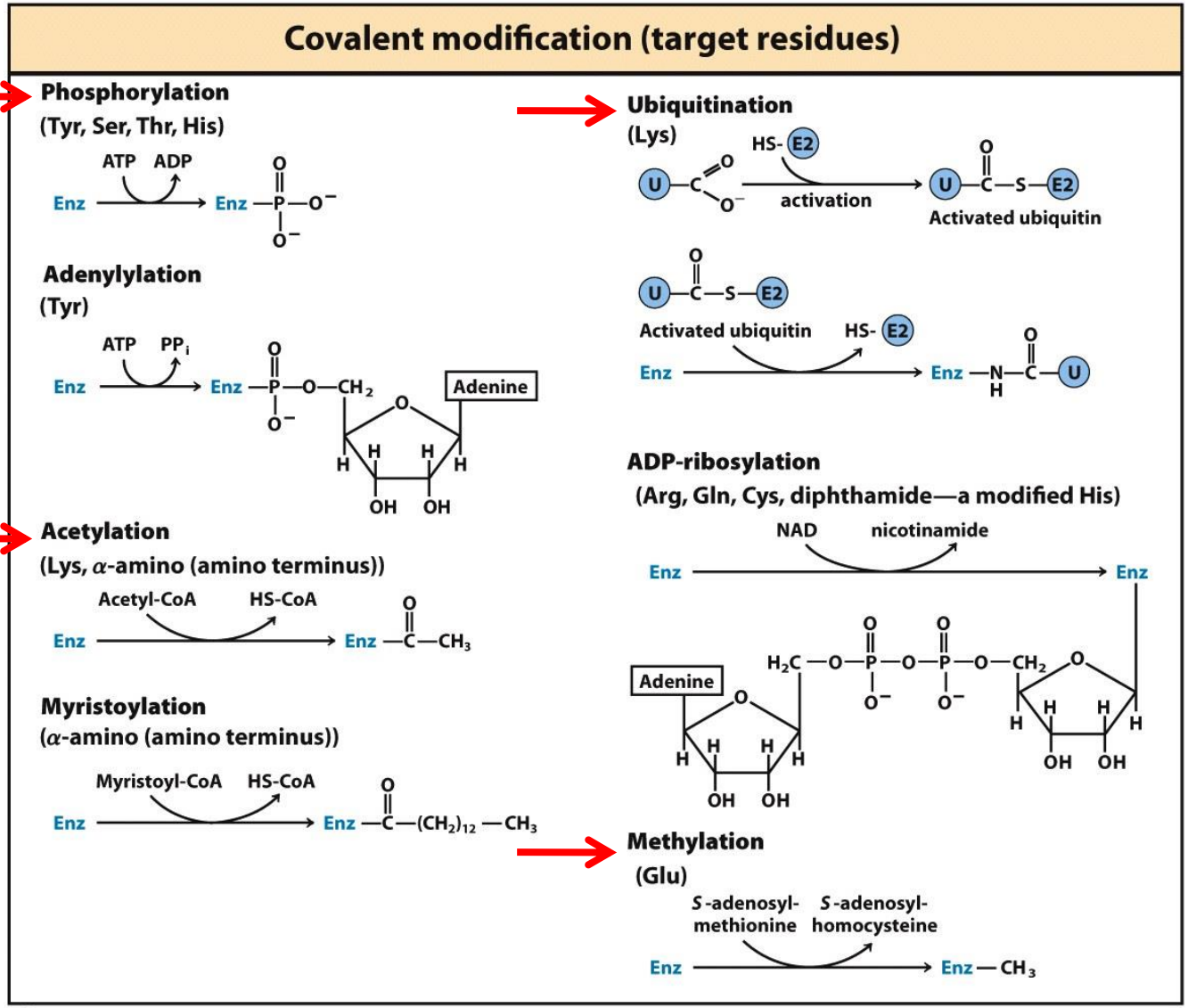


Figure 6-35

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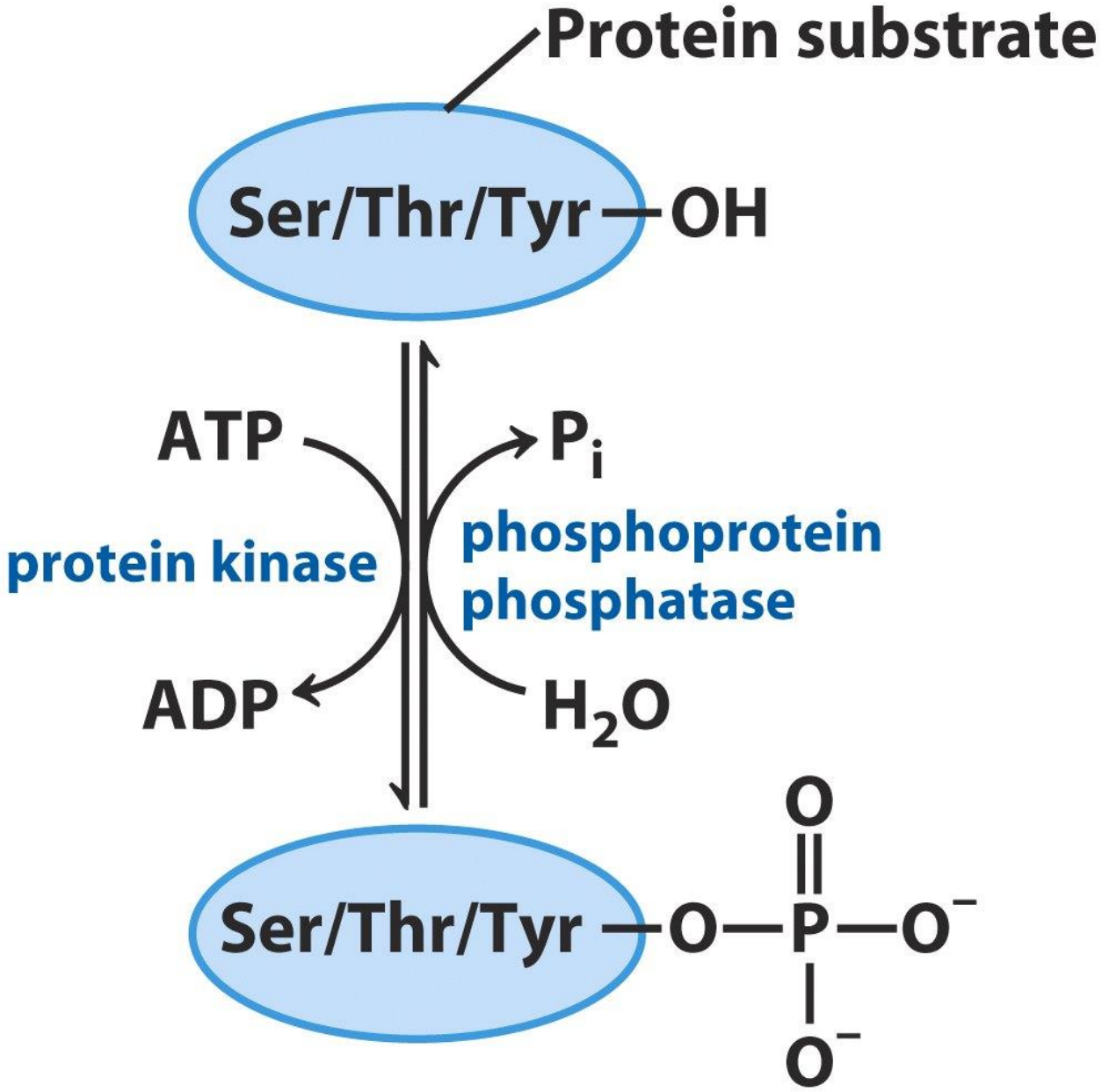
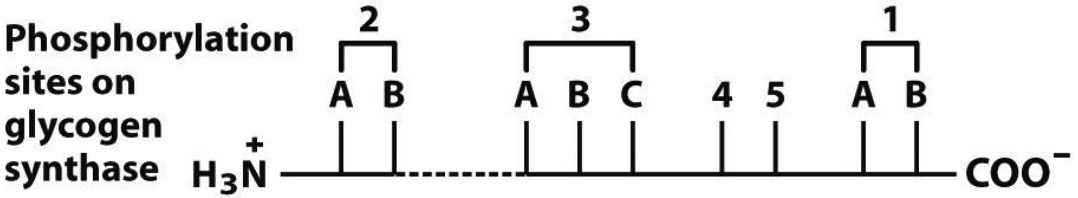
Some enzyme modification reactions



Protein phosphorylation and dephosphorylation



Kinase	Phosphorylation sites	Degree of synthase inactivation
Protein kinase A	1A, 1B, 2, 4	+
Protein kinase G	1A, 1B, 2	+
Protein kinase C	1A	+
Ca^{2+}/calmodulin kinase	1B, 2	+
Phosphorylase *b* kinase	2	+
Casein kinase I	At least nine	+ + + +
Casein kinase II	5	0
Glycogen synthase kinase 3	3A, 3B, 3C	+ + +
Glycogen synthase kinase 4	2	+

Figure 6-37
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Multiple regulatory phosphorylations of glycogen synthase

TABLE 6–10 Consensus Sequences for Protein Kinases

Protein kinase	Consensus sequence and phosphorylated residue*
Protein kinase A	-x-R-[RK]-x-[ST]-B-
Protein kinase G	-x-R-[RK]-x-[ST]-x-
Protein kinase C	-[RK](2)-x-[ST]-B-[RK](2)-
Protein kinase B	-x-R-x-[ST]-x-K-
Ca^{2+}/calmodulin kinase I	-B-x-R-x(2)-[ST]-x(3)-B-
Ca^{2+}/calmodulin kinase II	-B-x-[RK]-x(2)-[ST]-x(2)-
Myosin light chain kinase (smooth muscle)	-K(2)-R-x(2)-S-x-B(2)-
Phosphorylase *b* kinase	*-K-R-K-Q-I-S-V-R-*
Extracellular signal–regulated kinase (ERK)	-P-x-[ST]-P(2)-
Cyclin-dependent protein kinase (cdc2)	-x-[ST]-P-x-[KR]-
Casein kinase I	-[SpTp]-x(2)-[ST]-B†
Casein kinase II	-x-[ST]-x(2)-[ED]-x-
β-Adrenergic receptor kinase	-[DE](*n*)-[ST]-x(3)
Rhodopsin kinase	-x(2)-[ST]-E(*n*)-
Insulin receptor kinase	-x-E(3)-Y-M(4)-*K(2)-S-R-G-D-Y-M-T-M-Q-I-G-K(3)-L-P-A-T-G-D-Y-M-N-M-S-P-V-G-D-*
Epidermal growth factor (EGF) receptor kinase	*-E(4)-Y-F-E-L-V-*

Sources: Pinna, L.A. & Ruzzene, M.H. (1996) How do protein kinases recognize their substrates? *Biochim. Biophys. Acta* **1314**, 191–225; Kemp, B.E. & Pearson, R.B. (1990) Protein kinase recognition sequence motifs. *Trends Biochem. Sci.* **15**, 342–346; Kennelly, P.J. & Krebs, E.G. (1991) Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. *J. Biol. Chem.* **266**, 15,555–15,558.

*Shown here are deduced consensus sequences (in roman type) and actual sequences from known substrates (italic). The Ser (S), Thr (T), or Tyr (Y) residue that undergoes phosphorylation is in red; all amino acid residues are shown as their one-letter abbreviations (see Table 3–1). x represents any amino acid. B, any hydrophobic amino acid. Sp, Tp, and Yp are Ser, Thr, and Tyr residues that must already be phosphorylated for the kinase to recognize the site.

†The best target site has two amino acid residues separating the phosphorylated and target Ser/Thr residues; target sites with one or three intervening residues function at a reduced level.

Table 6-10

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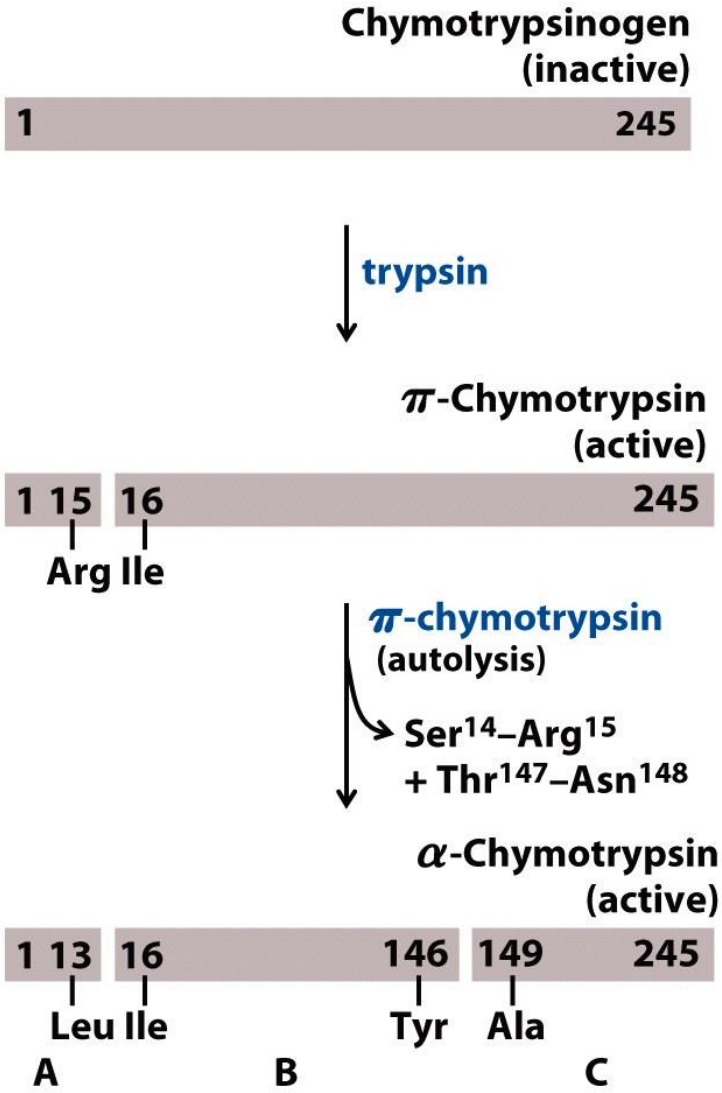

Figure 6-38
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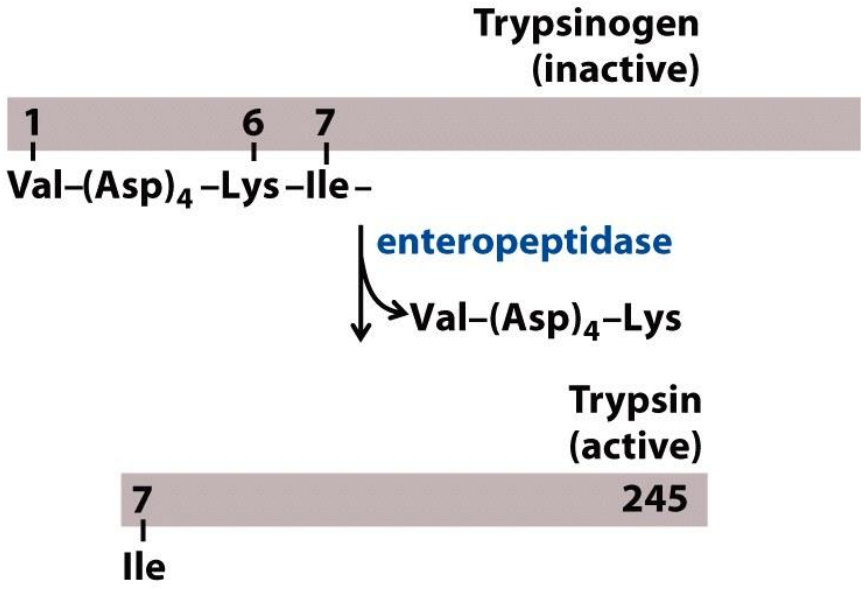

Many proteolytic enzymes are synthesized as inactive precursors called zymogens, which are activated by cleavage of small peptide fragments.

Activation of zymogens by proteolytic cleavage

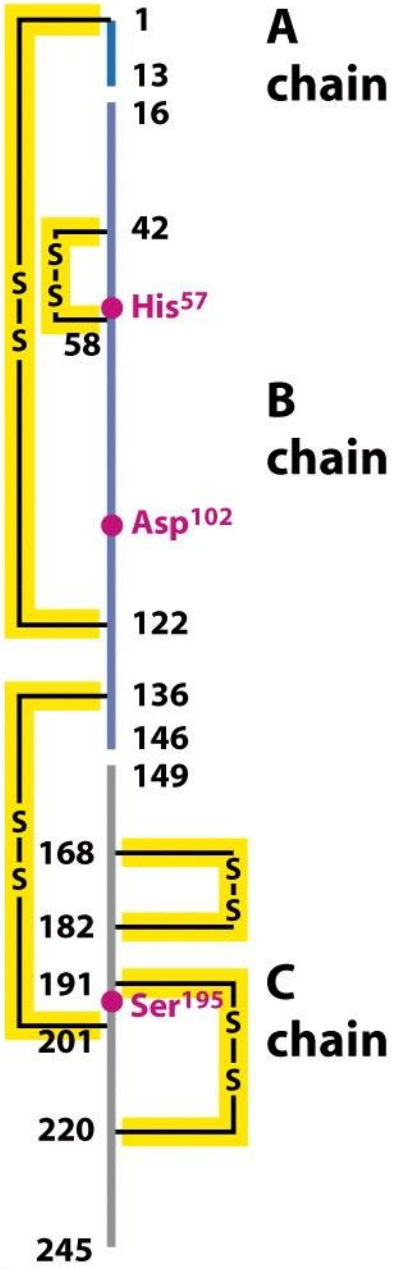

Figure 6-18a
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A representation of primary structure of chymotrypsin (a serine protease), showing disulfide bonds and the amino acid residues crucial to catalysis.
Chymotrypsin is specific for peptide bonds adjacent to Trp, Phe and Tyr.

Keywords

- **catalytic capacity**
- **specificity**
- K_m
- V_{max}
- **allosteric regulation**

Words of the week

- **allosteric**
- **covalent**
- **competitive**
- **uncompetitive**

Textbook-reading of the week

- **Summary 6.1**
- **Summary 6.2**
- **Summary 6.3**
- **Summary 6.5**
- **Enzyme kinetics as an approach to understanding mechanism (6.3)**
- **Transformation of the Michaelis-Menten equation: the double-reciprocal plot (Box 6.1)**
- **Kinetic tests for determining inhibition mechanism (Box 6.2)**

Summary

- **Enzymes are highly effective catalysts of biological systems.**
- **The function of enzymes is to lower the activation energy for a reaction and enhance the reaction rate.**
- **Weak binding interactions between the enzyme and the substrate provide a substantial driving force for enzymatic catalysis. The same binding energy also gives an enzyme its specificity.**
- **The Michaelis-Menten equation relates initial velocity to substrate concentration and V_{max} through the Michaelis constant K_m.**
- **Allosteric enzymes undergo conformational changes in response to modulator binding and do not obey Michaelis-Menten kinetics.**