

6 Enzymes

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Learning



Principle 1 (1 of 4)

Enzymes are powerful biological catalysts. Rate accelerations by enzymes are often far greater than those by synthetic or inorganic catalysts. Like all catalysts, enzymes increase reaction rates, lowering reaction activation barriers. Enzymes do not affect the equilibria of reactions.

Principle 2 (1 of 3)

Enzymes exhibit a very high degree of specificity. Each enzyme catalyzes only one chemical reaction, or sometimes a few closely related reactions. Reaction activation barriers are thus lowered selectively.

Principle 3 (1 of 3)

Enzymatic reactions occur in specialized pockets called active sites. These pockets are similar to ligand binding sites, except that a reaction occurs there—the conversion of a substrate, a molecule that is acted on by an enzyme, to a product.

Principle 4 (1 of 8)

Two concepts explain the catalytic power of enzymes. First, enzymes bind most tightly to the transition state of the catalyzed reaction, using binding energy to lower the activation barrier. Second, enzyme active sites are organized by evolution to facilitate multiple mechanisms of chemical catalysis simultaneously.

Principle 5 (1 of 3)

Many enzymes are regulated. Regulatory mechanisms include reversible covalent modification, binding of allosteric modulators, proteolytic activation, noncovalent binding to regulatory proteins, and elaborate regulatory cascades. Enzymes are often subject to multiple methods of regulation, which allows for exquisite control of every chemical process that occurs in a cell.

6.1 An Introduction to Enzymes

Enzymes

- in 1897, Eduard Buchner:
 - demonstrated cell-free yeast extracts could ferment sugar to alcohol
 - showed fermentation was promoted by molecules that continued to function when removed from cells
- the name **enzymes** were given to the molecules detected by Buchner

Principle 1 (2 of 4)

Enzymes are powerful biological catalysts. Rate accelerations by enzymes are often far greater than those by synthetic or inorganic catalysts. Like all catalysts, enzymes increase reaction rates, lowering reaction activation barriers. Enzymes do not affect the equilibria of reactions.

Most Enzymes Are Proteins

- catalytic activity depends on the integrity of the native protein conformation
- molecular weight = ranges from 12,000 to >1 million
- some enzymes require additional chemical components:
 - **cofactor** = 1+ inorganic ions, such as Fe^{2+} , Mg^{2+} , Mn^{2+} , or Zn^{2+}
 - **coenzyme** = complex organic or metalloorganic molecule that act as transient carriers of specific functional groups

Inorganic Ions as Cofactors

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
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Coenzymes as Transient Carriers of Atoms or Functional Groups

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 (vitamin B ₇)
Coenzyme A	Acyl groups	Pantothenic acid (vitamin B ₅) 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, vitamin B ₃)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B ₆)
Tetrahydrofolate	One-carbon groups	Folate (vitamin B ₉)
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B ₁)

Describing Enzymes and Their Additional Chemical Components

- **prosthetic group** = coenzyme or metal ion that is very tightly or covalently bound to the enzyme protein
- **holoenzyme** = complete catalytically active enzyme together with its bound coenzyme and/or metal ions
- **apoenzyme** or **apoprotein** = the protein part of a holoenzyme

Enzymes Are Classified by the Reactions They Catalyze

- enzymes are divided into seven classes, each with subclasses, based on the type of reaction catalyzed
- each enzyme has a four-part Enzyme Commission number (E.C. number) and a systematic name
- most enzymes have trivial names

Table 6-3 International Classification of Enzymes

Class number	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer
3	Hydrolases	Hydrolysis (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
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
7	Translocases	Movement of molecules or ions across membranes or their separation

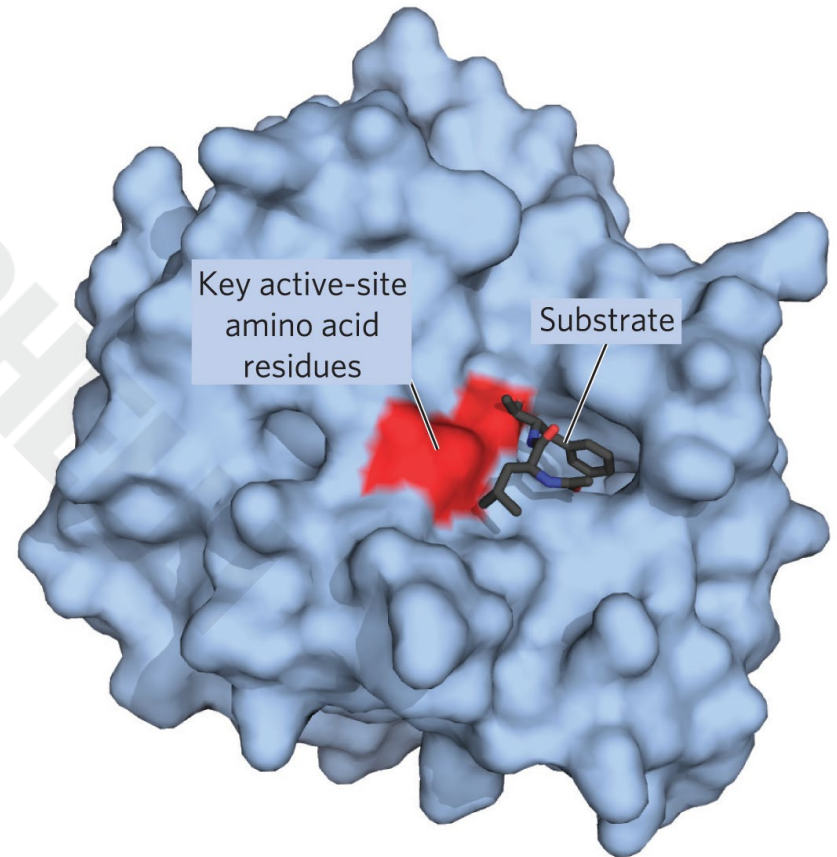
6.2 How Enzymes Work

Principle 3 (2 of 3)

Enzymatic reactions occur in specialized pockets called active sites. These pockets are similar to ligand binding sites, except that a reaction occurs there—the conversion of a substrate, a molecule that is acted on by an enzyme, to a product.

Enzyme-Catalyzed Reactions Take Place within the Active Site

- **active site** = provides a specific environment in which a given reaction can occur more rapidly
- **substrate** = the molecule that is bound to the active site and acted upon by the enzyme



Enzymes Affect Reaction Rates, Not Equilibria

- simple enzymatic reactions can be written as



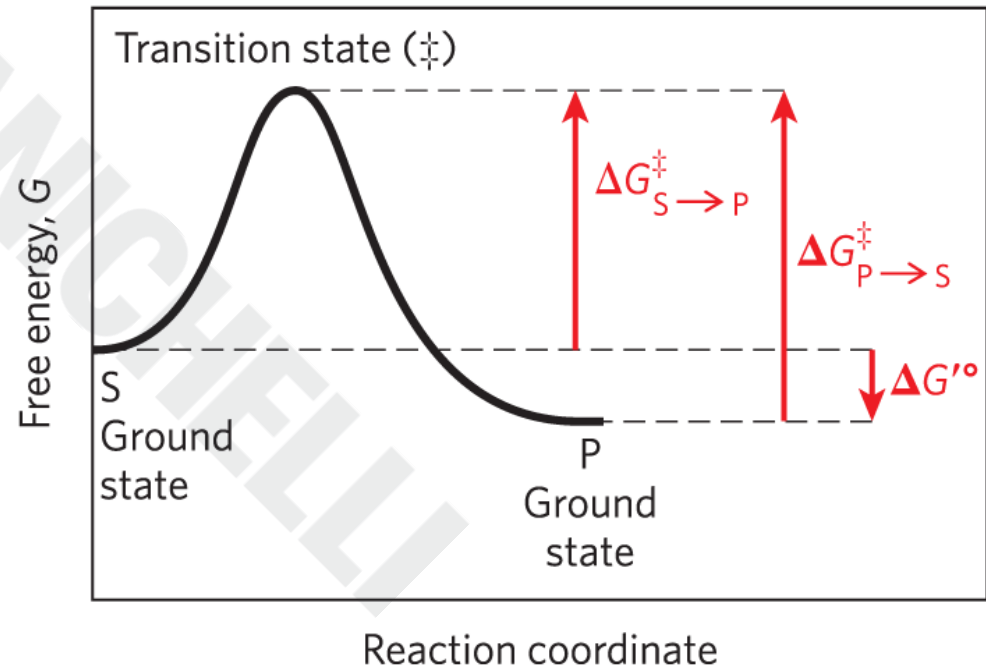
where E, S, and P represent the enzyme, substrate, and product and ES and EP are transient complexes of the enzyme

Principle 1 (3 of 4)

Enzymes are powerful biological catalysts. Rate accelerations by enzymes are often far greater than those by synthetic or inorganic catalysts. Like all catalysts, enzymes increase reaction rates, lowering reaction activation barriers. Enzymes do not affect the equilibria of reactions.

Ground State and Transition State

- **ground state** = starting point for either the forward or reverse reaction
- **transition state** ($\ddagger$) = the point at which decay to substrate or product are equally likely

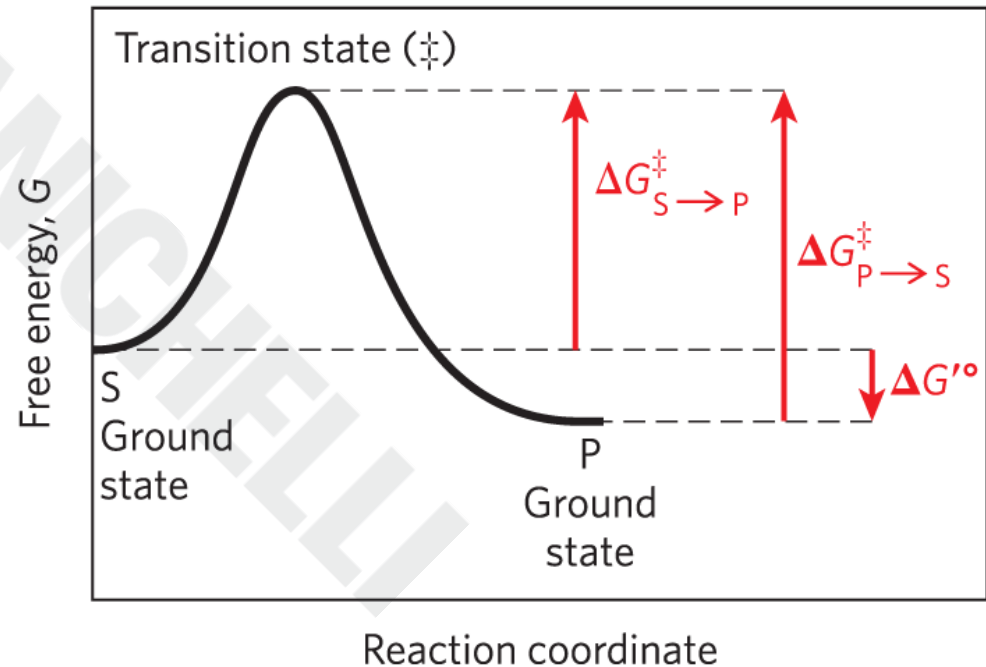


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Energy Changes in a Reaction Coordinate Diagram

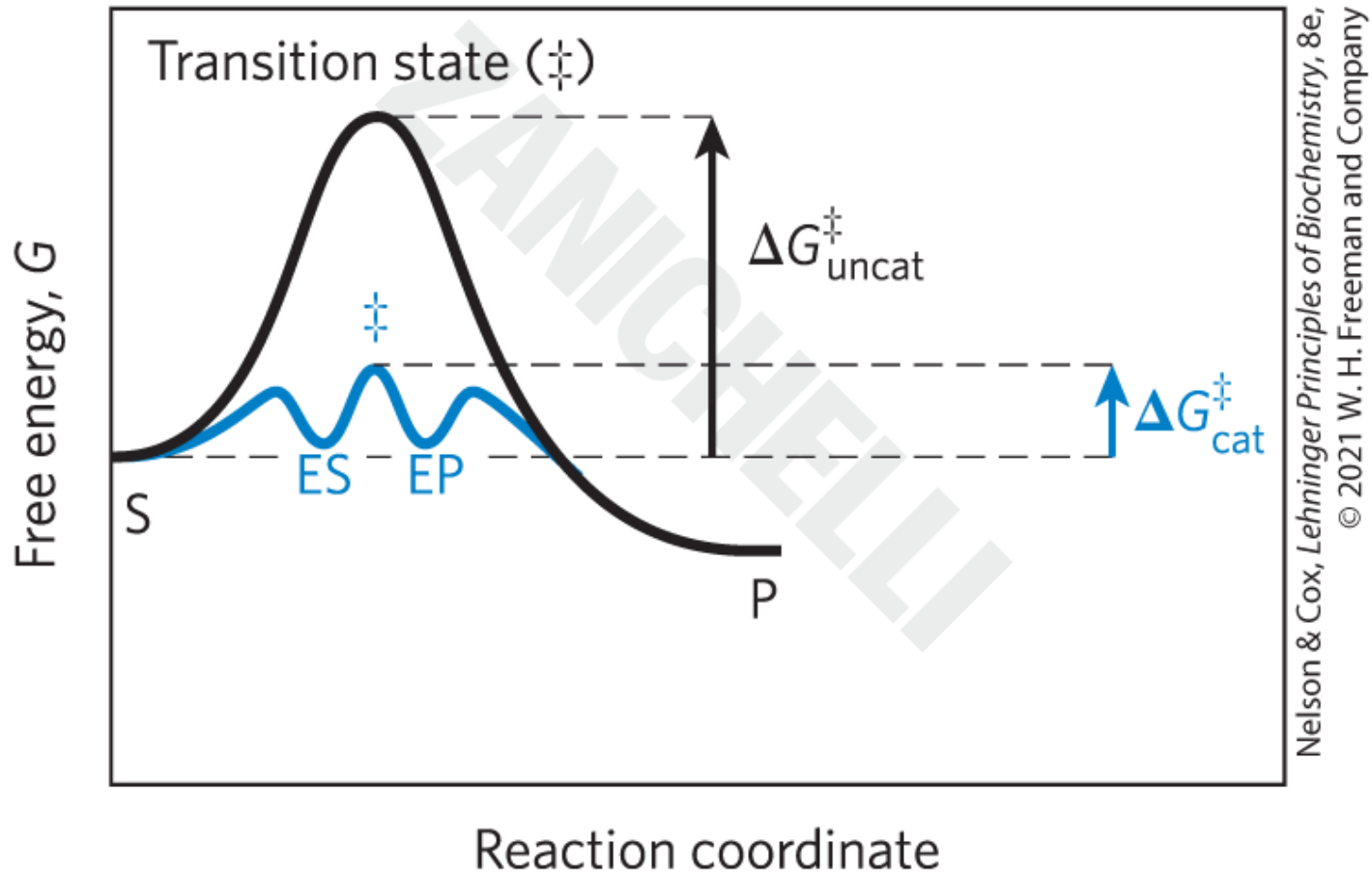
- **biochemical standard free-energy change** = $\Delta G'^{\circ}$ = the standard free-energy change at pH 7.0

- **activation energy** ($\Delta G^{\ddagger}$) = difference between the ground state energy level and the transition state energy level



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Catalysts Lower the Activation Energy and Increase the Reaction Rate

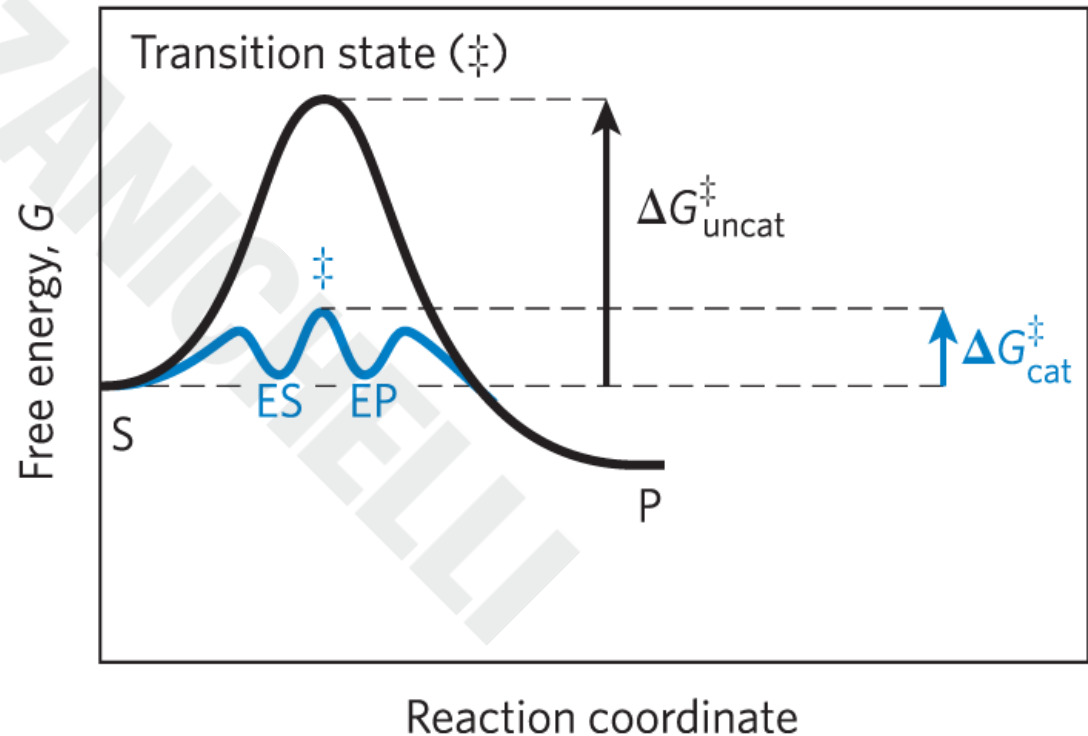


Catalysts Do Not Affect Reaction Equilibria

- any enzyme that catalyzes the reaction $S \rightarrow P$ also catalyzes the reaction $P \rightarrow S$
- enzymes accelerate the interconversion of S and P
- enzymes are not used up in the process
- the equilibrium point is unaffected

Reaction Intermediates

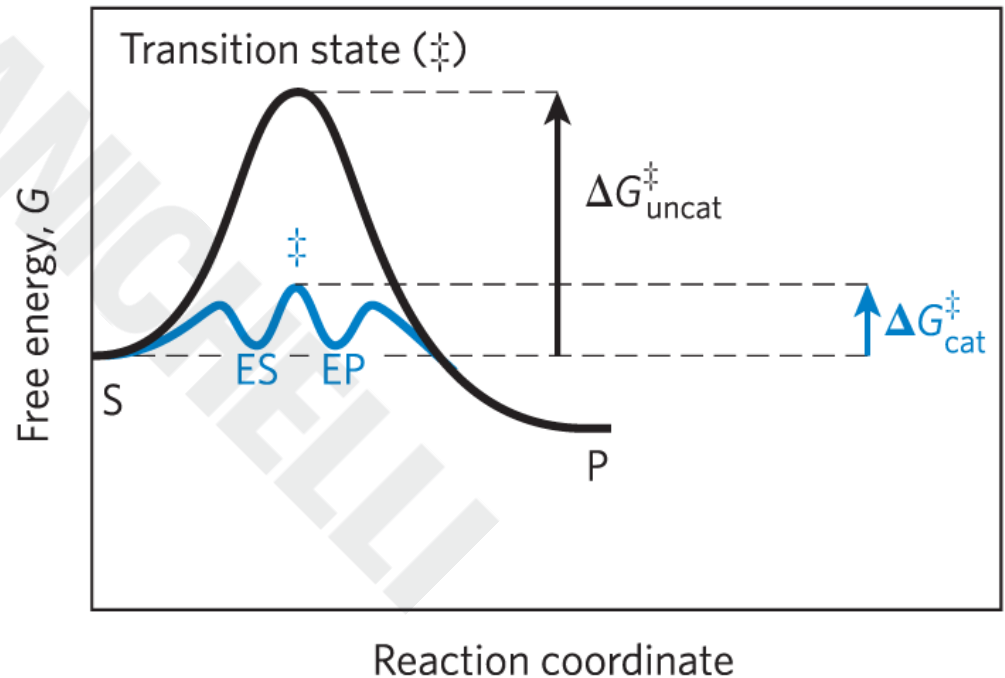
- **reaction intermediate** = any species on the reaction pathway that has a finite chemical lifetime
 - example: ES and EP complexes



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Rate-Limiting Steps

- **rate-limiting step**
= the step in a reaction with the highest activation energy that determines the overall rate of the reaction



- activation energies
are barriers to
chemical reactions

Principle 2 (2 of 3)

Enzymes exhibit a very high degree of specificity. Each enzyme catalyzes only one chemical reaction, or sometimes a few closely related reactions. Reaction activation barriers are thus lowered selectively.

Enzymes Lower Activation Energies Selectively

- enzymes have developed to lower activation energies *selectively* to increase rates for reactions needed for cell survival

Principle 1 (4 of 4)

Enzymes are powerful biological catalysts. Rate accelerations by enzymes are often far greater than those by synthetic or inorganic catalysts. Like all catalysts, enzymes increase reaction rates, lowering reaction activation barriers. Enzymes do not affect the equilibria of reactions.

Reaction Rates and Equilibria Have Precise Thermodynamic Definitions

- reaction *equilibria* are linked to the standard free-energy change for the reaction, $\Delta G'^{\circ}$
- reaction *rates* are linked to the activation energy, $\Delta G^{\ddagger}$

Thermodynamics Relates K'_{eq} and $\Delta G'^{\circ}$

- **equilibrium constant, K'_{eq}** = describes an equilibrium such as $S \rightleftharpoons P$
- under standard conditions:

$$K'_{eq} = \frac{[P]}{[S]} \quad (6-2)$$

- from thermodynamics:

$$\Delta G'^{\circ} = -RT \ln K'_{eq} \quad (6-3)$$

The Relationship between K'_{eq} and $\Delta G'^{\circ}$

Table 6-4 Relationship between K'_{eq} and $\Delta G'^{\circ}$

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^1	-5.7
10^2	-11.4
10^3	-17.1

Rate Constants and Rate Equations

- the rate of any reaction is determined by the concentration of reactant(s) and the **rate constant**, k
- for the unimolar reaction $S \rightarrow P$, a **rate equation** expresses the rate of the reaction

$$V = k[S] \quad (6-4)$$

where V is the velocity or rate of the reaction and $[S]$ is the concentration of the substrate

First-Order Reactions

- first-order reaction = rate depends only on the concentration of S
- k has units of reciprocal time, such as s^{-1}

Second-Order Reactions

- second-order reaction = rate depends on the concentration of two different compounds or the reaction is between two molecules of the same compound
- k has units of $\text{M}^{-1}\text{s}^{-1}$
- the rate equation becomes

$$V = k[S_1][S_2] \quad (6-5)$$

The Relationship between Rate Constants and Activation Energy

- from transition-state theory

$$k = \frac{kT}{h} e^{-\Delta G^\ddagger/RT} \quad (6-6)$$

where k is the Boltzmann constant and h is Planck's constant

- the relationship between the rate constant k and the activation energy $\Delta G^\ddagger$ is inverse and exponential

A Few Principles Explain the Catalytic Power and Specificity of Enzymes

- enzymes enhance rates in the range of 5 to 17 orders of magnitude

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}

Principle 4 (2 of 8)

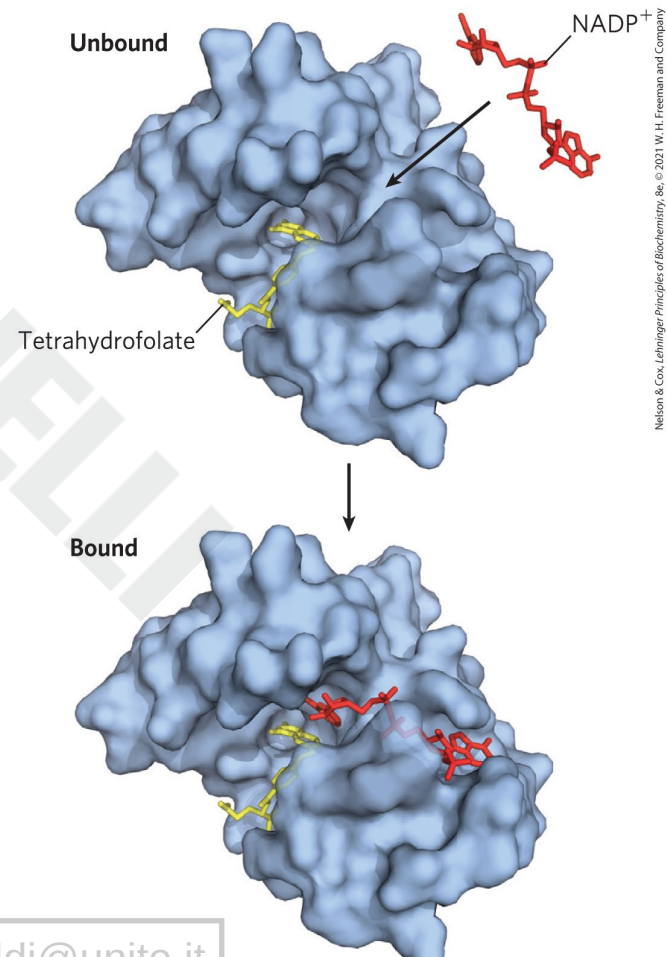
Two concepts explain the catalytic power of enzymes. First, enzymes bind most tightly to the transition state of the catalyzed reaction, using binding energy to lower the activation barrier. Second, enzyme active sites are organized by evolution to facilitate multiple mechanisms of chemical catalysis simultaneously.

Interactions between Enzymes and Substrates

- **binding energy, ΔG_B** = energy derived from noncovalent enzyme-substrate interaction
 - mediated by hydrogen bonds, ionic interactions, and the hydrophobic effect
 - major source of free energy used by enzymes to lower the activation energy
- covalent interactions between enzyme and substrate lower the activation energy

Noncovalent Interactions between Enzyme and Substrate Are Optimized in the Transition State

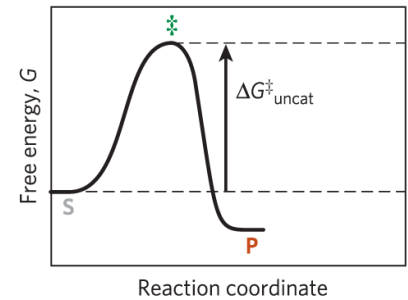
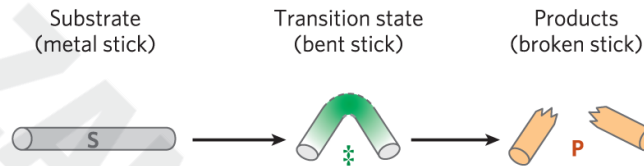
- “lock and key” hypothesis
= enzymes are structurally complementary to their substrates
 - would make for a poor enzyme



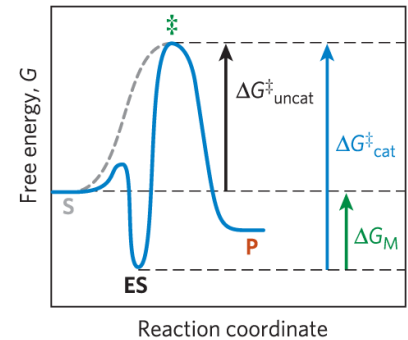
Enzymes Must Be Complementary to the Reaction Transition State

- the full complement of interactions between substrate and enzyme is formed only when the substrate reaches the transition state

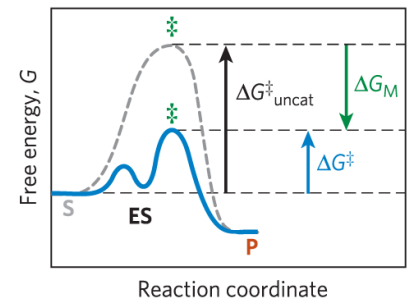
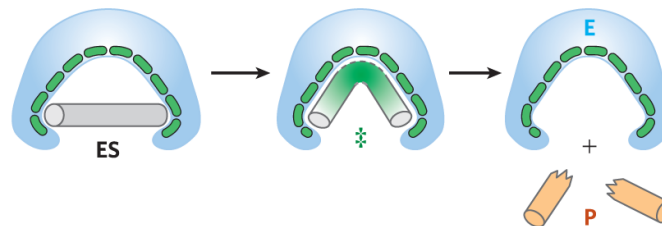
(a) No enzyme



(b) Enzyme complementary to substrate



(c) Enzyme complementary to transition state

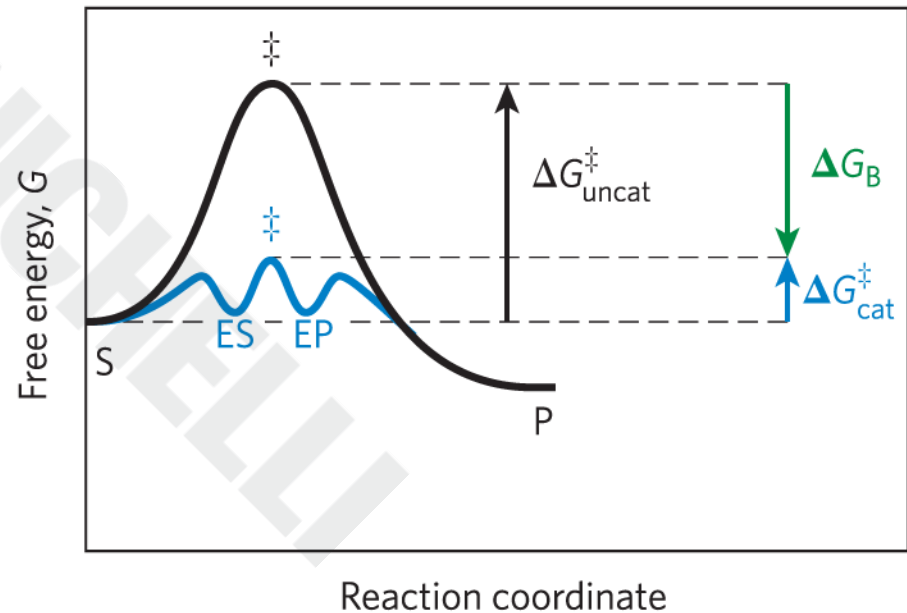


Principle 4 (3 of 8)

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The Role of Binding Energy in Catalysis

- the sum of the unfavorable activation energy $\Delta G^\ddagger$ and the favorable binding energy ΔG_B results in a lower *net* activation energy
- weak binding interactions between the enzyme and the substrate drive enzymatic catalysis



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Principle 3 (3 of 3)

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The Active Site of an Enzyme

- optimized binding energy in the transition state is accomplished by positioning a substrate in a cavity (the active site), removed from H₂O

Principle 2 (3 of 3)

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Enzyme Specificity

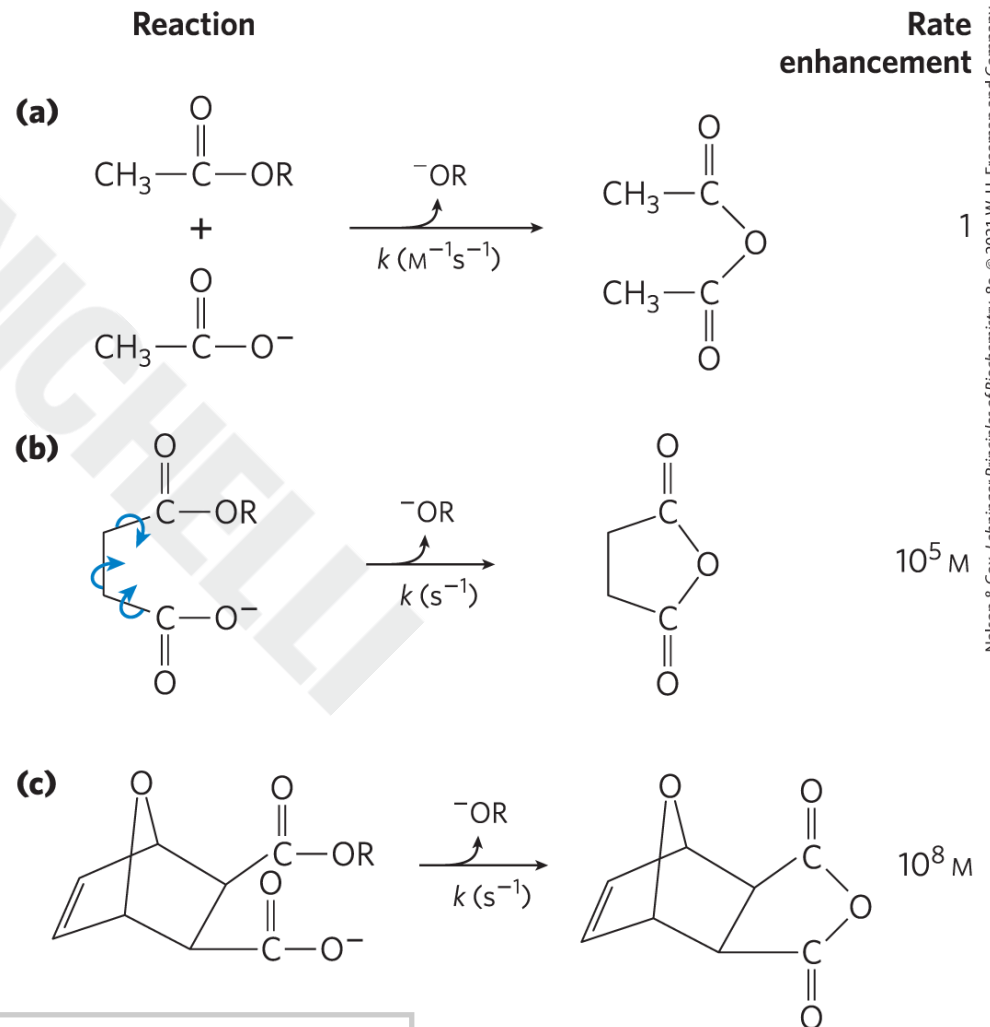
- **specificity** = ability to discriminate between a substrate and a competing molecule
 - given by binding energy

Binding Energy Overcomes the Barrier to Reaction

- barrier to reaction, $\Delta G^\ddagger$ includes:
 - the entropy of molecules in solution
 - the solvation shell of hydrogen-bonded water that surrounds and stabilizes most biomolecules in aqueous solution
 - the distortion of substrates that must occur in many reactions
 - the need for proper alignment of catalytic functional groups on the enzyme

Rate Enhancement by Entropy Reduction

- **entropy reduction** = large restriction in the relative motions of two substrates that are to react
- binding energy constrains substrates in the proper orientation to reaction



Desolvation of the Substrate

- **desolvation** = replacement of the solvation shell of structured water around the substrate with weak bonds between substrate and enzyme
 - replaces most or all hydrogen bonds between the substrate and H₂O

Distortion of the Substrate

- substrate must undergo distortion (electron redistribution) to react
 - causes an unfavorable free-energy change
- binding energy compensates thermodynamically for this

The Induced Fit Mechanism

- **induced fit** = mechanism by which the enzyme itself undergoes a conformational change when the substrate binds, induced by multiple weak interactions with the substrate
 - enhances catalytic properties

Principle 4 (4 of 8)

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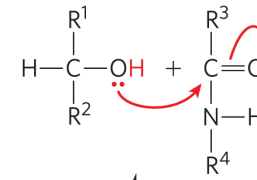
Covalent Interactions and Metal Ions Contribute to Catalysis

- catalytic functional groups aid in the cleavage and formation of bonds by a variety of mechanisms:
 - general acid-base catalysis
 - covalent catalysis
 - metal ion catalysis

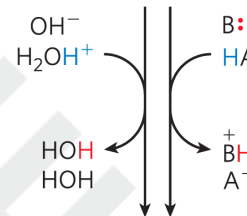
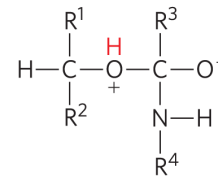
General Acid-Base Catalysis

- protons are transferred between an enzyme and a substrate or intermediate
- specific acid-base catalysis** = uses only the H^+ (H_3O^+) or OH^- ions present in water
- general acid-base catalysis** = mediated by weak acids or bases other than water

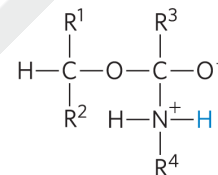
Reactants



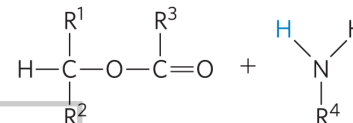
Without catalysis, unstable (charged) intermediate breaks down rapidly to form reactants.



Proton transfers to and from either water or weak acids and bases stabilize the intermediate and accelerate the reaction.

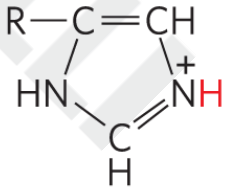
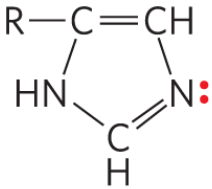
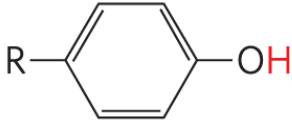
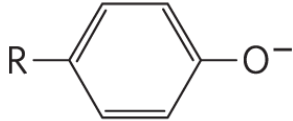


Products



Amino Acids in General Acid-Base Catalysis

- occurs in the vast majority of enzymes

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	$\text{R}-\text{COOH}$	$\text{R}-\text{COO}^-$
Lys, Arg	$\text{R}-\overset{\text{H}}{\overset{+}{\text{N}}}\text{H}$	$\text{R}-\ddot{\text{N}}\text{H}_2$
Cys	$\text{R}-\text{SH}$	$\text{R}-\text{S}^-$
His		
Ser	$\text{R}-\text{OH}$	$\text{R}-\text{O}^-$
Tyr		

Covalent Catalysis

- **covalent catalysis** = transient covalent bond forms between the enzyme and the substrate
 - catalysis only results when the new pathway has a lower activation energy than the uncatalyzed pathway
 - all new steps must be faster than the uncatalyzed reaction
- hydrolysis of a bond: $A-B \xrightarrow{H_2O} A + B$
- hydrolysis of a bond between A and B in the presence of a covalent catalyst:



Metal Ion Catalysis

- metals:
 - help orient the substrate for reaction
 - stabilize charged reaction transition states
 - mediate oxidation-reduction reactions by reversible changes in the metal ion's oxidation state
- nearly 1/3 of all known enzymes require 1+ metal ions for catalytic activity

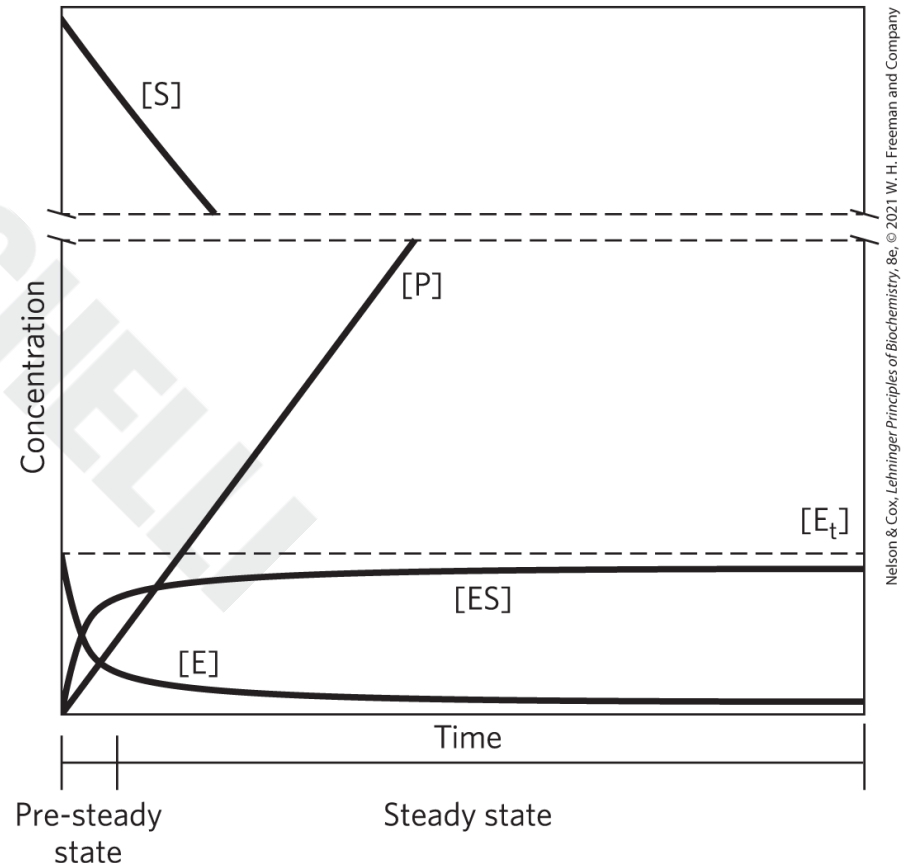
6.3 Enzyme Kinetics as an Approach to Understanding Mechanism

Enzyme Kinetics

- **enzyme kinetics** = the discipline focused on determining the *rate* of a reaction and how it changes in response to changes in experimental parameters

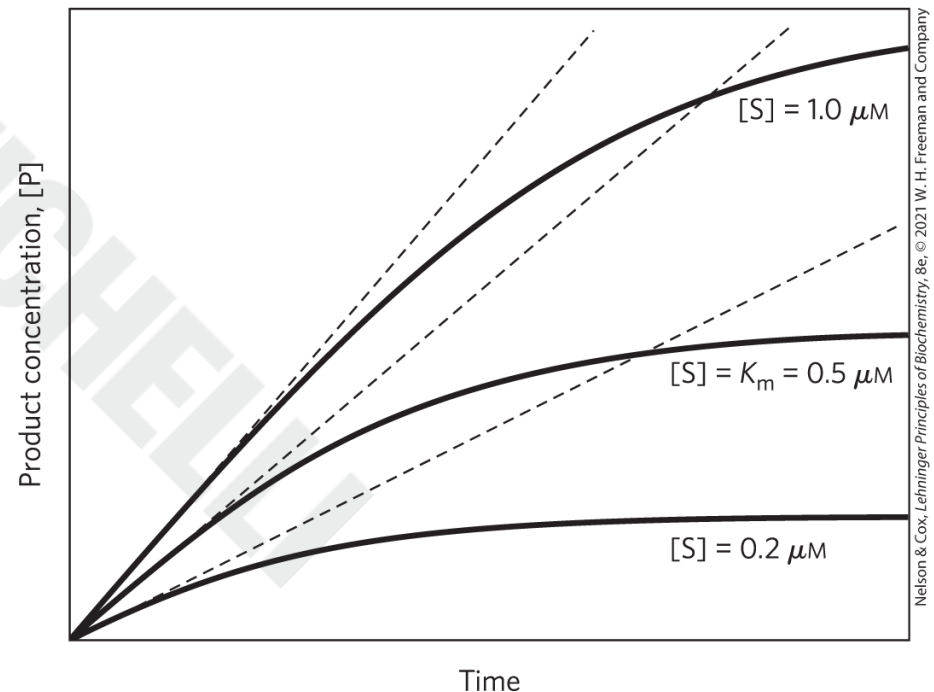
Substrate Concentration Affects the Rate of Enzyme-Catalyzed Reactions

- **pre-steady state** = initial transient period during which ES builds up
- **steady state** = period during which [ES] and other intermediates remain constant
- **steady-state kinetics** = the traditional analysis of reaction rates



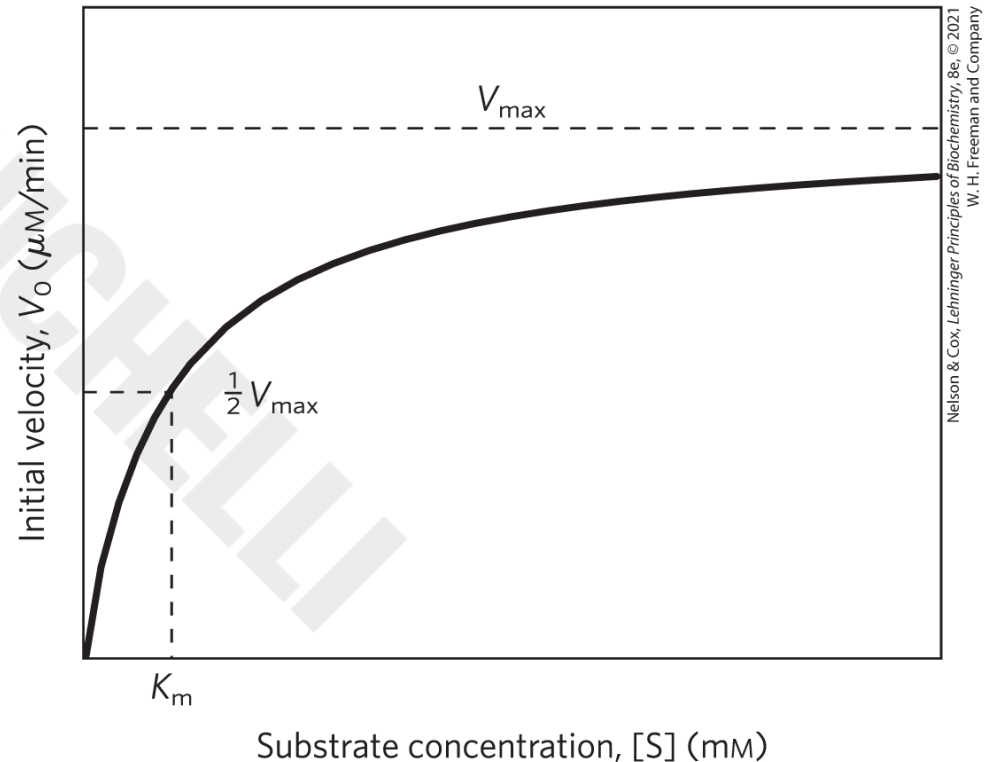
Initial Velocities of Enzyme-Catalyzed Reactions

- **initial rate (initial velocity), V_0** = tangent to each curve taken at time = 0
 - reflects a steady state
- at the beginning of the reaction, $[S]$ is regarded as constant



Effect of $[S]$ on the V_0 of an Enzyme-Catalyzed Reaction

- the plateau-like V_0 region is close to the **maximum velocity, $V_{\max}$**

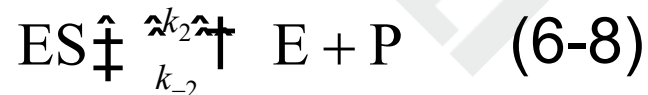


General Theory of Enzyme Action Proposed by Michaelis and Menten

- step 1 – enzyme and substrate combine to form a complex in a reversible, relatively fast step:



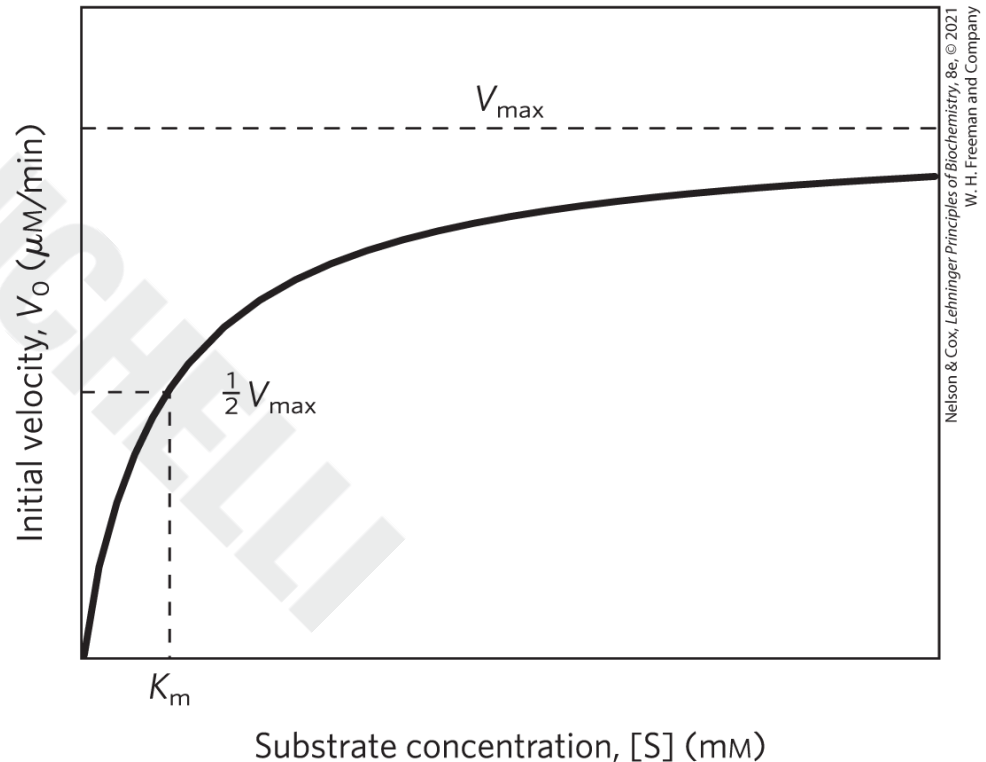
- step 2 – the complex breaks down to yield the free enzyme and the reaction product in a slower step:



- because the second step limits the overall reaction rate, the overall rate is proportional to [ES]

The Saturation Effect

- $V_{\max}$ is observed when virtually all the enzyme is present as the ES complex
 - further increases in $[S]$ have no effect on rate
 - responsible for the plateau observed



The Relationship between Substrate Concentration and Reaction Rate Can Be Expressed with the Michaelis-Menten Equation

- the curve expressing the relationship between $[S]$ and V_0 can be expressed by the **Michaelis-Menten equation**:

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]} \quad (6-9)$$

where V_0 is the initial velocity, $V_{\max}$ is the maximum velocity, $[S]$ is the initial substrate concentration, and K_m is a constant called the Michaelis constant

The Michaelis-Menten Equation

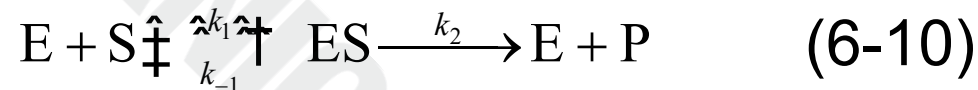
- the **Michaelis-Menten equation** is the **rate equation** for a one-substrate enzyme-catalyzed reaction:

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]} \quad (6-9)$$

- V_0 , $V_{\max}$, $[S]$, and K_m are readily measured experimentally

Deriving the Michaelis-Menten Equation

- the overall reaction for the formation and breakdown of ES simplifies to



- V_0 is determined by the breakdown of ES to form product, which is determined by [ES]:

$$V_0 = k_2[ES] \quad (6-11)$$

Deriving the Michaelis-Menten Equation, $[E_t]$

- $[ES]$ is not easily measured experimentally
- $[E_t]$ = the total enzyme concentration (the sum of free and substrate-bound enzyme)
- free or unbound enzyme $[E] = [E_t] - [ES]$
- because $[S] \gg [E_t]$, the amount of substrate bound by the enzyme at any given time is negligible compared with the total $[S]$

Deriving the Michaelis-Menten Equation, Step 1

- rate of ES formation = $k_1([E_t] - [ES])[S]$ (6-12)
- rate of ES breakdown = $k_{-1}[ES] + k_2[ES]$ (6-13)

Deriving the Michaelis-Menten Equation, Step 2

- **steady-state assumption** = the rate of formation of ES is equal to the rate of its breakdown
- when equated for the steady state:

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

Deriving the Michaelis-Menten Equation, Step 3

- solving for [ES]:

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

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

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

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

Deriving the Michaelis-Menten Equation, K_m

- the **Michaelis constant**, $K_m = (k_{-1} + k_2) / k_1$
- substituting K_m into the equation:

$$[ES] = \frac{[Et][S]}{K_m + [S]} \quad (6-19)$$

Deriving the Michaelis-Menten Equation, Step 4

- expressing V_0 in terms of $[ES]$:

$$V_0 = k_2[ES] \quad (6-11)$$

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]} \quad (6-20)$$

- given $V_{\max}$ occurs when the enzyme is saturated (when $[ES] = [E_t]$), $V_{\max} = k_2[E_t]$:

$$V_0 = \frac{V_{\max}[S]}{K_m + [S]} \quad (6-9)$$

$$K_m = [S], \text{ When } V_0 = \frac{1}{2}V_{\max}$$

- when $V_0 = \frac{1}{2}V_{\max}$:

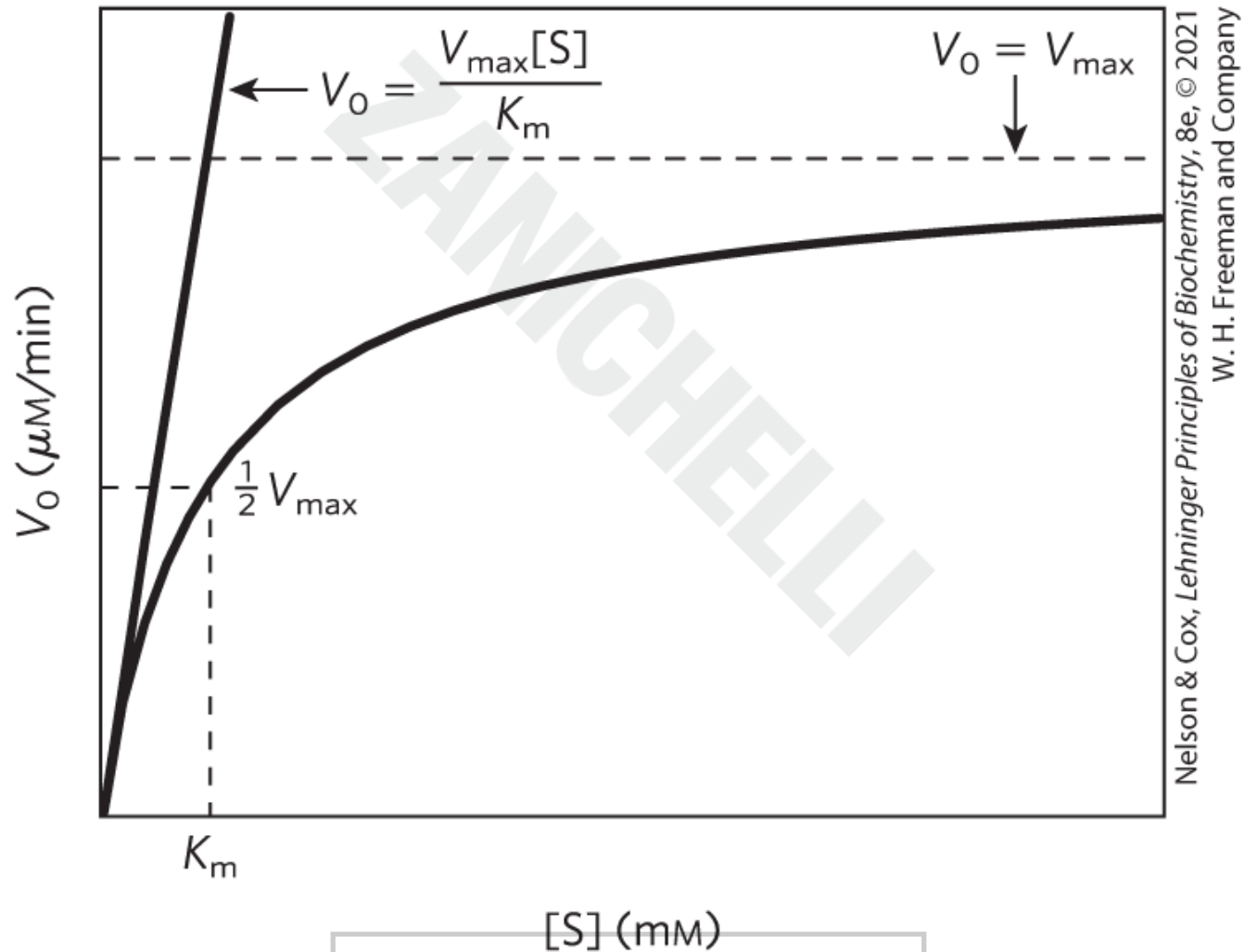
$$\frac{V_{\max}}{2} = \frac{V_{\max}[S]}{K_m + [S]} \quad (6-21)$$

$$\frac{1}{2} = \frac{[S]}{K_m + [S]} \quad (6-22)$$

solving for K_m :

$$K_m = [S] \quad \text{when} \quad V_0 = \frac{1}{2}V_{\max} \quad (6-23)$$

Dependence of V_0 on $[S]$



Michaelis-Menten Kinetics Can Be Analyzed Quantitatively

- an algebraic transformation of the Michaelis-Menten equation converts the hyperbolic curve into a linear form:

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

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{\max}[S]} \quad (6-24)$$

Deriving the Lineweaver-Burk Equation

- simplifying this equation gives the **Lineweaver-Burk equation**:

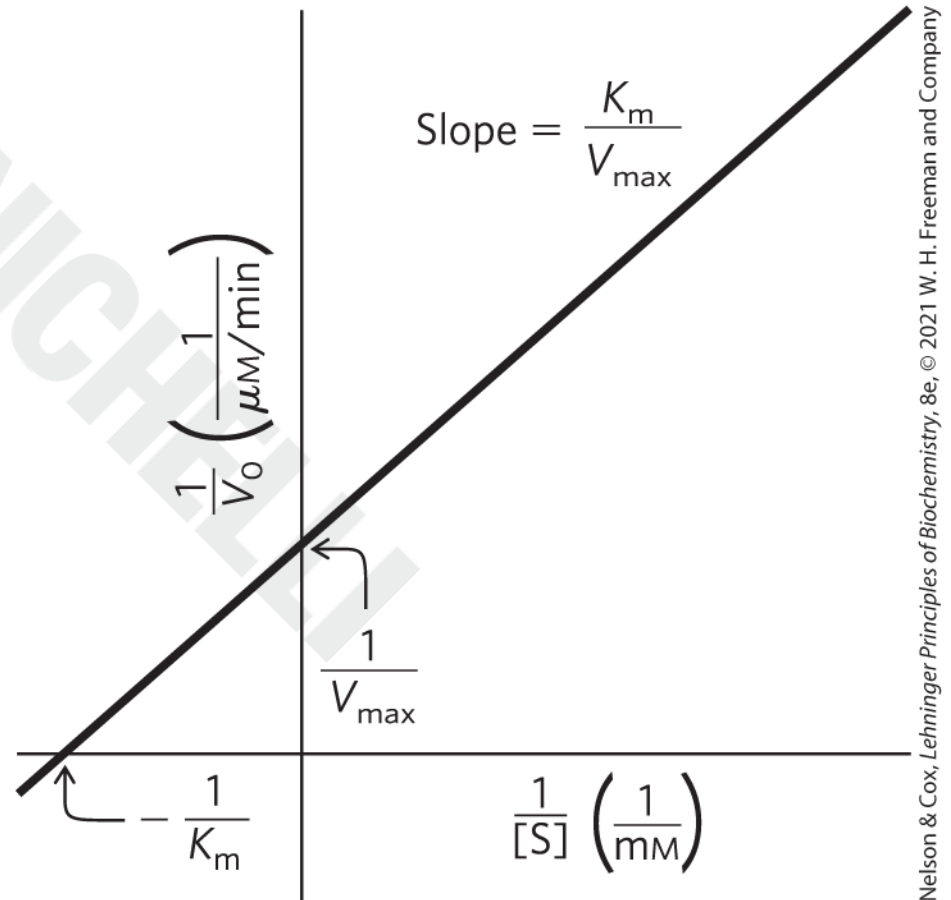
$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{\max}[S]} \quad (6-24)$$

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}[S]} + \frac{[S]}{V_{\max}[S]} \quad (6-25)$$

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}[S]} + \frac{1}{V_{\max}} \quad (6-26)$$

A Double-Reciprocal, or Lineweaver-Burk, Plot

- for enzymes obeying the Michaelis-Menten relationship, a plot of $1/V_0$ versus $1/[S]$ yields a straight line



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Kinetic Parameters Are Used to Compare Enzyme Activities

- all enzymes that exhibit a hyperbolic dependence of V_0 on $[S]$ follow **Michaelis-Menten kinetics** where:

$$K_m = [S] \quad \text{when} \quad V_0 = \frac{1}{2}V_{\max} \quad (6-23)$$

Interpreting K_m and V_{max}

- K_m can vary for different substrates of the same enzyme

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

The Dissociation Constant, K_d

- for reactions with two steps:

$$K_m = \frac{k_2 + k_{-1}}{k_1} \quad (6-27)$$

- when k_2 is rate-limiting, $k_2 \ll k_{-1}$, and K_m reduces to k_{-1}/k_1 , which is defined as the **dissociation constant, K_d** , of the ES complex
 - under these conditions, K_m represents a measure of the affinity

Interpreting $V_{\max}$

- the number of reactions steps and the identity of the rate-limiting step(s) varies from enzyme to enzyme
- for a two-step Michaelis-Menten mechanism,

$$V_{\max} = k_2[E_t]$$
- when product release, $EP \rightarrow E + P$, is rate-limiting:



The General Rate Constant, k_{cat}

- general rate constant, k_{cat} = describes the limiting rate of any enzyme-catalyzed reaction at saturation
 - if one step in a multistep reaction is clearly limiting, k_{cat} equals the rate constant for the step
 - more complex when several steps are rate-limiting
- in the Michaelis-Menten equation, $k_{\text{cat}} = V_{\text{max}}/[E_t]$:

$$V_0 = \frac{V_{\text{max}}[S]}{K_m + [S]} \quad (6-9)$$

$$V_0 = \frac{k_{\text{cat}}[E_t][S]}{K_m + [S]} \quad (6-29)$$

The Constant k_{cat} Is a First-Order Rate Constant

- k_{cat} = **turnover number** = the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated

Table 6-7 Turnover Number, k_{cat} , of Some Enzymes

Enzyme	Substrate	k_{cat} (s ⁻¹)
Catalase	H ₂ O 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

Comparing Catalytic Mechanisms and Efficiencies

- comparing the ratio k_{cat}/k_m for two reactions is the best way to compare catalytic efficiencies or turnover
- **specificity constant** = the rate constant for the conversion of E + S to E + P
- when $[S] \ll k_m$:

$$V_0 = \frac{k_{\text{cat}}}{K_m} [E_t][S] \quad (6-30)$$

The Constant k_{cat}/K_m Is a Second-Order Rate Constant

- k_{cat}/K_m has units of $\text{M}^{-1}\text{s}^{-1}$ with an upper limit of 10^8 to 10^9
 $\text{M}^{-1}\text{s}^{-1}$

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

Enzyme	Substrate	$k_{\text{cat}} (\text{s}^{-1})$	$K_m (\text{M})$	$k_{\text{cat}}/K_m (\text{M}^{-1}\text{s}^{-1})$
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

Many Enzymes Catalyze Reactions with Two or More Substrates

- nearly 2/3 of all enzymatic reactions have 2 substrates and 2 products
- example types of reactions:
 - a group is transferred from one substrate to the other
 - one substrate is oxidized while the other is reduced

Some Enzyme Reactions Involve a Ternary Complex

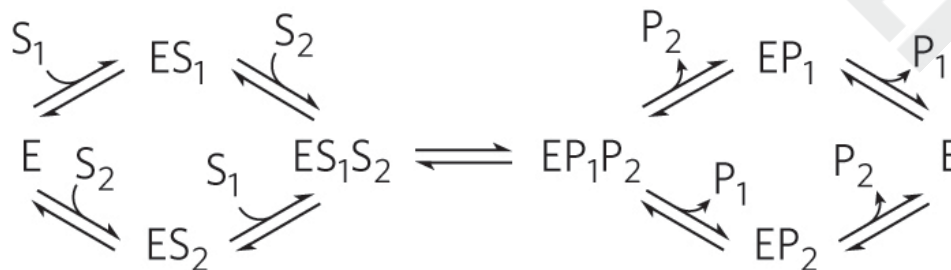
- substrates can bind in a random sequence or in a specific order

(a) Enzyme reaction involving a ternary complex

Ordered



Random order



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Some Enzyme Reactions Do Not Form a Ternary Complex

- the first substrate is converted to product and dissociates before the second substrate binds
 - example: Ping-Pong, or double-displacement, mechanism

(b) Enzyme reaction in which no ternary complex is formed



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Cleland Nomenclature

- **Cleland nomenclature** = shorthand notation for describing reactions with multiple substrates and products
 - substrates = A, B, C, and D, in the order in which they bind to the enzyme
 - products = P, Q, S, and T, in the order in which they dissociate
 - one, two, three, or four substrates = uni, bi, ter, and quad, respectively
 - the enzyme = E, but if modified during the reaction, successive forms = F, G, and so on

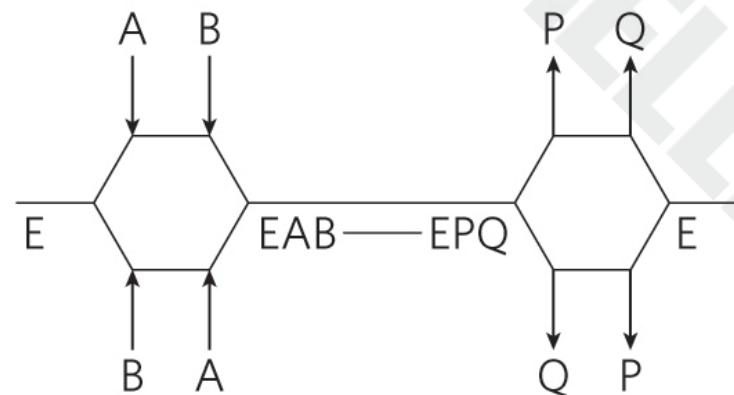
Cleland Nomenclature for a Bi Bi Reaction

(c) Cleland nomenclature

Ordered bi bi



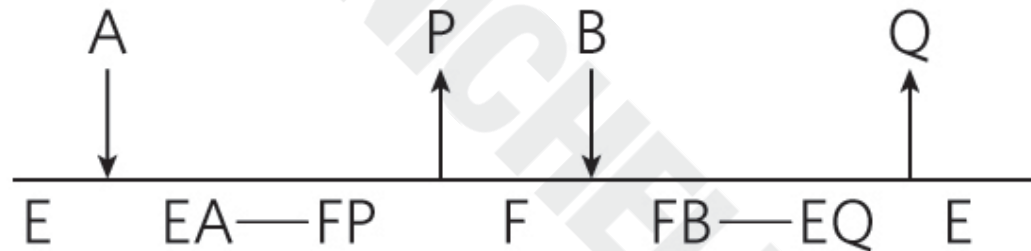
Random bi bi



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Cleland Nomenclature for a Ping-Pong Reaction

(d) Ping-Pong in Cleland nomenclature

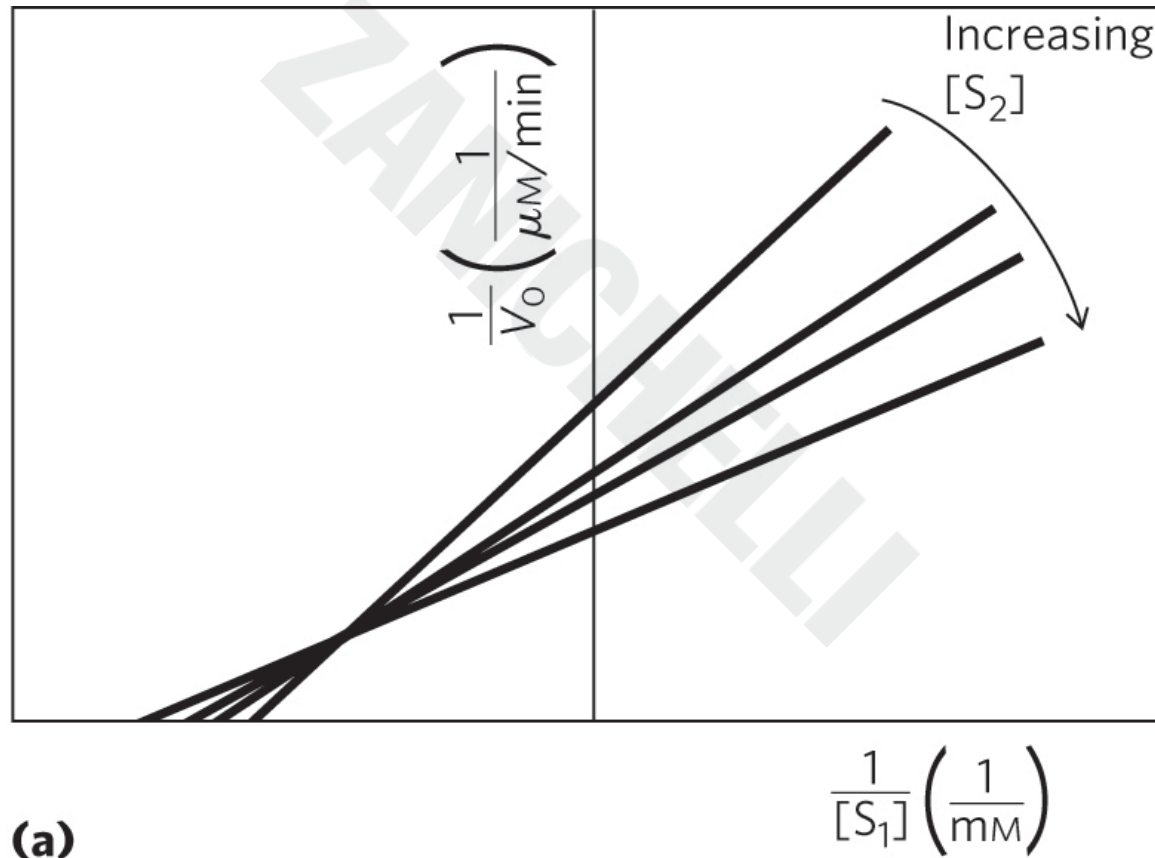


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Steady-State Kinetic Analysis of Bisubstrate Reactions

- Michaelis-Menten steady-state kinetics can distinguish between pathways that have a ternary intermediate and pathways that do not

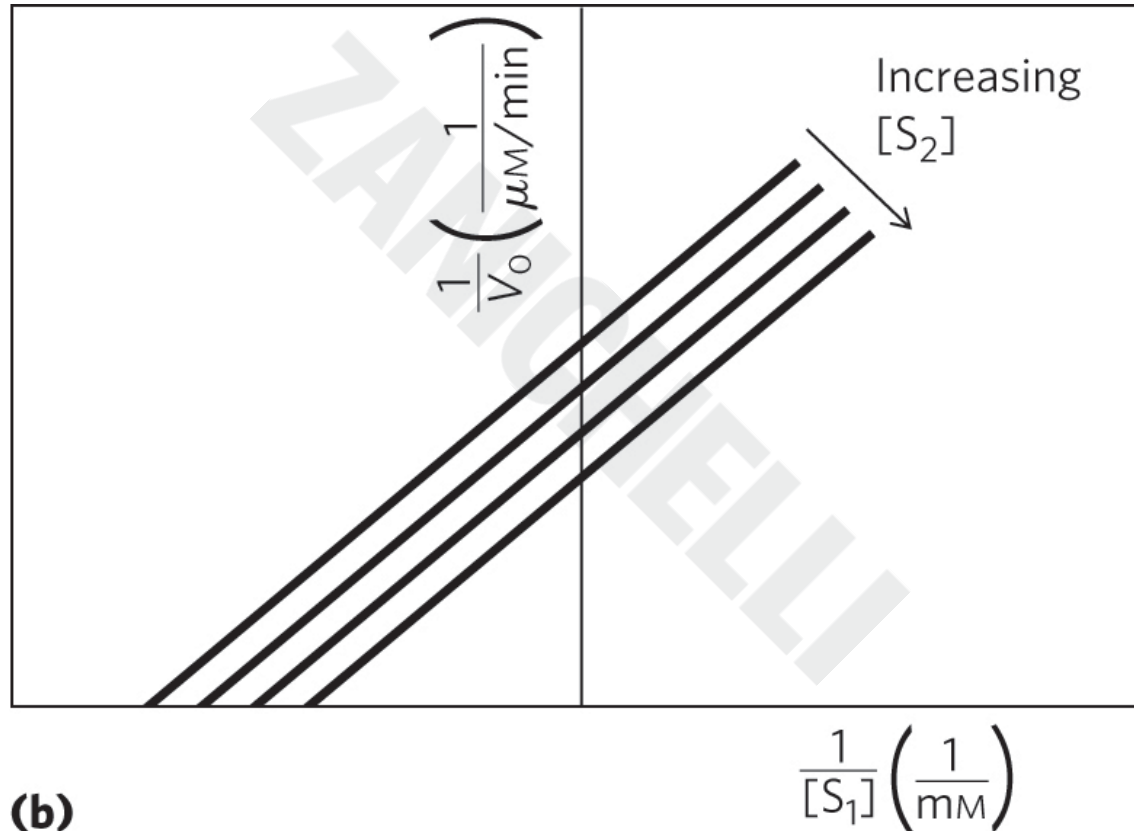
Intersecting Lines Indicate the Formation of a Ternary Complex



(a)

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Parallel Lines Indicate a Ping-Pong (Double-Displacement) Pathway

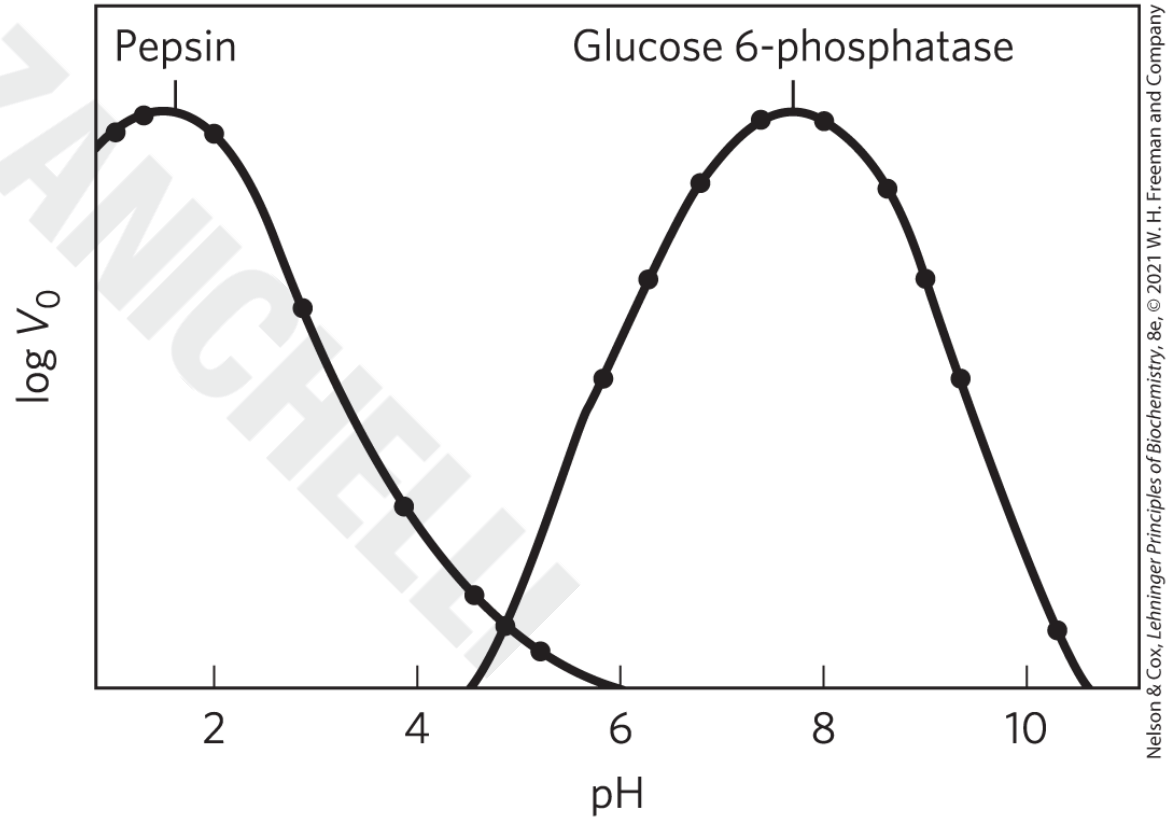


(b)

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Enzyme Activity Depends on pH

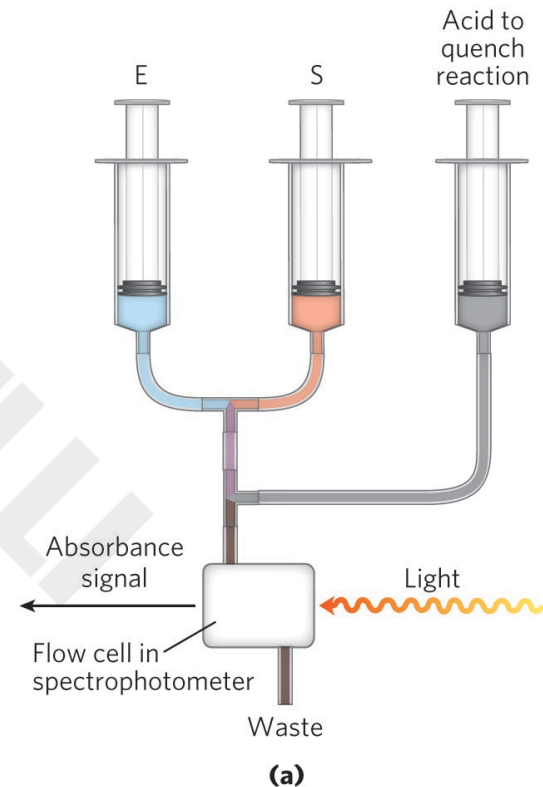
- the pH range over which an enzyme undergoes changes in activity can provide a clue to the type of amino acid residue involved



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Pre–Steady State Kinetics Can Provide Evidence for Specific Reaction Steps

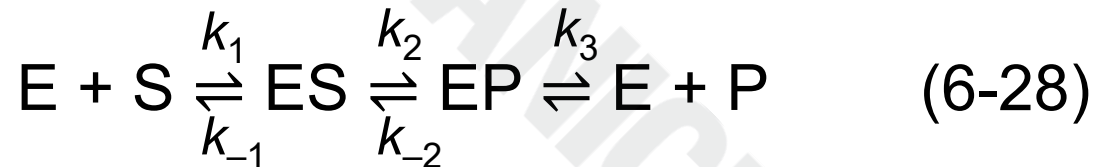
- used to measure rate constants of individual steps
- stopped-flow device allows mixing and sampling on a second or millisecond time scale



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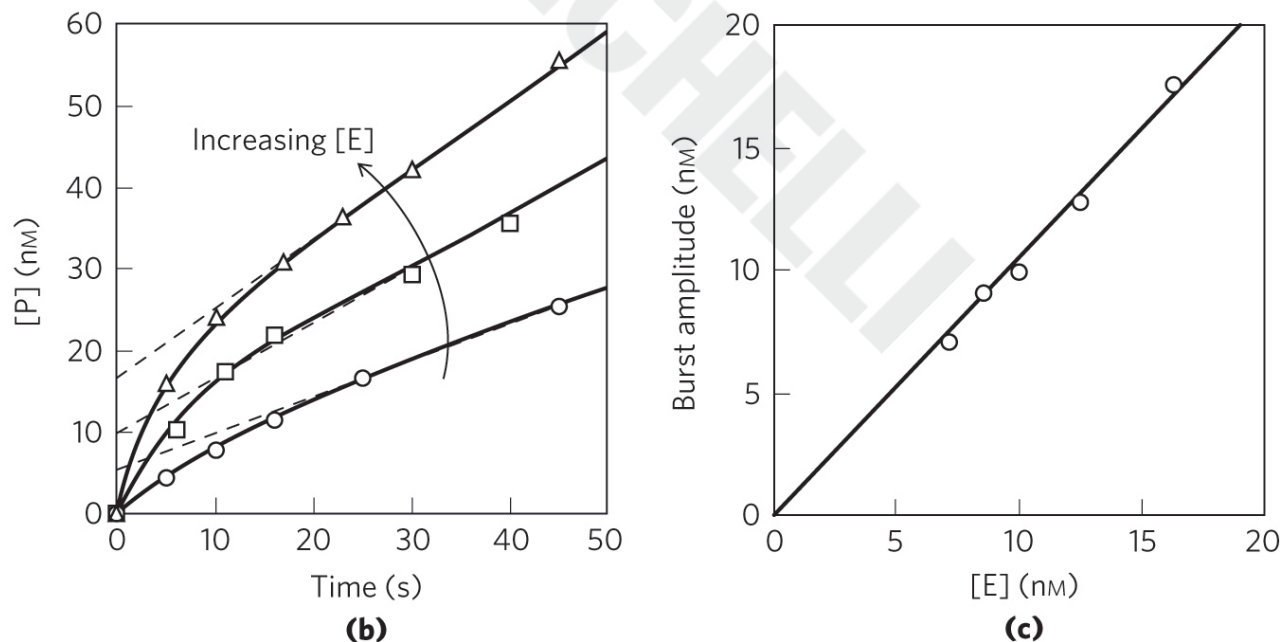
Dissociation of the Product is Rate-Limiting for Many Enzymes

- for an enzyme where product release, $EP \rightarrow E + P$, is rate-limiting (k_3 is rate-limiting):



Using Pre-Steady State Kinetics to Determine the Rate-Limiting Step

- the observation of a burst indicates that a rate-limiting step (product release, enzyme conformational change, etc.) occurs after formation of the product being monitored



Enzymes Are Subject to Reversible or Irreversible Inhibition

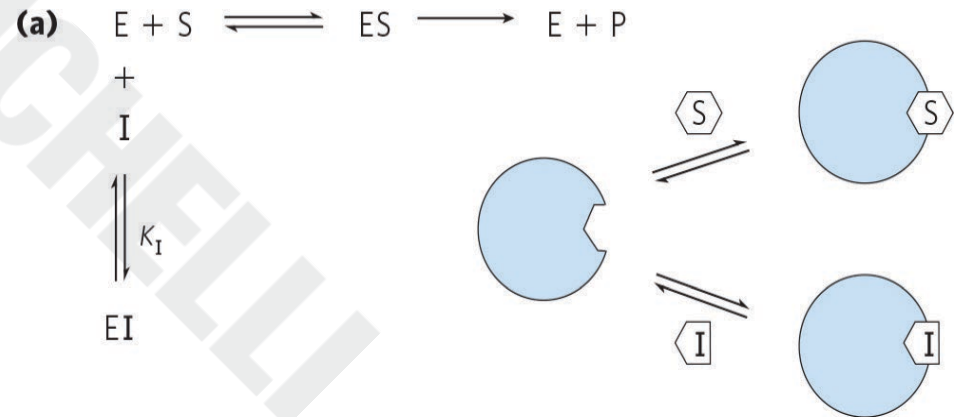
- enzyme inhibitors = molecules that interfere with catalysis, slowing or halting enzymatic reactions
- 2 classes of enzyme inhibitors:
 - reversible
 - irreversible

Reversible Inhibition

- types of reversible inhibition:
 - competitive inhibition
 - uncompetitive inhibition
 - mixed inhibition
 - noncompetitive inhibition

Competitive Inhibition

- **competitive inhibitor:**
 - competes with the substrate for the active site of an enzyme
 - type of **reversible inhibition**
 - by definition, the value of α' for a competitive inhibitor is 1



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Competitive Inhibitors Alter the Michaelis-Menten Equation

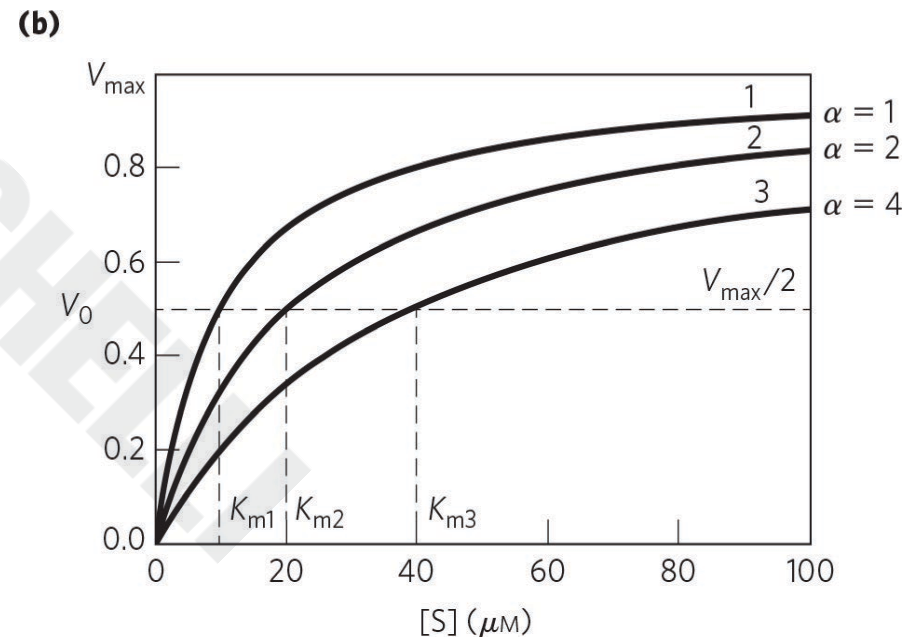
- competitive inhibition can be measured by steady-state kinetics
- the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{\max}[S]}{\alpha K_m + [S]} \quad (6-31)$$

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

Competitive Inhibitors Affect the Apparent K_m , but Not the V_{max}

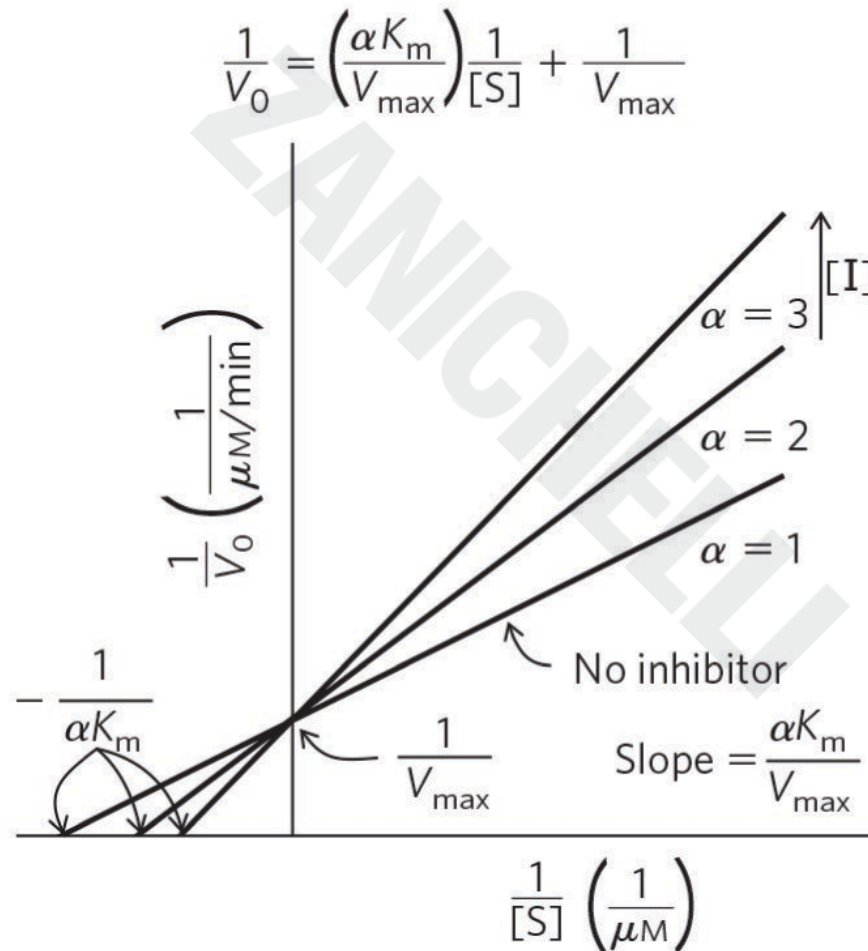
- “apparent” K_m = the experimentally determined variable αK_m
- when $[S] \gg [I]$, the reaction exhibits normal V_{max}
- in the presence of inhibitor, the $[S]$ at which $V_0 = \frac{1}{2}V_{max}$, the apparent K_m , increases by the factor α



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A Lineweaver-Burk Plot Reveals Competitive Inhibition

(c)

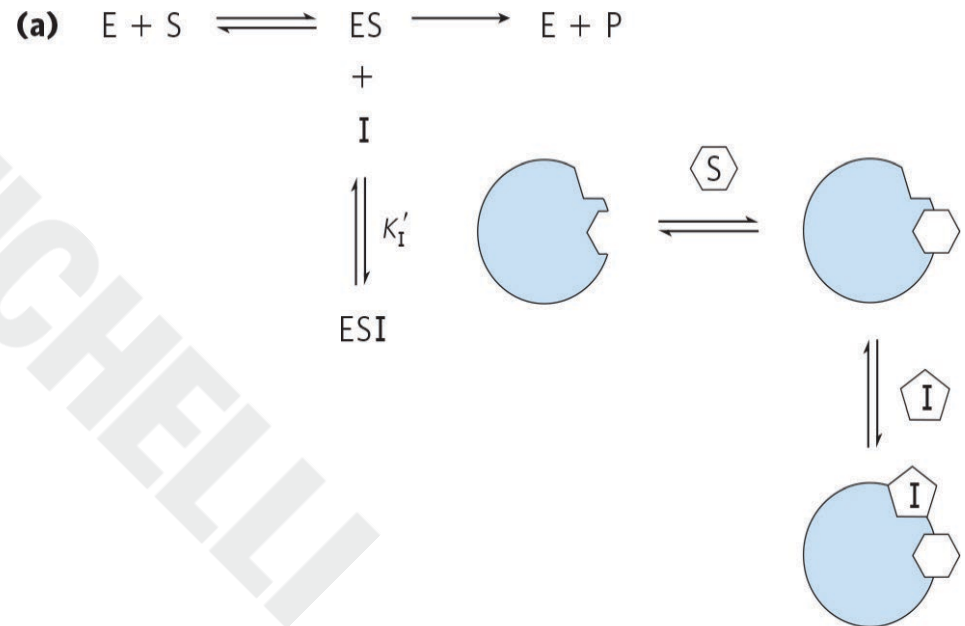


lines
intersect at
the y axis

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Uncompetitive Inhibition

- **uncompetitive inhibitor:**
 - binds at a site distinct from the substrate active site
 - unlike a competitive inhibitor, binds only to the ES complex
 - type of **reversible inhibition**



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Uncompetitive Inhibitors Alter the Michaelis-Menten Equation

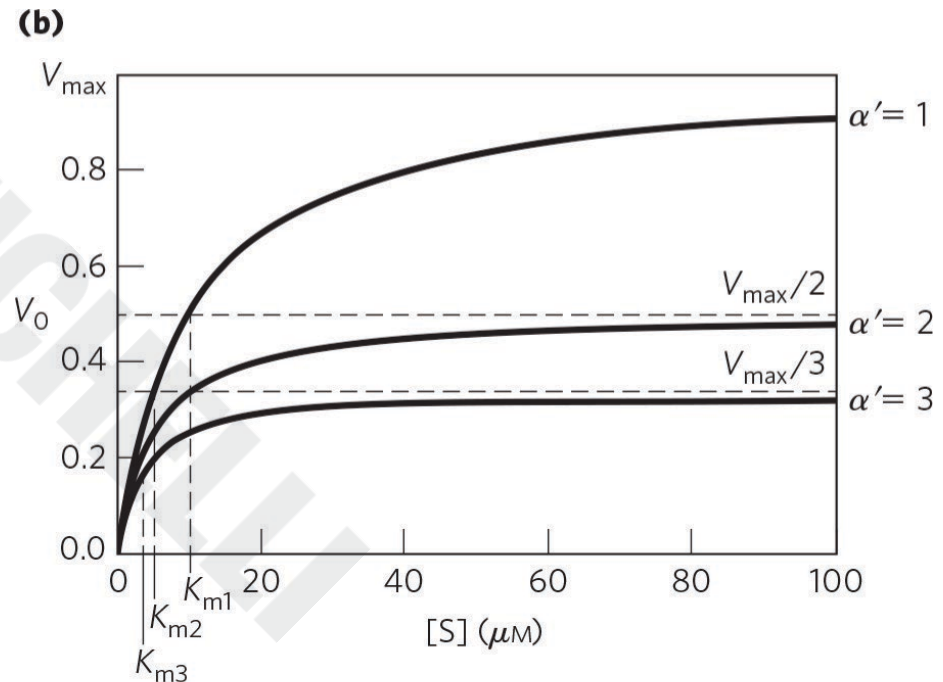
- the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{\max}[S]}{K_m + \alpha'[S]} \quad (6-32)$$

where $\alpha' = 1 + \frac{[I]}{K'_i}$ and $K'_i = \frac{[ES][I]}{[ESI]}$

Uncompetitive Inhibitors Affect Both the Apparent K_m and the V_{max}

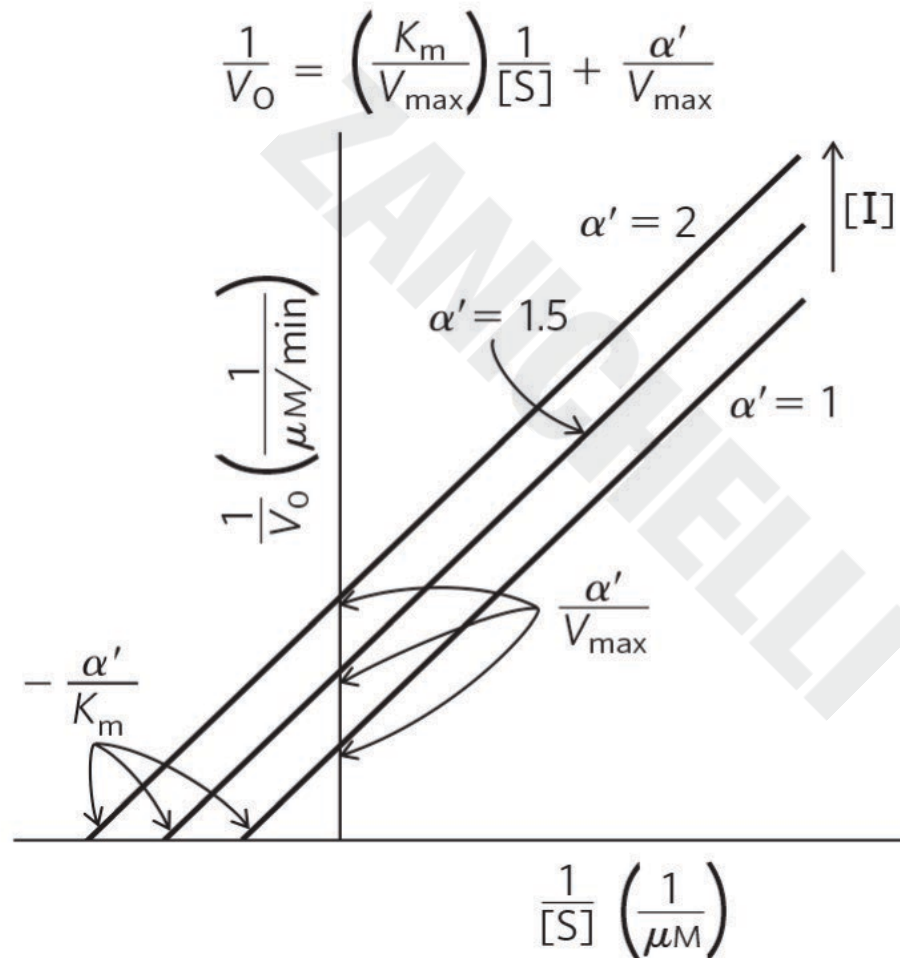
- the measured V_{max} decreases because at high $[S]$, V_0 approaches V_{max}/α'
- apparent K_m decreases because the $[S]$ required to reach $\frac{1}{2}V_{max}$ decreases by the factor α'



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A Lineweaver-Burk Plot Reveals Uncompetitive Inhibition

(c)



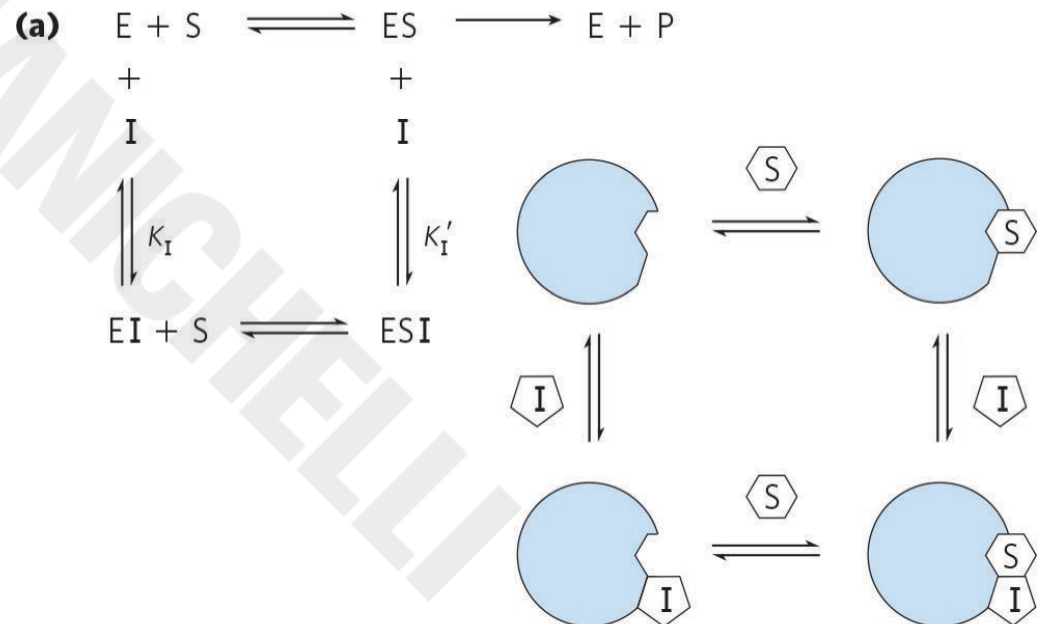
lines are
parallel

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Mixed Inhibition

- **mixed inhibitor:**

- binds at a site distinct from the substrate active site
- binds to either E or the ES complex
- type of **reversible inhibition**



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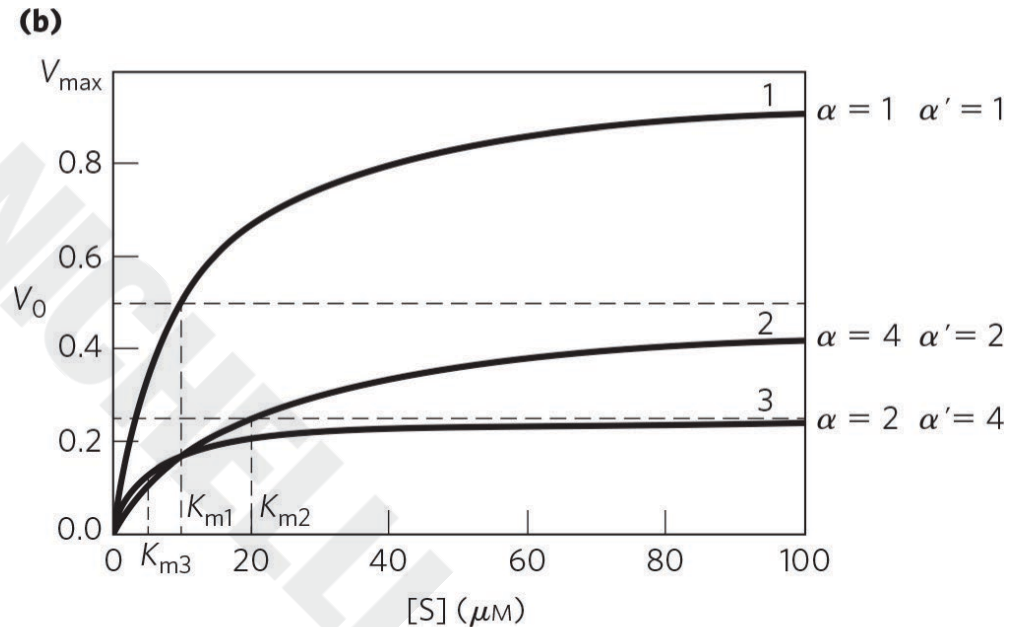
Mixed Inhibitors Alter the Michaelis-Menten Equation

- the Michaelis-Menten equation becomes

$$V_0 = \frac{V_{\max}[S]}{\alpha K_m + \alpha'[S]} \quad (6-33)$$

Mixed Inhibitors Usually Affect Both the Apparent K_m and the V_{max}

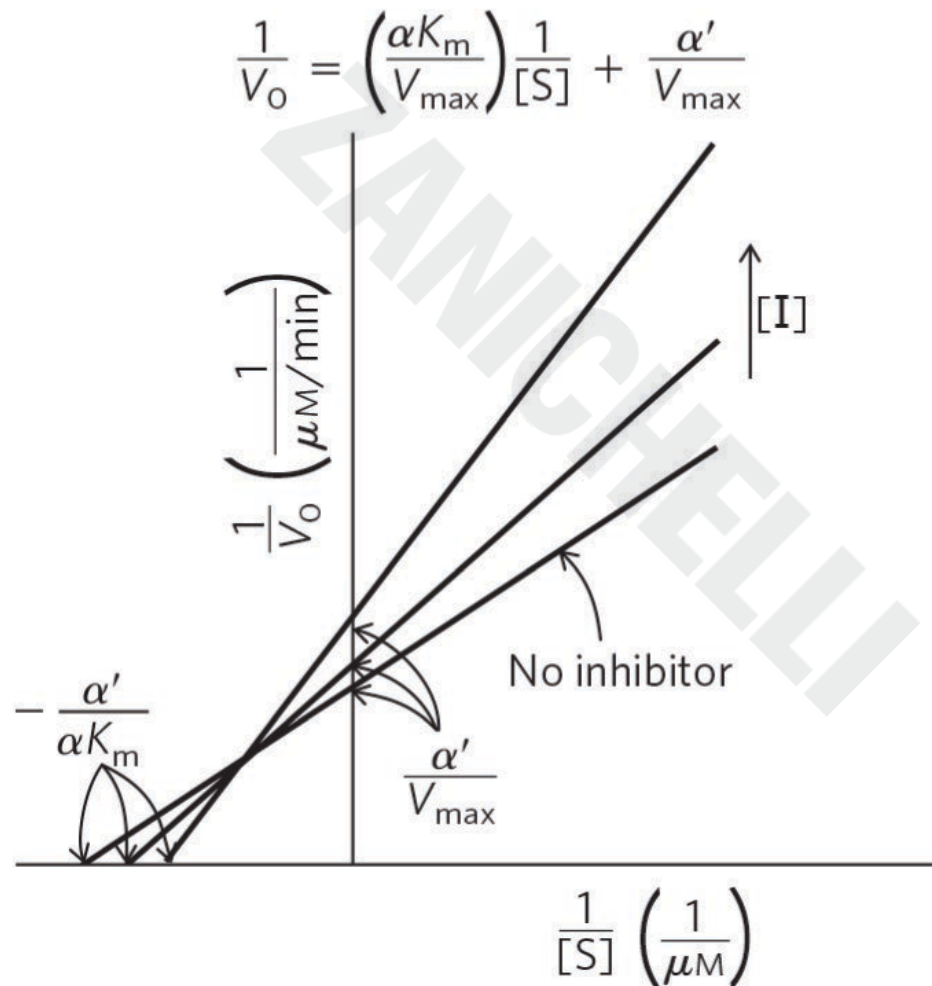
- V_{max} is affected because the effective $[E]$ on which V_{max} depends decreases
- apparent K_m may increase or decrease depending on which enzyme form the inhibitor binds most strongly



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A Lineweaver-Burk Plot Reveals Mixed Inhibition

(c)



lines
intersect to
the left of
the y axis

Noncompetitive Inhibition

- **noncompetitive inhibitor:**
 - special case of $\alpha = \alpha'$
 - affects the $V_{\max}$ but not the K_m

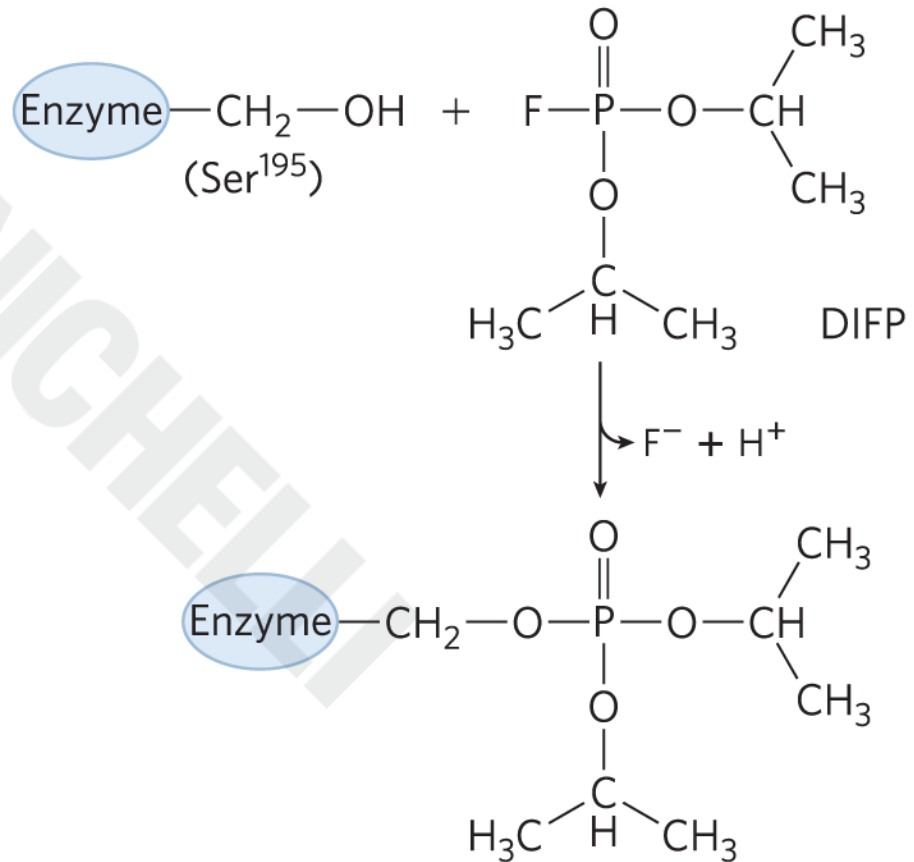
Effects of Reversible Inhibitors on Apparent $V_{\max}$ and Apparent K_m

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	$V_{\max}$	K_m
Uncompetitive	$V_{\max}/\alpha'$	K_m/α'
Mixed	$V_{\max}/\alpha'$	$\alpha K_m/\alpha'$

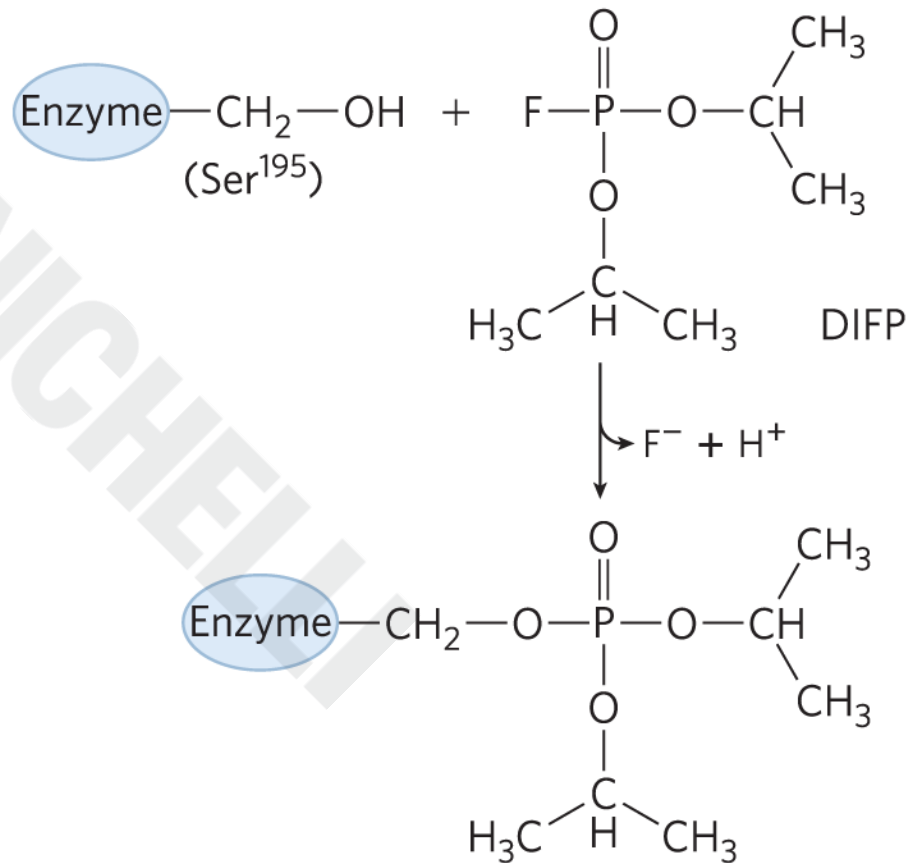
Irreversible Inhibition

- irreversible inhibitor** = bind covalently with or destroy a functional group on an enzyme that is essential for the enzyme's activity, or form a highly stable noncovalent association



Suicide Inactivators

- **suicide inactivator = mechanism-based inactivators** = undergo the first few steps until it is converted into a compound that combines irreversibly with the enzyme
 - class of irreversible inhibitors



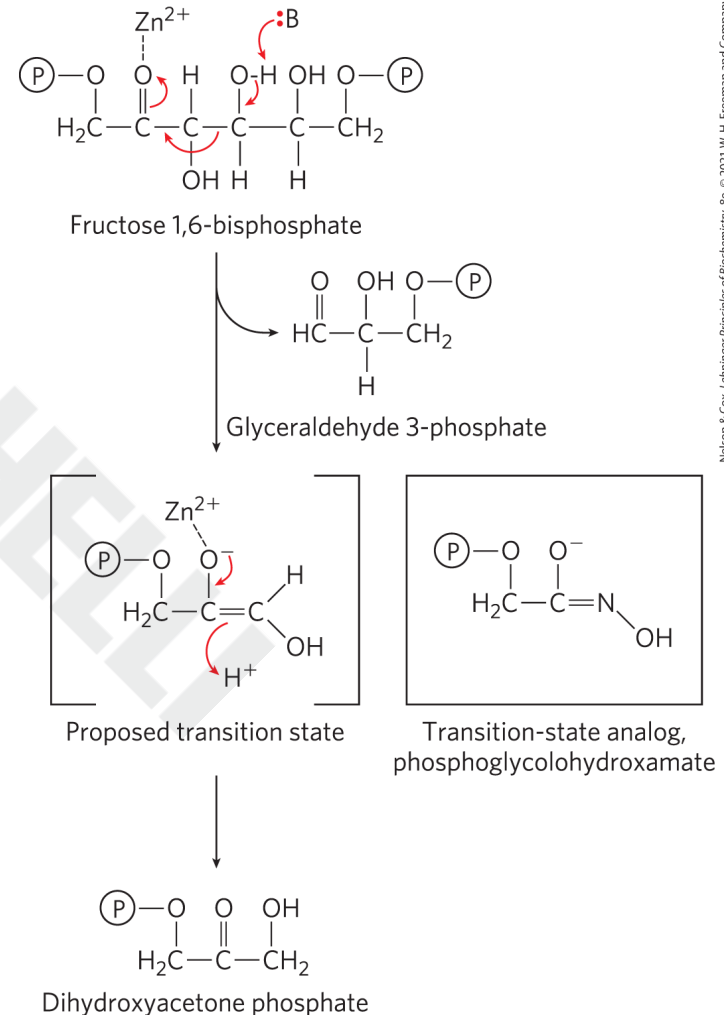
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Principle 4 (5 of 8)

Two concepts explain the catalytic power of enzymes. First, enzymes bind most tightly to the transition state of the catalyzed reaction, using binding energy to lower the activation barrier. Second, enzyme active sites are organized by evolution to facilitate multiple mechanisms of chemical catalysis simultaneously.

Transition-State Analogs

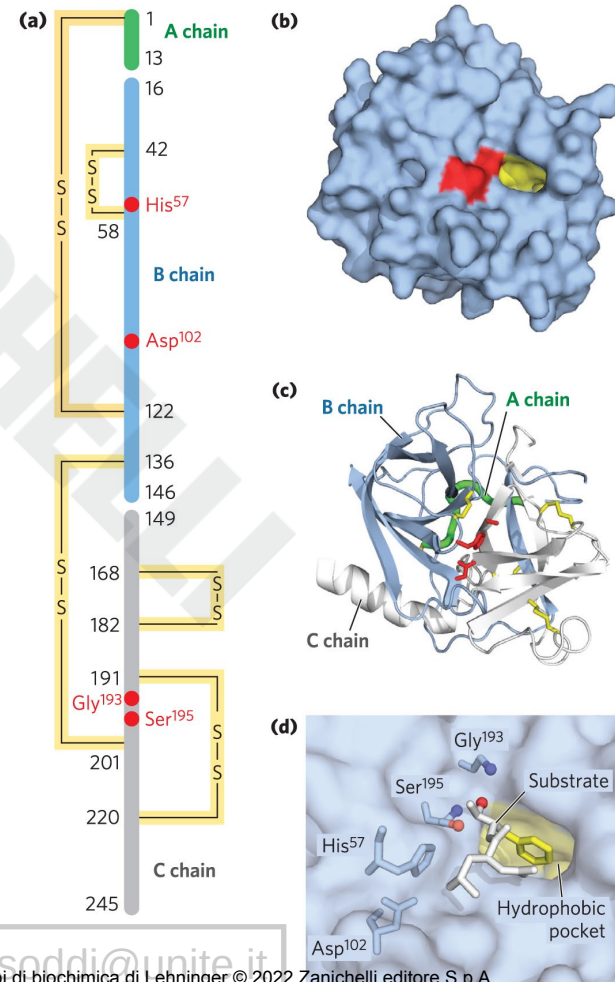
- **transition-state analogs** = stable molecules designed to resemble transition states
- type of irreversible inhibitors
- bind to an enzyme more tightly than does the substrate in the ES complex



6.4 Examples of Enzymatic Reactions

The Chymotrypsin Mechanism Involves Acylation and Deacylation of a Ser Residue

- **protease** = an enzyme that catalyzes the hydrolytic cleavage of peptide bonds
 - example = bovine pancreatic chymotrypsin



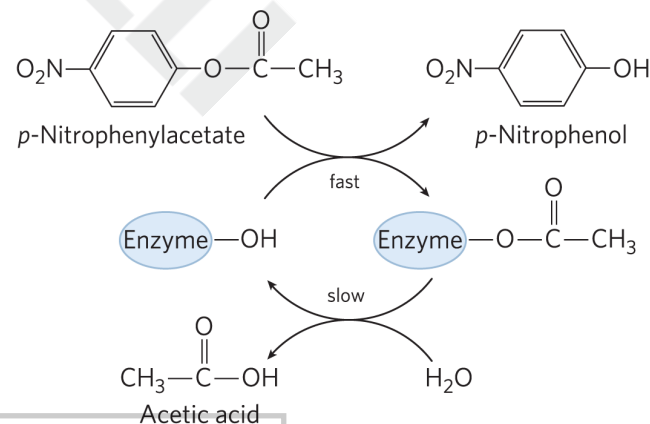
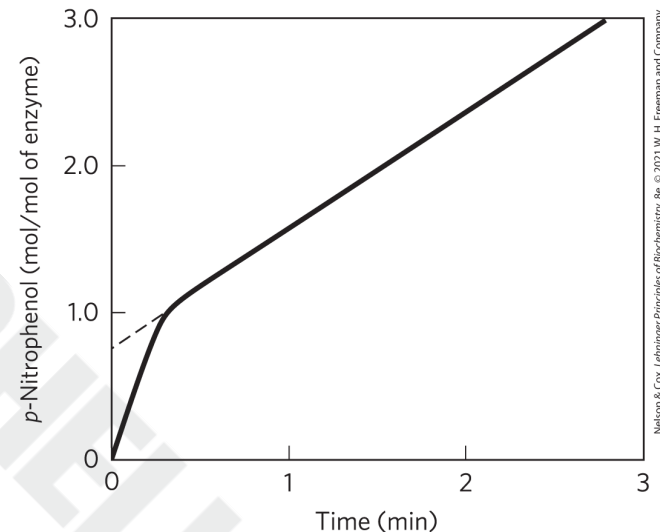
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The Chymotrypsin Mechanism Involves Two Phases

- acylation phase = the peptide bond is cleaved and an ester linkage is formed between the peptide carbonyl carbon and the enzyme
- deacylation phase = the ester linkage is hydrolyzed and the nonacylated enzyme is regenerated

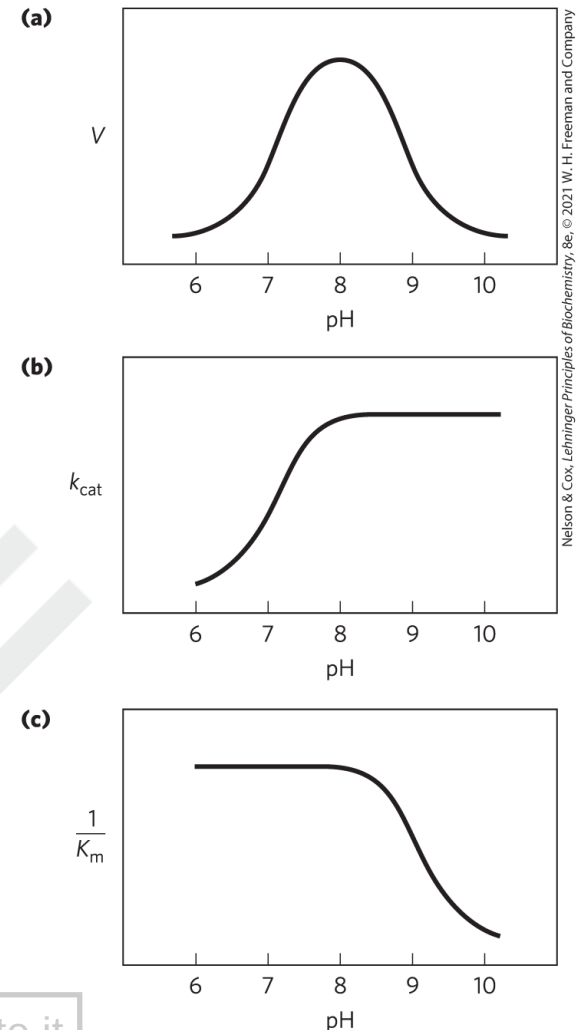
Pre-Steady State Kinetic Evidence for an Acyl-Enzyme Intermediate

- presence of a burst suggested that turnover of the enzyme was limited by a subsequent, slower deacylation step



The pH Dependence of Chymotrypsin-Catalyzed Reactions

- optimal activity at pH 8
 - His⁵⁷ is unprotonated and Ile¹⁶ is protonated
- the transition just above pH 7 is due to changes in k_{cat}
 - results from protonation of His⁵⁷
- the transition above pH 8.5 is due to changes in $1/K_m$
 - results from the ionization of the α -amino group of Ile¹⁶



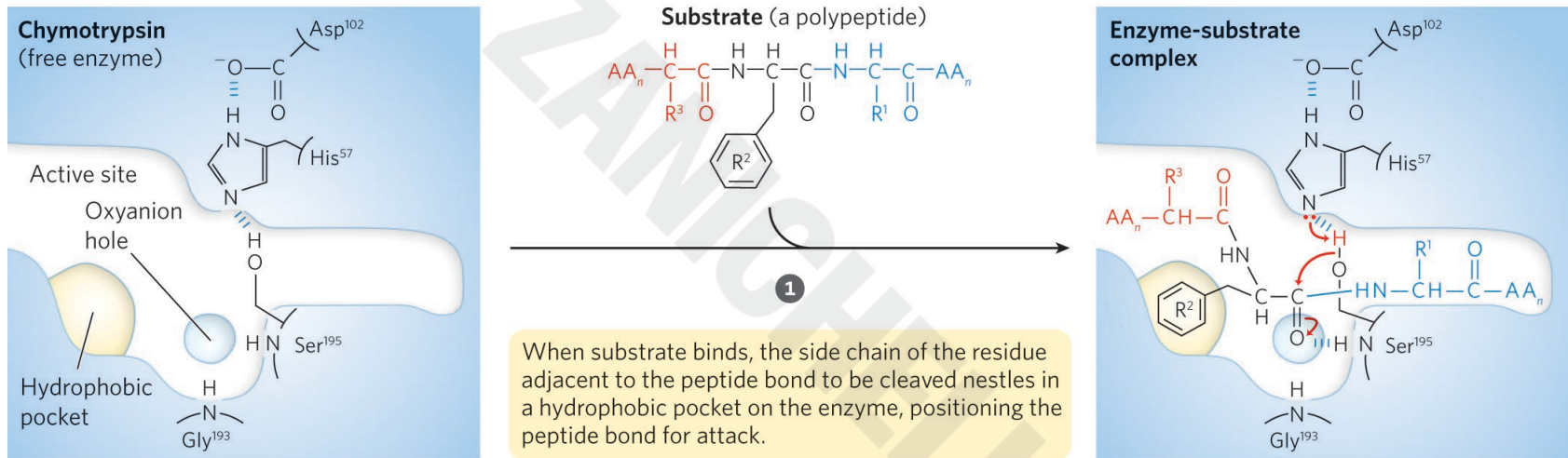
The Chymotrypsin Reaction

- **serine proteases** = proteases with a Ser residue that acts as the nucleophile
 - acylation phase nucleophile is the oxygen of Ser¹⁹⁵
- **catalytic triad** = hydrogen bonding network
 - in chymotrypsin, Ser¹⁹⁵ is linked to His⁵⁷ and Asp¹⁰²

Principle 4 (6 of 8)

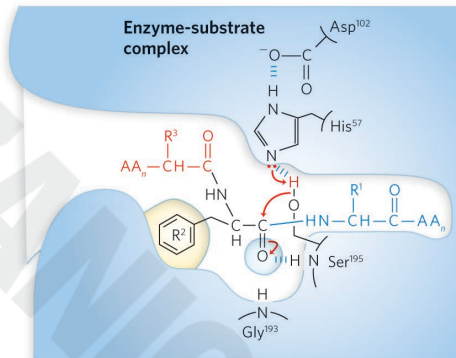
Two concepts explain the catalytic power of enzymes. First, enzymes bind most tightly to the transition state of the catalyzed reaction, using binding energy to lower the activation barrier. Second, enzyme active sites are organized by evolution to facilitate multiple mechanisms of chemical catalysis simultaneously.

Chymotrypsin Mechanism, Step 1



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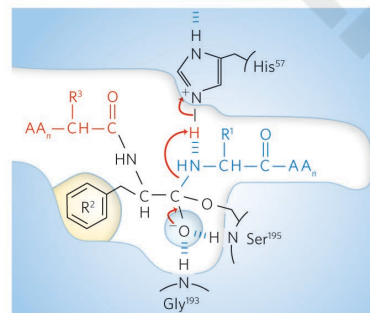
Chymotrypsin Mechanism, Step 2



2

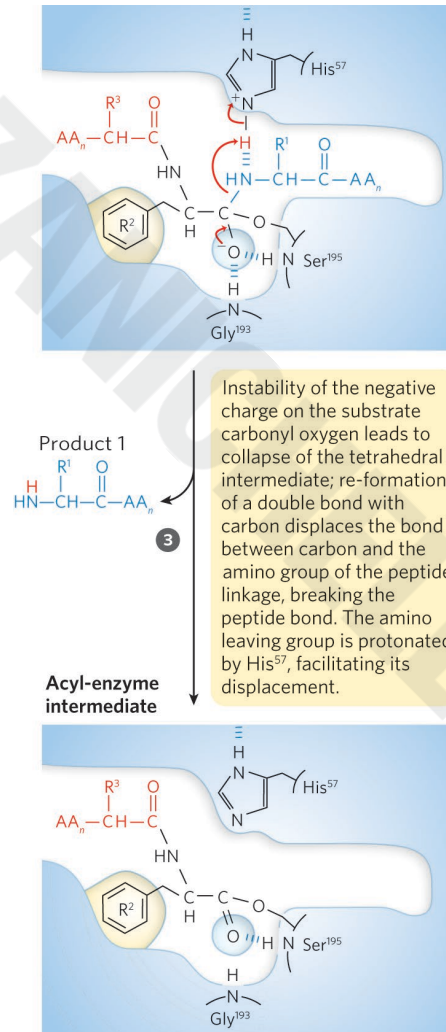
Short-lived intermediate* (acylation)

Interaction of Ser¹⁹⁵ and His⁵⁷ generates a strongly nucleophilic alkoxide ion on Ser¹⁹⁵; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of a short-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.



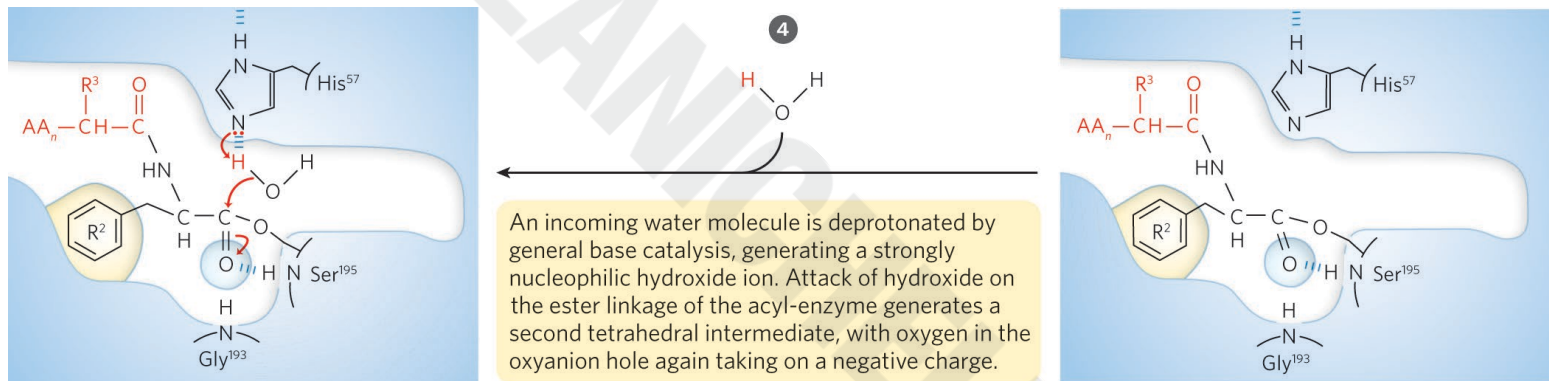
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Chymotrypsin Mechanism, Step 3



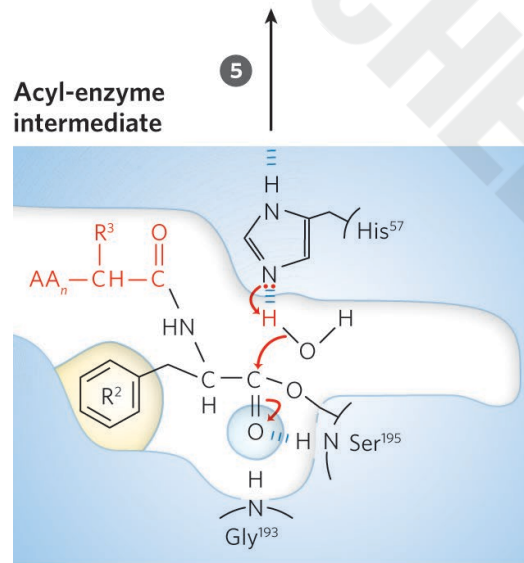
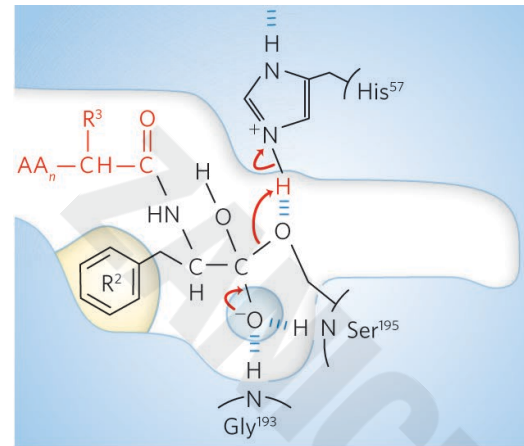
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Chymotrypsin Mechanism, Step 4



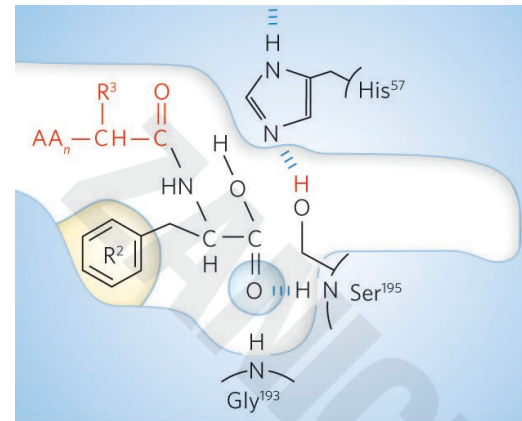
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Chymotrypsin Mechanism, Step 5



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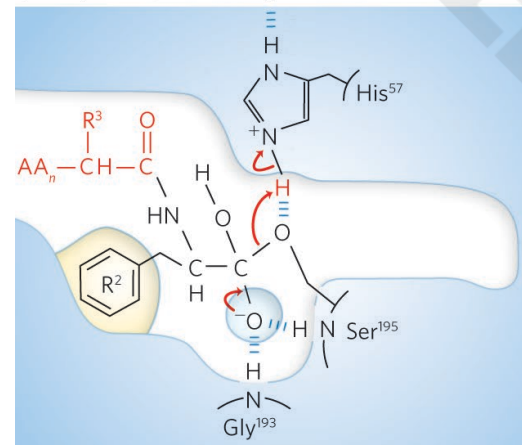
Chymotrypsin Mechanism, Step 6



Dissociation of the second product from the active site regenerates free enzyme.

Short-lived intermediate*
(deacylation)

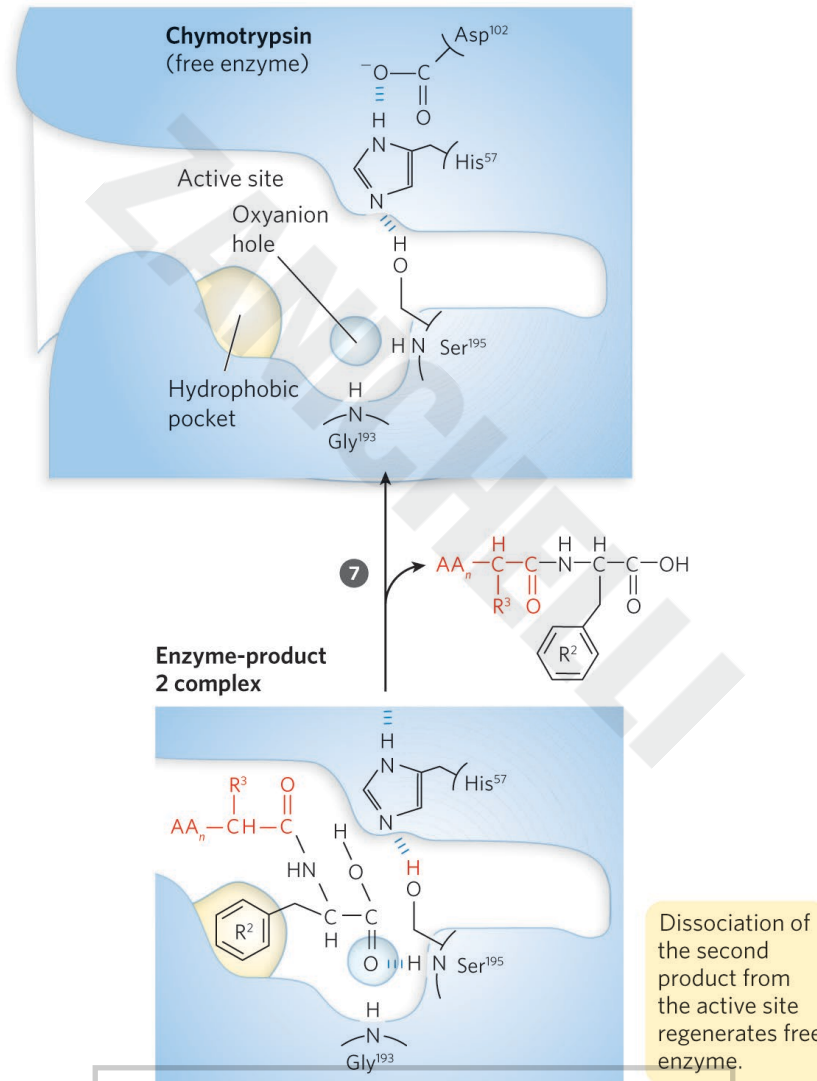
6



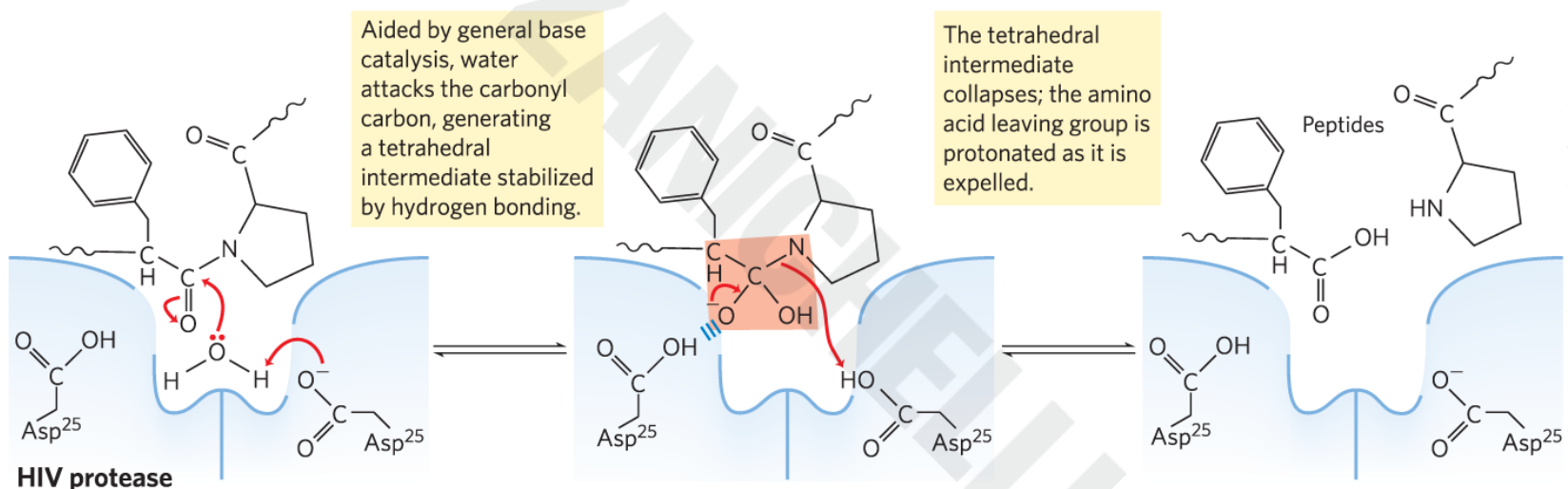
Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser¹⁹⁵.

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Chymotrypsin Mechanism, Step 7



Mechanism of Action of an HIV Protease



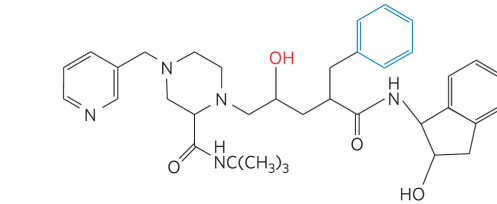
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Principle 4 (7 of 8)

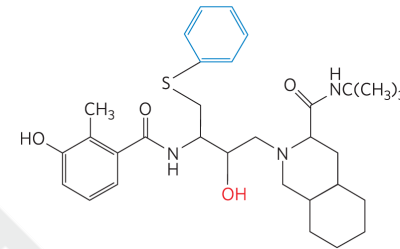
Two concepts explain the catalytic power of enzymes. First, enzymes bind most tightly to the transition state of the catalyzed reaction, using binding energy to lower the activation barrier. Second, enzyme active sites are organized by evolution to facilitate multiple mechanisms of chemical catalysis simultaneously.

HIV Protease Inhibitors

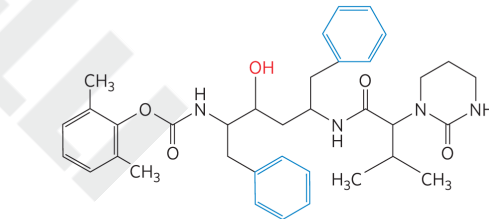
- HIV protease inhibitors form noncovalent complexes with the enzyme
 - designed as transition-state analogs
 - can be considered irreversible inhibitors



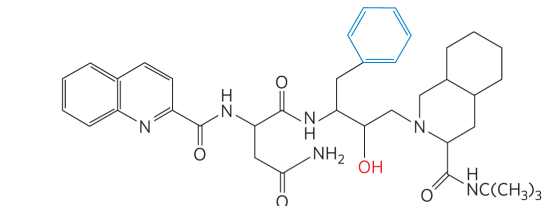
Indinavir



Nelfinavir



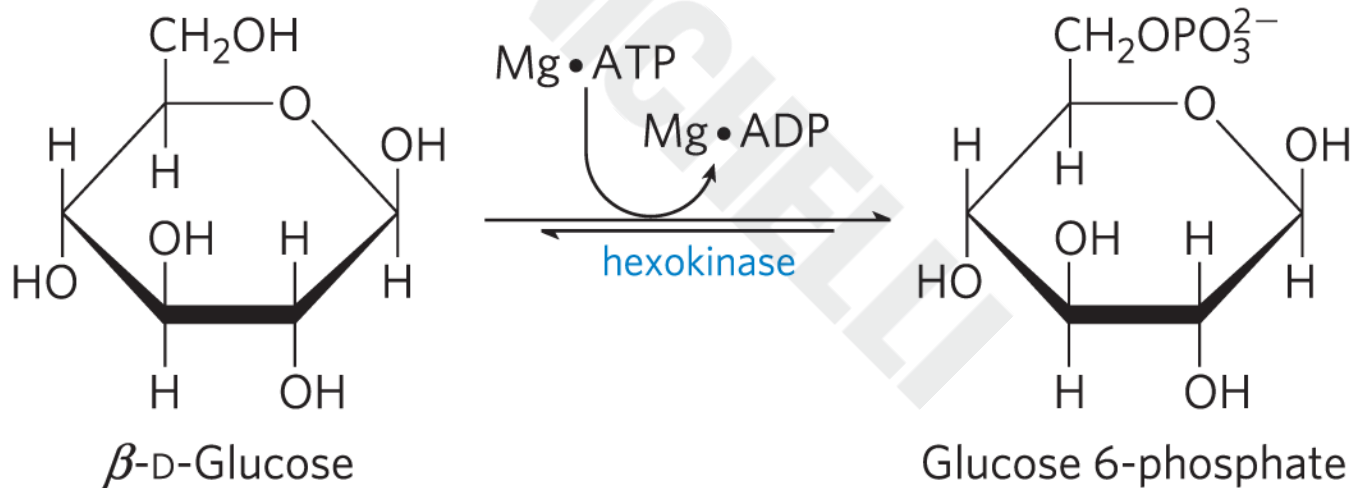
Lopinavir



Saquinavir

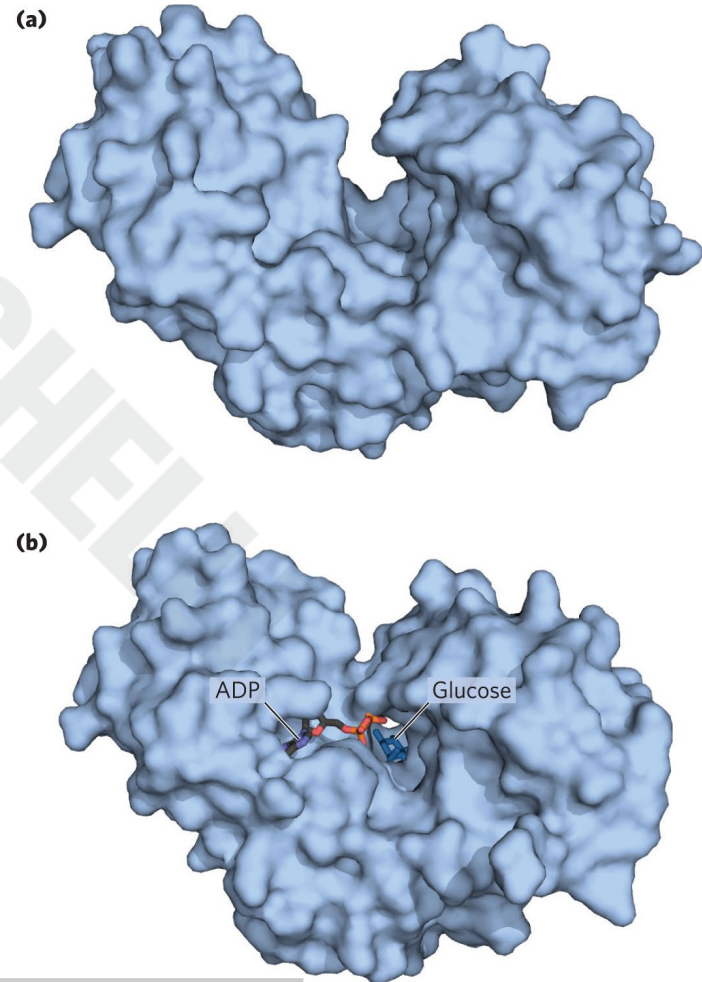
Hexokinase Undergoes Induced Fit on Substrate Binding

- yeast hexokinase = bisubstrate enzyme that catalyzes the reversible reaction:



Induced Fit in Hexokinase

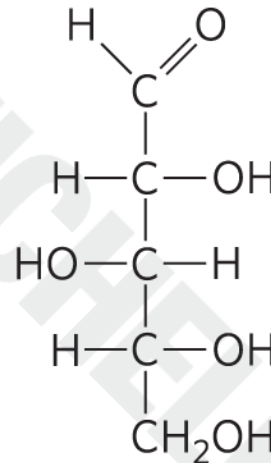
- when glucose (but not water) and $\text{Mg}\cdot\text{ATP}$ bind, the binding energy derived induces a conformational change in hexokinase to the catalytically active form



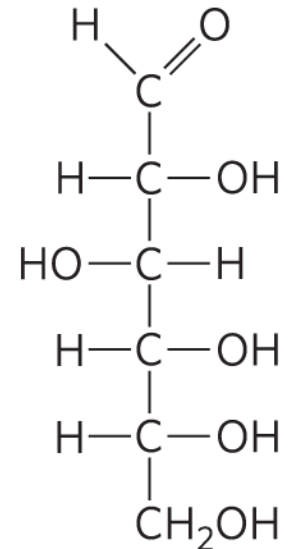
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Addition of Xylose Increases the Rate of ATP Hydrolysis

- xylose cannot be phosphorylated
- binding of xylose induces a change in hexokinase to its active conformation



Xylose



Glucose

Principle 4 (8 of 8)

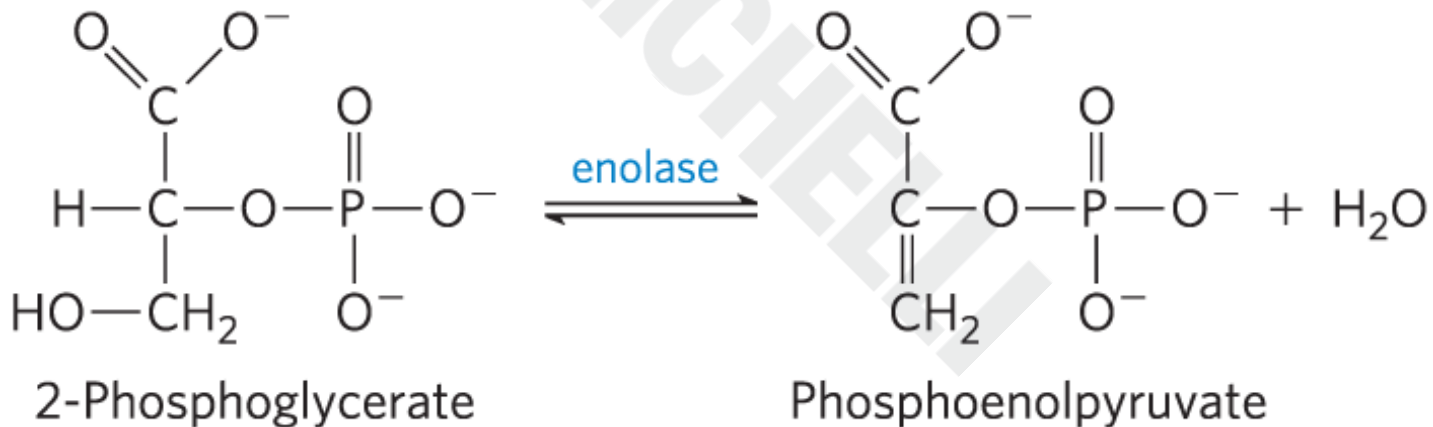
Two concepts explain the catalytic power of enzymes. First, enzymes bind most tightly to the transition state of the catalyzed reaction, using binding energy to lower the activation barrier. Second, enzyme active sites are organized by evolution to facilitate multiple mechanisms of chemical catalysis simultaneously.

The Catalytic Mechanism of Hexokinase

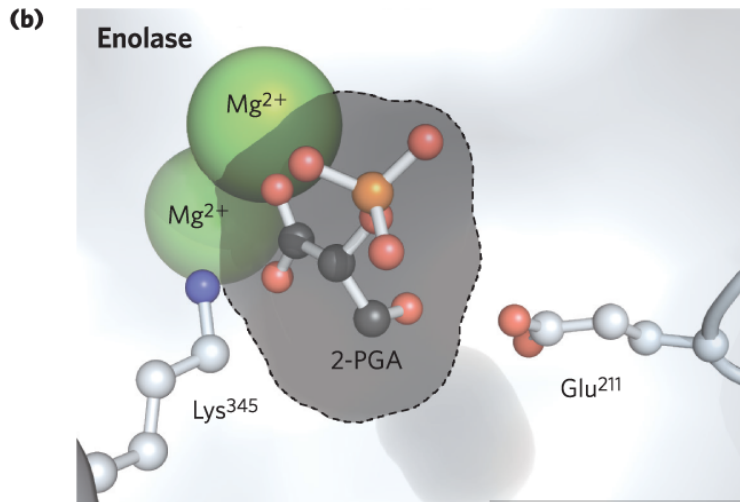
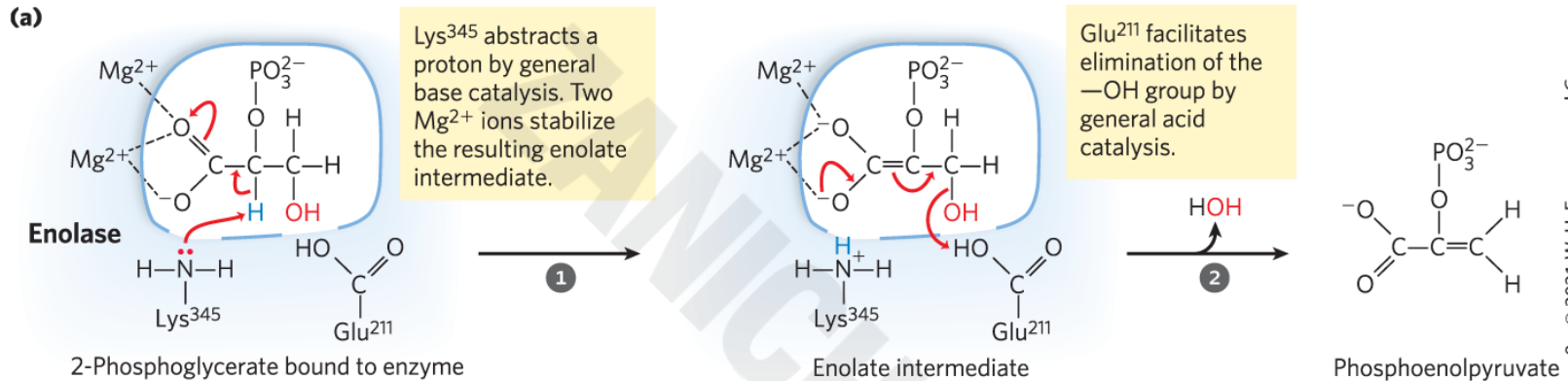
- active-site amino acid residues participate in general acid-base catalysis and transition-state stabilization

The Enolase Reaction Mechanism Requires Metal Ions

- enolase = catalyzes the reversible dehydration of 2-phosphoglycerate to phosphoenolpyruvate:

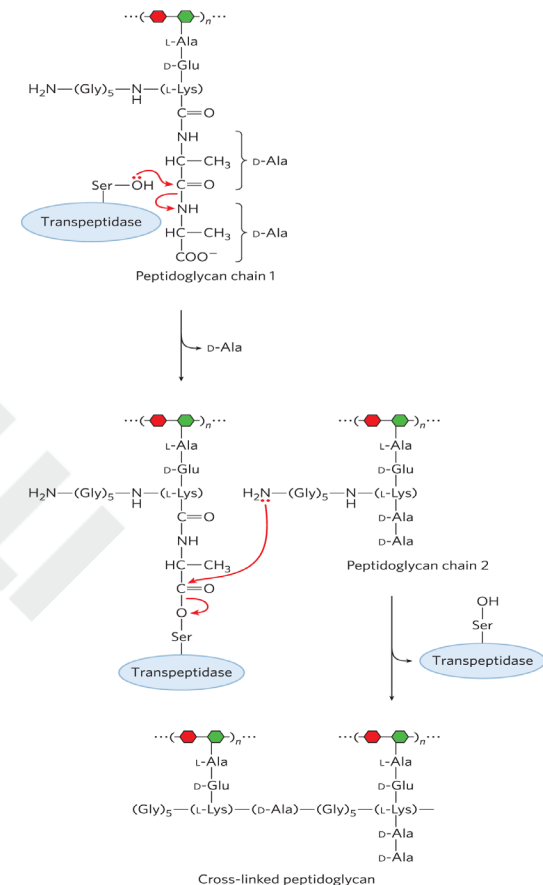


The Enolase Reaction Illustrates Metal Ion Catalysis



An Understanding of Enzyme Mechanism Produces Useful Antibiotics

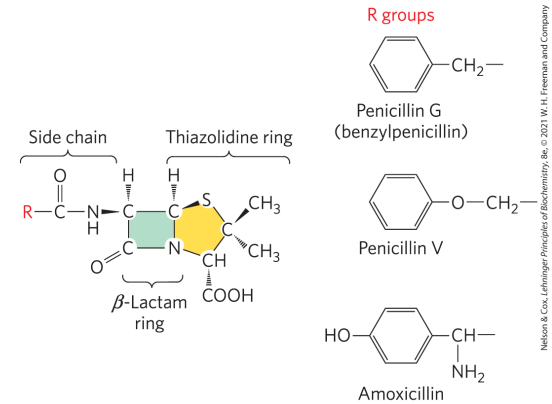
- peptidoglycan = major component of the bacterial cell wall
 - consists of polysaccharides and peptides cross-linked in several steps that include a transpeptidase reaction



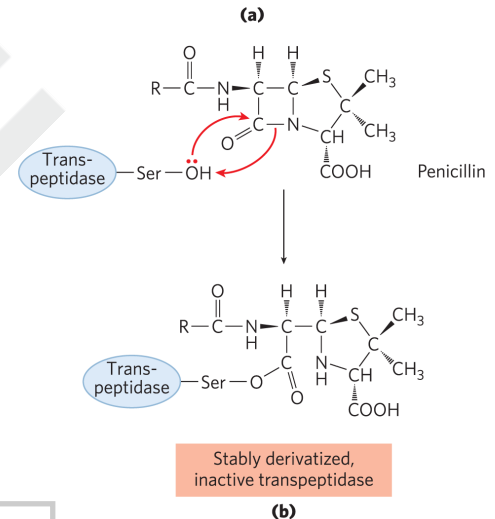
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Transpeptidase Inhibition by β -Lactam Antibiotics

- β -lactam antibiotics bind to the active site of transpeptidase
- the covalent complex irreversibly inactivates the enzyme, blocking synthesis of the cell wall

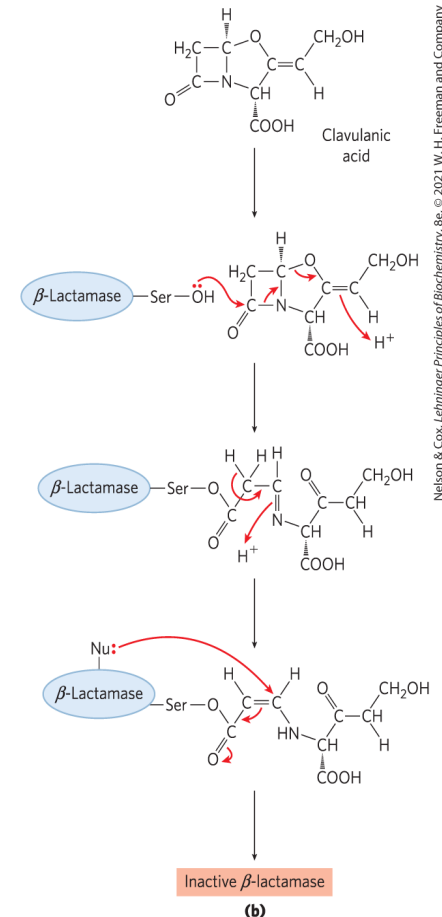
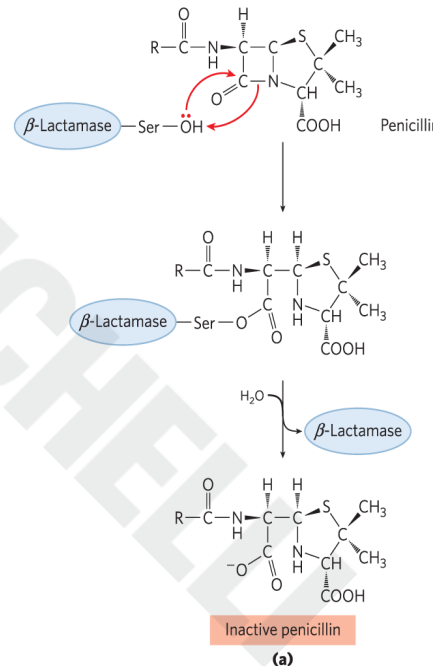


General structure of penicillins



β -Lactamases

- **β -lactamases** = enzymes that cleave β -lactam antibiotics
- bacteria that express β -lactamases become resistant to β -lactam antibiotics
- compounds that mimic the structure of a β -lactam antibiotic can inactivate β -lactamases



6.5 Regulatory Enzymes

Principle 5 (2 of 3)

Many enzymes are regulated. Regulatory mechanisms include reversible covalent modification, binding of allosteric modulators, proteolytic activation, noncovalent binding to regulatory proteins, and elaborate regulatory cascades. Enzymes are often subject to multiple methods of regulation, which allows for exquisite control of every chemical process that occurs in a cell.

Regulatory Enzymes

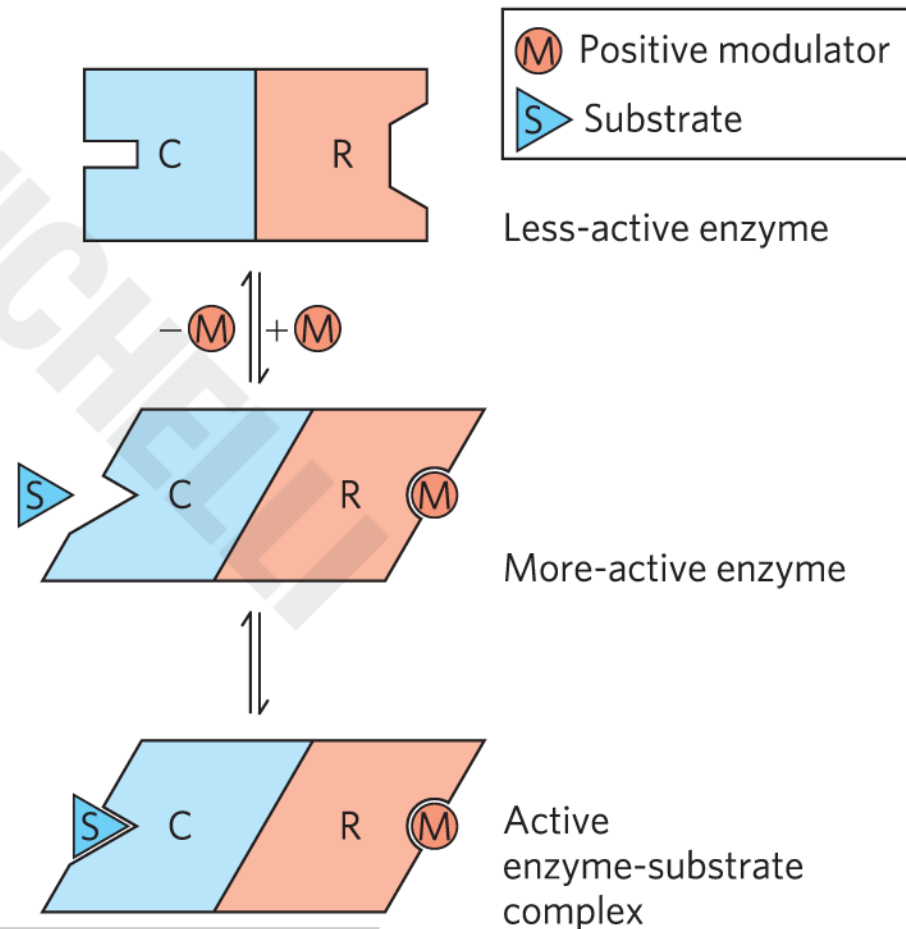
- **regulatory enzymes** = catalytic activity increases or decreases in response to certain signals
 - allows the cell to meet changing needs for energy and biomolecules

Modulation of Regulatory Enzymes

- activities of regulatory enzymes are modulated in a variety of ways:
 - **allosteric enzymes** = function through reversible, noncovalent binding of regulatory compounds called **allosteric modulators** or **allosteric effectors** (small metabolites or cofactors)
 - reversible **covalent modification**
 - binding of separate **regulatory proteins**
 - removal of peptide segments by **proteolytic cleavage**

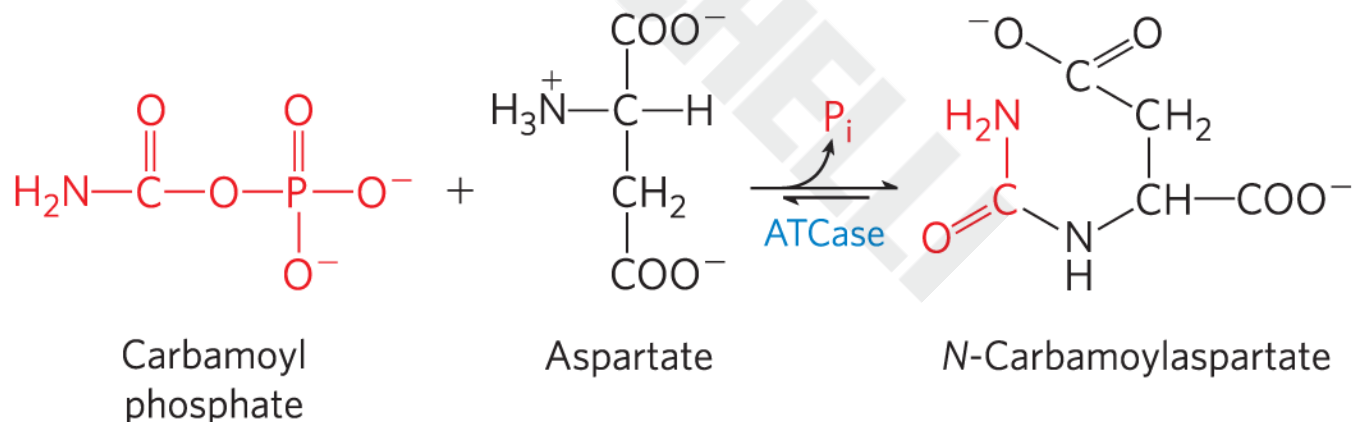
Allosteric Enzymes Undergo Conformational Changes in Response to Modulator Binding

- **homotropic** = regulation in which the substrate and modulator are identical
- **heterotropic** = regulation in which the modulator is a molecule other than the substrate



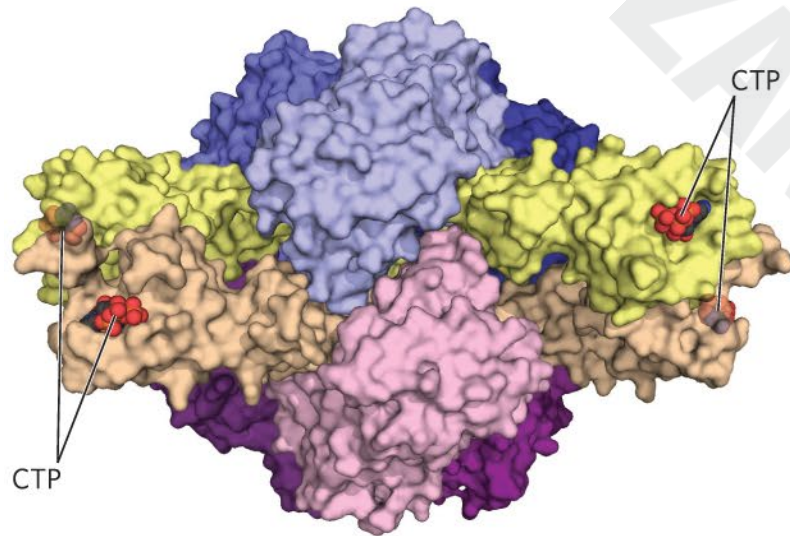
The Regulatory Enzyme Aspartate Transcarbamoylase

- aspartate transcarbamoylase (ATCase) = catalyzes the formation of carbamoyl aspartate, an early step in pyrimidine biosynthesis:

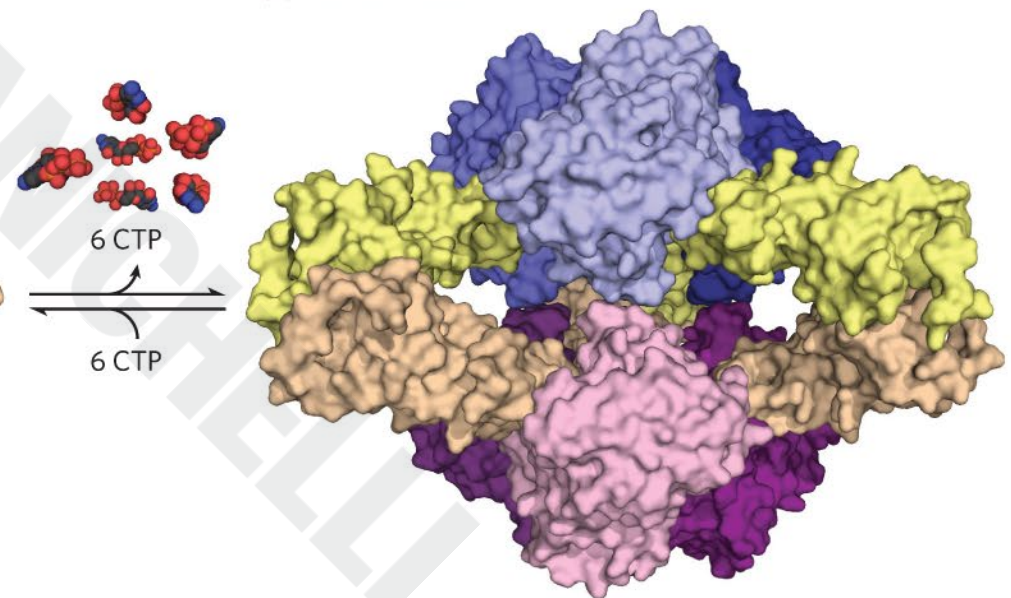


The Quaternary Structure of Aspartate Transcarbamoylase

(a) Inactive T state



(b) Active R state



ATP = Positive regulator

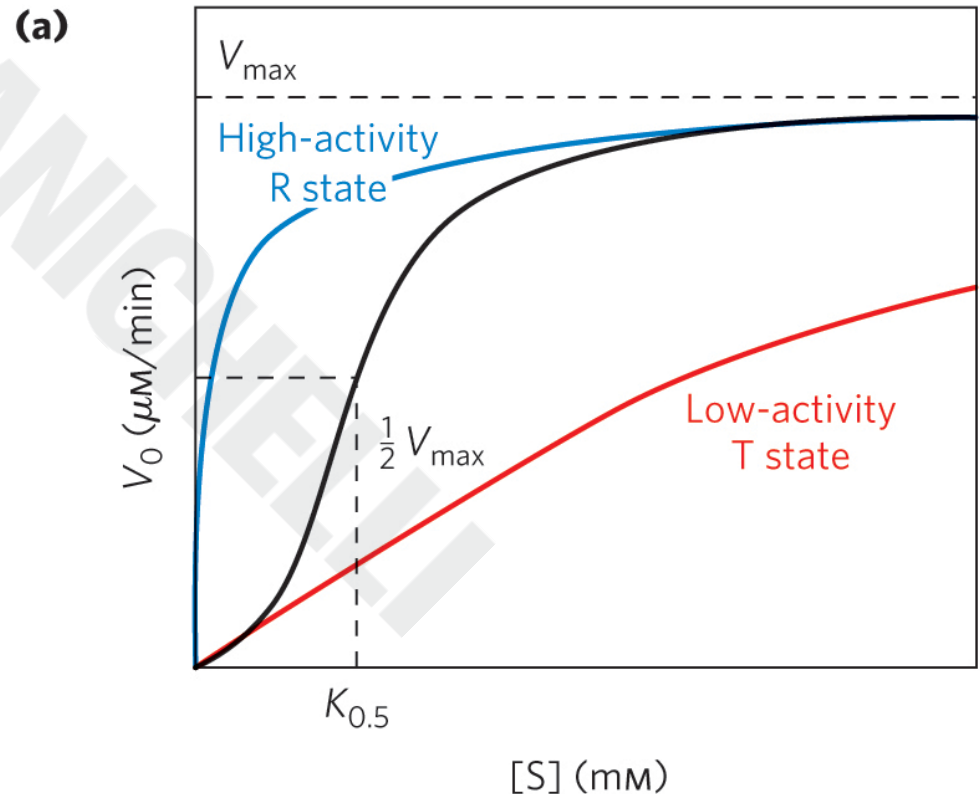
CTP = Negative regulator

The Kinetic Properties of Allosteric Enzymes Diverge from Michaelis-Menten Behavior

- plots of V_0 versus $[S]$ usually produce a sigmoid saturation curve, rather than a hyperbolic curve
- $[S]_{0.5}$ or $K_{0.5}$ represents the $[S]$ giving half-maximal velocity of the reaction

The Sigmoid Curve of a Homotropic Enzyme

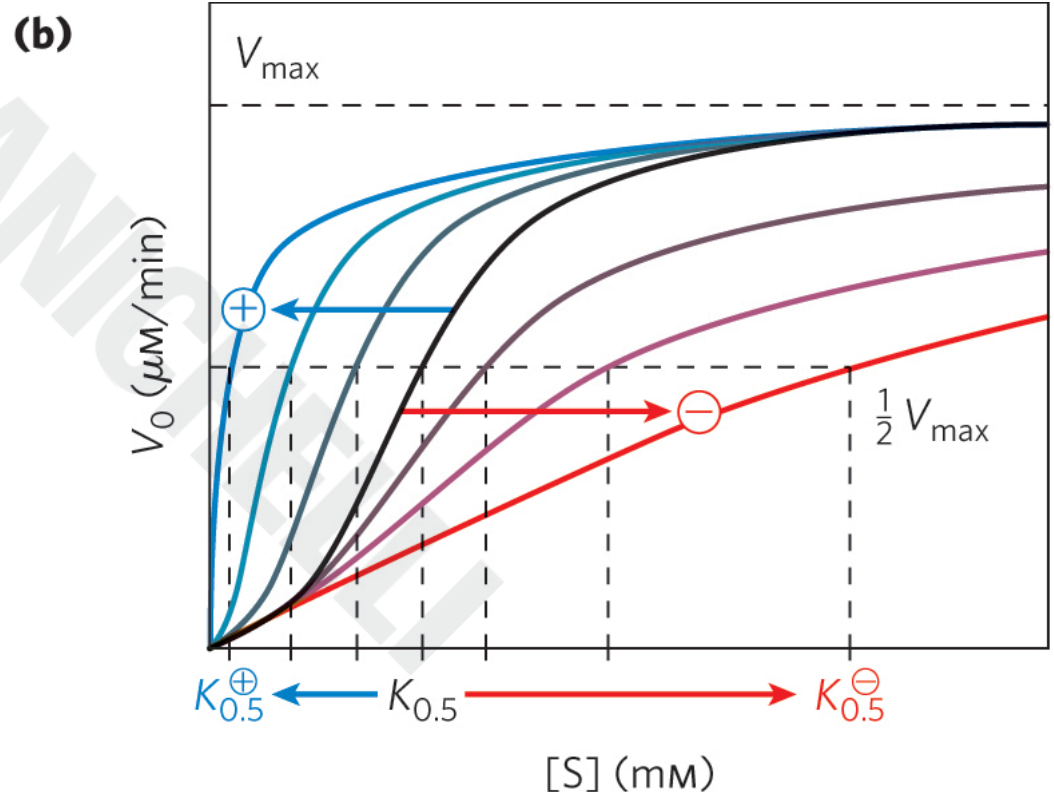
- a relatively small increase in $[S]$ in the steep part of the curve causes a comparatively large increase in V_0



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Modulation in Which $K_{0.5}$, but Not $V_{\max}$, Is Altered

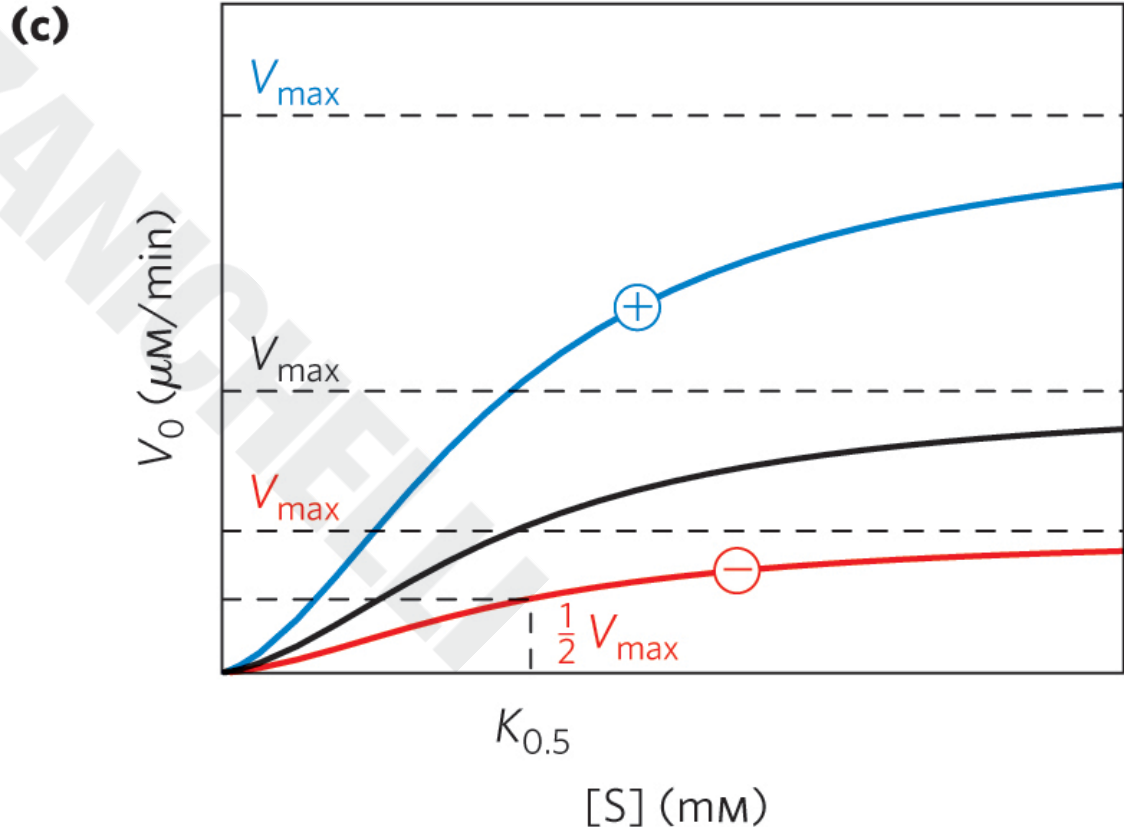
- for heterotropic allosteric enzymes:
 - activators may cause the curve to become more hyperbolic
 - inhibitor may cause the curve to become more sigmoidal



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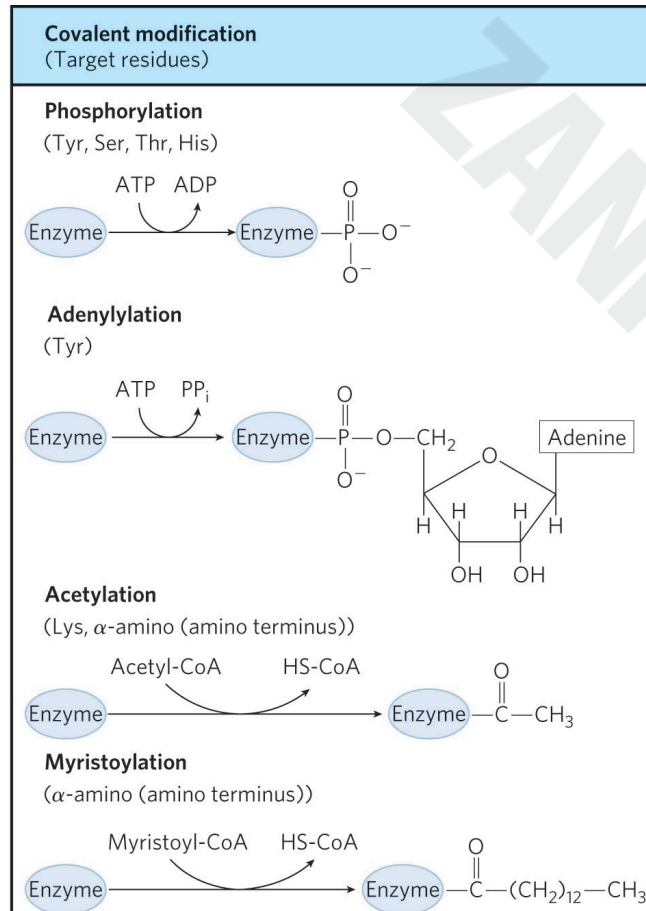
Modulation in Which $V_{\max}$, but Not $K_{0.5}$, Is Altered

- less common type of modulation for heterotropic allosteric enzymes

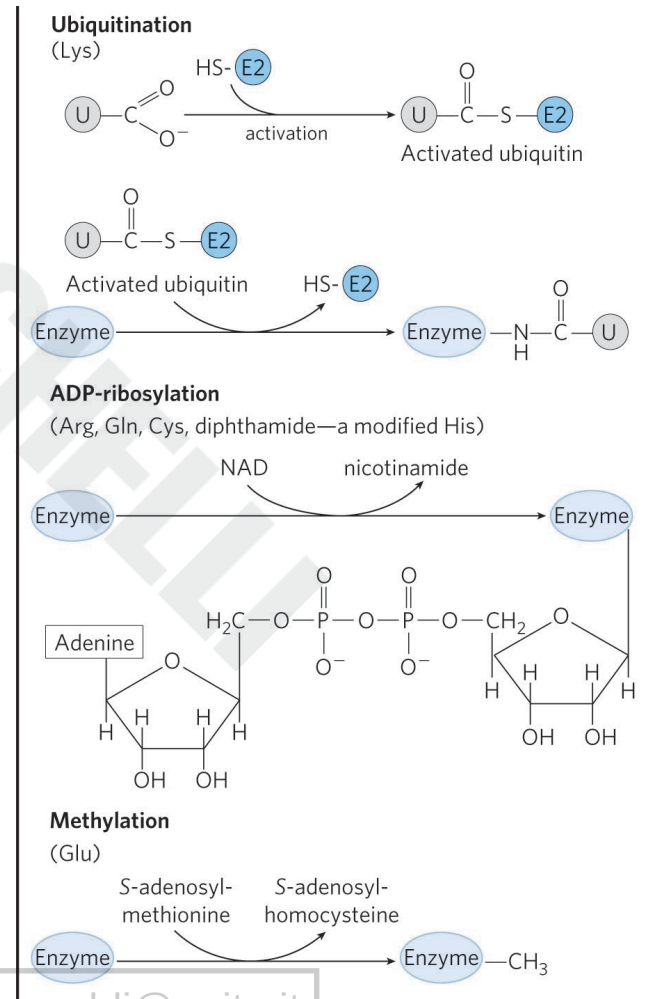


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Some Enzymes Are Regulated by Reversible Covalent Modification



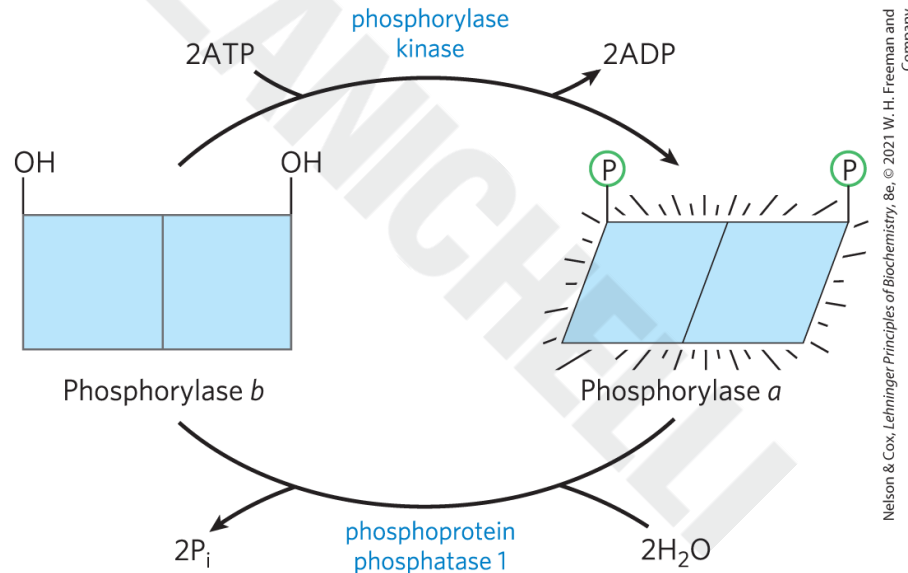
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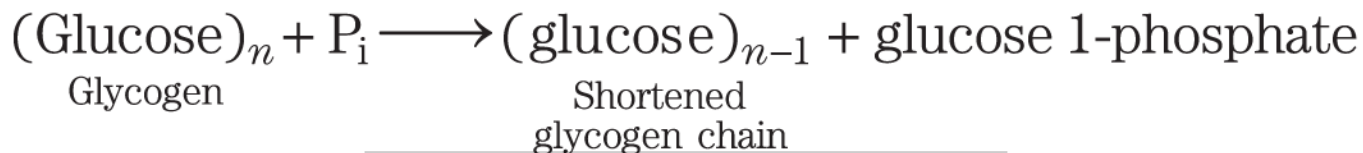
Phosphoryl Groups Affect the Structure and Catalytic Activity of Enzymes

- **protein kinases** = catalyze the attachment of phosphoryl groups to specific amino acid residues (Ser, Thr, Tyr, His)
- **phosphoprotein phosphatases** = **protein phosphatases**
= remove phosphoryl groups from the same target proteins

Regulation of Muscle Glycogen Phosphorylase Activity by Phosphorylation

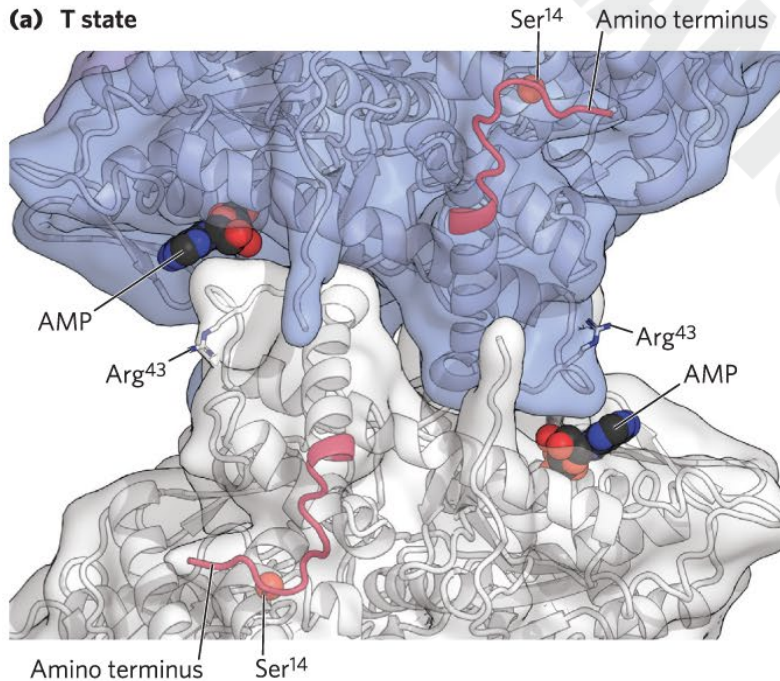


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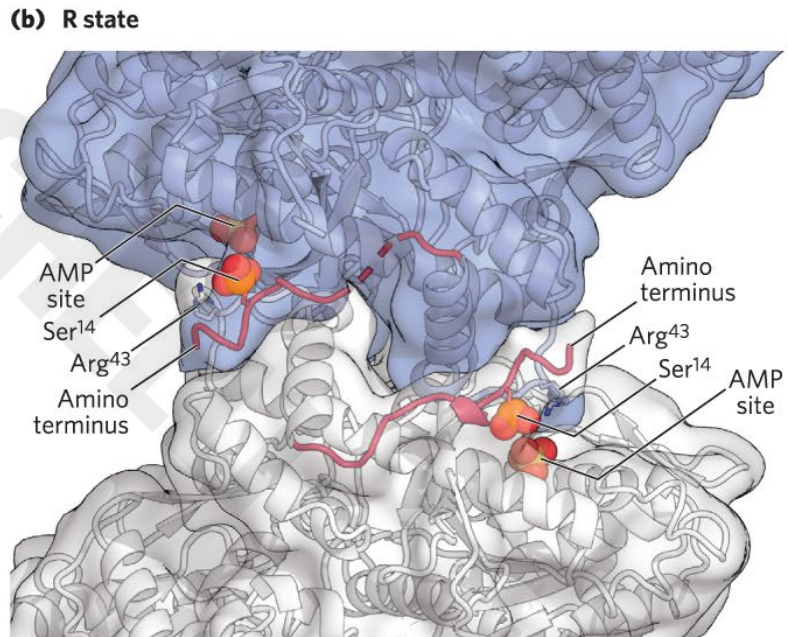


Phosphorylation in Glycogen Phosphorylase Causes a Conformational Change

(a) T state



(b) R state



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Multiple Phosphorylations Allow Exquisite Regulatory Control

- residues that are typically phosphorylated in regulated proteins occur within common structural motifs (consensus sequences)

Common Recognition Sequences for Protein Kinases

Table 6-10 Consensus Recognition Sequences for a Few Protein Kinases

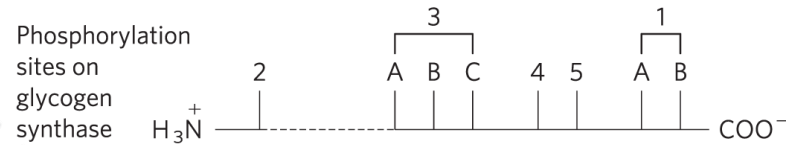
Protein kinase	Consensus sequence and phosphorylated residue
Protein kinase A	-x-R-[RK]-x-[ST]-B-
Protein kinase G	-x-R-[RK]-x-[ST]-X-
Protein kinase C	-[RK](2)-x-[ST]-B-[RK](2)-
Protein kinase B	R-x-x-R-x-[ST]-x-ψ-
Ca ²⁺ /calmodulin kinase I	-B-x-R-x(2)-[ST]-x(3)-B-
Ca ²⁺ /calmodulin kinase II	-B-x-[RK]-x(2)-[ST]-x(2)-
Myosin light chain kinase (smooth muscle)	-K(2)-R-x(2)- S -x-B(2)-
Phosphorylase <i>b</i> 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)-vABL-[YLV]- Y -X ₁₋₃ -[PF]-
Epidermal growth factor (EGF) receptor kinase	-E(4)- Y -F-E-L-V-

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Multiple Regulatory Phosphorylations

- provide the potential for extremely subtle modulation of enzyme activity
- sequential phosphorylation processes can be hierarchical

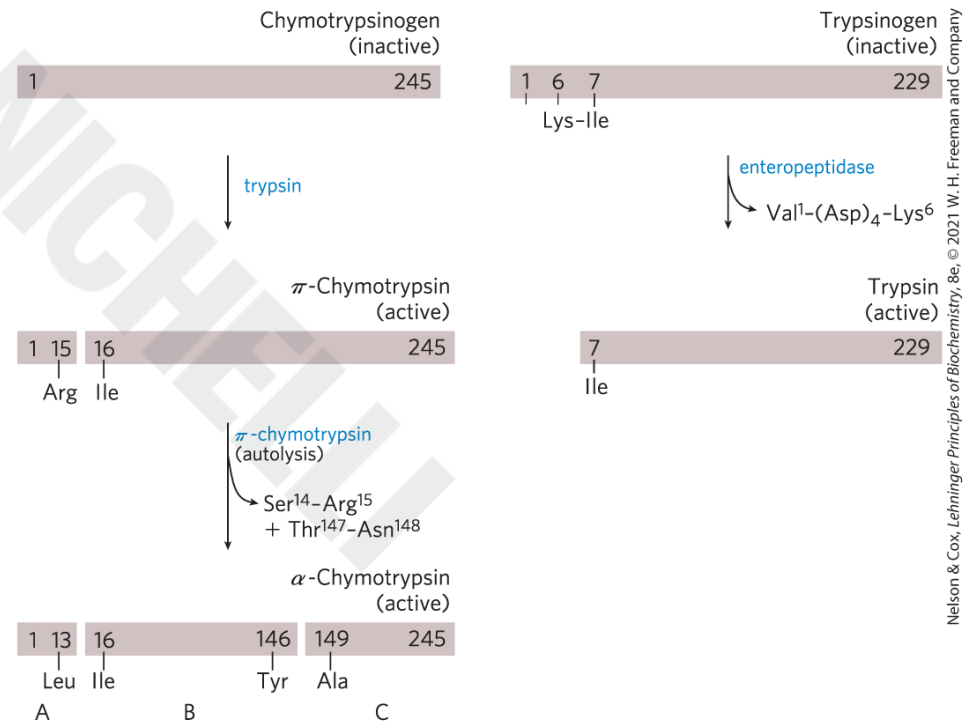


Kinase	Phosphorylation sites	Degree of synthase inactivation
Protein kinase A	1A, 1B, 2, 4	+
Protein kinase G	1A, 1B, 2	+
Protein kinase C	1A	+
Ca ²⁺ /calmodulin kinase	1B, 2	+
Phosphorylase <i>b</i> kinase	2	+
Casein kinase I	At least nine	+
Casein kinase II	5	0
Glycogen synthase kinase 3	3A, 3B, 3C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

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Some Enzymes and Other Proteins Are Regulated by Proteolytic Cleavage of an Enzyme Precursor

- **zymogen** = inactive precursor that is cleaved to form an active protease enzyme
- **proprotein** or **proenzyme** = precursors that are cleaved to form other proteins



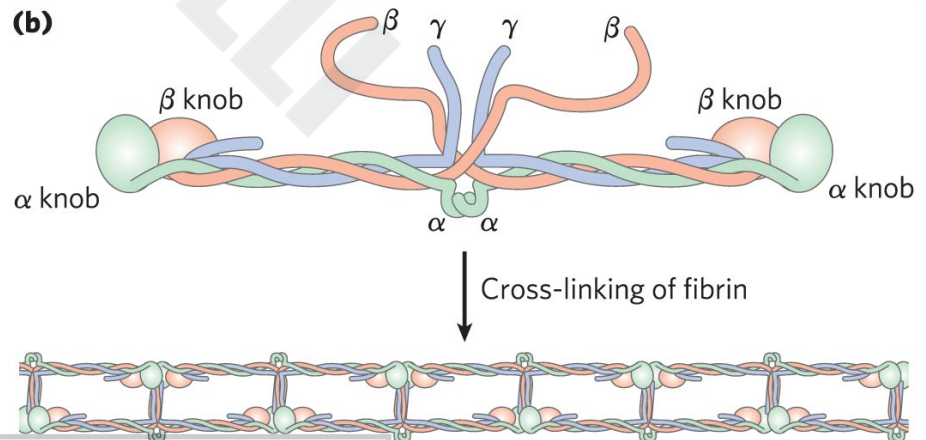
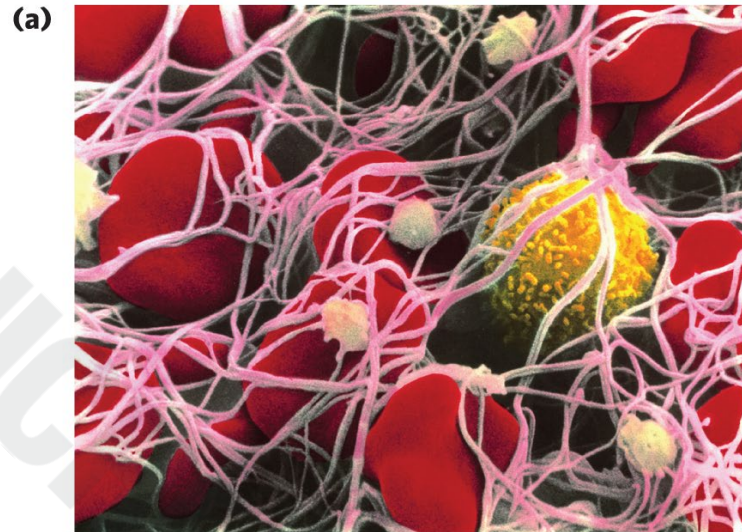
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A Cascade of Proteolytically Activated Zymogens Leads to Blood Coagulation

- **regulatory cascade** = a mechanism that allows a very sensitive response to—and amplification of—a molecular signal
 - example = formation of a blood clot

Blood Clots

- blood clot = aggregate of specialized cell fragments that lack nuclei (**platelets**) cross-linked and stabilized by proteinaceous fibers consisting mainly of the protein **fibrin** (derived from the soluble zymogen **fibrinogen**)



Platelet Activation

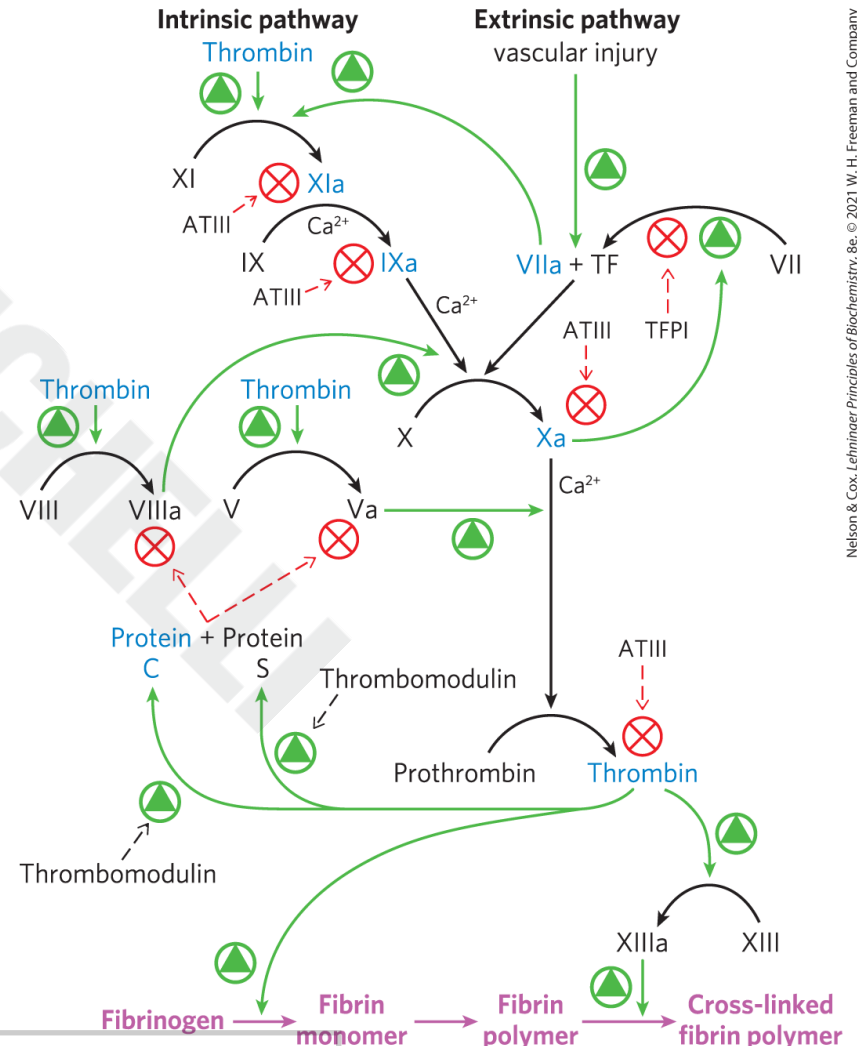
- caused by collagen exposure to blood
- causes the release of signaling molecules such as **thromboxanes** to stimulate the activation of additional platelets

The Cleavage of Fibrinogen to Fibrin

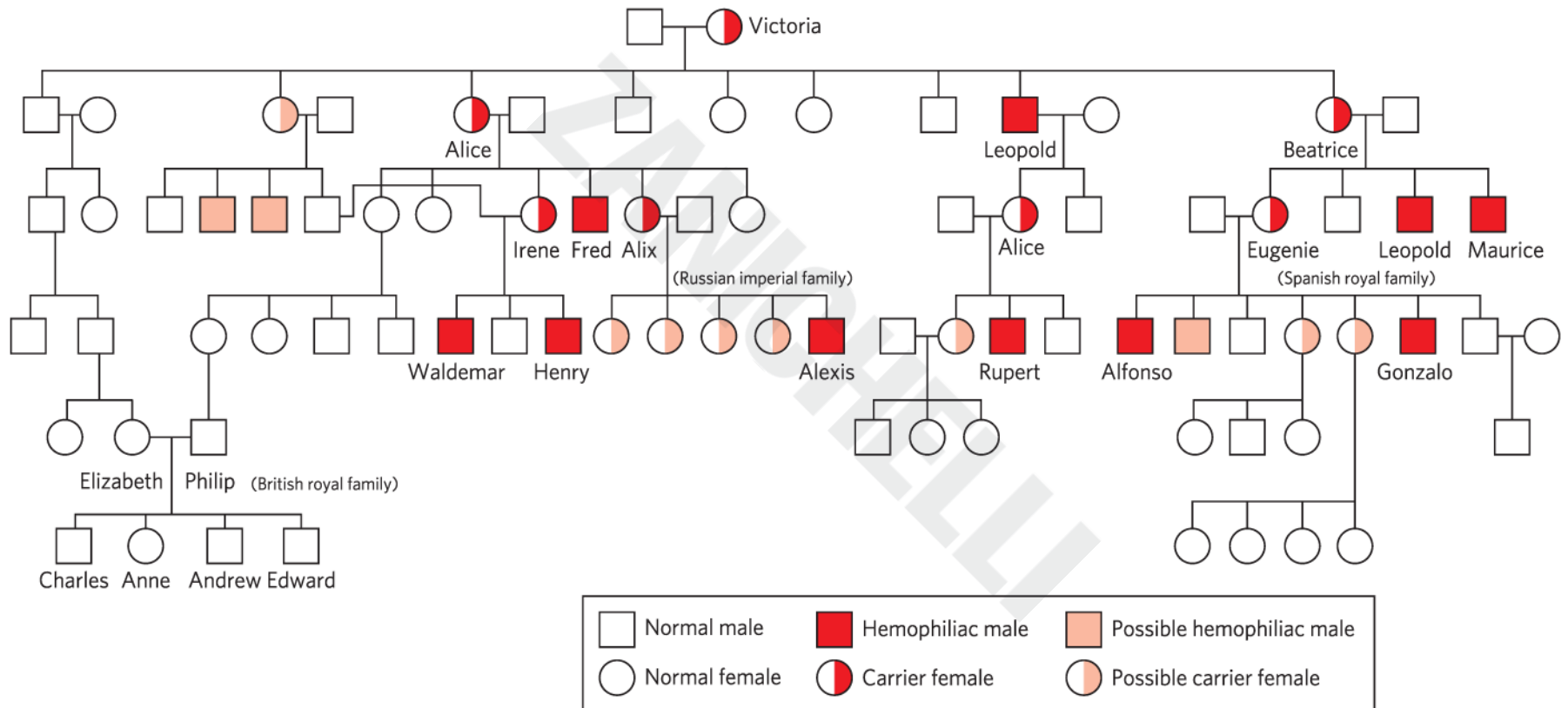
- fibrinogen is converted to fibrin by the proteolytic removal of amino acid residues
- **thrombin** = serine protease that catalyzes peptide removal
- **factor XIIIa** = transglutaminase enzyme that catalyzes the formation of covalent cross-links between fibrins

Two Regulatory Cascades Lead to Fibrinogen Activation

- **intrinsic pathway** = involves all components found in the blood plasma
- **extrinsic pathway** = tissue factor pathway = involves the protein **tissue factor (TF)** which is not present in blood



The Inheritance of Hemophilia B



Principle 5 (3 of 3)

Many enzymes are regulated. Regulatory mechanisms include reversible covalent modification, binding of allosteric modulators, proteolytic activation, noncovalent binding to regulatory proteins, and elaborate regulatory cascades. Enzymes are often subject to multiple methods of regulation, which allows for exquisite control of every chemical process that occurs in a cell.

Some Regulatory Enzymes Use Several Regulatory Mechanisms

- when chemical resources are plentiful, cells synthesize and store glucose and other metabolites
- when chemical resources are scarce, cells use these stores to fuel cellular metabolism
- the availability of specific catalysts allows for the regulation of these reactions