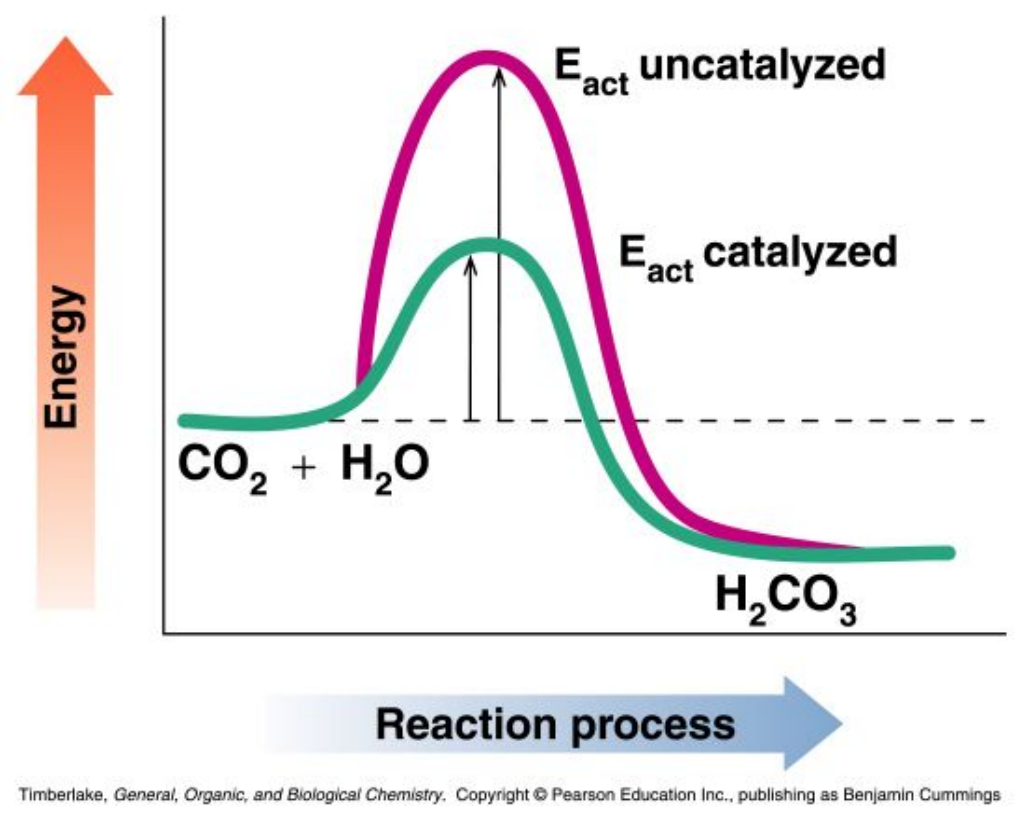
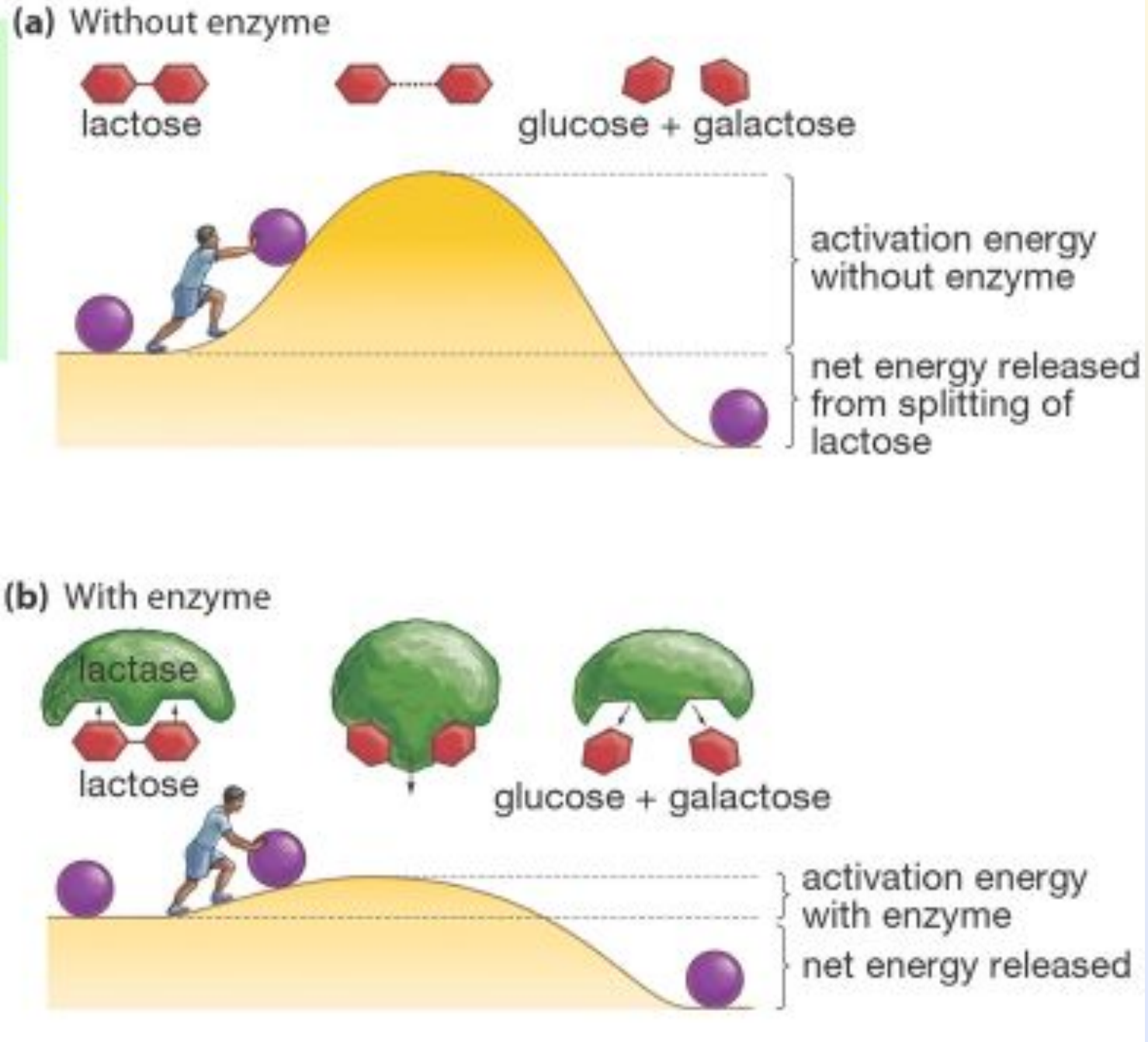
Enzymes

Enzymes as Biological Catalysts

- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation
- They catalyze nearly all the chemical reactions taking place in the cells of the body
- Enzymes have unique three-dimensional shapes that fit the shapes of reactants (**substrates**)



Enzymes Lower a Reaction's Activation Energy



Naming Enzymes

- The name of an enzyme identifies the reacting substance - usually ends in *–ase*
- For example, *sucrase* catalyzes the hydrolysis of sucrose
- The name also describes the function of the enzyme
- For example, *oxidases* catalyze oxidation reactions
- Sometimes common names are used, particularly for the digestion enzymes such as *pepsin* and *trypsin*
- Some names describe both the substrate and the function
- For example, *alcohol dehydrogenase* oxides ethanol

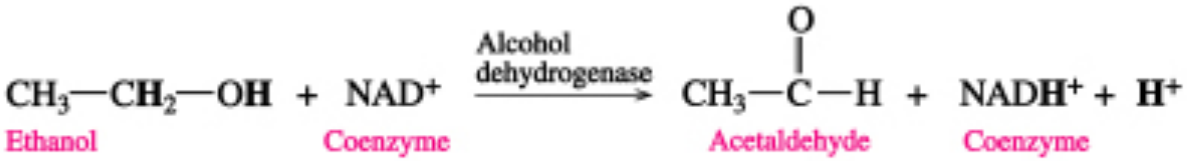
Classification of Enzymes

- Enzymes are classified according to the type of reaction they catalyze:

Class	**Reactions catalyzed**
Oxidoreductases	Oxidation-reduction
Transferases	Transfer groups of atoms
Hydrolases	Hydrolysis
Lyases	Add atoms/remove atoms to/from a double bond
Isomerases	Rearrange atoms
Ligases	Use ATP to combine molecules

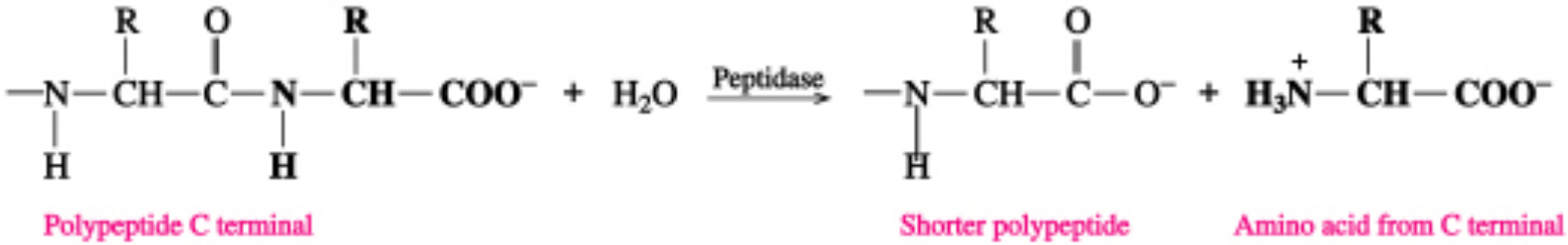
Oxidoreductases, Transferases and Hydrolases

Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds



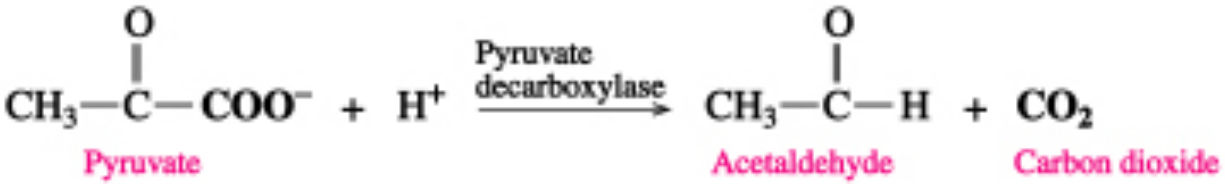
2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups

3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose



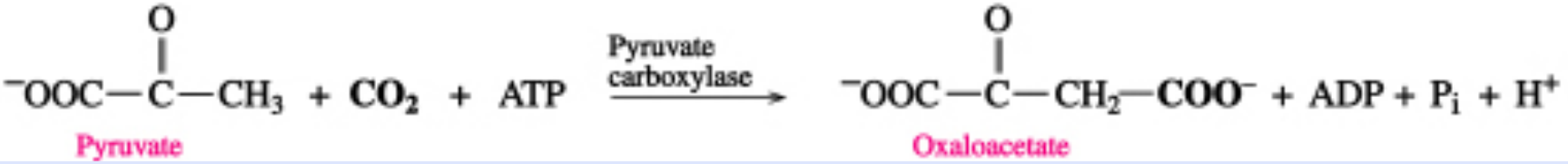
Lyases, Isomerases and Ligases

Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3



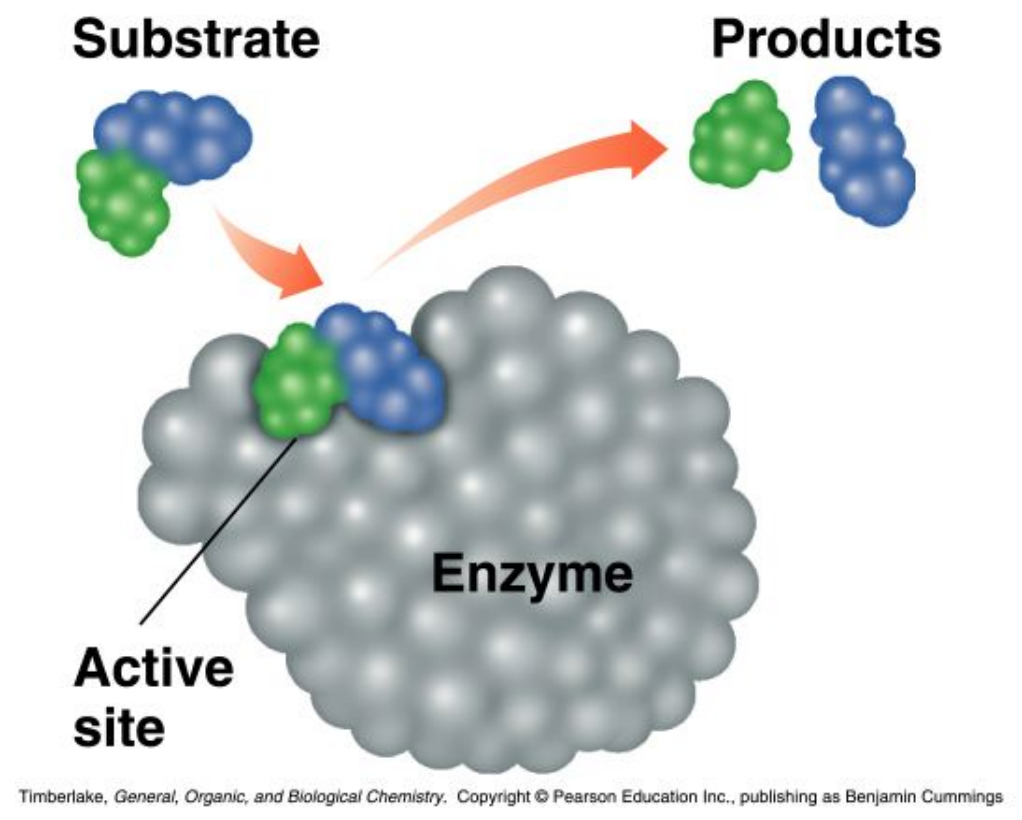
5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers

6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO_2



Active Site of an Enzyme

- The **active site** is a region within an enzyme that fits the shape of substrate molecules
- Amino acid side-chains align to bind the substrate through H-bonding, salt-bridges, hydrophobic interactions, etc.
- Products are released when the reaction is complete (they no longer fit well in the active site)



Enzyme Specificity

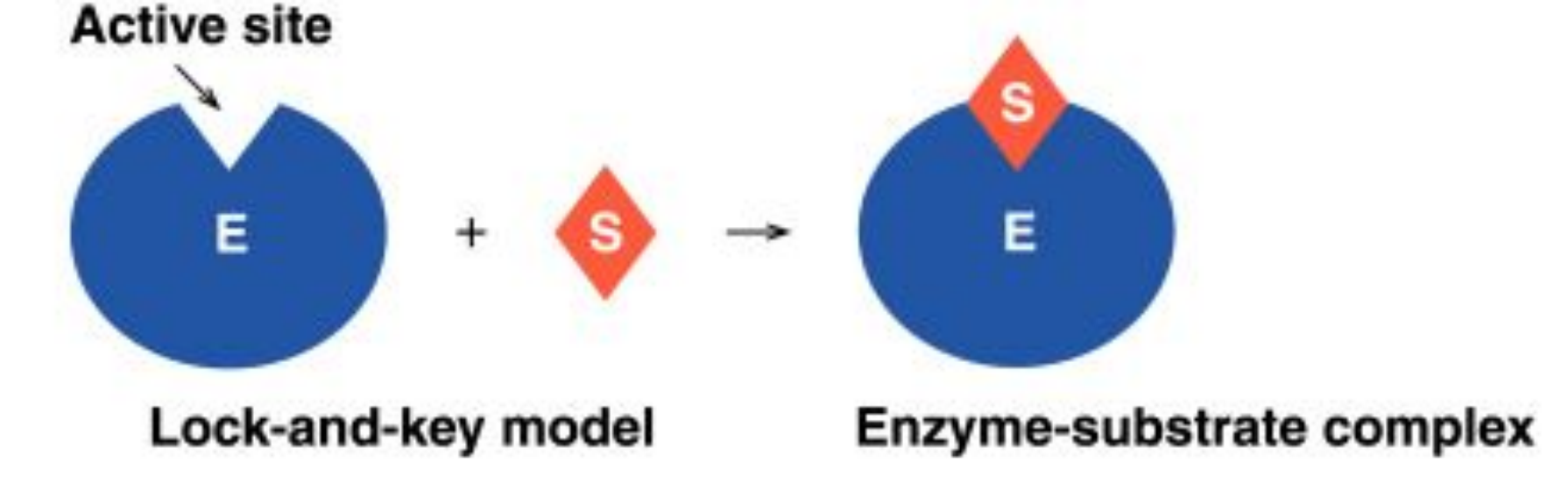
- Enzymes have varying degrees of **specificity** for substrates
- Enzymes may recognize and catalyze:
  - a single substrate
  - a group of similar substrates
  - a particular type of bond

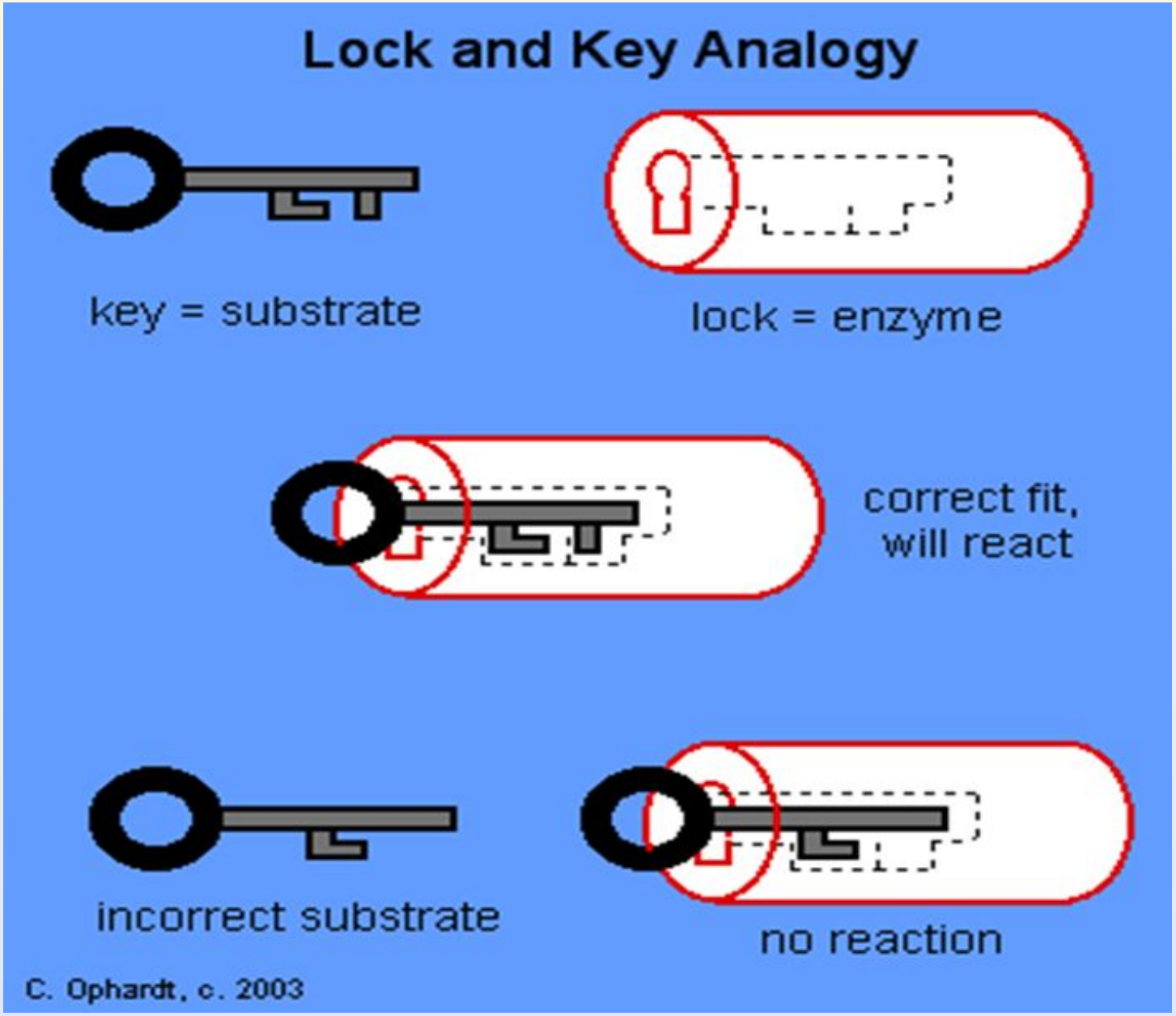
Table 21.2 Types of Enzyme Specificity

Type	Reaction Type	Example
Absolute	Catalyze one type of reaction for a single substrate	Urease catalyzes only the hydrolysis of urea
Group	Catalyze one type of reaction for similar substrates	Hexokinase adds a phosphate group to hexoses
Linkage	Catalyze one type of reaction for a specific type of bond	Chymotrypsin catalyzes the hydrolysis of peptide bonds

Lock-and-Key Model

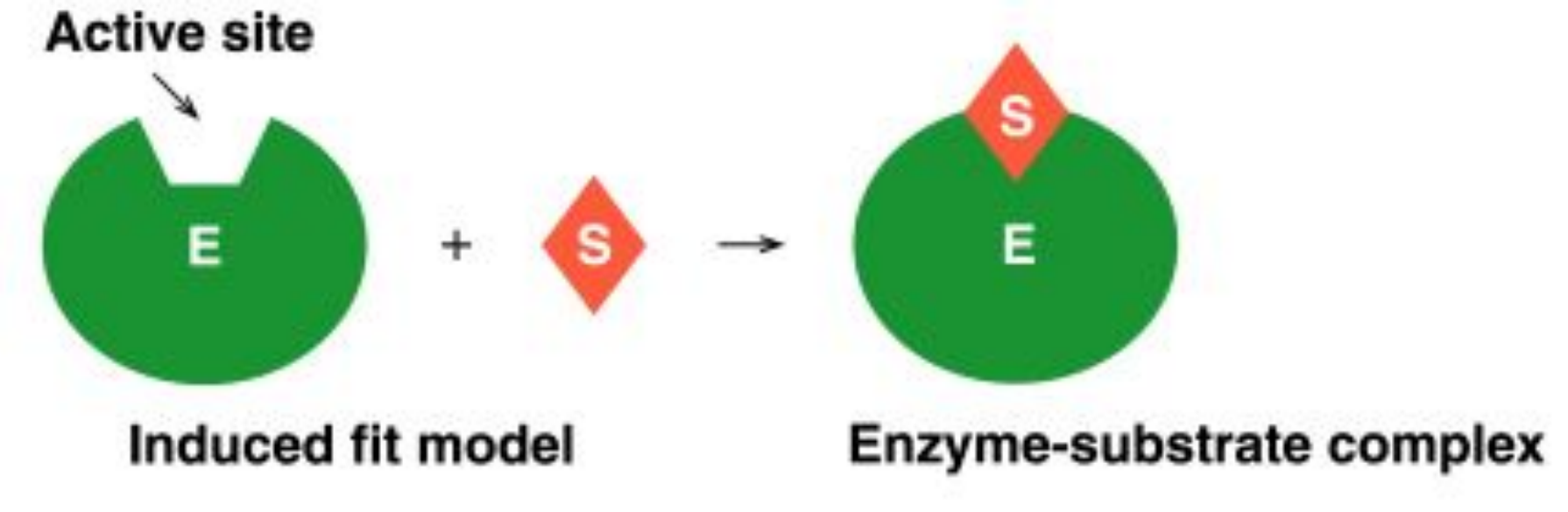
- In the **lock-and-key model** of enzyme action:
  - the active site has a rigid shape
  - only substrates with the matching shape can fit
  - the substrate is a key that fits the lock of the active site
- This is an older model, however, and does not work for all enzymes




Lock and Key Analogy
key = substrate
lock = enzyme
correct fit,
will react
incorrect substrate
no reaction
C. Ophardt, c. 2003

Induced Fit Model

- In the **induced-fit model** of enzyme action:
  - the active site is flexible, not rigid
  - the shapes of the enzyme, active site, and substrate adjust to maximumize the fit, which improves catalysis
  - there is a greater range of substrate specificity
- This model is more consistent with a wider range of enzymes



Enzyme Catalyzed Reactions

- When a substrate (S) fits properly in an active site, an **enzyme-substrate (ES) complex** is formed:

  $E + S \rightleftharpoons ES$

- Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

  $ES \rightarrow E + P$

- The products are then released, allowing another substrate molecule to bind the enzyme

  - this cycle can be repeated millions (or even more) times per minute

- The overall reaction for the conversion of substrate to product can be written as follows:

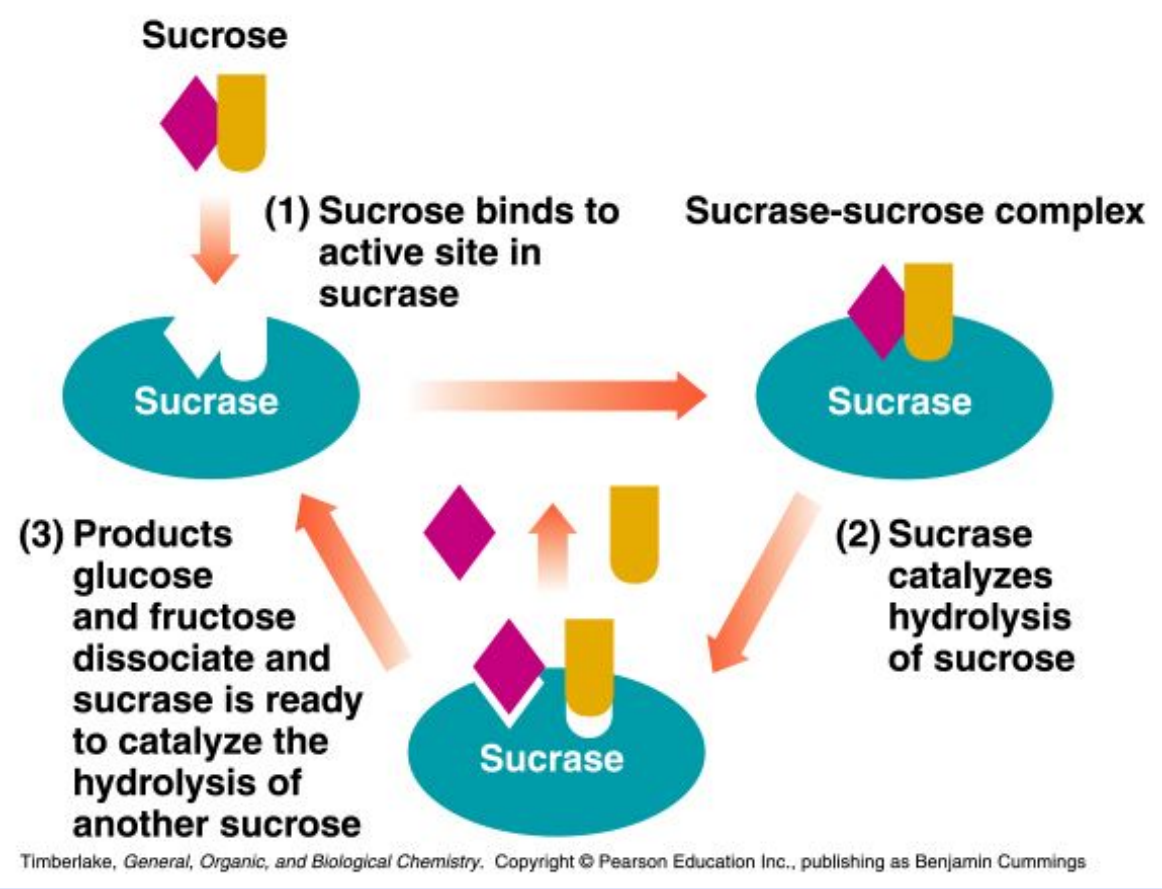
  $E + S \rightleftharpoons ES \rightarrow E + P$

Example of an Enzyme Catalyzed Reaction

- The reaction for the *sucrase* catalyzed hydrolysis of sucrose to glucose and fructose can be written as follows:

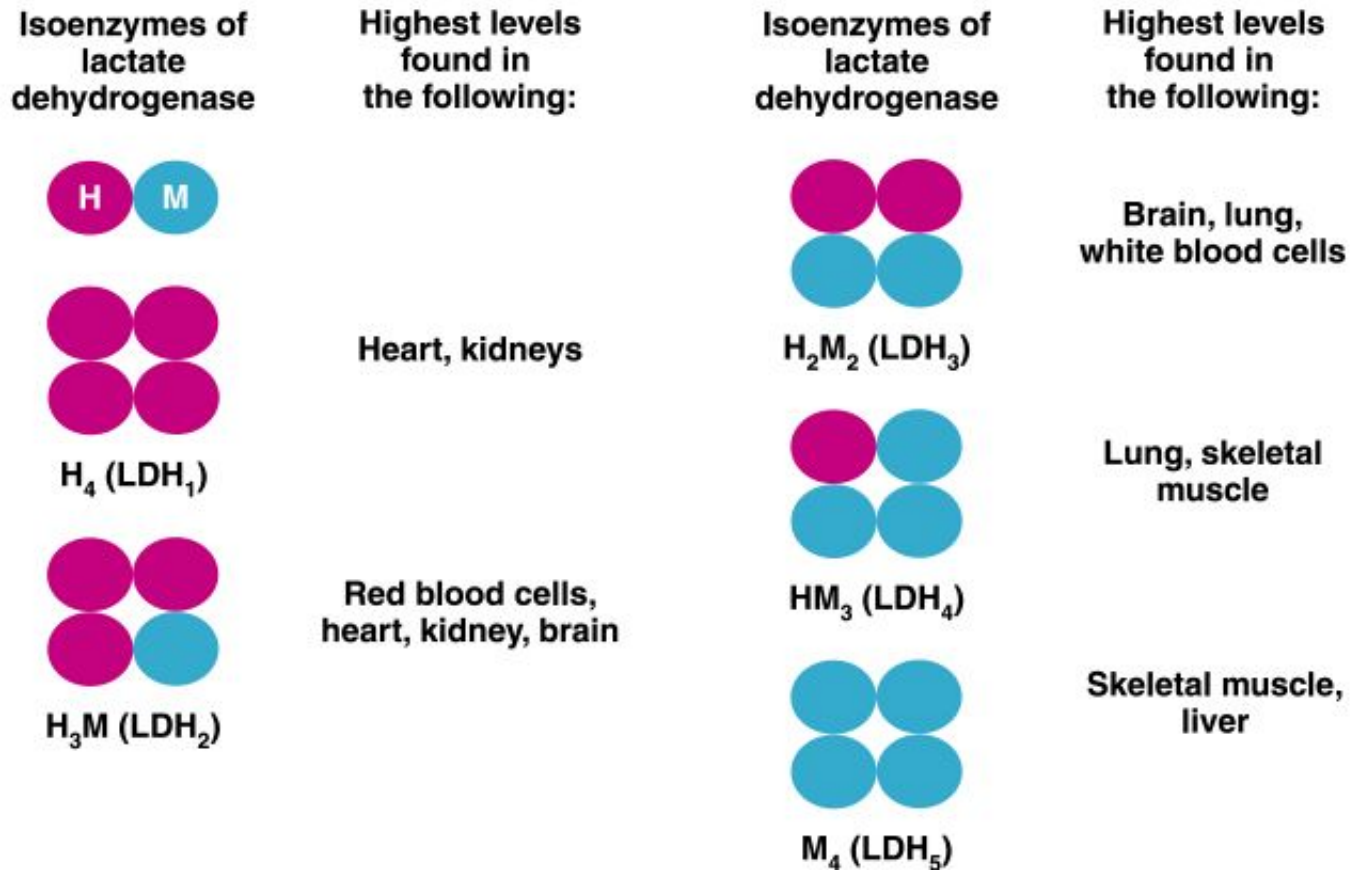
$$E + S \rightleftharpoons ES \rightarrow E + P_1 + P_2$$

where E = *sucrase*, S = sucrose, P_1 = glucose and P_2 = fructose



Isoenzymes

- **Isoenzymes** are different forms of an enzyme that catalyze the same reaction in different tissues in the body
  - they have slight variations in the amino acid sequences of the subunits of their quaternary structure
- For example, lactate dehydrogenase (LDH), which converts lactate to pyruvate, consists of five isoenzymes



Diagnostic Enzymes

- The levels of **diagnostic enzymes** in the blood can be used to determine the amount of damage in specific tissues

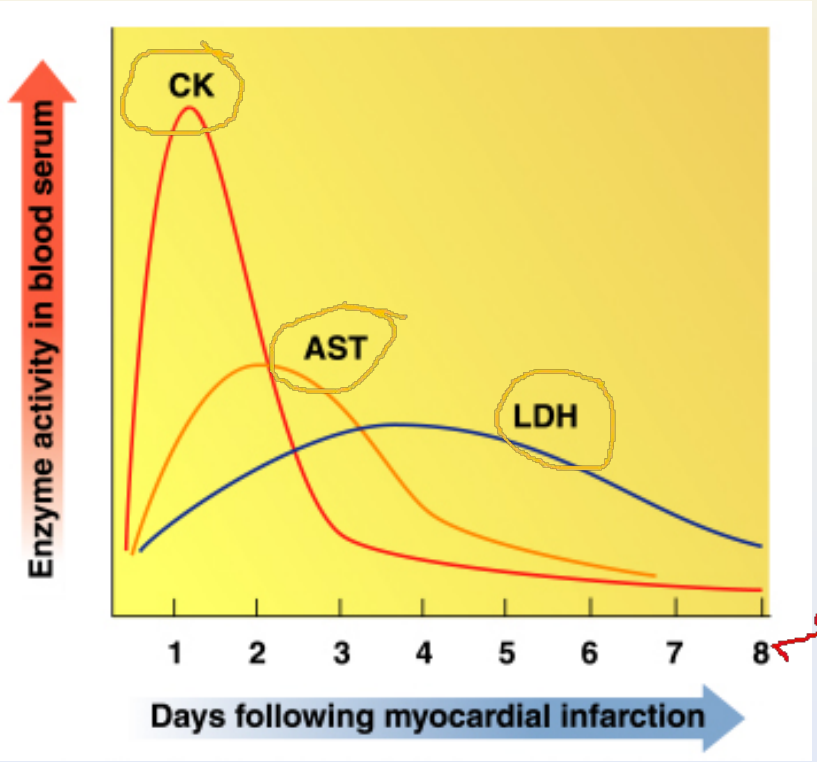
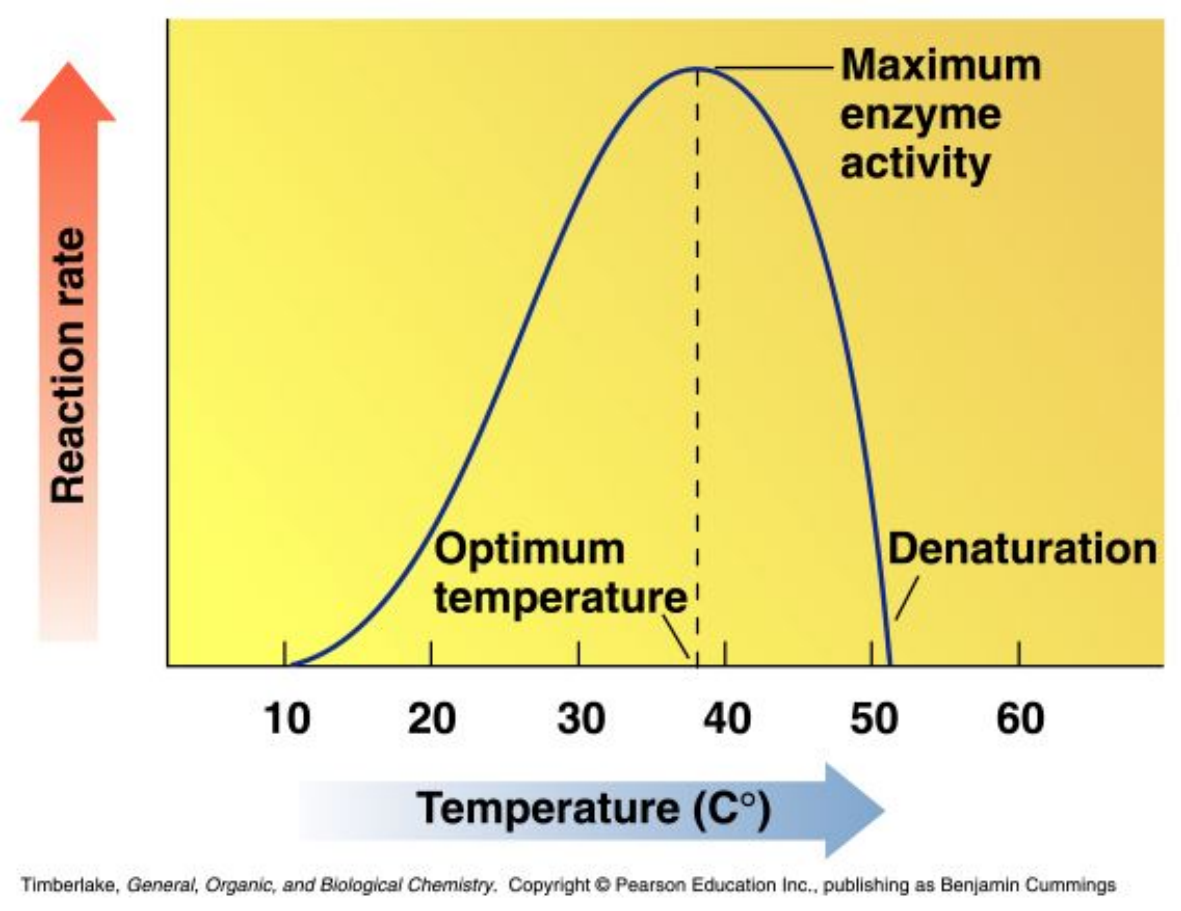


Table 21.4 Serum Enzymes Used in Diagnosis of Tissue Damage

Condition	Diagnostic Enzymes Elevated
Heart attack, or liver disease (cirrhosis, hepatitis)	Lactate dehydrogenase (LDH) Aspartate transaminase (AST)
Heart attack	Creatine kinase (CK)
Hepatitis	Alanine transaminase (ALT)
Liver (carcinoma) or bone disease (rickets)	Alkaline phosphatase (ALP)
Pancreatic disease	Amylase, cholinesterase
lipase (LPS)	
Prostate carcinoma	Acid phosphatase (ACP)

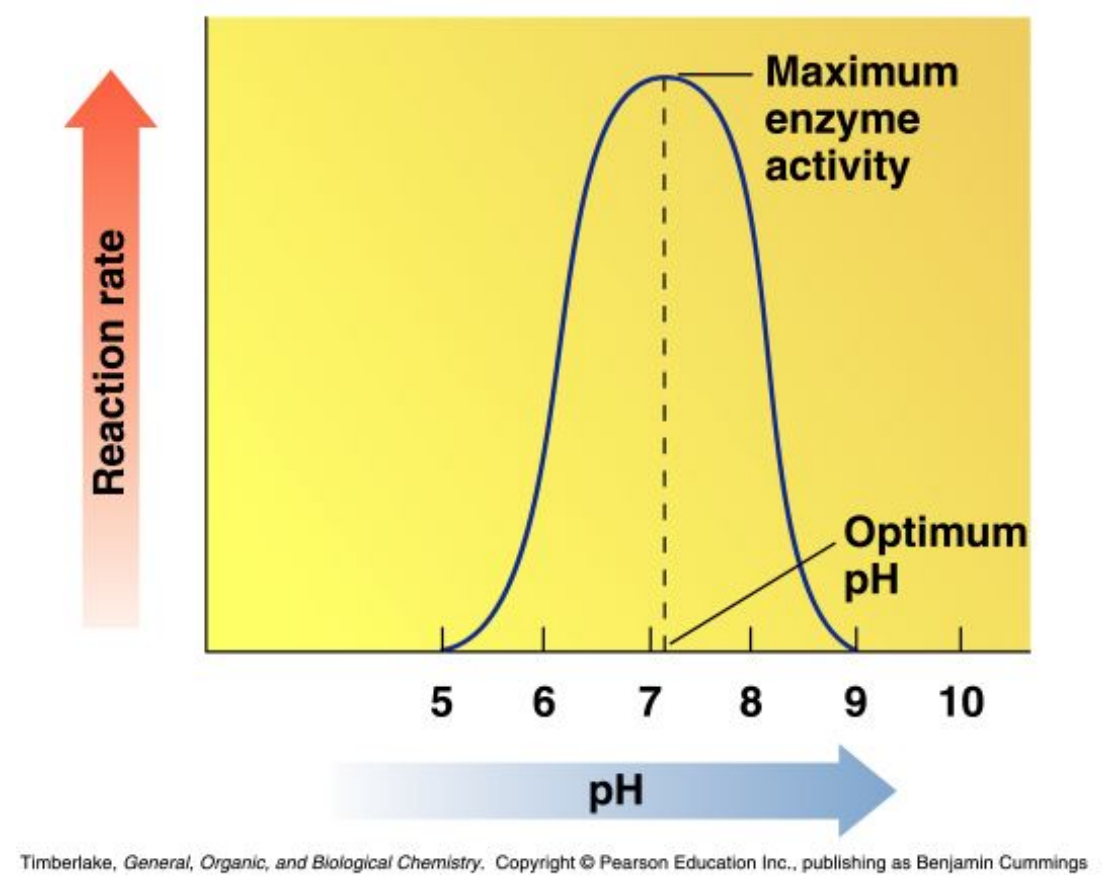
Temperature and Enzyme Activity

- Enzymes are most active at an optimum temperature (usually 37°C in humans)
- They show little activity at low temperatures
- Activity is lost at high temperatures as denaturation occurs



pH and Enzyme Activity

- Enzymes are most active at optimum pH
- Amino acids with acidic or basic side-chains have the proper charges when the pH is optimum
- Activity is lost at low or high pH as tertiary structure is disrupted



Optimum pH for Selected Enzymes

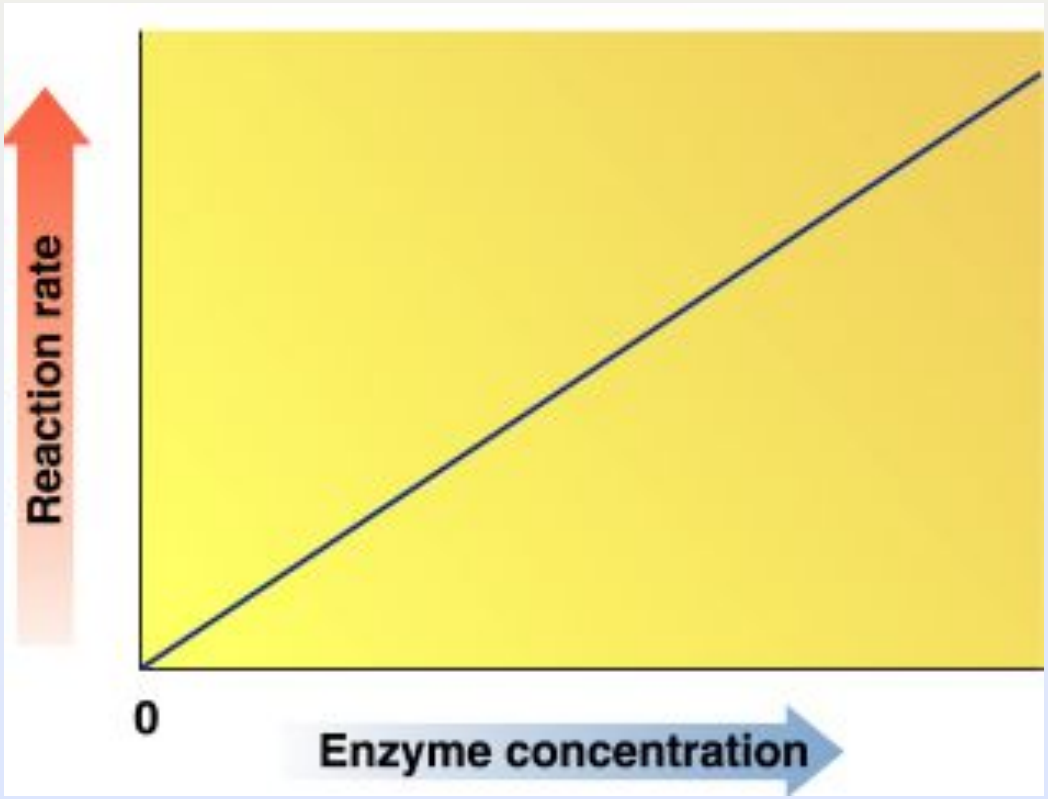
- Most enzymes of the body have an optimum pH of about 7.4
- However, in certain organs, enzymes operate at lower and higher optimum pH values

Table 21.5 Optimum pH for Selected Enzymes

Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	2
Urease	Liver	Urea	5
Sucrase	Small intestine	Sucrose	6.2
Pancreatic amylase	Pancreas	Amylose	7
Trypsin	Small intestine	Peptide bonds	8
Arginase	Liver	Arginine	9.7

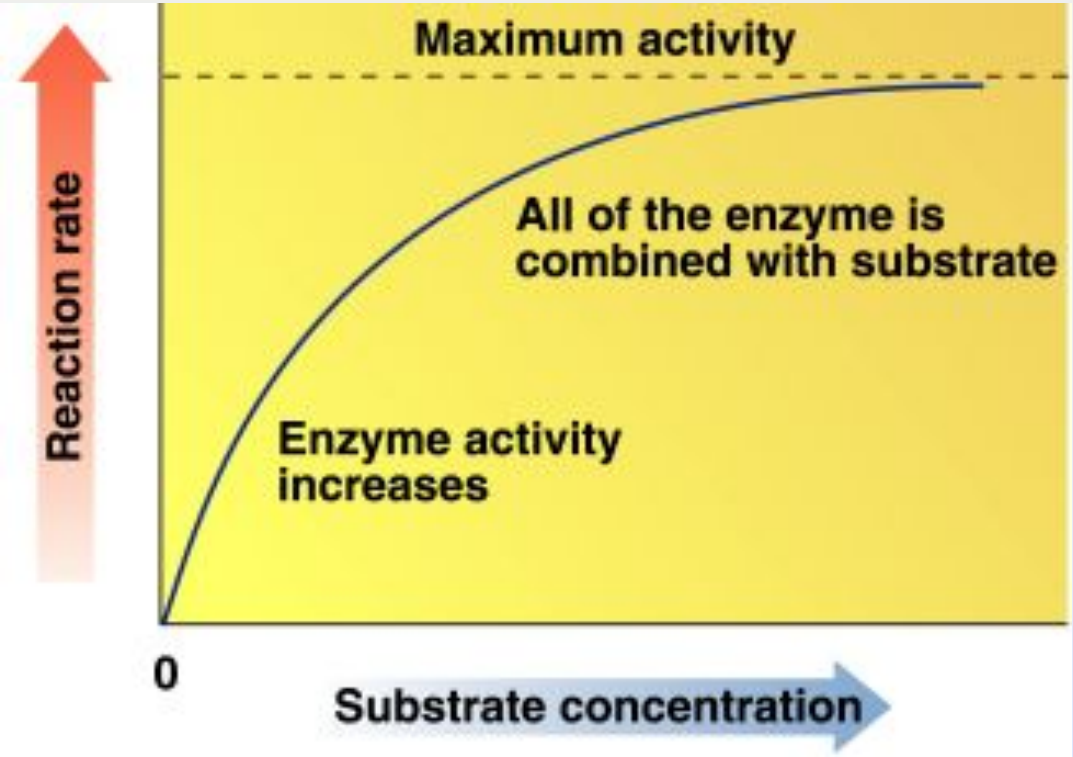
Enzyme Concentration and Reaction Rate

- The rate of reaction increases as enzyme concentration increases (at constant substrate concentration)
- At higher enzyme concentrations, more enzymes are available to catalyze the reaction (more reactions at once)
- There is a linear relationship between reaction rate and enzyme concentration (at constant substrate concentration)

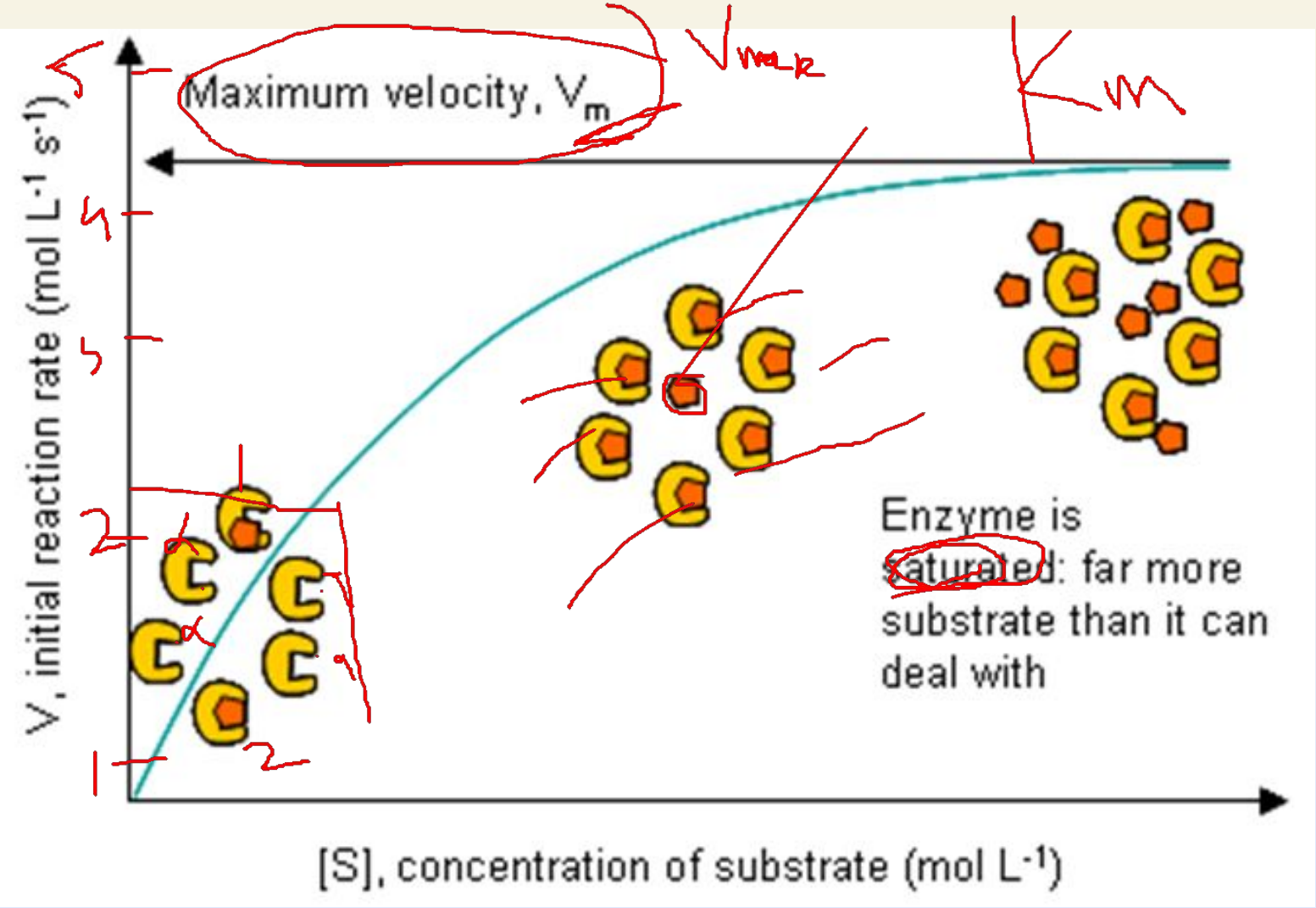


Substrate Concentration and Reaction Rate

- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)
- **Maximum activity** occurs when the enzyme is saturated (when all enzymes are binding substrate)
- The relationship between reaction rate and substrate concentration is exponential, and asymptotes (levels off) when the enzyme is saturated



Substrate Concentration and Reaction Rate



Enzyme Inhibitors

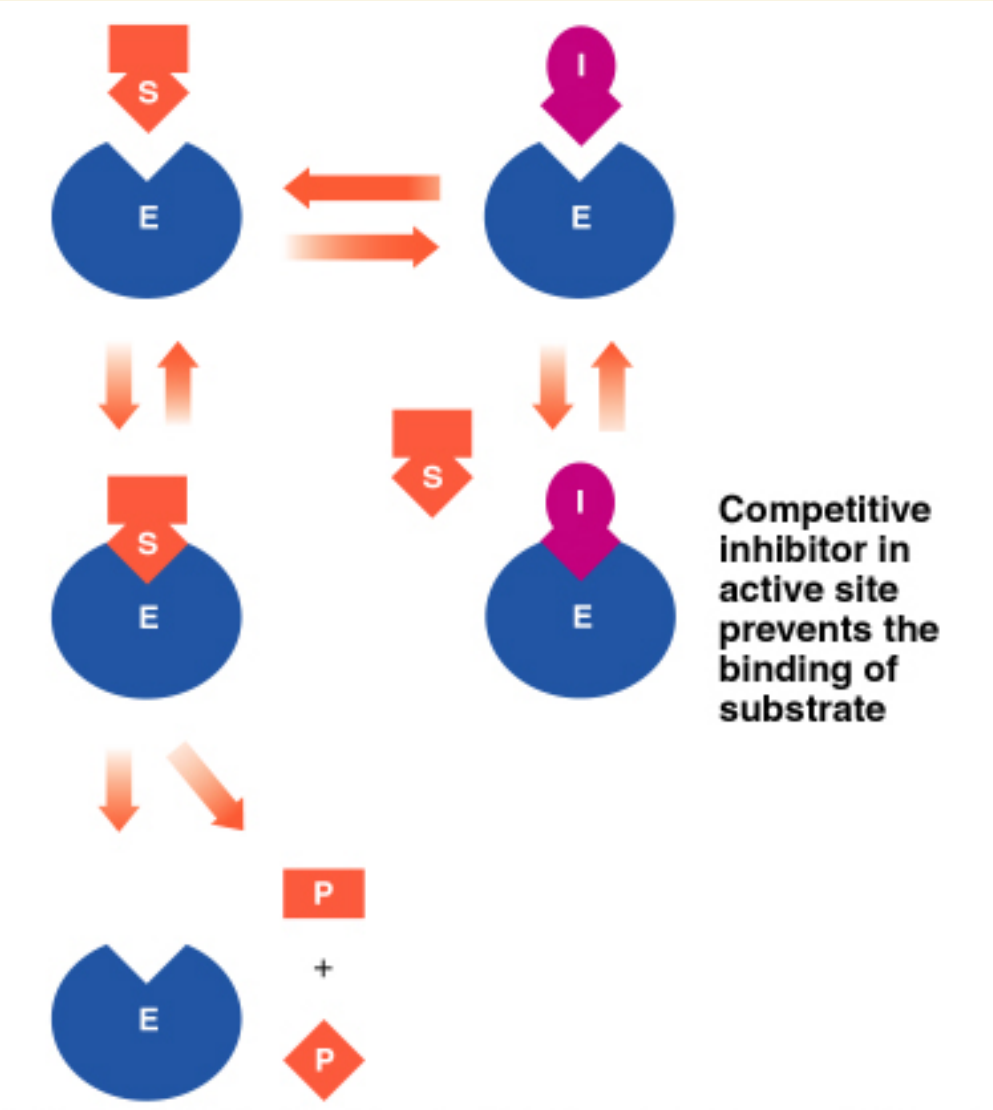
- **Inhibitors (I)** are molecules that cause a loss of enzyme activity
- They prevent substrates from fitting into the active site of the enzyme:

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

$$E + I \rightleftharpoons EI \rightarrow \text{no P formed}$$

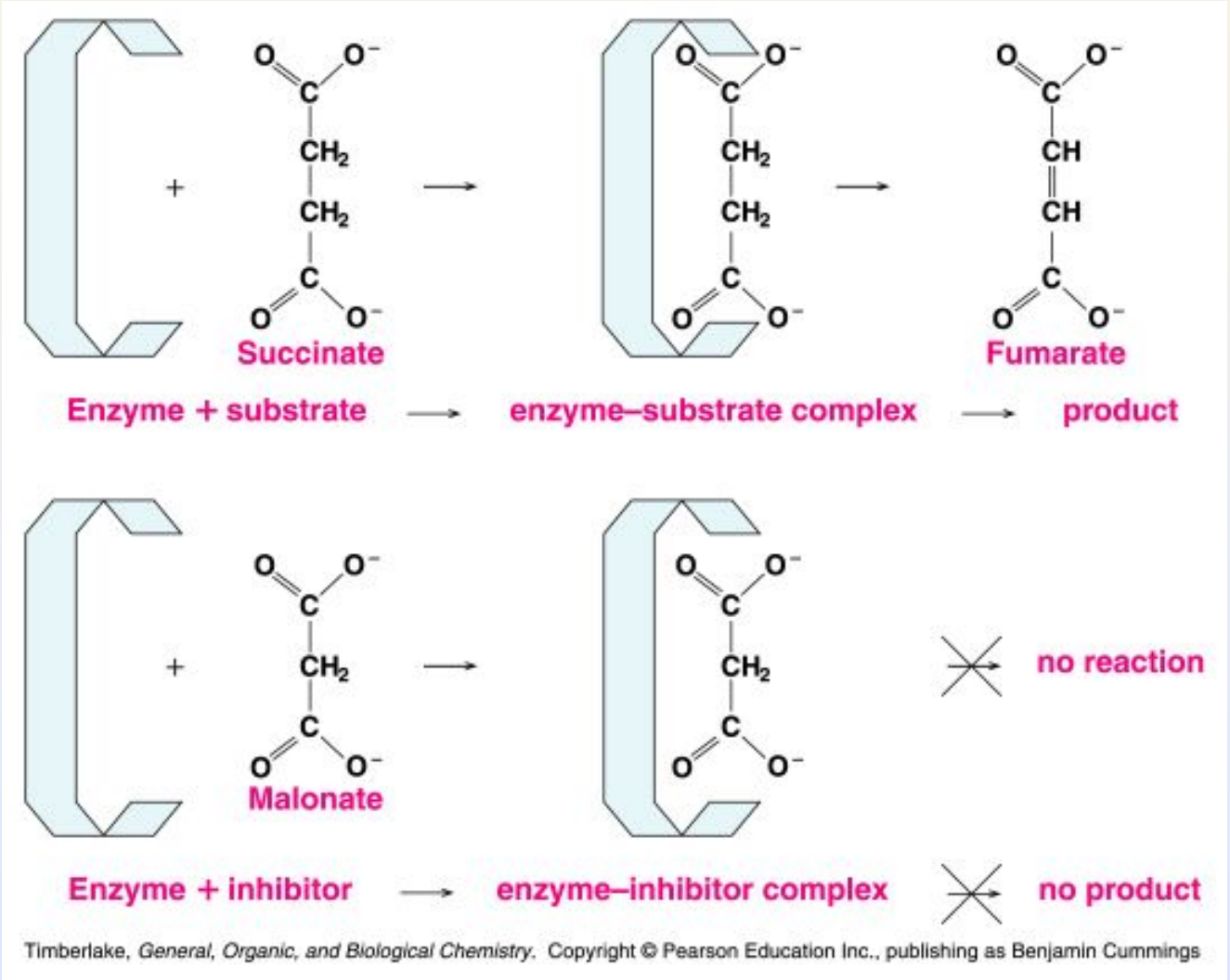
Reversible Inhibitors (Competitive Inhibition)

- A **reversible inhibitor** goes on and off, allowing the enzyme to regain activity when the inhibitor leaves
- A **competitive inhibitor** is reversible and has a structure like the substrate
  - it competes with the substrate for the active site
  - its effect is reversed by increasing substrate concentration



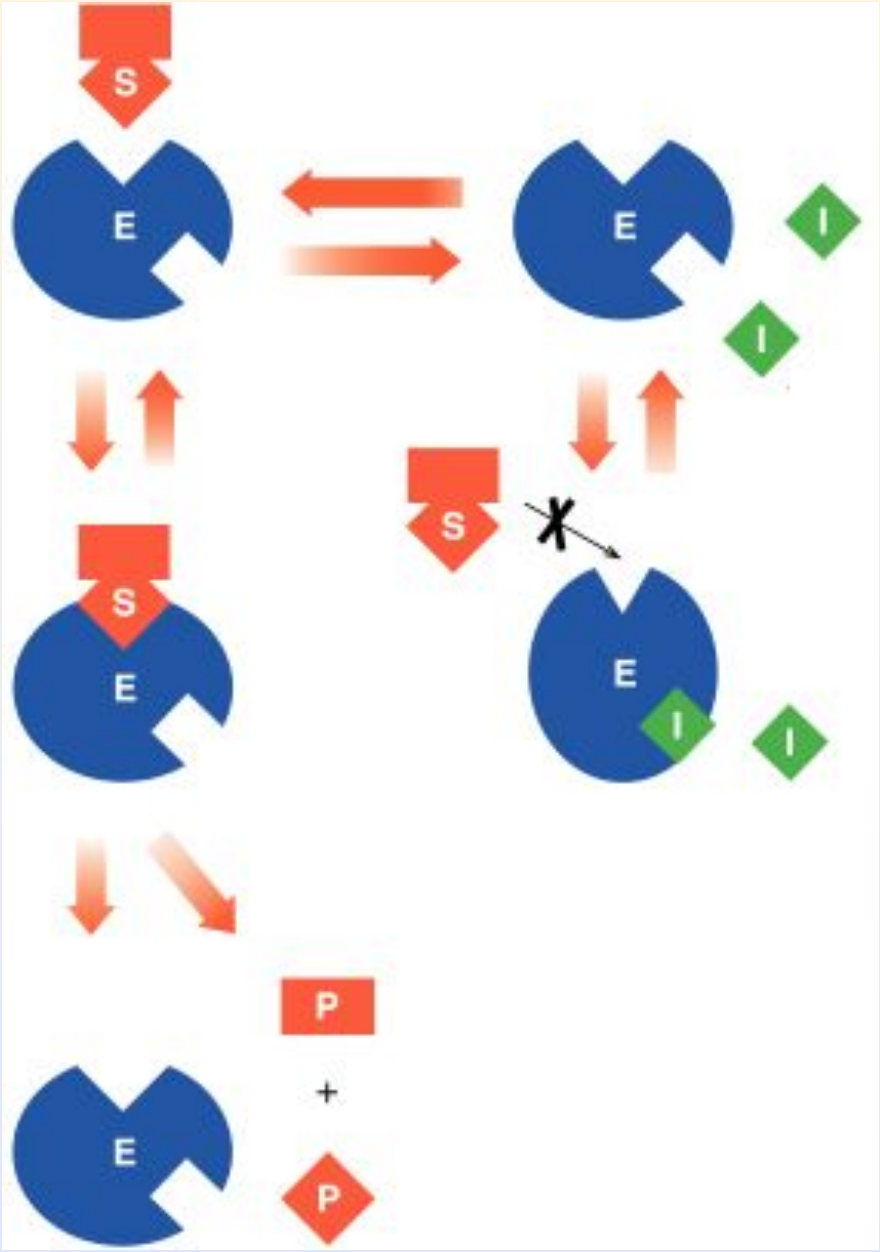
Example of a Competitive Inhibitor

- Malonate is a competitive inhibitor of *succinate dehydrogenase*
  - it has a structure that is similar to succinate
  - inhibition can be reversed by adding succinate



Reversible Inhibitors (Noncompetitive Inhibition)

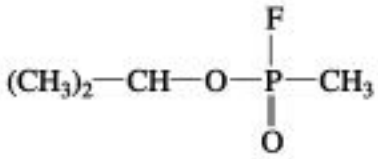
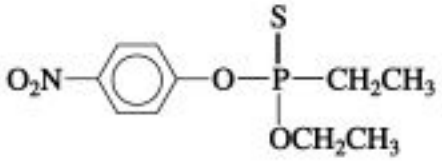
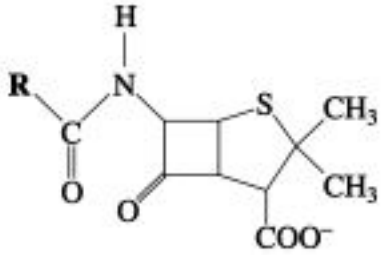
- A **noncompetitive inhibitor** has a structure that is different than that of the substrate
  - it binds to an **allosteric site** rather than to the active site
  - it distorts the shape of the enzyme, which alters the shape of the active site and prevents the binding of the substrate
- The effect can not be reversed by adding more substrate



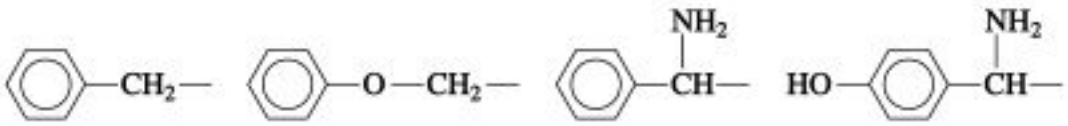
Irreversible Inhibitors

- An **irreversible inhibitor** destroys enzyme activity, usually by bonding with side-chain groups in the active site

Table 21.6 Selected Irreversible Enzyme Inhibitors

Name	Structure	Natural/Synthetic Source	Inhibitory Action
Cyanide	CN^-	Bitter almonds	Bonds to metal ions in enzymes in the electron transport chain
Sarin		Nerve gas	Similar to DFP
Parathion		Insecticide	Similar to DFP
Penicillin		*Penicillium* fungus	Inhibits enzymes that build cell walls in bacteria

R Groups for Penicillin Derivatives

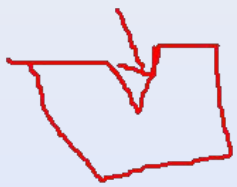


Ribozyme

- A is a **ribo**nucleic acid (RNA) en**zyme** that catalyzes a chemical reaction. The ribozyme catalyses specific reactions in a similar way to that of protein enzymes.
- Also called catalytic RNA, ribozymes are found in the ribosome where they join amino acids together to form protein chains. Ribozymes also play a role in other vital reactions such as RNA splicing, transfer RNA biosynthesis, and viral replication

- RNA may have played a crucial role in the evolution of self-replicating systems. This is referred to as the RNA World Hypothesis and today, many scientists believe that ribozymes are remnants of an ancient world that existed before the evolution of proteins. It is thought that RNAs used to catalyse functions such as cleavage, replication and RNA molecule ligation before proteins evolved and took over these catalytic functions, which they could perform in a more efficient and versatile way

- **Allosteric enzymes** are unique compared to other enzymes because of its ability to adapt various conditions in the environment due to its special properties. The special property of Allosteric enzymes is *that it contains an allosteric site on top of its active site which binds the substrate. The binding of a nonsubstrate molecule to the allosteric site functions to influences the activity of the enzyme.* In influencing the activity, it can either enhance or impair the activity of the enzyme. Another important property of allosteric enzymes is that it also contains many polypeptide chains with multiple active and allosteric sites. The nonsubstrate molecules that bind at the allosteric sites are called allosteric modulators.

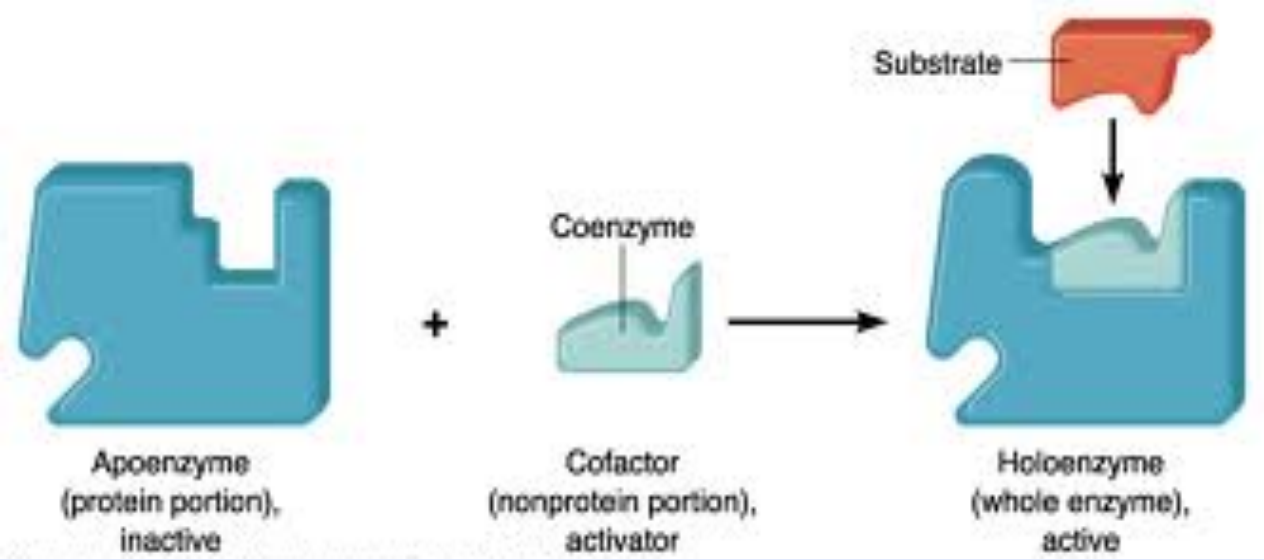


Properties of Allosteric Enzymes

- One is that allosteric enzymes do not follow the Michaelis-Menten Kinetics. This is because allosteric enzymes have multiple active sites. These multiple active sites exhibit the property of cooperativity,
- Allosteric Enzymes are influenced by substrate concentration
- Allosteric Enzymes are regulated by other molecules. (when the molecules 2,3-BPG, pH, and CO2 modulates the binding affinity of hemoglobin to oxygen)

APOENZYME and HOLOENZYME

- The enzyme without its non protein moiety is termed as apoenzyme and it is inactive.
- Holoenzyme is an active enzyme with its non protein component.



- **Cofactor:**
  - **A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.**
  - **Types of Cofactors:**
    - **Coenzymes.**
    - **Prosthetic groups.**

Types of Cofactors

- **Coenzyme:**
  The non-protein component, loosely bound to apoenzyme by non-covalent bond.
- **Examples :** vitamins or compound derived from vitamins.
- **Prosthetic group**
  The non-protein component, tightly bound to the apoenzyme by covalent bonds is called a Prosthetic group.

Phenylketonuria (PKU)

- Phenylketonuria (PKU) is a rare genetic condition that causes an amino acid called phenylalanine to build up in the body. Amino acids are the building blocks of protein. Phenylalanine is found in all proteins and some artificial sweeteners.
- Phenylalanine hydroxylase is an enzyme your body uses to convert phenylalanine into tyrosine, which your body needs to create neurotransmitters such as epinephrine, norepinephrine, and dopamine. PKU is caused by a defect in the gene that helps create phenylalanine hydroxylase. When this enzyme is missing, your body can't break down phenylalanine. This causes a buildup of phenylalanine in your body

- A musty odor in the breath, skin or urine, caused by too much phenylalanine in the body
- Neurological problems that may include seizures
- Skin rashes (eczema)
- Fair skin and blue eyes, because phenylalanine can't transform into melanin — the pigment responsible for hair and skin tone
- Abnormally small head (microcephaly)
- Hyperactivity
- Intellectual disability
- Delayed development
- Behavioral, emotional and social problems
- Psychiatric disorder

Alkaptonuria (AKU)

- Alkaptonuria is a rare inherited disorder. It occurs when your body can't produce enough of an enzyme called homogentisic dioxygenase (HGD). This enzyme is used to break down a toxic substance called homogentisic acid. When you don't produce enough HGD, homogentisic acid builds up in your body

- dark spots in the sclera (white) of your eyes
- thickened and darkened cartilage in your ears
- blue speckled discoloration of your skin, particularly around sweat glands
- dark-colored sweat or sweat stains
- black earwax
- kidney stones and prostate stones
- arthritis (especially hip and knee joints)

IMMBOLIZED ENZYME

- As enzymes are biological catalysts that promote the rate of reactions but are not themselves consumed in the reactions; they may be used repeatedly for as long as they remain active. However, in most of the processes, enzymes are mixed in a solution with substrates and cannot be economically recovered after the reaction and are generally wasted. Thus, there is an incentive to use enzymes in an immobilized or insolubilized form so that they may be retained in a biochemical reactor for further catalysis

- Enzyme immobilization may be defined as a process of confining the enzyme molecules to a solid support over which a substrate is passed and converted to products. The process whereby the movement of enzymes, cells, organelles, etc. in space is completely or severely restricted usually resulting in a water-insoluble form of the enzyme

- An immobilized enzyme is one whose movement in space has been restricted either completely or to a small limited region

- Protection from degradation and deactivation.
- Retention of enzyme, enzyme-free products.
- Recycling, repetitive use.
- Cost efficiency.
- Enhanced stability.
- Use as controlled release agents.
- The ability to stop the reaction rapidly by removing the enzyme from the reaction Solution (or vice-versa)
- Allows development of multi-enzyme reaction system.

Regulatory enzyme

- A regulatory enzyme is an enzyme in a biochemical pathway which, through its responses to the presence of certain other biomolecules, regulates the pathway activity. This is usually done for pathways whose products may be needed in different amounts at different times, such as hormone production.
- Regulatory enzymes exist at high concentrations (low Vmax) so their activity can be increased or decreased with changes in substrate concentrations.

- The enzymes which catalyse chemical reactions again and again are called regulatory enzymes.

zymogen

- A **zymogen** also called a **proenzyme** is an inactive precursoran inactive precursor of an enzymean inactive precursor of an enzyme. A zymogen requires a biochemicalan inactive precursor of an enzyme. A zymogen requires a biochemical change (such as a hydrolysis reaction revealing the active site, or changing the configuration to reveal the active site) for it to become an active enzyme.
- Trypsinogen
- Chymotrypsinogen
- Pepsinoge