Enzyme Structure, classification and mechanism of action

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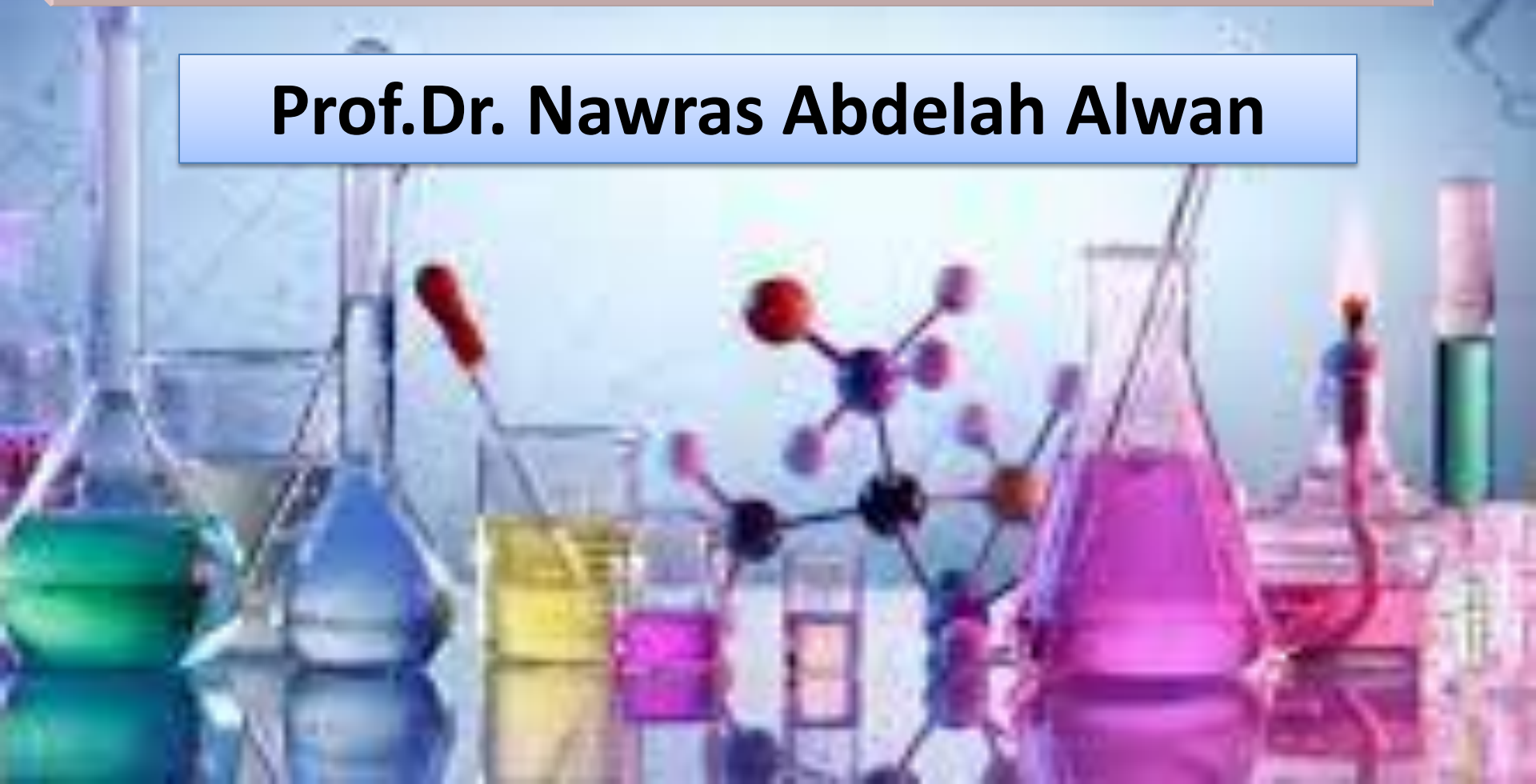


Define enzymes

(Enzymes as Biological Catalysts)

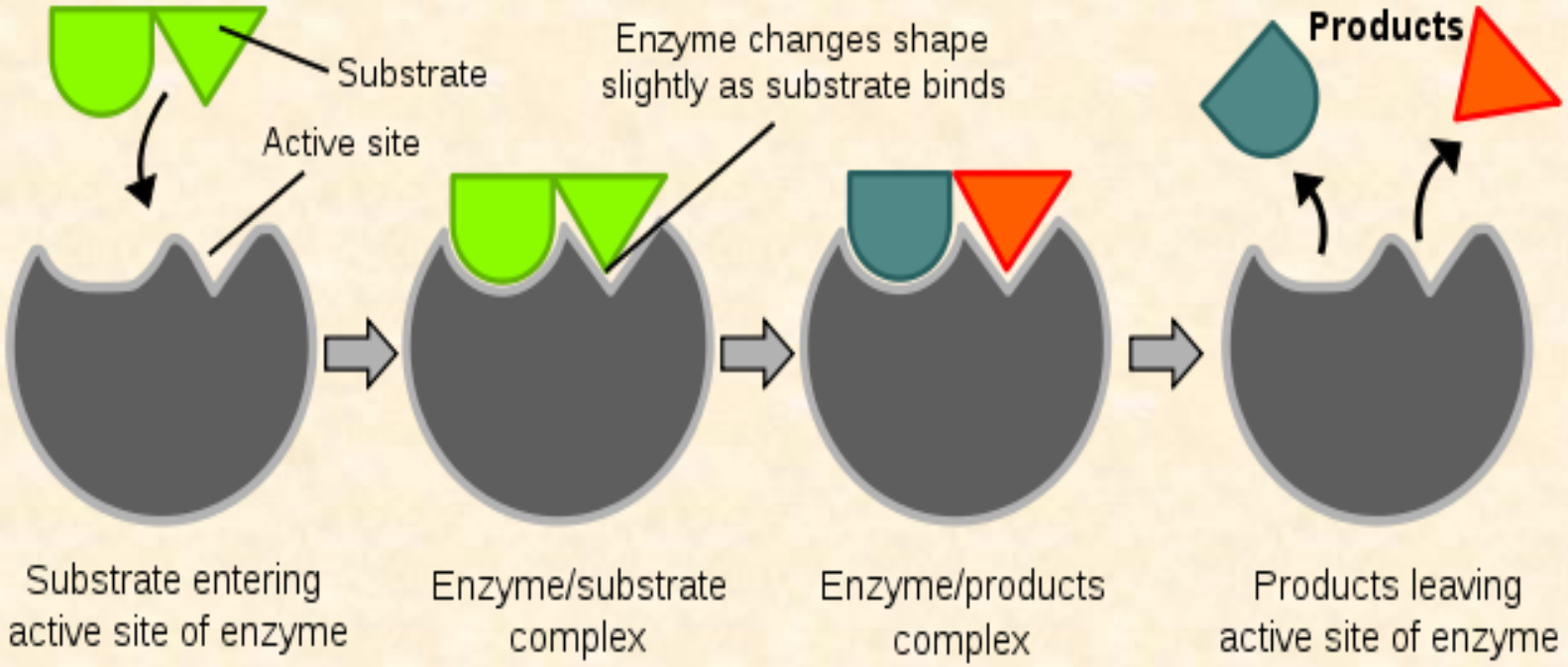
- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation
- They catalyze nearly all the chemical reactions taking place in the cells of the body.
- Not altered or consumed during reaction.
- Reusable

Importance

- Enzymes play an important role in Metabolism, Diagnosis, and Therapeutics.
- All biochemical reactions are enzyme catalyzed in the living organism.
- Level of enzyme in blood are of diagnostic importance e.g. it is a good indicator in disease such as myocardial infarction.
- Enzyme can be used therapeutically such as digestive enzymes.

ACTIVE SITES

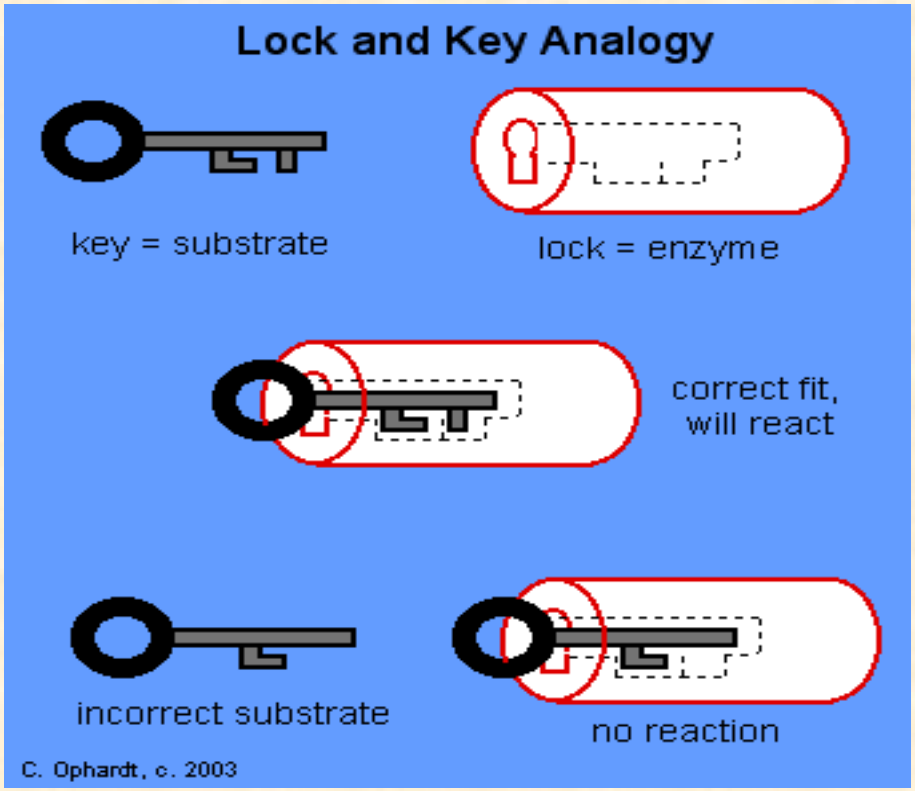
- Enzyme molecules contain a special pocket or cleft called the active sites.



Lock-and-Key Model

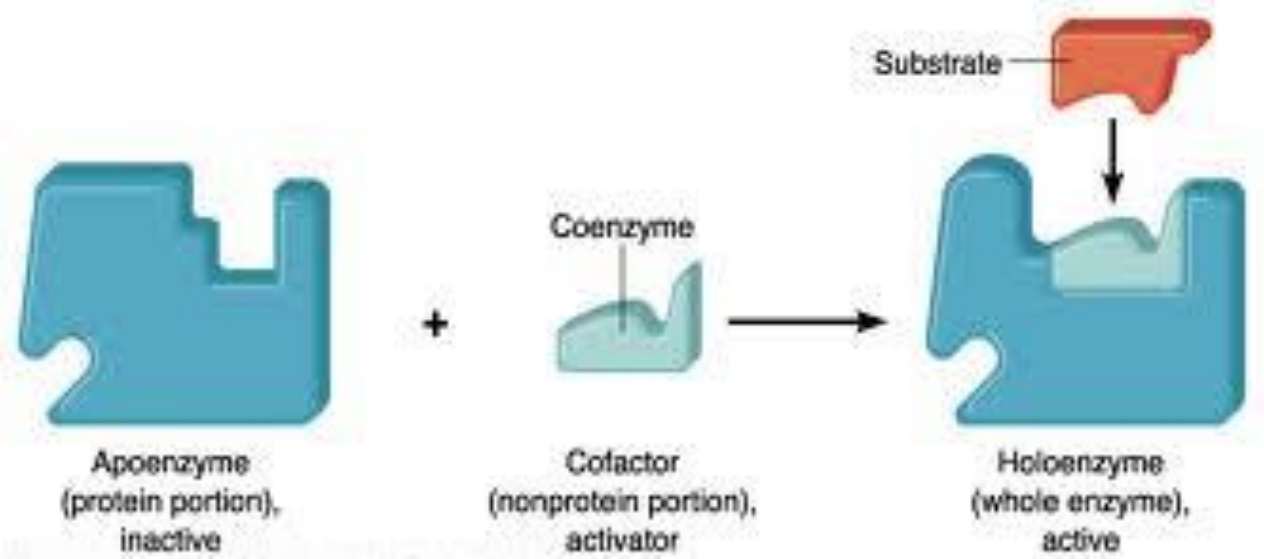
- **In the lock-and-key model of enzyme action:**
  - the active site has a rigid shape
  - only substrates with the matching shape can fit
  - the substrate is a key that fits the lock of the active site

This explains enzyme specificity
This explains the loss of activity when enzymes denature



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.



Important Terms to Understand Biochemical Nature And Activity of Enzymes

- **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.

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

Important Terms to Understand Biochemical Nature And Activity of Enzymes

Activation energy or Energy of Activation:

- All chemical reactions require some amount of energy to get them started.

OR

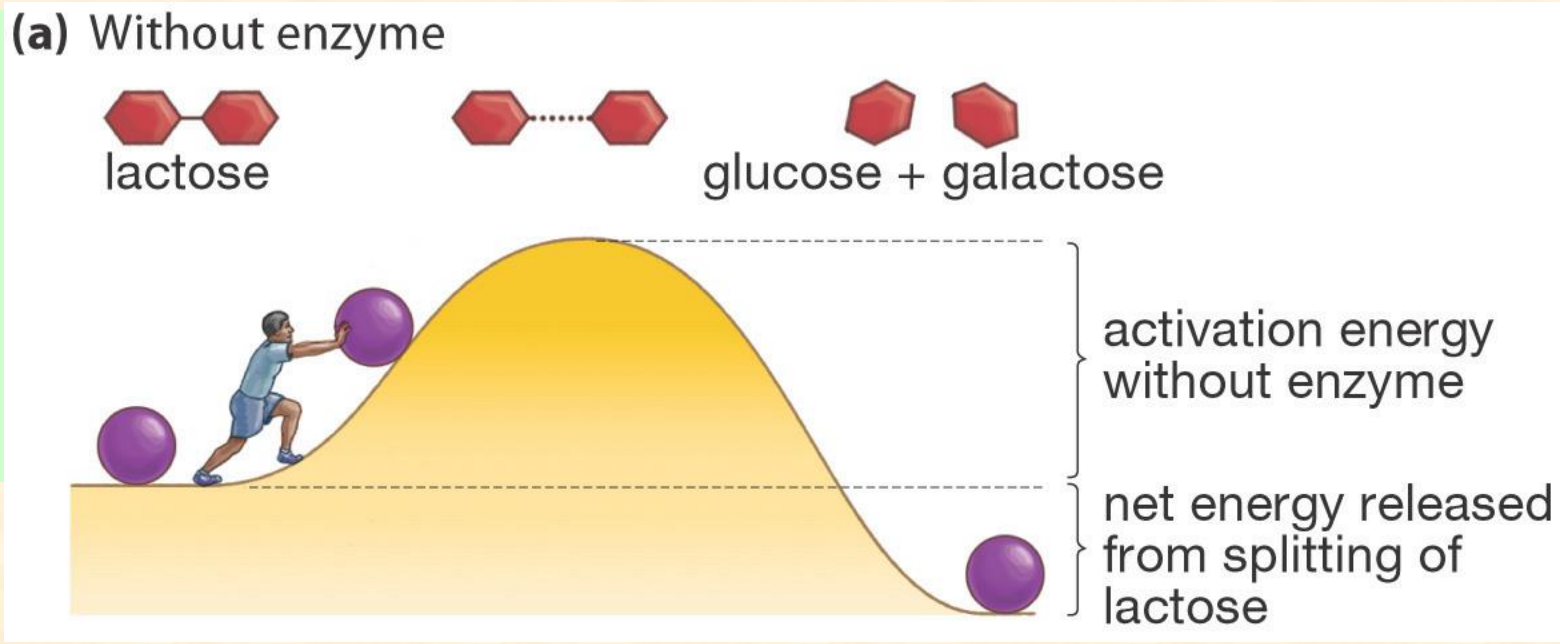
- It is First push to start reaction.

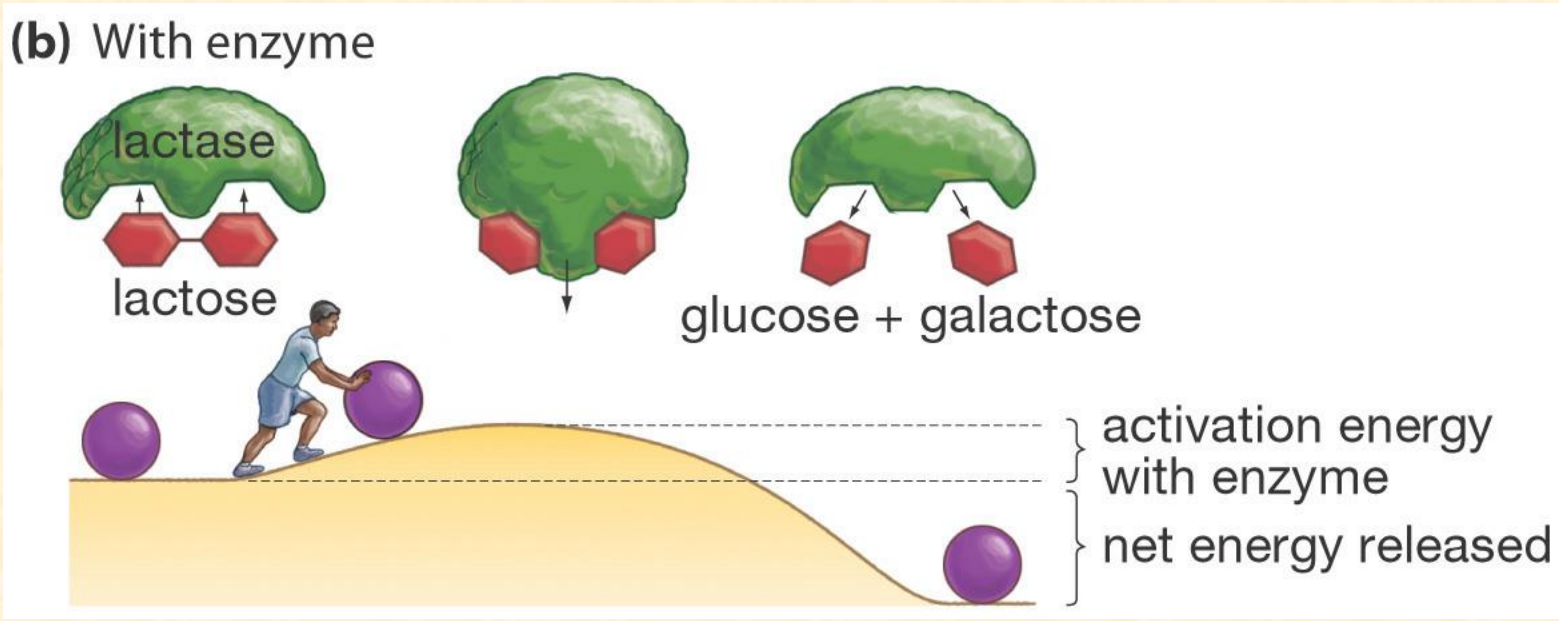
This energy is called activation energy.

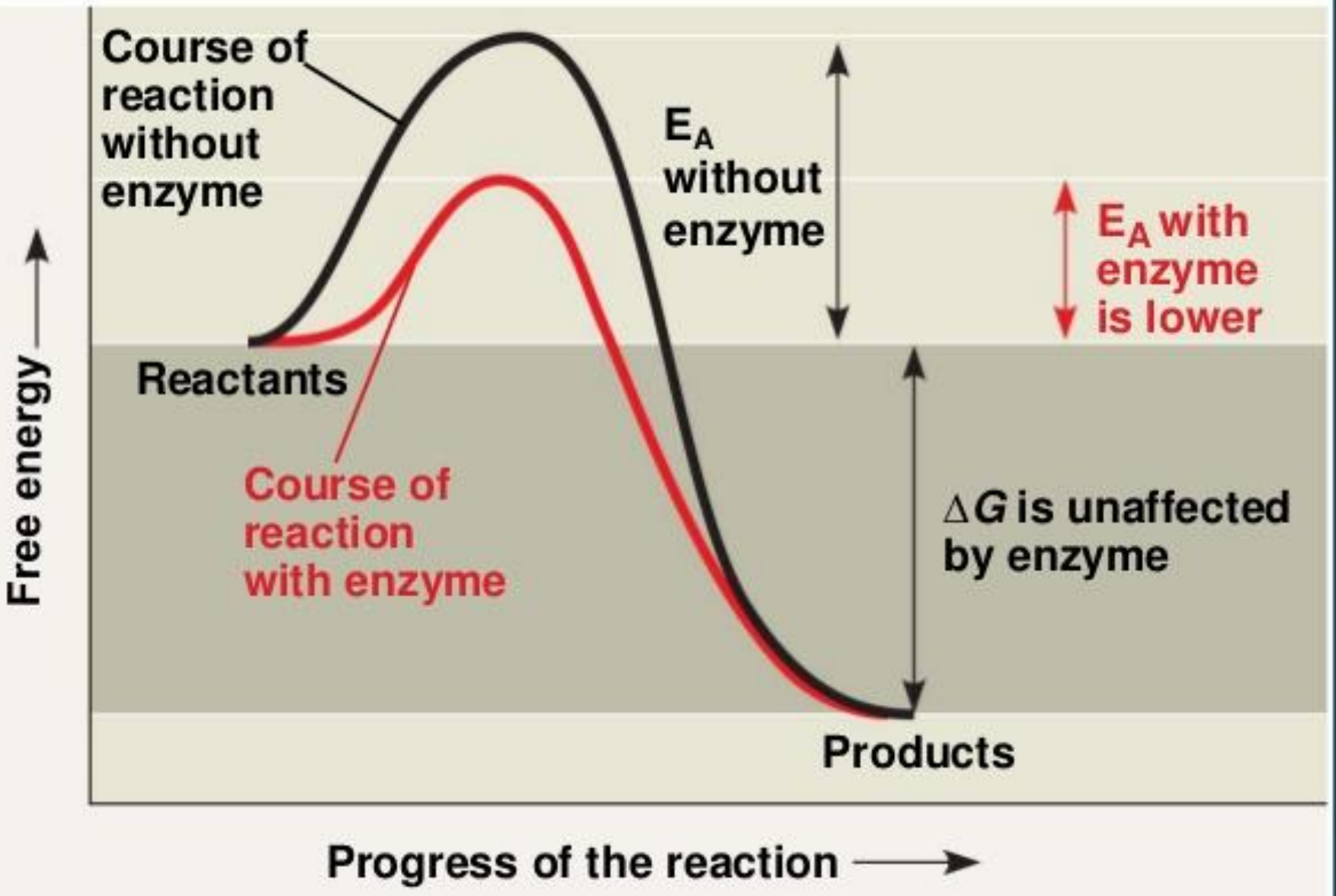
Mechanism of Action of Enzymes

- **Enzymes increase reaction rates by decreasing the Activation energy:**
- **Enzyme-Substrate Interactions:**
  - **Formation of Enzyme substrate complex by:**
  - Lock-and-Key Model
  - Induced Fit Model

Enzymes Lower a Reaction's Activation Energy





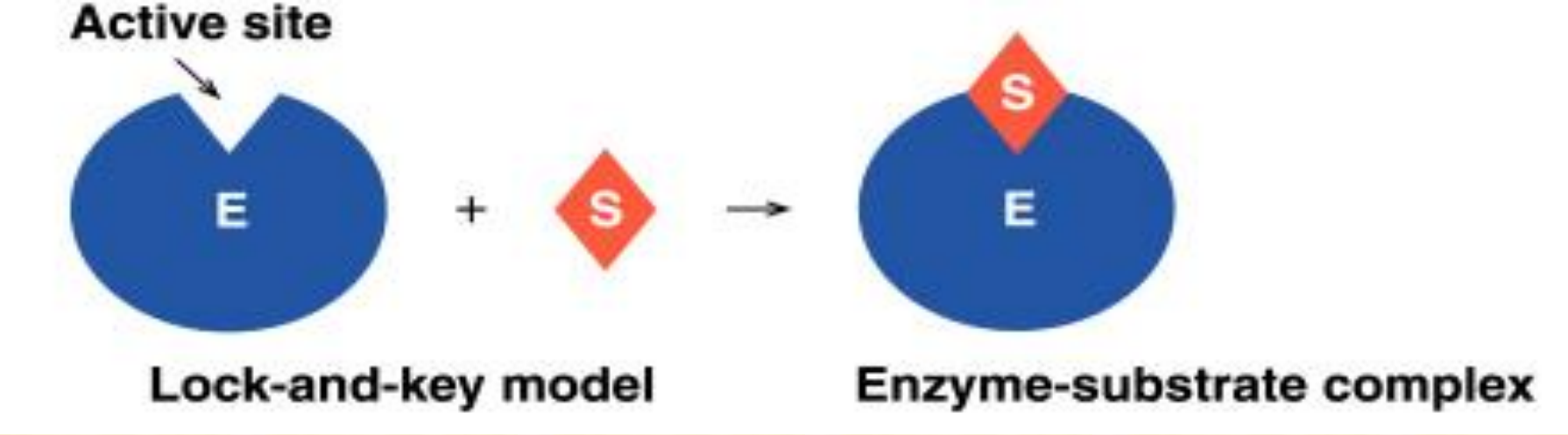

Course of reaction without enzyme
E_A without enzyme
E_A with enzyme is lower
Reactants
Course of reaction with enzyme
ΔG is unaffected by enzyme
Products
Free energy
Progress of the reaction

MECHANISM OF ACTION OF ENZYMES

1- Lock: Key model of enzyme action: implies that the active site of the enzyme is complementary in shape to that of its substrate, i.e. the shape of the enzyme molecule and the substrate molecule should fit each other like a lock and Key. In 1958, Daniel Koshland, postulated another model; which implies that the shapes & the active sites of enzymes are complementary to that of the substrate only after the substrate is bound.

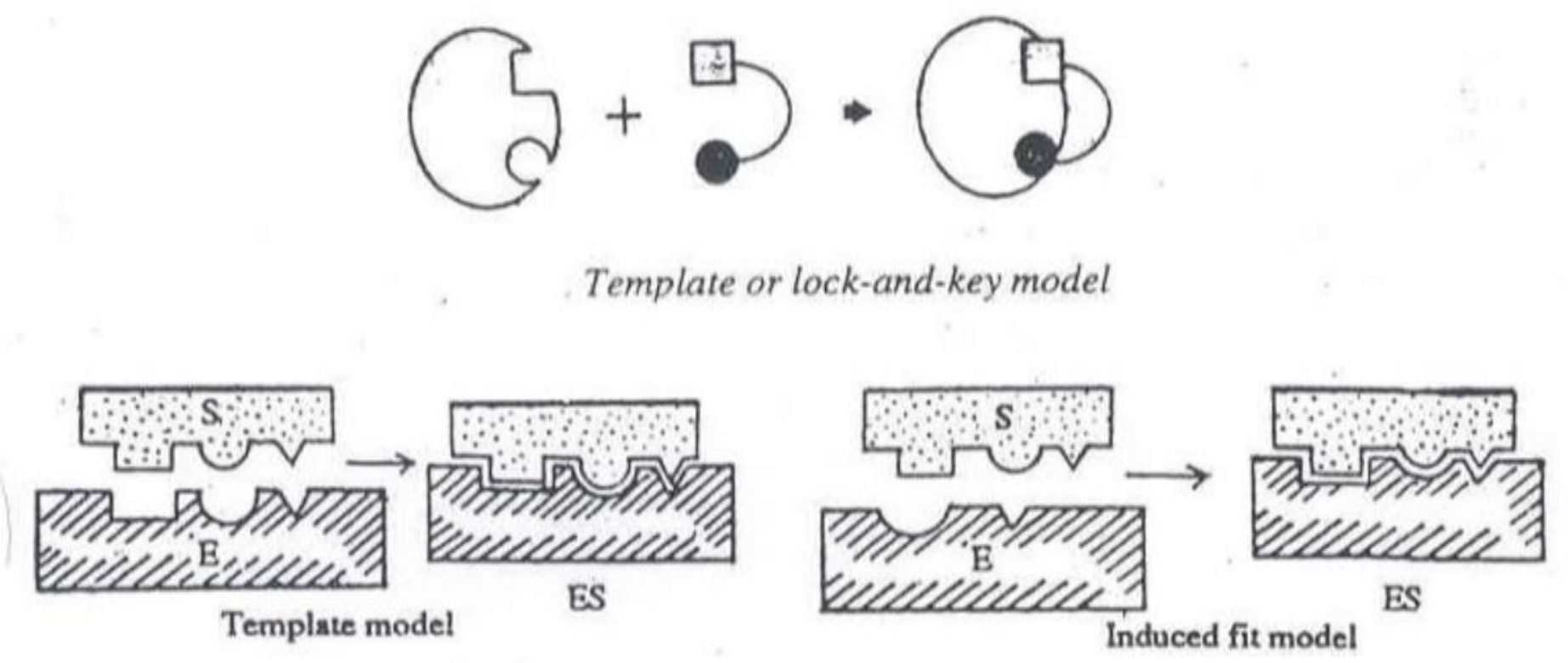
Lock-and-Key Model

- 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



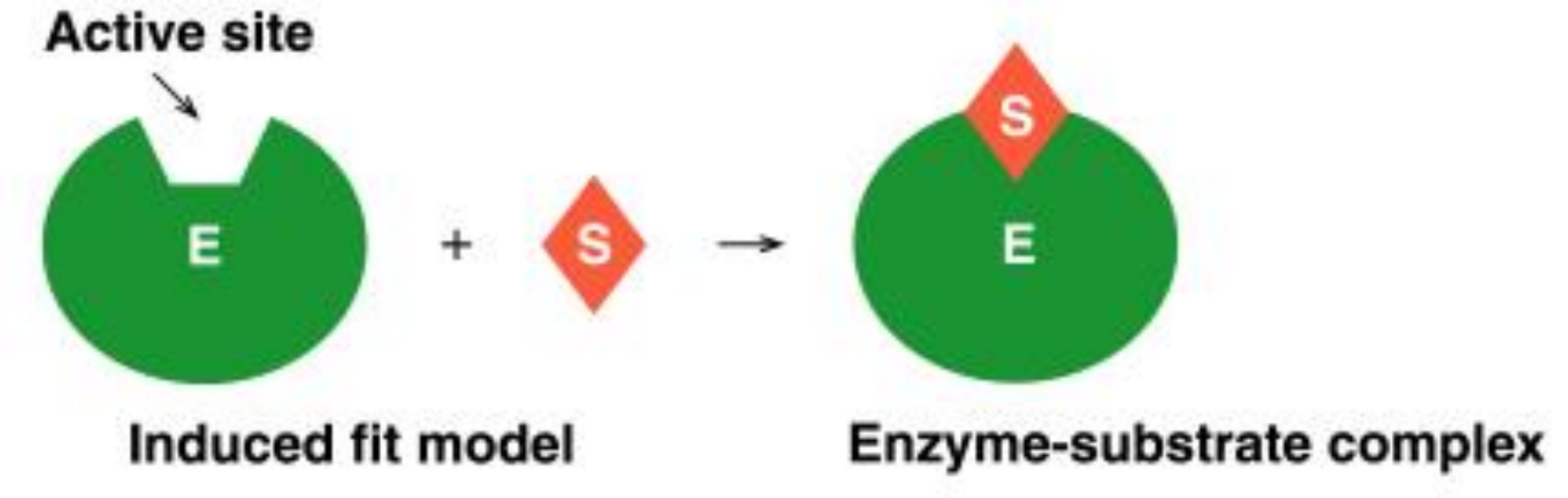
2-Induced fit Model :

is a model that implies the shapes & the active sites of enzymes are complementary to that of the substrate only after the substrate is bound.



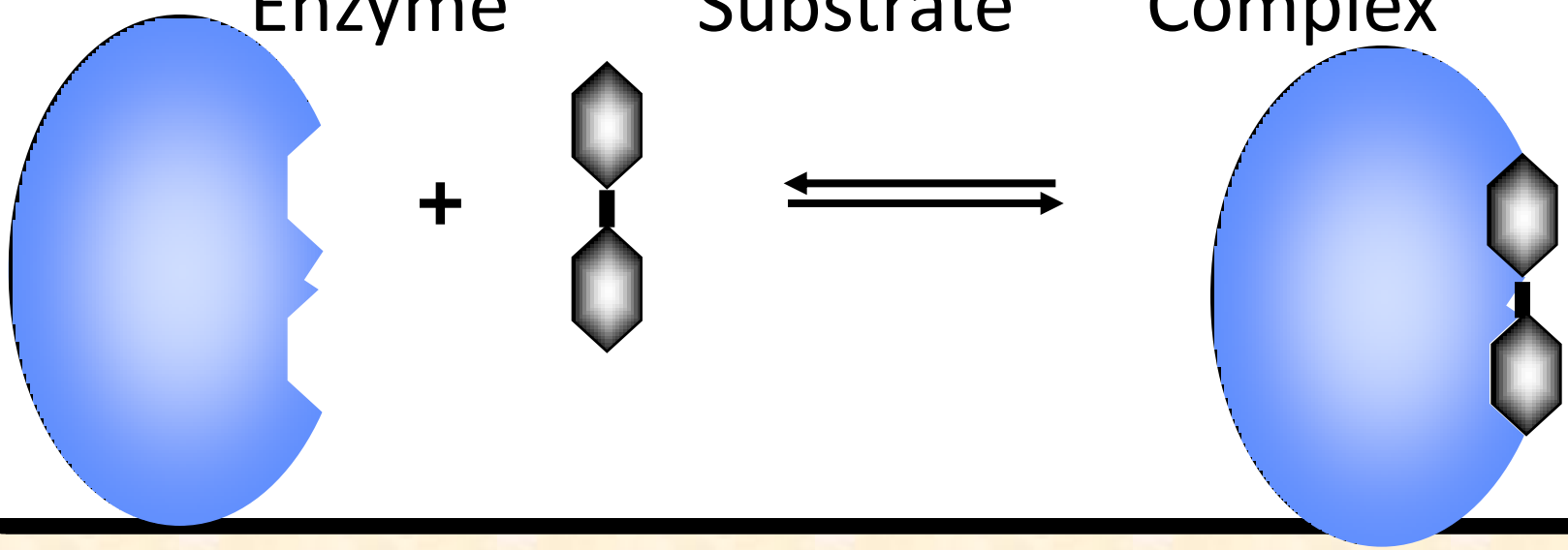
Induced Fit Model

- 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-substrate complex

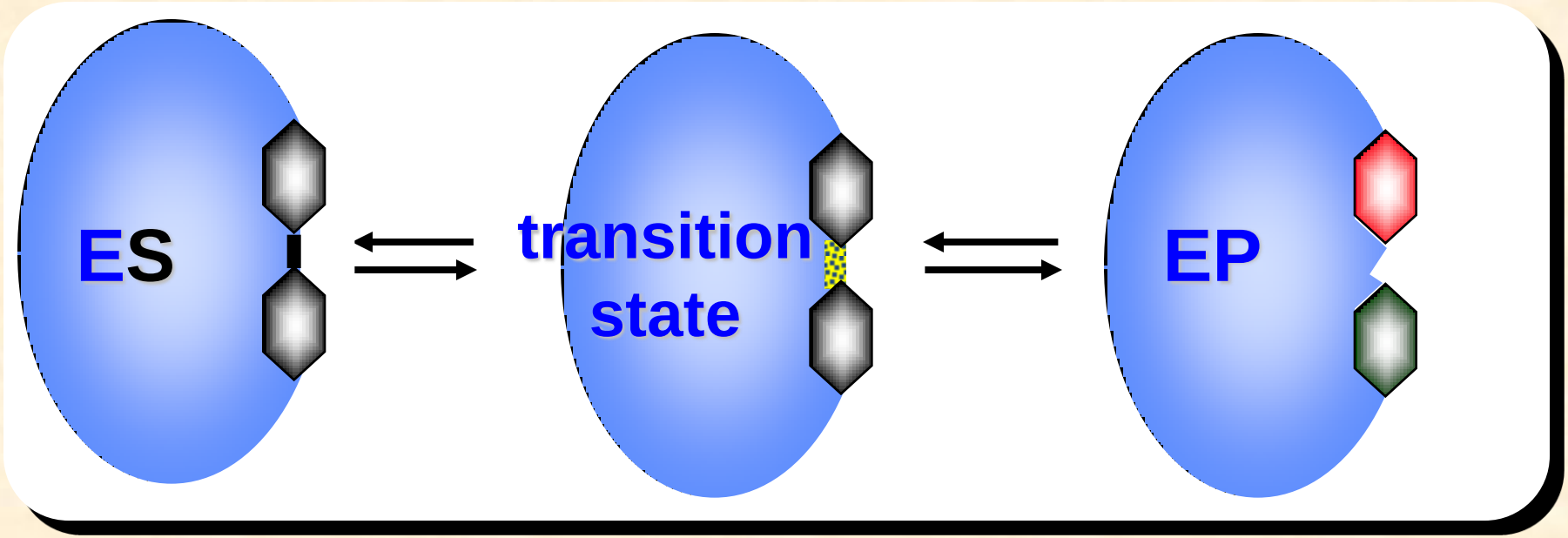
- Step 1:
- Enzyme and substrate combine to form complex
- E + S ⇌ ES
- Enzyme Substrate Complex



Enzyme-product complex

- Step 2:
- An enzyme-product complex is formed.

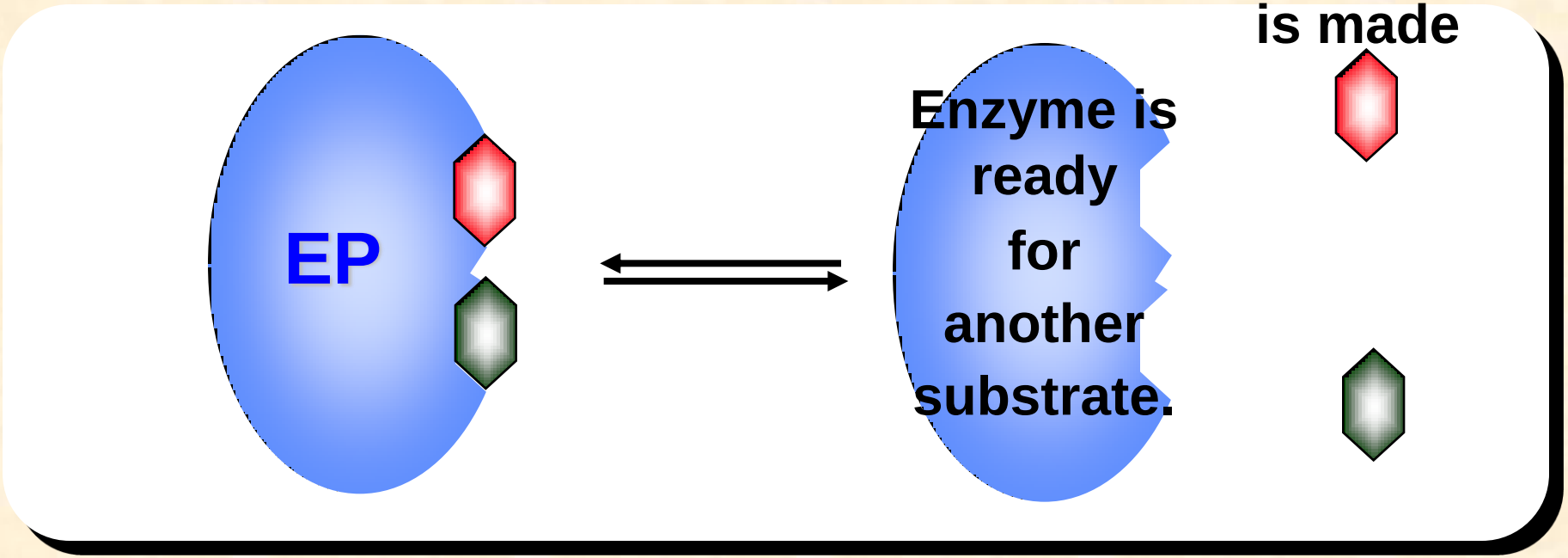
- ES ⇌ EP



Product

- The enzyme and product separate

- EP ⇌ E + P



What Affects Enzyme Activity?

- **Three factors:**
  1. **Environmental Conditions**
  2. **Cofactors and Coenzymes**
  3. **Enzyme Inhibitors**

1. Environmental Conditions

1. Extreme Temperature are the most dangerous

- high temps may denature (unfold) the enzyme.

2. pH (most like 6 - 8 pH near neutral)

3. substrate concentration .

2. Cofactors and Coenzymes

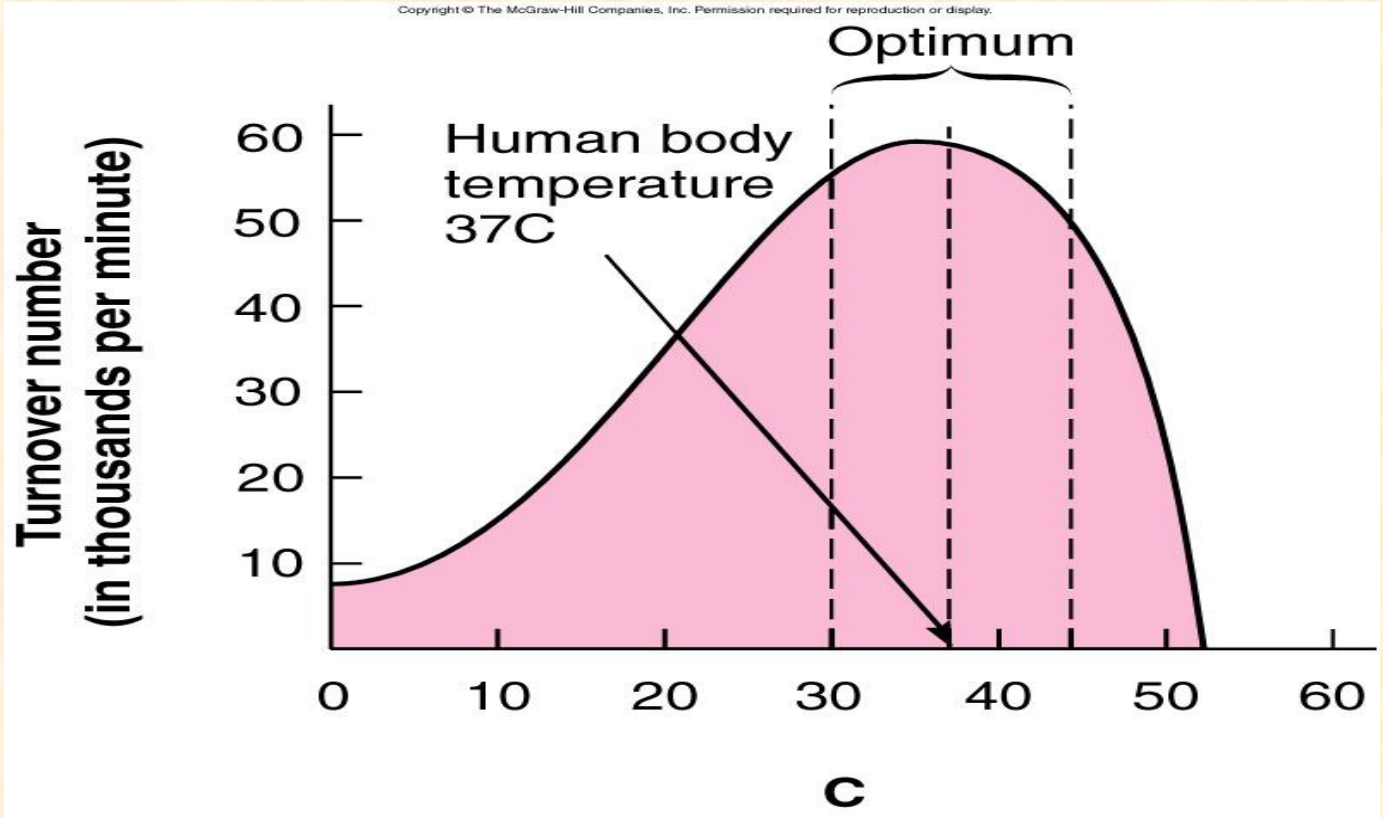
- **Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes need for proper enzymatic activity.**

- **Example:**

  Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.

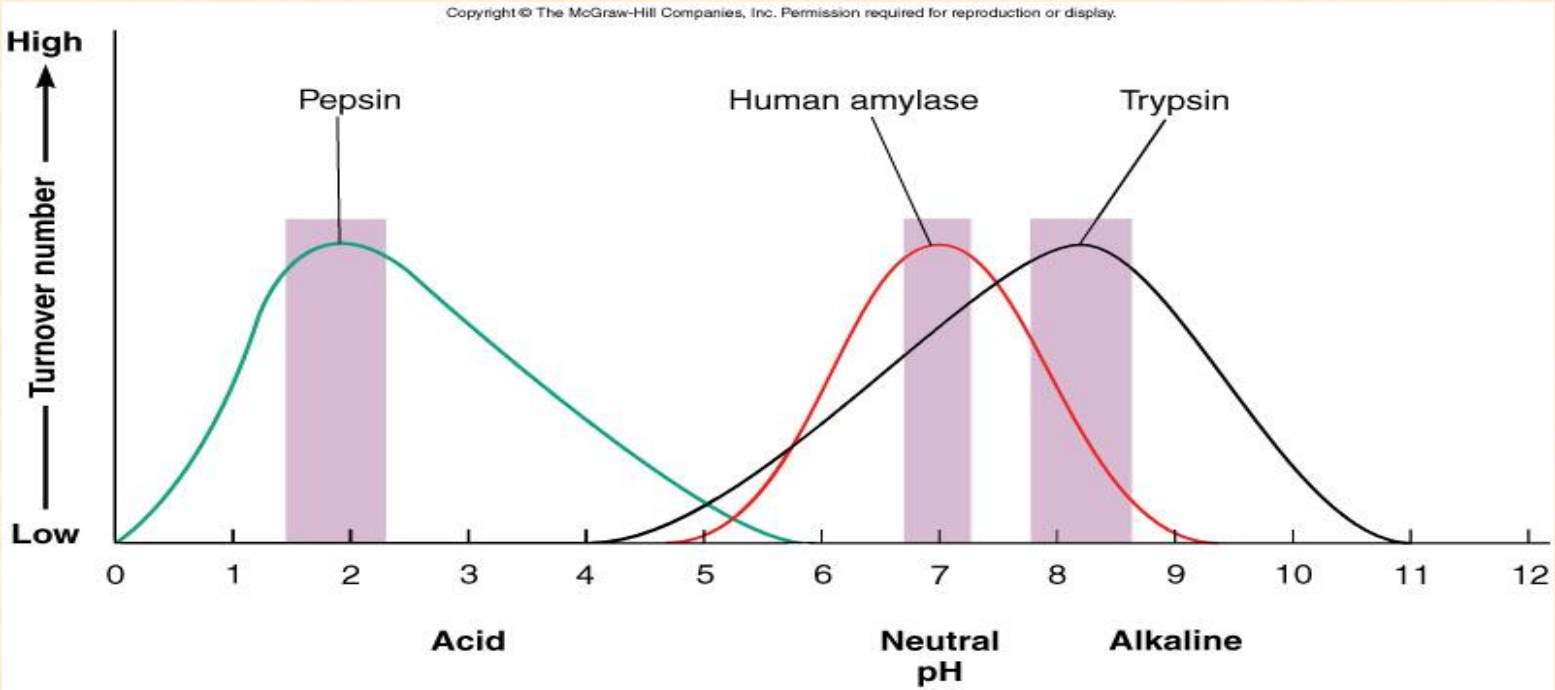
Environmental factors

- ***Optimum temperature*** The temp at which enzymatic reaction occur fastest.



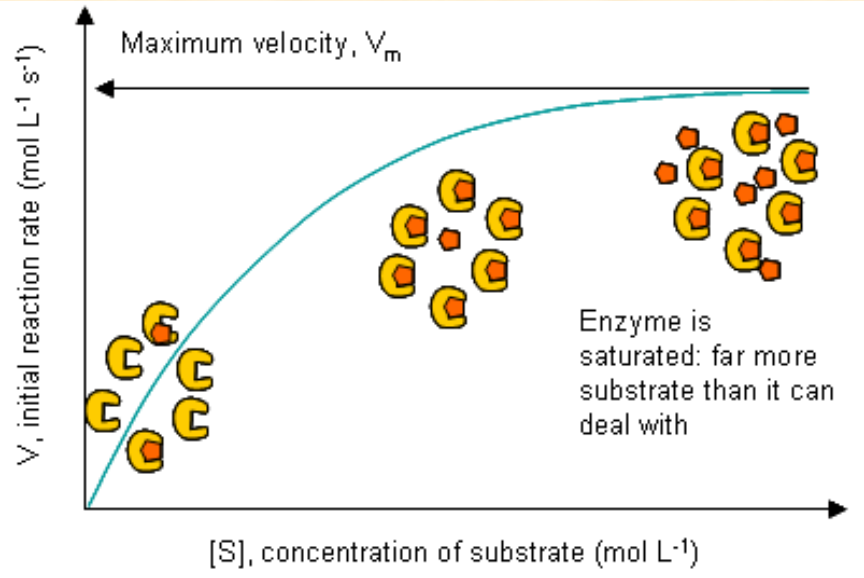
Environmental factors

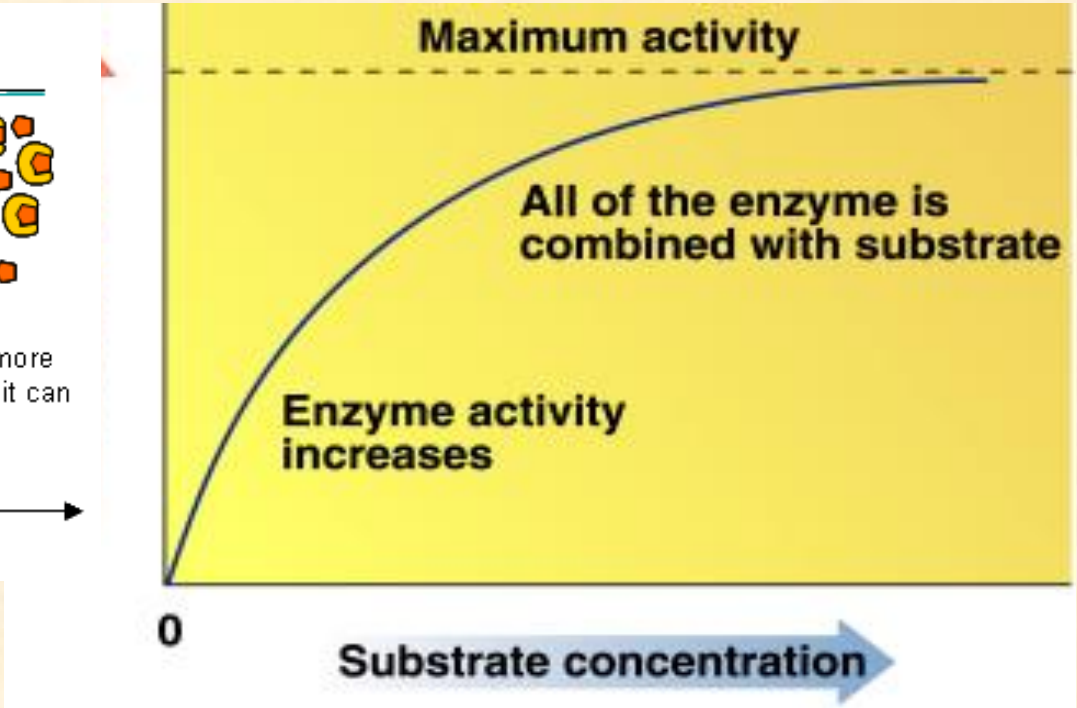
- pH also affects the rate of enzyme-substrate complexes
  - Most enzymes have an optimum pH of around 7 (neutral)
    - However, some prefer acidic or basic conditions



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)**





Enzyme Inhibition

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an inhibitor and the process is known as inhibition. There are two major types of enzyme inhibition, Irreversible and Reversible.

1- Irreversible Inhibition

The type of inhibition that can not be reversed by increasing substrate concentration or removing the remaining free inhibitor is called Irreversible inhibition. Example: Acetyl cholinesterase catalyzes the hydrolysis of Acetylcholin (to acetic acid and choline) a neurotransmitter substance functioning in certain portions of the nervous system

2-REVERSIBLE INHIBITION

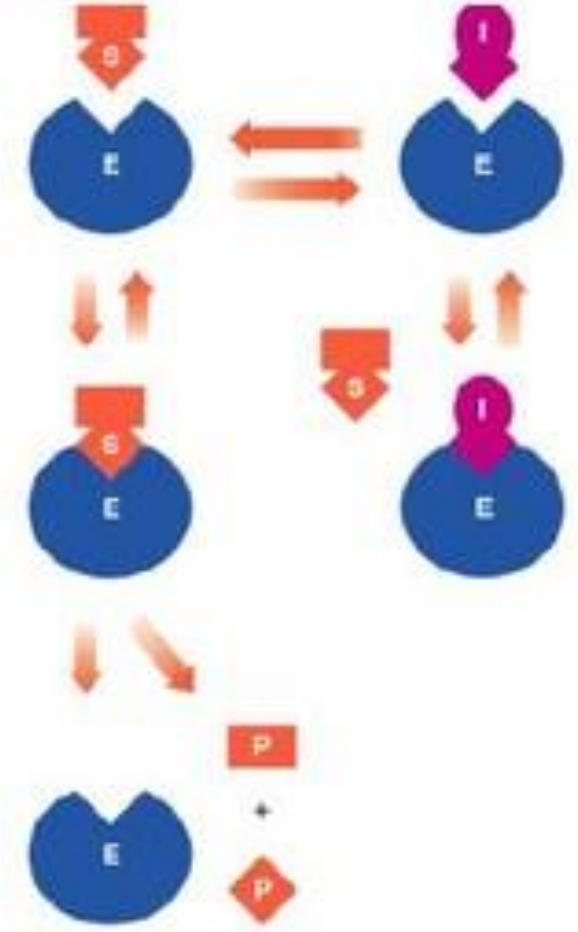
This type of inhibition can be Competitive, Non-competitive and uncompetitive Competitive Inhibition: This type of inhibition occurs when the inhibitor binds reversibly to the same site that the substrate would normally occupy, therefore, competes with the substrate for that site.

In competitive inhibition the inhibitor and substrate compete for the same active site on the enzyme as a result of similarity in structure. The enzyme substrate complex will be broken dawn to products (E+S → ES → E+P) where as enzyme inhibitor complex; (EI) will not be broken down to products.

Reversible Competitive Inhibition

A **competitive inhibitor**:

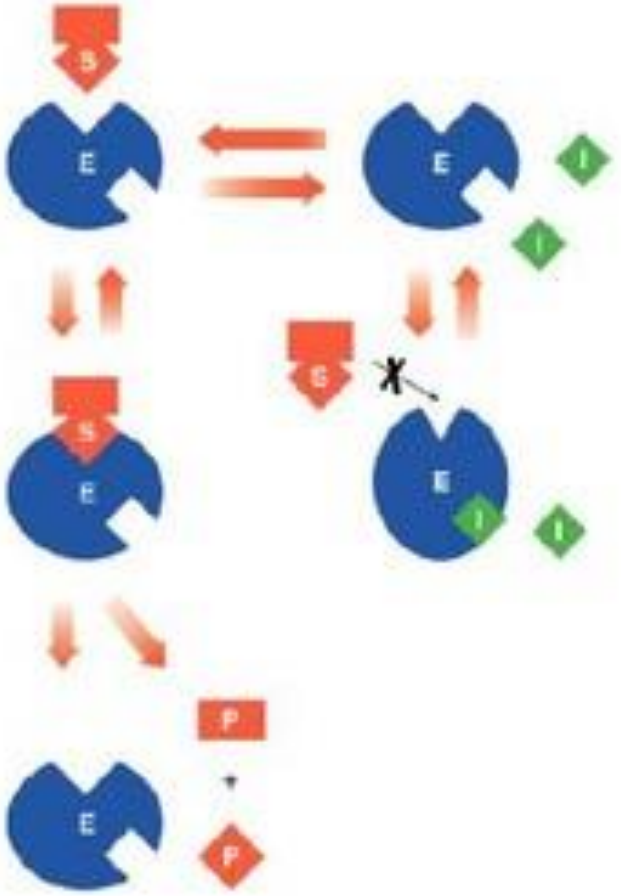
- Has a structure like the substrate.
- Competes with the substrate for the active site.
- Has its effect reversed by increasing substrate concentration.



Noncompetitive Inhibition

A **noncompetitive inhibitor**:

- Has a structure different than the substrate.
- Distorts the shape of the enzyme, which alters the shape of the active site.
- Prevents the binding of the substrate.
- Cannot have its effect reversed by adding more substrate.



Naming Enzymes

- **The name of an enzyme in many cases end in *–ase***
- **For example, *sucrase* catalyzes the hydrolysis of sucrose**

- **The name 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**

Enzymes Are Classified into six functional Classes (EC number Classification) by the International Union of Biochemists (I.U.B.). on the Basis of the Types of Reactions That They Catalyze

- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

Principle of the international classification

Each enzyme has **classification number**

consisting of four digits:

Example, **EC:** (**2.7.1.1**) **HEXOKINASE**

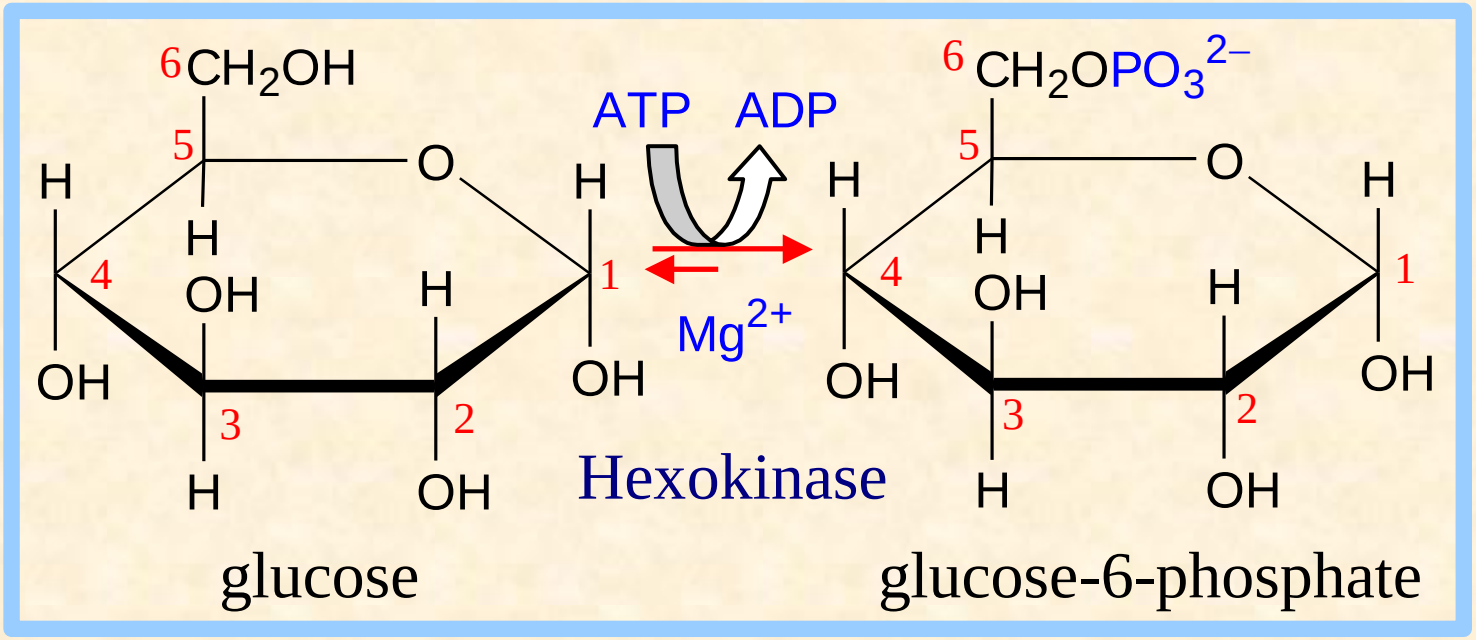
- **EC: (2.7.1.1) these components indicate the following groups of enzymes:**

- **2. IS CLASS (TRANSFERASE)**

- **7. IS SUBCLASS (TRANSFER OF PHOSPHATE)**

- **1. IS SUB-SUB CLASS (ALCOHOL IS PHOSPHATE ACCEPTOR)**
- **1. SPECIFIC NAME**

  ATP,D-HEXOSE-6-PHOSPHOTRANSFERASE (Hexokinase)



1. Hexokinase catalyzes:

Glucose + ATP → glucose-6-P + ADP

ENZYMES IN CLINICAL DIAGNOSIS:

Plasma enzymes can be classified into two major groups

1. Those relatively, small group of enzymes secreted into the plasma by certain organs (i.e. Enzymes those have function in plasma) For example: - the liver secretes zymogens of the enzymes involved in blood coagulation.

2. Those large enzyme species released from cells during normal cell turnover. These enzymes are normally intracellular and have no physiologic function in the plasma. In healthy individuals the levels of these enzymes are fairly constant and represent steady state in which the rate of release from cells into the plasma is balanced by an equal rate or removal from the plasma.

Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues.

The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Thus, the degree of elevation of a particular enzyme activity in plasma is often useful in evaluating the diagnosis and prognosis for the patient.

Measurement of enzymes concentration of mostly the latter type in plasma gives valuable informatio0n about disease involving tissues of their origin.

1. Lipase:

It is an enzyme catalyzing the hydrolysis of fats. It is secreted by pancreas and Liver. The plasma lipase level may be low in liver disease, Vitamin A deficiency, some malignancies, and diabetes mellitus. It may be elevated in acute pancreatitis and pancreatic carcinoma.

2. α- Amylase

α- amylase is the enzyme concerned with the break down of dietary starch and glycogen to maltose. It is present in pancreatic juice and saliva as well as in liver, fallopian tubes and muscles. The enzyme is excreted in the Urine. The main use of amylase estimations is in the diagnosis of acute pancreatitis. The plasma amylase level may be low in liver disease and increased in high intestinal obstruction, mumps, acute pancreatitis and diabetes.

3. Trypsin: Trypsin is secreted by pancreas. Elevated levels of trypsin in plasma occur during acute pancreatic disease.

4. Alkaline phosphates (ALP)

The alkaline phosphates are a group of enzymes, which hydrolyze phosphate esters at an alkaline pH. They are found in bone, liver, kidney, intestinal wall, lactating mammary gland and placenta. In bone the enzyme is found in osteoblasts and is probably important for normal bone function. The level of these enzymes may be increased in rickets and osteomalacia, hyperparathyroidism, obstructive jaundice, and metastatic carcinoma. Serum alkaline phosphatase levels may be increase in congestive heart failure result of injury to the liver.

5. Acid Phosphatase (ACP)

Acid phosphatases catalyzing the hydrolysis of various phosphate esters at acidic pH is found in the prostate, liver, red cells, platelets and bone. It may be elevated in metastatic prostatic carcinoma.

6. Transaminases:

Two transaminases are of clinical interest.

A- Aspartate Transaminase, AST or (Glutamate oxaloacetate transaminase, GOT):

catalyzes the transfer of the amino group of aspartic acid to α- ketoglutarate forming glutamate and oxaloacetate.

AST or GOT is widely distributed, with high concentration, in the heart, liver, skeletal muscle, kidney and erythrocytes, and damage to any of these tissues may cause raised levels.

B. Alanine transaminase, ALT (Glutamate pyruvate transaminase, GPT):

Transfer the amino group of alanine to α- ketoglutarate, forming glutamate and pyruvate. It is present in high concentration in liver and to a lesser extent in skeletal muscle, kidney and heart.

- Serum levels of glutamate- pyruvate transaminase (SGOT) and Glutamateoxaloacetate- transaminase (SGOT) are useful in the diagnosis of liver parenchymal damage and myocardial damage respectively. In liver damage, both enzymes are increased, but SGPT increases more. In myocardial infarction SGOT is increased

7. Lactate Dehydrogenase (LDH)

It catalyzes the reversible interconversion of lactate and pyruvate. It is widely distributed with high concentrations in the heart, skeletal muscle, liver, kidney, brain and erythrocytes. The enzyme is increased in plasma in myocardial infarction, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of it isoenzymes is more useful in clinical diagnosis to differentiate hepatic disease and myocardial infarction.

8. Creatine kinase (CK) or creatine phosphokinase (CPK):

CK (CPK) is found in heart muscle, brain and skeletal muscle. Measurement of serum creatine phosphokinase activity is of value in the diagnosis of disorders affecting skeletal and cardiac muscle. The level of CPK in plasma highly increased in myocardial infarction.