Enzymes

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Introduction

- Enzymes are *biological catalysts* that speed up the rate of the biochemical reaction.
- Most enzymes are three dimensional *globular proteins* (tertiary and quaternary structure).

Rates of reaction and their dependence on activation energy

- Activation Energy (Ea):

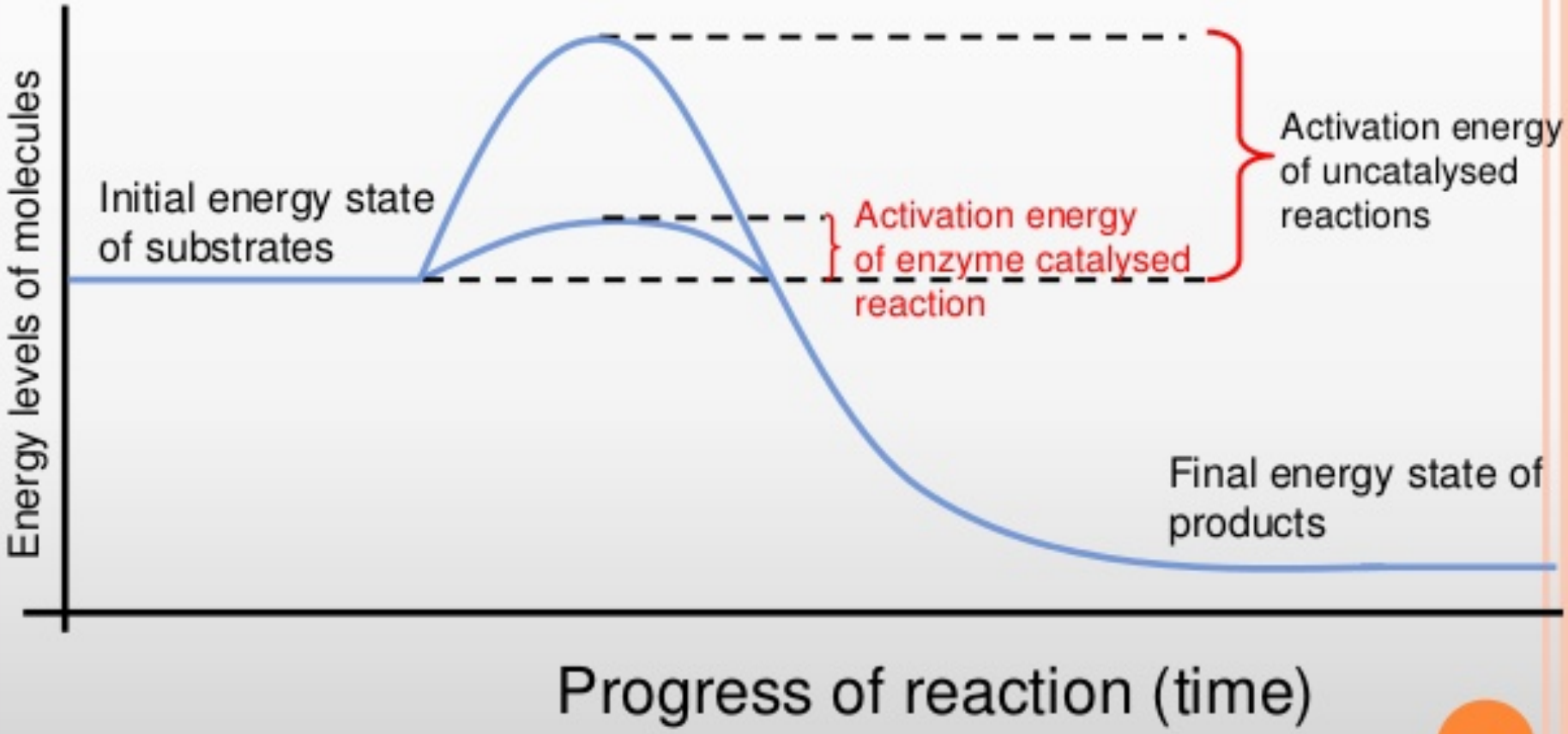
"The least amount of energy needed for a chemical reaction to take place."

- Enzyme (as a catalyst) acts on substrate in such a way that they lower the activation energy by changing the route of the reaction.
- The reduction of activation energy (Ea) increases the amount of reactant molecules that achieve a sufficient level of energy, so that they reach the activation energy and form the product.

Example:

- *Carbonic anhydrase* catalyses the hydration of 10^6 CO_2 molecules per second which is 10^7x faster than spontaneous hydration.

ENZYMES LOWER THE ACTIVATION ENERGY OF A REACTION



Location of enzymes

- **Intracellular** enzymes are *synthesized and retained in the cell for the use of cell itself.*
- They are found in the cytoplasm, nucleus, mitochondria and chloroplast.

Example :

- Oxydoreductase catalyses biological oxidation.
- Enzymes involved in reduction in the mitochondria.

- **Extracellular** enzymes are *synthesized in the cell but secreted from the cell to work externally.*

Example :

- Digestive enzyme produced by the pancreas, are not used by the cells in the pancreas but are transported to the duodenum.

Structure of Enzymes

- The *active site* of an enzyme is the region that binds substrates, co-factors and prosthetic groups and contains residue that helps to hold the substrate.

Protein structure

Scaffold to support and position active site

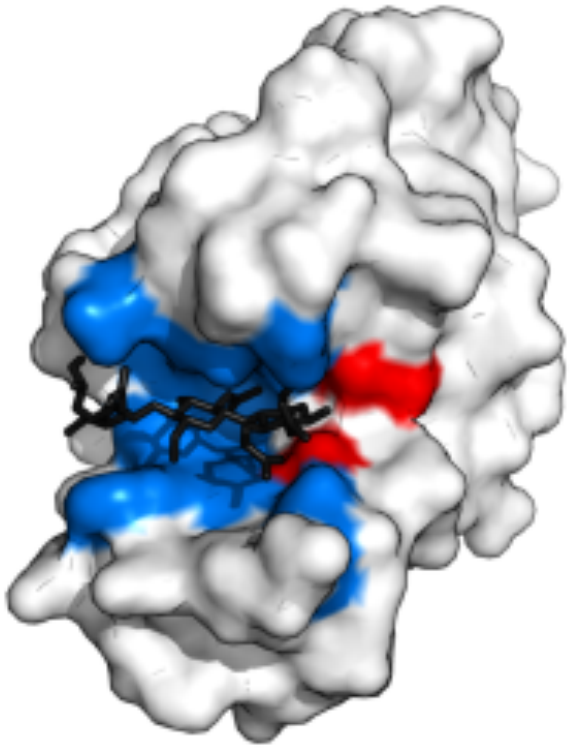
Active site

Binding sites

Bind and orient substrate(s)

Catalytic site

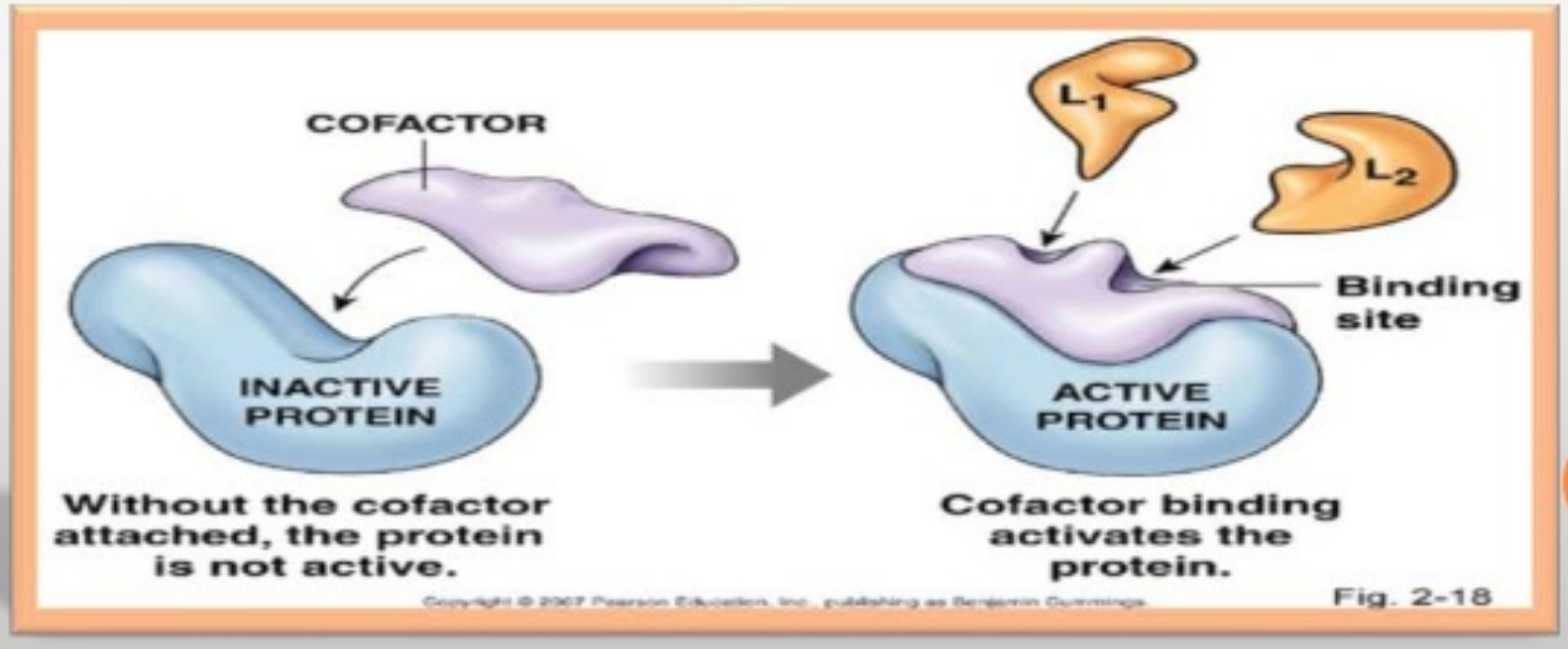
Reduce chemical activation energy



CO-FACTORS

- Co-factor is the non protein molecule which carries out chemical reactions that can not be performed by standard 20 amino acids.

- Co-factors are of two types:
  - Organic co-factors → Flavin, heme, Niacin
  - Inorganic cofactors → Zn^{+2}, Mg^{+2}



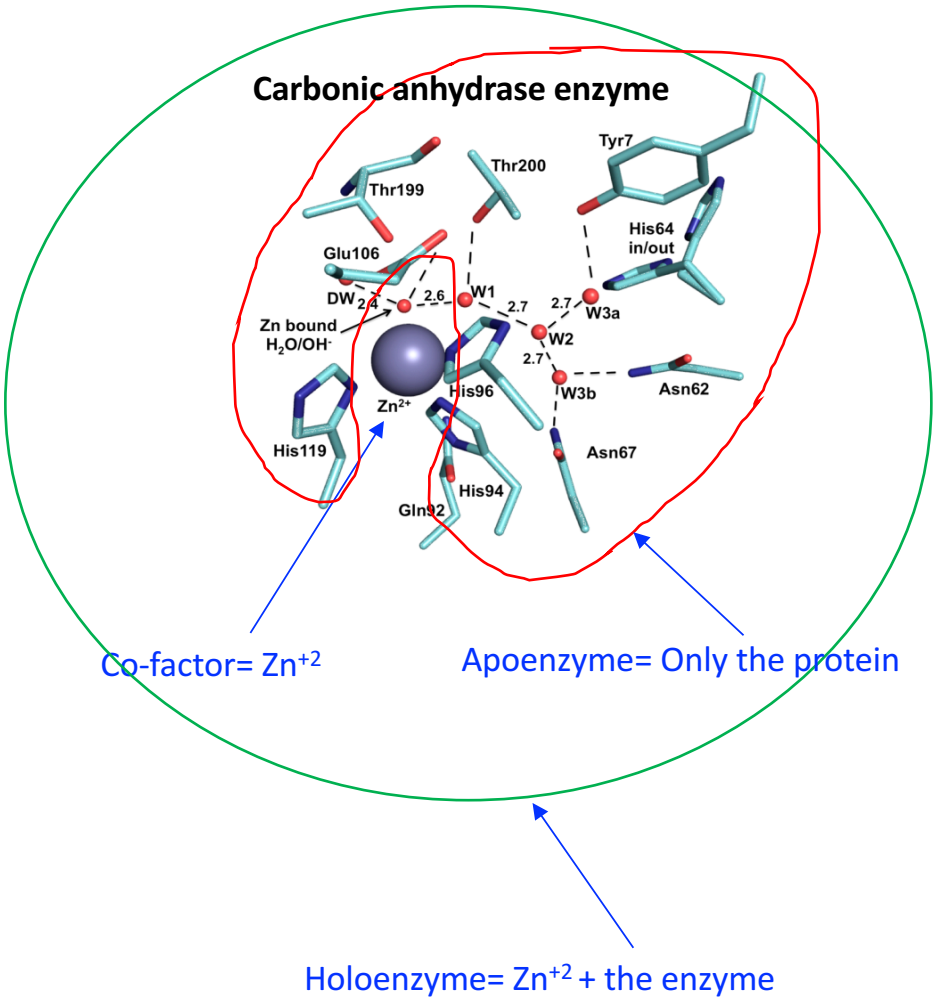
Cofactors:

The enzyme with its cofactor called **Holoenzyme** .

The protein portion of the holoenzyme is called **Apoenzyme**.

In the absence of the appropriate cofactor, the apoenzyme typically does not show biologic activity.

A prosthetic group is a tightly bound coenzyme that does not dissociate from the enzyme

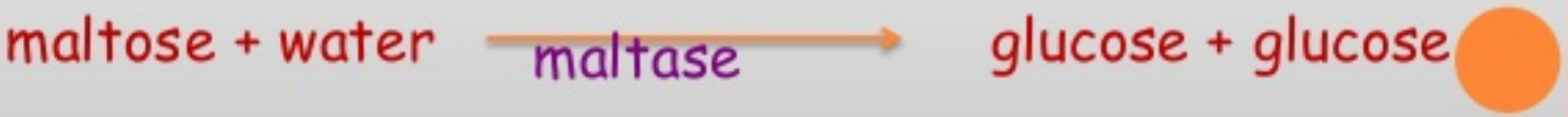


NOMENCLATURE OF ENZYMES

- An enzyme is named according to the name of the substrate it catalyses.
- Some enzymes were named before a systematic way of naming enzyme was formed.

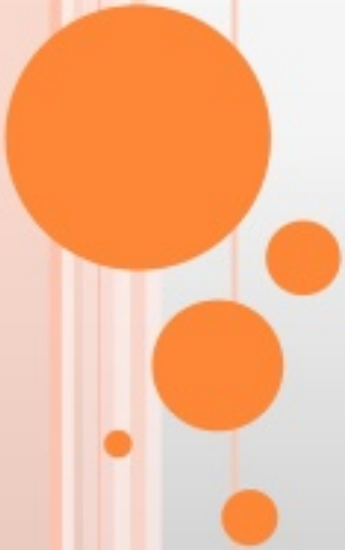
  Example: pepsin, trypsin and rennin
- By adding suffix ***-ase*** at the end of the name of the substrate, enzymes are named.
- Enzyme for catalyzing the hydrolysis is termed as hydrolase.

  Example :



EXAMPLES

substrate	enzymes	products
lactose	lact**ase**	glucose + galactose
maltose	malt**ase**	Glucose
cellulose	cellul**ase**	Glucose
lipid	lip**ase**	Glycerol + fatty acid
starch	amyl**ase**	Maltose
protein	prote**ase**	Peptides + polypeptide



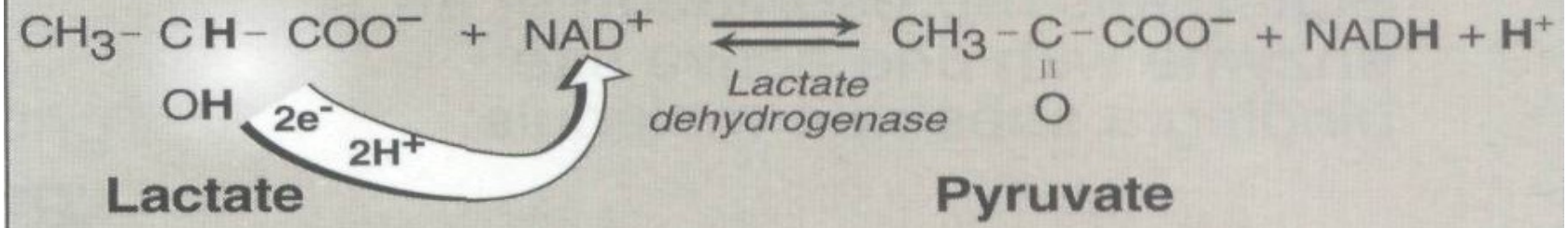
Classification

CLASSIFICATION OF ENZYMES

- A systematic classification of enzymes has been developed by *International Enzyme Commission*.
- This classification is based on the type of reactions catalyzed by enzymes.
- There are *six* major classes.
- Each class is further divided into sub classes, sub sub-classes and so on, to describe the huge number of different enzyme-catalyzed reactions.

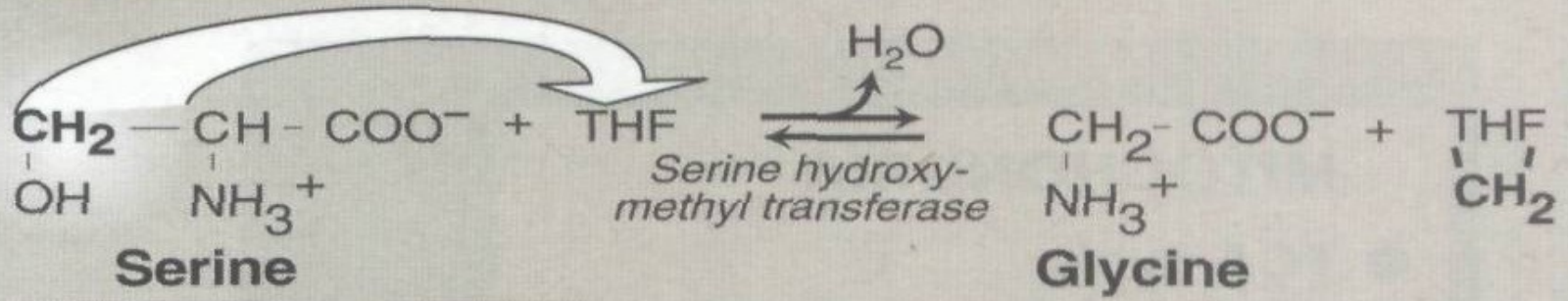
1. Oxidoreductases

Catalyze oxidation-reduction reactions, such as:



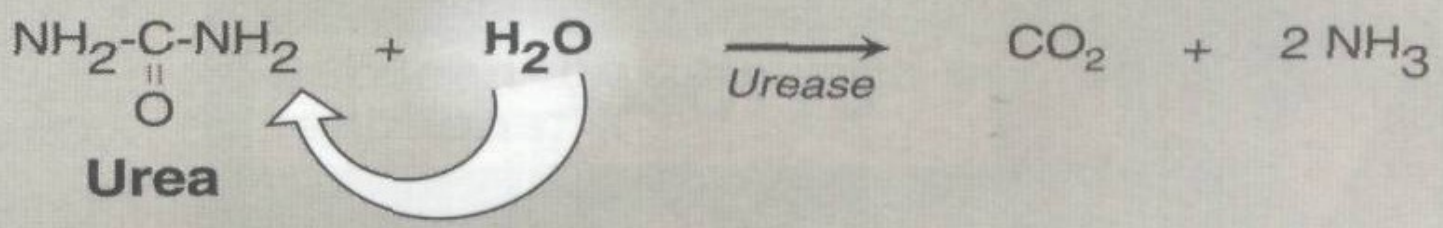
2. Transferases

Catalyze transfer of C-, N-, or P-containing groups, such as:



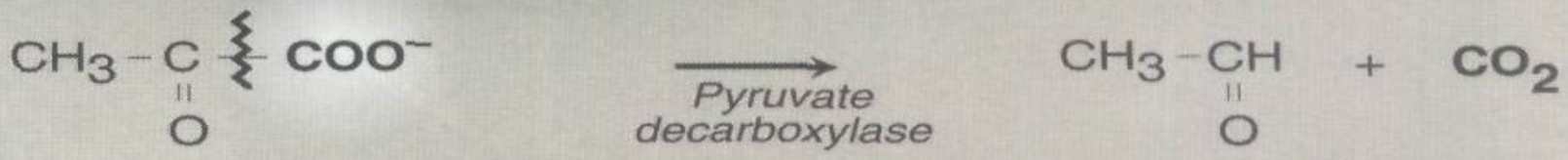
3. Hydrolases

Catalyze cleavage of bonds by addition of water, such as:



4. Lyases

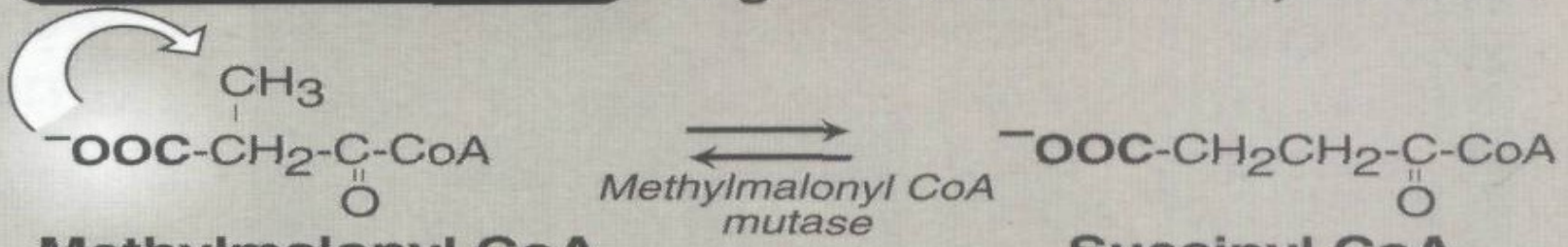
Catalyze cleavage of C–C, C–S and certain C–N bonds, such as:



Pyruvate **Acetaldehyde**

5. Isomerases

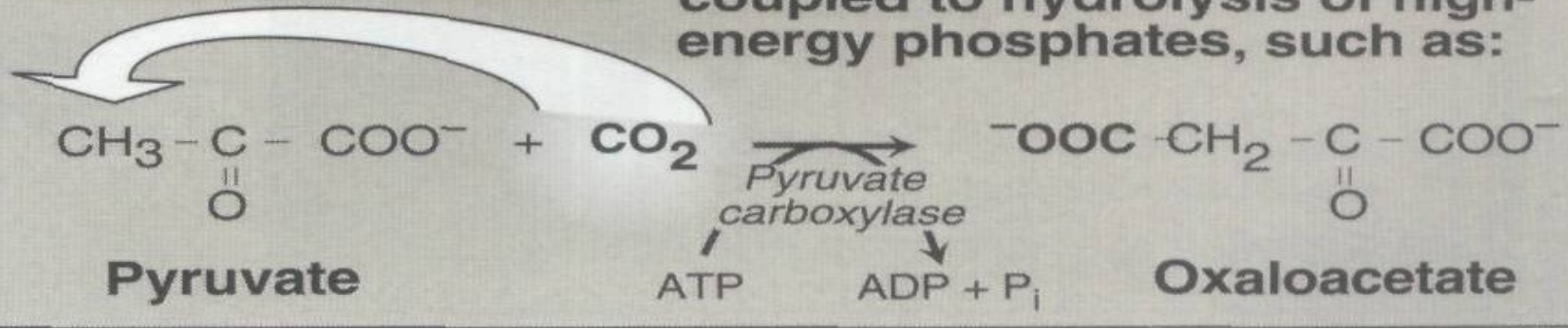
Catalyze racemization of optical or geometric isomers, such as:



Methylmalonyl CoA **Succinyl CoA**

6. Ligases

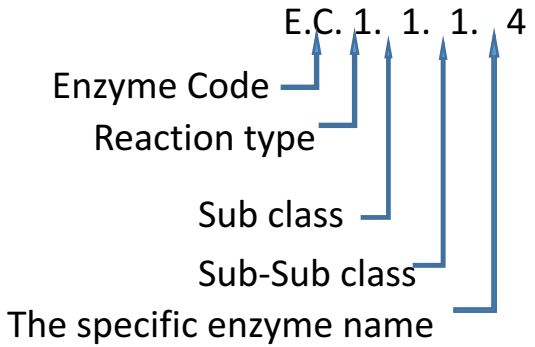
Catalyze formation of bonds between carbon and O, S, N coupled to hydrolysis of high-energy phosphates, such as:

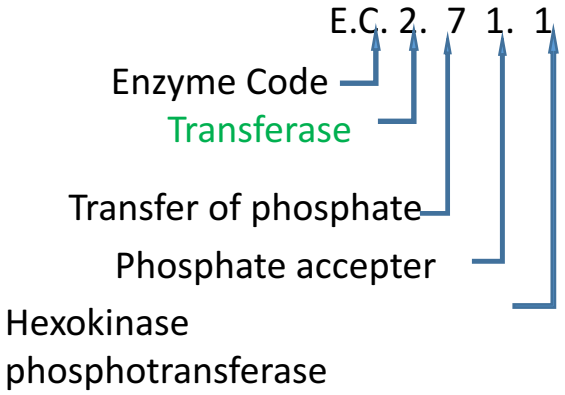


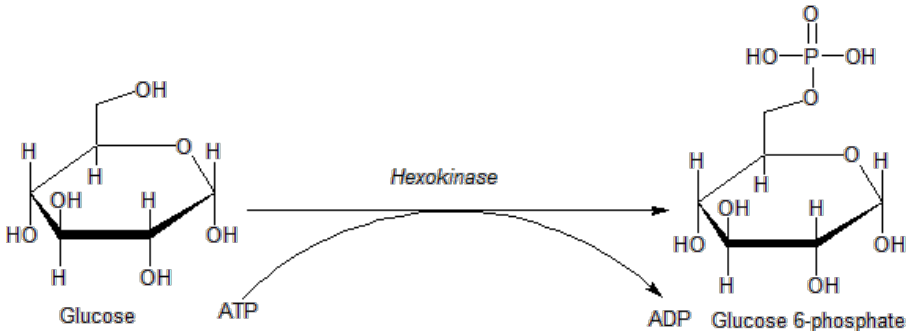
Pyruvate **Oxaloacetate**

The enzyme code number

The EC number : is a unique identifier for each enzyme classified according to this system. The EC number consists of 4 digits. The first digit represents the class of enzyme, the second digit strands for the subclass, the third digit represents the sub-subclass or subgroup and the fourth digit provides the particular enzyme





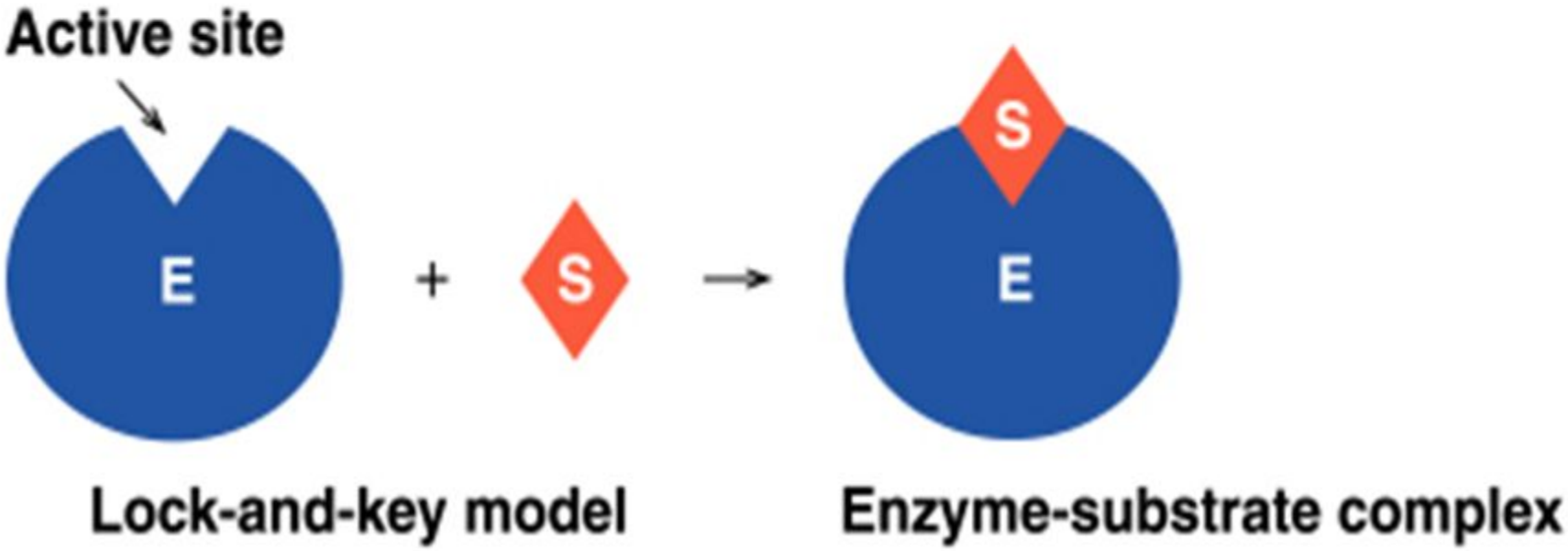


Substrate binding

Lock & Key Model

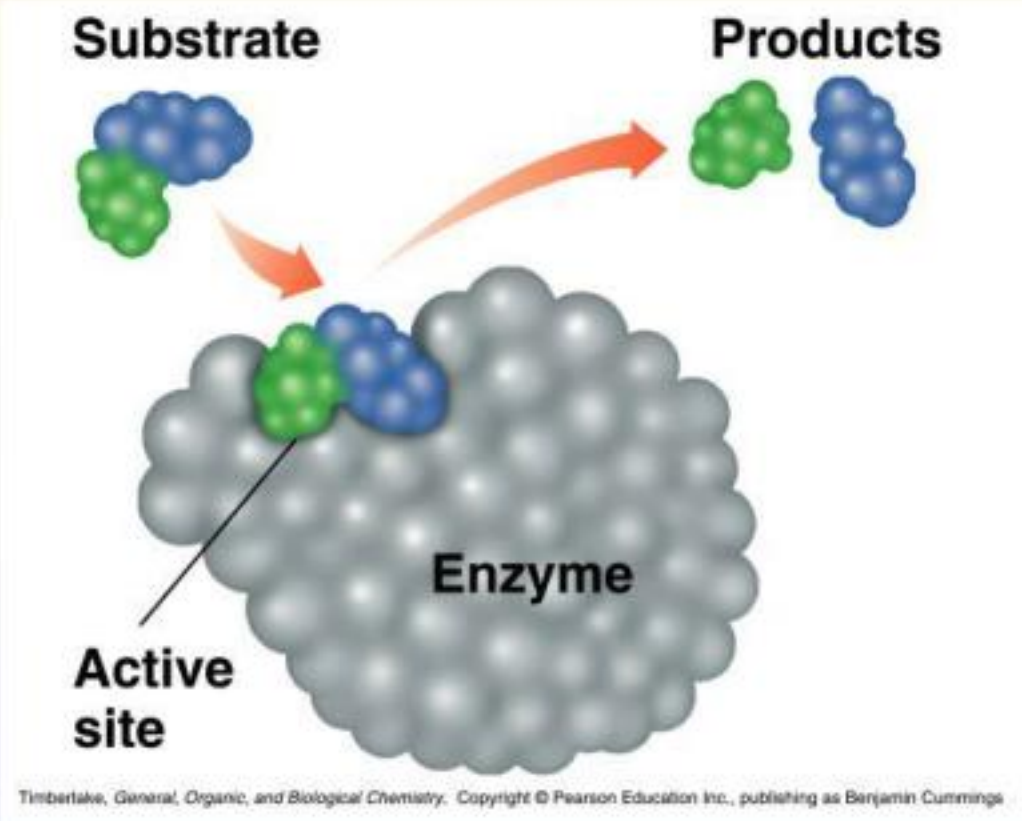
In the lock-and-key model of enzyme action:

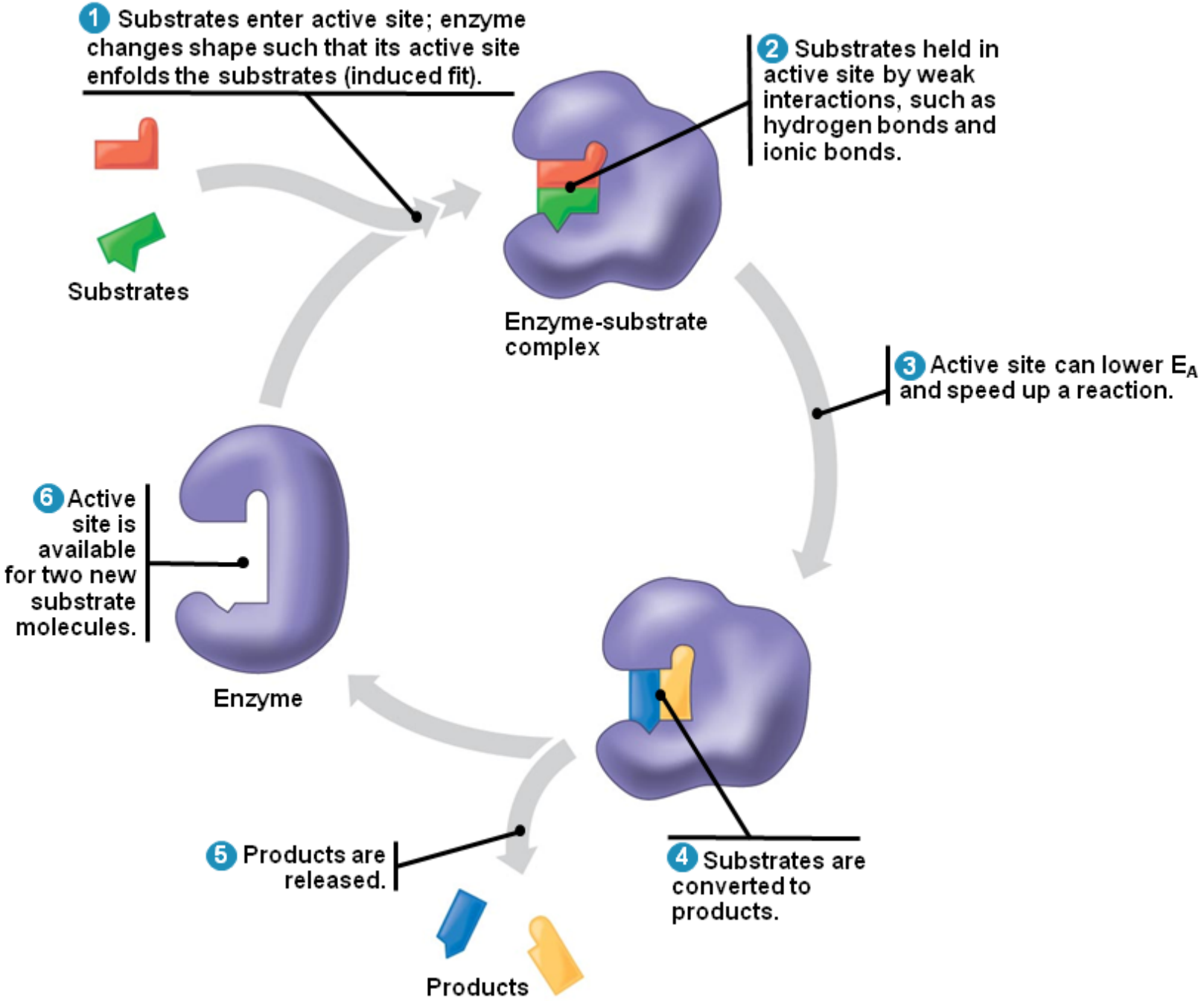
- The active site has a rigid shape.
- Only substrates with the matching shape can fit.
- The substrate is a key that fits the lock of the active site.



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)





Enzymes kinetics

INTRODUCTION

"It is a branch of biochemistry in which we study *the rate of enzyme catalyzed reactions*."

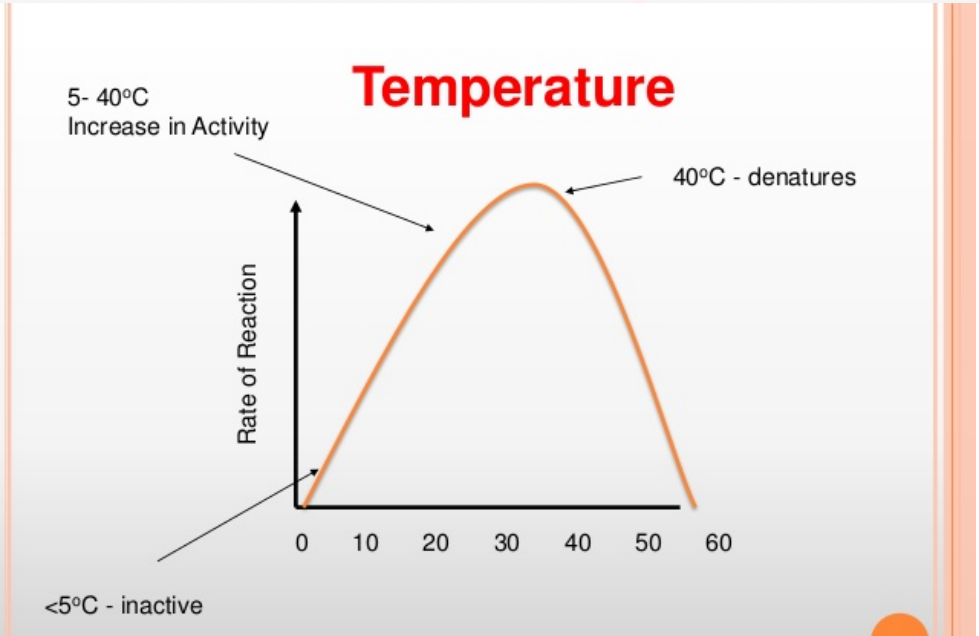
- Kinetic analysis reveals the number and order of the individual steps by which enzymes transform substrate into products
- Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of that enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme

Factors affecting rate of enzyme catalyzed reactions

- Temperature
- Hydrogen ion concentration(pH)
- Substrate concentration

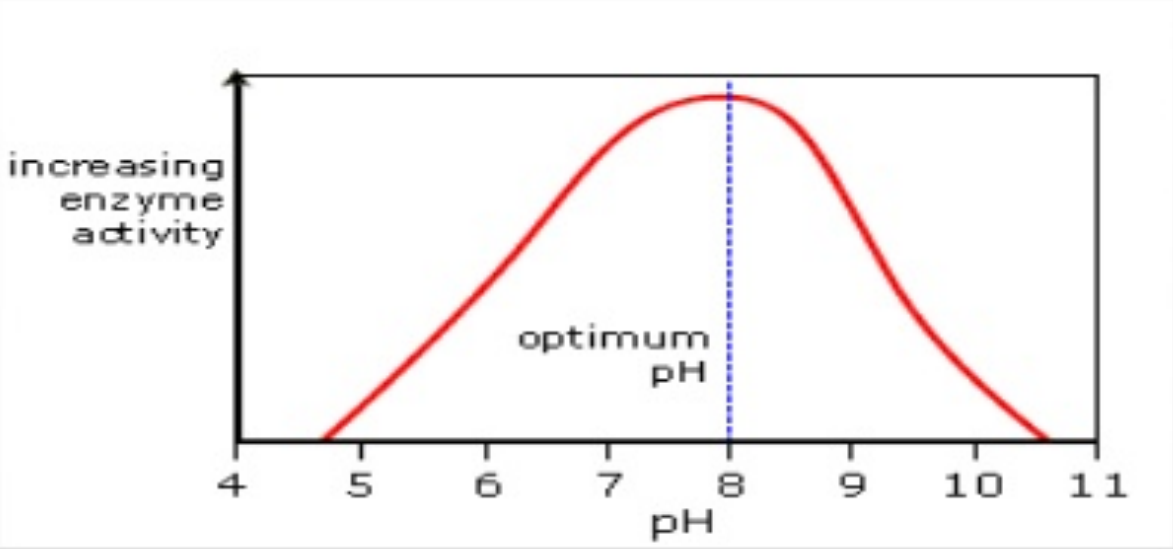
Effect of Temperature

- Raising the temperature increases the rate of enzyme catalyzed reaction by increasing kinetic energy of reacting molecules.
- Enzymes work maximum over a particular temperature known as *optimum temperature*. Enzymes for humans generally exhibit stability temperature up to 35-45 □ C



EFFECT OF pH

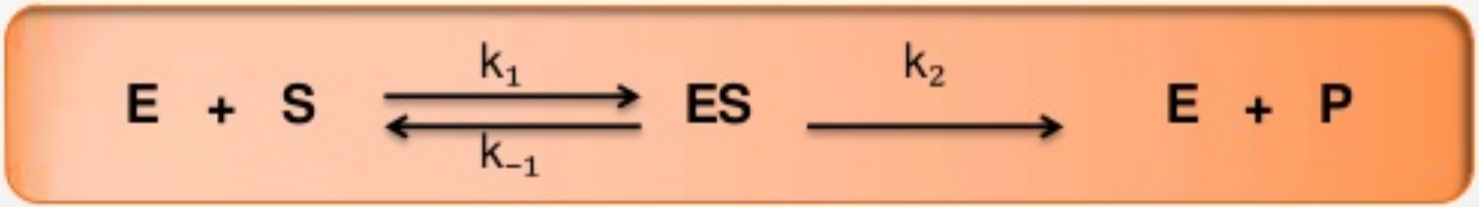
- Rate of almost all enzymes catalyzed reactions depends on pH
- Most enzymes exhibit optimal activity at pH value between *5 and 9*
- High or low pH value than optimum value will cause ionization of enzyme which result in denaturation of enzyme



Michaelis-Menten model & effects of substrate concentration

- Michaelis-Menten Model:

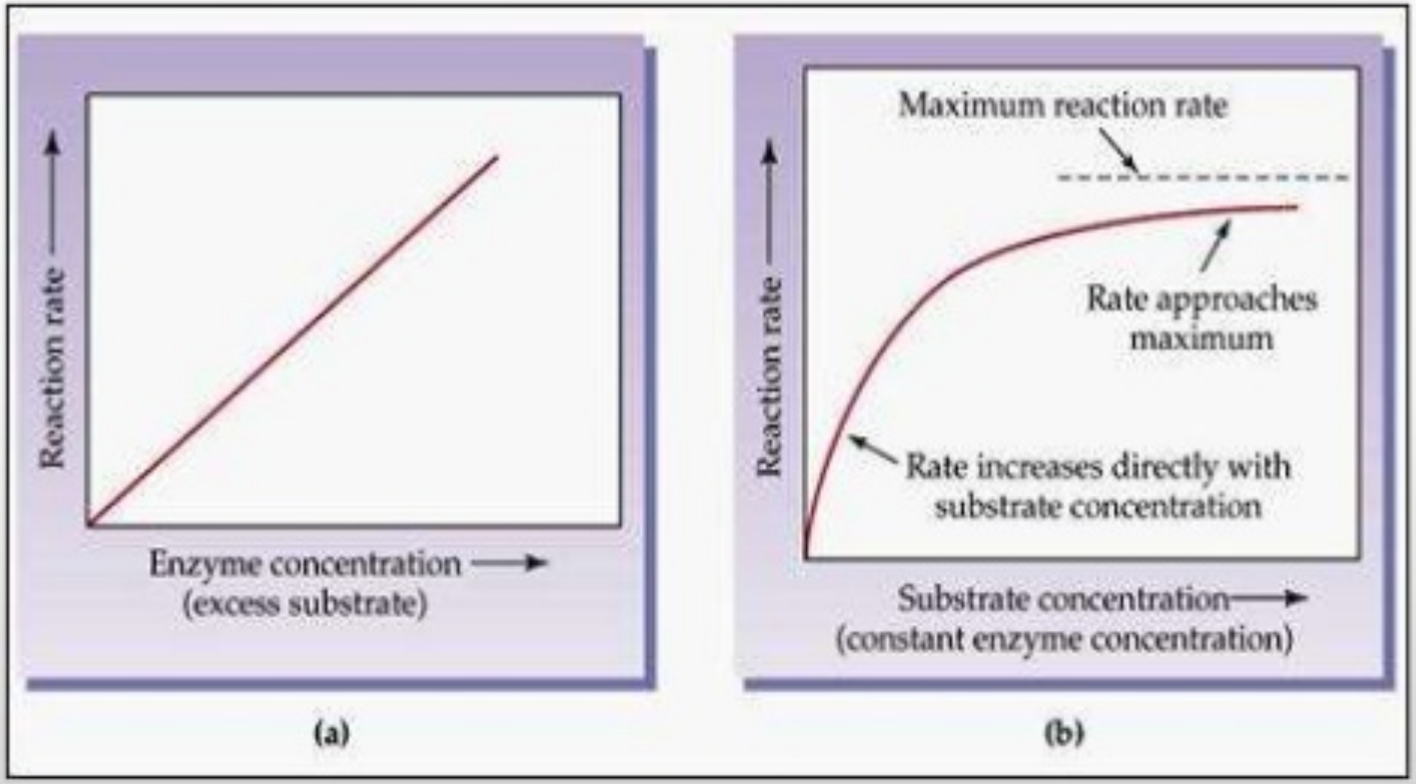
"According to this model the enzyme reversibly combines with substrate to form an ES complex that subsequently yields product, regenerating the free enzyme."



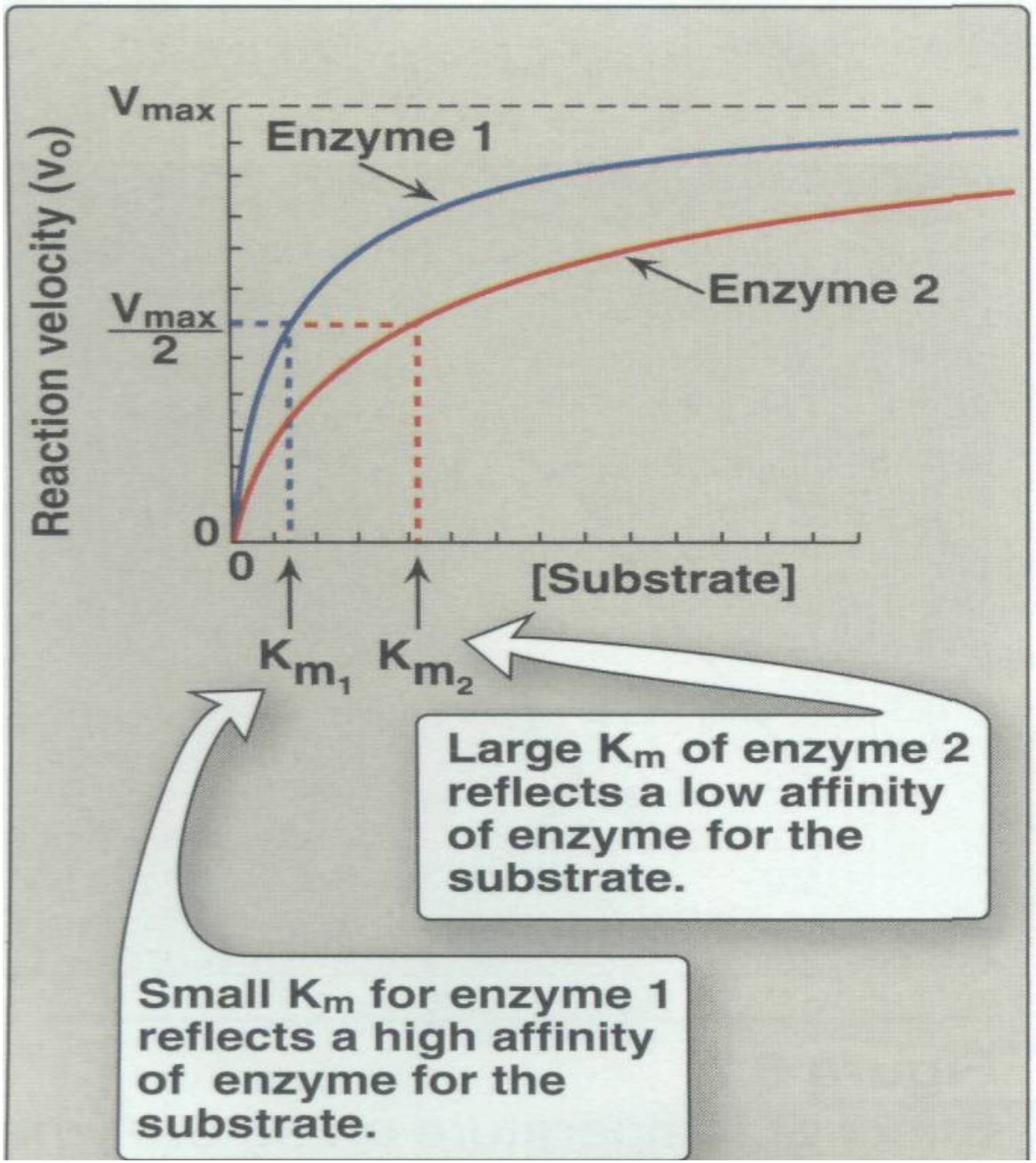
where:

- S is the substrate
- E is the enzyme
- ES-is the enzyme substrate complex
- P is the product
- K1,K-1 and K2 are rate constants

SUBSTRATE CONCENTRATION



Michaelis-Menten plot



Michaelis-Menten Equation

- Michaelis-Menten Equation:

"It is an equation which describes how reaction velocity varies with substrate concentration."

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

- Where
  - V_o is the initial reaction velocity.
  - V_{max} is the maximum velocity.
  - K_m is the Michaelis constant = $(k_{-1}+k_2)/k_1$.
  - [S] is the substrate concentration.

. Enzyme Denaturation

A. When proteins unravel and lose their original conformation
B. Can be caused by extreme temperatures or pH levels
C. Prevents substrate from binding by changing the active site
D. The proteins become inactive

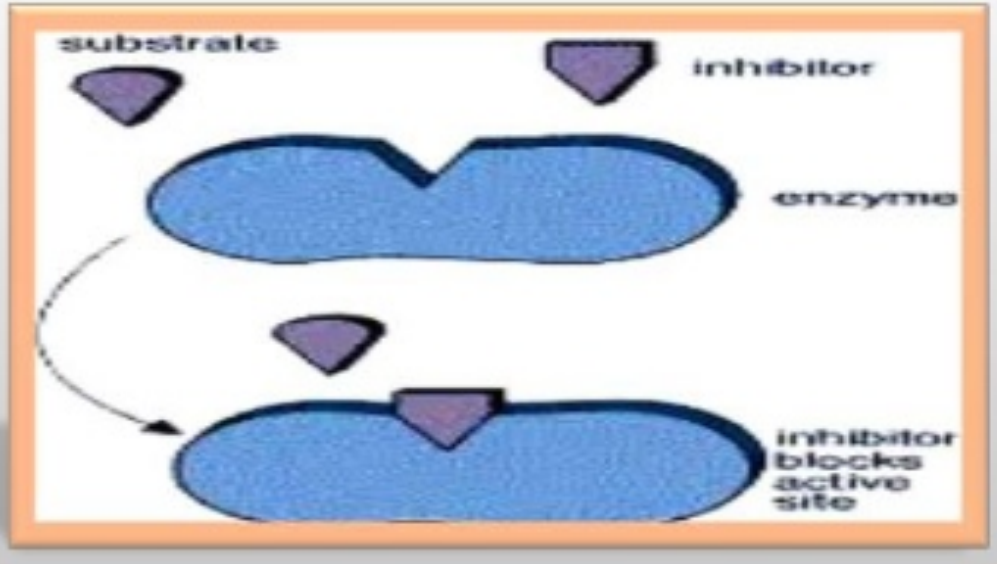
INHIBITION

INHIBITION

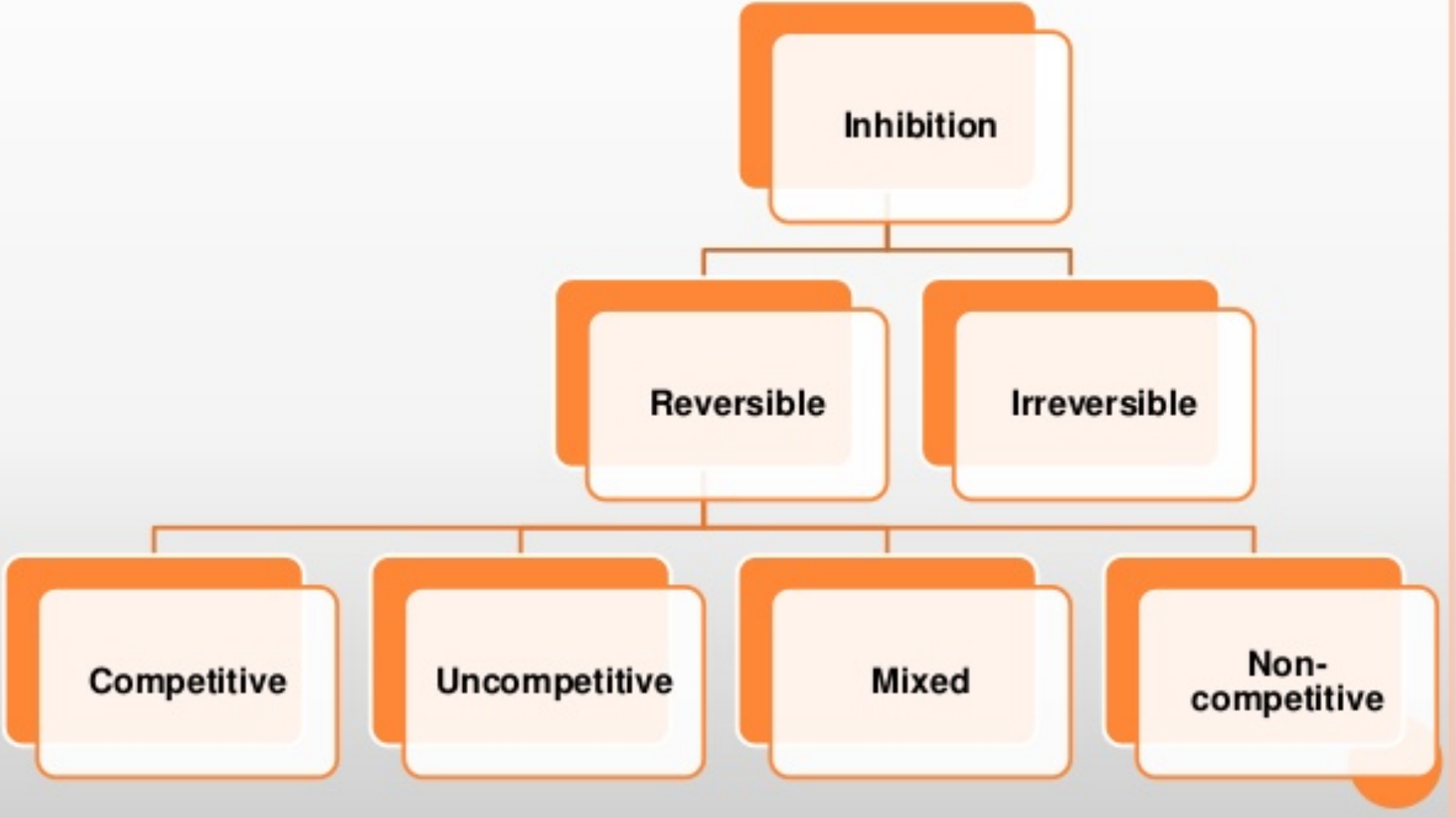
- The prevention of an enzyme process as a result of interaction of inhibitors with the enzyme.

- INHIBITORS:

  Any substance that can diminish the velocity of an enzyme catalyzed reaction is called an inhibitor.

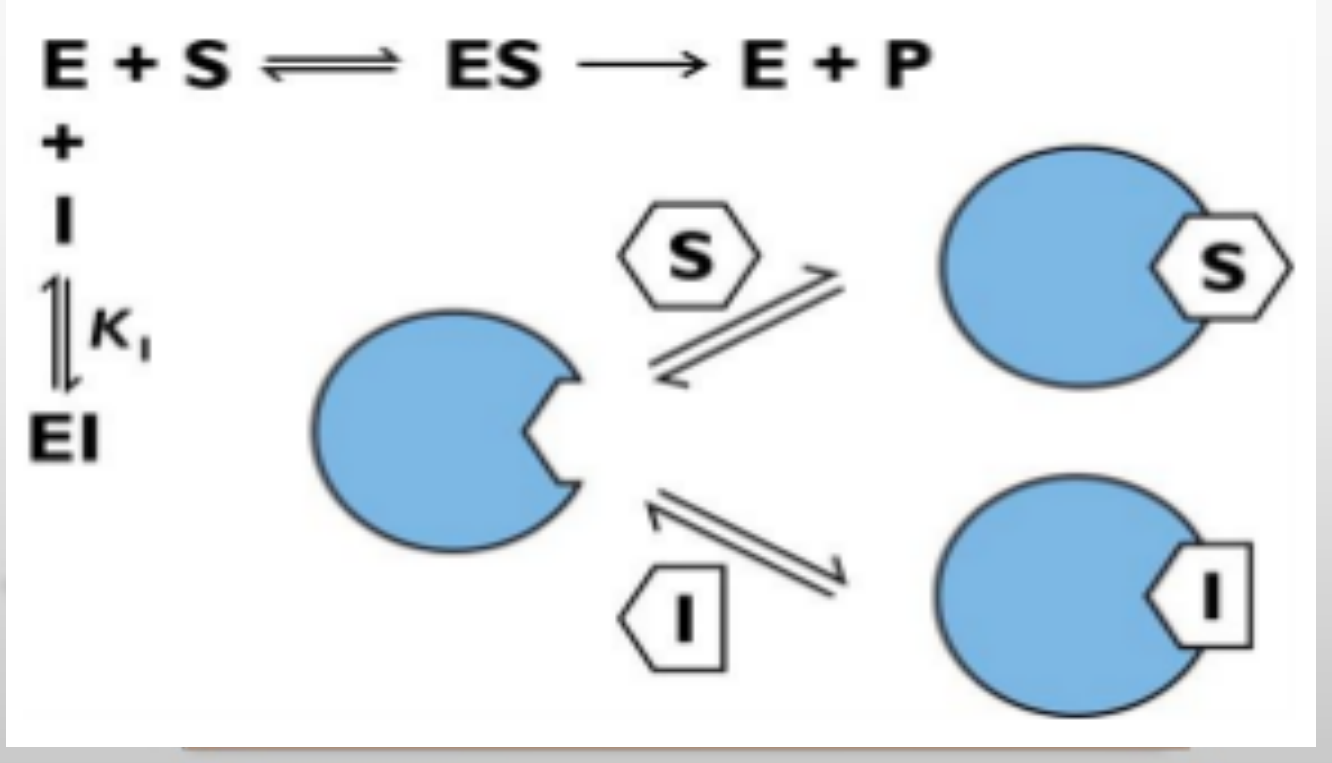


TYPES OF INHIBITION



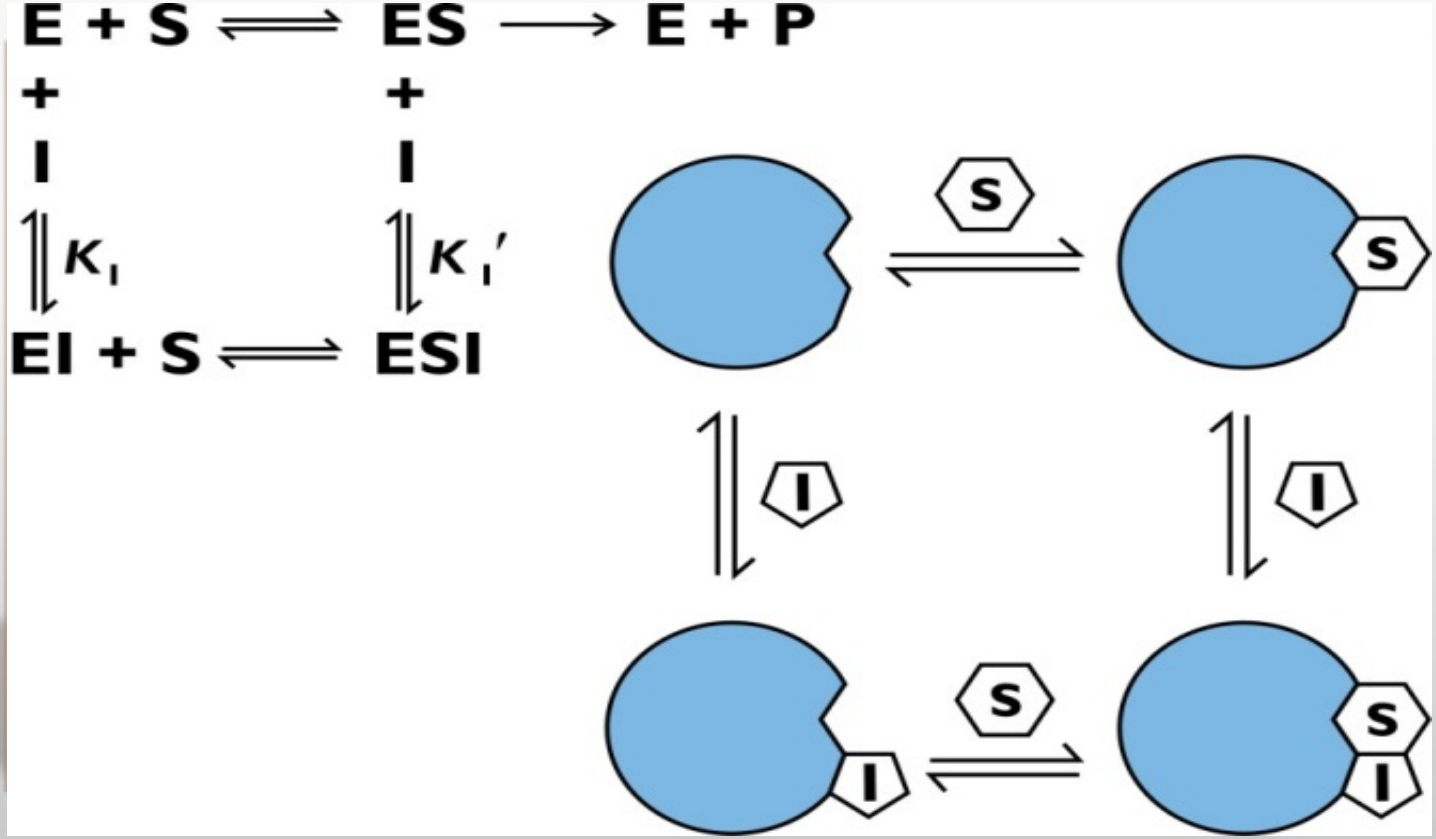
COMPETITIVE INHIBITION

- In this type of inhibition, the inhibitors compete with the substrate for the active site. Formation of E.S complex is reduced while a new E.I complex is formed.



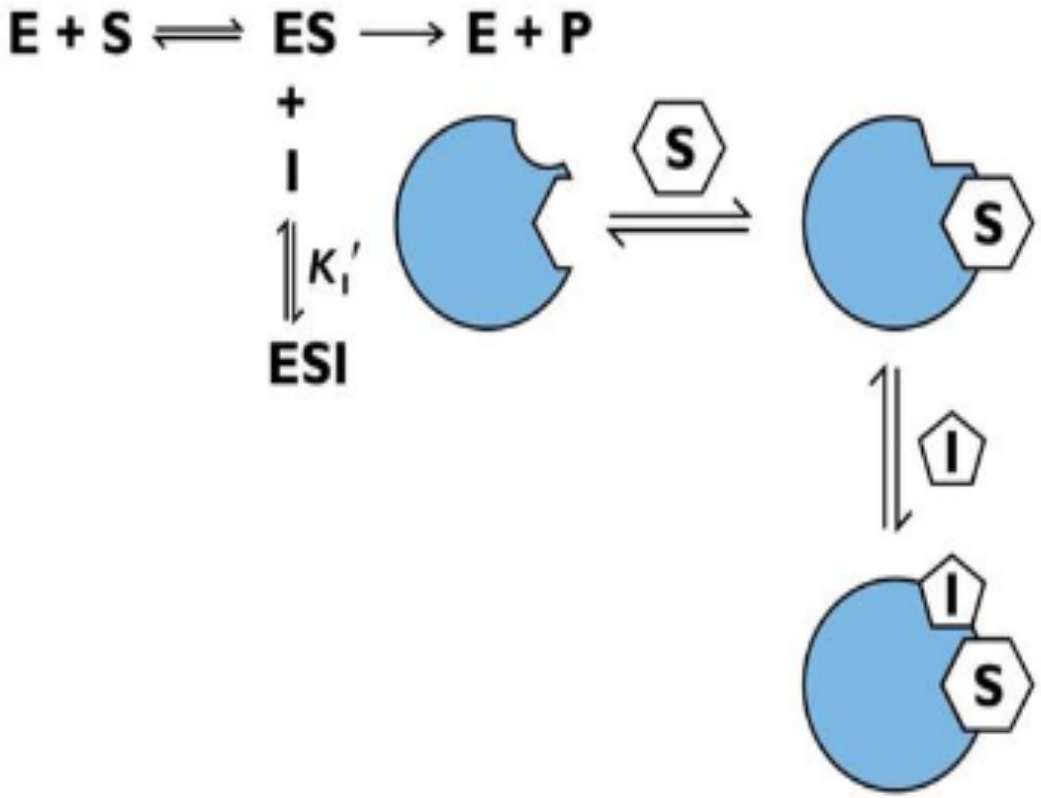
UNCOMPETITIVE INHIBITION

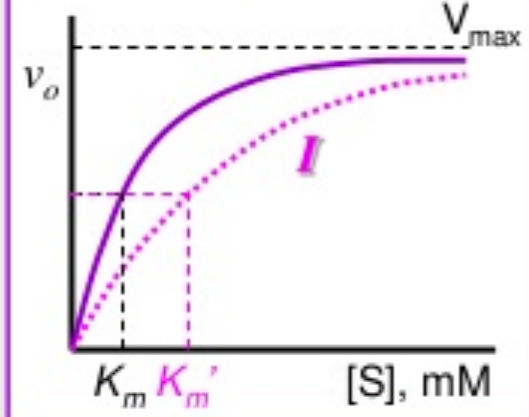
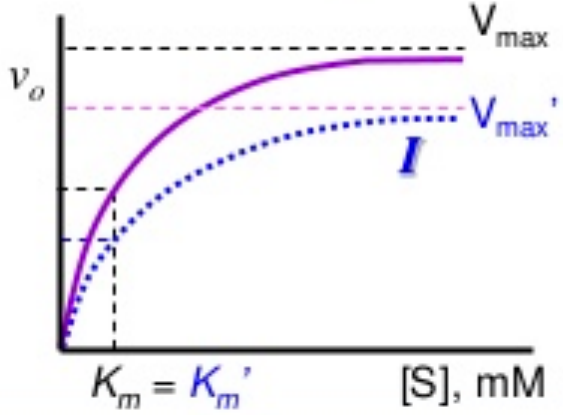
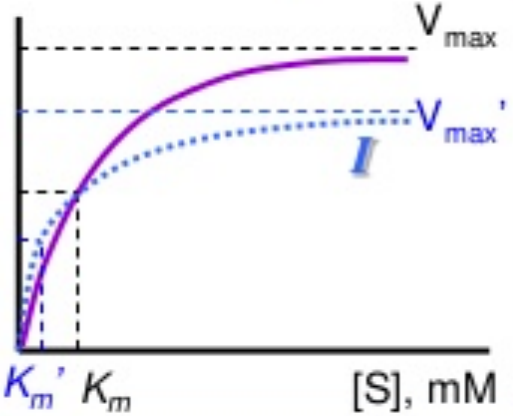
- In this type of inhibition, inhibitor does not compete with the substrate for the active site of enzyme instead it binds to another site known as *allosteric* site.



NON COMPETITIVE INHIBITION

- It is a special case of inhibition.
- In this inhibitor has the same affinity for either enzyme E or the E.S complex.



	Competitive	Non-Competitive	Un-Competitive
Direct Plots			
	V_{max} unchanged K_m increased	V_{max} decreased K_m unchanged	Both V_{max} & K_m decreased