BIOCHEMISTRY 2

2ND CLASS

UNIVERSITY OF ANBAR

COLLOGE OF SCIENCE

BIOLOGY DEPARTMENT

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Enzymes

Lecture Two (2)

Hameed Hussein Ali

Chemistry Department

College of Science

References:

Harper's Illustrated Biochemistry

Lippincott Biochemistry

Lehninger Principles of Biochemistry

Stryer Biochemistry

SYLABUSE

1- Enzymes
2- Vitamins and Coenzymes.
3- Nucleotides and Nucleic acids.
4- Carbohydrate Metabolism.
5- Lipids Metabolism.
6- Amino acids and Proteins Metabolism.
7- Nucleic acids metabolism.

Enzymes

Major Concepts

A. To know what are Enzymes and their biomedical importance.

B. To learn what are Enzyme Specificity

C. To learn the classification and properties of Enzymes.

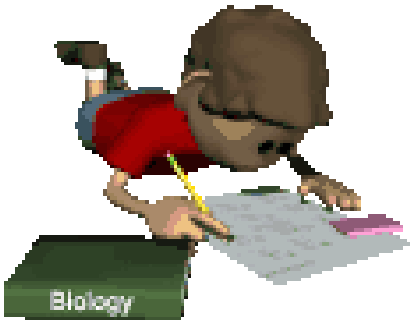
D. Learn the uses of Enzymes

E. study of reaction rate and Kinetics of Enzymes.

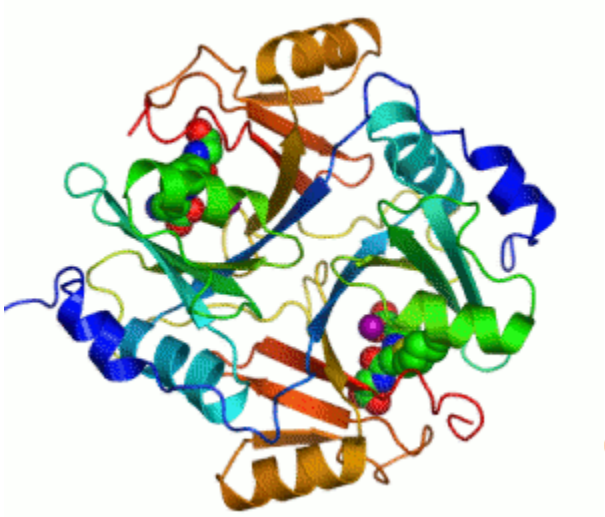
F-Define Catalyst and know the properties of a catalyst

G- Know the 6 classes of enzymes and there functions. Be able to classify a specific enzyme if given the reaction.

H- Define Coenzyme, apoenzyme, holoenzyme,



Enzymes: Introduction



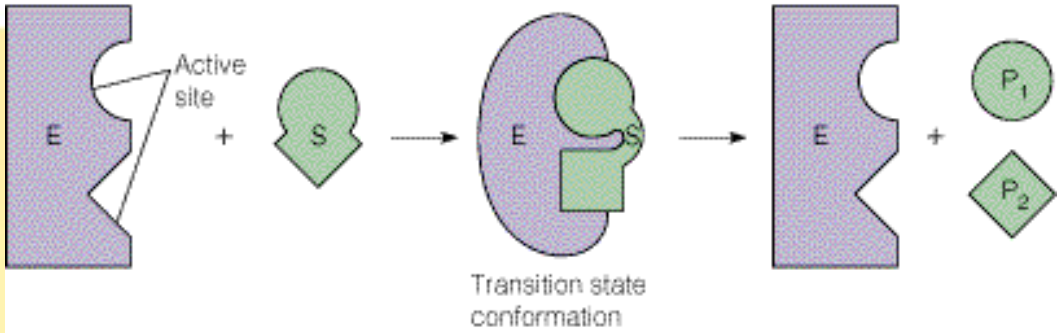
Catalyst

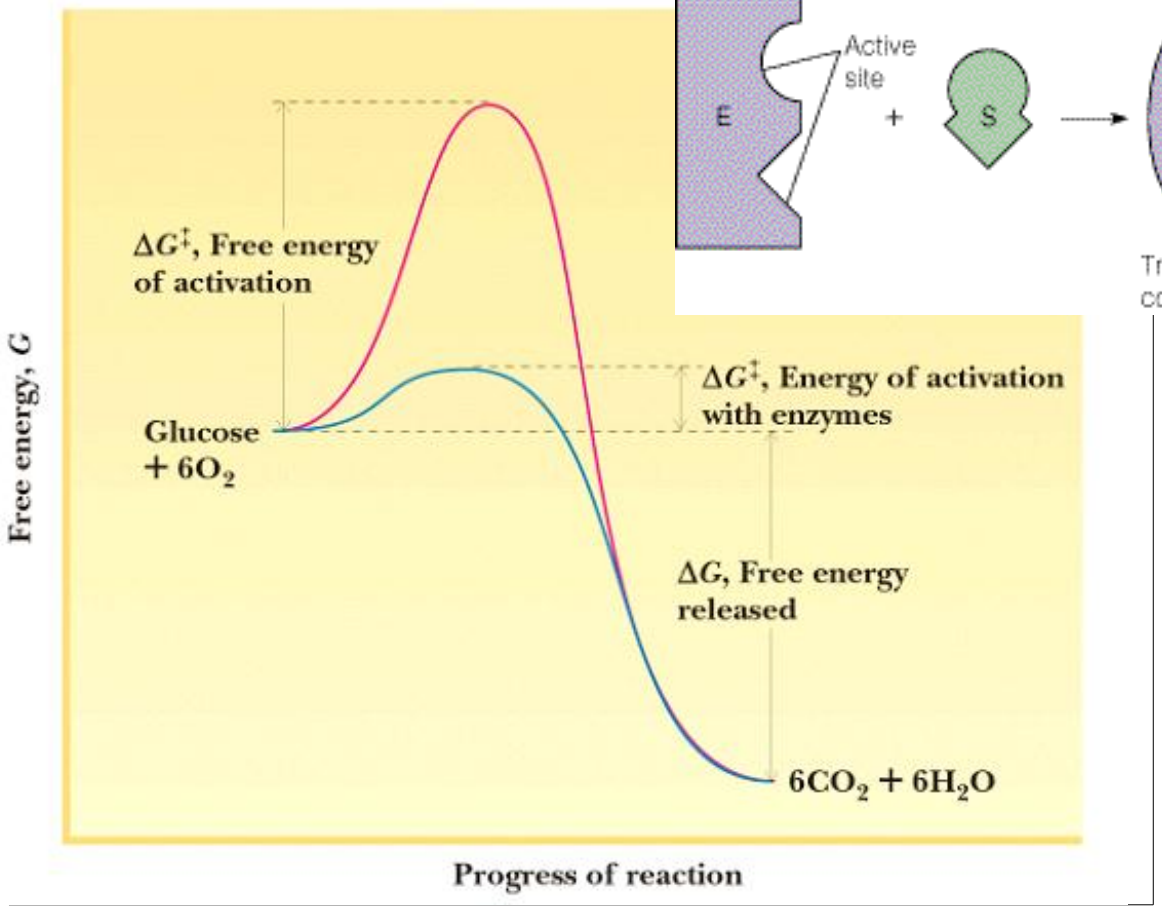
- substance that increase rates of a chemical reaction
- does not effect equilibrium
- remain unchanged in overall process
- reactants bind to catalyst, products are released

Catalysts increase product formation by

(1) lowering the energy barrier (activation energy) for the product to form

(2) increases the favorable orientation of reactant colliding molecules for product formation to be successful (stabilize transition state intermediate)





Catalytic Power

- Enzymes can accelerate reactions as much as 10^{16} over uncatalyzed rates!
- Urease is a good example:
  - Catalyzed rate: 3×10^{4}/sec
  - Uncatalyzed rate: 3×10^{-10}/sec
  - Ratio is 1×10^{14} !

*Chemical reactions in biological systems rarely occur in the absence of catalyst.

*Theses catalysts are specific proteins called enzymes.

*The striking characteristics of all enzymes are their catalytic power and specificity. Further more, the activity of many enzymes is regulated.

*Enzymes are biological catalysts.

Almost all enzymes are proteins .

*The basic function of an enzyme is to increase the speed of biochemical reactions .

*Almost all processes in a biological cell need enzymes to occur at significant rates .

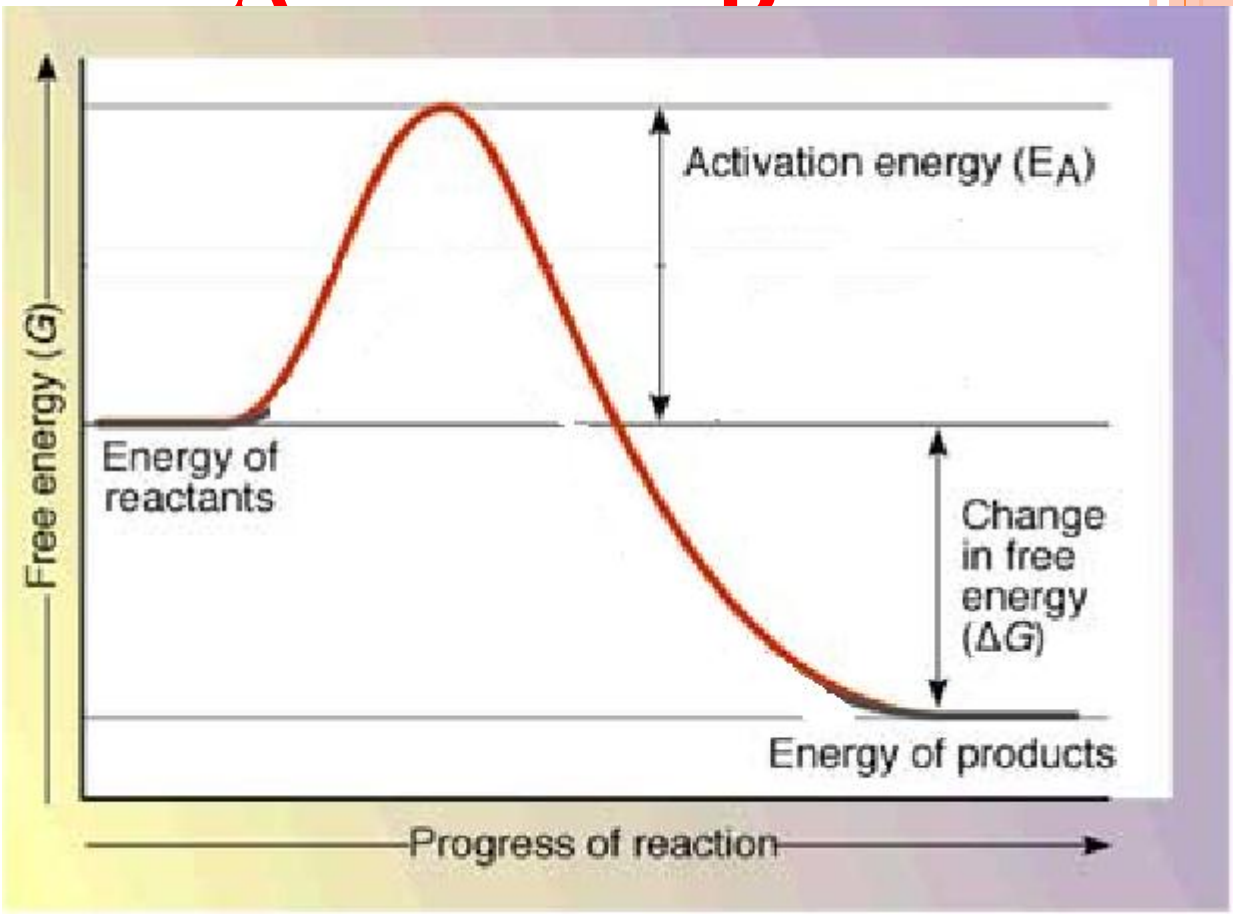
*Enzymes are present in all living cells, where they perform a vital function by controlling the metabolic processes.

The chemical reactions occur according to the thermo dynamical rule:

The energy of reactants (initial compounds - A) - must be higher then the energy of products (final compounds - B):

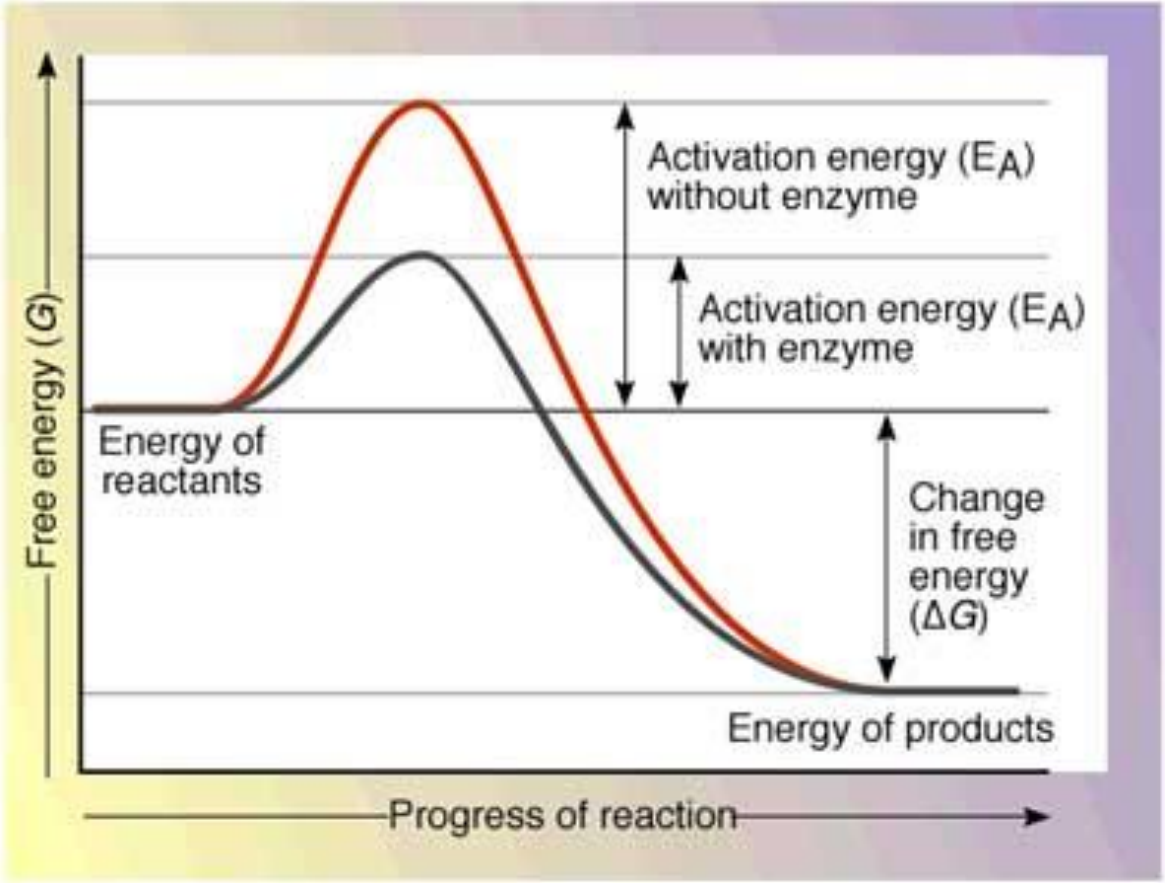
A ⟶ B

However, all chemical reactions require activation energy to begin. The activation energy acts as a barrier to the speed of the reaction.



Enzymes (as all catalysts) decrease the barrier by decreasing the required activation energy.

Thus, in the presence of enzymes, reactions proceed faster.



The similarities between enzymes and inorganic catalysts:

*Enzymes, as all catalysts, remain unchanged at the end of the reaction. They are "reusable".

*Enzymes, as all catalysts, are able to catalyze only the reactions that are thermo dynamically possible

*Enzymes, as all catalysts, don't change the direction of reaction

*Enzymes, as all catalysts, don't modify the equilibrium of a reversible reaction

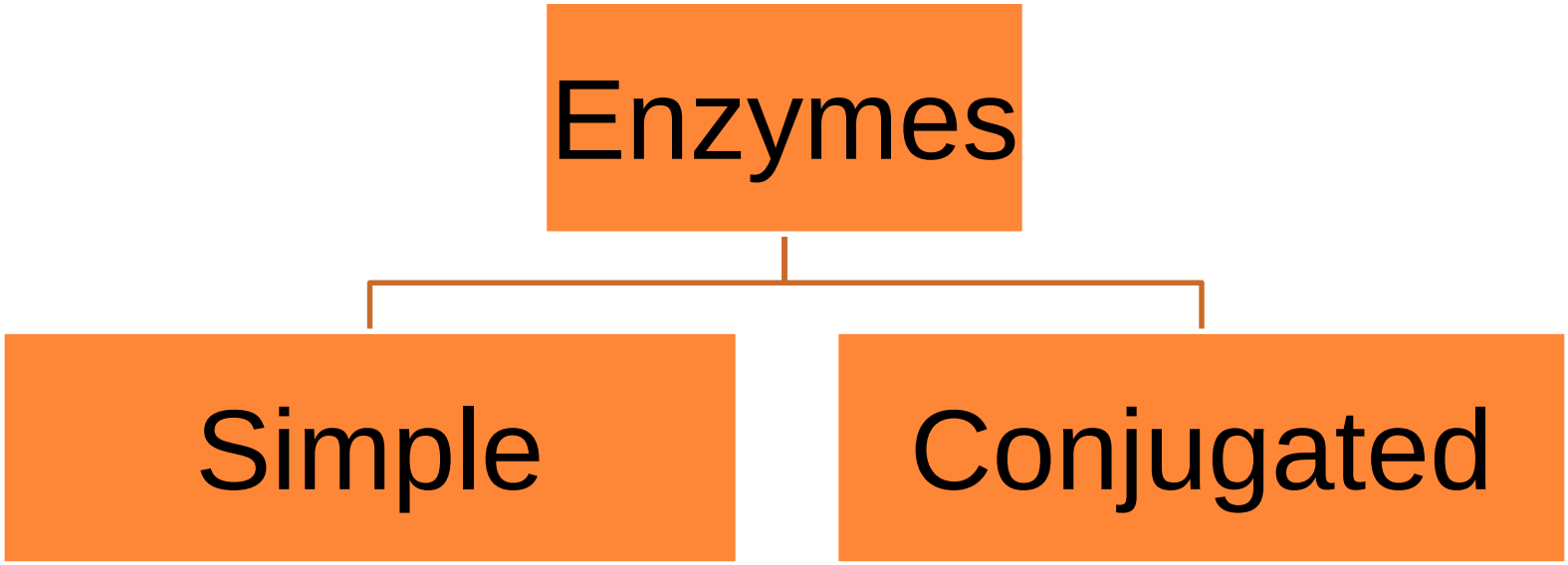
The differences between the enzyme and inorganic catalysts

*Enzymes are more efficient catalysts.
*Enzymes are highly specific – each enzyme is able to catalyze only a specific transformation of some specific compounds
*Enzymes work in mild conditions of temperature, pH and pressure.
*The activity of enzymes can be regulated
*The speed of the enzymatic reaction is directly proportional to the enzyme's quantity.
*Only a small amount of enzyme is needed to change the speed of reactions

Enzymes are proteins

- - their catalytic activity depends on the integrity of their native protein conformation. If an enzyme is broken down into amino acids or is denatured or dissociated into subunits, catalytic activity is usually lost.
- - thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity

The structure of enzymes



Consist of only amino acid chain (protein part). Require no chemical groups for activity other than their amino acid residues

Consist of a protein part and a nonprotein structure required for the activity of the enzyme

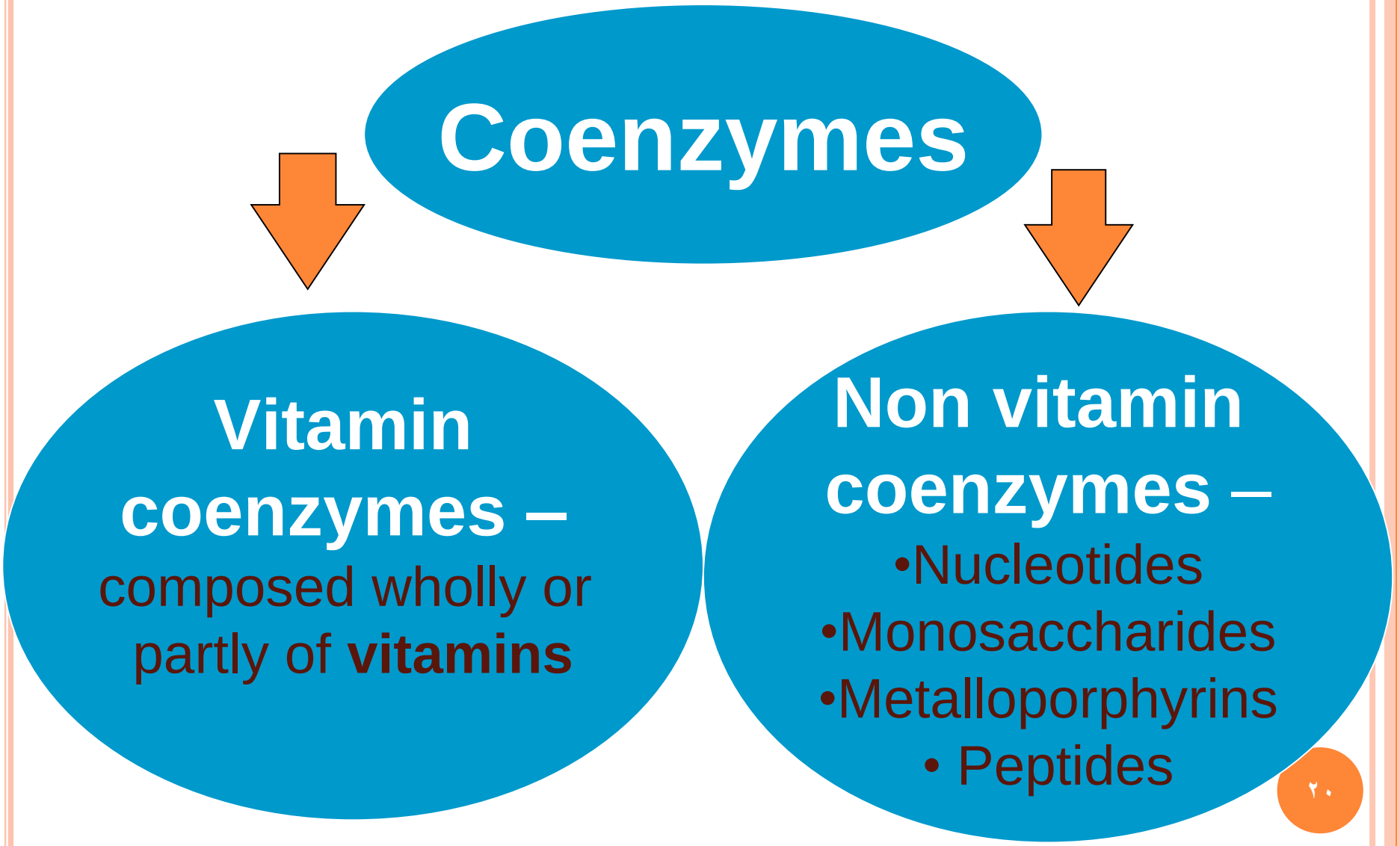
Conjugated enzymes

- The protein part of such an enzyme is called the apoenzyme or apoprotein.
- The non-proteic part can be: cofactor either one or more inorganic ions, such as Fe_2, Mg_2, Mn_2, or Zn_2
- a complex organic or metalloorganic molecule called a coenzyme.
- Some enzymes require *both* a coenzyme and one or more metal ions for activity.
- A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a prosthetic group.
- A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a holoenzyme.

Co-enzymes

- Non-protein molecules that help enzymes function
- Associate with active site of enzyme
- Enzyme + Co-enzyme = holoenzyme
- Enzyme alone = apoenzyme
- Organic co-enzymes – thiamin, riboflavin, niacin, biotin
- Inorganic co-enzymes – Mg^{++}, Fe^{++}, Zn^{++}, Mn^{++}

The chemical structure of coenzymes



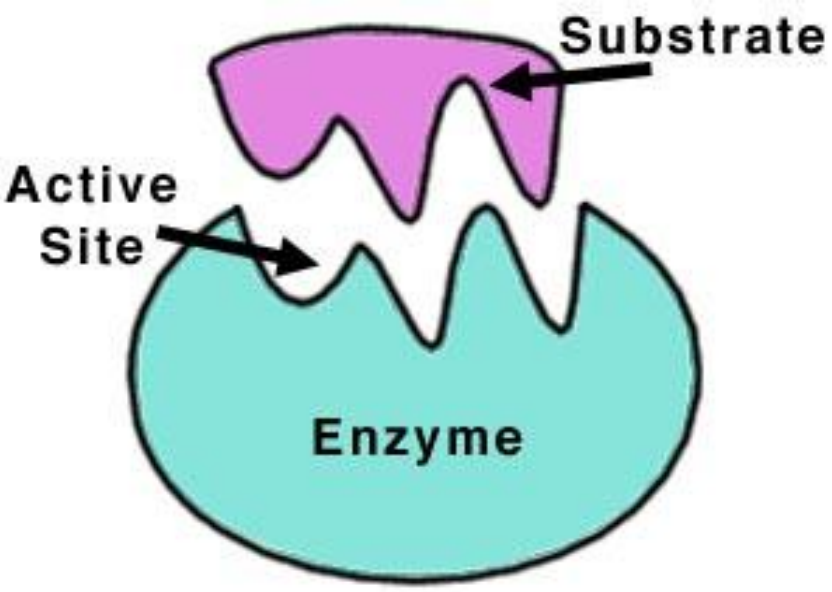
- The vitamins require conversion in the body - so they are not immediately available for use as coenzymes
- As usual the vitamin is converted by adding a phosphate group or other chemical groups in order to become an active coenzyme form.

Vitamins	Active coenzyme forms
Vitamin B1 (thiamin)	■ thiamin pyrophosphate
Vitamin B2 (riboflavin)	■ flavinmononucleotide (FMN) ■ flavinadenindinucleotide phosphate FAD
Vitamin PP (nicotinamide)	■ nicotinamide adeninedinudleotide NAD^+ ■ nicotinamide adeninedinudleotide phosphate ($NADP^+$)
Vitamin B6 (pyridoxin)	■ pyridoxal phosphate ■ pyridoxamine phosphate

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

Active Site and Substrate of enzymes



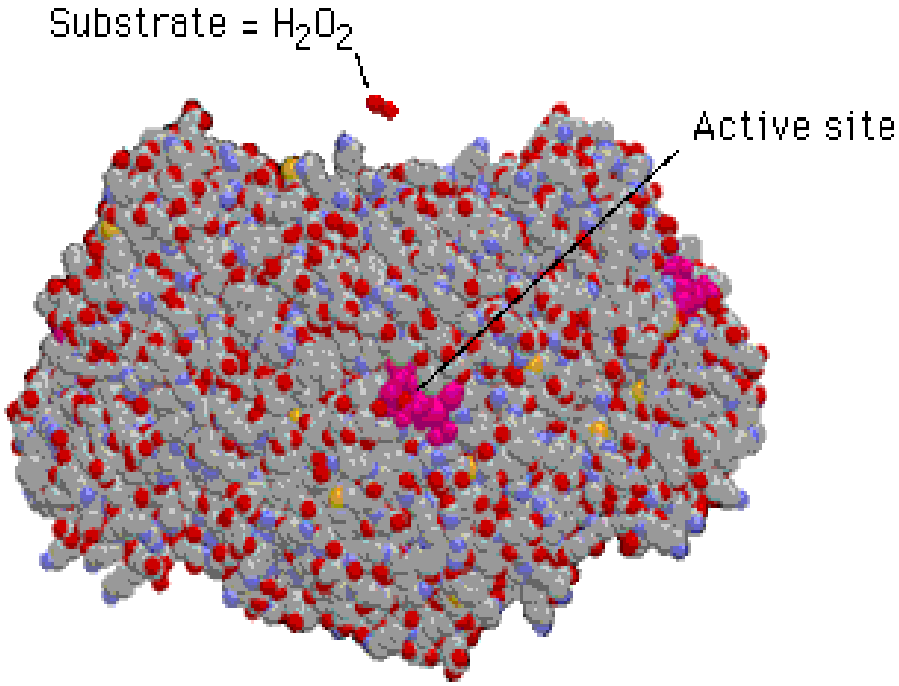
- Each enzyme interacts chemically with only one particular substance or type of substance, named a substrate.
- The substrate is bound to the enzyme in a special place named active site.
- The active site is a unique three-dimensional structure that is formed at the interaction of different regions of polypeptide chain in the tertiary level of protein structure.

*The active site is the location where the catalysis of chemical reaction takes place.

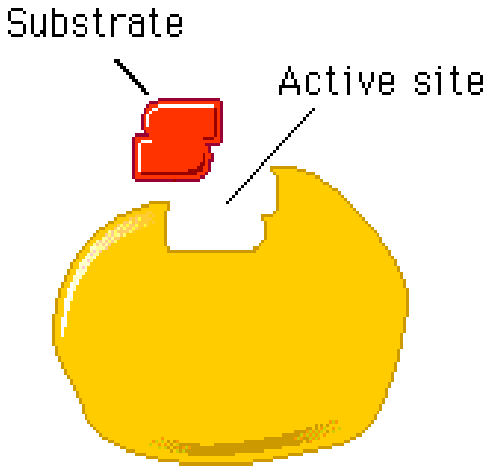
* In the active site of the conjugated enzymes the cofactor or coenzyme is situated.

*Enzyme and its active site have a specific three-dimensional complementary shape that enables the enzyme to fit with the substrate.

Most enzymes are much larger than the substrates they act on, and only a small portion of the enzyme (around 3–4 amino acids) is directly involved in catalysis.
The active site is the region that contains these catalytic residues, binds the substrate, and then carries out the reaction.



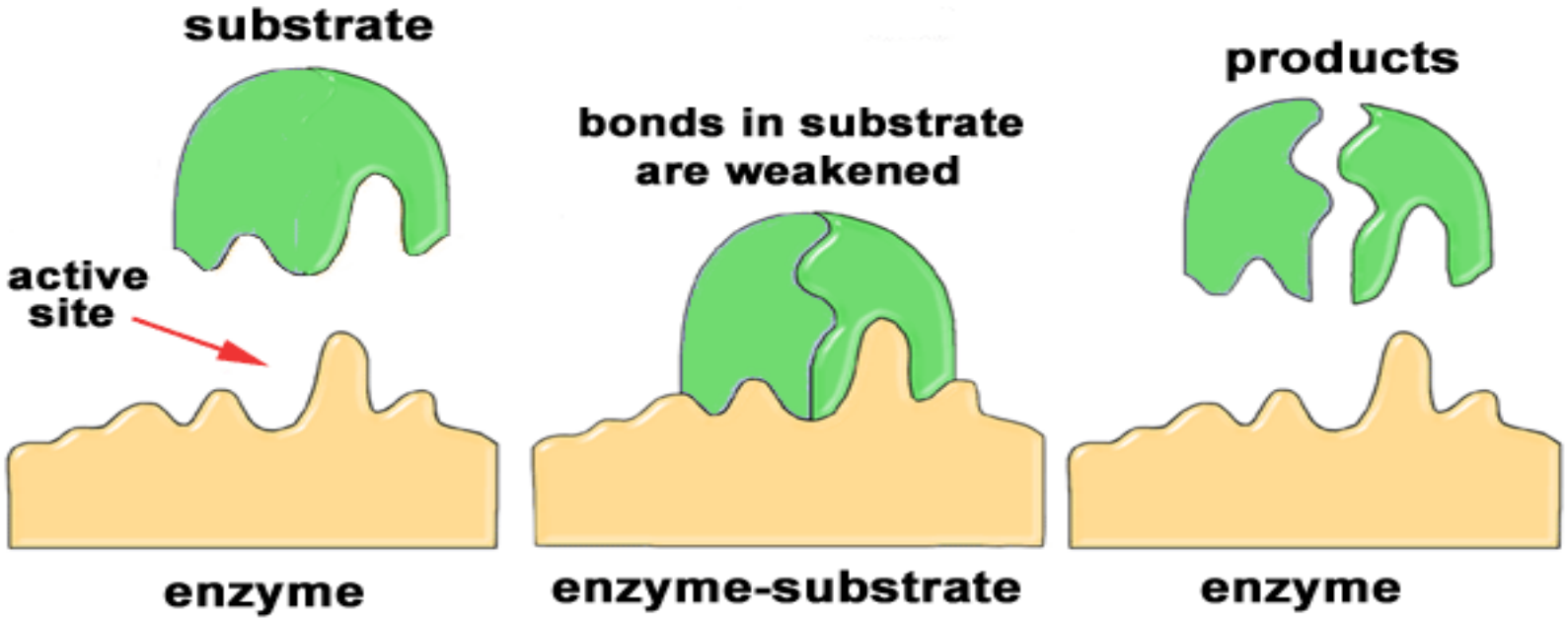
Molecular model of catalase



Schematic model of an enzyme

The mechanism of the enzyme's action

The substrate molecule attaches to the active site on the enzyme forming an enzyme-substrate complex. The enzyme causes a weakening of certain chemical bonds in the substrate molecule, resulting in a breakdown of the substrate into two smaller product molecules. The enzyme is unaltered during the reaction and is free to catalyze the breakdown of another substrate molecule.



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

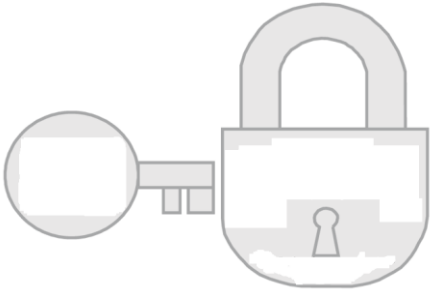
The enzyme action can be understand by different theories as

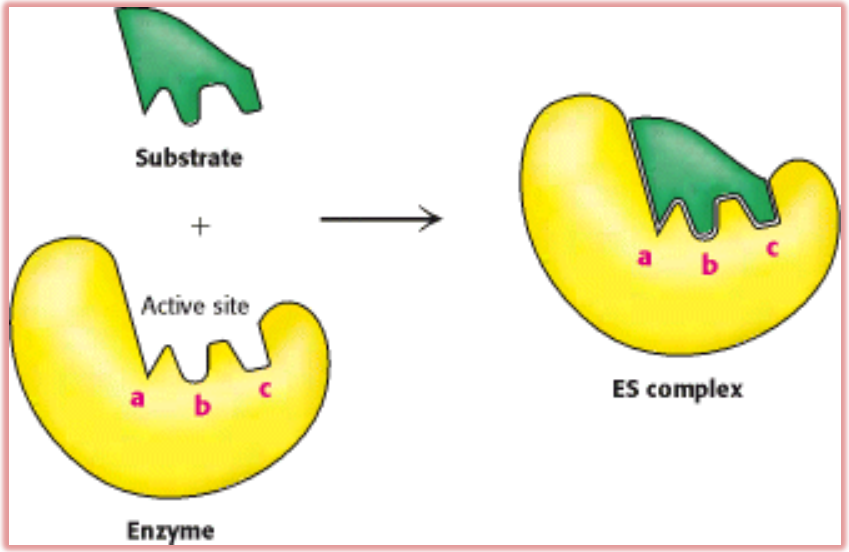
1. Fischer's lock and key model:

This model was proposed by Emil Fischer in 1898. It is also called the **template model**.

Enzyme has a particular shape into which the substrate fit exactly in a manner in which **a key fits in a lock**.

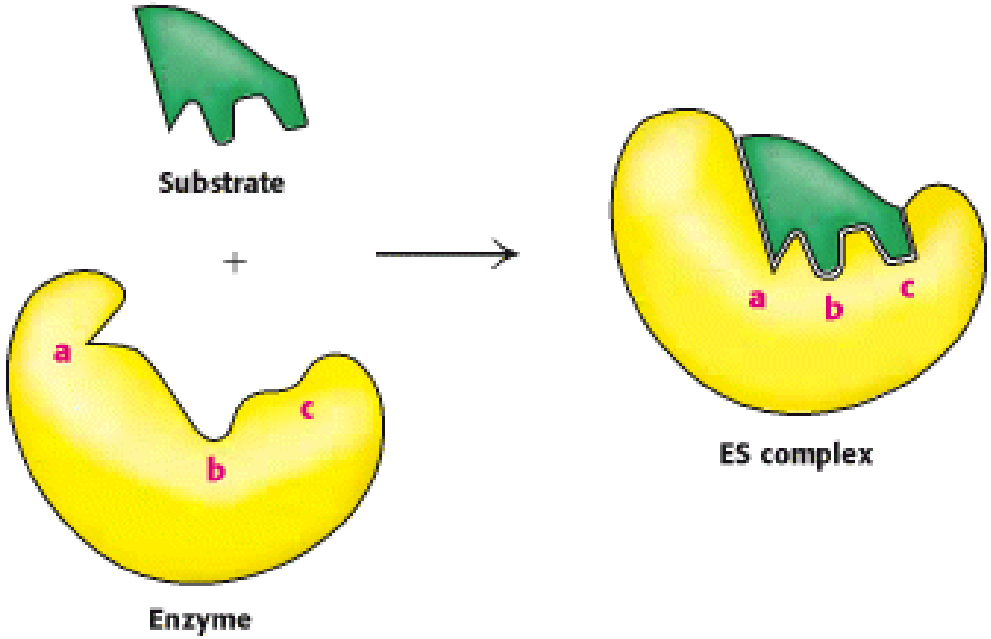
Key = substrate
Lock = enzyme



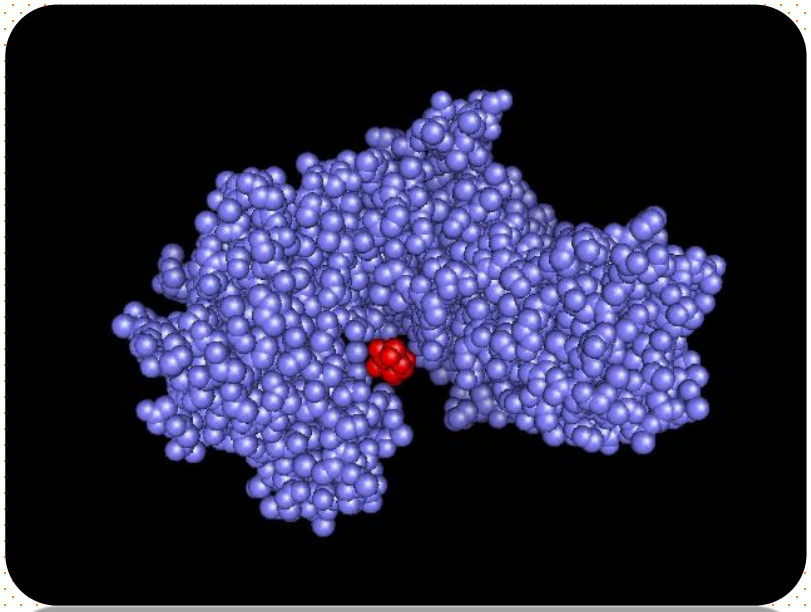


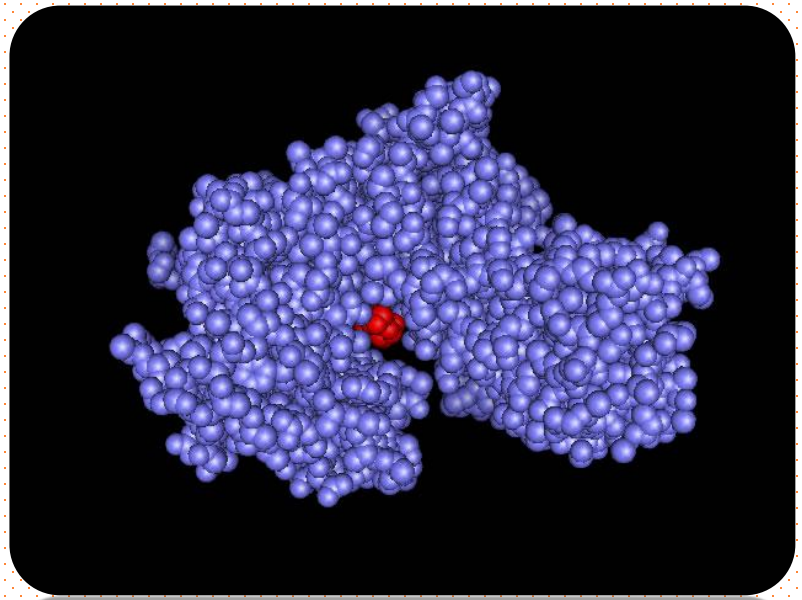
2. Koshland's Induced fit model:

- This model was proposed by Koshland in 1958. He demonstrated that the enzyme molecule does not retain its original shape and structure. The contact of the substrate induces some conformational changes in the active site of the enzyme molecule.
- Step by step the active center of the enzyme becomes completely fit to the configuration of the substrate.
- The change in the conformation of the enzyme in response to substrate binding renders the enzyme catalytically active.



Induced Fit and Hexokinase





Specificity Of Enzymes

- Enzymes selectively recognize proper substrates over other molecules
- Enzymes produce products in very high yields - often much greater than 95%
- Specificity is controlled by structure - the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield

The action of enzymes is usually very specific.

- Specificity is a property of enzymes to catalyze:

- the transformation of a particular type of substrate- substrate specificity

- a particular type of reaction - reaction specificity

Substrate specificity

There are different degree of substrate specificity:

- **1. Stereochemical specificity -** the enzyme will catalyze the transformation only of a particular steric or optical isomer of a substrate. This is the highest degree of specificity.

Example: fumarase catalyzes the hydration only of fumaric acid (trans-isomer) and doesn't act on the maleinic acid (cis-isomer);

- **2. Absolute Substrate specificity** - the enzyme will catalyze the transformation of only one substrate.

  Example: urease catalyzes the break-down only of the molecule of urea;

  These two types of specificity can be explained by **Fischer's lock and key model** of substrate-enzyme interaction

- **3. Absolute Group specificity** - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate or methyl groups.

  Example: alcoholdehydrogenase catalyzes the transformation of different kind of alcohols: methanol, ethanol, propanol, etc.

- **4. Relative Group specificity** - the enzyme will act on a particular type of chemical bond in the substrate.

  Example: proteolytic enzymes (pepsin, trypsin) that break down the peptide bonds in proteins.

- **5. Relative Substrate specificity** – the enzyme is able to transform a large number of different compounds. This is the lowest degree of specificity.

  Example: cytochrome P450 in liver microsoms participates in hydroxylation of different hydrophobic compounds.

These types of specificity can be explained by **Koshland's Induced fit model** of substrate-enzyme interaction.

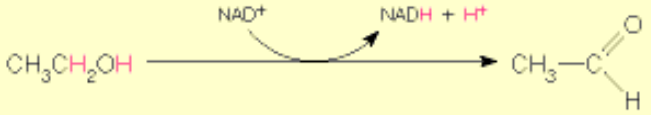
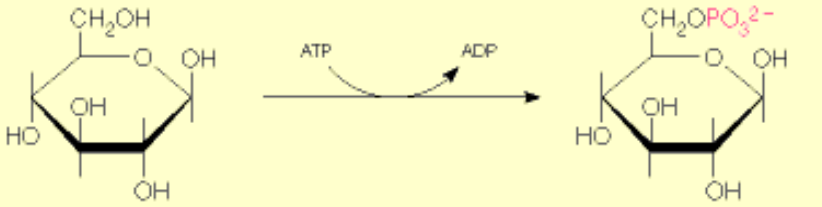
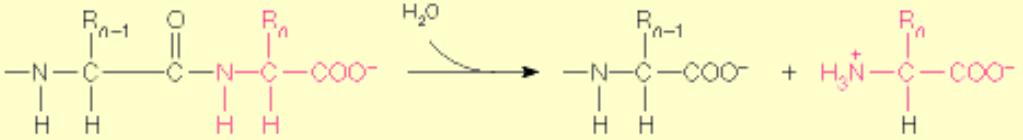
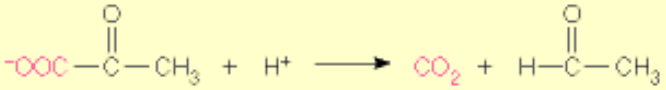
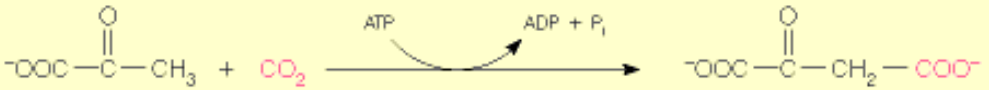
Classification of Enzymes

Based on catalyzed reactions, the nomenclature committee of the International Union of Biochemistry and Molecular Biology (IUBMB) recommended the following classification - enzymes are classified in 6 classes by the reactions they catalyze (reaction specificity):

Classes of enzymes

1. Oxidoreductases = catalyze oxidation-reduction reactions (NADH)
2. Transferases = catalyze transfer of functional groups from one molecule to another.
3. Hydrolases = catalyze hydrolytic cleavage
4. Lyases = catalyze removal of a group from or addition of a group to a double bond, or other cleavages involving electron rearrangement.
5. Isomerases = catalyze intramolecular rearrangement.
6. Ligases = catalyze reactions in which two molecules are joined.

Enzymes named for the substrates and type of reaction

Class	Example (reaction type)	Reaction Catalyzed
1. Oxidoreductases	Alcohol dehydrogenase (EC 1.1.1.1) (oxidation with NAD^+)	 **Ethanol** **Acetaldehyde**
2. Transferases	Hexokinase (EC 2.7.1.2) (phosphorylation)	 **D-Glucose** **D-Glucose-6-phosphate**
3. Hydrolases	Carboxypeptidase A (EC 3.4.17.1) (peptide bond cleavage)	 **C-terminus of polypeptide** **Shortened polypeptide** **C-terminal residue**
4. Lyases	Pyruvate decarboxylase (EC 4.1.1.1) (decarboxylation)	 **Pyruvate** **Acetaldehyde**
5. Isomerases	Maleate isomerase (EC 5.2.1.1) (*cis–trans* isomerization)	 **Maleate** **Fumarate**
6. Ligases	Pyruvate carboxylase (EC 6.4.1.1) (carboxylation)	 **Pyruvate** **Oxaloacetate**

The IUBMB committee also defines subclasses and sub-subclasses. Each enzyme is assigned an **EC** (Enzyme Commission) number. For example, the EC number of catalase is **EC1.11.1.6.** The first number indicates that the enzyme belongs to oxidoreductase (class 1). Subsequent numbers represent subclasses and sub-subclasses.

Factors affecting Enzyme activity

1- Effect of Temperature

2- Effect of pH

3- Effect of Substrate Concentration

4- Effect of Enzyme Concentration

5-Presence of activators andinhibitors

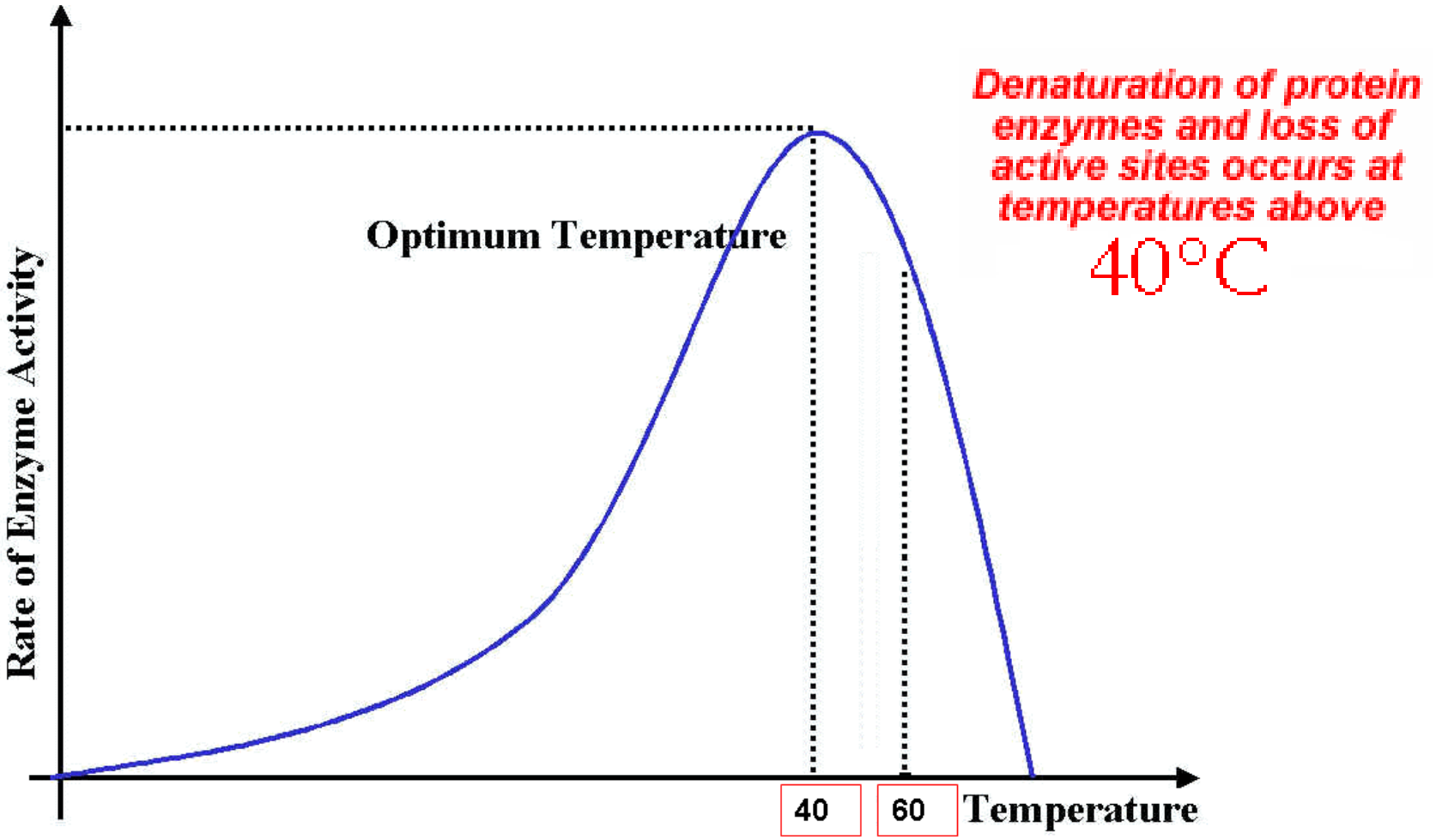
Enzyme activity and Temperature

At very low temperatures, enzymes are inactive.

As temperature **increases** - the rate of chemical reactions will increase. This rule is true for enzyme-substrate reactions in the temperature range of 10-40ºC.

However, as temperature rises above 40°C the rate of enzyme catalyzed reactions drops dramatically because of the complete **denaturation** of most enzymes at temperatures over 60°C.

Enzyme Activity and Temperature

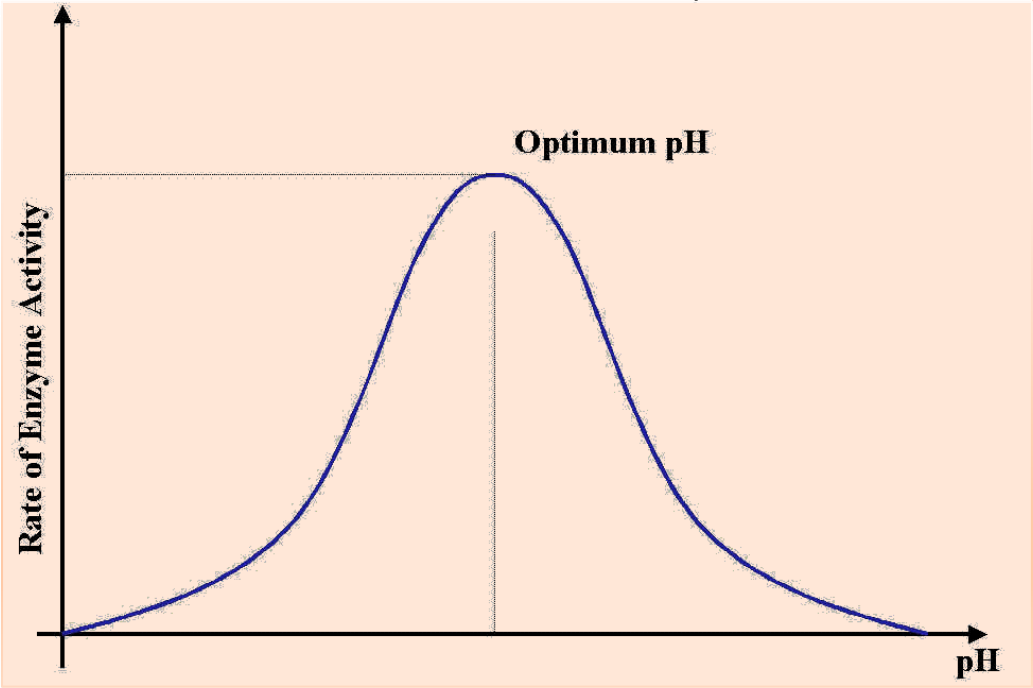


- Each enzyme has a special temperature that they are most active. This is the optimum temperature for that enzyme. Each enzyme has it's own optimum temperature.

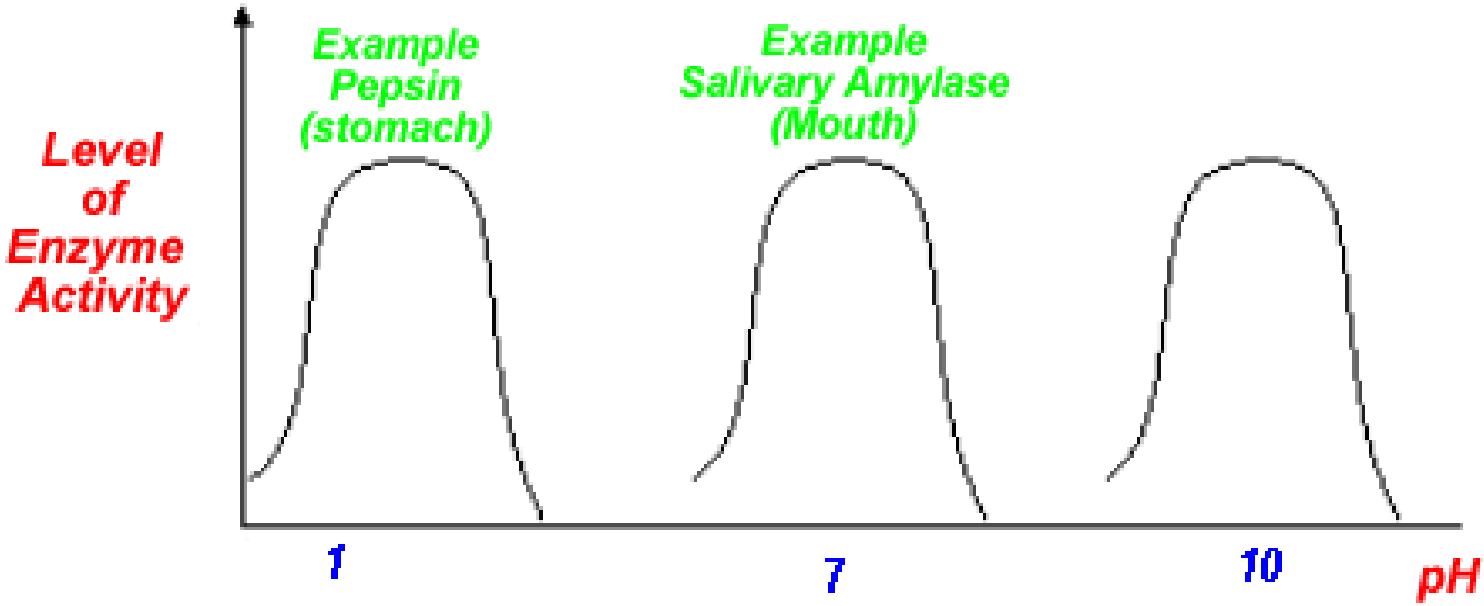
Enzyme activity and pH

Enzymes catalyze reactions in different conditions of pH. The rate of enzymatic reactions is highest at **an optimal pH**. Any change of pH below or above the optimum pH decreases the rate of enzyme action due to:

- changes in the ionization state of the enzyme, coenzyme and substrate
- extreme changes in pH lead to denaturation of the enzyme

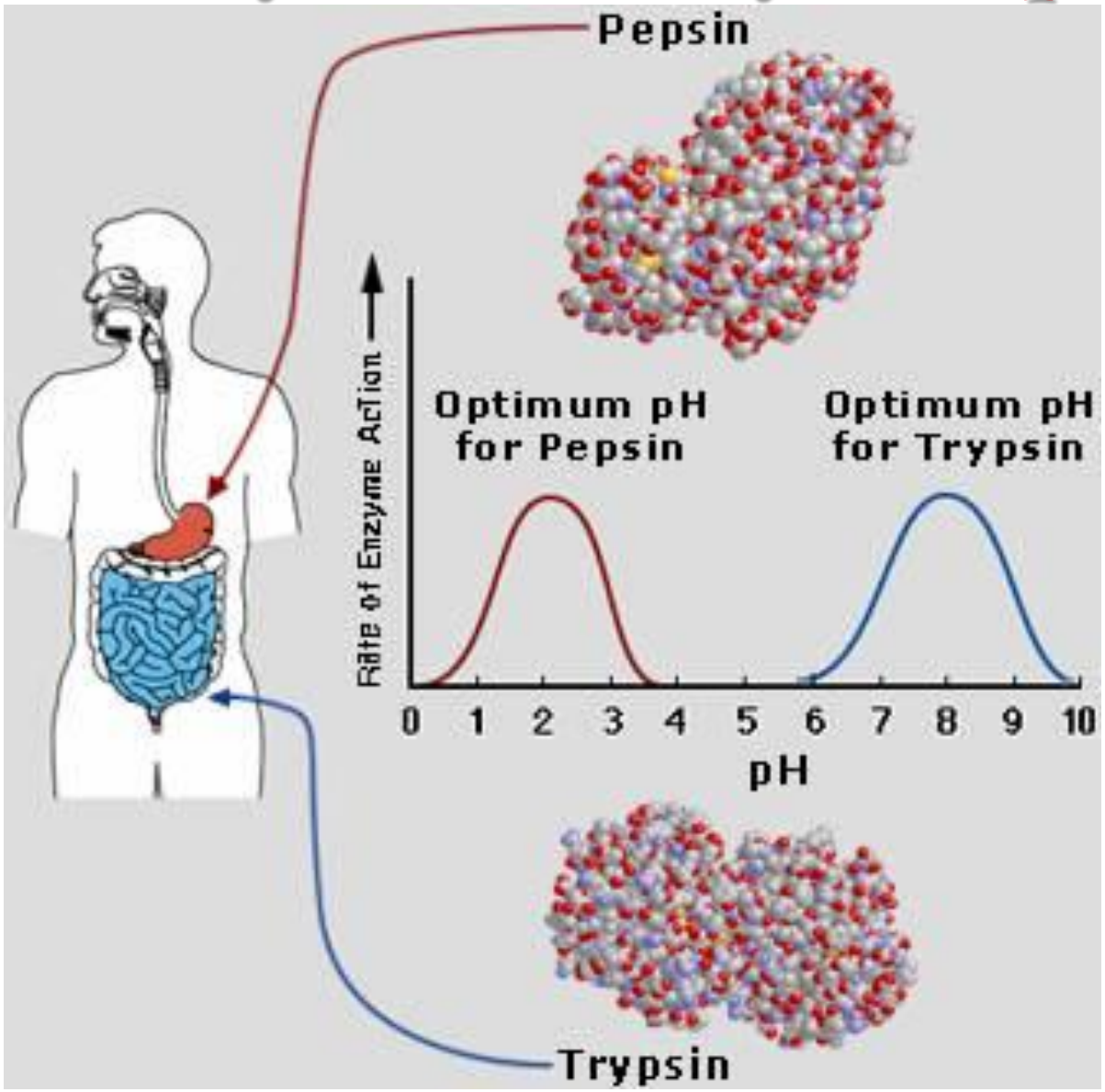


Enzyme Activity and pH

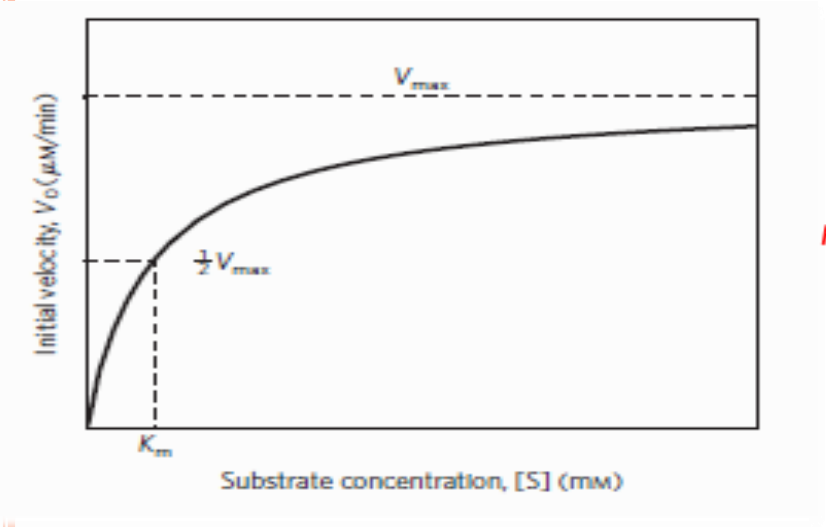


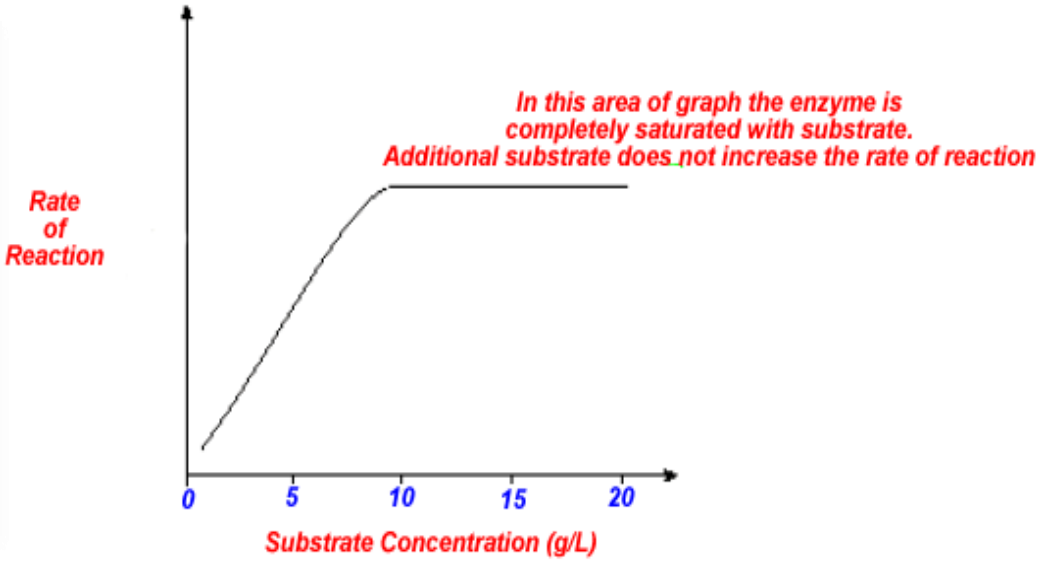
For example, salivary enzyme amylase acts at the neutral pH of the mouth, but enzyme pepsin requires the acidic pH of the stomach. In the low pH environment of the stomach, salivary amylase will be denatured.

Enzyme activity and pH



Enzyme activity and Substrate Concentration





1. Increasing substrate concentration increases reaction rate, as it increases the number of collisions between the enzyme and the substrate resulting in the formation of more enzyme-substrate complexes.
2. Eventually, the substrate concentration will be high enough to fill up or saturate all of the active sites on the enzyme. At this point, the maximum rate of reaction has been reached.
3. Increasing substrate concentration any more will not increase the rate of reaction.

Enzyme activity and Enzyme Concentration

The rate of an enzyme-catalyzed reaction depends directly on the concentration of the enzyme.

The rate of reaction increase also. By increasing the enzyme conc. the numbers of active sites increased and as a result the probability of E-S binding increases.

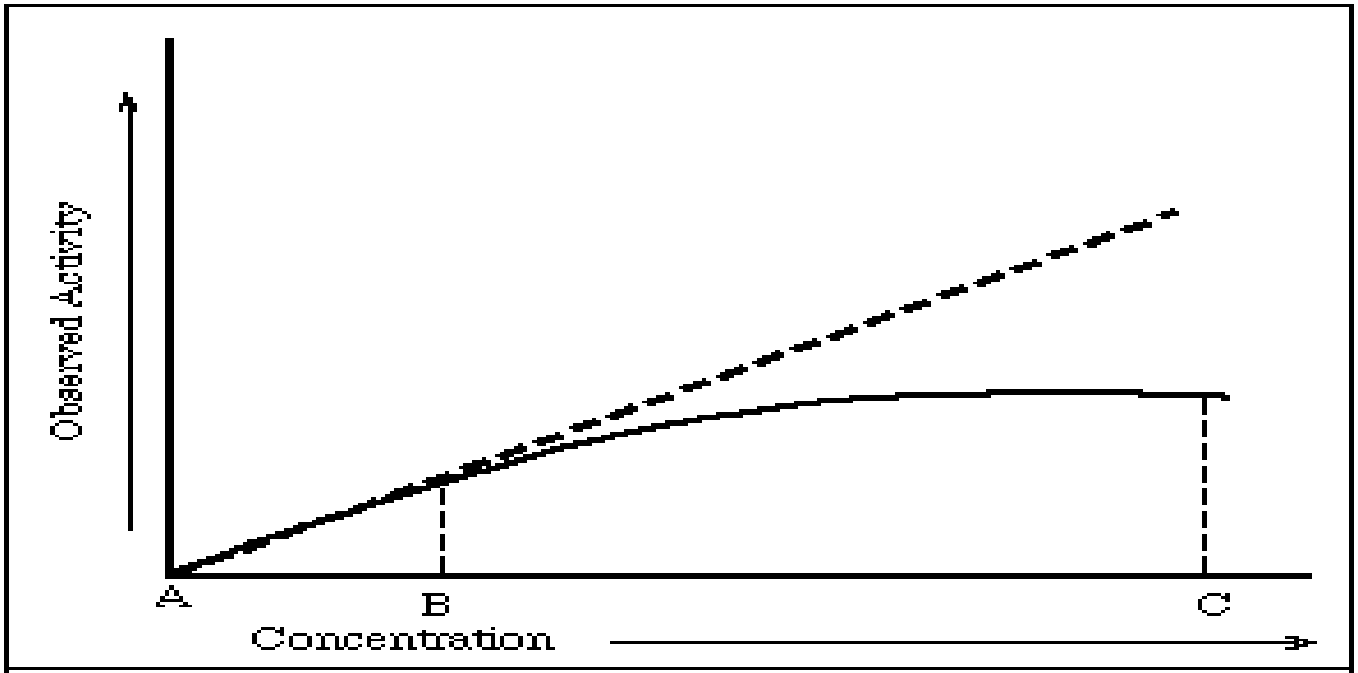


FIGURE 5
Activity vs. Concentration

Regulation of enzyme activity

- Enzyme activation
- Enzyme inhibition

Kinetics

- study of reaction rate
- determines number of steps involved
- determines mechanism of reaction
- identifies "rate-limiting" step

Enzyme activation

Conversion of an inactive form of an enzyme to one possessing metabolic activity.

It includes:

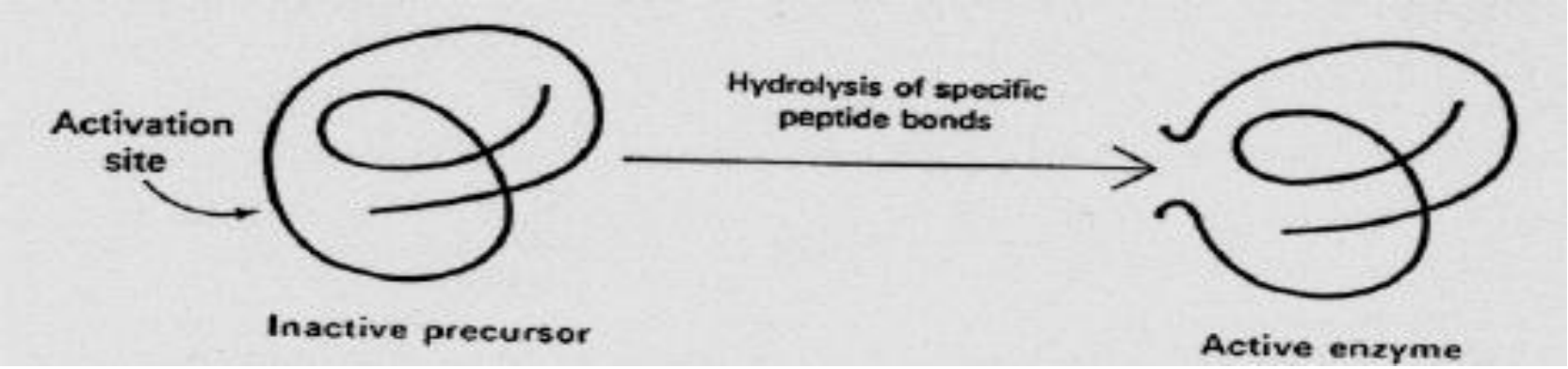
- 1. activation by ions (cations, anions);
- 2. activation by cofactors (coenzymes);
- 3. partial proteolysis;
- 4. allosteric activation;
- 5. activation by interconversion – phosphorylation-dephosphorylation;
- 6. quaternary assembling

Partial proteolysis

- Some **enzymes** are produced initially as inactive precursors (**proenzymes** or **zymogens**), that contain an additional portion of polypeptide chain that influence on the native structure of enzymes and make them inactive.
- Under the action of some activating factors a conversion of the **enzyme** precursor to an **active enzyme** by **hydrolysis** of specific peptide bond takes place.

Partial proteolysis

- Examples include digestive enzymes & blood clotting components



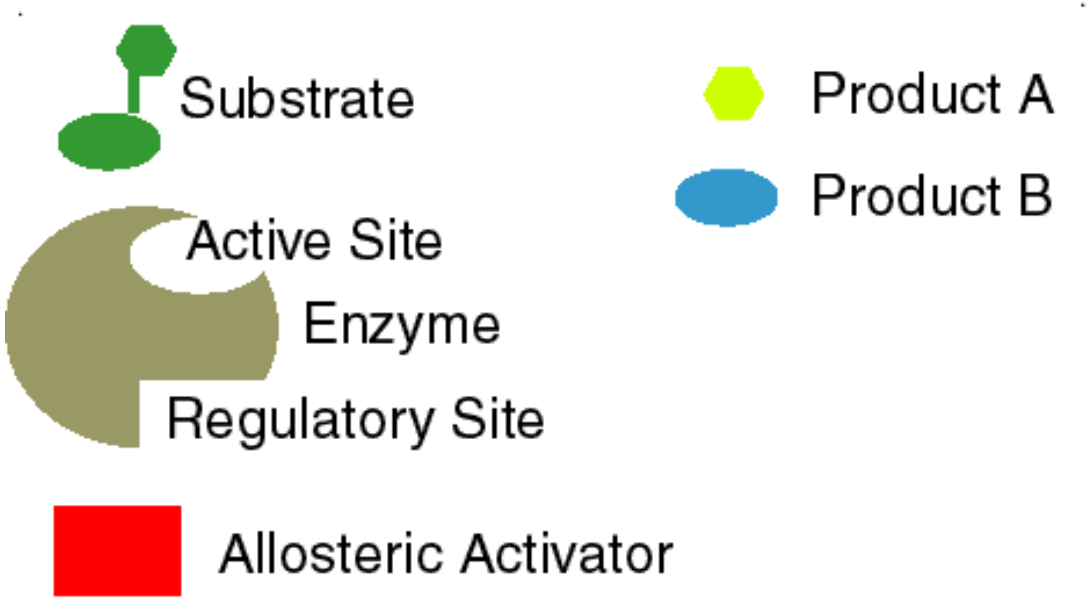
- Trypsinogen (produced in pancreas) subsequently cleaved and activated in the small intestine to form trypsin

Allosteric activation

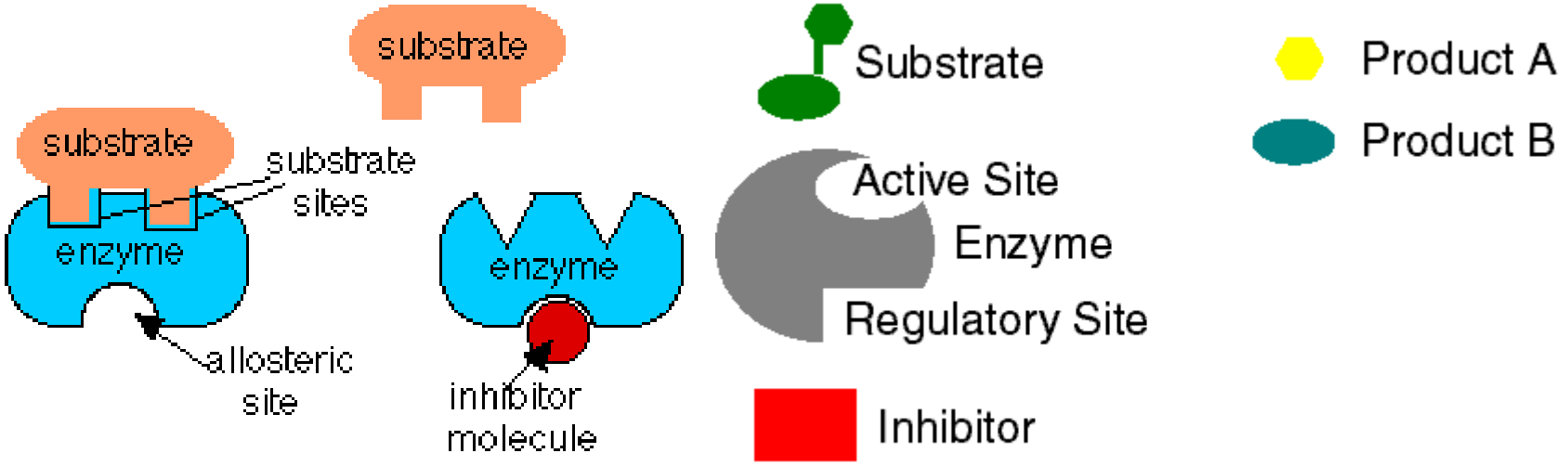
- There are some enzymes that besides the active site have another one - the regulatory (allosteric) site.
- In the allosteric site of the enzyme the specific allosteric modulators – activators and inhibitors are bound.
- While the modulators interact with the allosteric site – the conformation of enzyme changes and its activity also changes. This binding causes a change in the three dimension conformation of the protein, turning off (or turning on) the catalytic site.

Allosteric activation

- When the allosteric activator is not bound to the regulatory site, the active site of the enzyme is not able to bind substrate and catalyze the production of product. When the allosteric activator binds to the enzyme at the regulatory site, the shape of the active site changes so that it can bind its substrate and catalyze the production of products A and B. The enzyme will remain activated until the allosteric activator leaves the regulatory site.

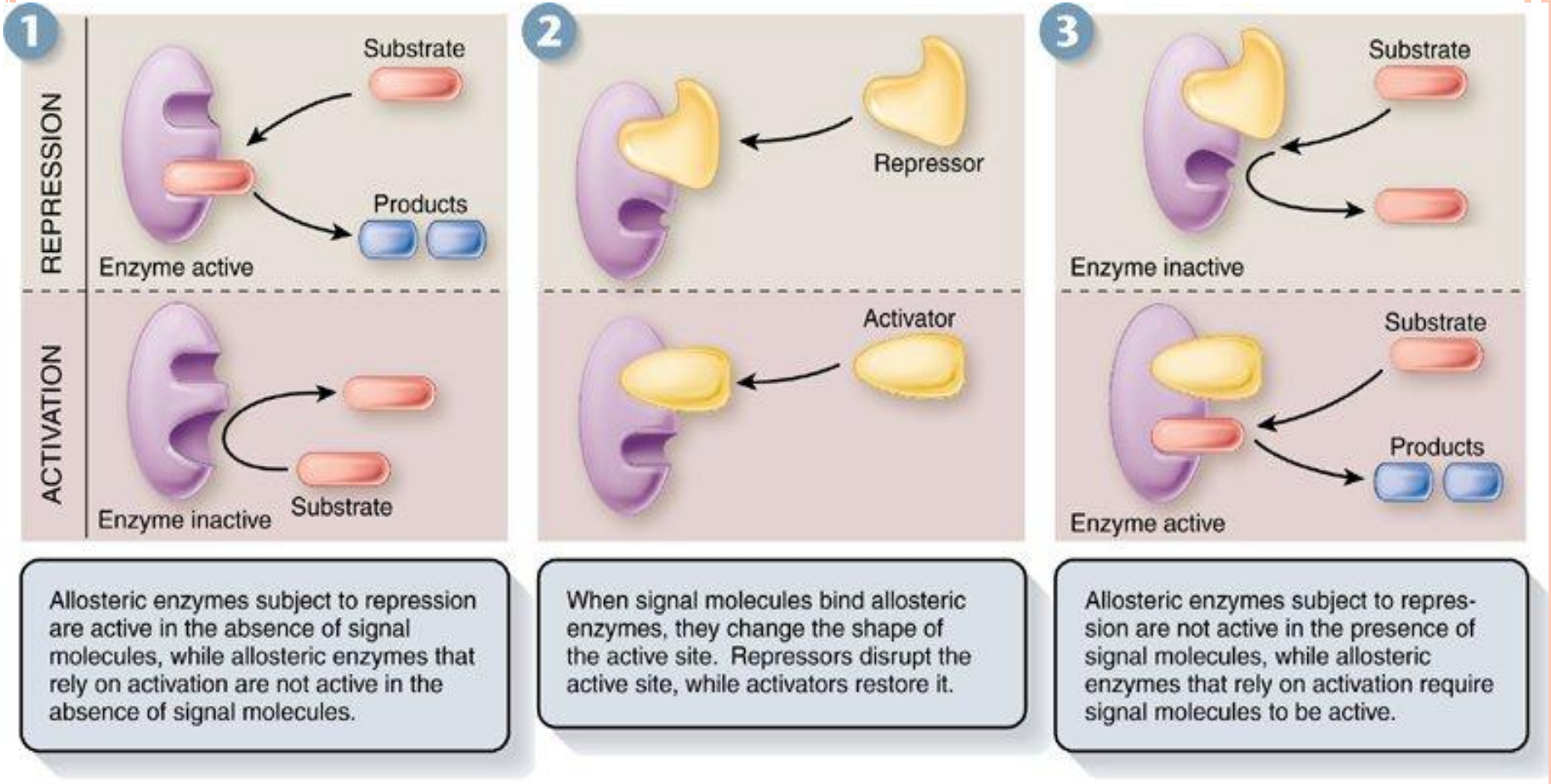


Allosteric inhibition



Allosteric inhibition results from a change in the shape of the active site when an inhibitor binds to an allosteric site. When this occurs the substrate cannont bind to it's active site due to the fact that the active site has changed shape and the substrate no longer fits.

Allosteric regulation



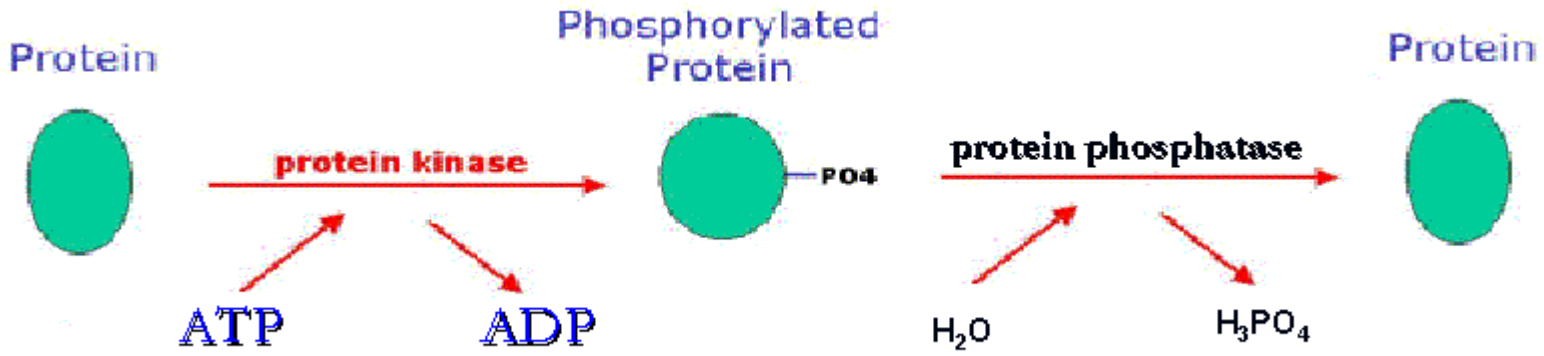
Allosteric enzymes subject to repression are active in the absence of signal molecules, while allosteric enzymes that rely on activation are not active in the absence of signal molecules.

When signal molecules bind allosteric enzymes, they change the shape of the active site. Repressors disrupt the active site, while activators restore it.

Allosteric enzymes subject to repression are not active in the presence of signal molecules, while allosteric enzymes that rely on activation require signal molecules to be active.

Activation by phosphorylation-dephosphorylation

- There are enzymes that are active in a phosphorylated state, and there are other enzymes that are active in a dephosphorylated state
- An enzyme - protein kinase transfers a phosphate group from ATP to the protein and phosphorylates it. A different enzyme - protein phosphatase, removes the phosphate group, thereby dephosphorylates the protein.



SUMMARY

- Life depends on powerful and specific catalysts: enzymes. Almost every biochemical reaction is catalyzed by an enzyme.
- With the exception of a few catalytic RNAs, all known enzymes are proteins. Many require nonprotein coenzymes or cofactors for their catalytic function.
- Enzymes are classified according to the type of reaction they catalyze. All enzymes have formal E.C. numbers and names, and most have trivial names.

- Enzymes are highly effective catalysts, commonly enhancing reaction rates by a factor of 10^5 to 10^{17}.
- Enzyme-catalyzed reactions are characterized by the formation of a complex between substrate and enzyme (an ES complex). Substrate binding occurs in a pocket on the enzyme called the active site.
- The function of enzymes and other catalysts is to lower the activation energy, $\Delta G^{\ddagger}$, for a reaction and thereby enhance the reaction rate. The equilibrium of a reaction is unaffected by the enzyme.

Thank you very much for your attention!