بسم الله الرحمن الرحيم

King Saud University

College of Science

Department of Biochemistry

Protein Biochemistry (BCH 303)

Chapter 4

Introduction to Enzymes

Prepared by Dr. Farid Ataya

http://fac.ksu.edu.sa/fataya

- https://www.youtube.com/watch?v=f7jRpniCsaw
- 1- ادخل الموقع مهم المحاضرة
- https://www.youtube.com/watch?v=26ho8zCSobI
- 2 المحاضرة
- https://www.youtube.com/watch?v=KCG5fDKr9HQ
- 3 المحاضرة
- https://www.youtube.com/watch?v=jUUeR40_2-0
- 4 المحاضرة(kinetics)
- https://www.youtube.com/watch?v=qJgEmewoPbw
- 5 المحاضرة(kinetics)

An Introduction to Enzymes

- Much of the history of biochemistry is the history of enzyme research.
- Biological catalysis was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach, and research continued in the 1800s with examinations of the conversion of starch to sugar by saliva and various plant extracts.
- In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments." He postulated that these ferments were inseparable from the structure of living yeast cells; this view, called vitalism, prevailed for decades.

Then in 1897 Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells.

W. Kühne called these molecules **enzymes. As ~~vitalistic~~ notions of life were** disproved, the isolation of new enzymes and the investigation of their properties advanced the science of biochemistry.

The isolation and crystallization of urease by James Sumner in 1926 provided a breakthrough in early enzyme studies. Sumner found that urease crystals consisted entirely of protein, and he postulated that all enzymes are proteins.

In the absence of other examples, this idea remained controversial for some time.

Only in the 1930s was Sumner's conclusion widely accepted, after John Northrop and Moses Kunitz crystallized pepsin, trypsin, and other digestive enzymes and found them also to be proteins.

During this period, J. B. S. Haldane wrote a treatise entitled *Enzymes. Although the molecular nature* of enzymes was not yet fully appreciated, Haldane made the remarkable suggestion that weak bonding interactions between an enzyme and its substrate might be used to catalyze a reaction. This insight lies at the heart of our current understanding of enzymatic catalysis.

Since the latter part of the twentieth century, research on enzymes has been intensive. It has led to the purification of thousands of enzymes, elucidation of the structure and chemical mechanism of many of them, and a general understanding of how enzymes work.

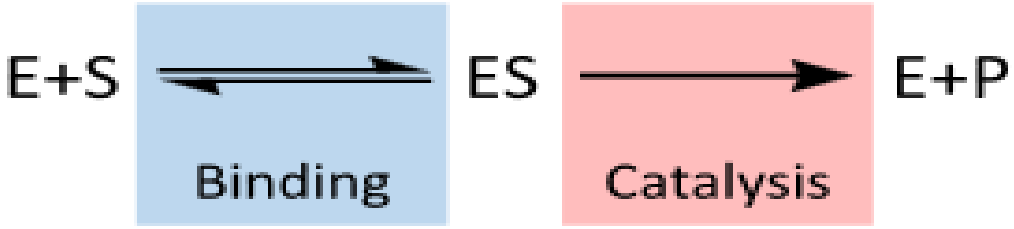
An Introduction to Enzymes

- Enzymes are macromolecules (mostly proteins) that catalyze more than 5,000 biochemical reaction types.
- They accelerate, or catalyze the biochemical reactions by converting the reactants (called substrate) into products.
- The study of enzymes is called *enzymology*.
- Like all catalysts, enzymes increase the reaction rate by lowering the activation energy required to initiate the conversion of reactants to products.
- So it increases the reaction velocity many millions of times faster than the non enzymatic process of the same reaction.

An Introduction to Enzymes (Cont.)

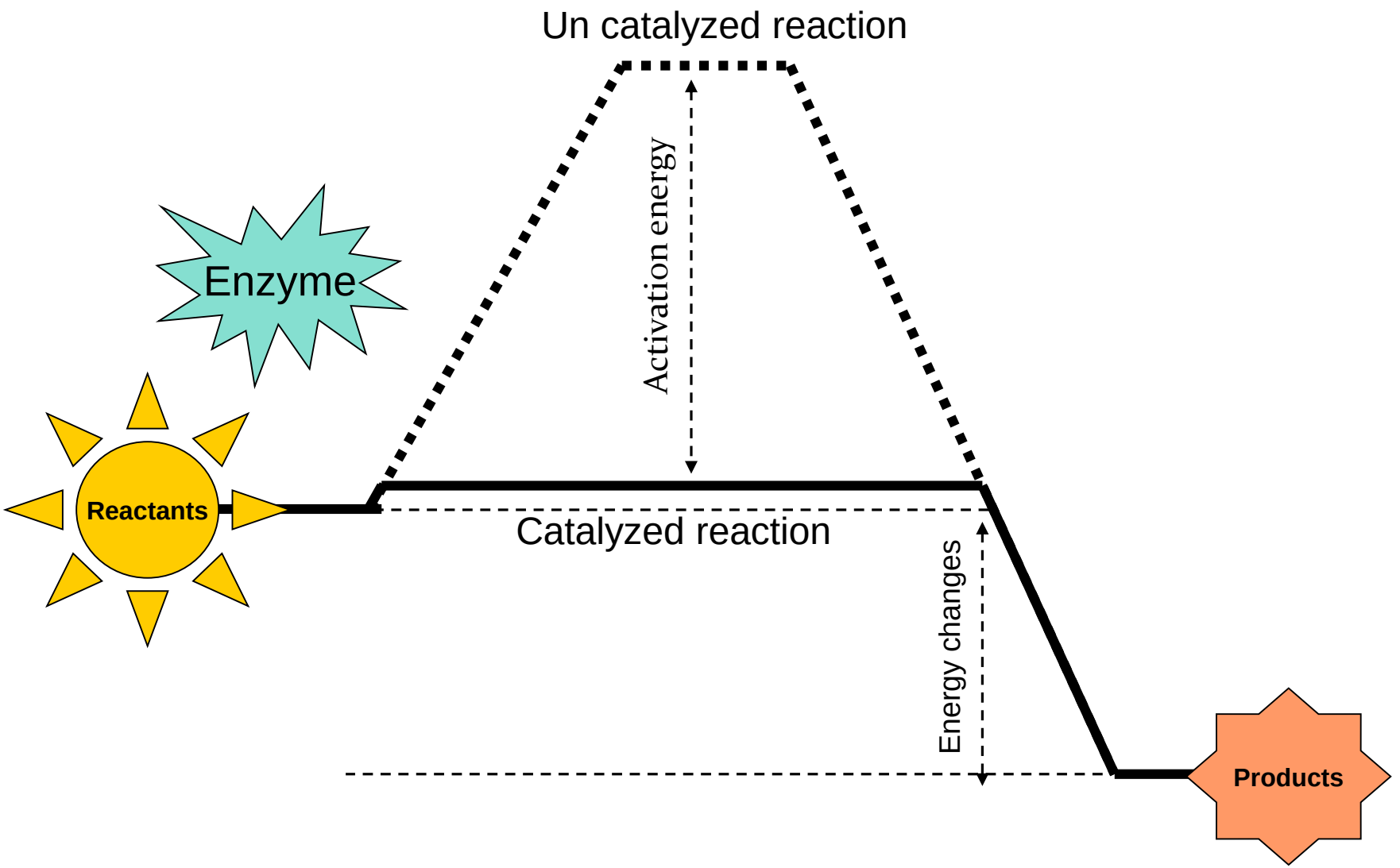
- Enzymes are not consumed in chemical reactions and are not part of the reactants or the products.

Enzyme + substrate → Enzyme/substrate complex → Enzyme + Product



- They does not alter the equilibrium of the reaction reaction.
- They differ from other non enzymatic reactions in that they are very specific to the substrates they bind and then to the chemical reaction catalyse.
- Enzyme activity is affected by pH, temperature, substrate concentration and the presence of activators or inhibitors.

Energy changes in chemical reactions



Enzyme specificity

Enzymes are very specific towards the reaction they catalyse and the substrate they react upon.

Enzyme specificity can be classified into the following;

1-**Absolute specificity;** Here the enzyme reacts with one substrate only , such as the glucokinase enzyme which acts on glucose only.

2- Group specificity; The enzyme acts upon a group of substrates (reactants) that share a common functional group,

Such as the polyphenoloxidase enzymes which act on di and tri hydroxy phenols.

3- **Bond specific;** The enzyme identifies a specific bond and acts upon it.

4-**sterio-isomer specific ;** The enzyme identifies a specific isomer. It is capable of differentiating between L- and D- isomers of a compound.

Property of enzymes

- With the exception of a small group of catalytic RNA molecules (ribozymes), all enzymes are proteins.
- Each enzyme has a distinctive 3D dimensional conformation depending on its amino acid composition .
- Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity.
- It is denatured and lose their native conformation and consequently lose their catalytic activity when exposed to heat or other denaturating agents.
- Denaturing agent include:

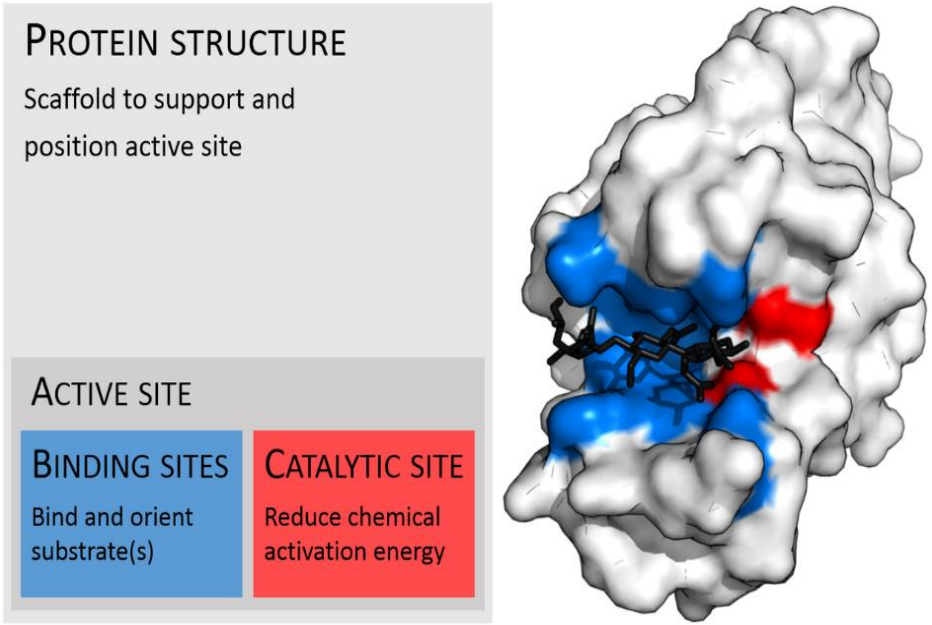
Extreme change in pH	8M urea
Heavy metals	Radiations
Detergents	

Property of enzymes (Cont.)

- Molecular weights range from 10000 to several hundred thousand
- Absorb UV light. Maximum absorption at 280nm due to aromatic a.a
- Solubility: soluble in water and diluted salts (globular proteins)
- Each enzyme has a distinctive pI depending on its amino acid composition .
- They are charged molecules depending on the pH of the solution.
  - Positively charged below pI, and
  - Negatively charged above pI.

Enzyme structure

- Most of the amino acids forming the enzyme serve as structural part and form the 3 D of the enzyme.
- Only few amino acids are incorporated in the catalysis and binding of the substrate.
- Catalysis occurs in specific site called "Active site"
- The shape and the chemical environment inside the active site permits a chemical reaction to proceed more easily.



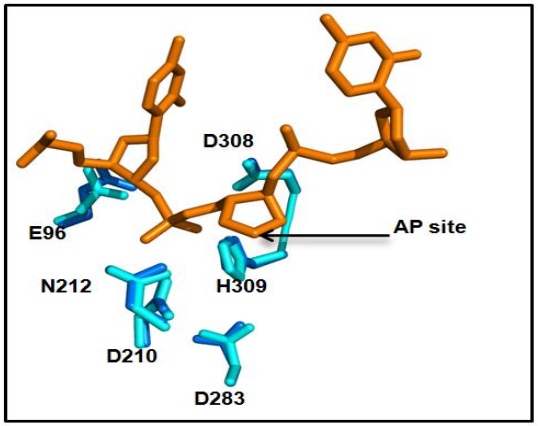
Enzyme structure

The active site is composed of two sites:

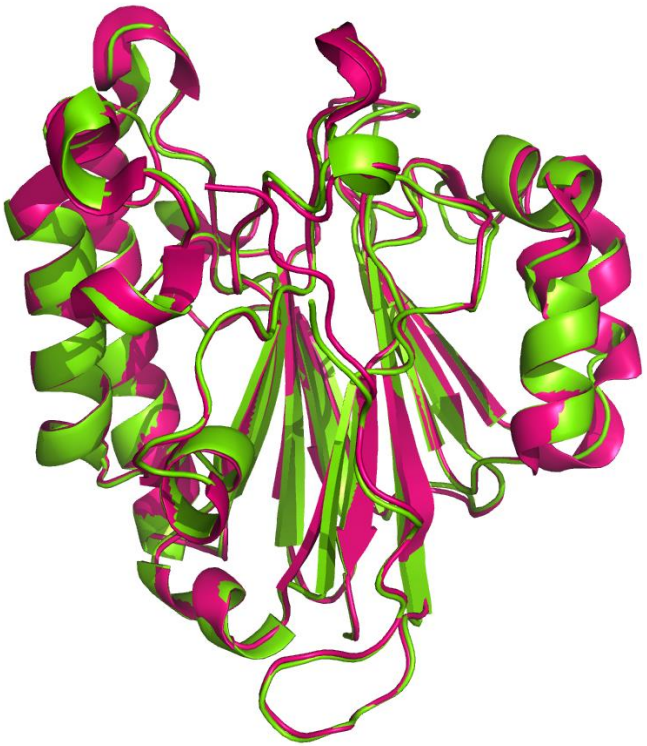
A- Binding site is some amino acids that binds the substrate and direct it to the catalytic site.

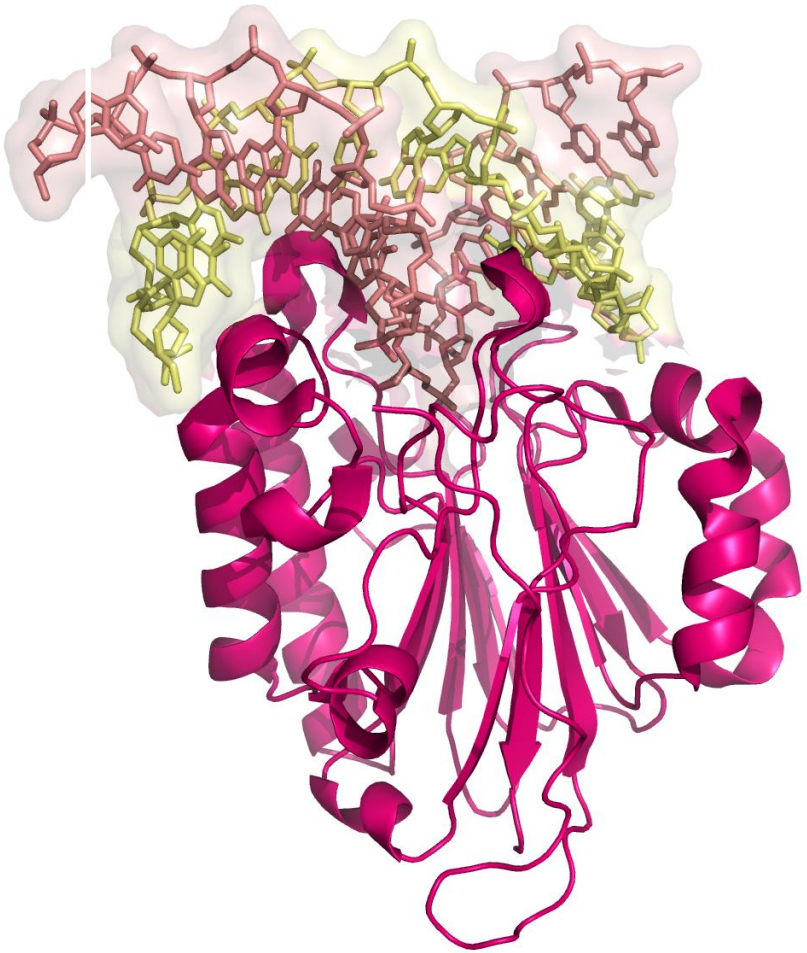
B- Catalytic site is the amino acid residues responsible for the chemical catalysis.

- Specificity of the enzyme is achieved by binding pockets with complementary shape, charge and hydrophilic/hydrophobic characteristics to the substrates
- The remaining majority of the enzyme structure serves to maintain the precise orientation and dynamics of the active site.



Protein structure, active site and substrate binding.
Ataya et al., *Int J Mol Sci.* 2012;13(7):8578-96





Enzyme activation

Some enzymes are synthesized in active form and require no chemical groups for activity other than their amino acid residues.

Other enzymes are produced in an inactive form due to either:

- Presence of excess polypeptide in their structure and is converted to active form after deletion of this part. The primary inactive form is called proenzyme or zymogen.

  Pepsin is produced as inactive pepsinogen which will cut to peptide + pepsin

- Lack of part of the enzyme and is converted to active form after addition of this part which is one of two forms:
  - **cofactor** - either one or more inorganic ions, such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+} or
  - **coenzyme** - a complex organic or metalloorganic molecule.

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.**

The protein part of such an inactive enzyme is called the **apoenzyme or apoprotein and** it has no activity by itself.

Coenzymes act as transient carriers of specific functional groups and it has no activity by itself.

Most coenzymes are derived from vitamins,

A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme.**

Apoenzyme	+	coenzyme	→	Holoenzyme
Inactive		inactive		active

Finally, some enzyme proteins are modified covalently by phosphorylation, glycosylation, and other processes. Many of these alterations are involved in the regulation of enzyme activity.

TABLE 1. Some Inorganic Elements That Serve as Cofactors for 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
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

TABLE 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
Coenzyme A	Acyl groups	Pantothenic acid 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)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

Enzymes Are Classified by the Reactions They Catalyze

Nomenclature:

Old names:

Enzymes were named by their discovers for a broad function. For example, an enzyme known to act in the digestion of foods was named pepsin, from the Greek *pepsis, "digestion,"* and lysozyme was named for its ability to lyse bacterial cell walls. Other were named for their source: trypsin, named in part from the Greek *tryein,* "to wear down".

By adding suffix -ase

Many enzymes have been named by adding the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity. E.g. urease catalyzes hydrolysis of urea, and DNA polymerase catalyzes the polymerization of nucleotides to form DNA.

Scientific name (Systematic name):

The International Union of Biochemistry and Molecular Biology (IUBMB) has developed a nomenclature for enzymes.

Enzyme classification (EC numbers);

- Each enzyme is described by four numbers preceded by "EC".
  - The first number classifies the enzyme based on its mechanism.
    - E.C. 1, Oxidoreductases: catalyze oxidation/reduction reactions
    - E.C. 2, Transferases: transfer a functional group (*e.g.* a methyl or phosphate group)
    - E.C. 3, Hydrolases: catalyze the hydrolysis of various bonds
    - E.C. 4, Lyases: cleave various bonds by means other than hydrolysis and oxidation
    - E.C. 5, Isomerases: catalyze isomerization changes within a single molecule
    - E.C. 6, Ligases: join two molecules with covalent bonds.
  - The second number indicates the groups that are added or deleted in the chemical reaction.
  - The third number is the coenzyme or cofactor bound to the enzyme.
  - The fourth is the kind of reactant (substrate)

TABLE 3. International Classification of Enzymes

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
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 ATP cleavage

Note: Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor.

Example:

E.C. 1.4.1.3 Glutamate: NAD(P) Oxidoreductase

L-Glutamate + $NAD(P)^+$ + H_2O ⟶ α- Ketoglutarate + NH_4^+ + NAD(P)H

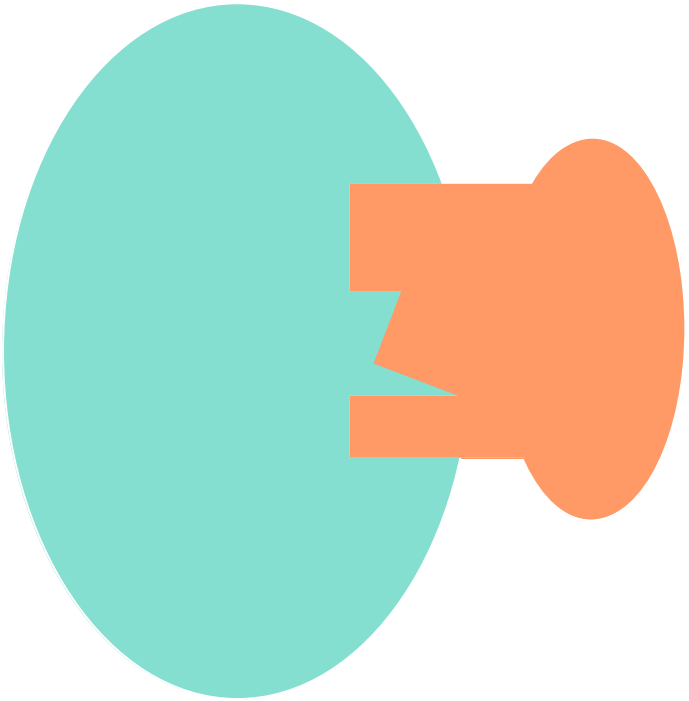
Enzyme with classification (E.C. number) 2.7.1.1 is .....

a- transferase b- oxidoreductase c- hydrolase d- ligase

Enzyme mechanism

1- Lock and key model

In 1894 Emil Fischer proposed that both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another.

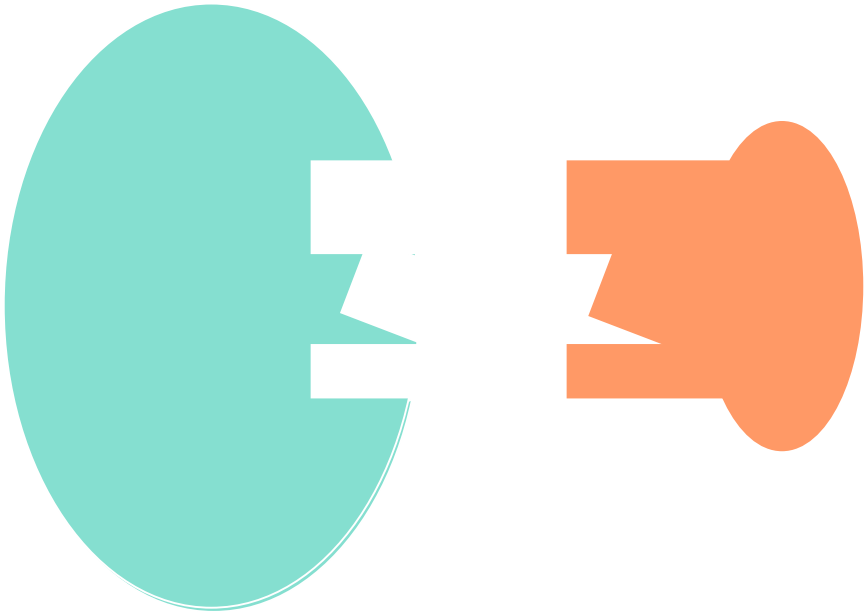


Enzyme Substrate

Enzyme mechanism

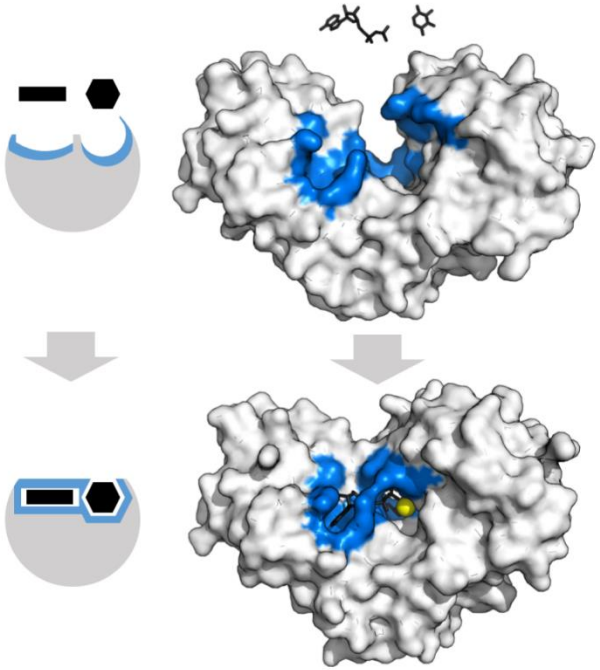
2-Induced fit model

In 1958, Daniel Koshland suggested that since enzymes are rather flexible structures, the active site is continuously reshaped by interactions of the substrate into the side chain of certain amino acid in the active site which changes its shape to fit the binding site to the substrate.



Enzyme

Substrate



Regulation of enzyme activity

- Some enzyme are synthesized in an inactive form.
- After accomplishing there functional role the enzymatic catalysis must be stopped.
- There are many ways to control the enzyme activity like: ????

Make a search about the control of enzyme activity

Quiz

- **The catalytically active complex of an apoenzyme and its prosthetic group is referred to .....**
- a. holoenzyme b- coenzyme c- cofactor

- **Protein that can act as a catalyst for certain cellular chemical reactions is .....**
- a-Enzyme b- carbohydrate c- lipid d- Non of the above

- **How many classes of enzymes are there???**
  a. 4 b. 5 c. 6 c. 7

- **Compound ends with the suffix –ase is .....**
  a. Sugar b. enzyme c. amino acid d. lipid

- **The chemical nature of most enzymes is .....**
- a. carbohydrate b. proteins c. lipids d. all of the above

- **All are distinctive features of enzymes EXCEPT:**
  a. regulation. b. catalytic activity. c. ability to change ΔG. d. specificity.

Quiz

- **What is a cofactor?**
- Typically small molecules that help promote catalysis. Many vitamins act as cofactors.

- **How do enzymes affect the energy of reactions?**
- **How do catalysts work to accelerate a chemical reaction?**
- They lower the activation energy.

- **Name the different types of enzyme specificities.**
- **Write the different classes of enzyme according to the IUBMB classification**

- **Compare between the lock/key and induced fit theory of enzyme catalysis.**

- **True or false**
- Apoenzyme is the active form of the enzyme ( )
- Enzyme with classification (E.C. number) 2.7.1.1 is transferase ( )