Enzymes

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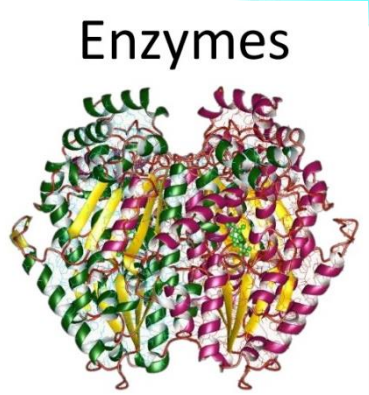
What is an Enzyme?



Enzyme is a substance that acts as a catalyst in living organisms (biocatalysts), regulating the rate of chemical reactions in the process.

Catalysts accelerate chemical reactions.

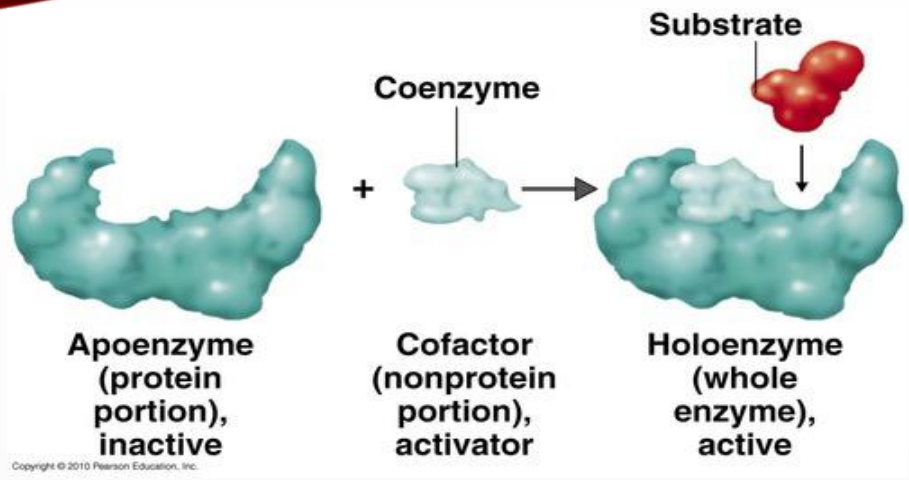
Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life

Like all catalysts, enzymes increase the reaction rate by lowering its activation energy.

Foods have natural digestive enzymes, our body creates the rest on its own.

Enzymes are naturally found in foods, an essential part of food digestion, besides they directly affect food quality in terms of color, flavor and appearance. Proteases, amylases and lipases are groups of digestive enzymes. They help break down the macronutrients of proteins, carbs and fats.

Structure of enyzmes



Apoenzyme + Cofactor = Holoenzim

Cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis (proper functioning of enzyme) .

Cofactors can be composed of 3 groups: **1. Coenzyme, 2. Metal ions, 3. Prostatic Group.**

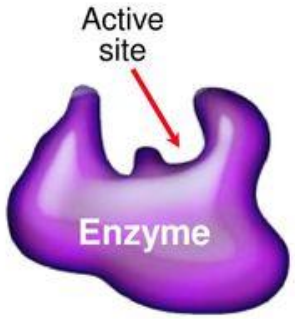
-Coenzymes are non-protein components, loosely bound to apoenzyme by non-covalent bond , e.g organic compounds like vitamins.

-Metal ions can be defined as inorganic ions that function as minerals, e.g Fe^{+2}, Mg^{+2}..

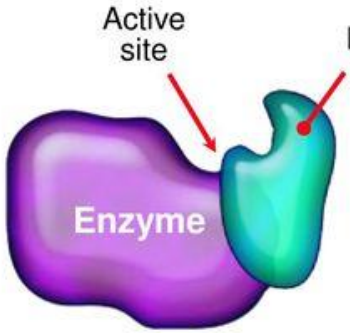
-The prostatic group has a coenzyme structure, but is bound to the enzyme with a tighter covalent bond than coenzymes.

Enzyme Cofactors

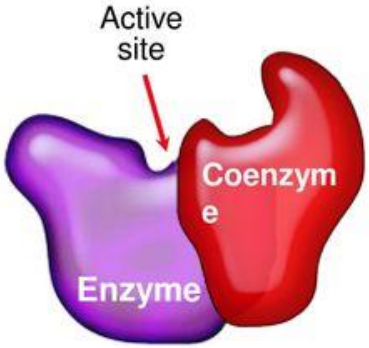
- Some enzymes require **cofactors** to be active.
- Cofactors are a **nonprotein** component of an enzyme. Cofactors can be:
  - **organic molecules** (coenzymes).
  - **inorganic ions** (e.g. Ca^{2+}, Zn^{2+}).
- Cofactors may be:
  - **Permanently attached**, in which case they are called **prosthetic** groups.
  - **Temporarily attached coenzymes**, which detach after a reaction, and may participate with another enzyme in other reactions.



Enzyme is protein only
Example: lysozyme



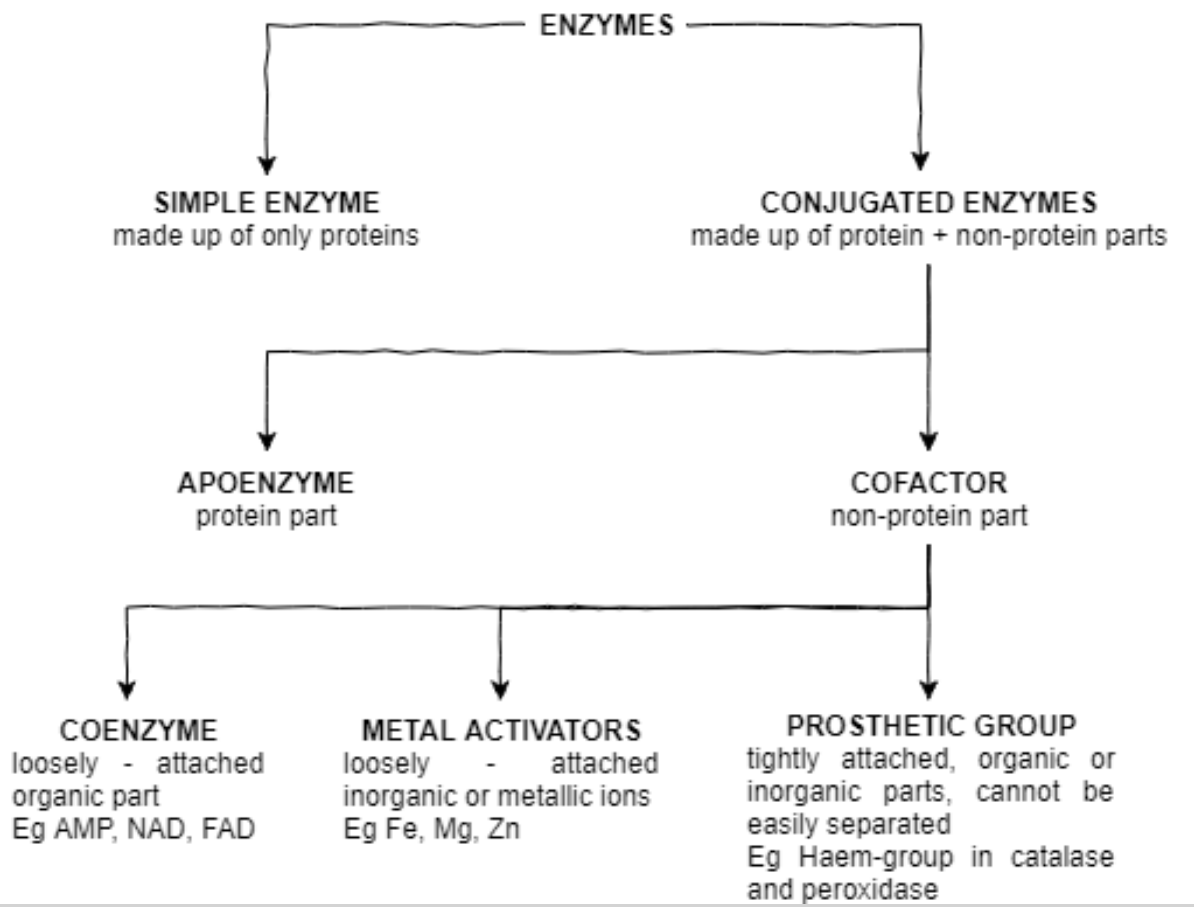
Enzyme + prosthetic group
Example:
flavoprotein + FAD



Enzyme + coenzyme
Example:
dehydrogenases + NAD

Apoenzyme is an inactive enzyme.

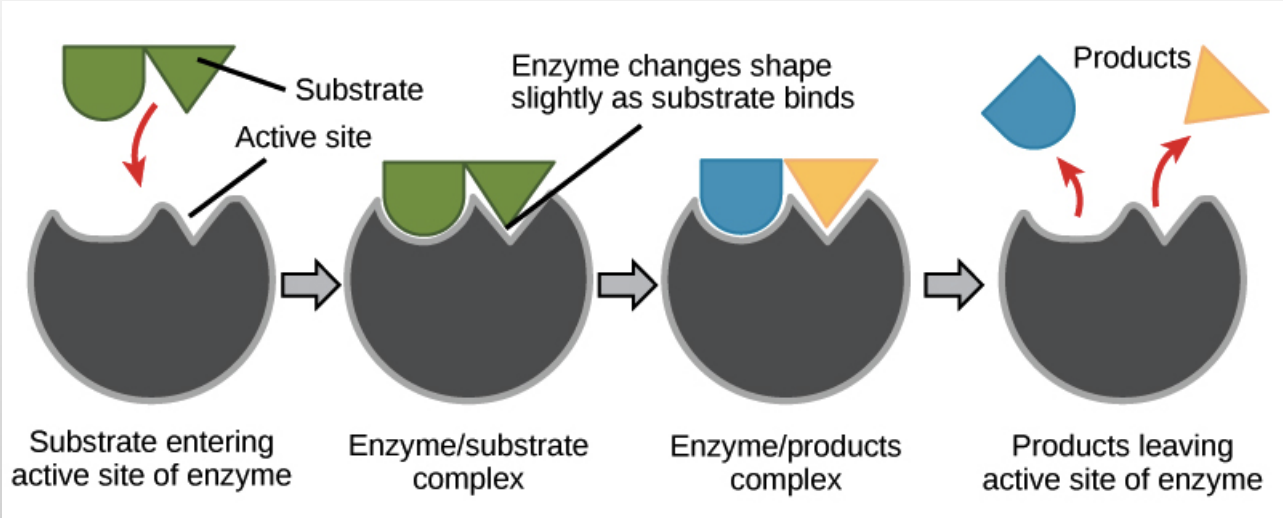
Holoenzyme is an active enzyme with its non-protein component.



Enzyme- Substrate Complex

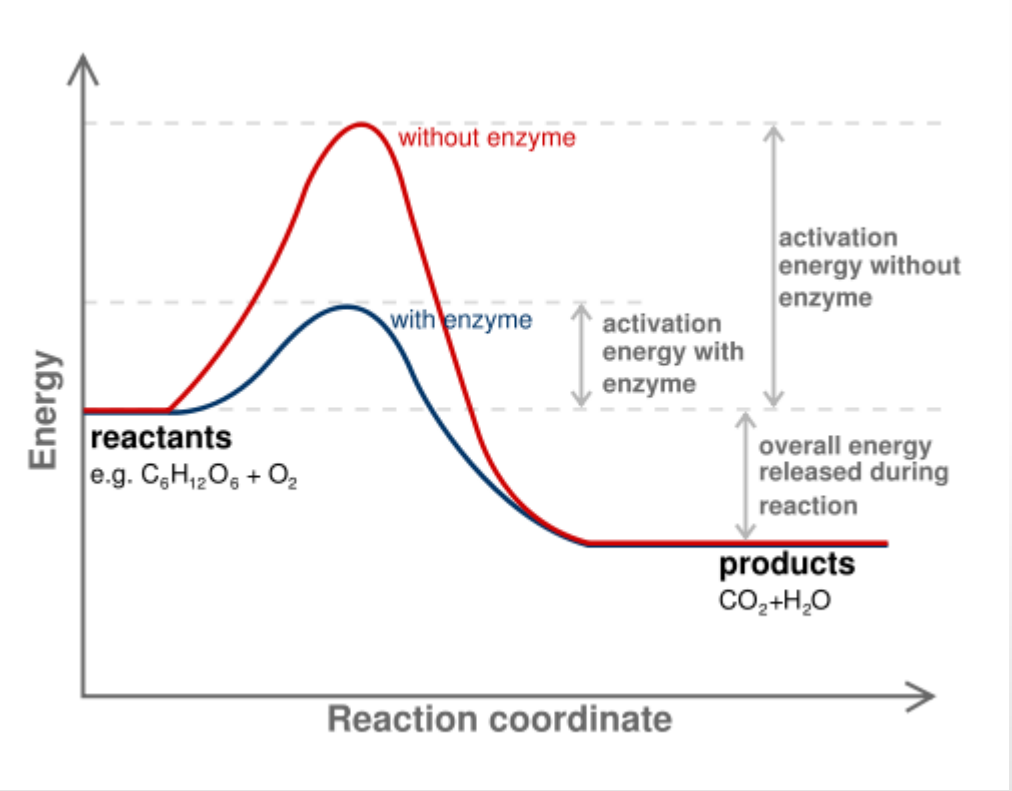
- The molecules upon which enzymes may act are called **substrates**, and the enzyme converts the substrates into different molecules known as **product**s.
- Each enzyme is specific to a particular substrate.
- The structure formed by binding the enzyme to the substrate is called the enzyme-substrate complex.
- Enzyme-substrate complex was called as the **lock-and-key model**.
- At the end of the reaction, the substrate turns into a product and the enzyme substrate complex is separated from each other
- Enzymes are reusable because they are not changed by the reactions that they catalyze.

$$E + S \longleftrightarrow ES \longleftrightarrow E + P$$



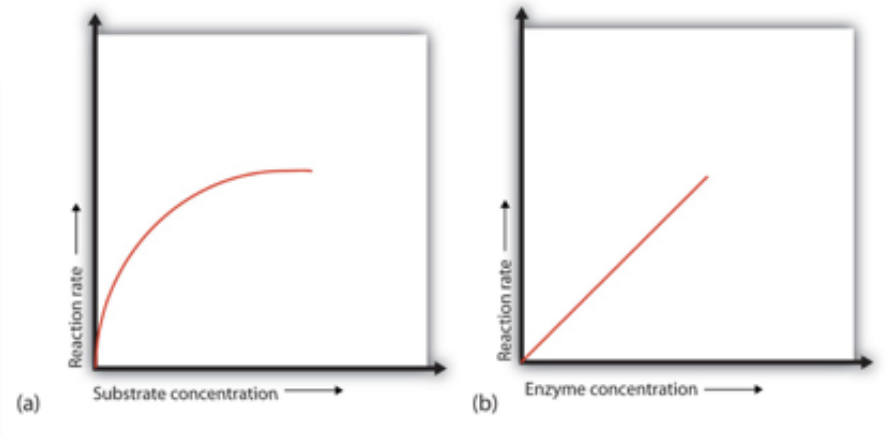
Enzyme Activity

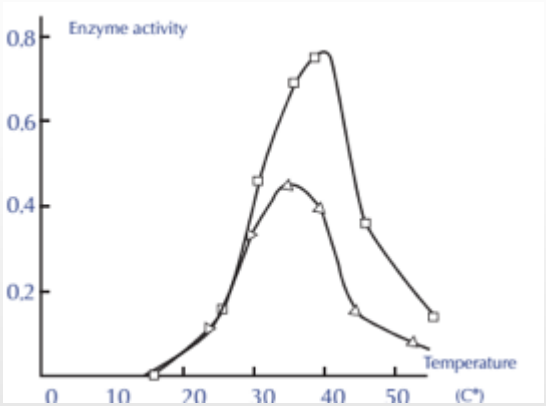
- In biochemical processes, molecules similarly require energy in order to start a reaction. This energy is called the **activation energy (Ea).**
- The lower the activation energy for a reaction, the faster the rate. Thus enzymes speed up reactions by lowering activation energy.

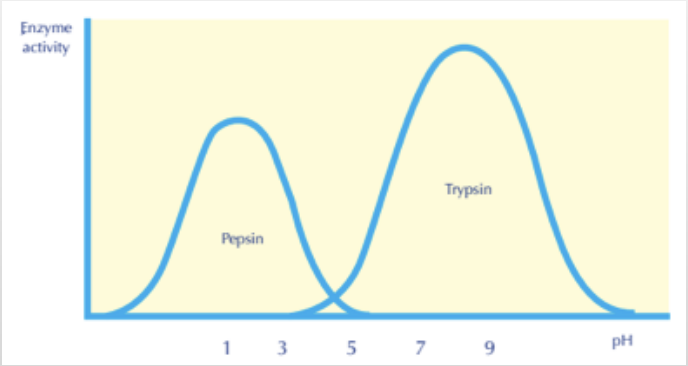


Factors affecting enzyme activity

1) **Substrate concentration**
2) **Enzyme concentration**
3) **Temperature**
4) **pH**
5) **Presence of Activators/Inhibitors**







Naming Enzymes

Enzymes are named by adding the suffix -ase to the name of the substrate that they modify (i.e., urease and tyrosinase), or the type of reaction they catalyze (e.g. dehydrogenase, decarboxylase).

Urease→ urea to ammonia and CO2

Alcohol dehidrogenase→dehydrogenation of alcohol

Lactasez→breaking down lactose into glucose and galactose

Except for some of the originally studied enzymes that are used with common names (particularly digestive/proteolytic enzymes) such as pepsin , rennin, and trypsin that ending with –in.

Classification of Enzymes

Enzymes can be classified in by the kind of chemical reaction catalyzed:

Oxidoreductases: Catalyze oxidation and reduction reactions, e.g. pyruvate dehydrogenase, catalysing the oxidation of pyruvate to acetyl coenzyme A.

Transferases: Catalyze transferring of the chemical group from one to another compound. An example is a transaminase, which transfers an amino group from one molecule to another.

Hydrolases: Catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes peptide bonds in proteins.

Lyases: Catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Isomerases: Catalyze the formation of an isomer of a compound (changing geometry or structure of a molecule)

Ligases: Joining two molecules through hydrolysis of pyrophosphate bond in ATP or other tri-phosphate

Specificity of Enzymes

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze.

In general, there are four distinct types of specificity:

Absolute specificity - the enzyme will catalyze only one reaction.

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

Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

Enzyme immobilization

Enzymes are produced by animals, plants or microorganisms.

Immobilization is defined as the imprisonment of cell or enzyme in a distinct support or matrix.

Enzyme immobilization can be defined as the confinement of enzyme molecules onto/within a support/matrix physically or chemically or both, in such a way that it retains its full activity or most of its activity. This can provide increased resistance to changes in conditions such as pH or temperature. Moreover, allows continuous use and reuse of the catalyst.

- Enzymes imprisoned in a matrix (resins, porous / non-porous polymeric matrices, gels) can be used over and over again.
- After imprisoned, enzyme activity and affinity to the substrate are generally negatively affected, while its stability (its ability to maintain its activity, does not lose its structure and catalytic activity) is positively affected. These changes depend on the enzyme's structure, the type of matrix and the conditions of arrest.

Methods of enzyme immobilization

1.Binding/support/attachment

2. Entrapment

3- Cross-linking

1.Binding:

Functional ionic groups and hydrophobic regions on the surface of the enzyme molecule play a role in binding. Many natural or synthetic organic / inorganic materials can be used.

Support binding can be physical or chemical, involving weak or covalent bonds in two ways:

a. **Adsorption (non-covalent):** use of the physical interactions generated between the carrier and enzyme that include van der Waals forces, ionic interactions and hydrogen bonding. Agarose, cefadex, cellulose derivatives, metal salts and minerals can be used as absorbents.
b. **Covalent binding/bonding:** stable complexes between functional groups on enzyme molecules and a support matrix are formed through covalent bondings.

2. Entrapment:

Entrapment requires the synthesis of the polymeric network in the presence of the enzyme. The most important difference from binding method is enzyme molecules are not bound by physical or chemical bonds.

Entrapment methods can also applied in 2 ways:

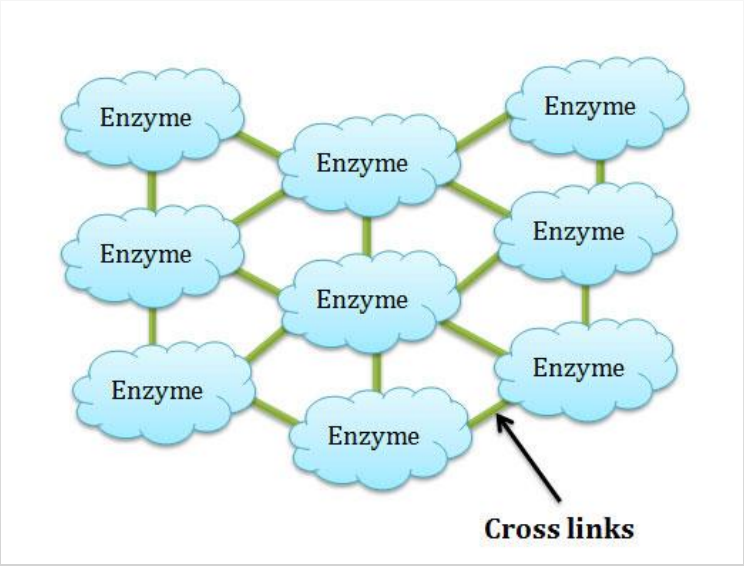
1-Matrix entrapment : Enzymes are entraped in natural or synthetic polymer cages. Substrate enters these meshes but product cannot exit, e.g carragenane and alginate

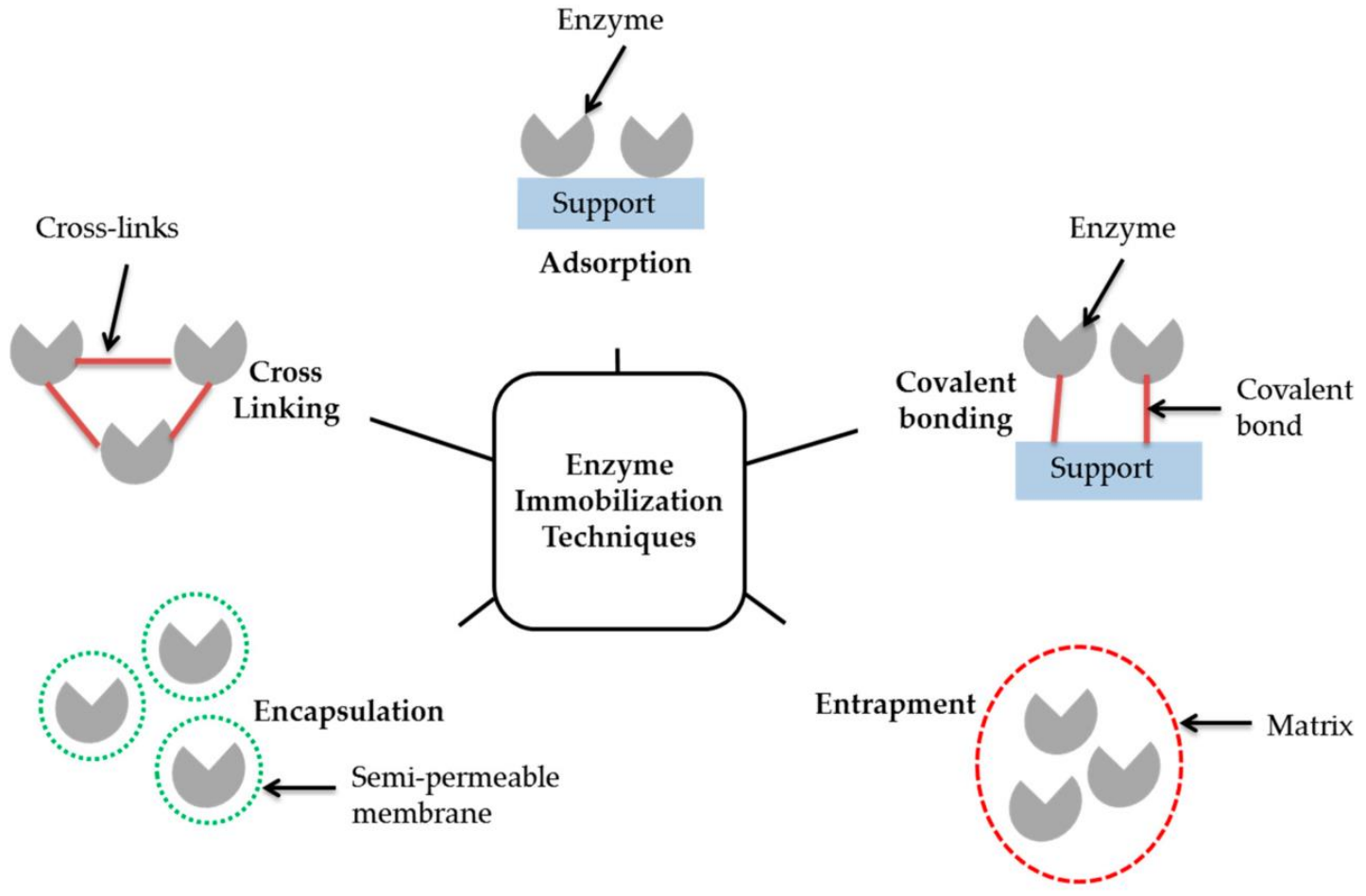
2- Membrane entrapment (encapsulation): Microcapsule entrapment encloses enzymes inside a microcapsule of semipermeable polymer membrane. The diameter of the microcapsule is generally from several micrometers to several hundred micrometers allowing both the entrance and exit of substrate (btw 1-100 μm).

3-Cross- linking:

Enzyme **immobilization** by **cross-linking** is an irreversible **method** performed by the formation of intermolecular **cross- linkages** between the enzyme molecules by covalent bonds.

The immobilized enzyme is present in the reaction mixture and not bound to any support.




Enzyme
Support
Adsorption
Cross-links
Cross
Linking
Enzyme
Immobilization
Techniques
Enzyme
Covalent
bonding
Covalent
bond
Support
Encapsulation
Semi-permeable
membrane
Entrapment
Matrix

Some advantages of the immobilized enzymes;

1) increased enzyme stability
2) Reuse of enzyme
3) continuous use of enzyme
4) reduced enzyme costs (due to the reuse of enzymes)
5) high enzyme-substrate ratio
6) easy product separation
7) Minimum reaction time

Disadvantages of immobilized enzymes;

1) lower stability
2) activity losses can be seen
3) higher cost than soluble enzymes (general cost due to the used materials)