LESSON 6. REACTIONS CATALYSED BY ENZYMES

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Adapted from a previous version from Dr. Herminia González Navarro

LESSON 6. ENZYME-CATALYSED REACTIONS

1. Concepts and the role of enzymes in chemical reactions
- **1.1.** Activation energy and transition state

2. Properties of enzymes as catalysts
- **2.1.** Properties of enzymes in chemical reactions
- **2.2.** Properties of enzymes due to their protein nature
- **2.3.** Properties of enzymes: specificity

3. Nomenclature and classification

4. Mechanisms of enzymatic action
- **4.1.** Characteristics of the active site
- **4.2.** Interaction models
- **4.3.** Types of enzymatic reaction

5. Enzymatic cofactors: characteristics, properties and types
- **5.1.** Classification of coenzymes
- **5.2.** Important coenzymes
- **5.3.** Metal cofactors

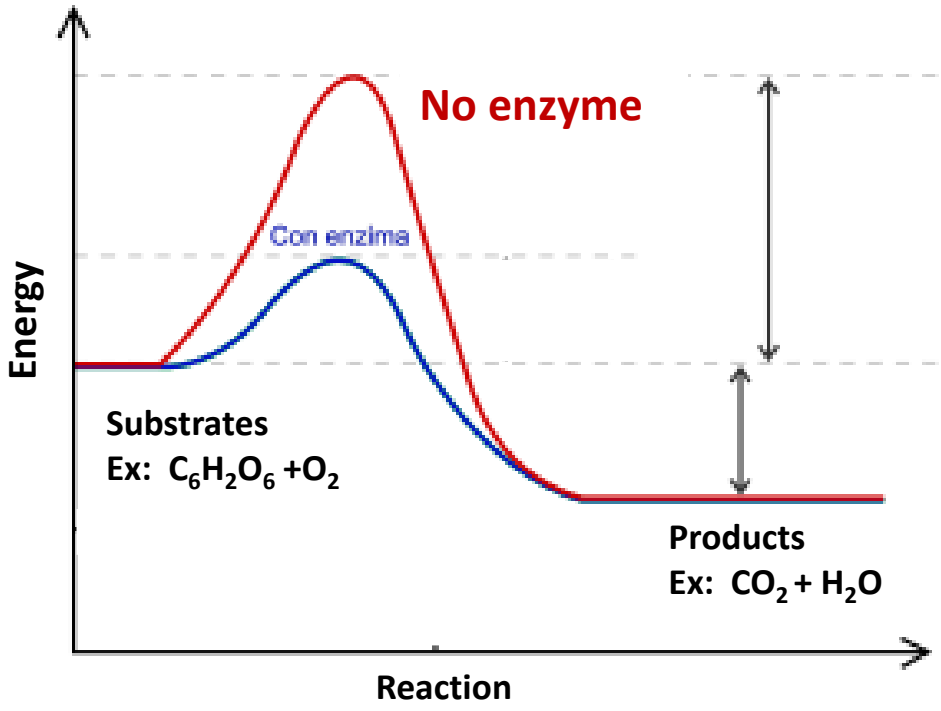
What is an enzyme?

https://www.menti.com/al4bajyqav1s

1. Concepts and the role of enzymes in chemical reactions

An enzyme is a biological catalyst; they are biological molecules that increase the speed of a chemical reaction

1. Concepts and the role of enzymes in chemical reactions



Source: Biochemistry, Stryer, 7th Edition

- **Biological functions** require chemical reactions and these must take place **quickly**.

- Enzymes are biological molecules that catalyse a chemical reaction**.**

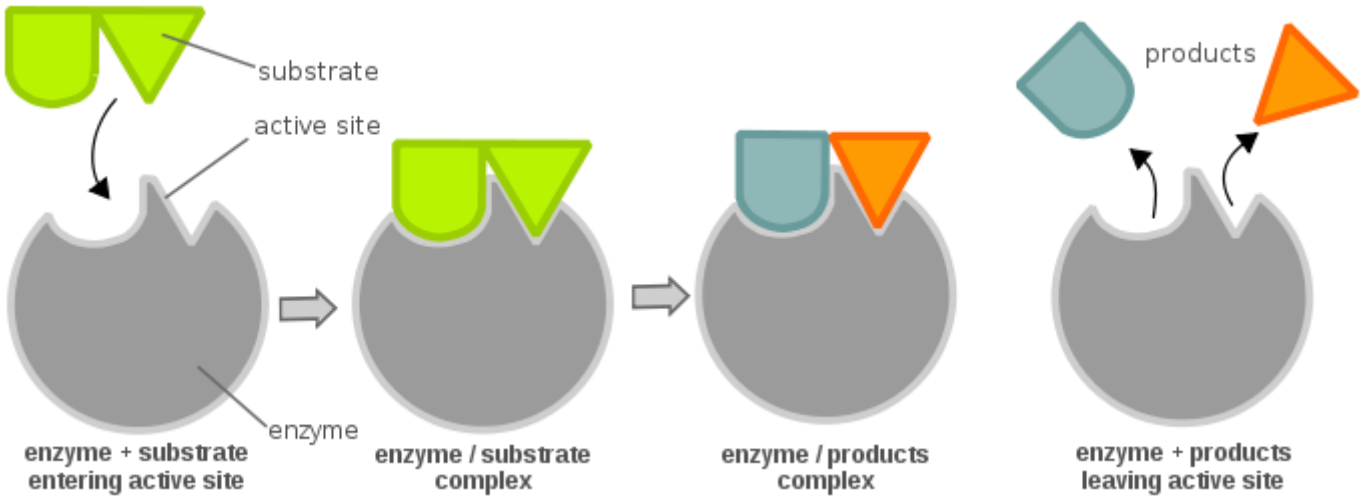
- They are proteins that facilitate biochemical reactions by accelerating them between 10^3 and 10^8 times.

- Among all the biochemical reactions that are energetically possible**, enzymes channel and modify the chemistry for greater efficiency in terms of time**.

- Enzymes are present in all metabolic processes.

1. Concepts and the role of enzymes in chemical reactions

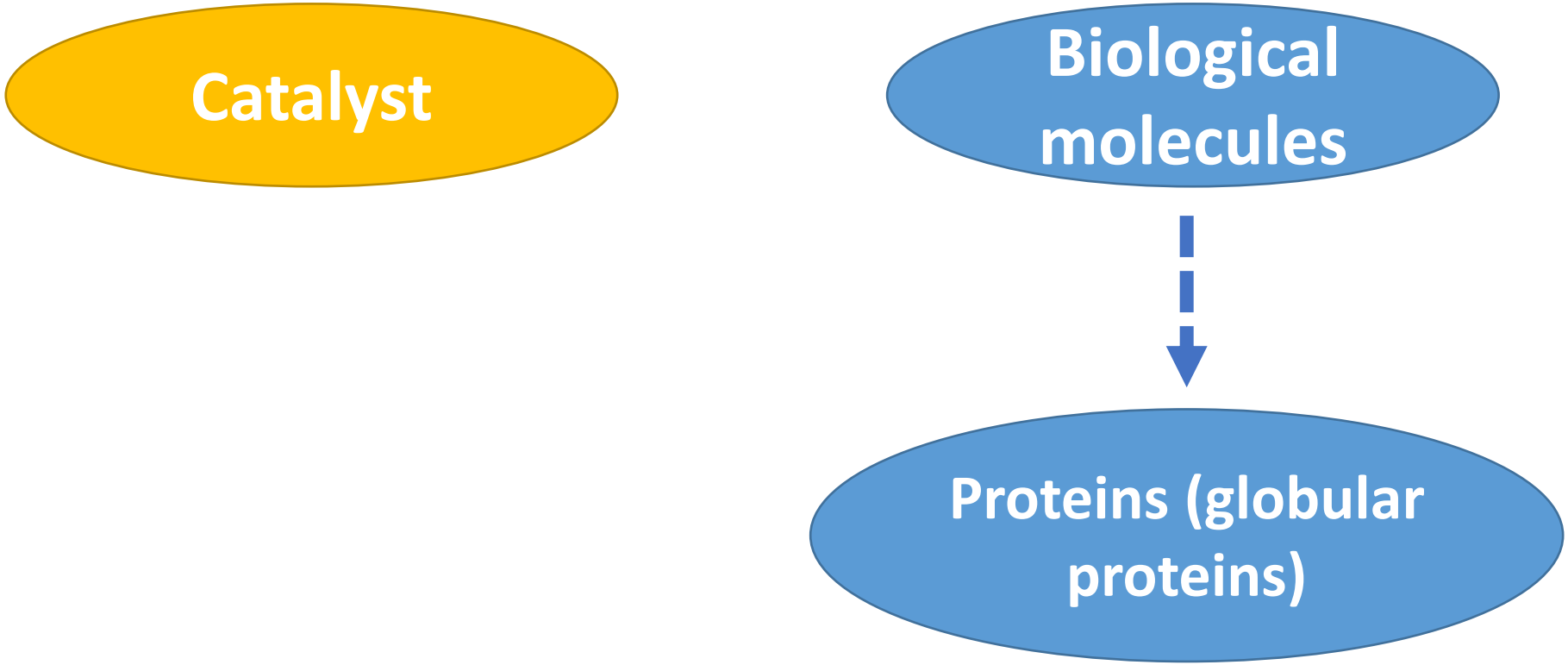
Enzymes in chemical reactions:
Enzymes bind to molecules called **substrates**.
Enzymes transform molecules into other ones called **products**.
The part of the enzyme the substrate binds to is called the **active site**.



Enzyme (E) + Substrate (S) → ES →EP → Product (P) +Enzyme (E)

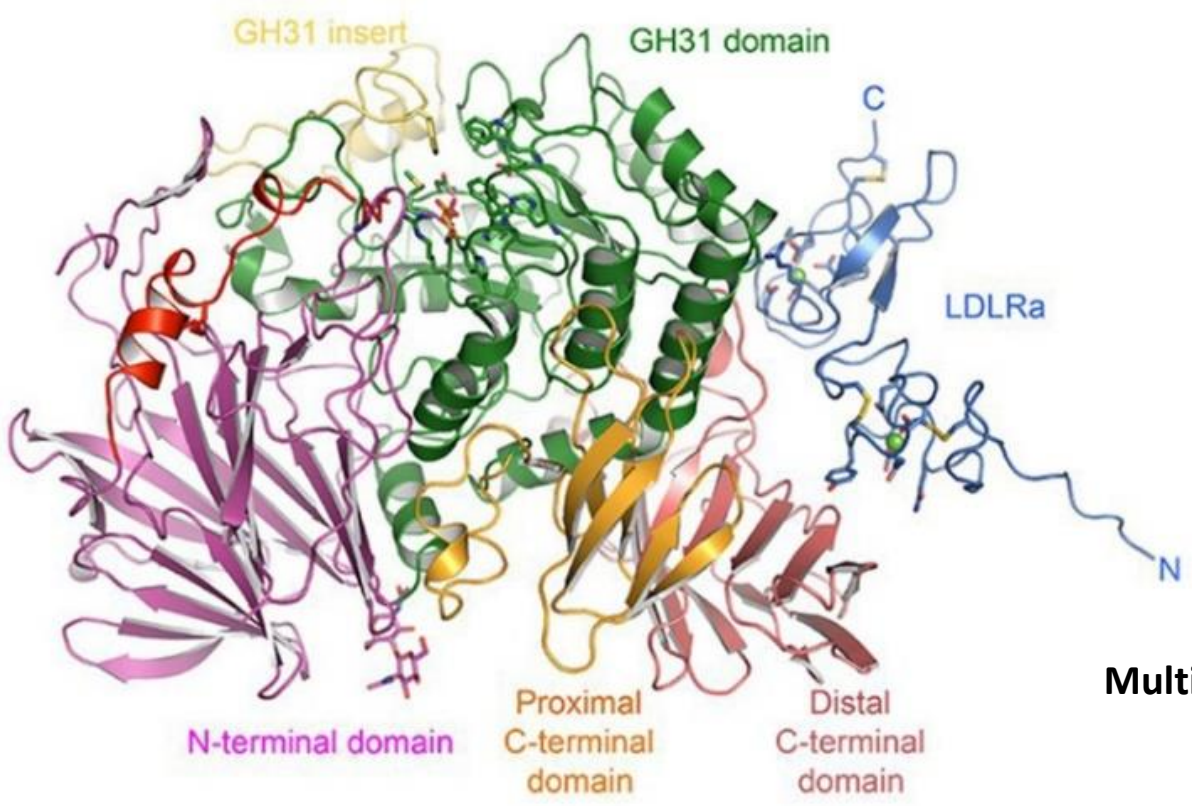
Diagram source: courseslumenlearning.com

What is an enzyme?



1. Concepts and the role of enzymes in chemical reactions

Enzymes are catalysts. They are proteins, mainly globular proteins.



Multidomain globular structure of an enzyme

PNAS July 26, 2016, doi: 10.1073/pnas.1604463113

What is an enzyme?

Catalyst

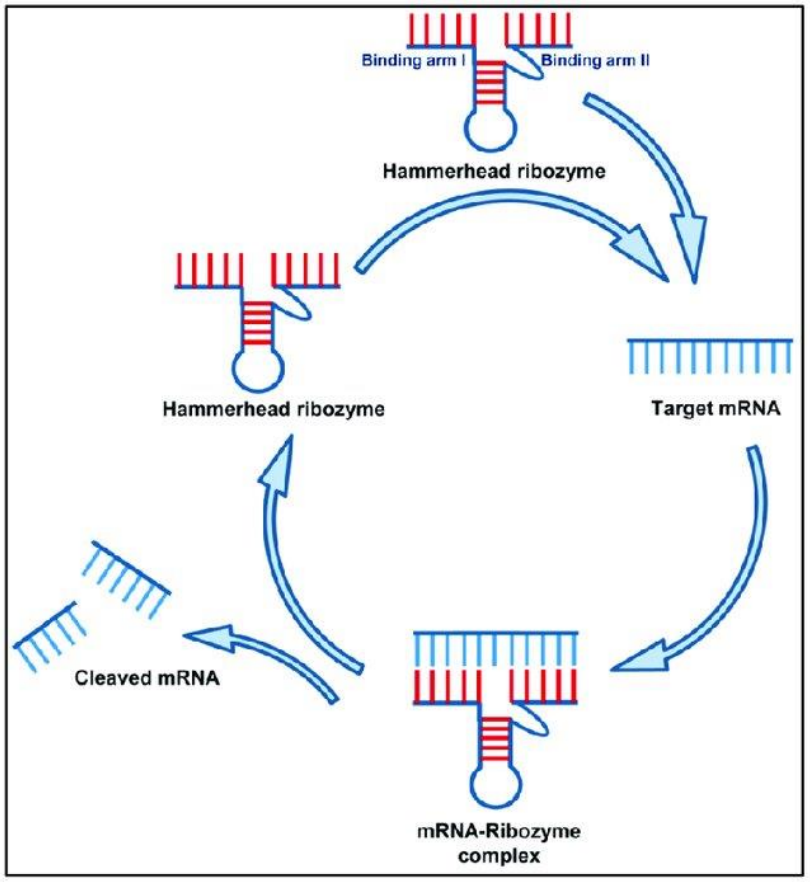
Biological molecules

Are all the catalysts that exist in nature proteins? Are they all globular proteins?

1. Concepts and the role of enzymes in chemical reactions

Ribozymes: Ribozymes are RNA molecules that can act as catalysts, i.e., they accelerate reactions in a specific way.

Deoxyribozymes: are DNA oligonucleotides that are capable of performing a specific chemical reaction,



Abzyme: Antibodies with enzymatic and therefore catalytic activity. Abzymes are normally artificial constructs but are also found in normal organisms and humans.

Synzymes: synthetic enzymes

Mechanism of action of ribozymes.
Source: Asha et al, Advances in Nucleid Acid Therapeutics against respiratory virus infections. Journal of Clinical Medicine, 2018, doi: 10.3390/jcm8010006

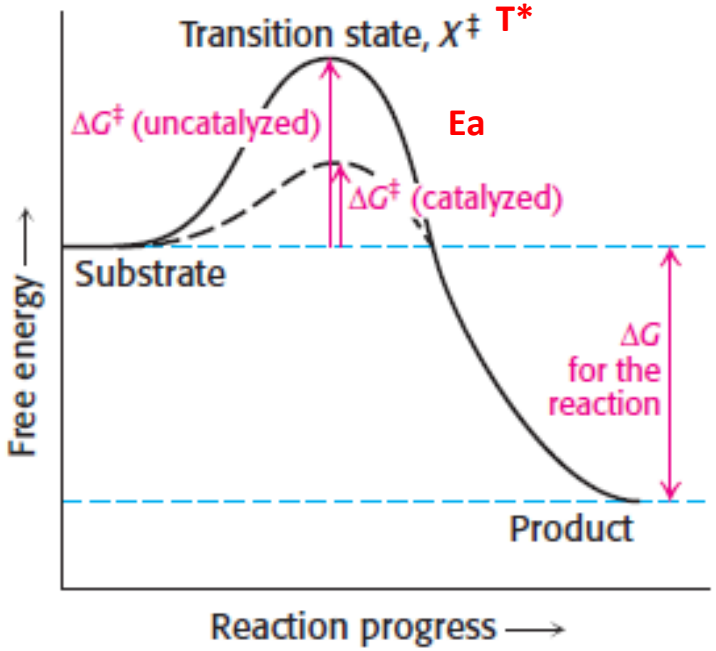
1. Concepts and the role of enzymes in chemical reactions

1.1. Activation energy and transition state

All chemical reactions have an **energy barrier** between substrates and products.

ACTIVATION ENERGY (Ea): This is the difference in energy between the substrates and the **reaction intermediate** that has the highest energy content. This intermediate with the greatest amount of energy is called the transition state.

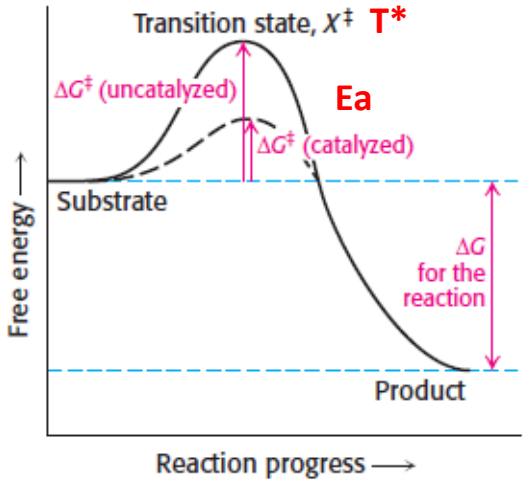
TRANSITION STATE (T*): is unstable and its high energy content allows the reaction to resume



Biochemistry Stryer 7th edition

1. Concepts and the role of enzymes in chemical reactions

1.1. Activation energy and transition state



The most useful energy magnitude to determine whether a chemical reaction will occur is the **change in Gibbs free energy (ΔG).**

Biochemistry Stryer 7th edition

ΔG<0 → indicates a favourable reaction

ΔG>0 → indicates a non-favourable reaction and if the system requires this reaction an input of energy is needed.

The enzyme facilitates the reaction because it decreases the value of **ΔG**

THE DIFFERENCE IN ENERGY BETWEEN THE PRODUCTS AND THE SUBSTRATES DOES NOT DEFINE OR DETERMINE THE REACTION VELOCITY.

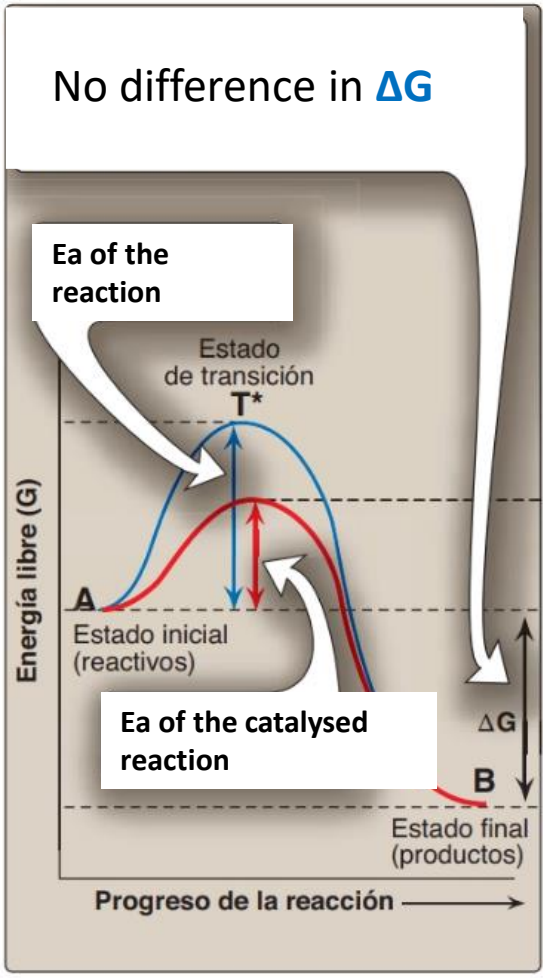
What is an enzyme?

Catalyst

Biological molecules (normally proteins)

It decreases the value of **ΔG**

2. Properties of enzymes as catalysts



S →T*→P

1. Diminishes the activation energy needed to achieve the transition state and favours the transition state.

2. Accelerates the chemical reactions. The enzyme is not altered during the reaction.

3. Does not enable reactions that are not thermodynamically favourable.

4. Does not modify the reaction's final equilibrium.

5.Does not modify the reaction's energy balance.

6. Is needed in small quantities.

From: Harvey, R., Ferrier, D. Bioquímica (Lippincot Illustrated Reviews) 7th Edition, 2017 Editorial Wolters Kluwer. ISBN: 9788416781805

2. Properties of enzymes as catalysts

2.1. Properties of enzymes in chemical reactions

- Efficiency: they can create **favourable positioning of substrates** through interactions (hydrophobic, electrostatic, and even covalent unions) that make collisions between substrates more likely, or they can strain a substrate in such a way that it breaks or splits.
Efficiency increases the reaction velocity by 10^3 -10^8 times.

- Specificity: the **complex globular structure** provides the enzyme with the capability to create catalytic sites that can accommodate different substrates in different ways. They may even discriminate between L and D enantiomers.

- Able to work in biological conditions: Temperature, physiological pH

- Variable activity and regulation capacity: Activators, inhibitors, etc.

- Cellular localization: Depending on their function, the needs of the products and the location of the substrates, enzymes are located in organelles such as mitochondria, ER, nucleus, lysosomes, etc.

2. Properties of enzymes as catalysts

2.2. Properties of enzymes due to their protein nature

1. Lability: Enzymes are sensitive to temperature and pH. They can undergo denaturation and loss of globular protein structure.

2. Ability to form non-covalent bonds: These non-covalent bonds are characterized by having specific chemical groups that are needed in the chemical reaction.

3. Requirements of cofactors and coenzymes: They require non-protein molecules to participate and assist in the enzymatic reaction (Zn, Fe, FADH, NADH).

4. Size: The enzyme is always much larger than the substrate and products

2. Properties of enzymes as catalysts

2.2. Properties of the enzymes due to their protein nature

4. Size: The enzyme is always much larger than the substrate and products

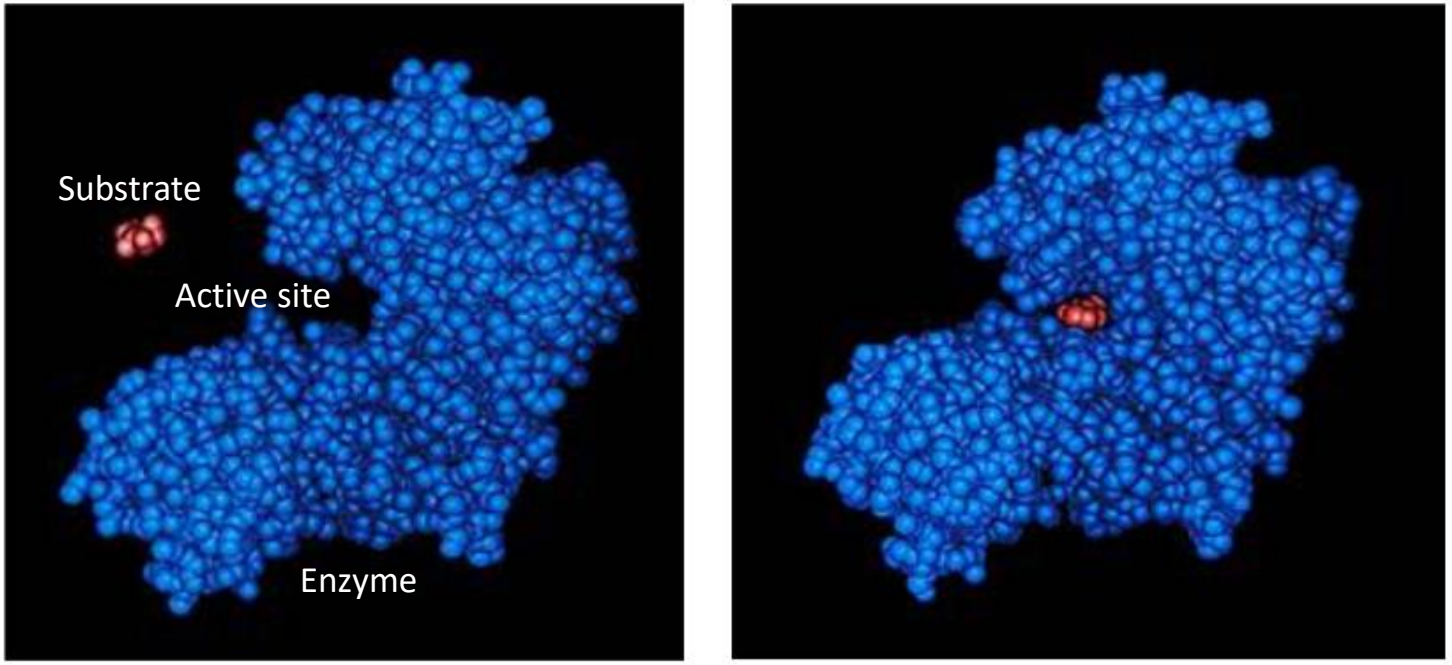


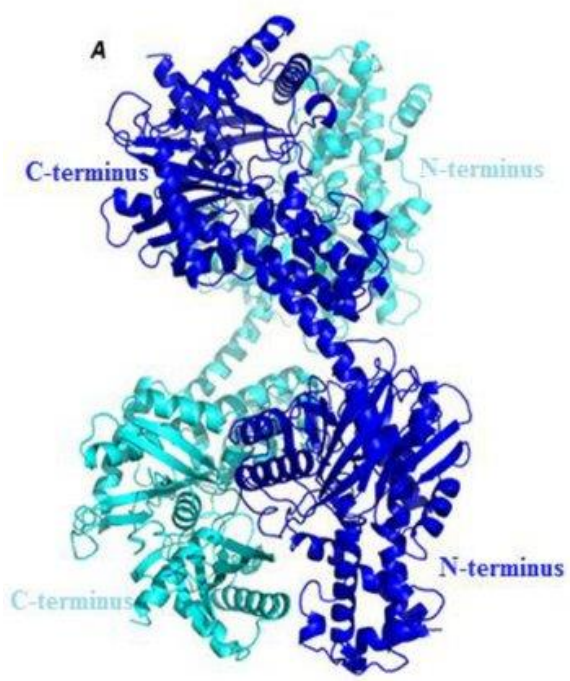
Diagram source: quizlet.com

2. Properties of enzymes as catalysts

2.3. Properties of enzymes: specificity

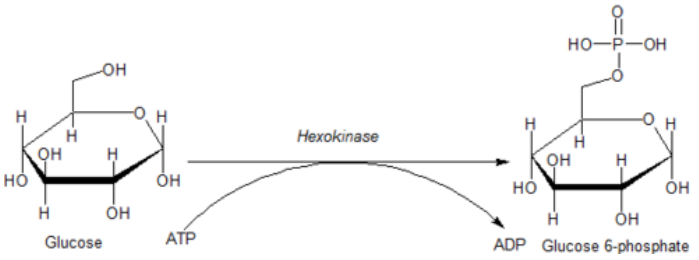
Substrate specificity: Enzymes differentiate between substrates with similar characteristics at different levels.

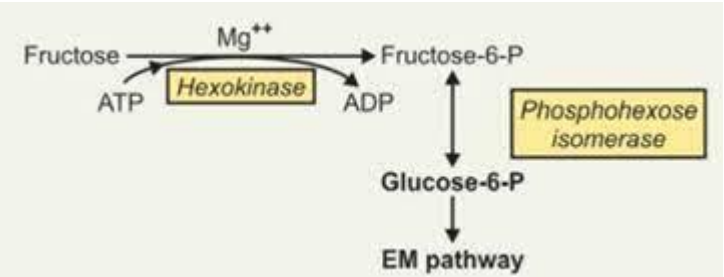
- **Group specificity:** enzymes that catalyse chemical reactions in a group of substrates that **share a chemical group (**PHOSPHATASES AND KINASES).



From Shah et al, Single Residue Determinants in the Binding of Recombinant Human Brain Hexokinase to the Mitochondrion, 2011

Hexokinase
EC 2.7.1.1





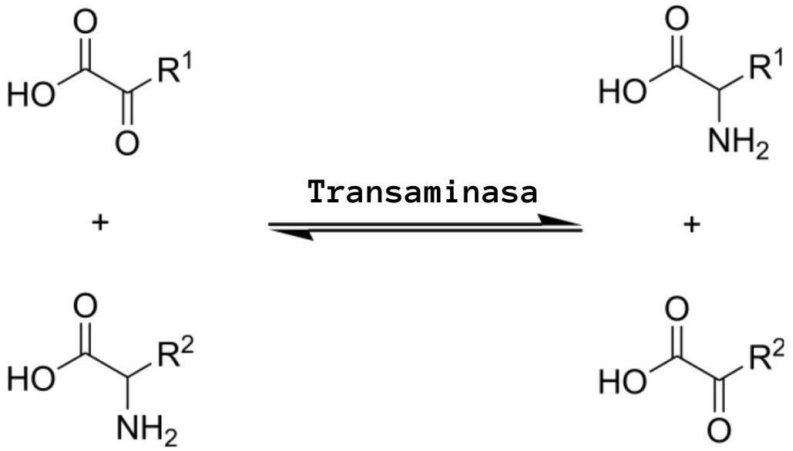
D-Glucose: K_M $5 \cdot 10^{-5}$ mol/L
D-Fructose: K_M $14 \cdot 10^{-4}$ mol/L

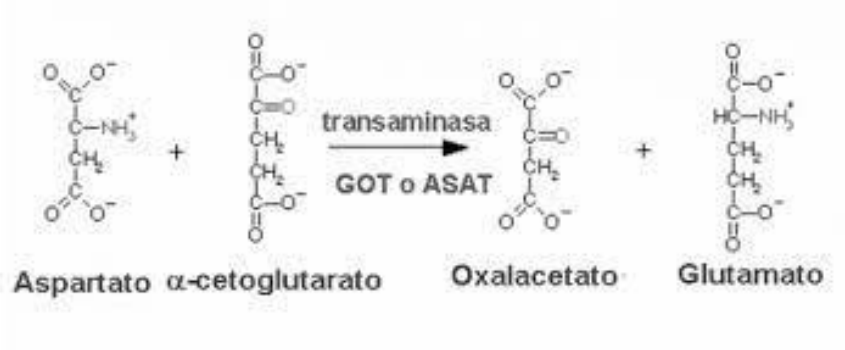
2. Properties of enzymes as catalysts

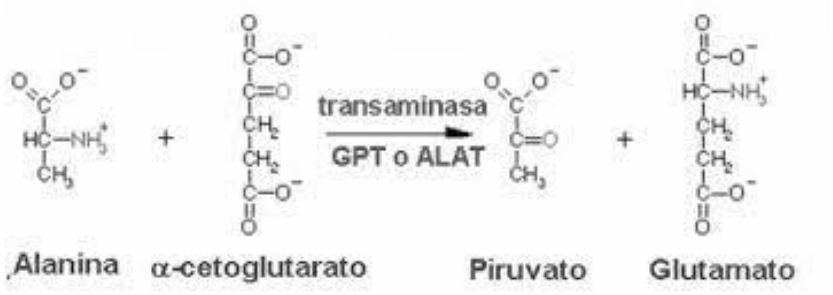
2.3. Properties of the enzymes: specificity

Substrate specificity: Enzymes differentiate between substrates with similar characteristics at different levels.

- **Bond specificity:** enzymes that catalyse the **transformation** of substances that have a **specific type of bond** (PEPTIDASES, TRANSAMINASES).





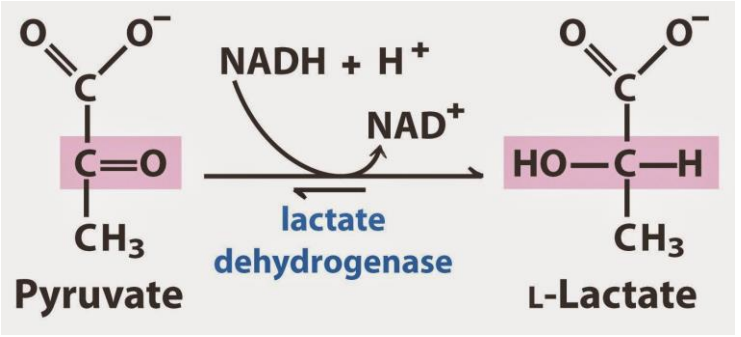


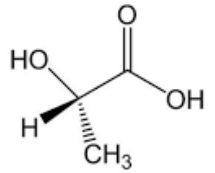
2. Properties of enzymes as catalysts

2.3. Properties of the enzymes: specificity

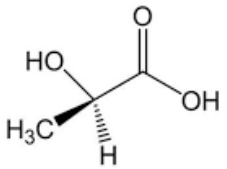
Substrate specificity: Enzymes differentiate between substrates with similar characteristics at different levels.

- **Stereospecificity:** Enzymes that catalyse chemical reactions that differentiate **between D and L isomers**.





L-lactic acid



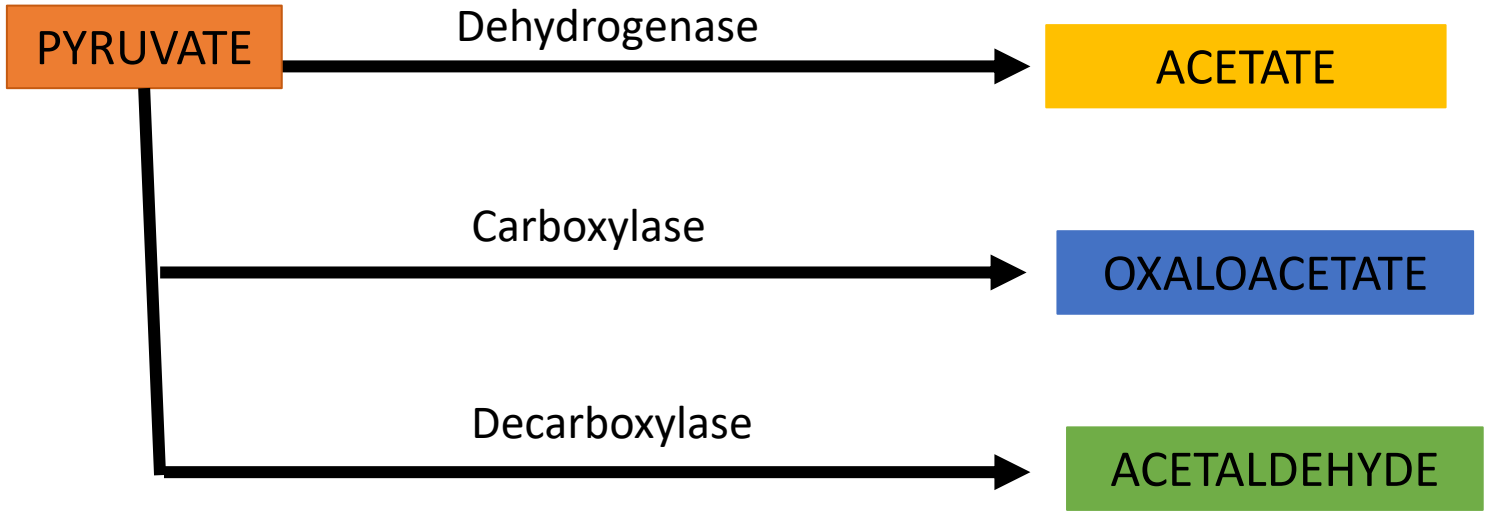
D-lactic acid

Lactate dehydrogenase dimer complex with cofactor NAD and pyruvate (PDB code 4nd4). Source: protopedia.org

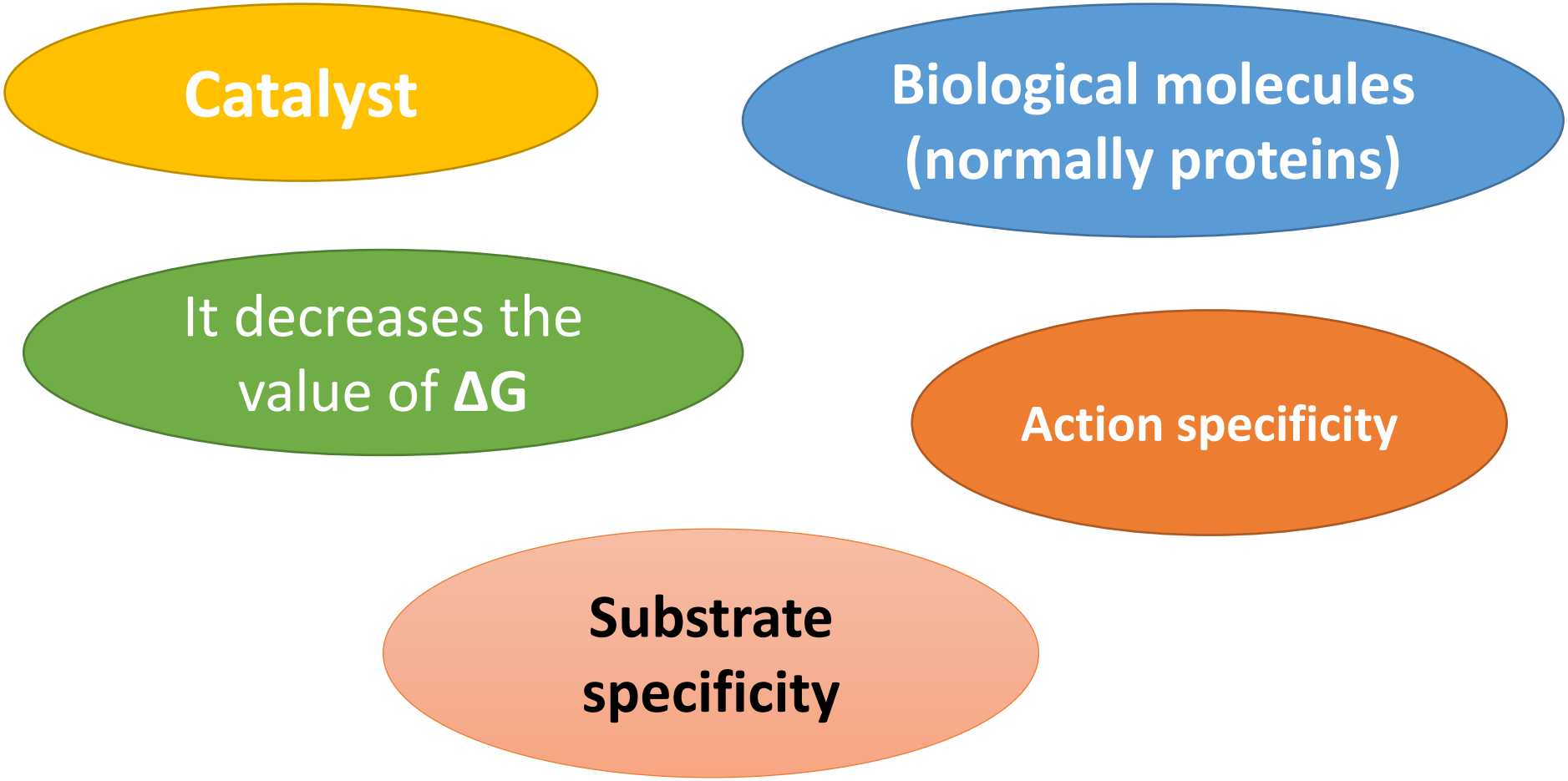
2. Properties of enzymes as catalysts

2.3. Properties of the enzymes: specificity

Action specificity: Enzymes that perform reactions in the same substrate but differ in the kind of transformation they perform in that substrate, e.g., pyruvate dehydrogenase, pyruvate kinase, pyruvate carboxylase and pyruvate decarboxylase.



What is an enzyme?



3. Nomenclature and classification

Traditional nomenclature: -ase is added to the catalyzed substrate, the type of reaction, or the organ that provides the enzyme.

- Organ: *Pancrease*
- Substrate name: *Protease*
- Type of reaction: *Hydrolase*

Current nomenclature: Current nomenclature follows the rules of the **Enzyme Commission** [EC] of the **IUBMB** (*International Union of Biochemistry and Molecular Biology).*

- **An EC assigned code E.C.: 1.1.1.1** meaning class (oxidoreductase); subclass (chemical groups); sub-subclass (transfer details); the fourth number specifically identifies the enzyme
- **Systematic name**, formed by writing substrate:cosubstrate, followed by the reaction and –ase, e.g.: **Ethanol:NAD^+ Oxidoreductase**

3. Nomenclature and classification

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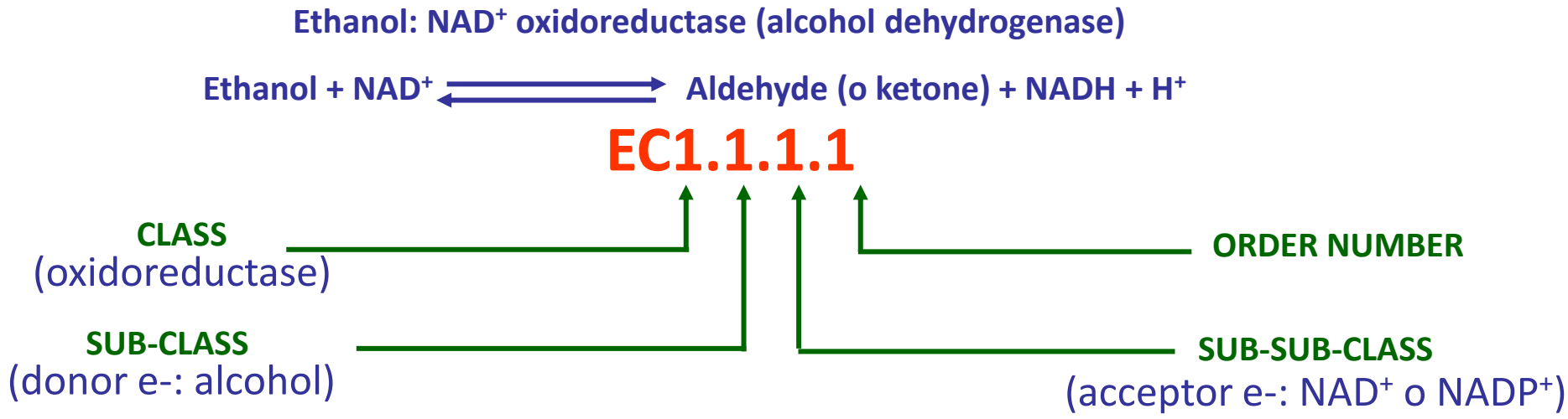


3. Nomenclature and classification

The classification of enzymes follows the format set by the ***International Union of Biochemistry and Molecular Biology [IUBMB]***. Enzymes are classified according to the general type of reaction they catalyse.

Enzyme class

N	Class
1.	OXIDOREDUCTASES
2.	TRANSFERASES
3.	HYDROLASES
4.	LYASES
5.	ISOMERASES
6.	LIGASES

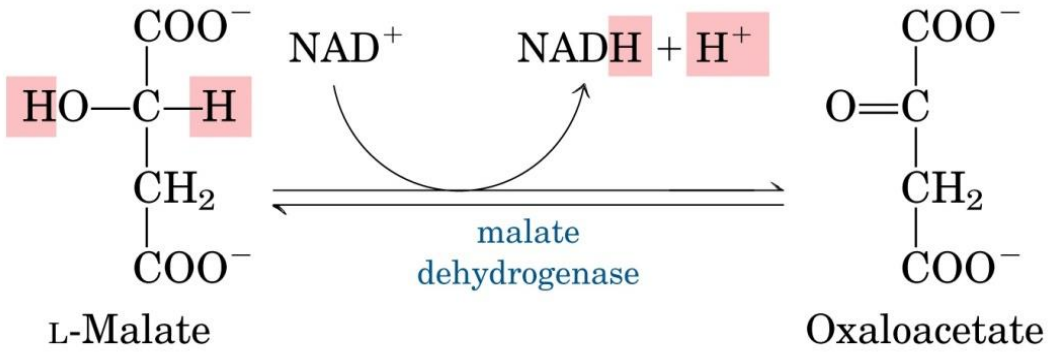
3. Nomenclature and classification

1. OXIDOREDUCTASES: REDOX reactions. An element is reduced, and another is oxidized. **ELECTRON TRANSFERENCE.**

$$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$$

L- MALATE + NAD^+ ⟶ OXALOACETATE + NADH

MALATE DEHYDROGENASE



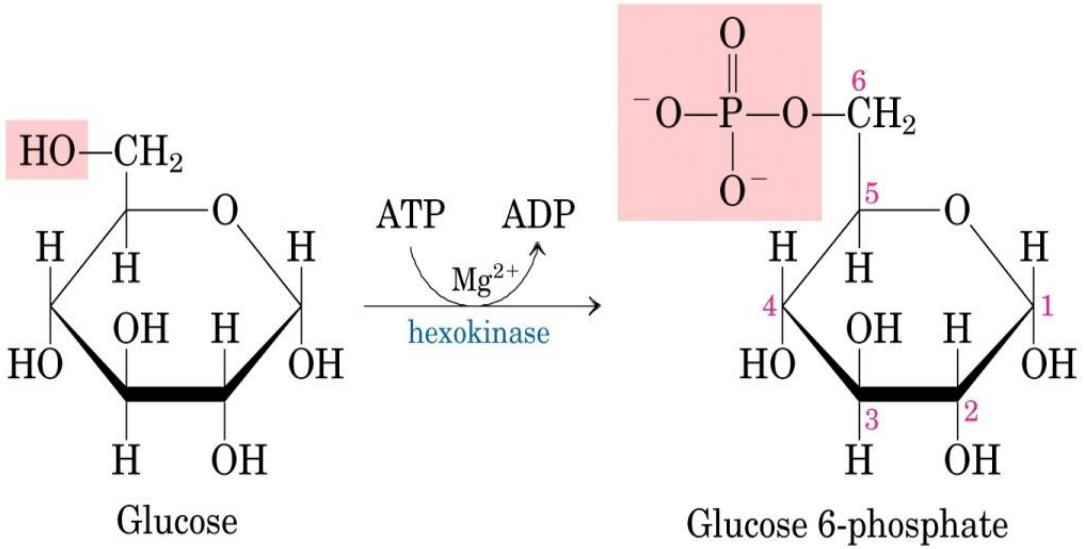
3. Nomenclature and classification

2. TRANSFERASES: Reactions that catalyse the **transfer of chemical groups** usually containing C, N or P.
Transference of a chemical groups: AMINOTRANSFERASES

S-grupo + S' → S'-grupo + S

Glucose + ATP ⟶ Glucose-6-P + ADP

HEXOKINASE

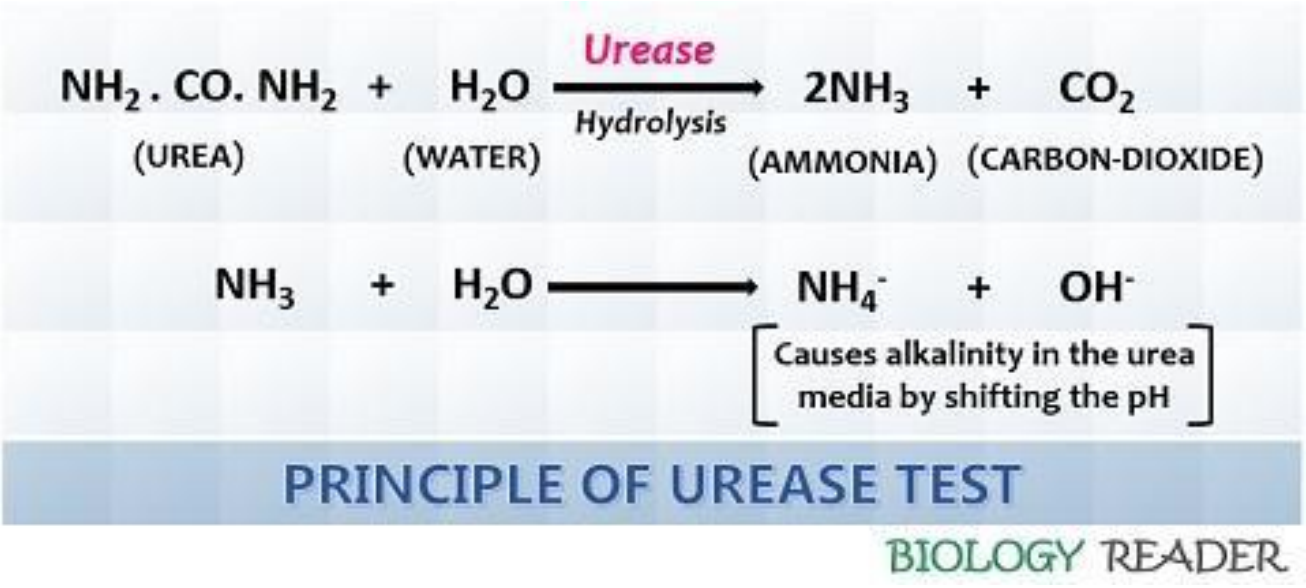


3. Nomenclature and classification

3. HYDROLASES: These break bonds using **water** (i.e., by adding water).

$$A\text{-}B + H_2O \longrightarrow A\text{-}H + B\text{-}OH$$

$$UREA + H_2O \rightarrow CO_2 + 2NH_3 \text{ (Urease)}$$



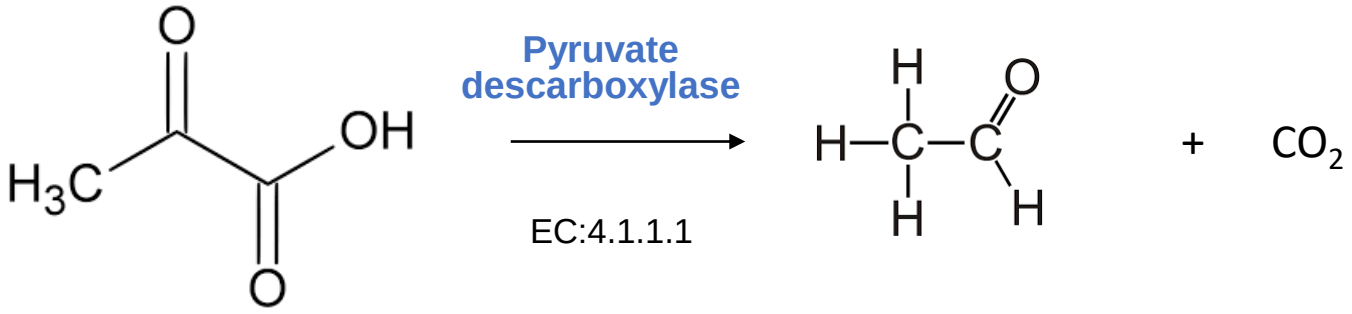
3. Nomenclature and classification

4. LYASES: These break covalent bonds such as C-C C-S and C-N, creating double bonds or rings, or the addition of groups to double bonds.

XC-CY ⇄ X-Y + C=C

D=E + H-I ⇄ H-D-E-I

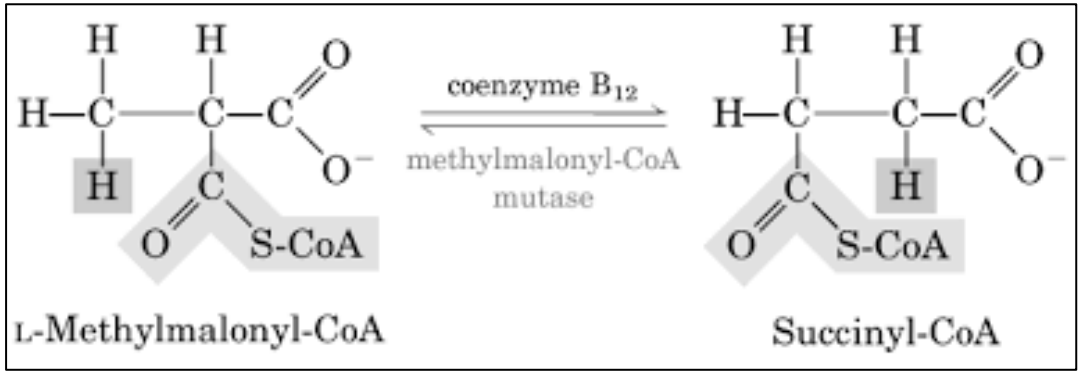
PYRUVATE → acetaldehyde + **CO_2 (pyruvate decarboxylase)**

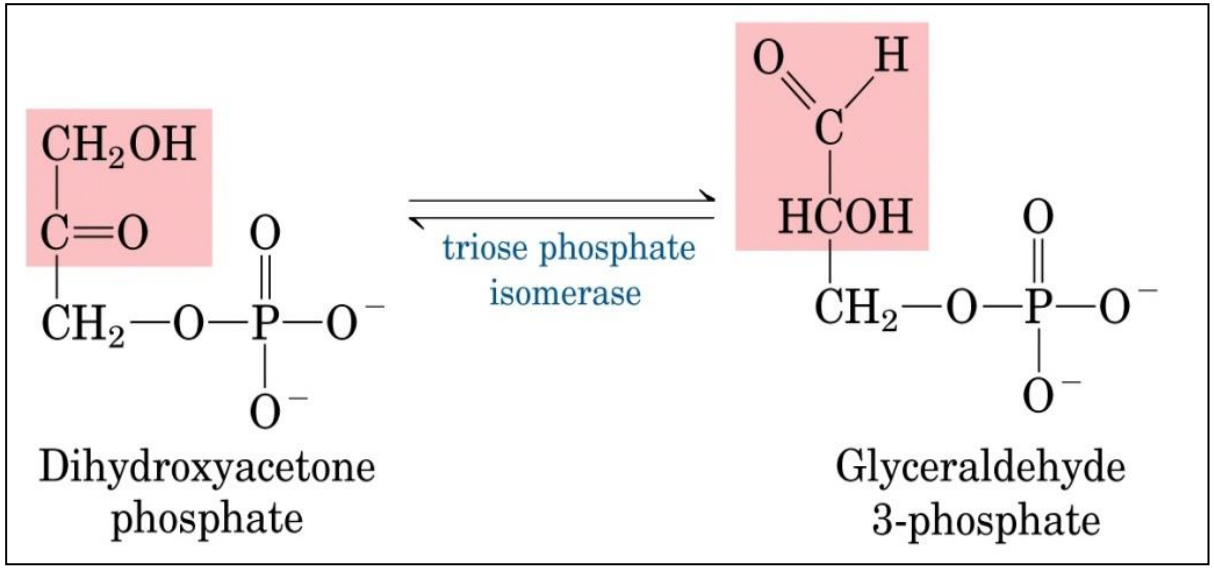


Piruvate **acetaldehyde**

3. Nomenclature and classification

5. ISOMERASES: These reorganize chemical groups inside a molecule.



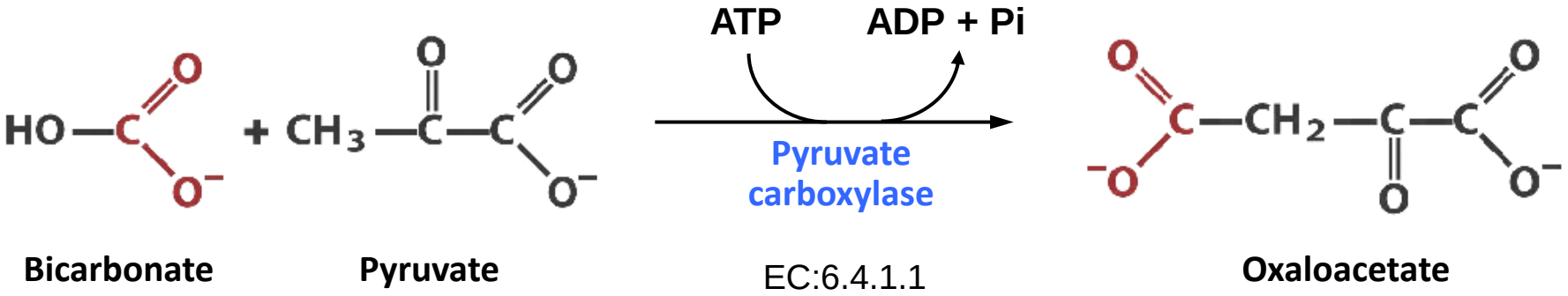


3. Nomenclature and classification

6. LIGASES: These enzymes fuse substrates by making covalent bonds between C and O, S, N. They usually have a high energy demand and need an ATP molecule.

$$A + B + XTP \rightleftharpoons A\text{-}B + XDP + P_i$$

PIRUVATO + CO_2+ATP→ oxalacetato + ADP+Pi



Bicarbonate **Pyruvate** EC:6.4.1.1 **Oxaloacetate**

4. Mechanisms of enzymatic action

4.1. Characteristics of the active site

The active site is a groove or pocket or split formed by the folding of a protein. It is usually hydrophobic in nature and **contains different AAs with lateral residues (chains)** that orient and bind the substrate.

1. Binding amino acids: these residues bind the substrate in a non-covalent way but orient and position it to facilitate the chemical attack or collision. They determine **substrate specificity**.

2. Catalytic amino acids: these directly participate in the reaction and provide the **specificity of the reaction** (type).

The active site environment: chemical groups that support the reaction by interacting with the substrate.

Specificity is determined by the shape and size of the active site

4. Mechanisms of enzymatic action

4.1. Characteristics of the active site

AAS OR BINDING RESIDUES: BINDING SITE

Stabilize
Orient
Recognize

CATALYTIC AAS OR RESIDUES: CATALYTIC SITE

Involved in the chemical reaction

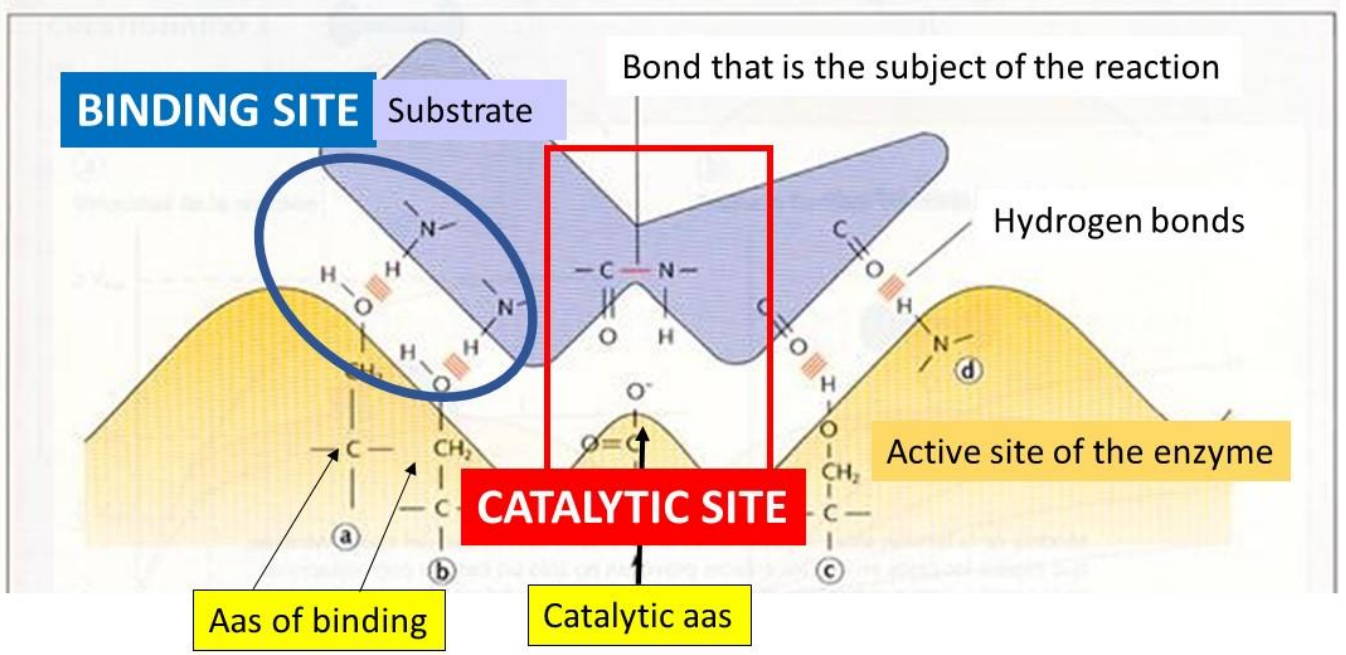


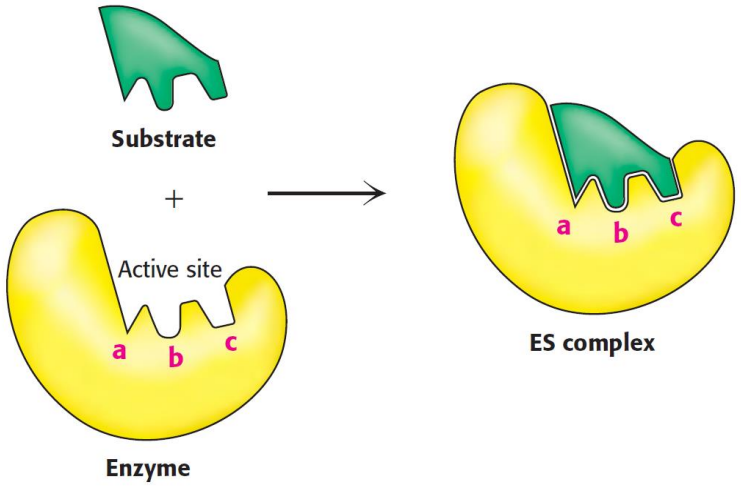
Diagram source: biologiasur.com

4. Mechanisms of enzymatic action

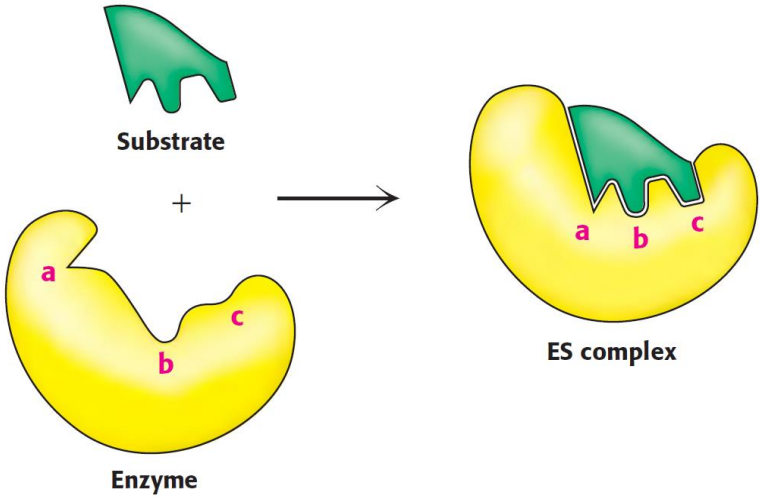
4.2. Interaction models

Enzyme-substrate interaction

1. Fisher Model (1890): (LOCK AND KEY MODEL). The substrate **perfectly fits** into the groove of the active site.



2. KOSHLAND-NEET MODEL (1968): (INDUCED-FIT MODEL). The coupling occurs like a hand in a glove. The binding of the substrate into the active site induces a conformational change.



Biochemistry Stryer 7th edition

4. Mechanisms of enzymatic action

4.3. Types of enzymatic reaction

Different **mechanisms** facilitate the formation of T* in the catalysed chemical reaction:

1. **Proximity and orientation** of the chemical groups: the enzyme-substrate binding induces conformational changes that favour the reaction between the chemical groups that undergo the catalytic process.

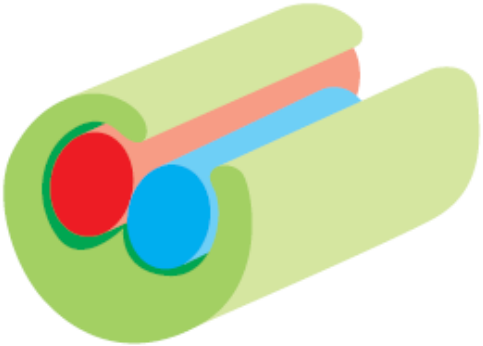
2. **Destabilization of substrate bonds.**

3. **Catalysis**
   3.a. Covalent catalysis: enzymes that bind to the substrate, form an ES complex with covalent interactions, and form an unstable intermediate.
   3.b. Acid-base catalysis: AAs in the catalytic site are H+ donors or acceptors (carboxyl, amine) for certain types of reactions.
   3.c. Electrostatic catalysis: electrostatic unions between the enzyme and the substrate that will generate an ES complex.

4. Mechanisms of enzymatic action

4.3. Types of enzymatic reaction

1. PROXIMITY AND ORIENTATION OF CHEMICAL GROUPS

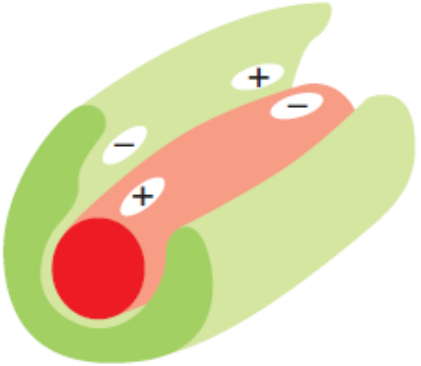


The enzyme facilitates the interaction by binding two substrates.

2. DESTABILIZATION

The enzyme interaction forces the substrate to acquire an unstable structure, T*, which favours the reaction.

3. CATALYSIS
3.3. ELECTROSTATIC CATALYSIS



The interaction of the enzyme, with charges + and – favours the reorganization of the charges in the substrate, facilitating the reaction.

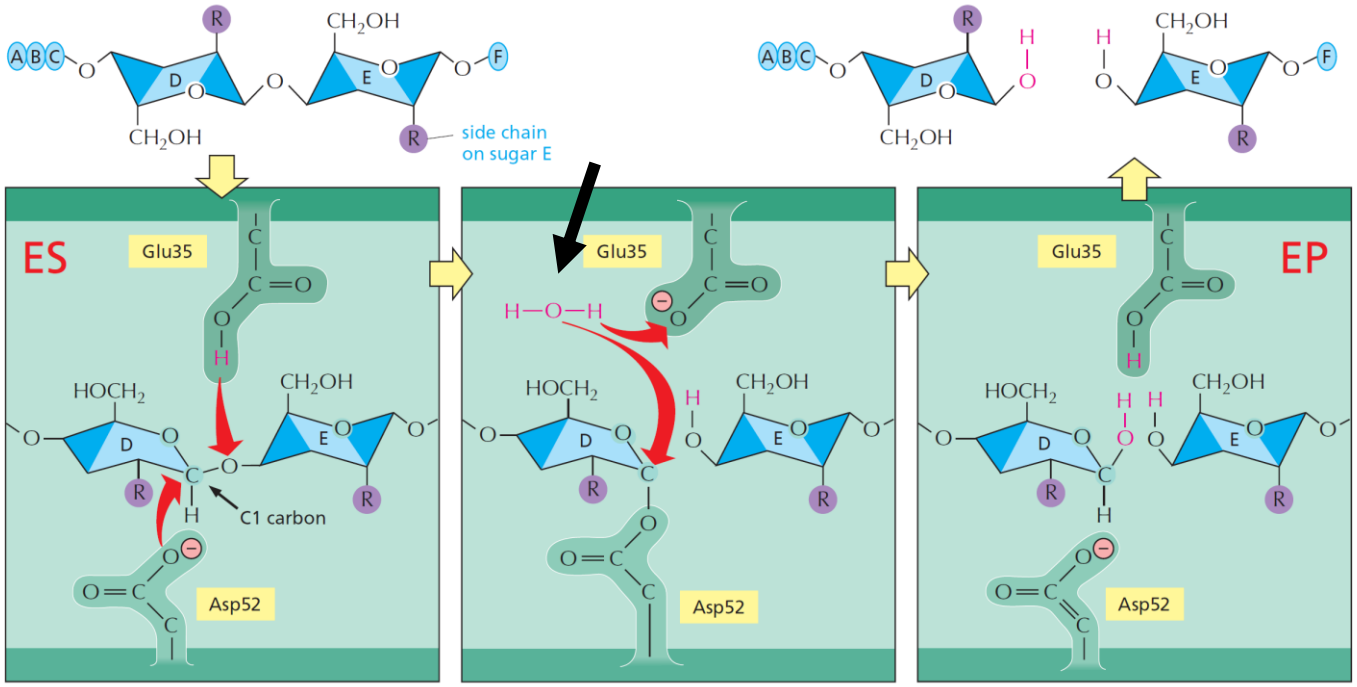
Molecular Biology of the Cell, 6th edition, Bruce Alberts

4. Mechanisms of enzymatic action

4.3. Types of enzymatic reaction

Two bound molecules **Two independent molecules**

ACTIVE SITE



Asp52: the covalent bond with C1 of one of the sugars. Glu35 gives up its H+ to the O of the other sugar.

The H2O molecule (hydrolase) enters and the Glu35 with its charge (-) polarizes the water which through O attacks C1.

The product has been formed and the active site of the enzyme has been regenerated

5. Enzymatic cofactors: characteristics, properties and types

1. Enzymatic cofactors are **not protein molecules; they have a low molecular weight and are thermostable.**
2. They are found at **low concentrations** in cells.
3. They can be shared by **several enzymes.**
4. They cooperate with enzymes, **can be altered** during catalysis, and are not recovered after the reaction. These types of cofactors (modified) are called **co-substrates (NADH/NAD+).**
5. Some of them have the chemical structure of a heterocyclic/cyclic compound with **highly reactive electrons.**
6. They are **highly reactive.**
7. Most of them are **vitamin-derived.**

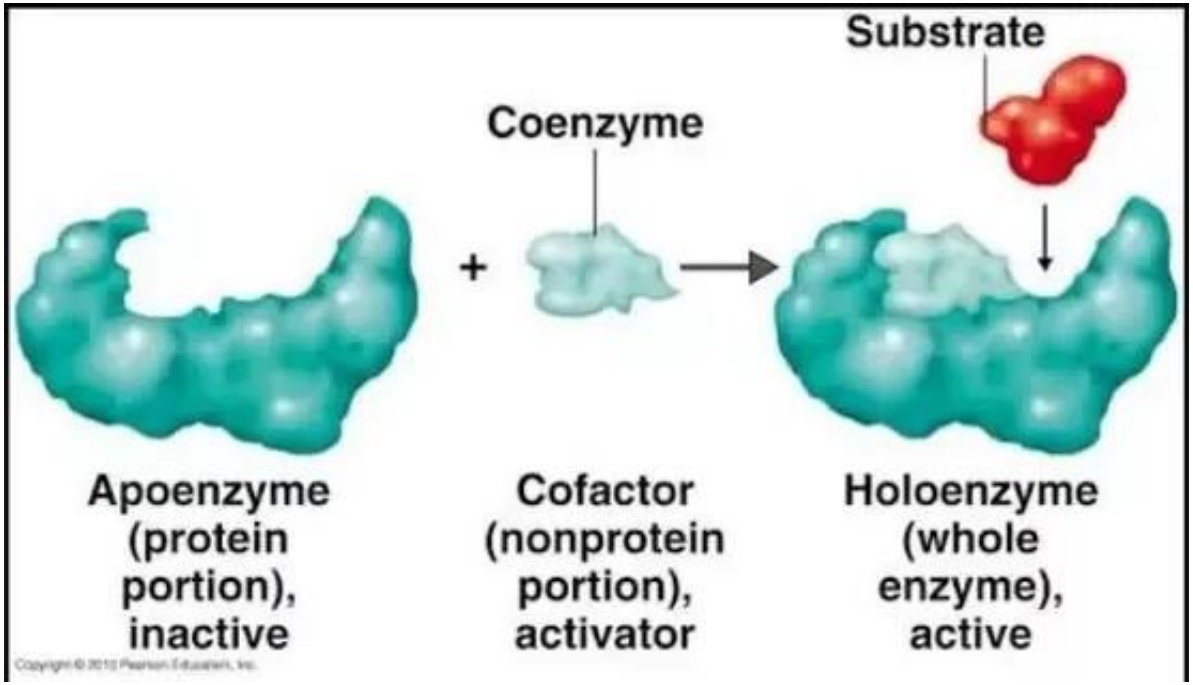
Enzymes that require a cofactor and are otherwise inactive are called
APOENZYMES

HOLOENZYME = APOENZYME + COFACTOR

5. Enzymatic cofactors: characteristics, properties and types

Enzymes that require a cofactor and are otherwise inactive are called APOENZYMES

HOLOENZYME = APOENZYME + COFACTOR



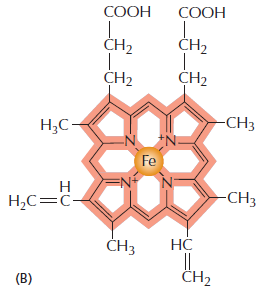
Source: quora.com

5. Enzymatic cofactors: characteristics, properties and types

1. Metal ions: (Fe2+, Cu2+, Mn2+, Mg2+)

2. Coenzymes, which have a very complex structure

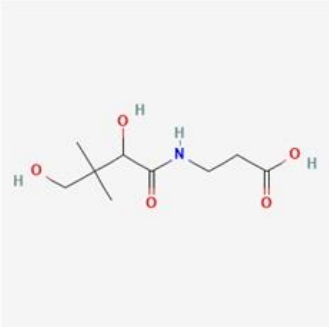
Haem/heme: prosthetic group



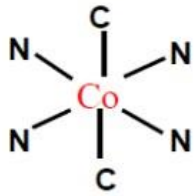
3. The group of coenzymes that are **covalently bound** to the enzyme are called a **PROSTHETIC group.**

Examples of coenzymes:

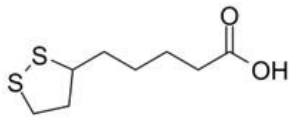
Pantothenic acid



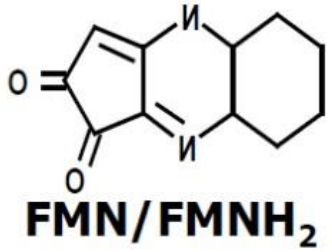
Cobalamin



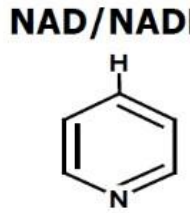
Lipoic acid



Flavin nucleotides



Nicotinamide nucleotide



5. Enzymatic cofactors: characteristics, properties and types

5.1. Classification of coenzymes

1. **Nutritional origin:** vitamin or non-vitamins

2. **Functional criteria:** transport of electrons.

3. **Enzymological criteria:** depending on **the type of reaction** in which they participate

5. Enzymatic cofactors: characteristics, properties and types

5.1. Classification of coenzymes

Vitaminic origin

Transfer of chemical groups **Acyl acid: Coenzyme A-pantothenic** Carboxyl: Biotin - **Vit H** Aldehyde: **Thiamine pyrophosphate- Vit B1** Alkyl: Cobalamin Amine: pyridoxal phosphate **Vit B6** Monocarbonates: Tetrahydrofolate acid	**Electron transfer** Nicotinamide nucleotides: NAD+/NADH; NADP+/NADPH **Vit B3** Flavin nucleotides: $FMN/FMNH_2$; $FAD+/FADH_2$ **Vit B2** Sugar-derived **Ascorbate Vit C**

5. Enzymatic cofactors: characteristics, properties and types

5.1. Classification of coenzymes

Non-vitaminic groups

Transfer of chemical groups	**Transfers of electrons**
Phosphoryl: nucleotide-derived XTP	**Coenzyme Q** (Ubiquinone)
Adenylate: Adenylate ATP	Tetrahydrobiopterin
Aminoalcohols and diacylglycerols bound to CDP –	Methoxantin Factor 420
Sugar Phosphate: XDP (ADP, CDP, **UDP**, GDP, dTDP)	
Methyl groups: S-Adenosyl-Methionine	
Acyl groups: Dihydrolipoic	

5. Enzymatic cofactors: characteristics, properties and types

5.2. Most important coenzymes

VITAMIN	COENZYME	REACTIONS	METABOLISM
Pantothenic acid	Coenzyme A	Acyl activation Acyl transfer	β-oxidation Fatty acid synthesis Amino acid decarboxylation
Biotin (vit H)	Biocytin	CO binding$_2$ /CO_3 H^- Carboxylation	Carboxylases: early stages of gluconeogenesis and fatty acid synthesis
--	Lipoic acid	Simultaneous acyl and electron transfer	Decarboxylation of a-ketoacids
Thiamine (vit B1)	Thiamine Pyrophosphate	C-C bond breaking	Decarboxylation of a-ketoacids
Pyridoxal (vit B6)	Pyridoxal Phosphate	Transaminations, α-decarboxylations.	Transaminases Glycogen phosphorylase
Cobalamin (vit B12)	5'-deoxyadenosyl Cobalamin	Racemisations, intermolecular rearrangements	Mutasas
Folic acid	Tetramethyl hydrofolate	Transfer of C- groups	Purine biosynthesis Inhibited by methotrexate
Niacin (vit B3)	NAD+/NADH NADP+/NADPH	Electron transfer	1 electron transfer
Riboflavin (vit B2)	FMN/FMNH2 FAD/FADH2	Electron transfer	2-electron transfer

5. Enzymatic cofactors: characteristics, properties and types

5.3. Metal cofactors

1. Have a greater concentration of **positive charge**. Their ions are bound to enzymes.

2. They have **directed valences, which** allow interaction with several ligands.

3. They can exist in **more than one oxidation state** (Fe^{+2} Fe^{+3}).

4. The most important cofactors are **transition metals,** e.g. Mn, Fe, Cu, Co, Mo.

Metallic ion	Enzyme
Cu^{+2}	Cytochrome Oxidase
Fe^{+2} Fe^{+3}	Catalase, Peroxidase
K^{+}	Pyruvate kinase
Mg^{+2}	Hexokinase, Glucose-6-phosphatase
Mn^{+2}	Arginase, Ribonucleotide Reductase
Zn^{+2}	Alcohol Dehydrogenase Carboxypeptidases A y B