ENZYMES - I

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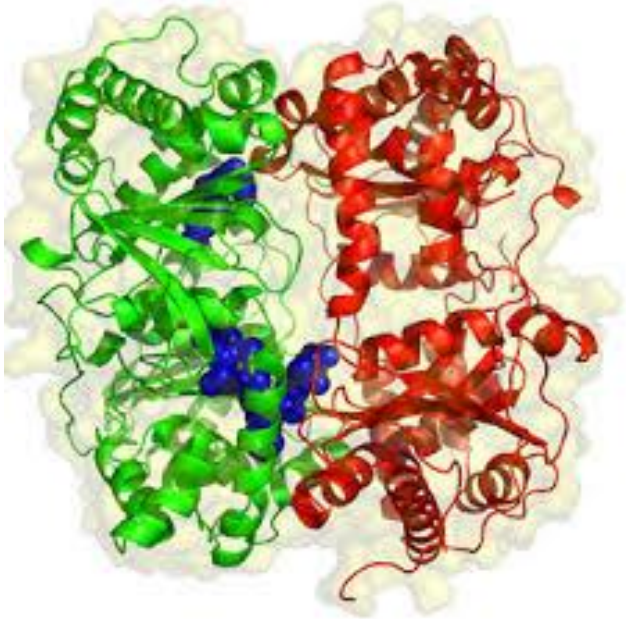
Enzymes are biologic polymers that catalyze the chemical reactions

In general with the exception of ***ribozymes*** *which are few RNA molecules with* enzymatic activity, ***all the enzymes are protein in nature.***

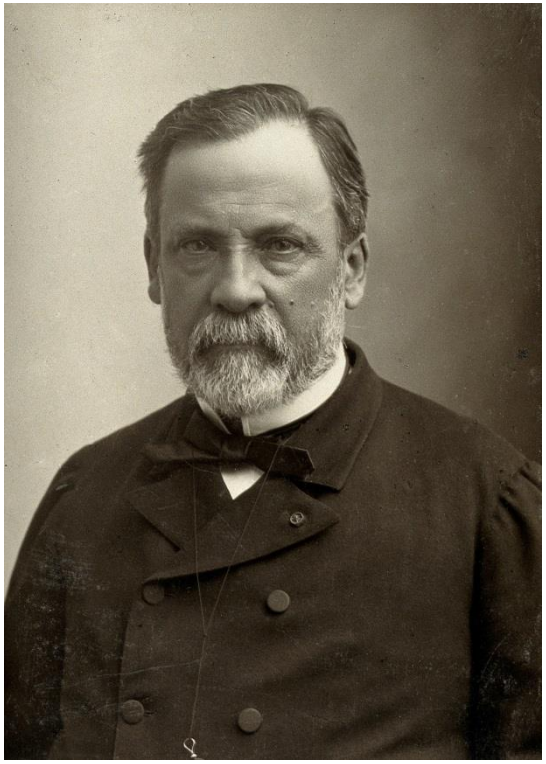


The history of **enzyme** research

Biological catalysis was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach.

Research continued in the 1800s with examinations of the conversion of starch to sugar by saliva and various plant extracts.

The history of **enzyme** research



In the 1850s, *L. Pasteur* concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments".

He postulated that the ferments were inseparable from the living yeast cells.

The history of **enzyme** research

In 1897 *E. 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. He named the enzyme that brought about the fermentation of sucrose "**zymase**". Following Buchner's example, enzymes are usually named according to the reaction they carry out: the suffix -**ase** is combined with a) the name of the substrate (e.g., **lactase** is the enzyme that cleaves **lactose**) or b) to the type of reaction (e.g., **DNA polymerase** forms **DNA polymers**)

In **1907**, he received **the Nobel Prize** in Chemistry for "his discovery of cell-free fermentation"

W. Kuhne later gave the name "**enzymes**" to the molecules detected by Buchner.

The history of **enzyme** research

In 1926 ***J. Sumner*** isolated ***urease*** and found that ***urease*** crystals consisted of protein, and he postulated that **all enzymes are proteins**.

In the 1930s ***J. Northrop*** and ***M. Kunitz*** crystallized ***pepsin***, ***trypsin***, and other digestive enzymes and found them also to be **proteins**.

1946 **Nobel Prize** in Chemistry

The history of **enzyme** research

During this period, *J. Haldane* made the suggestion that weak bonding interactions between an enzyme and its substrate might be used to catalyze a reaction.

Since the latter part of the 20th century thousands of enzymes have been purified, their structures elucidated, and their mechanisms explained.

Properties of enzymes

Properties of enzymes

General properties of enzymes :

1. **Not consumed in the reaction.**
2. **Exert their effects at low concentrations.**
3. **Do not affect the value of the equilibrium constant of the reaction.**
4. **Their action is subject to the law of mass action (**is the proposition that the rate of a chemical reaction is directly proportional to the product of the concentrations of the reactants. The **ratio** between the concentration of reactants and products is **constant**.**).**

Properties of enzymes

Differences from inorganic catalysts:

1. **Thermolability** of enzymes.
2. The dependence of enzyme activity on **pH**.
3. **Specificity** of action of enzymes.
4. Rate of enzymatic reactions is subject to certain **kinetic regularities**.

Properties of enzymes

Differences from inorganic catalysts:

5. **Enzyme activity depends on the action of regulators - activators and inhibitors.**
6. **Several enzymes in the formation of tertiary and quaternary structures are subjected to postsynthetic modification.**
7. **Size of enzyme molecules is usually much larger than their substrates.**

Structure

Enzyme-catalyzed reaction takes place within the confines of a pocket on the enzyme called the **active site**. The molecule that is bound in the **active site** and acted upon by the enzyme is called the **substrate**.

PROTEIN STRUCTURE

Scaffold to support and position active site

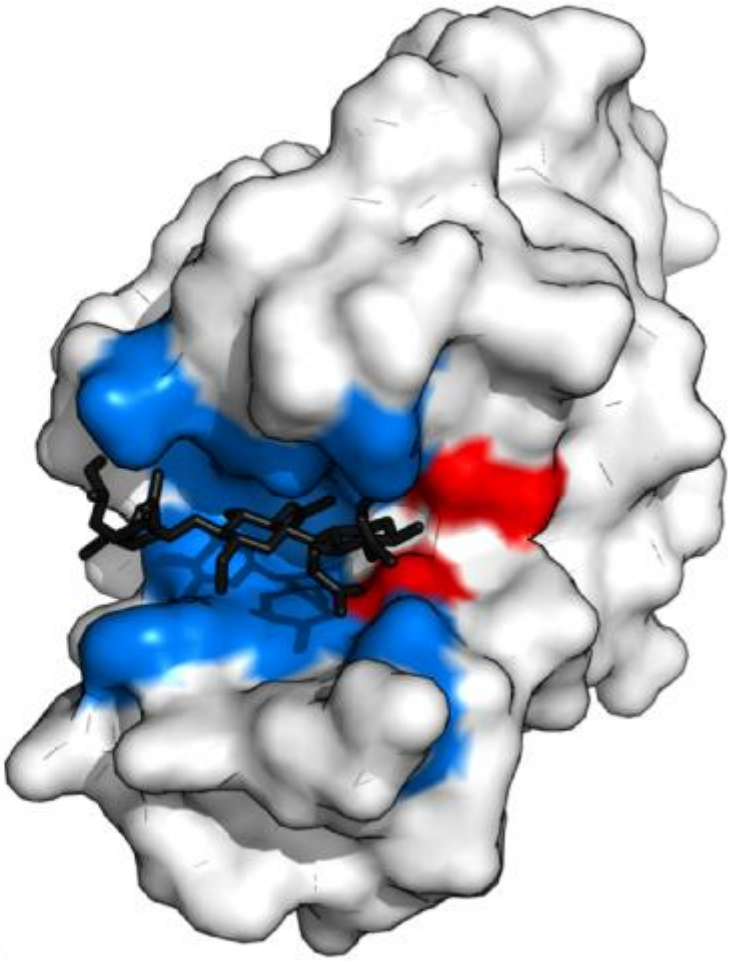
ACTIVE SITE

BINDING SITES

Bind and orient substrate(s)

CATALYTIC SITE

Reduce chemical activation energy



The enzyme active site (center)

The enzyme active center

Part of the enzyme molecule which specifically interacts with the substrate

is called an active center.

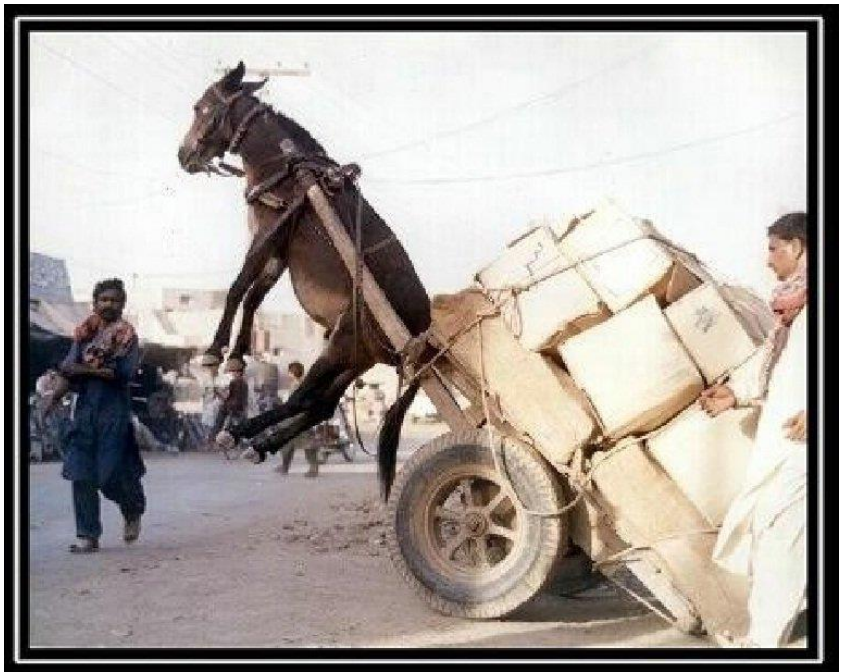
Active center is a unique combination of amino acid residues in the enzyme molecule, providing direct interaction with its substrate molecule and participating directly

in the act of catalysis.

In complex enzymes cofactor is a part of the active center.

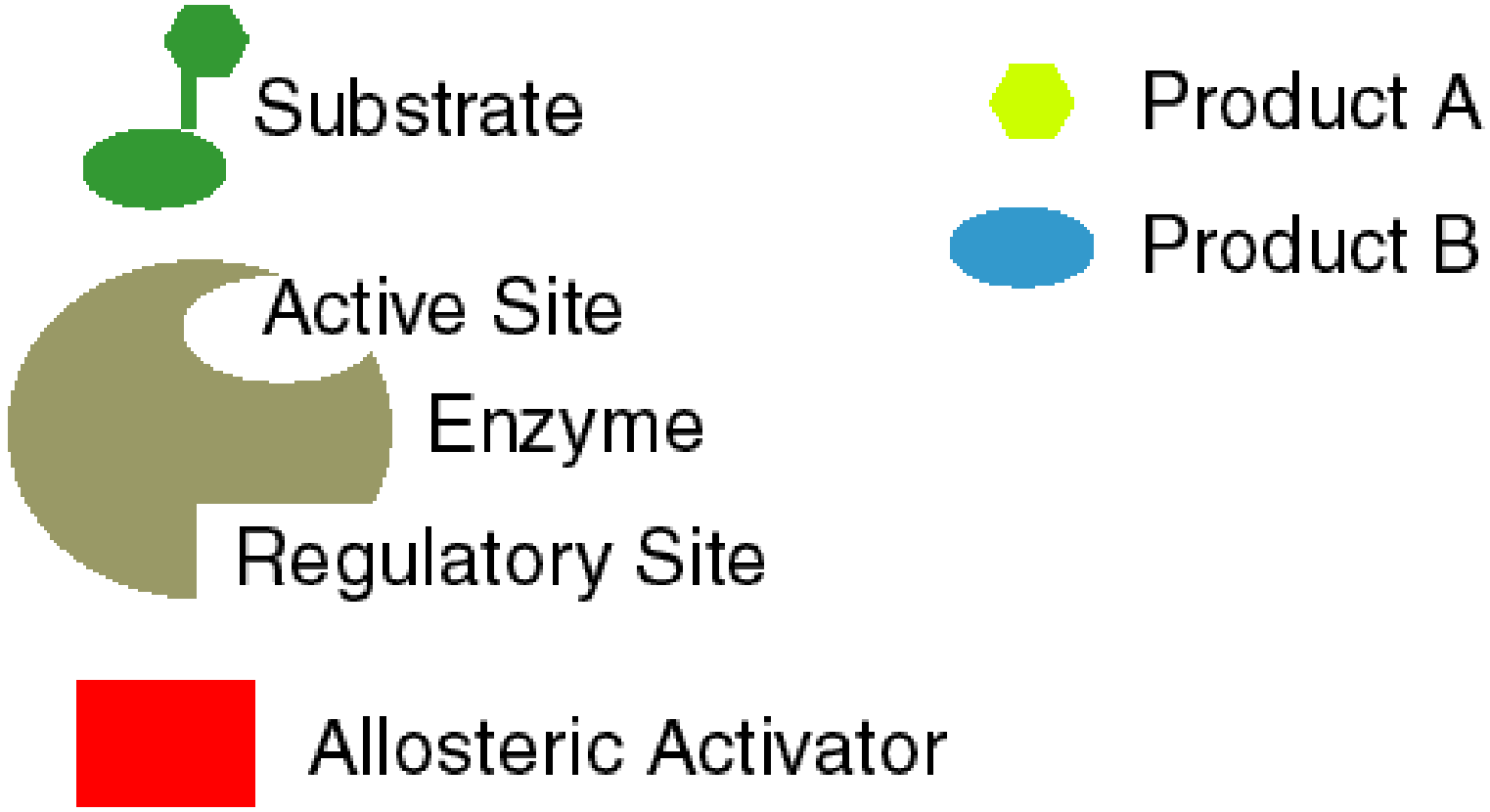
Allosteric center

or **regulatory site** - is the part of the enzyme molecule (**apart from active center**), which normally binds certain small molecules (allosteric regulators), which are not similar in structure to the substrate.



The allosteric site influences (***enhances*** or ***impairs***) the activity of the enzyme.

Allosteric center



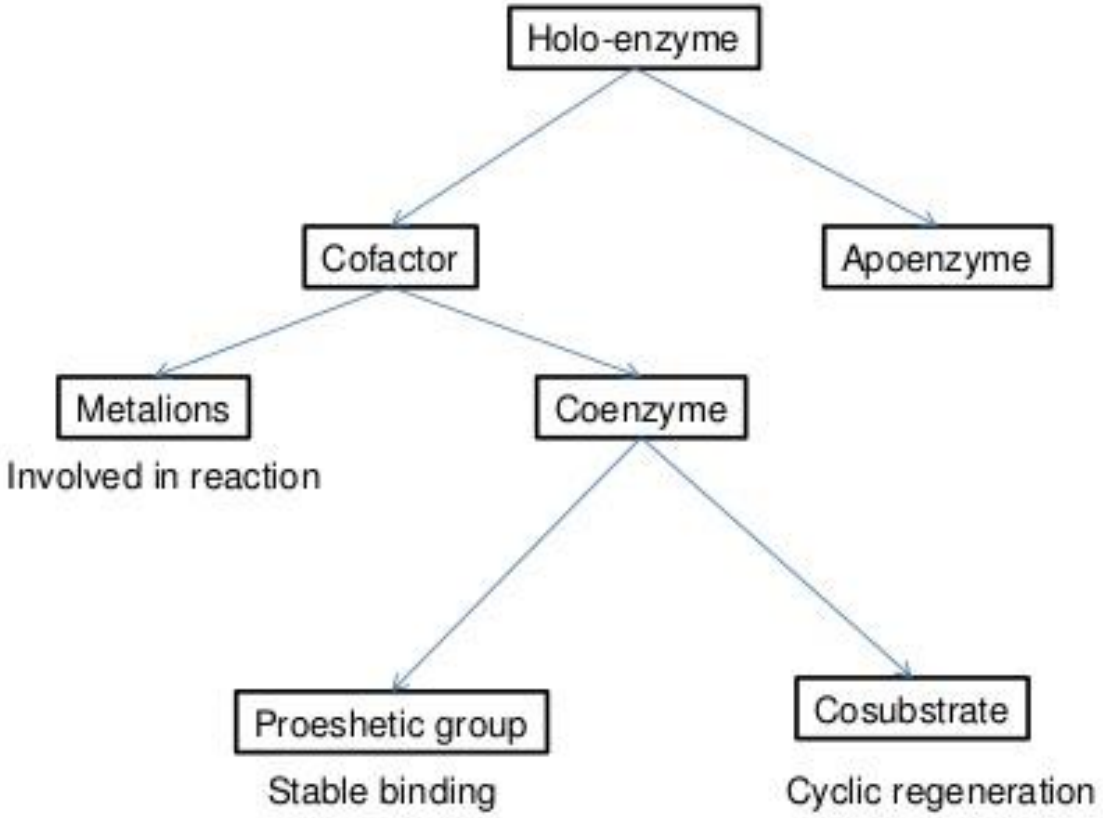
Simple and Conjugated Enzymes

Many enzymes contain small nonprotein molecules and metal ions that participate directly in substrate binding or in catalysis.

The protein part of conjugated enzymes is called apoenzyme.

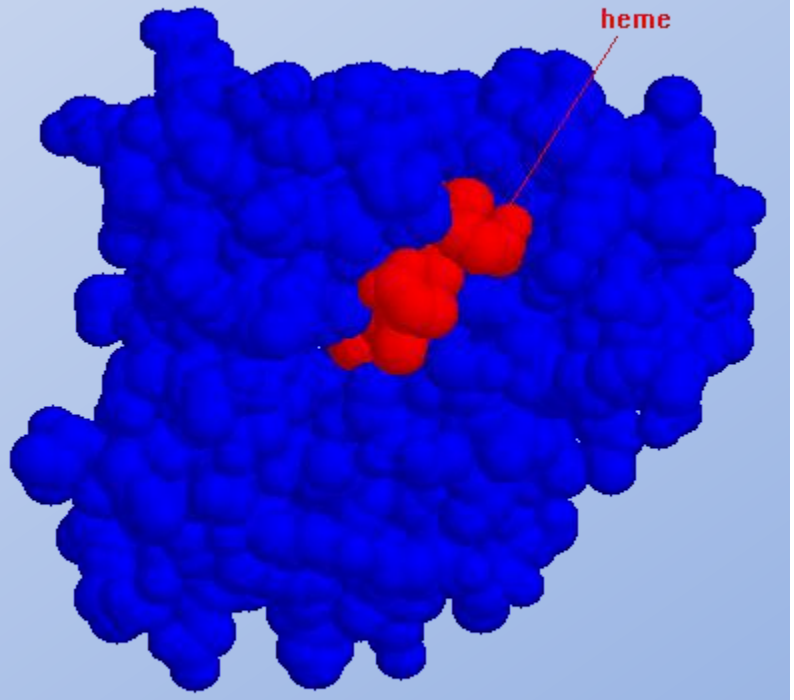
The whole molecule of conjugated enzymes is called holoenzyme.

Systematics of cofactor-containing enzymes



Prosthetic groups

- nonprotein part of enzymes that tightly and stably incorporated into a protein's structure by covalent or noncovalent forces.



Cofactors

is a non-protein chemical compound or metallic ion that is required for a enzyme's biological activity to happen.

Cofactors bind in a transient, dissociable manner either to the enzyme or to a substrate.

Cofactors must be present in the medium surrounding the enzyme for catalysis to occur.

Coenzymes

- serve as recyclable shuttles that transport substrates from one point within the cell to another.

The function of these shuttles is two fold:

- they **stabilize species** that are too reactive to persist for any significant time period (**NADH + H^+**).
- they serve as an **adaptor** that facilitates the recognition and binding of small chemical groups by their target enzymes - **Ac-CoA**, **N^5-methylTHF**, **N^{10}-formyl tetrahydrofolate**, etc.

Some **enzymes** or **enzyme complexes** require several **cofactors**.

For example, the multienzyme complex - **pyruvate dehydrogenase** at the junction of **glycolysis** and the **citric acid cycle** requires 5 organic **cofactors** and one **metal ion**:

- ✓ loosely bound thiamine pyrophosphate (**TPP**),
- ✓ covalently bound **lipoamide** and **flavin adenine dinucleotide** (**FAD**),
- ✓ **nicotinamide adenine dinucleotide** (**NAD^+**)
- ✓ a metal ion (**Mg^{2+}**).

and the **cosubstrates**

- ✓ coenzyme A (**CoA**).

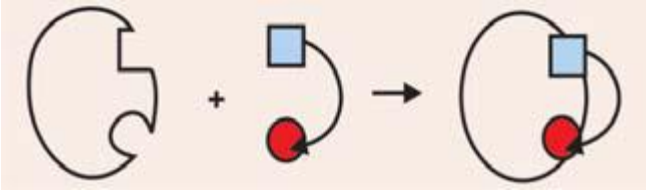
Many Coenzymes, Cofactors & Prosthetic Groups Are Derivatives of Vitamins

Vitamin	*Coenzyme*	*Enzyme*
B_1-thiamine	**thiamindiphosphate**	**transketolase, pyruvate dehydrogenase**
B_2-riboflavin	**FMN, FAD**	**flavin-dependent dehydrogenase**
B_5-pantothenic acid	**coenzyme A (CoA)**	**reaction of acylation**
B_6-pyridoxine	**pyridoxal-phosphate**	**aminotransferase**
B_3 (PP) - nicotinamide	**NAD,NADP**	**NAD (NADP)-dependent dehydrogenase**
B_9-folic acid	**tetrahydrofolic acid**	**transferring one-carbon groups**

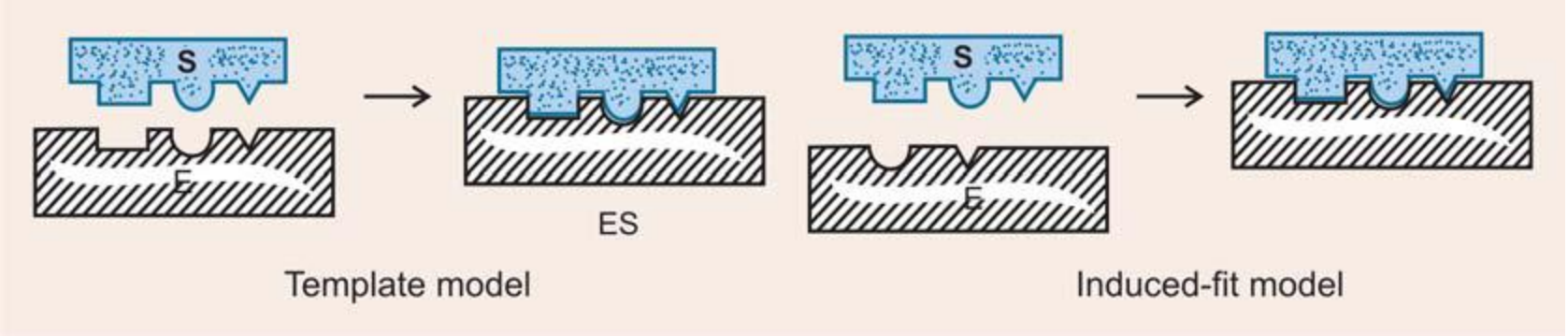
The mechanism of enzymes action

Any enzymatic reaction includes the following stages:

Emil Fischer proposed that enzymes and their substrates interact to form an enzyme–substrate (ES) complex analogous to the manner in which a mechanical lock distinguishes the proper key.

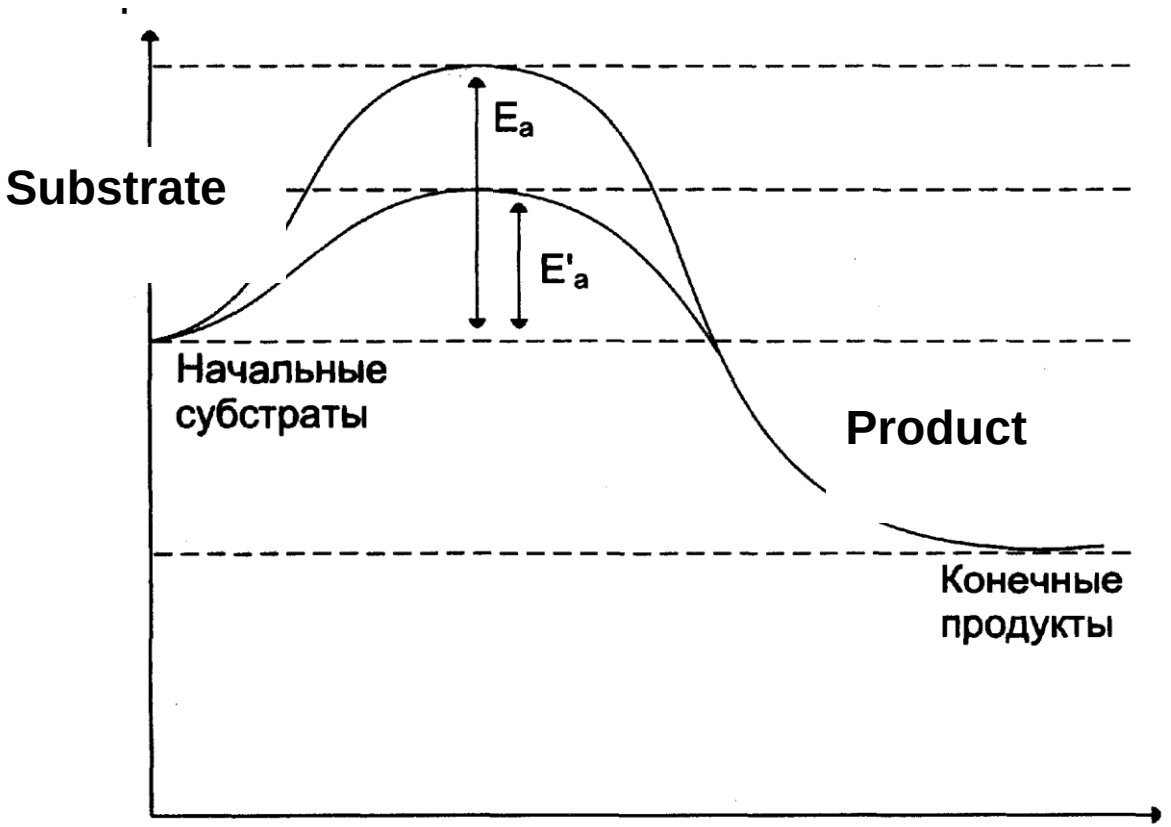


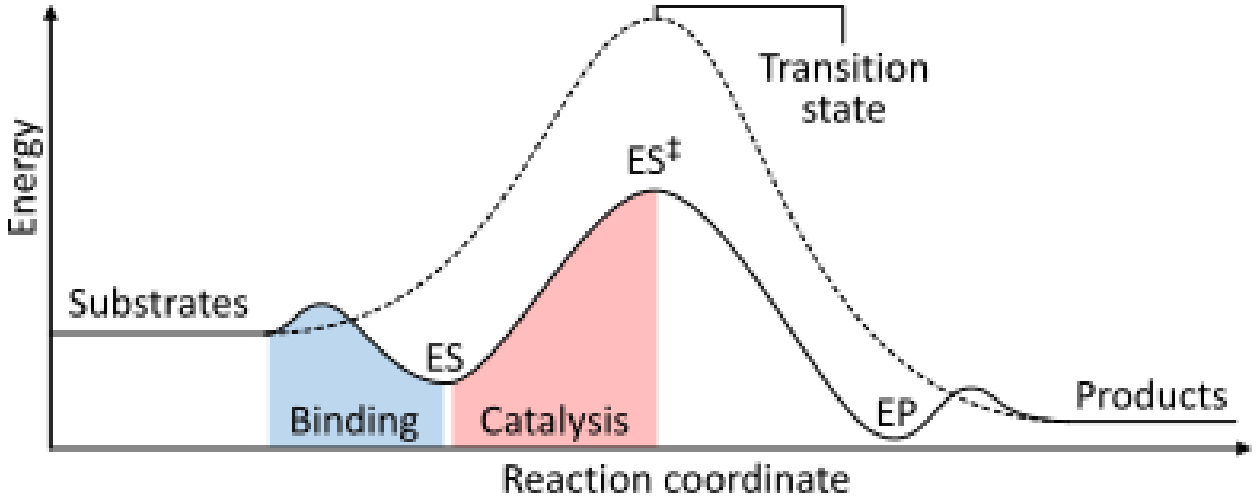
Daniel Koshland proposed the induced fit model, which states that when substrates bind to an enzyme they induce a conformational change analogous to placing a hand (substrate) into a glove (enzyme).



Before a chemical reaction can occur, the reacting molecules are required to gain a minimum amount of energy, this is called the **energy of activation (E_a).**

Enzymes accelerate reactions by *reducing the* energy of activation (E` a).





The energies of the stages of a chemical reaction.

Uncatalysed (dashed line), substrates need a lot of activation energy to reach a transition state, which then decays into lower-energy products. When **enzyme catalysed** (solid line), the enzyme binds the substrates (ES), then stabilizes the transition state (ES‡) to reduce the activation energy required to produce products (EP) which are finally released.

Specificity of enzymes

Specificity with respect to the substrate is enzyme's substrate preference for a particular structure in comparison with other substrates.

Specificity of enzymes

There are 4 types of substrate specificity of enzymes:

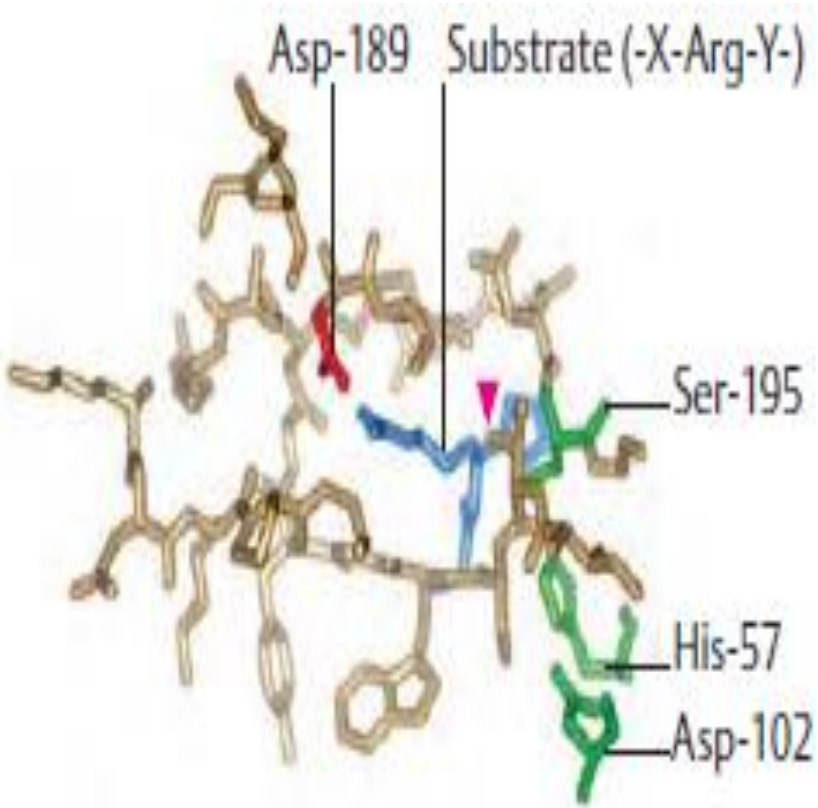
1. **Absolute specificity is the ability of the enzyme to catalyze the conversion of only one substrate.**
2. **Relative specificity is the ability of the enzyme to catalyze the conversion of several substrates having the same type of bond.**

Specificity of enzymes

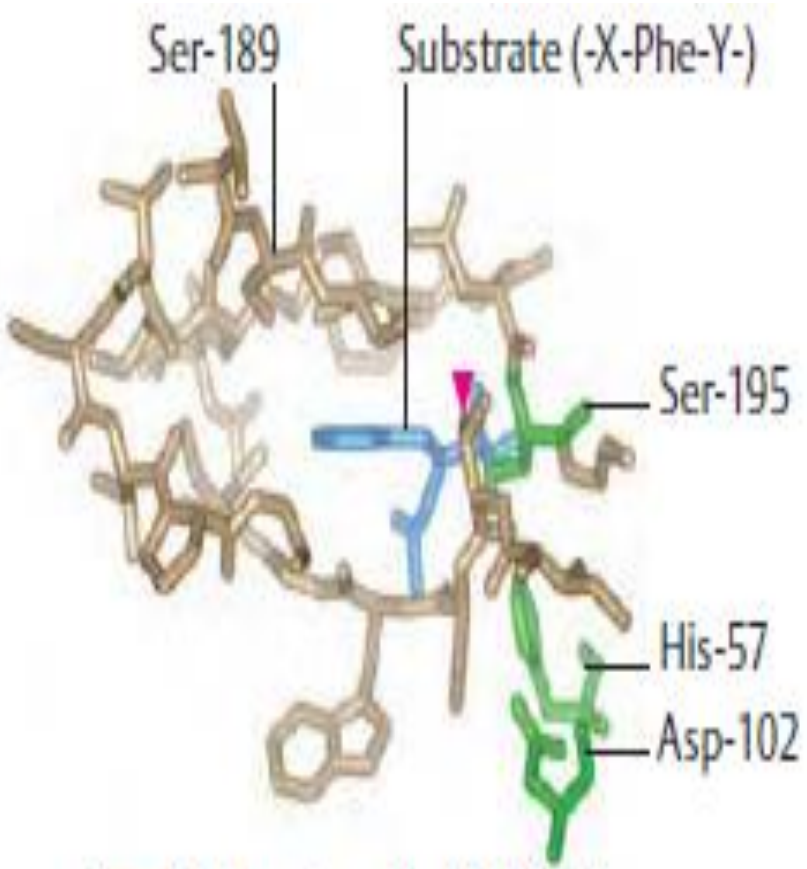
3. **Relative group specificity.** **The enzyme catalyzes the conversion of several substrates having one type of bond, but requires the presence of certain functional groups.**

4. Stereochemical specificity (stereospecificity) is the property of the enzyme to convert only one stereoisomer.

Specificity of enzymes



1 Trypsin (3.4.21.4)
-X-Y-Arg (Lys)-Z-



2 Chymotrypsin (3.4.21.1)
-X-Y-Tyr (Trp, Phe, Leu)-Z-

Classification of enzymes

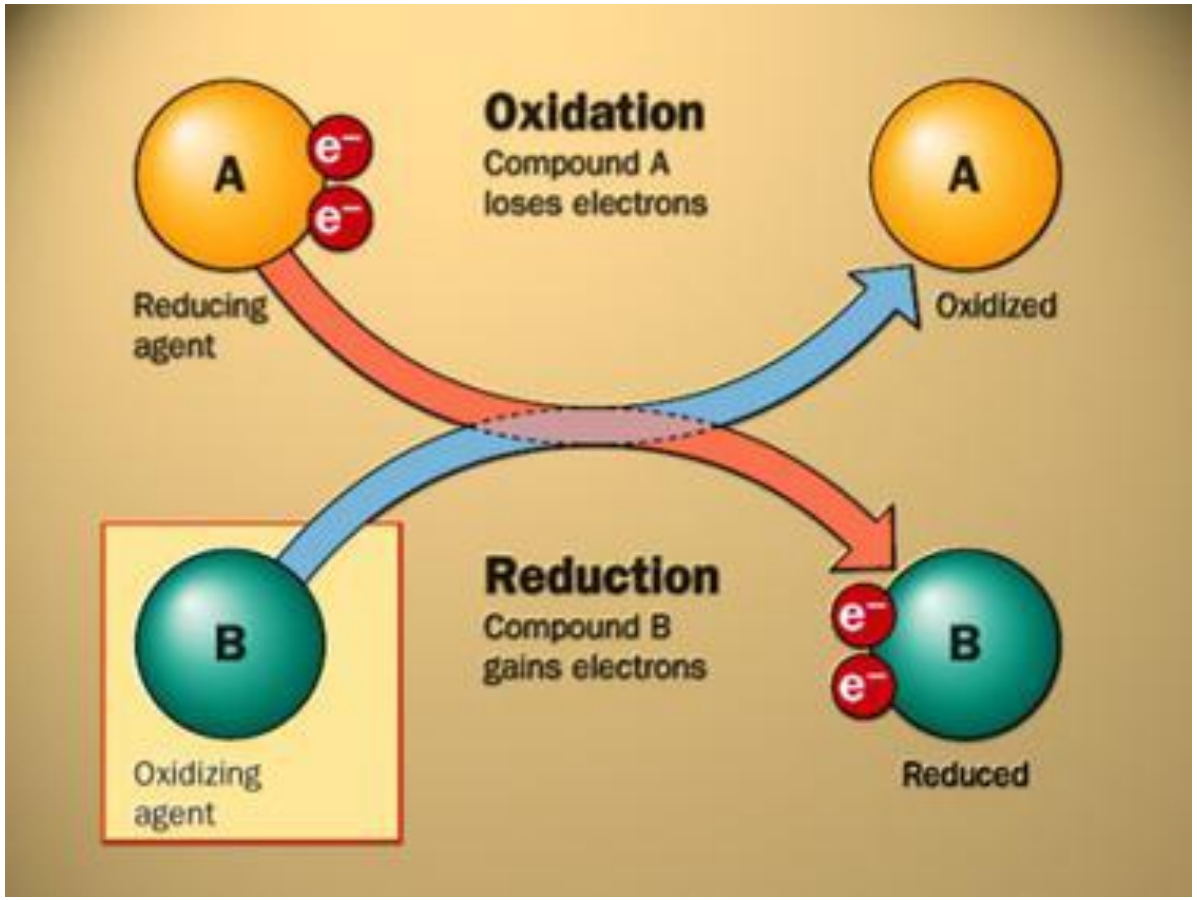
Specificity with respect to the reaction

Each **enzyme** catalyzes the reaction of one or a group of the same type of reactions. **Specificity for the type of reaction** is the basis of a uniform classification of enzymes.

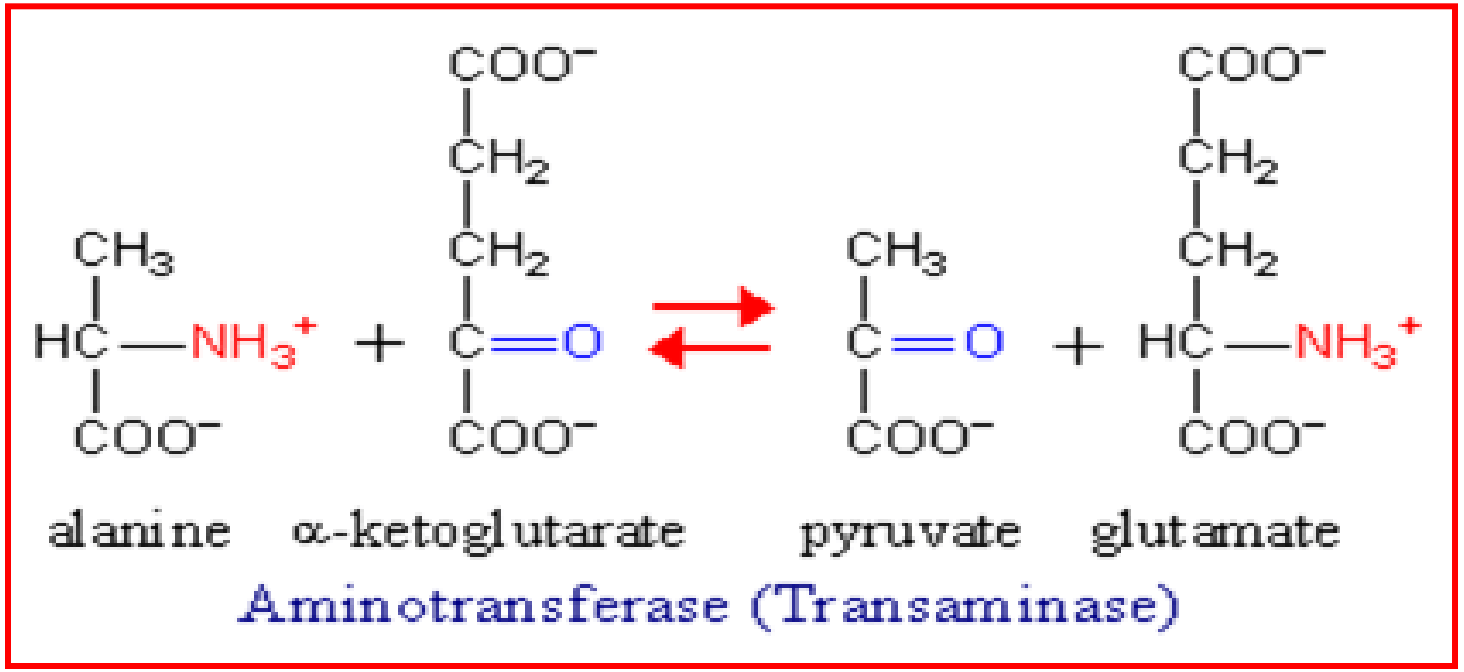
The **first** (fundamental) classification of enzymes appeared at the **Enzyme commission meeting** in **Moscow** in 1961.

Enzymes are grouped into the following six classes.

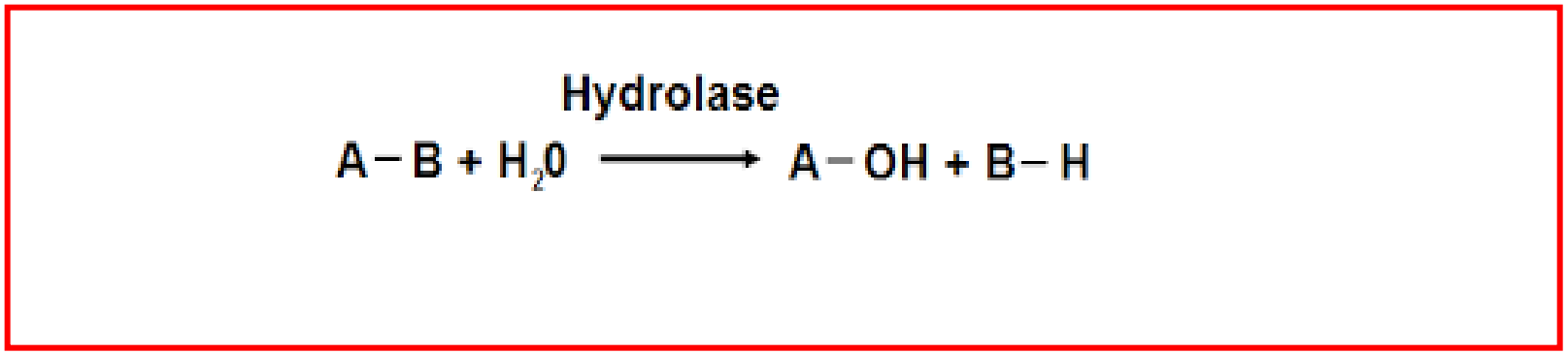
***1.Oxidoreductase*:** enzymes involved in oxidations and reductions of their substrates, e.g. *alcohol dehydrogenase, lactate dehydrogenase*.



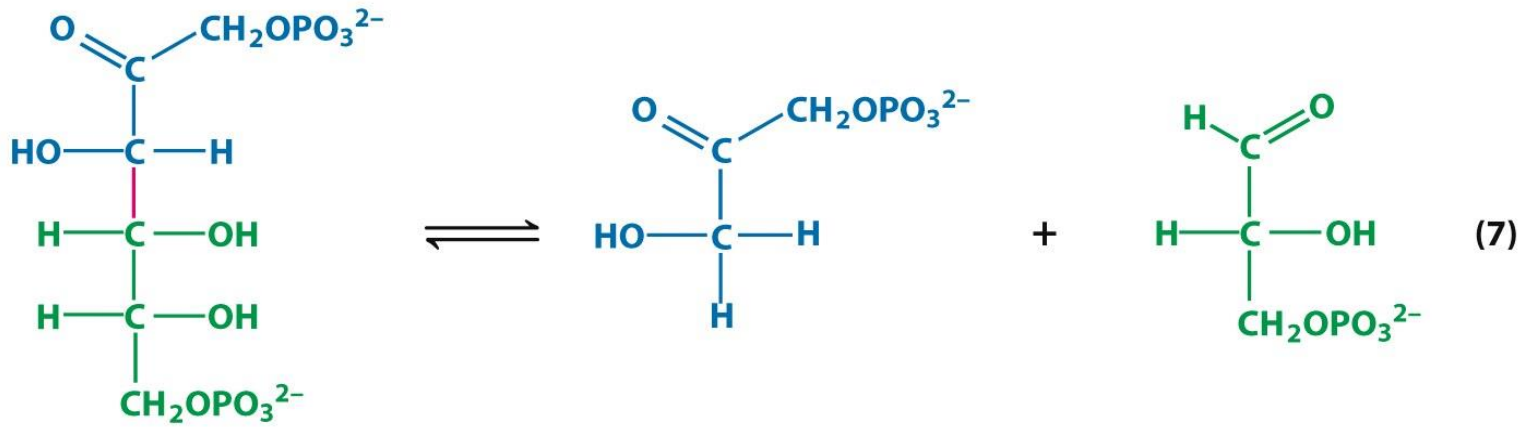
2. ***Transferases:*** *e*nzymes that catalyse transfer of a particular group from one substrate to another, e.g. *aspartate and alanine transaminase, hexokinase, etc.*



3. ***Hydrolases:*** *is an enzymes that catalyzes the hydrolysis of a chemical bond, e.g. glucose-6-phosphatase, pepsin, trypsin, etc.*



4. *Lyases:* enzymes that facilitate removal of small molecule from a large substrate, e.g. *aldolase A, -B, etc.*



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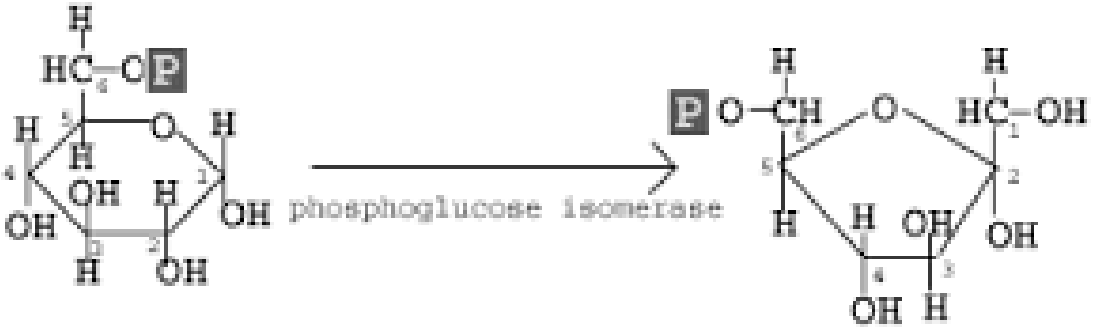
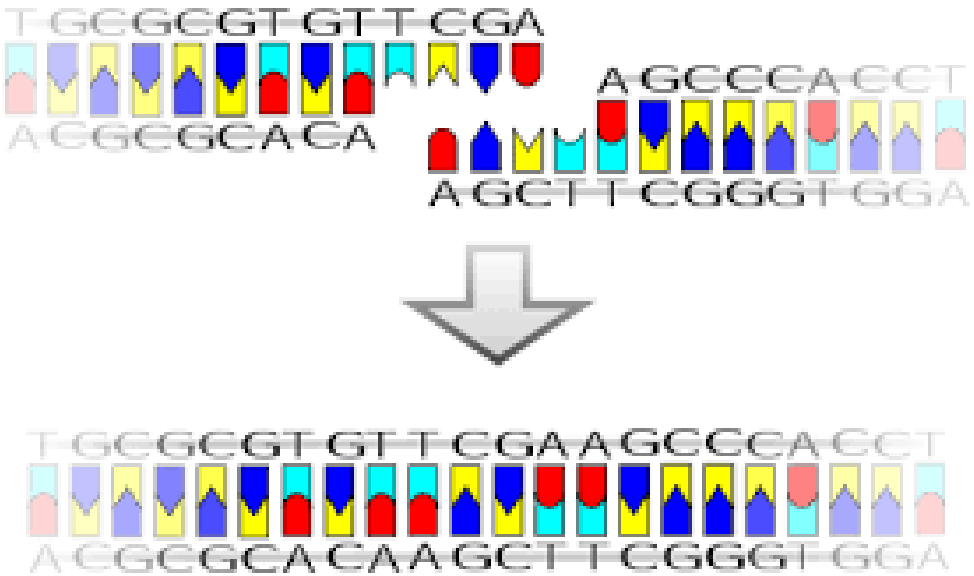
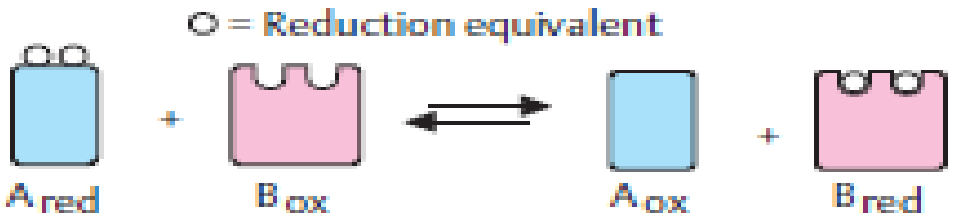
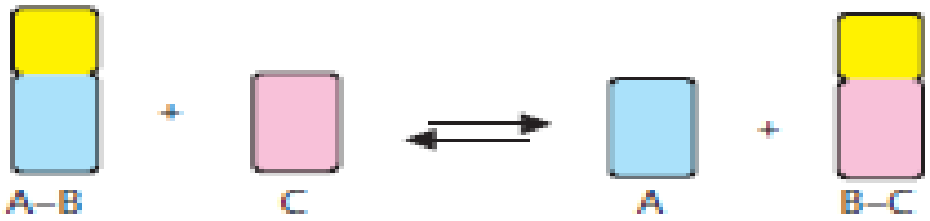
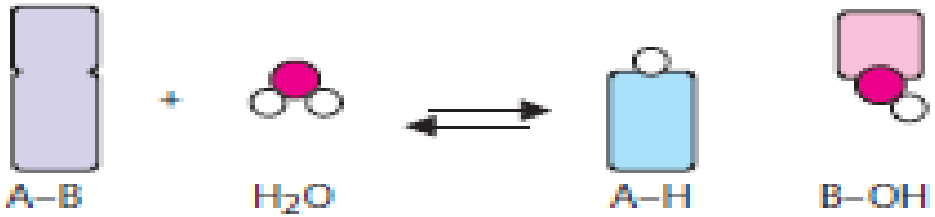
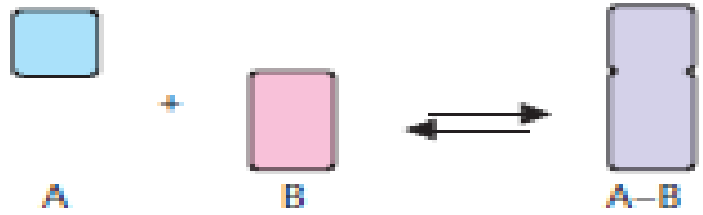
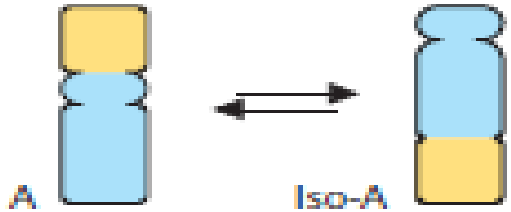
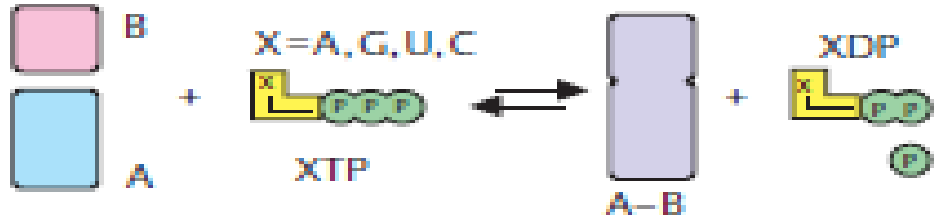
Biochemistry, Seventh Edition

5. ***Isomerases:*** enzymes involved in isomerisation of substrate, e.g. *UDP-glucose epimerase, retinal isomerase, etc.*



6. ***Ligases/synthetase:*** *e*nzymes involved in joining together two substrates, e.g. *alanyl-tRNA synthetase, glutamine synthetase, DNA ligases.*



1 Oxidoreductases	
2 Transferases	
3 Hydrolases	
4 Lyases	
5 Isomerases	
6 Ligases/ synthetase	

Nomenclature of enzymes

The commonly used names for most enzymes describe the type of reaction catalyzed, followed by the suffix – *ase*

Modifiers may precede the name to indicate

- the substrate (*xanthine* oxidase),

- the source of the enzyme (*pancreatic* ribonuclease),

- its regulation (hormone-sensitive lipase),

- feature of its mechanism of action (cysteine protease).

Where needed, alphanumeric designators

are added to identify multiple forms of an enzyme (RNA polymerase *III*; protein kinase *C*).

For many enzymes a trivial name is more commonly used (pepsin, tripsin)

Nomenclature of enzymes

There is the **Enzyme Catalogue**, in which each enzyme has formal specific number

(**Enzyme Comission** number - **E.C.** number).

This number represents a **four digit code**, and each part is separated by dot from each other.

(EC 2.1.1.3).

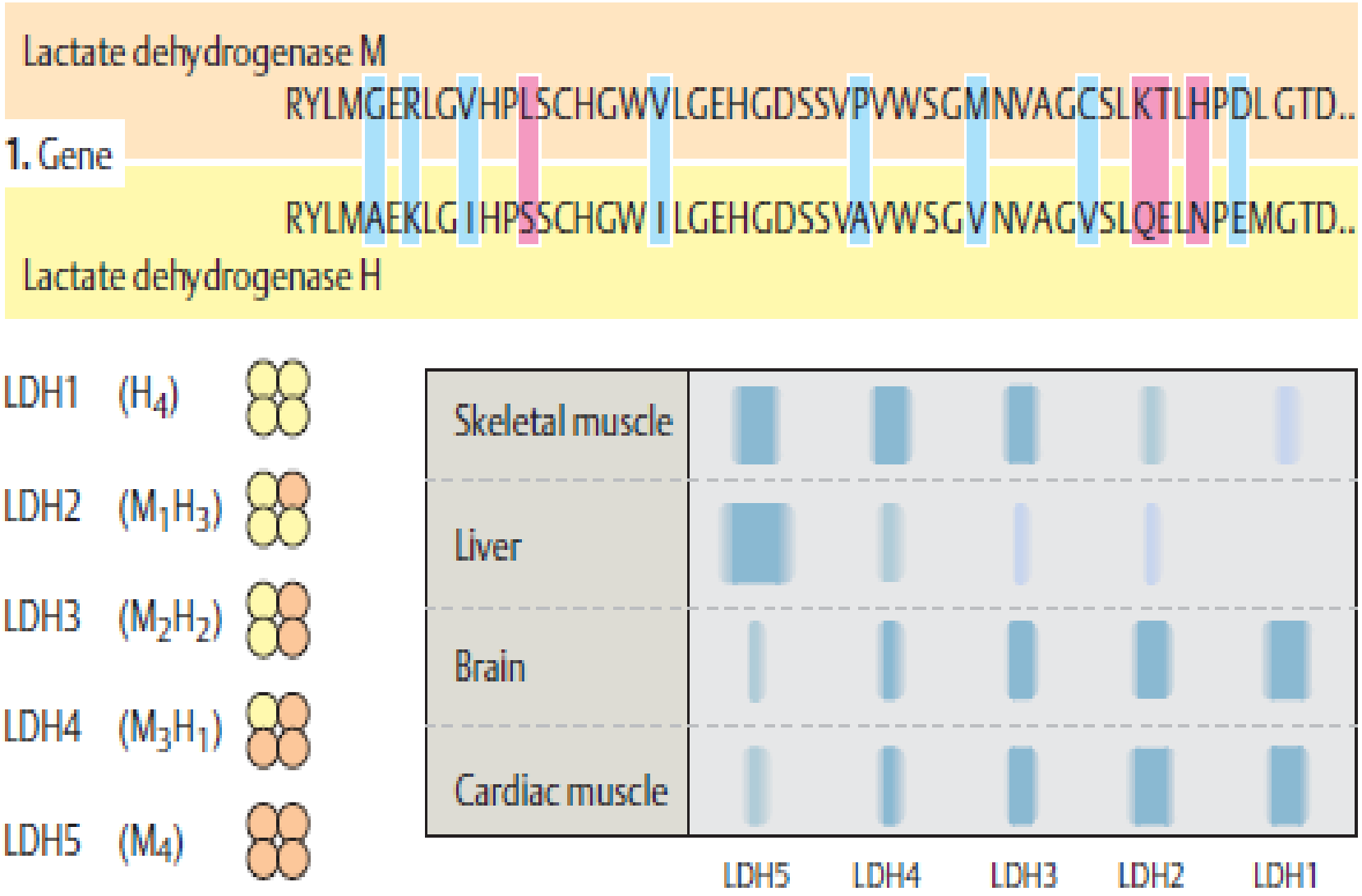
Thetin—homocysteine S-methyltransferase

- **The first digit denotes the class (one of the six)**
- **The second number indicates sub-class which characterizes the substrates involved in the given type of chemical conversions.**
- **The sub-classes are divided into sub-sub-classes which designate the nature of donor or acceptor.**
- **Finally, each enzyme has the ordinal number within the sub-sub-class.**

Isoenzymes (Isozymes)

are distinct enzyme forms that catalyze the same reaction.

Isozymes may exhibit subtle differences in properties such as sensitivity to particular regulatory factors or substrate affinity that adapt them to specific tissues or circumstances


Lactate dehydrogenase M
RYLMGERLGVHPLSCHGWVLGEHGDSSVPVWSGMNVAGCSLKTLHPDLGTD..
1. Gene
RYLMAEKLGIHPSSCHGWILGEHGDSSVAVWSGVNVAGVSLQELNPEMGTD..
Lactate dehydrogenase H
LDH1 (H_4)
LDH2 (M_1H_3)
LDH3 (M_2H_2)
LDH4 (M_3H_1)
LDH5 (M_4)
2. Forms
Skeletal muscle
Liver
Brain
Cardiac muscle
LDH5
LDH4
LDH3
LDH2
LDH1
3. Separation by gel electrophoresis

Units of enzyme activity

The catalytic action of an enzyme, its **activity**, is measured by determining the **increase in the reaction rate** under precisely defined conditions in a specific time interval.

Reaction rates are expressed as the ***change in concentration per unit of time*** (mol /L *s)

The **unit** used for enzymes is usually ***turnover per unit time***, expressed in **mol/s -**

katal (**kat**).

the **international unit (IU)** - more commonly used

micromol / **min**;

IU = 16.7 **nkat**.

(sek/min – 1/60 = 0,01666.....)

In order to remove the possibility of having the letter "**I**" confused with the digit "**1**", some hospitals have it as a stated policy to omit the "**I**", that is, to only use **U** or **E** when talking and writing about dosages.