Outline of biochemistry course

Topic	Likely number of lectures
Enzymes	3
Bioenergetics	1
Electron transport chain	1
Protein metabolism	4
Introduction to metabolism	With first CHO lecture
Carbohydrate metabolism	5
Lipid metabolism	3
Integration of metabolism	1-2

Aim: understand key (simplified) principles (important clinical correlations)

Biochemistry lecture 1: enzymes 1

Ahmed Salem, MD, MSc, PhD, FRCR

Topic	Lecture outline
Introduction	1. What is biochemistry? 2. Outlines of biochemistry application in medicine

What is biochemistry?

- **Biochemistry:** science of the chemical basis of life (Gk bios "life")
- It forms a bridge between biology and chemistry
- The cell is the structural unit of living systems
  - → biochemistry can also be described as the science of the chemical constituents of living cells & reactions and processes they undergo
- By this definition, biochemistry encompasses large areas of:
  - cell biology
  - molecular biology
  - molecular genetics

Biochemistry applications in medicine

- The biochemistry of the nucleic acids lies at the heart of **genetics**;
- The use of **genetic** approaches has been critical for elucidating many areas of biochemistry
- **Physiology**, the study of body function, overlaps with biochemistry almost completely
- **Immunology** employs numerous biochemical techniques, and many immunologic approaches have found wide use by biochemists

Biochemistry applications in medicine

- **Pharmacology** rest on a sound knowledge of biochemistry & physiology;
  - most drugs are metabolized by enzyme-catalyzed reactions
- Poisons act on biochemical reactions or processes; this is **toxicology**
- Biochemical approaches are being used increasingly to study basic aspects of **pathology** (the study of disease), such as inflammation, cell injury, and cancer
- Many workers in **microbiology**, **zoology**, and **botany** employ biochemical approaches almost exclusively

Enzymes I	1.Understanding enzymes a catalyst 2.The catalytic cycle 3. How enzymes accelerate cellular reactions? 4. The basis of enzyme classifications 5. Exploring the factors affecting the rate of enzymic reaction

Enzymes

- **Definition:** Enzymes are specific biocatalysts [mainly proteins in nature] that regulate (accelerate) the rate of biochemical reactions

- Proteins can be hydrolyzed with hydrochloric acid by boiling for a very long time; but inside the body, with the help of enzymes, proteolysis takes place within a short time at body temperature

- **Enzyme catalysis is very rapid;** usually 1 molecule of an enzyme can act upon about 1000 molecules of the substrate per minute

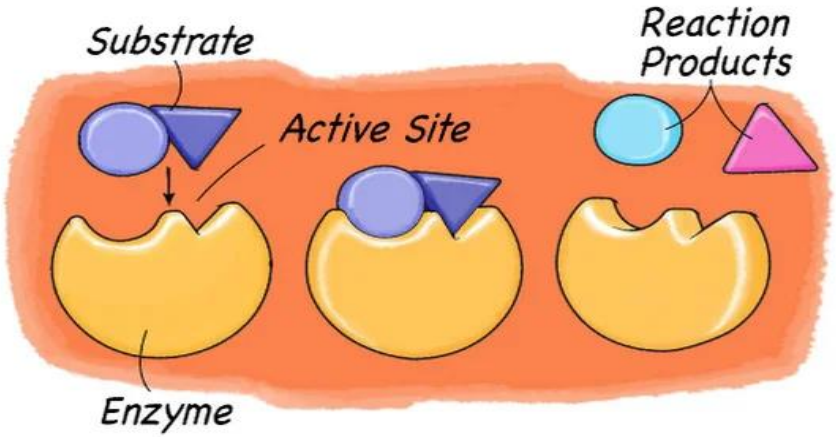
- Lack of enzymes will lead to block in metabolic pathways → **inborn errors of metabolism**

Enzymes

- The substance upon which an enzyme acts, is called the **substrate**

  Substrates are also called <u>reactants</u> because they are the molecules undergoing the reaction

- The enzyme will convert the substrate into the product or **products**



Nomenclature

- Most commonly used enzyme names have the suffix "-ase" attached to the substrate of the reaction (e.g. glucosidase, urease, sucrase)

or

- A description of the action performed (e.g. lactate dehydrogenase and adenylyl cyclase)

- Some enzymes retain their original trivial names, which give no hint of the associated enzymatic reaction, e.g. trypsin and pepsin

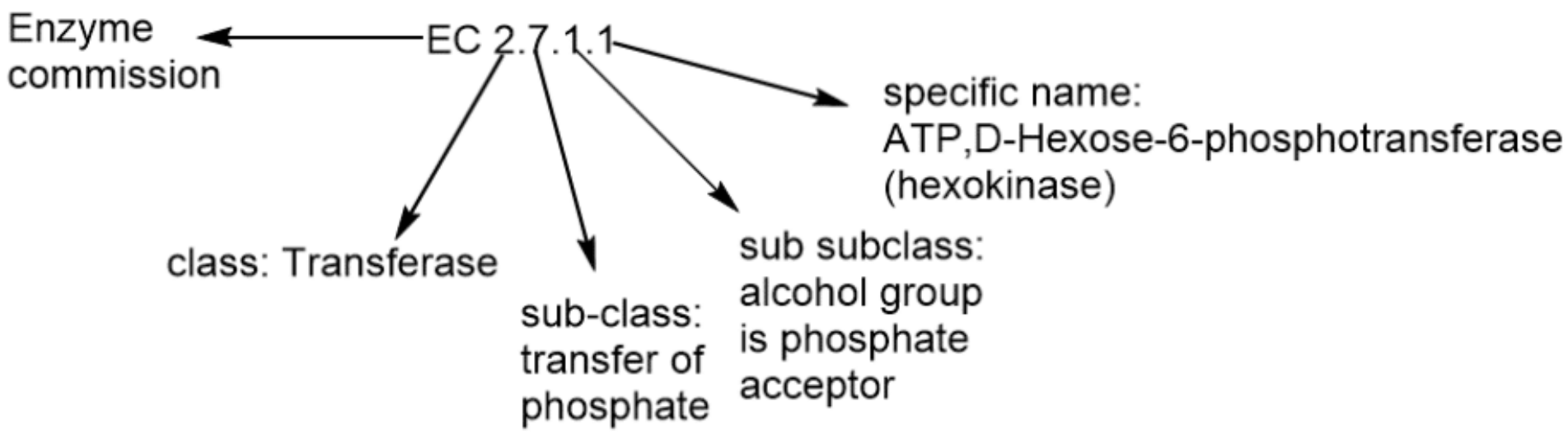
The basis of enzyme classifications

- International Union of Biochemistry and Molecular Biology (IUBMB) developed a system of nomenclature for enzymes

- It is complex and cumbersome; but unambiguous.

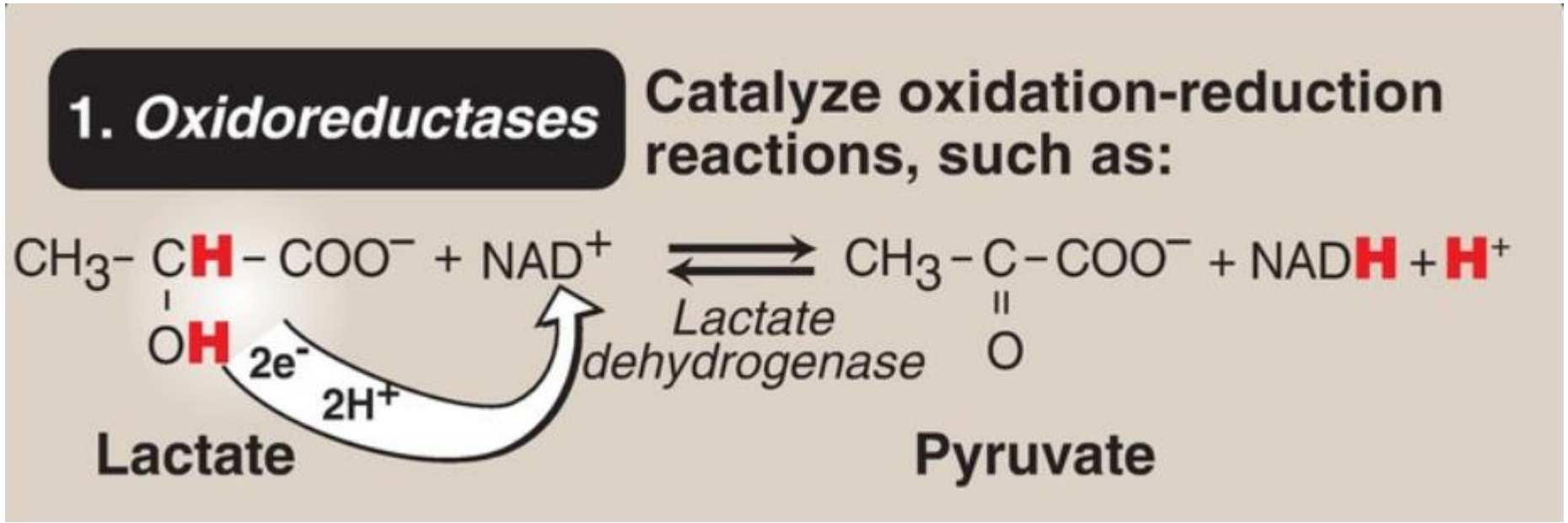
- The name starts with EC (enzyme class) followed by 4 digits:
  - **First digit represents the class (6 classes)**
  - Second digit stands for the subclass
  - Third digit is the sub-subclass or subgroup
  - Fourth digit gives the number of the particular enzyme in the list

https://www.enzyme-database.org/class.php


Enzyme commission
EC 2.7.1.1
specific name: ATP,D-Hexose-6-phosphotransferase (hexokinase)
class: Transferase
sub-class: transfer of phosphate
sub subclass: alcohol group is phosphate acceptor

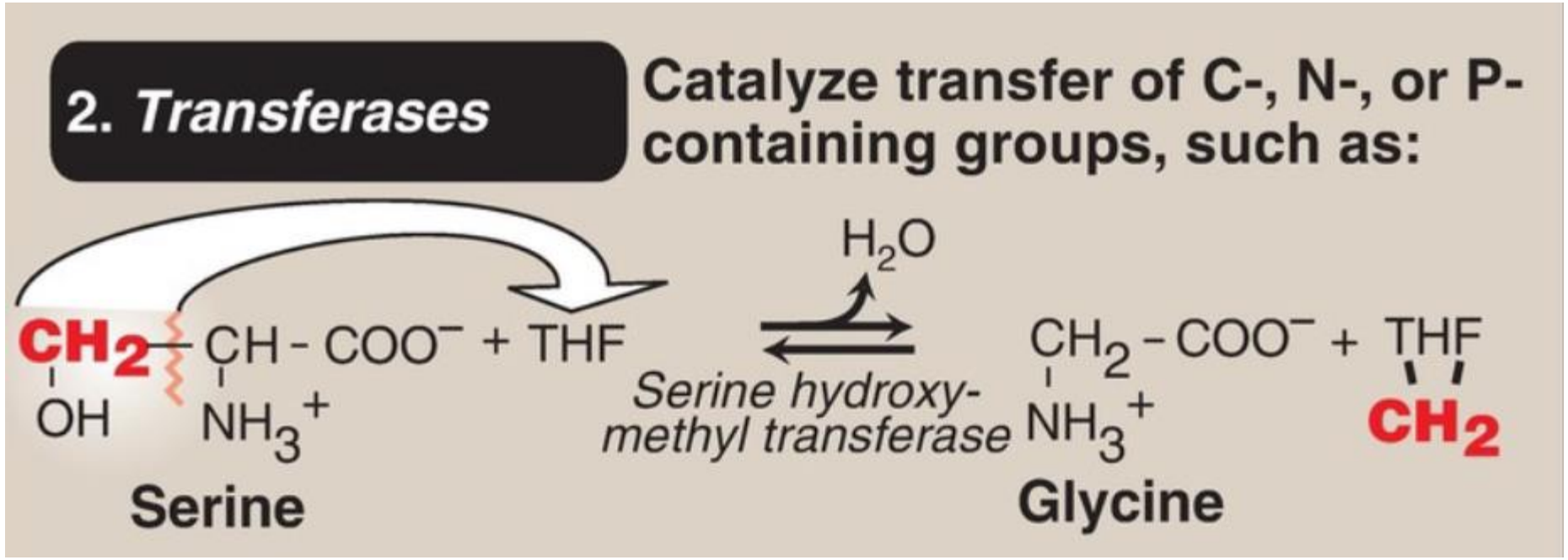
Class 1: Oxidoreductases

- This group of enzymes will catalyze oxidation of one substrate with simultaneous reduction of another substrate or co-enzyme
  - $AH2 + B \rightarrow A + BH2$



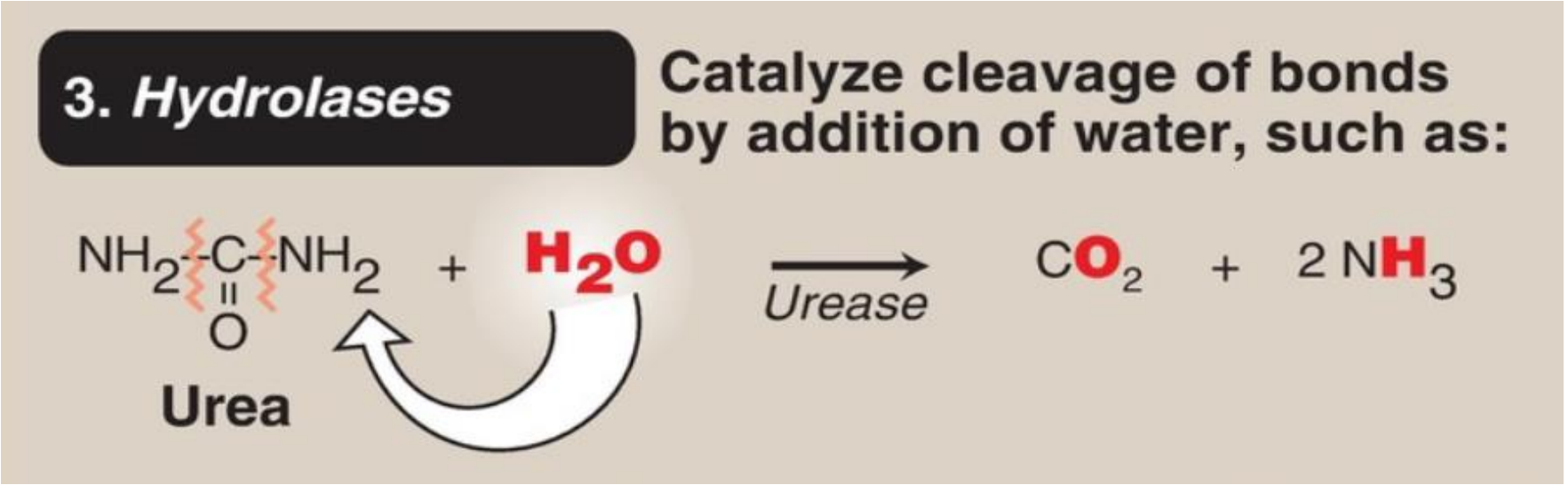
Class 2: Transferases

- This class of enzymes transfers one group (other than hydrogen) from the substrate to another substrate
  - This may be represented as:
    - A-R + B → A + B-R



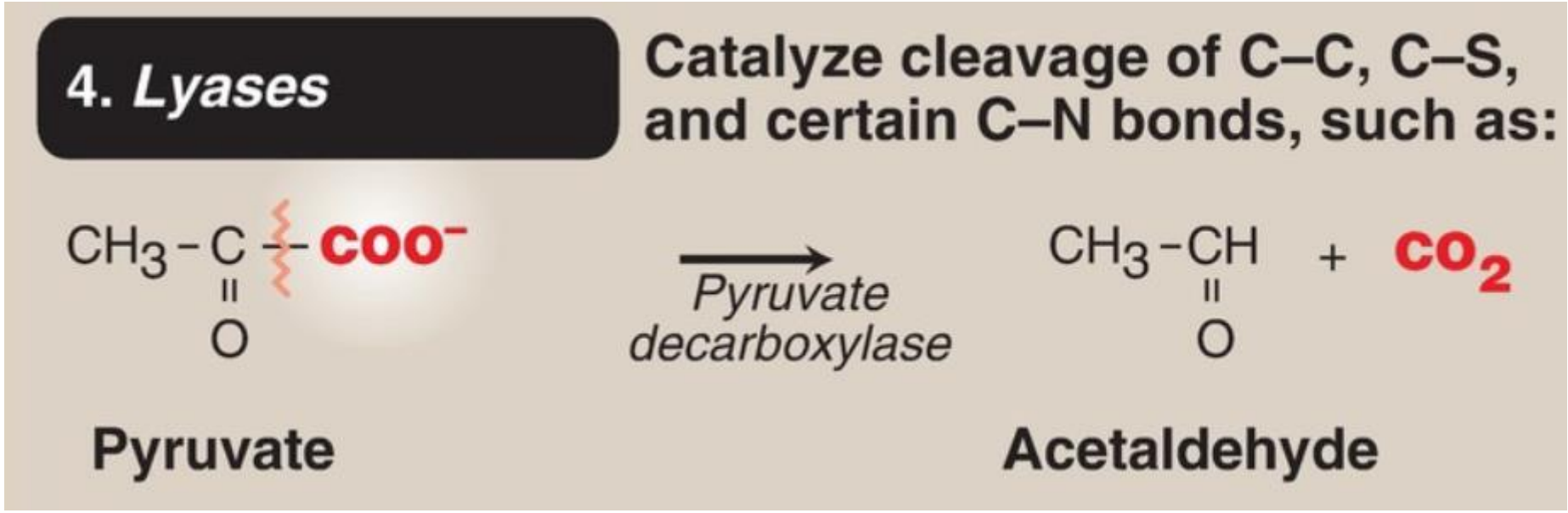
Class 3: Hydrolases

- This class of enzymes can hydrolyze ester, peptide or glycosidic bonds by adding water and then breaking the bond
- All digestive enzymes are hydrolases
- $A–B + H_2O \rightarrow A–OH + B–H$



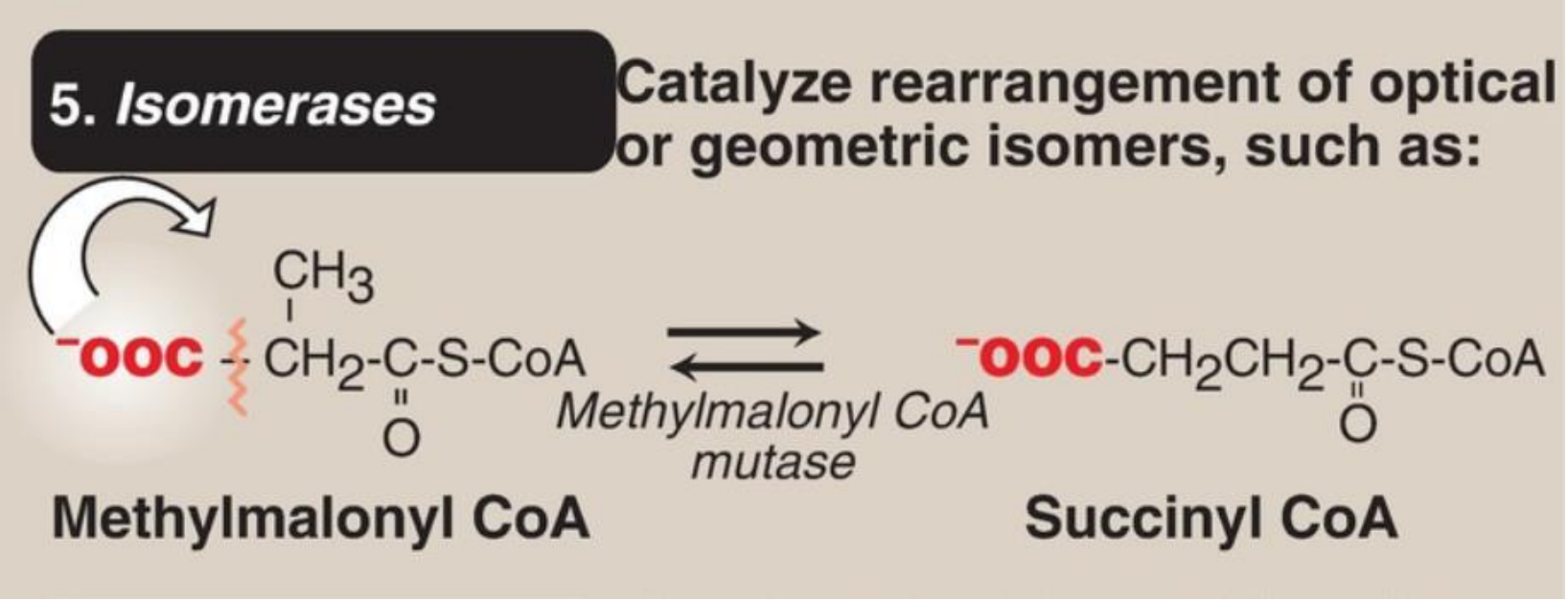
Class 4: Lyases

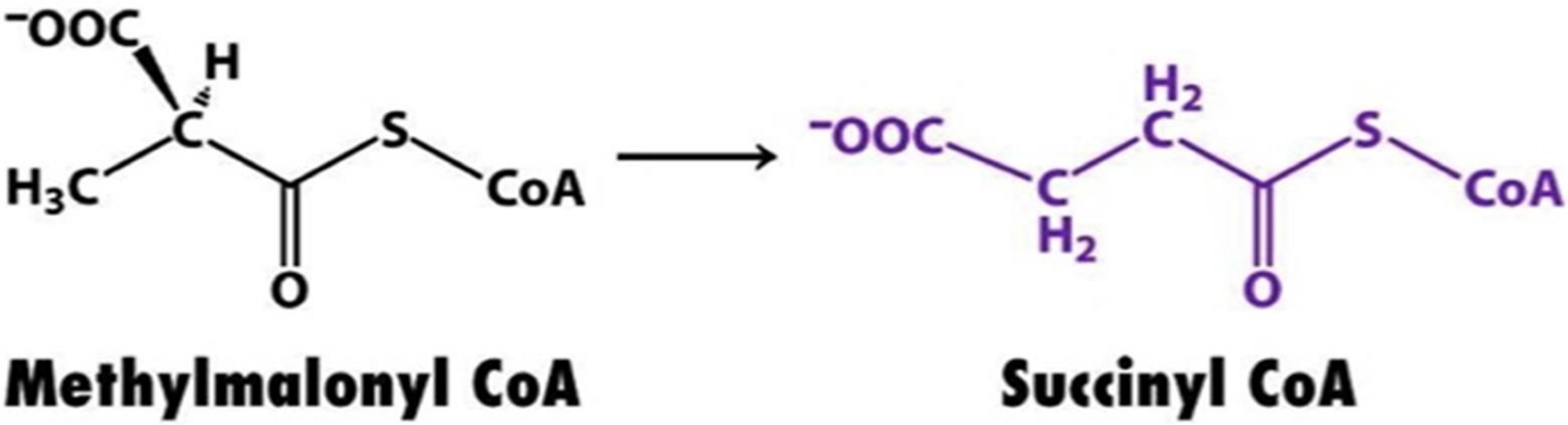
- These enzymes can remove groups from substrates or break bonds by mechanisms other than hydrolysis
- ATP → cAMP + PPi



Class 5: Isomerases

- These enzymes can produce isomers of substrates
- Racemases, epimerases, cis-trans isomerases are examples
- A–B → B–A

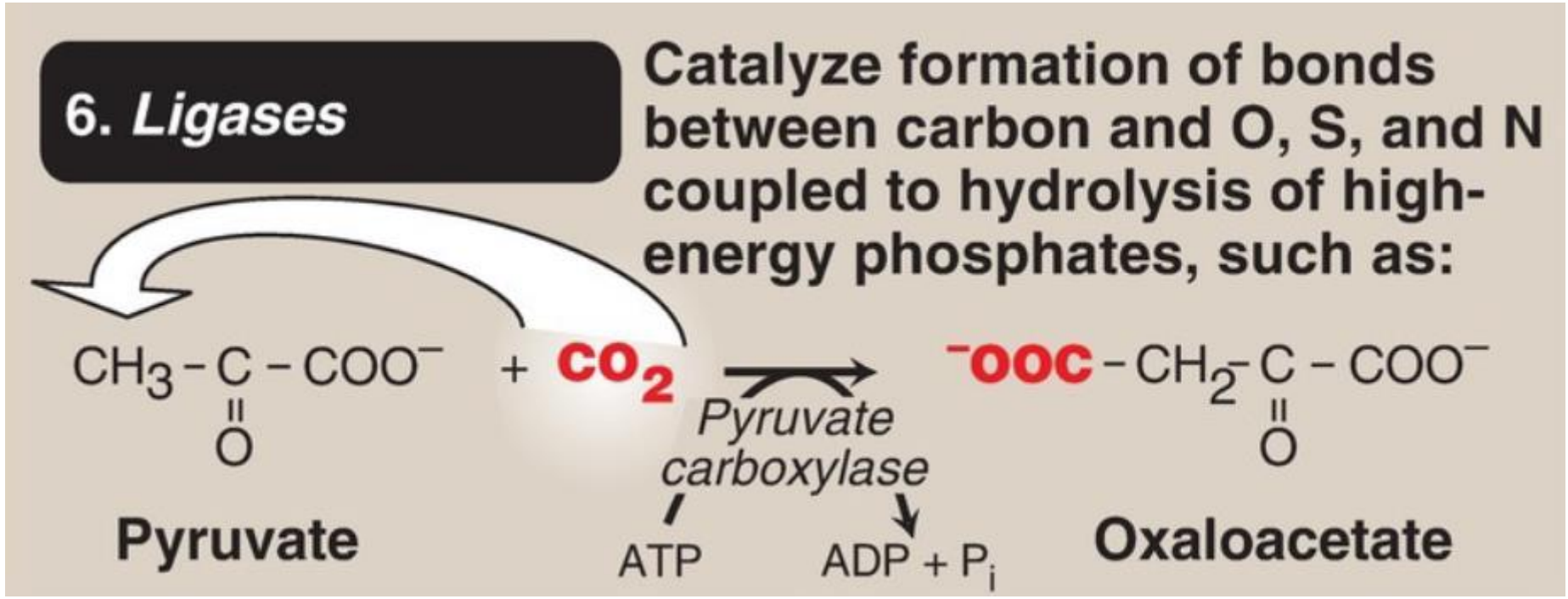




Methylmalonyl CoA → **Succinyl CoA**

Class 6: Ligases

- These enzymes link two substrates together, usually with the simultaneous hydrolysis of ATP (Latin, Ligare = to bind)
- A-OH + B-H → A–B + H_2O



Characteristics of Enzymes

- Almost all enzymes are proteins (either simple or conjugated)

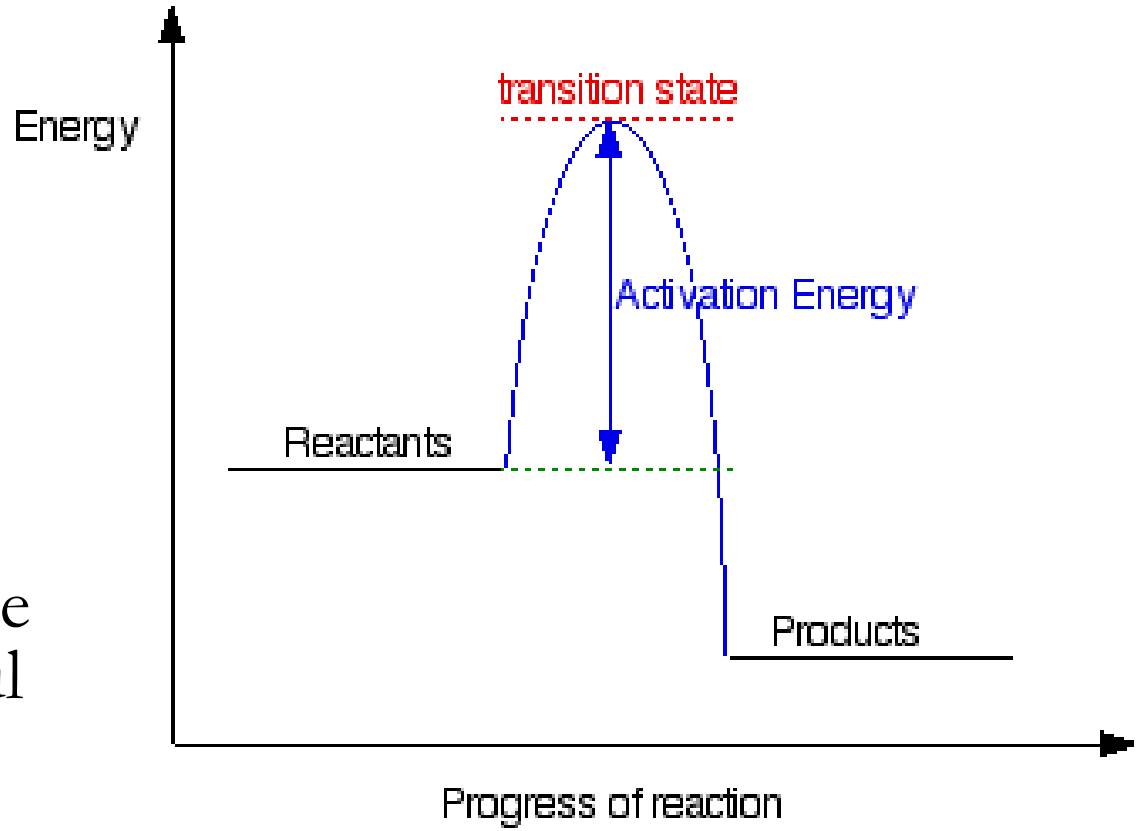
- **<u>Enzymes follow the physical and chemical reactions of proteins:</u>**
  - They are heat labile
  - They are water-soluble.
  - They can be precipitated by protein precipitating reagents (ammonium sulfate or trichloroacetic acid)

- They contain 16% weight as nitrogen

- They are needed in very small amounts

Mechanism of action of enzymes

- Virtually all chemical reactions have an **energy barrier.**
- This barrier is called the **energy of activation**.
- Many theories exist on MOA of enzymes but most accepted is the **lowering of activation energy.**
- Gibbs free energy (*G*), a measure of the amount of energy available to do useful work in a process.



Mechanism of action of enzymes

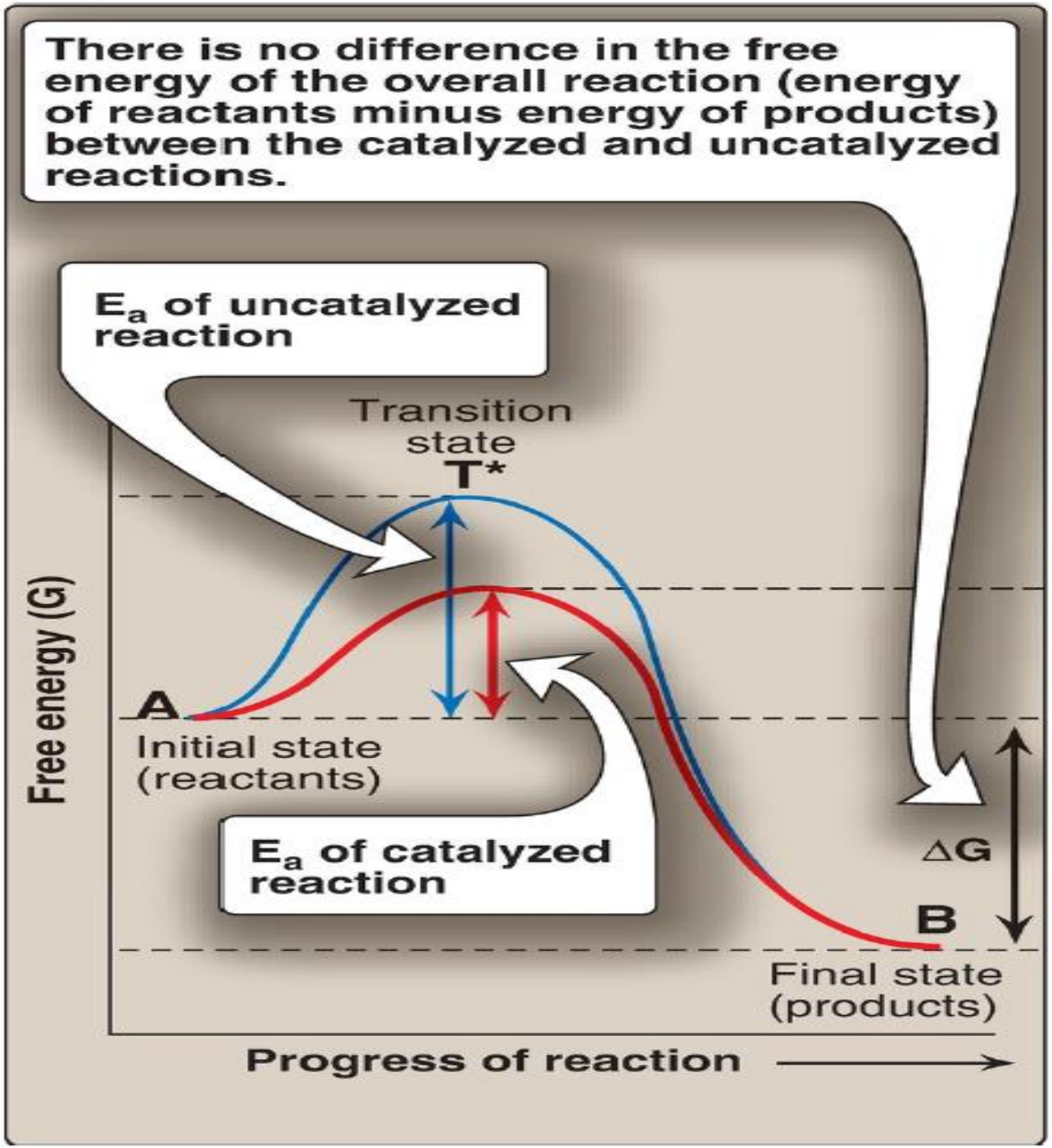
- To convert one or more substrate molecules into a product, some bonds must be broken, and new ones must be made.
- For example, the substrate molecule or molecules might have to be forced or bent into a form that will allow existing bonds to break or form, just as you might need to bend a stick to weaken it at the spot you want it to break.
- This contorted form of the reactants is called the **transition state**, and to reach it takes energy, just as you need to put in effort to bend a stick.

Mechanism of action of enzymes

- The **activation energy** is—the energy needed to get molecules to that transition state.

- The **activation energy** (ΔG^{++}), is the minimum amount of energy that is required to activate atoms or molecules to a condition in which they can undergo chemical transformation.

- When the activation energy is lower, many more substrate molecules reach the transition state at a given temperature, so the **conversion of substrate to product is correspondingly faster.**



The enzyme provides an ***alternate reaction pathway*** with a lower free energy of activation than that of the un-catalyzed reaction.

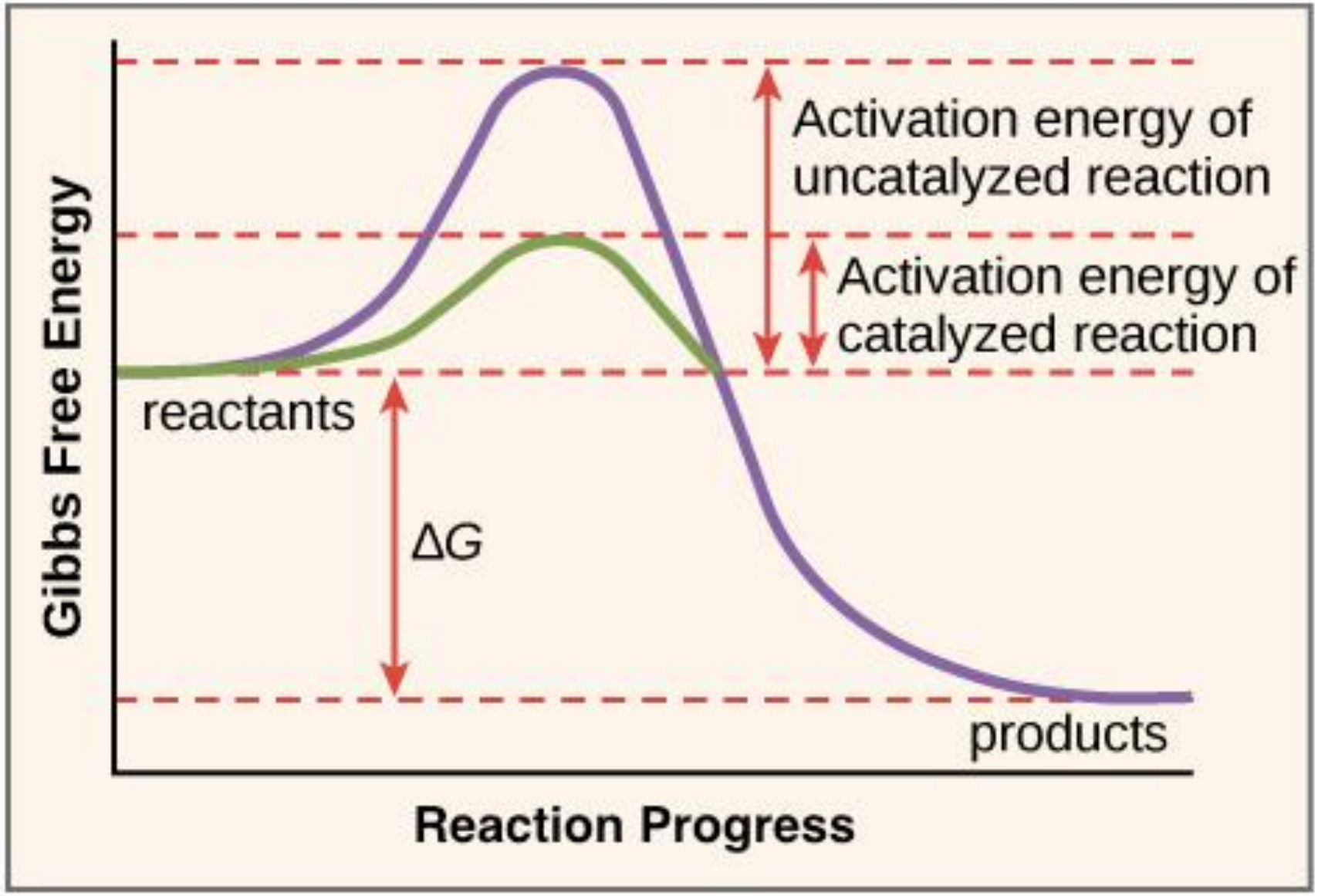
Note:
The enzyme does not affect the free energy change of the reaction (ΔG).

The change in Gibbs free energy (Δ*G*) is the maximum amount of free energy available to do useful work.

Does not change the equilibrium of the reaction*

It does, however, accelerate the rate with which equilibrium is reached.

*: equilibrium constant of reaction: **Equilibrium** is when the rate of the forward reaction equals the rate of the reverse reaction. All reactant and product concentrations are constant at equilibrium (Keq).


Activation energy of uncatalyzed reaction
Activation energy of catalyzed reaction
reactants
ΔG
products
Gibbs Free Energy
Reaction Progress

Enzyme kinetics

- **Velocity or rate of enzyme reaction** is assessed by the **rate of change of substrate to product per unit time** (product formation of disappearance of substrate/time).
- The **velocity** is proportional to the concentration of reacting molecules (**dependent upon the substrate concentration** [S]).
- At equilibrium, forward and backward reactions are equal.
- If Keq is >1, the forward reaction is favored (spontaneous & exothermic).
- Concentration of enzyme **does not** affect the Keq.

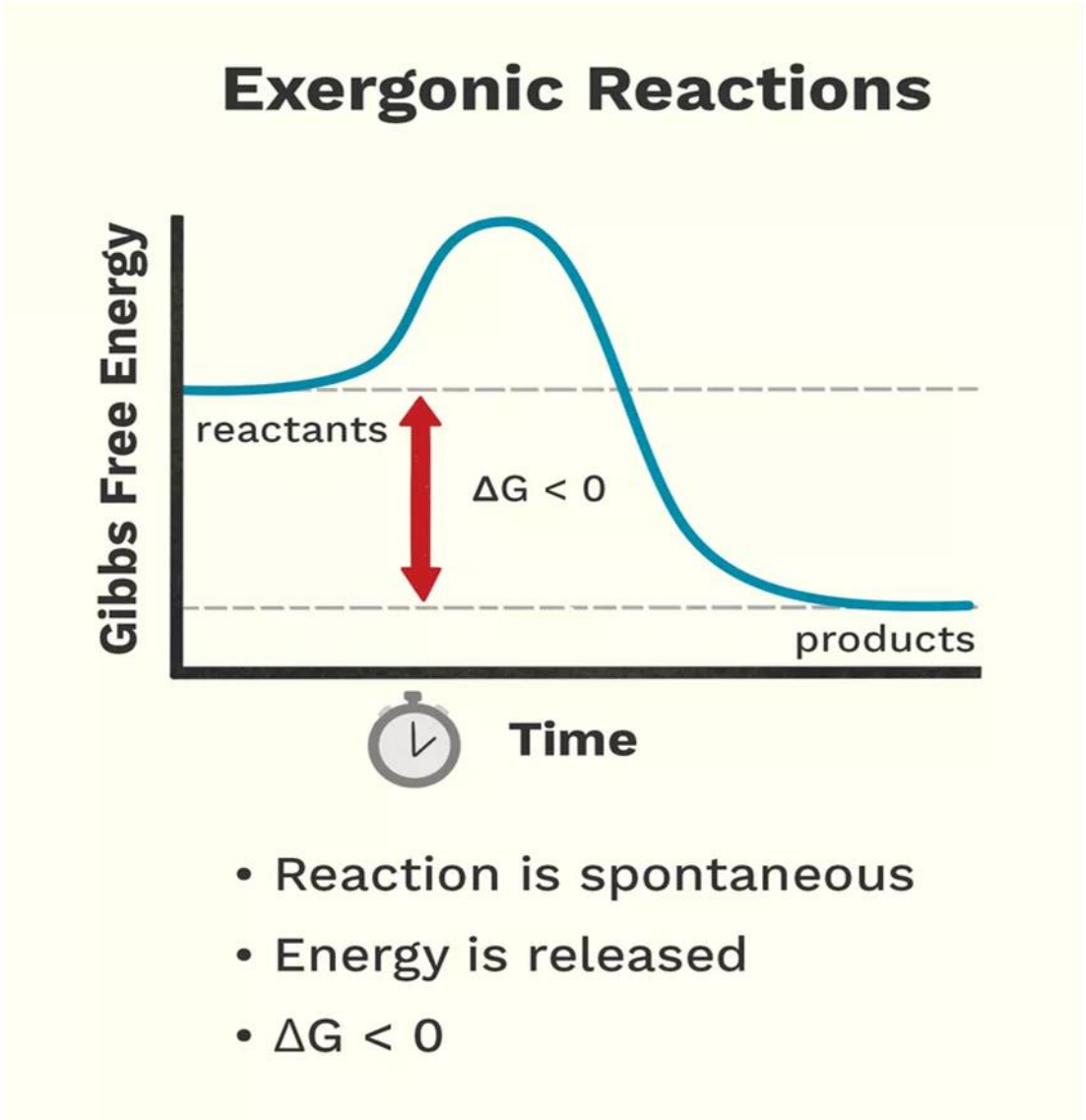
Types of reactions

According to the free energy changes delta G, there are three types of reactions:

1. **Exothermic reactions (Exergonic reactions)**

Accompanied with release of free energy; have **negative delta G** and are **irreversible.**

Urea → ammonia + CO2 + energy



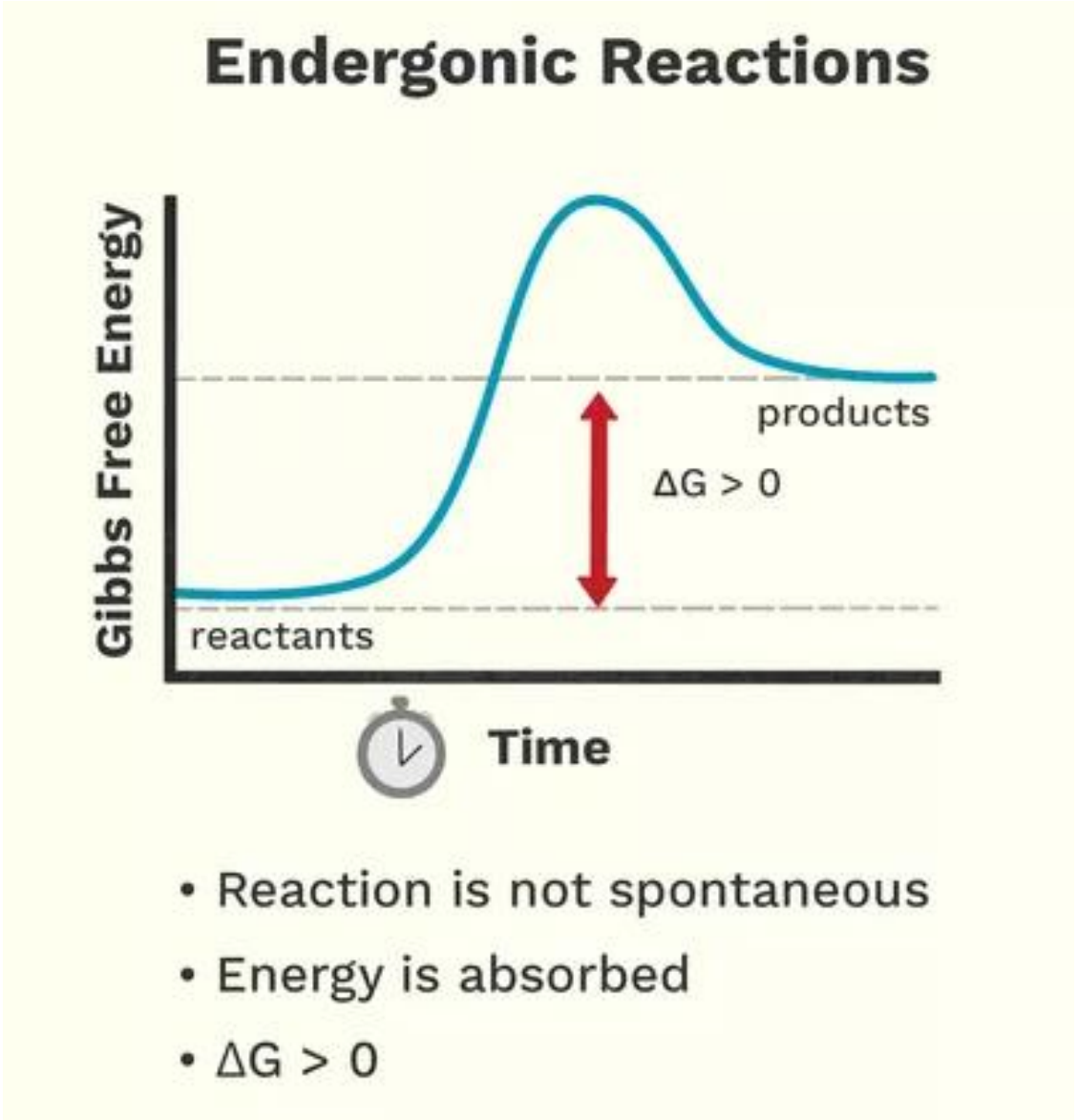
Types of reactions (continued)

2. Endergonic reaction (Endothermic)

Energy is consumed and external energy is to be supplied for these reactions; **positive delta G.**

e.g. Hexokinase catalyses the following reaction:

Glucose + ATP → Glucose-6-phosphate + ADP



Types of reactions

3. Isothermic reactions

These reactions are not accompanied with changes in free energy

(**delta G = zero** or is negligible)

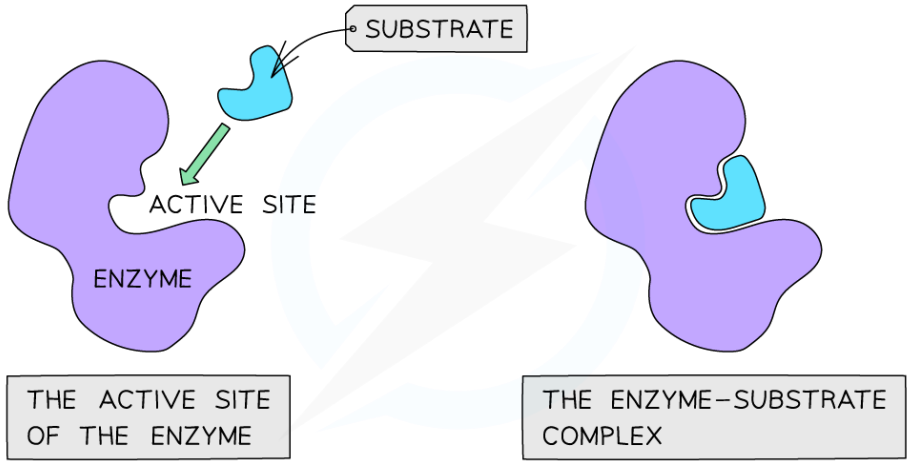
They are reversible

Pyruvate + 2H ↔ Lactate

Chemistry of enzyme active site

- The region of the enzyme where substrate binding and catalysis occurs is referred to as **active site** **[contain binding and catalytic site]**

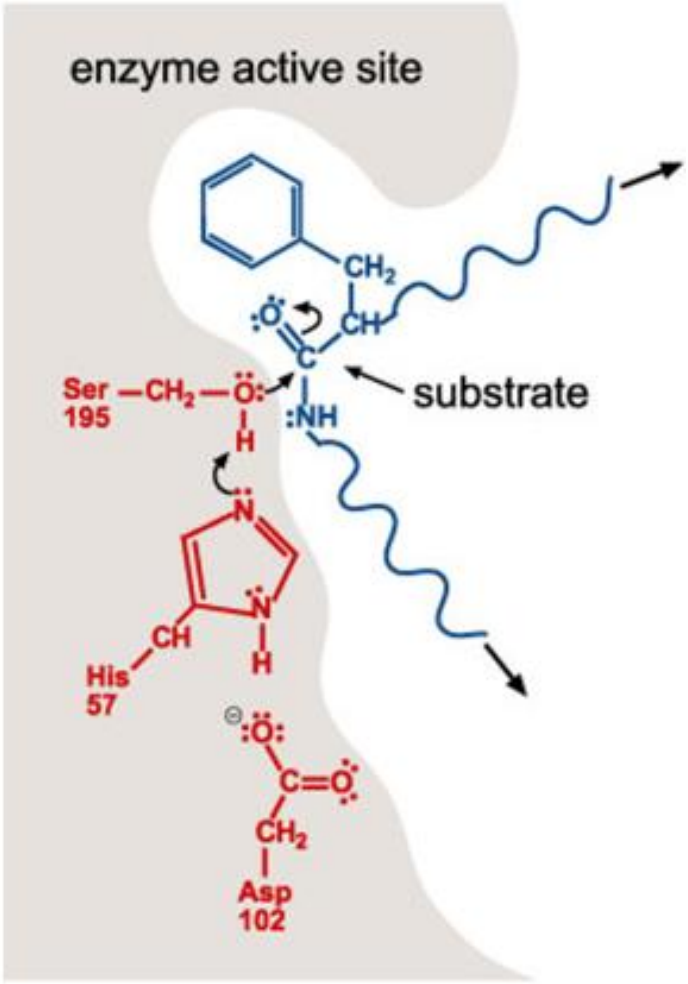
- The amino acids at the active site are arranged in a very precise manner so that only specific substrate or inter-related substrates can bind at the active site.



Chemistry of enzyme active site

- The **active site** of an enzyme is the part of the enzyme where substrate molecules bind, and a chemical reaction takes place.

- The active site is made up of amino acid residues that establish temporary bonds with the substrate (binding site) as well as residues that catalyze that substrate's reaction (catalytic site).



Chemistry of enzyme active site

- Usually serine, histidine, cysteine, aspartate and glutamate residues make up active site
    - The amino acids or groups that directly participate in making or breaking the bonds (present at the active site) are called **catalytic residues or catalytic groups.**

- The shape and the chemical environment inside the active site permit a chemical reaction to proceed more easily.

- Enzymes are named according to the active site amino acid
    - For example, **trypsin** is a serine protease and **papain** is cysteine protease

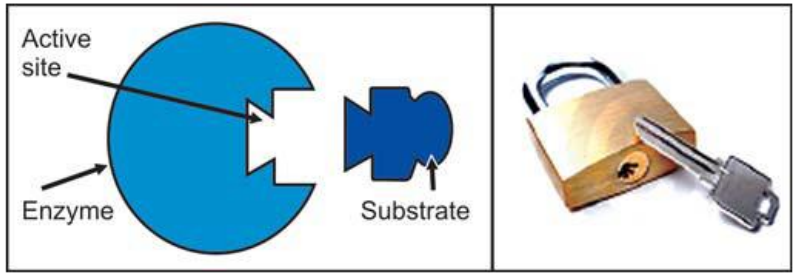
Name of enzyme	Important amino acid at the catalytic site
Chymotrypsin	His (57), Asp (102), Ser (195)
Trypsin	Serine, Histidine
Thrombin	Serine, Histidine
Phosphoglucomutase	Serine
Alkaline phosphatase	Serine
Acetyl cholinesterase	Serine
Carbonic anhydrase	Cysteine
Hexokinase	Histidine
Carboxypeptidase	Histidine, Arginine, Tyrosine
Aldolase	Lysine

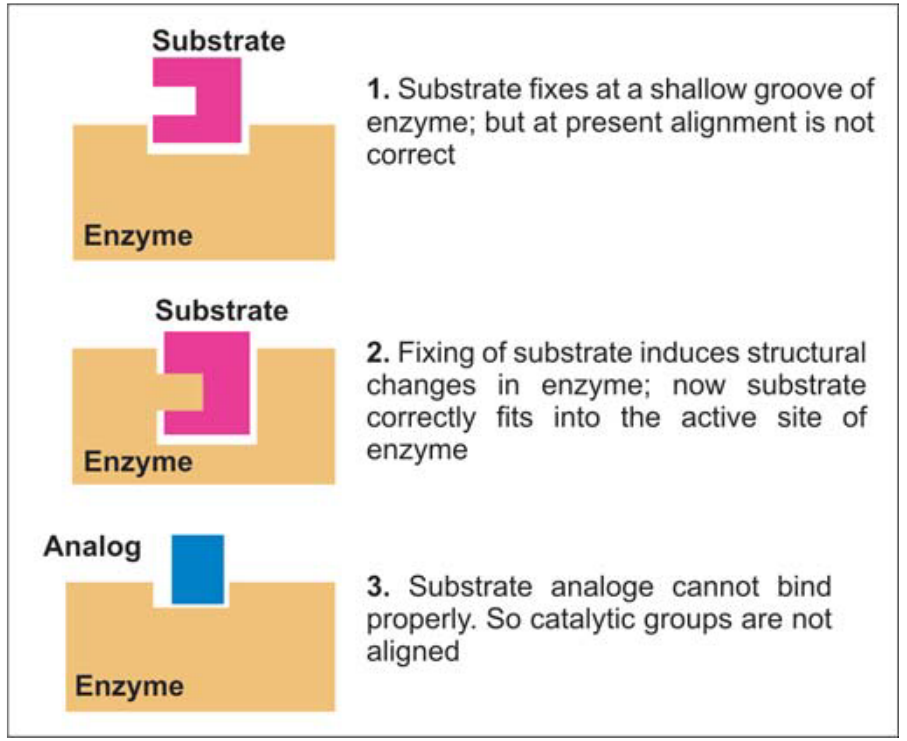
Enzyme Specificity

- The **induced fit model** (Koshland's theory) states that when substrates bind to an enzyme, they induce a conformational change analogous to placing a hand (substrate) into a glove (enzyme).

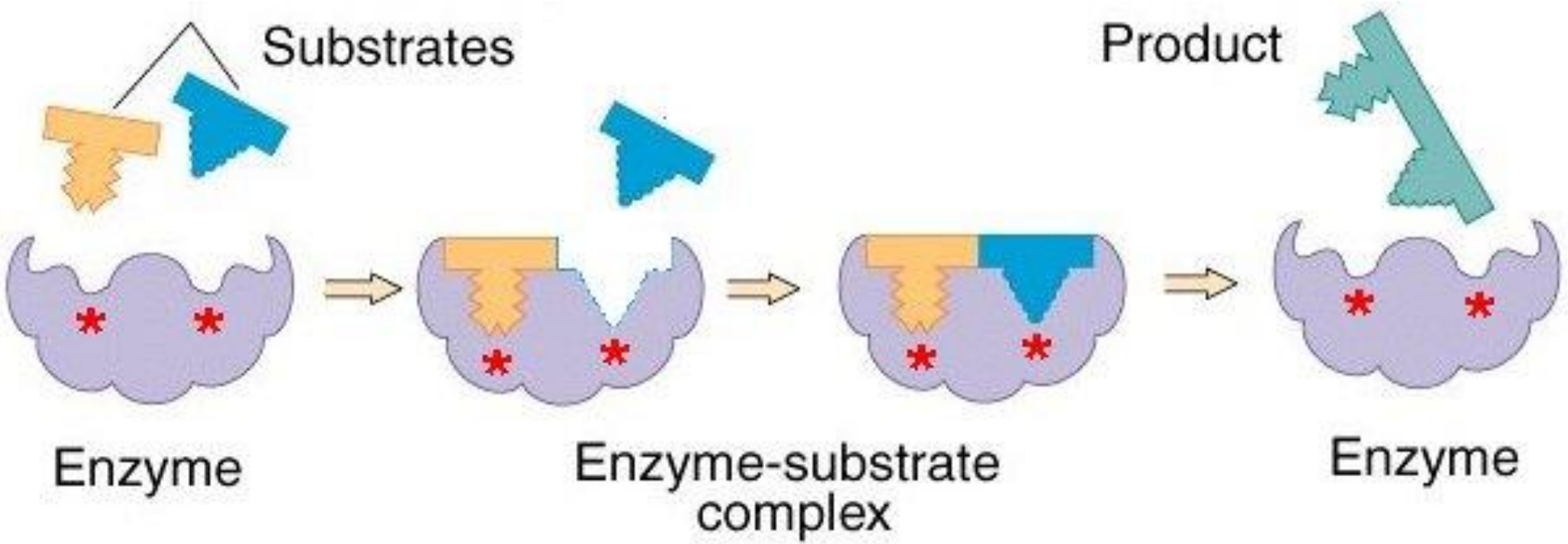
✖ Fischer's template theory (lock and key)

- could not explain the flexibility shown by enzymes

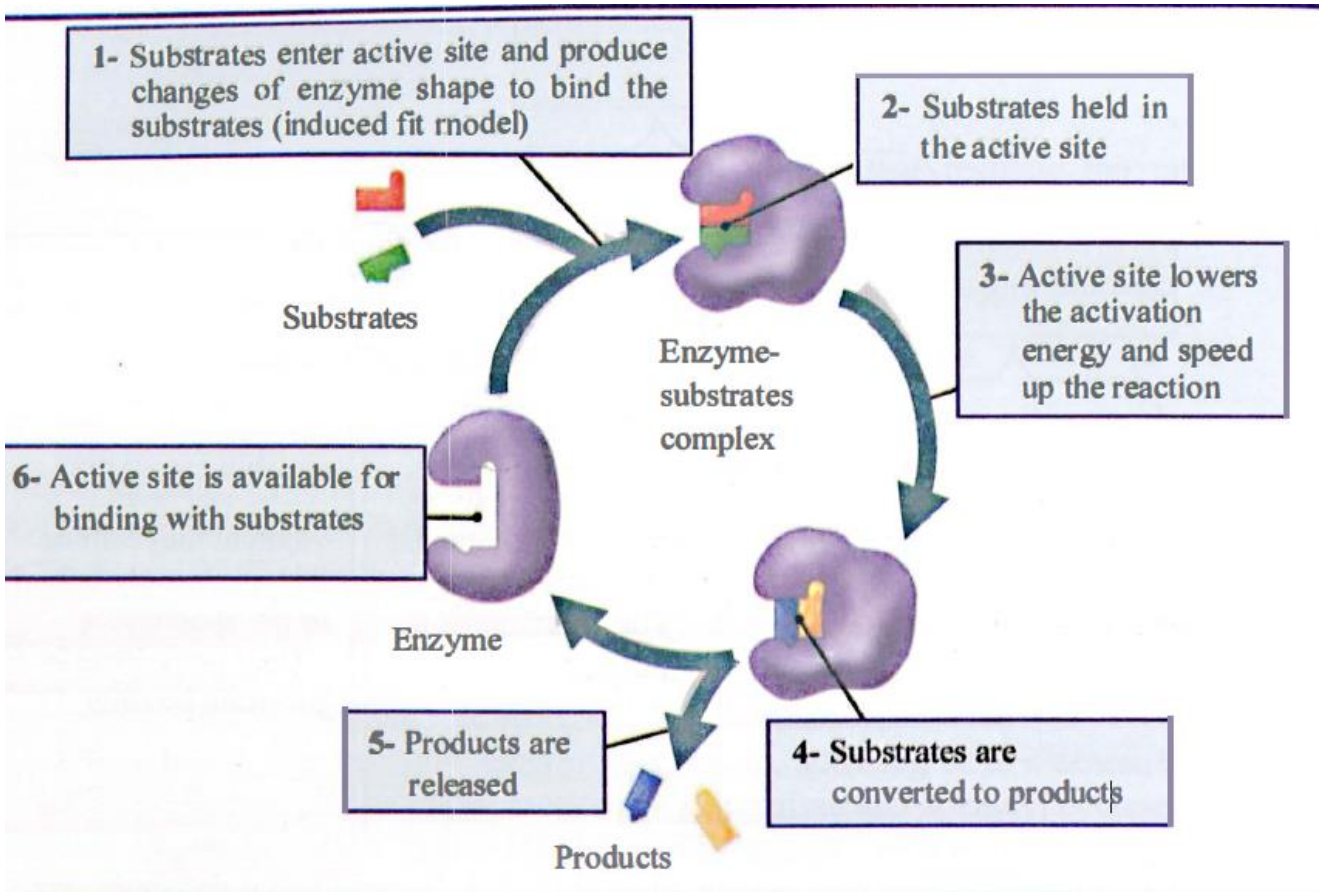


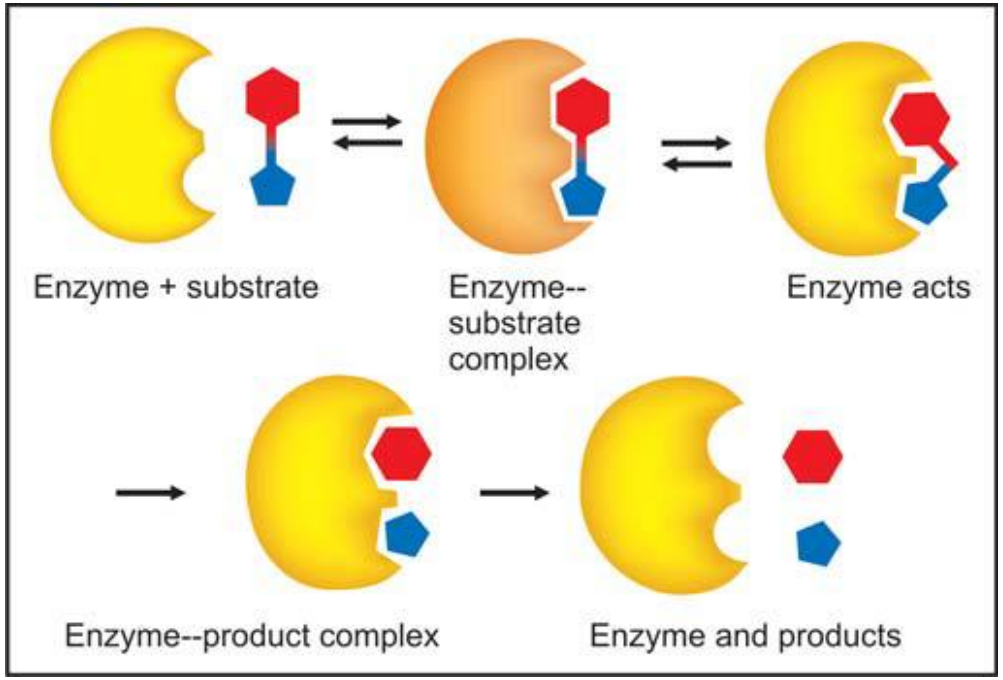


Induced fit model



The catalytic cycle





Substrate binding site & catalytic site may be separate

Co-factor/ Coenzymes

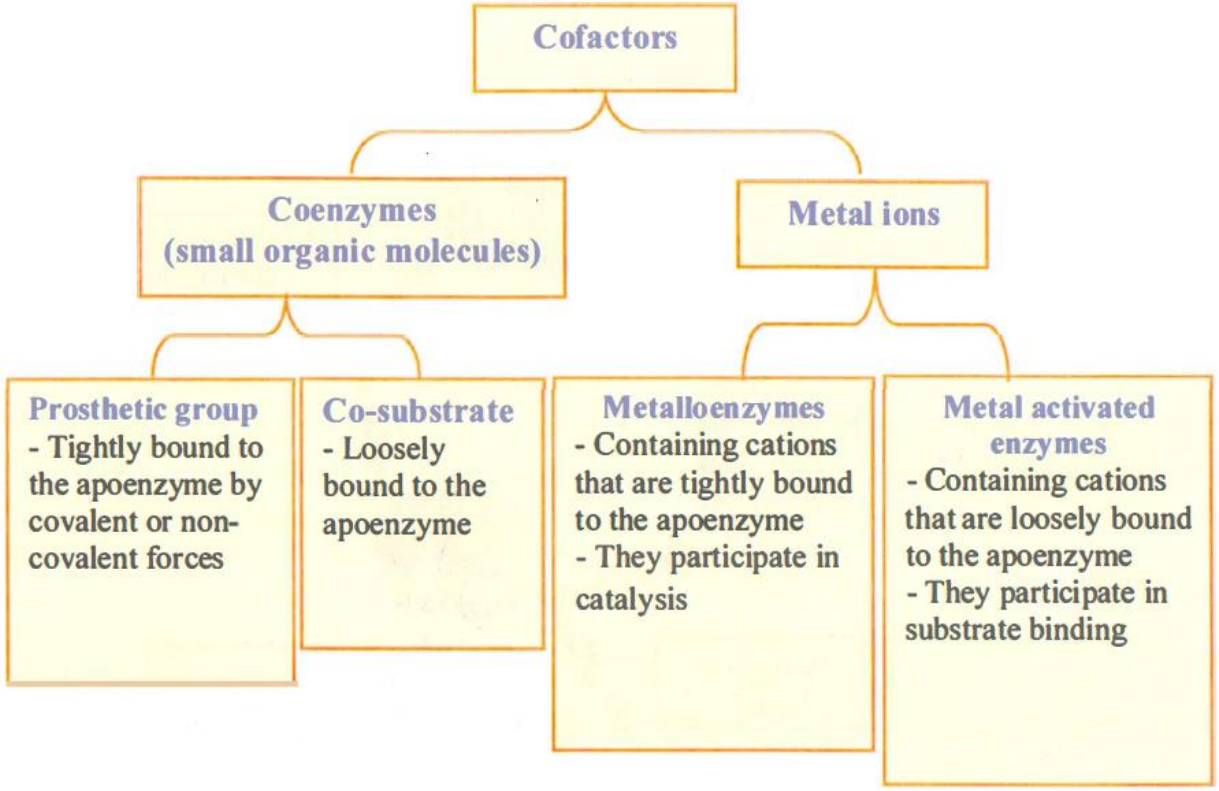
- Are heat stable, low molecular weight non-protein compounds.
- Strictly required by some enzymes for their actions.
- **Actions of coenzymes:** function as group transfer agents.
- **Important:** co-factor is used as a collective term to include co-enzymes and metal ions. Co-enzyme is an organic co-factor.

Cofactors

Cofactors: organic or inorganic molecules that are required for the activity of certain enzymes

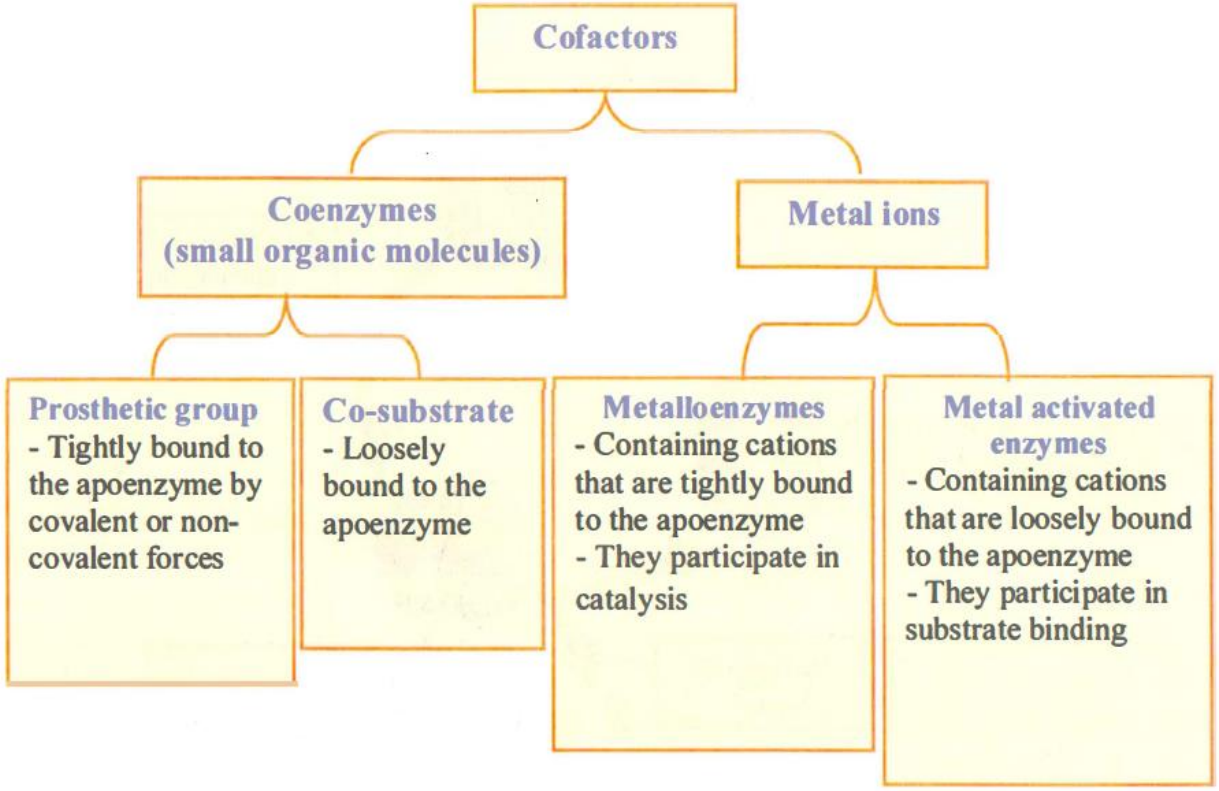
Holoenzyme: refers to the active enzyme with its non-protein component (cofactor)

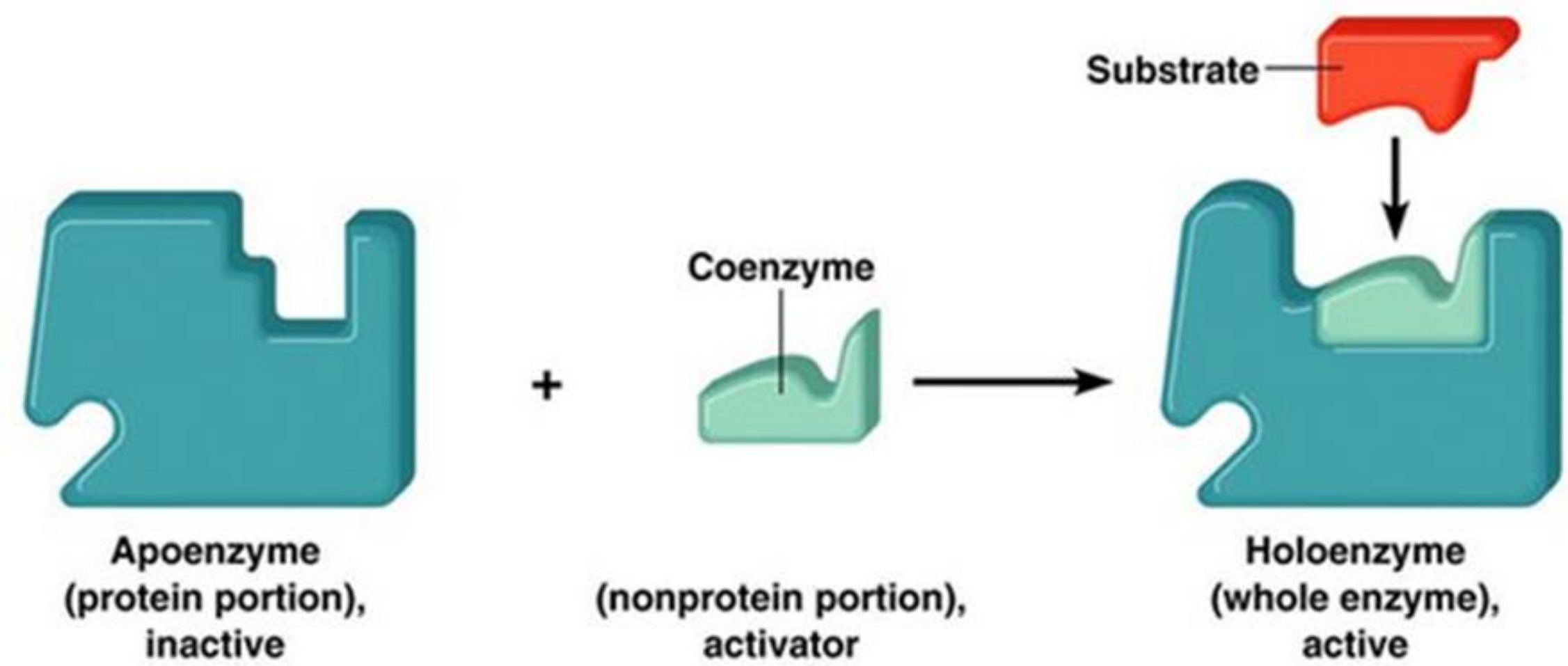
Apoenzyme: enzyme without its cofactor and is inactive

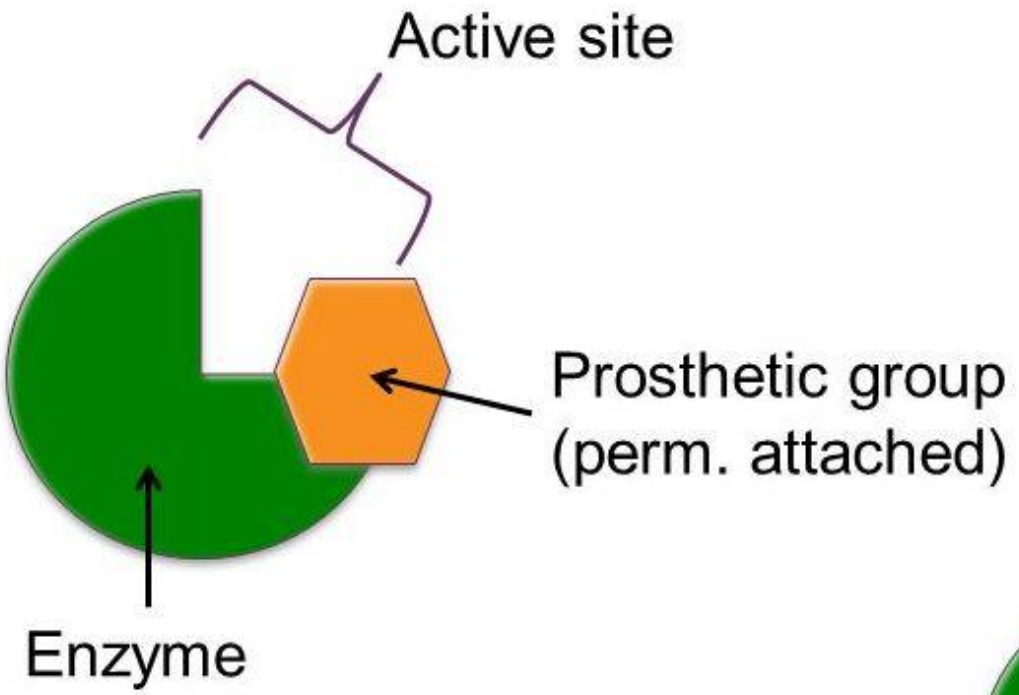


Cofactors

- Prosthetic group mainly provides a **structural property** to the enzyme
- Coenzyme (co-substrates) mainly provides a **functional property** to the enzyme




Substrate
Coenzyme
+
Apoenzyme
(protein portion),
inactive
(nonprotein portion),
activator
Holoenzyme
(whole enzyme),
active

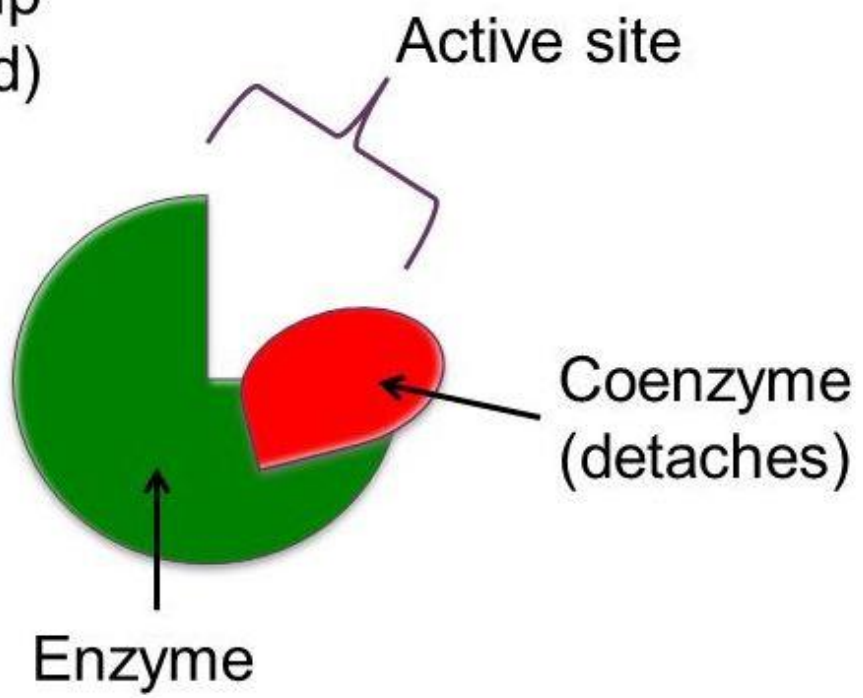


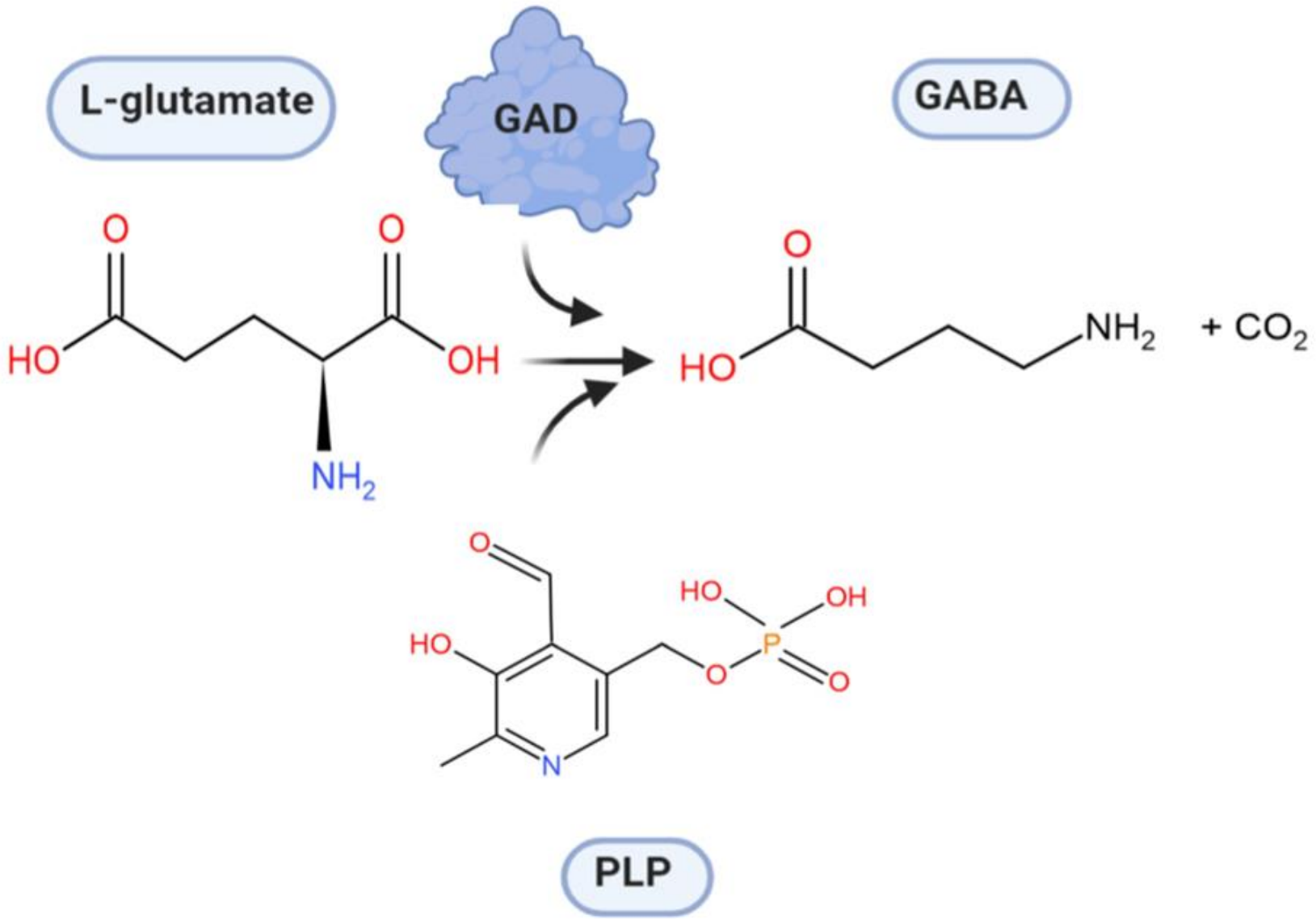
Prosthetic Groups

e.g., FAD

Coenzymes

e.g., NAD




L-glutamate
GAD
GABA
O
O
HO
OH
NH2
O
HO
NH2
+ CO2
O
HO
HO
OH
P
O
O
N
PLP

Coenzymes

- **Are regarded sometimes as second substrate**:
  - Chemical changes in co-enzymes are opposite the substrate (if substrate is oxidised coenzyme is reduced).
  - Reaction in coenzyme is sometimes of greater physiological importance than substrate.

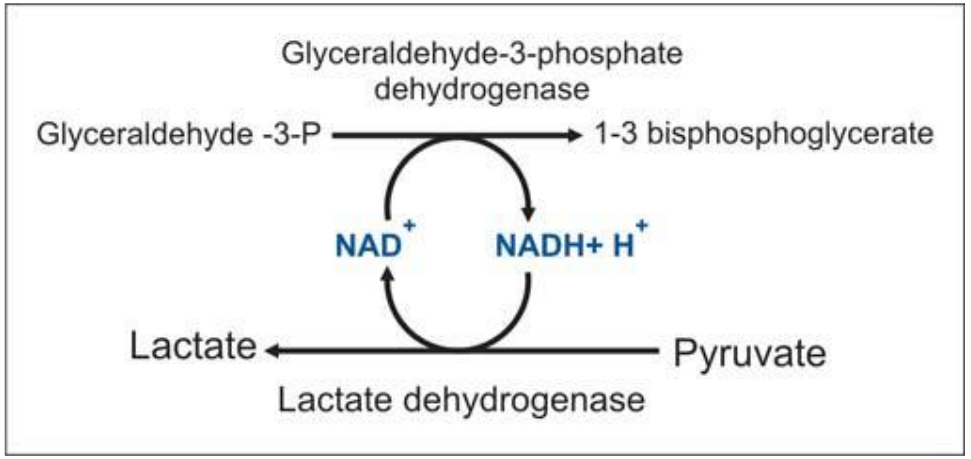
- **Coenzymes are required by:**
  - Oxidoreductases
  - Transferases
  - Isomerase
  - Ligase

- **Coenzymes are not required by**:
  - Hydrolases
  - Lyases

Coenzymes are classified into

- Involved in hydrogen or electron transfer
  - Nicotinamide nucleotides (NAD, NADP)
  - Flavin nucleotides (FMN, FAD)
  - Glutathione
  - Coenzyme Q

- Involved in transfer of other groups
  - Thiamine pyrophosphate (TPP) (carries alpha keto acids and glycoladehyde)
  - Pyridoxal phosphate (PLP) (carries amino acids and amino groups)
  - Coenzyme A (CoA) (carries carboxylic acid)
  - Biotin (carries carbon dioxide)
  - Tetrahydrofolic acid (THF) (carries one carbon unit)
  - Adenosine triphosphate (ATP) (carries phosphate)



One co-enzyme molecule can work with
different enzymes

Metalloenzymes: These are enzymes which require certain metal ions for their activity

Table 5.2. Metallo-enzymes

Metal	Enzyme containing the metal
Zinc	Carbonic anhydrase, carboxy peptidase, alcohol dehydrogenase
Magnesium	Hexokinase, phospho fructo kinase, enolase, glucose-6-phosphatase
Manganese	Phospho gluco mutase, hexokinase, enolase, glycosyl transferases
Copper	Tyrosinase, cytochrome oxidase, lysyl oxidase, superoxide dismutase
Iron	Cytochrome oxidase, catalase, peroxidase, xanthine oxidase
Calcium	Lecithinase, lipase
Molybdenum	Xanthine oxidase

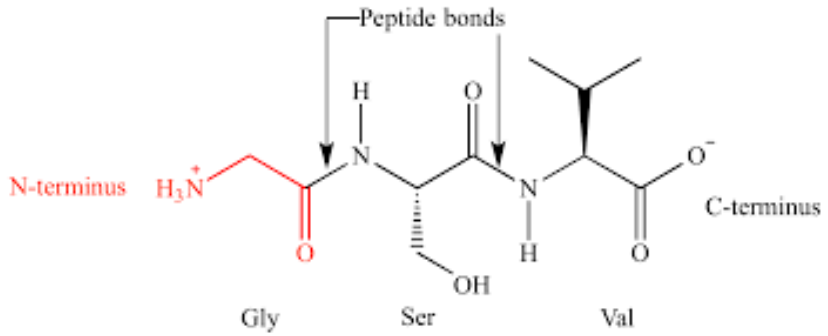
Specificity of enzymes

1. Absolute Specificity

- Some enzymes are absolutely specific.
- For example: hydrolysis of **urea** to **ammonia** and **carbon dioxide** is catalyzed by urease (urea is the only substrate for urease).

2. Bond Specificity

- Most of the proteolytic enzymes are showing group (bond) specificity.
- For example, trypsin can hydrolyse **peptide bonds** formed by carboxyl groups of **arginine** or **lysine** residues in any protein.



Specificity of enzymes

3. Group Specificity

- One enzyme can catalyse the same reaction on a group of structurally similar compounds.
- e.g. hexokinase can catalyse phosphorylation of glucose, galactose and mannose.

4. Stereospecificity

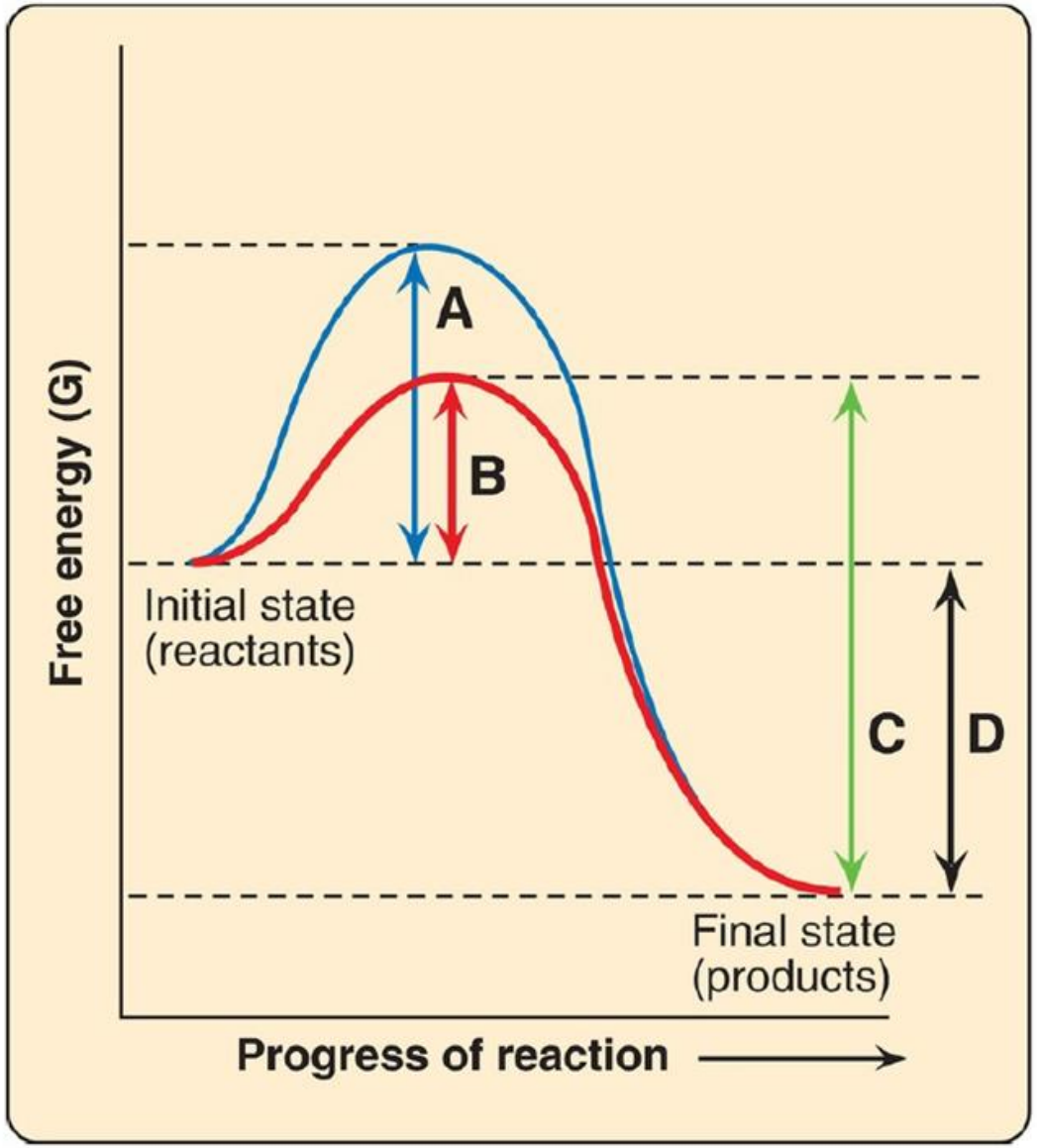
- Human enzymes are specific for **L-amino acids** and **D-carbohydrates**
- Lactate dehydrogenase, acting on pyruvate will form only L-lactate, but not the D variety
- Cellulose cannot be digested due to lack of β enzymes in humans.

Questions

Use the graph below that shows the changes in free energy when a reactant is converted to a product in the presence and absence of an enzyme. Select the letter that best represents:

1. the activation energy of the catalyzed forward reaction.

2. the free energy of the reaction.



Questions

Alcohol dehydrogenase (ADH) requires oxidized nicotinamide adenine dinucleotide (NAD+) for catalytic activity. In the reaction catalyzed by ADH, an alcohol is oxidized to an aldehyde as NAD+ is reduced to NADH and dissociates from the enzyme. The NAD+ is functioning as a/an:

A. apoenzyme.

B. coenzyme–cosubstrate.

C. coenzyme–prosthetic group.

D. cofactor.

E. heterotropic effector.