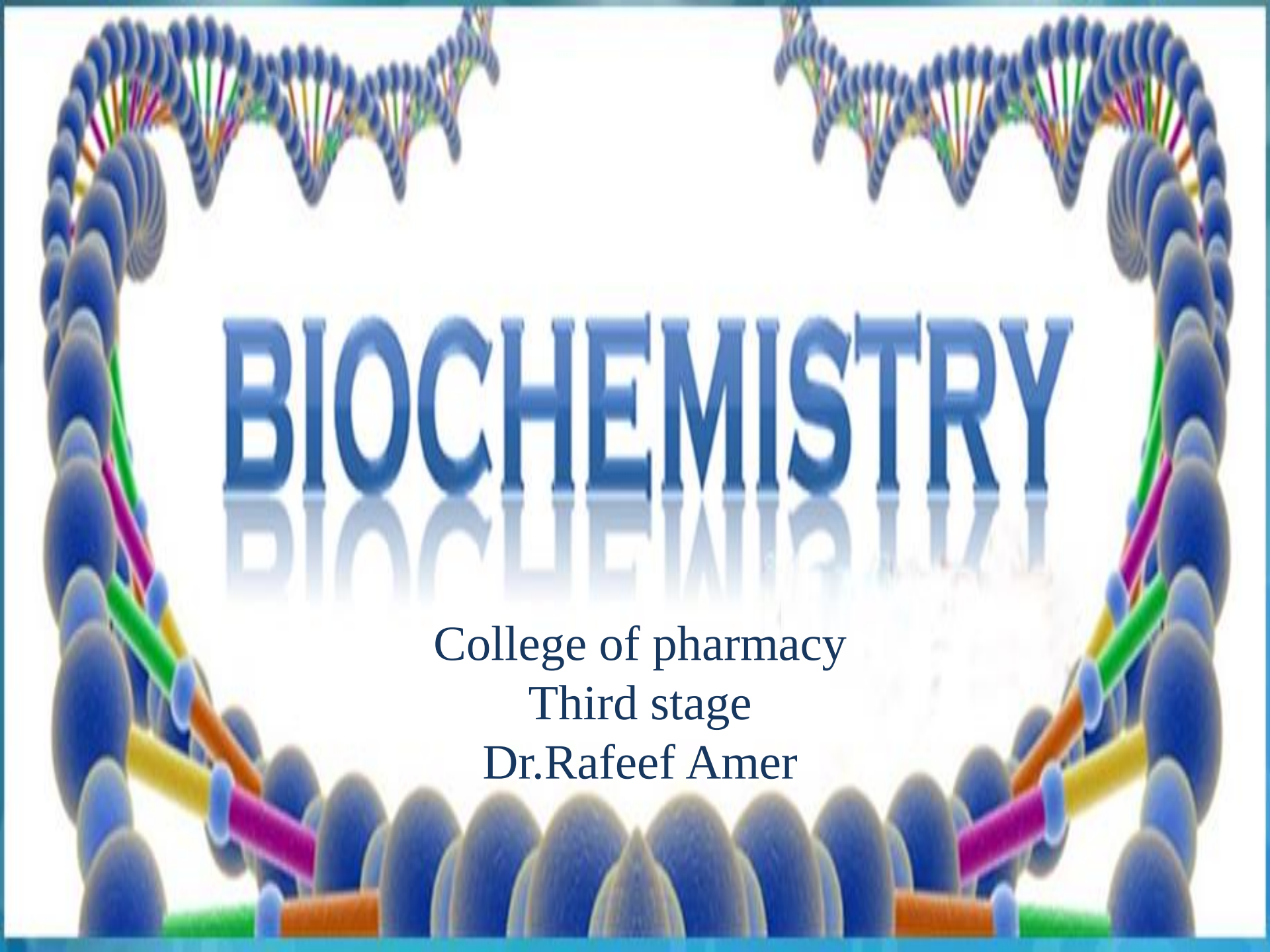
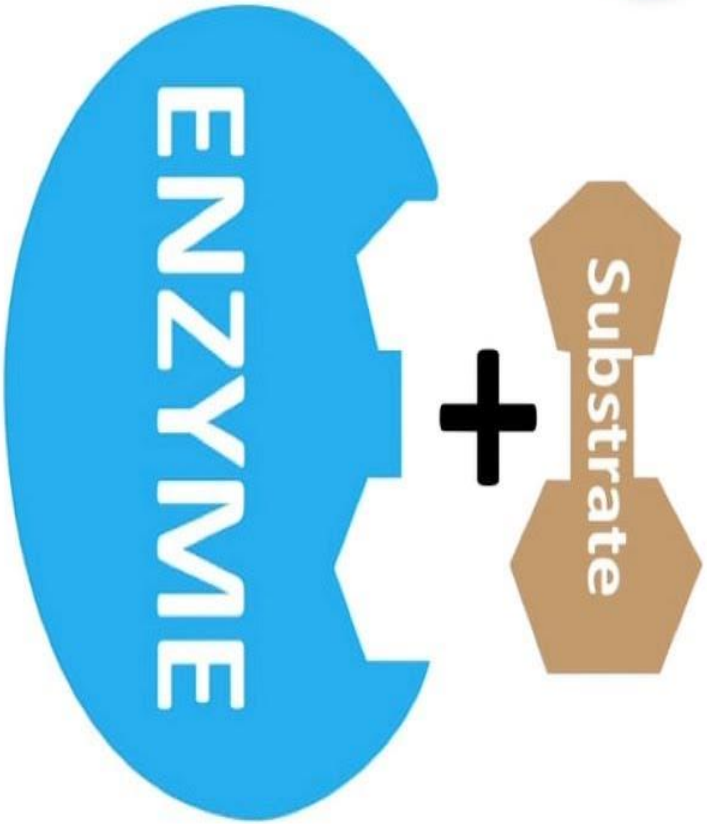


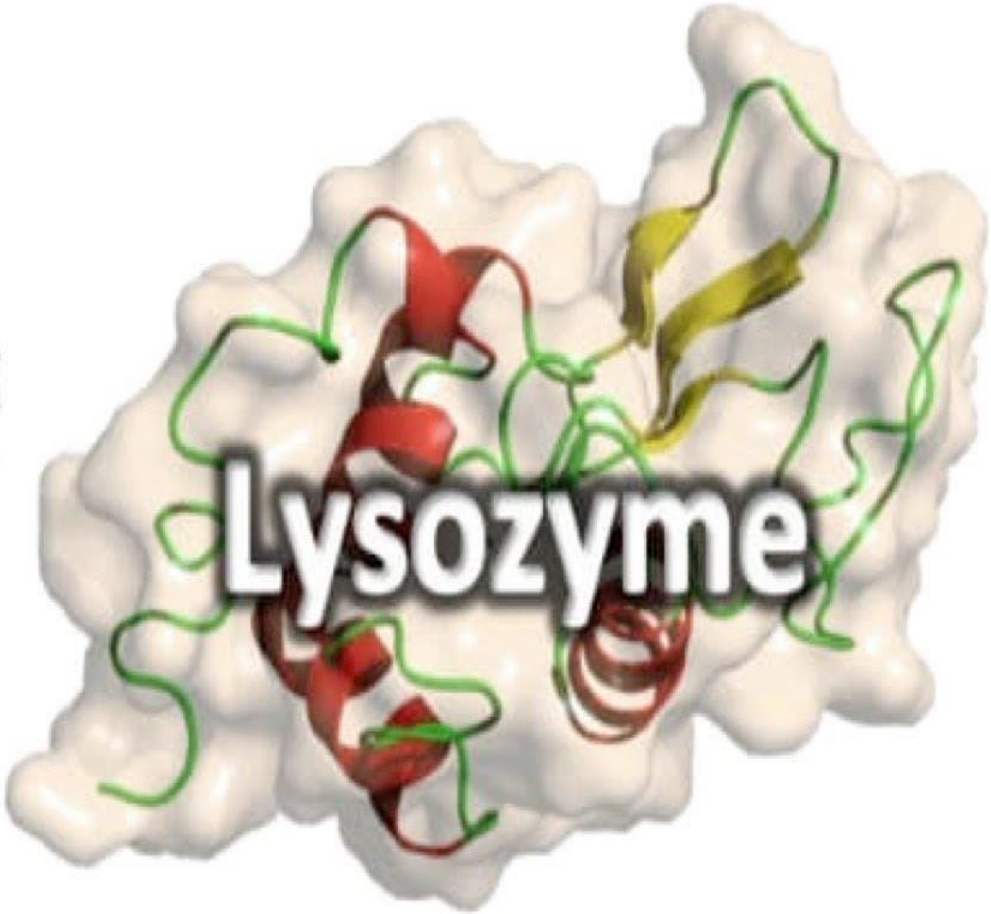
College of pharmacy

Third stage

Dr.Rafeef Amer

Enzymes!





Enzyme

- ***Enzymes*** are very efficient catalysts for biochemical reactions. present in the cell in small amounts their function are to speed up the chemical reactions.

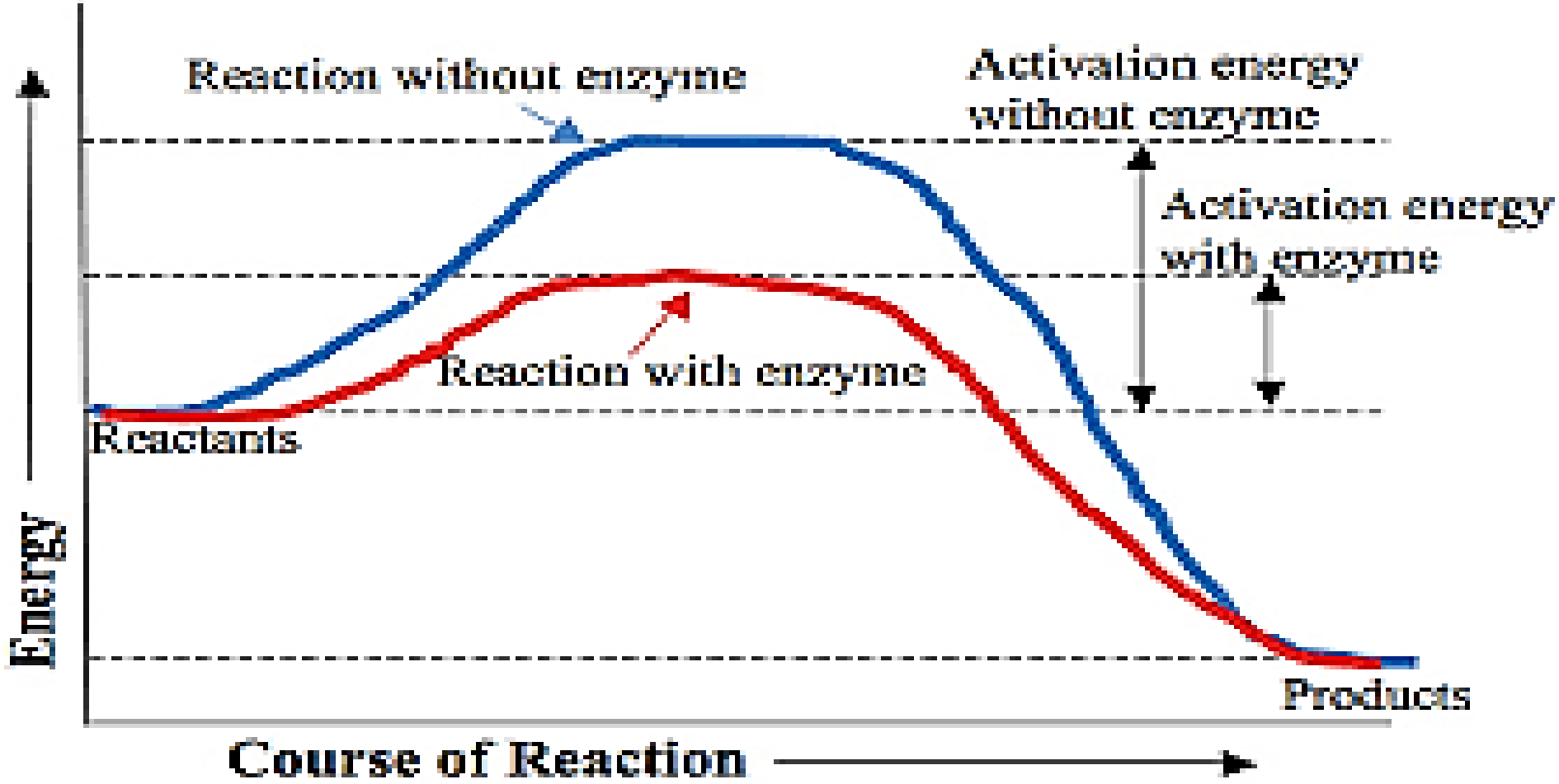
The Catalytic Sites:

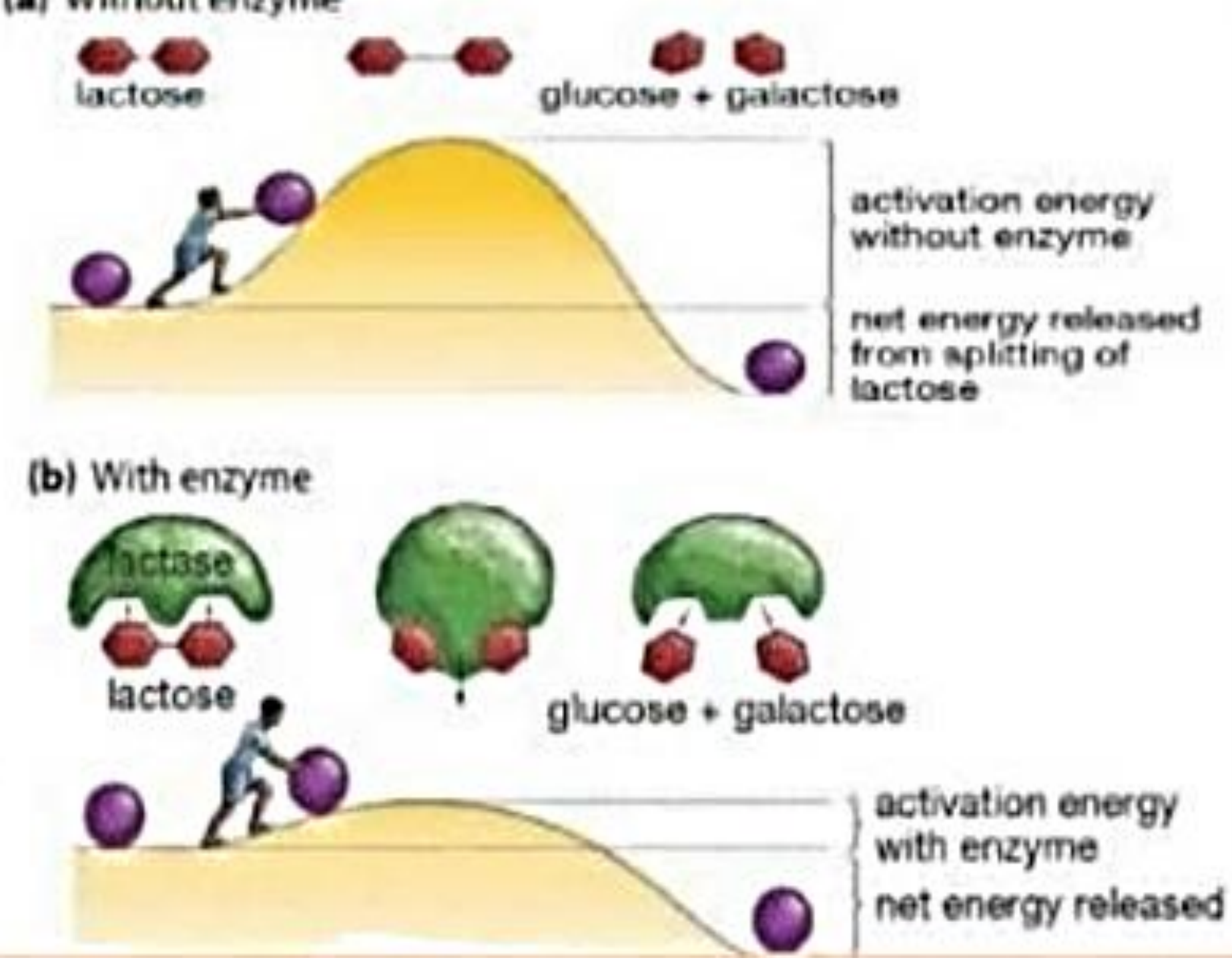
Enzymes are much larger compared to the substrate molecules. the substrate is in contact with only a very small area less than 5% of the total enzymic surface. This part of the enzyme comprising amino acid residues and peptide bonds that are in physical contact with the substrate but essential for catalytic activity put together constitute **an active site**, presently referred as the **catalytic site**.

Excluding the catalytic site, the rest of the enzyme molecule may be necessary for maintaining the correct three-dimensional conformation of the catalytic site or it may just be there without any functional role. The structure of a catalytic site has been studied in some enzymes. It is either a **crevice** on the enzyme as in papain and ribonuclease or a **deep pit** as in carbonic anhydrase. Whatever the shape of the catalytic site may be, it is believed that the correct substrate binds with the catalytic site producing a substrate-catalytic site complex . The term **productive binding** is often applied to this complex. In productive binding, both the enzymes and substrates show conformational changes with a reduction in **activation energy** so that the substrate is converted into a product.

Enzyme

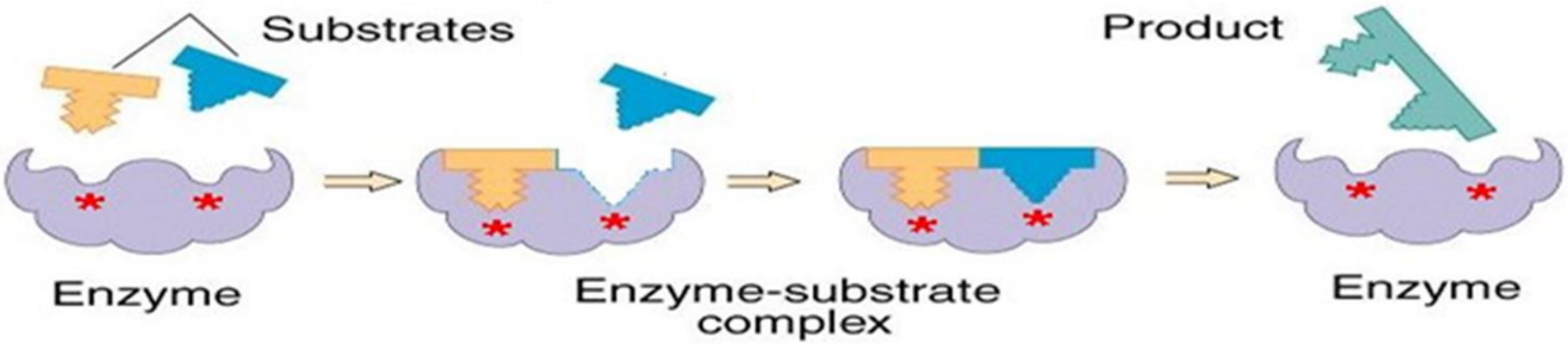
Enzymes allow chemical reactions to occur fast enough to support life by lowering the energy of activation for chemical reactions . Energy of activation is that minimal amount of energy which is required of a molecule to take part in a reaction.




lactose
glucose + galactose
activation energy without enzyme
net energy released from splitting of lactose
(b) With enzyme
lactose
lactose
glucose + galactose
activation energy with enzyme
net energy released

Enzyme

There are hundreds of different enzymes each speed up only one kind of reaction, **enzymes are specific.**



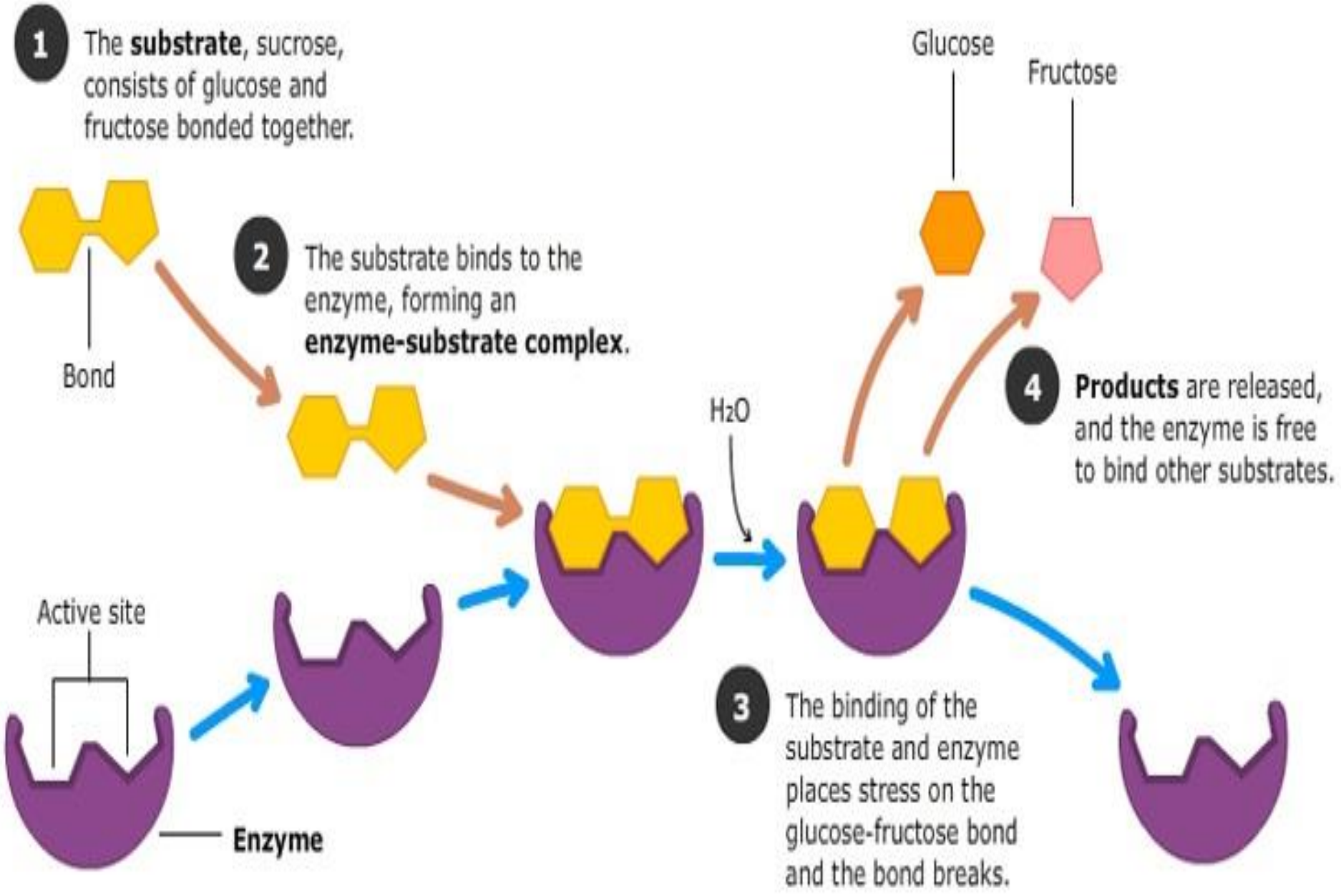
A few **enzymes** exhibit absolute **specificity**; that is, they will catalyze only one particular reaction. Other **enzymes** will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of **specificity.**

1.Absolute specificity

Absolute specificity in which an enzyme acts upon one **specific substrate**. Absolute specific enzymes will only catalyze one reaction with its specific substrate. For example, lactase is an enzyme specific for the degradation of lactose into two sugar monosaccharides, glucose and galactose.

2.Group specificity

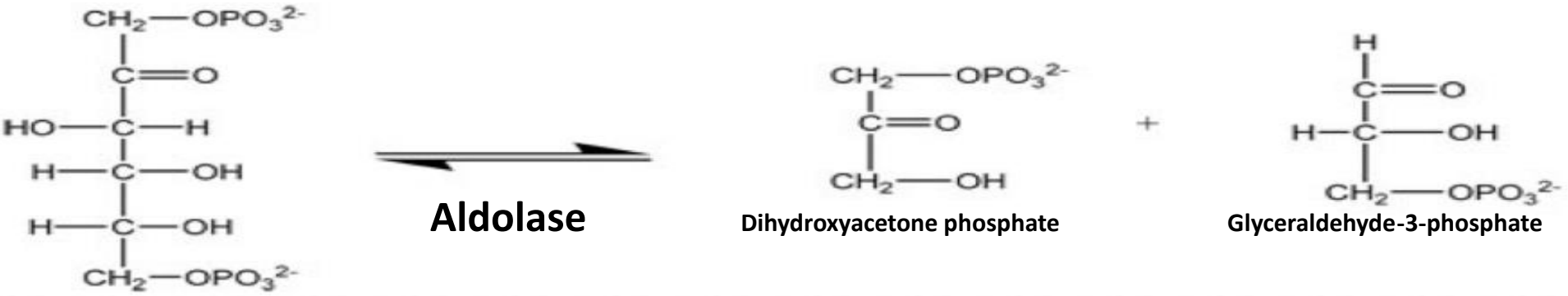
Group specificity occurs when an enzyme will only reacts with molecules that have specific functional groups, such **as aromatic structures, phosphate groups, and methyls**. One example is Pepsin, an enzyme that is crucial in digestion of foods ingested in our diet, that hydrolyzes peptide bonds in between hydrophobic amino acids, with recognition for aromatic side chains such as phenylalanine, tryptophan, and tyrosine.


1 The substrate, sucrose, consists of glucose and fructose bonded together.
Bond
2 The substrate binds to the enzyme, forming an enzyme-substrate complex.
Active site
Enzyme
H2O
3 The binding of the substrate and enzyme places stress on the glucose-fructose bond and the bond breaks.
Glucose
Fructose
4 Products are released, and the enzyme is free to bind other substrates.

Enzyme

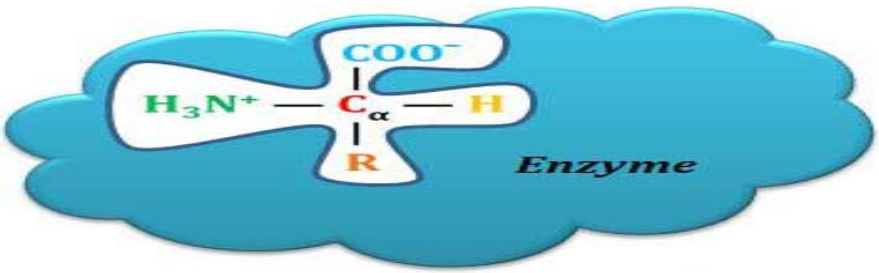
3. Bond specificity

Bond specificity, unlike group specificity, recognizes particular chemical bond types like the Figure below the reaction that illustrates an enzyme cleaving a specific bond of the reactant in order to create two products another example is the peptide bond).

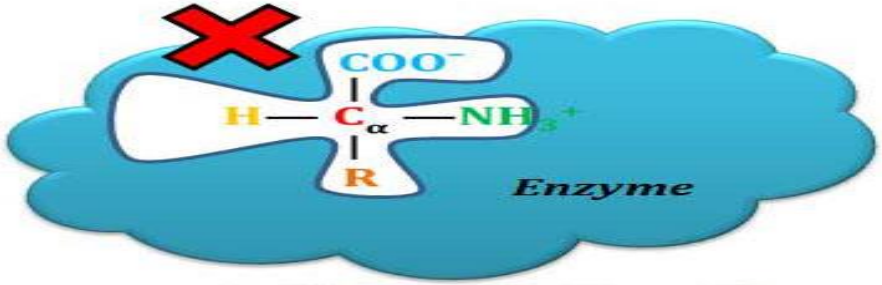


4.Stereochemical specificity

Enzymes that are stereochemically specific will bind substrates with these particular properties. For example, Sugars containing alpha-glycosidic linkages This type of specificity is sensitive to the substrate's optical activity of orientation. Stereochemical molecules differ in the way in which they rotate plane polarized light, or orientations of linkages (see alpha, beta glycosidic linkages). beta-glycosidase will only react with beta-glycosidic bonds which are present in cellulose, but not present in starch and glycogen, which contain alpha-glycosidic linkages. Another example in the Figure below

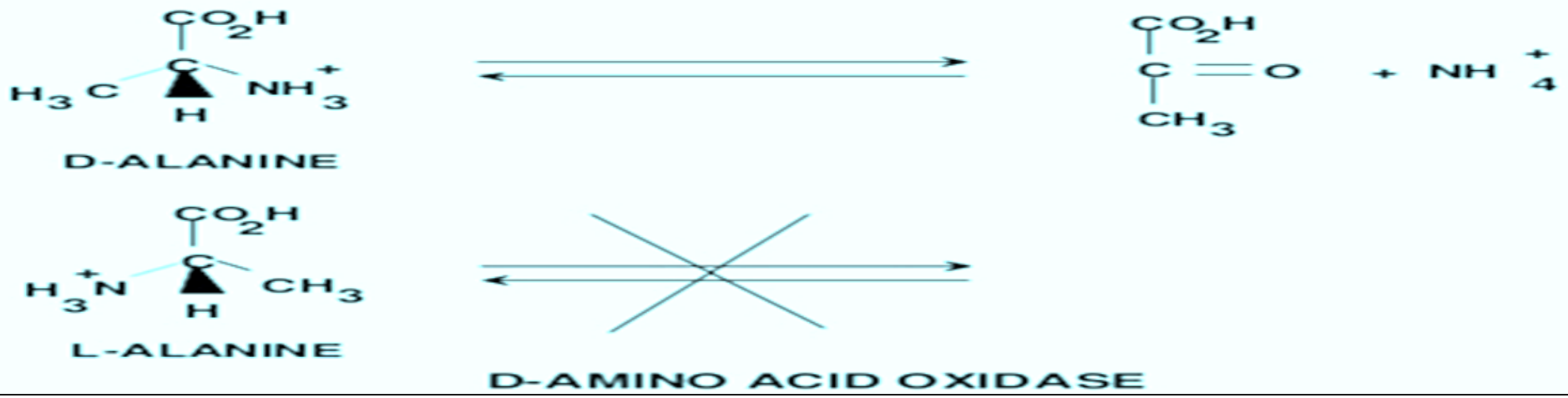


L-Alanine Oxidase & L-Alanine

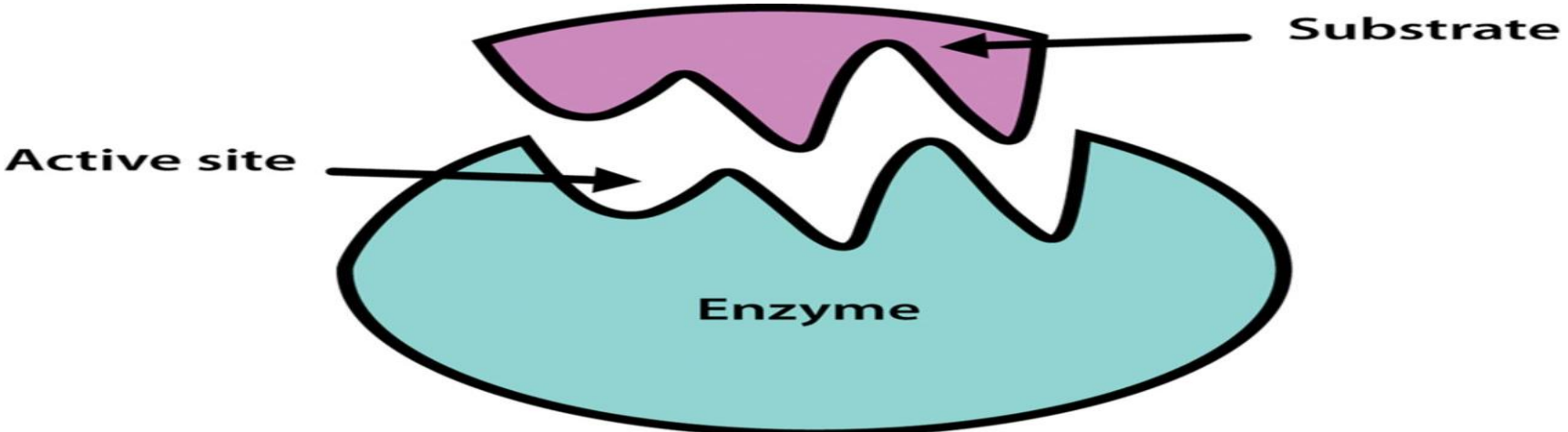


L-Alanine Oxidase & D-Alanine

Enzyme



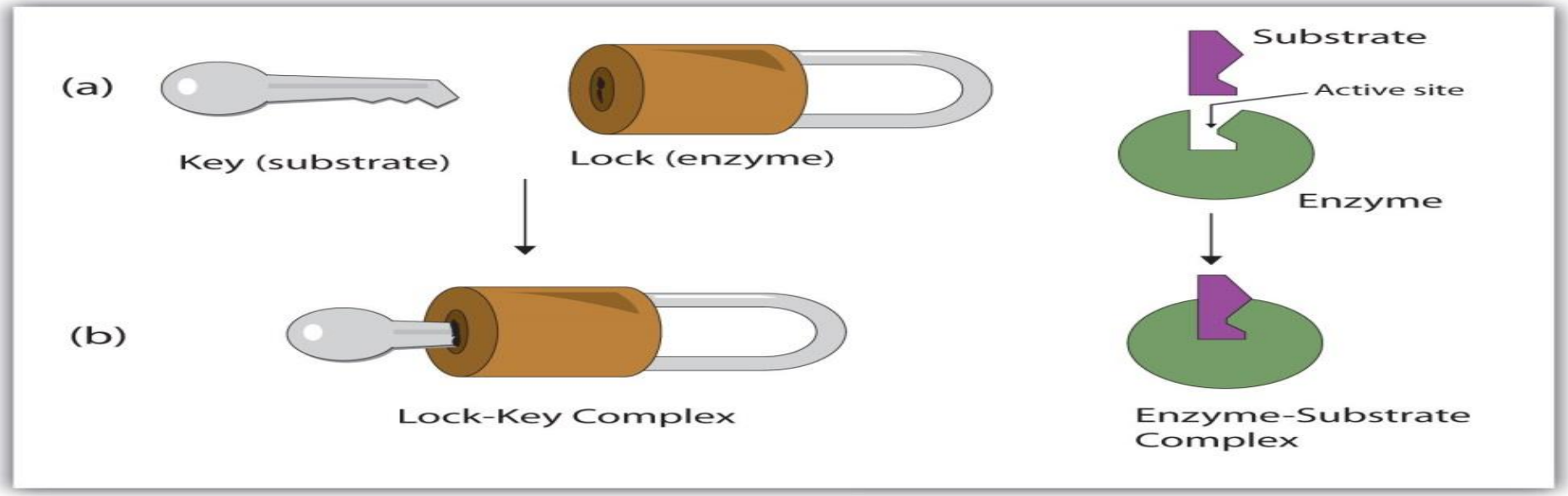
- **The active site** is the region of an enzyme where substrate molecules bind and undergo a chemical reaction.The active site consists of residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site).



lock and key model

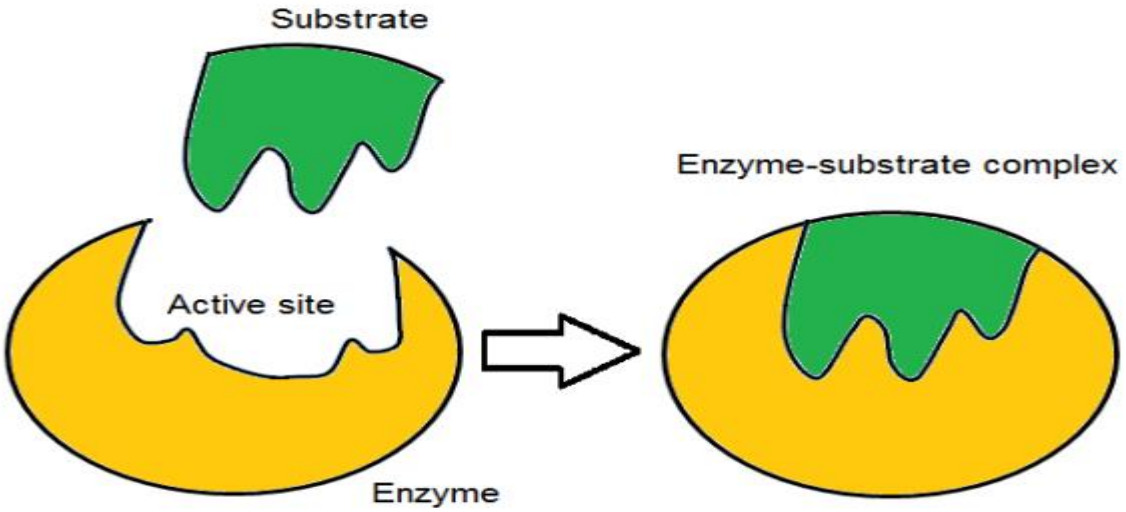
There are two theories that describe the binding of enzymes to the substrate:

- Emil Fischer in 1894 suggested that both a substrate and an enzyme have specific geometric shapes that fit exactly into each other.
- The problem with this hypothesis is that it doesn't explain the stabilization of the enzyme. When an enzyme has a substrate enter into its active site, the enzyme will change its shape slightly to match the substrate. If the enzymes were to be specifically designed to fit a substrate, then there would be no need for it to have to adjust its shape.
- Koshland suggested a slight modification to the lock and key hypothesis that since enzymes were so flexible, the active site is constantly being reshaped by its interaction with the substrate.



Induced Fit model

This theory maintains that the active site and the substrate are, initially, not perfect matches for each other. Rather, the substrate induces a change of shape in the enzyme.



Enzymes- The Induced Fit Model - YouTube.MP4

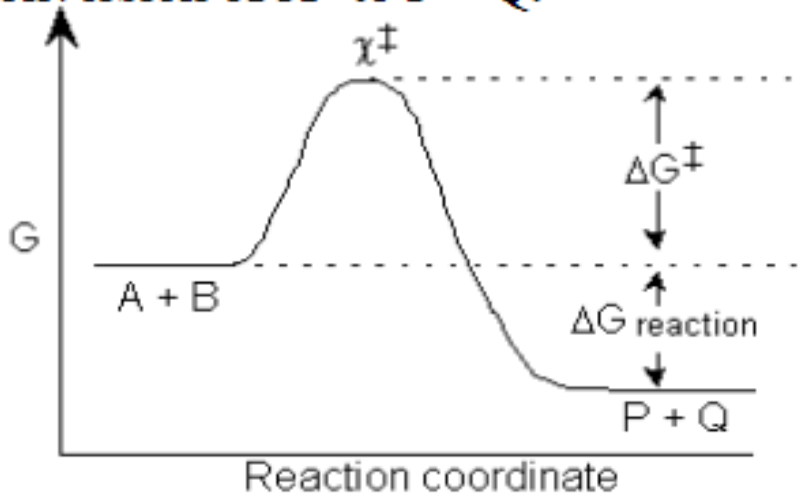
Transition state stabilization

I. General Aspects of enzymatic reactions and transition states

Consider a reaction $A + B \rightarrow P + Q$

$$A + B \overset{K^{\ddagger}}{\rightleftharpoons} X^{\ddagger} \overset{k'}{\rightarrow} P + Q$$

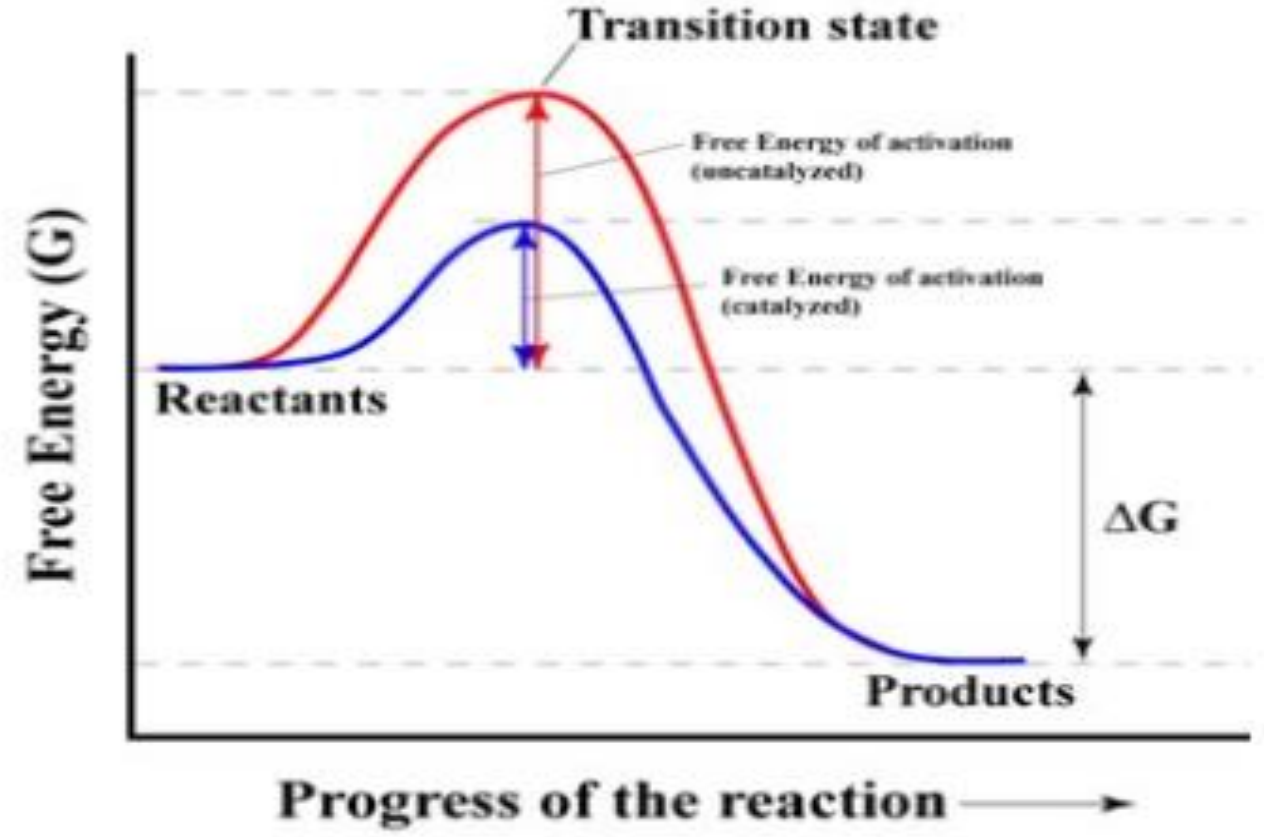
where A + B react through transition state, $X^{\ddagger}$, to form products P + Q. $K^{\ddagger}$ is the equilibrium constant between A + B and $X^{\ddagger}$ and k' is the rate constant for conversion of $X^{\ddagger}$ to P + Q.



The minimum energy pathway of the reaction is shown in the reaction coordinate, or transition state diagram, at left. Chemical conversion of A + B to P + Q proceeds through a transition state $X^{\ddagger}$ which is the least stable (least probable, highest free energy) species along the pathway. Molecules that achieve the activation energy, $\Delta G^{\ddagger}$, can go on to react while molecules that fail to achieve the transition state fall back to the ground state.

The transition state, $X^{\ddagger}$, is metastable with only a transient existence. The less stable the transition state, the more difficult it is for a reaction to proceed.

Transition state stabilization

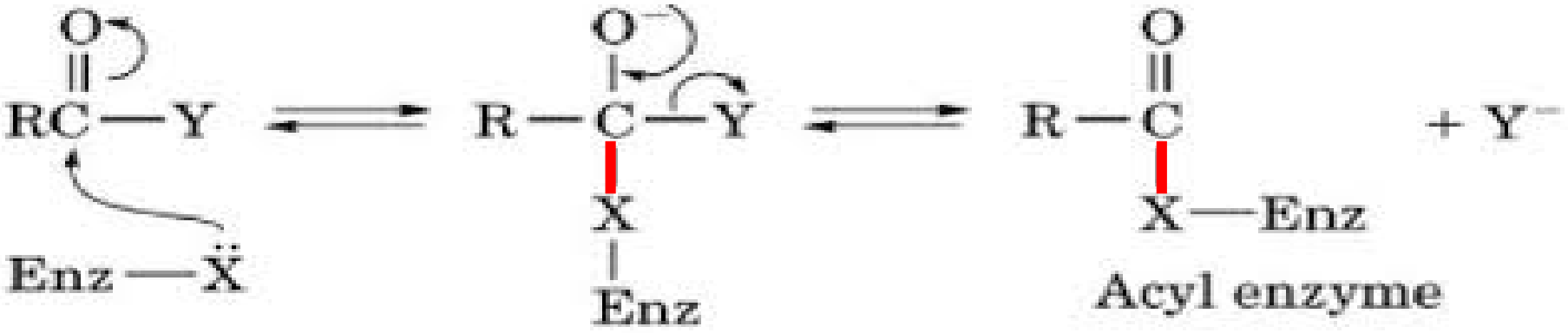


Enzyme Catalytic Mechanism

- **A catalyst lowers the activation energy of a reaction, which allows for a different reaction pathway to be followed.**
- **simply serves to speed up the reaction rate, often exponentially.**
- **it is not consumed and thus the same enzyme may be used to catalyze the same reaction again in the future.**

There are four strategies that enzymes use to catalyze specific reactions.

1. Covalent Catalysis: In Covalent Catalysis, the substrate forms a temporary covalent bond with a reactive group usually a good nucleophile, in the active site, and the complex is then incorporated into the catalysis of the reaction, The covalent complex is more reactive than the substrate itself originally was. This may serve to reduce the energy required for later states of the reaction. The enzyme, of course, is not used up during the reaction and thus must be regenerated at some point by breaking the temporary covalent bond. An example of an enzyme following such a mechanism is **chymotrypsin**, which is an enzyme that cleaves peptide bonds by a hydrolysis reaction, or a protease. Some examples of nucleophilic groups in proteins are serine and tyrosine (presence of a hydroxyl group), histidine (imidazole group), lysine (amino group), cystine (thiol group).

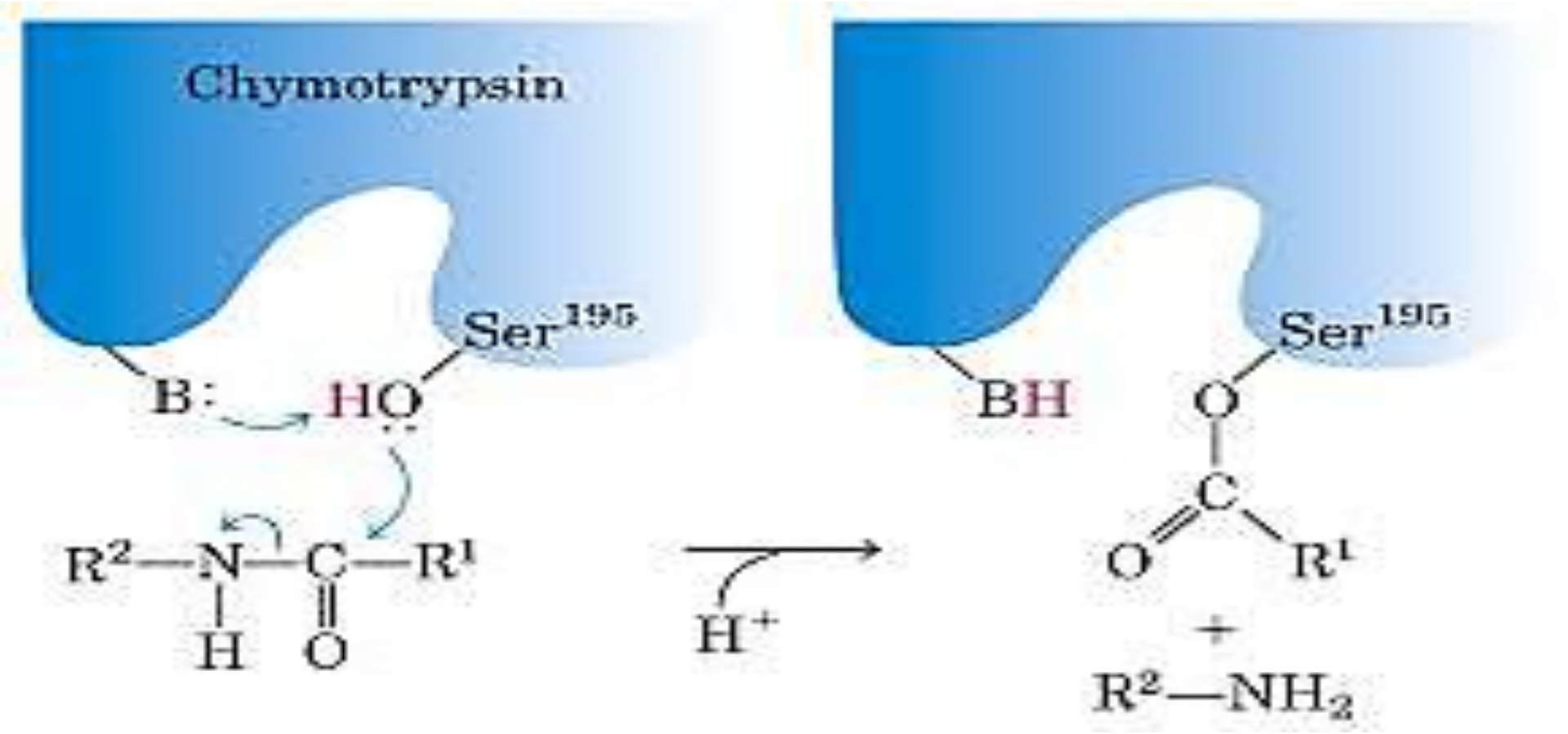


Enzyme Catalytic Mechanism

2.Acid-Base Catalysis: In Acid-Base Catalysis , an acid or base catalyzes a reaction by being a proton donor or acceptor. The acid is often a donor whereas the base is often an acceptor (e.g. a hydroxyl ion).

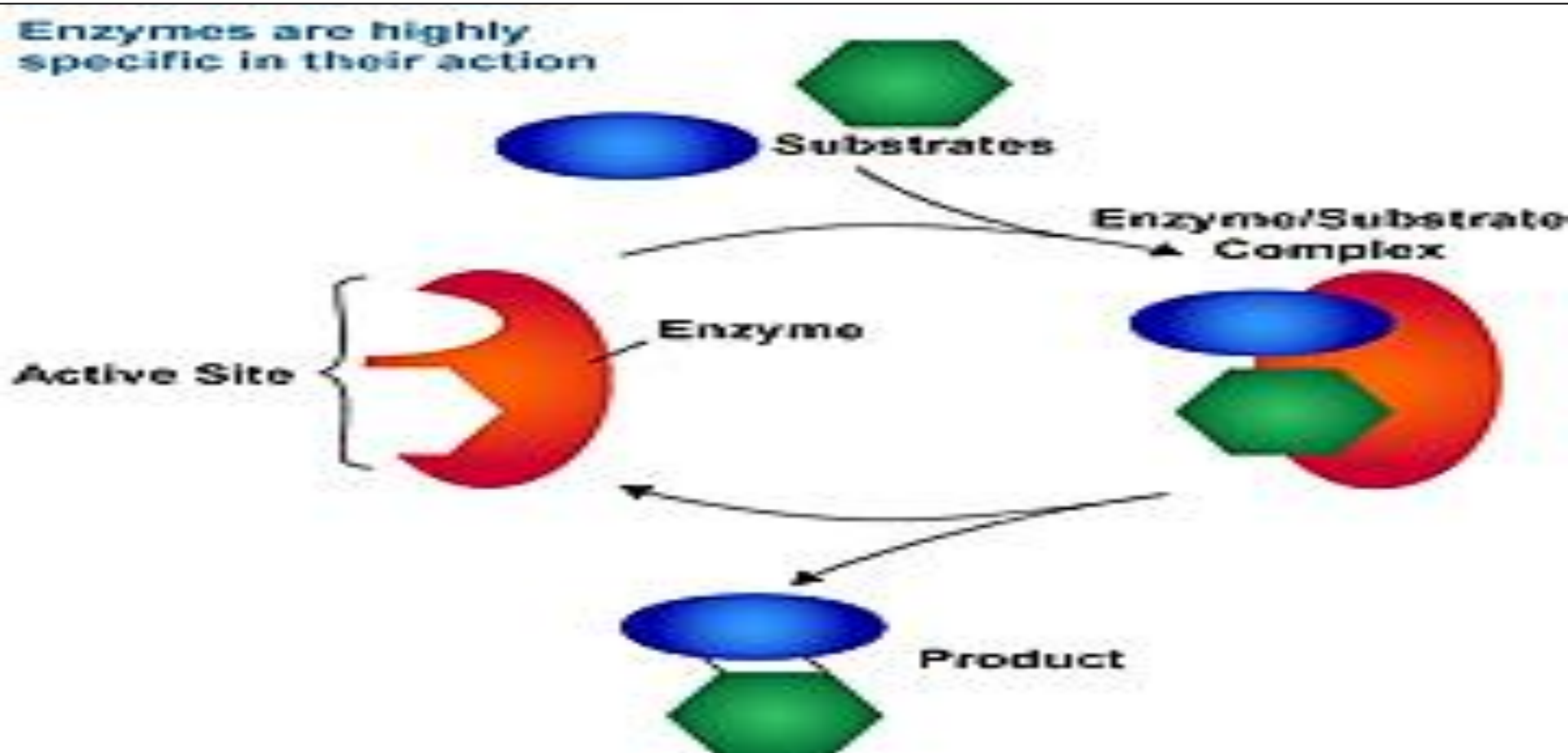
the catalyst donates or accepts a proton to create a better leaving group in order to jump start the reaction.

In general acid-base catalysis, molecules can take the role of the protone donor or acceptor Examples may be **cofactors**, or residues from amino acid side chains of the enzyme like the **Histidine** which is an effective catalyst. the enzyme is formed again at some point in the reaction and is not consumed.



Enzyme Catalytic Mechanism

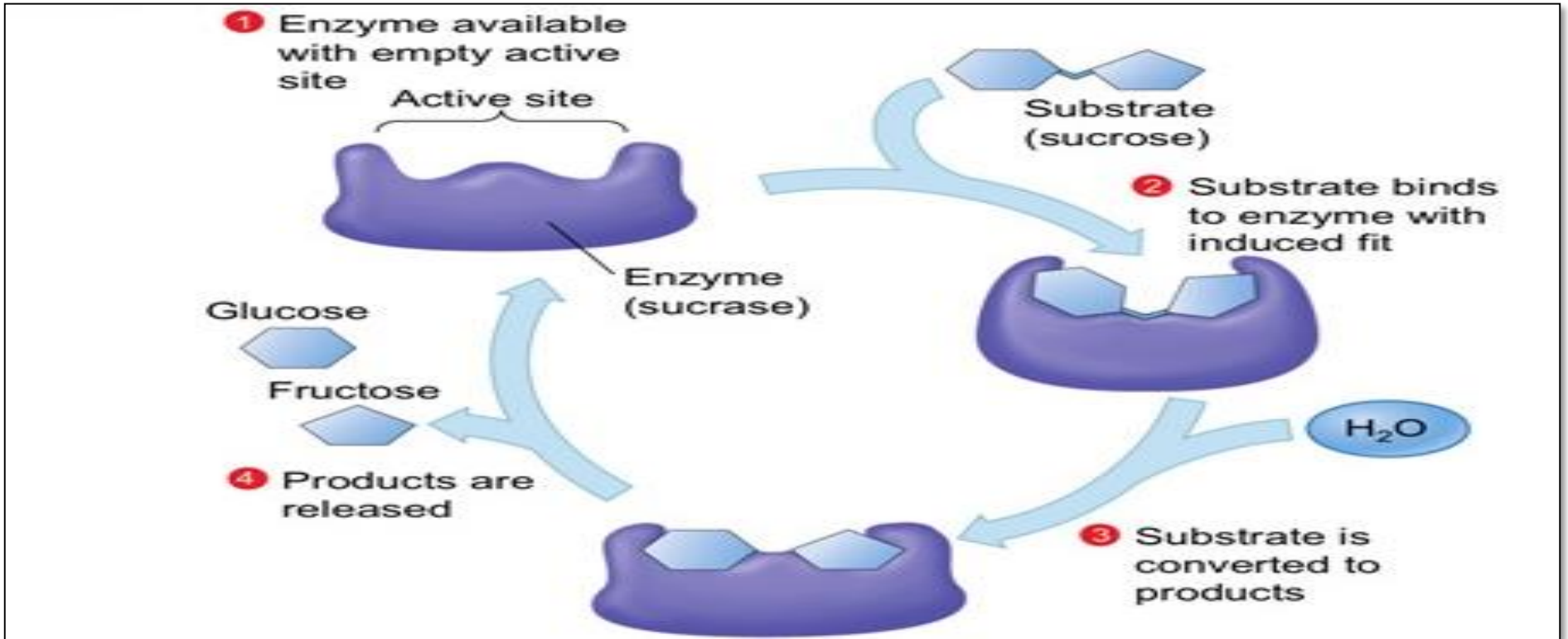
3.Catalysis by Approximation: is described by two substrates bound to one another so that they are close together near the site of reaction along the enzyme, thus increasing the reaction rate. A substrate may also be brought into contact with a catalytic group rather than with another substrate . This strategy takes advantage of binding energy and positions the substrates in the correct orientation for the reaction to proceed.



Enzyme Catalytic Mechanism

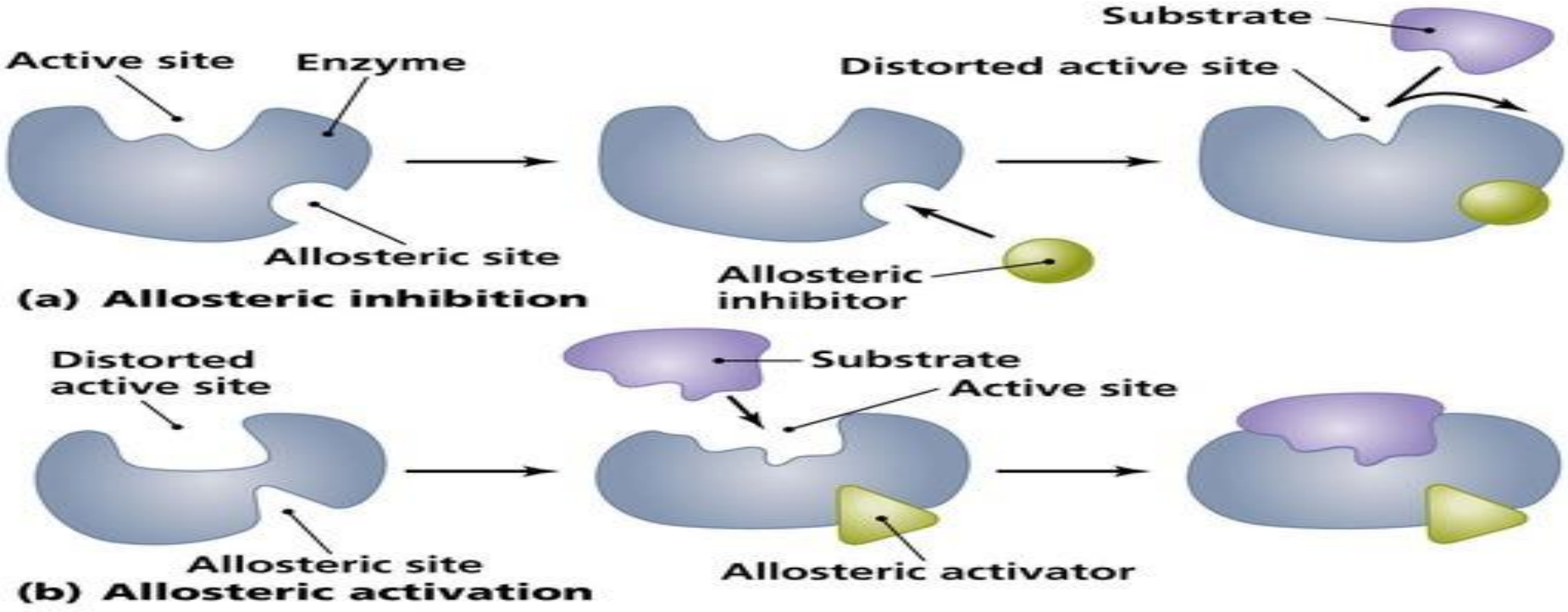
4.Catalysis by Bond Strain: In this form of catalysis, the induced structural rearrangements that take place with the binding of substrate and enzyme ultimately produce strained substrate bonds, which more easily reach the transition state. The new conformation often forces substrate atoms and bulky catalytic groups, such as aspartate and glutamate, into conformations that strain existing substrate bonds.

- Strain is created by binding to substrates in a conformation slightly unfavorable for the bond to undergo cleavage.
- The strain stretches or distorts the targeted bond. Weakening it more vulnerable to cleavage.



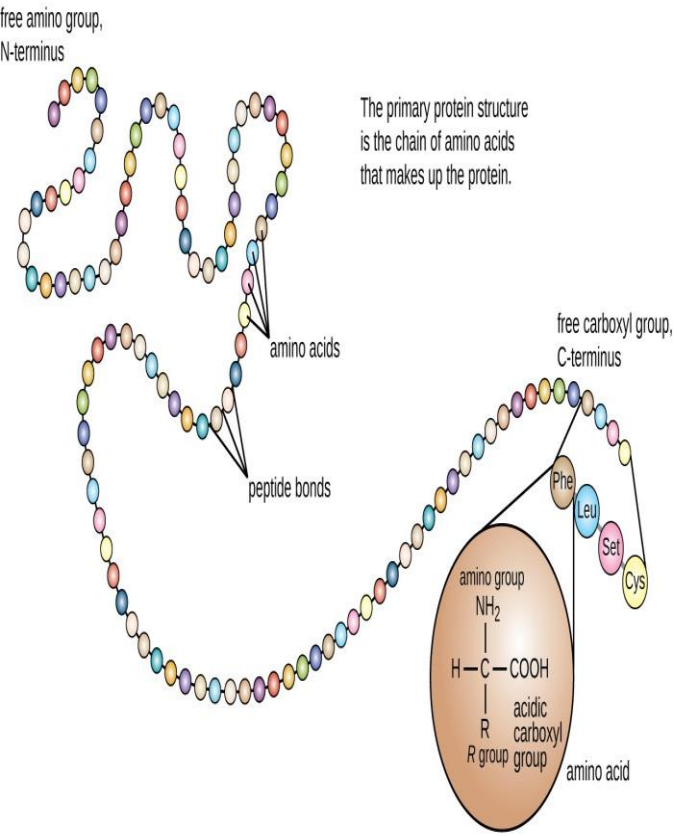
Enzymes allosteric modulation

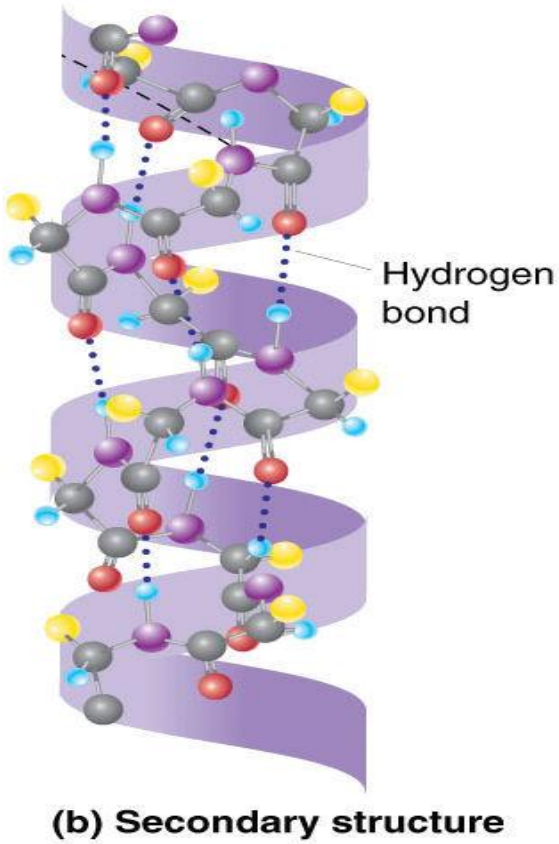
Regulatory enzymes exhibit increased or decreased catalytic activity in response to certain signals. By the action of such regulatory enzymes, the rate of each metabolic sequence is constantly adjusted to meet changes in the cell's demands for energy and for biomolecules required in cell growth and repair. In most multienzyme systems the first enzyme of the sequence is a regulatory enzyme. Catalyzing even the first few reactions of a pathway . One of the important regulatory enzymes in metabolic pathways are the **Allosteric enzymes their** function through reversible, noncovalent binding of a regulatory metabolite called a modulator . The regulatory enzymes tend to have multiple subunits, and in some cases the regulatory site(s) and the active site are on separate subunits.

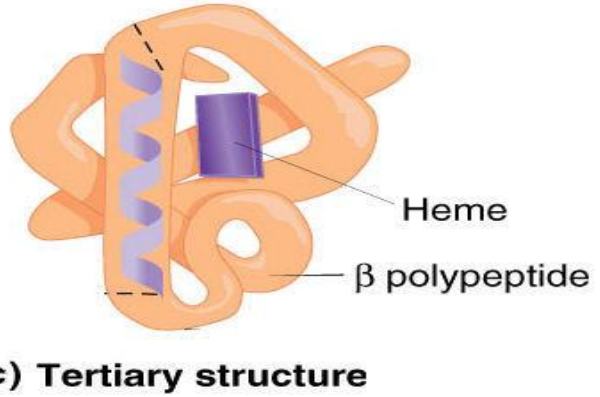


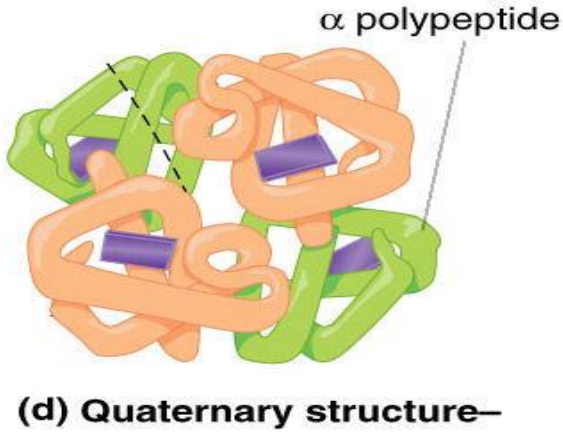
Chemical Nature of Enzymes

All known enzymes are proteins. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds.





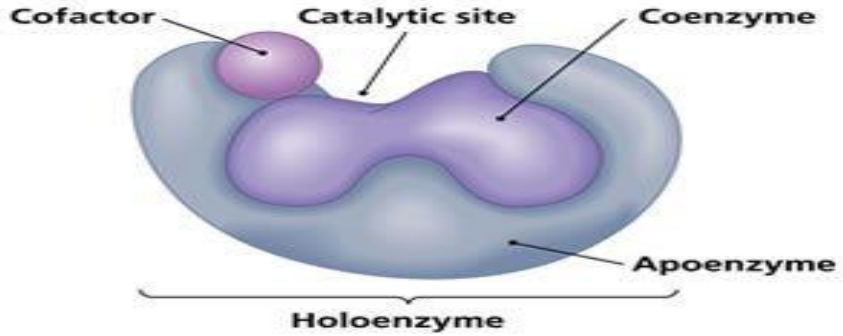




Some enzymes are simple proteins, i.e., on hydrolysis, they yield amino acids only . like Digestive enzymes such as **pepsin**, **trypsin** and **chymotrypsin** are of this nature

Chemical Nature of Enzymes

Many are conjugated enzymes consist of a protein and a non-protein (called the **cofactor**) .



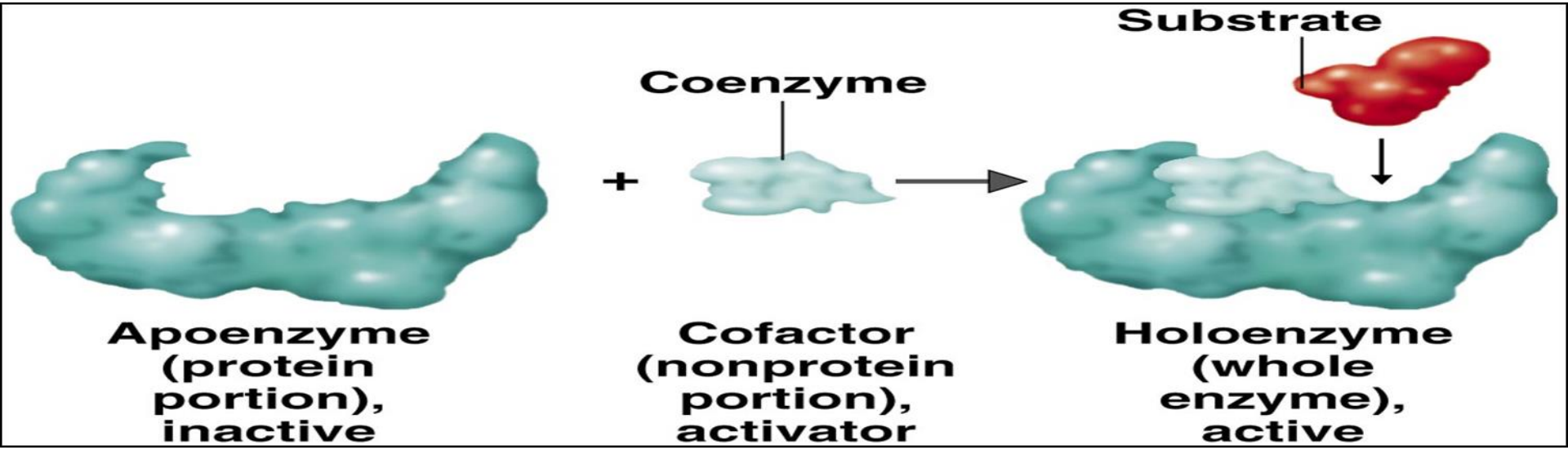
A cofactor is a non-protein chemical compound that is required for the protein's biological activity. Many enzymes require cofactors to function properly. Cofactors can be considered "helper molecules" that assist enzymes in their action. Cofactors can be

- cations - positively charged metal ions (**activators**), which temporarily bind to the active site of the enzyme, giving an intense positive charge to the enzyme's protein.
- organic molecules, usually vitamins or made from vitamins (water soluble vitamins) **(coenzymes)**, which are not permanently bound to the enzyme molecule, but combine with the enzyme-substrate complex temporarily which serve as carriers for chemical groups or electrons. NAD+, NADP+ and coenzyme A (CoA) are examples of coenzymes.

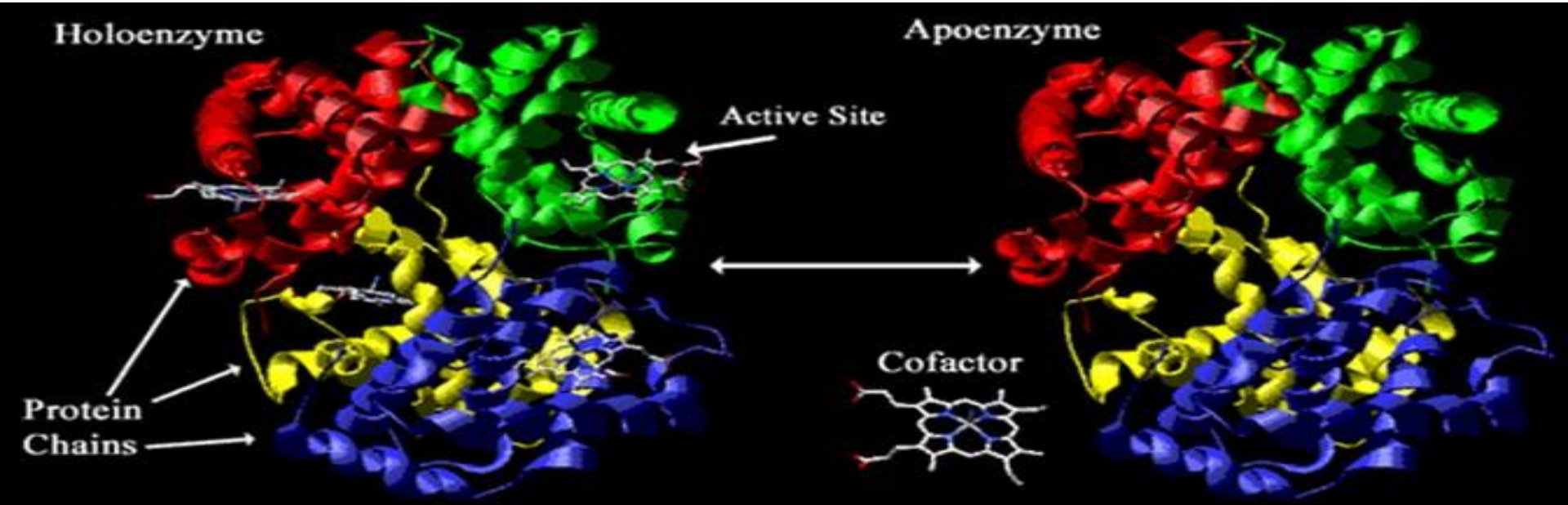
Mechanism of Action

Many cofactors will sit in the enzyme active site and assist the binding of the substrate. An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is called a holoenzyme.

Chemical Nature of Enzymes



Enzyme three dimensional structure

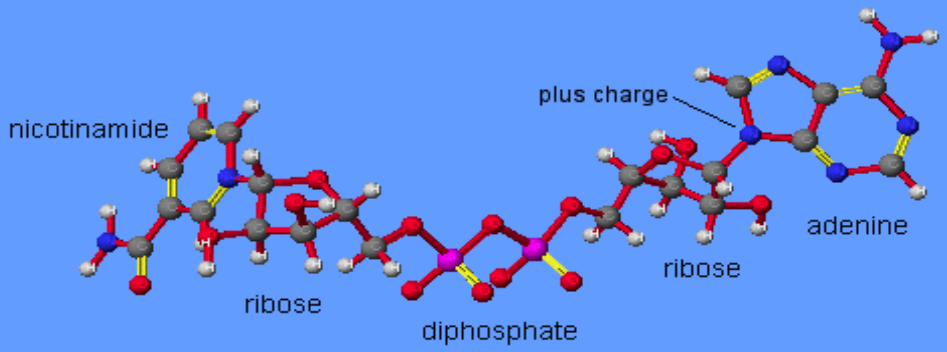


Chemical Nature of Enzymes

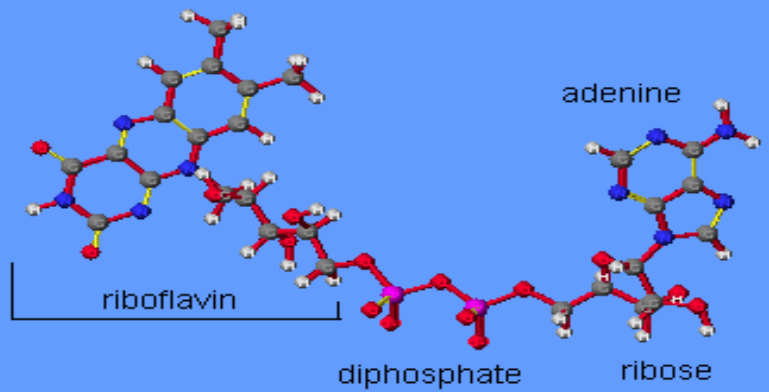
Function of coenzyme

- The coenzyme is **essential** for the biological activity of the enzyme.
- A coenzyme is a **low molecular weight organic substance**, without which the enzyme cannot exhibit any reaction.
- **One molecule** of the coenzyme is able to convert a **large number of substrate** molecules with the help of enzyme.

Nicotinamide Adenine Dinucleotide, NAD+
Coenzyme



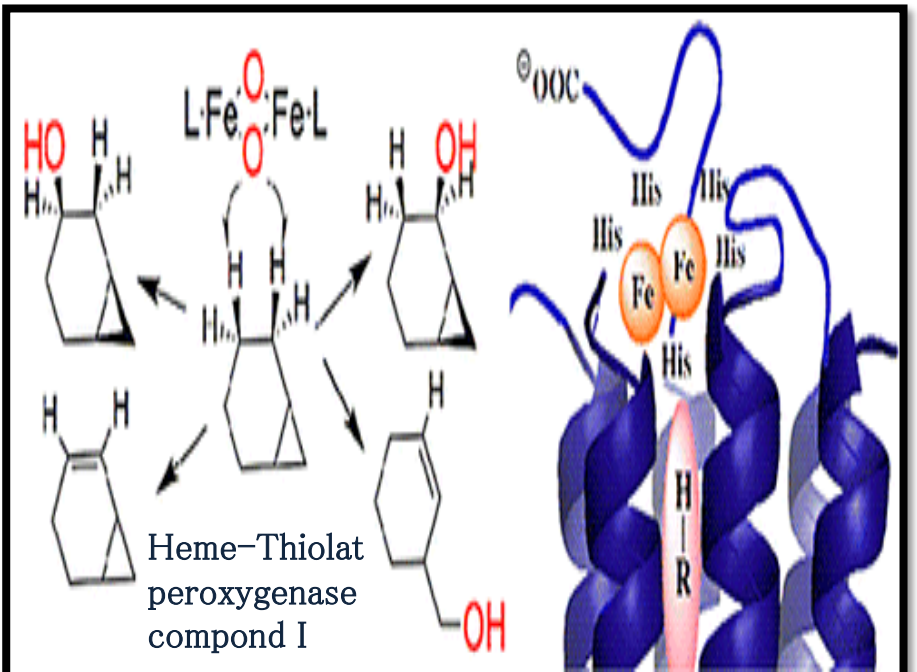
FAD - Flavin Adenine Dinucleotide

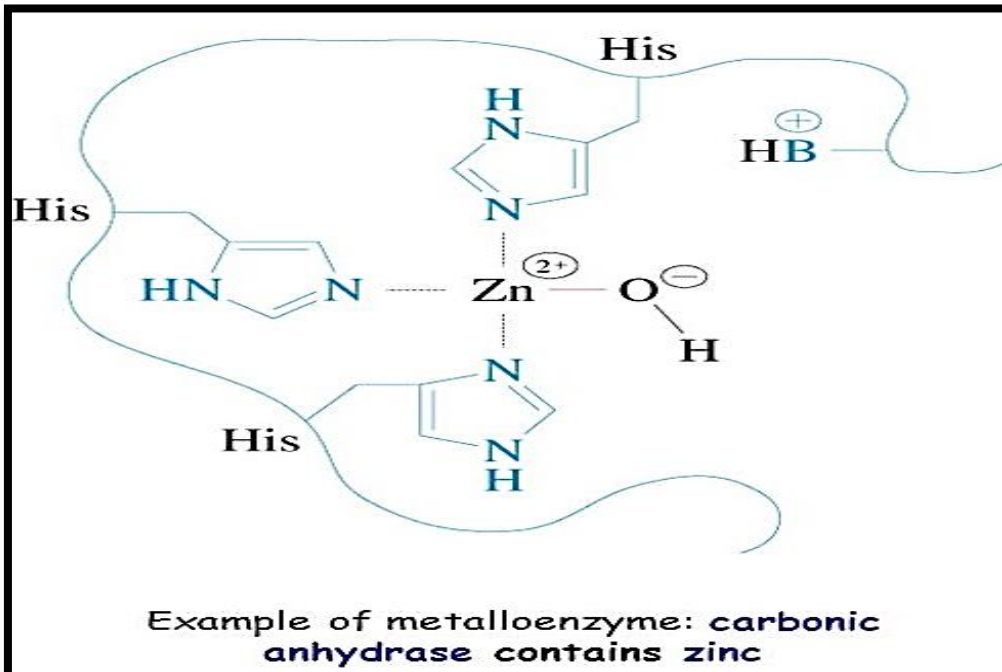


Chemical Nature of Enzymes

organic groups that are permanently bound to the enzyme (**prosthetic groups)** like Heme and Flavin.

Metallo-enzymes: An enzyme containing a metal (ion) as an integral part of which are directly bound to its active structure (protein) or to enzyme-bound nonprotein components (prosthetic groups); e.g., **cytochromes (Fe, Cu), aldehydeoxidase (Mo), catechol oxidase (Cu), carbonic anhydrase (Zn).** About one-third of all enzymes known so far are metalloenzymes. metal ion allows metalloenzymes to perform functions such as redox reactions that cannot easily be performed by the limited set of functional groups found in amino acids. The iron atom in most cytochromes is contained in a heme group.





Example of metalloenzyme: carbonic anhydrase contains zinc

Chemical Nature of Enzymes

Isoenzymes (Isozymes):

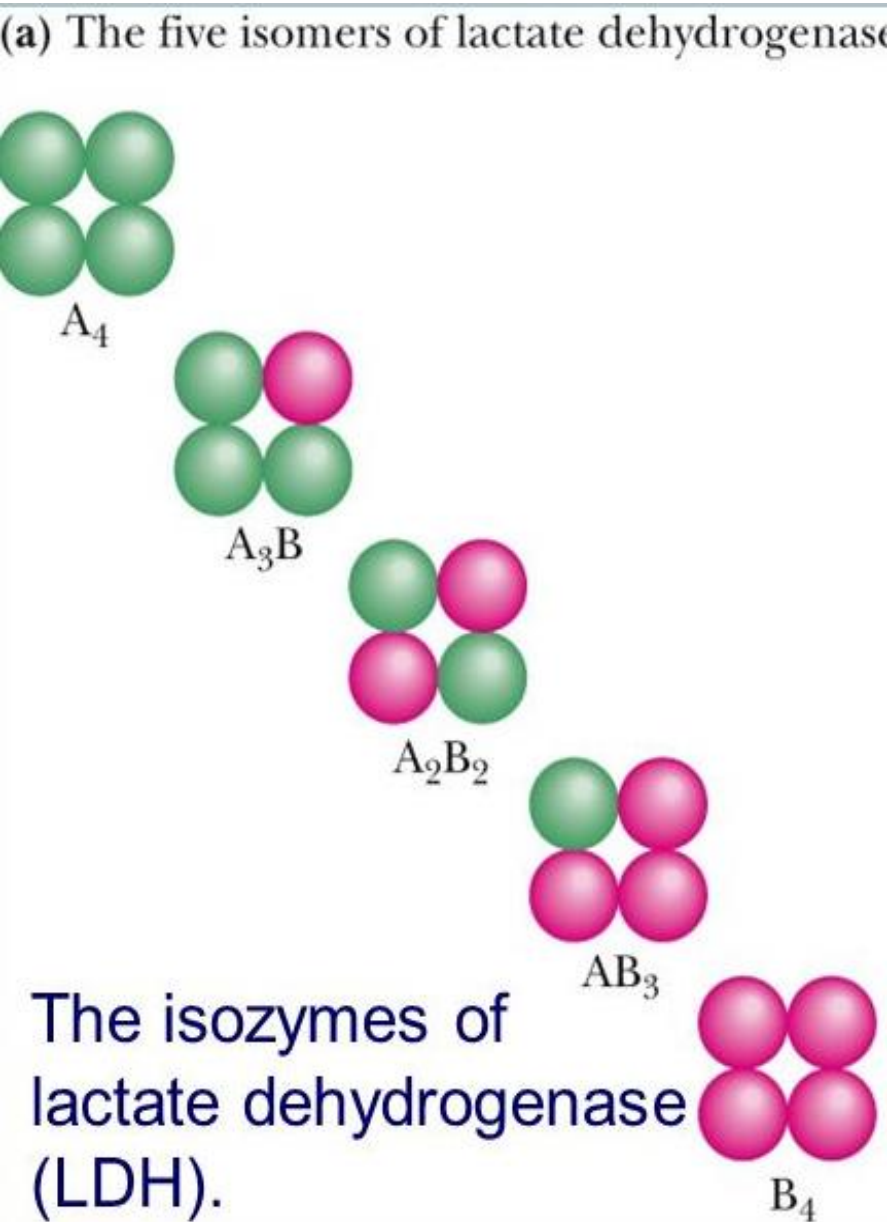
At one time it was believed that an organism has only a single enzyme for a given step of a metabolic reaction. It was later discovered that a substrate may be acted upon by a number of variants of an enzyme producing the same product.

The multiple molecular forms of an enzyme occurring in the same organism and having a similar substrate activity are called isoenzymes or isozymes. Over 100 enzymes are known to have isoenzymes. Thus a-amylase of wheat endosperm has 16 isozymes.

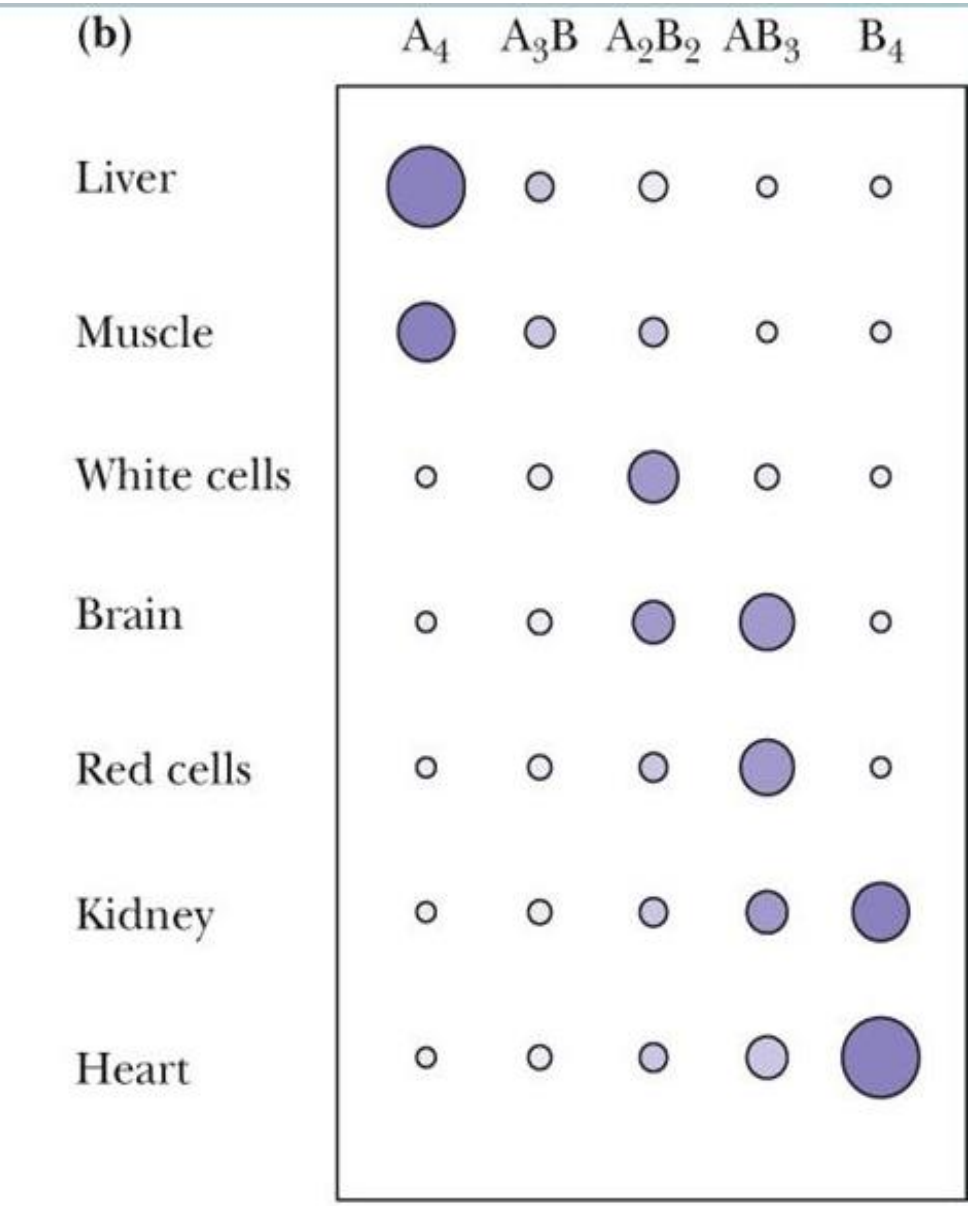
ISOENZYMES

- Isoenzymes or isozymes are mutiple forms of same enzyme that catalyse the same chemical reaction
- Different chemical and physical properties:
- Electrophoretic mobility
- Kinetic properties
- Amino acid sequence
- Amino acid composition

Chemical Nature of Enzymes



The isozymes of lactate dehydrogenase (LDH).



Enzymes Nomenclature & Classification

International Commission on Enzymes in its report in 1961recognised that each enzyme should consist of: (1) name of the substrate and (2) a word ending in 'ase' specifying one kind of catalytic reaction as in **succinic dehydrogenase, pyruvate transaminase**.

The modern system of enzyme classification was introduced by International Union of Biochemistry (IUB) in 1961. It groups enzymes into the following six categories.

1. Oxidoreductases: They take part in oxidation and reduction reactions or transfer of electrons. Like **oxidases**, **dehydrogenases** and **reductases.**

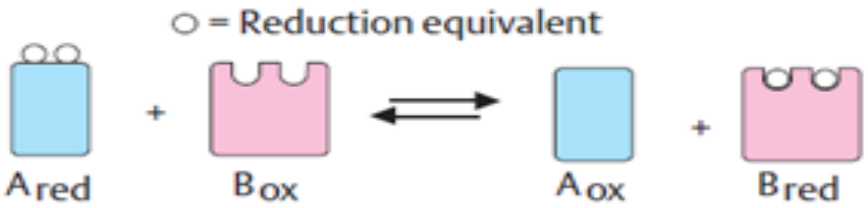
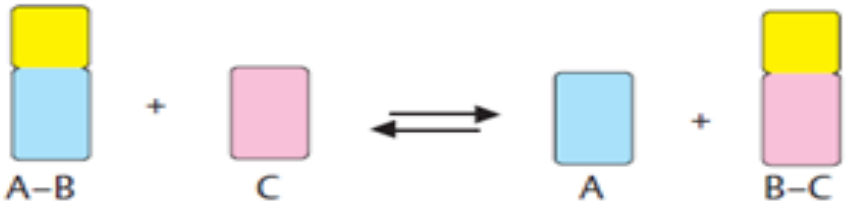
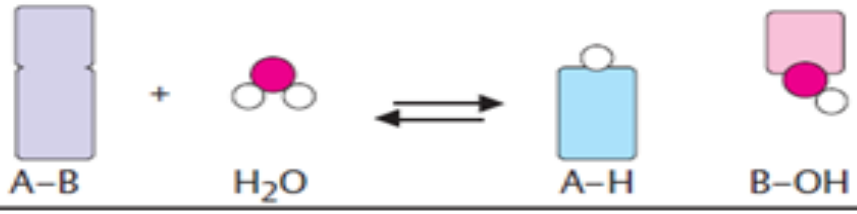
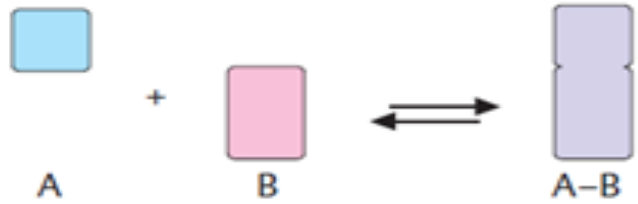
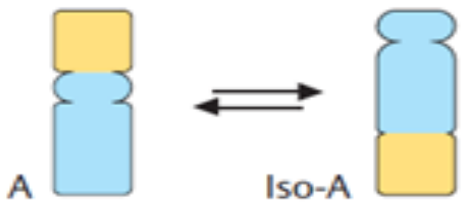
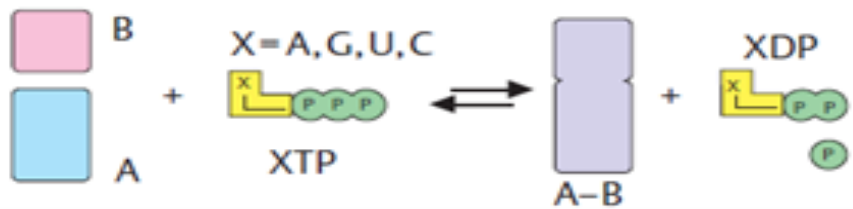
2. Transferases: They transfer a group from one molecule to another e.g**., glutamate-pyruvate transaminase**.

3. Hydrolases: They break up large molecules into smaller ones with the help of hydrogen and hydroxyl groups of water molecules. The phenomenon is called hydrolysis. Like **sucrase**.

4. Lyases: The enzymes cause cleavage, removal of groups without hydrolysis, addition of groups to double bonds or reverse, e.g., **histidine decarboxylase** (breaks histidine to histamine and CO_2).

5. Isomerases: The enzymes cause rearrangement of molecule structure to effect isomeric changes. They are of three types**, isomerases , epimerases and mutases**.

6. Ligases: (Synthetases). The enzymes catalyse bonding of two chemicals with the help of energy obtained from ATP, e.g**., phosphenol pyruvate PEP carboxylase**.

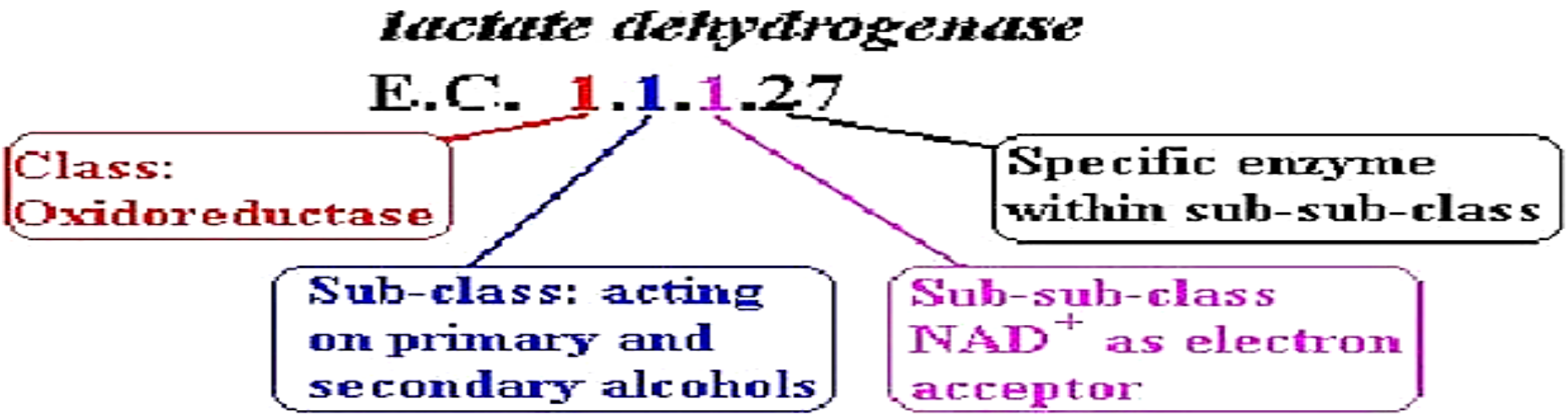
Class	Reaction type	Important subclasses
1 Oxidoreductases		Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases		C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases		Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")		C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases		Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")		C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

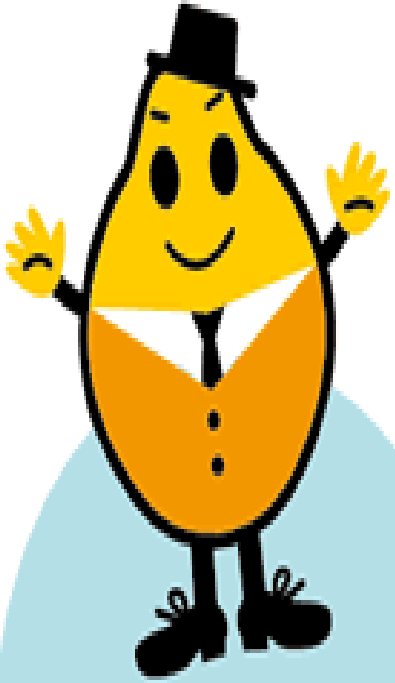
EC Numbers

- NC-IUBMB developed the Enzyme Classification number system (EC)
- Classification system is based on the reactions the enzymes catalyzed

- Classification:
  - Classes
  - Subclasses
  - Sub-subclasses
  - EC numbers (**E**nzyme **C**ommission)

- Example EC 1.1.1.1

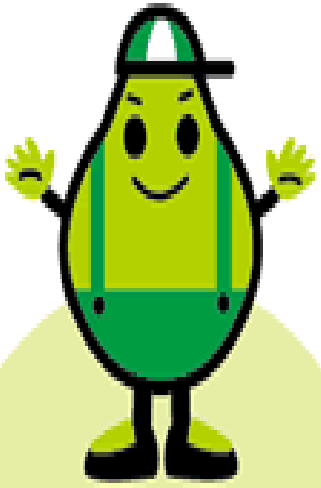




Proteolytic enzymes

Protease

Meat, seafood, soybean, etc



Fats-degrading enzyme

Lipase

Beef, foie gras and fresh cream, egg yolk, cheese, etc

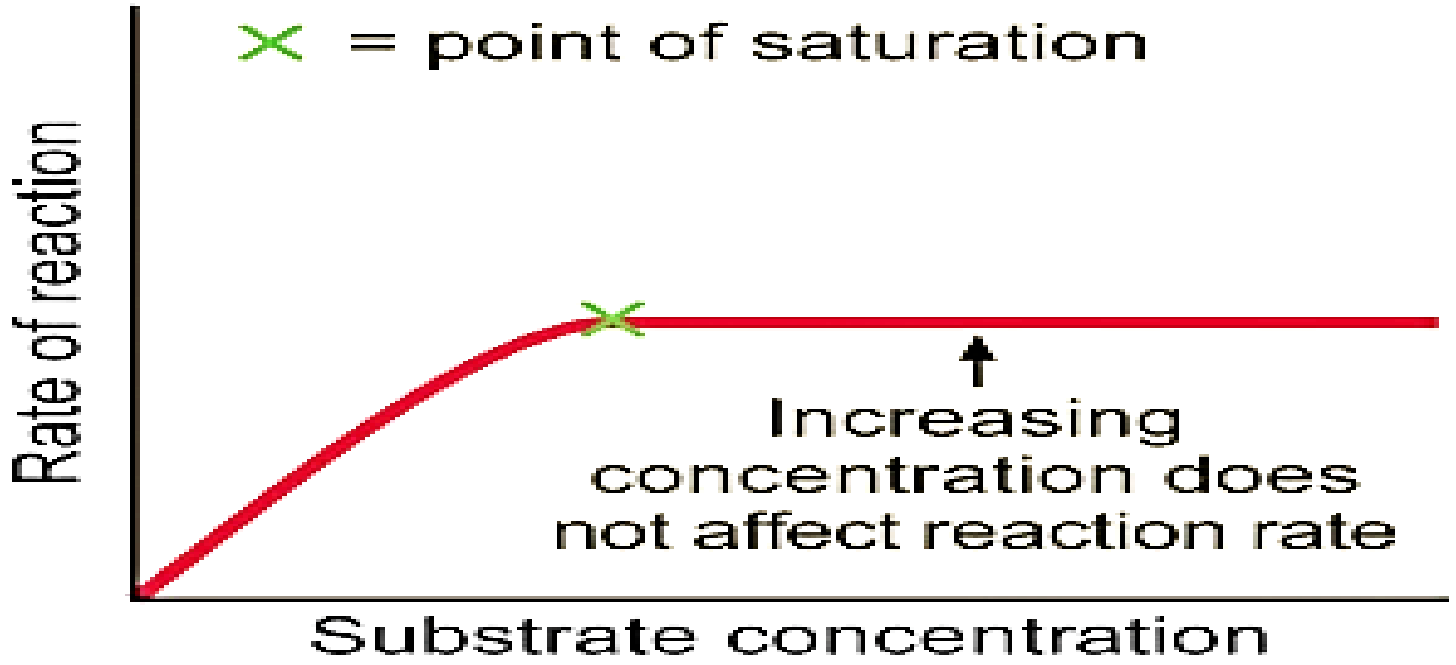
Sugar-degrading enzyme

Amylase

Chocolate, cake, biscuits, cookies, soft drinks, alcohol

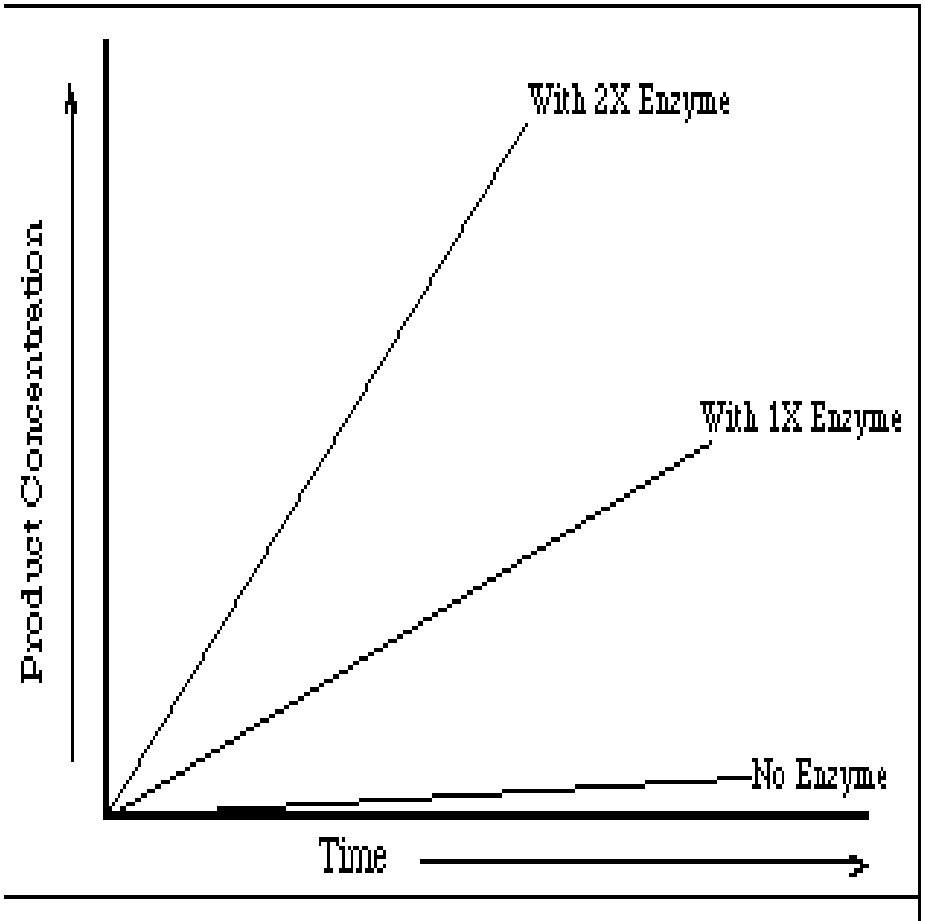
Factors that affect enzyme catalyzed reactions

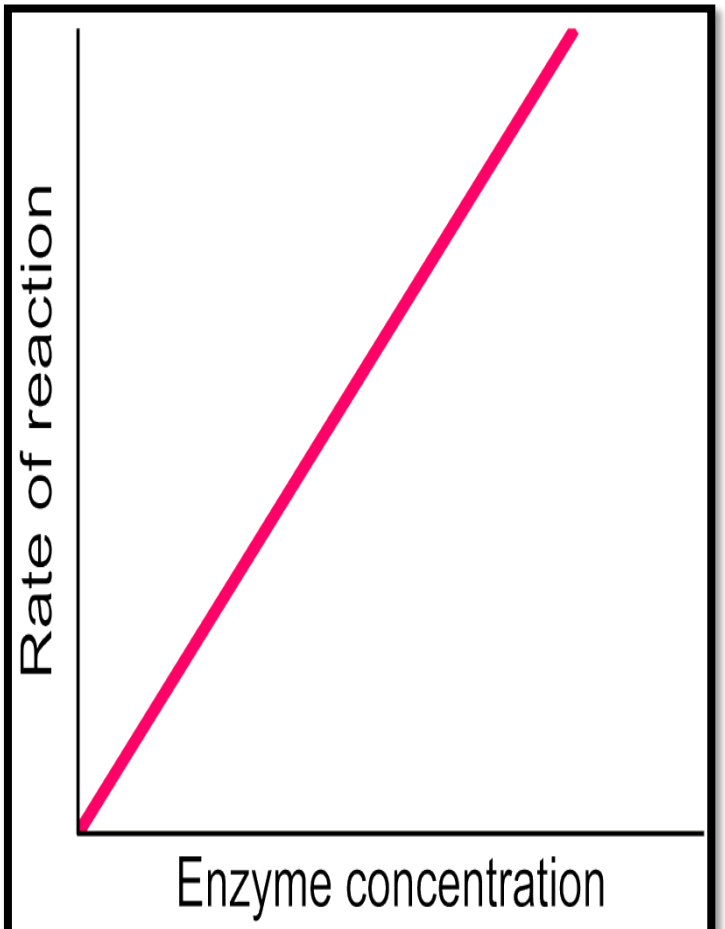
1. **Substrate concentration:** the rate of reaction increases with substrate concentration until a maximal velocity (Vmax) is reached. At low substrate concentration the active sites of the enzymes molecules are not used up. There are not enough substrate molecules to occupy all the active sites. As the substrate concentration increases more and more active sites come into use until all are being used (saturation) .any further increase in substrate concentration cannot increase the rate of the reaction.



Factors that affect enzyme catalyzed reactions

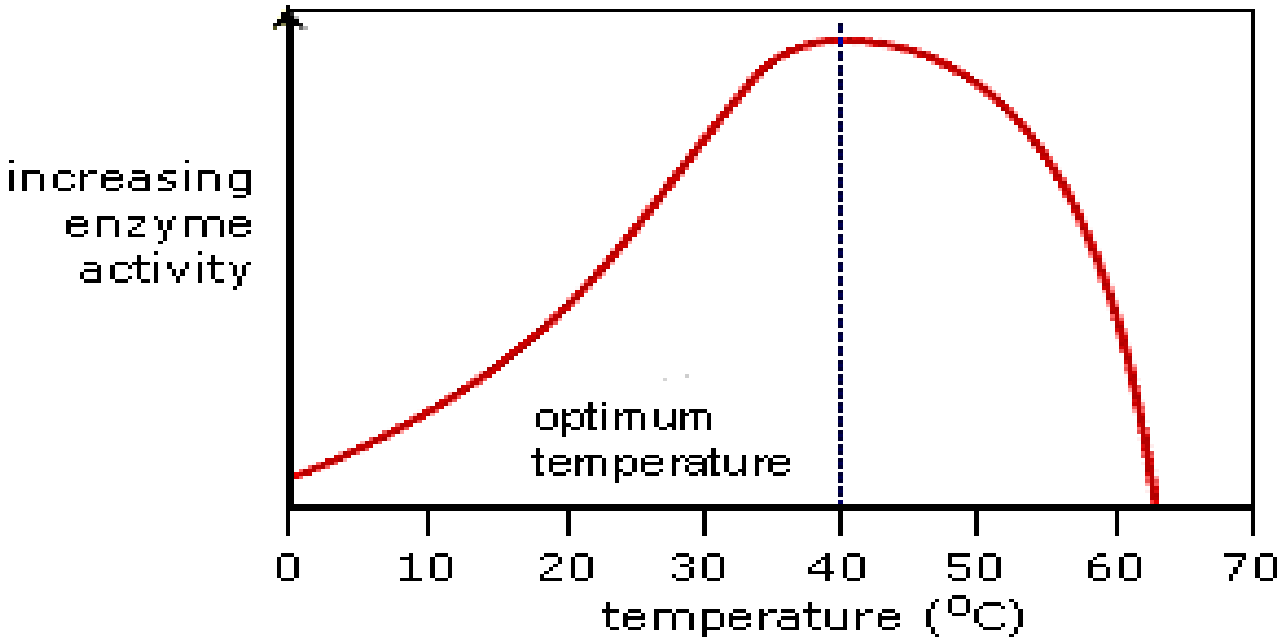
2. Enzyme concentration: the active site of an enzyme maybe used over and over. Enzymes work efficiently at low concentrations. The rate of enzyme reaction is proportional to the enzyme concentration once substrate concentration is high and pH and temperature are kept constant.





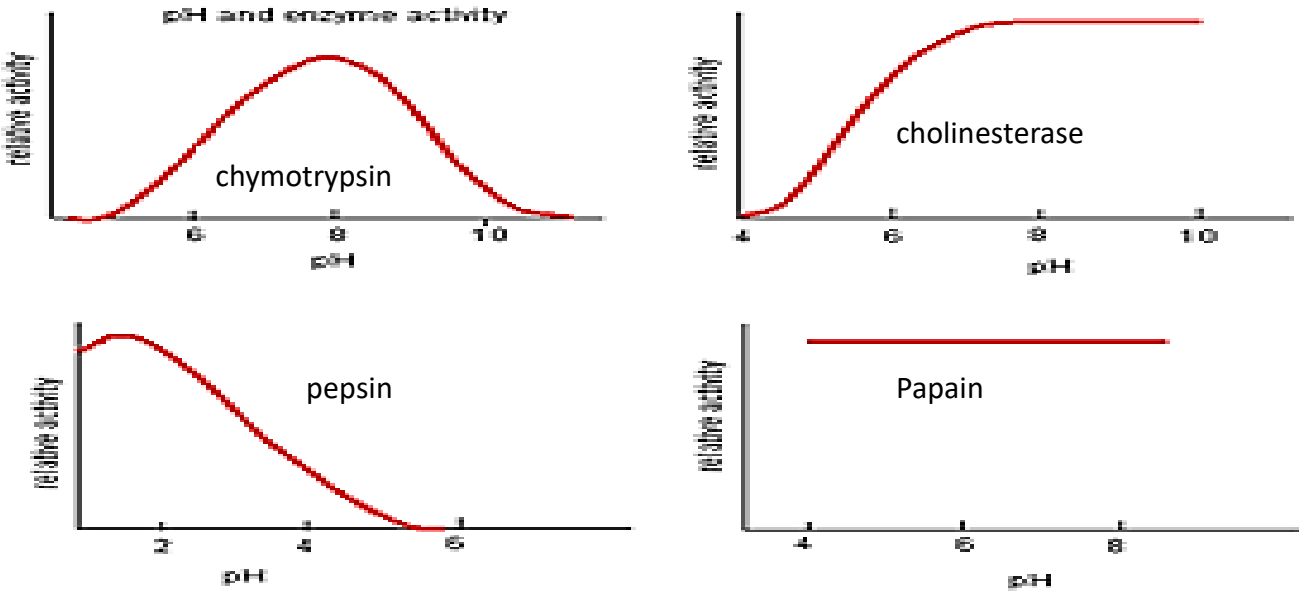
Factors that affect enzyme catalyzed reactions

3. Temperature: the reaction velocity is increased until a peak velocity is reached to the optimum. This is due to an increased number of molecules having the activation energy to pass over the energy barrier. Also there is an increase in collision frequency of the molecules. There is a decrease of velocity with higher temperature because the high temperature results in denaturation of the enzyme. 35 ^{o}C – 40^{o}C is the optimum temperature required for human enzymes.



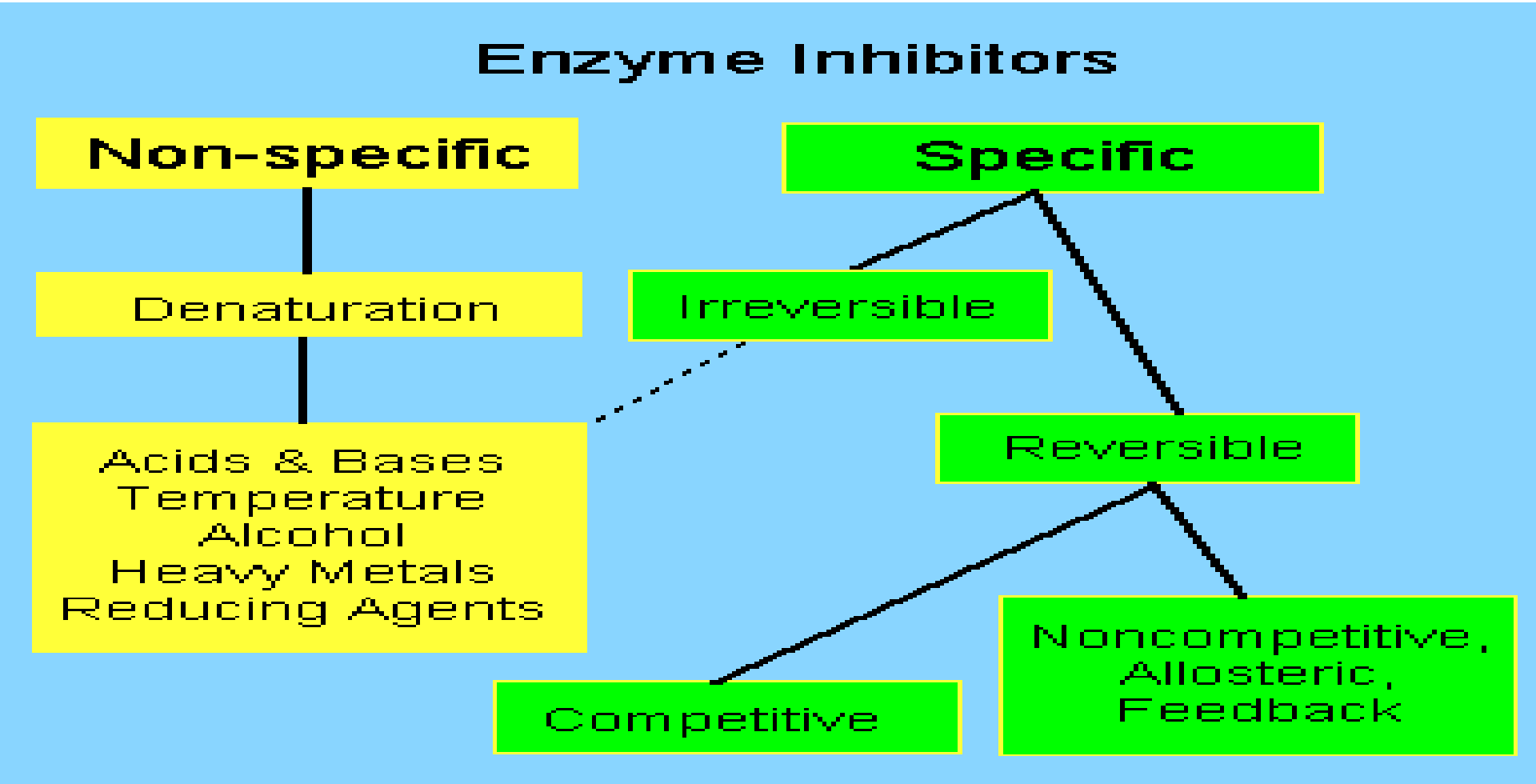
Factors that affect enzyme catalyzed reactions

4. pH-enzymes : each enzyme have a optimum pH range at which they work best and they function within a narrow pH range. The optimum pH is that where the maximum rate of reaction is achieved. When pH is altered above or below this value the rate of enzyme reactivity decreases. As pH decreases the acidity increases. Therefore increasing the number of positive charge. Changes in pH alter the ionic charge of the acidic and basic side groups. This disrupts the bonding that maintains the specific shape of the enzyme. Therefore leading to a change in shape of the enzyme and active site. Extremes in pH cause the enzyme to be denatured.



Enzyme inhibitors

Enzyme inhibitors: Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. There are a variety of types of inhibitors including: nonspecific, irreversible, reversible - competitive and noncompetitive. Poisons and drugs are examples of enzyme inhibitors.



Enzyme inhibitors

Specific Inhibitors:

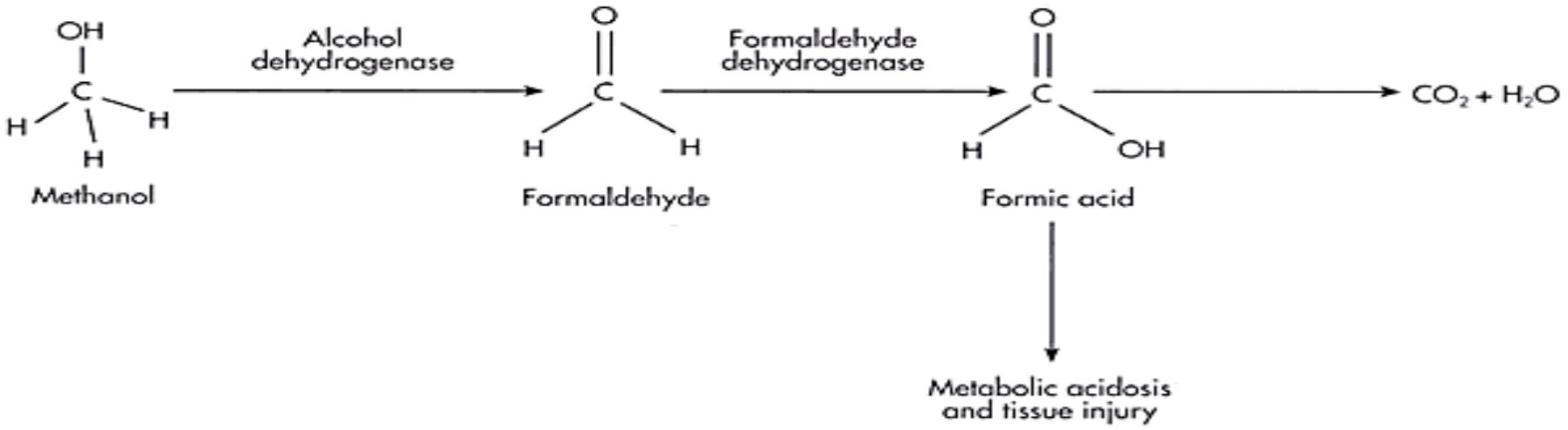
Specific Inhibitors exert their effects upon a single enzyme. Most poisons work by specific inhibition of enzymes. Many drugs also work by inhibiting enzymes in bacteria, viruses, or cancerous cells .

Competitive Inhibitors:

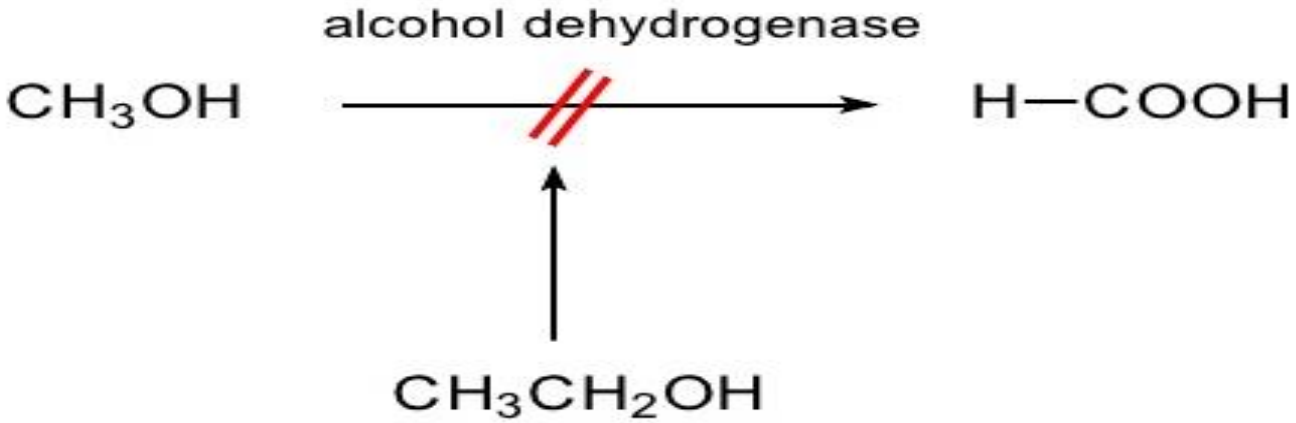
A competitive inhibitor is any compound which closely resembles the chemical structure and molecular geometry of the substrate. The inhibitor competes for the same active site as the substrate molecule. The inhibitor may interact with the enzyme at the active site, but no reaction takes place. The inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor.

A drug, **disulfiram (Antabuse) inhibits** the aldehyde dehydrogenase which causes the accumulation of acetaldehyde with subsequent unpleasant side-effects of nausea and vomiting. This drug is sometimes used to help people overcome the drinking habit.

Methanol poisoning occurs because methanol is oxidized by Alcohol dehydrogenases (ADH) enzyme to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol. Ethanol is oxidized in preference to methanol and consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.



Methanol poisoning is treated by ethanol



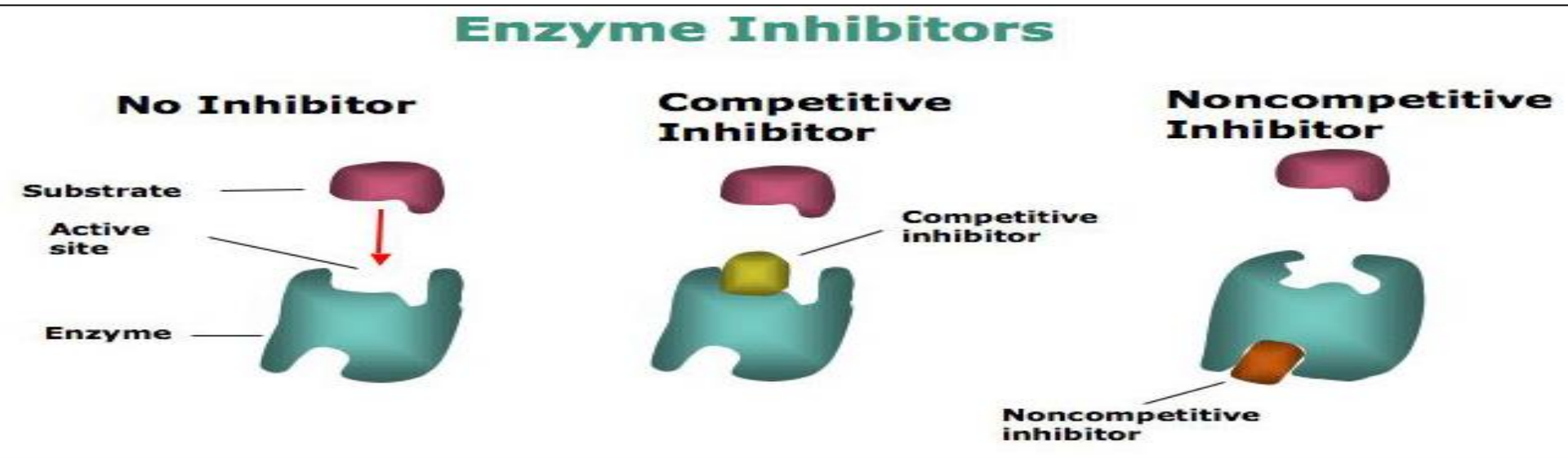
ethanol and methanol are similar molecules

they compete for active site in enzyme

Enzyme inhibitors

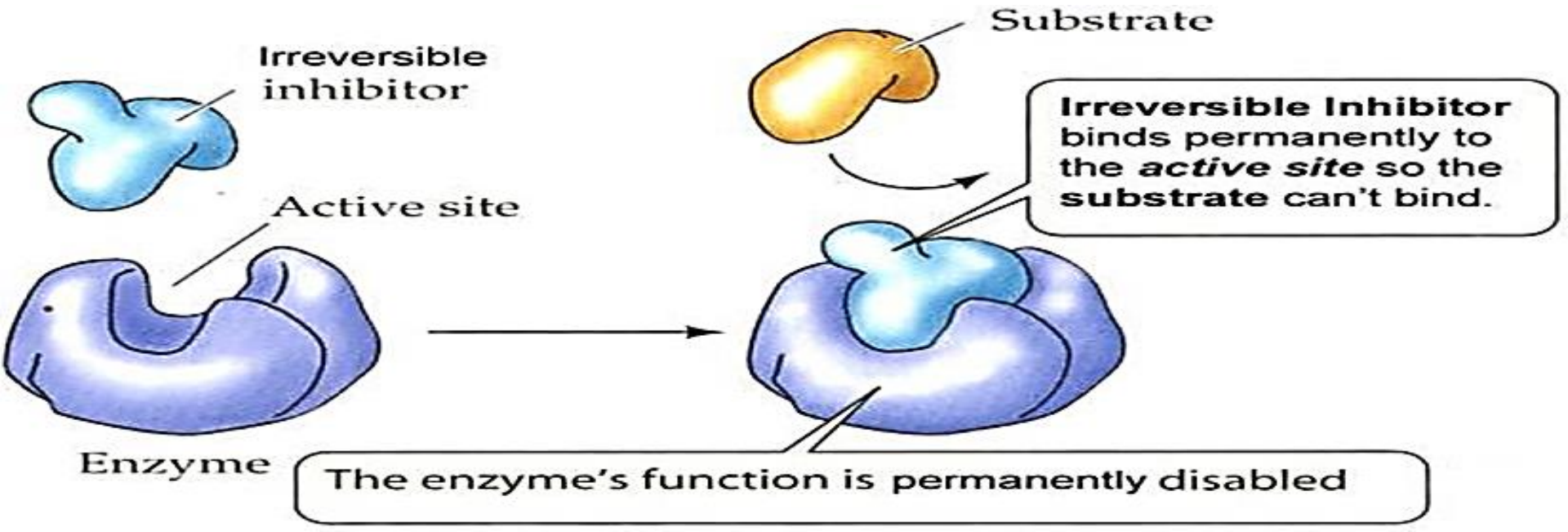
- **Non competitive Inhibitors:**
- A noncompetitive inhibitor is a substance that interacts with the enzyme, but usually not at the active site. The noncompetitive inhibitor reacts either isolated from or very close to the active site. The net effect of a non competitive inhibitor is to change the shape of the enzyme and thus the active site, so that the substrate can no longer interact with the enzyme to give a reaction. Non competitive inhibitors are usually reversible. For example in the enzyme-catalyzed reactions, phosphoenol is catalyzed by **pyruvate kinase** into pyruvate. Alanine is an amino acid that inhibits the enzyme pyruvate kinase during glycolysis. In glycolysis, the end product is pyruvate. However alanine is a non-competitive inhibitor, therefore it doesn't need an active site to bind to the substrate to still become the final product.

$$\text{PhophoenolPyruvate} \xrightarrow[\text{kinase}]{\text{Pyruvate}} \text{Pyruvate} \xrightarrow[\text{inhibitor}]{\text{noncompetitive}} \text{Alanine}$$



Enzyme inhibitors

- **Irreversible Inhibitors** form strong covalent bonds with an enzyme. These inhibitors may act at, near, or distant from the active site. Consequently, they may not be displaced by the addition of excess substrate. In any case, the basic structure of the enzyme is modified to the degree that it stops to work.
- Since many enzymes contain sulfhydral (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor. Heavy metals such as Ag^{+}, Hg^{2+}, Pb^{2+} have strong affinities for -SH groups.
- Nerve gases such as **diisopropylfluorophosphate (DFP)** inhibit the active site of acetylcholine esterase by reacting with the hydroxyl group of serine to make an ester.

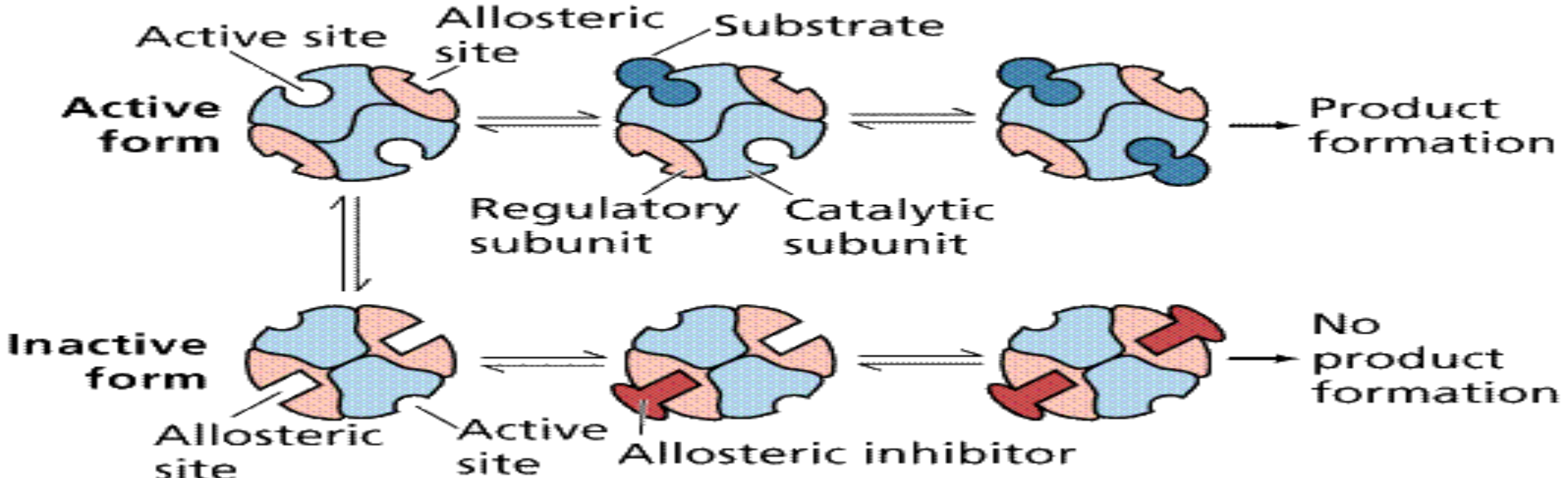


Regulation of enzyme activity

Mechanisms for Regulating Enzyme Activity

1. Allosteric Enzymes

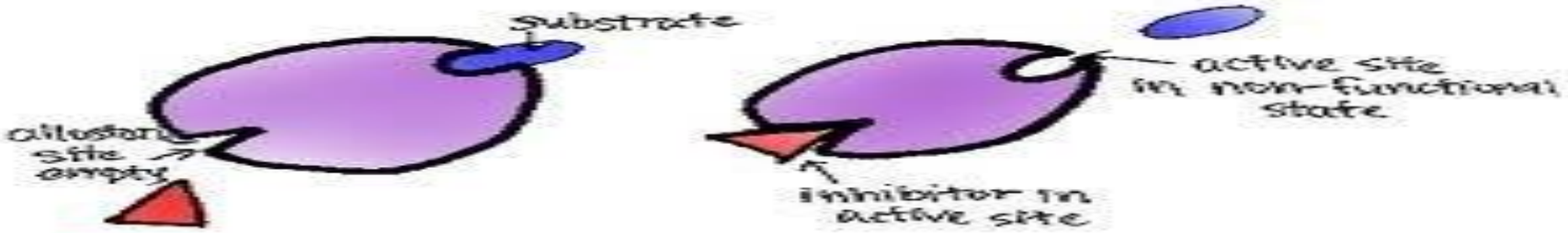
- Enzymes whose activity can be changed by molecules (effector molecules) other than substrate.



Regulation of enzyme activity

2 Processes Involving the Allosteric Enzyme

1. Negative Allosterism
   - effector binding sites alters the shape of the active site of the enzyme making it to an inactive configuration.



2. Positive Allosterism
   - effector binding sites that alters the shape of inactive site of enzyme to an active configuration.

Therefore, binding of the effector molecule regulates enzyme activity by determining whether it will be active or not.

Regulation of enzyme activity

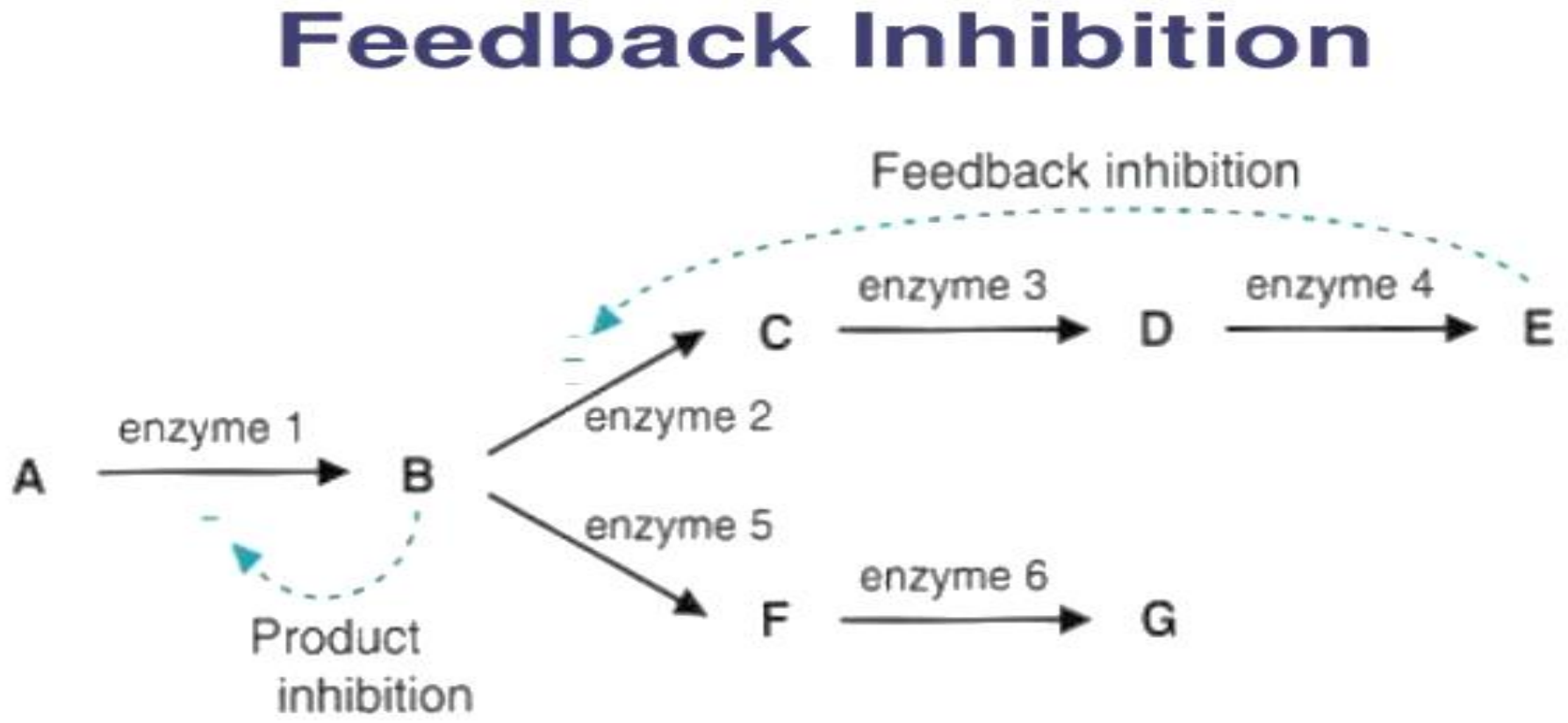
2. Feedback Inhibition

- An enzyme regulation process in which formation of a product inhibits an earlier reaction in the sequence. It controls the allosteric enzymes.

- This occurs when an end-product of a pathway accumulates as the metabolic demand for it declines.

- This end-product in turn binds to the regulatory enzyme at the start of the pathway and decreases its activity - the greater the end-product levels the greater the inhibition of enzyme activity.

Regulation of enzyme activity



Regulation of enzyme activity

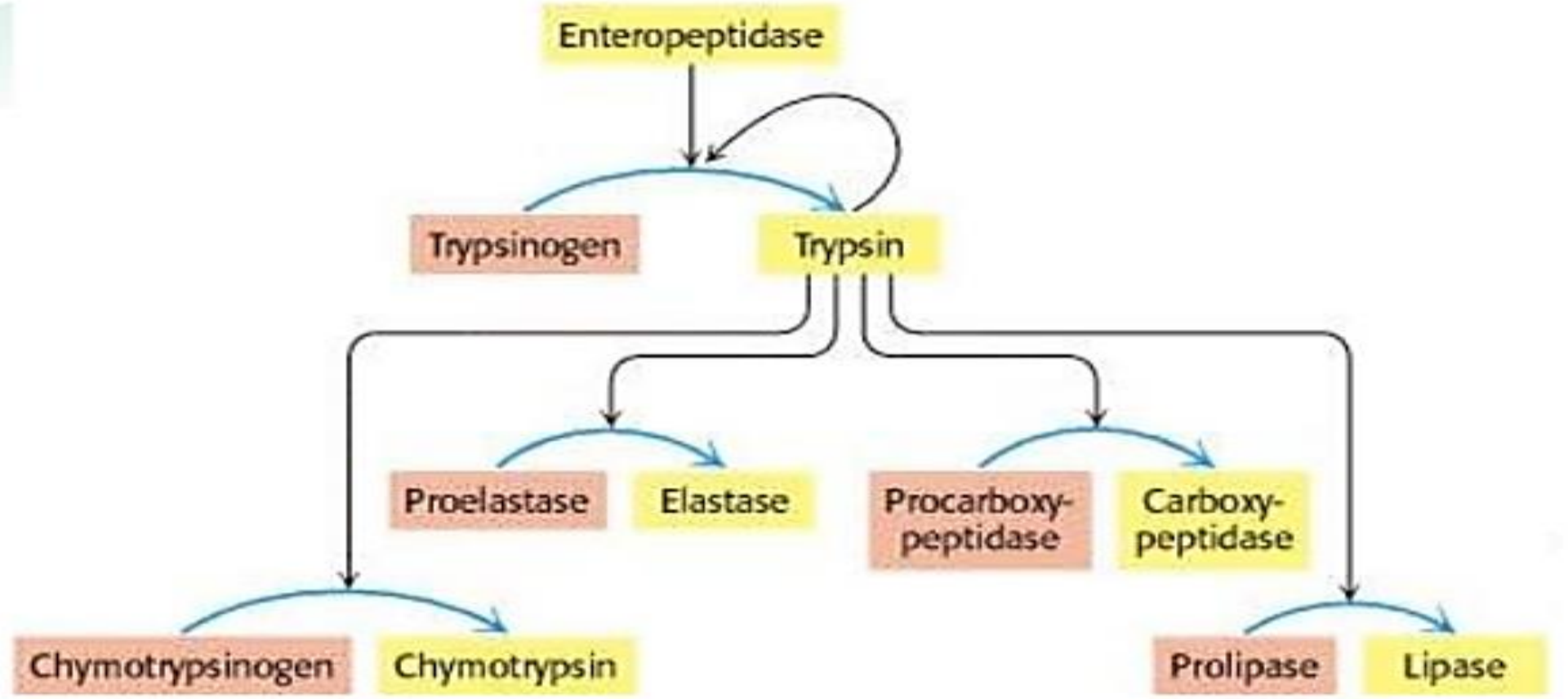
Covalent modification:

3. Proenzymes (Zymogen)

- The inactive form of enzyme which can be activated by removing a small part on their polypeptide chain.
- Mostly are the digestive enzymes and blood clotting enzymes.
- Why is it that digestive enzymes are in inactive state before it becomes active?
- This is necessary to prevent digestion of pancreatic and gastric tissues.

Regulation of enzyme activity

Zymogen		Active Form of Enzyme
• Pepsinogen	→	• Pepsin
• Trypsinogen	→	• Trypsin
• Prothrombin	→	• Thrombin



Regulation of enzyme activity

4. Protein Modification

- Another mechanism that can on and off the enzyme.
- This a process in which a chemical group is covalently added or removed from the protein.
- **Phosphorylation**, whereby a phosphate is transferred from an activated donor (usually ATP) to an amino acid on the regulatory enzyme, is the most common example of this type of regulation.