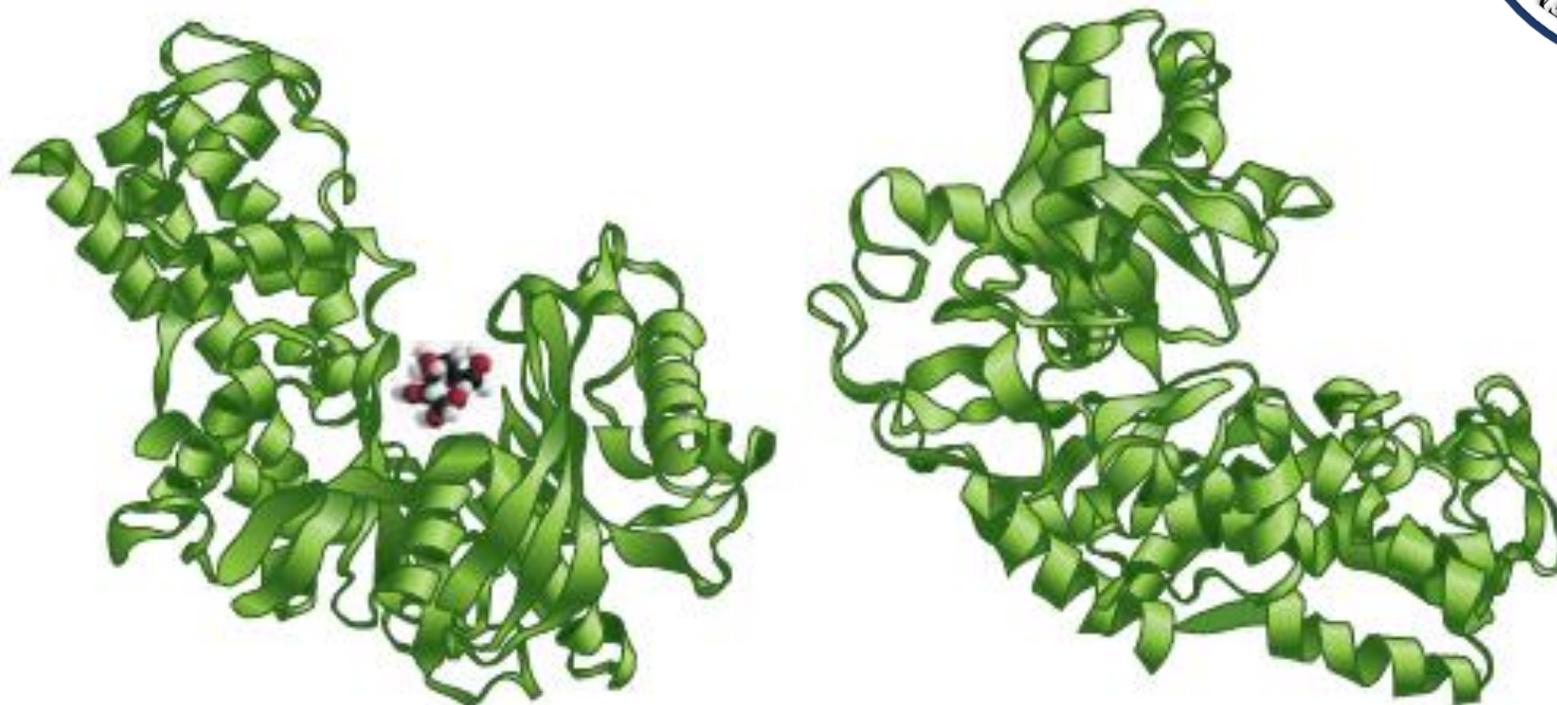




Enzyme Chemistry



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Enzymes

Frederick W. Kühne coined the name **enzymes**

The word enzyme comes from the Greek words en, which means “in,” and zyme, which means “yeast.” Long before their chemical nature was understood, yeast enzymes were used in the production of bread and alcoholic beverages.

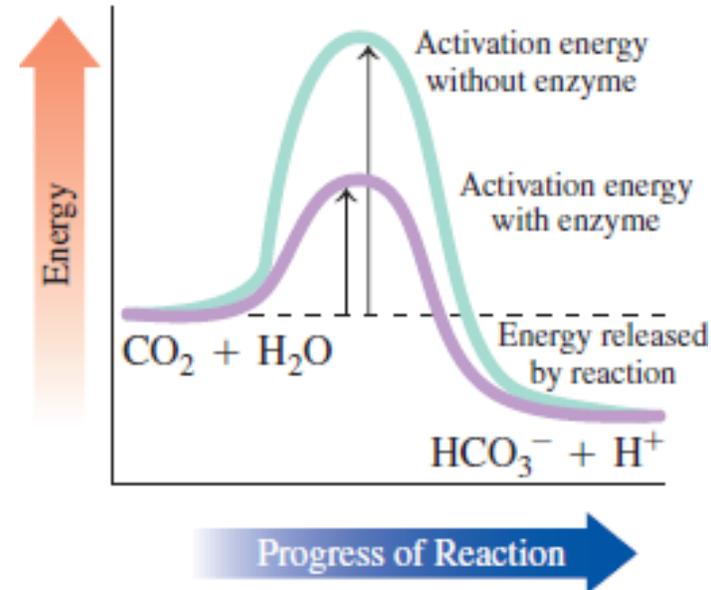
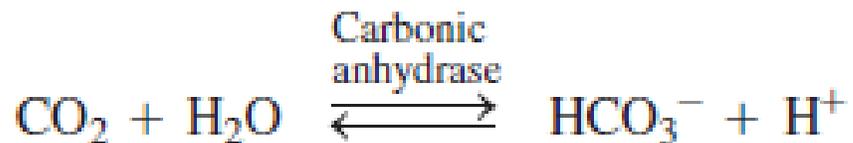
Catalysis can be described formally in terms of a stabilization of the transition state through tight binding to the catalyst.

—*William P. Jencks, article in Advances in Enzymology, 1975*

Enzymes

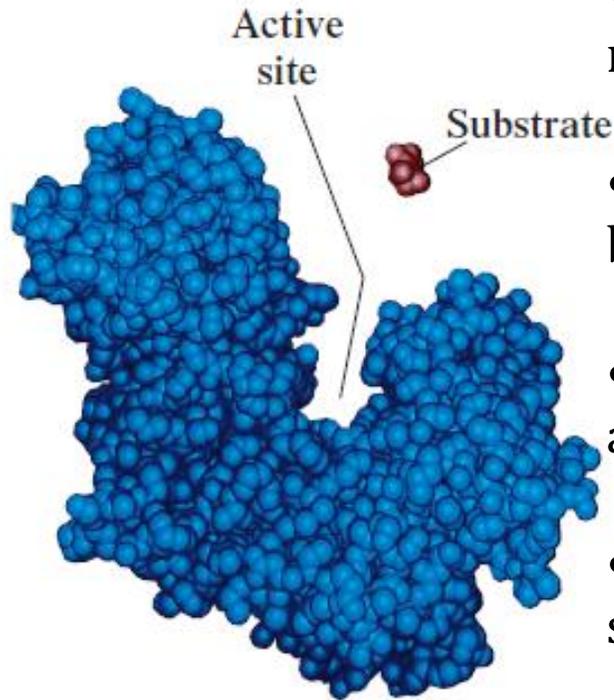
Enzymes are the biological macromolecules which speed up the rate of biochemical reactions without undergoing any change. They are also called as biological catalysts.

An enzyme is a highly selective catalyst that greatly accelerates both the rate and specificity of metabolic reactions.



Most enzymes are three dimensional globular proteins (tertiary and quaternary structure).

Characteristics of Enzyme



- Nearly all enzymes are proteins, although a few catalytically active RNA molecules have been identified.
- Enzymes are catalysts that increase the rate of a chemical reaction without being changed themselves in the process.
- Enzymes are highly specific with respect to the substrates on which they act and the products that they form.
- Enzyme activity can be regulated, varying in response to the concentration of substrates or other molecules.
- Enzyme catalyzed reactions usually take place under relatively mild conditions (temperatures well below 100°C , atmospheric pressure and neutral pH) as compared with the corresponding chemical reactions.
- They function under strict conditions of temperature and pH in the body.

Nomenclature and Classification of Enzymes

Enzymes are named according to the type of reaction they catalyze and/or their substrate on which they act upon.

- **Suffix of an enzyme–ase**

- Lactase, amylase, lipase or protease

- **Some digestive enzymes have the suffix –in**

- Pepsin, trypsin & chymotrypsin

- (These enzymes were the first ones to be studied)

- **Prefix denotes the type of reaction the enzyme catalyzes**

- Oxidase: redox reaction

- Hydrolase: Addition of water to break one component into two parts*

- **Substrate identity is often used together with the reaction type**

- Pyruvate carboxylase* (carboxylation of pyruvate to form oxaloacetate), *lactate dehydrogenase* (catalyze the reversible conversion of lactate to pyruvate with the reduction of NAD⁺ to NADH and vice versa)

Nomenclature and Classification of Enzymes

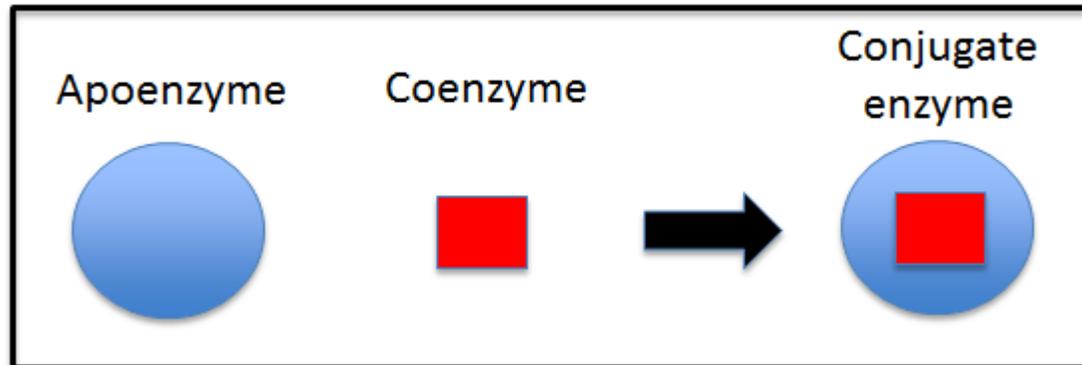
Recently, a systematic method of classifying and naming enzymes has been established. The name and class of each indicates the type of reaction it catalyzes. There are six main classes of enzymes

Class	Reaction Catalyzed	Examples
1. Oxidoreductases	Oxidation–reduction reactions	<i>Oxidases</i> oxidize a substance. <i>Reductases</i> reduce a substance. <i>Dehydrogenases</i> remove 2H atoms to form a double bond.
2. Transferases	Transfer a group between two compounds	<i>Transaminases</i> transfer amino groups. <i>Kinases</i> transfer phosphate groups.
3. Hydrolases	Hydrolysis reactions	<i>Proteases</i> hydrolyze peptide bonds in proteins. <i>Lipases</i> hydrolyze ester bonds in lipids. <i>Carbohydrases</i> hydrolyze glycosidic bonds in carbohydrates. <i>Phosphatases</i> hydrolyze phosphoester bonds. <i>Nucleases</i> hydrolyze nucleic acids.
4. Lyases	Add or remove groups involving a double bond without hydrolysis	<i>Carboxylases</i> add CO ₂ . <i>Deaminases</i> remove NH ₃ .
5. Isomerases	Rearrange atoms in a molecule to form an isomer	<i>Isomerases</i> convert cis to trans isomers or trans to cis isomers. <i>Epimerases</i> convert D to L stereoisomers or L to D.
6. Ligases	Form bonds between molecules using ATP energy	<i>Synthetases</i> combine two molecules.

Enzyme Structure

The enzymes can be divided into two types on its structural aspect

- i. Simple Enzyme: Enzyme composed only of proteins
- ii. Conjugate Enzyme: They have two basic components:
 - a. Apo enzyme : Protein component (which alone cannot catalyze any reaction)
 - b. Co-factors/ Coenzyme : Non protein component



Co-Factors

Co-factor is the non protein molecule which carries out chemical reactions that can not be performed by simple enzymes.

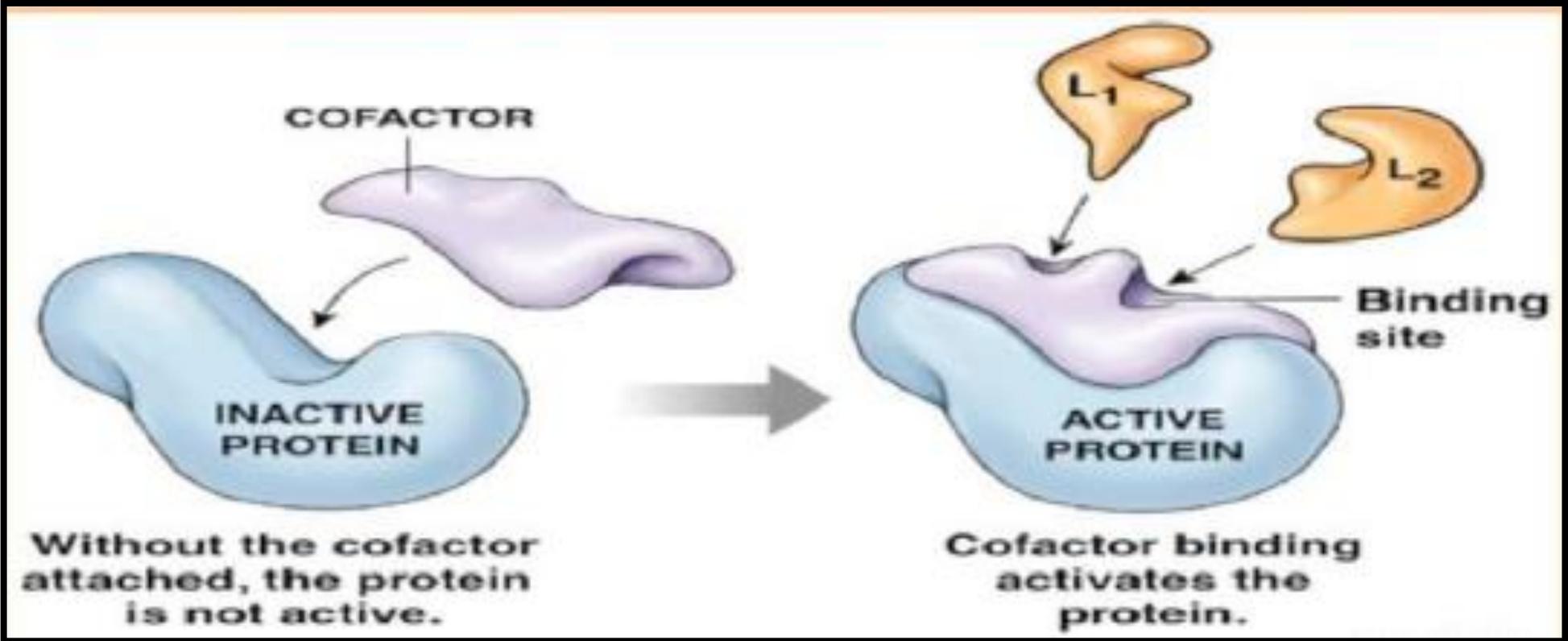
Co-factors are of two types:

i. Organic co-factors

<i>Coenzyme</i>	<i>Examples of chemical groups transferred</i>
Biocytin	CO ₂
Coenzyme A	Acyl groups
5'-Deoxyadenosylcobalamin (coenzyme B ₁₂)	H atoms and alkyl groups
Flavin adenine dinucleotide	Electrons
Lipoate	Electrons and acyl groups
Nicotinamide adenine dinucleotide	Hydride ion (:H ⁻)
Pyridoxal phosphate	Amino groups
Tetrahydrofolate	One-carbon groups
Thiamine pyrophosphate	Aldehydes

ii. Inorganic co-factors

Cu ²⁺	Cytochrome oxidase
Fe ²⁺ or Fe ³⁺	Cytochrome oxidase, catalase, peroxidase
K ⁺	Pyruvate kinase
Mg ²⁺	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn ²⁺	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni ²⁺	Urease
Se	Glutathione peroxidase
Zn ²⁺	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B



Co-Factors

Inorganic co-factors : These are the cofactors which incorporates some metal (Zn^{2+} , Mg^{2+} , Mn^{2+} & Fe^{2+}) ions for the required activity .

Example: i. Carbonic anhydrase requires Zn^{2+}
ii. Hexokinase requires Mg^{2+}

Organic co-factors/Coenzyme : These are the organic molecules required for the desired action of the enzymes.

Example: Glycogen phosphorylase requires the small organic molecule pyridoxal phosphate.

Prosthetic Groups: If the cofactor (Inorganic /Organic) is tightly bound to the enzyme (i.e. integrated to enzyme) via covalent bonds are termed as prosthetic groups.

Example: Flavin mononucleotide (FMN), Flavin adenine dinucleotide (FAD)

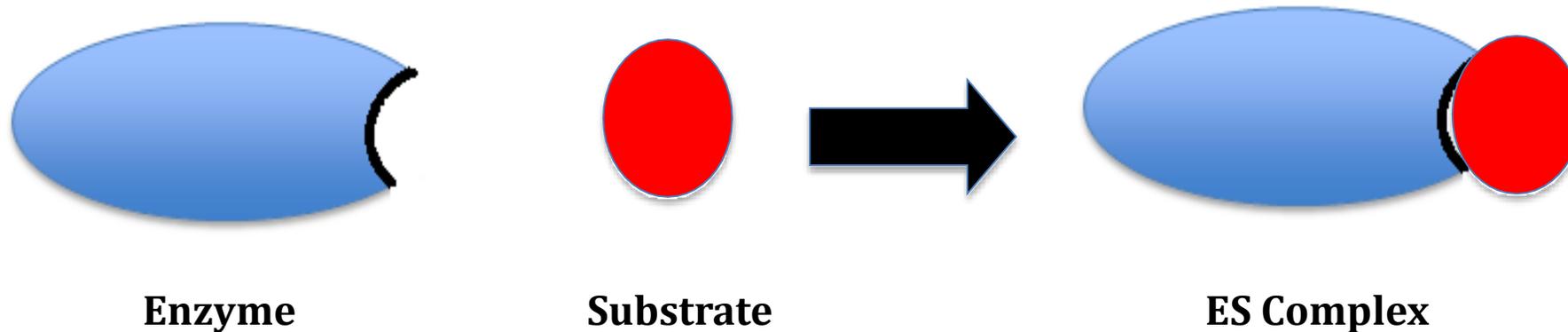
Term	Definition
Enzyme (simple)	Protein only enzyme that facilitates a chemical reaction
Coenzyme	Compound derived from a vitamin (e.g. NAD ⁺) that assists an enzyme in facilitating a chemical reaction
Cofactor	Metal ion (e.g. Mg ²⁺) that that assists an enzyme in facilitating a chemical reaction
Apoenzyme	Protein only part of an enzyme (e.g. isocitrate dehydrogenase) that requires an additional coenzyme to facilitate a chemical reaction (not functional alone)
Holoenzyme	Combination of the apoenzyme and coenzyme which together facilitating a chemical reaction (functional)

Enzyme Catalyzed Reaction

For a biological reaction to occur a substrate and enzyme should interact with each other to form an enzyme-substrate (ES) complex

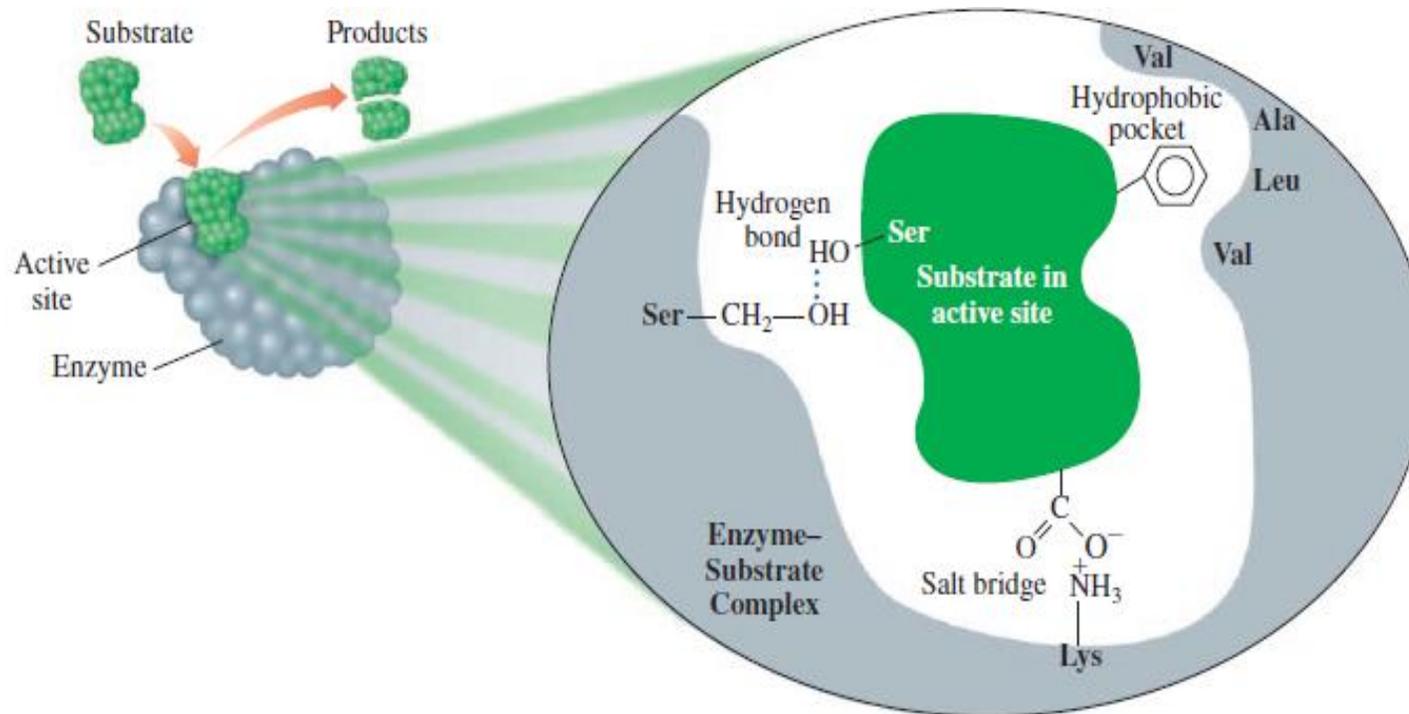
Substrate: any reactant on which the specific enzyme acts upon

Enzyme-substrate complex (ES): it is a resultant of interaction among enzyme and substrate

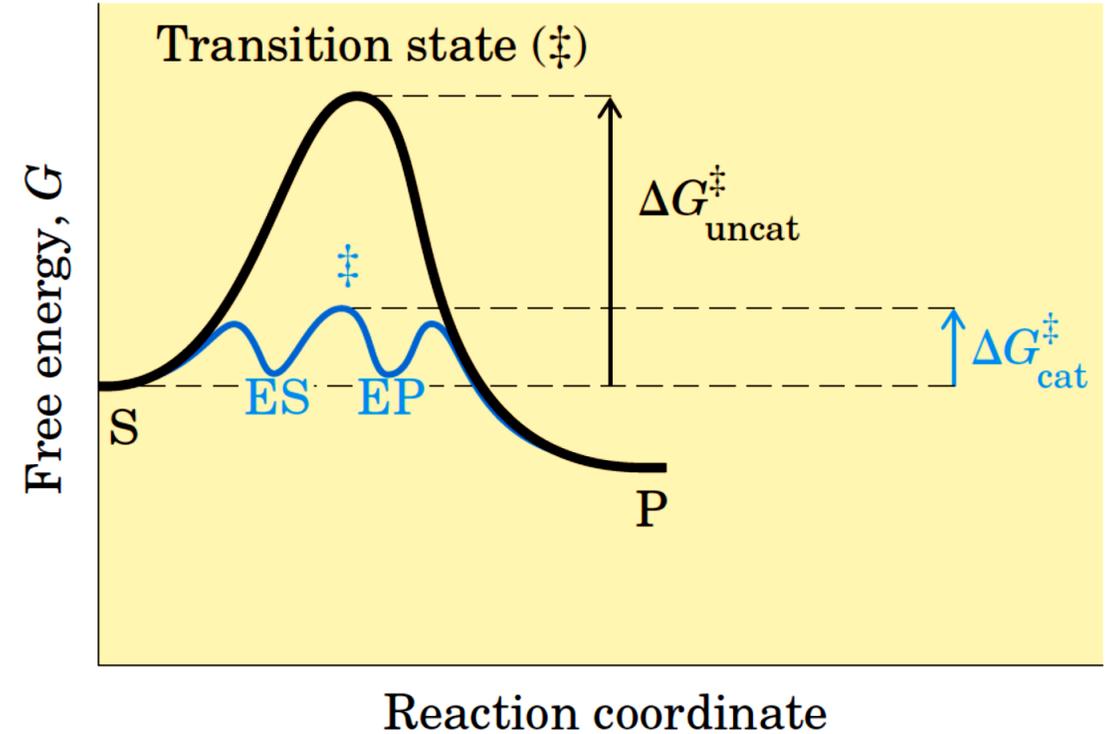
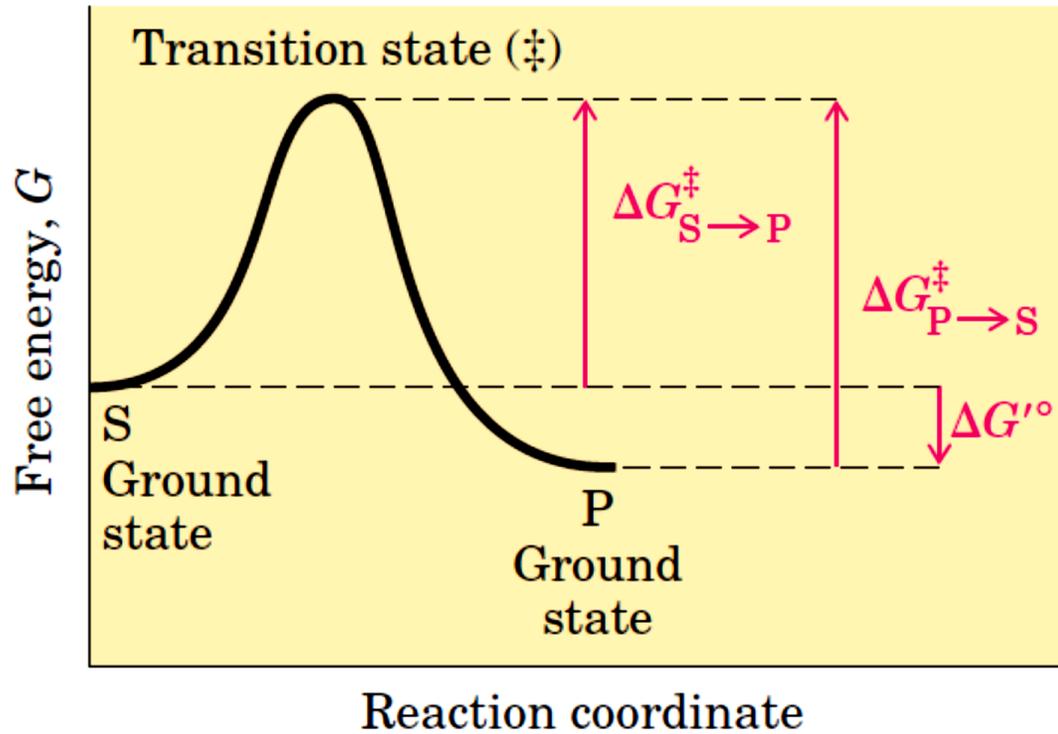


Active Site of Enzyme

- The **active site** of an enzyme is the region that binds the substrate and converts it into product.
- It is usually a relatively **small part** of the whole enzyme molecule and is a three-dimensional entity formed by amino acid residues that can lie far apart in the linear polypeptide chain.
- The active site is often a cleft or crevice on the surface of the enzyme that forms a predominantly **nonpolar** environment which enhances the binding of the substrate.
- The substrate(s) is bound in the active site by **multiple weak forces** (electrostatic interactions, hydrogen bonds, van der Waals bonds, hydrophobic interactions; and in some cases by reversible covalent bonds).



How Does Enzyme Works??

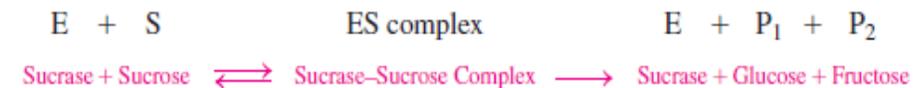
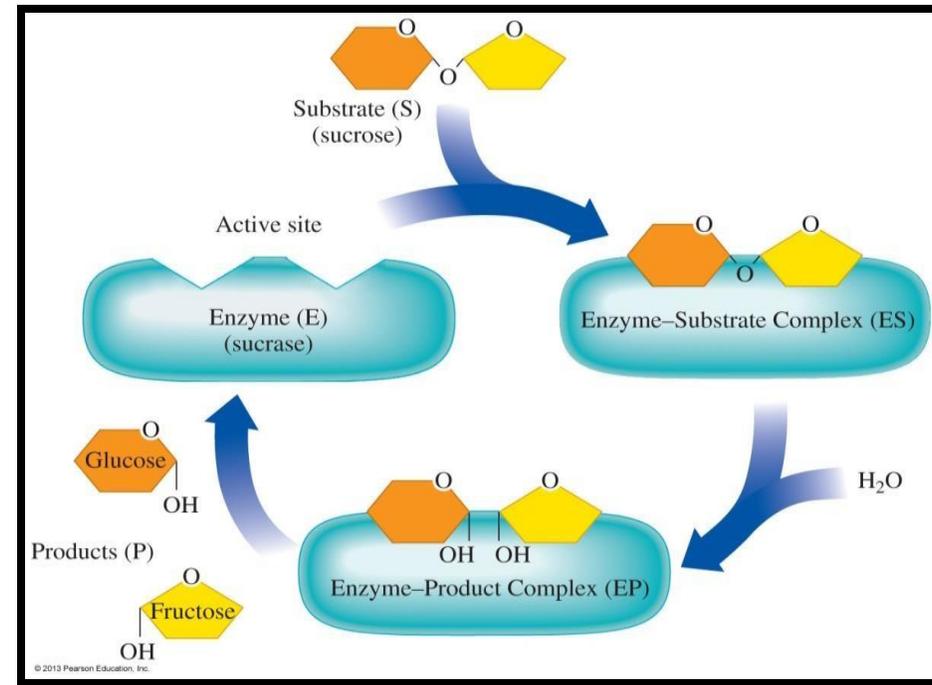
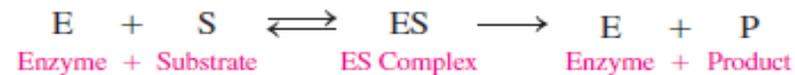
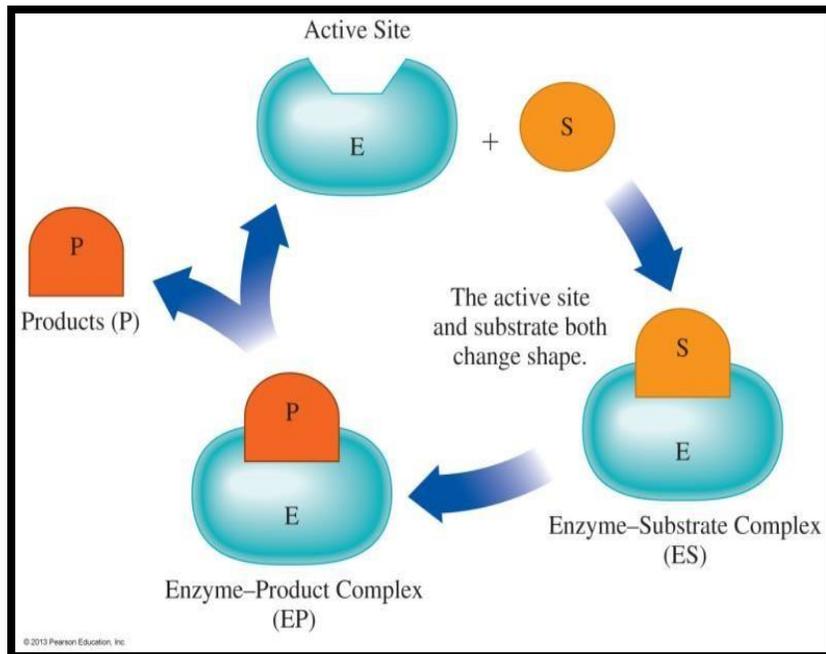


Reaction coordinate diagram comparing enzyme catalyzed and uncatalyzed reactions

Enzyme – Substrate Complex

When the substrate binds to the enzyme active site an Enzyme-Substrate Complex is formed temporarily

-Allows the substrate to undergo its chemical reaction much faster

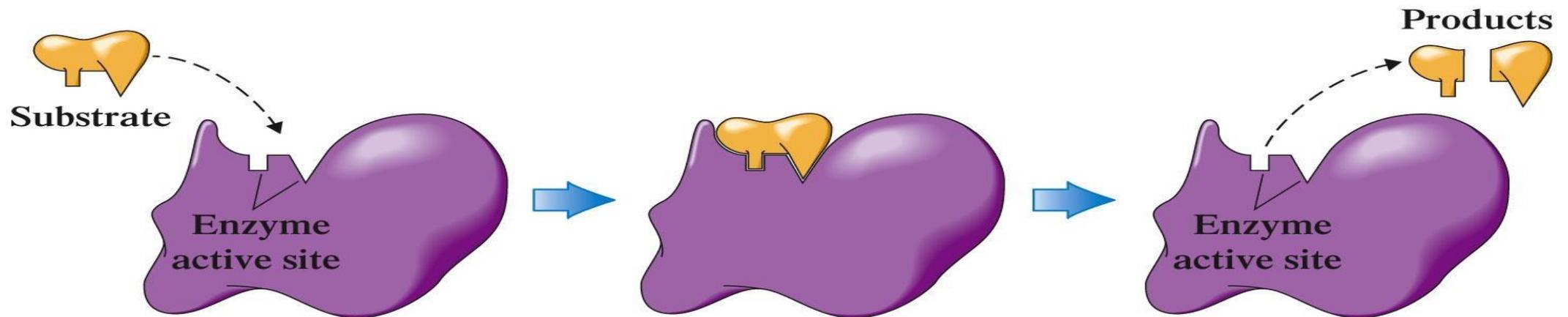


Mode of Enzyme action

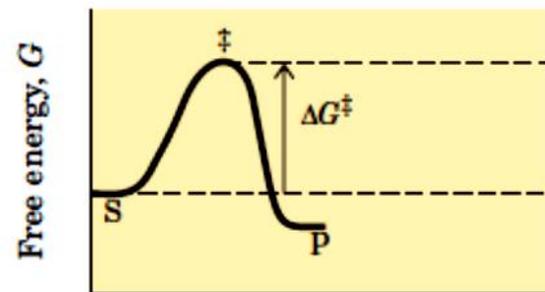
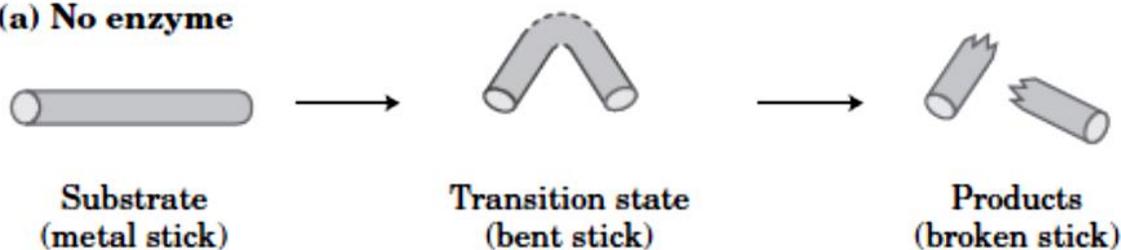
The action of the enzyme can be categorized into two possible pathways depending on the static or dynamic nature of the active site of an enzyme:

i. Lock and Key Model:

- In the lock-and-key model proposed was proposed by Emil Fischer in 1894.
- According to the model, the shape of the substrate and the active site of the enzyme are thought to fit together like a key into its lock.
- The two shapes are considered as rigid and fixed, and perfectly complement each other when brought together in the right alignment.



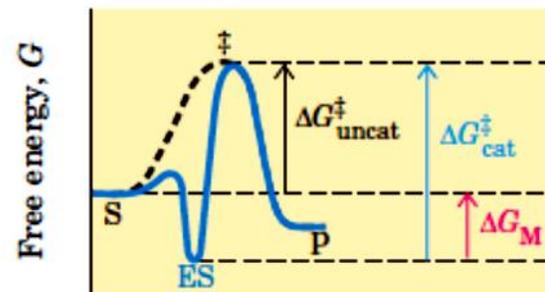
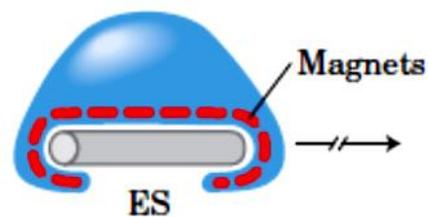
(a) No enzyme



An imaginary enzyme (stickase) designed to catalyze breakage of a metal stick.

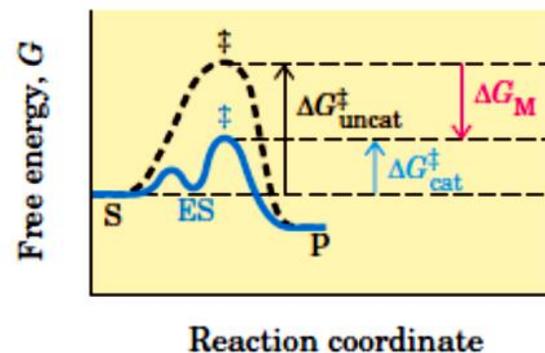
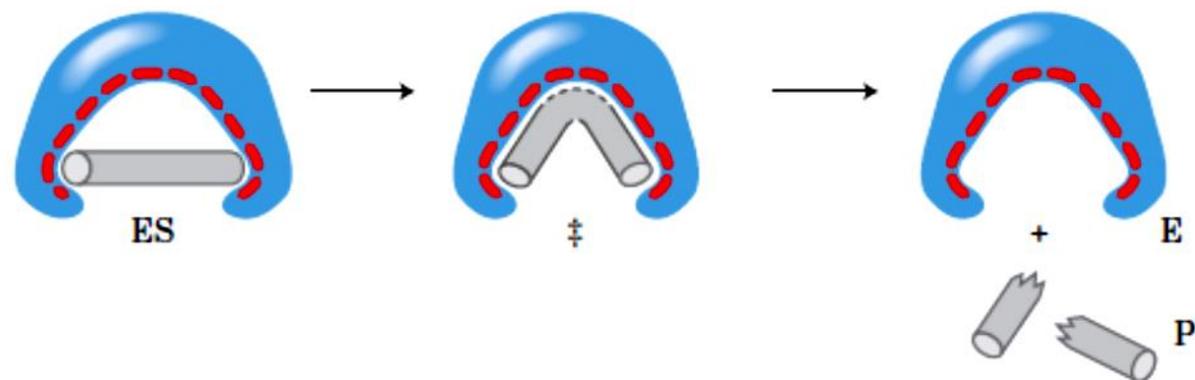
(a) Before the stick is broken, it must first be bent (the transition state). In both stickase examples, magnetic interactions take the place of weak bonding interactions between enzyme and substrate.

(b) Enzyme complementary to substrate



(b) A stickase with a magnet-lined pocket complementary in structure to the stick (the substrate) stabilizes the substrate. Bending is impeded by the magnetic attraction between stick and stickase.

(c) Enzyme complementary to transition state

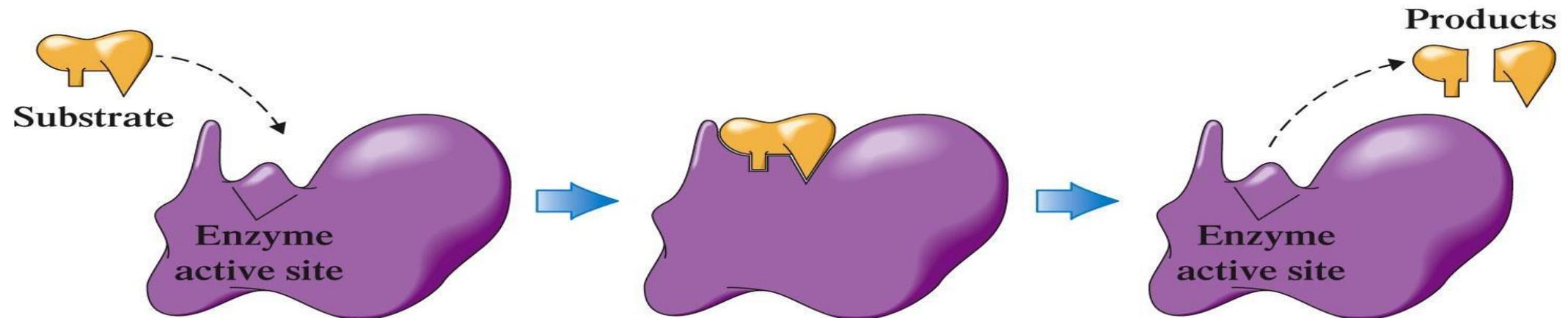


(c) An enzyme with a pocket complementary to the reaction transition state helps to destabilize the stick, contributing to catalysis of the reaction. The binding energy of the magnetic interactions

Mode of Enzyme action

The Induced Fit Model

- In the induced-fit model was proposed by Daniel E. Koshland, Jr., in 1958.
- It states that the binding of substrate induces a conformational change in the active site of the enzyme.
- In addition, the enzyme may distort the substrate, forcing it into a conformation similar to that of the transition state.



Enzyme Specificity

- **Absolute Specificity**
 - An enzyme will catalyze a particular reaction for only one substrate
 - Most restrictive of all specificities
 - Not common
 - *Catalase* has absolute specificity for hydrogen peroxide (H_2O_2) Decomposition of Hydrogen peroxide into water and oxygen.
 - *Urease* catalyzes only the hydrolysis of urea
- **Group Specificity**
 - The enzyme will act only on similar substrates that have a specific functional group
 - *Carboxypeptidase* cleaves amino acids one at a time from the carboxyl end of the peptide chain

Enzyme Specificity

- **Linkage Specificity**
 - The enzyme will act on a particular type of chemical bond, irrespective of the rest of the molecular structure
 - The most general of the enzyme specificities
 - *Phosphatases* hydrolyze phosphate–ester bonds in all types of phosphate esters
 - *Chymotrypsin* catalyzes the hydrolysis of peptide bonds
- **Stereochemical Specificity**
 - The enzyme can distinguish between stereoisomers
 - Chirality is inherent in an active site (as amino acids are chiral compounds)
 - *L-Amino-acid oxidase* catalyzes reactions of L-amino acids but not of D-amino acids

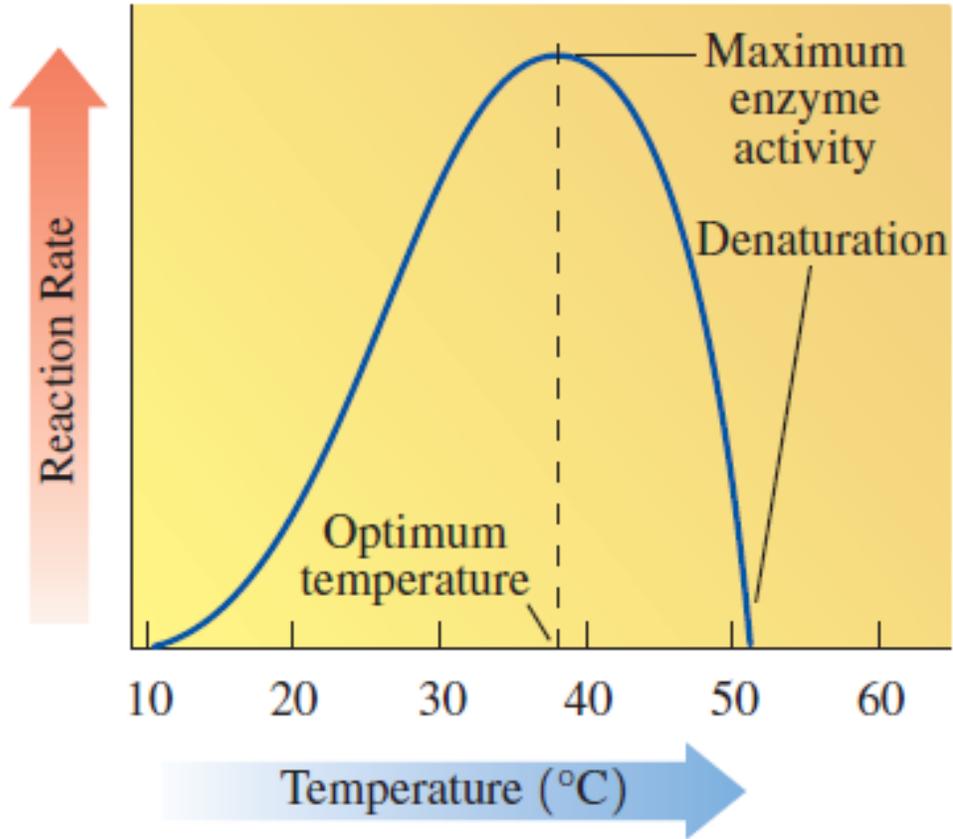
Factors Affecting Enzyme Activity

Enzyme activity is a measure of the rate at which an enzyme converts substrate to products in a biochemical reaction.

Four factors affect enzyme activity:

1. Temperature
2. pH
3. Substrate concentration
4. Enzyme concentration.

Temperature (t)



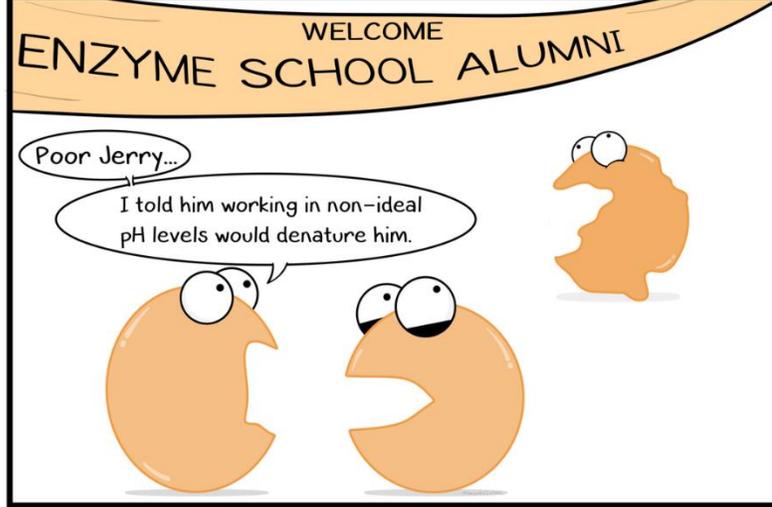
With increased t the E_{KIN} increases

- More collisions
- Increased reaction rate

When the t increases beyond t_{OPT} (**Optimum Temperature**)

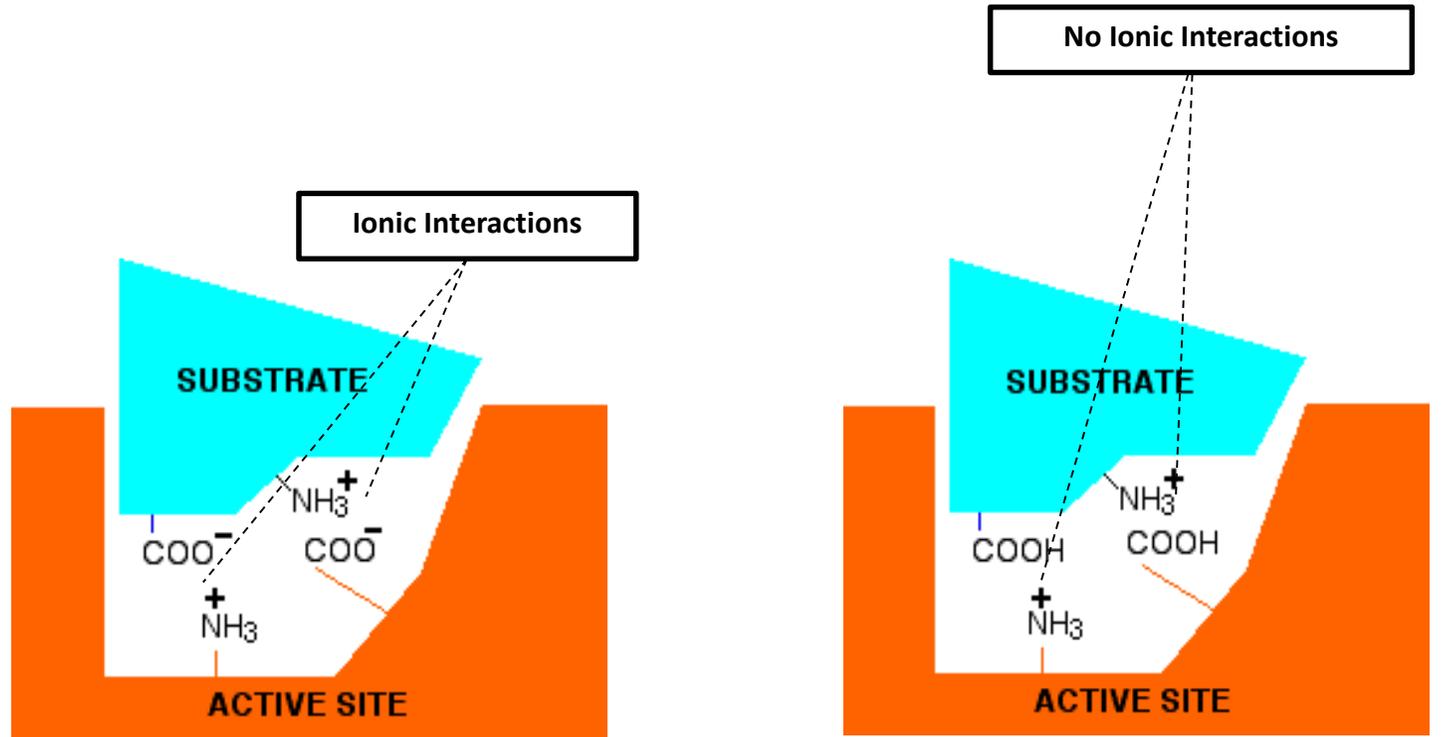
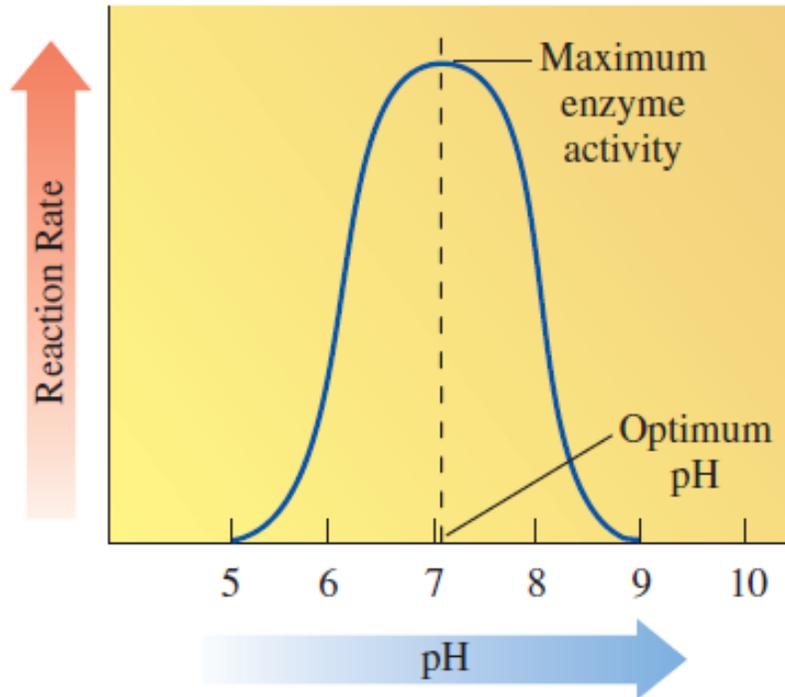
- Changes in the enzyme's tertiary structure occur, inactivating & denaturing it (e.g. fever)

An enzyme attains maximum activity at its optimum temperature, usually 37°C. Lower temperatures slow the rate of reaction, and temperatures above 50 °C denature most enzymes with a loss of catalytic activity.



pH

Enzymes are most active at their optimum pH. At a higher or lower pH, denaturation of the enzyme causes a loss of catalytic activity.



pH

Optimum pH for Selected Enzymes

Enzyme	Location	Substrate	Optimum pH
Pepsin	Stomach	Peptide bonds	1.5–2.0
Sucrase	Small intestine	Sucrose	6.2
Amylase	Pancreas	Amylose	6.7–7.0
Urease	Liver	Urea	7.0
Trypsin	Small intestine	Peptide bonds	7.7–8.0
Lipase	Pancreas	Lipid (ester bonds)	8.0
Arginase	Liver	Arginine	9.7

Substrate Concentration

If [enzyme] is kept constant & the [substrate] is increased
–The reaction rate increases until a **saturation point** is met

- At saturation the reaction rate stays the same even if the [substrate] is increased

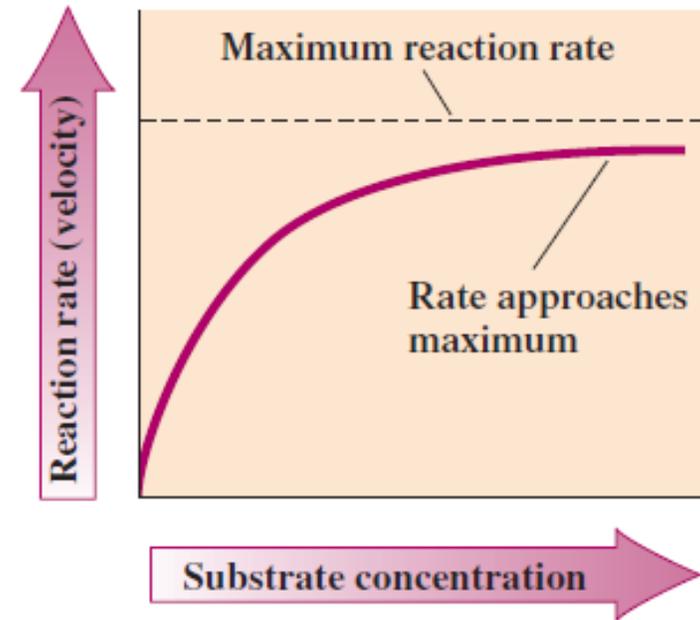
At saturation point substrate molecules are bound to all available active sites of the enzyme molecules

- Reaction takes place at the active site

–If they are all active sites are occupied the reaction is going at its maximum rate

- Each enzyme molecule is working at its maximum capacity

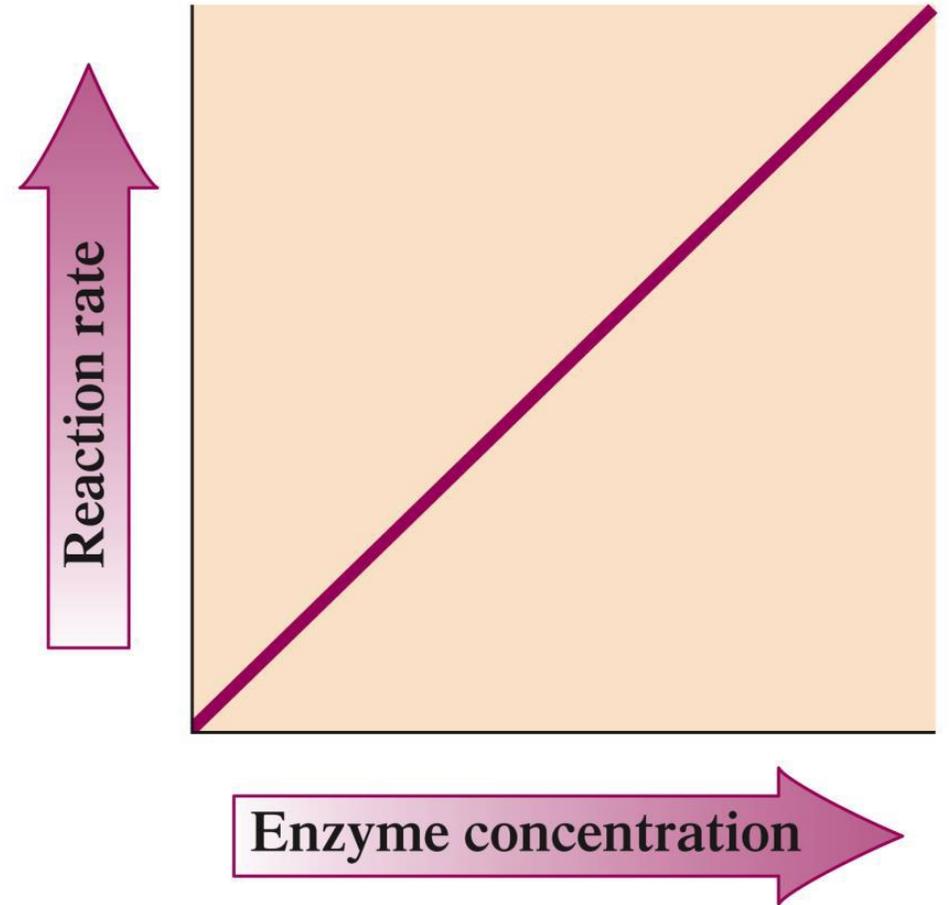
–The incoming substrate molecules must “wait their turn”



Concentration of Enzyme

If the **[substrate]** is kept constant and the **[enzyme]** is increased

- The reaction rate increases
- The greater the [enzyme], the greater the reaction rate
- RULE:
 - The rate of an enzyme-catalyzed reaction is always directly proportional to the amount of the enzyme present
- In a living cell:
 - The [substrate] is much higher than the [enzyme]
- Enzymes are not consumed in the reaction
- Enzymes can be reused many times



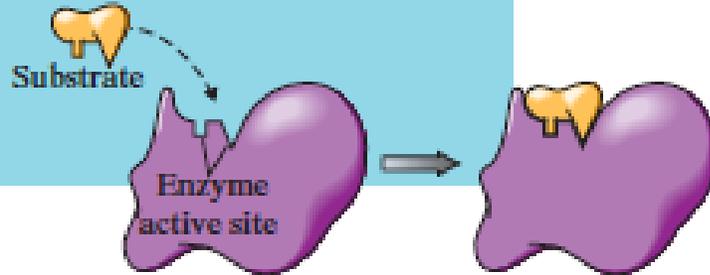
Enzyme Activity

THE MECHANISM OF ENZYME ACTION

Formation of an enzyme–substrate complex as an intermediate species provides an alternative pathway, with lower activation energy, through which a reaction can occur.

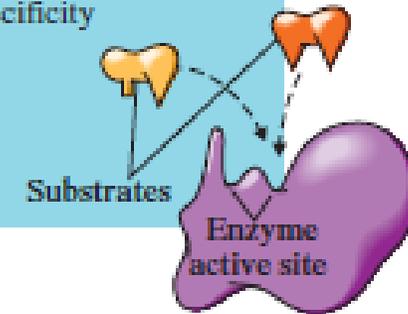
Lock-and-Key Model

The active site has a fixed geometric shape. Only a substrate with a matching shape can fit into it.



Induced-Fit Model

The active site has a flexible shape that can change to accept a variety of related substrates. Enzymes vary in their degree of specificity for substrates.



FACTORS THAT AFFECT THE RATE OF ENZYME ACTIVITY

Temperature

Reaction rate increases with temperature until the point at which the protein is denatured and activity drops sharply.

pH

Maximum enzymatic activity is possible only within a narrow pH range; outside this pH range, the protein is denatured and activity drops sharply.

Concentration of Substrate

Reaction rate increases with substrate concentration until full saturation occurs; then the rate levels off.

Concentration of Enzyme

Reaction rate increases with increasing enzyme concentration, assuming enzyme concentration is much lower than that of substrate.

AMINO ACIDS, PROTEINS, AND ENZYMES

Proteins

contain

Amino Acids

that have

**Ammonium,
Carboxylate,
and R Groups**

contain peptide bonds between

Amino Acids

in a specific order as

Primary Structure

and hydrogen bond as

Secondary Structure

with interactions that give

**Tertiary and
Quaternary
Structures**

are active as

Enzymes

are used for structure,
transport, hormones,
storage, and as

Enzymes

bind substrate at

Active Site

to form an

ES Complex

that is blocked by

Inhibitors

undergo

Denaturation

by

**Heat, Acids, Bases,
Organic Compounds**

that change

**Secondary or
Tertiary Structures
of Proteins**