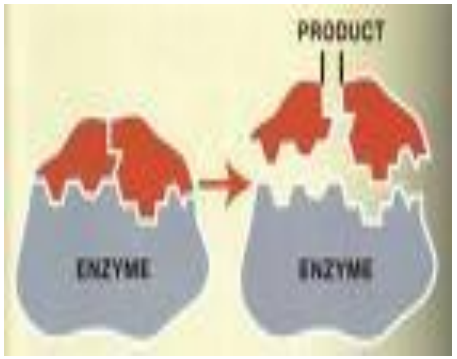
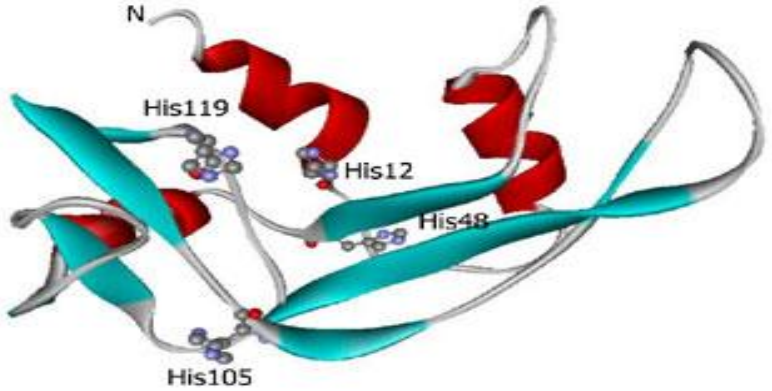
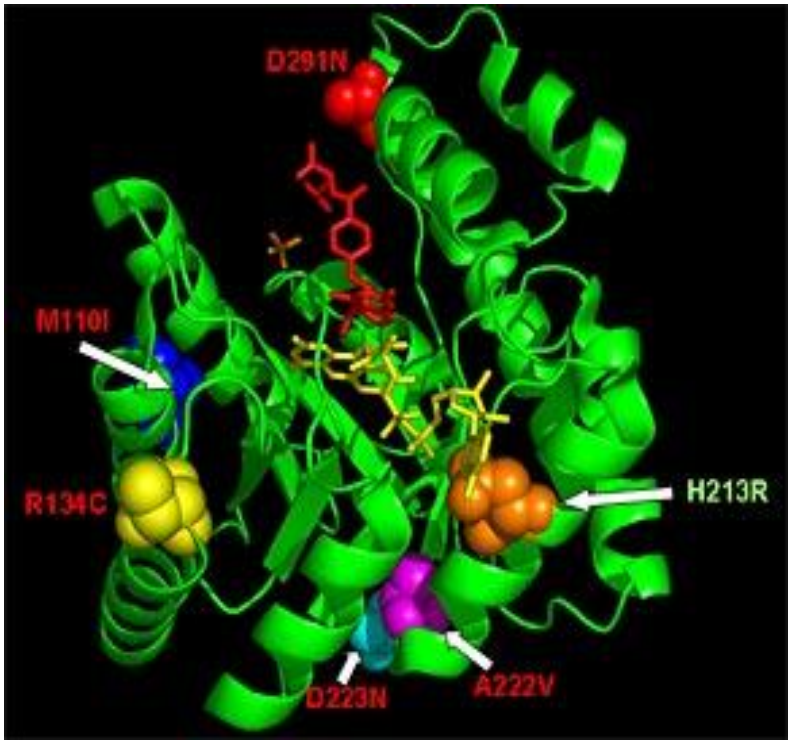




ENZYMOLOGY



Presented By:-

Prof. Katarnavare A.B

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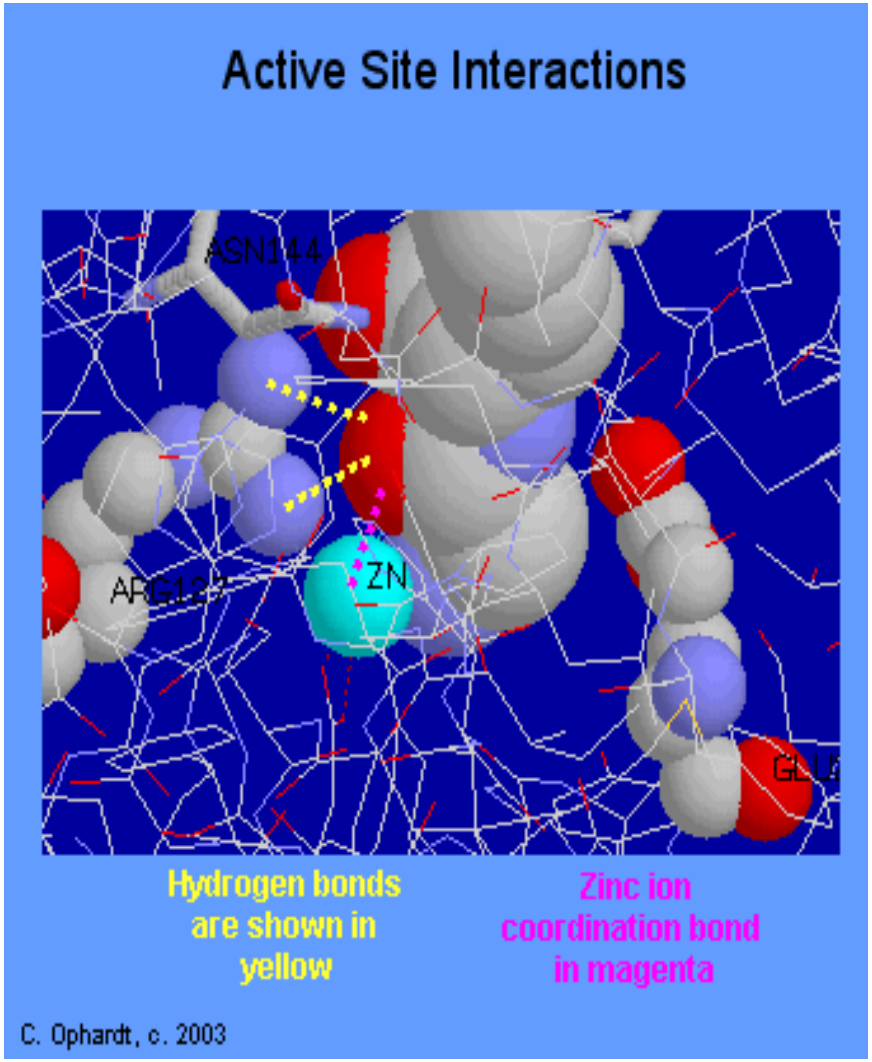

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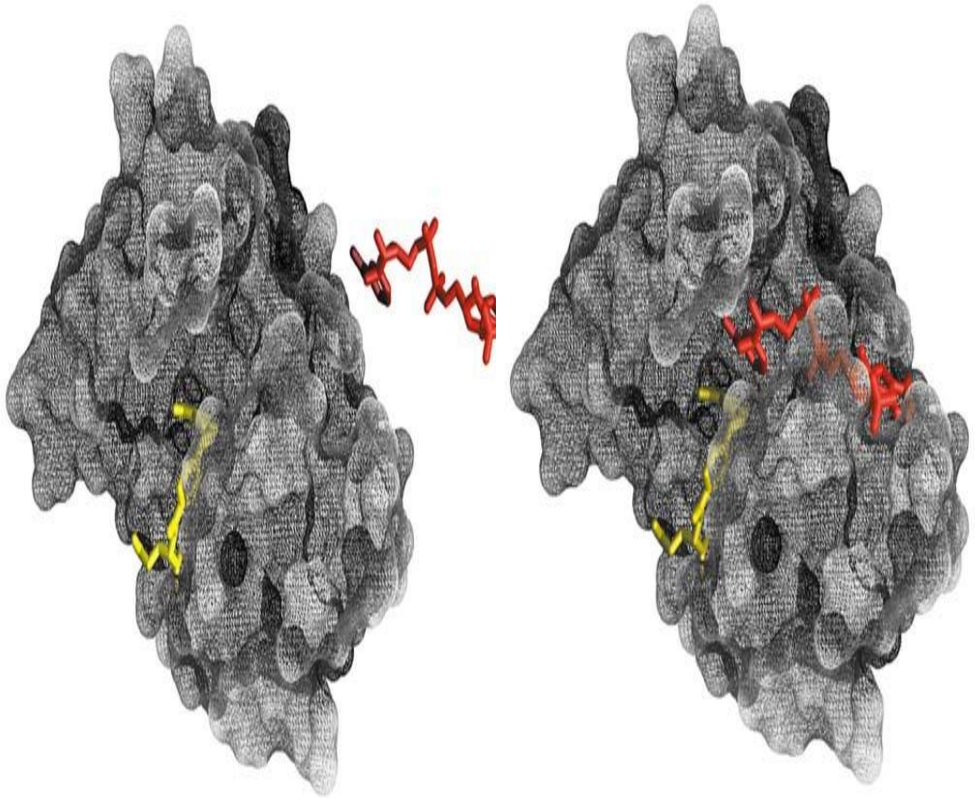
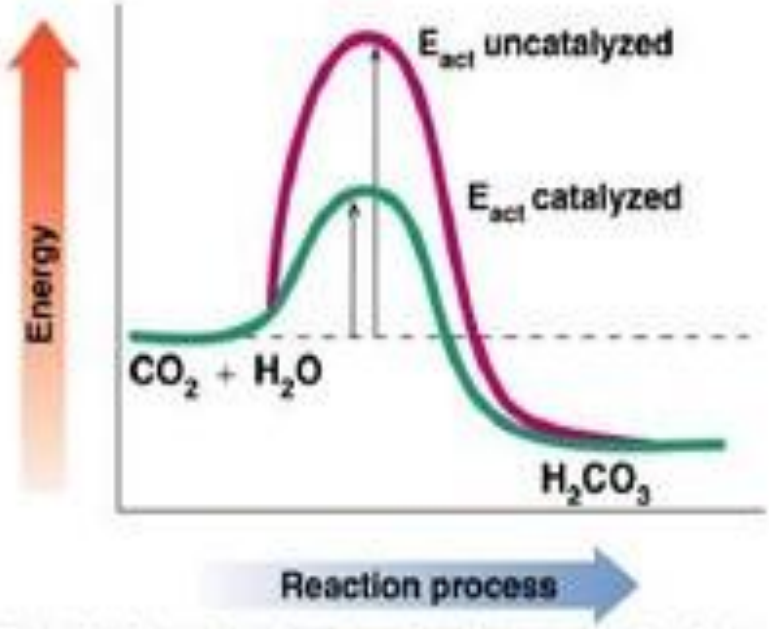


Enzymes are Biological Catalysts

Enzymes are proteins that:

- Increase the rate of reaction by lowering the energy of activation.
- Catalyze nearly all the chemical reactions taking place in the cells of the body.
- Have unique three-dimensional shapes that fit the shapes of reactants.



History

DATE	INVESTIGATORS	CONTRIBUTION
1833	Payen and Persoz	First observation of enz. activity in test tube
1878	Kuhne	word:"enzyme"
1898	Duclaux	Ist suggestion foe enz. nomenclature
1890	Fisher	Lock and key model
1913	Michaelis and Menton	Equation
1926	Sumner	Ist isolation of an enz. urease
1986	Cech	Discovery of catalytic RNA
1986	Lerner and Schultz	Catalytic abs

Enzymes vs. Catalysts

FEATURES	ENZYMES	NONBIOLOGICAL CATALYSTS
Specificity	Highly specific	Nonspecific
Rate of reaction	Enhanced by a factor of 10^{3}- 10^{6}	Only a fraction of that by enzymes
Saturation	Shows	Do not
Tp, Pressure,pH	Mild, biologically compatible	High tp. and high pressure
Chemical Nature	Proteins	Metal and non-metal inorganic molecules

Nomenclature of Enzymes

- End in –'*ase*'
- Identifies a reacting substance
  - sucrase – reacts on sucrose
  - lipase - reacts on lipid
- Describes function of enzyme
  - oxidase – catalyzes oxidation
  - hydrolase – catalyzes hydrolysis
- Common names of digestive enzymes use –'*in*'
  - pepsin, trypsin

CLASSIFICATION OF ENZYMES

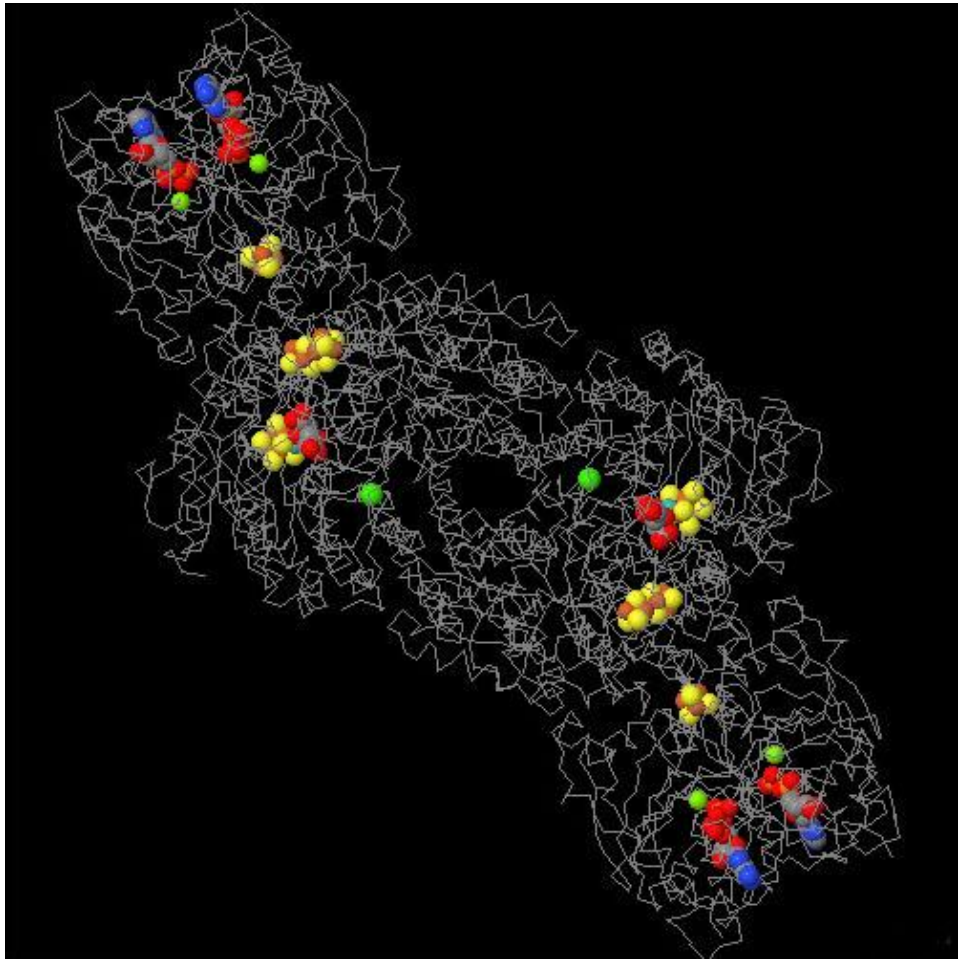
Group of Enzyme	Reaction Catalysed	Examples
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another.	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl etc.) from one substrate to another.	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate.	Estrases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate.	Phospho hexo isomerase, Fumarase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate.	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds.	Citric acid synthetase

Structure of Enzyme

- ✓Cofactors
- ✓Coenzymes
- ✓Apoenzyme
- ✓Holoenzyme

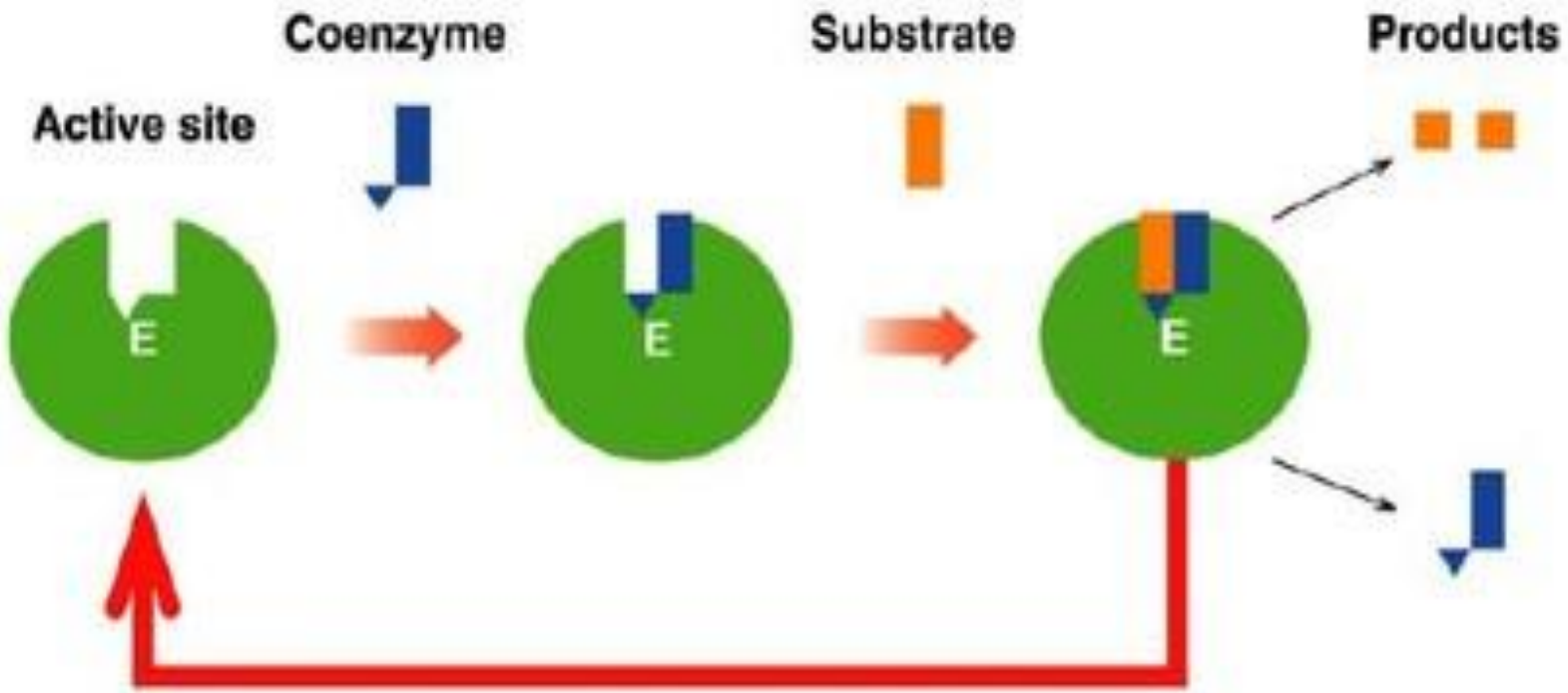
Cofactors

- An additional non-protein molecule that is needed by some enzymes to help the reaction
- Tightly bound cofactors are called **prosthetic** groups
- Cofactors that are bound and released easily are called coenzymes
- Many vitamins are coenzymes



Function of Coenzymes

- A coenzyme prepares the active site for catalytic activity.

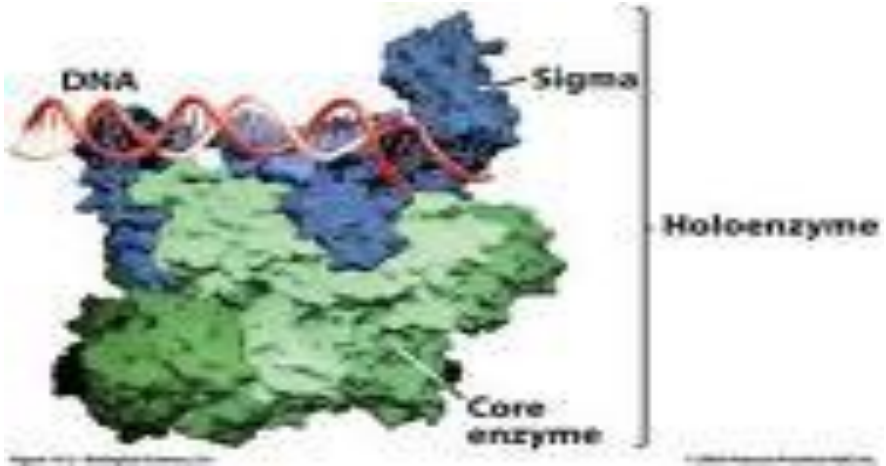


APOENZYME

- The inactive protein component of an enzyme.
- Cofactor and coenzyme may be covalently or non-covalently attached to the protein molecule called apoenzyme.
- When a cofactor is so tightly bound to the apoenzyme thai it is difficult to remove it without damaging the enzyme, the cofactor is called a prosthetic group.

HOLOENZYME

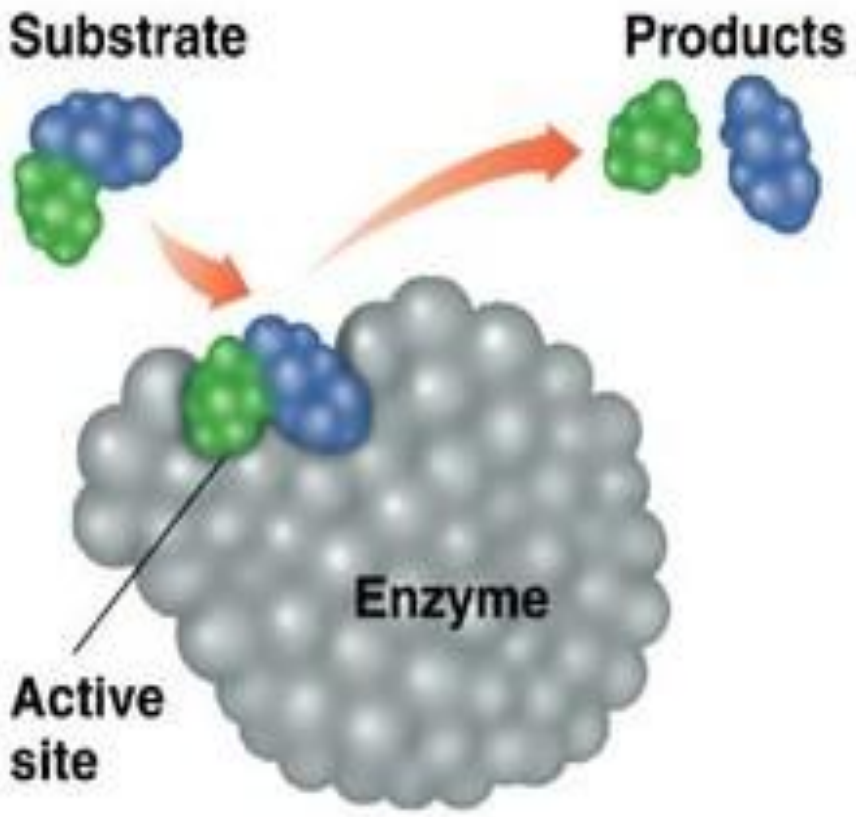
Apoenzyme + Cofactor→ Holoenzyme



Active Site

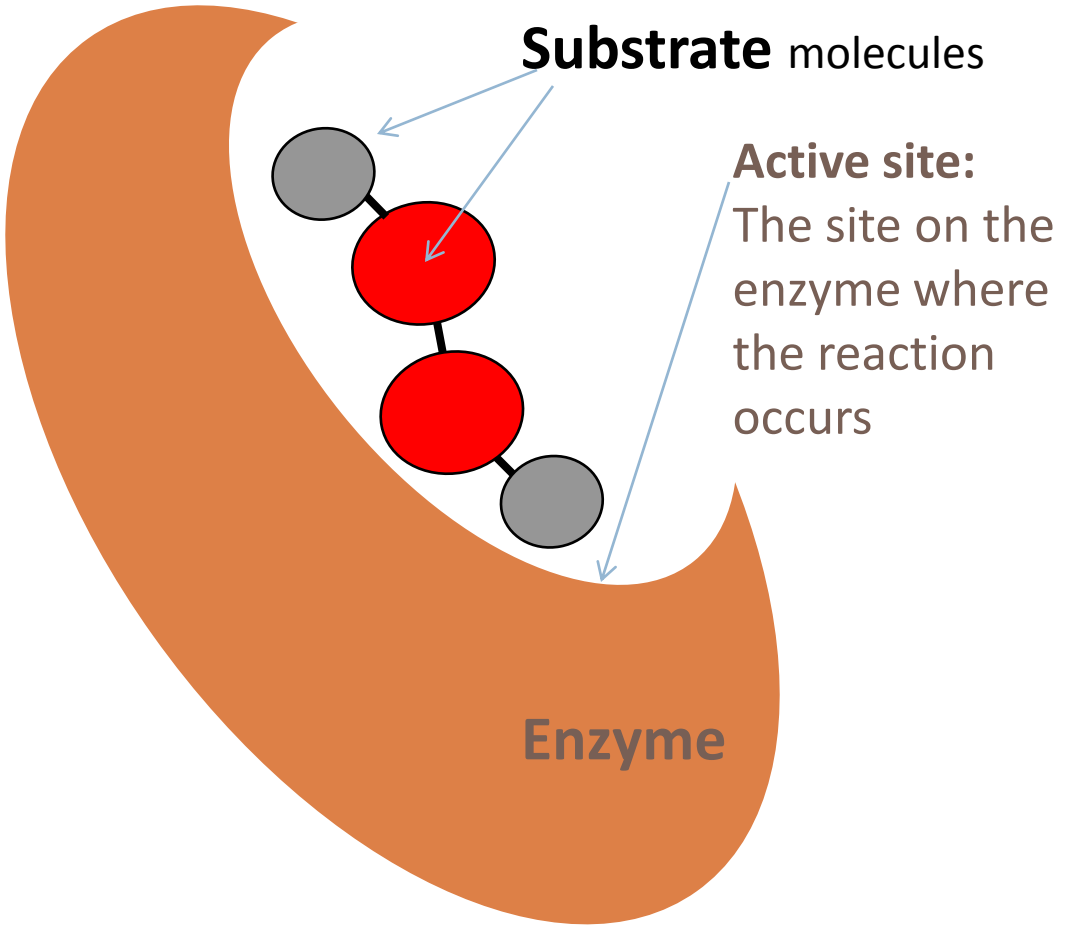
The **active site**:

- Is a region within an enzyme that fits the shape of molecules called **substrates**.
- Contains amino acid R groups that align and bind the substrate.
- Releases products when the reaction is complete.

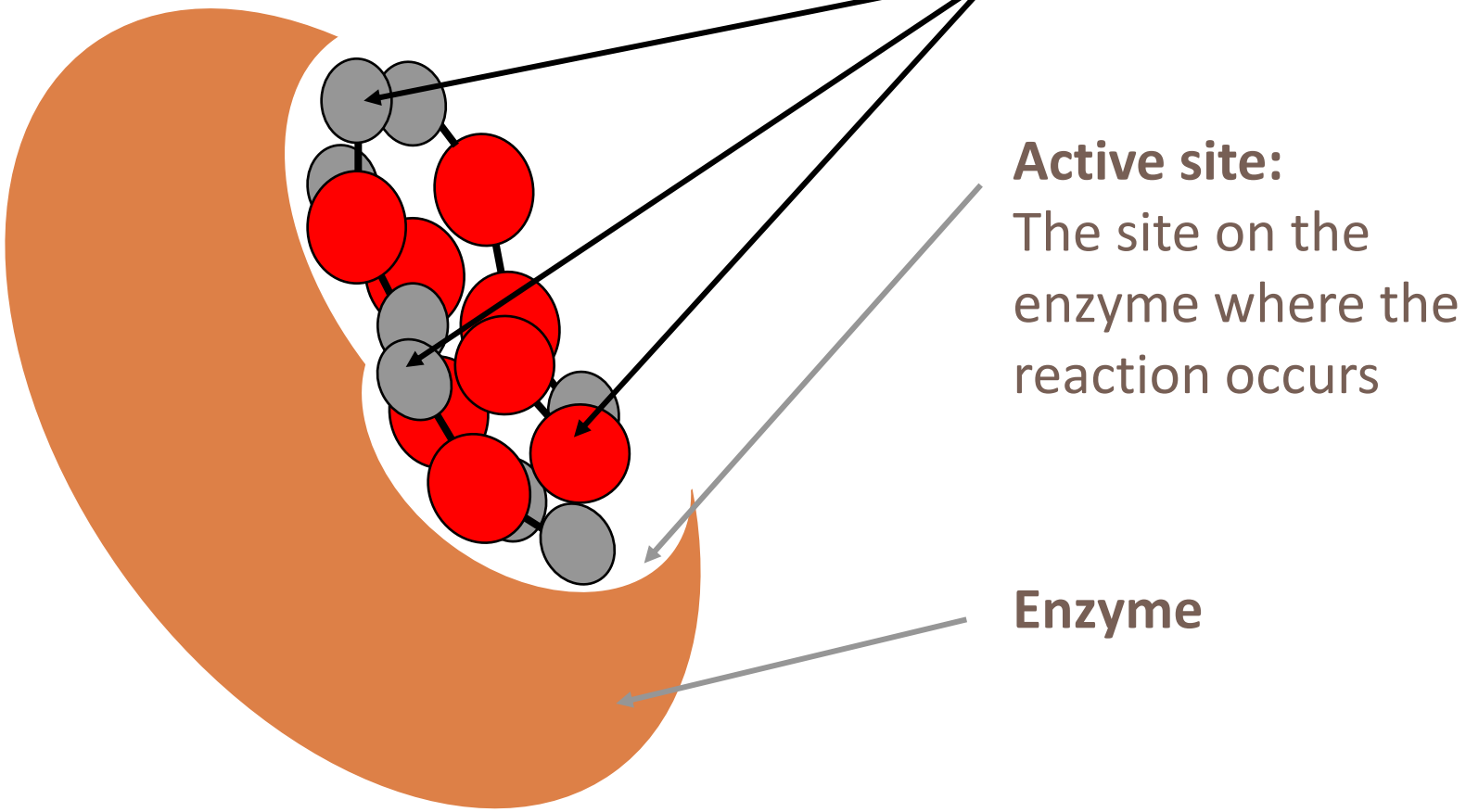


The substrate

- The substrate of an enzyme are the **reactants** that are activated by the enzyme
- Enzymes are **specific** to their substrates
- The specificity is determined by the **active site**



The molecule, the enzyme produces is known as the ***product*** molecule



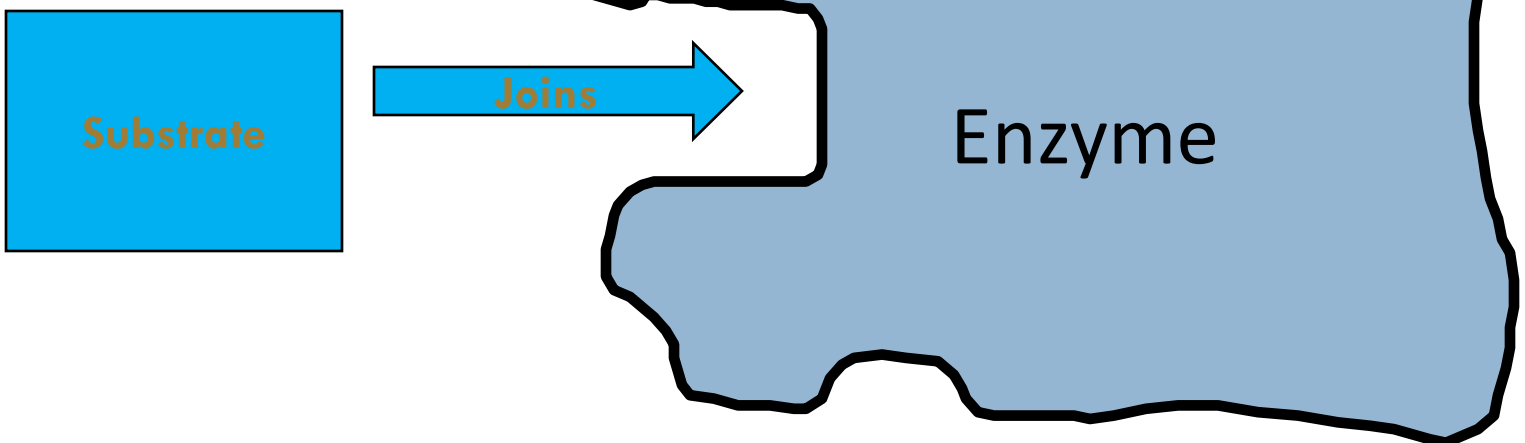
Enzyme reactions

enzyme + substrate → enzyme-substrate complex

E +S → ES

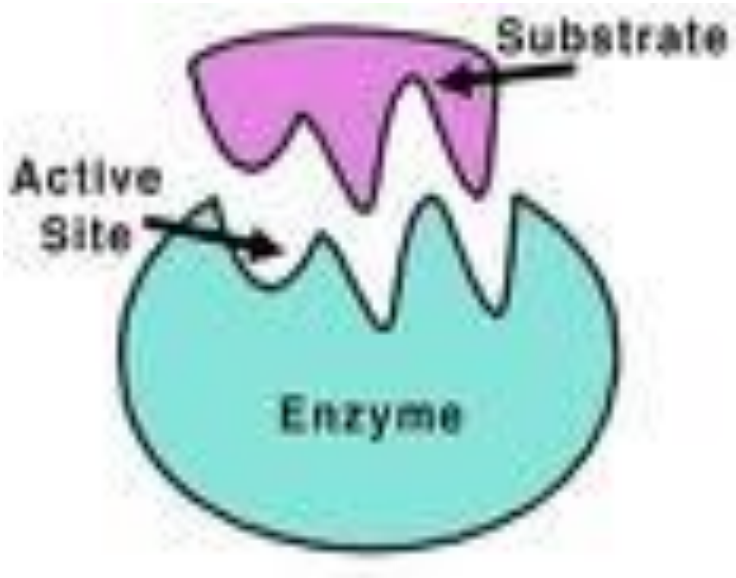
enzyme-substrate complex → enzyme + product

ES → E +P



Specificity and its types

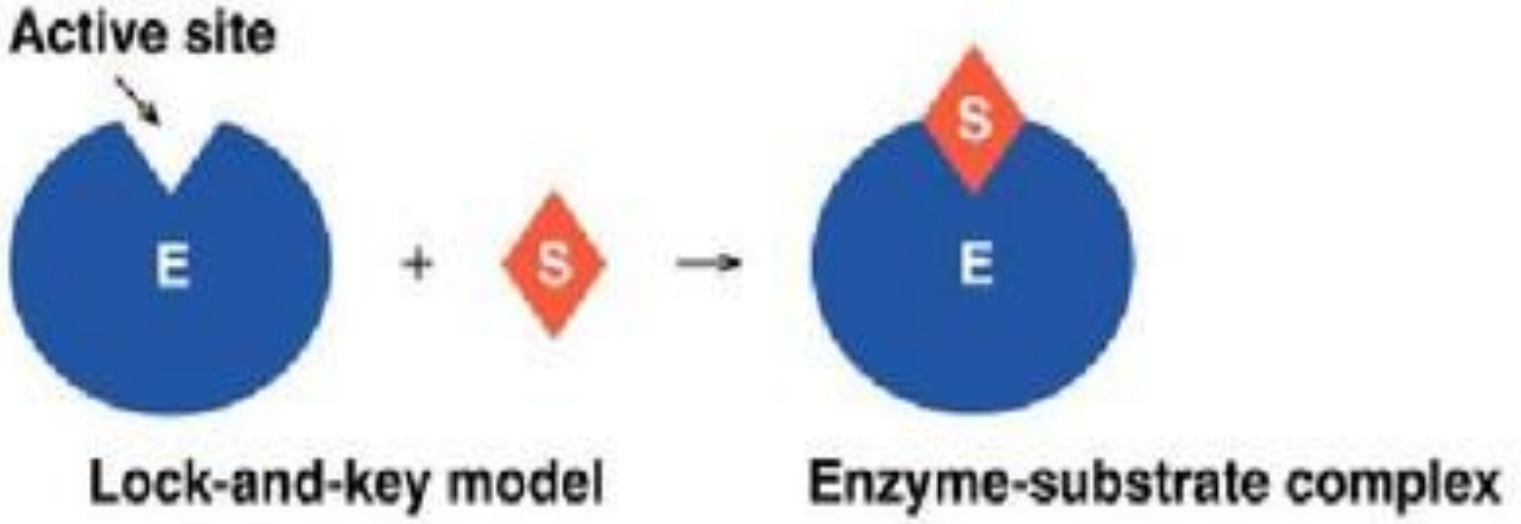
- Each enzyme is specific to one substrate molecule or type of molecule
- Complimentary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrate are responsible.
- ***Types:-***
- 1.Absolute Specificity
- 2.Group Specificity
- 3.Optical Specificity
- 4.Geometrical Specificity



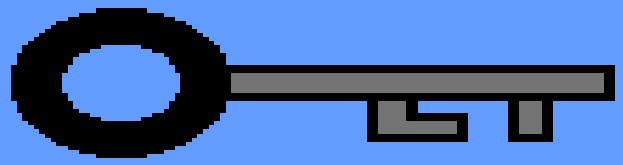
Lock-and-Key Model

In the lock-and-key model of enzyme action:

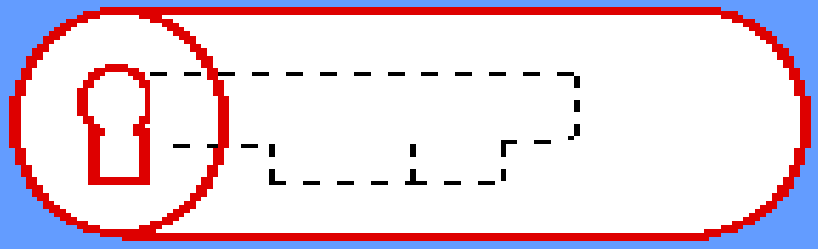
- The active site has a rigid shape.
- Only substrates with the matching shape can fit.
- The substrate is a key that fits the lock of the active site.



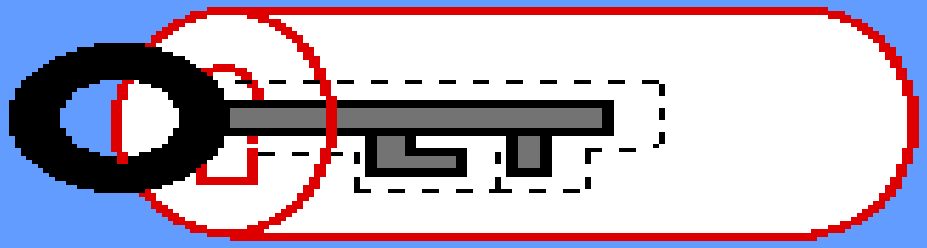
Lock and Key Analogy



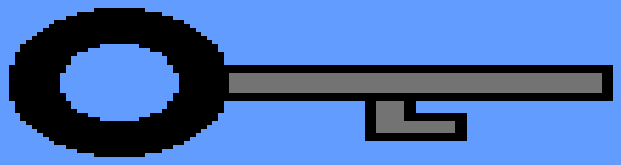
key = substrate



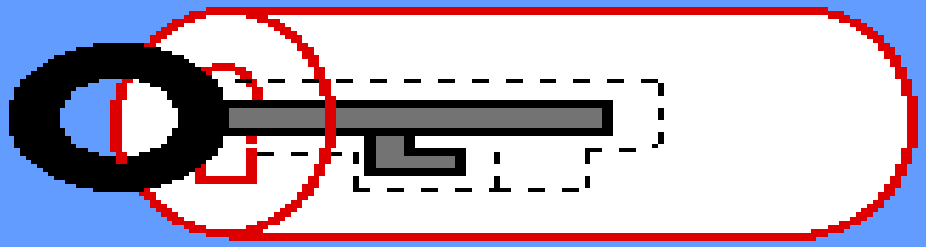
lock = enzyme



correct fit,
will react



incorrect substrate

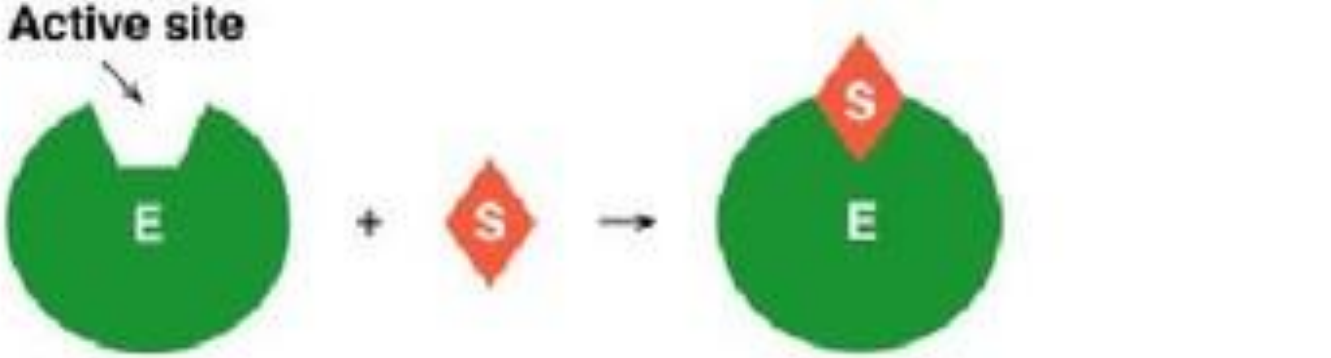


no reaction

Induced-fit Model

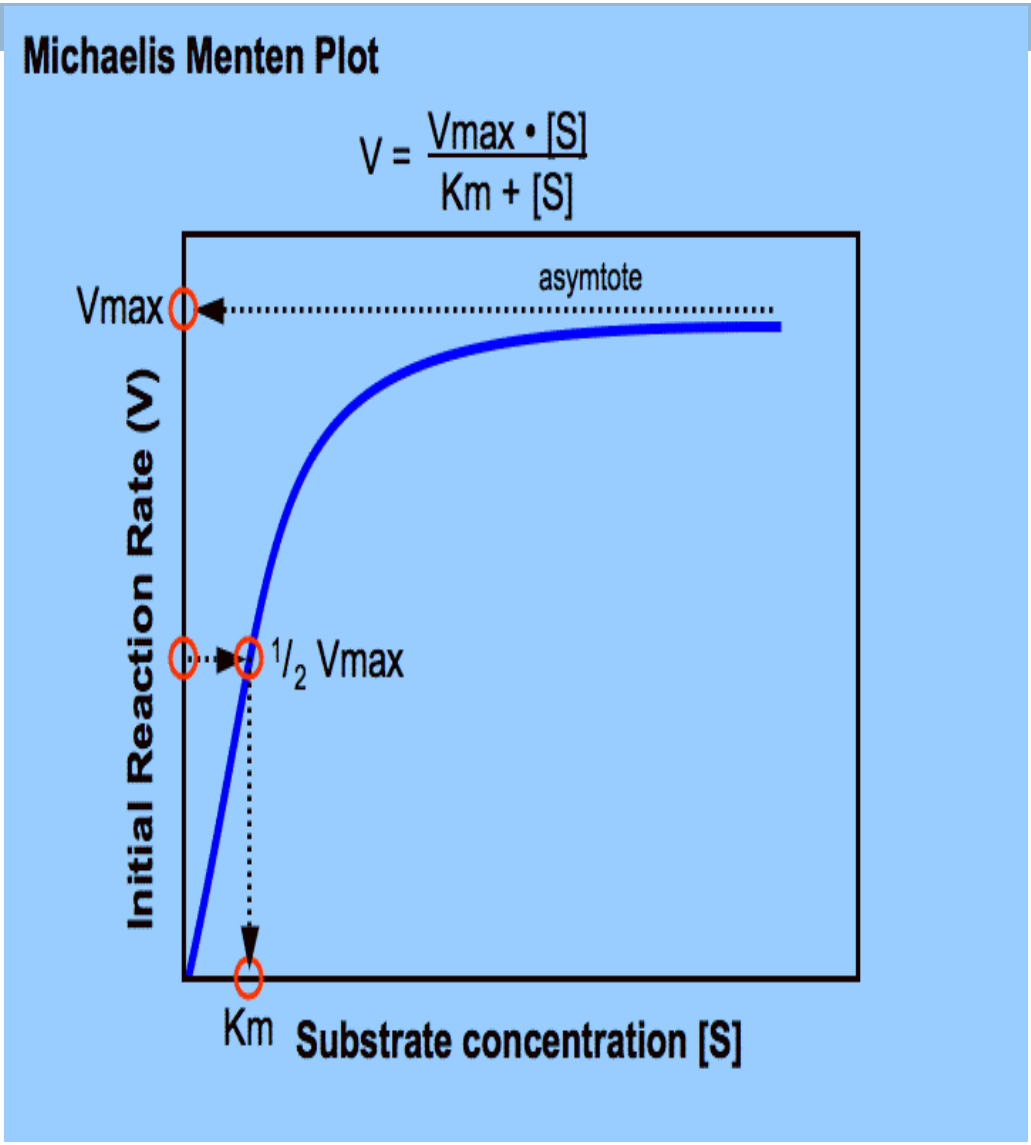
In the induced-fit model of enzyme action:

- The active site is flexible, not rigid.
- The shapes of the enzyme, active site, and substrate adjust to maximum the fit, which improves catalysis.
- There is a greater range of substrate specificity.



ENZYME KINETICS

- It is the investigation of how enzymes bind substrates and turn them into products.
- It is the quantitative study of enzyme catalysis.
- Measures reaction rates and the affinity of enzymes for substrates and inhibitors.
- **The rate of reaction catalyzed by an enzyme, increases linearly with the substrate concentration upto a point and reaches the maximum value *Vmax*.**



- **SUBSTRATE SATURATION:-**
- Beyond **Vmax** there is no further increase in reaction rate, this is known as **substrate saturation**.
- It is described by the…

$$V_1 = \frac{V_{max}[S]}{K_m + [S]}$$

where

V_t = the velocity at any time

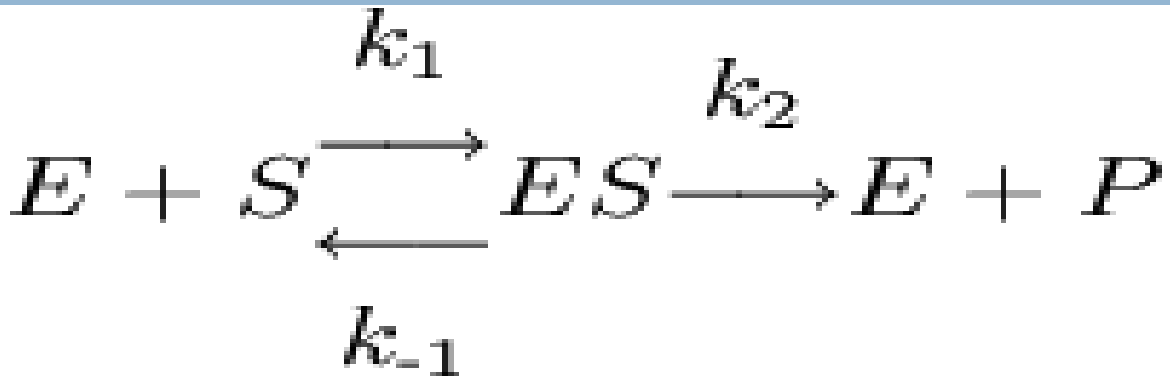
$[S]$ = the substrate concentration at this time

V_{max} = the highest under this set of experimental conditions (pH, temperature, etc.)

K_m = the Michaelis constant for the particular enzyme being investigated

Michaelis – Menton equation

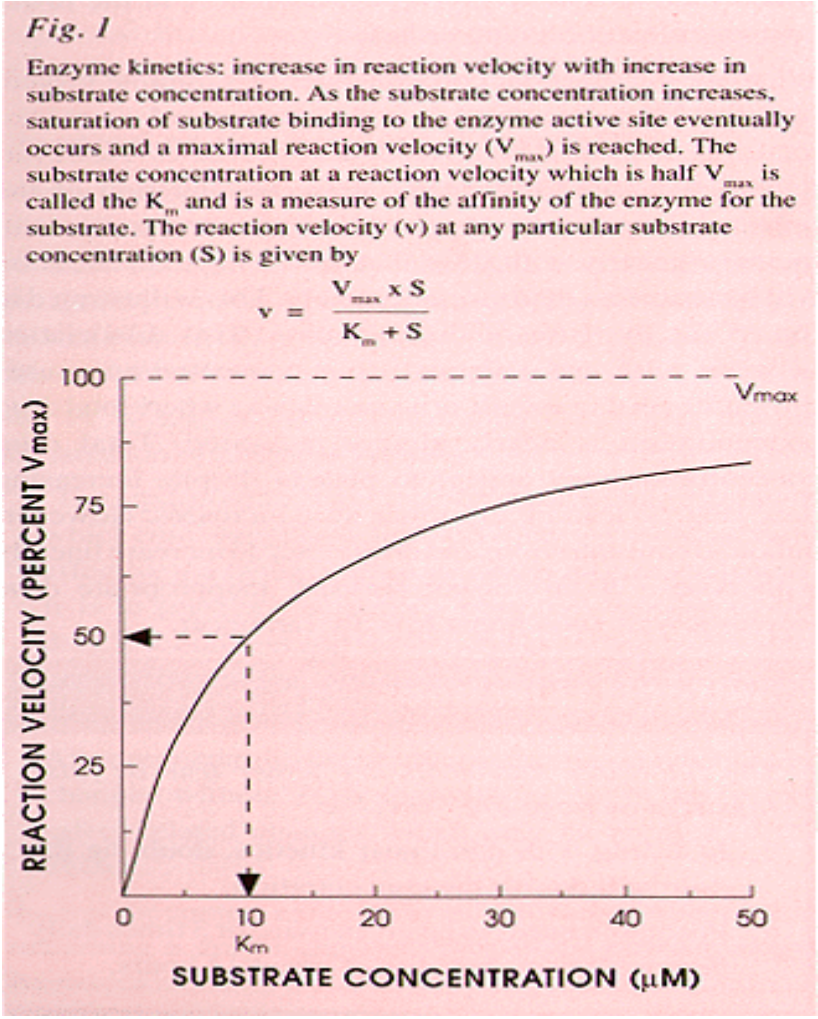
- Model for the kinetics of enzyme-catalysed reaction was devised in **1913** by **L.Michaelis** and **M.Menten.**
- They described the *relationship between reaction velocity and substrate concentration.*



where:-

- **k**1- rate constant for the formation of the enzyme-substrate complex
- **k**-1 – rate constant for the reverse reaction
- **k**2- rate constant for the conversion of the ES-complex to product P

- Initial rate of the reaction-V_{init} or Vo
- At infinite substrate concentration, the reaction would proceed at its maximum velocity- Vmax.
- The substrate concentration at which the reaction proceeds at one half of its Vmax is -Km



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

ASSUMPTION I: $k_{-2} = 0$

$$V = \frac{d[P]}{dt} = k_2[ES] - k_{-2}(\underbrace{([E]-[ES])}_{\text{FREE } [E]})[P]$$

ASSUMPTION II: $[S] \gg [E]$ ($[S] \gg [ES]$)

$$\text{rate of formation of } [ES] = k_1(\underbrace{[E]-[ES]}_{\text{FREE } [E]})(\underbrace{[S]-[ES]}_{\text{FREE } [S]})$$

$$\text{rate of breakdown of } [ES] = k_{-1}[ES] + k_2[ES]$$

ASSUMPTION III: STEADY STATE → rate of formation of $[ES]$ = rate of breakdown of $[ES]$

$$k_1([E]-[ES])[S] = (k_{-1}+k_2)[ES]$$

which, when rearranged gives:

$$[ES] = [E]\frac{[S]}{[S]+K_M} \quad \text{where } K_M = \frac{k_{-1}+k_2}{k_1}$$

$$\text{or } V = k_2\frac{[E][S]}{[S]+K_M}$$

maximum V (call this V_{max}) when $[S]$ very high ($[S] \gg K_M$)

$$V_{max} = k_2[E]$$

So,

$$\boxed{V = V_{max}\frac{[S]}{[S]+K_M}}$$

Assumptions

- *Michaelis –Menton model is based on the following assumptions:-*

→*The concentration of substrate [S] is much greater than the concentration of enzyme [E].*

→*The rate of formation of [ES] is equal to that of the breakdown of [ES] (steady state assumption).*

$$E+S = E\text{-}S$$

→*Very little accumulation of P, so formation of [ES]complex from E+P is negligible.*

Km, Vmax and Kcat

- ***Km***

- ✓ *Varies from one E to another and with different S for the same E.*
- ✓ *It is equal to the S concentration at half the maximum rate.*
- ✓ *Represents the S concentration at which half the enzyme active sites are filled by substrate molecules.*
- ✓ *Depends on tp, nature of the S, pH, ionic strength.*
- ✓ *Any variation in Km is due to the +nce of and inhibitor or activator.*
- ✓ *Its value is sometimes equated with the dissociation constant(Ks) of the ES-Complex.*

$$Km = \frac{k\text{-}1}{k1} = Ks$$

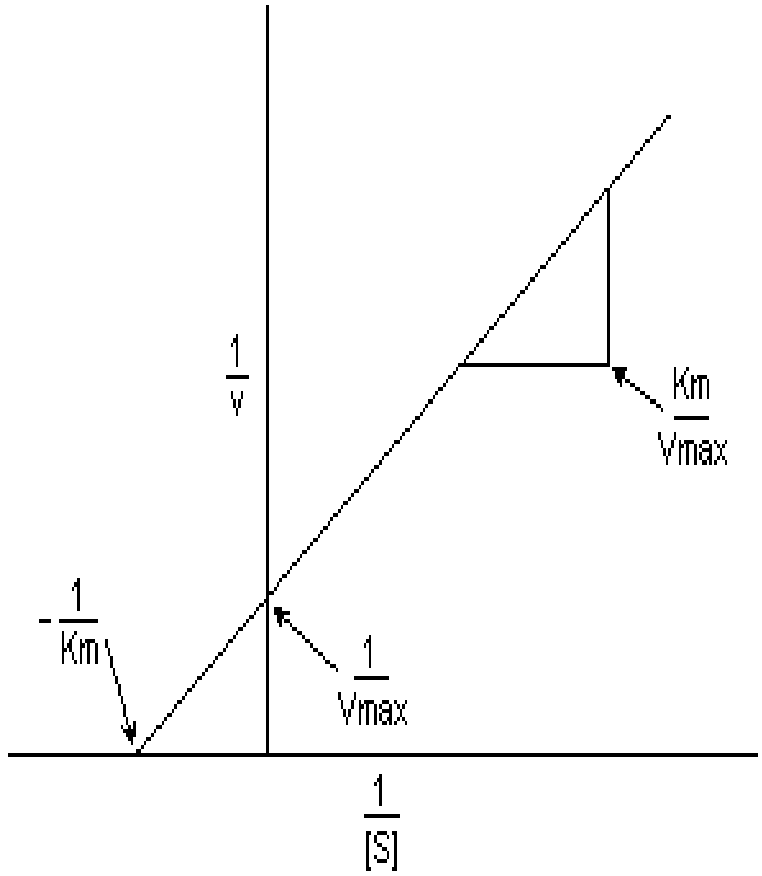
- ***Vmax***
  - $V_{max} = K_2[E_t]$
  - [Et]=total enzyme concentration
- ***Kcat(Turn over number)***
  - *It* is the number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.
  - Represents the kinetic efficiency of the enzyme.
  - Unit- sec^{-1}

$$K_2 = \frac{V_{max}}{E_t} = K_{cat}$$

Lineweaver-Burk Equation

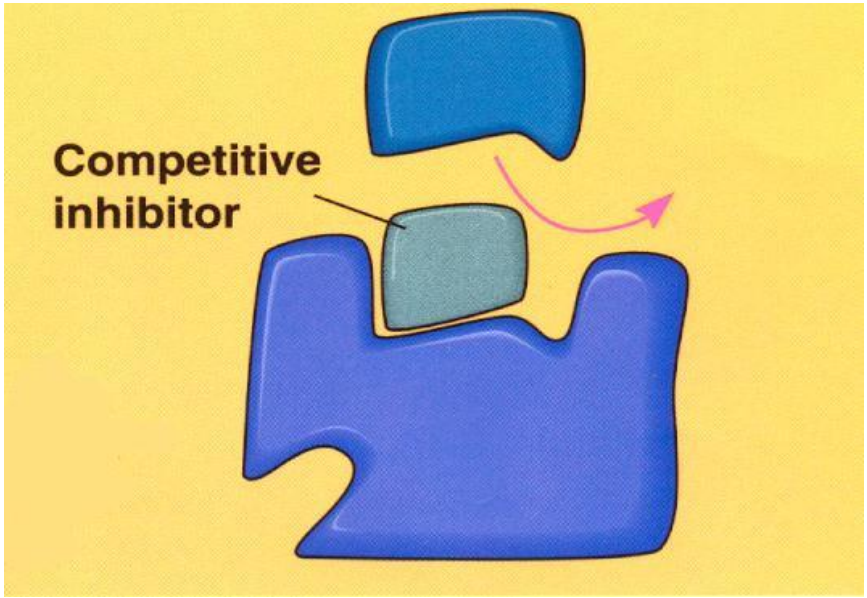
- Plot between the reciprocal of V and [S] is known as *Lineweaver-Burk plot* or *double reciprocal plot*.

$$\frac{1}{v} = \frac{K_m}{v_{max}} \frac{1}{[S]} + \frac{1}{v_{max}}$$



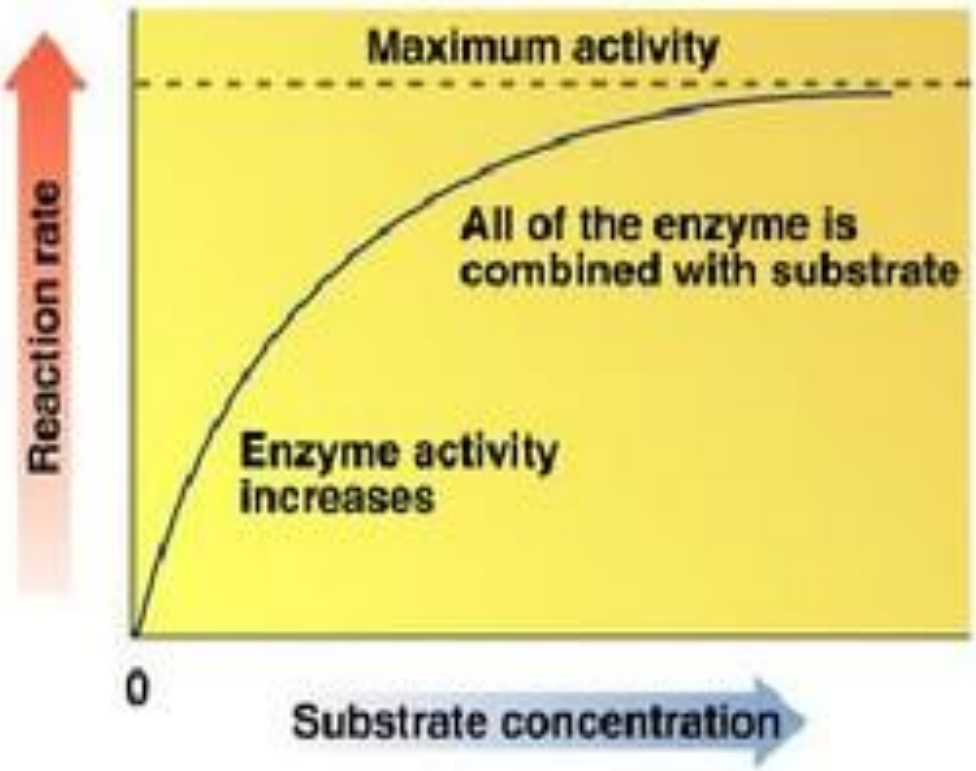
Factors affecting Enzyme Reaction

- substrate concentration
- pH
- temperature
- inhibitors



Substrate Concentration

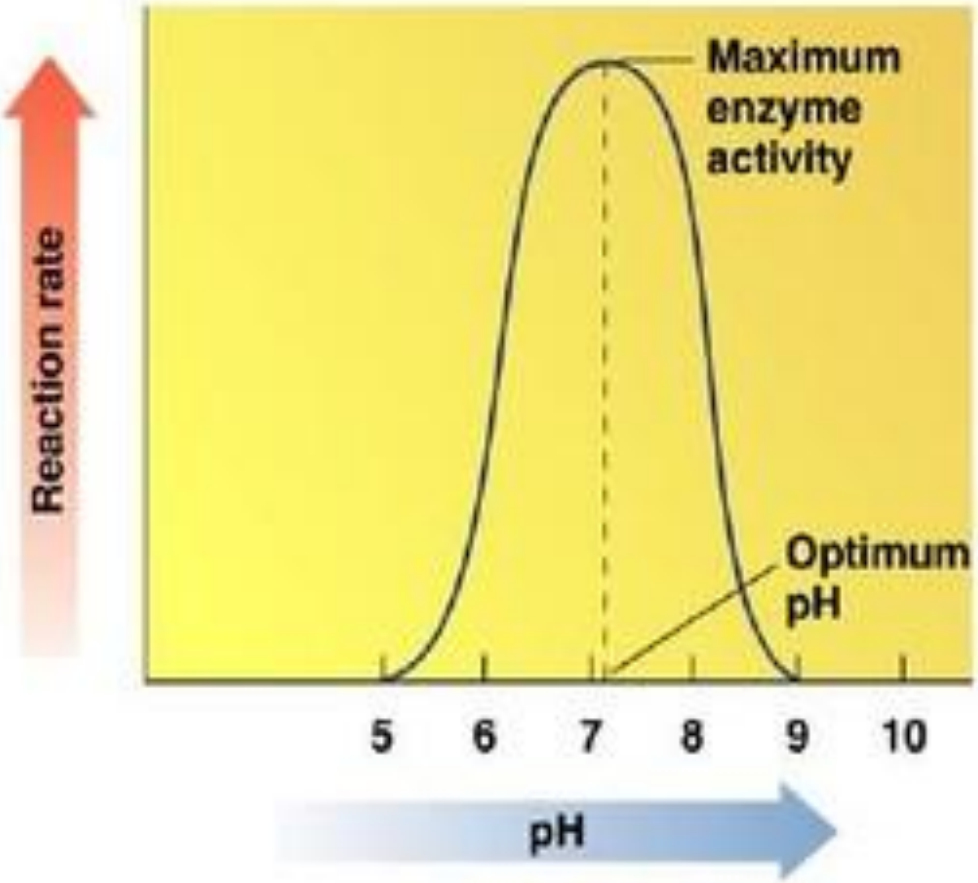
- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration).
- Maximum activity occurs when the enzyme is saturated.



pH and Enzyme Action

Enzymes:

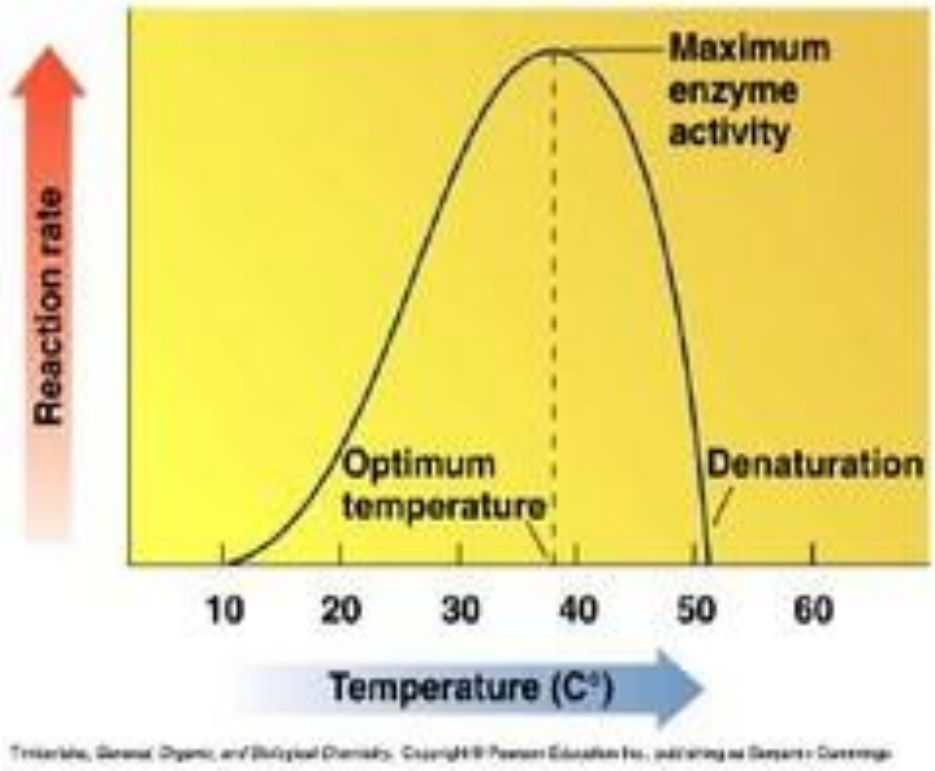
- Are most active at optimum pH.
- Contain R groups of amino acids with proper charges at optimum pH.
- Lose activity in low or high pH as tertiary structure is disrupted.



Temperature and Enzyme Action

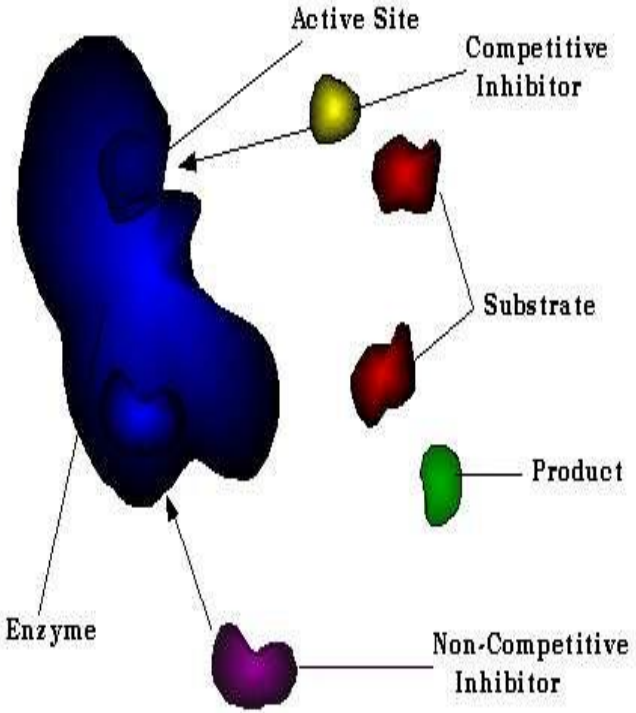
Enzymes:

- Are most active at an optimum temperature (usually 37°C in humans).
- Show little activity at low temperatures.
- Lose activity at high temperatures as denaturation occurs.



INHIBITORS

- *Inhibitors are chemicals that reduce the rate of enzymatic reactions.*
- *They are usually specific and they work at low concentrations.*
- *They block the enzyme but they do not usually destroy it.*



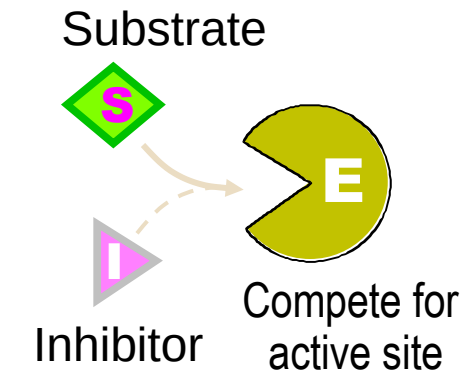
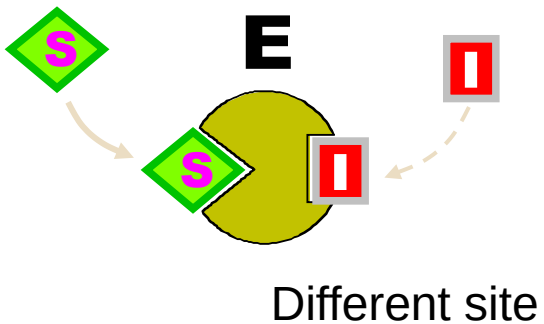
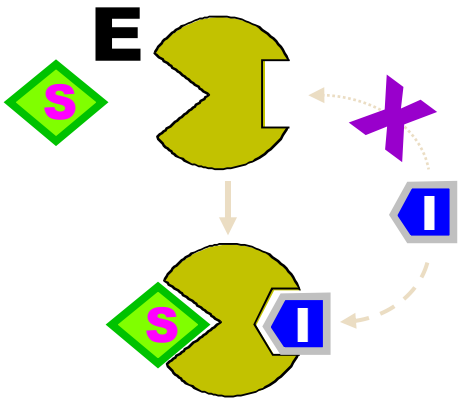
Classes of enzyme inhibition:-

1. ***Reversible inhibitors***

   A. Competitive reversible inhibitors
   B. Non-competitive reversible inhibitors
   C. Un-competitive reversible inhibitors

2. ***Irreversible inhibitors***

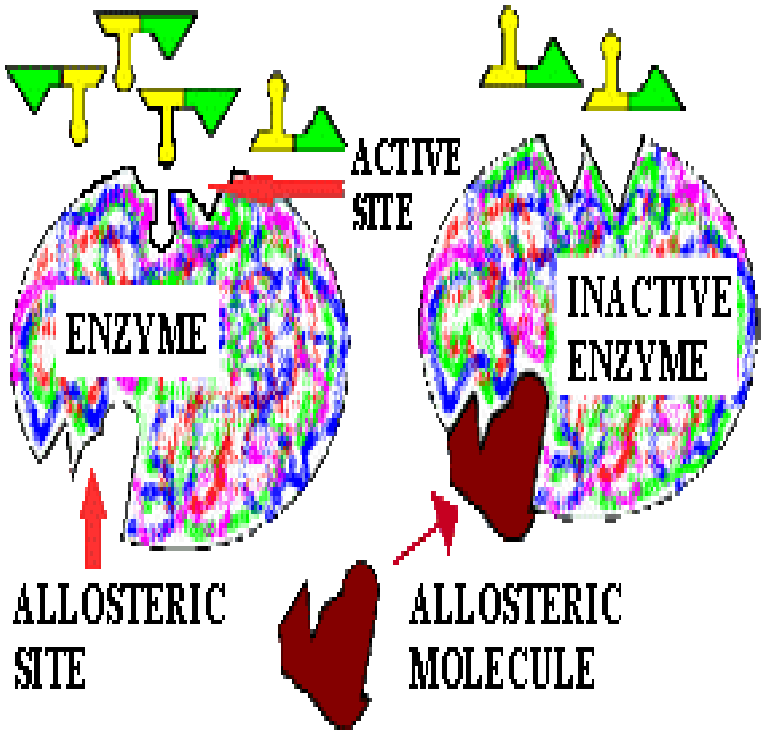
Reversible inhibitors

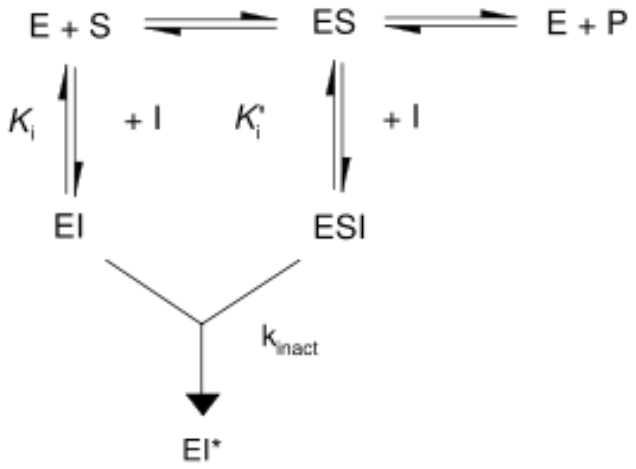
	Competitive	Non-competitive	Uncompetitive
Picture			
Equation and Description	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EI [*I*] binds to free [E] only, and competes with [S]; increasing [S] overcomes Inhibition by [*I*].	$E + S \rightleftarrows ES \rightarrow E + P$ $+ \quad +$ $I \quad I$ $\downarrow\uparrow \quad \downarrow \quad \uparrow$ $EI + S \rightarrow EIS$ [*I*] binds to free [E] or [ES] complex; Increasing [S] can not overcome [*I*] inhibition.	$E + S \rightleftarrows ES \rightarrow E + P$ $+$ I $\downarrow\uparrow$ EIS [*I*] binds to [ES] complex only, increasing [S] favors the inhibition by [*I*].

Irreversible inhibitors:

These molecules bind permanently with the enzyme molecule and so effectively reduce the enzyme concentration, thus limiting the rate of reaction.

For example- Cyanide irreversibly inhibits the enzyme cytochrome oxidase found in the electron transport chain used in respiration. If this cannot be used, death will occur.

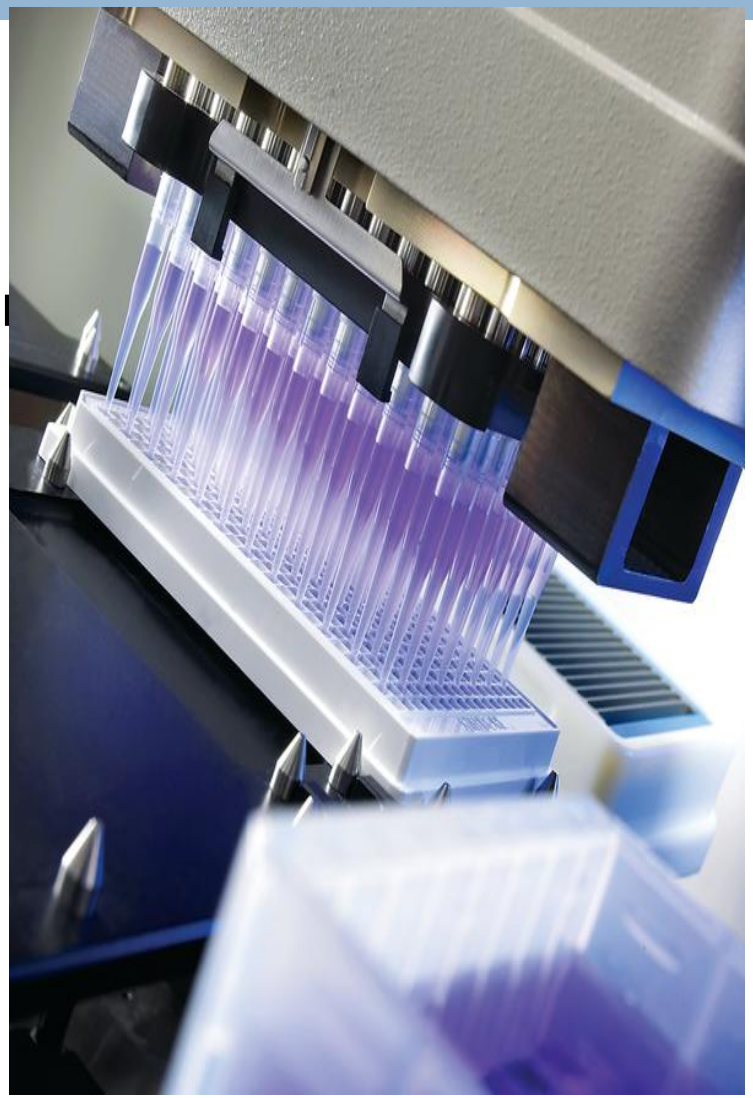




Enzyme Assays

- Laboratory methods for measuring enzymatic activity.
- Measure either the consumption of substrate or production of product.
- It can be :- *a. Continuous*

  b. Discontinuous



- **Continuous Assay:** gives a continuous reading of activity.
- **Discontinuous Assay**: reaction stops, then the concentration of substrates/products are determined.

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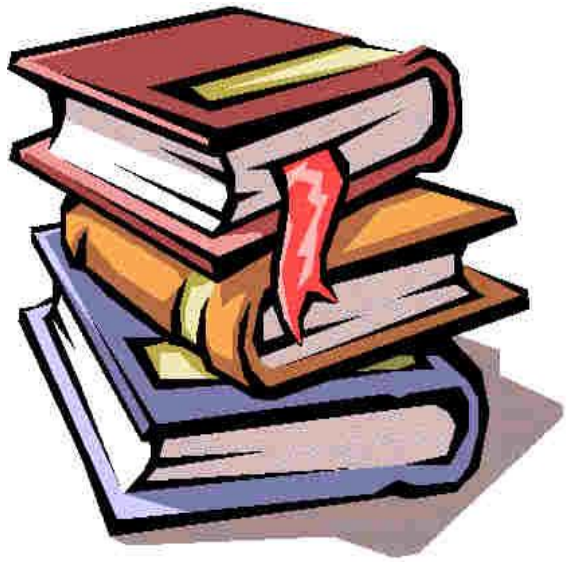


Thank You!