# Module-3 : Enzyme Structure, Classification & mechanism of action



## # Importance

- Enzymes play an important role in Metabolism, Diagnosis and Therapeutics.
- All biochemical reactions are enzyme catalyzed in the living organism.
- Level of enzyme in blood are of diagnostic importance eg. It is a good indicator in disease such as myocardial infarction.
- Enzyme can be used therapeutically such as digestive enzymes

## # Define Enzymes
[Enzymes as Biological catalysts]

- Enzymes are proteins that increase the rate of reaction by lowering the energy of activation.
- They catalyze nearly all the chemical reactions taking place in the cells of the body.
- Not altered or consumed during reaction.
- Reusable.

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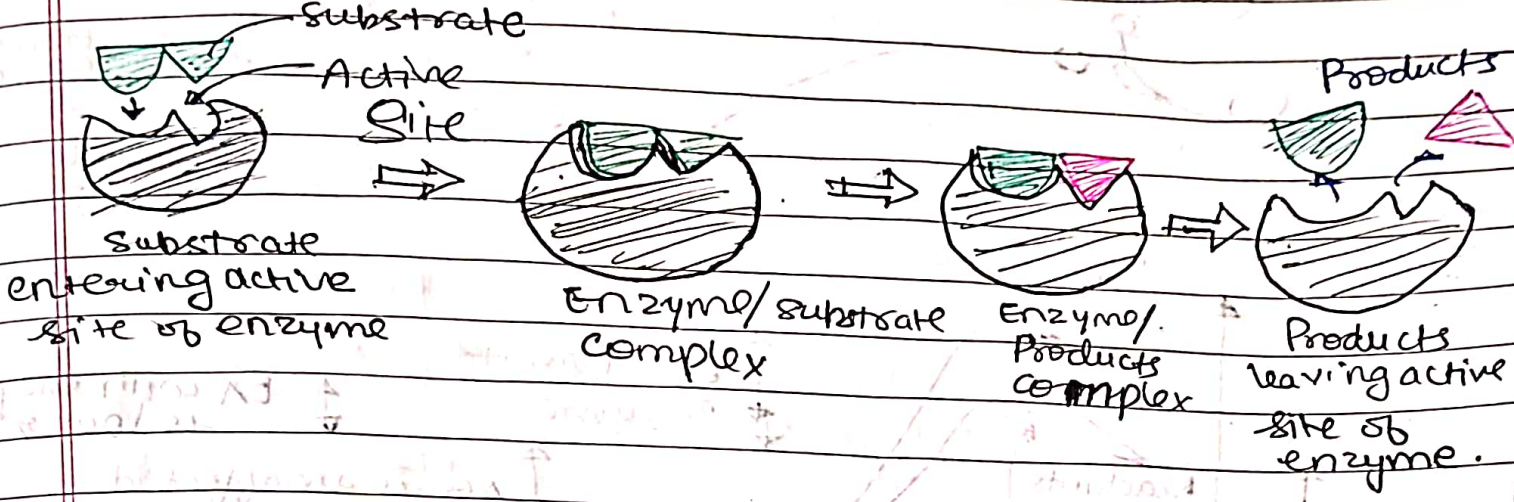
- Enzyme molecules contain a special packet or deft called the active sites.




# Activation energy :—

- All chemical reactions require some amount of energy to get them started.

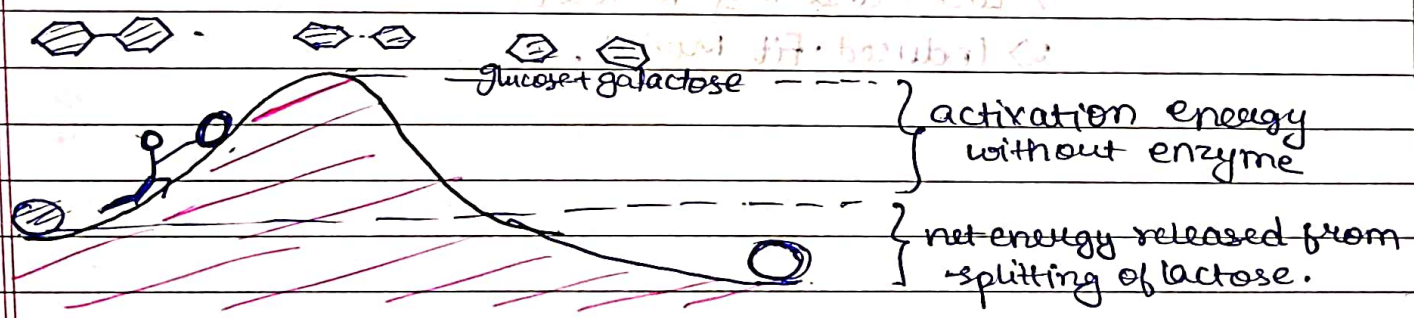
or

- It is first Push to start reaction.

This energy is called activation energy.

- Enzymes lower a reaction's activation energy.

(a) Without Enzyme

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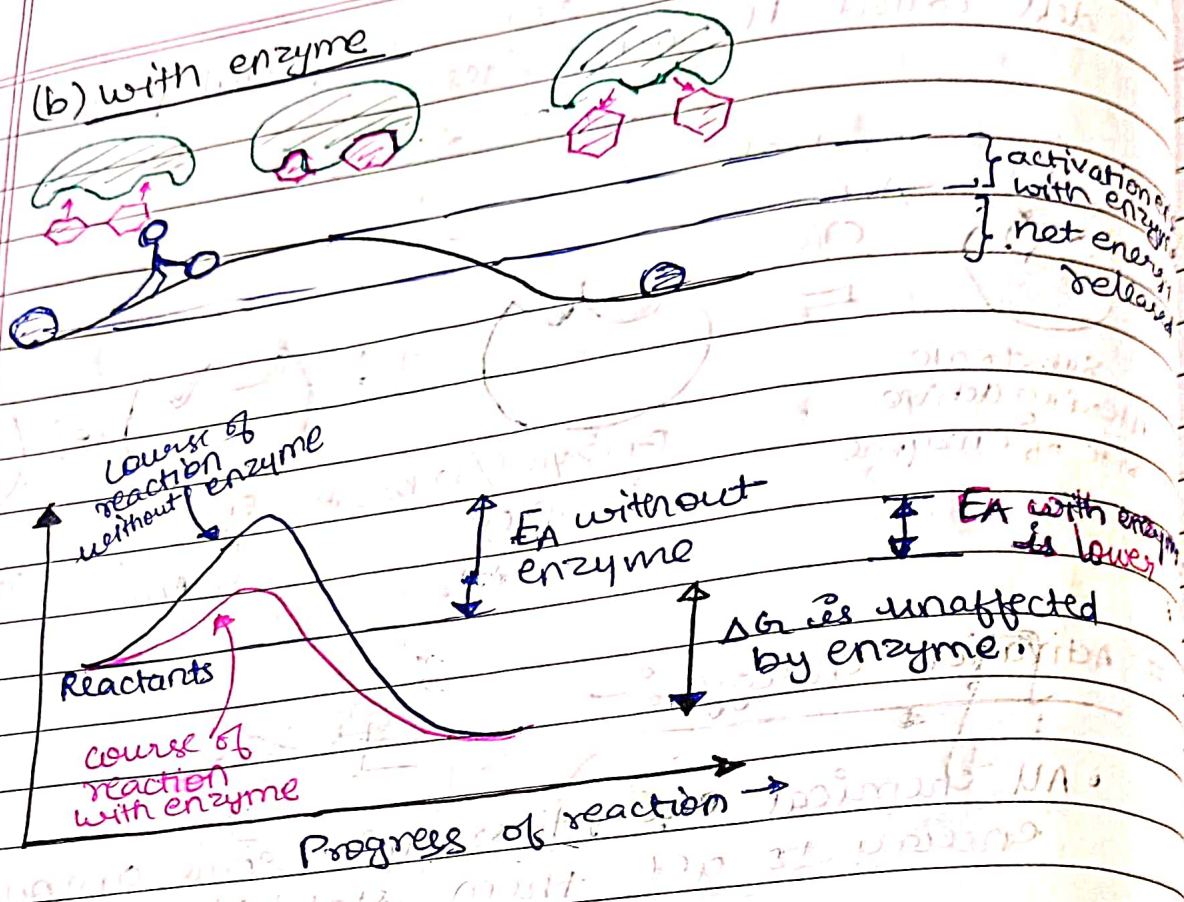
(b) with enzyme




# Mechanism of Action of Enzymes :-

- Enzymes increase reaction rates by decreasing the activation energy.
- Enzyme-substrate Interactions :
  - Formation of Enzyme substrate complex by :
    1) Lock-and-key model
    2) Induced-Fit Model.

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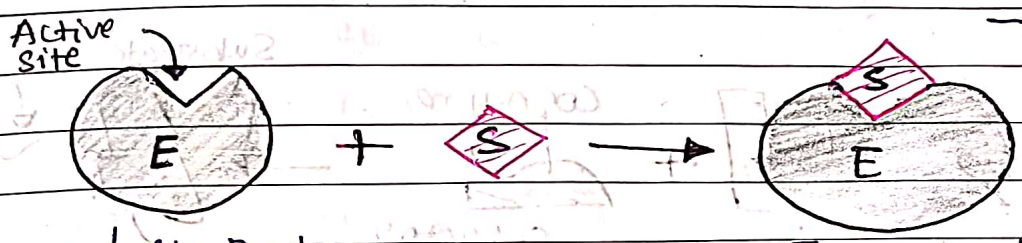
## 1) Lock-And-key Model :-

- 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.

- This is an older model, however and does not work for all enzymes.

→ This explains enzyme specificity.

→ This explains the loss of activity when enzymes denature



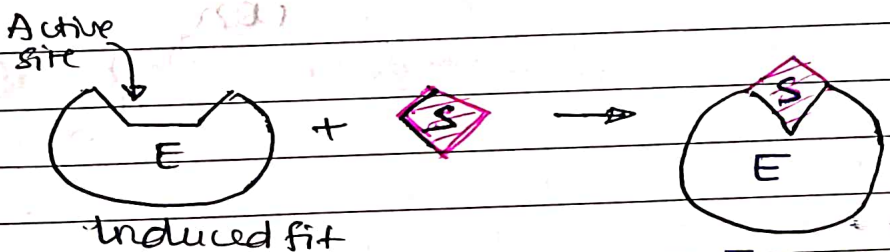

Lock-And-key Model

Enzyme-substrate Complex

## 2) Induced fit Model :-

- In the Induced-fit model of enzyme action.
  - the active site is flexible; not rigid.
  - the shape of the enzyme, active site and substrate adjust to maximum the fit, which improves catalysis
  - There is a greater range of substrate specificity.

- This model is more consistent with a wider range of enzym

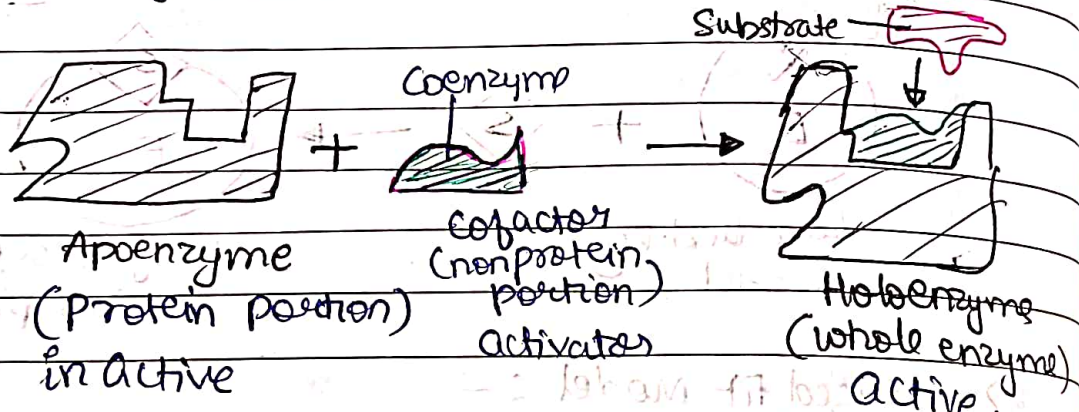


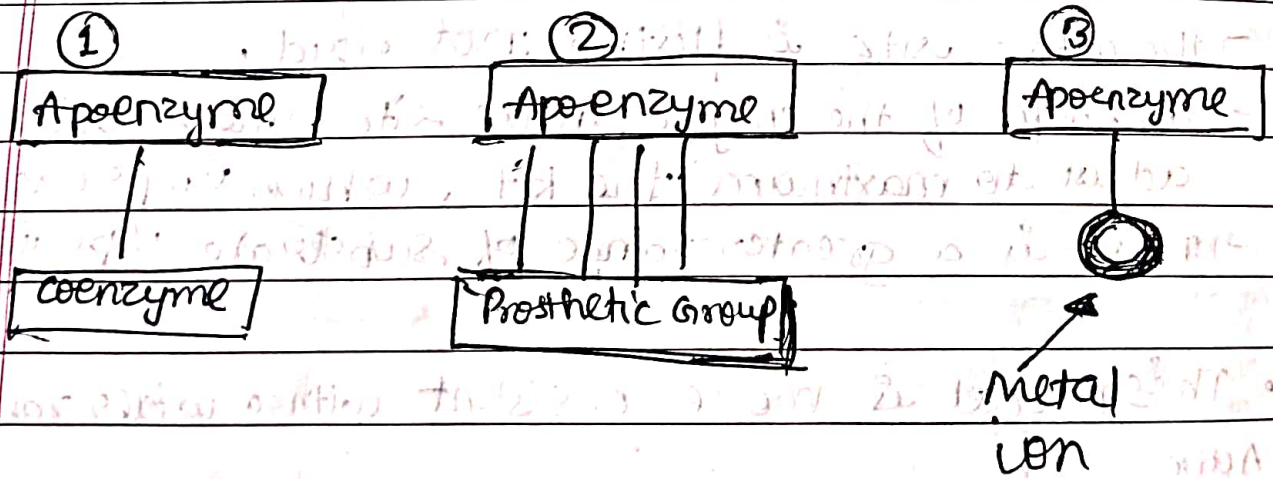

Induced fit

Enzyme-substrate Complex

# Apoenzyme and Holoenzyme :-

- The enzyme without its non-protein moiety is termed as **apoenzyme** & it is inactive.
- **Holoenzyme** is an active enzyme with its non-protein component.
- A **cofactor** is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.

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According to Holum, the cofactor may be:

1. A coenzyme: a non-protein organic substance which is dialyzable, thermostable, and loosely attached to the protein part.

2. A Prosthetic group: an organic substance which is dialyzable & thermostable which is firmly attached to the protein or apoenzyme portion.

3. A metal-ion activator: these include $K^+$, $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Mn^{2+}$, $Mg^{2+}$, $Ca^{2+}$ & $Mo^{3+}$.

# Enzyme Specificity :-

- Enzymes have varying degrees of specificity for substrates.
- Enzymes may recognize & Catalyze:
  - a single substrate
  - a group of similar substrates
  - a particular type of bond.

# Type of Enzyme Specificity :-

| Type | Reaction Type | Example |
|---|---|---|
| Absolute | Catalyze one type of reaction for a single substrate. | urease catalyzes only the hydrolysis of urea. |
| Group | Catalyze one type of reaction for similar substrates | Hexokinase adds a phosphate group to hexoses |
| Linkage | Catalyze one type of reaction for a specific type of bond | chymotrypsin catalyzes the hydrolysis of peptide bonds |

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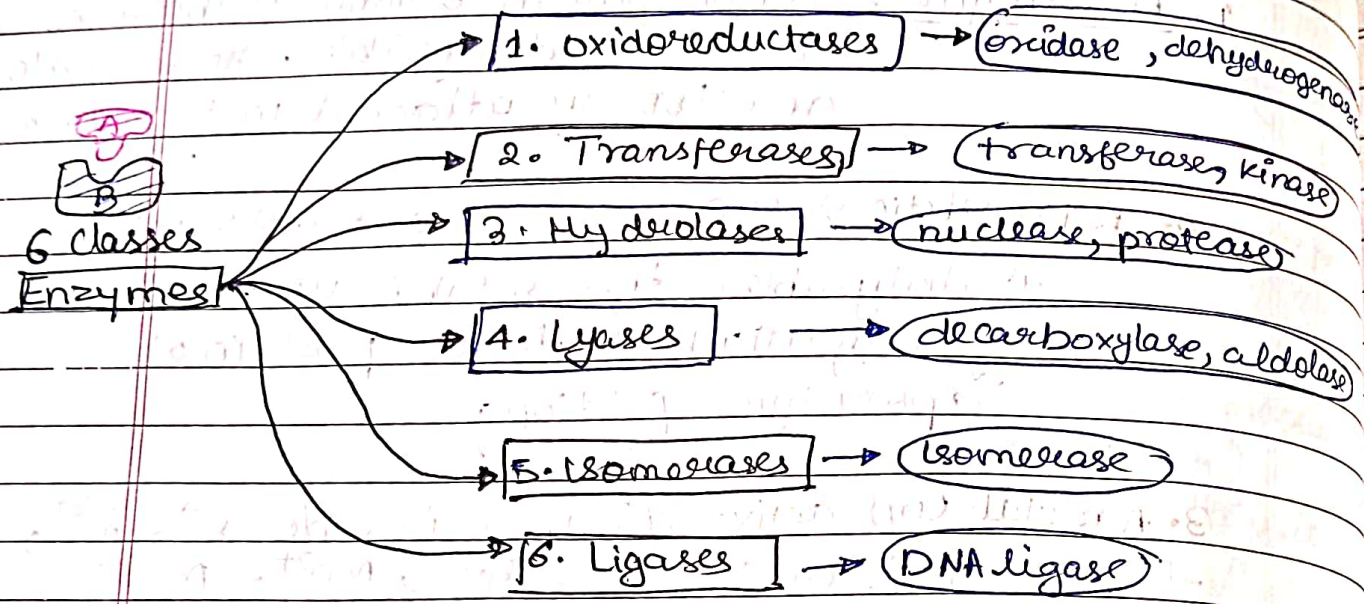
# Classification of Enzymes :-




| | Enzyme Class | Reaction Catalyzed | Example |
|---|---|---|---|
| 1. | Hydrolase | Hydrolysis (Catabolic) | Lipase, protease |
| 2. | Isomerase | Rearrangement of atoms with a molecule | Phosphohexoisomerase |
| 3. | Lyase | Splitting chemicals into smaller parts without using water (Catabolic | Decarboxylases, aldolases. |
| 4. | Synthetases | Joining of two molecules by the formations of new bonds (anabolic) | DNA ligase, DNA polymerase |
| 5. | Oxidoreductase | Transfers electrons or hydrogen atoms from one molecule to another | Dehydrogenases oxidases |
| 6. | Transferase | Moving a functional group from one molecule to another | Kinases, transaminase. |

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~~Enzyme catalyzed Rxn~~

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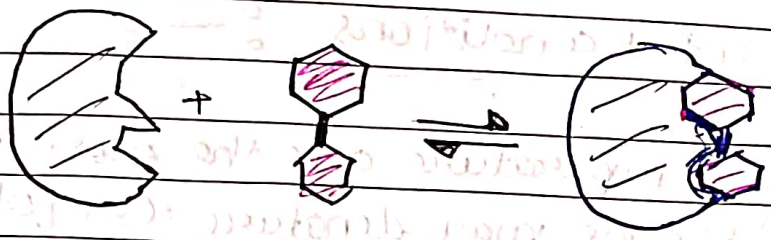
# Enzyme - substrate complex :-

~~when a substrate~~

- Step 1 : Enzyme and substrate combine to form complex

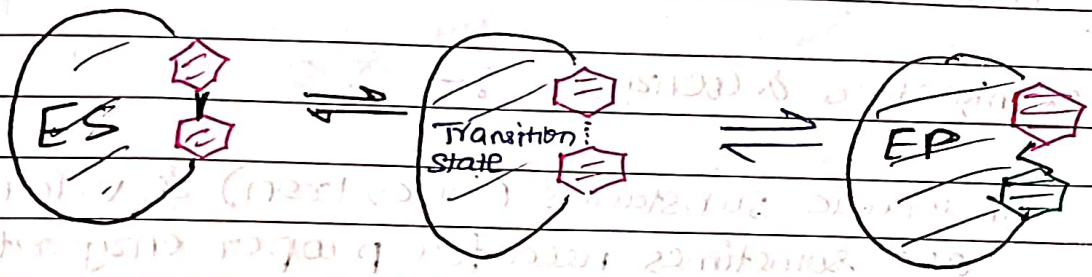
$$E + S \rightleftharpoons ES$$

Enzyme



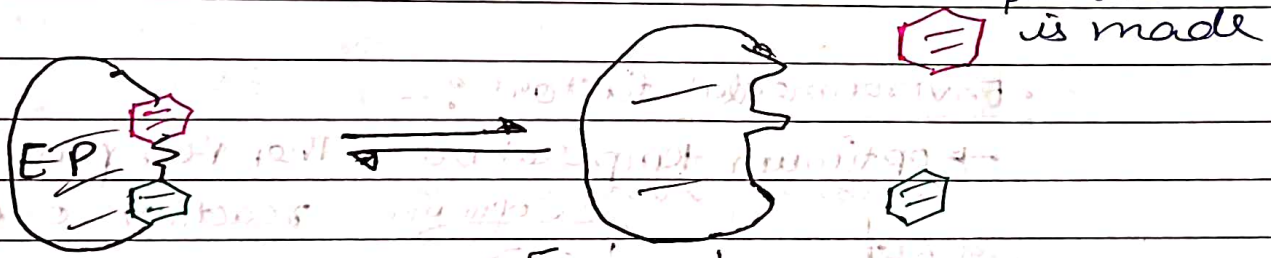
- Step-2 : An enzyme-product complex is formed.

- $ES \rightleftharpoons EP$




- Step-3 : The enzyme & product separate.

- $EP \rightleftharpoons E + P$.

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# What affects Enzyme Activity?

- Three factors:
  1. Environmental conditions
  2. cofactors & coenzymes
  3. Enzyme Inhibitors.

## 1. Environmental conditions :—

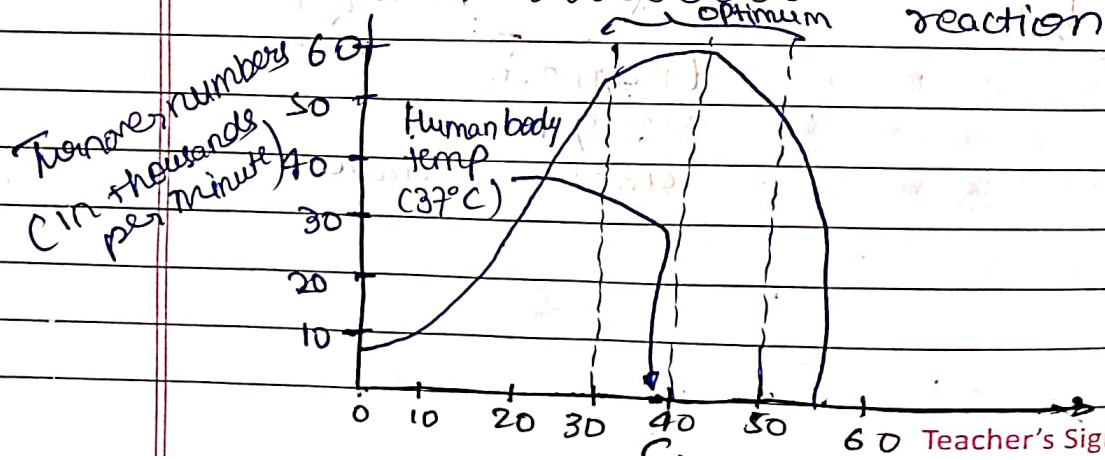
i) Extreme Temperature are the most dangerous -high temps may denature (unfold) the enzyme
ii) pH (most like 6-8 pH near neutral.
iii) Substrate concentration.

## 2. cofactors & coenzymes :—

- Inorganic substances (zinc, Iron) & vitamins (respectively) are sometimes need for proper enzymatic activity.
- example: Iron must be present in the quaternary structure of hemoglobin in order for it to pickup oxygen.

## • Environmental factors :—

→ optimum temperature : The temp at which enzymatic reaction occur fastest.

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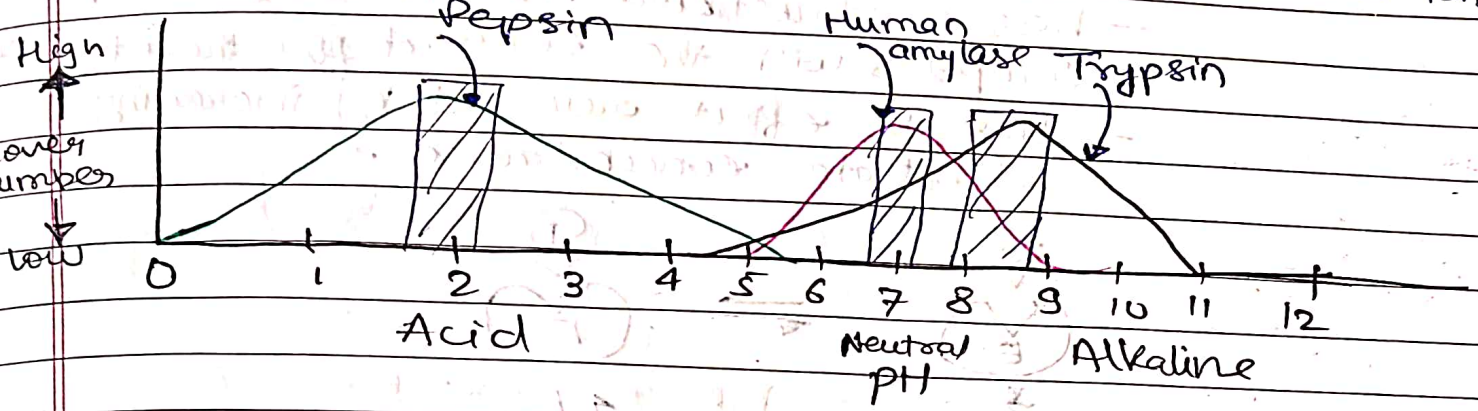
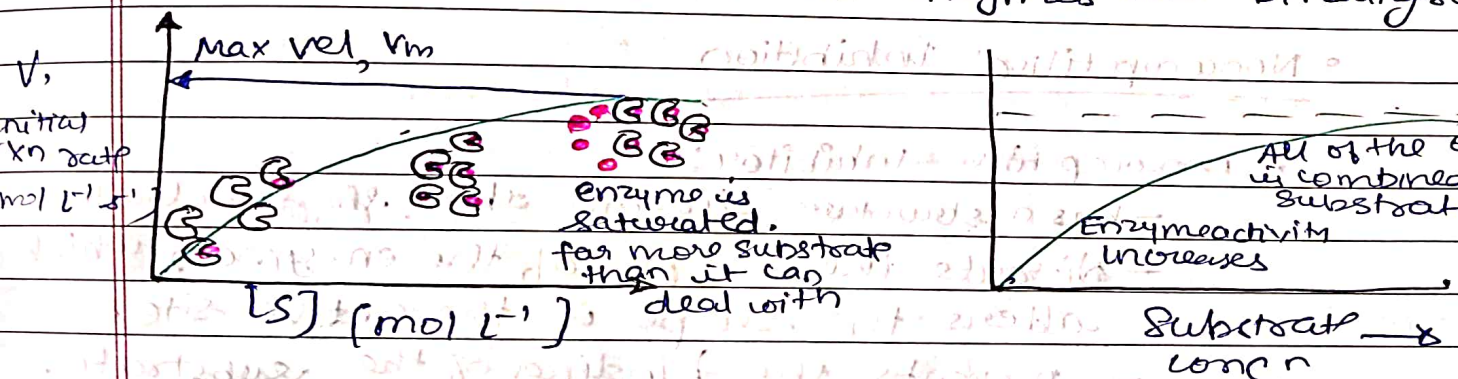
- pH also affects the rate of enzyme – substrate complexes.
  - Most enzymes have an optimum pH of around 7 (neutral).
  - However, some prefer acidic or basic conditions




iii) Substrate concentration & Reaction Rate

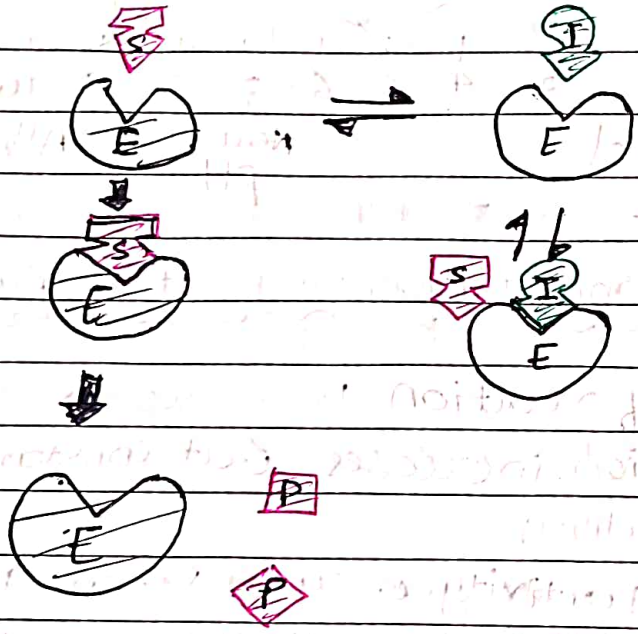
- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)
- Maximum activity occurs when the enzyme is saturated (when all enzymes are binding substrate).



3. Enzyme Inhibitors

- Reversible competitive inhibition

A competitive inhibitor:
- has a structure like the substrate.
- competes with the substrate for the active site.
- Has its effect reversed by increasing substrate concentration.




- Noncompetitive inhibition:

A noncompetitive inhibitor:
- has a structure different than the substrate
- Distorts the shape of the enzyme, which alters the shape of the active site.
- prevents the binding of the substrate.
- cannot have its effect reversed by adding more substrate.

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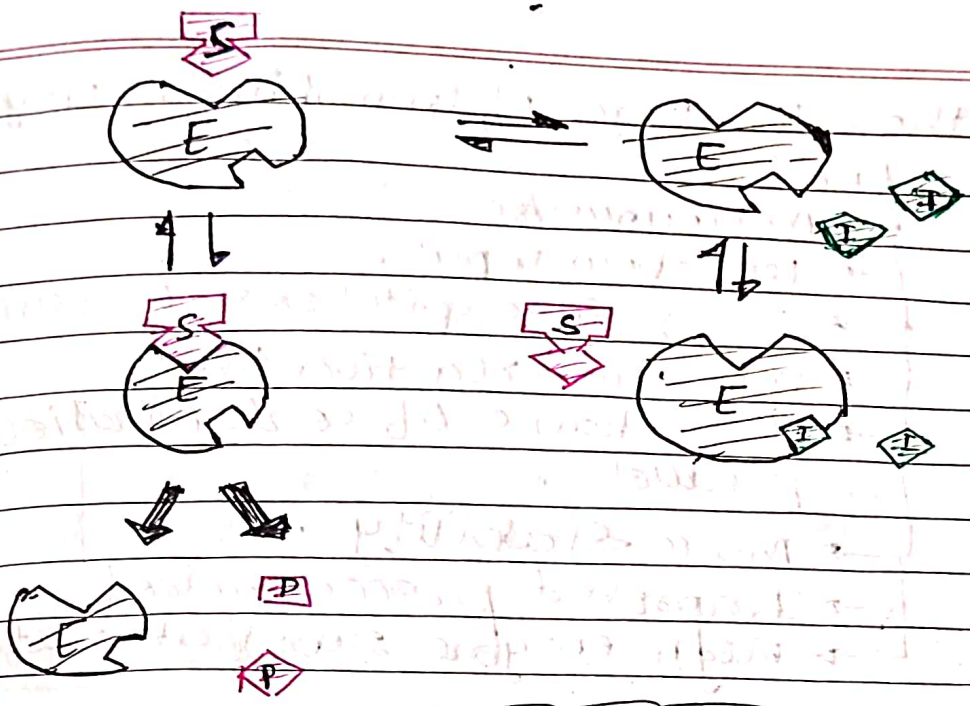



# Immobilization of Enzymes

## Immobilization Techniques

### What is Immobilization?

- **Definition:** Imprisonment of cell or enzyme in a distinct support/matrix.
- the support/matrix allows exchange of medium.
- The medium contains substrate or effecter or inhibitor molecules.

- First Immobilization technology: amino acylases by Aspergillus Oryzae for the production of L-amino acids in Japan.

- Two main advantages of enzyme immobilization
  - 1. Increased functional efficiency.
  - 2. Enhanced reproducibility.

Q. What are the advantages of immobilized enzymes?

- Reuse
- Continuous use
- Less labour input
- Saving in capital cost/investment
- Minimum reaction time
- Less chance of contamination in products.
- More stability
- Improved process control
- High enzyme substrate ratio.

Q. What are the disadvantages of enzyme immobilization?

- Uses in industrial applications are limited
- Loss of catalytic properties in some enzymes
- Some enzymes become unstable.
- Enzymes are inactivated by heat generated in the system
- High cost for isolation, purification and recovery of active enzyme.

Q. What are the applications of enzyme immobilization?

Ans ▶
- Industrial Production eg. Antibiotics, beverages, amino acids etc
- Biomedical applications: treatment, diagnoses & drug delivery
- Food Industry: production of Jams, Jellies & syrups
- Research: HRP in blotting experiments, proteases for cell lysis
- Production of biodiesel: from vegetable oils.
- Waste water management: treatment of sewage & industrial effluents.
- Textile Industry: Scouring, bio-polishing & desizing of fabrics
- Detergent Industry: Immobilization of lipase for effective dirt removal.

## Support/Matrix used in Immobilization technology :-

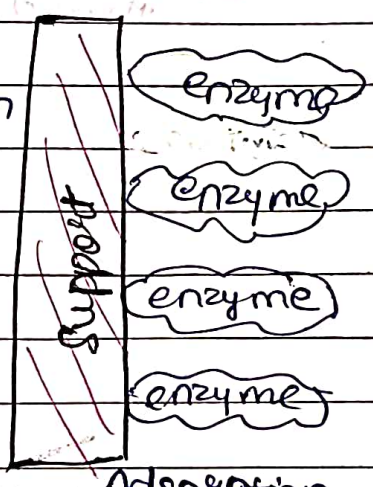
- The matrix/supports hold the enzyme.
- The matrix used should be cheap and easily available.
- The matrix/supports are grouped into three major categories :-
  1. Natural Polymers (Chitosan, Collagen, Alginate)
  2. Synthetic Polymers (PVC, PEG)
  3. Inorganic Material (Ceramics, Glass, Silica)

## # Types/Methods of Immobilization :-

5 different methods of Immobilization of enzyme/cells
1. Adsorption
2. covalent bonding
3. Entrapment
4. Copolymerization (Cross-linking)
5. Encapsulation.

### 1. Adsorption :-

- oldest method of enzyme immobilization
- simplest method of enzyme immobilization
- Nelson & Griffin used charcoal to adsorb Invertase
- Enzymes are adsorbed to external surface of support.
- Support/carriers may be
  1. Mineral support (aluminium oxide, clay)
  2. organic support (Starch)
  3. Modified Sepharose & Ion exchange resins.



Adsorption

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- Weak bonds stabilize enzymes to the support/ carrier.
- Bonds involved are low energy bonds such as
  - Ionic interaction
  - Hydrogen bonds
  - Van der Waal forces

- Carrier particles size must be small (for appreciable surface bonding).
- Particle size used : 500 Å to 1mm diameter.

- No pore diffusion limitations (since enzyme are immobilized externally)

**Advantages of adsorption method**

- Easy to carryout
- No reagents are required.
- Minimum activation steps involved.
- Comparatively cheap method.
- less disruptive to protein than chemical methods.

**Disadvantages of adsorption method**

- Desorption of enzymes from the carrier
- Efficiency is less.

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## (2) Covalent bonding :-

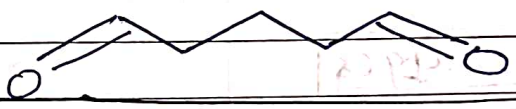
- Involves the formation of covalent bonds between enzyme and support
- widely used method of enzyme immobilization.
- chemical groups in enzymes that forms covalent bonds with support are:
  - → Amino groups, imino groups
  - → Hydroxyl groups
  - → Carboxyl groups
  - → Thiol groups & methyl thiol groups
  - → Guanidyl groups & Imidazole groups
  - → Phenol rings.


### Methods of covalent bonding

→ 1. Diazotization : Bonding b/w amino group of support and thyrosil or histidyl group of enzyme.

→ Peptide bond : b/w amino/carboxyl groups of support and enzyme.

→ Poly functional reagents : use of a bi-functional or multifunctional reagent (glutaraldehyde) which forms bonding between the amino group of the support & amino group of the enzym



**Advantages**

- Strong linkage of enzyme to the support
- No leakage or desorption problem
- Comparatively simple method.

**Disadvantages**

- Chemical modification of enzyme leading to functional conformation loss.
- Enzyme inactivation by changes in the conformation when undergoes reactions at active sites.

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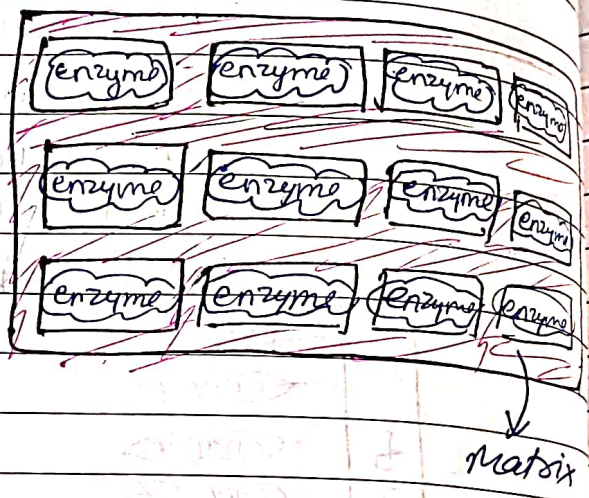
## (3) Entrapment

- Enzymes are physically entrapped inside a matrix.
- Bonds involved may be covalent or non-covalent.
- Matrix used will be water soluble polymer.
  - eg. of matrix:
    * Polyacrylamide gels
    * Cellulose triacetate
    * Agar
    * Gelatin
    * Carrageenan
    * Alginate.




### Methods of entrapment

1. **Inclusion in the gels:**
   enzymes trapped in gels

2. **Inclusion in fibers:**
   enzymes supported on the fiber format

3. **Inclusion in microcapsules:**
   enzymes Entrapped in microcapsules formed by monomer mixtures such as polyamine, calcium alginate.

**Advantages**

- Fast
- Cheap (low cost matrix available)
- Mild conditions are required.
- less chance of conformational changes in enzyme.

**Disadvantages**

- Leakage of enzyme
- Pore diffusion limitation
- Chance of microbial contamination

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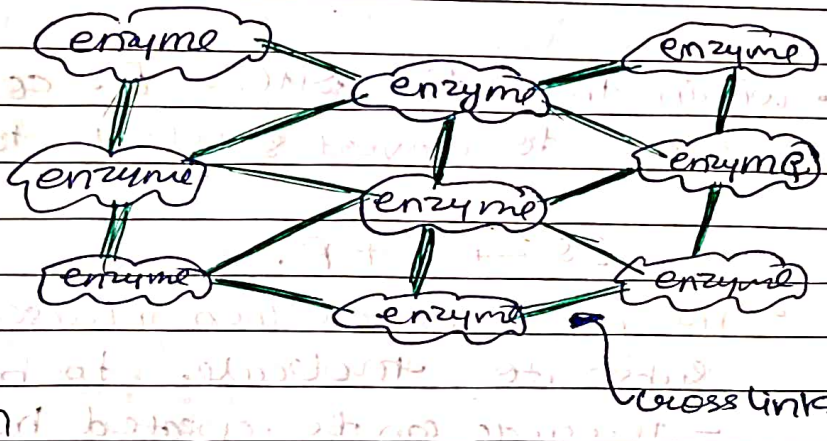
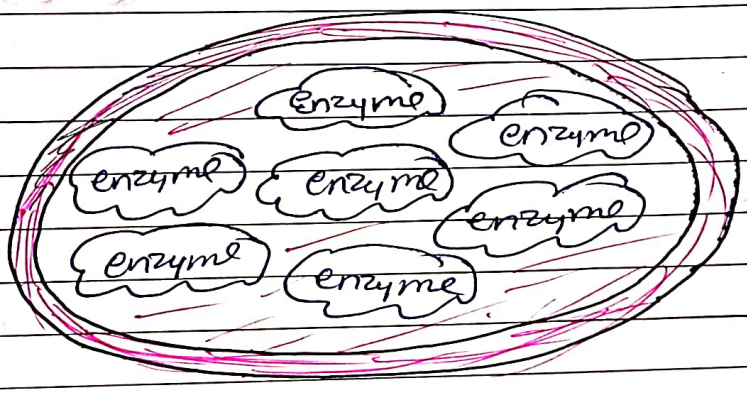
## (4) Cross Linking (copolymerization)

- Cross linking : covalent bonding b/w various groups of enzymes via polyfunctional reagents.
- NO matrix or support are involved.




## (5) Encapsulation

- Enclosing enzymes in a semi-permeable membrane capsule.
- Capsule is made up of nitro cellulose or nylon
- Effectiveness depends upon the stability of enzymes.




**Advantages**
- cheap & simple method
- large quantity of enzymes can be immobilized by encapsulation

**Disadvantages**
- Pore size limitation
- only small substrate molecule is able to cross the membrane.

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# Enzyme Catalyzed Reactions:

- when a substrate(S) fits properly in an active site, an enzyme-substrate (ES) complex is formed.

$$E + S \rightleftharpoons ES$$

- within the active site of ES complex the reaction occurs to convert substrate to product (P):

$$ES \rightarrow E + P.$$

- The products are then released allowing another substrate molecule to bind the enzyme.
  - This cycle can be repeated millions (or even more) times per minute.

- The overall reaction for the conversion of substrate to product can be written as follows

$$E + S \rightleftharpoons ES \rightarrow E + P$$

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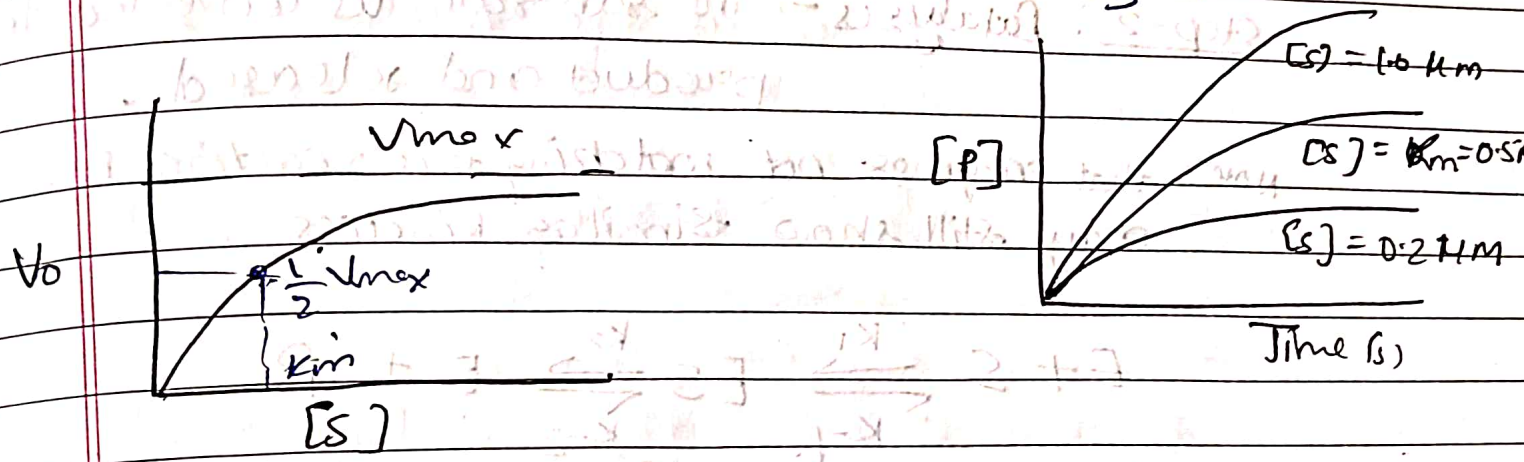
# Enzyme kinetics

The oldest approach to understand the enzyme mechanism & the one that remain the most important is to determine the rate of rxn & how it changes in responses to changes in experimental parameters. This is the discipline known as enzyme kinetics.




$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$K_m$: Michaelis const.

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## Michaelis Menten · (Steady-State) Kinetics

The M-M model for enzyme kinetics presumes a simple 2-step reaction.

Step-1: Binding - The substrate binds to the enzyme.

Step-2: Catalysis - The substrate is converted to product and released.

Note: that enzymes not matching this reaction scheme may still show similar kinetics.

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \underset{K_{-2}}{\overset{K_2}{\rightleftharpoons}} E + P$$

Binding Catalysis

M-M eqn shows how initial rate of this reaction $V_0$ depends on substrate conc$^n$ [S]

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Several simplifying assumptions allow for derivation of M-M eqn.

(1) The binding step ($E + S \rightleftharpoons ES$) is fast allowing the reaction to quickly reach equilibrium ratios of [E], [S] & [ES].

The catalytic step ($ES \rightleftharpoons E + P$) is slower & thus rate limiting.

(2) At early time points where initial velocity is measured → [P] ≈ 0

(3) ES immediately comes to steady state so [ES] is constant (Through the measured portion of the reaction)

(4) [S] >> [$E_T$] so fraction of S that binds to E (to form ES) is negligible & [S] is constant at early time points.

(5) The enzyme exists in only two forms free (E) & substrate-bound (ES).
Thus, total enzyme conc$^n$ ($E_T$) is sum of free & substrate bound conc$^n$.

$$[E_T] = [E] + [ES]$$

A derivation of M-M eq$^n$ shows how to use the above assumptions to describe the rate of the enzyme catalyzed reaction in terms of measurable quantities.

From (1) we know the overall rate of r x n is determined by rate of catalytic step.

$$V_o = k_2[ES] - k_{-2}[E][P]$$

$$[P] = 0$$

$$V_o = k_2[ES]$$

Rate of formation of ES = Rate of breakdown of ES

$$k_1[E][S] + k_{-2}[E][P] = k_{-1}[ES] + k_2[ES]$$

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

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

$$[E][S] = [ES]\left[\frac{k_{-1} + k_2}{k_1}\right]$$

The ratio of rate constants is defined as the Michaelis constant ($K_m$)

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

$$[E][S] = [ES] K_m$$

$$\{[E_T] - [ES]\}[S] = [ES] K_m$$

$$[E_T][S] - [ES][S] = [ES] K_m$$

$$[E_T][S] = [ES] K_m + [ES][S]$$

$$[E_T][S] = [ES](K_m + [S])$$

$$[ES] = \frac{[E_T][S]}{K_m + [S]}$$

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

$$\left(V_{max} = \frac{k_2 [E_T][S]}{[S]} = k_2 [E_T]\right)$$

$$V_0 = k_2 [ES] = k_2 \frac{[E_T][S]}{K_m + [S]}$$

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

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