SEM II MICROBIOLOGY CORE

Paper- MBIOCC203
Biochemistry

Topic- Enzymes

By-
Dr. Niti Yashvardhini
Dept. of Microbiology
Patna Women's College

Introduction to Enzymes

The living cell is the site of tremendous biochemical activity called metabolism.

"Enzymes can be defined as biological polymers that catalyze biochemical reactions."

Eduard Buchner submitted his first paper on the study of yeast extracts in 1897.

An overview of Enzymes

- Enzymes are **biological catalysts**
- Enzymes are specialized proteins (Except – Ribozymes)
- Chemical reactions in the cell are catalyzed by the enzymes
- Enzymes have extraordinary catalytic power
- Enzymes accelerate reactions up to **1014 to 1020 times**
- Enzyme **reduce the activation energy** of reaction
- High degree of **specificity** for **substrates** and **reactants**
- Function in aqueous solution
- Work under mild condition of **temperature** and **pH**

Properties of Enzymes

1. Catalytic property
2. Specificity
3. Reversibility
4. Sensitiveness to heat and temperature
5. Specific to pH

Enzymes 3D structure

Enzymes are proteins and their activities depends on the 3D structure of the amino acids that compose them (note: also some RNAs have catalytic activity but they won't be covered in this course)

Primary structure

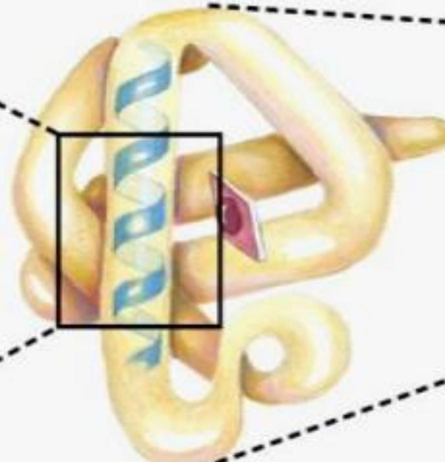
Lys
Lys
Gly
Gly
Leu
Val
Ala
His

Amino acid residues

Secondary structure

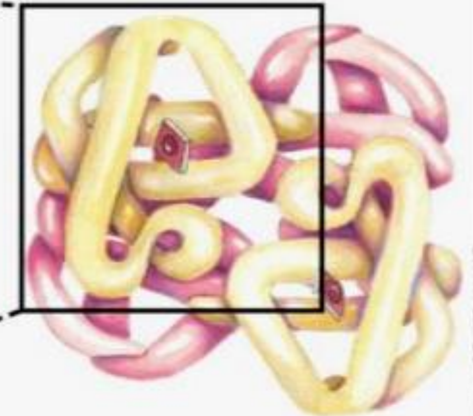
α Helix

Tertiary structure



Polypeptide chain

Quaternary structure



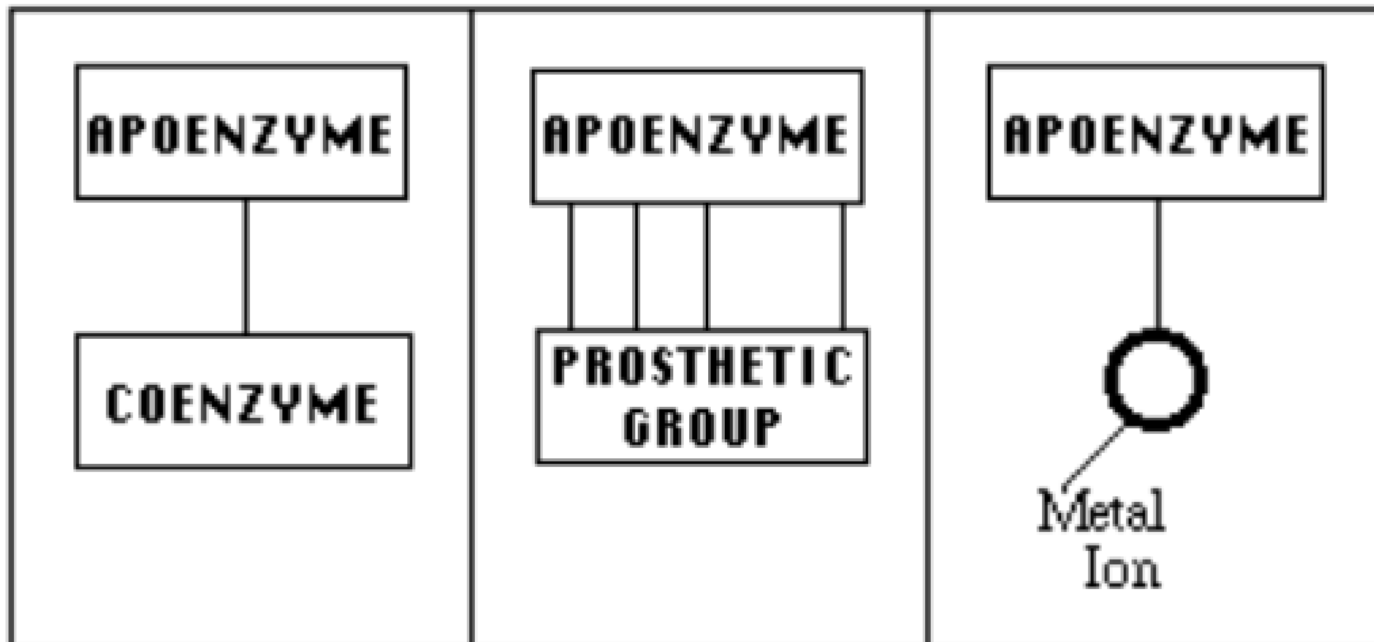
Assembled subunits

Structure of enzymes

➢ Enzymes are a linear chain of amino acids that generate the three-dimensional structure.

➢The sequence of amino acids enumerates the structure, which in turn identifies the catalytic activity of the enzyme.

➢The structure of the enzyme denatures when heated, leading to loss of enzyme activity, which is typically connected to the temperature.



Cofactors

Cofactors are non-proteinous substances that associate with enzymes. A cofactor is essential for the functioning of an enzyme.
An enzyme without a cofactor is called an apoenzyme. An apoenzyme and its cofactor together constitute the **holoenzyme**.

There are three kinds of cofactors present in enzymes:

1. **Prosthetic groups**: These are cofactors tightly bound to an enzyme at all times. A fad is a prosthetic group present in many enzymes.

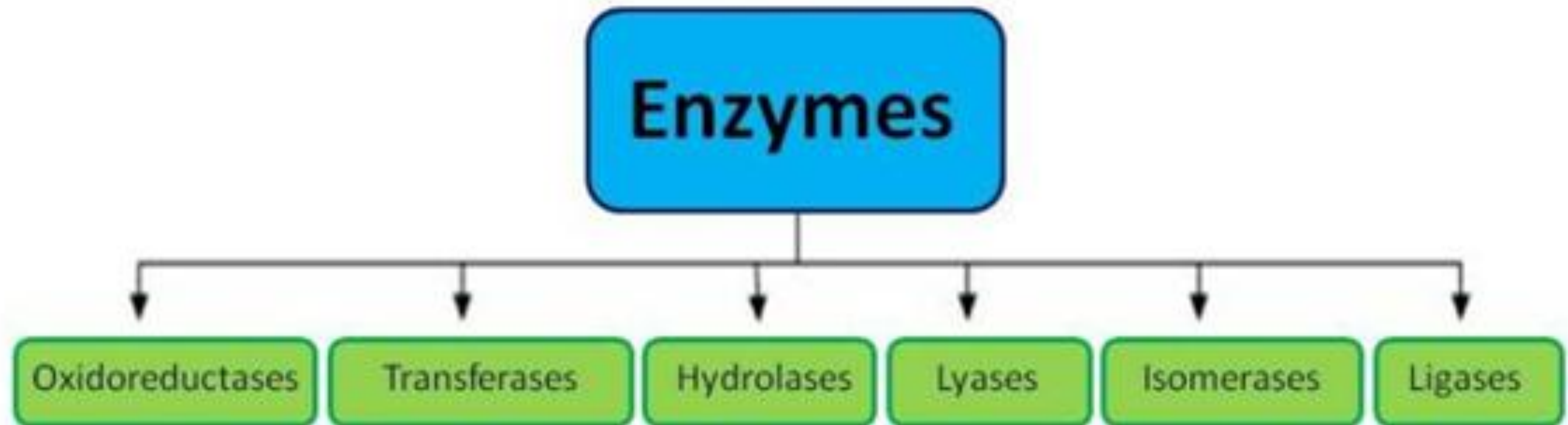
2. Coenzyme: A coenzyme is bound to an enzyme only during catalysis. At all other times, it is detached from the enzyme. NAD^+ is a common coenzyme.

3. Metal ions: For the catalysis of certain enzymes, a metal ion is required at the active site to form coordinate bonds. Zn^{2+} is a metal ion cofactor used by a number of enzymes.

Coenzymes and the reaction catalyzed

Co-enzyme	Entity transferred
Thyamin pyrophosphate	Aldehydes
Tetrahydrofolate	Other one carbon groups
Pyridoxal phosphate	Amino groups
Nicotinamide adenine dinucleotide	Hydrogen atoms (electrons)
Flavin adenine dinucleotide	Hydrogen atoms (electrons)
Co-enzyme A	Acyl groups
Biocytin	CO_2
3′-deoxyadenorylcohalamine (co-enzyme B12)	H atoms and alkyl groups

Classification of Enzymes

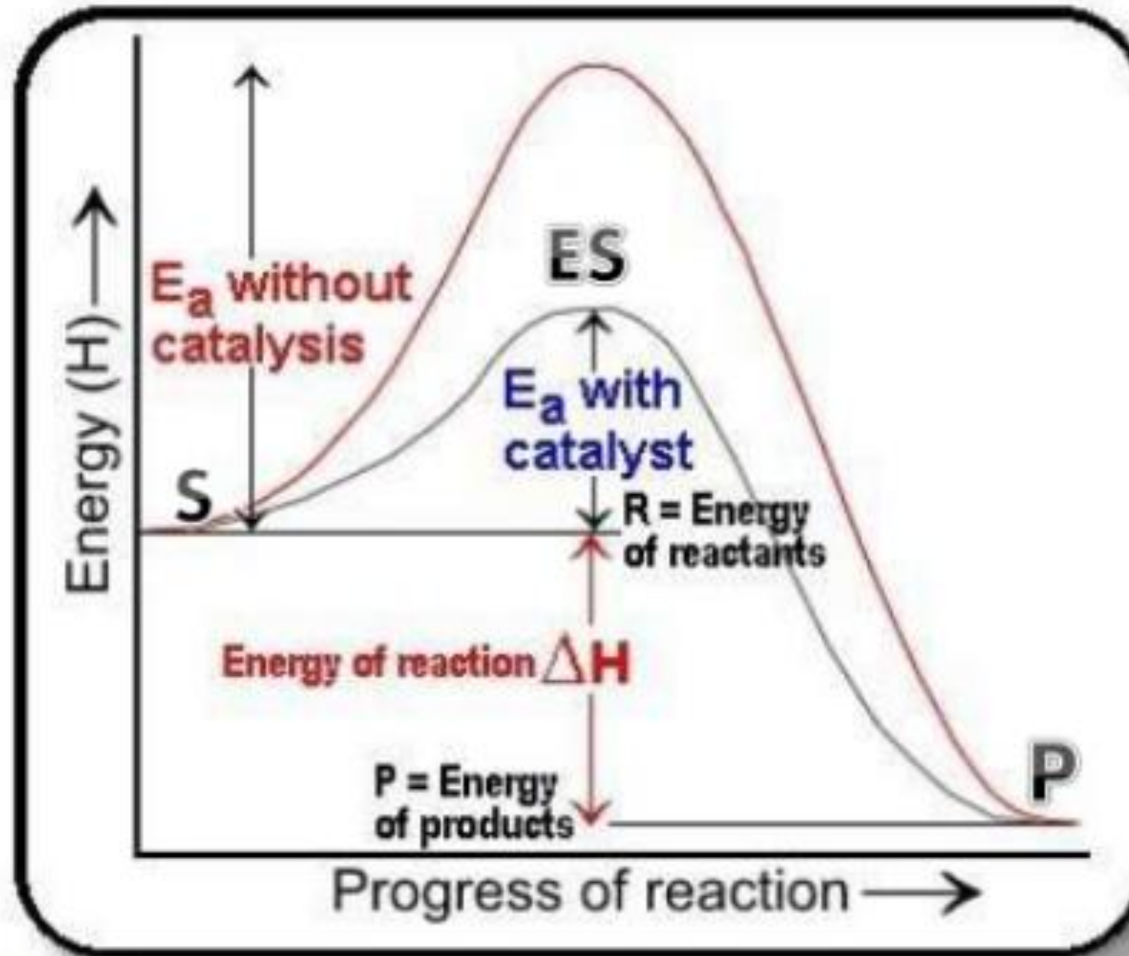


Classification of Enzymes….. Contd

Types	Biochemical Property
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electrons tend to travel from one form of a molecule to the other.
Transferases	The Transferases enzymes help in the transportation of the functional group among acceptors and donors molecules.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, thus causing the change in the shape of the molecule.
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

What Enzymes do?

Lower the activation energy of the reaction

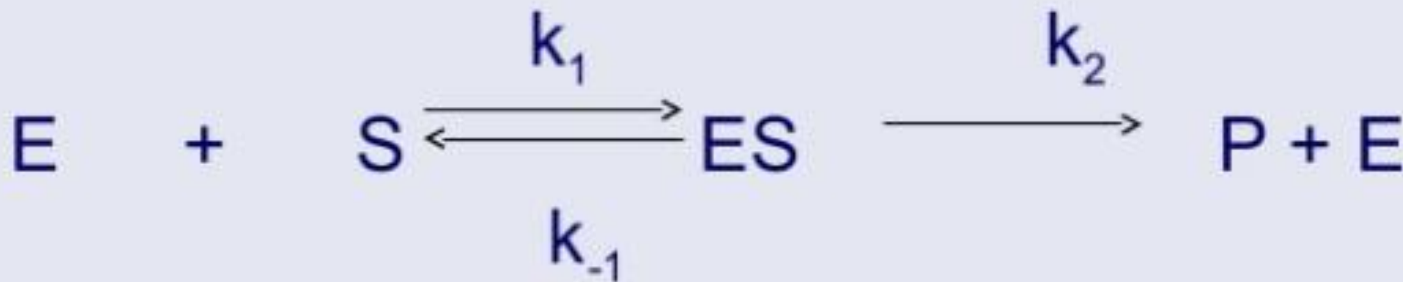


Enzyme kinetics

❖Enzyme kinetics is the quantitative measurement of the rates of enzyme catalyzed reactions.

❖Started with Brown's proposal- overall reaction is composed of two elementary reactions in which substrate forms a complex with the enzyme which is decomposed to products and enzymes.

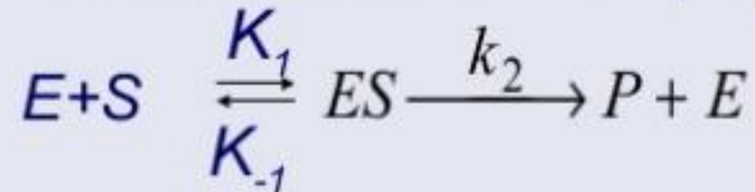
Here E,S, ES and P symbolizes the enzyme, substrate, enzyme- substrate complex and products.



Michaelis- Menten Kinetics

The rapid equilibrium assumption:

- Assumes a rapid equilibrium between the enzyme and substrate to form an [ES] complex.



$$E+S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P+E$$

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

- The equilibrium constant K_m can be expressed by the following equation in a dilute system.

$$K_m=\frac{k_{-1}}{k_1}=\frac{[E][S]}{[ES]}$$

Assumptions for Michaelis- Menten Equation

1. Relative concentration of E and S
2. Steady state assumptions
3. Initial velocity

- Since the enzyme is not consumed, the conservation equation on the enzyme yields

$$[E] = [E_0] - [ES]$$

- Then rearrange the equilibrium constant equation

$$K_m = \frac{k_{-1}}{k_1} = \frac{[E][S]}{[ES]} \qquad [ES] = \frac{[E][S]}{K_m}$$

- Substituting [E] in the above equation with enzyme mass conservation equation

$$[ES] = \frac{([E_0] - [ES])[S]}{K_m}$$

$$[ES] = \frac{([E_0] - [ES])[S]}{K_m}$$

$$[ES]K_m = [E_0][S] - [ES][S]$$

$$[ES]K_m + [ES][S] = [E_0][S]$$

$$[ES](K_m + [S]) = [E_0][S]$$

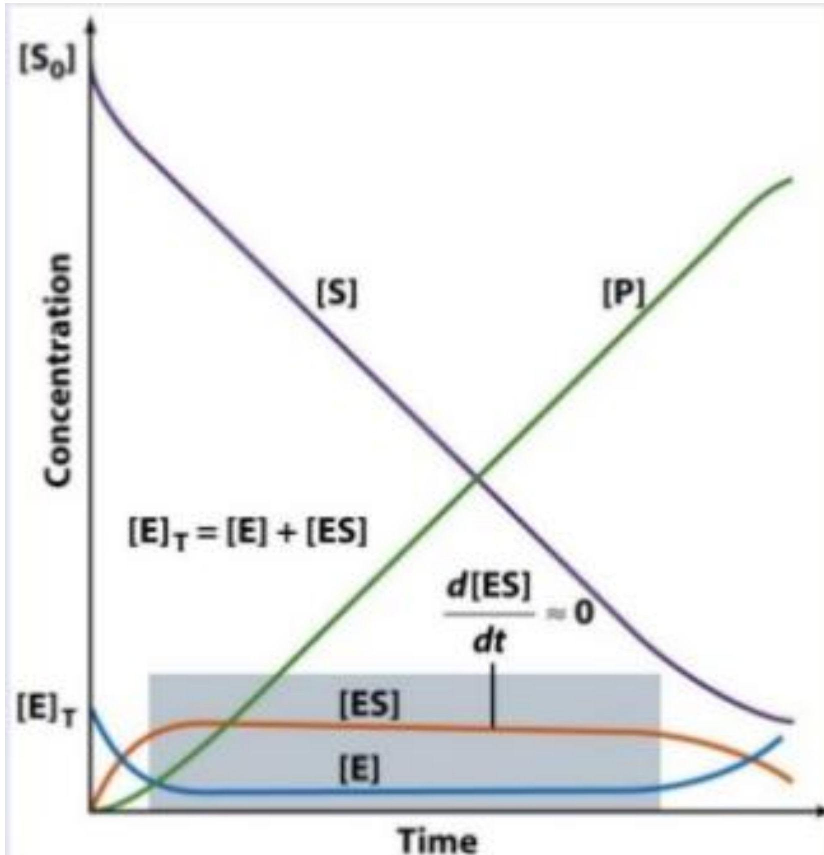
$$[ES] = \frac{[E_0][S]}{K_m + [S]}$$

- Then the rate of production formation v can be expressed in terms of [S]

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

- Where $V_{max} = k_2[E_0]$

Steady- State Assumptions



- Progress curve for the components of a simple michaelis-Menten reaction
- Except the transition phase of the reaction (before shaded block) [ES] remains constant until the substrate is nearly exhausted.
- Hence synthesis of ES must equals to its consumption over the course of reaction i.e. ES maintain **steady state**

SSA leads to the following equation of Michaelis-Menten

$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

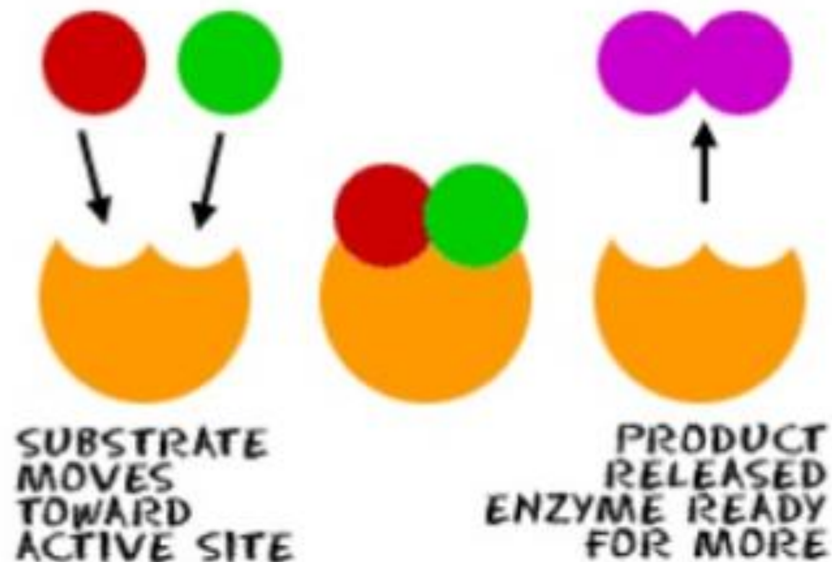
- Where $V_{max} = k_2[E_0]$

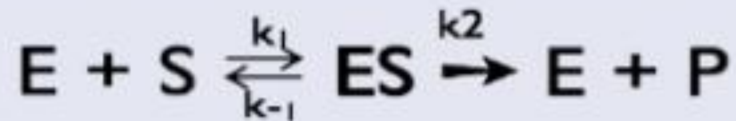
- Michaelis Menten Equation

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

Significance of Km

- K_M is the concentration of substrate at which half the active sites are filled. *It* provides a measure of the substrate concentration required for significant catalysis to occur.



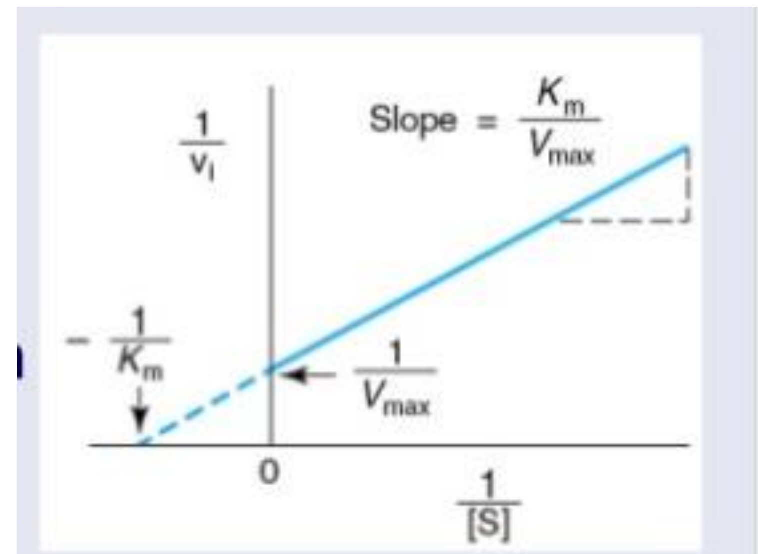


- Assumes the formation of Enzyme substrate complex
- Assumes that the ES complex is in rapid equilibrium with free enzyme
- Breakdown of ES to form products assumed to be slower than
  1. Formation of ES and
  2. Breakdown of ES to reform E and S

$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Lineweaver -Burk plot

- Starting with the MM equation
$$v_0 = \frac{V_{\max}[S]}{K_m + [S]}$$
- Reciprocal of MM equation
$$\frac{1}{v_0} = \frac{K_m}{V_{\max}[S]} + \frac{1}{V_{\max}}$$
- Lineweaver-Burk Equation
$$\frac{1}{v_0} = \left(\frac{K_m}{V_{\max}}\right)\frac{1}{[S]} + \frac{1}{V_{\max}}$$
- Equation is the equation for a straight line, *y = ax +* b, where *y = 1/*v_0 *and x = 1/[S].*



Reference books for further study:

1. Lehninger

2. Voet and Voet

3. Stryer