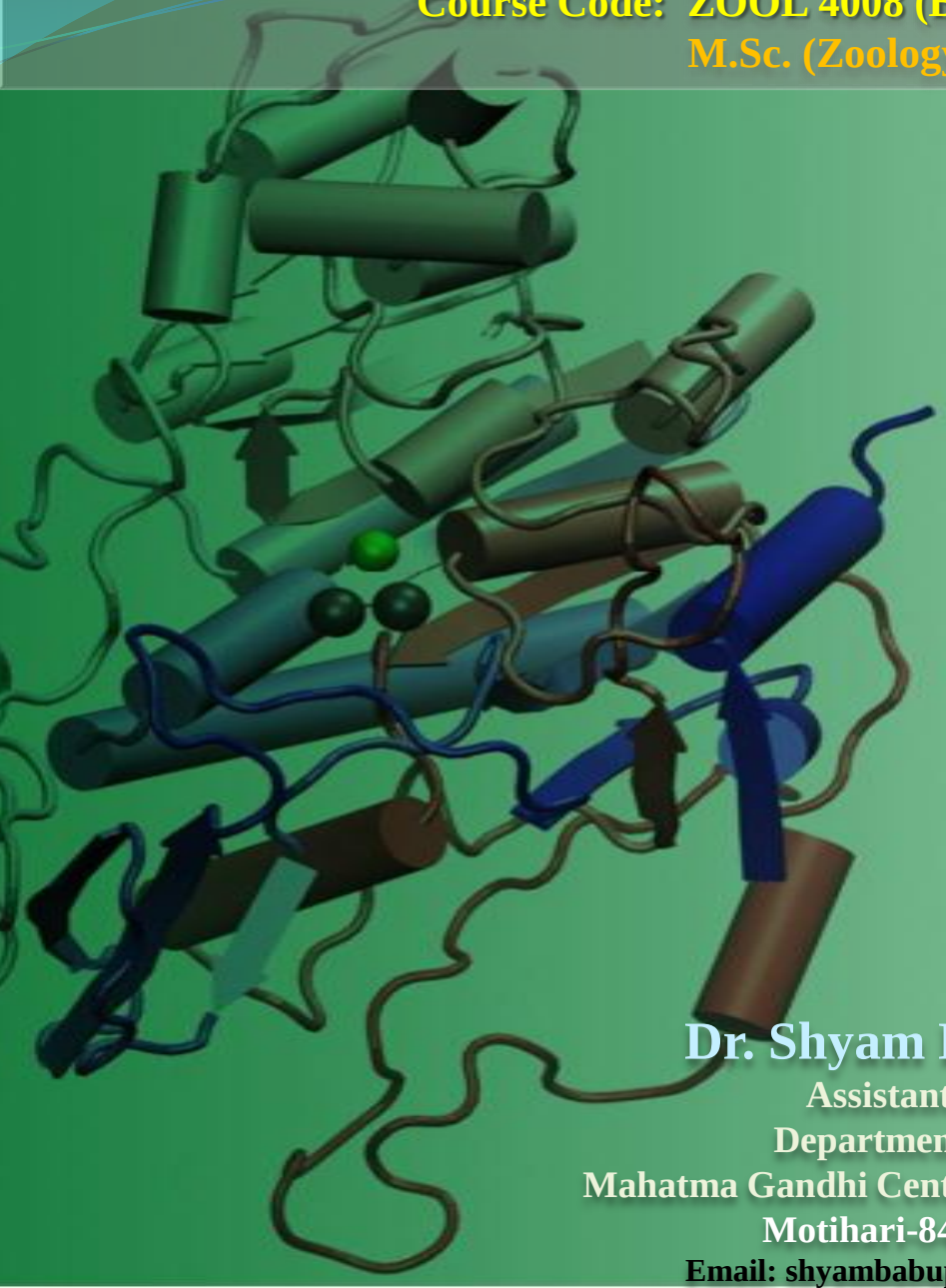
Enzyme Kinetics

Course Code: ZOOL 4008 (Biochemistry and Metabolism)

M.Sc. (Zoology), Semester –II



$$v_0 = \frac{v_{max}[S]}{K_M + [S]}$$

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Basic concepts of the Enzyme

•**Enzymes** are both **proteins** and biological **catalysts** (biocatalysts), that accelerates **specific chemical reactions** in biological system. It alters the rate of reaction in biological process.

•They are high molecular weight compounds made up mainly of chains of amino acids linked together by peptide bonds.

•Enzymes are to a large extent protein molecules, although some enzymes are made from RNA and are referred to as ribozymes.

•Many **enzymes** require the presence of other compounds - cofactors - before their catalytic activity can be exerted.

•Enzyme kinetics is concerned with the influence of enzymes on chemical reaction rates.

•**Holoenzyme**- An **apoenzyme** together with its cofactor (**apoenzyme** is an inactive enzyme activation requires cofactors).

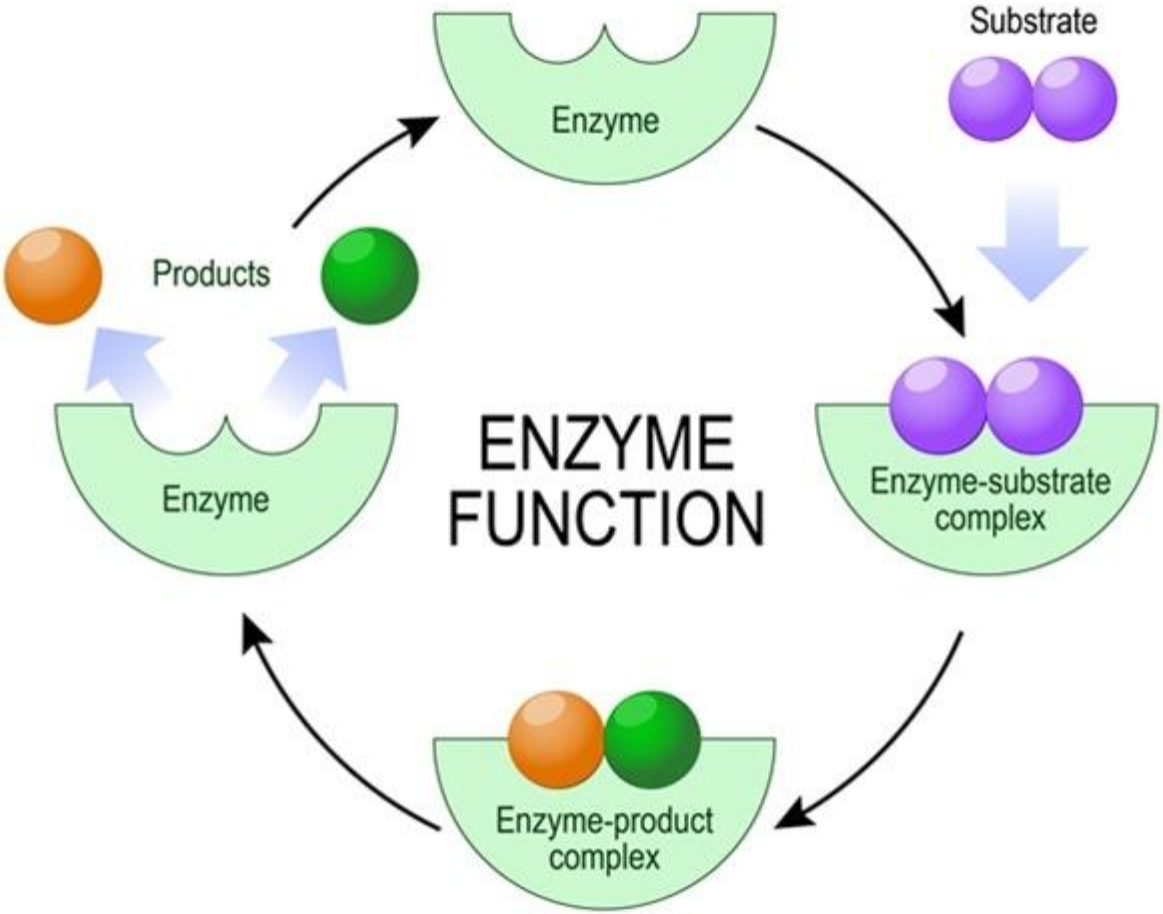
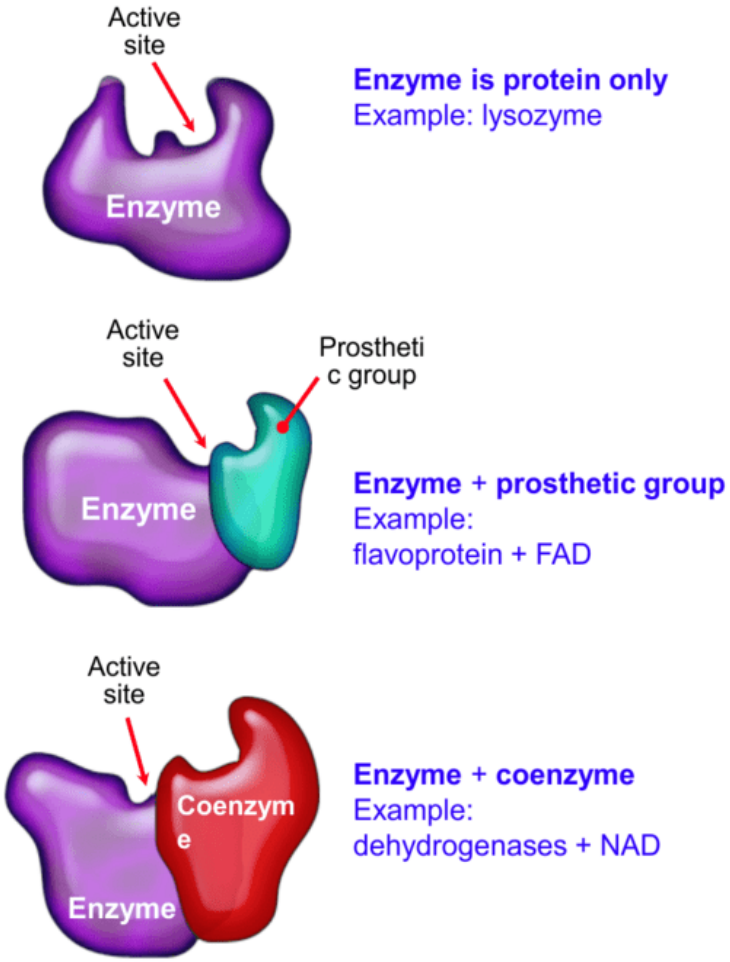


Fig. Schematic diagram of enzyme substrate reaction

Enzyme Cofactors

- Some enzyme require cofactors to be active.
- Cofactors are a non-protein components of the enzyme.
  - Organic Molecules (Coenzymes)
  - Inorganic ions e.g., Ca^{2+} , Zn^{2+} **(Prosthetic group)**
- Cofactors may be:
  - The Permanently attached cofactors, are called **Prosthetic group** (such as a vitamin, sugar, or lipid or inorganic such as a metal ion)
  - Temporarily attached cofactors are called coenzyme, its detach after a reaction and may participate in the reaction with other enzyme.
  - Coenzymes involves in oxidation and reduction in biological reactions.

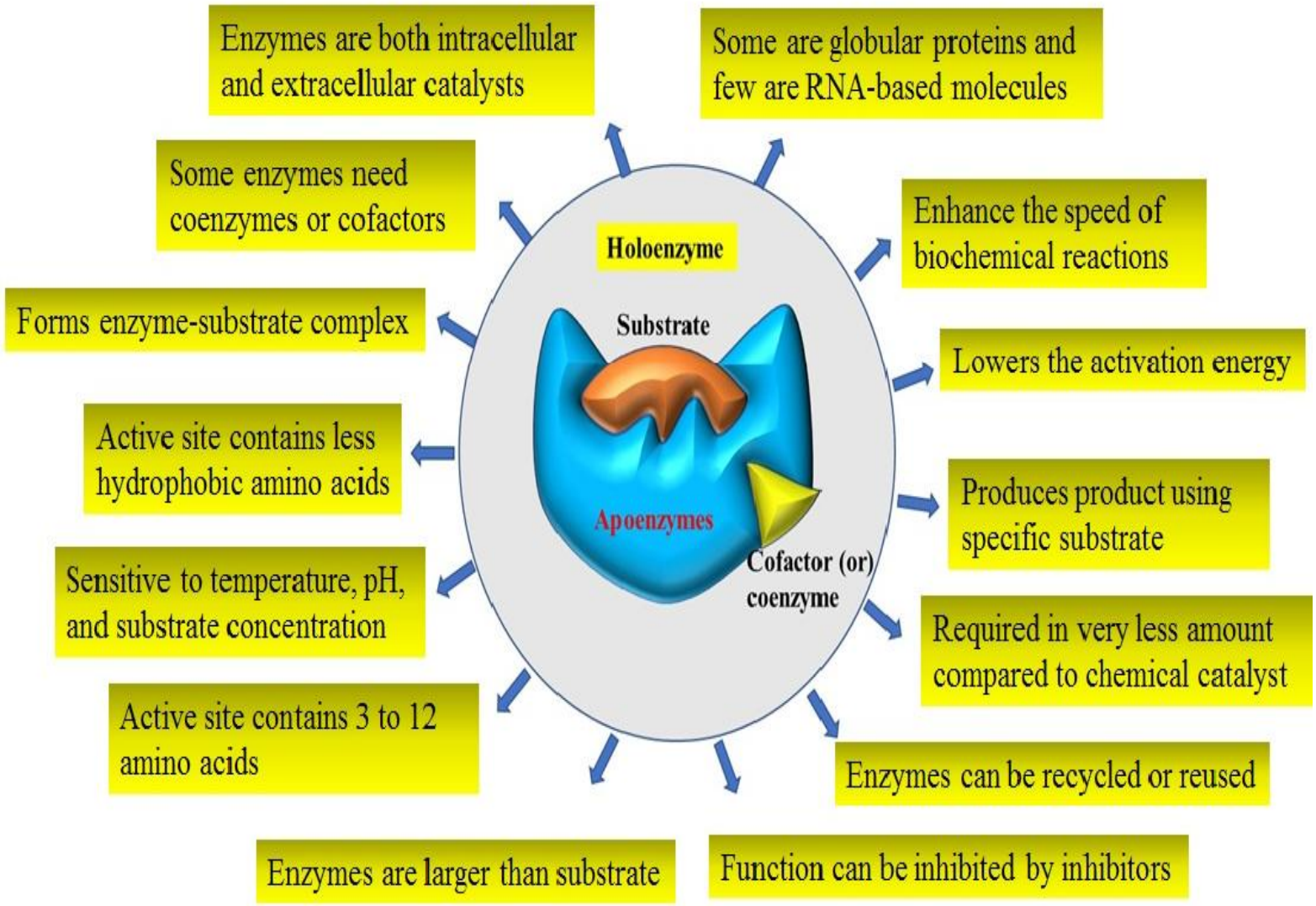


Important Prosthetic Group and Coenzymes

Prosthetic Group	Enzymes/ Proteins
Zn^{++}	Carbonic anhydrase , Alcohol dehydrogenase
Fe^{+++} or Fe^{++}	Hemoglobin, Cytochromes, ferrodoxin
Cu^{++} or Cu^{+++}	Cytochrome oxidase
K^{+} and Mg^{++}	Pyruvate Phosphokinase

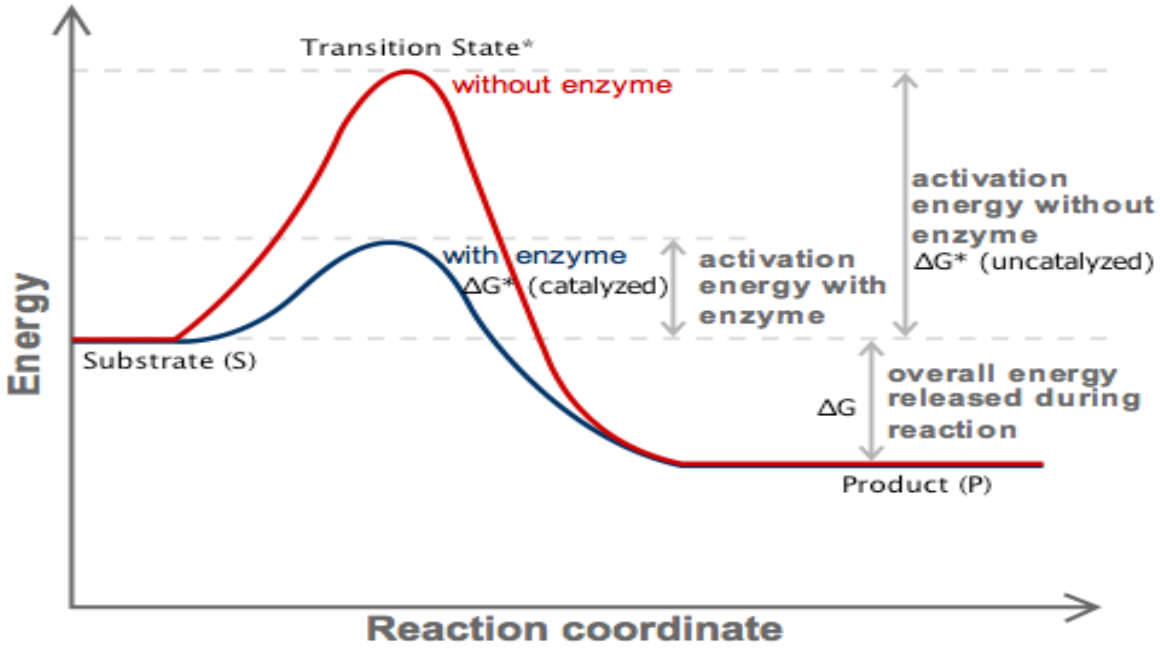
Coenzymes	Vitamins
Nicotinamide adenine dinucleotide (NAD^{+})or nicotinamide adenine dinucleotide phosphate ($NADP^{+}$)	vitamin B_3 (niacin)
Flavin mononucleotide (FMN^{+}) or flavin adenine dinucleotide(FAD^{+})	vitamin B_2 (riboflavin)
Pyridoxal phosphate	vitamin B_6 (pyridoxine)
Coenzyme A	Pantothenic Acid

Characteristics of the enzymes



Mechanism of Enzymatic reaction

•Enzymes work by lowering the activation energy (Ea or ΔG✳) for a reaction. This increases the rate of reaction.
•Enzymes decrease the Gibbs free energy of activation, but they have no effect on the free energy of reaction.



Factors that affects Enzyme Activity

The are several factors that affects the enzyme catalyse reaction, these are following:

1. Substrate Concentration
2. Enzyme Concentration
3. pH of the Medium
4. Temp of the Medium
5. Effects of Inhibitors on Enzyme Activity (Competitive inhibition and Non-Copetitive Inhibitors)

Enzyme Kinetics

•**Enzyme kinetics** is the study of the chemical reactions that are catalysed by enzymes.

•In enzyme kinetics, the **reaction rate is measured** and how get changes in response to changes in experimental parameters such as substrate concentration, enzyme concentration etc.

•This is the oldest approach to understanding enzyme mechanisms and remains the most important.

• **The initial rate** (or **initial velocity**), designated ***Vo*, when** [S] is much greater than the concentration of enzyme [E] can be measured by **Michaelis–Menten kinetics.** It is one of the simplest and best-known models of enzyme kinetics.

Note# Michaelis-Menten equation, the rate equation for a **one-substrate enzyme-catalyzed** reaction.

Effect of Substrate Concentration on Enzyme Kinetics

• The concentration of substrate [S] is a key factor affecting the rate of a reaction catalyzed by an enzyme .

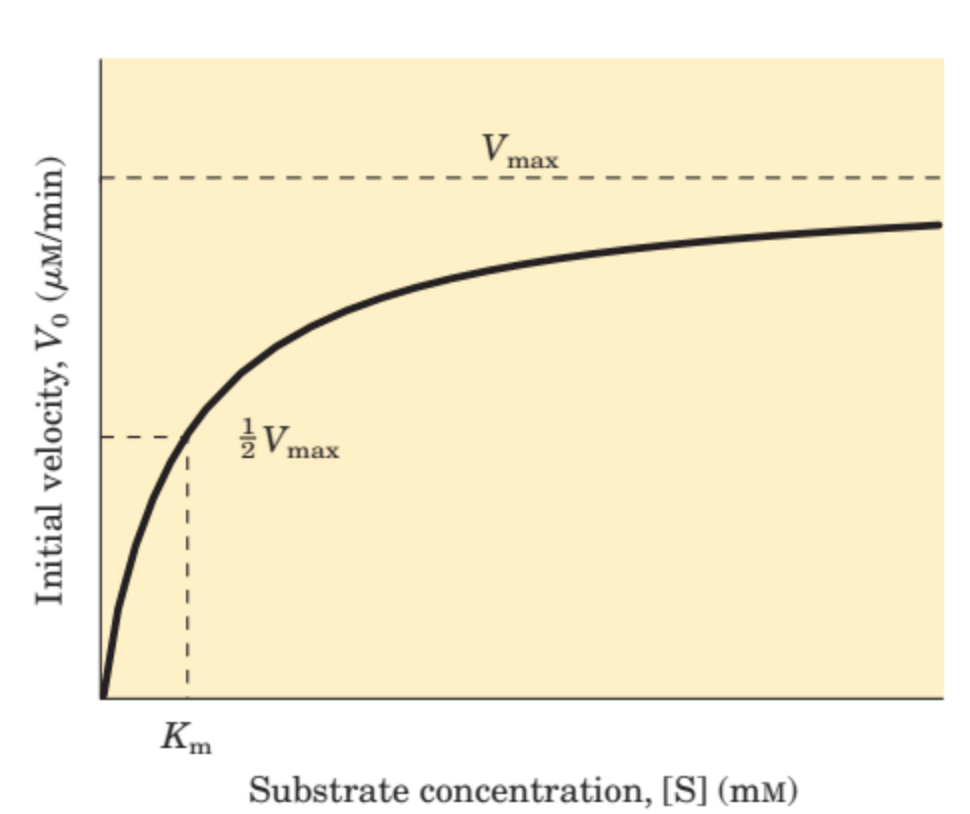


Fig. Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction.

A General Enzymatic reaction

$$E + S \underset{K_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \text{ ---------- 1}$$

V_o is determined by the breakdown of ES to form product, which is determined by [ES]:

$$Vo = \frac{d\,[P]}{d\,t} = k2[ES] \text{ ---------- 2}$$

*W*here as k_1= rates of formation of ES, $K_{-1} + k_2$ = breakdown of ES

Rate of formation of [ES] =$k1$[E] [S]
Rate of dissociation of [ES]= $k\text{-}1$[ES] + $k2$[ES]

So in the steady sate ,

$$k1[E]\,[S] = k\text{-}1[ES] + k2[ES] \text{ ---------- 3}$$

$$[E]\,[S]/[ES] = (k\text{-}1 + k2)/k1 \text{ ---------- 4}$$

$$k\text{-}1 + k2)/k1 = Km$$

Note# Here E, S, ES and P symbolize the enzyme, substrate, enzyme-substrate complex and products respectively

Note# Km is called **Michaelis constant**, it is a substrate concentration at which *V*o= ½*V*max and half of the active sites on the enzymes are filled . Different enzymes have different Km values. They typically range from 10^{-1} to 10^{-7} M. The unit of *K*m is concentration. Km is affected by: pH, temperature, ionic strengths and the nature of the substrate. If *V*o= ½*V*max, then *K*m=[S]. Km is a measure of a substrate's affinity for the enzyme.

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

$$[E] = [Eo]-[ES]$$

$$Km= [E]\,[S]/[ES] \qquad \text{---------- 5}$$

So putting the value of [E] in to the equation no -5

$$Km= [Eo]-[ES]\,[S]/[ES]$$

$$[ES]= [Eo]-[ES]\,[S]/\,Km$$

So finally, $[ES]= [Eo][S] / Km +[S]$----------6

Then putting the value of [ES] from equation no-5 to equation no -2, the rate *V*o can be expressed in terms of [S].

$$V_o = k_2 [E_o][S] / Km + [S] \text{----------} 7$$

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

Finally, $V_o = V_{max} [S] / Km + [S] \text{----------} 8$

The equation -8 is called the **Michaelis-Menten equation,** for a **one-substrate enzyme-catalyzed** reaction.

Note # when *V*o is exactly one-half *V*max:

$$V_o/2 = V_{max} [S] / Km + [S]$$

Graphical Representation of Michaelis-Menten equation

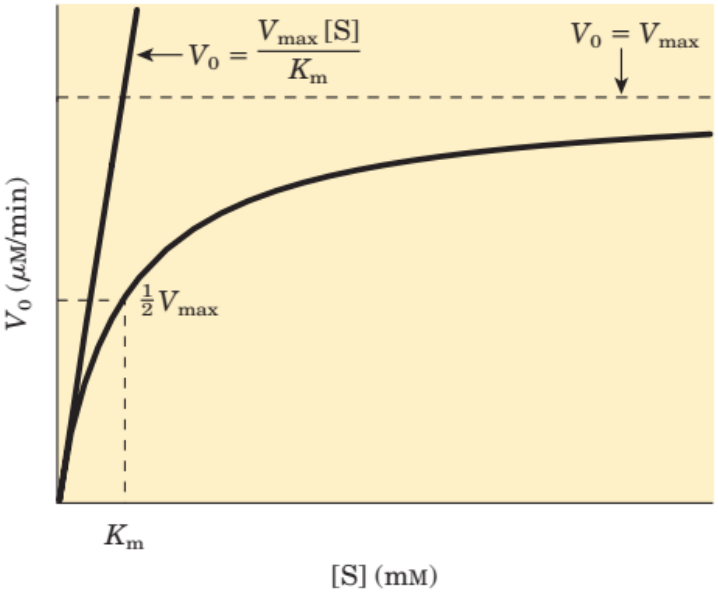


Fig. Dependence of initial velocity (Vo) on substrate concentration.

- The equation describes the kinetic behavior of all enzymes that exhibit a hyperbolic dependence of *V*o on [S] are said to follow **Michaelis Menten kinetics.**
- This equation practically determine the value of *K*m and *V*max and, also describe the analysis of inhibitor action.
- But **double-reciprocal plot is** more convenient procedure, to determine an approximate value of Km.

Transformations of the Michaelis-Menten Equation: The Double-Reciprocal Plot :

•The Michaelis-Menten equation can be algebraically transformed into equations that are more useful in plotting **experimental data.**

*V*o = *V*max [*S*] / Km +[S]--- Michaelis-Menten equation

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* $x = 1/[S]$.

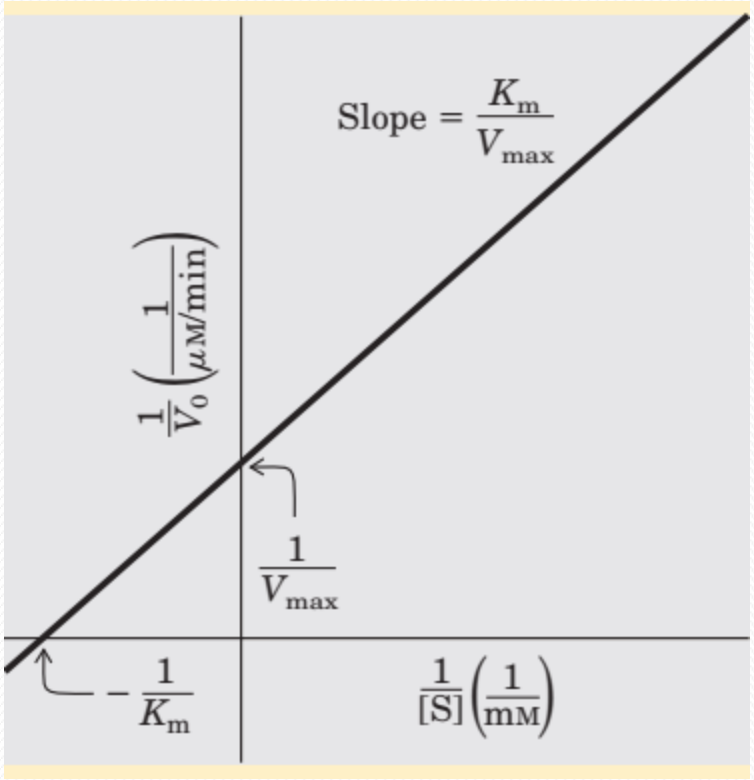


Fig. A double-reciprocal or Lineweaver-Burk plot.

- Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of Vmax, and Km.
- The double-reciprocal plot is very useful to determined the mechanism of enzymatic reaction
- This line has a slope of *K*m/*V*max, an intercept of 1/*V*max on the 1/*V*o y -axis, and an intercept of -1/*K*m on the 1/[S] x-axis .

References:

•Stryer, Lubert. *Biochemistry (Third Edition)*.New York, NY: W.H. Freeman and Company, 1988. Page 187-191 .

• Lehninger principles of biochemistry (4th ed.): Nelson, D., and Cox, M, W.H. Freeman and Company, New York, 2005 .

Thank You