Enzymes and Coenzymes I

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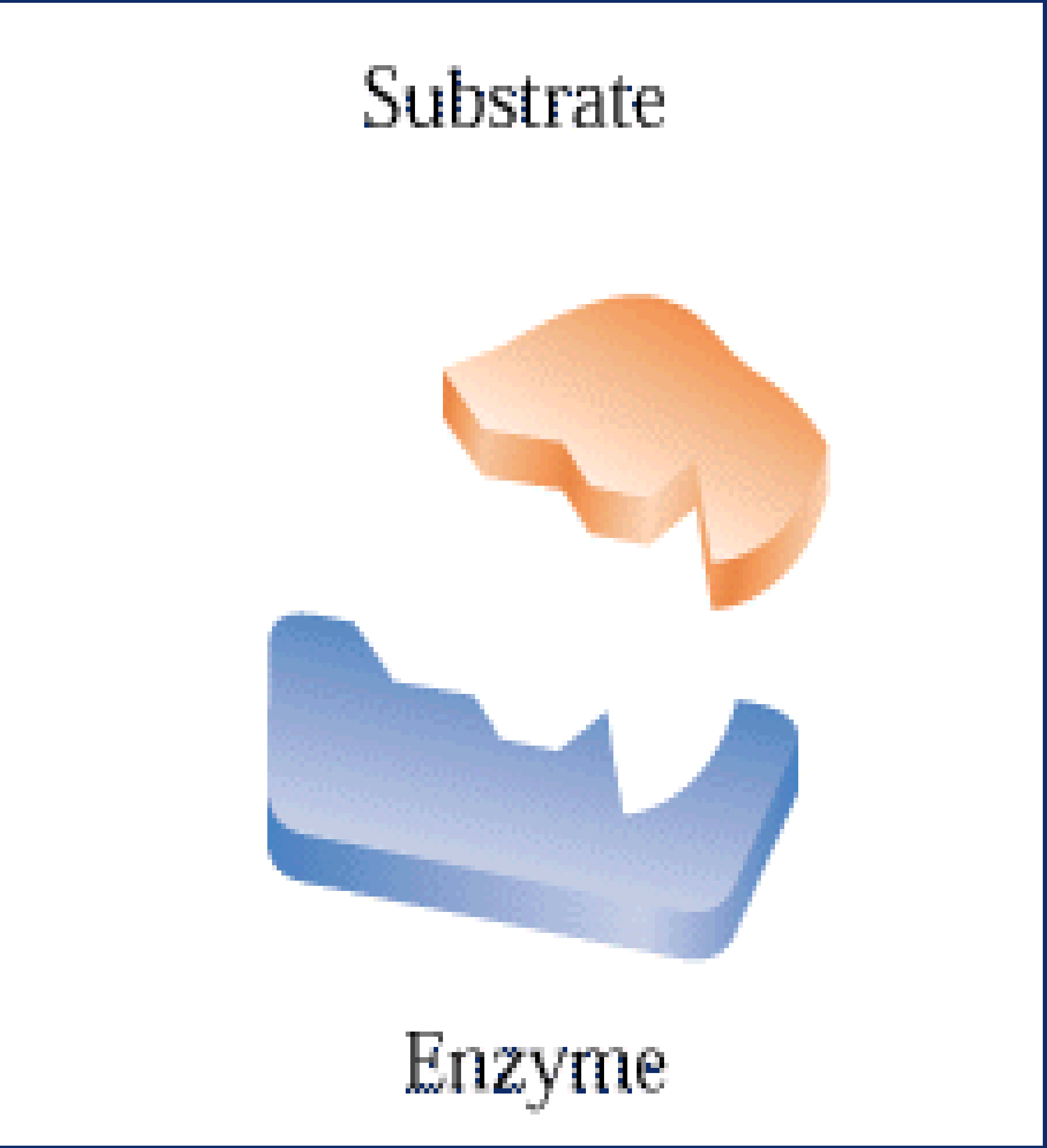
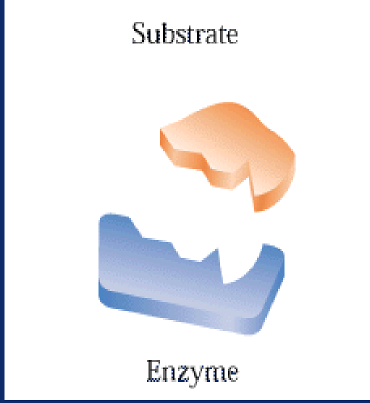
What are Enzymes?

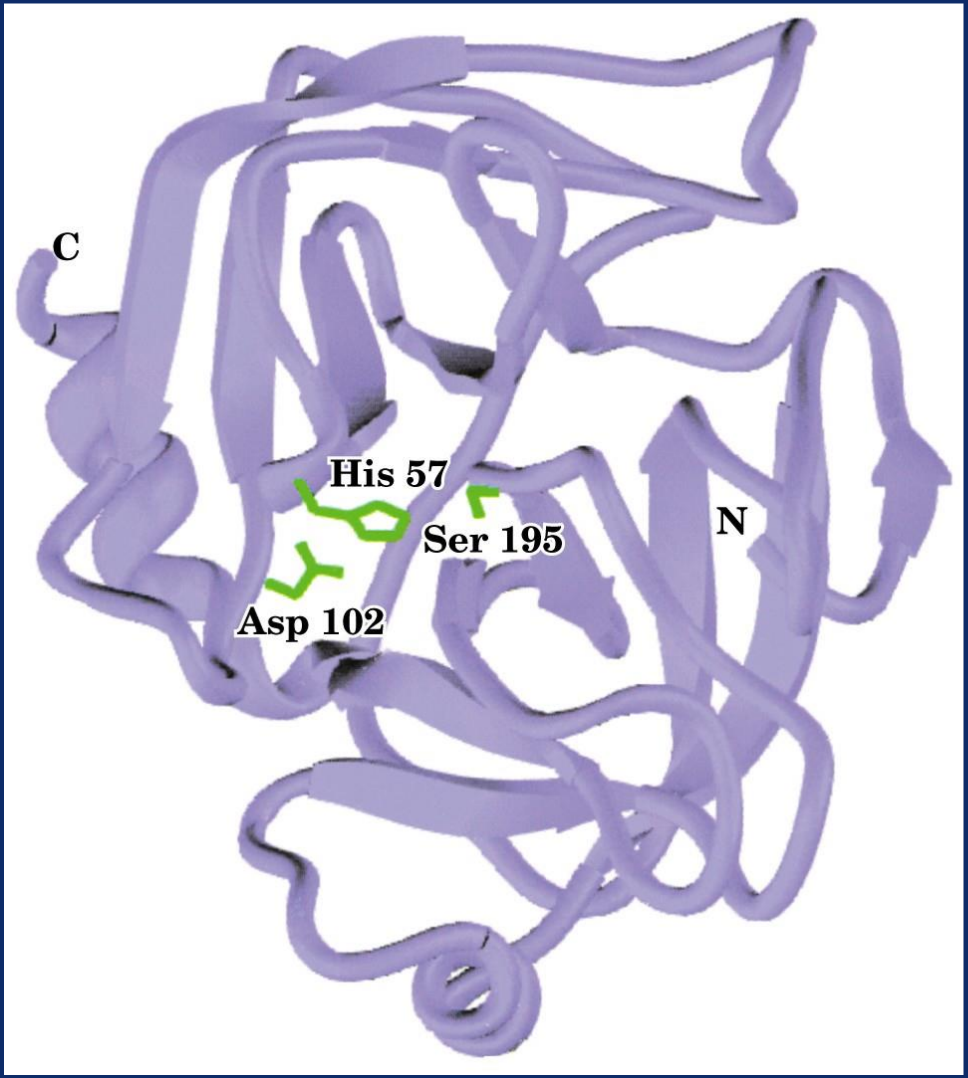
- Enzymes are biological catalysts that speed up the rate of a reaction without being changed in the reaction
- All enzymes are protein in nature
- But all proteins are not enzymes
- Substance upon which the enzymes act are called substrates
- Enzyme converts substrates into product(s)


Substrate
Enzyme

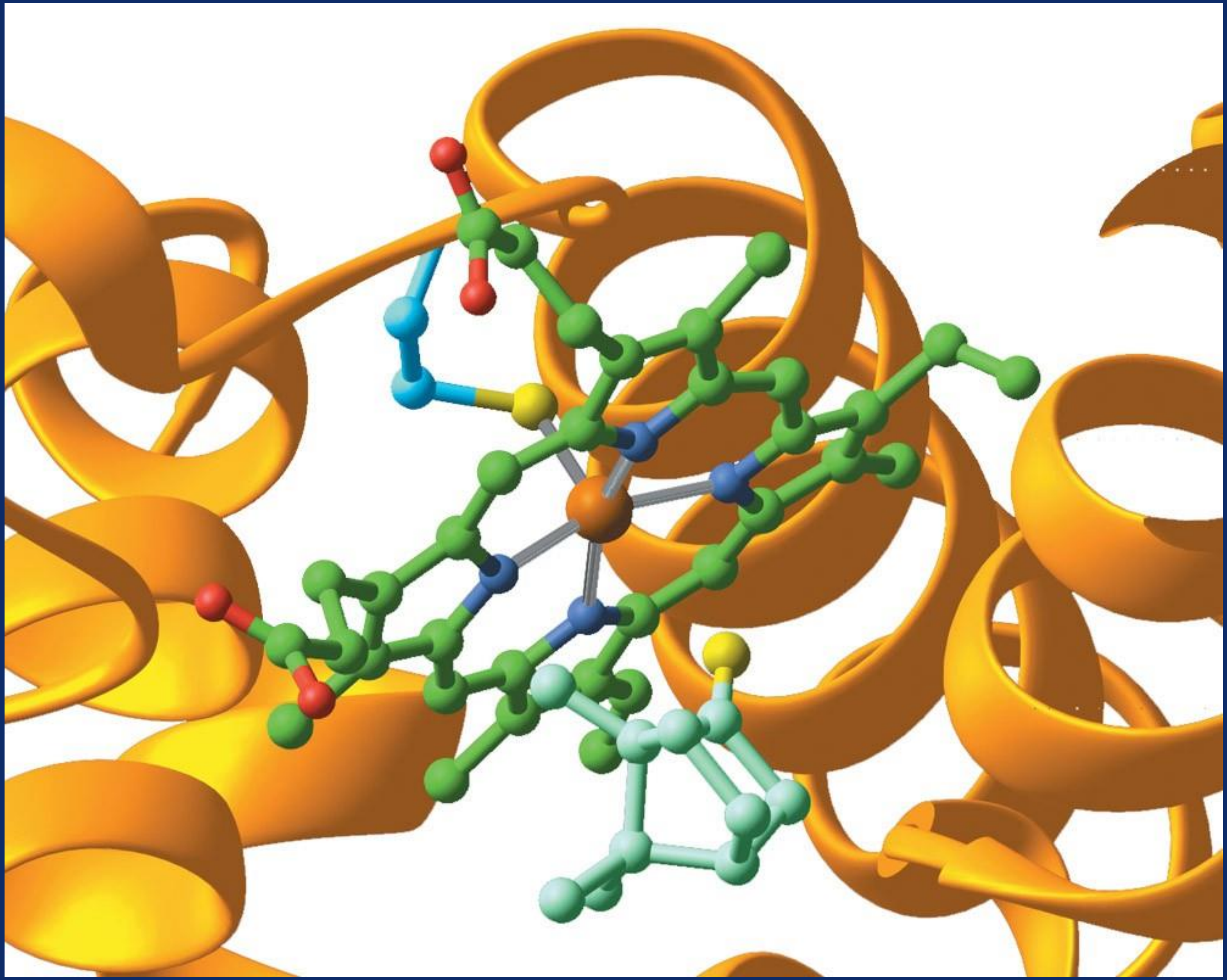
Properties of Enzymes



- **Active site-**
  - The region of enzyme that binds with the substrate and where catalysis occurs
  - All enzymes have one or more active sites
- **Specificity-**
  - Enzymes bind to their specific substrates in the active site to convert them to product(s)
- **Regulation-**
  - Enzymes can be activated or inhibited so that the rate of product formation responds to the need of the cell



Structure of trypsin enzyme



An enzyme with its active site

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Enzyme specificity

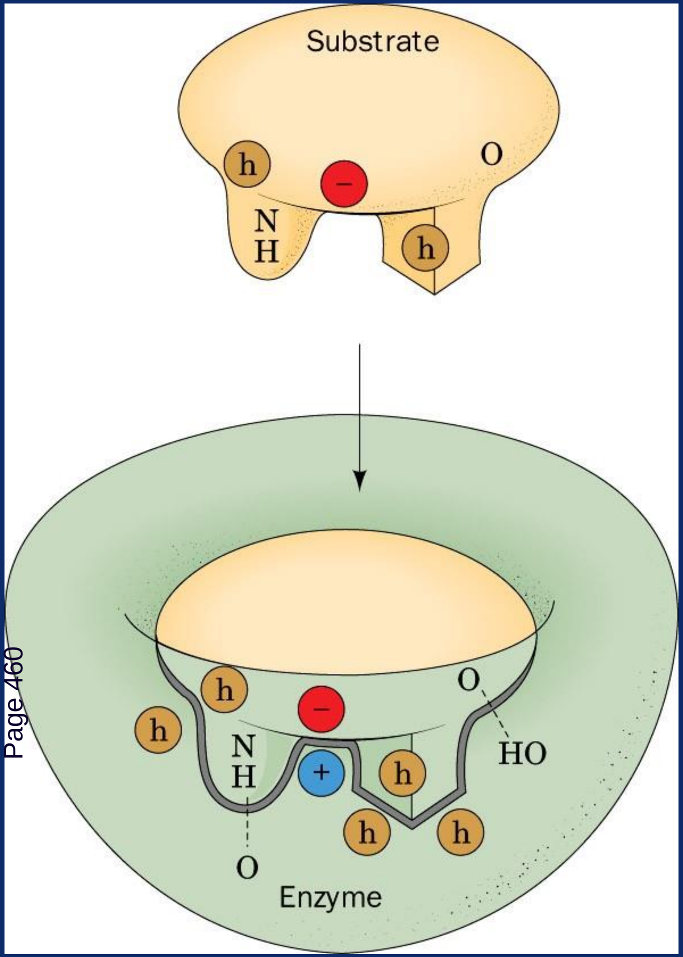
- Enzymes are highly specific
- Interact with only one or a few of the substrates
- Catalyze only one type of reaction

Enzyme-substrate binding

- Two models have been proposed
  - Lock and key binding
  - Induced fit binding

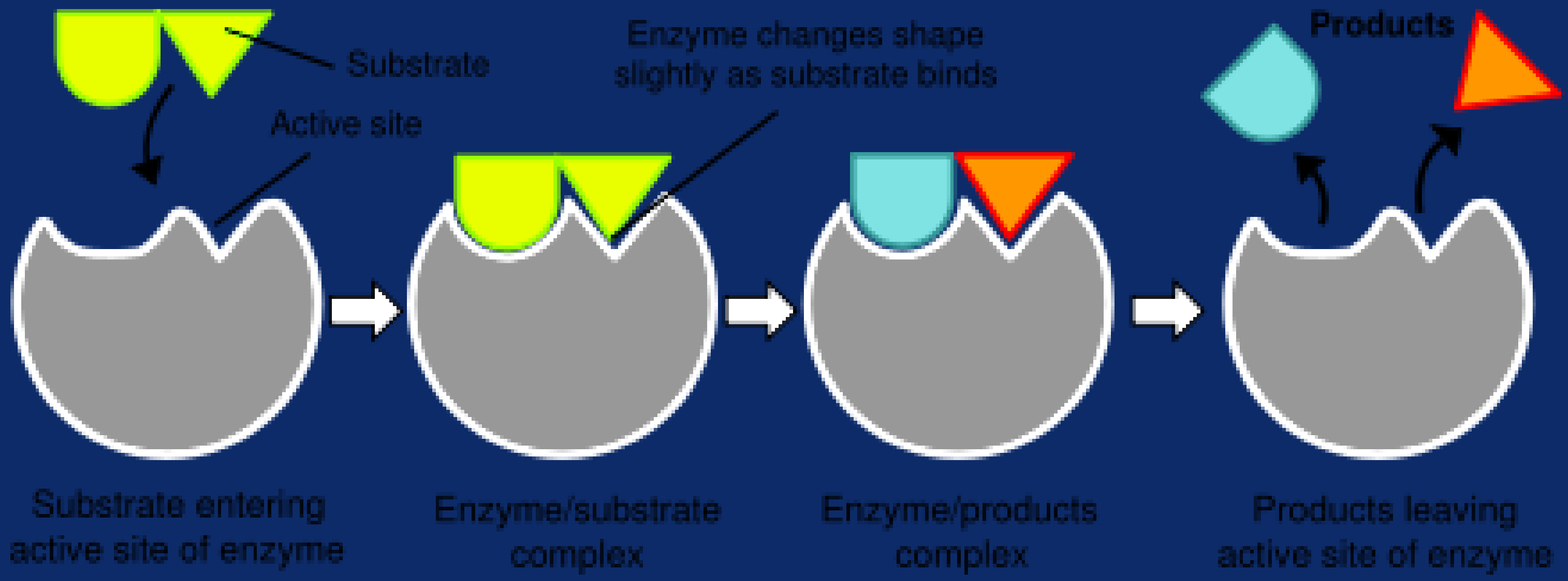
Lock and key binding

The enzyme has an active site that fits the exact dimensions of the substrate



Induced fit binding

- After the binding of substrate, the enzyme changes its shape to fit more perfectly with substrate



Classification of Enzymes

Classified into six types according to the reaction catalyzed

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Classification	Type of Reaction Catalyzed
1. Oxidoreductases	Oxidation–reduction reactions
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions
4. Lyases	Group elimination to form double bonds
5. Isomerases	Isomerization
6. Ligases	Bond formation coupled with ATP hydrolysis

Enzyme nomenclature (Naming)

- Enzyme nomenclature is based on the rules given by IUBMB (International Union of Biochemistry and Molecular Biology)
- EC 3.4.17.1 (carboxypeptidase A)

  EC = Enzyme Commission

Class.Subclass.Subsubclass.Enzyme number

Holoenzymes

- Some enzymes require non-protein groups to become active

- The inactive form of enzyme without its non-protein part is called an apoenzyme

- Apoenzyme (inactive) + nonprotein part = Holoenzyme (active)

Cofactors and Coenzymes

- If the non-protein part is a metal ion such as Cu^{2+}, Fe^{3+}, Zn^{2+}, etc., it is called cofactor
- If small organic molecules, known as coenzymes such as NAD^{+}
  - Prosthetic groups
  - Cosubstrates

Coenzymes

- **Coenzymes that are permanently associated with an enzyme known as prosthetic groups** e.g. FAD

- **Coenzymes that only temporarily associate with an enzyme known as cosubstrates** e.g. NAD

Apoenzyme (inactive) + Cofactor/coenzyme = Holoenzyme (active)

Ribozymes, Isoenzymes and zymogens

- **Ribozymes** are RNAs with enzyme activity
- **Isoenzymes** are enzymes that catalyze the same chemical reaction but they have slightly different structures
- **Zymogens** are inactive enzyme precursors that require a biochemical change to become active e.g. cleavage of a peptide blocking the active site

Enzymes decrease activation energy of a reaction

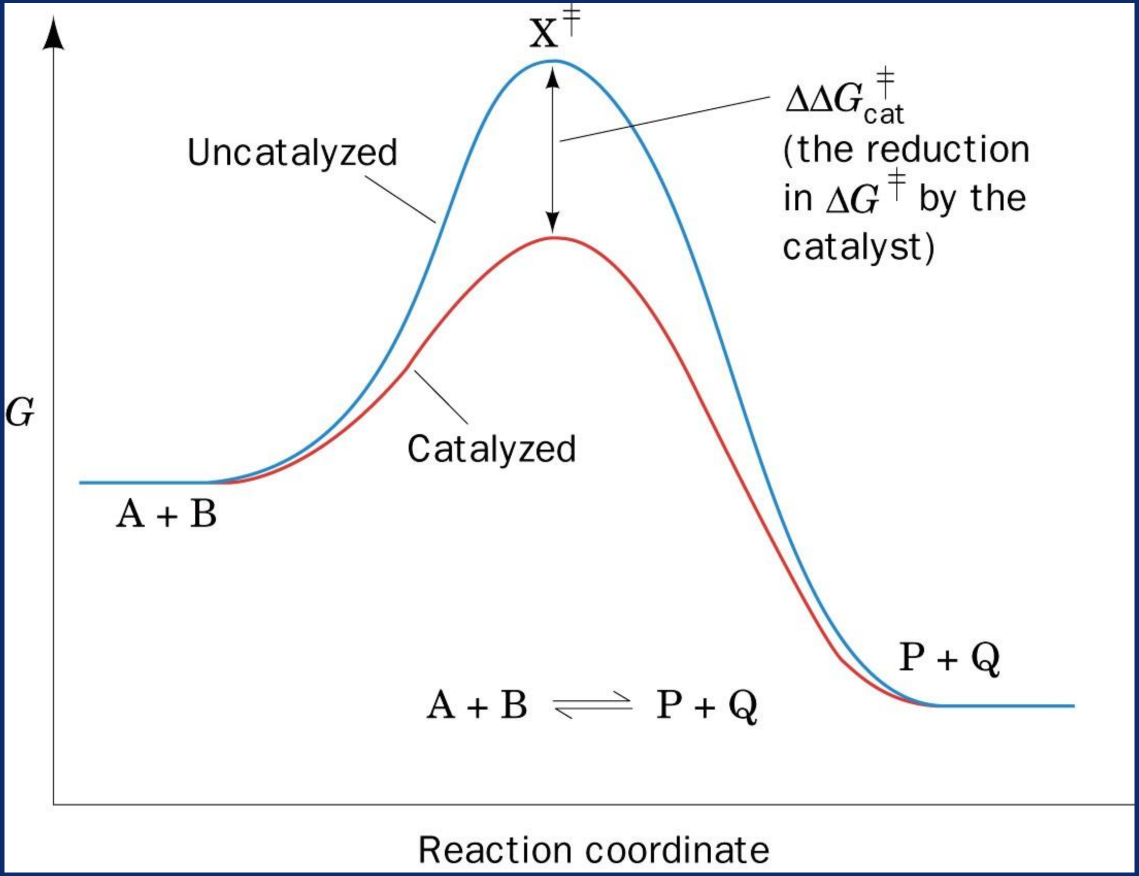
- In every chemical reaction, the reactants pass through a transition state that has greater energy than that of the reactants or products alone
- The difference in energy between the reactants and the transition state is called the activation energy
- If the activation energy is available then the reaction can proceed forming products

- **An enzyme reduces the activation energy required for a reaction**

- **It provides an alternative transition state of lower energy called the enzyme-substrate complex and thus speeds up the reaction**

- **Enzymes decrease the activation energy but they do not alter the change in the free energy (ΔG)**

The effect of a catalyst on the transition state diagram of a reaction



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Enzyme Activity or Velocity

- Velocity is the rate of a reaction catalyzed by an enzyme

- Enzyme activity is expressed as:

*m*moles of product formed/min/mg enzyme

Factors that affect enzyme activity

- <u>Effect of temperature</u>
  - Every enzyme has an optimal temp. for catalyzing a reaction
  - The rate of an enzyme reaction initially increases with rise in temperature
  - At high temp. enzymes are denatured and become inactive
  - In humans most enzyme have an optimal temp. of 37^{o}C

Factors that affect enzyme activity

- Effect of pH
  - Effect of pH on the ionizable groups in the active site of enzyme or in the substrate affect catalysis
  - Every enzyme has an optimal pH for catalyzing a reaction
  - Most enzymes have highest activity between pH 6 and pH 8
  - Pepsin has highest activity at pH 2

Effect of pH on the initial rate of the reaction catalyzed by most enzymes (the bell-shaped curve)

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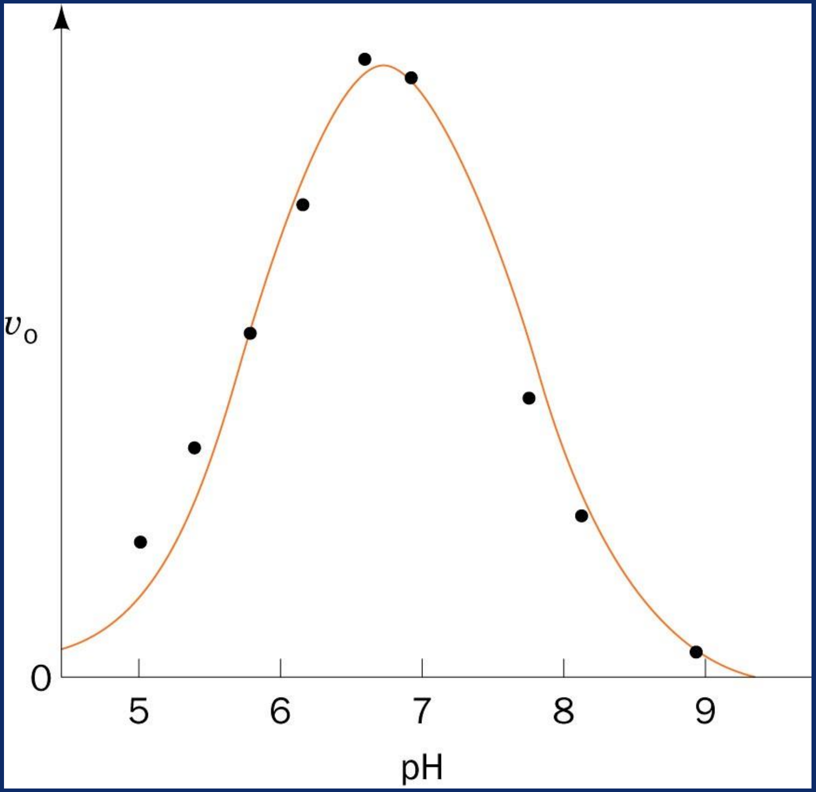


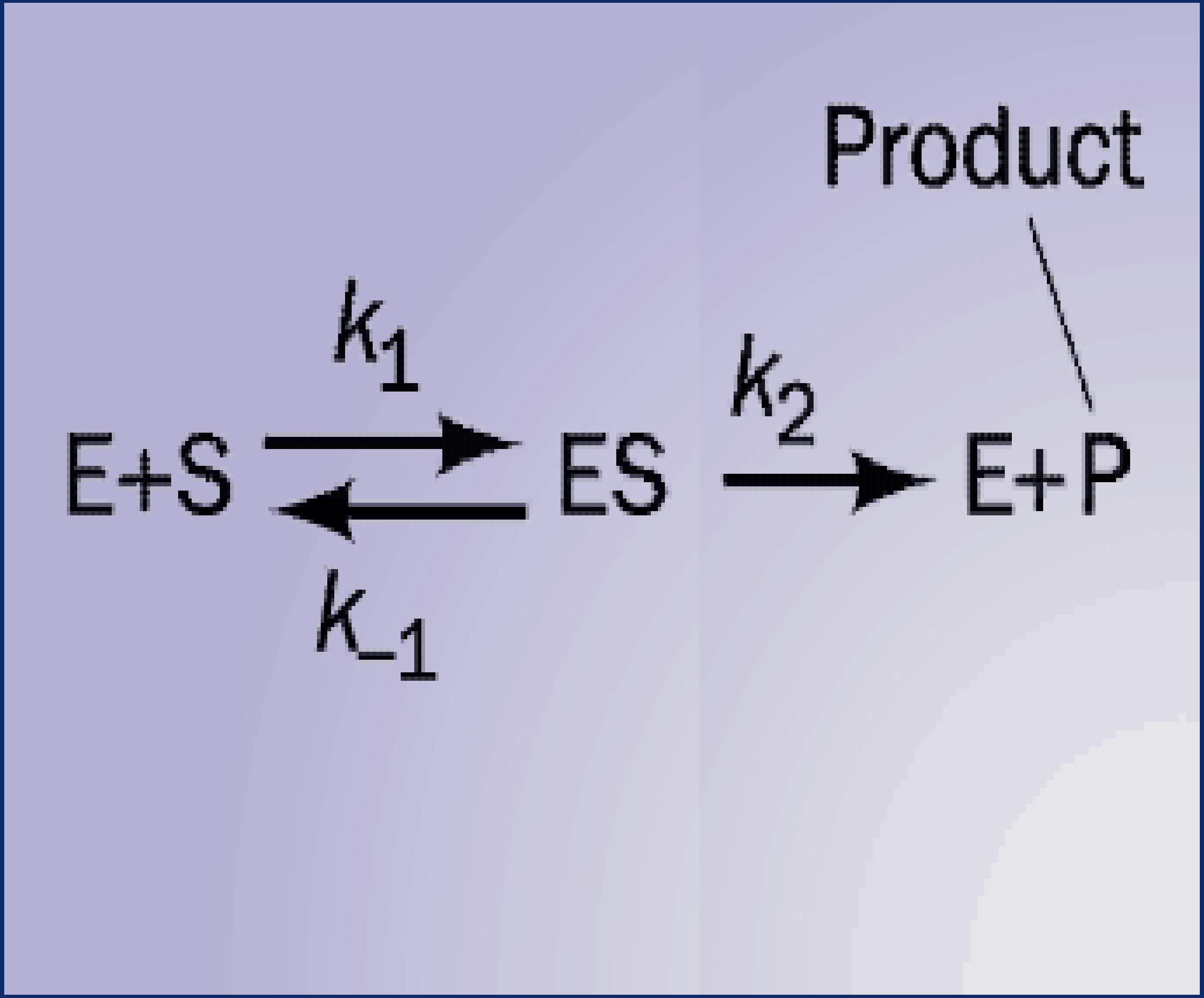
Factors that affect enzyme activity

- **Effect of [E] and [S]**
  - The reaction velocity increases initially with increasing [S]
  - At low [S], the reaction rate is proportional to [S]
  - Further addition of substrate has no effect on enzyme velocity (*v*)
  - The rate of an enzyme reaction is directly proportional to the conc. of enzyme if the substrate concentration [S] is higher than enzyme

Enzyme kinetics

- The model of enzyme kinetics was first proposed by Michaelis and Menten in 1913 and later modified by Briggs and Haldane

- The Michaelis Menten equation describes the relationship of initial rate of an enzyme reaction to the [S]


Product
k1
k2
E+S
ES
E+P
k−1

Initial rate of enzyme reaction

Pre-steady state kinetics

- When an enzyme is mixed with high concentration of substrate, there is an initial short period of time (a few hundred microseconds) during which intermediates leading to the formation of product gradually build up

- **Steady state kinetics**

  - After initial state, the reaction rate and the concentration of intermediates change slowly with time called steady state reaction
  - An intermediate is said to be steady state when its rate of synthesis is equal to its rate of degradation

Michaelis Menten Equation

- It measures the initial velocity (v_o) of an enzyme reaction

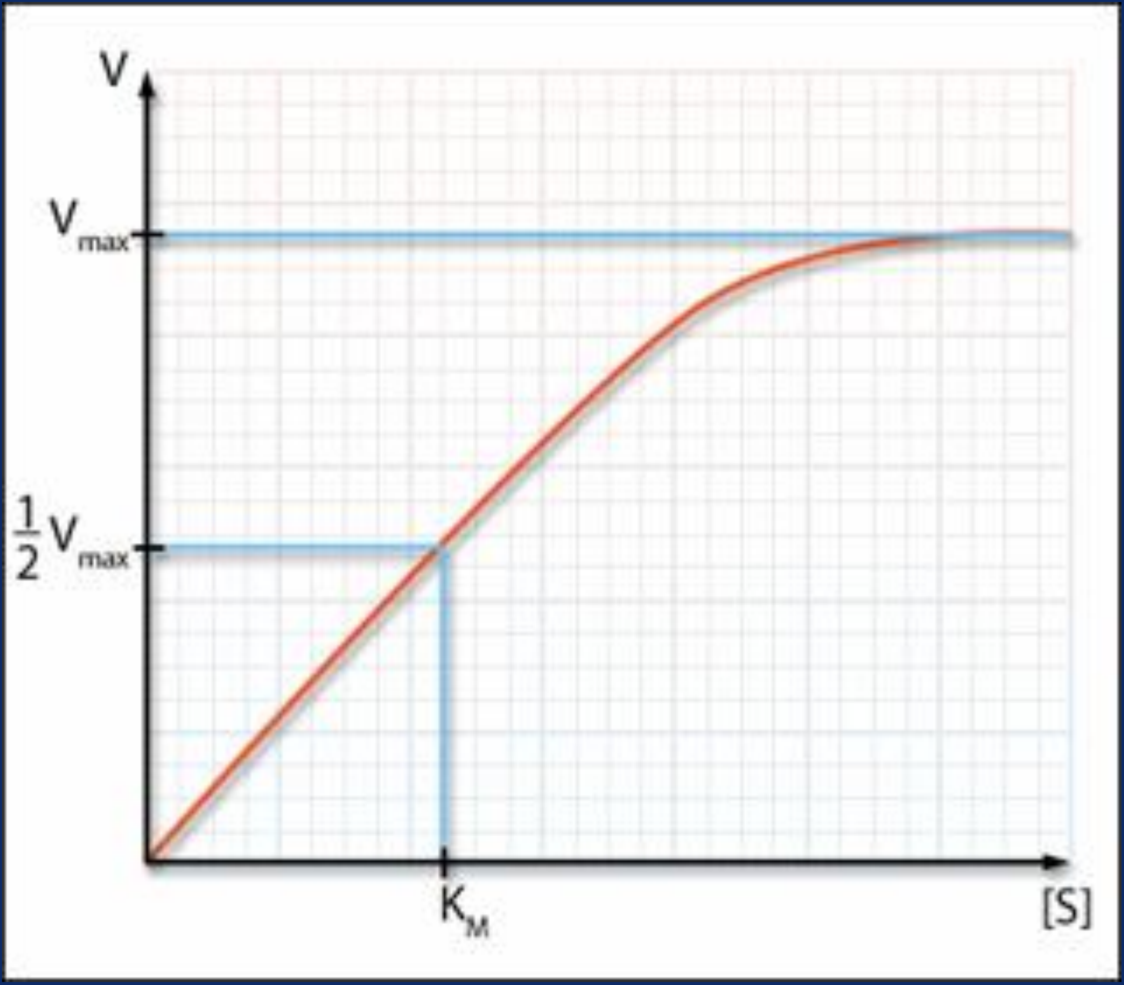
$$v_o = \frac{V_{max}\,[S]}{K_m + [S]}$$

[S] = substrate concentration

V_{max} = maximum velocity

K_m = Michaelis constant

Initial velocity v_o of a simple Michaelis–Menten reaction versus the substrate concentration[S]



K_m (Michaelis Constant)

- K_m is the substrate concentration at which the initial rate is one-half of the maximum rate (½ V_{max})

- It is the [S] required to saturate half of all of the active sites of an enzyme

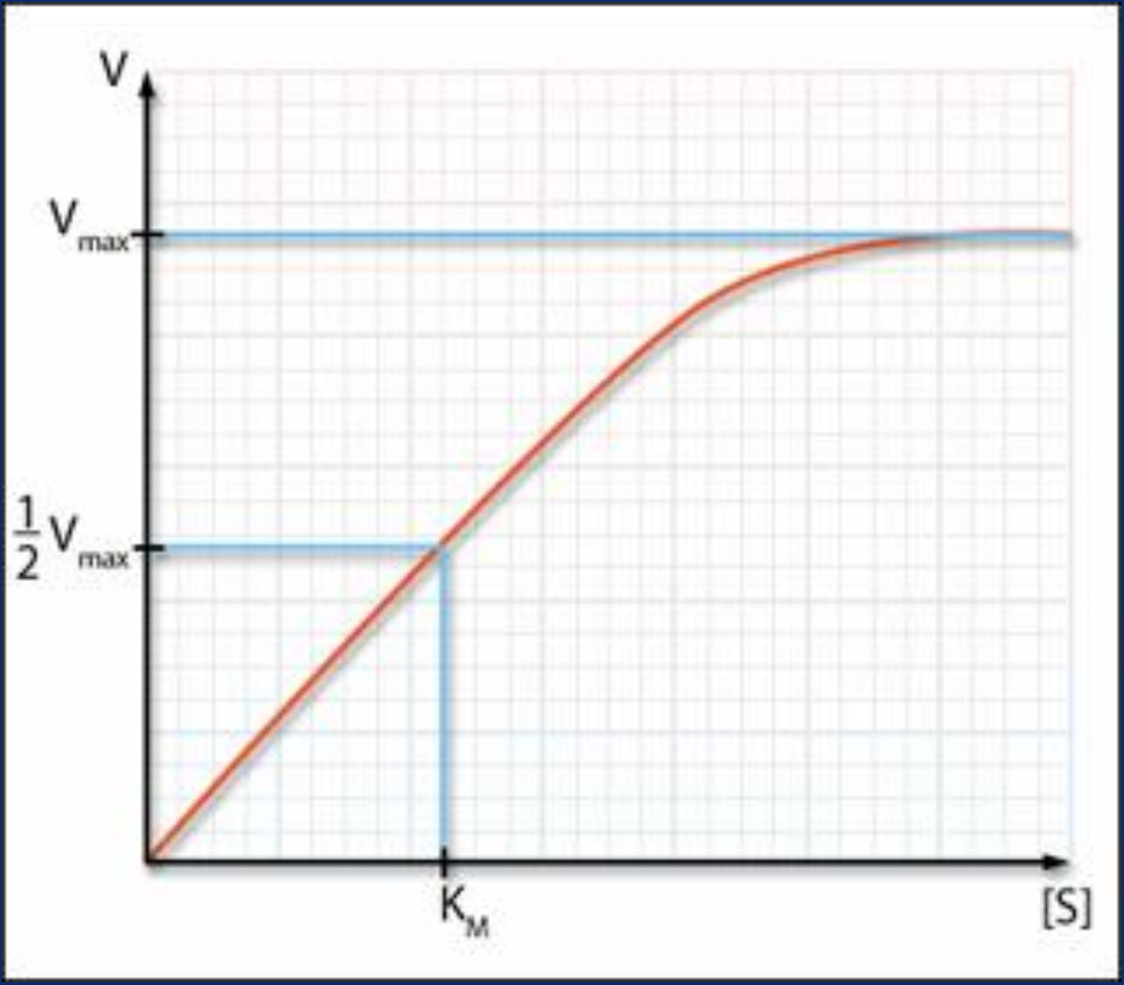
- **The K_m value of a substrate depends on its affinity with the enzyme**
  - **<u>High K_m</u> means low affinity with enzyme (more substrate needed to saturate the enzyme)**
  - **<u>Low K_m</u> means high affinity with enzyme (less substrate needed to saturate the enzyme)**

Lineweaver-Burk plot

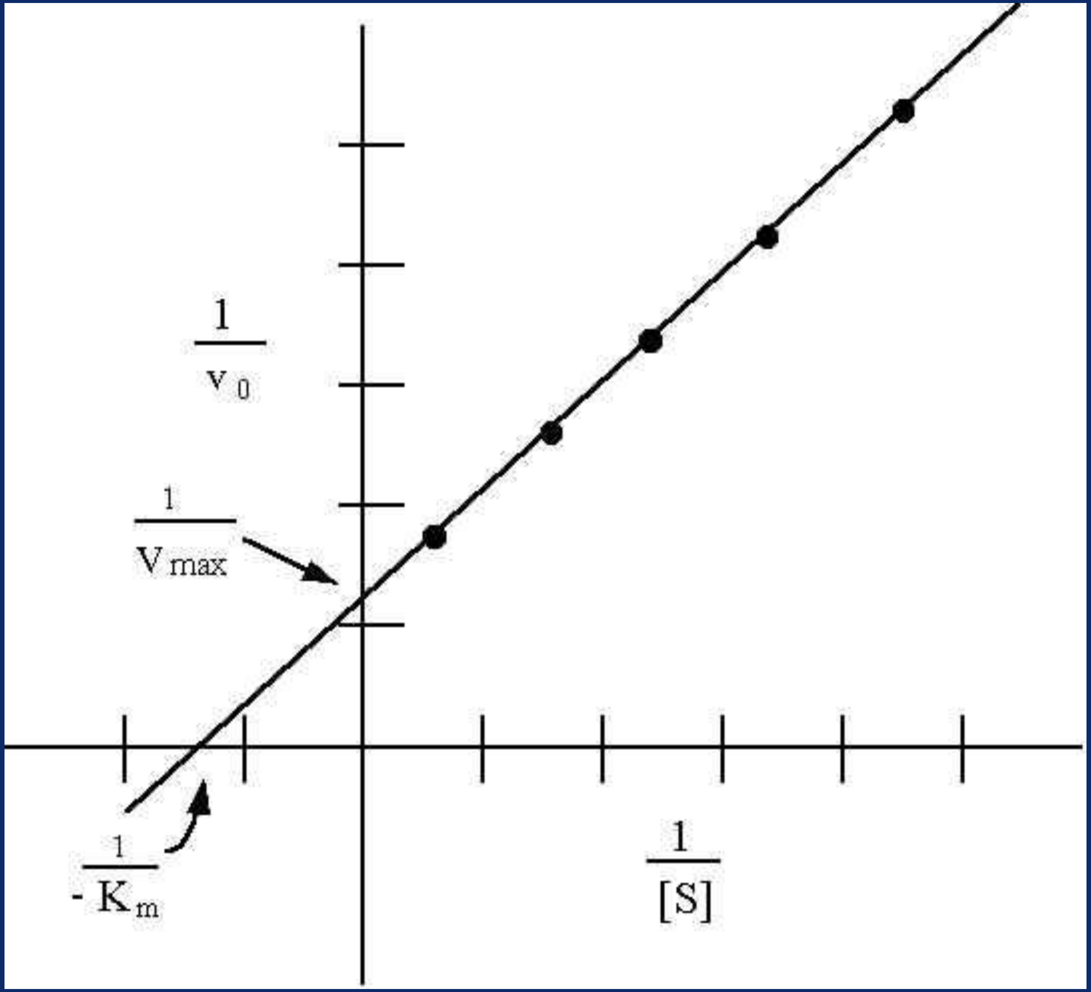
- Also called the double-reciprocal plot, obtained by taking reciprocals of the Michaelis Menten equation

- It is plotted to calculate the Km and Vmax values and to determine the mechanism of action of enzyme inhibitors

Initial velocity v_o of a simple Michaelis–Menten reaction versus the substrate concentration[S]



Lineweaver-Burk plot



References

- **Illustrated Reviews in Biochemistry by Lippincott**