Enzymes (Foundation Block)

Two Lectures

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Overview

- What are enzymes?
  - Classification of enzymes and naming
  - Coenzymes, Cofactors, Isoenzymes
- Enzyme activity and specificity
- Factors affecting enzyme activity

Overview

- Enzyme kinetics (Michaelis Menten equation)
- Enzyme inhibition and types
- Regulation of enzyme activity
- Enzymes in clinical diagnosis

Objectives

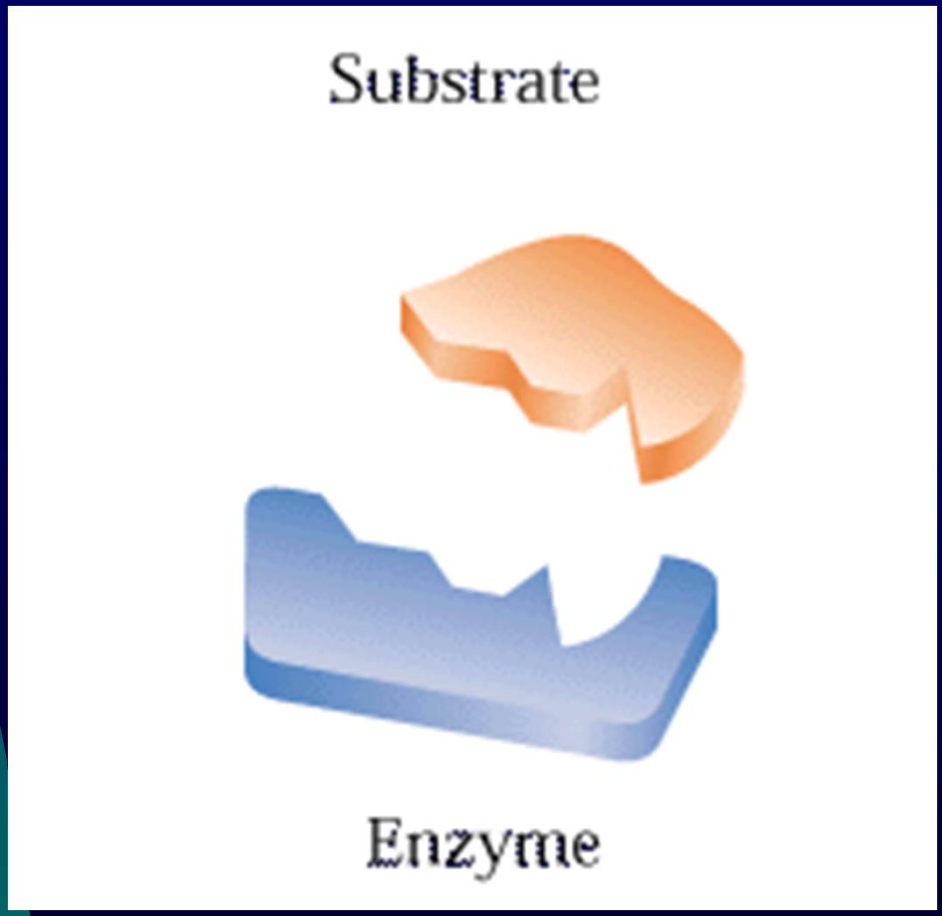
By the end of this lecture the First Year students will be able to:

- Understand how enzymes are able to speed up the rate of biochemical reactions in the body
- Identify classes of enzymes based on the type of reactions they catalyze
- Comprehend the basic terms of coenzymes, isoenzymes, enzyme activity and specificity along with factors affecting their activity
- Understand the enzyme kinetics, types of inhibition and regulation of enzyme activity
- Discuss the clinical role enzymes in the diagnosis of diseases

What are Enzymes?

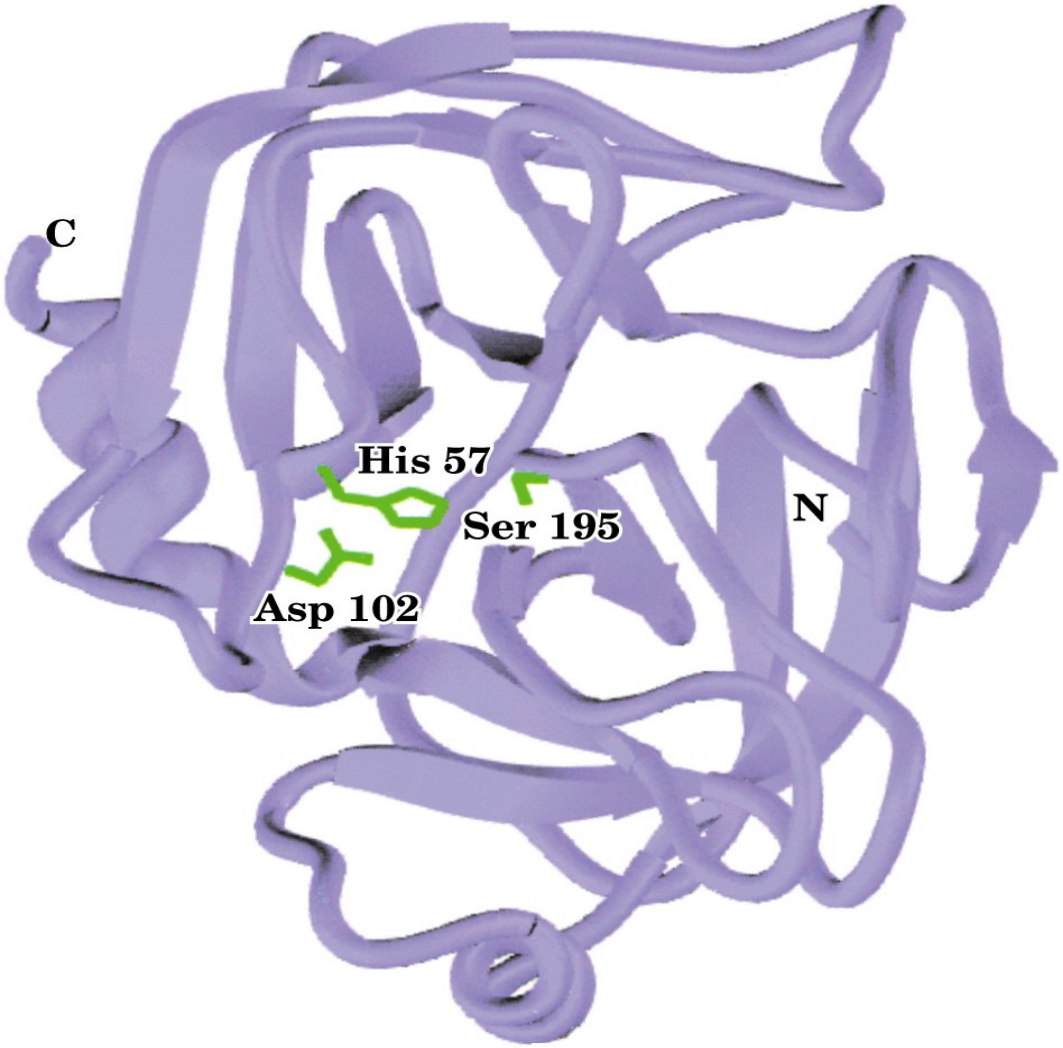
- Enzymes are biological catalysts that speed up the rate of a reaction without being changed in the reaction
- All enzymes are protein in nature
- But all proteins are not enzymes
- All enzymes have one or more active sites
- Some enzymes have both active and regulatory sites
- Enzymes bind to their specific substrates in the active site to convert them to product(s)

Enzymes

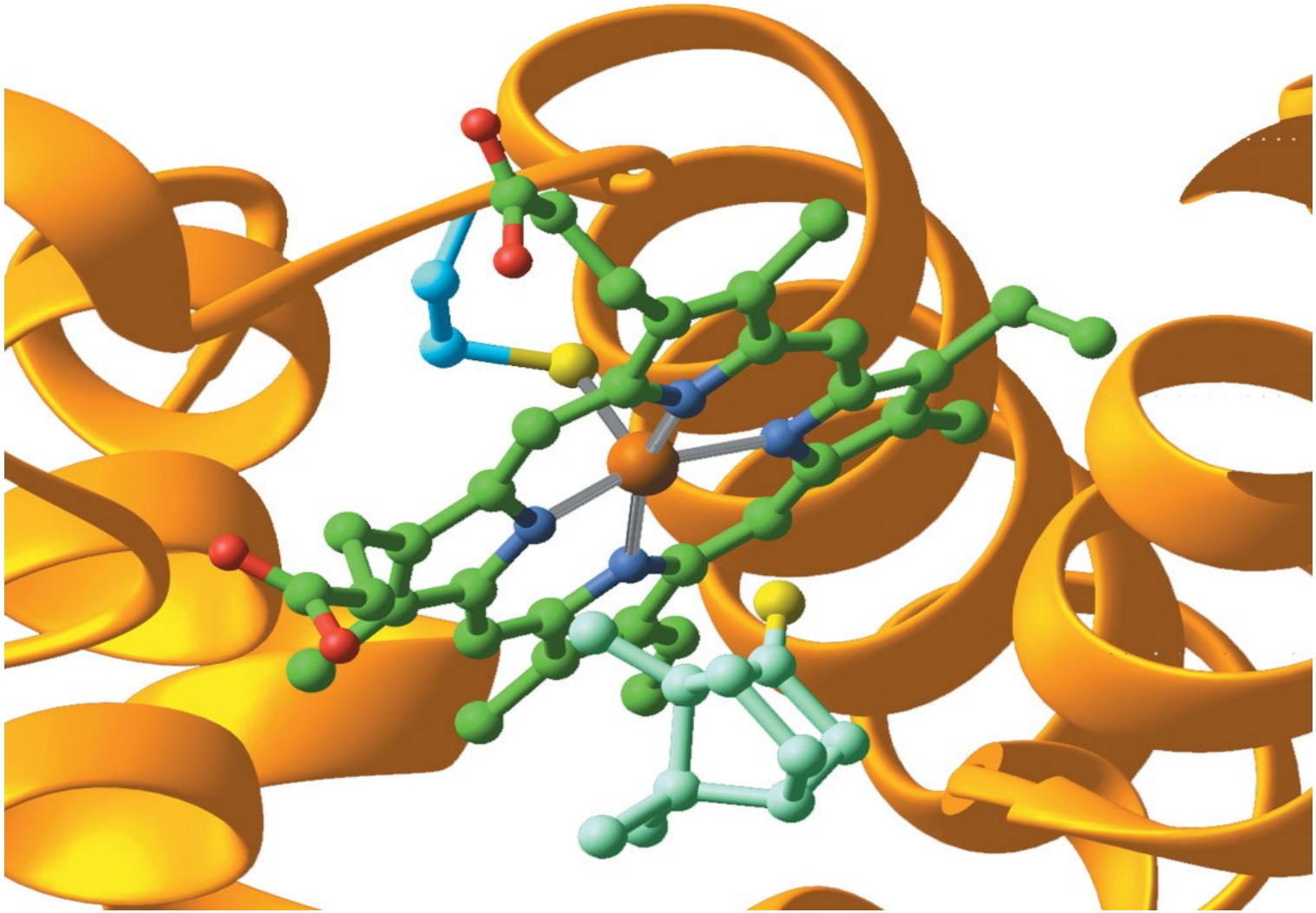


Active site

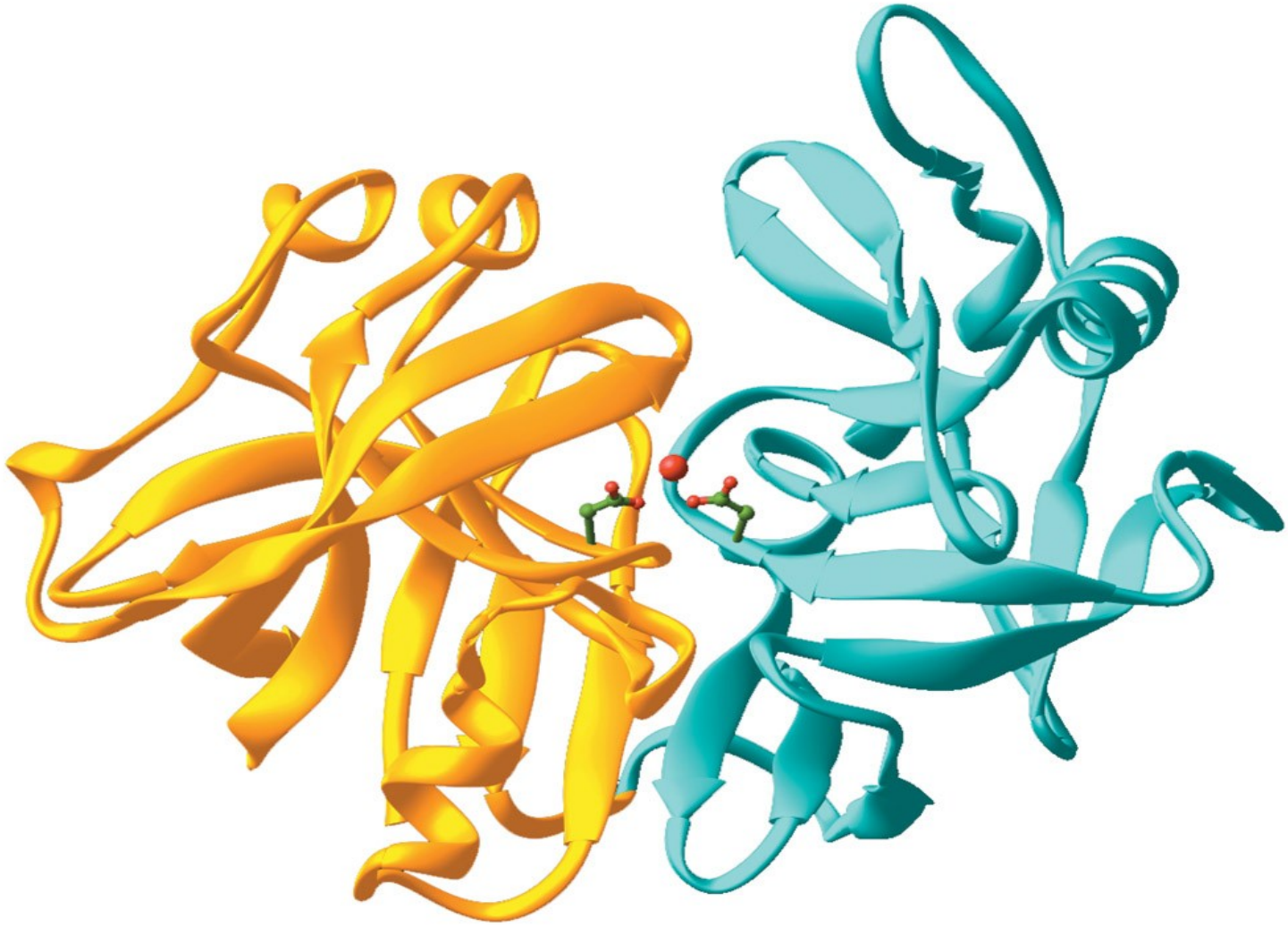
- The region of enzyme where a substrate binds is called active site
- Once the substrate is bound, catalysis takes place
- All enzymes have one or more active sites



Structure of trypsin enzyme



An enzyme with its active site



Structure of pepsin enzyme

Classification of Enzymes

- Enzymes are classified into six types according to the type of reaction catalyzed

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 Classification According to Reaction Type

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

  EC Class . Subclass . Subsubclass . Enzyme number

EC = Enzyme Commission

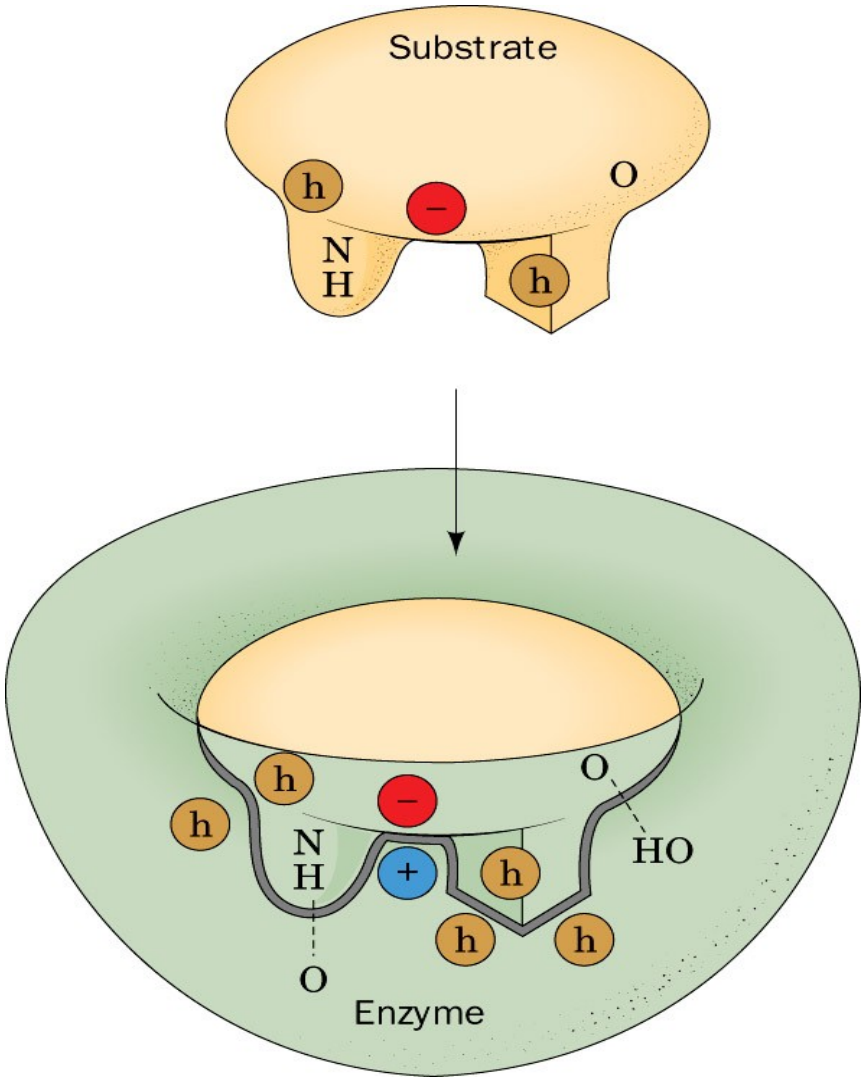
Enzyme specificity

- Enzymes are highly specific to their substrate
- They catalyze only one type of reaction

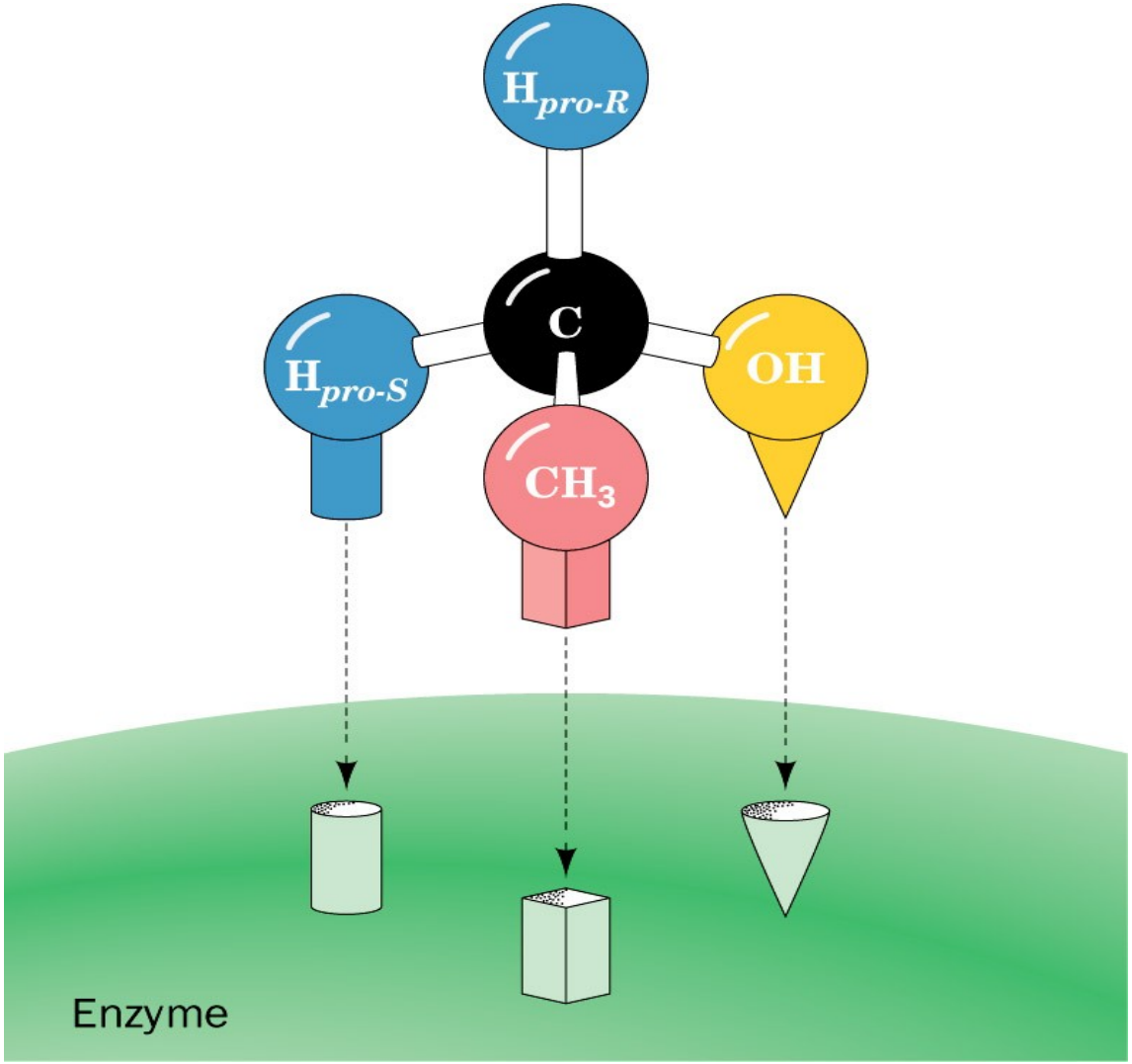
Enzyme –substrate binding

Two models have been proposed:

- Lock and key binding
  - The enzyme has an active site that fits the exact dimensions of the substrate

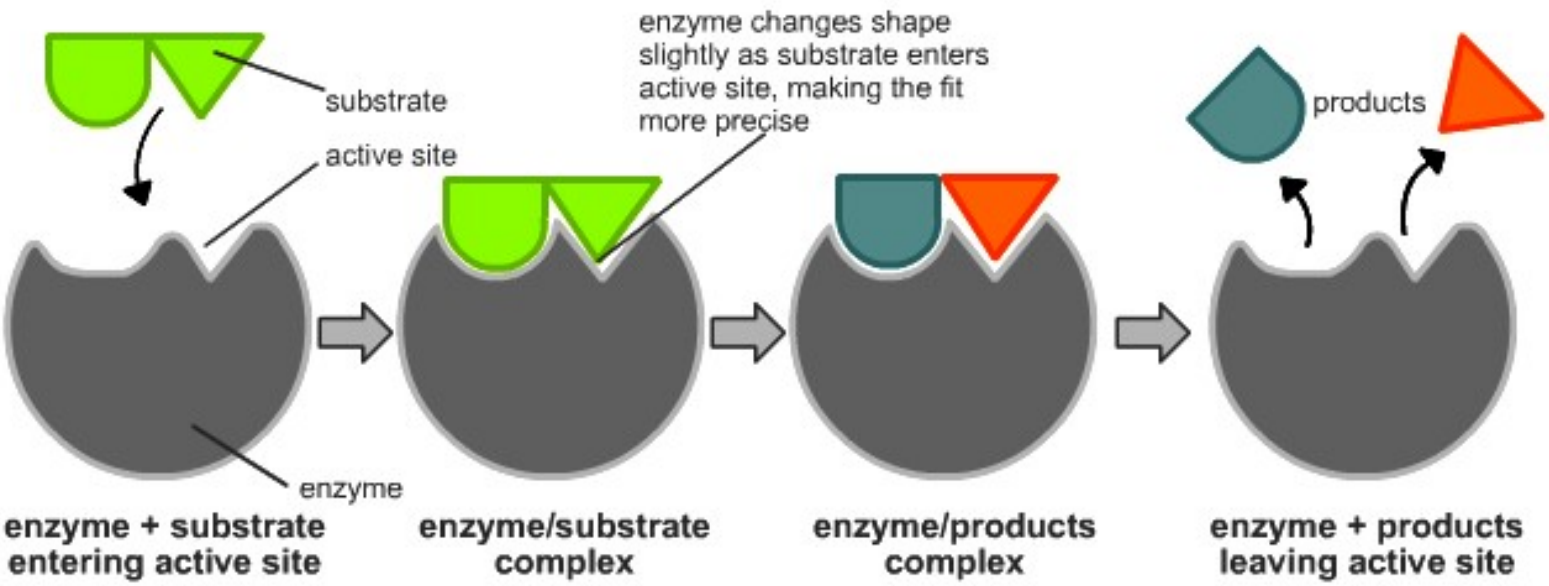


An enzyme–substrate complex illustrating both the geometric and the physical complementarity between enzymes and substrates



Enzyme –substrate binding

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



Enzyme Activity or Velocity

- Velocity is the rate of a reaction catalyzed by an enzyme
- Enzyme activity is expressed as:

μmoles of product formed/min/mg enz

Cofactors, Coenzymes, Isoenzymes

- Cofactors are small molecules or metal ions such as Cu^{2+}, Fe^{3+}, Zn^{2+}, etc. which help an enzyme to catalyze a reaction
- Cofactors may also be organic molecules known as coenzymes such as NAD^{+}
- Some cofactors are only temporarily associated with an enzyme known as cosubstrates

Cofactors, Coenzymes, Isoenzymes

- Some cofactors are permanently associated with an enzyme known as prosthetic groups
- An active enzyme-cofactor complex is called a holoenzyme
- The inactive form of an enzyme without its cofactor/coenzyme is called an apoenzyme
- Isoenzymes catalyze the same chemical reaction but they have slightly different structures

Apoenzyme (inactive) + Cofactor = Holoenzyme (active)

Apoenzyme (inactive) + Coenzyme = Holoenzyme (active)

Ribozymes

- Ribozymes are RNA (ribonucleic acid) with enzymatic activity

Zymogens

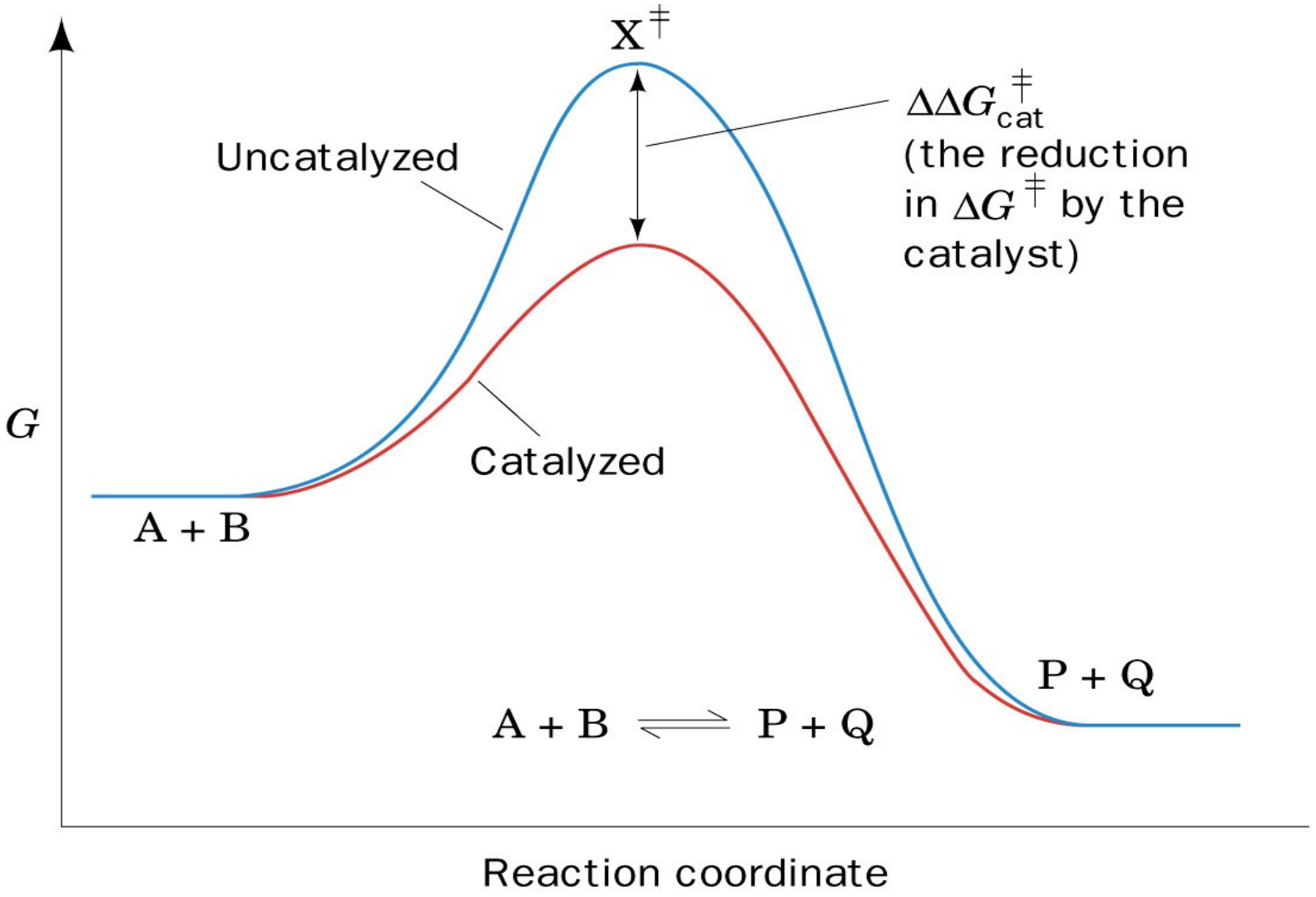
- Zymogens are inactive forms of enzyme
- They are activated when needed

How do enzymes work?

- 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

How do enzymes work?

- 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 cause a change in the free energy (ΔG) (available energy)



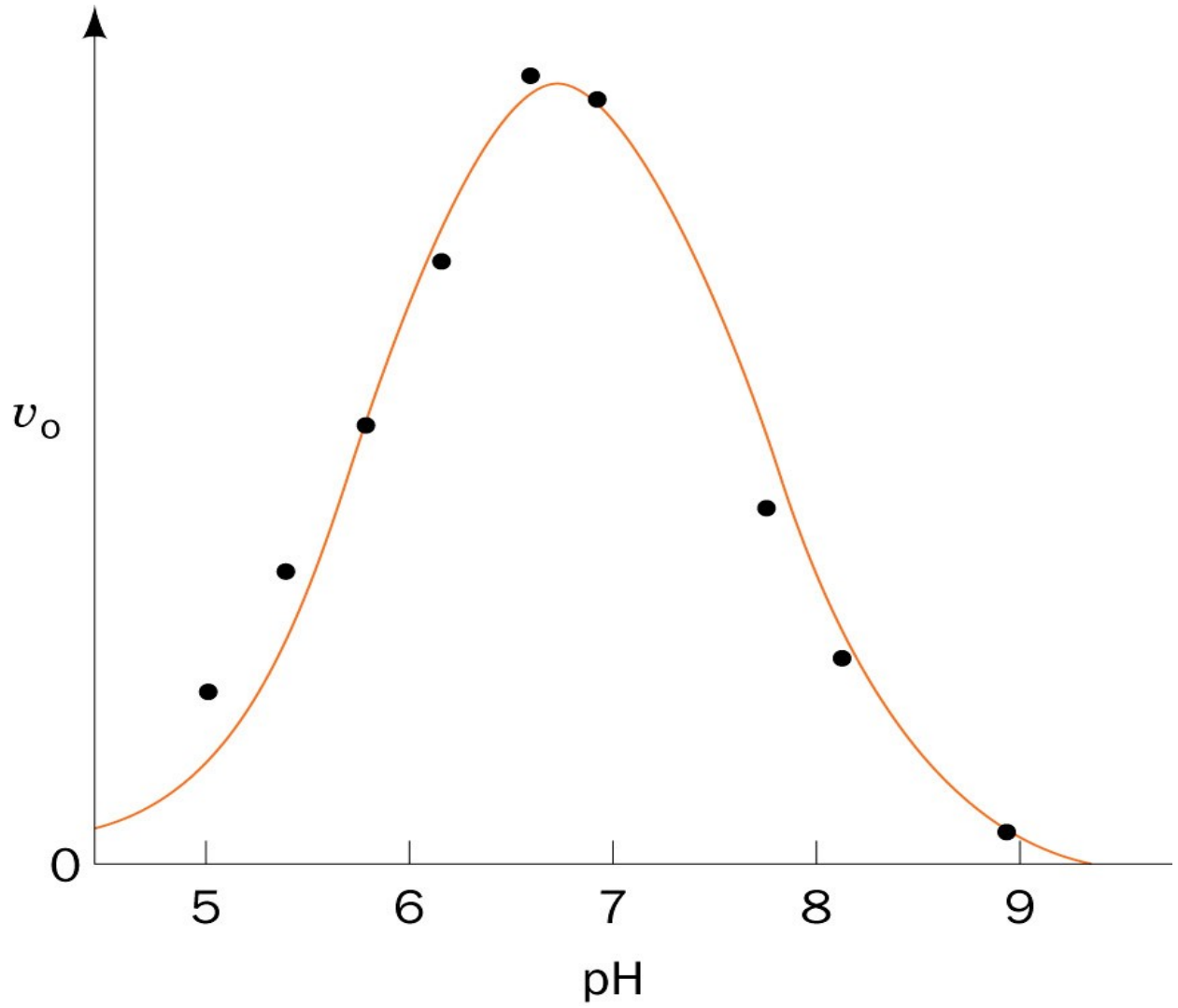
The effect of a catalyst on the transition state diagram of a reaction.

Factors affecting enzyme activity

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

Factors affecting enzyme activity

- Effect of pH
  - pH changes the ionizable groups in the active site – this affects 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



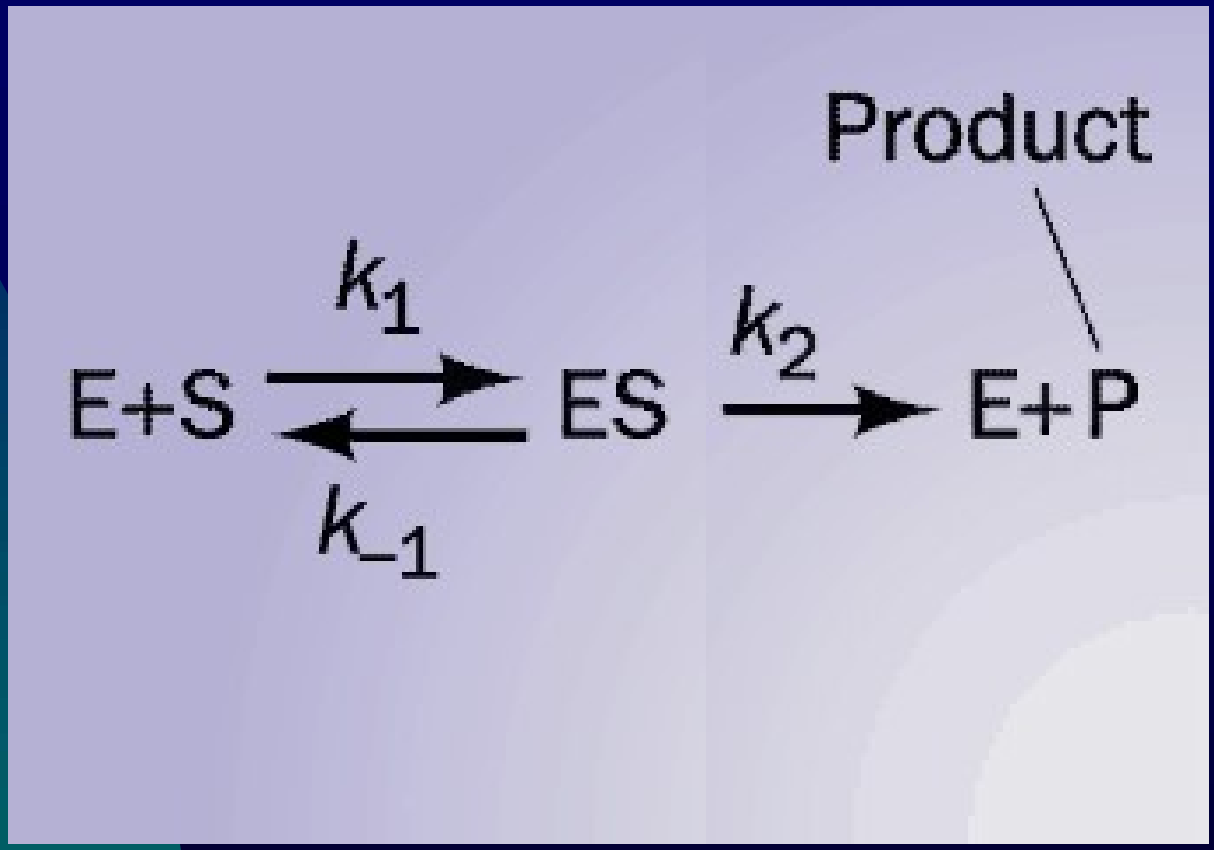
Effect of pH on the initial rate of the reaction catalyzed by most enzymes (the bell-shaped curve)

Factors affecting enzyme activity

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

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
k_1
k_2
E+S
ES
E+P
k_{-1}

Initial rate of enzyme reaction

Pre-steady state kinetics

- When an enzyme is mixed with high [S]
- There is an initial short period of time (a few hundred microseconds) in which intermediates of product are formed
- This is called pre-steady state reaction

- Steady state kinetics
  - After the initial state, the reaction rate and the conc. of intermediates change slowly with time
  - This is called steady state reaction
- Michaelis-Menten equation measures the initial velocity (v_o) of an enzyme reaction

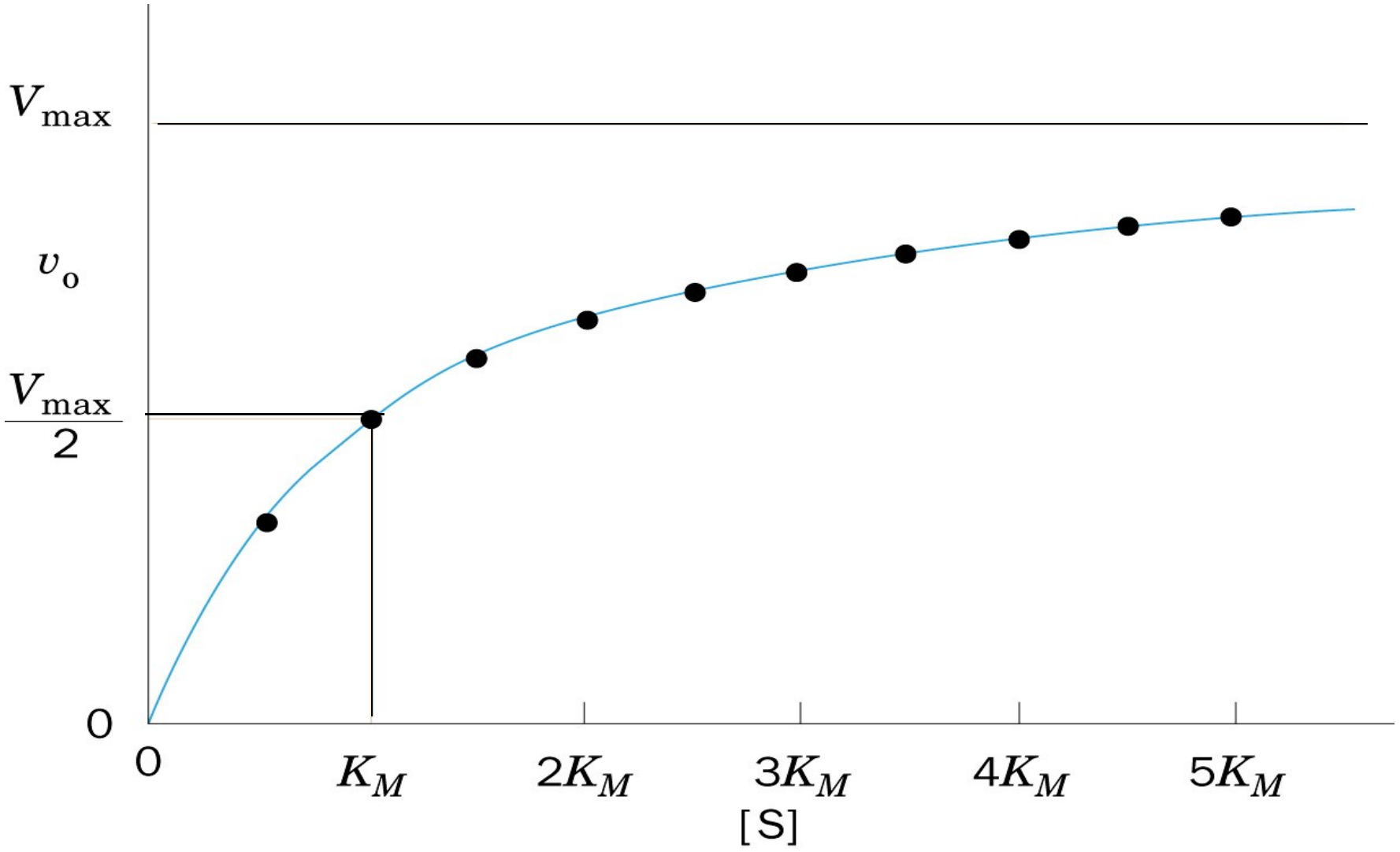
Michaelis Menten Equation

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

[S] = substrate concentration

V_{max} = maximum velocity

K_m = Michaelis constant



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

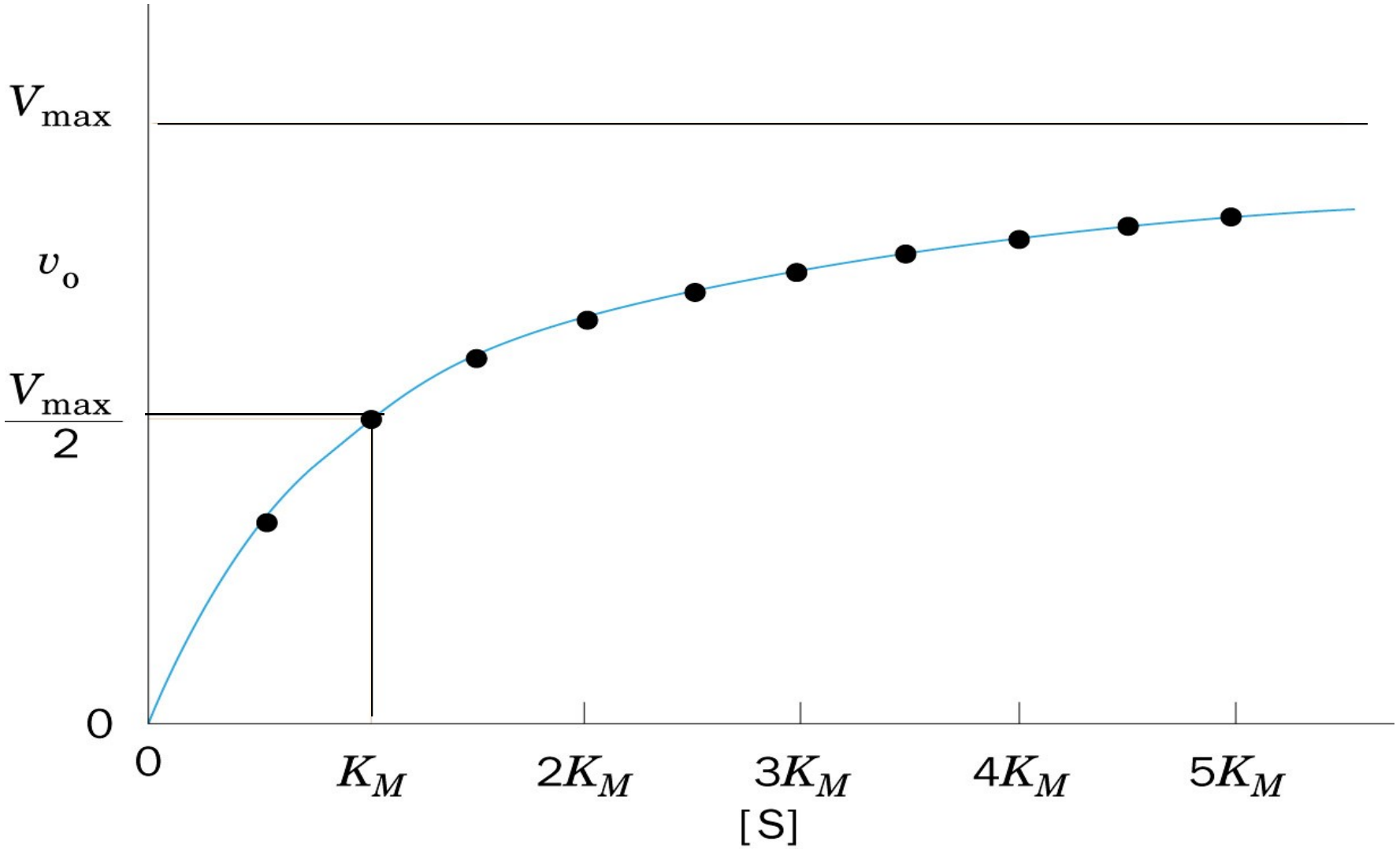
K_m (Michaelis Constant)

- K_m is the [S] at which the initial rate is one-half of the maximum rate (½ V_{max})
- It is the [S] required to saturate all of the active sites of an enzyme

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

Lineweaver-Burk plot

- The Lineweaver-Burk plot is a double-reciprocal plot, obtained by taking reciprocals of the Michaelis Menten equation



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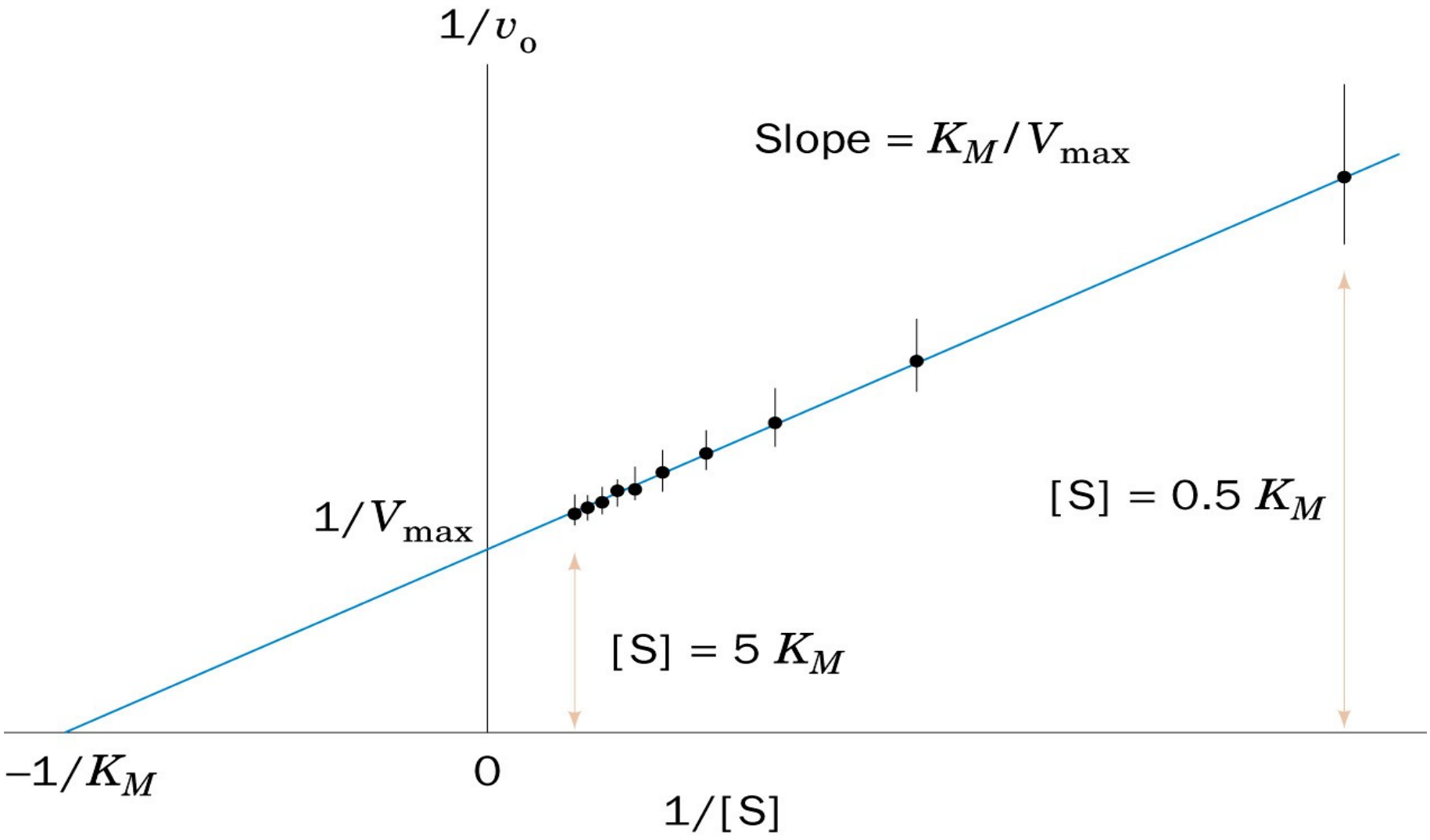

A double reciprocal Lineweaver–Burk plot