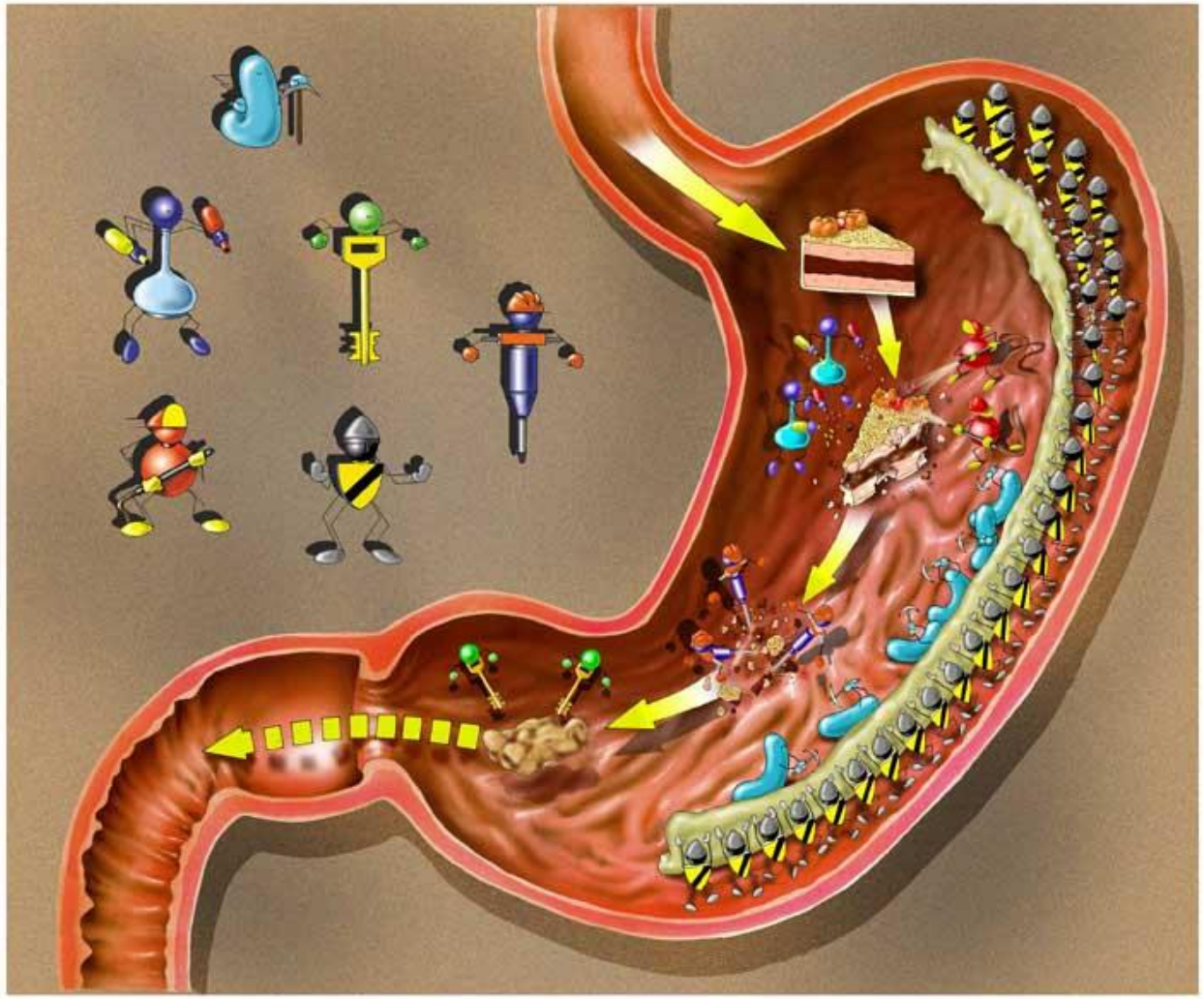
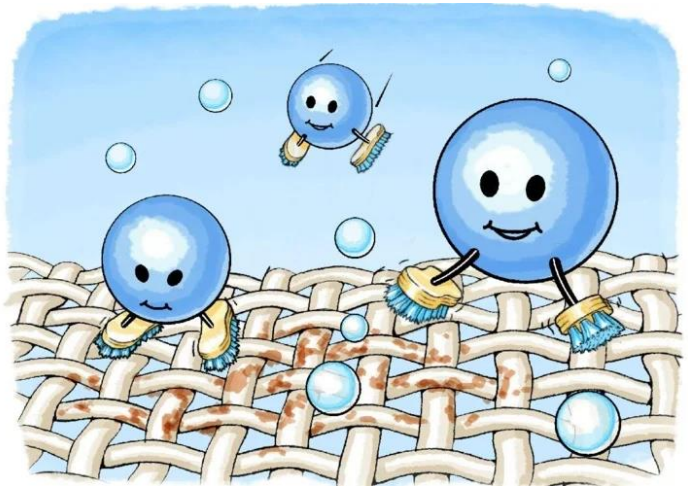
Enzymes



Clean the dishes/clothes.

Otto Rohm



1.What are enzymes?

2.How do enzymes work?

3.How are enzyme activities regulated?

Reactants & Products

What is energy? *Physicists vs Biochemists*

Two basic types of energy

The Laws of thermodynamics

- First, Energy is neither created nor destroyed
- Second, Disorder tends to increase (Not all energy can be used)
  - enthalpy (H) and free energy (G): $H = G + TS$
  - $\Delta G = G_{product} - G_{reatants}$

- ΔG tells us the equilibrium point of the reaction lies but tells us nothing about the rate of a reaction.
- No catalyst makes a reaction occur that cannot otherwise occur!

What are enzymes?

Enzymes are biological catalysts produced by **living cells**. Most enzymes are proteins, and a few are catalytic RNAs called ribozymes（核酶）.

How to verify that an enzyme is a protein?

What are enzymes?

Most Enzymes Are Proteins（大多数酶是蛋白质）

- In 1926, James B. Sumner first crystallized urease（脲酶） from jack bean seeds（刀豆种子），showing that urease has a protein nature.
- In the 1930s, John H. Northrop crystallized several digestive enzymes（消化酶），such as pepsin（胃蛋白酶），trypsin（胰蛋白酶），and chymotrypsin（胰凝乳蛋白酶），further supporting that enzymes are proteins.

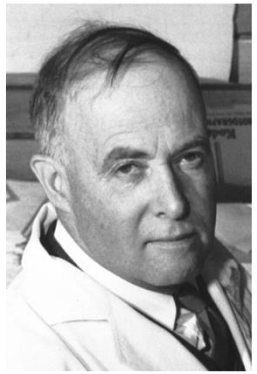


Photo from the Nobel Foundation archive.
James Batcheller Sumner
Prize share: 1/2

Photo from the Nobel Foundation archive.
John Howard Northrop
Prize share: 1/4

Photo from the Nobel Foundation archive.
Wendell Meredith Stanley
Prize share: 1/4

The Nobel Prize in Chemistry 1946 was divided, one half awarded to James Batcheller Sumner "for his discovery that enzymes can be crystallized", the other half jointly to John Howard Northrop and Wendell Meredith Stanley "for their preparation of enzymes and virus proteins in a pure form"

What are enzymes?

Some enzymes are RNA: ribozymes / 核酶

- In 1982, Thomas Cech（切赫） discovered that precursor rRNA from Tetrahymena（四膜虫） had self-splicing catalytic activity（自我剪接催化活性）.
- Catalytic RNA molecules are called ribozymes（核酶）: RNA with catalytic activity（具有催化活性的RNA）.

Photo: Yale University. From the Nobel Foundation archive

Sidney Altman

Prize share: 1/2

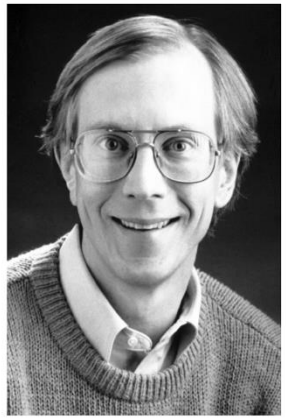


Photo from the Nobel Foundation archive.

Thomas R. Cech

Prize share: 1/2

The Nobel Prize in Chemistry 1989 was awarded jointly to Sidney Altman and Thomas R. Cech "for their discovery of catalytic properties of RNA"

What are enzymes?

Characteristics of Enzyme Catalysis

- High efficiency（高效性）
- Specificity（专一性）
- Regulability（可调控性）
- Easy denaturation（易变性 / 易失活）
- Cofactor requirement（常需辅助因子）
- Mild working conditions（作用条件温和）

Enzymes are efficient, specific, regulatable biological catalysts that usually work under mild conditions but depend strongly on proper structure and cofactors.

What are enzymes?

High catalytic efficiency（高效性）

Enzymes greatly accelerate biochemical reactions by lowering activation energy

Urease(脲酶) accelerates urea hydrolysis by about 10^{14}–10^{15} times compared with the uncatalyzed reaction.

What are enzymes?

Specificity（专一性）

Enzymes show high specificity for their substrates and reactions.

- **Absolute specificity（绝对专一性）: one enzyme acts on one substrate.**
- **Group specificity（基团专一性）: one enzyme acts on a group of related substrates.**
- **Bond specificity（键专一性）: one enzyme acts on a specific type of chemical bond.**
- **Stereospecificity（立体专一性）: one enzyme recognizes a specific stereoisomer.**

Urease（脲酶） acts specifically on urea（尿素）.

What are enzymes?

Regulation of enzyme activity（酶活性的可调控性）

Enzyme activity can be regulated according to cellular needs.

- **Allosteric regulation（变构调节）**
- **Covalent modification（共价修饰）, such as phosphorylation（磷酸化）**
- **Feedback inhibition（反馈抑制）**
- **Changes in enzyme synthesis or degradation（酶合成或降解的改变）**
- **Substrate or product concentration（底物或产物浓度）**

This allows metabolism to respond to changes in energy status（能量状态）and nutrient availability（营养供应）.

What are enzymes?

Easy inactivation / denaturation（易失活 / 易变性）

Most enzymes are proteins, so their activity depends on a specific three-dimensional structure.

They can lose activity under conditions such as:

- **High temperature（高温）**
- **Extreme pH（极端pH）**
- **Heavy metal ions（重金属离子）**
- **Organic solvents（有机溶剂）**
- **Strong acids or bases（强酸或强碱）**

What are enzymes?

Requirement for cofactors（常需要辅助因子）

Many enzymes require non-protein components（非蛋白成分） for activity. These are called **Cofactors**（辅助因子）

Types include:

- **Metal ions（金属离子）, such as Mg^{2+}, Zn^{2+}, Fe^{2+}/Fe^{3+}**
- **Coenzymes（辅酶）, often derived from vitamins（维生素）**
- **Prosthetic groups（辅基）, tightly bound cofactors**

Definitions:

- **Apoenzyme（脱辅酶）: protein part alone, inactive**
- **Holoenzyme（全酶）: apoenzyme + cofactor, active**

What are enzymes?

Mild reaction conditions（作用条件温和）

Enzymes usually function under mild physiological conditions:

- ❖Normal temperature（常温或体温）
- ❖Near-neutral pH（接近中性的pH）
- ❖Aqueous environment（水环境）
- ❖Normal pressure（常压）

This is different from many industrial chemical catalysts, which may require high temperature, high pressure, or strong acid/base conditions.

What are enzymes?

Naming of Enzymes

A. Common names（习惯命名）

1. Substrate name（底物名称）

- **Urease（脲酶）** — acts on urea（尿素）
- **Amylase（淀粉酶）** — acts on starch（淀粉）
- **Protease（蛋白酶）** — acts on proteins（蛋白质）

2. Reaction type（反应性质）

- **Dehydrogenase（脱氢酶）**
- **Oxidase（氧化酶）**
- **Transaminase / Aminotransferase（转氨酶 / 氨基转移酶）**
- **Decarboxylase（脱羧酶）**
- **Kinase（激酶）**

3. Source of the enzyme（酶的来源）

- **Pepsin（胃蛋白酶）**
- **Papain（木瓜蛋白酶）**

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

The Enzyme Commission, EC（酶学委员会） classifies enzymes according to the type of reaction they catalyze. Enzymes are divided into seven major classes.

1. Oxidoreductases（氧化还原酶类）：Catalyze oxidation–reduction reactions

$$AH_2 + B \Longleftrightarrow A + BH_2$$

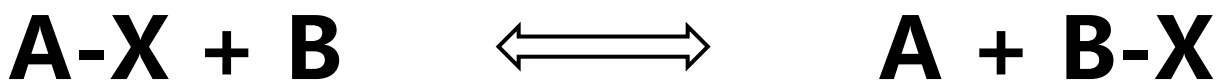
$$AH_2 + O_2 \Longleftrightarrow A + H_2O_2$$

Examples: Succinate dehydrogenase（琥珀酸脱氢酶）；Lactate dehydrogenase（乳酸脱氢酶）；Cytochrome oxidase（细胞色素氧化酶）

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

2. Transferases（转移酶类）：Catalyze transfer of functional groups（基团转移）



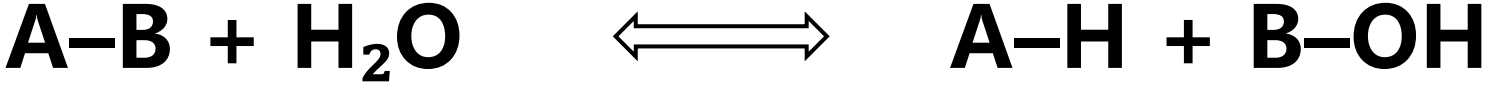
Examples:

- **Aminotransferase / transaminase（氨基转移酶 / 转氨酶）**
- **Kinase（激酶）**
- **Methyltransferase（甲基转移酶）**

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

3. Hydrolases（水解酶类）：Catalyze bond cleavage using water（水解反应）



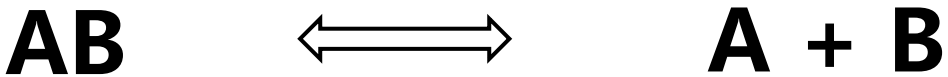
Examples:

- **Protease（蛋白酶）**
- **Lipase（脂肪酶）**
- **Amylase（淀粉酶）**
- **Urease（脲酶）**

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

4. Lyases（裂合酶类）：Catalyze non-hydrolytic, non-oxidative bond cleavage or addition（非水解、非氧化方式的裂解或加成）.



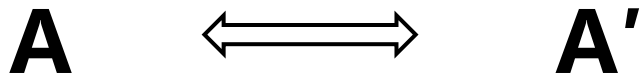
Examples:

- **Decarboxylase（脱羧酶）**
- **Aldolase（醛缩酶）**
- **Fumarase（延胡索酸酶）**

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

5. Isomerases（异构酶类）：Catalyze intramolecular rearrangements（分子内重排）.



Examples:

- **Phosphoglucose isomerase（磷酸葡萄糖异构酶）**
- **Triose phosphate isomerase（磷酸丙糖异构酶）**
- **Racemase（消旋酶）**

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

6. Ligases（连接酶类）：Catalyze joining of two molecules, usually coupled to ATP or another NTP（通常偶联ATP或其他NTP水解）.

$$A + B + ATP \Longleftrightarrow A\text{–}B + ADP + Pi$$

$$A + B + ATP \Longleftrightarrow A\text{–}B + AMP + PPi$$

Examples:

- **DNA ligase（DNA连接酶）**
- **Aminoacyl-tRNA synthetase（氨酰-tRNA合成酶）**
- **Glutamine synthetase（谷氨酰胺合成酶）**

What are enzymes?

B. Systematic nomenclature（国际系统命名法）

7. Translocases（转位酶类 / 易位酶类）：Catalyze movement of ions or molecules across membranes or their separation within membranes（催化离子或分子跨膜转运或在膜内分离）. The IUBMB EC 7 subclasses include translocation of hydrons, inorganic cations, inorganic anions, amino acids/peptides, carbohydrates, and other compounds..

A [side 1] ⟺ **A [side2]**

Examples:

- **Na^+/K^+-ATPase（钠钾ATP酶）**
- **Ca^{2+}-ATPase（钙泵）**
- **ABC transporters（ABC转运蛋白）**
- **H^+-transporting ATP synthase / ATPase（H^+转运ATP合酶 / ATP酶）**

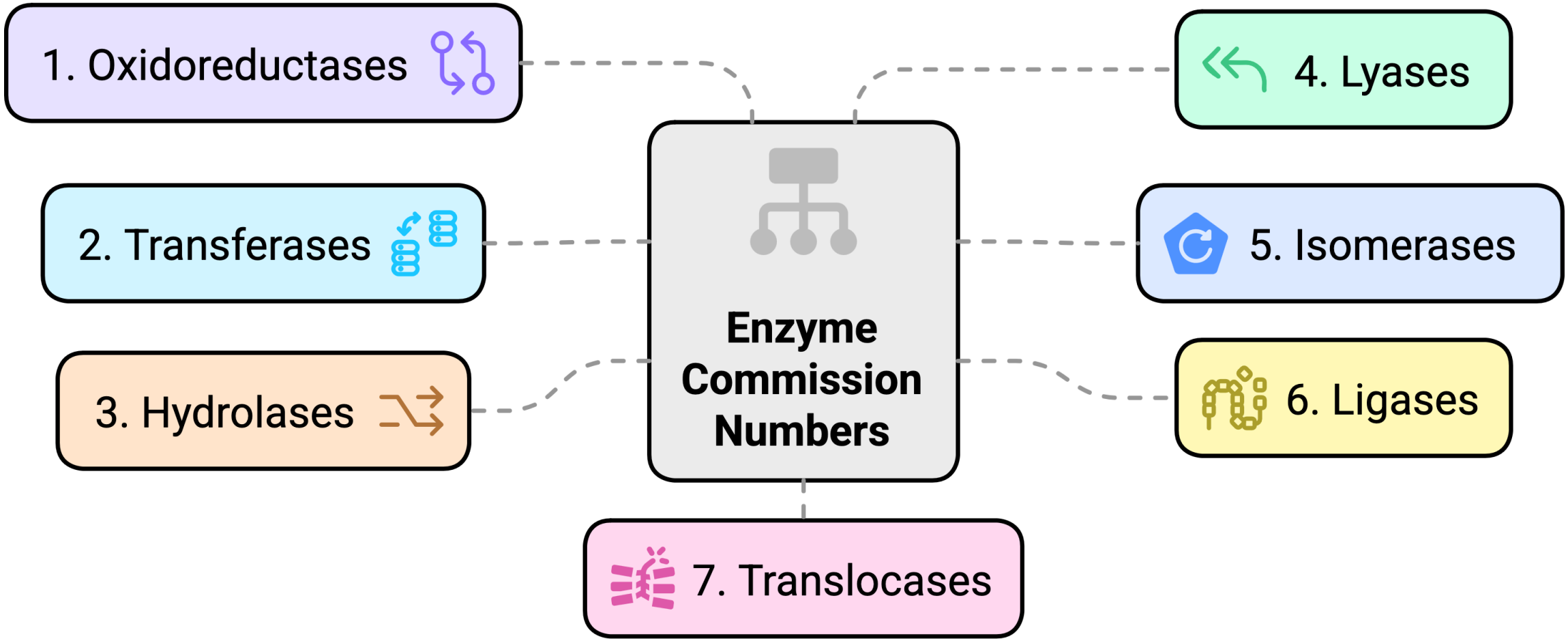
What are enzymes?

EC Numbering System (https://iubmb.qmul.ac.uk/enzyme/)

- **First digit: Main class,** Indicates the broad type of reaction catalyzed.
- **Second digit: Subclass,** Specifies the type of bond acted on or functional group transferred.
- **Third digit: Sub-subclass,** Gives more detailed reaction features, such as the coenzyme or acceptor involved.
- **Fourth digit: Serial number,** Identifies the specific enzyme within that sub-subclass.

What are enzymes?

EC Numbering System (https://iubmb.qmul.ac.uk/enzyme/)

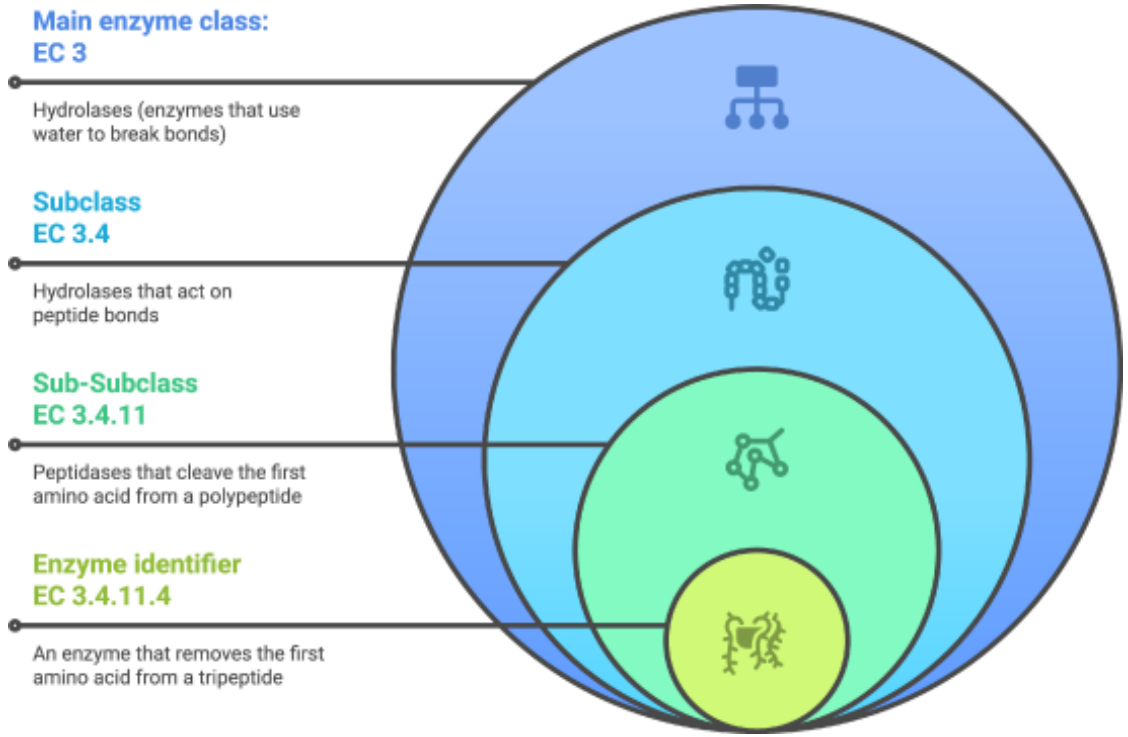


What are enzymes?

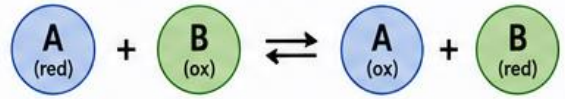
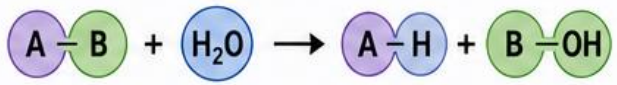
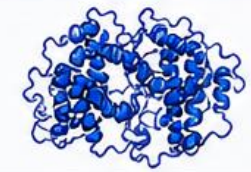
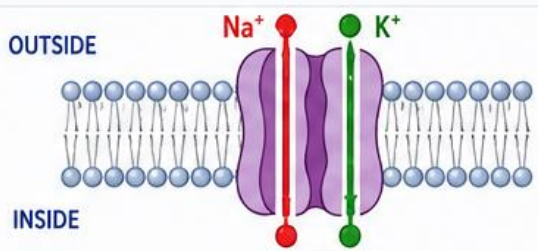
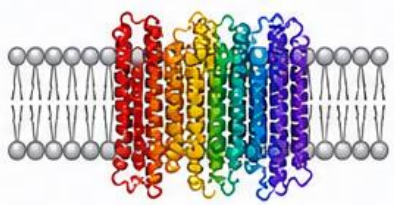
EC Numbering System (https://iubmb.qmul.ac.uk/enzyme/)

Example: *Alcohol dehydrogenase* has the designation **EC 1.1.1.1**

- **EC 1:** It is an Oxidoreductase
- **EC 1.1:** It acts on the CH-OH group of electron donors.
- **EC 1.1.1:** It specifically uses NAD+ or NADP+ as the electron acceptor.
- **EC 1.1.1.1:** It is the first specific enzyme cataloged in this exact group.



7 TYPES OF ENZYMES

	REACTION TYPE (general form)	EXAMPLE ENZYME (EC) / EXAMPLE REACTION	COMMON NAME	STRUCTURE (example)
1 Oxidoreductases Catalyze oxidation–reduction reactions.		**Lactate dehydrogenase (EC 1.1.1.27)** L-lactate + NAD^+ ⇌ Pyruvate + NADH + H^+	Dehydrogenases	
2 Transferases Transfer functional groups between molecules.		**Hexokinase (EC 2.7.1.1)** Glucose + ATP → Glucose-6-phosphate + ADP	Kinases	
3 Hydrolases Catalyze hydrolysis reactions.		**Trypsin (EC 3.4.21.4)** Peptide bond + H_2O → Peptides	Proteases	
4 Lyases Add or remove groups to form double bonds, no hydrolysis or oxidation.		**Fumarase (EC 4.2.1.2)** Fumarate ⇌ Malate	Synthases	
5 Isomerases Catalyze isomerization within a molecule.		**Triose phosphate isomerase (EC 5.3.1.1)** Dihydroxyacetone phosphate ⇌ Glyceraldehyde 3-phosphate	Isomerases	
6 Ligases Join two molecules using the energy of ATP.		**DNA ligase (EC 6.5.1.1)** DNA + ATP → DNA (phosphodiester bond) + AMP + PP_i	Synthetases	
7 Translocases Move ions or molecules across membranes or separate compartments.		**Na^+/K^+-ATPase (EC 7.2.2.13)** ATP-driven transport: 3 Na^+ out of the cell 2 K^+ into the cell	Transporters	

What are enzymes?

Composition of Enzymes（酶的组成）

1. Molecular Organization of Enzymes（酶的分子组织形式）

- **Monomeric enzymes（单体酶）** contain only one polypeptide chain. *i.e.* ***Lysozyme***（溶菌酶）& Some ***proteases***（某些蛋白酶）
- **Oligomeric enzymes（寡聚酶）** consist of two or more subunits.
- **Multienzyme complexes（多酶复合体）** are organized assemblies of several different enzymes that catalyze consecutive reactions（连续反应）in a metabolic pathway. *i.e.* ***Pyruvate dehydrogenase complex***, PDC（丙酮酸脱氢酶复合体）& ***Fatty acid synthase complex***（脂肪酸合成酶复合体）

What are enzymes?

Composition of Enzymes（酶的组成）

2. Chemical Composition of Enzymes（酶的化学组成）

- **Simple enzymes（单纯酶）** are composed only of protein.
- **Conjugated enzymes（结合酶）** contain a protein part and a non-protein component（非蛋白成分）.

  - ✓ The non-protein component is called a: **Cofactor（辅助因子）**
  - ✓ The complete active enzyme is called a: **Holoenzyme（全酶）**
  - ✓ The protein part alone is called an: **Apoenzyme（酶蛋白 / 脱辅酶）**

What are enzymes?

Composition of Enzymes（酶的组成）

3. Apoenzyme, Cofactor, and Holoenzyme（酶蛋白、辅助因子与全酶）

Holoenzyme（全酶） = Apoenzyme（酶蛋白） + Cofactor（辅助因子）

- **Apoenzyme determines substrate specificity and catalytic efficiency.**
- **Cofactor transfers electrons, atoms, or functional groups during the reaction.**

What are enzymes?

Composition of Enzymes（酶的组成）

4. Types of Cofactors

- **Metal ions（金属离子）：** Mg^{2+}, Zn^{2+}, Fe^{2+}/Fe^{3+}, Cu^{2+}, Ca^{2+}
- **Coenzymes（辅酶）：** NAD^{+}, $NADP^{+}$, CoA, ATP
- **Prosthetic groups（辅基）：** FAD, FMN, heme（血红素）, biotin（生物素）, PLP（磷酸吡哆醛）

✓ **Coenzymes are small organic molecules that bind loosely to enzymes.**

✓ **Prosthetic groups are tightly bound cofactors.**

1.What are enzymes?

2.How do enzymes work?

3.How are enzyme activities regulated?

How do enzymes work?

Essential groups

Essential groups（必需基团） are chemical groups required for enzyme activity. If they are chemically modified or removed, enzyme activity is lost or greatly reduced.

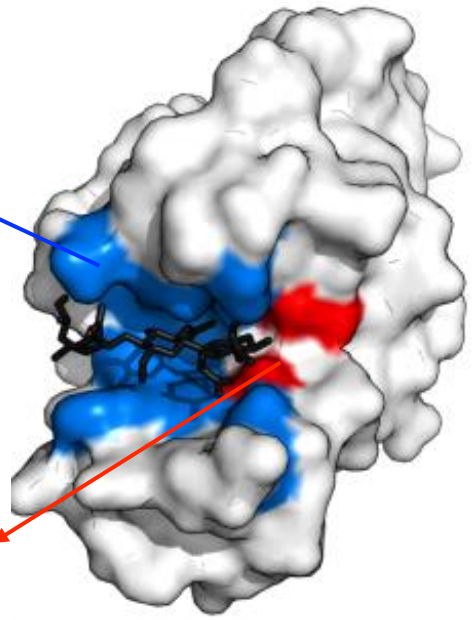
How do enzymes work?

Active Site of Enzymes

The active site（活性中心） is the region of an enzyme where the substrate binds and the reaction is catalyzed.

Active site

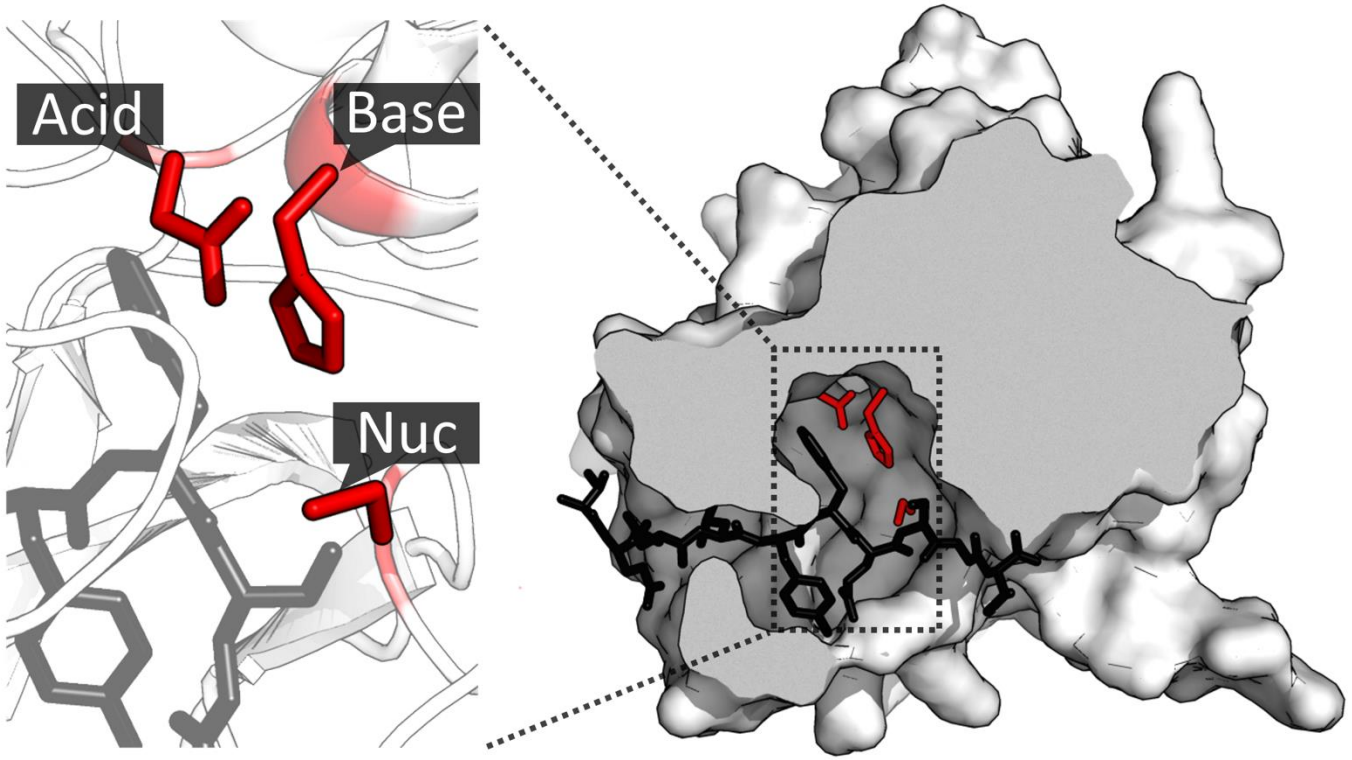
- **Binding site**
  - Recognizes and binds the substrate
  - Determines substrate specificity
- **Catalytic site**
  - Break old bonds and form new bonds
  - Determines catalytic efficiency



How do enzymes work?

Formation of the active site

The active site is formed when the polypeptide chain folds into a specific three-dimensional structure, bringing key amino acid residues together. 酶蛋白多肽链折叠形成特定三维结构，使相关氨基酸残基聚集形成活性中心。



How do enzymes work?

Formation of the active site

Cleft or pocket
裂隙或口袋

- Most active sites are located in a **cleft or pocket** on the enzyme surface.

Specific microenvironment
特定微环境

Helps to:

- Bind substrates（结合底物）
- Exclude water when needed（排除水分子）
- Stabilize transition states（稳定过渡态）

Flexibility
柔性

- Substrate binding often induces a small conformational change that improves catalysis.
- This is called i**nduced fit（诱导契合）**

Weak interactions with substrate
与底物通过弱相互作用结合

- Hydrogen bonds（氢键）
- Ionic interactions（离子相互作用）
- Hydrophobic interactions（疏水相互作用）
- van der Waals forces（范德华力）

How do enzymes work?

Zymogen and Zymogen Activation（酶原与酶原激活）

Zymogen（酶原），also called proenzyme（酶前体），is an inactive enzyme precursor that requires activation before it becomes catalytically active.

Zymogen activation （酶原激活） is the conversion of a zymogen into an active enzyme. It usually occurs through limited proteolysis（有限蛋白水解），which removes specific peptide segments and forms or exposes the active site.

How do enzymes work?

Activation energy（活化能）

Activation energy is the energy required for reactants to reach the transition state（过渡态）. Molecules that reach this energy level are called activated molecules（活化分子）.

Ways to increase activated molecules:

- **Add energy directly（直接提供能量）**

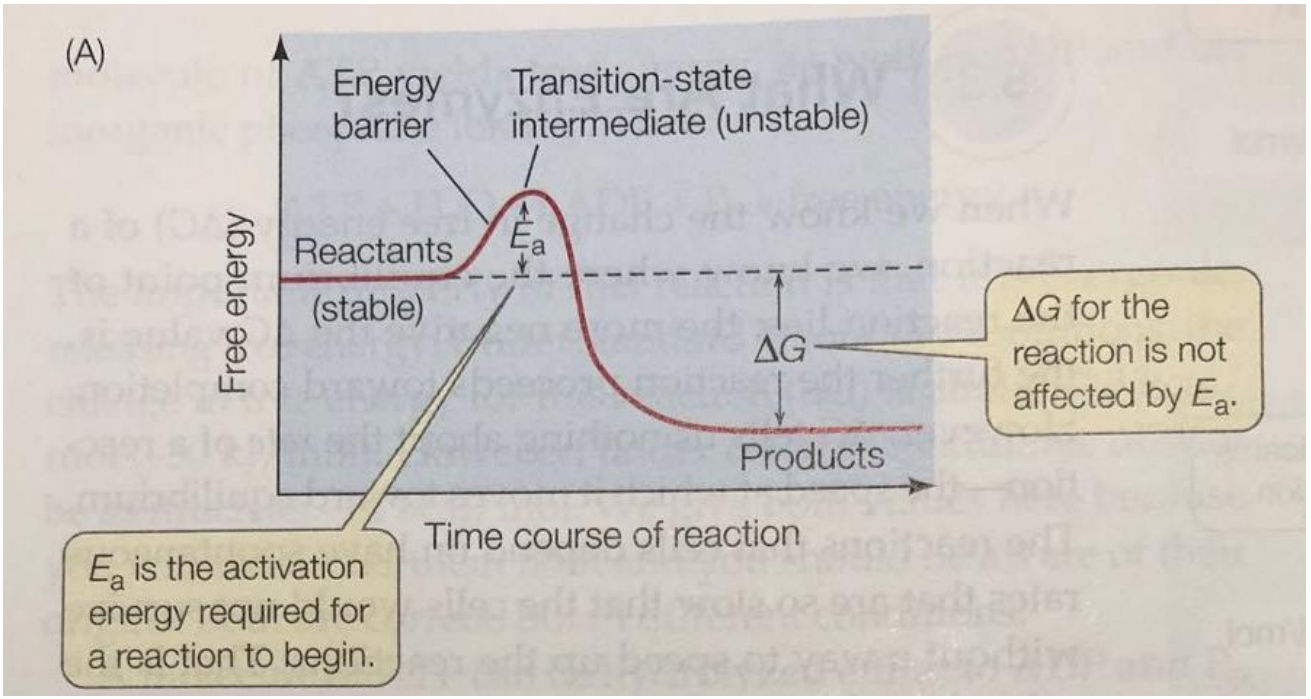
  Example: heating or irradiation.

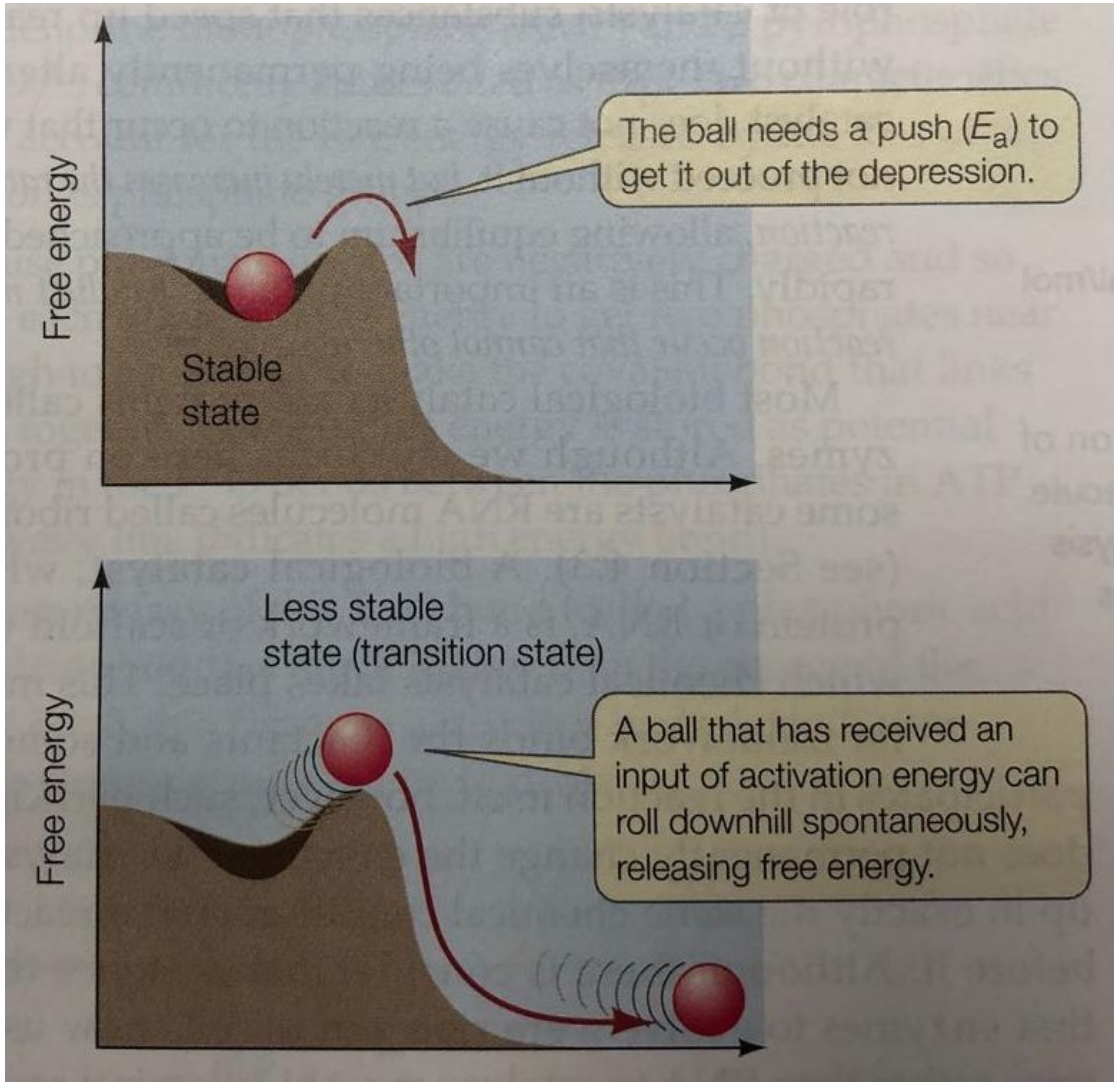
- **Lower the activation energy（降低活化能）**

  Enzymes lower the energy barrier and increase reaction rate.

How do enzymes work?

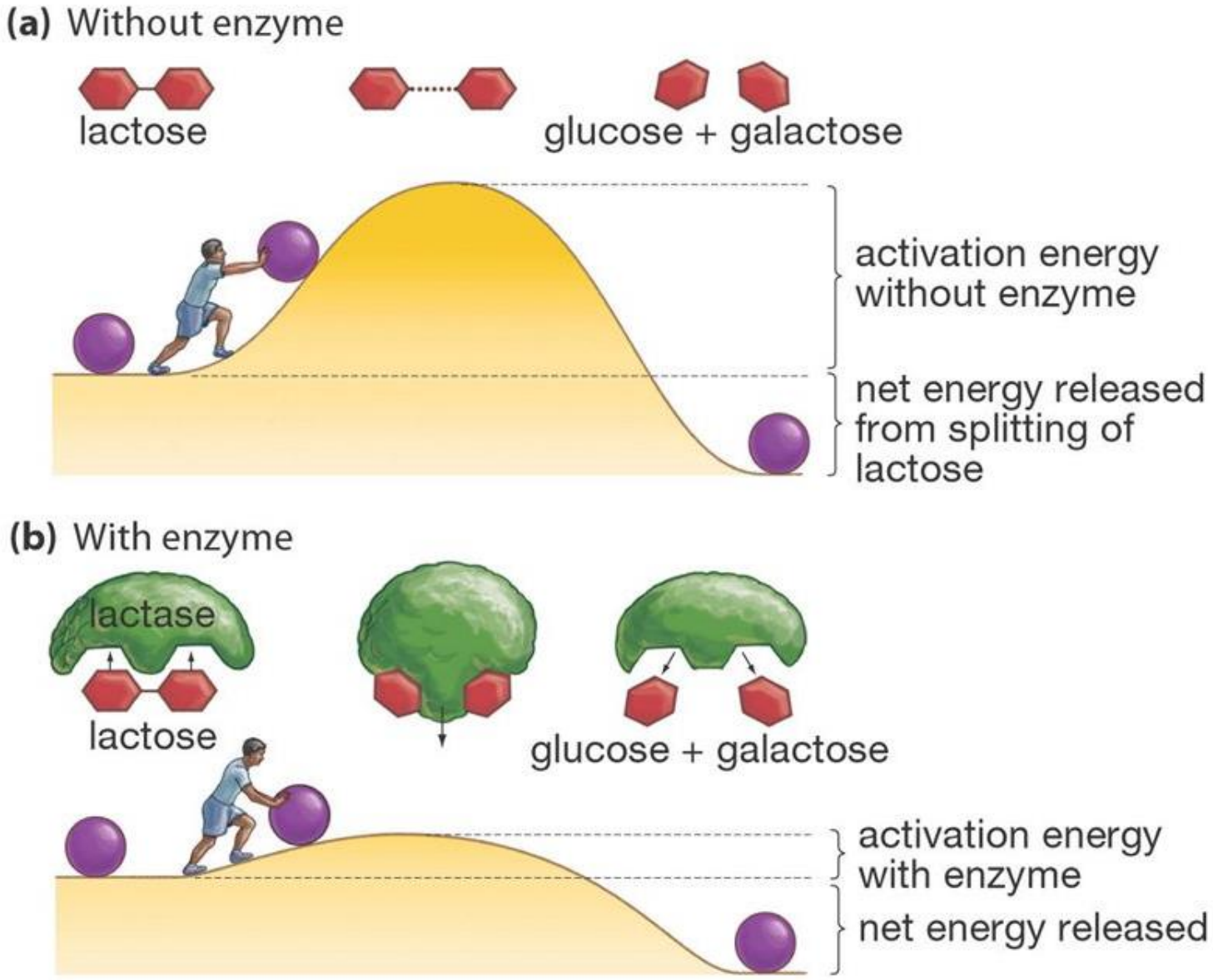
Activation energy（活化能）





How do enzymes work?

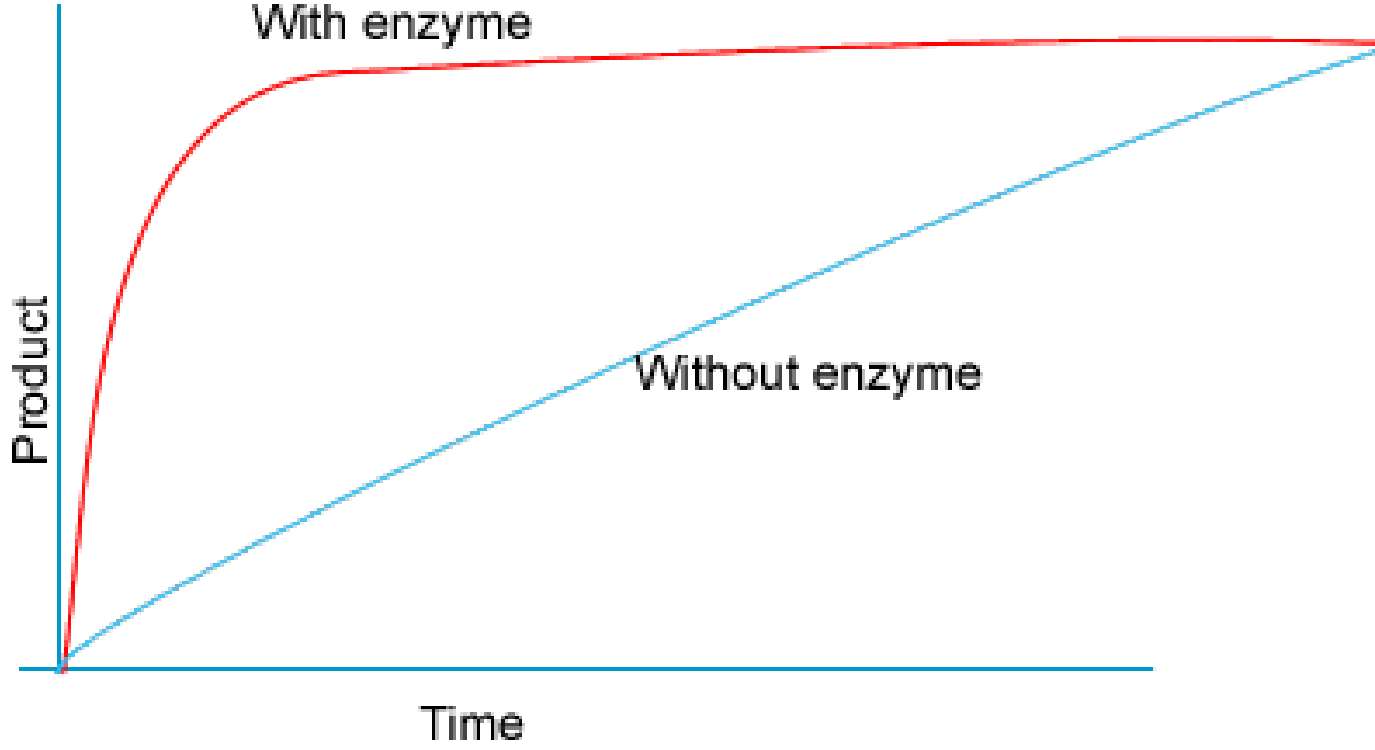
Enzymes Lower Activation Energy（酶降低活化能）



How do enzymes work?

Enzymes Change Rate, Not Equilibrium（酶改变速率，不改变平衡）

- Increase reaction rate
- Lower activation energy
- Do not change ΔG
- Do not change equilibrium constant
- Do not change reaction direction



Enzymes accelerate reactions by lowering activation energy, not by changing reaction direction or equilibrium.

How do enzymes work?

Intermediate Product Theory（中间产物学说）

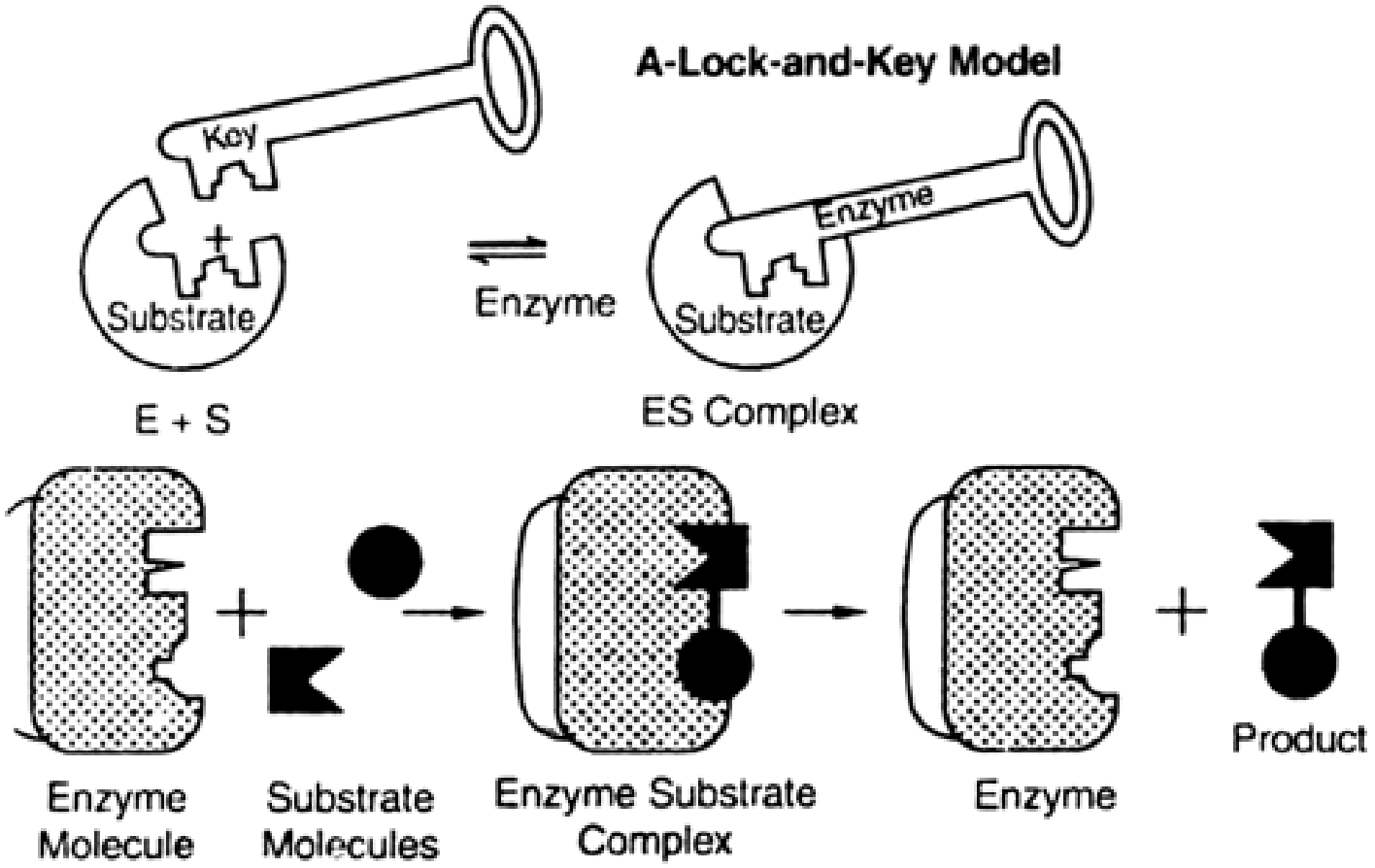
Non-enzymatic reaction（非酶反应）

Enzymatic reaction（酶促反应）

The enzyme first binds substrate to form an ES complex. This stabilizes the transition state（稳定过渡态） and lowers the activation energy. The product is then formed and released, while the enzyme is regenerated.

How do enzymes work?

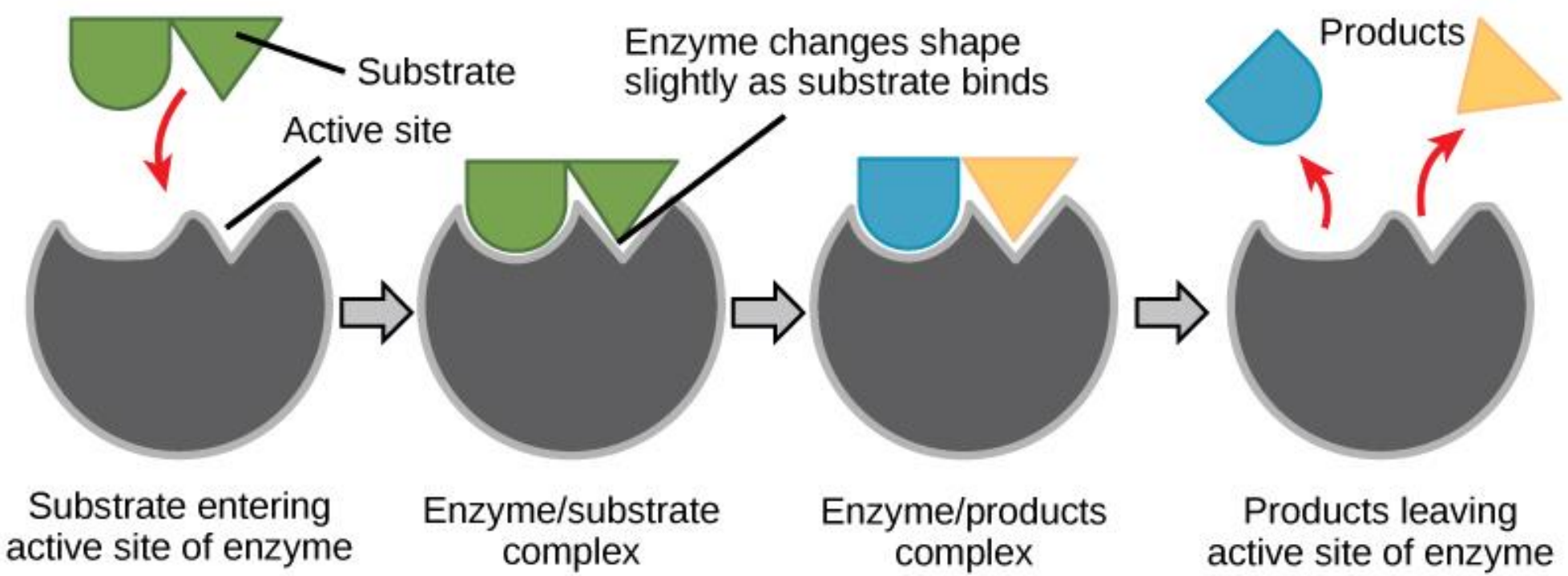
Lock-and-Key Model（锁钥学说）



The lock and key model is a classical biochemical theory proposed by Emil Fischer in 1894 to explain enzyme specificity.

How do enzymes work?

Induced-Fit Model（诱导契合学说）



Substrate binding induces a conformational change（构象变化） in the enzyme active site, making the enzyme and substrate fit more closely.

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

Enzyme reaction rate, *v*（酶促反应速度）, is expressed as:

$$v = \Delta[P] / \Delta t$$

or

$$v = -\Delta[S] / \Delta t$$

In practice, enzyme activity is measured by the initial velocity, v_0（初速度）, from the initial linear phase of the reaction curve.

How do enzymes work?

Enzyme Activity

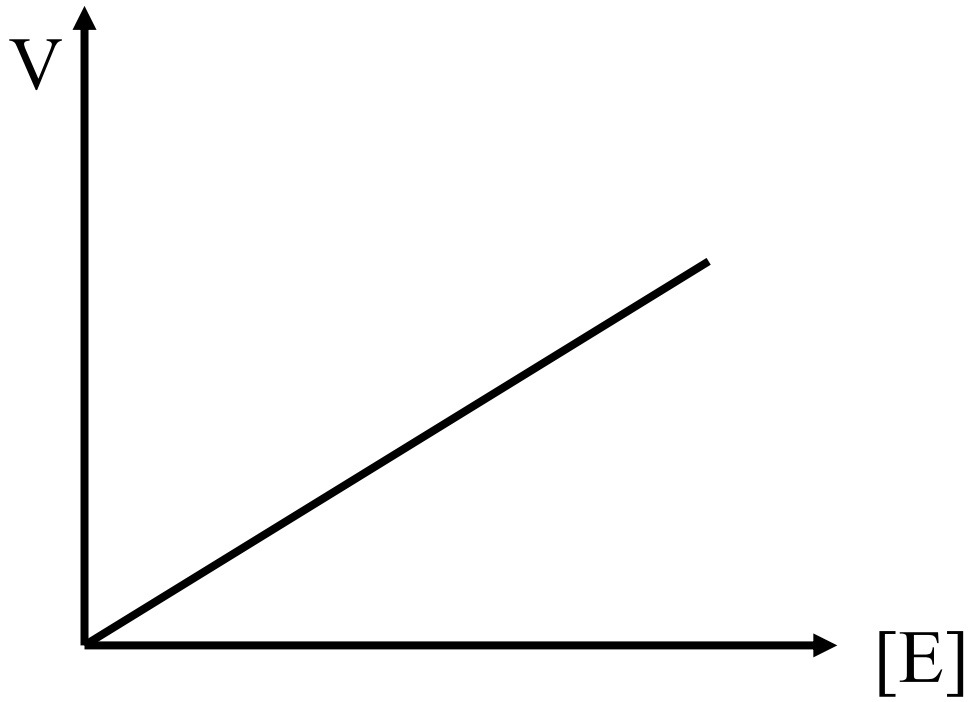
Enzyme activity（酶活力, V） refers to the catalytic capacity of an enzyme. It is usually expressed as the rate of an enzyme-catalyzed reaction（酶促反应速度） under specified conditions.

- **International Unit, IU（国际单位）, 1 IU is defined as the amount of enzyme that converts 1 μmol of substrate per minute under specified conditions.**
- **Turnover number（转换数, k_{cat}） is the number of substrate molecules converted into product by each enzyme molecule per unit time when the enzyme is saturated with substrate.**

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

[S] >> [E]

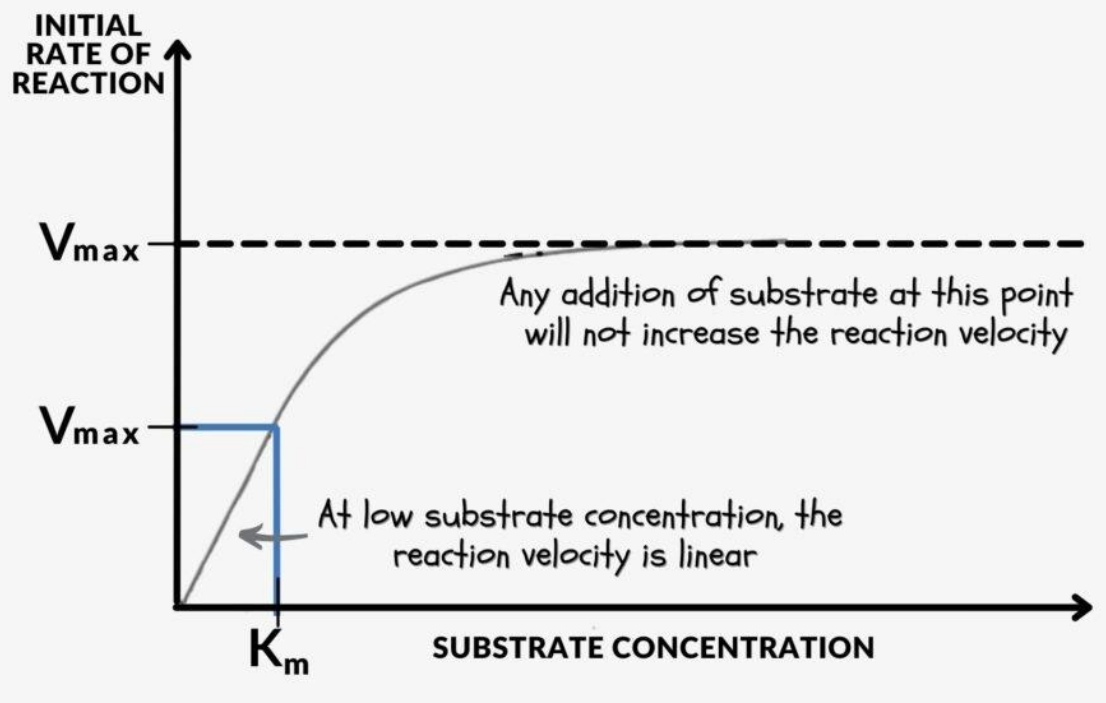


When substrate is in excess, the reaction rate is proportional to enzyme concentration

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

When **pH, temperature, and enzyme concentration** are constant, the reaction rate **v increases with substrate concentration [S]**.



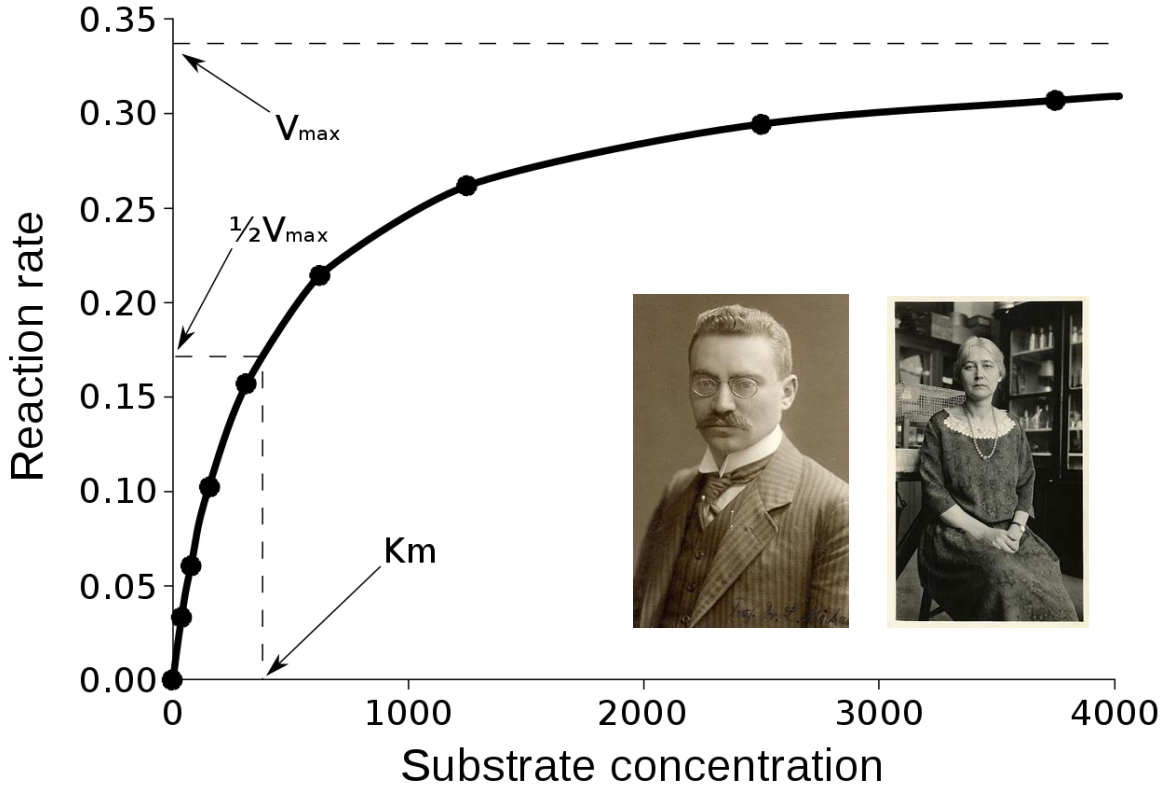
- At low substrate concentration: **v increases almost linearly with [S].**
- At high substrate concentration: **the enzyme becomes saturated, and v approaches V_{max}.**

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

Michaelis-Menten equation

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



- v_0（初速度）: initial reaction velocity
- [S]（底物浓度）: substrate concentration
- V_{max}（最大反应速度）: maximum velocity when enzyme is saturated with substrate
- K_m（米氏常数）: substrate concentration at which v_0 = 1/2 Vmax

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

Michaelis-Menten equation

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

- **Initial velocity is measured（测定初速度）**

  Product concentration is very low, so the reverse reaction can be ignored.

- **Substrate is in excess（底物过量）**

  [S] ≫ **[E]**, so substrate consumption during measurement is negligible.

- **ES reaches steady state（ES达到稳态）**

  The rate of ES formation equals the rate of ES breakdown.

- **pH, temperature, and enzyme concentration are constant（pH、温度、酶浓度恒定）**

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

Limitation of Michaelis–Menten equation

The Michaelis–Menten equation describes the quantitative relationship between initial velocity and substrate concentration for simple single-substrate enzyme reactions.

Many enzyme reactions do not follow simple Michaelis–Menten kinetics, such as allosteric enzymes (变构酶) and many multi-substrate enzymes.

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

Meaning of K_m

K_m is the substrate concentration at which the reaction velocity equals half of V_{max}.

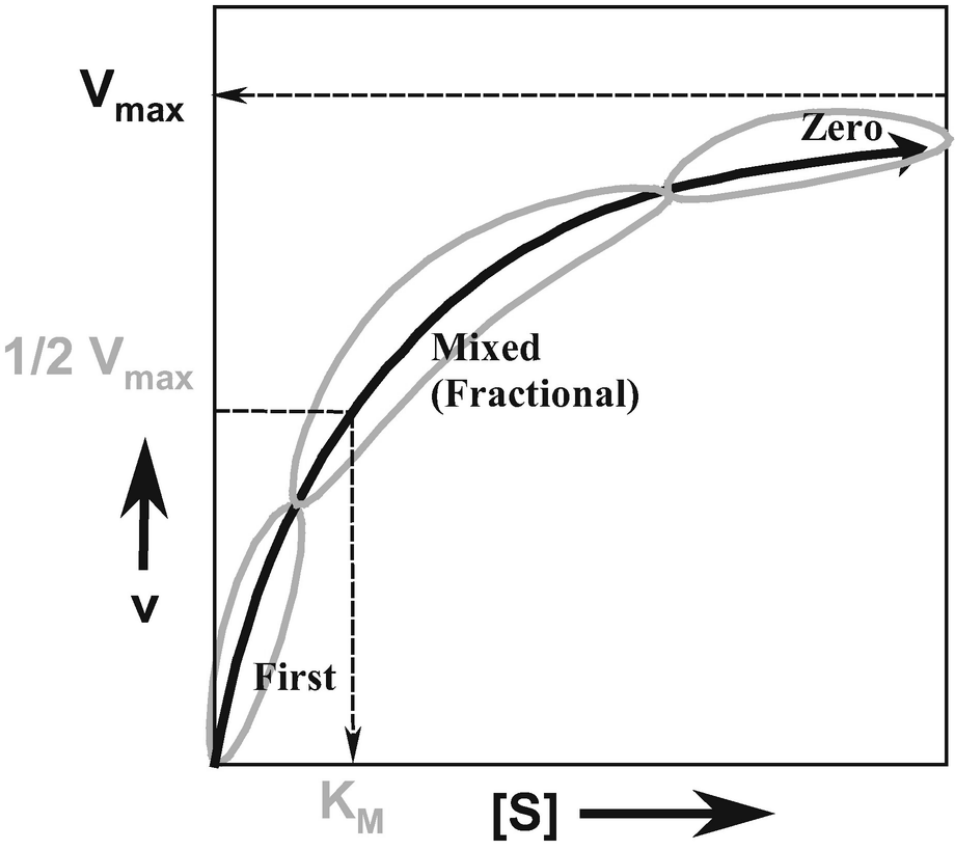
Under Michaelis–Menten conditions, K_m reflects the apparent affinity between enzyme and substrate（酶与底物的表观亲和力）.

- Low K_m: high apparent affinity（表观亲和力高）
- High K_m: low apparent affinity（表观亲和力低）

How do enzymes work?

Kinetics of Enzyme-Catalyzed Reactions（酶促反应动力学）

Reaction order（反应级数） describes how strongly the reaction rate depends on the concentration of a reactant.



When [S] ≫ Km　　Zero-order reaction（零级反应）

$$v_0 = V_{max}$$

When [S] ≈ Km

Mixed-order kinetics（混合级数反应）

When [S] ≪ Km　　First-order reaction（一级反应）

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

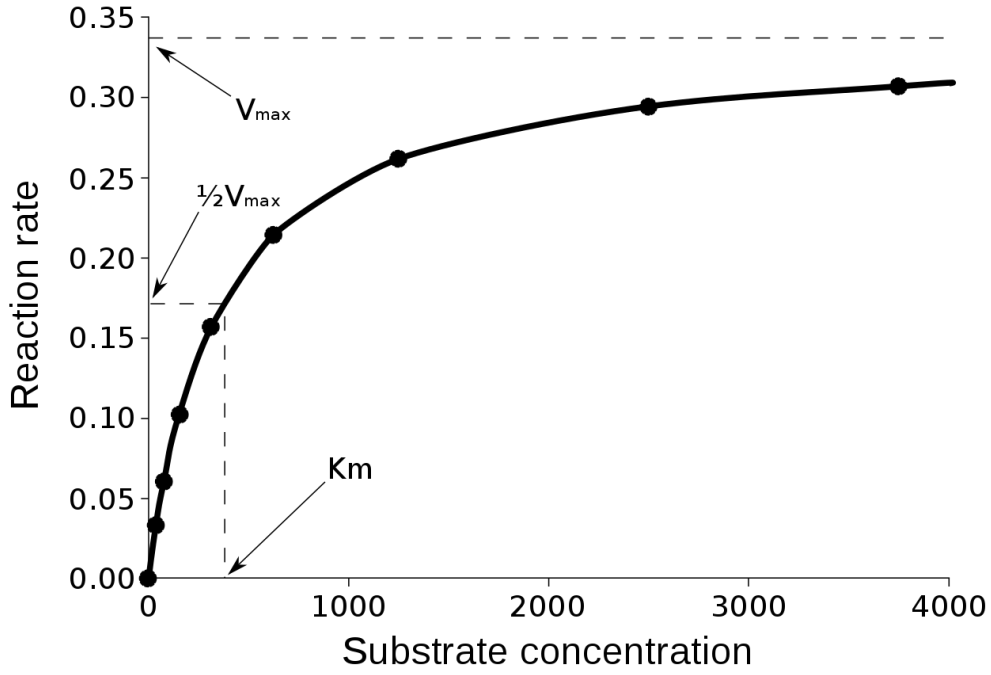
How do enzymes work?

Determination of K_m and V_{max}

1. Direct Michaelis–Menten plot（米氏曲线）

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

Based on the relationship between initial velocity v and substrate concentration [S], both V_{max} and K_m can be estimated, but this method is not very accurate.



How do enzymes work?

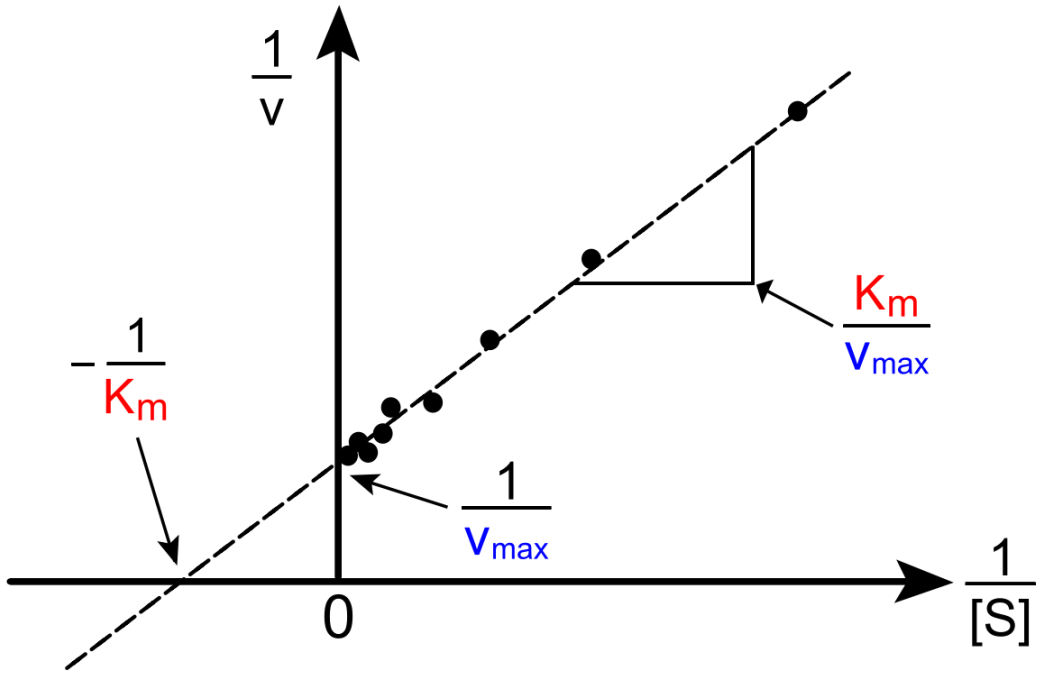
Determination of K_m and V_{max}

Lineweaver–Burk plot

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max}[S]} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}$$

y = ax + b

- y = 1/v
- x = 1/[S]
- Slope = Km / Vmax
- y-intercept = 1 / Vmax
- x-intercept = −1 / Km



How do enzymes work?

Determination of K_m and V_{max}

Lineweaver–Burk plot

[S] (mol L^{-1})	v (nmol L^{-1} min^{-1})
8.33×10^{-6}	13.8
1.00×10^{-5}	16.0
1.25×10^{-5}	19.0
1.67×10^{-5}	23.6
2.00×10^{-5}	26.7

Vmax = 80 **Km = 4.0×10^{-5}**

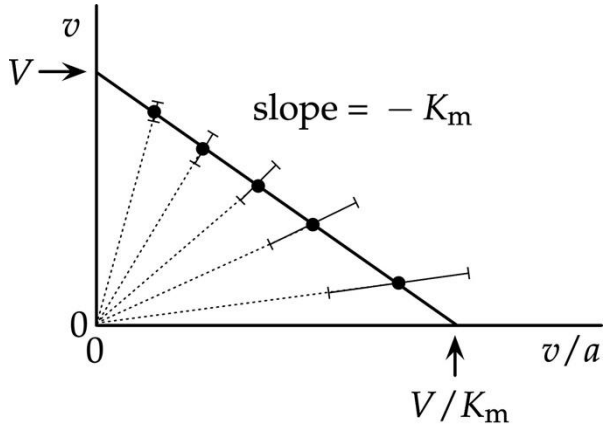
How do enzymes work?

Determination of K_m and V_{max}

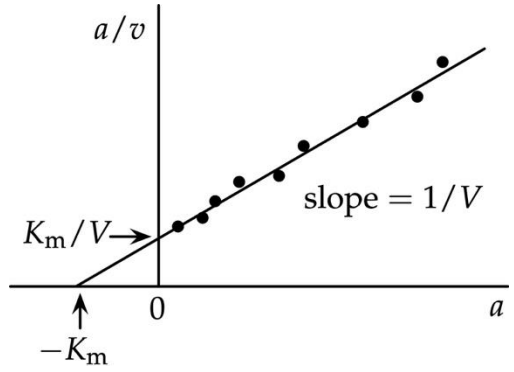
The Lineweaver–Burk plot is easy to understand, but it can magnify errors at low substrate concentrations.

Better modern methods include:

- **Eadie–Hofstee plot**
- **Hanes–Woolf plot**
- **Nonlinear regression**



Eadie–Hofstee plot



Hanes–Woolf plot

1.What are enzymes?

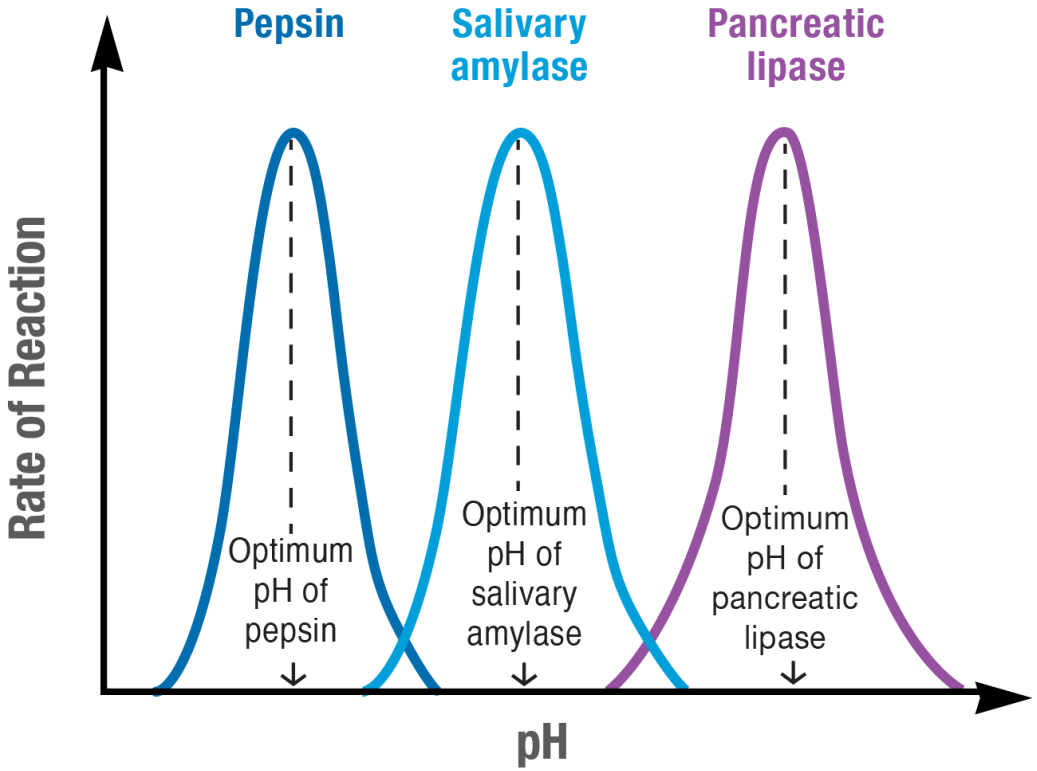
2.How do enzymes work?

3.How are enzyme activities regulated?

How are enzyme activities regulated?

Effect of pH on enzyme activity

Each enzyme has an optimum pH, at which its catalytic activity is maximal.



- Pepsin（胃蛋白酶）: optimum pH about 1.5–2.0
- Trypsin（胰蛋白酶）: optimum pH about 8.0
- Cholinesterase（胆碱酯酶）: near neutral to slightly alkaline pH

How are enzyme activities regulated?

Why pH affects enzyme activity

pH affects enzyme activity mainly by changing the ionization state（解离状态 / 电离状态） and structure of the enzyme and substrate.

- **Extreme pH can denature proteins（极端pH可使蛋白质变性)**
  Strong acid or alkali may disrupt enzyme structure and cause loss of activity.
- **pH changes the ionization state of the substrate（改变底物的解离状态)**
  This affects substrate binding to the active site.
- **pH changes the ionization state of active-site groups（改变活性中心基团的解离状态)**
  Catalytic amino acid residues must often be in a specific protonation state to function.
- **pH can affect cofactors（影响辅助因子)**
  Some cofactors or metal ions bind differently at different pH values.

How are enzyme activities regulated?

Effect of temperature on enzyme activity

Each enzyme has an optimum temperature（最适温度）, where activity is highest.

Temperature affects enzyme activity in two opposite ways:

A. Before the optimum temperature（达到最适温度前）

Increasing temperature raises molecular kinetic energy（分子动能）, increasing the number of activated molecules（活化分子） and increasing reaction rate.

B. Above the optimum temperature（超过最适温度后）

High temperature disrupts enzyme structure, causing **denaturation（变性）** and loss of activity.

How are enzyme activities regulated?

Temperature coefficient Q10 (温度系数)

Q10 is the ratio of reaction rates when the temperature is increased by 10°C within a certain temperature range.

$$Q10 = \frac{V_{T+10}}{V_t}$$

Q10 is often around 2

How are enzyme activities regulated?

Effect of activators on enzyme activity（激活剂对酶活性的影响）

Activators are substances that increase enzyme activity.

1.Inorganic ions（无机离子）: Mg^{2+}, K^{+}, Ca^{2+}, Cl^{-}, Fe^{2+}/Fe^{3+}

2.Organic molecules（有机分子）: GSH, cysteine, vitamin C, EDTA

3.Biological macromolecules（生物大分子）: calmodulin, regulatory proteins

- ❖ Stabilize enzyme structure（稳定酶结构）
- ❖ Help substrate binding（促进底物结合）
- ❖ Restore essential groups（恢复必需基团）
- ❖ Remove inhibitory ions（解除抑制）

How are enzyme activities regulated?

Effect of Inhibitors on Enzyme Activity

Inhibition occurs when an inhibitor binds to an enzyme and reduces its catalytic activity, usually without changing the total amount of enzyme protein.

- Inhibitor
  - Irreversible inhibition（不可逆抑制）
  - Reversible inhibition（可逆抑制）
    - 1.Competitive inhibition（竞争性抑制）
    - 2.Noncompetitive inhibition（非竞争性抑制）
    - 3.Uncompetitive inhibition（反竞争性抑制）

How are enzyme activities regulated?

Effect of Inhibitors on Enzyme Activity

Irreversible inhibition（不可逆抑制）

Irreversible inhibitors（不可逆抑制剂） bind covalently or very tightly to essential groups（必需基团） of enzymes and permanently inactivate them.

- **Organophosphorus compounds（有机磷化合物）**
  Modify Ser–OH in acetylcholinesterase（乙酰胆碱酯酶）.
- **Heavy metals and organic mercurials/arsenicals（重金属及有机汞、有机砷）**
  Bind Cys–SH groups（半胱氨酸巯基）.
- **Cyanide, sulfide, CO（氰化物、硫化物、一氧化碳）**
  Bind metal ions in enzymes and block respiration.
- **Penicillin（青霉素）**
  Inhibits bacterial transpeptidase（细菌转肽酶）, blocking cell wall synthesis.

How are enzyme activities regulated?

Effect of Inhibitors on Enzyme Activity

Reversible inhibition（可逆抑制）

In reversible inhibition, the inhibitor binds to the enzyme noncovalently and reversibly. The inhibition can usually be removed by dialysis or dilution (透析或稀释).

1.Competitive inhibition（竞争性抑制）

2.Noncompetitive inhibition（非竞争性抑制）

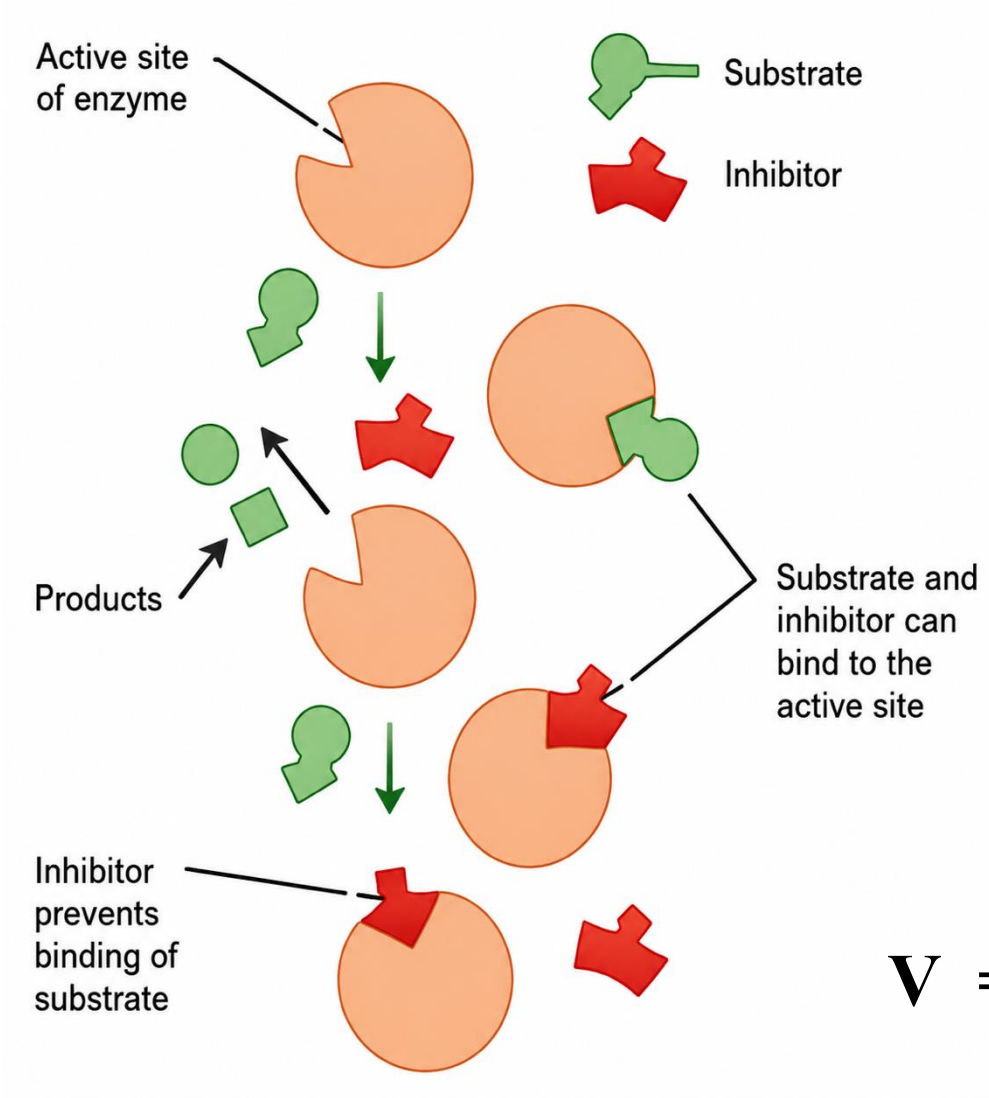
3.Uncompetitive inhibition（反竞争性抑制）

1. Competitive inhibition（竞争性抑制）

Competitive inhibition occurs when an inhibitor resembles the substrate and competes with the substrate for the enzyme active site.

- The inhibitor is often structurally similar to the substrate. 抑制剂常与底物结构相似。
- The inhibitor competes with substrate for the active site. 抑制剂与底物竞争酶的活性中心。
- Inhibition depends on the ratio [I]/[S]. 抑制强弱取决于 [I]/[S]。
- Increasing substrate concentration can overcome inhibition. 增加底物浓度可减弱或消除竞争性抑制。

1. Competitive inhibition（竞争性抑制）



$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_3} P + E$$

$$+$$

$$I$$

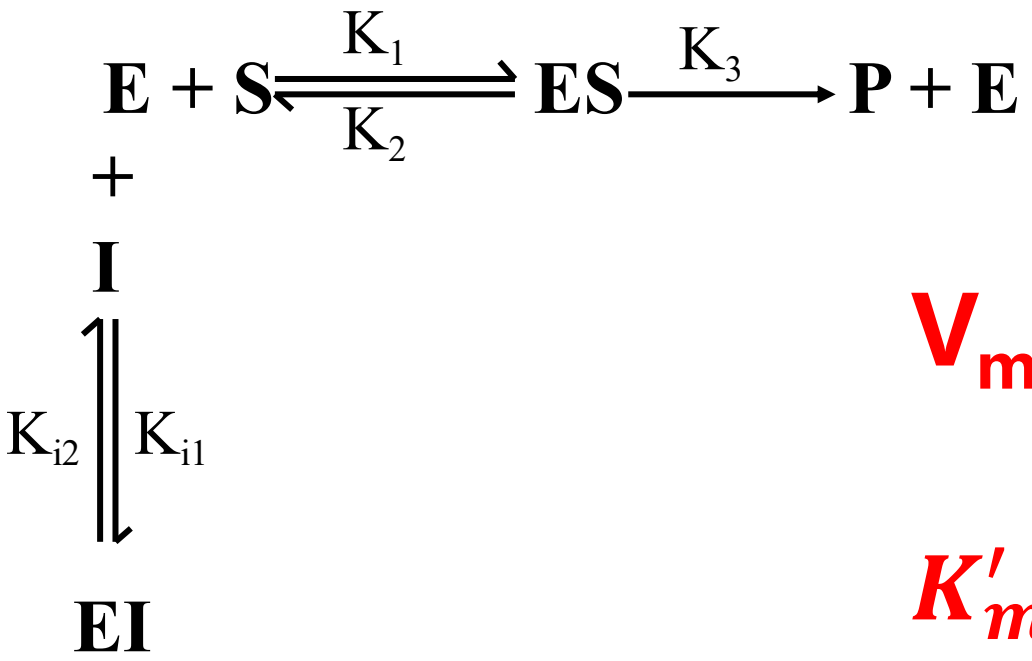
$$K_{i2} \quad K_{i1}$$

$$EI$$

$$Ki = Ki2 / Ki1 = [E][I] / [EI]$$

$$V = \frac{Vmax[S]}{Km(1+[I]/Ki)+[S]} \qquad Km' = Km\left(1+\frac{[I]}{Ki}\right)$$

1. Competitive inhibition（竞争性抑制）

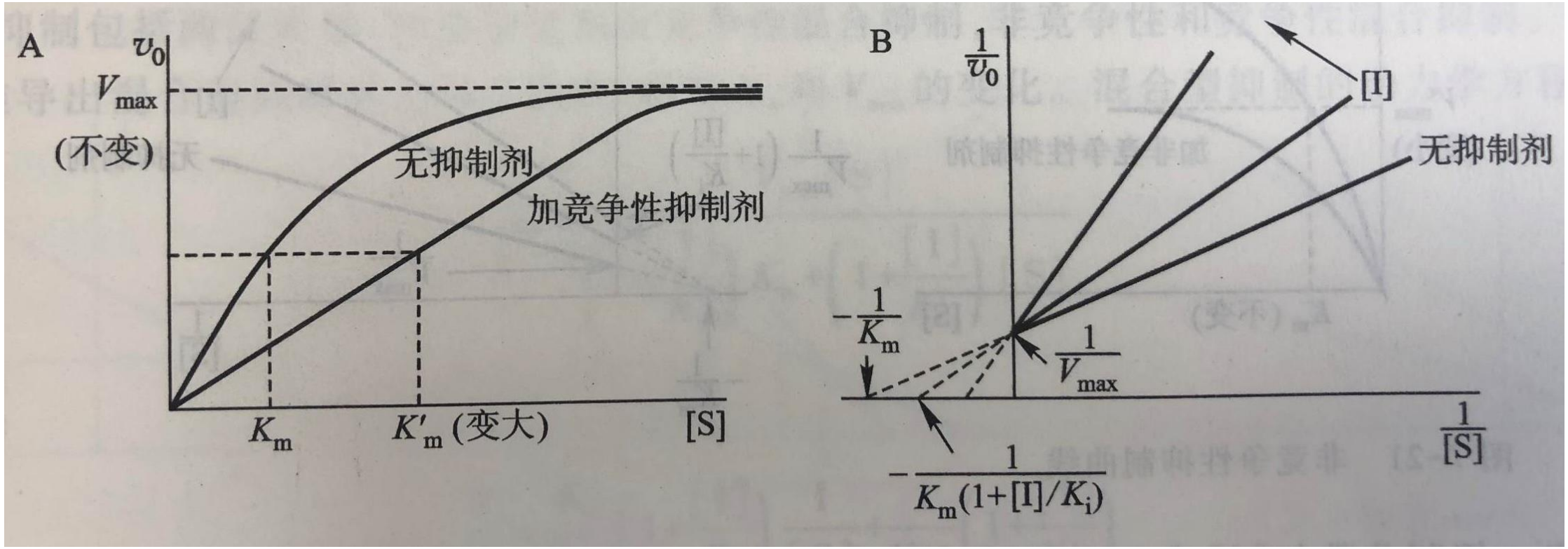


$$V_{max}' = V_{max}$$

$$K_m' = K_m\left(1 + \frac{[I]}{K_i}\right)$$

- V_{max} unchanged
- Apparent K_m increases
- Inhibition can be overcome by increasing [S]

1. Competitive inhibition（竞争性抑制）

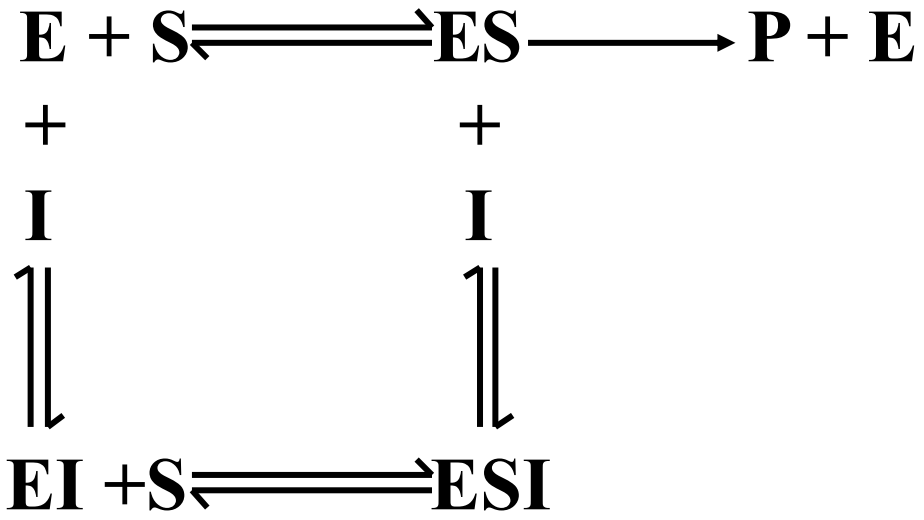


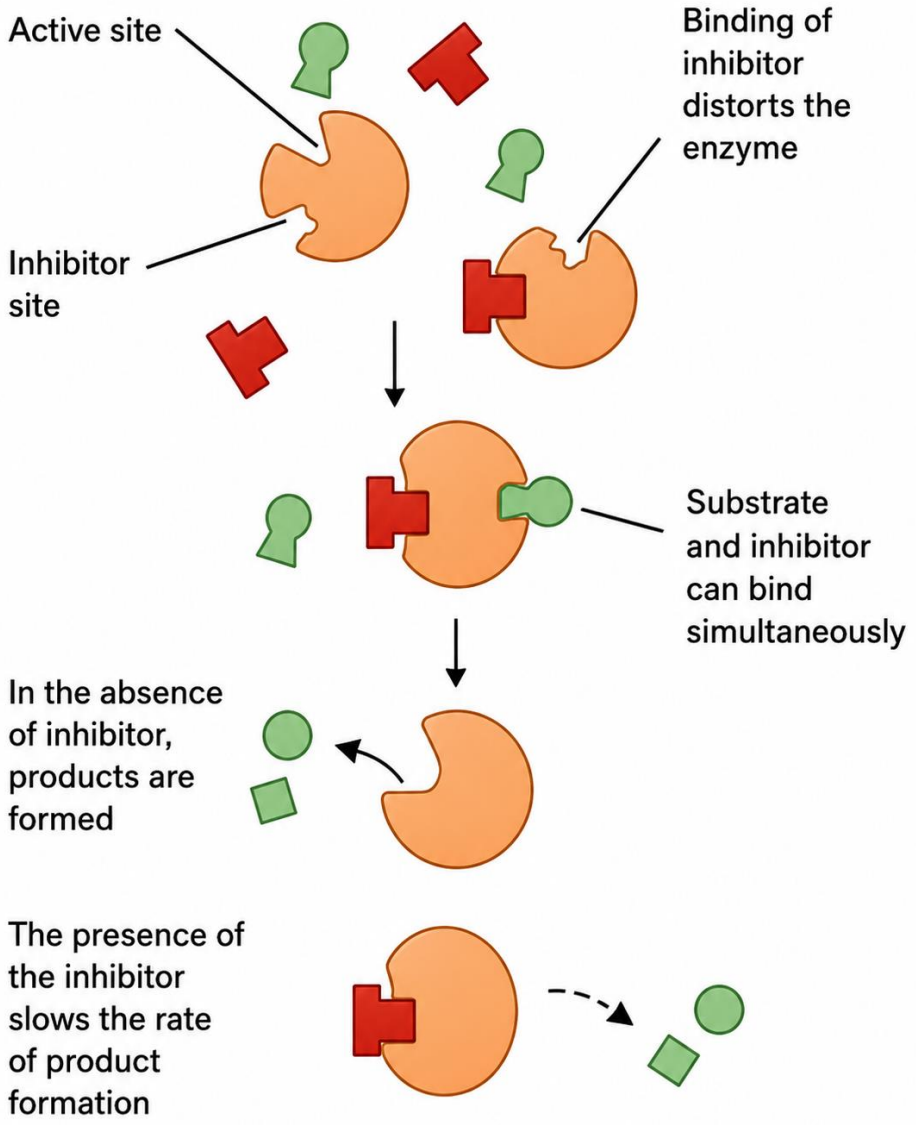
2. Noncompetitive inhibition（非竞争性抑制）

Noncompetitive inhibition occurs when an inhibitor binds to a site other than the active site, usually called an allosteric site（变构部位）.

- **The inhibitor binds outside the active site.**
- **The inhibitor can bind both E and ES.**
- **The strength of inhibition mainly depends on [I]（抑制剂浓度）.**
- **Increasing substrate concentration cannot eliminate inhibition.**

2. Noncompetitive inhibition（非竞争性抑制）





The inhibitor binds outside the active site and reduces catalytic efficiency by changing enzyme conformation.

2. Noncompetitive inhibition（非竞争性抑制）

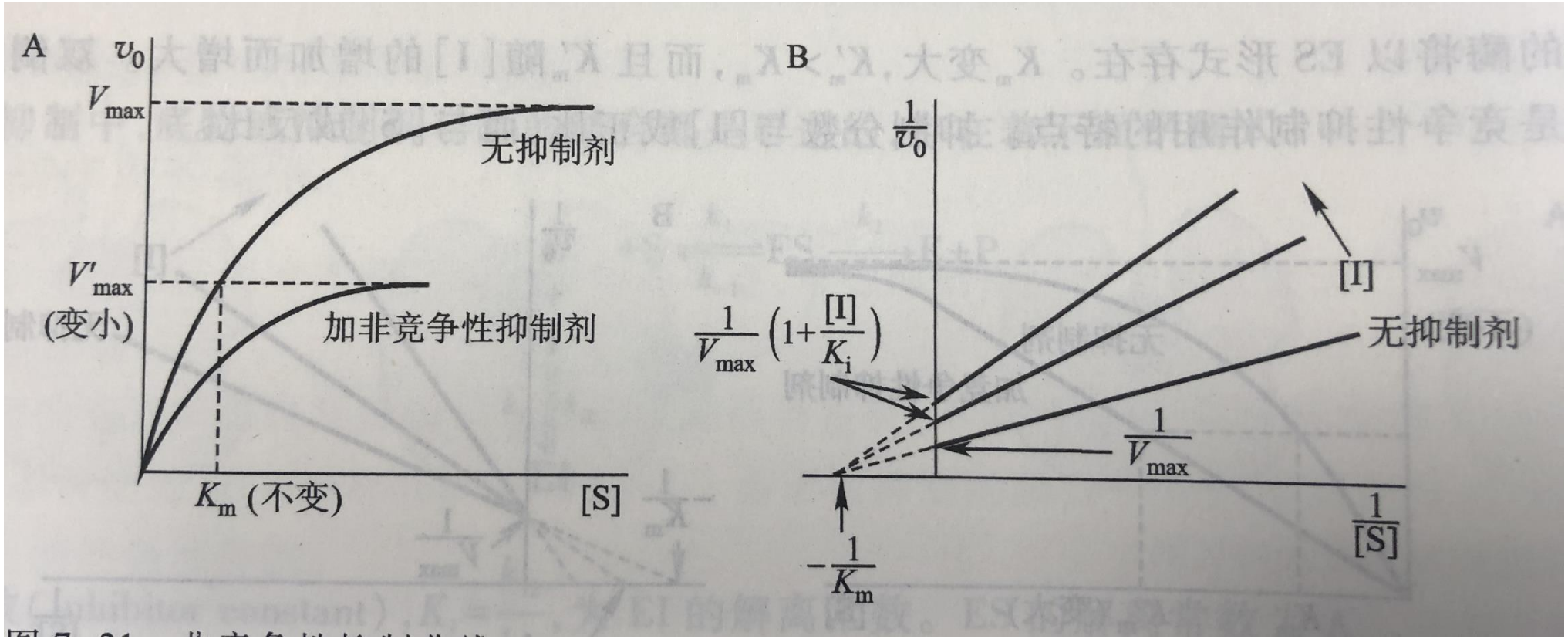
For pure noncompetitive inhibition:

$$v = \frac{V'_{max}[S]}{K_m + [S]} \qquad V'_{max} = \frac{V_{max}}{1 + \frac{I}{K_i}}$$

- V_{max} decreases
- K_m unchanged

- The inhibitor reduces the amount of catalytically active enzyme, so the maximum reaction rate decreases.
- But because substrate binding affinity is not changed in pure noncompetitive inhibition, Km remains unchanged.

2. Noncompetitive inhibition（非竞争性抑制）



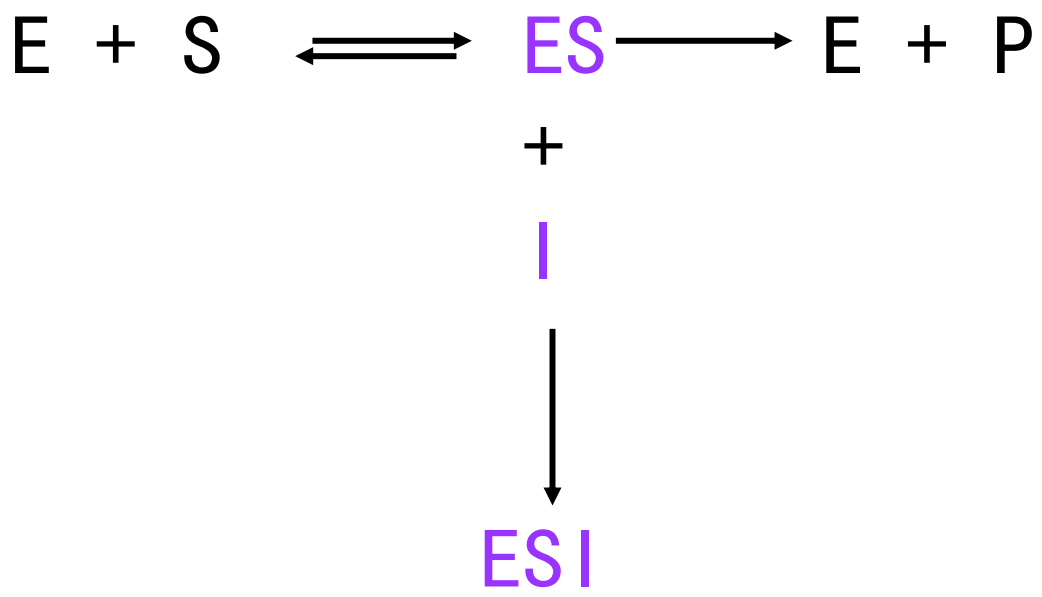
3. Uncompetitive Inhibition（反竞争性抑制作用）

Uncompetitive inhibition occurs when the inhibitor binds only to the enzyme–substrate complex, ES, not to free enzyme.

- ❖ **Inhibitor binds only to ES complex**
- ❖ **Forms inactive ESI complex**
- ❖ **Cannot be overcome by increasing [S]**

3. Uncompetitive Inhibition（反竞争性抑制作用）

Kinetic effects



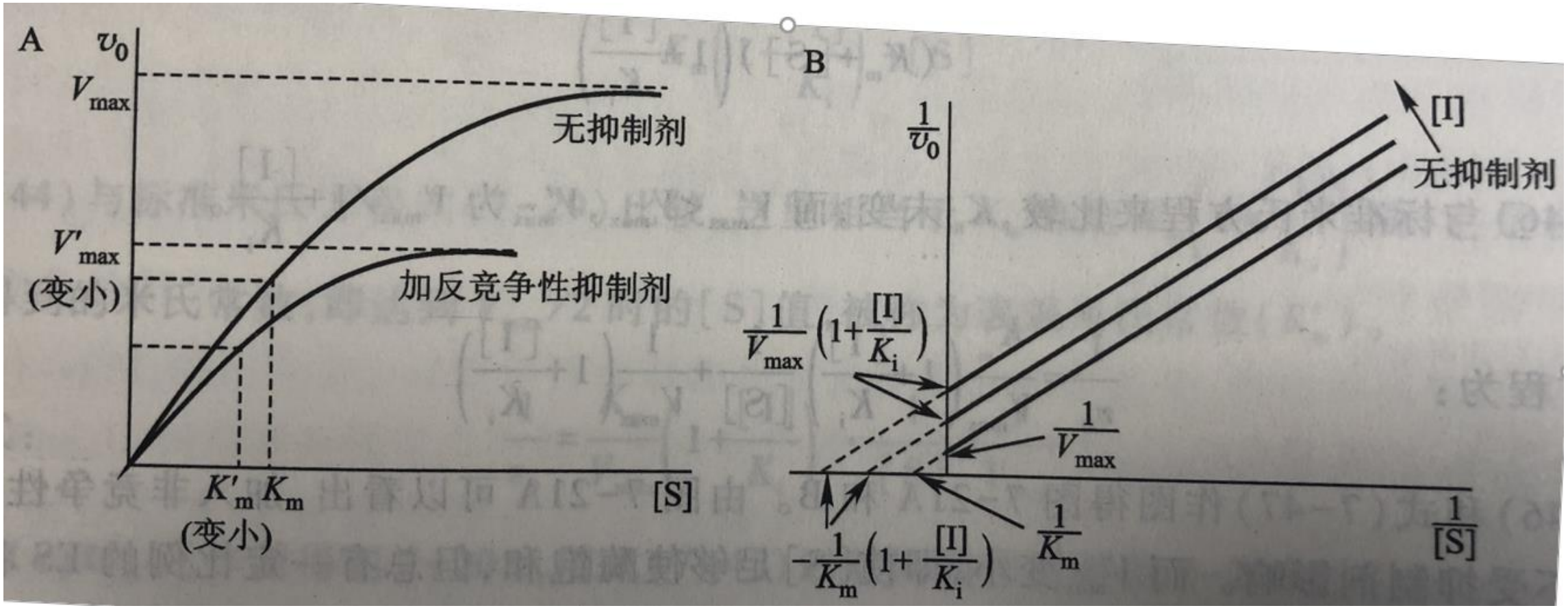
$$V = \frac{Vmax[S]}{Km+[S](1+[I]/Ki)}$$

$$Km' = \left(\frac{Km}{1+\frac{[I]}{Ki}}\right)$$

$$Vmax' = \left(\frac{Vmax}{1+\frac{[I]}{Ki}}\right)$$

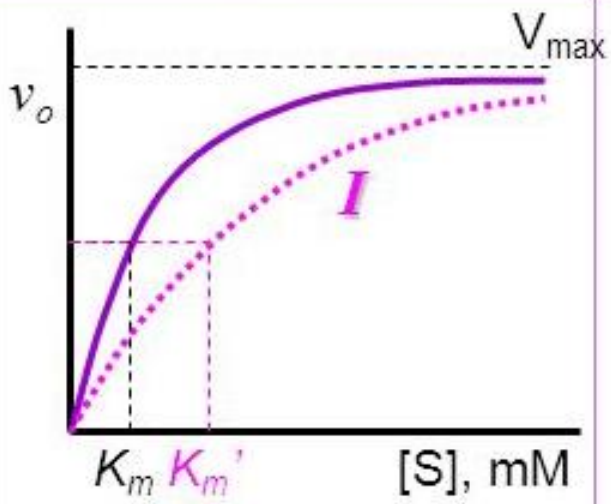
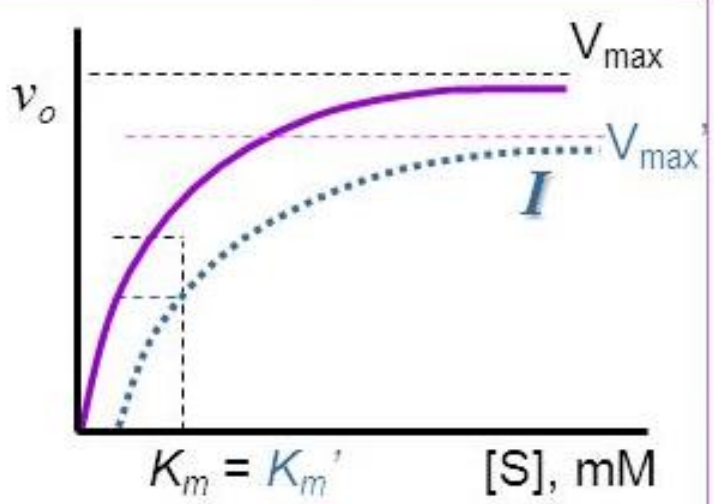
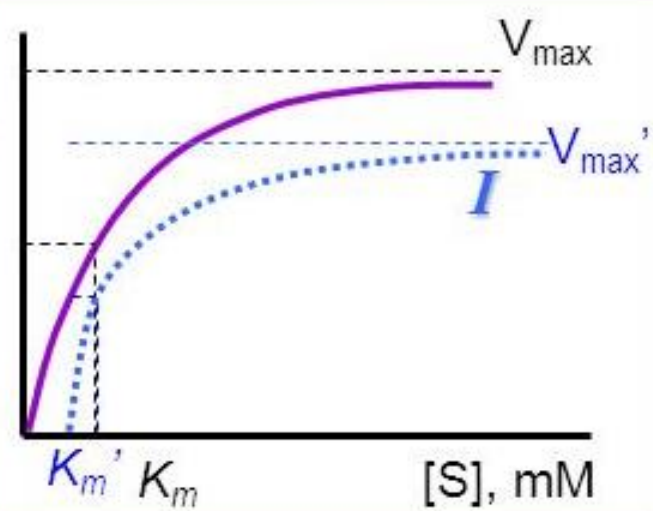
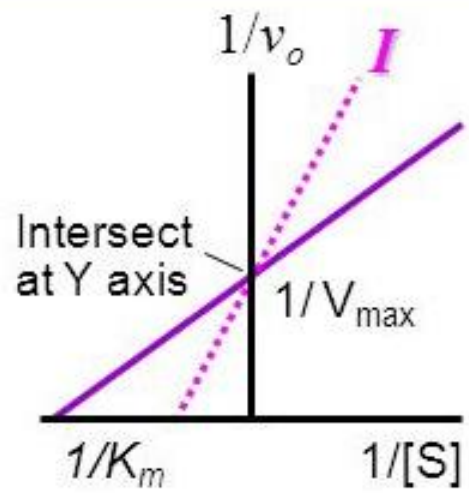
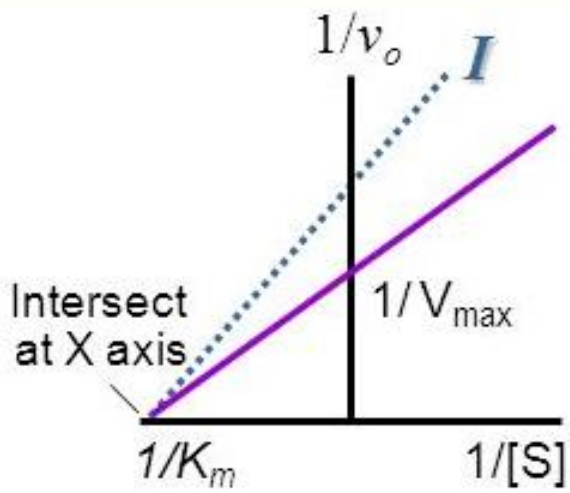
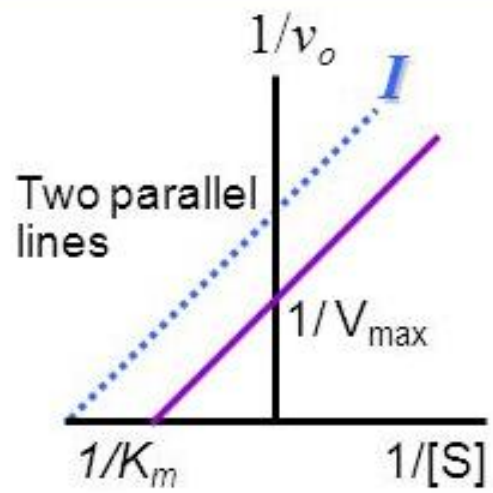
- V_{max} decreases
- K_m decreases
- V_{max} and K_m decrease by the same factor

3. Uncompetitive Inhibition（反竞争性抑制作用）



Reversible inhibition（可逆抑制）

Type	Equation	Vmax	Km
No inhibitor	$v = \frac{V_{max}[S]}{K_m + [S]}$	Vmax	Km
Competitive inhibition	$v = \frac{V_{max}[S]}{K_m(1 + \frac{[I]}{K_i}) + [S]}$	Unchanged	Increased
Noncompetitive inhibition	$v = \frac{V_{max}[S]}{(1 + \frac{[I]}{K_i})(K_m + [S])}$	Decreased	Unchanged
Uncompetitive inhibition	$v = \frac{V_{max}[S]}{K_m + (1 + \frac{[I]}{K_i})\,[S]}$	Decreased	Decreased

Competitive	Non-competitive	Uncompetitive
		
V_{max} unchanged K_m increased	V_{max} decreased K_m unchanged	Both V_{max} & K_m decreased
		

How are enzyme activities regulated?

Regulation of Enzyme Activity

1. Allosteric Enzymes（变构酶）

Allosteric enzymes have both an active site and regulatory site(s).

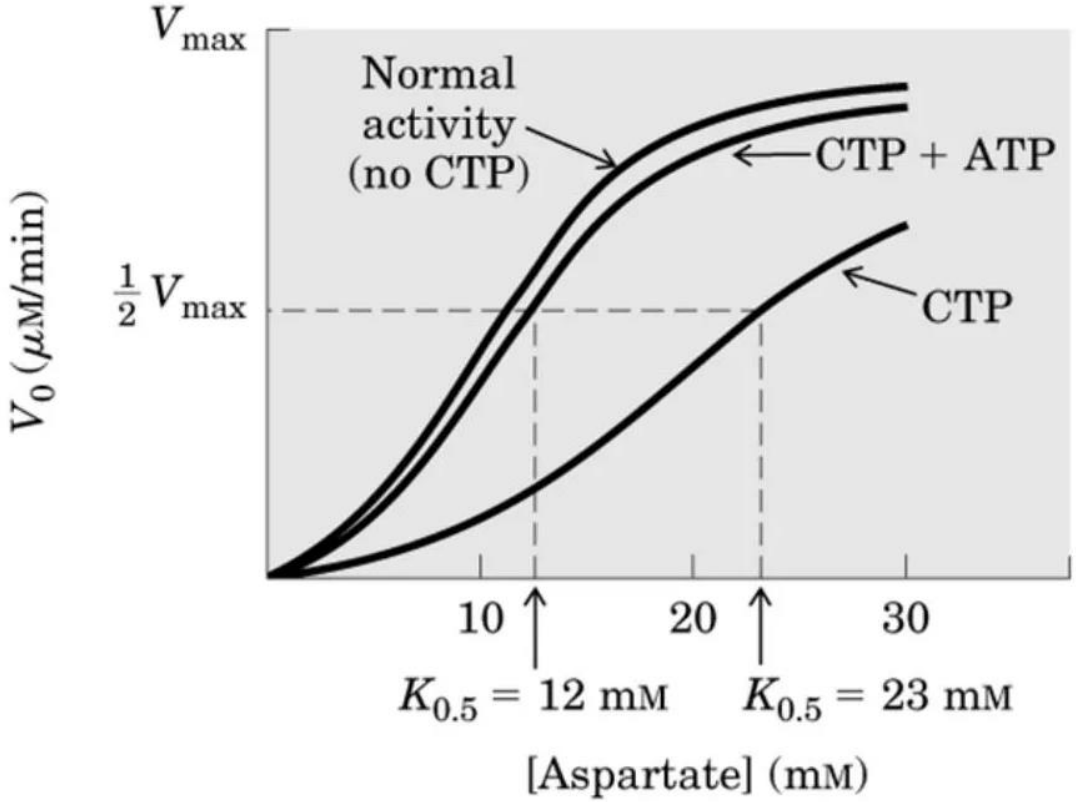
- ❖Usually composed of multiple subunits（多亚基）
- ❖Regulated by allosteric effectors（变构效应物）
- ❖Show sigmoidal kinetics（S形动力学曲线）
- ❖Often key enzymes in metabolic pathways（代谢途径中的关键酶）

How are enzyme activities regulated?

Regulation of Enzyme Activity

1. Allosteric Enzymes（变构酶）

ATCase（天冬氨酸转氨甲酰酶 / aspartate transcarbamoylase） is a classic allosteric enzyme.



- ❖ **The S-shaped curve indicates cooperative substrate binding.**
- ❖ **Binding of CTP changes enzyme conformation(构象), decreases affinity for substrate, and slows the reaction.**
- ❖ **Binding of ATP changes enzyme conformation, increases affinity for substrate, and accelerates the reaction.**

How are enzyme activities regulated?

Regulation of Enzyme Activity

2. Isoenzymes / Isozymes（同工酶)

Isoenzymes catalyze the same reaction but differ in molecular structure, tissue distribution, or regulation.

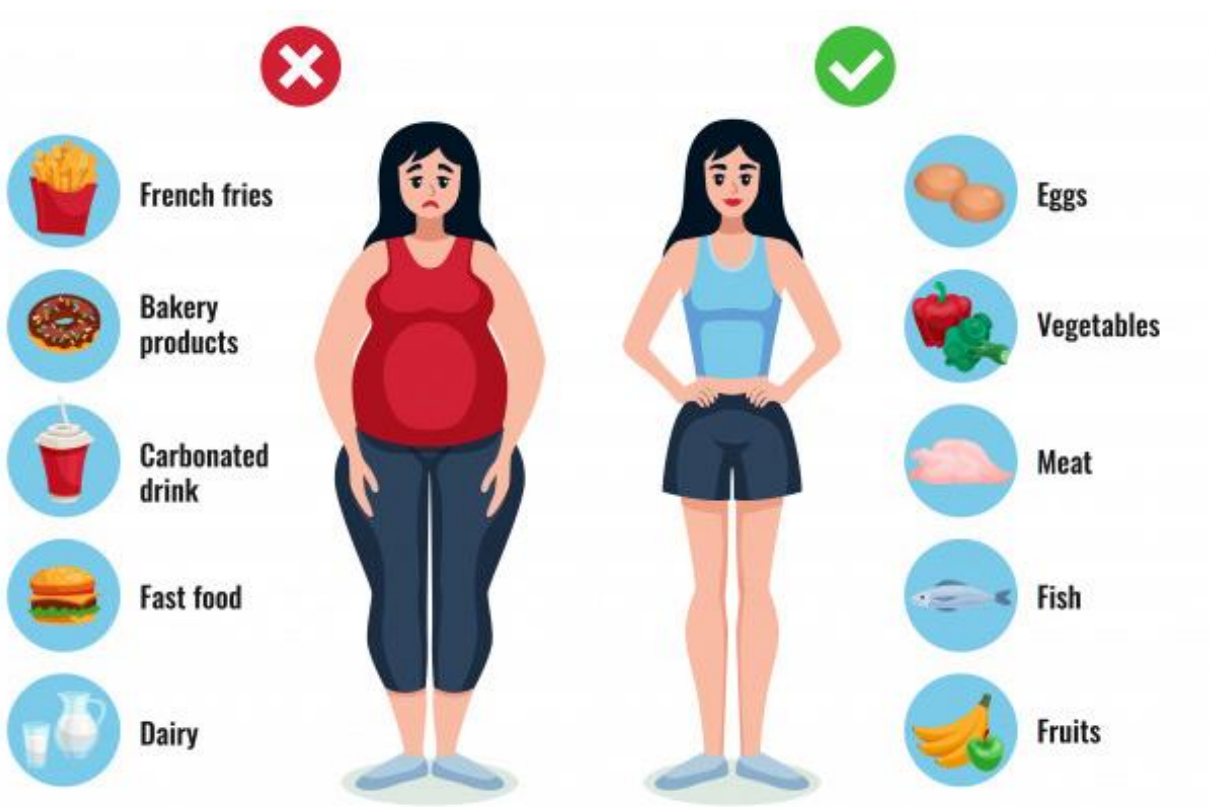
3. Inducible Enzymes（诱导酶)

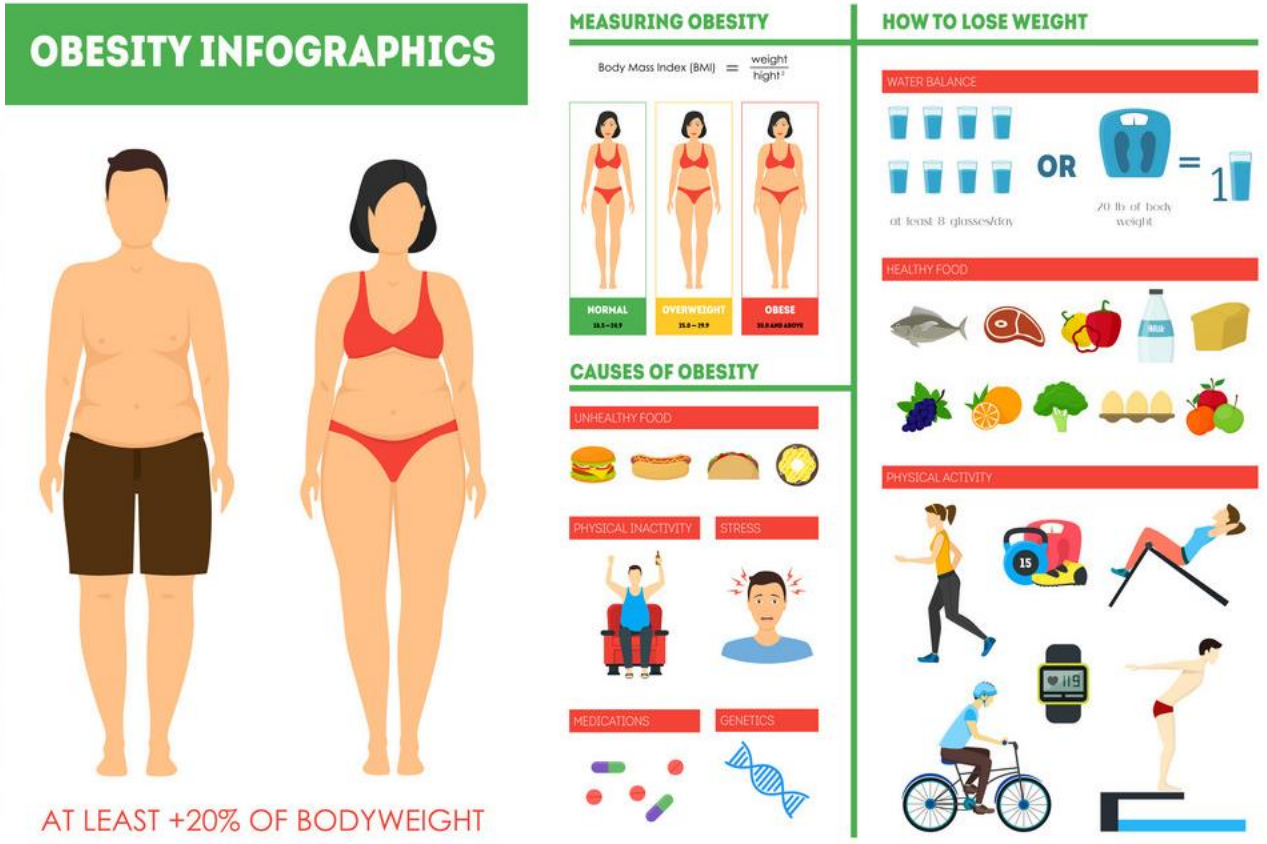
Inducible enzymes are synthesized in larger amounts when an inducer is present.

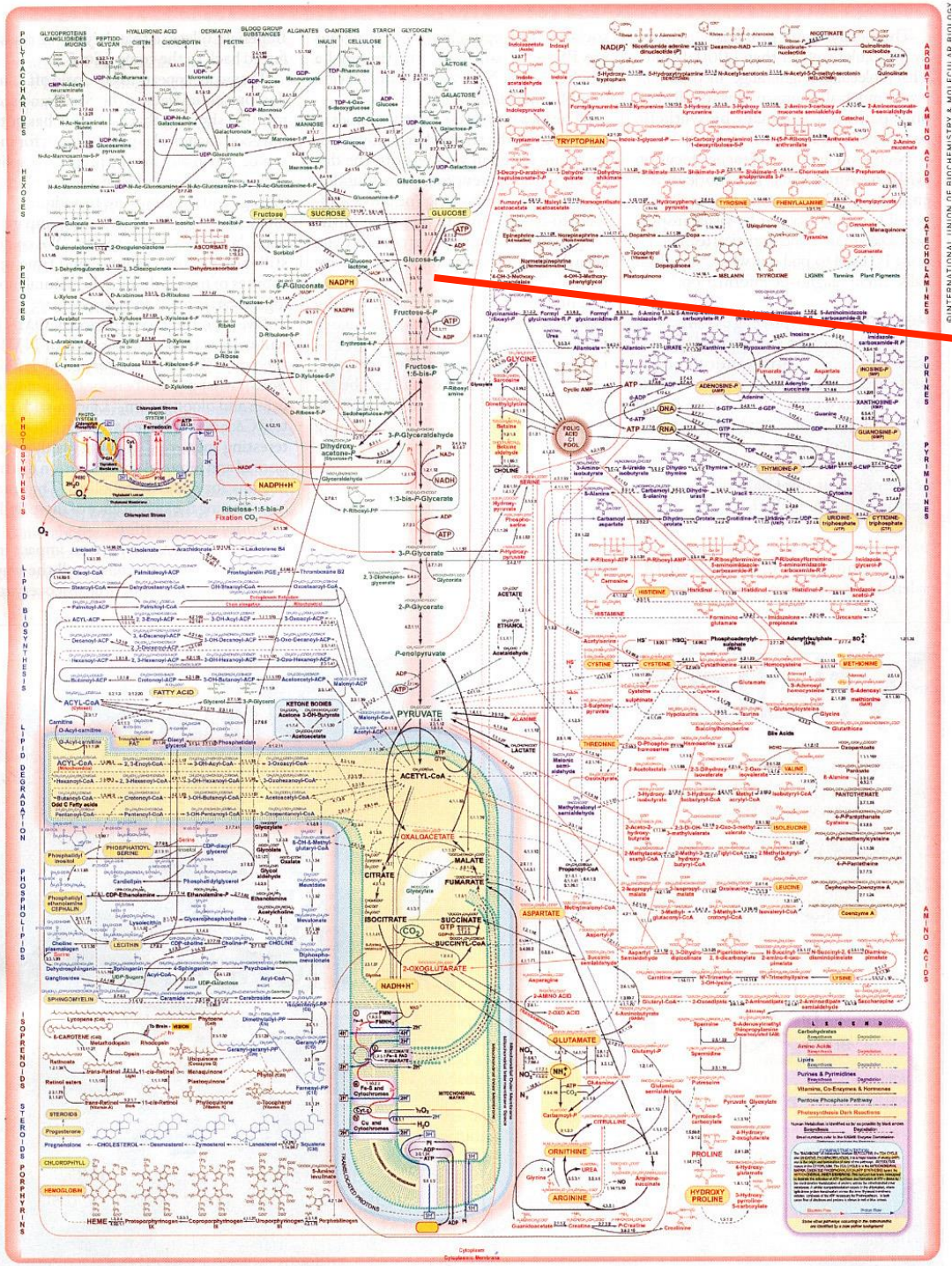
4. Catalytic Antibodies / Abzymes(催化性抗体 / 抗体酶)

Catalytic antibodies are antibodies with catalytic activity.

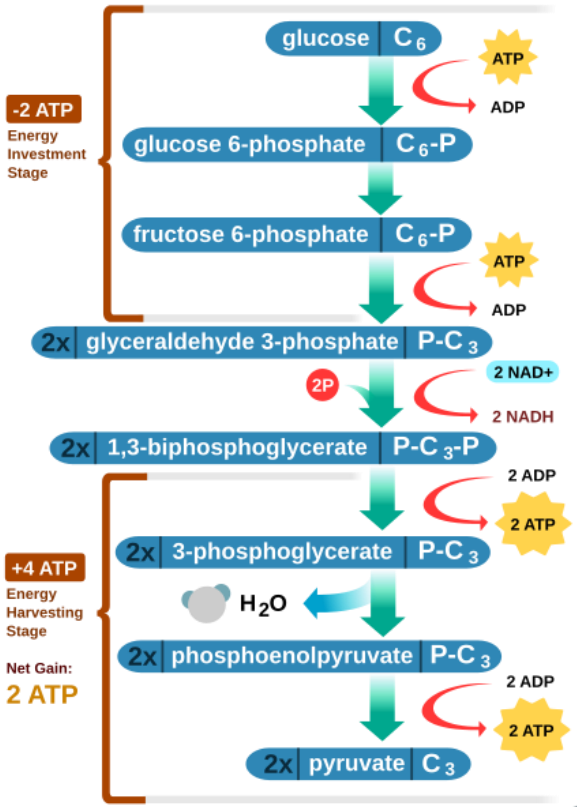
From Enzymes to Metabolism



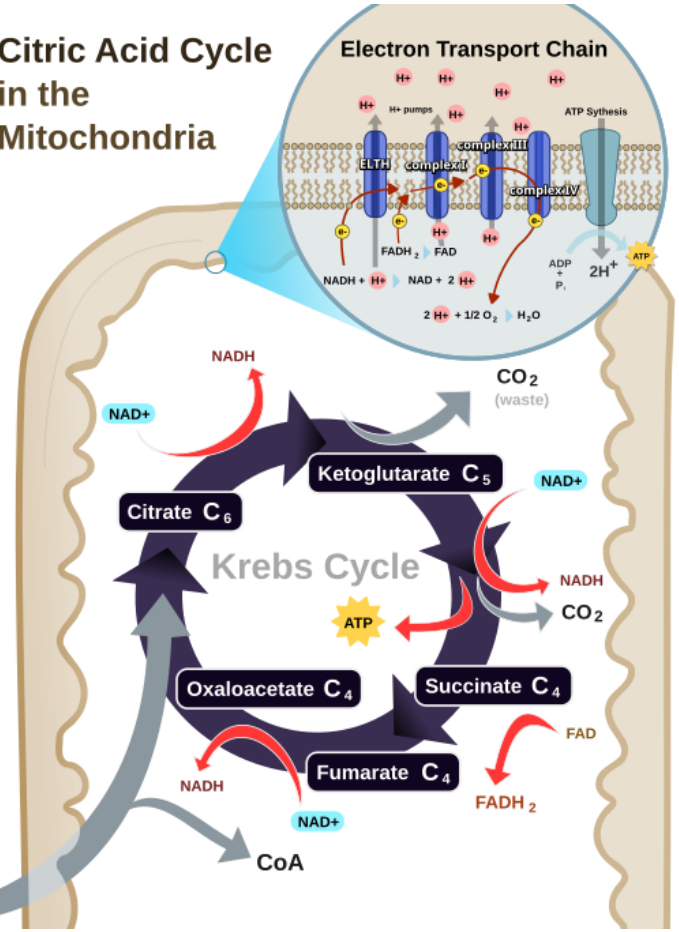




Glycolysis in the Cytoplasm



Citric Acid Cycle in the Mitochondria



General Concept of Metabolism

Metabolism (新陈代谢) refers to the exchange of matter and energy between a living organism and its environment.

At the cellular level, metabolism consists of all enzyme-catalyzed chemical reactions that transform substrates into products through metabolic intermediates (代谢中间产物).

Metabolism is not a random collection of reactions; it is an enzyme-organized network that coordinates matter transformation, energy conversion, and cellular regulation.

General Concept of Metabolism

In plants:

- **Carbon assimilation (碳同化):** conversion of CO_2 into organic compounds through photosynthesis.
- **Nutrient assimilation (营养同化):** incorporation of inorganic ions such as NO_3^-, NH_4^+, SO_4^{2-}, and PO_4^{3-} into biomolecules.
- **Respiratory metabolism (呼吸代谢):** oxidation of organic compounds to generate ATP, reducing power, and biosynthetic precursors.
- **Biosynthesis and storage (生物合成与贮藏):** production of carbohydrates, lipids, proteins, nucleic acids, and specialized metabolites.

General Concept of Metabolism

Anabolism and Catabolism

Anabolism (合成代谢；同化作用) uses energy and reducing power to convert simple molecules into complex cellular components, such as carbohydrates, proteins, lipids, and nucleic acids.

- **CO_2 fixation in the Calvin–Benson cycle**
- **Nitrate reduction and ammonium assimilation**
- **Starch and sucrose biosynthesis**
- **Fatty acid and amino acid biosynthesis**

Catabolism (分解代谢；异化作用)
Catabolism breaks down complex molecules into smaller compounds, releasing energy, reducing equivalents, and precursor metabolites.

- **Glycolysis**
- **Tricarboxylic acid cycle, TCA cycle**
- **Mitochondrial respiration**
- **Starch and lipid mobilization during seed germination**

General Concept of Metabolism

Energy Metabolism

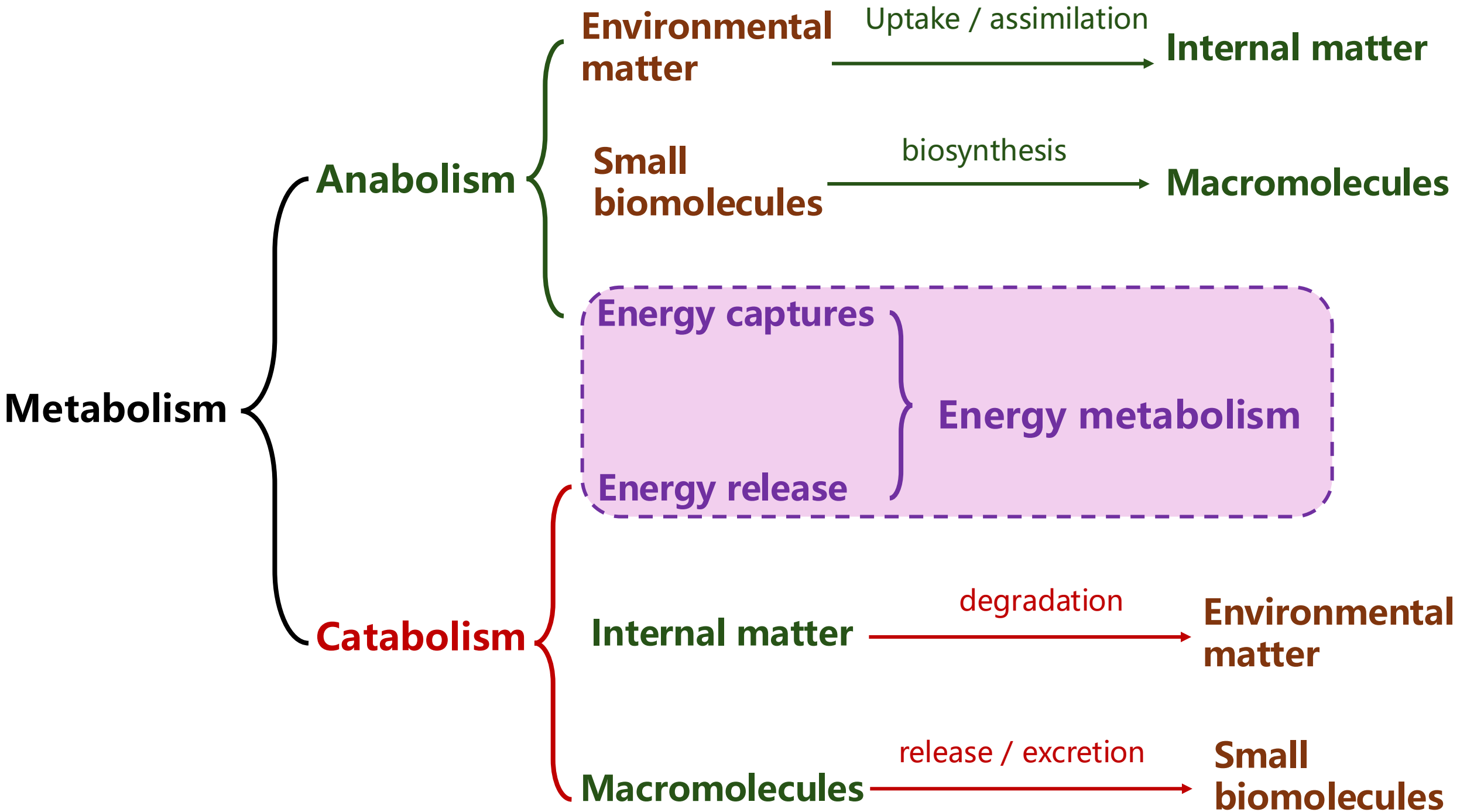
Energy metabolism (能量代谢) refers to the capture, conversion, storage, and utilization of energy during metabolic processes.

In living cells, energy is mainly transferred through:

- ATP/ADP cycling
- Redox cofactors such as NADH, NADPH, and $FADH_2$
- H^+ gradients across membranes
- High-energy phosphorylated intermediates

In plants, major energy-converting systems include:

- Photosynthetic electron transport in chloroplasts
- Photophosphorylation (光合磷酸化)
- Mitochondrial electron transport (线粒体电子传递)
- Oxidative phosphorylation (氧化磷酸化)
- Glycolysis and the TCA cycle (糖酵解与三羧酸循环)


Metabolism
Anabolism
Environmental matter
Uptake / assimilation
Internal matter
Small biomolecules
biosynthesis
Macromolecules
Energy captures
Energy release
Energy metabolism
Catabolism
Internal matter
degradation
Environmental matter
Macromolecules
release / excretion
Small biomolecules

Research Approaches in Metabolism

Approach	Experimental material	What it tells us
In vivo **(活体研究)**	intact plants, organs, cells	physiological function
In vitro **(离体研究)**	extracts, organelles, purified enzymes	mechanism and kinetics
In situ **(原位研究)**	tissues, cells, compartments	spatial regulation
In planta **(植物体内研究)**	intact plant systems	whole-plant relevance

Experimental Methods for Studying Plant Metabolism

1. Isotope tracing (同位素示踪)
Radioisotopes such as ^{14}C, ^{32}P, and ^{35}S, or stable isotopes such as ^{13}C and ^{15}N, are used to trace metabolite movement, pathway direction, and nutrient assimilation.

2. Enzyme inhibitors and activators (酶抑制剂与激活剂)
Specific inhibitors or activators perturb enzyme activity, allowing researchers to infer the role of a pathway or rate-limiting step.

3. Metabolomics and metabolic profiling (代谢组学与代谢谱分析)
LC–MS, GC–MS, and NMR are used to quantify metabolites and identify pathway-level responses.

4. Subcellular and organ-level analysis (亚细胞与器官水平分析)
Chloroplasts, mitochondria, peroxisomes, vacuoles, leaves, roots, and seeds can be analyzed separately to reveal compartment-specific metabolism.

5. Metabolic flux analysis (代谢通量分析)
Flux analysis estimates the rate of metabolite flow through pathways, often using isotope labeling and computational models.

6. Genetic and molecular approaches (遗传学与分子生物学方法)
Mutants, transgenic lines, gene editing, transcriptomics, and proteomics are used to connect enzyme genes with metabolic phenotypes.

The End