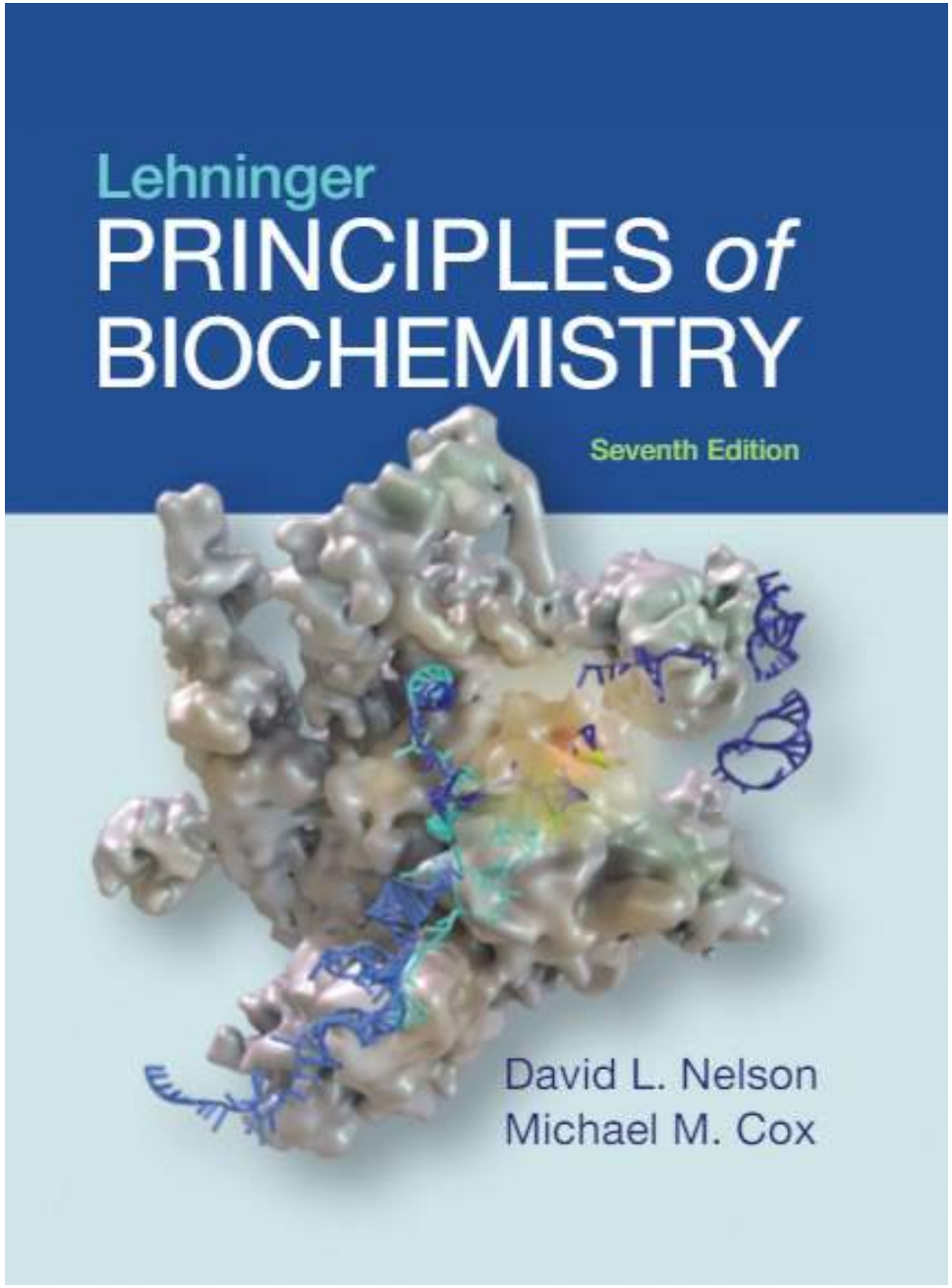
6| Enzymes

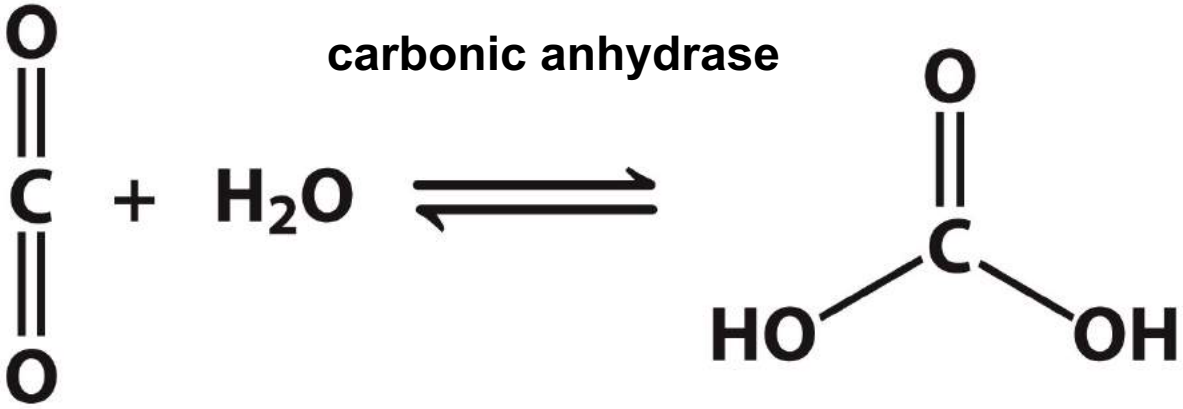


What are enzymes

- Enzymes are catalysts.
  - increase reaction rates without being used up
- Most enzymes are proteins.
  - However, some RNA (ribozymes and ribosomal RNA) also catalyze reactions.
- The study of enzymatic processes is the oldest field of biochemistry, dating back to late 1700s.
- The study of enzymes has dominated biochemistry in the past and continues to do so.

Enzymes are macromolecular biological catalysts

碳酸酐酶，效率超级高~

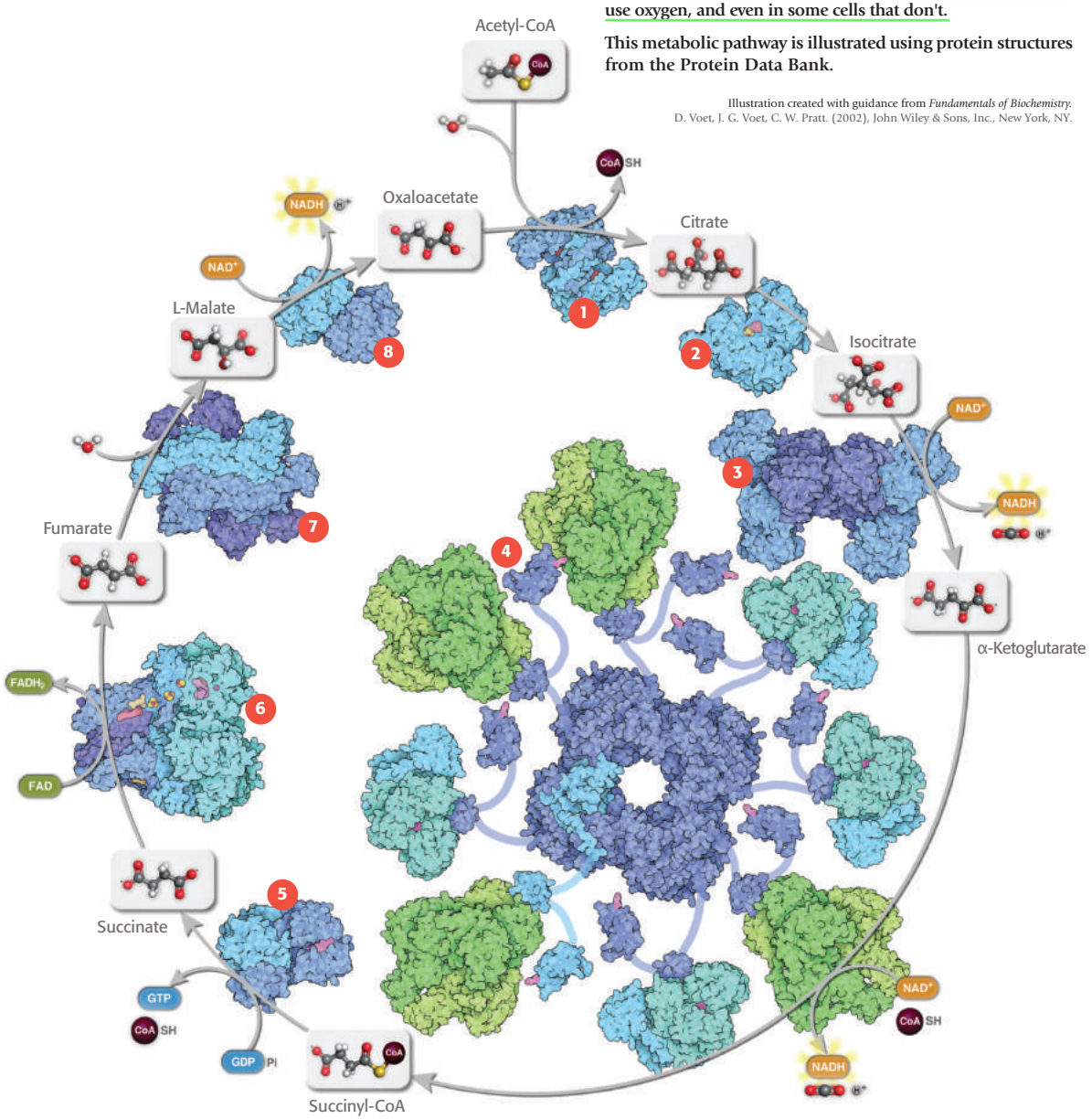


The Structures of the Citric Acid Cycle

Also known as the Krebs cycle or the tricarboxylic acid cycle, the *citric acid cycle* is at the center of cellular metabolism. It plays a starring role in both the process of energy production and biosynthesis. The cycle finishes the sugar-breaking job started in glycolysis and fuels the production of ATP in the process. It is also a central hub in biosynthetic reactions, providing intermediates that are used to build amino acids and other molecules. Citric acid cycle enzymes are found in all cells that use oxygen, and even in some cells that don't.

This metabolic pathway is illustrated using protein structures from the Protein Data Bank.

Illustration created with guidance from *Fundamentals of Biochemistry.* D. Voet, J. G. Voet, C. W. Pratt. (2002), John Wiley & Sons, Inc., New York, NY.



Enzymes are extraordinary catalysts

高效催化剂！

TABLE 6–5 Some Rate Enhancements Produced by Enzymes

Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

乳清酸脱羧酶（？）

Most enzymes are proteins

The Nobel Prize in Chemistry 1946

James Batcheller Sumner

Prize share: 1/2

John Howard Northrop

Prize share: 1/4

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"*.

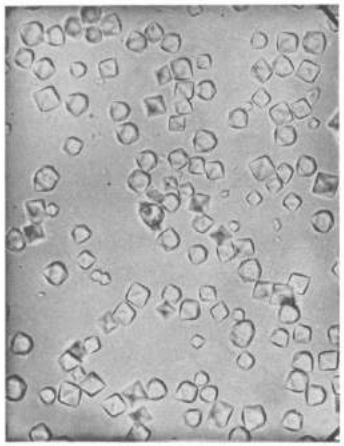
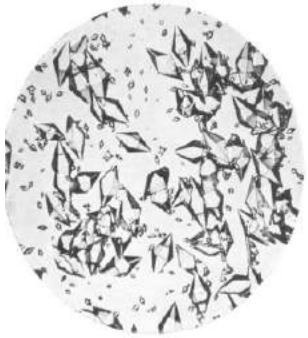


Fig. 1. Photomicrograph of urease crystals magnified 728 diameters.

Octahedral crystals of jack bean urease



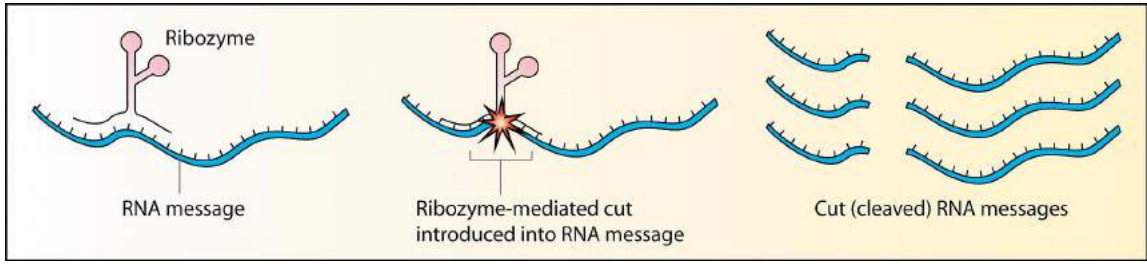
Pepsin crystals

Some RNA can function as enzyme (Ribozyme): implications in the origin of life

The Nobel Prize in Chemistry 1989

Sidney Altman
Prize share: 1/2

Thomas R. Cech
Prize share: 1/2



RNase P

自剪切RNA（不算是真正的合酶，反应前后化学性质改变了）

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

RNA催化功能的发现支持了RNA世界假说

Vocabularies

- Reactants in enzyme-catalyzed reactions are called **substrates**.
- Enzymes bring substrates together to form an enzyme-substrate complex on a particular region of the enzyme called the **active site** (catalytic site + binding site).
- Many enzymes require cofactors for activity. Cofactors are small molecules that some enzymes require for activity. The two main classes of cofactors are **coenzymes** (organic molecules derived from vitamins) and metals.
- Tightly bound coenzymes are called **prosthetic groups**.
- An enzyme with its cofactor is a **holoenzyme**. Without the cofactor, the enzyme is called an **apoenzyme**.

apo：empty（?）

Protein structure

Scaffold to support and position active site

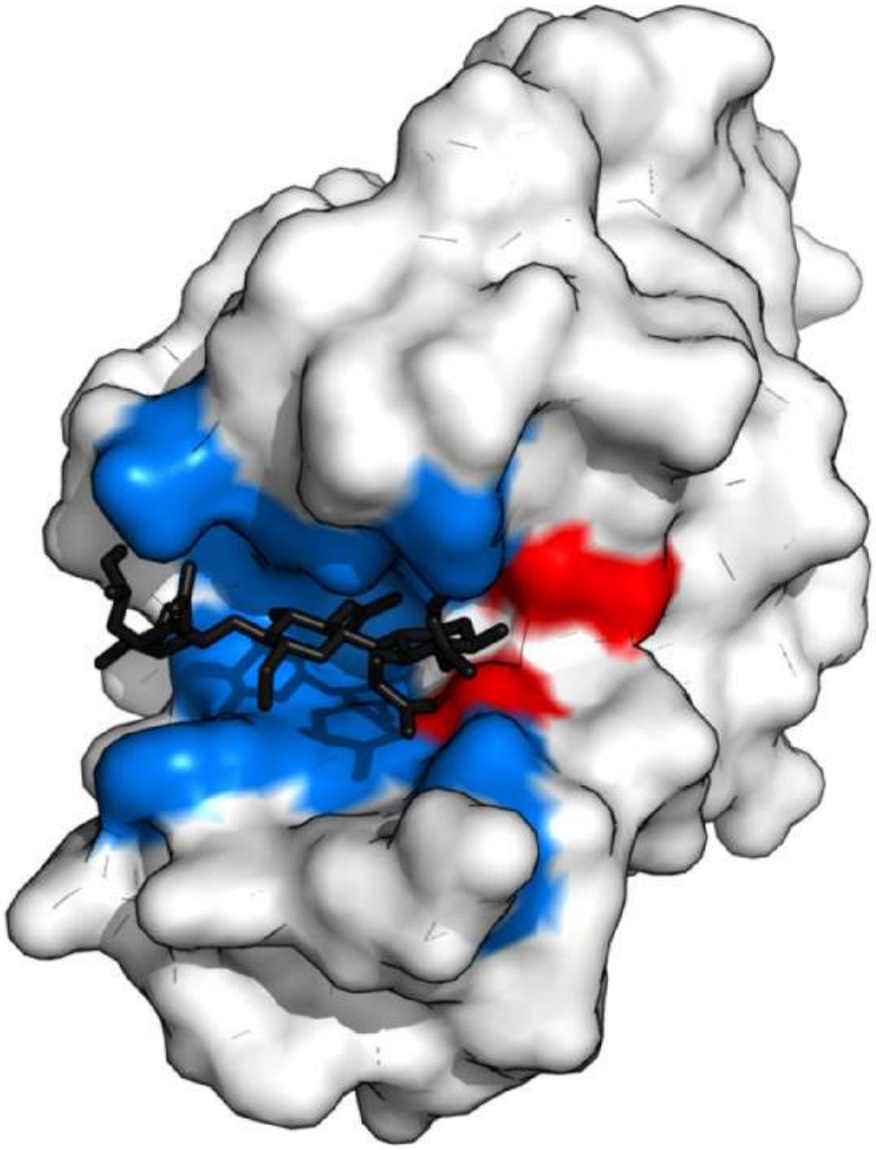
Active site

Binding sites

Bind and orient substrate(s)

Catalytic site

Reduce chemical activation energy



Cofactors and Coenzymes

TABLE 6–2 Some Coenzymes That Serve as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin 生物胞素	CO_2 一碳单位载体！	Biotin 泛酸
Coenzyme A	Acyl groups 乙酰基	Pantothenic acid and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups H原子、烷基	Vitamin B_{12}
Flavin adenine dinucleotide FAD	Electrons	Riboflavin (vitamin B_2)
Lipoate 硫辛酸	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide NAD	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
Pyridoxal phosphate 磷酸吡哆醛	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate 四氢叶酸	One-carbon groups	Folate
Thiamine pyrophosphate TPP	Aldehydes 醛基	Thiamine (vitamin B_1)

Note: The structures and modes of action of these coenzymes are described in Part II.

Enzymes can be classified by the reactions they catalyze

TABLE 6–3 International Classification of Enzymes

Class no.	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leavin… bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to cleavage of ATP or similar cofactor

脱氢酶
转移酶
水解酶
裂合酶
异构酶
连接酶

IUBMB Enzyme Nomenclature

EC 2.7.1.1

2: class name (transferase);
7: subclass (phosphotransferase);
1: a hydroxyl group as acceptor; 羟基作为磷酸受体
1: D-hexose D-己糖

Accepted name: hexokinase

Reaction: ATP + D-hexose = ADP + D-hexose 6-phosphate

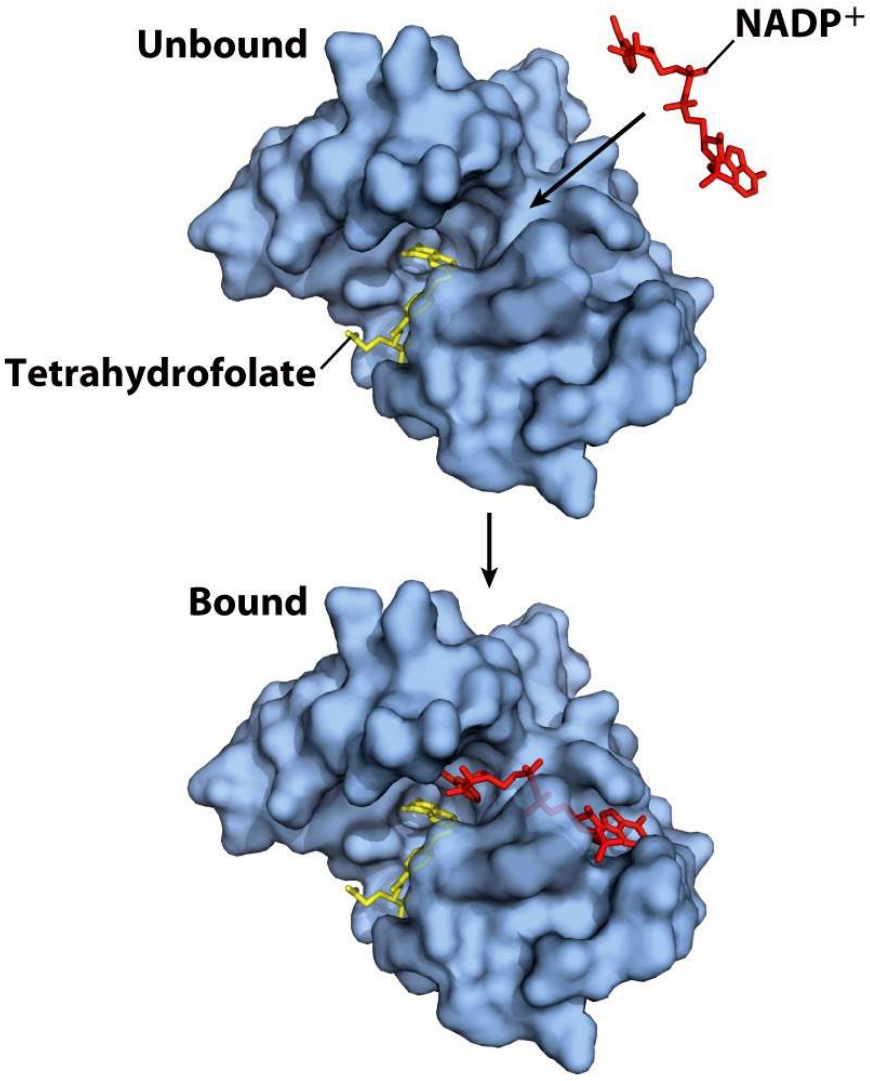
Other name(s): hexokinase type IV glucokinase; hexokinase D; hexokinase type IV; hexokinase (phosphorylating); ATP-dependent hexokinase; glucose ATP phosphotransferase

Systematic name: ATP:D-hexose 6-phosphotransferase

Comments: D-Glucose, D-mannose, D-fructose, sorbitol and D-glucosamine can act as acceptors; ITP and dATP can act as donors. The liver isoenzyme has sometimes been called glucokinase.

Links to other databases: BRENDA, EXPASY, GTD, KEGG, Metacyc, PDB, UM-BBD, CAS registry number: 9001-51-8

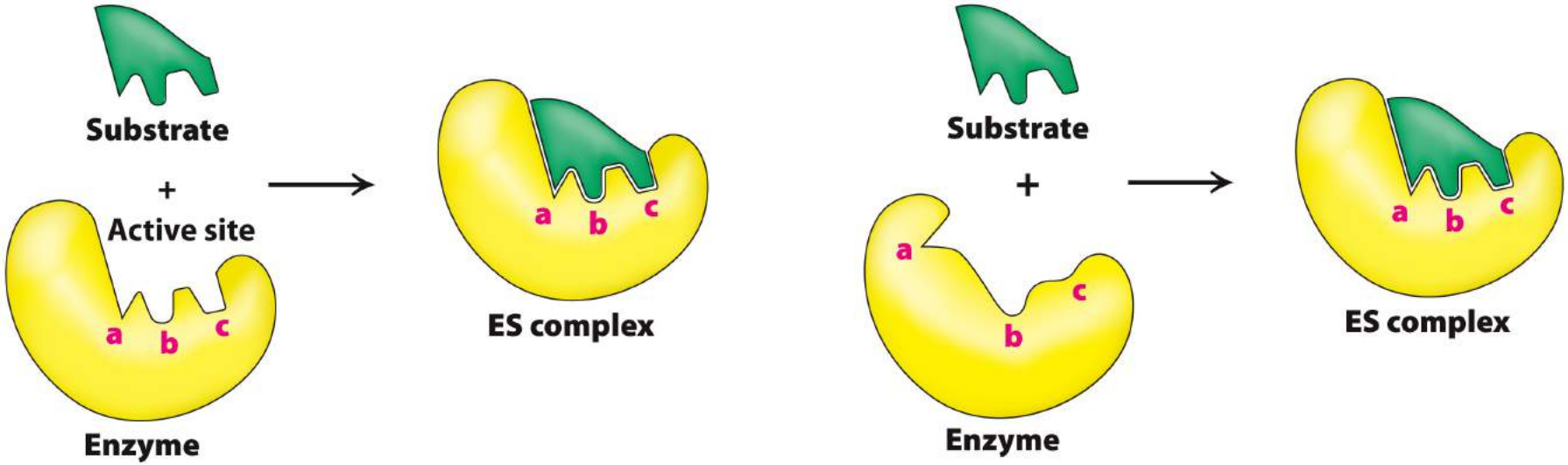
Enzymes display a high degree of specificity



Dihydrofolate reductase

The specificity is due to the precise interaction of the enzyme and its substrate, achieved by binding pockets with complementary shape, charge and hydrophilic/hydrophobic properties of the substrates.

“Lock and key” vs “induced fit”

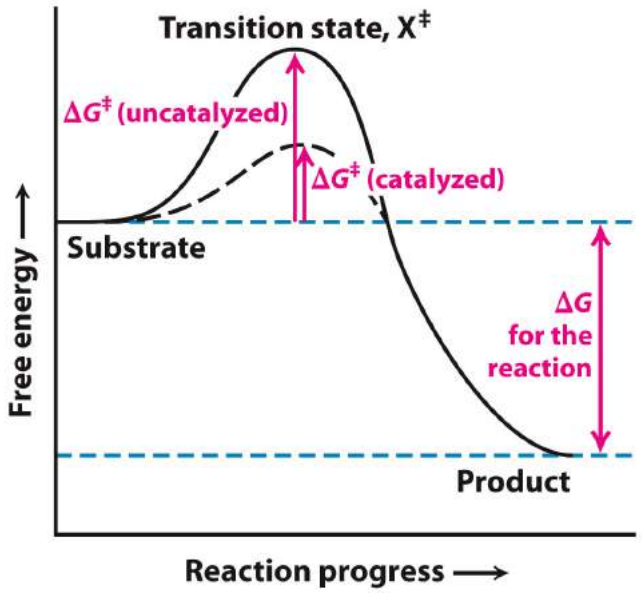


If so, there will not be catalysis.

Enzymes do not interact with their substrates like a lock and key.
Rather, the enzyme changes shape upon substrate binding, a phenomenon called induced fit.

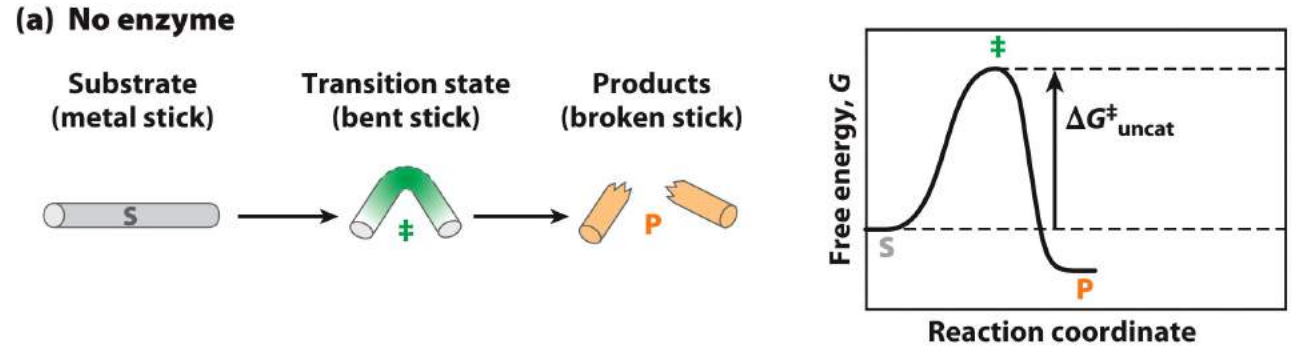
Enzymes alter reaction rate, not equilibrium

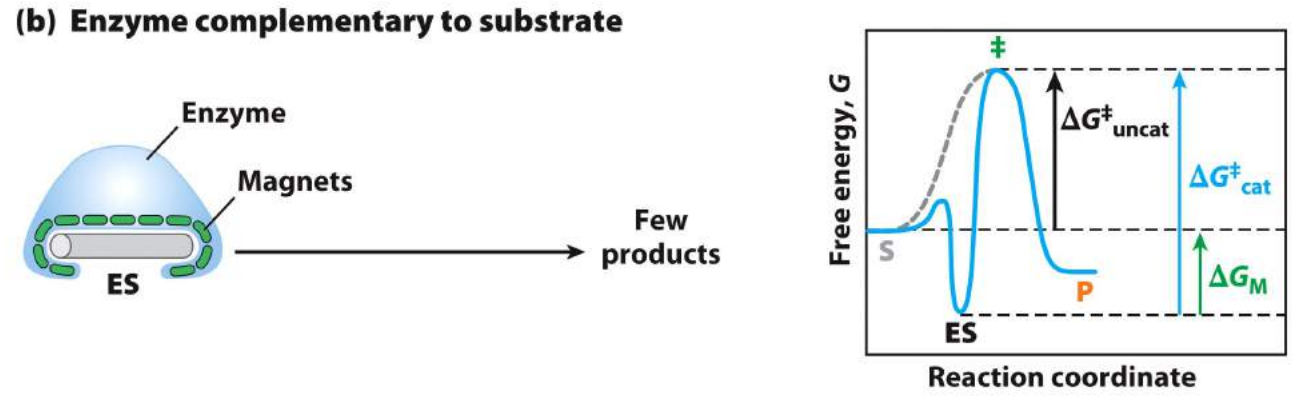
- A chemical reaction proceeds through a **transition state**, a molecular form that is no longer substrate but not yet product.
- The **activation energy** ($\Delta G^{\ddagger}$) is the difference between the energy levels of the ground state and the transition state.

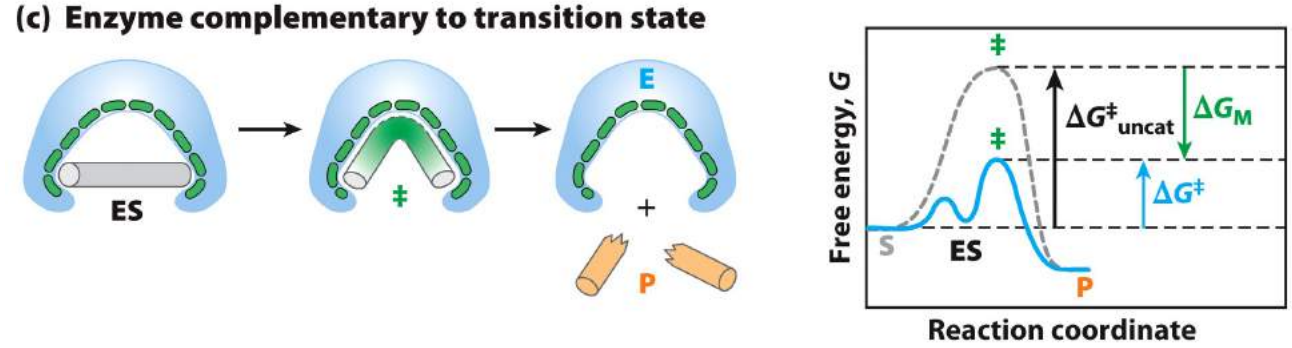


- Enzymes catalyze reaction by lowering down the activation energy.
- The reaction equilibrium is determined only by the free-energy difference between the products and reactants. Enzymes cannot alter this difference.
- In the presence of an enzyme, the reaction runs in the same direction as it would without the enzyme, just more quickly.

Enzymes facilitate formation of the transition state







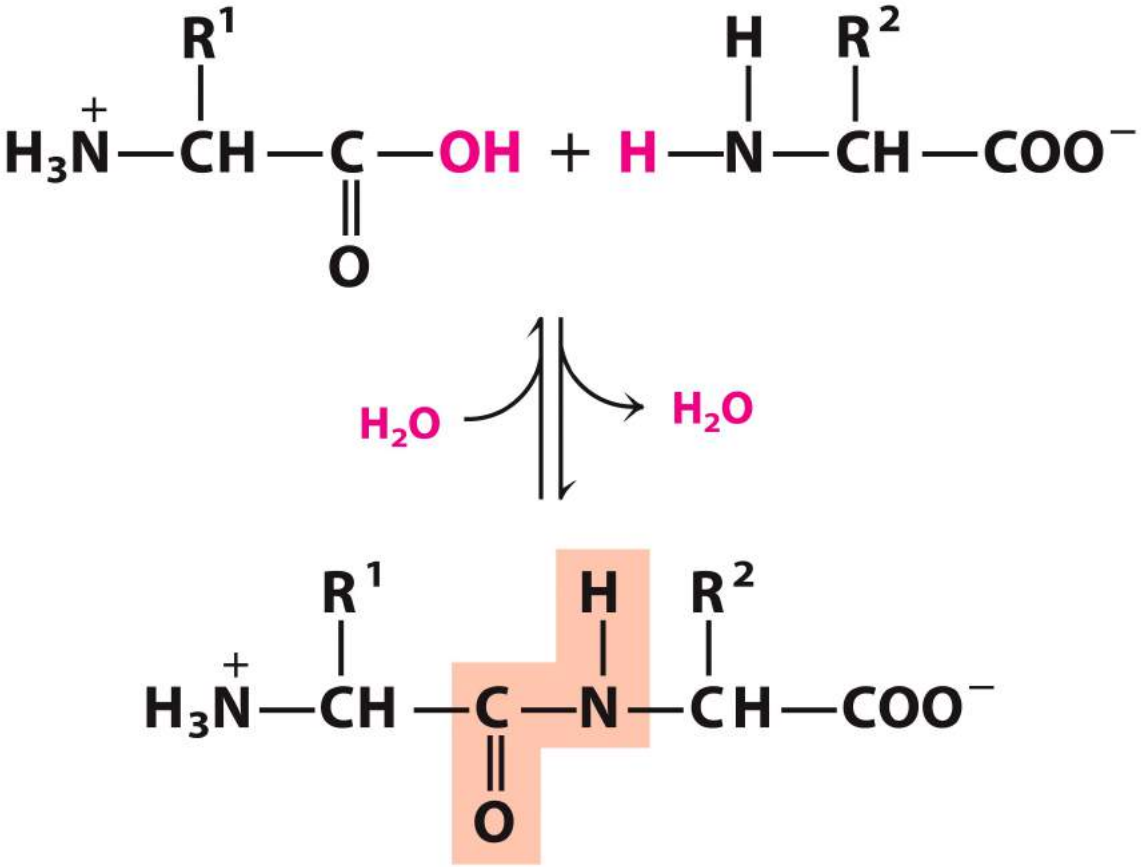
Common catalytic strategies

- General acid-base catalysis: A molecule other than water donates or accepts a proton. 广义酸碱催化
- Covalent catalysis: The active site contains a nucleophile that is briefly covalently modified. 亲核基团 共价催化
- Metal ion catalysis: Metal ions function in a number of ways, including serving as an electrophilic catalyst. 亲电催化 金属离子催化
- Catalysis by approximation: The enzyme brings two substrates together in an orientation that facilitates catalysis. 临近定向效应

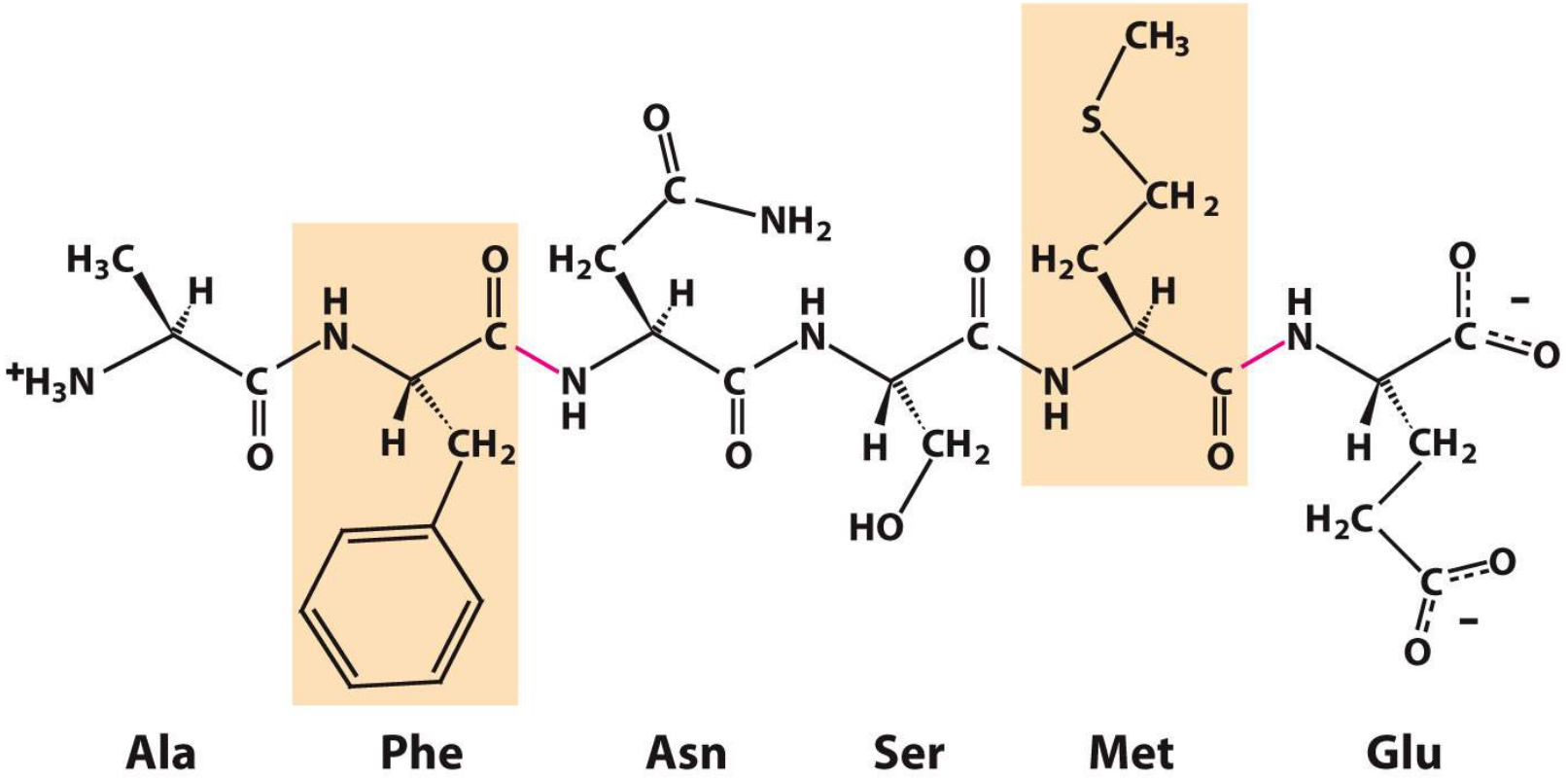
蛋白水解酶

Proteases catalyze an energetically favorable, but kinetically difficult reaction

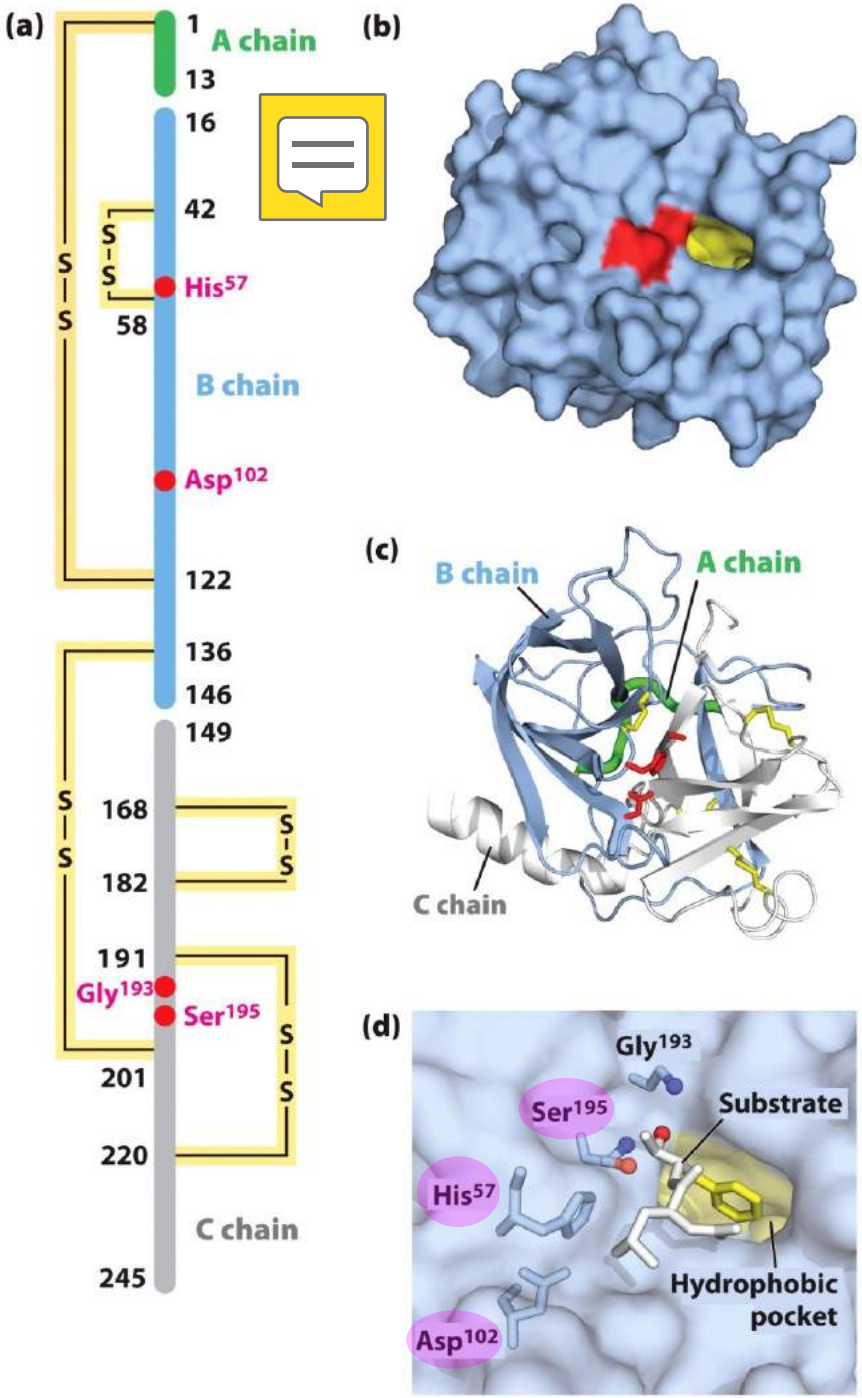
能量上有利，但是肽键稳定，不易反应



Chymotrypsin selectively hydrolyzes peptide bonds on the carboxyl side of large hydrophobic amino acids



Chymotrypsin is a proteolytic enzyme secreted by the pancreas that hydrolyzes peptide bonds selectively on the carboxyl side of large hydrophobic amino acids

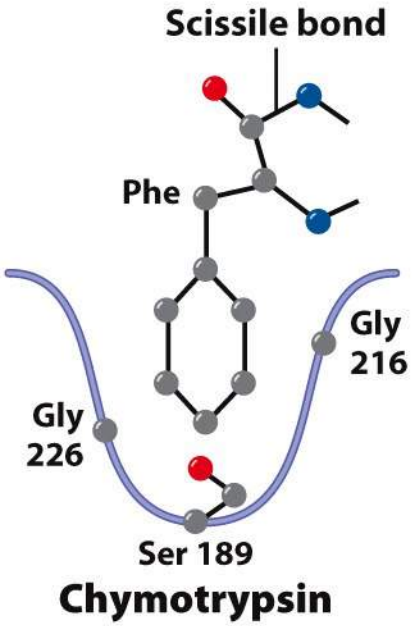


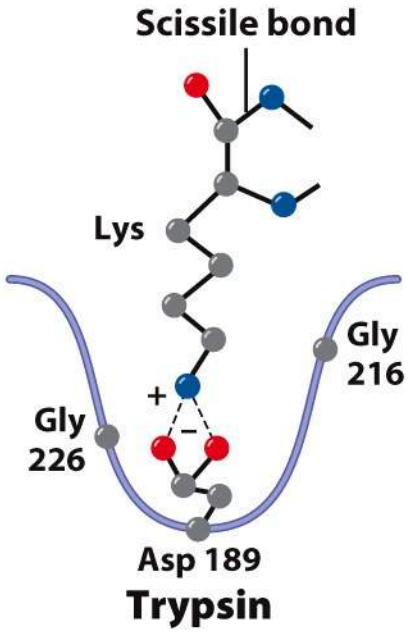
Chymotrypsin structure

- A binding site recognizes a large hydrophobic amino acids especially aromatic residues;
- A catalytic site contains a catalytic triad (Ser, His, Asp),which acts via a mixture of covalent and general acid-base catalysis to cleave a peptide bond.

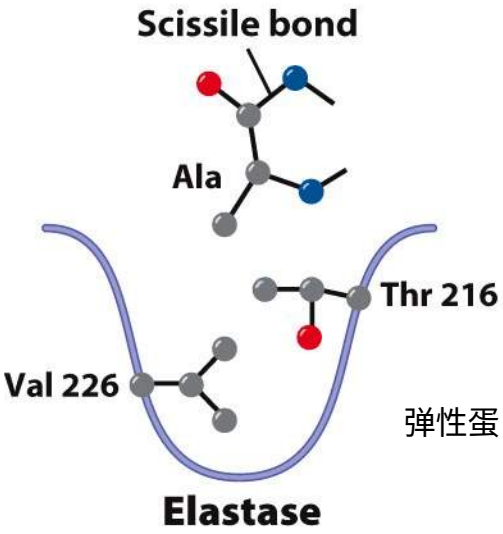
Specificity of the serine proteases

口袋的形状及周围的aa决定了ser prE家族蛋白酶的特异性



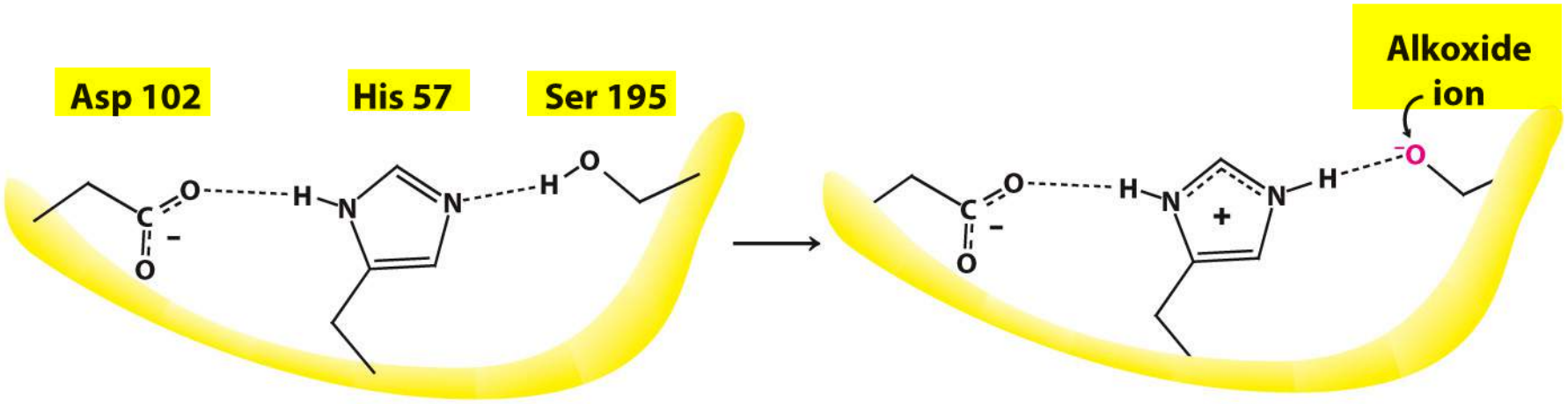


胰蛋白酶
就Lys最合适，Arg都大了点

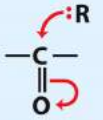
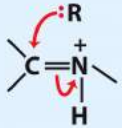
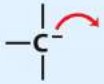
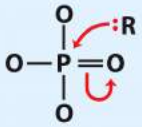
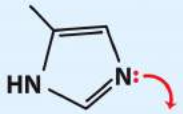


弹性蛋白酶，poket只装得下小aa

Catalytic triad 催化三联体



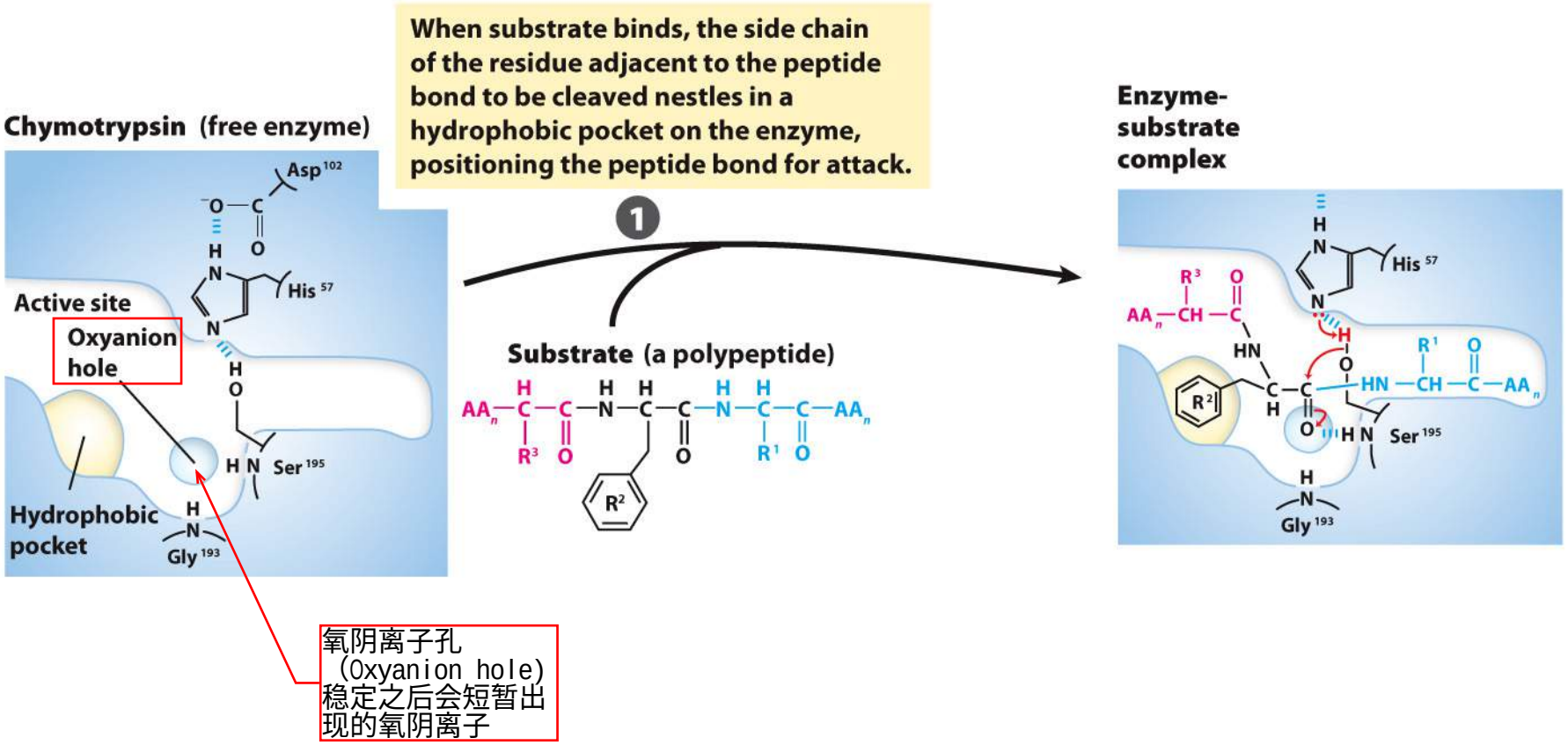
- Aspartate orients the histidine and renders it a better proton acceptor.
- Histidine removes a proton from serine 195, generating a highly reactive alkoxide ion (RO^-).
- The alkoxide ion attacks the peptide bond of the substrate.

Nucleophiles	Electrophiles
 Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)	 Carbon atom of a carbo (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)
 Negatively charged sulfhydryl	 Protonated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)
Carbanion 	Phosphorus of a phosphate group 
 Uncharged amine group	 Proton
Imidazole 	
 Hydroxide ion	

- An electron-rich atom is called a nucleophile (Lewis base).
- An electron-deficient atom is called an electrophile (Lewis acid).
- Nonbonded electrons are designated by dots.
- Curved arrows represent the movement of electron pairs. (A single-headed arrow represents the movement of a single electron.)

Chymotrypsin Mechanism

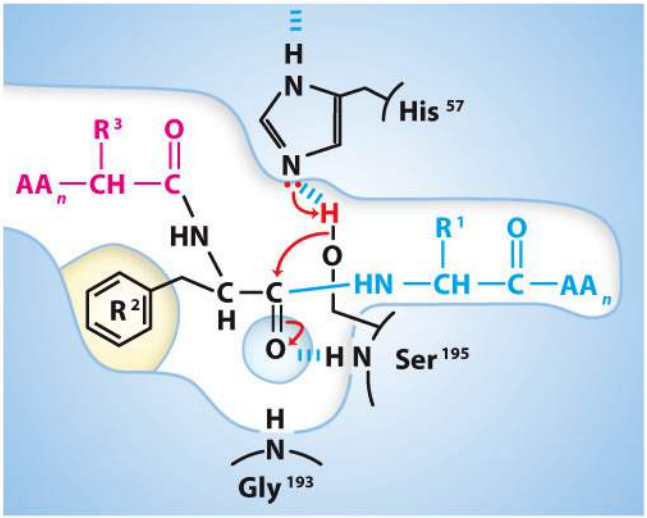
Step 1: Substrate Binding



Step 2: Nucleophilic Attack

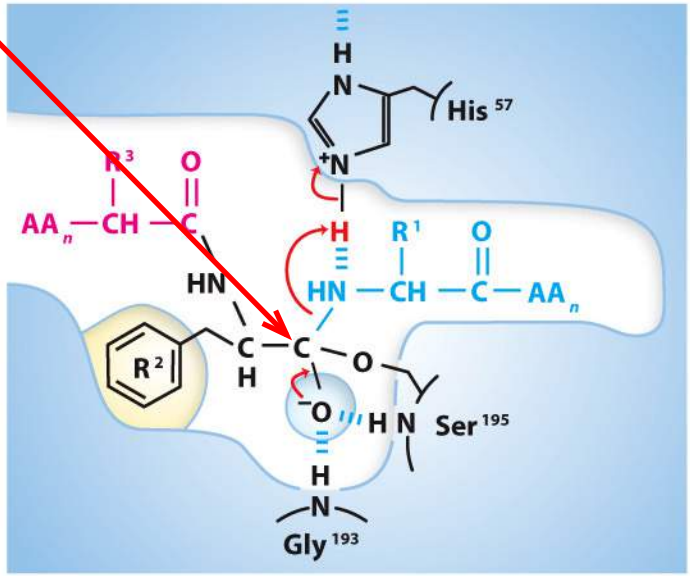
Enzyme-substrate complex

Interaction of Ser[195] and His[57] generates a strongly nucleophilic alkoxide ion on Ser[195]; the ion attacks the peptide carbonyl group, forming a tetrahedral acyl-enzyme. This is accompanied by formation of ashort-lived negative charge on the carbonyl oxygen of the substrate, which is stabilized by hydrogen bonding in the oxyanion hole.



2

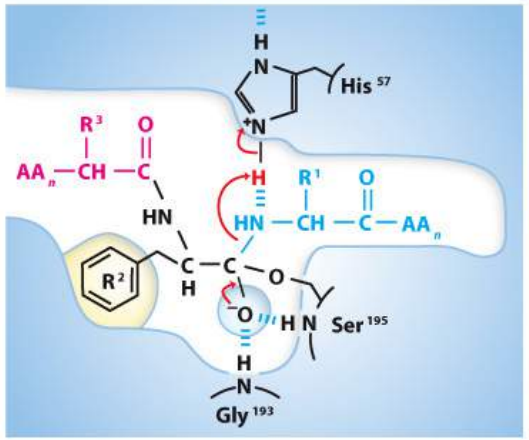
Short-lived intermediate* (acylation)



Step 3: Substrate Cleavage

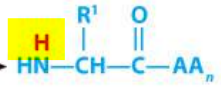
Short-lived intermediate* (acylation)

Instability of the negative charge on the substrate carbonyl oxygen leads to collapse of the tetrahedral intermediate; re-formation of a double bond with carbon displaces the bond between carbon and the amino group of the peptide linkage, breaking the peptide bond. The amino leaving group is protonated by His57, facilitating its displacement.



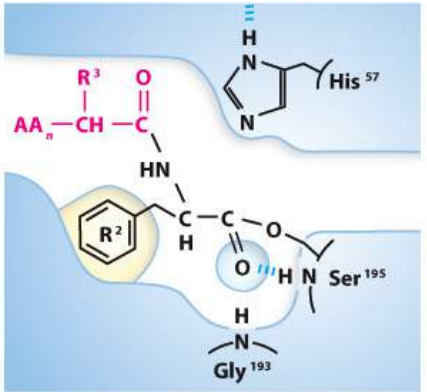
compared to C-O,C-N is easily to break(peptide bond)

Product 1

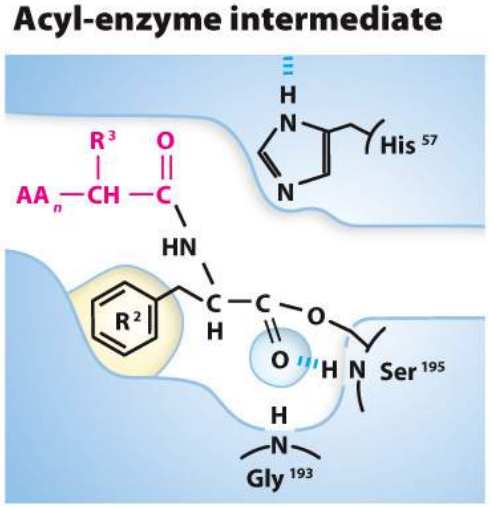


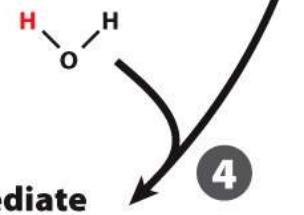
3

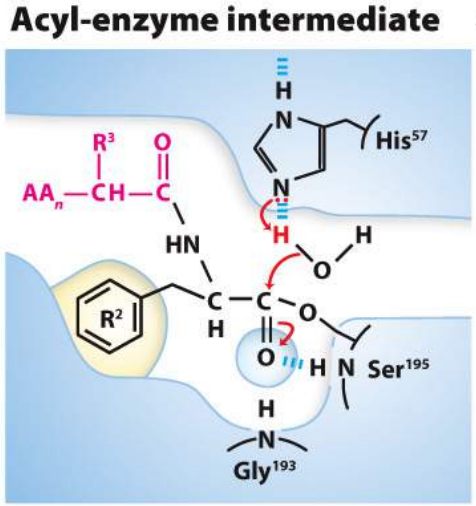
Acyl-enzyme intermediate

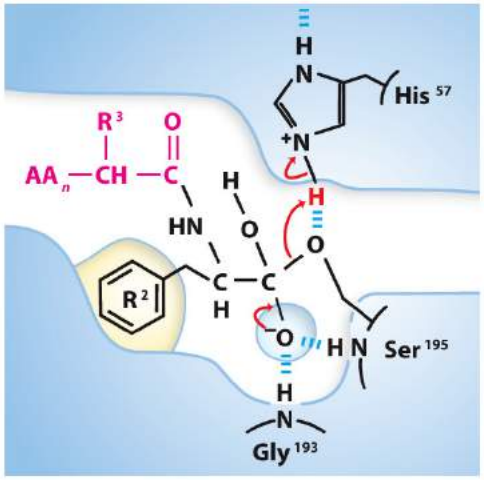


Step 4&5: Water Comes In and attacks





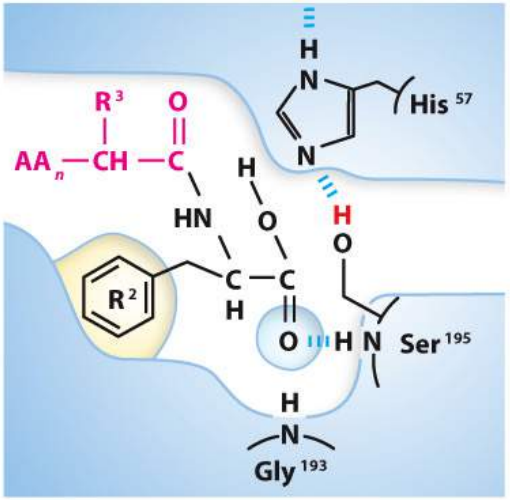




An incoming water molecule is deprotonated by general base catalysis, generating a strongly nucleophilic hydroxide ion. Attack of hydroxide on the ester linkage of the acyl-enzyme generates a second tetrahedral intermediate, with oxygen in the oxyanion hole again taking on a negative charge.

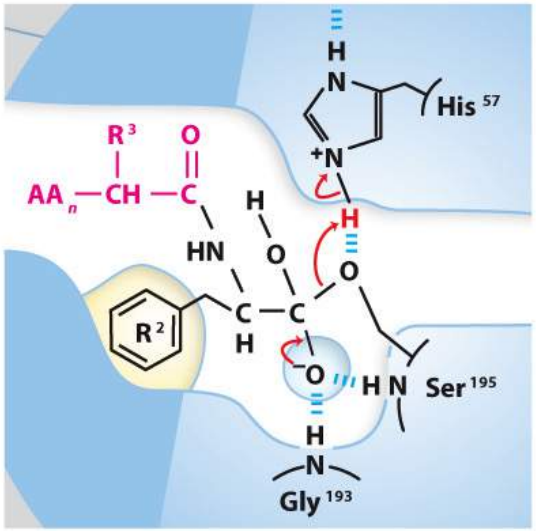
Step 6: Break-off from the Enzyme

Enzyme-product 2 complex



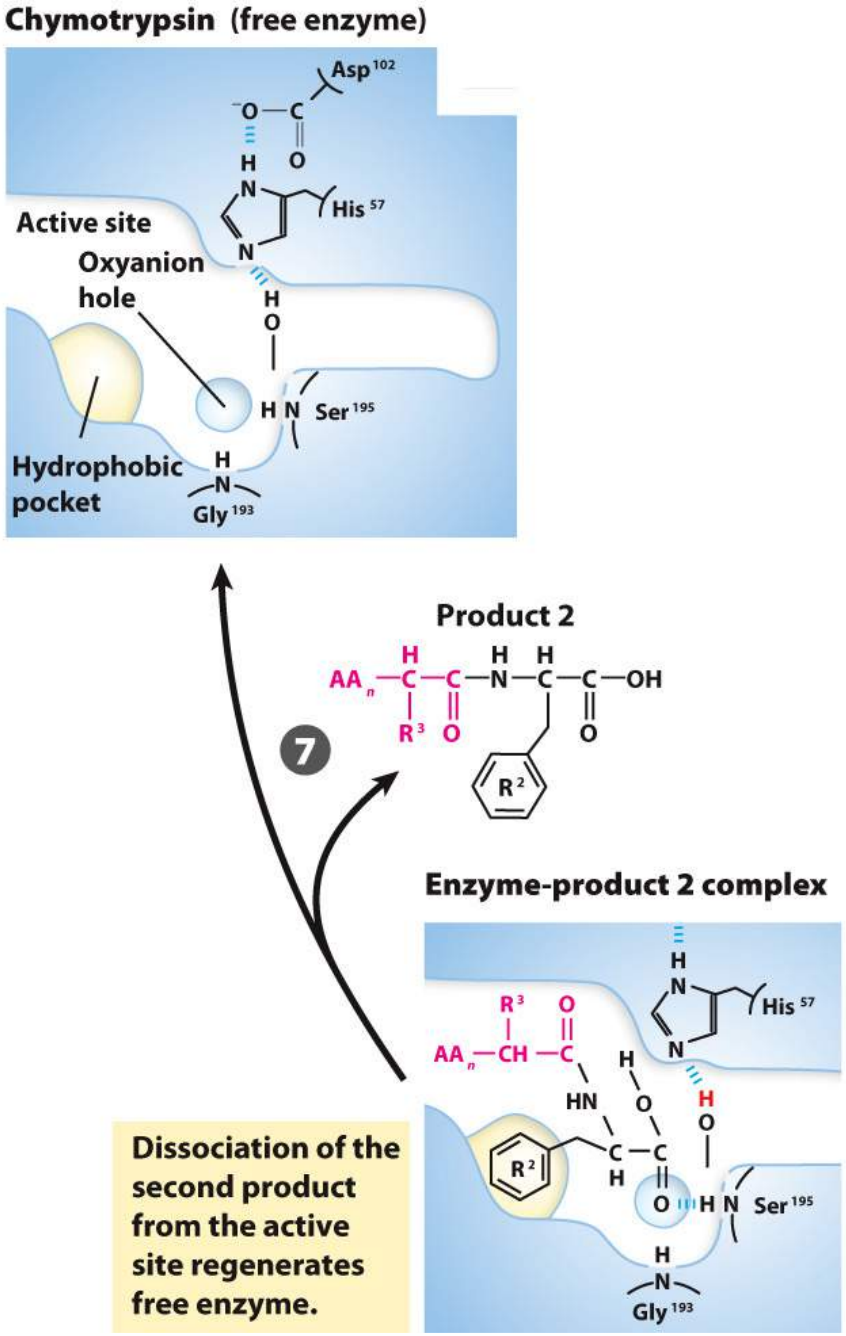
6

Short-lived intermediate *
(deacylation)



Collapse of the tetrahedral intermediate forms the second product, a carboxylate anion, and displaces Ser_{195}.

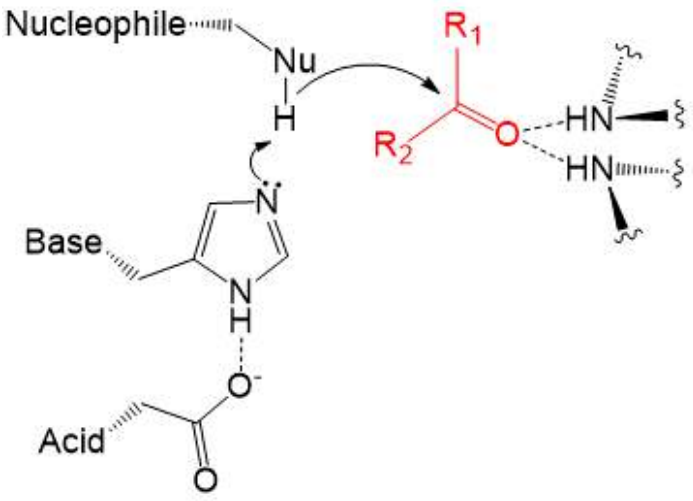
Step 7: Product Dissociates



Catalytic triad

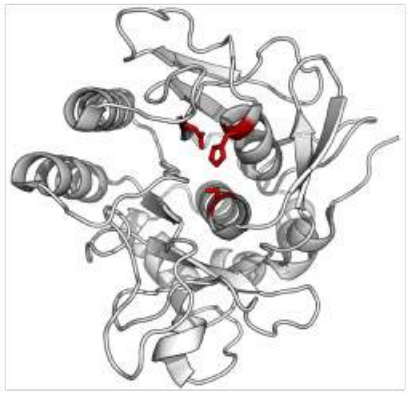
- An **Acid-Base-Nucleophile triad** is a common motif for generating a nucleophilic residue for covalent catalysis
- The acid residue (Asp, Glu) aligns and polarizes the base (His, Lys), which activates the nucleophile (Ser, Cys, Thr). The nucleophilic residue then attacks the substrate.
- An **oxyanion hole** in the enzyme (usually formed by backbone amides) stabilizes the negative charge on the **alkoxide** (RO^-) during catalysis.
- Present in many hydrolases (水解酶), including proteases, amidases, esterases, acylases, lipases, β-lactamases, etc.

生成共价催化的亲核残基

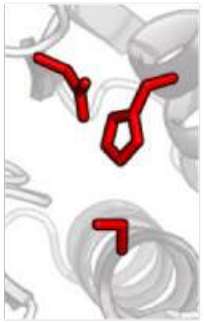


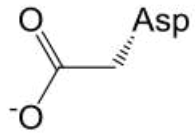
Catalytic triad and Convergent evolution

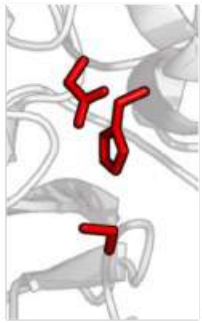
催化三联体空间位置存在趋同演化

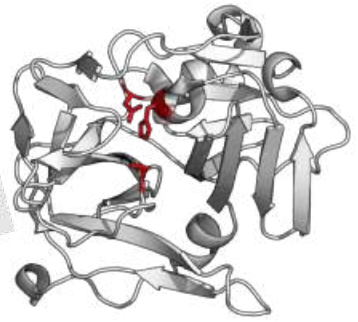


Subtilisin



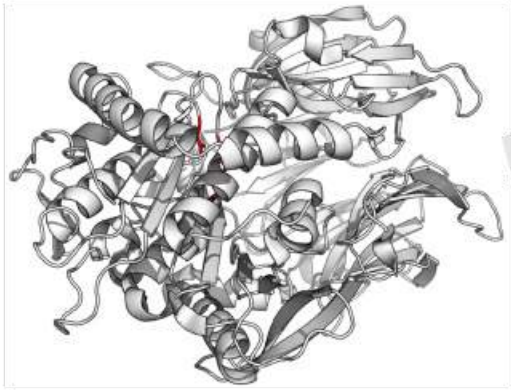


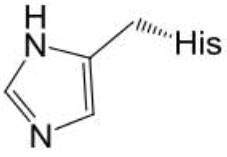


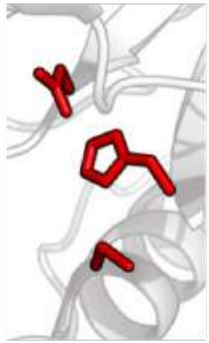


TEV protease

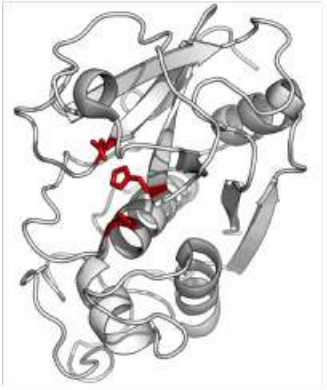
Prolyl oligopeptidase



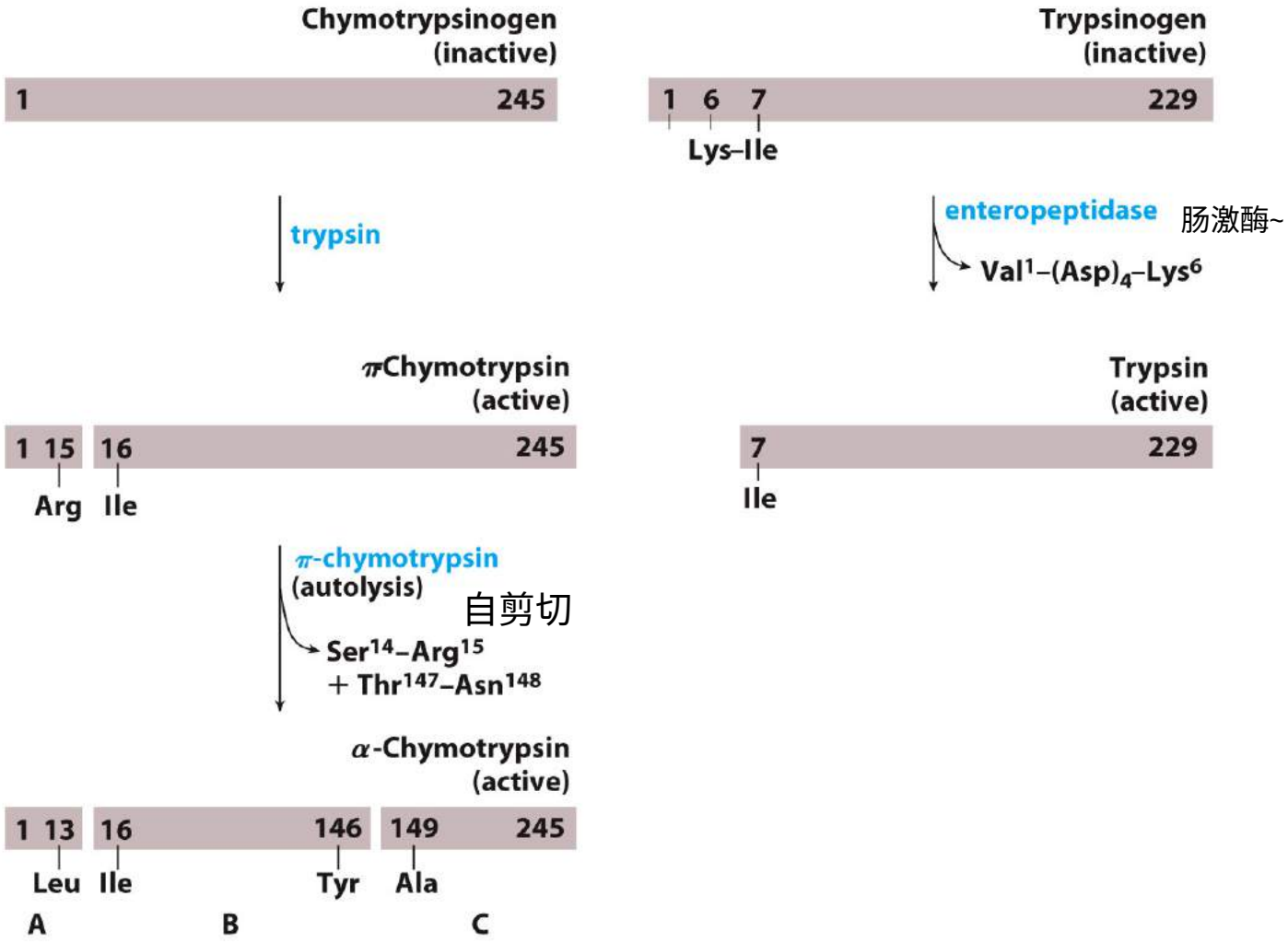




Papain



Zymogens are activated by irreversible covalent modification



Control of Enzymes

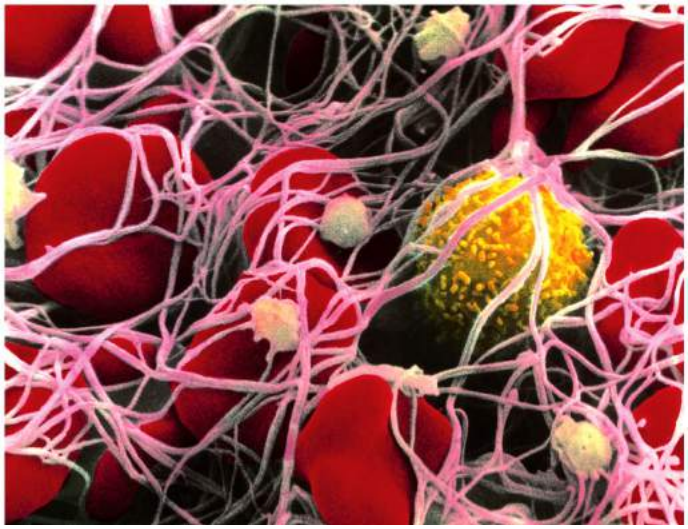
Five means to regulate enzymatic activity:

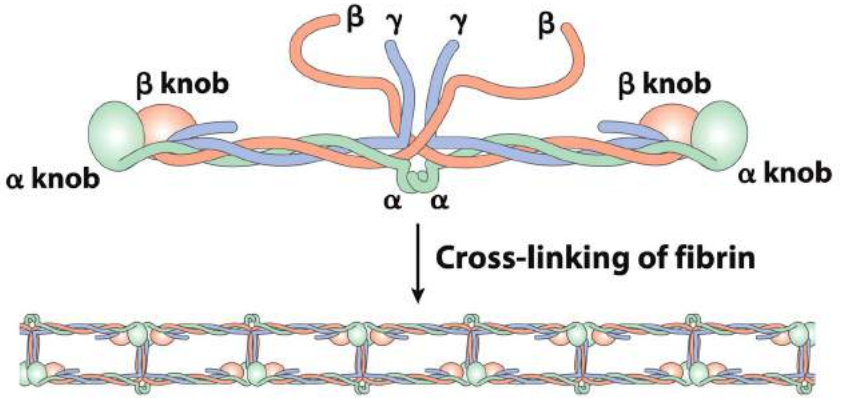
1. Multiple Forms of Enzymes 同工酶
2. Controlling the Amount of Enzyme Present
3. **Proteolytic Activation**
4. **Reversible Covalent Modification**
5. **Allosteric Control**

Many enzymes are regulated by multiple mechanisms.

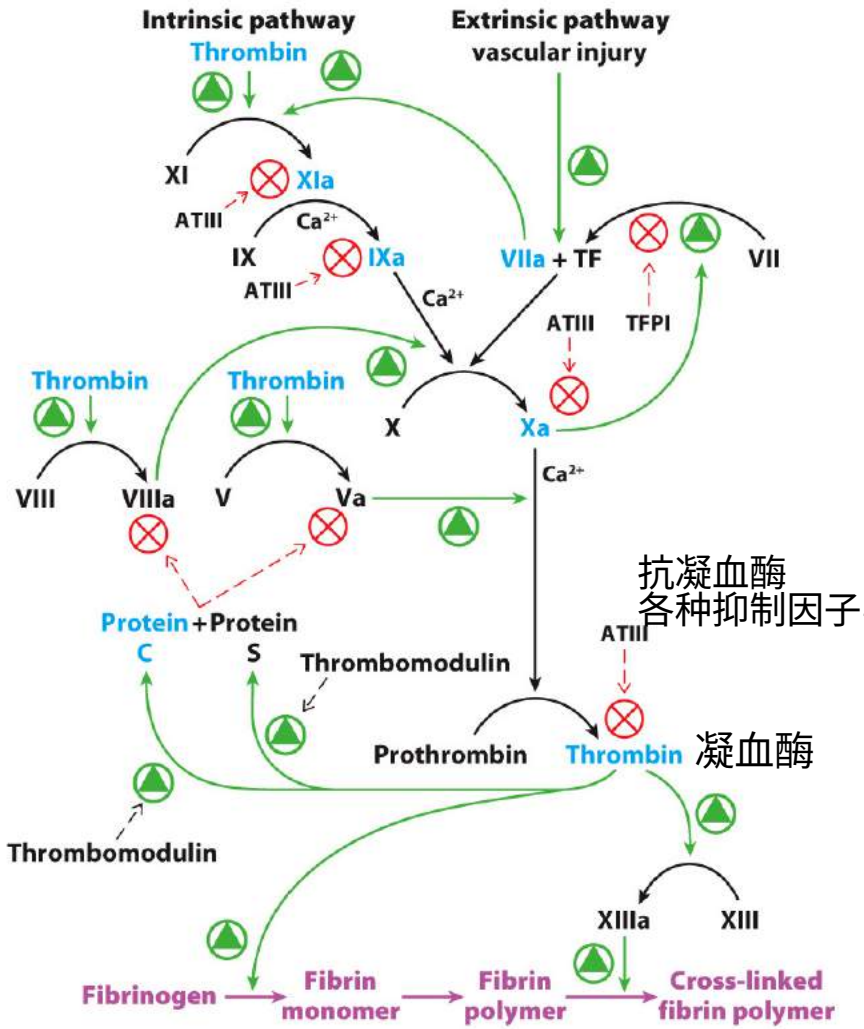
Blood clotting (Coagulation)

- Coagulation (also known as clotting) is the process by which blood changes from a liquid to a gel, forming a blood clot.
- A blood clot consists of aggregated platelets and red blood cells (erythrocytes), which are tied together by cross-linked fibrin.





The coagulation cascade



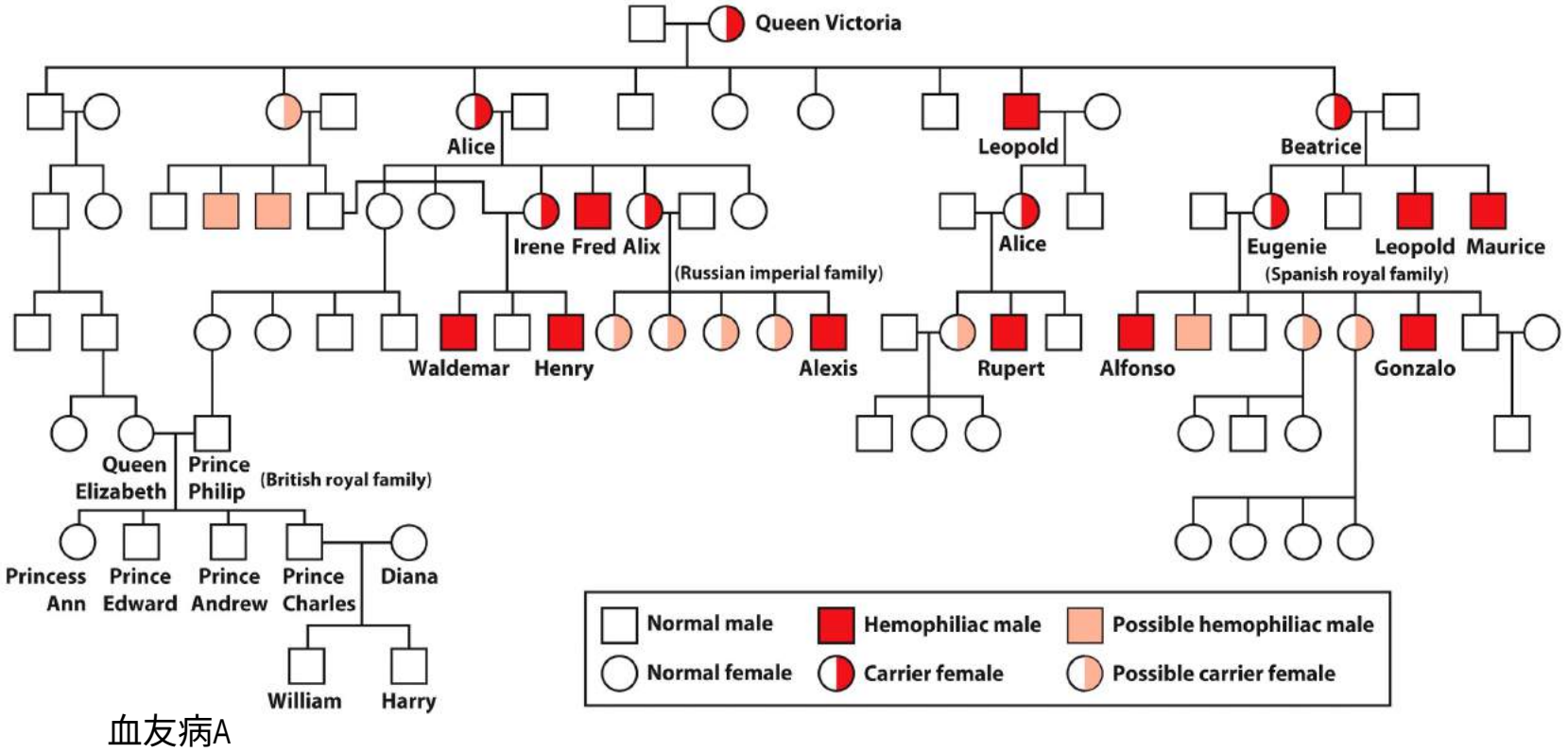
Blood clotting cascades can be initiated in two different ways that lead to a common end point.

- The extrinsic pathway is activated by the the tissue factor (TF).
- The intrinsic pathway is initiated by exposure of anionic surfaces of damaged tissue.

Most of the protein factors in the coagulation cascade are designated by Roman numerals.

Many of these factors are chymotrypsin-like serine proteases that have zymogen precursors (the activated forms are indicated by a subscript “a”).

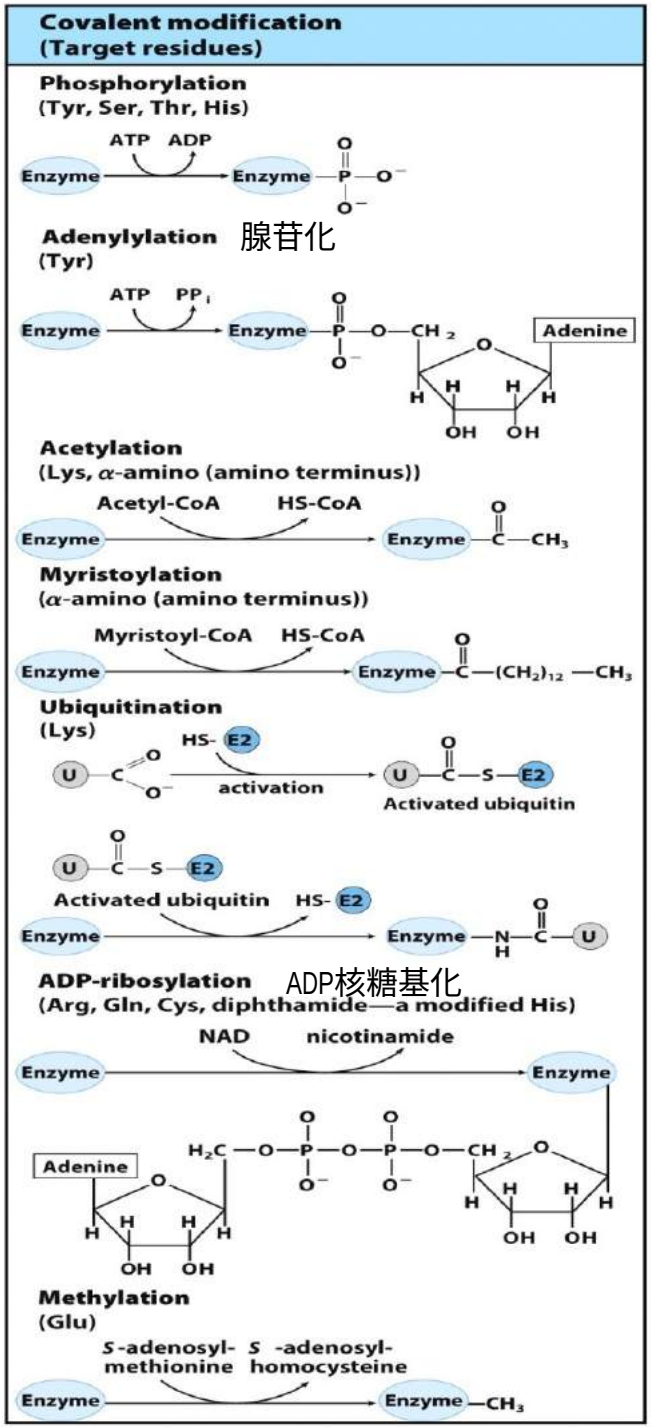
Coagulation defects can cause hemorrhage or thrombosis

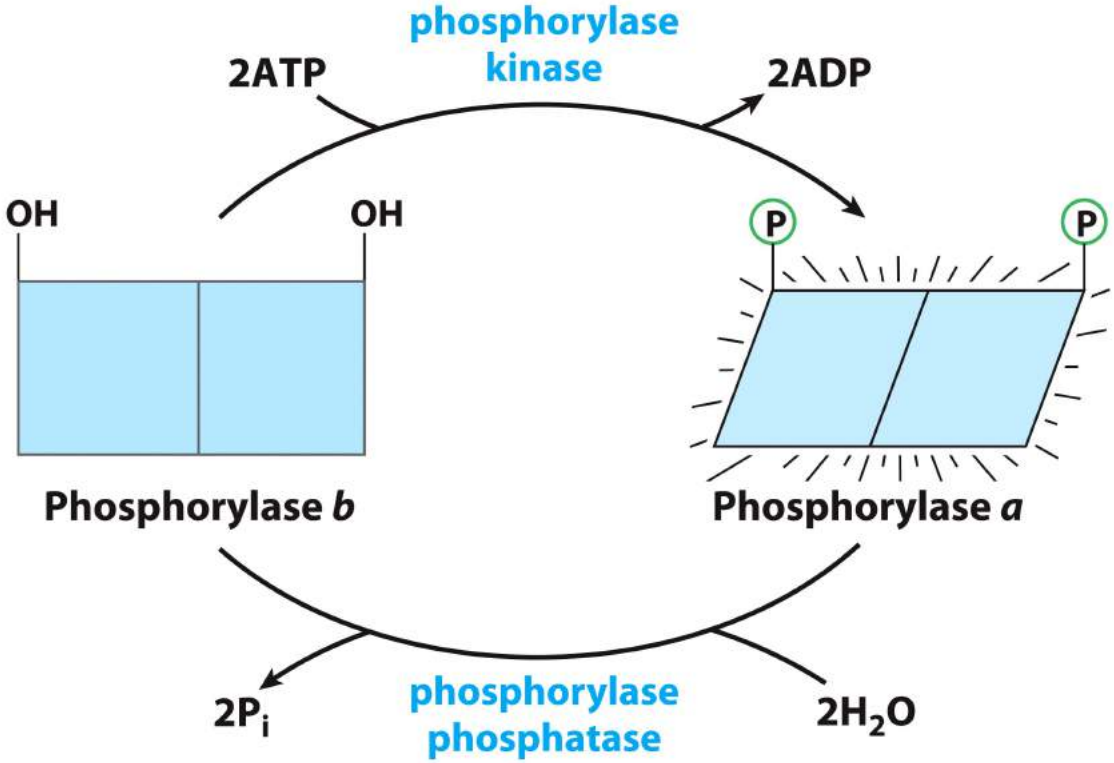


血友病A

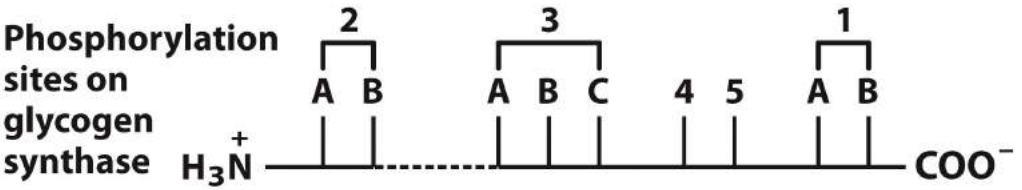
Hemophilia A, the royal disease, is caused by defect in the gene for factor VIII, which is located on the X chromosome

Enzyme regulation by covalent modification





Multiple phosphorylation allow exquisite regulatory control

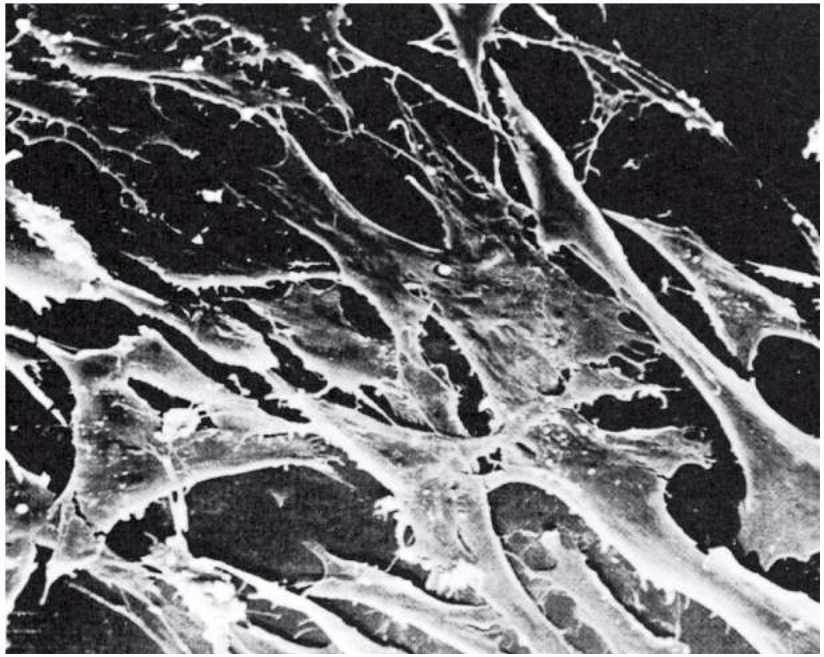


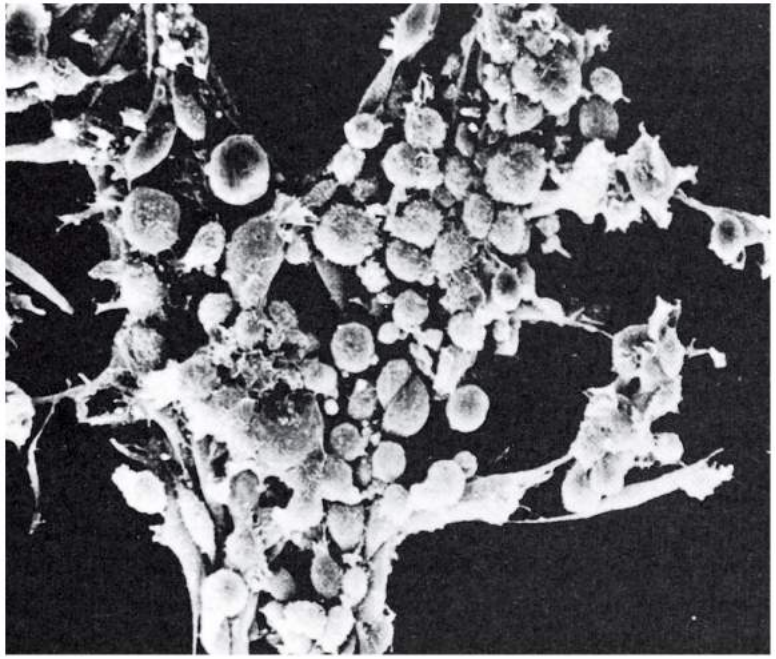
Kinase	Phosphorylation sites	Degree of synthase inactivation
Protein kinase A	1A, 1B, 2, 4	+
Protein kinase G	1A, 1B, 2	+
Protein kinase C	1A	+
Ca^{2+}/calmodulin kinase	1B, 2	+
Phosphorylase *b* kinase	2	+
Casein kinase I	At least nine	+ + + +
Casein kinase II	5	0
Glycogen synthase kinase 3	3A, 3B, 3C	+ + +
Glycogen synthase kinase 4	2	+

多种磷酸化位点
多种激酶
可以实现对酶活性的精细调控

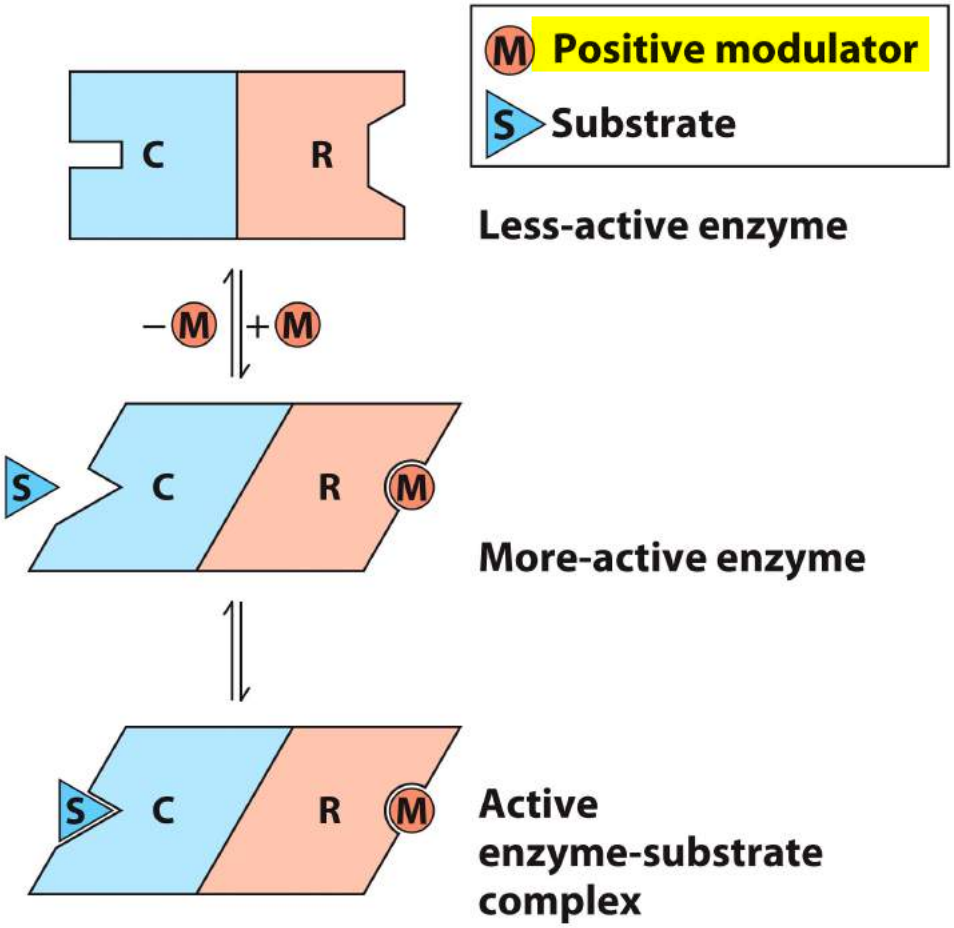
Loss of normal regulatory control can cause cancer

eg.some kinase lose control,stay active, the cell may keep mitoticing

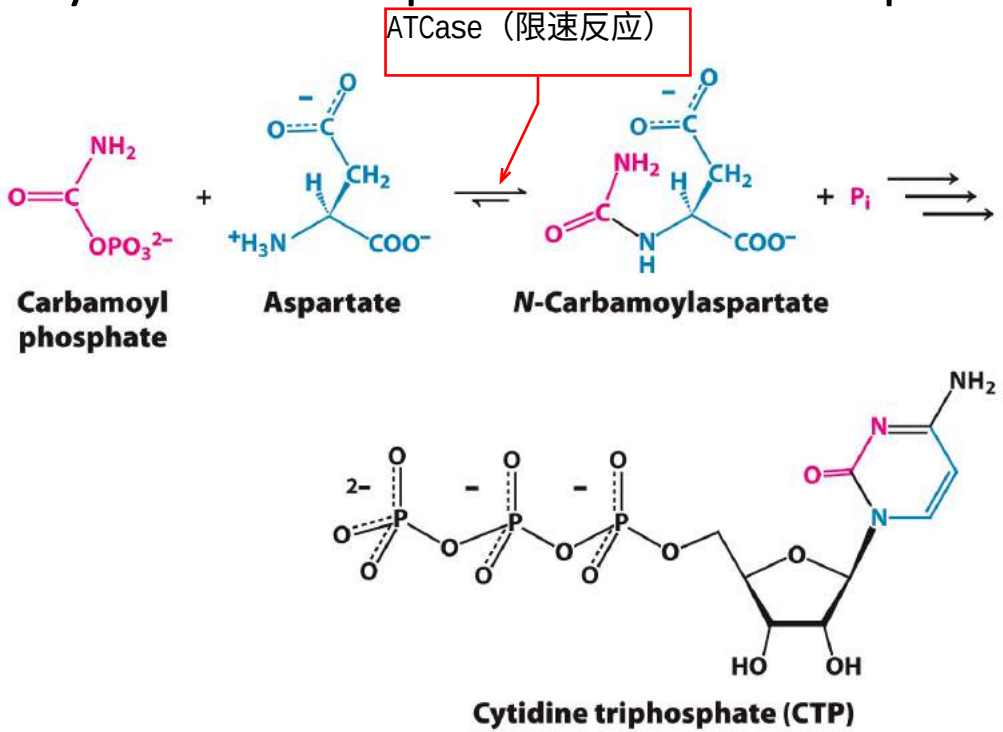




Allosteric enzymes

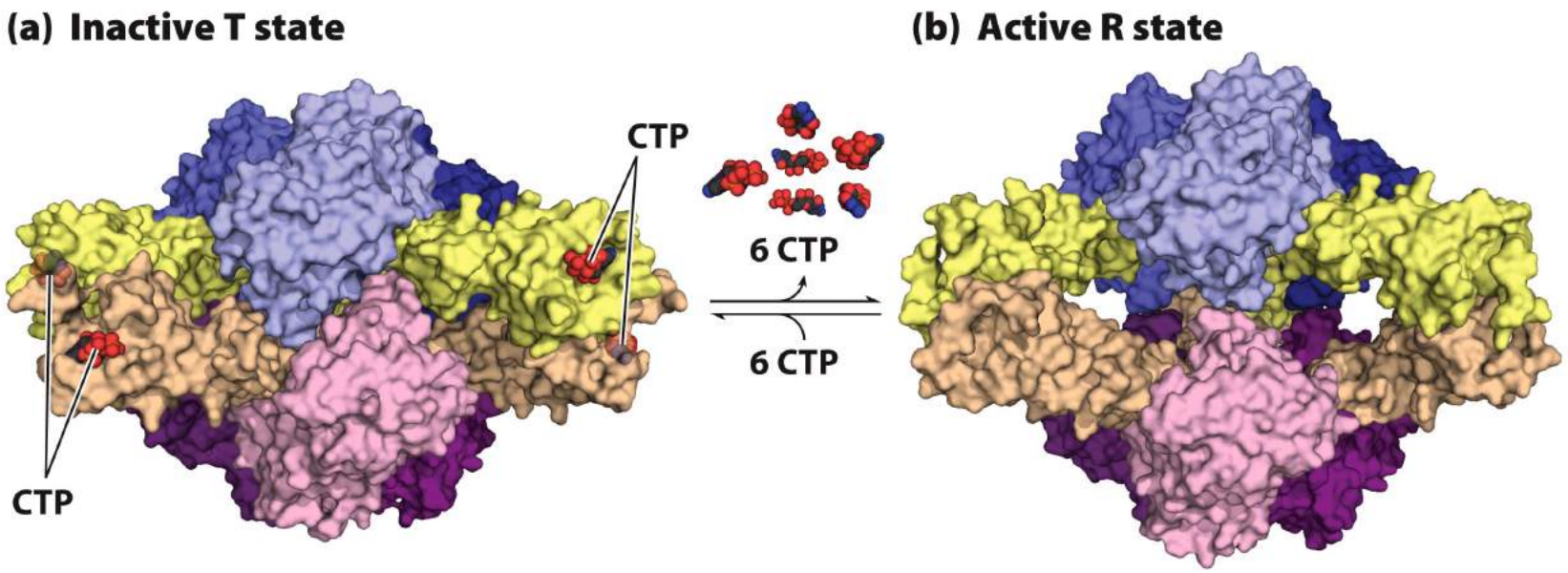


Aspartate transcarbamoylase is allosterically inhibited by the end product of its pathway



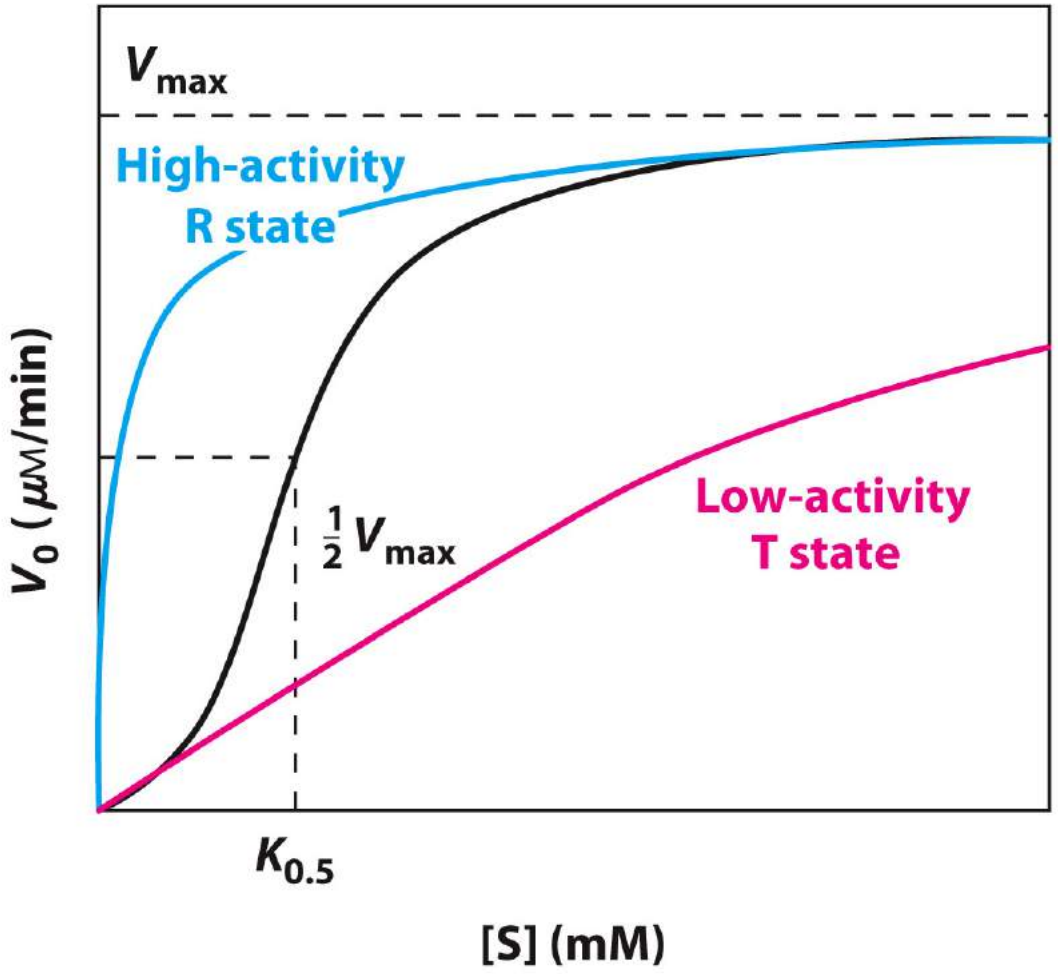
- Aspartate transcarbamoylase catalyzes the committed step, the condensation of aspartate and carbamoyl phosphate to form *N*-carbamoylaspartate, in pyrimidine synthesis.
- ATCase is activated by ATP and Asp (indicators of robust cellular metabolism), and inhibited by the end product of the pathway, CTP. 实现ATP\CTP数量的平衡
- ATP and CTP exert their effects by binding at distinct regulatory or allosteric sites on ATCase.

Allosteric regulation is mediated by conformational changes



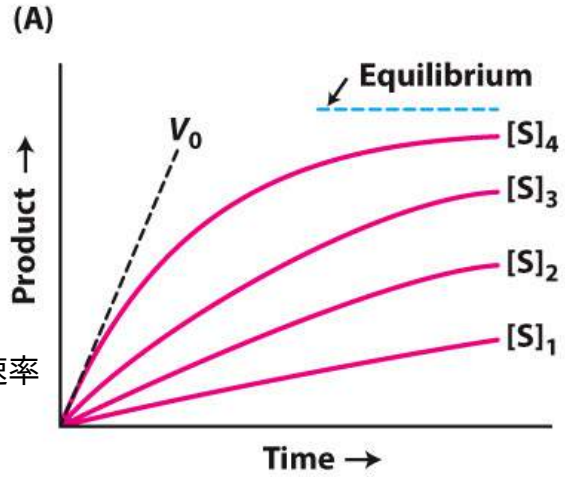
- Native ATCase consists of six catalytic and six regulatory subunits (c_6r_6).
- Free ATCase is in equilibirum between the T state (low activity) and the R state (high activity), with the T state being more favored.
- Binding of CTP to the regulatory site of ATCase alters the T-to-R equilibrium in favor of the T state.
- ATP and Asp alters the T-to-R equilibrium in favor of the R state.

The kinetic properties of allosteric enzymes can diverge from Michaelis-Menten behavior



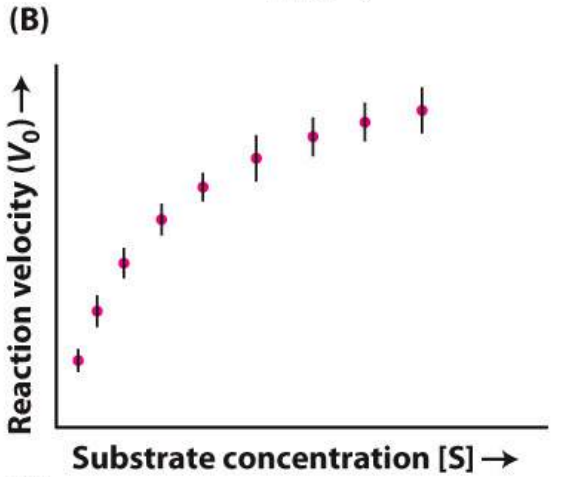
Kinetics is the study of reaction rates

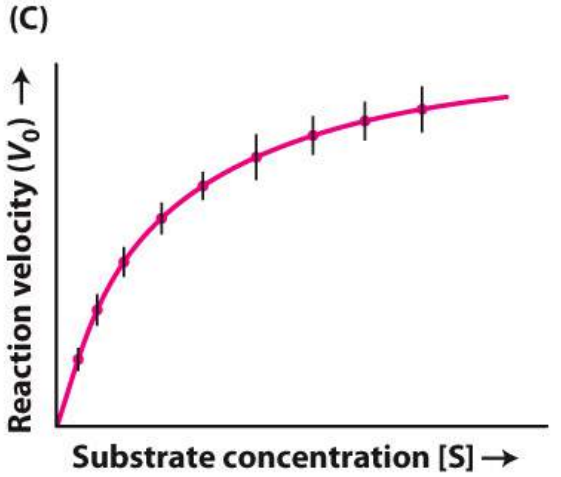
估算v0时的反应速率



Experiment:

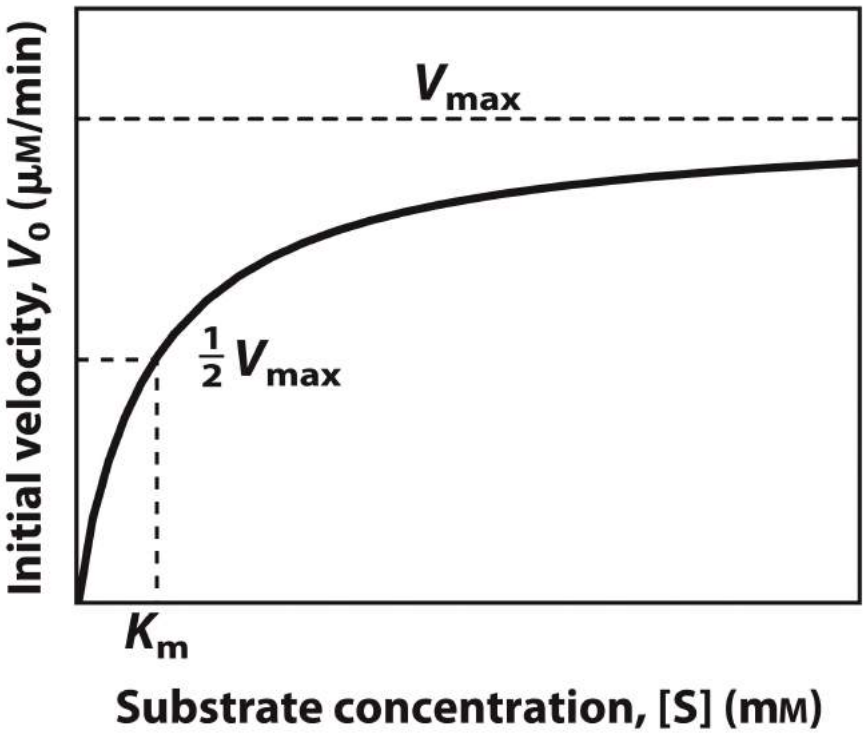
1. Mix enzyme + substrate.
2. Record rate of substrate disappearance and/or product formation as a function of time (the velocity of reaction).
3. Plot initial velocity versus substrate concentration.





The Michaelis-Menten equation

$$V_0 = V_{\max}\frac{[S]}{[S] + K_M}$$



When V_o = ½ V_{max}, K_M =[S].
K_M is the substrate concentration that yields ½ V_{max}.

Leonor Michaelis
1875–1949

Maud Menten
1879–1960

deduce(推导)

Consider a simple reaction in which the enzyme E catalyzes the conversion of S→P.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

with k_1, k_2 k_{-1} and k_{-2} being the rate constants for the indicated reaction steps. So as to ignore the reverse reaction of P→S, we measure activity when $[P] \approx 0$.

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

Under these conditions, the velocity is called the **initial velocity** or **V_o**.

初速度

$$V_0 = k_2[ES]$$

用产物生成的速率代表V0

At the **steady-state**, the rate of formation of ES is equal to the rate of its breakdown. Thus

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

This equation can be rearranged to

$$[E][S]/[ES] = (k_{-1} + k_2)/k_1$$

We can define a new constant, K_M, called the Michaelis constant.

$$K_{\mathrm{M}} = \frac{k_{-1} + k_2}{k_1}$$

Km的动力学含义

Thus

$$[\mathrm{E}][\mathrm{S}]/[\mathrm{ES}] = (k_{-1} + k_2)/k_1$$

becomes

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_{\mathrm{M}}}$$

The amount of free enzyme is equal to the total amount of enzyme present minus any enzyme bound to substrate.

$$[\mathrm{E}] = [\mathrm{E}]_\mathrm{T} - [\mathrm{ES}]$$

Substituting this equation for [E] in $[\mathrm{ES}] = \dfrac{[\mathrm{E}][\mathrm{S}]}{K_\mathrm{M}}$

yields $$[\mathrm{ES}] = \frac{([\mathrm{E}]_\mathrm{T} - [\mathrm{ES}])[\mathrm{S}]}{K_\mathrm{M}}$$

This equation can be rearranged to yield $$[\mathrm{ES}] = [\mathrm{E}]_\mathrm{T}\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

Recall that $$V_0 = k_2[\mathrm{ES}]$$

Thus, $$V_0 = k_2[\mathrm{E}]_\mathrm{T}\frac{[\mathrm{S}]}{[\mathrm{S}] + K_\mathrm{M}}$$

The Michaelis-Menten equation

The maximal rate of catalysis, V_{max}, occurs when all of the enzyme is bound to substrate

$[ES] = [E]_T$

$$V_{max} = k_2[E]_T$$

Substituting the equation for V_{max} into $$V_0 = k_2[E]_T \frac{[S]}{[S] + K_M}$$

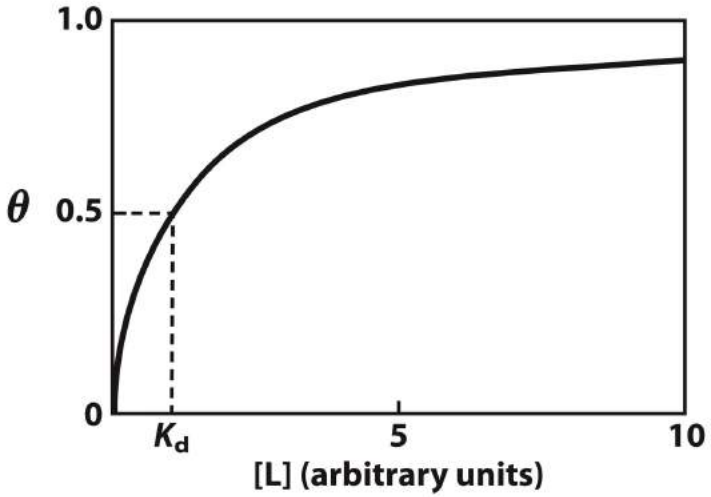
yields the **Michaelis-Menten equation**

$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

Still remember K_d?

K_d equals to the molar concentration of free ligand concentration at which half of available binding sites are occupied.

$$\theta = \frac{[\mathrm{L}]}{[\mathrm{L}] + K_d}$$



K_M vs. K_d

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

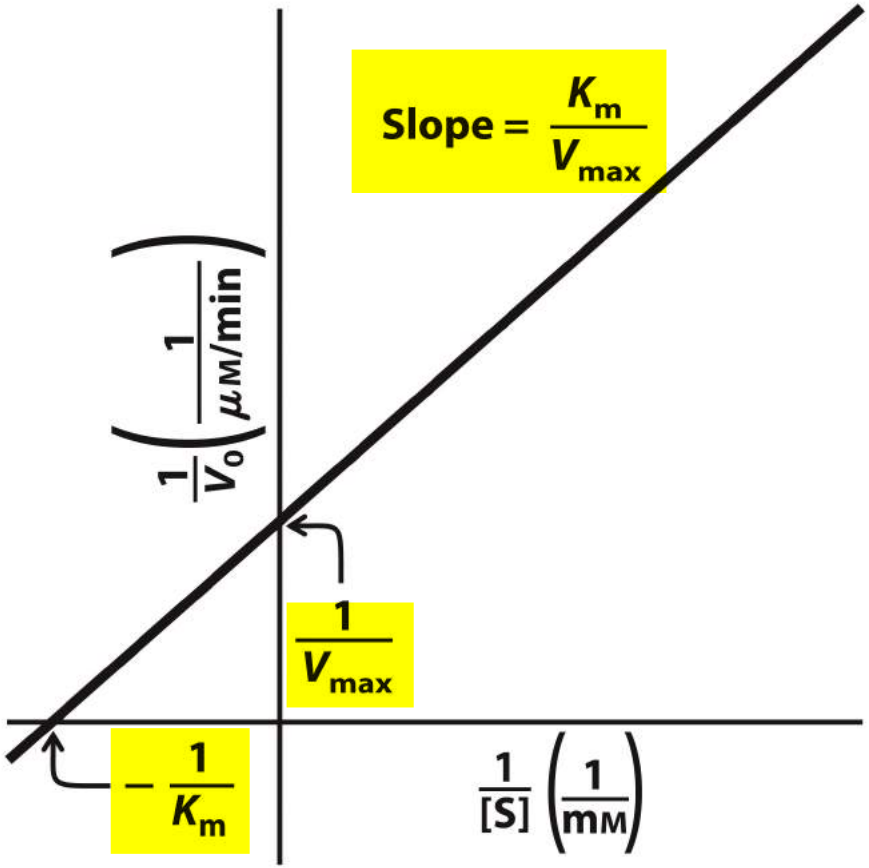
$$K_M = \frac{k_{-1} + k_2}{k_1}$$

Kd=k-1/k1

- If k_2 is rate-limiting, $k_2 << k_{-1}$, $K_M = k_{-1}/k_1$, which equals to the dissociation constant (K_d) of the ES complex. **Under this condition**, K_M represents a measure of affinity of the enzyme for its substrate in the ES complex (lower K_M, higher affinity and vice versa).
- If $k_2 >> k_{-1}$, then $K_M = k_2/k_1$.
- If k_2 and k_{-1} are comparable, K_M is a complex function of all three rate constants.

The Lineweaver-Burk plot (Double-Reciprocal Plot)

双倒数作图



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

Taking the reciprocal gives

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

Lineweaver & Burk (1934) The Determination of Enzyme Dissociation Constants, *J. Ame. Chem.Soc.* 56: 658–666.

Dean Burk
(1904-1988)

Hans Lineweaver
(1907-2009)

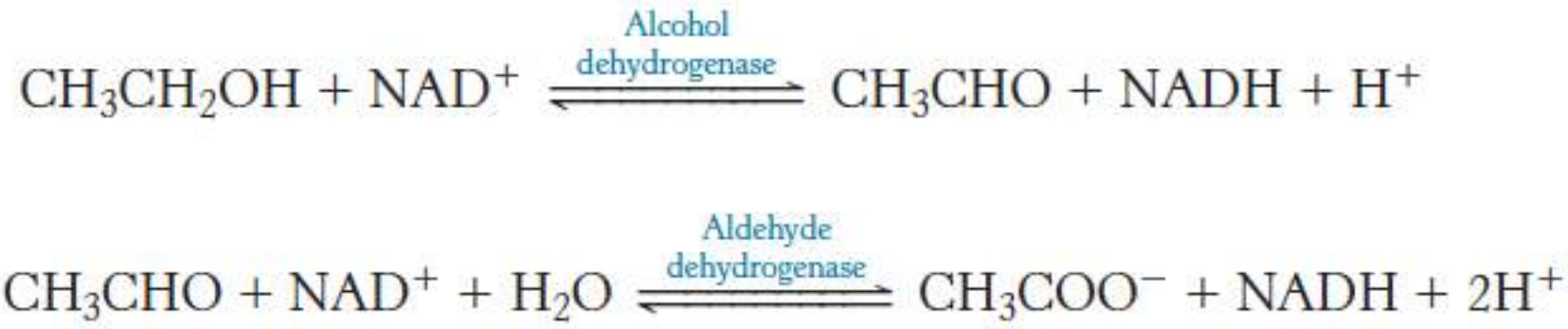
K_M is a constant for each substrate of an enzyme at a defined condition

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain) 己糖激酶	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Physiological implications of K_M: an example

Two enzymes play a key role in the metabolism of alcohol.



Some people respond to alcohol consumption with facial flushing and rapid heart beat, symptoms caused by excessive amounts of acetaldehyde in the blood. There are two different acetaldehyde dehydrogenase in most people, one with a low K_M and one with a high K_M. The low K_M enzyme is inactivated in susceptible individuals. The enzyme with the high K_M cannot process all of the acetaldehyde, and so some acetaldehyde appears in the blood.

The turnover number

$k_{cat} = V_{max}/[Enzyme]$

Km与酶浓度无关，但是Vmax与酶浓度有关
把酶浓度除了就是常数了！

k_{cat} is also called the **turnover number**. It is equivalent to the number of substrate molecules that are converted to product in a given unit of time on a single enzyme (when the enzyme is saturated with substrate)

TABLE 6–7 Turnover Number, k_{cat}, of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

k_{cat}/K_M (specificity constant) is a measure of catalytic efficiency

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

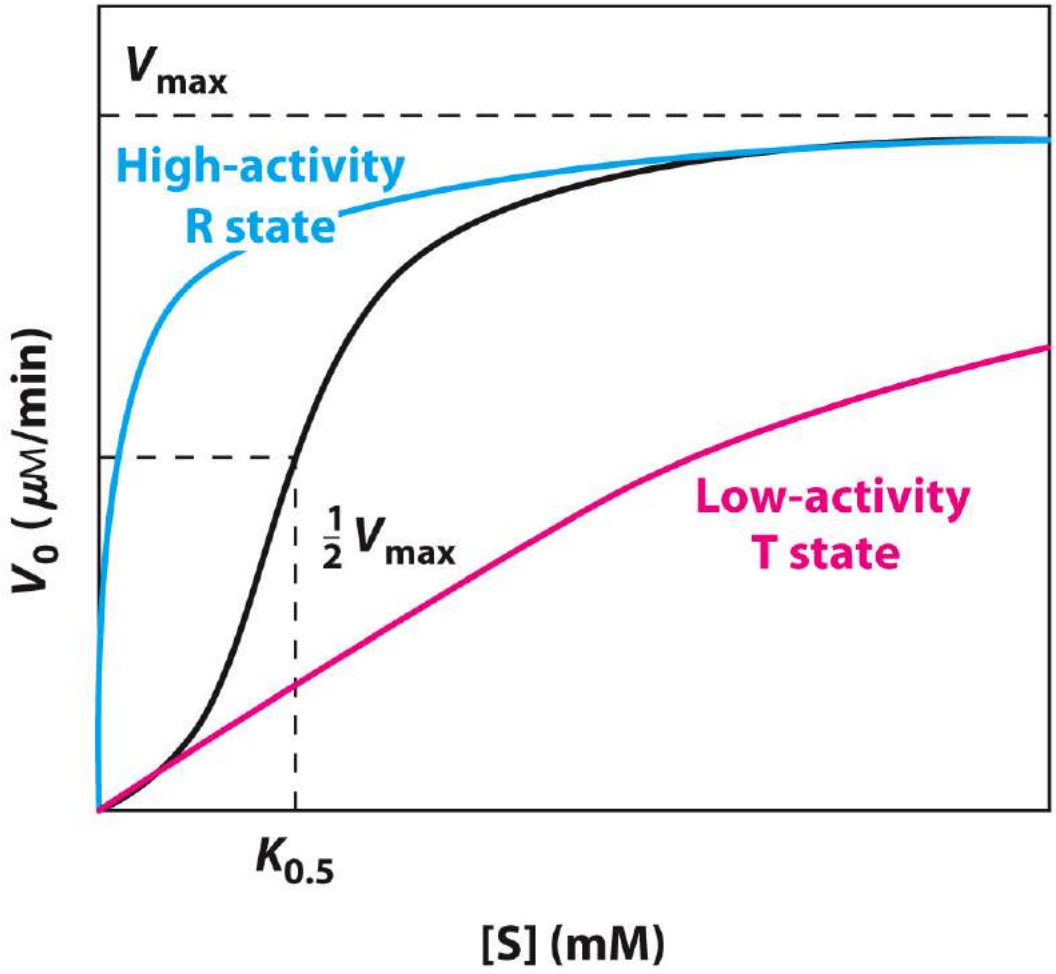
Enzyme	Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, New York.

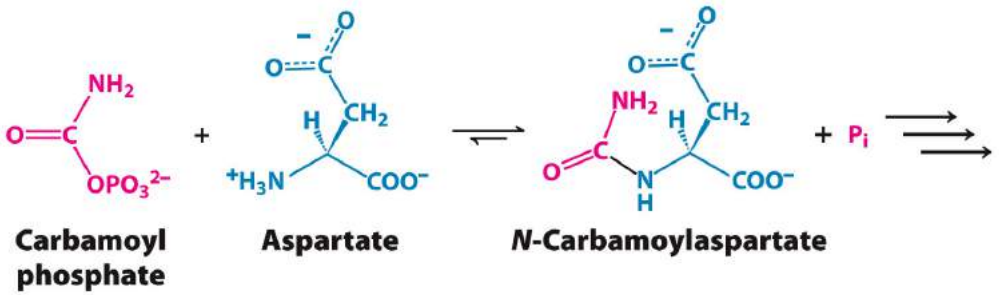
- The value of k_{cat}/K_M has an upper limit. It can be no greater than the rate at which E and S can diffuse together to form ES (k_1).
- The most efficient enzymes have k_{cat}/K_M values near the diffusion-controlled limit of 10^8 to 10^9 $M^{-1}S^{-1}$. Such enzymes are catalytically perfect.

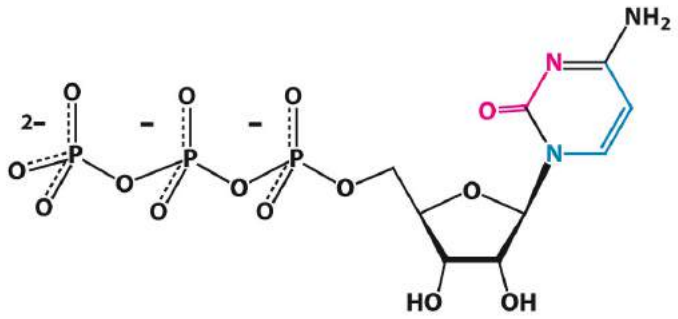
Not all enzymes display Michaelis-Menten behavior

别构酶不遵循米氏方程！！



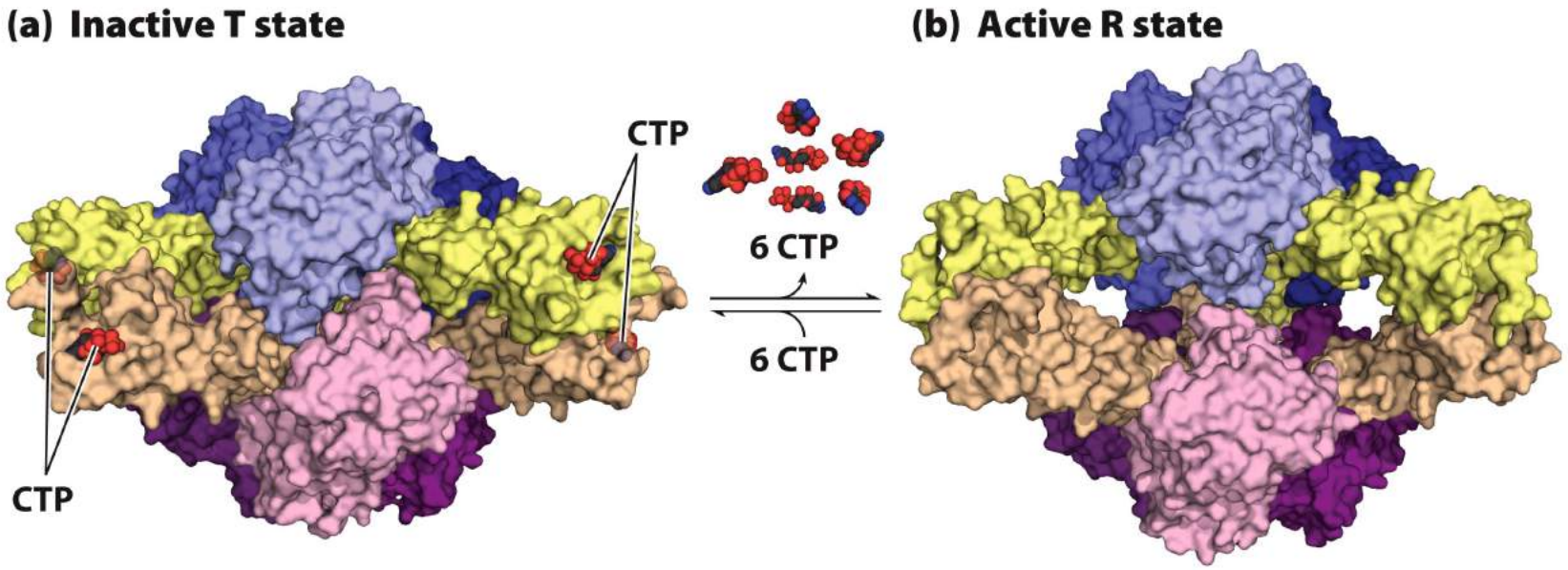
Aspartate transcarbamoylase is allosterically inhibited by the end product of its pathway





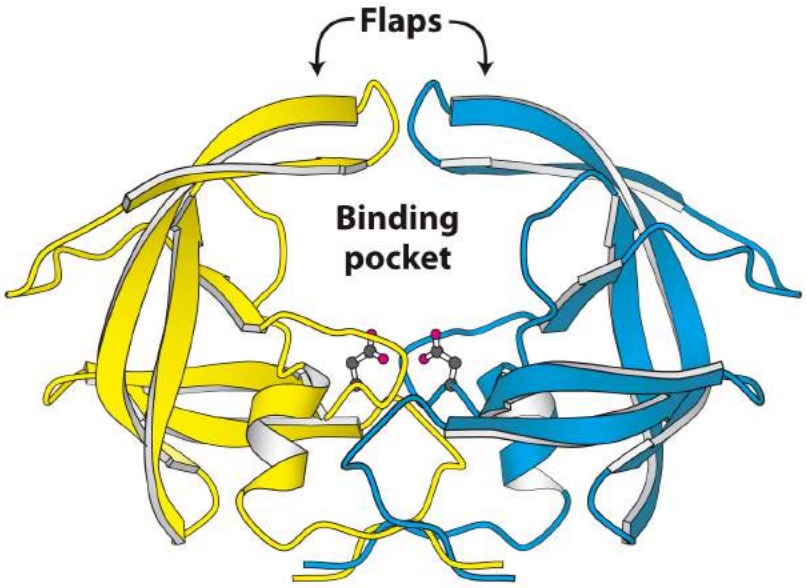
- Aspartate transcarbamoylase catalyzes the committed step, the condensation of aspartate and carbamoyl phosphate to form *N*-carbamoylaspartate, in pyrimidine synthesis.
- ATCase is activated by ATP and Asp (indicators of robust cellular metabolism), and inhibited by the end product of the pathway, CTP.
- ATP and CTP exert their effects by binding at distinct regulatory or allosteric sites on ATCase.

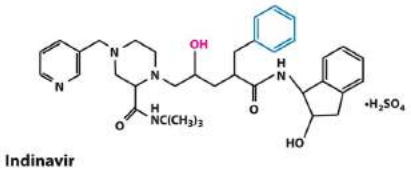
Allosteric regulation is mediated by conformational changes

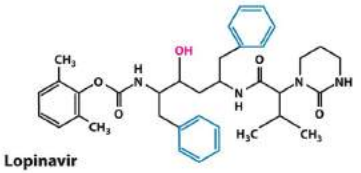


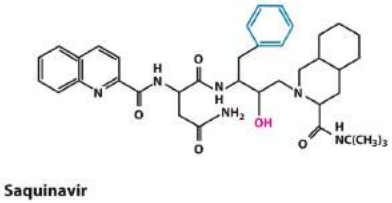
- Native ATCase consists of six catalytic and six regulatory subunits (c_6r_6).
- Free ATCase is in equilibirum between the T state (low activity) and the R state (high activity), with the T state being more favored.
- Binding of CTP to the regulatory site of ATCase alters the T-to-R equilibrium in favor of the T state.
- ATP and Asp alters the T-to-R equilibrium in favor of the R state.

Enzyme inhibitors







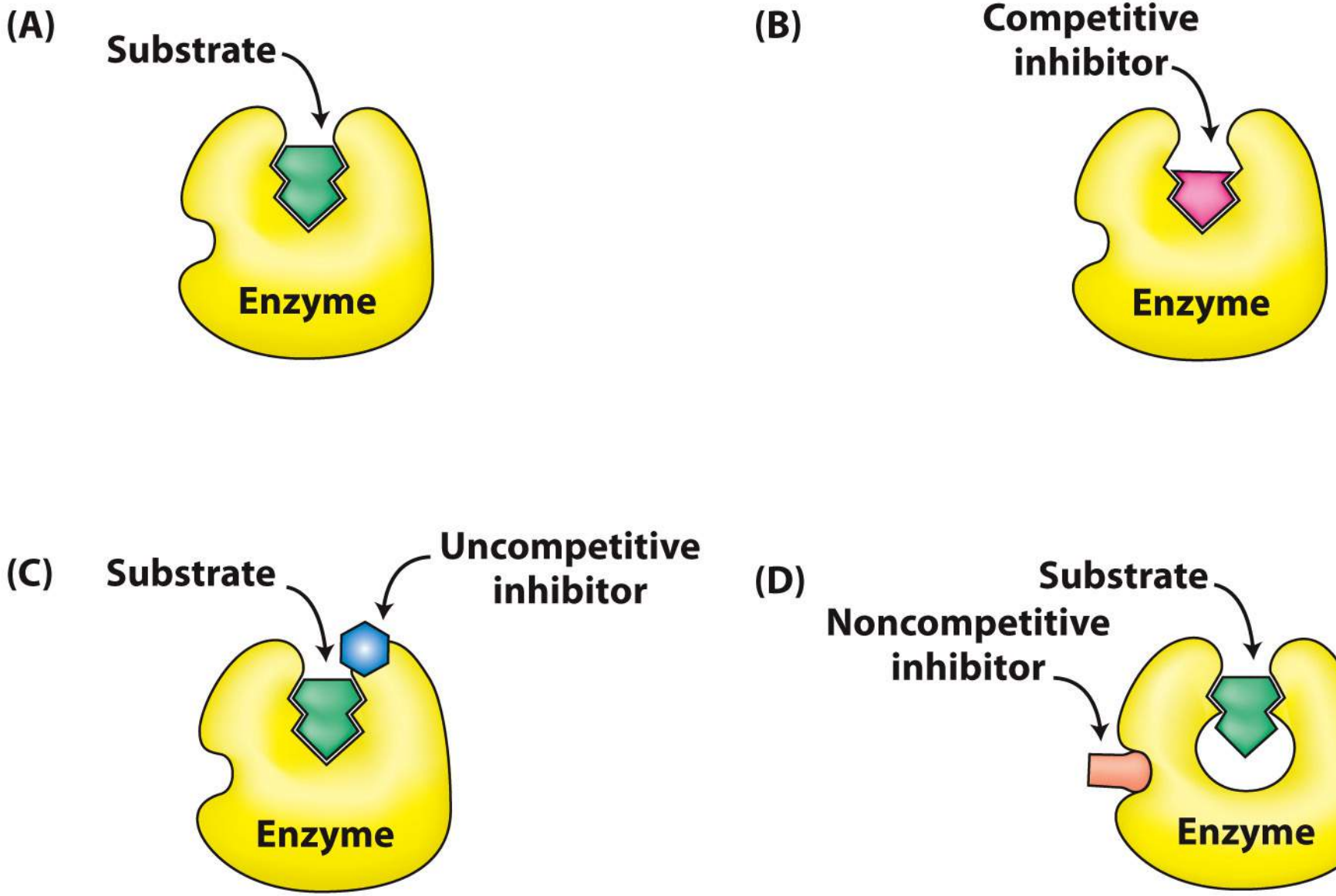


Enzymes can be inhibited by specific molecules

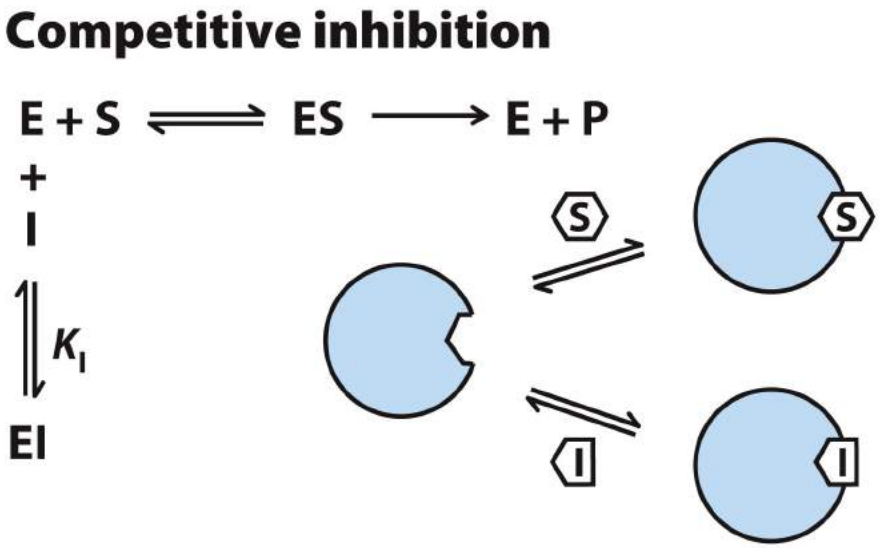
Reversible inhibition is characterized by a rapid dissociation of the enzyme-inhibitor complex.
There are three common types of reversible inhibition:

1. **Competitive inhibition**: The inhibitor is structurally similar to the substrate and can bind to the active site, preventing the actual substrate from binding.
2. **Uncompetitive inhibition**: The inhibitor binds only to the enzyme-substrate complex in what is essentially substrate-dependent inhibition.
3. A **mixed inhibitor** also binds to an allosteric site, but it binds to either E or ES. In **Noncompetitive inhibition**, the inhibitor binds to either E or ES equally well.

Irreversible enzyme inhibitors bind covalently, or noncovalently to the enzyme but with a negligible dissociation constant.


(A)
Substrate
Enzyme
(B)
Competitive inhibitor
Enzyme
(C)
Substrate
Uncompetitive inhibitor
Enzyme
(D)
Substrate
Noncompetitive inhibitor
Enzyme

Competitive Inhibition

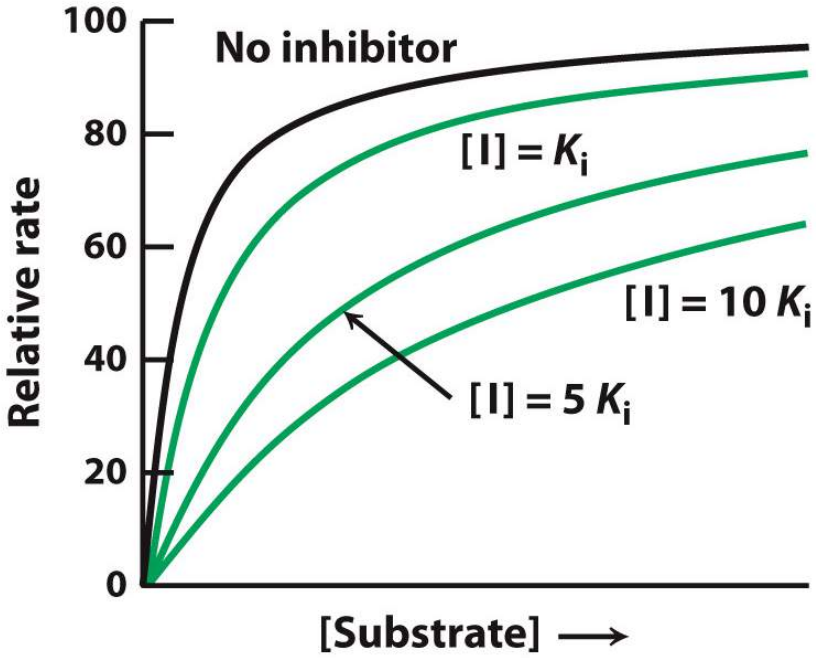


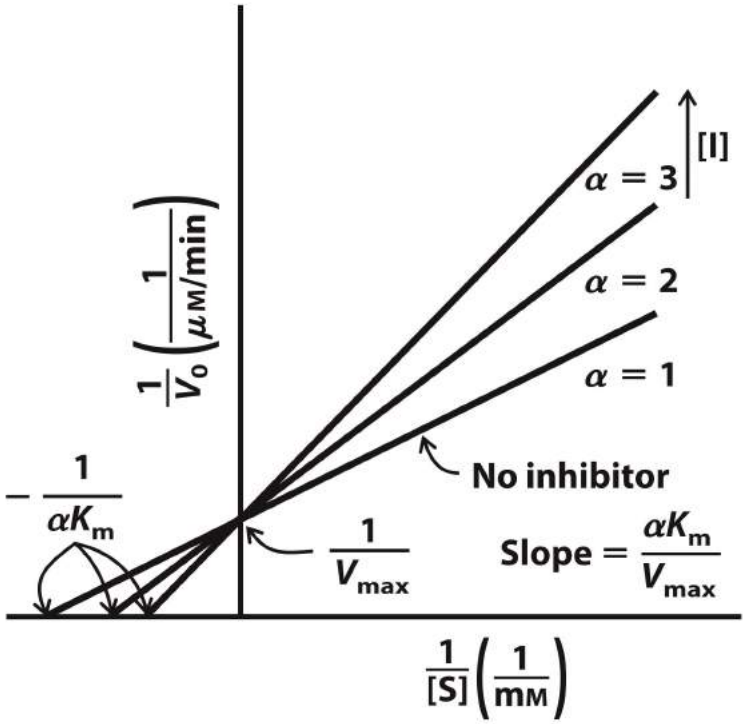
- Competes with substrate for binding
- Does not affect catalysis
- No change in V_{max} (because the inhibition can be overcome by a sufficiently high concentration of substrate)
- Increase in apparent K_M

Competitive Inhibition

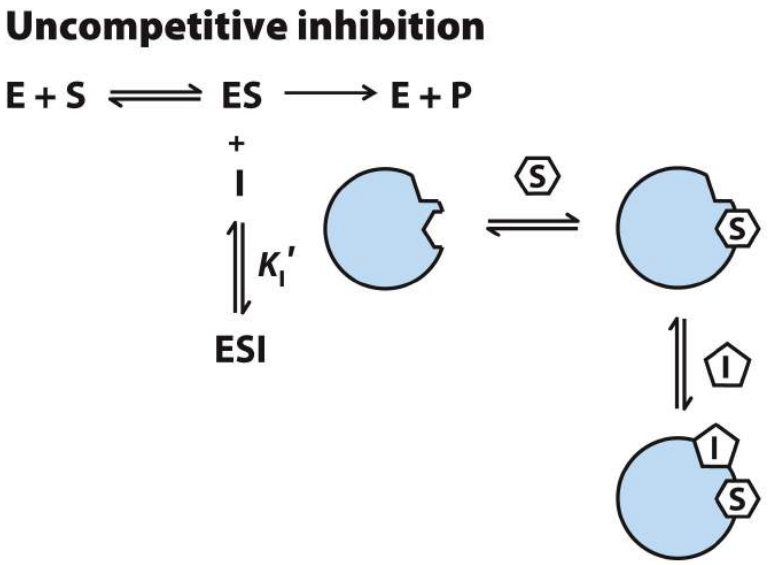
α = （1+【I】）/Ki

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



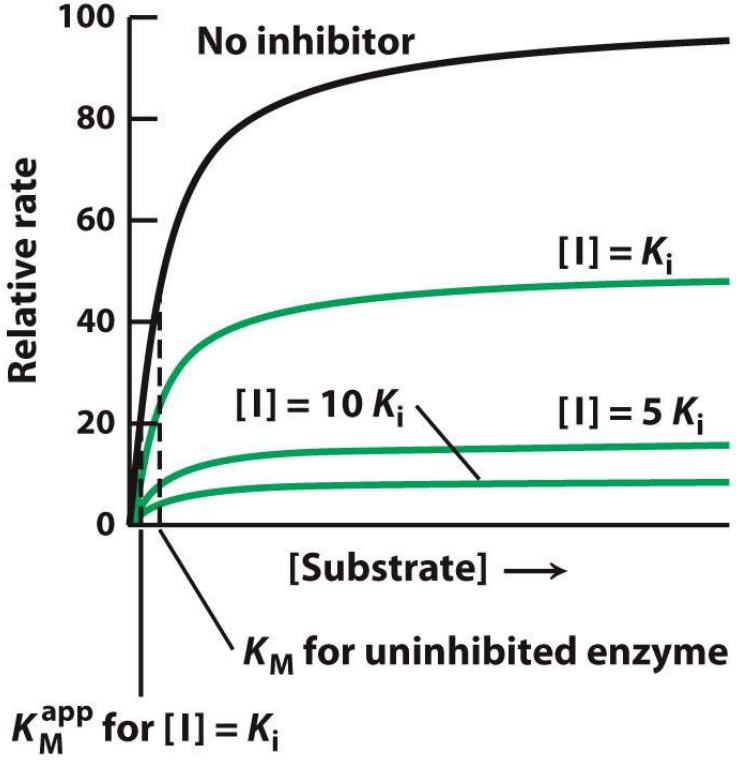


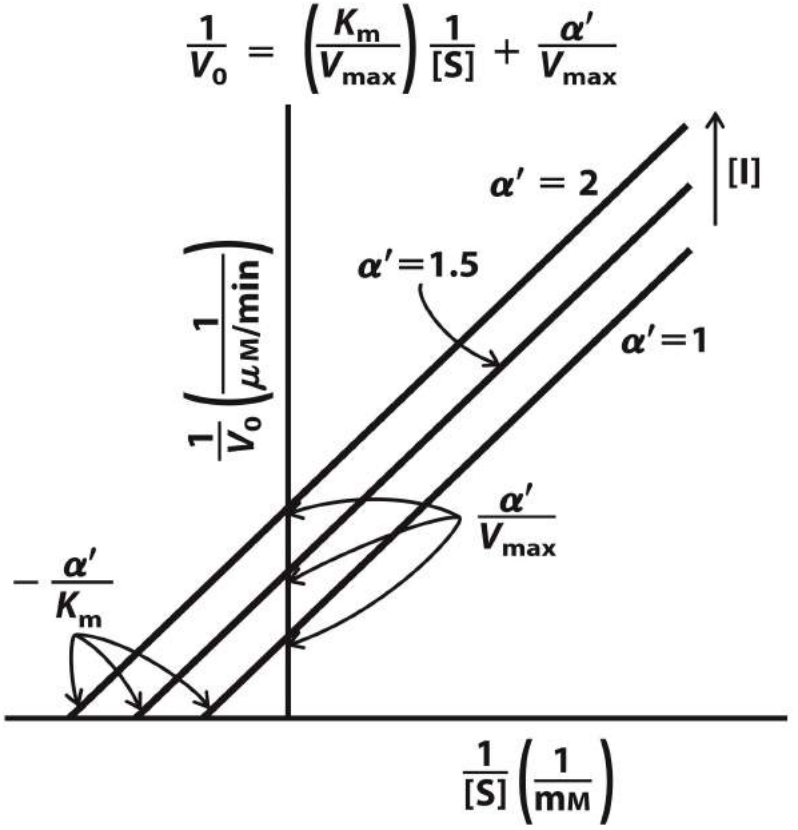
Uncompetitive Inhibition 反竞争性抑制剂



- Only binds to ES complex
- Decrease in V_{max}
- Apparent decrease in K_M

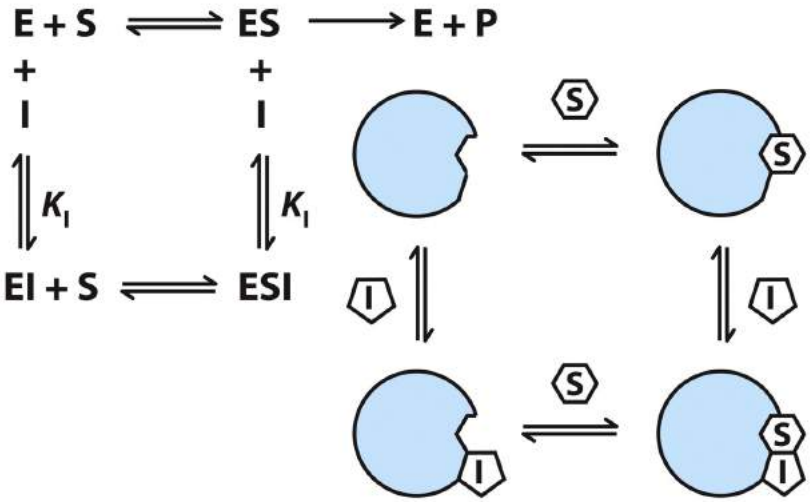
Uncompetitive Inhibition

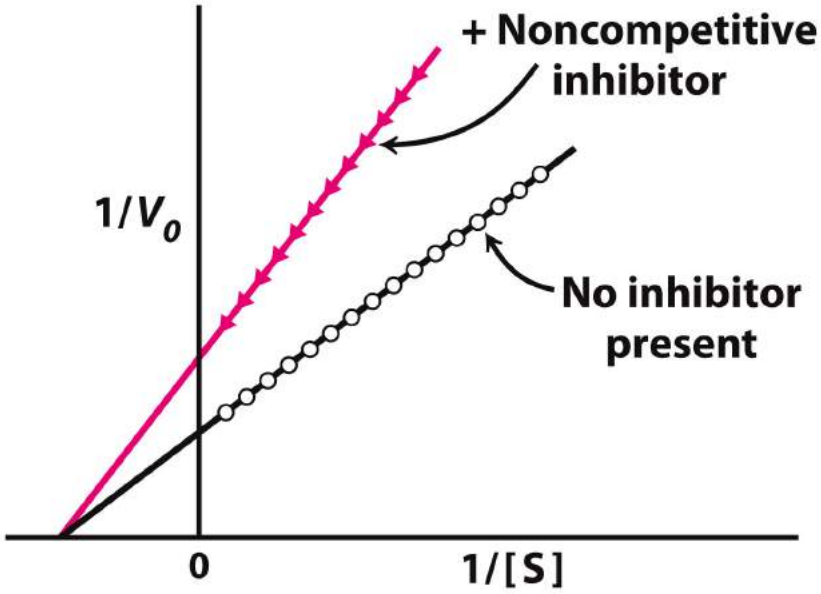




Noncompetitive inhibition 非竞争性抑制剂

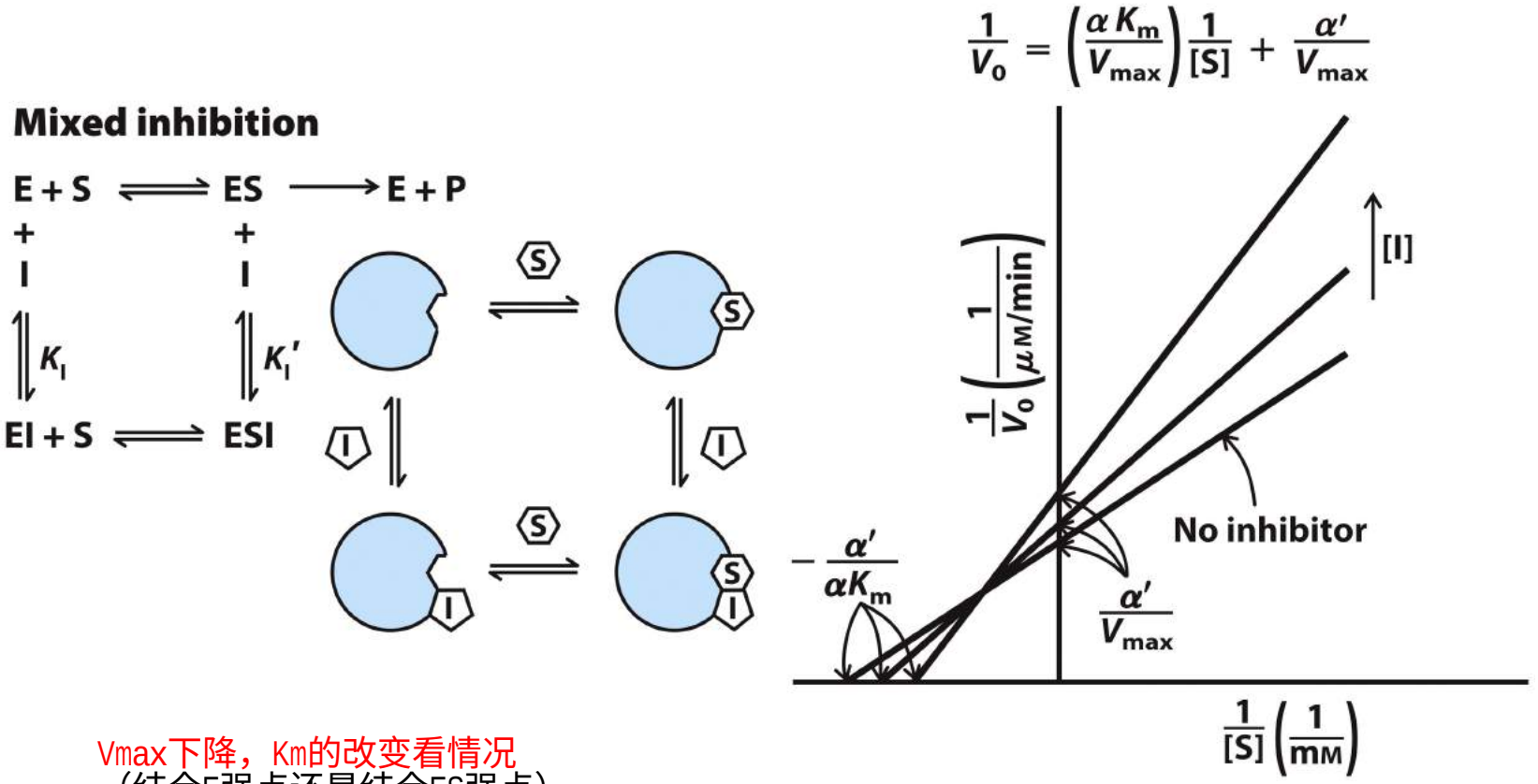
Noncompetitive inhibition



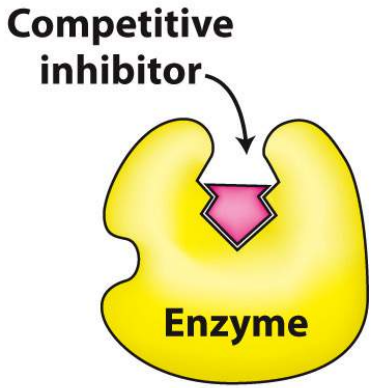


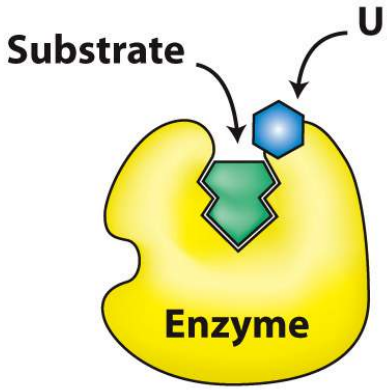
- K_M is not changed.
- V_{max} is lower.
- Lineweaver-Burk: lines intersect at the *x*-axis.

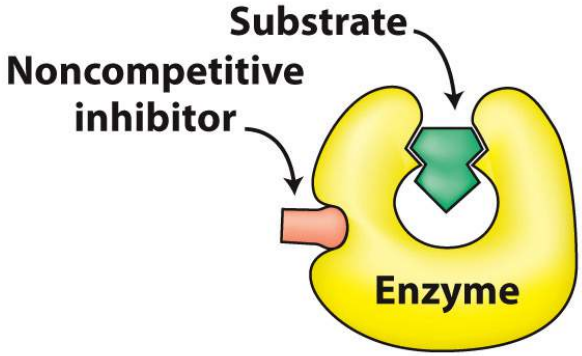
A mixed inhibitor binds to either E or ES

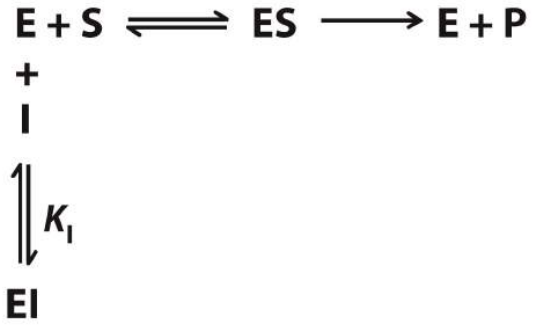


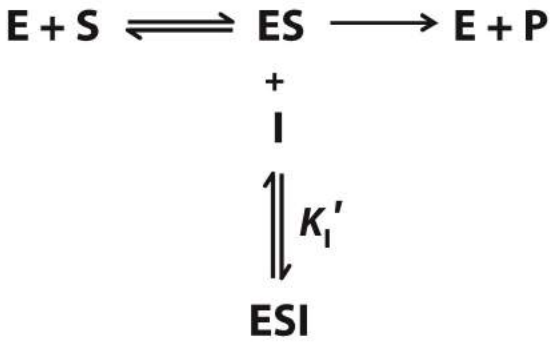
Vmax下降，Km的改变看情况
（结合E强点还是结合ES强点）
结合E强：Km升高，交点在第二象限
结合ES强：Km降低，交点在第三象限

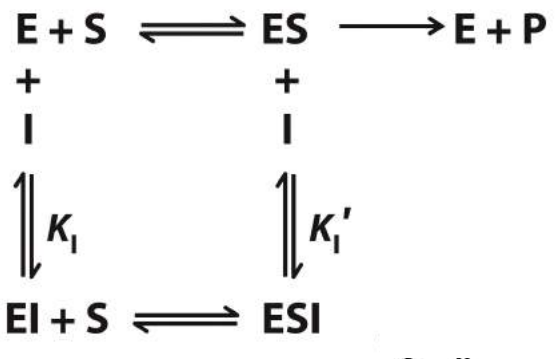

Competitive inhibitor
Enzyme

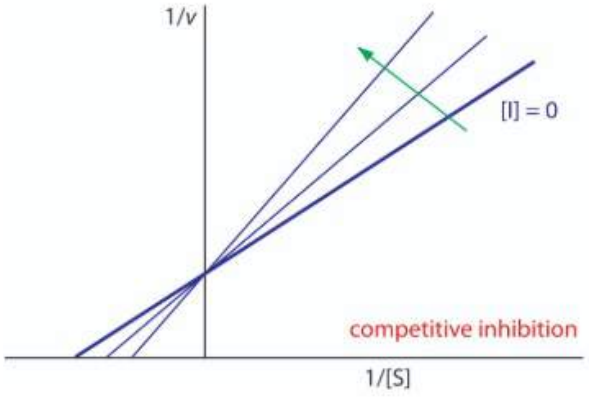

Substrate
Uncompetitive inhibitor
Enzyme

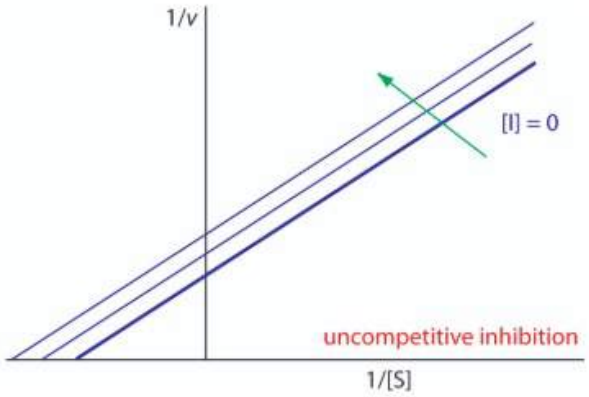

Substrate
Noncompetitive inhibitor
Enzyme

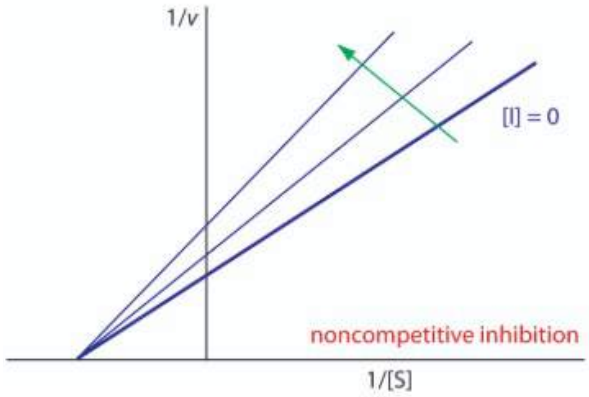

E + S ⇌ ES → E + P
+
I
⇅ K_I
EI


E + S ⇌ ES → E + P
+
I
⇅ K_I'
ESI

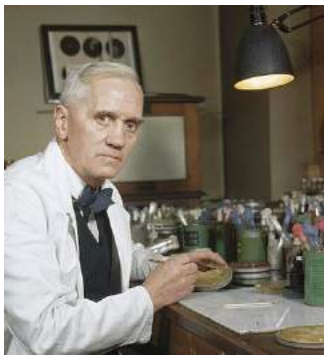

E + S ⇌ ES → E + P
+ +
I I
⇅ K_I ⇅ K_I'
EI + S ⇌ ESI


1/v
[I] = 0
competitive inhibition
1/[S]

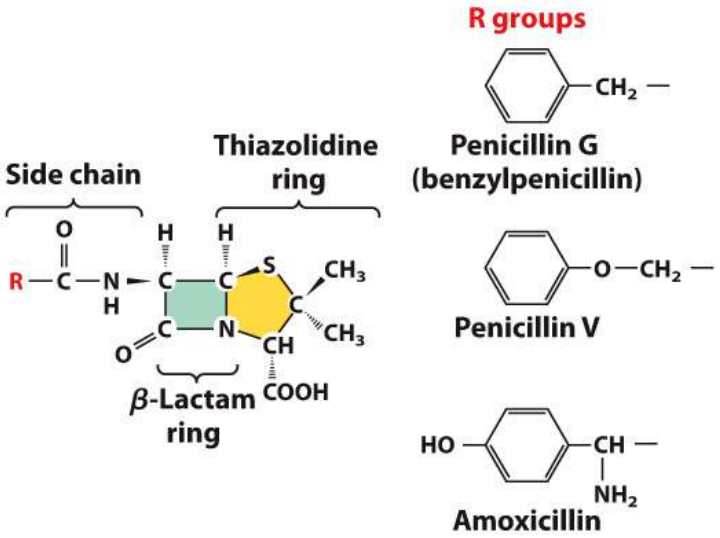

1/v
[I] = 0
uncompetitive inhibition
1/[S]

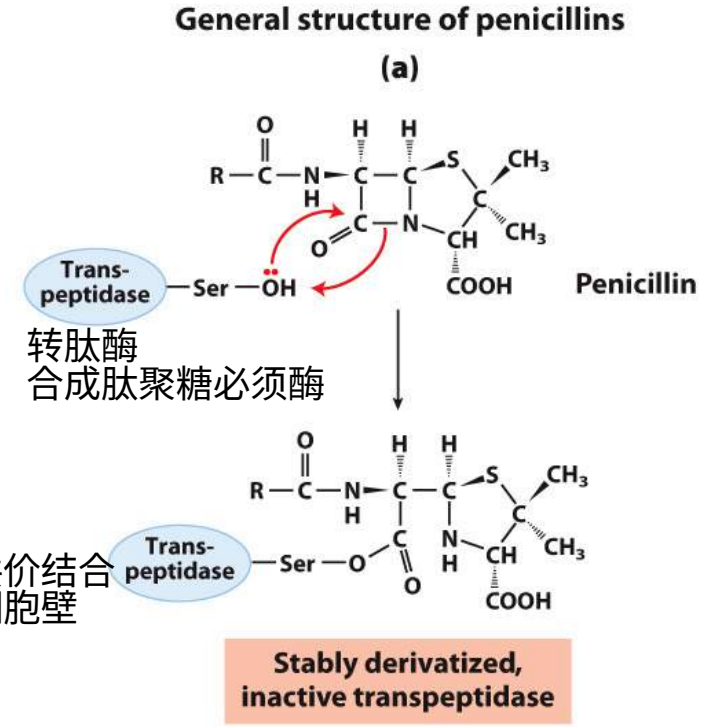

1/v
[I] = 0
noncompetitive inhibition
1/[S]

An irreversible inhibitor: Penicillin 青霉素！

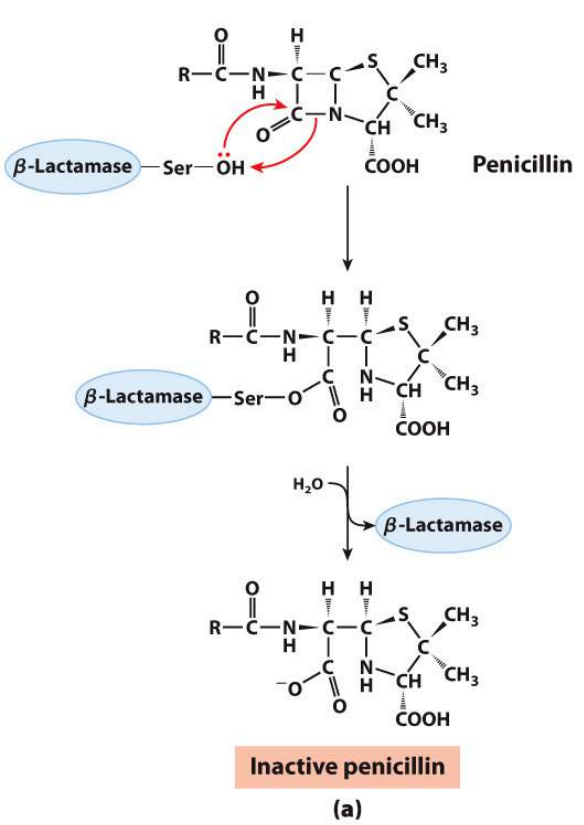


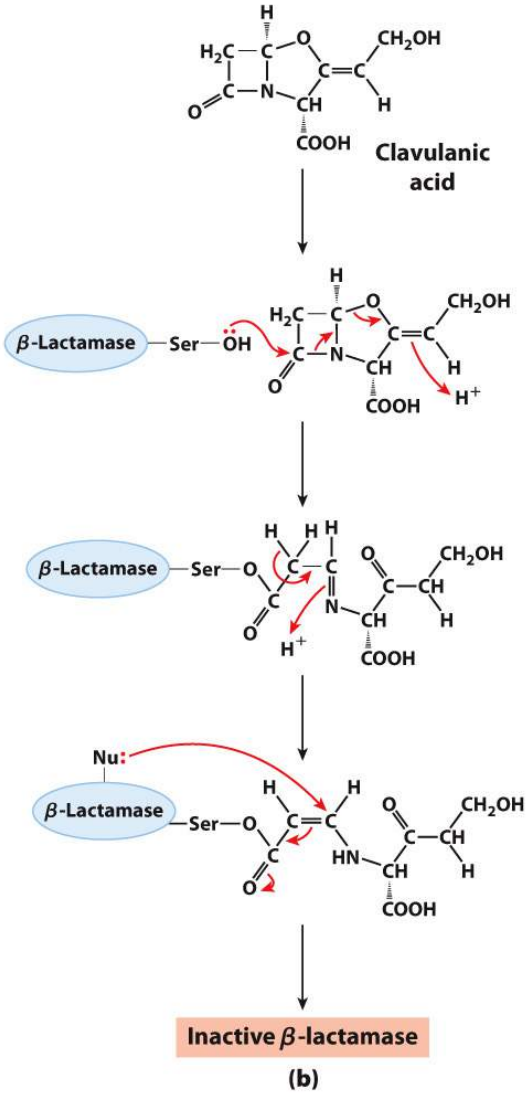
Alexander Fleming
1945 Nobel Prize





Chemical warfare between us and bacteria continues





细菌产生β-内酰胺酶，干死青霉素
人再搞出Clavulanic acid，干死β-内酰胺酶

Most biochemical reactions include multiple substrates

$$A + B \rightleftharpoons P + Q$$

There are two classes of multiple substrate reactions: sequential and double-displacement reactions.

Sequential reactions, which may be random or ordered, are characterized by the formation of a ternary complex consisting of the enzyme and both substrates.

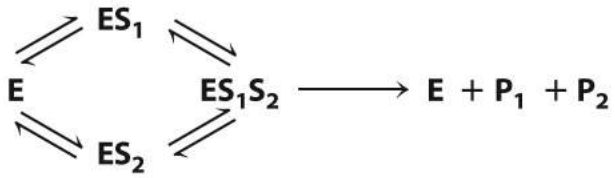
Double-displacement reactions are characterized by the formation of a substituted enzyme intermediate.

(a) Enzyme reaction involving a ternary complex

Ordered

$$E + S_1 \rightleftharpoons ES_1 \overset{S_2}{\rightleftharpoons} ES_1S_2 \longrightarrow E + P_1 + P_2$$

Random order



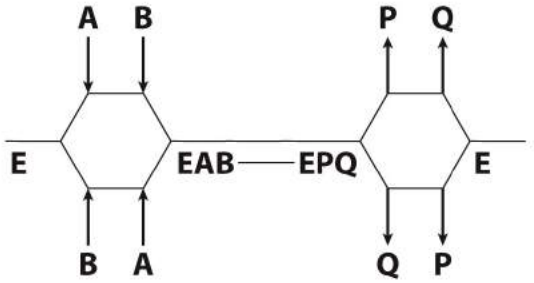
(b) Enzyme reaction in which no ternary complex is formed

$$E + S_1 \rightleftharpoons ES_1 \rightleftharpoons E'P_1 \overset{P_1}{\rightleftharpoons} E' \overset{S_2}{\rightleftharpoons} E'S_2 \longrightarrow E + P_2$$

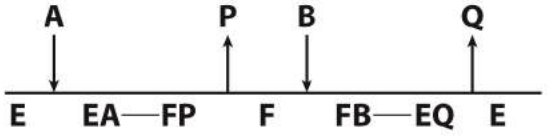
(c) Cleland nomenclature

Ordered bi bi

Random bi bi



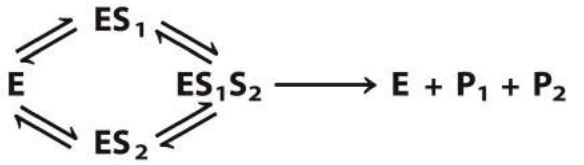
(d) Ping-Pong in Cleland nomenclature



Enzyme kinetics allows to distinguish between different kinetic mechanisms

(a) Enzyme reaction involving a ternary complex

Random order



Ordered

(b) Enzyme reaction in which no ternary complex is formed