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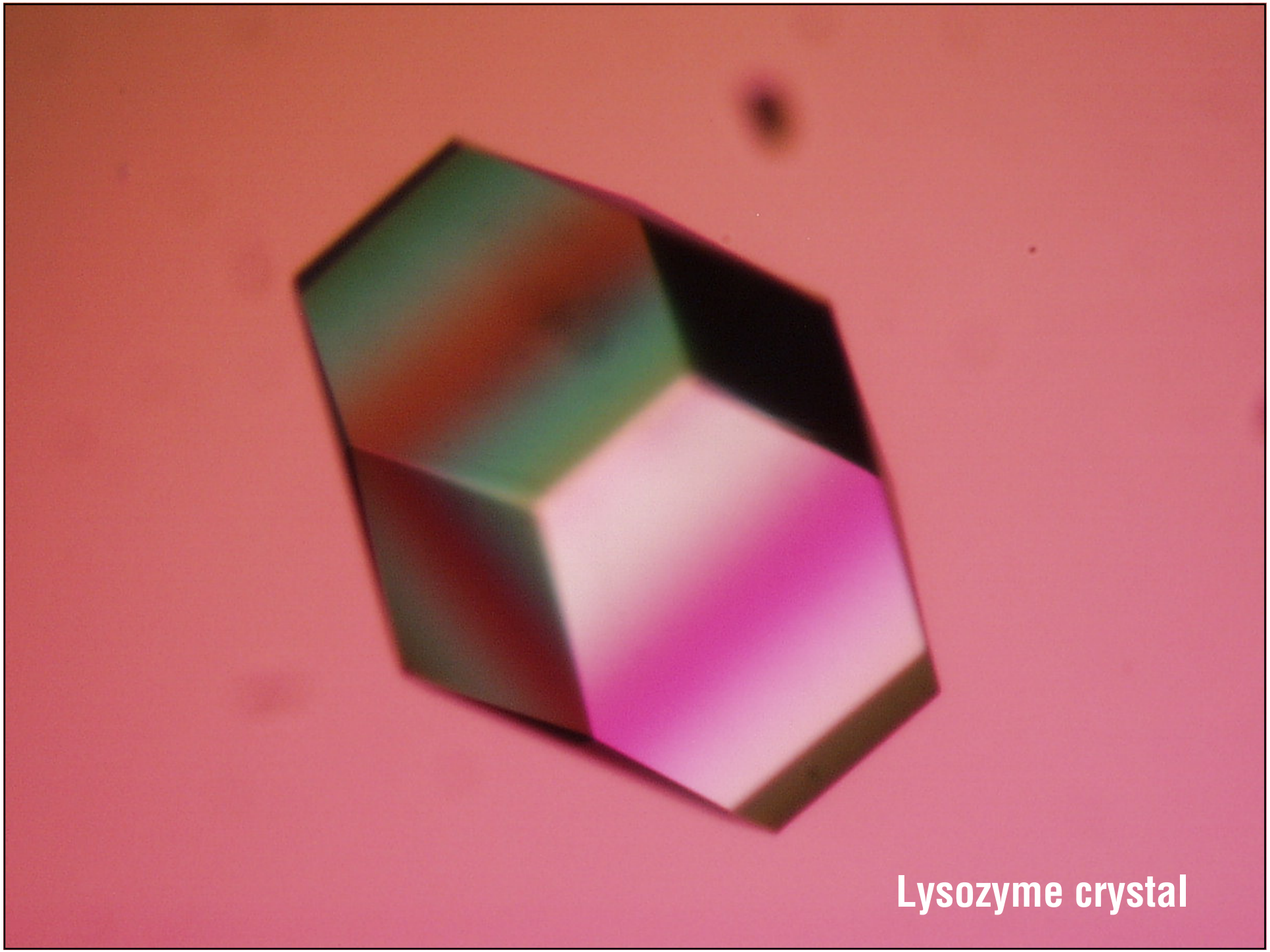
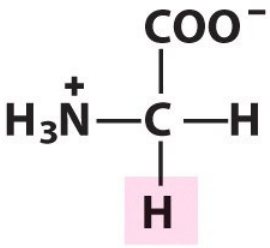
The specificity of enzyme catalytic mechanism

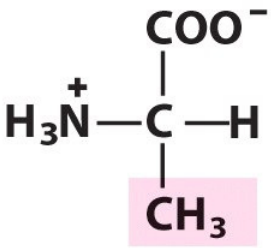
- In 1926, James B. Sumner showed that the enzyme **urease** was a pure protein, and he crystallized it.
- Northrop and Stanley, who worked on the digestive enzymes pepsin (1930), trypsin and chymotrypsin, also proved that pure proteins can be enzymes.
- These three scientists above were awarded the 1946 Nobel Prize in Chemistry. *"for his discovery that enzymes can be crystallized" and "for their preparation of enzymes and virus proteins in a pure form".*
- Lysozyme was the second protein structure and the first enzyme structure to be solved via X-ray diffraction methods by David Chilton Phillips group and published in 1965. This high-resolution structure of lysozyme revealed how enzymes work at an atomic level of detail.
- Many enzymes have been named by adding the suffix **-ase** to the name of their substrates (*e.g.*, urease catalyzes the hydrolysis of urea) or the type of reaction (*e.g.*, DNA polymerase forms DNA polymers).


Lysozyme crystal

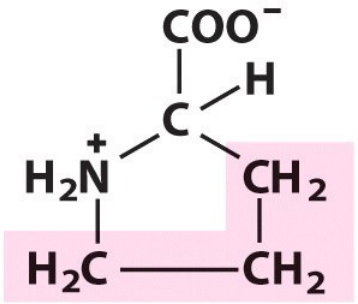
Nonpolar, aliphatic R groups



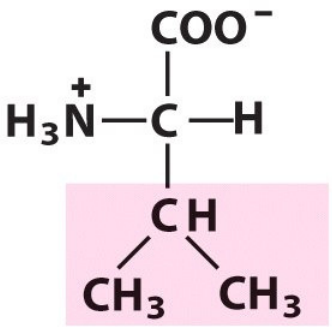
Glycine
甘胺酸



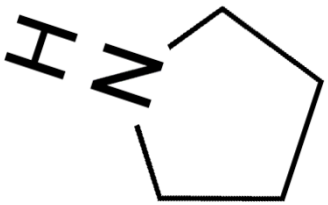
Alanine
丙胺酸



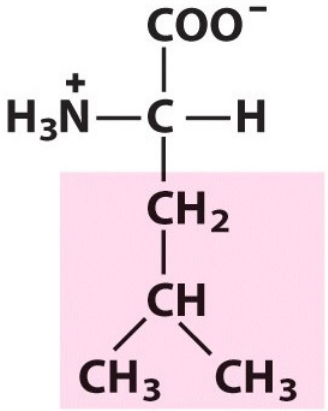
Proline
脯胺酸



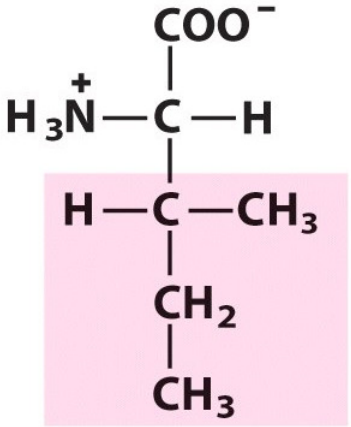
Valine
纈胺酸



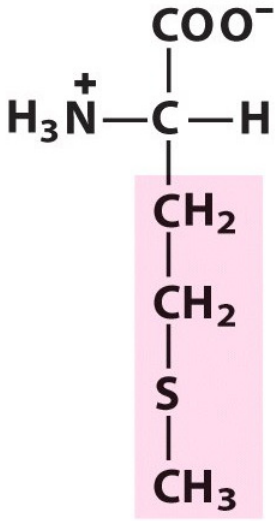
Pyrrolidine ring



Leucine
白胺酸



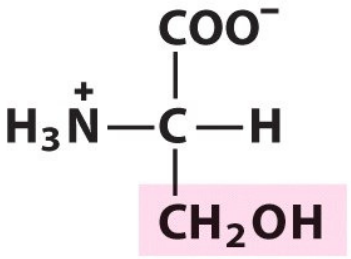
Isoleucine
異白胺酸



Methionine
甲硫(丁)胺酸

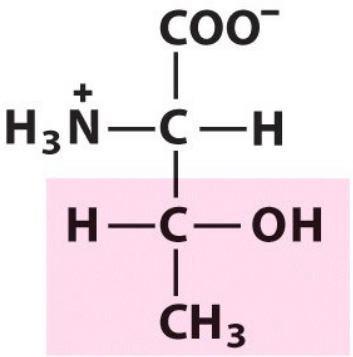
Lehninger Principles of biochemistry, 5th edition (2008)

Polar, uncharged R groups



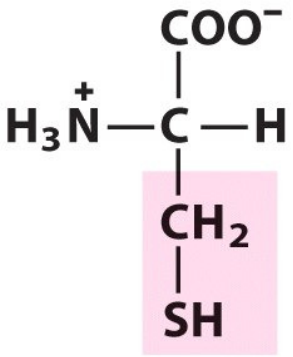
Serine

絲胺酸



Threonine

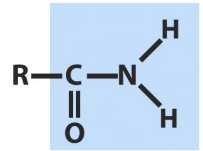
蘇胺酸

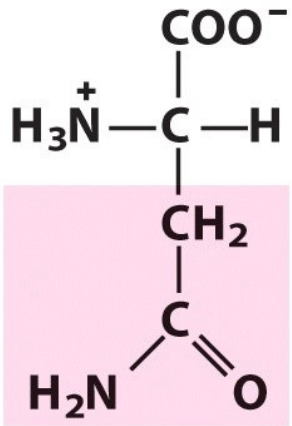


Cysteine

半胱胺酸

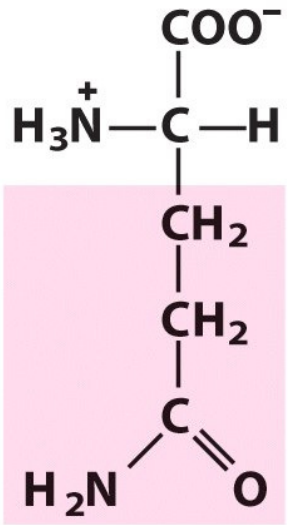
Amido





Asparagine

天門冬醯胺酸

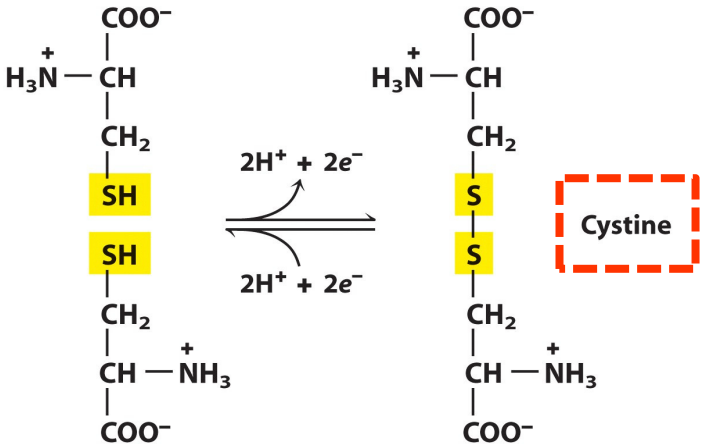


Glutamine

麩醯胺酸

Cysteine

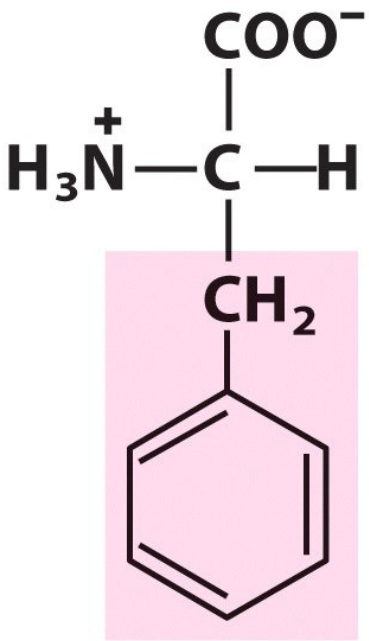
Cysteine

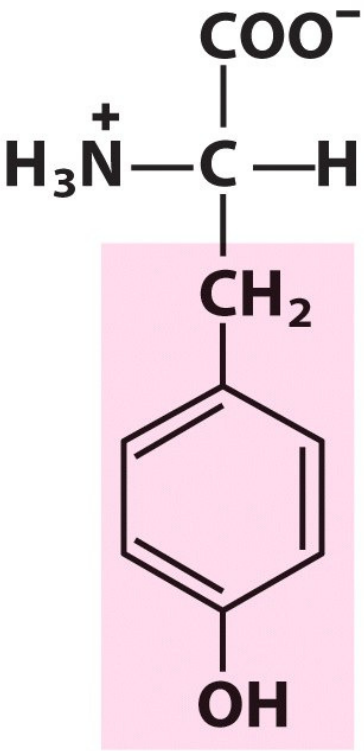


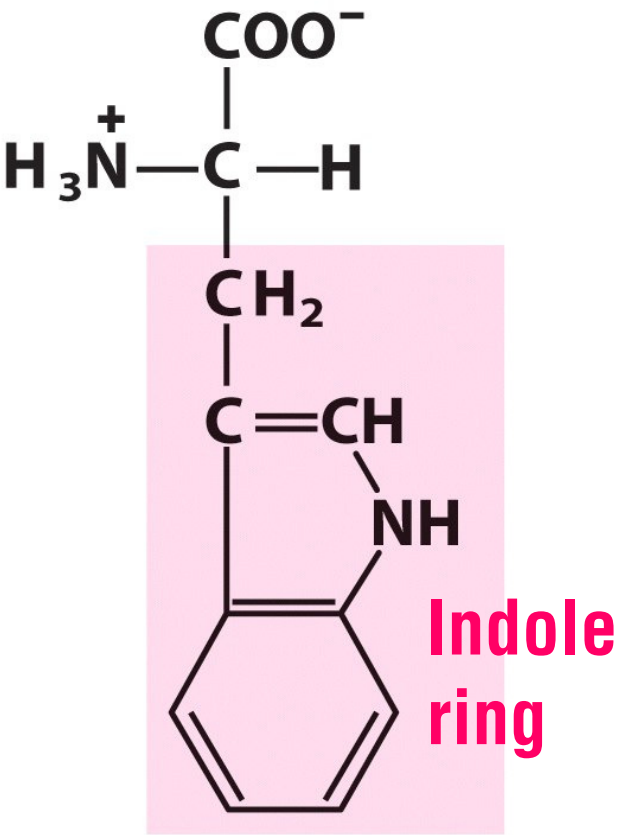
胱胺酸

Lehninger Principles of biochemistry, 5th edition (2008)

Aromatic R groups







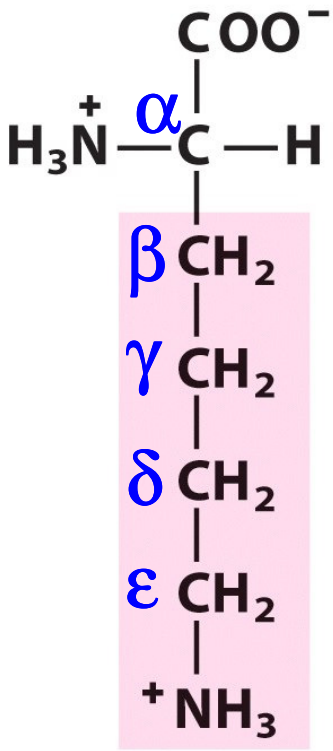
Phenylalanine 苯丙胺酸

Tyrosine 酪胺酸

Tryptophan 色胺酸

Lehninger Principles of biochemistry, 5th edition (2008)

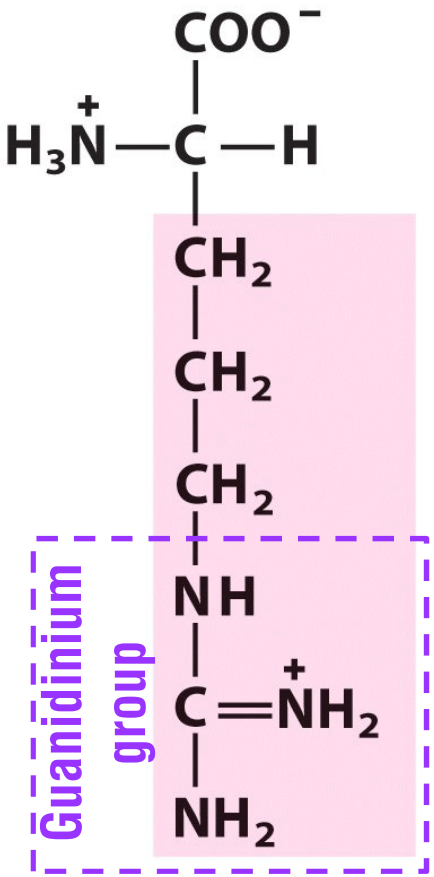
Positively charged R groups



Lysine

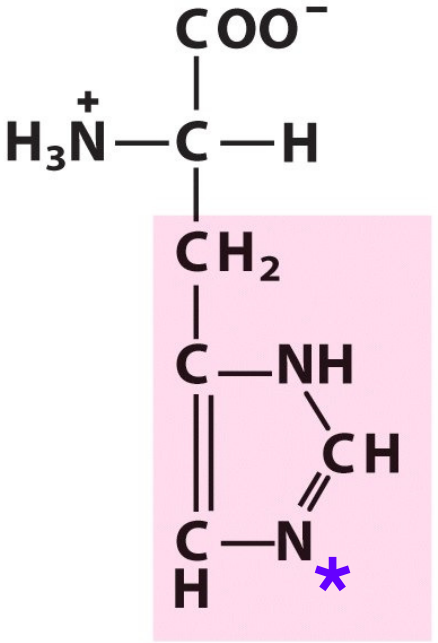
離胺酸

賴胺酸



Arginine

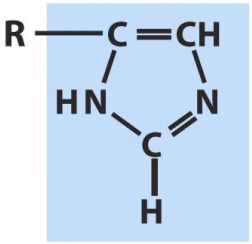
精胺酸

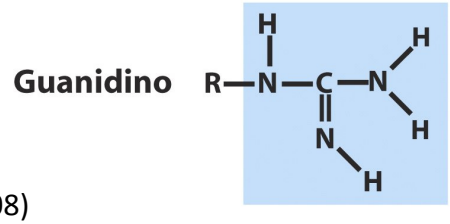


Imidazole ring

Histidine

組胺酸





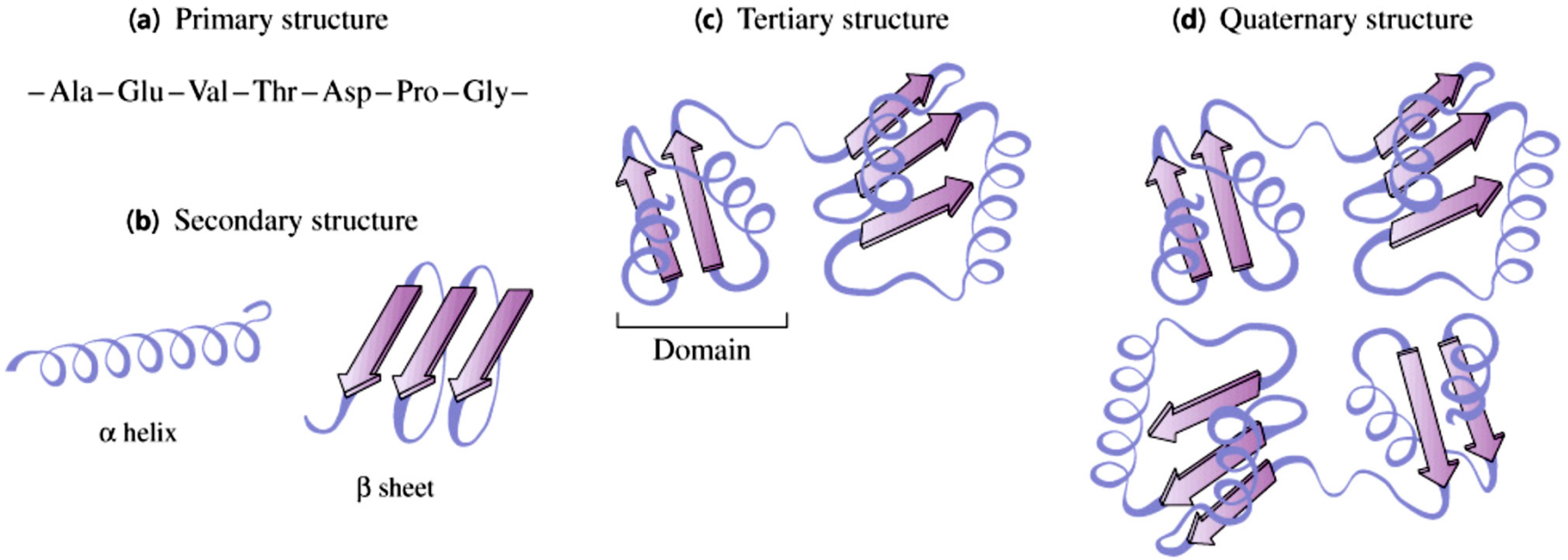
Guanidine group

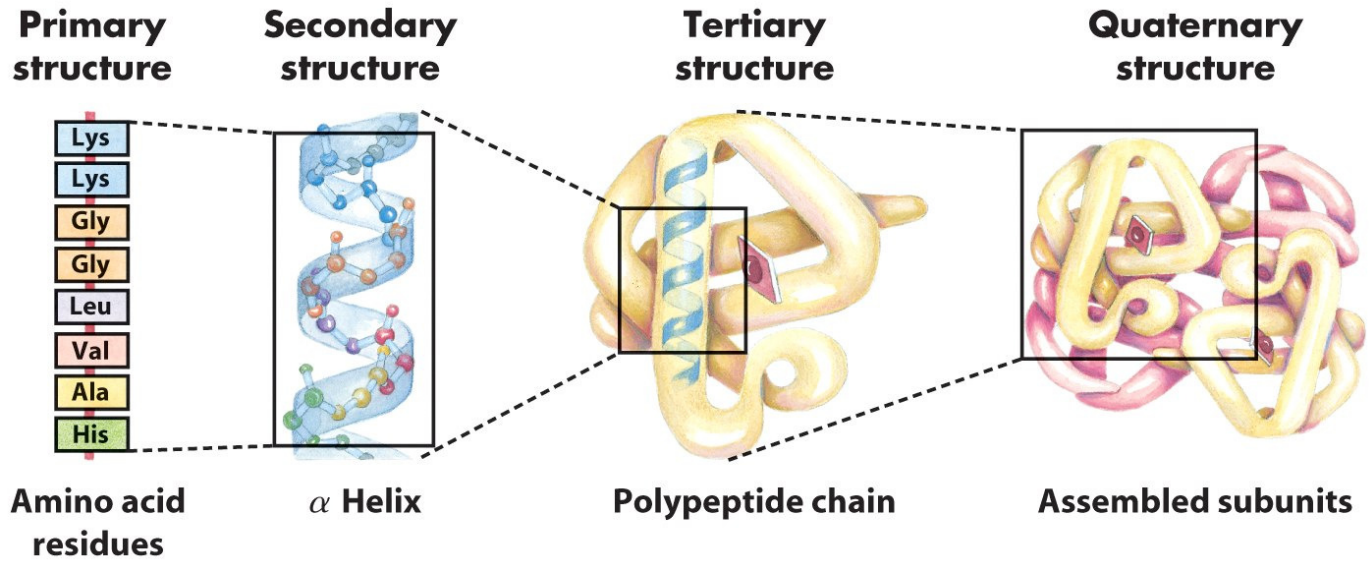
Lehninger Principles of biochemistry, 5th edition (2008)

Four Levels of Protein Structure

- **Primary structure** - amino acid linear sequence
- **Secondary structure** - regions of regularly repeating conformations of the peptide chain, such as α-helices and β-sheets
- **Tertiary structure** - describes the overall three-dimensional arrangement of all atoms in a protein and the shape of the fully folded polypeptide chain
- **Quaternary structure** - arrangement of two or more polypeptide chains, which may be identical or different, into multisubunit molecule

Four Levels of Protein Structure





Principles of biochemistry, 3rd edition, Horton, Moran, Ochs , Rawn, Scrimgeour

與蛋白質摺疊缺失有關的疾病

- A soluble protein is secreted in a misfolded state and converted into an insoluble extracellular amyloid fiber.
- The diseases are collectively referred to as amyloidoses.

◆ Amyloid 類澱粉蛋白/澱粉樣蛋白 ◆ Amyloidosis 類澱粉變性症/澱粉樣變性病 ◆ Amyloid plaque 澱粉樣蛋白斑

Type II diabetes

Amyloid deposition near the pancreatic islet β cells

Alzheimer's disease

Extracellular amyloid deposition by neuron amyloid β-peptide derived from amyloid β-peptide precursor protein or APP

- Intracellular aggregation of misfolded proteins

Parkinson's disease

α-synuclein aggregates into Lewy bodies

Huntington's disease

Huntingtin with a long polyglutamine repeat

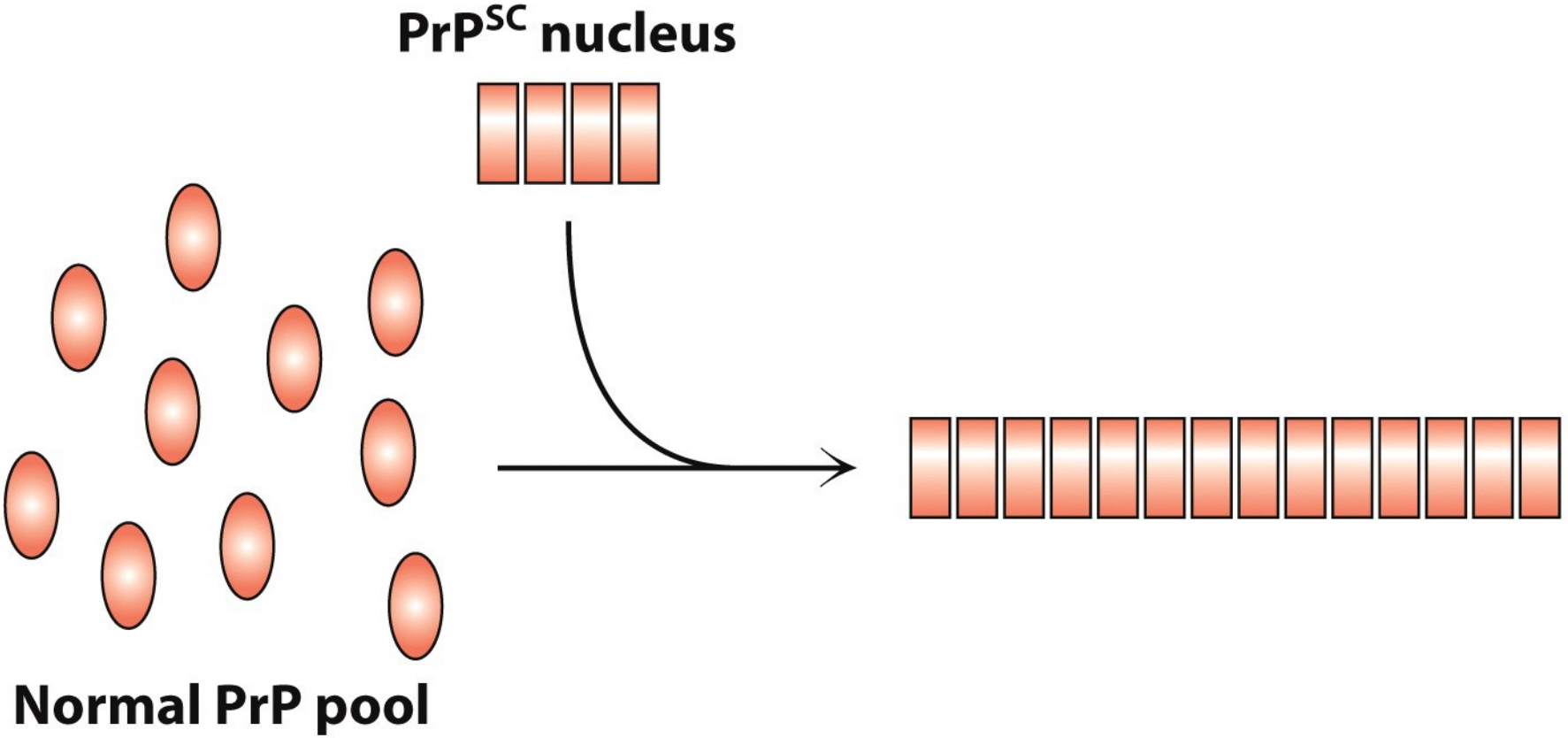
- Misfolded leads to degradation and loss of function

Cystic fibrosis 囊性纖維化病

Defects in a membrane-bound protein called CFTR

CFTR: Cystic fibrosis transmembrane conductance regulator, which act as a channel for chloride ions

The protein-only model for prion-disease transmission

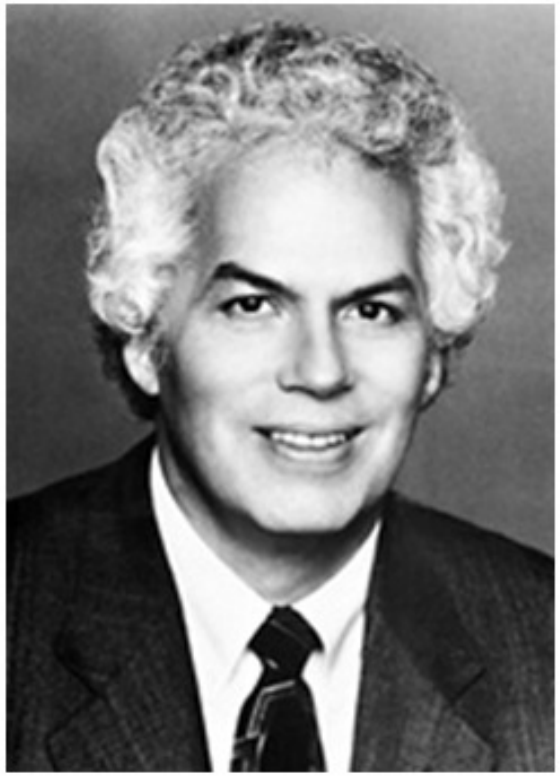


PrP, the **pr**ion **p**rotein
Prp^{c}, normal **c**ellular prion protein
PrP^{sc}, **sc**rapie form

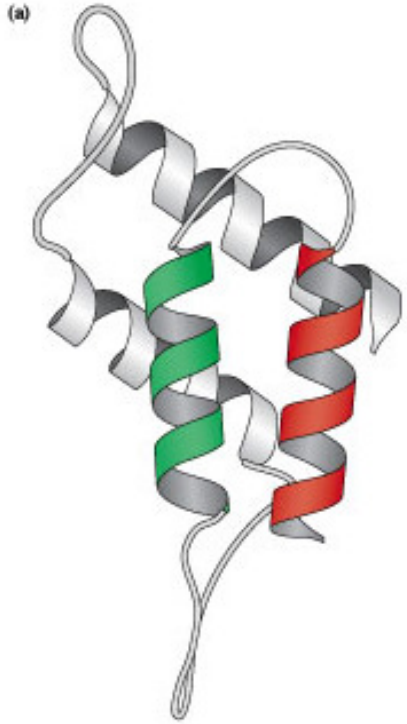
The prion (proteinaceous infectious particle) disease

coined in 1982 by Dr. Stanley B. Prusiner

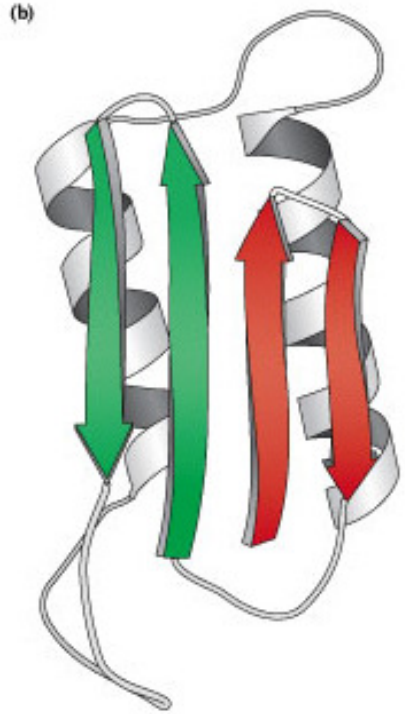
Tikvah Alper suggested the hypothesis in 1960s that some transmissible spongiform encephalopathies are caused by an infectious agent consisting solely of proteins.



Stanley B. Prusiner



左邊為正常的 PrP^{c}蛋白質結構，多由 α helices 組成



右邊則是呈現 β sheets 構型的致病性 PrP^{sc} 蛋白質結構

Prusiner, S. B. (1996). Molecular biology and the pathogenesis of prion diseases. Trends in Biochemical Sciences 21:482-487

The Nobel Prize in Physiology or Medicine 1997 was awarded to Stanley B. Prusiner *"for his discovery of Prions - a new biological principle of infection"*.

PrP, the prion protein, comes in various forms, such as Prp^{c}, the normal cellular prion protein, and PrP^{sc}, the scrapie form

Prion

Proteinaceous infectious particles

Novel Proteinaceous Infectious Particles Cause Scrapie

Stanley B. Prusiner

Summary. After infection and a prolonged incubation period, the scrapie agent causes a degenerative disease of the central nervous system in sheep and goats. Six lines of evidence including sensitivity to proteases demonstrate that this agent contains a protein that is required for infectivity. Although the scrapie agent is irreversibly inactivated by alkali, five procedures with more specificity for modifying nucleic acids failed to cause inactivation. The agent shows heterogeneity with respect to size, apparently a result of its hydrophobicity; the smallest form may have a molecular weight of 50,000 or less. Because the novel properties of the scrapie agent distinguish it from viruses, plasmids, and viroids, a new term "prion" is proposed to denote a small *pro*teinaceous *in*fectious particle which is resistant to inactivation by most procedures that modify nucleic acids. Knowledge of the scrapie agent structure may have significance for understanding the causes of several degenerative diseases.

A major, unanswered question in mo- senile dementia, was shown by Gibbs, ek, and co-workers to be caused ansmissible agent (*6, 7*). cent study suggests that there may ilarities between the agents caus- apie and CJD (*8*). Goats inoculat- brain tissue from demented pa- lying of CJD developed a neuro- disorder 3 to 4 years after inocula- g. 1). Five out of ten CJD inocula roduced disease in goats (*9*). Ex- ntal CJD in goats is indistinguish- th clinically and neuropathologi- rom natural scrapie. Monkeys

ibation period, the scrapie agent us system in sheep and goats. Six ses demonstrate that this agent . Although the scrapie agent is vith more specificity for modifying shows heterogeneity with respect ; the smallest form may have a vel properties of the scrapie agent new term "prion" is proposed to hich is resistant to inactivation by dge of the scrapie agent structure ses of several degenerative dis-

Scrapie Agent Contains Protein

Six separate and distinct lines of evidence show that the scrapie agent contains a protein that is required for infectivity: (i) inactivation as a result of digestion with proteinase K, (ii) inactivation by chemical modification with diethyl pyrocarbonate, (iii) inactivation by SDS, (iv) inactivation by chaotropic salts such as guanidinium thiocyanate, (v) inactivation by phenol, and (vi) inactivation by urea (*60*). The cumulative evidence for a protein within the scrapie agent appears to be compelling (Table 1).

animals were vaccinated against louping ill virus with a formalin-treated suspension of ovine brain and spleen that, as was shown subsequently, had been contaminated with the scrapie agent (*2*). Two years later, 1500 sheep developed scrapie. Subsequently, studies on CNS diseases (including scrapie) of sheep provided the foundation for Sigurdsson's concept of slow infections (*3*). In 1959, Hadlow suggested that kuru, a CNS degenerative disease of New Guinea highlanders, might be similar to scrapie because the pathologies of these disorders share many features (*4*). The transmission of kuru to chimpanzees in 1965 by Gajdusek, Gibbs, and Alpers forced a major reconsideration of the etiology of all degenerative disorders and made scrapie a subject of intense medical interest (*5*). Subsequently, Creutzfeldt-Jakob disease (CJD), a progressive, pre-

have been used as a common experimental host for scrapie and CJD; curiously, chimpanzees are susceptible to CJD but not scrapie (*10*). Numerous attempts to link scrapie epidemiologically to CJD have been unsuccessful (*11*). At present, there is no direct evidence that the scrapie agent causes disease in humans.

In contrast to CJD which occurs worldwide, kuru is found only in a small mountainous region of Papua New Guinea. Epidemiological studies of kuru provide evidence for incubation periods of 20 to 30 years (*12, 13*). Although considerable evidence implicates cannibalism in the spread of kuru, no direct observations of cannibalistic acts in the "endemic" region have been recorded. Attempts to transmit kuru by feeding infected brain tissue to chimpanzees have been unsuccessful although one monkey developed a kuru-like illness 36 months after oral ingestion of the kuru agent (*14*). In contrast, goats fed scrapie-infected tissue frequently develop disease (*15*). Recently, we have taken advantage of the natural cannibalistic activities of hamsters to develop an experimental model of scrapie transmitted by cannibalism (*16*). Oral transmission of the scrapie agent appears to be extremely inefficient. Cannibalism requires a dose of agent 10^9 times greater than that needed to produce scrapie by intracerebral injection. These results provide compelling evidence for oral transmission of the scrapie agent and may offer new insights into the spread of kuru by cannibalism among the Fore people and their neighboring tribes.

Bioassay of the Scrapie Agent

Studies on the scrapie, kuru, and CJD agents have been greatly limited by the slow, tedious, and costly bioassays used to detect these agents. Since tissue culture systems are not available for the replication and assay of these agents and they appear to be nonantigenic in their native forms, animal bioassays must be used. For many years all assays for the scrapie agent were performed in sheep and goats (*17*). In 1961, transmission of the scrapie agent to mice transformed research (*18*), but the murine end-point titration assay was still heroic. Quantifying a single sample required eight to ten serial tenfold dilutions and injection of each dilution into six mice (*19*). Then 50 to 60 mice were held for 1 year and examined weekly for signs of scrapie. The number of animals developing scrapie at the highest dilution was used to calculate an end point. The time required for titration of a sample was reduced to 200 days when a more rapid form of the disease in hamsters was discovered (*20, 21*).

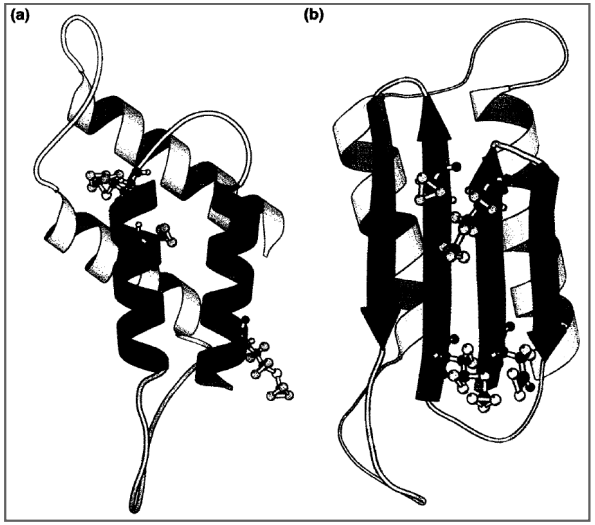
Several investigators have estimated scrapie titers by measuring the time interval from inoculation to onset of illness (incubation period) in mice (*22, 23*). Reluctance to refine such measurements has prevented its wide use in mice.

With hamsters, studies on the scrapie agent have been accelerated by development of a bioassay based on measurements of incubation time (*24, 25*). It is now possible to assay samples with the use of four animals in 60 to 70 days if the titers of the scrapie agent are high. As is shown in Fig. 2, the interval from inocu-

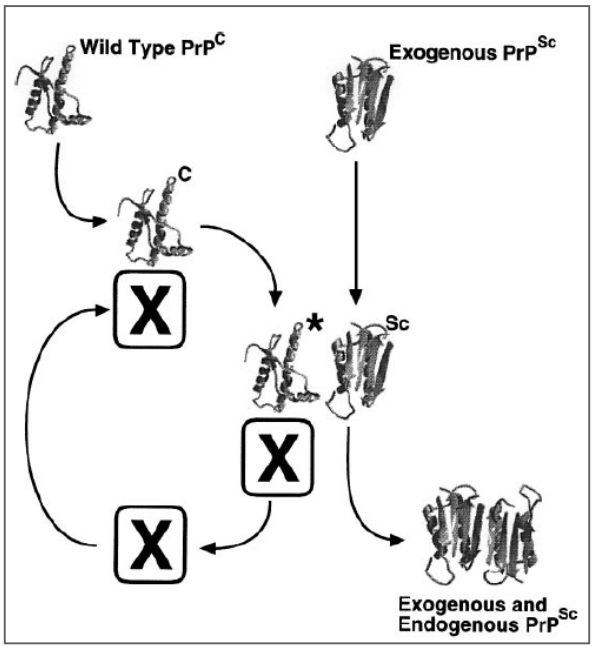
The author is an associate professor in the Departments of Neurology and Biochemistry and Biophysics at the School of Medicine, University of California, San Francisco 94143.

0036-8075/82/0409-0136$01.00/0

Novel proteinaceous infectious particles cause scrapie, Stanley B. Prusiner, Science. 1982 Apr 9;216(4542):136-44.

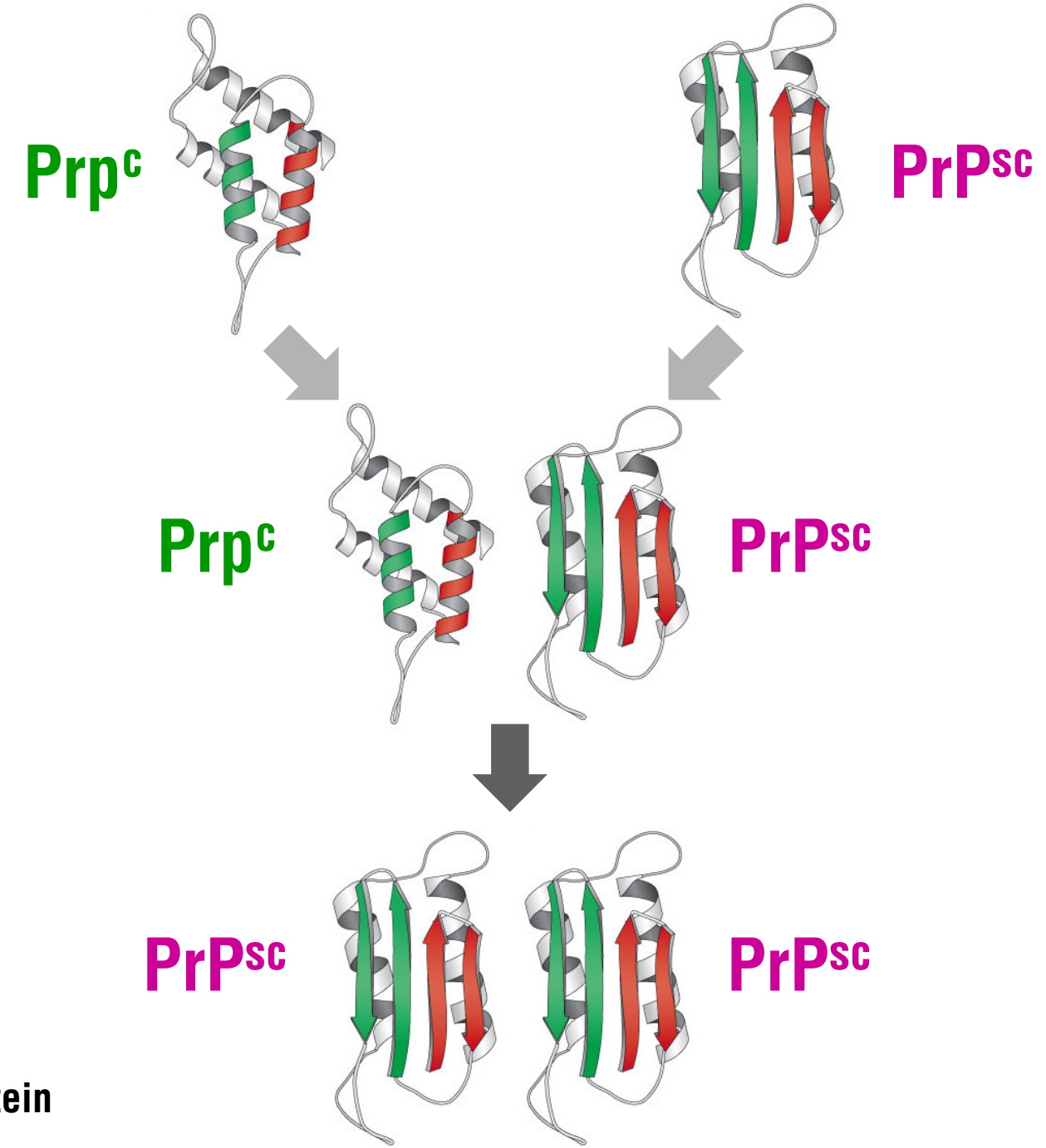


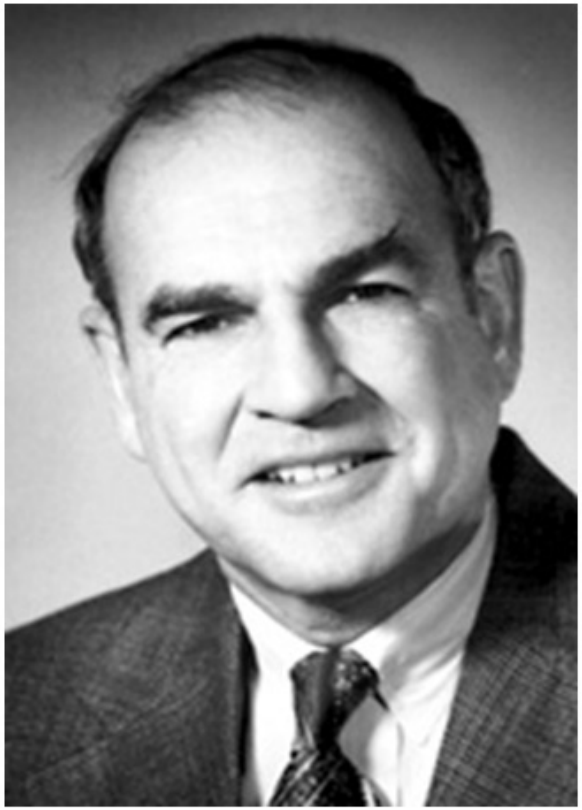
TIBS 21 - December 1996



Annu. Rev. Biochem. 1998. 67:793–819

Prp^{c}, normal cellular prion protein
PrP^{sc}, scrapie form





Baruch S. Blumberg

D. Carleton Gajdusek

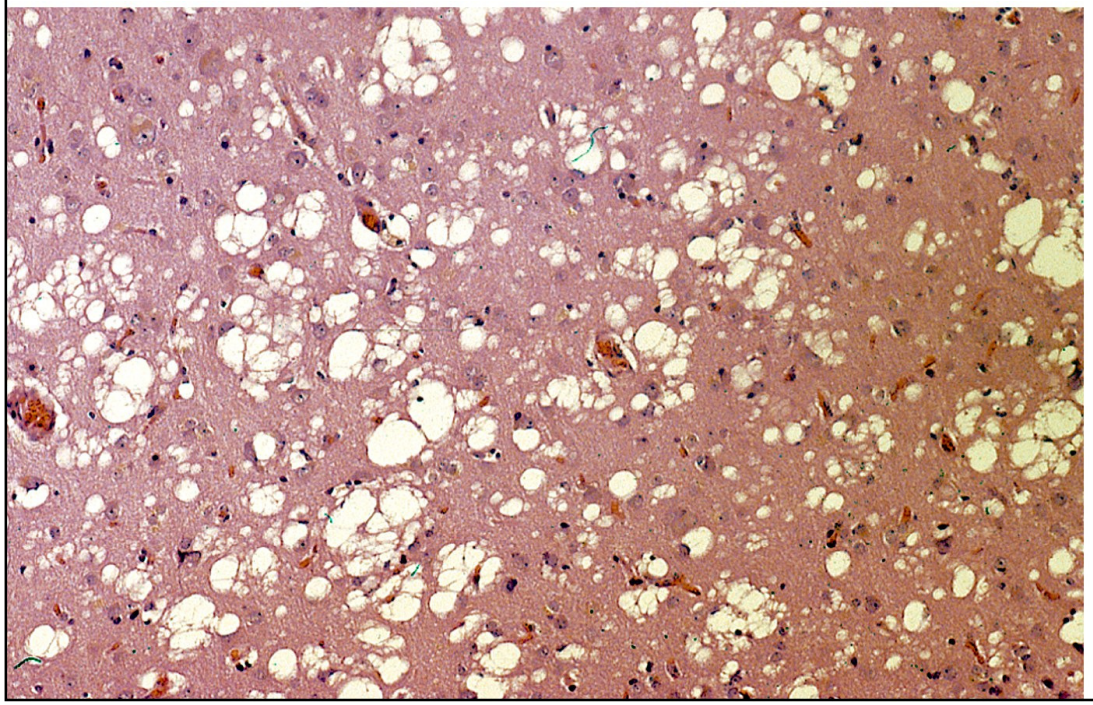
在研究Prion蛋白的領域已經產生兩位諾貝爾醫學暨生理學獎得主 (1976及1997)。第一位是 Gajdusek，他於巴布亞新幾內亞的食人族部落發現庫魯 (kuru) 症，並推測病源可能是一種慢性作用病毒 (slow-acting virus)。Prusiner 則是第二位研究該領域獲獎的科學家，他發現造成羊搔癢症的病原並不是病毒，它是一種不含 DNA 或 RNA 的物質，而是一種變異的蛋白質，Prusiner 並將此具感染力病原命名為 Prion。

The Nobel Prize in Physiology or Medicine 1976 was awarded jointly to Baruch S. Blumberg and D. Carleton Gajdusek *"for their discoveries concerning new mechanisms for the origin and dissemination of infectious diseases"*

B型肝炎表面抗原　　**Kuru**

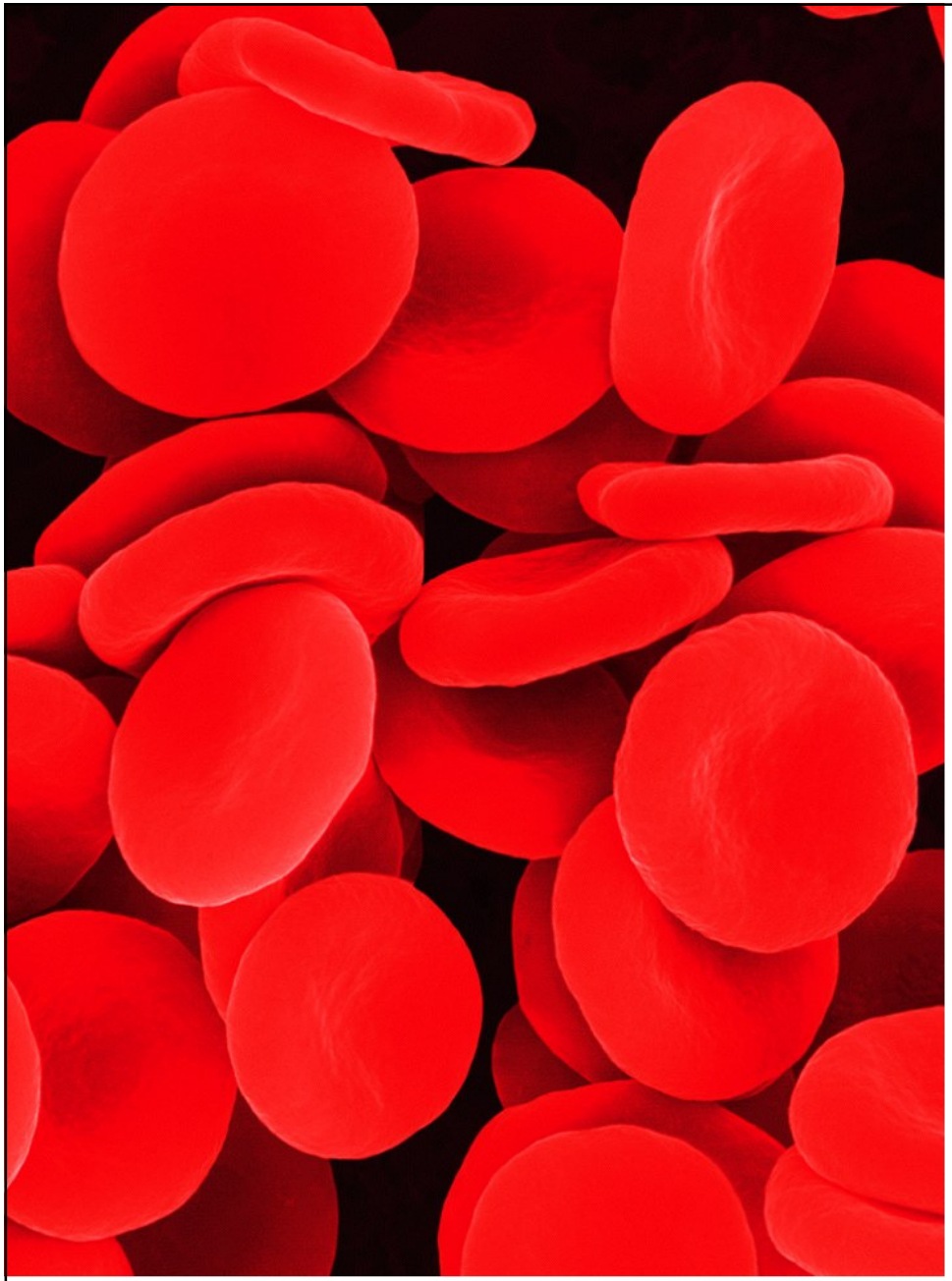
The prion-related diseases

- Creutzfeldt-Jakob Disease and kuru in human
- Bovine spongiform encephalopathy, BSE (Mad Cow Disease) 牛海綿狀腦病
- Scrapie in sheep
- Chronic Wasting Disease in deer and elk



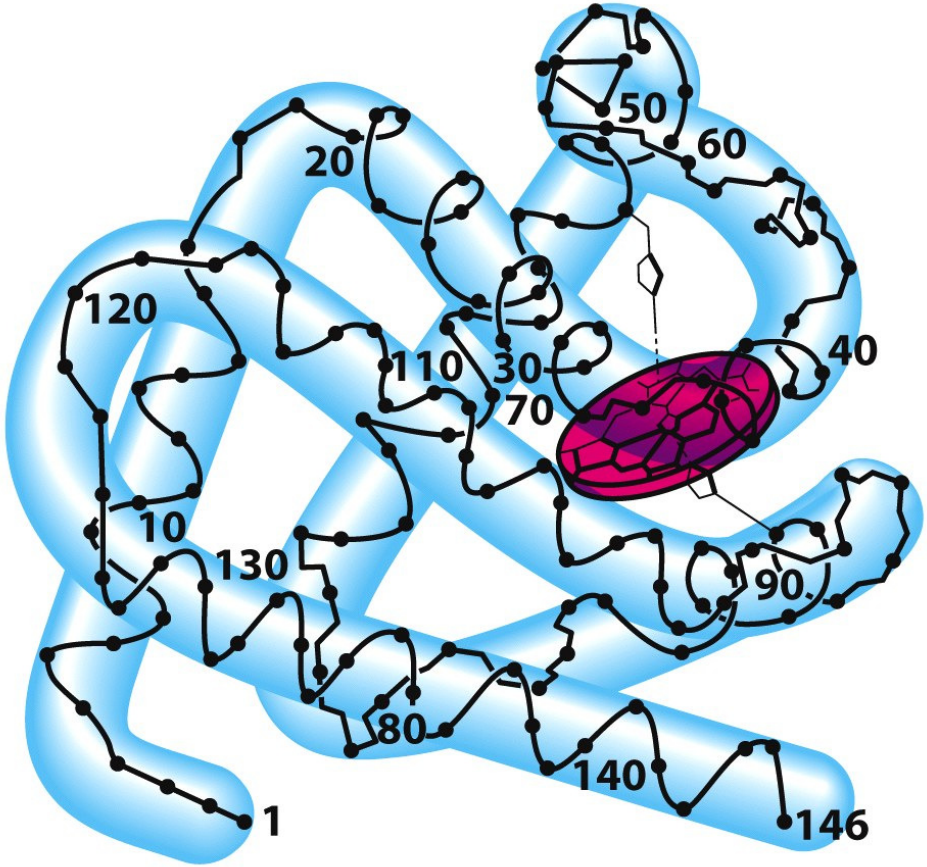
■ Stained section of cerebral cortex from autopsy of a patient with CJD showes spongiform degeneration, the most characteristic neurohistological feature

Lehninger Principles of biochemistry, 5th edition (2008)



Red Blood Cell

Hemoglobin gives blood its red color



Beta chain of hemoglobin

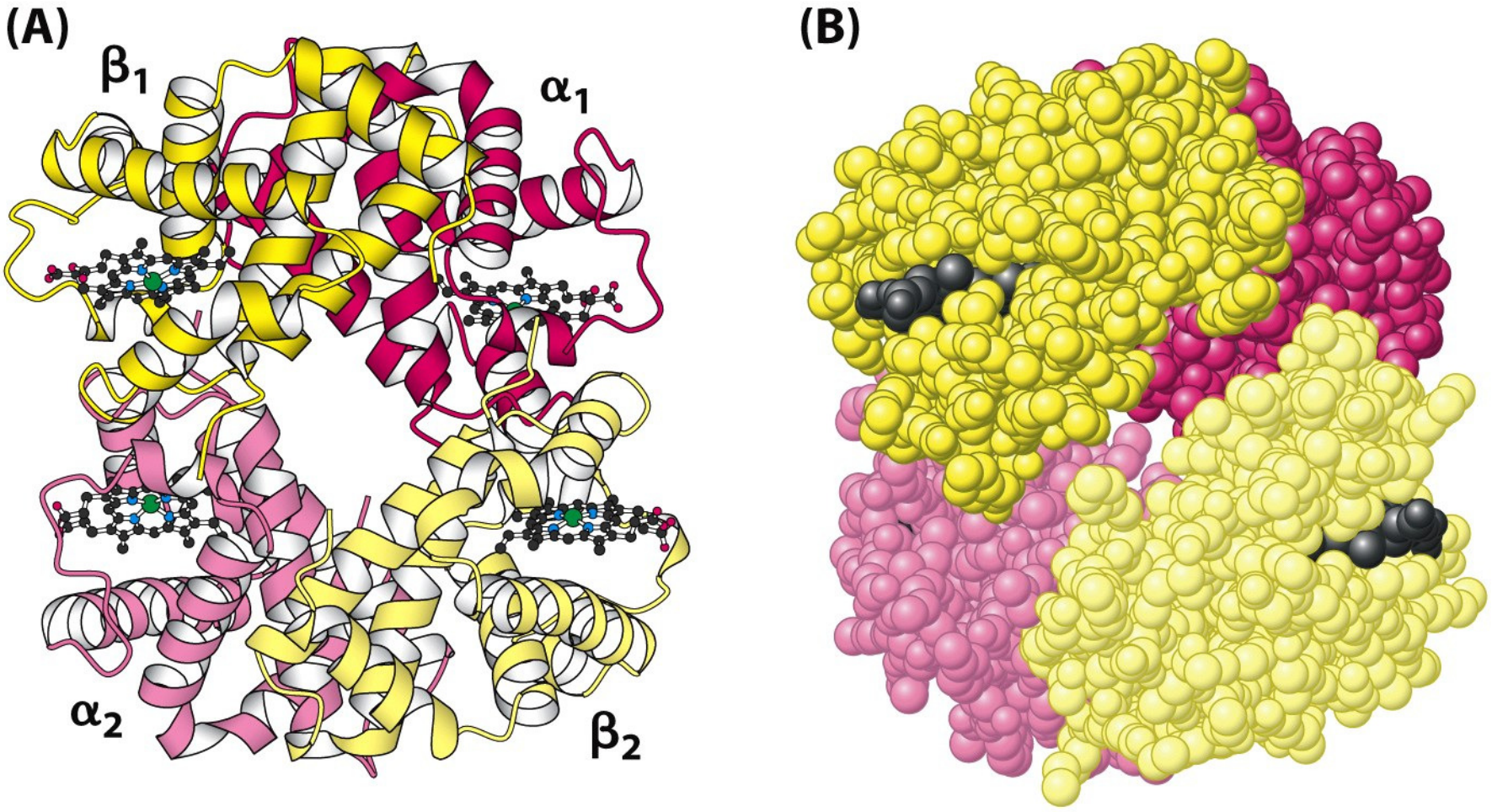
For their studies of the structures of globular proteins

1962

Max Perutz, 1914-2002 (left) John kendrew, 1917-1997 (right)

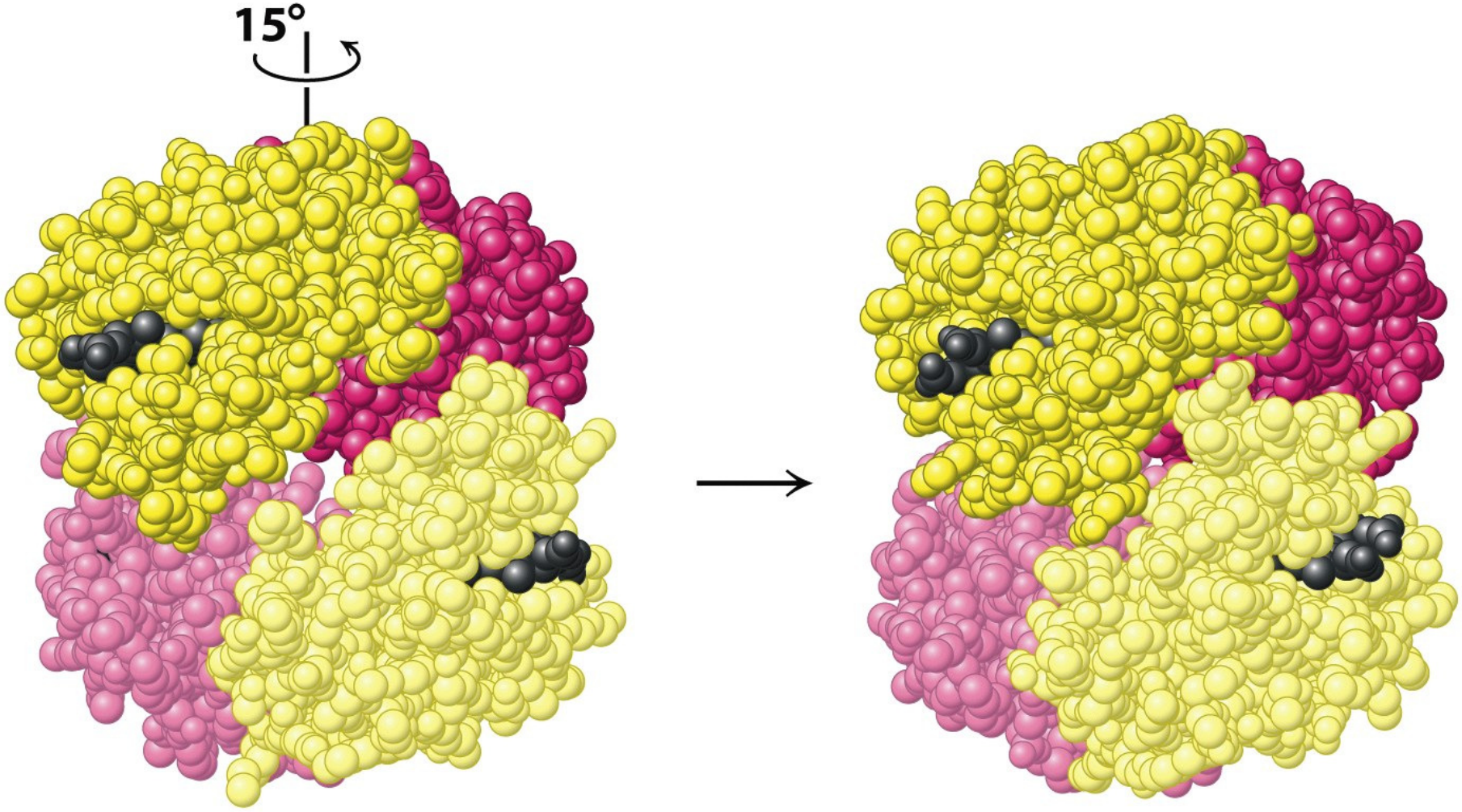
Perutz and Kendrew won the Nobel Prize in 1962 for solving the structures of hemoglobin (Perutz) and myoglobin (Kendrew). This is the same year that Watson, Crick, and Wilkins won for the structure of DNA. Recall that Watson & Crick were working in the Perutz lab at the time of their discovery and Crick was actually working on the structure of hemoglobin as part of his Ph.D. thesis

Quaternary structure of deoxyhemoglobin



Biochemistry, 7th edition (2010), Berg, Tymoczko and Stryer

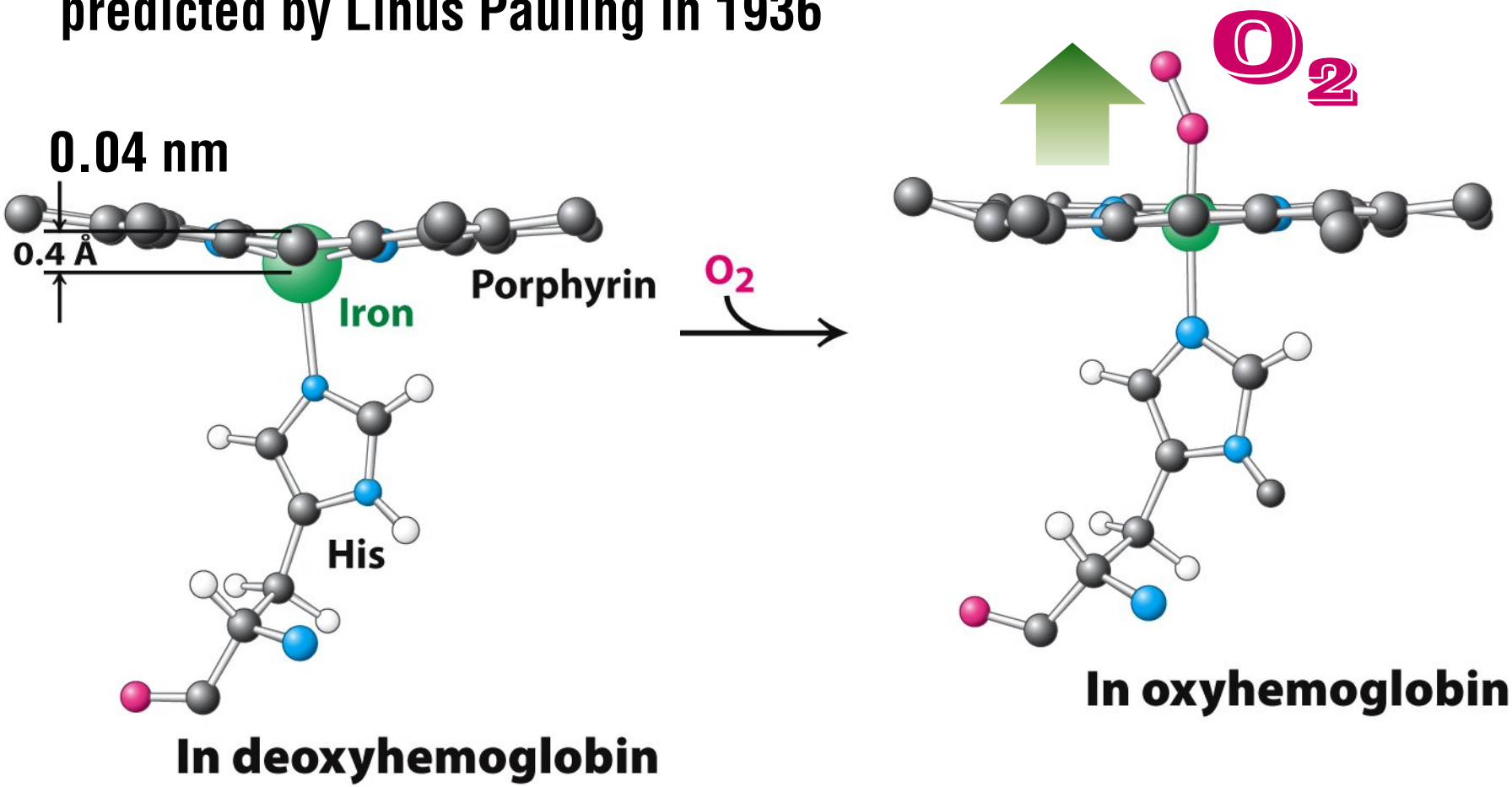
On oxygenation, one αβ dimer shifts with respect to the other by a rotation of 15 degrees



Biochemistry, 7th edition (2010), Berg, Tymoczko and Stryer

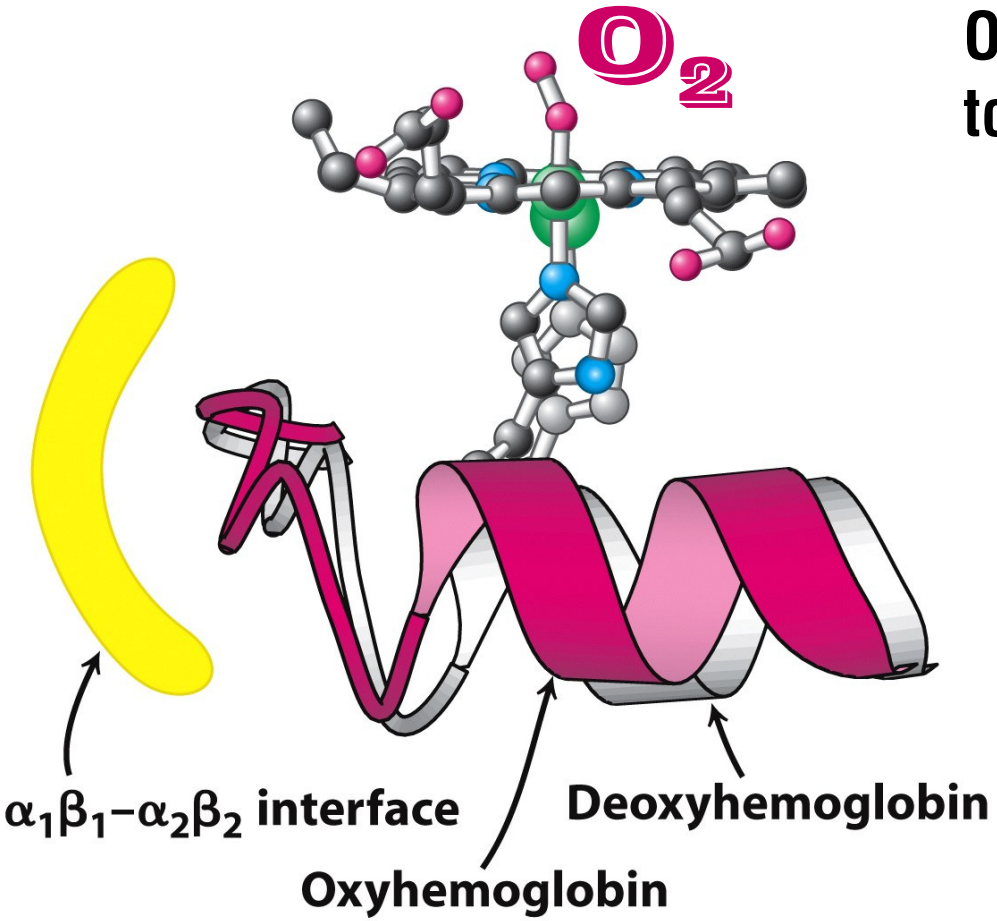
Oxygen binding changes the position of the iron ion

- **Iron ion moves into the plane of the heme on oxygenation**
  predicted by Linus Pauling in 1936

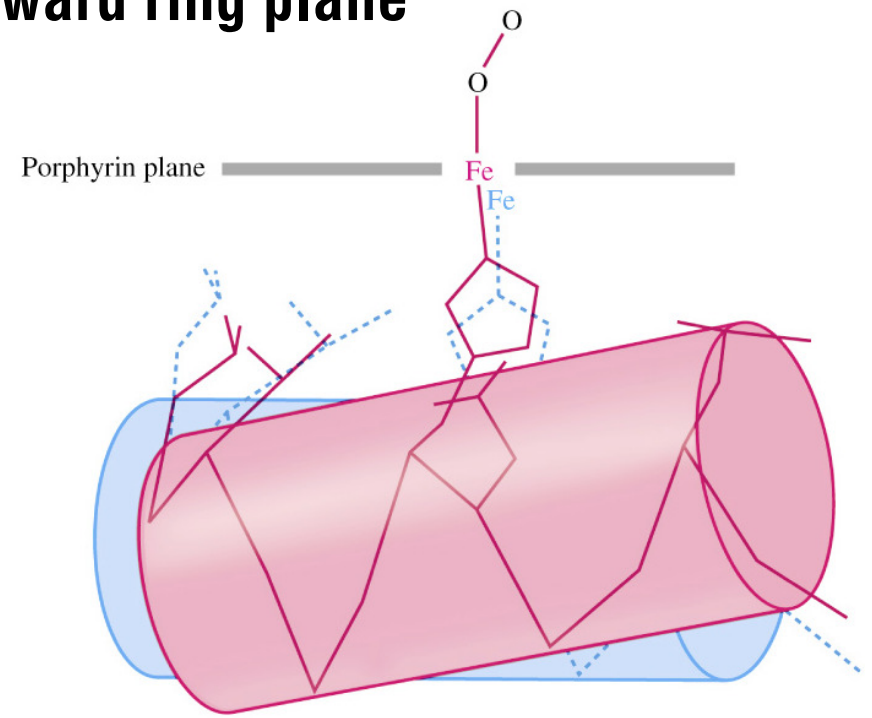


Biochemistry, 7th edition (2010), Berg, Tymoczko and Stryer

Conformational changes in a hemoglobin chain induced by oxygenation



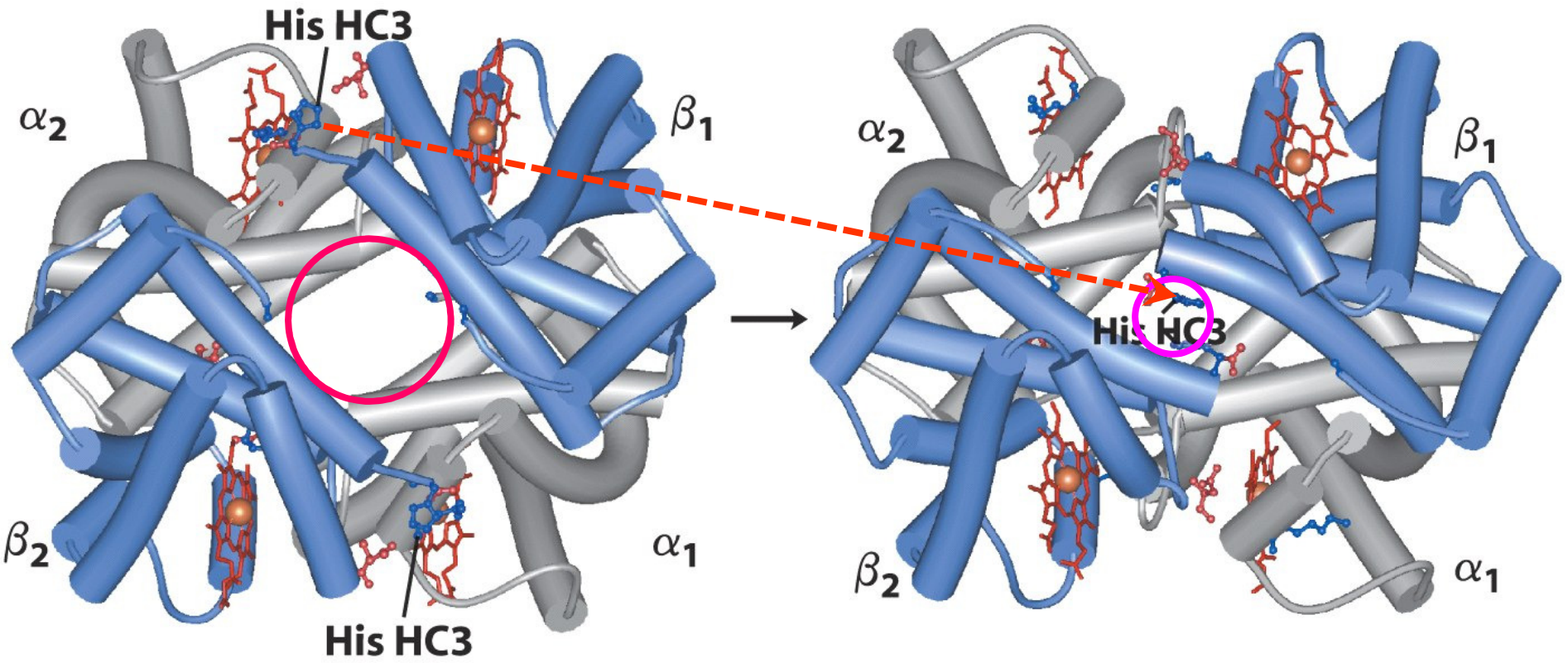
Oxygen binding to Fe pulls the His toward ring plane



- For Mb, this small change has little consequence.
- But a similar change in Hb initiates a series of conformational changes that are transmitted to adjacent subunits.

The T → R transition

Two conformations of Hb: T state (inactive) and R state (active)



T state

Deoxyhemoglobin

T: tense (low-affinity state)

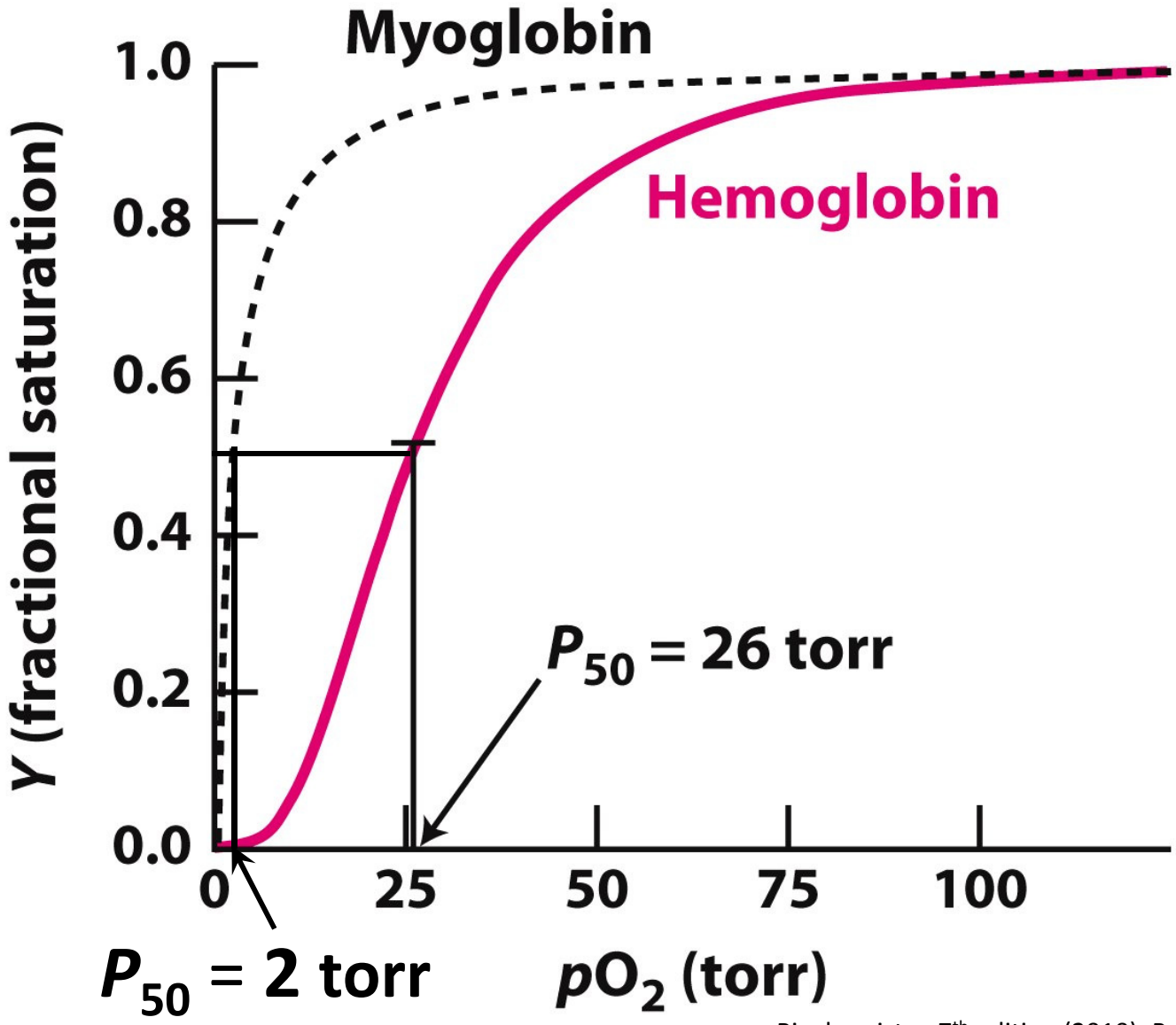
R state

Oxyhemoglobin

R: relaxed (high-affinity state)

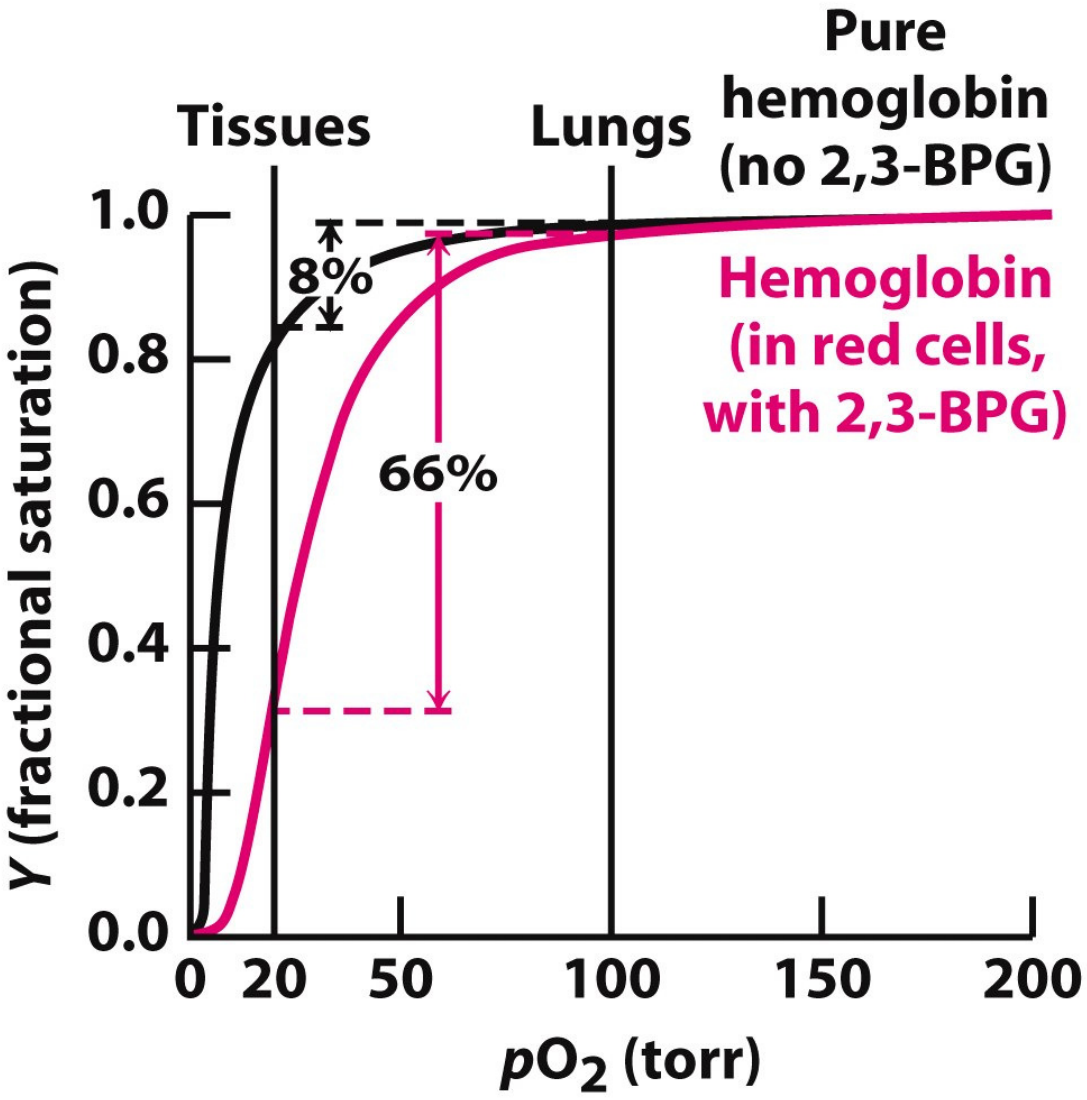
Lehninger Principles of biochemistry, 5th edition (2008)

Oxygen binding curves of Mb and Hb



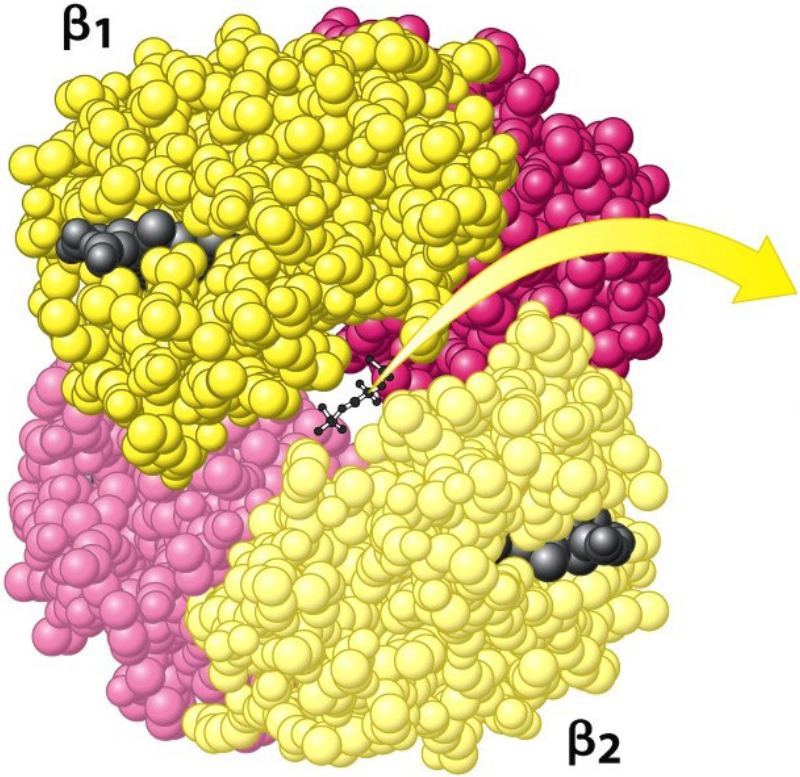
Biochemistry, 7th edition (2010), Berg, Tymoczko and Stryer

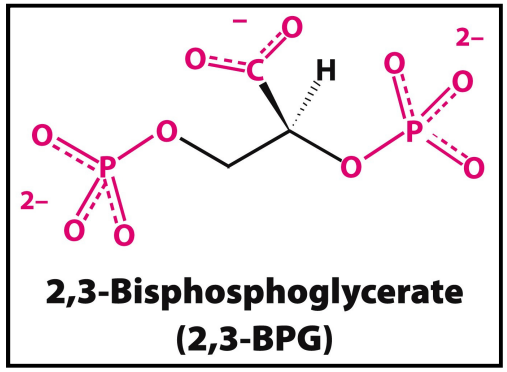
Oxygen Binding by pure hemoglobin compared with hemoglobin in RED BLOOD CELLS

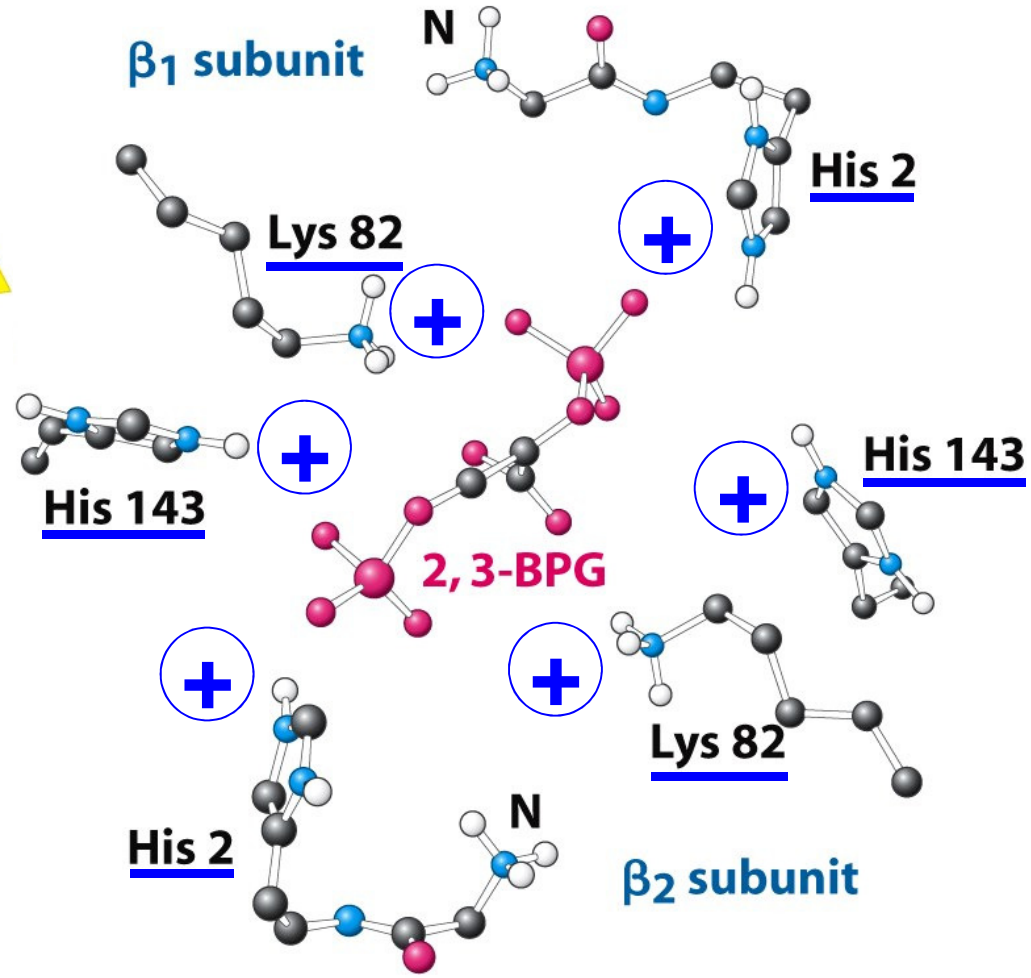


- Oxygen binding to Hb is regulated by 2,3-bisphosphoglycerate (2,3BPG)
- 2,3BPG is an allosteric effector of Hb.
- 2,3BPG lowers the affinity of deoxyHb for oxygen (raises the P_{50} of Hb from ~12 to ~26 torr).

Binding of 2,3BPG to the central cavity of Hb stabilizes the DeoxyHb form



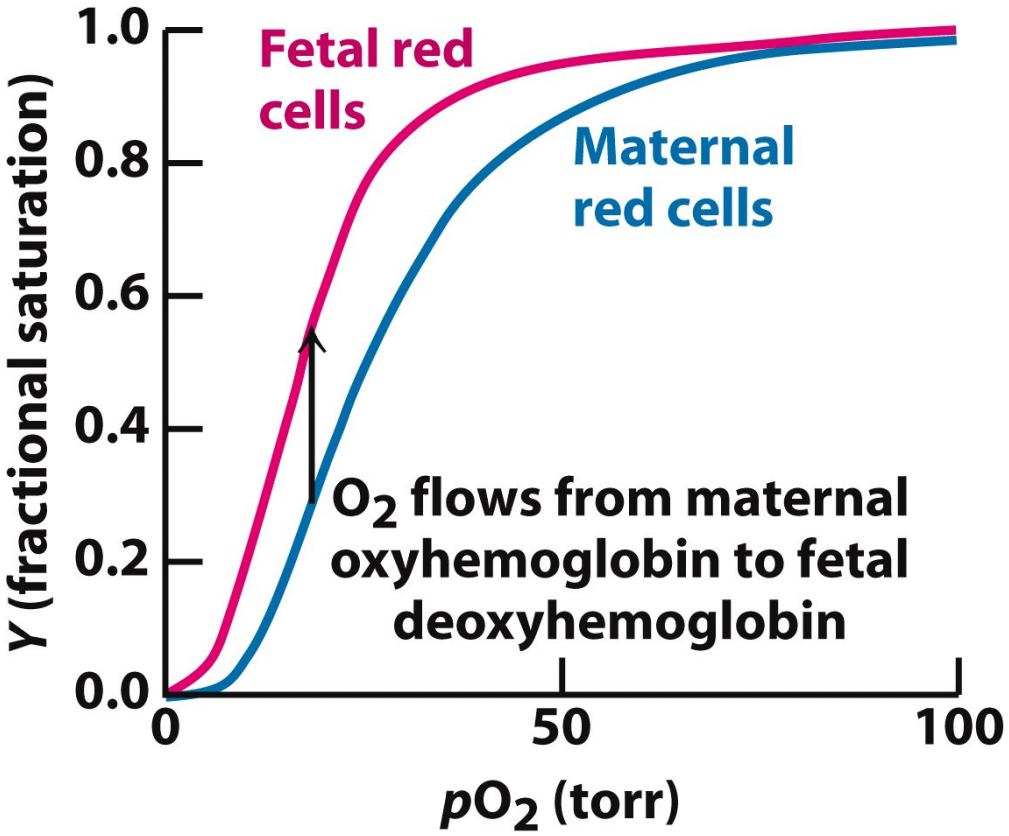




- Negatively charged 2,3BPG is bound to six (+) charged residues of deoxyhemoglobin.

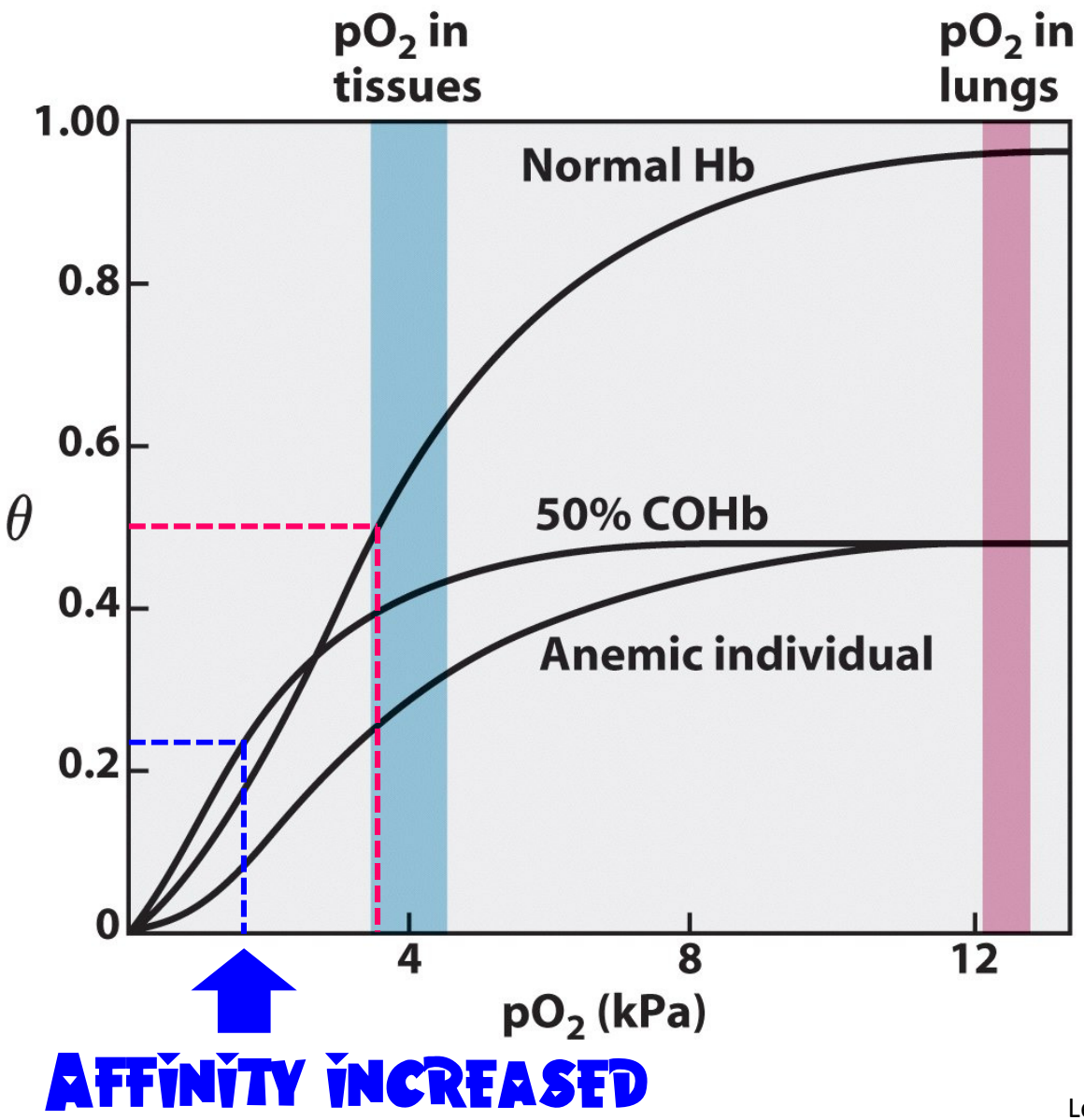
Biochemistry, 7th edition (2010), Berg, Tymoczko and Stryer

Fetal red blood cells have a higher oxygen affinity than do maternal red blood cells



- The fetus synthesizes γ subunits rather than β subunits, forming α2γ2 hemoglobins. His-143 in β chains, part of the BPG-binding site, is substituted to a Ser residue in γ subunits. This change removes two positive charges from the BPG-binding site (one from each chain). Consequently, α2γ2 has a much lower affinity for BPG than normal hemoglobin, and a corresponding higher

Binding of carbon monoxide to Hb



- **Binding of CO increases the affinity of Hb for oxygen**
- **Fetal $\alpha_2\gamma_2$ Hb has a higher affinity for CO than adult Hb**

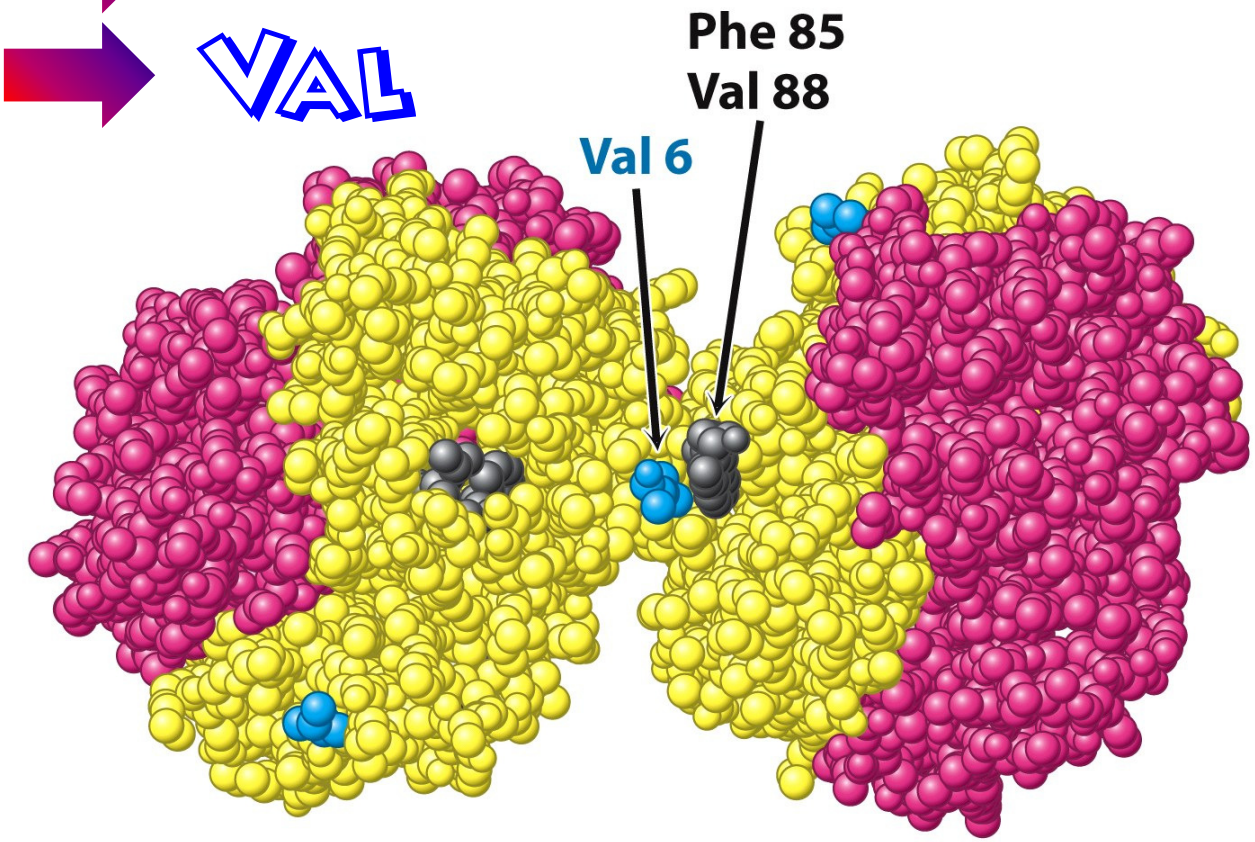
Lehninger Principles of biochemistry, 5th edition (2008)

Sickle-cell anemia results from the aggregation of mutated deoxyhemoglobin molecules (HbS)

GAA → GTA

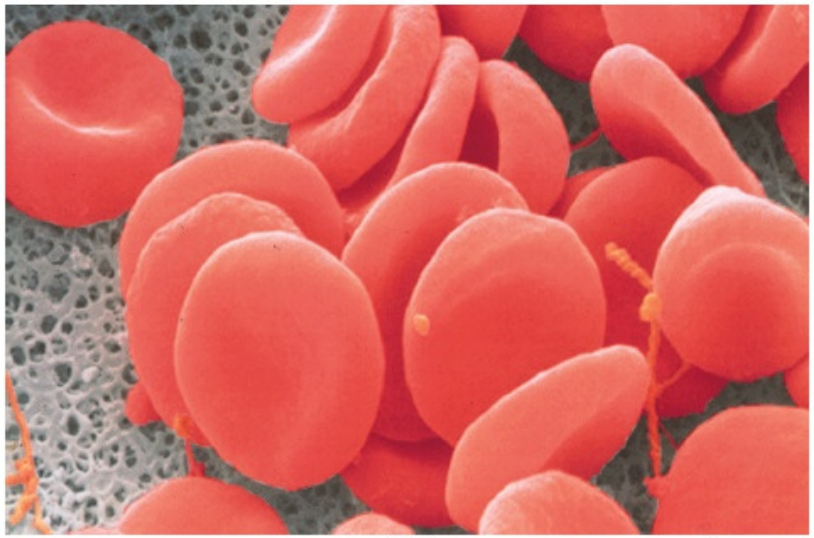
GLU → VAL

Demonstrated by Vernon Ingram in 1956

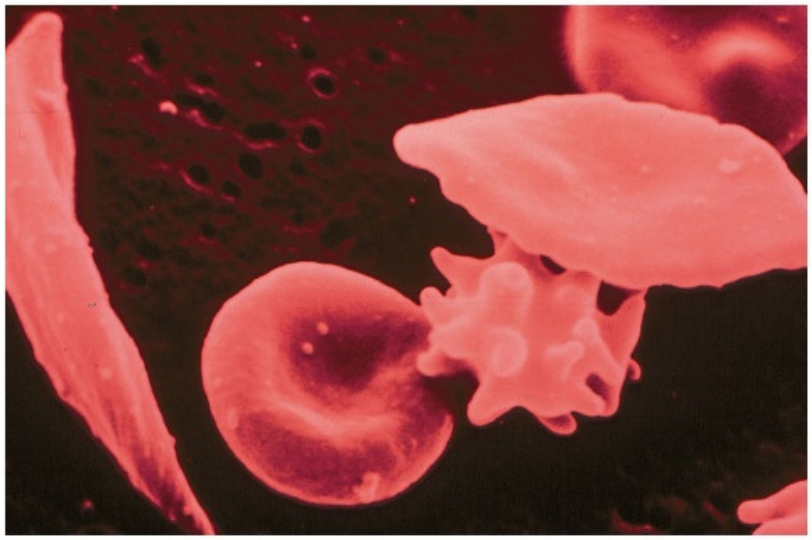


- This was the first time a researcher demonstrated that a single amino acid exchange in a protein can cause a disease or disorder. As a result, Vernon Ingram is sometimes referred to as "The father of Molecular Medicine".

Sickle-cell anemia ("lack of blood")

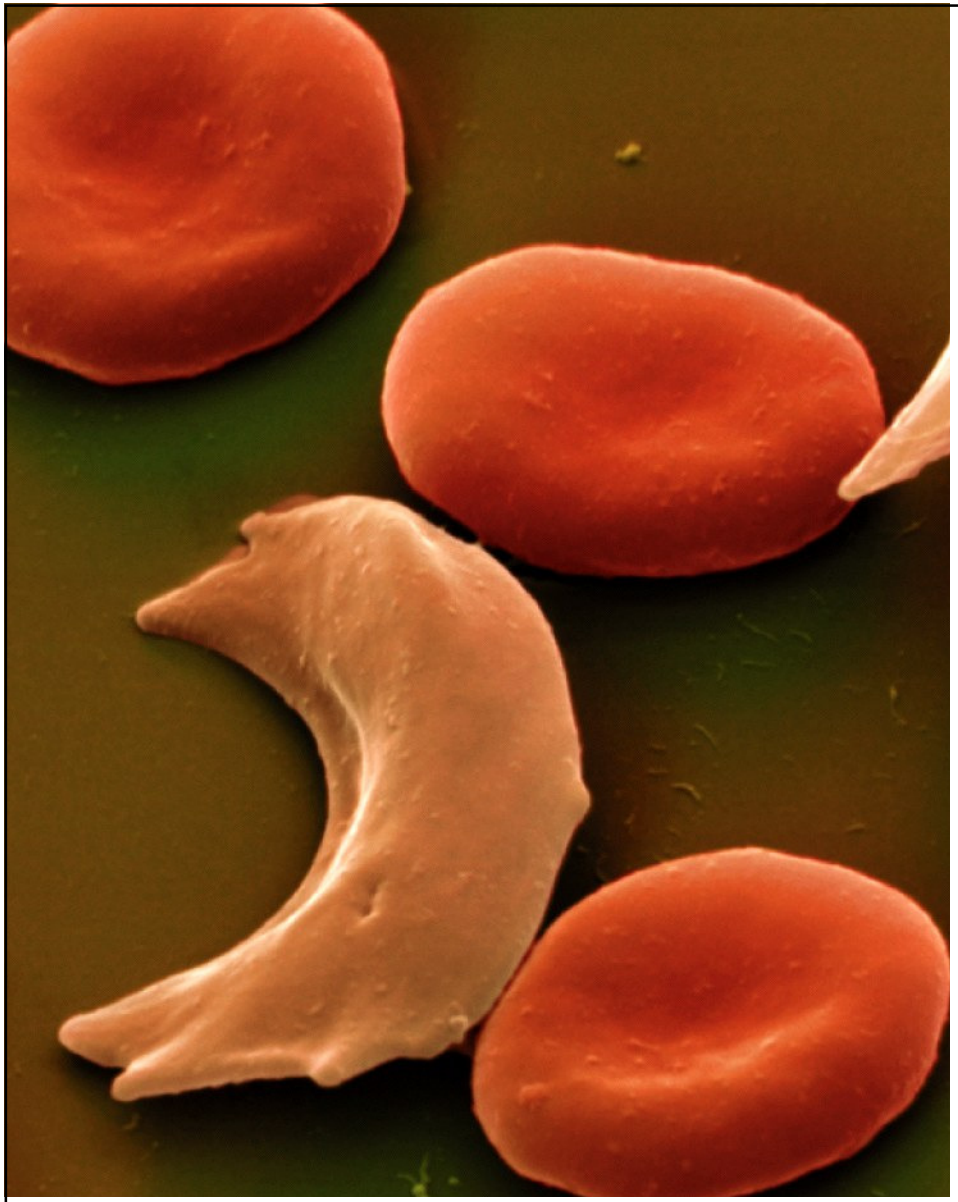


(a)



(b)

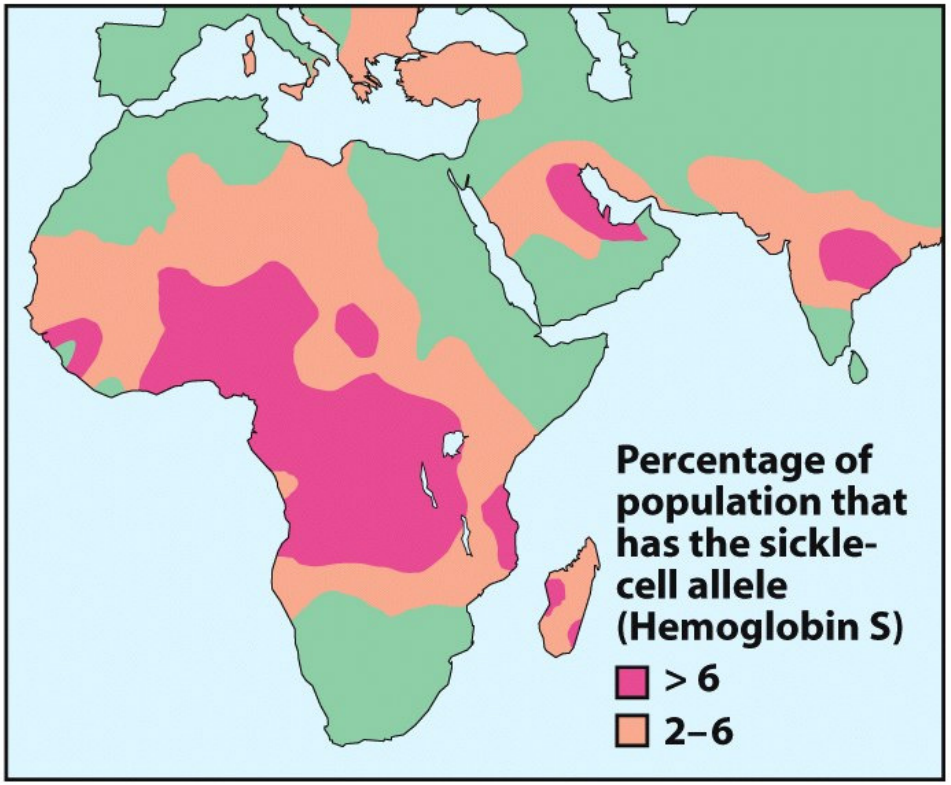
- A genetic disease in which an individual has inherited the allele for sickle-cell hemoglobin from both parents. The erythrocytes of these individuals are fewer and also abnormal.
- Long, thin, crescent-shaped erythrocytes that look like the blade of a sickle.
- Sickle-cell trait (heterozygous): about 1% of erythrocytes become sickled on deoxy.
- Frequency of the sickle-cell allele in populations is unusually high in certain parts of Africa. Investigation into this matter led to the finding that in heterozygous individuals, the allele confers a small but significant resistance to lethal forms of malaria.

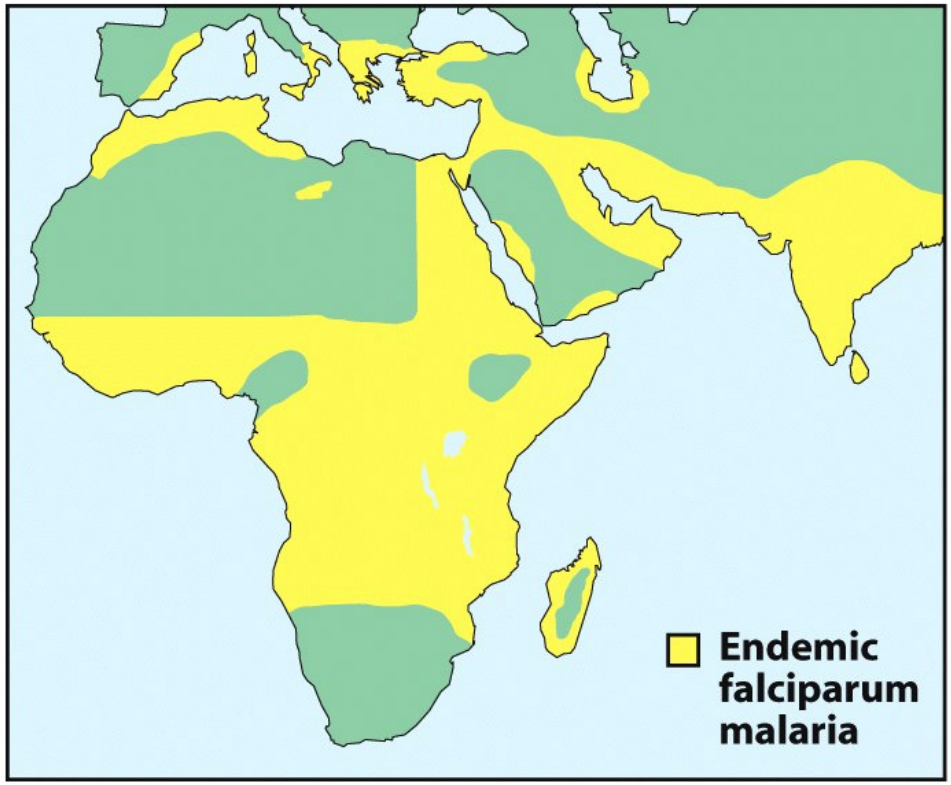


Sickled red blood cells

Sickle-cell hemoglobin fibers

Sickle-cell trait and malaria (瘧疾)





鐮刀型與地中海型貧血患者比具正常性徵的人更不容易罹患瘧疾。因為瘧疾原蟲需要在紅血球裡孵化，而這些貧血患者的紅血球容易破裂死亡，而使瘧疾原蟲無法順利繁衍。然而由於瘧疾盛行區域存活下來的人多為鐮刀型與地中海型貧血的患者或帶有隱性的突變基因者，而使該區域持續具有高比例的鐮刀型與地中海型貧血患者。

- In the 1850s, Louis Pasteur concluded that the fermentation of sugar to alcohol by yeast was catalyzed by a vital force contained within the yeast cells called "ferments", which were thought to be inseparable from the organisms. This view, called vitalism, prevailed for decades.
- In 1877, Wilhelm Frederick Kühne first used the term **enzyme**, which comes from Greek, "in yeast", to describe this process. Note that, in 1876, Kühne discovered the protein-digesting enzyme trypsin.
- In 1897, Eduard Buchner found that the sugar was fermented even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sucrose "zymase". In 1907, he received the Nobel Prize in Chemistry *"for his biochemical research and his discovery of cell-free fermentation"*.

- In 1926, James B. Sumner showed that the enzyme **urease** was a pure protein, and he crystallized it.
- Northrop and Stanley, who worked on the digestive enzymes pepsin (1930), trypsin and chymotrypsin, also proved that pure proteins can be enzymes.
- These three scientists above were awarded the 1946 Nobel Prize in Chemistry. *"for his discovery that enzymes can be crystallized" and "for their preparation of enzymes and virus proteins in a pure form".*
- Lysozyme was the second protein structure and the first enzyme structure to be solved via X-ray diffraction methods by David Chilton Phillips group and published in 1965. This high-resolution structure of lysozyme revealed how enzymes work at an atomic level of detail.
- Many enzymes have been named by adding the suffix **-ase** to the name of their substrates (*e.g.*, urease catalyzes the hydrolysis of urea) or the type of reaction (*e.g.*, DNA polymerase forms DNA polymers).

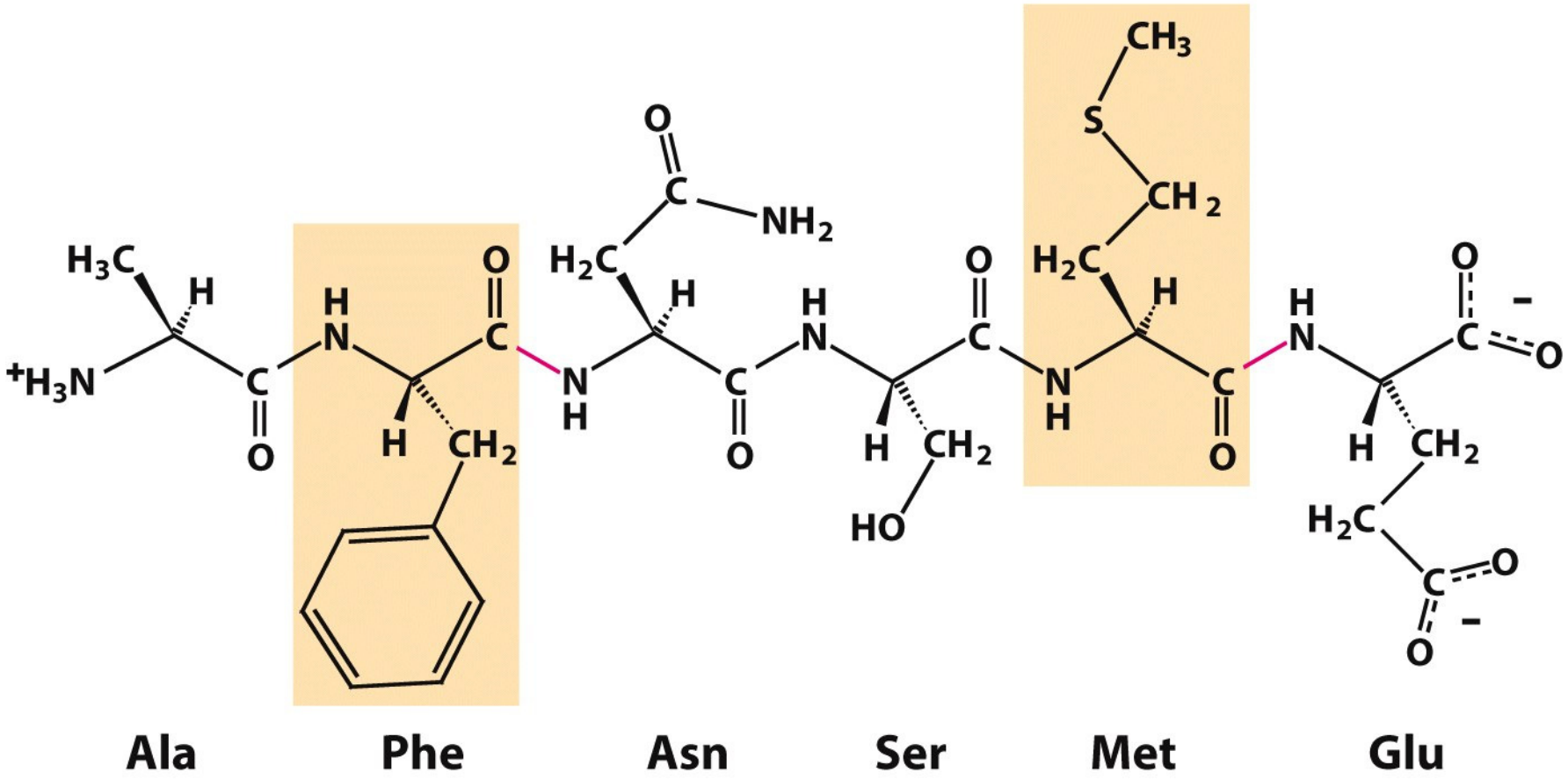
Understanding the enzymatic reactions

- **Understanding of the complete catalytic mechanism of a purified enzyme requires identification of all substrates, cofactors, products, and regulators. Moreover, it requires a knowledge of:**

  1) the temporal sequence in which enzyme-bound reaction intermediates form,
  2) the structure of each intermediate and each transition state,
  3) the structural relationship of the enzyme to each intermediate,
  4) the rates of interconversion between intermediates,
  5) the energy contributed by all reacting and interacting groups to intermediate complexes and transition states.

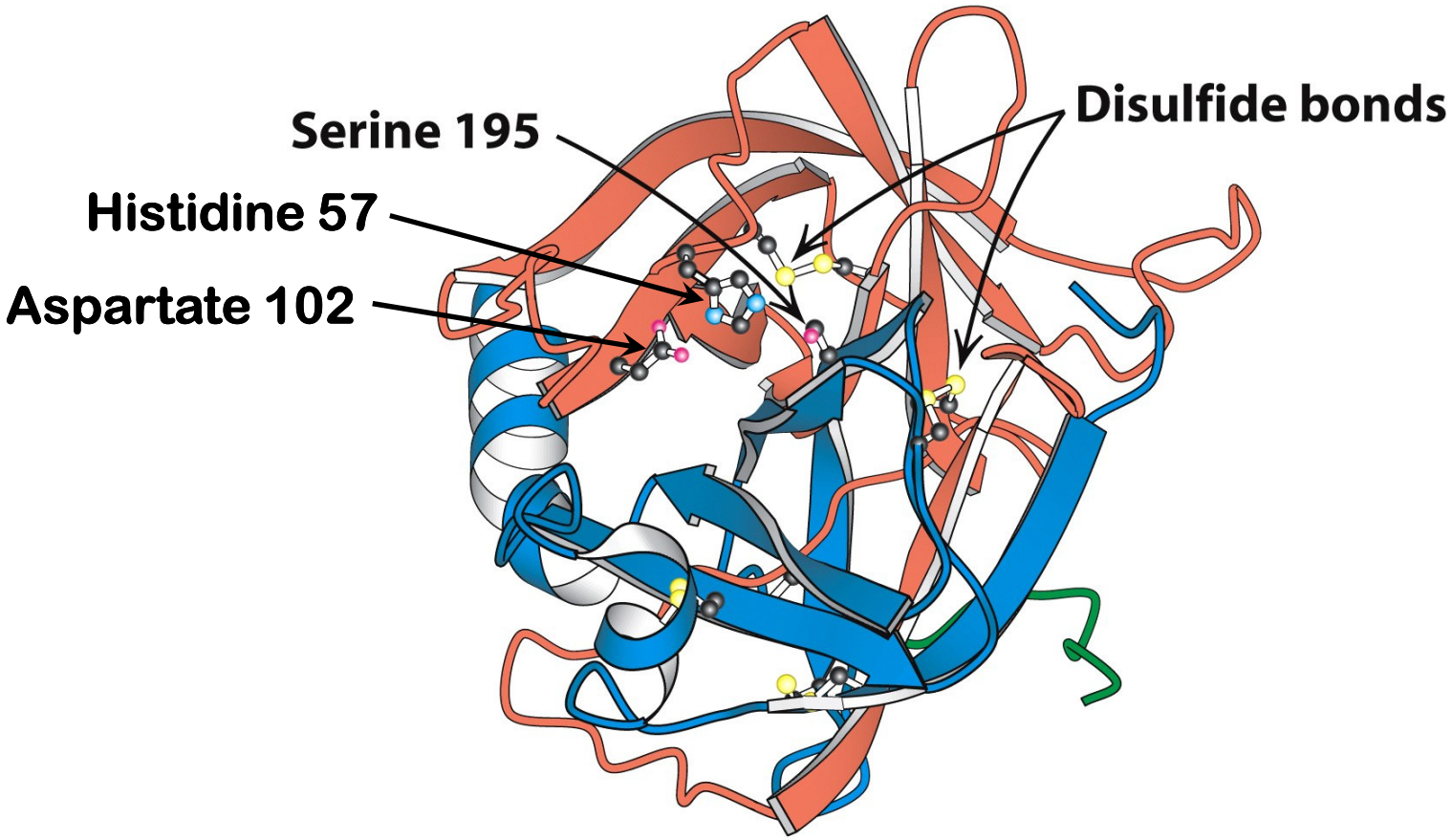
Specificity of chymotrypsin 胰凝乳蛋白酶

- Chymotrypsin cleaves on the carboxyl-terminal side of the large hydrophobic amino acids: Trp, Tyr, Phe, and Met



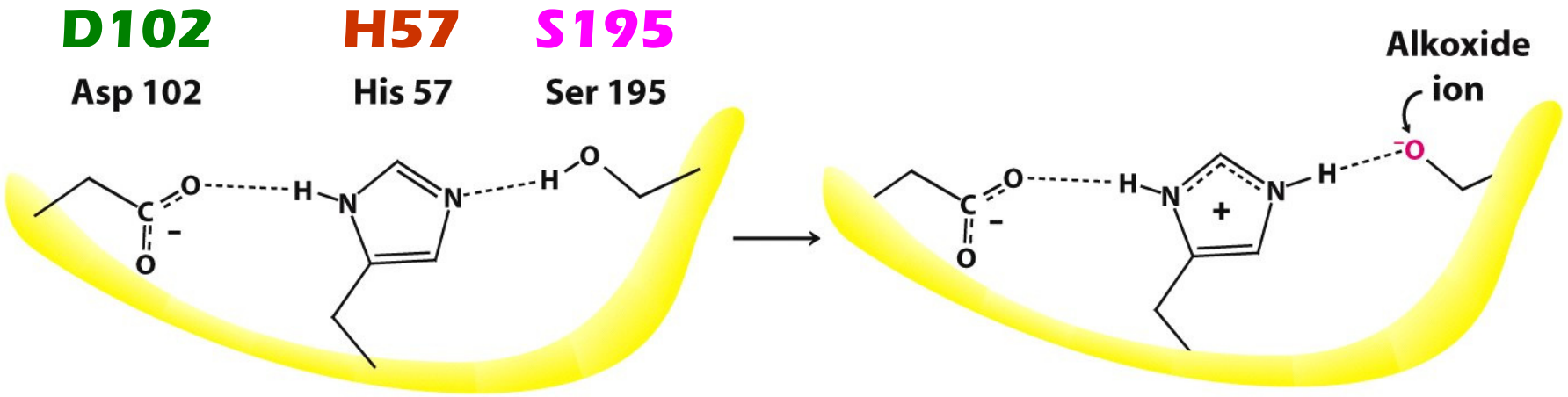
The three-dimensional structure of chymotrypsin

- Chymotrypsin is synthesized as a single polypeptide, termed chymotrypsinogen, which is activated by proteolytic cleavage to yield three chains linked by disulfide bonds.



Solved by David Blow in 1967

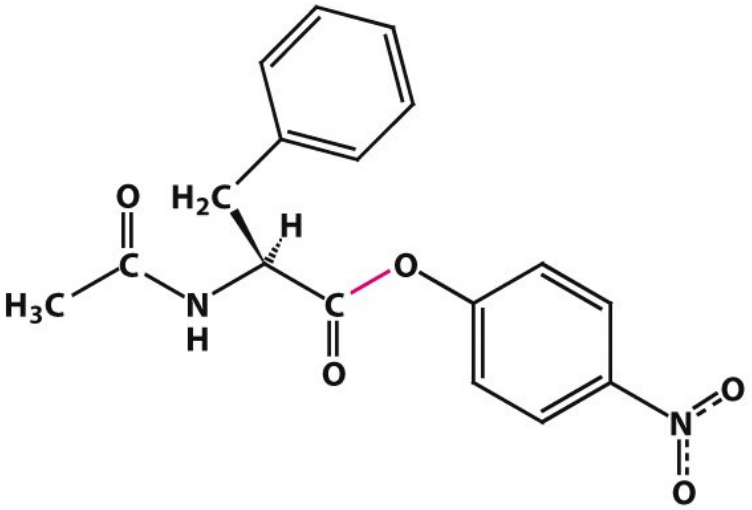
The catalytic triad converts Ser-195 into a potent nucleophile



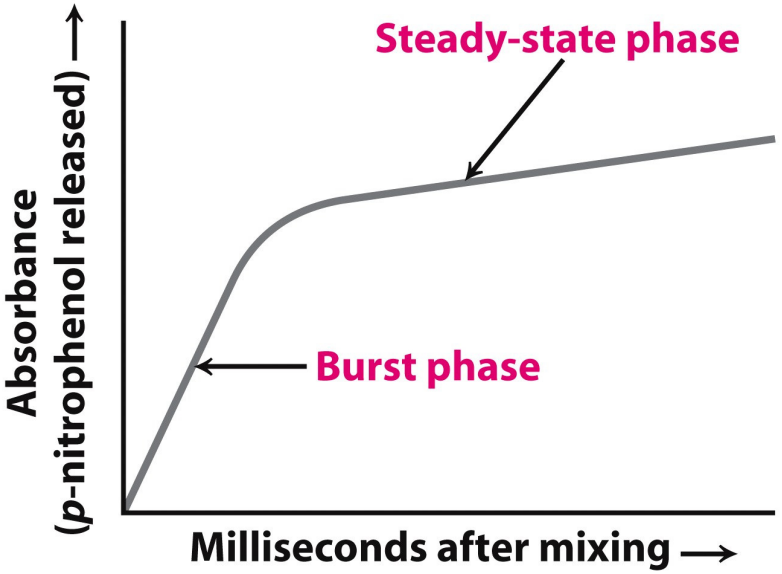
a low-barrier hydrogen bond

CHARGE-RELAY

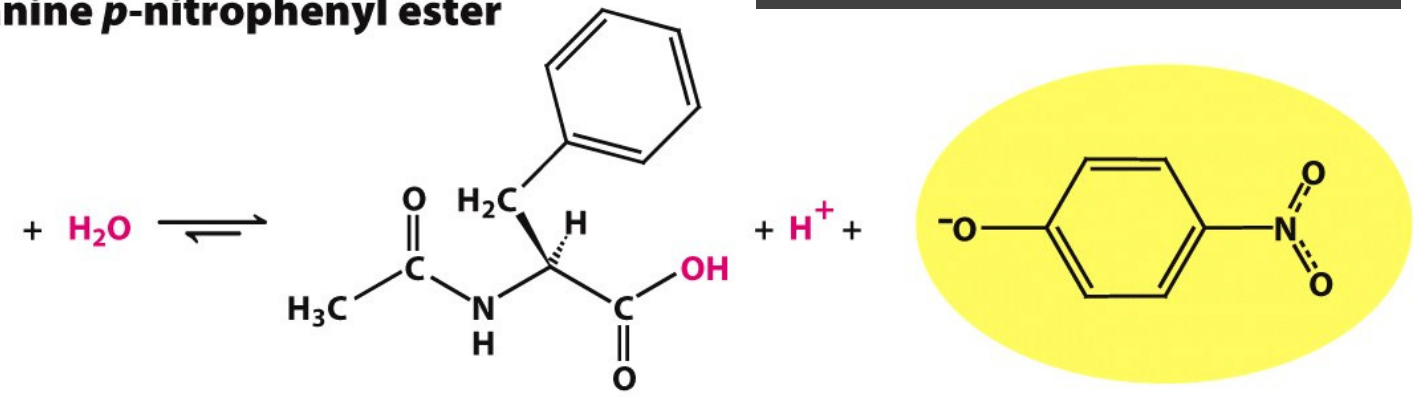
A chromogenic substrate of chymotrypsin



N-Acetyl-L-phenylalanine *p*-nitrophenyl ester



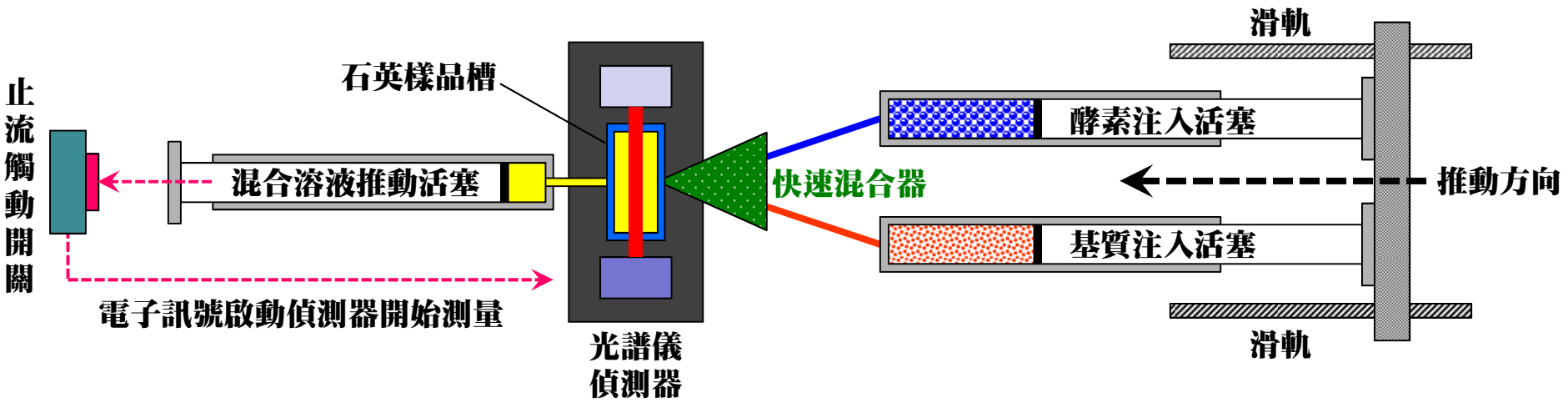
Examined by stopped-flow method



p-Nitrophenolate

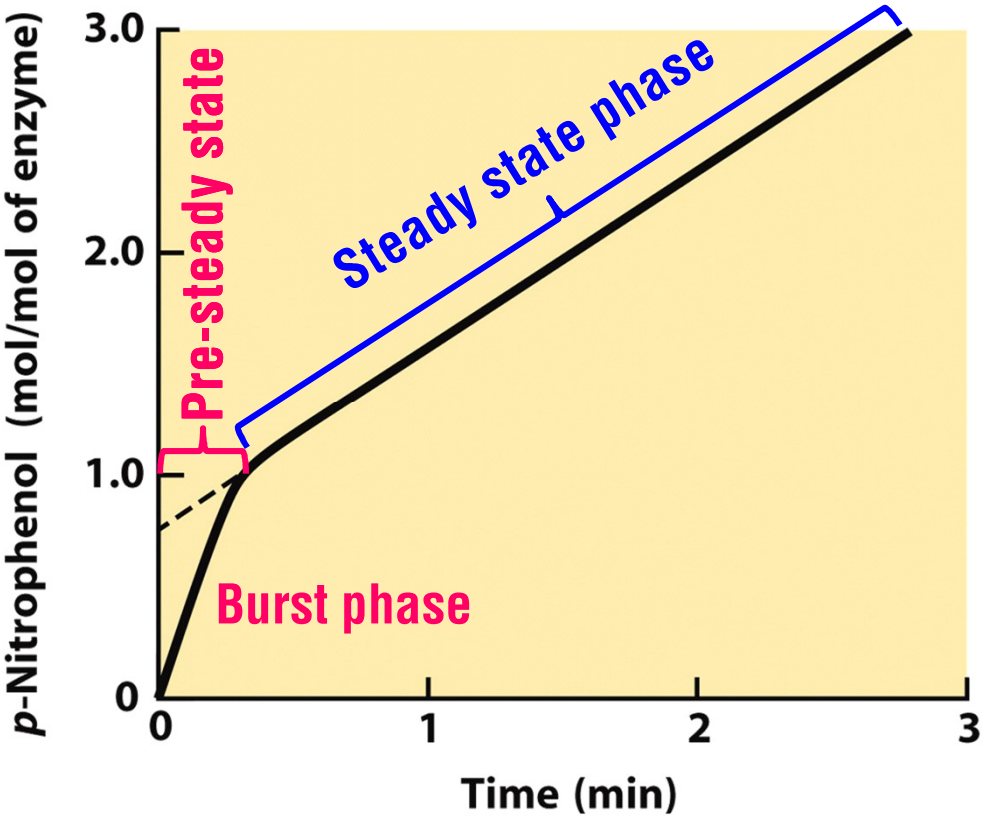
Chymotrypsin: a hydrolase type of enzyme

Stopped-flow method 止流法裝置簡圖

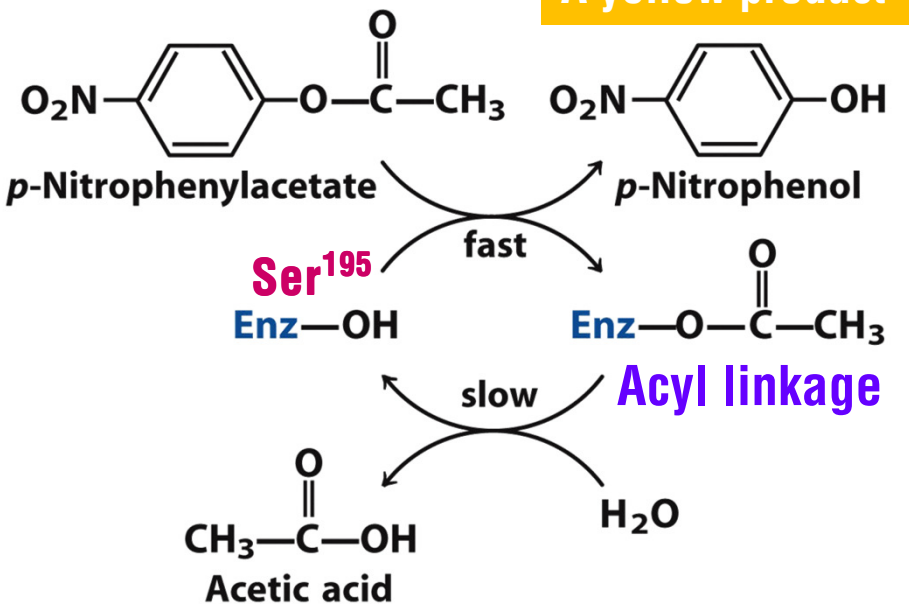


- 兩種樣品溶液藉由活塞的推動注入快速混合器中，混合後立即通過觀測槽進入一裝有活塞之玻璃管中並推動活塞，活塞後方有一觸動開關，當活塞被溶液推至此開關時即觸動止流開關，活塞被迫停止運動，反應物溶液此時即無法再注入。
- 在活塞觸及開關前，觀測槽中（即快速混合槽出口處）之溶液由於反應物溶液不斷地向前推送更新，因此隨時保持初始混合時之狀態，亦即反應時間約等於零。
- 當活塞觸及開關並停止運動時，觀測槽內之溶液不再更新，混合液停留於此處並開始進行反應。活塞觸及開關時亦送出電子訊號控制光譜儀開始測量觀測槽內溶液吸收度隨時間之變化，如此即可得到某一反應物（或產物）濃度隨時間變化之情形。
- 反應之起始時間，則由前述觸動開關所送出之訊號決定。
- 止流法之偵測極限主要仍受限於反應物之混合時間，一般約數個至數十毫秒左右。
- 除了吸收光譜與螢冷光之外，止流法尚可與其他的偵測技術結合，常見的有放射光譜、光散射、導電度等等。

Pre-steady state kinetic evidence for an acyl-enzyme intermediate



A yellow product



Acylation at Ser^{195}

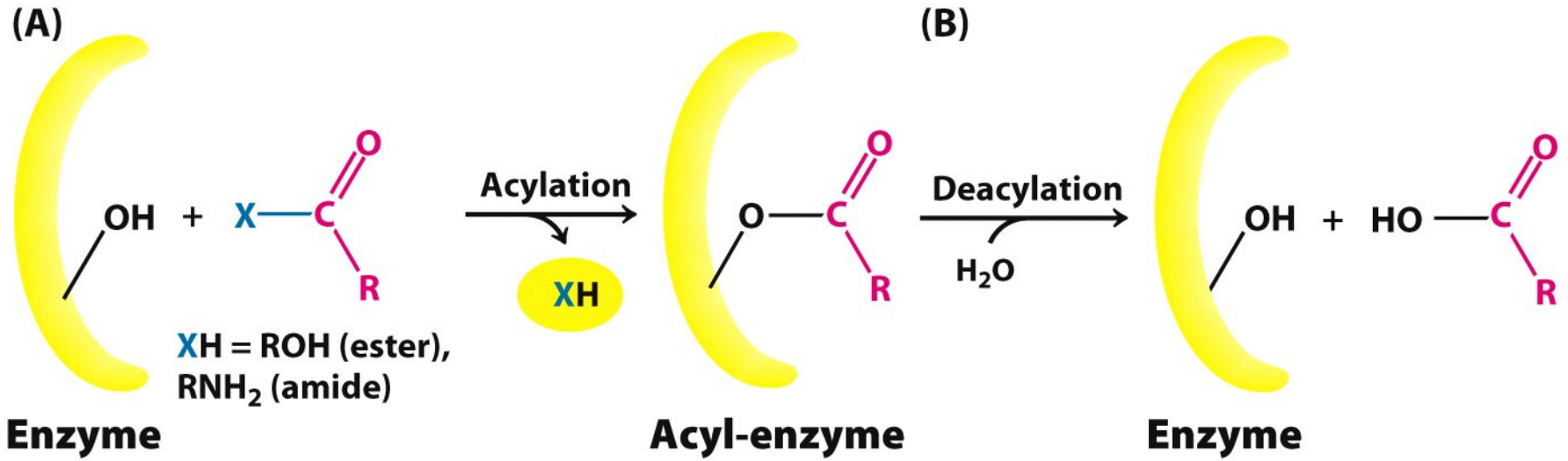
- The reaction releases a rapid burst of *p*-nitrophenol nearly stoichiometric with the amount of enzyme present. This reflects the fast acylation phase of the reaction. The subsequent rate is slower, because enzyme's turnover number is limited by the rate of the slower deacylation phase.

Lehninger Principles of biochemistry, 5th edition (2008)

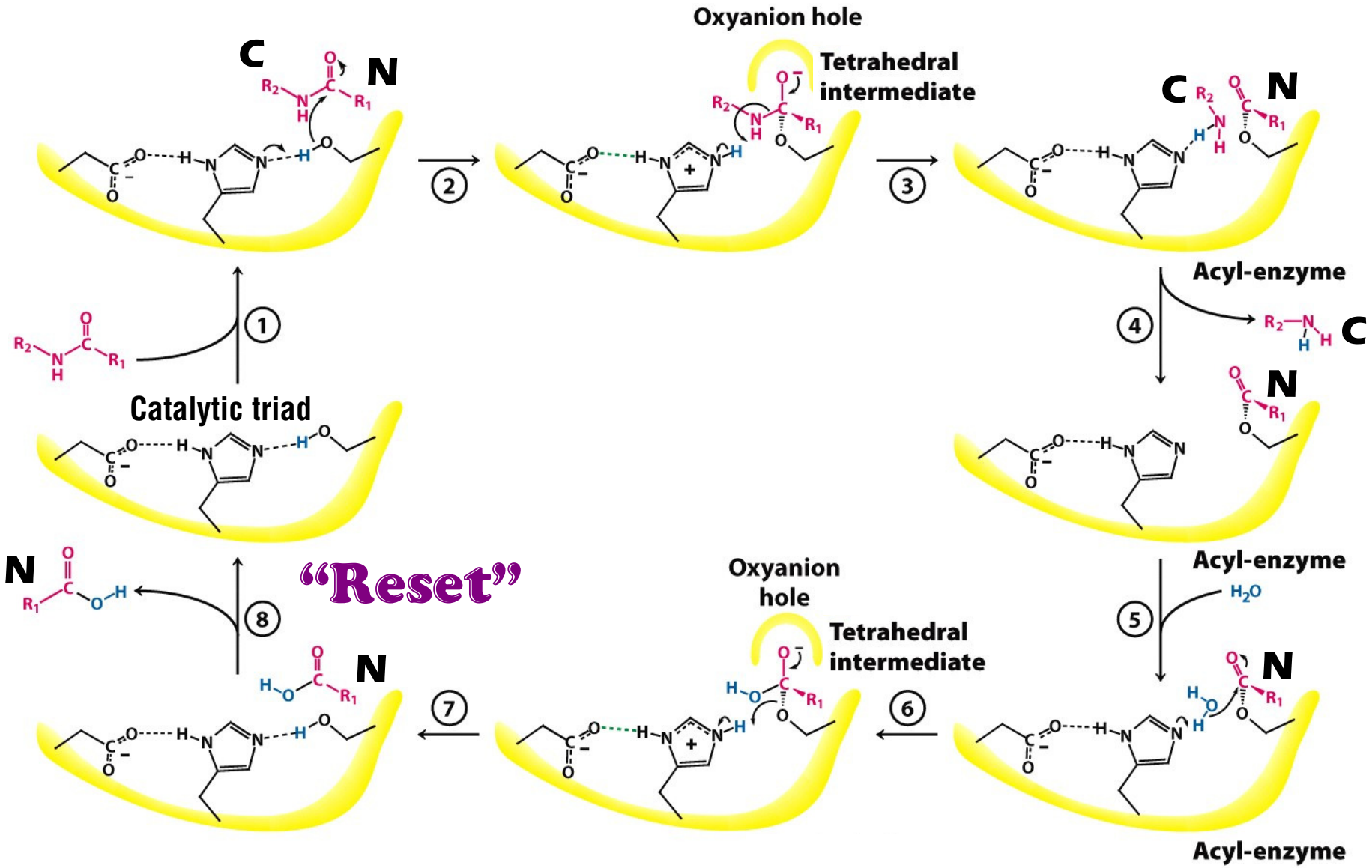
Hydrolysis of the specific ester or peptide bond by chymotrypsin

Acylation to form the acyl-enzyme intermediate

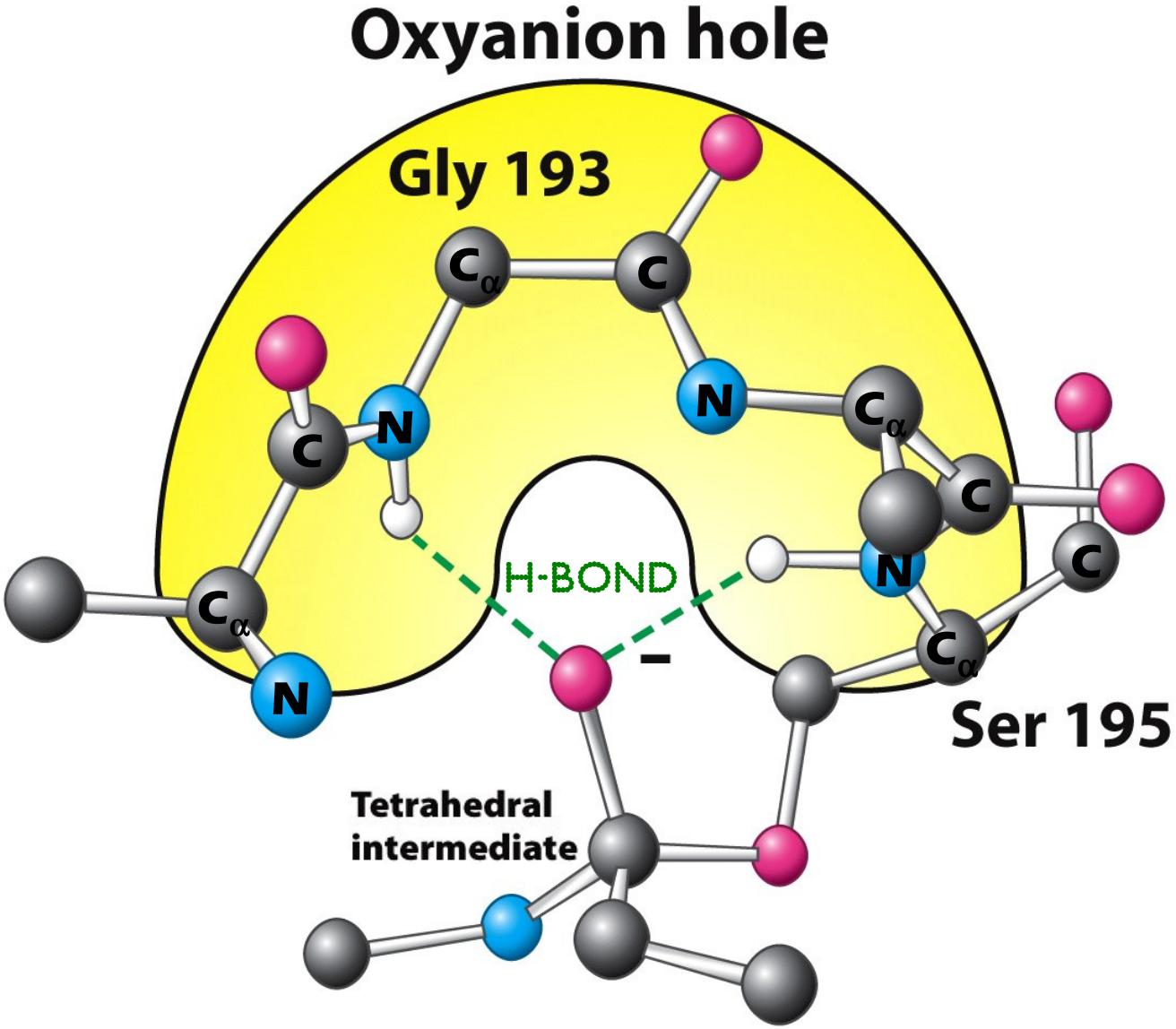
Deacylation to generate the free enzyme



Peptide hydrolysis of chymotrypsin

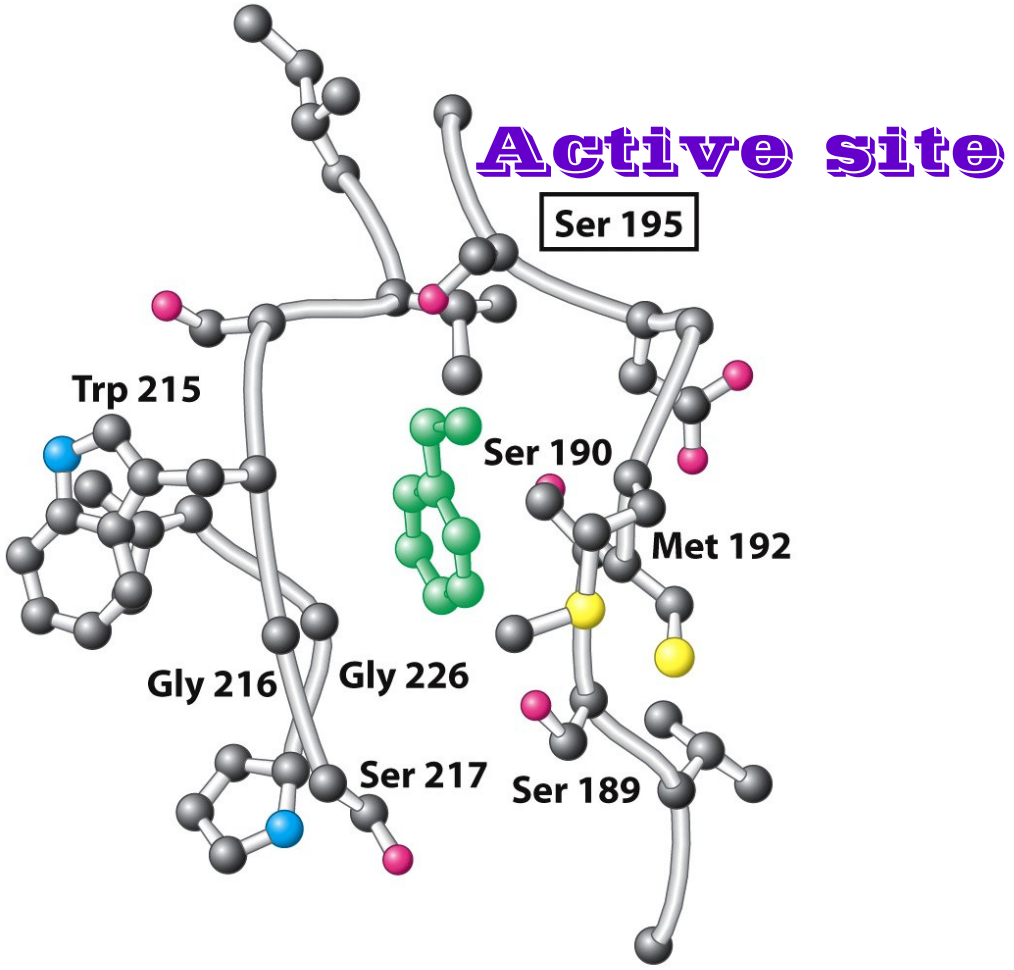


The structure stabilizes the tetrahedral intermediate of chymotrypsin reaction

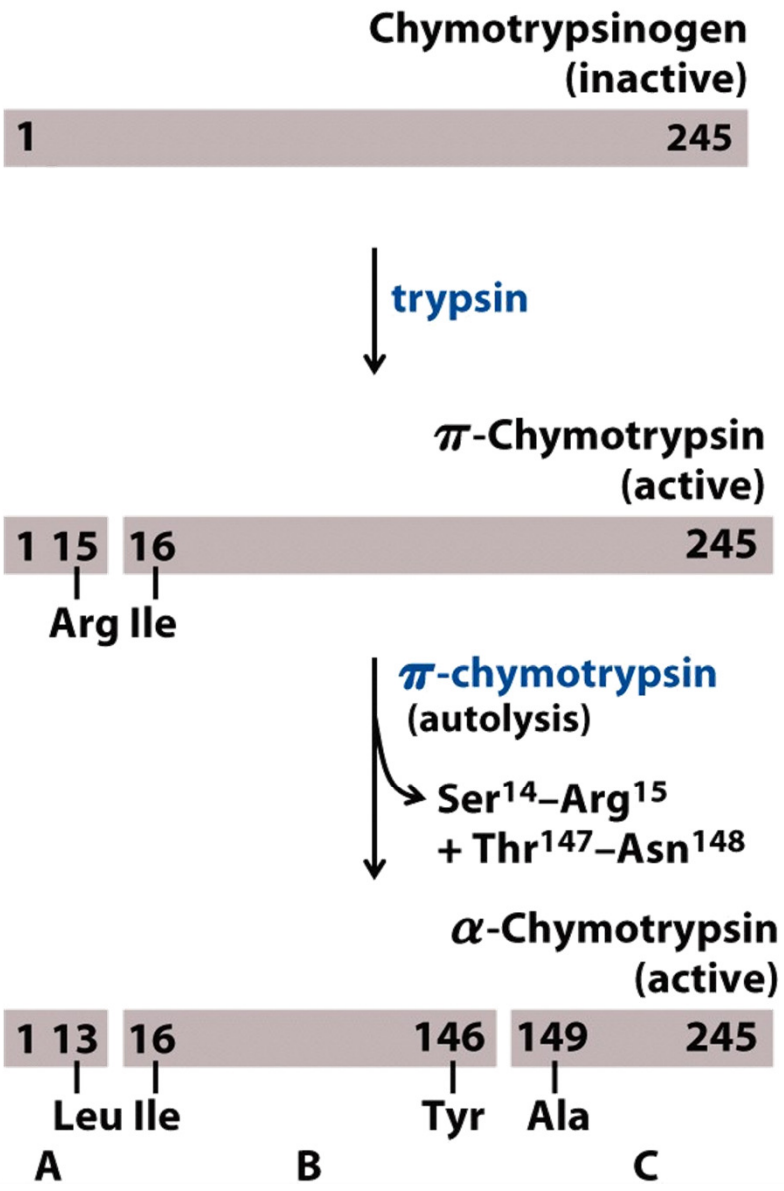


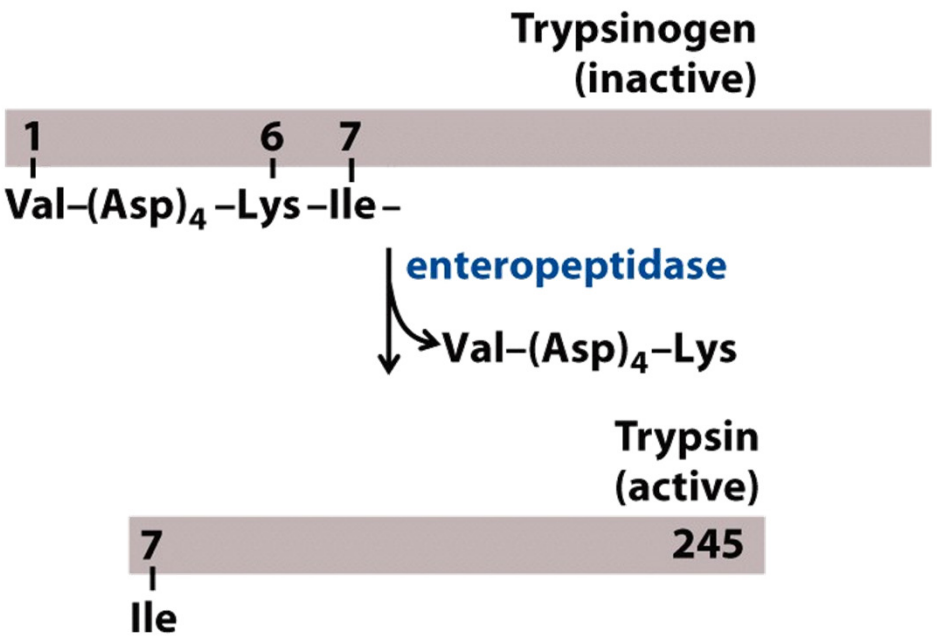
Specific substrate binding pocket of chymotrypsin

The substrate binding pocket of chymotrypsin is lined with hydrophobic residues and is deep.



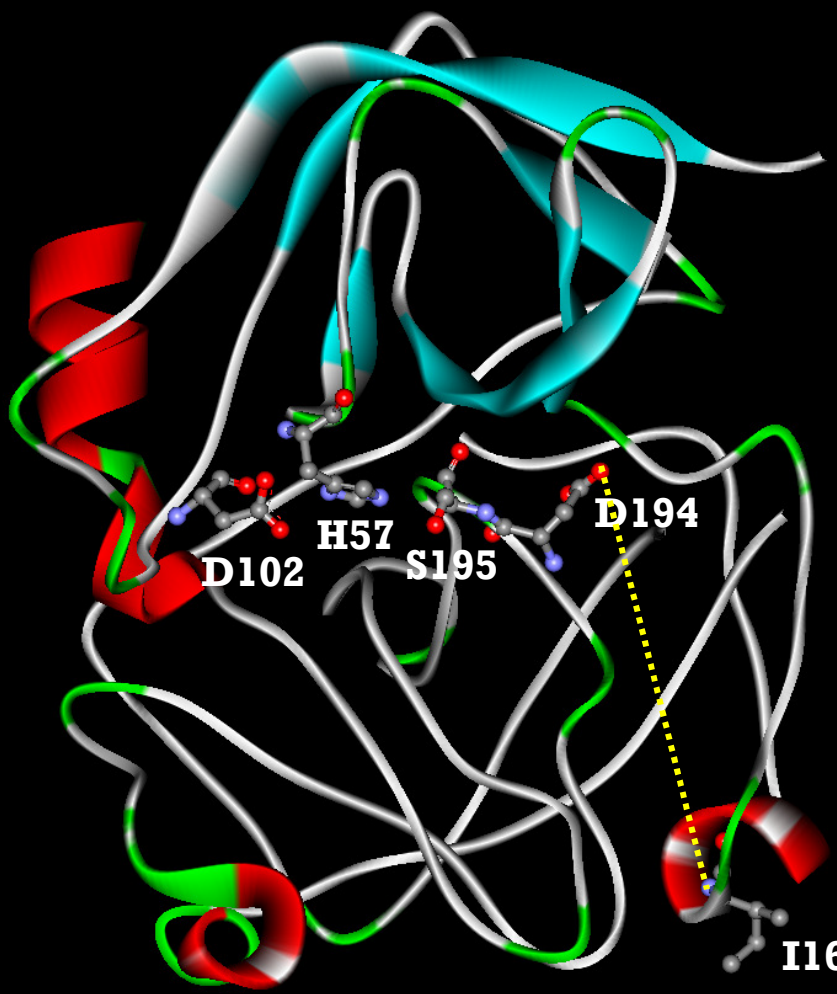
Activation of zymogens by proteolytic cleavage





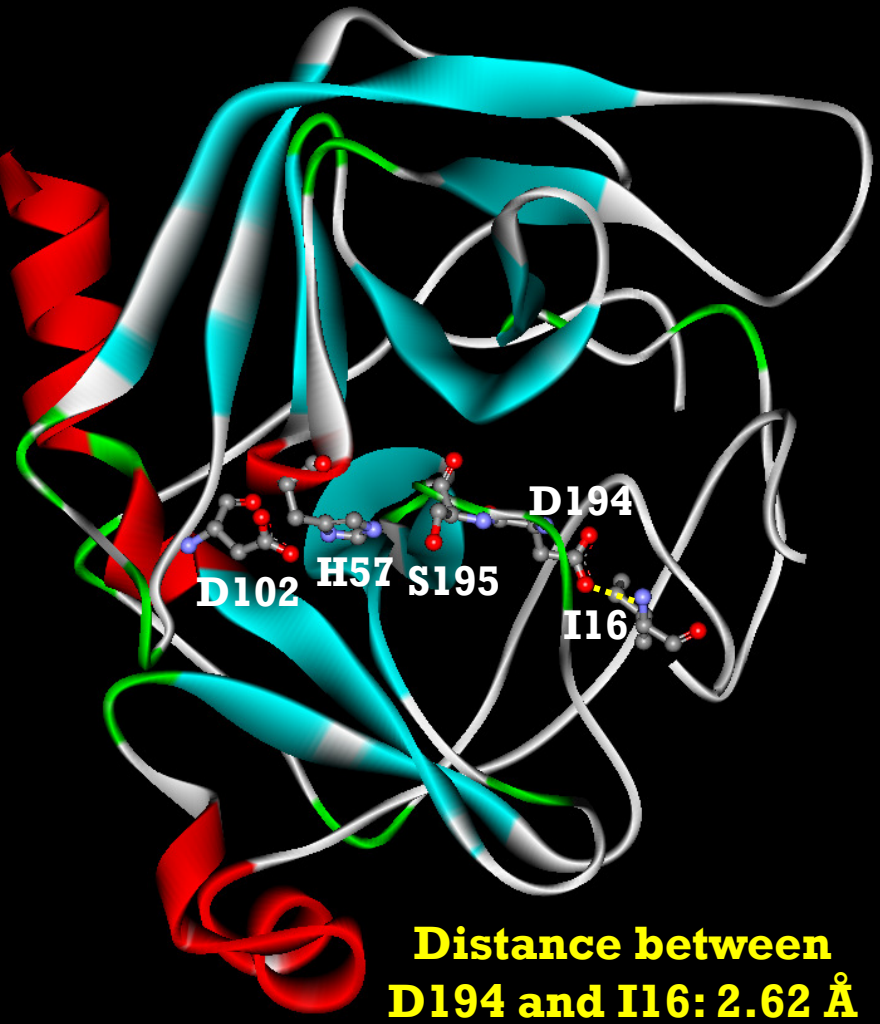
- Three polypeptide chains (A, B, and C) of chymotrypsin are linked by disulfide bonds
- Protonated Ile^{16} forms salt-bridge with Asp^{194}

Chymotrypsinogen



Distance between D194 and I16: 18.28 Å

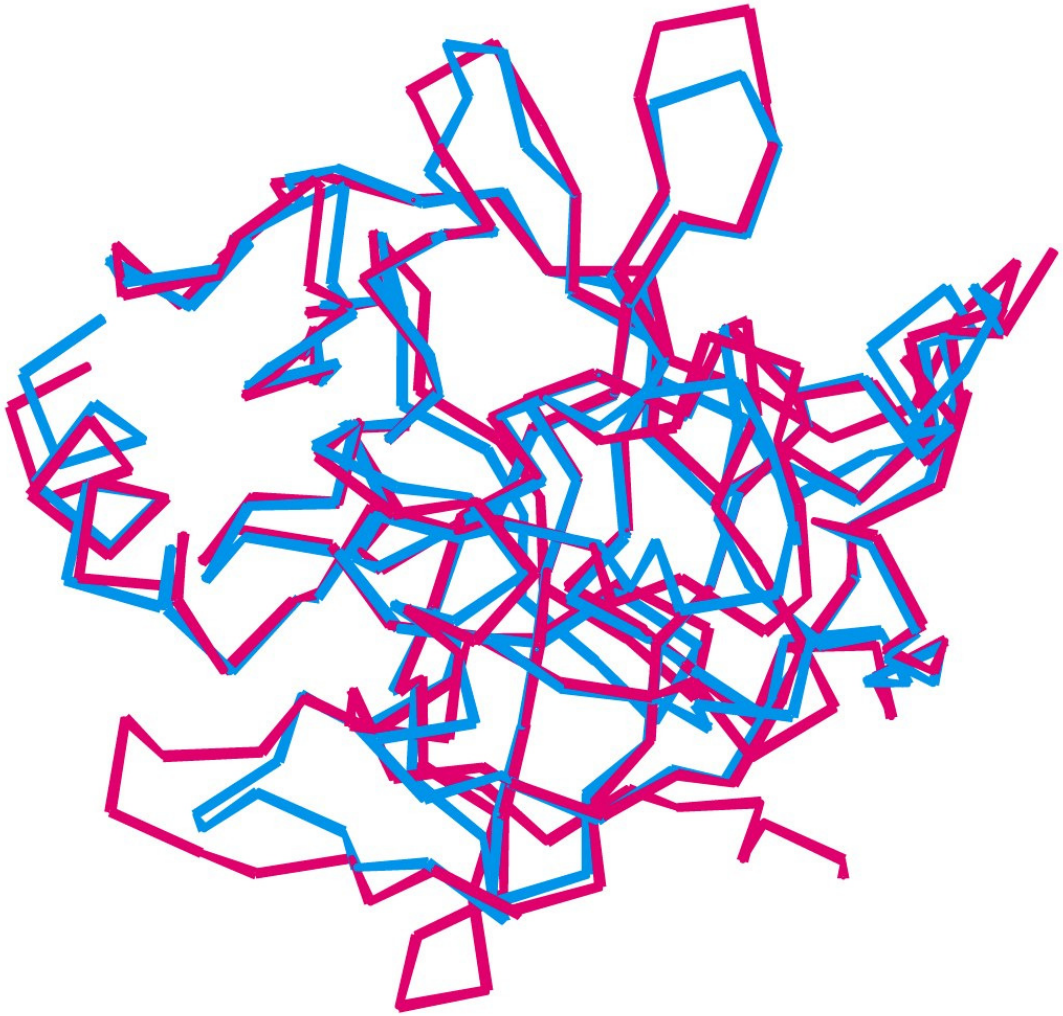
Chymotrypsin



Distance between D194 and I16: 2.62 Å

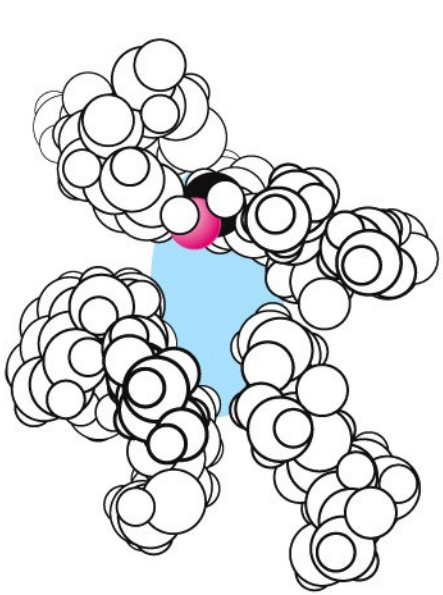
Structural similarity of trypsin and chymotrypsin

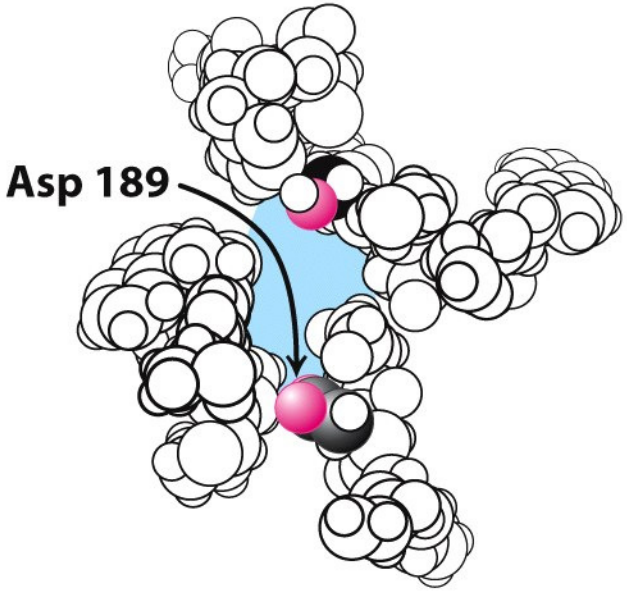
Chymotrypsin (red) Trypsin (blue)

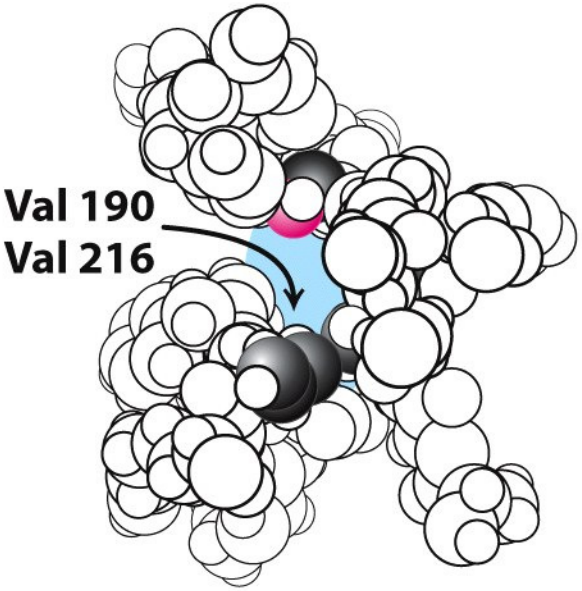


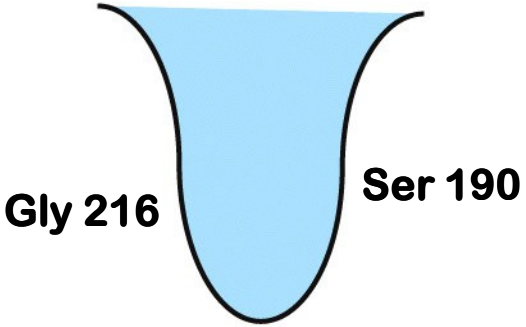
- ~40% sequence identity
- Similar structure
- Similar catalytic mechanism
- Different substrate specificity

The S_1 pocket of chymotrypsin, trypsin, and elastase



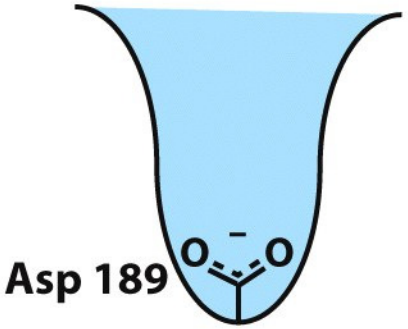






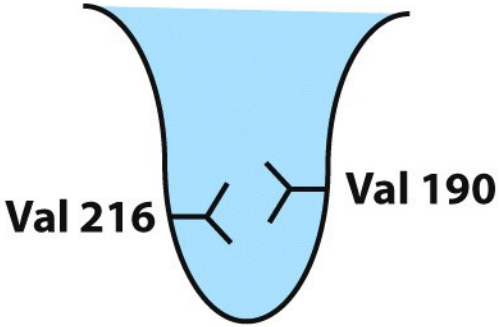
Chymotrypsin

Aromatic or long nonpolar side chain



Trypsin

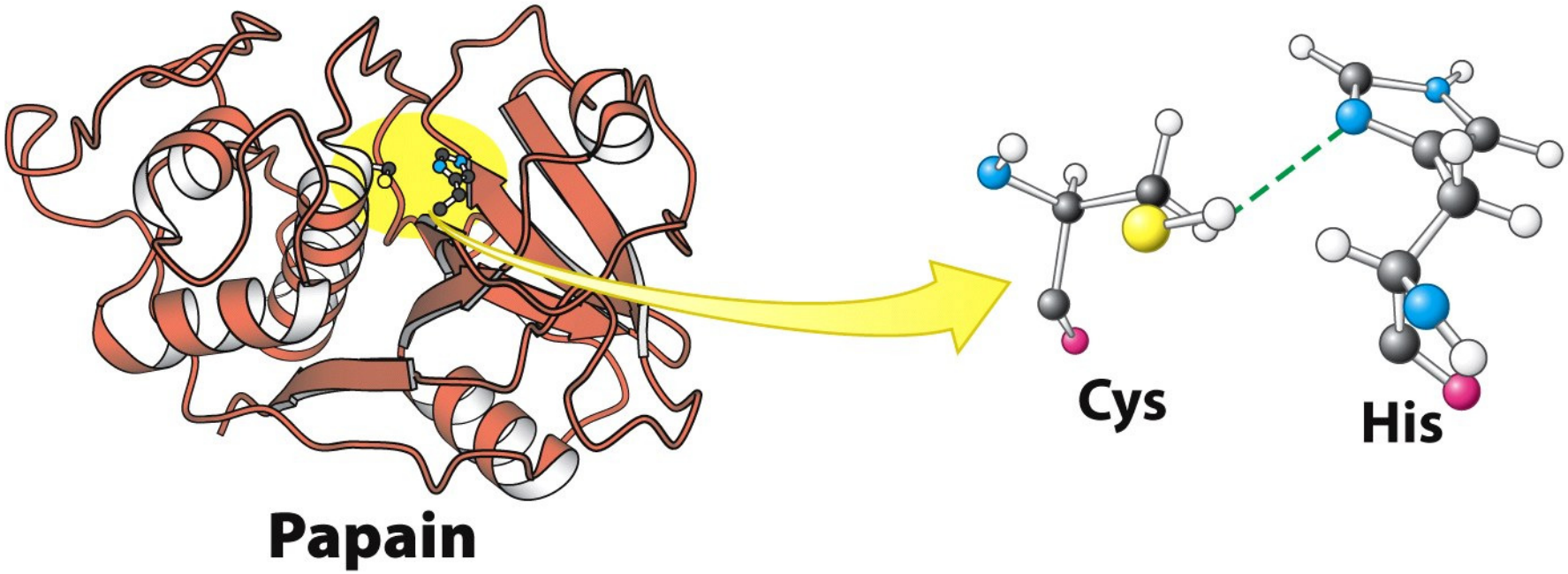
Long, positively charged side chain



Elastase

Small side chain

Cysteine protease



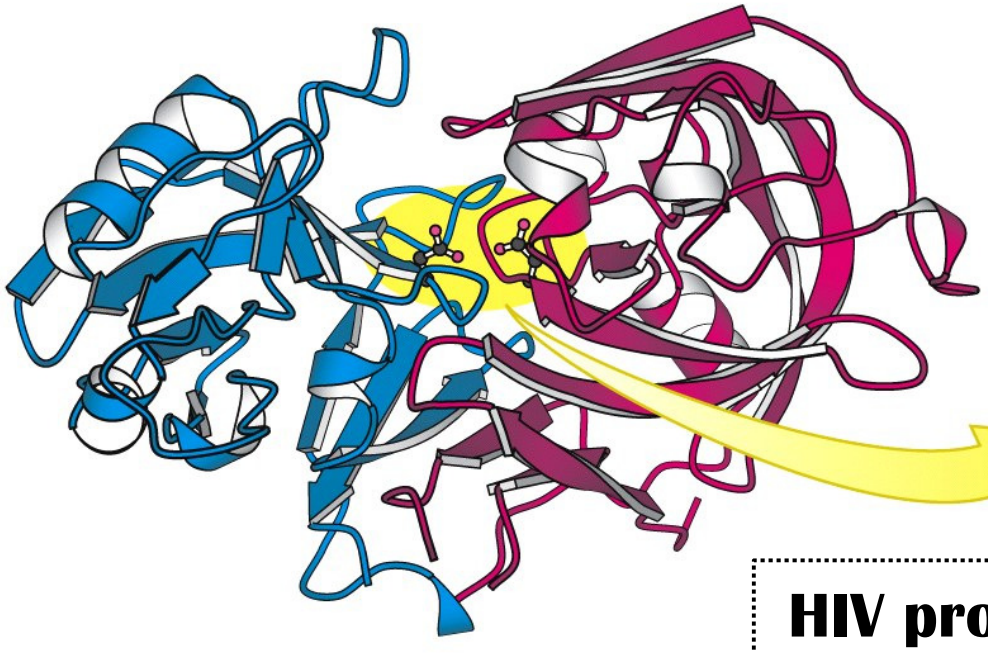
Cathepsin

Caspase

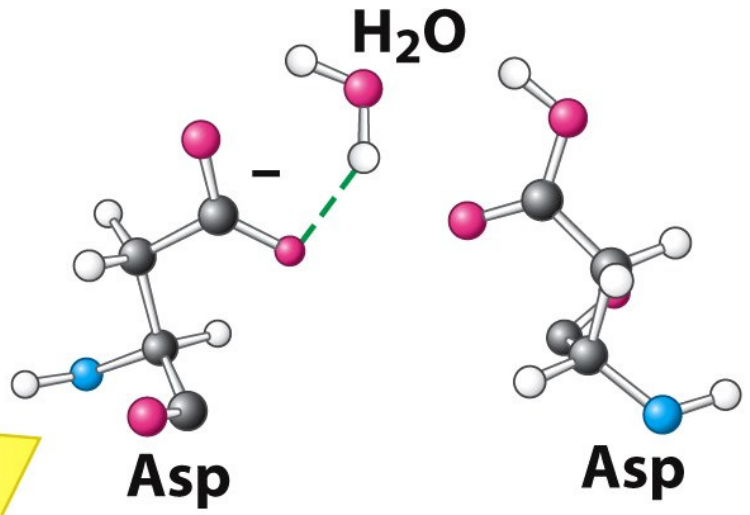
Aspartyl protease

Twofold symmetry

Each contributes an Asp to the active site



Renin

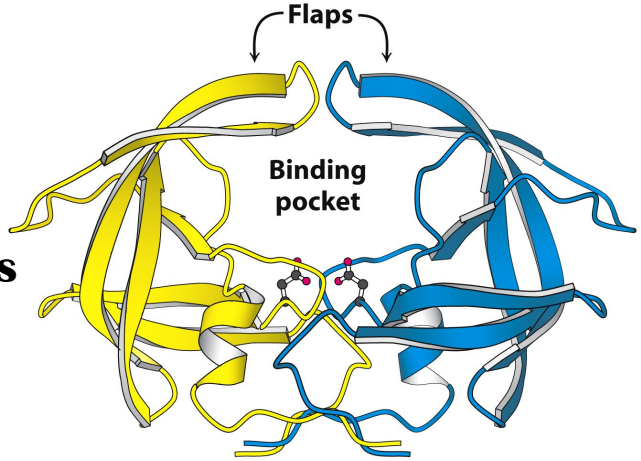


Pepsin

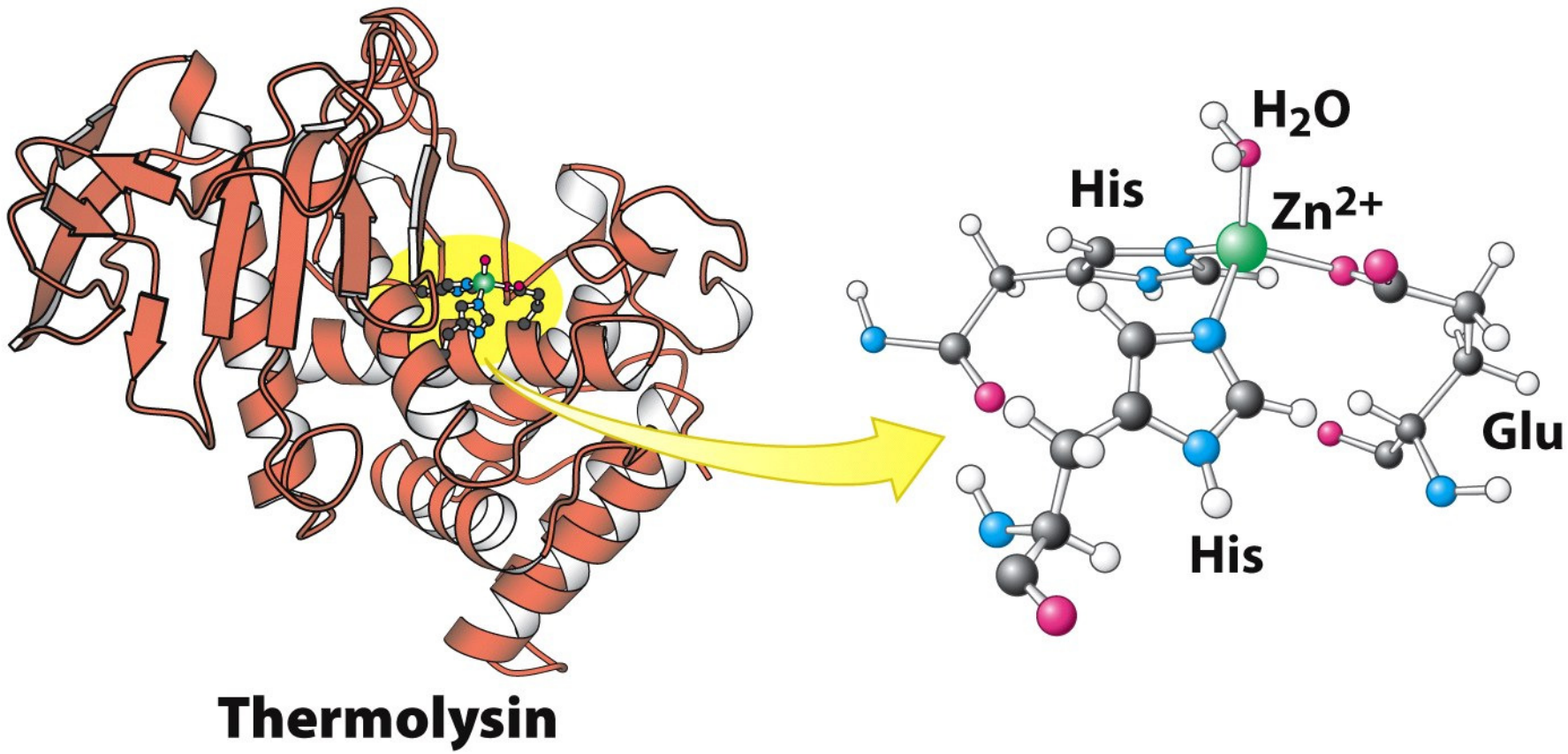
HIV protease

HIV protease

cleaves multidomain viral protein into their active forms; blocking this process completely prevents the virus from being infectious.



Metalloprotease

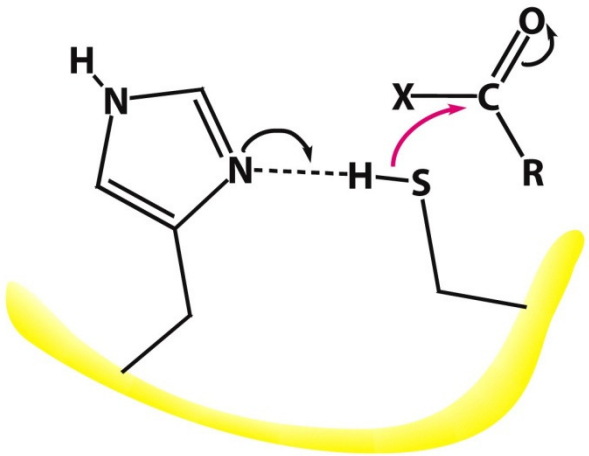


Carboxypeptidase A
Matrix metalloprotease

The different activation strategies for various proteases

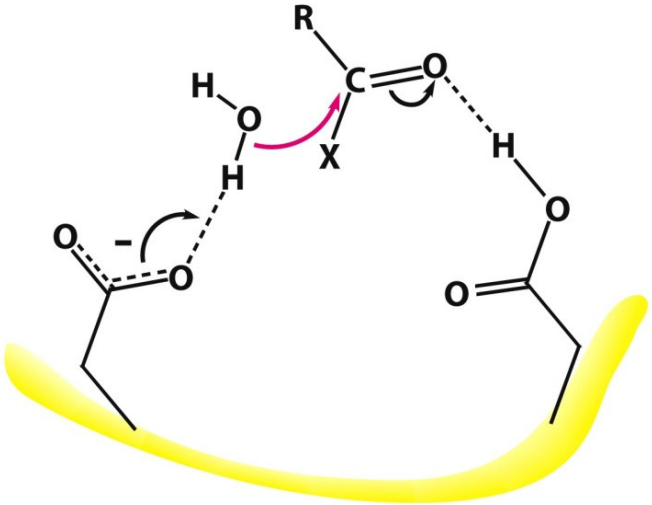
The peptide carbonyl group is attacked by:

CYSTEINE PROTEASES



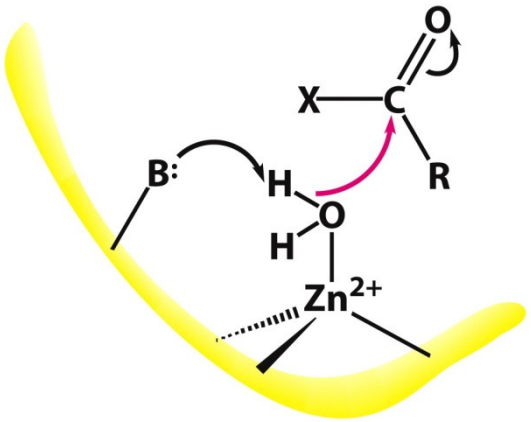
(A) A histidine-activated cycteine

ASPARTYL PROTEASES



(B) An aspartate-activated water molecule

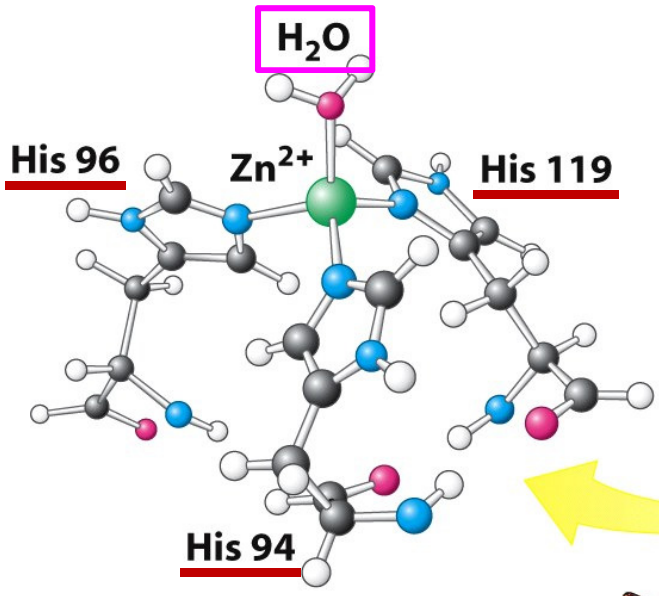
METALLOPROTEASES

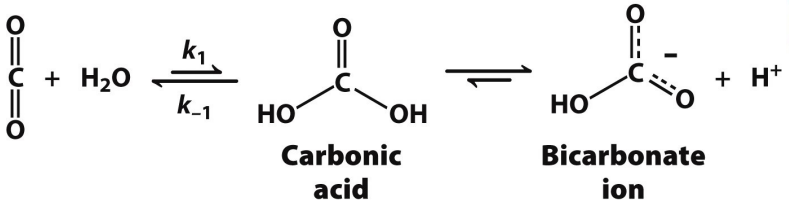


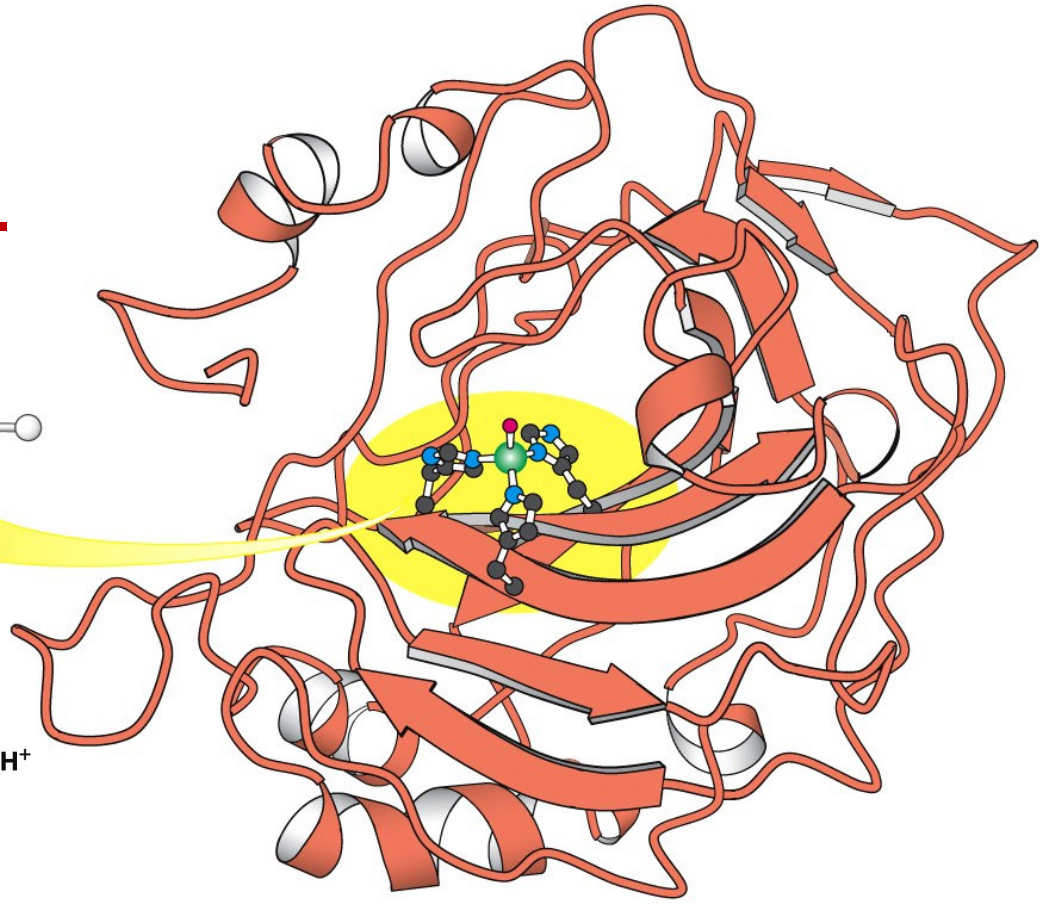
(C) A metal-activated water molecule. “B” represents a base (often glutamate) that helps deprotonate the metal-bound water.

The structure of human carbonic anhydrase II and its zinc site

Carbonic anhydrase—the first known zinc-containing enzyme







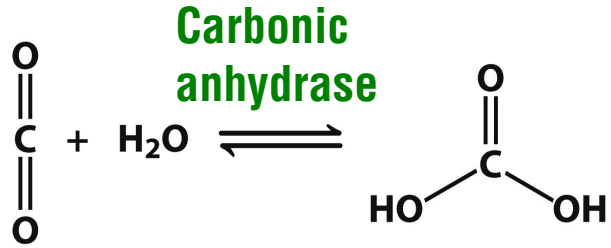
- At least seven homologous carbonic anhydrases are present in human beings.
- Carbonic anhydrase II is a major protein component of red blood cells, and is also one of the most active carbonic anhydrases.

Enzymes accelerate reactions by factors of as much as a million or more

Table 8.1 Rate enhancement by selected enzymes

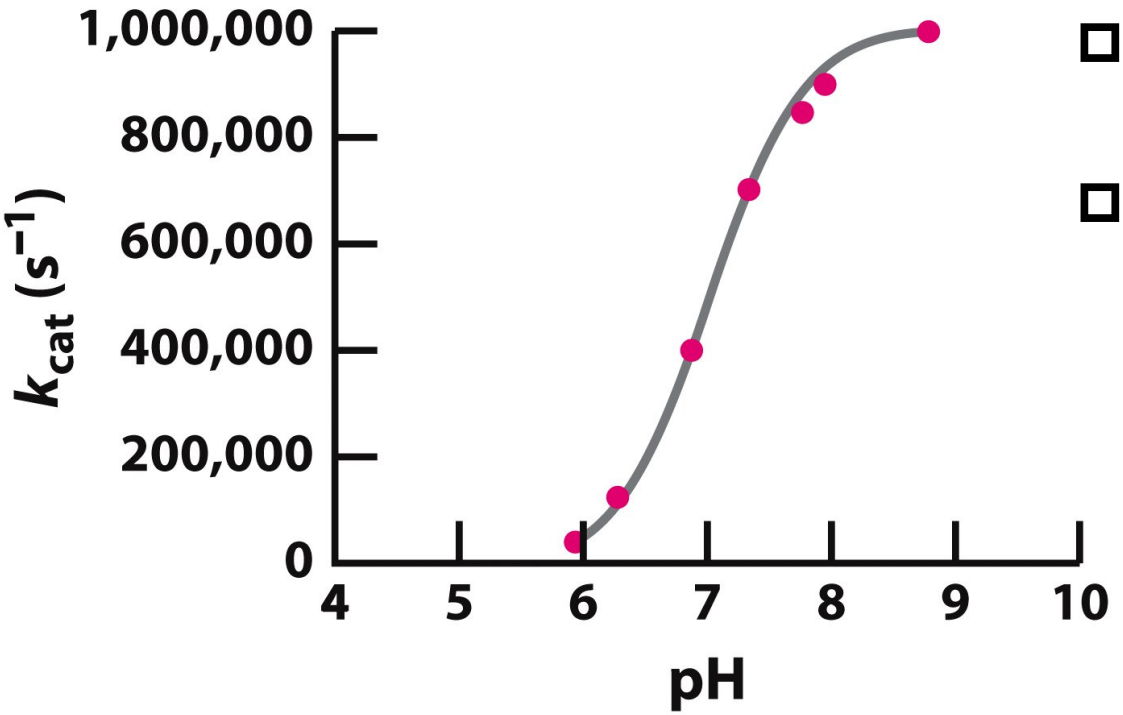
Enzyme	Nonenzymatic half-life		Uncatalyzed rate (k_{un} s^{-1})	Catalyzed rate (k_{cat} s^{-1})	Rate enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000	years	2.8×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000	years	1.7×10^{-13}	95	5.6×10^{14}
AMP nucleosidase	69,000	years	1.0×10^{-11}	60	6.0×10^{12}
Carboxypeptidase A	7.3	years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7	weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9	days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4	hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5	seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations: OMP, orotidine monophosphate; AMP, adenosine monophosphate.
Source: After A. Radzicka and R. Wolenden. *Science* 267:90–93, 1995.

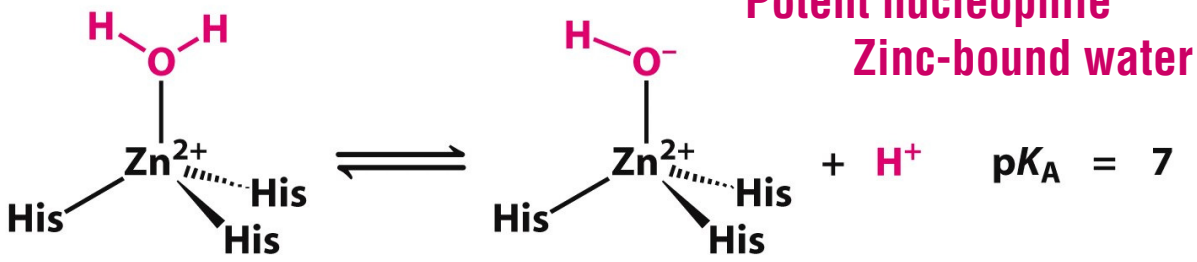


Biochemistry, 7th edition (2010), Berg, Tymoczko and Stryer

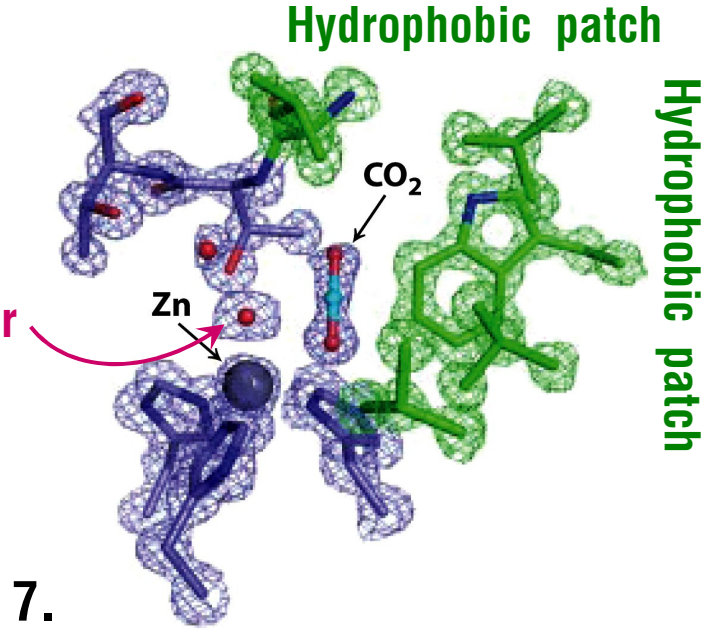
Effect of pH on carbonic anhydrase activity



- Carbonic anhydrase II is maximally active at high pH.
- The midpoint is near pH 7, suggesting that a group loses a proton at pH 7 plays an important role in the activity of carbonic anhydrase.



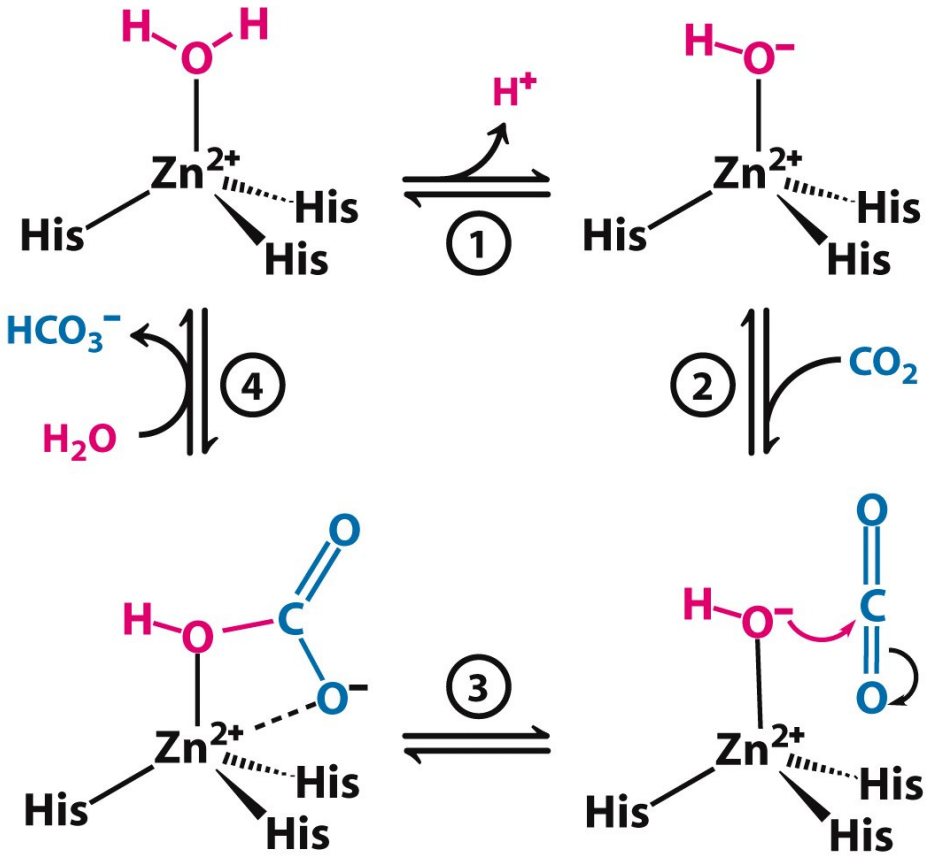
Potent nucleophile
Zinc-bound water



- Binding to zinc lowers the pKa of water from 15.7 to 7.

The catalytic mechanism of carbonic anhydrase

Acid-base catalysis



① The zinc ion facilitates the release of a proton from a water molecule, which generates a hydroxide ion.

② The carbon dioxide substrate binds to the enzyme's active site and is positioned to react with the hydroxide ion.

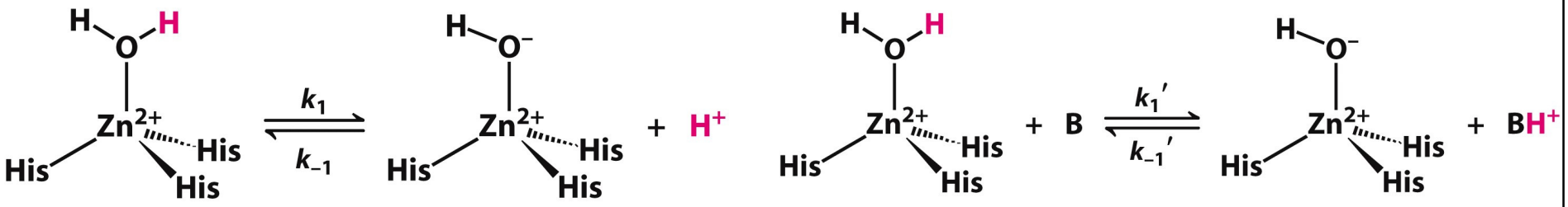
③ The hydroxide ion attacks the carbon dioxide, converting it into bicarbonate ion, HCO_3^-.

④ The catalytic site is regenerated with the release of HCO_3^-, and the binding of another molecule of water.

Histidine proton shuttle of carbonic anhydrase

- The rate of proton diffusion may limit the rate of carbonic anhydrase

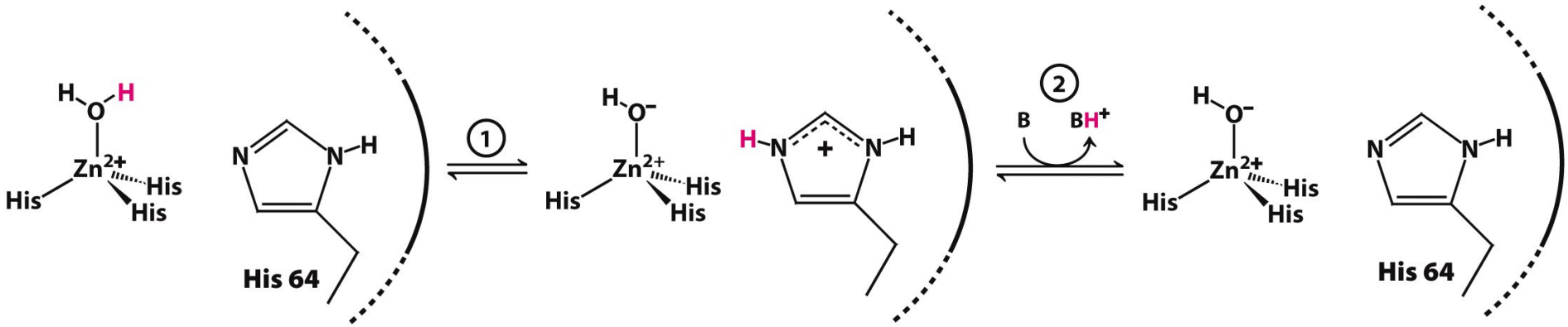
In the presence of "B"uffer



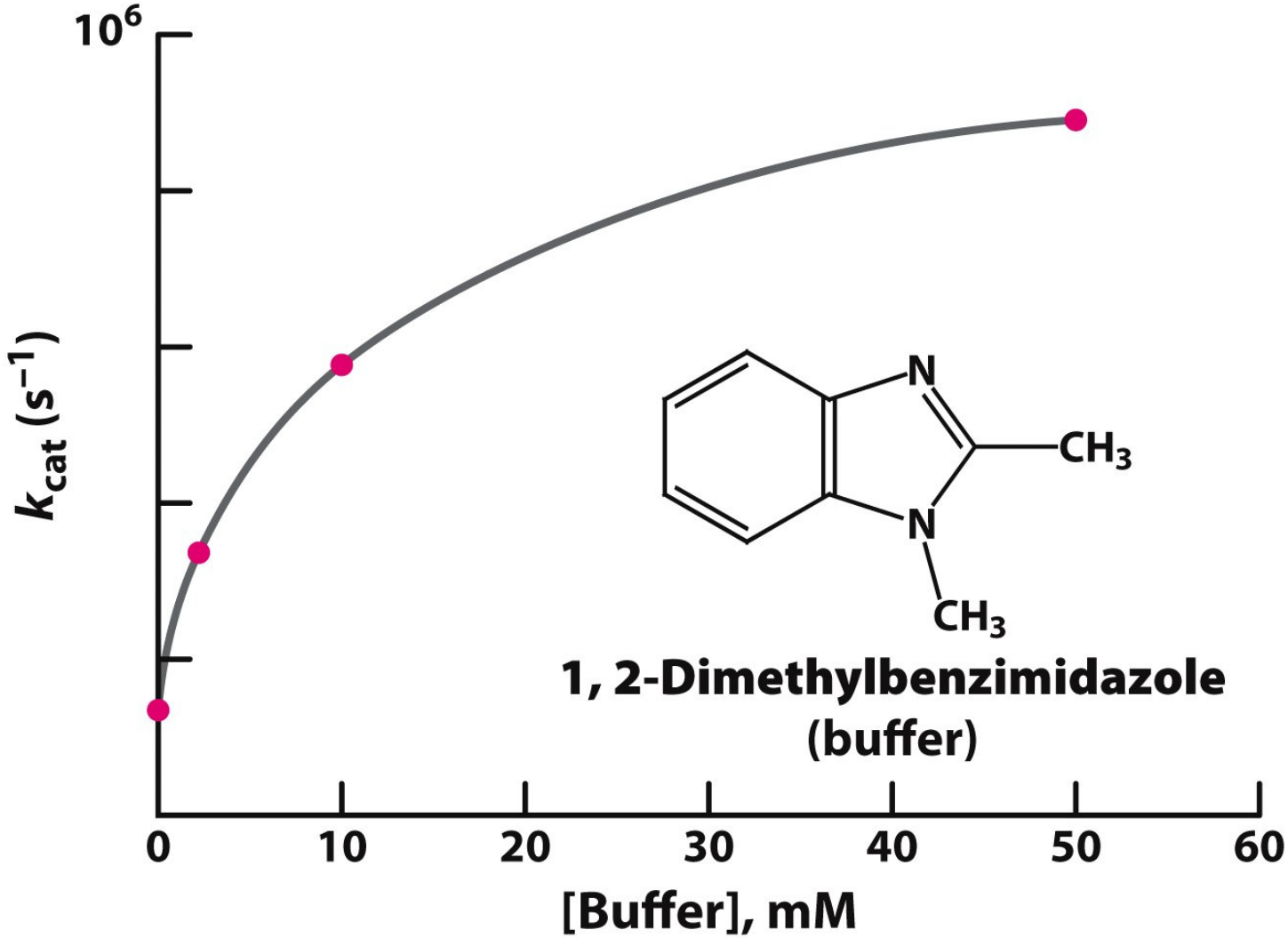
$K = k_1/k_{-1} = 10^{-7}$

$K = k_1'/k_{-1}' \oplus 1$

- His-64 transfers protons from the zinc-bound water to the protein surface and then to the buffer

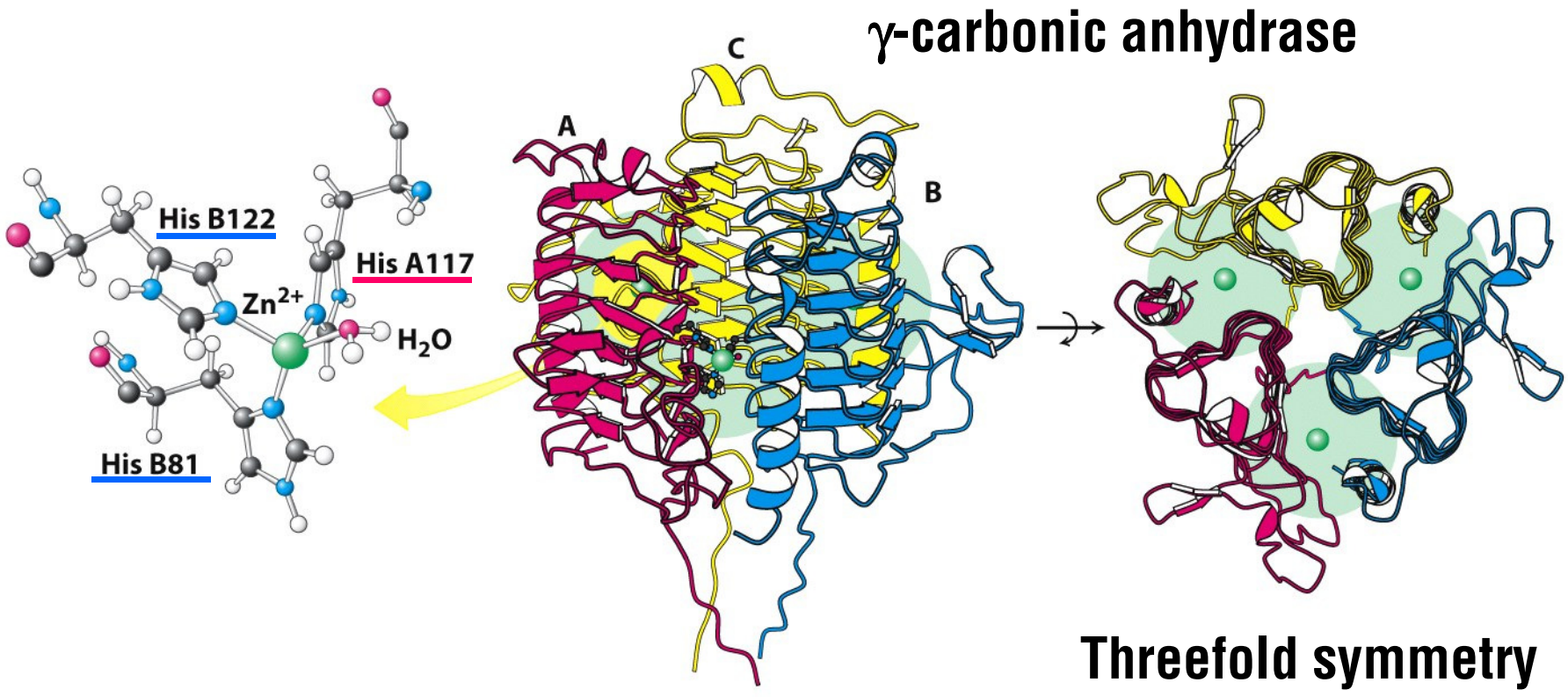


The effect of buffer concentration on the rate of carbon dioxide hydration by carbonic anhydrase



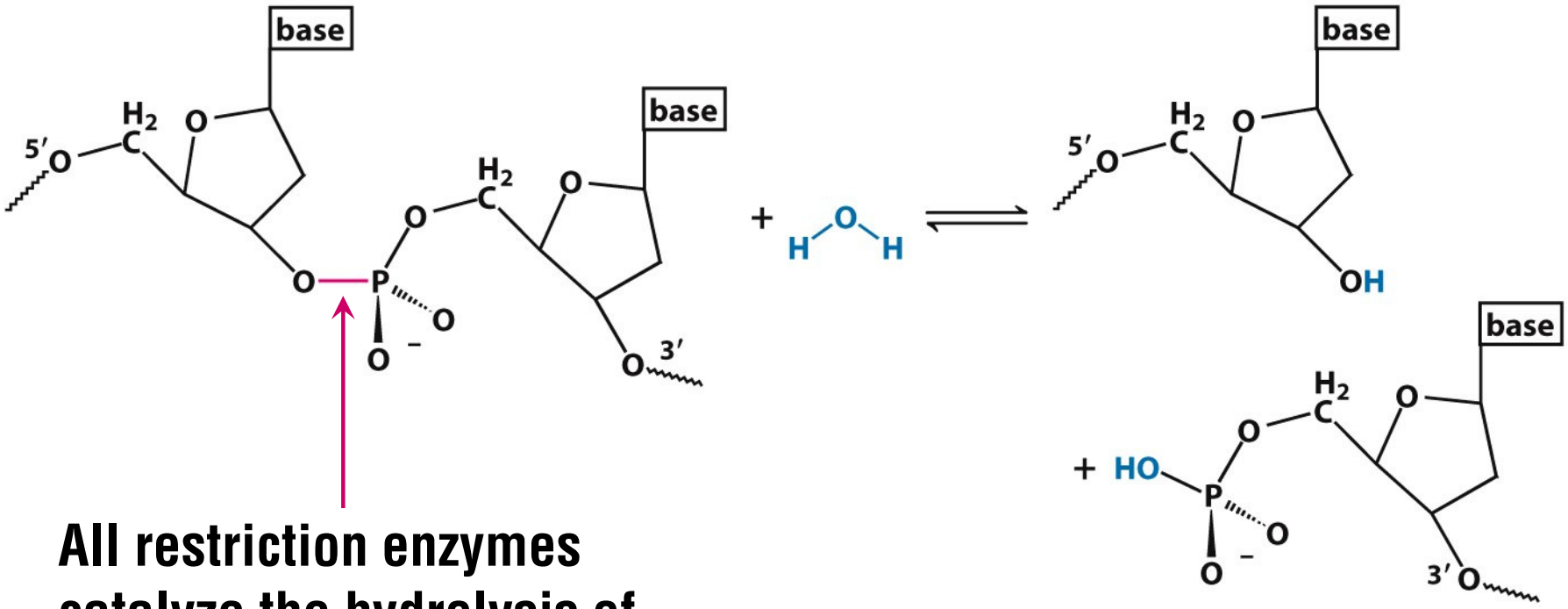
Convergent evolution 趨同演化 has generated zinc-based active sites in different carbonic anhydrase

- α-carbonic anhydrase: human, animals, some bacteria and algae
- β-carbonic anhydrase: higher plants, and many bacterial species
- γ-carbonic anhydrase: archaeon



Restriction enzyme (restriction endonuclease)

- must NOT degrade host DNA containing the recognition sequences
- must cleave only DNA molecules that contain recognition sites without cleaving DNA molecules that lack these sites



All restriction enzymes catalyze the hydrolysis of DNA phosphodiester bonds

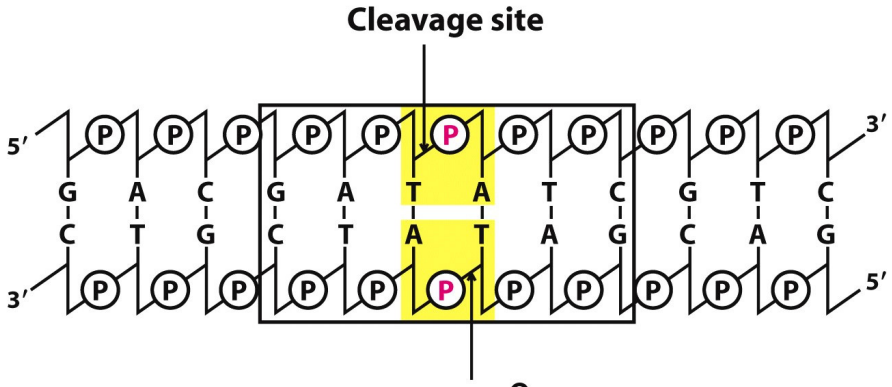
A phosphoryl group attaches to the new 5’ end

To determine which mechanism is correct, the stereochemistry at the phosphorus atom is examined

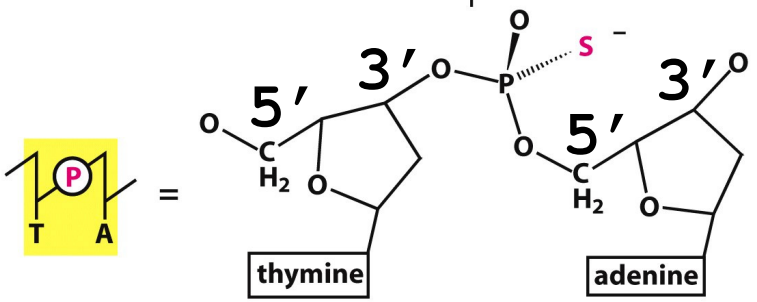
*Eco*RV

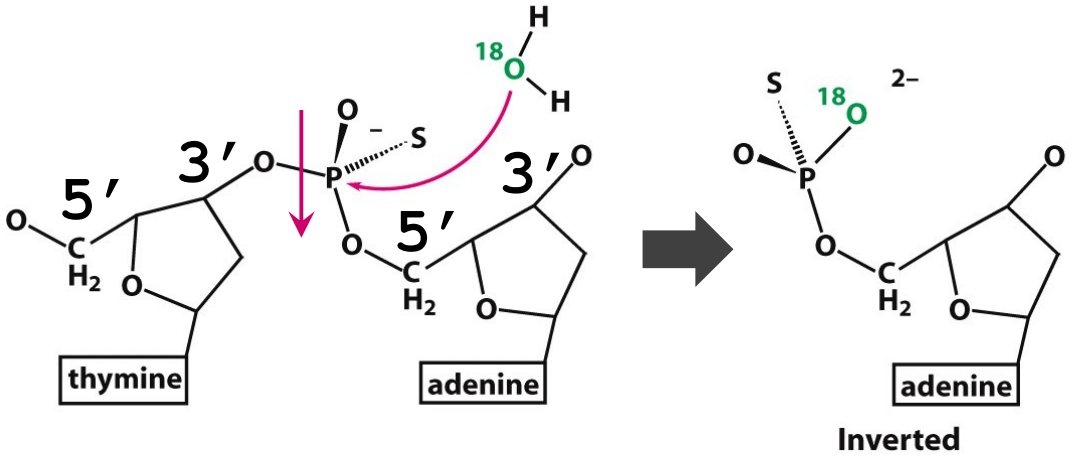
5′…GATATC…3′

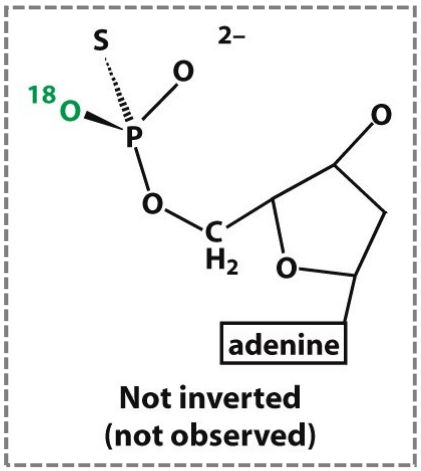
3′…CTATAG…5′



- The stereochemistry configuration at the phosphorus atom is inverted only once with cleavage.





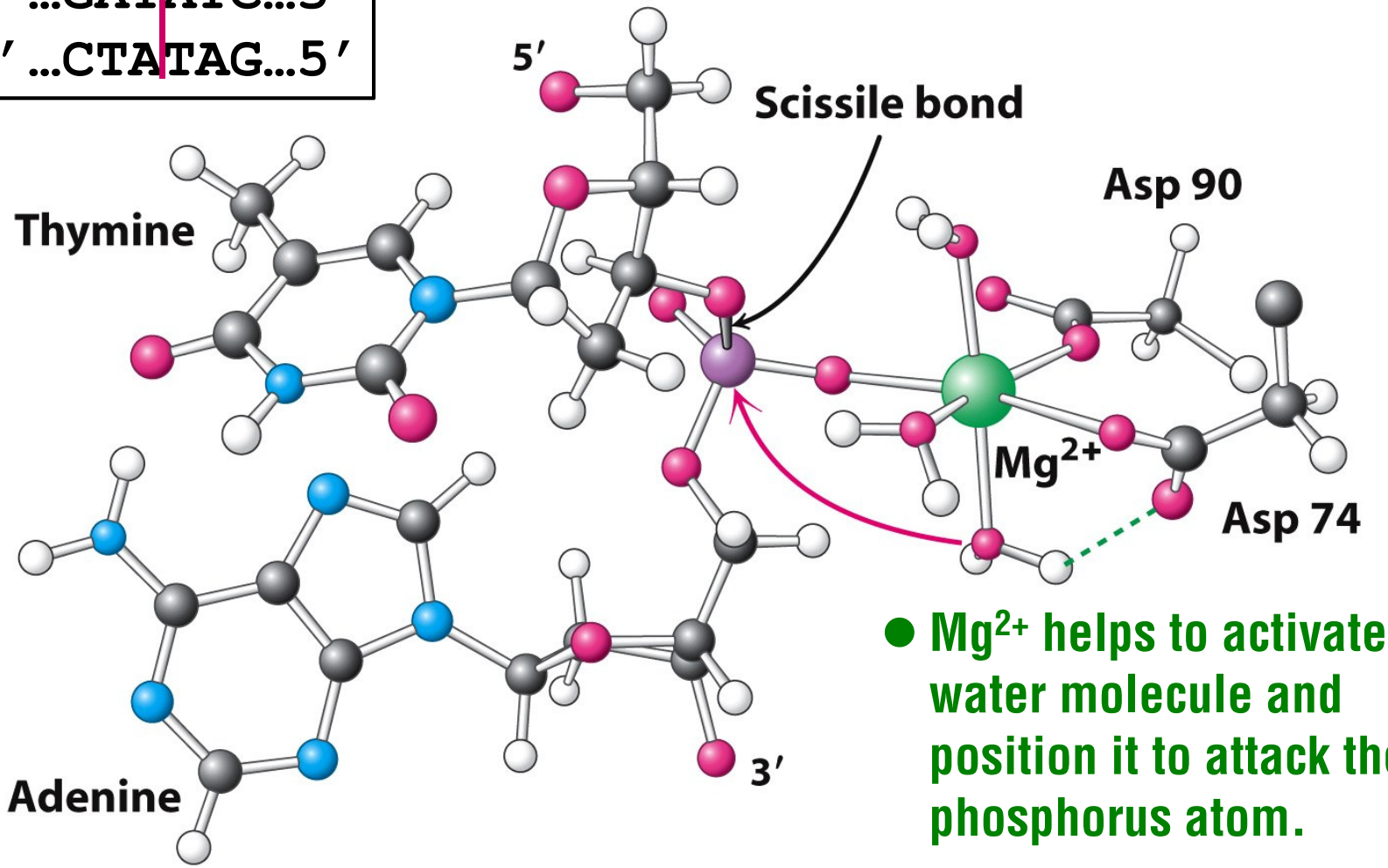


A magnesium ion-binding site in *Eco*RV

***Eco*RV**

5′…GATATC…3′

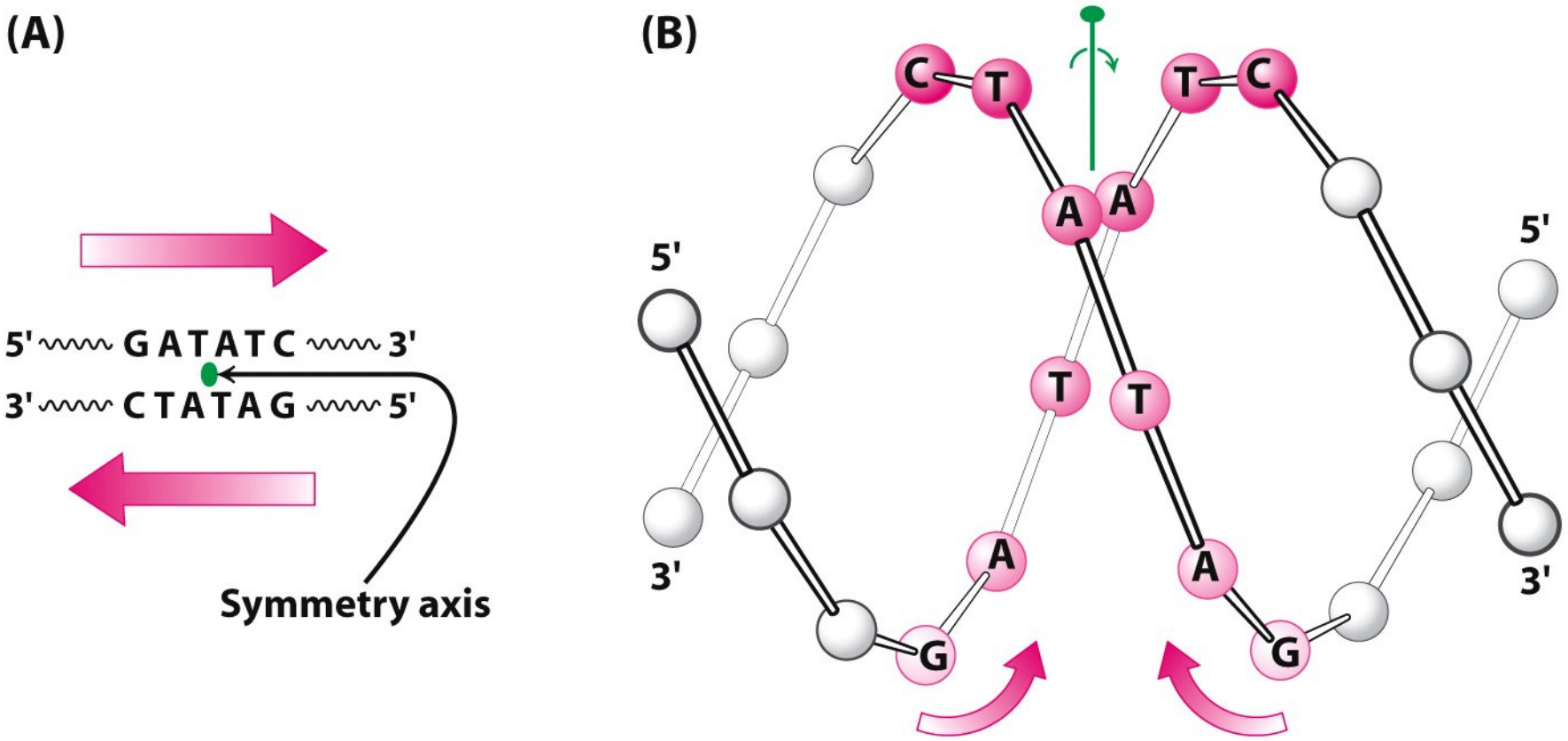
3′…CTATAG…5′



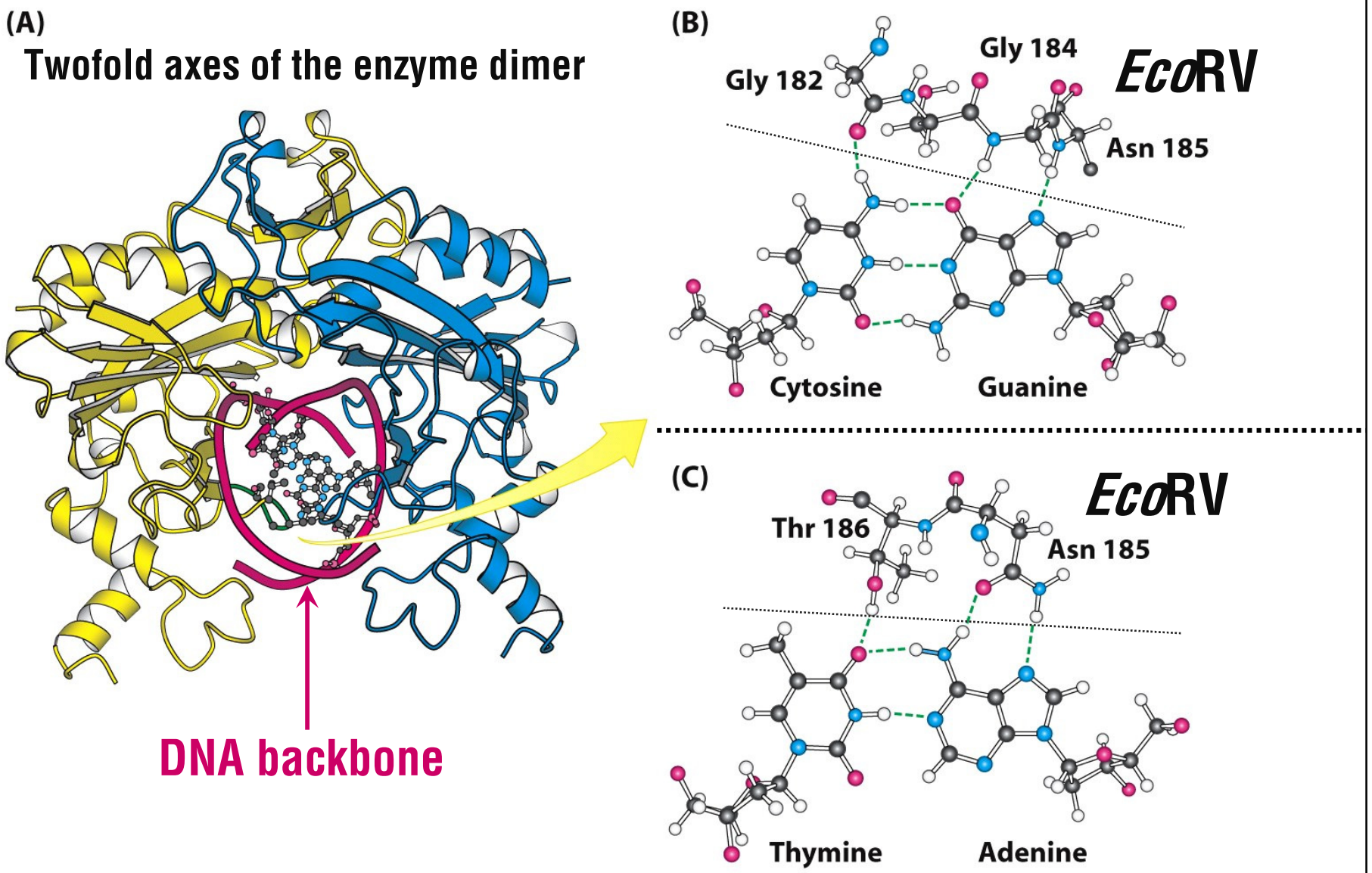
- Mg^{2+} helps to activate a water molecule and position it to attack the phosphorus atom.

Sturcture of the recognition site of *Eco*RV

- The recognition sequences for most restriction endonucleases are inverted repeats (palindrome).
- This arrangement gives the three-dimensional structure of the recognition site a twofold rotational symmetry

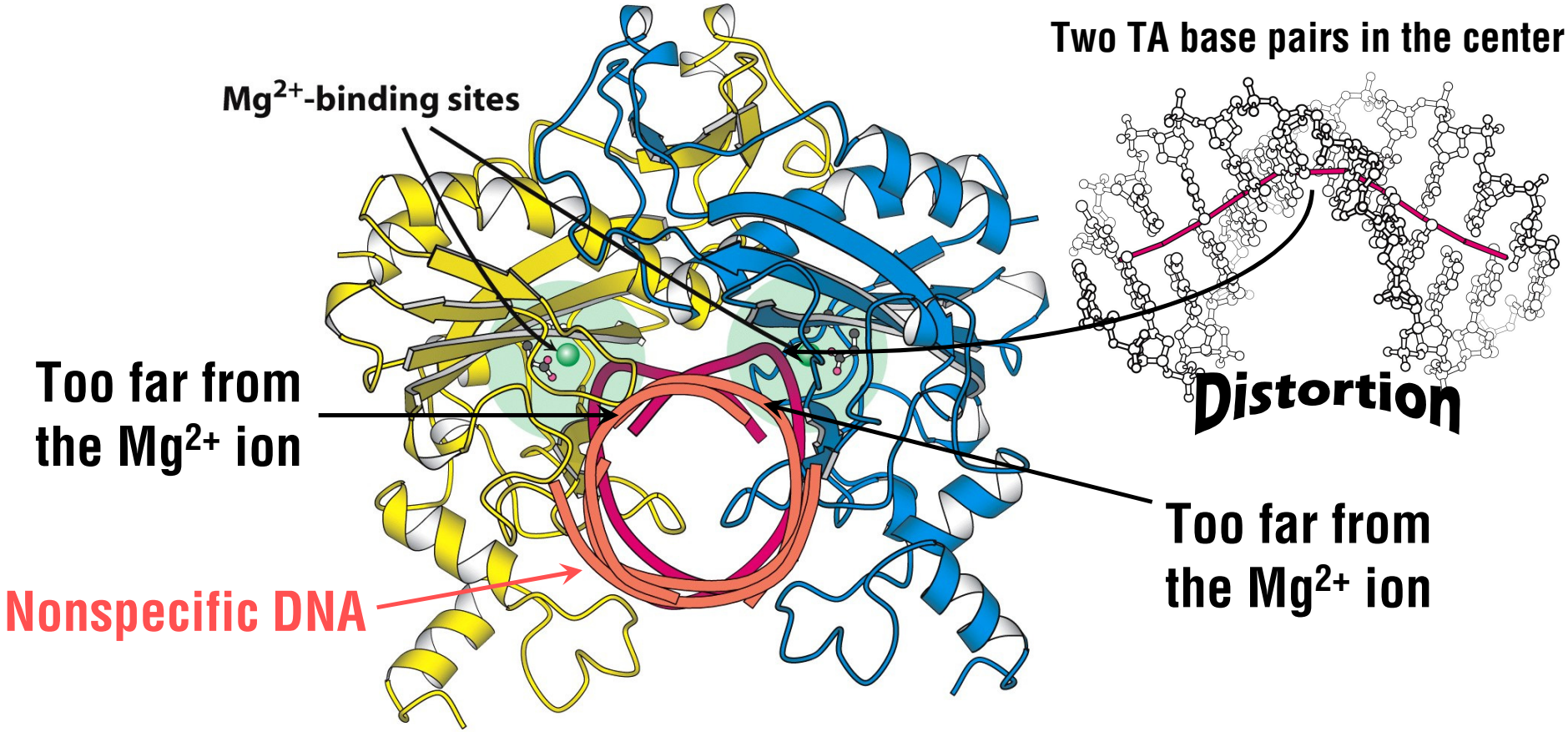


*Eco*RV embracing a cognate DNA molecule



Nonspecific and cognate DNA within *Eco*RV

- The nonspecific DNA backbone is too far from the enzyme to complete the magnesium ion-binding sites.



- Actually, in the absence of Mg^{2+}, *Eco*RV binds to all sequences.
- Enzyme specificity may be determined by the specificity of enzyme action rather than the specificity of substrate binding.

Host-cell DNA is protected by the addition of methyl groups to specific bases

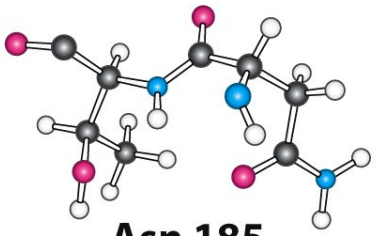
Cleaved

5′ ~~~~ GATATC ~~~~ 3′
3′ ~~~~ CTATAG ~~~~ 5′

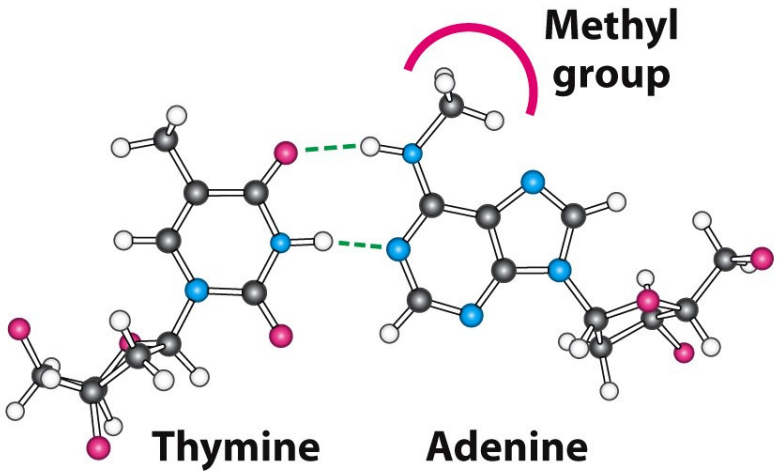
Not cleaved

5′ ~~~~ G$\overset{*}{A}$TATC ~~~~ 3′
3′ ~~~~ CTATA$\overset{*}{G}$ ~~~~ 5′

***Eco*RV**



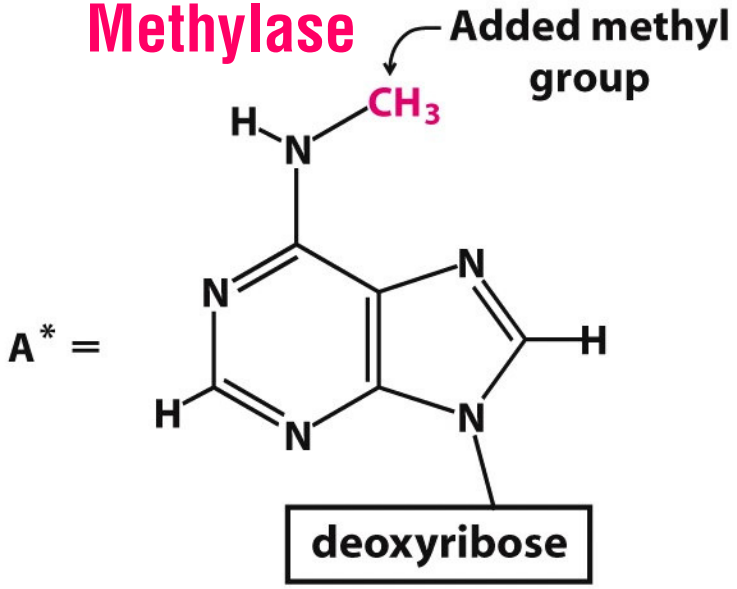
Asn 185



Methylated DNA

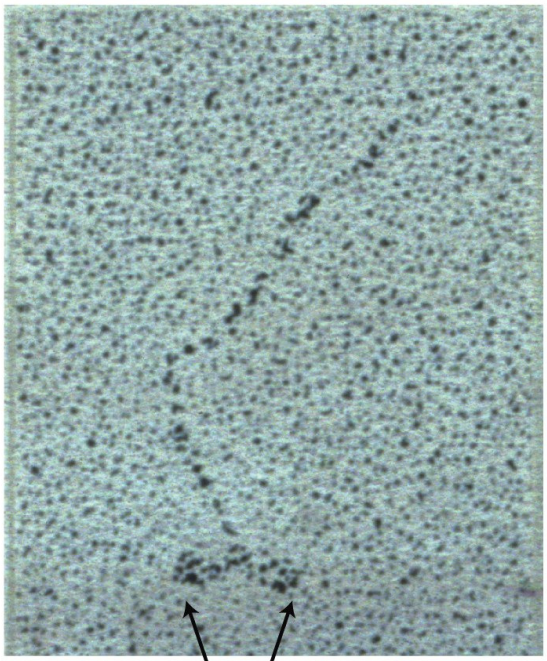
Restriction-modification systems

Methylase



- The methylation of adenine blocks the formation of hydrogen bond between EcoRV (Asn-185) and cognate DNA molecules and prevents their hydrolysis.

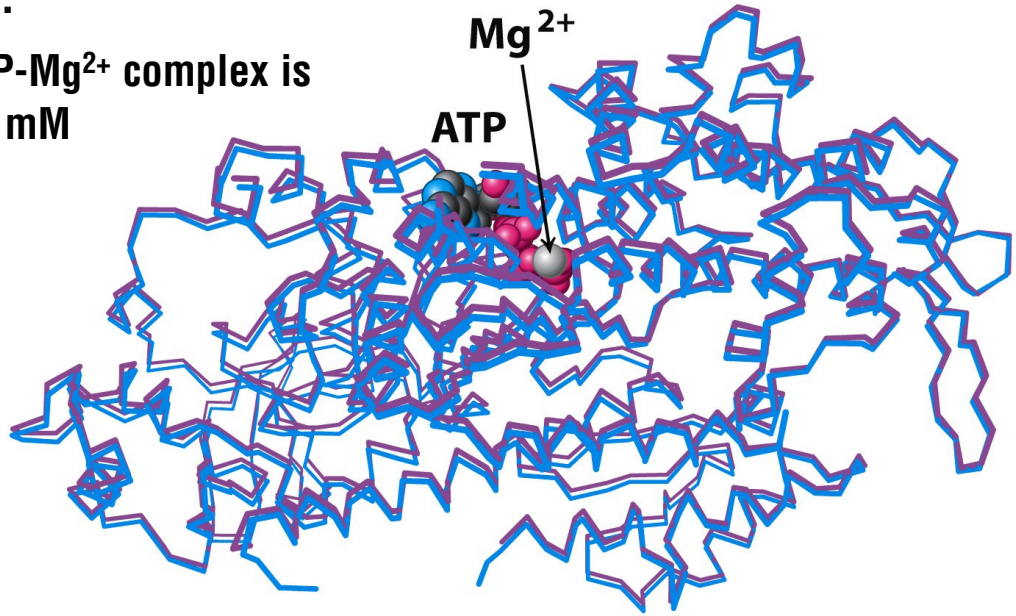
Myosin- ATP complex structure



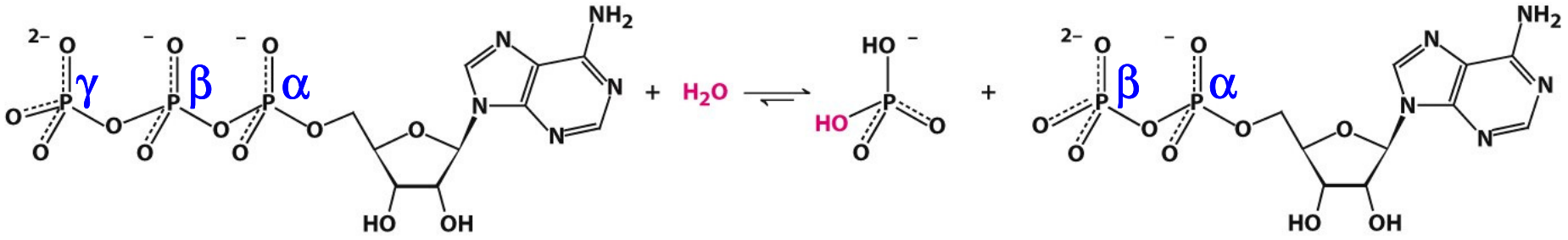
Globular ATPase domains

- Two structures are extremely similar to one another.
- ATP bound in the active site with very little change in the overall structure.

K_d for ATP-Mg^{2+} complex is about 0.1 mM



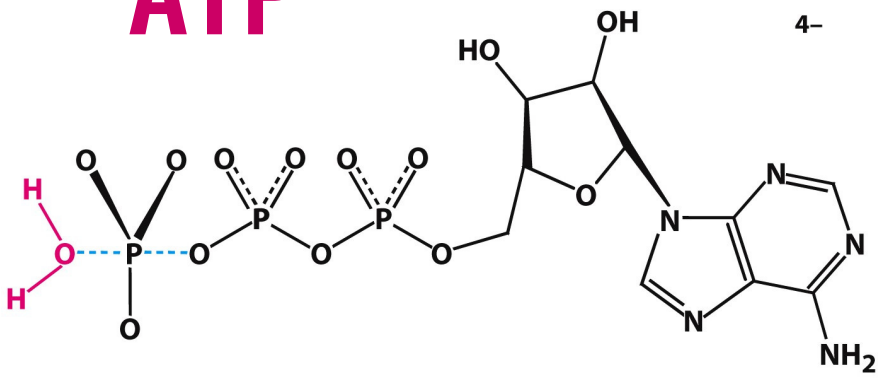
☐ No ligands bound ☐ With ATP and Mg^{2+} bound



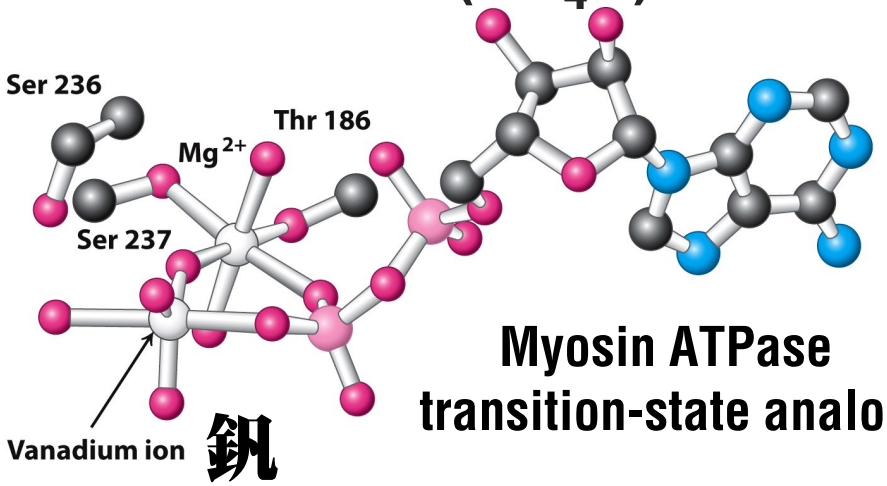
Adenosine triphosphate (ATP) Inorganic phosphate (P_i) Adenosine diphosphate (ADP)

Myosin ATPase transition-state analog

ATP

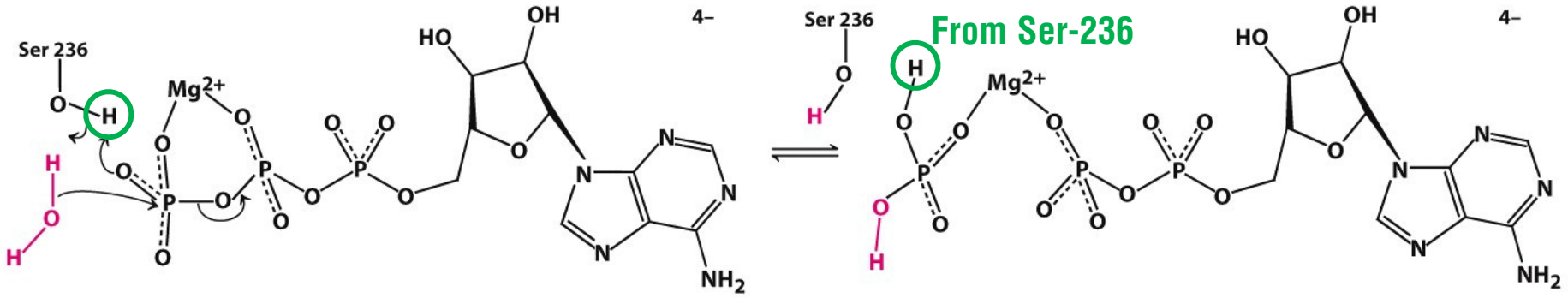


Vanadate (VO_4^{3-})-ADP



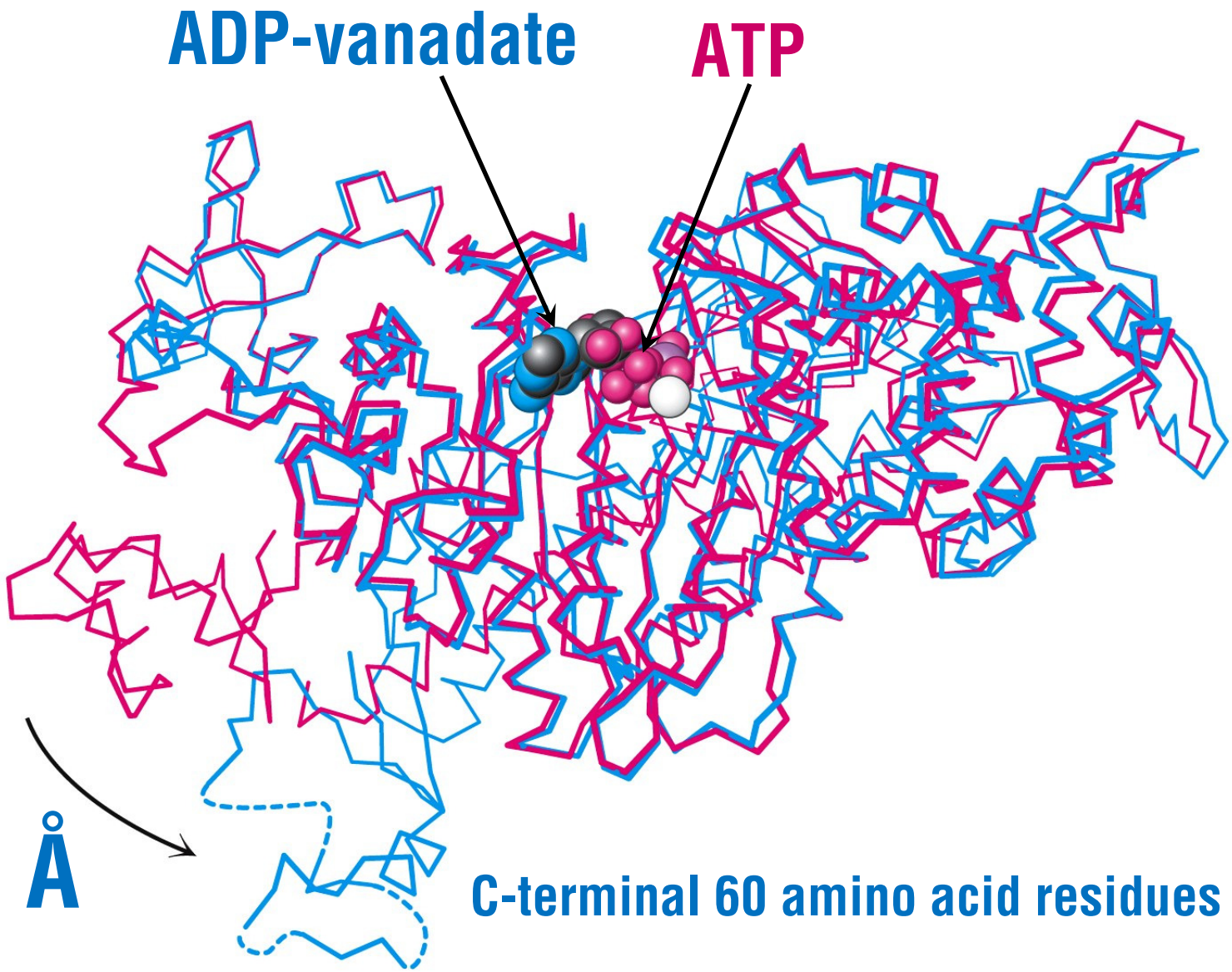
Myosin ATPase transition-state analog

- The water molecule attacking the γ-phosphoryl group of ATP is deprotonated by the hydroxyl group of Ser-236, which, in turn, is deprotonated by one of the oxygen atoms of the γ-phosphoryl group forming the $H_2PO_4^-$ product.

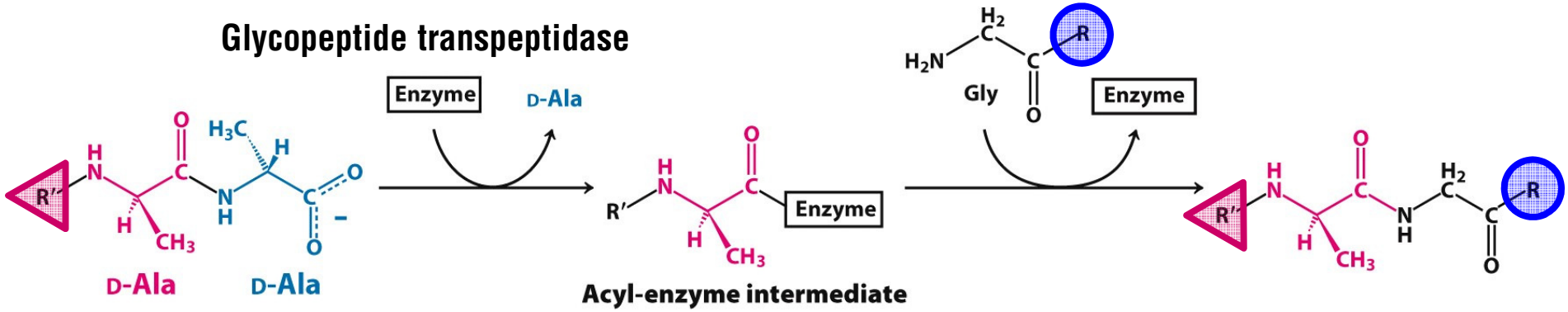


ATP serves as a base to promote its own hydrolysis

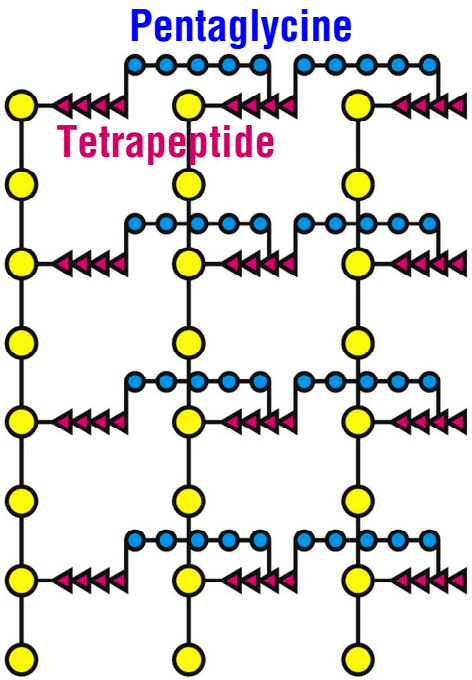
Myosin conformational changes

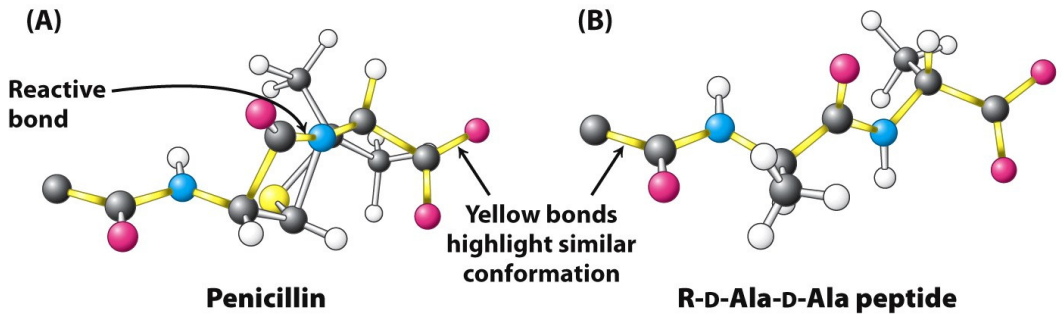


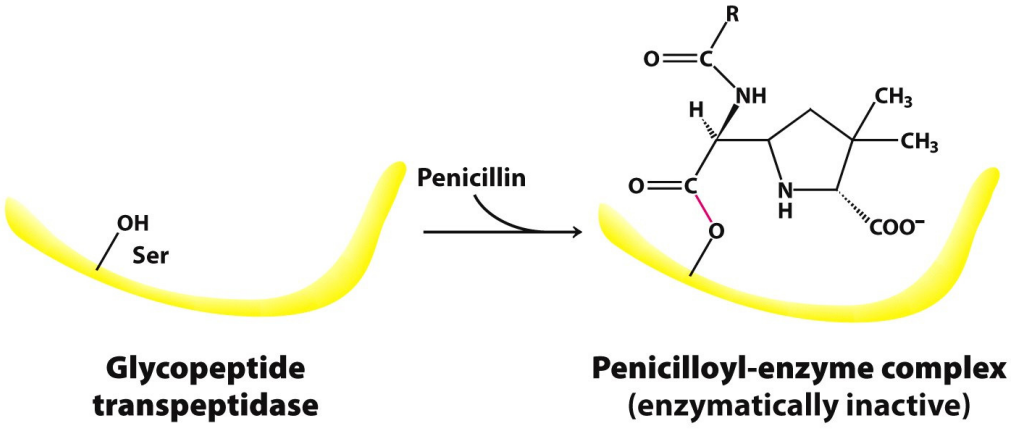
Penicillin irreversibly inactivates glycopeptide transpeptidase in bacterial cell-wall synthesis



The peptidoglycan in *Staphylococcus aureus*





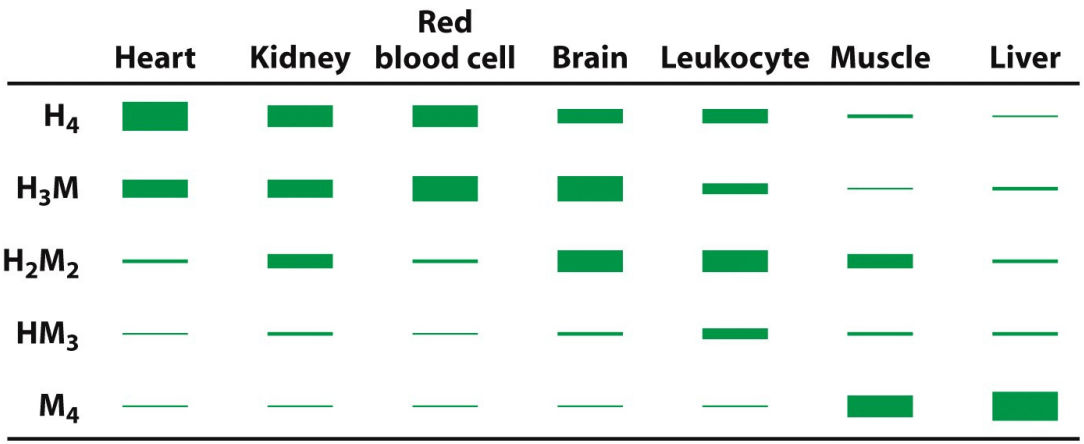


- **Methotrexate** is an potent competitive inhibitor of the enzyme **dihydrofolate reductase**, which catalyzes the biosynthesis of purines and pyrimidines.
- **Statins** are drugs that reduce high cholesterol levels by competitively inhibiting a key enzyme, **HMG-CoA reductase**, in cholesterol biosynthesis.
- **Ibuprofen (布洛芬) or Aspirin (阿斯匹靈)**, a nonsteroidal anti-inflammatory drug (NSAID), works as a competitive inhibitor by inhibiting the enzyme **cyclooxygenase (COX)**, which converts arachidonic acid to prostaglandin H_2 (PGH_2). PGH_2, in turn, is converted by other enzymes to several other prostaglandins (which are mediators of pain, inflammation, and fever) and to thromboxane A_2 (which stimulates platelet aggregation, leading to the formation of blood clots).
- **Acetaminophen (Tylenol 普拿疼)**, a drug for reducing pain and fever, and relieving the symptoms of allergies, cold, cough, and flu. The proposed main mechanism of acetaminophen is the inhibition of **cyclooxygenase**.
- The herbicide **glyphosate** works as an uncompetitive inhibitor by inhibiting the enzyme **5-enolpyruvylshikimate-3-phosphate synthase** in the biosynthesis pathway of shikimate for aromatic amino acids. The shikimate pathway is not present in animals, which instead obtain aromatic amino acids from their diet.
- **Deoxycycline** (去氧羥四黴素), an antibiotic, functions at low concentrations as a noncompetitive inhibitor of a proteolytic enzyme collagenase.
- Some of the toxic effects of **lead poisoning** may be due to lead's ability to act as a noncompetitive inhibitor of a host of enzymes with crucial sulfhydryl groups.
- **Penicillin** acts by covalently modifying the enzyme **transpeptidase**, thereby preventing the synthesis of bacterial cell walls and thus killing the bacteria.

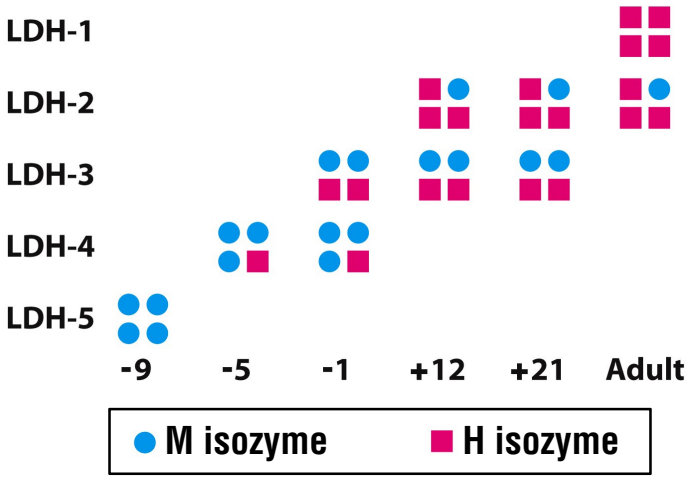
Isozymes of lactate dehydrogenase

☐ Isozymes provide a means of regulation specific to distinct tissues and developmental stages

■ Rat LDH isozyme content varies by tissue

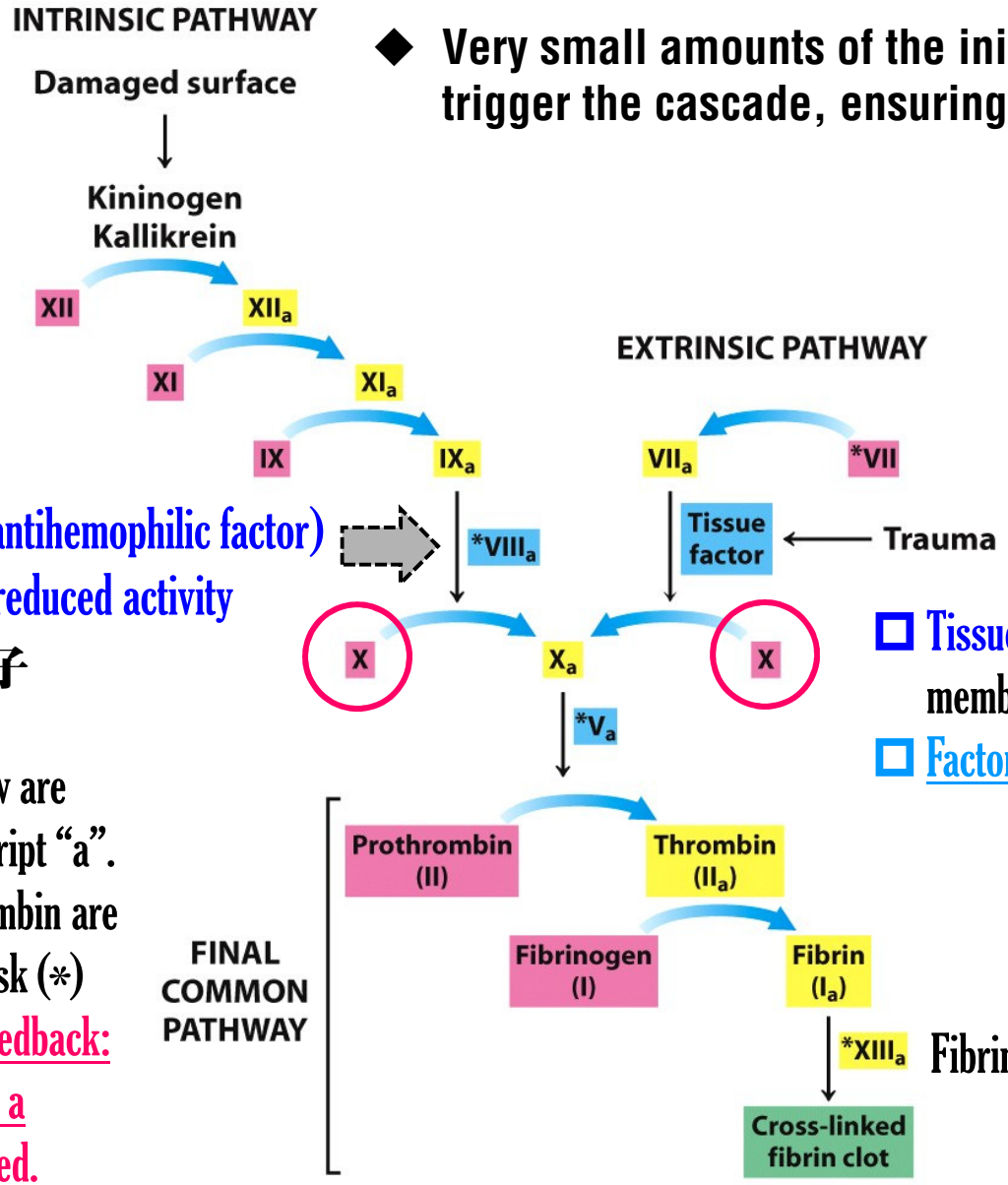


■ The rat heart LDH isozyme profile changes in the course of development



- ☐ H isozyme is highly expression in heart muscle and the M isozyme is expressed in skeletal muscle.
- ☐ The H_4 isozyme has a higher affinity for substrate than does the M_4 isozyme.
- ☐ The M_4 isozyme functions optimally in the anaerobic environment of hard-working muscle, whereas the H_4 does so in the aerobic environment of heart muscle.

Blood clots are formed by a cascade of zymogen activations



◆ Very small amounts of the initial factors suffice to trigger the cascade, ensuring a rapid response.

Hemophilia A: Factor VIII (antihemophilic factor) is missing or has markedly reduced activity

Factor VIII: 抗血友病因子

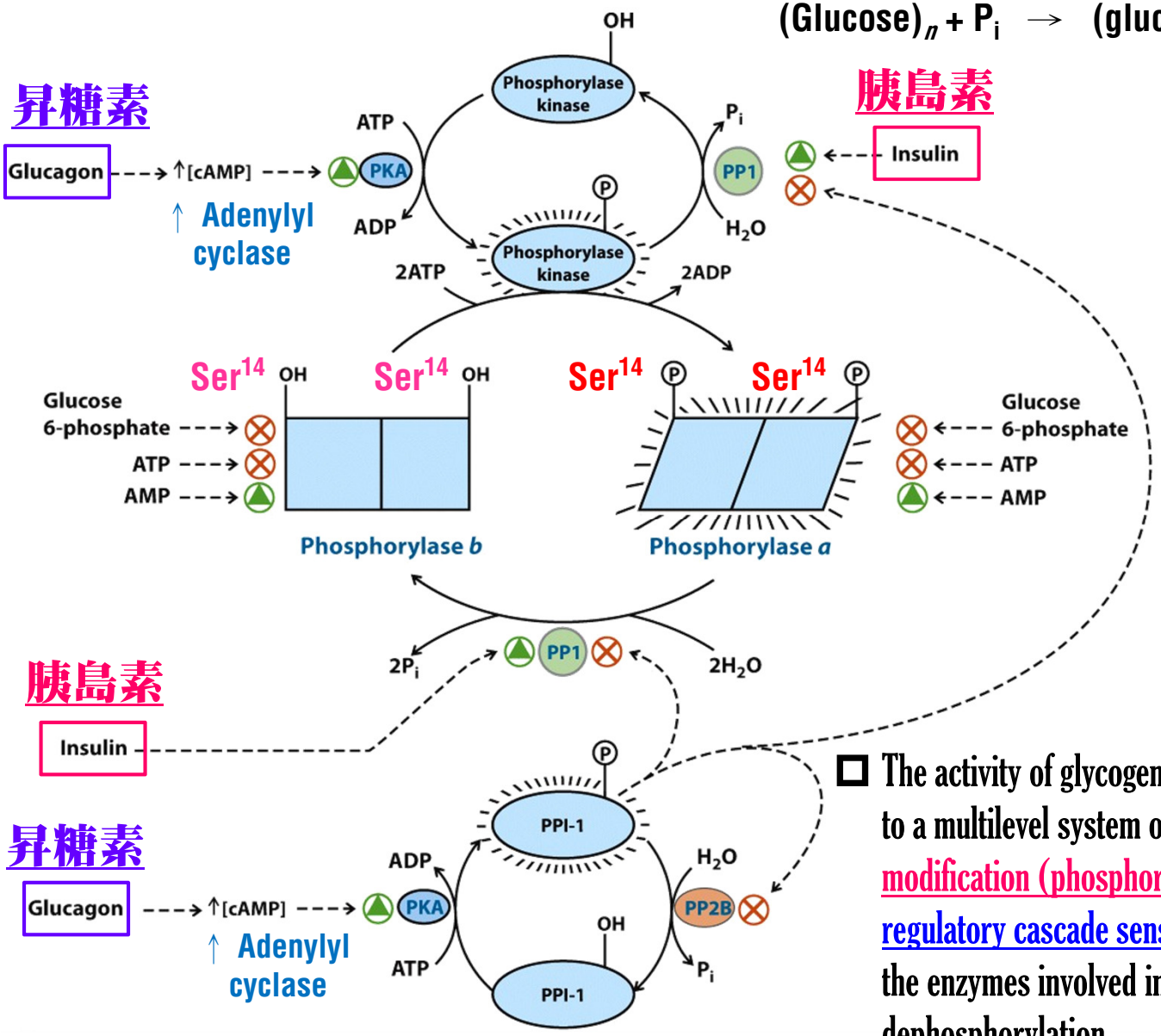
- The active forms in yellow are designated with an subscript "a".
- Factors activated by thrombin are designated with an asterisk (*)
- An example of positive feedback: accelerate reactions after a threshold has been reached.

- Tissue factor is an integral membrane glycoprotein.
- Factors in blue are not enzymes.

凝血因子

Regulation of muscle glycogen phosphorylase activity by multiple mechanisms

$$(\text{Glucose})_n + P_i \rightarrow (\text{glucose})_{n-1} + \text{glucose 1-phosphate}$$



GP

肝醣磷解酶

- The attachment of phosphoryl groups to specific residues of a protein is catalyzed by protein kinases ; removal of phosphoryl groups is catalyzed by protein phosphatases .

- The activity of glycogen phosphorylase in muscle is subjected to a multilevel system of regulation, involving covalent modification (phosphorylation), allosteric regulation, and a regulatory cascade sensitive to hormonal status that acts on the enzymes involved in phosphorylation and dephosphorylation.