

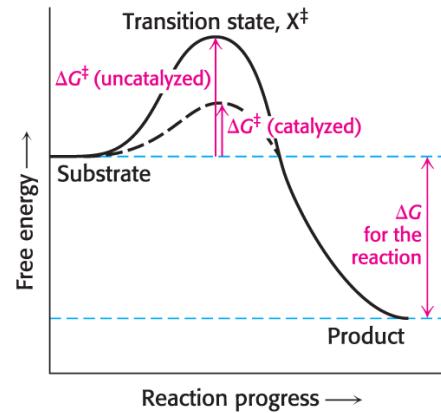
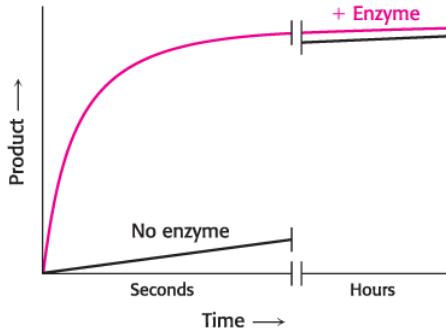


# Lecture 4

## Mechanisms and Inhibitors

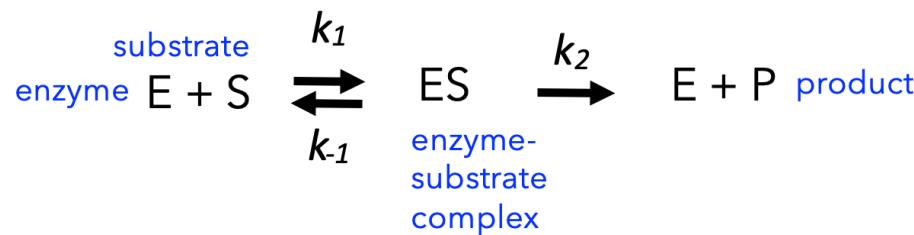
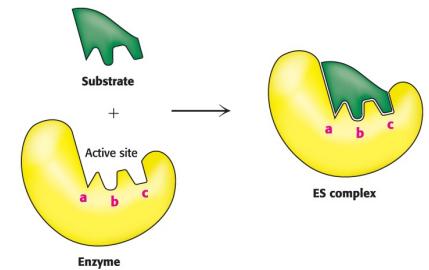
### Hemoglobin

# From last week's lecture...

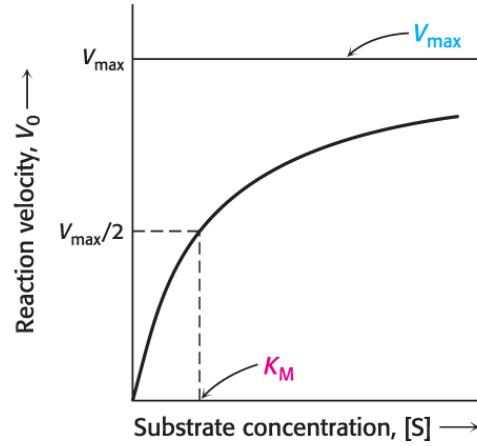


$$\Delta G^\circ' = -RT \ln K'_{eq}$$

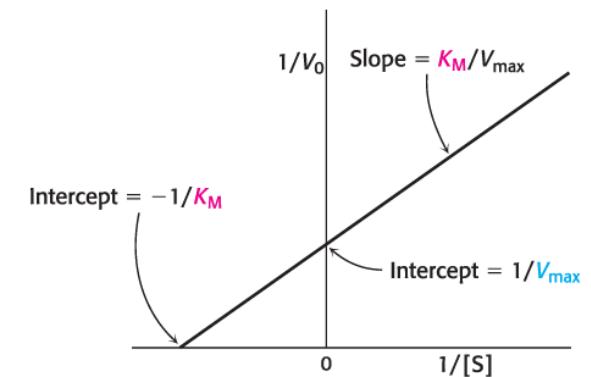
$$K'_{eq} = e^{-\Delta G^\circ' / 2.47}$$



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

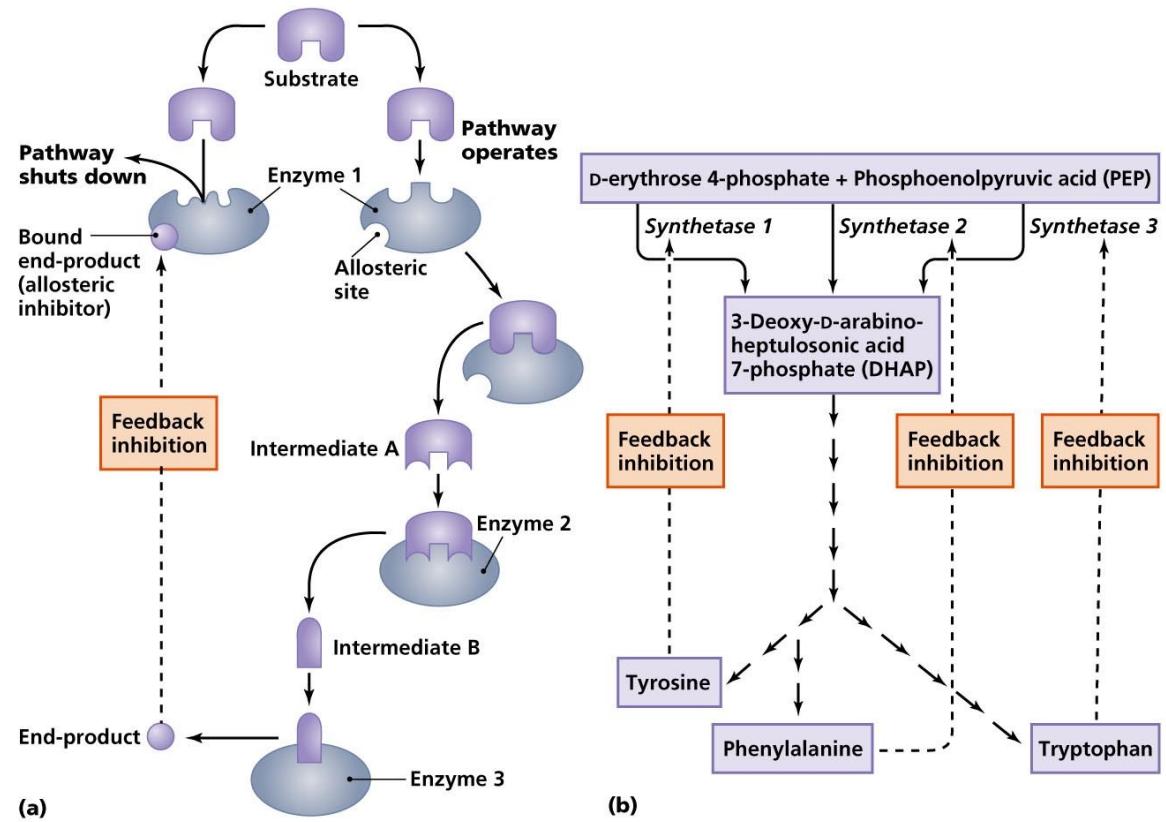


$$\frac{1}{V_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{S} + \frac{1}{V_{max}}$$



# Allosteric enzymes - Feedback Inhibition

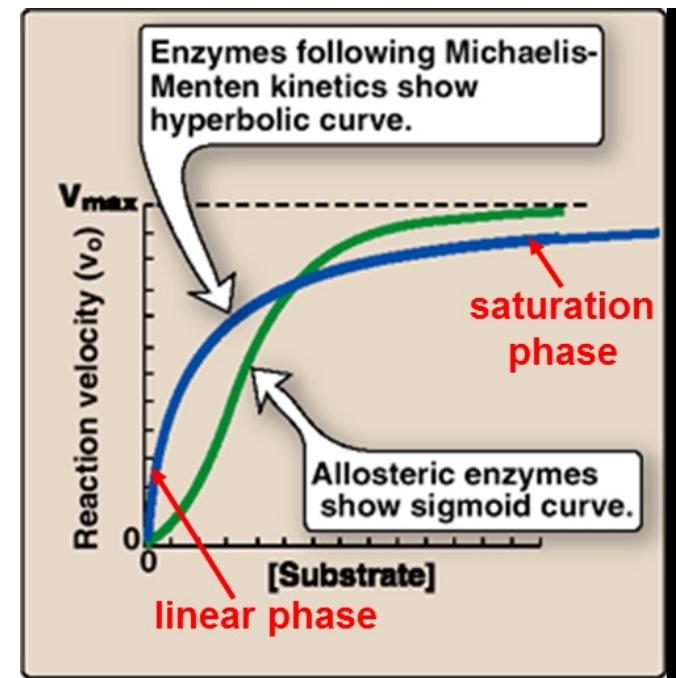
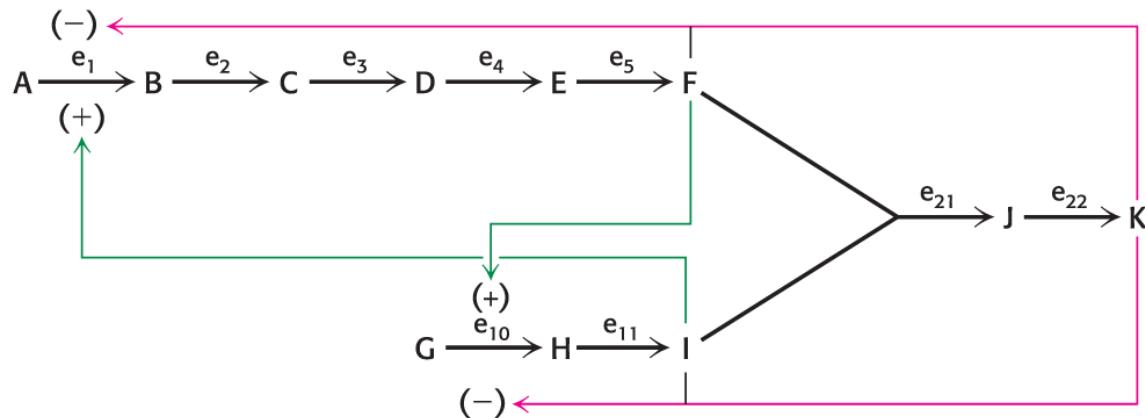
- Allosteric enzymes control the flux of biochemical reactions in metabolic pathways.
- Feedback inhibition prevents unnecessary overproduction of metabolites.
- It relies on allosteric regulation, where the product controls the first (or an early) committed step of its own biosynthesis.
- In branched pathways, multiple feedback control points can act in parallel, each one attuned to a different product requirement.



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# Allosteric enzymes

- The regulation of metabolic pathways can be quite complex.
- Allosteric enzymes may be inhibited or stimulated by several regulatory molecules.



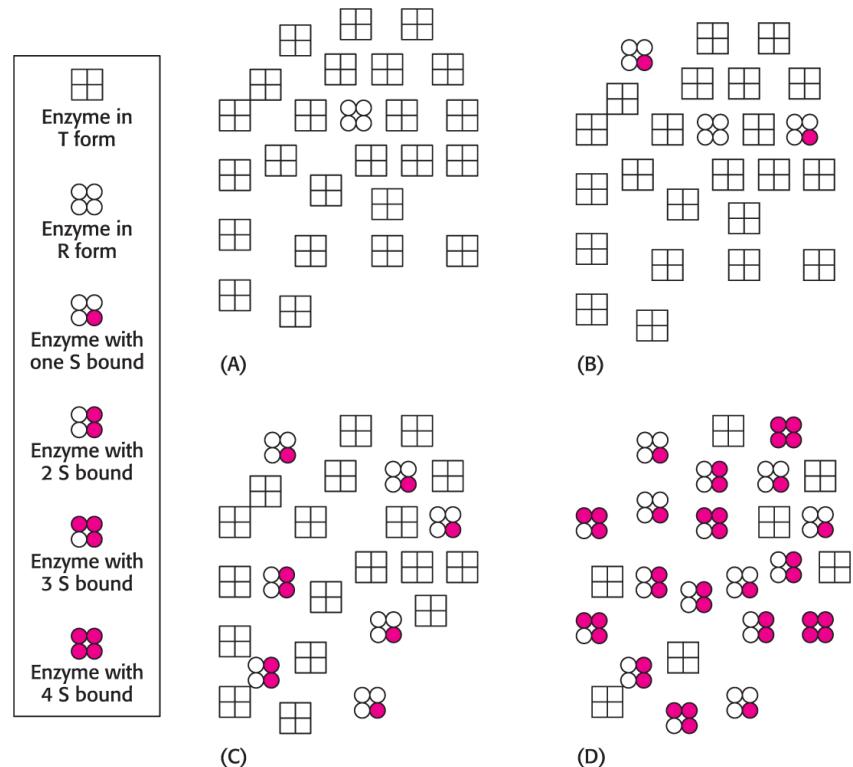
- The reaction velocity of allosteric enzymes displays a sigmoidal relationship to substrate concentration.

# Allosteric Enzymes and Quaternary Structure

- All allosteric enzymes display quaternary structure with multiple active sites and regulatory sites.
- One model that explains the behavior of allosteric enzymes is the **concerted model**.

## Features of the concerted model:

- The enzyme exists into two different quaternary structures, designated **T(tense)** and **R (relaxed)**.
  - T and R are in equilibrium, with T being the more stable state.
  - The R state is enzymatically more active than the T state.
  - All active sites must be in the same state.
- The **sequential model** for allosteric enzymes proposes that subunits undergo sequential changes in structure



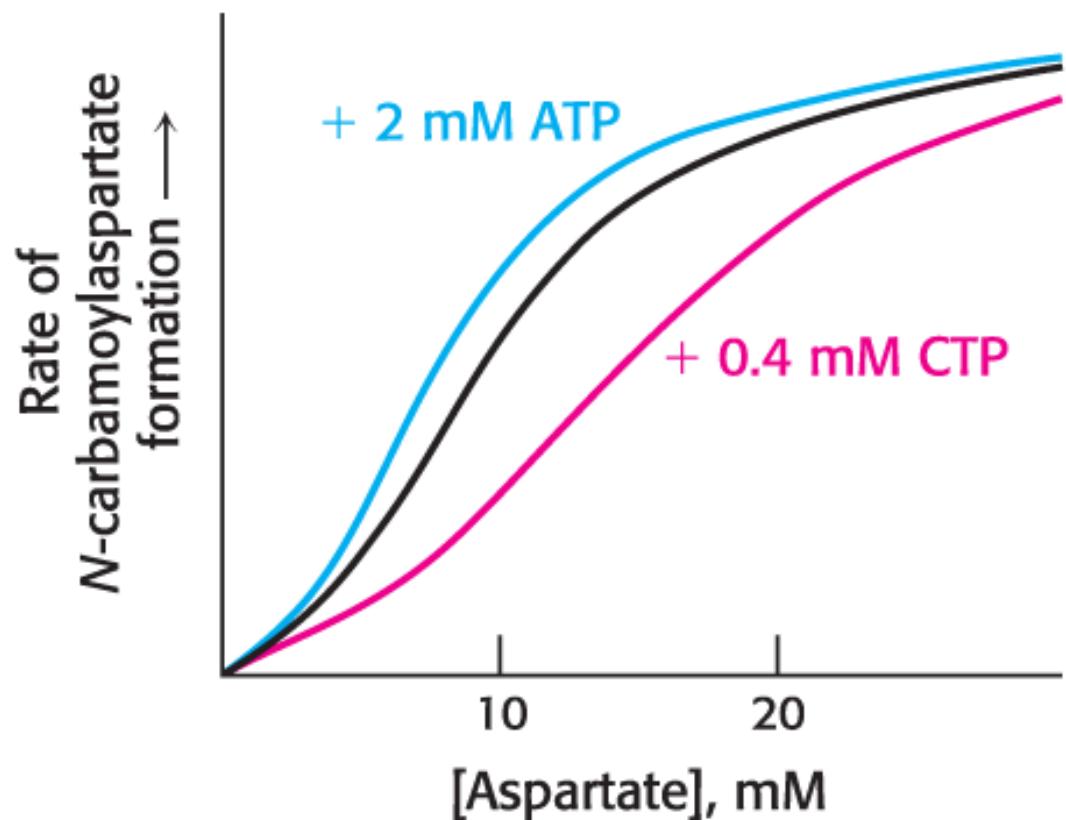
## Quick Quiz 5

An allosteric enzyme can exist in two states, \_\_\_\_\_ and \_\_\_\_\_.

- A. tight; responsive
- B. tense; responsive
- C. tense; relaxed
- D. tight; relaxed
- E. turgid; relaxed

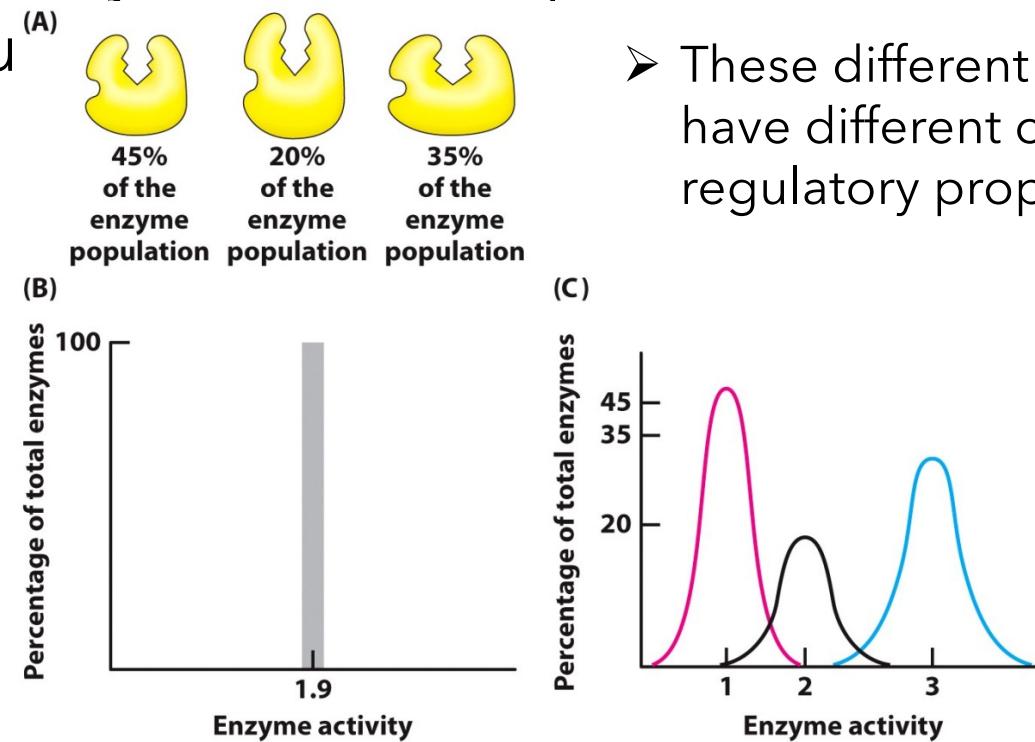
# Regulator Molecules Modulate the $R \rightleftharpoons T$ Equilibrium

- Allosteric regulators disrupt the  $R \rightleftharpoons T$  equilibrium when they bind the enzyme.
- Inhibitors stabilize the T state while activators stabilize the R state.
- The disruption of the  $T \rightleftharpoons R$  equilibrium by substrates is called the **homotropic** effect.
- The disruption of the  $T \rightleftharpoons R$  equilibrium by regulators is called the **heterotropic** effect.



# Enzymes Can Be Studied One Molecule at a Time

- Studies of individual enzyme molecules suggest that some enzymes may exist in multiple conformations that are in equilibrium<sup>(A)</sup>



- These different conformations may have different catalytic or regulatory properties.

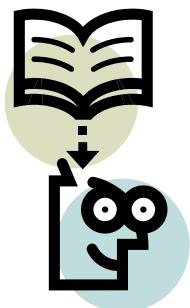
# Mechanisms and Inhibitors Hemoglobin

## Lecture Outline:

- Enzymes and Their Catalytic Strategy
- Factors That Modulate Enzyme Activity
- Chymotrypsin and Its Catalytic Activity
- Introduction to Hemoglobin
- Hemoglobin and Myoglobin
- An Allosteric Regulator Determines the Oxygen Affinity of Hemoglobin
- Hydrogen Ions and Carbon Dioxide Promote the Release of Oxygen

## Readings:

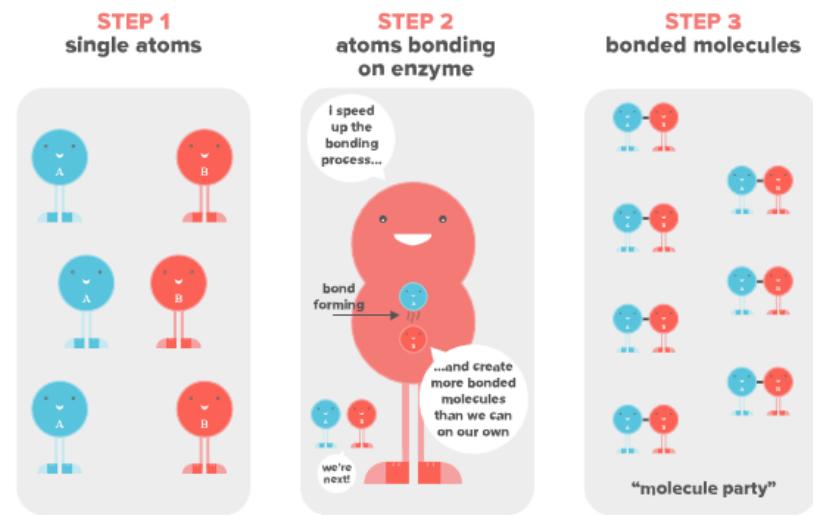
Tymochko, Berg, Stryer,  
Biochemistry, Ch. 8 - 9  
2<sup>nd</sup> Edition, pp. 125 - 154  
3<sup>rd</sup> Edition, pp. 131 - 163  
4<sup>th</sup> Edition, Ch. pp. 143 - 171



# Enzymes Catalytic Strategies

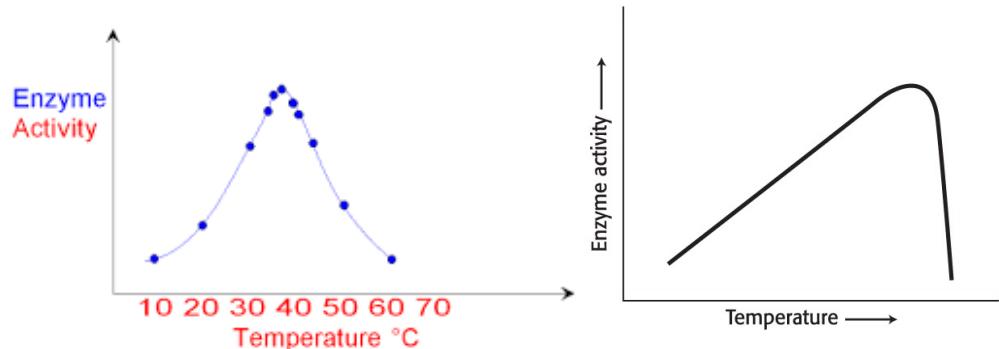
- Common catalytic strategies include:

1. **Covalent catalysis:** The active site contains a nucleophile that is briefly covalently modified.
2. **General acid-base catalysis:** A molecule other than water donates or accepts a proton.
3. **Metal ion catalysis:** Metal ions function in a number of ways including serving as an electrophilic catalyst/change acidity/binds to the substrate
4. **Catalysis by approximation and orientation:** The enzyme brings two substrates together in an orientation that facilitates catalysis.



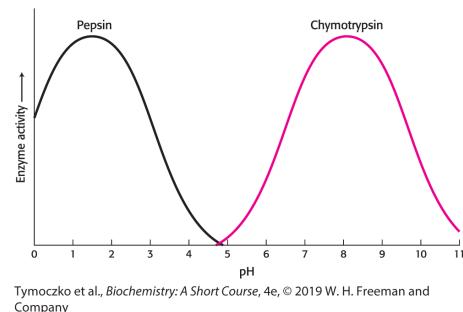
# Factors That Modulate Enzyme Activity

- Temperature enhances the rate of enzyme-catalyzed reactions.



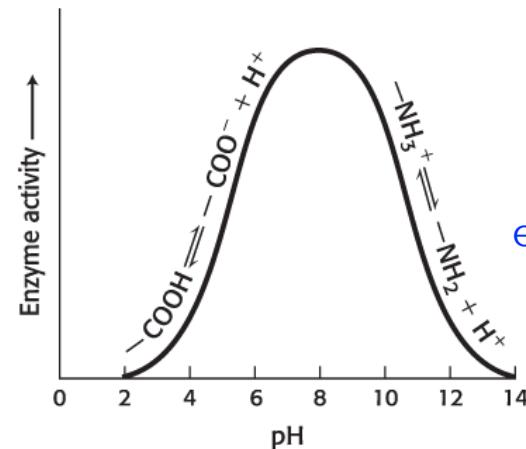
Lizard adjusts their body temp → rate of biochemical reactions

- Most enzymes have an optimal pH.



Tymoczko et al., *Biochemistry: A Short Course*, 4e, © 2019 W. H. Freeman and Company

Pepsin operates at very acidic pH!

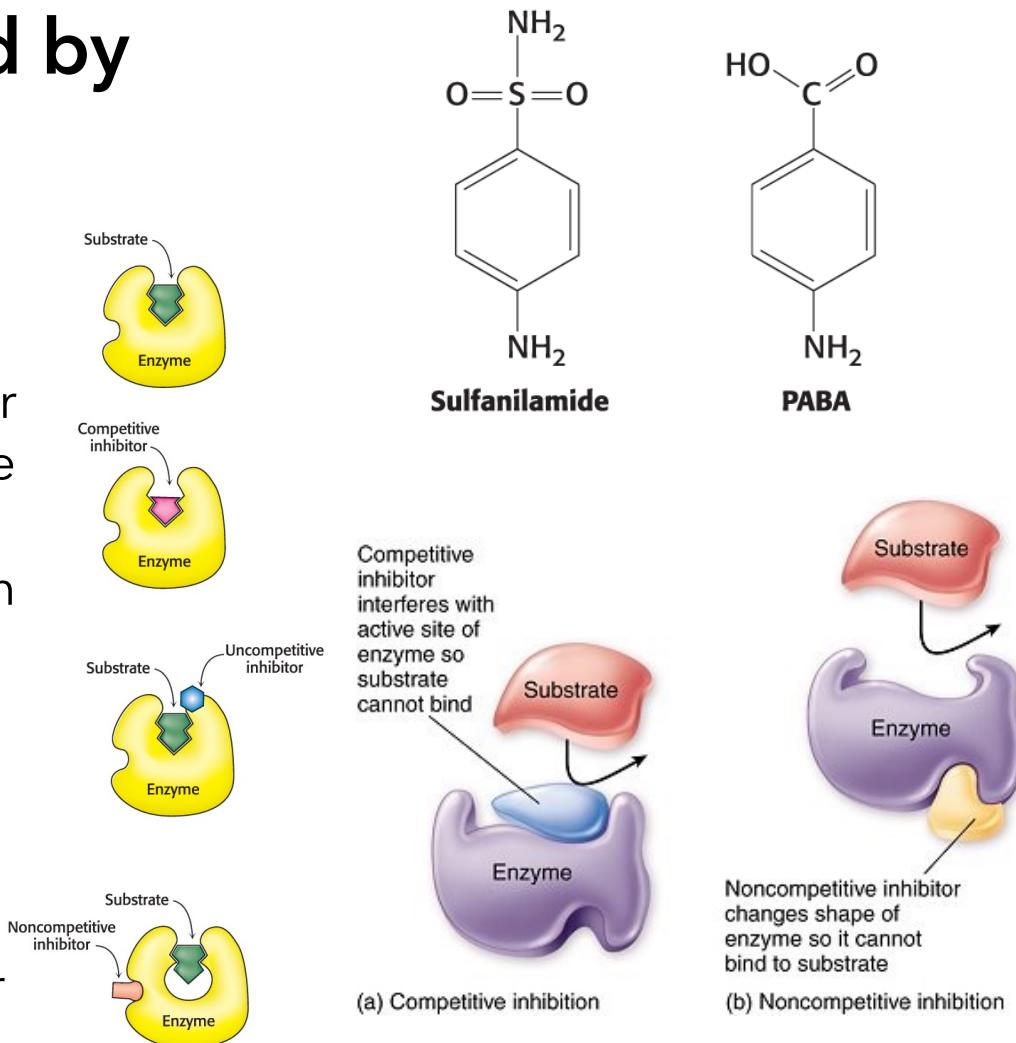


pH dependence of enzymes → ionizable groups

Figure 8.2  
Biochemistry: A Short Course, Second Edition  
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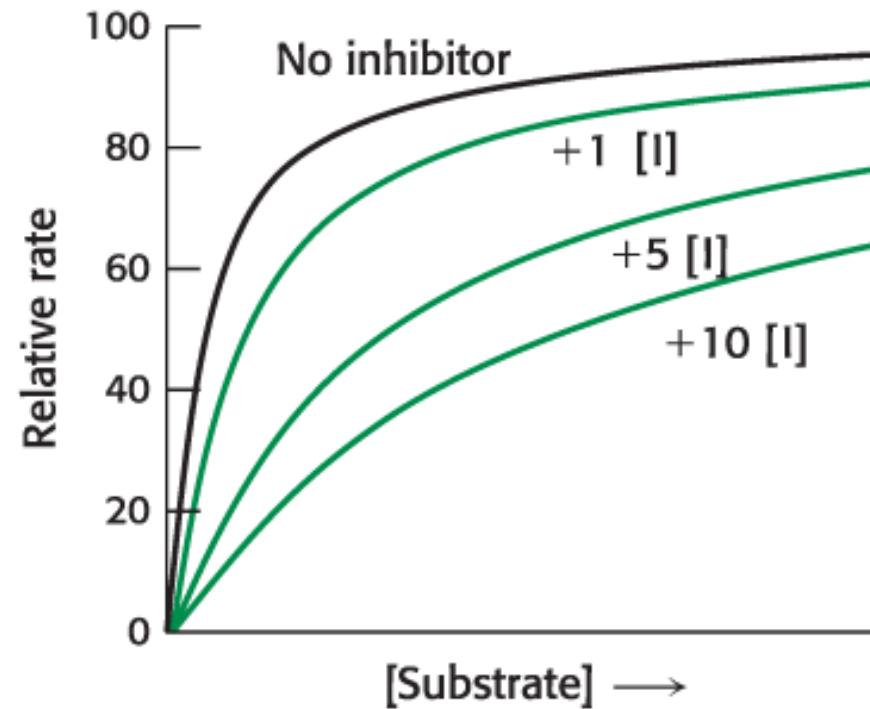
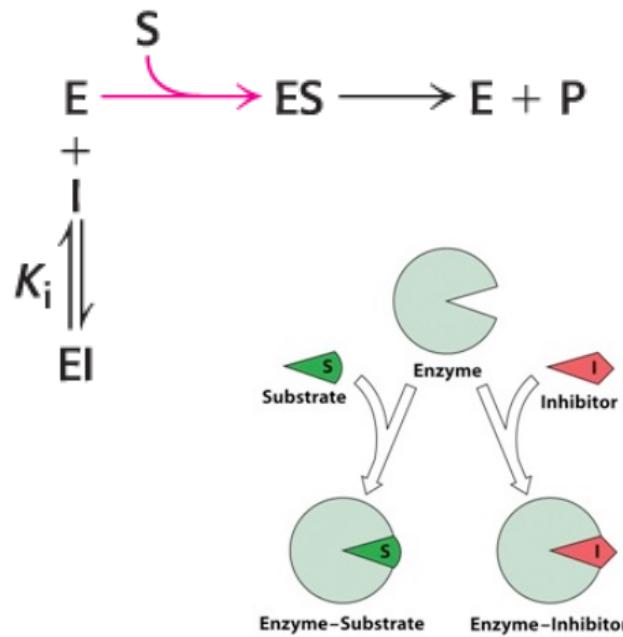
# Enzymes can be inhibited by specific molecules

- 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.
  3. **Noncompetitive inhibition:** The inhibitor binds either the enzyme or enzyme-substrate complex.



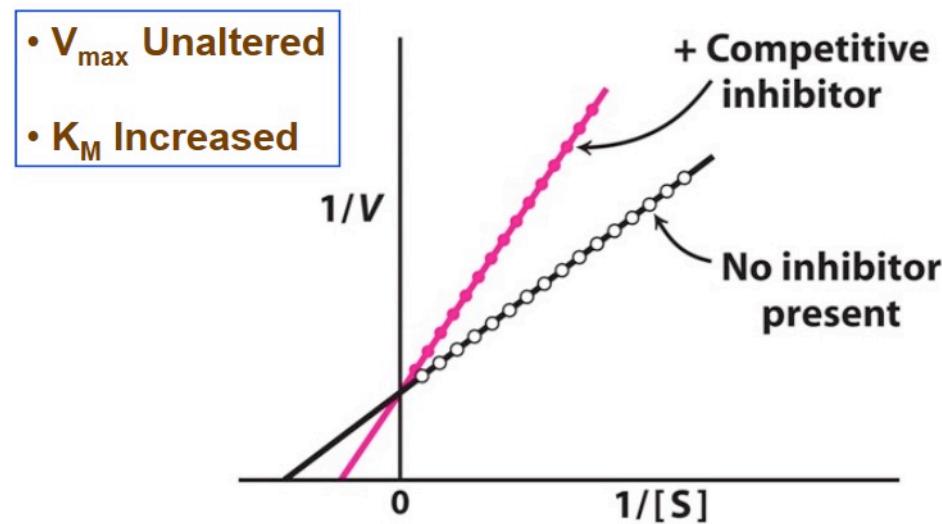
# Reverse Inhibitors Are Kinetically Distinguishable

- In **competitive inhibition**,  $V_{max}$  of the enzyme is unchanged because the inhibition can be overcome by a sufficiently high concentration of substrate.
- However, **apparent  $K_M$  is increased** in the presence of inhibitor.



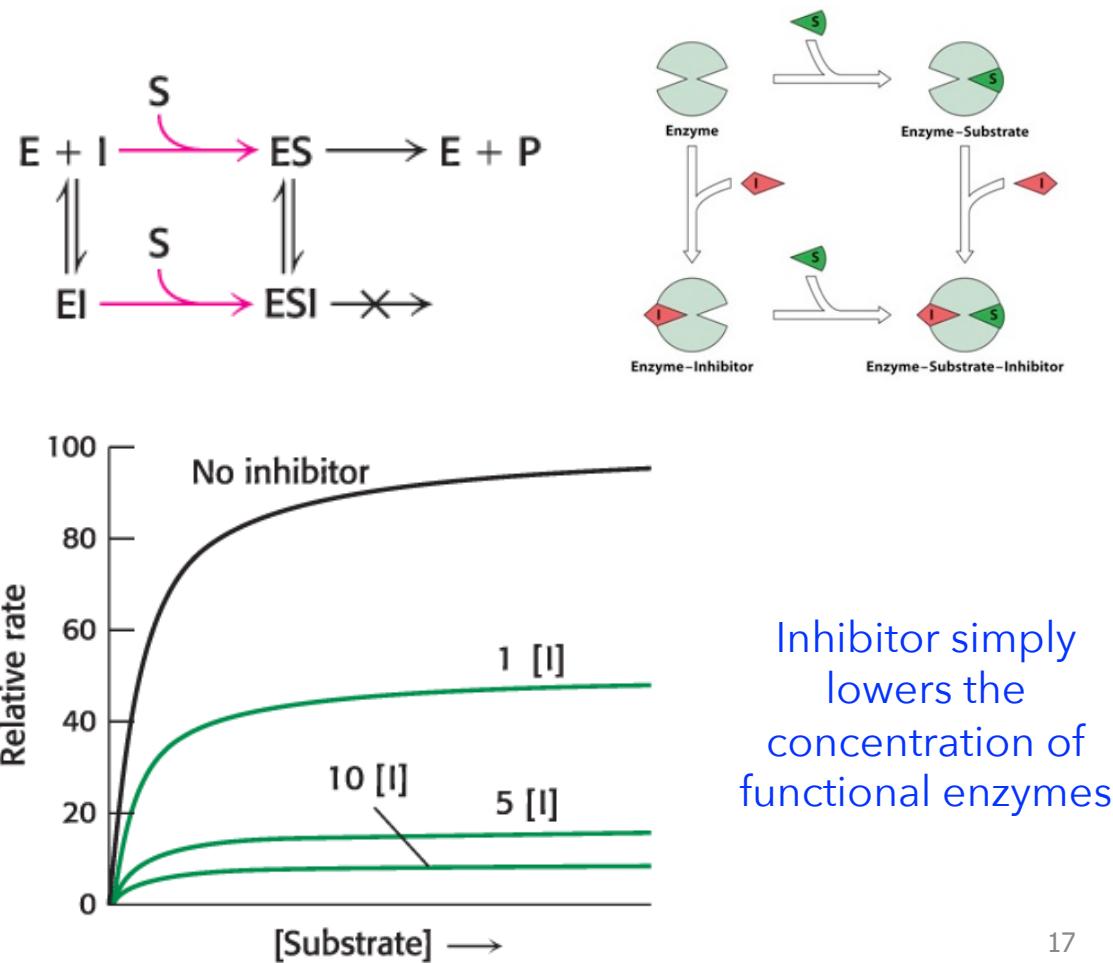
# Competitive Inhibition

- Substrate can out compete inhibitor  $\rightarrow V_{max}$  is unchanged
- Inhibitor binds to the active site  $\rightarrow K_M$  increases
- A double-reciprocal plot (Lineweaver-Burk plot)
- An example - treating methanol poisoning with ethanol



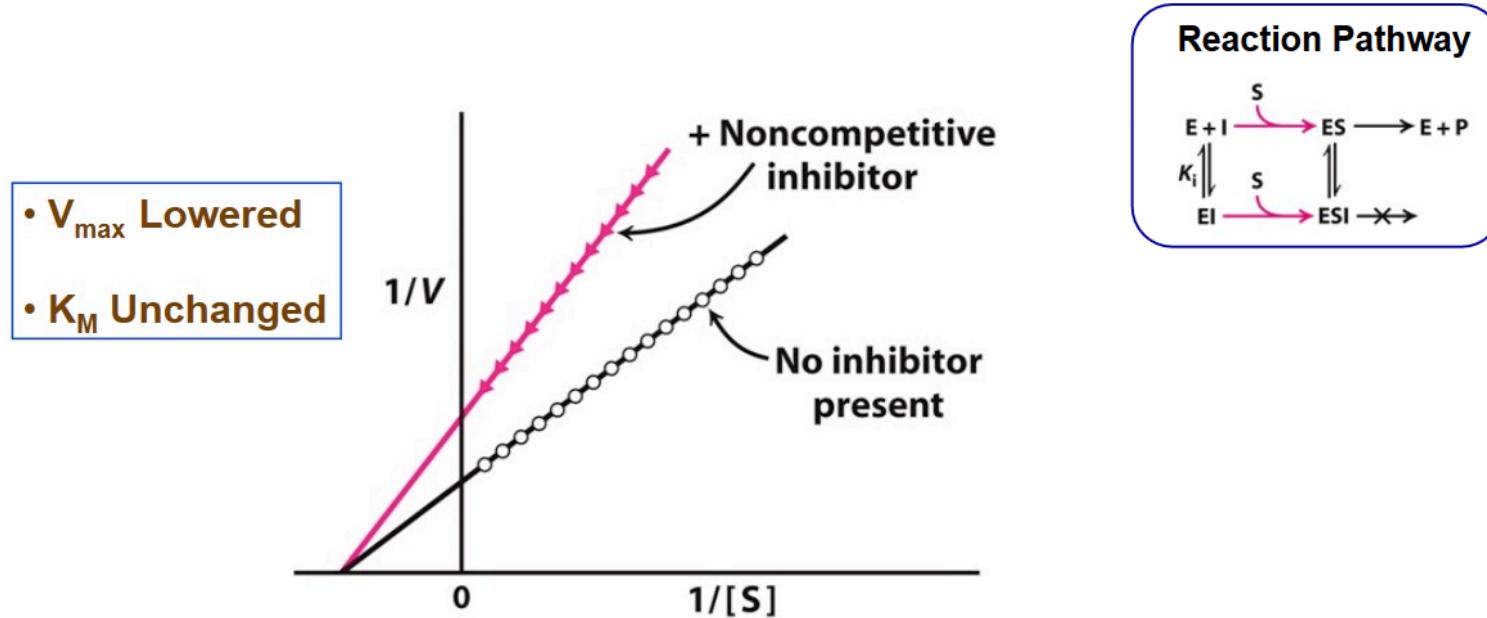
# Reverse inhibitors are kinetically distinguishable

- In **noncompetitive inhibition**, the inhibitor can bind to free enzyme or to the enzyme-substrate complex. In either case, the binding of inhibitor prevents the formation of product.
- **$V_{max}$  is lower** in the presence of a **noncompetitive** inhibitor.
- **$K_M$  is not changed** by the presence of a noncompetitive inhibitor.
- Noncompetitive inhibition cannot be overcome by increasing substrate concentration.



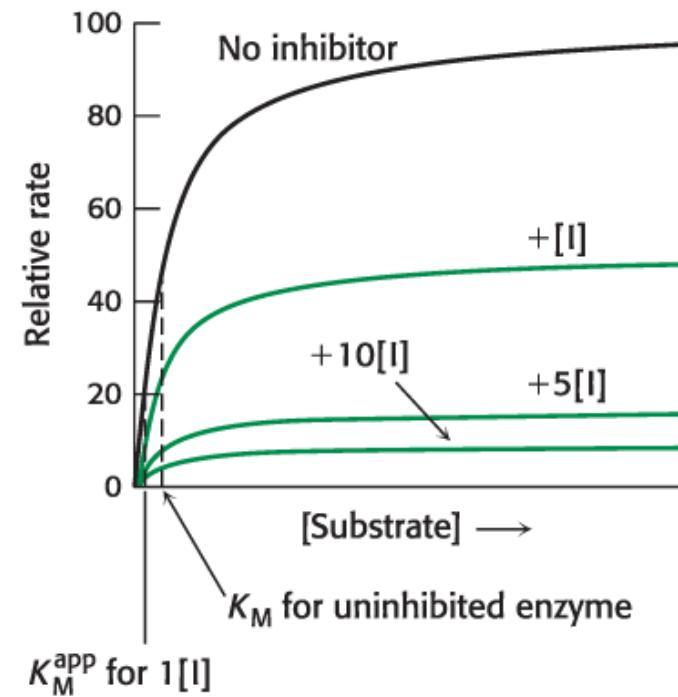
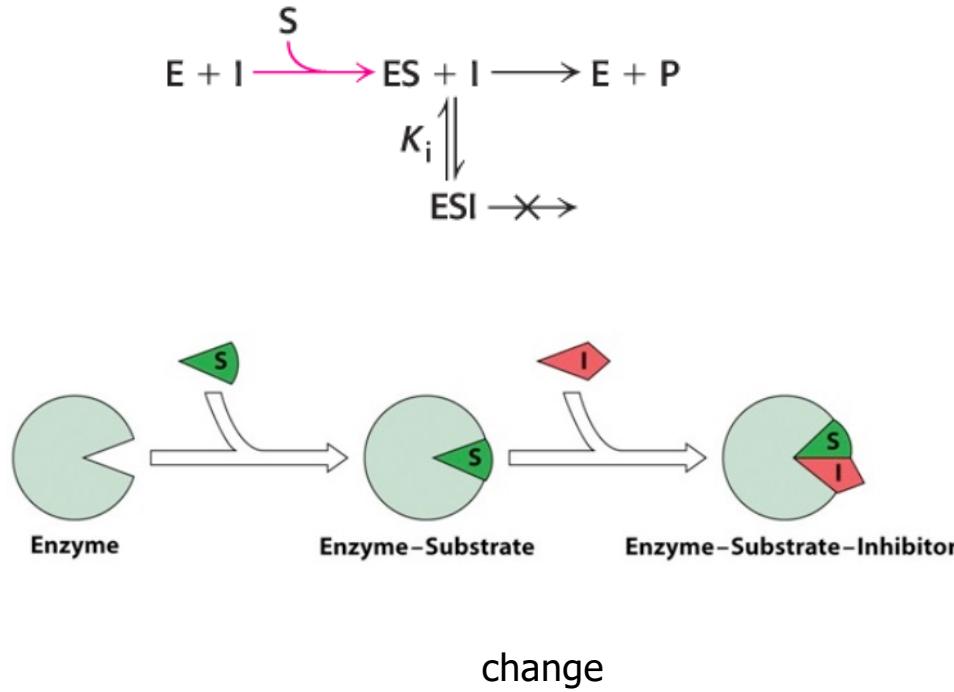
# Noncompetitive Inhibition

- Taking enzyme out of circulation  $\rightarrow V_{max}$  is lowered
- Inhibitor binds both E and ES  $\rightarrow K_M$  unchanged
- A double-reciprocal plot (Lineweaver-Burk plot)



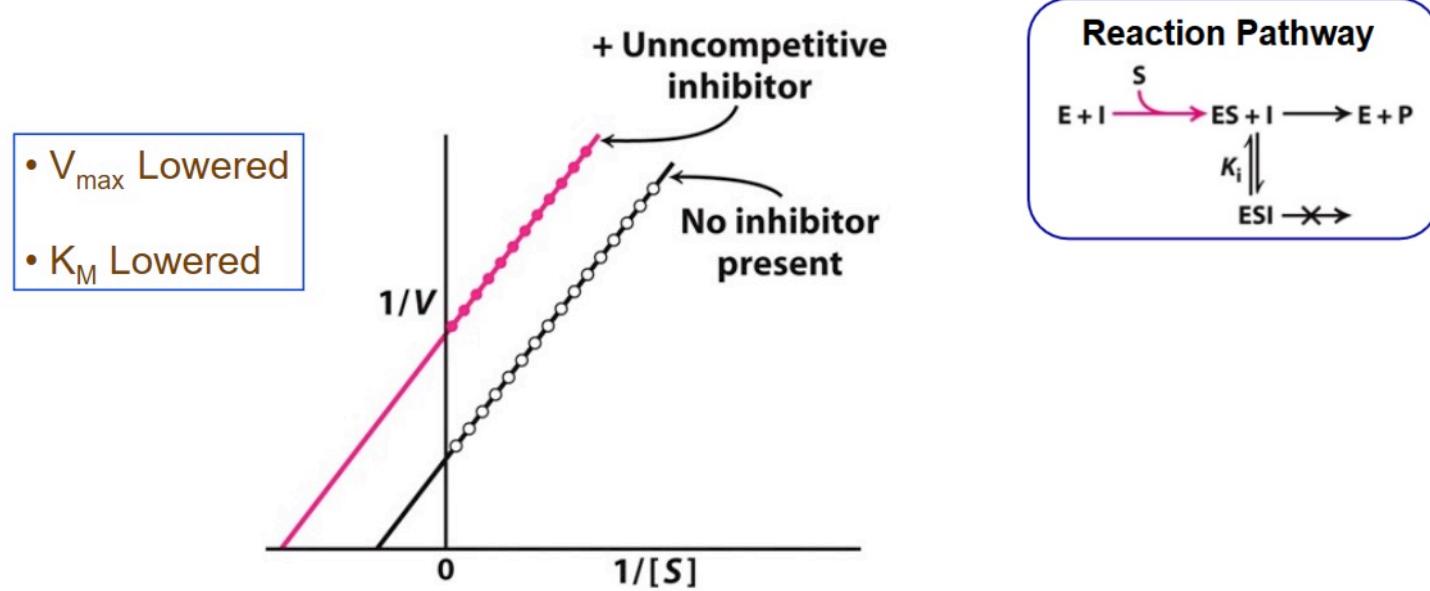
# Reverse Inhibitors Are Kinetically Distinguishable

- An **uncompetitive inhibitor** binds at a site distinct from the substrate active site and, unlike competitive inhibitor, binds only to the ES complex.



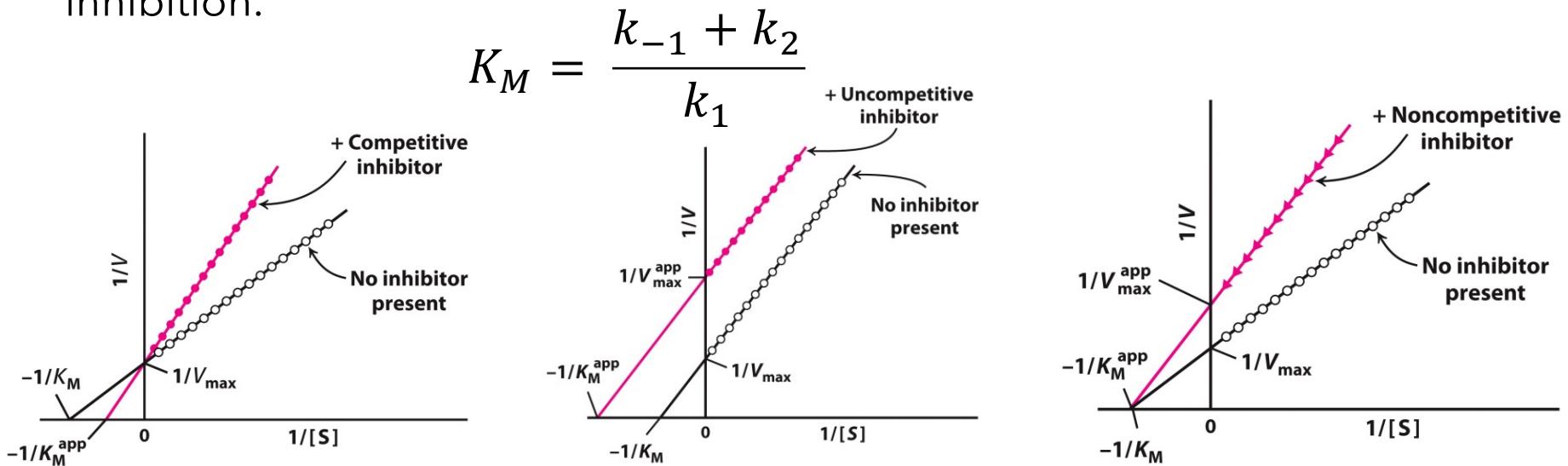
# Uncompetitive Inhibition

- Taking enzyme out of circulation  $\rightarrow V_{max}$  is lowered
- Inhibitor binds to E after the substrate binds  $\rightarrow K_M$  decreased
- A double-reciprocal plot (Lineweaver-Burk plot)



# Reverse Inhibitors Are Kinetically Distinguishable

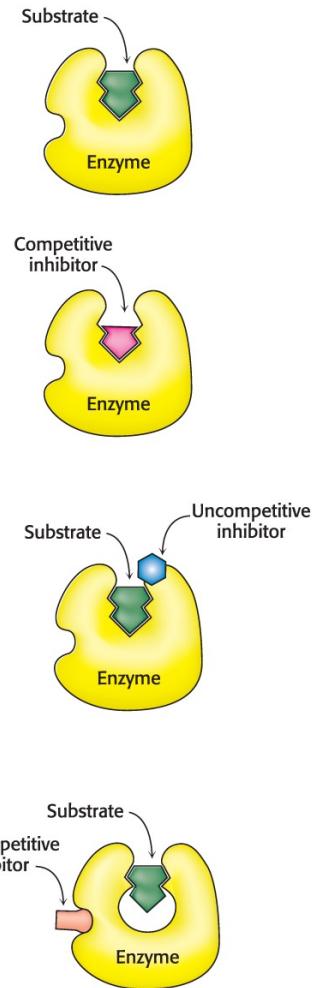
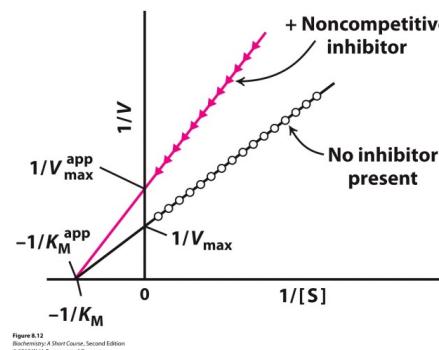
- Double reciprocal plots highlight the differences in the types of reversible inhibition.



# Quick Quiz 1

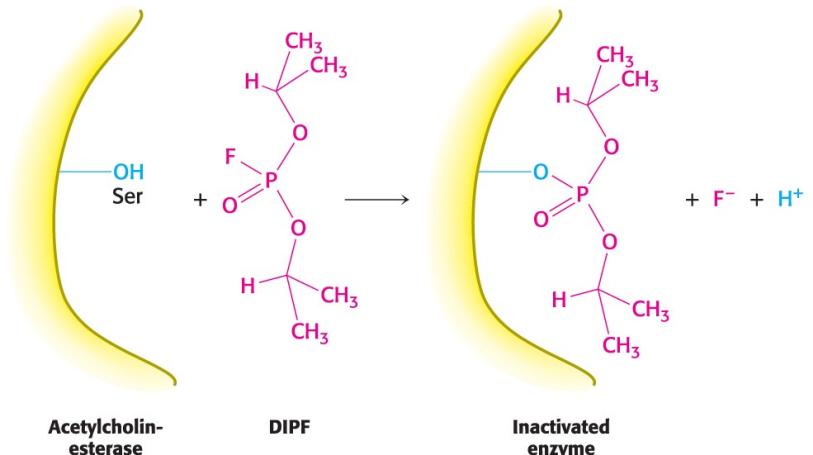
Which of the following statements are true for BOTH noncompetitive inhibitors and uncompetitive inhibitors?

- A. They both bind to the active site of the enzyme.
- B. They both block the ability of substrate to bind the active site of the enzyme.
- C. They both yield a  $K_M^{app}$  that is lower than the  $K_M$ .
- D. All of the above.
- E. none of the above.



# Irreversible Inhibitors Can Be Used to map the Active Site

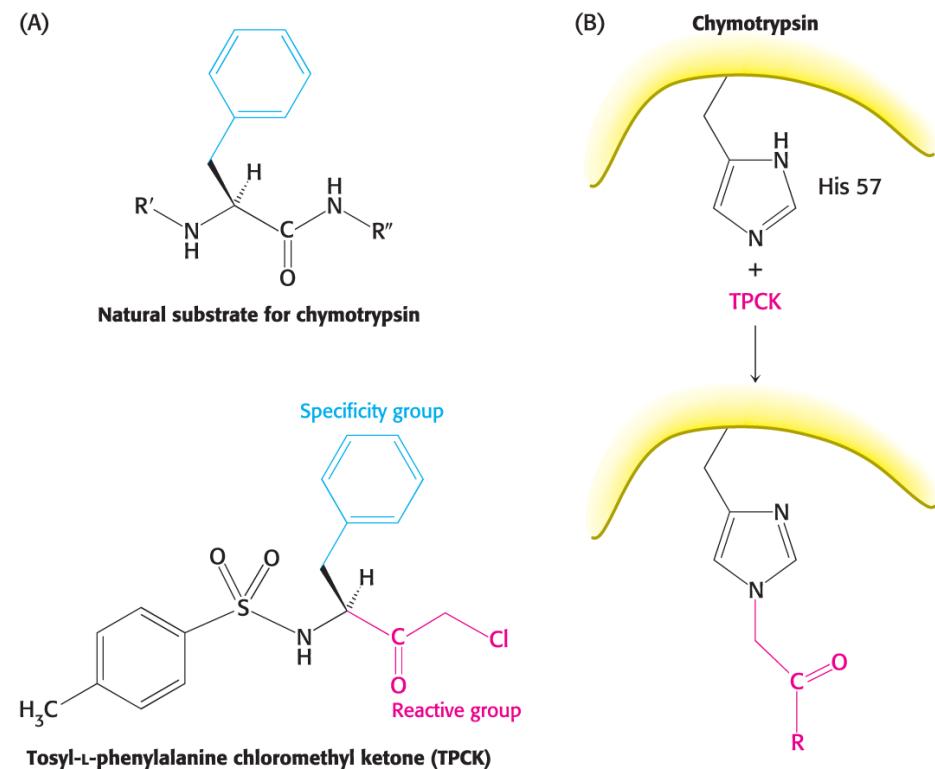
- Irreversible inhibitors bind very tightly to enzymes.
- Irreversible inhibitors that bind the enzyme covalently are powerful tools for elucidating the mechanisms of enzyme action.
- Four categories of irreversible inhibitors are known: **group-specific reagents**, **affinity labels**, **suicide inhibitors**, and **transition-state analogs**.
- Group specific reagents react with particular R-groups of amino acids.



DIPF inhibits by covalently modifying a crucial serine residue.

# Irreversible Inhibitors Can Be Used to map the Active Site

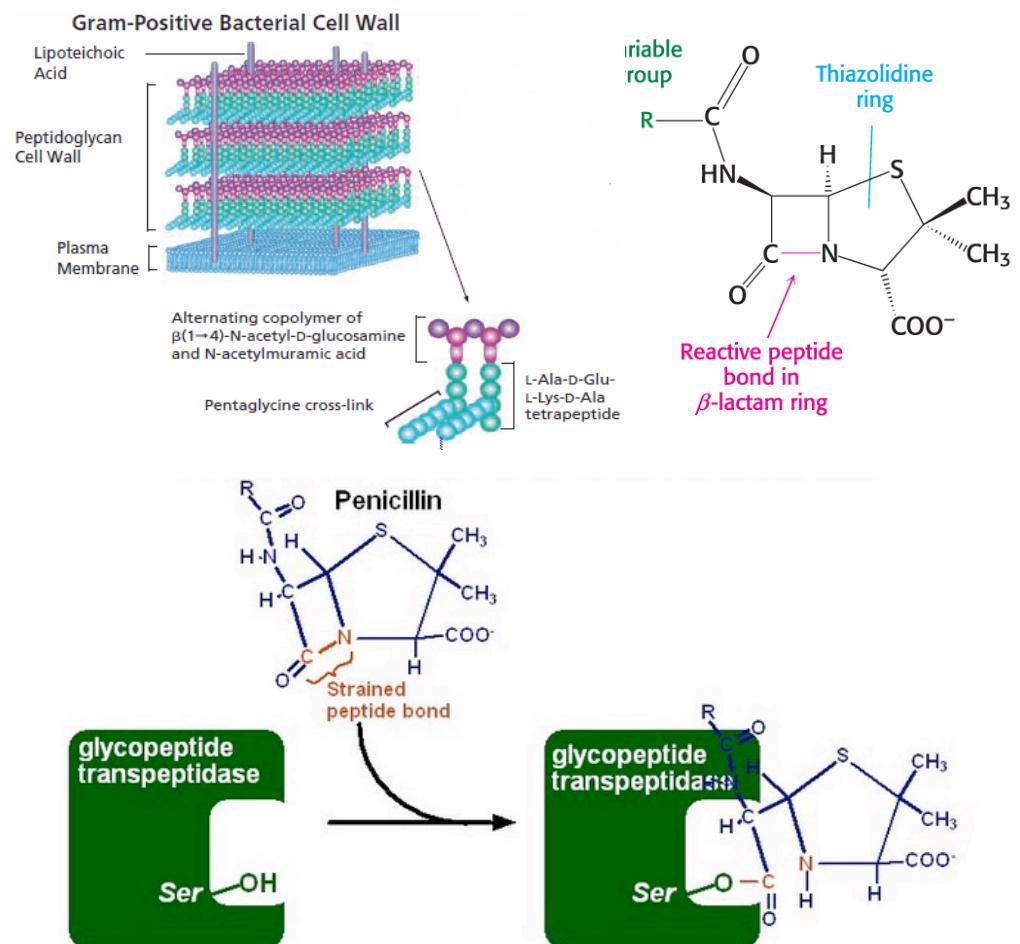
- **Affinity labels** or **substrate analogs** are structurally similar to the enzyme's substrate but inhibit the enzyme by covalently modifying an amino acid in the active site.
- **Suicide inhibitors**, or **mechanism-based inhibitors**, are chemically modified substrates.
- **Transition-state analogs** resembled the transition state of the enzyme but are not capable of being acted on by enzyme.



Affinity Labeling: TPCK binds at the chymotrypsin active site and modifies an essential histidine residue.

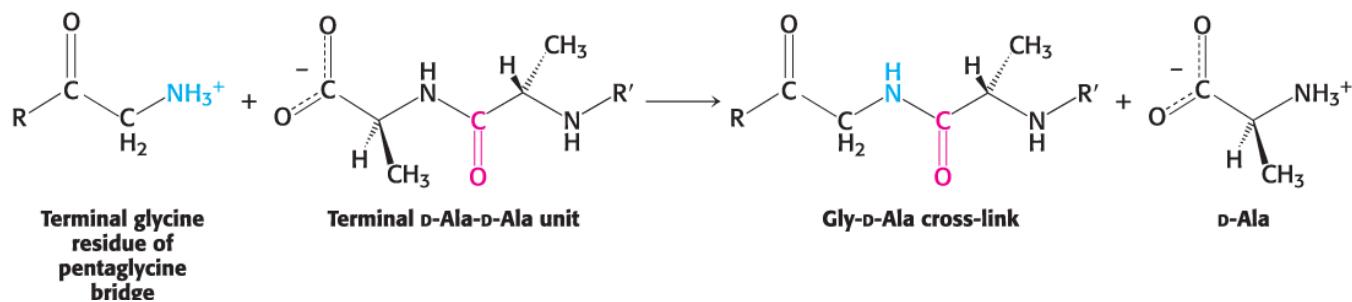
# Penicillin is an example of an irreversible inhibitor

- Penicillin is an antibiotic that consists of thiazolidine ring fused to a very reactive  $\beta$ -lactam ring.
- Penicillin inhibits the formation of cell walls in certain bacteria such as *S. aureus*.
- Penicillin inhibits the enzyme **transpeptidase** that catalyzes the last step in bacterial cell wall biosynthesis.
- When penicillin binds to the peptidase, a serine residue at the active site attacks the carbonyl carbon of the lactam ring as if penicillin were a substrate.
- The defective walls cause bacterial cells to burst. Humans are not affected because our cells have cell membranes, not cell walls.

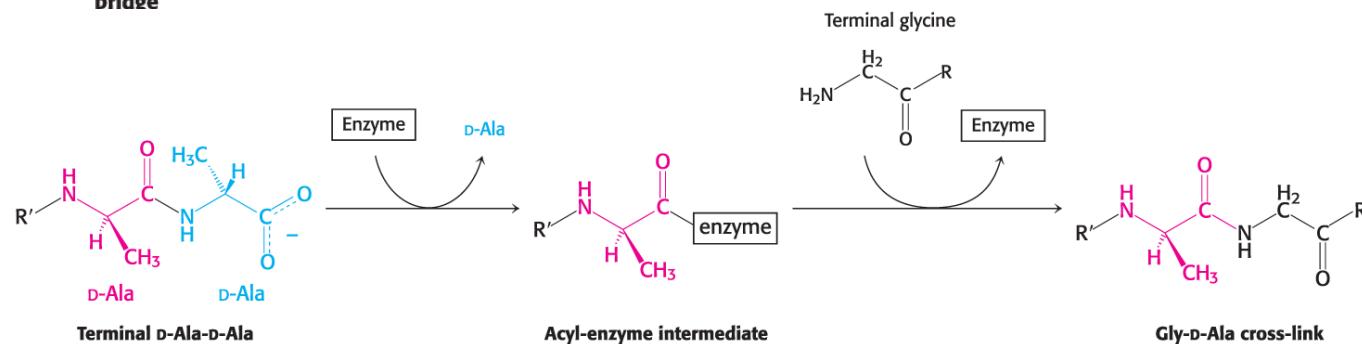


# Penicillin is an example of an irreversible inhibitor

- A penicillinoyl-serine derivative is formed which is inactive and very stable.
- Thus, the antibiotic penicillin is a **suicide inhibitor** of the enzyme that synthesizes bacterial cell walls.



Normal cross-linking in bacterial cell walls

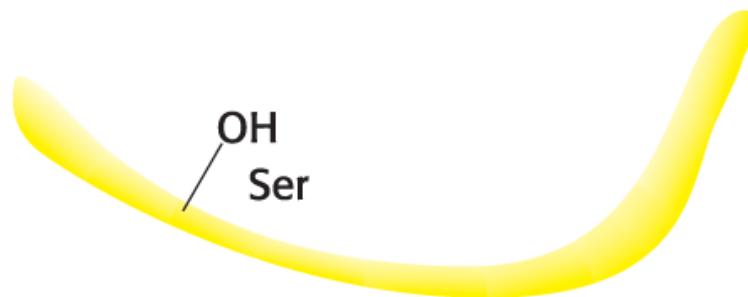


Enzyme transpeptidase facilitating the cross-links formation

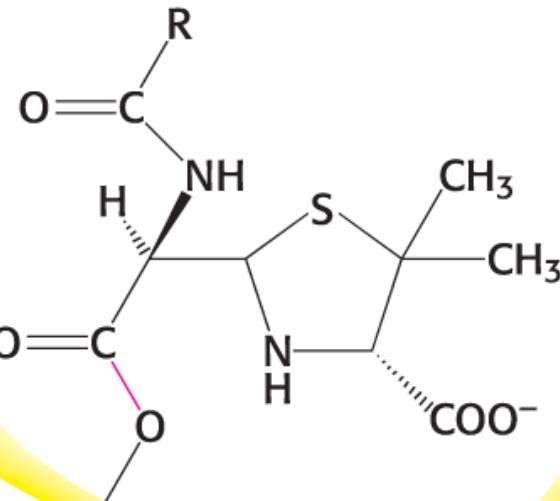
# Penicillin is an example of an irreversible inhibitor

- A penicillinoyl-serine derivative is formed which is inactive and very stable.
- Thus, the antibiotic penicillin is a **suicide inhibitor** of the enzyme that synthesizes bacterial cell walls.

Penicillin reacts with the enzyme transpeptidase to form an inactive complex, which is indefinitely stable.



Glycopeptide transpeptidase



Penicilloyl-enzyme complex (enzymatically inactive)

## Quick Quiz 2

Transition state analogs:

- A. have lower free energy than the transition states that they mimic.
- B. would inhibit a catalytic antibody if that antibody was first raised against the transition-state analog.
- C. can be sources of insight into catalytic mechanisms.
- D. All of the above.
- E. B & C

# Chymotrypsin and its catalytic activity

- Chymotrypsin is a proteolytic enzyme secreted by the pancreas that hydrolyzes peptide bonds selectively on the carboxyl side of large hydrophobic amino acids.
- In the process of catalysis, serine 195 becomes a nucleophile that attacks the carbonyl group of protein substrate.
- The group-specific reagent diisopropylphosphofluoridate (DIPF) modifies only serine 195, one of 28 serine residues in chymotrypsin, and inhibits the enzyme

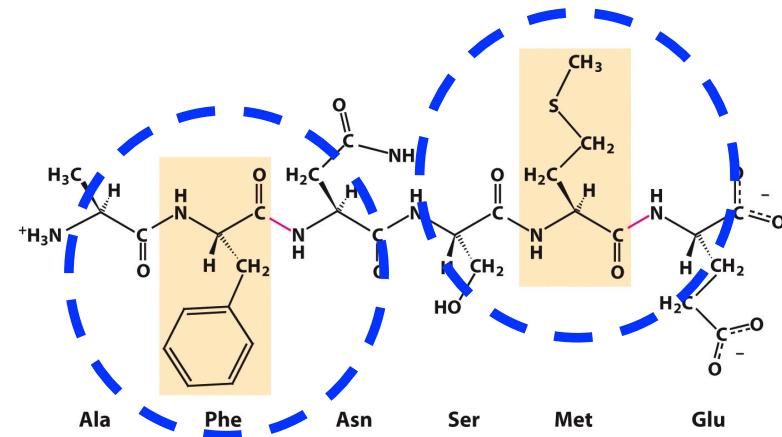
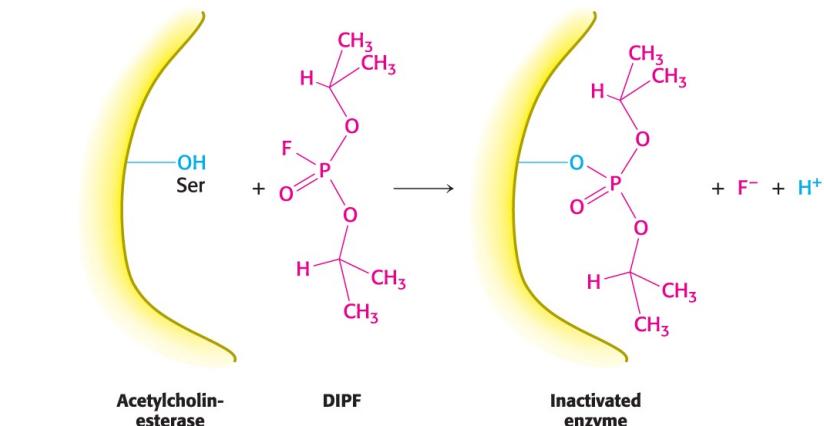
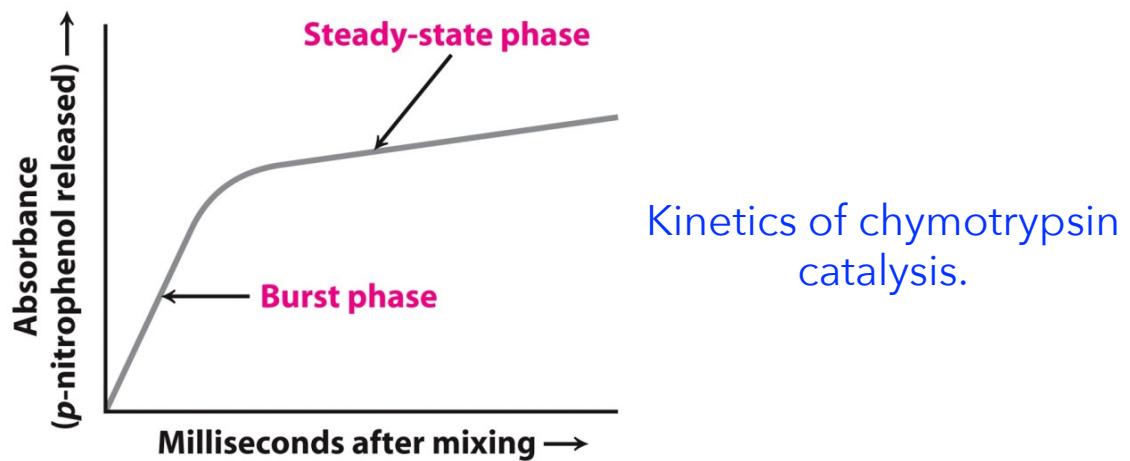
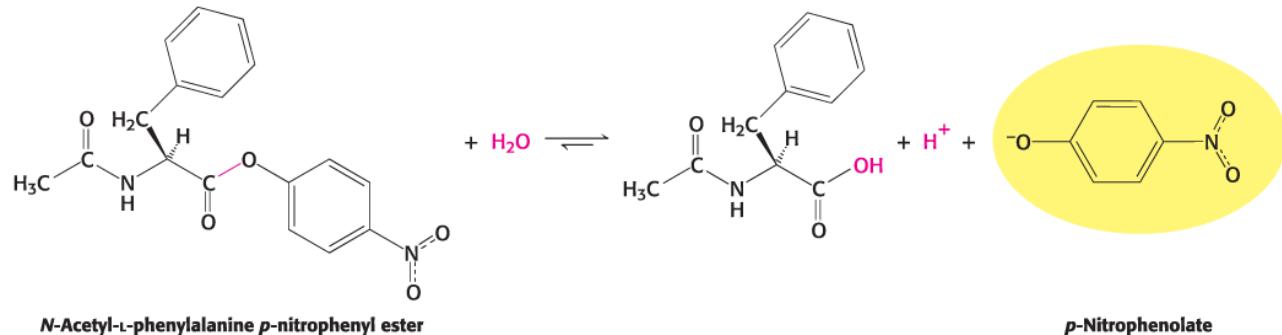


Figure 8.20  
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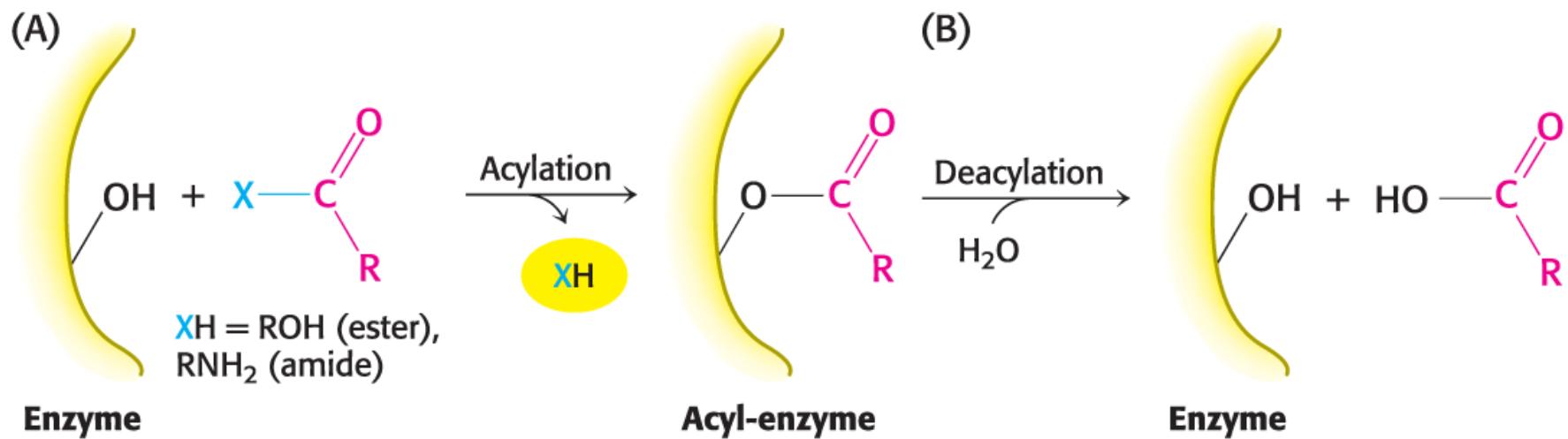


# Chymotrypsin Action...proceeds in two steps

- Chromogenic substrates generate colored products, facilitating enzymatic studies.
- N-Acetyl-L-phenylalanine p-nitrophenyl ester is a *chromogenic substrate* for chymotrypsin.
- Studies with the chromogenic substrate reveal that catalysis by chymotrypsin occurs in two stages: a rapid step (pre-steady state) and a slower step (steady state).



# Chymotrypsin Action...proceeds in two steps



An example of covalent catalysis. Chymotrypsin hydrolysis starts with acylation to form the covalently-bound acyl-enzyme intermediate, followed by deacylation.

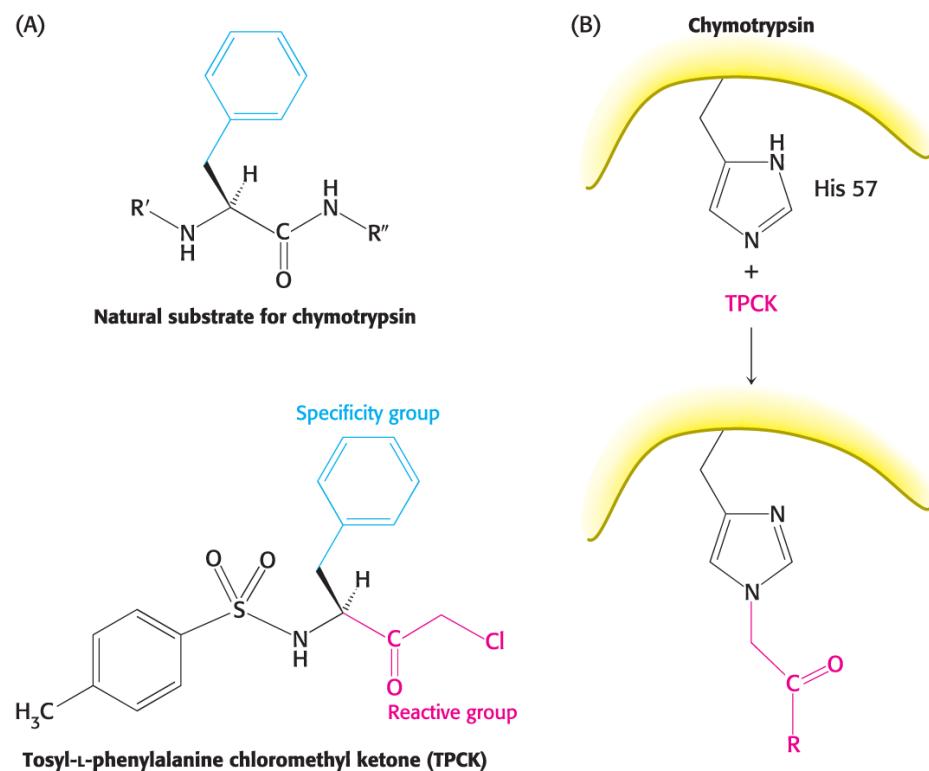
## Quick Quiz 3

N-acetyl-L-phenylalanine p-nitrophenyl ester is useful for the study of chymotrypsin because \_\_\_\_.

- A. it is a fluorescent substrate of the enzyme
- B. it is a chromogenic substrate of the enzyme
- C. it is a competitive inhibitor of the enzyme
- D. it is an affinity label for the enzyme
- E. C & D

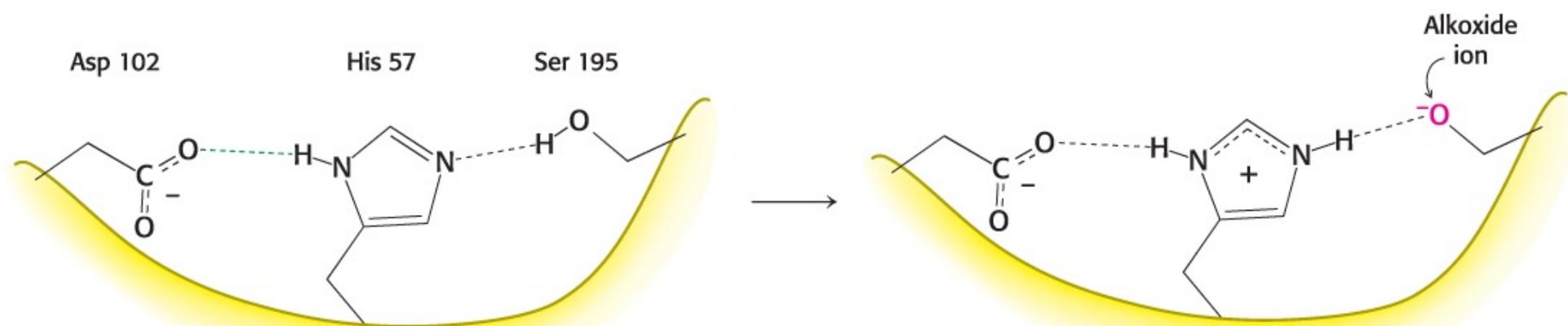
# Chymotrypsin Action

- Second residue important to chymotrypsin's catalysis was identified via **affinity labelling**.
  - molecule should specifically bind to the active site of the enzyme
  - should form a stable covalent bond with a group on the enzyme that is in proximity
- The affinity label tosyl-L-phenylalanine chloromethyl ketone (TPCK) covalently modifies histidine 57 in chymotrypsin, leading to a loss of enzyme activity.



**Affinity labels** or **substrate analogs** are structurally similar to the enzyme's substrate but inhibit the enzyme by covalently modifying an amino acid in the active site.

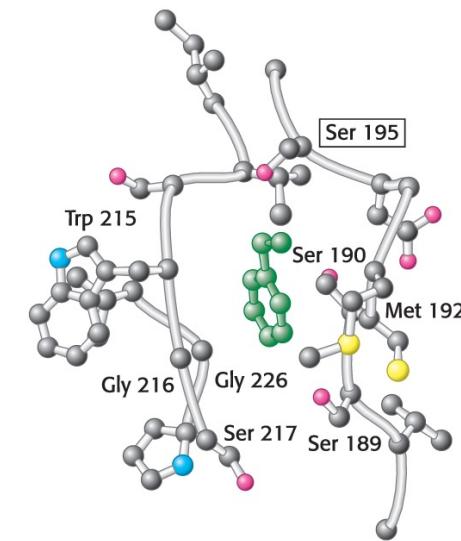
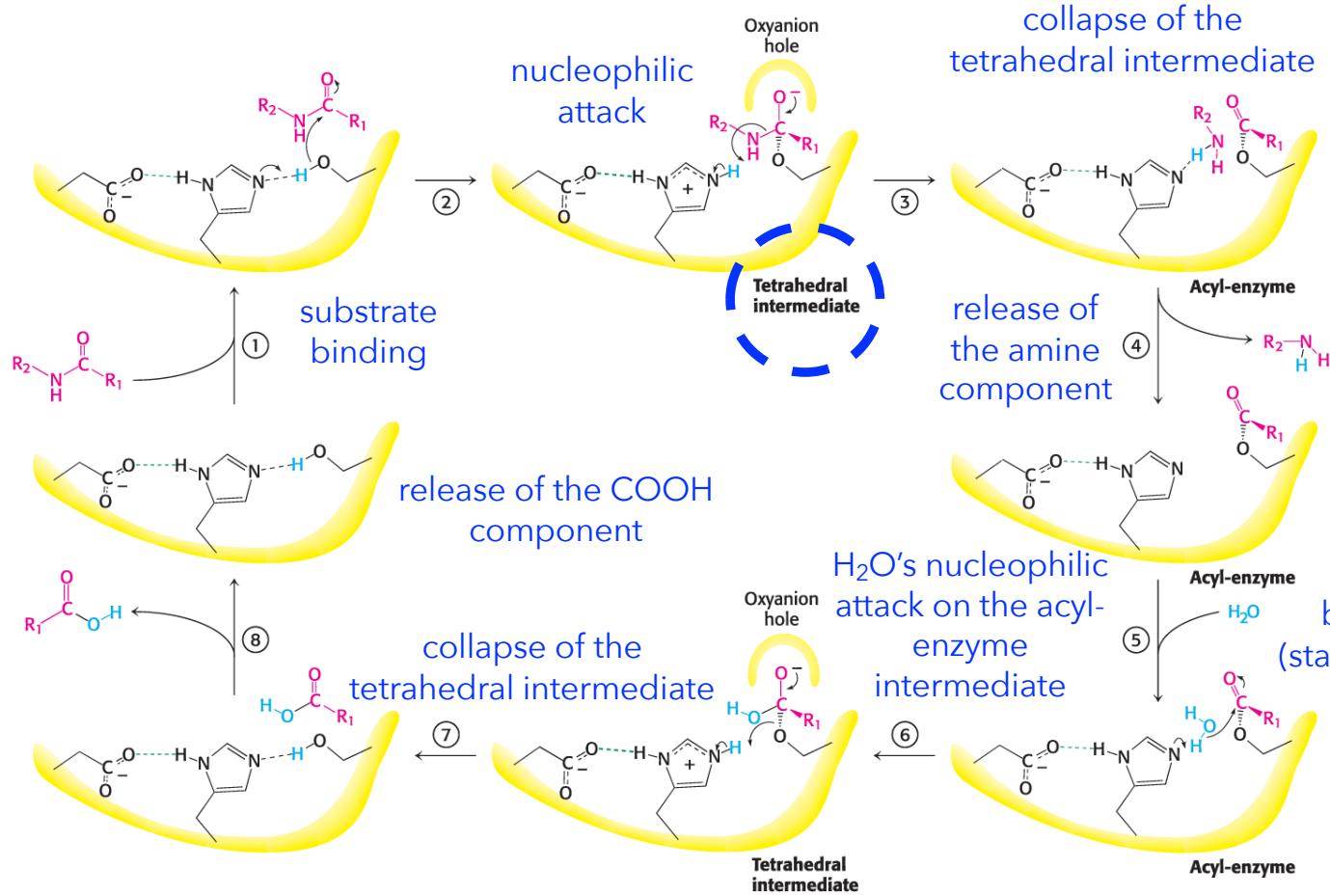
## Serine is Part of a Catalytic Triad That Includes Histidine and Aspartic Acid



### The Catalytic Triad

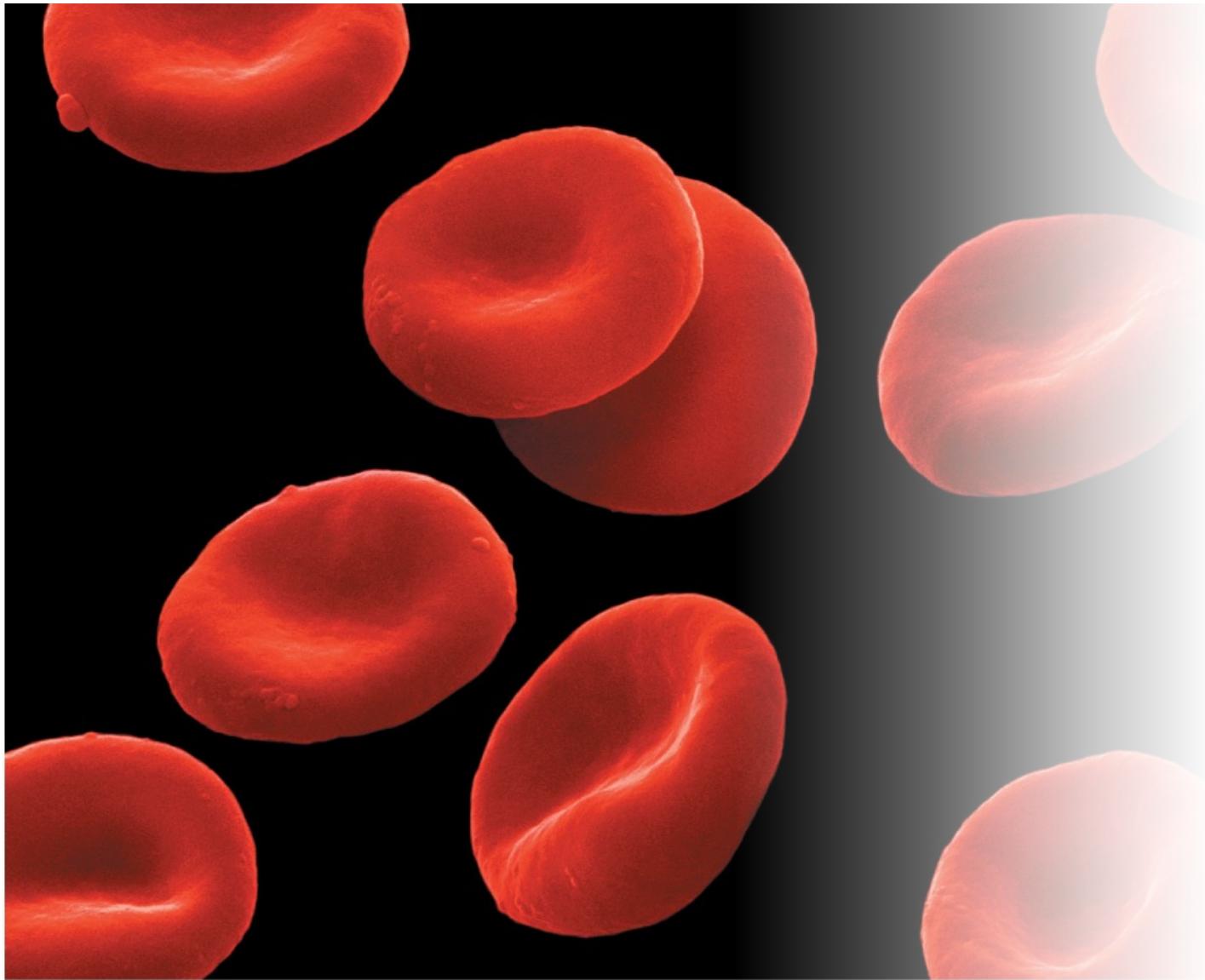
- His 57 polarizes Ser 195's hydroxyl group and accepts its  $\text{H}^+$  (His acts as a general base catalyst)
- more reactive alkoxide is generated (making Ser 195 a more potent nucleophile)
- Asp 102 helps with the orientation

# Peptide Hydrolysis by Chymotrypsin



The specificity pocket of chymotrypsin.

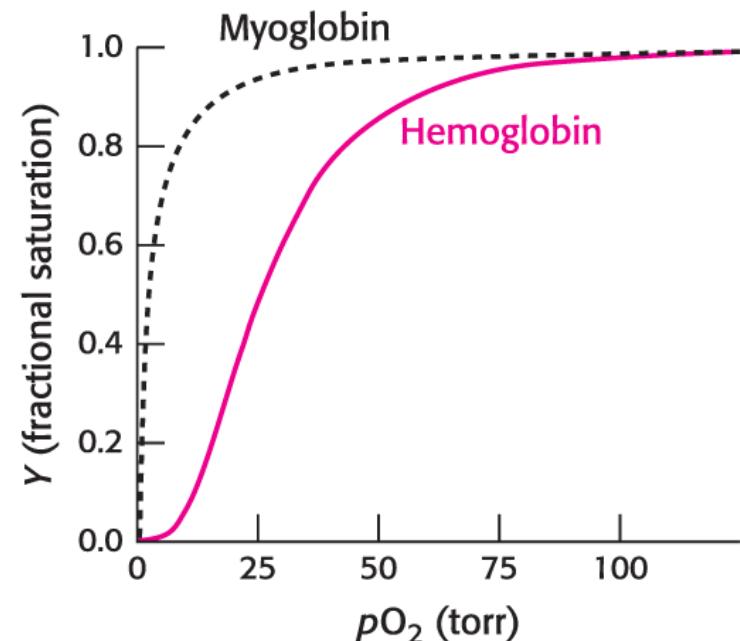
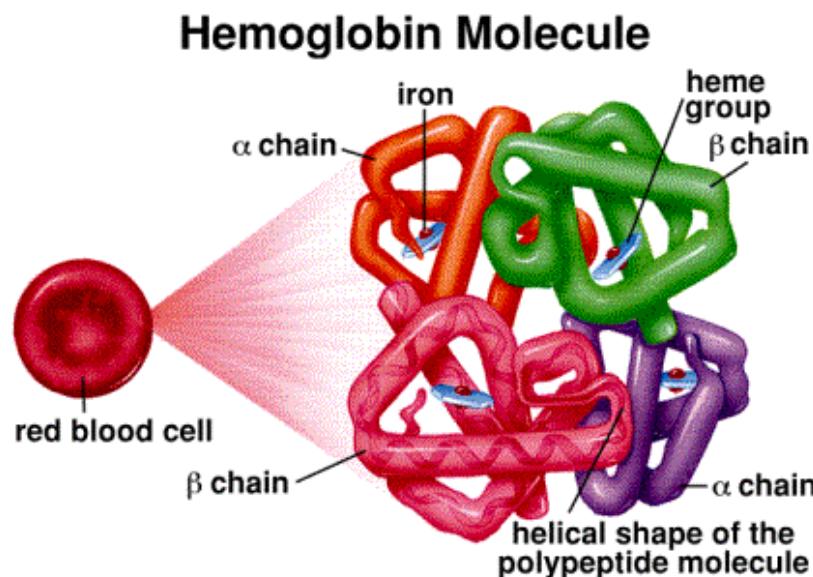
➤ covalent and acid-base catalyst



**Hemoglobin –  
an “honorary  
enzyme”**

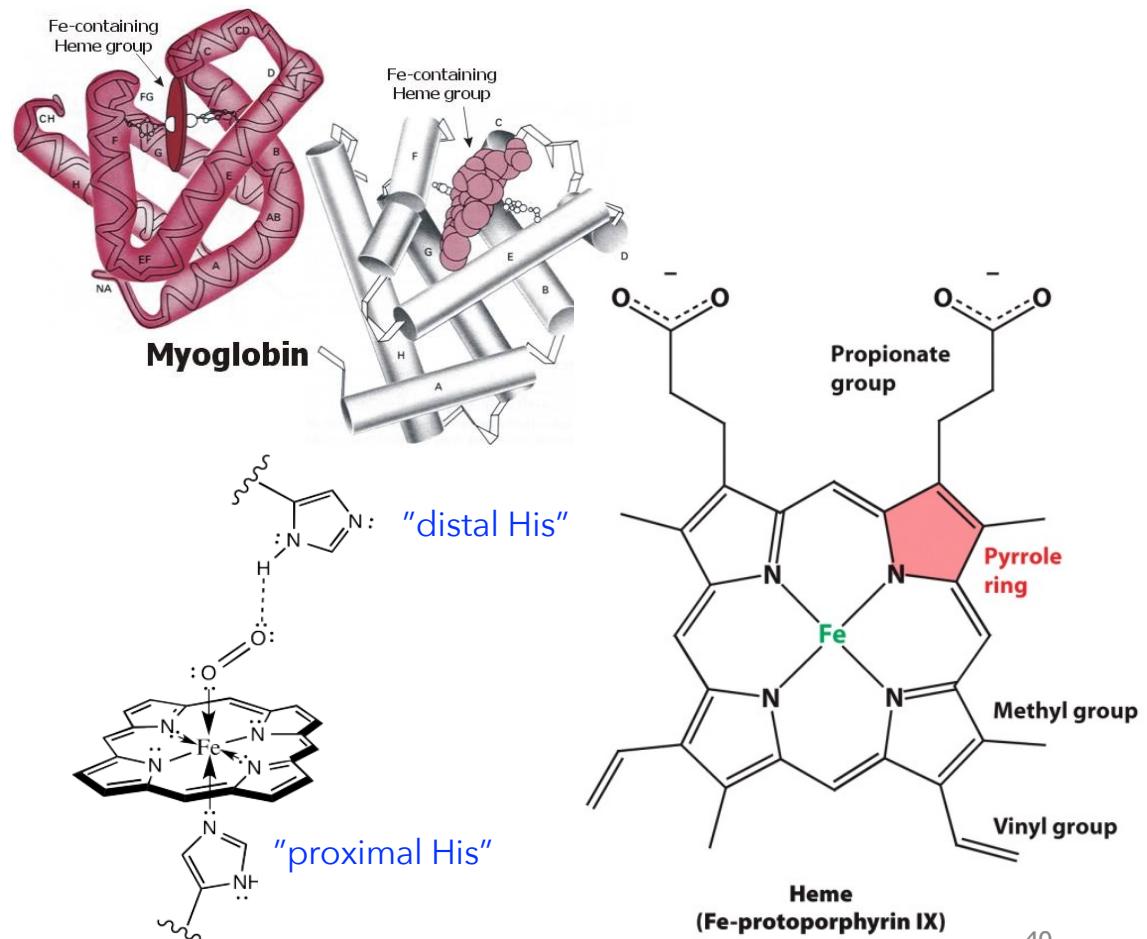
# Hemoglobin displays cooperative behavior

- Hemoglobin is a red blood cell protein that carries  $O_2$  from the lungs to the tissues.
- Hemoglobin is an **allosteric protein** that displays cooperativity in  $O_2$  binding and release.
- Myoglobin binds  $O_2$  in muscle cells. The binding of  $O_2$  by myoglobin is not cooperative.
- Oxygen binding is measured as a function of the partial pressure of oxygen ( $pO_2$ ).



# Myoglobin and Hemoglobin Bind Oxygen in Heme Groups

- Myoglobin is a single polypeptide chain consisting mainly of  $\alpha$ -helices arranged to form a globular structure.
- Myoglobin, like hemoglobin, binds oxygen at a heme, a bound prosthetic group.
- Iron can form two additional bonds, called the fifth and sixth coordination sites.
- The fifth coordination site is occupied by an imidazole ring of a histidine called the proximal histidine.
- The sixth coordination site binds oxygen.
- Upon oxygen binding, the iron moves into the plane of the protoporphyrin ring.



# Myoglobin and Hemoglobin Bind Oxygen in Heme Groups

- The magnetic properties of the heme iron change when it moves into the plane of the protoporphyrin ring.
- Functional magnetic resonance imaging (fMRI) can distinguish the relative amounts of oxy- and deoxyhemoglobin.
- Functional magnetic resonance can be used to monitor activity in specific regions of the brain by measuring the increase in oxyhemoglobin (more active region is rich in oxy form).

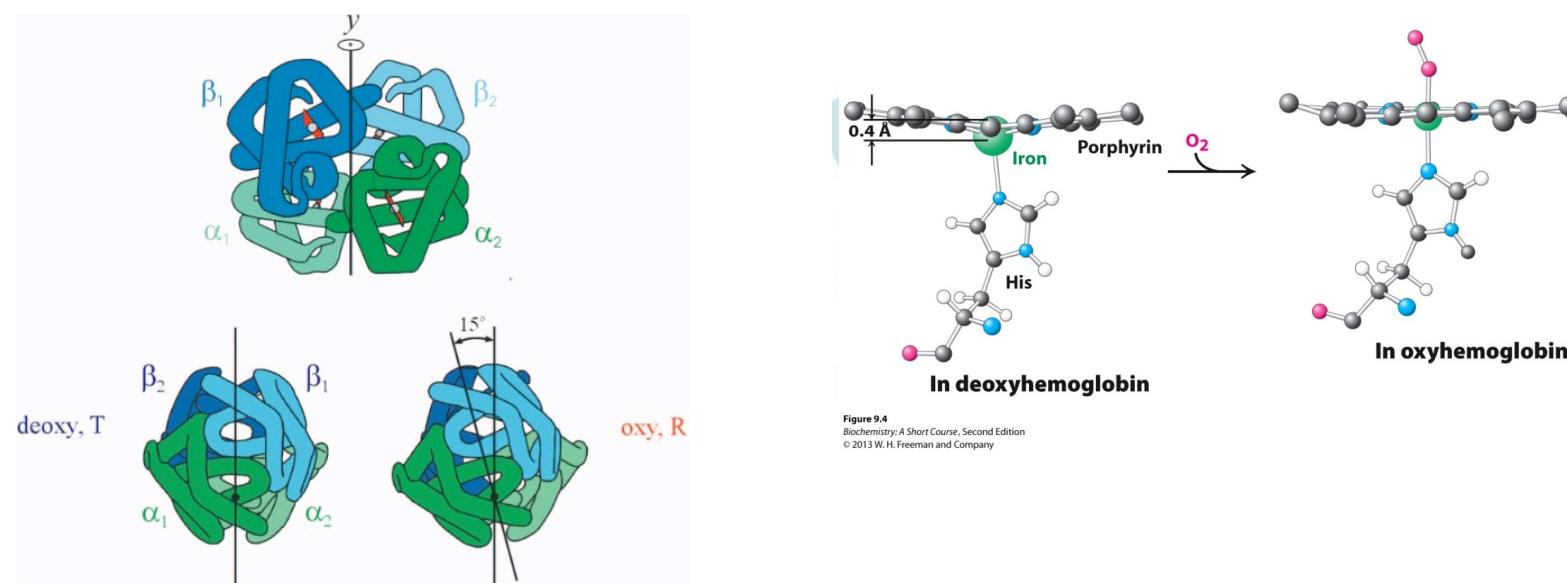
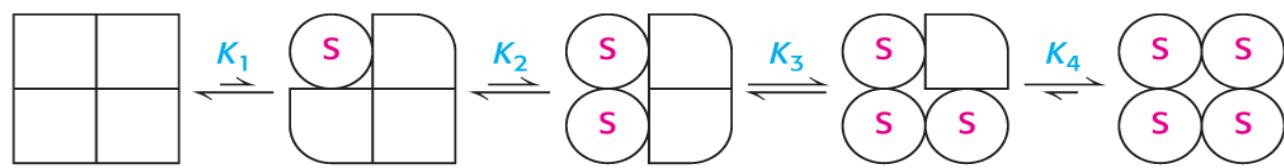
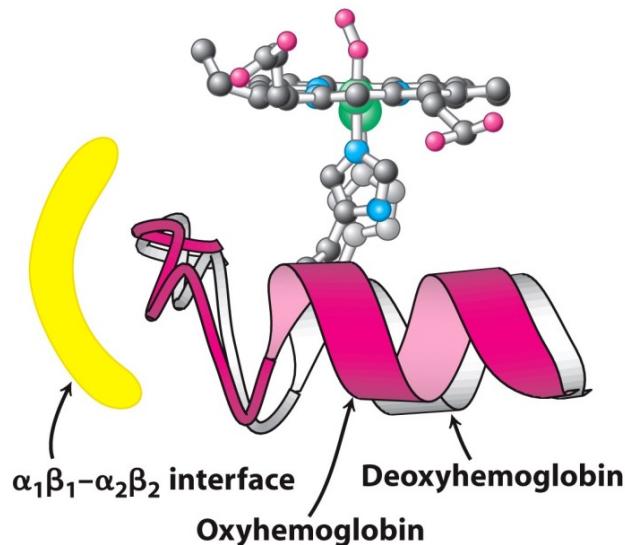


Figure 9.4  
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# Hemoglobin Binds Oxygen Cooperatively

- The transition from deoxyhemoglobin (T state) to oxyhemoglobin (R state) occurs upon oxygen binding.
- The iron ion moves into the plane of the heme when oxygen binds. The proximal histidine, which is a member of an  $\alpha$ -helix, moves with the iron.
- The resulting structural change is communicated to the other subunits so that the two  $\alpha\beta$  dimers rotate with respect to one another, resulting in the formation of the R state.



Imagine "S" to be O<sub>2</sub>. The binding of one O<sub>2</sub> molecule causes a change in conformation allowing the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> O<sub>2</sub> molecules to bind more efficiently to hemoglobin.

Figure 9.7  
Biochemistry: A Short Course, Second Edition  
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# An Allosteric Regulator Determines the Oxygen Affinity of Hemoglobin

- 2,3-Bisphosphoglycerate (2,3-BPG) stabilizes the T state of hemoglobin and thus facilitates the release of oxygen.
- 2,3-BPG binds to a pocket in the hemoglobin tetramer that exists only when hemoglobin is in the T state.

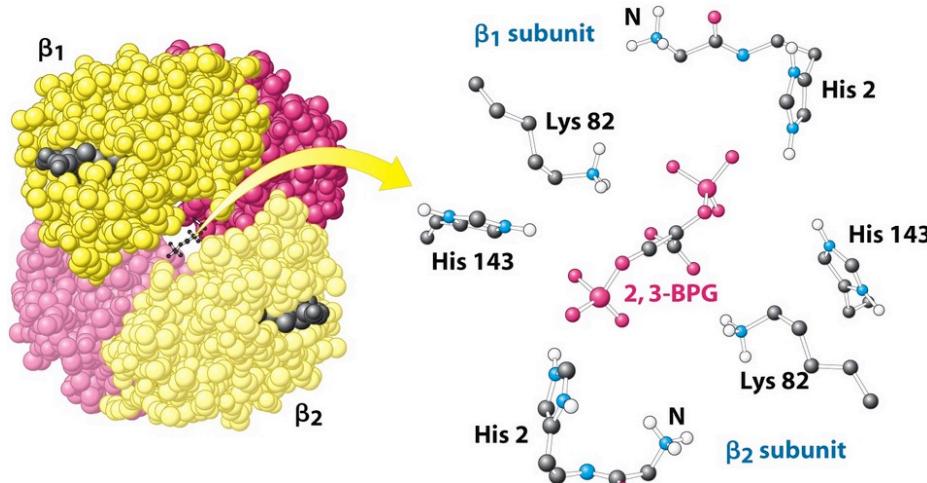
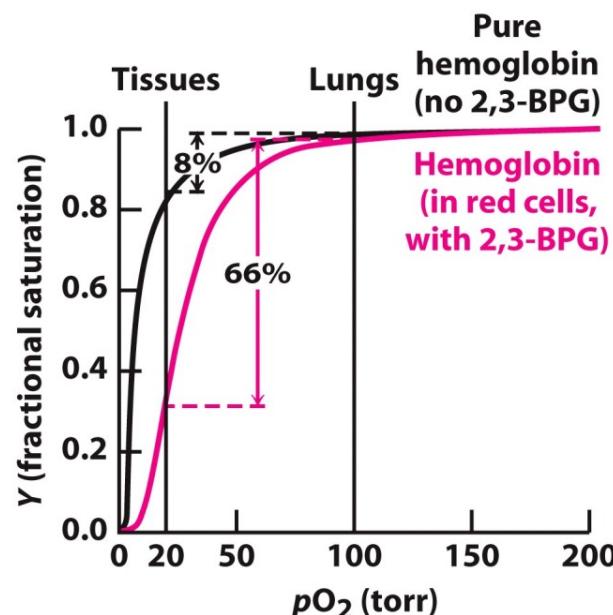
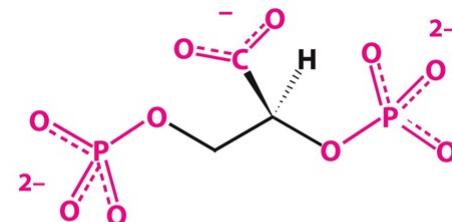


Figure 7.17  
Biochemistry, Seventh Edition  
© 2012 W. H. Freeman and Company



# Hemoglobin Adaptations Allow Oxygen Transport in Response to Environmental Needs

- Fetal hemoglobin must bind oxygen when the mother's hemoglobin is releasing oxygen.
- In fetal hemoglobin, the  $\alpha$  chain is replaced with a  $\gamma$  chain.
- The fetal  $\alpha_2\gamma_2$  hemoglobin does not bind 2,3-BPG as well as adult hemoglobin. The reduced affinity for 2,3-BPG results in fetal hemoglobin having a higher affinity for oxygen, binding oxygen when the mother's hemoglobin is releasing oxygen.
- The bar-headed goose can fly over Mt. Everest, where the oxygen concentration is low.
- Changes in hemoglobin that facilitate the formation of the R state may account in part for this remarkable ability.

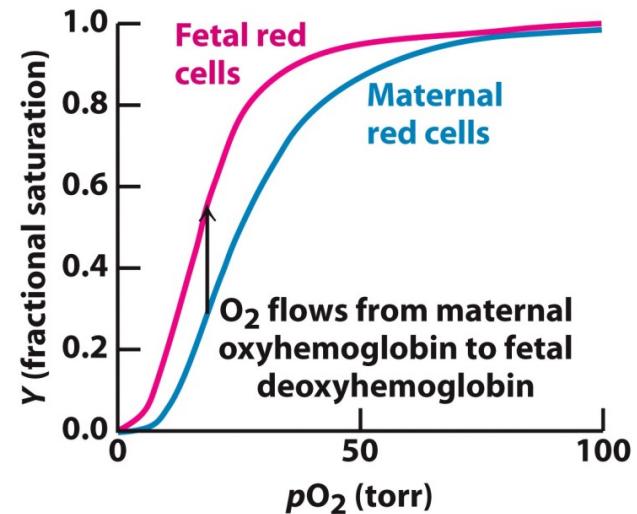
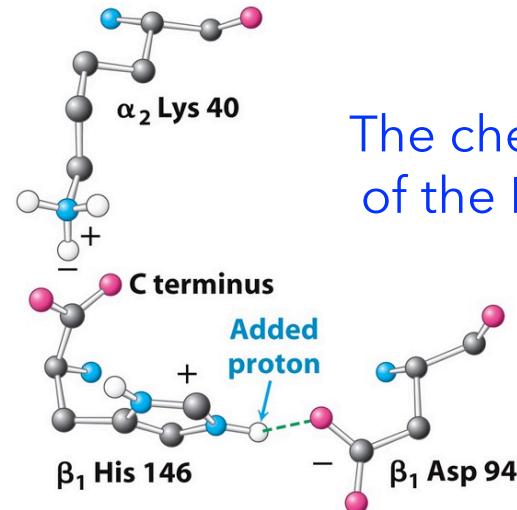
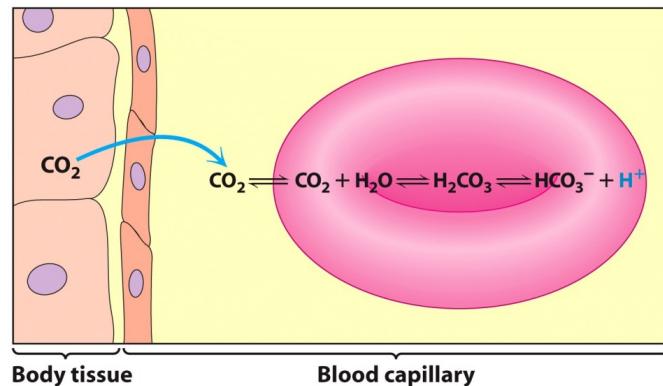
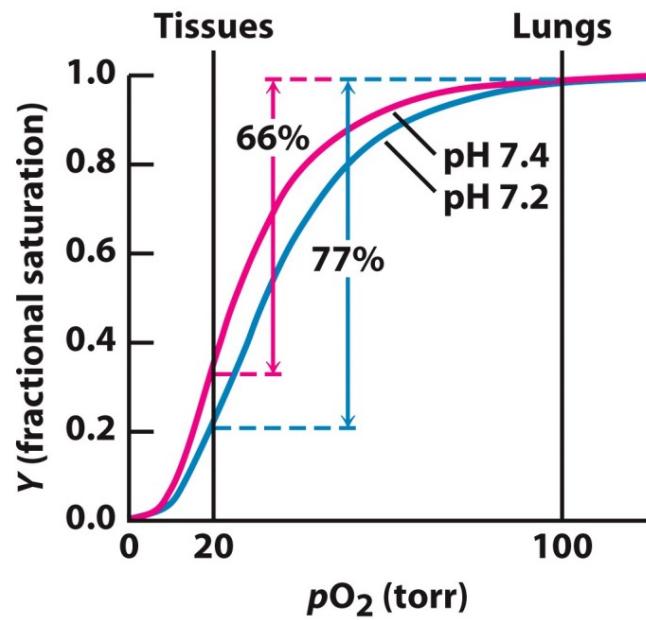


Figure 14.13  
Anatomical & Physiological Adaptations to High Altitude

# $\text{H}^+$ and $\text{CO}_2$ Promote the Release of Oxygen

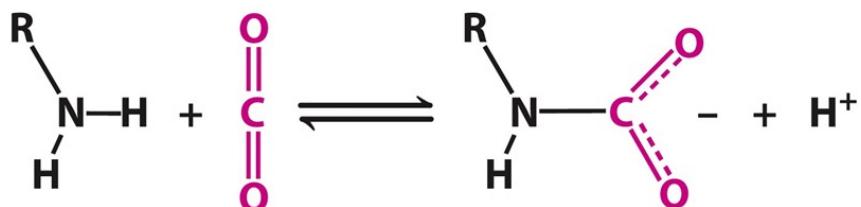
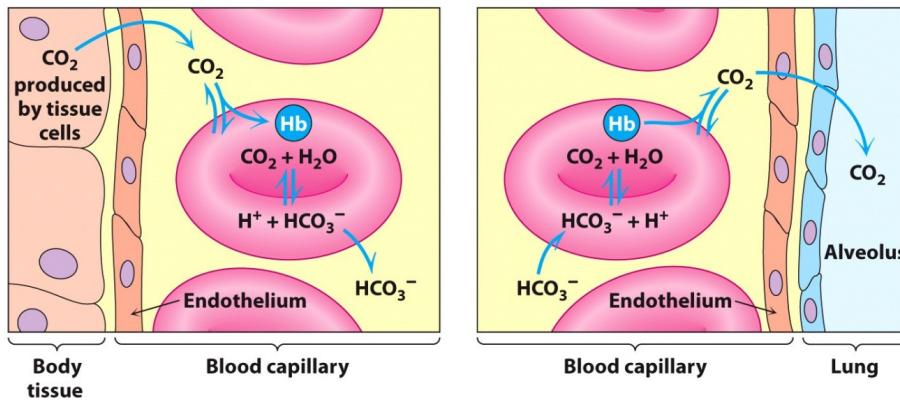
- Carbon dioxide and  $\text{H}^+$ , produced by actively respiring tissues, enhance oxygen release by hemoglobin.
- The stimulation of oxygen release by carbon dioxide and  $\text{H}^+$  is called the **Bohr effect**.



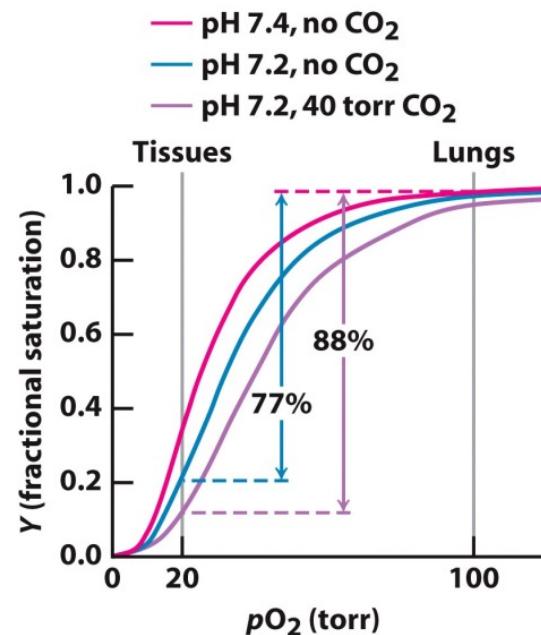
The chemical basis of the Bohr effect.

# $\text{H}^+$ and $\text{CO}_2$ Promote the Release of Oxygen

- Low pH allows the formation of ionic interactions that stabilize the T state of hemoglobin, enhancing oxygen release.
- Carbon dioxide reacts with terminal amino groups to form negatively charged carbamate groups. The carbamate forms salt bridges that stabilize the T state.
- Carbon dioxide and  $\text{H}^+$  are heterotropic regulators of oxygen binding by hemoglobin.



**Carbamate**



$\text{CO}_2$  decreases the affinity of hemoglobin for  $\text{O}_2$  due to a decrease in pH  $\rightarrow$  more efficient  $\text{O}_2$  transport from tissues to lungs.

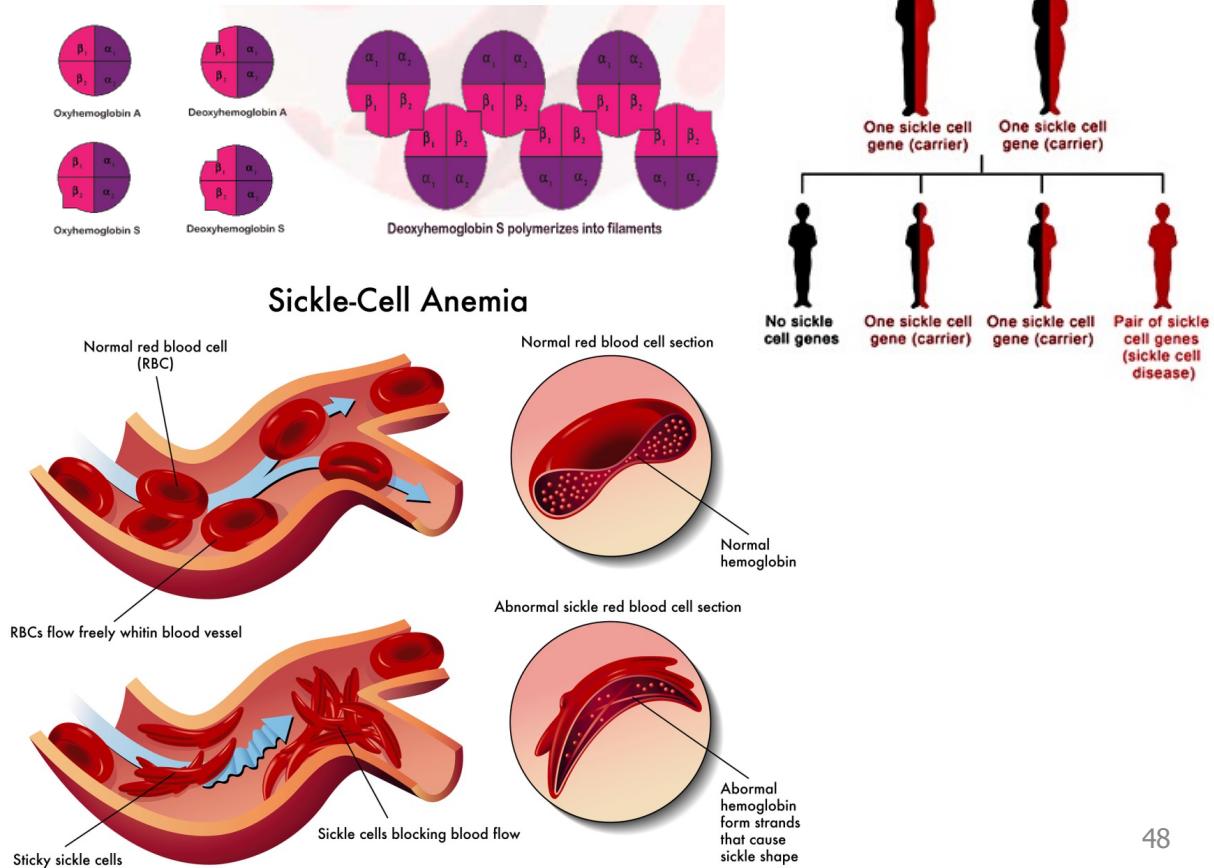
## Quick Quiz 4

The oxygen affinity of hemoglobin \_\_\_\_\_ when pH is slightly \_\_\_\_\_ and CO<sub>2</sub> concentration is \_\_\_\_\_.

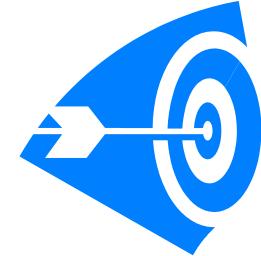
- A. increases; lowered; decreased
- B. is unaltered; lowered; lowered
- C. increases; raised; increased
- D. decreases; lowered; increased
- E. decreases; lowered; lowered

# Mutations in Genes Encoding Hemoglobin Subunits Can Result in Disease

- Sickle-cell anemia is a genetic disease caused by a mutation resulting in the substitution of valine for glutamate at position 6 of the  $\beta$  chains.
- Sickle-cell anemia can be fatal when both alleles of the  $\beta$  chain are mutated.
- In sickle cell trait, one allele is mutated and one is normal. Such individuals are asymptomatic.



# Assigned Problems



<b>Chapter</b>	Tymochko, Berg, Stryer, Biochemistry, 2 <sup>nd</sup> Edition,	<b>Chapter</b>	Tymochko, Berg, Stryer, Biochemistry, 2 <sup>nd</sup> Edition,
8	2, 3, 5, 7, 8, 9, 14	9	1, 2, 3, 8, 13, 15, 16, 17, 22
<b>Chapter</b>	Tymochko, Berg, Stryer, Biochemistry, 3 <sup>rd</sup> Edition, 4 <sup>th</sup> Edition (second line)	<b>Chapter</b>	Tymochko, Berg, Stryer, Biochemistry, 3 <sup>rd</sup> Edition, 4 <sup>th</sup> Edition (second line)
8	2, 3, 5, 7, 8, 9, 16 2, 3, 5, 7, 8, 9, 16	9	1, 2, 3, 8, 13, 16, 17, 18, 23 1, 2, 3, 8, 13, 16, 17, 18, 23