5 Protein Function

Proteins Function by Interacting Dynamically with Other Molecules

- two types of interactions:
  - protein acting as a reaction catalyst, or **enzyme**, alters the chemical configuration or composition of a bound molecule
  - neither the chemical configuration nor the composition of the bound molecule is changed

P1 Principle 1 (1 of 4)

The functions of many proteins involve the reversible binding of other molecules. A molecule bound reversibly by a protein is called a **ligand**. A ligand may be any kind of molecule, including another protein. The transient nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic circumstances.

P2 Principle 2 (1 of 4)

A ligand binds a protein at a binding site that is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. The interaction is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few types. A given protein may have separate **binding sites** for several different ligands. These specific molecular interactions are crucial in maintaining the high degree of order in a living system.

P3 Principle 3 (1 of 2)

Proteins are flexible. Changes in conformation may be subtle, reflecting molecular vibrations and small movements of amino acid residues throughout the protein. Changes in conformation may also be more dramatic, with major segments of the protein structure moving as much as several nanometers. Specific conformational changes are frequently essential to a protein's function.

P4 Principle 4 (1 of 2)

The binding of a protein and a ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**.

P5 Principle 5 (1 of 4)

In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.

P6 Principle 6 (1 of 3)

Interactions between ligands and proteins may be regulated.

5.1 Reversible Binding of a Protein to a Ligand: Oxygen-Binding Proteins

P1 Principle 1 (2 of 4)

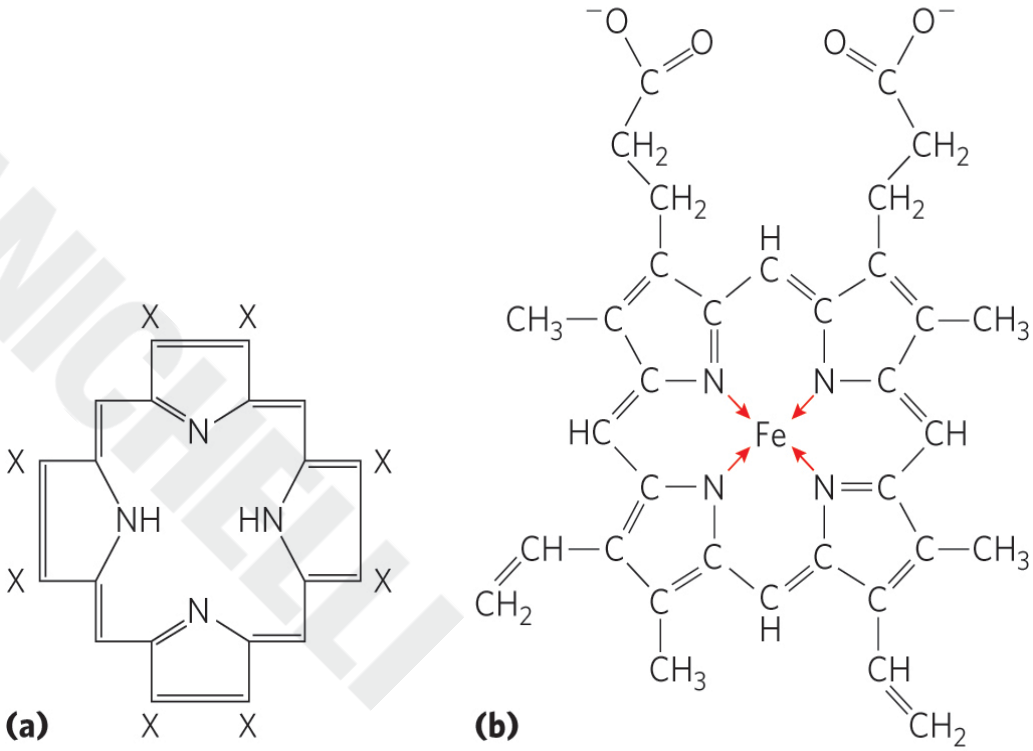
The functions of many proteins involve the reversible binding of other molecules. A molecule bound reversibly by a protein is called a **ligand**. A ligand may be any kind of molecule, including another protein. The transient nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic circumstances.

Oxygen Can Bind to a Heme Prosthetic Group

- oxygen:
  - poorly soluble in aqueous solutions
  - diffusion through tissues is ineffective over large distances
  - transition metals have strong tendency to bind (iron, copper)

Heme Prosthetic Group

- heme = protein-bound prosthetic group
  - present in myoglobin and **hemoglobin**
  - consists of a complex organic ring structure, **protoporphyrin**, with a bound Fe^{2+} atom



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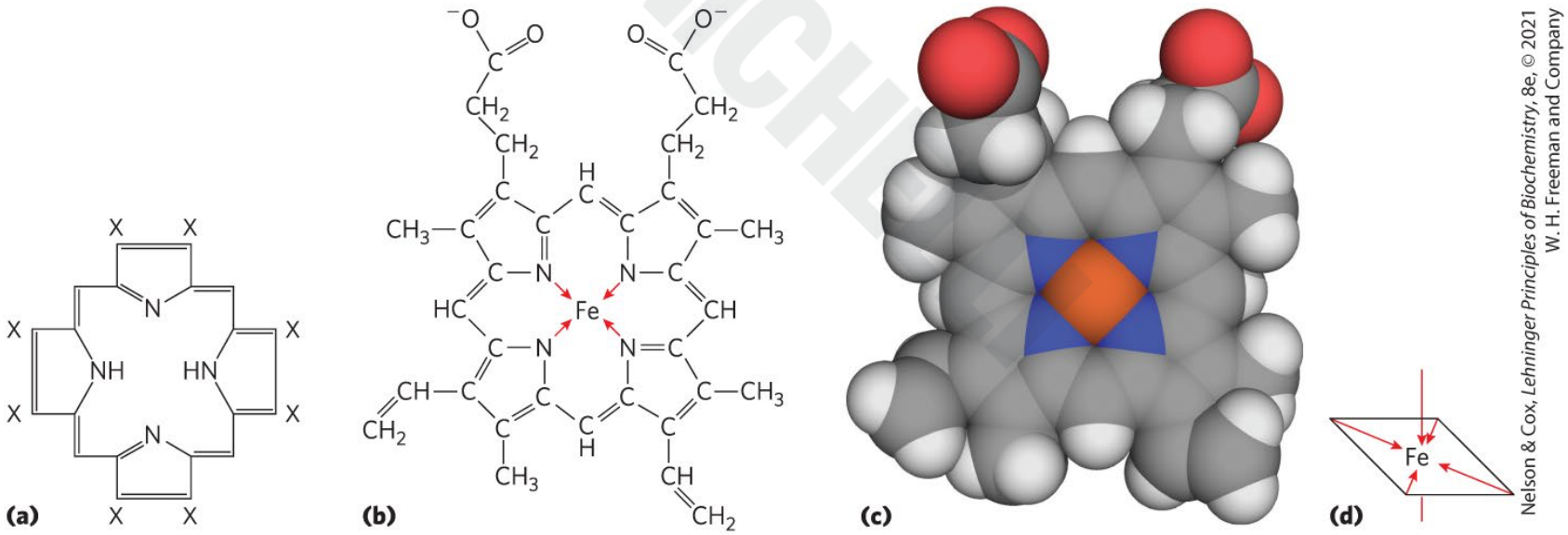
Coordination Bonds of Iron

- six coordination bonds:
  - four to nitrogen atoms in the flat **porphyrin ring**
  - two perpendicular to the porphyrin

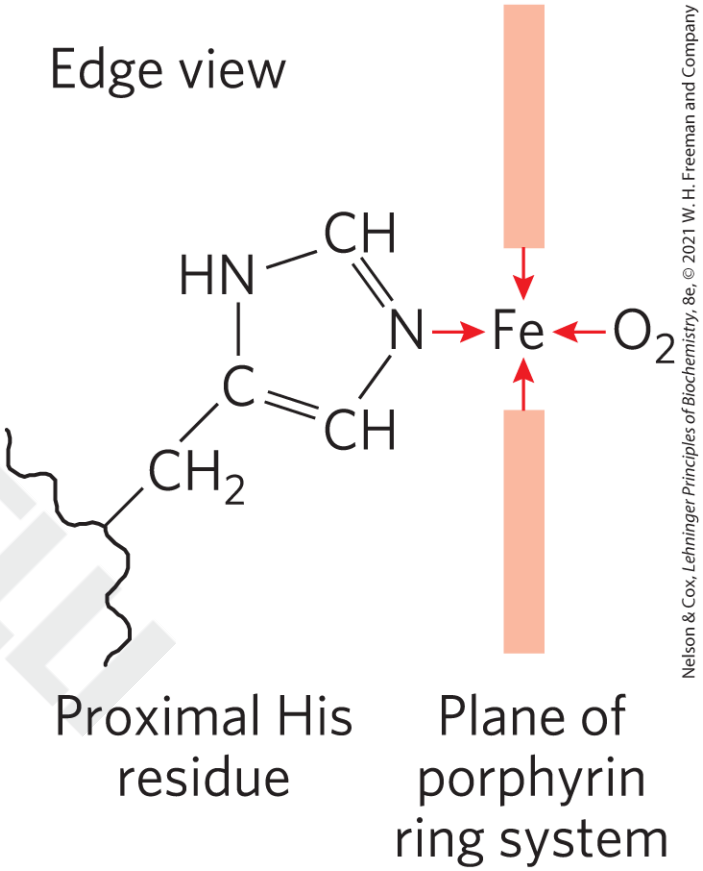


P2 Principle 2 (2 of 4)

A ligand binds a protein at a binding site that is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. The interaction is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few types. A given protein may have separate **binding sites** for several different ligands. These specific molecular interactions are crucial in maintaining the high degree of order in a living system.

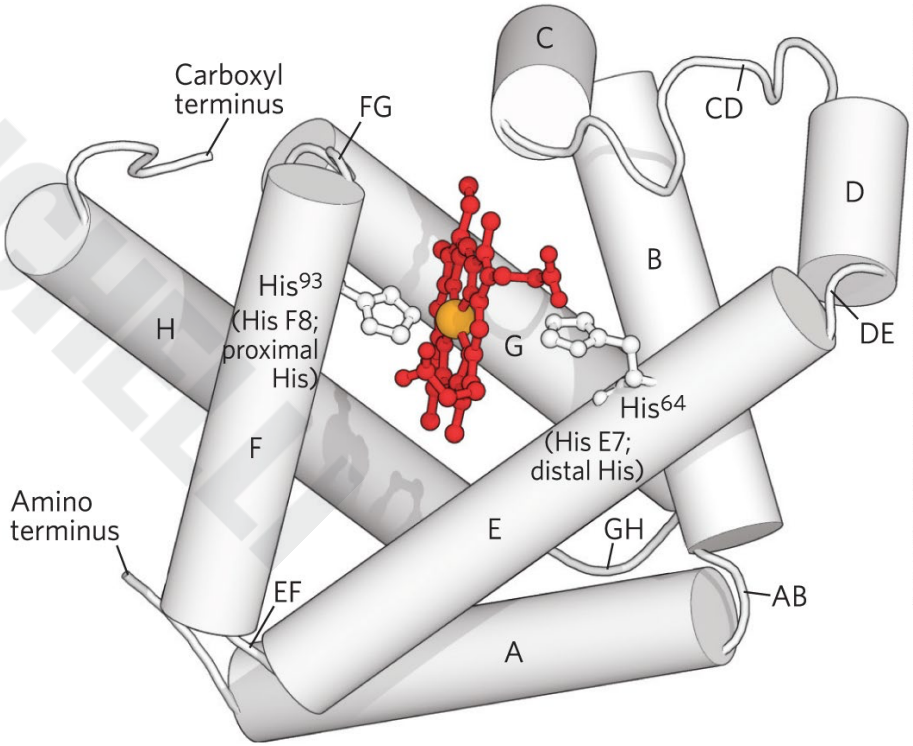
Perpendicular Coordination Bonds

- two perpendicular coordination bonds:
  - one is occupied by a side-chain nitrogen of a highly conserved **proximal His** residue
  - one is the binding site for molecular oxygen (O_2)
    - Fe^{2+} binds O_2 reversibly
    - Fe^{3+} does not bind O_2



Globins Are a Family of Oxygen-Binding Proteins

- **globins** = widespread protein family
  - highly conserved tertiary structure: eight *α*-helical segments connected by bends (**globin fold**)
  - most function in O_2 transport or storage



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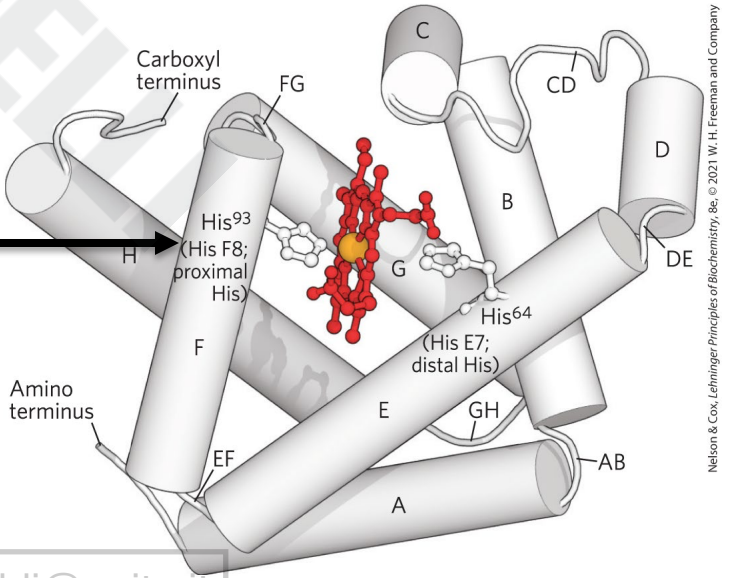
Types of Globins

- four types in humans and other mammals:
  - myoglobin = monomeric, facilitates O_2 diffusion in muscle tissue
  - hemoglobin = tetrameric, responsible for O_2 transport in the bloodstream
  - neuroglobin = monomeric, expressed largely in neurons to protect the brain from low O_2 or restricted blood supply
  - cytoglobin = monomeric, regulates levels of nitric oxide, a localized signal for muscle relaxation

Myoglobin Has a Single Binding Site for Oxygen

- myoglobin:
  - 153 residues + one molecule of heme
  - bends named after the *α*-helical segments they connect

- His^{93} = ninety-third reside from the amino terminal end
- His F8 = eighth residue in *α* helix F



Protein-Ligand Interactions Can Be Described Quantitively

- a simple **equilibrium expression** describes the reversible binding of a protein (P) to a ligand (L):

$$P + L \rightleftharpoons PL \quad (5\text{-}1)$$

Association Constant

- **association constant (K_a)** = provides a measure of the affinity of the ligand L for the protein
  - higher K_a = higher affinity
  - equivalent to the ratio of the rates of the forward (association) and the reverse (dissociation) reactions that form the PL complex

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d} \quad (5\text{-}2)$$

[L] Remains Constant

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d} \quad (5\text{-}2)$$

$$K_a[L] = \frac{[PL]}{[P]} \quad (5\text{-}3)$$

- when [L] >>> [ligand-binding sites], the binding of the ligand by the protein does not appreciably change [L]

Binding Equilibrium

$$Y = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[PL]}{[PL] + [P]} \qquad (5\text{-}4)$$

substituting $K_a[L][P]$ for $[PL]$:

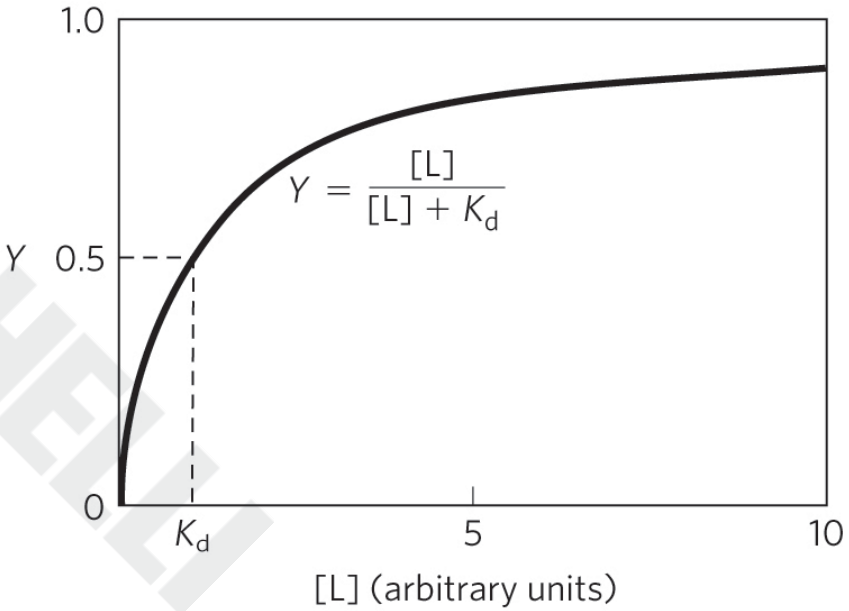
$$Y = \frac{K_a[L][P]}{K_a[L][P] + [P]} = \frac{K_a[L]}{K_a[L] + 1} = \frac{[L]}{[L] + \frac{1}{K_a}} \qquad (5\text{-}5)$$

$x = y/(y + z)$ describes a hyperbola

Graphical Representations of Ligand Binding

$$Y = \frac{[L]}{[L] + \frac{1}{K_a}} \qquad (5\text{-}5)$$

- [L] at which ½ of the available ligand-binding sites are occupied (Y = 0.5) corresponds to $1/K_a$



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Dissociation Constant

- **dissociation constant (K_d)** = reciprocal of K_a
  - equilibrium constant for the release of ligand
  - lower K_d = higher affinity

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_d}{k_a} \qquad (5\text{-}6)$$

$$[PL] = \frac{[P][L]}{K_d} \qquad (5\text{-}7)$$

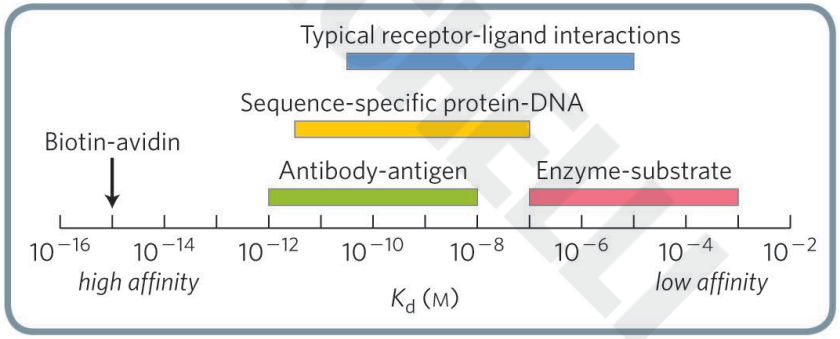
$$Y = \frac{[L]}{[L] + K_d} \qquad (5\text{-}8)$$

- when [L] = K_d, ½ of the ligand-binding sites are occupied

Representative K_d Values

TABLE 5-1 Protein Dissociation Constants: Some Examples and Range

Protein	Ligand	K_d (M)[a]
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)[b]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)[c]	Ca^{2+}	3×10^{-6}
		2×10^{-5}



Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

[a]A reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in the solution's salt concentration, pH, or other variables.

[b]This immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

[c]Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

Binding of O_2 to Myoglobin

- substituting the $[O_2]$ for [L]:

$$Y = \frac{[O_2]}{[O_2] + K_d} \qquad (5\text{-}9)$$

- K_d equals the $[O_2]$ at which ½ of the available ligand-binding sites are occupied, or $[O_2]_{0.5}$:

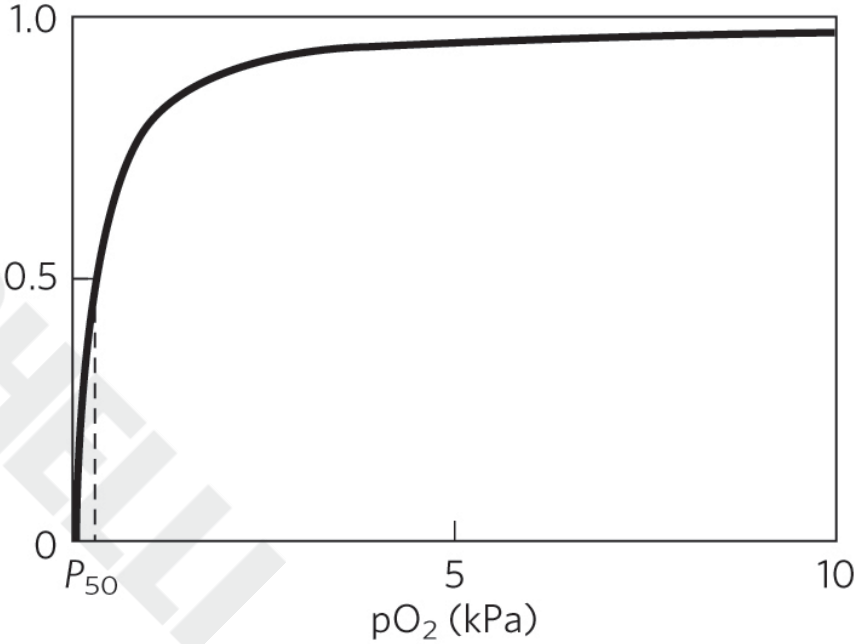
$$Y = \frac{[O_2]}{[O_2] + [O_2]_{0.5}} \qquad (5\text{-}10)$$

Partial Pressure of O_2

- partial pressure of O_2 (pO_2) is easier to measure than $[O_2]$
- defining the partial pressure of oxygen at $[O_2]_{0.5}$ as P_{50}:

$$Y = \frac{pO_2}{pO_2 + P_{50}}$$

(5-11)



(b)

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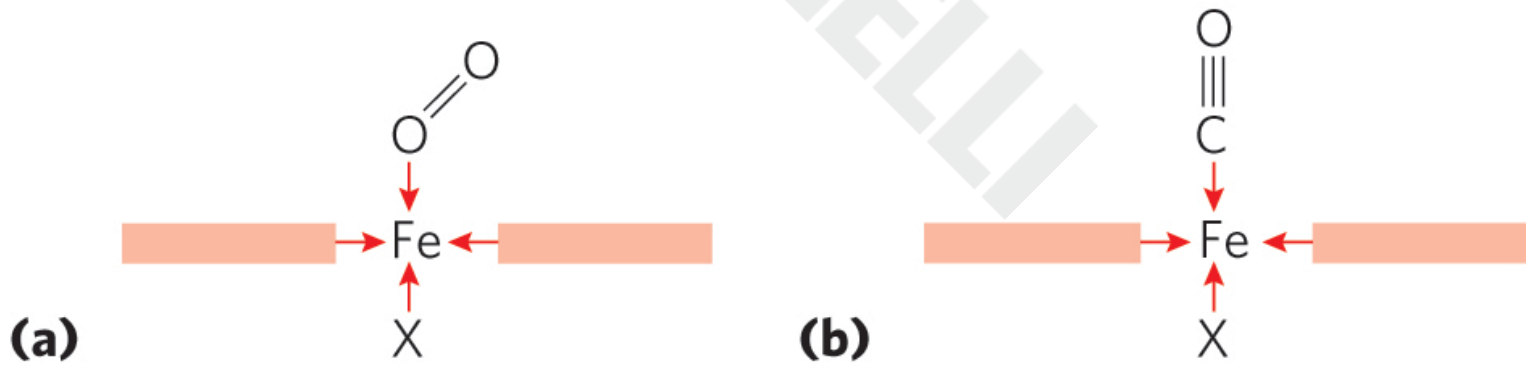
P3 Principle 3 (2 of 2)

Proteins are flexible. Changes in conformation may be subtle, reflecting molecular vibrations and small movements of amino acid residues throughout the protein. Changes in conformation may also be more dramatic, with major segments of the protein structure moving as much as several nanometers. Specific conformational changes are frequently essential to a protein's function.

Protein Structure Affects How Ligands Bind

- carbon monoxide (CO) binds free heme more than 20,000 times better than does O_2
  - differences in the orbital structures affect binding geometries



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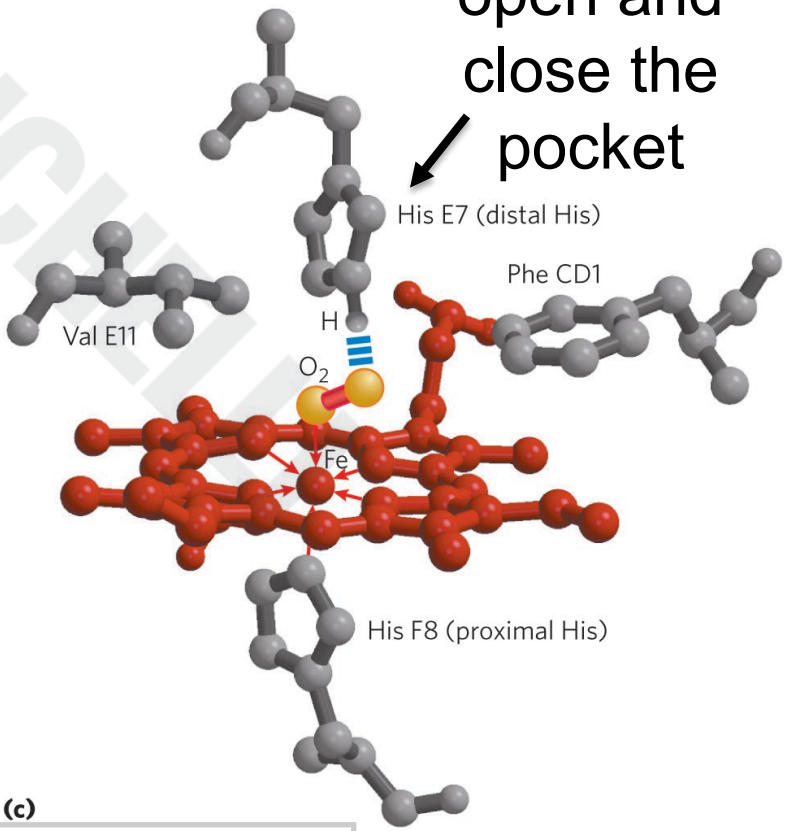
Myoglobin's Distal His Increases Heme's Affinity for O_2

- hydrogen bond between the imidazole side chain of His E7 and bound O_2 electrostatically stabilizes the Fe-O_2 polar complex
- 20,000-fold stronger binding affinity of free heme for CO compared with O_2 declines to ~40-fold



Hemoglobin Transports Oxygen in Blood

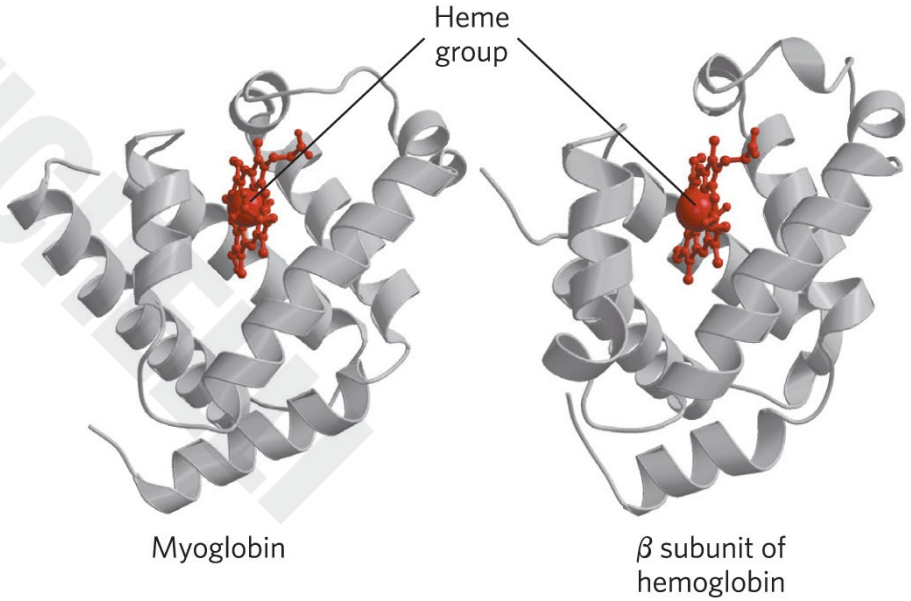
- erythrocytes (red blood cells) transport O_2
  - formed from **hemocytoblasts** (precursor stem cells)
  - main function is to carry hemoglobin
- arterial blood = ~96% saturated with O_2
- peripheral blood = ~64% saturated with O_2

P5 Principle 5 (2 of 4)

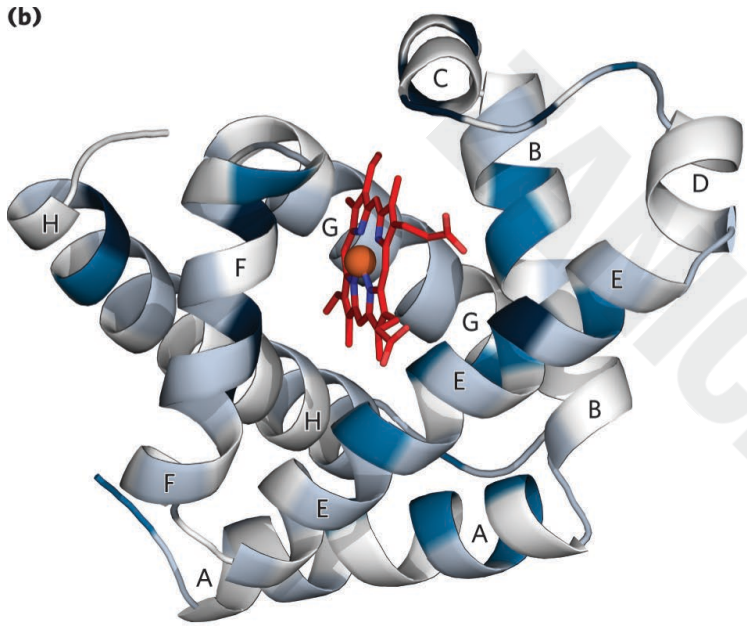
In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Hemoglobin Subunits Are Structurally Similar to Myoglobin

- hemoglobin:
  - tetrameric protein with 4 heme groups
  - adult hemoglobin has two globin types: two α chains (141 residues each) and two β chains (146 residues each)



Structural Conservation of Globins



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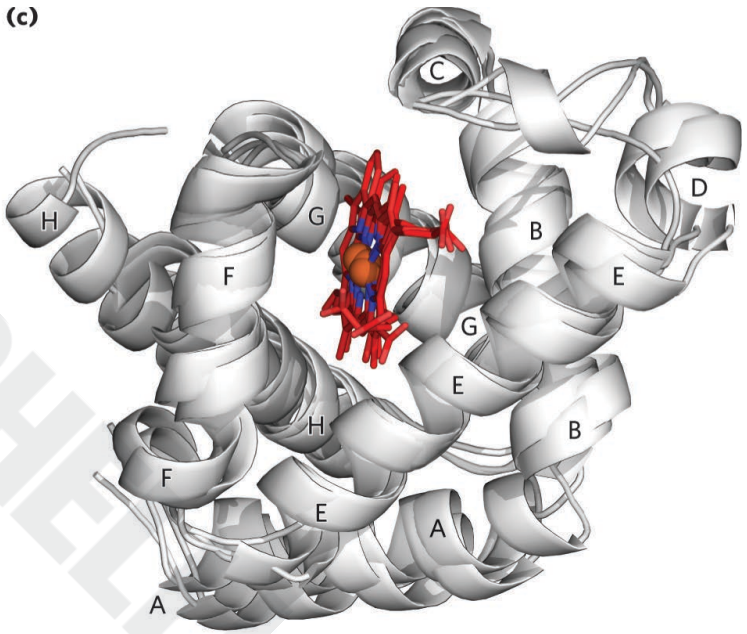


Low sequence similarity

High structural similarity

The Quaternary Structure of Hemoglobin

- strong interactions between unlike subunits
  - hydrophobic effect
  - hydrogen bonds
  - ion pairs (salt bridges)

- $\alpha_1\beta_1$ (and $\alpha_2\beta_2$) interface involves >30 residues

- $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface involves 19 residues

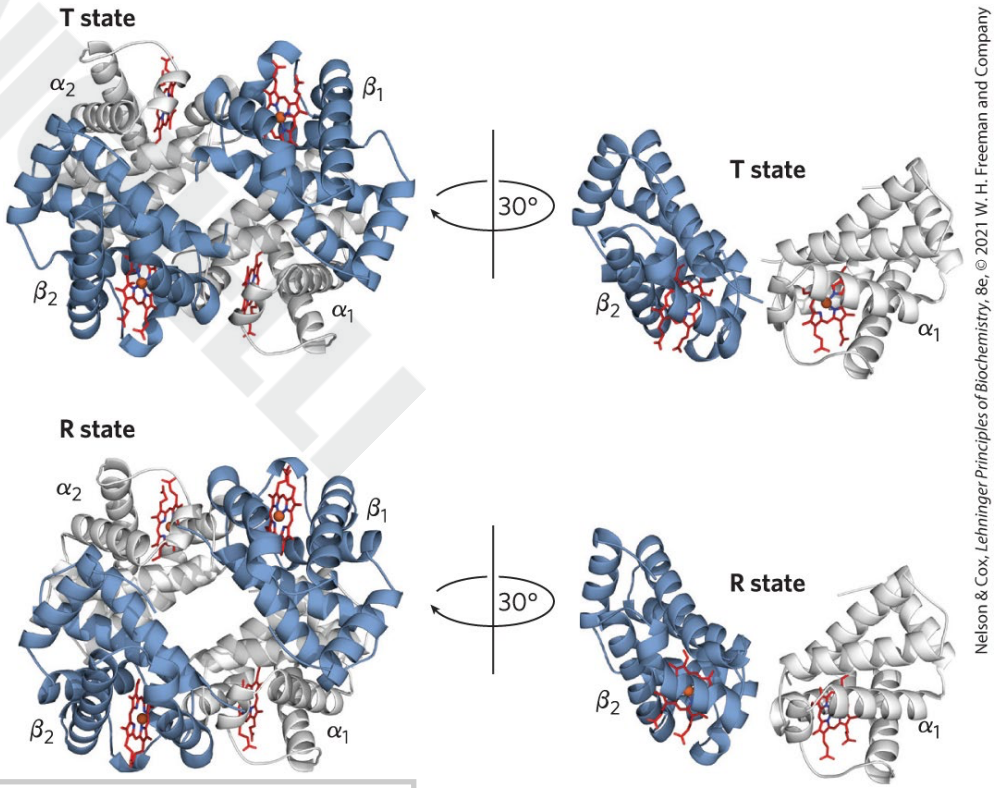
P4 Principle 4 (2 of 2)

The binding of a protein and a ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**.

Hemoglobin Undergoes a Structural Change on Binding Oxygen

- two conformations of hemoglobin:
  - **R state** = O_2 has a higher affinity for hemoglobin
  - **T state** = more stable when O_2 is absent, predominant conformation of **deoxyhemoglobin**

Greater number of ion pairs



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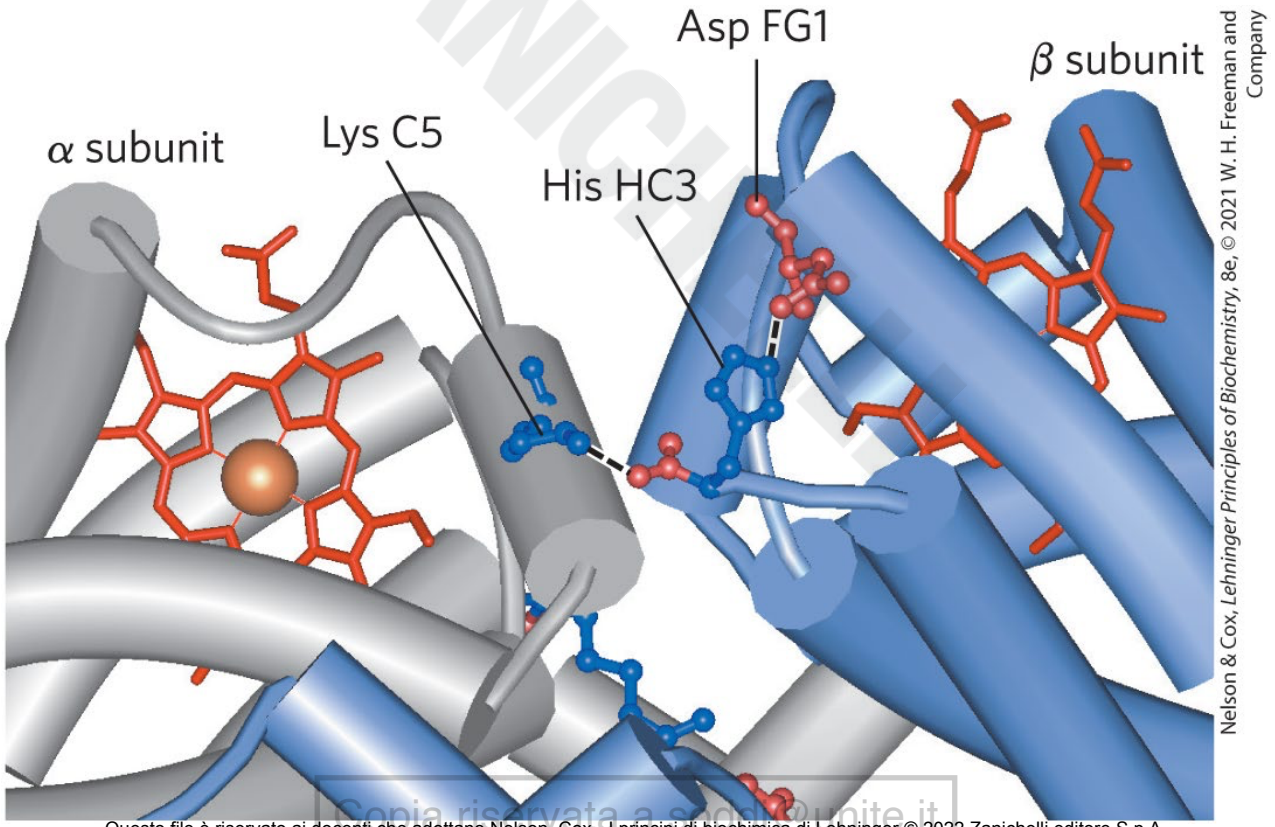
Ion Pairs Stabilize the T State

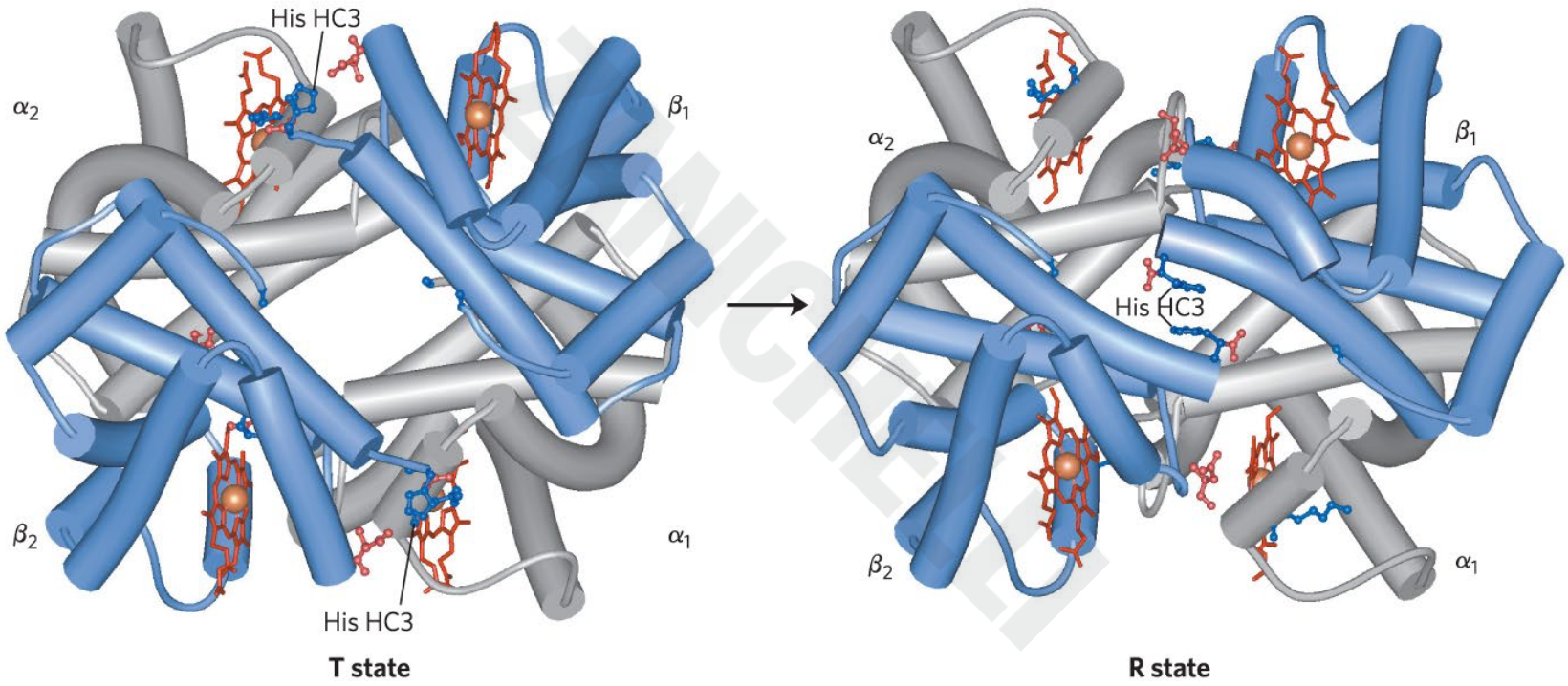
- T state is stabilized by a greater number of ion pairs, many of which lie at the $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface



Conformational Change in Hemoglobin

- O_2 binding to hemoglobin in the T state triggers a conformational change to the R state
  - *αβ* subunit pairs slide past each other and rotate
  - the pocket between the *β* subunits narrow
  - some ion pairs that stabilize the T state break and some new ones form

The T → R Transition



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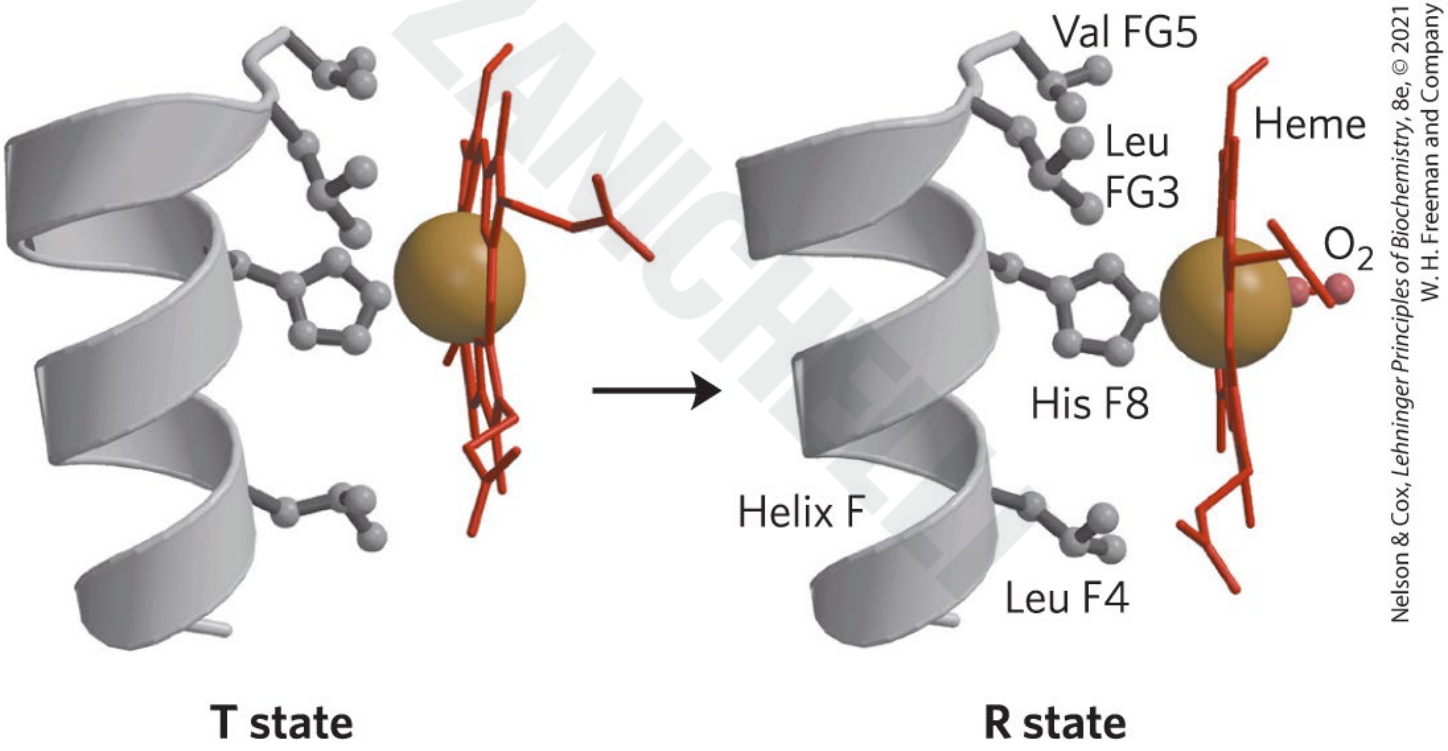
Changes in Conformation Near Heme

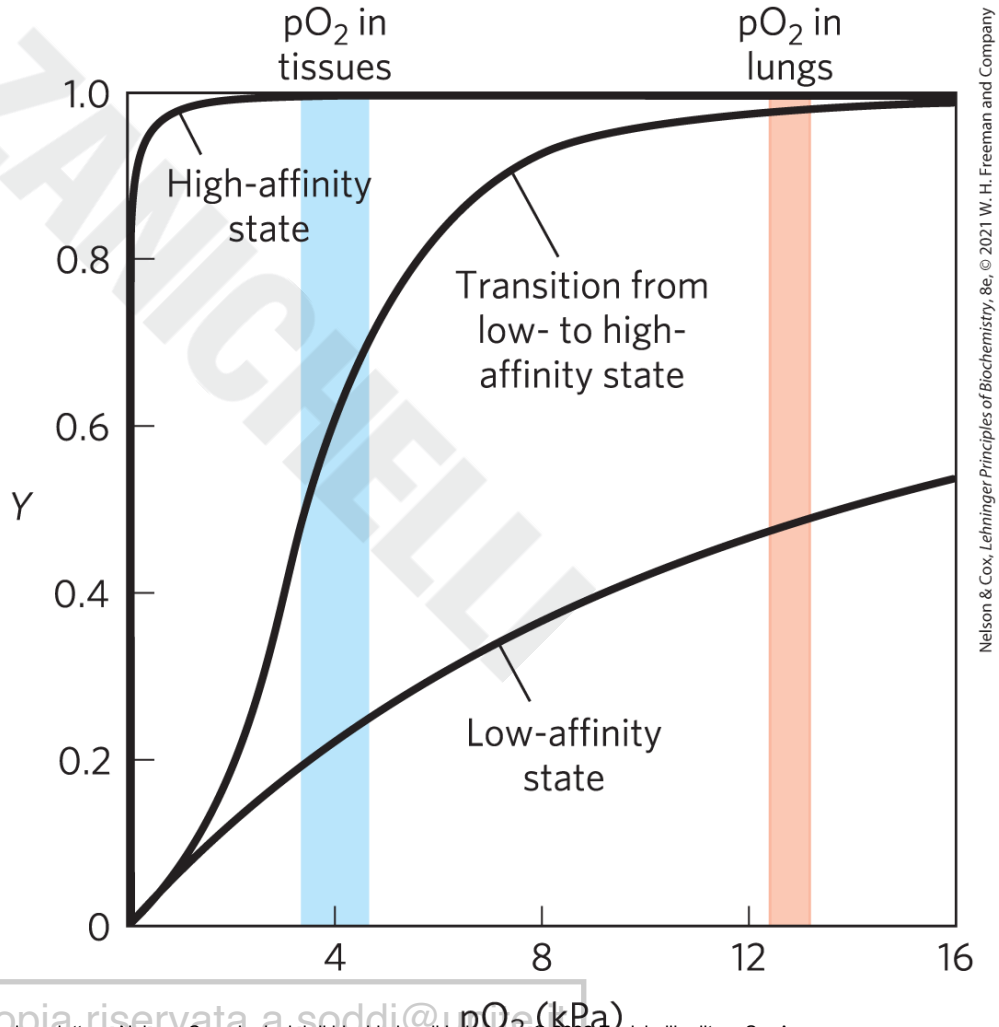


P5 Principle 5 (3 of 4)

In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Hemoglobin Binds Oxygen Cooperatively

- hemoglobin has a hybrid sigmoid binding curve for oxygen



P6 Principle 6 (2 of 3)

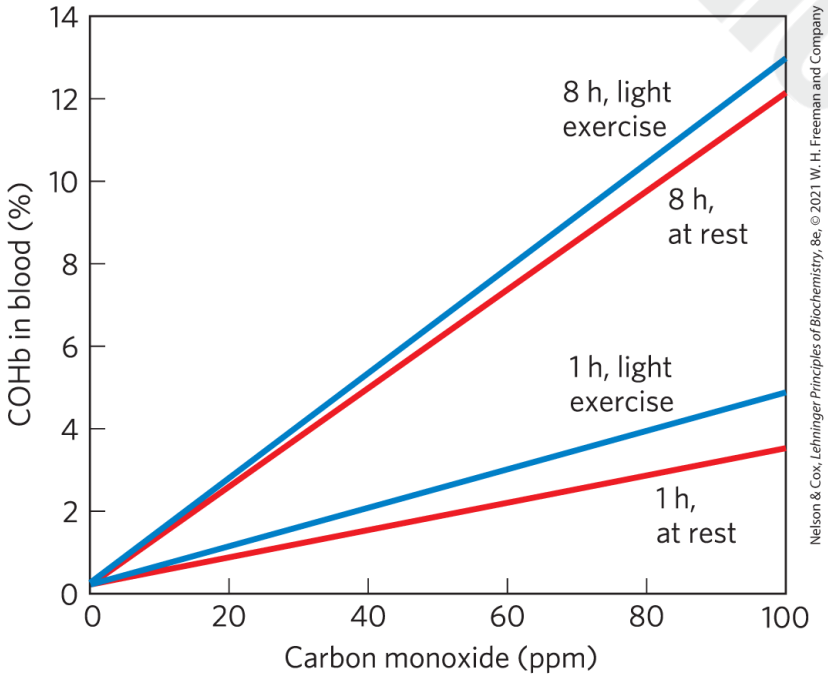
Interactions between ligands and proteins may be regulated.

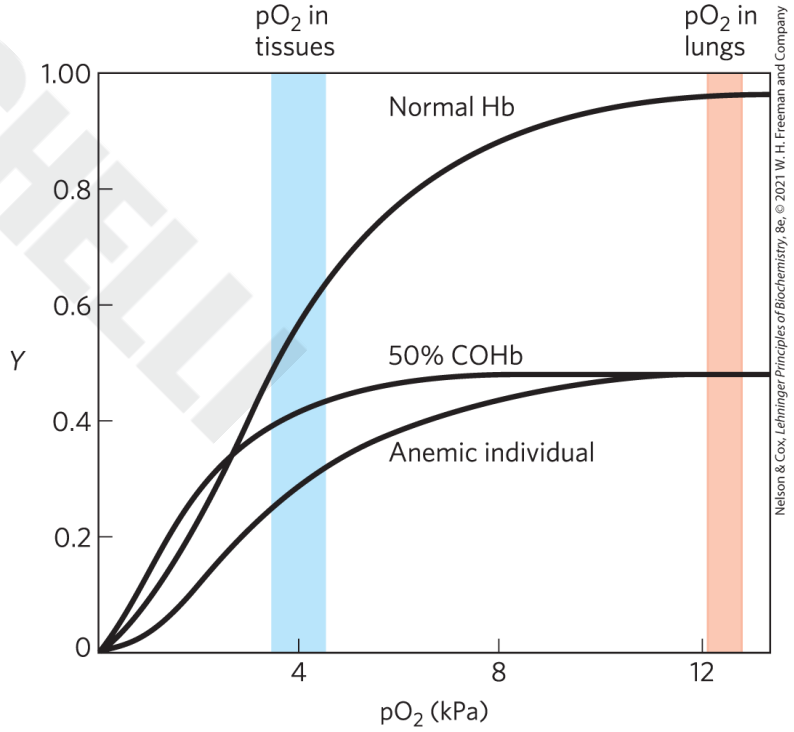
Allosteric Proteins

- **allosteric protein** (e.g., hemoglobin) = binding of a ligand to one site affects the binding properties of another site on the same protein
  - **modulators** = ligands that bind to an allosteric protein to induce a conformational change
  - **homotropic** = normal ligand and modulator are identical
  - **heterotropic** = modulator is a molecule other than the normal ligand

Carbon Monoxide Can Bind to Hemoglobin

- CO has ~250-fold greater affinity for hemoglobin than does O_2





Cooperative Ligand Binding Can Be Described Quantitatively

- for a protein with *n* binding sites, the equilibrium becomes:

$$P + nL \rightleftharpoons PL_n \qquad (5\text{-}12)$$

The K_a and Y for Cooperative Ligand Binding

- the expression for the association constant becomes:

$$K_a = \frac{[PL_n]}{[P][L]^n} \qquad (5\text{-}13)$$

- the expression for Y is:

$$Y = \frac{[L]^n}{[L]^n + K_d} \qquad (5\text{-}14)$$

The Hill Equation

$$Y = \frac{[L]^n}{[L]^n + K_d} \qquad (5\text{-}14)$$

- rearranging, then taking the log of both sides, yields:

$$\frac{Y}{1 - Y} = \frac{[L]^n}{K_d} \qquad (5\text{-}15)$$

the **Hill equation**:

$$\log\left(\frac{Y}{1 - Y}\right) = n \log [L] - \log K_d \qquad (5\text{-}16)$$

Hill Plots and Hill Coefficients

- **Hill plot** = plot of log [Y/(1 − Y)] versus log [L]

- **Hill coefficient** = n_H = slope of a Hill plot
  - If n_H = 1, ligand binding is not cooperative
  - n_H > 1 indicates positive cooperativity
  - n_H < 1 indicates negative cooperativity

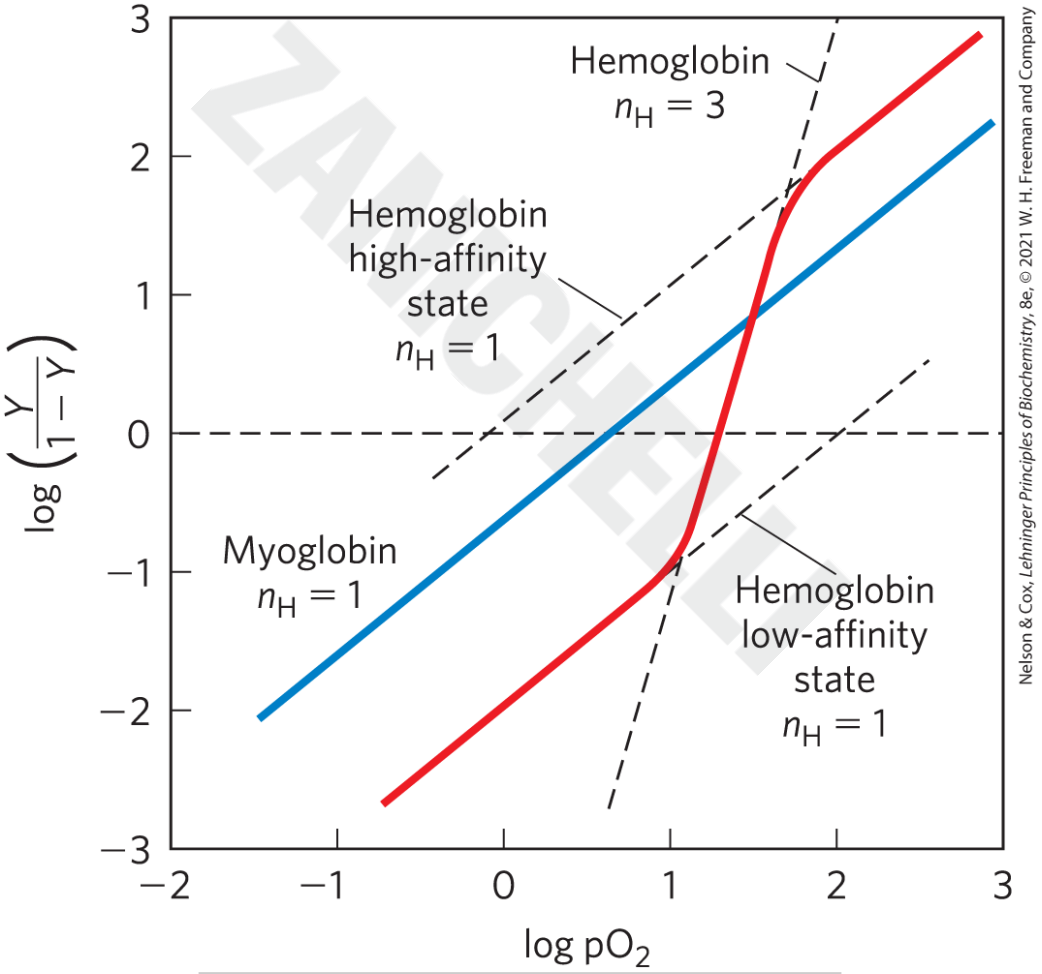
Adapting the Hill Equation to the Binding of O_2 to Hemoglobin

- substituting pO_2 for [L] and P_{50}^{n} for K_d:

$$\log\left(\frac{Y}{1-Y}\right) = n \log pO_2 - n \log P_{50} \qquad (5\text{-}17)$$

- **Hill coefficient** = n_H = slope of a Hill plot
  - If $n_H = 1$, ligand binding is not cooperative
  - $n_H > 1$ indicates positive cooperativity
  - $n_H < 1$ indicates negative cooperativity

Hill Plots for Myoglobin and Hemoglobin



P5 Principle 5 (4 of 4)

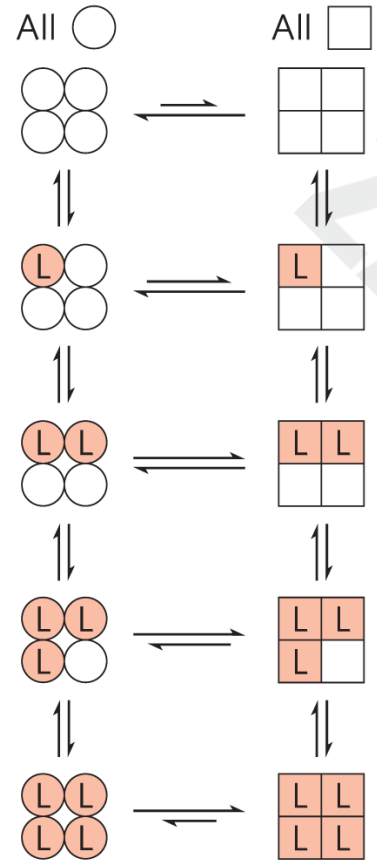
In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Two Models Suggest Mechanisms for Cooperative Binding

- **MWC model = concerted model**
  - all subunits in the same conformation
  - ligand binds more tightly to the R state

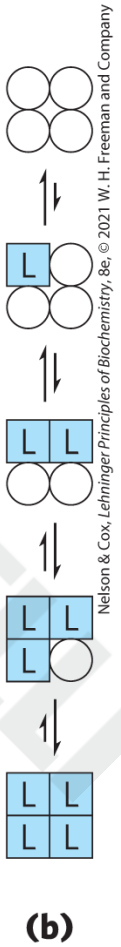
- **sequential model**
  - each subunit can be in either conformation
  - equilibrium is altered as additional ligands are bound, progressively favoring the R state

Concerted and Sequential Models



(a)

MWC model (concerted model)



(b)

Sequential model

○ Low affinity or inactive

□ High affinity or active

Hemoglobin Also Transports H^+ and CO_2

- hemoglobin carries two end products of cellular respiration: H^+ and CO_2
- **carbonic anhydrase** catalyzes the hydration of CO_2 to bicarbonate:

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

P6 Principle 6 (3 of 3)

Interactions between ligands and proteins may be regulated.

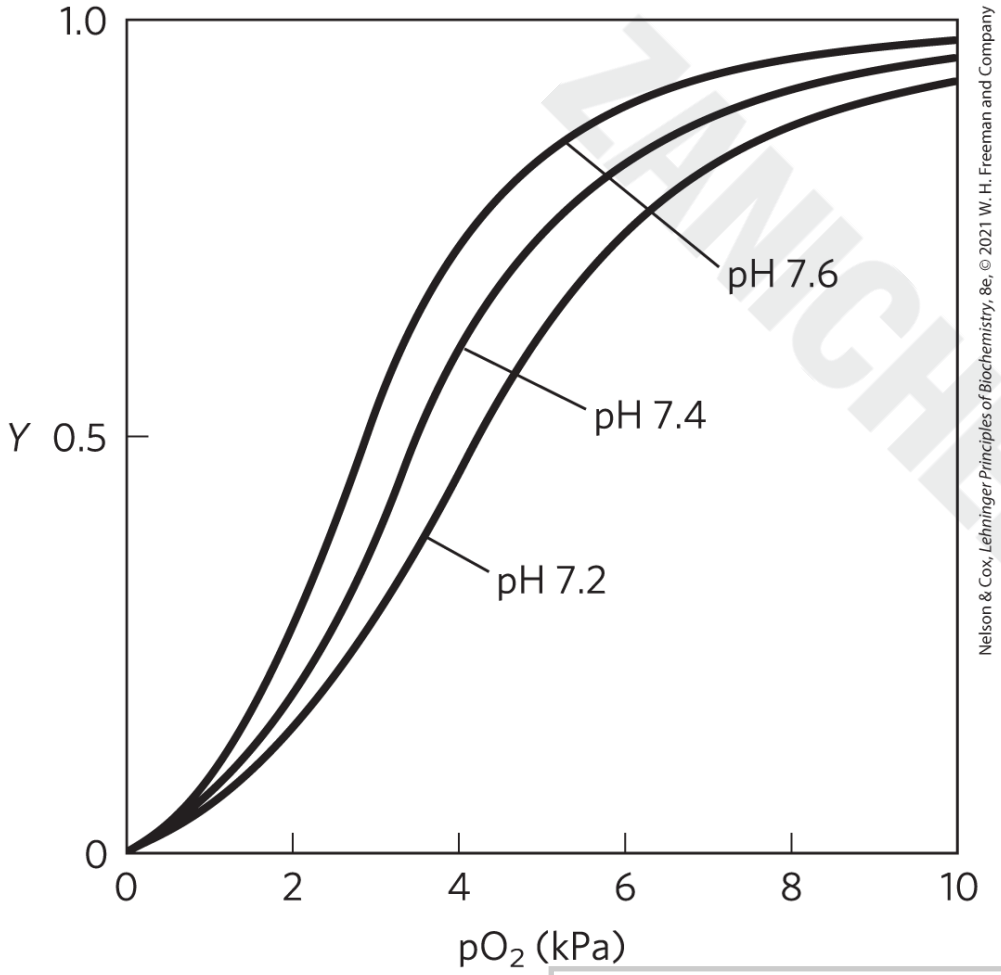
The Bohr Effect

- the structural effects of H^+ and CO_2 binding to hemoglobin favor the T state
  - the binding of H^+ and CO_2 is inversely related to the binding of O_2

- **Bohr effect** = describes the effect of pH and [CO_2] on the binding and release of O_2 by hemoglobin

$$HHB^+ + O_2 \rightleftharpoons HbO_2 + H^+$$

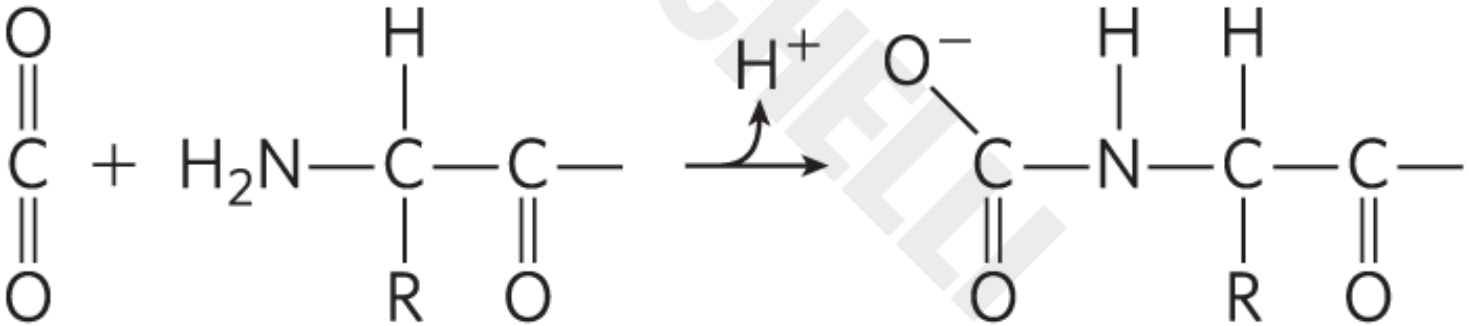
The Effect of pH on O_2 Binding to Hemoglobin



- when $[O_2]$ is high, hemoglobin binds O_2 and releases H^+
- when $[O_2]$ is low, hemoglobin releases O_2 and binds H^+

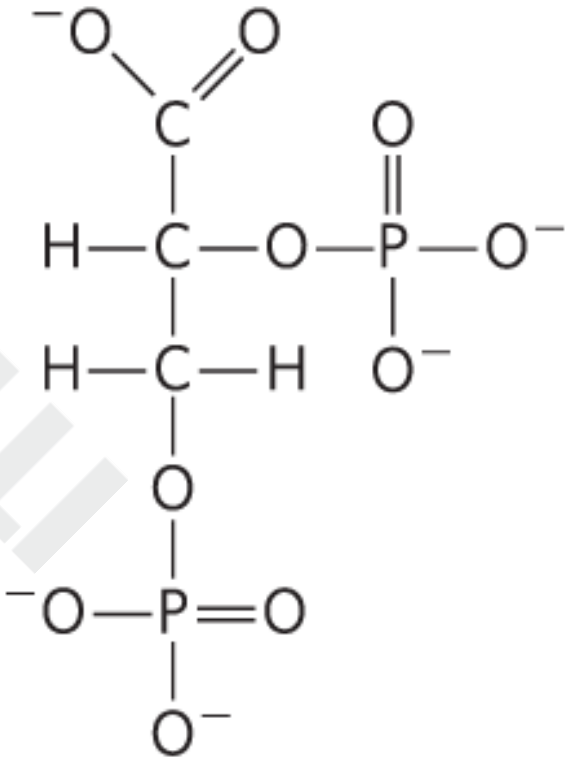
Hemoglobin Binds CO_2

- CO_2 binding to hemoglobin is inversely related to binding of O_2
  - contributes to the Bohr effect by producing H^+



Oxygen Binding to Hemoglobin Is Regulated by 2,3-Bisphosphoglycerate (1 of 2)

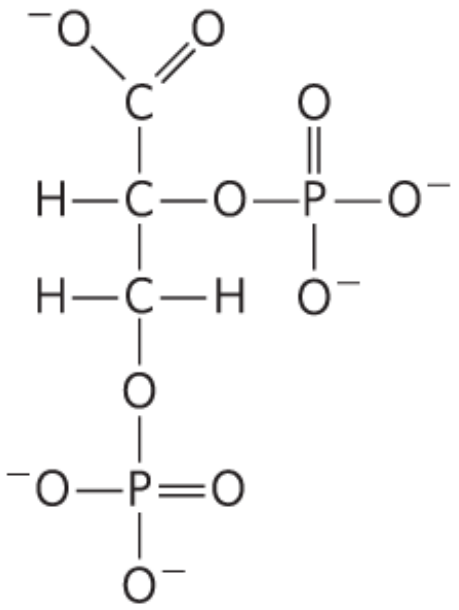
- **2,3-bisphosphoglycerate (BPG):**
  - example of heterotropic allosteric modulation
  - binds to a site distant from O_2-binding site
  - greatly reduces the affinity of hemoglobin for oxygen



2,3-Bisphosphoglycerate

Oxygen Binding to Hemoglobin Is Regulated by 2,3-Bisphosphoglycerate (2 of 2)

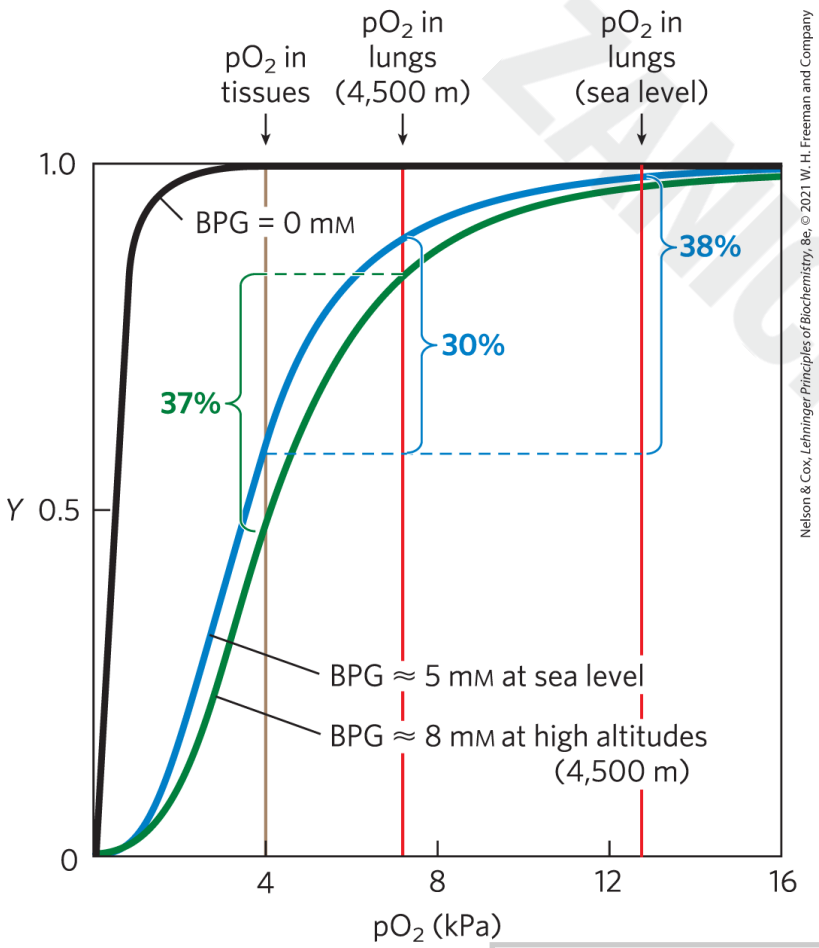
- **2,3-bisphosphoglycerate (BPG):**
  - example of heterotropic allosteric modulation
  - binds to a site distant from O_2-binding site
  - greatly reduces the affinity of hemoglobin for oxygen



2,3-Bisphosphoglycerate

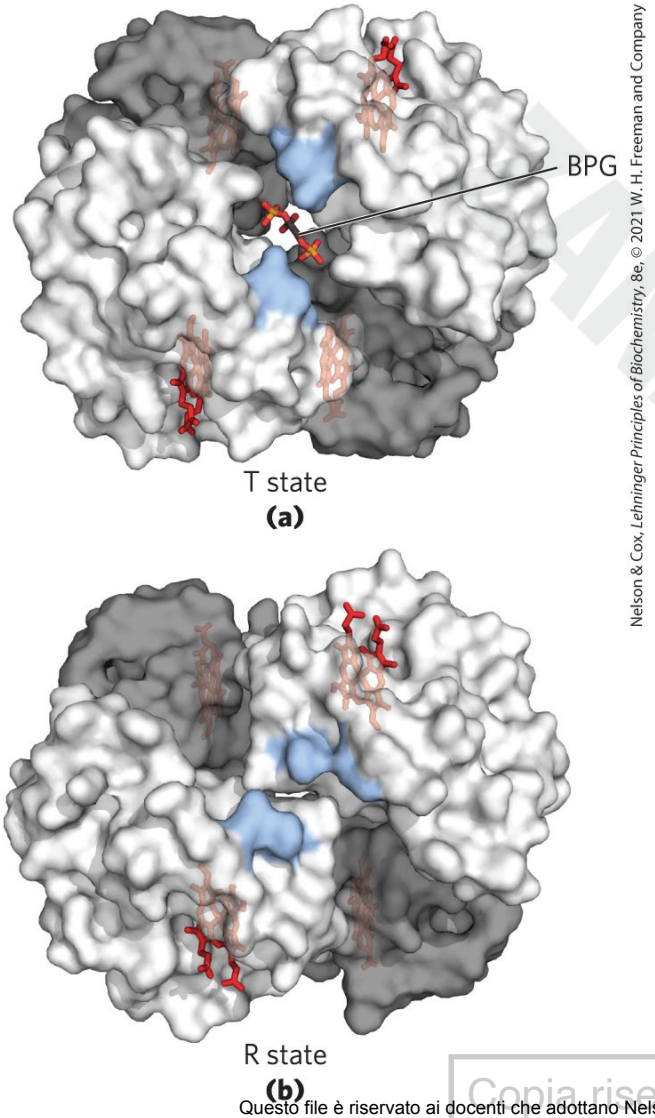
$$HbBPG + O_2 \rightleftharpoons HbO_2 + BPG$$

Effect of BPG on O_2 Binding to Hemoglobin



- BPG increases at high altitudes
- **hypoxia** = lowered oxygenation of peripheral tissues
  - causes BPG increases

Binding of BPG to Deoxyhemoglobin



Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

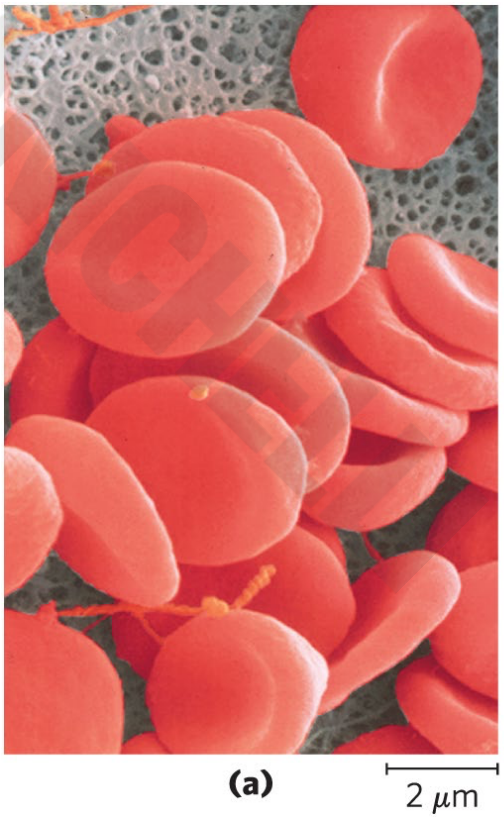
- BPG binds to the cavity between the β subunits in the T state
  - cavity is lined with positively charged residues
  - BPG stabilizes the T state

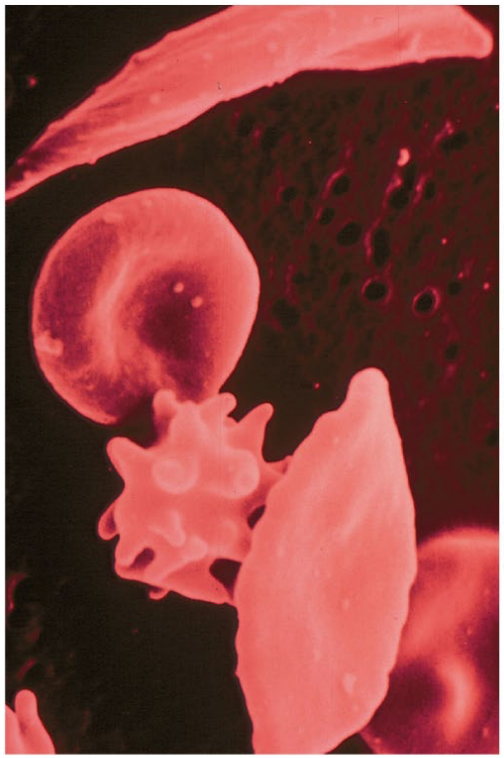
Fetal Hemoglobin

- fetus synthesizes $\alpha_2\gamma_2$ hemoglobin
  - lower affinity for BPG than normal adult hemoglobin
  - higher affinity for O_2 than normal adult hemoglobin

Sickle Cell Anemia Is a Molecular Disease of Hemoglobin

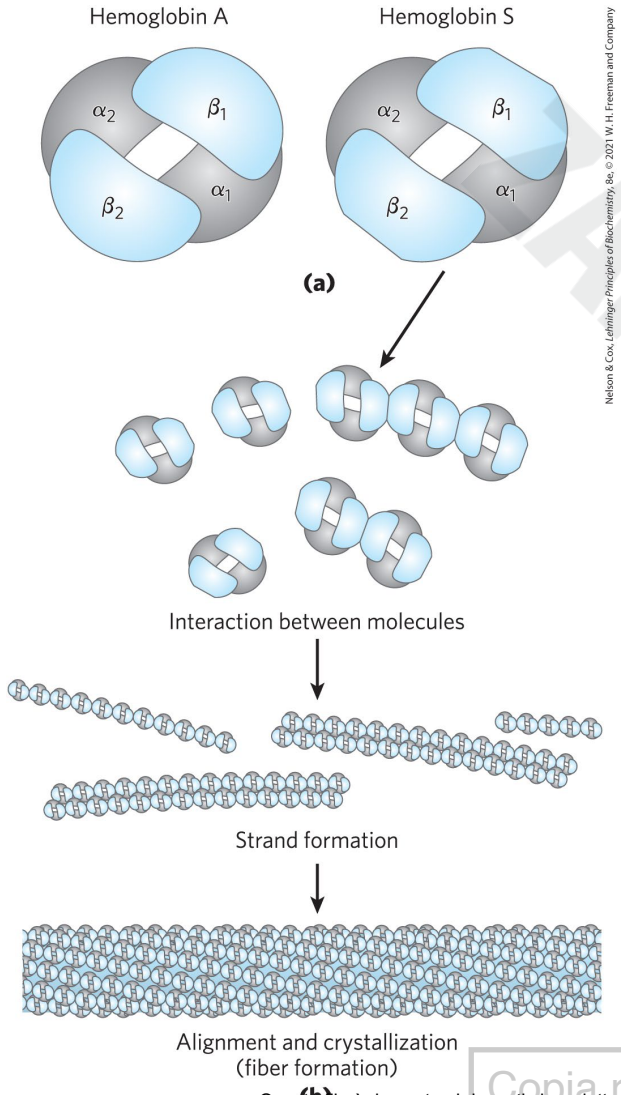
- sickle cell anemia:
  - homozygous condition
  - single amino acid substitution (Glu^6 to Val^6) β chains produces a hydrophobic patch





(a) A. Syred/Science; (b) Jackie Lewin, Royal Free Hospital/Science Source.

Normal and Sickle Cell Hemoglobin



- deoxygenated hemoglobin becomes insoluble and forms polymers that aggregate
- normal hemoglobin remains soluble upon deoxygenation