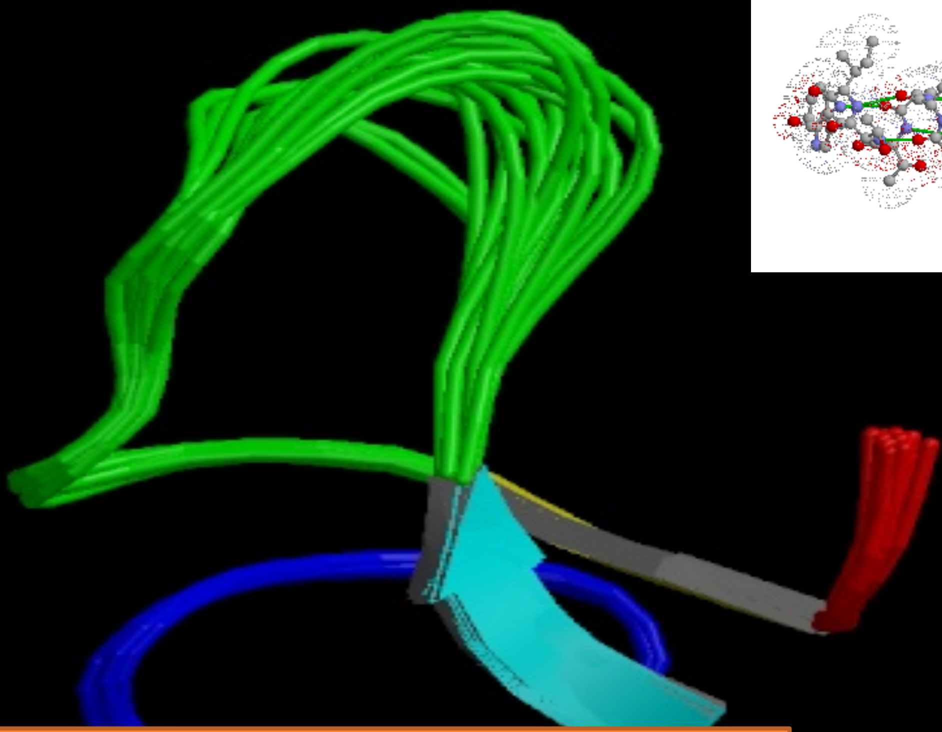
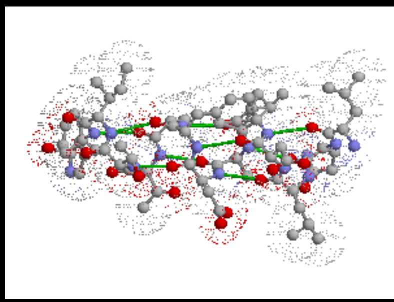




Protein Structure, Function and Engineering

M. Fayyaz ur Rehman

Course Outline

- Overview of Protein Structure
  - Primary, Secondary, Tertiary and Quaternary
  - Protein Folds
- Classification of Proteins
- Protein Families
  - CATH
  - SCOP
  - FSSP
- Secondary and Tertiary Structure Prediction
- Protein X-Ray Crystallography
- NMR Spectroscopy of Protein
- Molecular Modeling and Other Techniques
- Genes and Proteins

Cont….

Course Outline

- Protein Folding Pathway
- Protein Denaturation
- Protein Engineering
- Recombinant Proteins
- Post Translational Modifications
- Site Directed Mutagenesis
- Gene Reshuffling
- Chimeric Enzyme
- *In vitro* Enzyme Production

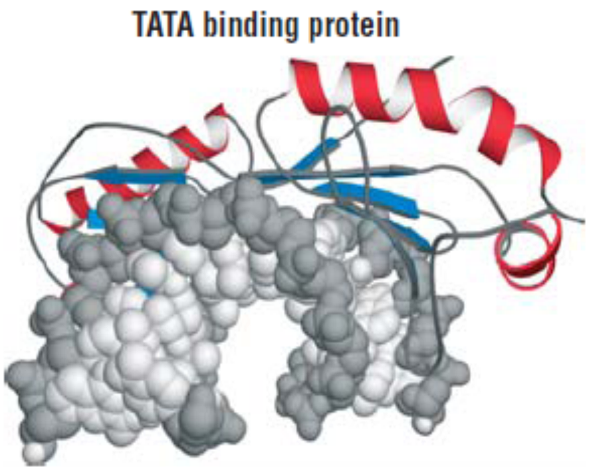
Student Evaluation

- Assignment Topics
  - Presentations
- Research Paper
  - Presentation
  - Poster
- Quizzes

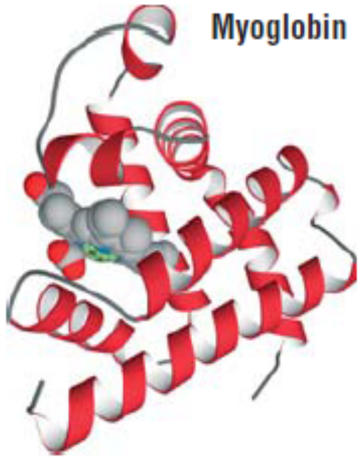
Protein Function

- Protein are most versatile macromolecule of the cell.
  - Binding
  - Catalysis
  - Switching
  - Structural Proteins

BINDING



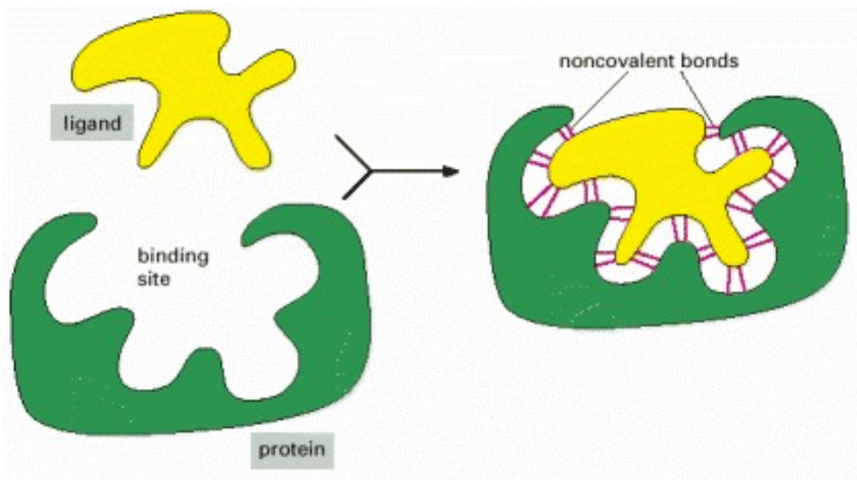
The TATA binding protein binds a specific DNA sequence and serves as the platform for a complex that initiates transcription of genetic information. (PDB 1tgh)



Myoglobin binds a molecule of oxygen reversibly to the iron atom in its heme group (shown in grey with the iron in green). It stores oxygen for use in muscle tissues. (PDB 1a6k)

BINDING

- All Proteins Bind to Other Molecules
  - antibodies attach to viruses or bacteria to mark them for destruction
  - enzyme hexokinase binds glucose and ATP so as to catalyze a reaction between them
  - actin molecules bind to each other to assemble into actin filaments
- all proteins stick, or *bind*, to other molecules
  - binding always shows great *specificity*
  - each protein molecule can usually bind just one or a few molecules
  - The substance that is bound by the protein—no matter whether it is an ion, a small molecule, or a macromolecule— is referred to as a ligand for that protein



- The region of a protein that associates with a ligand, known as the ligand's binding site
  - Consists of a cavity in the protein surface formed by a particular arrangement of amino acids

Interaction with Other Molecules

- Reversible, transient process of chemical equilibrium:

$$A + B \leftrightarrow AB$$

- A molecule that binds to a protein is called a ligand.
  - typically a small molecule
- A region in the protein where the ligand binds is called the binding site.
- Ligand binds via same noncovalent interactions that dictate protein structure.
- Allows the interactions to be transient

Example: Oxygen Binding to Myoglobin

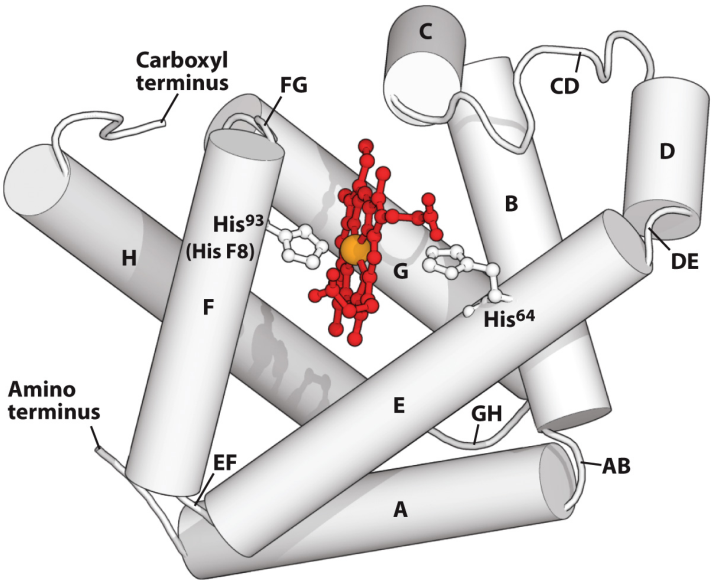


Figure 5-3
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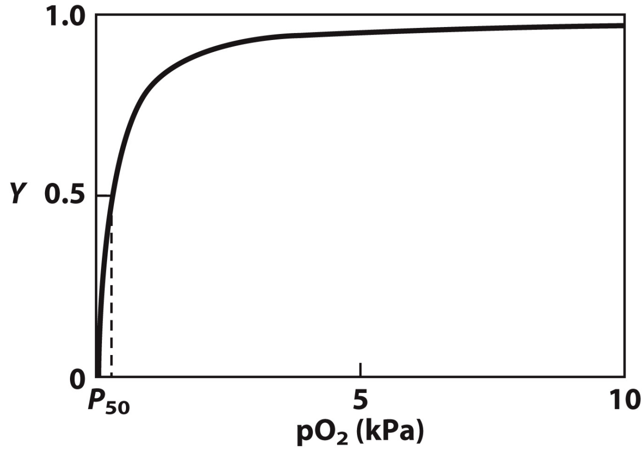

Figure 5-4b
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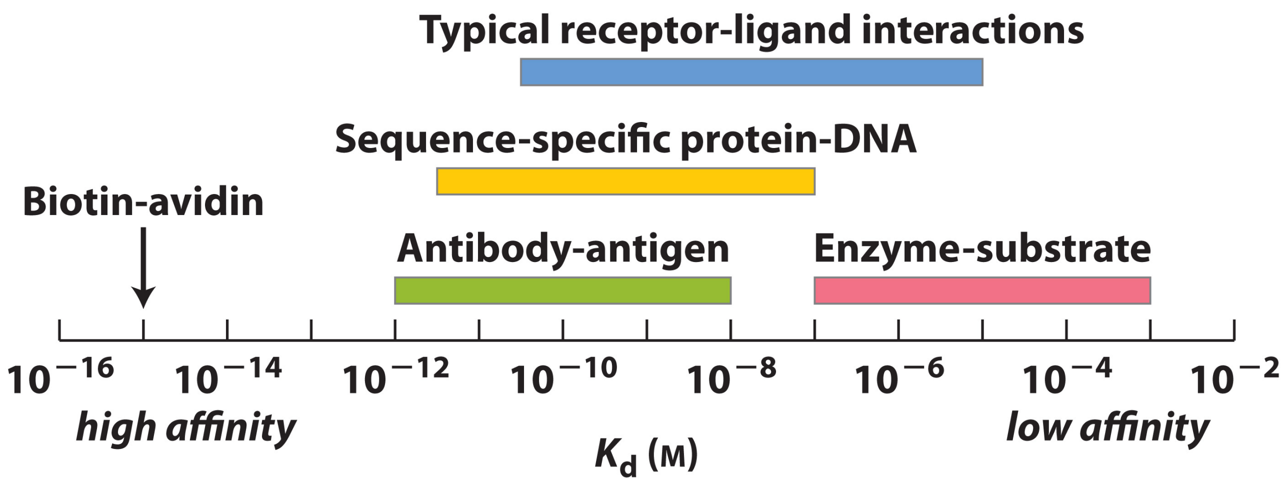
When a ligand is a gas, binding is expressed in terms of partial pressures.

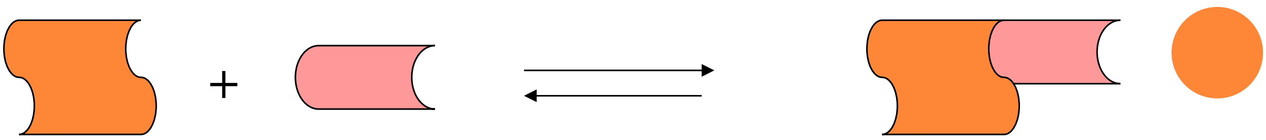
$$\theta = \frac{[L]}{K_d + [L]} \longrightarrow \theta = \frac{pO_2}{p_{50} + pO_2}$$

Examples of Binding Strength



Specificity: Lock-and-Key Model

- Proteins typically have high specificity: only certain ligands bind.
- High specificity can be explained by the complementary of the binding site and the ligand.
- Complementary in:
  - size
  - shape
  - charge
  - hydrophobic/hydrophilic character
- The "lock and key" model by Emil Fisher (1894) assumes that complementary surfaces are preformed.



Case Study I: Globins Are Oxygen-Binding Proteins

Biological problems:

- Protein side chains lack affinity for O_2.
- Some transition metals bind O_2 well but would generate free radicals if free in solution.
- Organometallic compounds such as heme are more suitable, but Fe^{2+} in free heme could be oxidized to Fe^{3+} (very reactive!).

Biological solution:

- Capture the oxygen molecule with heme that is protein bound.

Myoglobin (storage) and hemoglobin (transport) can bind oxygen via a protein-bound heme.

Structures of Porphyrin and Heme

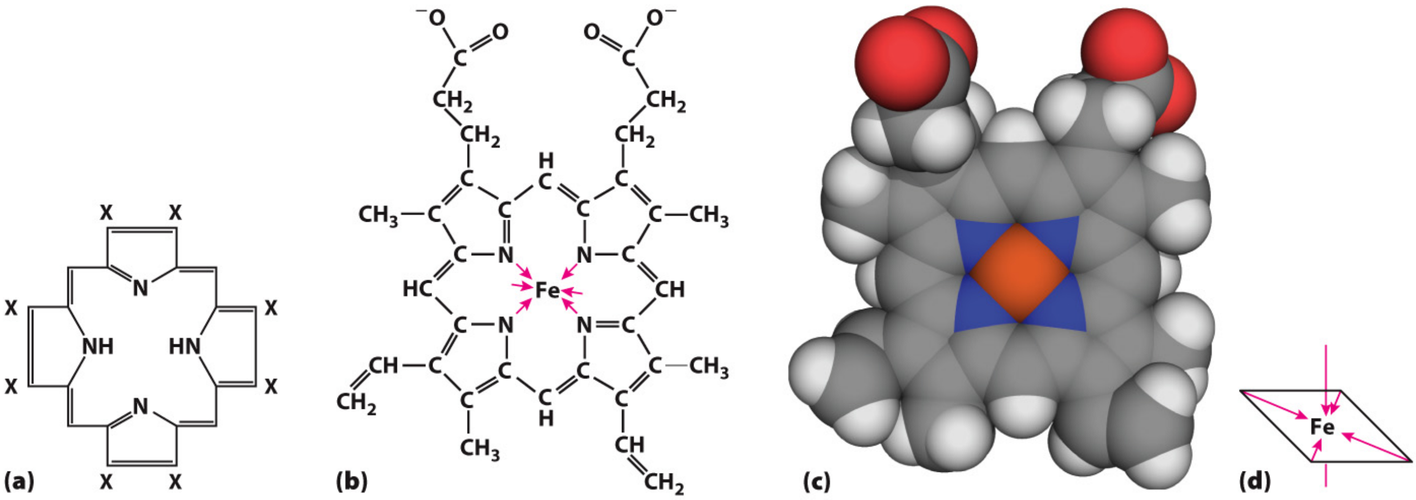


Figure 5-1
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Structure of Myoglobin

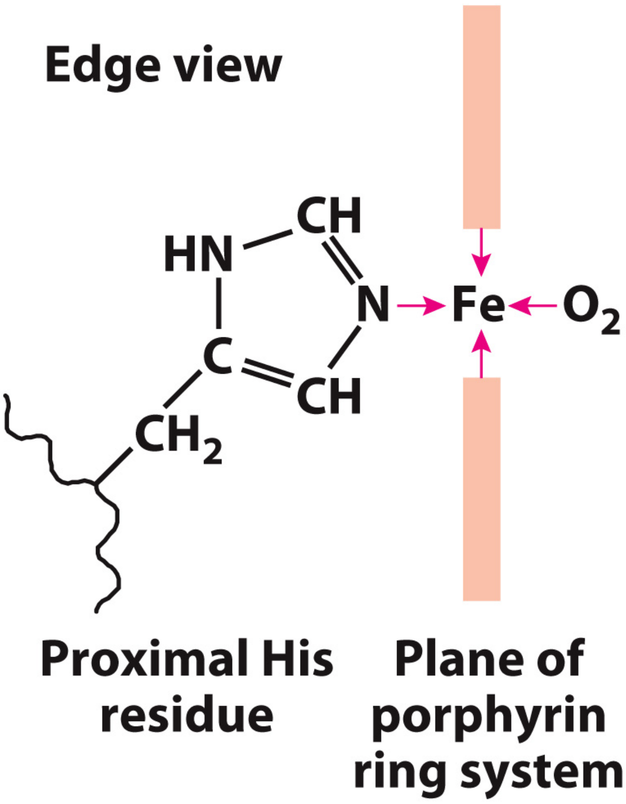

Figure 5-2
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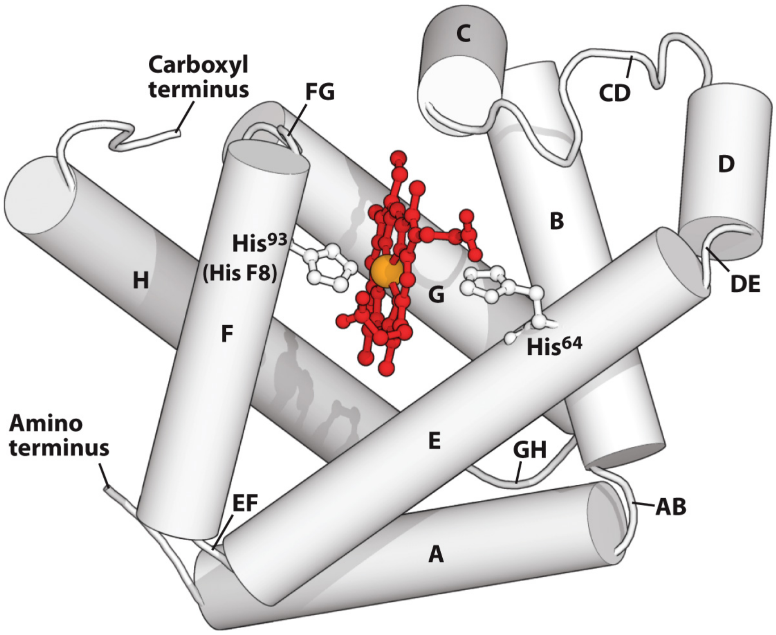

Figure 5-3
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Binding of Carbon Monoxide

- CO has similar size and shape to O_2; it can fit to the same binding site.
- CO binds heme over 20,000 times better than O_2 because the carbon in CO has a filled lone electron pair that can be donated to vacant *d*-orbitals on the Fe^{2+}.
- The protein pocket decreases affinity for CO, but it still binds about 250 times better than oxygen.
- CO is highly toxic, as it competes with oxygen. It blocks the function of myoglobin, hemoglobin, and mitochondrial cytochromes that are involved in oxidative phosphorylation.

CO vs. O_2 Binding to Free Heme

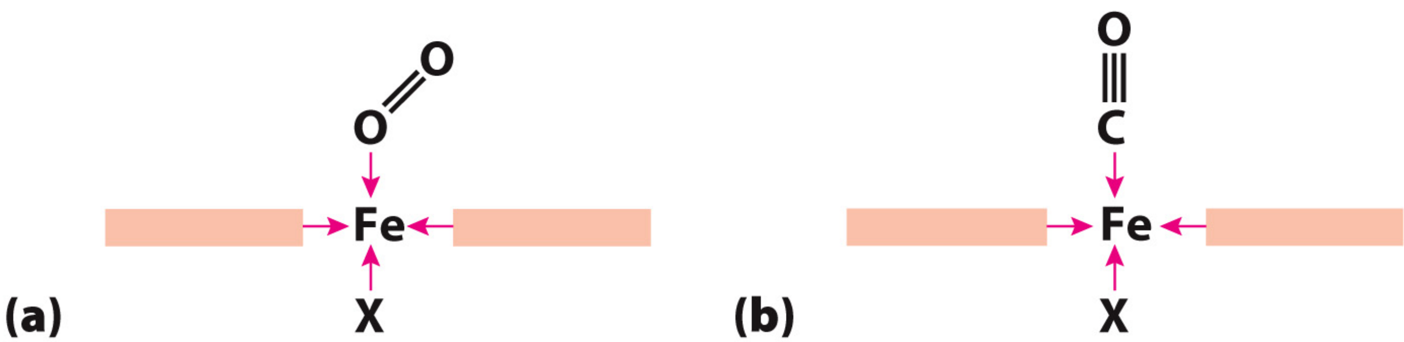

Figure 5-5ab
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Heme Binding to Protein Affects CO vs. O_2 Binding

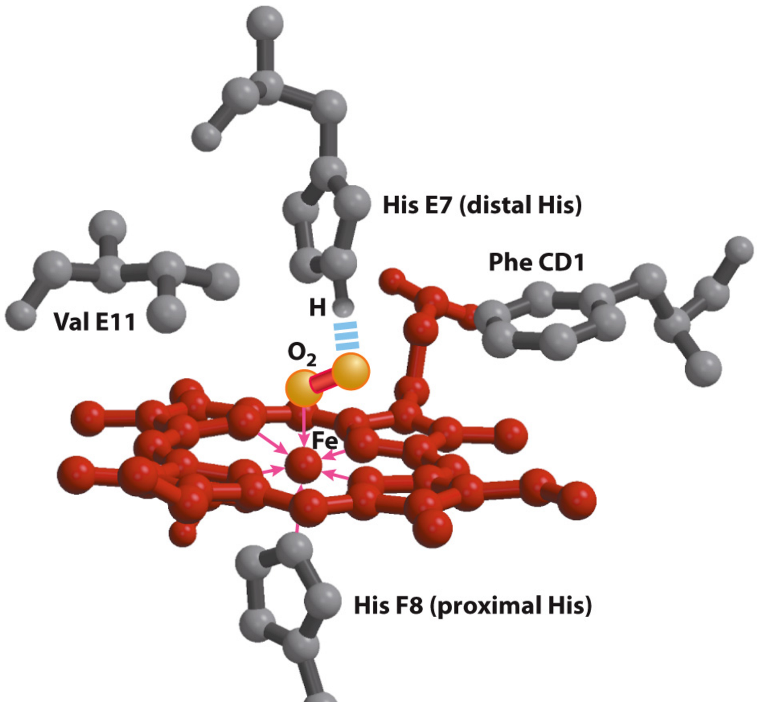

Figure 5-5c
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Spectroscopic Detection of Oxygen Binding to Globins

- The heme group is a strong chromophore that absorbs both in ultraviolet and visible range.
- Ferrous form (Fe^{2+}) without oxygen has an intense Soret band at 429 nm.
- Oxygen binding alters the electronic properties of the heme and shifts the position of the Soret band to 414 nm.
- Binding of oxygen can be monitored by UV-Vis spectrophotometry.
- Deoxyhemoglobin (in venous blood) appears purplish in color and oxyhemoglobin (in arterial blood) is red.

Could Myoglobin Transport O_2?

- pO_2 in lungs is about 13 kPa: it sure binds oxygen well.
- pO_2 in tissues is about 4 kPa: it will not release it!

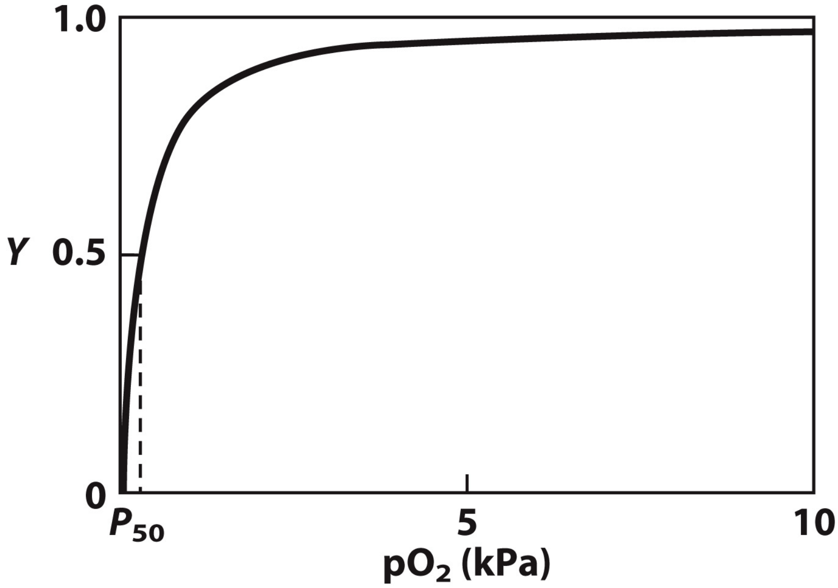

Figure 5-4b
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Would lowering the affinity (P_{50}) of myoglobin to oxygen help?

For Effective Transport Affinity Must Vary with pO_2

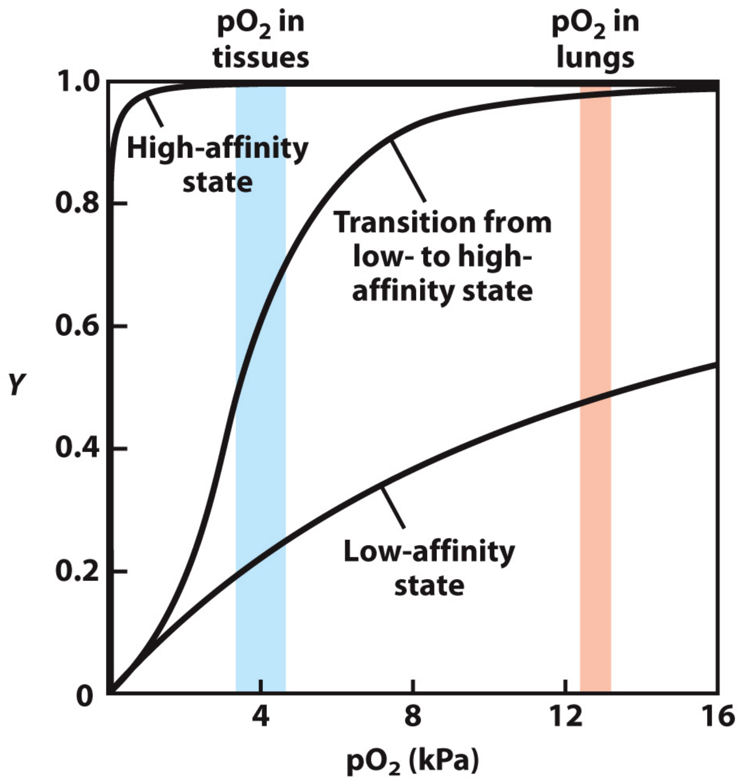

Figure 5-12
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How Can Affinity to Oxygen Change?

- It must be a protein with multiple binding sites.
- Binding sites must be able to interact with each other.
- This phenomenon is called cooperativity.
  - positive cooperativity
    - first binding event increases affinity at remaining sites
    - recognized by sigmoidal binding curves
  - negative cooperativity
    - first binding event reduces affinity at remaining sites

Cooperativity

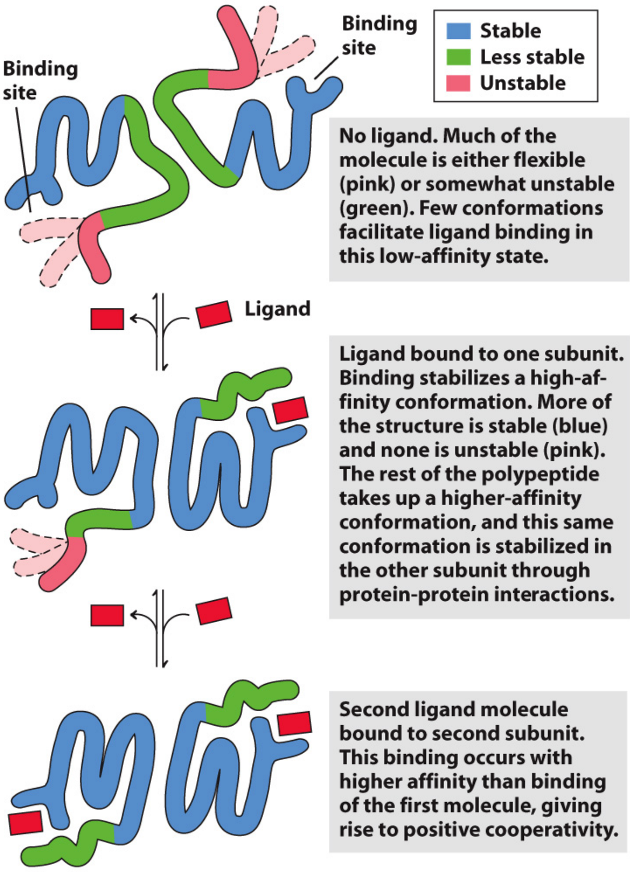

Figure 5-13
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Hemoglobin Binds Oxygen Cooperatively

- Hemoglobin (Hb) is a tetramer of two subunits ($\alpha 2 \beta 2$).
- Each subunit is similar to myoglobin.

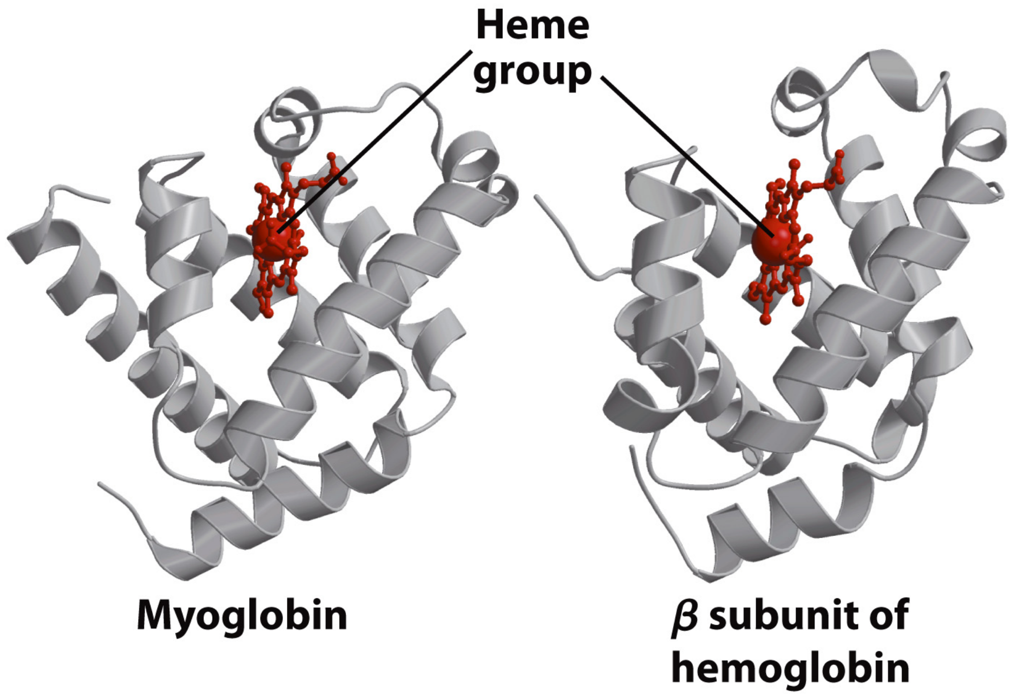

Figure 5-6
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Subunit Interactions in Hemoglobin

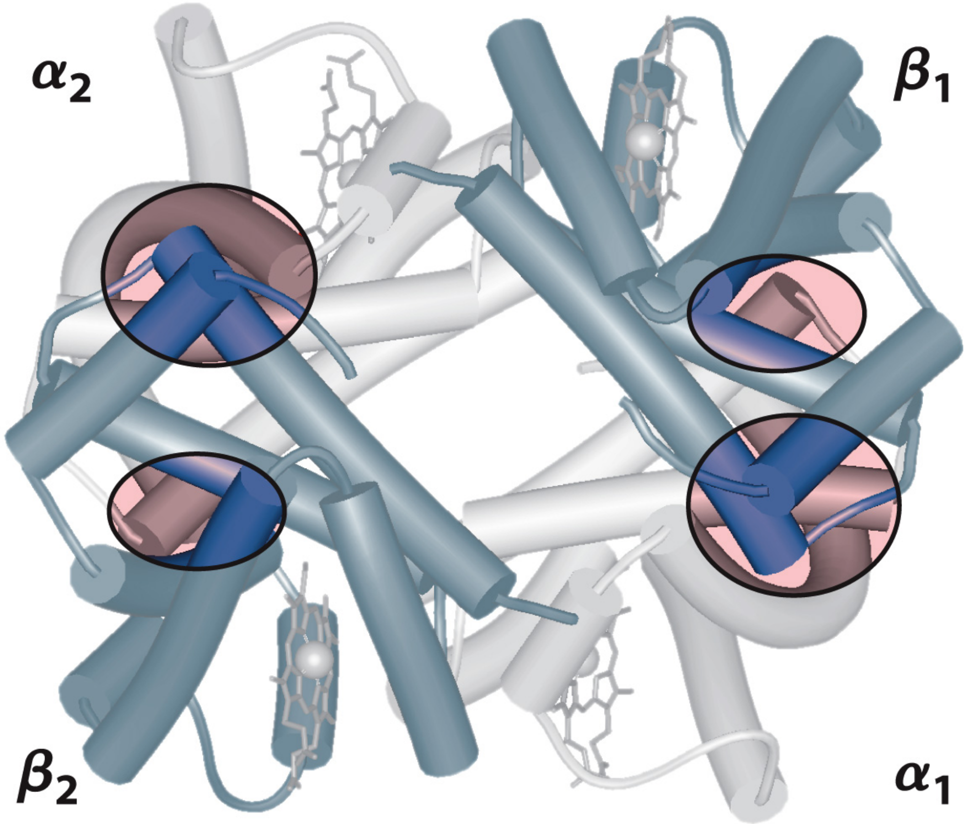

Figure 5-8
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Subunit Interactions: Details

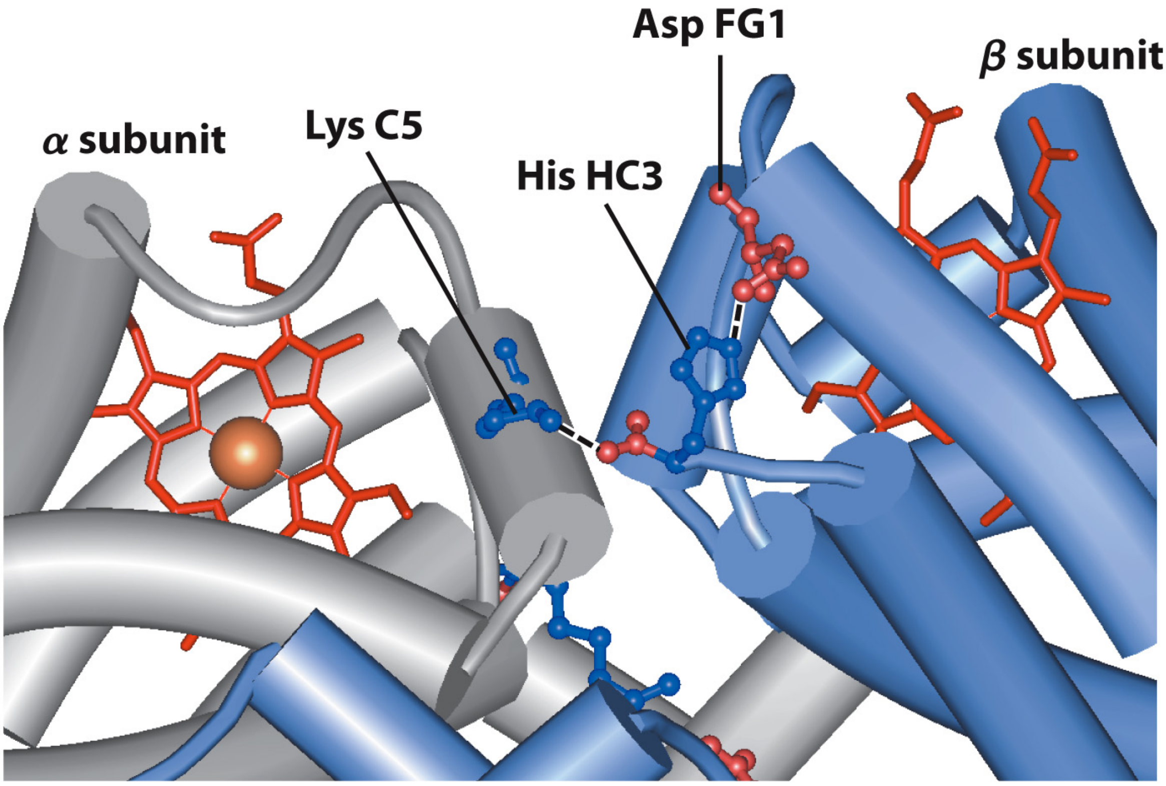

Figure 5-9a
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Subunit Interactions: Details

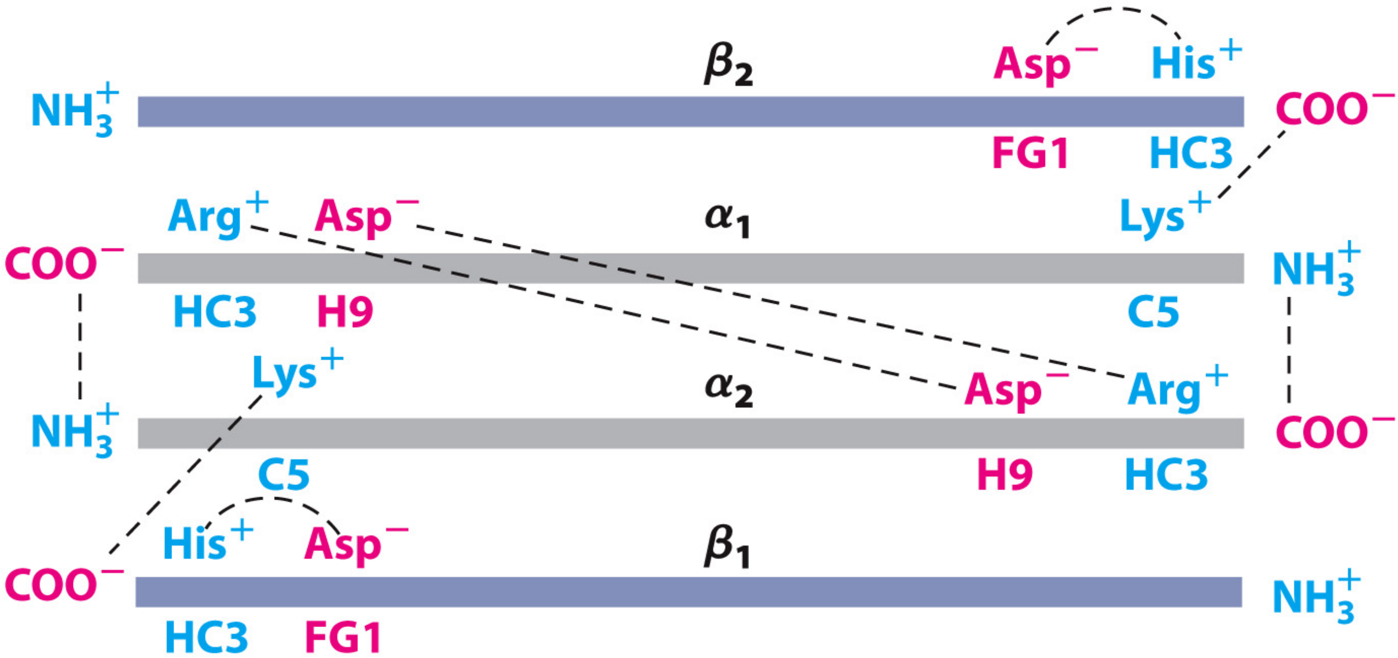

Figure 5-9b
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R and T States of Hemoglobin

- T = tense state
  - more interactions, more stable
  - lower affinity for O_2
- R = relaxed state
  - fewer Interactions, more flexible
  - higher affinity for O_2
- O_2 binding triggers a **T** → **R** conformational change.
- Conformational change from the T state to the R state involves breaking ion pairs between the α1-β2 interface.

R and T States of Hemoglobin

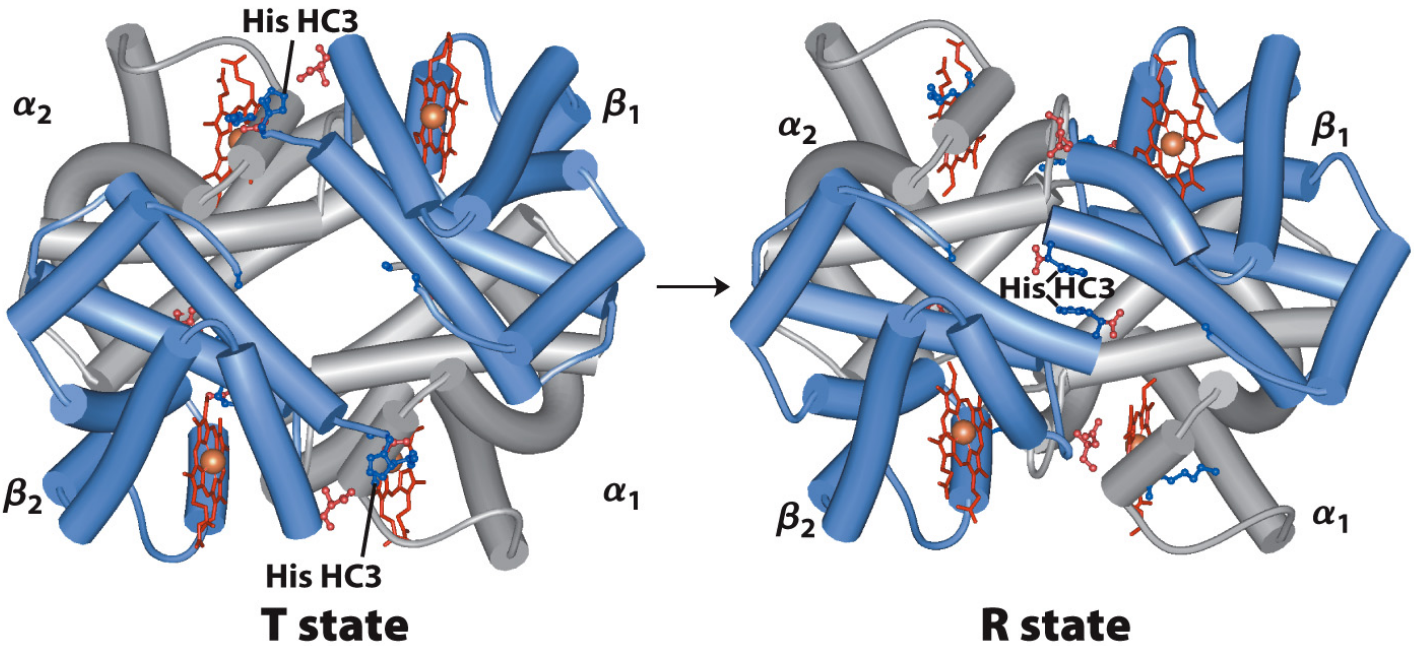

Figure 5-10
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Conformational Change Is Triggered by Oxygen Binding

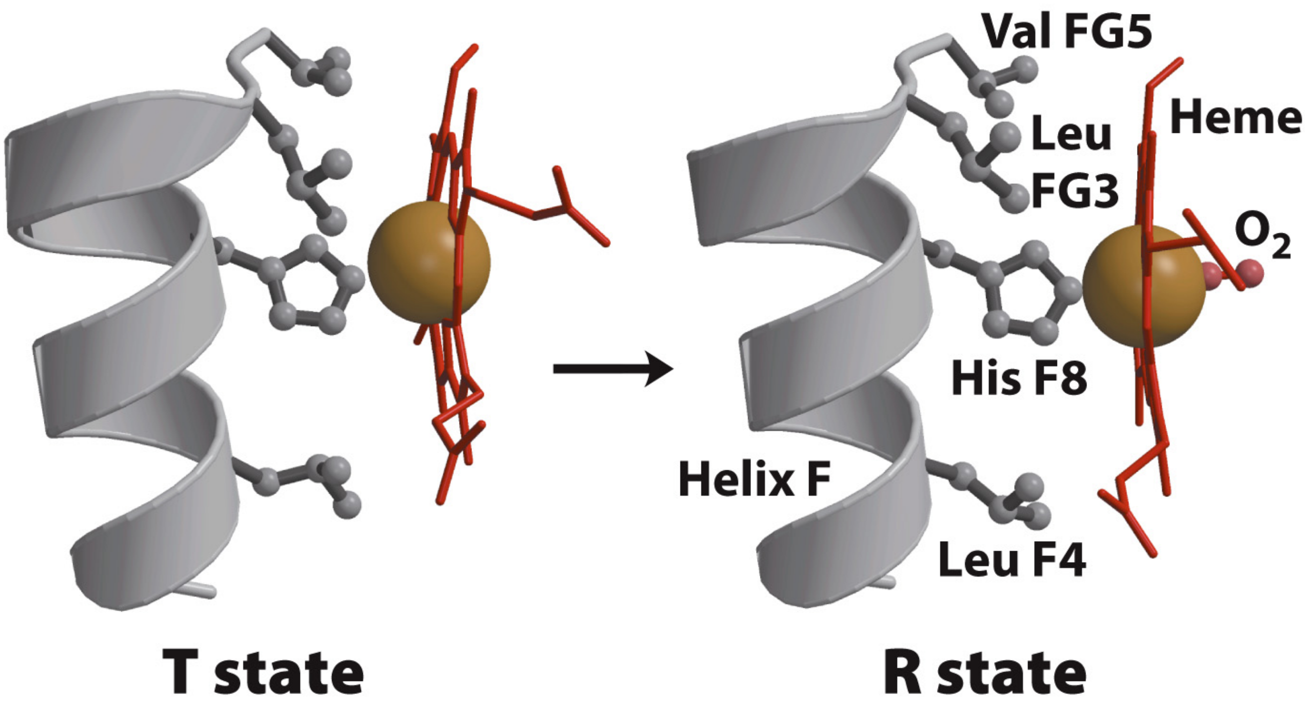

Figure 5-11
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pH Effect on O_2 Binding to Hemoglobin

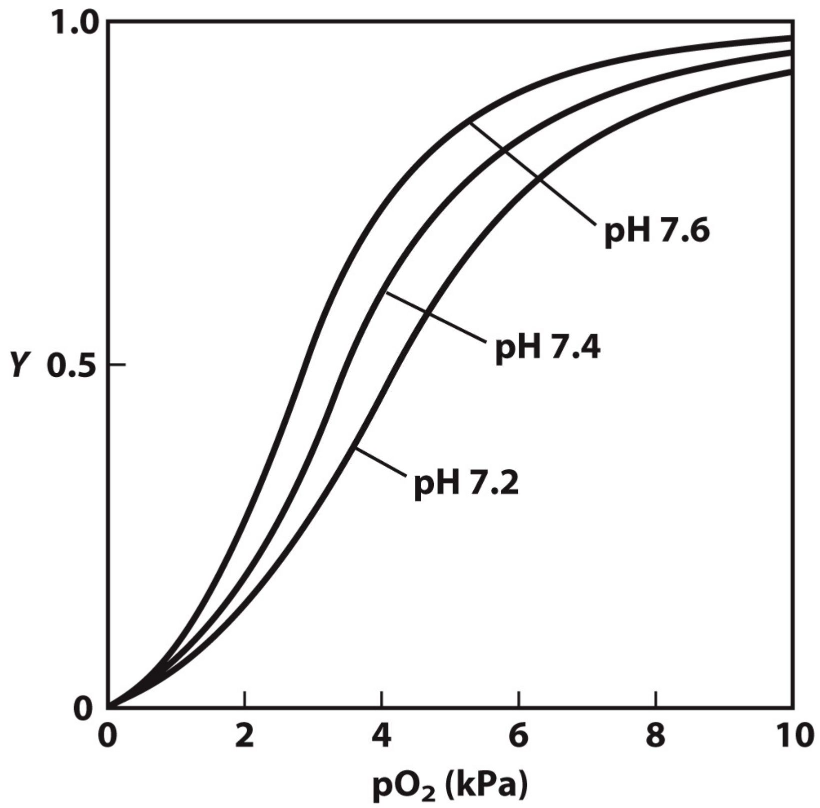

Figure 5-16
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Does acidity increase or decrease the K_d?

pH Effect on O_2 Binding to Hemoglobin

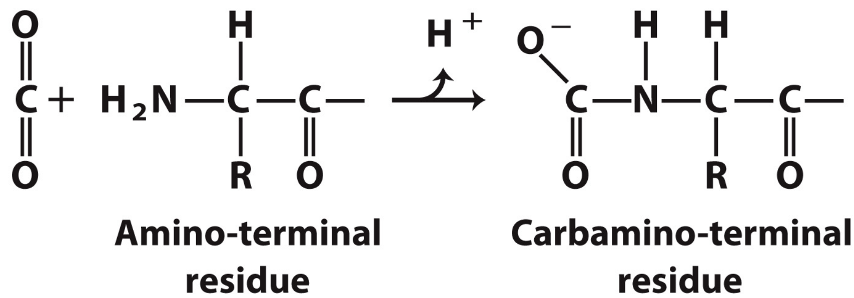
- Actively metabolizing tissues generate H^+, lowering the pH of the blood near the tissues relative to the lungs (catalyzed by carbonic anhydrase).

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

- Hb Affinity for oxygen depends on the pH.
  - H^+ binds to Hb and stabilizes the T state.
    - protonates His146, which then forms a salt bridge with Asp94
    - leads to the release of O_2 (in the tissues)
- The pH difference between lungs and metabolic tissues increases efficiency of the O_2 transport.
- This is known as the Bohr effect.

Hemoglobin and CO_2 Export

- CO_2 is produced by metabolism in tissues and must be exported.
- 15–20% of CO_2 is exported in the form of a carbamate on the amino t eptide subunits.



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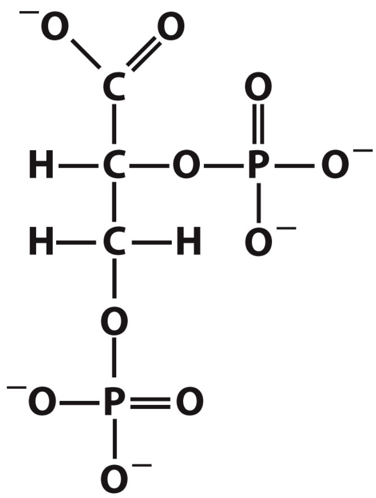
- Notice:
  - The formation of a carbamate yields a proton that can contribute to the Bohr effect.
  - The carbamate forms additional salt bridges, stabilizing the T state.

2,3-Bisphosphoglycerate Regulates O_2 Binding

- Negative heterotropic regulator of Hb function
- Present at mM concentrations in erythrocytes
  - produced from an intermediate in glycolysis
- Small negatively charged molecule, binds to the positively charged central cavity of Hb
- Stabilizes the T states



2,3-Bisphosphoglycerate

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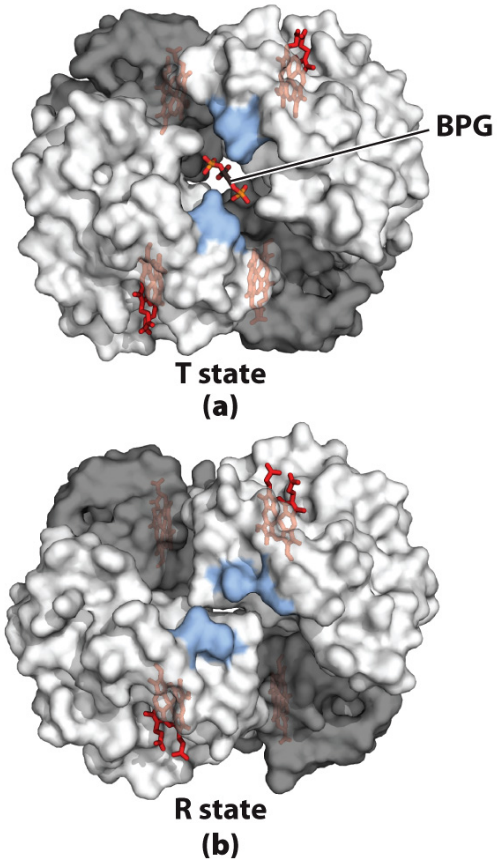
2,3-BPG Binds to the Central Cavity of hB



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2,3-BPG Allows for O_2 Release in the Tissues and Adaptation to Changes in Altitude

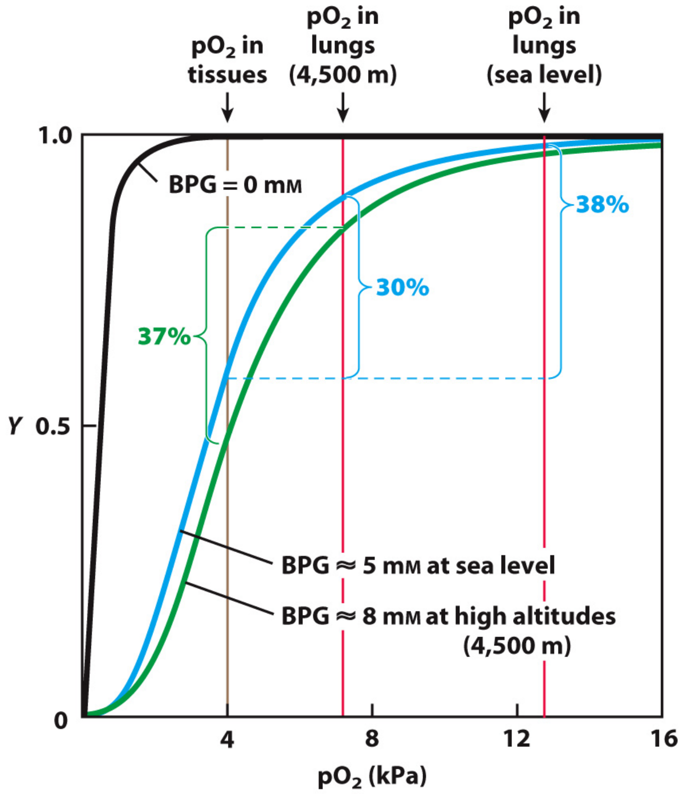

Figure 5-17
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