Protein Function

- Methods of binding ligands and proteins
- Quantitative and graphical modeling of protein-ligand interactions
- Interaction of globins with oxygen and non-oxygen ligands
- Physiological regulation of oxygen binding

Function of Globular Proteins

- Reversible binding of ligands is essential.
  - specificity of ligands and binding sites
  - Ligand binding is often coupled to conformational changes, sometimes quite dramatically (**induced fit**).
  - In multisubunit proteins, conformational changes in one subunit can affect the others (**cooperativity**).
  - Interactions can be regulated.
- Examples:
  - hemoglobin, antibodies, and muscle proteins

Globular Proteins

Functions

- Storage of ions and molecules
  - myoglobin, ferritin
- Transport of ions and molecules
  - hemoglobin, serotonin transporter
- Defense against pathogens
  - antibodies, cytokines
- Muscle contraction
  - actin, myosin
- Biological catalysis
  - chymotrypsin, lysozyme

Interactions

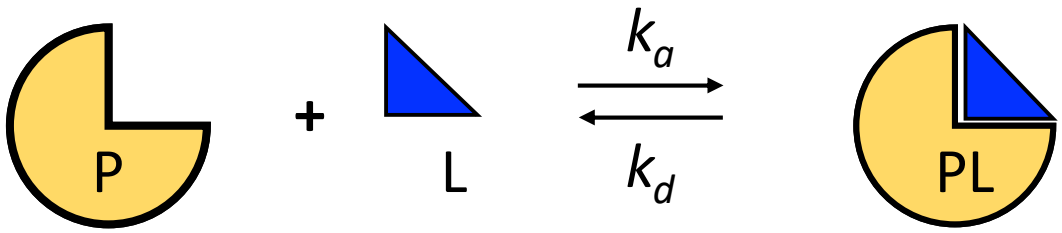
- Reversible, transient process of chemical equilibrium:

$$A + B \leftrightarrow AB$$

- A molecule that binds to a protein is called a ligand.
- A region in the protein where the ligand binds is called the binding site.
- Ligand binds via noncovalent interactions: allows the interactions to be transient

Binding: Quantitative Description

- Consider a process in which a ligand (L) binds reversibly to a site in a protein (P).



- This interaction can be described quantitatively by the association rate constant k_a or the dissociation rate constant k_d.
- After some time, the process will reach the equilibrium where the association and dissociation rates are equal.

$$k_a[\mathrm{P}]\cdot[\mathrm{L}] = k_d[\mathrm{PL}]$$

- The equilibrium composition is characterized by the equilibrium association constant K_a or the equilibrium dissociation constant, K_d.

$$\mathrm{K_a} = \frac{[\mathrm{PL}]}{[\mathrm{P}]\cdot[\mathrm{L}]} = \frac{1}{\mathrm{K_d}}$$

Binding: Analysis of the bound fraction

- In practice, we can often determine the fraction of occupied binding sites (θ).

$$\theta = \frac{[\mathrm{PL}]}{[\mathrm{PL}]+[\mathrm{P}]}$$

$$K_a = \frac{[\mathrm{PL}]}{[\mathrm{P}]\cdot[\mathrm{L}]}$$

- Substituting [PL] with K_a[L][P], eliminate [PL].

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

- Eliminating [P] and rearranging gives the result in terms of equilibrium association constant.

$$\theta = \frac{[\mathrm{L}]}{[\mathrm{L}]+\frac{1}{K_a}}$$

- In terms of the more commonly used equilibrium dissociation constant:

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

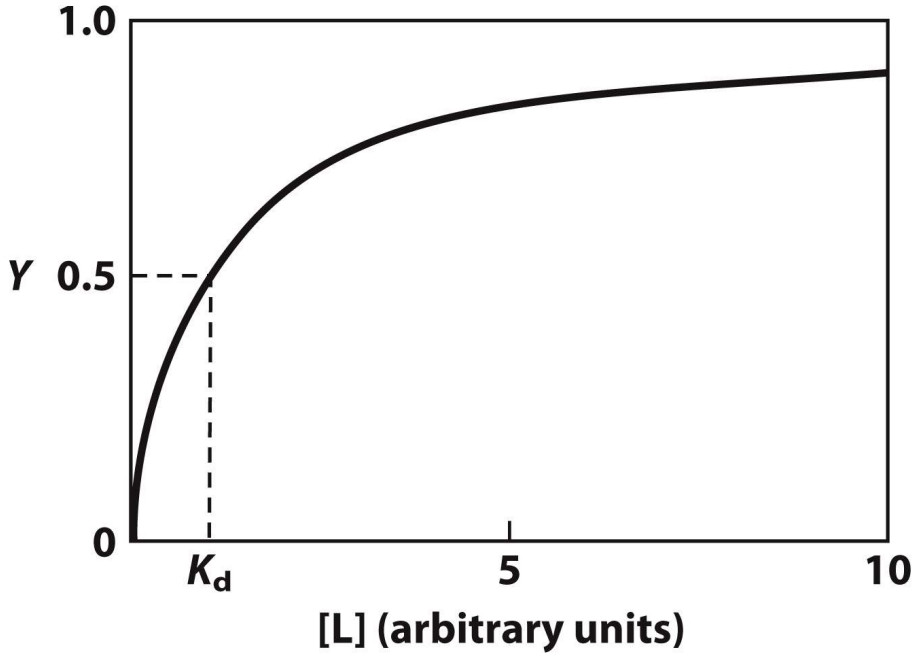
Binding: Graphical Analysis

- The fraction of bound sites depends on the free ligand concentration and K_d.

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

- Experimentally:
  - ligand concentration is known
  - K_d can be determined graphically or via least-squares regression

$$[\mathrm{L}] \approx [\mathrm{L}]_{\text{total}}$$



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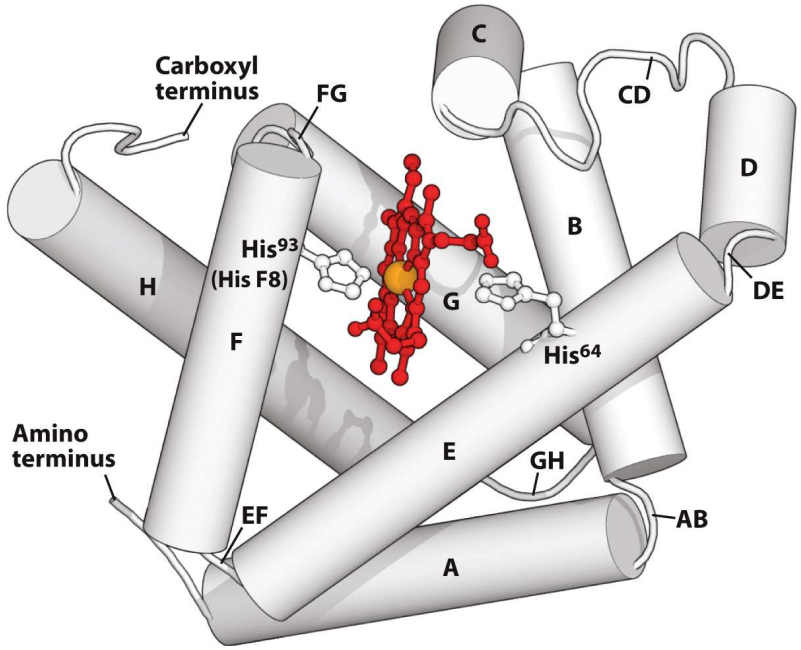
Example: Oxygen Binding to Myoglobin



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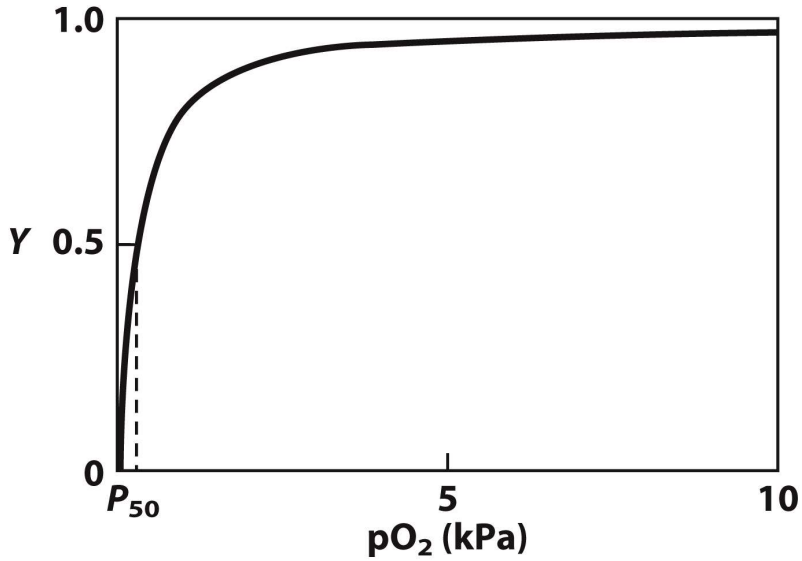

Figure 5-4b
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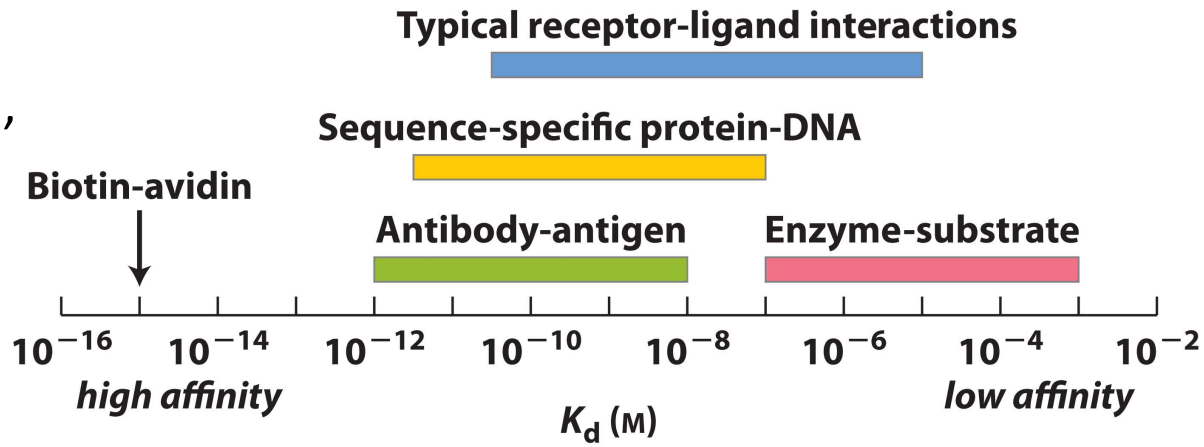
When a ligand is a gas, binding is expressed in terms of partial pressures.

$$\theta = \frac{[\mathrm{L}]}{K_\mathrm{d} + [\mathrm{L}]} \longrightarrow \theta = \frac{p\mathrm{O}_2}{p_{50} + p\mathrm{O}_2}$$

Binding: Thermodynamic Connections

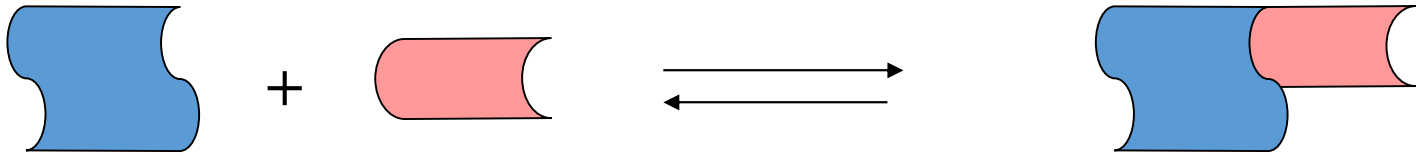
- Interaction strength can be expressed as:
  - association (binding) constant K_a, units M^{-1}
  - dissociation constant K_d, units M, $K_d = 1/K_a$
  - interaction (binding) free energy ΔG^o, units: kJ/mol

  Definitions
  - $\Delta G^o = \Delta H^o - T\Delta S^o$: enthalpy and entropy
  - $K_a = [PL]/[P][L]$ $K_d = [P][L]/[PL]$
- Relationships
  - $\Delta G^o = -RT \ln K_a = RT \ln K_d$ (RT at 25°C is 2.48 kJ/mol.)
- Magnitudes
  - strong: $K_d < 10$ nM,
  - weak: $K_d > 10$ μM



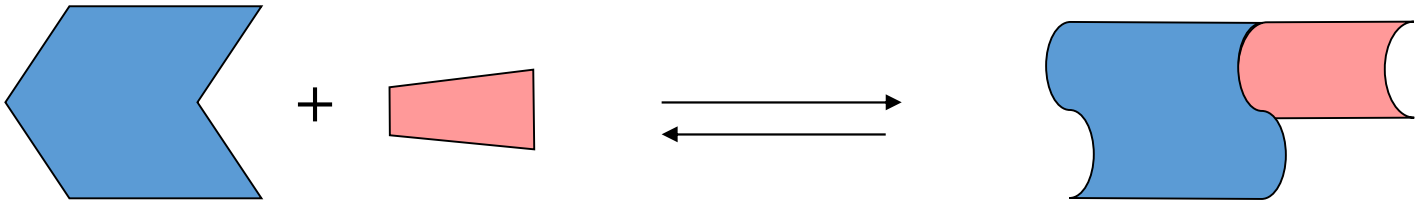
Specificity: Lock-and-Key Model

- 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.



Specificity: Induced Fit

- Conformational changes may occur upon ligand binding (Daniel Koshland in 1958).
  - This adaptation is called the induced fit.
  - Induced fit allows for tighter binding of the ligand.
  - Induced fit allows for high affinity for different ligands.
- Both the ligand and the protein can change their conformations.



Globins: Oxygen-Binding Proteins

Biological problems:

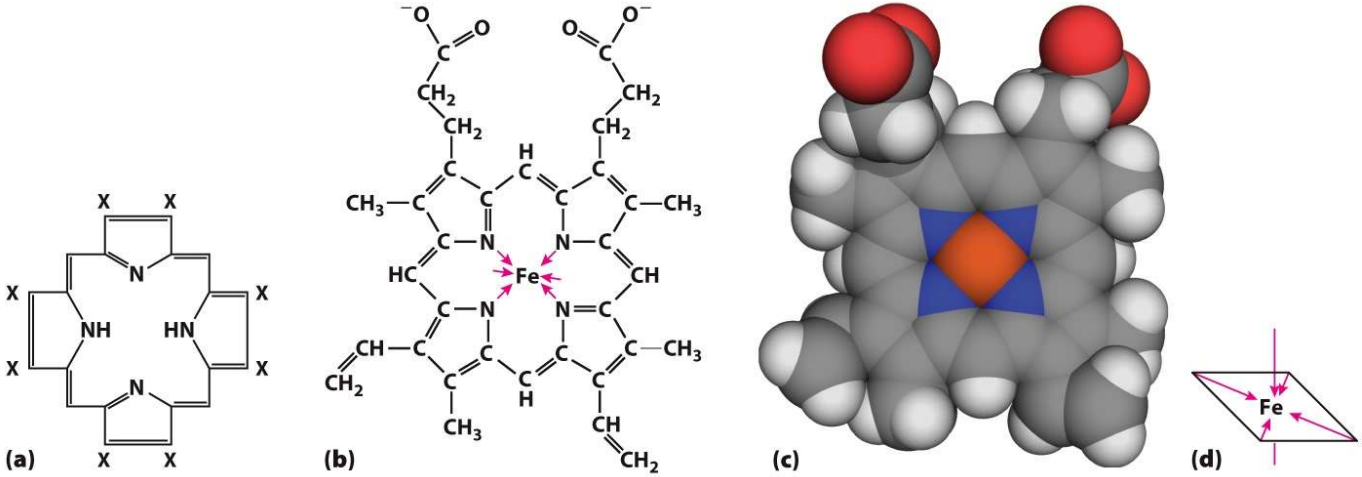
- Protein side chains lack affinity for O_2.
- Some transition metals bind O_2 well but would generate free radicals if free in solution.
- Organometallic compounds such as heme are more suitable, but Fe^{2+} in free heme could be oxidized to Fe^{3+} (very reactive!).

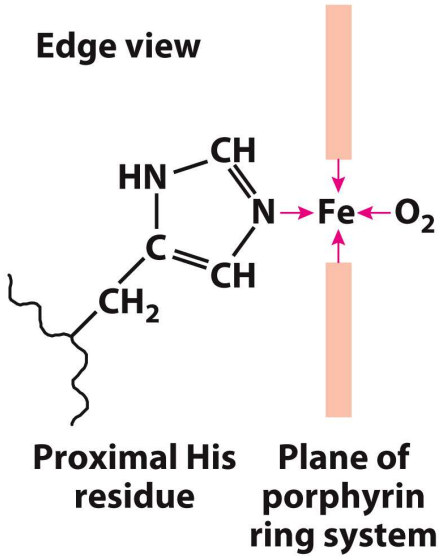
Biological solution:

- 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, Heme, Myoglobin





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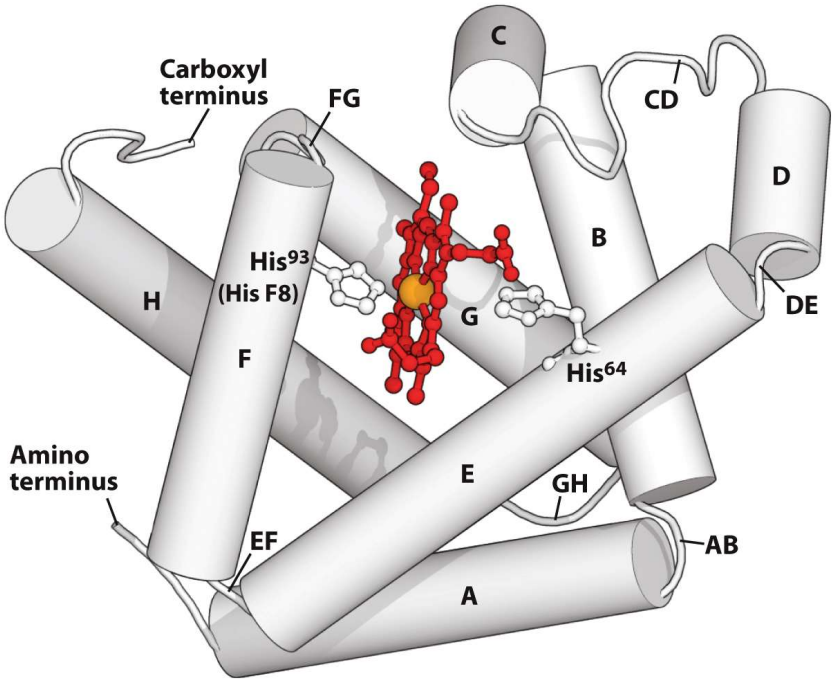

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CO vs O_2 binding to heme

- 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.

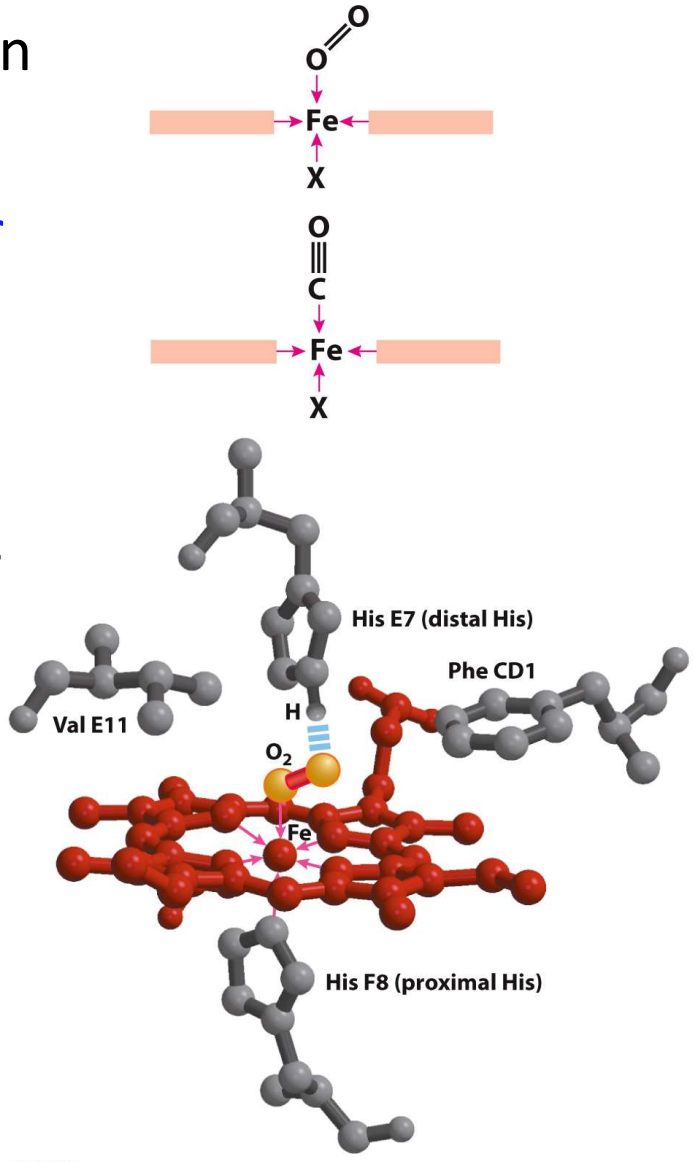

Figure 5-5c
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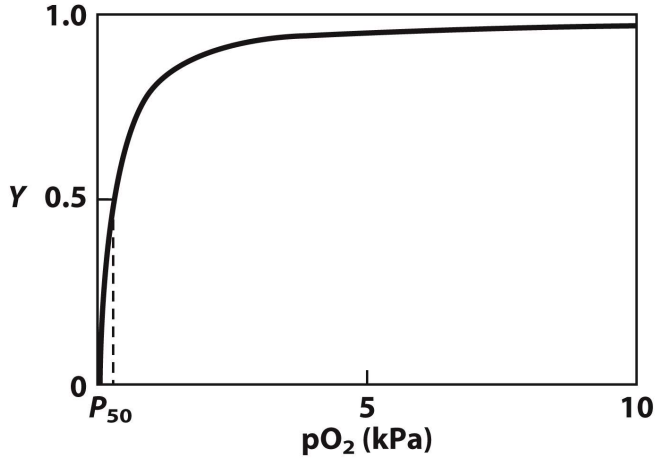
Could Myoglobin Transport O_2?

- pO_2 in lungs is about 13 kPa
- pO_2 in tissues is about 4 kPa



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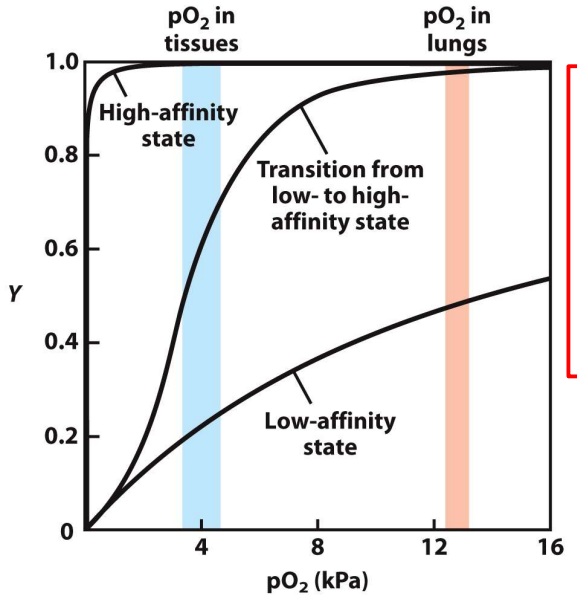

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- Cooperativity
- Two states
- BPG
- pH
- CO_2

Would it work? Why or why not?

If not, how to make it work?: Cooperativity

* cooperativity: multiple binding sites with mutual interactions.

positive (sigmoidal binding curve) and negative cooperativity

Cooperativity

- multiple binding sites: K_a=

$$K_a = \frac{[PL_n]}{[P][L]^n}$$

- θ =

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

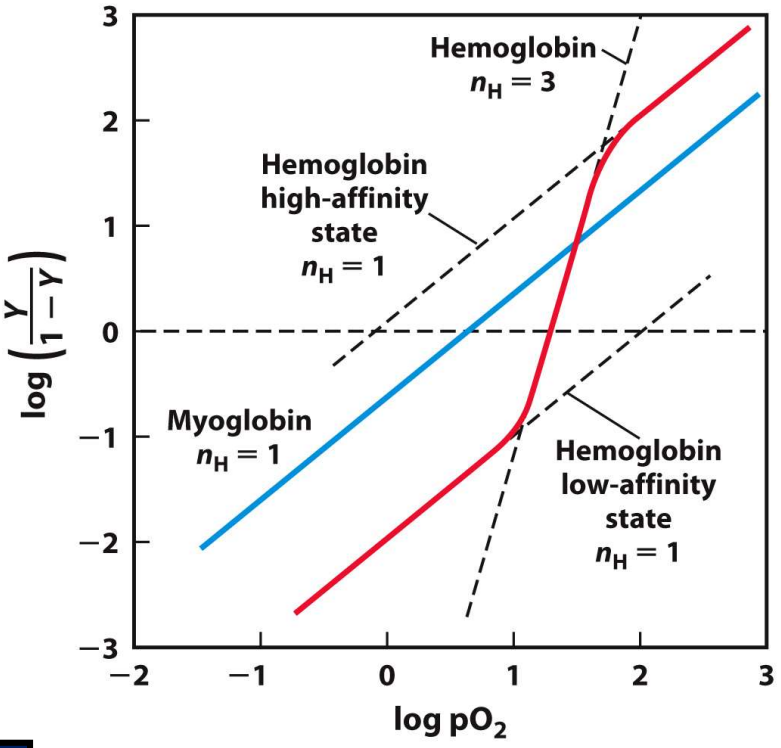
- Taking the log of both sides
- gives the **Hill Equations**:

$$\log\left(\frac{\theta}{1-\theta}\right) = n\log[L] - \log K_d$$

Hill Coefficient (the degree of cooperativity)

n: the n=1: no cooperativity
n>1 : positive cooperativity
n<1 : negative cooperativity

Hill plot of cooperativity



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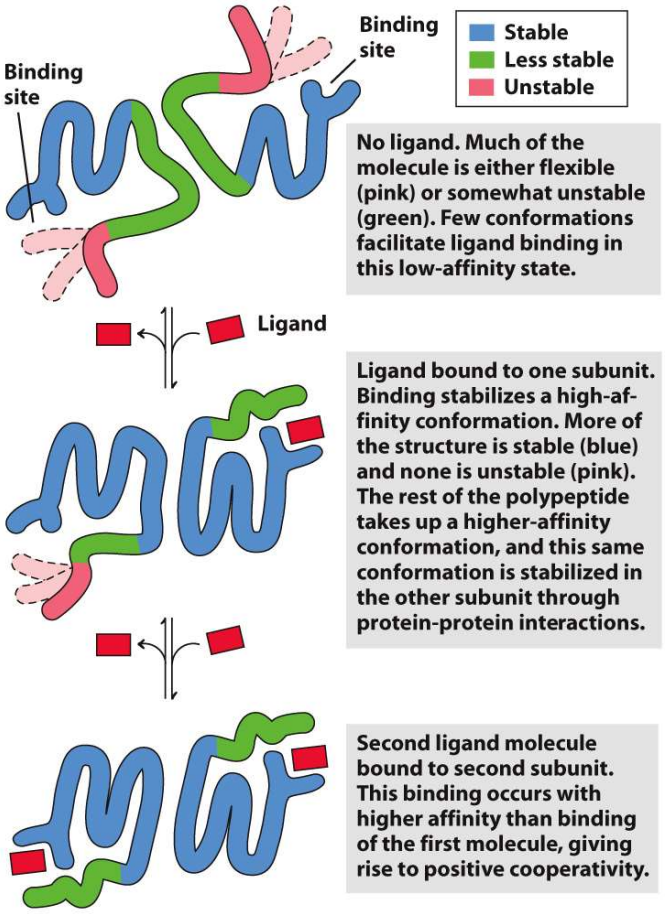
Two Models of Cooperativity: Concerted vs. Sequential



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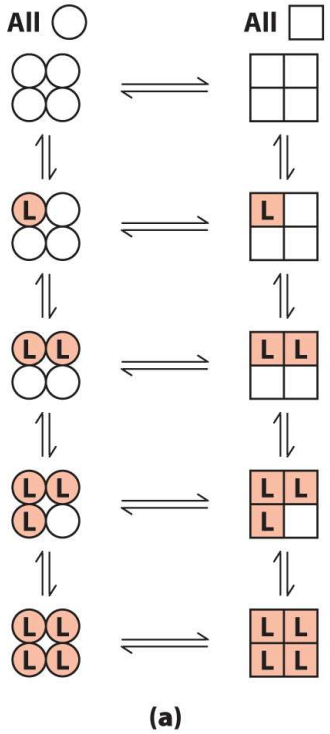

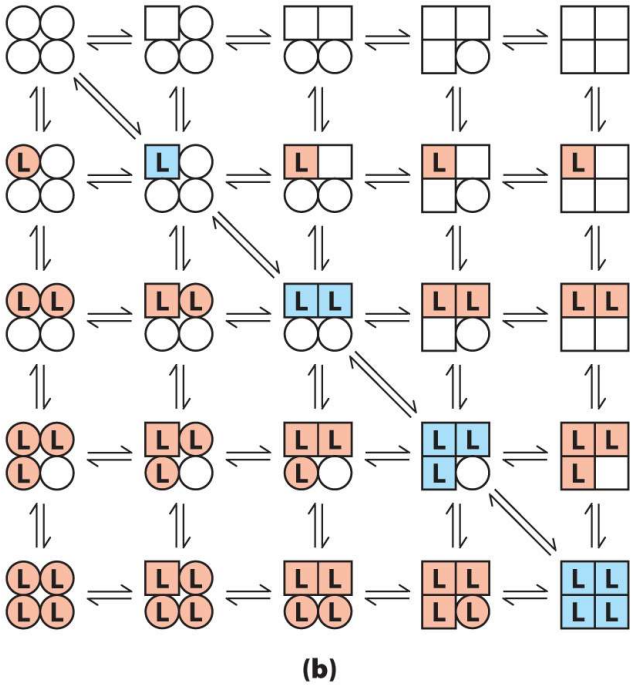

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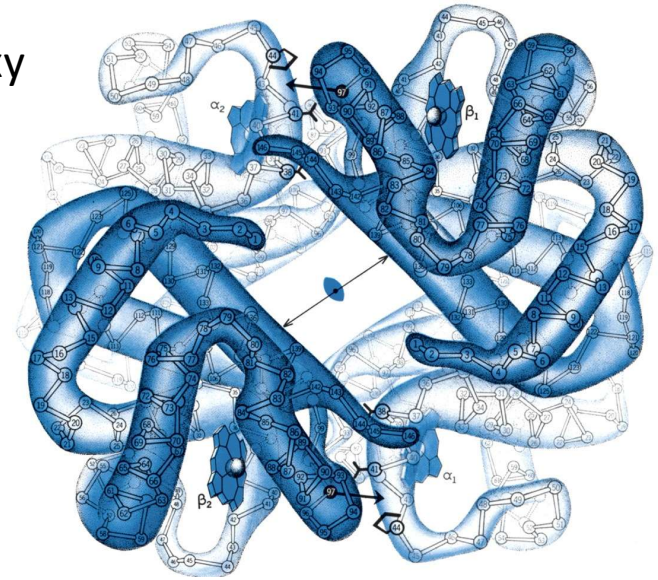
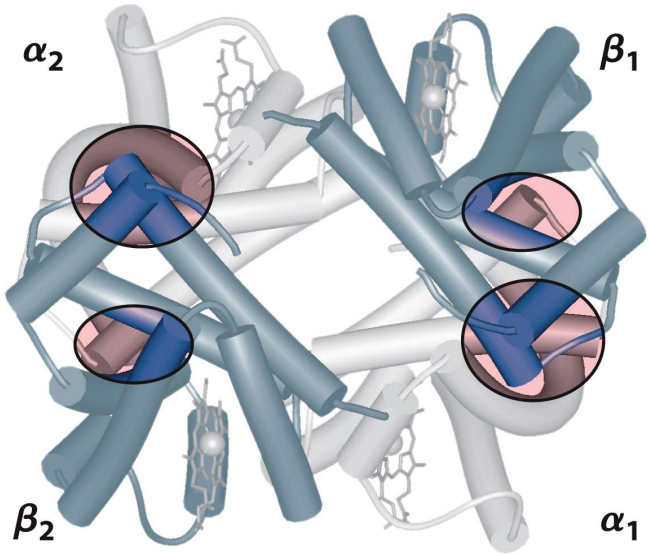
Cooperativity Is a Special Case of Allosteric Regulation

- Allosteric protein
  - Binding of a ligand to one site affects the binding properties of a different site on the same protein.
  - can be positive or negative
  - homotropic
    - The normal ligand of the protein is the allosteric regulator.
  - heterotropic
    - A different ligand affects binding of the normal ligand.
- Cooperativity = positive homotropic regulation
- Hemoglobin (Hb) is a tetramer ($\alpha 2 \beta 2$) with two conformations.
- Hb binds oxygen cooperatively.

Subunit Interactions in Hemoglobin

deoxy





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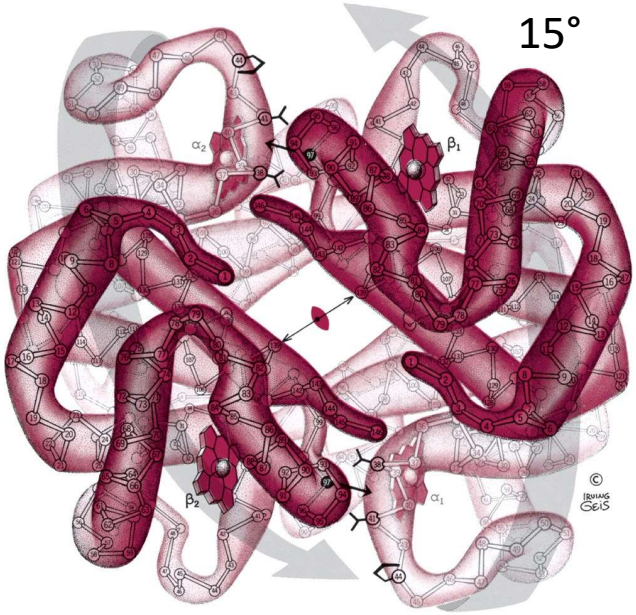
oxy

15°



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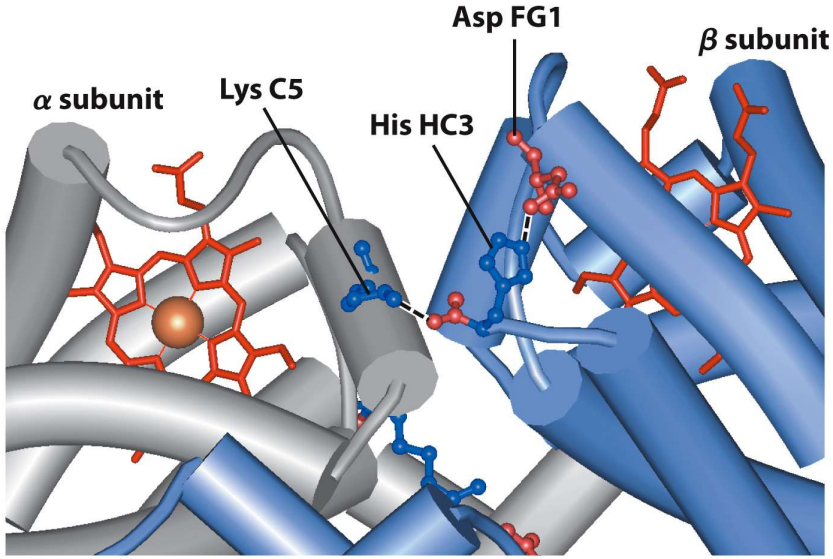

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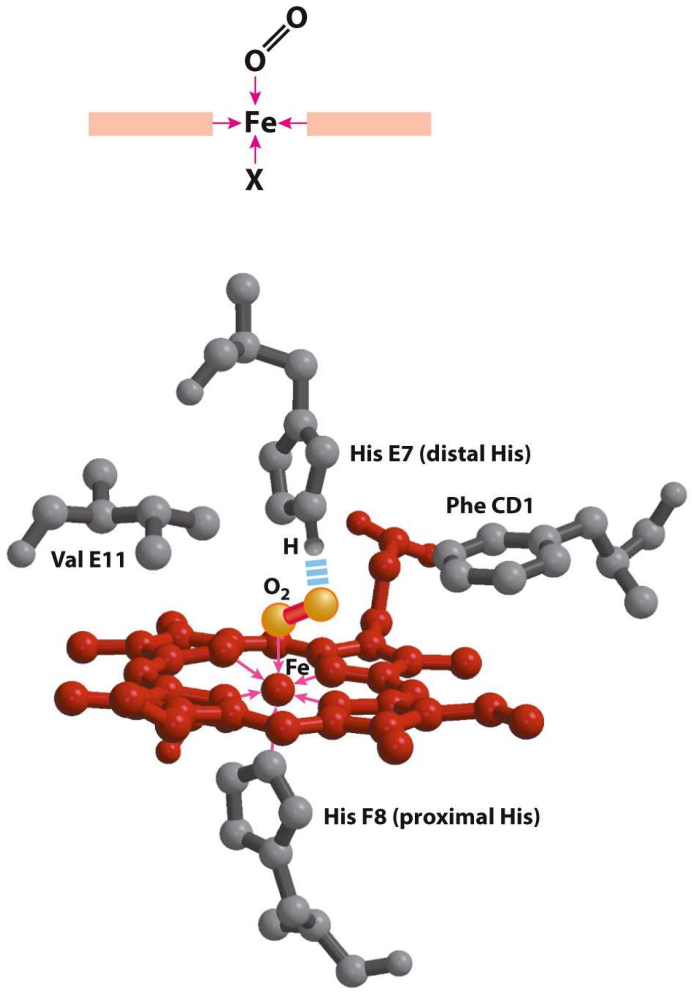
O_2 binding to heme causes shift from T to R



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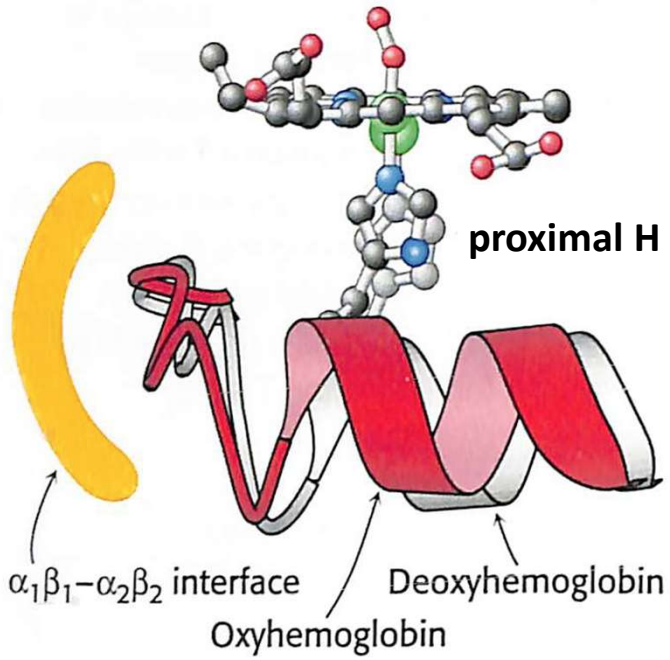


Two States of Hb

- 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 $\alpha 1$-$\beta 2$ interface.

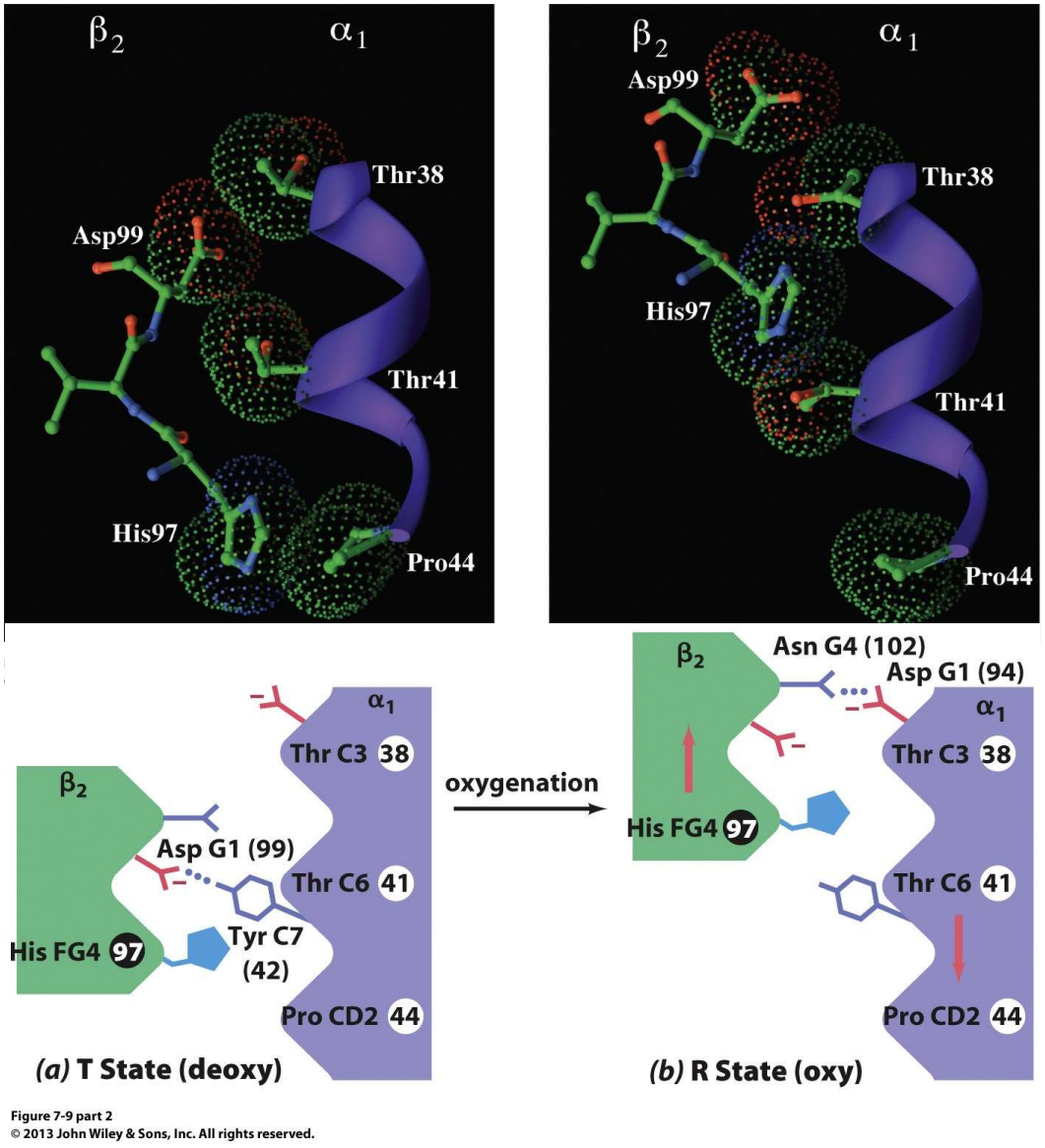


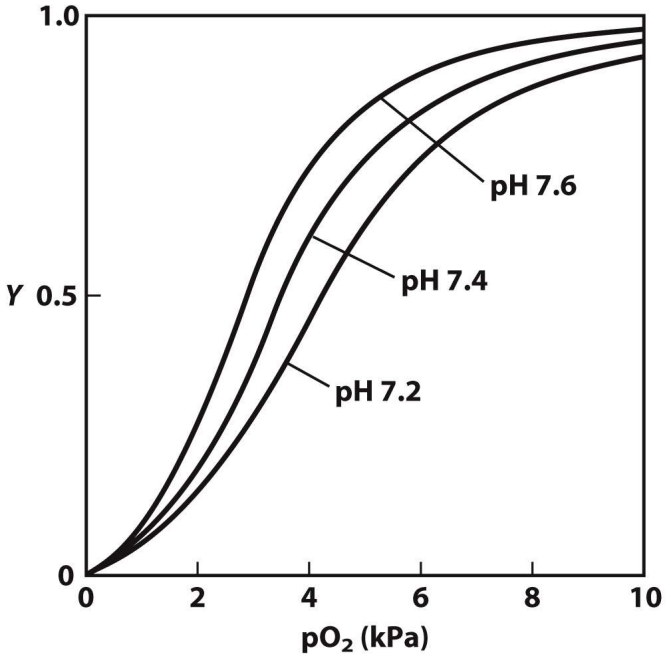
Figure 7-9 part 2

pH Effect on O_2 Binding to Hemoglobin – Bohr effect

- Actively metabolizing tissues (7.4 vs 7.2) 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: Bohr effect



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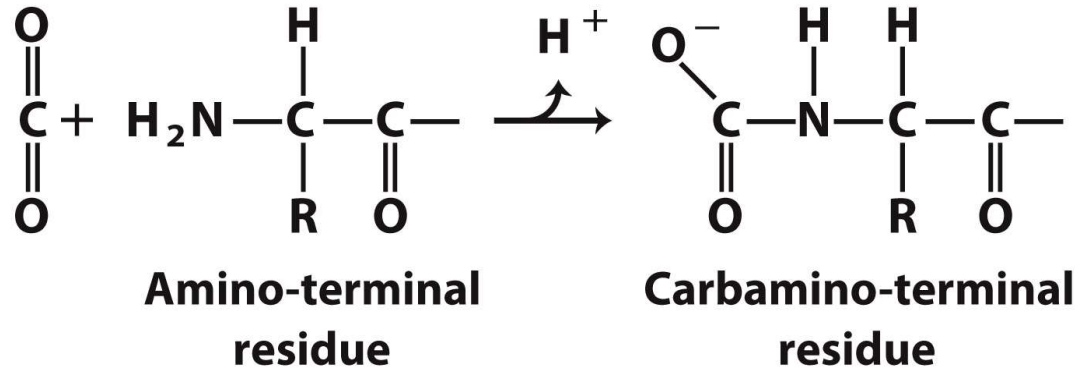
Q: Does acidity increase or decrease the K_d?

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 terminal residues of each of the polypeptide subunits.



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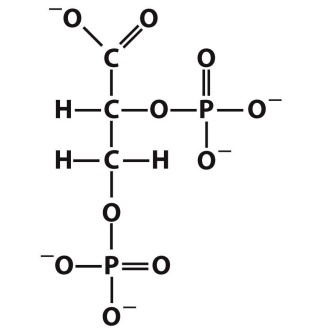
- Notice:
  - The formation of a carbamate yields a proton that can contribute to the Bohr effect.
  - The carbamate forms additional salt bridges, stabilizing the T state.

2,3-BPG Binds to central cavity of Hb

Adaptation to altitude

- Negative regulator – Present at mM concentrations in erythrocytes: an intermediate in glycolysis
- Small negatively charged molecule, binds to the positively charged central cavity of Hb and stabilizes the T states



2,3-Bisphosphoglycerate

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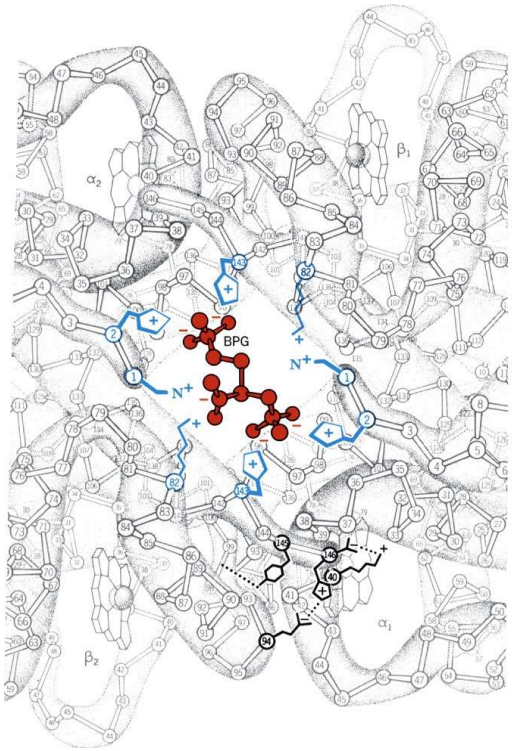

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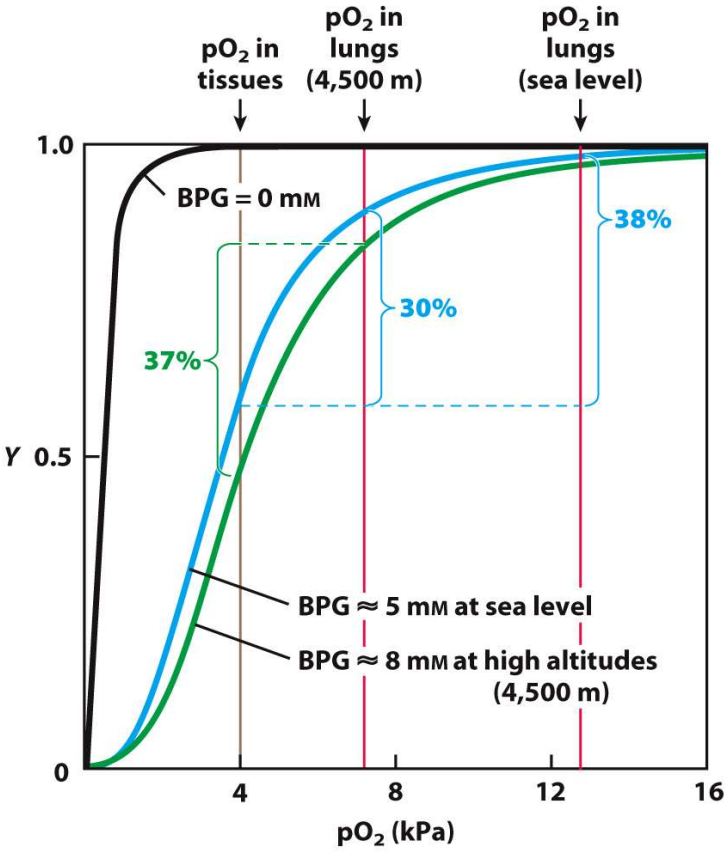

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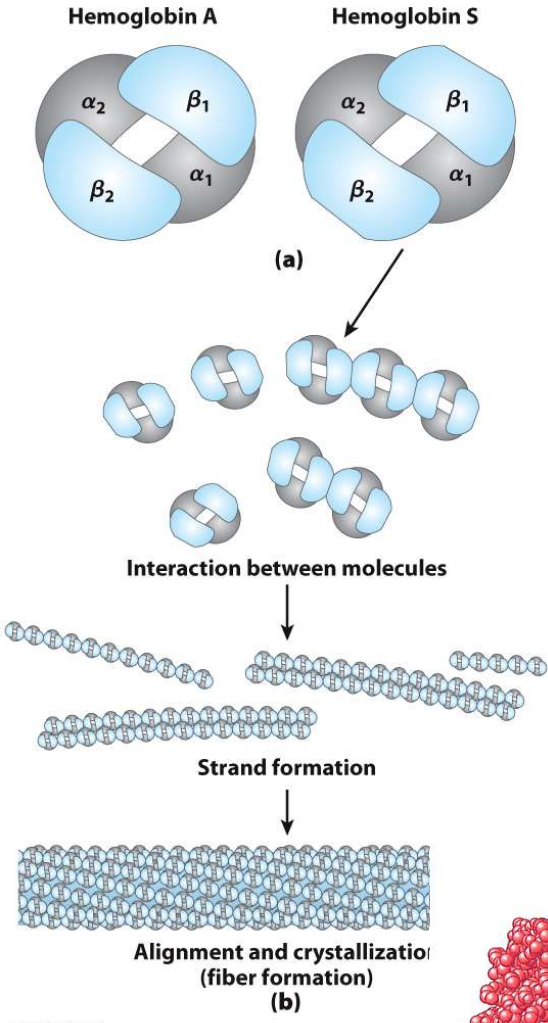
Sickle-Cell Anemia

- Glu6 → Val in the β chain
- The new Val side chain can bind to a different Hb molecule to form a strand similar to the amyloidgenic proteins.
- This sickles the red blood cells.
- Untreated homozygous individuals generally die in childhood.
- Heterozygous individuals exhibit a resistance to malaria.



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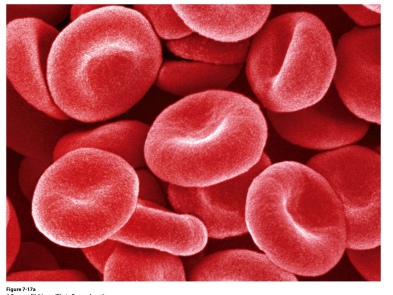

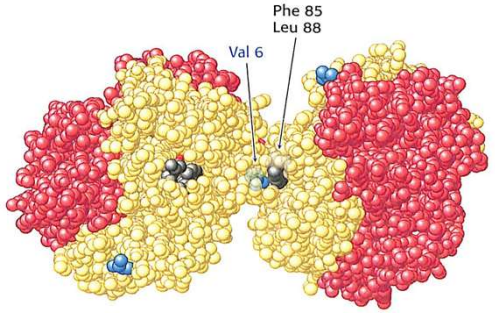

Antibody – Antigen Interaction

: two immune systems

- Cellular immune system
  - targets own cells that have been infected
  - also clears up virus particles and infecting bacteria
  - key players: macrophages, killer T cells (T_c), and inflammatory T cells (TH_1)

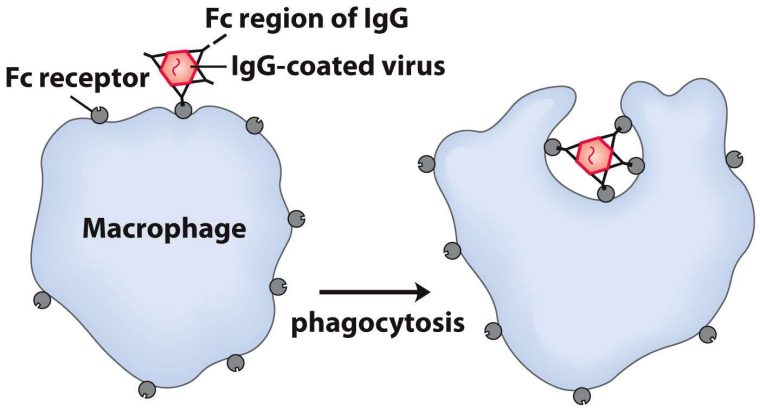

Figure 5-24
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- Humoral "fluid" immune system
  - targets extracellular pathogens
  - can also recognize foreign proteins
  - makes soluble antibodies
  - keeps "memory" of past infections
  - key players: B-lymphocytes and helper T-cells (TH_2)

Humoral Immune System

Fight infections with antibodies that specifically bind antigens.

- Antigens are substances that stimulate production of antibodies.
  - typically macromolecular in nature
  - recognized as foreign by the immune system
  - coat proteins of bacteria and viruses
  - surface carbohydrates of cells or viruses
- Antibodies are proteins that are produced and secreted by B cells and that specifically bind to antigens.
  - Binding will mark the antigen for destruction or interfere with its function.
  - A given antibody will bind to a small region (epitope) of the antigen.
  - One antigen can have several epitopes.

Antibodies: Immunoglobulin G

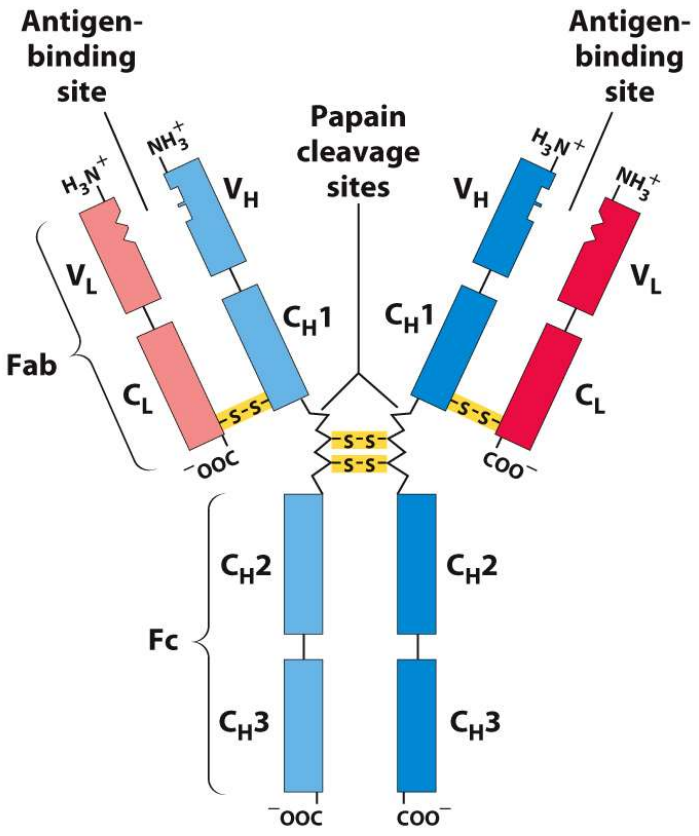
Two **heavy chains** and two **light chains**

- composed of constant domains and variable domains

Light chains: one constant and one variable domain

Heavy chains: three constant and one variable domain

Variable domains of each chain make up the antigen-binding site (two per antibody) and are hypervariable, which confers antigen specificity.



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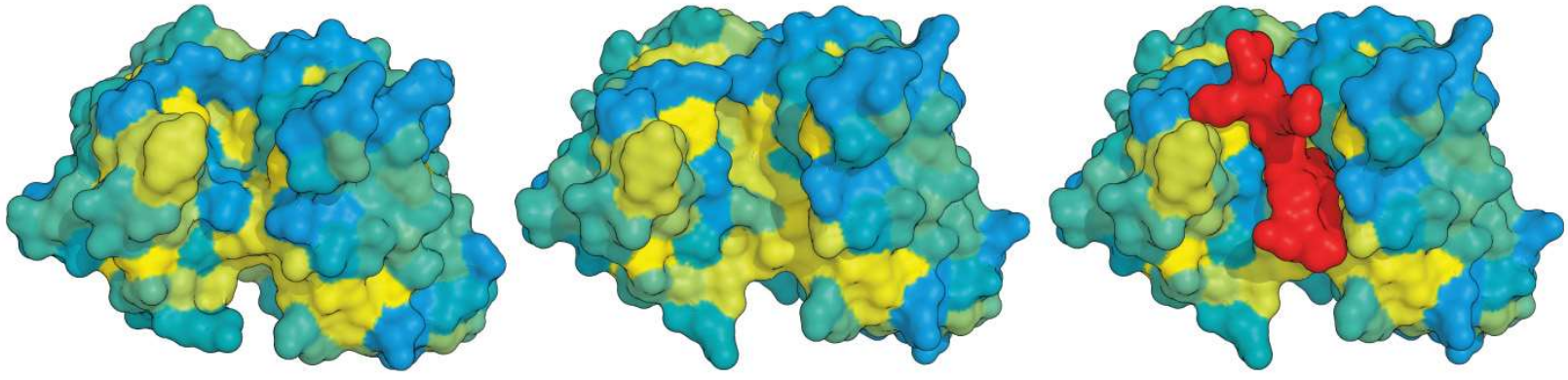
Antigen binding via Induced Fit



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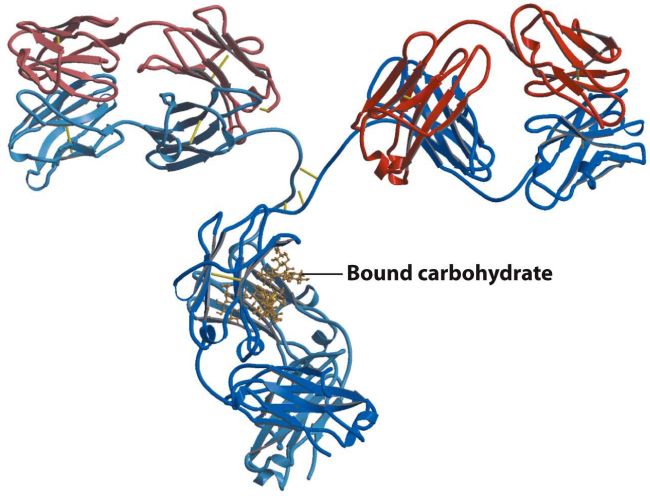

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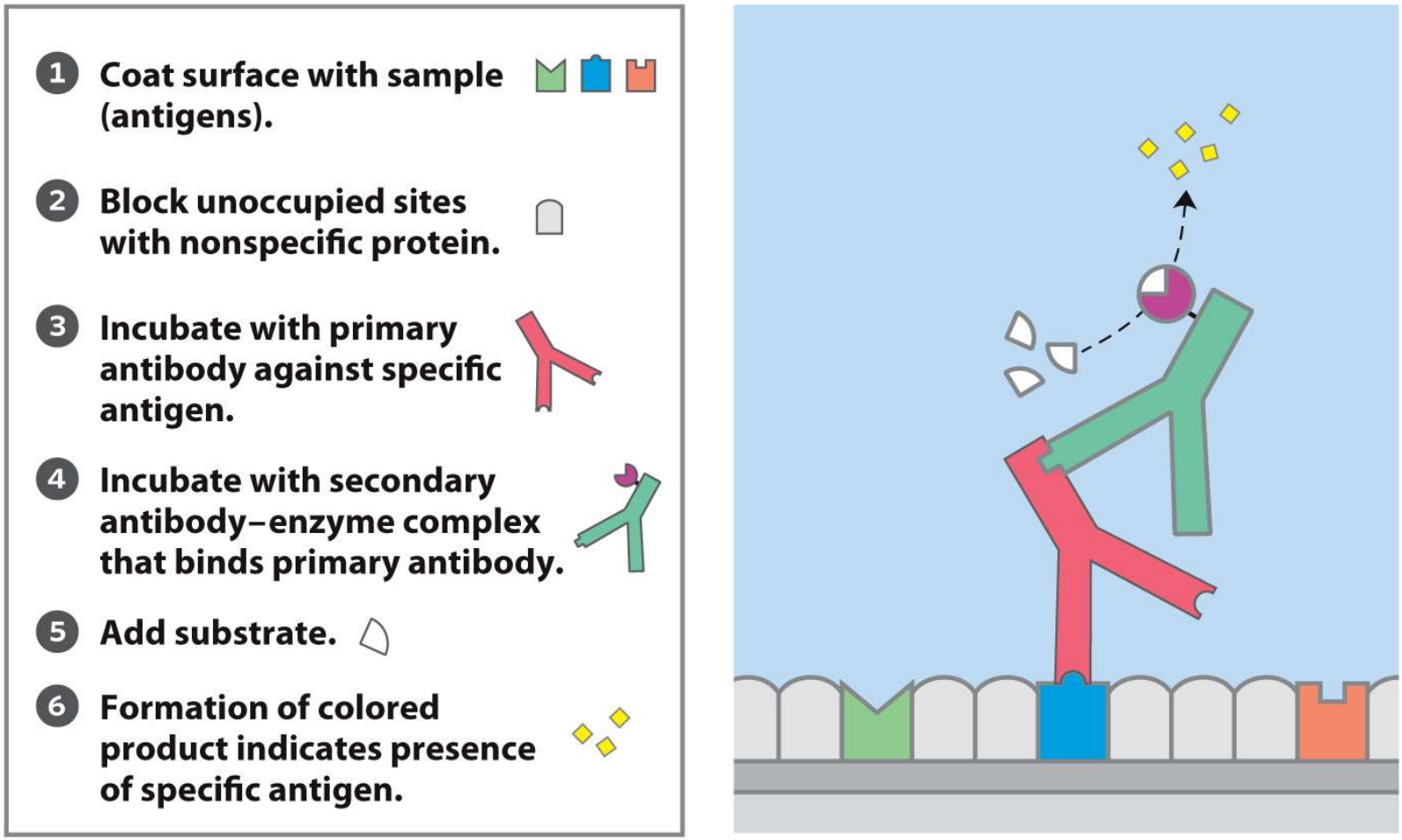
Antibody Specificity Is an Important Analytical Reagent



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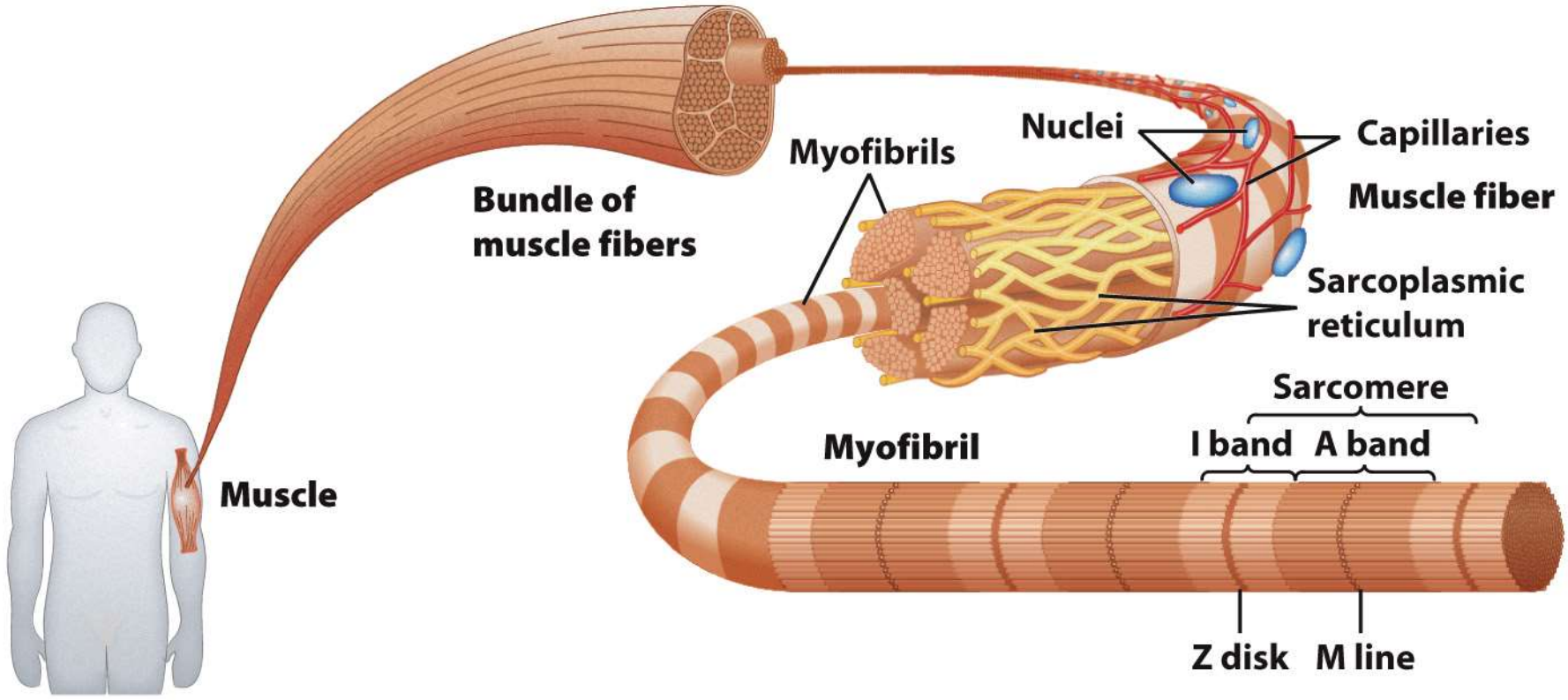
Muscle Proteins

- Muscle fiber: large, single, elongated, multinuclear cell
- Each fiber contains about 1,000 myofibrils.



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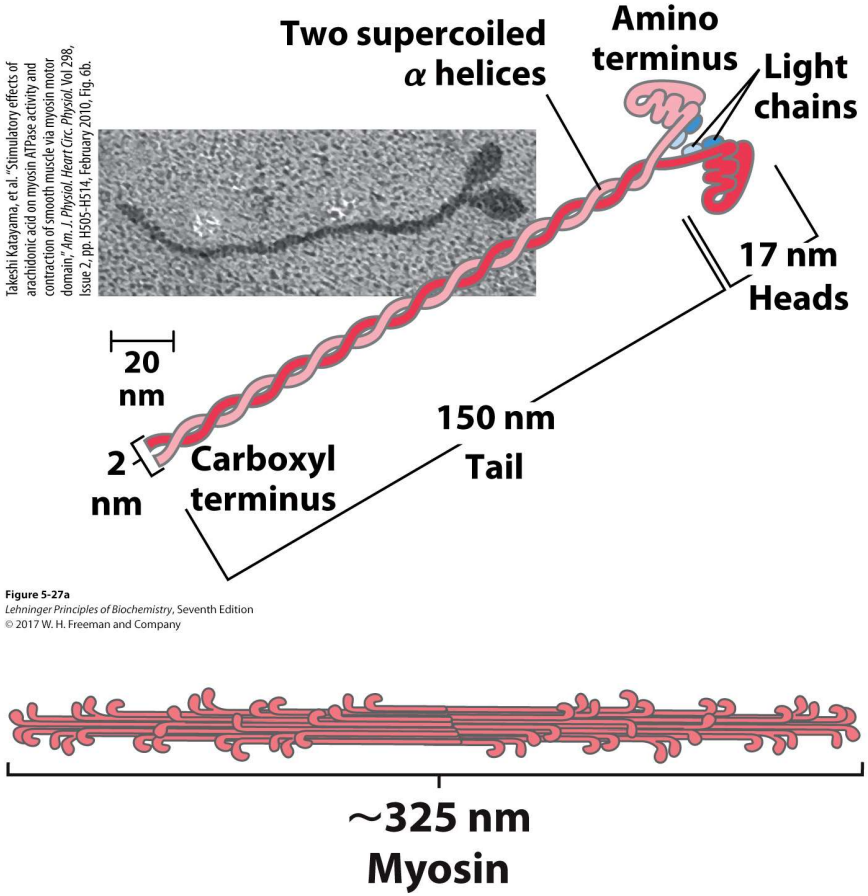
Myofibrils = Myosin filament (thick) + Actin filament (thin)



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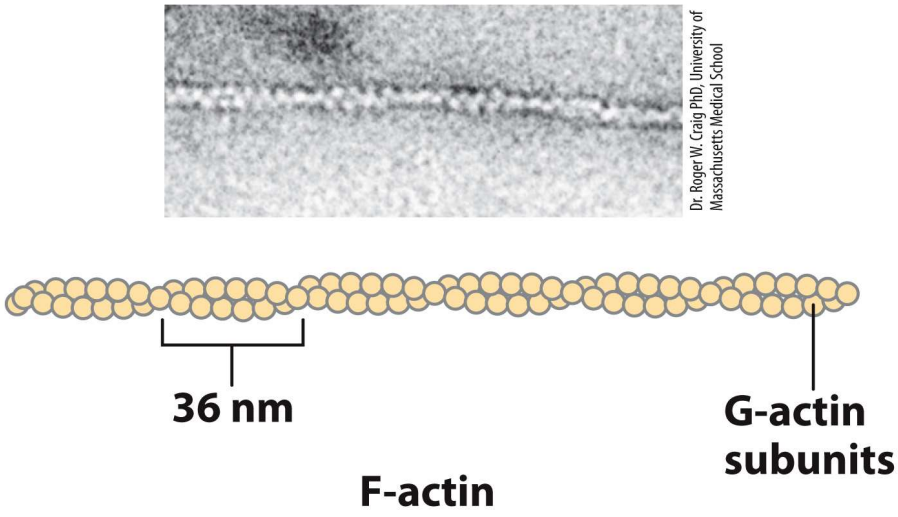

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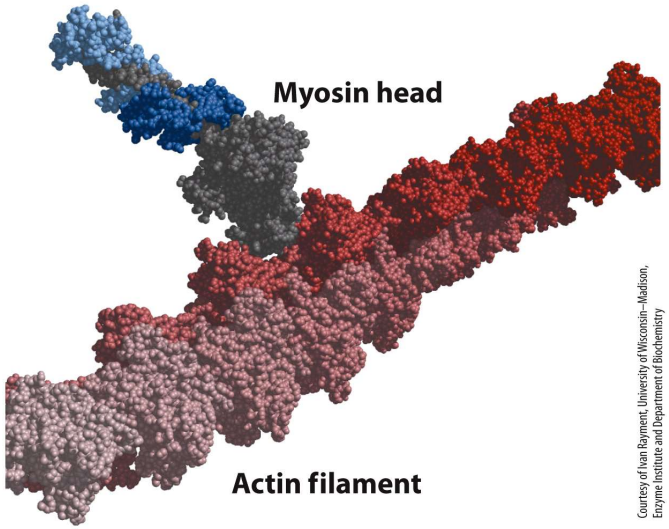
Myosin Thick Filaments Slide Along Actin Thin Filaments



Courtesy of Ivan Rayment, University of Wisconsin–Madison, Enzyme Institute and Department of Biochemistry

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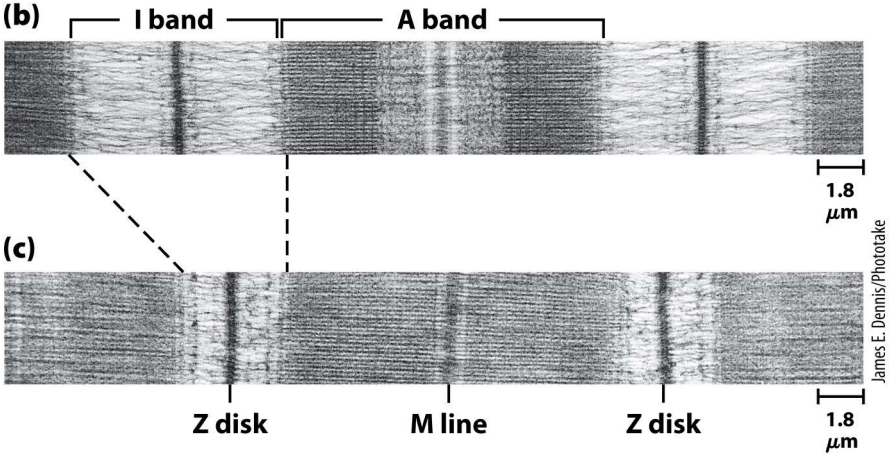

James E. Dennis/Phototake

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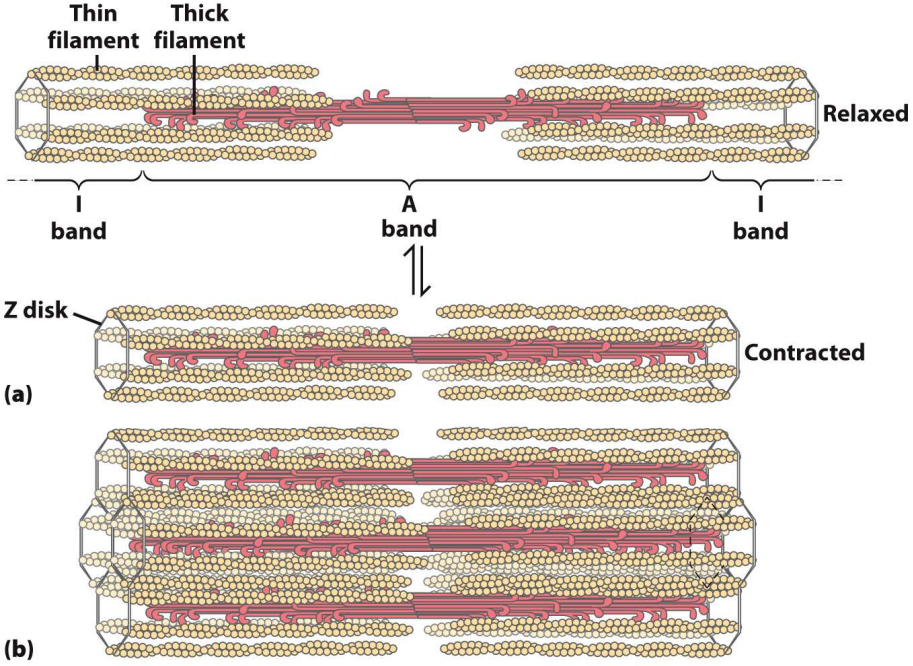

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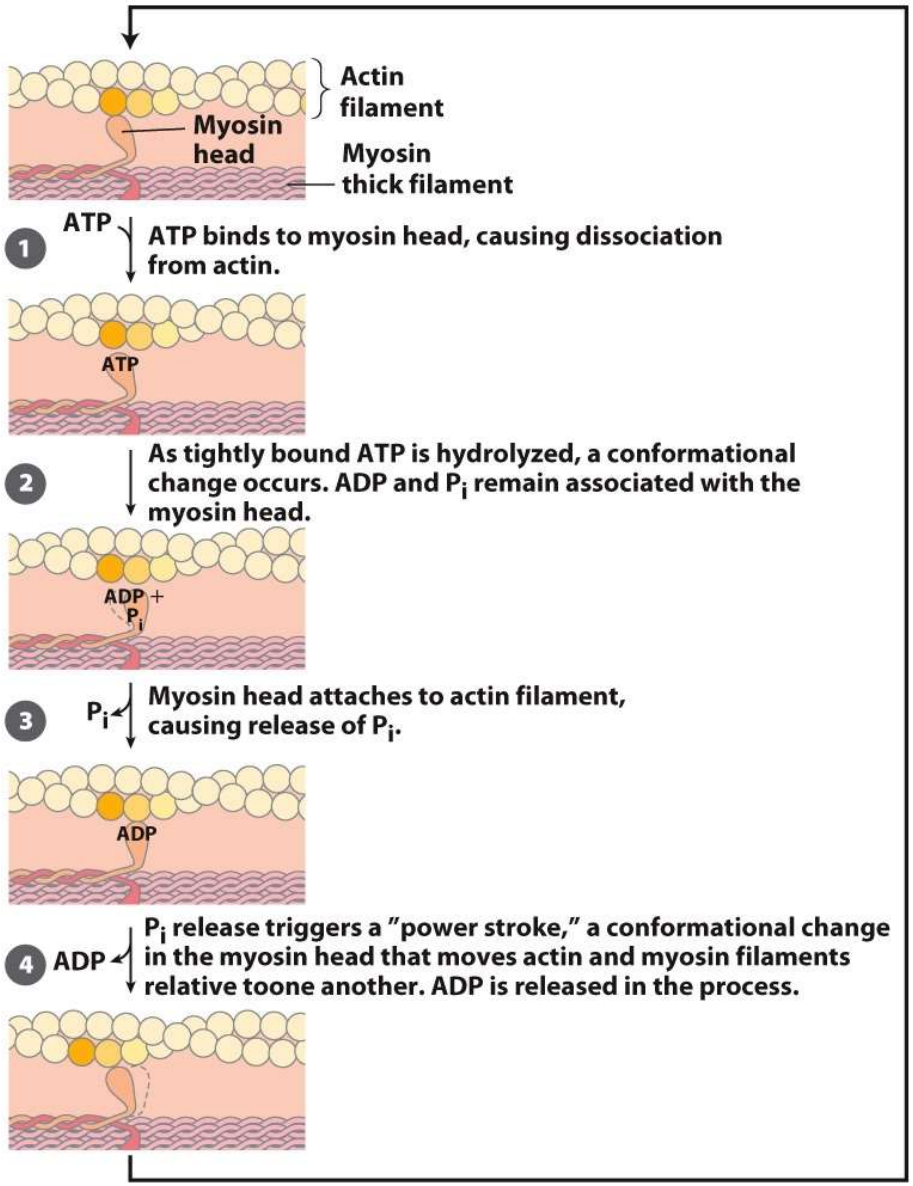
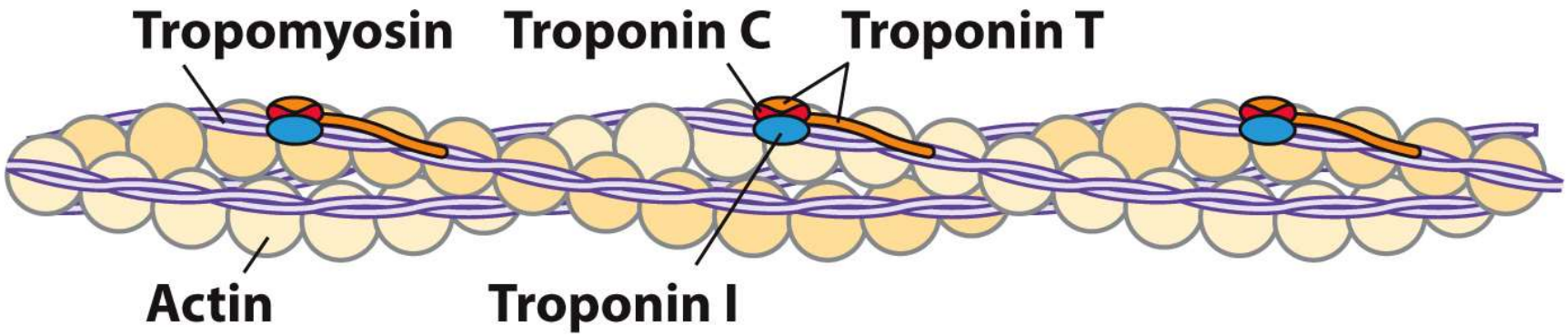
Actomyosin Cycle by ATP

- Use of chemical energy (ATP) can cause conformational changes in proteins, generally required for their function.
- Especially in motor proteins
  - control movement of cells and organelles within cells
  - Muscle contraction occurs through a series of conformational changes to protein structure due to binding, hydrolysis, and release of ATP and ADP.



Regulation of Muscle Contraction

- Availability of myosin-binding sites on actin is regulated by troponin and tropomyosin.
  - avoids continuous muscle contraction
- Nerve impulse triggers release of Ca^{2+}.
  - causes conformational changes to tropomyosin-troponin complex, exposing myosin-binding sites



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Summary

- how ligand binding can affect protein function
- how to quantitatively analyze binding data
- how myoglobin stores oxygen
- how hemoglobin transports O_2, protons, and CO_2
- how antibodies recognize foreign structures
- how muscle works