Lehninger

SIXTH EDITION

Principles of Biochemistry

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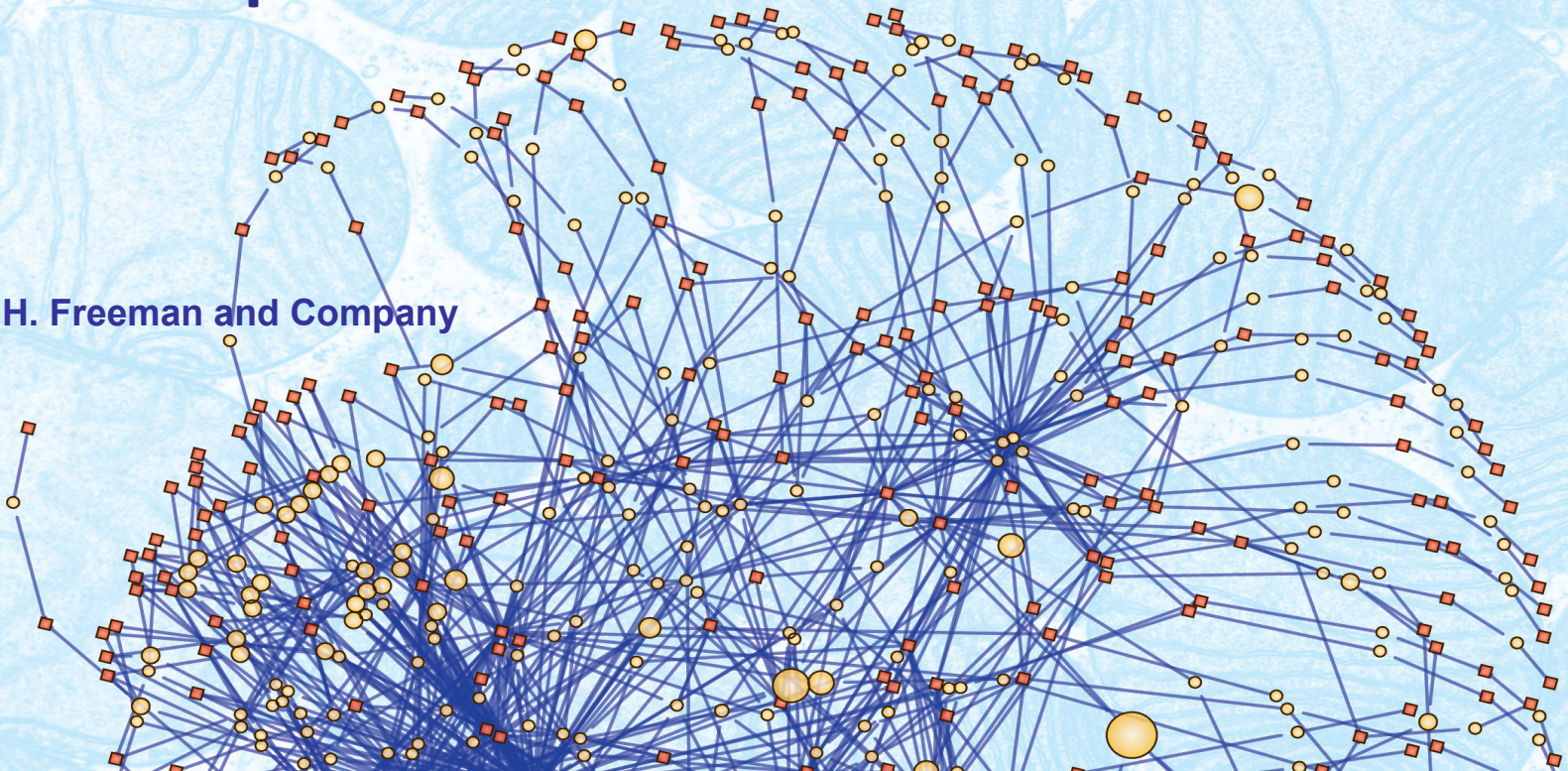
5| Function of Globular Proteins



Functions of Globular Proteins

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

Interaction with Other Molecules

- Reversible, transient process of chemical equilibrium:

  $$A + B \rightleftarrows AB$$

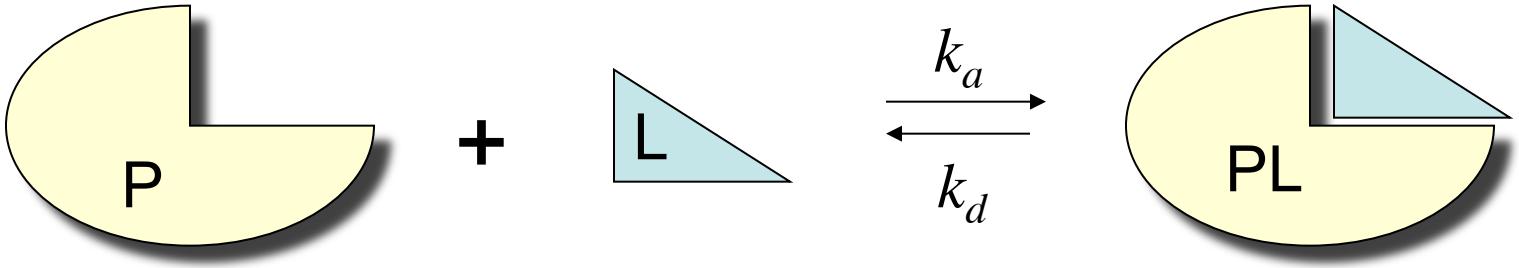
- A molecule that binds to a protein is called a ligand
  - Typically a small molecule
- A region in the protein where the ligand binds is called the binding site
- Ligand binds via same noncovalent forces that dictate protein structure (see Chapter 4)
  - Allows the interactions to be transient
  - (this is key to life → organism can respond quickly and reversibly to changes)

Interaction with Other Molecules

- When ligands bind to proteins, some conformational changes occur permitting tighter binding → this is called **induced fit**
  – In multisubunit proteins, a conformational change of one subunit often affects the others
- Enzymes are special kinds of proteins. They bind and transform other molecules. Enzyme ligands are called **substrates**
- The binding site is called **catalytic site (active site)**

Binding: Quantitative Description

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



- The kinetics of such a process is described 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

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

Binding:
Analysis in Terms of the Bound Fraction

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

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

- Substituting [PL] with K_a[L][P], we'll 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

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

Protein-Ligand Interactions

- Plotting θ as a function of [L] can give the value of K_a
- At $\theta = 0.5$ ➔ [L] = $1/K_a$
- Normally we use the **dissociation constant** ($K_d = 1/K_a$) ➔ θ = [L] / [L] + K_d)
- When [L] > K_d by 9 x → 90% of sites are occupied
- Note: **↑ K_d ↓ affinity of L for P**
- *K_d is the molar concentration of ligand at which half of the binding sites are occupied*
- The more tightly L is bound to P, the lower [L] needed for ½ binding sites to be filled → lower value of K_d

Binding: Graphical Analysis

- The fraction of bound sites depends on the free ligand concentration and K_d
- Experimentally
  - Ligand concentration is known
  - K_d can be determined graphically

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

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

In cells, normally [L] >> binding sites for L → binding of L to P does not change [L]

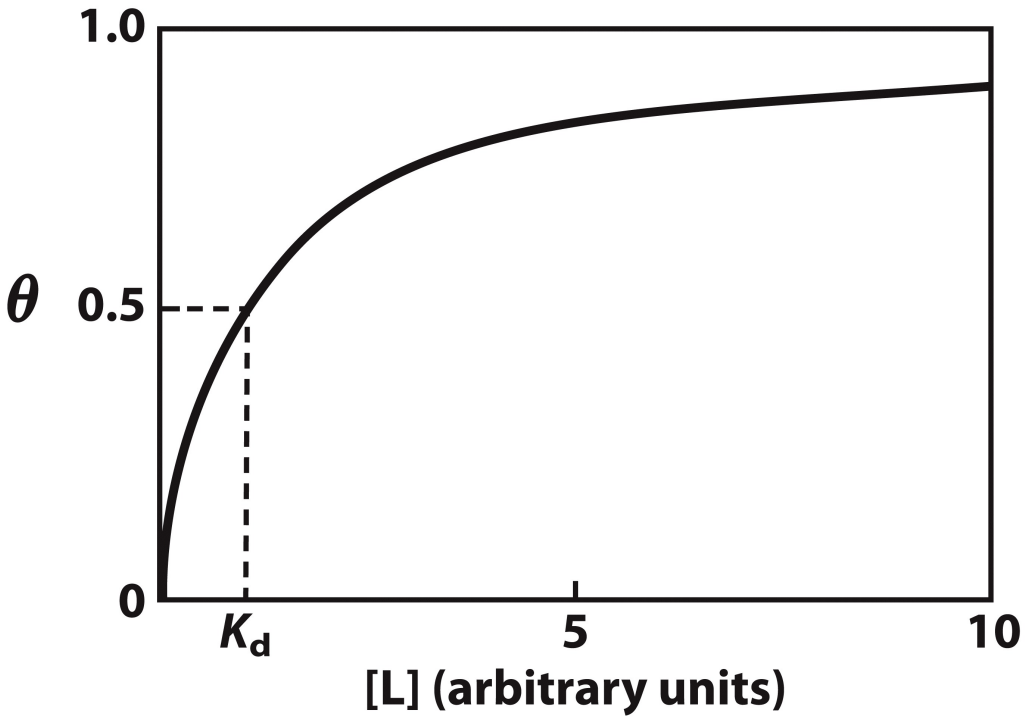


Figure 5-4a
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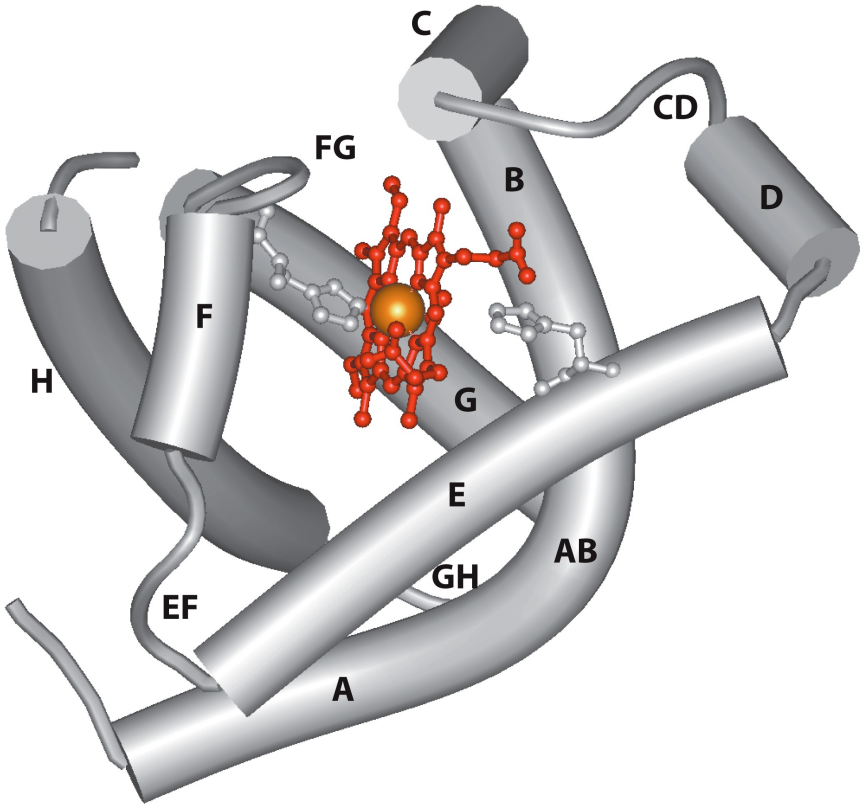
Example: Oxygen Binding to Myoglobin

When ligand is a gas, binding is expressed in terms of **partial pressures**



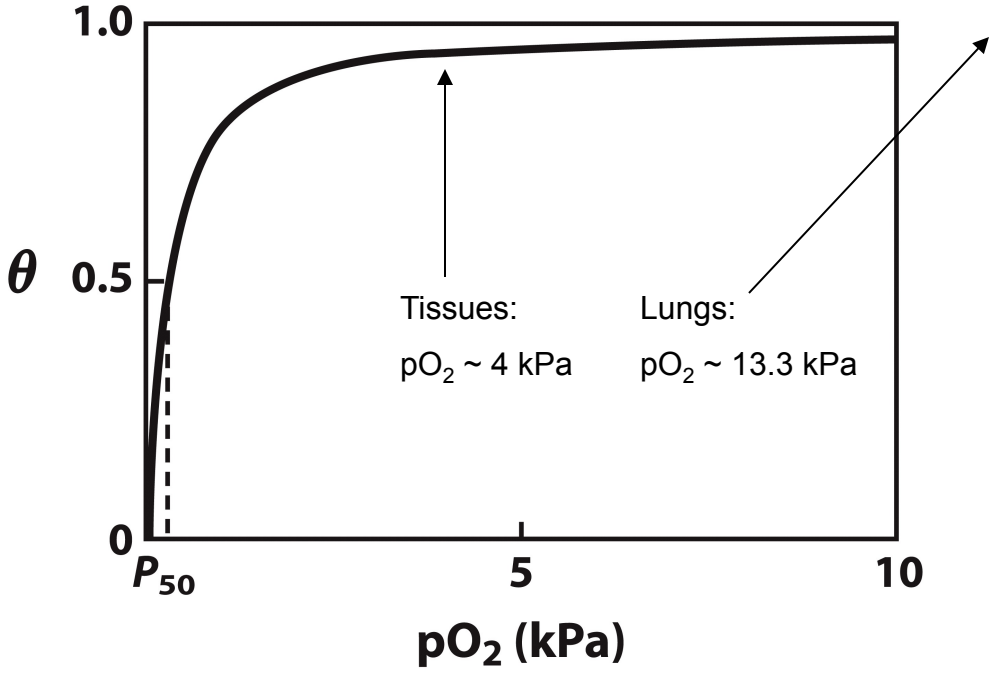


Figure 5-4b
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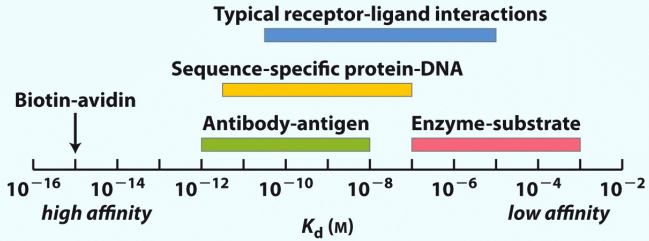
$$\theta = \frac{[\mathrm{L}]}{K_\mathrm{d} + [\mathrm{L}]} \longrightarrow \theta = \frac{p\mathrm{O}_2}{p_{50} + p\mathrm{O}_2}$$

Examples of Binding Strength

TABLE 5–1 Some Protein Dissociation Constants

Protein	Ligand	K_d (M)*
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)†	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)‡	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 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.

†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.

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

Table 5-1

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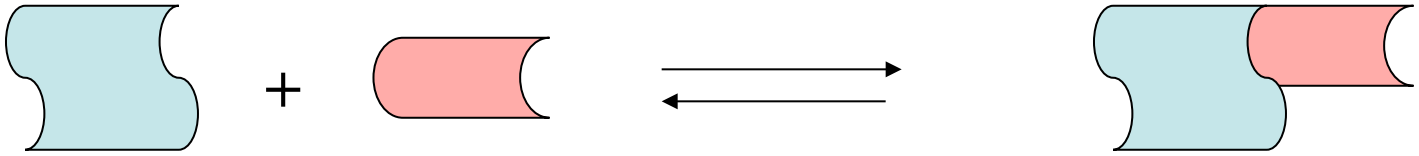
Specificity: Lock-and-Key Model

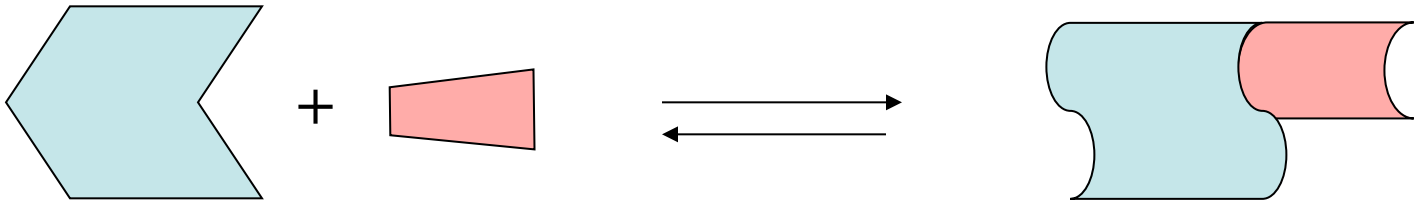
- Proteins typically have high specificity: only certain ligands bind
- High specificity can be explained by the complementary of the binding site and the ligand.
- Complementary in
  - size,
  - shape,
  - charge,
  - or hydrophobic/hydrophilic character
- "Lock and Key" model by Emil Fisher (1894) 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 are oxygen-binding proteins

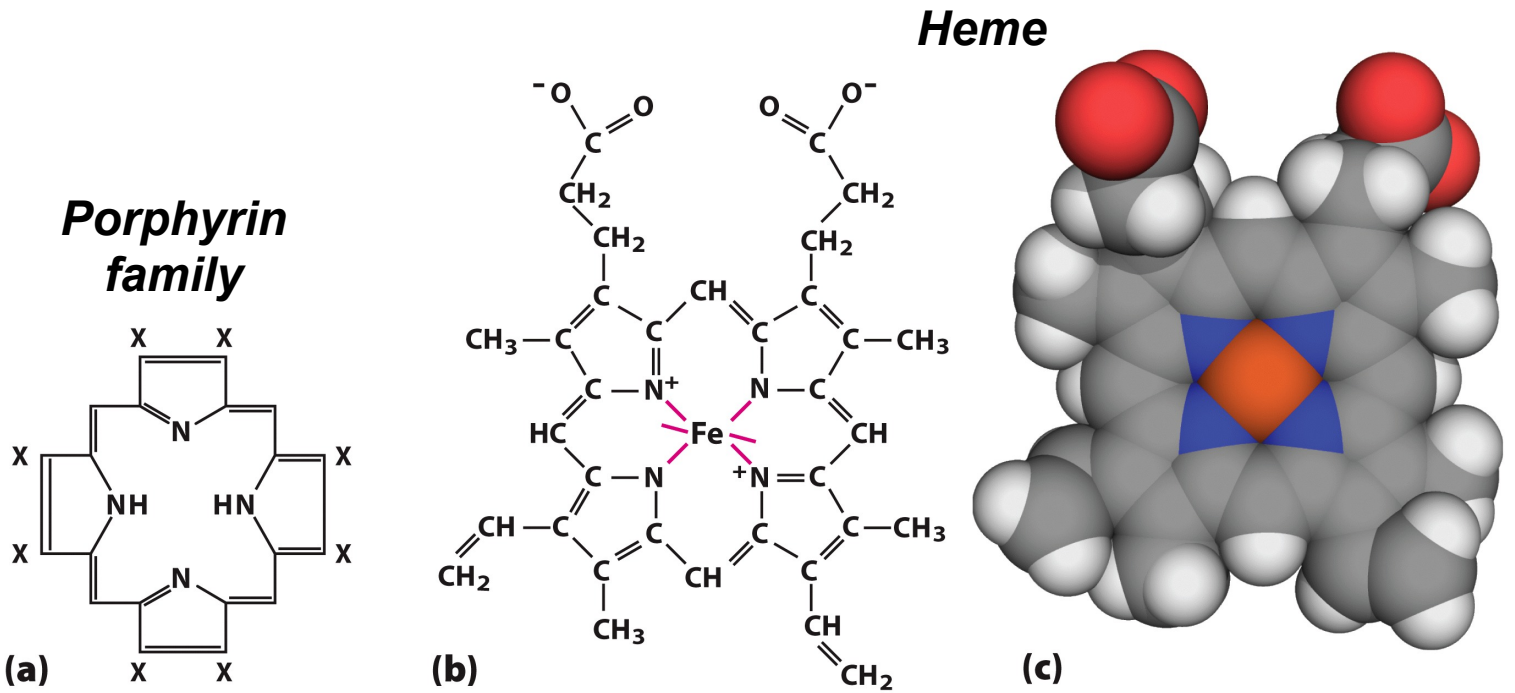
- Protein side chains lack affinity for O_2
- Some transition metals bind O_2 well but would generate free radicals if free in solution
- Organometallic compounds such as heme are more suitable, but Fe^{2+} in free heme could be oxidized to Fe^{3+}

- Solution
  - Capture the oxygen molecule with heme that is protein bound
  - Myoglobin is the main oxygen storage protein
  - Hemoglobin is a circulating oxygen-binding protein

Example: Oxygen Binding to Myoglobin

- O_2 dissolves poorly in aqueous solutions
- Its diffusion is not effective
- Evolution of larger animals needed evolution of proteins that carry O_2
- However, none of the aa can reversibly bind O_2
- There is a need for transition elements like Fe and Cu which can do that
- Fe is incorporated into a protein-bound prosthetic group called **heme**

Structures of Porphyrin and Heme



four pyrrole rings linked by methene bridges

The iron atom of heme has six coordination bonds: four in the plane of, and bonded to, the flat porphyrin ring system, and two perpendicular to it

Figure 5-1
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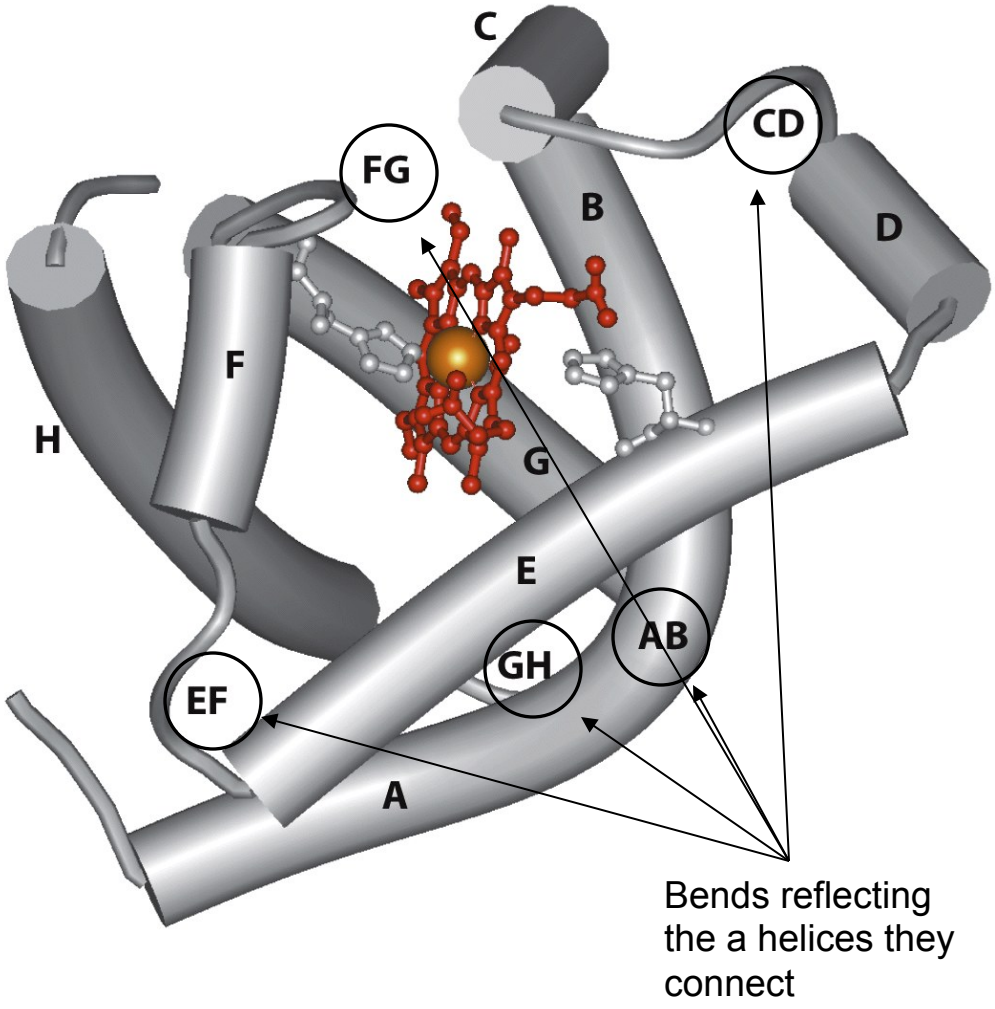
Example: Oxygen Binding to Myoglobin

- Free heme molecules not bound in proteins → 2 open coordination bonds
- Reaction of 1 O_2 molecule with two hemes will lead to irreversible conversion of Fe^{2+} to Fe^{3+} which does not bind O_2
- This reaction is prevented in heme-containing proteins because one of the coordination bonds is attached to a His side chain and the other is free to bond O_2
- When O_2 binds, electronic properties of heme changes (color changes from dark purple to bright red)
- CO and NO bind more tightly to heme than O_2 → toxic to aerobic organisms

Structure of Myoglobin

- Mb is a single polypeptide of 153 aa and 1 heme molecule
- It is part of a family of proteins called **globins**
- 8 α helices
- His residue coordinated heme is His^{93} (or His F8)



Binding of Carbon Monoxide

- CO has similar size and shape to O_2; it can fit to the same binding site
- CO binds 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+}
- 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

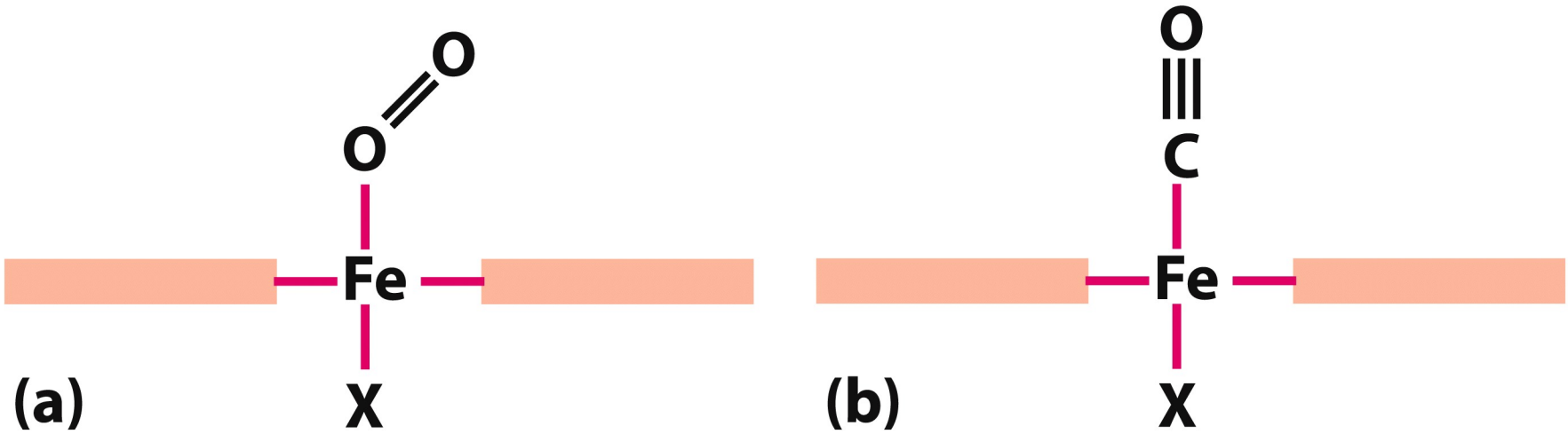


Figure 5-5ab
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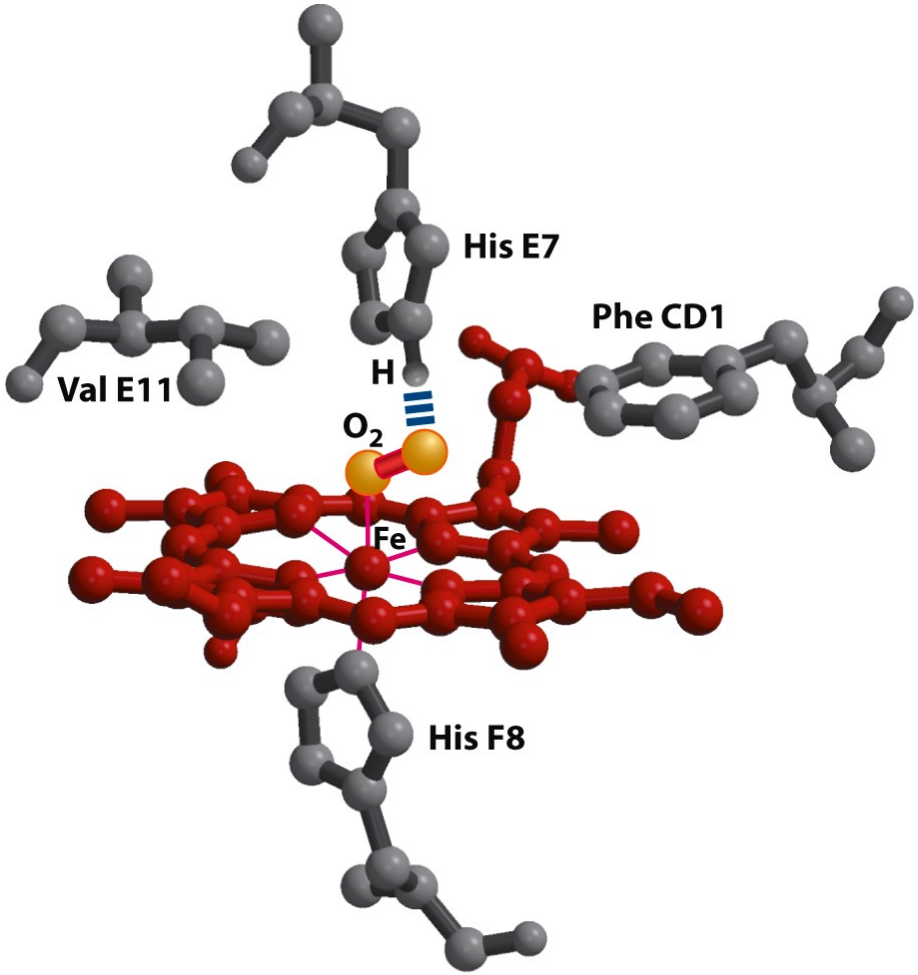
Heme binding to protein affects CO vs. O_2 binding



When binding to the heme in myoglobin, CO is forced to adopt a slight angle because the perpendicular arrangement is sterically blocked by His E7, the distal His. This effect weakens the binding of CO to myoglobin.

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

- 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!

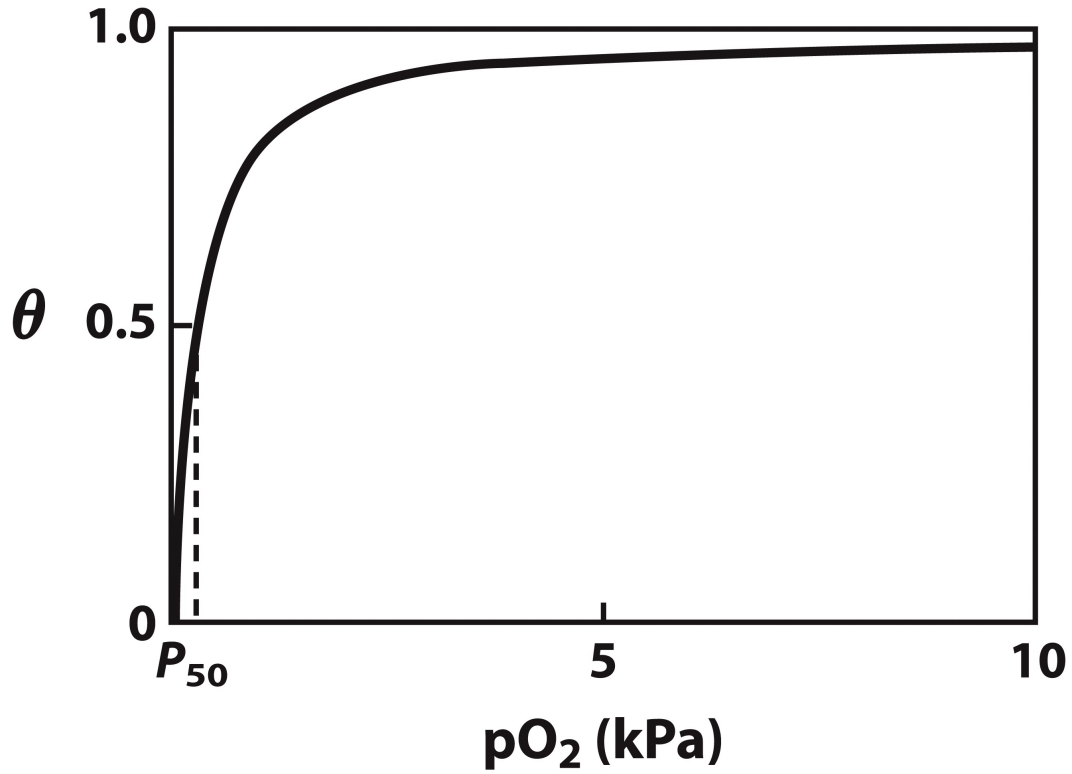

Figure 5-4b
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- Would lowering the affinity (P_{50}) of myoglobin to oxygen help?

For effective transport affinity must vary with pO_2

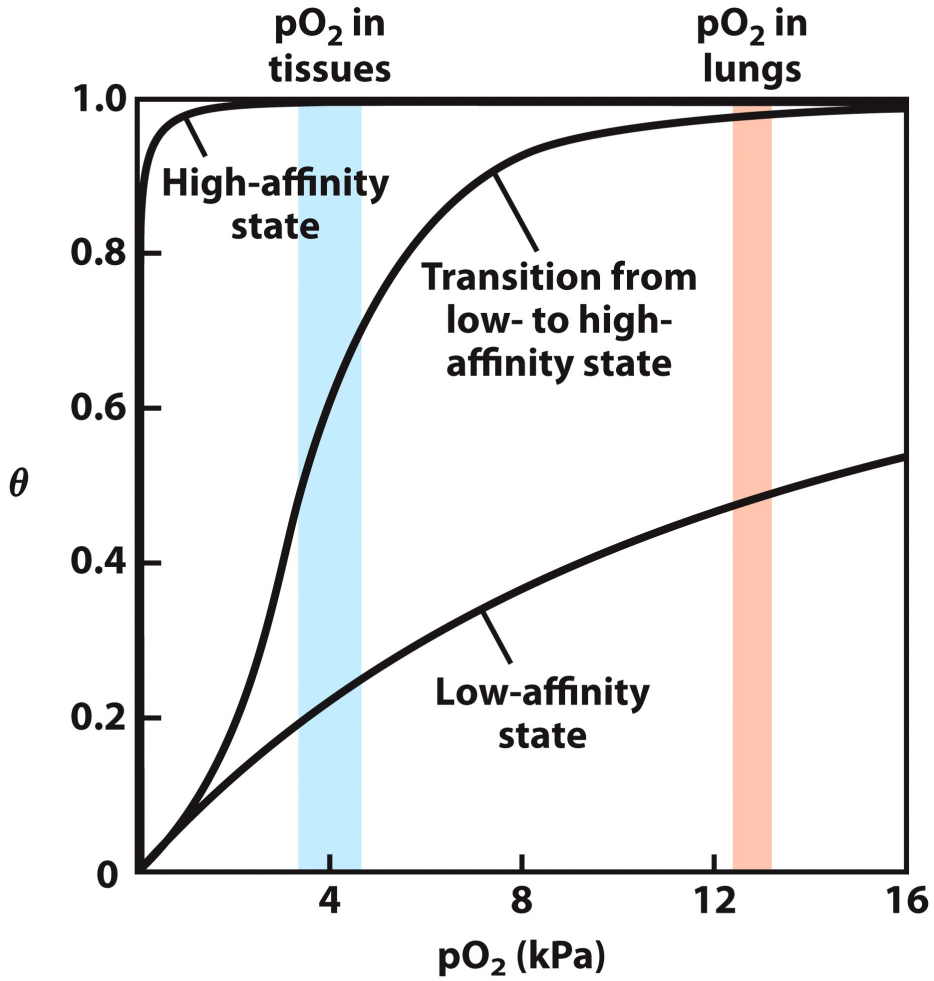

Figure 5-12
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How can affinity to oxygen change?

- 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

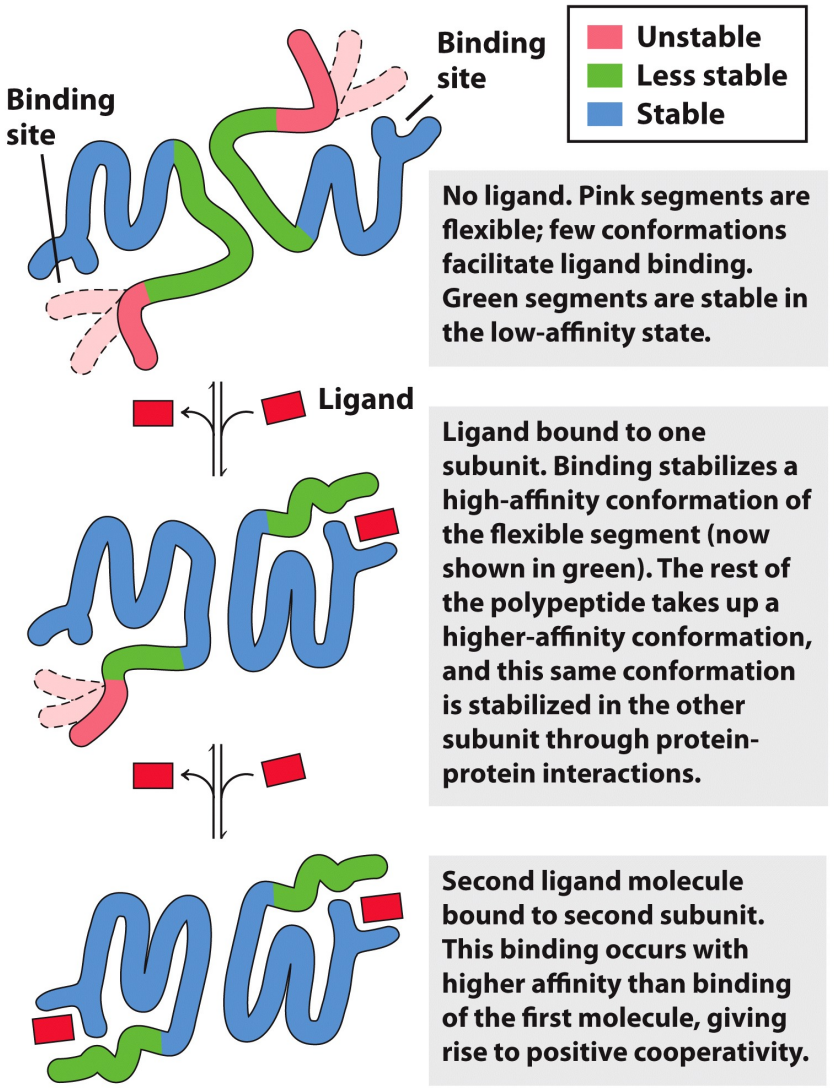

Figure 5-13
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Cooperativity: Quantitative Description

- Cooperative proteins have multiple ligand-binding sites
- so K_a becomes: $$K_a = \frac{[\mathrm{PL_n}]}{[\mathrm{P}][\mathrm{L}]^n}$$
- And θ becomes: $$\theta = \frac{[\mathrm{L}]^n}{[\mathrm{L}]^n + K_d}$$
- Taking the log of both sides gives the Hill Equation:

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

  - n = the Hill Coefficient (the degree of cooperativity)
  - n = 1 → no cooperativity
  - **Hill plot:** plotting log (θ / 1 – θ) vs. log [L]. Gives the Hill coefficient (n_H) which measures the degree of cooperativity

The Hill Plot of Cooperativity

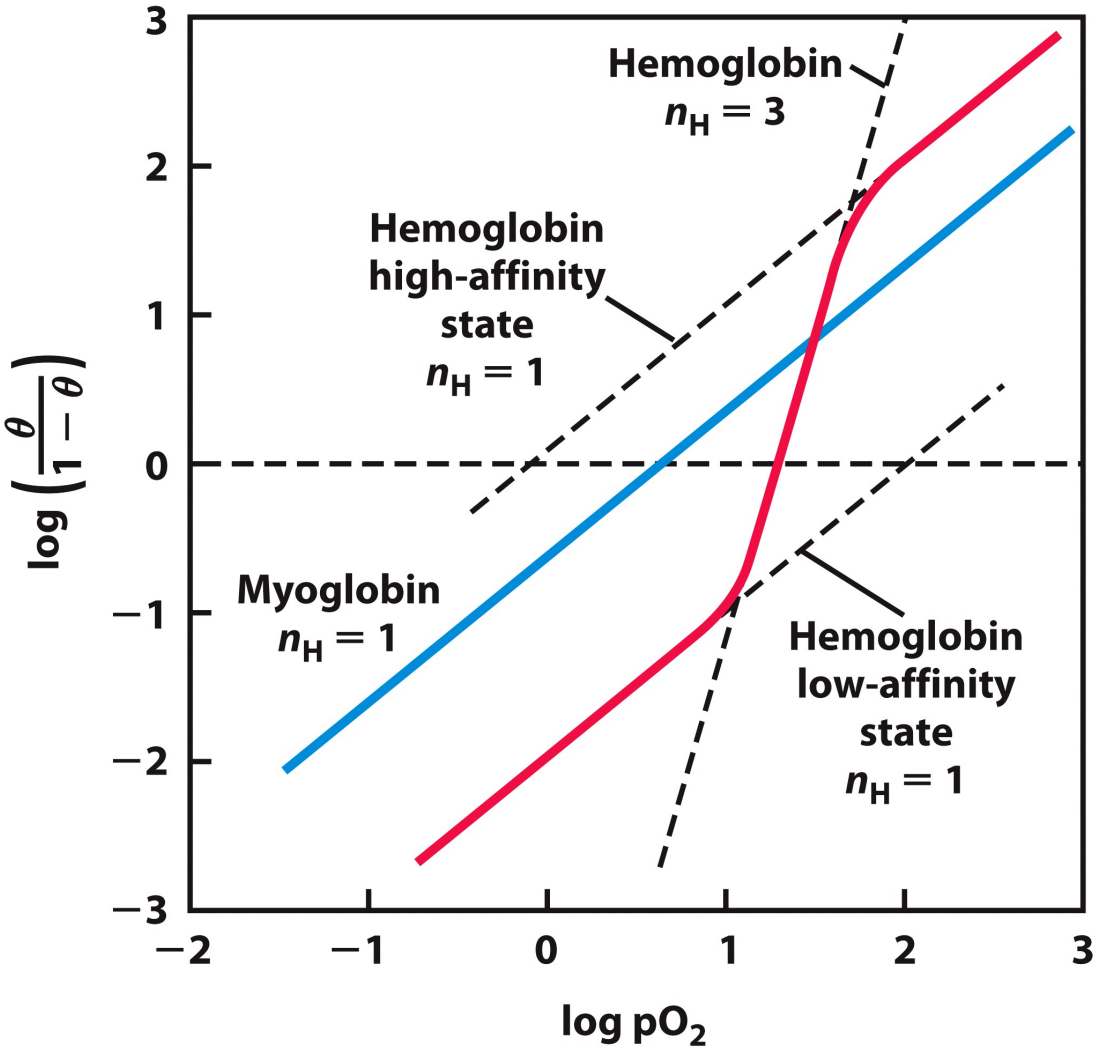

Figure 5-14
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Cooperativity is a special case of allosteric regulation

- **Allosteric protein**
  - Binding of a ligand (a modulator) to one site affects the binding properties of a different site, on the same protein
  - Can be positive or negative
  - Homotropic
    - Normal ligand of the protein is the allosteric regulator
  - Heterotropic
    - Different ligand affects binding of the normal ligand

- Cooperativity = positive homotropic regulation

Hemoglobin binds oxygen cooperatively

- Red blood cells (erythrocytes) are special incomplete cells filled with Hb (and no nucleus or organelles). They are biconcave discs. Their lifespan is 120 days
- In arterial blood (from the lungs), Hb is 96% saturated with O_2. In venous blood (to the heart and lungs), Hb is ~64%
- Mb is insensitive to small changes in [O_2] (O_2-storage protein)
- Hb is sensitive to small changes → O_2-transport protein (multiple subunits)

Hemoglobin binds oxygen cooperatively

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

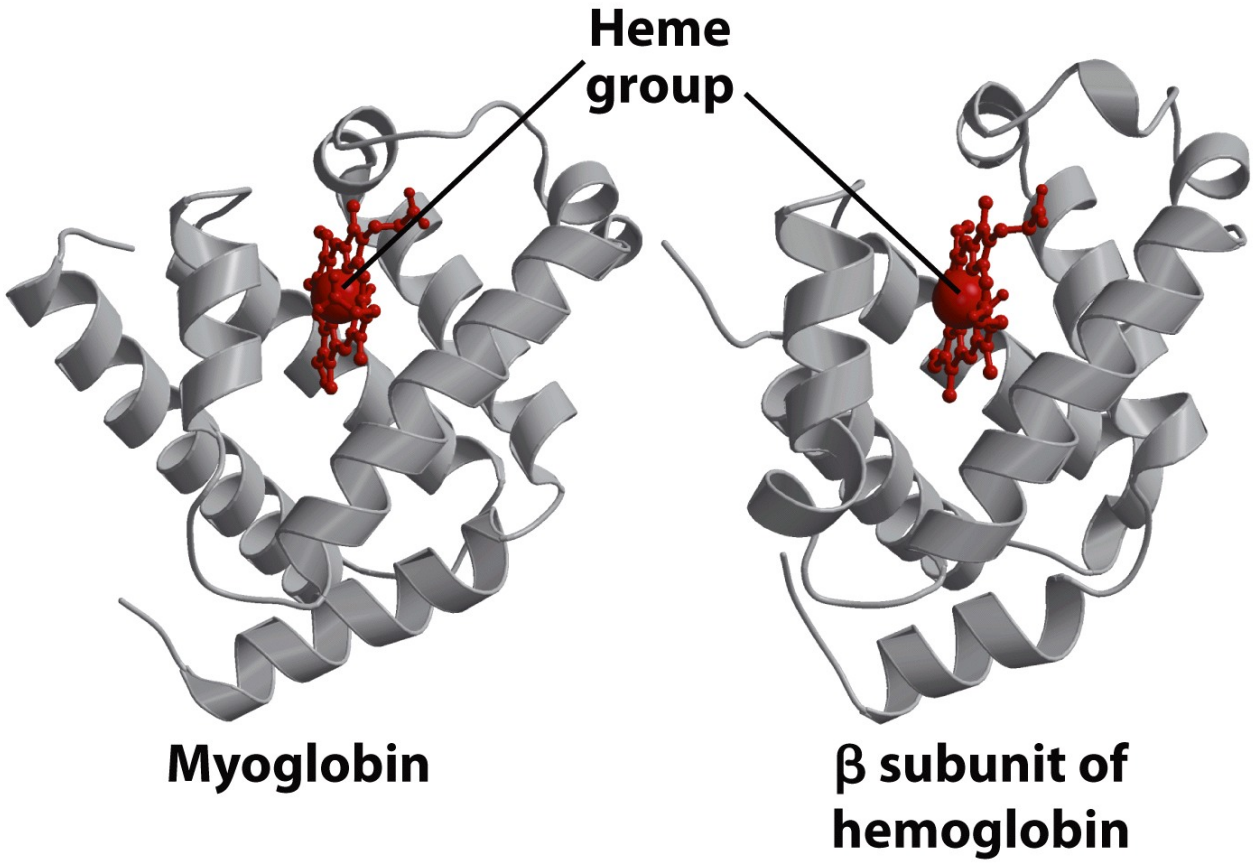

Figure 5-6
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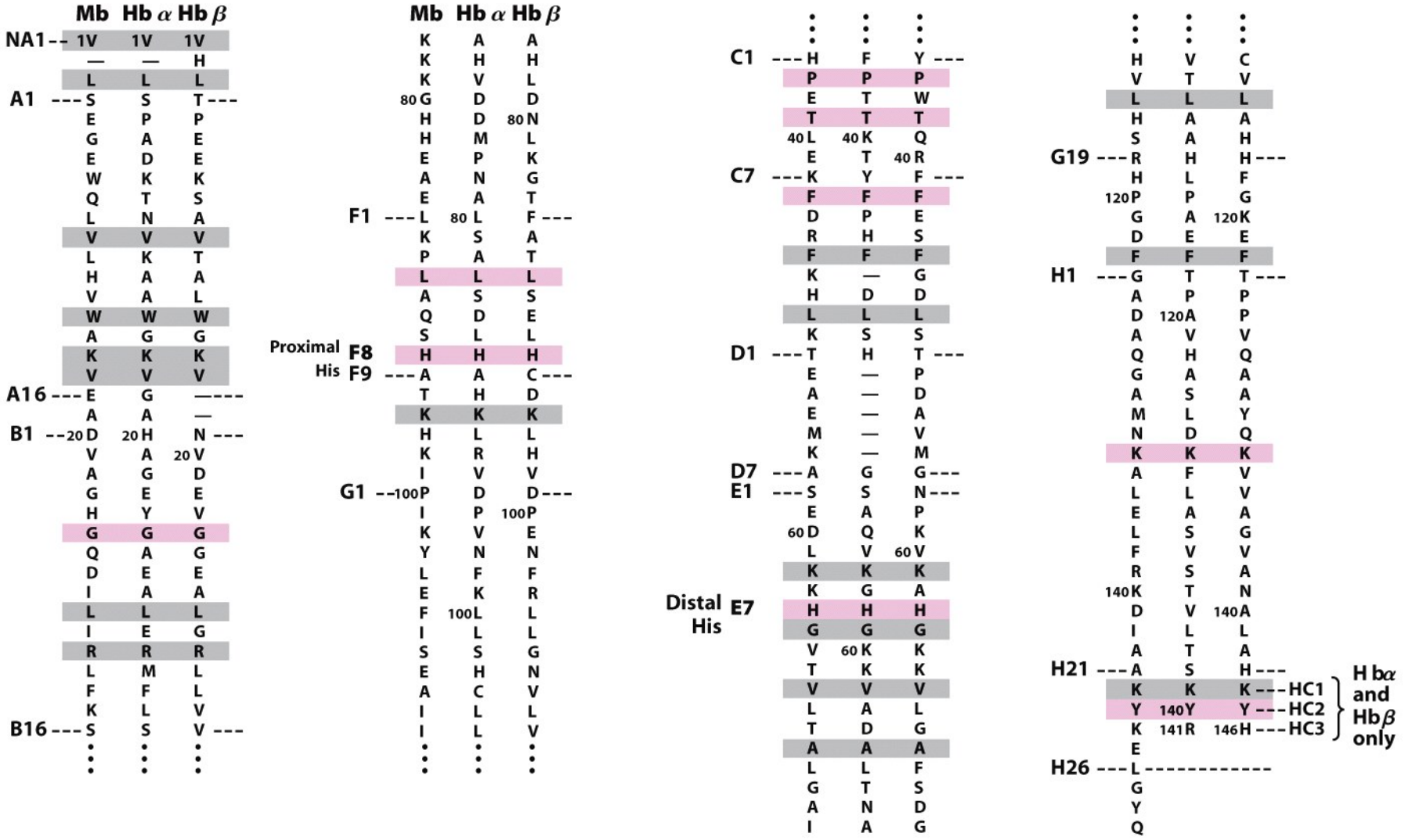
Hb Subunits are Similar to Mb

- Hb (M_r 64,500) is spherical
- Tetramer
- 4 heme prosthetic groups
- 2 α chains (141 aa each) and 2 β chains (146 aa each)
- 3D structure of both α and β is similar
- aa sequences of Mb and α and β Hb are identical in 27 positions
- The helix-naming system for Mb is also used for Hb polypeptides
- Hbα does not have D helix

Sequence Similarity between Hemoglobin and Myoglobin



Hb is a dimer of two $\alpha\beta$ protomers

- 4° structure of Hb shows strong interactions between unlike subunits

- The $\alpha_1\beta_1$ interface (and also $\alpha_2\beta_2$) involve > 30 aa

- The $\alpha_1\beta_2$ interface (and also $\alpha_2\beta_1$) involve 19 aa

- These interfaces make strong interactions → mild treatment of Hb with urea breaks the tetramer into $\alpha\beta$ dimers

Subunit Interactions in Hemoglobin

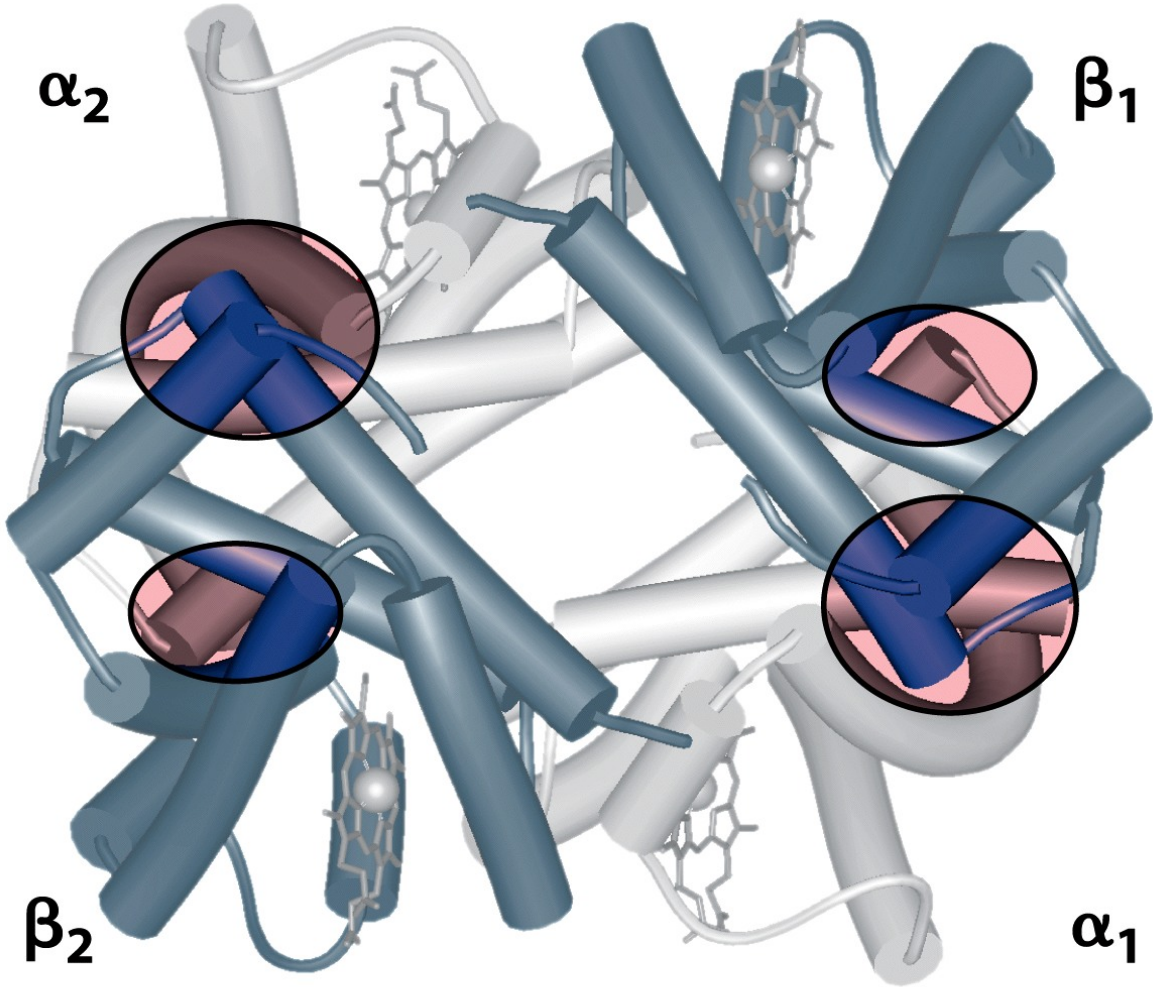


Figure 5-8
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R and T States of Hemoglobin

- Two major conformations of Hb:
  R state and **T state**
- O_2 binds to Hb in either one, but it has to R state

- T = Tense state
  - More interactions, more stable
  - Lower affinity for O_2
- R = Relaxed state
  - Fewer Interactions, more flexible
  - Higher affinity for O_2

Hb Changes Structure after O_2 Binding

- O_2 binding stabilizes R state
- T state is more stable when not bound to O_2 (deoxyhemoglobin)
- *O_2 binding to a Hb subunit at the T state converts the subunit to R state*
- Therefore, 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

R and T States of Hemoglobin

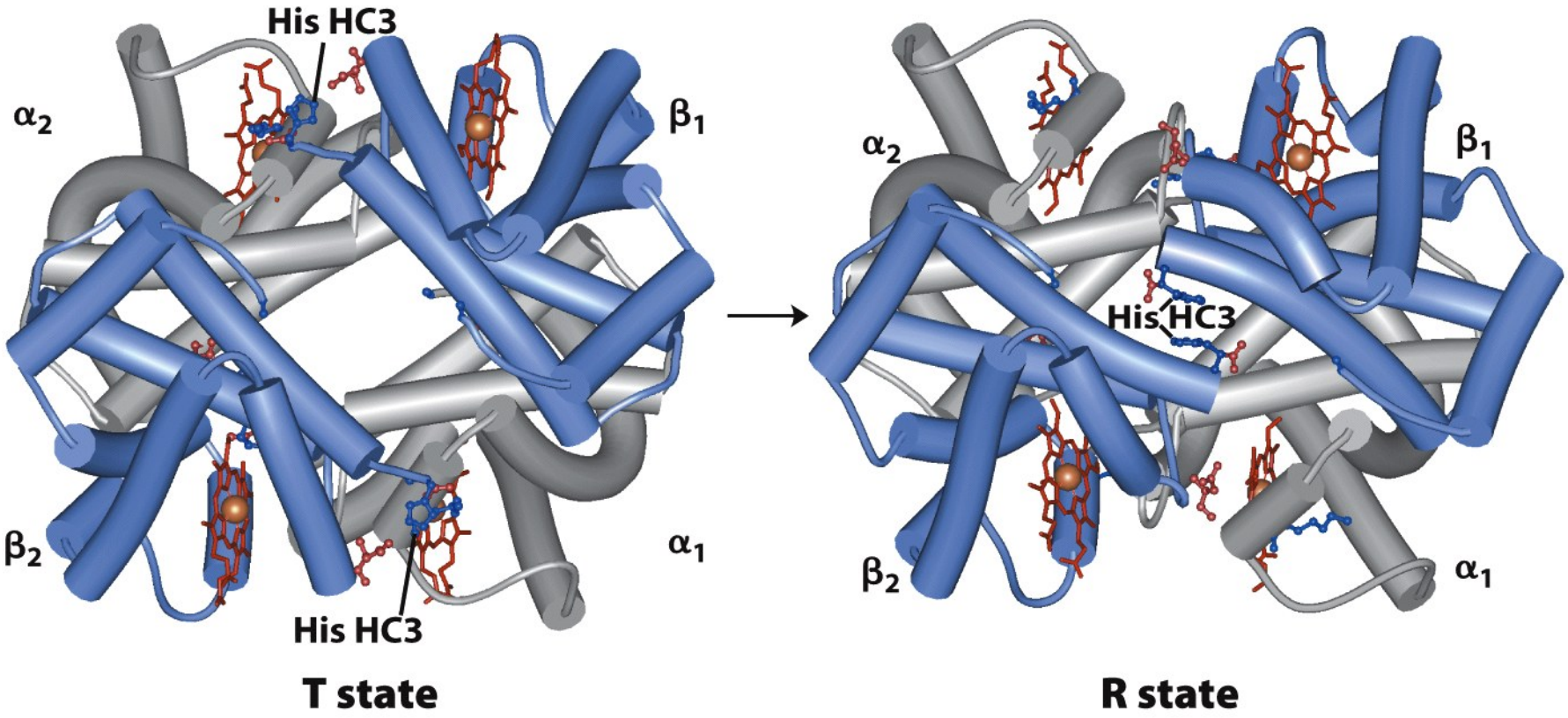

Figure 5-10
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The transition from the T state to the R state shifts the subunit pairs, affecting certain ion pairs. Most noticeably, the His HC3 residues at the carboxyl termini of the *β* subunits, which are involved in ion pairs in the T state, rotate in the R state toward the center of the molecule, where they are no longer in ion pairs. Another dramatic result of the T → R transition is a narrowing of the pocket between the *β* subunits.

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

- 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

pH Effect on O_2 Binding to Hemoglobin

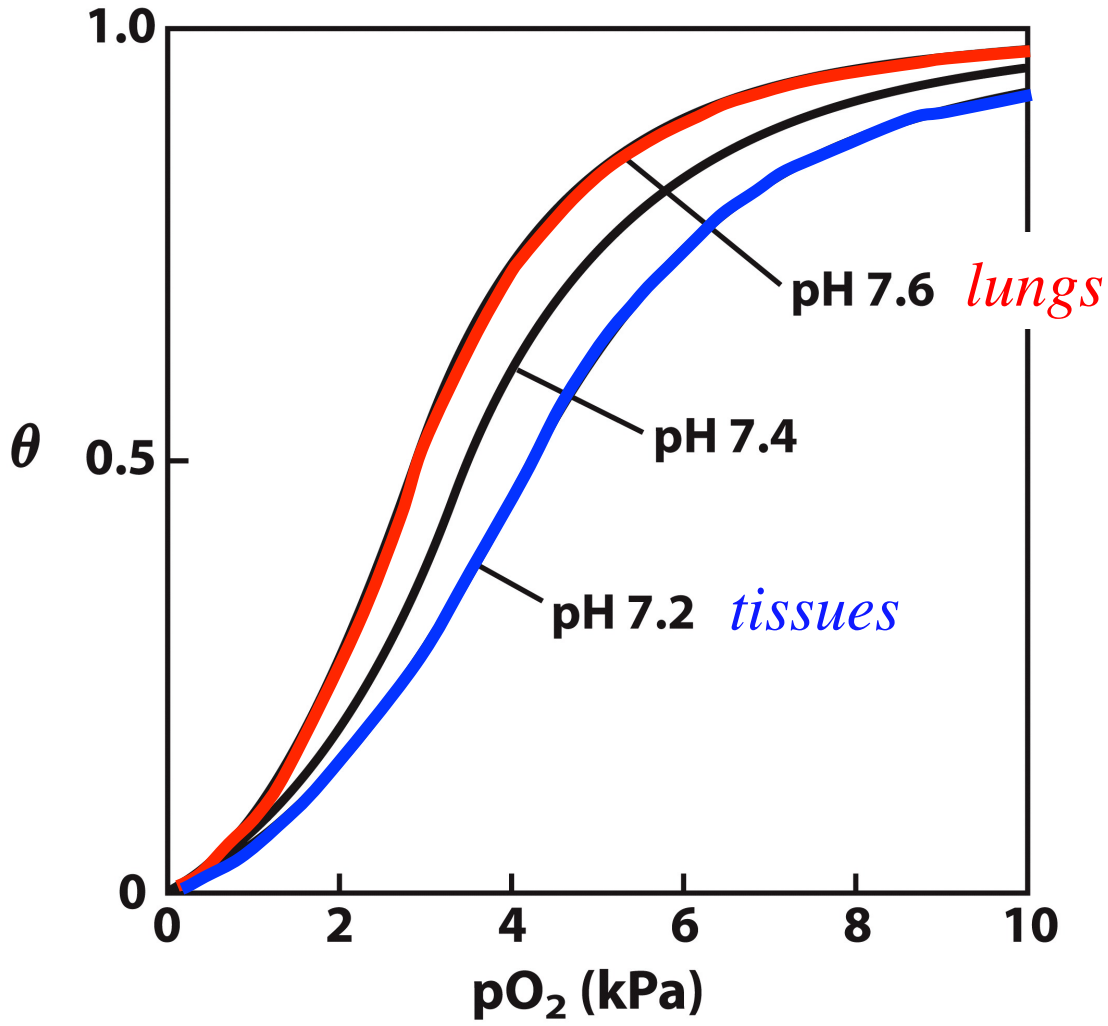

Figure 5-16
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Hemoglobin and CO_2 Export

- 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.

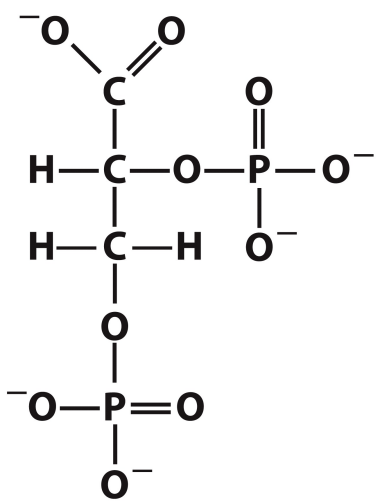
- Notice:
  - the formation of a carbamate yields a proton which can contribute to the Bohr Effect
  - the carbamate forms additional salt bridges stabilizing the T state

- The rest of the CO_2 is exported as dissolved bicarbonate
  - Formed by carbonic anhydrase, and also producing a proton

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
  - Plays an important role in physiological adaptations for low oxygen concentration (like at high altitudes or in cases of **hypoxia**)

- Small negatively charged molecule, binds to the positively charged central cavity of Hb
- **Stabilizes the T states**



2,3-Bisphosphoglycerate

2,3-BPG binds to the central cavity of hB

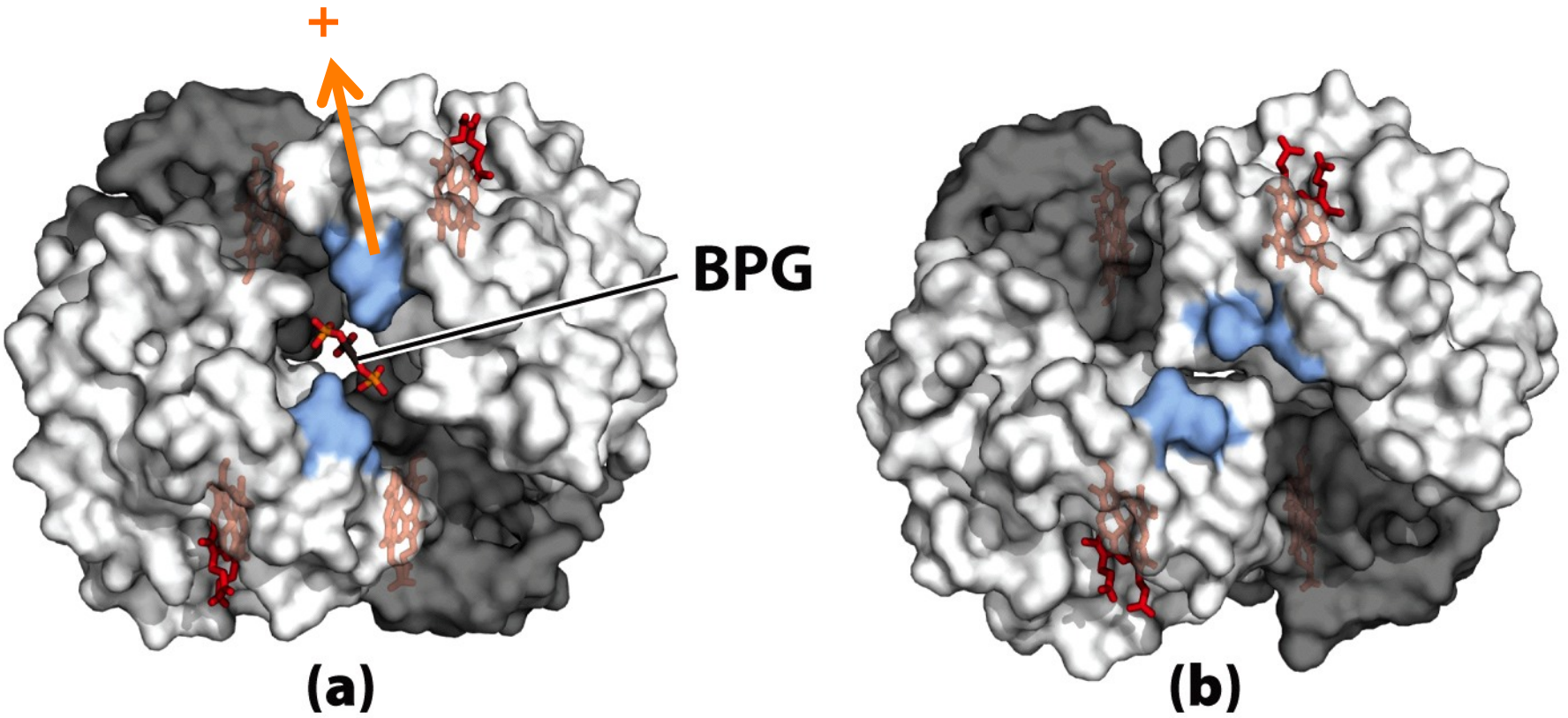


Figure 5-18
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BPG binding stabilizes the T state of deoxyhemoglobin

The binding pocket for BPG disappears on oxygenation

2,3-BPG allows for O_2 release in the tissues and adaptation to changes in altitude

- ★ At sea level, Hb is nearly saturated with O_2 in the lungs
- ★ Hb is just over 60% saturated in the tissues
- ★ The amount of O_2 released in the tissues is about 38% of the maximum that can be carried in the blood

- ★ At high altitudes, O_2 delivery declines to 30% of maximum
- ★ An increase in [BPG] decreases the affinity of Hb for O_2, so ~ 37% of what can be carried is again delivered to the tissues

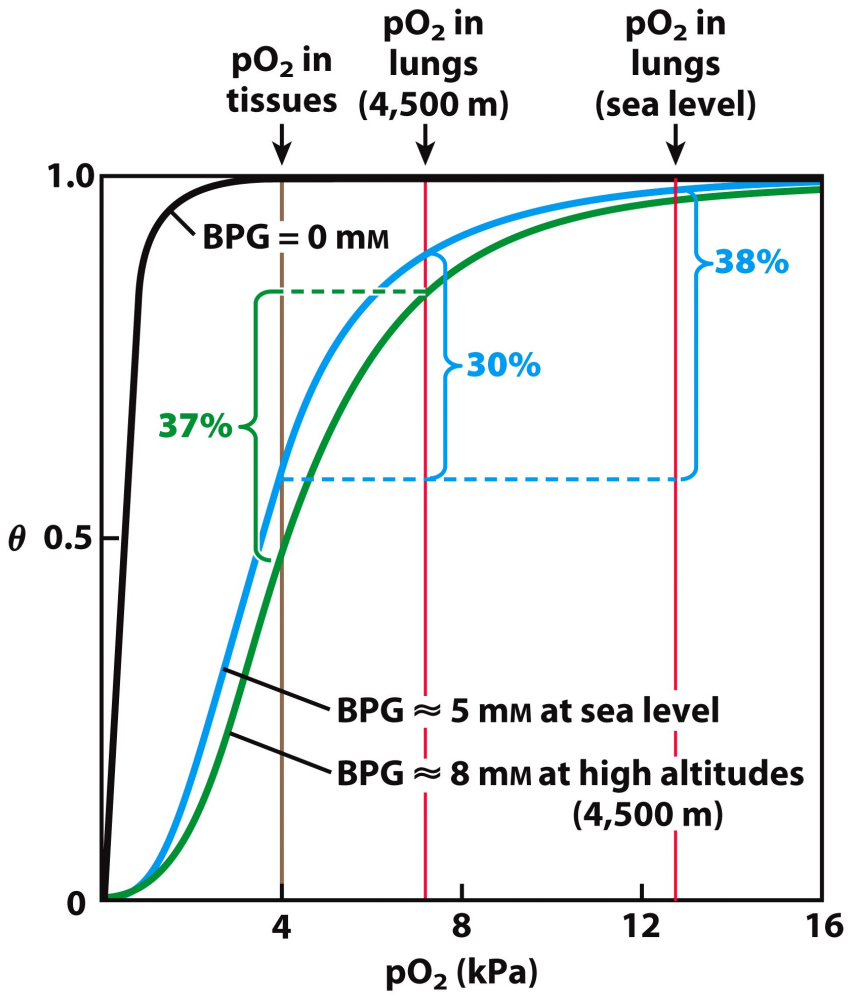

Figure 5-17
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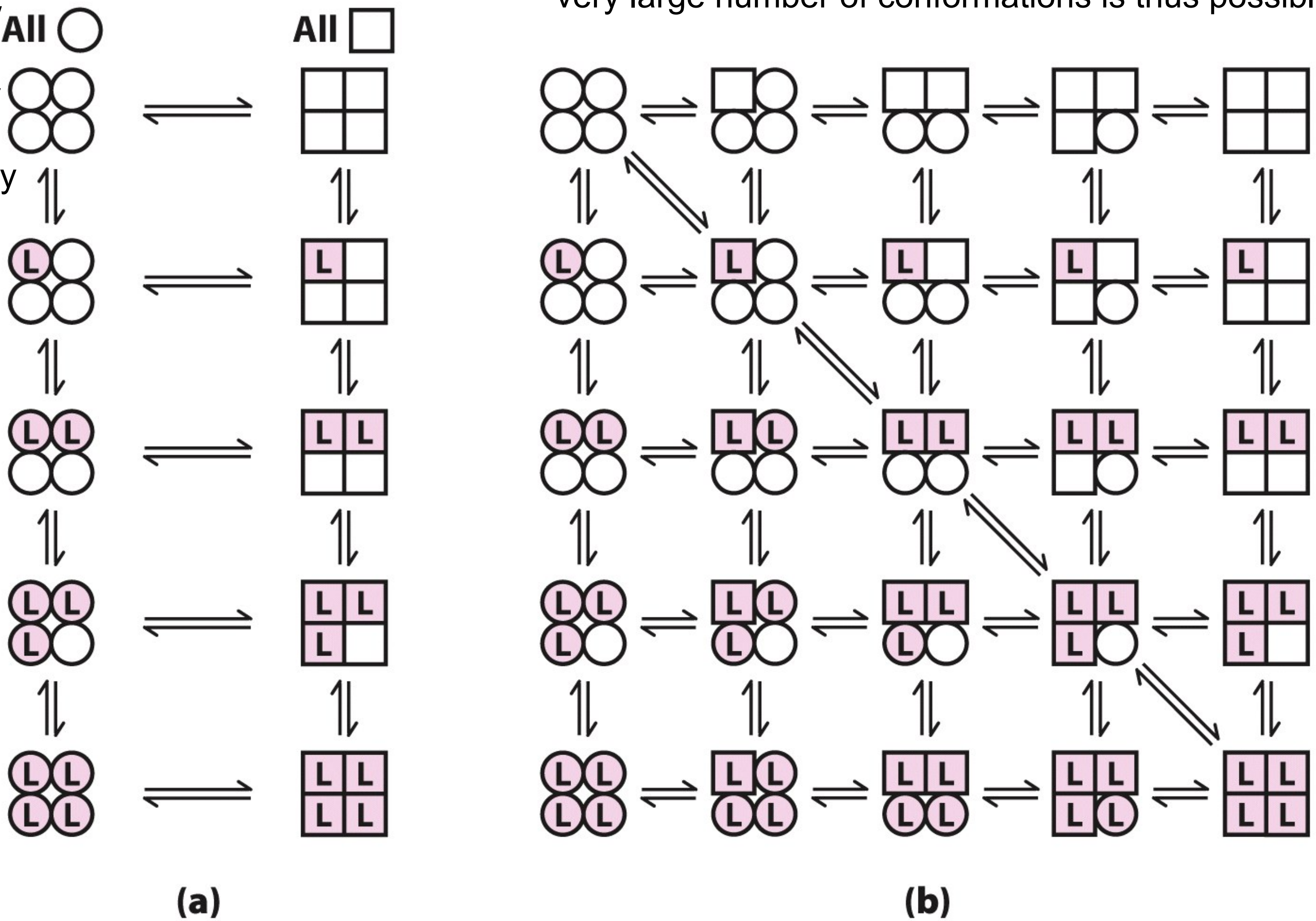
Two Models of Cooperativity: Concerted (MWC) vs. Sequential

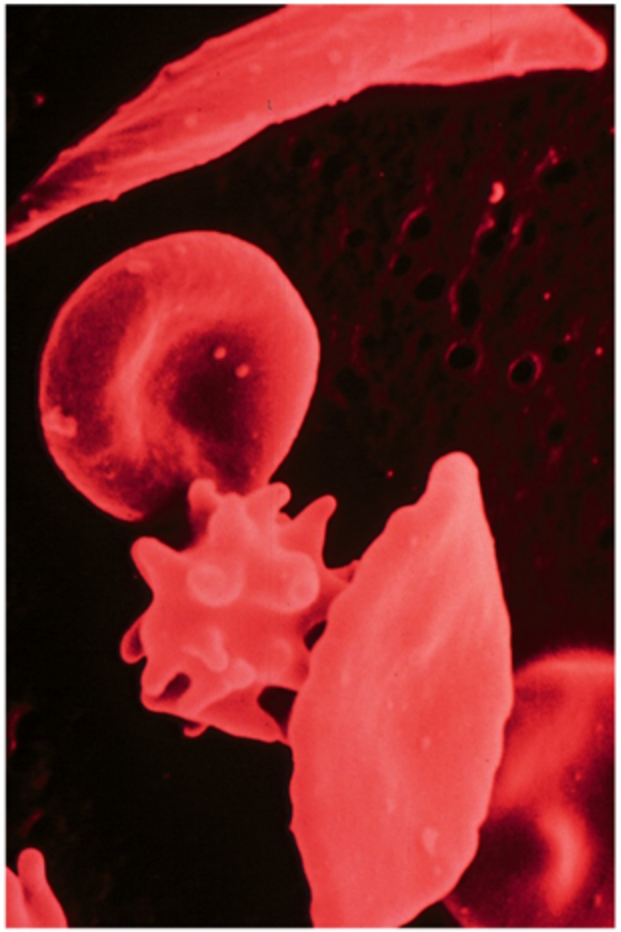
Either all circles (low affinity or inactive) or all squares (high affinity or active).

Each individual subunit can be in either the or form. A very large number of conformations is thus possible



Sickle-cell anemia is due to a mutation in hemoglobin

- Sickle-cell disease occurs in individuals *homozygous* for the sickle cell allele of the gene encoding the β subunit of Hb
- When Hb from a sick patient is deoxygenated (Hb S) it aggregates and precipitates (normal Hb, Hb A does not precipitate upon deoxygenation)
- The difference is a single aa substitution Glu6 → Val in the β chain of Hb
- The new Val (hydrophobic) side chain can bind to a different Hb molecule to form a strand



(b)

- Untreated homozygous individuals generally die in childhood
- Heterozygous individuals exhibit a resistance to malaria

Formation of Hb Strands in Sickle-Cell Anemia

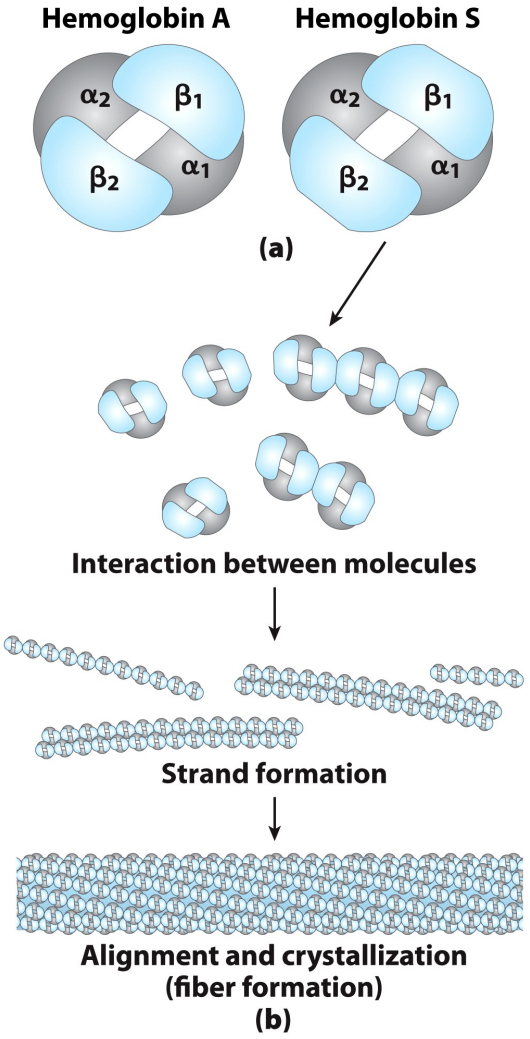


Figure 5-20
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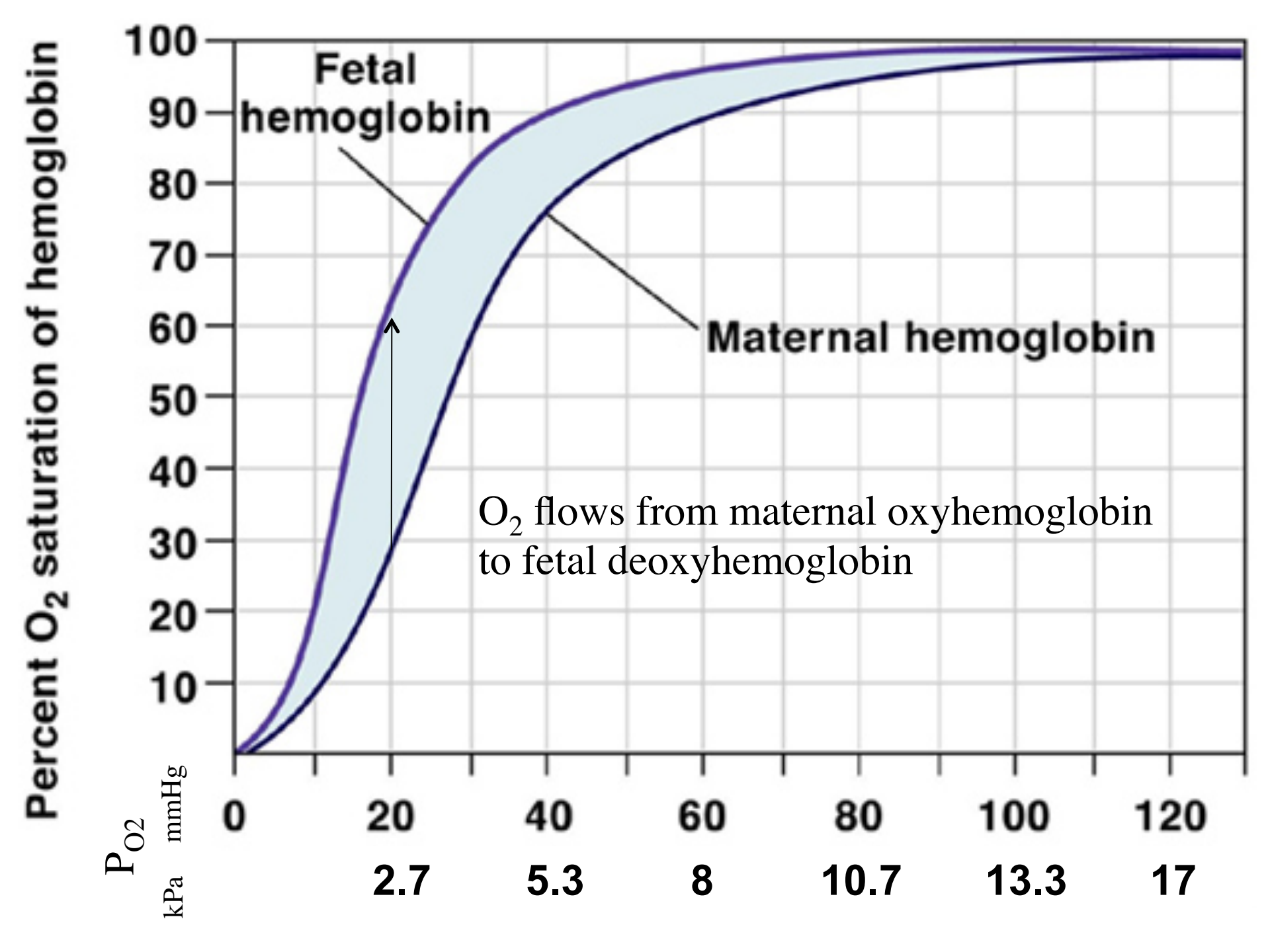
deoxyhemoglobin S has a hydrophobic patch on its surface, which causes the molecules to aggregate into strands that align into insoluble fibers

Fetal Hemoglobin (HbF)

- The main oxygen transport protein in the fetus during the last seven months of development in the uterus and in the newborn until ~ 6 months old
- **2 α, 2 γ** subunits (fewer positive charges than the adult hemoglobin β subunit; *2,3-BPG binds less*)
- **Binds O_2 at a greater affinity that HbA** (adult)
  → fetus can extract O_2 from his/her mother bloodstream easily
- The affinity of HbF for oxygen > that of HbA
  (P_{50} HbF ~ 2.5 kPa; P_{50} HbA ~ 3.7 kPa)
- The oxygen saturation curve is shifted to the left for HbF
- HbF does not interact with 2,3-BPG (which decreases the affinity of HbA for oxygen) → **HbF binds O_2 tighter than HbA**


Percent O_2 saturation of hemoglobin
100
90
80
70
60
50
40
30
20
10
Fetal hemoglobin
Maternal hemoglobin
O_2 flows from maternal oxyhemoglobin to fetal deoxyhemoglobin
P_{O2} mmHg
0
20
40
60
80
100
120
kPa
2.7
5.3
8
10.7
13.3
17

5.2 The Immune System and Immunoglobulins

- Most interactions between a P and L is in pockets in the protein lined with aa arranged to make this interaction ***specific***

- The immune system in vertebrates can discriminate between "self" and "nonself" entities and can destroy the nonself ones

- **Leukocytes** (WBC) are immunity cells developing in the bone marrow (including **macrophages** and **lymphocytes**)

- Any molecule that can induce an immune response is called an **antigen**

Two Types of Immune Systems

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

- **Humoral "fluid" immune system**
  - targets extracellular pathogens and infectious agents like bacteria and viruses
  - can also recognize foreign proteins
  - makes soluble antibodies
  - keeps "memory" of past infections
  - key players: B-lymphocytes and helper T-cells (TH_2)
  - soluble proteins called **antibodies** (immunoglobulins, Ig)

TABLE 5–2 Some Types of Leukocytes Associated with the Immune System

Cell type	Function
Macrophages	Ingest large particles and cells by phagocytosis
B lymphocytes (B cells)	Produce and secrete antibodies
T lymphocytes (T cells)	
Cytotoxic (killer) T cells (T_C)	Interact with infected host cells through receptors on T-cell surface
Helper T cells (T_H)	Interact with macrophages and secrete cytokines (interleukins) that stimulate T_C, T_H, and B cells to proliferate.

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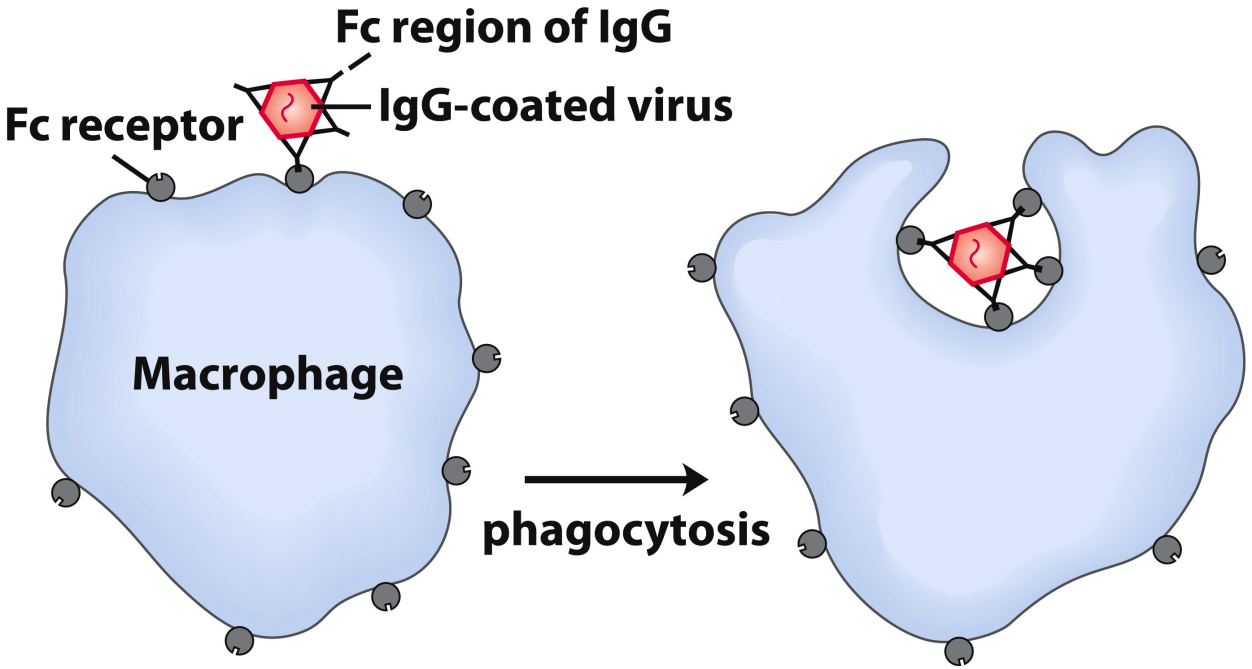
Cellular Immune System

- Antibodies bind to fragments displayed on the surface of invading cells
- **Phagocytes:** specialized cells that eat invaders
- **Macrophages:** large phagocytes that ingest bacteria that are tagged by antibodies



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Humoral Immune System

- Vertebrates also fight infections with soluble 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 by B cells and 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

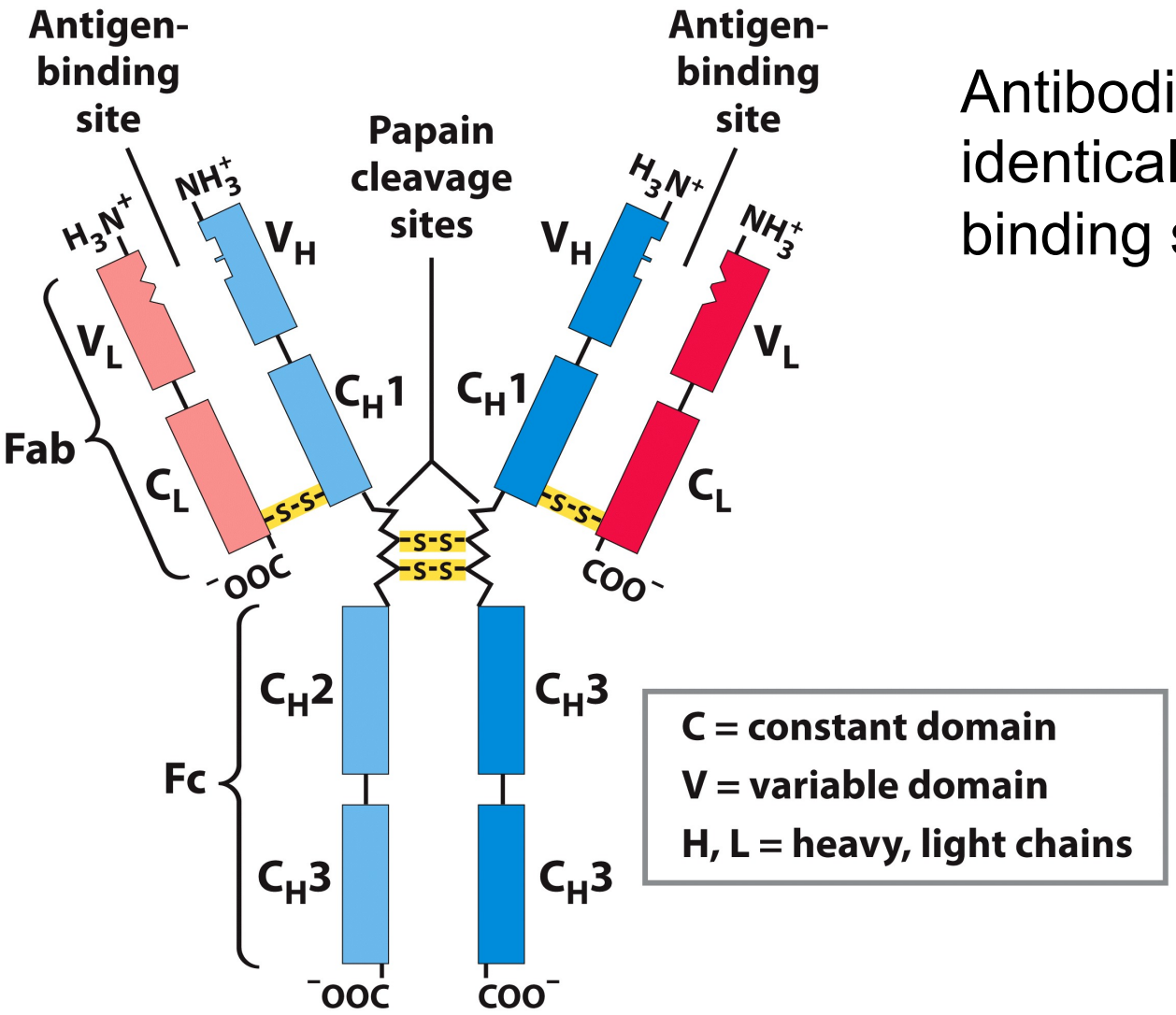
Antibodies: Immunoglobulin G

- Composed of 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 antigen-binding site (two/antibody)
- Variable domains contain regions that are hypervariable (specifically the antigen-binding site)
    - Confers high antigen specificity

Antibodies: Immunoglobulin G



Antibodies have 2 identical antigen-binding sites

Figure 5-21a
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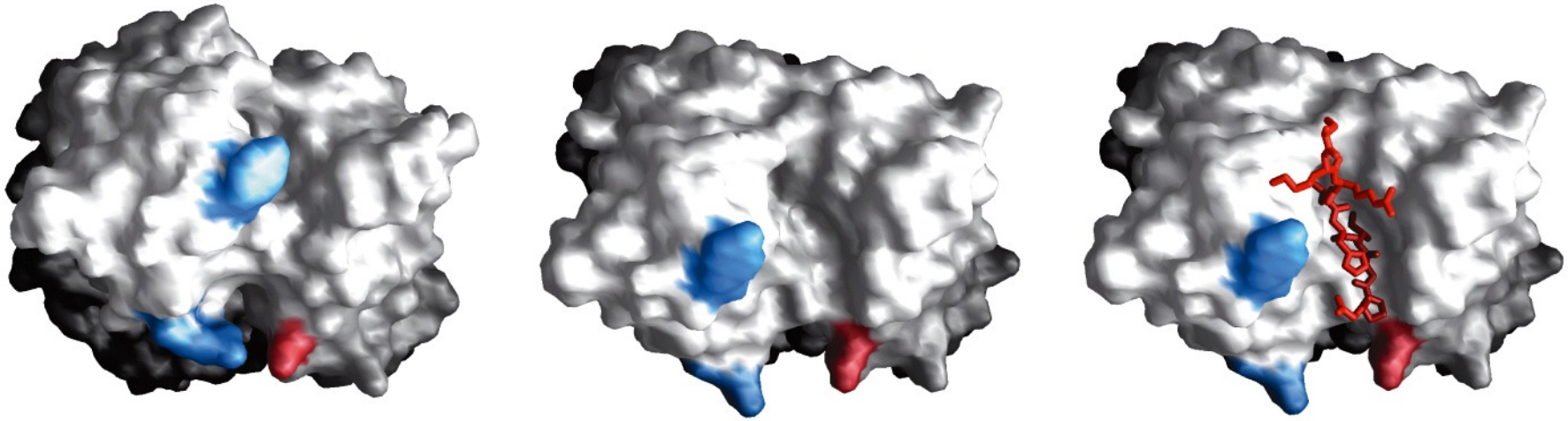
Antigens bind via induced fit

Antigen binding causes significant structural changes to the antibody



(a) Conformation with no antigen bound

(b) Antigen bound (but not shown)

(c) Antigen bound (shown)

Figure 5-25
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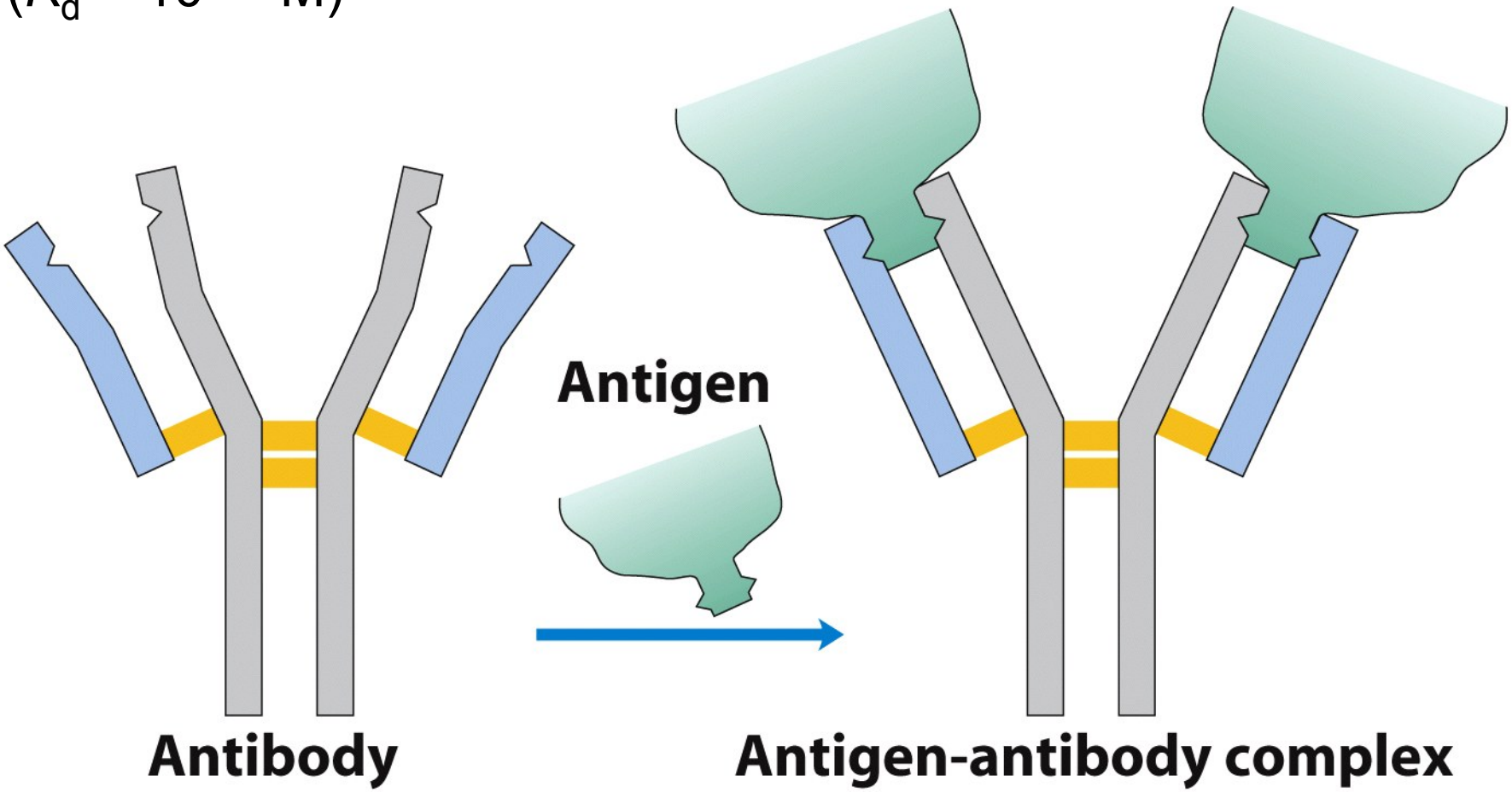
Antibodies bind tightly and specifically to antigens ($K_d \sim 10^{-10}$ M)



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Antigen-Antibody Interactions in Analytical Procedures

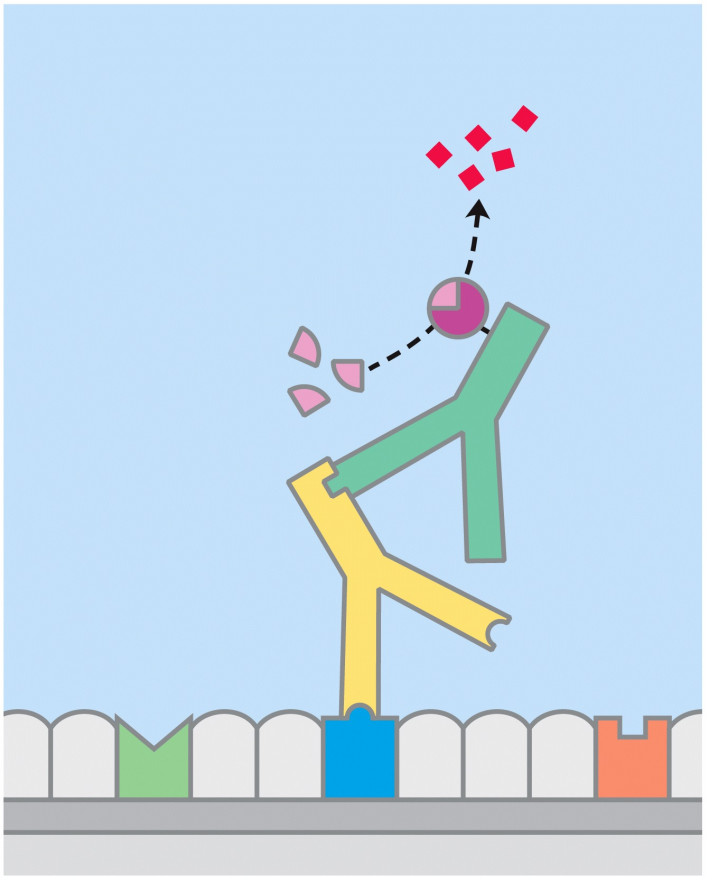
- **Polyclonal antibodies** – produced by different B lymphocytes responding to one antigen (e.g. antigen injected in an animal)
  – contain a mixture of Ab that recognize different parts of the antigen

- **Monoclonal antibodies** – produced by identical (cloned) B cells grown in cell culture
  – Homogeneous, recognize the same part of the antigen

- Can be used for affinity chromatography (attached to beads)
- Also in **ELISA** and **immunoblot** assays

Antibody specificity is an important analytical reagent

1. Coat surface with sample (antigens).
2. Block unoccupied sites with nonspecific protein.
3. Incubate with primary antibody against specific antigen.
4. Incubate with secondary antibody–enzyme complex that binds primary antibody.
5. Add substrate.
6. Formation of colored product indicates presence of specific antigen.



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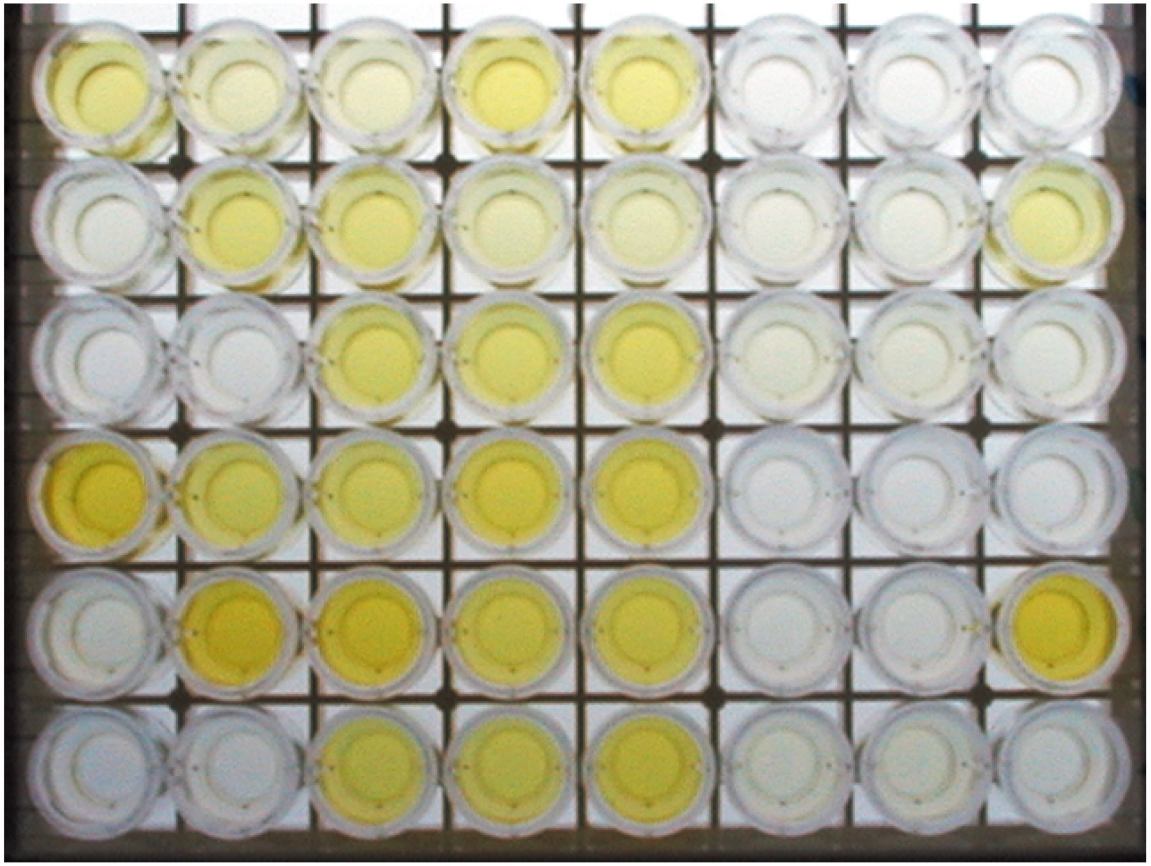
Antibody detection can be colormetric or luminescent



ELISA

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Immunoblot

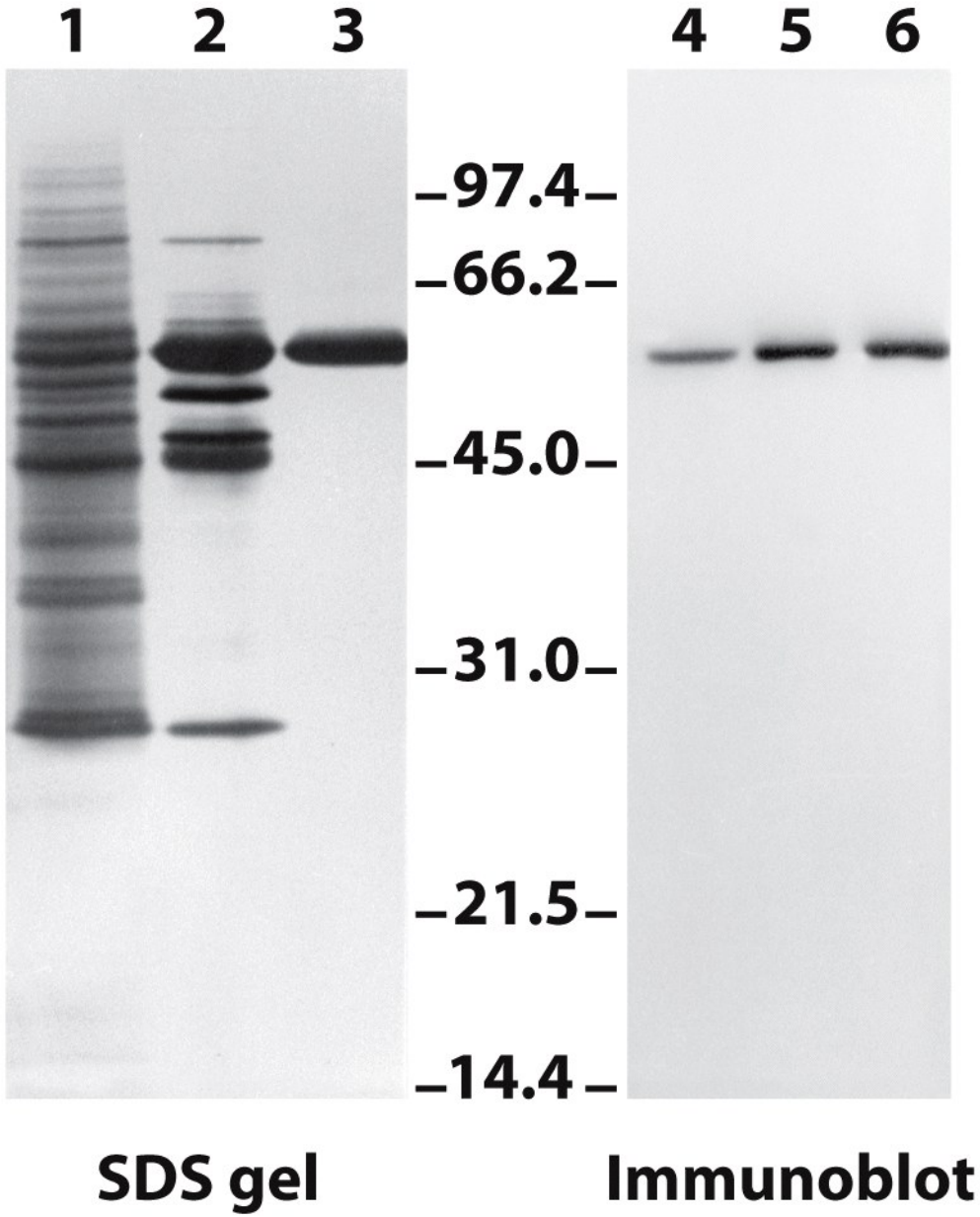

Figure 5-26c
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