

Lehninger

SIXTH EDITION

Principles of Biochemistry

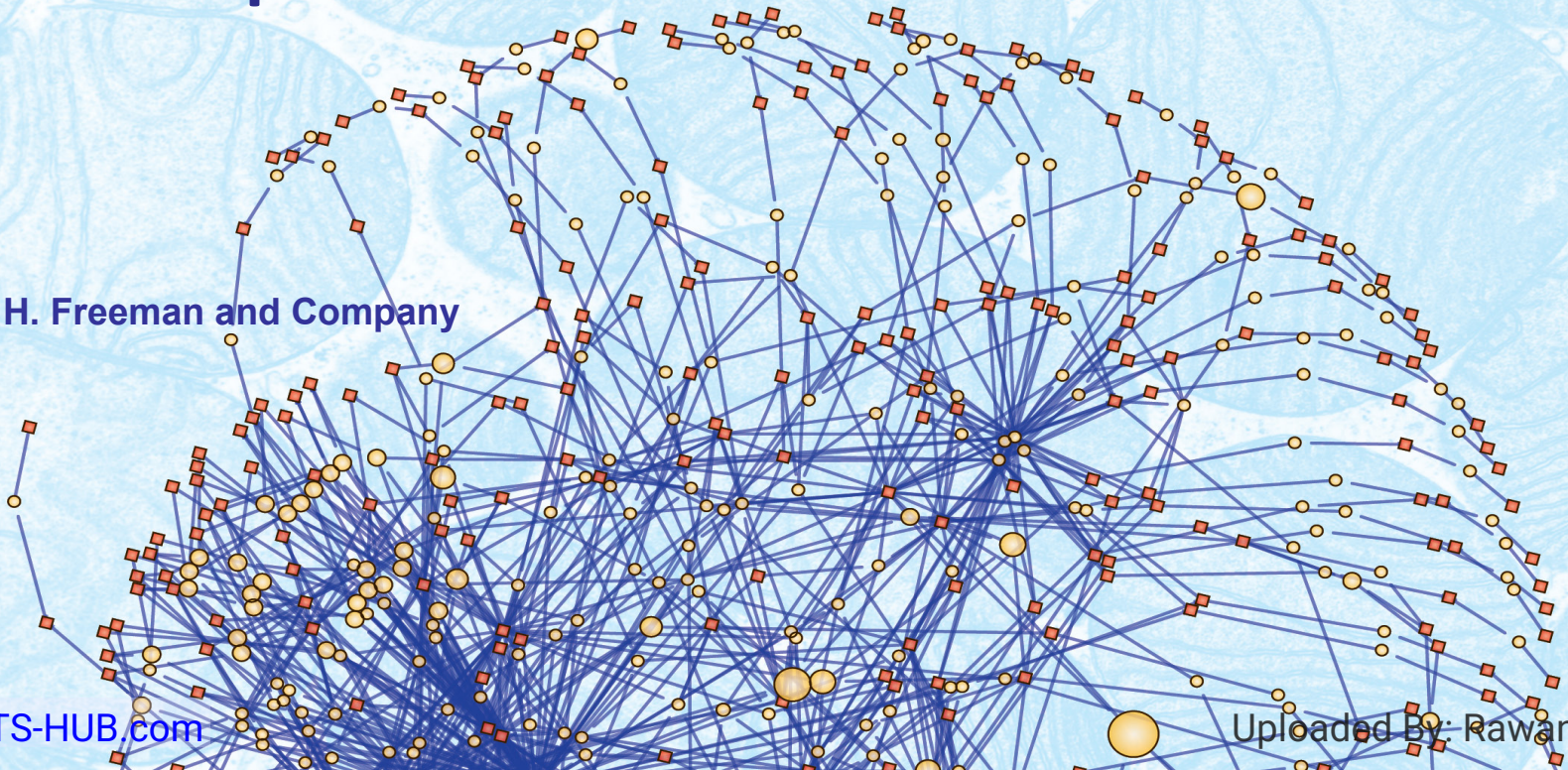
David L. Nelson | Michael M. Cox

5 | Function of Globular Proteins

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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

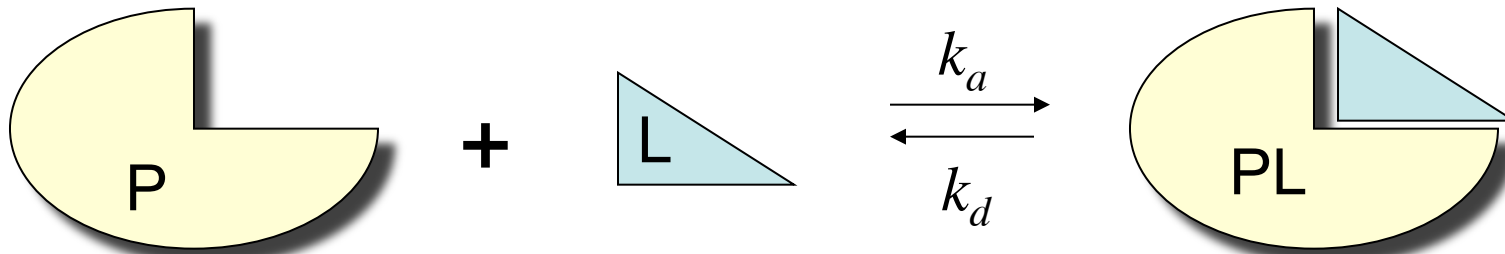
- Reversible, transient process of chemical equilibrium:
$$A + B \rightleftharpoons AB$$
- A molecule that binds to a protein is called a **ligand**
 - Typically a small molecule
- A region in the protein where the ligand binds is called the **binding site**
- Ligand binds via same **noncovalent** forces 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 $k_a[P] \cdot [L] = k_d[PL]$
- The **equilibrium composition** is characterized by the **equilibrium association constant** K_a

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

Binding:

Analysis in Terms of the Bound Fraction

- In practice, we can often determine the **fraction of occupied binding sites (θ)**
- Substituting $[PL]$ with $K_a[L][P]$, we'll eliminate $[PL]$
- Eliminating $[P]$ and rearranging gives the result in terms of equilibrium association constant
- In terms of the more commonly used **equilibrium dissociation constant**

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

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

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

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

Protein-Ligand Interactions

- Plotting θ as a function of $[L]$ can give the value of K_a
- At $\theta = 0.5 \rightarrow [L] = 1/K_a$
- Normally we use the **dissociation constant** ($K_d = 1/K_a$) $\rightarrow \theta = [L] / [L] + K_d$
- When $[L] > K_d$ by 9 x $\rightarrow$ 90% of sites are occupied
- Note: $\uparrow K_d \downarrow$ **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 $\frac{1}{2}$ binding sites to be filled $\rightarrow$ 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

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

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

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

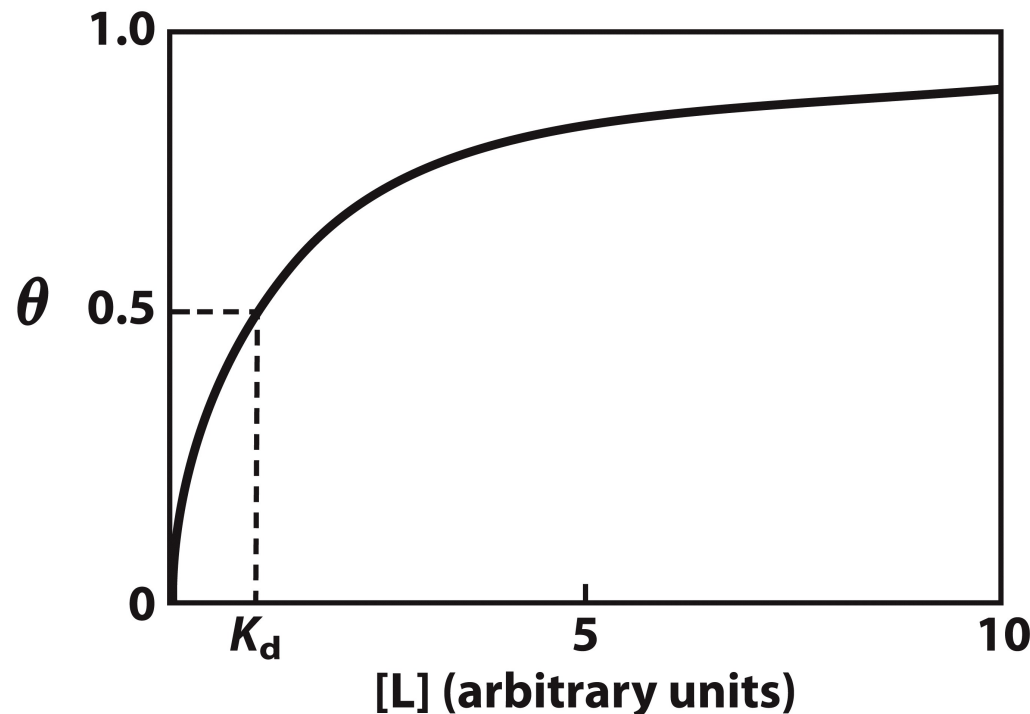


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Example: Oxygen Binding to Myoglobin

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

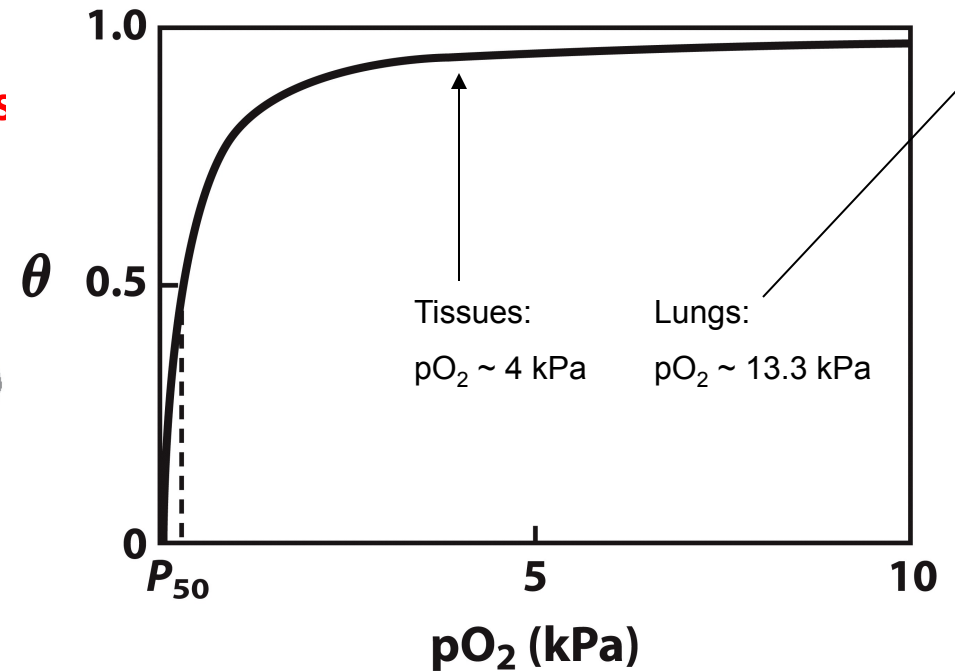
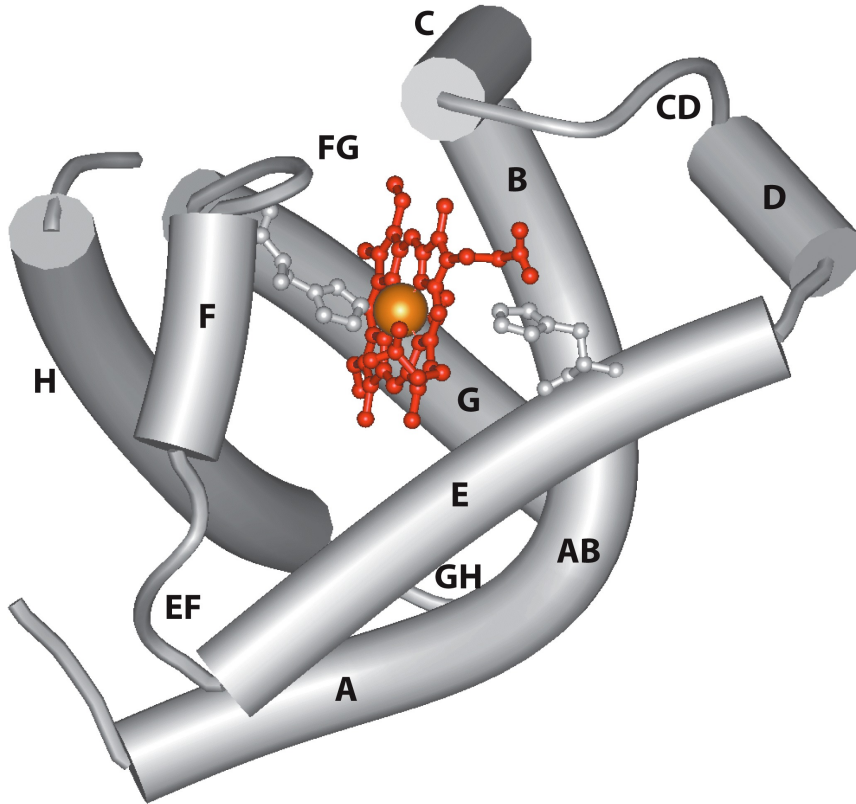


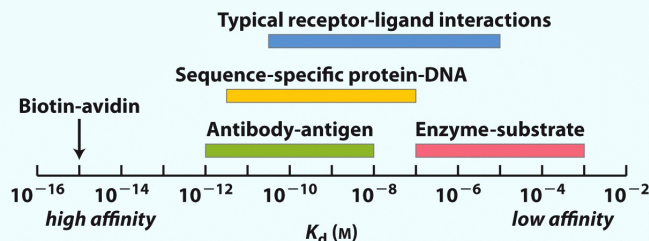
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$$\theta = \frac{[L]}{K_d + [L]} \longrightarrow \theta = \frac{pO_2}{p_{50} + pO_2}$$

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 (<i>E. coli</i>)	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.

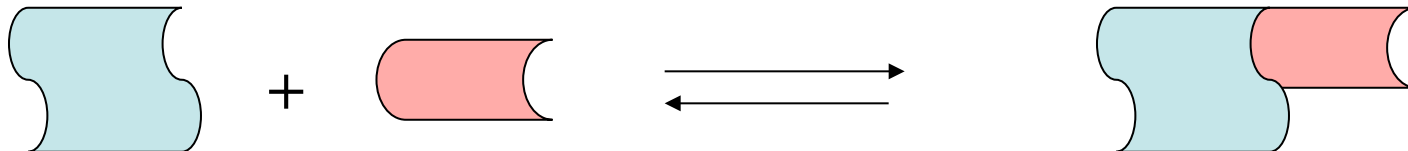
[‡]Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

Table 5-1

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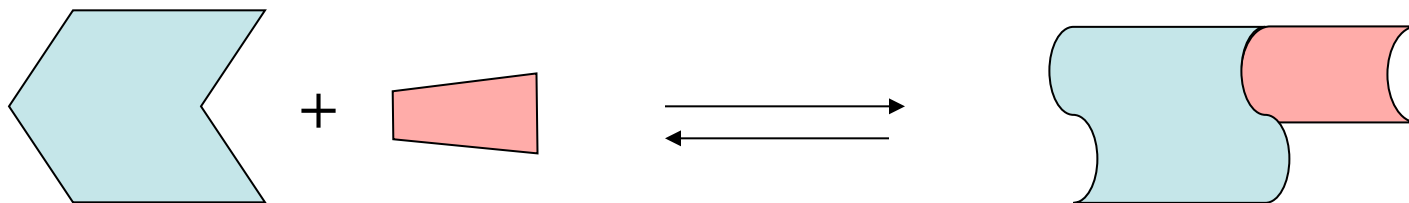
Specificity: Lock-and-Key Model

- 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



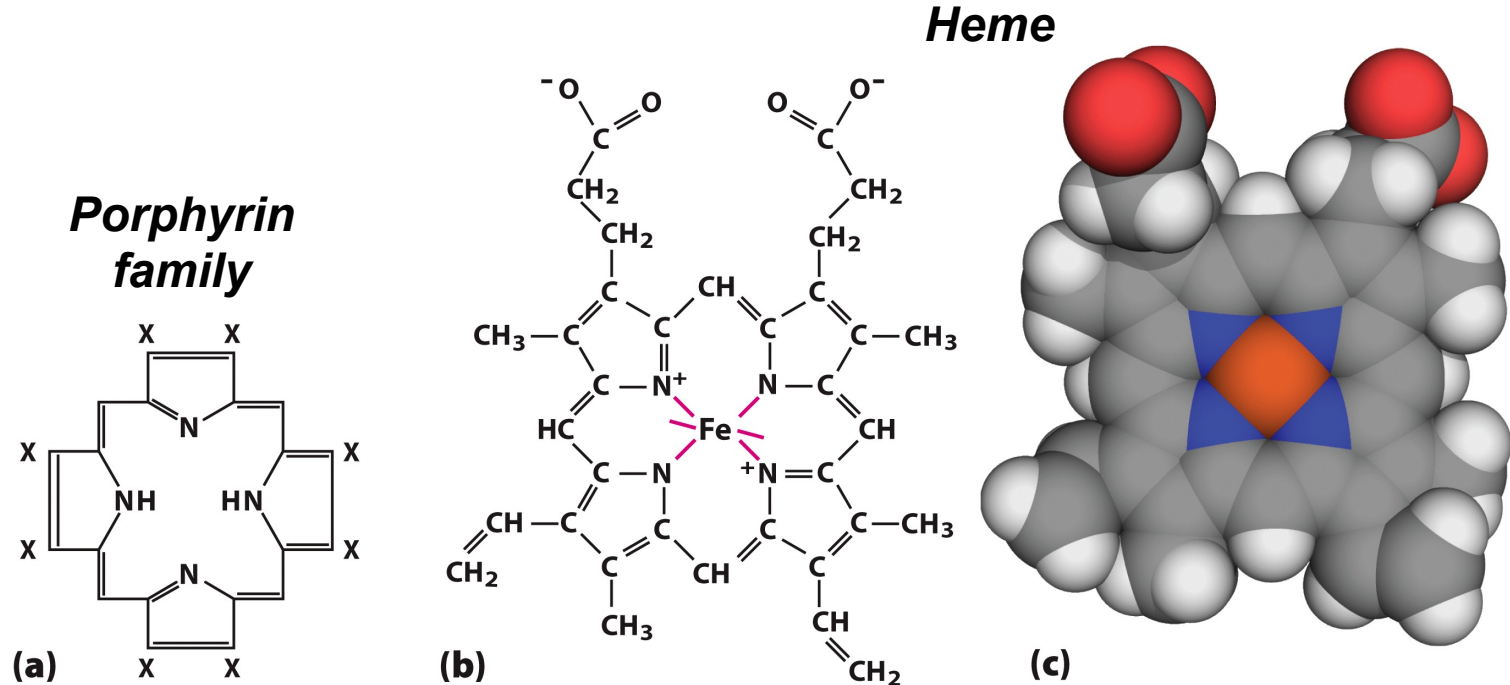
Globins are oxygen-binding proteins

- 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

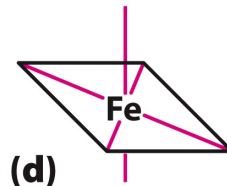
Example: Oxygen Binding to Myoglobin

- 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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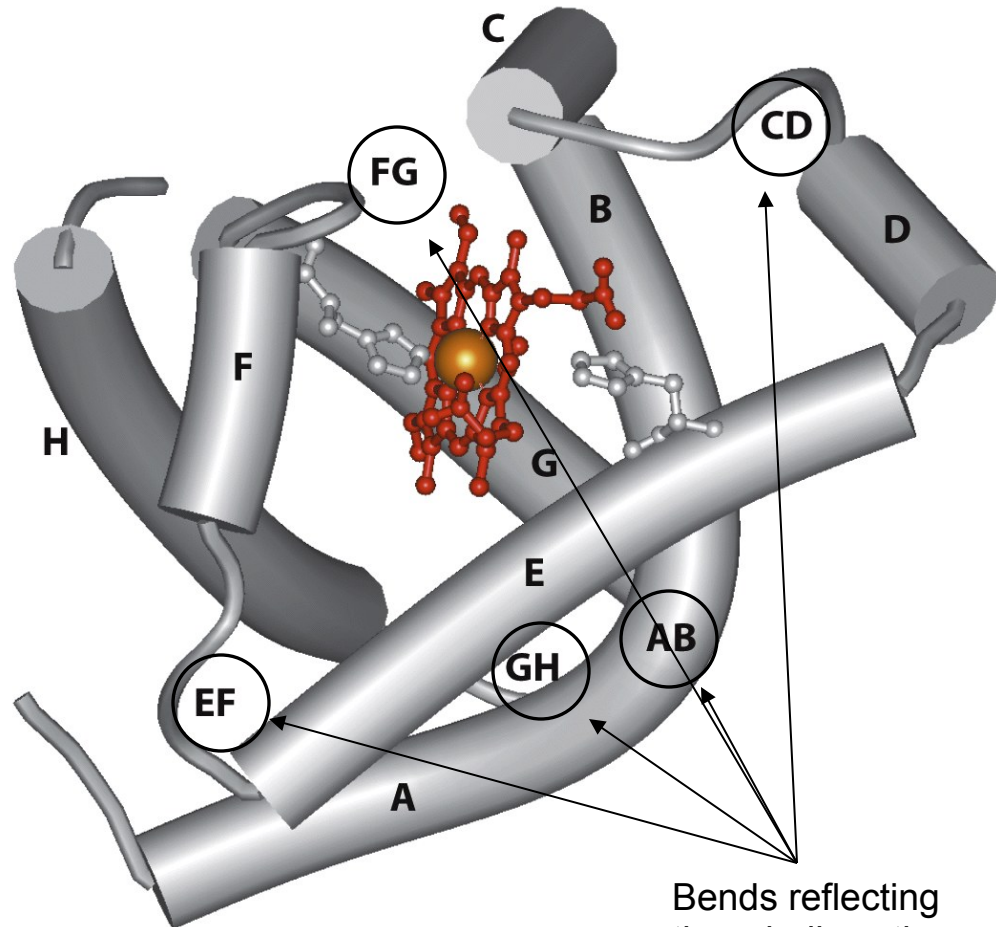
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Example: Oxygen Binding to Myoglobin

- Free heme molecules not bound in proteins → 2 open coordination bonds
- Reaction of 1 O₂ molecule with two hemes will lead to irreversible conversion of Fe²⁺ to Fe³⁺ which does not bind O₂
- 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 O₂
- When O₂ binds, electronic properties of heme changes (color changes from dark purple to bright red)
- CO and NO bind more tightly to heme than O₂ → 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 His⁹³ (or His F8)



Bends reflecting
the α helices they
connect

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Binding of Carbon Monoxide

- CO has similar size and shape to O₂; it can fit to the same binding site
- CO binds over 20,000 times better than O₂ because the carbon in CO has a filled lone electron pair that can be donated to vacant d-orbitals on the Fe²⁺
- 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. O₂ Binding to Free Heme

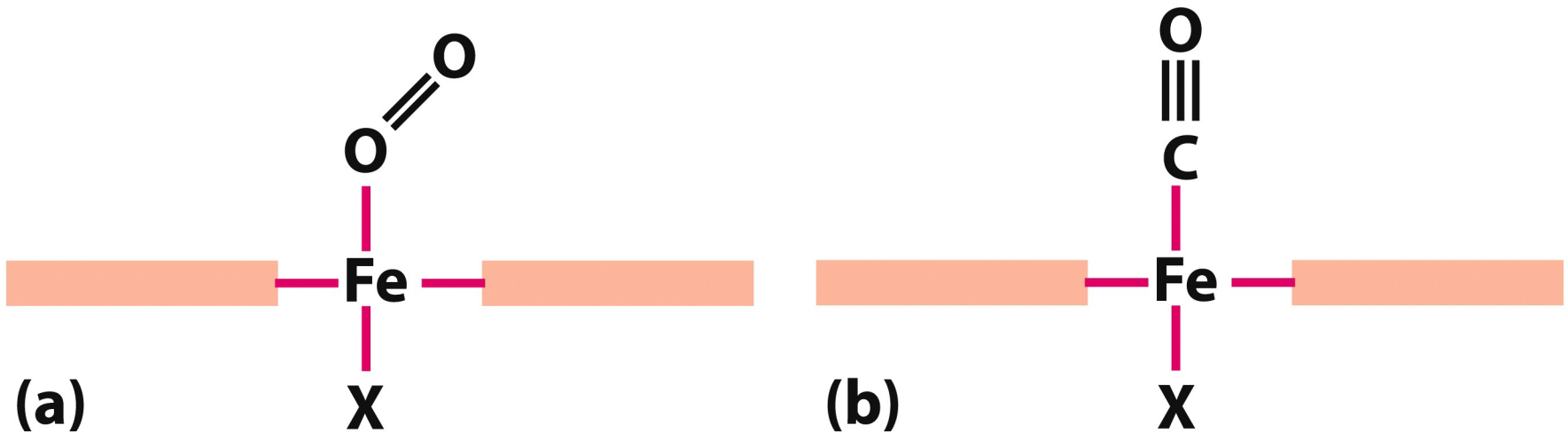
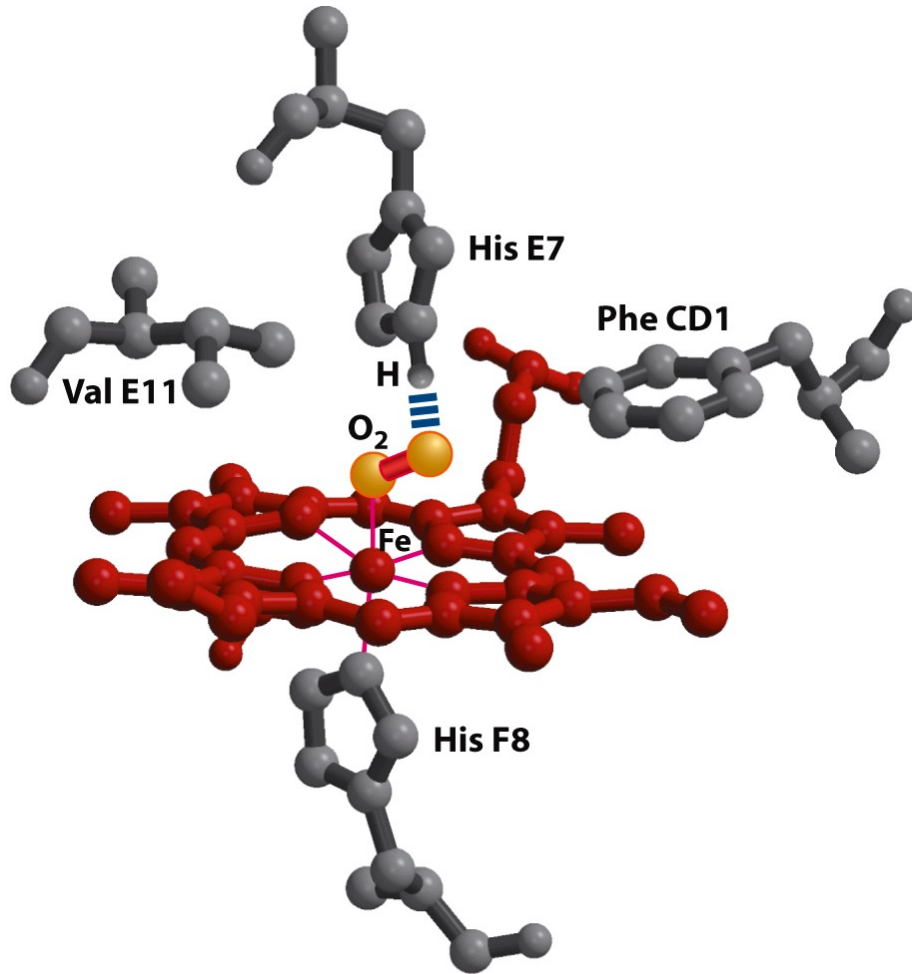


Figure 5-5ab

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Heme binding to protein affects CO vs. O₂ 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.

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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 O₂?

- pO₂ in lungs is about 13 kPa: it sure binds oxygen well
- pO₂ in tissues is about 4 kPa: it will not release it!

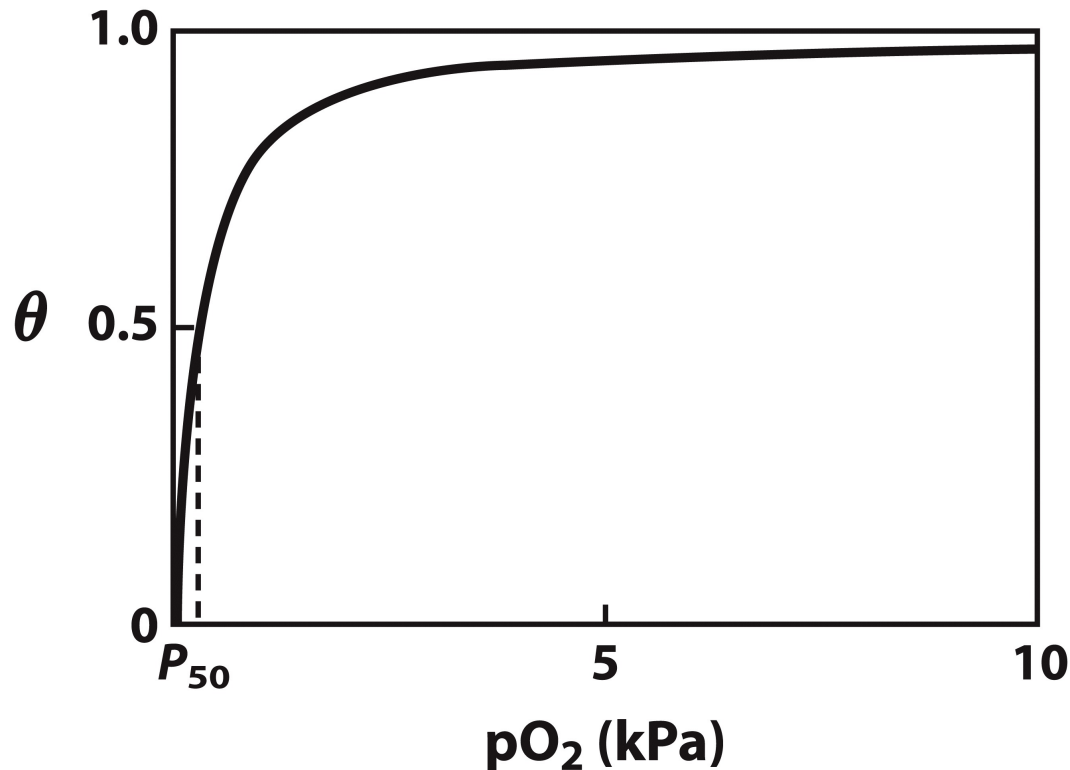


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- Would lowering the affinity (P₅₀) of myoglobin to oxygen help?

For effective transport affinity must vary with pO_2

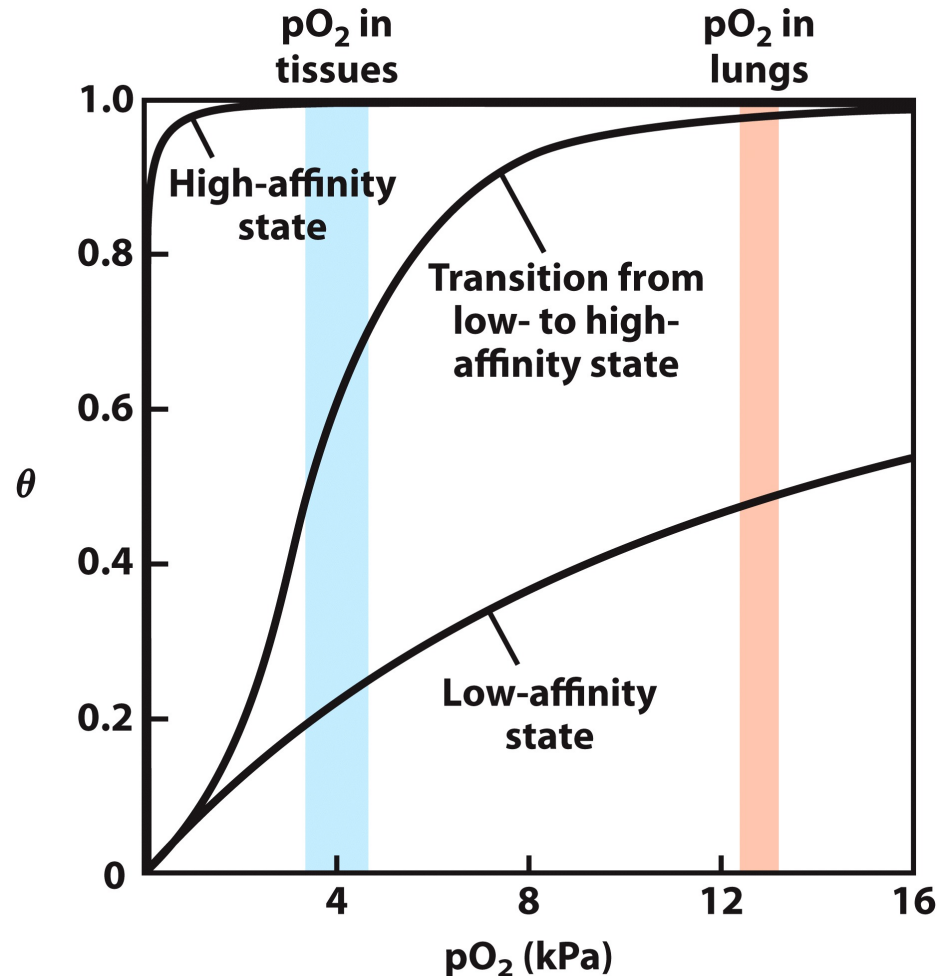


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How can affinity to oxygen change?

- 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

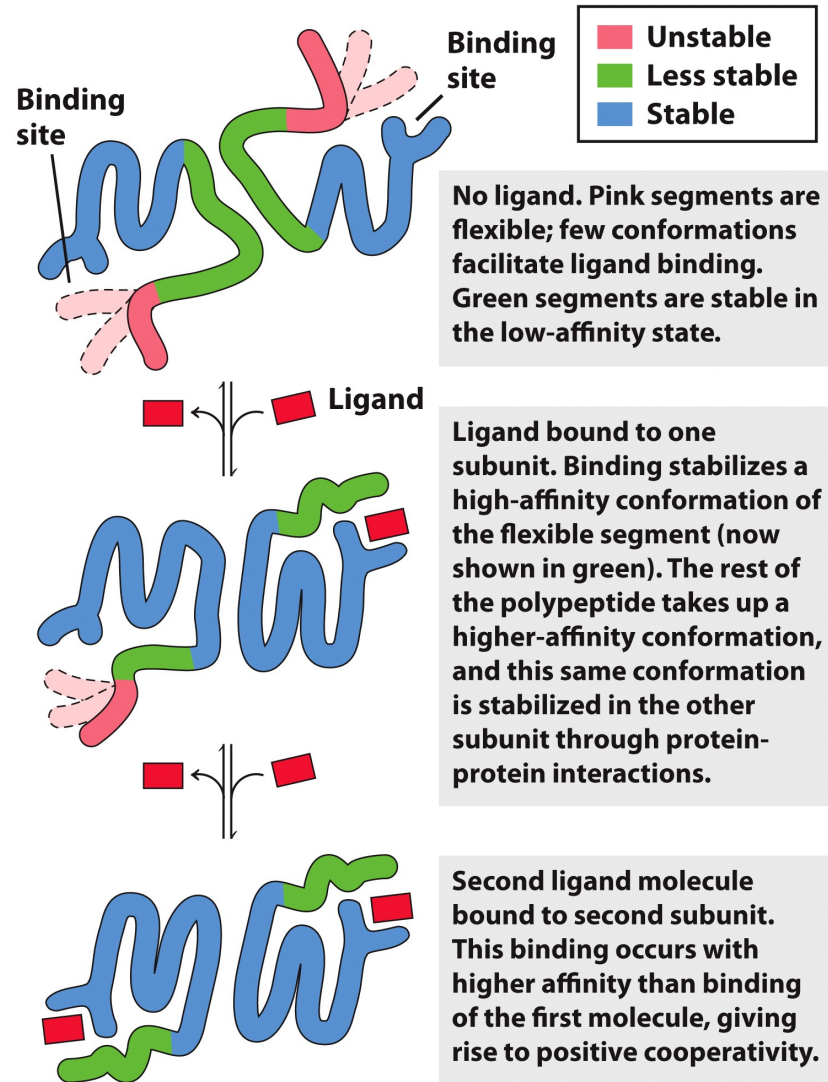


Figure 5-13

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Cooperativity: Quantitative Description

- Cooperative proteins have multiple ligand-binding sites

- so K_a becomes:

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

- And θ becomes:

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

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

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

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

The Hill Plot of Cooperativity

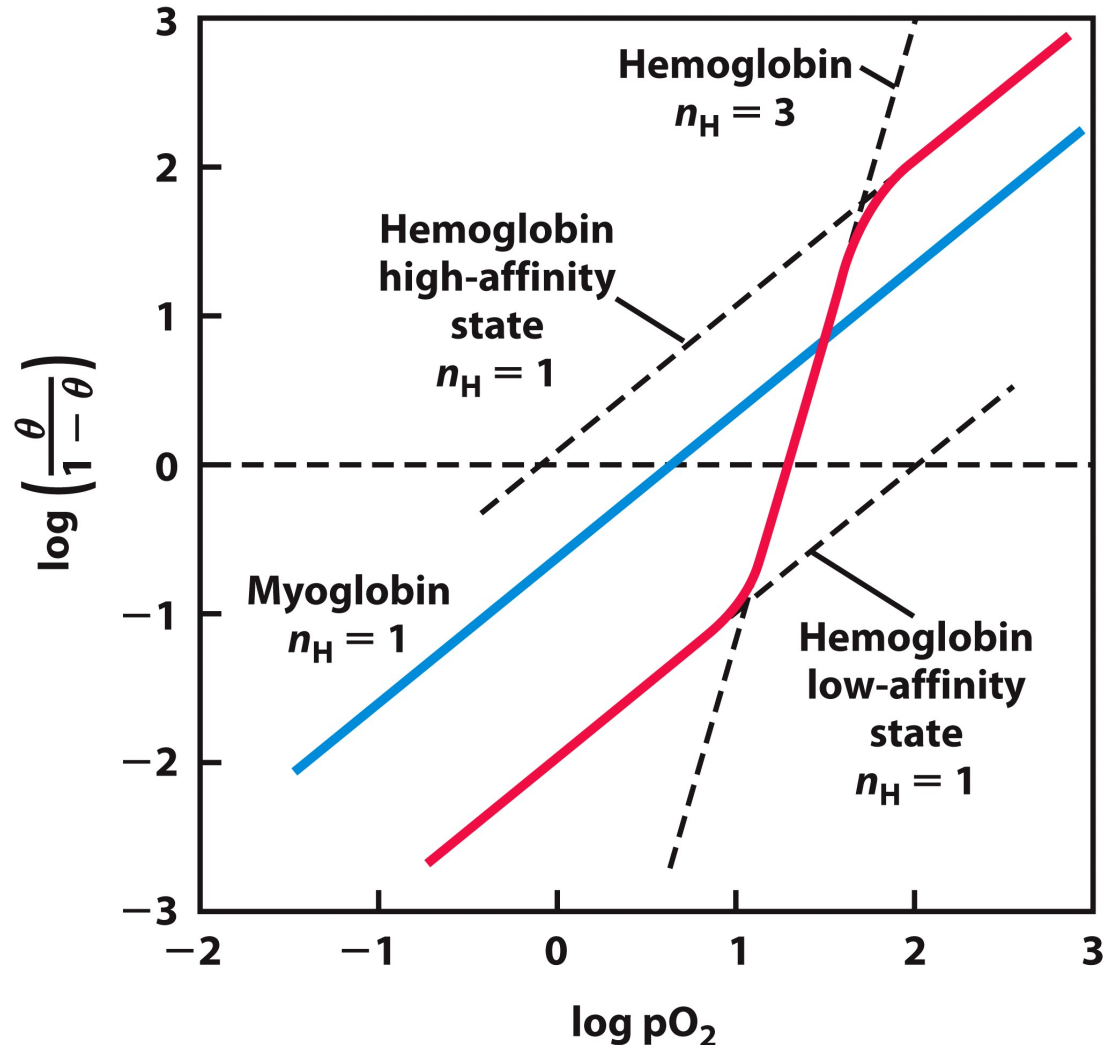


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Cooperativity is a special case of allosteric regulation

- **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 $\sim 64\%$
- Mb is insensitive to small changes in $[O_2]$ (O_2 -storage protein)
- Hb is sensitive to small changes $\rightarrow O_2$ -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

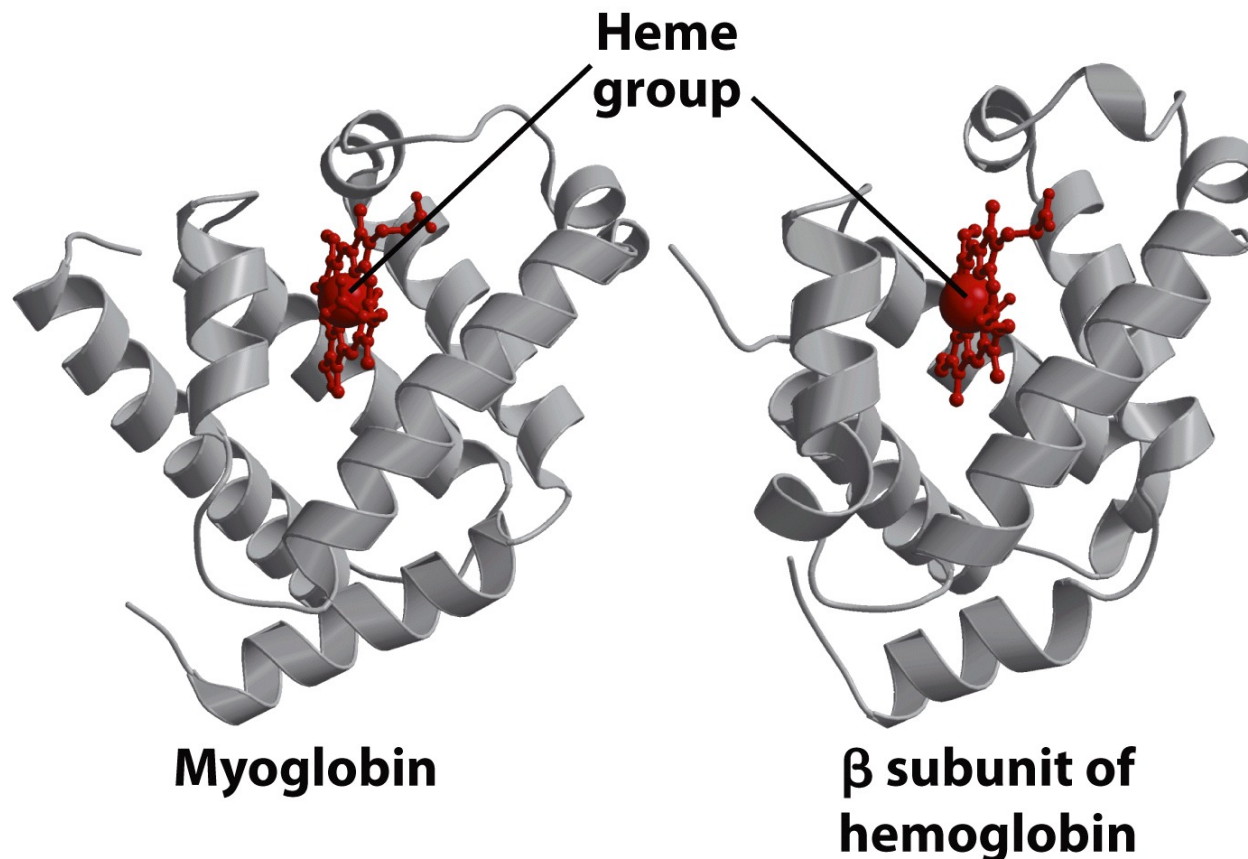


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Hb Subunits are Similar to Mb

- 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
- Hb α does not have D helix

Sequence Similarity between Hemoglobin and Myoglobin

	Mb	Hb α	Hb β
NA1	-- 1V	1V	1V
	—	—	H
	L	L	L
A1	---S	S	T---
	E	P	P
	G	A	E
	E	D	E
	W	K	K
	Q	T	S
	L	N	A
	V	V	V
	L	K	T
	H	A	A
	V	A	L
	W	W	W
	A	G	G
	K	K	K
	V	V	V
A16	---E	G	----
	A	A	—
B1	--20D	20H	N----
	V	A	20V
	A	G	D
	G	E	E
	H	Y	V
	G	G	G
	Q	A	G
	D	E	E
	I	A	A
	L	L	L
	I	E	G
	R	R	R
	L	M	L
	F	F	L
	K	L	V
B16	---S	S	V----
	:	:	:
	:	:	:
	:	:	:

	Mb	Hb α	Hb β
	K	A	A
	K	H	H
	K	V	L
80G	D	D	D
	H	D	80N
	H	M	L
	E	P	K
	A	N	G
	E	A	T
F1	---L	80L	F---
	K	S	A
	P	A	T
	L	L	L
	A	S	S
	Q	D	E
	S	L	L
	H	H	H
Proximal His F8	---	A	C---
F9	---	A	C---
	T	H	D
	K	K	K
	H	L	L
	K	R	H
	I	V	V
G1	--100P	D	D---
	I	P	100P
	K	V	E
	Y	N	N
	L	F	F
	E	K	R
	I	L	L
	S	S	G
	E	H	N
	A	C	V
	I	L	V
	:	:	:
	:	:	:
	:	:	:

	:	:	:
C1	---H	F	Y---
	P	P	P
	E	T	W
	T	T	T
40L	E	40K	Q
	E	T	40R
C7	---K	Y	F---
	F	F	F
	D	P	E
	R	H	S
	F	F	F
	K	—	G
	H	D	D
	L	L	L
	K	S	S
D1	---T	H	T---
	E	—	P
	A	—	D
	E	—	A
	M	—	V
	K	—	M
D7	---A	G	G---
E1	---S	S	N---
	E	A	P
60D	Q	60K	K
	L	V	60V
	K	K	K
	K	G	A
	H	H	H
	G	G	G
	V	60K	K
	T	K	K
	V	V	V
	L	A	L
	T	D	G
	A	A	A
	L	L	F
	G	T	S
	A	N	D
	I	A	G

	:	:	:
	H	V	C
	V	T	V
	L	L	L
	H	A	A
	S	A	H
G19	---S	A	H---
	H	L	F
120P	P	P	G
	G	A	120K
	D	E	E
	F	F	F
H1	---G	T	T---
	A	P	P
	D	120A	P
	A	V	V
	Q	H	Q
	G	A	A
	A	S	A
	M	L	Y
	N	D	Q
	K	K	K
	A	F	V
	L	L	V
	E	A	A
	L	S	G
	F	V	V
	R	S	A
140K	T	140A	N
	D	V	140A
	I	L	L
	A	S	A
H21	---A	S	H---
	K	K	K
	Y	140Y	HC1
	K	141R	HC2
	E	146H	HC3
	L	-----	
H26	---	-----	
	G	-----	
	Q	-----	

Hb α and Hb β only

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

- 4^o 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

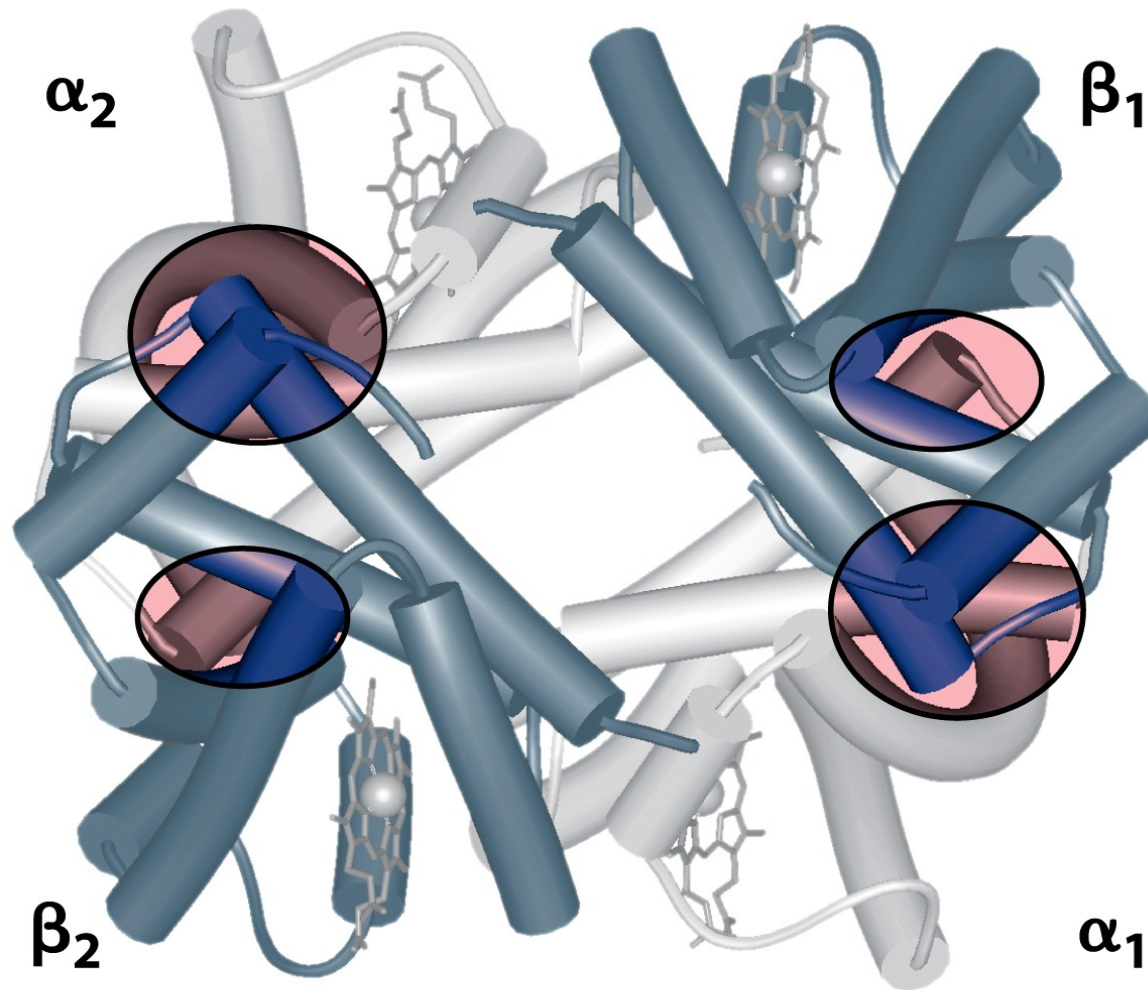


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R and T States of Hemoglobin

- Two major conformations of Hb:
R state and **T state**
- O₂ binds to Hb in either one, but it has to R state
- **T = Tense** state
 - More interactions, more stable
 - **Lower affinity** for O₂
- **R = Relaxed** state
 - Fewer Interactions, more flexible
 - **Higher affinity** for O₂

Hb Changes Structure after O₂ Binding

- O₂ binding stabilizes R state
- T state is more stable when not bound to O₂ (deoxyhemoglobin)
- *O₂ binding to a Hb subunit at the T state converts the subunit to R state*
- Therefore, O₂ 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

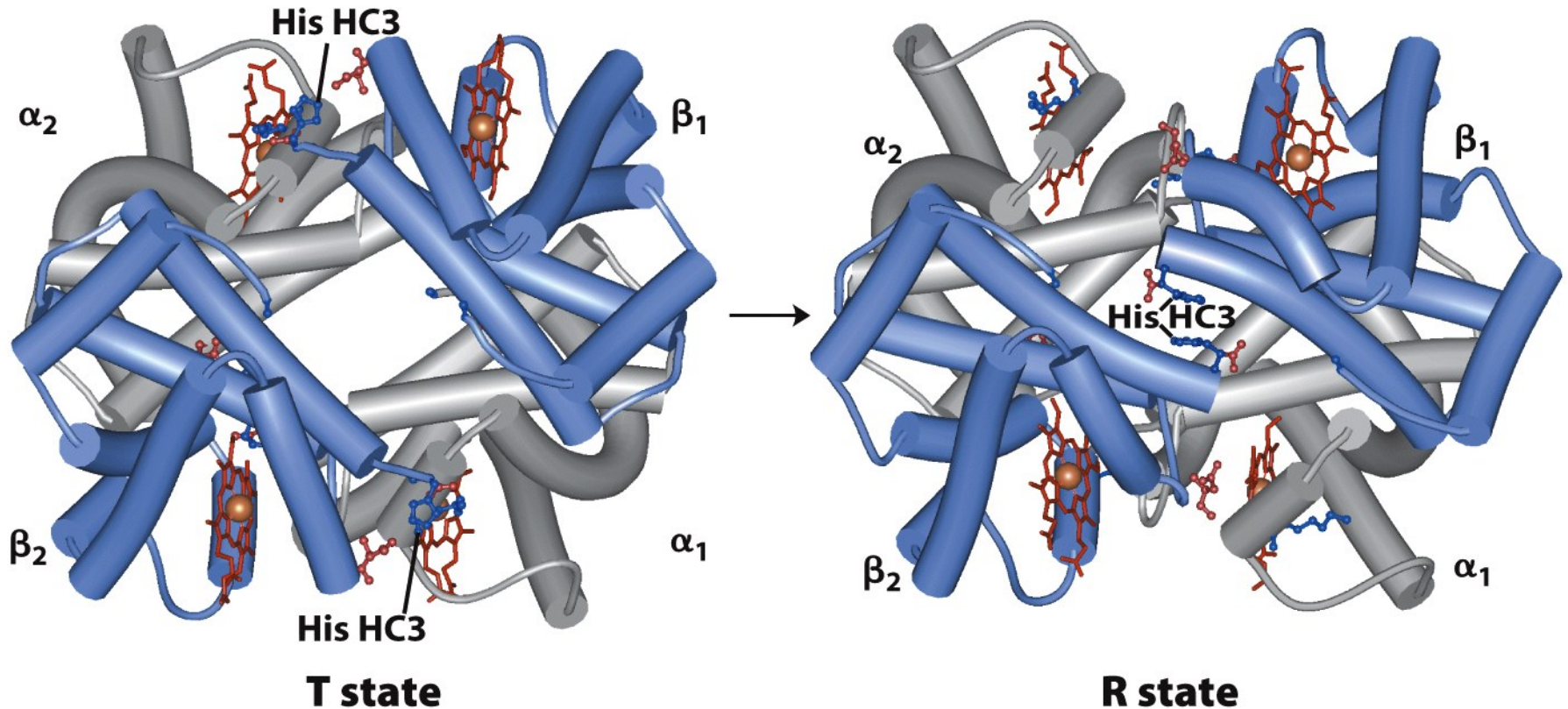


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The transition from the T state to the R state shifts the subunit pairs, affecting certain ion pairs. Most noticeably, the His HC3 residues at the carboxyl termini of the β subunits, which are involved in ion pairs in the T state, rotate in the R state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

pH Effect on O₂ 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 O₂ (in the tissues)
- The pH difference between lungs and metabolic tissues increases efficiency of the O₂ transport
- This is known as the **Bohr effect**

pH Effect on O₂ Binding to Hemoglobin

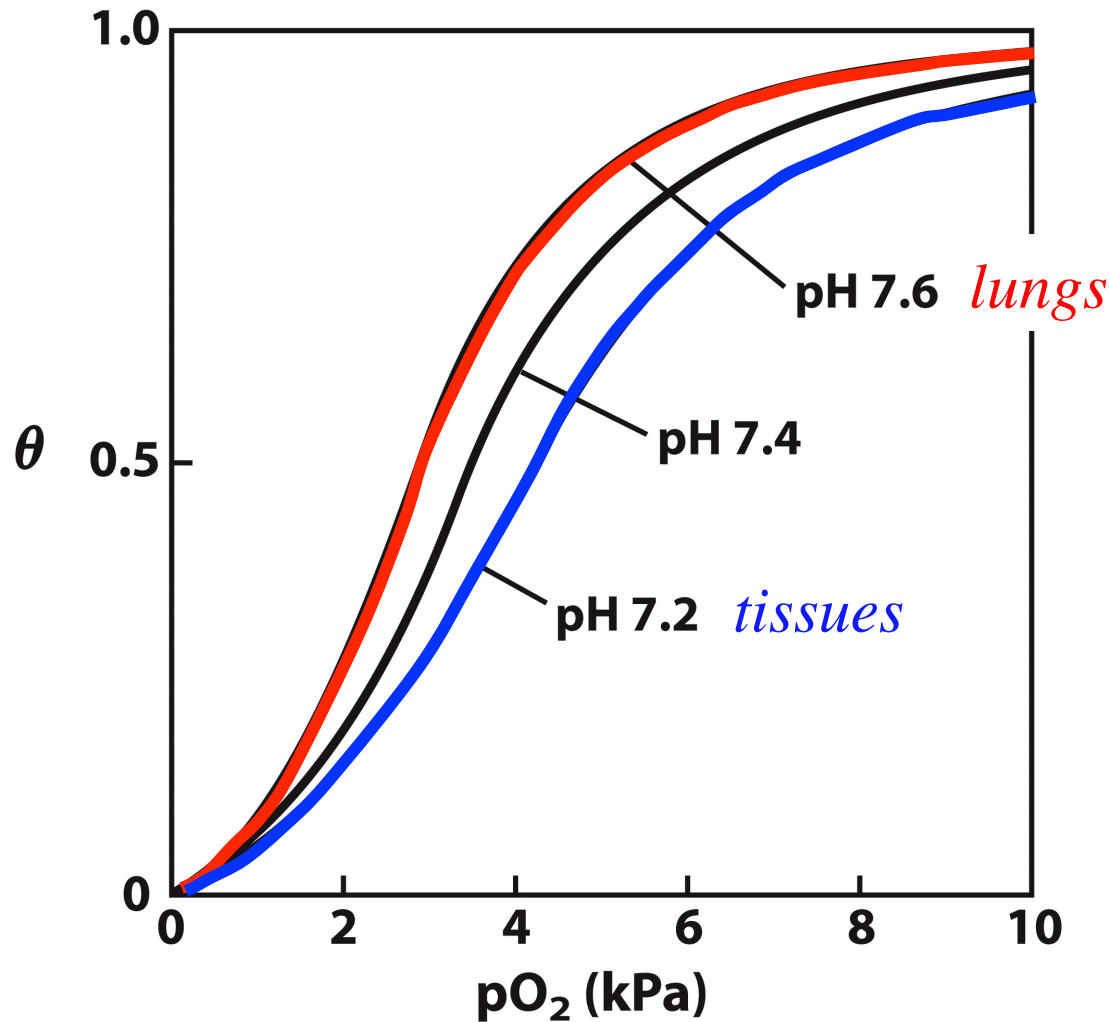


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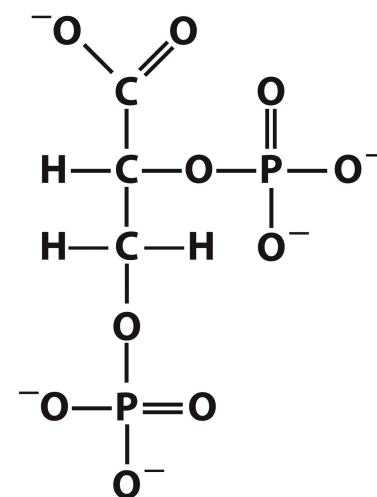
Hemoglobin and CO₂ Export

- CO₂ is produced by metabolism in tissues and must be exported
- 15–20% of CO₂ 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 CO₂ is exported as dissolved bicarbonate
 - Formed by carbonic anhydrase, and also producing a proton

2,3-Bisphosphoglycerate regulates O₂ 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



2,3-Bisphosphoglycerate

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

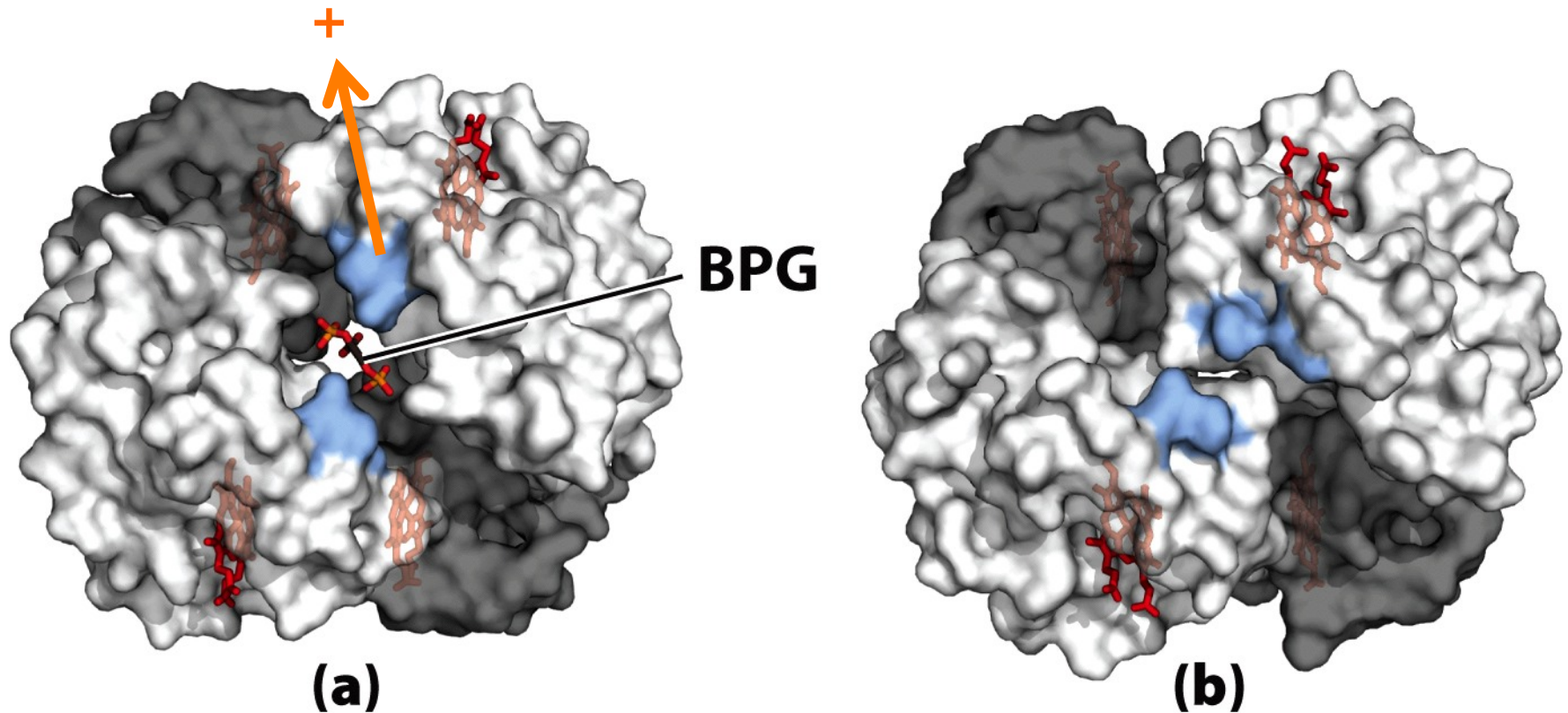


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BPG binding stabilizes the T state of deoxyhemoglobin

The binding pocket for BPG disappears on oxygenation

2,3-BPG allows for O₂ release in the tissues and adaptation to changes in altitude

- ★ At sea level, Hb is nearly saturated with O₂ in the lungs
- ★ Hb is just over 60% saturated in the tissues
- ★ The amount of O₂ released in the tissues is about 38% of the maximum that can be carried in the blood
- ★ At high altitudes, O₂ delivery declines to 30% of maximum
- ★ An increase in [BPG] decreases the affinity of Hb for O₂, so ~ 37% of what can be carried is again delivered to the tissues

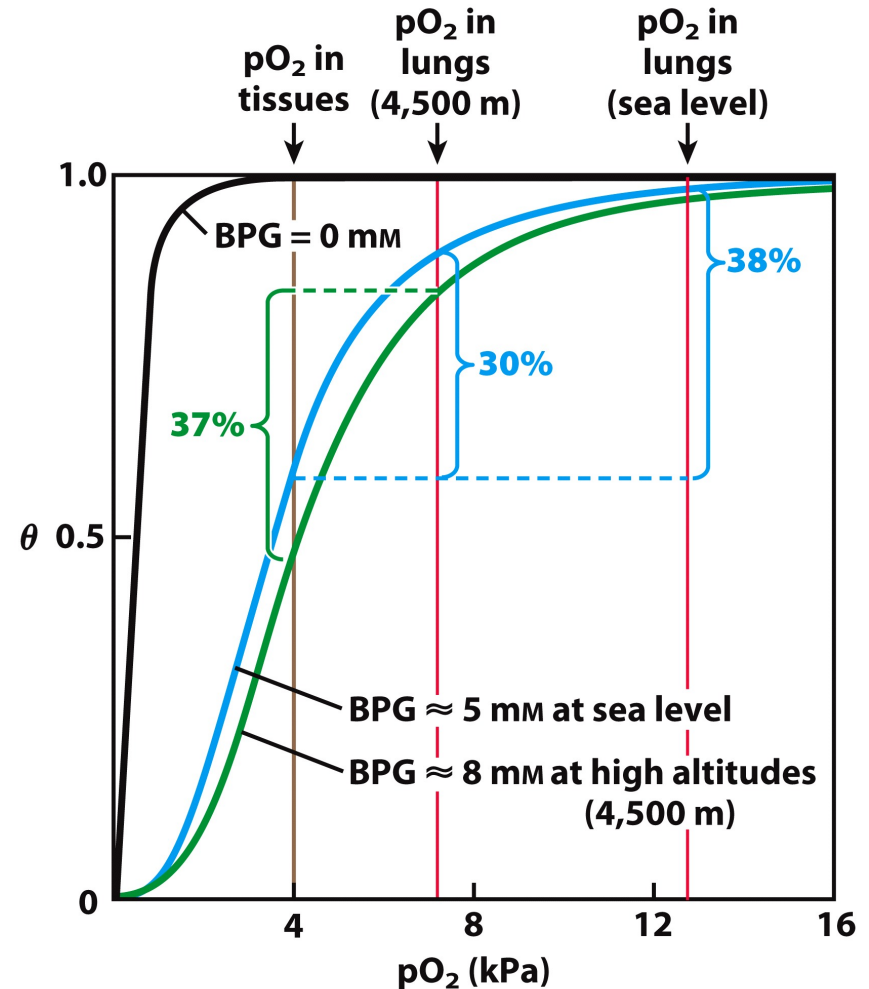
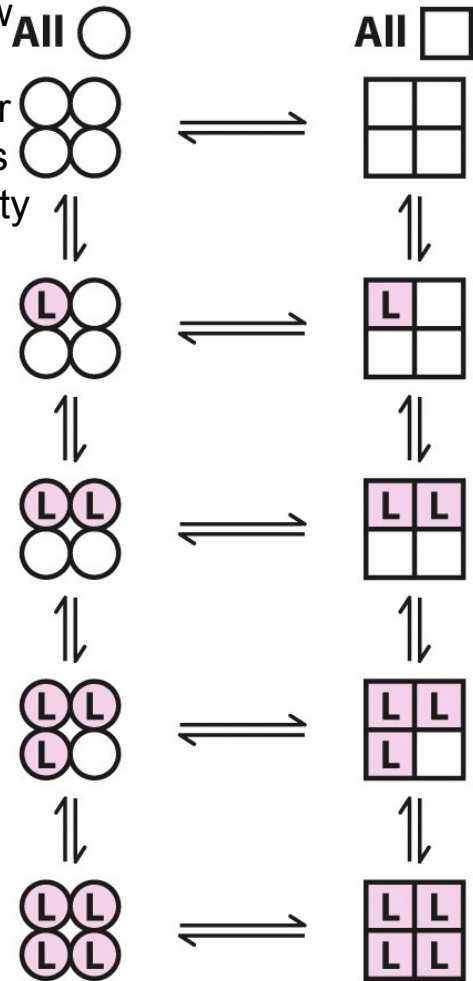


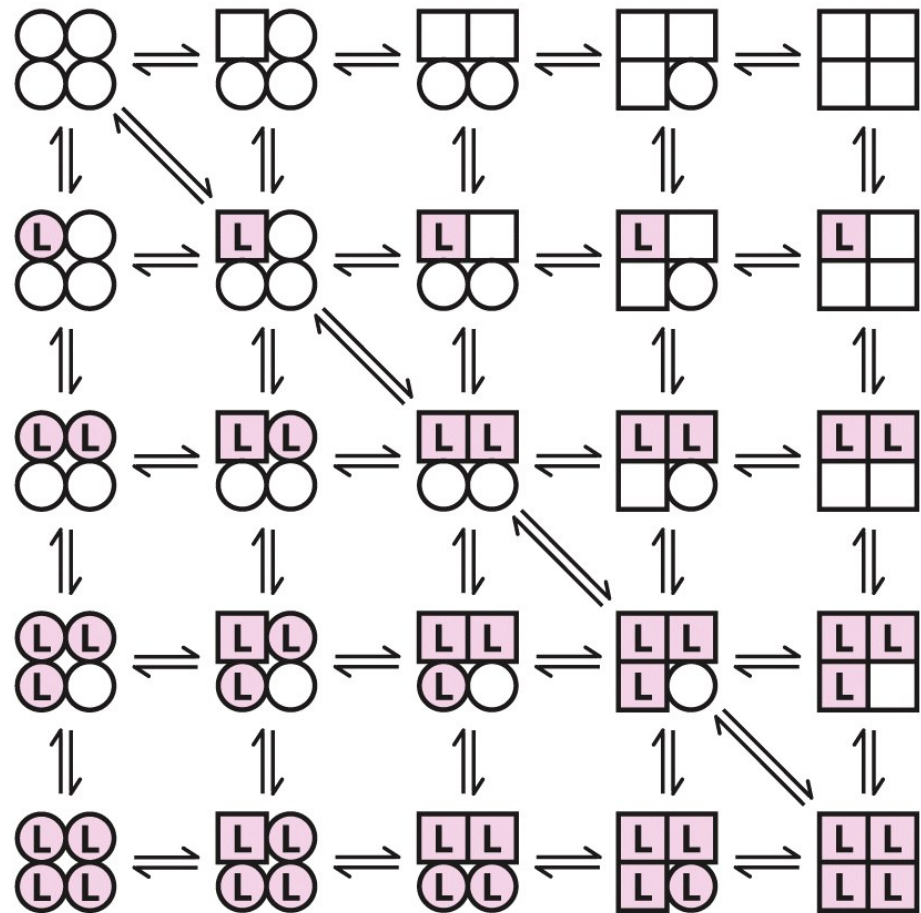
Figure 5-17
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Two Models of Cooperativity: Concerted (MWC) vs. Sequential

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

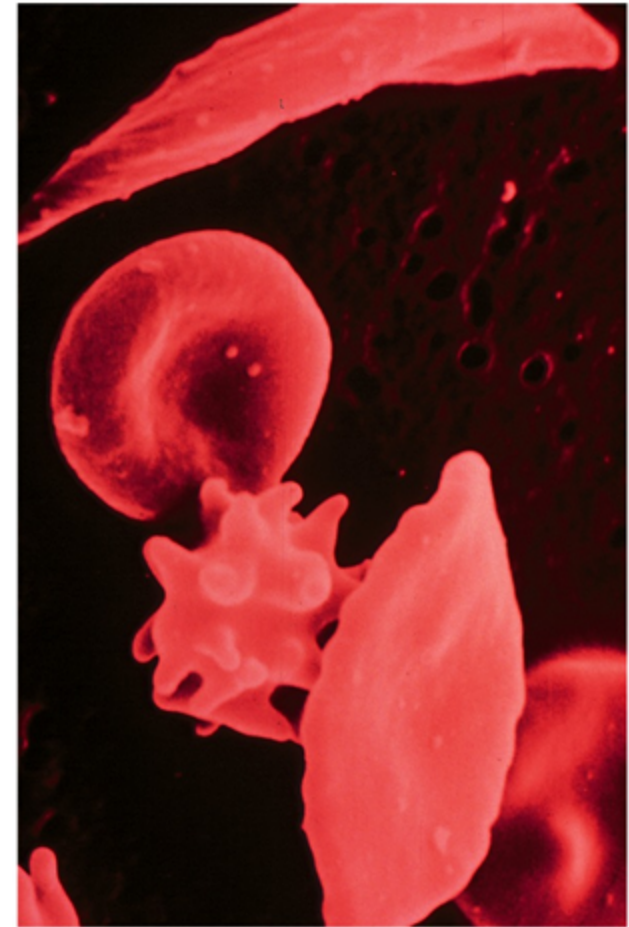


Each individual subunit can be in either the or form. A very large number of conformations is thus possible



Sickle-cell anemia is due to a mutation in hemoglobin

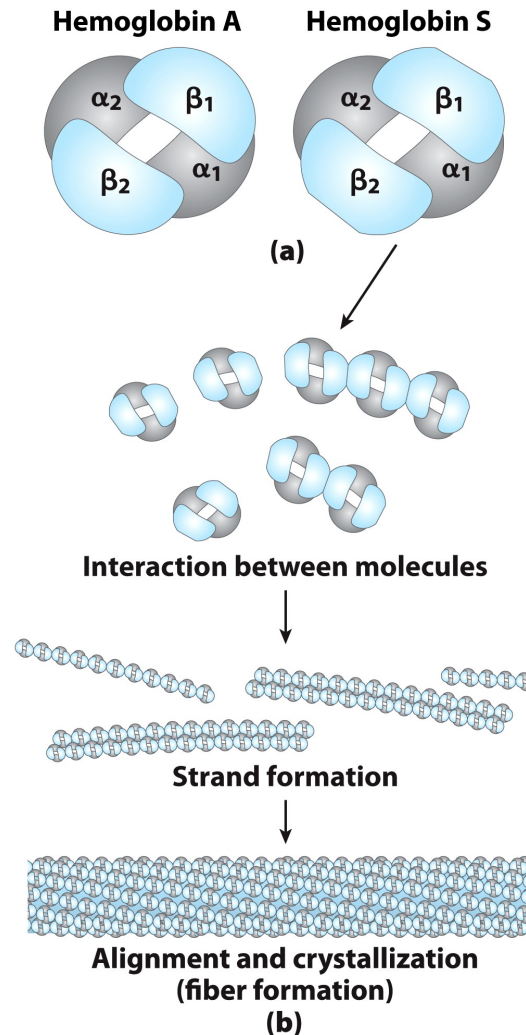
- Sickle-cell disease occurs in individuals *homozygous* for the sickle cell allele of the gene encoding the β subunit of Hb
- When Hb from a sick patient is deoxygenated (Hb S) it aggregates and precipitates (normal Hb, Hb A does not precipitate upon deoxygenation)
- The difference is a single aa substitution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



(b)

- Heterozygous individuals exhibit a resistance to malaria

Formation of Hb Strands in Sickle-Cell Anemia



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

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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 than 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 O₂ saturation of hemoglobin

P_{O₂}
kPa mmHg

Fetal
hemoglobin

Maternal hemoglobin

O₂ flows from maternal oxyhemoglobin
to fetal deoxyhemoglobin

0 20 40 60 80 100 120
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.

Table 5-2

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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

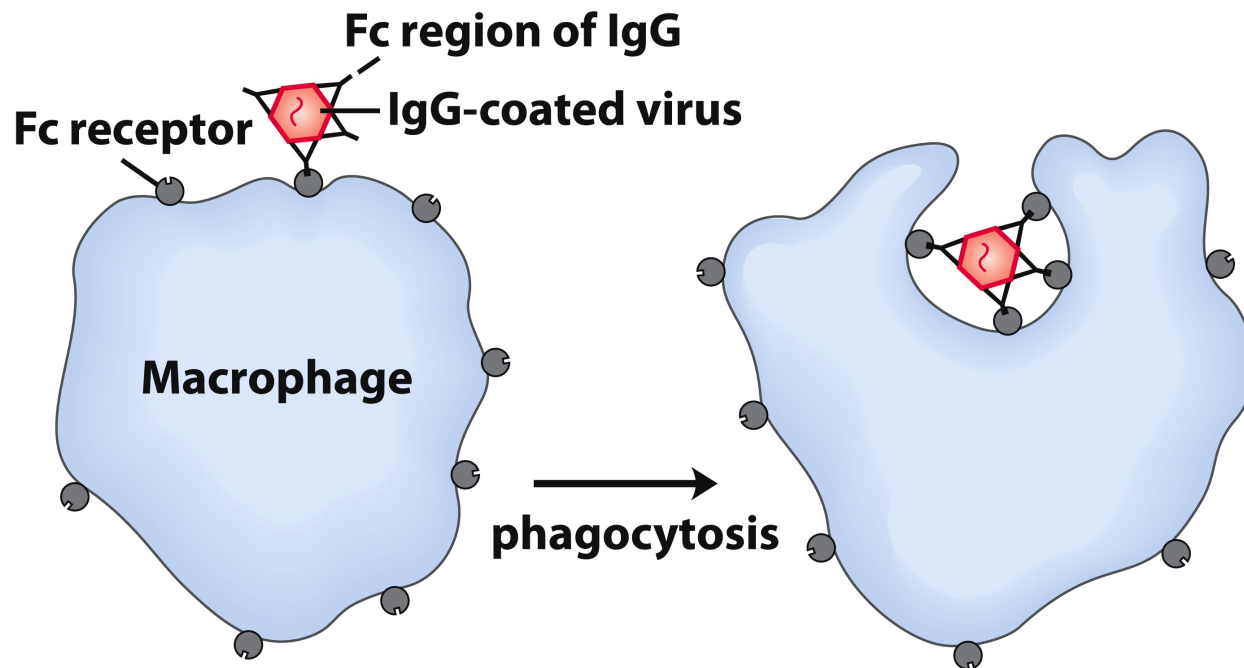


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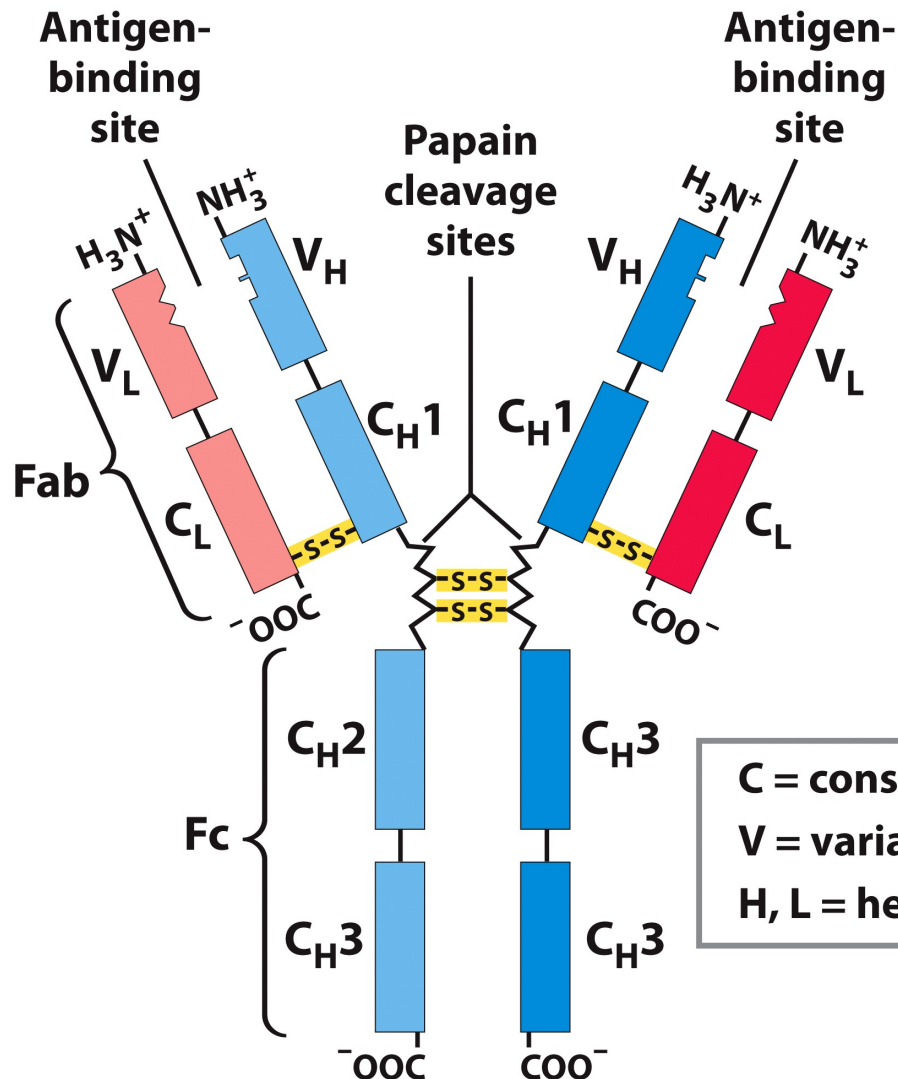
Humoral Immune System

- 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

C = constant domain
V = variable domain
H, L = heavy, light chains

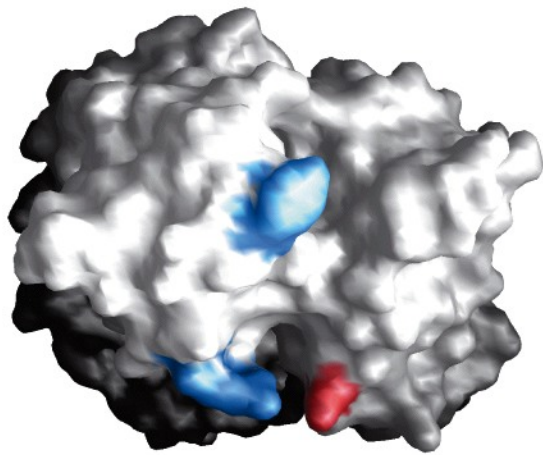
Figure 5-21a

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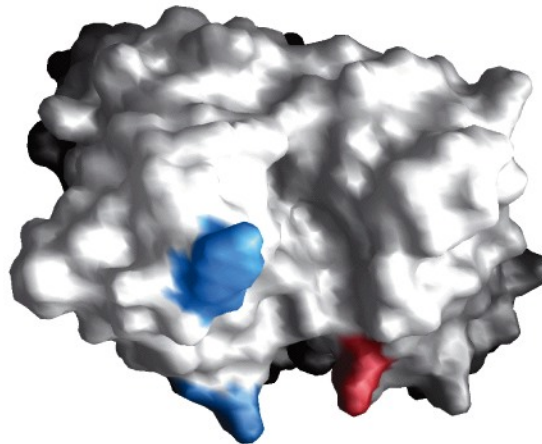
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Antigens bind via induced fit

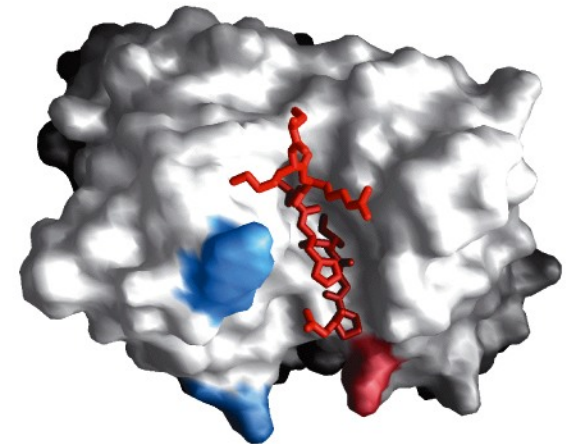
Antigen binding causes significant structural changes to the antibody



(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)

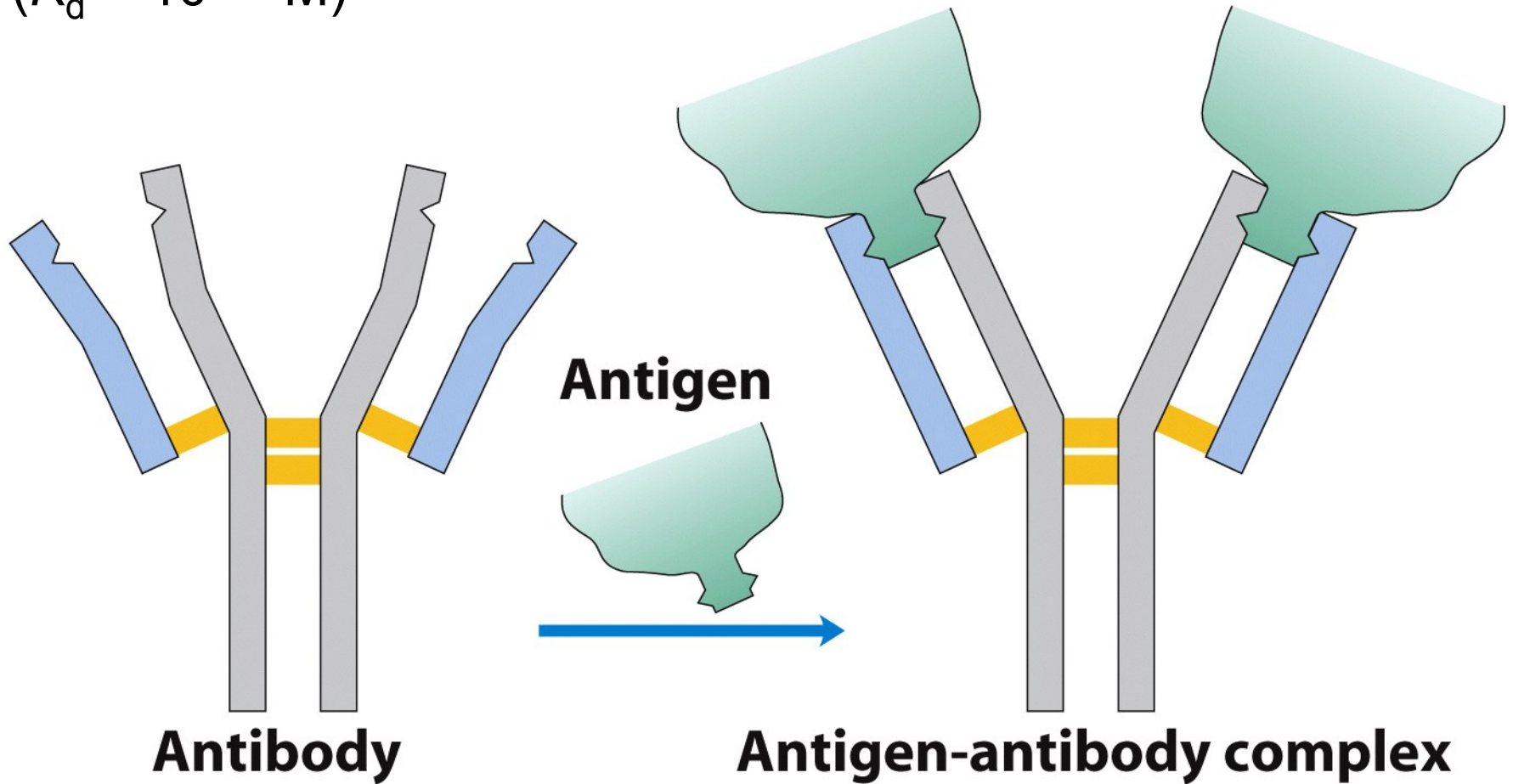


Figure 5-22

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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)

Antibody specificity is an important analytical reagent

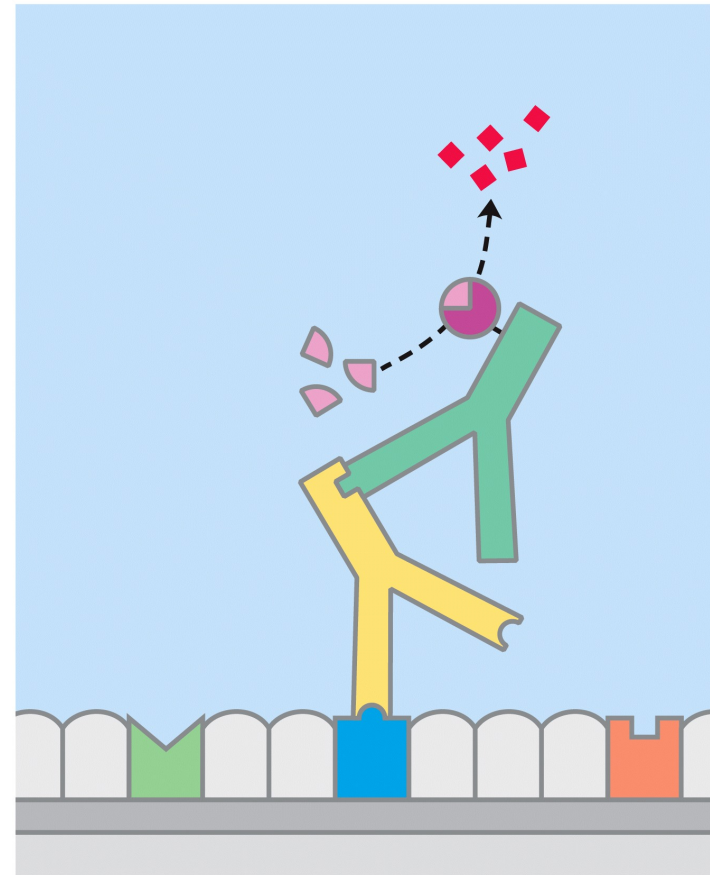
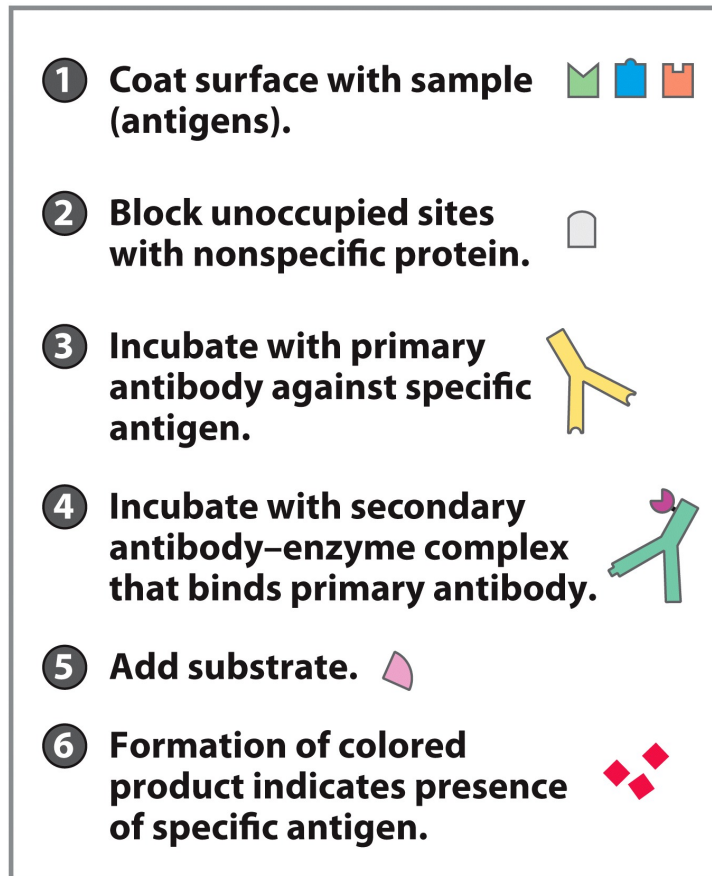
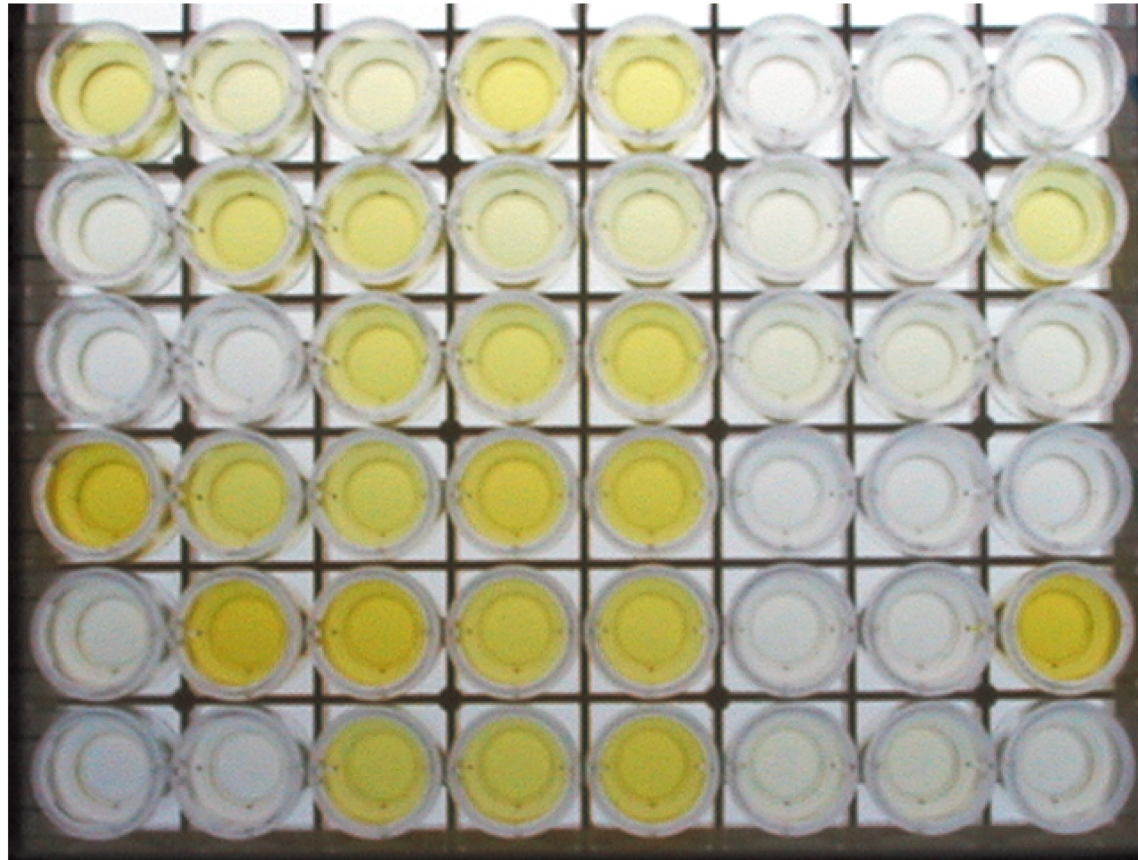


Figure 5-26a

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Antibody detection can be colormetric or luminescent

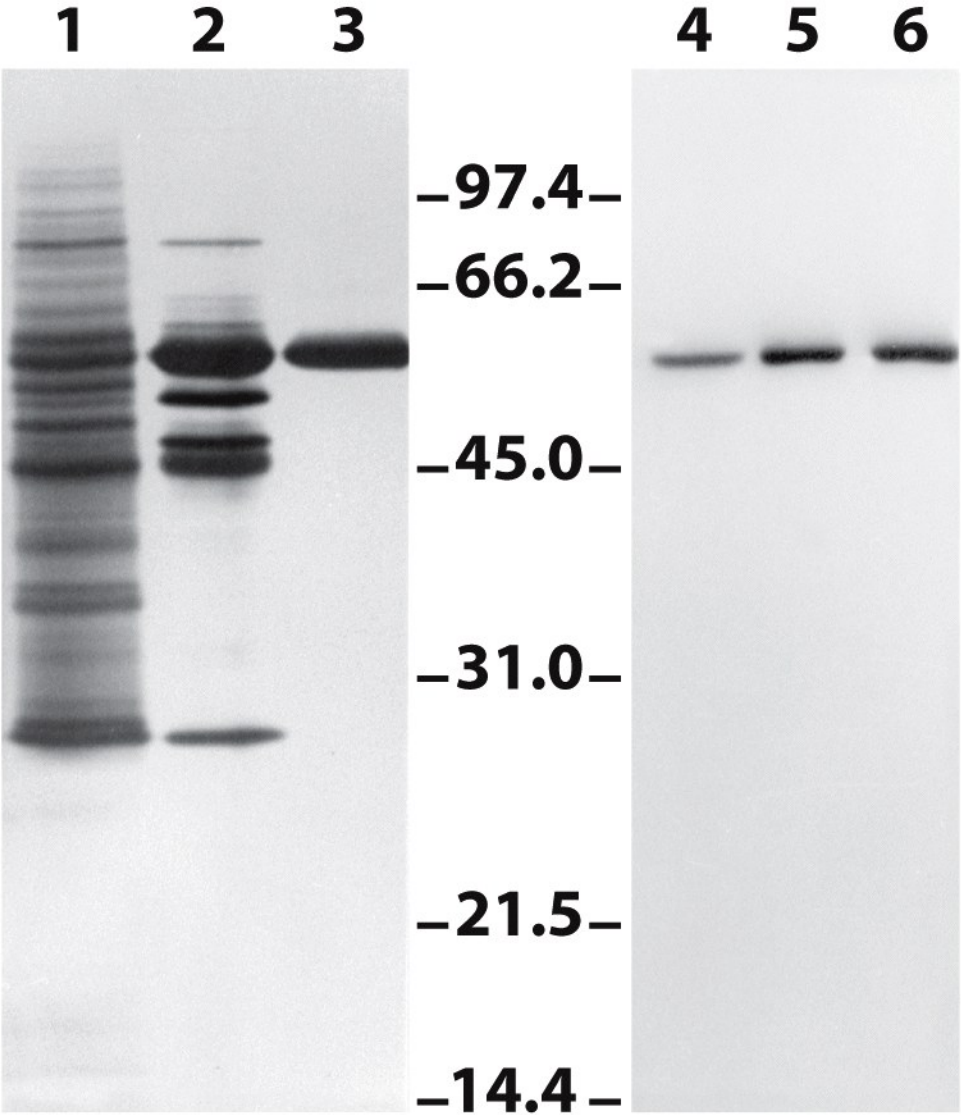


ELISA

Figure 5-26b

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Immunoblot



SDS gel

Immunoblot

Figure 5-26c
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