Lehninger

SIXTH EDITION

Principles of Biochemistry

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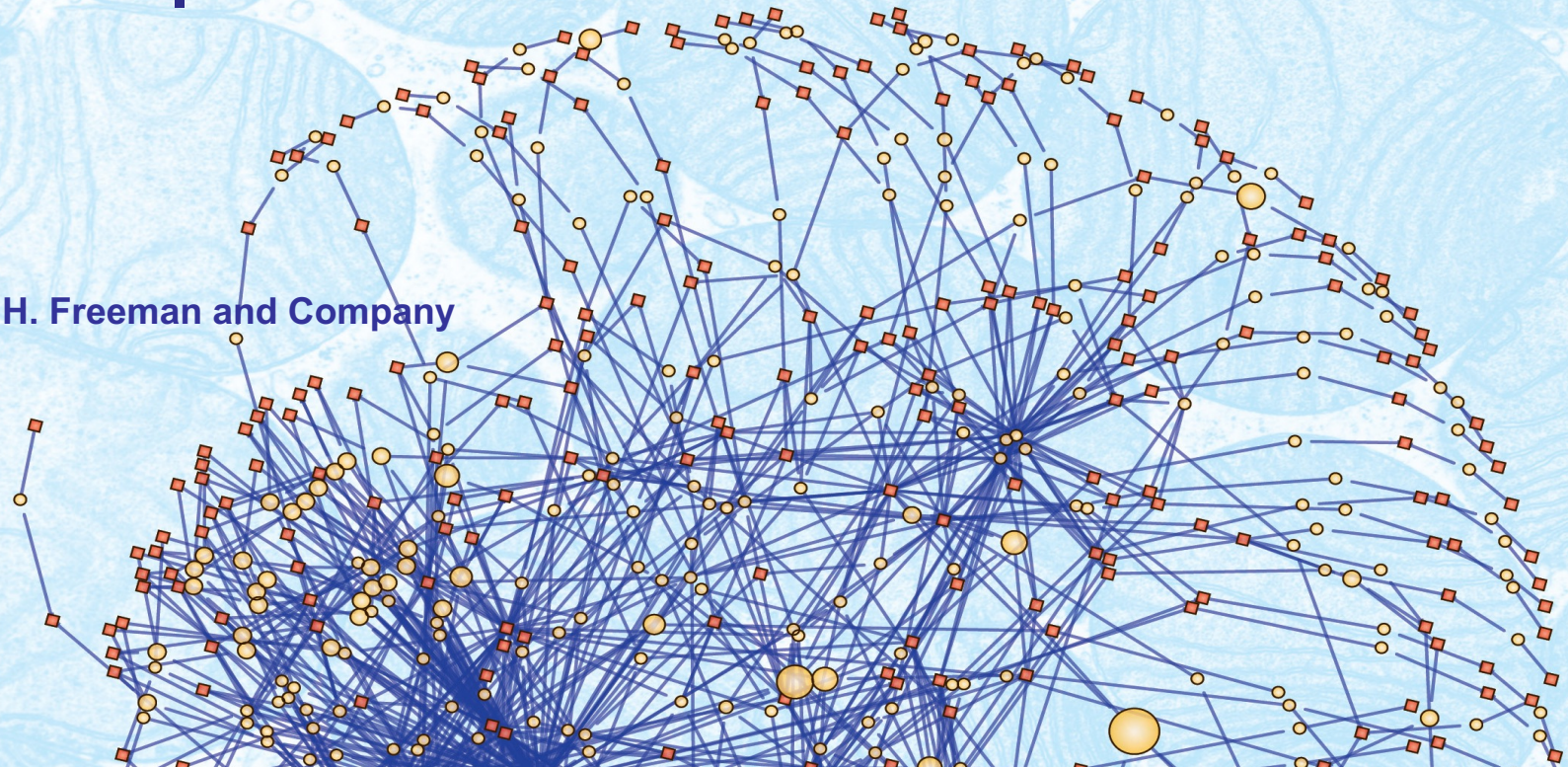
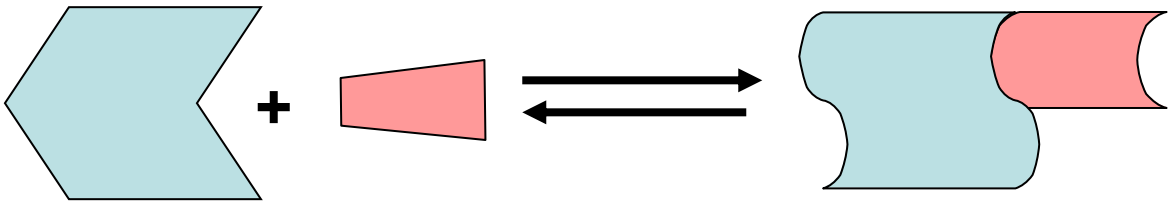
5| Function of Globular Proteins



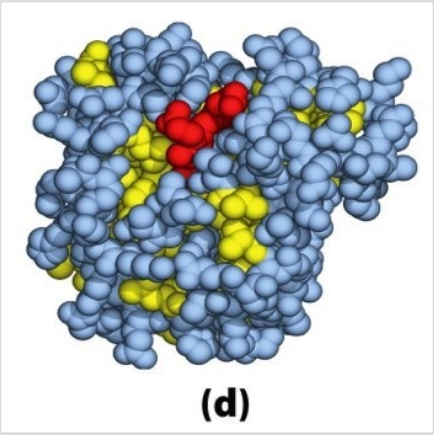
Key Topics in Protein Function

- Reversible binding of ligands is essential.
  - Specificity of ligands and binding sites.
  - Ligand binding is often coupled to conformational changes, sometimes quite dramatic (**Induced Fit**).
  - In multi-subunit proteins, conformational changes in one subunit can affect others (**Cooperativity**).
- Illustrated by:
  - Hemoglobin, antibody, and muscle contraction.



Protein Function Involves Binding

- Ligand: a molecule bound reversibly by a protein.
  - **Transient** interaction.
- Binding Site: where ligand binds on protein.
  - Complementary.
  - **Specific**.
- In Enzyme.
  - Ligand -> Substrate.
  - Binding Site -> Active or Catalytic Site.



Function of Globular Proteins

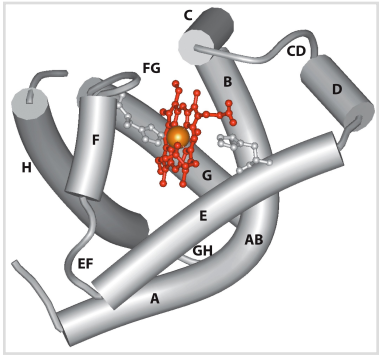
5.1 Reversible Binding to Ligand

5.2 Complementary Interaction between Protein and Ligand

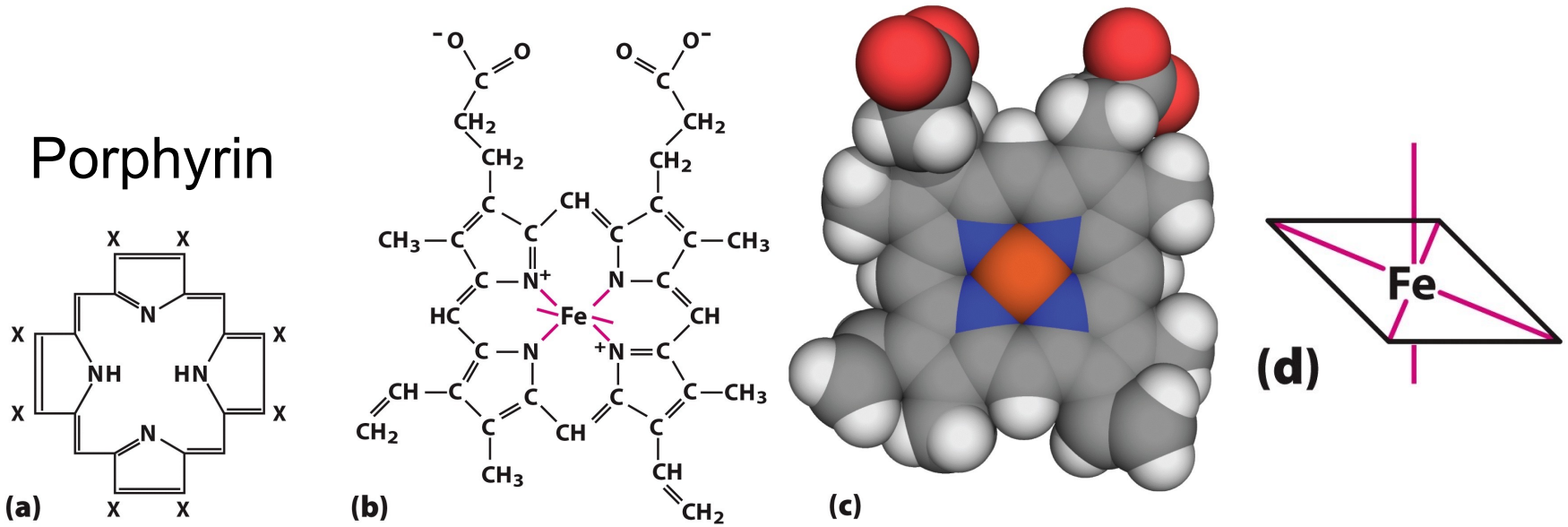
5.3 Interaction Modulated by Chemical Energy

Globins Are Oxygen-Binding Proteins

- Problem
  - 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.

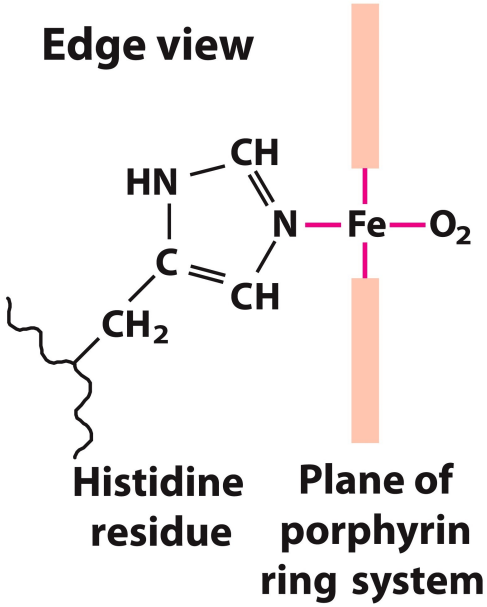


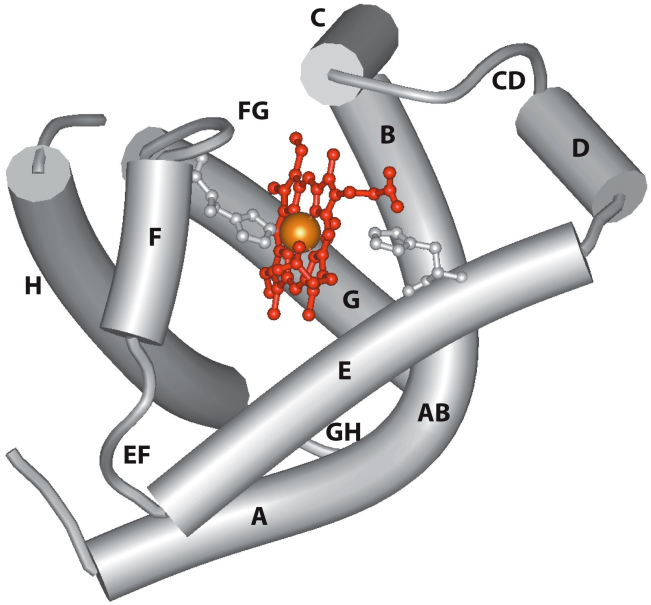
Structures of Porphyrin and Heme



- Protoporphyrin bound to a single iron atom.
- Iron atom has six coordination bonds.
  - Four in the plane of, and bonded to, porphyrin ring.
  - Two perpendicular.
- Iron in different states bind oxygen differently.
  - Iron in ferrous (Fe^{2+}) state binds oxygen reversibly.
  - Iron in ferric (Fe^{3+}) state does not bind oxygen.

Structure of Myoglobin



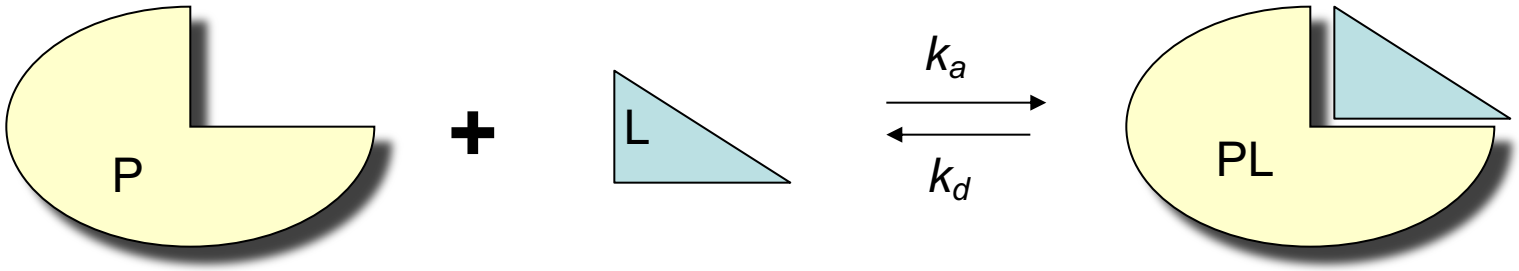


- Single polypeptide
- 153 residues
- 8 helices (A-H)
- His93 = His F8
- Heme as **prosthetic** group

- When oxygen binds, electronic properties of heme iron change.
  - Bright red color of oxygen-rich **arterial** blood.
  - Dark purple color of oxygen-depleted **venous** blood.
- Some small molecules bind to heme iron with greater affinity.
  - Carbon monoxide (CO) is highly toxic.

Binding: Quantitative Description

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



- Equilibrium constant ***Ka*** (M^{-1})

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

- Kinetics of such a process
  - Association rate constant *ka*
  - Dissociation rate constant *kd*

$$k_a[P]\cdot[L] = k_d[PL]$$

  - At equilibrium the association and dissociation rates are equal

Binding: Bound Fraction

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

$$\theta = \frac{[PL]}{[PL]+[P]} \quad (1)$$

2. Substituting [PL] with K_a[L][P], we will eliminate [PL]

$$\theta = \frac{K_a[L][P]}{K_a[L][P]+[P]} \quad (2)$$

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

$$\theta = \frac{[L]}{[L]+\frac{1}{K_a}} \quad (3)$$

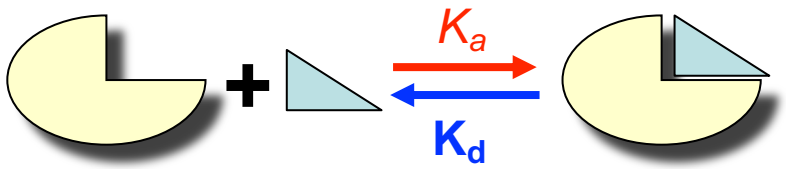
- In terms of the more commonly used equilibrium dissociation constant K_d (M)

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

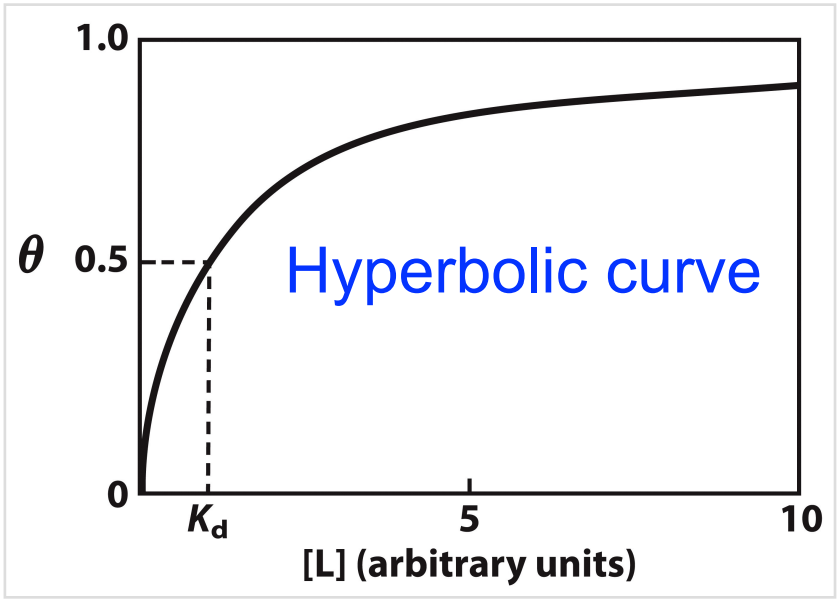
$$\theta = \frac{[L]}{[L]+K_d}$$

Binding: Graphical Analysis

- K_d is equivalent to molar concentration of free ligand at which half of ligand-binding sites on protein is occupied
  - protein reaches half-saturation
  - the more tightly a ligand binds a protein
  - the lower the concentration of ligand required to reach half-saturation
  - the lower the value of K_d

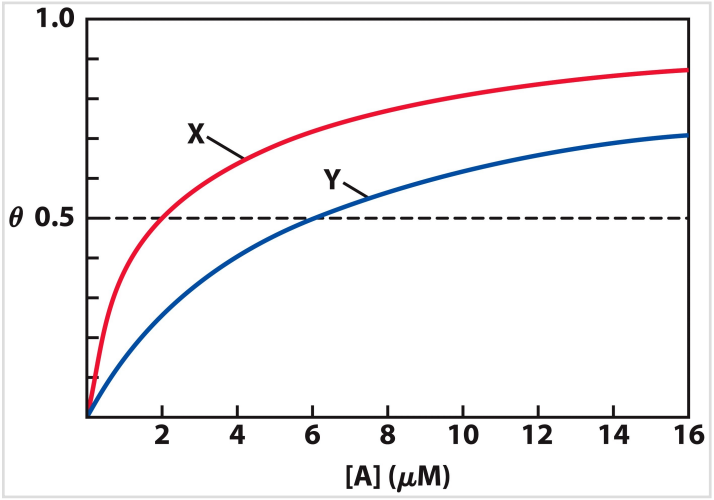


$$\theta = \frac{[L]}{[L] + K_d} = \frac{[L] + K_d - K_d}{[L] + K_d} = 1 - \frac{K_d}{[L] + K_d}$$



Example Protein-Ligand K_d

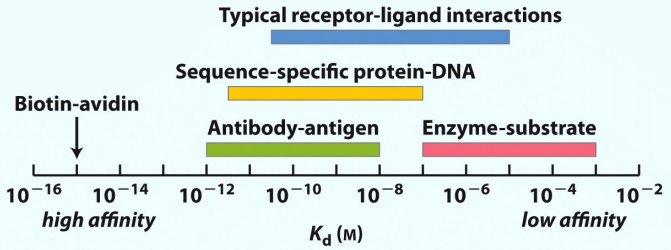
- Protein X and Y bind to the same ligand A
- Which protein has a greater affinity for ligand A?



10^{-3} M = 1 mM
10^{-6} M = 1 μM
10^{-9} M = 1 nM
10^{-12} M = 1 pM
10^{-15} M = 1 fM

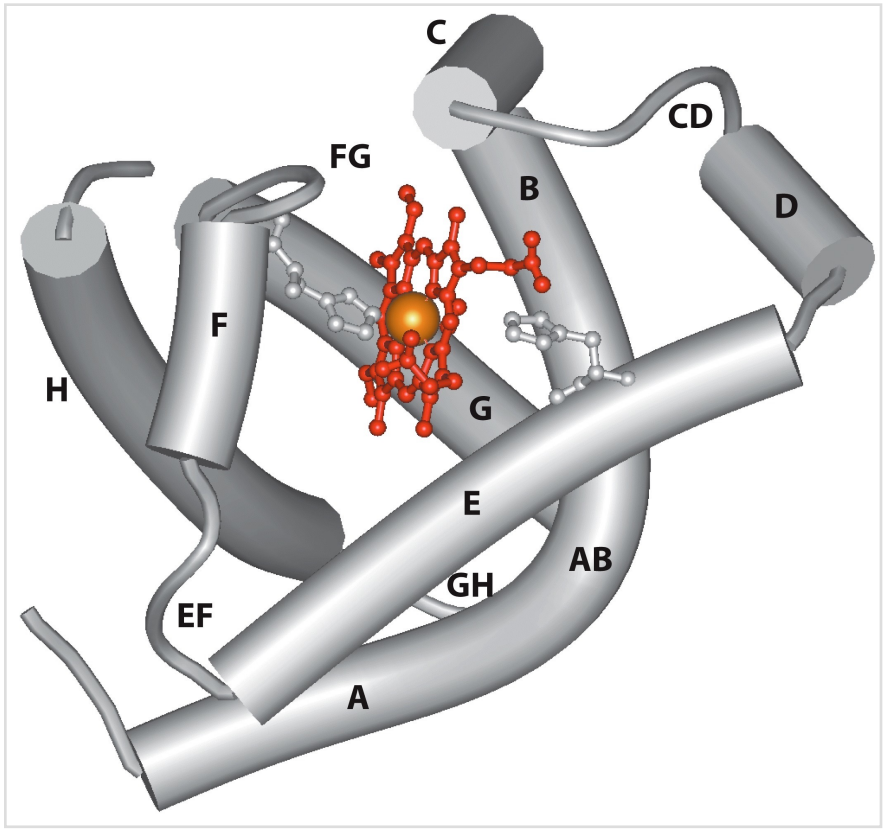
TABLE 5–1 Some Protein Dissociation Constants

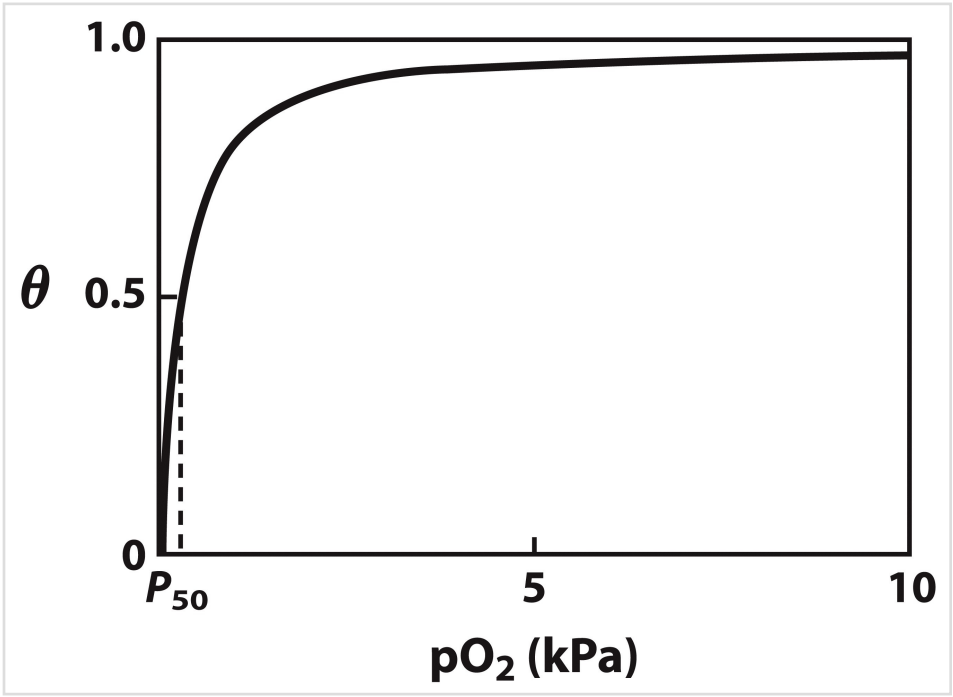
Protein	Ligand	K_d (M)*
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)†	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)‡	Ca^{2+}	3×10^{-6}
		2×10^{-5}



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.

Example: Oxygen Binding to Myoglobin



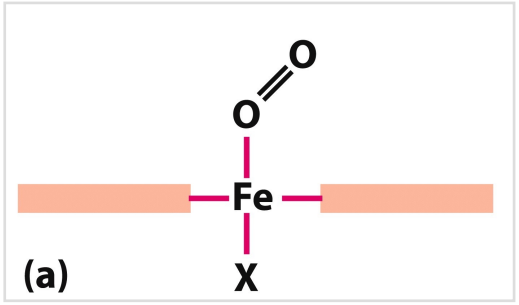


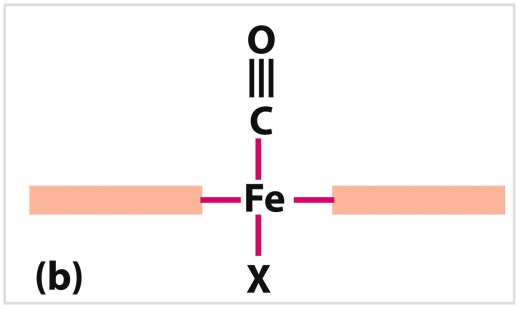
When ligand is a gas, binding is expressed as **partial pressures**

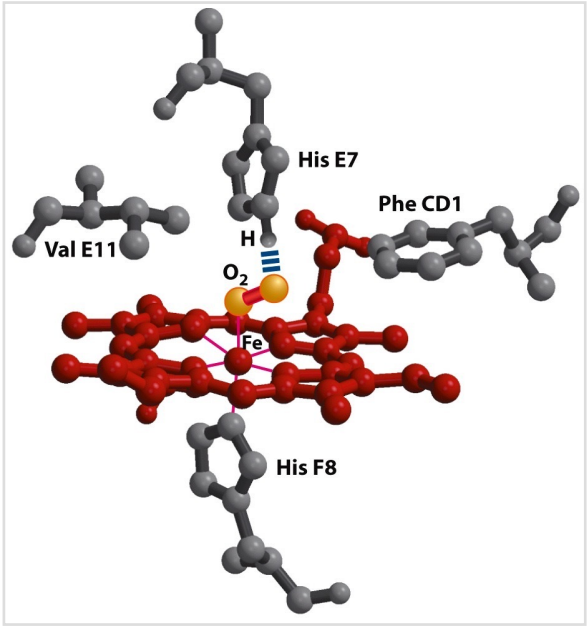
$$\theta = \frac{[\mathrm{L}]}{K_\mathrm{d} + [\mathrm{L}]} \longrightarrow \theta = \frac{p\mathrm{O}_2}{p_{50} + p\mathrm{O}_2}$$

Binding of Carbon Monoxide

- CO has similar size and shape to O_2; can fit to same binding site.
- CO binds to free heme over 20,000 times better than O_2
- Protein pocket decreases selectivity for CO, but it still binds to heme about 40 times better than oxygen in myoglobin
- CO is highly toxic as it **directly competes** with oxygen





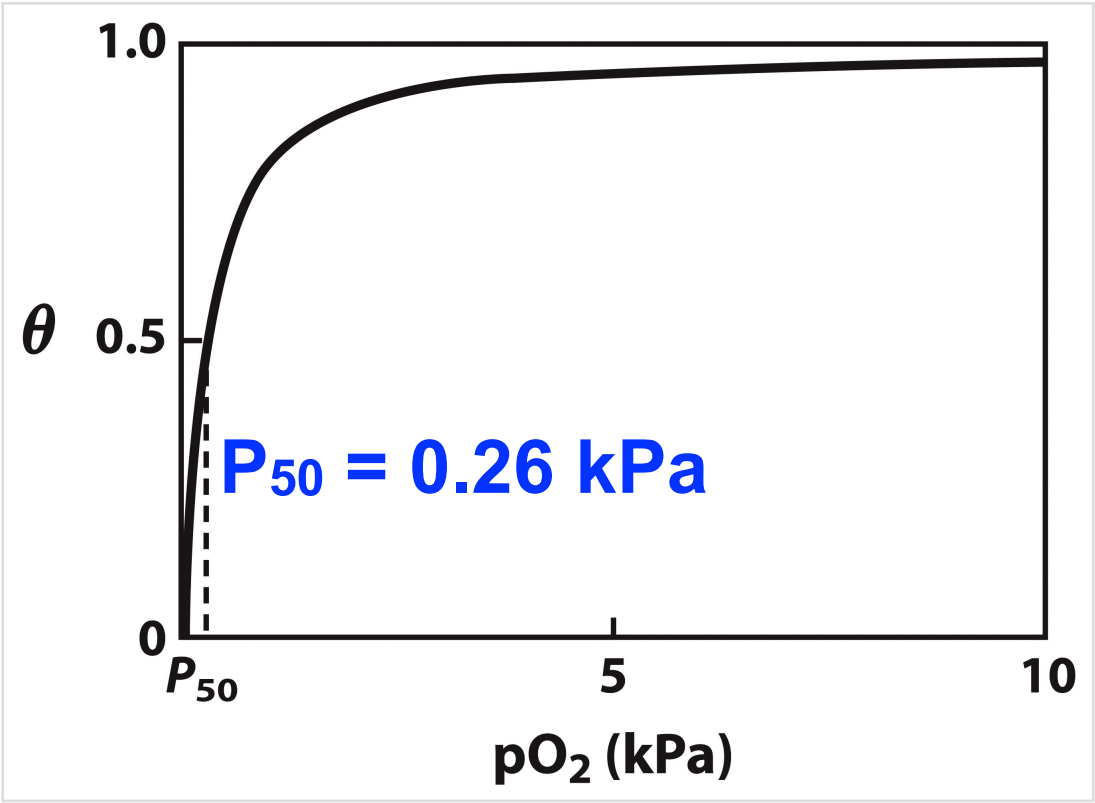


- Partial negative charge on oxygen
- H-bond between His E7 and oxygen
- No such effect for CO

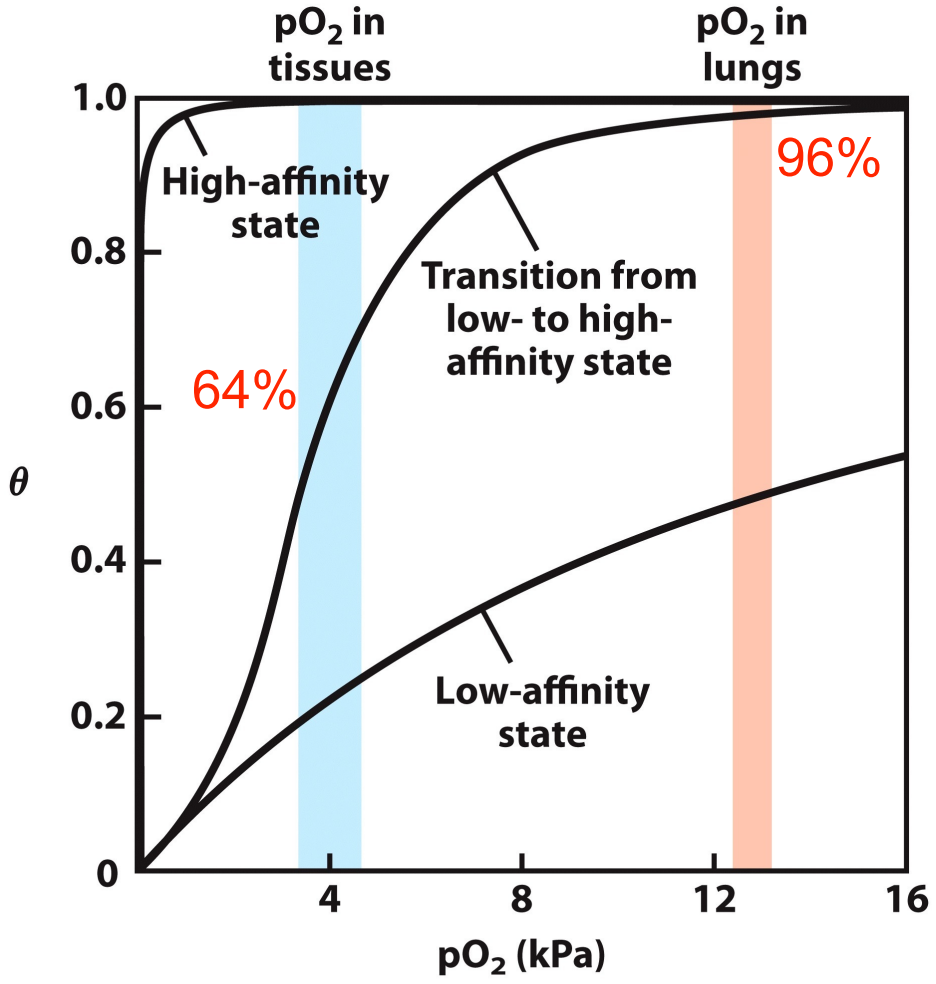
- O_2 binds heme at an angle
- Readily accommodated in myoglobin
- CO binds heme in a linear fashion
- Cause steric clash with His E7

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



Affinity Varies with pO_2



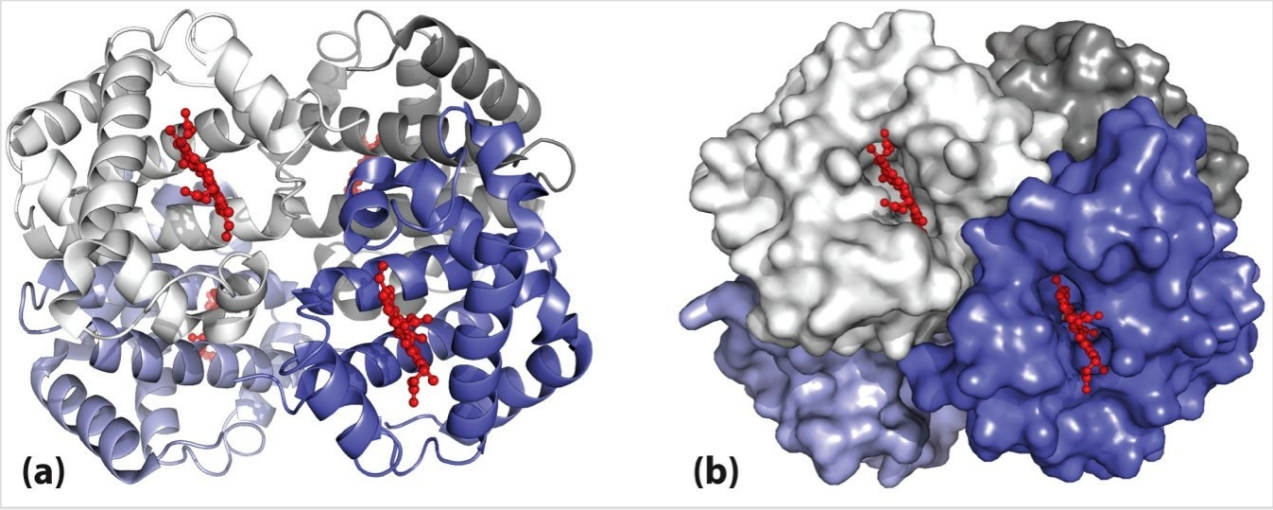
For effective transport,

affinity must **vary** with pO_2

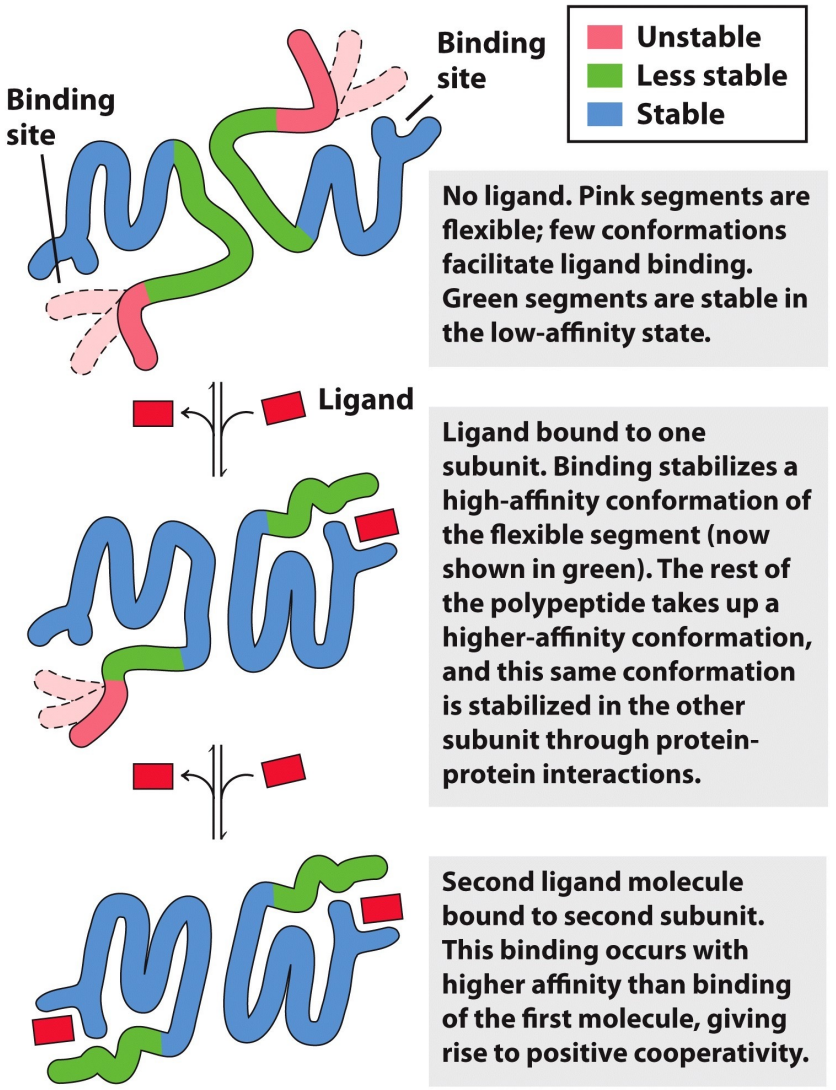
- Bind oxygen where pO_2 is high
- Release oxygen where pO_2 is low

How Can Affinity to Oxygen Change?

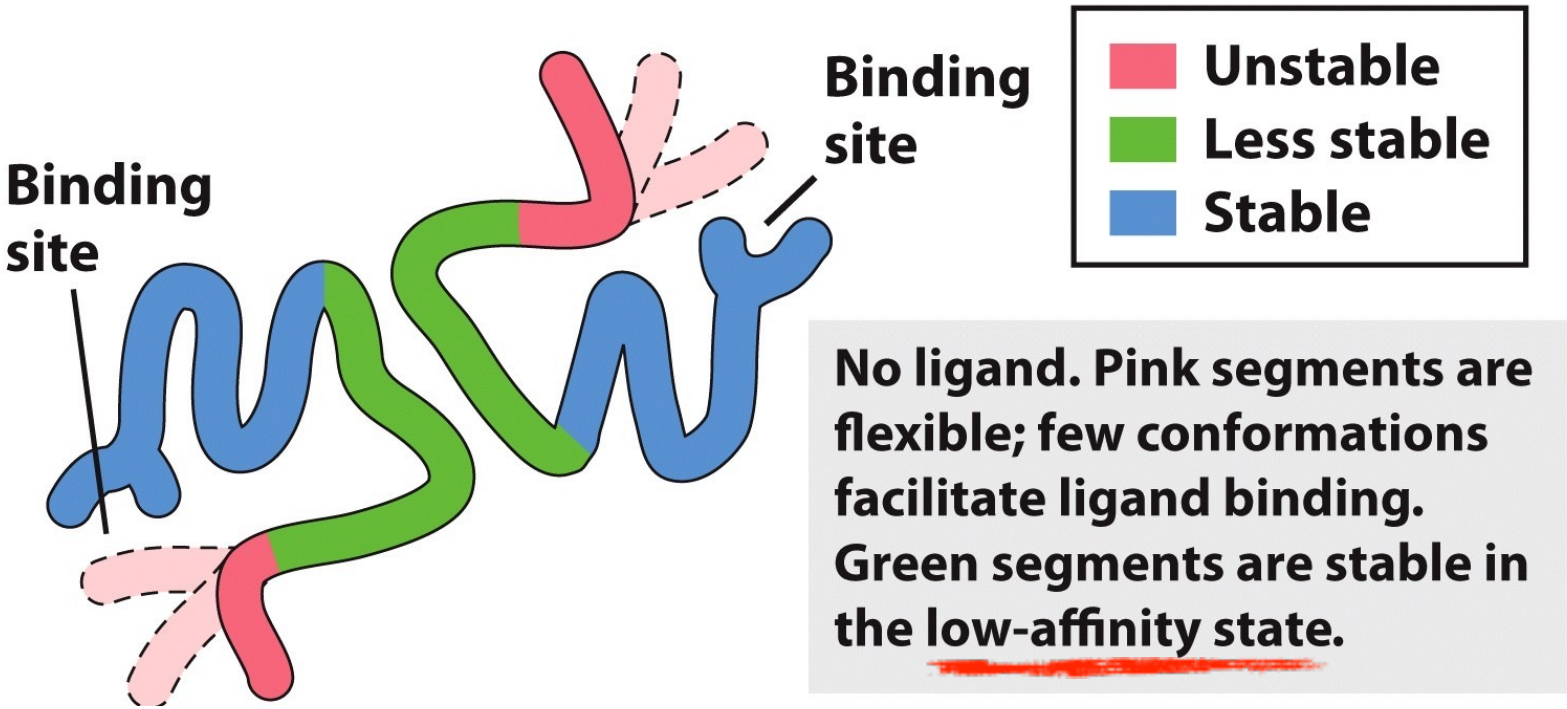
- Must be a protein with multiple binding sites
- Binding sites must be able to interact with each other
- This phenomenon is called **cooperativity**
  - Positive cooperativity
    - First binding event increases affinity at remaining sites
  - Negative cooperativity
    - First binding event reduces affinity at remaining sites



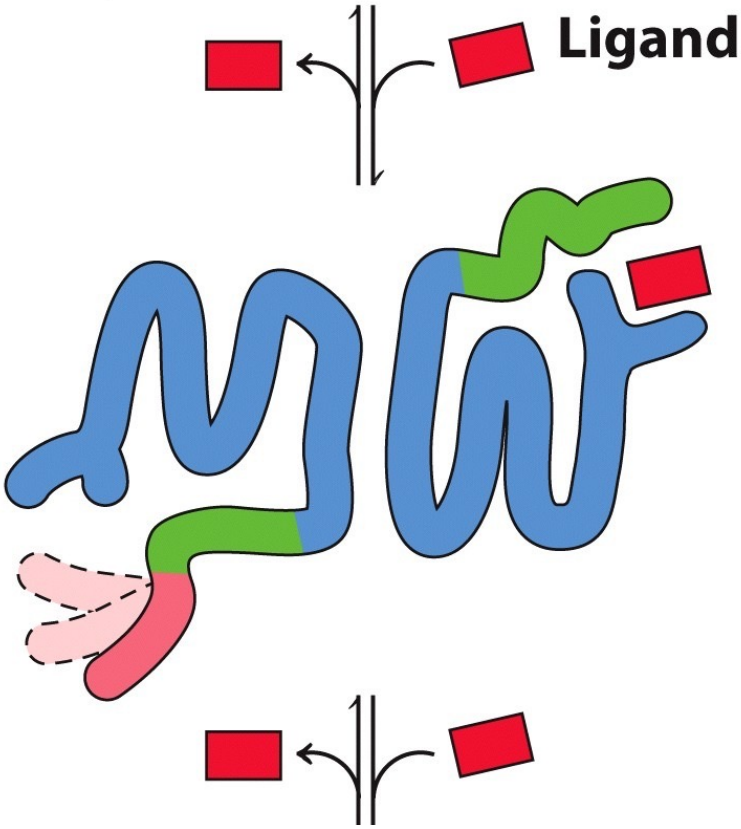
Cooperativity



Cooperativity



Cooperativity



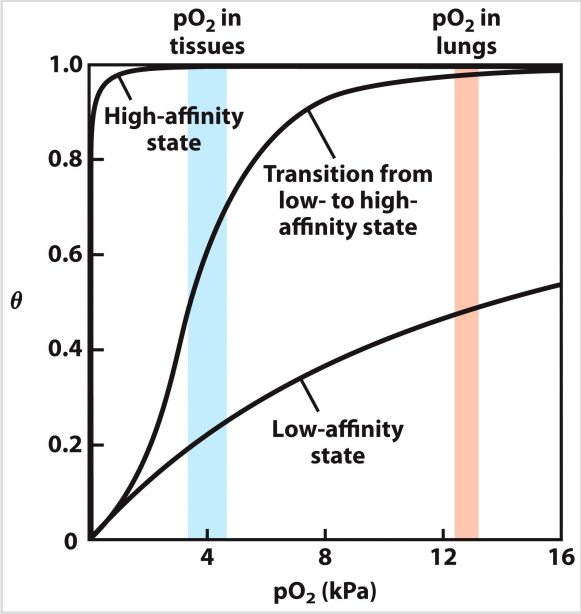
Ligand bound to one subunit. Binding stabilizes a high-affinity conformation of the flexible segment (now shown in green). The rest of the polypeptide takes up a higher-affinity conformation, and this same conformation is stabilized in the other subunit through protein-protein interactions.

Cooperativity

Second ligand molecule bound to second subunit. This binding occurs with higher affinity than binding of the first molecule, giving rise to positive cooperativity.

Hemoglobin Binds Oxygen Cooperatively

- Undergoes a transition as more O_2 are bound
  - From low-affinity to high-affinity state
  - First O_2 interacts with deoxyhemoglobin weakly
  - First O_2 binding makes it easier for additional O_2 to bind
  - Fourth O_2 binds with much higher affinity than first O_2



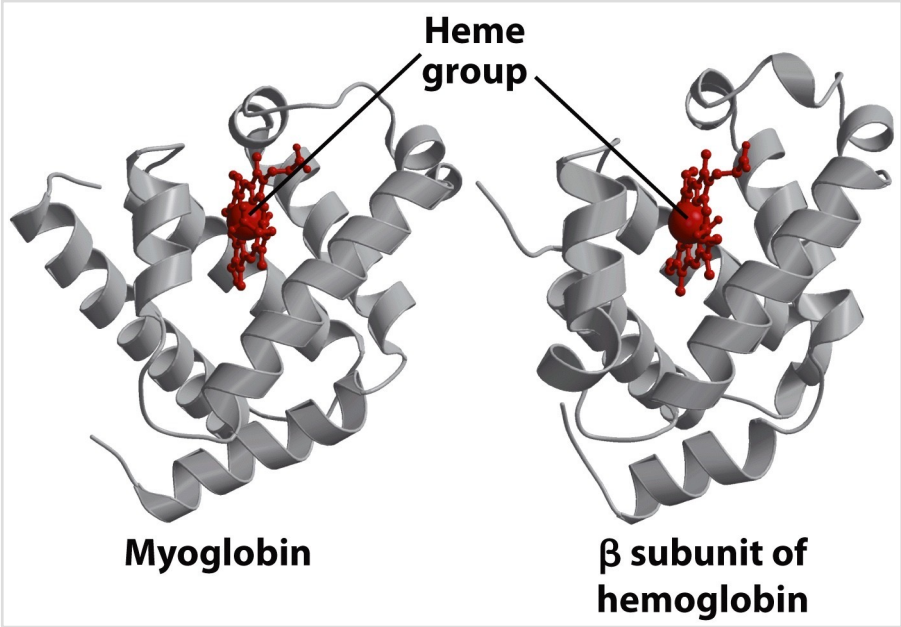
- Cooperative binding
  - Sigmoid (S-shaped) curve
  - Hybrid (low- and high-affinity)

Hemoglobin Binding to O_2 is Allosteric

- Allosteric protein
  - Binding of a ligand to one site affects the binding properties of a different site, on the same protein
  - Ligand, referred to as modulator
  - Can be positive or negative
- Modulator
  - Allosteric activator or allosteric inhibitor
  - modulator = ligand, homotropic interaction
  - modulator ≠ ligand, heterotropic interaction

Hemoglobin Similar to Myoglobin

- Hemoglobin is a tetramer of two subunits ($\alpha 2 \beta 2$)
- Each subunit is structurally similar to myoglobin
- Amino acid sequences are identical at **27** positions
  - Myoglobin 153 residues
  - Hemoglobin α chain 141 residues
  - Hemoglobin β chain 146 residues

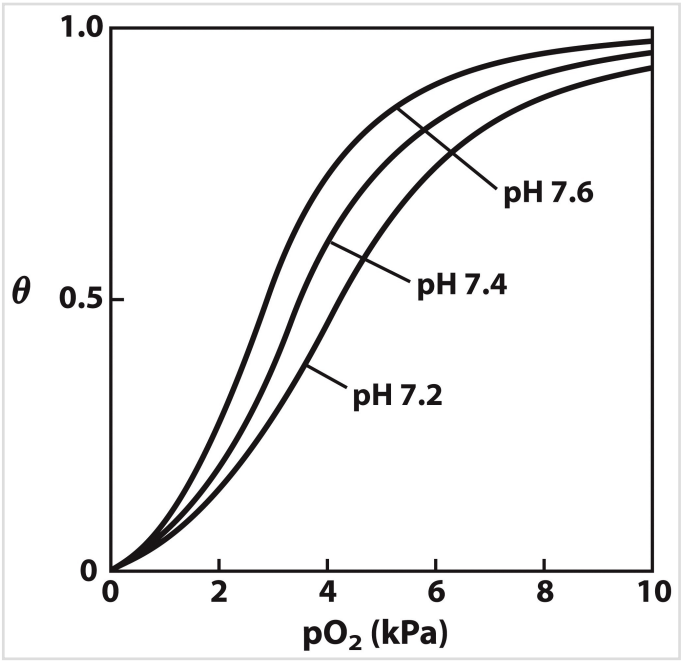


	Mb	Hbα	Hbβ
NA1	1V	1V	1V
	—	—	H
	L	L	L
A1	S	S	T
	E	P	P
	G	A	E
	E	D	E
	W	K	K
	Q	T	S
	L	N	A
	V	V	V
	L	K	T
	H	A	A
	V	A	L
	W	W	W
	A	G	G
	K	K	K
	V	V	V
A1	6 E	G	—
	A	A	—
B1	20D	20H	N
	V	A	20V
	A	G	D
	G	E	E
	H	Y	V
	G	G	G
	Q	A	G
	D	E	E
	I	A	A
	L	L	L
	I	E	G
	R	R	R
	L	M	L
	F	F	L
	K	L	V
B16	S	S	V

pH Effect on O_2 Binding to Hemoglobin

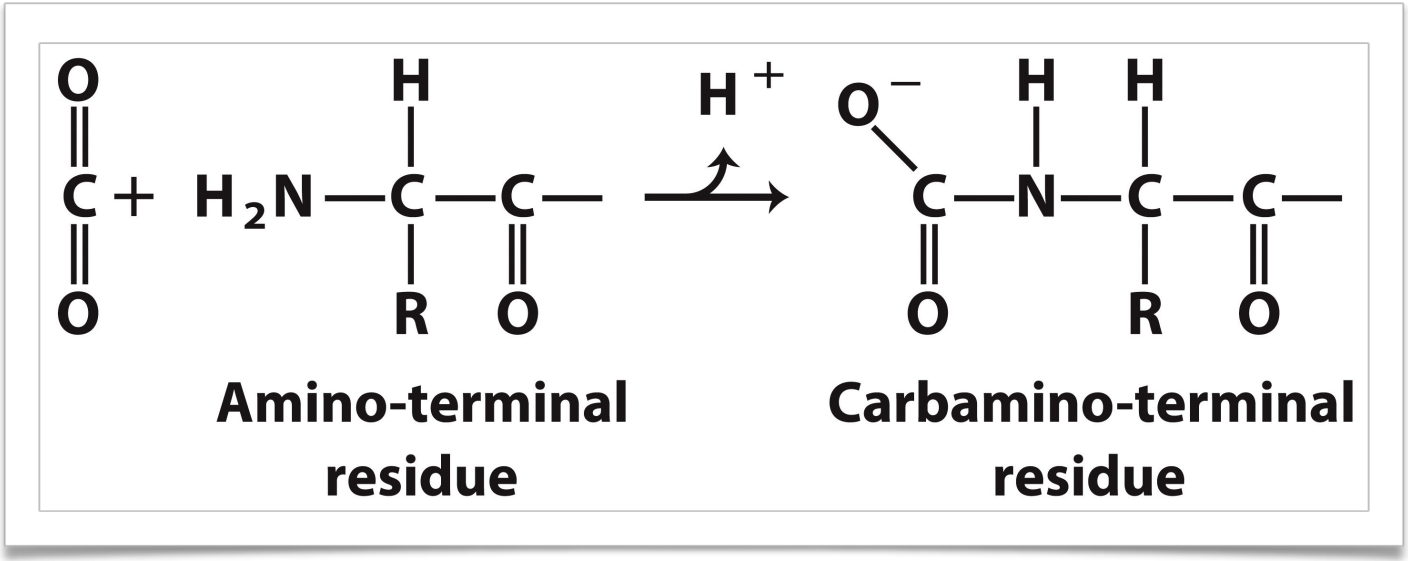
- Actively metabolizing tissues generate H^+
  - Blood pH near the tissues is lower relative to the lungs
- Hemoglobin affinity for oxygen depends on pH
  - H^+ binds to hemoglobin and stabilizes low-affinity state
  - Leads to release of O_2 in the tissues

- pH difference between lungs and metabolic tissues **increases** efficiency of the O_2 transport



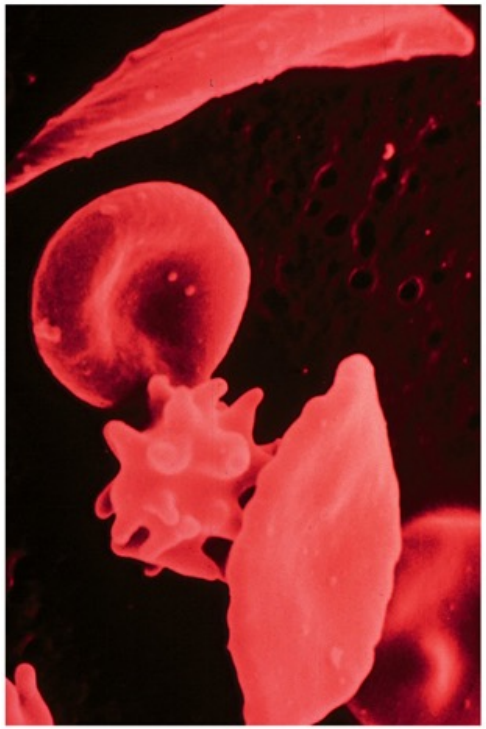
Hemoglobin and CO_2 Export

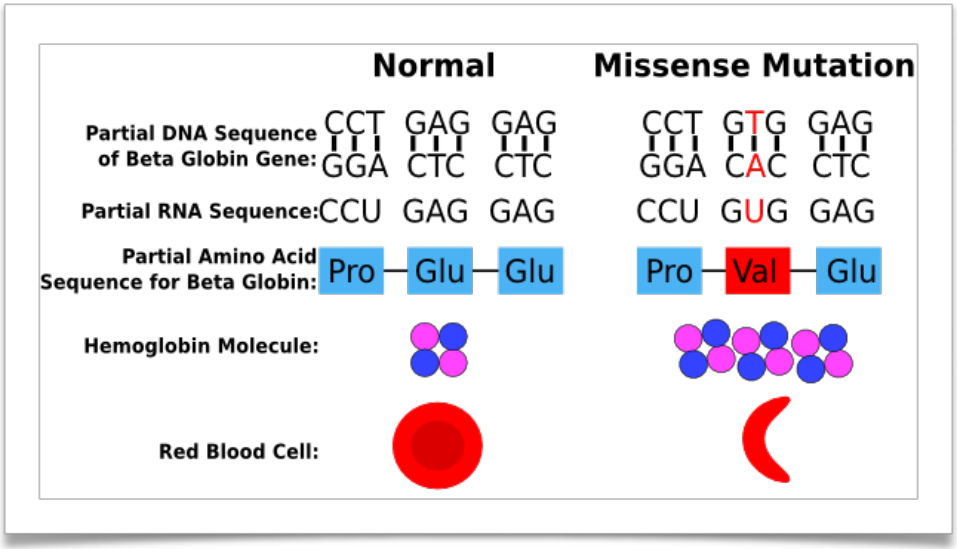
- CO_2 is produced by metabolism in tissues
- CO_2 forms carbamate on the amino terminal residues of each of the hemoglobin polypeptide subunits
  - Yield a proton which again leads to release of O_2



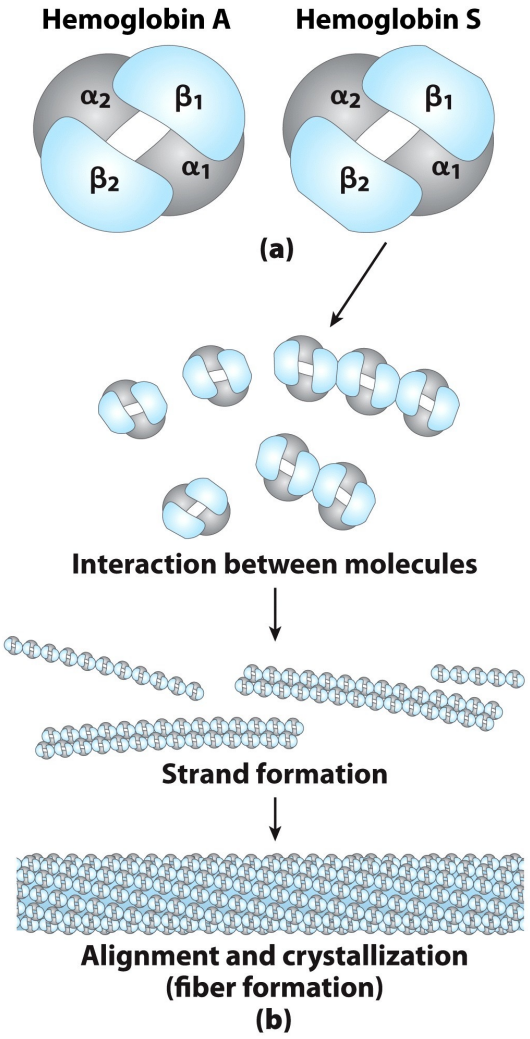
Sickle-Cell Anemia

- Glutamate at position 6 mutated to valine in β chains of hemoglobin
  - Residue #6 on outer surface of protein
- E6V mutation creates "sticky" **hydrophobic** contact point on protein surface
  - Cause multiple hemoglobin molecules to associate abnormally and form long, fibrous aggregates
- Insoluble fibers cause the sickle shape of red blood cells





Formation of Hemoglobin Strands



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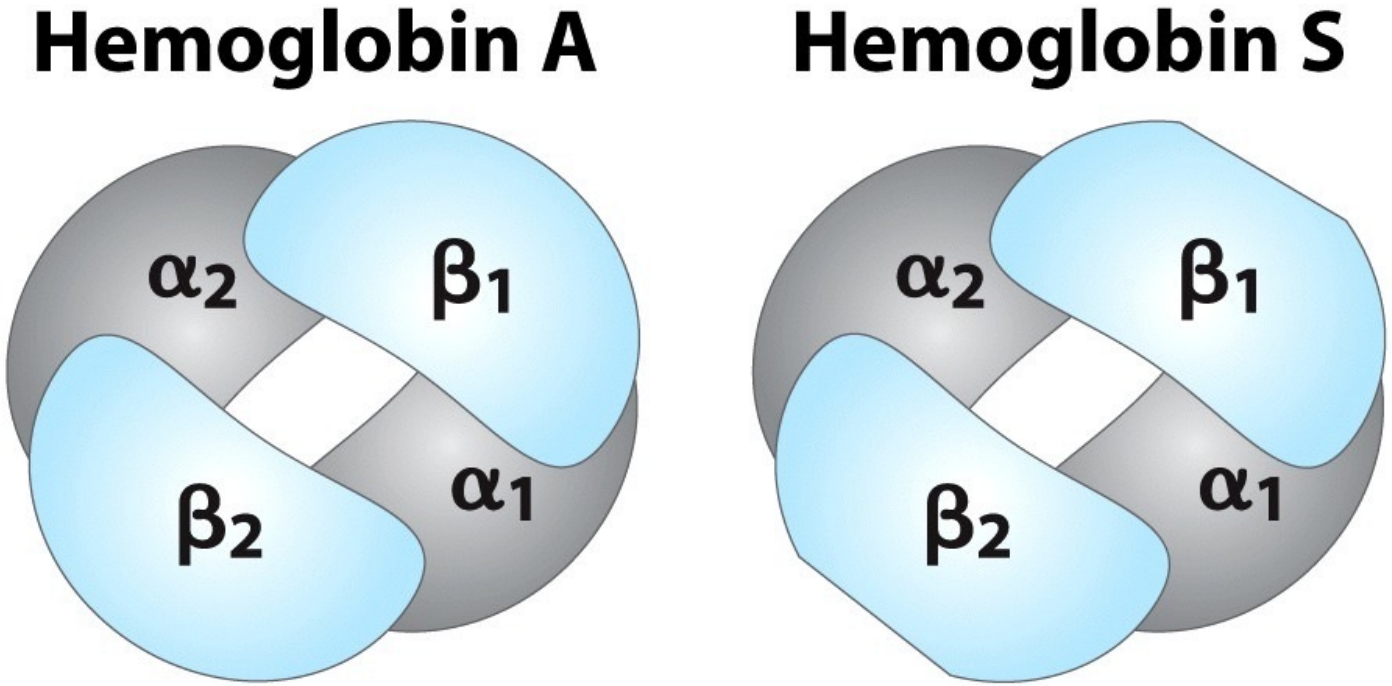

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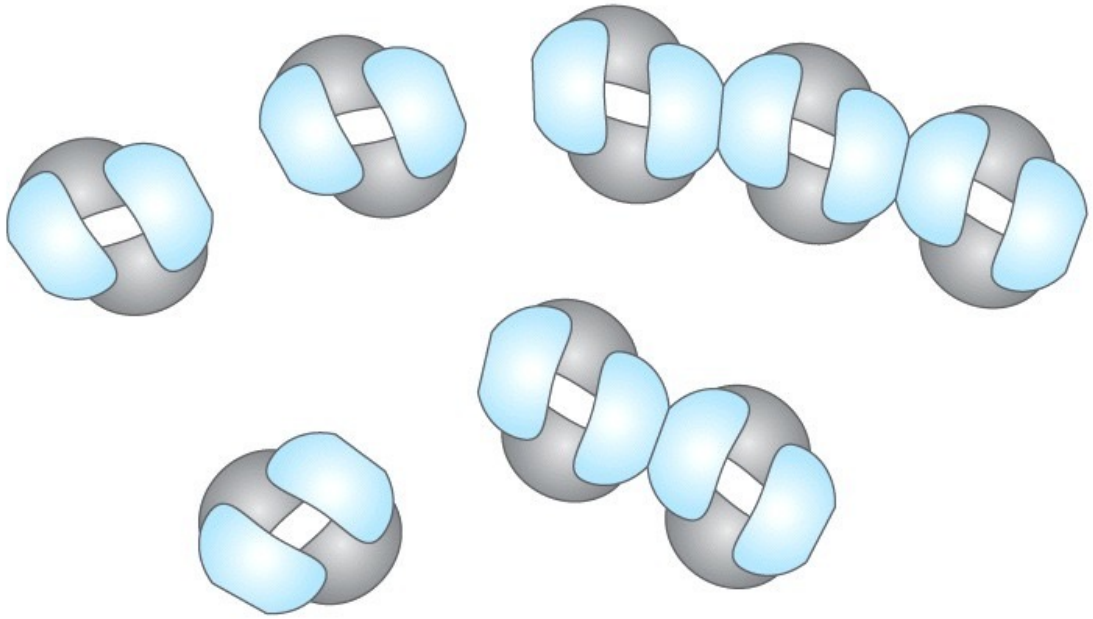
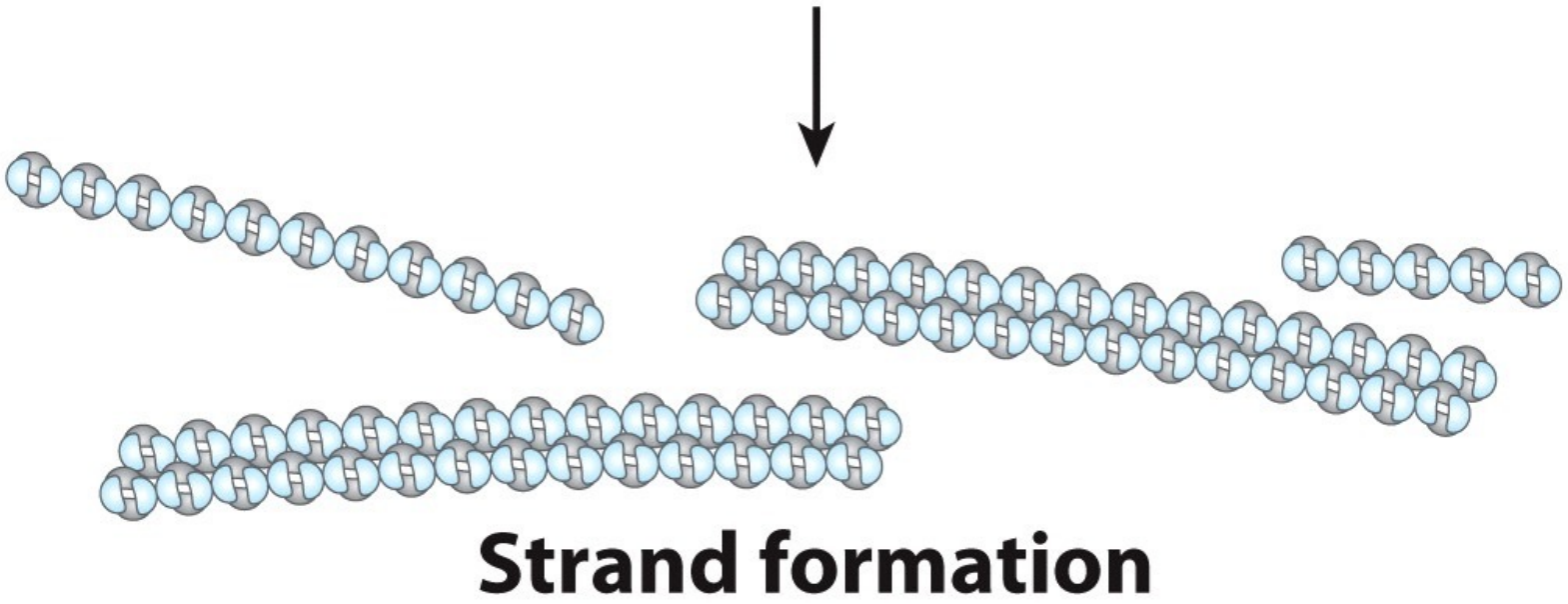


Interaction between molecules

Formation of Hemoglobin Strands



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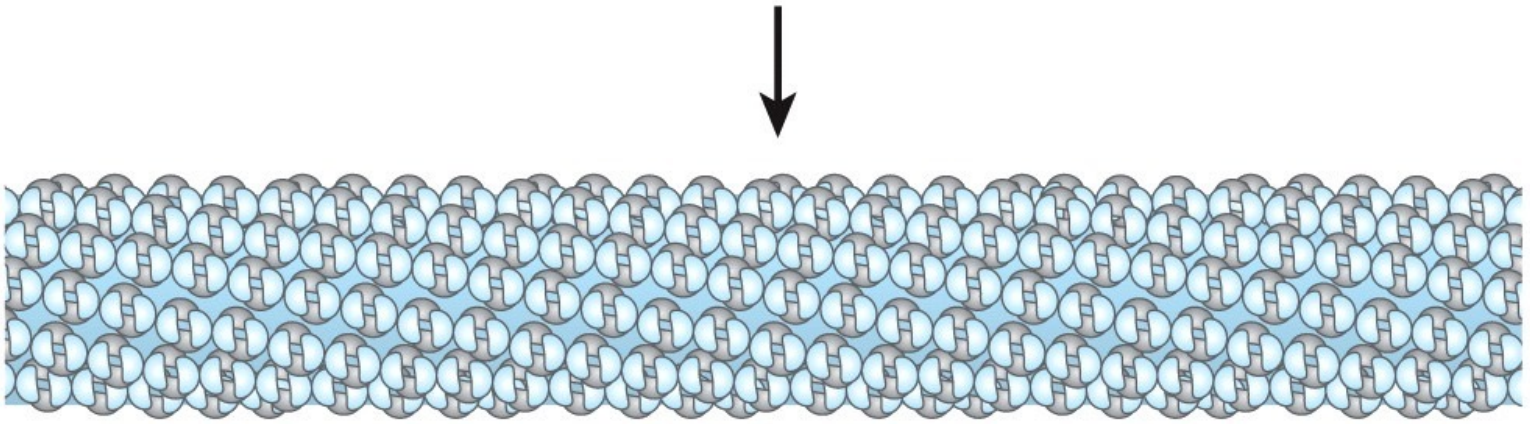
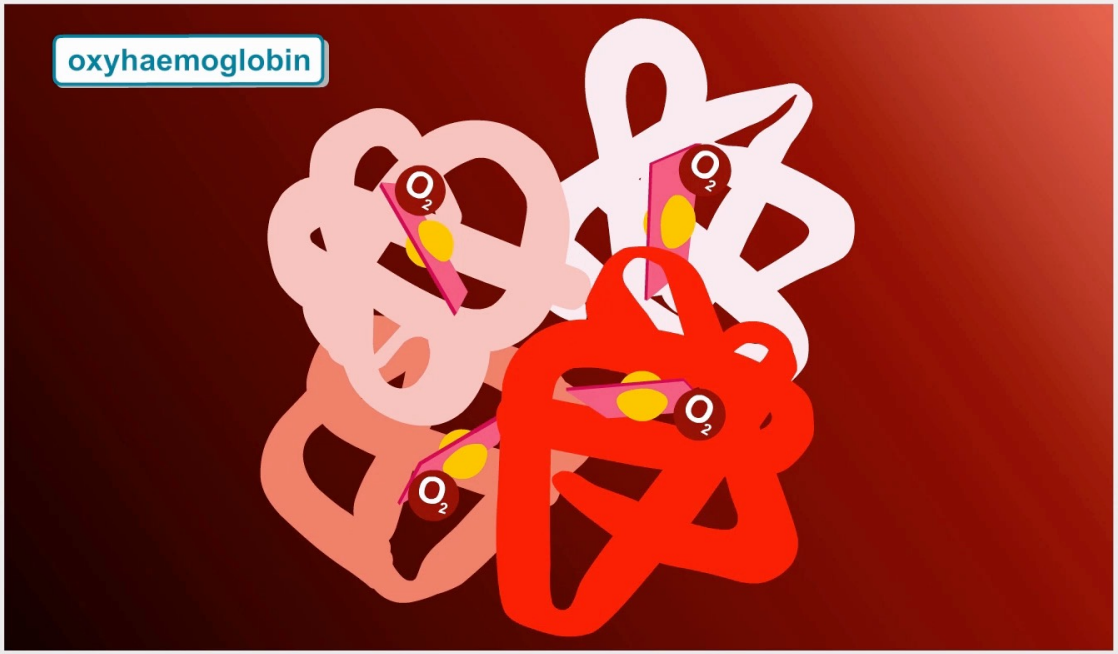


Alignment and crystallization (fiber formation)

Hemoglobin Review



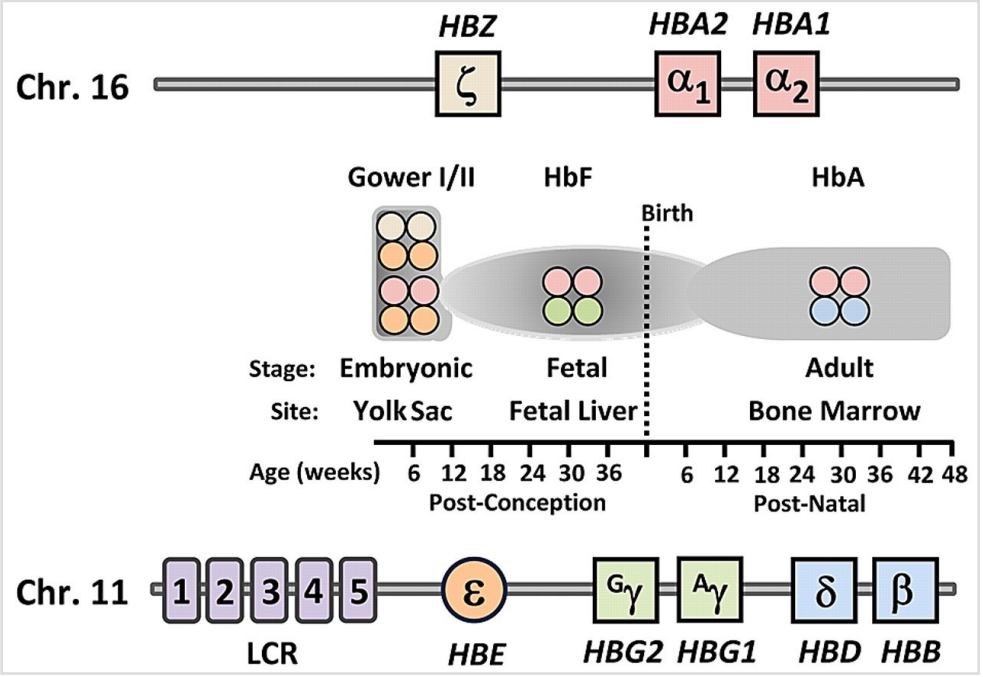
Oxygen unloads from haemoglobin one molecule at a time and haemoglobin returns to its deoxyhaemoglobin structure.

- H^+ or CO_2 increases, hemoglobin affinity decreases
- Fetal hemoglobin has higher affinity than adult hemoglobin
- At high altitude, hemoglobin affinity increases

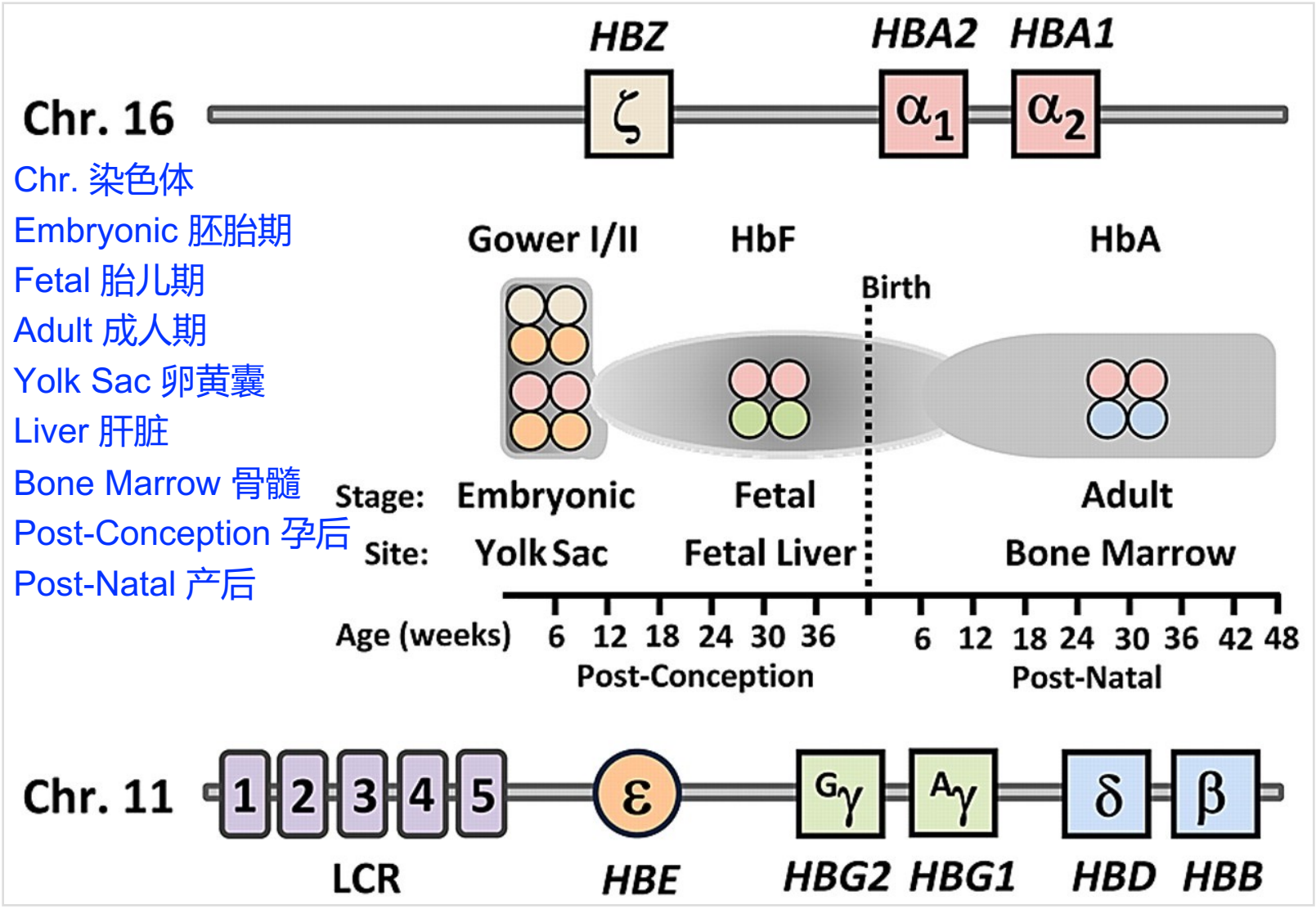
Organization of Globin Genes

- α gene cluster on chromosome 16
  - Embryonic ζ (zeta) gene, adult α1 and α2 genes
- β gene cluster on chromosome 11
  - Embryonic ε (epsilon) gene, fetal γ genes, and adult δ (delta) and β genes

- Temporal expression
  - Embryonic ζ + ε, α + ε
  - Fetal α + γ
  - Adult α + β

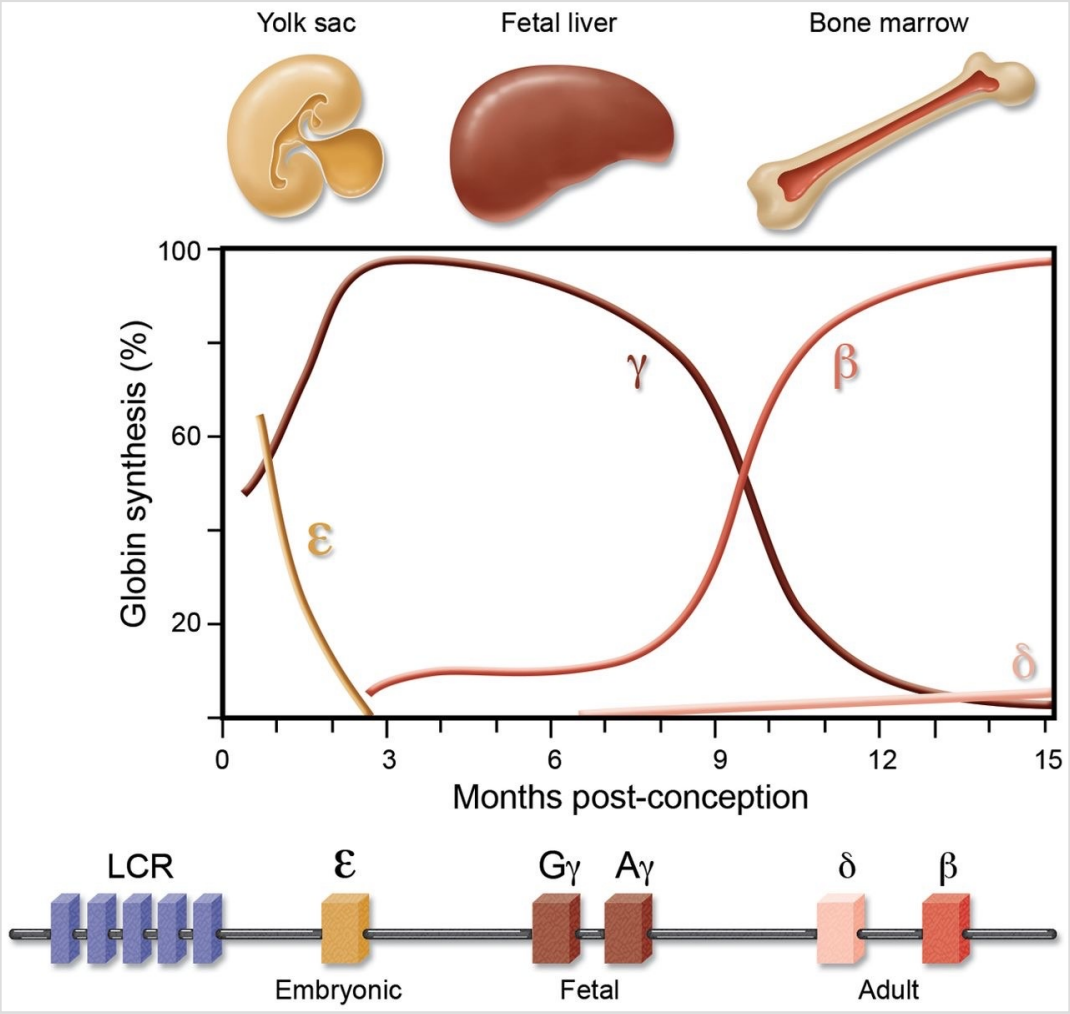


Organization of Globin Genes



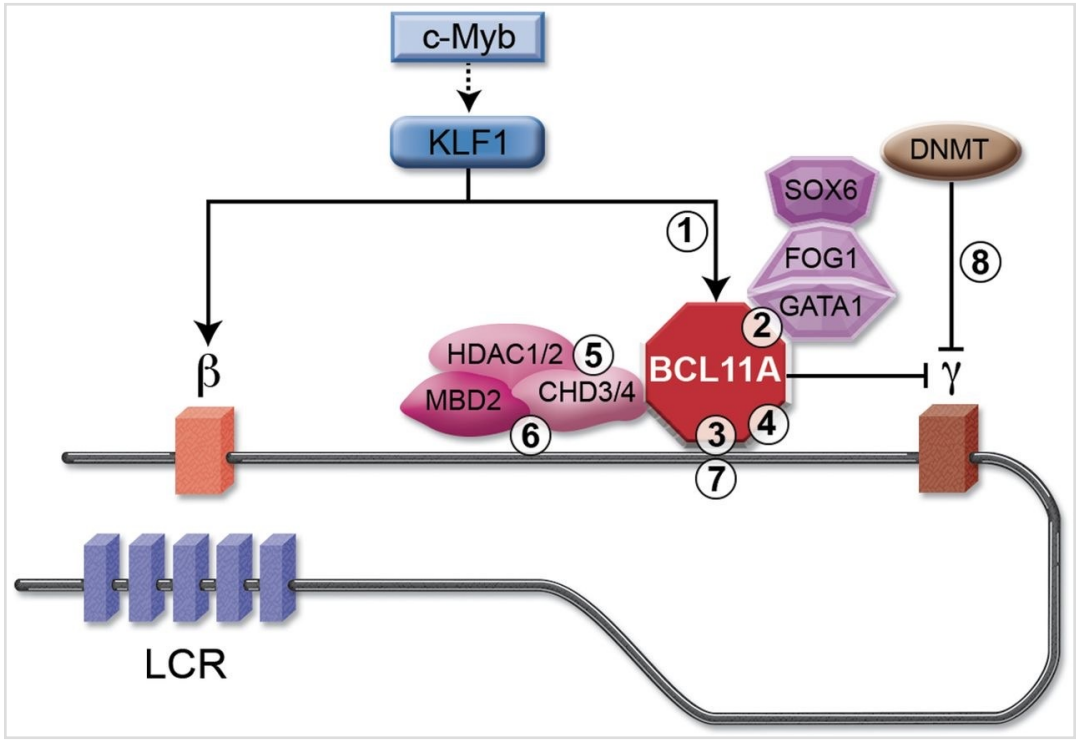
Developmental Control of β-Globins

- 1st switch:
  - 3 months
  - embryonic-to-fetal
  - ε-to-γ
- 2nd switch:
  - birth
  - fetal-to-adult
  - γ-to-β



Regulatory Network of β-Globins

- KLF1 promotes β-globin expression
- KLF1 activates BCL11A
- BCL11A silences γ-globin



How to Treat Sickle-Cell Anemia

Five major strategies

Strategy	Mechanism	Example	Status
Increase HbF	Inhibit BCL11A (reactivate fetal hemoglobin)	Hydroxyurea	✅ Approved
Stabilize Oxygenated State	Prevent deoxygenation (no aggregation)	Voxelotor	✅ Approved
Gene Therapy	Repair β-globin gene in stem cells	Casgevy	✅ Approved
Bone Marrow Transplant	Replace diseased stem cells with healthy donor cells	Allogeneic transplant	⚠️ Limited by matching
Anti-aggregation Peptides	Block hydrophobic patch interaction	5-HMF	🔬 In research

Summary 5.1 Reversible Binding to Ligand

- Protein binds ligand reversibly. Protein may undergo conformational changes when a ligand binds (induced fit). Binding of a ligand to one subunit may affect ligand binding to other subunits (cooperativity).
- Myoglobin, heme, oxygen, and K_d.
- Hemoglobin, two affinity states, allosteric and cooperative, H^+ and CO_2, and sickle-cell anemia.

Example Question

In the binding of oxygen to myoglobin, the relationship between the concentration of oxygen and the fraction of binding sites occupied can best be described as:

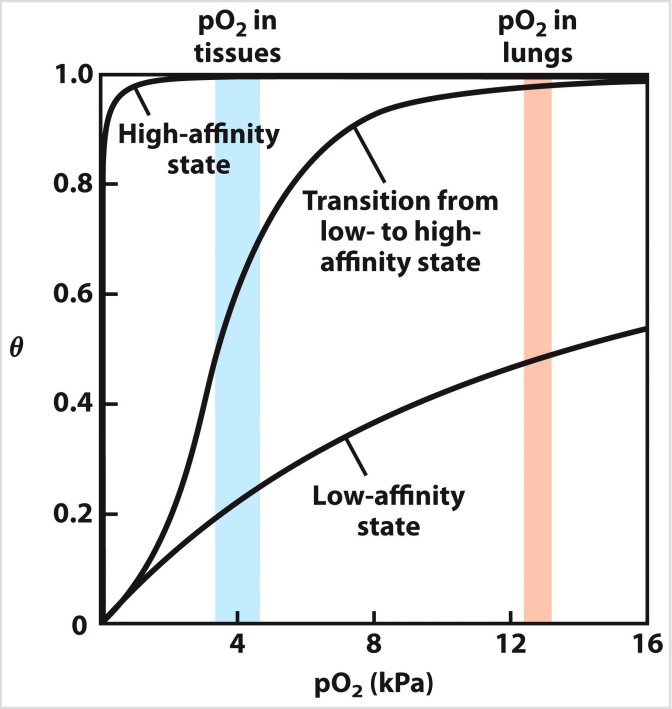
A) hyperbolic.

B) linear with a negative slope.

C) linear with a positive slope.

D) random.

E) sigmoidal.



Example Question

Which of the following statements about protein-ligand binding is correct?

A)The K_a is equal to the concentration of ligand when all of the binding sites are occupied.

B)The K_a is independent of such conditions as salt concentration and pH.

C)The larger the K_a (association constant), the weaker the affinity.

D)The larger the K_a, the faster is the binding.

E)The larger the K_a, the smaller the K_d (dissociation constant).

Example Question

Myoglobin and the subunits of hemoglobin have:

A) no obvious structural relationship.

B) very different primary and tertiary structures.

C) very similar primary and tertiary structures.

D) very similar primary structures, but different tertiary structures.

E) very similar tertiary structures, but different primary structures.

Example Question

Which of the following is *not* correct concerning cooperative binding of a ligand to a protein?

A) It is usually a form of allosteric interaction.

B) It is usually associated with proteins with multiple subunits.

C) It rarely occurs in enzymes.

D) It results in a sigmoidal binding curve.

E) Binding of the first ligand to the protein and binding the next ligand to the protein are not independent.

Example Question

What is the effect of the following changes on the oxygen affinity of hemoglobin?

i) A drop in the pH.

ii) A decrease in the partial pressure of CO_2.

iii) An increase in CO concentration.

i) Affinity decreases.

ii) Affinity increases.

iii) Affinity decreases.

Example Question

The fundamental cause of sickle-cell disease is a change in the structure of:

A) blood.

B) capillaries.

C) hemoglobin.

D) red blood cells.

E) the heart.

Function of Globular Proteins

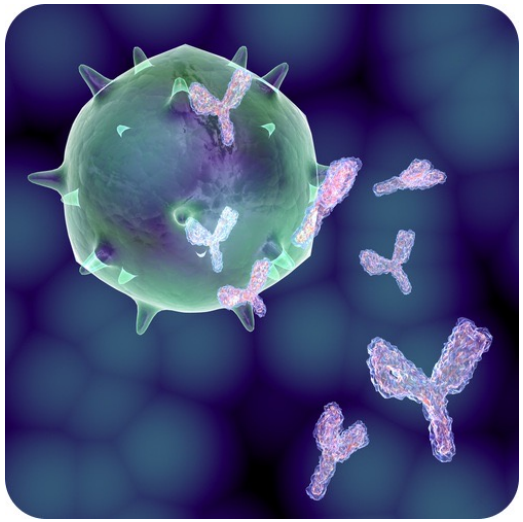
5.1 Reversible Binding to Ligand

5.2 Complementary Interaction between Protein and Ligand

5.3 Interaction Modulated by Chemical Energy

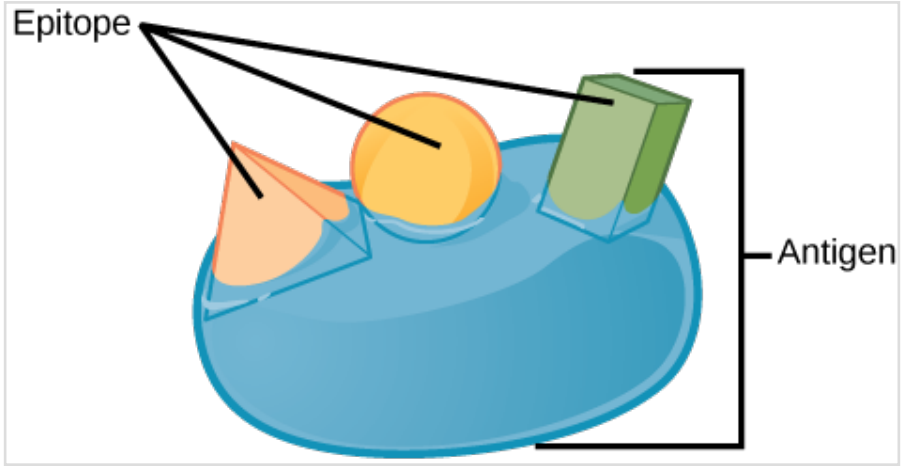
Immune System and Immunoglobulin

- Distinguishes self from non-self, and destroys non-self.
- Cellular immune system.
  - Targets own cells that have been infected
- Humoral “fluid” immune system.
  - Targets extracellular pathogens such as bacteria and viruses.
  - Can also recognize foreign proteins.
  - Recognition achieved by antibody.

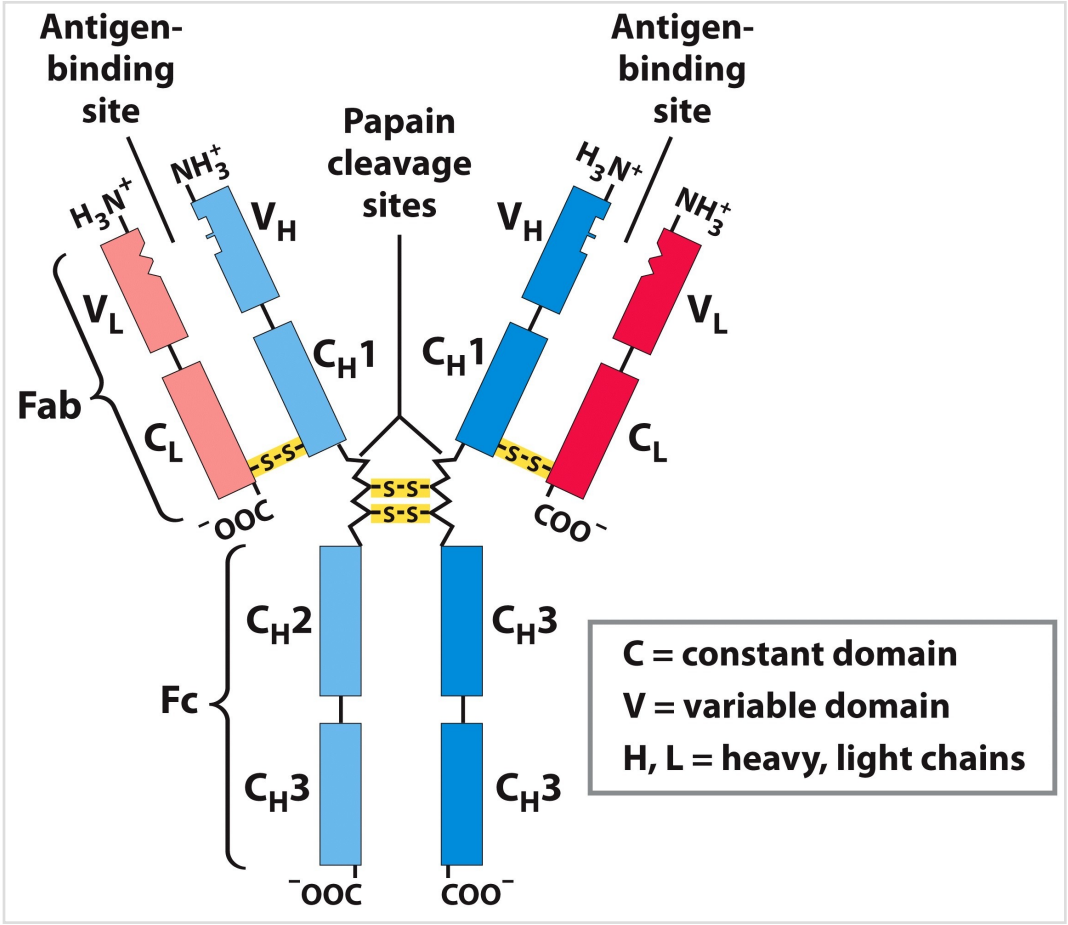


Antibody & Antigen

- Antibody or immunoglobulin.
  - Produced by B lymphocytes, or B cells.
- Antigen.
  - Molecule or pathogen that elicits immune response.
  - Could be a virus, a bacterial cell wall, a protein or other macromolecule.
  - Epitope: site on antigen where antibody binds.



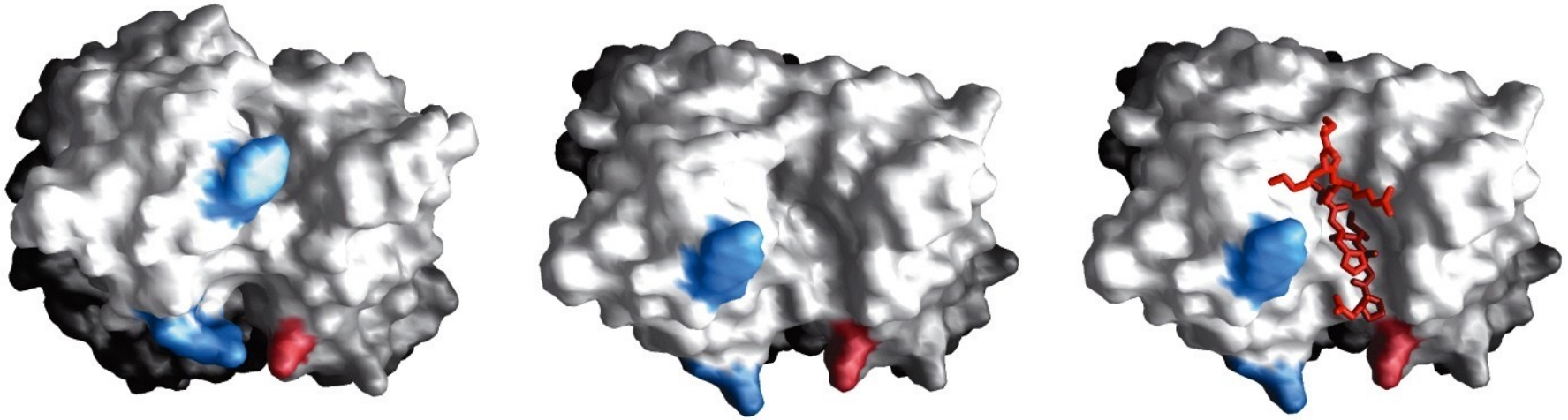
Antibody: Immunoglobulin G



- Y-shaped (Disulfide Bonds)
- Fab (antigen-binding)
- Fc (crystalize easily)
- 2 heavy chains
- 2 light chains
- Light chains: one V domain and one C domain
- Heavy chains: one V domain and three C domains
- **Variable** domains make up antigen-binding site (two per antibody).
  - Confers antigen specificity.

Antigens Bind via Induced Fit

- Antigen binding causes significant structural changes to antibody.
  - Residues in variable domains are hypervariable.
  - Specificity conferred by chemical complementarity.



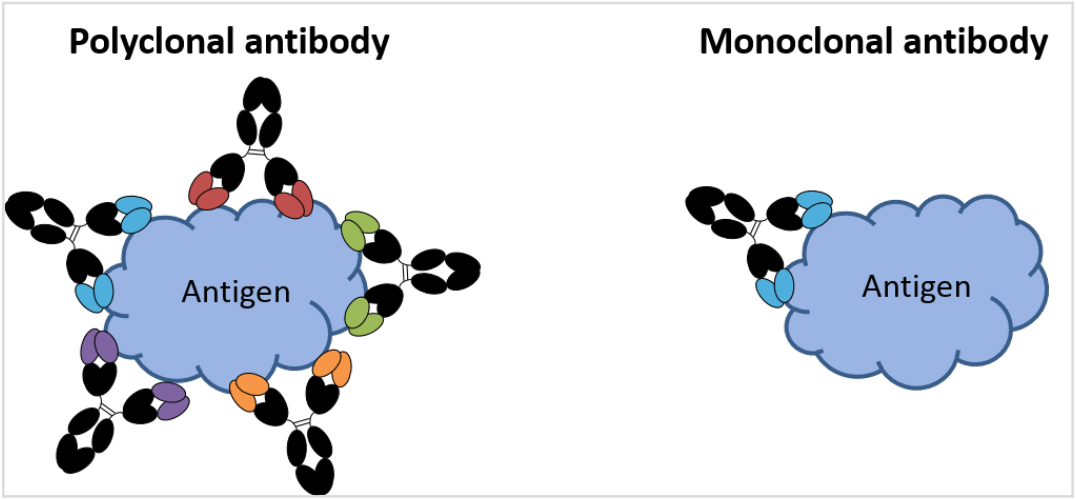
(a) Conformation with no antigen bound

(b) Antigen bound (but not shown)

(c) Antigen bound (shown)

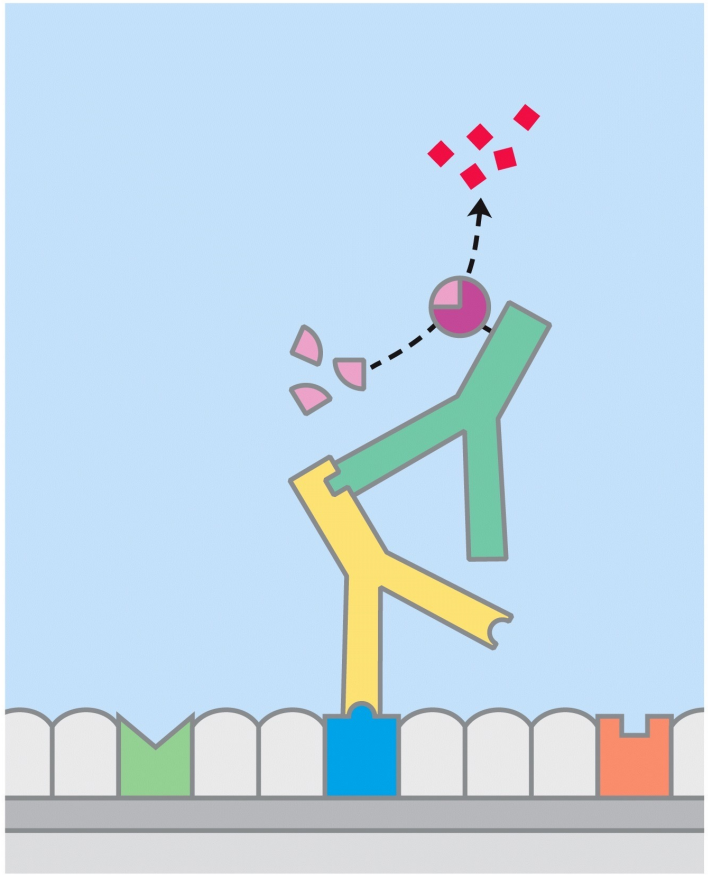
Antibody Preparation

- Polyclonal antibody
  - A mixture of antibodies.
  - Produced by many different B cells responding to one antigen.
  - Recognize different parts of antigen.
- Monoclonal antibody
  - Produced by a population of **identical** B cells.
  - Recognize the same epitope.



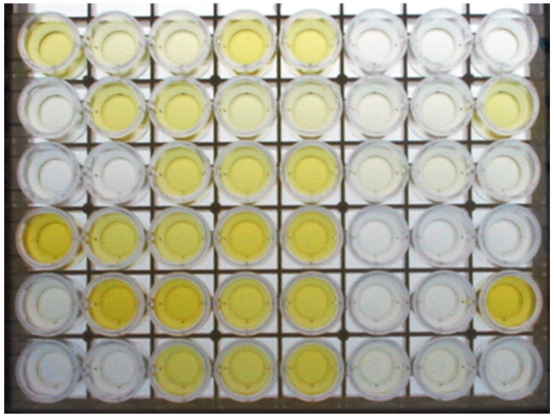
Antibody Technique

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.



Colormetric Antibody Detection

1. Proteins absorbed to surface.
2. Surface washed with nonspecific protein.
   - **Block** protein-binding sites.
3. Surface treated with primary antibody.
4. Unbound antibody washed away.
5. Surface treated with secondary antibody.
   - Secondary antibody linked to an **enzyme**.
6. Secondary antibody washed away.
7. Substrate added.
   - Antibody-linked enzyme converts substrate to colored product.
8. Product formed and monitored as color intensity.



ELISA

Summary 5.2 Complementary Interaction

- Y-shaped antibody structure. Heavy and light chains. Constant and variable domains. Fab and Fc fragments.
- Antibody-antigen binding. Epitope. Induced fit. Polyclonal vs monoclonal antibody.
- Antibody techniques. Antibody specificity as basis. Immunoaffinity chromatography, ELISA and immunoblot assay (Western blot)

Example Question

Which of the following statements about antibody is NOT true?

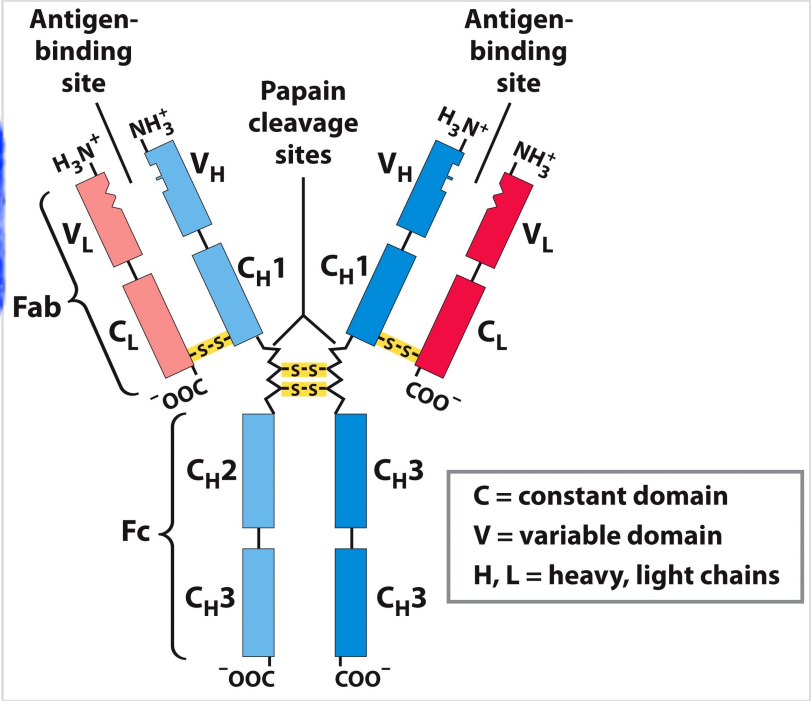
A) Disulfide linkages strengthen the quaternary protein structure.

B) Because the antigen-binding domain has high affinity, there is no observed induced fit.

C) Amino acid variability leads to specific epitope recognition.

D) There are two binding sites on the same molecule.

E) The Fc fragment does not directly participate in antigen binding.



Function of Globular Proteins

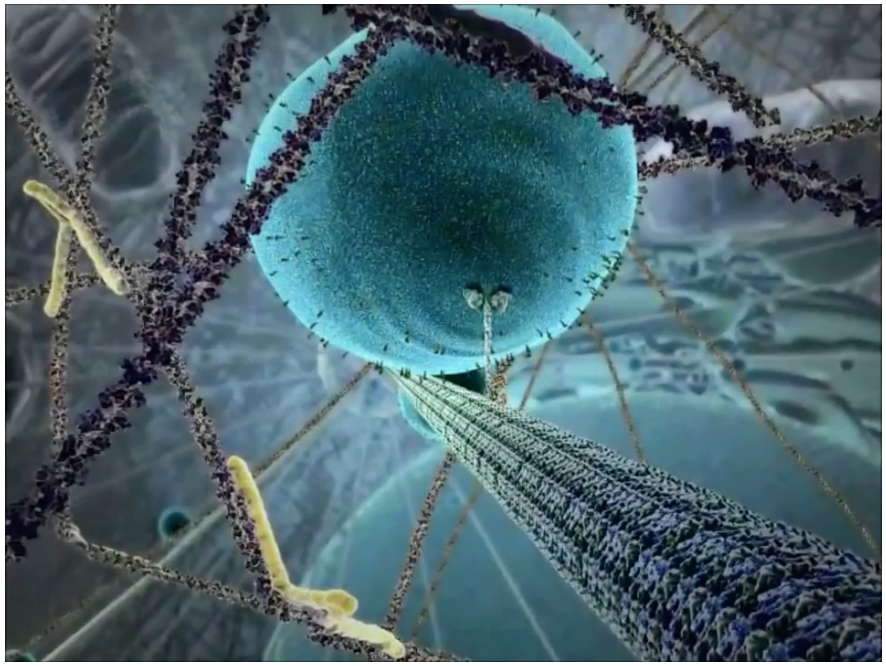
5.1 Reversible Binding to Ligand

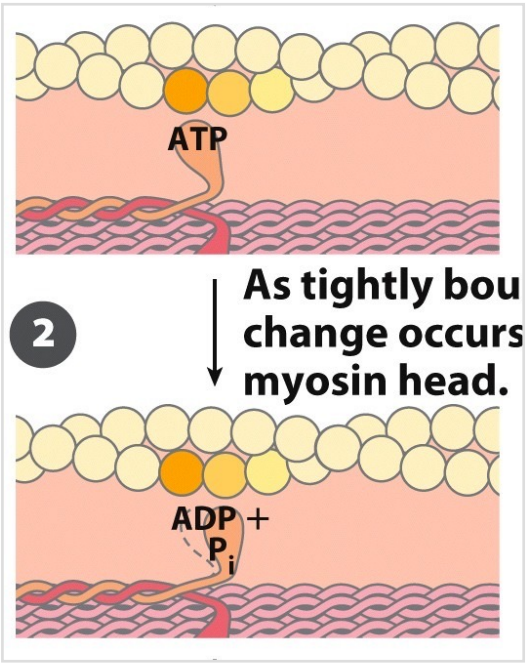
5.2 Complementary Interaction between Protein and Ligand

5.3 Interaction Modulated by Chemical Energy

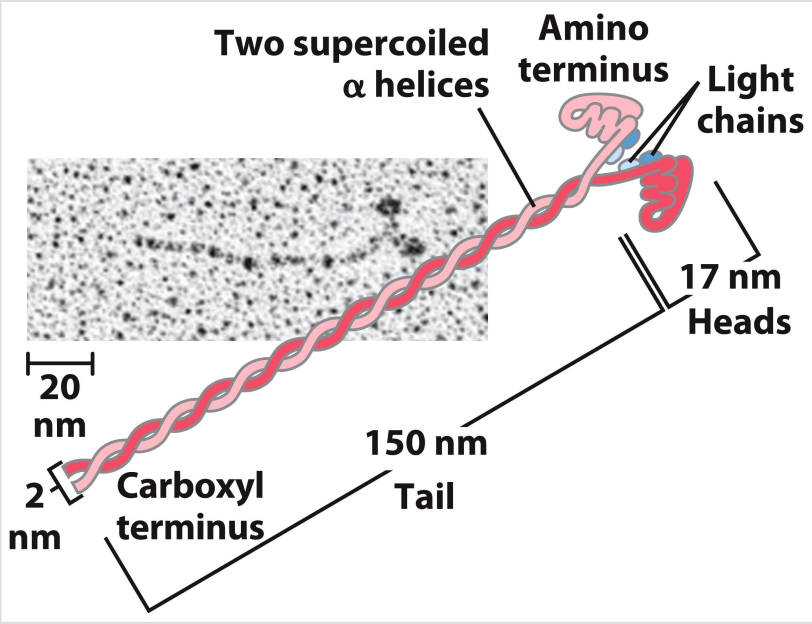
Interaction Modulated by Chemical Energy

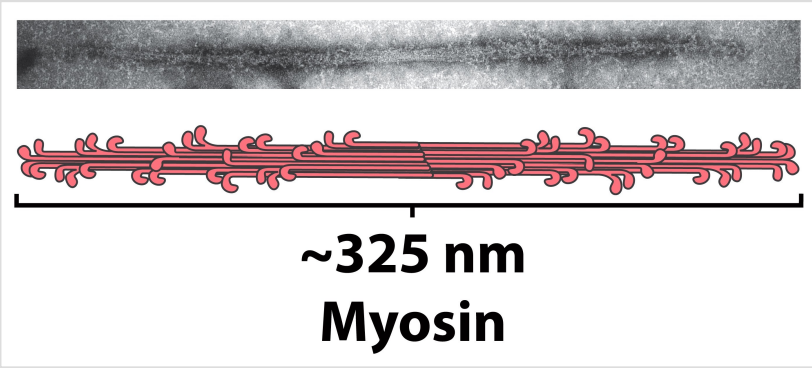
- Use of chemical energy (ATP) can cause conformational changes in proteins.
- Especially in motor proteins
  - Convert chemical energy to kinetic energy





Major Proteins of Muscle: Myosin

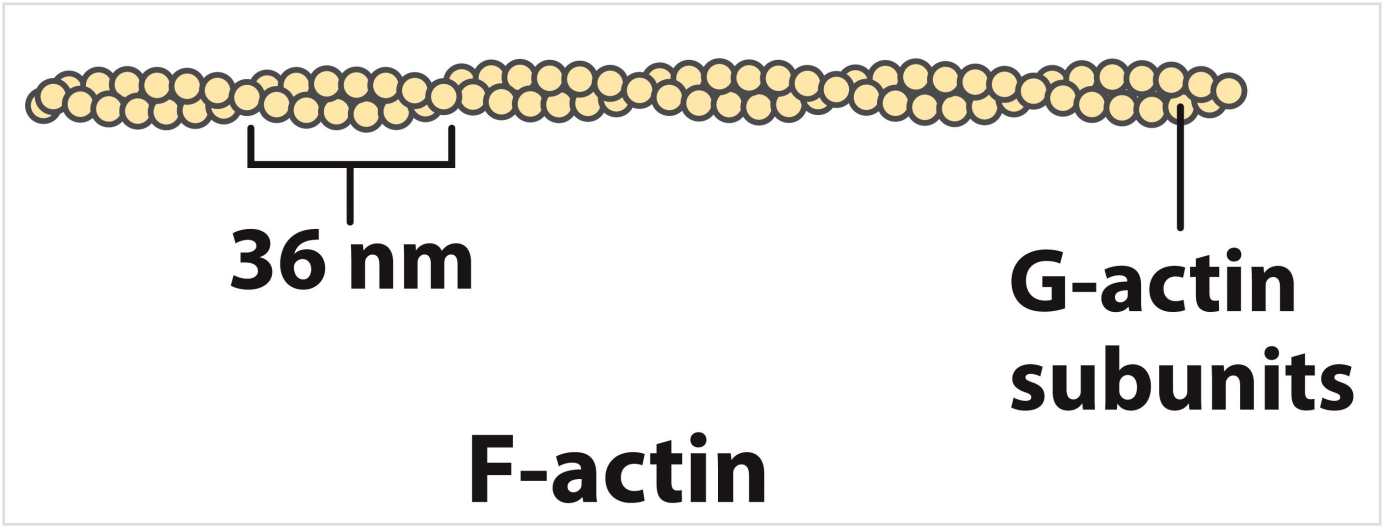




- Six subunits.
  - Two heavy chains.
  - Four light chains.
- Heavy chain.
  - C terminus α **helices** arranged as left-handed coiled coil "tail".
  - N terminus forms globular domain "head".
    - Site of ATP hydrolysis and where light chains associate.
- Multiple myosin proteins aggregate to form thick filament.

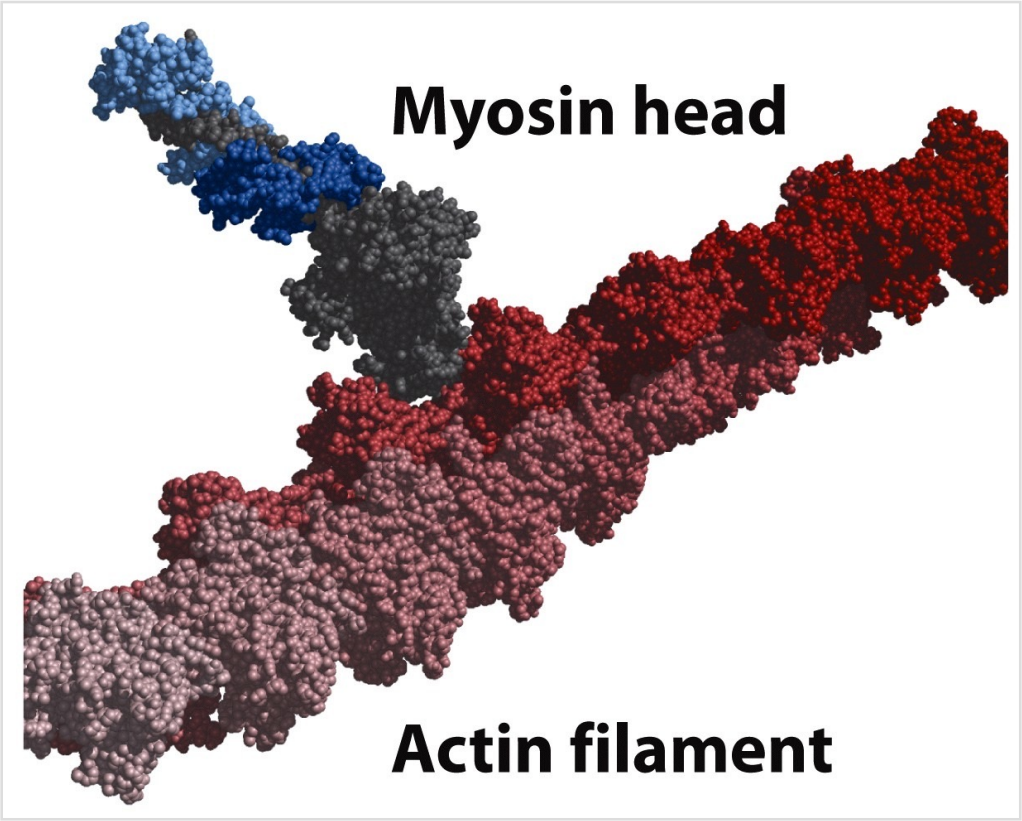
Major Proteins of Muscle: Actin

- Monomeric actin (G-actin, globular actin) molecules associate to form a long polymer (F-actin, filamentous actin).
  - Each monomer binds ATP, which is then hydrolyzed to ADP.
  - ATP hydrolysis functions only in assembly, NOT in muscle contraction.
- Along with other proteins, F-actin forms thin filament.



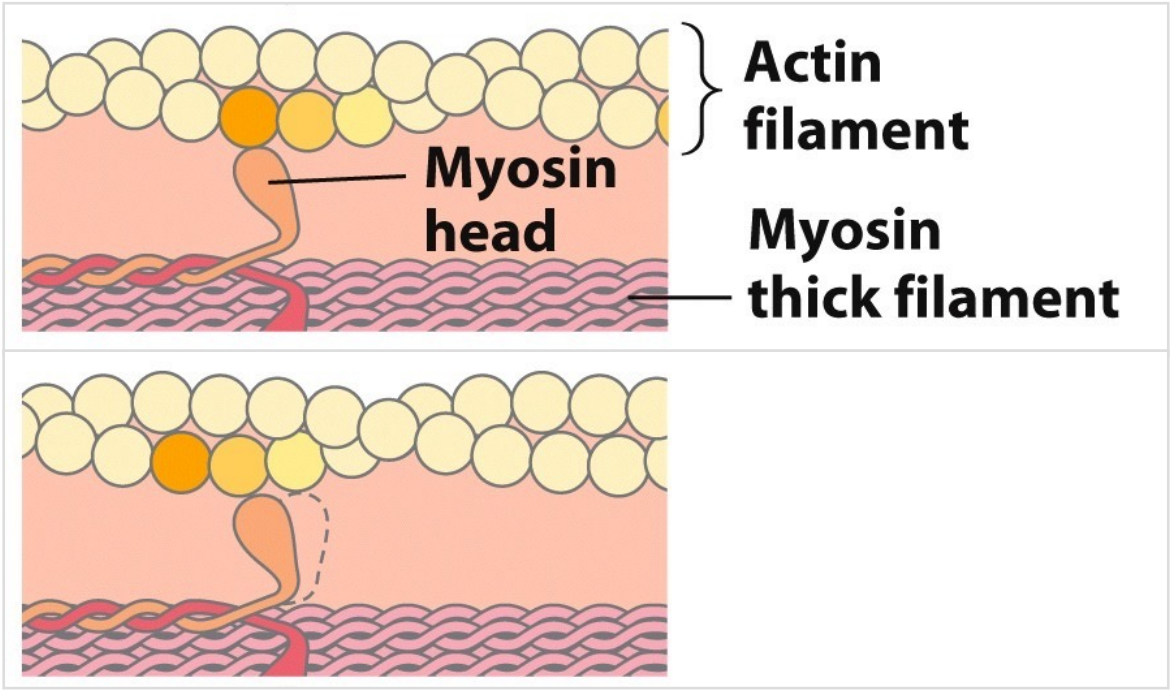
Myosin Binds Actin

- Each actin monomer in thin filament can bind tightly and specifically to one myosin head group in thick filament.

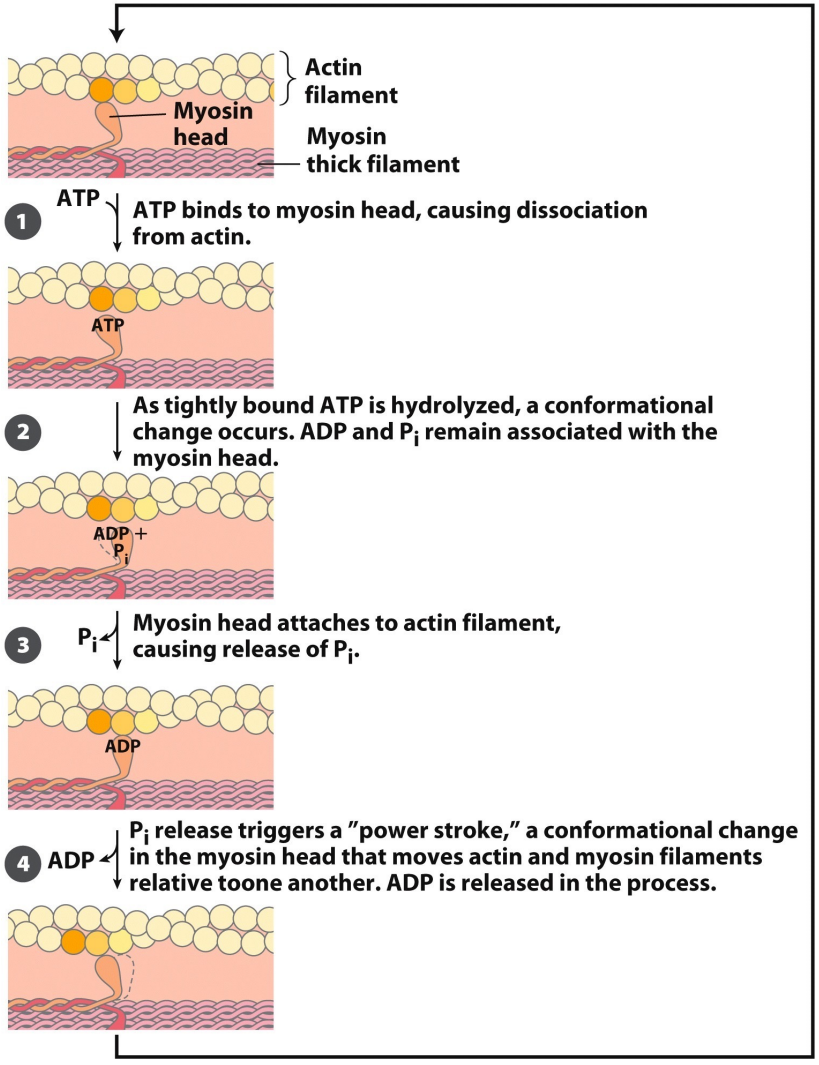


Thick Filaments Slide Along Thin Filaments

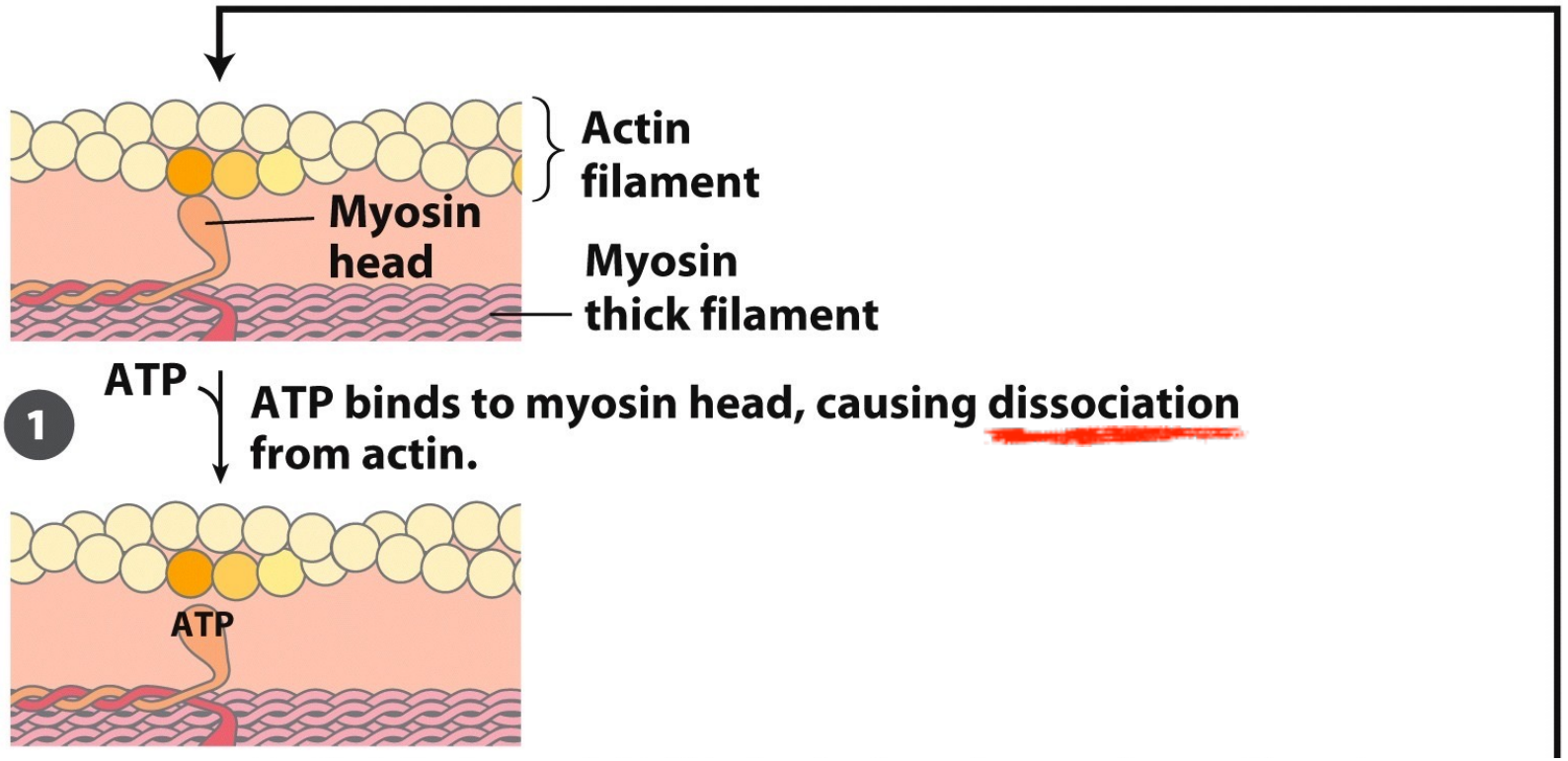
- When ATP is not bound, myosin binds tightly to actin.
- When ATP binds to myosin and is hydrolyzed, myosin releases F-actin subunit and binds another subunit.



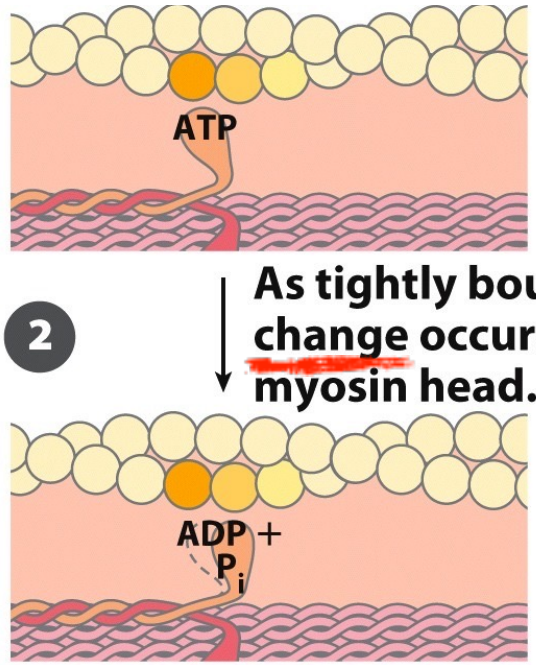
Thick Filaments Slide Along Thin Filaments



Thick Filaments Slide Along Thin Filaments

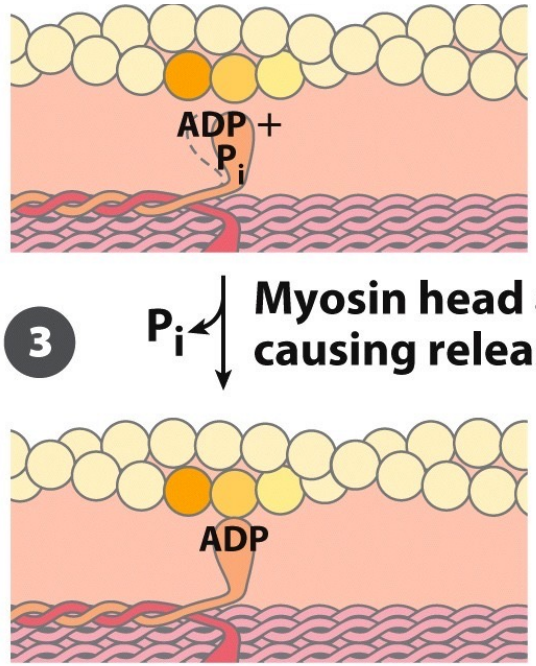


Thick Filaments Slide Along Thin Filaments

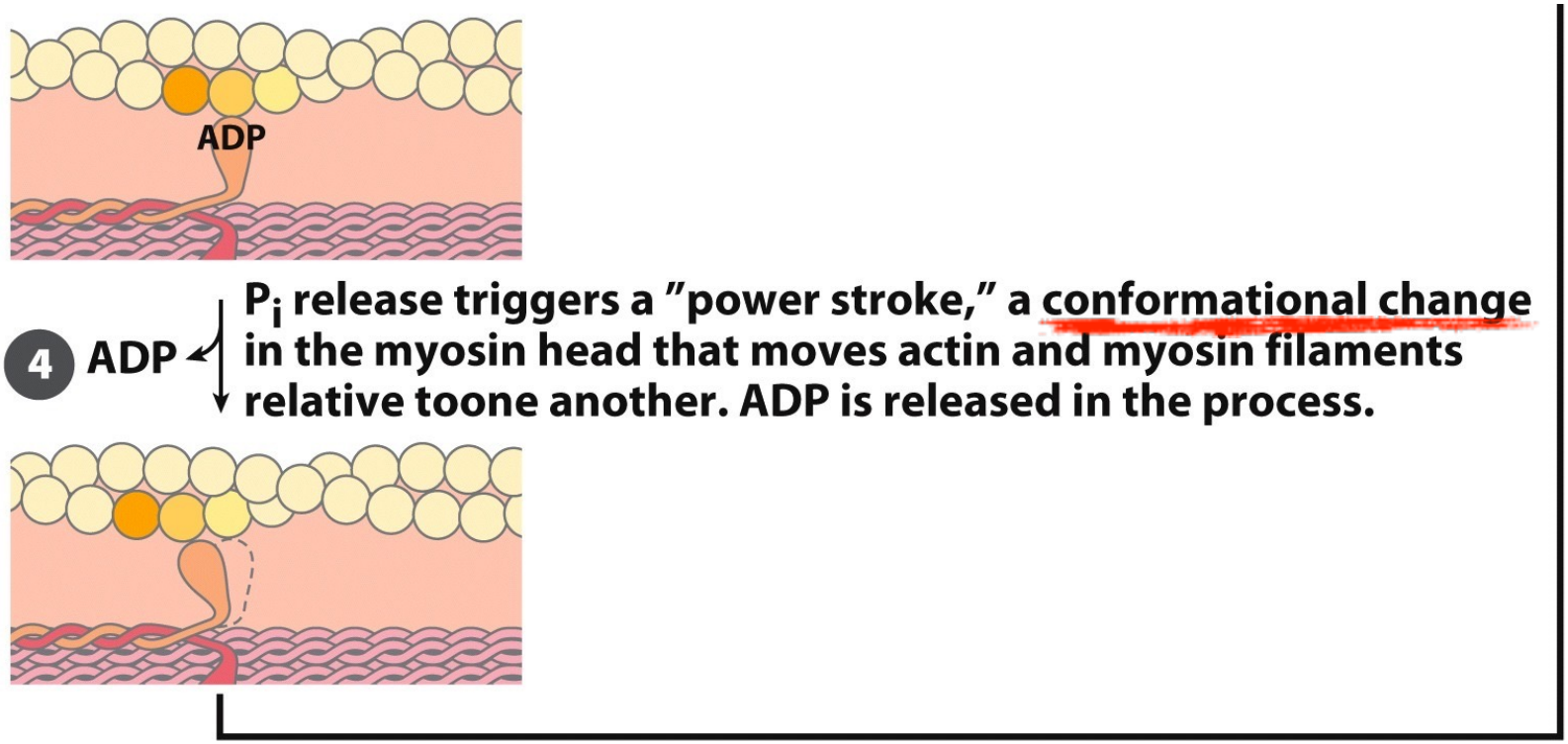


As tightly bound ATP is hydrolyzed, a conformational change occurs. ADP and P_i remain associated with the myosin head.

Thick Filaments Slide Along Thin Filaments



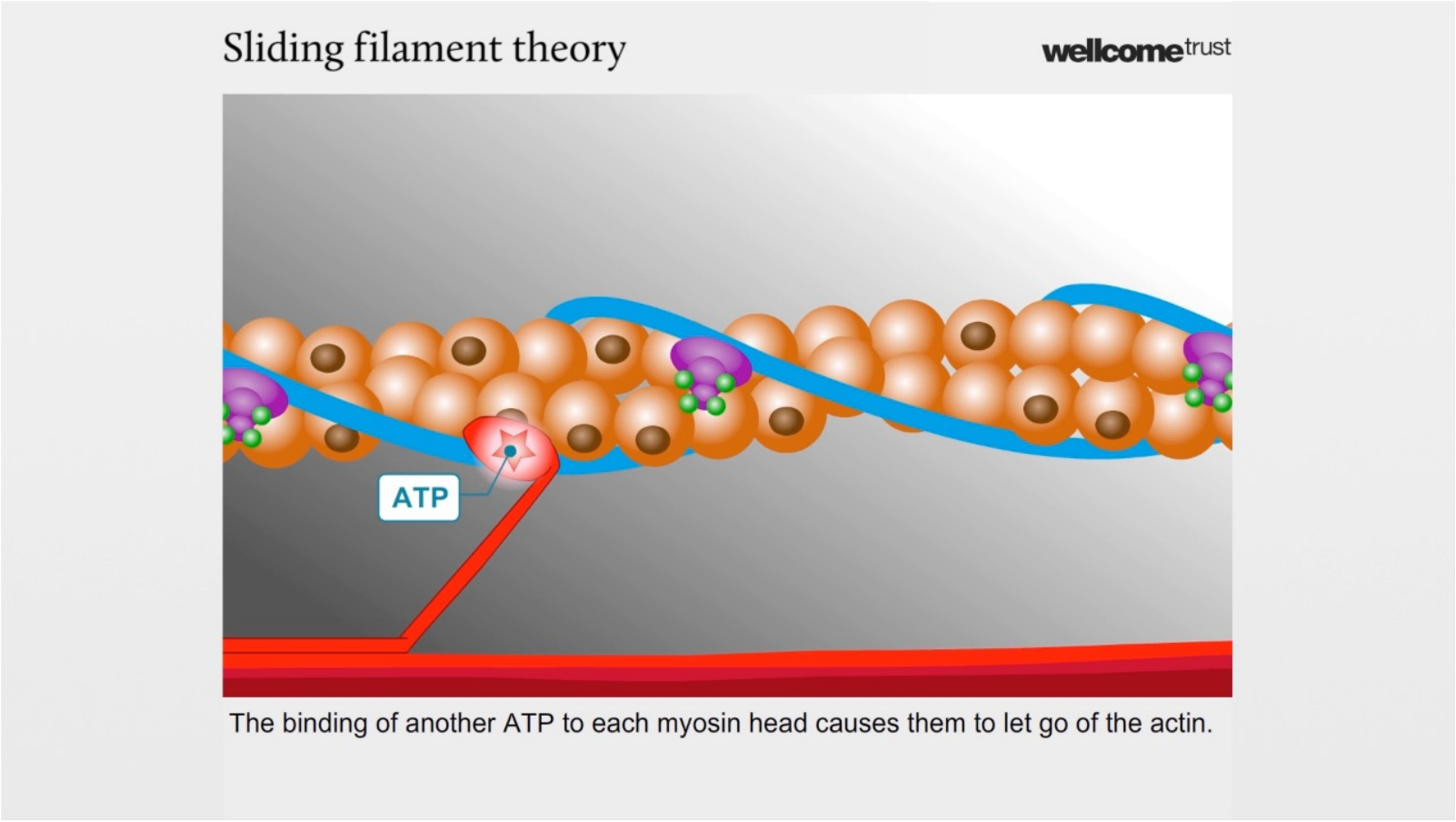
Thick Filaments Slide Along Thin Filaments



Summary 5.3 Interaction Modulation

- Muscle contraction results from interaction between myosin and actin, coupled to ATP hydrolysis.
- Myosin consists of two heavy and four light chains, forming a coiled coil tail domain and a globular head domain. Myosin molecules are organized into thick filaments.
- Thick filaments slide along thin filaments.

Muscle Contraction Review



- ATP hydrolysis causes a conformational change of myosin.

Example Question

The energy that is released by the hydrolysis of ATP by actin is used for:

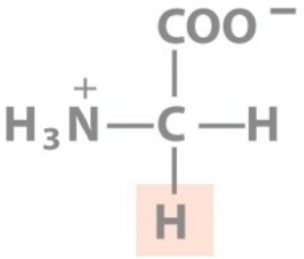
A) actin filament assembly.

B) actin filament disassembly.

C) actin-myosin assembly.

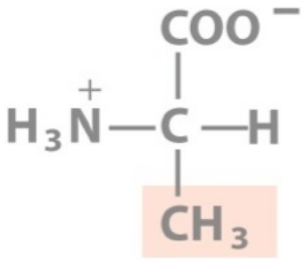
D) actin-myosin disassembly.

E) muscle contraction.



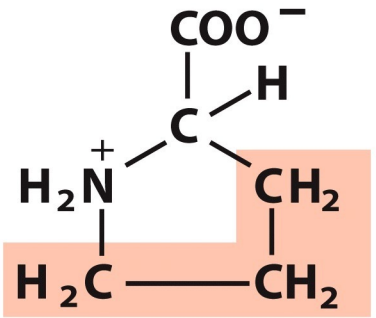
Glycine

Gly, G



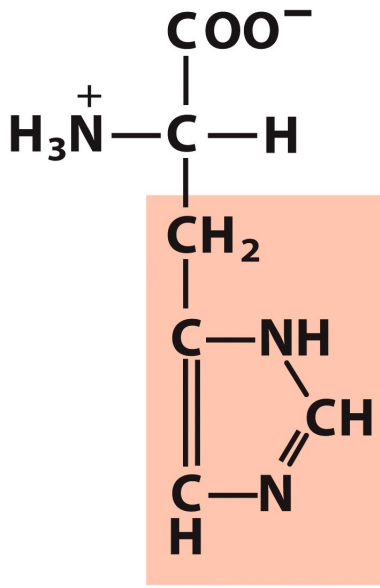
Alanine

Ala, A



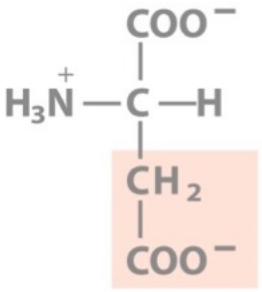
Proline

Pro, P



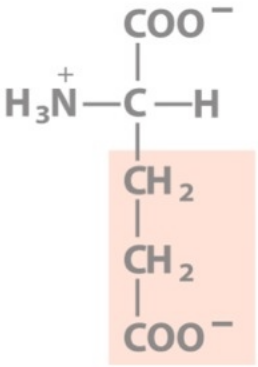
Histidine

His, H



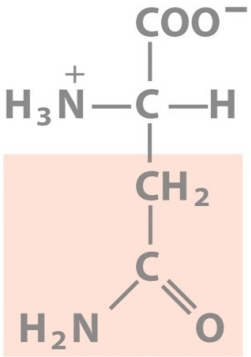
Aspartate

Asp, D



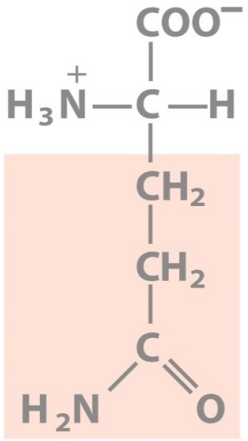
Glutamate

Glu, E



Asparagine

Asn, N



Glutamine

Gln, Q

Amino acid for 4th week

Chapter 5: Summary

In this chapter, we learned:

- 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