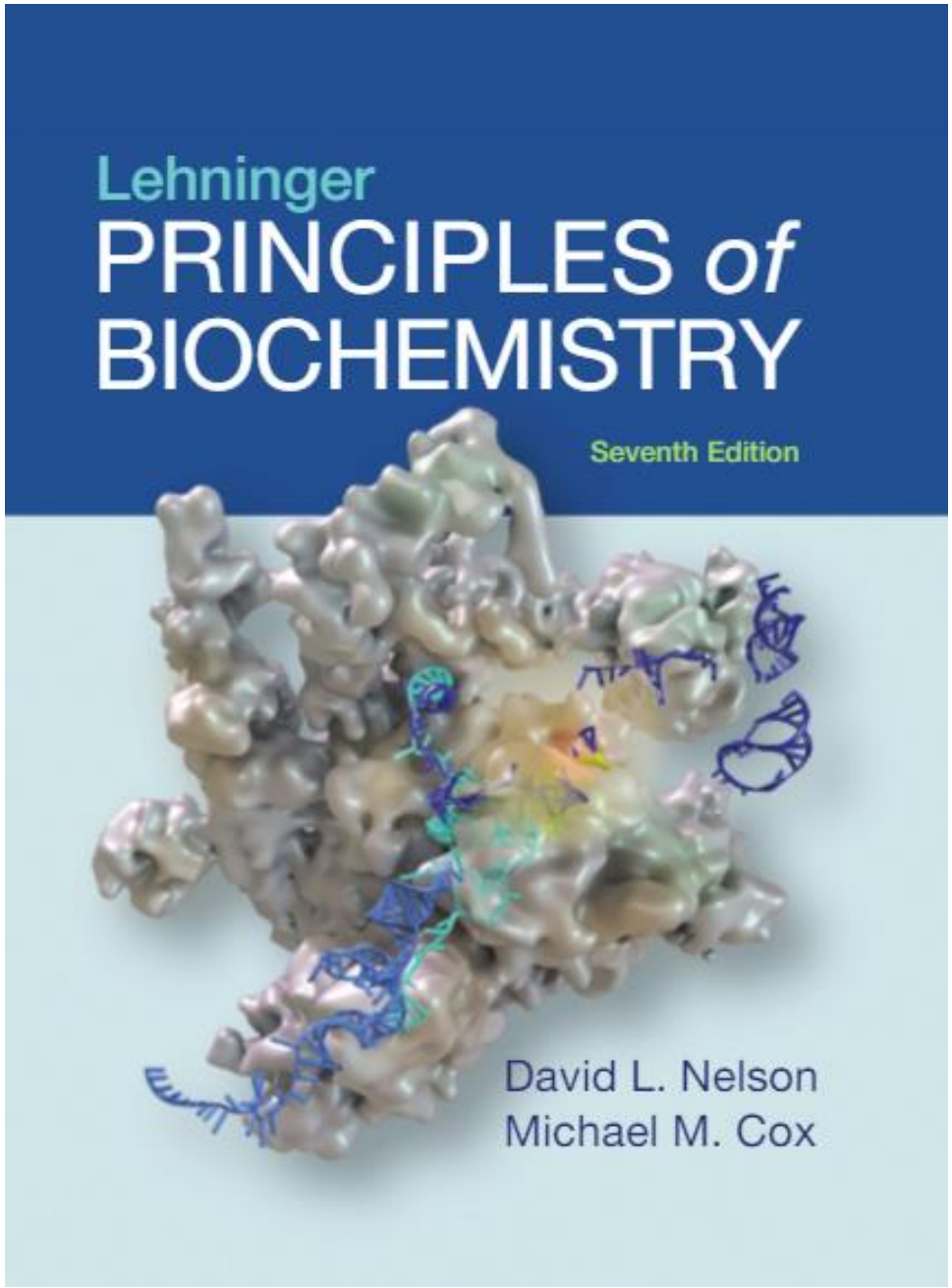
5| Protein Function



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CHAPTER 5:
Protein Function

Learning goals:

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

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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

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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)

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Interaction with Other Molecules

- When ligands bind to proteins, some conformational changes (sometimes quite dramatically) occur permitting tighter binding → this is called **induced fit**

  – In multisubunit proteins, a conformational change of one subunit often affects the others (**cooperativity**)

- 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)**

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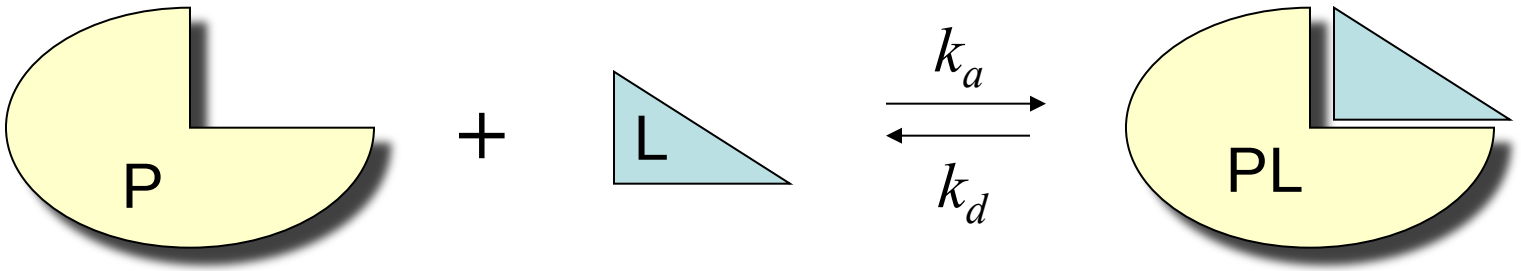
Binding: Quantitative Description

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



- The interaction can be 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 or the equilibrium dissociation constant, K_d

$$K_a = \frac{[\mathrm{PL}]}{[\mathrm{P}]\cdot[\mathrm{L}]} = \frac{k_a}{k_d} = 1/K_\mathrm{d}$$

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Binding:
Analysis in Terms of the Bound Fraction

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

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

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

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

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

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

- In terms of the more commonly used equilibrium dissociation constant

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

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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

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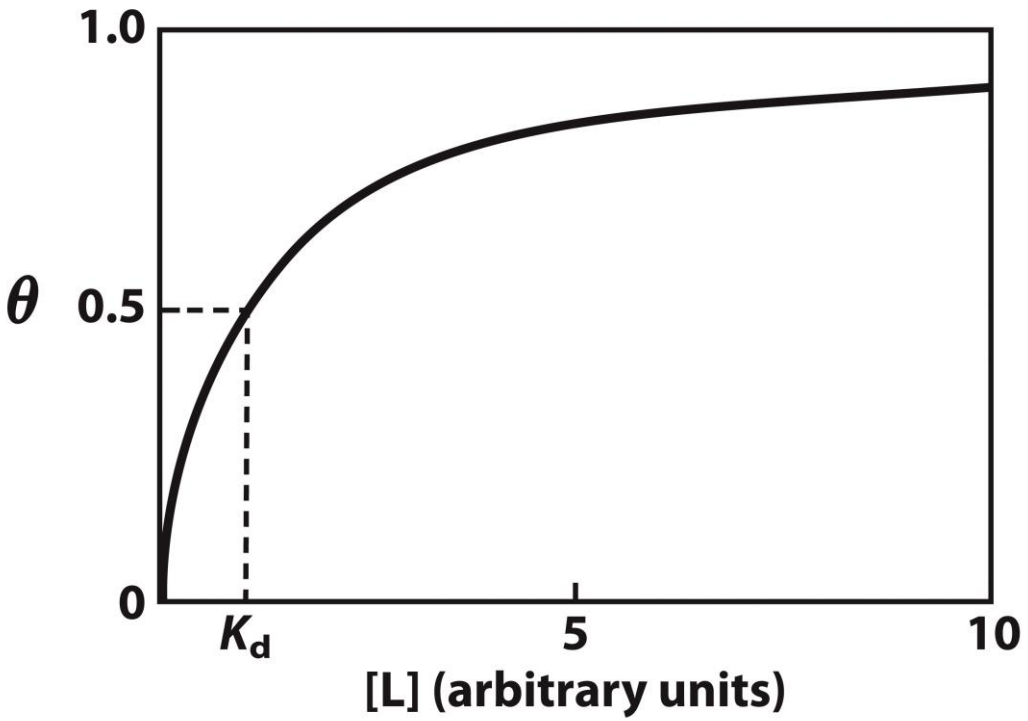
Binding: Graphical Analysis

- 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]



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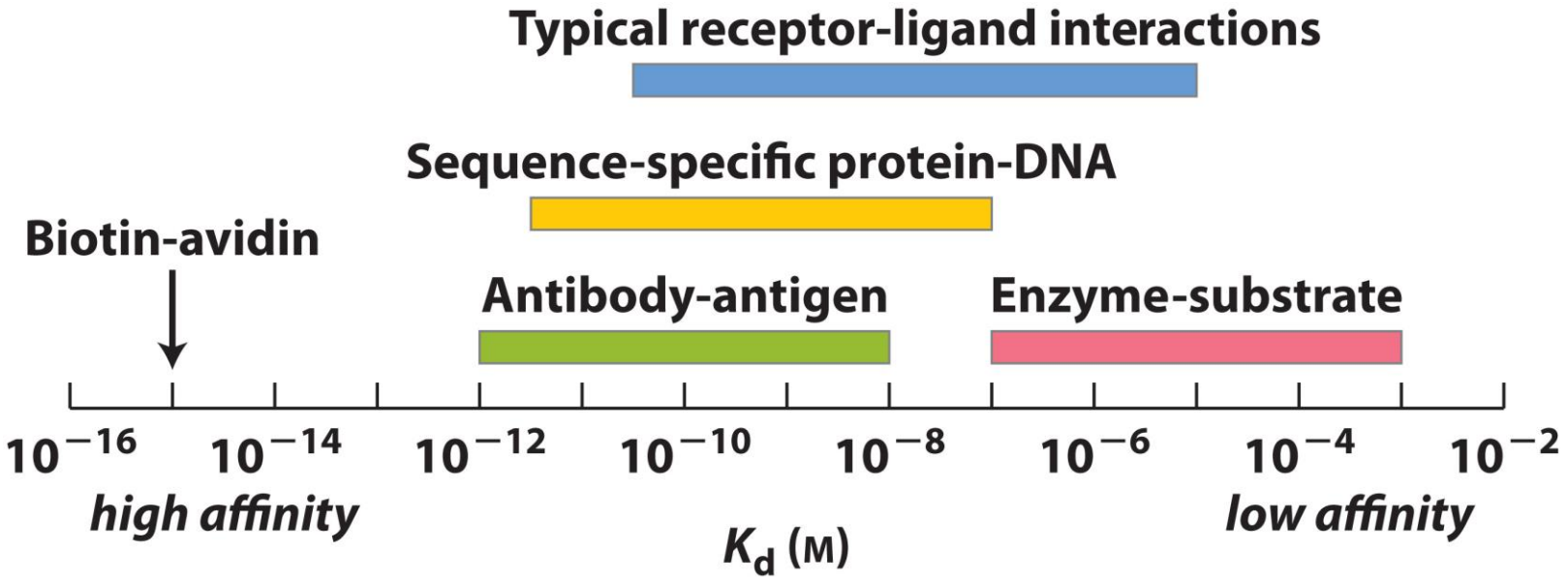
Examples of Binding Strength



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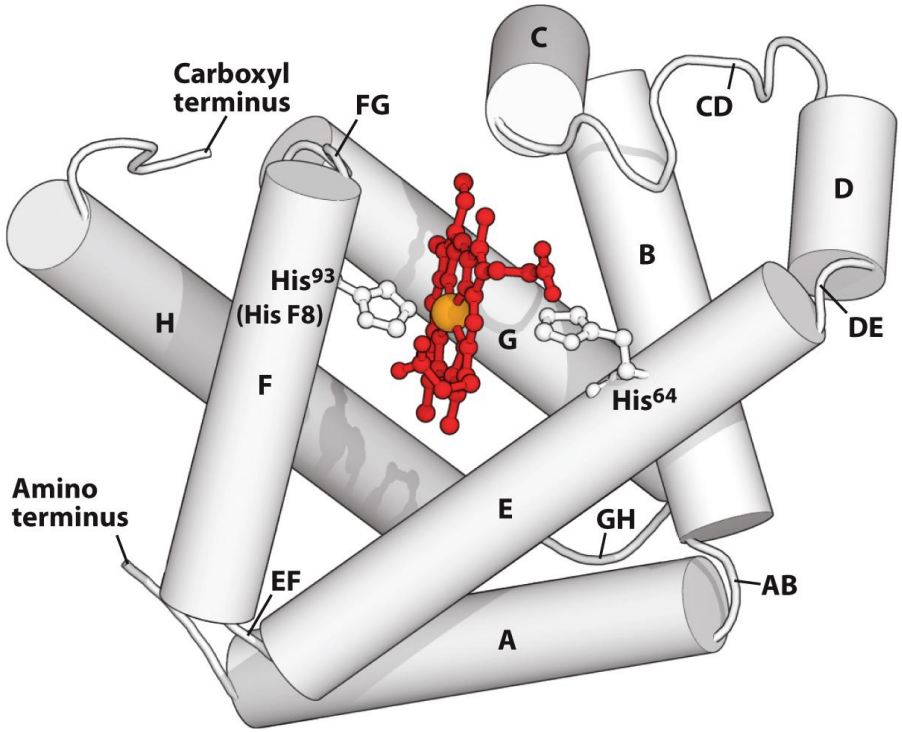
Example: Oxygen Binding to Myoglobin

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

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



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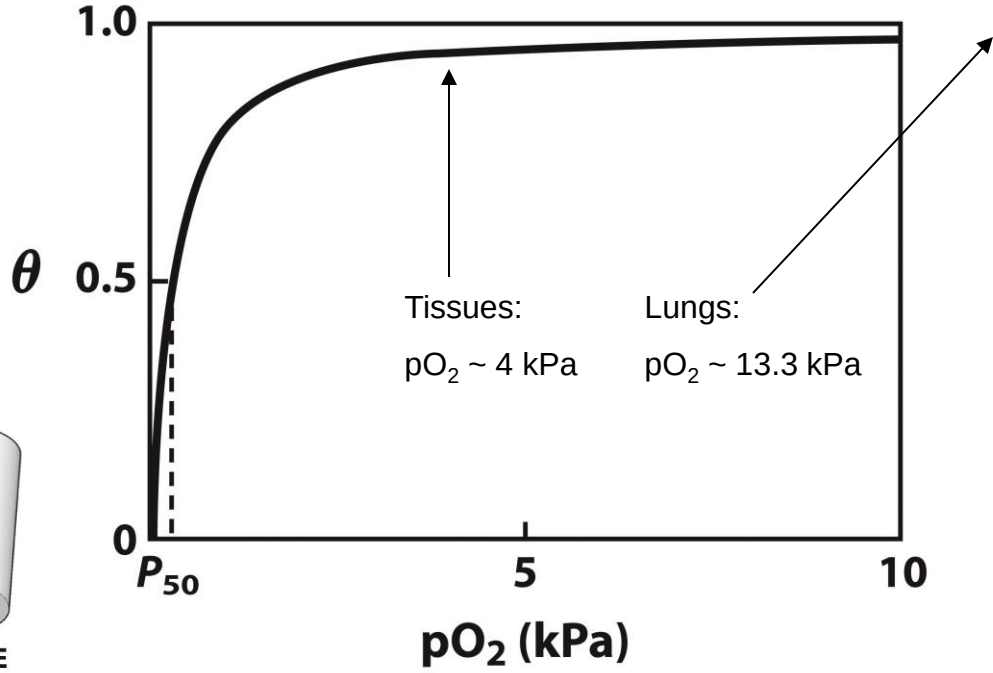

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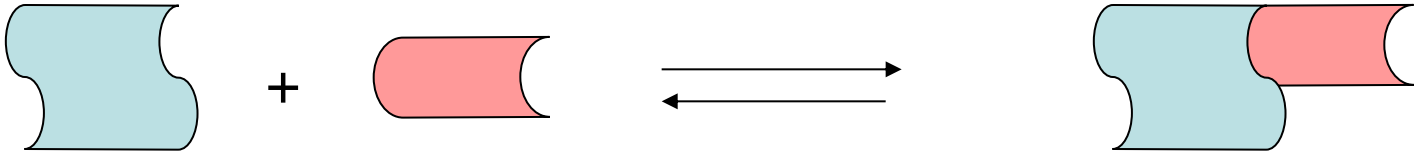
Specificity: Lock-and-Key Model

- 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.



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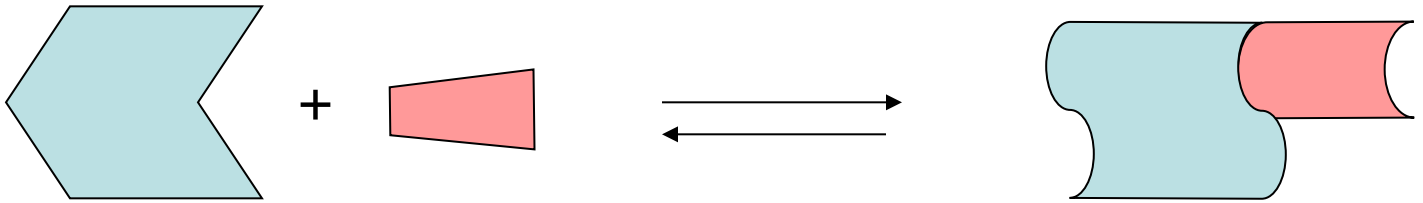
Specificity: Induced Fit

- 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



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Case Study I: Globins Are Oxygen-Binding Proteins

Biological problems:

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

Biological solution:

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

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

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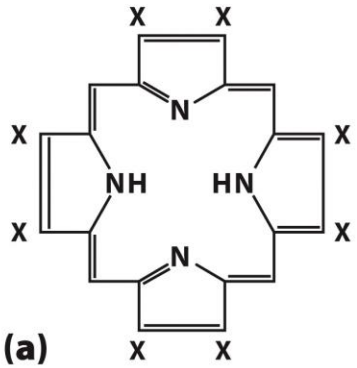
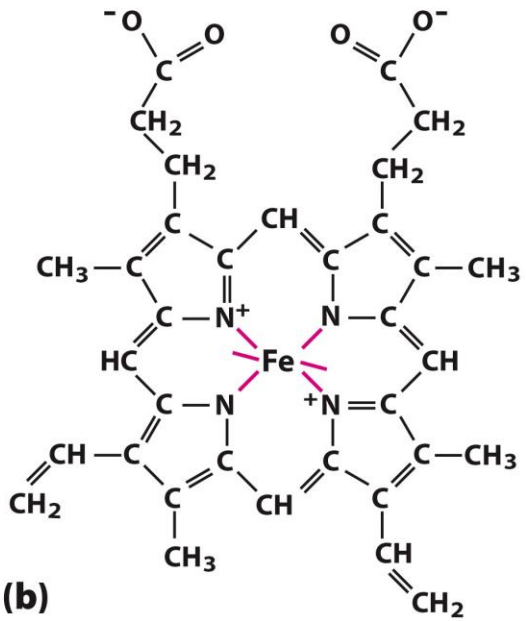
Structures of Porphyrin and Heme

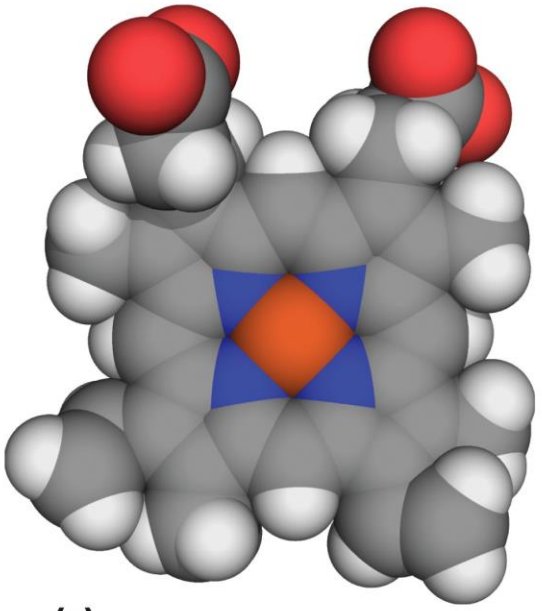
Porphyrin family

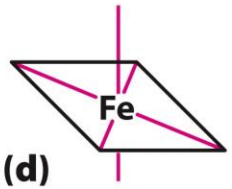


four pyrrole rings linked by methene bridges

Heme







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

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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

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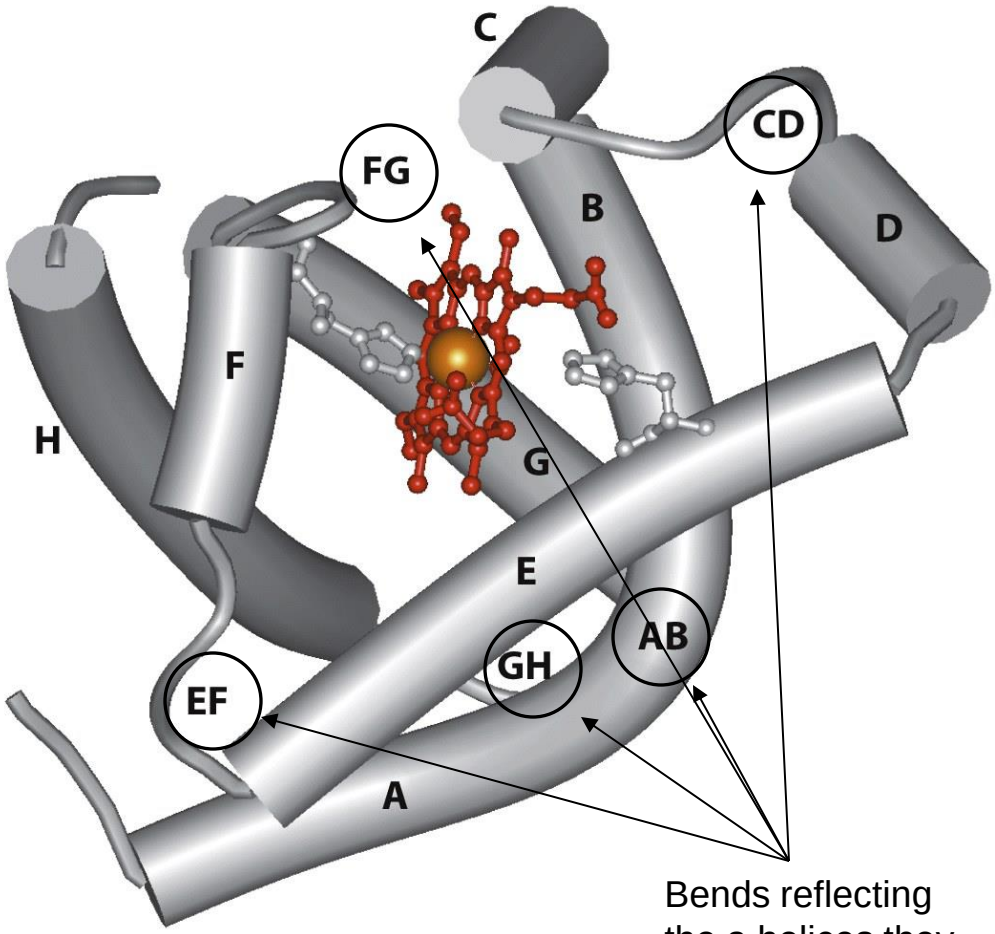
Structure of Myoglobin

- 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)



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Binding of Carbon Monoxide

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

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CO vs. O_2 Binding to Free Heme

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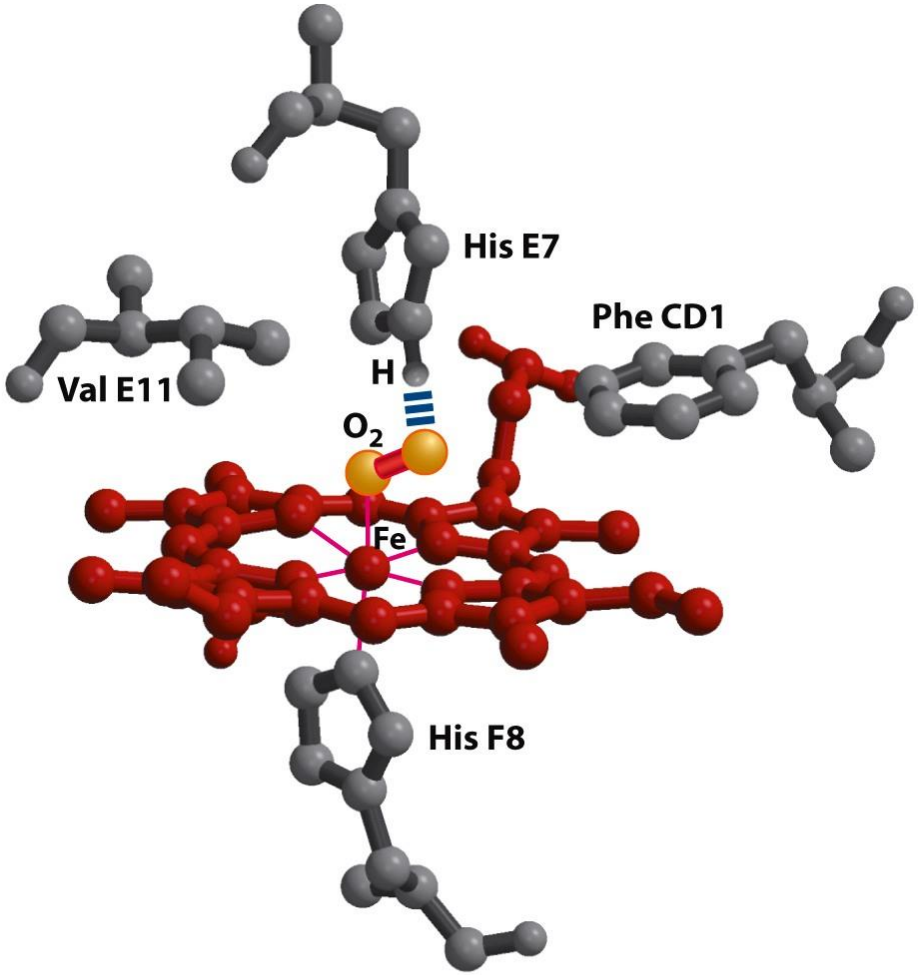
Heme binding to protein affects CO vs. O_2 binding



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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

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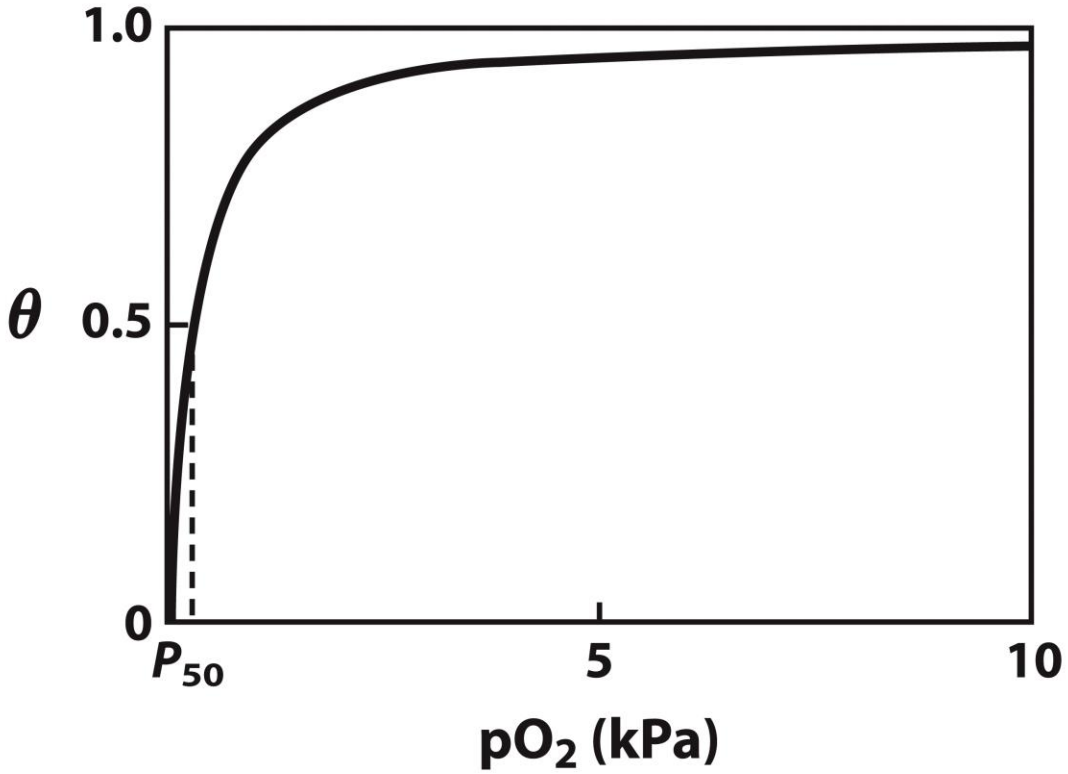
Could myoglobin transport O_2?

- 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!



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

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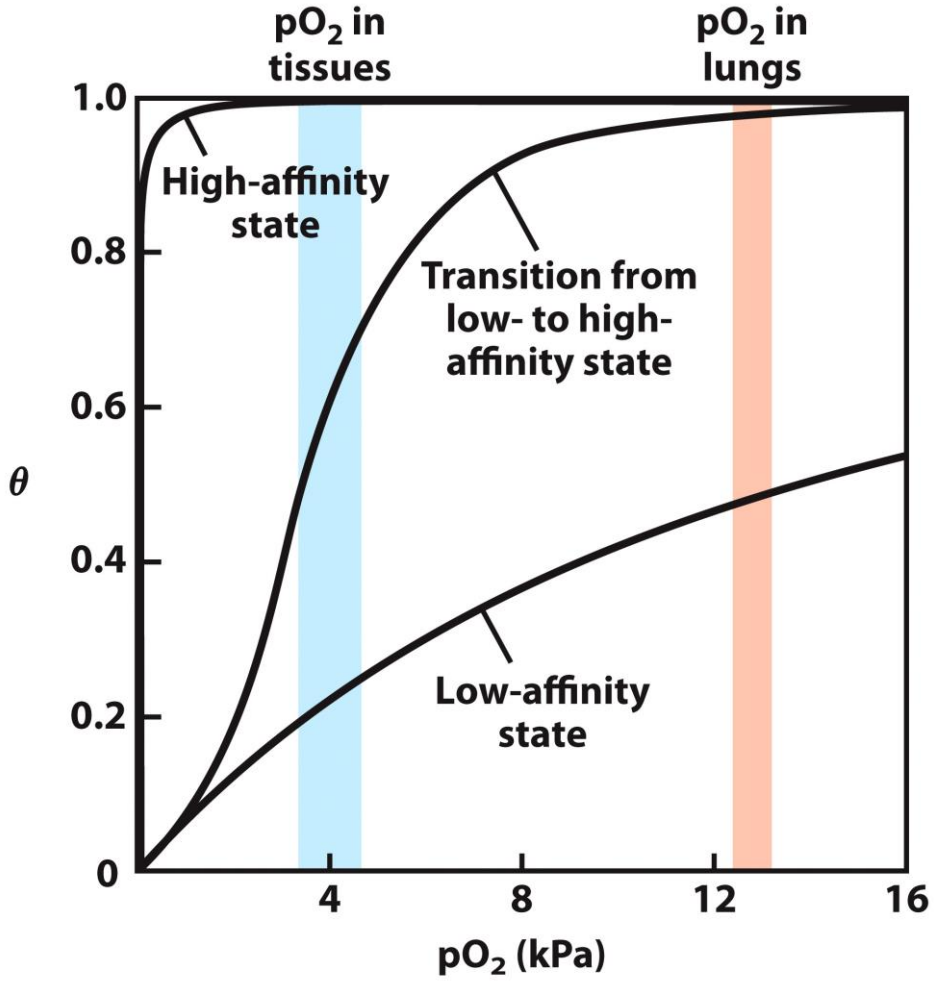
For effective transport affinity must vary with pO_2



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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

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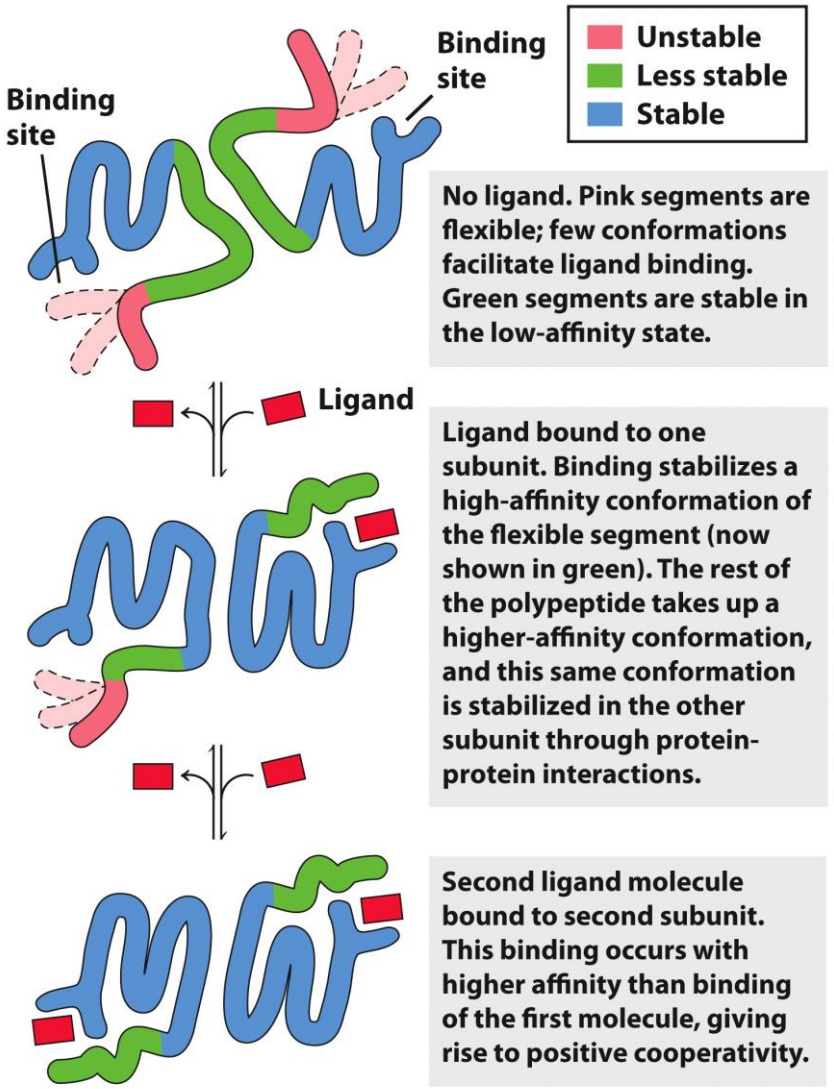
Cooperativity



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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; n>1 → +ve coop.; n<1 → -ve coop.
  - **Hill plot:** plotting log (θ / 1 – θ) vs. log [L]. Gives the Hill coefficient (n_H) which measures the degree of cooperativity

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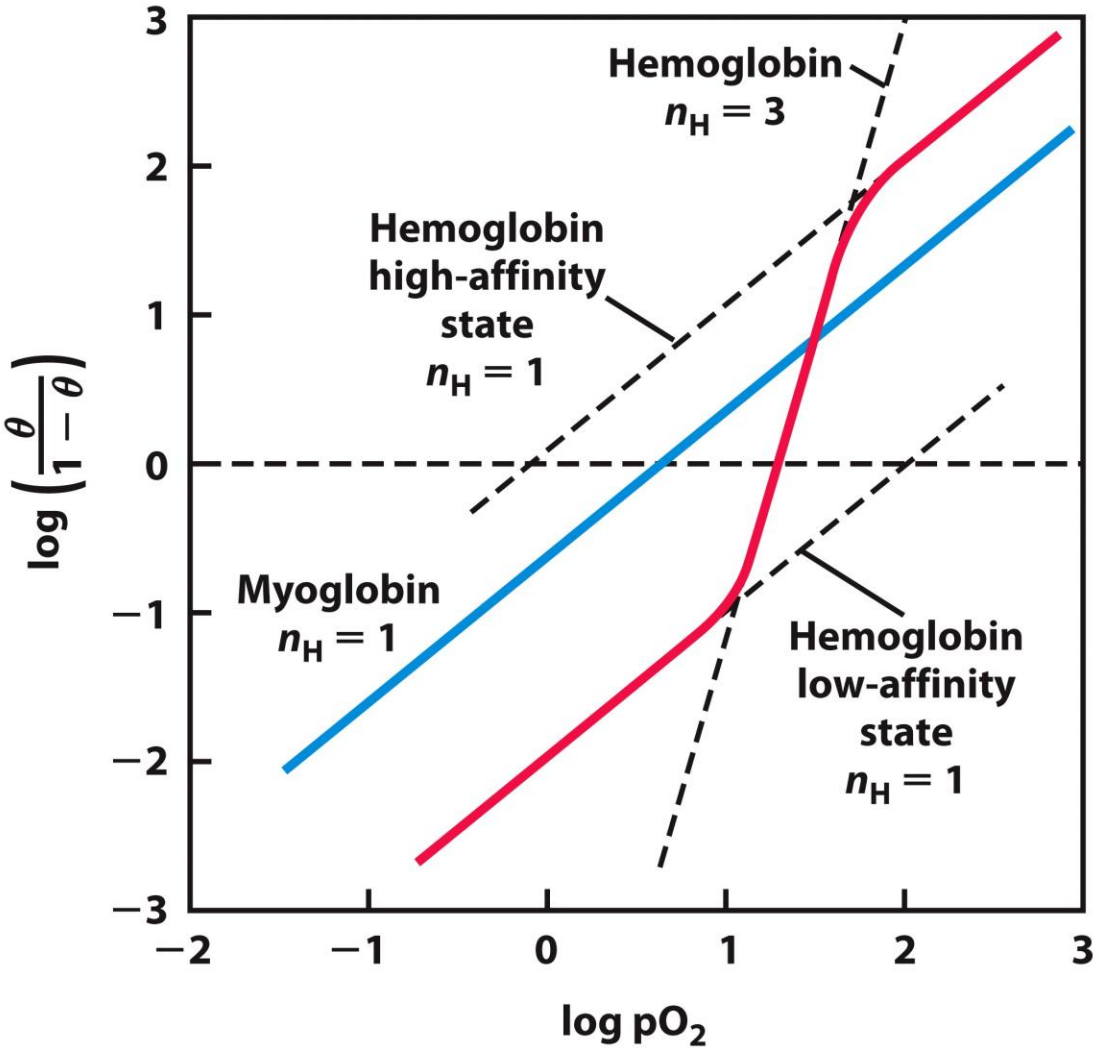
The Hill Plot of Cooperativity



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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

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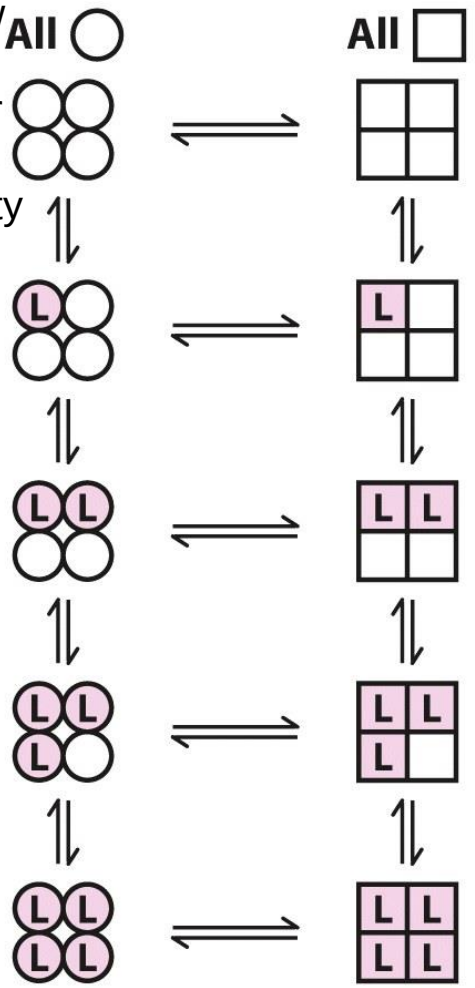
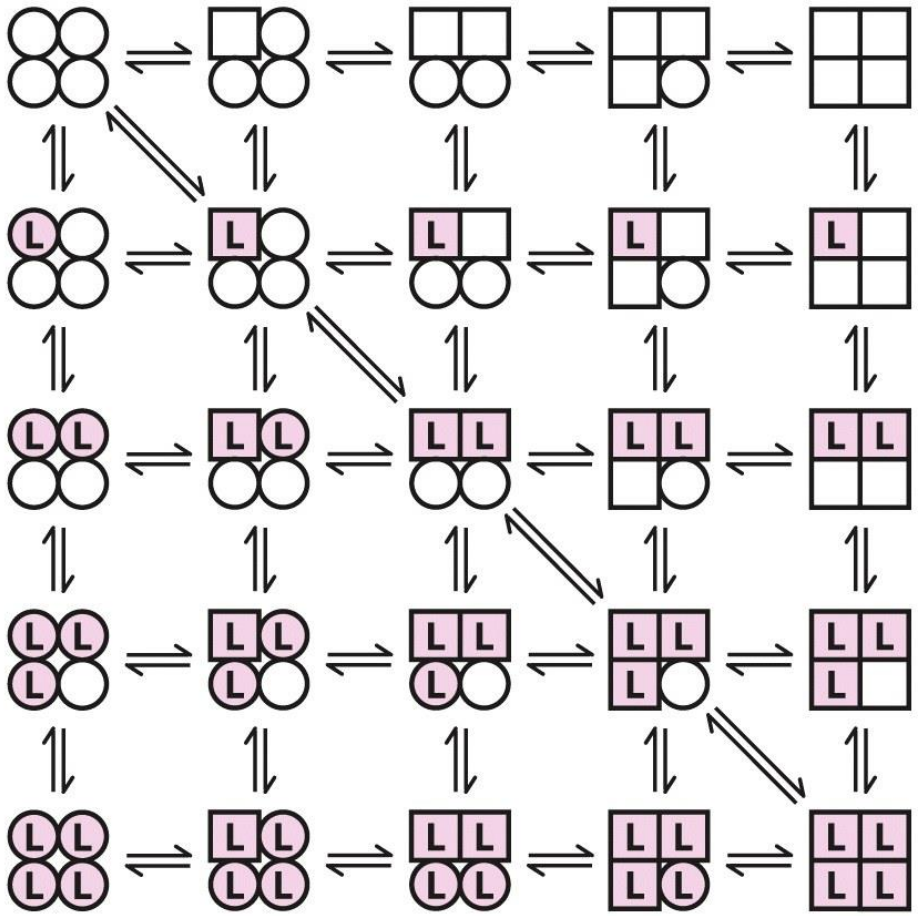
Two Models of Cooperativity: Concerted (MWC) vs. Sequential

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



(a)

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



(b)

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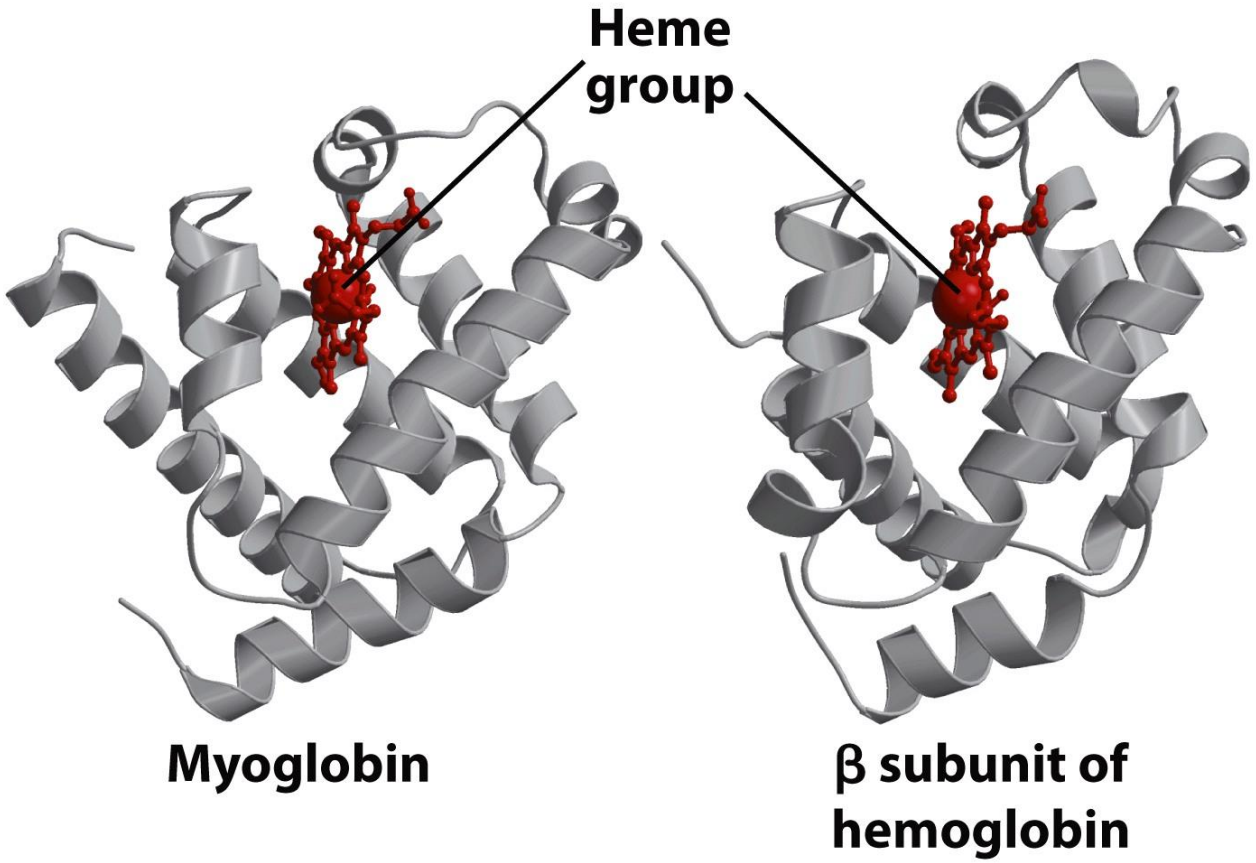
Hemoglobin binds oxygen cooperatively

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



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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)

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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

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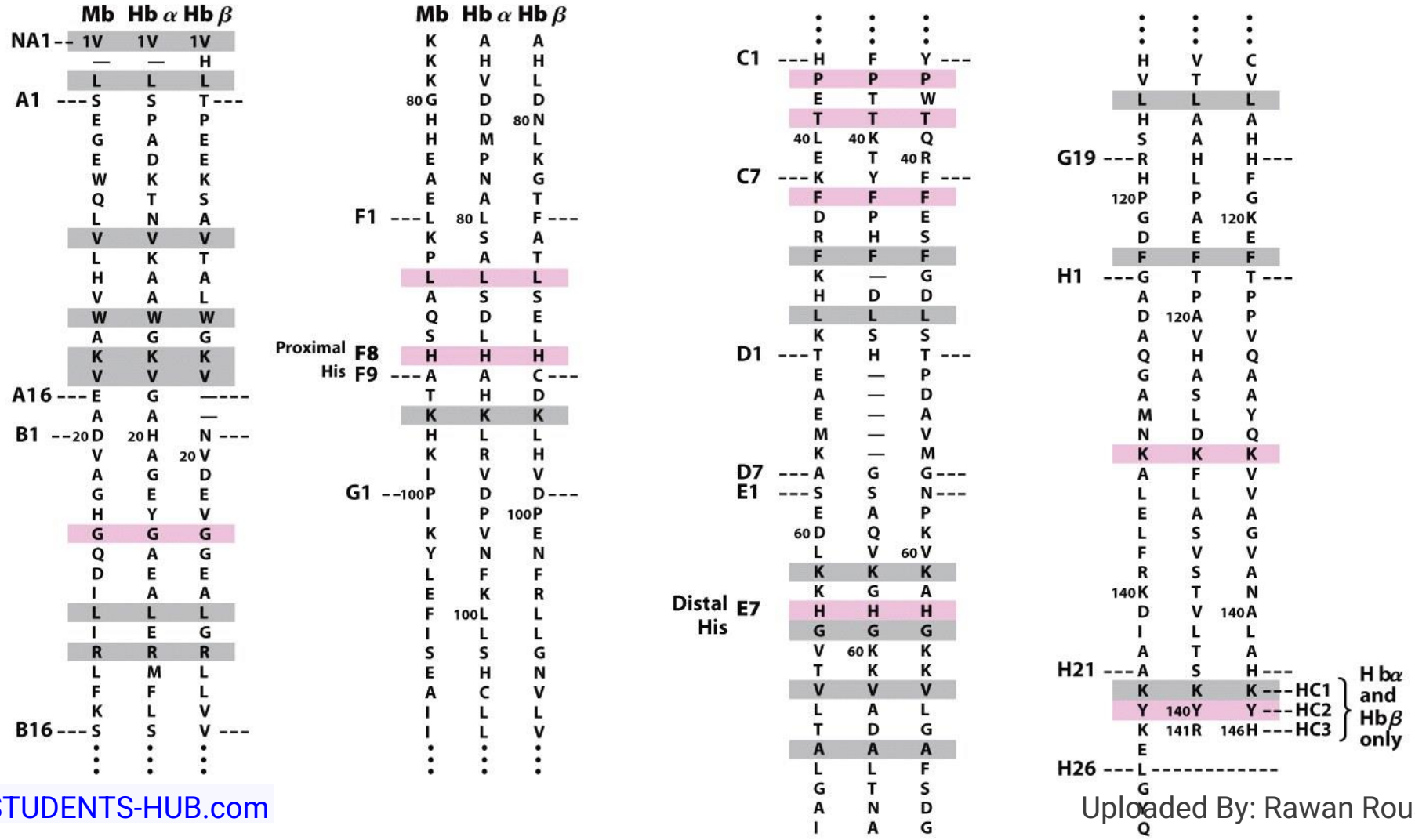
Sequence Similarity between Hemoglobin and Myoglobin



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Hb is a dimer of two αβ 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 αβ dimers

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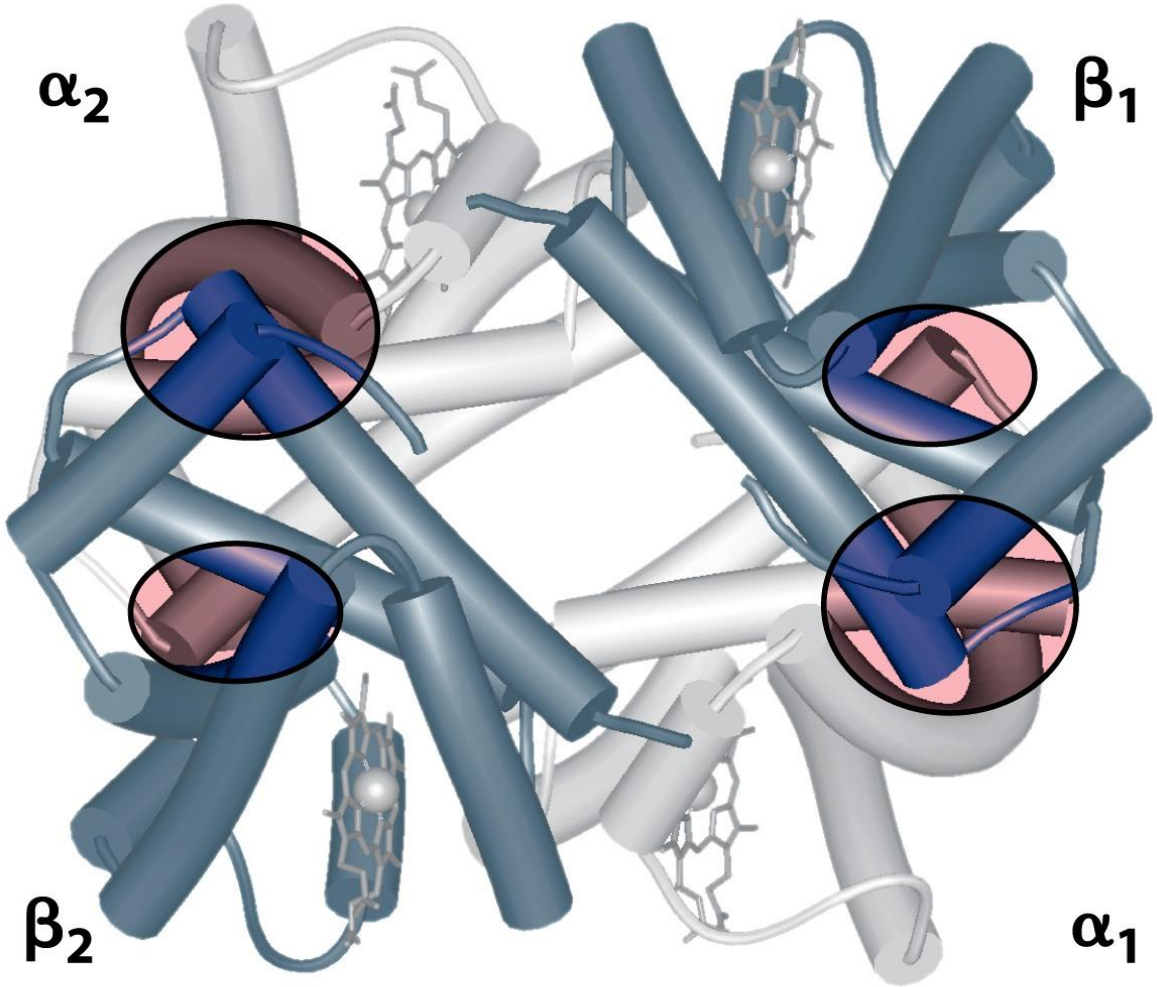
Subunit Interactions in Hemoglobin



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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 higher affinity 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

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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

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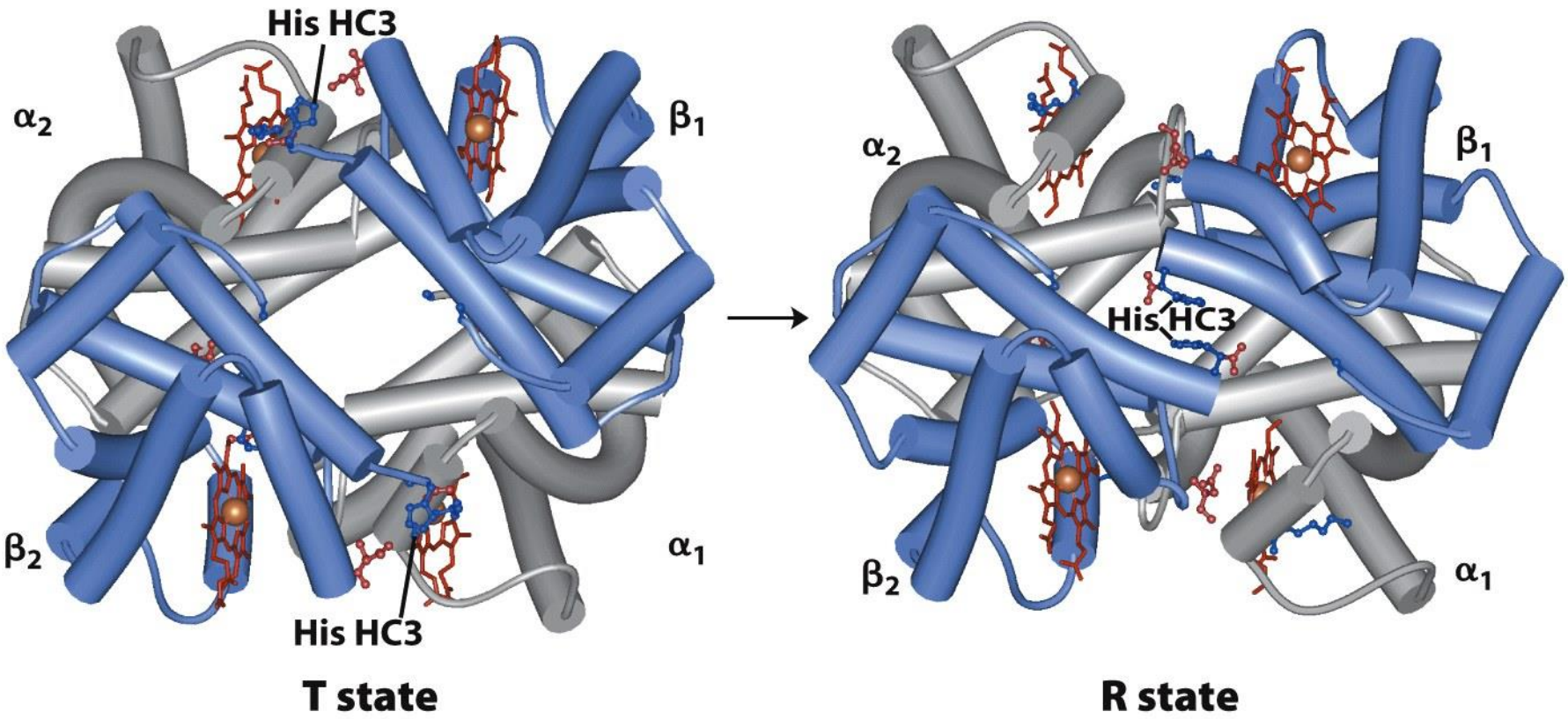
R and T States of Hemoglobin



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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 → R transition is a narrowing of the pocket between the *β* subunits.

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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

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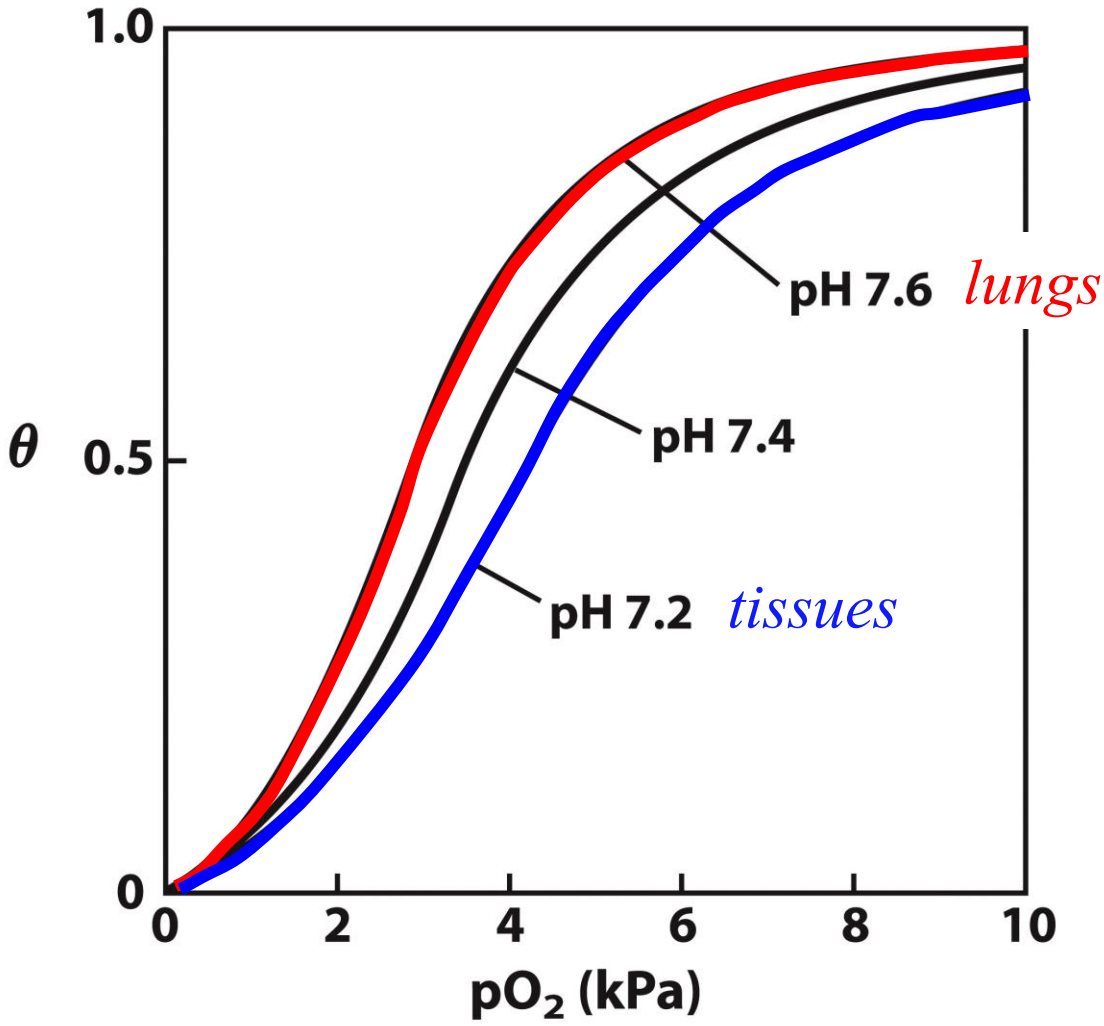
pH Effect on O_2 Binding to Hemoglobin



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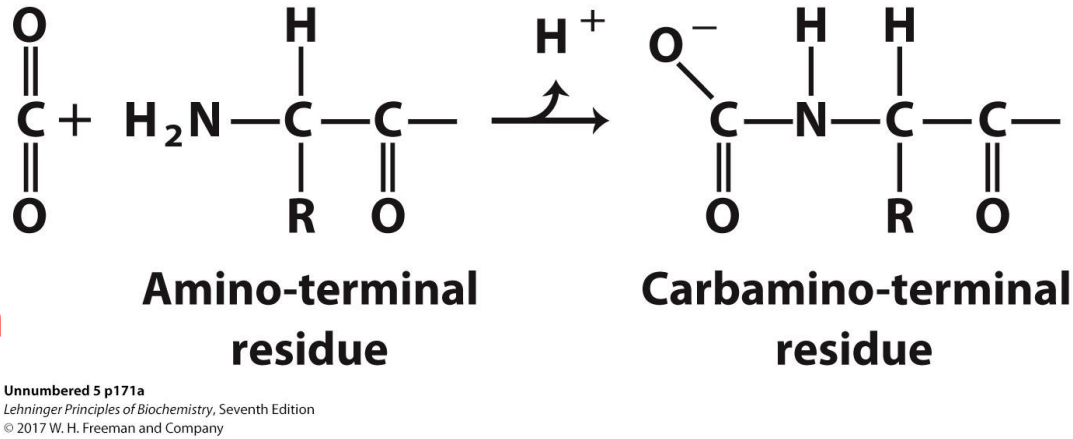
Hemoglobin and CO_2 Export

- 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.



Unnumbered 5 p171a
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- 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

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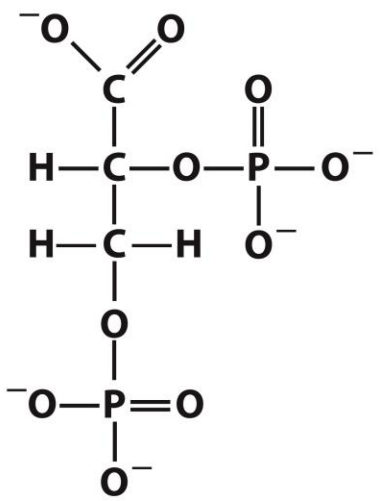
2,3-Bisphosphoglycerate regulates O_2 binding

- 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

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

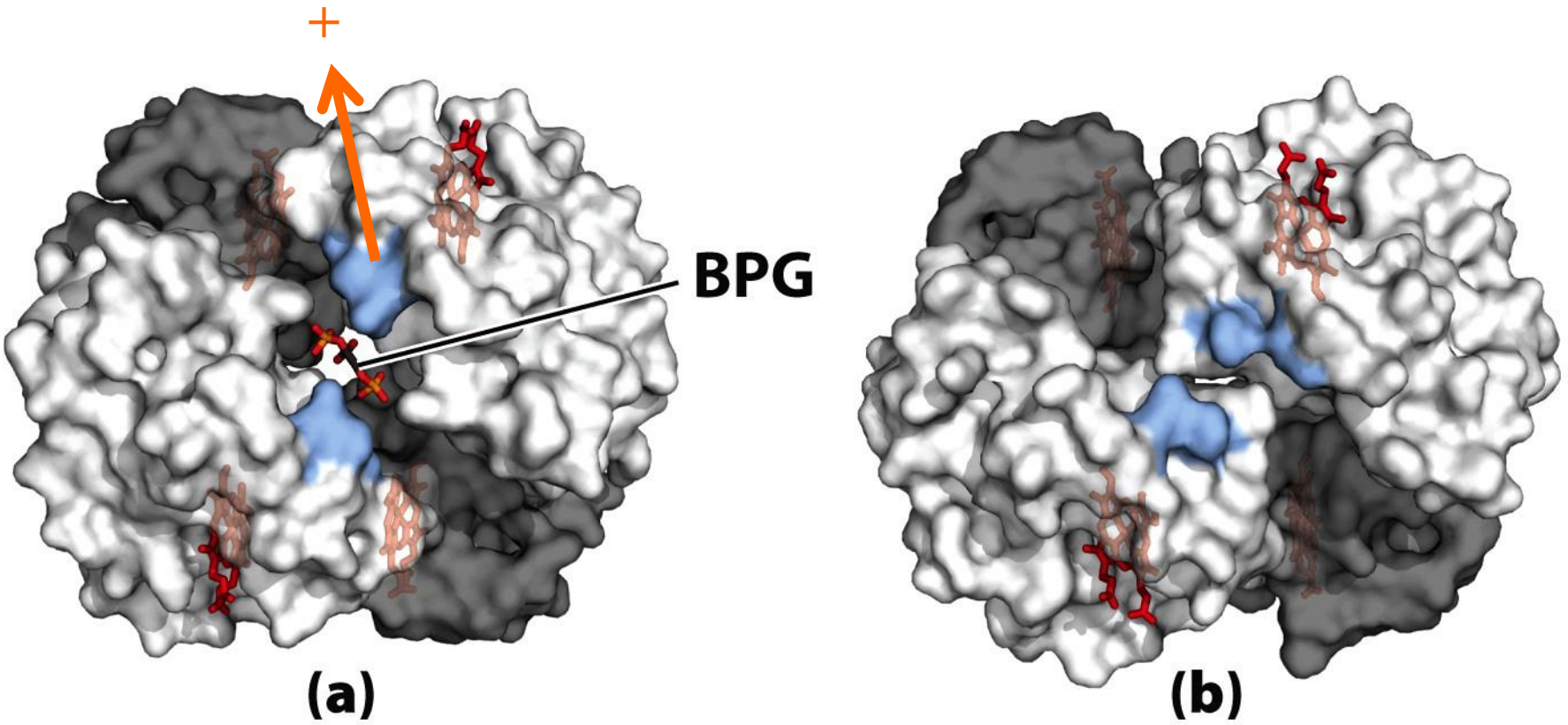

Figure 5-18
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BPG binding stabilizes the T state of deoxyhemoglobin

The binding pocket for BPG disappears on oxygenation

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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

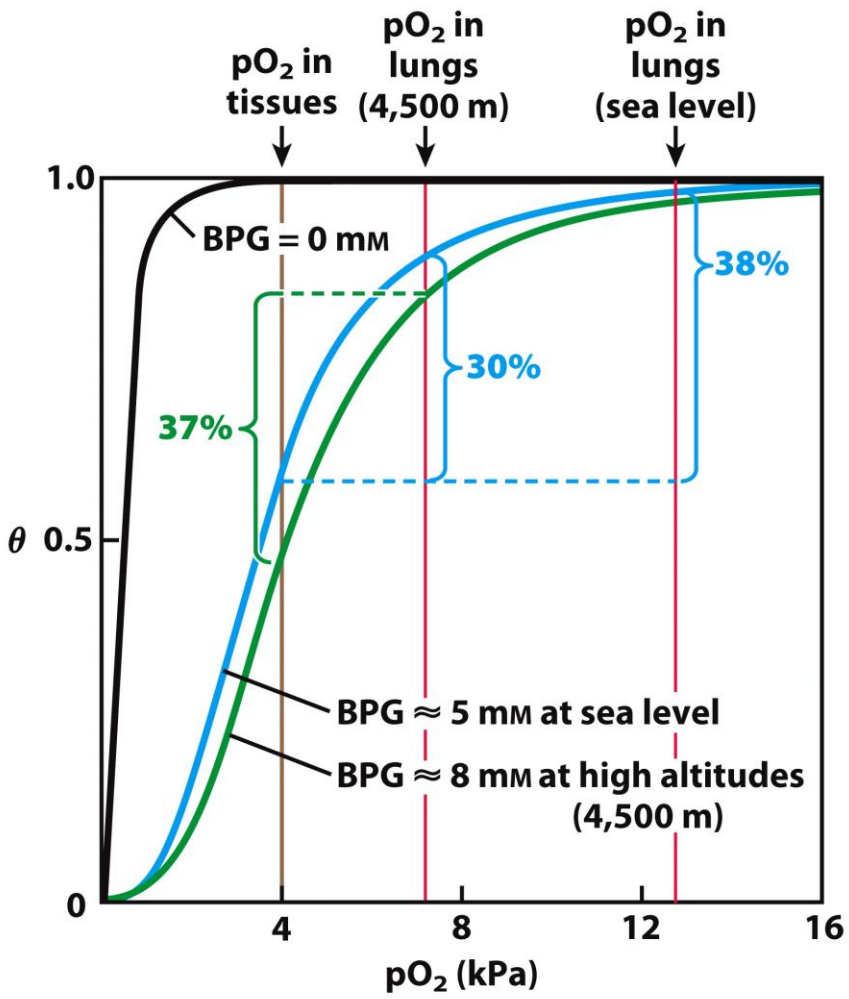

Figure 5-17
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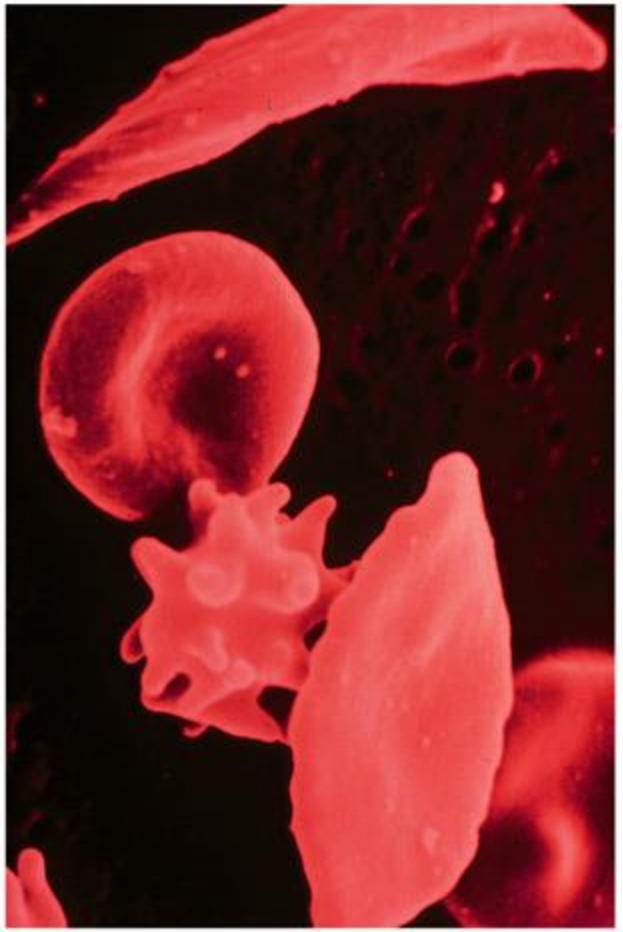
Sickle-cell anemia is due to a mutation in hemoglobin

- 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

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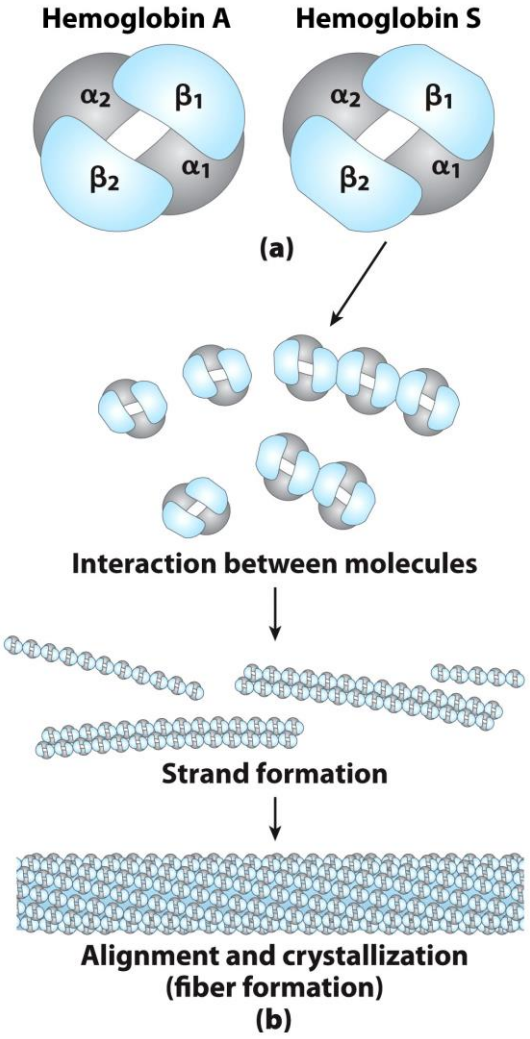
Formation of Hb Strands in Sickle-Cell Anemia



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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

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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 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**

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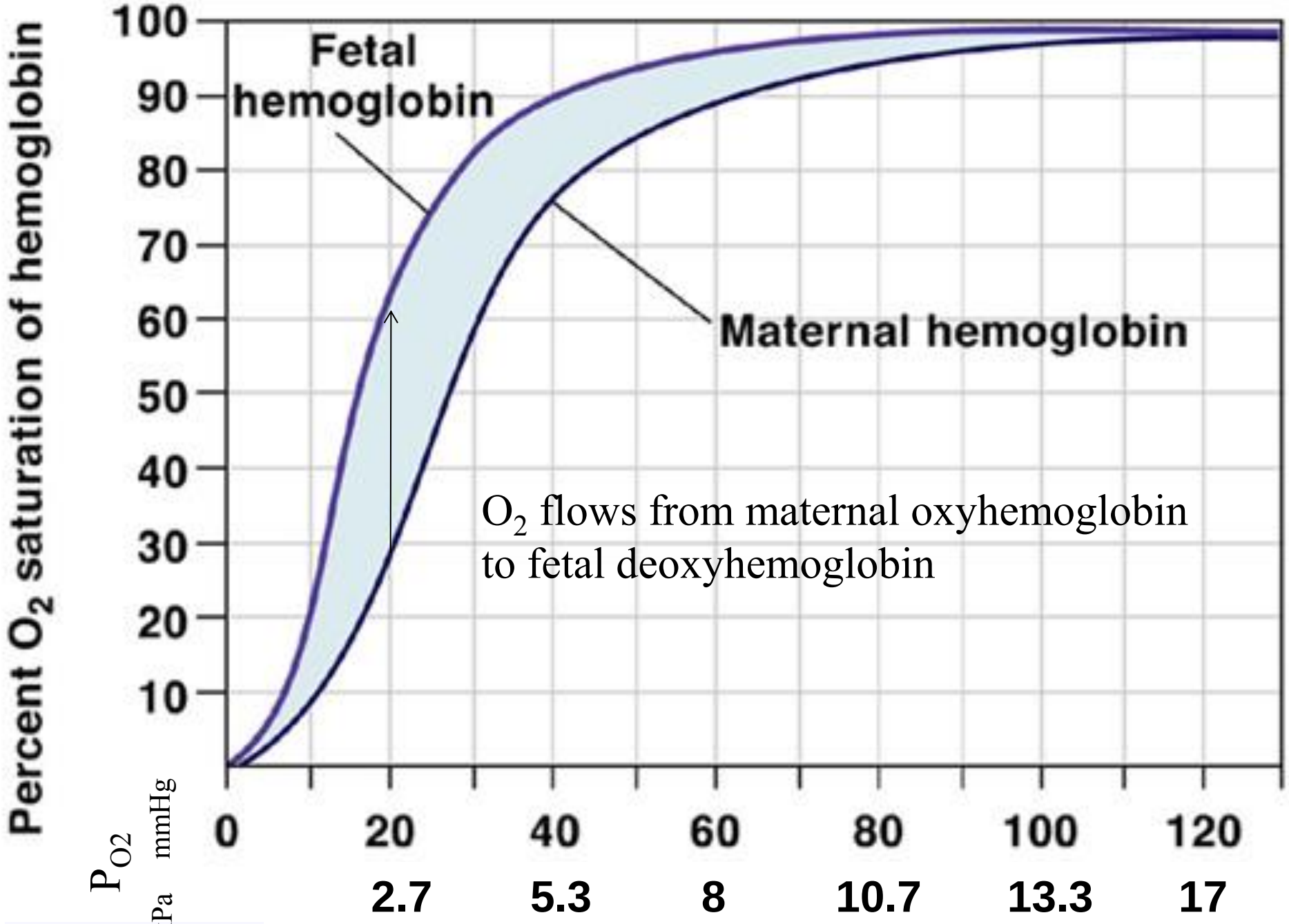

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