ENZYMES: Binding & Catalysis
A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
1. Transition State Theory
2. Catalytic & Mechanistic strategies
E. Quantifying the Catalytic Power: Kinetics
1. Review of kinetics
2. Enzyme Kinetics (M-M equation; L-B plot; inhibition)
a. M-M equation
b. Lineweaver-Burk; double reciprocal
c. Inhibition
d. Uses of Steady-state kinetics
e. Active-site identification
f. Energetics of Catalysis
F. Enzyme Mechanisms
1. Proteases
2. Serine Proteases
a. Active Site Determination
b. Proposed mechanism
c. Specificity; Chymotrypsin *versus* elastase
3. Other protease mechanisms
Enzyme Regulation
A. Introduction
B. Strategies
1. Gene Regulation
2. Covalent Modification
3. Allosteric Control
C. Covalent Modification
1. Proteolysis
2. Protein modification
a. Phosphorylation
i. Kinases
ii. Phosphatase
i. Example: PKA
D. Allosteric Control
1. Regulation nomenclature
a. Example: ATCase
2. Review; binding curves; why sigmoidal
3. Hill Equation
4. Physical models; 3° and 4° conf. changes
a. T_0 vs. R_6
b. Sequential; KNF
c. Concerted (symmetry); MWC
d. Examples of measuring these parameters

Lecture 19 (10/27/25)

- Reading: Ch5; 148-149,152-157,160-164
- Homework #19

NEXT

- Reading: Ch4; 128-136
- Homework #20

"Enzyme" Regulation: Hemoglobin

A. Roles of Hb
1. Oxygen transport
2. CO_2 binding
3. Blood buffer: Bohr effect
B. Oxygen Binding/role of protein
C. Binding curves
1. oxygen
2. Allosteric effectors (BPG)
3. Bohr effect; (protons)
4. Carbon dioxide
D. Structure-Function; Structural basis for physiology (T & R states)
E. Mechanism of Cooperativity

Protein Structure

A. Stability
1. Two-state model
2. Energetics
3. Denaturation
4. Methods to study
B. Protein Folding

Hemoglobin (Hb)

Best understood example of an allosteric protein

Evolution of oxygen transporters Hb and myoglobin (Mb):

- Serum [Oxygen] ≈ 2.3 mL/L; Blood [Oxygen] ≈ 200 mL/L
- Metabolism:

  Needs O_2 → $C_6H_{12}O_6 + 6O_2 \leftrightarrows 6CO_2 + 6H_2O$

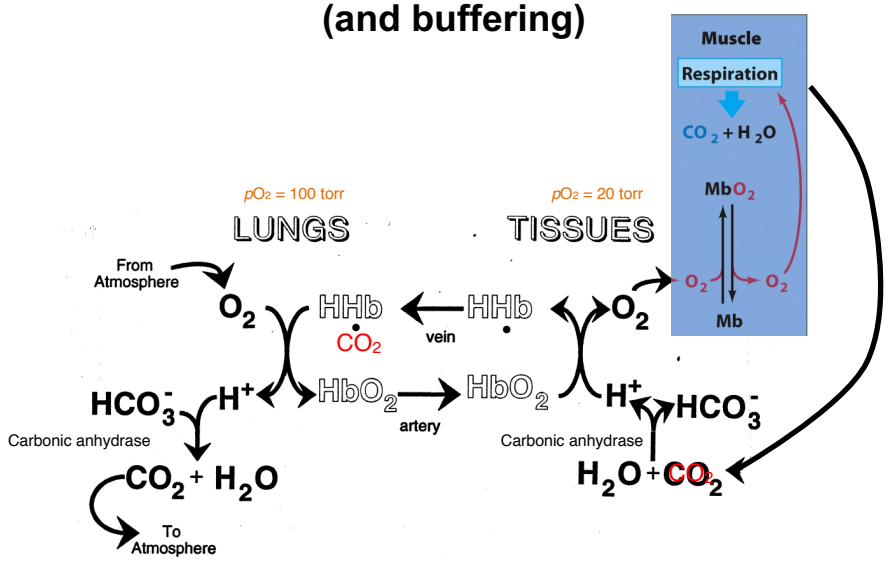
  Oxidation of sugars → metabolic acids and from $CO_2 + H_2O \leftrightarrows H_2CO_3$

Roles of Hb:

- Oxygen transport
- Proton transport-Blood buffer
- Carbon dioxide transport

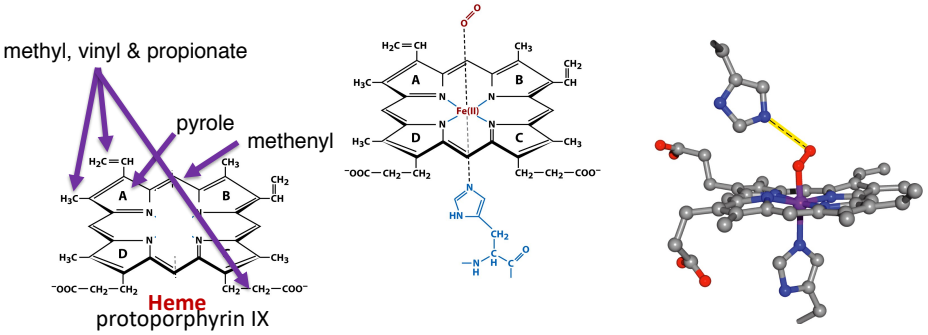
5

Hemoglobin & Myoglobin in O_2 & CO_2 Transport
(and buffering)



Oxygen transport

Heme



- Heme is protoporphyrin IX plus Fe^{2+}
- Heme prosthetic group in globins binds oxygen via Fe^{2+}
- Heme itself is not a good oxygen transporter because of oxidation to Fe^{3+}

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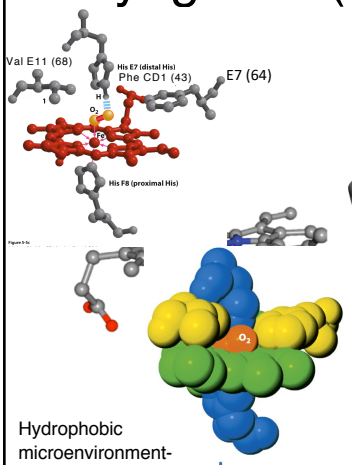
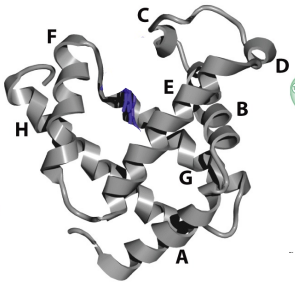
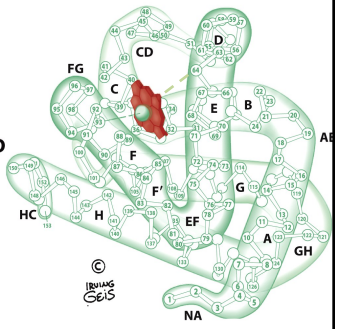
Oxygen transport

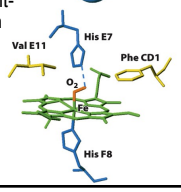
Myoglobin (Mb): O_2-binding protein



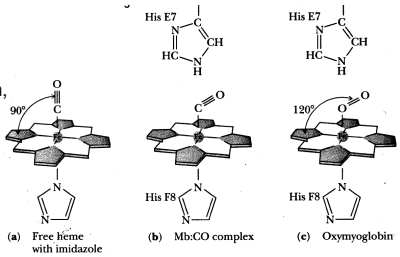




Hydrophobic microenvironment- prevent oxidation to ferric-Fe(III). Provides site for 6th ligand
Limits binding by other analogs

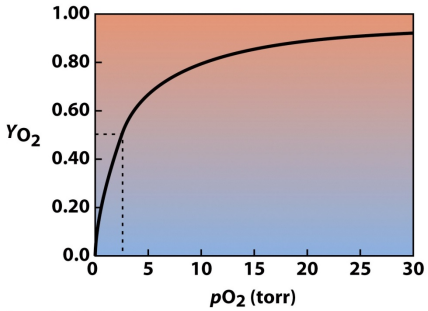


Carbon monoxide binds heme 20,000 better than dioxygen, but in Mb/Hb it only binds 200 times better.



Oxygen transport

Quantitative measure of O_2 binding



$$[O_2] \Rightarrow pO_2$$

$$K_D \Rightarrow p_{50}$$

$$M_bO_2 \underset{K_d}{\rightleftharpoons} M_b + O_2$$

$$K_d = \frac{[M_b]\,[O_2]}{[M_bO_2]} \Rightarrow [M_bO_2] = \frac{[M_b]\,[O_2]}{K_d}$$

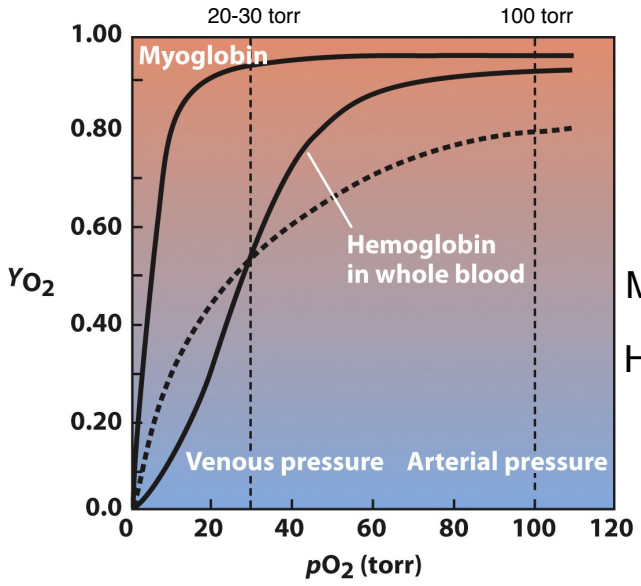
Same as :

$$Y = \frac{[L]}{K_D + [L]}$$

$$Y_{O_2} = \frac{[M_bO_2]}{[M_bO_2] + [M_b]} = \frac{\frac{[M_b]\,[O_2]}{K_d}}{\frac{[M_b]\,[O_2]}{K_d} + [M_b]} = \frac{[O_2]}{K_d + [O_2]}$$

Oxygen transport

Oxygen Binding Curve of Hemoglobin



Mb → K_d= 3-4 torr

Hb → K_d= 26 torr

Cooperativity: Hill coefficient

$$H_b + n\ O_2 \rightleftharpoons_{K_d} H_b(O_2)_n$$

- Positive cooperativity: n > 1
- Negative cooperativity: n < 1
- Non-cooperative: n = 1

- Theoretical maximum cooperativity = # of binding sites

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Cooperativity: Hill coefficient

$$\mathbf{H_b} + \mathbf{n\ O_2} \underset{K_d}{\rightleftharpoons} \mathbf{H_b(O_2)_n}$$

$$K_d = \frac{[\mathrm{H_b}]\,[\mathrm{O_2}]^n}{[\mathbf{H_b(O_2)_n}]} \qquad Y_{O2} = \frac{(p\mathrm{O_2})^n}{(p_{50}) + (p\mathrm{O_2})^n}$$

- Positive cooperativity: n > 1
- Negative cooperativity: n < 1
- Non-cooperative: n = 1

- Theoretical maximum cooperativity = # of binding sites

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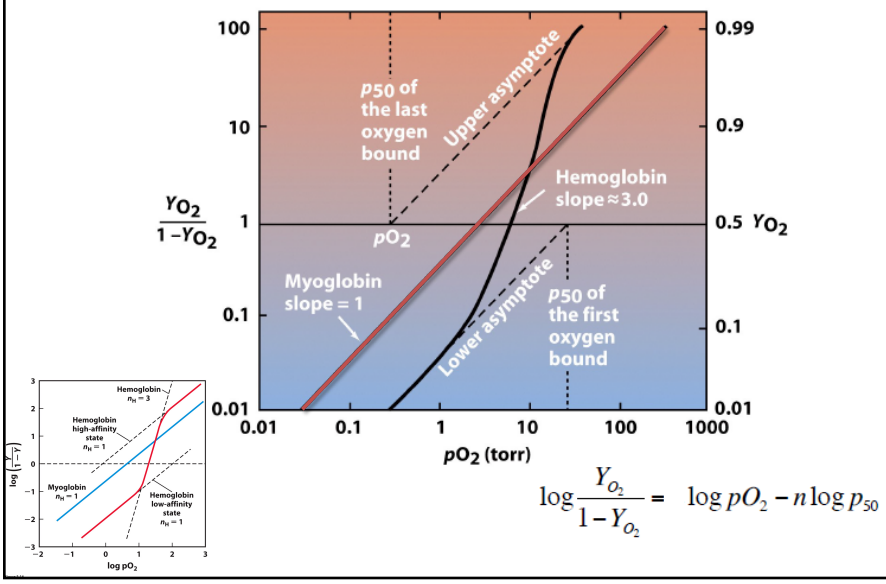
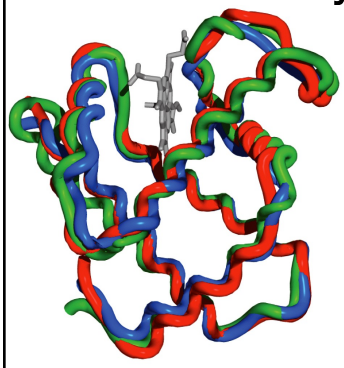
Hill Plot for Mb & Hb

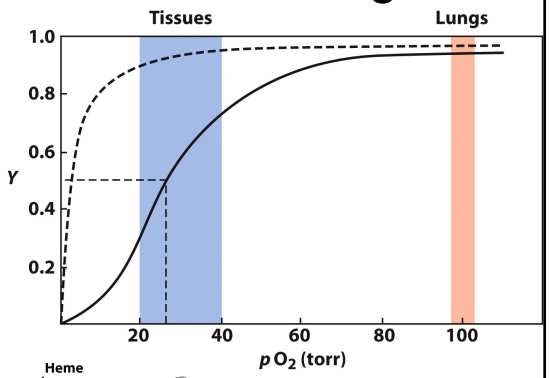


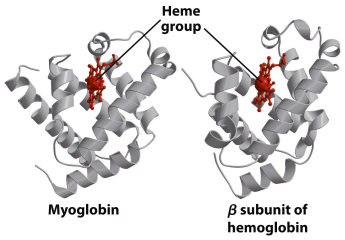
$$\log\frac{Y_{O_2}}{1-Y_{O_2}} = \log pO_2 - n\log p_{50}$$

Mb and Hb-subunits: structural similarity, differential binding



- Mb, Hb-α, Hb-β overlay





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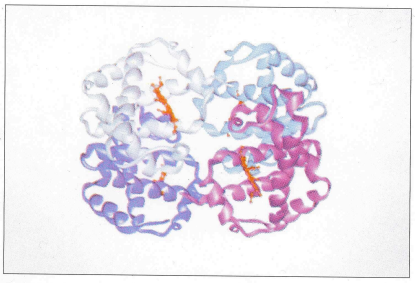
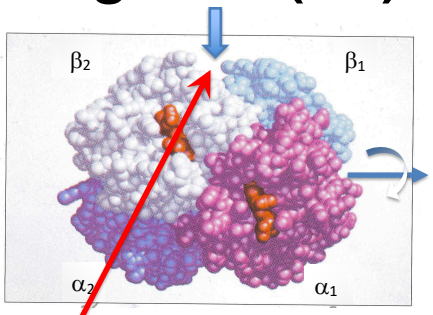
Structure of Hemoglobin (Hb)

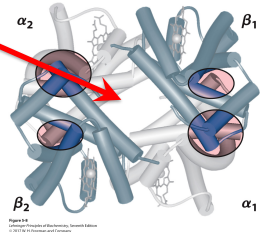




Tetramer has 2α and 2β subunits
But, the α & β have more affinity for each other than either αα or ββ
Therefore, Hb is best described as a dimer of αβ dimers = $(\alpha\beta)_2$

Notice nice pocket at β–β interface

This view is looking down from the top



Structure of Hemoglobin (Hb)

Changes during binding!

De-oxy Hemoglobin **Oxy Hemoglobin**

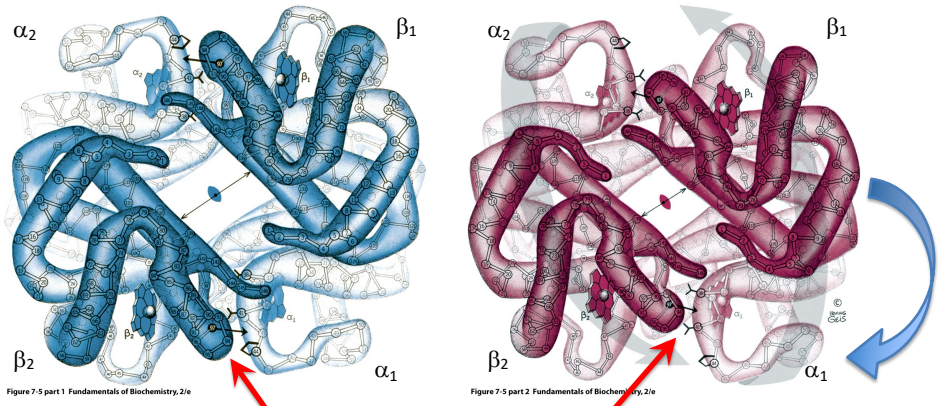


Figure 7-5 part 1 Fundamentals of Biochemistry, 2/e

Figure 7-5 part 2 Fundamentals of Biochemistry, 2/e

Notice residue 97 (His)

Lets look at this conformational change from the top of one αβ dimer

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Structure of Hemoglobin (Hb)

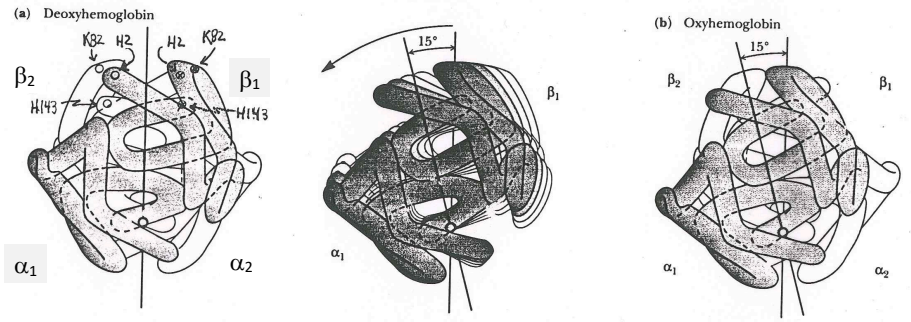

Figure **12.17** Subunit motion in hemoglobin when the molecule goes from the (a) deoxy to the (b) oxy form.

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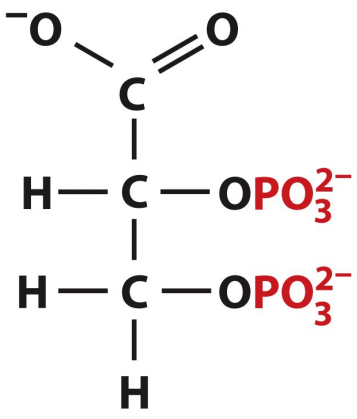
What binds to Hb in addition to O_2?

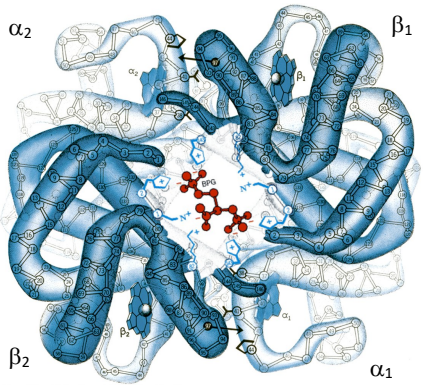
1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

Structure of BPG

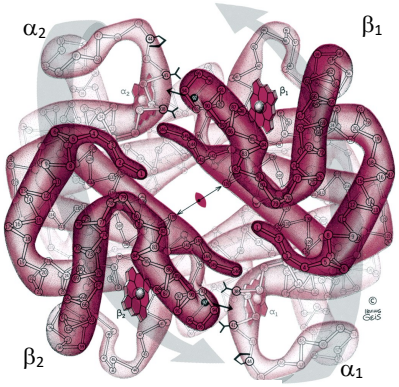


D-2,3-Bisphosphoglycerate (BPG)

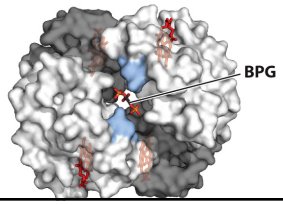
BPG Binds to Deoxyhemoglobin

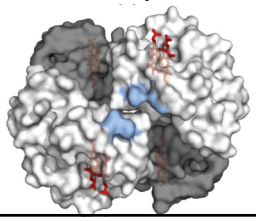


Binds to deoxy-Hb



Does not bind to oxy-Hb





21

BPG Binds to Deoxyhemoglobin

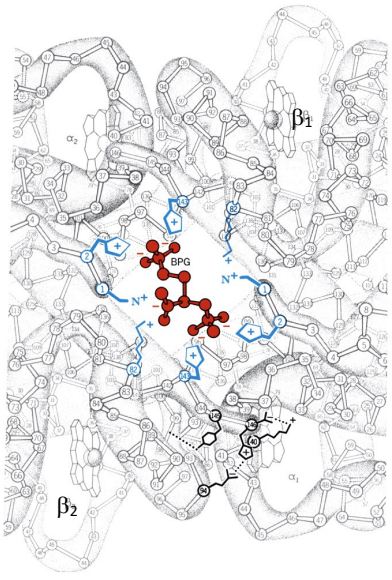
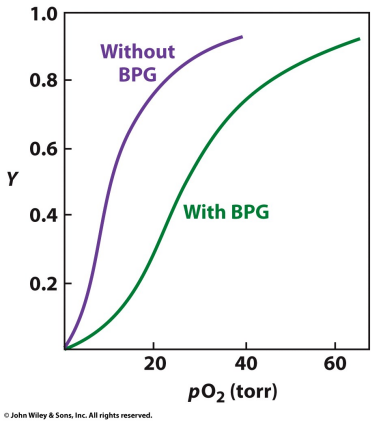


Figure 7-14
Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.



[BPG] is 5 mM in blood cells

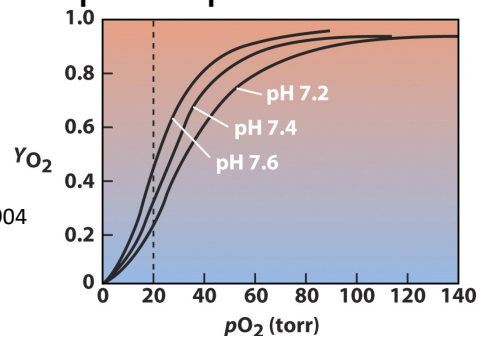
What binds to Hb in addition to O_2?

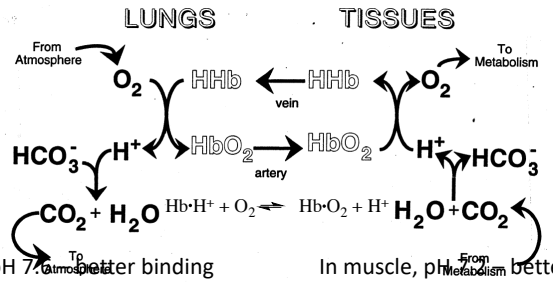
1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

Proton transport-Blood buffer

Bohr Effect: pH dependence of O_2 binding

Christian Bohr-1904





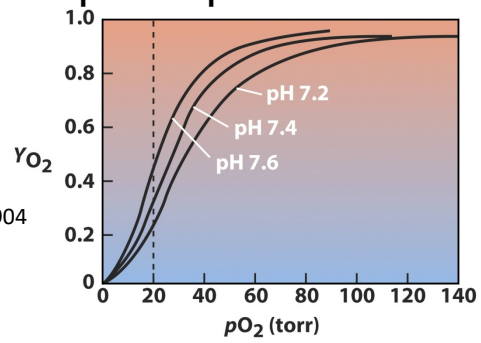
In lungs, pH 7.4 better binding

In muscle, pH 7.2 better release

Proton transport-Blood buffer

Bohr Effect: pH dependence of O_2 binding

Christian Bohr-1904



$$Hb{\cdot}H^+ + O_2 \rightleftharpoons Hb{\cdot}O_2 + H^+$$

In lungs, pH 7.6 – better binding

In muscle, pH 7.2 – better release

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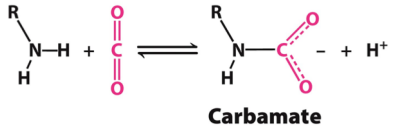
What binds to Hb in addition to O_2?

1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

Carbon Dioxide binds via Carbamate formation



Carbamate

Carbon dioxide binds better to the form of Hb that is not bound to oxygen; deoxy-Hb
Therefore, oxygen binding releases CO_2,
and CO_2 binding releases oxygen

$$Hb{\cdot}CO_2^- + O_2 \rightleftharpoons Hb{\cdot}O_2 + CO_2$$

Consequences of carbamate:

- charge change
- contributes to acidity: when CO_2 increases, carbamate formation increases, which is conducive to the Bohr effect (oxygen released at low pH)
- The negative charge interacts with the other subunit; tighter structure

CO_2 Binds to Deoxyhemoglobin

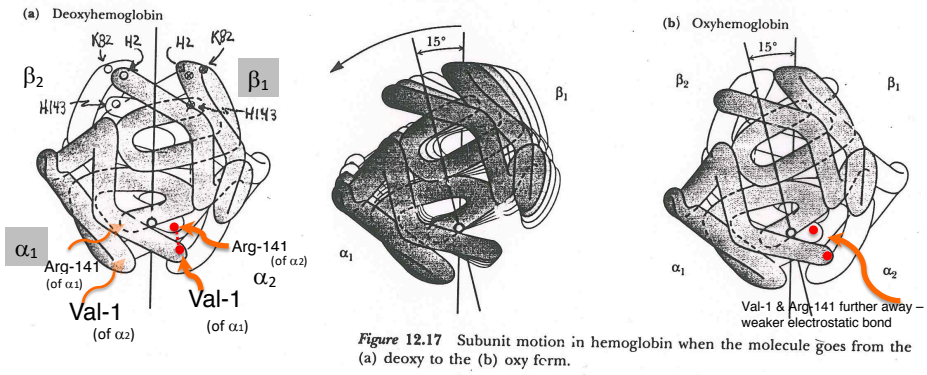


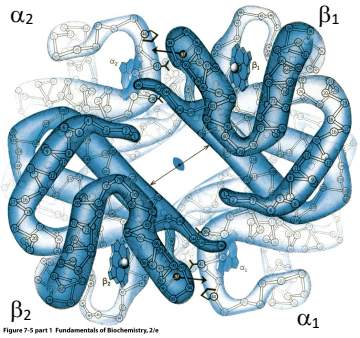
Figure 12.17 Subunit motion in hemoglobin when the molecule goes from the (a) deoxy to the (b) oxy form.

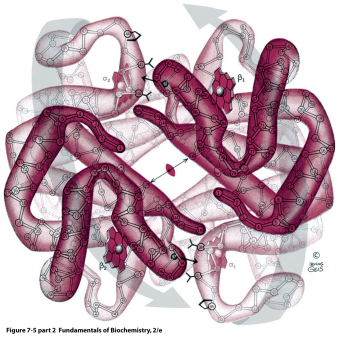
What binds to Hb in addition to O_2?

1. **D-2,3-Bisphosphoglycerate (BPG)**
2. **Protons**
3. **Carbon Dioxide**

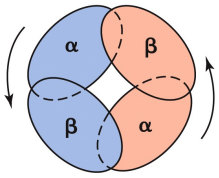
So, BPG, protons, and CO_2 bind specifically to deoxy-Hb.
Do these stabilize a possible T-state? Lets look at these states more closely….

T-state and R-state of Hemoglobin



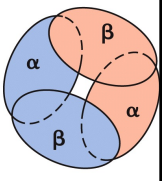


- T state
- Less active



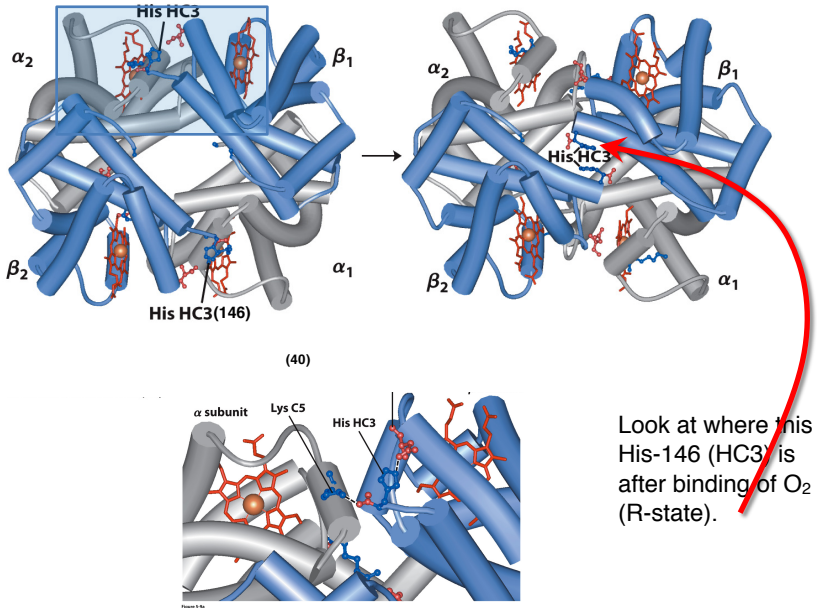
Deoxy

- R state
- More active



Oxy

R and T States of Hemoglobin



Look at where this His-146 (HC3) is after binding of O_2 (R-state).

Effects of BPG & CO_2 on Hb's O_2 Dissociation Curve

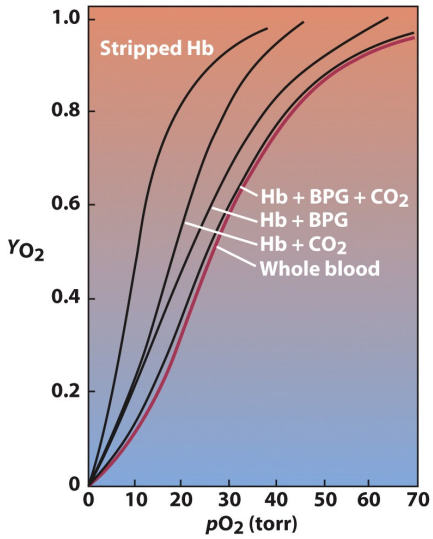


Figure 7-13

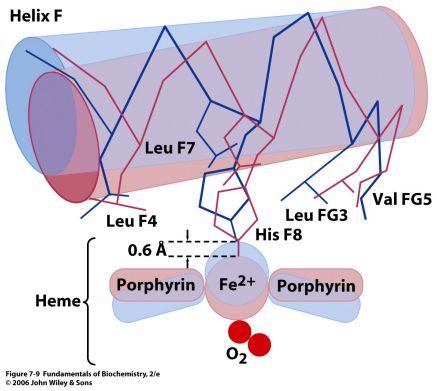
O_2 binds to Hb

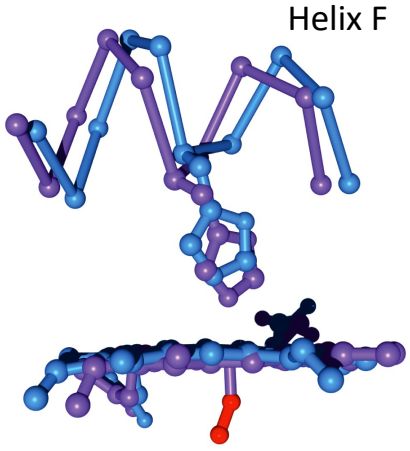
1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

How are all these related to the cooperative binding mechanism ?

Lets look at binding of oxygen to the T-state, which its most likely to encounter (L=300,000).

Structural Basis of Cooperativity





Deoxy — High-spin (paramagnetic)(larger) — Deoxy
Oxygen bound — Oxygen bound

Low-spin (diamagnetic)(smaller with Fe-N bonds 0.1 Å shorter)

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Conformational Change Is Triggered by Oxygen Binding

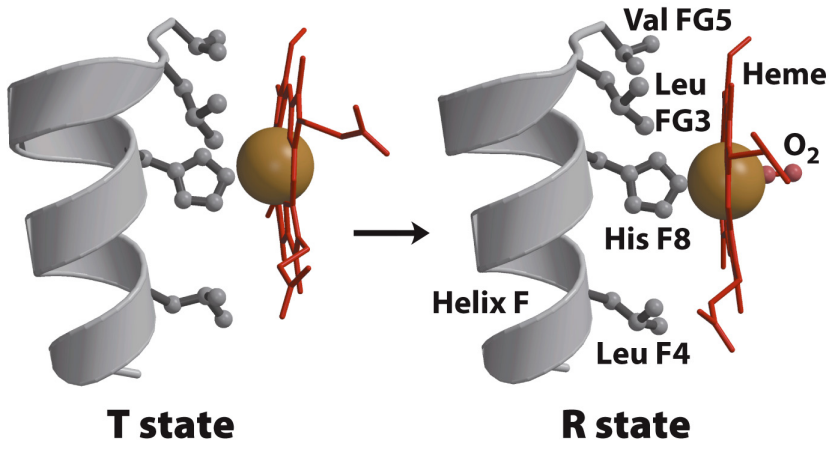

Figure 5-11
Lehninger Principles of Biochemistry, Seventh Edition
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Structural Basis of Cooperativity

What causes the conformational change?

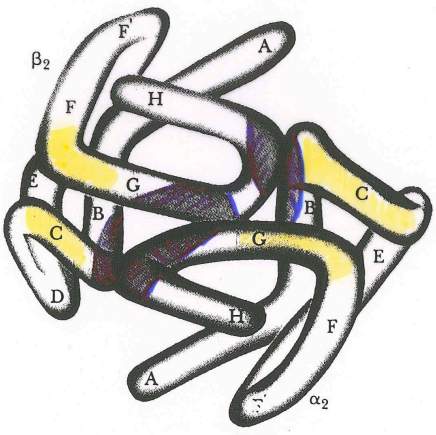

Figure **12.16** Side view of one of the two $\alpha\beta$ dimers in Hb, with packing contacts indicated in blue. The sliding contacts made with the other dimer are shown in yellow. The changes in these sliding contacts are shown in Figure 12.17.

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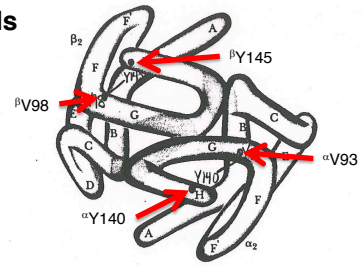
Structural Basis of Cooperativity

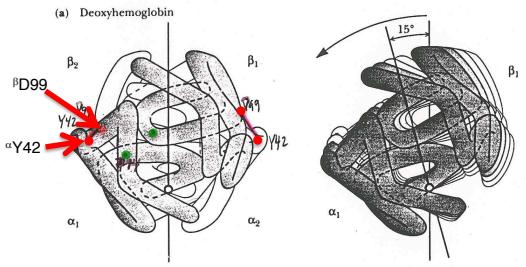
Changes in H-bonds when an oxygen binds to a single subunit



Changes within each subunit

The change at the F-helix changes the C-term stability within each subunit

Changes between dimers (α1 and β2)



The change at the F-helix changes the C-term stability between subunits

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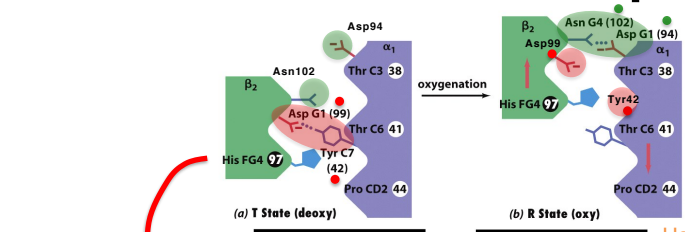
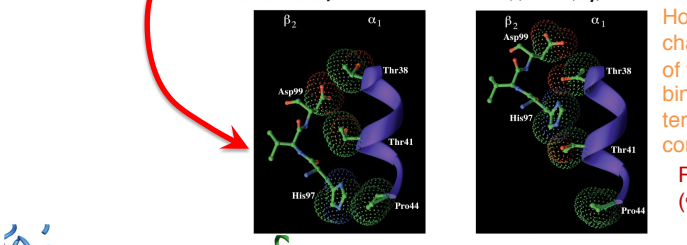
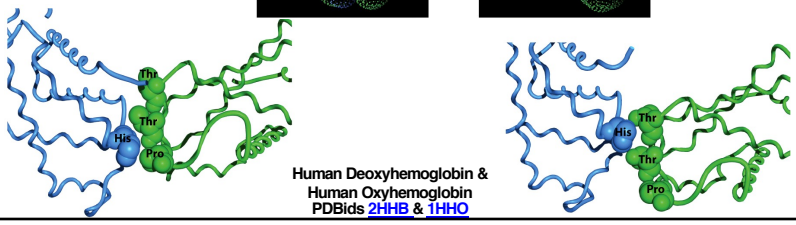
Structural Basis of Cooperativity





How do these slight changes due to movement of the F-helix upon O_2 binding destabilize the C-termini and lead to conformational change?

Recall H-bonds broken (αY140 & βY145)



Human Deoxyhemoglobin & Human Oxyhemoglobin PDBids 2HHB & 1HHO

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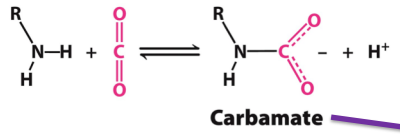
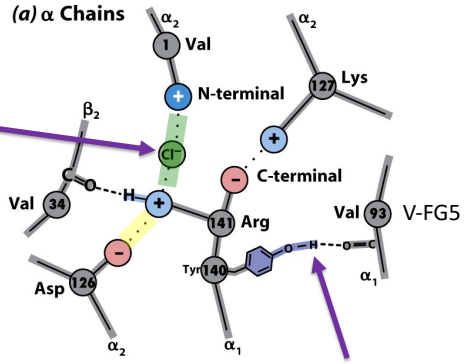
Carbamate formation favors the T-state

Changes at C-term of α_1

Recall H-bonds broken ($^{\alpha}$Y140)

$$Hb{\cdot}CO_2^- + O_2 \rightleftharpoons Hb{\cdot}O_2 + CO_2$$





Changes between dimers (Salt-bridge interactions at C-term of α_1)

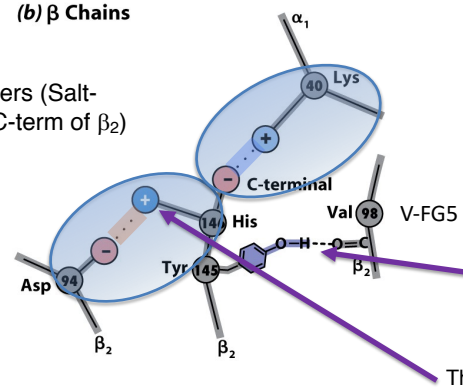
Recall, this is the first thing we saw destabilized

Protonation favors the T state

Changes at C-term of β_2

$$Hb{\cdot}H^+ + O_2 \rightleftharpoons Hb{\cdot}O_2 + H^+$$

Recall H-bonds broken ($^{\beta}$Y145)



Changes between dimers (Salt-bridge interactions at C-term of β_2)

Recall, this is the first thing we saw destabilized

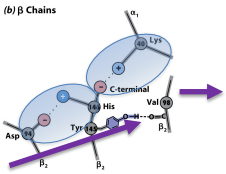
The Bohr effect: when these interactions are lost, the proton is released (pK_a drops)

But, what actually causes the dimers to rotate the 15°?

Oxygen-Binding affects Bonds to C-terminus

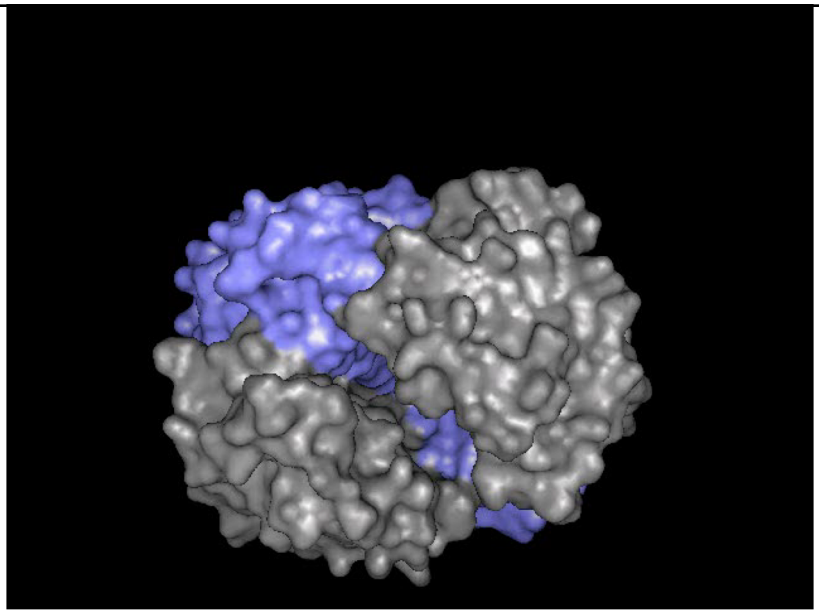
- Hemoglobin Dynamics at C-term of beta-subunit

See:
- O_2 binds
- The salt-bridge between His-146 and Asp-94 on the same β-subunit breaks
- The salt-bridge between the C-term carboxylate of β-subunit loses contact with Lys-40 of α-subunit
- "Anchor" is lost and subunits move



DON'T See:
- Fe moving into plane of heme when O_2 binds
- Helix F and FG loop moving when His-91 (F8) on helix-F moves
- H-bond with Tyr-145 on and Val-98 (on FG loop) on β-subunit breaking
- NONE of the comparable changes at the C-term of the α-subunit, due to binding the β-subunit
- E.g., the H-bond between the Asp-99 of β-subunit and Tyr-42 of α-subunit breaking

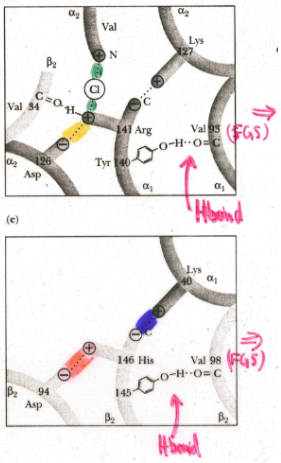
- The T- and R- states of Hb



β in gray
α in blue

- O_2 binds
- The salt-bridge between His-146 and Asp-94 on the same β-subunit breaks
- The salt-bridge between the C-term carboxylate of β-subunit loses contact with Lys-40 of α-subunit
- "Anchor" is lost and subunits move

Summary of changes in Hb T-state to R-state



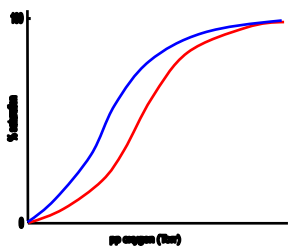
Bond	Subunit	Identity (residue)	Broken / Made.
H-bond	$\alpha_1\alpha_1$ $\alpha_2\alpha_2$	FG5 (Val 93) — HC2 (Y140)	broken
H-bond	$\beta\beta$ $\beta_2\beta_2$	FG5 (Val 98) — HC2 (Y145)	broken
H-bond	$\alpha_1\beta_2$	$C7_\alpha$ (Y42) — $G1_\beta$ (D99)	broken
H-bond	$\alpha_1\beta_2$	$G1_\alpha$ (D94) — $G4_\beta$ (N102)	made
Salt bridge	$\alpha_1\alpha_2$	HC3 (R141) — NA1 (V1) (Cl^- or carbamate)	broken
Salt bridge	$\alpha_1\alpha_2$	HC3 (R141) — H9 (D126)	broken
Salt bridge	$\beta_1\beta_2$ $\beta_2\beta_2$	HC3 (H146) — FG1 (D94)	broken
Salt bridge	$\beta_2\alpha_1$	HC3 (H146) — C5 (K40)	broken

Fetal Hemoglobins

Hb is always a tetramer of α-type and β-type subunits $(\alpha\text{-}\beta)_2$

Hb name	Symbol	4° structure	AA (α-type/β-type)	Chromosome (α-type/β-type)
Major adult	HbA	$(\alpha\text{-}\beta)_2$	141/146	16/11
Minor adult	HbA_2	$(\alpha\text{-}\delta)_2$	141/146	16/11
Fetal	HbF	$(\alpha\text{-}\gamma)_2$	141/146	16/11
Embryonic	HbG	$(\alpha\text{-}\varepsilon)_2$ & $(\zeta\text{-}\varepsilon)_2$	141/146	16/11

The γ-subunit of HbF does not bind BPG as well as the β-subunit



This shifts the T←→R equilibrium to the right.

This shifts the oxygen binding curve to the left such that at all pO_2, HbF binds oxygen better than HbA

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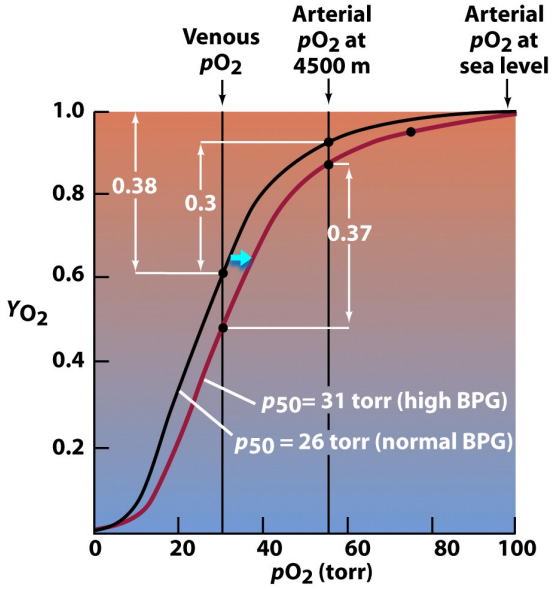
High altitude adaptation

[BPG] goes up to 8 mM

This shifts the T←→R equilibrium to the left.

This shifts the oxygen binding curve to the right such that at all pO_2, Hb binds oxygen worse and compensates for the lower pO_2 at high altitudes.



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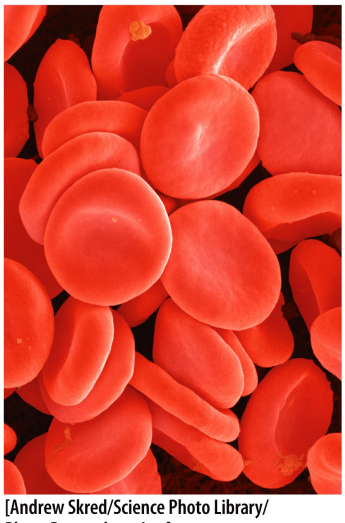
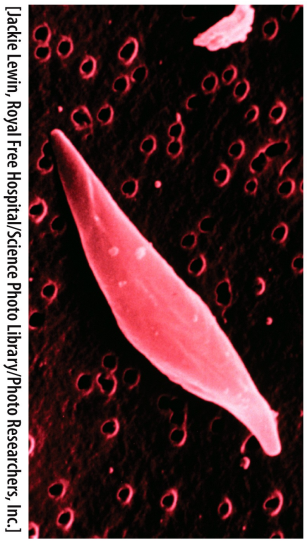
Abnormal Hemoglobins



[Andrew Skred/Science Photo Library/ Photo Researchers, Inc.]

Sickle-Cell Anemia



[Jackie Lewin, Royal Free Hospital/Science Photo Library/Photo Researchers, Inc.]

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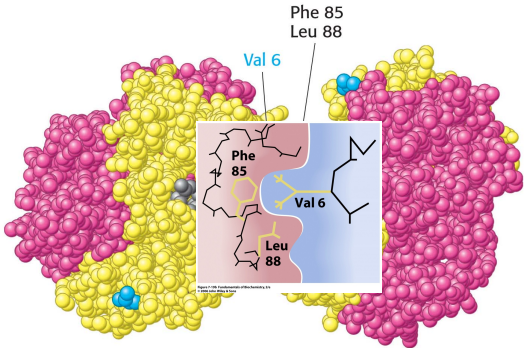
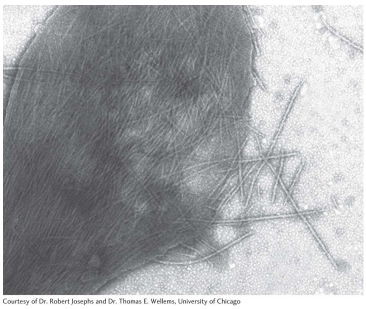
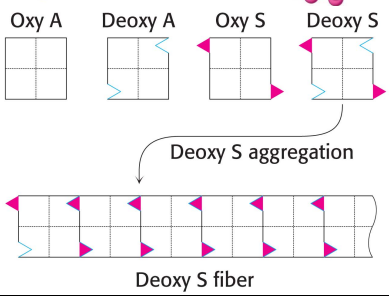
Hemoglobin S variant

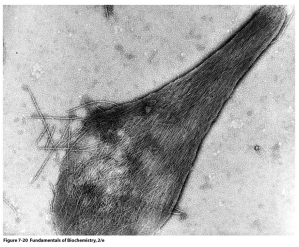




Courtesy of Dr. Robert Josephs and Dr. Thomas E. Wellems, University of Chicago

Sickle-cell hemoglobin fibers





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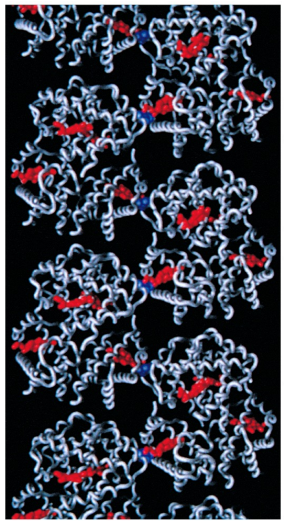
Formation of Hb Strands in Sickle-Cell Anemia

Structure of Deoxyhemoglobin S Fiber



[Courtesy W. Royer and D.Harrington, J. Mol. Biol222, 398-407 (1992)]

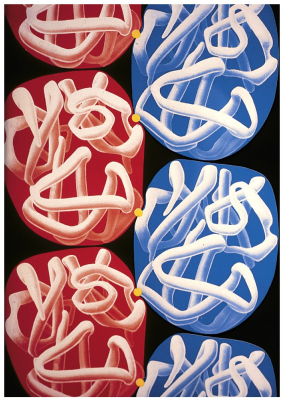


Figure 7-18a
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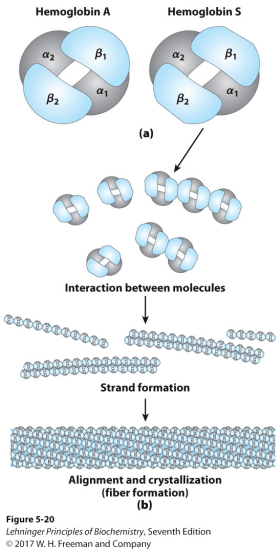

Figure 5-20
Lehninger Principles of Biochemistry, Seventh Edition
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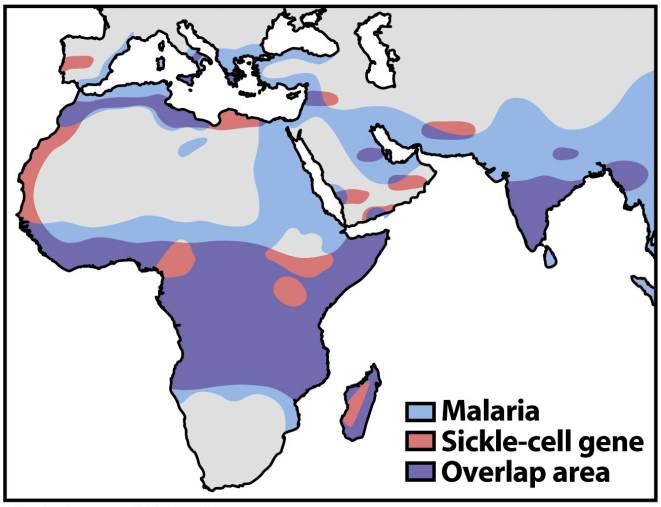
Why does hemoglobin S persist in the population?



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