Lecture 19 (10/28/24)

## "Enzyme" Regulation: Hemoglobin

1. Roles of Hb
   a. Oxygen transport
   b. $CO_2$ binding
   c. Blood buffer: Bohr effect
2. Oxygen Binding/role of protein
3. Binding
   a. Oxygen; curves
   b. Allosteric effectors
      i. BPG
      ii. Bohr effect; (protons)
      iii. Carbon dioxide
4. Structure-Function; Structural basis for physiology (T & R states)
5. Mechanism of Cooperativity
6. Isoforms during development
7. Adaptations for high altitude
8. Molecular disease: sickle-cell anemia

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

**NEXT**
- Reading: Ch4; 128-136
- Homework #20
- Quizzes 7-10

# Hemoglobin (Hb)

Best understood example of an allosteric protein

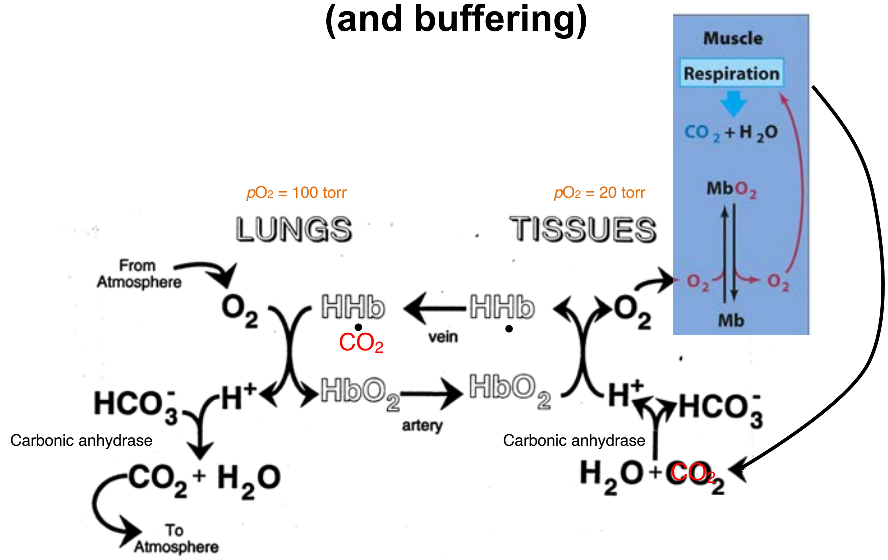
Evolution of oxygen transporters Hb and myoglobin (Mb):
- Serum [Oxygen] ≈ 2.3 mL/L; Blood [Oxygen] ≈ 200 mL/L
- Metabolism:
  Needs $O_2$ → $C_6H_{12}O_6 + 6O_2 \leftrightarrows 6CO_2 + 6H_2O$
  Oxidation of sugars → metabolic acids, and from $CO_2 + H_2O \leftrightarrows H_2CO_3$

Roles of Hb:
- Oxygen transport
- Proton transport-Blood buffer
- Carbon dioxide transport

4

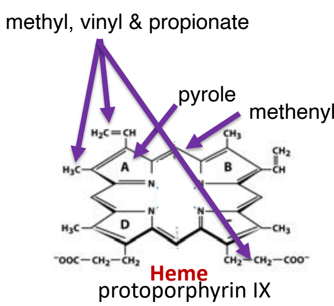
# Hemoglobin & Myoglobin in $O_2$ & $CO_2$ Transport

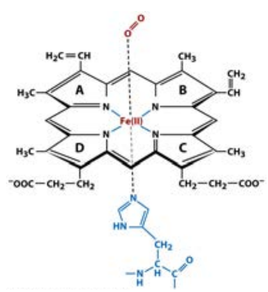
**(and buffering)**

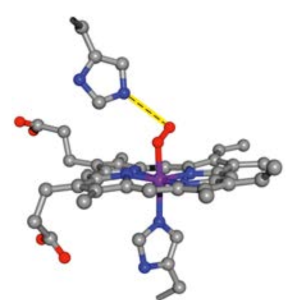



**Oxygen transport**

# Heme



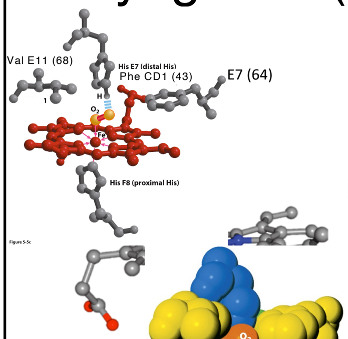


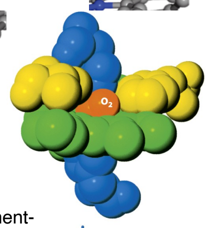
- Heme is protoporphyrin IX plus $Fe^{2+}$
- Heme is a prosthetic group in globins; it binds oxygen via $Fe^{2+}$
- Heme itself is not a good oxygen transporter because of oxidation to $Fe^{3+}$

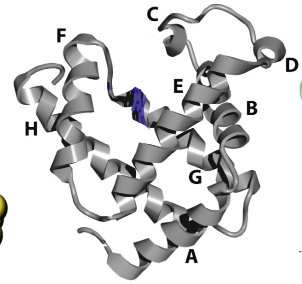
7

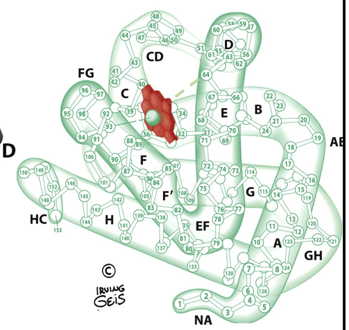
Oxygen transport

# Myoglobin (Mb): $O_2$-binding protein



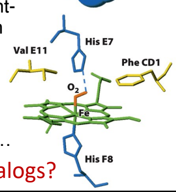



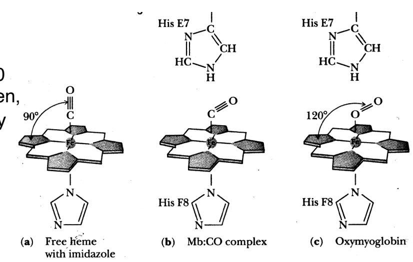

Hydrophobic microenvironment- prevent oxidation to ferric-Fe(III). Provides site for 6[th] ligand

Limits binding by other analogs……



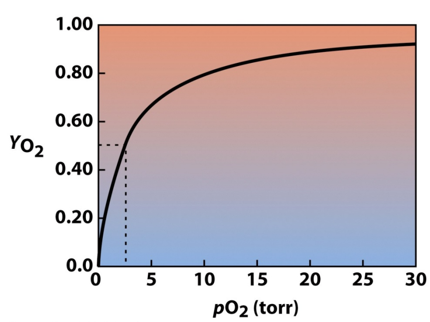

What other analogs?

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




$$[O_2] \Rightarrow pO_2$$

$$K_D \Rightarrow p_{50}$$

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

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

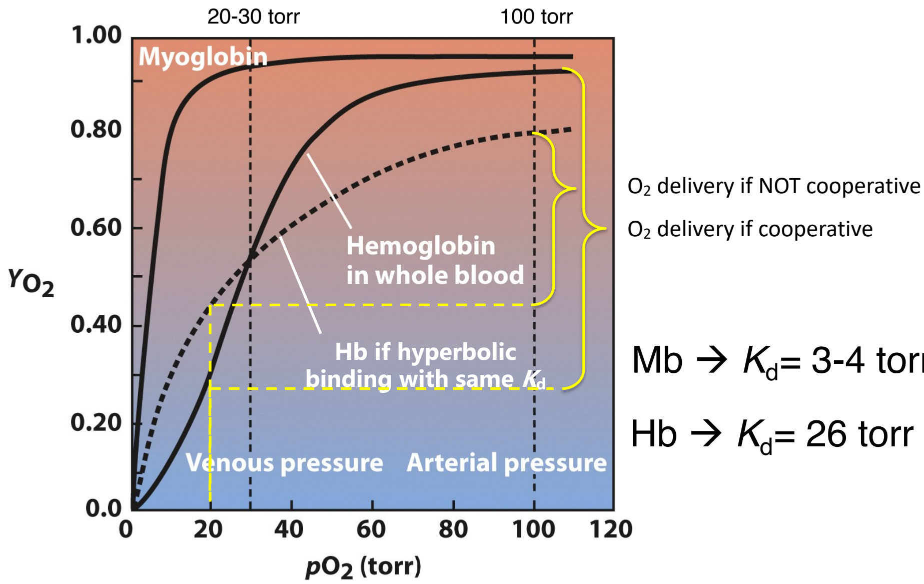
Same as :

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

$$Y_{O2} = \frac{[M_bO_2]}{[M_bO_2] + [M_b]} = \frac{\frac{[M_b][O_2]}{K_d}}{\frac{[M_b][O_2]}{K_d} + [M_b]} = \frac{[O_2]}{K_d + [O_2]}$$

Oxygen transport

## Oxygen Binding Curve of Hemoglobin




Mb → $K_d$= 3-4 torr

Hb → $K_d$= 26 torr

## Cooperativity: Hill coefficient

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

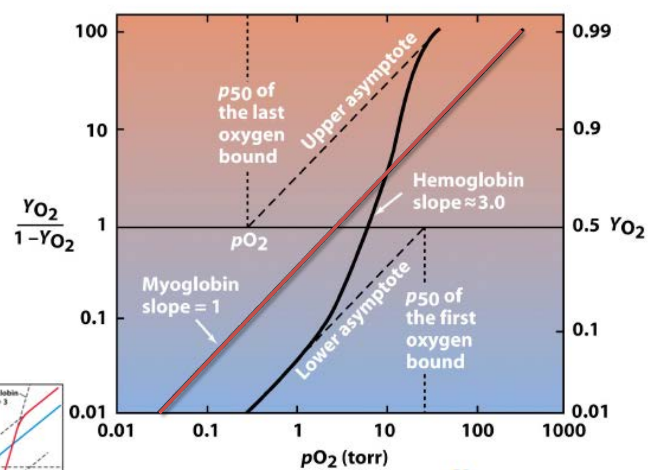
$$K_d = \frac{[H_b]\,[O_2]^n}{[\mathbf{H_b(O_2)_n}]} \qquad Y_{O_2} = \frac{(pO_2)^n}{(p_{50}) + (pO_2)^n}$$

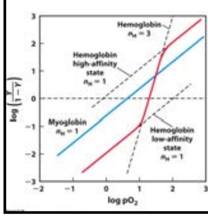
- Positive cooperativity: n > 1
- Negative cooperativity: n < 1
- Non-cooperative: n = 1

- Theoretical maximum cooperativity = # of binding sites

12

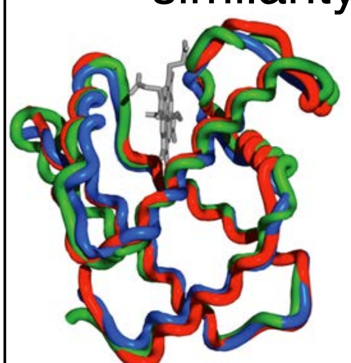
## Hill Plot for Mb & Hb

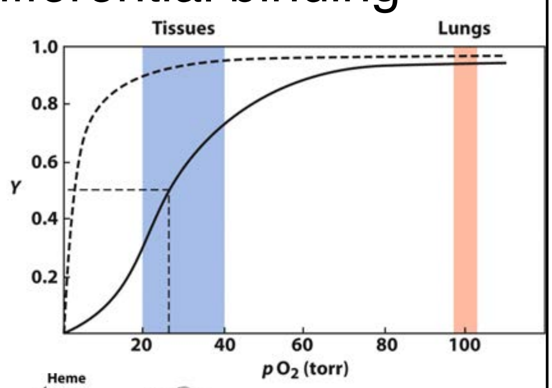


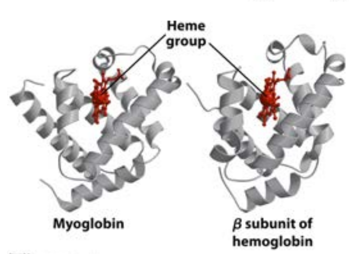
$$\log \frac{Y_{O_2}}{1-Y_{O_2}} = \log pO_2 - n \log p_{50}$$

## Mb and Hb-subunits: structural similarity, differential binding



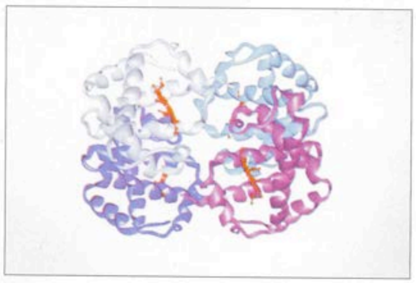


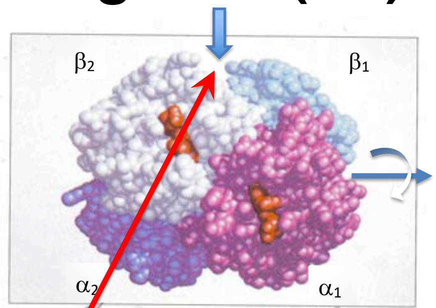

- Mb, Hb-α, Hb-β overlay




14

# Structure of Hemoglobin (Hb)

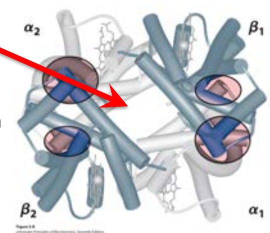





Tetramer has 2$\alpha$ and 2$\beta$ subunits
But, the $\alpha$ & $\beta$ have more affinity for each other than either $\alpha\alpha$ or $\beta\beta$
Therefore, Hb is best described as a dimer of $\alpha\beta$ dimers = $(\alpha\beta)_2$

Notice nice pocket at $\beta$–$\beta$ interface

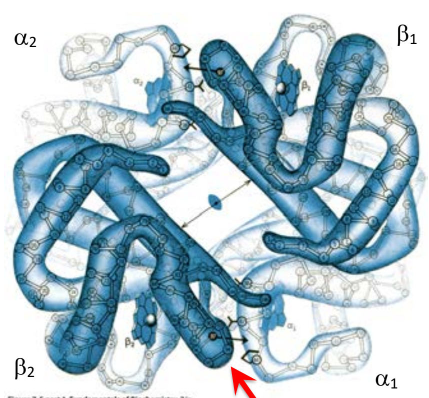
This view is looking down from the top

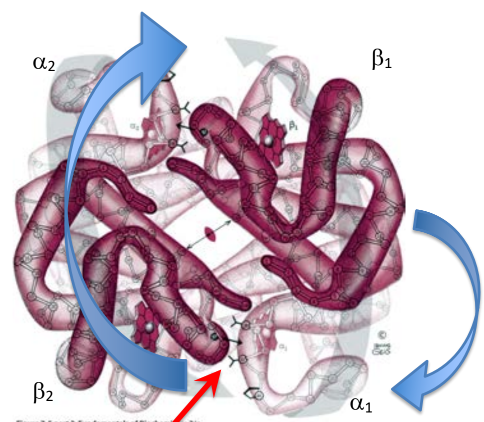



# Structure of Hemoglobin (Hb)

## Changes during binding!

**De-oxy Hemoglobin** **Oxy Hemoglobin**

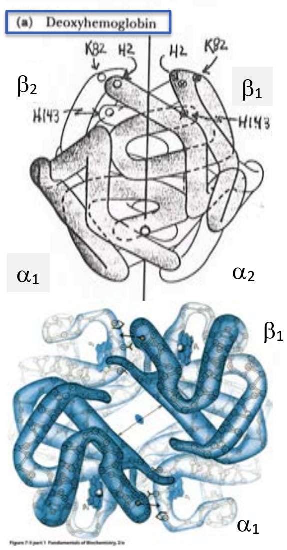



Notice residue 97 (His)

Lets look at this conformational change from the top of one $\alpha\beta$ dimer

## Structure of Hemoglobin (Hb)



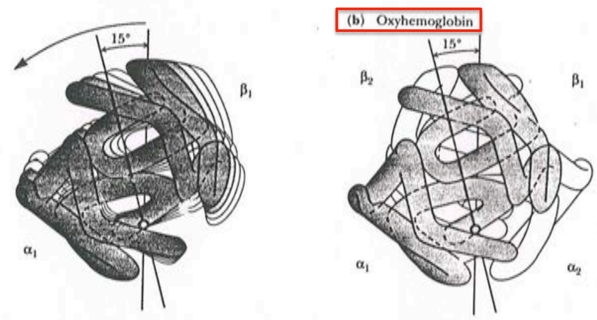
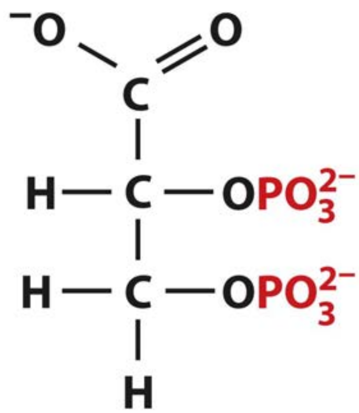




*Figure* 12.17 Subunit motion in hemoglobin when the molecule goes from the (a) deoxy to the (b) oxy form.

## What binds to Hb in addition to $O_2$?

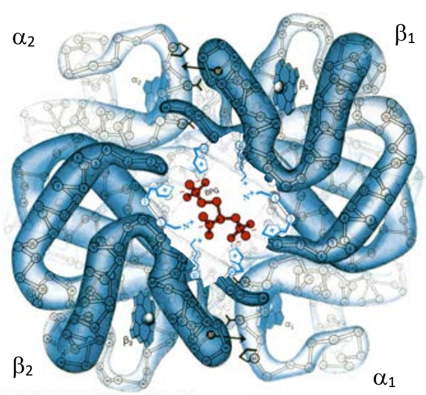
1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

## Structure of BPG

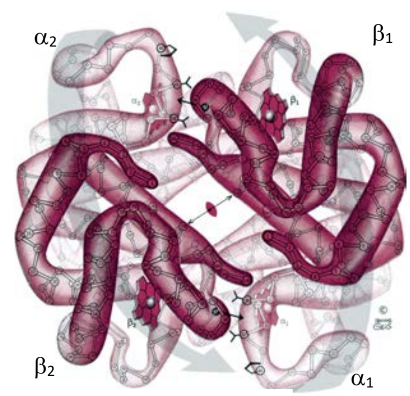



D-2,3-Bisphosphoglycerate (BPG)

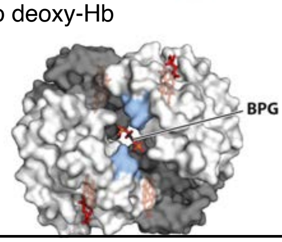
## BPG Binds to Deoxyhemoglobin

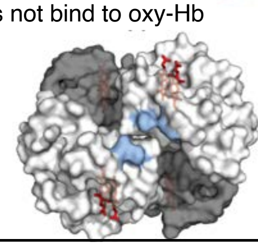



Binds to deoxy-Hb



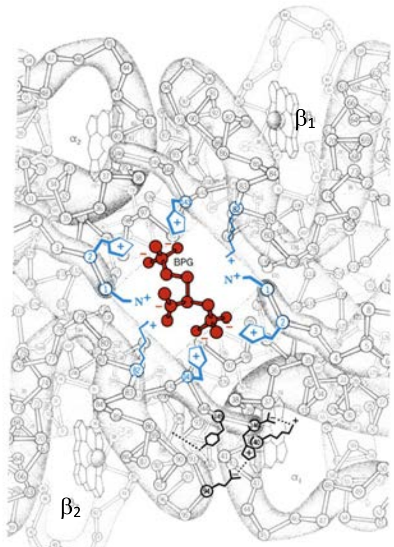

Does not bind to oxy-Hb



21

## BPG Binds to Deoxyhemoglobin



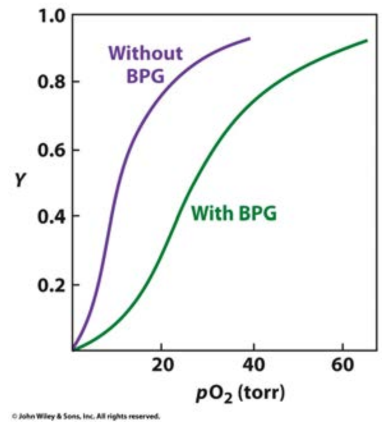




Figure 7-14
Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.

[BPG] is 5 mM in blood cells

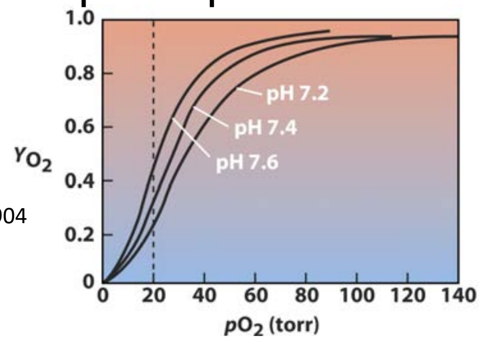
## What binds to Hb in addition to $O_2$?

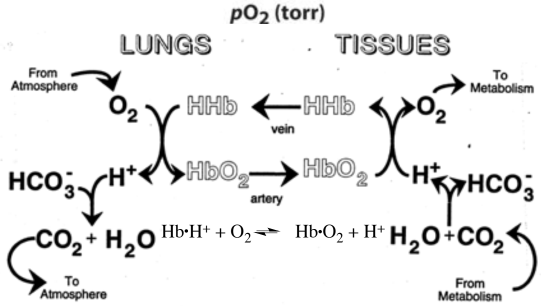
1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

**Proton transport-Blood buffer**

## Bohr Effect: pH dependence of $O_2$ binding

Christian Bohr-1904



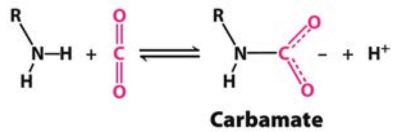




24

## 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




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

# $CO_2$ Binds to Deoxyhemoglobin

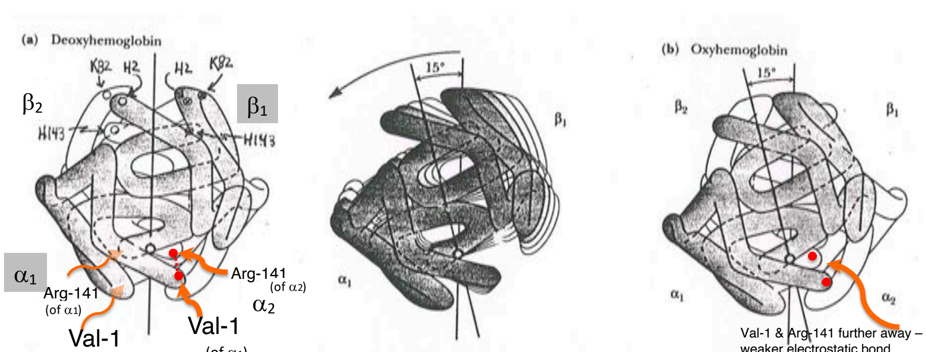


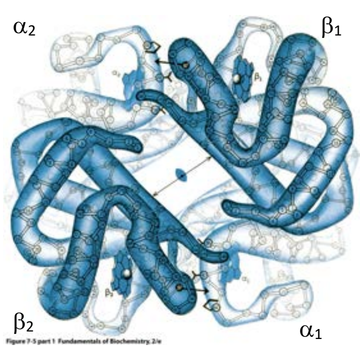

*Figure* **12.17** Subunit motion in hemoglobin when the molecule goes from the (a) deoxy to the (b) oxy form.

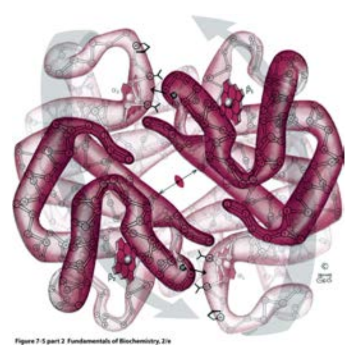
# What binds to Hb in addition to $O_2$?

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

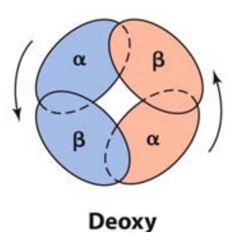
So, BPG, protons, and $CO_2$ bind specifically to deoxy-Hb.
Do these stabilize a possible T-state? Lets look at these states more closely….

# T-state and R-state of Hemoglobin

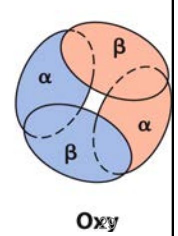


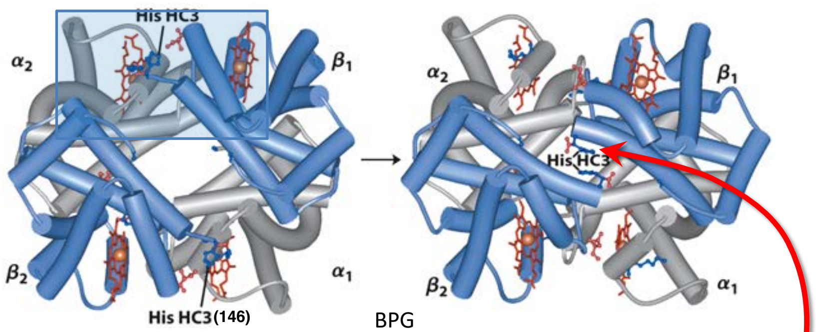
- T state
- Less active




- R state
- More active

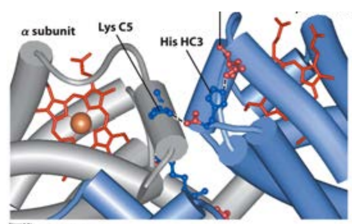


## R and T States of Hemoglobin



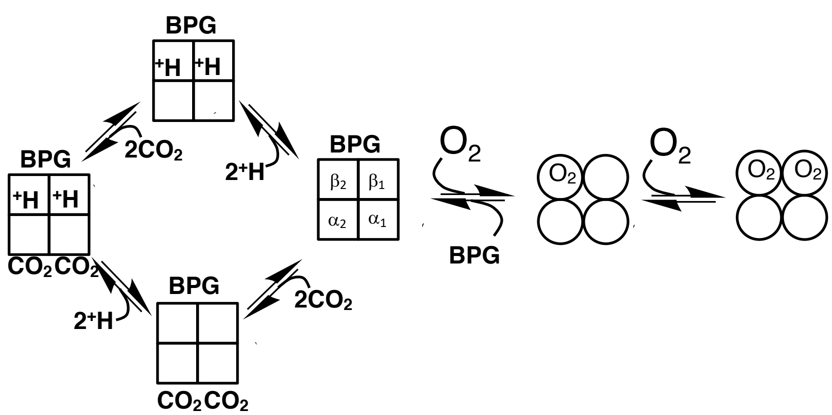

BPG

(40)




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

## Influences on T→R Transition




T-state R-state

Recall: L ($\frac{[T_0]}{[R_0]}$) = 300,000
and
C ($\frac{[K_R]}{[K_T]}$) = 0.01

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

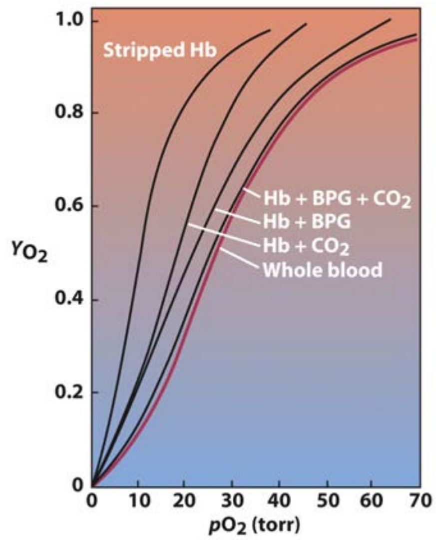



Figure 7-13


## $O_2$ binds to Hb

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

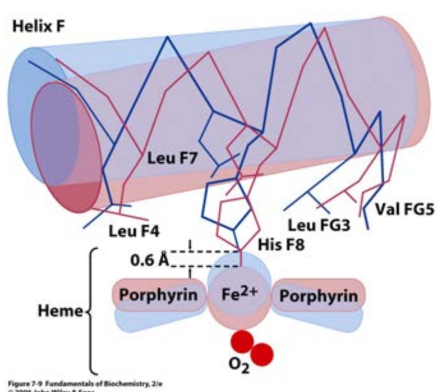
**How are all these related to the cooperative binding mechanism ?**

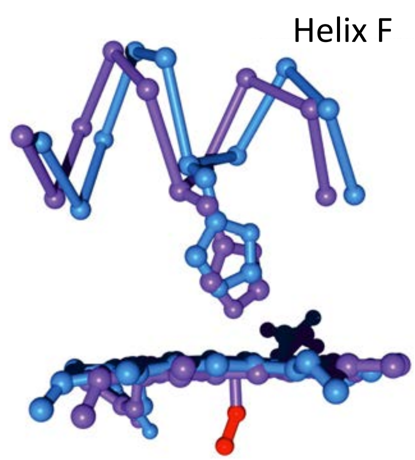
Lets look at binding of oxygen to the T-state, which its most likely to encounter (L=300,000).

Oxygen triggers Hb to switch from its low affinity (T) state to its high affinity (R) state. What kind of allosteric effector is oxygen for Hb?
What kind of allosteric effector is BPG for Hb?

A. Heterotropic positive allosteric effector
B. Homotropic negative allosteric effector
C. Heterotropic negative allosteric effector
D. Homotropic positive allosteric effector

# Structural Basis of Cooperativity




Deoxy
Oxygen bound

High-spin (paramagnetic)(larger)

Deoxy
Oxygen bound

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

36

## Conformational Change Is Triggered by Oxygen Binding

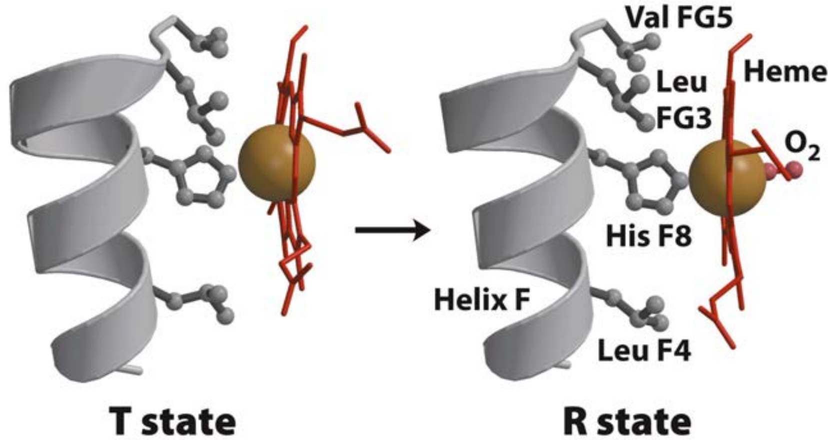
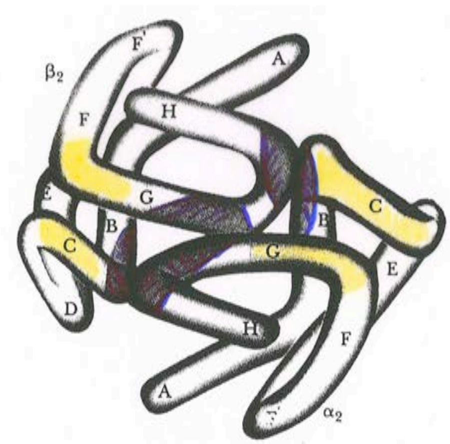



Figure 5-11
*Lehninger Principles of Biochemistry*, Seventh Edition


## Structural Basis of Cooperativity



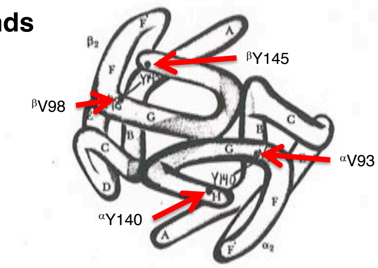

What causes the conformational change?

*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.

38

# Structural Basis of Cooperativity

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

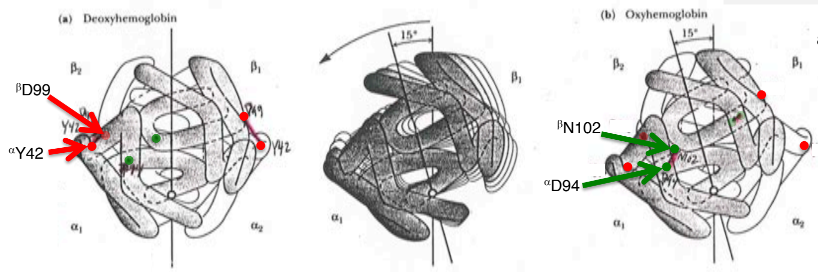



Changes within each subunit

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

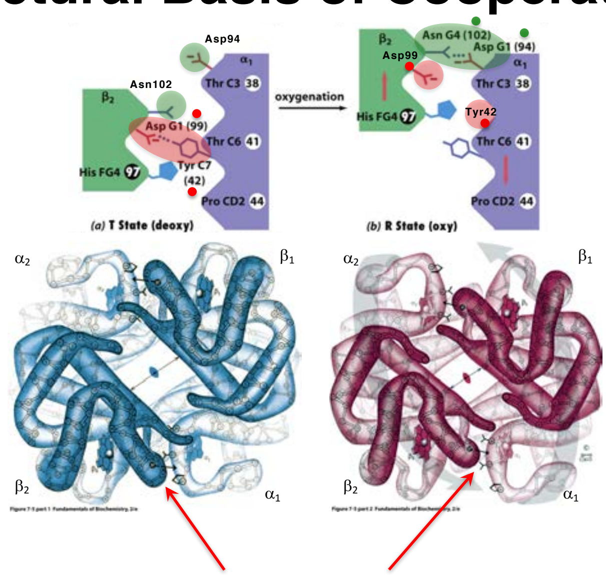
Changes between dimers ($\alpha$1 and $\beta$2)

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



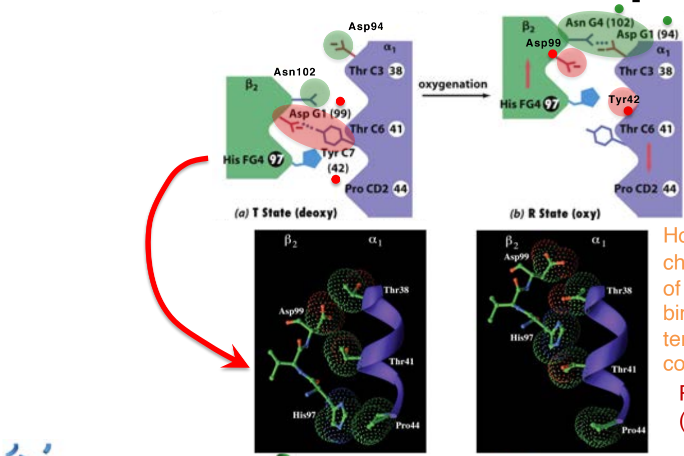

40

# Structural Basis of Cooperativity



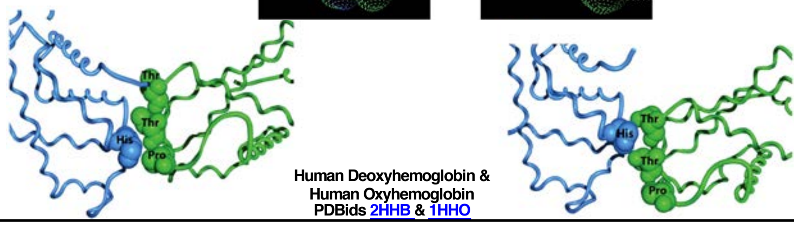

Notice residue 97 (His)

# 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 ($^{\alpha}$Y140 & $^{\beta}$Y145)




Human Deoxyhemoglobin &
Human Oxyhemoglobin
PDBids 2HHB & 1HHO

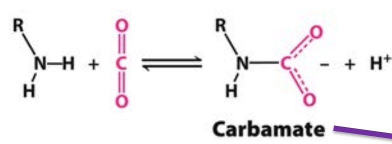
43

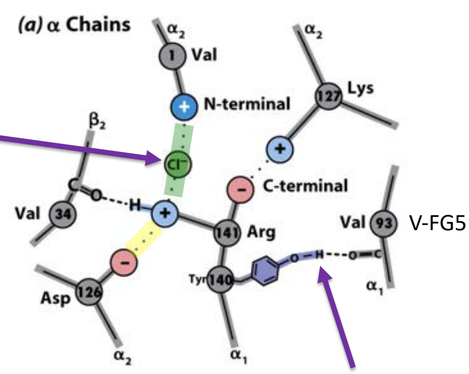
# Carbamate formation favors the T-state

Changes at C-term of $\alpha_1$

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 $\alpha_1$)

Recall, this is the first thing we saw destabilized

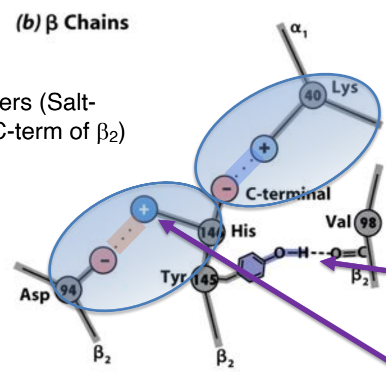
# Protonation favors the T state

Changes at C-term of $\beta_2$

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

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

Changes between dimers (Salt-bridge interactions at C-term of $\beta_2$)




V-FG5

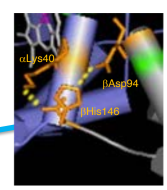
Recall, this is the first thing we saw destabilized

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

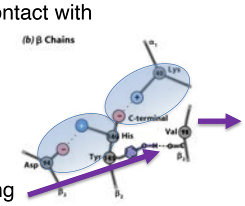
But, what actually causes the dimers to rotate the 15°?

# Oxygen-Binding affects Bonds to C-terminus

- Hemoglobin Dynamics at C-term of beta-subunit



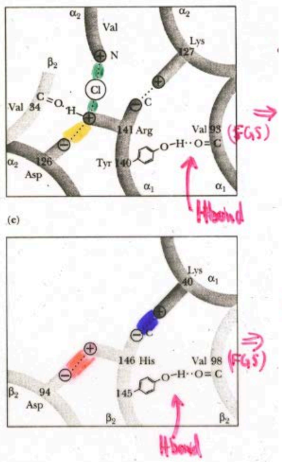

See:
- $O_2$ binds
- The salt-bridge between His-146 and Asp-94 on the same β-subunit breaks
- The salt-bridge between the C-term carboxylate of β-subunit loses contact with Lys-40 of α-subunit
- "Anchor" is lost and subunits move




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

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



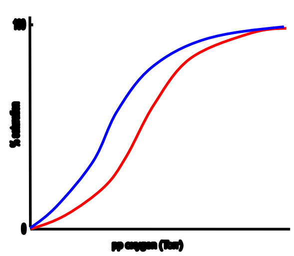

| Bond | Subunit | Identity (residue) | Broken / Made. |
|---|---|---|---|
| H-bond | $\alpha_1\alpha_1$, $\alpha_2\alpha_2$ | FG5 (Val93) — HC2 (Y140) | broken |
| H-bond | $\beta_1\beta_1$, $\beta_2\beta_2$ | FG5 (Val98) — HC2 (Y145) | broken |
| H-bond | $\alpha_1\beta_2$ | $C7_\alpha$ (Y42) — $G1_\beta$ (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

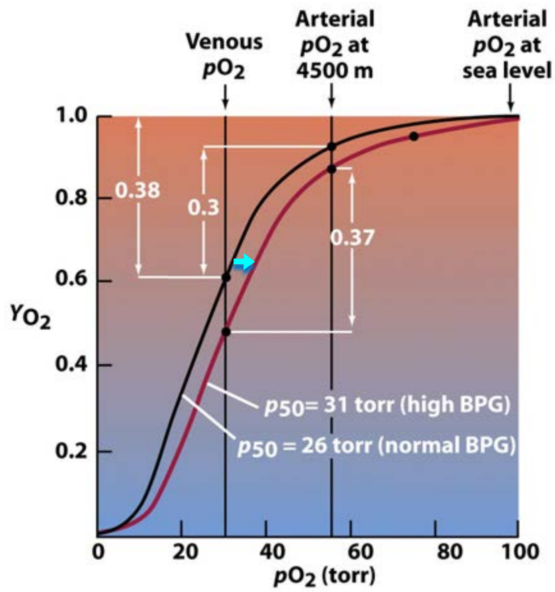
50

# 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.

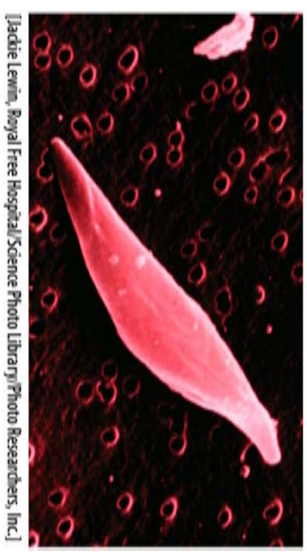



51

# Abnormal Hemoglobins

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

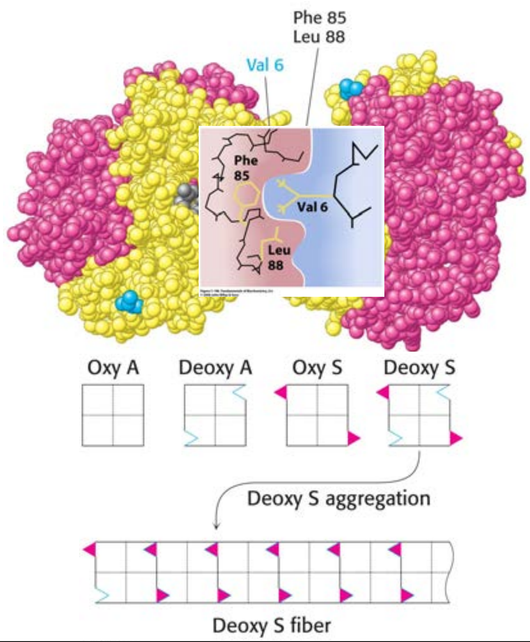
**Sickle-Cell Anemia**



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

52

# Hemoglobin S variant

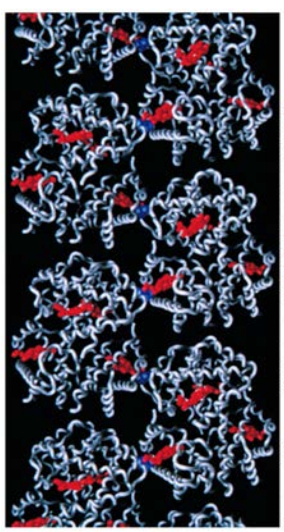



Sickle-cell hemoglobin fibers

53

# 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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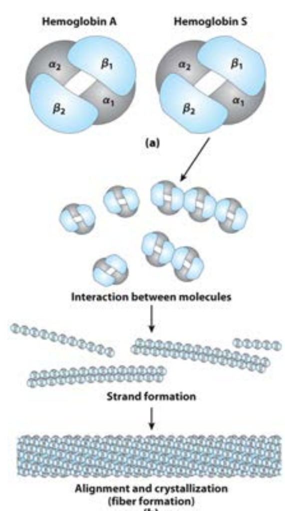


Figure 5-20
*Lehninger Principles of Biochemistry*, Seventh Edition
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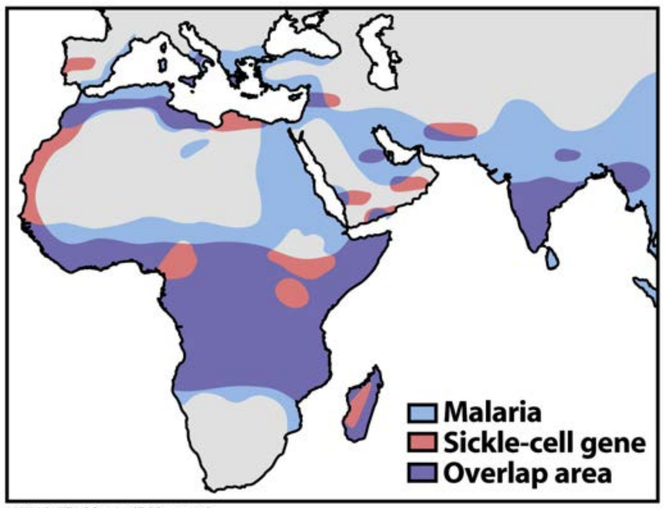
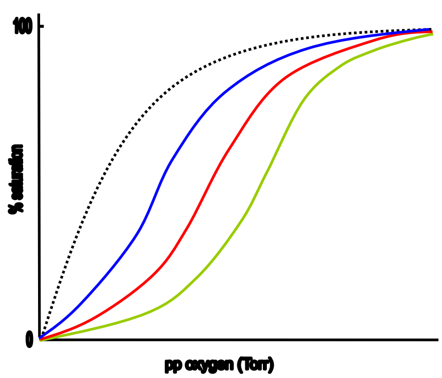
# Why does hemoglobin S persist in the population?






55

Which of the following lines represents the binding of oxygen to fetal Hb if the red line represents the binding of oxygen to maternal Hb?

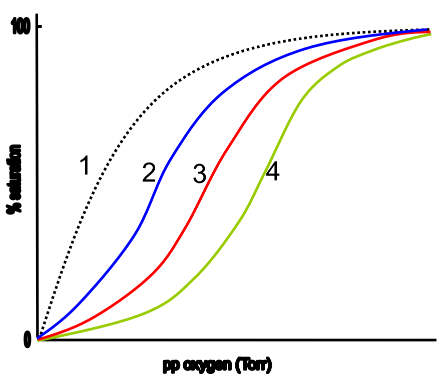



A. The red line
B. The green line
C. The blue line
D. The dotted line

Which of the following is true regarding the ability of Hb to bind oxygen?

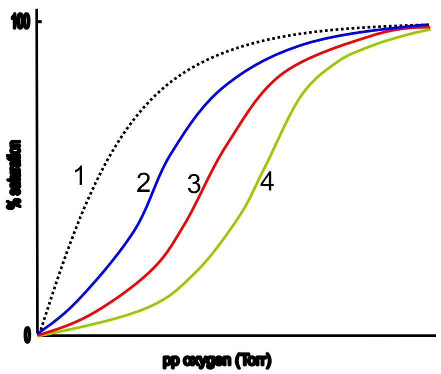
A. $CO_2$ promotes the release of oxygen
B. Salt bridges stabilize the deoxy form of Hb.
C. $H^+$ and BPG stabilize the deoxy form of Hb.
D. B and C are true.
E. All the above are true.

Which of the curves below show cooperative binding?




A. 2 and 3
B. 3 and 4
C. 2, 3 and 4
D. All of them show cooperative binding.
E. None show cooperative binding

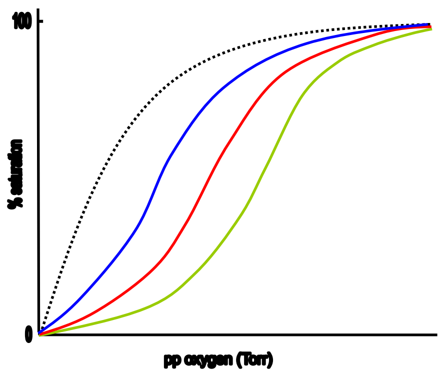
**If** curve 3 represents the binding behavior of normal Hb in the presence of 5 mM BPG, which curve represents the binding behavior of Hb at 8 mM BPG?




A. 1
B. 2
C. 3
D. 4
E. None of the above

**Proton transport-Blood buffer**

Which of the following lines represents the binding of oxygen to Hb at pH 7.8 if the red line represents the binding of oxygen to Hb at pH 7.2?




A. The red line
B. The green line
C. The blue line
D. The dotted line