Lecture 19 (10/26/20)

- Reading: Ch5; 168-169, 158-159,162-166
  169-174
- Problems: Ch5 (text); 3,7,8,10
  Ch5 (study guide-facts); *1,2,3,4,5,8*
  Ch5 (study guide-apply); **2,3**

Remember Tuesday at 7:30 in MOR-101 is the first MB lecture & quiz

NEXT

- Reading: Ch4; 142-151
- Problems: Ch4 (text); 14,16
  Ch6 (text); 1, 4

ENZYMES:

A. "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 curves
   a. oxygen
   b. Allosteric effectors (BPG)
   c. Bohr effect; (protons)
   d. Carbon dioxide
4. Structure-Function; Structural basis for physiology (T & R states)
5. Mechanism of Cooperativity

Hemoglobin (Hb)

<u>Best understood example of an allosteric protein</u>

Evolution of oxygen transporters Hb and myoglobin (Mb):

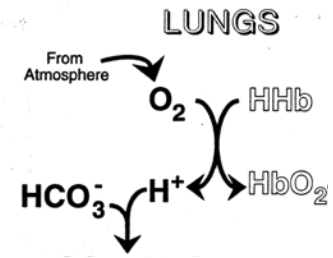
- Serum [Oxygen] ≈ 2.3 mL/L; Blood [Oxygen] ≈ 200 mL/L
- Metabolism:
  Needs O_2 → $C_6H_{12}O_6 + 6O_2 \leftrightarrows 6CO_2 + 6H_2O$
  Oxidation of sugars → metabolic acids and from $CO_2 + H_2O \leftrightarrows H_2CO_3$

Roles of Hb:

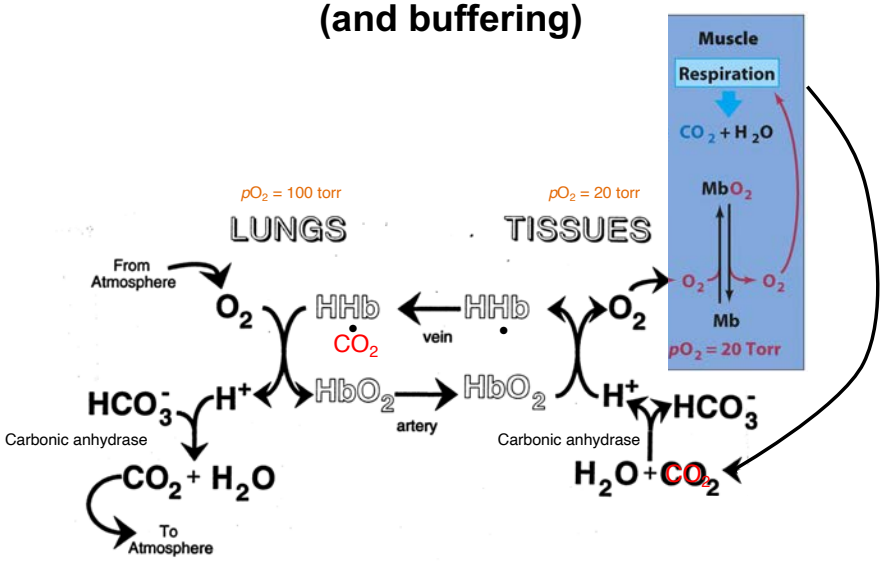
- Oxygen transport
- Proton transport-Blood buffer
- Carbon dioxide transport

2

Hemoglobin & Myoglobin in O_2 & CO_2 Transport

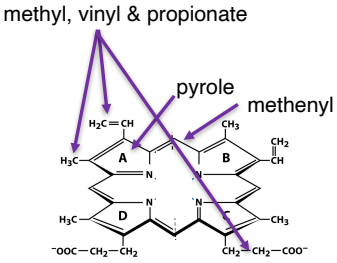


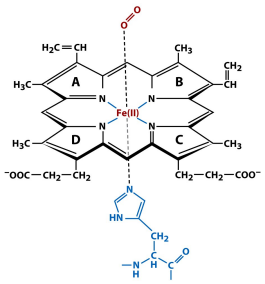
Hemoglobin & Myoglobin in O_2 & CO_2 Transport (and buffering)

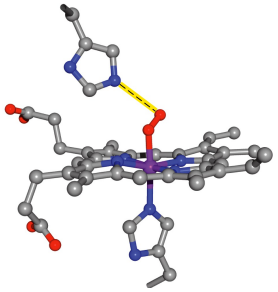


Oxygen transport

Heme





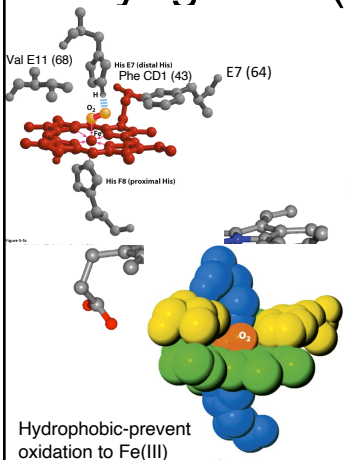


- Heme is protoporphyrin IX plus Fe^{2+}
- Heme prosthetic group in globins binds oxygen via Fe^{2+}
- Heme itself is not a good oxygen transporter because of oxidation to Fe^{3+}

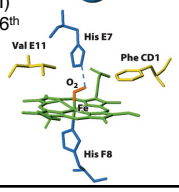
5

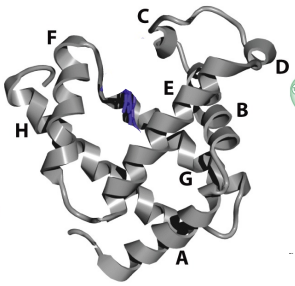
Oxygen transport

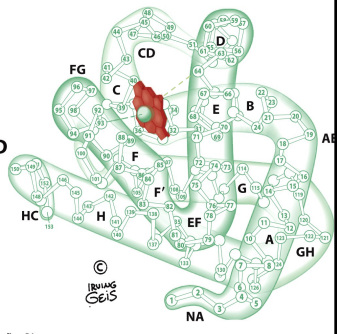
Myoglobin (Mb): O_2-binding protein



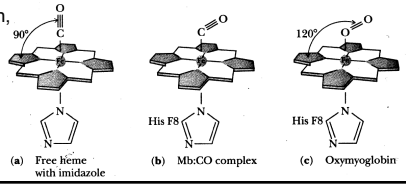
Hydrophobic-prevent oxidation to Fe(III)
Provides site for 6th ligand
Limits binding by other analogs





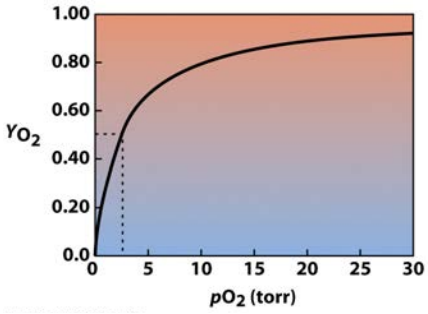


Carbon monoxide binds heme 20,000 better than dioxygen, but in Mb/Hb it only binds 200 times better.



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 :

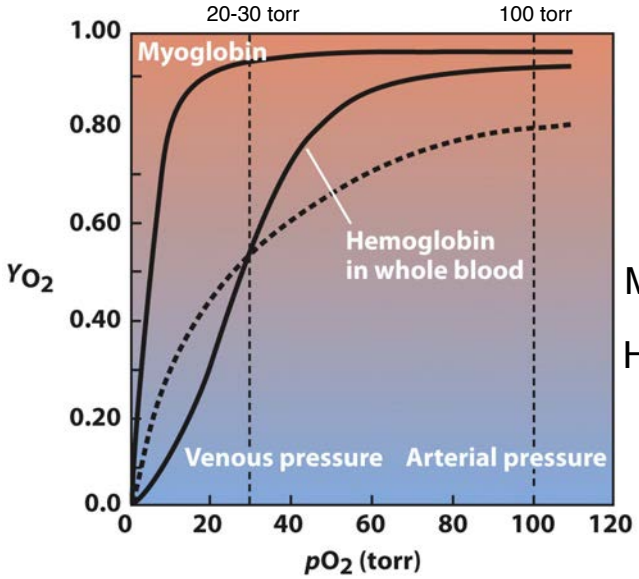
$$Y = \frac{[L]}{K_D + [L]}$$

$$Y_{o_2} = \frac{[M_bO_2]}{[M_bO_2] + [M_b]} = \frac{\frac{[M_b]\,[O_2]}{K_d}}{\frac{[M_b]\,[O_2]}{K_d} + [M_b]} = \frac{[O_2]}{K_d + [O_2]}$$

7

Oxygen transport

Oxygen Binding Curve of Hemoglobin



Mb → K_d= 3-4 torr

Hb → K_d= 26 torr

Cooperativity: Hill coefficient

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

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

- Theoretical maximum cooperativity = # of binding sites

9

Cooperativity: Hill coefficient

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

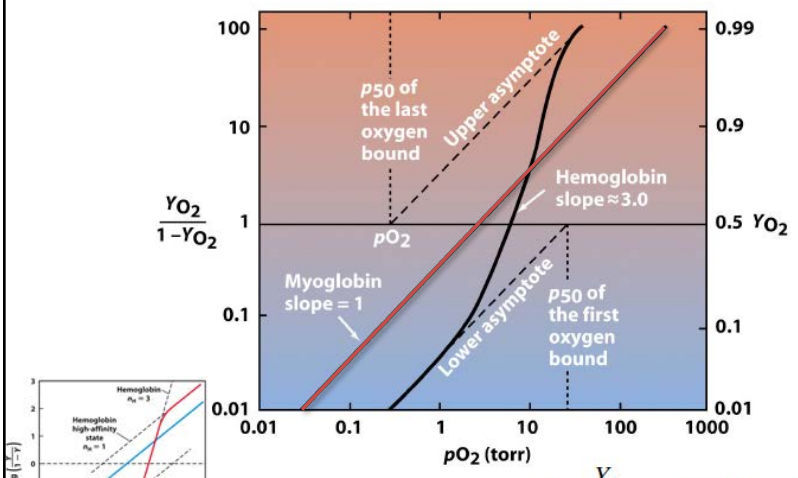
$$K_d = \frac{[\mathrm{H_b}]\,[\mathrm{O_2}]^n}{[\mathbf{H_b(O_2)_n}]} \qquad Y_{o2} = \frac{(p\mathrm{O_2})^n}{(p_{50}) + (p\mathrm{O_2})^n}$$

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

- Theoretical maximum cooperativity = # of binding sites

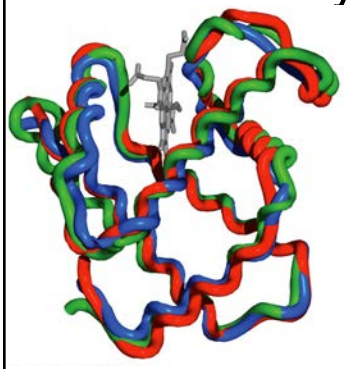
10

Hill Plot for Mb & Hb

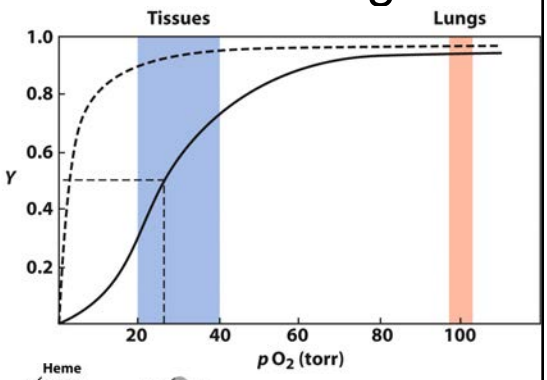


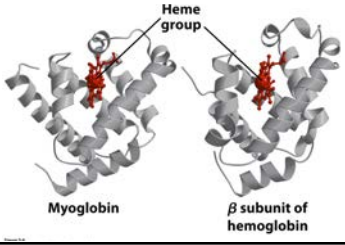
$$\log \frac{Y_{O_2}}{1-Y_{O_2}} = \log pO_2 - n \log p_{50}$$

Mb and Hb-subunits: structural similarity, differential binding



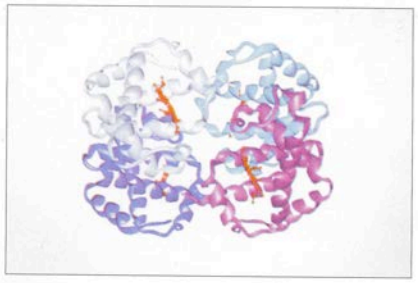
- Mb, Hb-α, Hb-β overlay

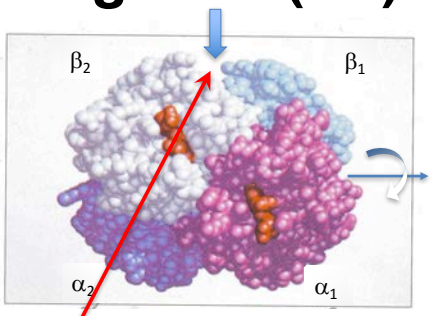




12

Structure of Hemoglobin (Hb)

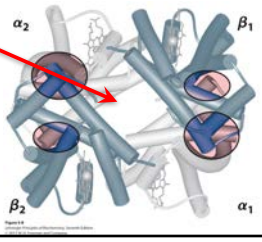




Tetramer has 2α and 2β subunits
But, the α & β have more affinity for each other than either $\alpha\alpha$ or $\beta\beta$
Therefore, Hb is best described as a dimer of $\alpha\beta$ dimers = $(\alpha\beta)_2$

Notice nice pocket at β–β interface

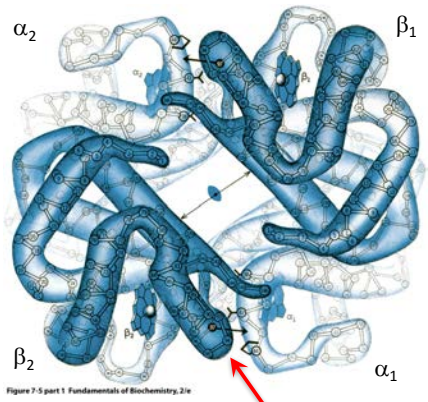
This view is looking down from the top

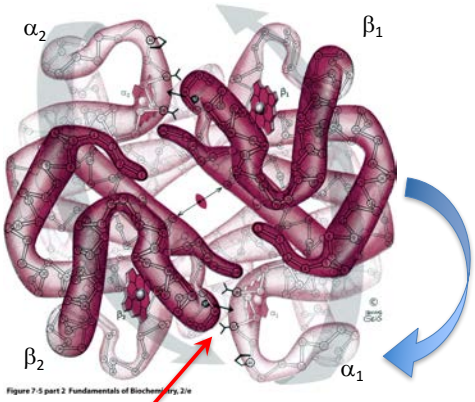


Structure of Hemoglobin (Hb)

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

14

Structure of Hemoglobin (Hb)

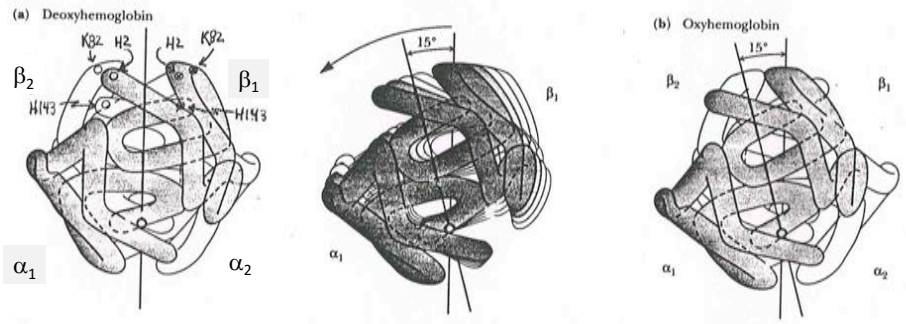


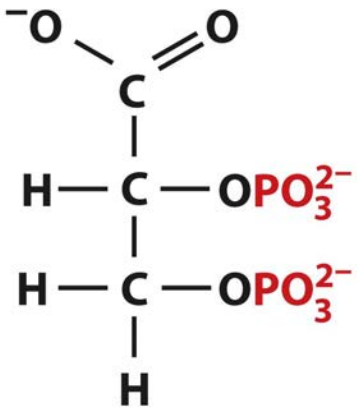
Figure 12.17 Subunit motion in hemoglobin when the molecule goes from the (a) deoxy to the (b) oxy form.

15

What binds to Hb in addition to O_2?

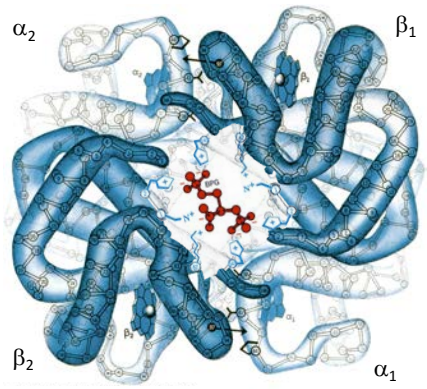
1. D-2,3-Bisphosphoglycerate (BPG)
2. Protons
3. Carbon Dioxide

Structure of BPG

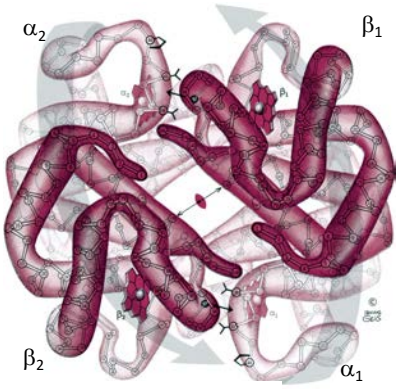


D-2,3-Bisphosphoglycerate (BPG)

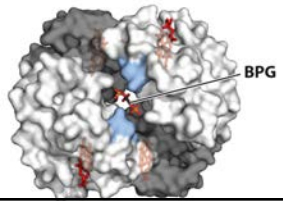
BPG Binds to Deoxyhemoglobin

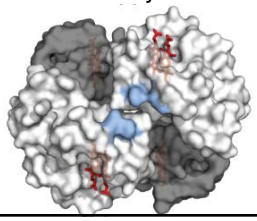


Binds to deoxy-Hb



Does not bind to oxy-Hb





18

BPG Binds to Deoxyhemoglobin

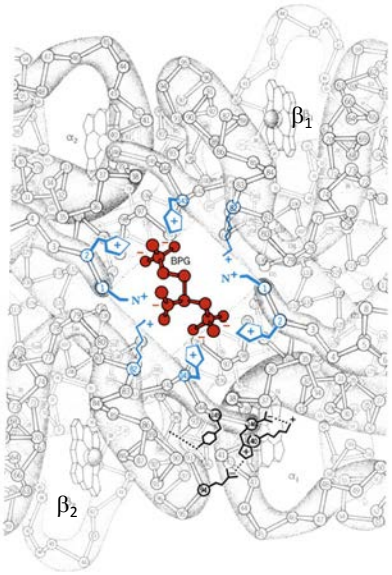
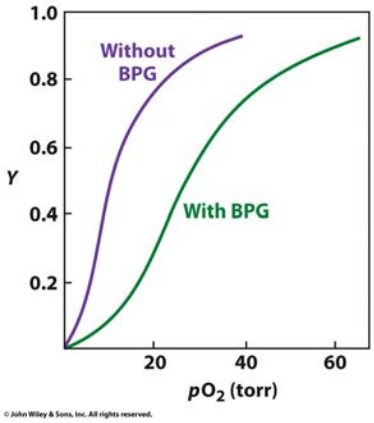


Figure 7-14
Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.



[BPG] is 5 mM in blood cells

What binds to Hb in addition to O_2?

✔ 1. D-2,3-Bisphosphoglycerate (BPG)

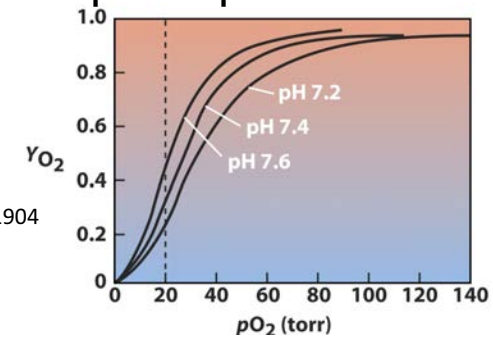
2. Protons

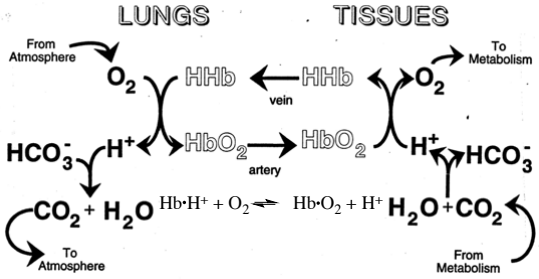
3. Carbon Dioxide

Proton transport-Blood buffer

Bohr Effect: pH dependence of O_2 binding

Christian Bohr-1904





21

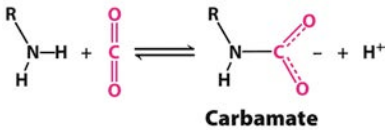
What binds to Hb in addition to O_2?

✔ 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

CO_2 Binds to Deoxyhemoglobin

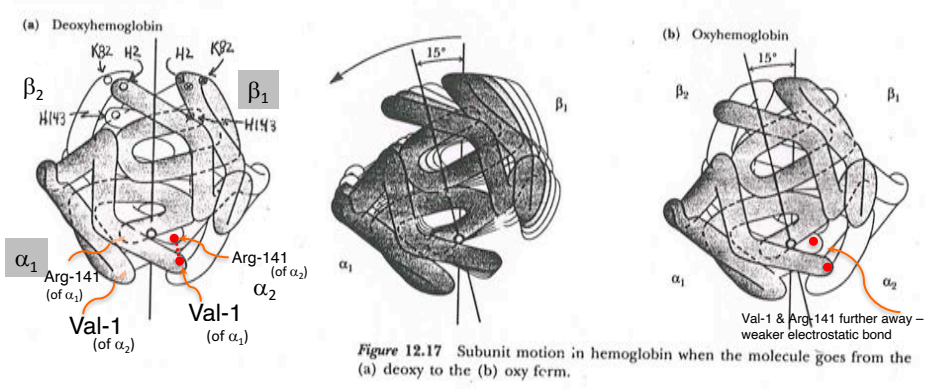


Figure 12.17 Subunit motion in hemoglobin when the molecule goes from the (a) deoxy to the (b) oxy form.

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

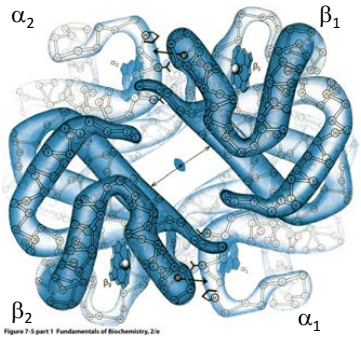
What binds to Hb in addition to O_2?

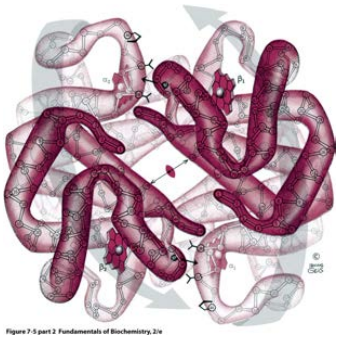
✔1. D-2,3-Bisphosphoglycerate (BPG)

✔2. Protons

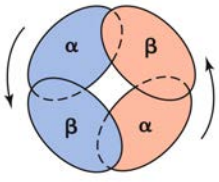
✔3. Carbon Dioxide

T-state and R-state of Hemoglobin



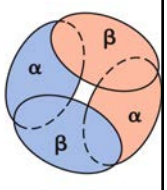


- T state
- Less active



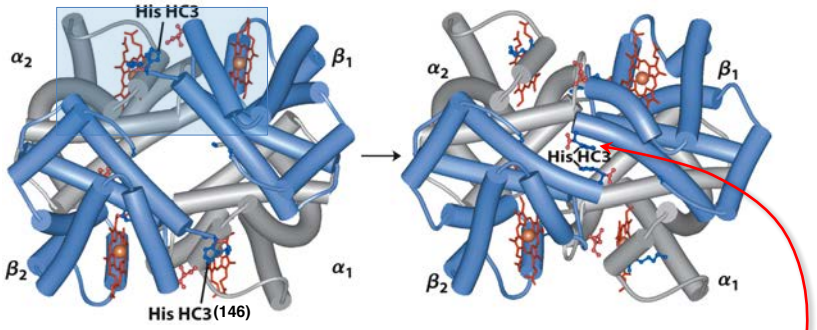
Deoxy

- R state
- More active

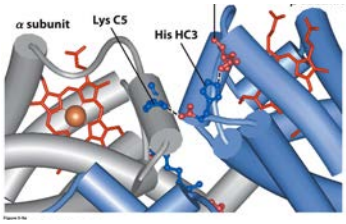


Oxy

R and T States of Hemoglobin

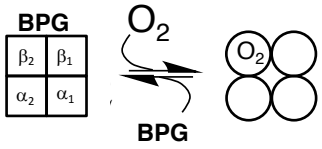


(40)



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

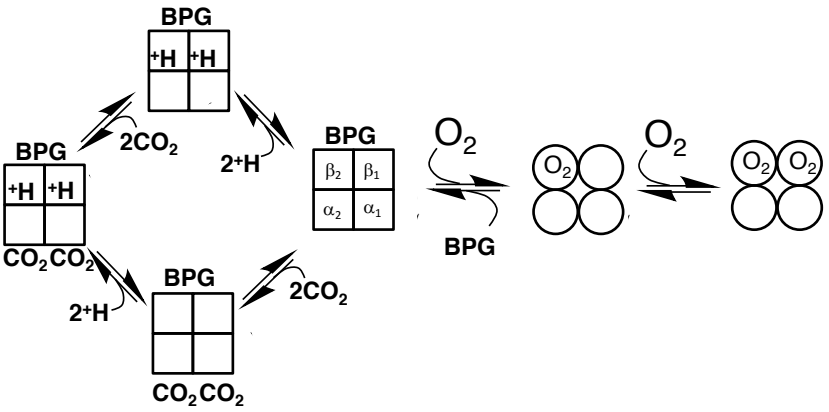
Influences on T→R Transition



T-state R-state

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

Influences on T→R Transition



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

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

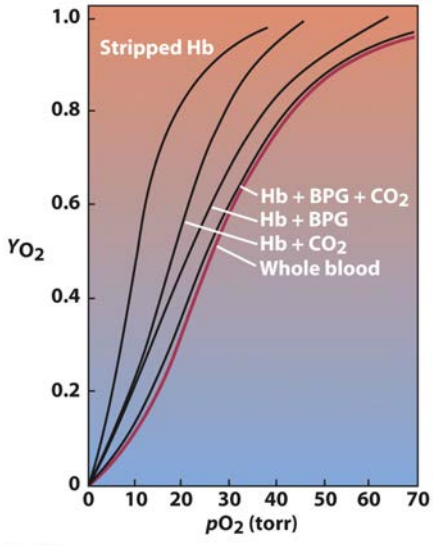


Figure 7-13

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- Reading: Ch5; 168-169, 158-159,162-166
  169-174
- Problems: Ch5 (text); 3,7,8,10
  Ch5 (study guide-facts); *1,2,3,4,5,8*
  Ch5 (study guide-apply); **2,3**

Remember Tuesday at 7:30 in MOR-101 is the first MB lecture & quiz

NEXT

- Reading: Ch4; 142-151
- Problems: Ch4 (text); 14,16
  Ch6 (text); 1, 4

ENZYMES:

A. "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 curves
   a. oxygen
   b. Allosteric effectors (BPG)
   c. Bohr effect; (protons)
   d. Carbon dioxide
4. Structure-Function; Structural basis for physiology (T & R states)
5. Mechanism of Cooperativity

✔ **O_2 binds to Hb**

✔ **1. D-2,3-Bisphosphoglycerate (BPG)**

✔ **2. Protons**

✔ **3. Carbon Dioxide**

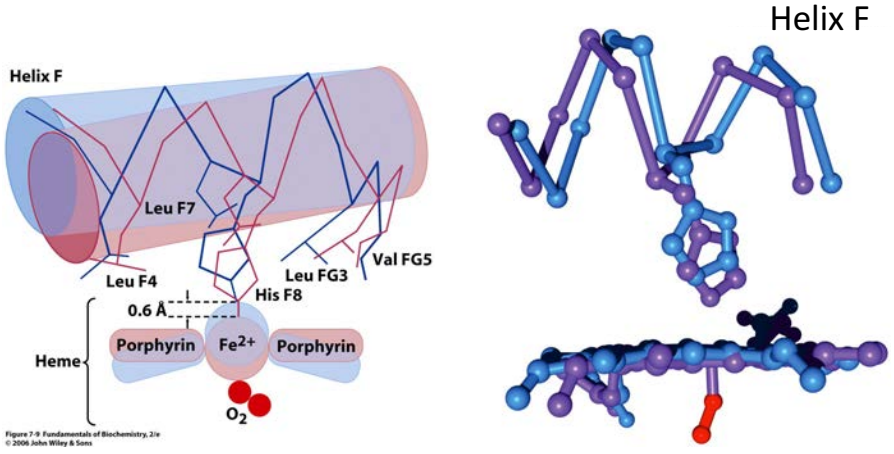
How are all these related to the cooperative binding mechanism ?

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

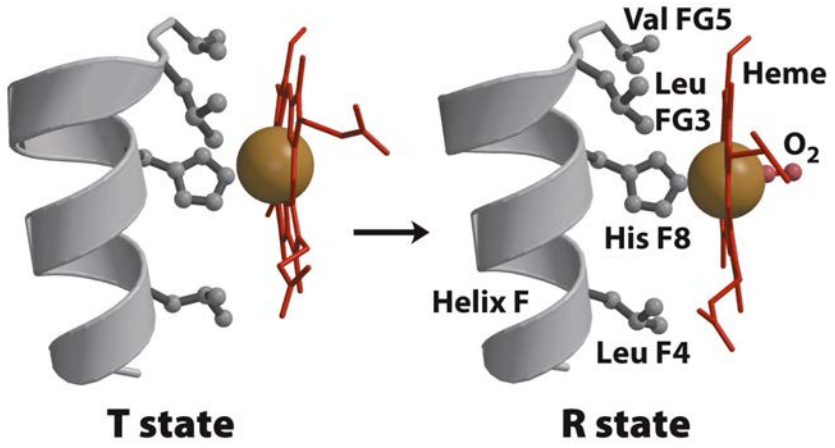


Figure 5-11
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Structural Basis of Cooperativity

What causes the conformational change?

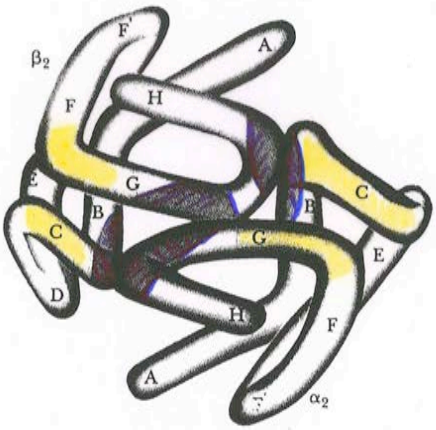
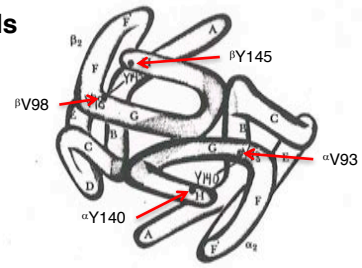


Figure 12.16 Side view of one of the two $\alpha\beta$ dimers in Hb, with packing contacts indicated in blue. The sliding contacts made with the other dimer are shown in yellow. The changes in these sliding contacts are shown in Figure 12.17.

38

Structural Basis of Cooperativity

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



Changes within each subunit

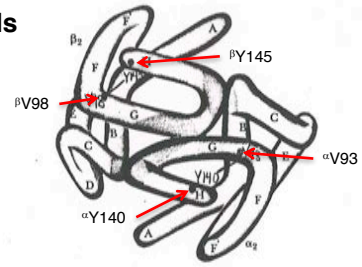
The change at the F-helix changes the C-term stability within each subunit

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

exercises

Structural Basis of Cooperativity

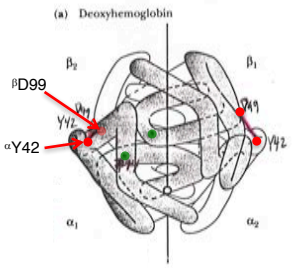
Changes in H-bonds when an oxygen binds to a single subunit

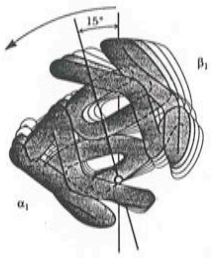


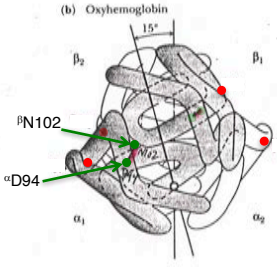
Changes within each subunit

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

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

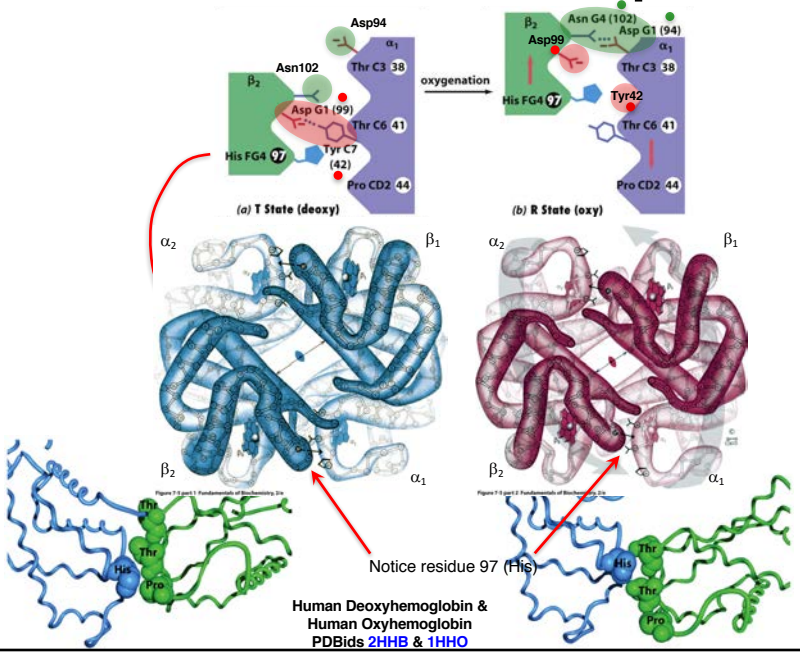






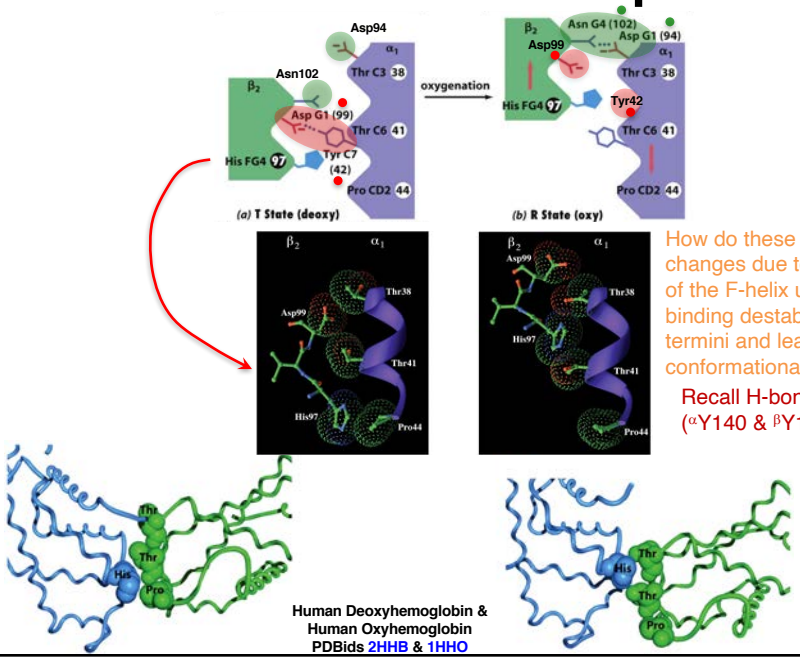
The change at the F-helix changes the C-term stability between subunits

Structural Basis of Cooperativity



Human Deoxyhemoglobin &
Human Oxyhemoglobin
PDBids 2HHB & 1HHO

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)

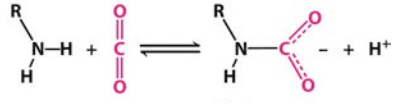
Human Deoxyhemoglobin &
Human Oxyhemoglobin
PDBids 2HHB & 1HHO

Carbamate formation favors the T-state

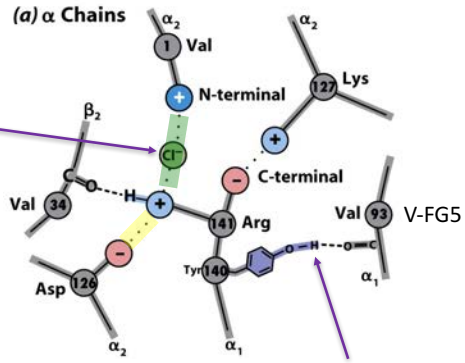
Changes at C-term of α_1

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

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



Carbamate



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

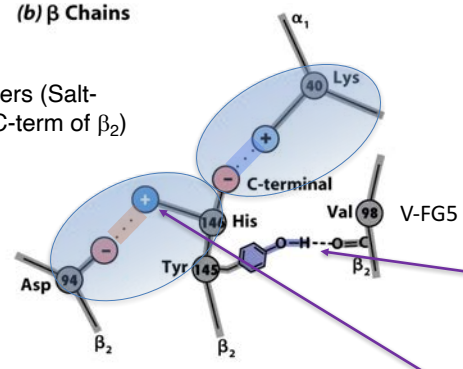
Recall, this is the first thing we saw destabilized

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

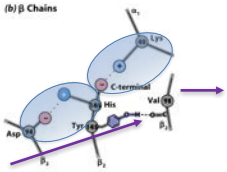
The Bohr effect: when these interactions are lost, the proton is released (pK_a drops)

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

Oxygen-Binding affects Bonds to C-terminus

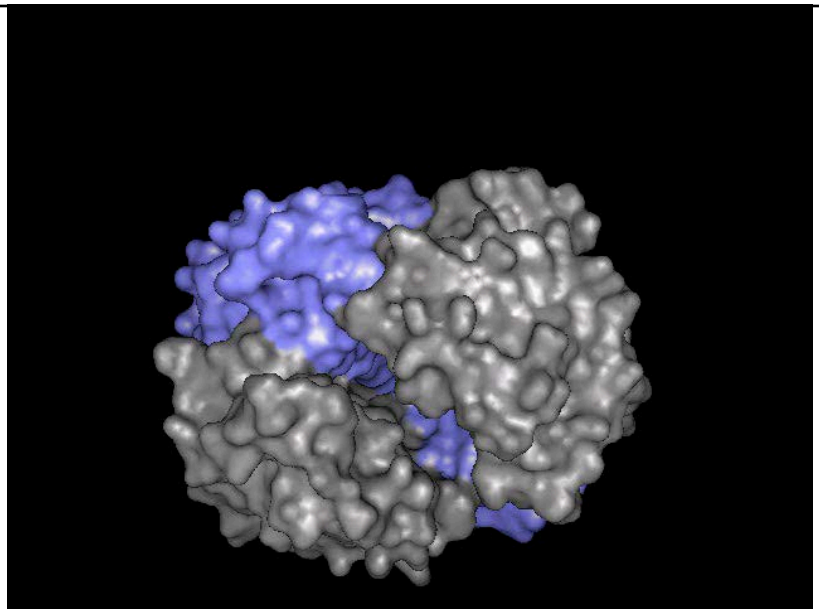
- Hemoglobin Dynamics at C-term of beta-subunit

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

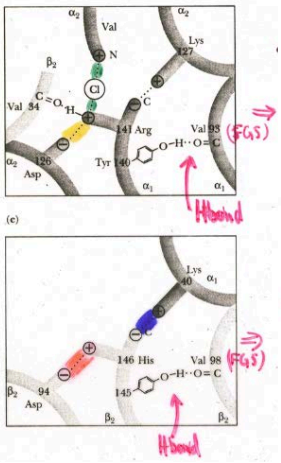
- The T- and R- states of Hb



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



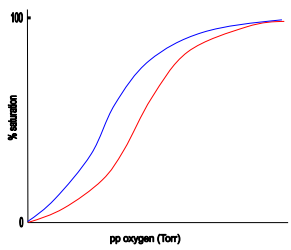
Bond	Subunit	Identity (residue)	Broken / Made
H-bond	$\alpha_1\alpha_1$ $\alpha_2\alpha_2$	FG5 (Val 93) — HC2 (Y140)	broken
H-bond	$\beta_1\beta_1$ $\beta_2\beta_2$	FG5 (Val 98) — HC2 (Y145)	broken
—	—	—	—
H-bond	$\alpha_1\beta_2$	$C7_\alpha$ (Y42) — $G1_\beta$ (D99)	broken
H-bond	$\alpha_1\beta_2$	$G1_\alpha$ (D94) — $G4_\beta$ (N102)	made
—	—	—	—
Salt bridge	$\alpha_1\alpha_2$	HC3 (R141) — NA1 (V1) (Cl^- in carbamate)	broken
Salt bridge	$\alpha_1\alpha_2$	HC3 (R141) — H9 (D126)	broken
Salt bridge	β_1/β_2 β_2/β_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

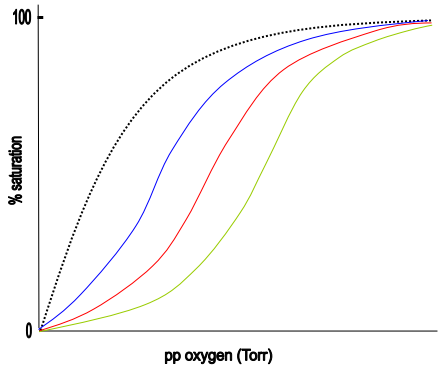


This shifts the T←→R equilibrium to the right.

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

48

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?

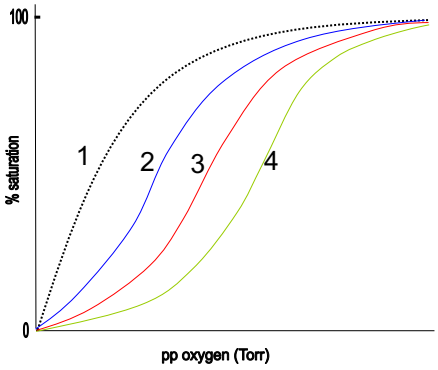


A. The red line
B. The green line
C. The blue line
D. The dotted line

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

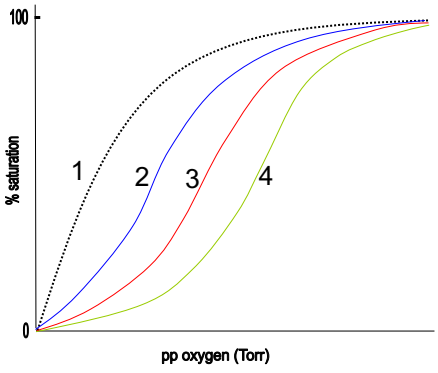
A. CO_2 promotes the release of oxygen
B. Salt bridges stabilize the deoxy form of Hb.
C. H^+ and BPG stabilize the deoxy form of Hb.
D. B and C are true.
E. All the above are true.

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

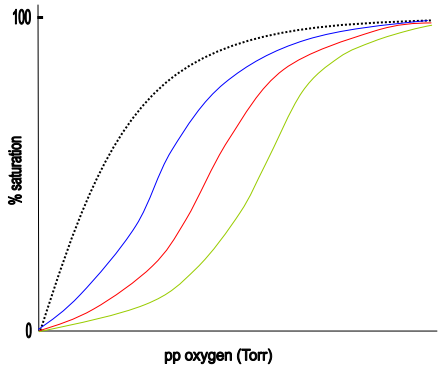
If curve 3 represents the binding behavior of normal Hb in the presence of 5 mM BPG, which curve represents the binding behavior of Hb at 8 mM BPG?



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?



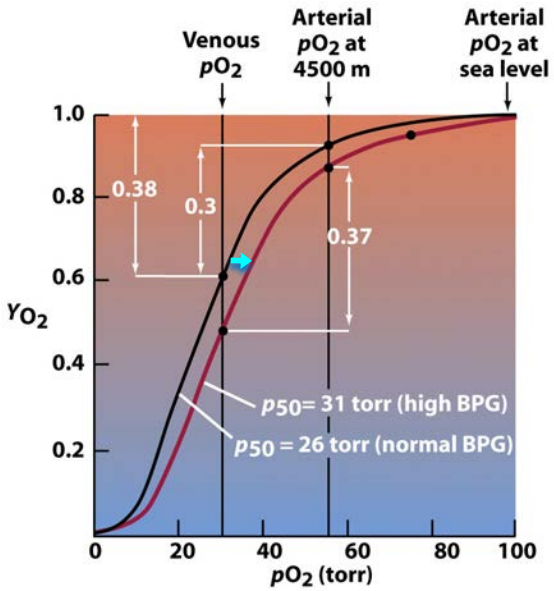
A. The red line
B. The green line
C. The blue line
D. The dotted line

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.



54

Protein Structure VIII

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

B. Protein Folding
   1. Evidence-Anfinsen
   2. Protein Folding Pathways
   3. Mechanism; *in vitro* vs. *in vivo*
      a. Kinetics
      b. Thermodynamics
   4. Diseases

C. Prediction

D. Dynamics

Protein Stability, Folding, and Dynamics

Two-state model:

(denatured) (native)

What is favored in this equilibrium, D or N?

What forces operate?

Which forces are the most important?

Protein Stability, Folding, and Dynamics

D ⇌ N

(denatured) (native)

What is the equilibrium?

Lies to the right

Therefore, ΔG is negative

What forces operate?

Non covalent:

H-bonds?	yes, definitely, but those with water in D-state
Ionic (salt-bridges)?	yes, but not that many and non specific
van der Waals?	yes, but not a driving force until there is compaction
Hydrophobic?	YES, bury hydrophobic residues

Covalent:

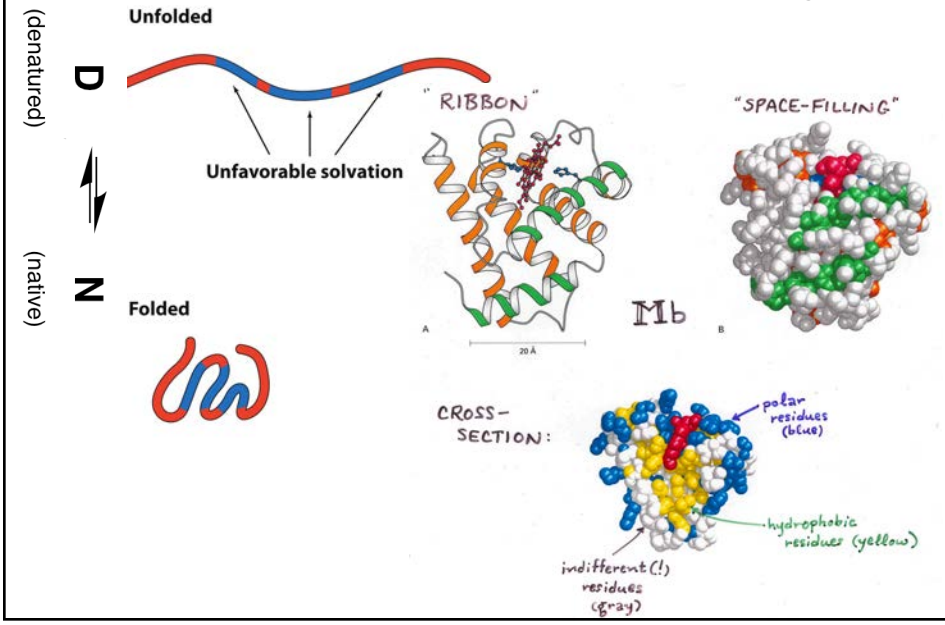
Disulfide bonds?	yes, but most proteins don't have any

Which force(s) are the most important?

Hydrophobic!

Protein Stability, Folding, and Dynamics

Hydrophobic effect drives protein folding



Protein Stability, Folding, and Dynamics

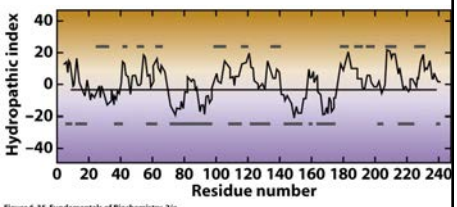
Hydrophobic effect drives protein folding

[TABLE 4-3] Hydrophobicity Scales

Residue	Scale A[a]	Scale B[b]
Phe	2.8	3.7
Met	1.9	3.4
Ile	4.5	3.1
Leu	3.8	2.8
Val	4.2	2.6
Cys	2.5	2.0
Trp	−0.9	1.9
Ala	1.8	1.6
Thr	−0.7	1.2
Gly	−0.4	1.0
Ser	−0.8	0.6
Pro	−1.6	−0.2
Tyr	−1.3	−0.7
His	−3.2	−3.0
Gln	−3.5	−4.1
Asn	−3.5	−4.8
Glu	−3.5	−8.2
Lys	−3.9	−8.8
Asp	−3.5	−9.2
Arg	−4.5	−12.3

[a]Scale A is from Kyte, J., and Doolittle, R.F., *J. Mol. Biol.* **157**, 105–132 (1982).

[b]Scale B is from Engelman, D.M., Steitz, T.A., and Goldman, A., *Annu. Rev. Biophys. Chem.* **15**, 321–353 (1986).



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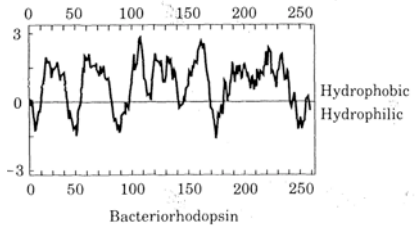
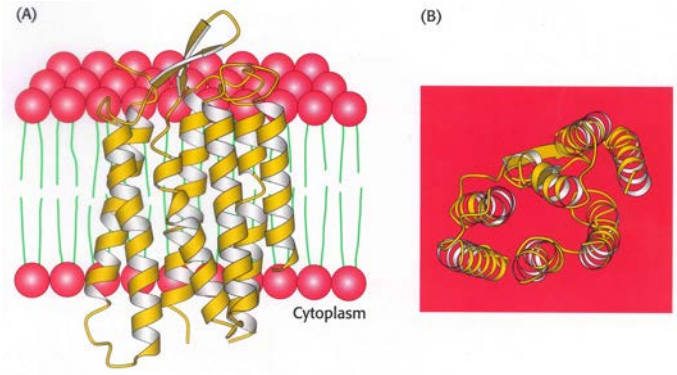
64

Protein Stability, Folding, and Dynamics

Hydrophobic effect drives protein folding

What about membrane proteins?





Protein Stability, Folding, and Dynamics

D ⇌ N

(denatured) (native)

What is the magnitude?

About ⁻9-10 kcal/mole = ΔG°'

$$K_{eq} = \frac{[N]}{[D]} = e^{-\Delta G°'/RT}$$

At 25 °C;

$$= e^{-(-10)/0.59}$$

$$= 2 \times 10^{7}$$

Protein Stability, Folding, and Dynamics

Degradation ← D ⇌ N

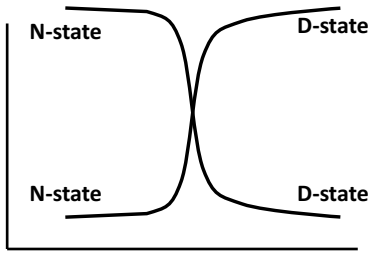
Aggregates ← D

(denatured) (native)

What other routes are there for the D-state?

Degradation (turnover)
Aggregates (precipitation)

What does the change in equilibrium, or transition, look like?



Observable

Conditions that perturb equilibrium

What are some observables?

CD, fluorescence, activity, viscosity, etc.

Protein Stability, Folding, and Dynamics

What are these conditions?

Disrupt forces that hold protein in tertiary structure

1) Temperature (affects both enthalpy and entropy (-**T**ΔS)
2) pH (changes charges; affects all polar interactions)
3) Detergents (creates micelles; turns proteins inside-out)
4) Chemicals:
   - urea, guanidine HCl (chaotropic agents; complicated)
   - mercaptoethanol, DTT (cleaves disulfide bonds)
   - Salts: Hofmeister Series (stabilizing/destabilizing salts)

Cations: $^{+}NH_4 > {}^{+}Cs > {}^{+}K > {}^{+}Na > {}^{+}Li > {}^{+2}Mg > {}^{+2}Ca > {}^{+2}Ba$

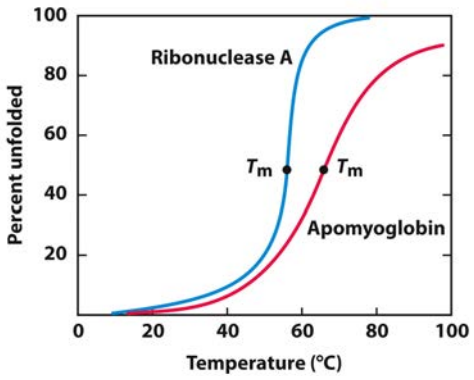
Anions: $^{-2}SO_4 > {}^{-2}PO_4 > CH_3COO^{-} > {}^{-}Cl > {}^{-}Br > {}^{-}I > {}^{-}ClO_4 > {}^{-}SCN$

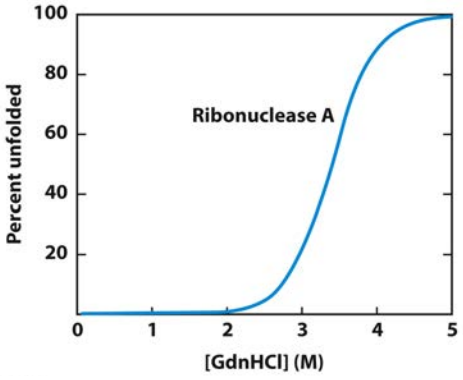
Chaotropic Agents Denature Proteins: Can you see how?

Guanidinium ion **Urea**

Protein Stability, Folding, and Dynamics

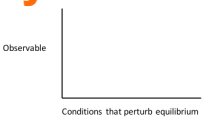
Examples of Protein Denaturation

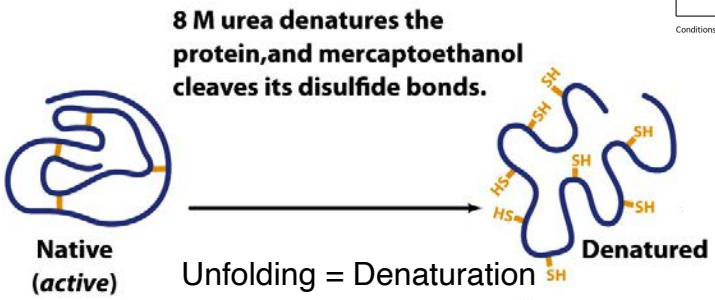




Protein Stability, Folding, and Dynamics

What are these Observables?





Unfolding = Denaturation

Any techniques that can measure a difference between these two states:

Optical rotation, CD, fluorescence (native (Trp) or ANS), UV absorbance, viscosity, shape