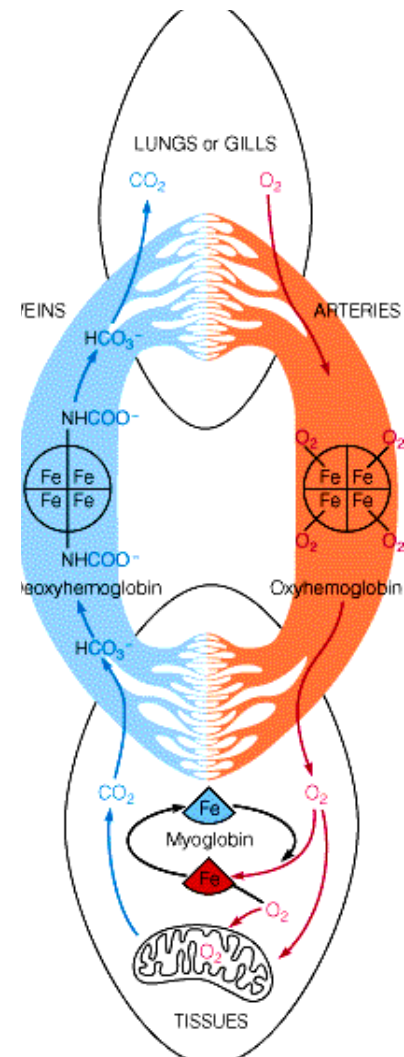


Chapter 7--Proteins that Bind

- **Oxygen Binders--Hb and Mb**
 - **Binding Equilibria and P_{50} 's**
 - **Hill Coefficients and Cooperativity**
 - **pH, Bohr effect and protons, CO_2 , BPG**
- **Structure**
 - **Porphyrin**
 - **Allostery, concerted, MWC**
 - **Molecular Diseases and Evolution**



O₂ Partial Pressures (Torr = mm Hg)

100

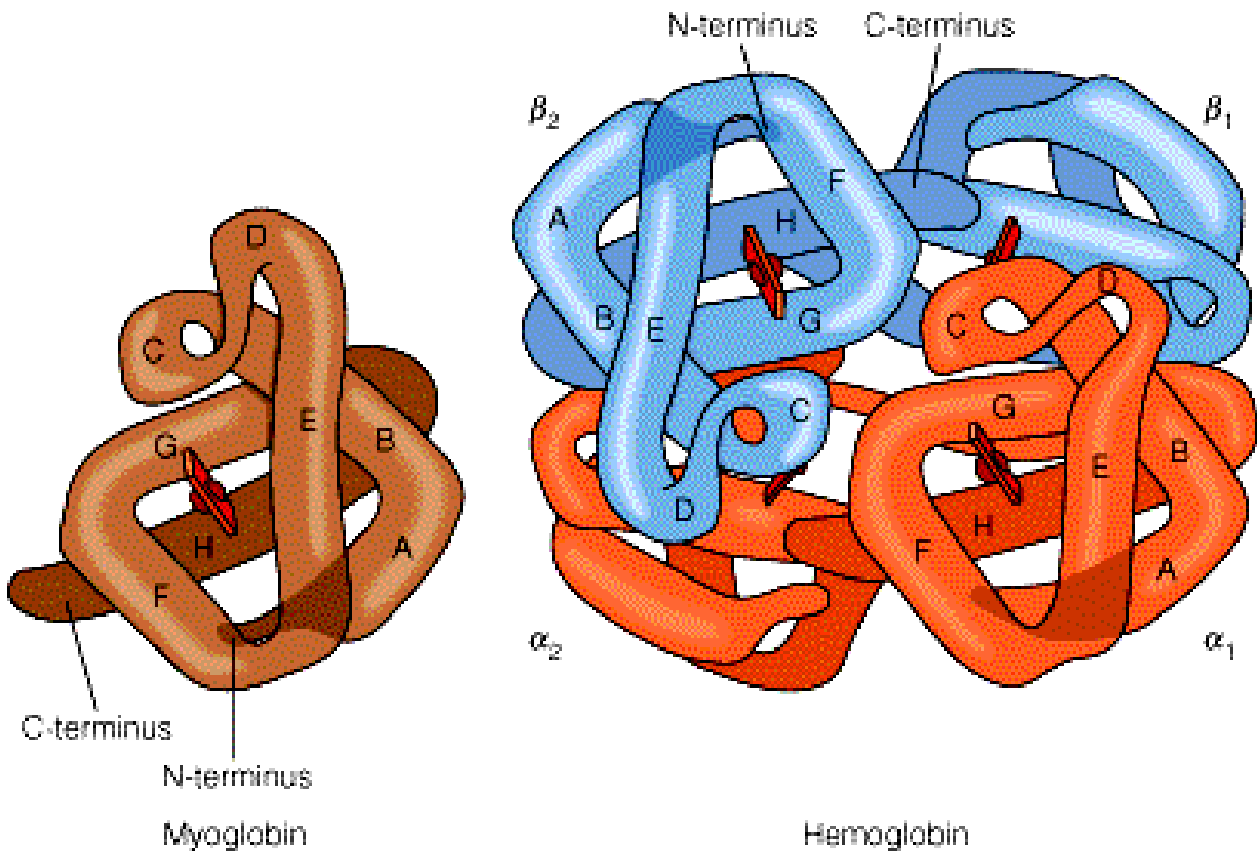
Half saturation

P_{50} Hb ~ 40

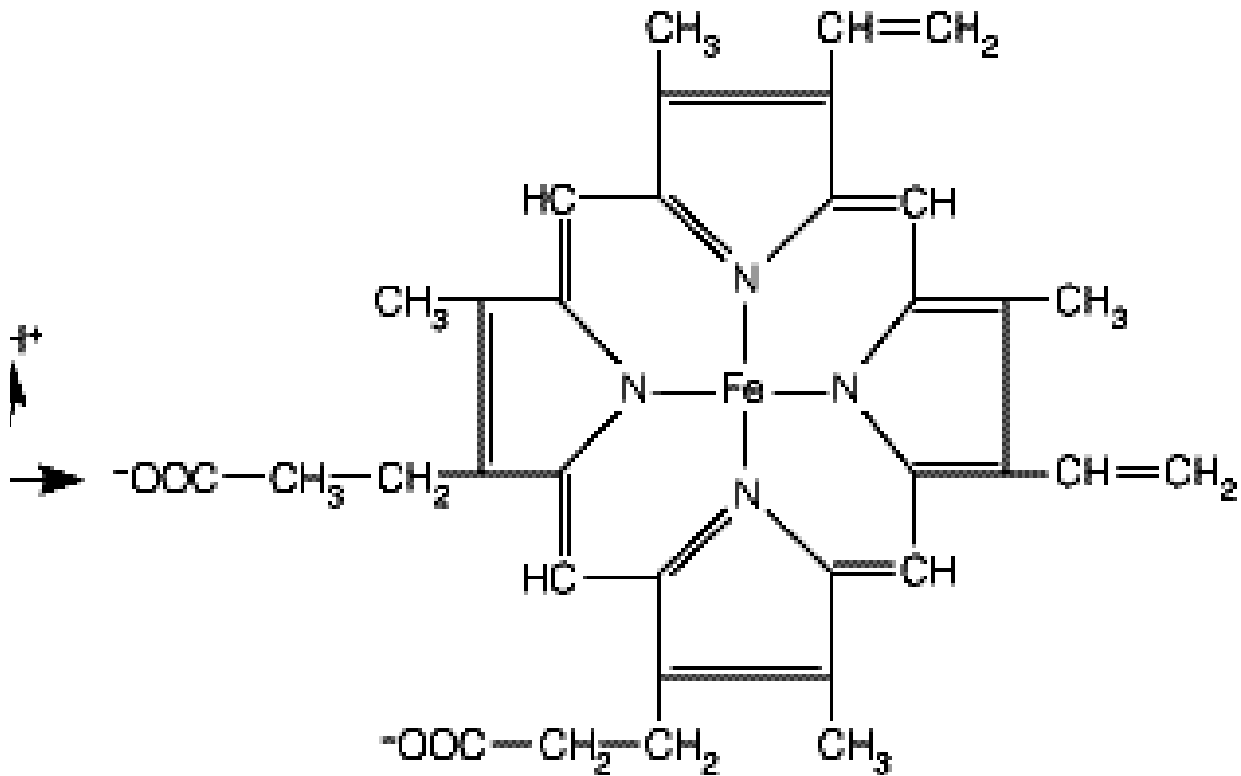
30 (capillaries)

P_{50} Mb = 4

2 (tissues/cells)



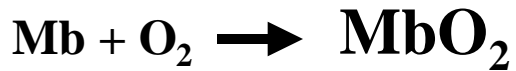
**Quaternary Structure ($\alpha\beta$)₂
Salt Bridges and H bonds**



(c) Ferroporphyrin (heme)

Prosthetic Group within protein--prevents

- 1) irreversible oxygen binding
- 2) Iron oxidation



$$K = \frac{[\text{MbO}_2]}{[\text{Mb}][\text{O}_2]}$$

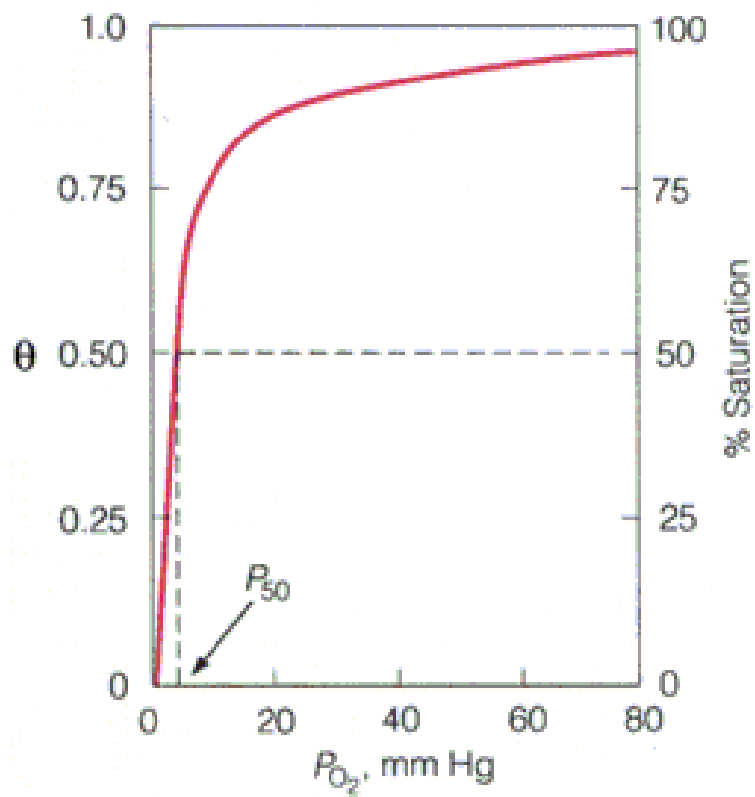
$$\theta = \frac{\text{sites occupied}}{\text{total available sites}}$$

$$\theta = \frac{[\text{MbO}_2]}{[\text{Mb}] + [\text{MbO}_2]} = \frac{K[\text{Mb}][\text{O}_2]}{[\text{Mb}] + K[\text{Mb}][\text{O}_2]}$$

$$\theta = \frac{[\text{O}_2]}{[\text{O}_2]_{1/2} + [\text{O}_2]}$$

Measure Henry's Law

$$\theta = \frac{P_{\text{O}_2}}{P_{50} + P_{\text{O}_2}}$$



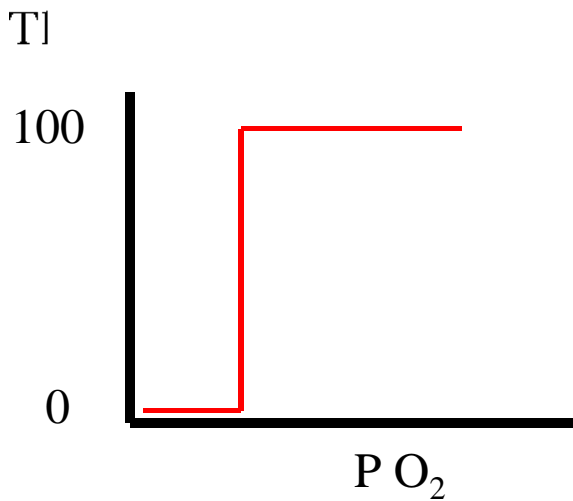
Measure
Spectroscopically

$$\theta = \frac{P_{O_2}}{P_{50} + P_{O_2}}$$

Hb design requirements--P₅₀ needs to depend on Saturation???

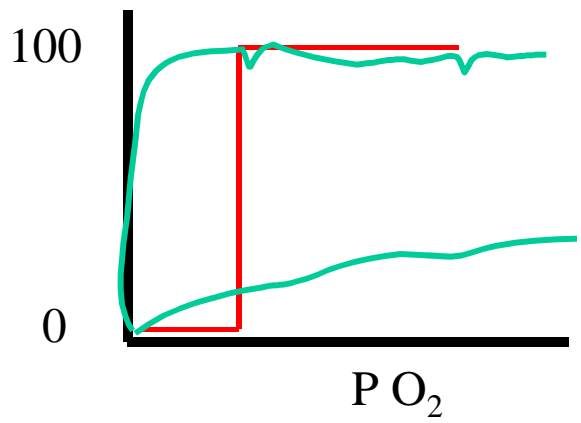
Should be fully saturated at P O₂ = 100 mm Hg (Theta = 100%)

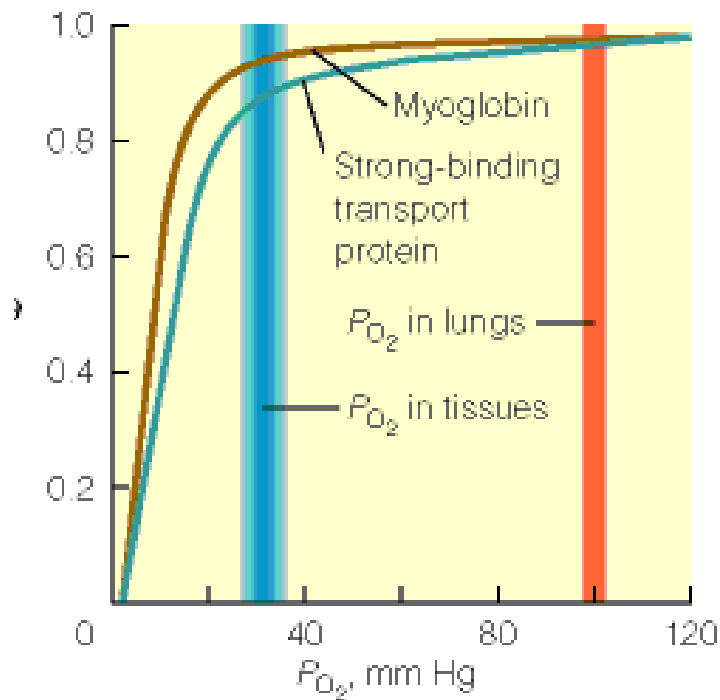
Should bind no O₂ at 40 mm Hg (Theta = 0%)



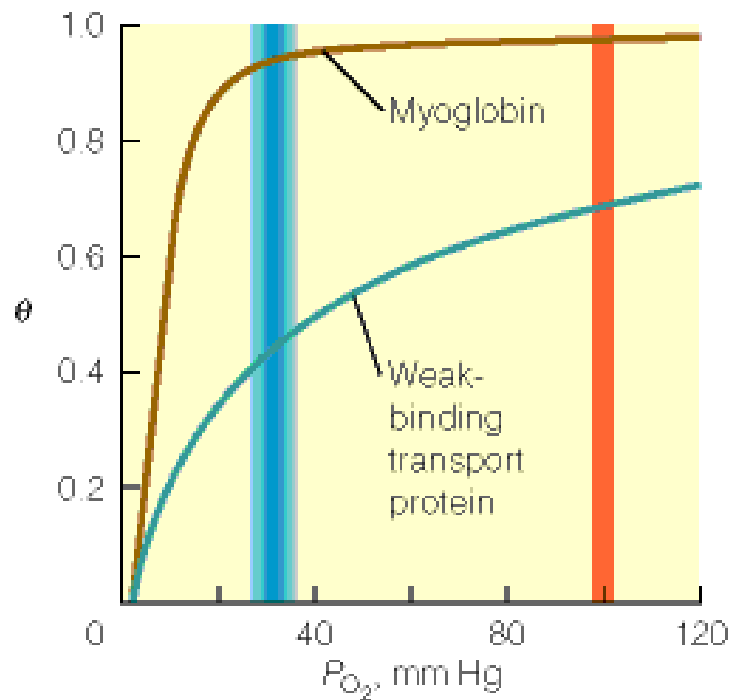
P₅₀ Low

P₅₀ High

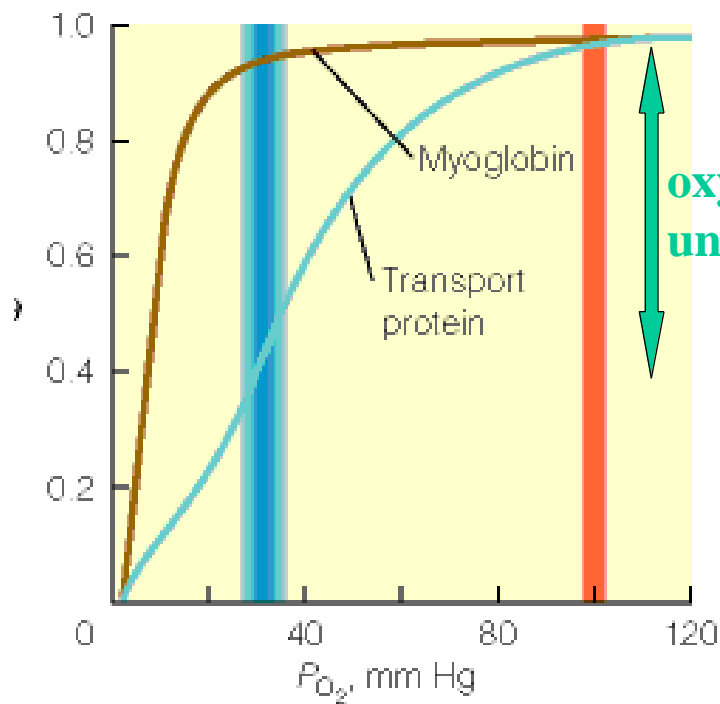




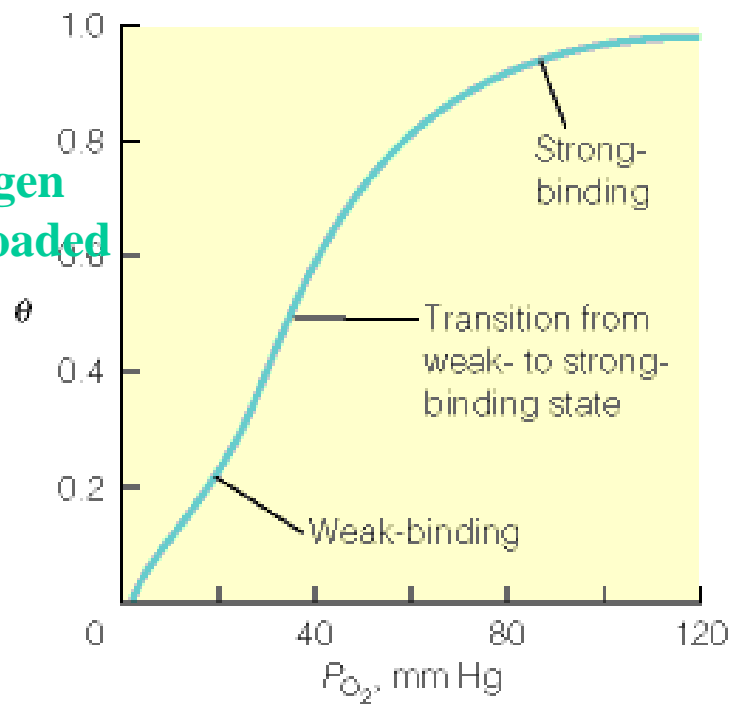
(a) Transport protein efficient in binding but inefficient in unloading (hyperbolic binding curves)



(b) Transport protein efficient in unloading but inefficient in binding (hyperbolic binding curves)



(c) Transport protein efficient in both binding and unloading, because it has a sigmoidal binding curve.



(d) Switch from weak- to strong-binding state explains the sigmoidal curve

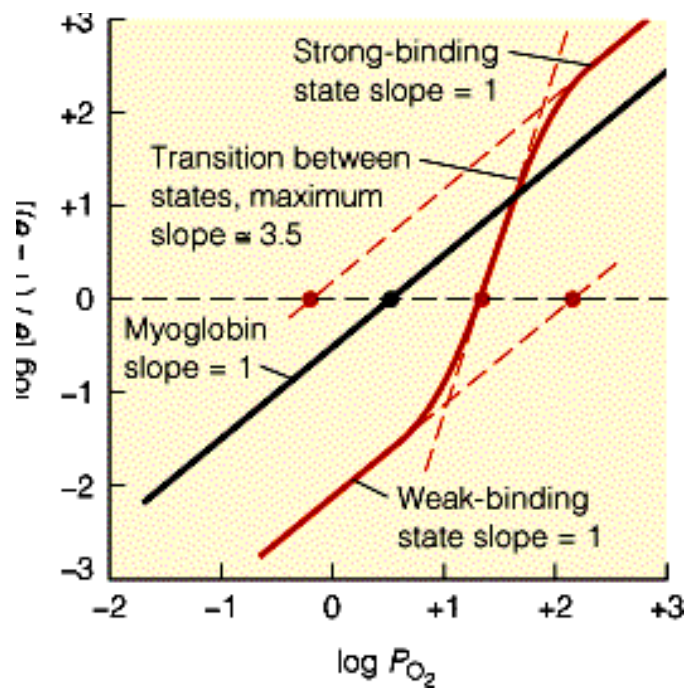


$$K = [\text{Hb} (\text{O}_2)_n] / [\text{Hb}][\text{O}_2]^n$$

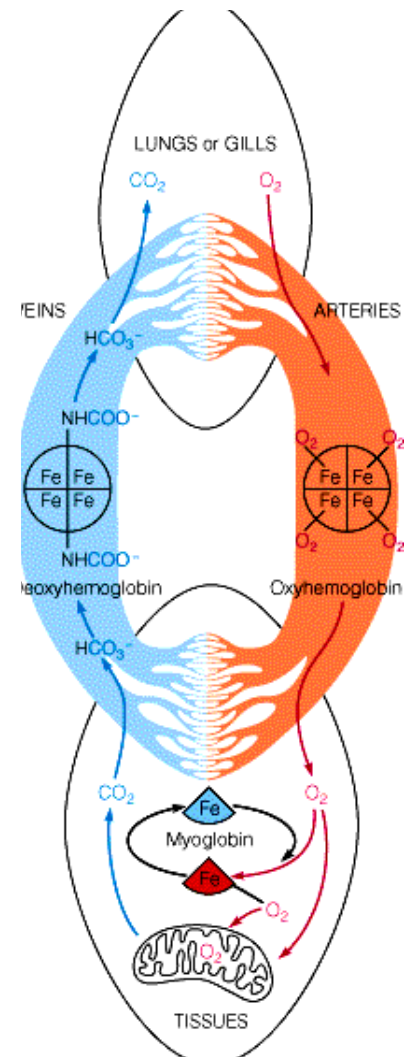
$$\theta = \frac{P_{\text{O}_2}^n}{P_{50}^n + P_{\text{O}_2}^n} \quad \frac{\theta}{1 - \theta} = \frac{P_{\text{O}_2}^n}{P_{50}^n}$$

$$\log \frac{\theta}{1 - \theta} = \log P_{\text{O}_2}^n - \log P_{50}^n$$

$$\log \frac{\theta}{1 - \theta} = n \log P_{\text{O}_2} - n \log P_{50}$$



N measures cooperativity-- the steepness of the transition from weak to strong binding near 50% saturation.



O_2 Partial Pressures (Torr = mm Hg)

100

Half saturation

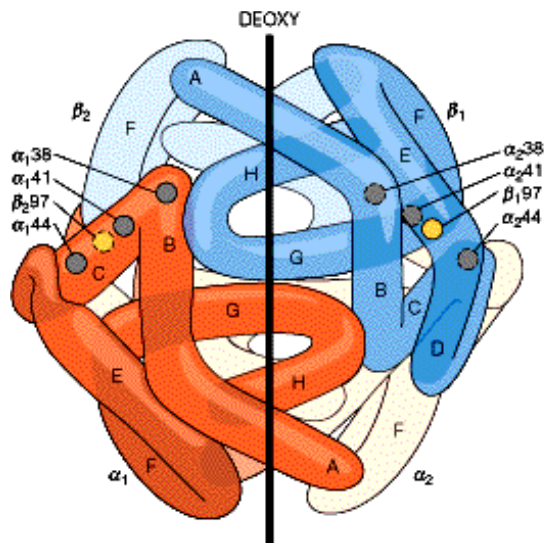
$P_{50} \text{ Hb} \sim 40$

30 (capillaries)

$P_{50} \text{ Mb} = 4$

2 (tissues/cells)

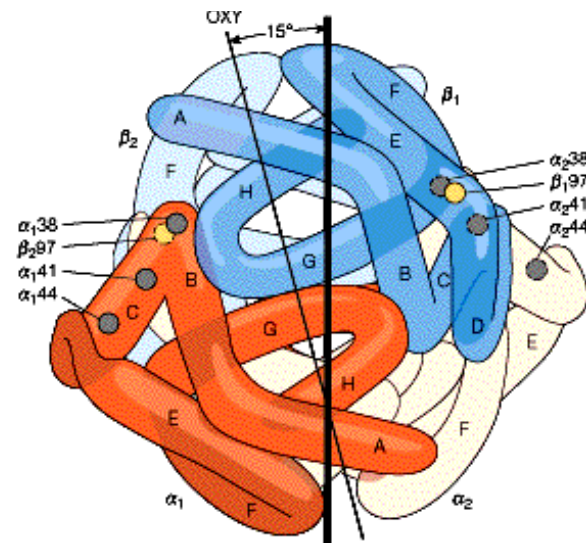
T = Deoxy



(a)

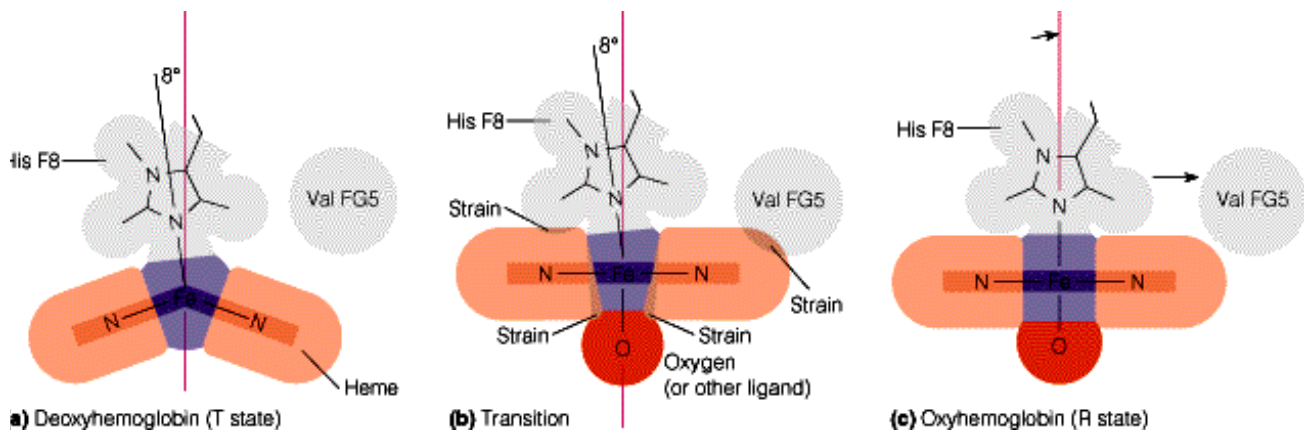
His 97 / Thr 41
Asp 99 / Tyr 42

R = Oxy



His 97 / Thr 38
Asp 102 / Asp 94

Roots of Allostery

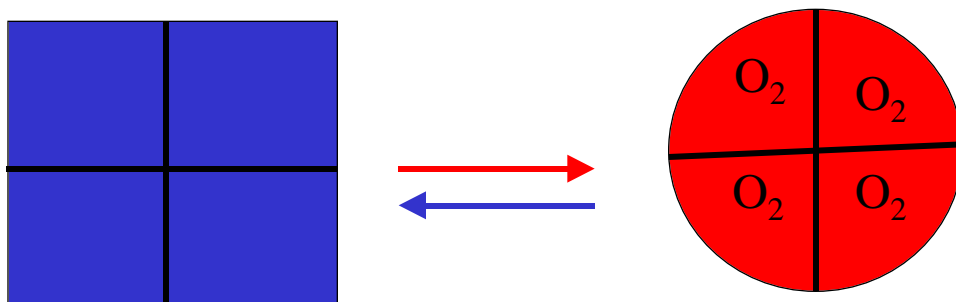


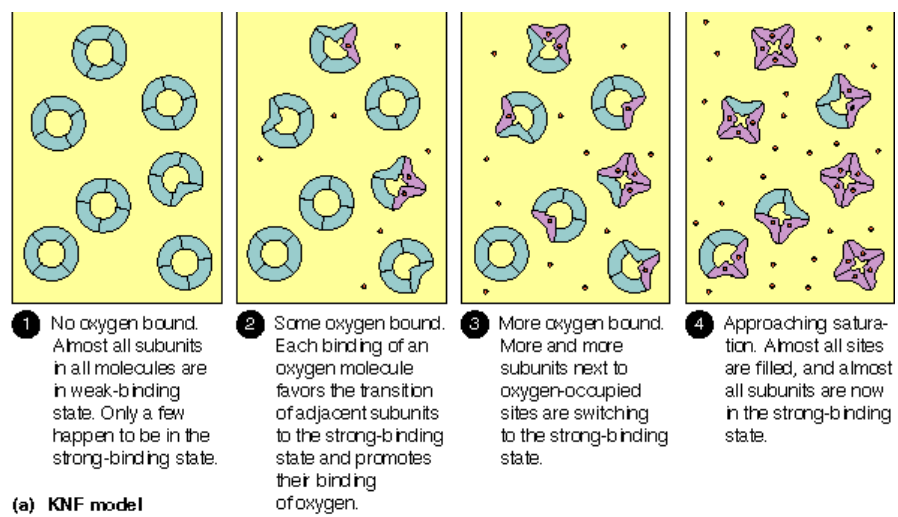
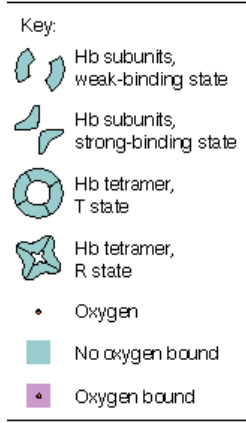
Domed Deoxy Heme >>>> Flat Oxy Heme clashes with valine >>>>
More rearrangements

MWC MODEL of Allostery

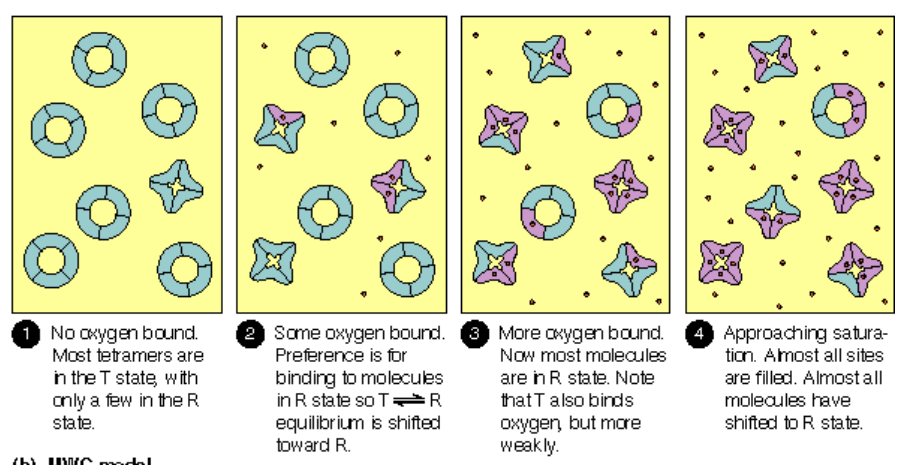
- Strong and weak binding conformations
 - Multiple Subunits
 - Subunit Interfaces communicate conformational change
 - **Concerted conformational change**
-
- Allosteric ligands shift conformational equilibria by preferentially binding one conformation

Allosteric Effectors bind the strong binding form
Allosteric Inhibitors bind the weak binding form



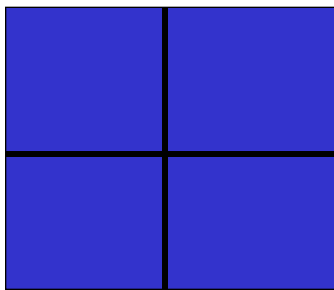


(a) **KNF model**



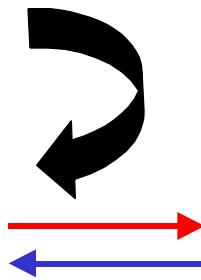
(b) **MWC model**

146 His+ :::Asp 94
Salt bridge T form only



Taut form

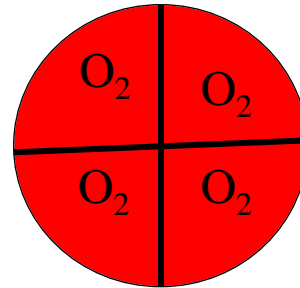
H^+
 CO_2



pK_a His 146 deoxy $>< =$

pK_a His 146 oxy

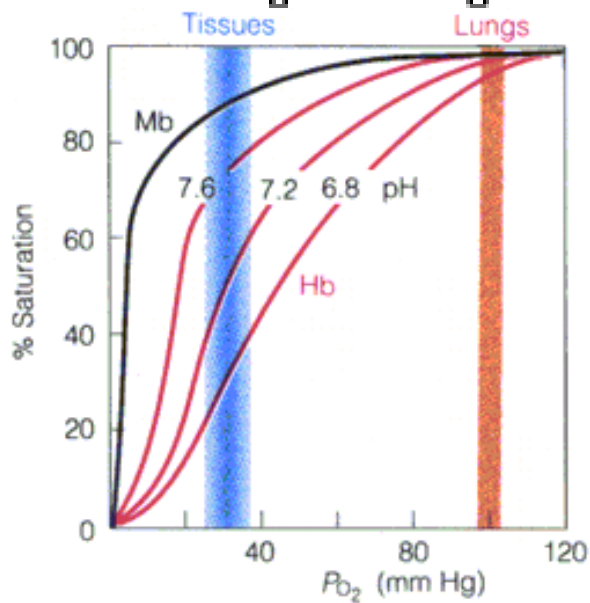
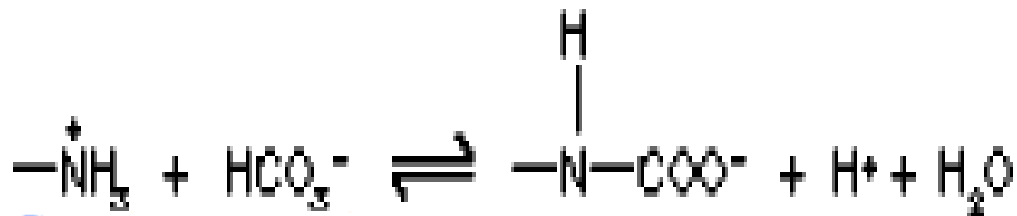
Why???



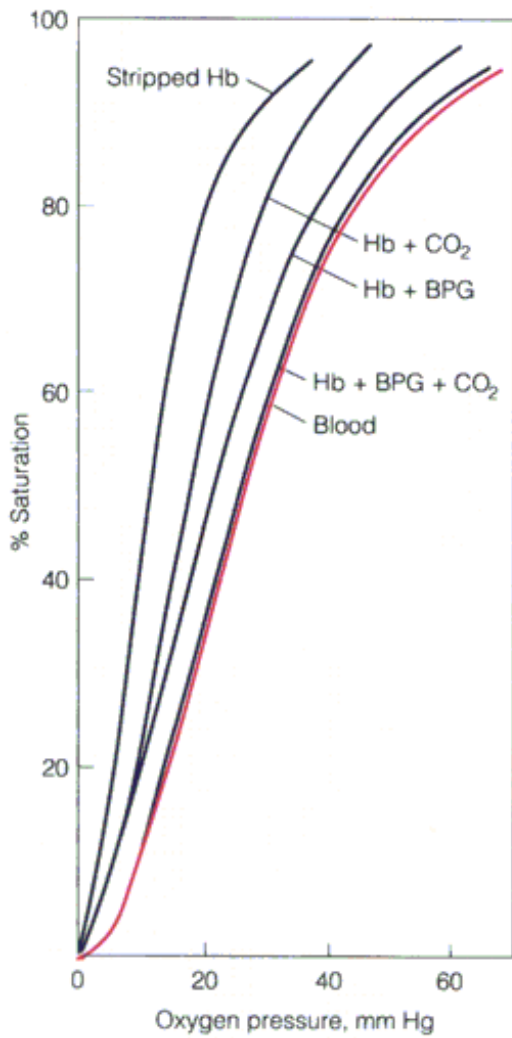
Relaxed form

Allosteric “other place” ligands bind to T form and favor Oxygen release

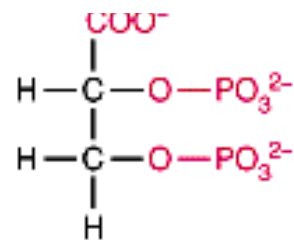
Bohr Effect--acidic pH weakens O₂ binding



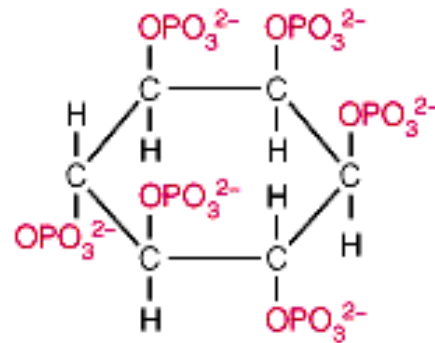
- charge at amino terminus favors deoxy / salt bridge



BPG weakens oxygen binding and is found in individuals living in low oxygen environments



a) 2,3-Bisphosphoglycerate



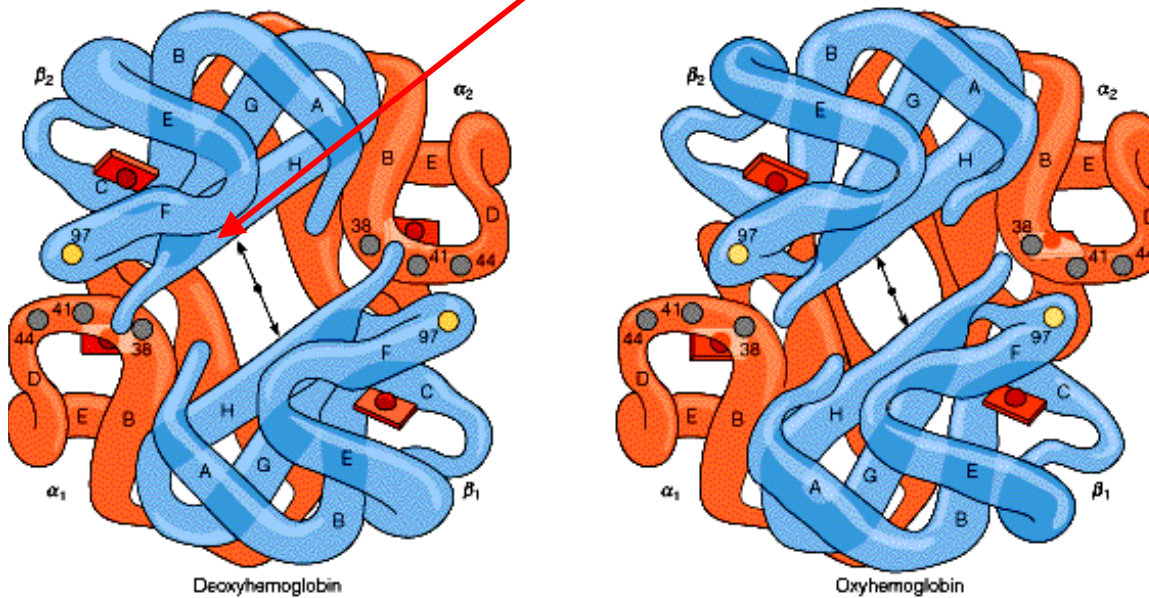
b) Inositol hexaphosphate

Athletes often feel muscular pain during a strenuous workout due to the build up of lactic acid. The lactic acid is a product of glucose metabolism and is produced when the body's demand for oxygen outstrips the supply.

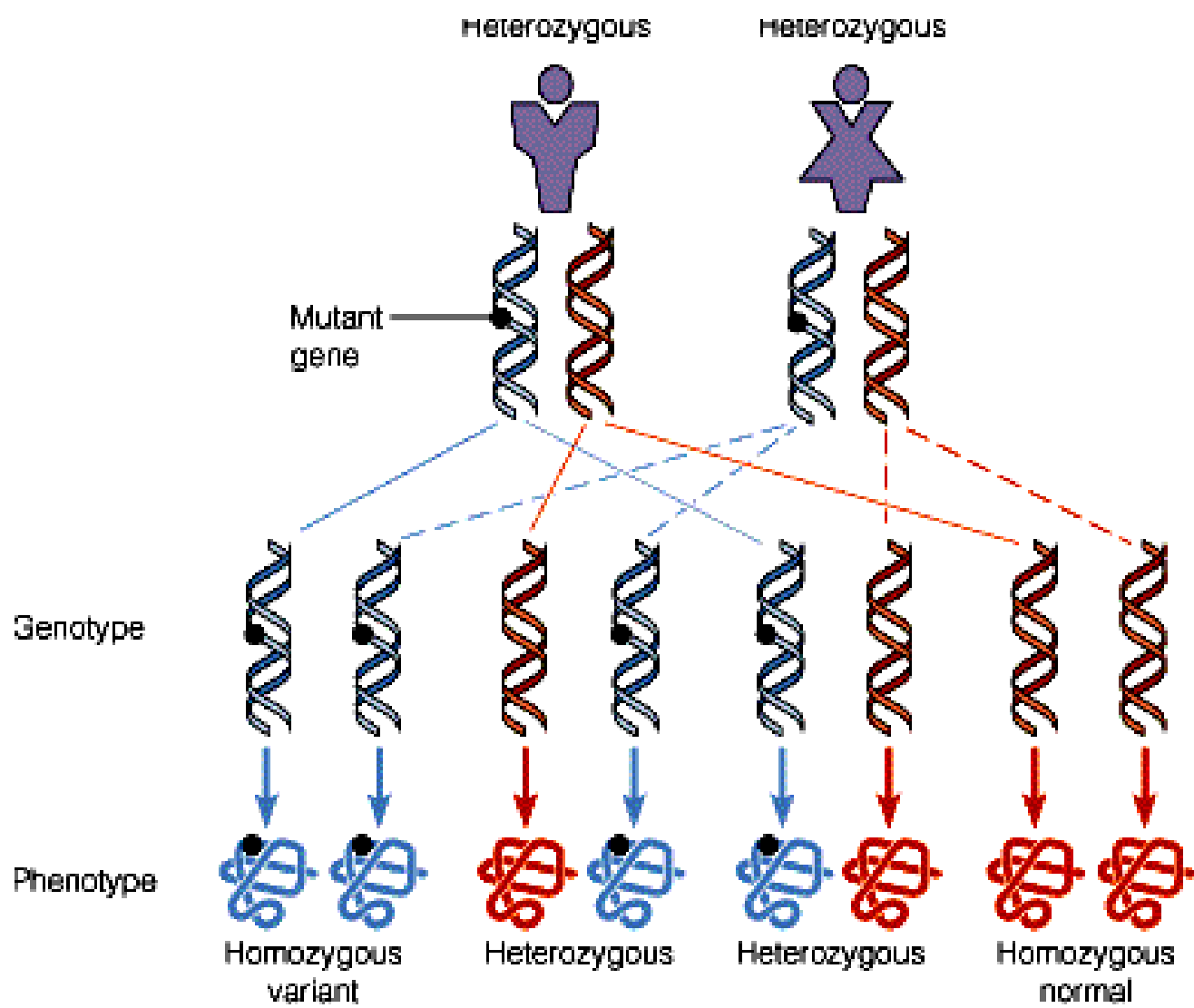
How does acid production help or hinder a return to oxidative metabolism?

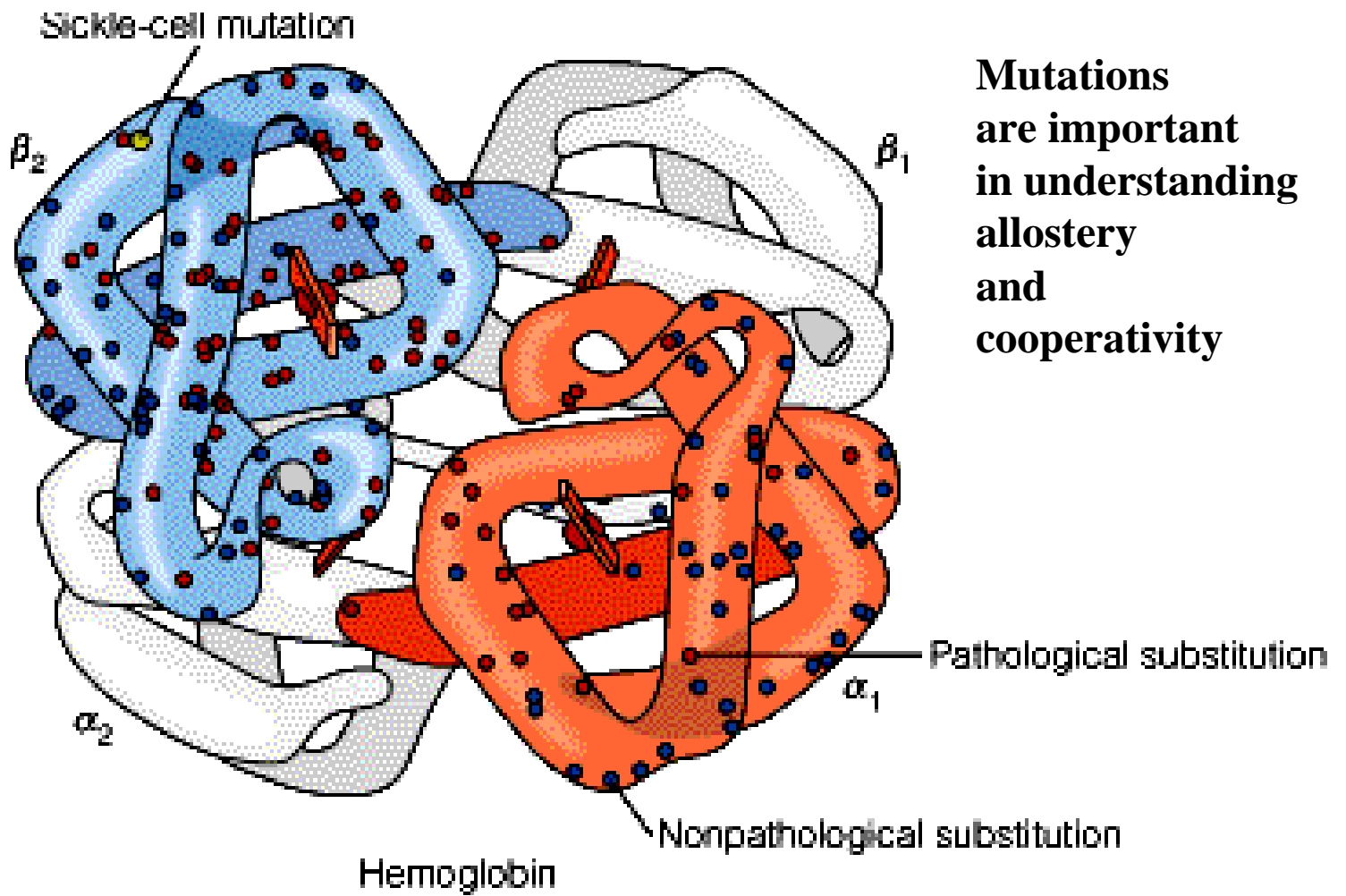
Deoxy binds DPG

In fetal γ 143His⁺ is replaced by serine to weaken DPG binding and strengthen oxygen binding.



Residue number		1	2	3	4	5	6	7	8	9	10	
Normal β gene	...A T G	G T G Val	C A C His	C T G Leu	A C T Thr	C C T Pro	G A G Glu	G A G Glu	A A G Lys	T C T Ser	G C C Ala	...
(a) Missense mutation		G T G Val	C A C His	C T G Leu	A C T Thr	C C T Pro	G T G Val	G A G Glu	A A G Lys	T C T Ser	G C C Ala	...
(b) Nonsense mutation		G T G Val	C A C His	C T G Leu	A C T Thr	C C T Pro	G A G Glu	G A G Glu	T A G Stop	T C T Ser	G C C Ala	...
(c) Frameshift mutation by deletion		G T G Val	C A C His	C T G Leu	A C C	C T G Leu	A G G Arg	A G A Arg	A A G Ser	T C T Ser	G C C Ala	...

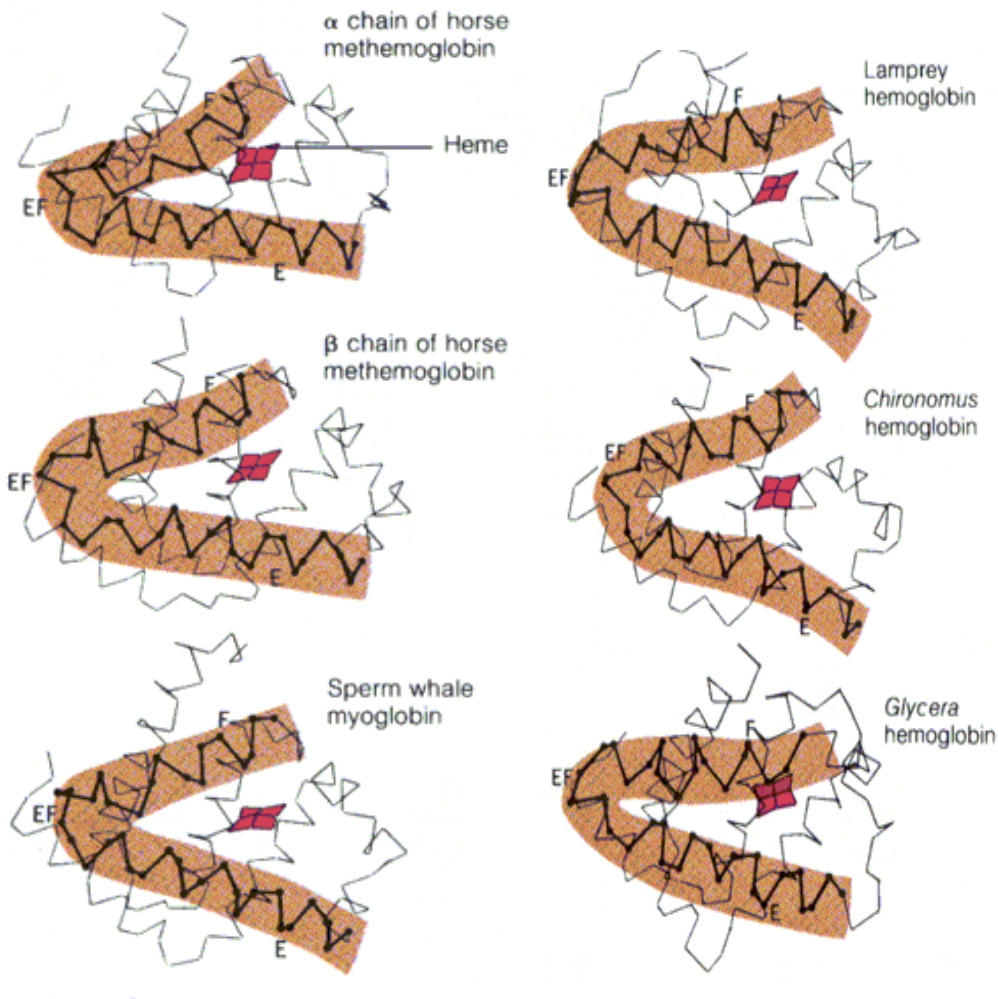




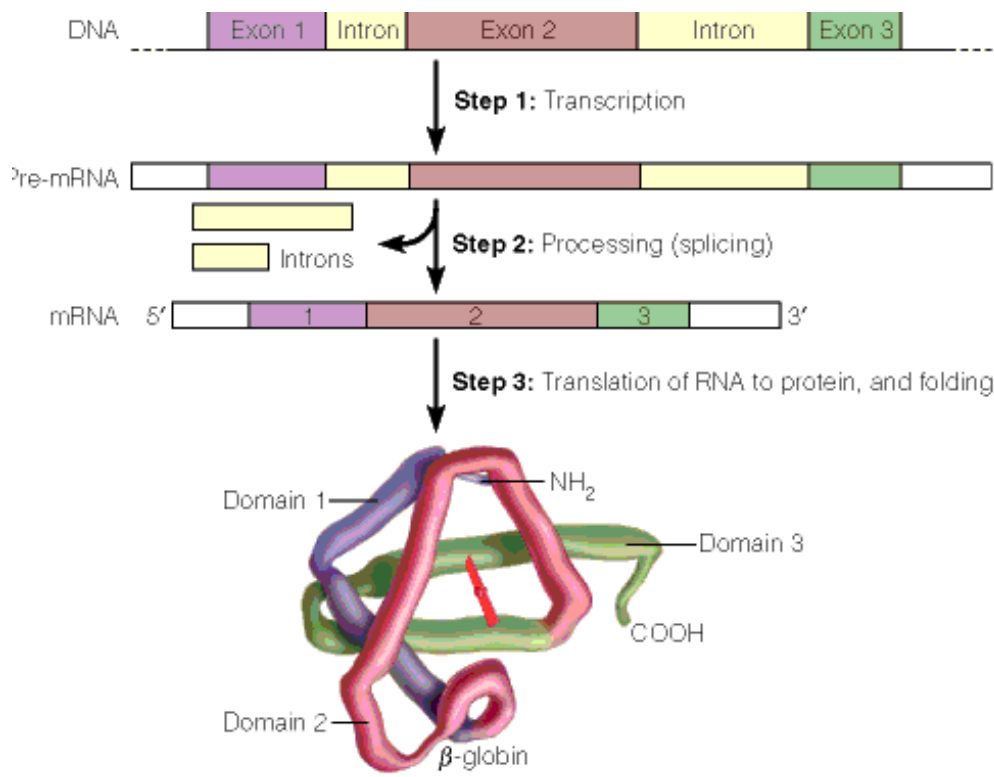
Mutations are important in understanding allostery and cooperativity

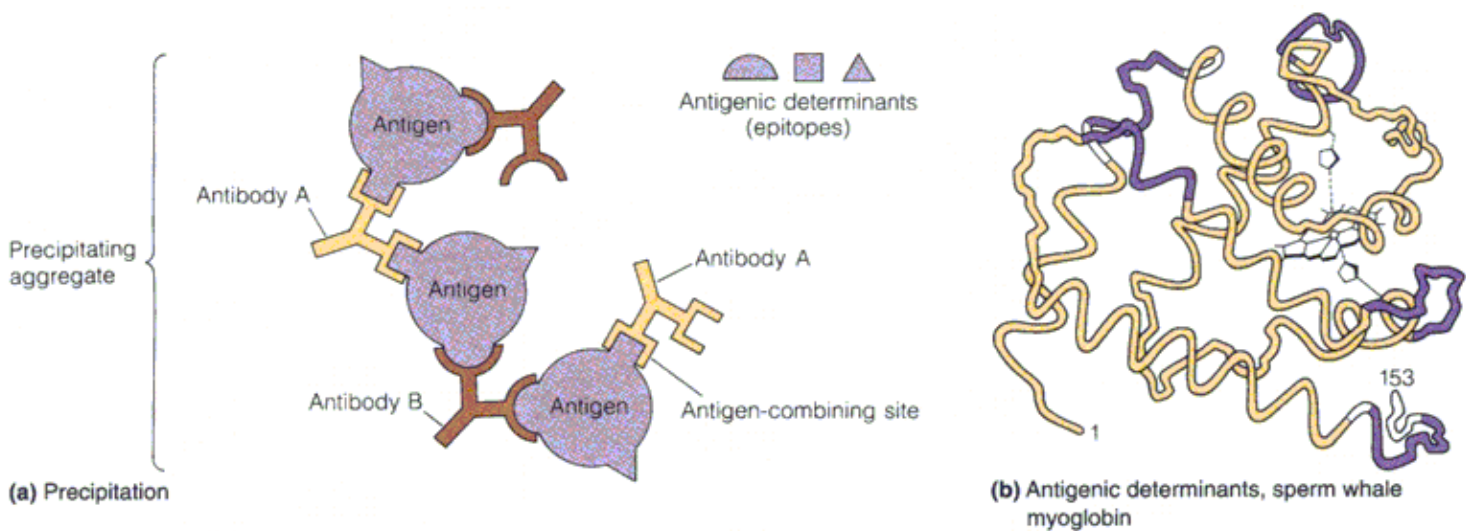


Gene duplications and mutations lead to diversification

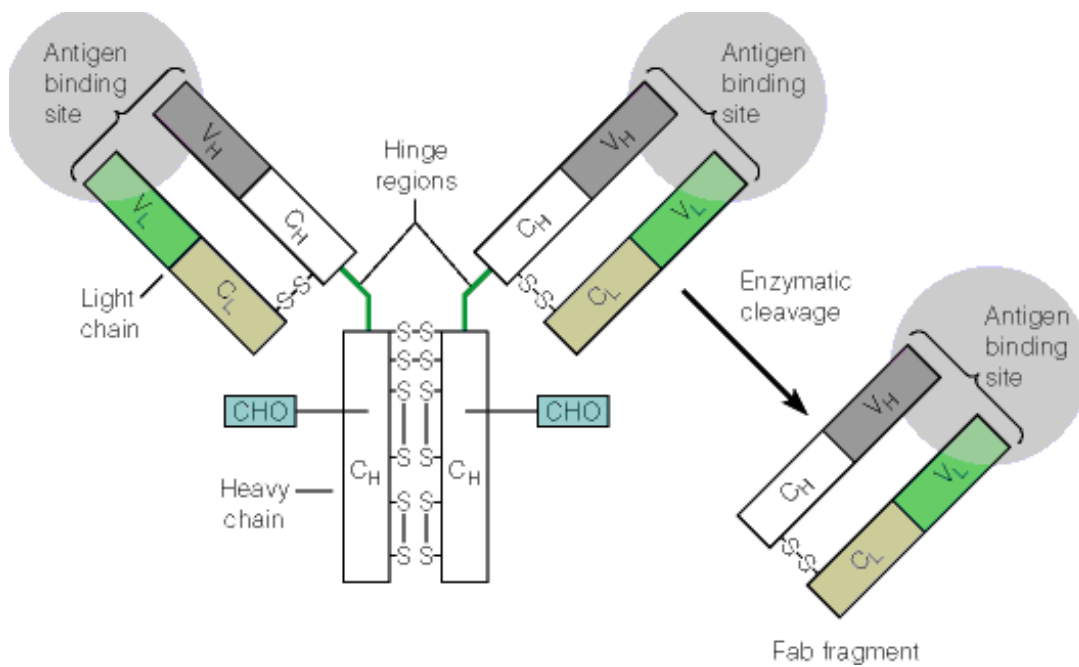


**Evolution
 acts on
 function and
 thus conserves
 3D structures**

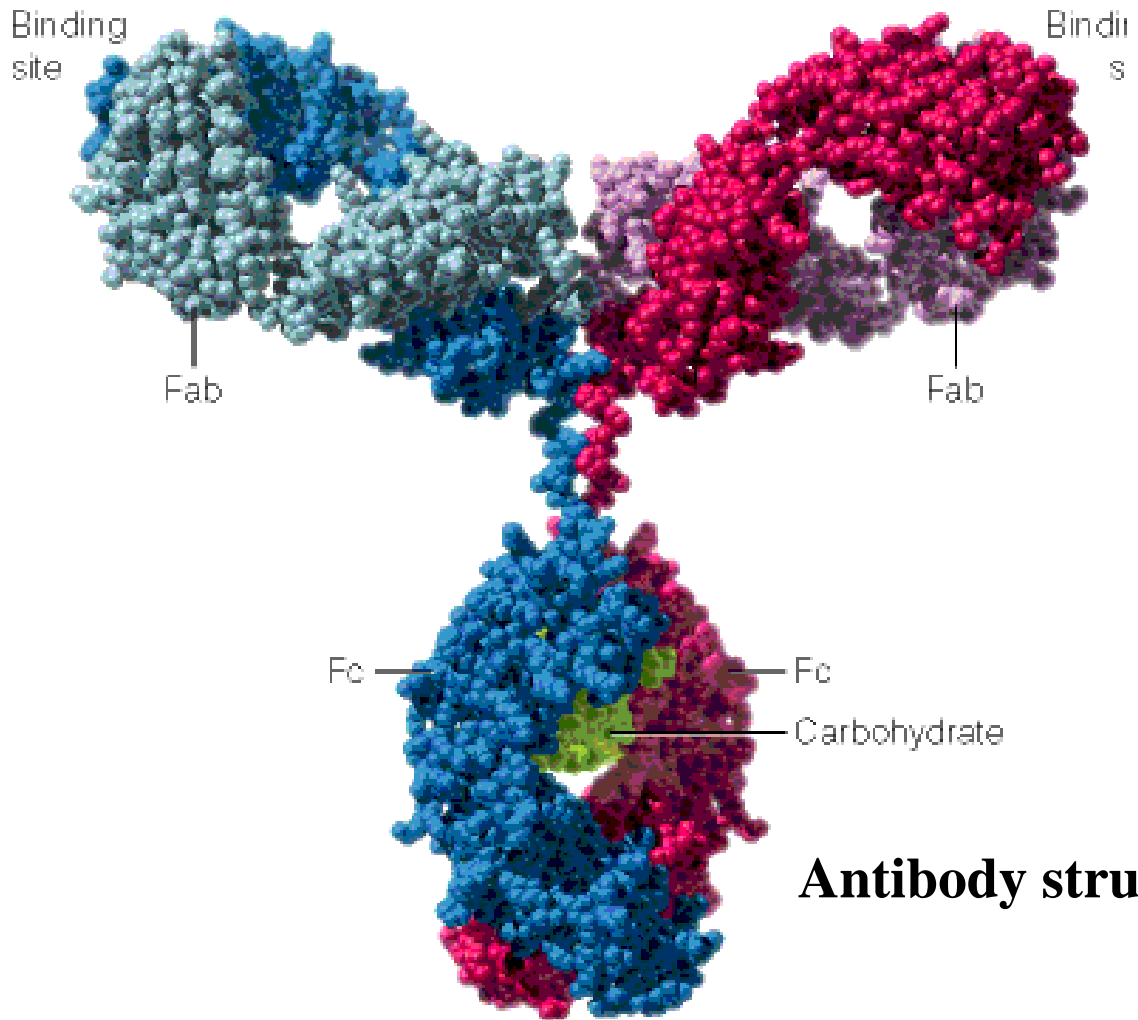




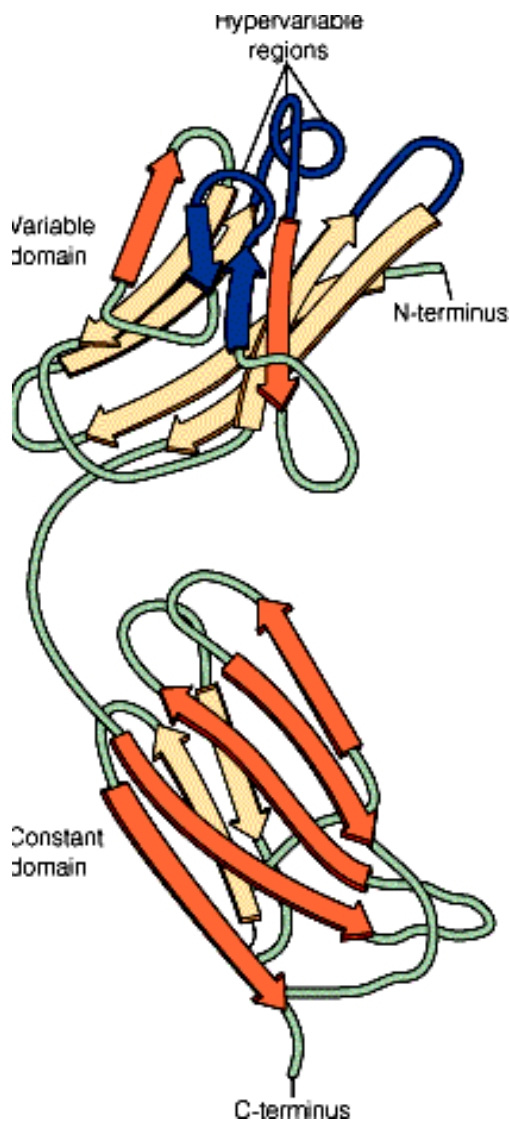
An individual's antibody proteins bind to foreign antigens



**Each antibody has 2 heavy chains and 2 light chains.
Antigens bind the variable regions.**

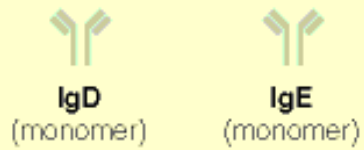
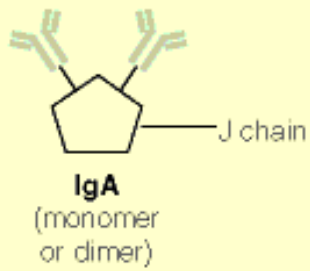
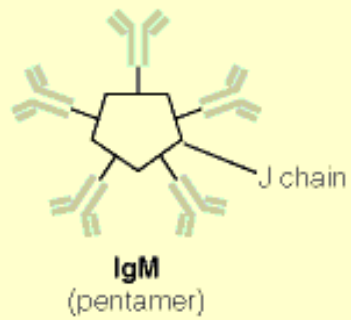


Antibody structure

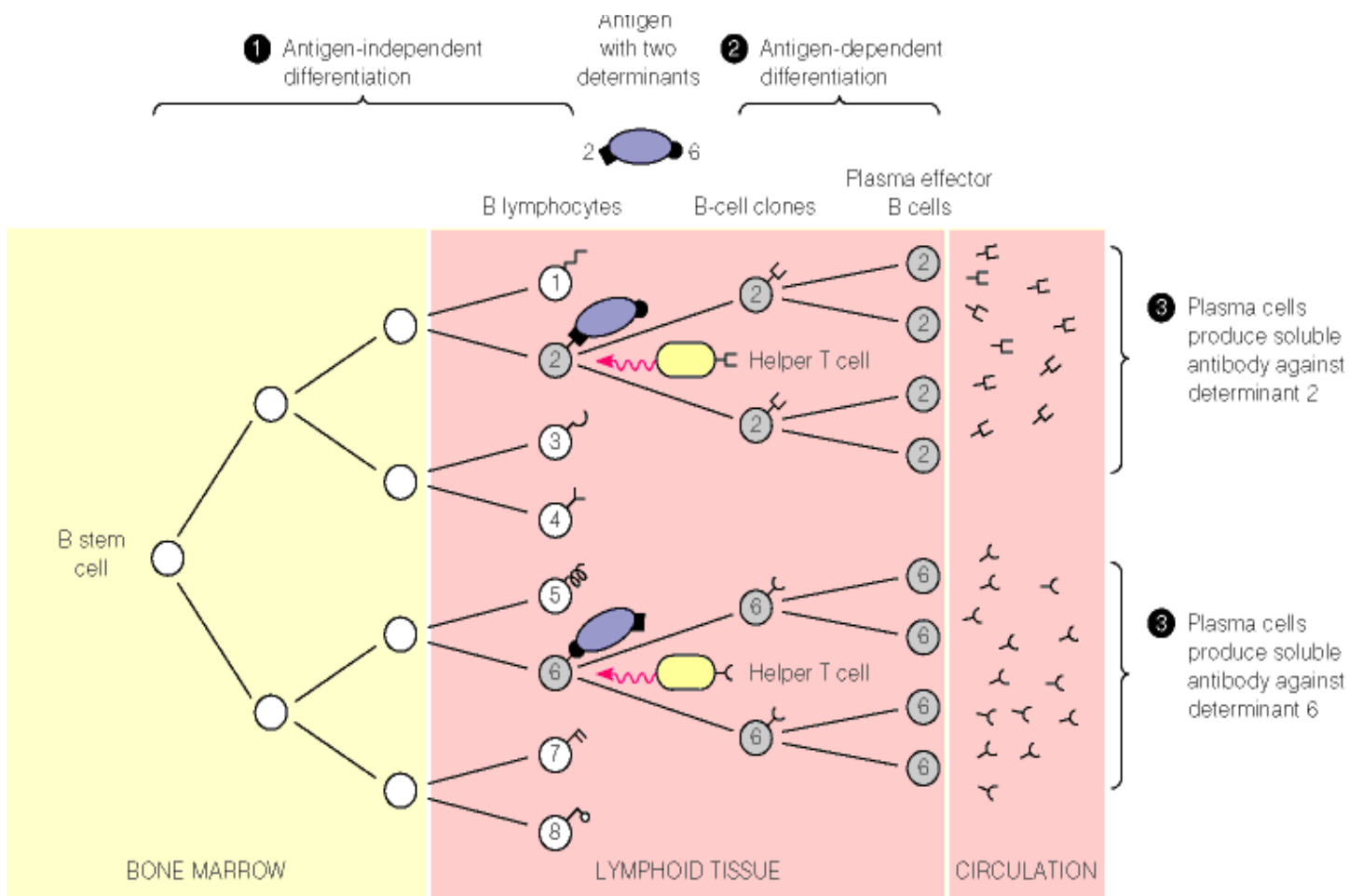


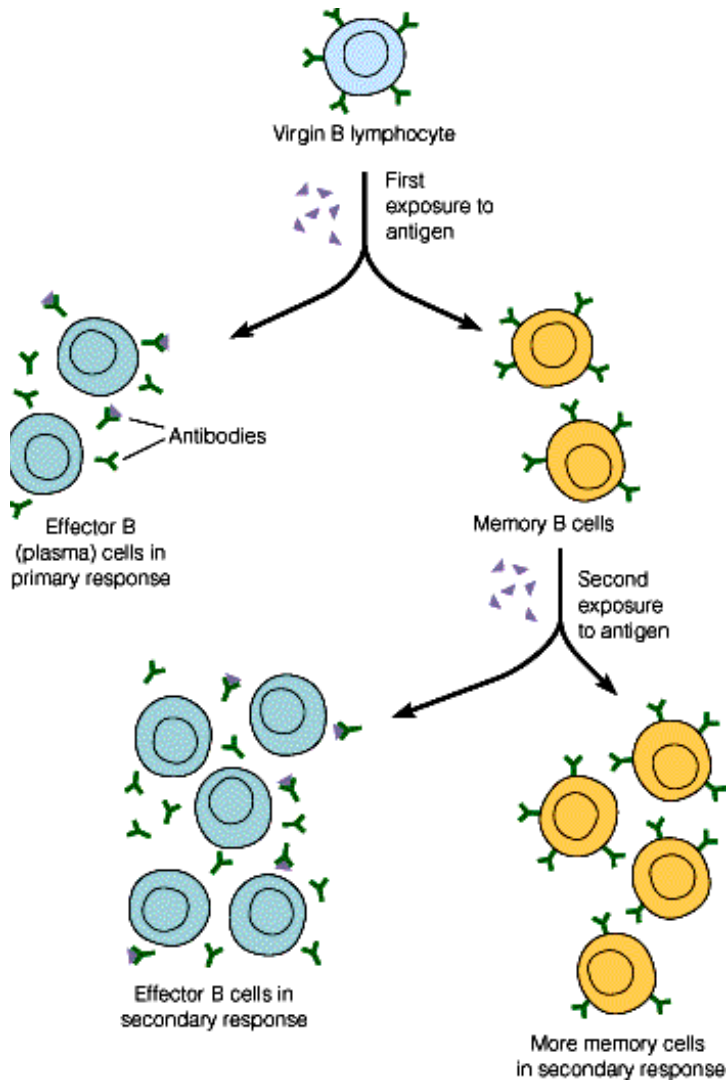
The antibody fold, two beta sheets packed against each other, occurs repeatedly in immune system proteins. This is the result of gene duplication throughout Evolution.

Variety in the hypervariable Regions is generated by combinatorially (at the DNA level) and by a high rate of somatic mutation.



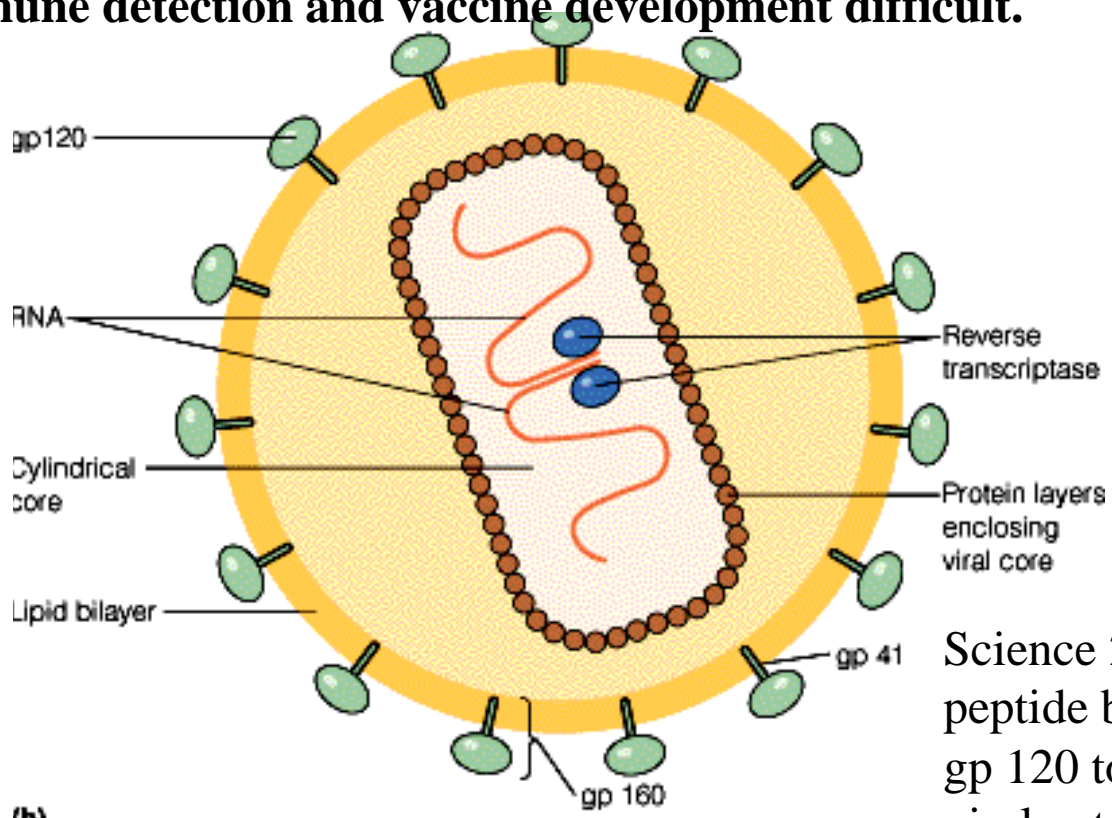
Immunoglobulin proteins



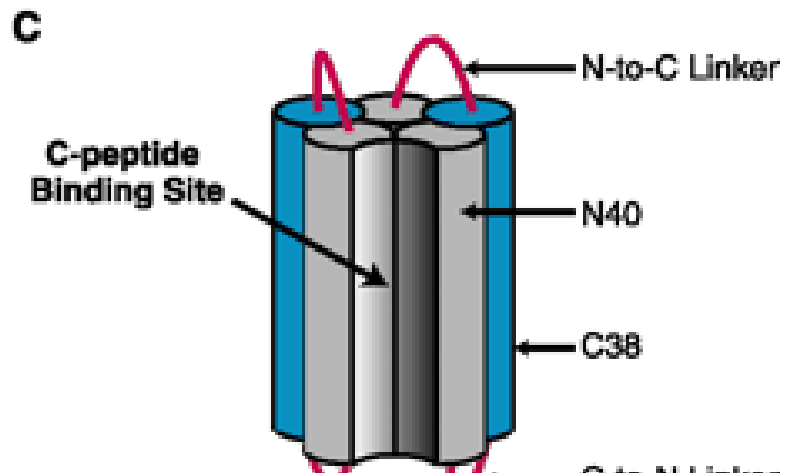
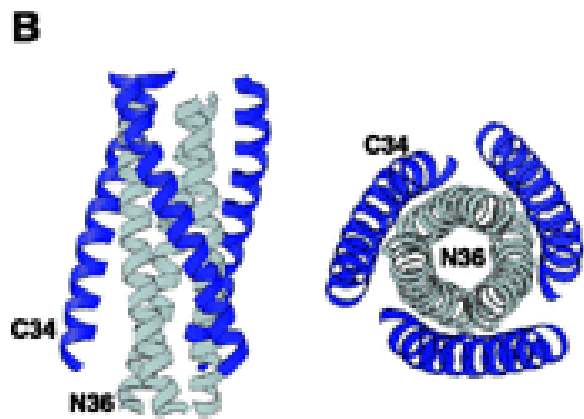
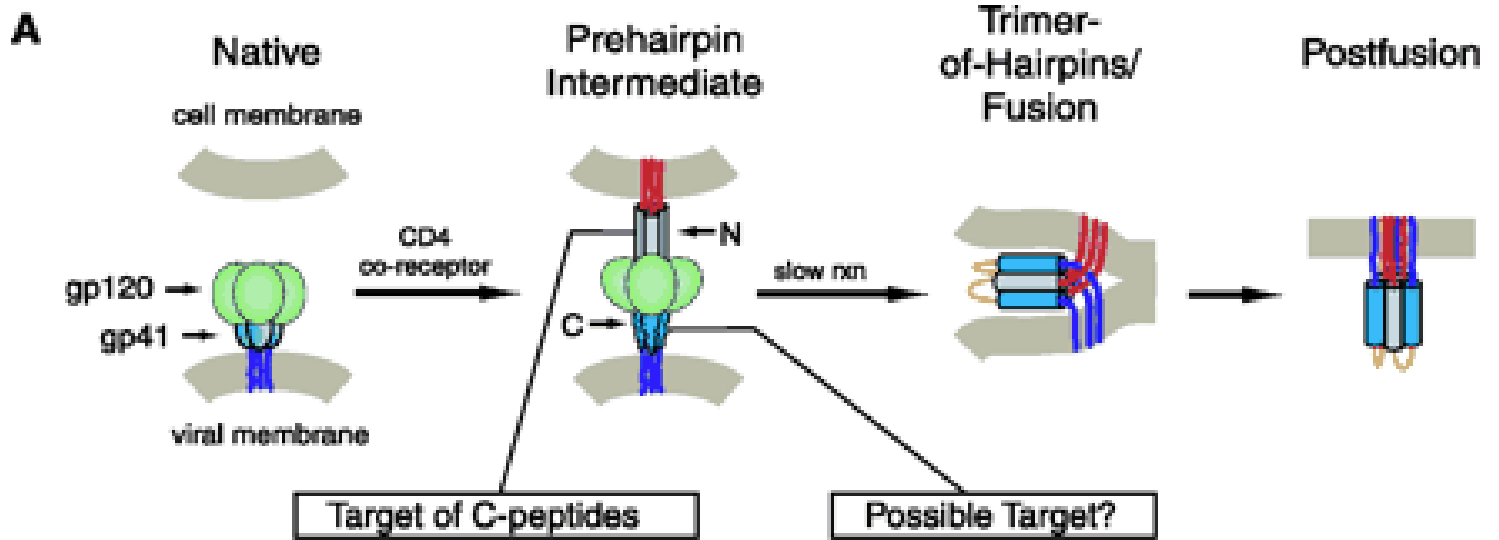


Antigens stimulate production of antibodies and B cells which bind that antigen. Second exposures quickly mobilize the immune system.

**HIV invades the immune system's T cells.
Its rapid rate of mutation of surface proteins makes
immune detection and vaccine development difficult.**



Science 2/2/01
peptide binds
gp 120 to block
viral entry.



Problem 6--Chap. 7 Page 251

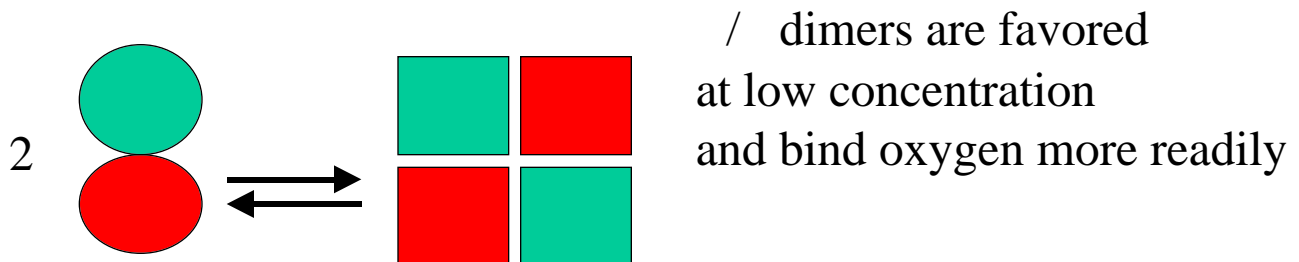
Ackers et al. have observed the the P_{50} of purified hemoglobin decreases as the concentration of hemoglobin in solution is decreased.

Suggest an explanation.

Problem 6--Chap. 7 Page 251

Ackers et al. have observed the the P_{50} of purified hemoglobin decreases as the concentration of hemoglobin in solution is decreased.

Suggest an explanation.



Problem 8

Suggest probable consequences of the following real or possible hemoglobin mutations.

Consult Figures 7.12, 7.18 and Table 7.2

A) At β 146 His to Asp

C) At β 2 His to Asp

B) At B92 His to Leu

In each case tell whether a single base change

Is sufficient for this mutation.

Problem 8

A) At β 146 His to Asp

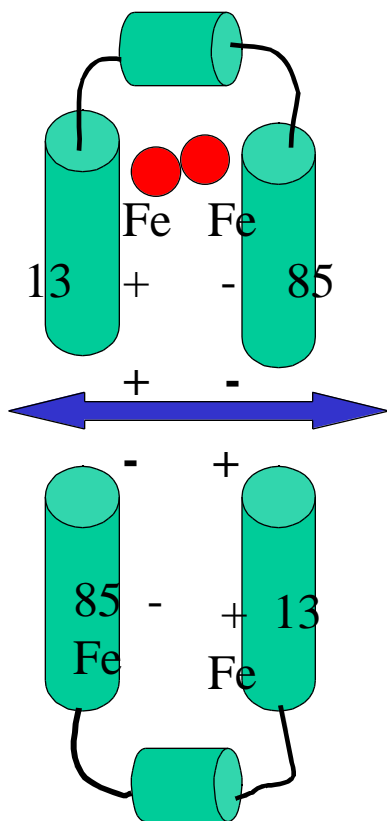
C) At β 2 His to Asp

Figure 7.18

Both mutations replace a positive charge in the DPG binding pocket with a negative charge. DPG binding will be greatly reduced and thus the R form will be stabilized and oxygen binding facilitated. Yes, can be caused by CAU to GAU.

B) At B92 His to Leu

Table 7.2--This removes the N which is the 5th ligand. Porphyrin will bind more weakly. Yes, can be caused by CAU to CUU.

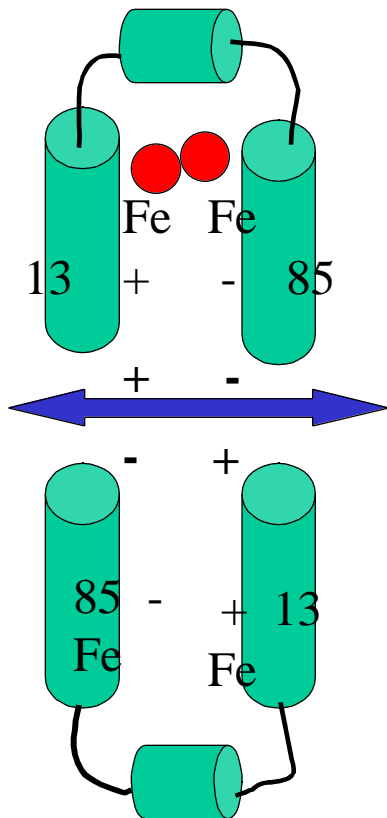


Problem 12

Assume that a new oxygen-transport protein has been discovered in certain invertebrate animals. X-ray diffraction of the deoxy protein reveals that it has the dimeric structure shown here with a salt bridge between residues His 13 and Asp 85.

The two monomers interact by salt bridges between carboxyl termini and N-termini.

The O_2 site lies between the two iron atoms shown, which are rigidly linked to a helices. In the deoxy form, the space between the iron atoms is too small to hold O_2 , and so the Fe atoms must be forced apart when O_2 is bound.



Problem 12

- Will the molecule show cooperative oxygen binding?
- Will this molecule exhibit a Bohr effect?
- What would be the effect of a mutation which replaced Asp 85 by Lys?

Problem 12

A) Will the molecule show cooperative oxygen binding?

Yes, the first oxygen will force apart the two subunits and because the subunits are linked by a salt bridge the second monomer will follow, thus facilitating oxygen binding.

B) Will this molecule exhibit a Bohr effect?

yes, a neutral His at basic pH will favor oxygen binding.

C) What would be the effect of a mutation which replaced

Asp 85 by Lys?

This will eliminate the 13/85 salt bridge, facilitating oxygen binding, and decreasing cooperativity.

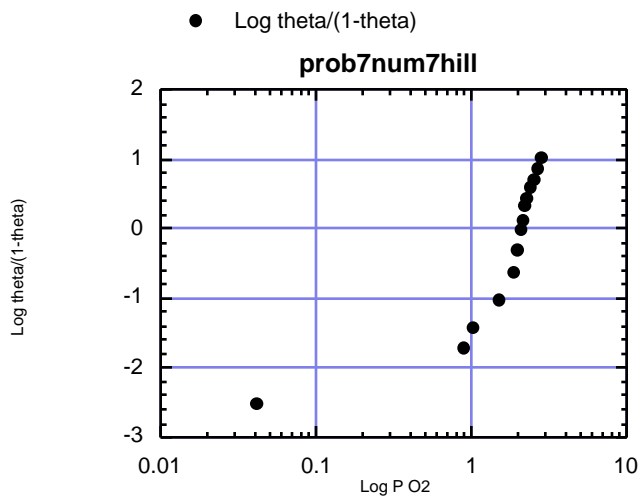
Problem 7 (Hints)

Oxygen binding by the hemocyanin of the shrimp *Callinectes* has been measured. Using the following data, prepare a Hill plot and determine

A) P_{50} (P at 50% saturation or $\log \theta/(1-\theta) = 0$)

B) n_H (the Hill coefficient) (slope at $\log \theta/(1-\theta) = 0$)

C) minimum number of oxygen binding sites ($\geq n_H$)



Log P O2	Log $\theta/(1-\theta)$
0.041393	-2.5216
0.88649	-1.7129
1.0294	-1.4405
1.5011	-1.0376
1.8567	-0.62973
2.0022	-0.30953
2.0910	-0.022588
2.1358	0.099451
2.2222	0.31347
2.3079	0.44081
2.4186	0.58595
2.5145	0.70106
2.6551	0.84510
2.8673	1.0210

