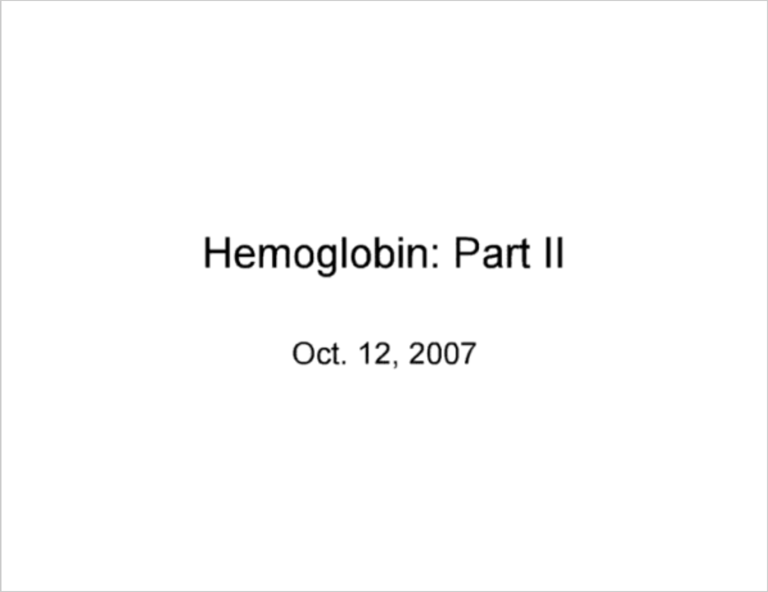
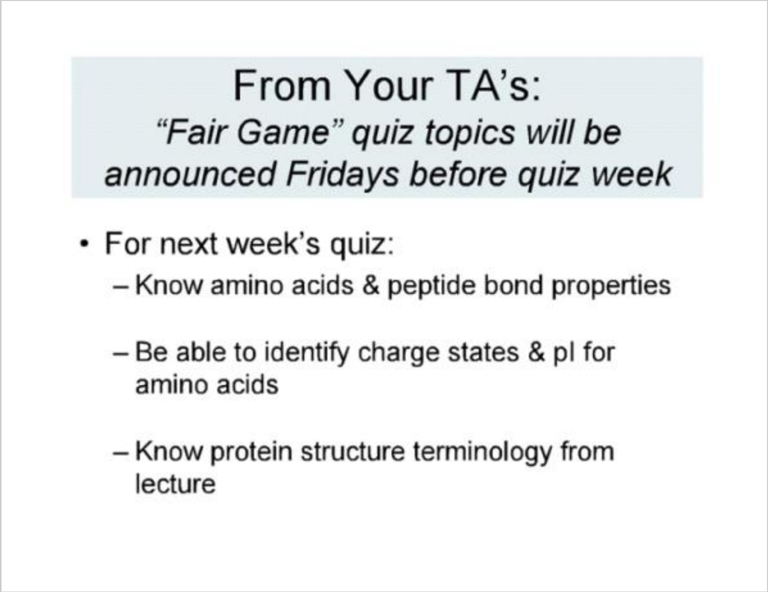
Notes 10/12

Friday, October 12, 2007
10:00 AM



Hemoglobin: Part II

Oct. 12, 2007



From Your TA's:

"Fair Game" quiz topics will be announced Fridays before quiz week

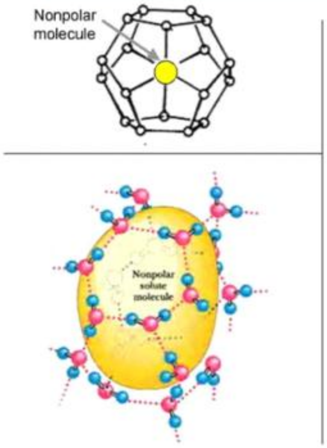
- For next week's quiz:
    - Know amino acids & peptide bond properties
    - Be able to identify charge states & pI for amino acids
    - Know protein structure terminology from lecture

Clathrate vs. Micelle

- Clathrate
- Cage-like complexes of **nonpolar** compounds with water in which the nonpolar molecules are enclosed

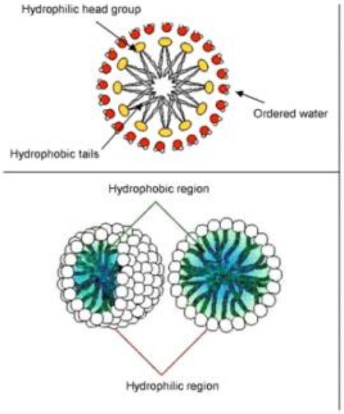
- Micelle
- Composed of **amphipathic** - compound w/ both hydrophilic & hydrophobic properties molecules (amphiphiles)
- Eliminates unfavorable contacts between water and hydrophobic tails of amphiphiles

Clathrate (cage)



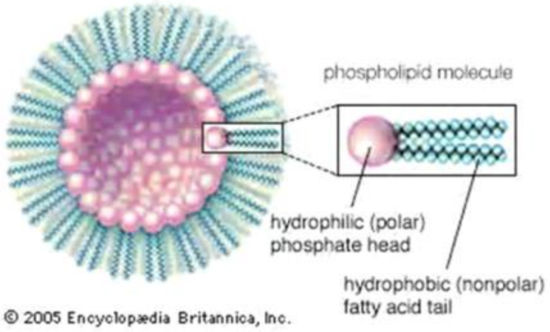
- Water is so powerful that nonpolar solutes are usually "sequestered"
- Water can enclose the unwanted group in a cage (clathrate)

Micelle



- Phospholipids are amphipathic
- In aqueous soln they form micelles
- Micelle: globular aggregate whose hydrocarbon groups are out of contact with water

Water dropped in oil results in an inverse micelle



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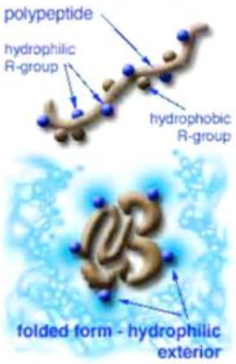
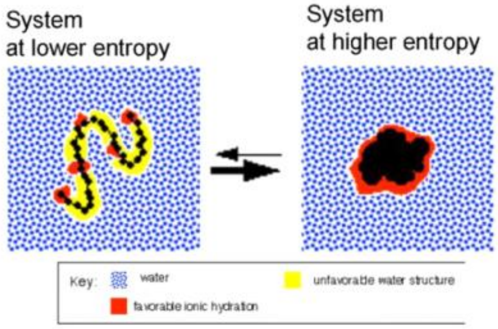
Polypeptides are Amphipathic



- If a molecule is amphipathic the hydrophobic group(s) will be accommodated in an aqueous soln in the energetically most favorable manner

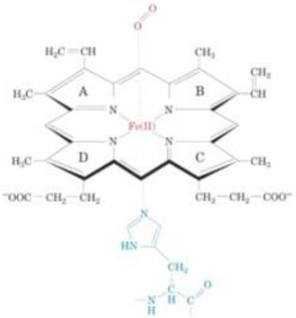
Hydrophobic effect helps drive protein folding



Hemoglobin

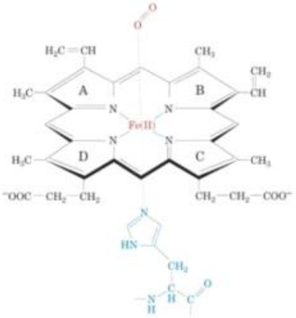
The heme group

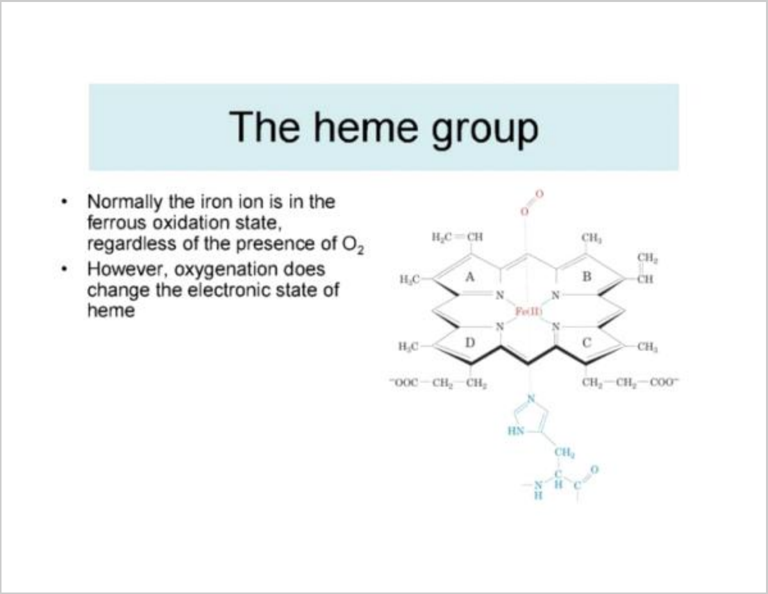
- Prosthetic group: a non-protein component of a conjugated protein
- Conjugated protein: a protein that functions in interaction with other chemical groups attached by covalent bonds or by weak interactions



The heme group

- In the oxygenated state, the iron ion (ferrous or ferric) forms "coordinate covalent bonds" to the N's of the pyrrole rings, the N of histidine's imadizole group, and oxygen molecule
- Coordinate covalent bond: bonding between two atoms in which both electrons shared in the bond come from the same atom
- Voet and Voet call it "coordinate bonding"
- Each ligand acts as a Lewis base, while iron ion acts a Lewis acid





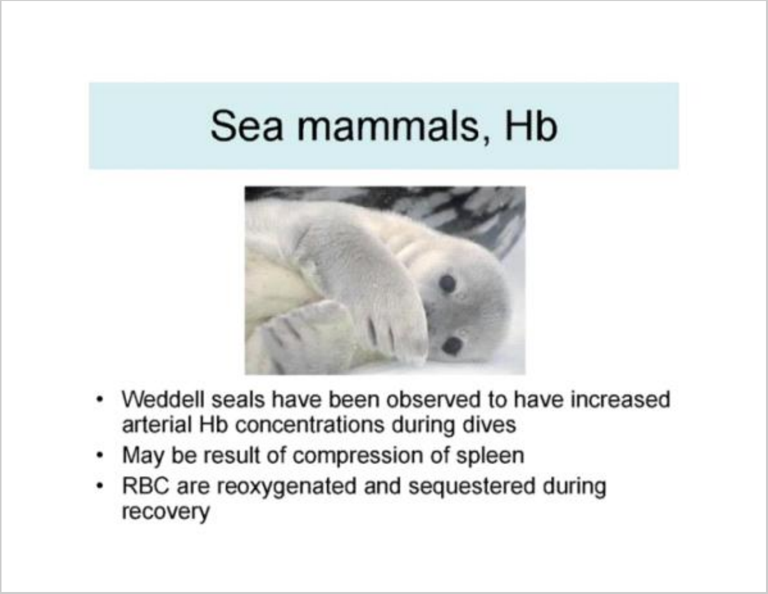
The heme group

- Normally the iron ion is in the ferrous oxidation state, regardless of the presence of O_2
- However, oxygenation does change the electronic state of heme

What happens when Fe(II) is oxidized to Fe(III)?

- Heme no longer can bind O_2
- Methemoglobin (metHb)
- Metmyoglobin (metMb)
- Methemoglobin reductase: an enzyme in RBC that reduces Fe(III) back to Fe(II)
- When concentration is elevated in RBCs a functional anemia and tissue hypoxia may occur

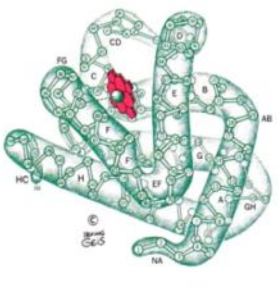
RBC - red blood cell



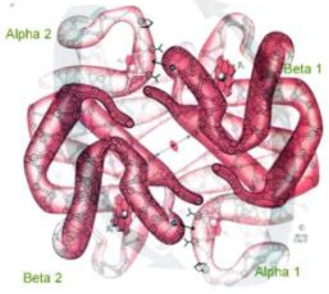
Sea mammals, Hb

- Weddell seals have been observed to have increased arterial Hb concentrations during dives
- May be result of compression of spleen
- RBC are reoxygenated and sequestered during recovery

The aa sequences of the α and β chains of human Hb and human Mb are only 18% identical, yet the 3° structure of the α and β subunits are remarkably similar



Myoglobin



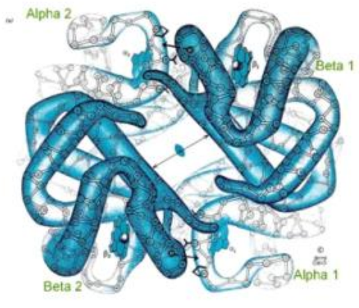
Oxyhemoglobin

Hemoglobin

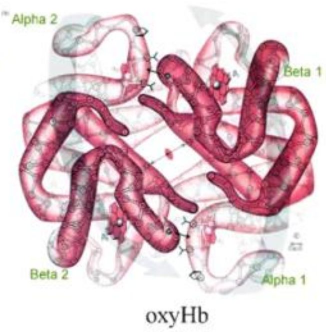
- Spherical 64 x 55 x 50 Å
- two fold rotation of symmetry
- α and β subunits are similar and are placed on the vertices of a tetrahedron
- There is no D helix in the α chain of hemoglobin.
- Extensive interactions between unlike subunits:
  - $\alpha 2$-$\beta 2$ or $\alpha 1$-$\beta 1$ interface has 35 residues
  - $\alpha 1$-$\beta 2$ and $\alpha 2$-$\beta 1$ have 19 residue contact
  - positive cooperativity of O_2 binding to Hb is observed

4° structure of deoxy- and oxyhemoglobin

Oxygenation causes a considerable structural conformational change



deoxyHb
T-state

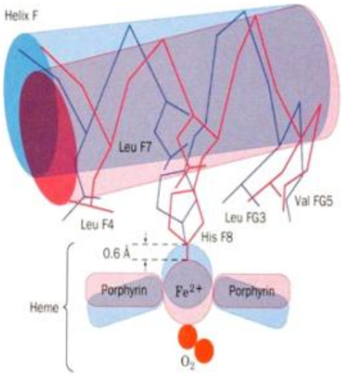


oxyHb
R-state

Max Perutz

- What causes the differences in the conformation states?
- It is somehow associated with the binding of oxygen, but how?
- The heme to heme distance in Hb is 25 to 37 **Å** so interactions can't be electronic in nature.
- X-ray structures of Hb allowed Max Perutz to formulate the "Perutz mechanism"
- A mechanical model

T ⇒ R Conformational Switch

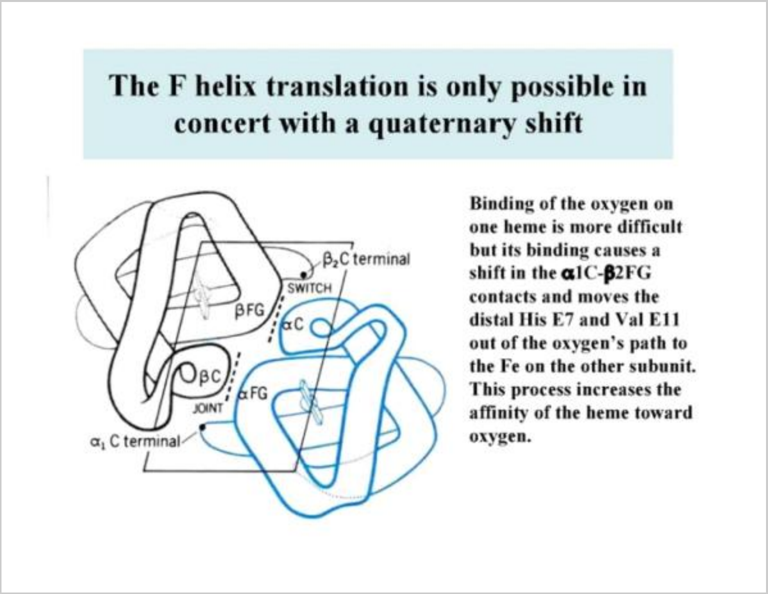


- **The Fe ion is about 0.6 Å out of the heme plane in the deoxy state**
- **When oxygen binds it pulls the iron back into the heme plane**
- **The proximal His F8 is attached to the Fe ion and pulls the complete F helix**
- **The F helix translation causes a quaternary shift**

Bohr Effect

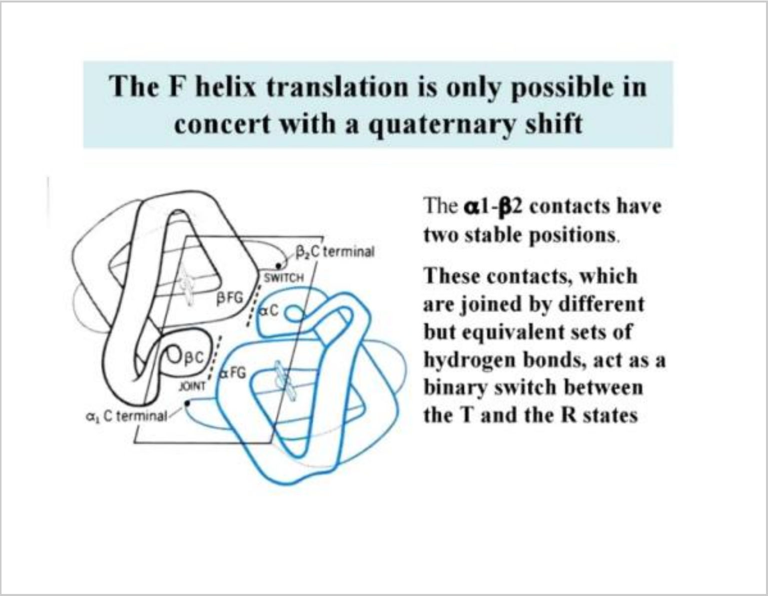
The T state is stabilized by a network of salt bridges that must break to form the R state.

The breakage of salt bridges is driven by the Fe-O_2 bonds' energy of formation



The F helix translation is only possible in concert with a quaternary shift

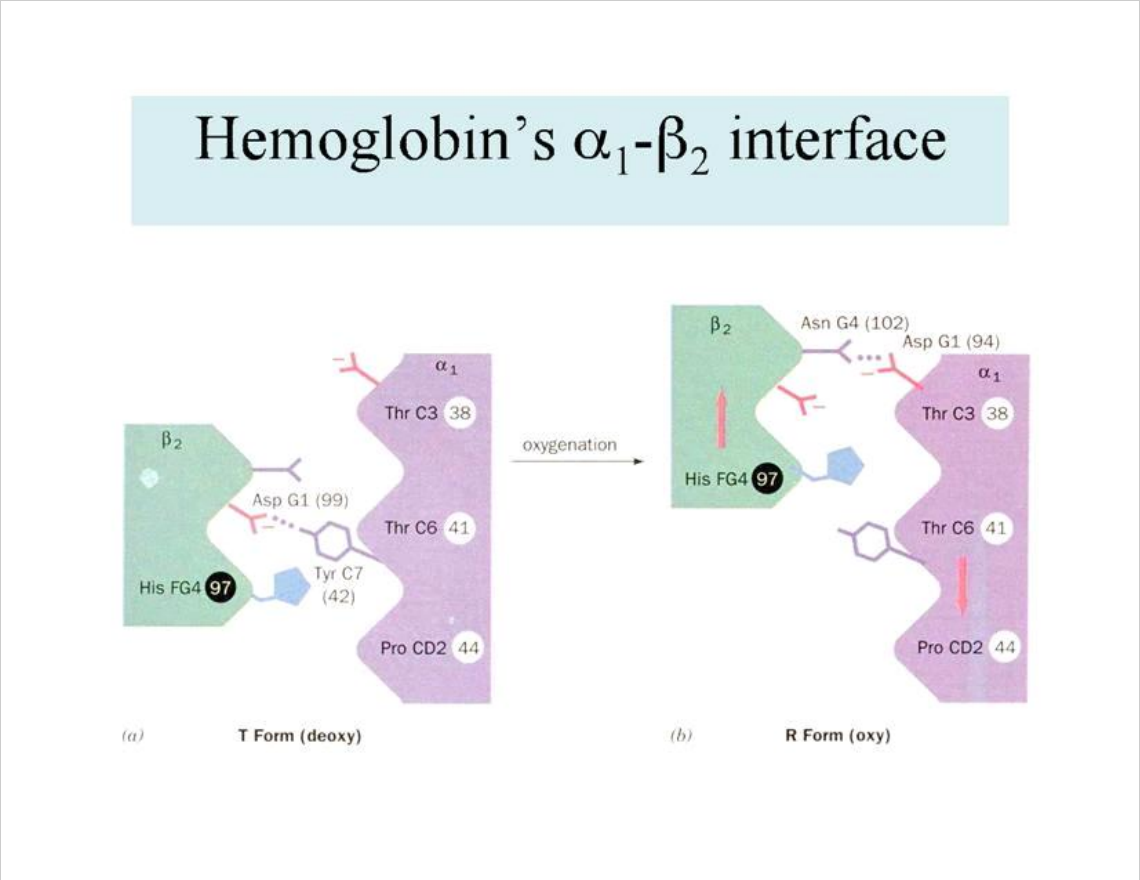
Binding of the oxygen on one heme is more difficult but its binding causes a shift in the α1C-β2FG contacts and moves the distal His E7 and Val E11 out of the oxygen's path to the Fe on the other subunit. This process increases the affinity of the heme toward oxygen.



The F helix translation is only possible in concert with a quaternary shift

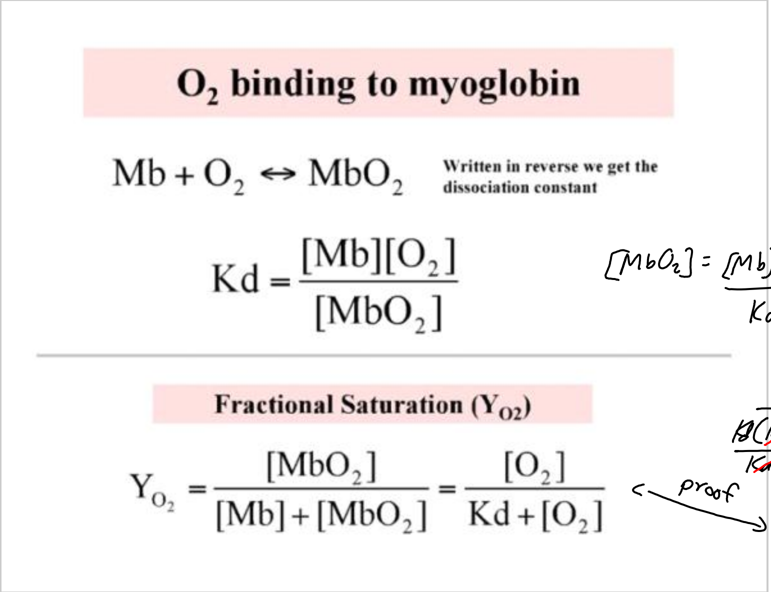
The **α1-β2 contacts have two stable positions**.

These contacts, which are joined by different but equivalent sets of hydrogen bonds, act as a binary switch between the T and the R states



Hemoglobin's α_1-β_2 interface

O_2 binding



O_2 binding to myoglobin

$$Mb + O_2 \leftrightarrow MbO_2$$

Written in reverse we get the dissociation constant

$$Kd = \frac{[Mb][O_2]}{[MbO_2]}$$

Fractional Saturation (Y_{O2})

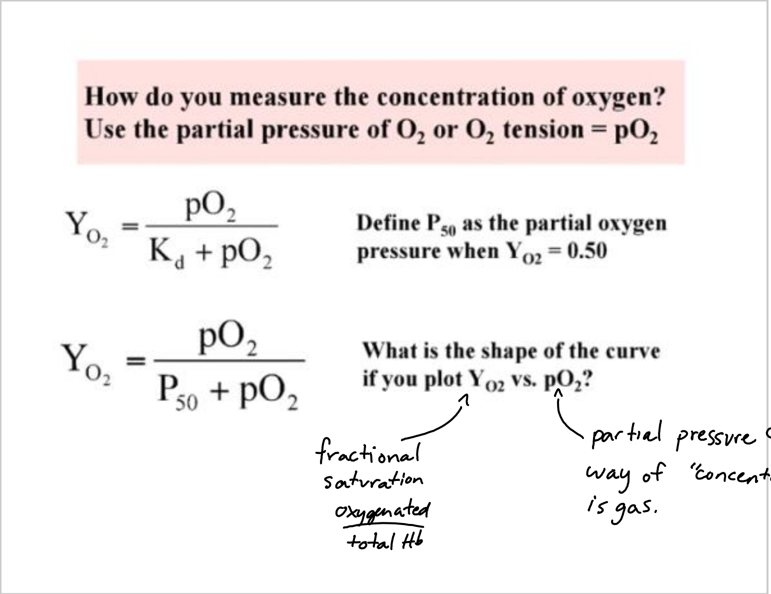
$$Y_{O_2} = \frac{[MbO_2]}{[Mb] + [MbO_2]} = \frac{[O_2]}{Kd + [O_2]}$$

$$[MbO_2] = \frac{[Mb][O_2]}{Kd}$$

$$\frac{\frac{[\cancel{Mb}][O_2]}{\cancel{Kd}}}{\frac{\cancel{Kd}[\cancel{Mb}]}{\cancel{Kd}} + \frac{[\cancel{Mb}][O_2]}{\cancel{Kb}}}$$

proof

$$\frac{[O_2]}{Kd + [O_2]}$$



How do you measure the concentration of oxygen?
Use the partial pressure of O_2 or O_2 tension = pO_2

$$Y_{O_2} = \frac{pO_2}{K_d + pO_2}$$

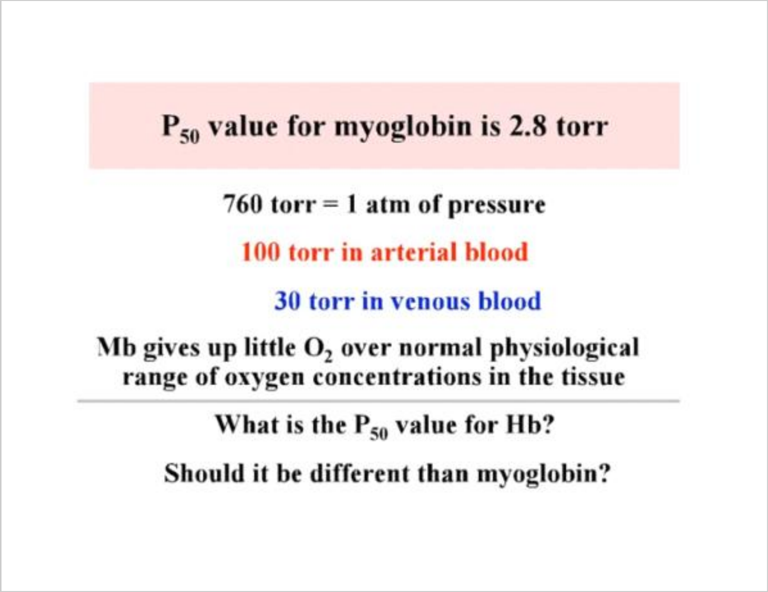
Define P_{50} as the partial oxygen pressure when $Y_{O2} = 0.50$

$$Y_{O_2} = \frac{pO_2}{P_{50} + pO_2}$$

What is the shape of the curve if you plot Y_{O2} vs. pO_2?

fractional saturation $\frac{\text{oxygenated}}{\text{total Hb}}$

partial pressure O_2
way of "concentration" since it
is gas.



P_{50} value for myoglobin is 2.8 torr

760 torr = 1 atm of pressure

100 torr in arterial blood

30 torr in venous blood

Mb gives up little O_2 over normal physiological range of oxygen concentrations in the tissue

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What is the P_{50} value for Hb?

Should it be different than myoglobin?

Contrast Mb O_2 binding to Hemoglobin

$$Y_{O2} = 0.95 \text{ at } 100 \text{ torr}$$

but

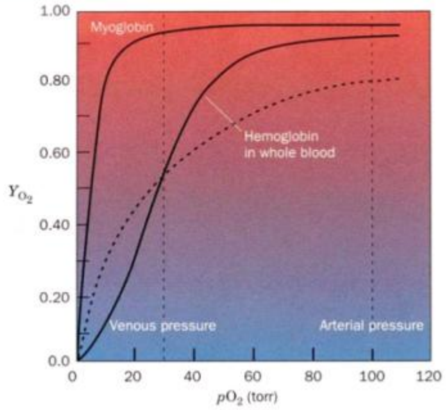
$$Y_{O2} = 0.55 \text{ at } 30 \text{ torr}$$

$$\Delta Y_{O2} = 0.40$$

Hb dissociates more readily from O_2 than Mb

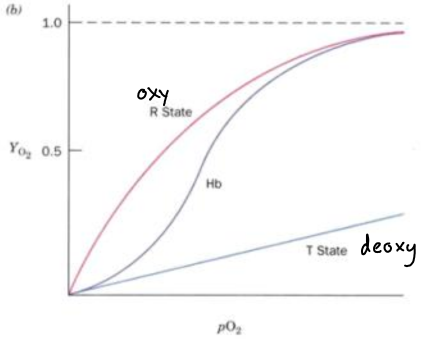
The binding of Hb to O_2 is cooperative

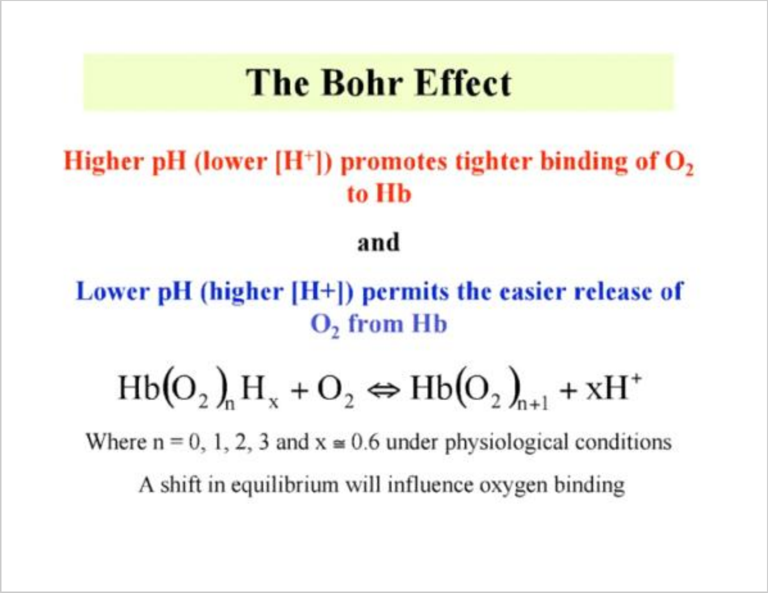
Oxygen dissociation curves of Mb and Hb in whole blood



"S shaped"

Sigmoidal binding curve as a composite of the R state binding and the T state binding.





The Bohr Effect

Higher pH (lower [H^+]) promotes tighter binding of O_2 to Hb

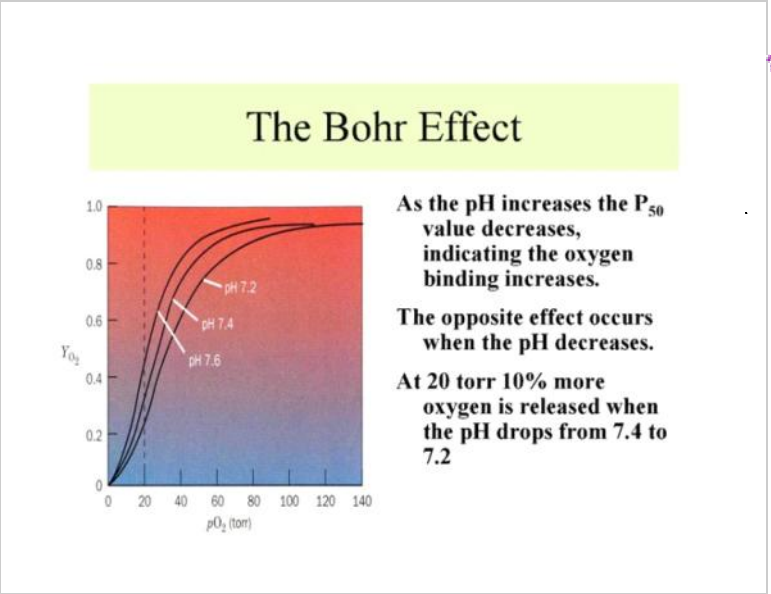
and

Lower pH (higher [$H+$]) permits the easier release of O_2 from Hb

$$Hb(O_2)_n H_x + O_2 \Leftrightarrow Hb(O_2)_{n+1} + xH^+$$

Where n = 0, 1, 2, 3 and x ≅ 0.6 under physiological conditions

A shift in equilibrium will influence oxygen binding



The Bohr Effect

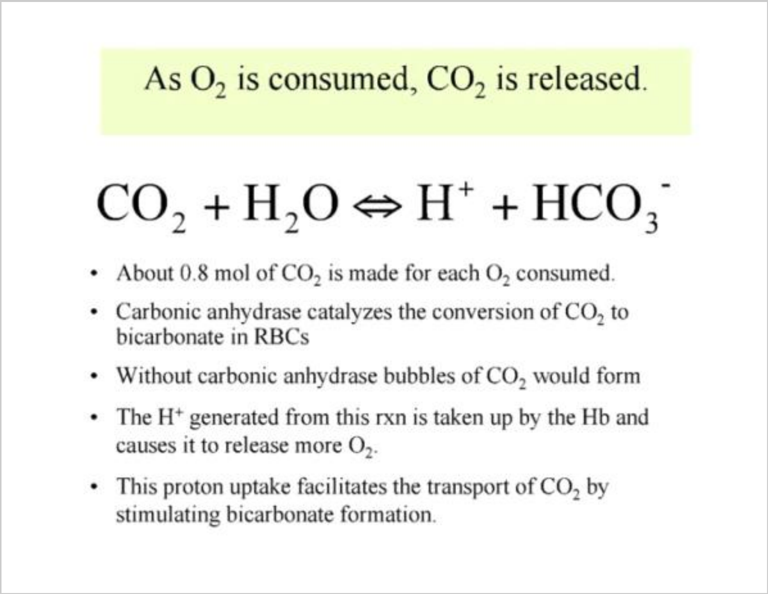
As the pH increases the P_{50} value decreases, indicating the oxygen binding increases.

The opposite effect occurs when the pH decreases.

At 20 torr 10% more oxygen is released when the pH drops from 7.4 to 7.2

P_{50} should be K... P_{50} is only applicable to Mb and by the sigmoidal shape of graph, this must be Hb

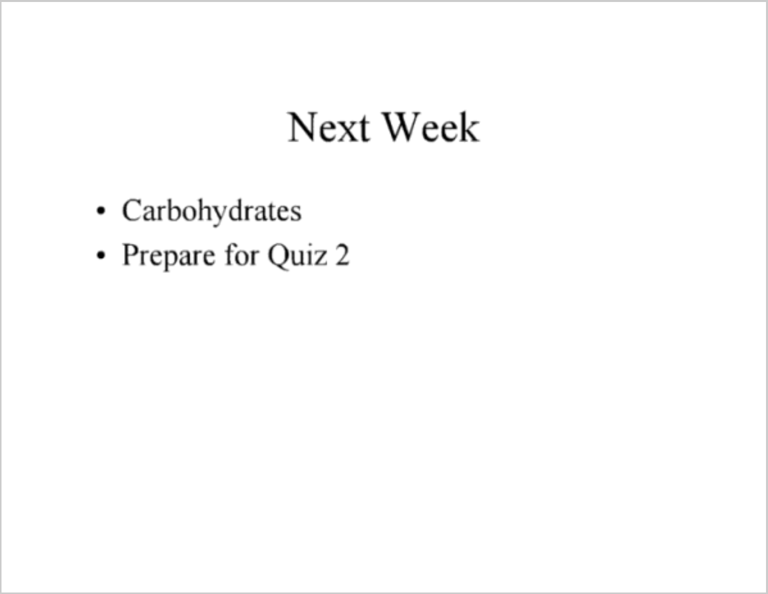
- ph ↑	- ph ↓
- OH ↑	- H^+ ↑
- P_{50} ↓	- P_{50} ↑
- O binding ↑	- O binding ↓



As O_2 is consumed, CO_2 is released.

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$

- About 0.8 mol of CO_2 is made for each O_2 consumed.
- Carbonic anhydrase catalyzes the conversion of CO_2 to bicarbonate in RBCs
- Without carbonic anhydrase bubbles of CO_2 would form
- The H^+ generated from this rxn is taken up by the Hb and causes it to release more O_2.
- This proton uptake facilitates the transport of CO_2 by stimulating bicarbonate formation.



Next Week

- Carbohydrates
- Prepare for Quiz 2