STRUCTURE-FUNCTION RELATIONSHIP: GLOBULAR PROTEINS

Myoglobin and hemoglobin

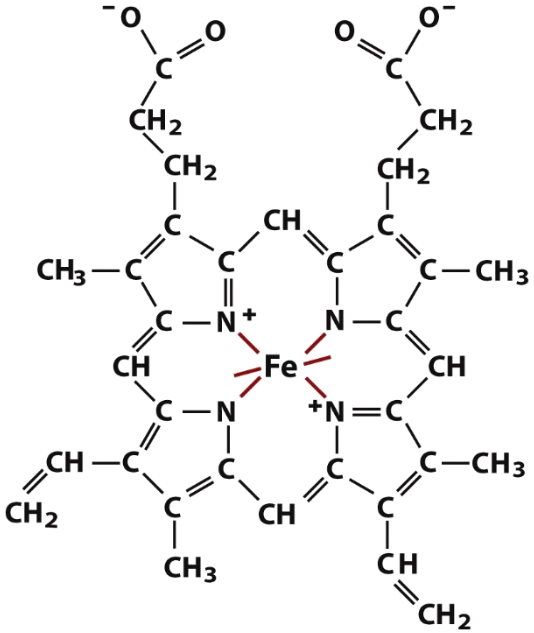
FUNCTIONS OF MYOGLOBIN AND HEMOGLOBIN

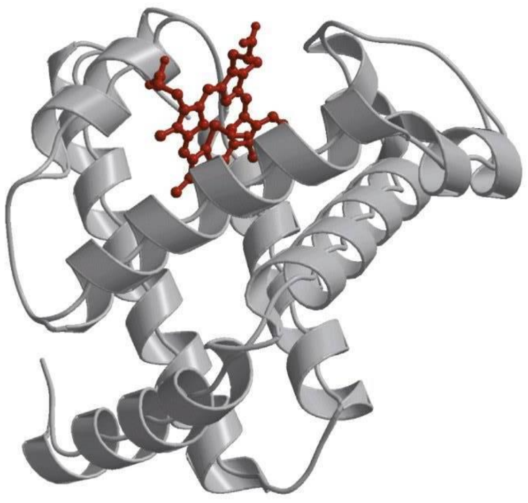
- Myoglobin is storage of O_2. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen.

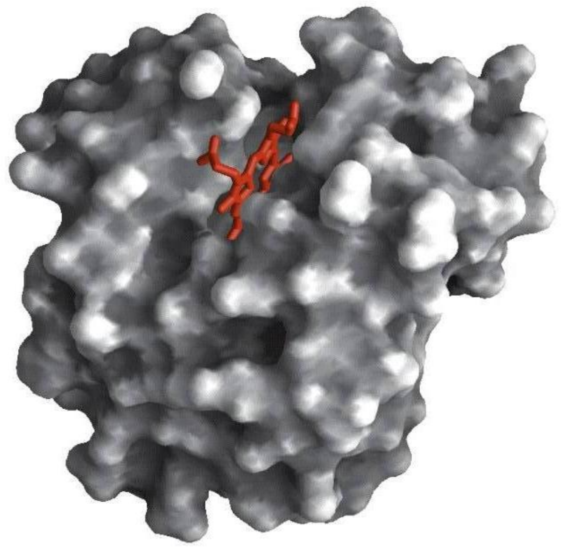
- Hemoglobin:
  - Transport of O_2 and CO_2
  - Blood buffering

HEMOPROTEINS

- Both myoglobin and hemoglobin are hemoproteins (a group of specialized proteins containing heme as a tightly bound non-protein group known as a prosthetic group).
- The protein environment dictates the function of the heme.





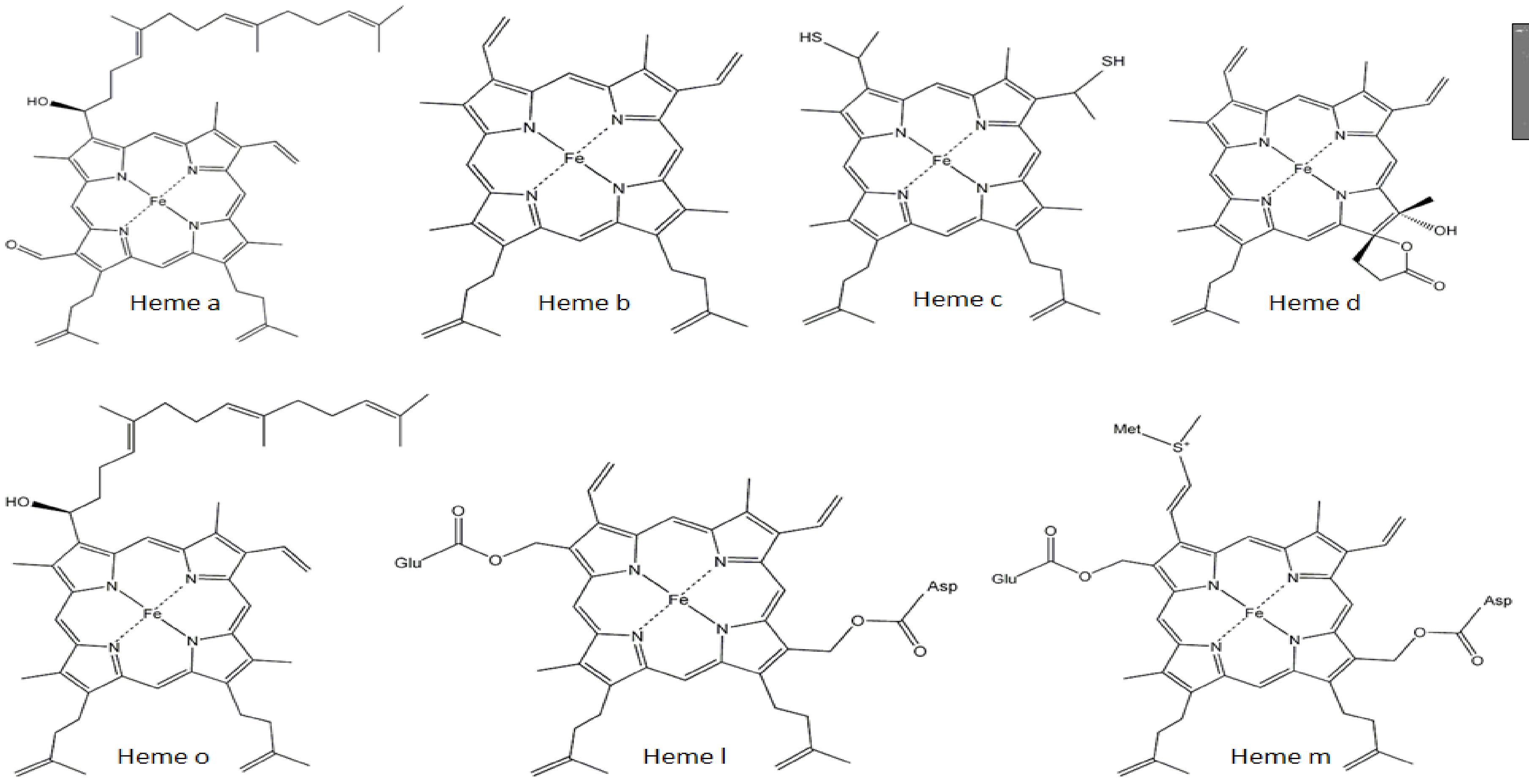


HEMOPROTEINS

- The protein environment dictates the function of the heme

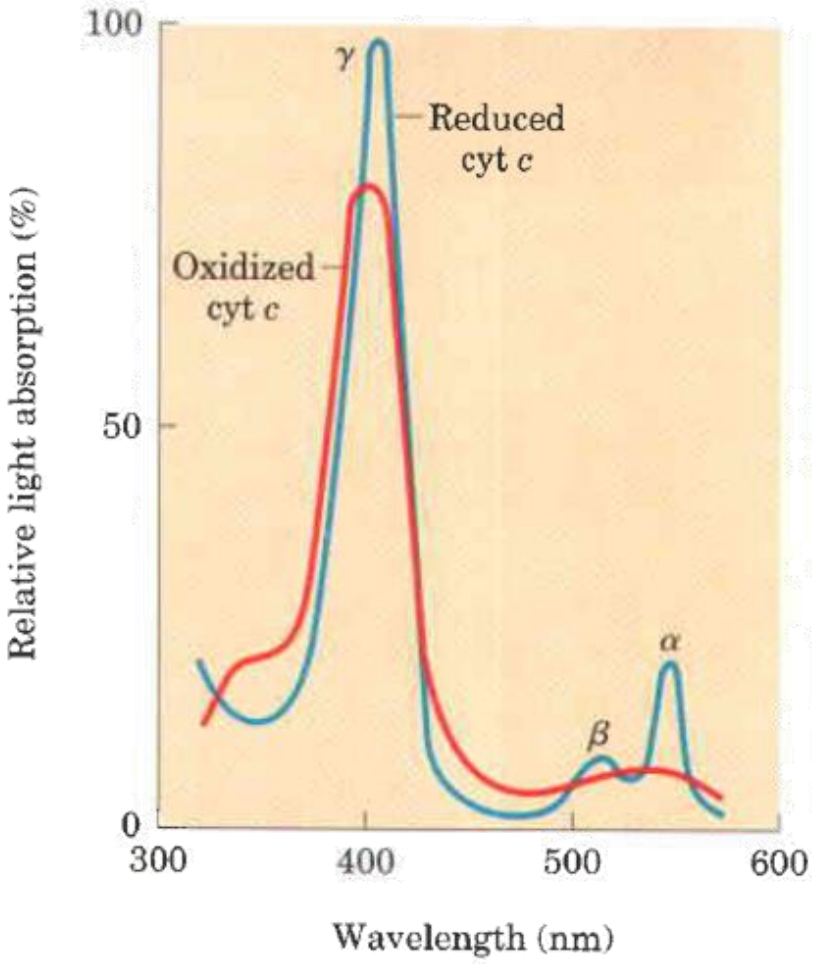
Cytochromes	**Cytochromes** • **An electron carrier (alternately oxidized & reduced)**
Catalytic enzymes	**Catalase** • **Catalyzes the breakdown of O_2 peroxide**
Binding proteins	**Hemoglobin & Myoglobin (conjugated)** • **Reversibly bind O_2**

STRUCTURES OF PORPHYRIN & HEME



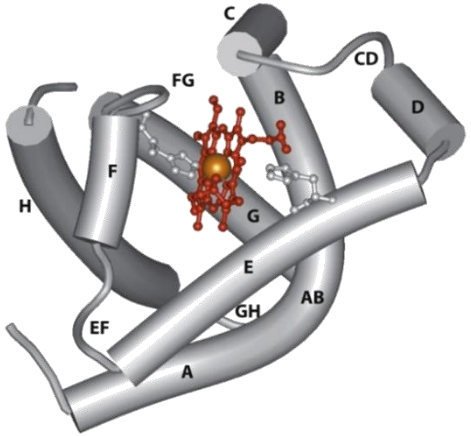
- Pyrrole, Porphyrin, Fe, Heme, Ligation

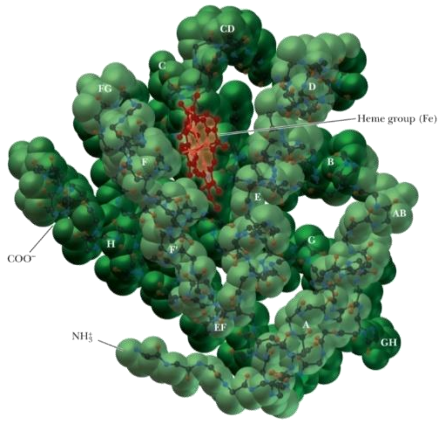
HEME – SPECTROSCOPIC FEATURES



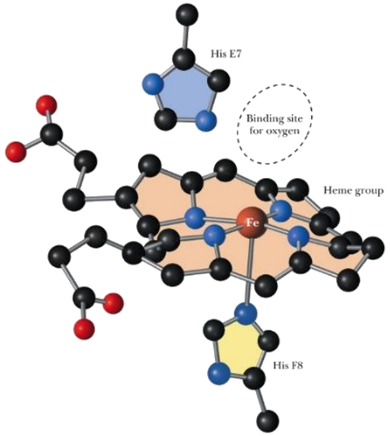
- Characteristic strong absorption of visible light (Fe-containing heme prosthetic groups)
- Classification based on light absorption & Mode of binding (a, b, c)
- Light absorption: Each cytochrome in its reduced (F^{+2}) state has 3 absorption bands in the visible range
- α band : near 600 nm in type a; near 560 nm in type b, & near 550 nm in type c
- Heme can carry one electron

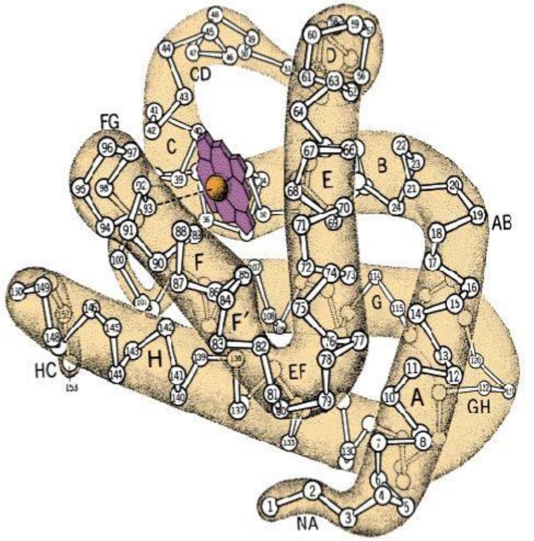
STRUCTURE & FUNCTION OF MYOGLOBIN





- Myoglobin is a monomeric protein (153 aa)
- It includes a prosthetic group, the heme group
- It can be present in two forms:
  - oxymyoglobin (oxygen-bound)
  - deoxymyoglobin (oxygen-free)

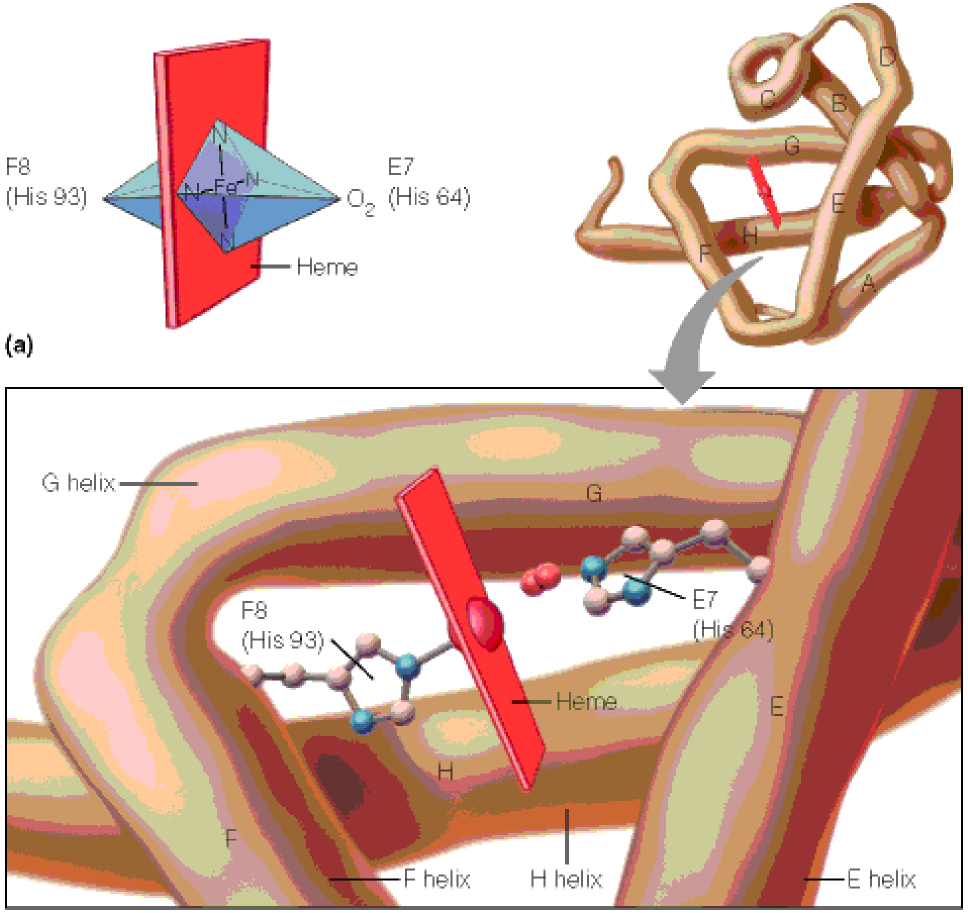


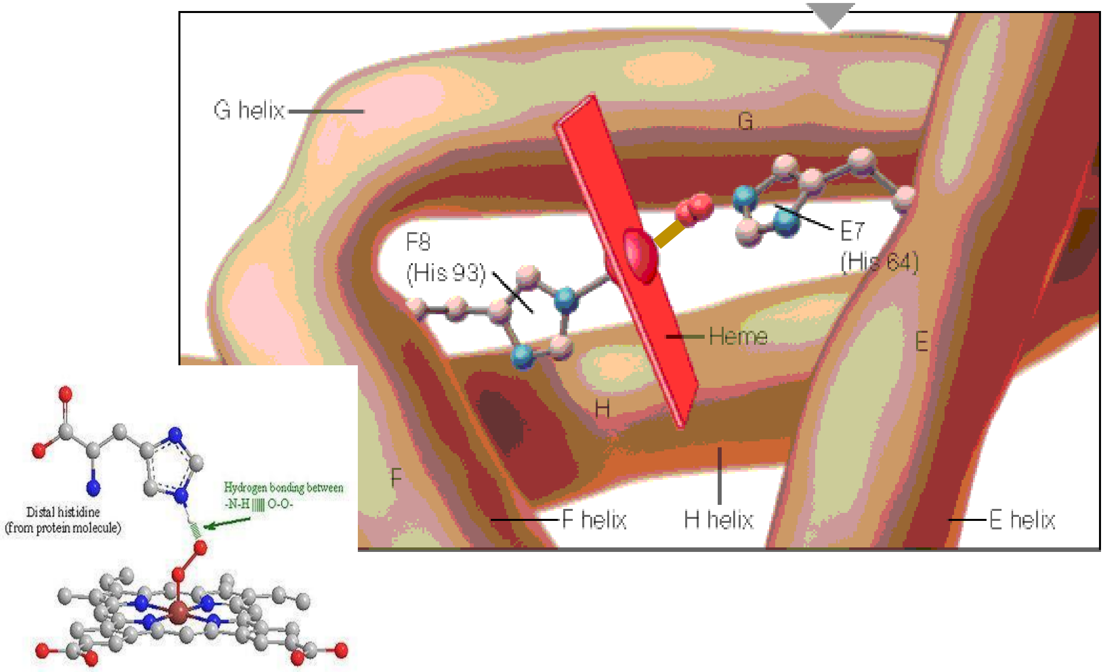


- The tertiary structure of myoglobin 8 α-helices, designated A through H, that are connected by short non-helical regions.

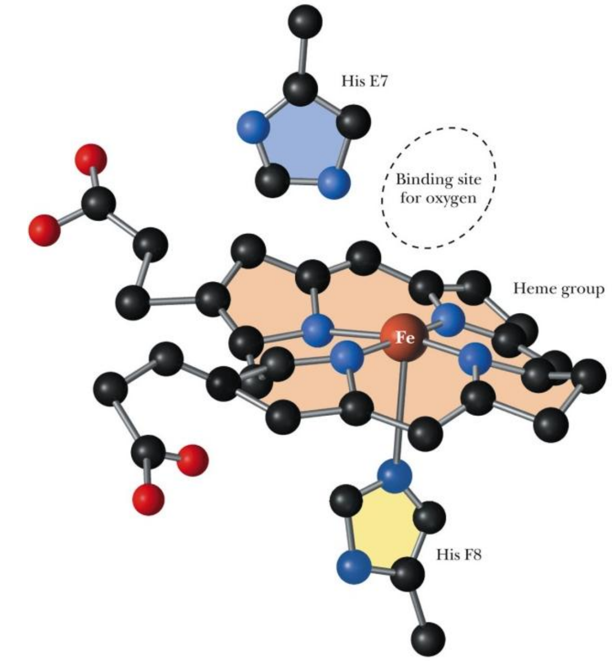
ARRANGEMENT OF AMINO ACIDS

- Like other globular protein, amino acid R-groups exposed on the surface of the molecule are generally hydrophilic, while those in the interior are predominantly hydrophobic.
- Except for two histidine residues in helices E and F (known as E7 and F8)
- F8 His is designated as proximal His, whereas E7 His is known as distal His

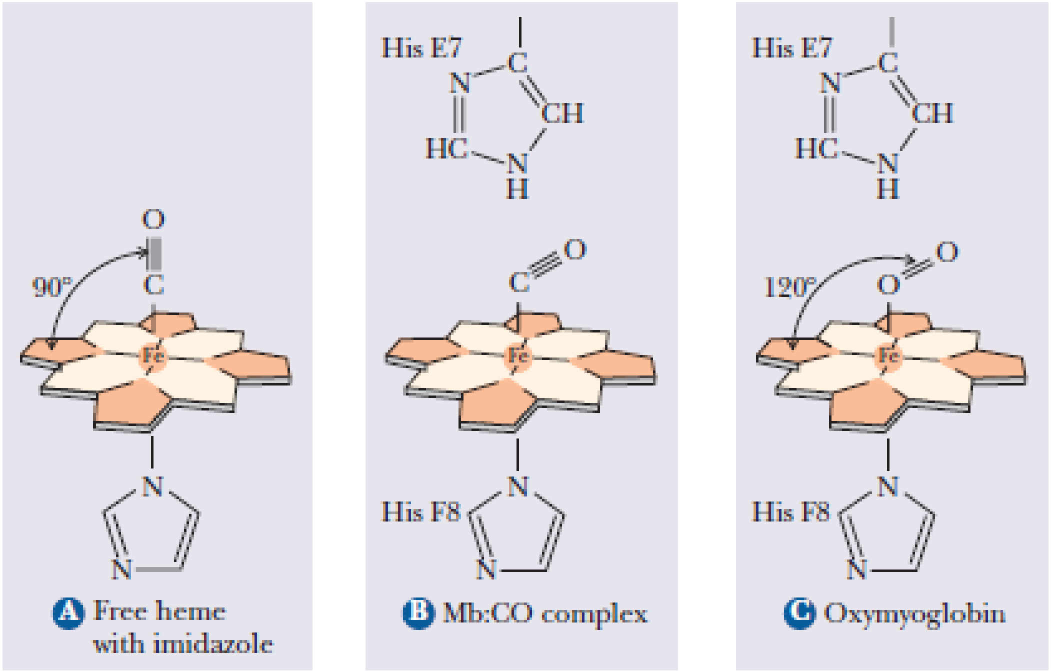




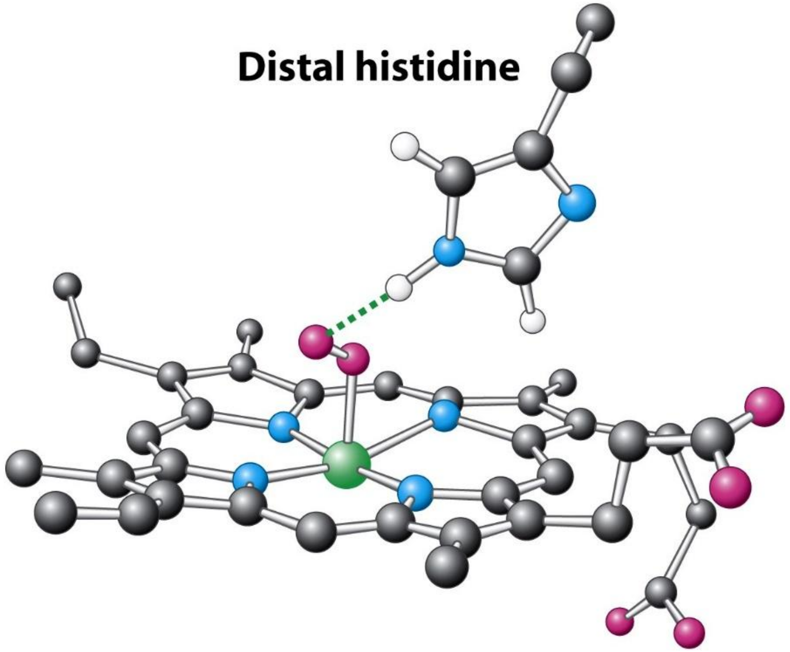
G helix
G
F8
(His 93)
E7
(His 64)
Heme
E
H
F
F helix
H helix
E helix
Distal histidine
(from protein molecule)
Hydrogen bonding between
-N-H O-O-



His E7
Binding site
for oxygen
Heme group
Fe
His F8



O
C
90°
Fe
N
N
A Free heme
with imidazole
His E7
N
C
CH
HC
N
H
C
O
Fe
His F8
N
N
B Mb:CO complex
His E7
N
C
CH
HC
N
H
120°
O
O
Fe
His F8
N
N
C Oxymyoglobin



Distal histidine

STRUCTURE-FUNCTION RELATIONSHIP

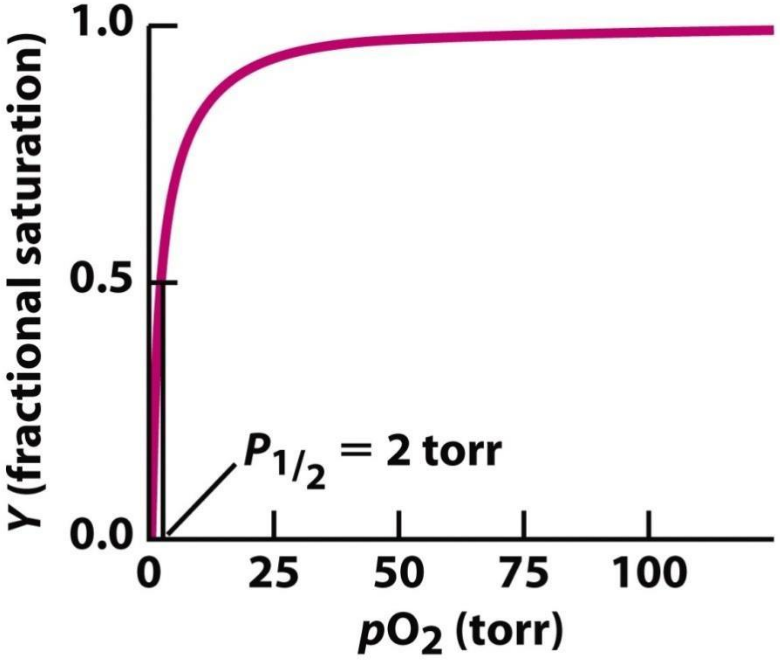
- The planar heme group fits into a **hydrophobic pocket** of the protein and the myoglobin-heme interaction is stabilized by hydrophobic attractions.
- The **heme group** stabilizes the tertiary structure of myoglobin.
- The **distal histidine** acts as a gate that opens and closes as O2 enters the hydrophobic pocket to bind to the heme.
- The **hydrophobic interior** of myoglobin (or hemoglobin) **prevents the oxidation of iron**, and so when O2 is released, the iron remains in the Fe(II) state and can bind another O2.

OXYGEN BINDING TO MYOGLOBIN

Myoglobin binds O2 with high affinity.

The P50 (oxygen partial pressure required for 50% of all myoglobin molecules) for myoglobin ~2.8 torrs or mm Hg.

Given that O2 pressure in tissues is normally 20 mm Hg, it is almost fully saturated with oxygen at normal conditions.

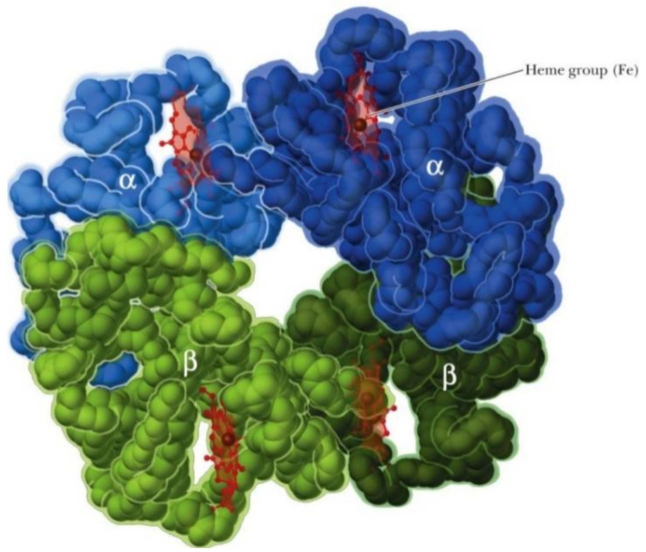


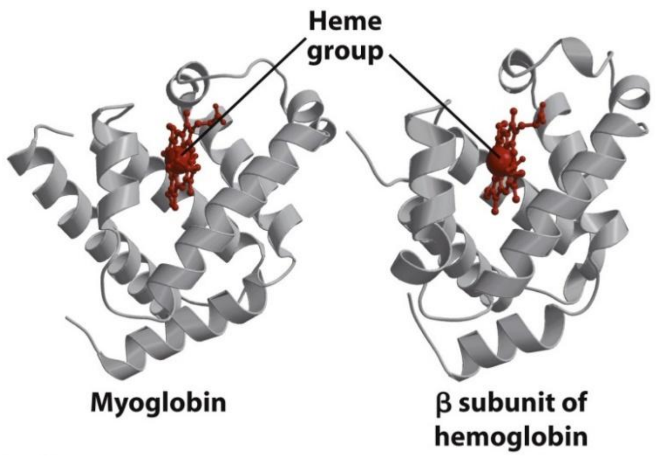
The binding of $\mathbf{O_2}$ to myoglobin follows a hyperbolic saturation curve

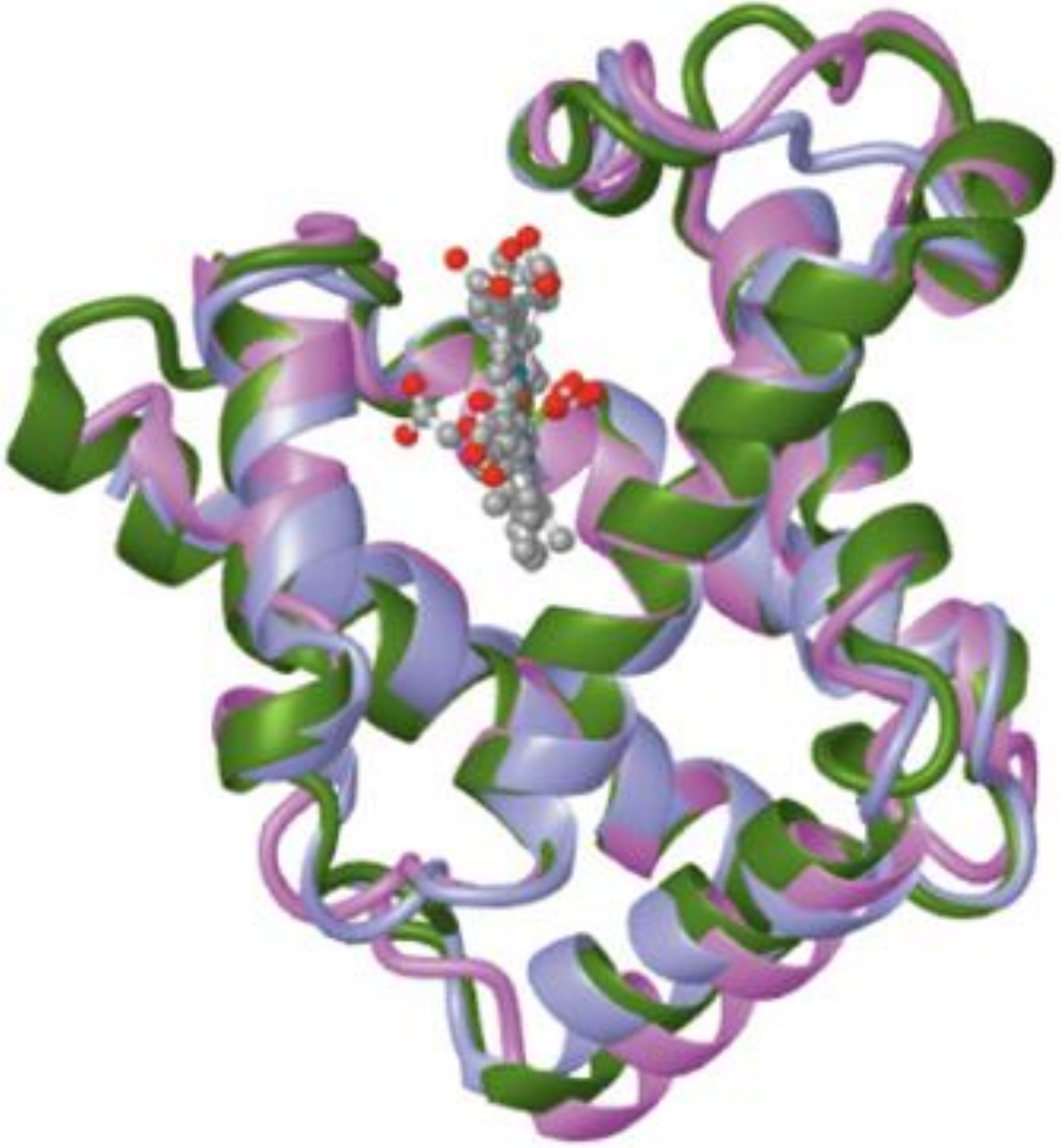
HEMOGLOBIN

STRUCTURE & FUNCTION OF HEMOGLOBIN

- Why do we need it? RBC/ml: (5×10^9); Hemoglobin/RBC: (2.8×10^8); O_2/hemoglobin: (4); O_2/100ml: $(5 \times 10^9)(2.8 \times 10^8)(4)(100) = (5.6 \times 10^{20})$
- Hemoglobin A: a tetramer $\alpha2\beta2$: α-chains (141 a.a, 7 helices) & β-chains (146 a.a, 8 helices)
- Non-covalent hydrophobic interactions
- Ionic and hydrogen bonds also occur
- Each subunit is similar to myoglobin
- 1 heme group in each $(4O_2)$. Can transport H^+ and CO_2
- Hemoglobin is an allosteric protein





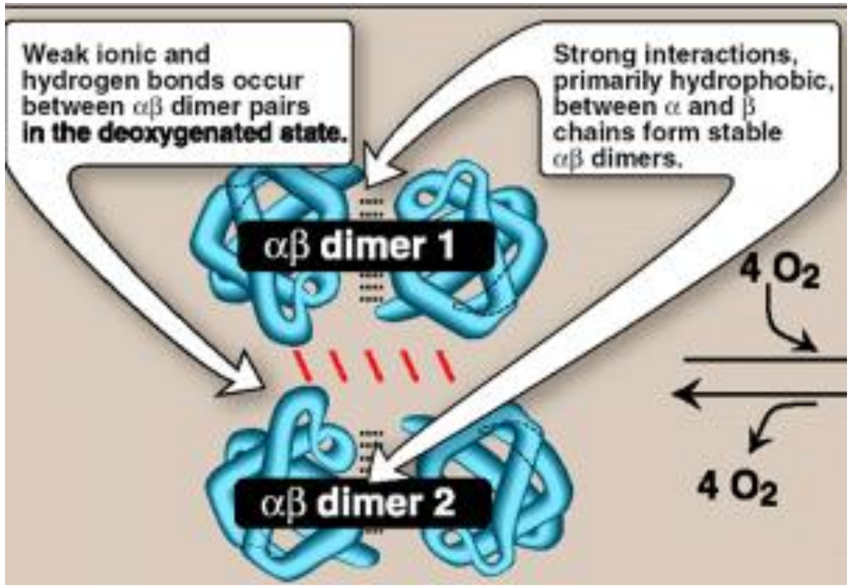


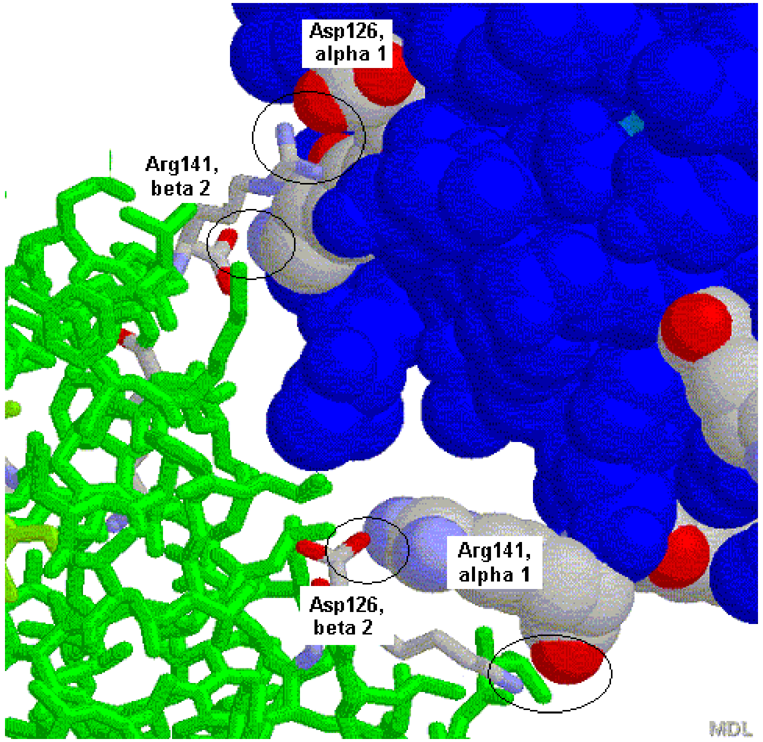
3D STRUCTURE OVERLAP MYOGLOBIN, α-GLOBIN AND β-GLOBIN

- **α-Globin (blue)**
- **β-Globin (violet)**
- **Myoglobin (green)**

STRUCTURE & FUNCTION OF HEMOGLOBIN CHAIN INTERACTION

- The chains interact with each other via hydrophobic interactions.
- Therefore, hydrophobic amino acids are not only present in the interior of the protein chains, but also on the surface.
- Electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two different chains.

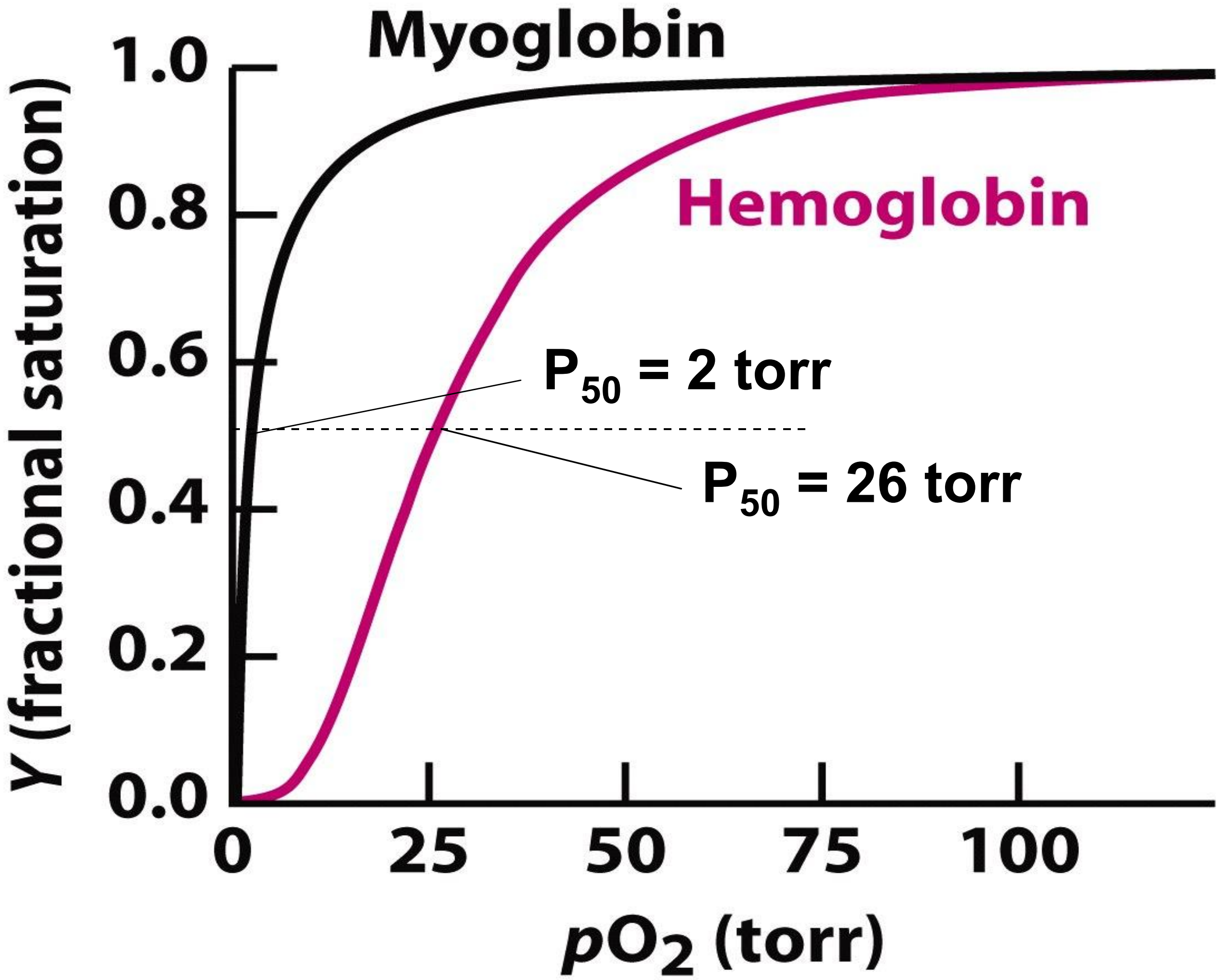


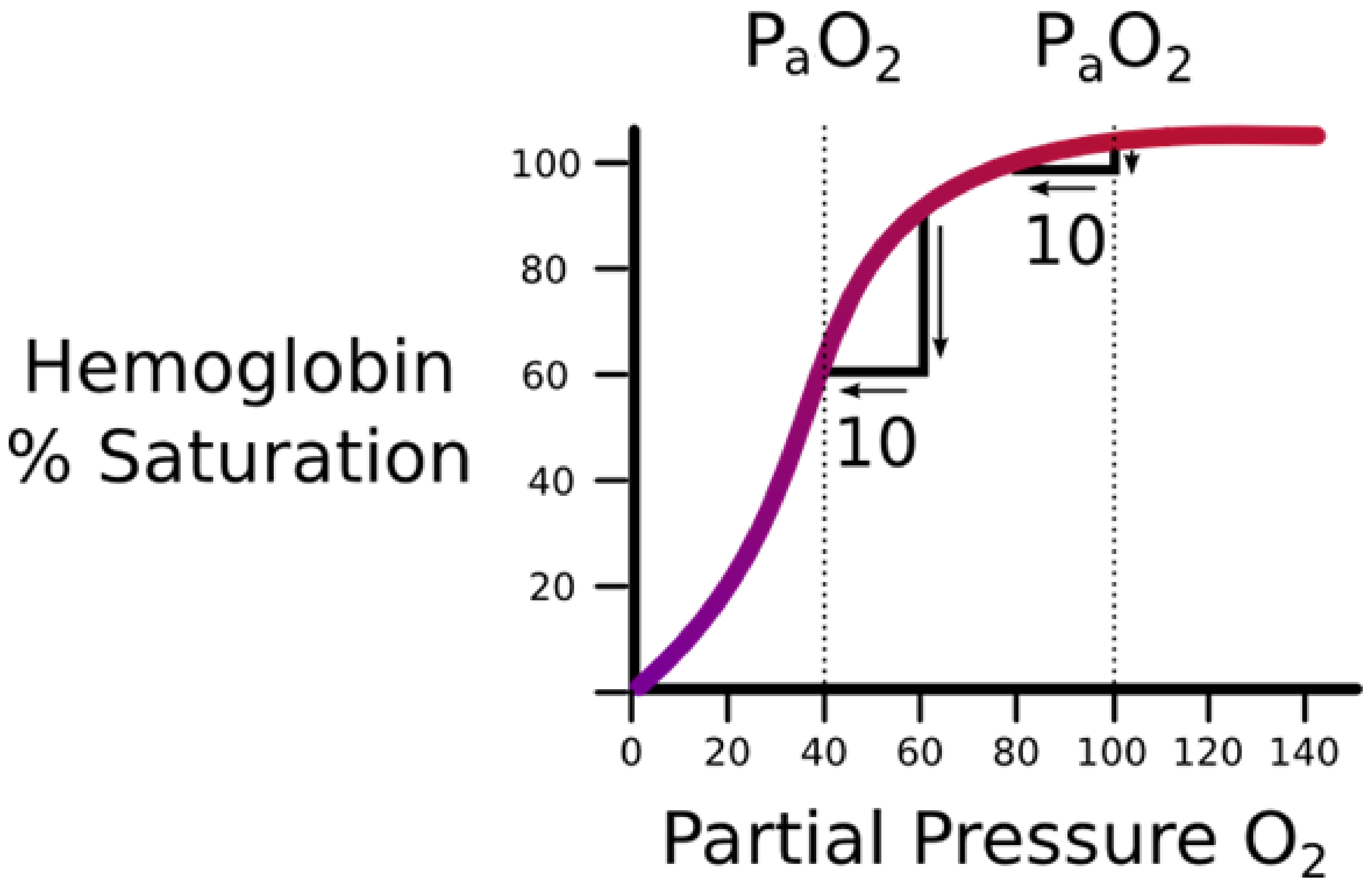


OXYGEN BINDING

HEMOGLOBIN

- How is the function related to pressure?
  - Lungs vs. tissues (100 vs. 20 mmHg)
- Cooperativity and sigmoidal plot
- At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin)



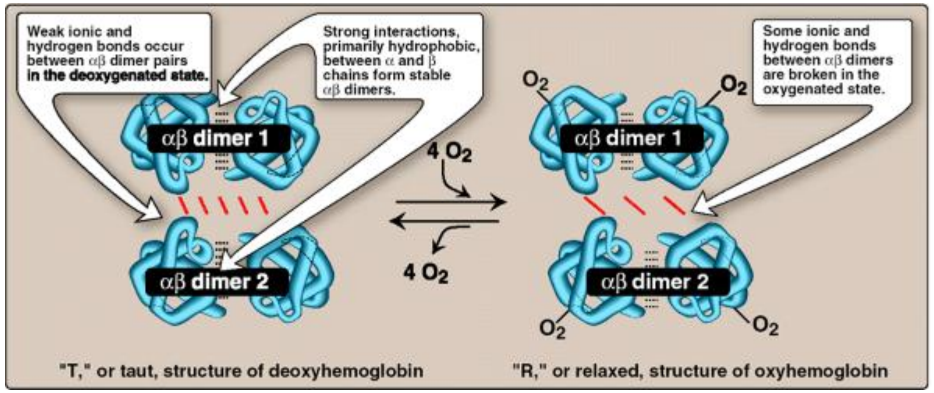


IT IS A PROTECTIVE MECHANISM

- A sudden drop in pulmonary capillary oxygen tension does not affect hemoglobin saturation
  - High altitudes

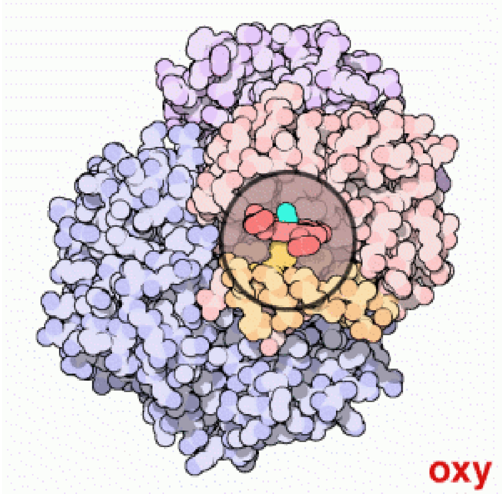
DEOXYHEMOGLOBIN & OXYHEMOGLOBIN

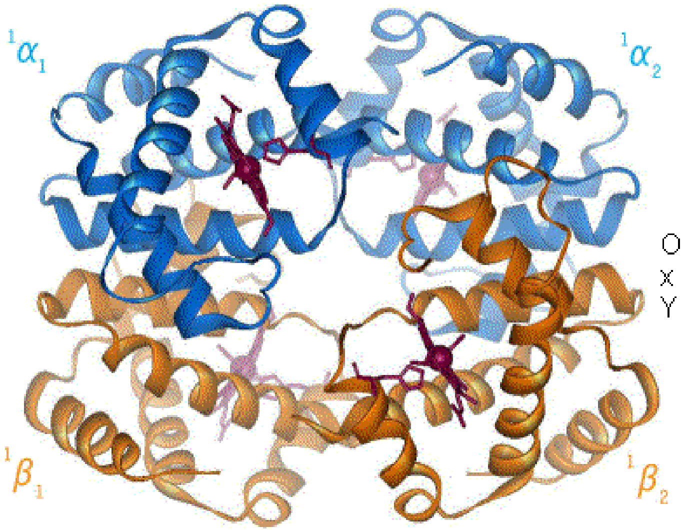
- T form
  - ✓ Taut (tense)
  - ✓ Deoxy
  - ✓ The two dimers movement is constrained (ionic & H-bonds)
  - ✓ Low-oxygen-affinity

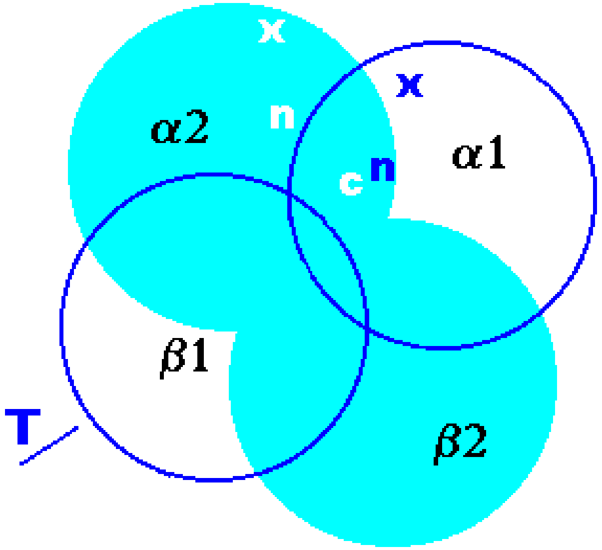


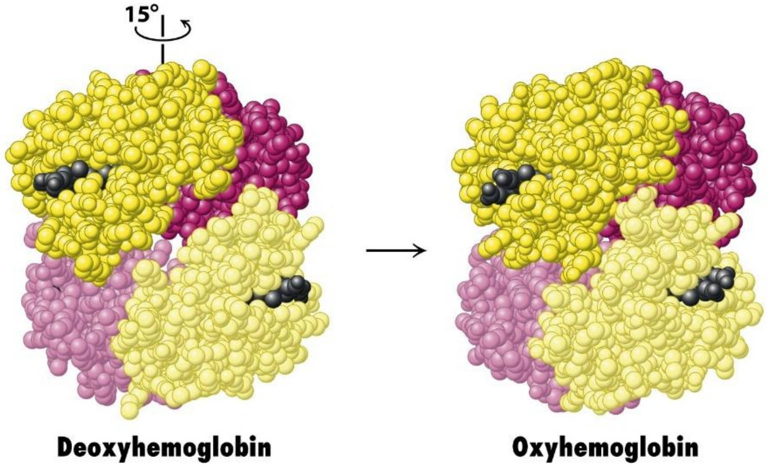
- R form:
  - ✓ Relaxed, Oxy
  - ✓ Breakage of some ionic & H-bonds
  - ✓ More freedom of movement
  - ✓ High-oxygen-affinity (500 times higher affinity)

DEOXYHEMOGLOBIN & OXYHEMOGLOBIN



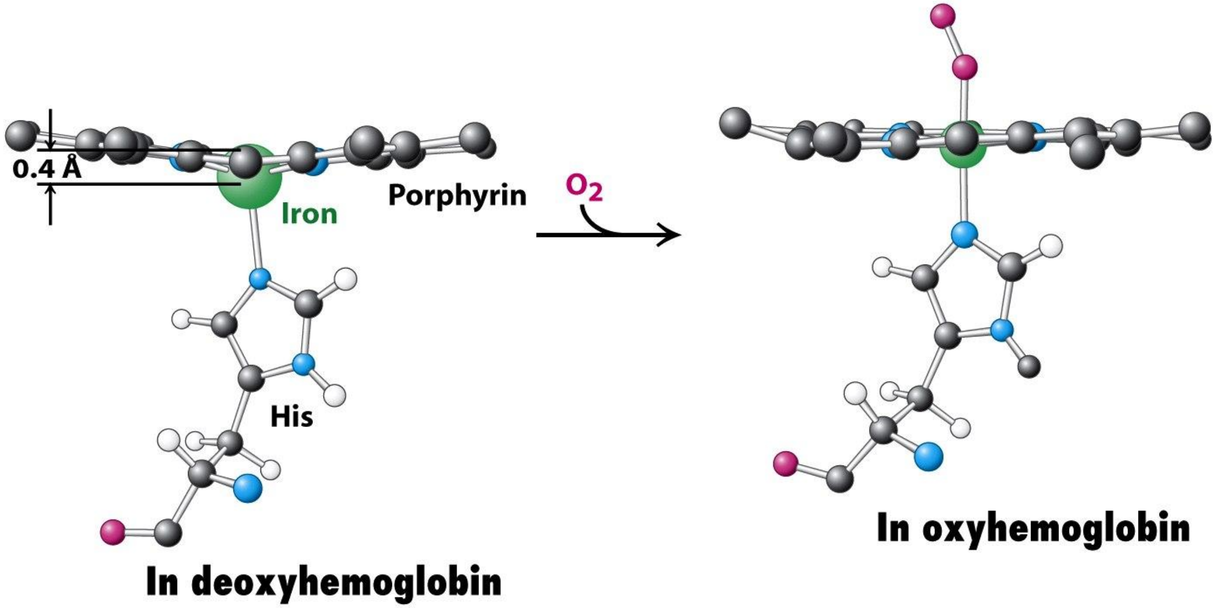


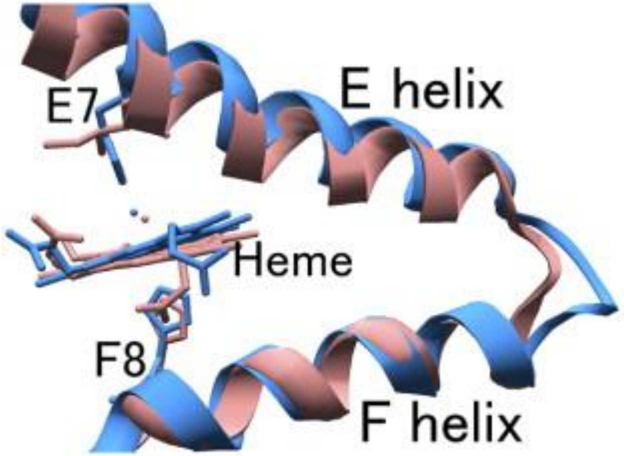


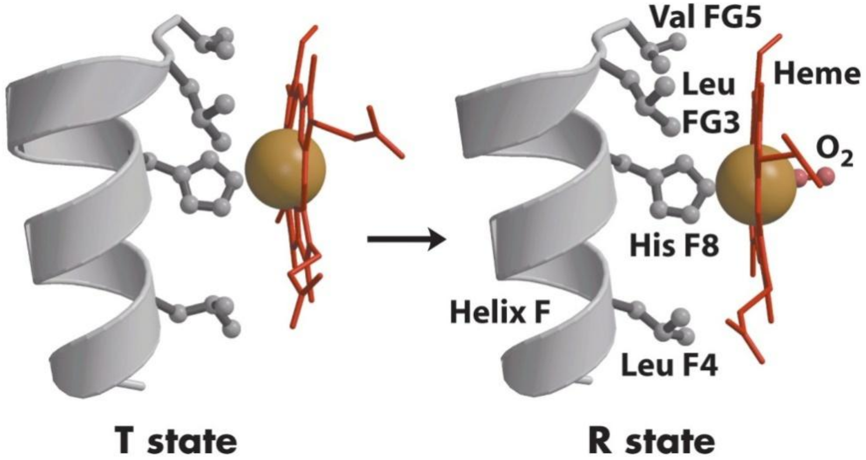


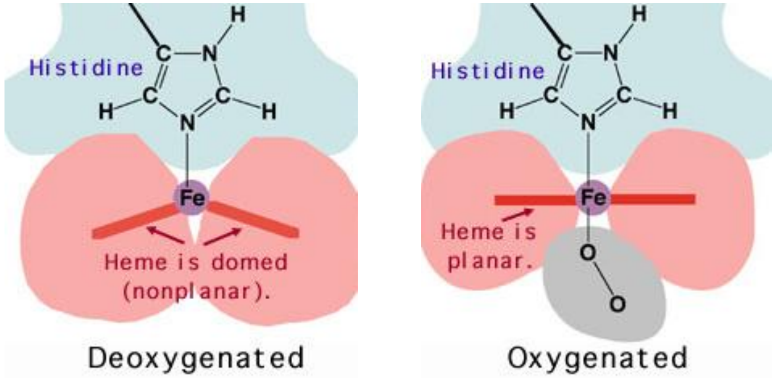
HOW DOES THE SWITCH OCCUR?

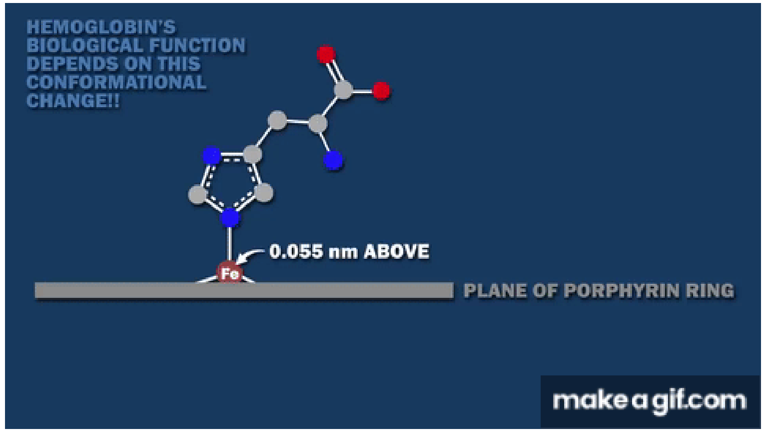
AFFECTS HEMOGLOBIN FUNCTION BUT NOT MYOGLOBIN





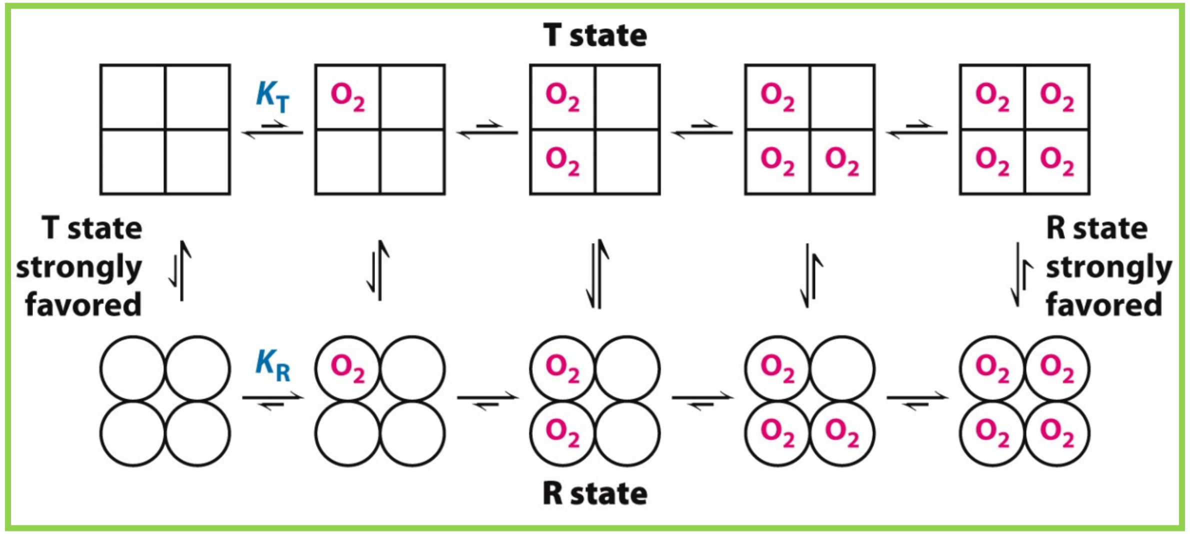


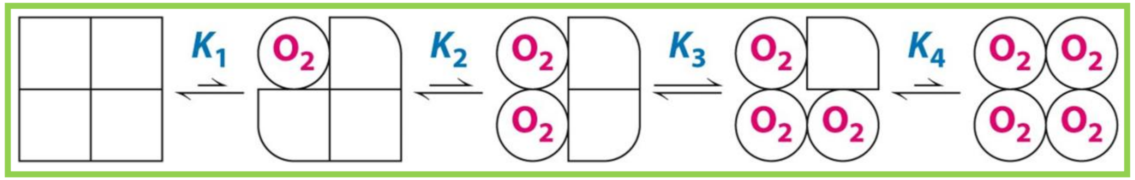




THE MECHANISM OF THE SWITCH

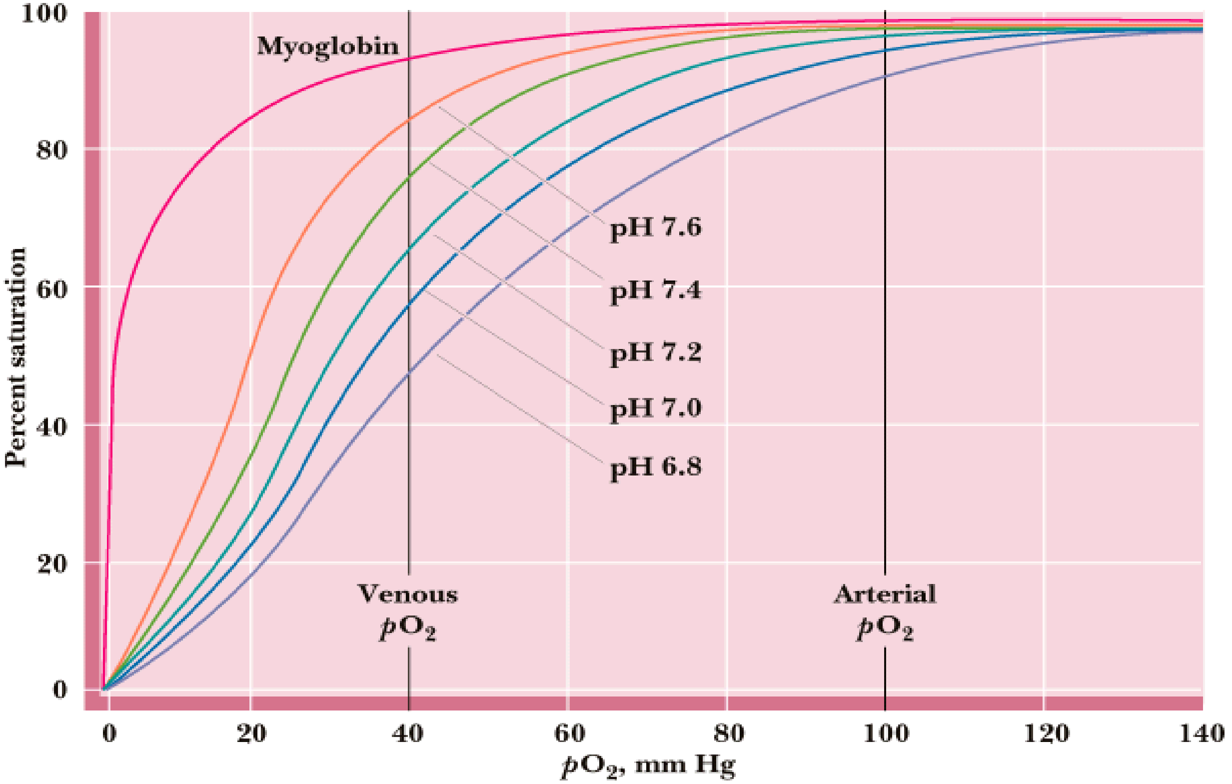
- Do all the chains transform at one stage (a quaternary change) or each chain by itself (a tertiary change)?
- Two models: Concerted vs. Sequential
- Concerted:
  - In the absence of O2 (T is favored)
  - In the presence of O2 (R is favored)
  - Does not depend on the number of O2 bound to tetramer
- Sequential:

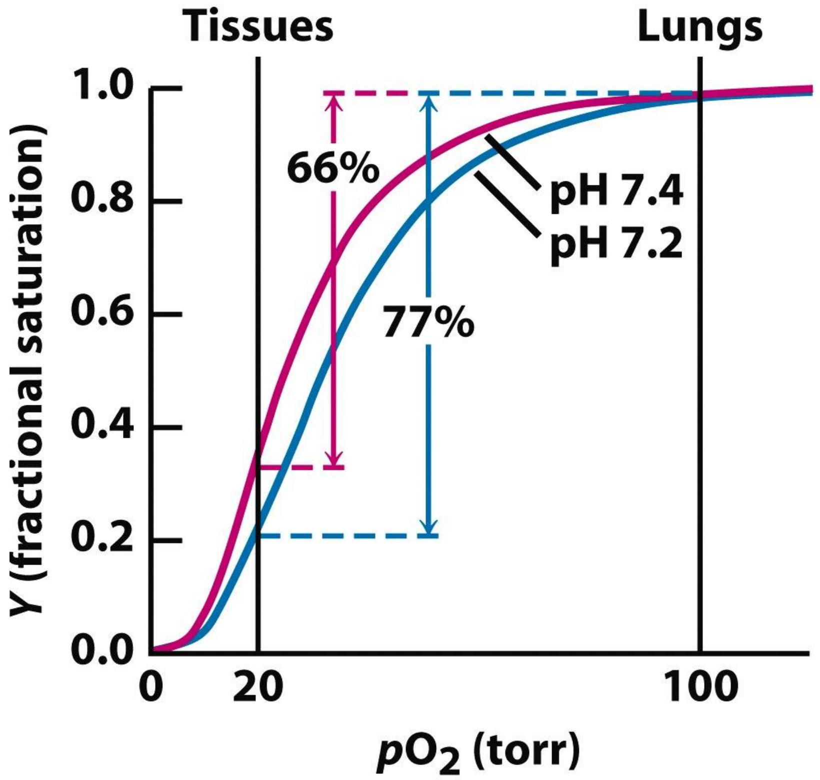




HEMOGLOBIN IS AN ALLOSTERIC PROTEIN

- Allosteric effectors (modulators) bind to a protein at a site separate from the functional binding site (modulators may be activators or inhibitors)
- Oxygen binding and release from Hb are regulated by allosteric interactions
- Hemoglobin cooperativity behaves as a mix of the above two models
- Homotropic vs. heterotropic effectros
- The major heterotropic effectors: hydrogen ion (H+), carbon dioxide (CO2), and red-cell 2,3-bisphosphoglycerate (2,3-BPG)



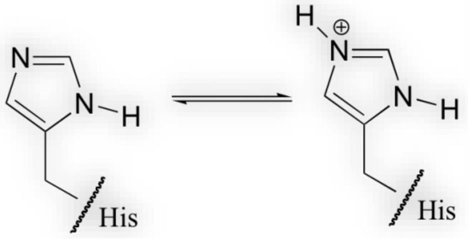


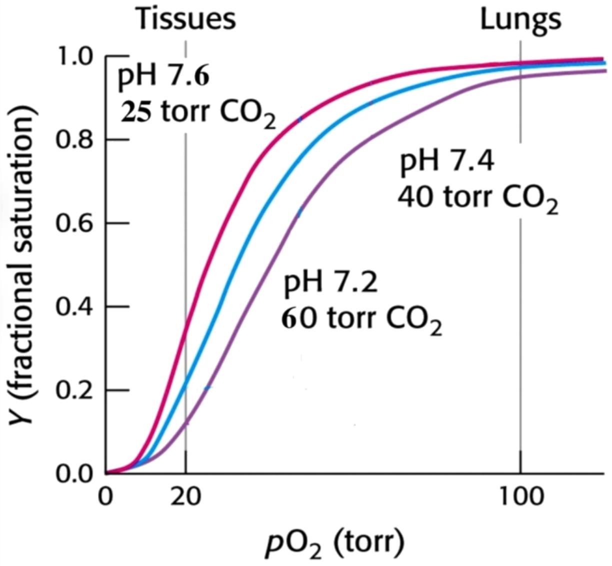
BOHR EFFECT

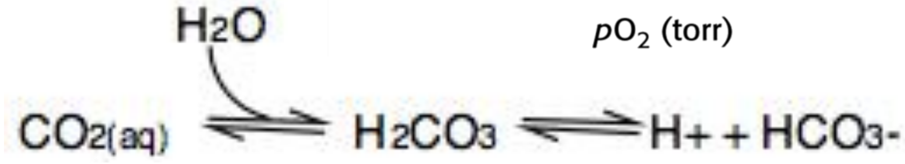
- Binding of protons favors loss of O_2
- So; Lowering the pH decreases the affinity of oxygen for Hb
- Is this physiologically relevant? (lung vs. tissues)

MECHANISM OF THE BOHR EFFECT

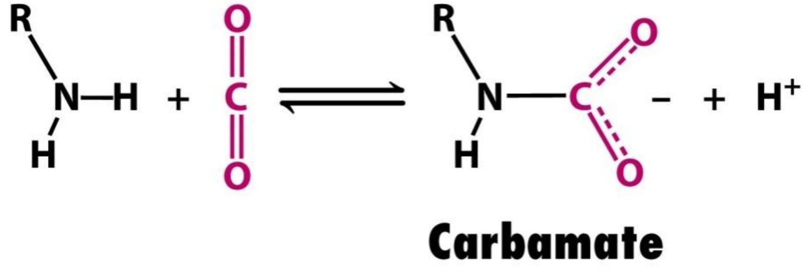
- $CO2$ and H+ help in favoring and stabilizing the T-form
- pH: More protons will protonate His allowing for more salt bridges which help in forming and stabilizing the T-form (O_2 release)
- CO_2:
  - Metabolism produces CO_2 in tissues (pH)
  - CO_2 binds the N-terminus (pH & salt bridges)



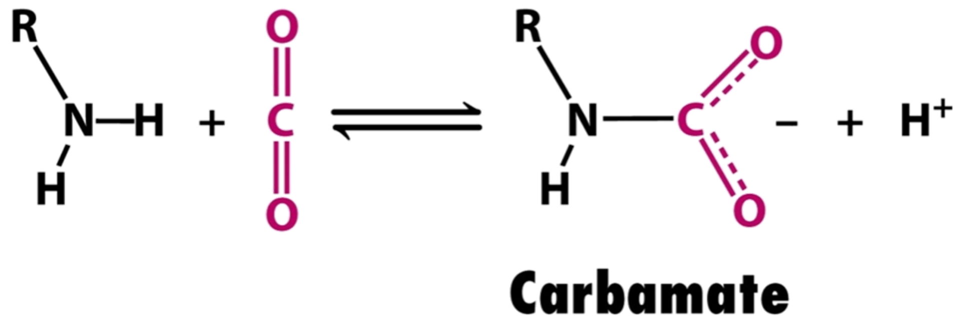


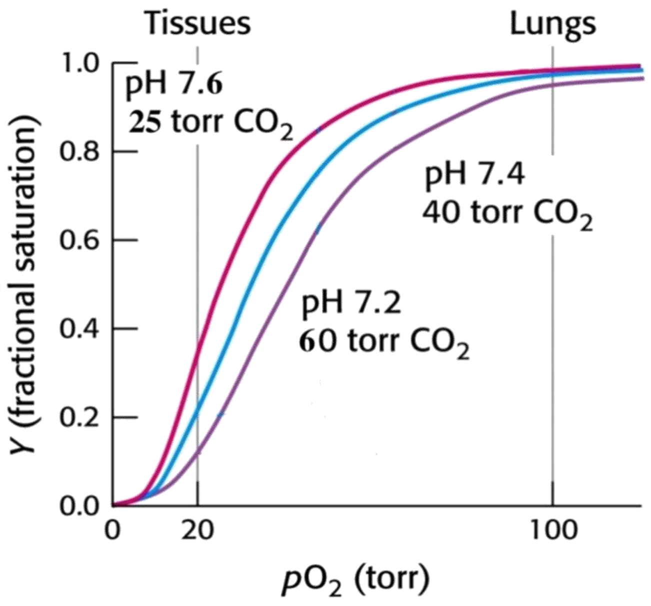


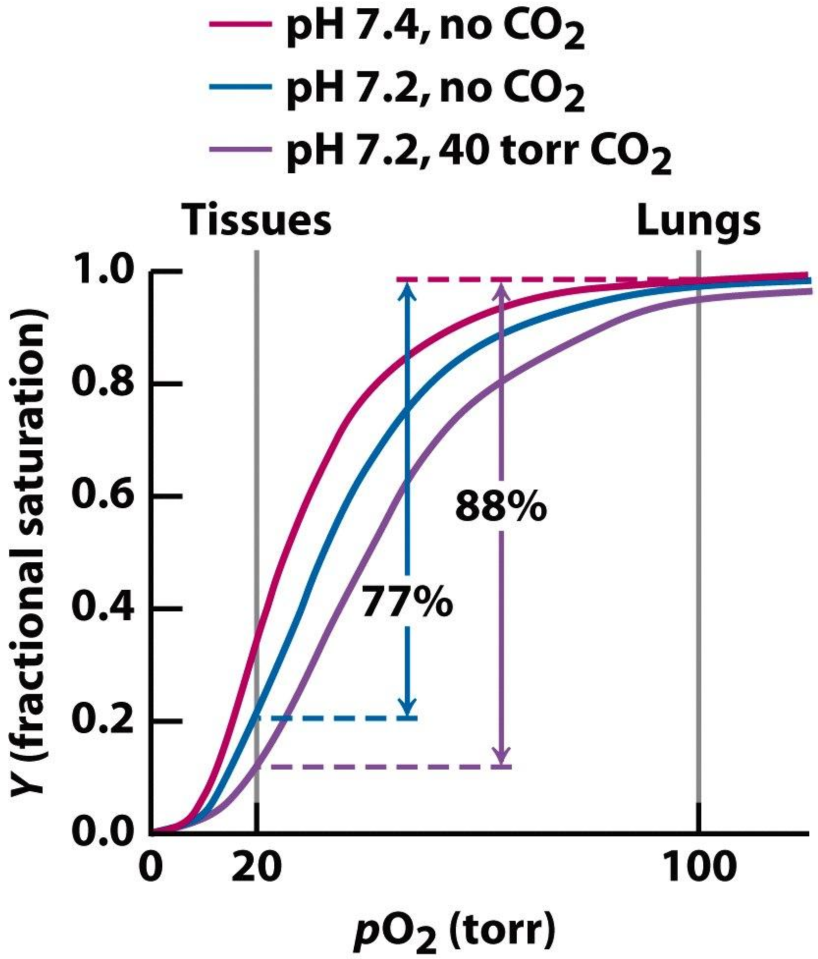
pK_a of H_2CO_3 is 6.35

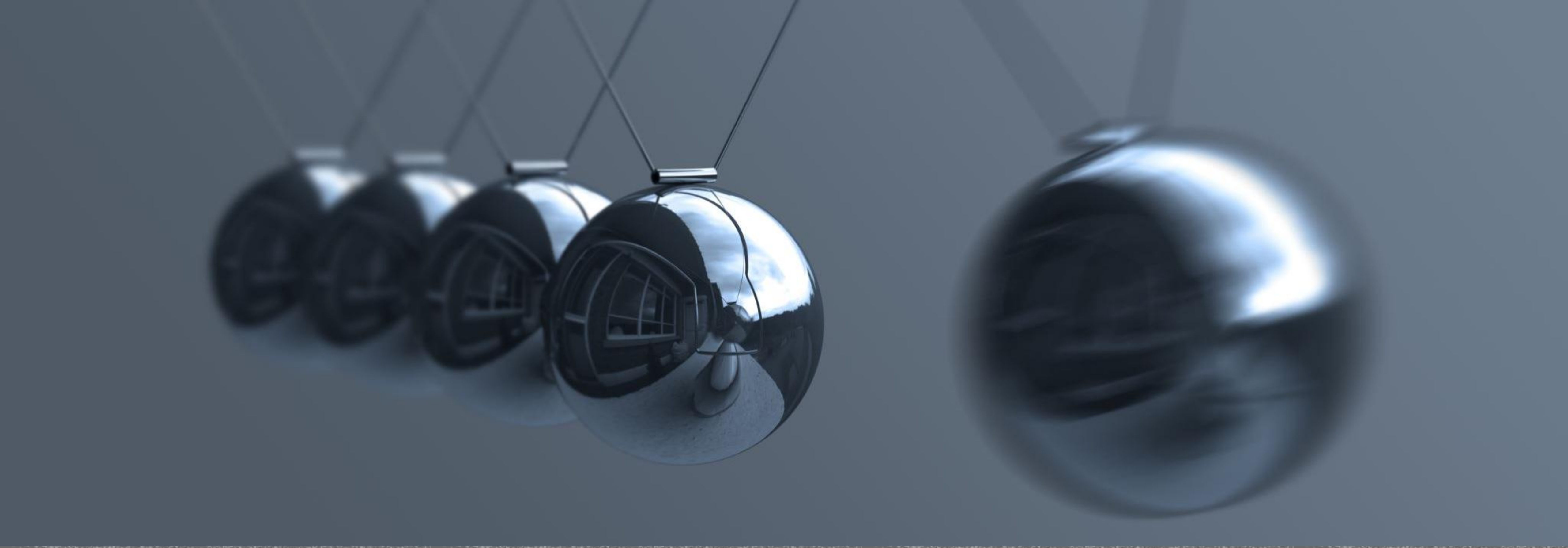


IS THERE A REAL CO2 EFFECT OTHER THAN THE PH?



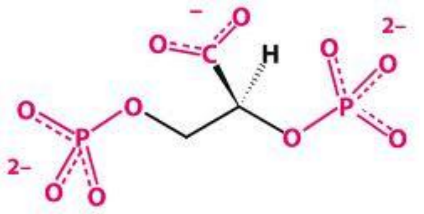






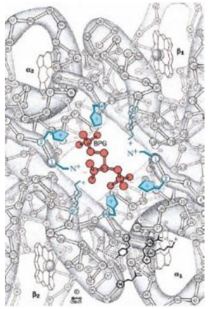
OTHER ALLOSTERIC EFFECTORS

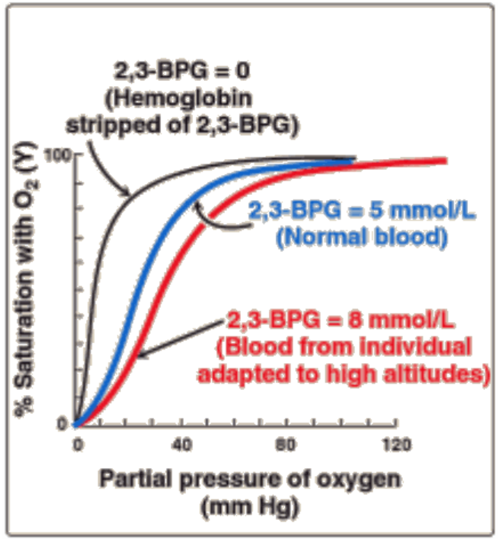
2,3-BISPHOSPHOGLYCERATE (BPG)

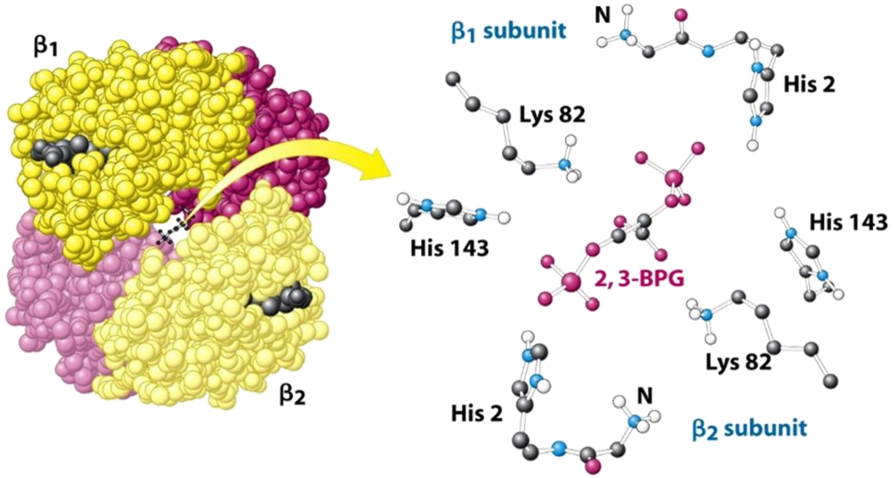


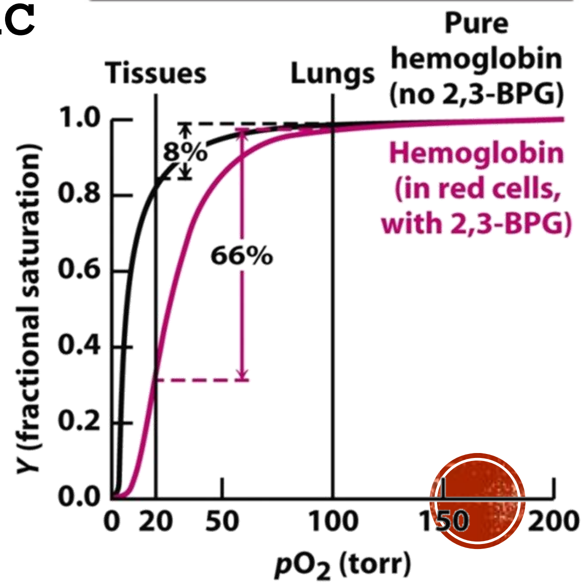
2,3-Bisphosphoglycerate
(2,3-BPG)

- Is a glycolytic intermediate, negatively charged
- Reduce the oxygen affinity of hemoglobin
- Binds in the central cavity (1BPG/Hb)
- Binding creates salt bridges which favor the deoxy form. Physiological relevance?
- Conc. of BPG increases at high altitudes, emphysema, & chronic anemia
- Consideration on transfusion

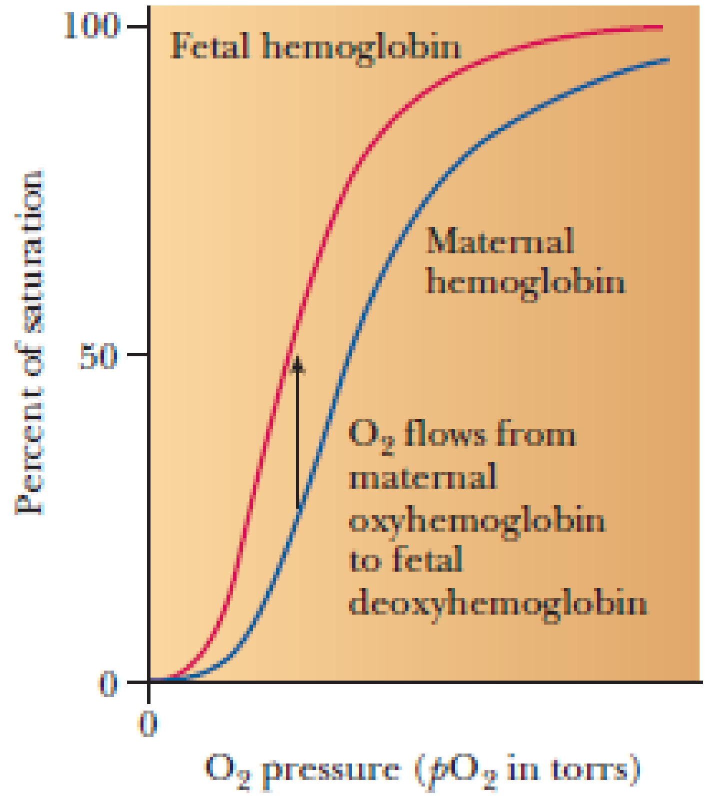




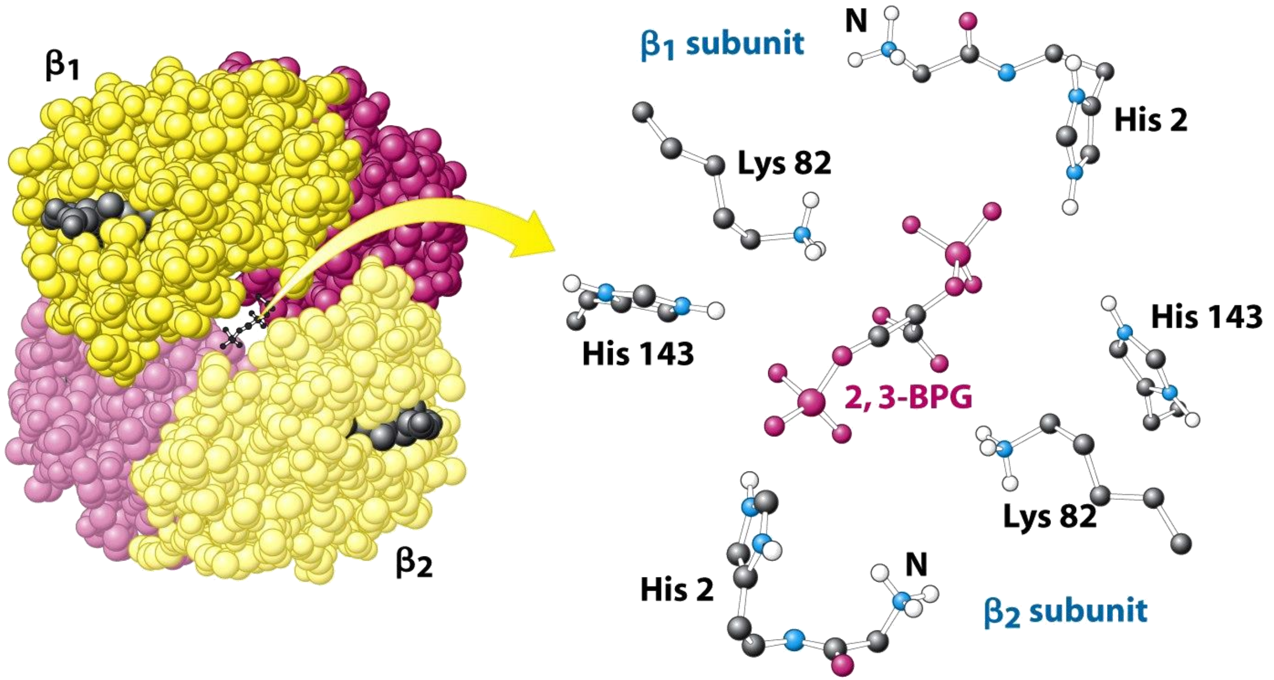


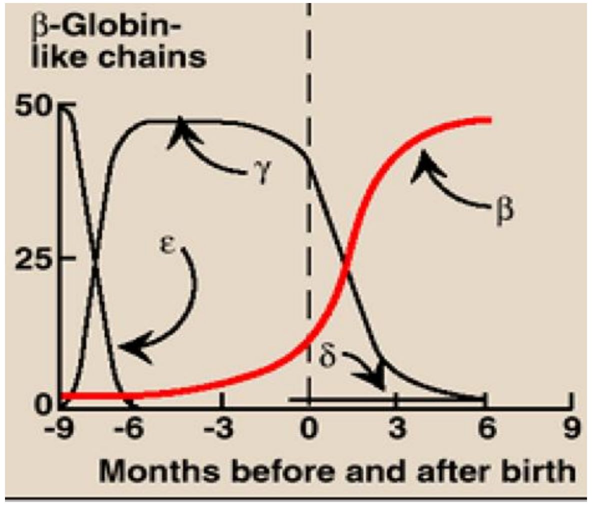


FETAL HEMOGLOBIN



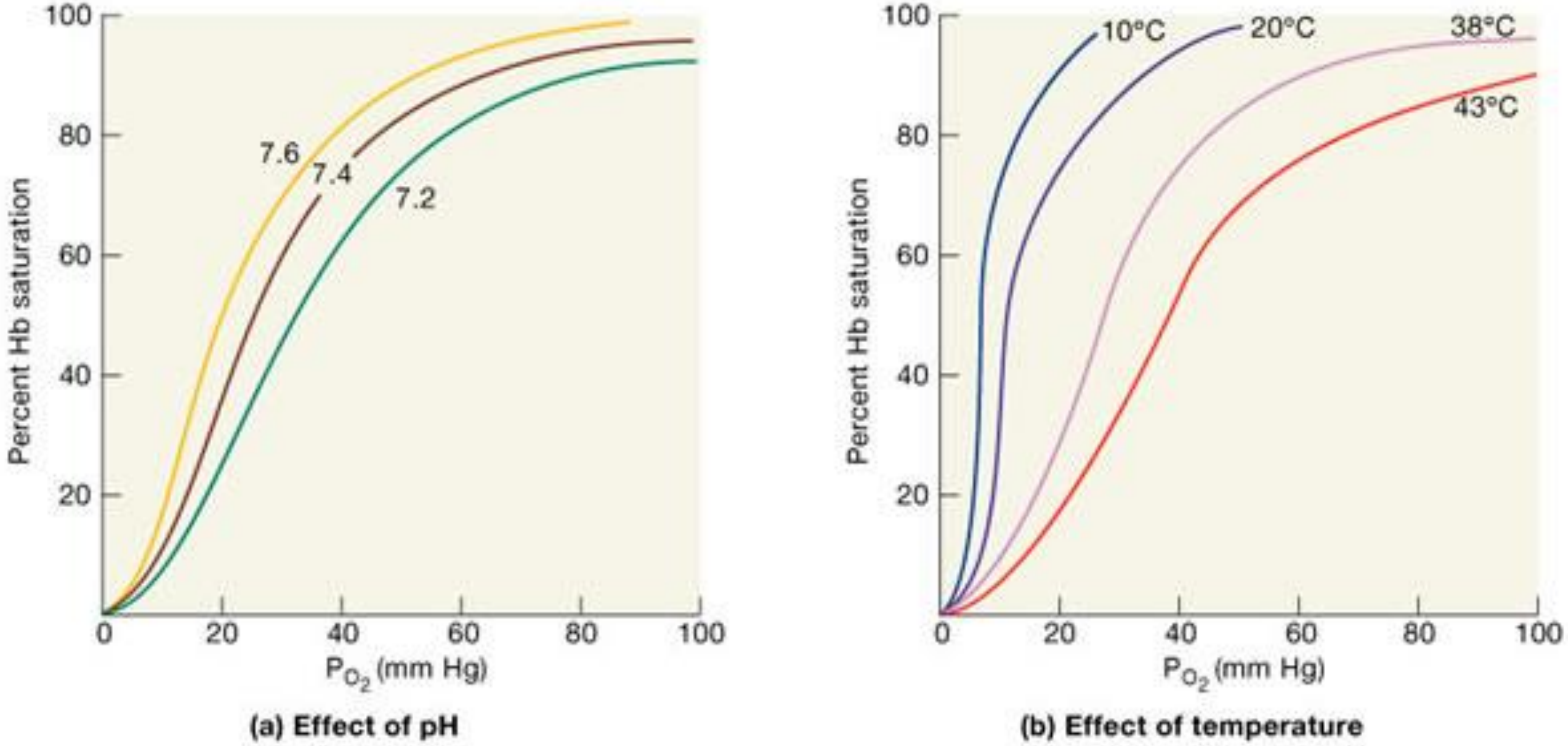
- Fetal Hb has higher affinity towards oxygen
  - α2γ2
  - Adult hemoglobin, βHis143-BPG salt bridge, fetal hemoglobin, the γ –Ser instead of His)





EFFECT OF TEMPERATURE

- As temperature rises, O2 affinity decreases
- Temperature affects both myoglobin and hemoglobin



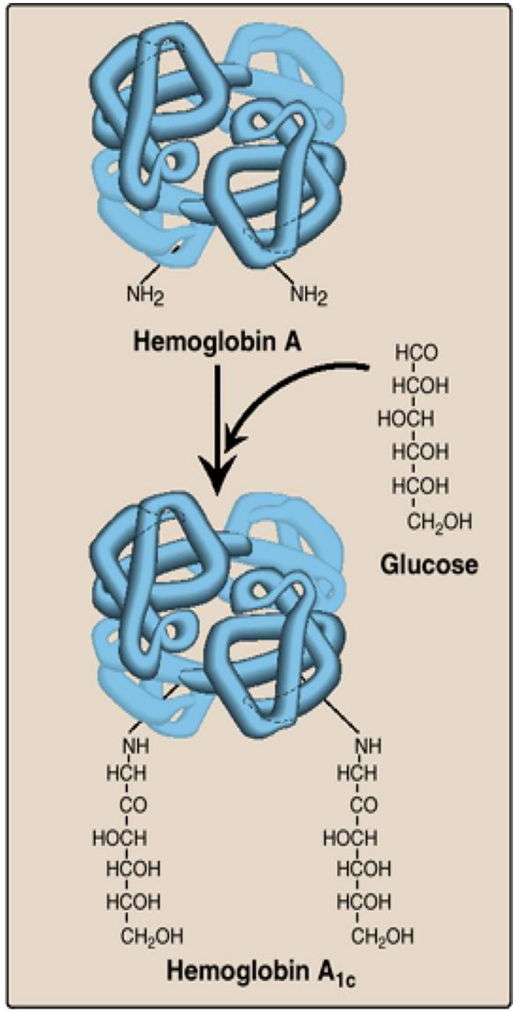
(a) Effect of pH

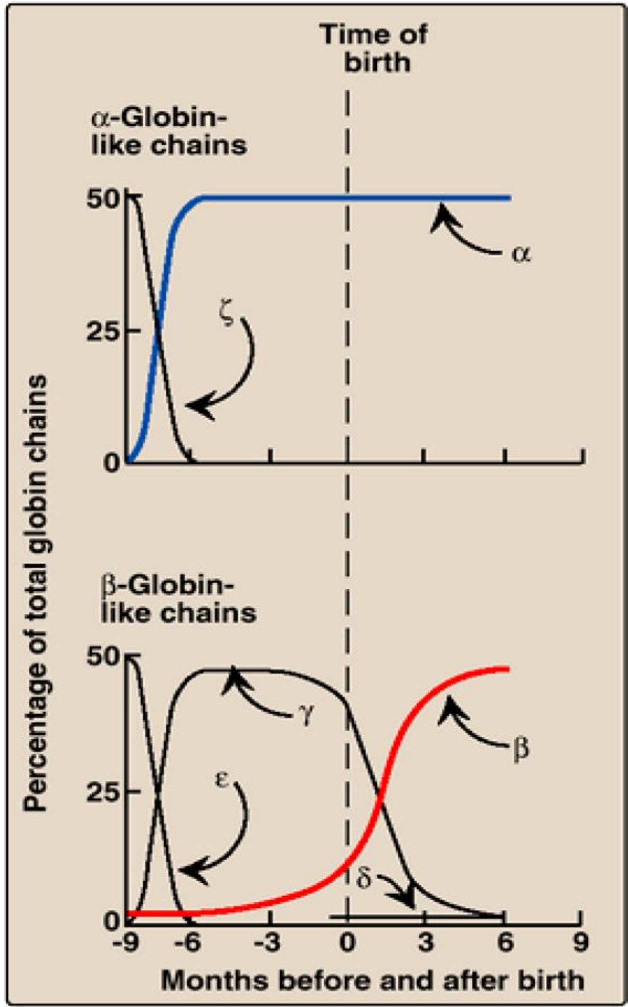
(b) Effect of temperature

MINOR HEMOGLOBINS

- Hemoglobin A_2 (Hb A_2)
- Hemoglobin A_{1c} (HbA_{1c})
- HbA

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3–9%





THE GLOBIN GENES

- α-Gene family: located on chromosome 16 & contains 2 genes for the α-globin chains plus others (remains on throughout life)
- β-Gene family: located on chromosome 11 & contains a single gene for the β-globin chain. Also contains 2γ genes and the δ gene