Globular proteins

Myoglobin and hemoglobin

Summer, 2024

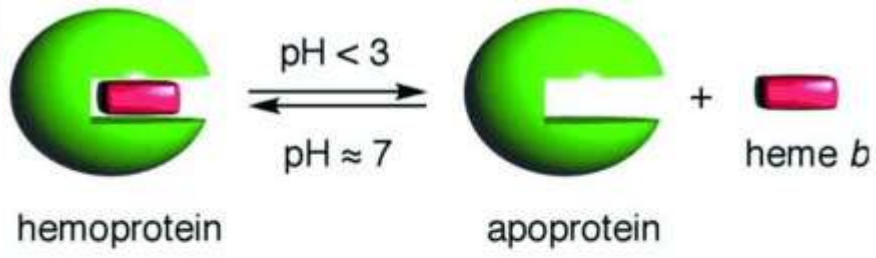
Functions of myoglobin and hemoglobin

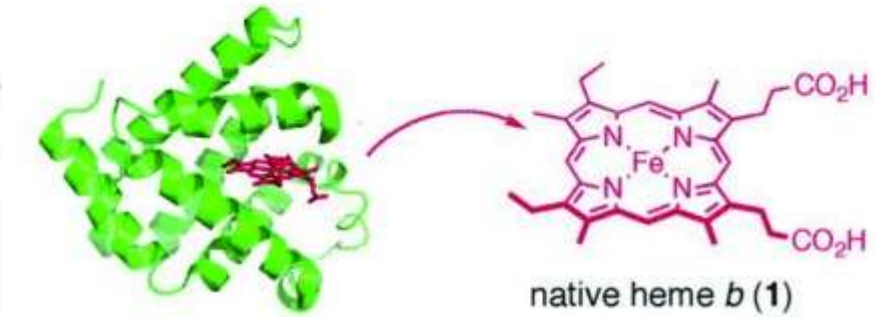
- Myoglobin functions in storing O2 in muscles. During periods of oxygen deprivation, oxymyoglobin releases its bound oxygen.

- Hemoglobin:
  - transport of O_2 and CO_2
  - blood buffering

Hemoproteins

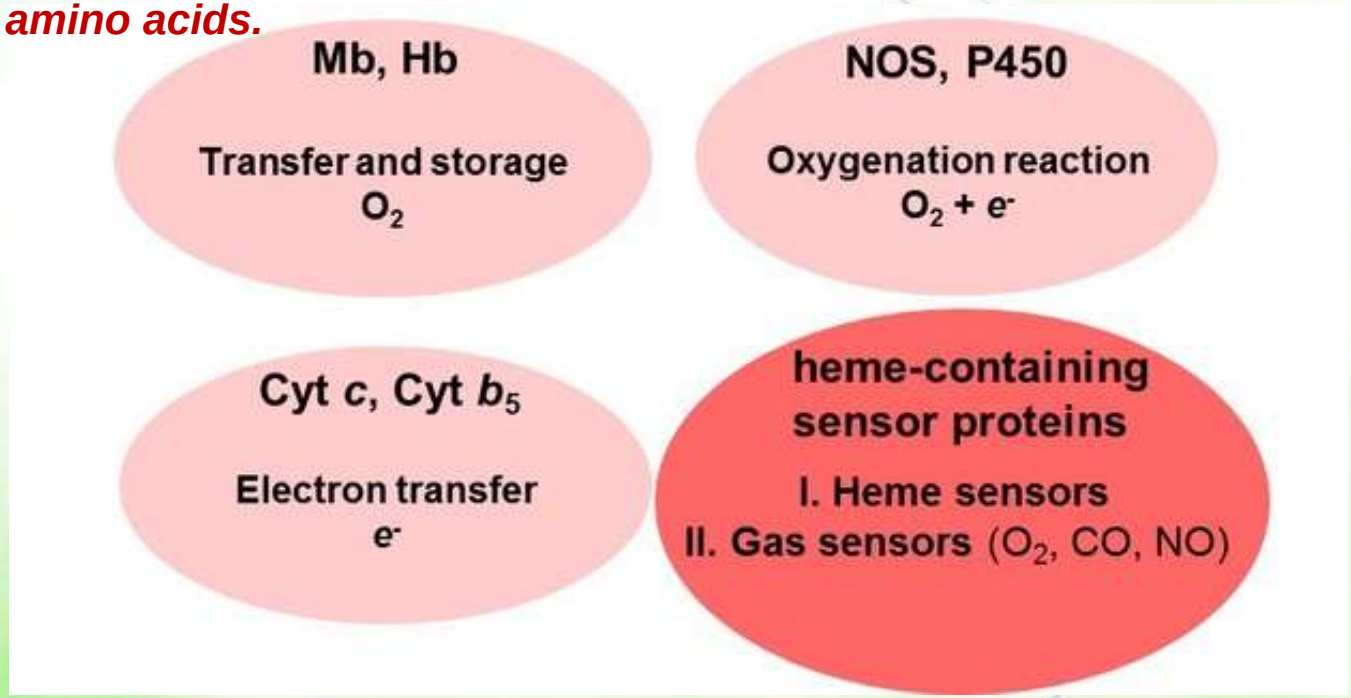
- Many proteins have heme as a prosthetic group called hemoproteins.





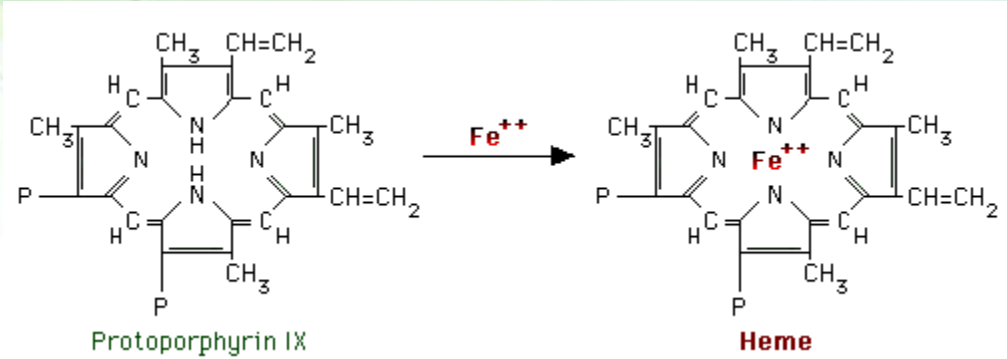
The protein environment dictates the function of the heme.

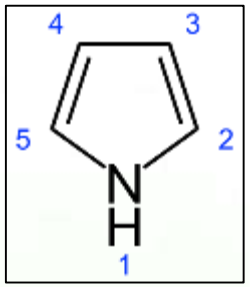
A prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.

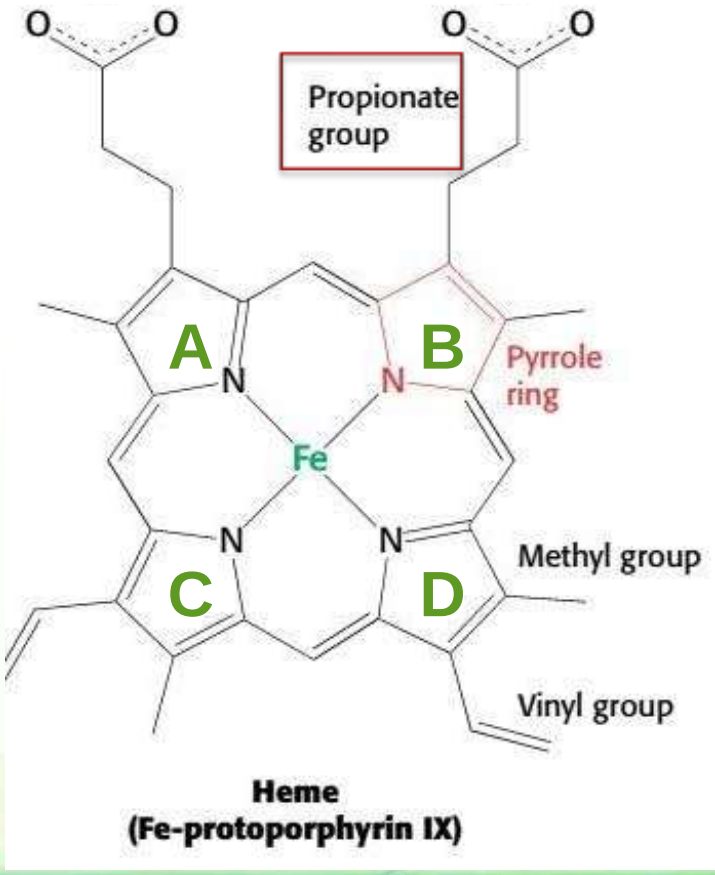


Heme structure

- It is a complex of protoporphyrin IX + Iron (Fe^{2+}).
- The porphyrin is planar and consists of four rings (designated A-D) called pyrrole rings.
- Each pyrrole can bind two substituents.
- Two rings have a propionate group each.
- *Note: the molecule is hydrophobic.*
- Fe has six coordinates of binding.

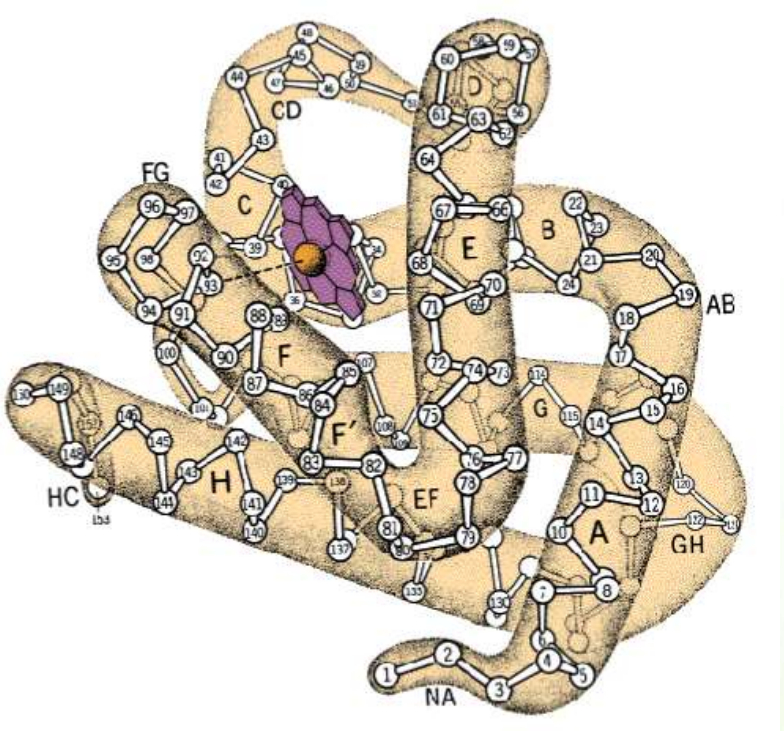






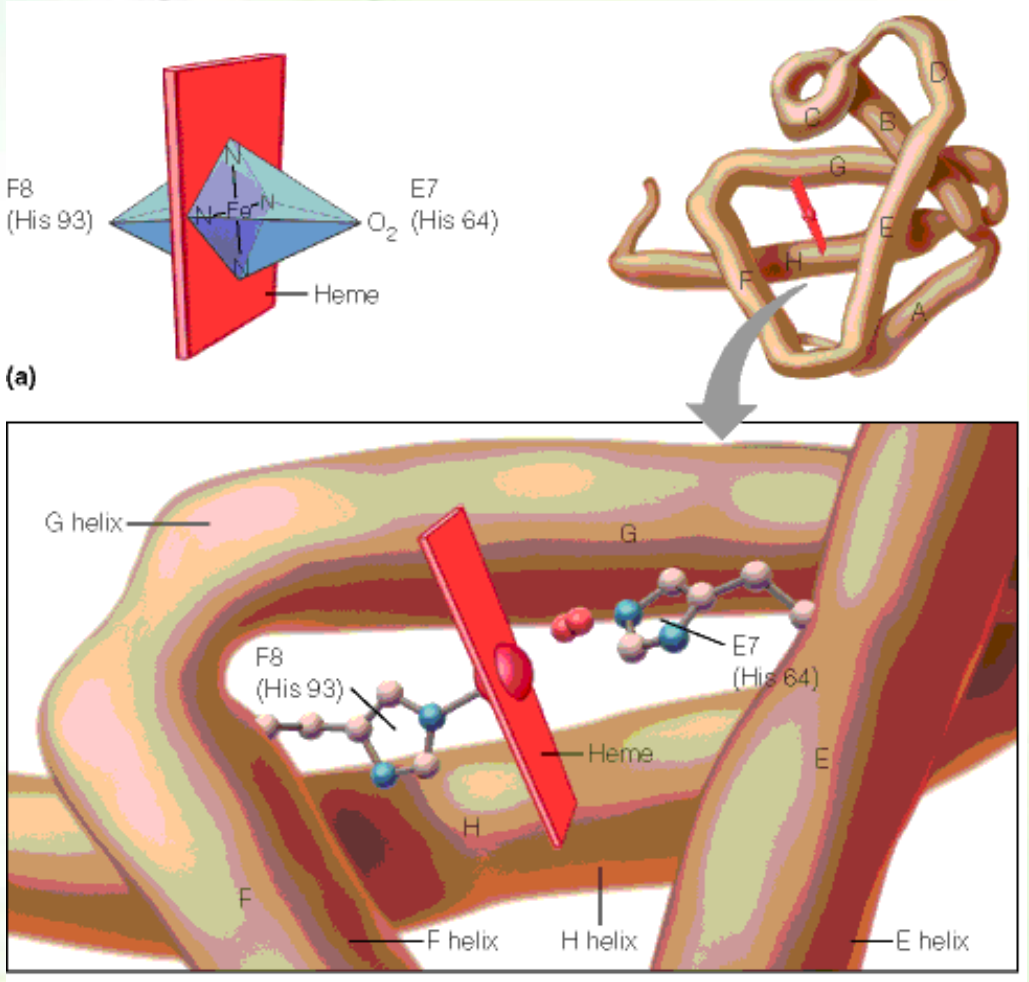
Structure of myoglobin

- Myoglobin is a monomeric protein that is mainly found in muscle tissue.
- The tertiary structure of myoglobin 8 α-helices, designated A through H, that are connected by short non-helical regions.
- The α-helices are connected by short coils, a structure that is known as the globin fold, which is a hydrophobic O_2-binding pocket.
- It contains heme as a prosthetic group internally.
- Myoglobin can be present in two forms:
  - oxymyoglobin (oxygen-bound)
  - deoxymyoglobin (oxygen-free)



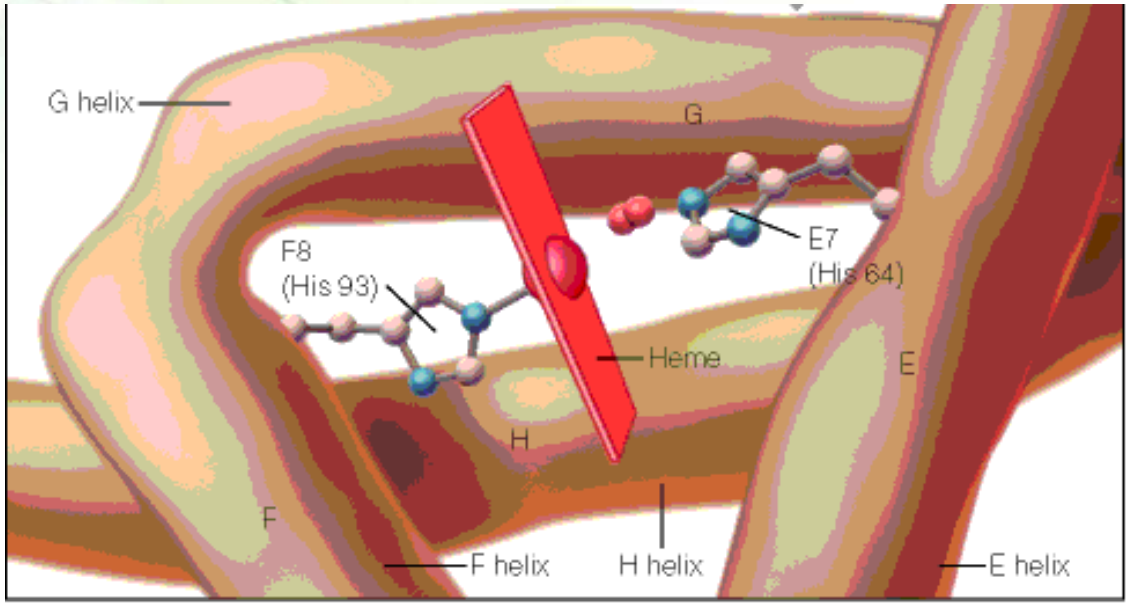
Arrangement of amino acids

- Like other globular proteins, the hydrophilic amino acids are generally on the surface, while hydrophobic amino acids are predominantly internal.
  - 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.



Iron

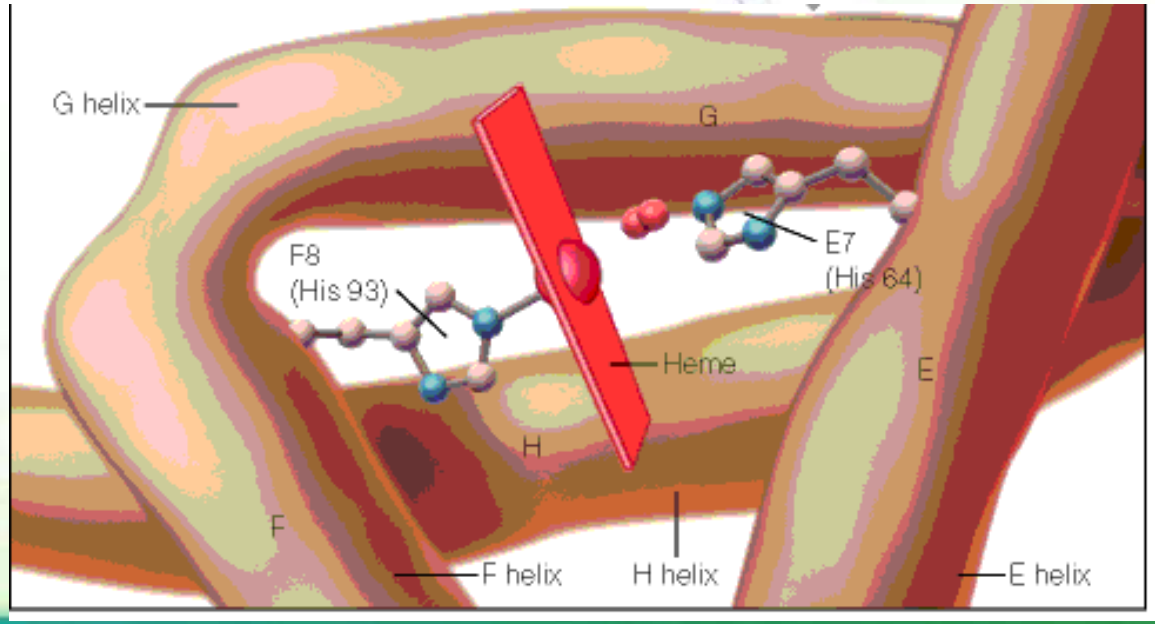
- Iron can bind in the center of the four rings.
- Fe is in the ferrous state (Fe^{2+}) and can form 6 bonds:
  - 4 bonds with the nitrogen of the rings,
  - One bond (known as the fifth coordinate) with the nitrogen of the proximal His.
  - A last one with O_2 (the sixth coordinate) when O_2 is there

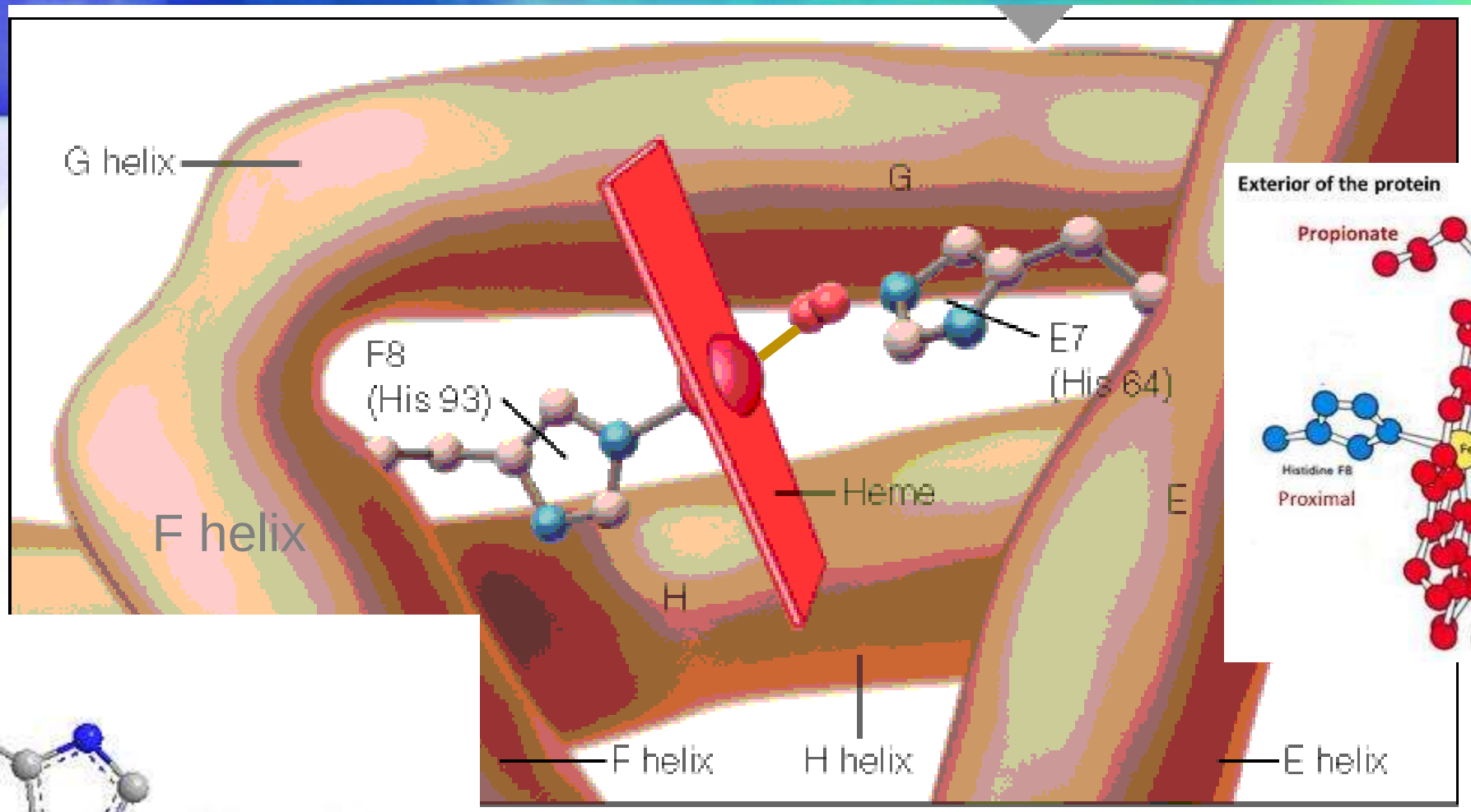


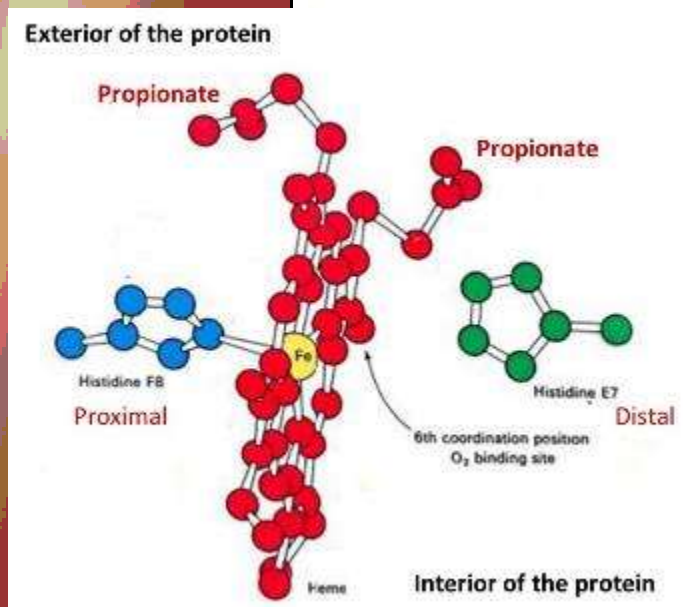
- Oxidation of iron to the Fe^{3+}, ferric, state makes the molecule incapable of normal O_2 binding.
- Upon absorption of light, heme gives a deep red color.

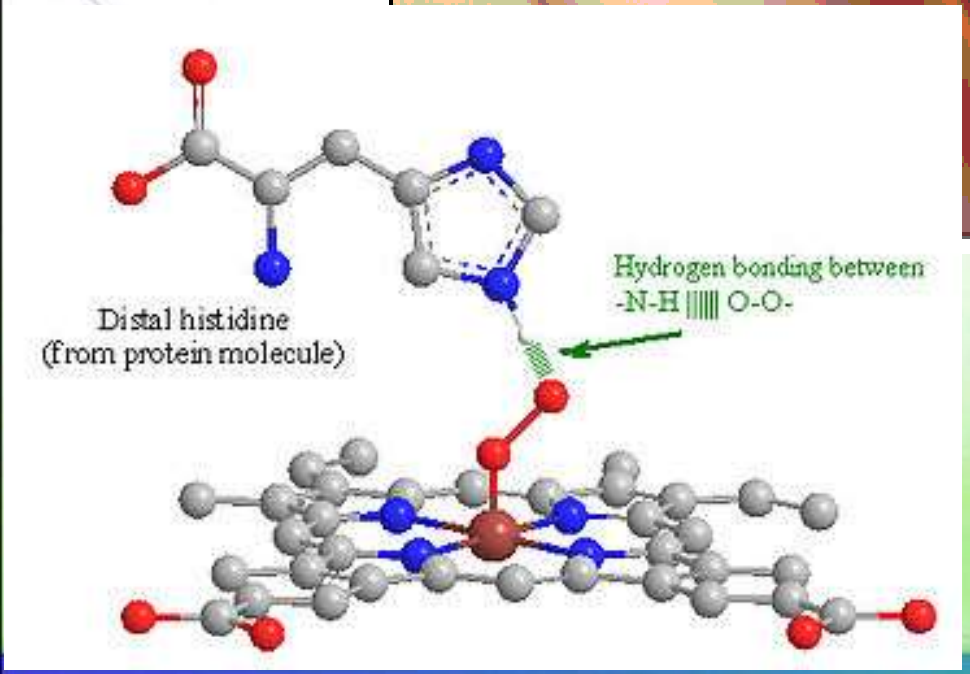
Structure-function relationship

- 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 hydrophobic interior of myoglobin (or hemoglobin) prevents the oxidation of iron, and so when O_2 is released, the iron remains in the Fe(II) state and can bind to another O_2.
- The distal histidine acts as a gate that opens and closes as O_2 enters the hydrophobic pocket to bind to the heme.
- It also stabilizes the interaction with oxygen.



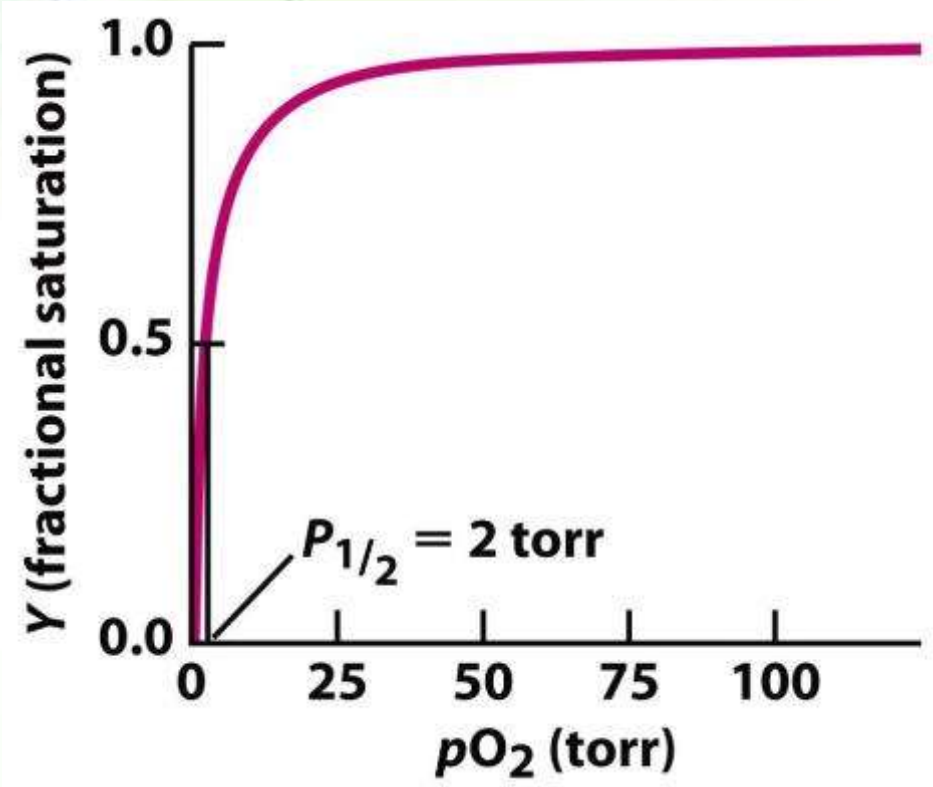

G helix
G
E7
(His 64)
F8
(His 93)
Heme
F helix
H
E
F helix
H helix
E helix


Exterior of the protein
Propionate
Propionate
Histidine F8
Proximal
Histidine E7
Distal
6th coordination position
O_2 binding site
Heme
Interior of the protein


Distal histidine
(from protein molecule)
Hydrogen bonding between
-N-H ||||| O-O-

Oxygen binding to myoglobin

- Myoglobin binds O_2 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 O_2 pressure in tissues is normally 20-40 mm Hg, it is almost fully saturated with oxygen at normal conditions.

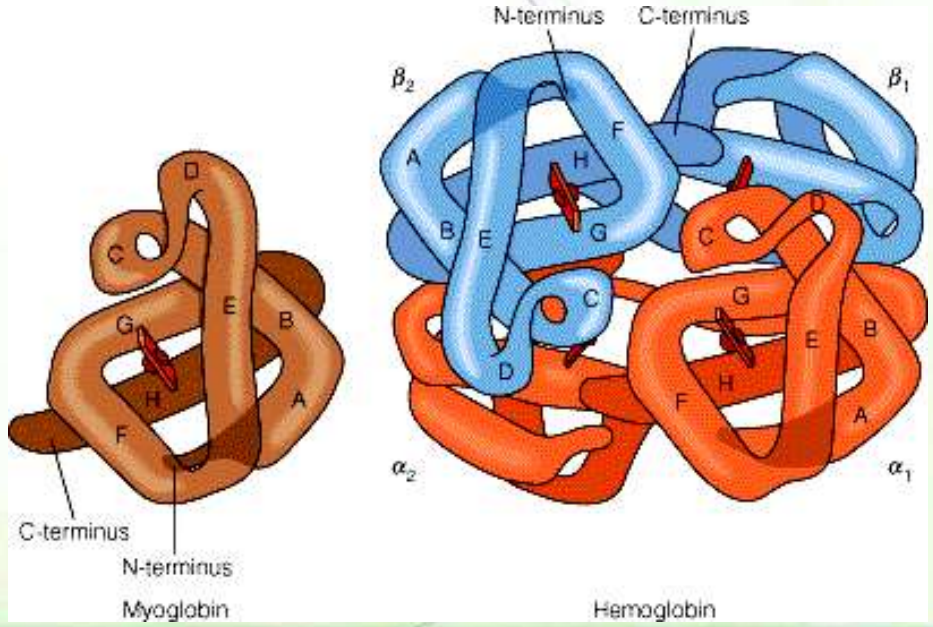


The binding of **O2** to myoglobin follows a hyperbolic saturation curve.

Hemoglobin

Hemoglobin structure

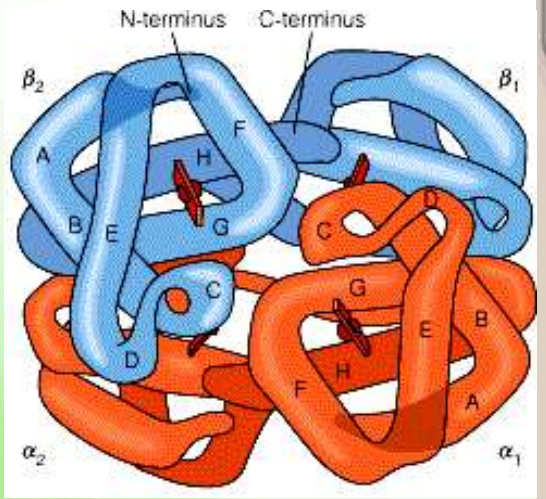
- Hemoglobin is a tetrameric hemeprotein (four globin protein chains with each bound to heme).
- In adults, the four globin proteins are of two different types known as α and β, so a hemoglobin protein is termed α2β2 globin protein.
  - α polypeptide = 141 amino acids (Val1 & Arg141)
  - β polypeptide = 146 amino acids (His146)

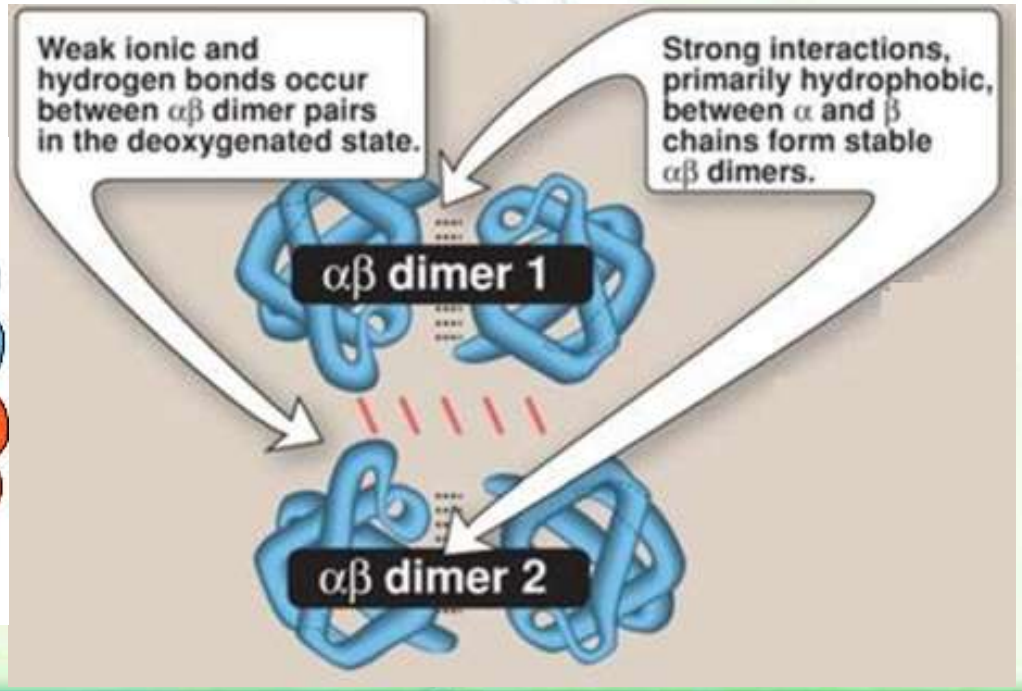


How are the subunits bound?

- A dimer of dimers (I made up this term) OR two αβ-protomers
  - $(\alpha\text{-}\beta)_2$
- The chains interact with each other via hydrophobic interactions.
  - Therefore, hydrophobic amino acids can also be present on the surface.

- Electrostatic interactions (salt bridges) and hydrogen bonds also exist between the two different chains.

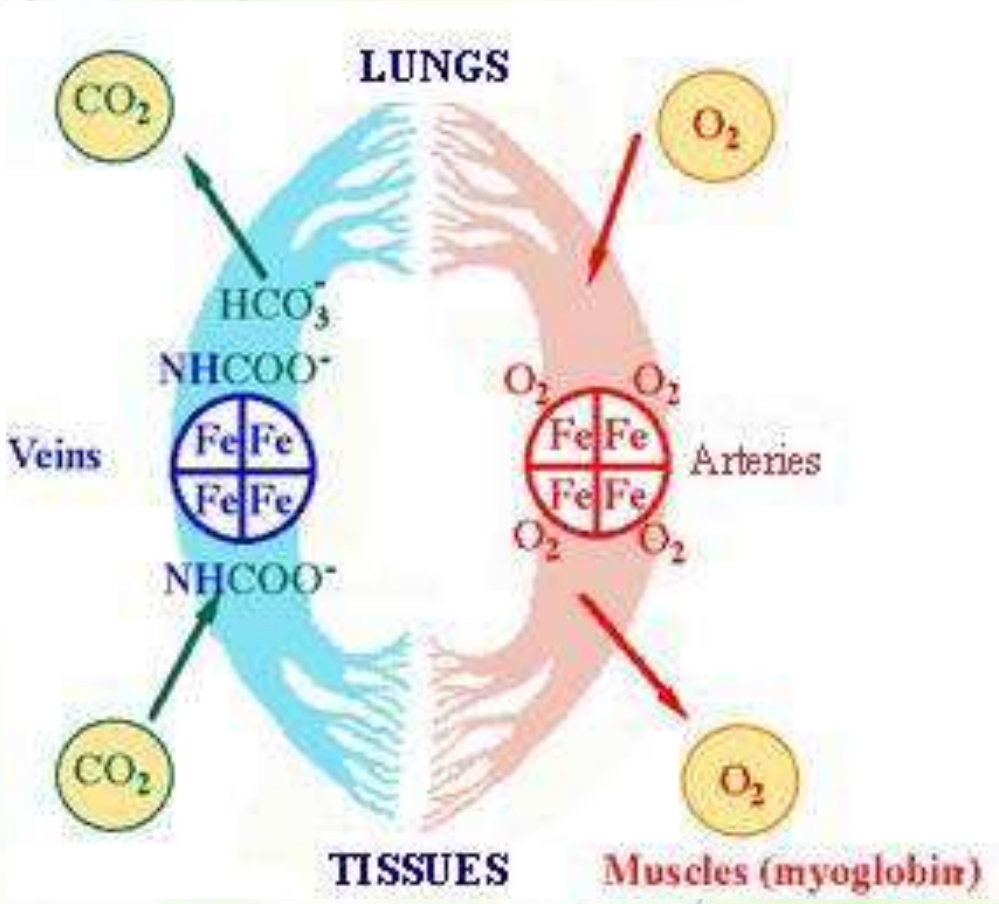




Oxygen binding to hemoglobin

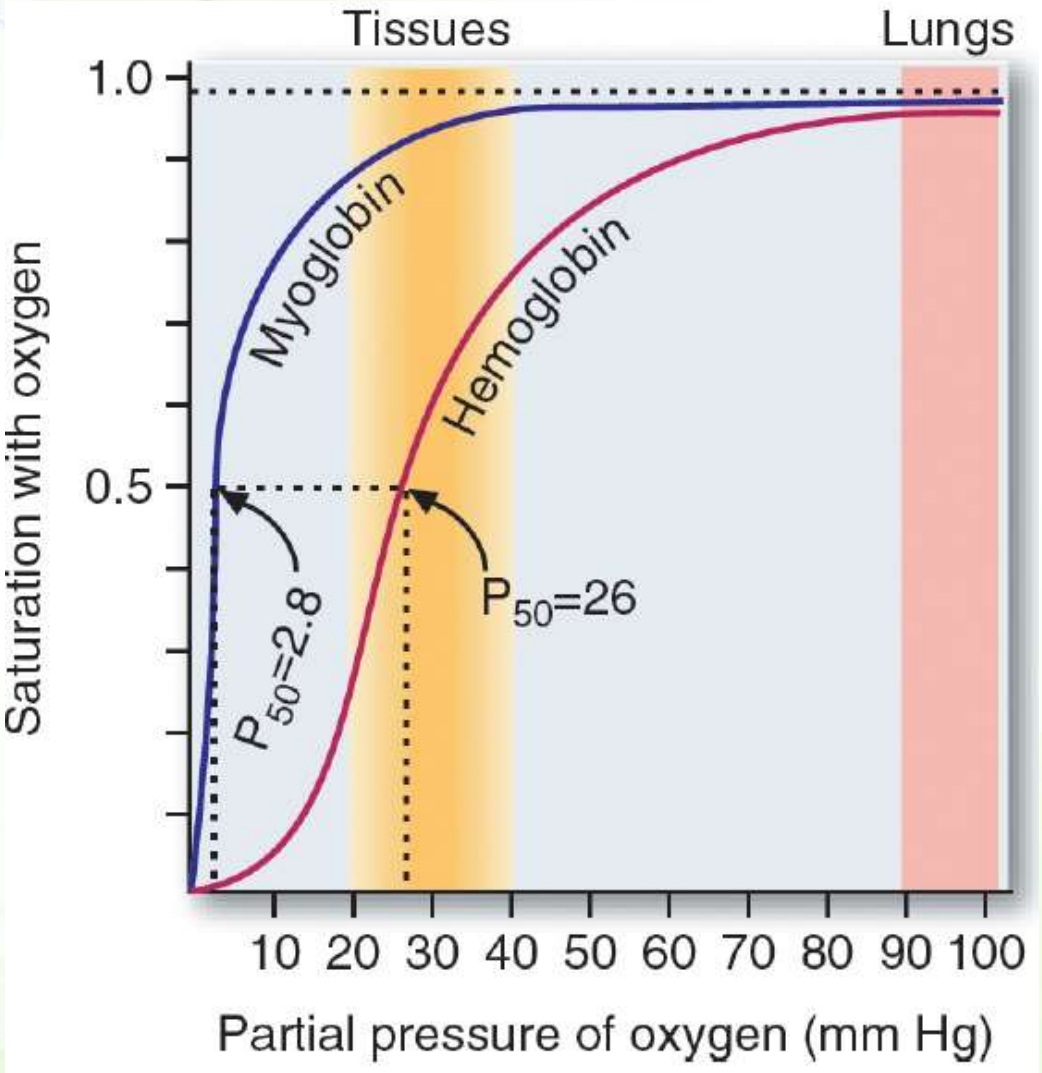
- Hemoglobin must bind oxygen efficiently and become saturated at the high oxygen pressure found in the lungs (approximately 100 mm Hg).
- Then, it must release oxygen and become unsaturated in tissues where the oxygen pressure is low (about 30 mm Hg).

Do you expect hemoglobin to have a high or low affinity for oxygen?

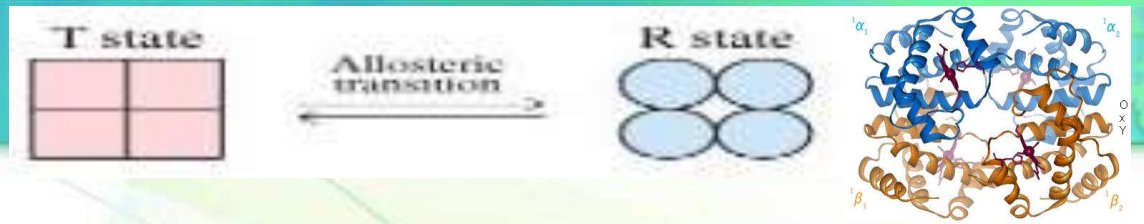


The saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
  - A sigmoidal curve indicates that the protein has different structures.
- At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin).
- As the oxygen pressure falls, oxygen is released to the cells.
- In contrast to a low p50 for myoglobin, the p50 of hemoglobin is approximately 26 mm.
  - *Relate the value of p50 to affinity*

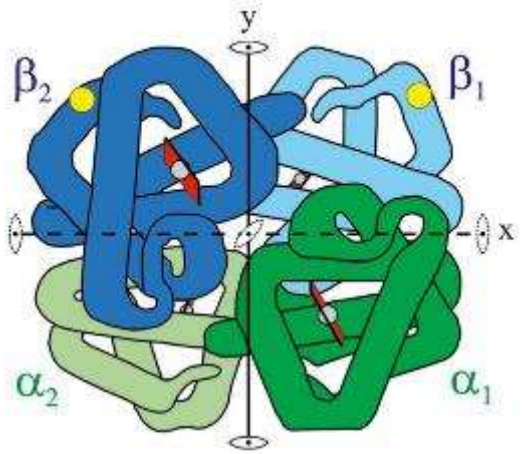


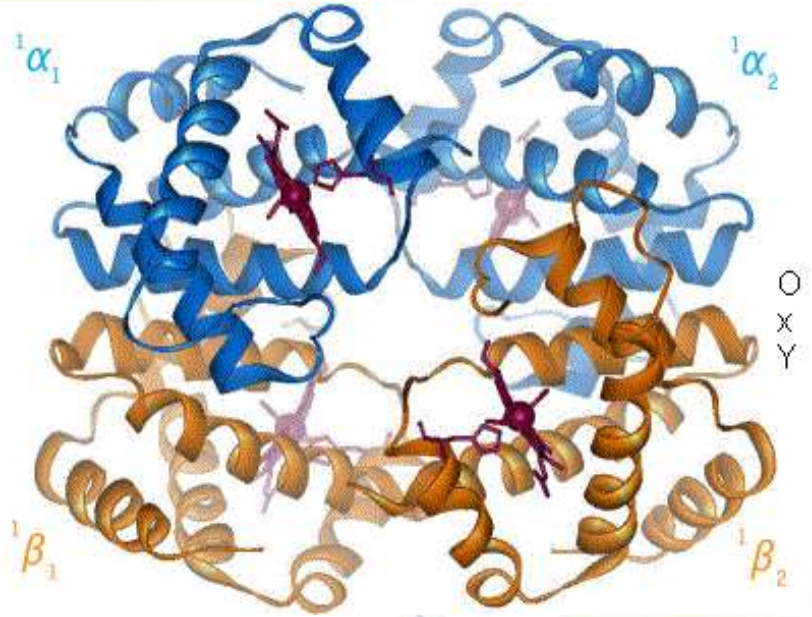
Hemoglobin is allosteric

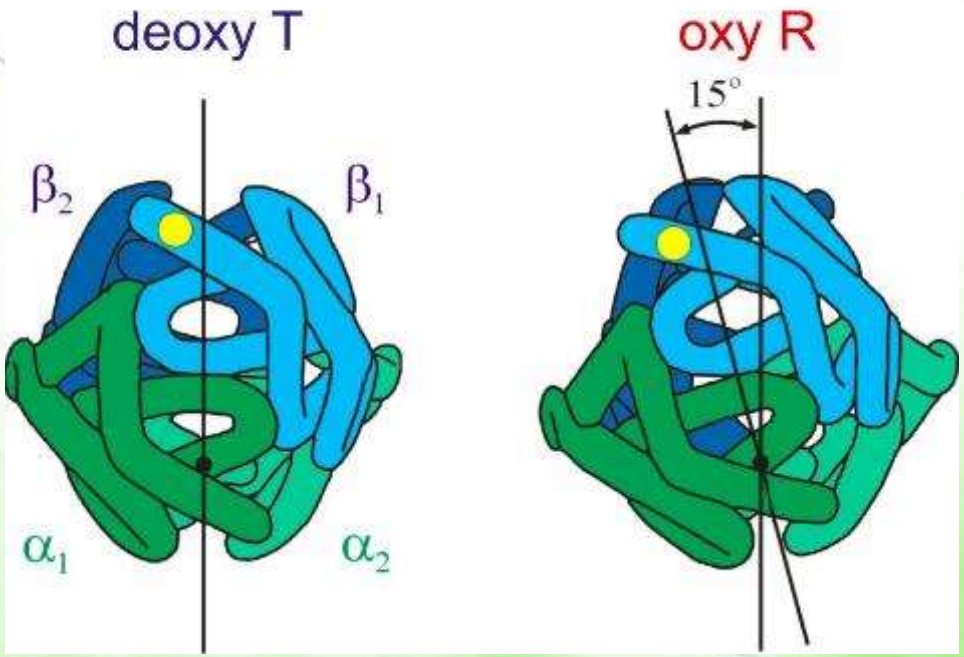


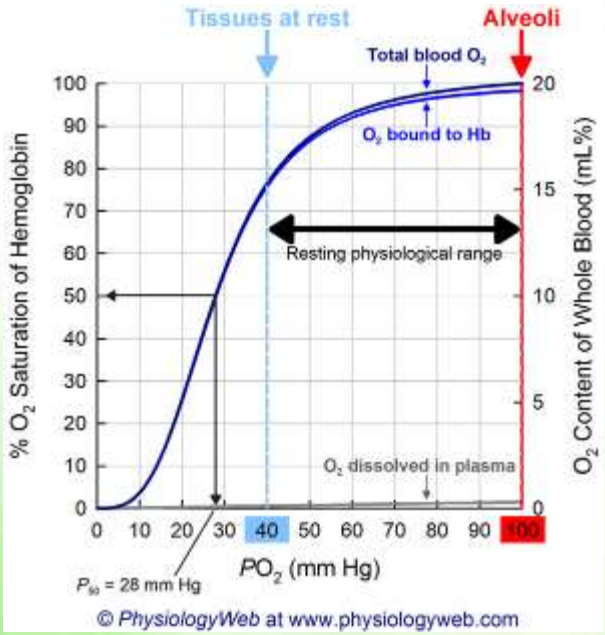
- Hemoglobin is an allosteric protein (from Greek "allos" = "other", and "stereos" = "shape").
  - An allosteric protein: a multi-subunit protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein by changing its structure slightly.
- Hemoglobin exists in two allosteric forms, T-state and R-state
  - The T-state is also known as the "taut" or "tense" state and it has a low binding affinity to oxygen.
  - The R-state is known as the "relaxed" state, and it has 500 times higher affinity to oxygen than the T conformation.
- Binding of O_2 causes conformational changes in hemoglobin, converting it from the low-affinity T-state to the high-affinity R-state .

Structural change of hemoglobin



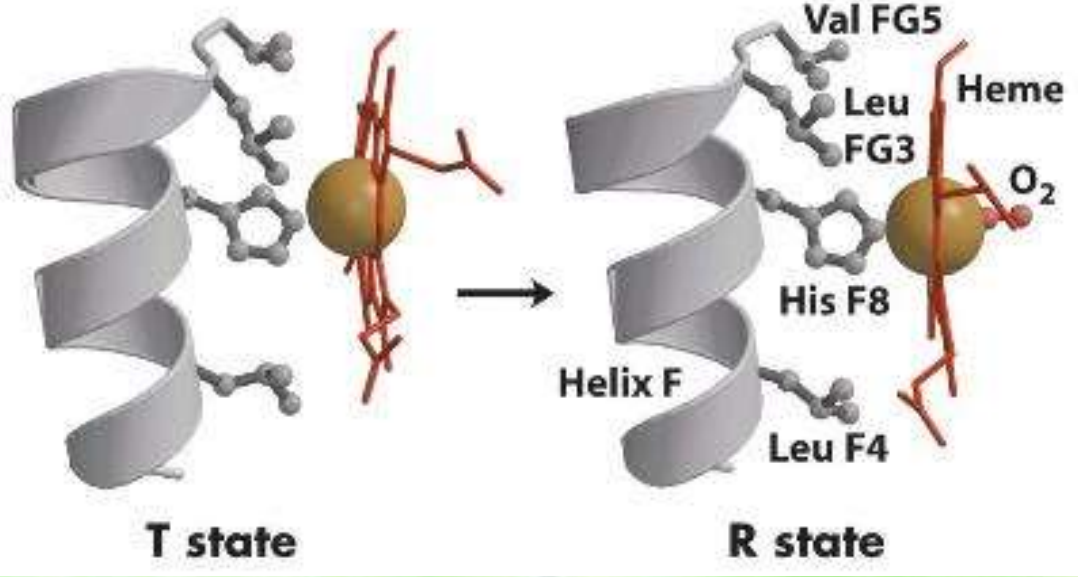


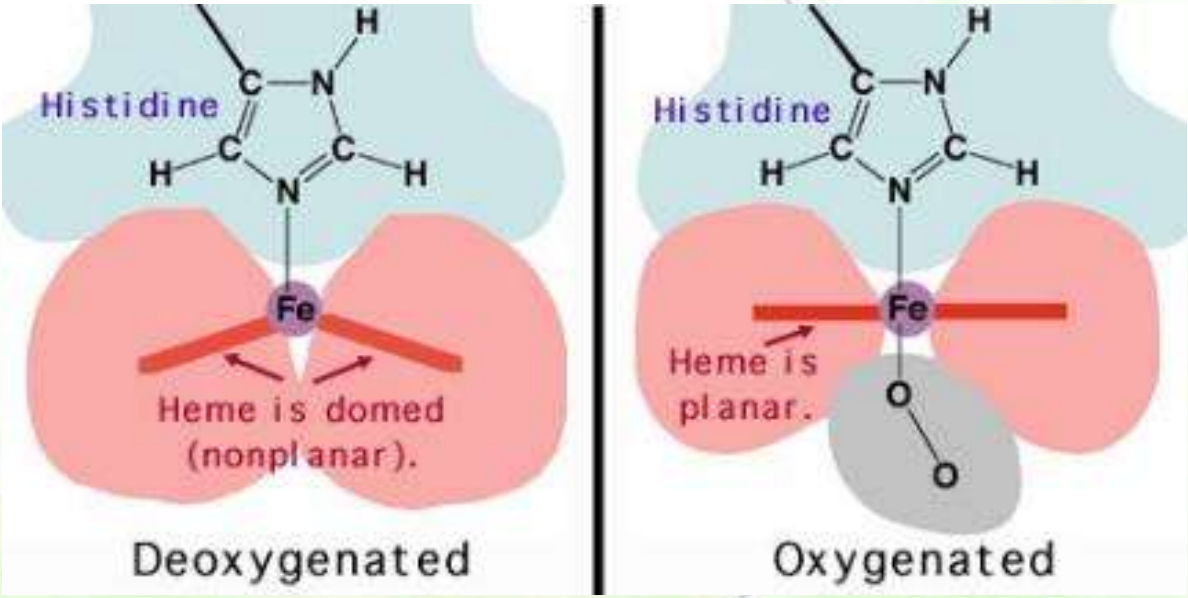




How does the structure change? (1)

- When heme is free of oxygen, it has a domed structure and iron is outside the plane of the heme group.
  - Because the hydrophobic heme is repelled by the proximal His.
- When oxygen binds to an iron atom, heme adopts a planar structure and the iron moves into the plane of the heme pulling proximal histidine (F8)

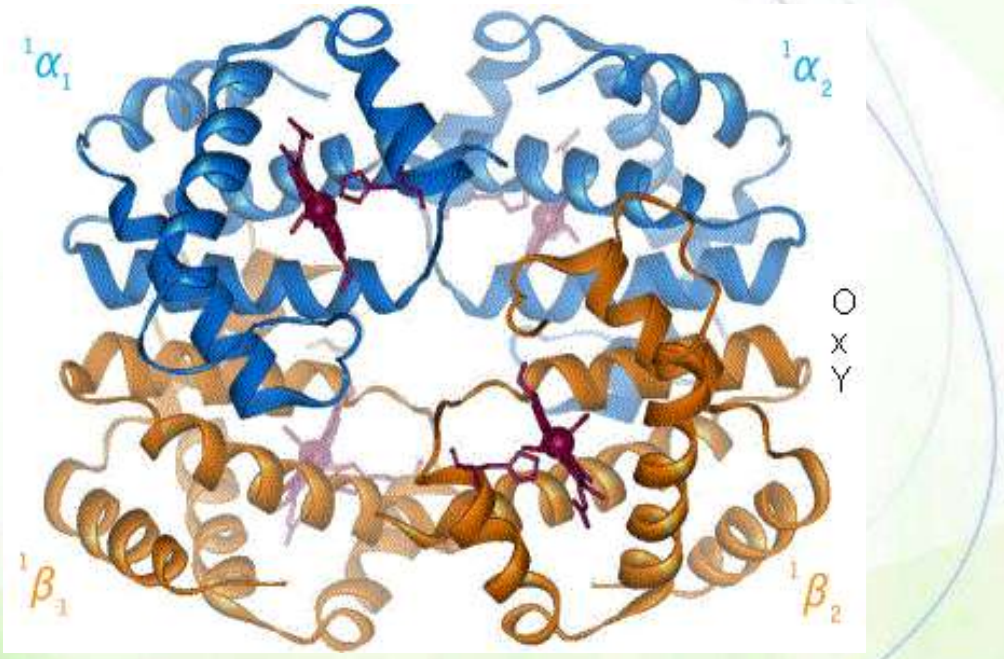




How does the structure change? (2)

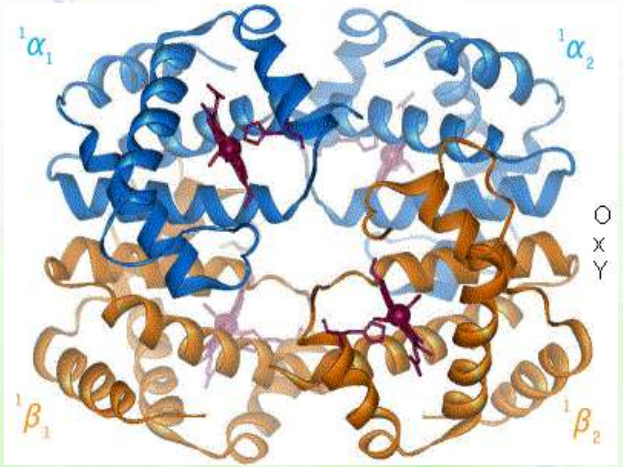
- This movement triggers
  - changes in tertiary structure of individual hemoglobin subunits
  - breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.

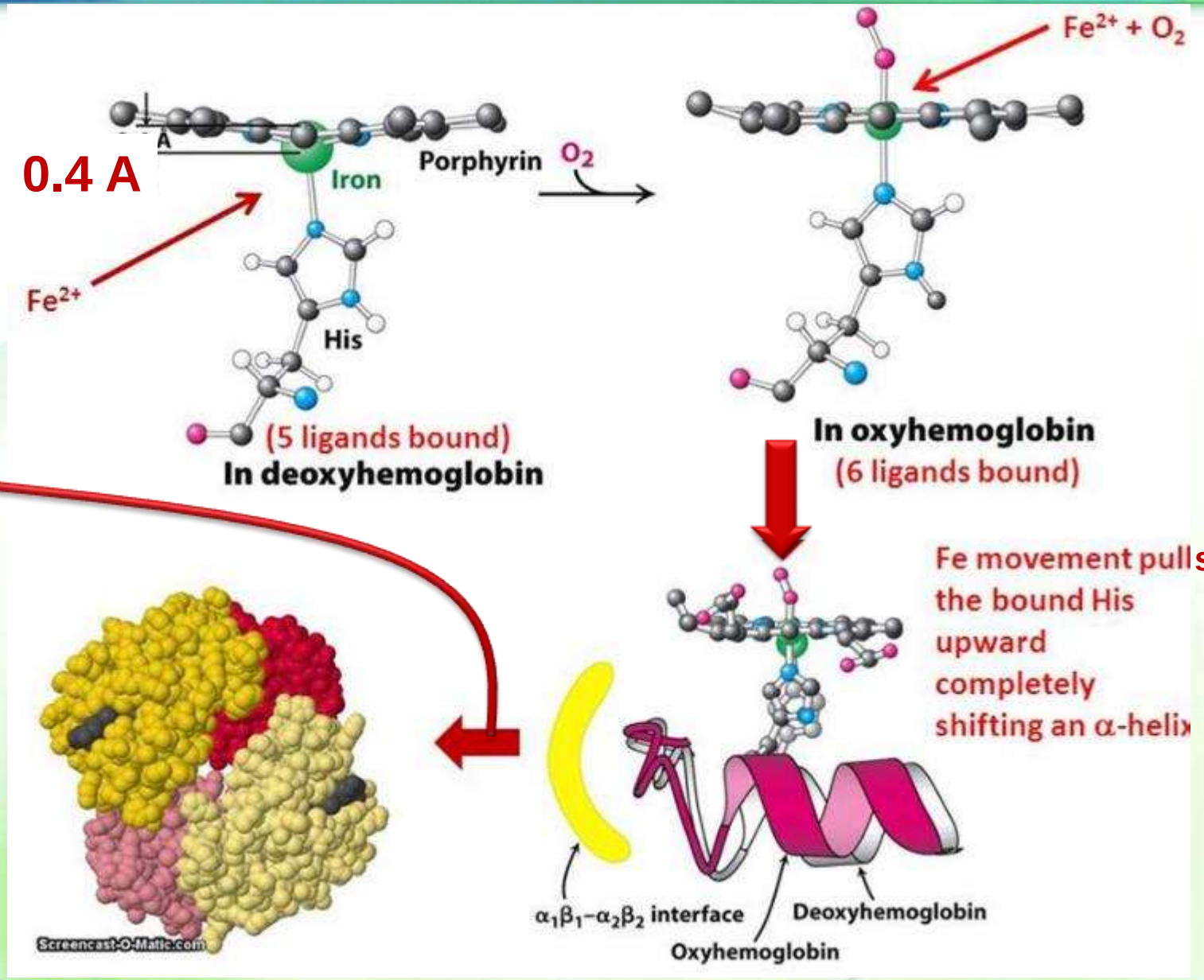
In myoglobin, movement of the helix does not affect the function of the protein.



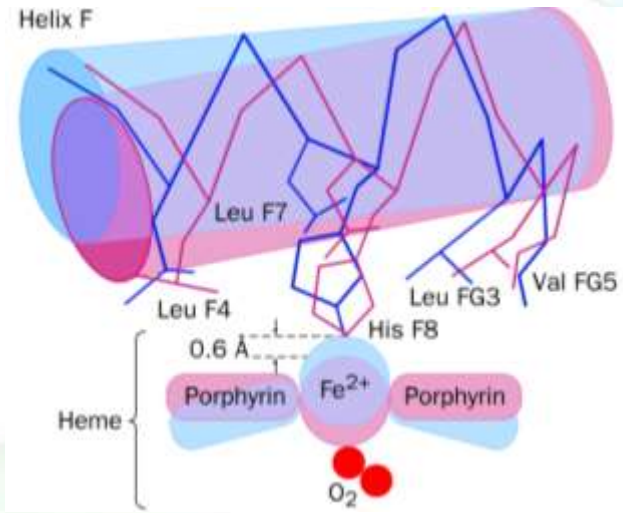
Structural amplification change

- **Changes in tertiary structure of individual hemoglobin subunits**
- **Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.**



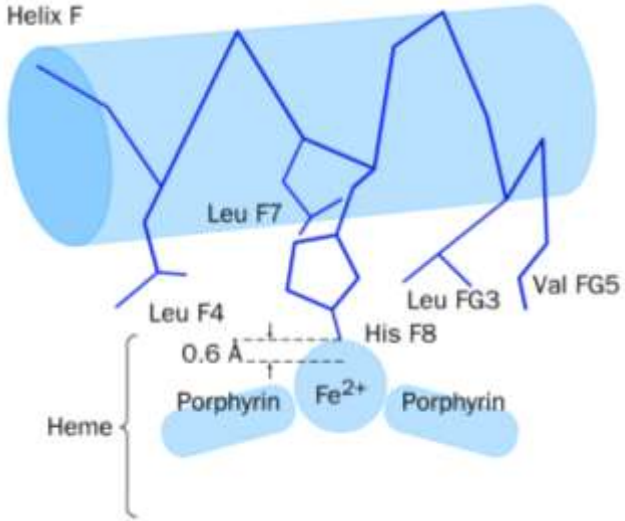


Another look at it

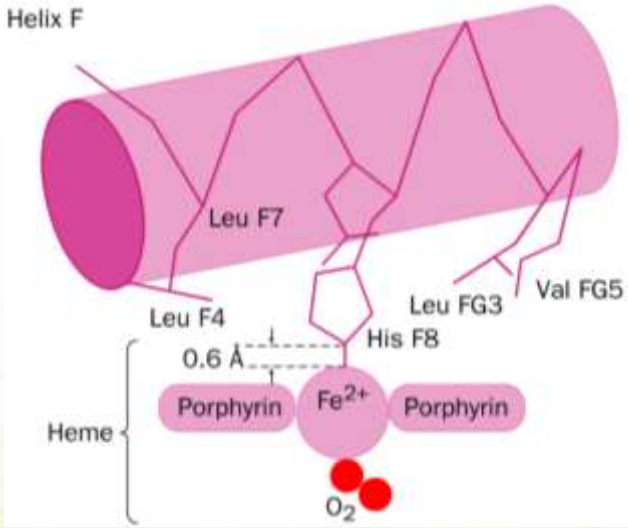


Movements of the hemoglobin's heme and F helix during the T → R transition.

Fig. 7-9 diagrams how the binding of O_2 to one hemoglobin site induces conformation changes that influence the O_2 -binding affinity of the other sites.

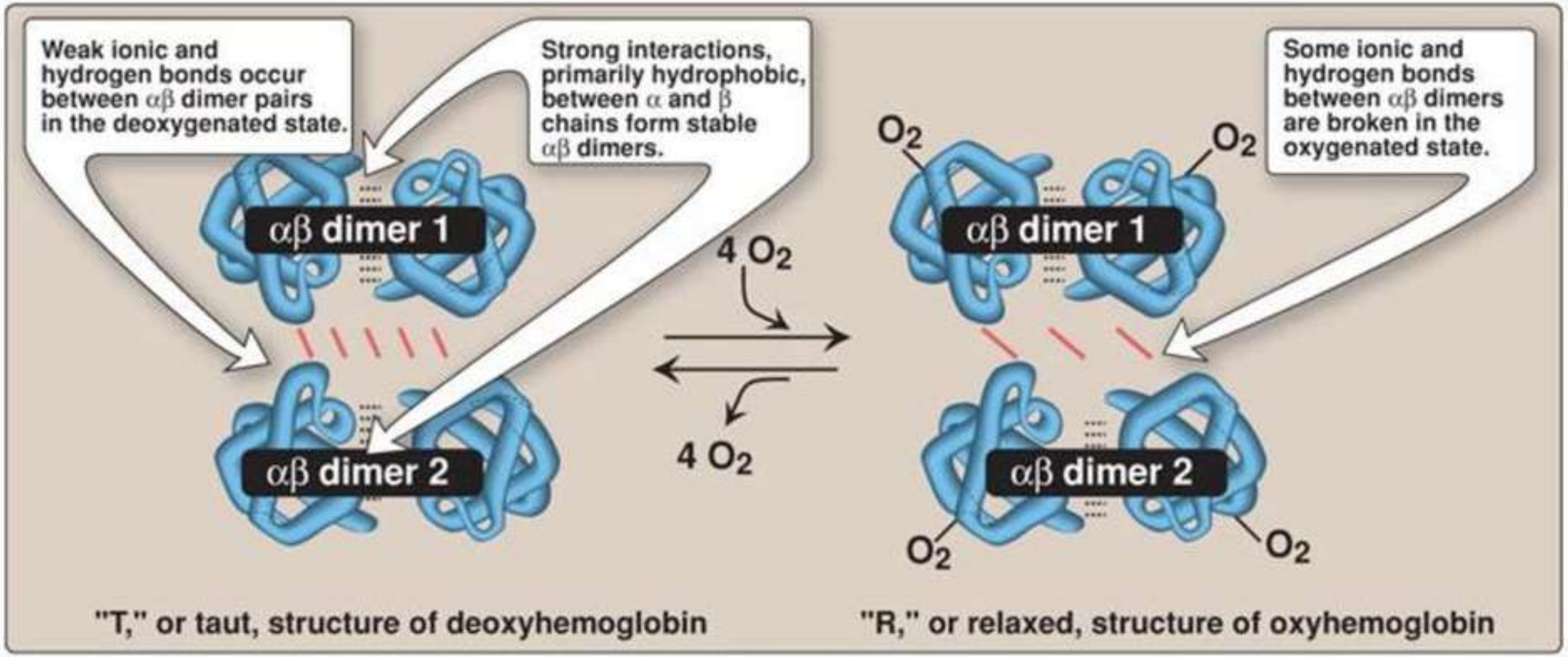


In the absence of bound O_2 ,the Fe(II) lacks a sixth ligand, and resides about 0.6 Angstrom out of the plane of the heme toward its His ligand (the proximal His).



Upon binding O_2 ,the Fe(II) is pulled towards the O_2 into the plane of the heme. This also pulls the attached proximal His towards the heme. Since the proximal His is part of the F helix, this entire helix is also pulled toward the heme. These conformational changes induce a rearrangement of the alpha and beta subunits in the hemoglobin tetramer.

Electrostatic interactions are broken

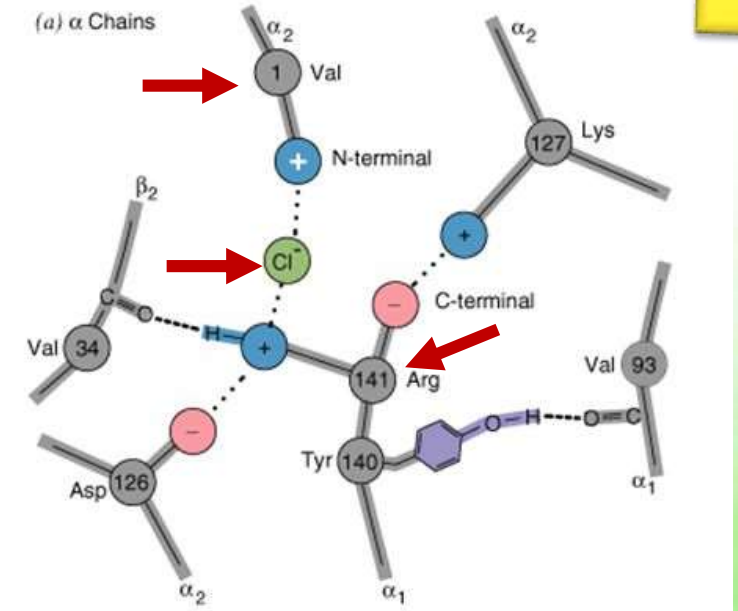


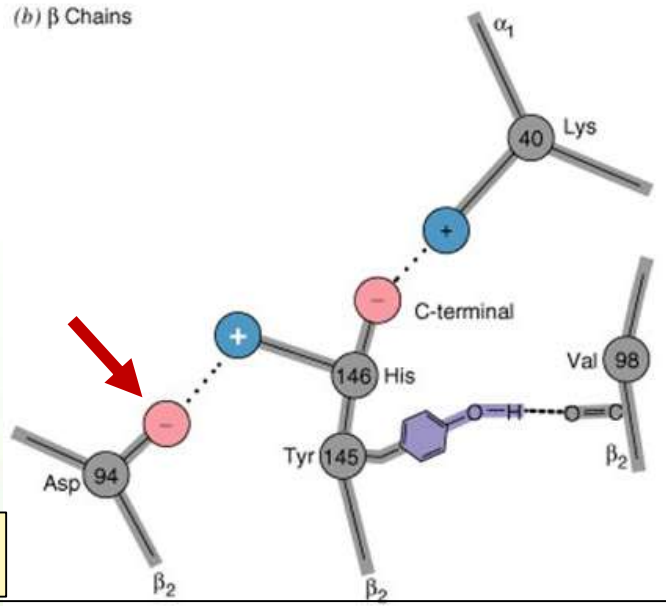
The broken interactions

- Electrostatic interactions and hydrogen bonds that stabilize the T-form of hemoglobin are broken upon movement of polypeptides.

N^+ of Val1 (α2) …….Cl^- ……. R^+ of Arg141 (α1) ……. C=O of Val34 (β2) & Asp126 (α2)
R^+ of Lys127 (α2) ……. C^- of Arg141 (α1)
R^+ of His146 (β2) ……. R^- of Asp94 (β2)

Do not memorize





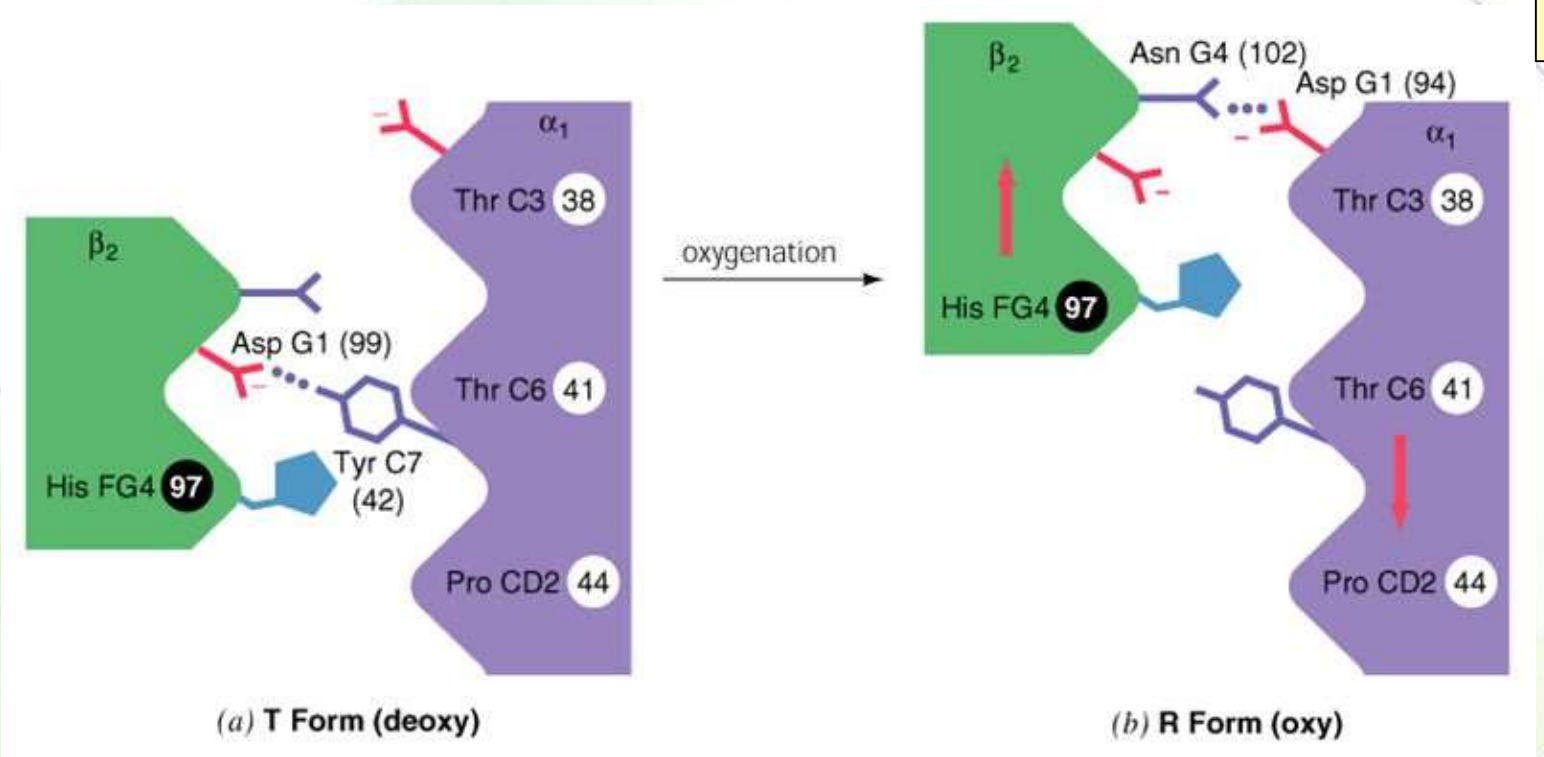
Do not memorize

C^- of His146 (β2) ……. Lys127 (α2)

Reformation of hydrogen bonds

- T-state hemoglobin (deoxyhemoglobin) is stabilized by a hydrogen bond between Asp G1 (99) of β2 with Tyr C7 (42) of α1.
- When O_2 binds, the α1 surface slides, and a hydrogen bond is formed between Asn G4 (102) of β chain and Asp G1 (94) of α chain stabilizing the R form of hemoglobin.

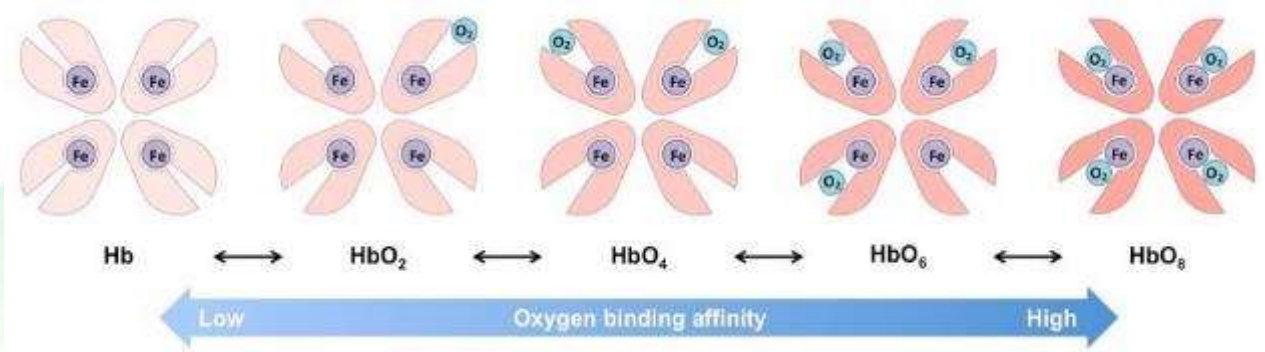
Do not memorize

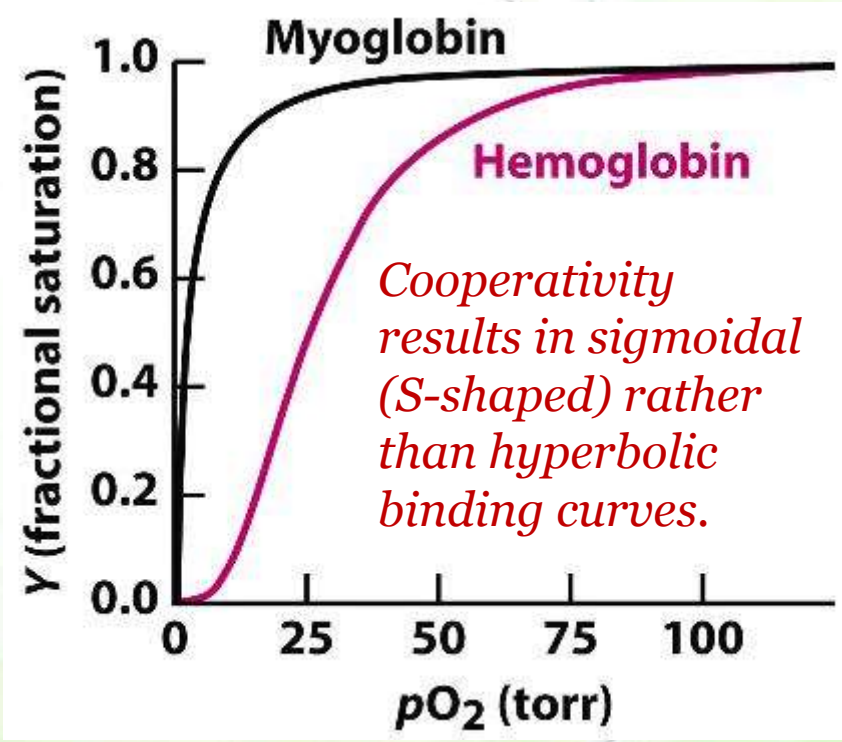


(a) T Form (deoxy) (b) R Form (oxy)

Binding is cooperative

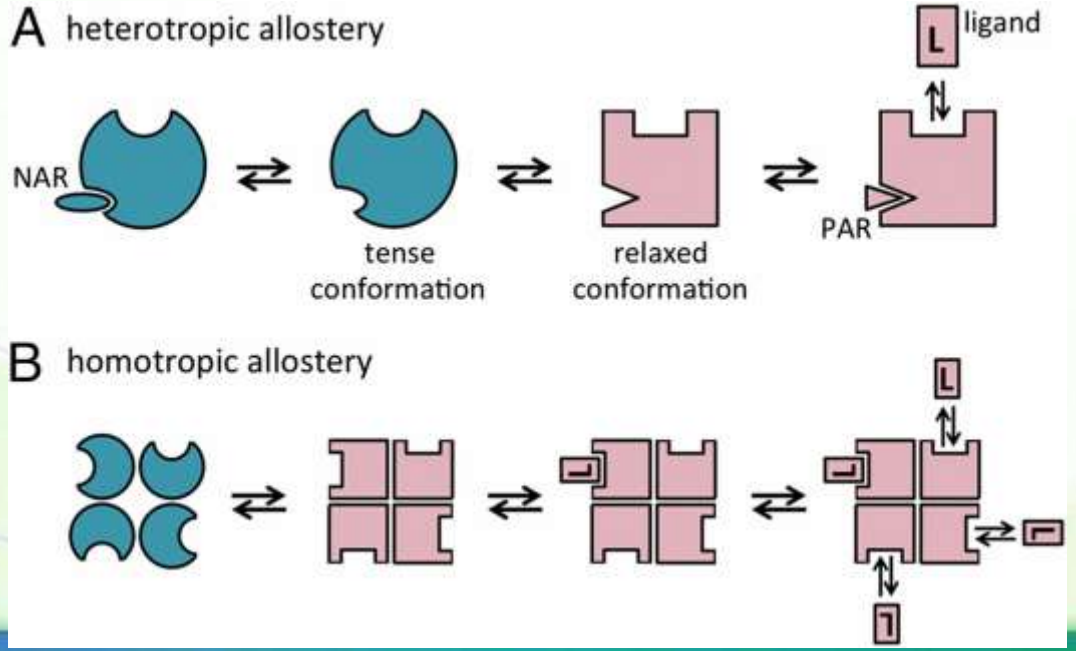
- Conformational changes lead to cooperativity among binding sites.
- Binding of the first O_2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule.
- Binding of the second O_2 molecule breaks more salt bridges increasing the affinity towards binding of a third O_2 even more, and so on.
- Binding is cooperative.
- Oxygen is a homotropic effector (the allosteric modulator is the substrate itself).





Some terminologies

- Homotropic allosteric regulator/effector: effector and ligand regulated by the effector are the same molecule (e.g., O_2 binding affects subsequent O_2 binding).
- Heterotropic allosteric regulator: effector and ligand are different molecules (e.g., H^+ or BPG binding affects O_2 binding).
- Positive allosteric interaction: effector binding increases affinity for ligand.
- Negative allosteric interaction: effector binding decreases affinity for ligand.



The Hill constant (coefficient)

Do not memorize

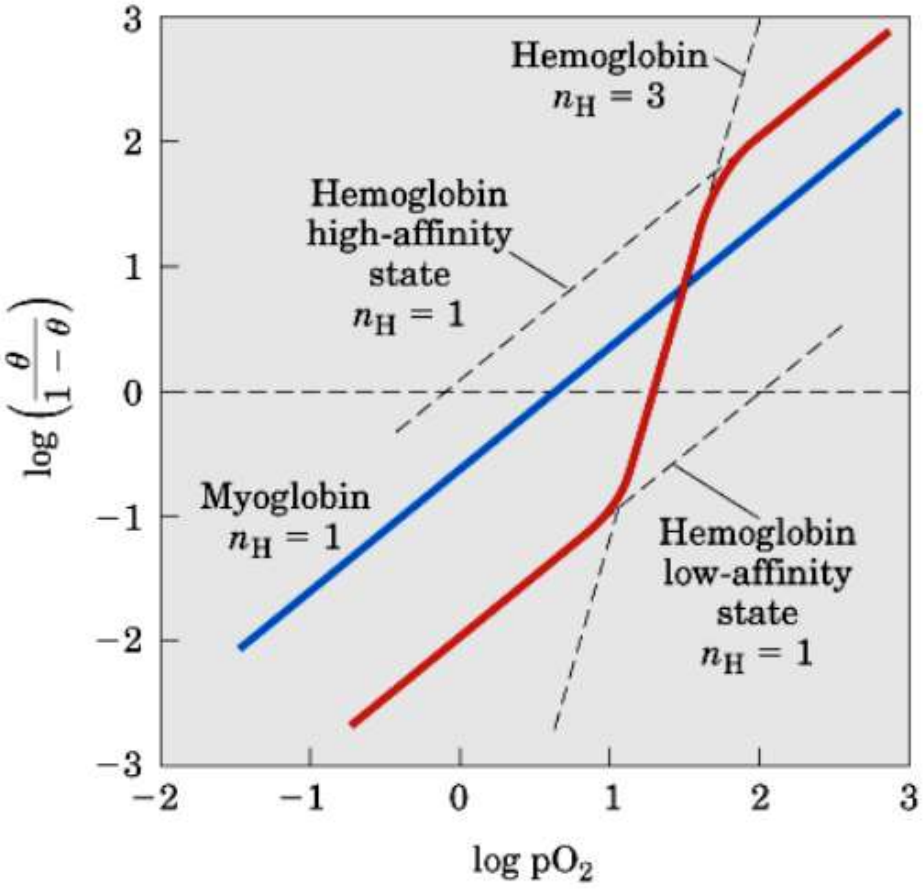
$$\log \frac{Y}{1-Y} = n \log pO_2 - n \log P_{50}$$

Y or θ is the fraction of oxygen-bound Hb

→ Y = mX + b (linear plot)

- The Hill plot is drawn based on an equation (you do not have to know it).
- n = Hill constant - determined graphically by the Hill plot
- n is the slope at the midpoint of binding of log (Y/1-Y) vs. log of pO_2.
  - if n = 1 then non cooperativity
  - if n < 1 then negative cooperativity
  - if n >1 then positive cooperativity

- *The slope reflects the degree of cooperativity, not the number of binding sites.*

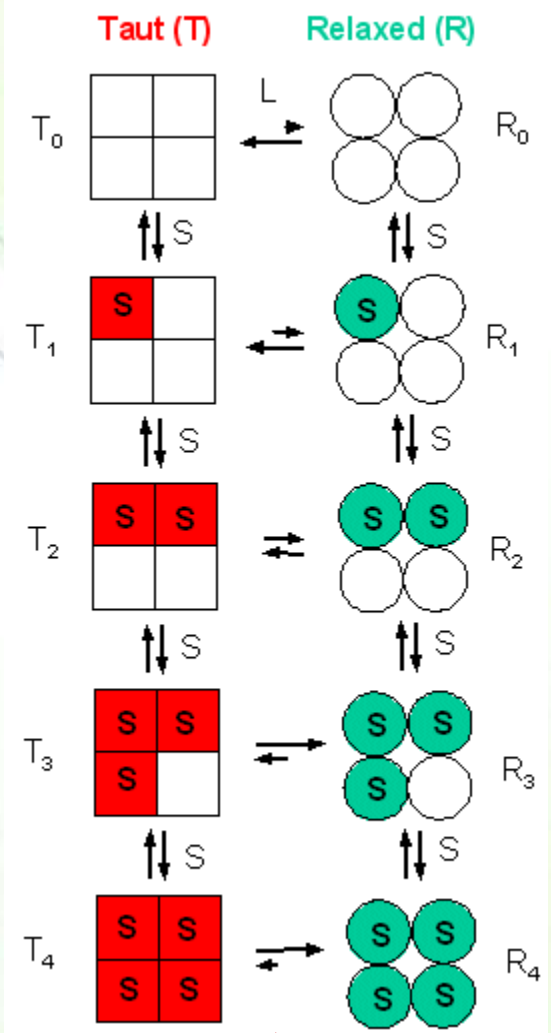


Cooperativity models

- Two models of cooperativity that could explain the observed data
  - Concerted model – all subunits undergo the conformational change simultaneously
    - There are only two states, R and T.
  - Sequential model – the subunits undergo the conformational change one at a time.
    - There are multiple states between full T and full R.

The concerted model (MWC model)

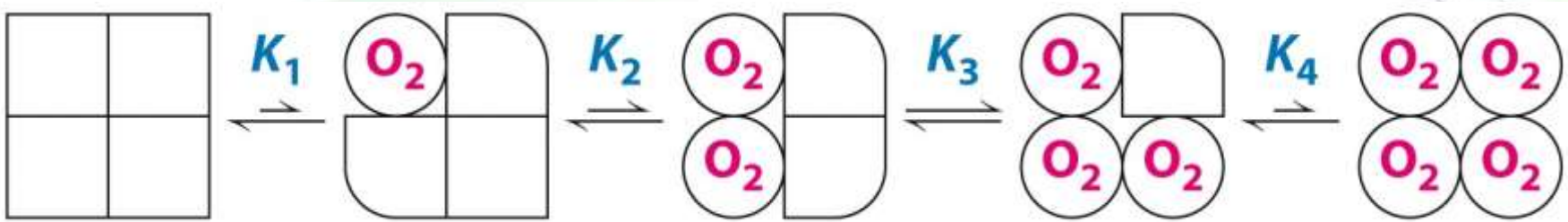
- The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.
- Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.
- This allows unoccupied subunits to adopt the high affinity R-state.



Note direction of arrows

The sequential, induced fit, or KNF model

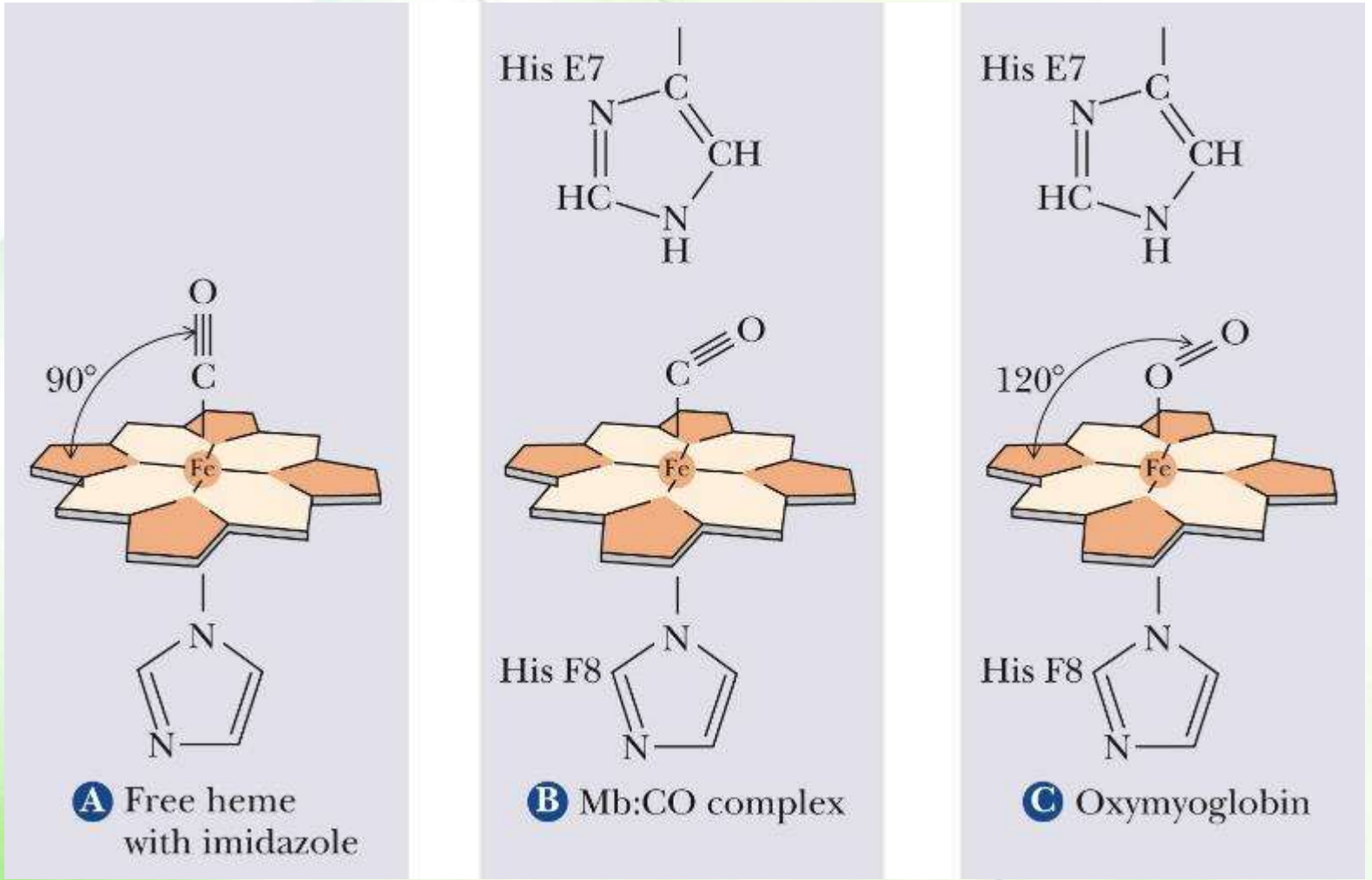
- The subunits go through conformational changes independently of each other, but they make the other subunits more likely to change, by reducing the energy needed for subsequent subunits to undergo the same conformational change.



- Which one is better? Both can explain the sigmoidal binding curve.

Another significance of distal histidine

- CO prefers straight bonding, but O_2 prefers bent bonding.
- CO binds to free heme with higher affinity (thousands folds more) than O_2.
- The affinity of CO to myoglobin-bound heme is only 250 times more than O_2.
- Yet, CO occupies 1% of hemoglobin, but 99% if distal His does not exist.



Accidents

وفاة أب وأم وابنهما إختناقاً بـ "صوبة غاز"

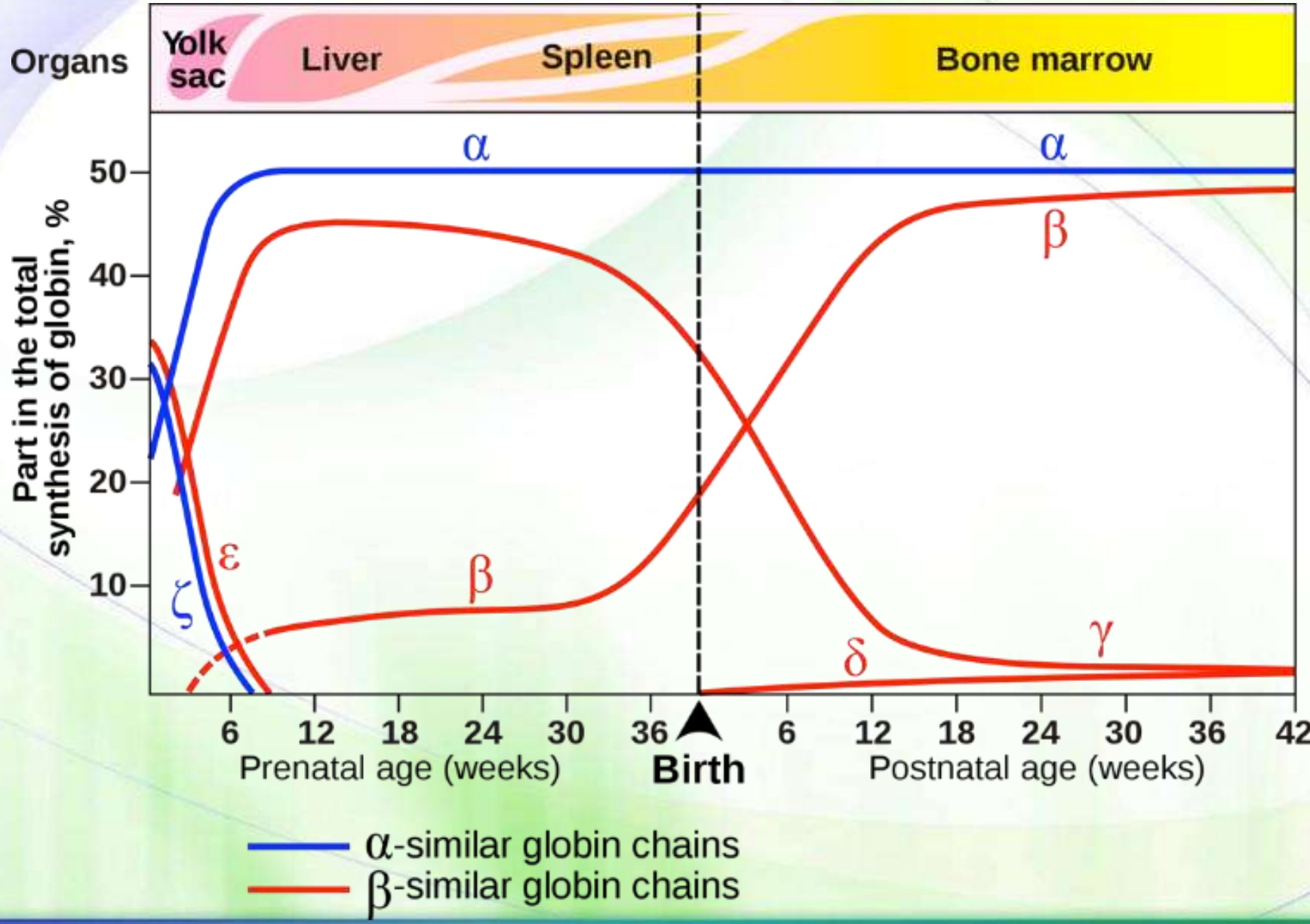
وفاة مواطن وزوجته اختناقا بصوبة الغاز في بيرين

وفاة 3 اشخاص من عائلة واحدة اختناقا بسبب صوبة غاز في الموقر

24 وفاة اختناقاً بالصوبات منذ بداية الشتاء.. و«الدفاع المدنى» تحذّر

It is not only one hemoglobin

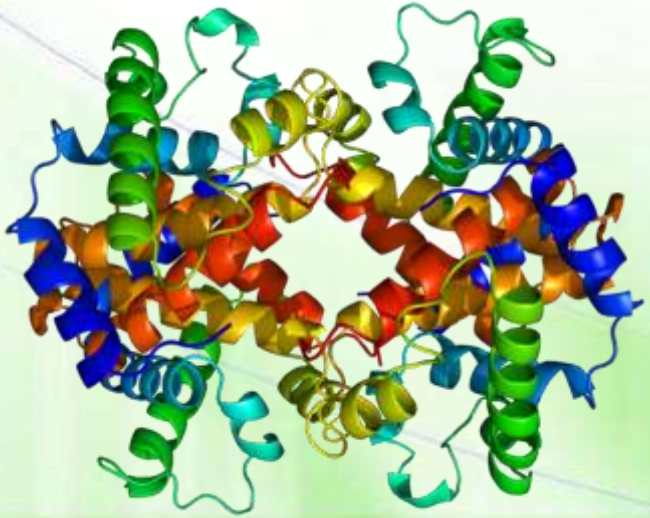
Developmental transition of hemoglobins

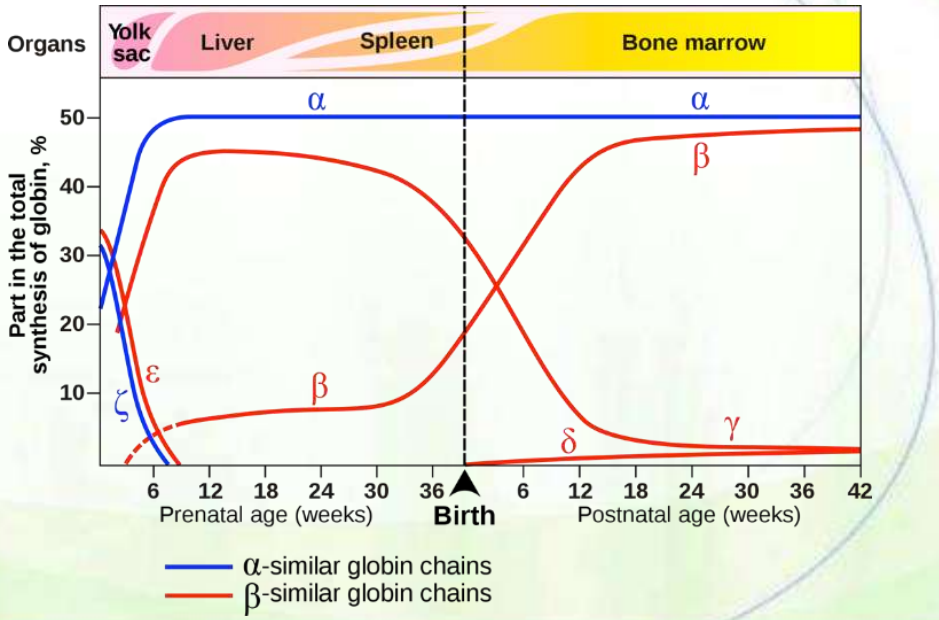


Alpha	Α α
Beta	Β β
Gamma	Γ γ
Delta	Δ δ
Epsilon	Ε ε
Zeta	Ζ ζ

The embryonic stage

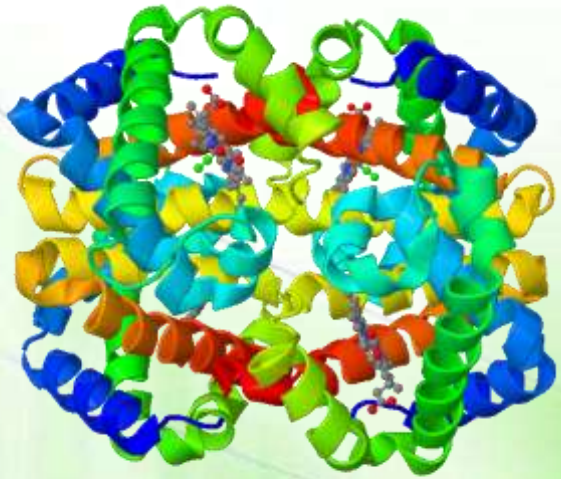
- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1) is a tetramer composed of 2 zeta (ξ) chains and 2 epsilon (ε) chains.
- Other forms exist (*do not memorize*): HbE Gower 2 (α2ε2), HbE Portland 1 (ζ2γ2), HbE Portland 2 (ζ2β2).

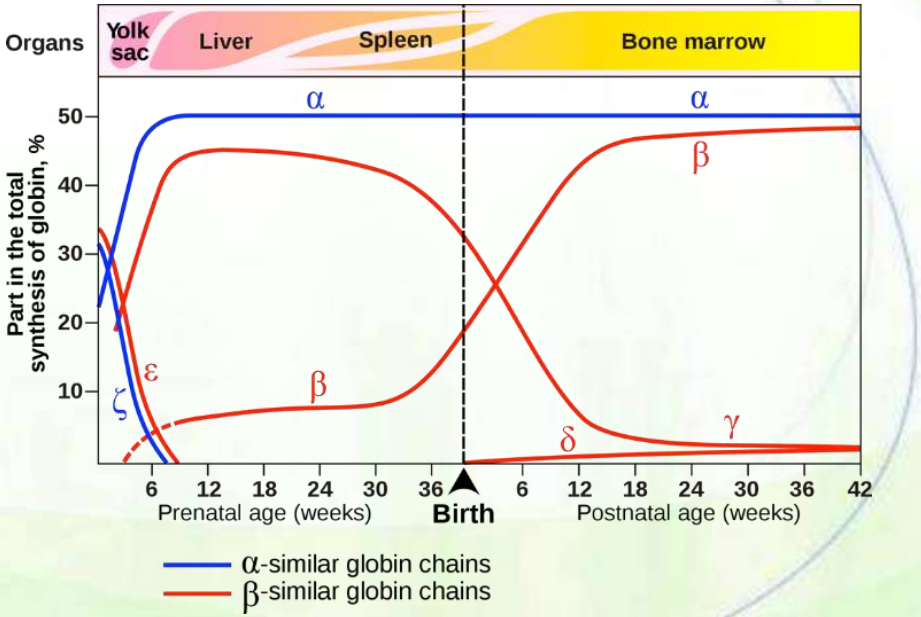




The fetal stage

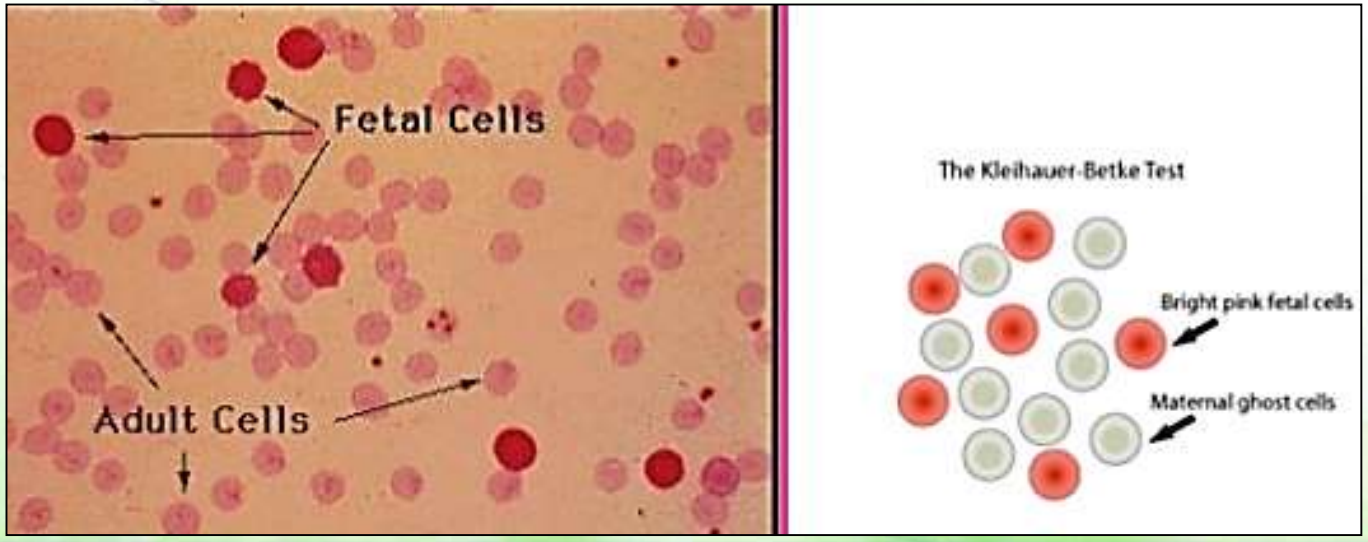
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines and fetal hemoglobin synthesis starts.
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides ($\alpha 2 \gamma 2$)
- The gene expression of the α polypeptides is active throughout life.

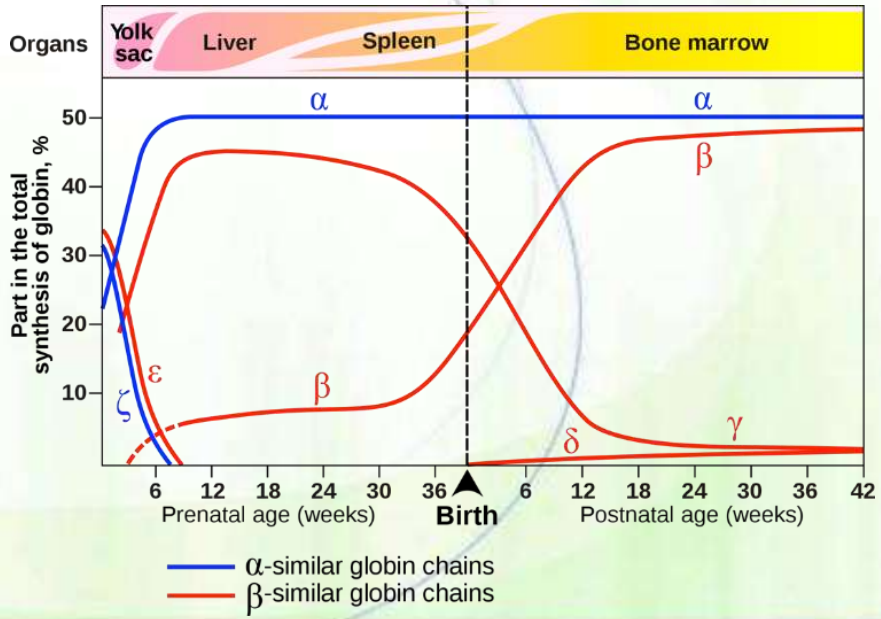


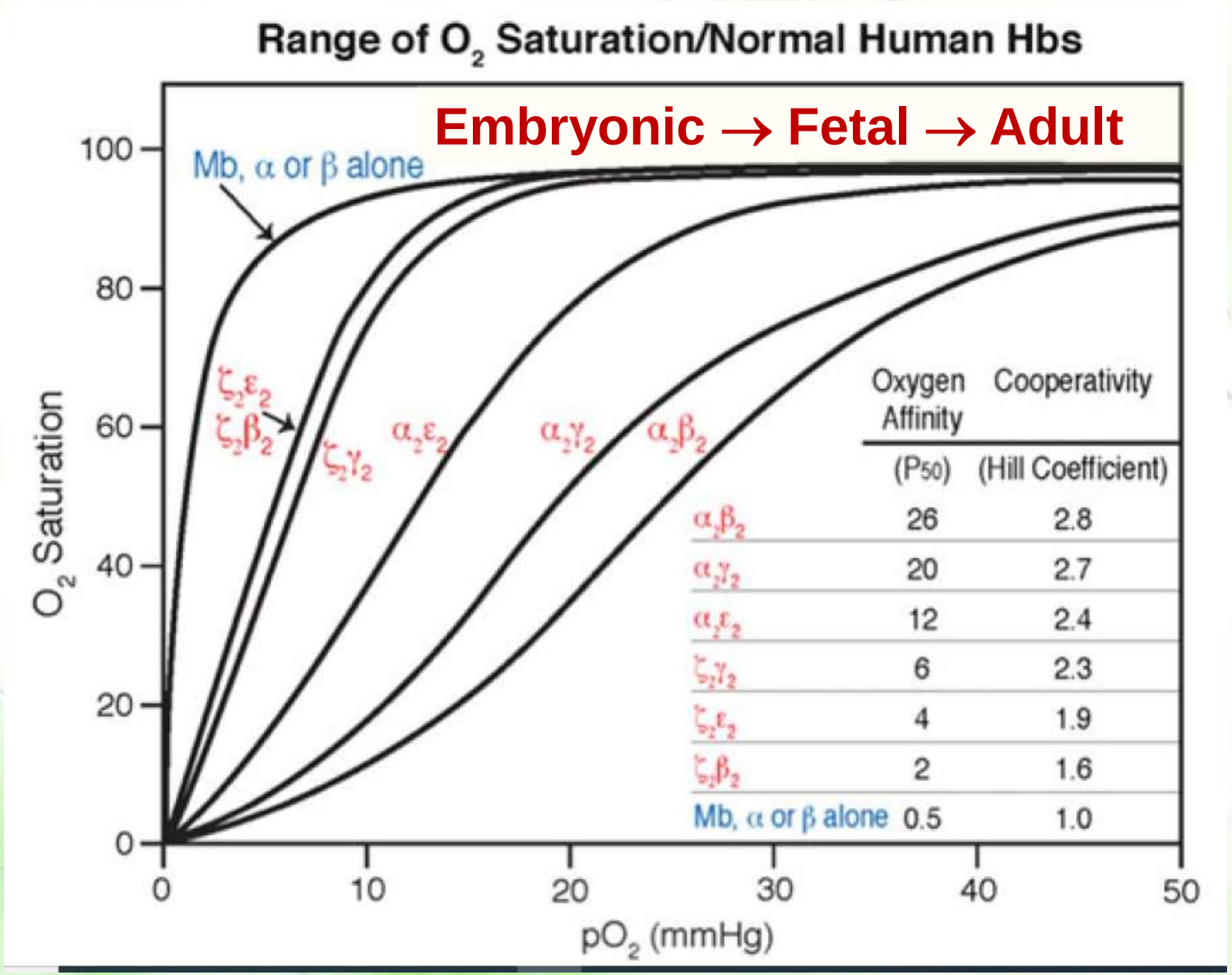


The adult stage

- Shortly before birth, there is a gradual switch to adult β-globin.
- Still, HbF makes up 60% of the hemoglobin at birth, but 1% of adults.
- At birth, synthesis of both γ and β chains occurs in the bone marrow.
- The major hemoglobin is HbA1 (a tetramer of 2 α and 2 β chains).
  - A minor adult hemoglobin, HbA2, is a tetramer of 2 α chains and 2 delta (δ) chains.

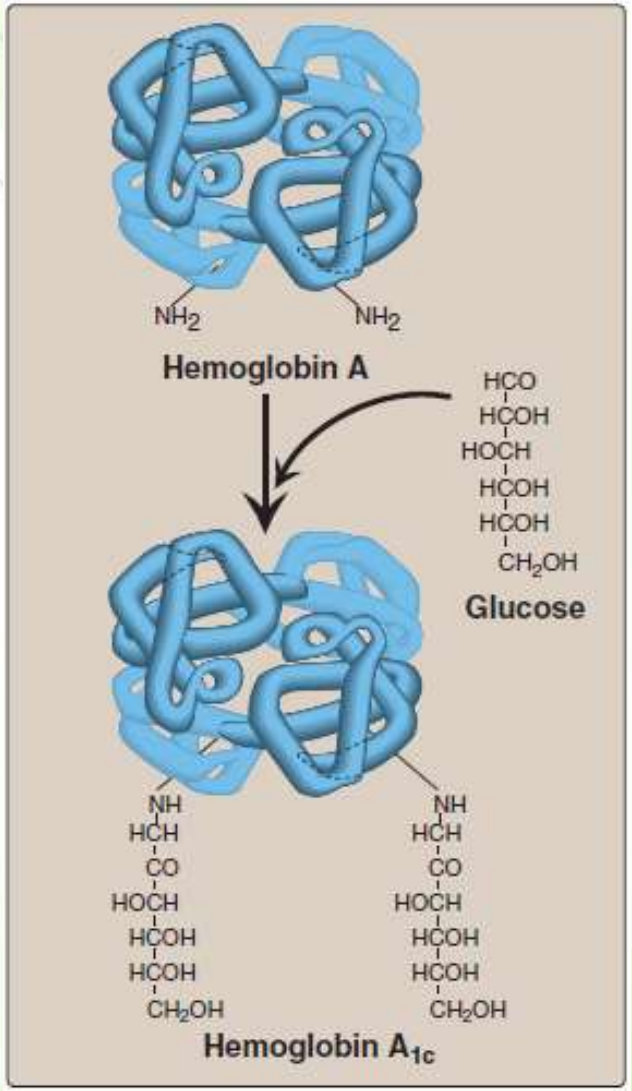





Range of O_2 Saturation/Normal Human Hbs
Embryonic → Fetal → Adult
Mb, α or β alone
$\zeta_2\varepsilon_2$
$\zeta_2\beta_2$
$\zeta_2\gamma_2$
$\alpha_2\varepsilon_2$
$\alpha_2\gamma_2$
$\alpha_2\beta_2$
O_2 Saturation
100
80
60
40
20
0
0
10
20
30
40
50
pO_2 (mmHg)
Oxygen Affinity (P50)
Cooperativity (Hill Coefficient)
$\alpha_2\beta_2$ 26 2.8
$\alpha_2\gamma_2$ 20 2.7
$\alpha_2\varepsilon_2$ 12 2.4
$\zeta_2\gamma_2$ 6 2.3
$\zeta_2\varepsilon_2$ 4 1.9
$\zeta_2\beta_2$ 2 1.6
Mb, α or β alone 0.5 1.0

Adult hemoglobins

- HbA1 can be glycosylated non-enzymatically with a hexose and is designated as HbA1c.
  - The major form (HbA1c) has glucose molecules attached to valines of β chains.
  - HbA1c is present at higher levels in patients with diabetes mellitus.



Advantages of HbA1c testing

- Blood fasting glucose level is the concentration of glucose in blood at a single point in time when fasting for a few hours.
- **HbA1c** level provides a longer-term trend, similar to an average, of how high blood sugar levels have been over a period of time (2-3 months).
  - HbA1c can be expressed as a percentage (DCCT unit, used in the US) or as a value in mmol/mol (IFCC unit).

Table

BLOOD GLUCOSE		STATUS	HbA1c	
mmol/L	mg/dL		%	mmol/mol
5.4	97	Normal	5	31
7.0	126		6	42
8.6	155	Pre-Diabetes	7	53
10.2	184	Diabetes	8	64
11.8	212	Diabetes	9	75
13.4	241		10	86
14.9	268	Diabetes	11	97
16.5	297		12	108