HLS

MODIFIED NO. 1

BIOCHEMISTRY

كتابة: إبراهيم الشوابكة و سلطان غيث

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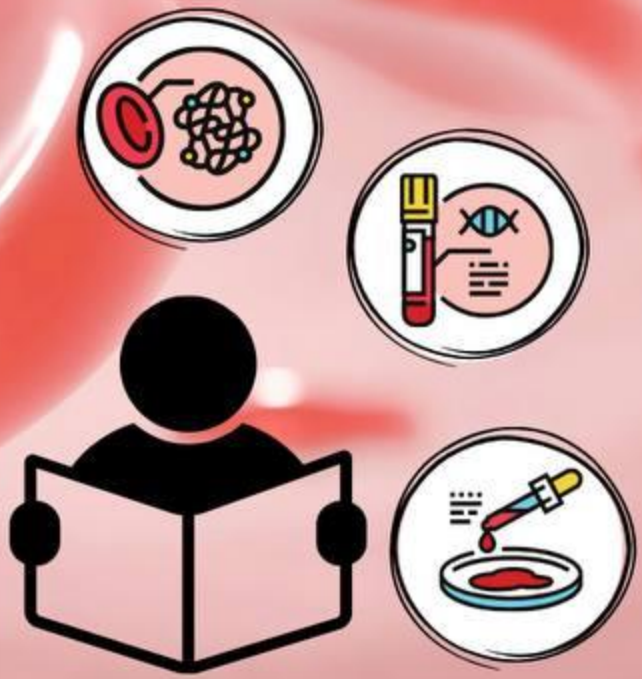


Hemoglobin
An overview and more

Prof. Mamoun Ahram
Hematopoietic-lymphatic system

Color code
Slides
Doctor
Additional info
Important

Resources

- This lecture
- Myoglobin/Hemoglobin O2 Binding and Allosteric Properties of Hemoglobin (http://home.sandiego.edu/~josephprovost/Chem331%20Lect%207_8%20Myo%20Hemoglobin.pdf)
- Lecture 3: Cooperative behaviour of hemoglobin (https://www.chem.uwec.edu/chem452_f12/pages/lecture_materials/unit_III/lecture-3/overheads/Chem452-lecture_3-part_1-overheads.pdf)

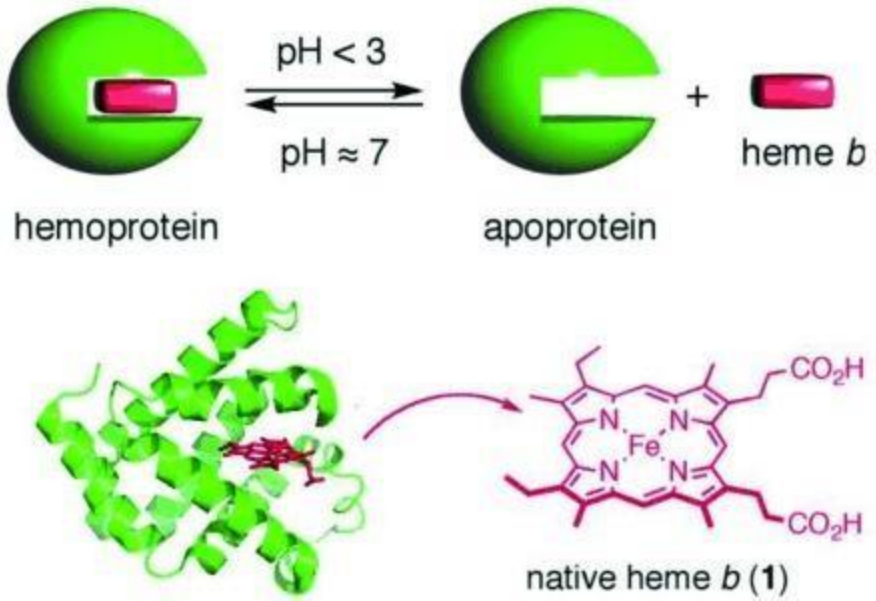
Let's breakdown some concepts:

1) Holoprotein is a protein that is associated with a non-protein group, such as hemoproteins.
2) Hemoproteins are a group of proteins that are bound to a heme group. The heme molecule itself does not determine the function, as it is the same in all hemoproteins. It is the protein part that dictates the specific function of a particular hemoprotein.
3) Prosthetic group is a non-protein group that is tightly bound to a protein.
4) Hemoglobinopathies are genetic disorders affecting the structure or production of the hemoglobin molecule.
5) Heme group is an organic molecule that is covalently bonded to the polypeptide part of hemoglobin and consists of four pyrrole rings (those four rings are attached to each other forming protoporphyrin IX.). Two rings (A and B) have a propionate side chain.
6) Allosteric protein is a protein that undergoes a conformational change when it binds to a particular molecule.
7) Globular protein is a protein that is spherical in shape.

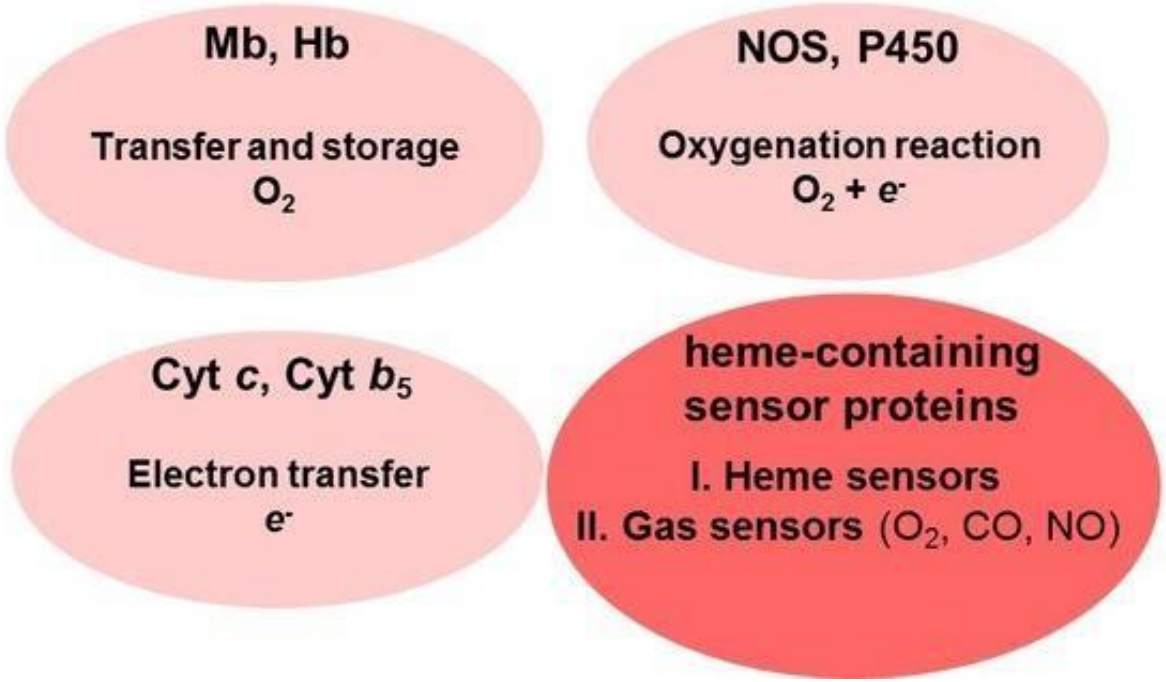
Hemoproteins

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

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.*

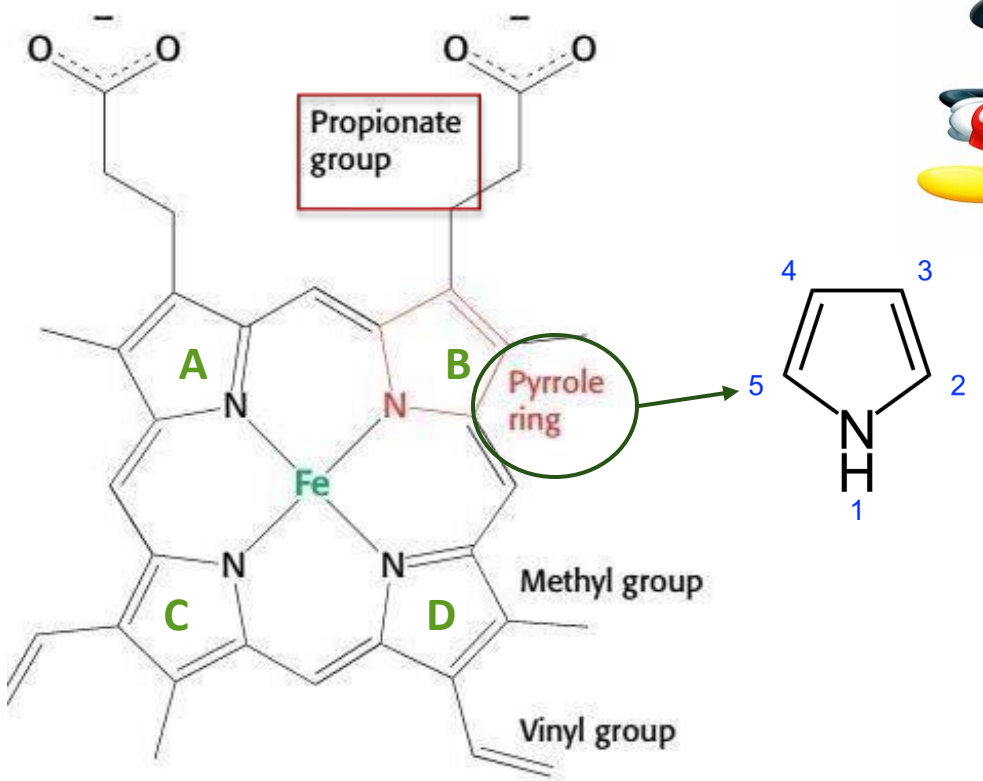


Functions of different hemoproteins:

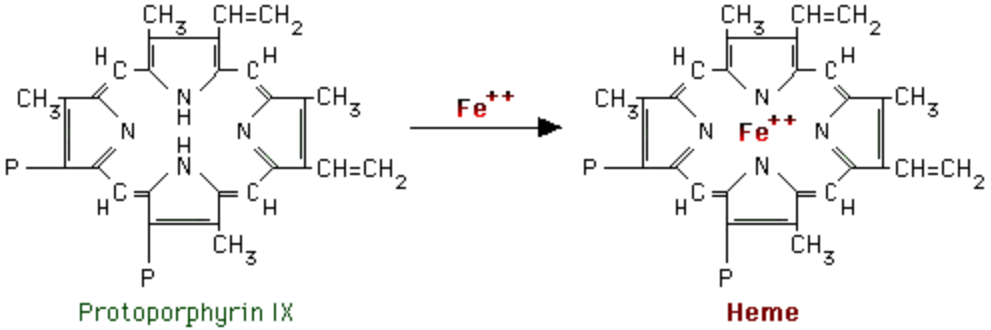


Heme structure

- 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 except for propionate groups that extend upwards.*
- Fe^{2+} has six coordinates of binding. Four coordinates are with nitrogen atoms of pyrrole rings. The 5th coordinate is with proximal histidine and the 6th one is with molecular oxygen.



Heme
(Fe-protoporphyrin IX)



propionate group is a hydrophilic group that interacts with hydrophilic amino acids on the surface.

Structure of hemoglobin

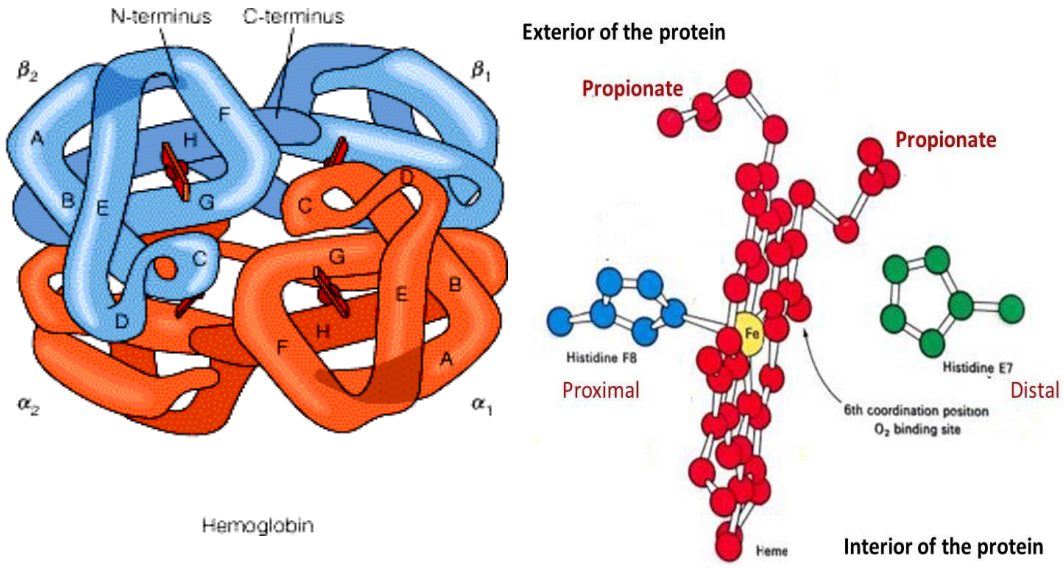
Let's make it easy :

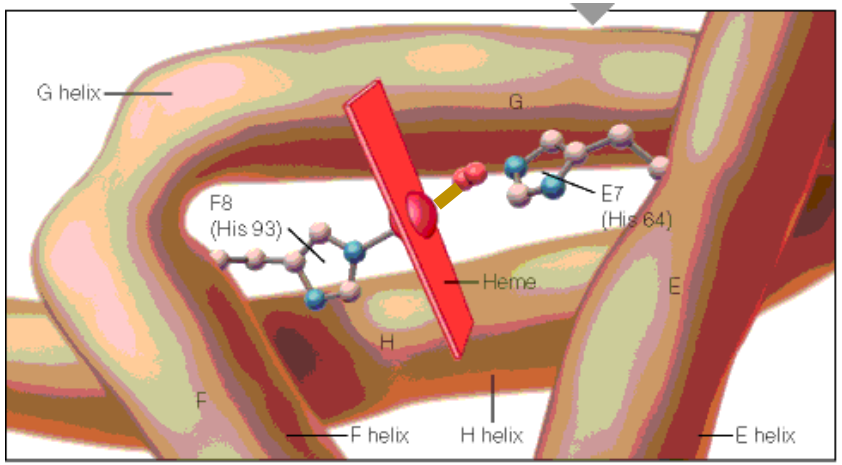
A) Hemoglobin is a major protein of RBCs (erythrocytes) that is made of four polypeptide subunits, thus it has a quaternaty structure.
B) Adult hemoglobin is composed of :
    1) 2α and 2β subunits.
    2) 4 heme groups.
 *Each heme group is attached to a subunit.
C) α and β subunits interact with each other by hydrophobic interactions.
D) αβ and αβ heterodimers interact with each other by electrostatic interactions.
E) Hemoglobin is an allosteric protein that assumes different conformations that are influenced by several factors, mainly by oxygen binding.
F) Hemoglobin has two states:
    1) T (tense or taut) state when Hb is deoxygenated.
    2) R (relaxed) state when Hb is oxygenated.
G) There are two histidine residues that play a critical role in heme group stablization:
    1) Proximal histidine which is covalently bonded to iron.
    2) Distal histidine which isn't covalently bonded to iron.

Structure of hemoglobin

- Hb is a globular protein.
  - Typical amino acid distribution
    - Positions of two histidine residues
    - Proximal and distal
- It is an allosteric protein.
  - Multiple subunits (2α + 2β) (for adult Hb)
    - α polypeptide = 141 amino acids (Arg141)
    - β polypeptide = 146 amino acids (His146)
    - The first amino acid in both is valine.
  - Altered structure depending on bound molecules
  - Positive cooperativity towards oxygen
  - Regulated by allosteric effectors

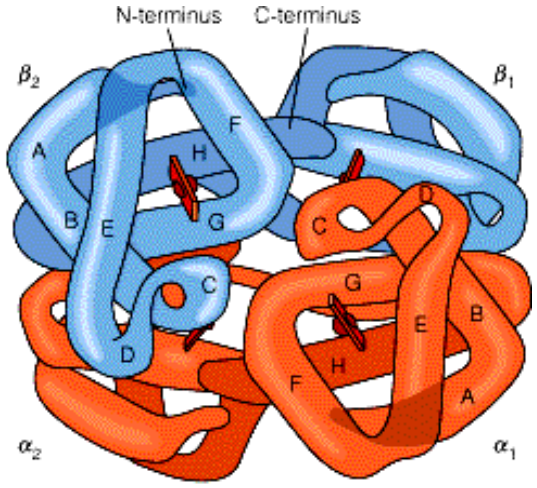
V3: Just added the pic

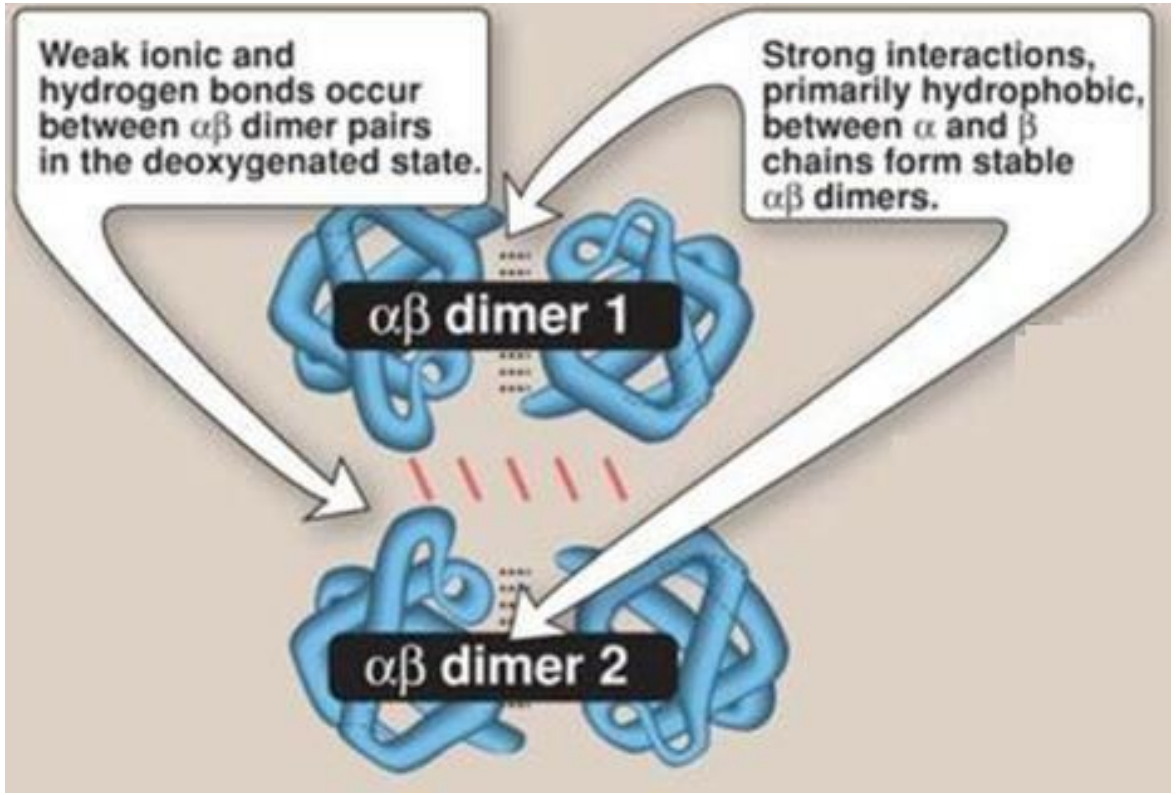




How are the subunits bound?

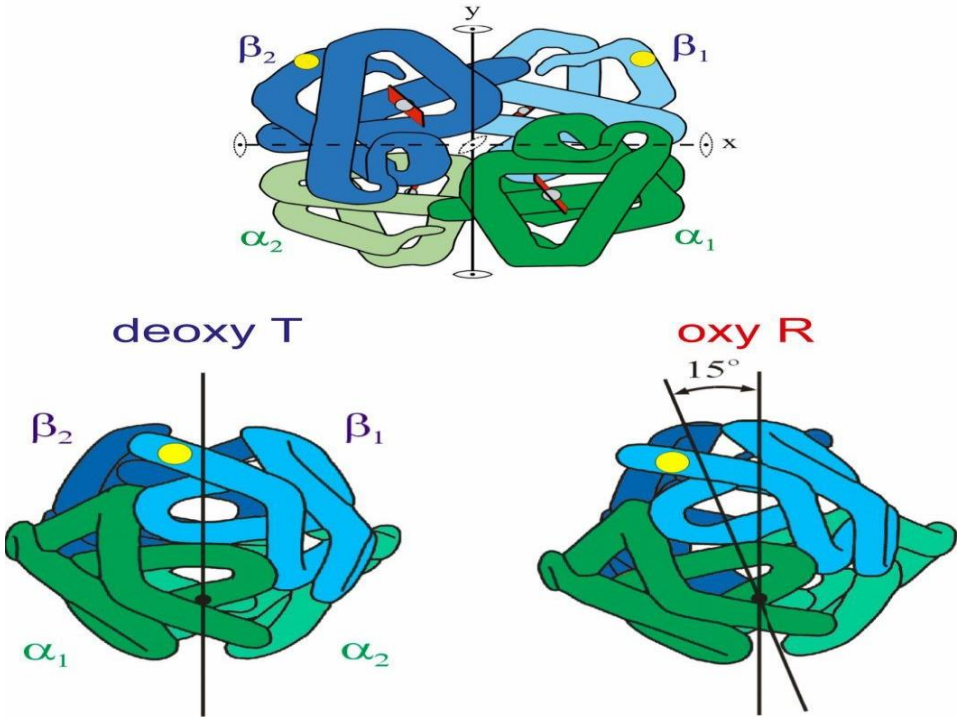
- A dimer of dimers (I made up this term)
  - (α-β)2
  - Note how they interact with each other.

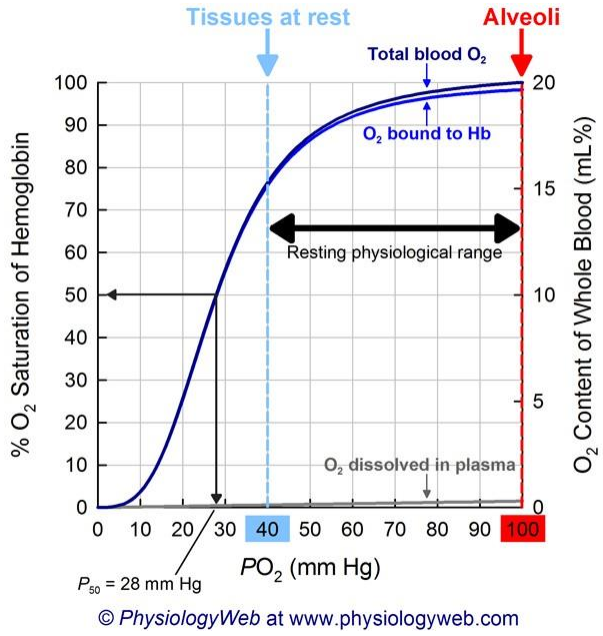


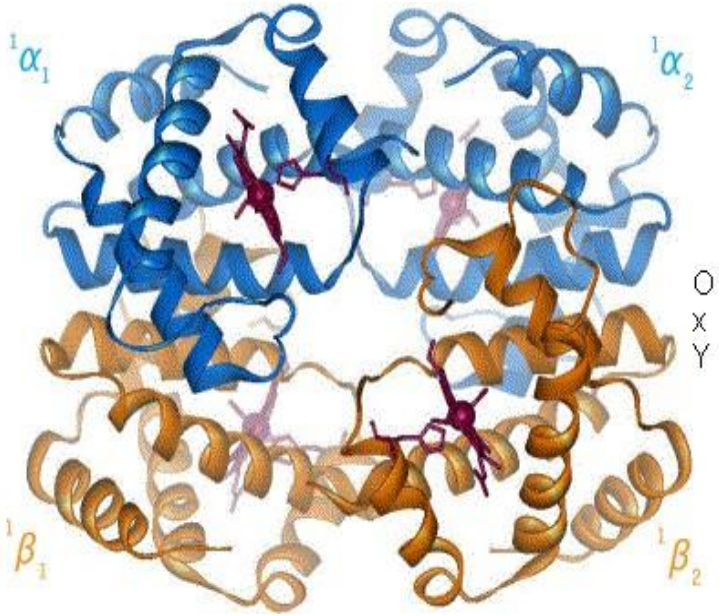


Structural change of hemoglobin

Distal histidine is a " Gate keeper ", it only allows oxygen to pass and bind to iron preventing passage of other molecules like CO2.



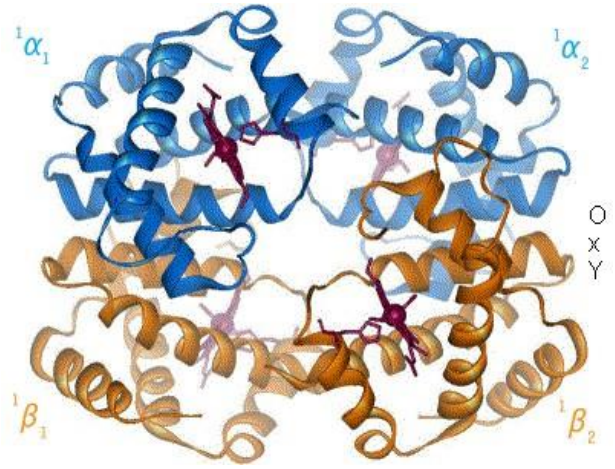


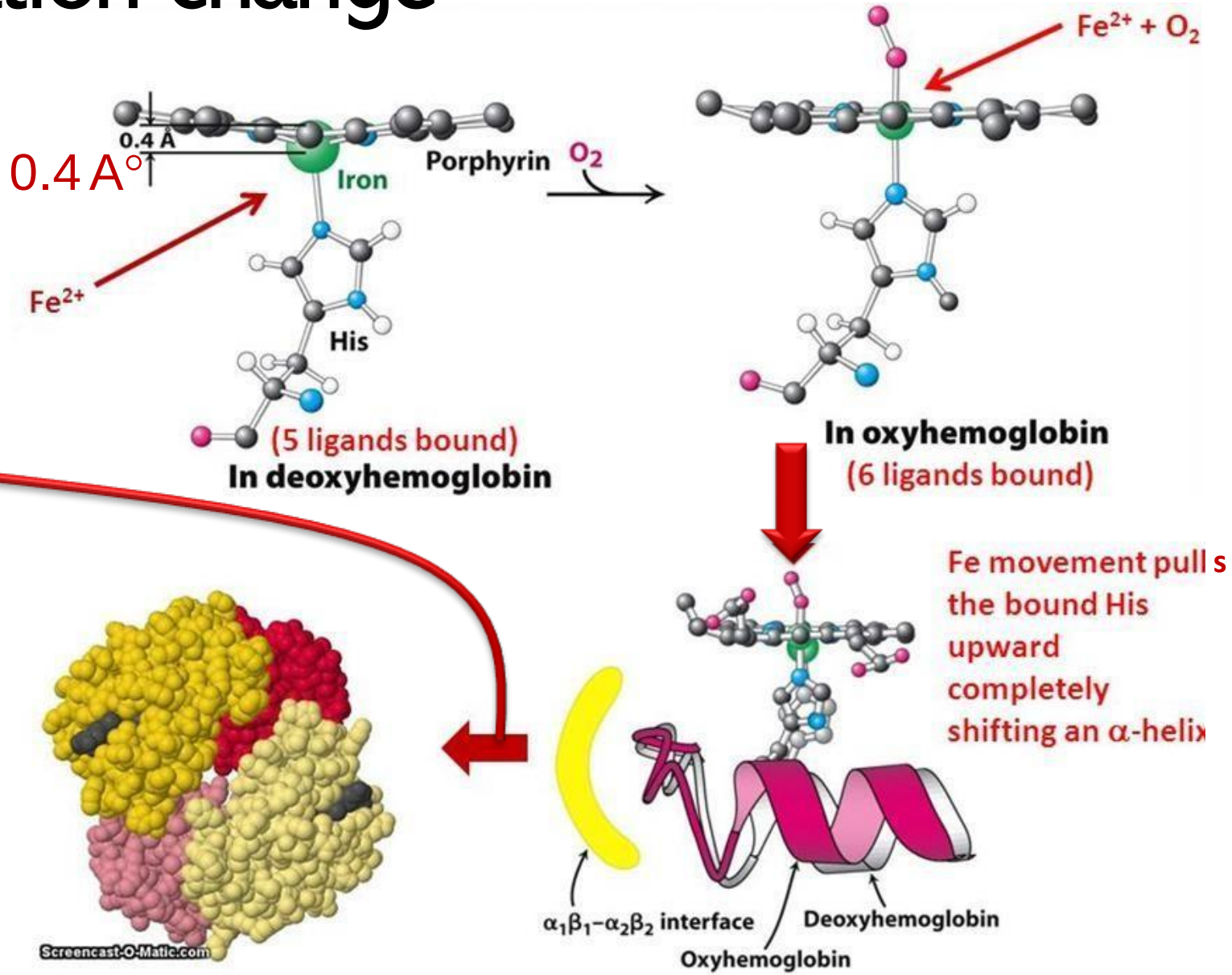


	state	shape	Oxygen affinity
oxygenated	R (relaxed) state	Heme group assumes a planar shape as oxygen forms hydrogen bonds with distal histidine leading to a slight pull of proximal histidine. This conformational change disrupts electrostatic interactions, hence named a "relaxed state" (fewer interactions). **More oxygen binding -->** more breakdown of electrostsatic interactions --> **more relaxed state.**	**High affinity.** When the first oxygen binds, it makes it easier for the second one to bind and this characteristic is termed "**positive cooperativity".**
deoxygenated	T *(tense or taut)* state	**Heme group is dome shaped** as there are repulsive forces between proximal histidine and hydrophobic parts of the molecule.	**Low affinity**

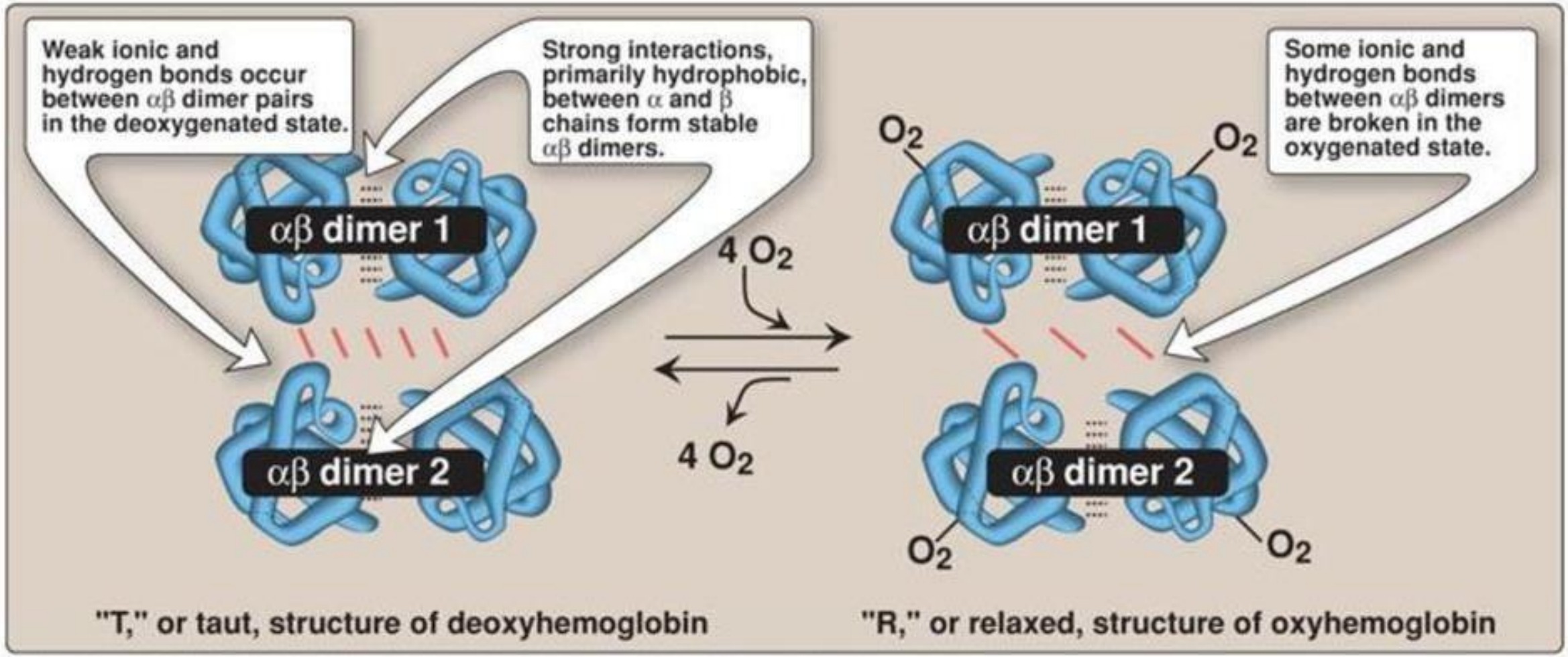
Structural amplification change

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





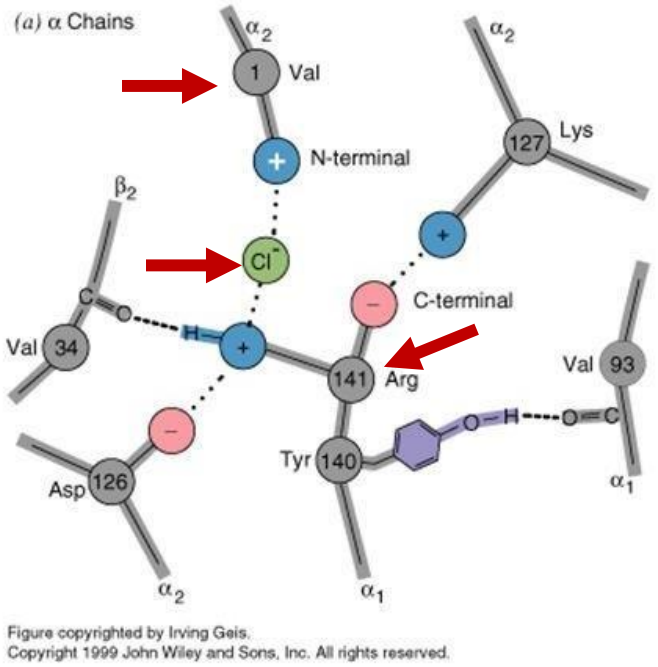
Broken electrostatic interactions and H-bonds

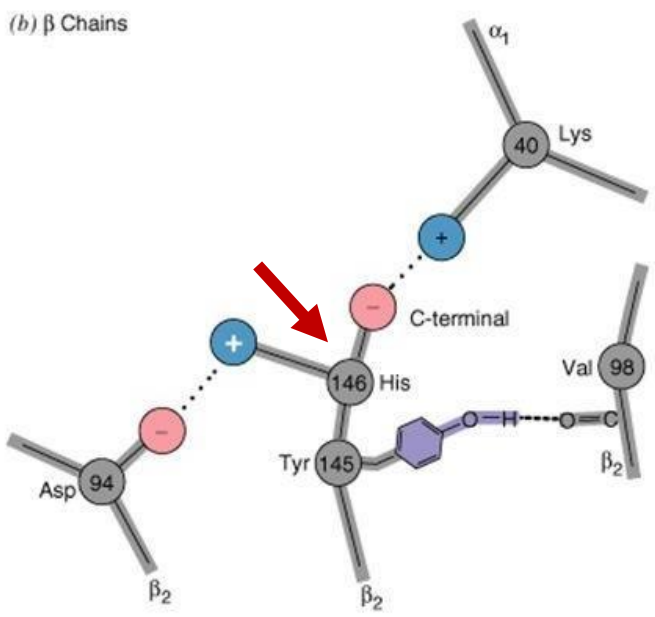


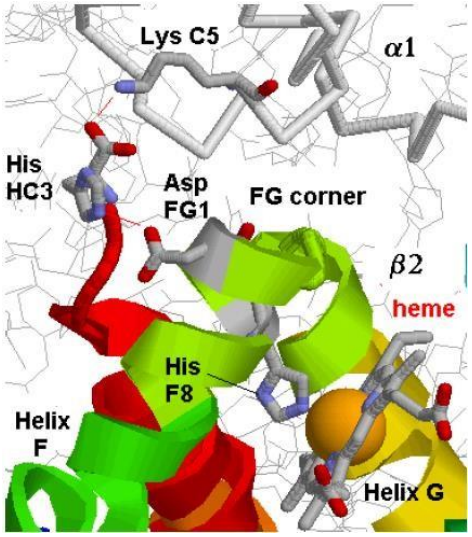
The broken interactions

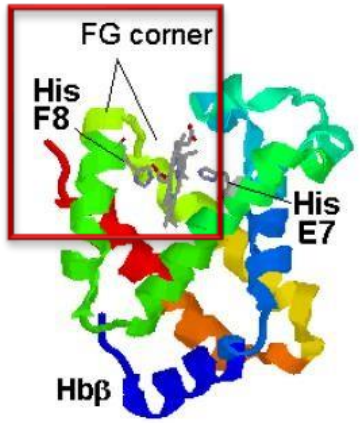
Electrostatic interactions (mainly) and hydrogen bonds that stabilize the T-form of hemoglobin are broken upon movement of polypeptides and they will be reformed but the total number will decrease.

*Note the groups, the protonation status, and the allosteric effectors









Hemoglobin was the first molecule for which the 3D structure was determined. Scientists have studied each amino acid in its structure, their locations and interactions. Because any alteration in any amino acid can lead to a specific disease. Hemoglobinopathies are the most common diseases worldwide.

There are 600 different disorders associated with hemoglobin. 😱

Let's take a look into some interactions:

1) His 146 is the terminal amino acid in β chain. It is positively charged, and it has 2 ionized groups:
   A) C terminus (- charge) : forms electrostatic interaction with lysine residue from a different polypeptide chain.
   B) R group (+ charge) : forms electrostatic interaction with aspartate residue within the same polypeptide chain.
2) Arg 141 is the terminal amino acid in α chain. It is positively charged, and it has 2 ionized groups:
   A) C terminus (- charge) : forms electrostatic interaction with lysine.
B) R group (+ charge) : forms electrostatic interaction with aspartate residue.

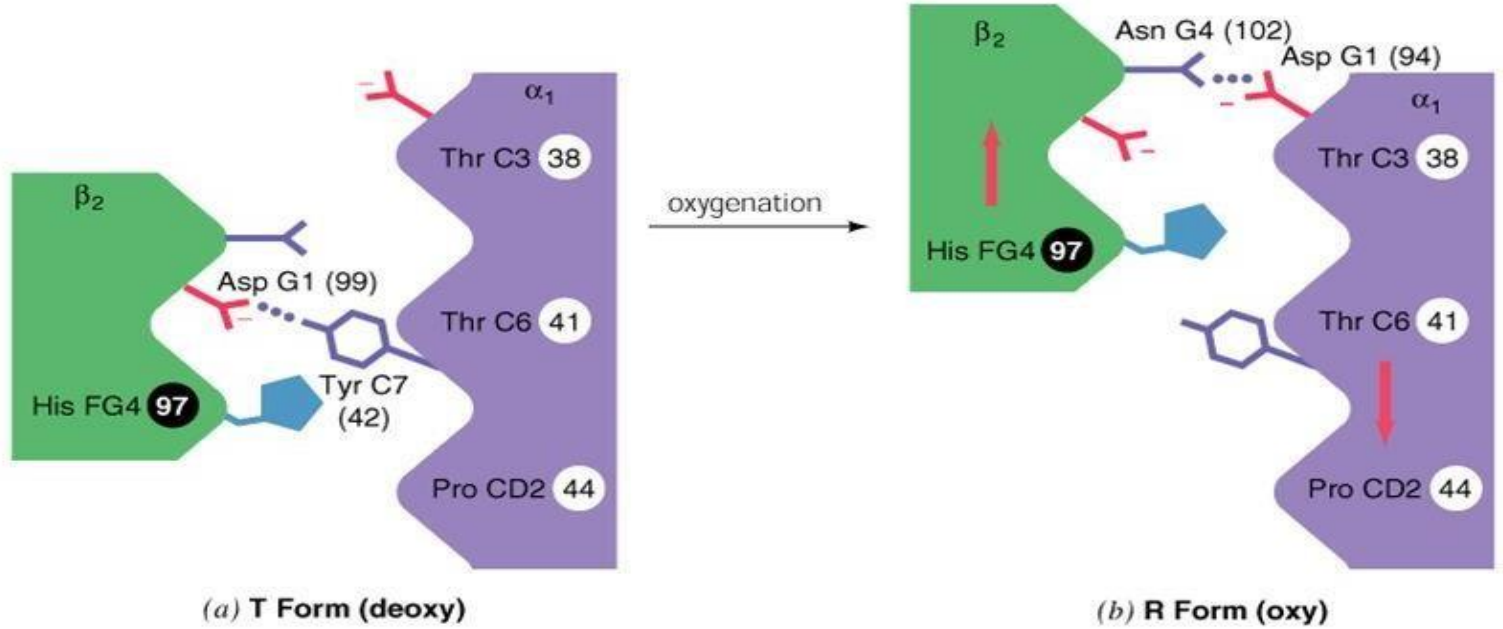
Notice that the C terminus of His 146 and C terminus of Arg 141 interact with lysine, and the R group of Arg 141 and R group of His 146 interact with aspartate.

Those electrostatic interactions stabilize the structure, making it locked in either T or R state.

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.

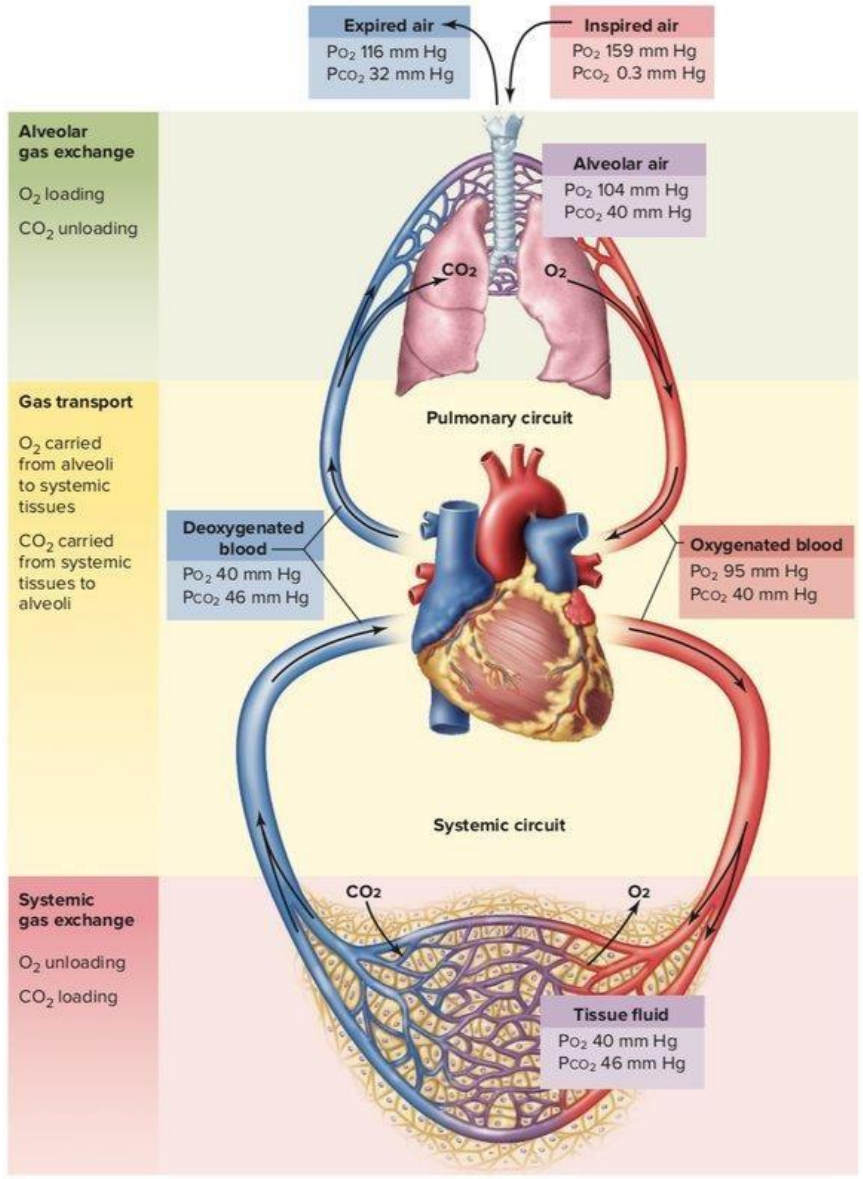
Alpha1 surface sliding implies that the position of amino acids has changed.



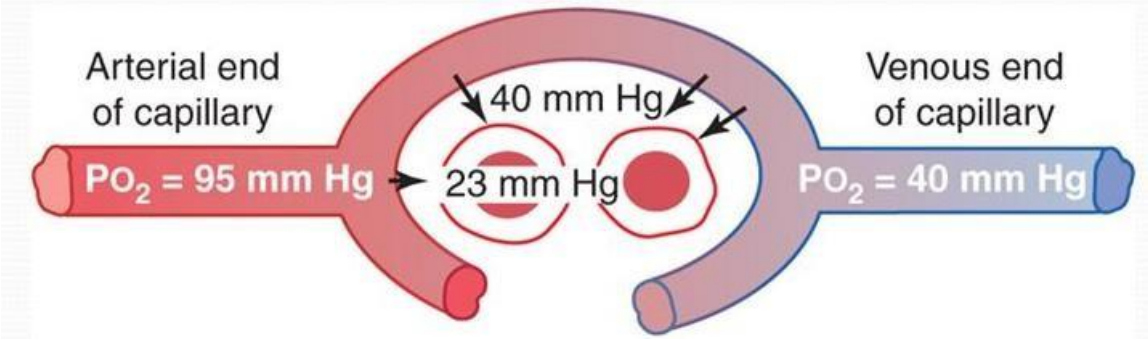
(a) T Form (deoxy)

(b) R Form (oxy)

Oxygen distribution in blood versus tissues



As an allosteric protein, hemoglobin can transition between a high-affinity "R" state (as in the lungs, where it is almost fully saturated with oxygen) and a low-affinity "T" state (as in different body tissues, facilitating the release of oxygen).



So, the allostery (the change in the structure of the hemoglobin molecule) serves the purpose of oxygen delivery to tissues.

Oxygen saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
  - It is allosteric.
- At 100 mm Hg, hemoglobin is 97% saturated (oxyhemoglobin).
- As the oxygen pressure falls, oxygen is released to the cells.
- *Note: at high altitude (~5000 m), alveolar pO2 = 75 mmHg.*

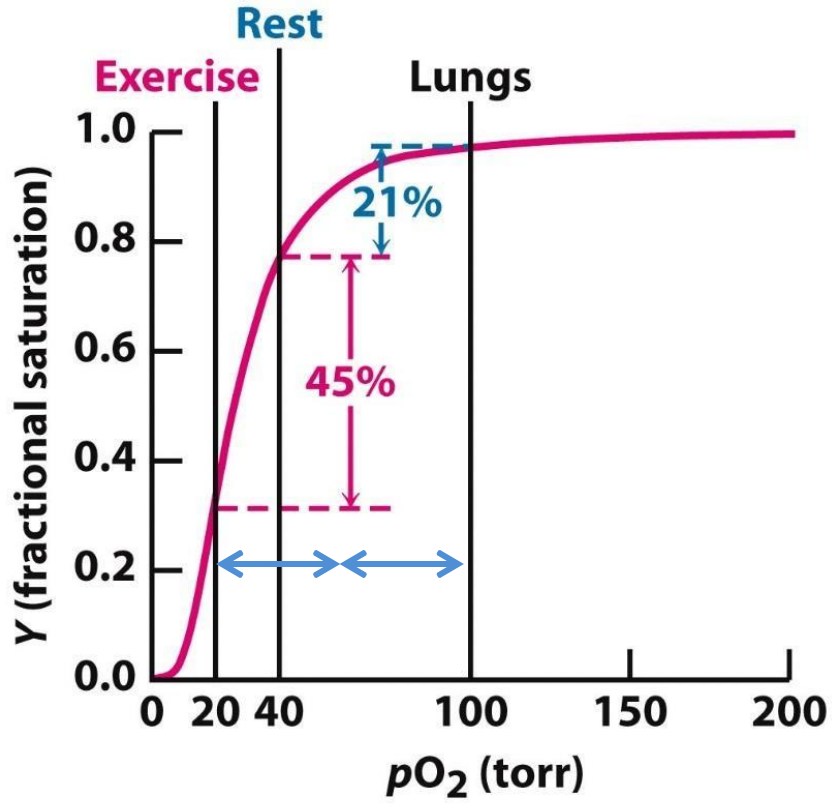


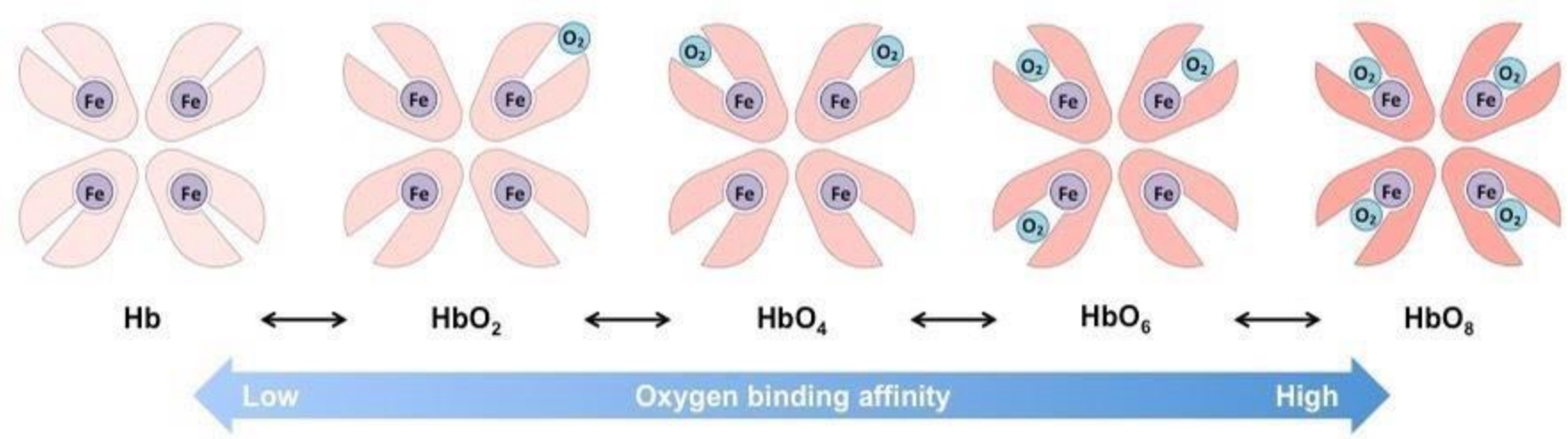
Figure 7.10
Biochemistry, Seventh Edition

Notice that when there is high pO2 (pressure of oxygen) as in lungs, the affinity of hemoglobin for oxygen is high, therefore oxygen is loaded on hemoglobin readily. However, in a state of low pO2, oxygen release into tissues is favored over its binding.

Important notes about oxygen saturation curve:

Notice that in case of high oxygen levels (high pO2), a change in the levels of oxygen, for example, from 100 to 80 mmHg, will not cause that dramatic change in the percentage of saturated hemoglobin molecules. However, in case of low levels of oxygen (low pO2), any change in the levels of oxygen, for example, from 40 to 20 mmHg, will cause a huge change in the fraction of saturated hemoglobin molecules, and that is an advantage that allosteric proteins have.

Positive cooperativity



- Increasing ligand concentration drives the equilibrium between R and T toward the R state (**positive cooperativity)** ⟶ **sigmoidal curve**
  - The effect of ligand concentration on the conformational equilibrium is a **homotropic effect (oxygen)**.
  - Other effector molecules that bind at sites distinct from the ligand binding site and thereby affect the R and T equilibrium in either direction are called **heterotropic effectors (e.g., CO_2)**.

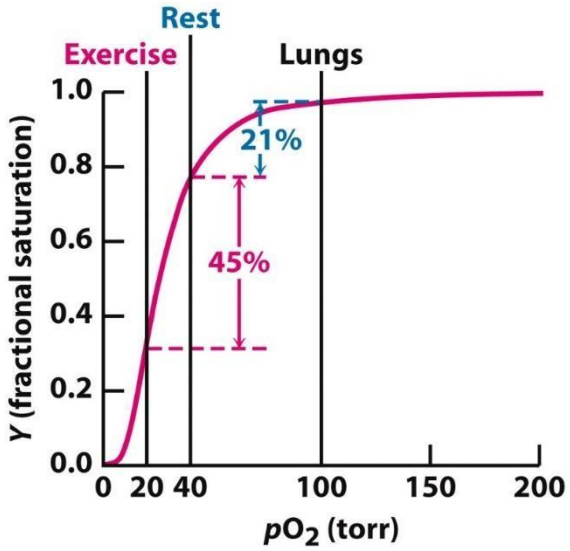


Figure 7.10
Biochemistry, Seventh Edition

Positive cooperativity means that when one oxygen binds to hemoglobin, the affinity of binding for the second oxygen will get higher.

Homotropic effector: it changes the affinity towards another molecule of the same type.
Heterotropic effector: it changes the affinity towards another molecule of a different type. For example, the binding of CO2 affects oxygen binding.

The Hill constant (coefficient)

The Hill plot is drawn based on an equation (you do not have to know it).

n = Hill constant - determined graphically

from 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

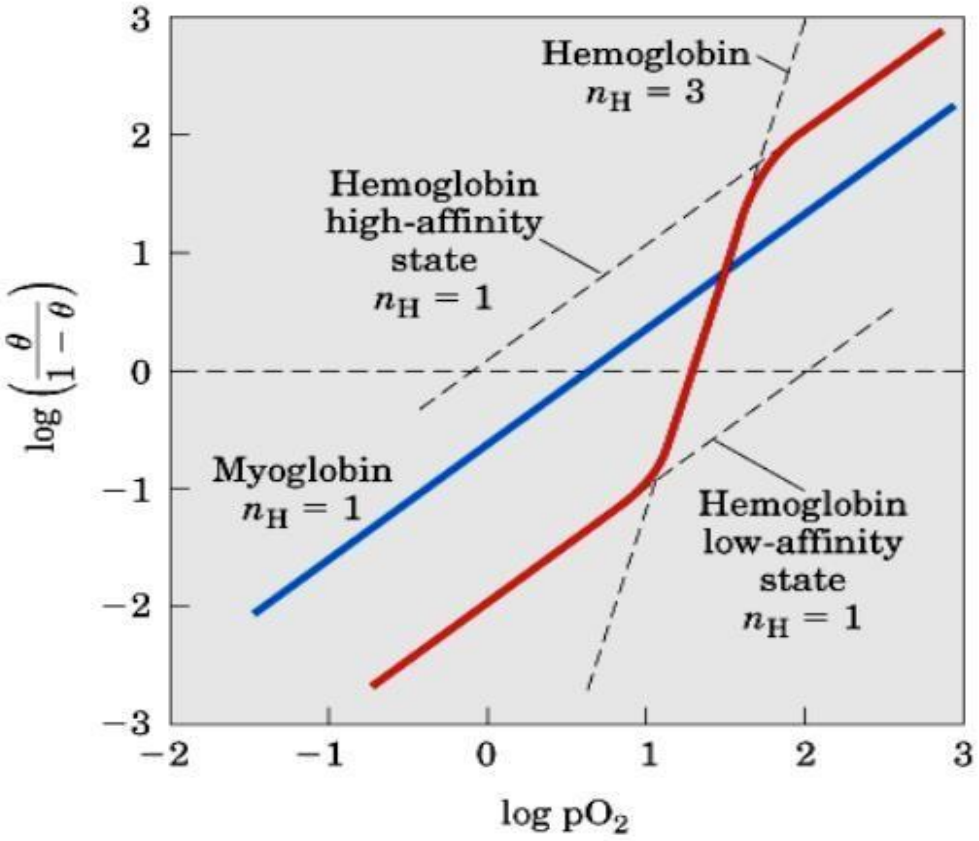
if n >1 then positive cooperativity

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

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

Y or θ is the fraction of oxygen-bound Hb

→ Y = mX + b (linear plot)



Negative cooperativity means that binding of one molecule makes it harder for another molecule to bind.

About Hill coefficient (n):
Let's say that there are two allosteric molecules, one has (n=2) and the other one has (n=3), what does that mean?

1) Both molecules have positive cooperativity.
2) The degree of cooperativity of the molecule that has (n=3) is higher than the molecule that has (n=2), let's take an example for better understanding…let's say that the molecule that has (n=3) has an affinity that is equal to 10 , when the first oxygen binds, the affinity of binding for the second oxygen will increase from 10 to 50 (numbrs are just for understanding)….and the molecule that has (n=2) has an affinity that is equal to 8 , when the first oxygen binds, the affinity of binding for the second oxygen will increase from 8 to 20….notice how the degree of cooperativity is higher in the first molecule.

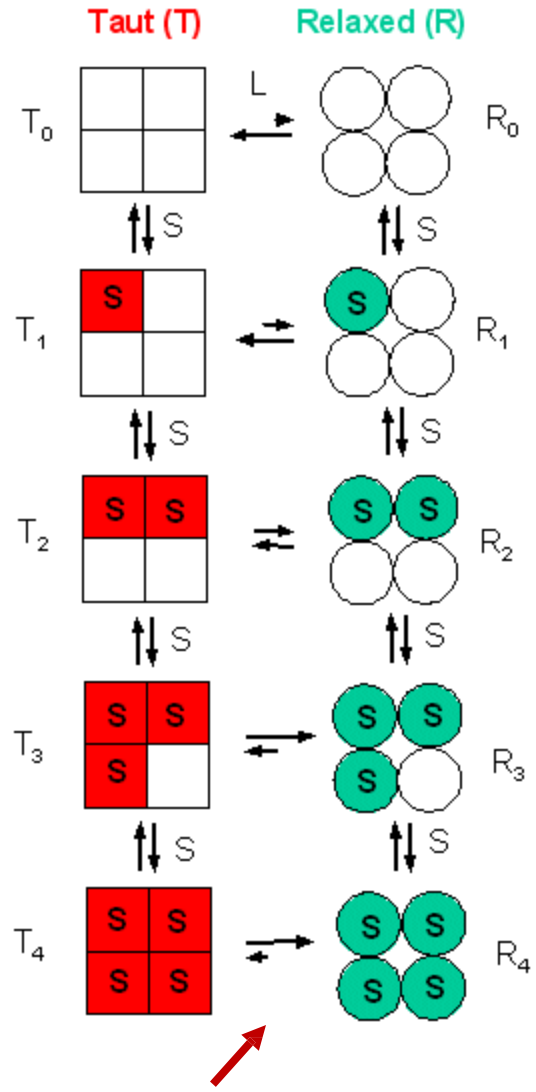
Cooperativity models

- 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.

Scientists wondered about how the structure of hemoglobin changes, does it change suddenly from R state to T state (just two states R and T with no intermediates) ? Or change gradually from R to T with gradient (there are intermediate states between R and T) ?
They postulated two hypotheses (two models).

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

About concerted model:

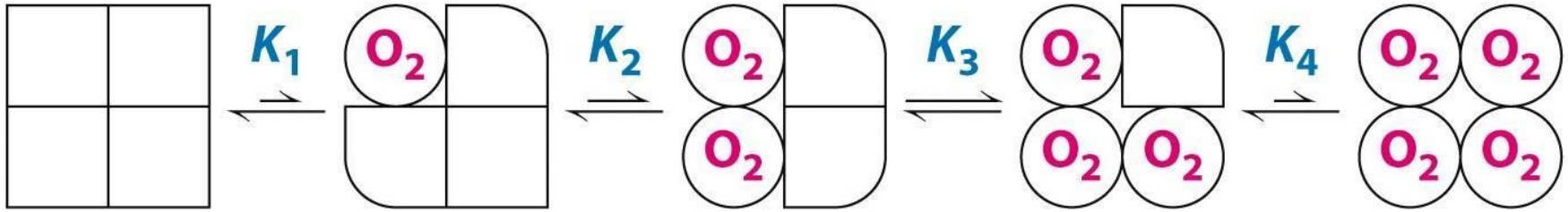
Let's say that we have a container full of hemoglobin, when there is no oxygen, we will have 95% of hemoglobin in the T state and 5% in the R state….so does that mean that it is possible to find a hemoglobin molecule in the R state when there is no oxygen bound to it ? According to concerted model..YES.
When we increase oxygen level, we will find that 75% of hemoglobin molecules are in the T state and 25% are in the R state….so notice how the EQUILIBRIUM shifts towards the R state whenever we increase oxygen level.
When increasing the level of oxygen a bit more, we will find that 50% of hemoglobin molecules are in the T state and 50% are in the R state…. with further increase in oxygen level, 75% of hemoglobin molecules are in the R state and the 25% are in the T state… with further increase in oxygen level, 95% of hemoglobin molecules are in the R state and 5% are in the T state…so does that mean that it is possible to find a hemoglobin molecule in the T state when it is fully saturated with oxygen? According to concerted model..YES.

So we conclude that in the concerted model, hemoglobin can either be in the R or T state with no intermediate states in between.

The sequential, induced fit, or KNF model

Experiments show that both models are correct

- 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.

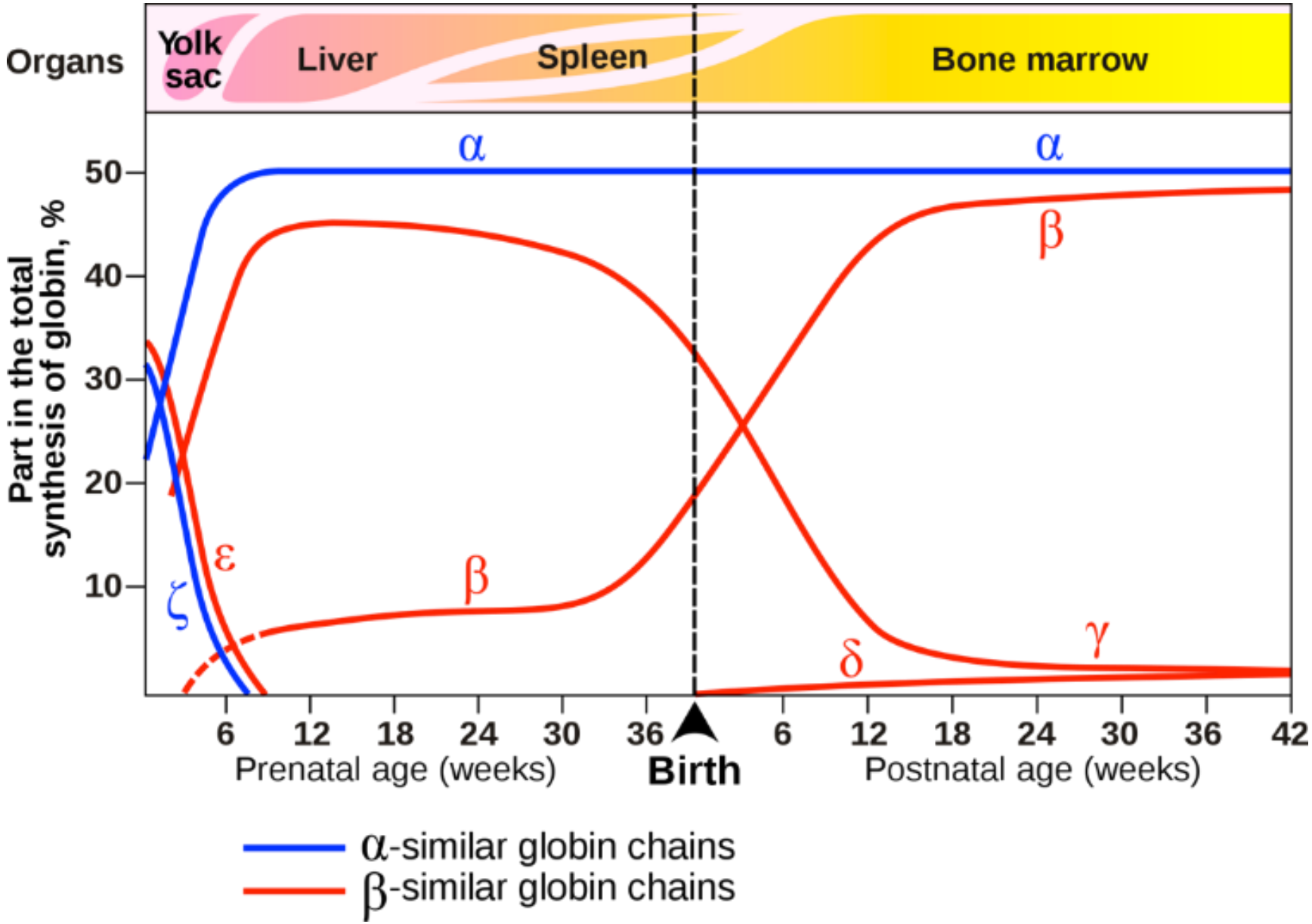


- Which one is better? Both can explain the sigmoidal binding curve.

In the sequential model, it isn't just (on/off) between the T and R states as in the case of the concerted model, there are intermediate states between the T and R states (there is a sort of intensity).

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

We said that hemoglobin has 2 alpha chains & 2 beta chains mmmm…that's not really true, actually we have different types of hemoglobin molecules.

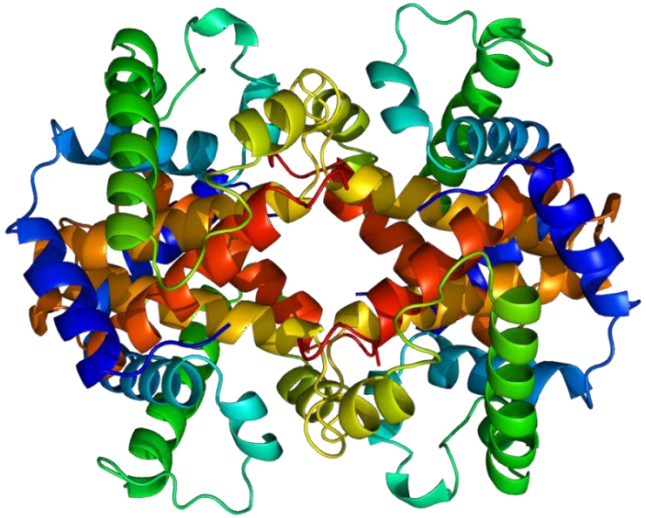
During development, when the zygote is formed….2 zeta chains & 2 epsilon chains are produced by the yolk sac, and they are called Embryonic hemoglobin (2 zeta/2 epsilon).
Later, the expression/activity of the genes that encode for zeta and epsilon chains will decrease over time while the expression of the genes that encode for alpha chains will increase. In addition, there will be a production of another chain which is called gamma chain (both alpha and gamma chains are produced from the liver then later from the spleen and a small amount is produced form bone marrow)…..forming what is known as Fetal hemoglobin (2 alpha/2 gamma). Then gamma chains production will decrease and beta chains start to be produced until we end up with what is called Adult hemoglobin (2 alpha/2 beta).

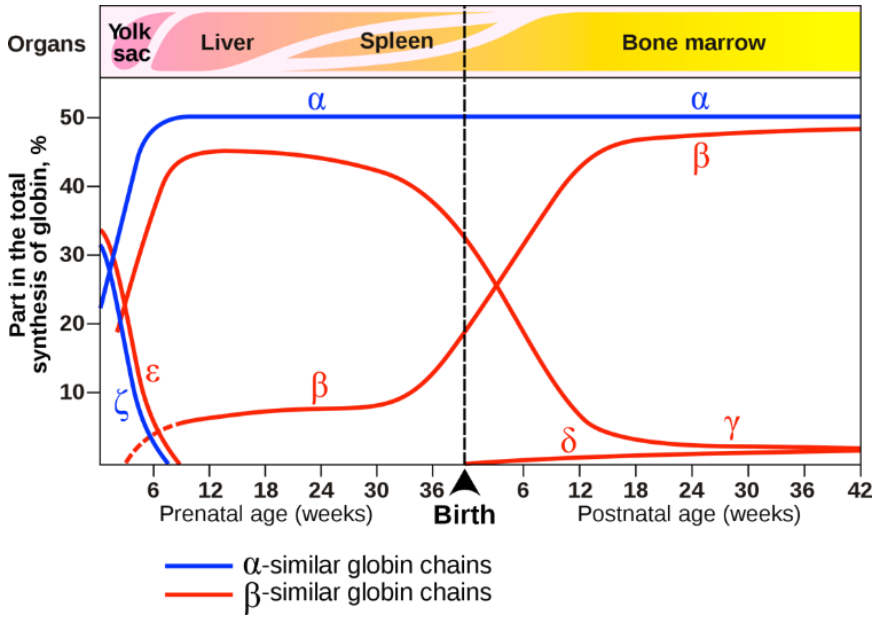
At birth, we mainly have fetal hemoglobin (2 alpha/2 gamma).
We also have adult hemoglobin (HbA1) (2 alpha/2 beta) to a lesser extent and a minority of adult hemoglobin (HbA2) (2 alpha/2 delta).

Notice that at the embryonic stage, we have different types of chains all existing at the same time….mainly embryonic hemoglobin which is called HbE Gower 1 that is composed of (2 zeta/2 epsilon) as mentioned above…..don't worry about the names of other hemoglobin molecules, just keep in mind that embryonic hemoglobin is called HbE Gower 1.

The embryonic stage

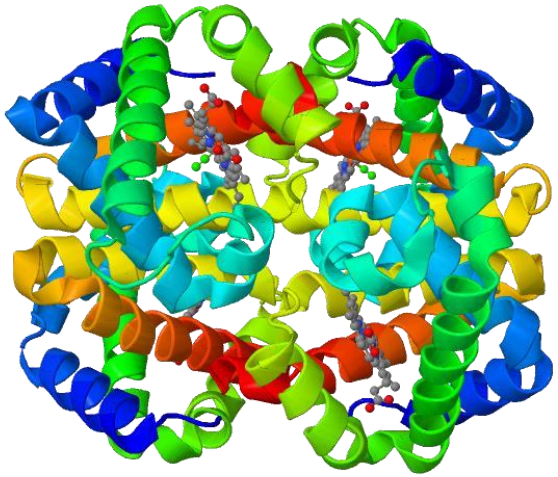
- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1) is a tetramer composed of 2 zeta (ξ) chains and 2 epsilon (ε) chains
- Other forms exist: HbE Gower 2 (α2ε2), HbE Portland 1 (ζ2γ2), HbE Portland 2 (ζ2β2).

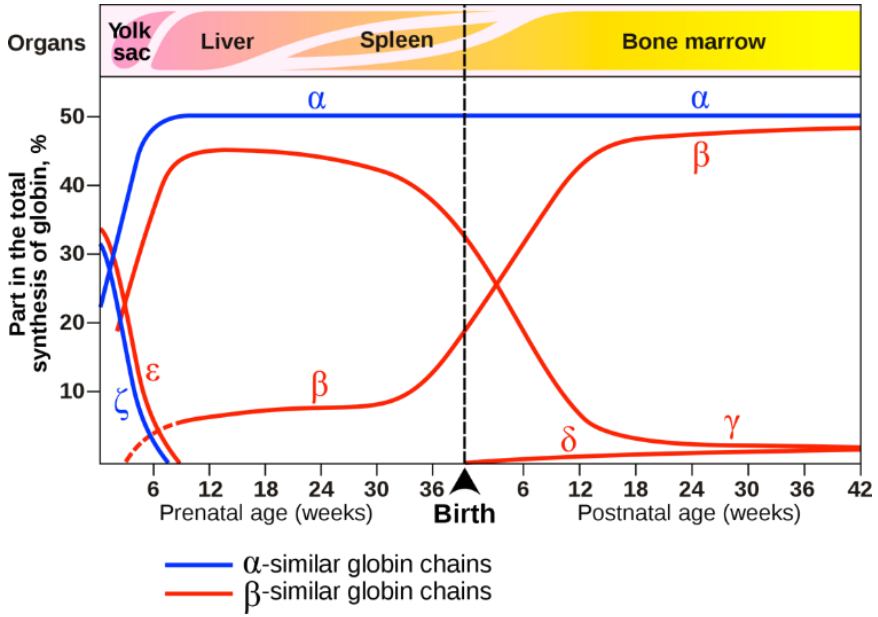




The fetal stage

- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines dramatically and fetal hemoglobin synthesis starts from the liver.
- 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.

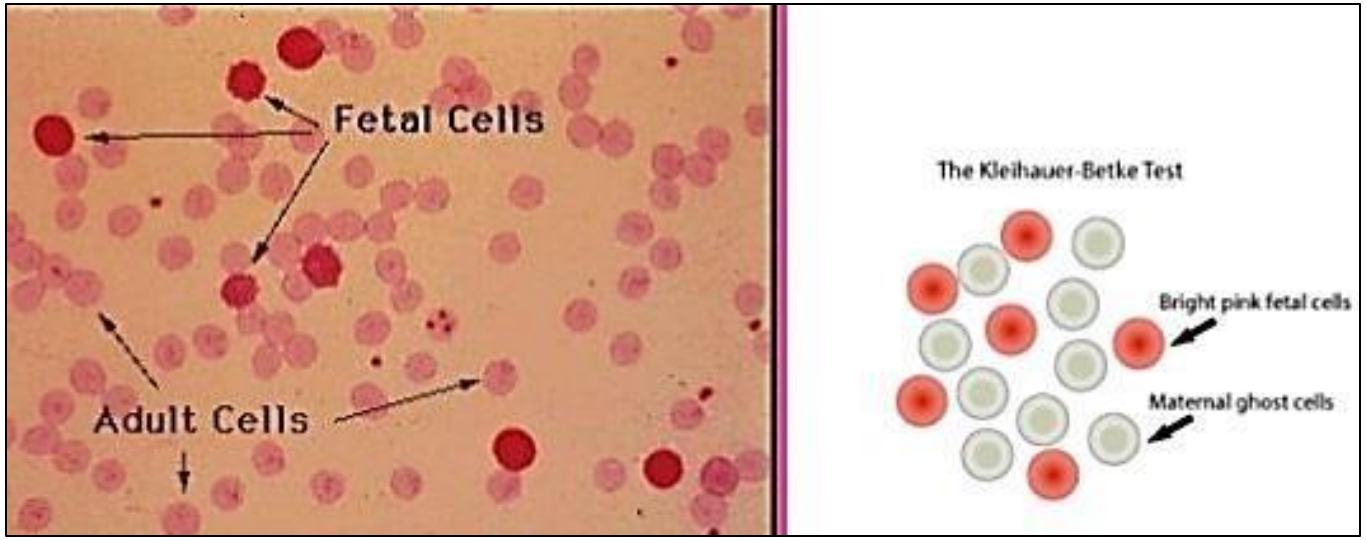


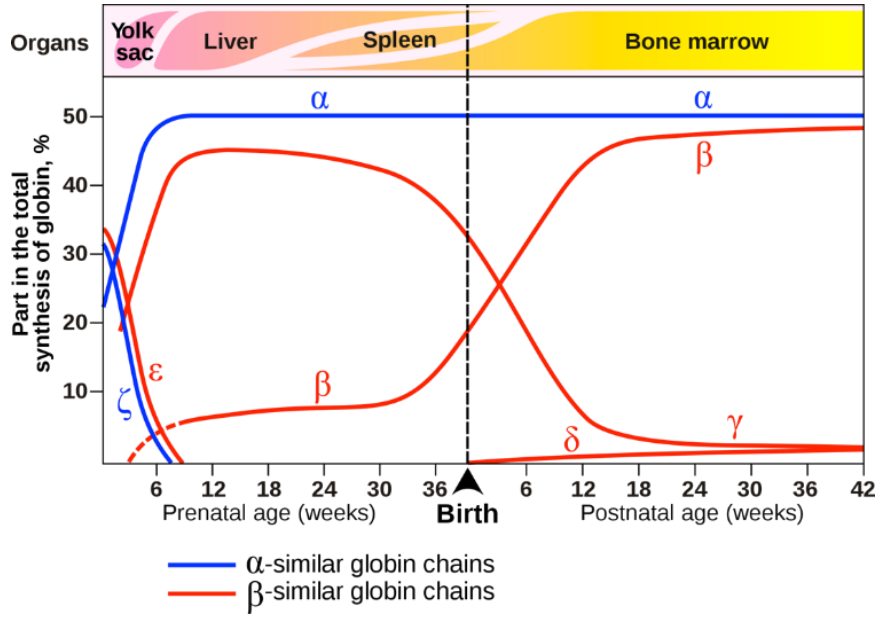


The adult stage

- 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.

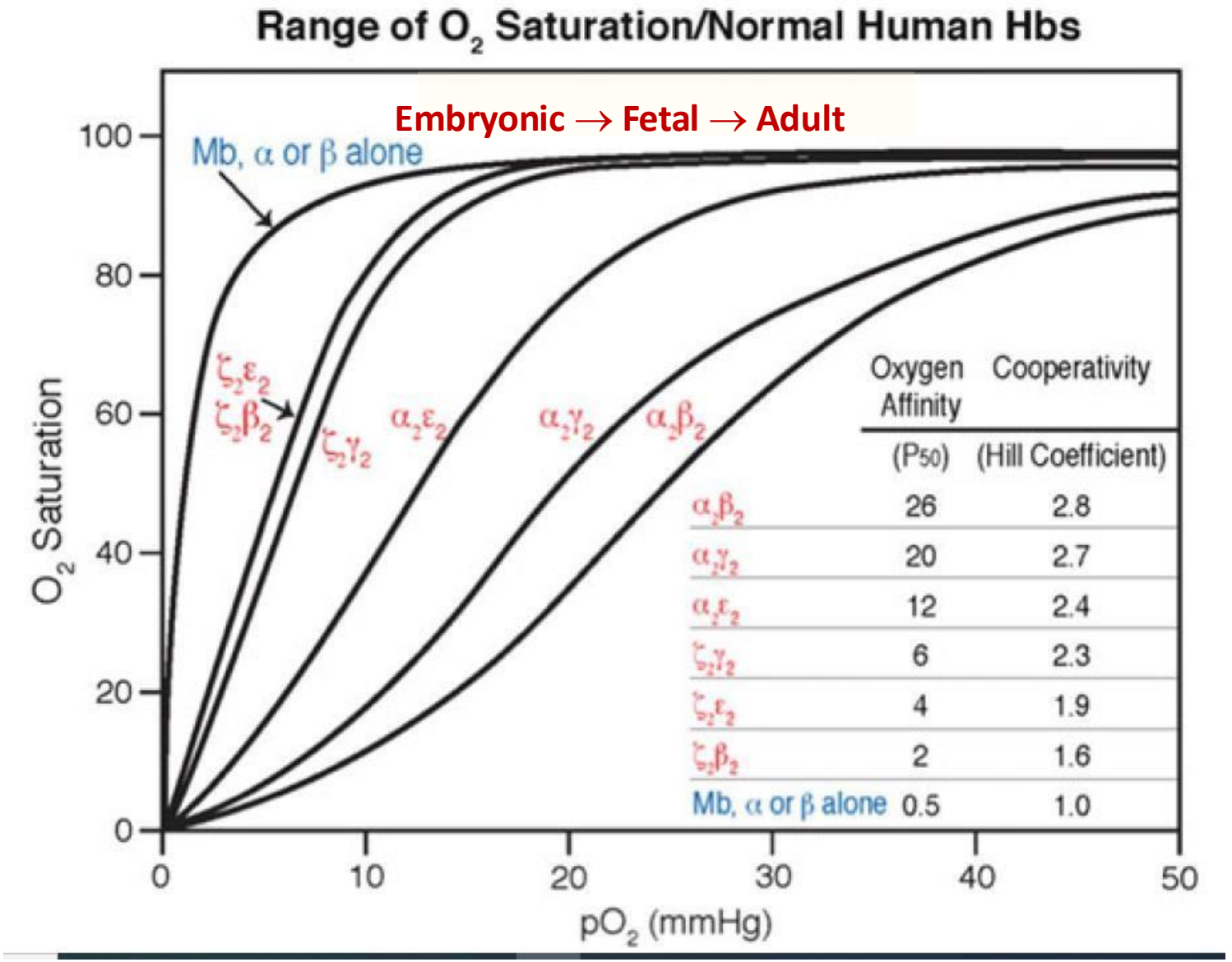
  At birth, synthesis of both γ and β chains occurs in the bone marrow.
- The major hemoglobin is HbA1 (a tetramer of 2 α and 2 β chains).
  - A minor adult hemoglobin, HbA2, is a tetramer of 2 α chains and 2 delta (δ) chains.





At the embryonic stage, the embryonic hemoglobin (2 zeta/2 epsilon) binds to oxygen with really high affinity and that matches the embryonic requirements of high metabolism rate and cell division…and all of these require high levels of oxygen.

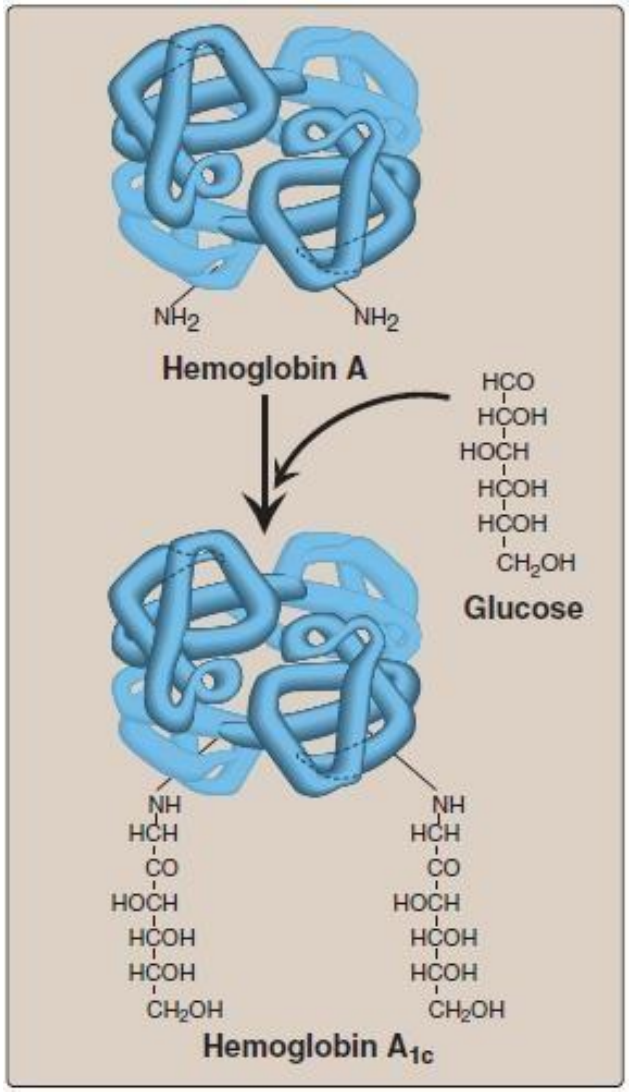
The order of hemoglobin types based on affinity to oxygen from higher to lower:
2 zeta/2 epsilon (highest)
2 alpha/2 gamma
2 alpha/2 beta (lowest)



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 on β and α chains.
  - HbA1c is present at higher levels in patients with diabetes mellitus.

- In both alpha and beta chains, the first amino acid is valine.
- Whenever there is an increase in blood glucose level, the level of glycosylated adult hemoglobin (HbA1c) will increase.
- The value of HbA1c can be measured and is of great clinical significance in diabetic patients.



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

	Hemoglobin A1C (HbA1c)	Fasting Blood Sugar Test	Random Blood Sugar Test
Normal	< 5.7%	< 100 mg/dL	N/A
Prediabetes	5.7 - 6.4%	100 - 125 mg/dL	N/A
Diabetes	≥ 6.5%	> 125 mg/dL	≥ 200 mg/dL

additional sources

1. https://youtu.be/HNi_xkbBOlY?feature=shared

2. https://youtu.be/jVUwn4wWTXI?feature=shared

VERSIONS	SLIDE #	BEFORE CORRECTION	AFTER CORRECTION
V1→ V2	Grammatical mistakes were corrected.	---------- -	---------- -
V2→V3	1) Slide 8 2) Slide 15 3) Slide 35	1)---------- 2) Arg 141 (*2) 3) Glycosylation occurs on β chain	1) just added a pic 2) His 146 3) Glycosylation occurs on β and α chains .

امسح الرمز و شاركنا بأفكارك لتحسين أدائنا !!