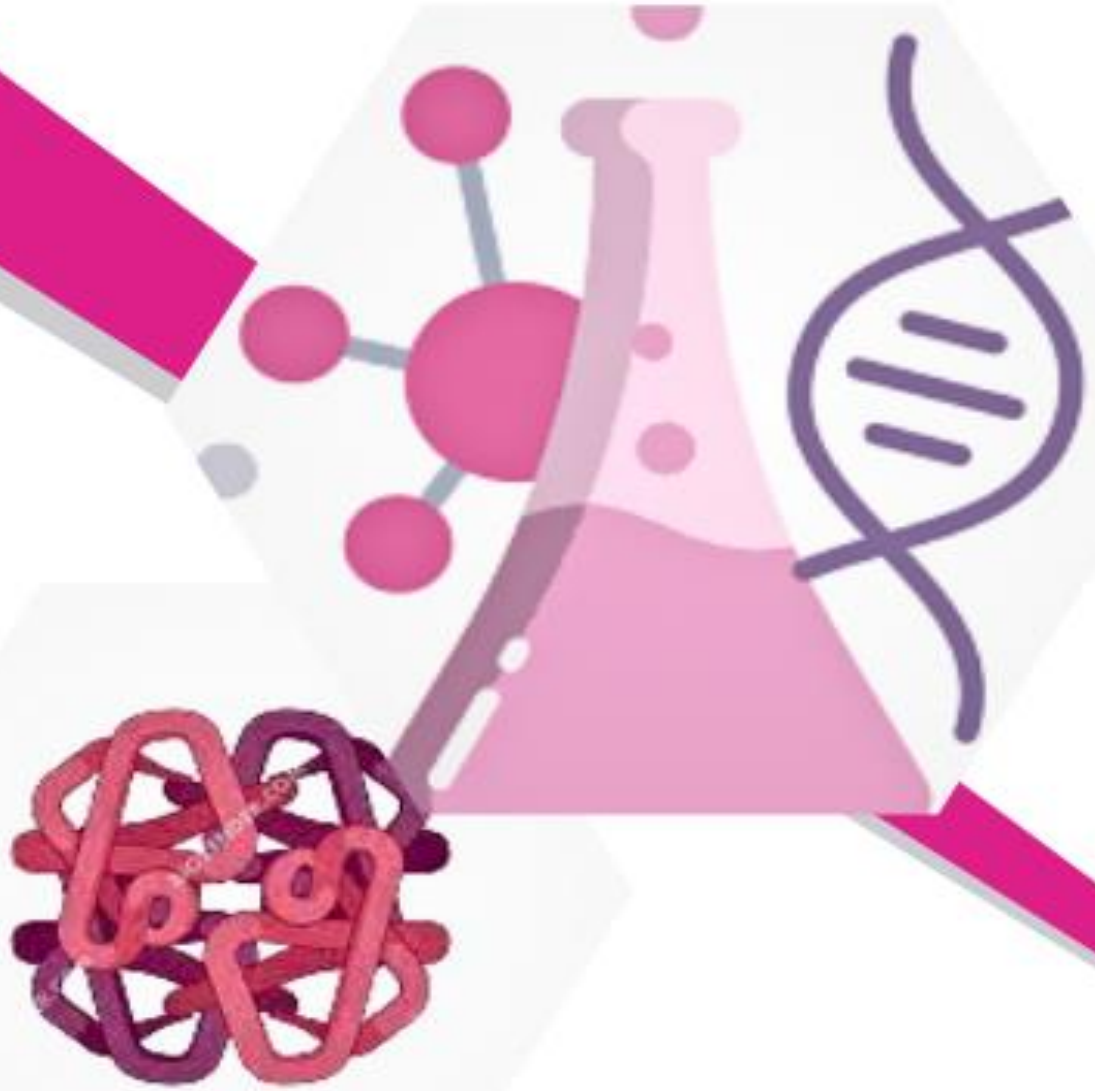
Doctor.021
no. 1

HLS
BIOCHEMISTRY



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HEMOGLOBIN

AN OVERVIEW AND MORE

The doctor started the lecture by asking “Why Hemoglobin is important?”

Hemoglobinopathies are the most widespread diseases in the world and Jordan (thalassemia for example).

Hemoglobin is the most studied protein, its whole structure is known and studied.

Hemoglobin is a holoprotein (protein associated with a non-protein groups), and specifically Hb is a hemoprotein (associated with a heme group).

Heme: an organic hydrophobic molecule associated almost with the center of hemoglobin, surrounded by hydrophobic amino acids.

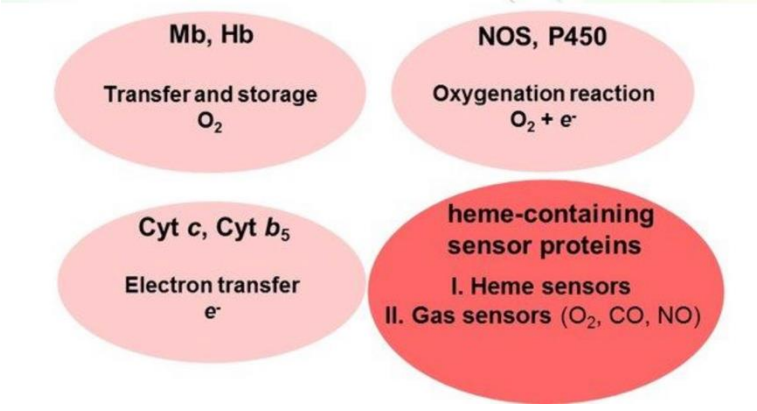
In hemoglobin heme is associated with iron covalently (4 bonds between iron and heme).

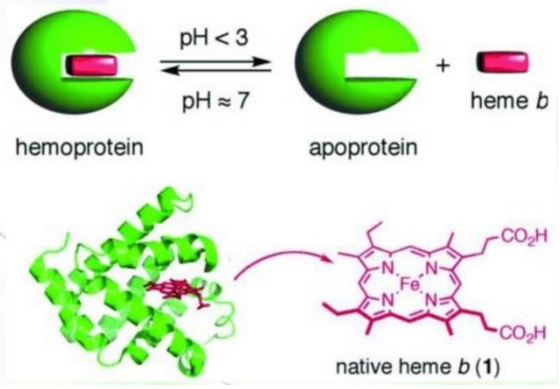
HEMOPROTEINS

Many proteins have heme as a prosthetic group called hemoproteins.

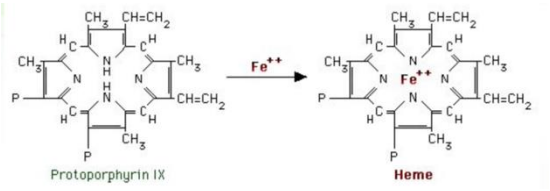
A prosthetic group is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.

The function the heme adds to the protein is determined by the amino acids surrounding the heme:





HEME STRUCTURE

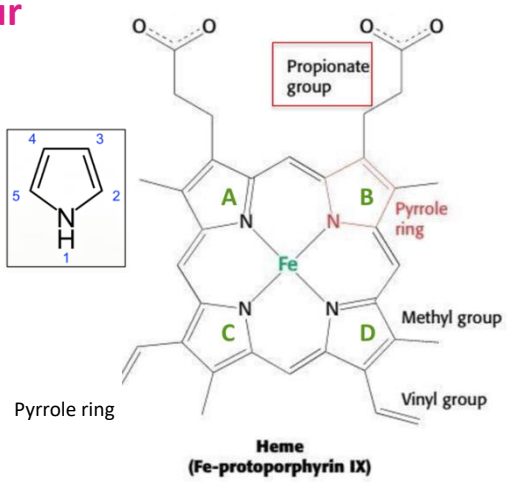


- **It is a complex of protoporphyrin IX + iron (Fe2+).**
- **The porphyrin is planar and consists of four rings (designated A-D) called pyrrole rings.**
- **Each pyrrole can bind two substituents.**
- **Two rings have a propionate group each.**

Although heme is hydrophobic it has two hydrophilic propionate groups.

- **Note: the molecule is hydrophobic.**
- **Fe has six coordinates of binding.**

(4 with the pyrrole rings).



STRUCTURE OF HEMOGLOBIN

- **Hb is a globular protein.**

- Typical amino acid distribution

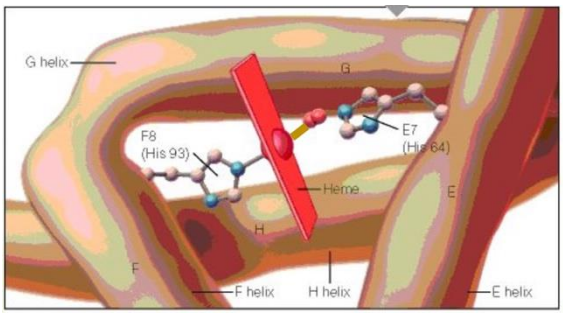
Like any other globular protein, hydrophobic amino acids are projecting toward the inside and hydrophilic amino acids to the surface.((but that does not necessarily mean that there are no hydrophobic or nonpolar AAs on the surface. Remember that heme is a hydrophobic molecule, so it makes sense)).

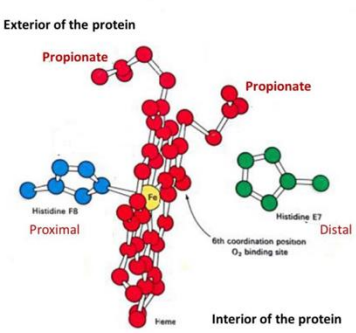
>>pyrrole rings are surrounded by the hydrophobic amino acids in the inside.

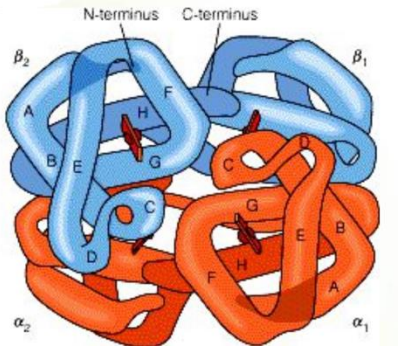
>>propionate groups are extending outside the protein and associate with the hydrophilic amino acids at the surface of the protein.

- Positions of two histidine residues. (Hydrophilic AAs located in the inside)

- Proximal and distal. Proximal histidine is associated with the iron forming the 5th bond (remember: iron forms 6 bonds. 4 with the pyrrole rings in the middle of the heme molecule, the 5th with the proximal histidine in the protein, and the 6th bond with oxygen).







- **It is an allosteric protein.**

1) Multiple subunits (2α + 2β)

Adult Hemoglobin (HbA) consists of 4 polypeptide chains.

- α polypeptide = 141 amino acids (Arg141)
- β polypeptide = 146 amino acids (His146)
- The first amino acid in both is valine.

2) Altered structure depending on bound molecules

3) Positive cooperativity towards oxygen

4) Regulated by allosteric effectors.

(Allo: means different , steric: means structure) so it has two structures depending on oxygen binding.

Hemoglobin is an allosteric protein, which means:

1- It has multiple subunits (polypeptides) 2alpha and 2beta.

2- It has altered structures (configurations) R & T depending on bound molecules.

R(relaxed)	T (taut or tight)
High affinity for oxygen	Low affinity for oxygen

3- Its saturation curve is sigmoidal in shape

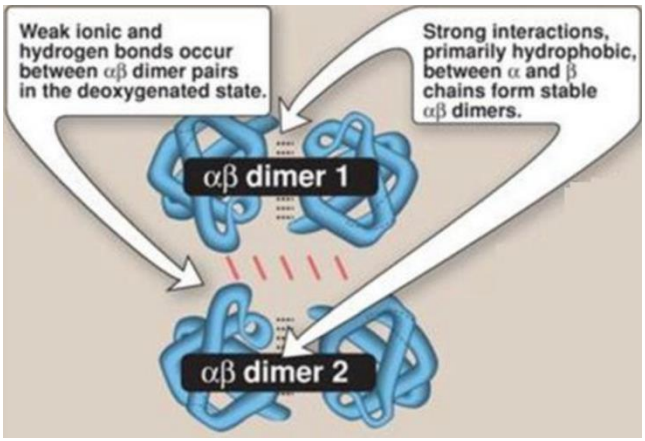
4- Cooperativity: when the first oxygen binds to the molecule it makes it easier for the 2nd oxygen to bind, then easier for the 3rd oxygen and finally easier for the 4th and last oxygen to bind.

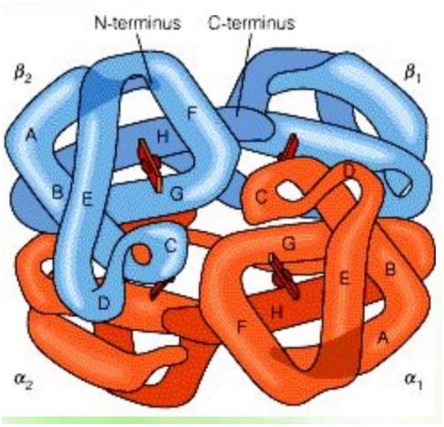
Each Hb molecule contains 4 heme molecules >> one Hb can bind 4 oxygens.

HOW ARE THE SUBUNITS BOUND?

Hemoglobin is a tetramer (dimer of dimers as the doctor said).

- **(α-β)2**
- **Note how they interact with each other.**





Ionic and hydrogen bonds that occur between αβ dimer pairs are very important in conversion between R and T states. When oxygen binds to Hb the structure switches from T state to R state making it easier for the other oxygen to bind.

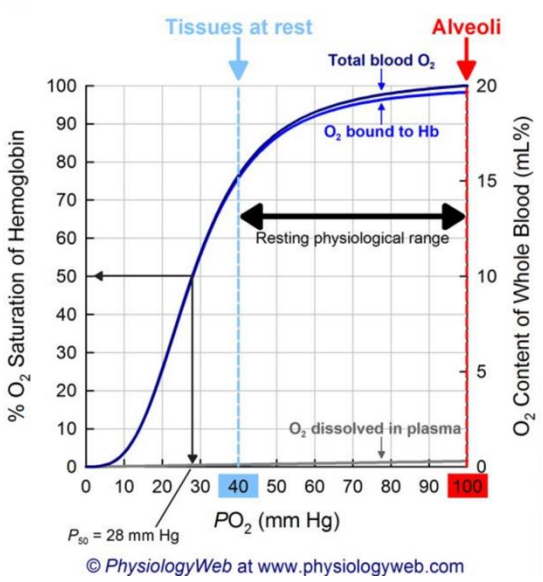
STRUCTURAL CHANGE OF HEMOGLOBIN

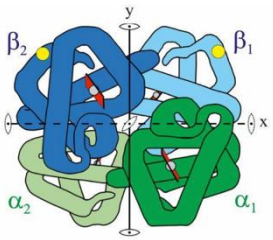
Oxygen saturation curve looks sigmoidal, high oxygen pressure increases the probability that oxygen will bind to Hb.

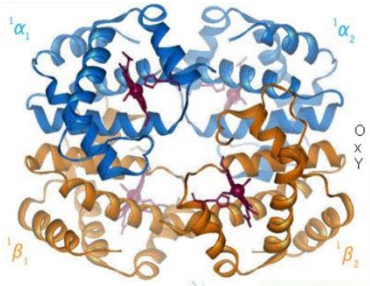
Notice that at low oxygen pressure the saturation (affinity) of oxygen is low but there is a sudden transition where the affinity becomes higher.

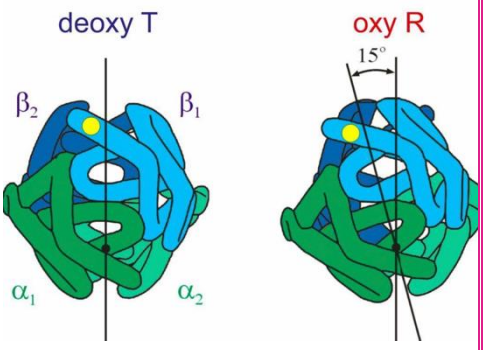
At 100 mmHg in the lungs >> 95% of Hb is saturated with oxygen.

P50= 26mmHg >> this means that when oxygen pressure is 26 torr, 50% of oxygen binding sites are filled. (This is the point where the transition from T to R state occur).



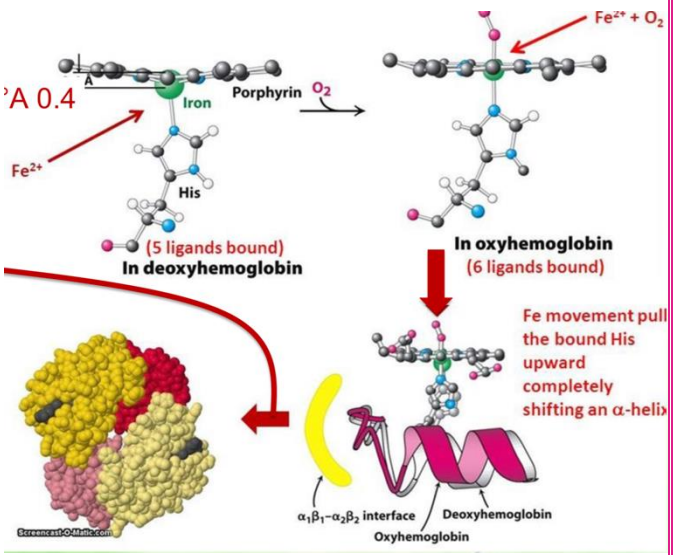






STRUCTURAL AMPLIFICATION CHANGE

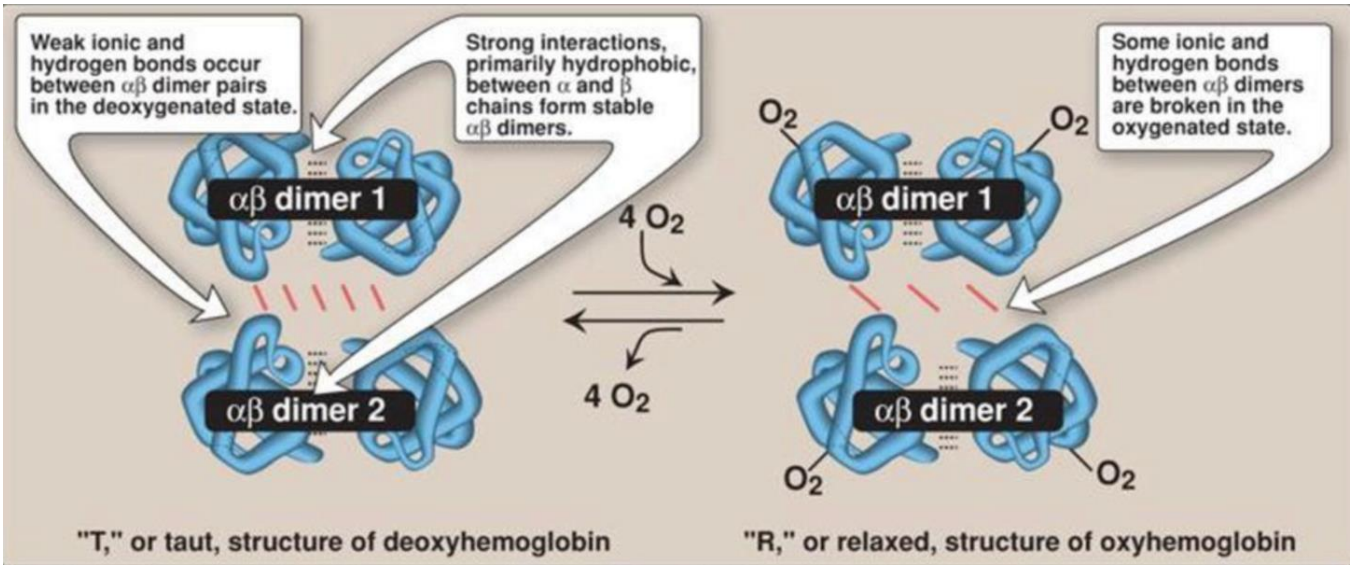
- **Changes in tertiary structure of individual hemoglobin subunits**
- **Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.**



How Hb goes from T state to R state? Heme molecule has a dome like structure (because the proximal histidine is hydrophilic and the heme is hydrophobic so there is repulsion forces between the ring structure of the heme and the proximal histidine resulting in the dome shape).

When oxygen binds to iron at the 6th coordinate, the oxygen interacts with the distal histidine (oxygen is pulled towards the distal histidine and pulls the heme so it becomes flat and pulls the proximal histidine upward toward the oxygen which makes changes in the alpha helix making the hemoglobin molecule relaxed with high affinity toward oxygen (R state) .

BROKEN ELECTROSTATIC



INTERACTIONS AND H-BONDS

Upon the movement and changes of the alpha helix some weak electrostatic and hydrogen bonds between the dimers are broken. At the T (taut) state there is more electrostatic and hydrogen bonds which makes the Hb more stressed>> the breakage and reforming of some bonds in the Hb makes it relaxed with high affinity for oxygen.

THE BROKEN INTERACTIONS

Let's zoom in and see the exact altered and broken bonds

• Electrostatic interactions and hydrogen bonds (at the C-termini of the alpha and beta chains) that stabilize the T-form of hemoglobin are broken upon movement of the alpha-helix.

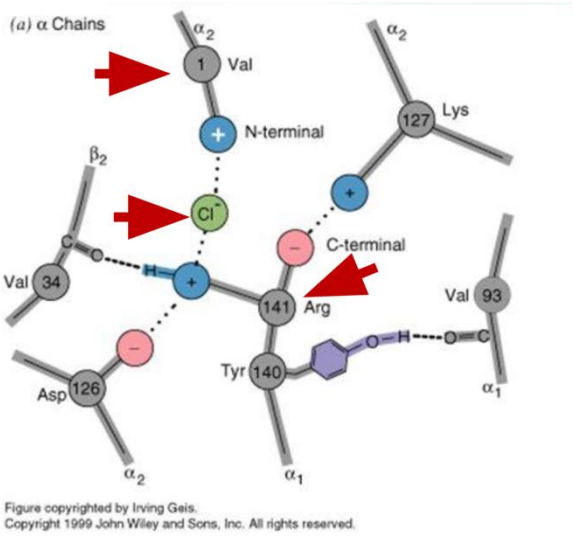
• Note the groups, the protonation status, and the allosteric effectors.

1. Alpha interactions:

there's a chloride ion in the alpha chain in RBCs which makes an interaction with the surrounding amino acids:

1) With the N-terminus of the valine (the 1st amino acid in the alpha chain).
2) With the R group of the 141 amino acid of the alpha chain (arginine, the last amino acid of this chain).

In the lungs there's less chloride ions which means that most of these electrostatic bonds are broken forming the R state).



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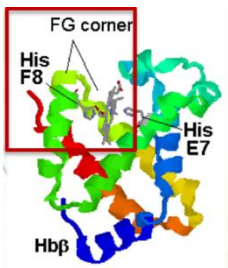
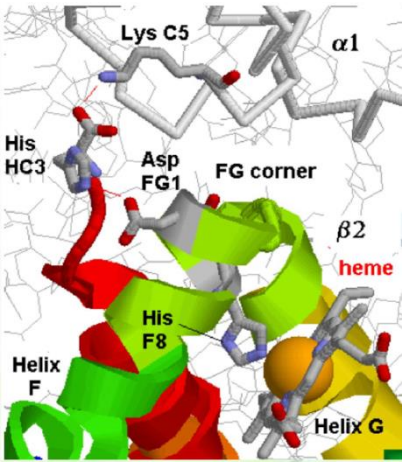
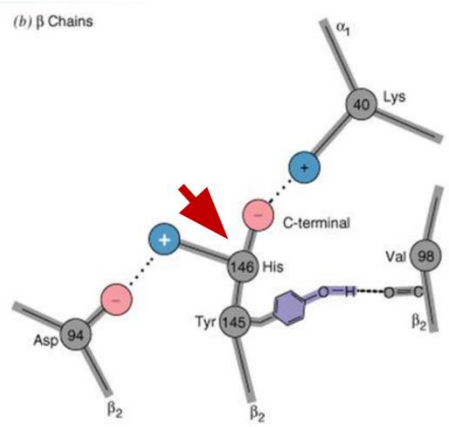




2. Beta interactions
   The last amino acid of the beta chain (Histidine 146) makes interactions with surrounding amino acids, Histidine has two charged groups:
   1) Negatively charged C-terminus>> interacts with the positively charged lysine on alpha chain.
   2) Positively charged R group>> interacts with Aspartate amino acid on the same beta chain.

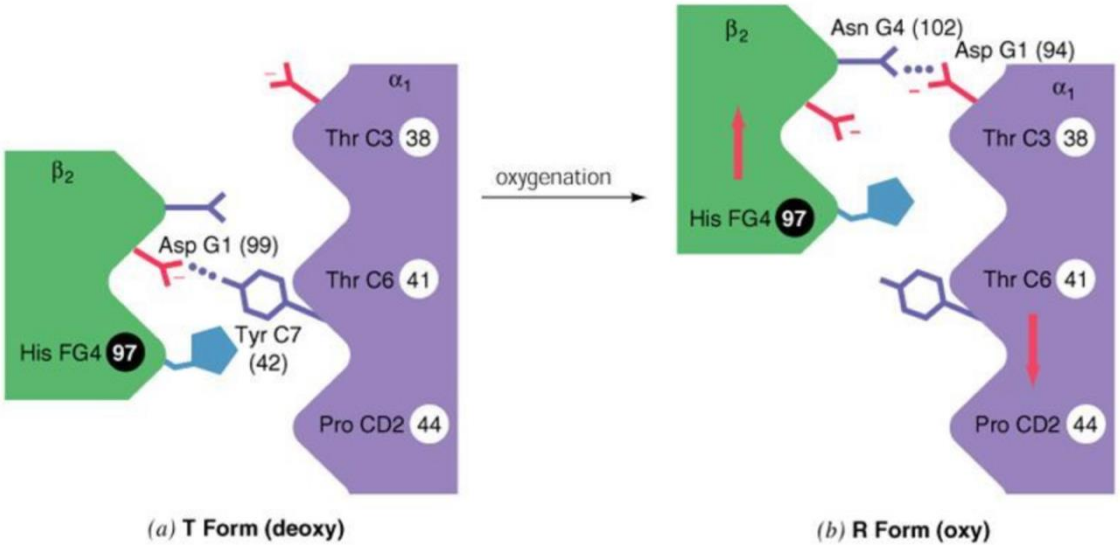


REFORMATION OF HYDROGEN BONDS

There's over 30 differences of electrostatic interactions in Hb when it binds to oxygen, here's an example:

• T-state hemoglobin (deoxyhemoglobin) is stabilized by a hydrogen bond between Asp G1 (99) of β2 with Tyr C7(42) of α1.

• When O2 binds, the α1 surface slides and a hydrogen bond is formed between Asn G4 (102) of β chain and Asp 1 (94) of α chain stabilizing the R form of hemoglobin.



(a) T Form (deoxy)

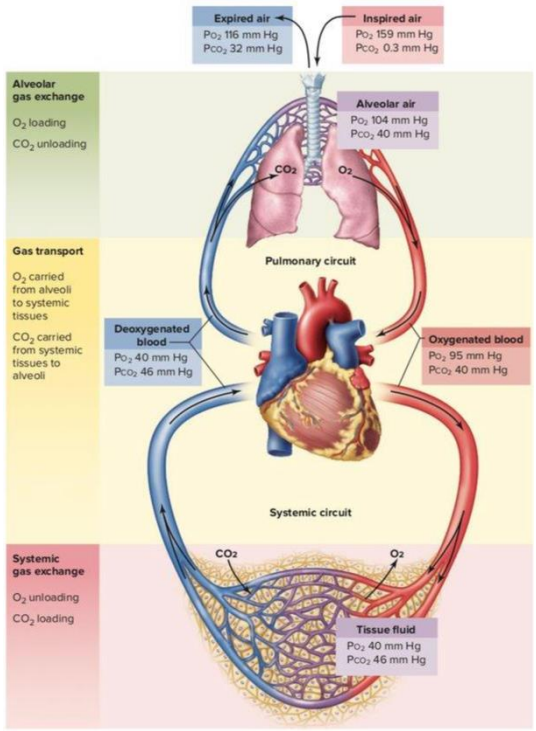
(b) R Form (oxy)

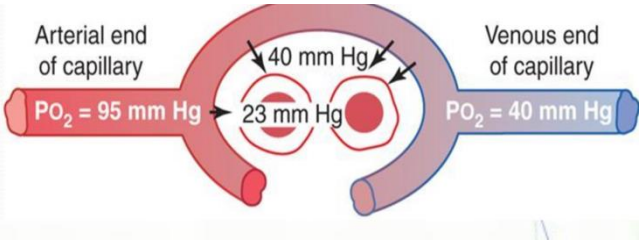
OXYGEN DISTRIBUTION IN BLOOD VERSUS

In the lungs there's high pressure of oxygen and in tissues there's low pressure of oxygen.

In the lungs the pressure of oxygen in about 100torr and in tissues its 30-40 torr.

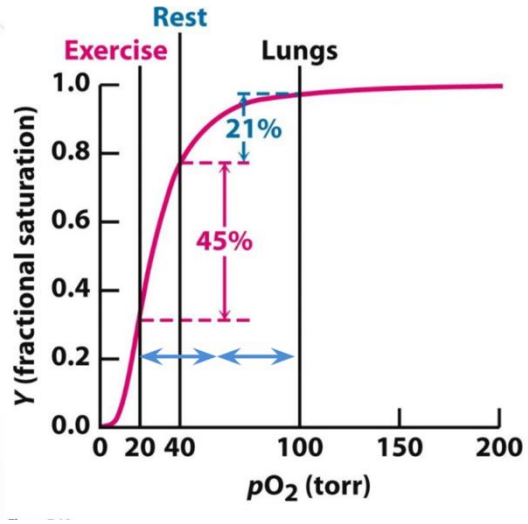
(Even on 40 tor most of Hb is still saturated with oxygen).



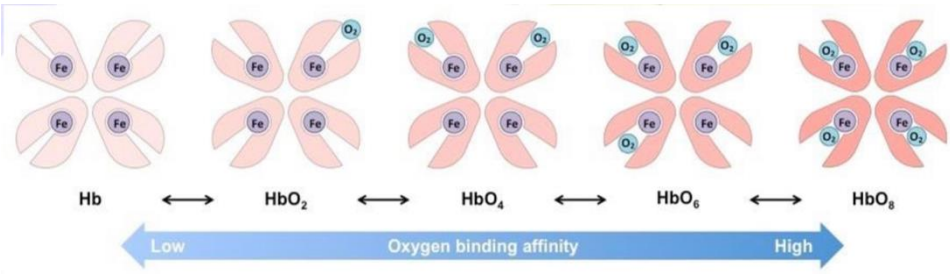


OXYGEN SATURATION CURVE

- **The saturation curve of hemoglobin binding to O2 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.**



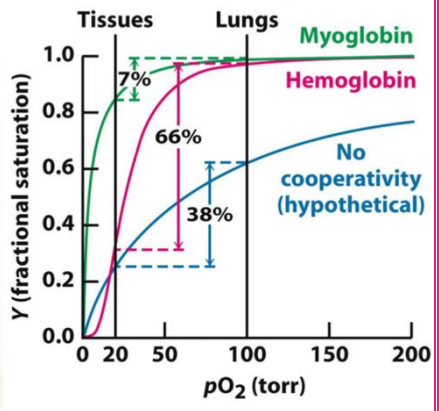
POSITIVE COOPERATIVITY



•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. CO2).



Oxygen is an allosteric positive homotropic effector:

Allosteric effectors>> (allosteric) because Hb in an allosteric protein,(effectors) because they change Hb structure/status.

These effectors can be positive or negative effectors.

Positive effector>> increases the affinity for the other ligands to bind oxygen.

Homotropic>> because it affects binding of another molecule of the same type.

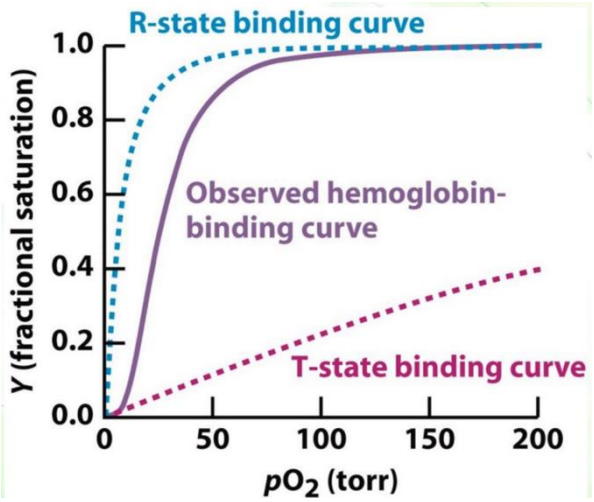
Heterotropic effectors are mostly negative effectors meaning that they reduce the affinity of Hb towards oxygen. (Like protons and CO2)

The Advantage that Hb is allosteric:

We need two states (high affinity toward oxygen in lungs and at the same time low affinity for oxygen in the tissues) and that's why the saturation curve of oxygen looks sigmoidal.

If it wasn't sigmoidal or allosteric, this means that if the Hb is in the T state all the time (always low affinity for oxygen) even in lungs there will be only 20% of Hb is saturated (which is not enough).

On the other hand, if it is always in the R state (always high affinity for oxygen) even in tissues, the oxygen won't be released to tissues)>> like the curve of myoglobin. See the curves on the photo.



Later when we talk about hemoglobinopathies we will see that mutations may happen at any single amino acid in Hb molecule making Hb molecule either always in the R state or always in the T state.

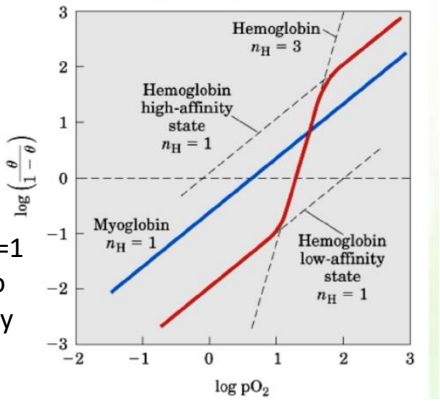
THE HILL CONSTANT (COEFFICIENT)

- **The Hill plot is drawn based on an equation (you do not have to know it).**
- **n = Hill constant - determined graphically by the hill plot.**
- **n is the slope at the midpoint of binding of log (Y/1-Y) vs. log of pO2.**
- **if n = 1 then non cooperativity**
- **if n < 1 then negative cooperativity**
- **if n >1 then positive cooperativity**

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

Y or θ is the fraction of oxygen-bound Hb

→ Y = mX + b (linear plot)



Myoglobin n=1 meaning no cooperativity

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

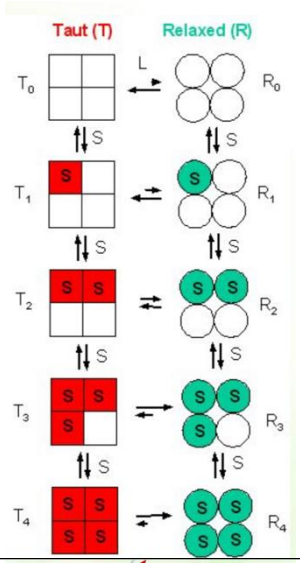
**very important to know that it measures the degree of cooperativity not the number of binding sites.

COOPERATIVITY MODELS

- **Two models of cooperativity that could explain the observed data:**

1) **Concerted model – all subunits undergo the conformational change simultaneously. (There are only two states, R and T).**
2) **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)



Note the arrows

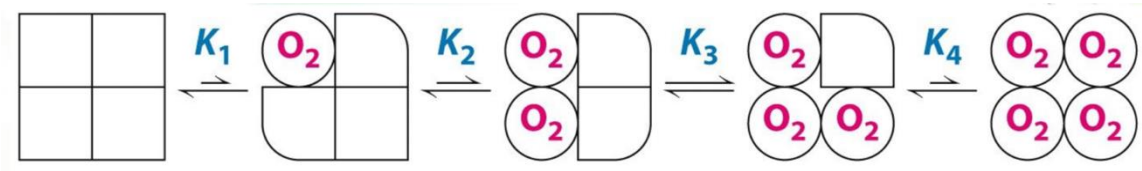
- **The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.**
- **Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.**
- **This allows unoccupied subunits to adopt the high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

Oxygen changes the equilibrium, when there's more oxygen the equilibrium shifts to the R state.

1. When there's no oxygen most of hemoglobin exists in the T structure (there's small amount of R state)
2. When one oxygen binds the majority still exists in T state but the probability of R state becomes higher than when there was no O2 at all.
3. When two O2 bind T and R state have the same probabilities to exist.
4. When three O2 bind the equilibrium shifts to the R state with few T structure.
5. When 4O2 bind the majority exist in R structure with few T state.

The sequential, induced fit, or KNF model

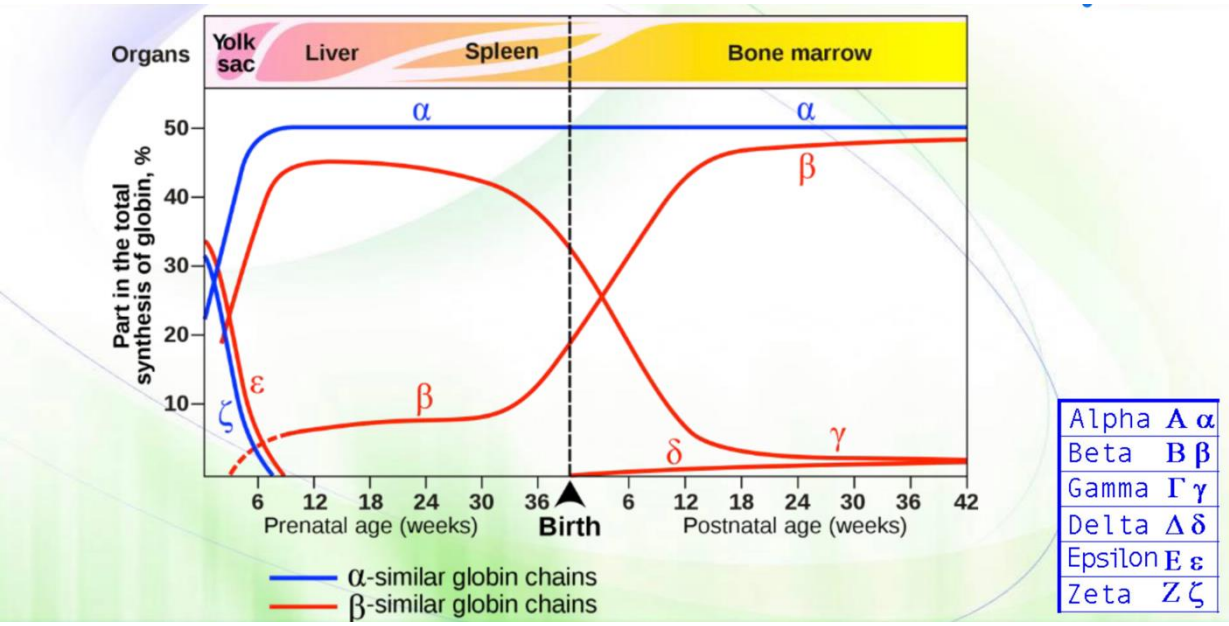
•The subunits go through conformational changes independently of each other, but they make the other subunits more likely to change, by reducing the energy needed for subsequent subunits to undergo the same conformational change.



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

It is not only one hemoglobin

DEVELOPMENTAL TRANSITION OF HEMOGLOBINS



Through out developing and embryogenesis we have different hemoglobin molecules not only alpha and beta. Initially in embryonic stage from the yolk sac we have Epsilon and Zeta. After 6-8 weeks their expression decrease and alpha expression starts and continues throughout life.

In the fetal stage gamma subunit starts to be produced from the liver and the alpha subunit switch to be produced from the liver rather than the yolk sac.

Right before birth gamma expression starts to decrease and beta expression starts. (But notice that at birth gamma expression is still higher than beta). Delta subunit starts to appear after birth but its still very low at the adult stage.

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 (α2γ2)**

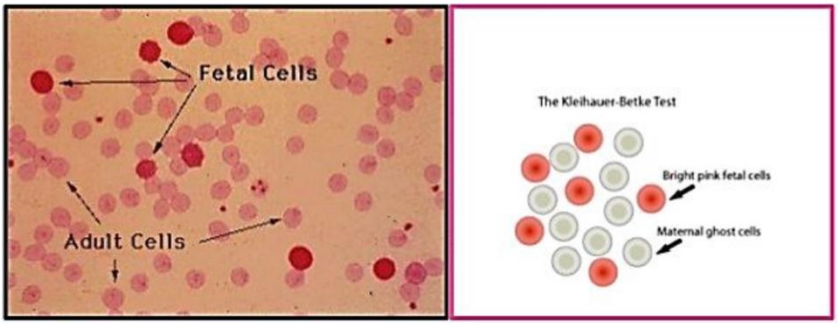
There's a little (α2β2)

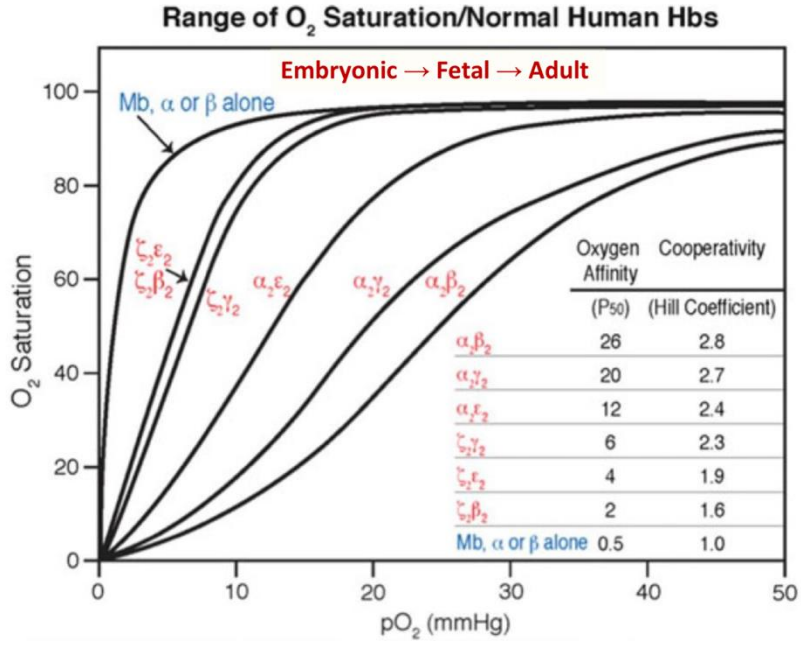
- **The α polypeptides remain on throughout life.**

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.**

There's (α2β2)=HbA1(major) ,minor (α2delta2)=HbA2, a little bit of (α2γ2).

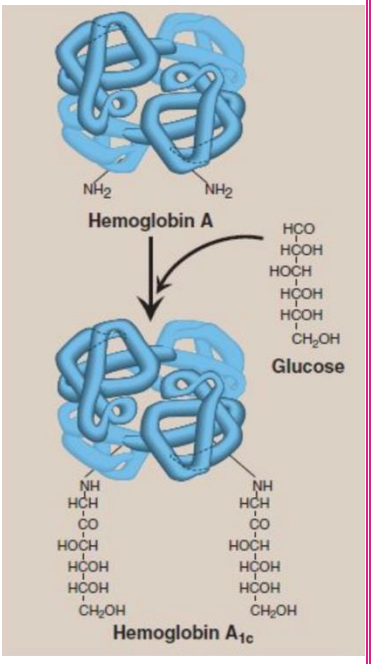




In the embryonic stage HbE(ξ2ε2) has a really high affinity towards oxygen compared to fetal and adult Hb. That's why pregnant women experience shortness of breath because the baby has higher affinity to oxygen.

(α2γ2) also has higher affinity than(α2β2).

ADULT HEMOGLOBINS



- **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.**
- **HbA1 can be glycosylated 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.**

Valine can be glycosylated.

When sugar concentration increases in the blood it enters the RBCs and the Hb gets glycosylated. So, it can be used as a glucose level monitor.

ADVANTAGES OF HBA1C TESTING

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).**

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

We should memorize the blood glucose level in mg/dL and HbA1c percentage .

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V1

Page 8, we correct the statement that is highlighted in yellow, its tyr c7(42) NOT thr C6(41).