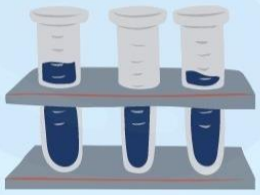
Sheet No.

1

Biochemistry



hematolymphatic system

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Hello all and welcome to our first Biochemistry lecture this year!

It's a relatively long one, but a fun one nonetheless!

Try to go through it all in one sitting, understand the concepts provided, mark the needed to be memorized info then comeback to it later when in the mood :)

Hope you enjoy!!

Let's start this lecture with a quick revision about **proteins**:

What are proteins??

Proteins are compose of amino acid (AA) sequences starting from the N-terminus and ending with the C-terminus. These termini are usually charged under normal physiological pH. N-terminus being positively charged, while the C-terminus is negatively charged.

Proteins have 4 levels of structure:

1- **Primary structure:** It is the simple sequence of AA. (N-Met-Ala-Val-…-C). This chain is called a polypeptide.
2- **Secondary structure:** It is the arrangement of this simple strand and interaction between the back bones of AA. Proteins have 2 main secondary structures: *α-helices* and *β-sheets*.
3- **Tertiary structure:** It is the interaction between the side chains (R groups) of AA within the same polypeptide chain.
4- **Quaternary structure:** This level is only present in proteins made up from multiple polypeptide chains. It is the interactions between multiple, different polypeptides and their orientation around each other.

End of revision

Heads up! This lecture is about Hemoglobin which is considered a Holoprotein. So, what is a Holoprotein??

Now, the lecture:

Holoproteins: Are proteins that have non-protein parts integrated in them and covalently bound to them. (whole protein)

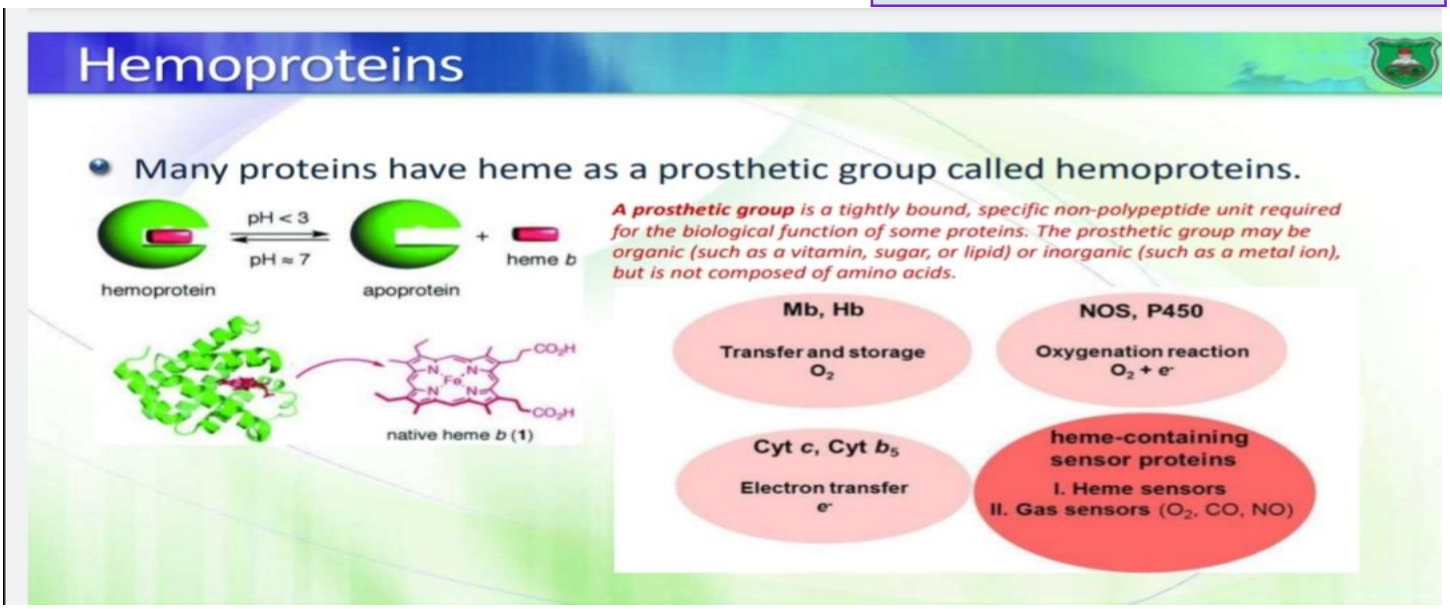
Prosthetic group: The non-protein (non-polypeptide) part which is tightly and covalently bound to the protein and . It may be organic (such as a vitamin, sugar, or a lipid) or inorganic (such as a metal ion).

Apoprotein: Holoprotein stripped away from its prosthetic group. (naked protein)

(((Holoprotein = Apoprotein + prosthetic group)))

Hemoproteins are Holoproteins having heme group (which is an organic non-protein molecule) as a prosthetic group <u>covalently bound</u> to it.

Note If a protein is <u>non-covalently</u> bound to a heme group it is called a heme associated protein.



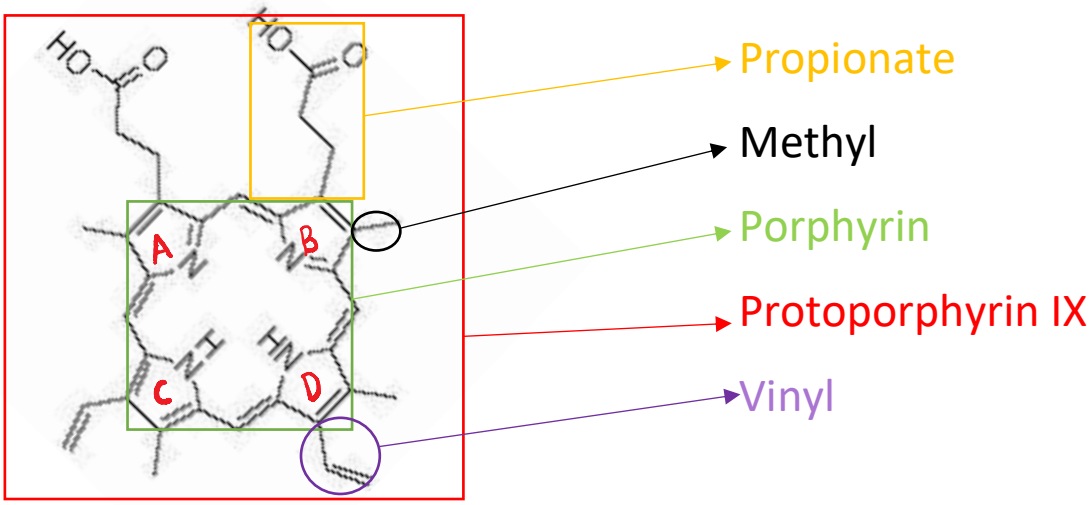
- Hemoproteins vary in type and function. They can be:

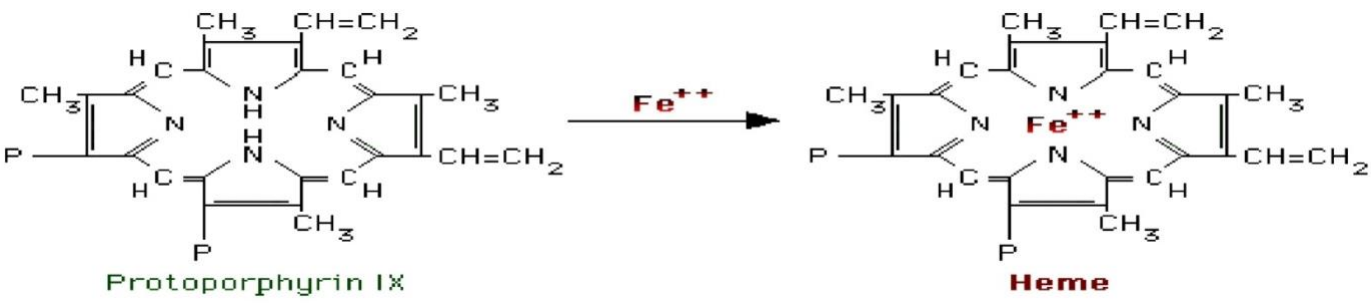
1- Oxygen carriers: For O_2 transfer and storage. (Hemoglobin & Myoglobin)
2- Detoxification of xenomolecules (foreign molecules): Via oxygenation reactions. (NOS, cyt P450)
3- Some are e^- carriers: Present in the e^- transfer chain. (cyt c, cyt b_5)
4- Heme-containing sensor proteins: Heme sensors, Gas sensors.

- Heme structure:

It is composed of **Protoporphyrin IX (9) + iron atom** in the middle.

Protoporphyrin IX is basically a **Porphyrin** molecule with added side chains in a specific order. Some of these side chains are hydrophobic (methyl, vinyl) and some are negatively charged and therefore hydrophilic (propionate groups).





The Porphyrin molecule is planar and consists of 4 rings (designated A-D) called pyrrole rings. Each pyrrole ring can bind two subunits or side chains. Two of the four pyrrole rings have a propionate group and a methyl group each. The other two pyrrole rings have methyl and vinyl groups each.

The iron atom can form 6 covalent bonds (6 coordinates). 4 of which are made with the nitrogen atoms of the pyrrole rings leaving 2 still to be made.

Even though there is two hydrophilic side chains in the structure of heme the overall heme molecule is hydrophobic meaning that it needs to be embedded inside the globin protein interacting with hydrophobic AA making the hemoglobin. The two propionate groups protrude and reach the surface of the globin interacting with hydrophilic AA.

To summarize:

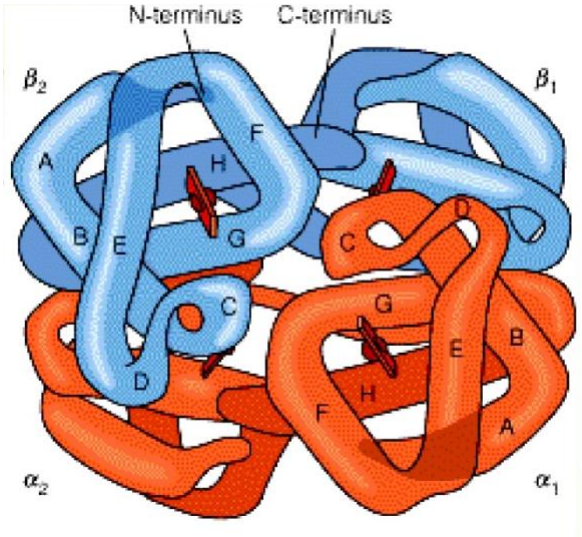
- The heme group is cyclic overall.
- It has four 5-sided rings called pyrrole rings.
- It has braches/sidechains/subunits. (methyl, vinyl, propionate)
- Even though propionate is hydrophilic the overall heme is hydrophobic.
- Fe can make 6 bonds 4 of which are made with the pyrrole rings.

<<The Hemoglobin protein>>

Hemoglobin is a tetramer, meaning that it is formed by 4 polypeptides: 2α chains, 2β chains.

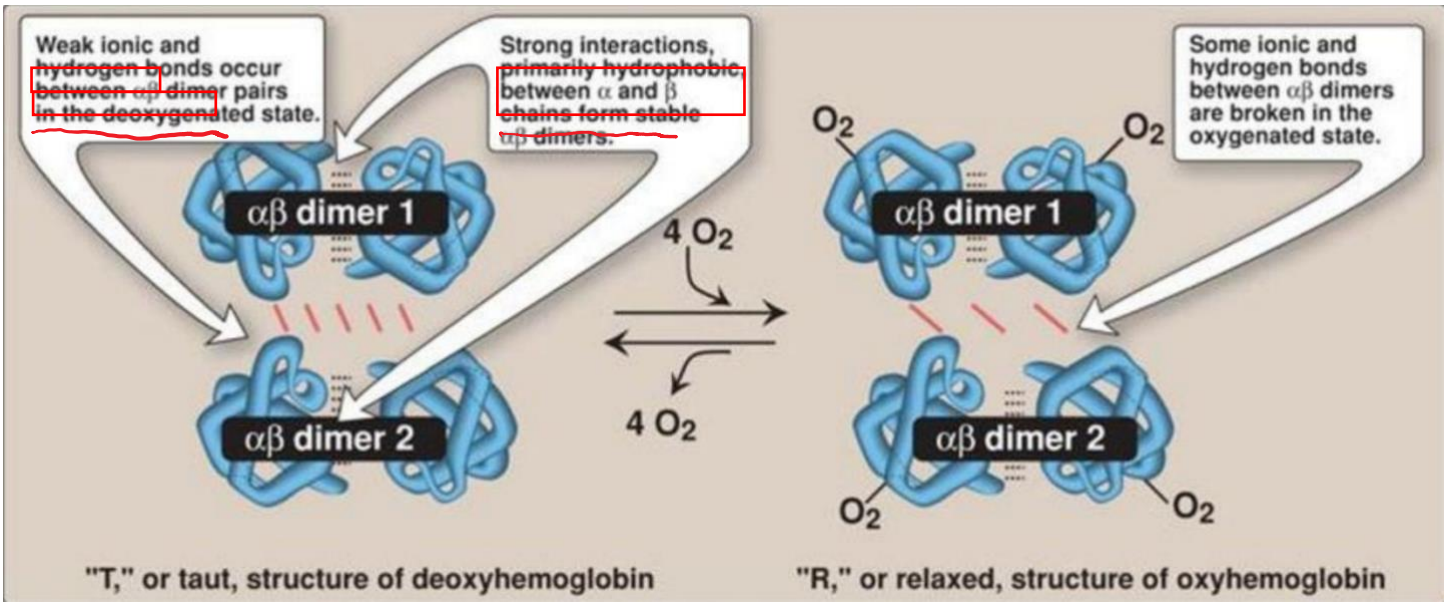
-α chain has 141 AA residues the first one is Valine (Val 1) and the last one being Arginine (Arg 141).

-β chain has 146 AA residues the first one is Valine (Val 1) last one being Histidine (His 146).



Both chain's secondary structure have α-helices, exactly 8 of them (A, B, C, D, E, F, G, H).

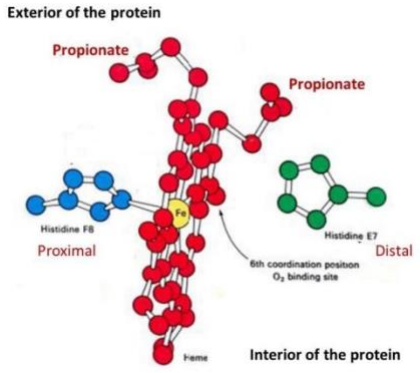
Each α chain binds via strong hydrophobic interactions with a β chains making an αβ dimer. The two dimers interact with each other via weak electrostatic interactions (ionic interactions) and hydrogen bonds.



So, the Hemoglobin is a tetramer or a dimer of dimers (4 polypeptides).

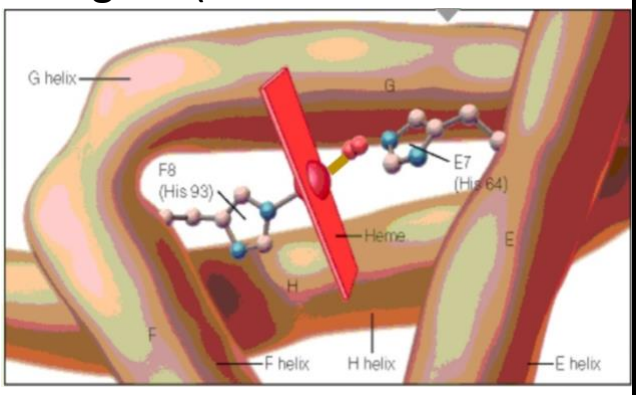
It's also a globular protein meaning that it is shaped like a globe and its AA are distributed in away that makes hydrophilic ones on the surface facing the aqueous environment, while the hydrophobic ones are in the core (inside it) surrounded by hydrophobic AA. EXCEPT for 2 histidine residues (which are positively charged thereby hydrophilic) inside.

One of these Histidines is closer to the heme group and thus called **Proximal Histidine** on position F8 (the 8th AA within helix F). Proximal Histidine binds covalently with the iron atom of heme forming the 5th bond (coordinate).



The other histidine residue is called the **Distal Histidine** on position E7 (7th AA within helix E) it DOESN'T bind with the iron rather it controls

what gets to the heme molecule acting as a gate (the 6th coordinate is guarded by Distal Histidine).



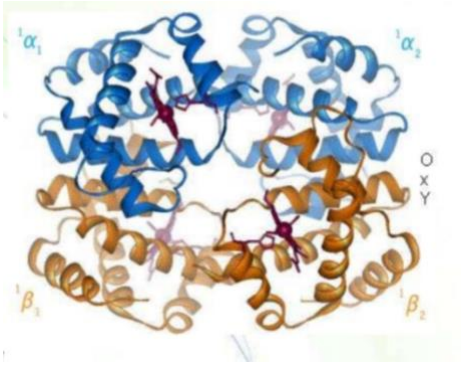
❖ Hemoglobin is an allosteric protein, that means:

1- It has multiple subunits/polypeptides (it has a quaternary structure)
2- It has different/altered structures (configurations) R & T depending on bound molecules (allosteric effectors/modulators)
3- Its saturation curve is sigmoidal in shape
4- Cooperativity

Let's elaborate on each of the previous points!

1- Multiple subunits:

As mentioned earlier, Hemoglobin is a tetramer (dimer of dimers) so how are the subunits bound to each other?



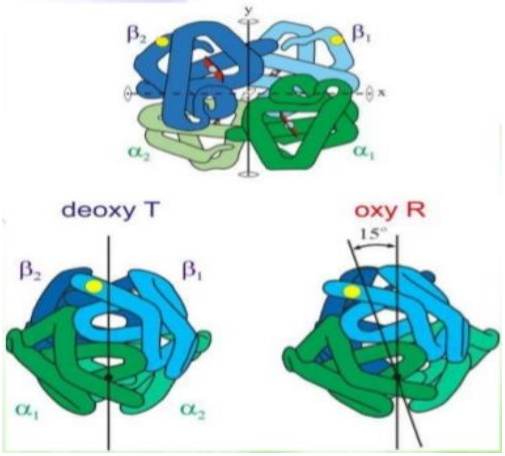
Hemoglobin has 2 α chains and 2 β chains. α1 is bound to β1 via strong hydrophobic interactions forming a stable dimer (αβ1). (αβ2) is formed in the same manner.

Now we have two αβ dimers (αβ1) &(αβ2). The two dimers are bound together via weak electrostatic (ionic) and hydrogen bonds.

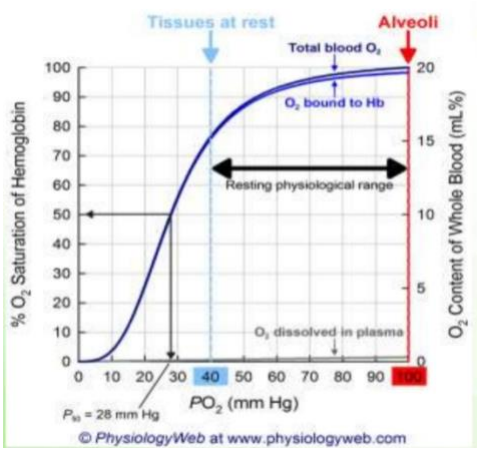
2- Different structures:

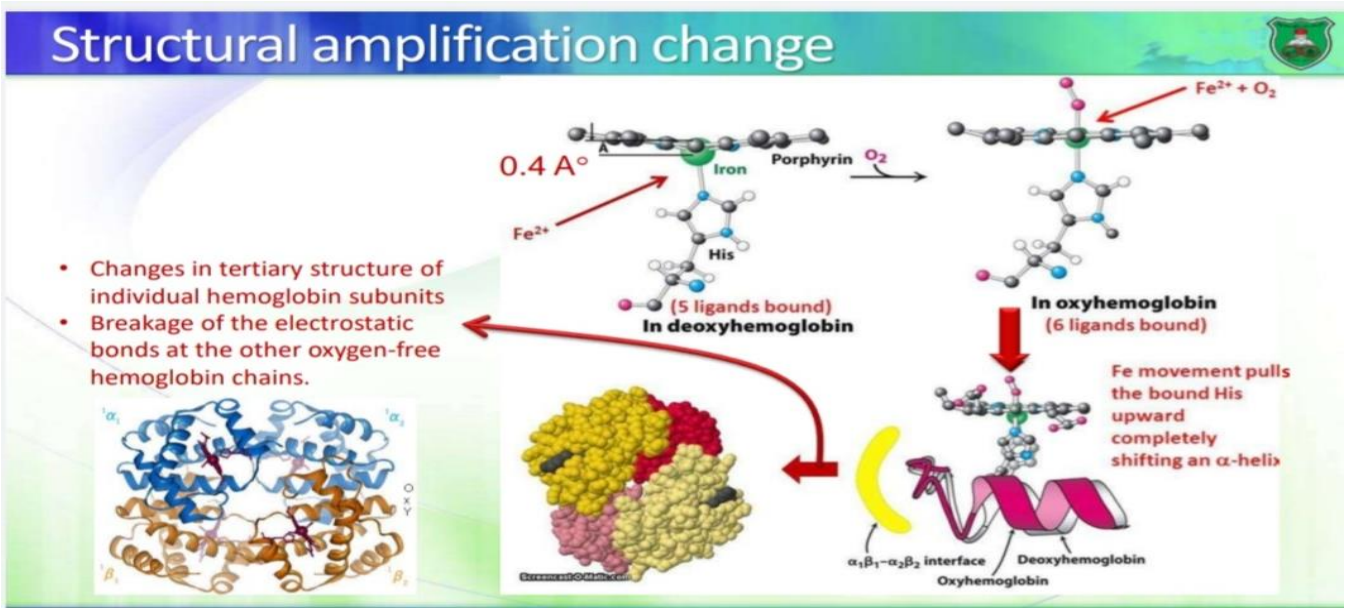
It has two structures: **T** (tout/tensed) & **R** (relaxed).

These structures are different in their 3D orientation (15 degree rotation around its axis) and in their affinity towards oxygen (T has less affinity towards O2. R has more affinity towards O2).



Heme group is domed (bent) due to the repulsion forces caused by the Proximal Histidine (F8). When O2 binds with the iron it makes the heme flat. When the heme gets flattened, it pulls the covalently bound Proximal Histidine (F8) upward pulling the entire F α-helix causing a change in the tertiary structure of the subunit (change in the structure of individual subunits). This tertiary structure change induces a quaternary structure change, because that change causes breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains (increasing their chances of binding oxygen molecules).





To summarize:

We can say that hemoglobin without O2 is stressed (in the T structure) WHY??! Due to the repulsion forces between the Proximal Histidine (F8) and the heme group. When O2 binds it flattens the heme group which in its part pulls the F α-helix upward making the hemoglobin "relaxed" (in the R structure) which has more O2 affinity!

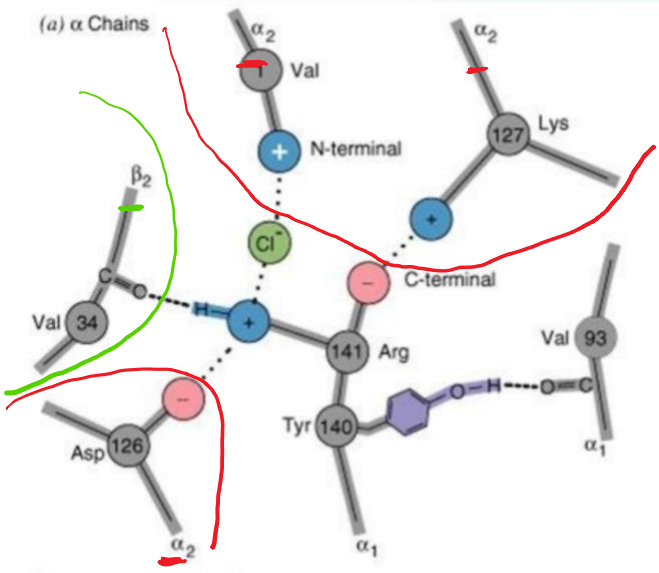
When that T→R change occurs some **weak electrostatic and hydrogen bonds** that stabilize the T-form will end up breaking down upon movement of the α-helix (F α-helix).

-:The broken bonds:-

A- The broken electrostatic interactions:

i. α interactions

The last residue of α1 chain (Arg 141) has a negatively charged C-terminus that interacts



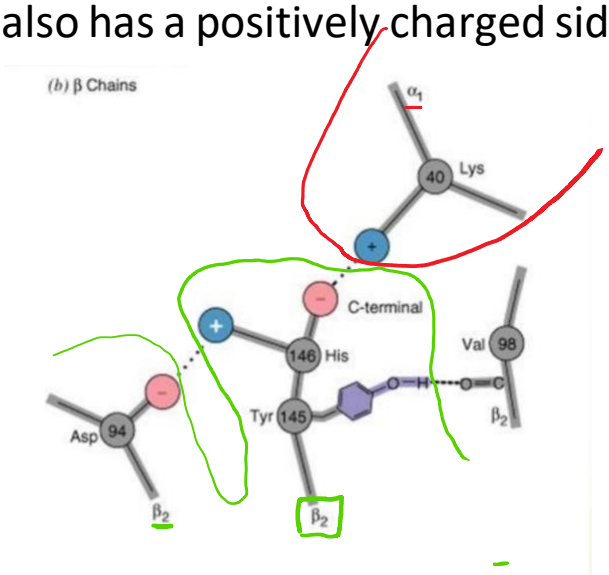
Note C-terminus under physiological pH it will acquire a negative charge and the N-terminus will have a positive charge (protonated).

Note The way α1 interacts with α2 & β2, is the same way α2 interacts with α1&β1.

with a lysine residue within α2 chain. (Arg 141) also has a positively charged side chain that forms a hydrogen bond with β2, it also interacts with α2 at two sites: (Val 1) through a chloride atom, (Asp 126) which has a negatively charged sidechain.

ii. β interactions

The last residue of β2 chain (His 146) has a negatively charged C-terminus that interacts with a Lysine residue within α1 chain. (His 146) also has a positively charged side chain that interacts with the negatively

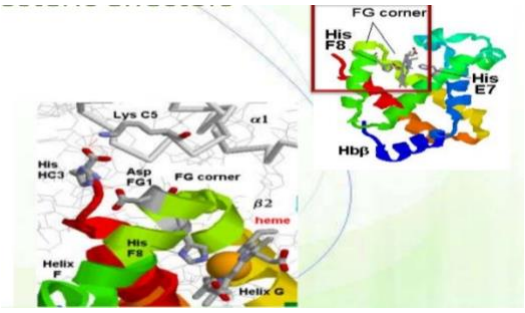


Note The way β2 interacts with α1 & β2, is the same way β1 interacts with α2 & β1.

charged sidechain of an aspartate (Asp 94) residue on β2 chain (more on it next lecture…)

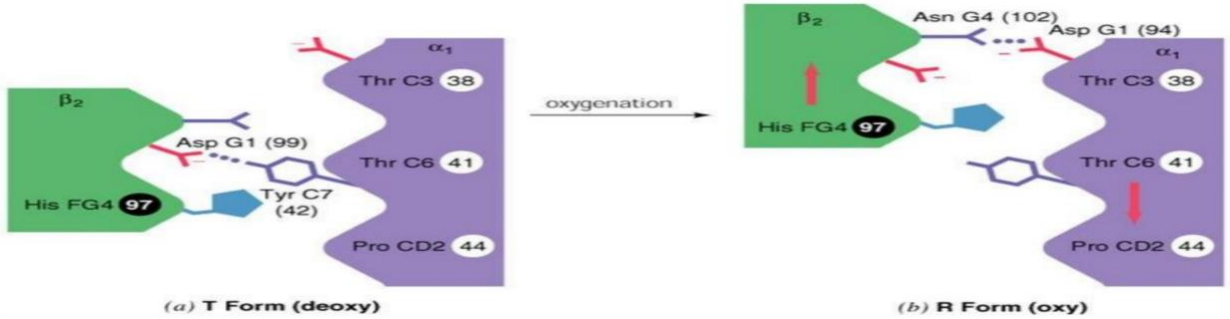
All the previously mentioned bonds break when T→R. Other electrostatic bonds will form.

B- Breakage & reformation of hydrogen bonds:



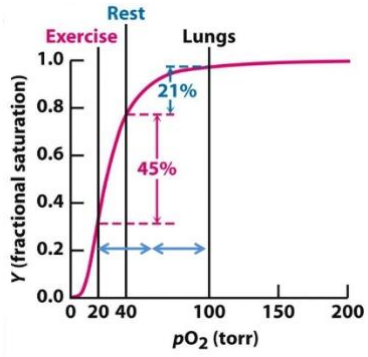
T-state hemoglobin (deoxyhemoglobin) is stabilized by a hydrogen bond between Asp G1 (99) of β2 chain with Tyr C7 (42) of α1 chain.

When O2 binds, the α1 surface slides and a hydrogen bond is formed between Asp G1 (94) of α1 chain with Asn G4 (102) of β2 chain stabilizing the R form of hemoglobin.



3- Its saturation curve is sigmoidal in shape:

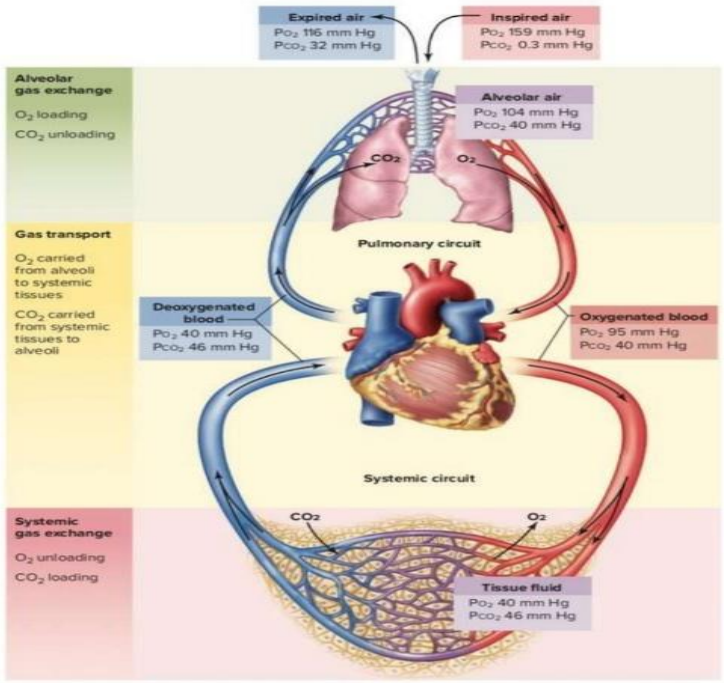
- Sigmoidal because → it is allosteric because → it has two structures with different O2 affinity (T & R).
- Sigmoidal in shape means S-shaped curve.
- At 100 mm Hg (torr), which is the same pO2 in blood leaving the lungs, Hb is saturated (95%-98% is saturated).

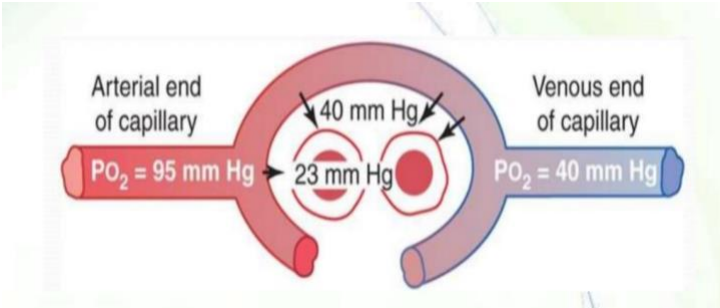


- When the O2 pressure falls in tissues, O2 is released. For example in exercise pO2 (in tissue) drops and there will be more deoxyhemoglobin (T-state) to supply the muscles.

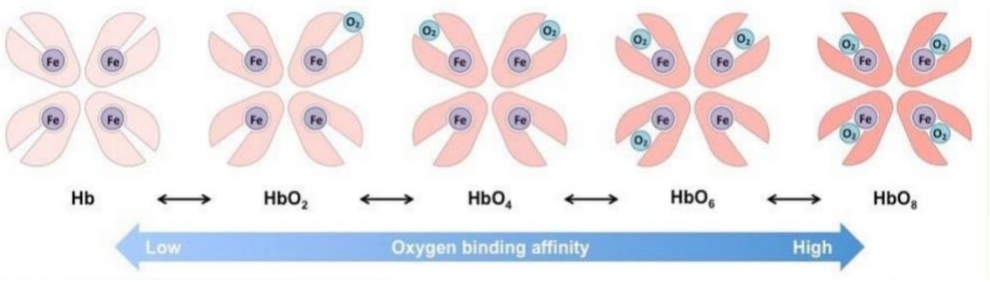
- *Note: at high altitude (~5000 m), alveolar pO2 = 75 mmHg.*

- Blood leaving lungs pO2 is 100 mm Hg (torr) in this pressure Hemoglobin is saturated (mostly bound to O2) **How does hemoglobin release O2 to tissues??** In the presence of high concentration of CO2 hemoglobin changes it structure from R (high O2 affinity) to T (low O2 affinity) and thus releasing the carried O2 and binding to the abundant CO2 to exhale it.



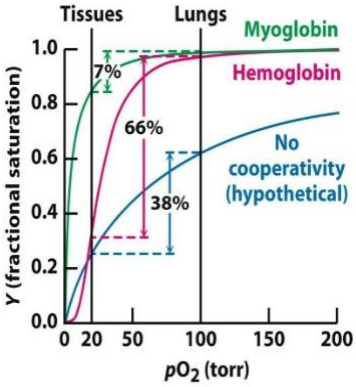


4- **Cooperativity**:



The ligand / homotropic effector / modulator for hemoglobin is O2. O2 binding increases the chances of more O2 to bind (drives the formation of R state from the T state) this is called positive cooperativity.

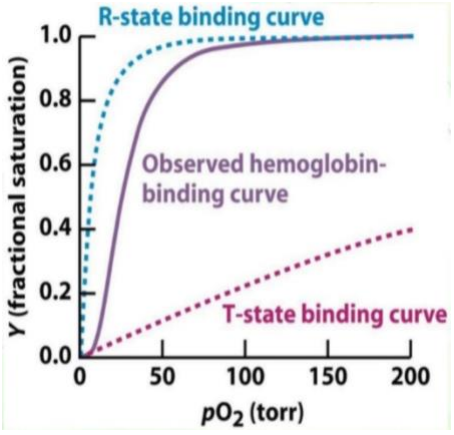
Other molecules (that are not the ligand) can bind at the binding site causing an altered equilibrium in either direction (favors one of the two structures). These molecules are called heterotropic effectors.



If cooperativity didn't exist, 100 mm Hg (torr) won't be sufficient for Hb saturation.

Note the curve of myoglobin. It is hyperbolic thus it shows no cooperativity and therefore is not an allosteric protein.

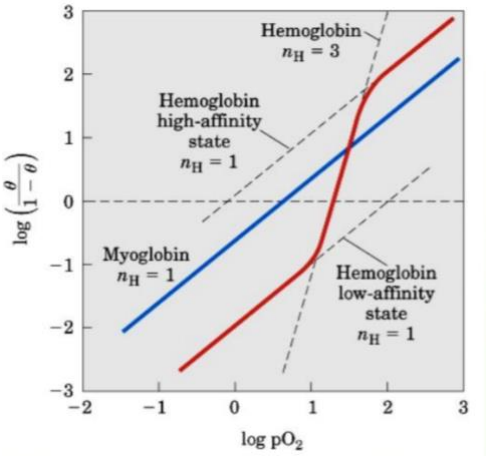
The Hill constant / coefficient (n):



We use the slope (n) of the Hill equation to show the extent or the degree of cooperativity.

The hill equation reflects the binding ligands to macro molecules.

In this graph, y-axis is the log of saturation over the x-axis log of O2 pressure (which refers to the amount of O2). Myoglobin shows no sign of cooperativity. It has a slope (n) of 1. Hemoglobin (which has two structures and demonstrates cooperativity) has a slope of more than 1 at the mid point of log(y/1-y) vs log pO2. The slope of the hemoglobin curve when log pO2 is less than one is equal to one and that tells us that there is no cooperativty here when hemoglobin is in the T structure. The slope of the hemoglobin curve when log pO2 is greater than 2 is also equal to one and that tells us that there is no cooperativty here when hemoglobin is in the R structure either. However, in the mid point when the switch from T→R happens the slope is greater than one and thus there is cooperativty.



The slope of hemoglobin → (2 - (-1) / 2 - 1) = 3

- <u>n can have three values:</u>

1. If n=1 there is no cooperativity as myoglobin shows.
2. If n>1 it shows that the enzyme or protein has positive cooperativity
3. If n<1 it shows that the enzyme or protein has negative cooperativity.

If we had two proteins: X and Y. X has n of 2 while Y has n value of 4. That means that protein Y exhibits cooperativity more evidently or more promptly than X.

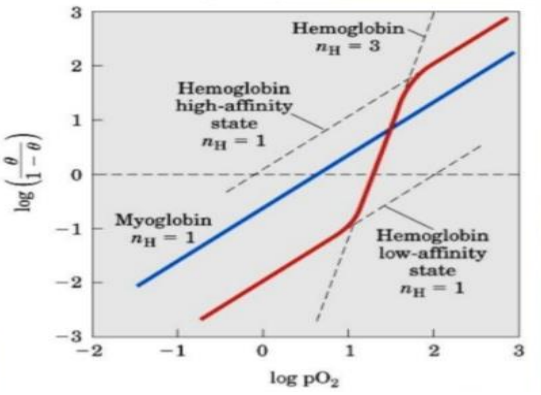
The Hill constant (coefficient)

- 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 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
- *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)



Models explaining cooperativity

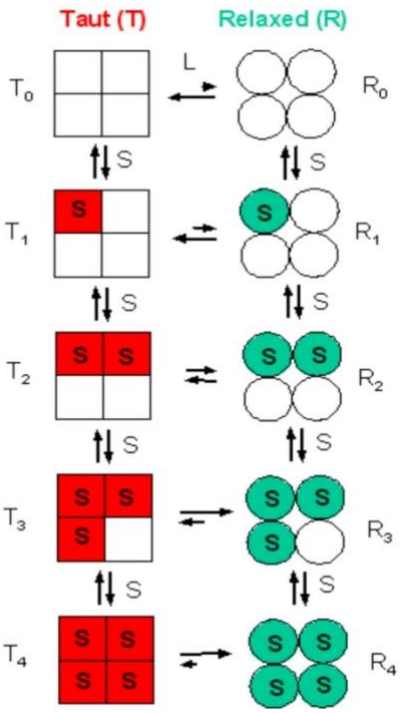
1-The concerted model (MWC model): (most accurate for Hb)

Suggests that there is only two states a protein (such as hemoglobin) can exist in → T structure and R structure. Both exist at the same time in equilibrium with a shift towards one structure depending on O2 occupancy (for hemoglobin).

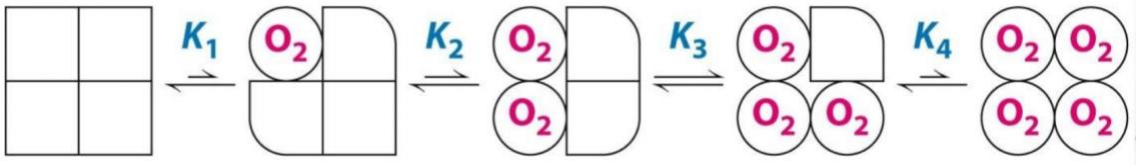
When hemoglobin is bound to no O2 molecules, most of hemoglobin exists in the T structure (there is some small amount in R structure). When one O2 binds, still the majority of Hb is in T state however slightly more Hb will be in R state than when there was no O2 bound. When two O2 molecules bind, to Hb the amount of Hb in R and T structure will be around the same. In case of three O2 bound, the equilibrium will be shifted towards the R state. In case 4 O2 bound to Hb (saturation), most of the Hb will be in the R structure (still there will be some in the T state).

Further explanation:

Let's say that T or R structures are structures of the subunits. The concerted model suggests that all the subunits may have T or R structure at a given time. So, all subunits may adapt the high affinity state (R) without being bound to O2. More O2 bound, increases the chance of Hb (all subunits) to exist in the relaxed high affinity sate.



2-The sequential model, Induced fit (KNF): (better for negative cooperativity)



Suggests that the subunits go through conformational changes independently of each other (makes it possible for subunits to coexist in different structures at a given time, for example two in the R and two in the T at the same time, that wasn't possible in the concerted model). When a subunit changes in structure, that doesn't mean that the other subunits have to change as well, but the changed ones make other subunits more likely to change by reducing the energy needed for subsequent units to undergo the same conformational change. (For example: if Hb had 3 subunits in the R structure that would increase the chance of the fourth sub unit to change as well from T to R) (3 in T, 1 in R that would

decrease the energy needed for the R subunit to undergo change becoming a T itself)

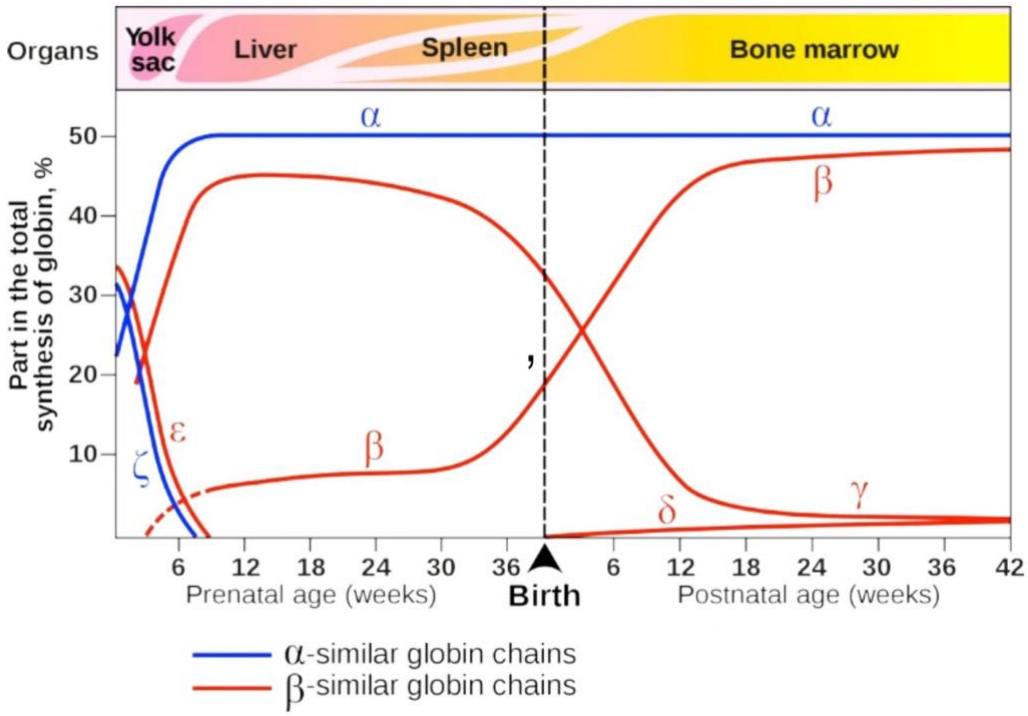
which model is better?? Both can explain the sigmoidal binding curve, <u>but in general:</u>

**The concerted model explains positive cooperativity better and oxygen-Hb binding.

**The sequential model works well for negative cooperativity.

So far we've been only concerned with Hb having 2α chains and 2β chains in what's known as the Adult Hemoglobin 1 (HbA1). It's not the only hemoglobin though…

Developmental transition of hemoglobins:



First 4-6 weeks of gestation the yolk sac produces embryonic globulin chains (ζ & ε),giving the rise of embryonic Hb like HbE gower 1 (2ζ 2ε).

After week 6 the liver, alongside the spleen to some extent, start to produce other types of chains (α, γ, β) mainly α & γ, forming other Hb like HbF (2α 2γ). Near birth (γ) production starts to decrease while (β) production starts to increase. At birth fetal Hb will be 60% of whole Hb. After week 6 of birth HbA1 (2α 2β) will be the predominant Hb present and hemoglobin production gets carried out by bone marrow throughout life. Another chain is also being produced called the δ chain forming HbA2.

❖Embryonic stage:

Embryonic hemoglobin (HbE) production in this stage is carried out by **the yolk sac**.

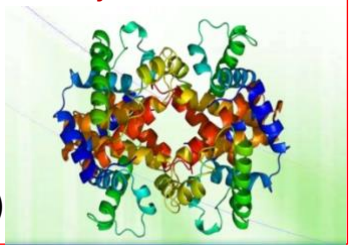
-Types of Hb at this stage:

1- HbE gower 1 (ζ2 ε2) the major one

2- HbE gower 2 (α2 ε2)

3- HbE portland 1 (ζ2 γ2)

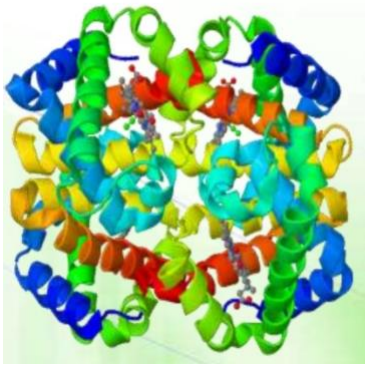
4- HbE portland 2 (ζ2 β2)



❖Fetal stage:

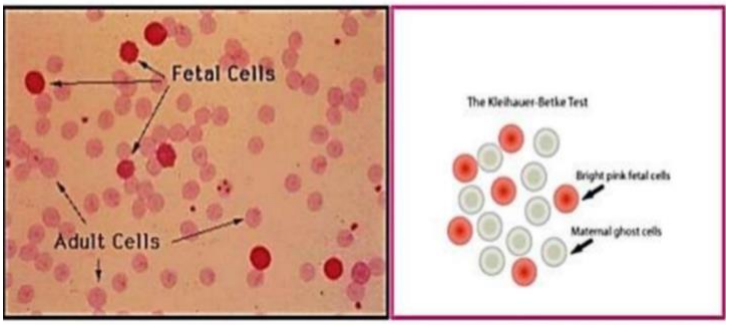
By 6-8 weeks of gestation embryonic hemoglobin expression declines dramatically and fetal hemoglobin (HbF) synthesis is initiated by **the liver**.

HbF (α2 γ2)



❖Adult stage:

- Shortly before birth gradual switch from fetal γ chain to adult β chain occurs.
- At birth still the fetal Hb will be the predominant one 60% and only 1% present in adults.

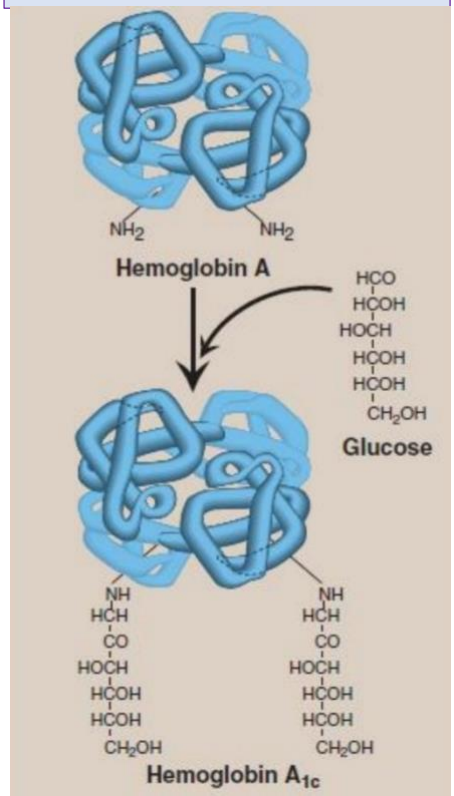


- After birth the production of α, β, γ and δ chains will be carried out by **the bone marrow** to the rest of the life.
- The α chain remains throughout life. Produced by the liver and spleen in fetus and by the bone marrow after birth.
- MNEMONIC: Alpha Always, Beta Becomes, Gamma Goes

Adult hemoglobin (HbA)

There are two: HbA1 (α2 β2)major one, HbA2 (α2 δ2)

The promoter of delta chain is not that active so it is found in small



Hemoglobin Glycosylation:

- HbA can be glycosylated with a hexose (six carbon sugar) designated as HbAc.
- Major form HbA1c has glucose molecules attached to a valine residues of β chains.
- HbA1c are present in higher levels in patients with diabetes mellitus.
- More glucose in the blood → More glycosylated HbA

Blood sugar can be tested in two ways:

Blood fasting glucose:

Tests for the amount of glucose in the blood at time of testing.

#uses:

To see how the body is handling sugar at an instant.

HbA1c levels:

provides a longer-term (glycosylation takes time) trend of blood glucose content over periods of 2-3 months. It can be presented in two units:

a- DCCT unit (percentage) used in US + Jordan (less accurate)
b- IFCC unit (mmol/mol) new used in Europe (more accurate)

>> Limitations of HbA1c testing:

- Doesn't capture short-term variations in blood glucose.
- Doesn't capture Exposure to hypo or hyperglycemia.
- Doesn't capture the impact of blood glucose variations on individuals' quality of life (consistency of diet).

>> Uses HbA1c Levels test:

- To see if the patient is taking drugs correctly.
- If the medication is working or not.
- To monitor the patients dietary habits over a period of time.

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

It's for memorization :(But you can do it :)

That's the sheet done!

Good luck!!!