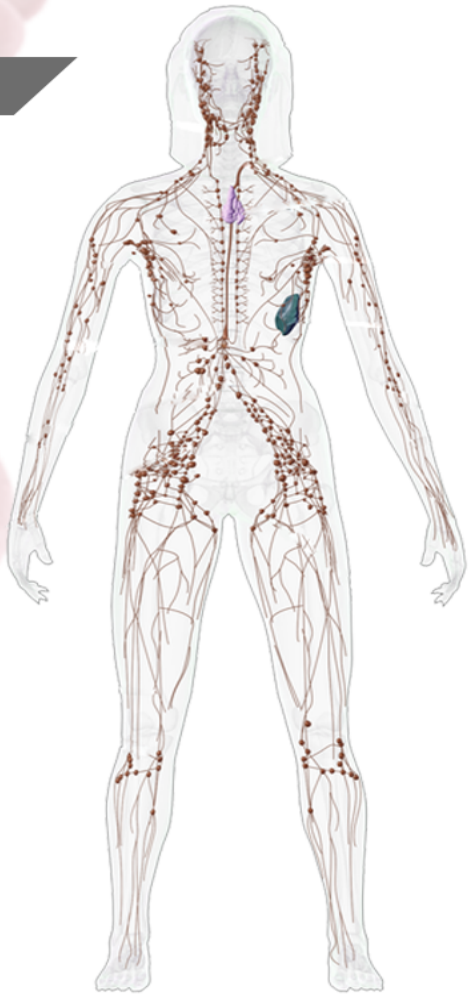
Sheet 1

Hematology and Lymphatic system



Subject | Biochemistry

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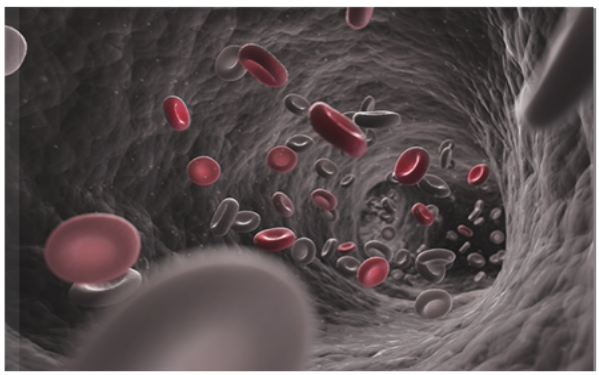
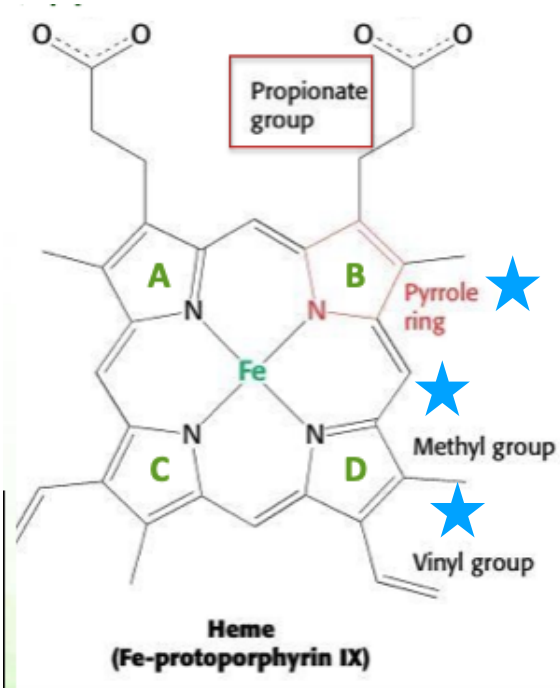


Structure of heme

It is a complex of protoporphyrin IX + Iron (Fe +2). Not Fe+3
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 whole molecule is hydrophobic except for the 2 propionate groups which are hydrophilic (charged) which has an important implication on how heme interacts with hemoglobin.

The iron has six coordinates of binding.(four of which are with the pyrrole, the other two are with the oxygen and the proximal histidine of the hemoglobin).

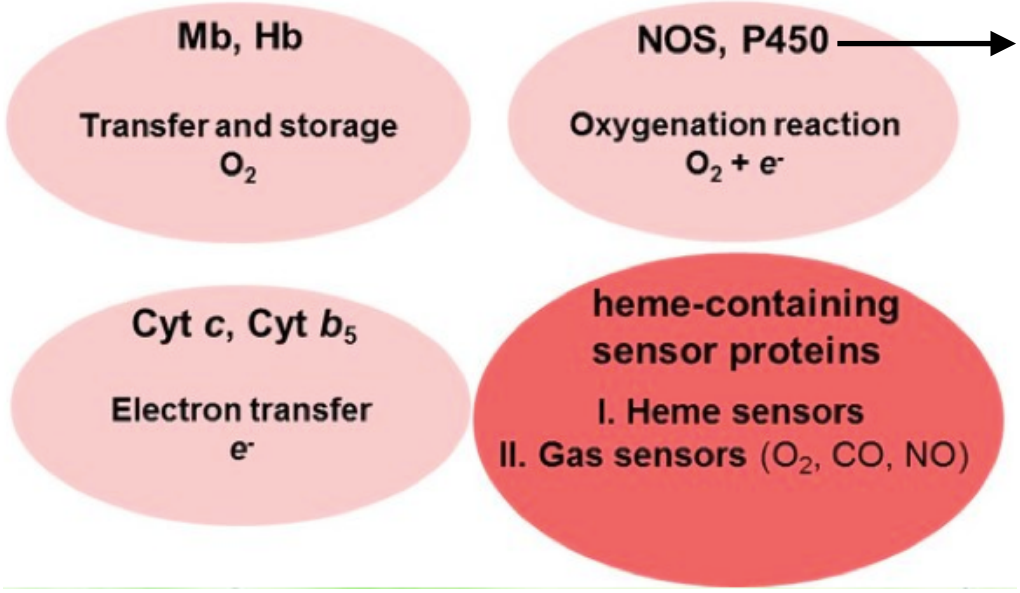


Heme
(Fe-protoporphyrin IX)

★ Check out the 3 groups attached to the heme molecule.

***** Prosthetic group : a large non protein group that interacts covalently with a protein**
So heme is a prosthetic group

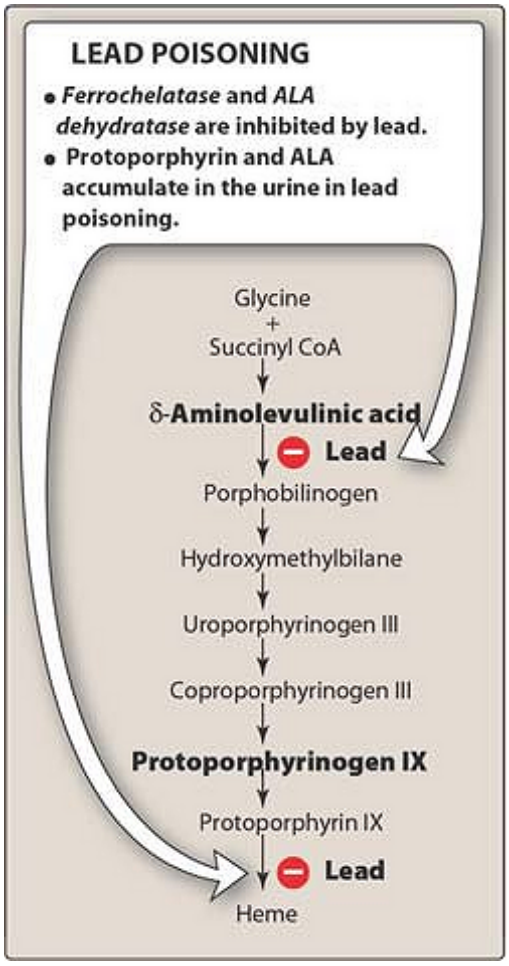
Examples of heme proteins:



In liver/ important in metabolism

Synthesis of of heme

- Liver and erythroid tissues are main sites of synthesis.
- **The first reaction is the rate limiting and committed step.**
- It requires **vitamin B6** (pyrodoxal phosphate). It is regulated by **hemin**.
- The last reaction is **spontaneous**, but can be catalyzed by **ferrochelatase.**



Explanation of some of the steps:
The heme production starts with the amino acid **glycine and succinylCoA,** and these two components are used by an enzyme known as **ALA synthase** or aminolevulinic acid synthase this process happens in the mitochondria to produce aminolevulinic acid - **rate limiting step**
Now in the hepatic system, **hemin** which is a byproduct of heme, **inhibits ALA synthase**. While reduced **hepatic heme up-regulates ALA synthase**, you can have reduced hepatic heme if you over utilize the the cytochrome p450 system. how? by say for instance ingesting too much of some drug. this requires more heme production.(in the previous page we said that heme is a prosthetic group of p450)
Heme and erethropoetin are the activators of this enzyme
The next step involves **ALA dehydrase** and occurs in the cytosol, the product is **porphobilinogen**. This enzyme requires **zinc** *and because it* requires a metal, it can be inhibited by **lead**, this is why we always hear the lead can cause anemia.
In the last step of the reaction, The **iron** binds to **protoporphyrin** by the enzyme **ferrochelatase** and this occurs in the mitochondria, also because we are using a metal in this reaction it could be inhibited by **lead** (so the second and last step both are inhibited by heme)

You can memorize the steps of the pathway using **APHUCPP**

Disorders related to heme biosynthesis

We are required to know the names, genetic pattern, enzyme, clinical manifestations (photosensitivity)

* any step before hydroxymethylbilane —> NOT photosensitive
* any step after hydroxymethylbilane —> photosensitive
* Main affected organs are: CNS, digestive system, liver and connective tissue.

Name	Genetic pattern	enzyme dificient	Clinical manifestations	Photosensitivity
Acute intermittent porphyria	**Autosomal dominant**	**Hydroxymethylbilane**	**Porphobilinogen and amino levulinic acid accumulate in urine which darkens in light and air**	**NO**
Congenital erythropoetic porphyria	**autosomal recessive**	**Urophorphyrinogen 3 synthase**	**Uroporphyrinogen 1 and coporphyrinogen accumulate in urine**	**YES**
Porphyria Cutanea tarda	**Autosomal dominant**	**Urophorphyrinogen decarboxylase**	**MOST COMMON porphyria (80%) Uroporphyrinogen accumulates in urine**	**YES**
Hereditary coproporhyria	**Autosomal dominant**	**Coproporphyrinogen oxidase**	**Coproporphyrinogen 3 and other intermediates accumulate in urine**	**YES**
Variegate porphyria	**Autosomal dominant**	**Protoporphyrinogen oxidase**	**Protoporphyrinogen IX and other intermediates accumulate in urine**	**YES**
Erythropoietic protoporphyria	**X-linked**	**Ferrochelatase**	**Protoporhyrin Accumulates in erythrocytes, bone marrow and plasma**	**YES**
lead poisoning	**NOT GENETIC**	**Ferrochelatase and ALA dehdrase are inhibited by lead**	**Coproporphyrin III and ALA accumulate in urine**	

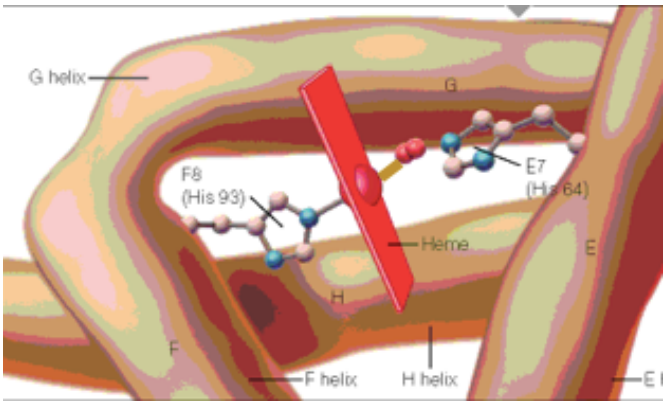
Treatment of **porphyria cutanea trada** :

- Intravenous injection of hemin and glucose: hemin is injected because it is the inhibitor of the very first reaction.
- Protection from sunlight: because patients are photosensitive.
- Ingestion of beta-carotene: because its an antioxidant and these intermediates lead to the production of free radicals

Hemoglobin

- Hb is a ***globular protein*** (characterized by having the polar amino acids on the surface and the hydrophobic non-polar amino acids in the core)
  *** exception: in the core of hemoglobin we have 2 positively charged histidines. Also, we have some non polar amino acids on the surface as we'll see later on this lecture.

- Notice the positions of the two ***histidine residues*** (proximal and distal)



- The histidines are important in:
  1. binding to heme
  2. Stabilizing the binding of O2 to heme

—> the proximal histidine binds covalently to iron, if the oxygen binds to the iron on the 6th coordinate, the distal histidine stabilizes the reaction.

—> In your opinion, whats the result of a mutation affecting the previously mentioned histidines ?
- it may affect the binding affinity of hemoglobin to oxygen.

Hemoglobin is an ***allosteric protein***

Additional revision: An allosteric protein is a protein with multiple ligand-binding sites such that ligand binding at one site affects ligand binding at another.

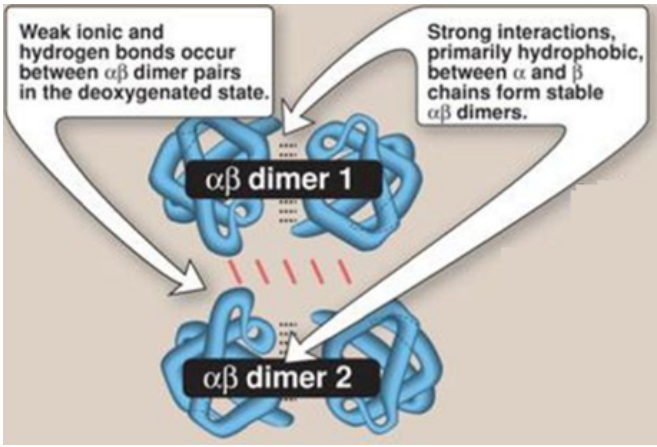
1. Multiple subunit (2a + 2β) :The predominant hemoglobin is made of 2 chains; in adults they are **α** and **β.** Each one of the chains has a heme bound to it, while each heme is bound to an oxygen molecule. So each hemoglobin has 4 oxygen molecules bound to it .

2. Altered structure depending on bound molecules:
1. The R state (relaxed state)(high affinity to oxygen)
2. The T state (tense state) (low affinity to oxygen)

3. Positive cooperativity towards oxygen:
I just talked about the allosteric behaviour of hemoglobin; the second point mentions how oxygen binding to one site effects oxygen binding to other sites ; So scientists found that the binding of oxygen to the first site is the hardest , but as soon as it binds , it makes the binding of oxygen to the

second site easier , and when oxygen is bound to the 1st and 2nd sites , it makes the 3rd site more and more easy and finally when the 1st 2nd and 3rd sites are occupied by oxygen , the binding to the fourth site is the easiest.

4. Regulated by allosteric effectors
Either negative or positive effect.



How are the subunits bound?

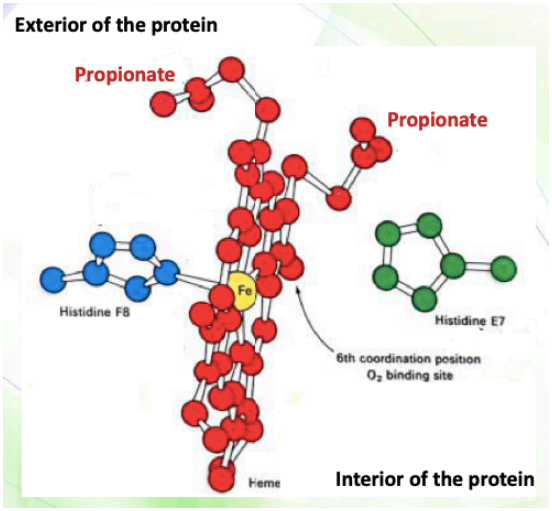
Its a tetramer - A dimer of a dimer $(a\text{-}\beta)_2$

*The interaction between the 2 polypeptide chains -> **hydrophobic interactions**
*The interaction between the two dimers -> **electrostatic interactions/hydrogen bonds.**

The electrostatic interactions are very important in changing the structure of hemoglobin from T state to R state and vice versa.

Now you must be wondering , shouldn't the hydrophobic(non polar) amino acids be hidden inside the subunit and the hydrophilic amino acids are the ones protruding outwards and interacting with the surroundings ? That's Definitely true , most of the amino acids on the surfaces of the subunits are hydrophilic , but we have some Hydrophobic regions which are responsible of this strong Alpha-Beta attraction.

Heme binding to hemoglobin



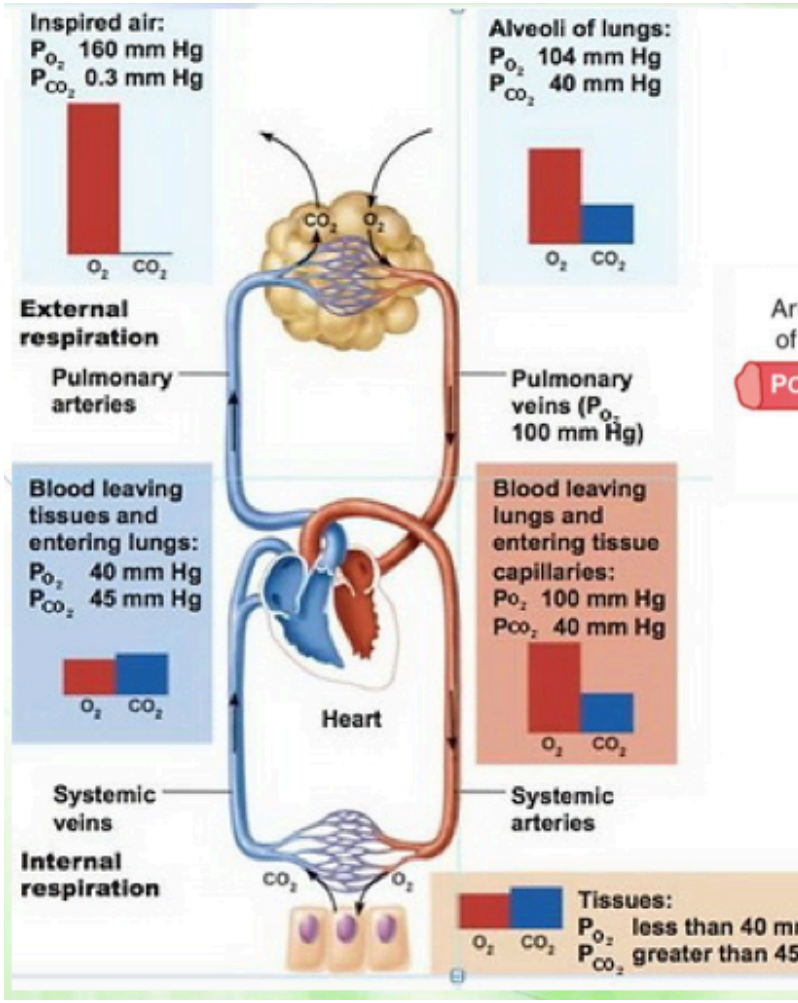
Remember how we said that heme is manly hydrophobic except for the 2 propionate groups ? Well this is how heme fits into the hemoglobin molecule.
The hydrophobic part of Heme is embedded inside the hemoglobin molecule. When you have this interaction with the proximal histidine and its also near by the distal histidine.
The propionate structure is on top they are oriented outwards interacting with the polar amino acids on the surface of the protein.

The interaction of heme molecule is stabilized by **2 forces**, the **hydrophobic** interactions *inside* as well as the **hydrophilic electrostatic** interactions with the propionate structures.
This has really important implications when its comes to hemoglobinopathies that is changing the hydrophobic environment that surrounds heme can affect the interaction between heme and the protein and that can also affect oxygen affinity toward hemoglobin.

Oxygen distribution in blood versus tissues

Here, just notice the difference between the pressure of oxygen between different states

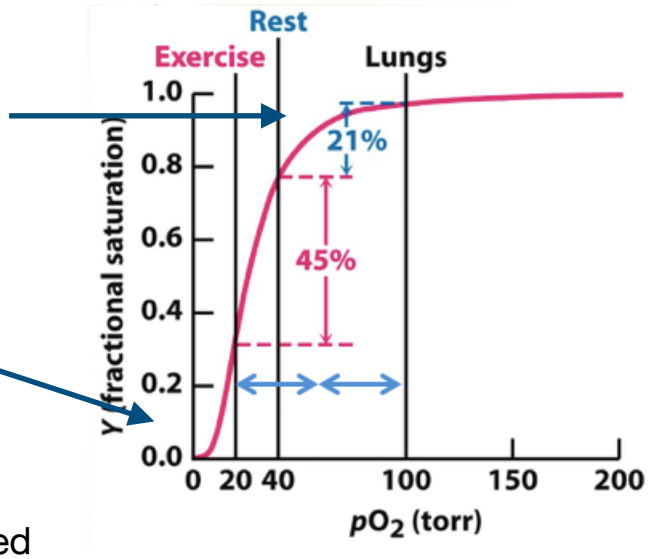
What oxygen does to hemoglobin is that its gives it this red color. when hemoglobin isnt bound to oxygen it has a blue color.



The saturation curve of hemoglobin binding to O2 has a sigmoidal shape. Not hyperbolic like myoglobin's. This curve indicates that this protein has 2 structures (allosteric)

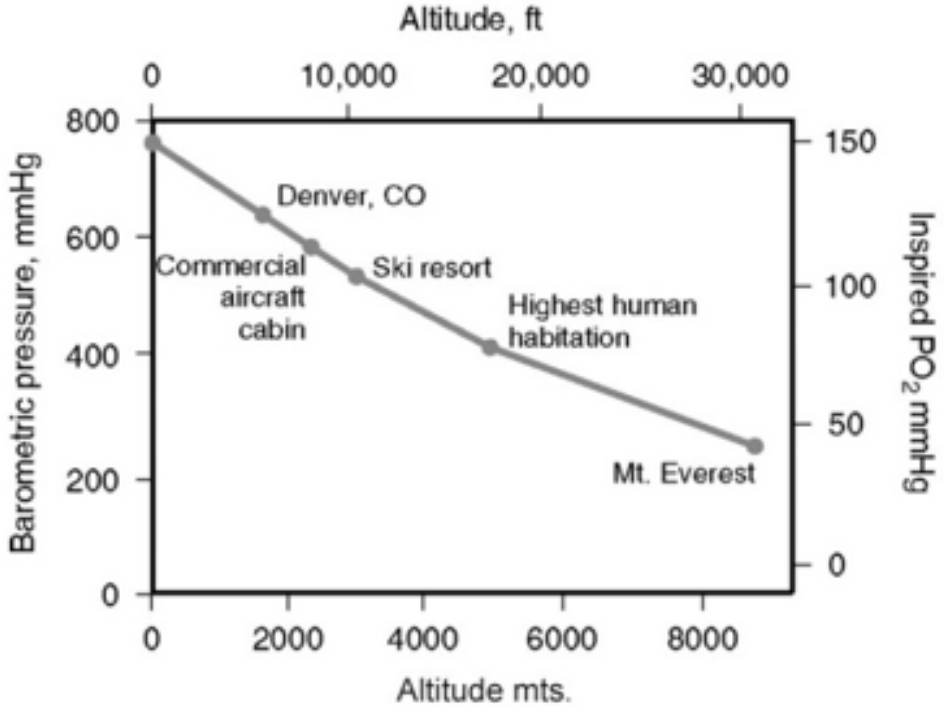
High affinity state (R state) (so it would be able to bind oxygen in the lungs)

Low affinity state (T state)
So it would be able to release oxygen when reaching tissues



*** notice the drop in saturation between the resting and the exercising state.
At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin).
As the oxygen pressure falls, oxygen is released to the cells.
Note: at high altitude (~5000 m) . alveolar pO2 = 75 mmHg.

Altitude (feet)	Atmospheric Pressure (mm/Hg)	PAO_2 (mm/Hg)	PVO_2 (mm/Hg)	Pressure Differential (mm/Hg)	Blood Saturation (%)
Sea Level	760	100	40	60	98
10,000	523	60	31	29	87
18,000	380	38	26	12	72
22,000	321	30	22	8	60
25,000	282	7	4	3	9
35,000	179	0	0	0	0

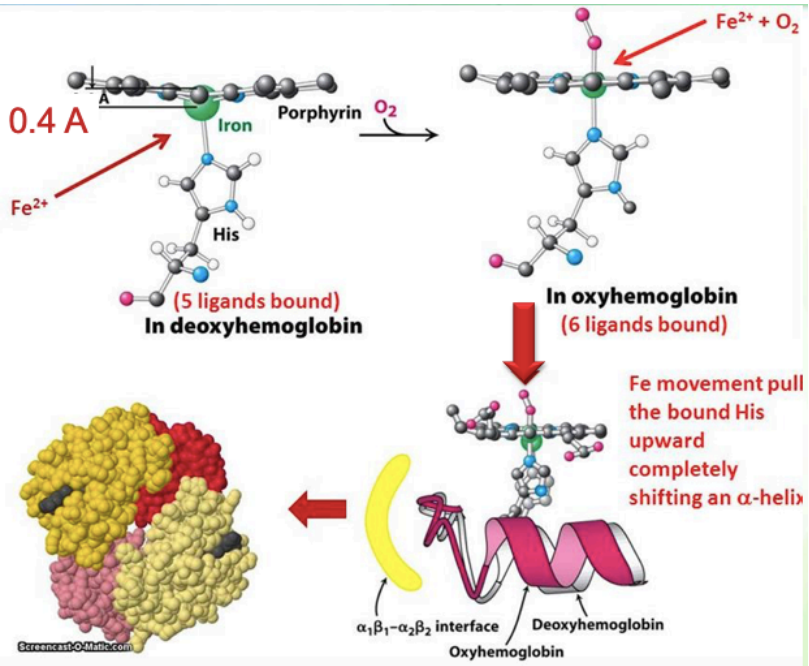


pO2 at different altitudes

The numbers here are NOT for memorization
And these examples are for understanding purposes :

When the atmospheric pressure is 760 (mm/hg)
The arterial and venous pressure of oxygen are 100 and 40 respectively so the level of saturation is almost 100 (the amount of oxygen released is 60 torr)

So what you should really focus on here is how blood saturation decreases with high altitude/ low atmospheric pressure —> hard time breathing.



Structural amplification change
How does the transition from T to R / R to T take place ?

Its all about little structural changes !

- When the iron of the heme binds to the proximal histidine on hemoglobin , repulsion forces occur between the hydrophobic heme and the charged distal histidine due to both being positively charged leading to heme becoming dome shaped with iron siting out of heme (iron is not in the middle).
- When oxygen is bound repulsion is reduced due to the interaction of the distal histidine with oxygen, So its goes back to being flat and oxygen sits in the middle of the molecule again.
- The change in the structure above is very mynute, only a one-nanometer change but with great effects. When heme becomes flat the proximal histidine is pulled upwards towards the heme molecule leading to changing of the interactions between that histidine and the amino acids in the alpha helix (change of the secondary structure) —> change in tertiary structure —> changes in the quaternary structure (overall structure).

Its mainly breaks in the electrostatic interactions that take place in the center of the hemoglobin , so the molecule becomes relaxed i.e. the R state

So over all this movement triggers:
1.changes in tertiary structure of individual hemoglobin subunits
2. 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.

Cooperativity

Go back to the 3rd point on page 4 and recall what I just said about positive cooperativity

This behaviour can be noticed through the dramatic change in Hemoglobin's affinity, so by comparing the affinity of empty hemoglobin (deoxyhemoglobin) to bind the first oxygen to the affinity of a nearly full hemoglobin bound to 3 oxygen molecules to bind the last oxygen , the nearly full hemoglobin is having 300% more affinity than the empty one!

This Property is called ***Cooperative binding of oxygen to hemoglobin*** , *and it's the reason behind the* ***sigmoidal*** *shape of the curve* (1st oxygen needs high increase in PO2 thus not steep curve at the beginning , but as soon as the Oxygen starts to bind , affinity to oxygen is increasing thus steeper curve, thus the Sigmoidal S shape)

but what exactly happens to hemoglobin when it binds oxygen ??

The concerted model (MWC model)

Most accurate

The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.

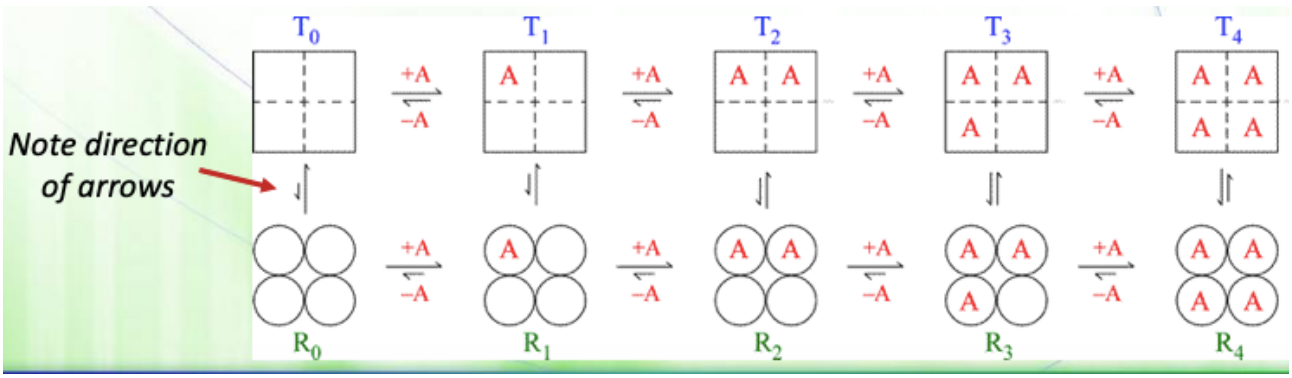
Increasing ligand concentration drives the equilibrium between R and T toward the R state (**positive cooperativity)**

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.**

The important things to focus on in this model:

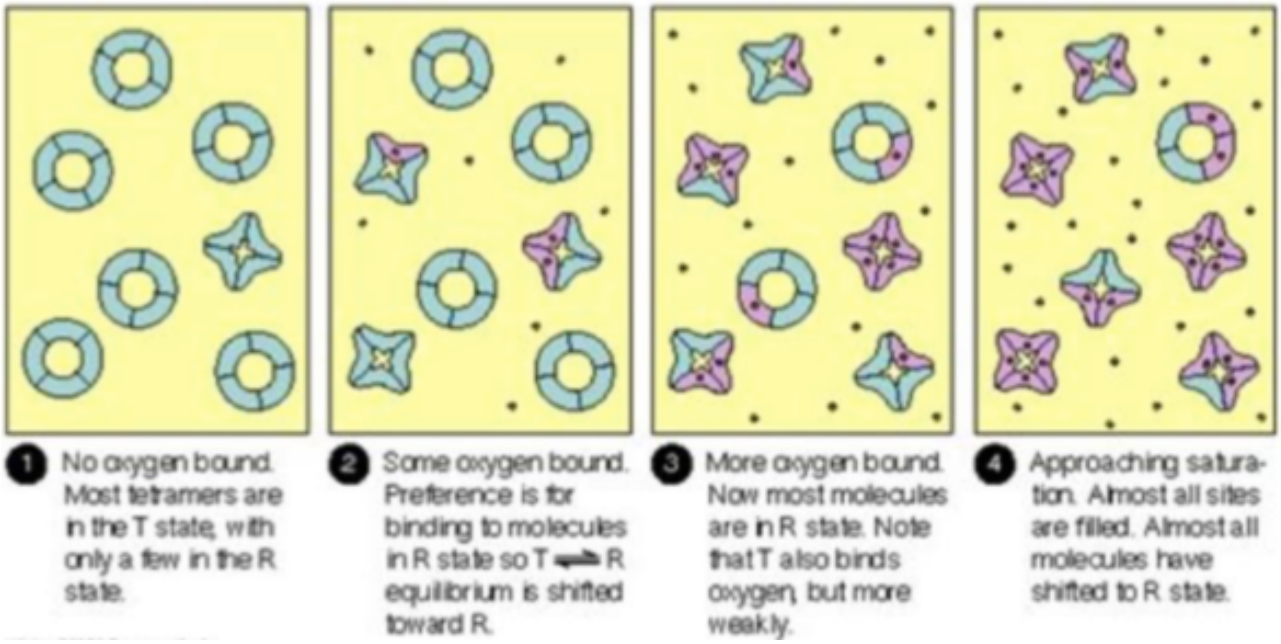
1. **Hemoglobin exists in 2 states *only* ; T and R**
2. **These two states are in equilibrium**
3. **Oxygen shifts the equilibrium *toward the R state***



let me explain more…

Now this model is all about probabilities ; it states that always the allosteric protein is in a state of equilibrium between the R state and T state ,, however , whenever a subunit binds to its ligand , the equilibrium shifts towards the more affinity R state ,making it more probable and likely to fully convert the whole protein to the R state , And the more subunits bind to their ligand ,the more probable the molecule will fully convert to the R state and the more probable the equilibrium will shift to the R state

I had to include this picture from 2016 sheets with the explanation under because I found it really helpful, you may skip it if like.



In picture 1 , There isn't a ligand (Oxygen), so the probability that you will find an R state Hemoglobin is very low , however , still , there is a small amount in the R state , but keep in mind that they are very unstable (usually ignored amount). **In picture 2** , Some binding to oxygen is happening , this is increasing the chance of a hemoglobin molecule that is bound to oxygen to fully convert to the R state , that's why we are having more proportion of R state hemoglobin . **In picture 3** , all molecules have been bound to oxygen , the ones that have more subunits bound to oxygen , are the ones having the biggest chance to convert to the R state. **Finally in Picture 4** , almost all hemoglobin is in the R state because mostly they either bound 3 or 4 oxygen molecules , but still , we can find some molecules , despite binding to 2 or 3 molecules of oxygen didn't convert to the R state . And the same idea to picture 1 , If hemoglobin binds to 4 molecules a very little amount will be in the T state and they will be unstable (usually ignored amount).

The sequential, induced fit, or KNF model

Less accurate but true as well!

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. Ligand binding may also result in negative cooperativity.

The MWC model only suggests only positive cooperativity. thats why we still use this model. (more details are not included, that's all we have to know)

In this model , The full transformation from the T state to R state is sequential , because in this model , to transform an allosteric protein to the R state , all subunits should bind to the ligand ,because remember when we took in biochem 1 about the induced fit model? And how the ligand causes structural changes in the subunit so it would fit? Same thing here, when a ligand binds to a subunit , it causes a conformational change in it , but this change does not spread to adjacent subunits, so not T and R states only but partially converted molecules as well . However , in the Concerted Model , there isn't an intermediate state between the T and R stat.

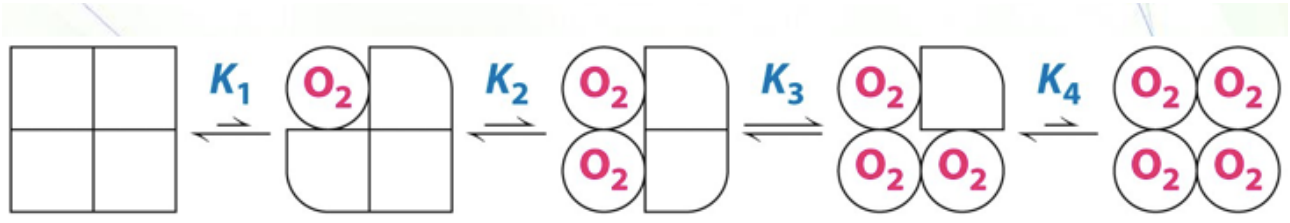
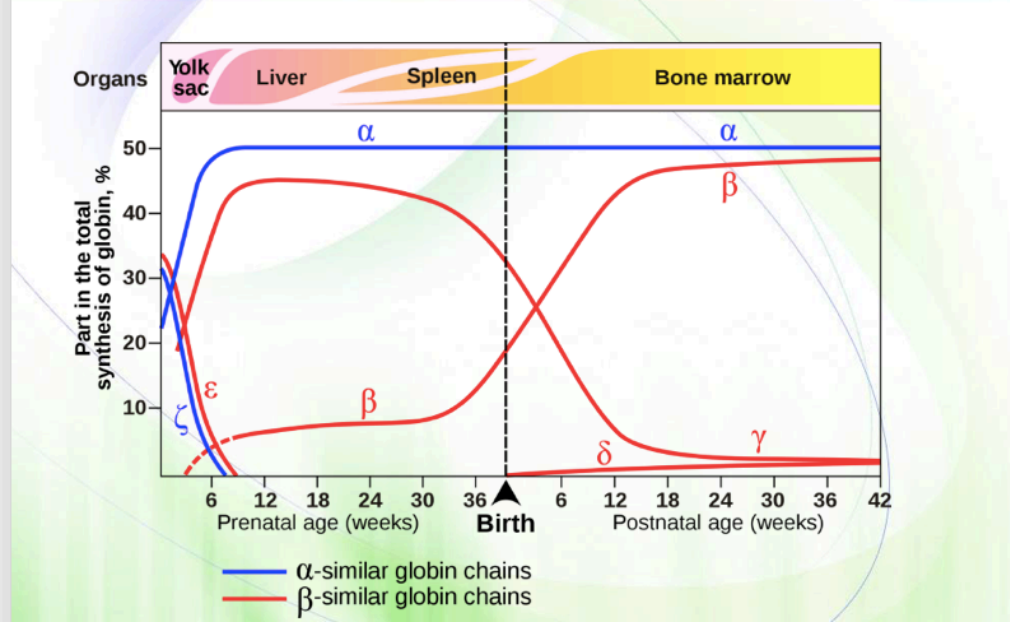


Figure 7.14
Biochemistry, Seventh Edition

Developmental transition of hemoglobins

whats written next is only an explanation of the picture below



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 (**x**)** chains and **2 epsilon (**e**)** chains
Other forms exist: **HbE Gower 2 ($\alpha_2\varepsilon_2$)**, **HbE Portland 1 ($\zeta_2\gamma$)**, **HbE Portland 2 ($\zeta_2\beta$)**.

Beginning of 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 (HbF) consists of **two** alpha polypeptides and **two gamma** (g) polypeptides **(a2g2)**
The alpha polypeptides remain on throughout life.

Beginning of adult stage

(from slides)
Shortly before birth, there is a gradual switch from gamma to adult b-globin.
Still, **HbF** makes up 60% of the hemoglobin at birth, but **1% of adults.**
At birth, both synthesis of g and b chains occurs in the **bone marrow.**
* In adults: (HbA1- a2b2) (HbA2- a2 delta2) (HbF-a2g2)