Hemoglobin
An overview and more

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Hematopoietic-lymphatic system
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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)

What are we covering

- In this lecture we will be covering:
  - 1- Heme and Hemoglobin structures
  - 2- Structural changes upon binding Oxygen
  - 3- The 2 models of cooperativity
  - 4- Types of Hemoglobin
  - 5- Hemoglobin for detecting Diabetes
  - 6- The genetics of Globin synthesis

1- Heme and Hemoglobin structures

Hemoproteins

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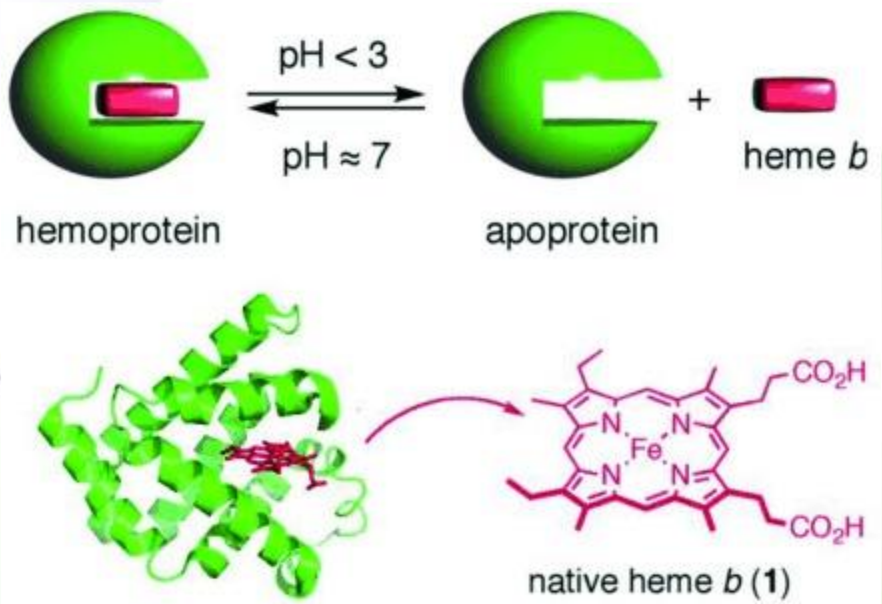

***A prosthetic group** is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.*

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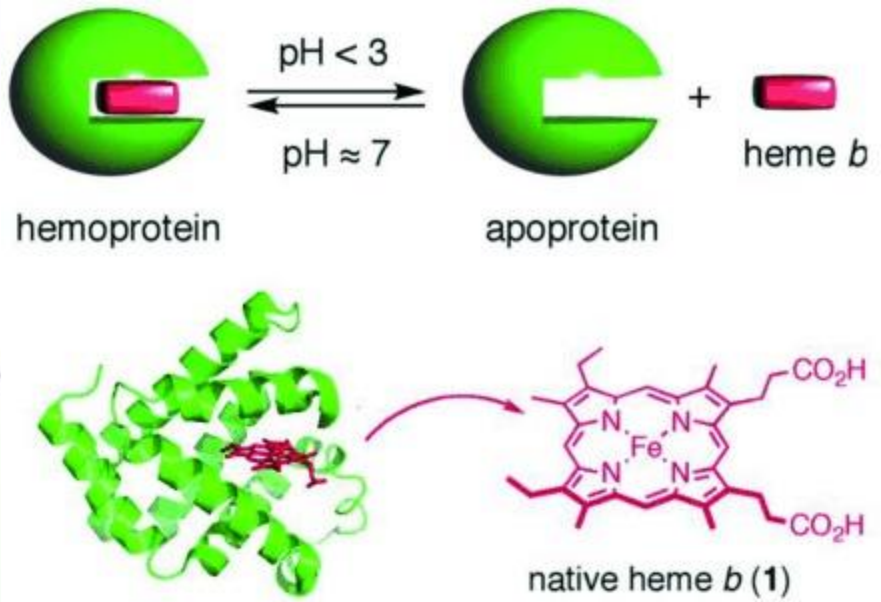

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Heme is present in:

Hemoproteins

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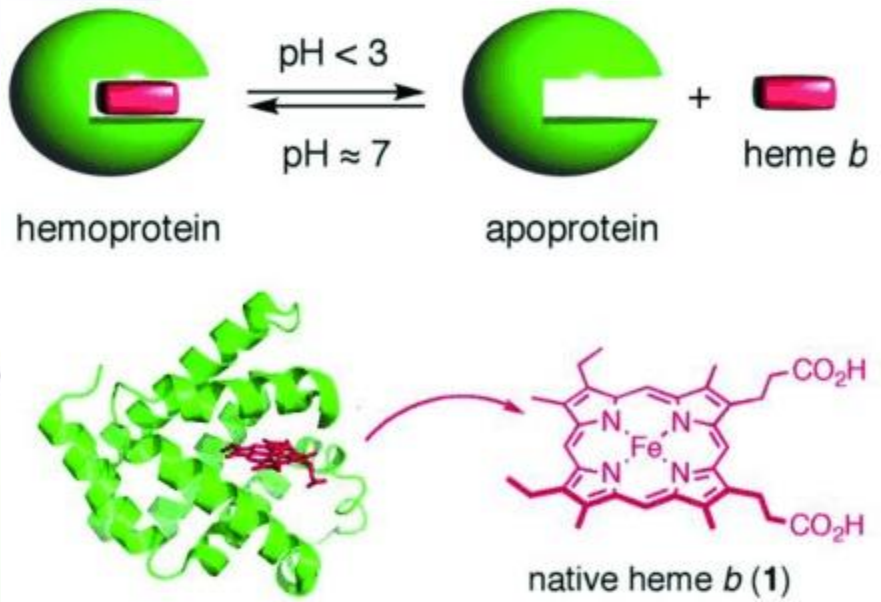

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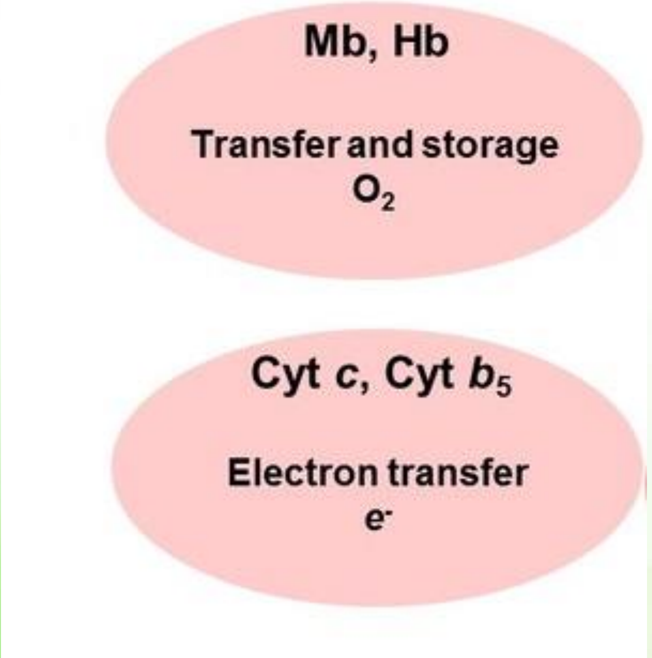

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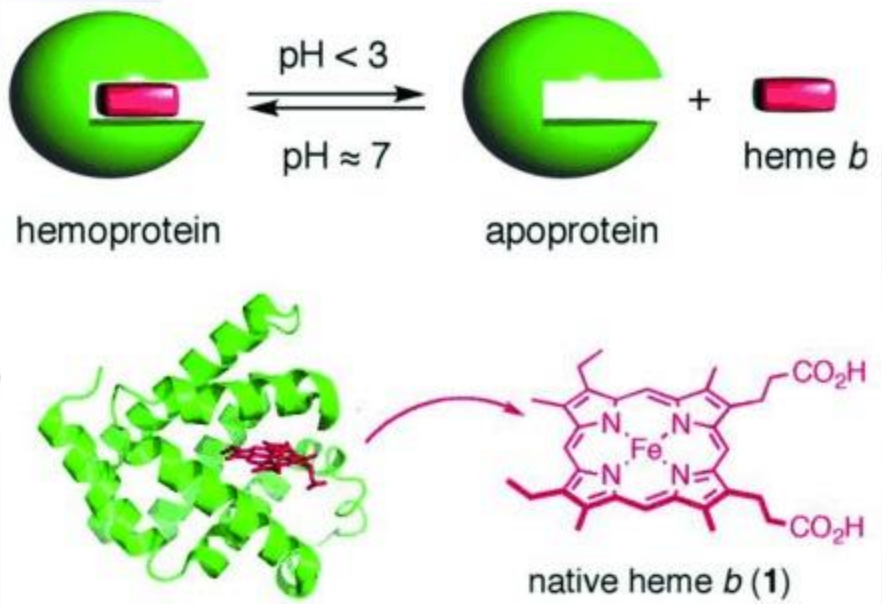

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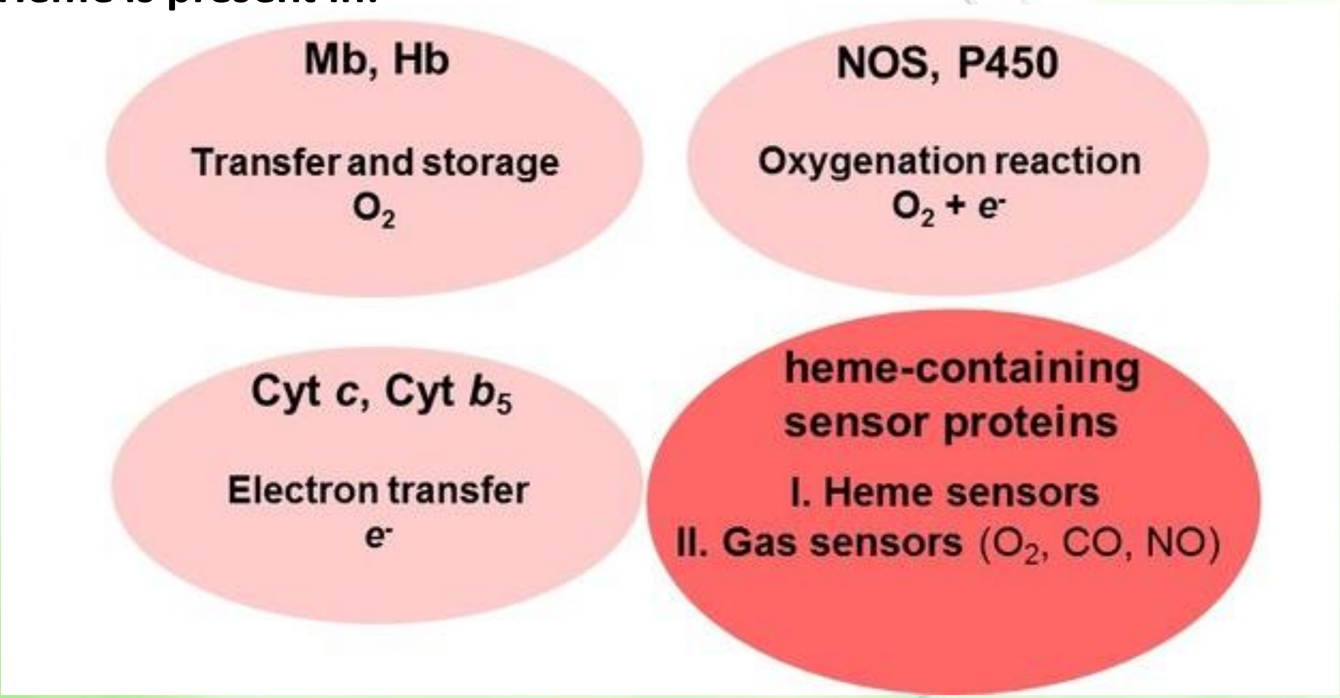

Heme structure

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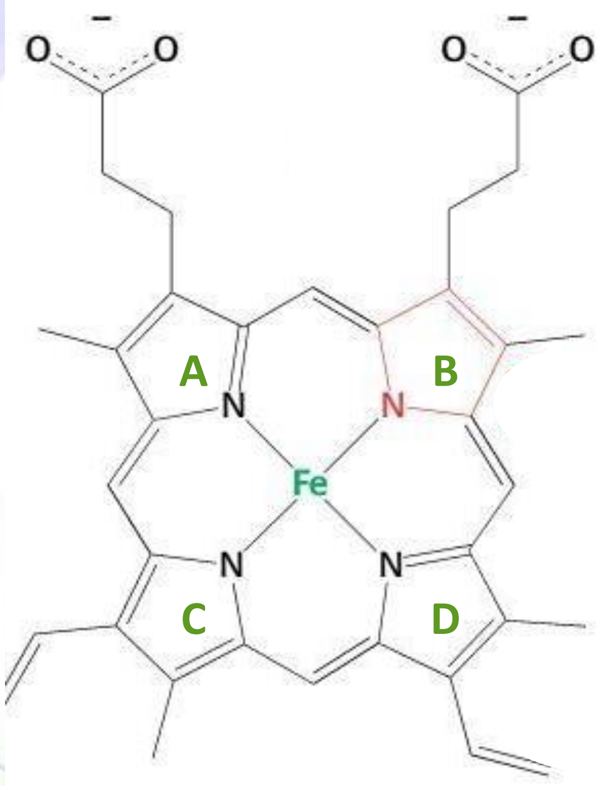
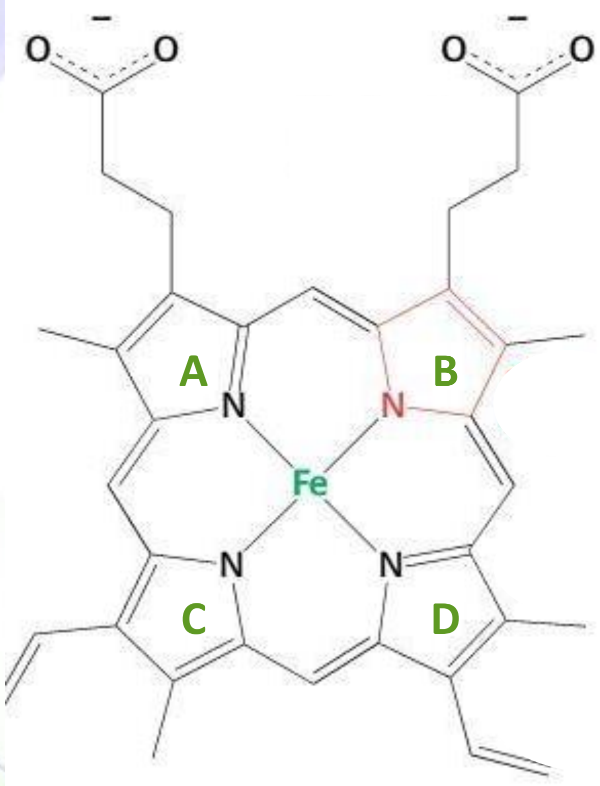


- The heme is composed of:
- 1-Fe+2

Heme structure

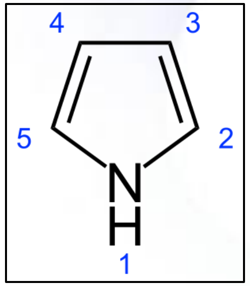


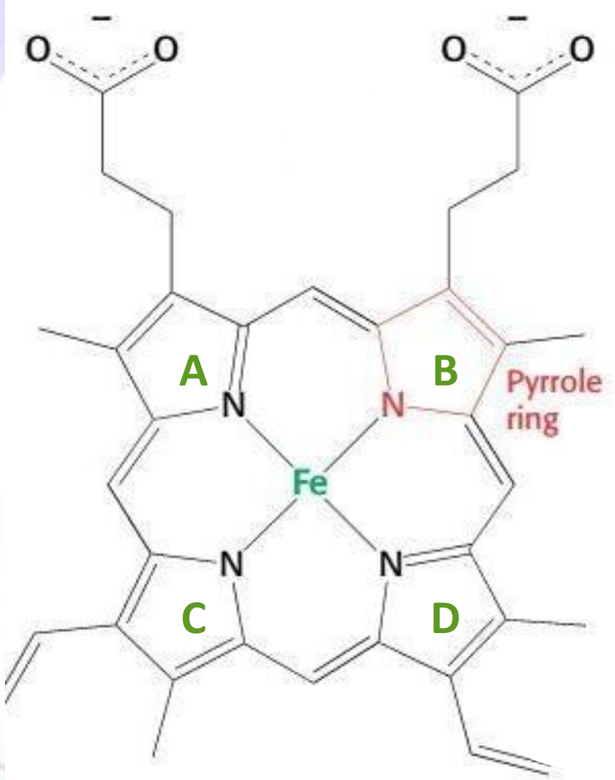
Heme
(Fe-protoporphyrin IX)

- The heme is composed of:
- 1-Fe+2
- 2-Protoporphyrin (which is composed of):

Heme structure

Pyrrole ring





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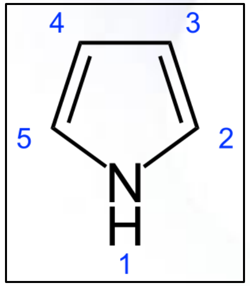

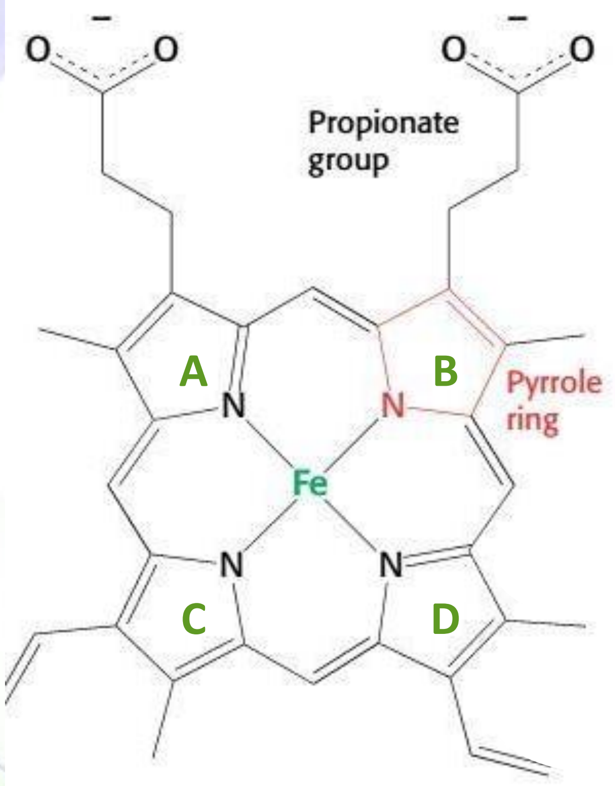

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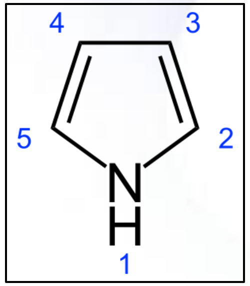

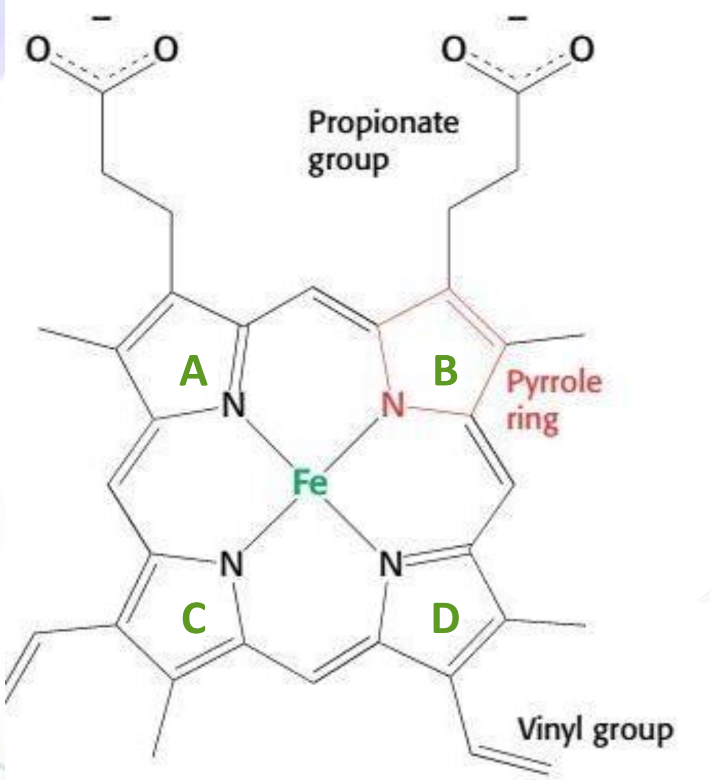

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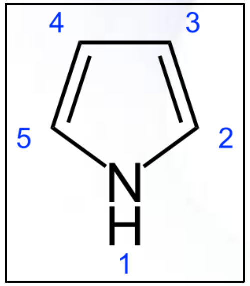

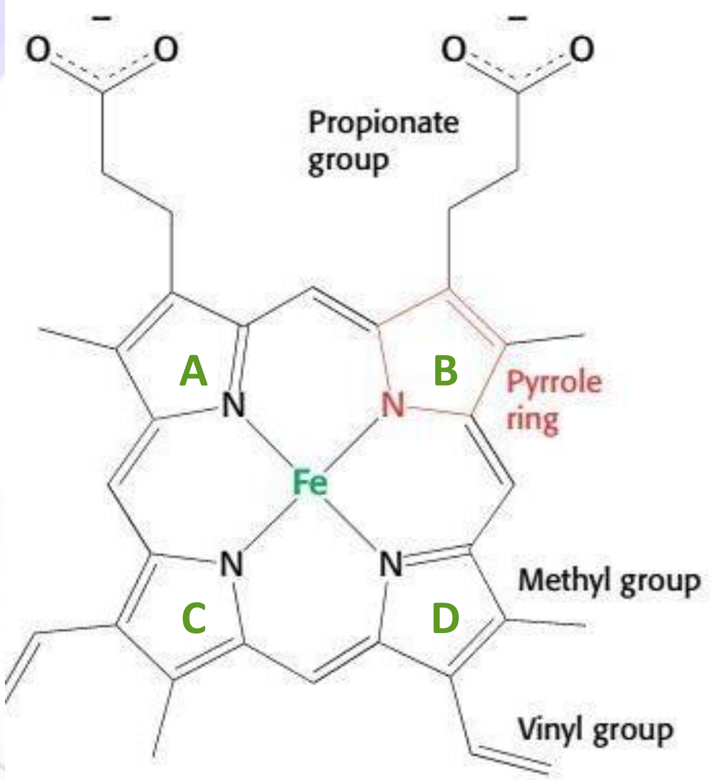

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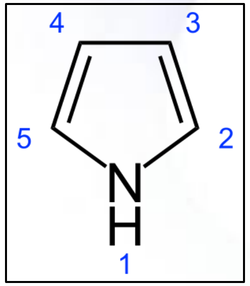

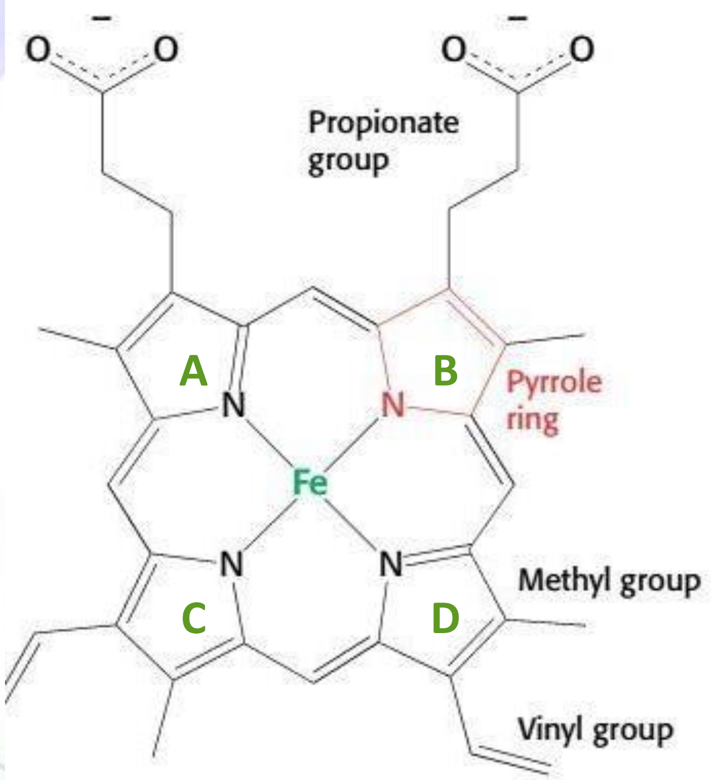

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- *Note: the molecule is hydrophobic.*

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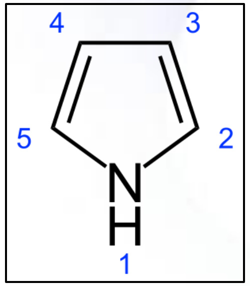

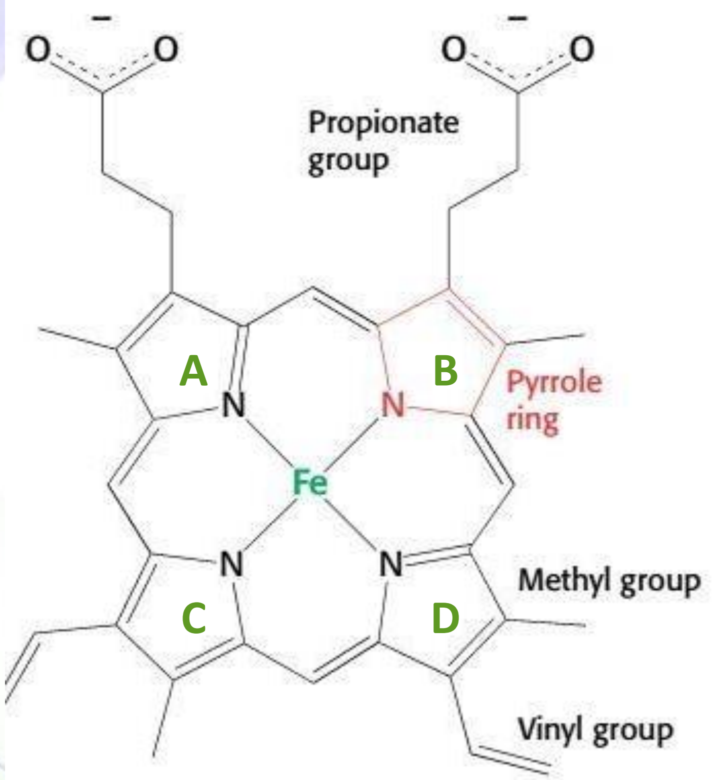

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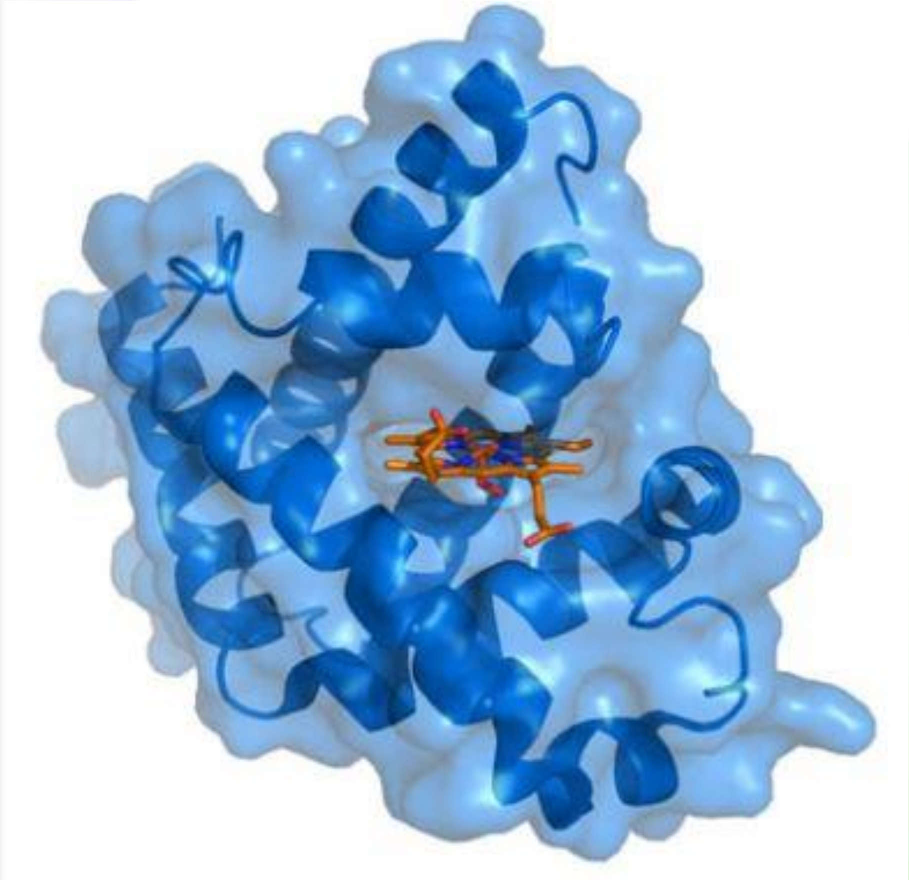
- *Note: the molecule is hydrophobic.*
- Fe has six coordinates of binding.

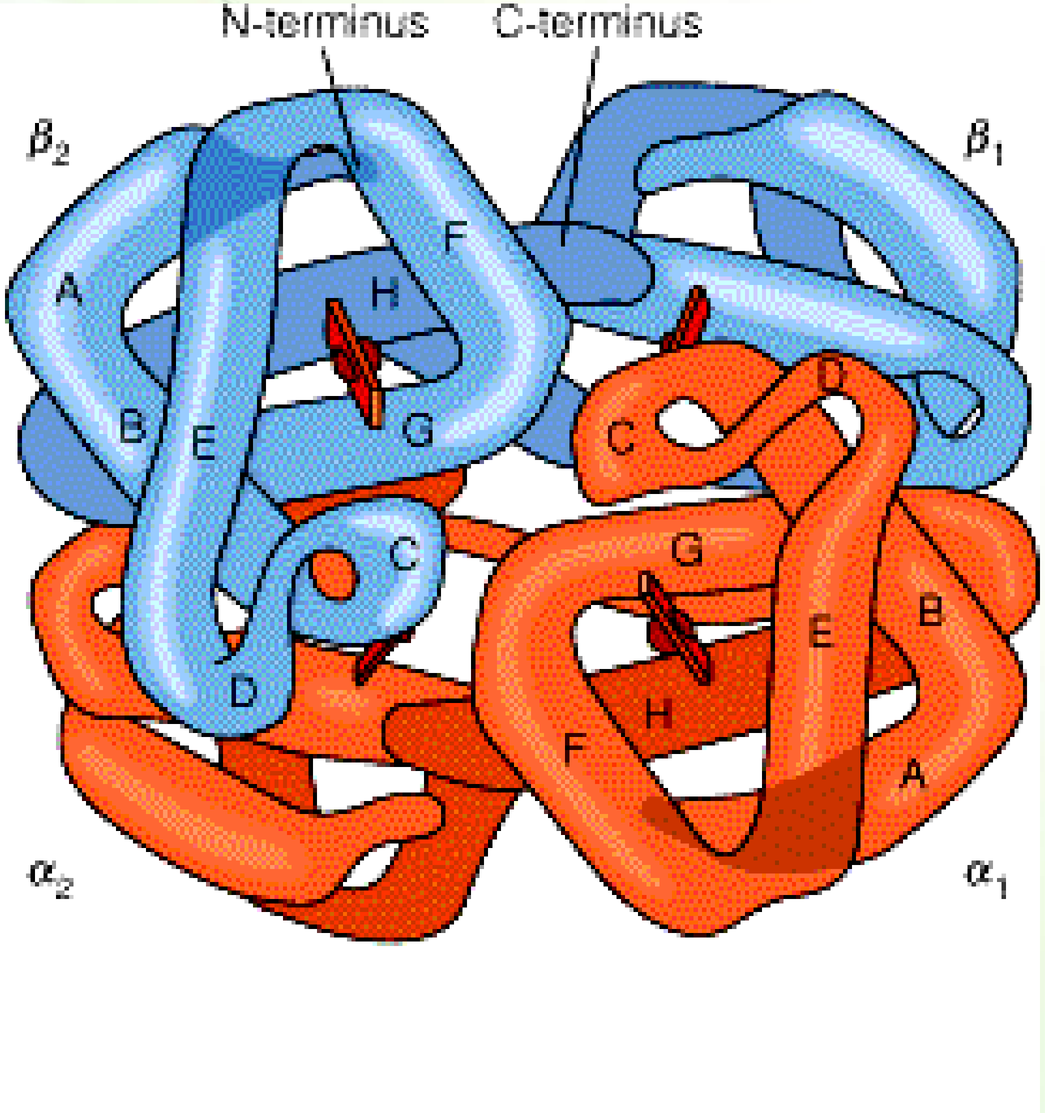
Heme inside a hydrophobic pocket



Heme is held inside a hydrophobic pocket (hydrophobic interactions), but the propionate groups (ionic bonds) also participate in the stabilizing the heme inside the Hemeglobin along with other interactions.

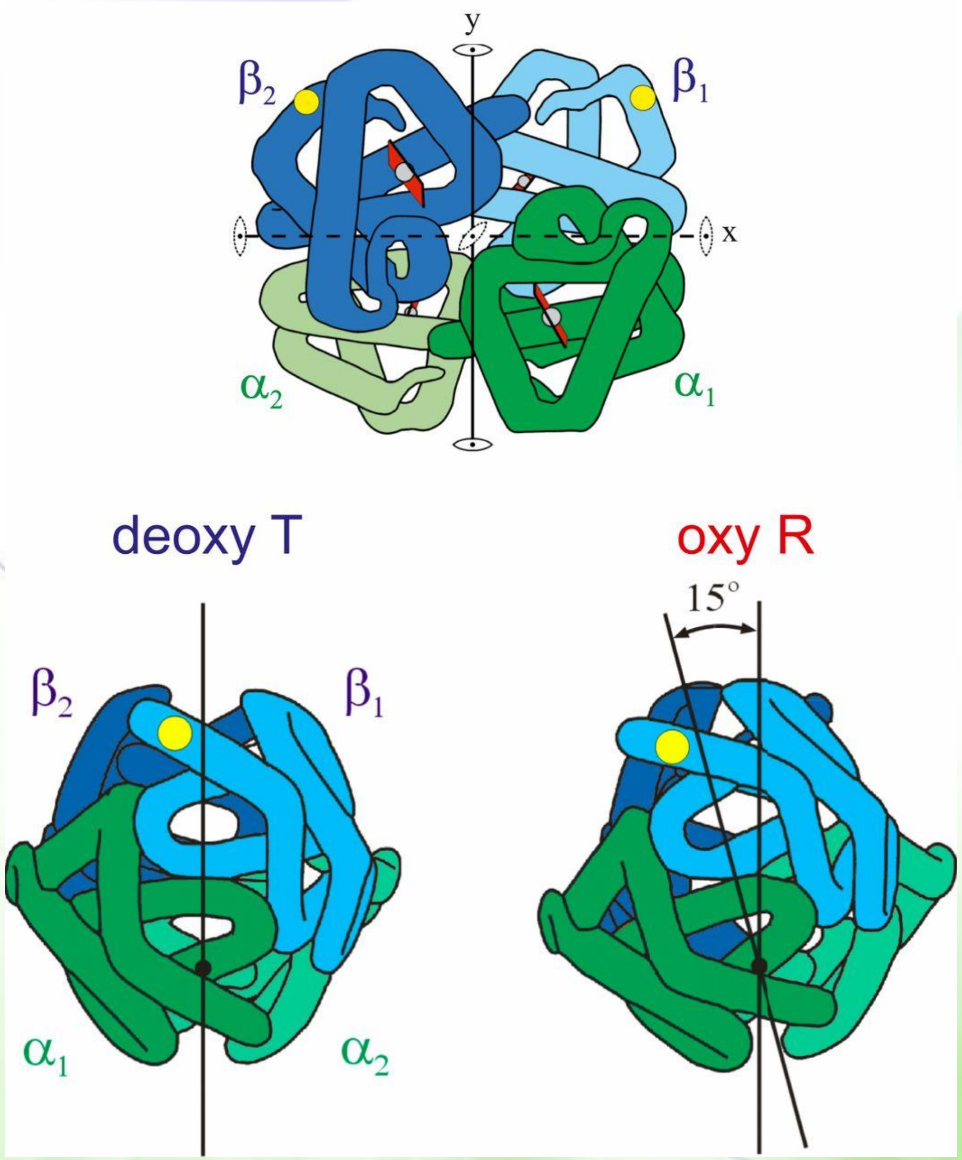
Structure of hemoglobin

- Hb is a globular protein.
- Multiple subunits (2α + 2β)
  - α polypeptide = 141 amino acids (Last aa Arg141)
  - β polypeptide = 146 amino acids (Last aa His146)
  - The first amino acid in both is valine.
- *Positive cooperativity towards oxygen. The last Oxygen has 100-250 fold stronger propensity for binding to hemoglobin than the first hemoglobin.*
- Regulated by allosteric effectors

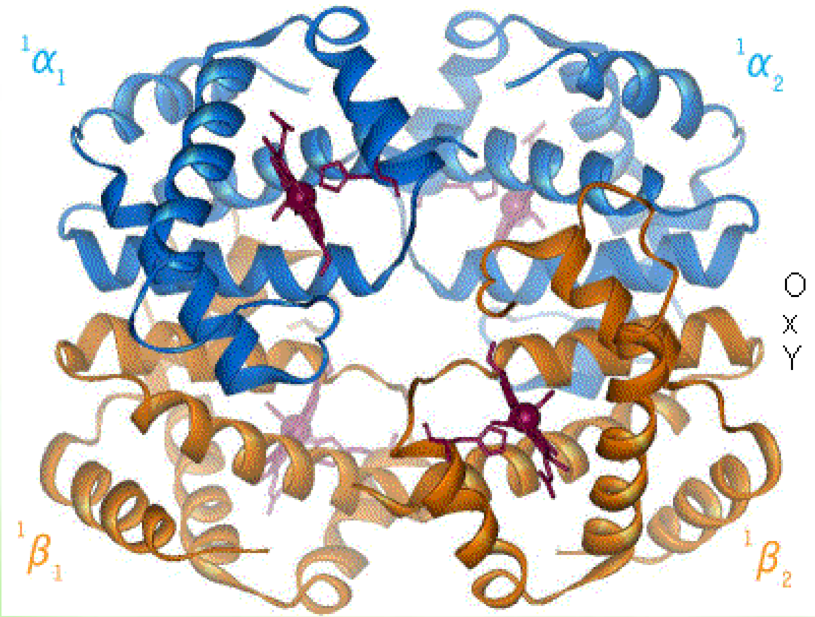


Hemoglobin

Structural change of hemoglobin

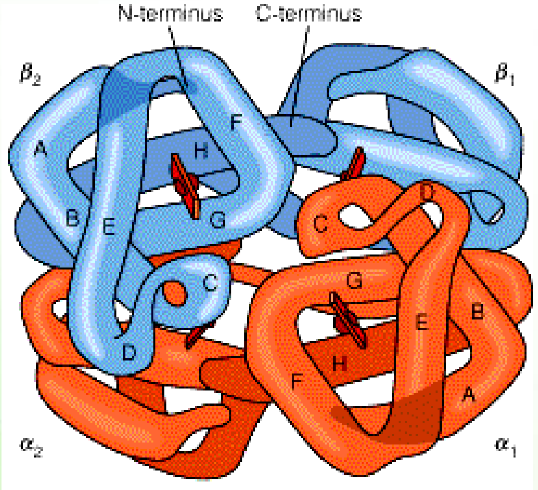


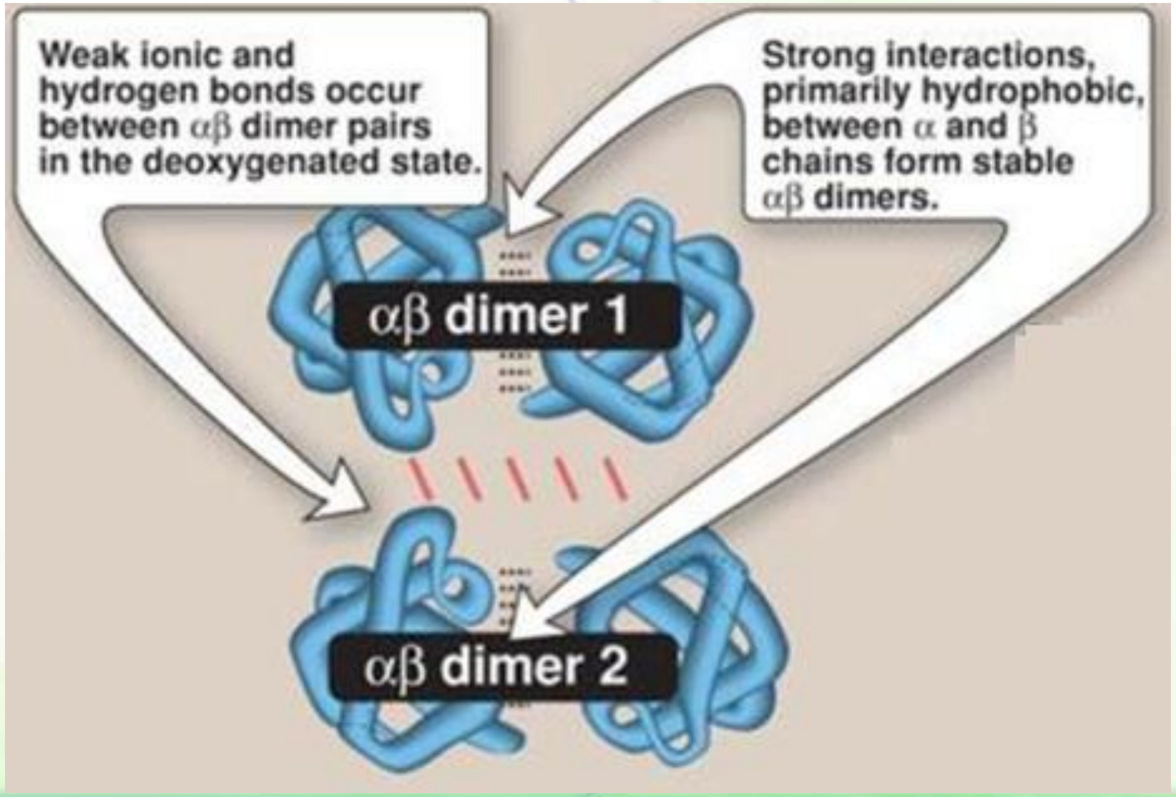
Hemoglobin could be in the deoxy T state (Tense or Taut) where it does not prefer to bind to Oxygen, or Oxy R state (Relaxed) where it is open to binding to Oxygen.



How are the subunits bound?

- A dimer of dimers (I made up this term)
  - $(\alpha\text{-}\beta)2$
  - Note how they interact with each other.





2- Structural changes upon binding Oxygen

Fe+2 attachments

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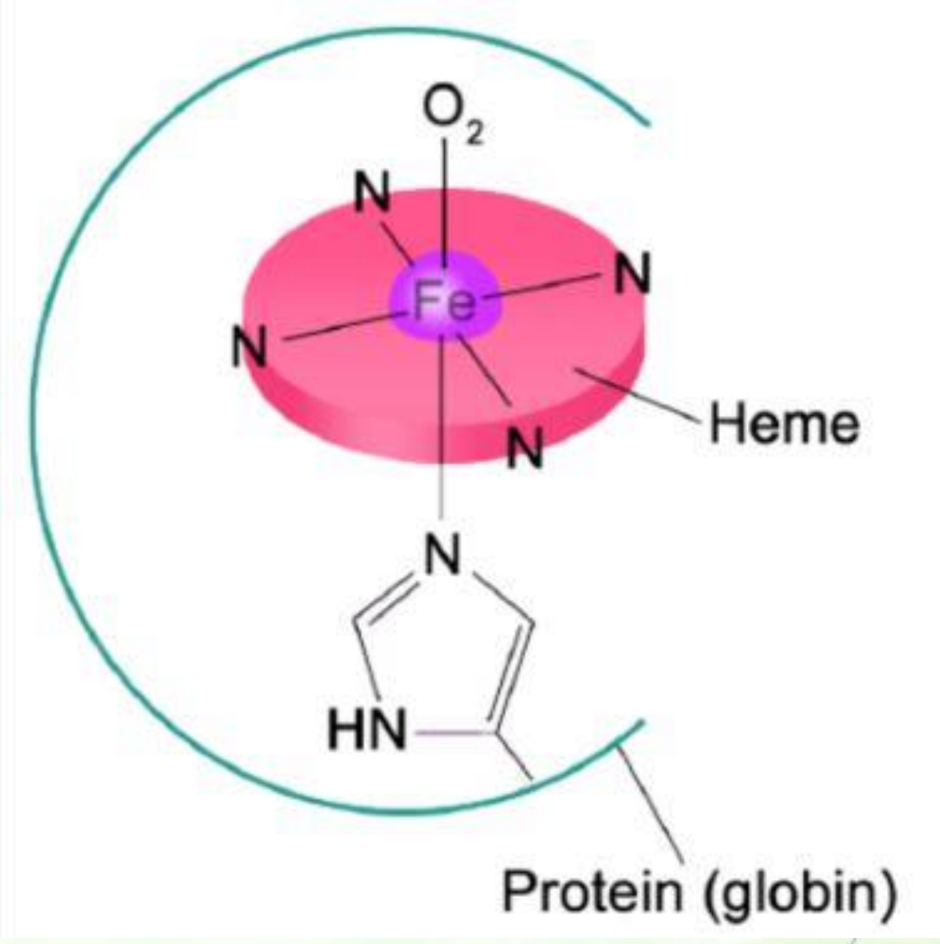
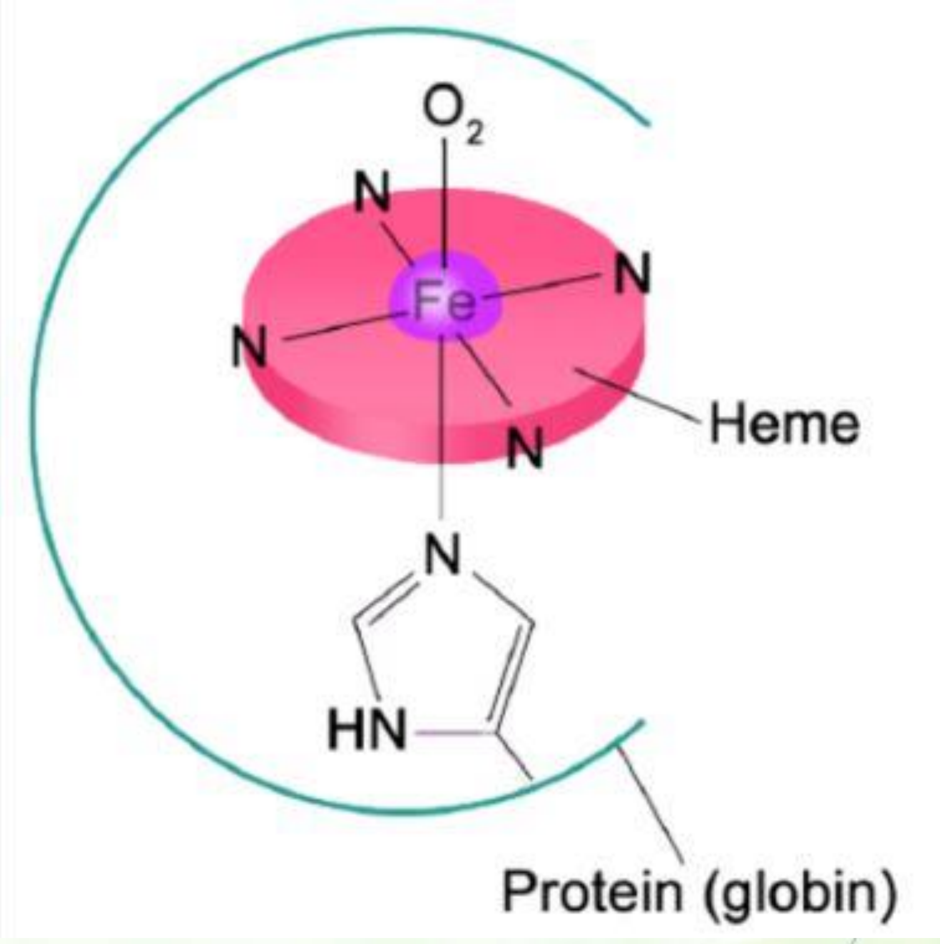


Fe+2 attachments

- Fe+2 makes 5 bonds. 4 with the protoporphoryn and the 5th with the globin protein through the proximal Histidine, it is a covalent bond (This is a very important bond)
- In the presence of Oxygen, Fe+2 makes a 6th bond with Oxygen.



Structural amplification change

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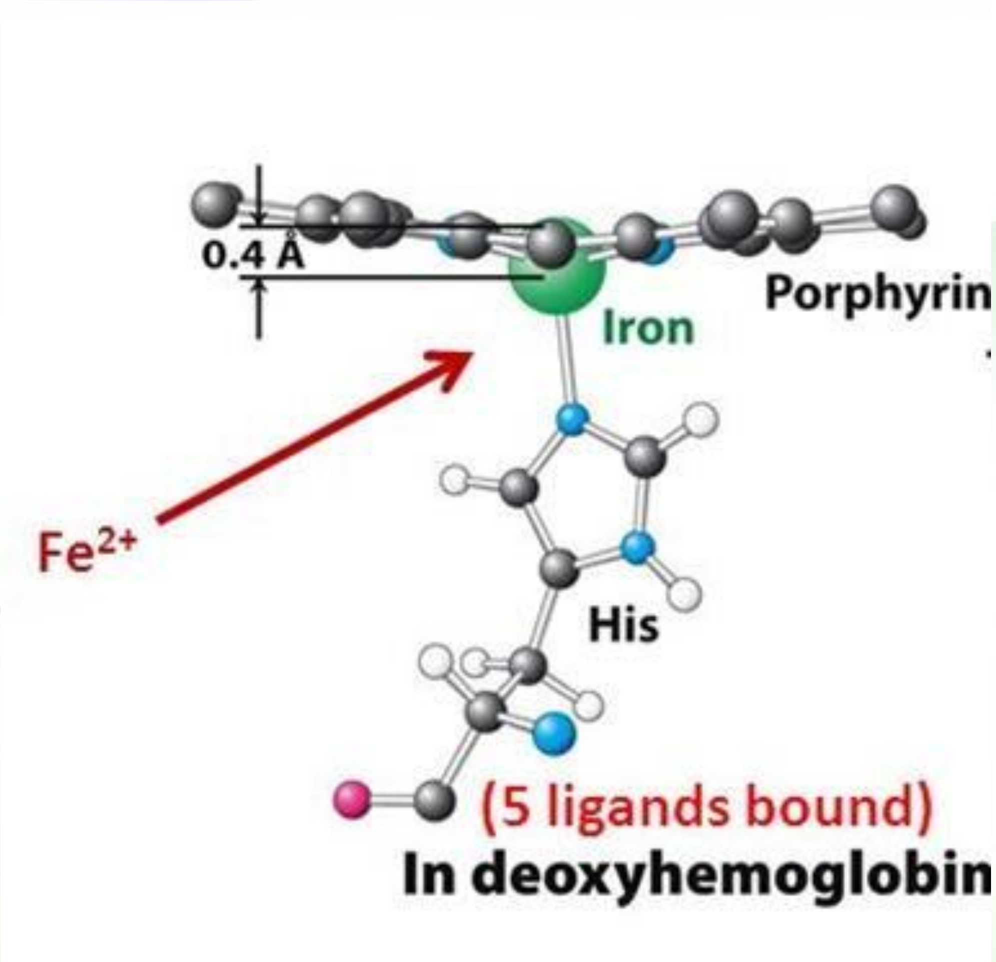

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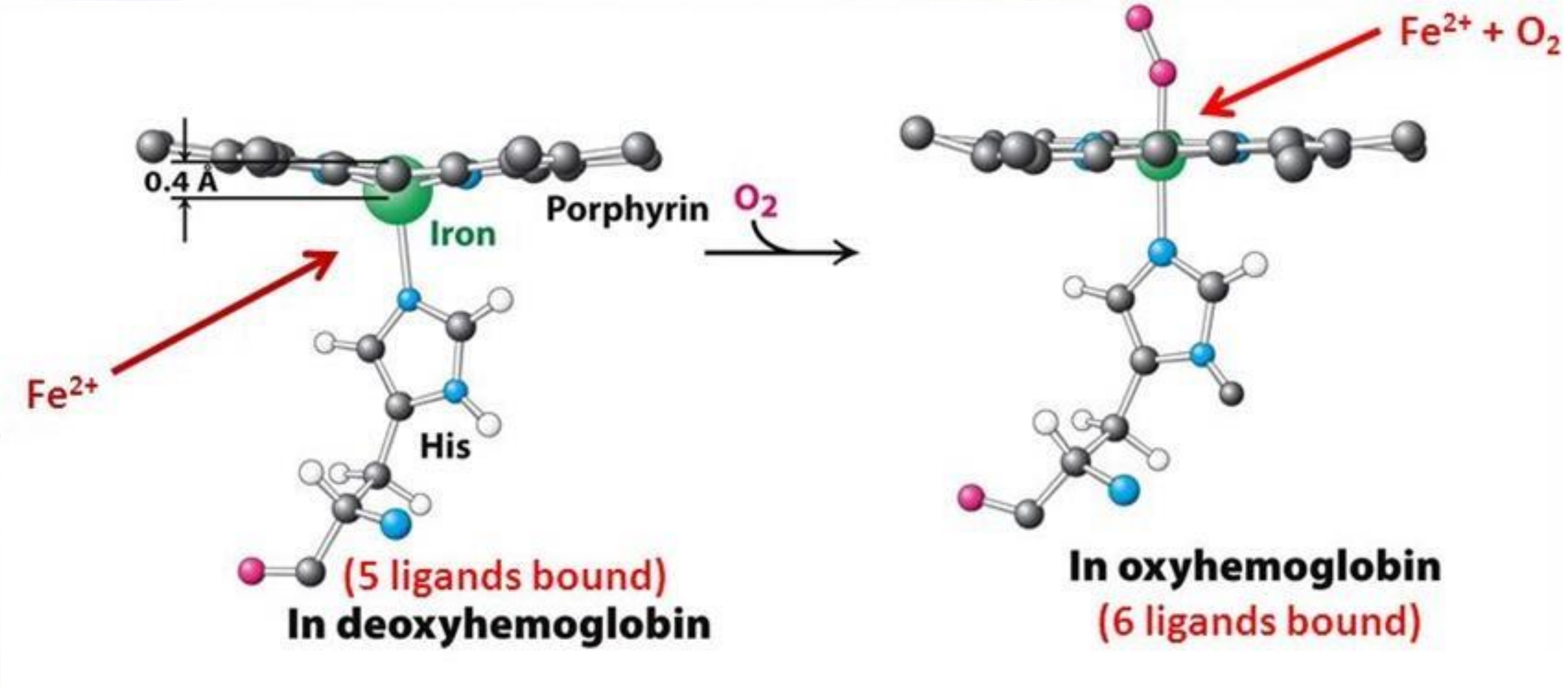
Structural amplification change



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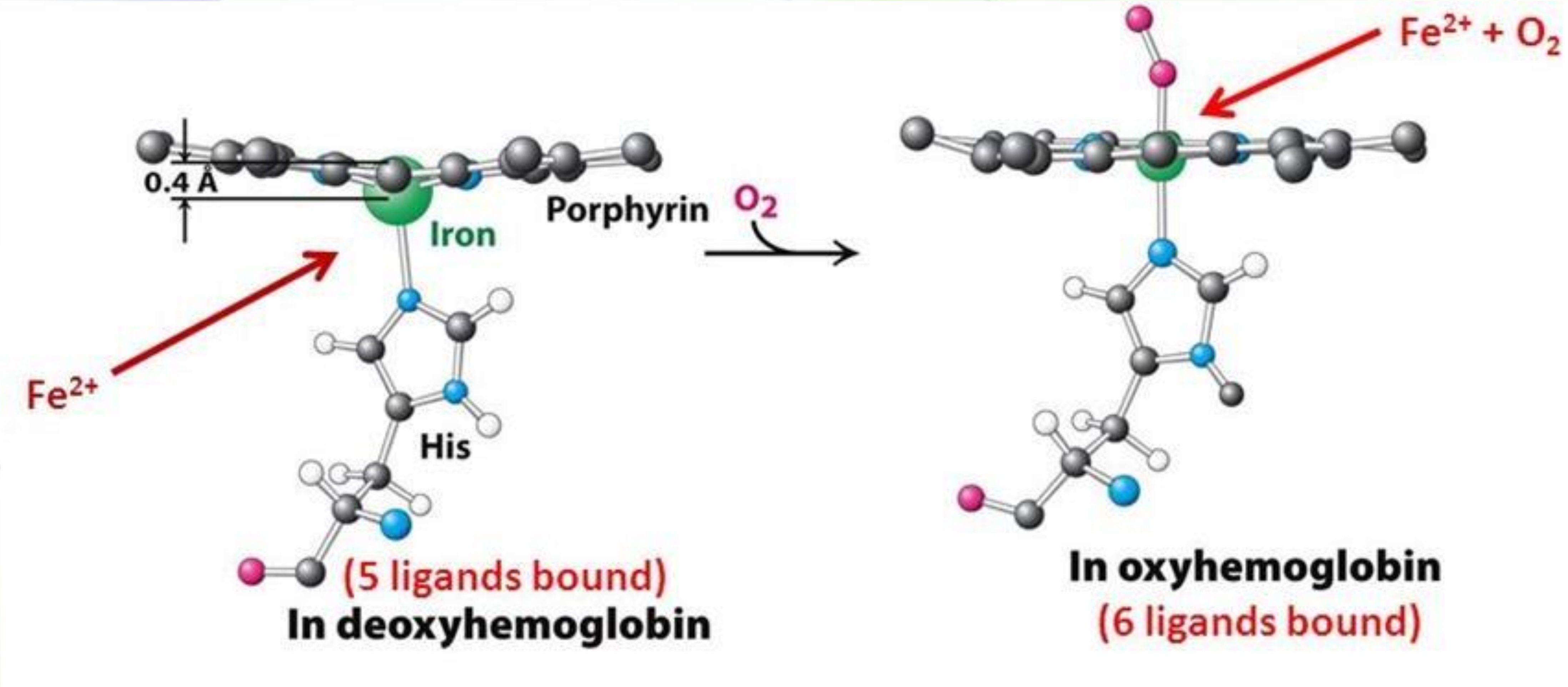
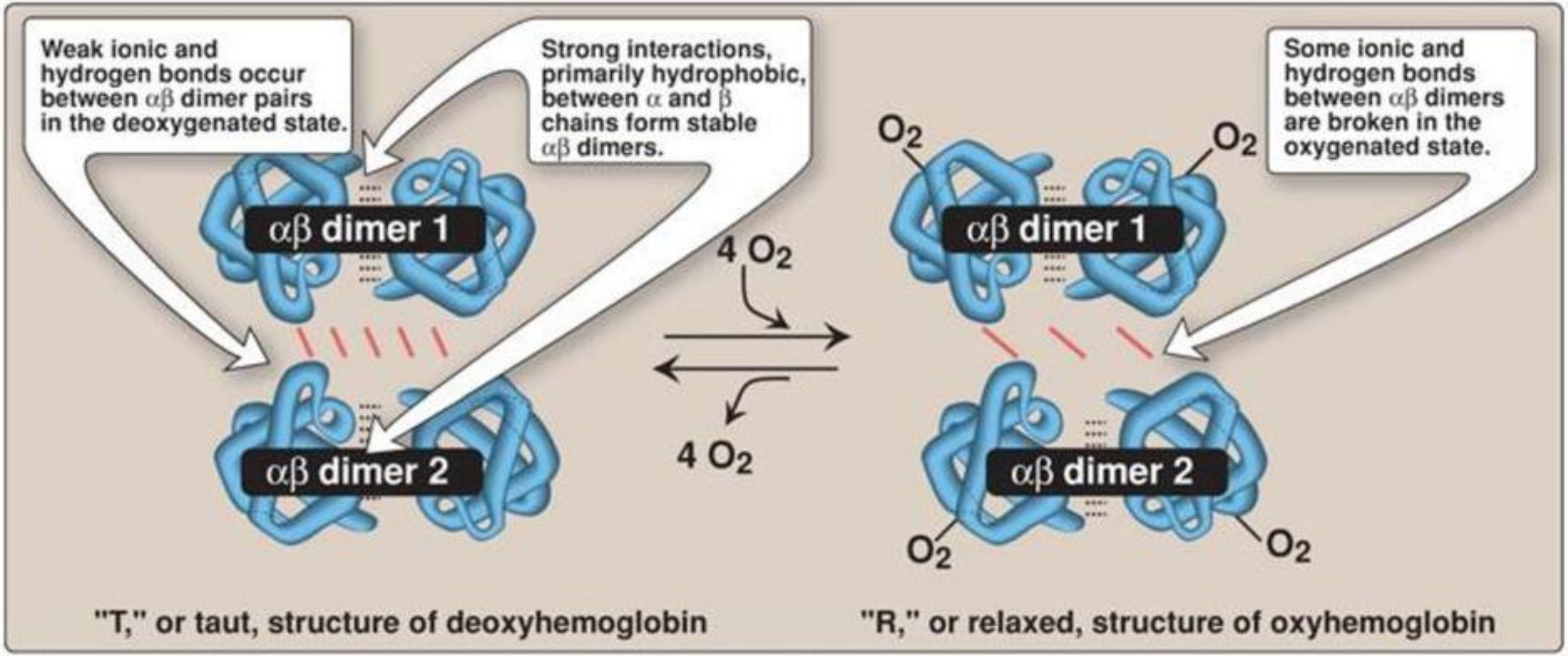


- The small move of Iron upon binding to Oxygen and the pull on the proximal His a.a. propagates through the protein and causes breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.
- It causes a change in the tertiary structure of individual hemoglobin subunits, allowing them to bind to Oxygen more readily.

Broken electrostatic interactions and H-bonds



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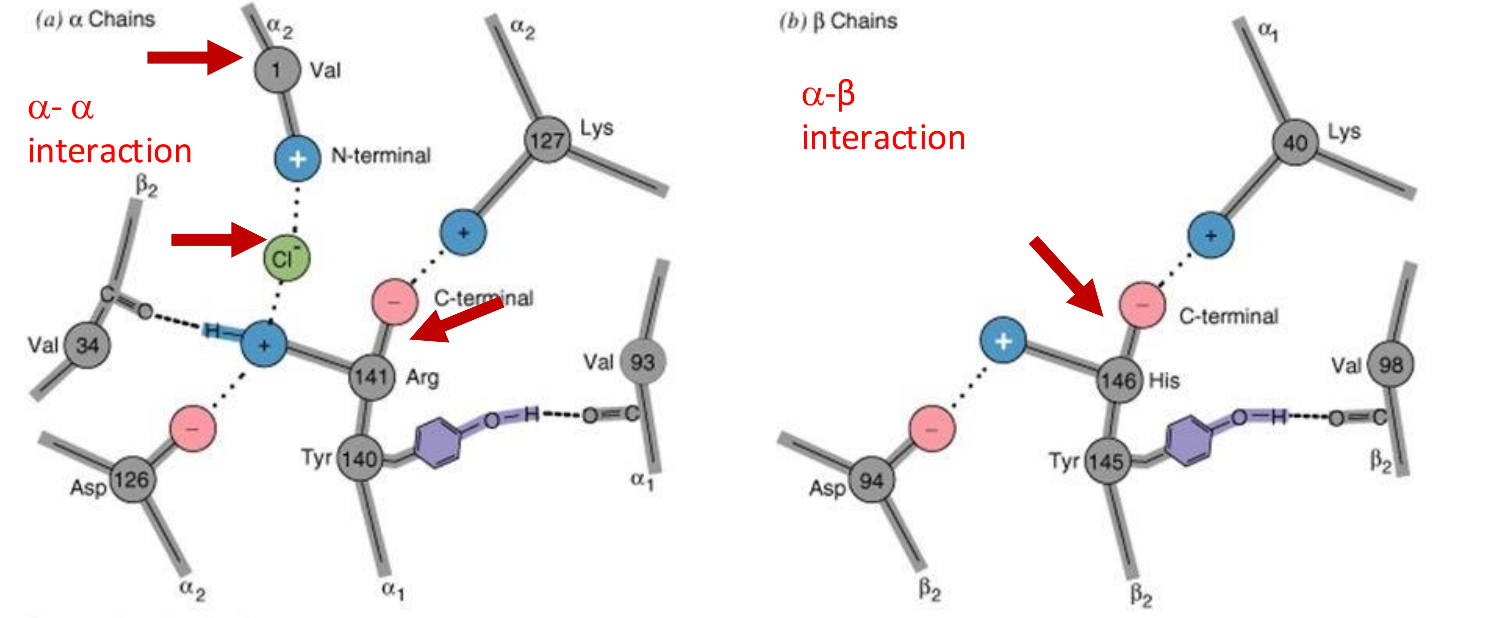

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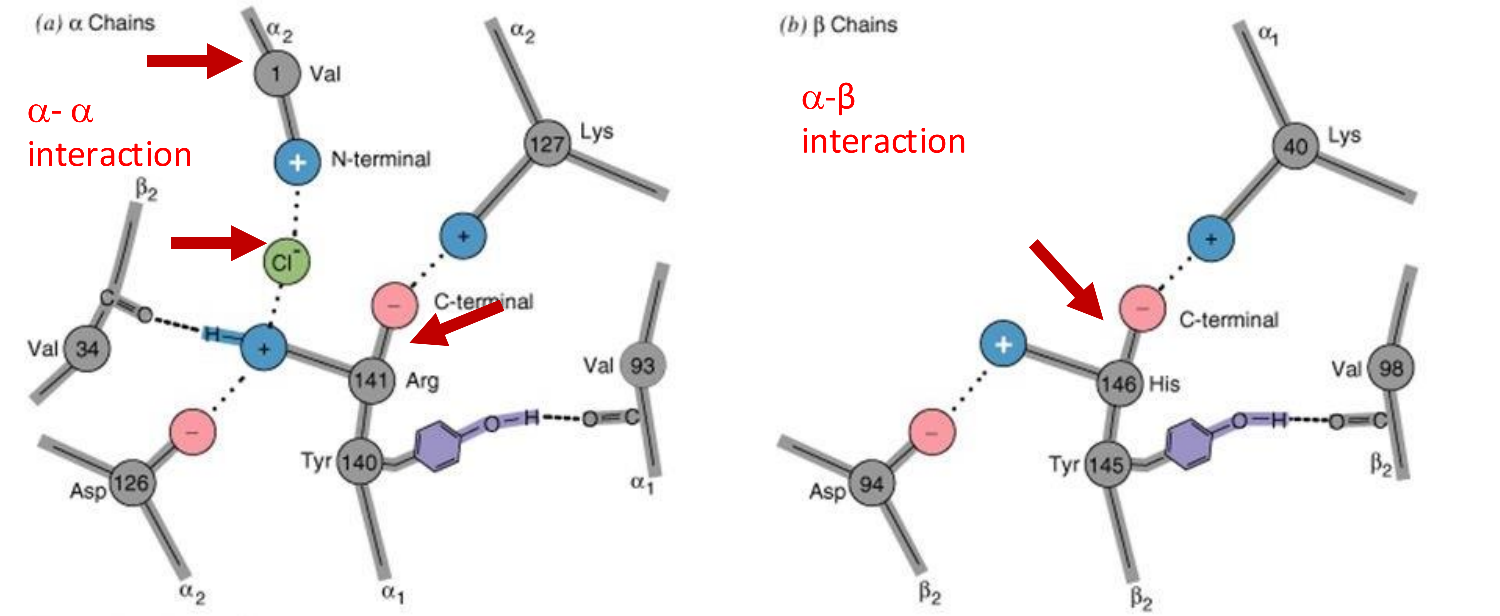
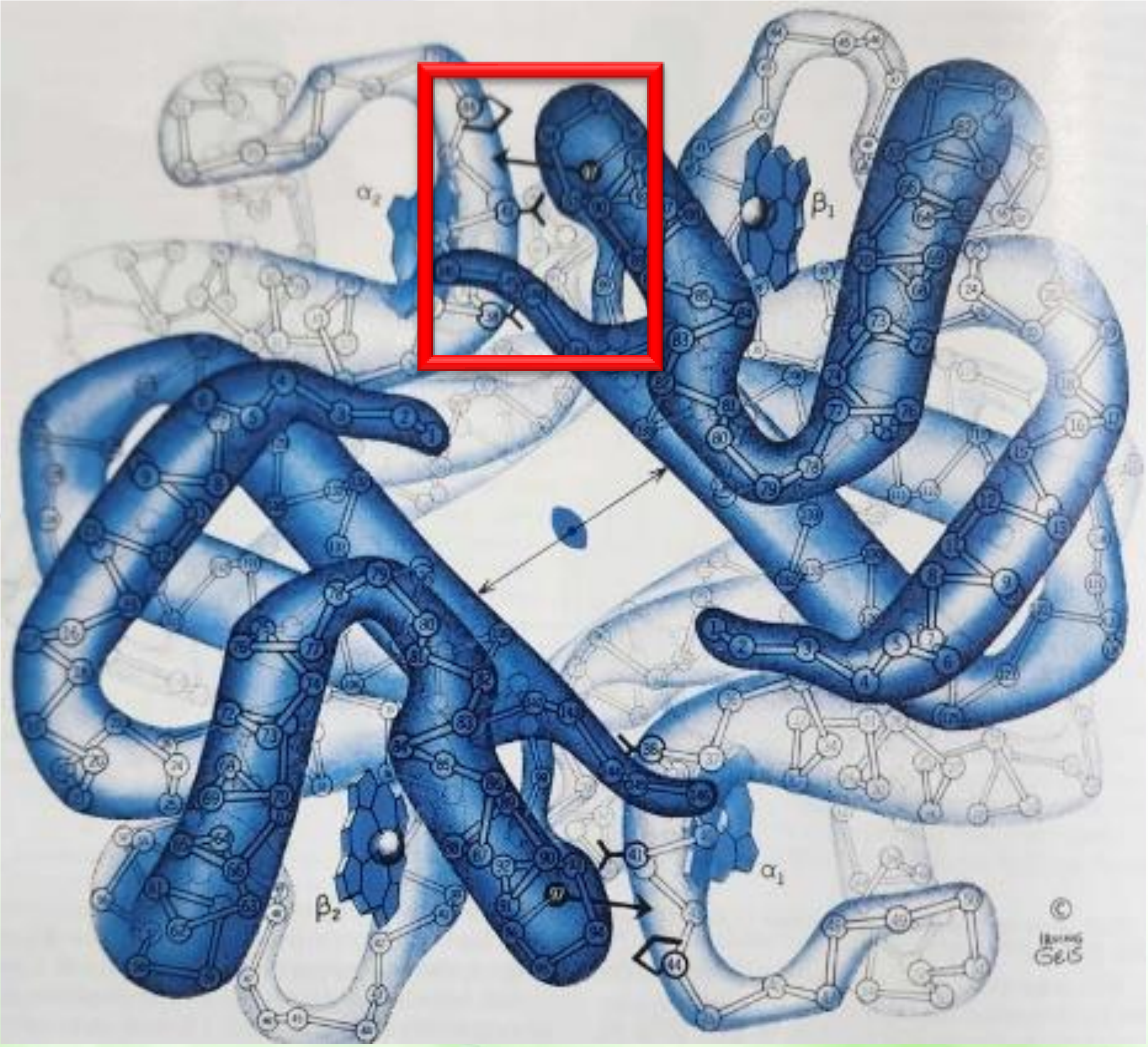


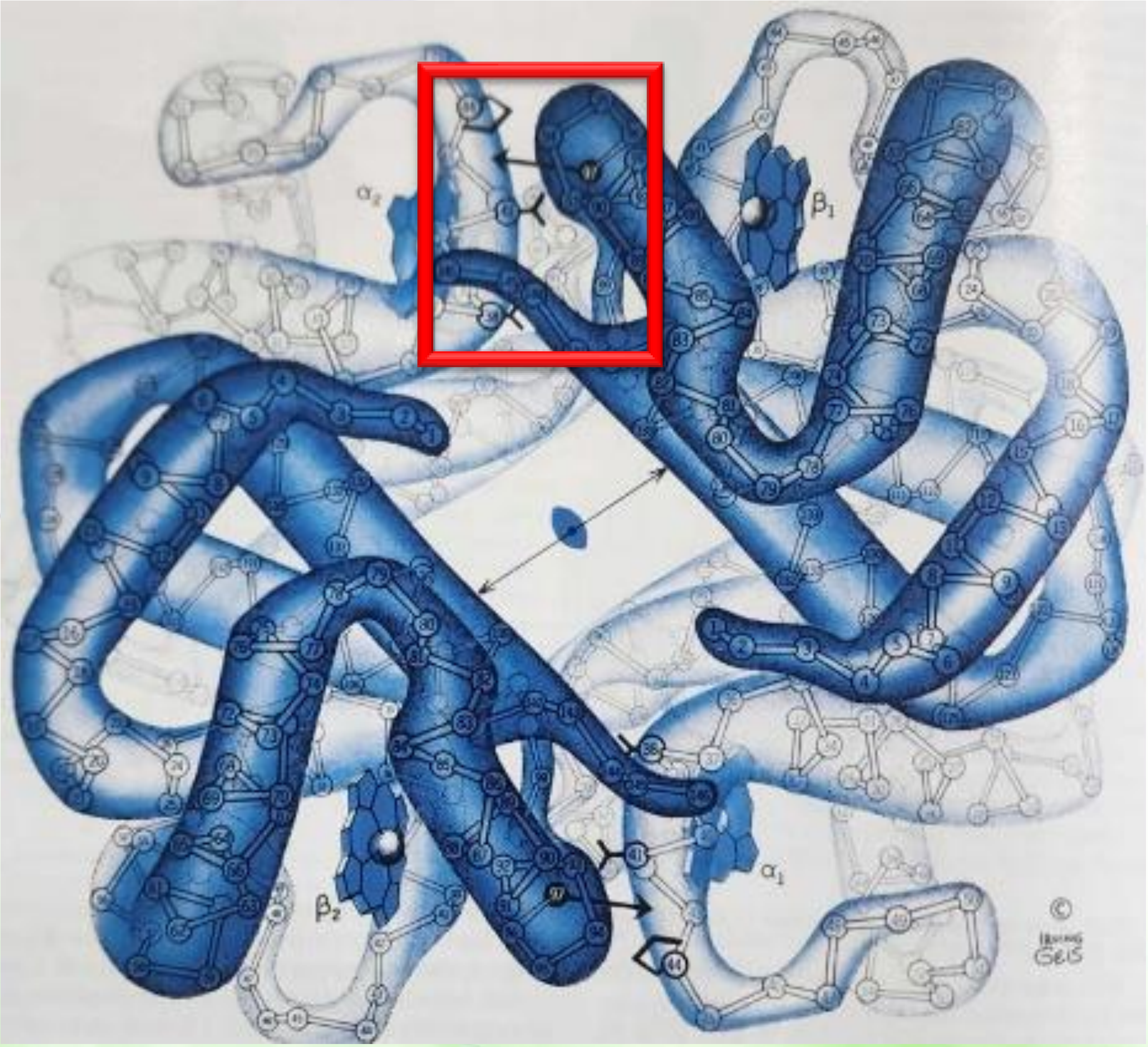
- When Oxygen binds, it causes the breakage of the electrostatic interaction and the Hydrogen bonds causing a movement in the polypeptides.

Reformation of H bonds



Notice how (aa 97) from B1 chain is between aa 41 and 44 ***(T State)***

Reformation of H bonds



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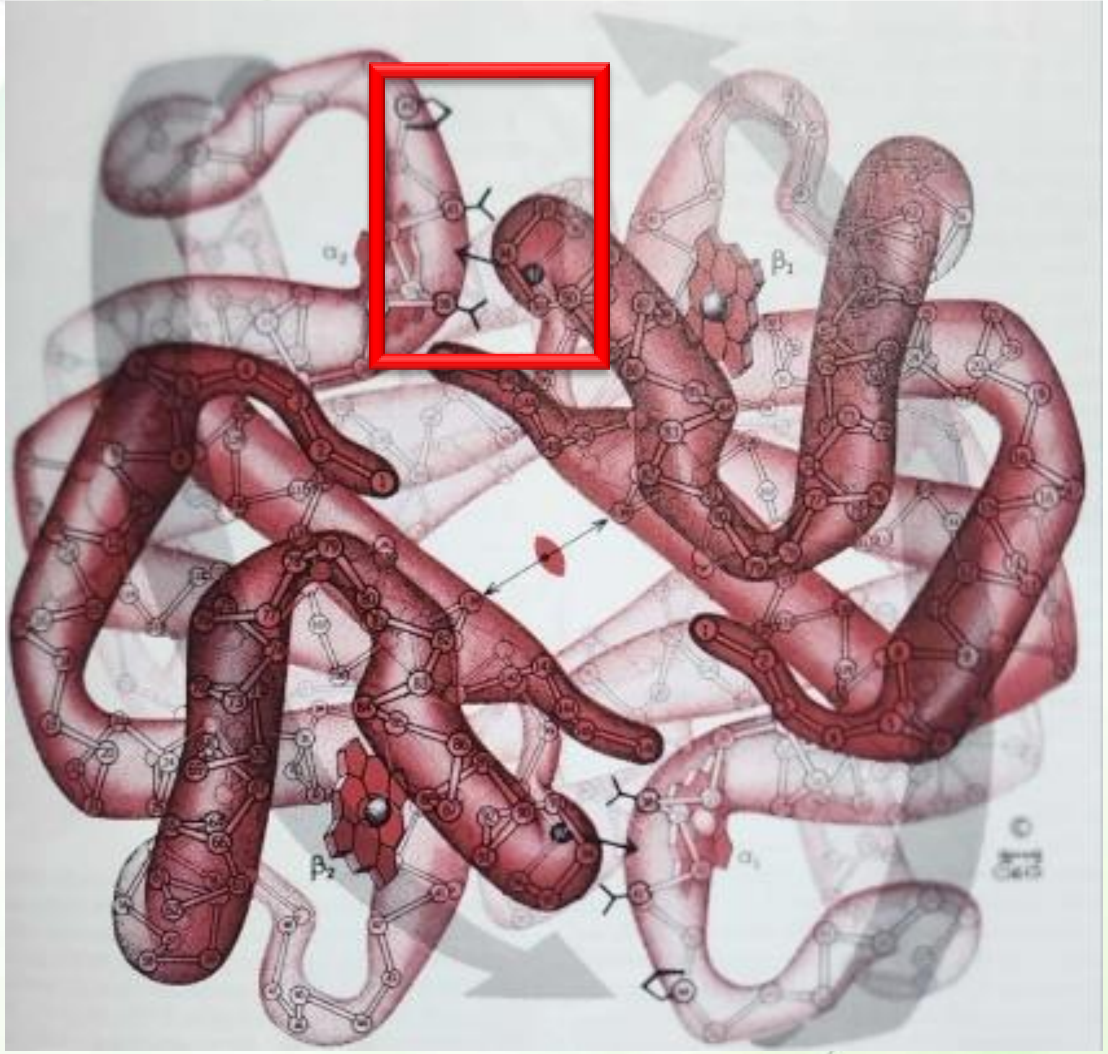


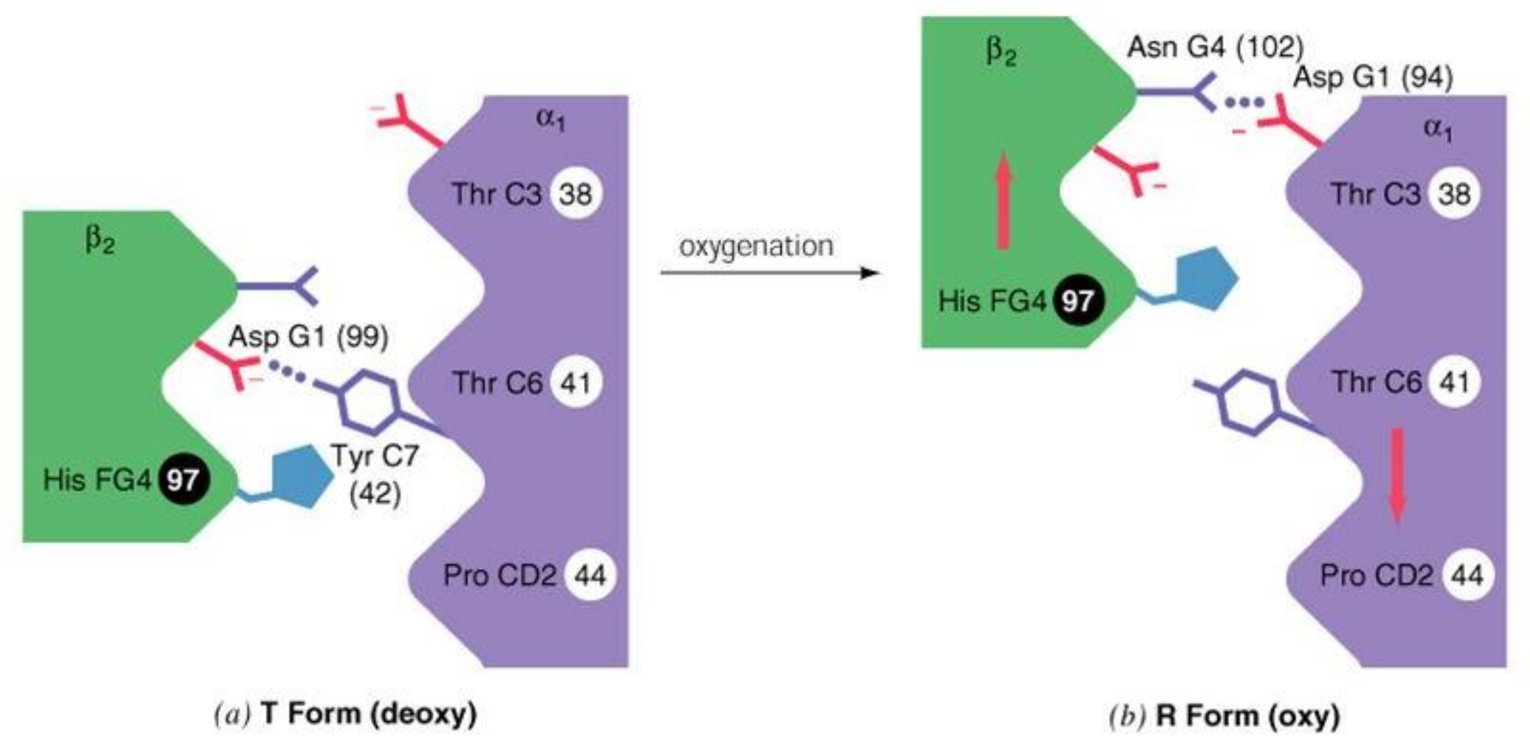
Notice how (aa 97) from B1 chain is now between aa 38 and 41 ***(R State)***

Reformation of H bonds

- The figures in the slide before are the closest to the actual orientation of the subunits where one alpha subunit is binding mainly to 2 beta subunits and making strong connections with them to stabilize the structure. The alpha-alpha subunits are bound with longer and weaker bonds, the same applies to the beta-beta interactions.

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.
- (No need to memorize the aa numbers or types)

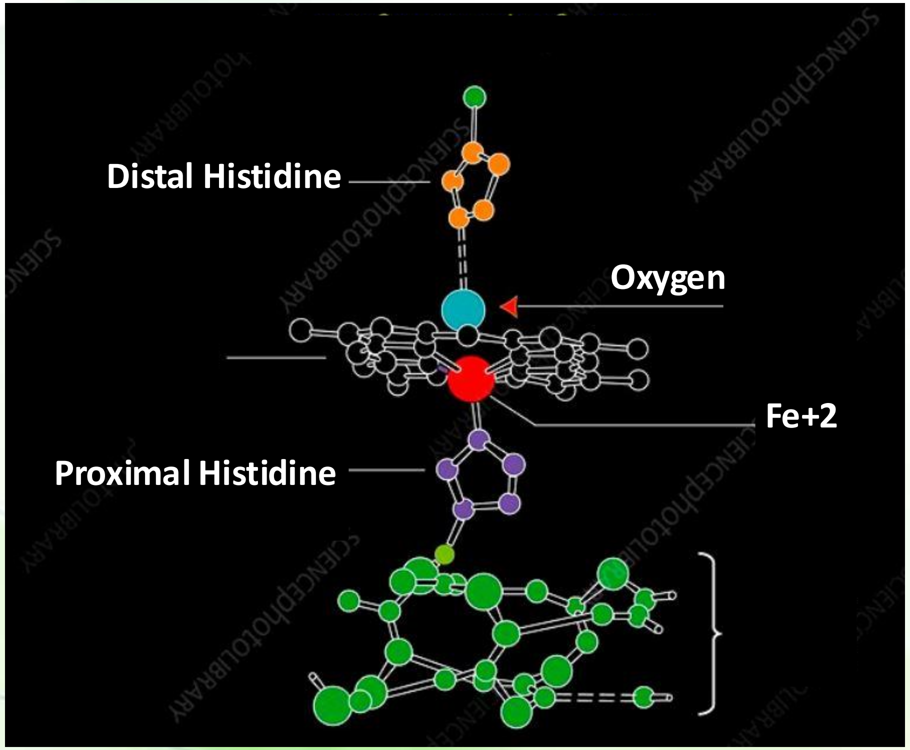


Reformation of hydrogen bonds

- Note: It is not the sliding that causes the Oxygen to bind more strongly to the other subunits. It is the total 3-D conformational change that does so. It could be a change to the binding site on each subunit where the Fe+2 becomes situated closer to the plane of the heme, or it could be a better opening of the Oxygen gate to allow better passage of Oxygen, or both.

Last piece of information about the structure

- There is another Histidine called the distal Histidine that has several functions:
  - It works as a gate keeper that allows the passage of Oxygen only.
  - It binds to Oxygen and prevents it (the Oxygen) from oxidizing the Fe+2 (Ferrous) iron to Fe+3 (Ferric) by stabilizing the Oxygen (sharing an electron).



3- The 2 models of cooperativity

Oxygen saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
  - It is cooperative.
- At 100 mm Hg (torr), hemoglobin is 97% saturated (oxyhemoglobin).
- As the oxygen pressure falls to 40 torr at rest in the tissues, hemoglobin is saturated at 77%.
- With exercise where the oxygen drops to 20 torr, hemoglobin becomes at 37% saturation which is a big drop to satisfy tissues' needs at exercise.
- *Note: at high altitude (~5000 m), alveolar pO2 = 75 mmHg.*

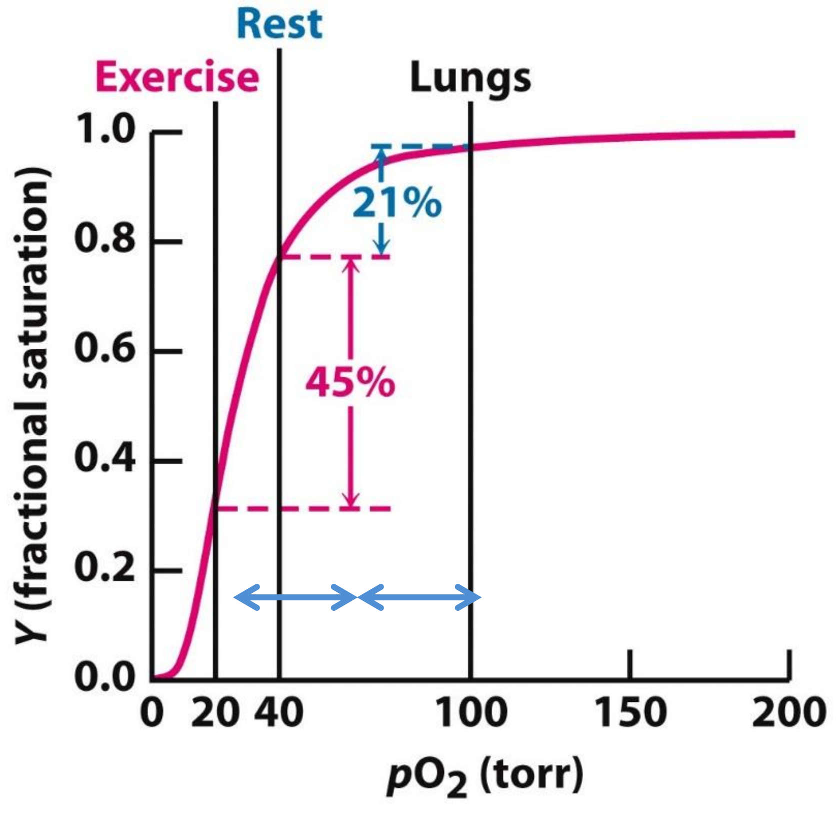


Figure 7.10
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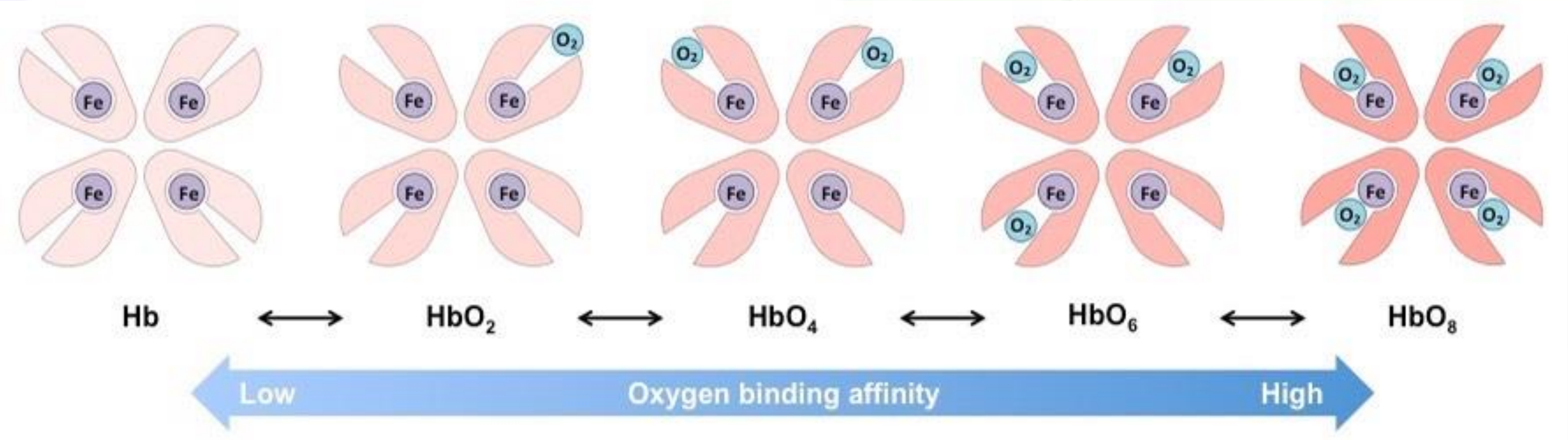
Oxygen saturation curve

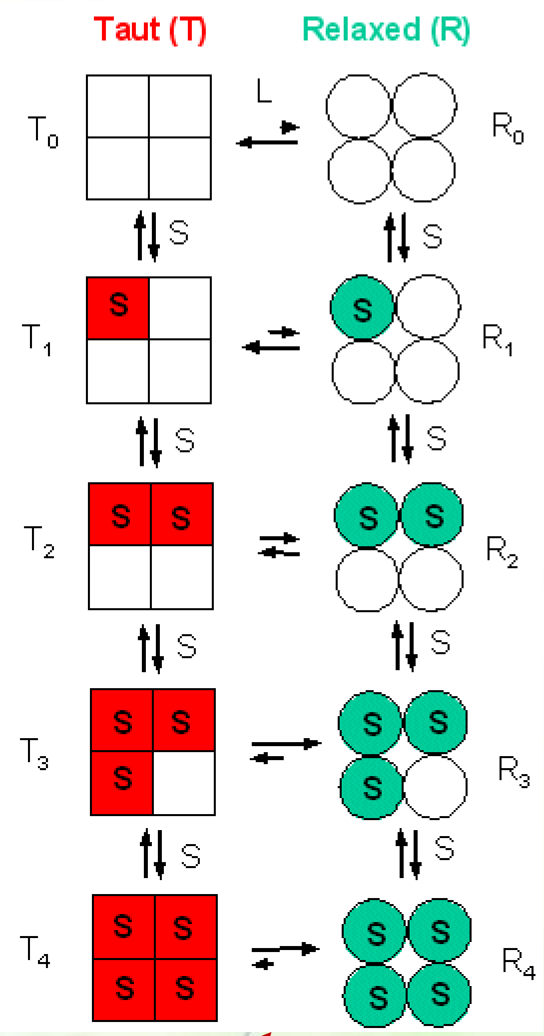


- Increasing ligand concentration drives the equilibrium between R and T toward the R state (**positive cooperativity) sigmoidal curve**
- The effect of ligand concentration on the conformational equilibrium is a **homotropic effect (oxygen)**.
- Other effector molecules that bind at sites distinct from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

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 for the hemoglobin as a whole.
- Sequential model – the subunits undergo the conformational change one at a time.
  - There are multiple states between full T and full R for the hemoglobin as a whole.

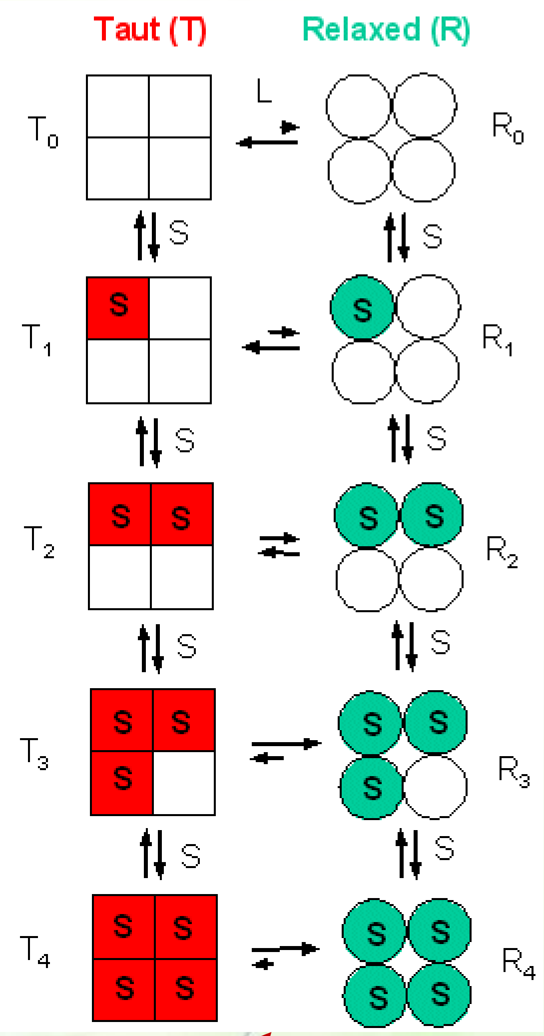
The concerted model (MWC model)



Note direction of arrows

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.



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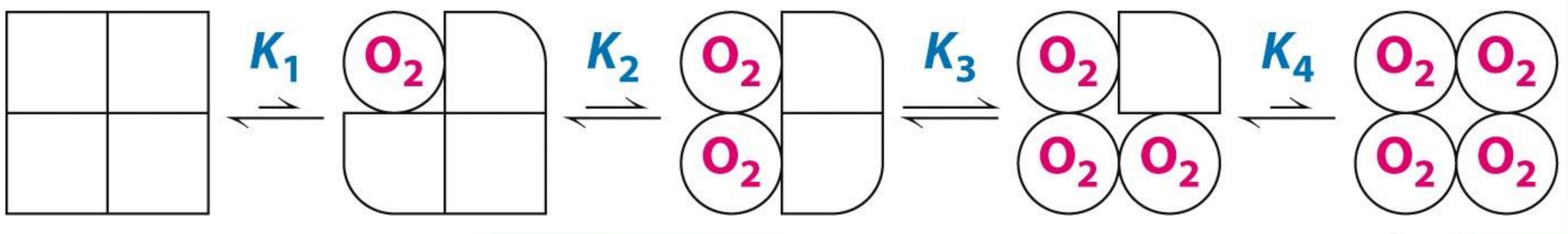
The sequential, induced fit, or KNF model



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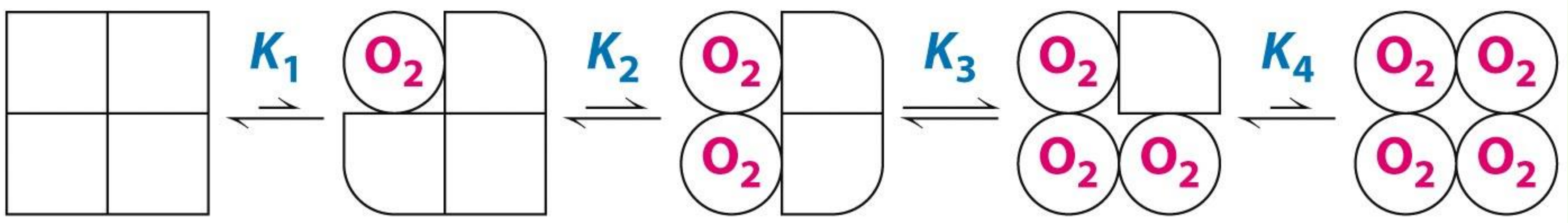


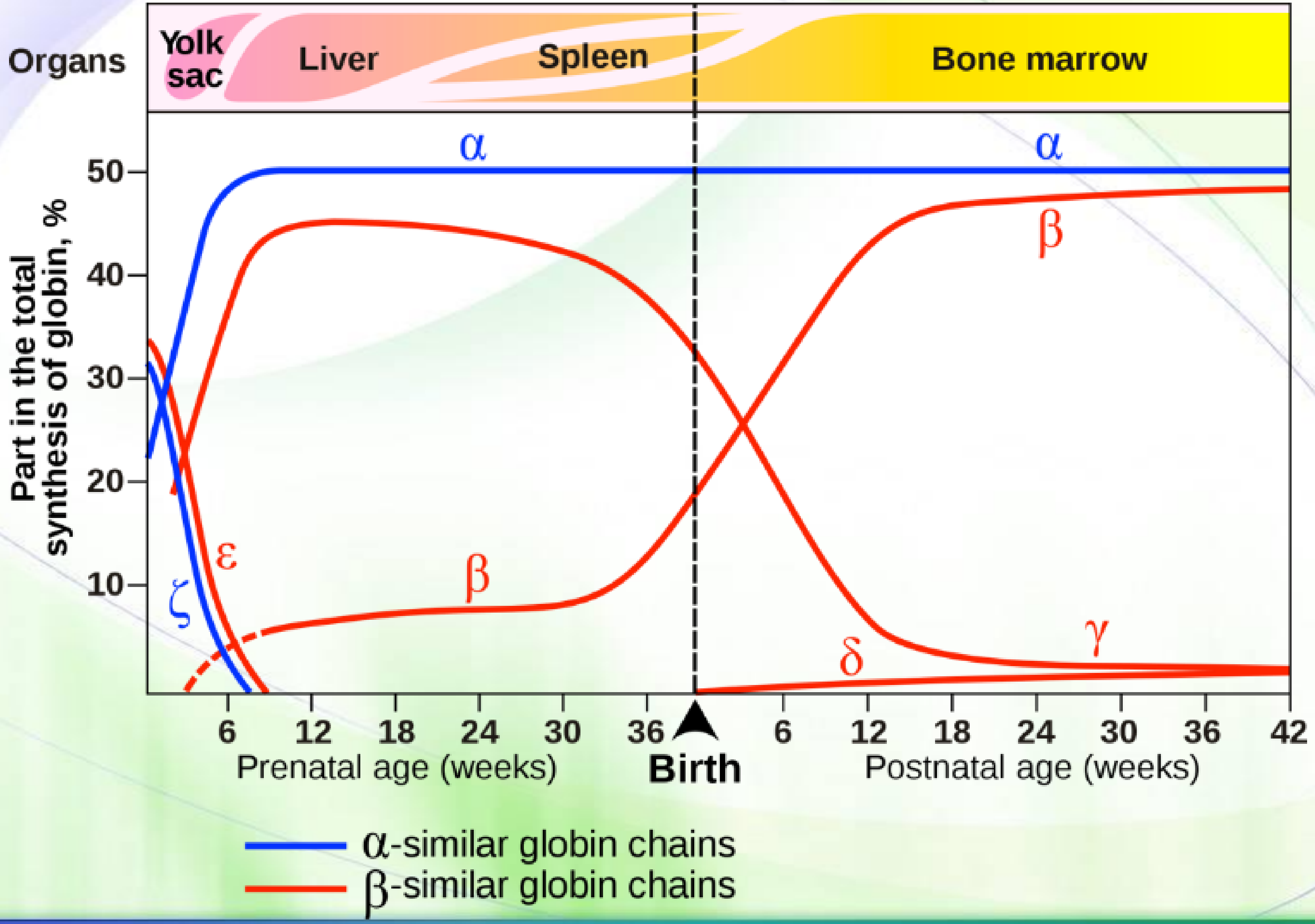
- The subunits go through conformational changes independently of each other, but they make the other subunits more likely to change, by reducing the energy needed for subsequent subunits to undergo the same conformational change.

The sequential, induced fit, or KNF model

- Which one is better? Both can explain the sigmoidal binding curve.
- It seems that binding one Oxygen to one subunit puts strain on that subunit and the surrounding subunits. Binding of a second Oxygen adds the strain further. This causes the whole molecule to snap to the R conformation.
- This might be the connection between both models, with the answer being a bit closer to the sequential model.

4- Types of Hemoglobin

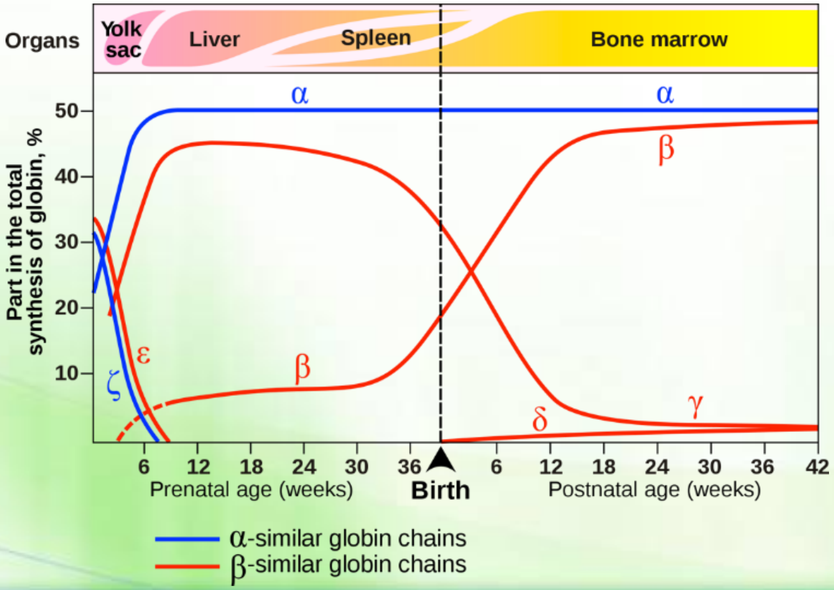
Developmental transition of hemoglobins



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

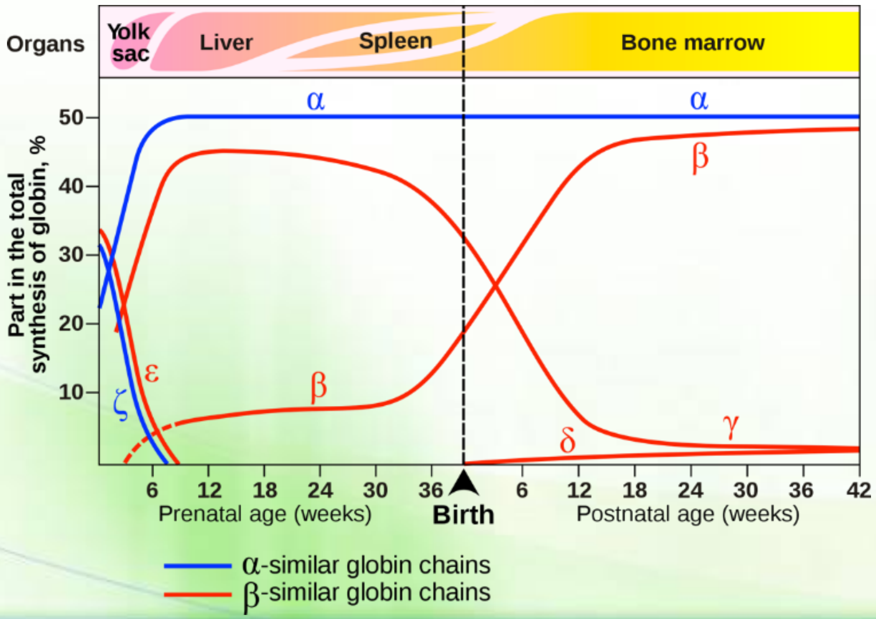
The embryonic stage

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



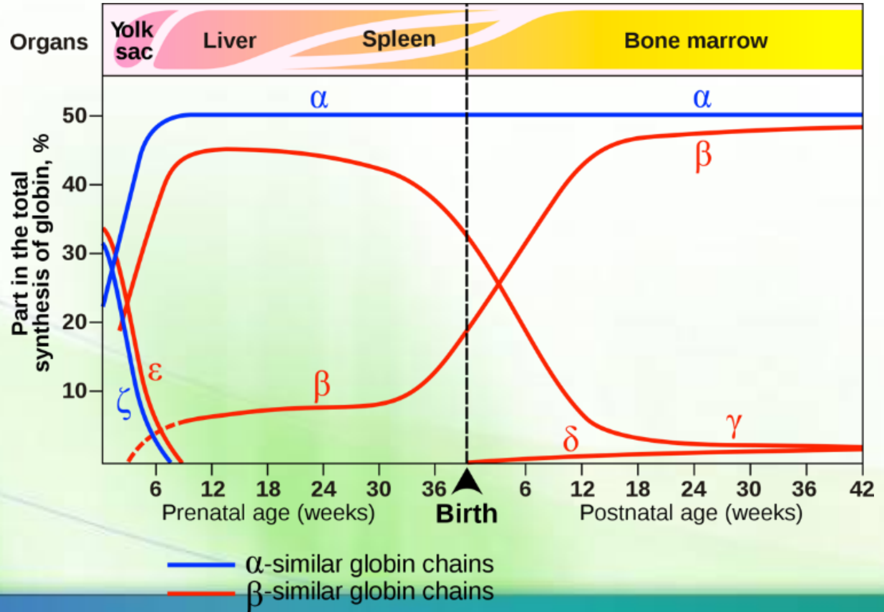
The fetal stage

- 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.



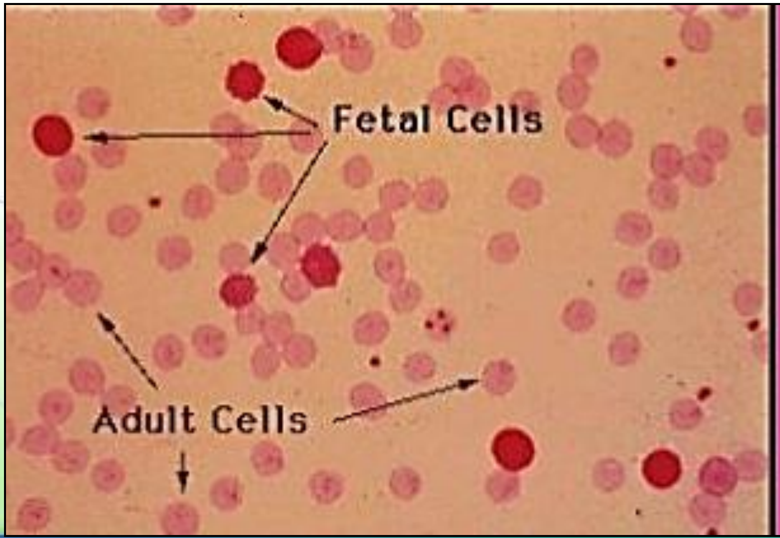
The adult stage

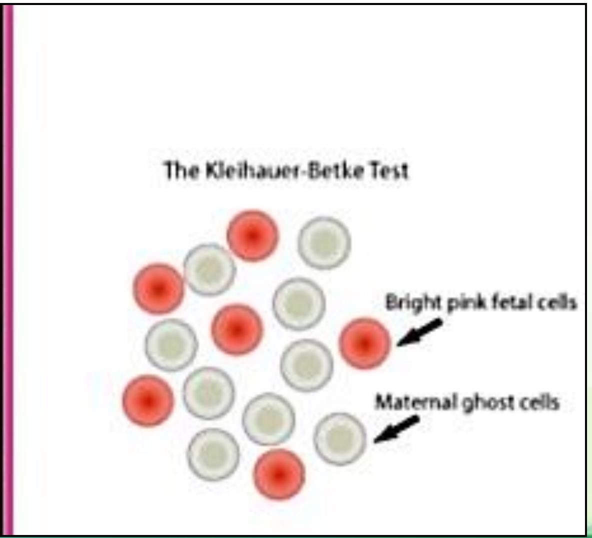
- Shortly before birth, there is a gradual switch to adult β-globin.
- Still, HbF makes up 60% of the hemoglobin at birth, but 1% of adults.
- At birth, synthesis of both γ and β chains occurs in the bone marrow.
- The major hemoglobin is HbA1 (a tetramer of 2 α and 2 β chains).
  - A minor adult hemoglobin,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



Testing fetal RBCs in mother's blood

- The Kleihauer Betke tests for fetal RBCs. An acid is added which causes the adult hemoglobin to wash away leaving fetal hemoglobin. When the sample is stained, it shows the fetal cells in bright pink while adult cells as being pale.
- It is used to determines the amount of fetal cells in the mother's blood (for anemia in the fetus, Rh factor)

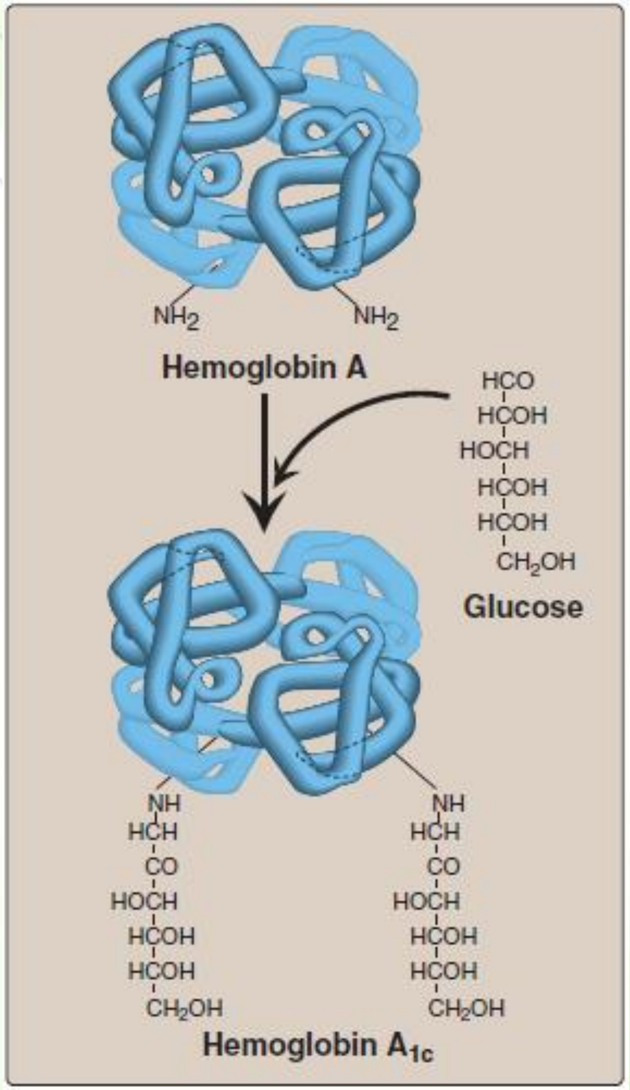




5- Hemoglobin for detecting Diabetes

Adult hemoglobins and diabetes

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



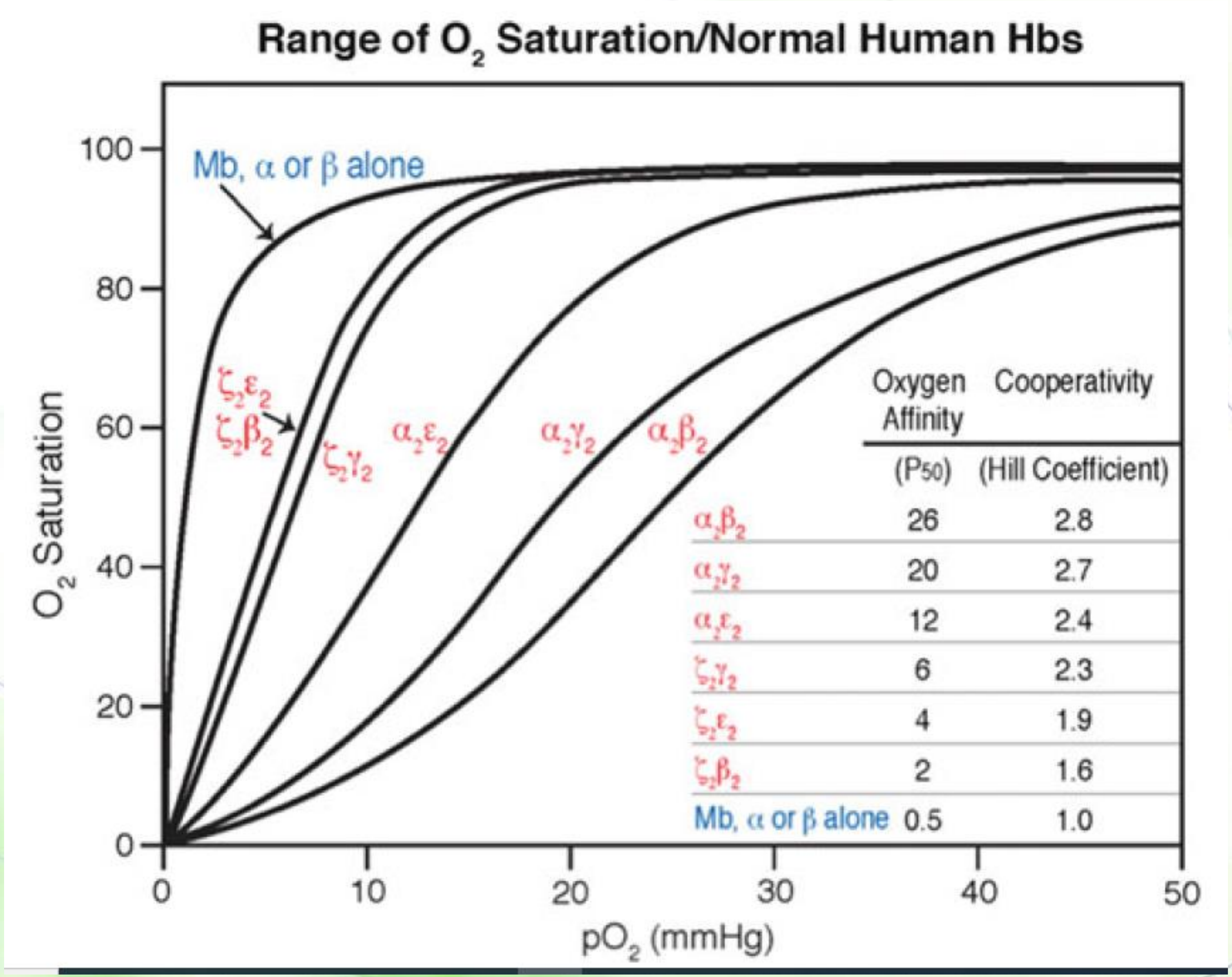
Advantages of HbA1c testing

- Blood fasting glucose level is the concentration of glucose in blood at a single point in time when fasting for a few hours.
- **HbA1c** level provides a longer-term trend, similar to an average, of how high blood sugar levels have been over a period of time (2-3 months) as the lifecycle of the RBCs is around 120 days.
- 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	$\geq 6.5\%$	> 125 mg/dL	≥ 200 mg/dL

Different Hemoglobin types binding to Oxygen

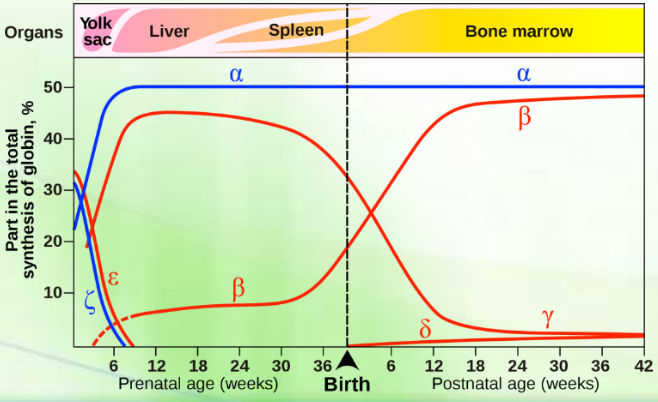


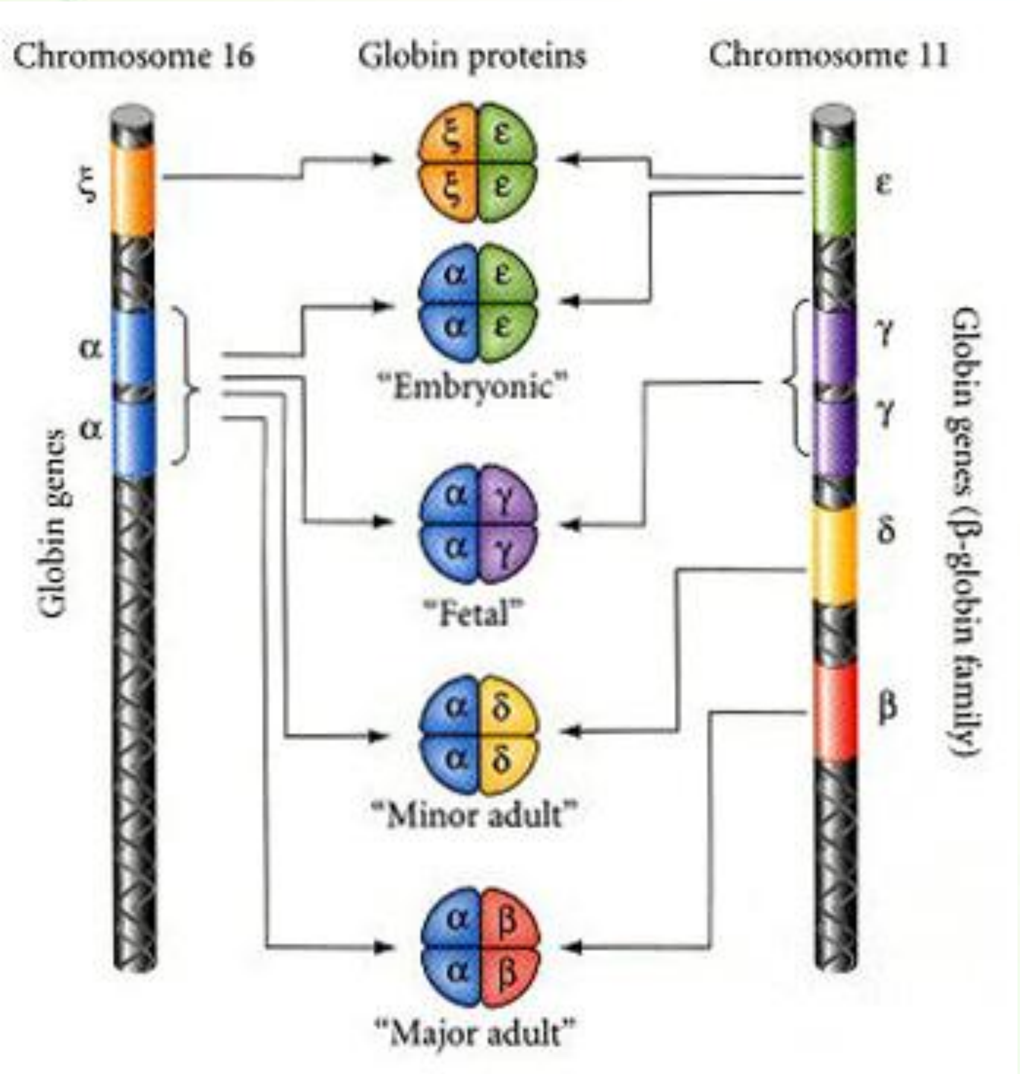
What are we covering

6- The Genetics of Globin synthesis

The genes

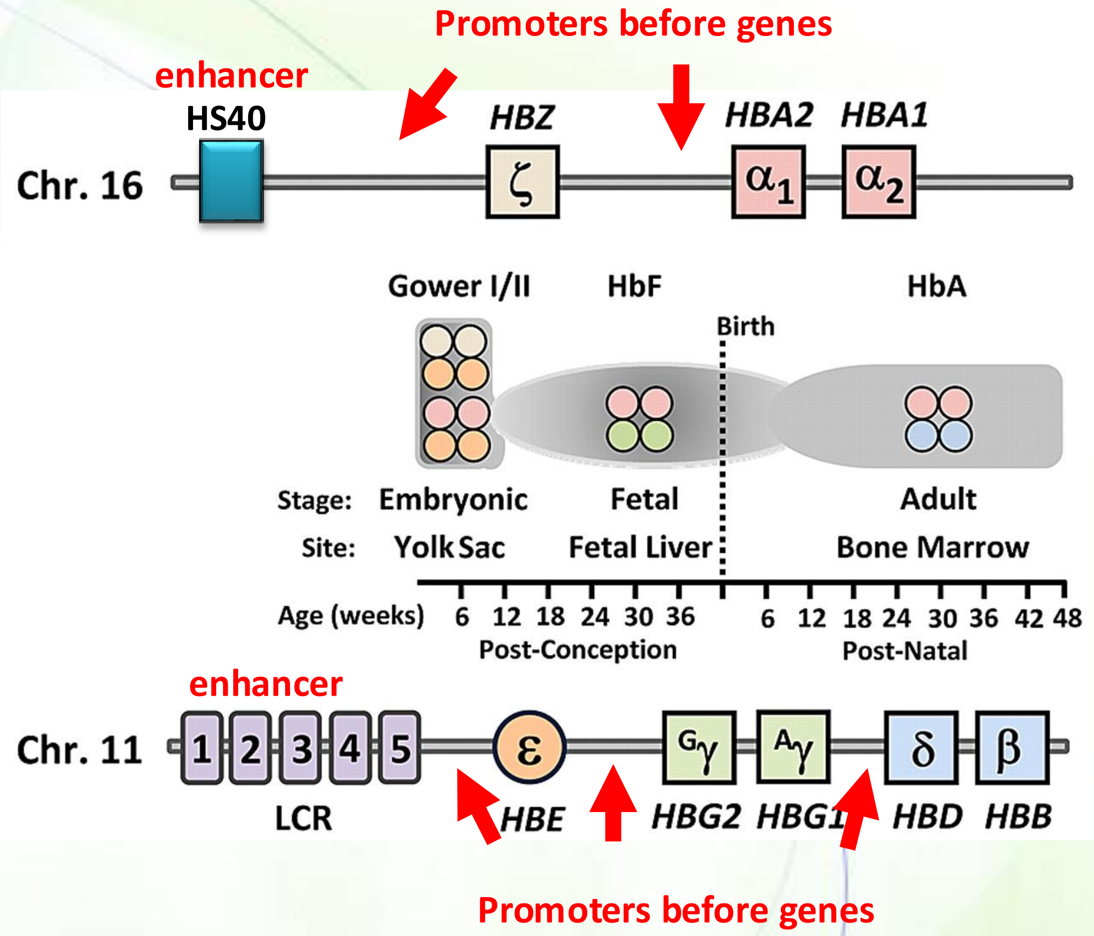
- The α gene cluster contains three genes: two α genes (α1 and α2 (they are similar)), and ζ (zeta) gene.
- The β gene cluster contains five genes: β gene, ε (epsilon) gene, two similar γ (gamma) genes, and δ (delta) gene.
- Genetic switching is controlled by a transcription factor-dependent developmental clock, independent of the environment.
- *Premature newborns follow their gestational age.*





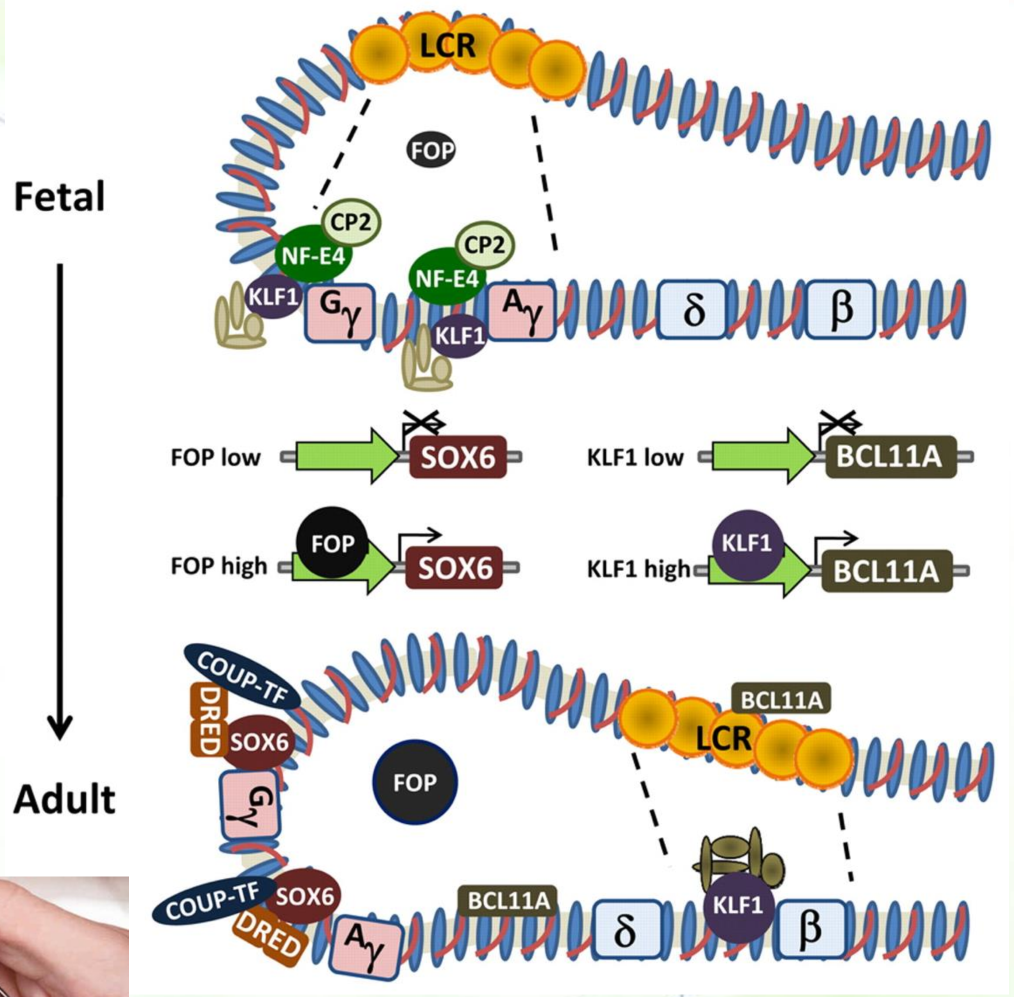
Locus structure

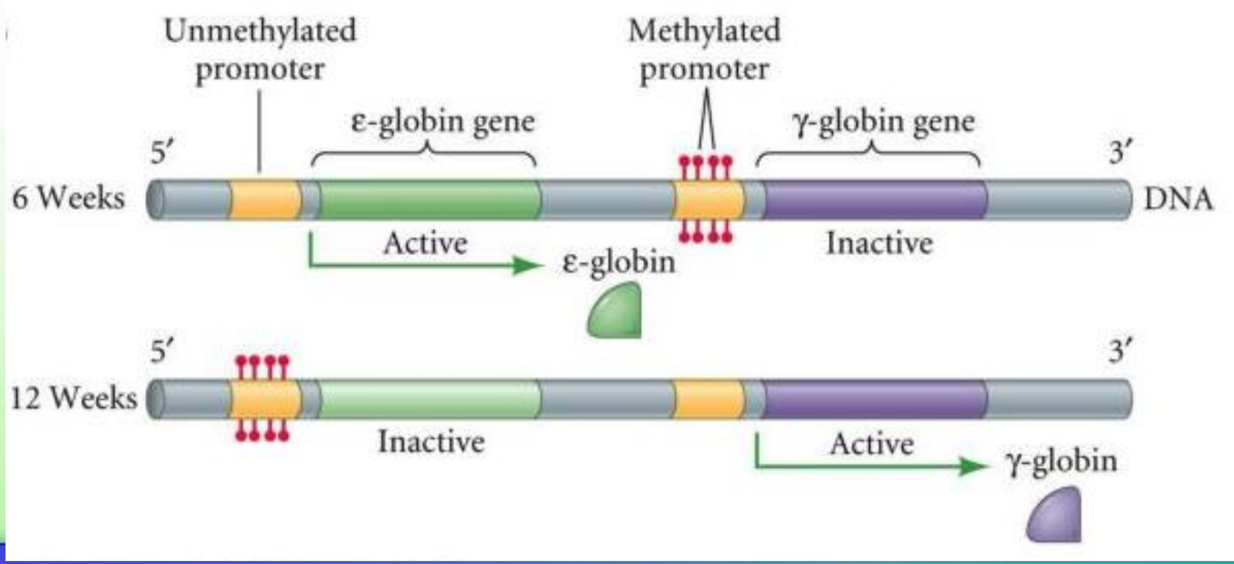
- Each gene has its promoter and regulatory sequences (enhancer, silencers) that are bound to proteins (activators and repressors respectively)
- The α gene cluster is controlled by the HS40 region (enhancer).
- The β-globin cluster is controlled by a master enhancer called locus control region (LCR).



The mechanism of regulation

- The mechanism requires *timed* expression of regulatory transcription factors for each gene, epigenetic regulation (e.g., acetylation, methylation), chromatin looping, and non-coding RNA (e.g., long non-coding RNA, microRNA, etc.).





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Activation of γ-globin expression by hypoxia-inducible factor 1α

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Abstract

Around birth, globin expression in human red blood cells (RBCs) shifts from γ-globin to β-globin, which results in fetal haemoglobin (HbF, $\alpha_2\gamma_2$) being gradually replaced by adult haemoglobin (HbA, $\alpha_2\beta_2$)[1]. This process has motivated the development of innovative approaches to treat sickle cell disease and β-thalassaemia by increasing HbF levels in postnatal RBCs[2]. Here we provide therapeutically relevant insights into globin gene switching obtained through a CRISPR–Cas9 screen for ubiquitin–proteasome components that regulate HbF expression. In RBC precursors, depletion of the von Hippel–Lindau (VHL) E3 ubiquitin ligase stabilized its ubiquitination target, hypoxia-inducible factor 1α (HIF1α)[3,4], to induce γ-globin gene transcription. Mechanistically, HIF1α–HIF1β heterodimers bound cognate DNA elements in *BGLT3*, a long noncoding RNA gene located 2.7 kb downstream of the tandem γ-globin genes *HBG1* and *HBG2*. This was followed by the recruitment of transcriptional activators, chromatin opening and increased long-range interactions between the γ-globin genes and their upstream enhancer. Similar induction of HbF occurred with hypoxia or with inhibition of prolyl hydroxylase domain enzymes that target HIF1α for ubiquitination by the VHL E3 ubiquitin ligase. Our findings link globin gene regulation with canonical hypoxia adaptation, provide a mechanism for HbF induction during stress erythropoiesis and suggest a new therapeutic approach for β-haemoglobinopathies.