بسم الله الرحمن الرحيم

Biochemistry

﴿ وَقُل رَّبِّ أَدْخِلْنِي مُدْخَلَ صِدْقٍ وَأَخْرِجْنِي مُخْرَجَ صِدْقٍ وَاجْعَل لِّي مِن لَّدُنكَ سُلْطَانًا نَّصِيرًا ﴾

ربنا آتنا من لدنك رحمة وهيئ لنا من أمرنا رشدًا

MID | Lecture 1

Hemoglobin Overview (pt.1)

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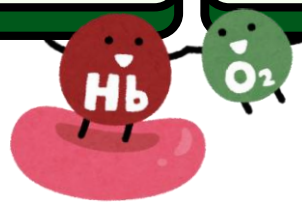
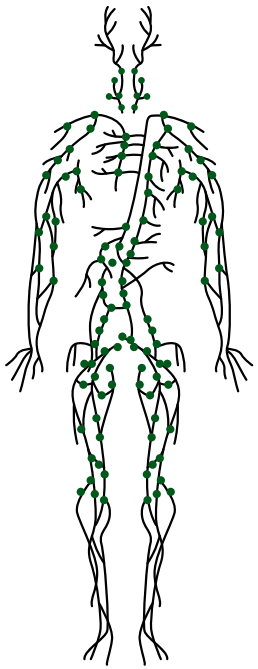




Feel free to use it as a summary.

Jog Your memory, ft. Summer Biochemistry course(1)

- **Cooperative binding:** Binding of one O_2 ↑ affinity for the next → sigmoidal curve.
  - **Mechanism:** O_2 binding breaks salt bridges → conformational shift (T → R state).O_2 = homotropic effector (substrate = modulator).
- **Allosteric types:**
  - Homotropic: Effector = ligand (O_2).
  - Heterotropic: Effector ≠ ligand (H^+, BPG).
- **Positive**: ↑ affinity; **Negative**: ↓ affinity.
- **Concerted model (MWC):** All subunits switch T↔R together; equilibrium shifts toward R as O_2 binds.
- **Sequential model (KNF):** Subunits change one at a time → intermediate states.

Jog Your memory, ft. Summer Biochemistry course(2)

- **Hemoglobin forms:**
  - **E**mbryonic (Hb**E** $\zeta_2\varepsilon_2$ – Major) – early yolk sac.
  - **F**etal (Hb**F** $\alpha_2\gamma_2$) – higher O_2 affinity; dominant before birth.
  - **A**dult (Hb**A1** $\alpha_2\beta_2$, Hb**A2** $\alpha_2\delta_2$) – main postnatal forms.
- **O_2 affinity trend:** HbE > HbF > HbA.
  - ➢ This explains fetal O_2 uptake from mother.
- **HbA1c:** Glycosylated HbA; ↑ in diabetes; reflects long-term glucose trend (2–3 months).

عذرًا، المو ديفايد تأخر بسبب تأخر نزول السلايدات، وإعادة تصوير المحاضرة في سكشن آخر.
المحاضرة سهلة إن شاء الله وأخذناها بالفصل الصيفي بسنة أولى فبإذن الله بتخلص بسرعة، بالتوفيق.

Hemoglobin
An overview and more

Prof. Mamoun Ahram,
Hematopoietic-lymphatic system
Minor edits by **Dr. Motamed Qadan**

Resources

- This lecture
  - Myoglobin/Hemoglobin O2 Binding and Allosteric Properties of Hemoglobin (http://home.sandiego.edu/~josephprovost/Chem331%20Lect%207_8%2_0Myo%20Hemoglobin.pdf)
  - Lecture 3: Cooperative behaviour of hemoglobin (https://www.chem.uwec.edu/chem452_f12/pages/lecture_materials/uni_t_III/lecture-3/overheads/Chem452-lecture_3-part_1-overheads.pdf)

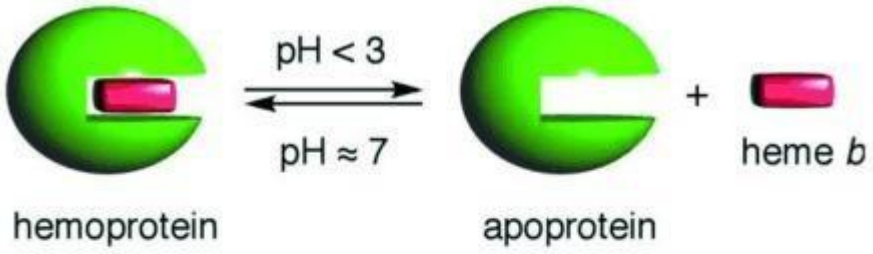
What are we covering

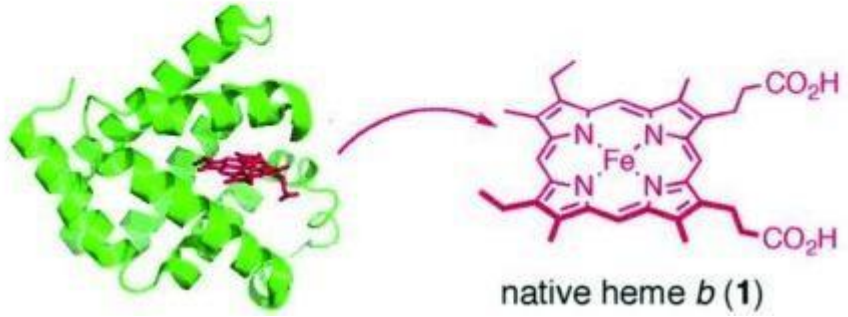
- In this lecture we will be covering:
  1. Heme and Hemoglobin structures **- (slides 7-14).**
  2. Structural changes upon binding Oxygen **- (slides 15-24).**
  3. The 2 models of cooperativity **- (slides 25-33).**
  4. Types of Hemoglobin **- (slides 34-42).**
  5. Hemoglobin for detecting Diabetes **- (slides 43-48).**
  6. The genetics of Globin synthesis **- (slides 49-57).**

1- Heme and Hemoglobin structures

Hemoproteins

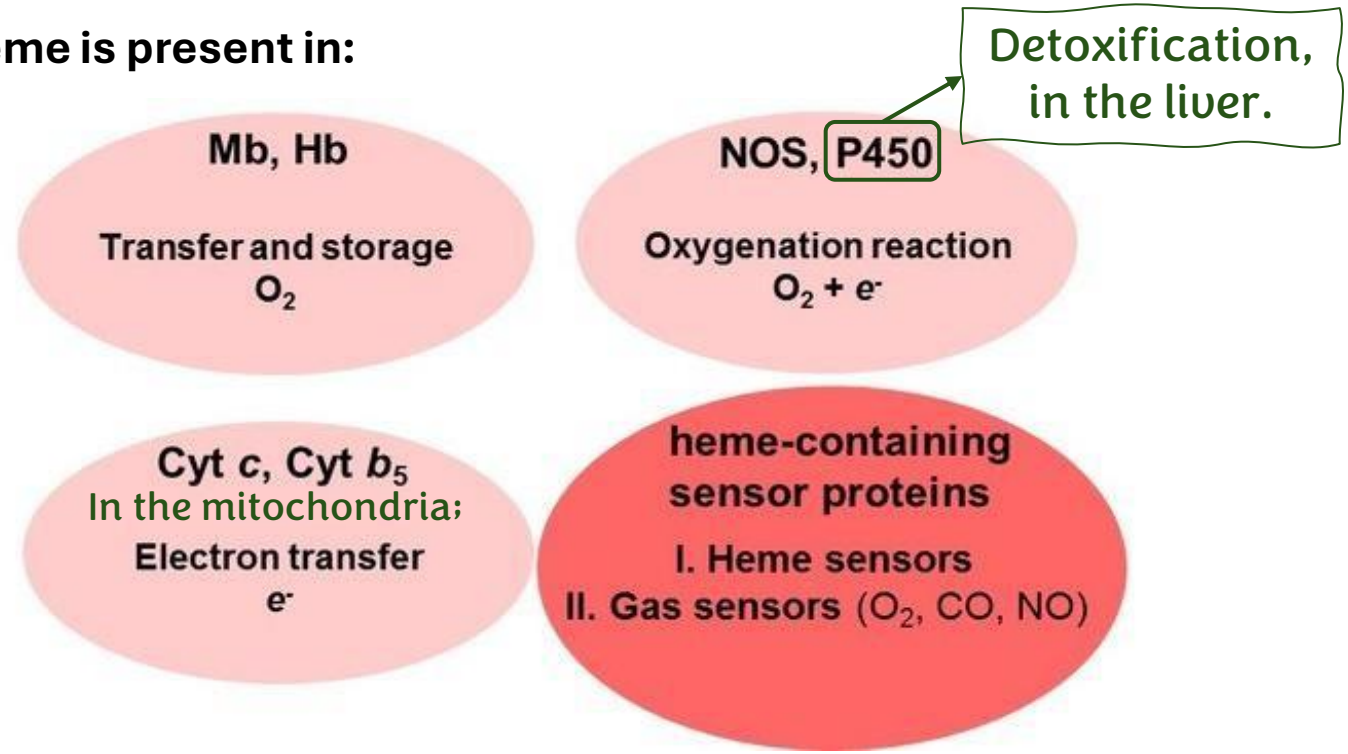
- Many proteins have heme as a prosthetic group called hemoproteins.





- ***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.*

- **Heme is present in:**



Heme structure

❖ The heme is composed of:

1- Ferrous ion (Fe^{+2}), which remains in the (+2) oxidation state, whether it is bound to oxygen or not.

2- Protoporphyrin (which is composed of):

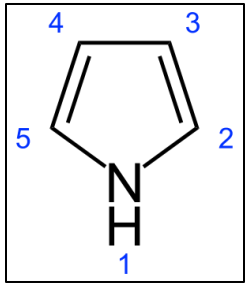
a. 4 Pyrrole rings

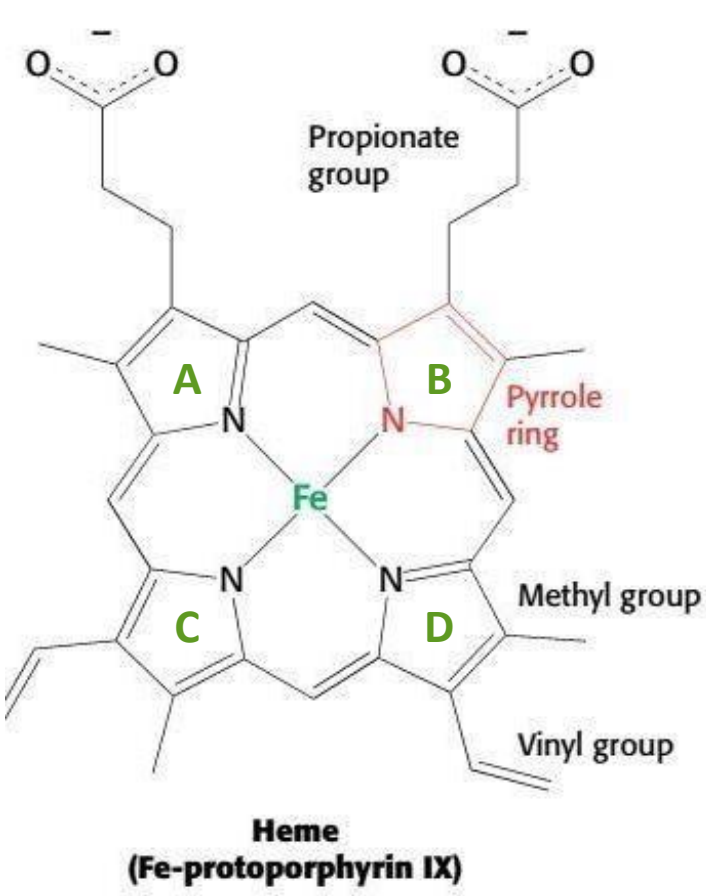
b. Two of the pyrrole rings have a carboxylic group each, named propionate group.

c. Vinyl groups

d. Methyl groups

Pyrrole ring



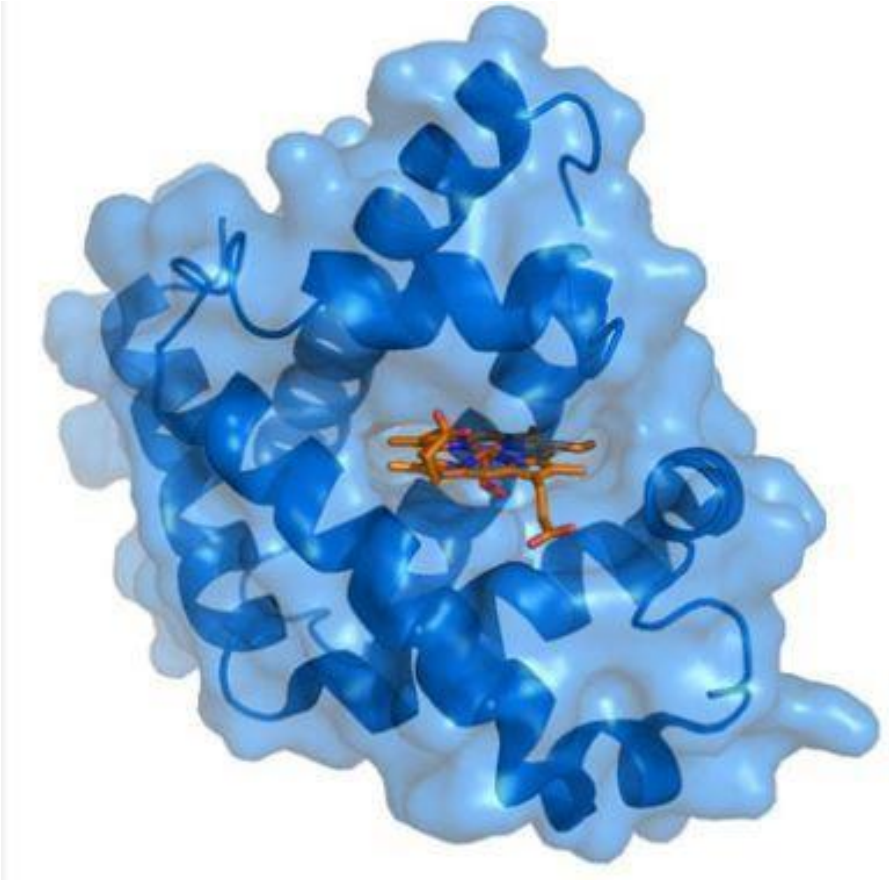


Heme (Fe-protoporphyrin IX)

✓ *Note: the molecule is hydrophobic, regardless –ve charges.*

- Fe has **six** coordinates of binding, four with the nitrogen atoms of the porphyrin rings, one with the proximal histidine residue, and one available for oxygen binding, which is stabilized by the distal histidine.

Heme inside a hydrophobic pocket



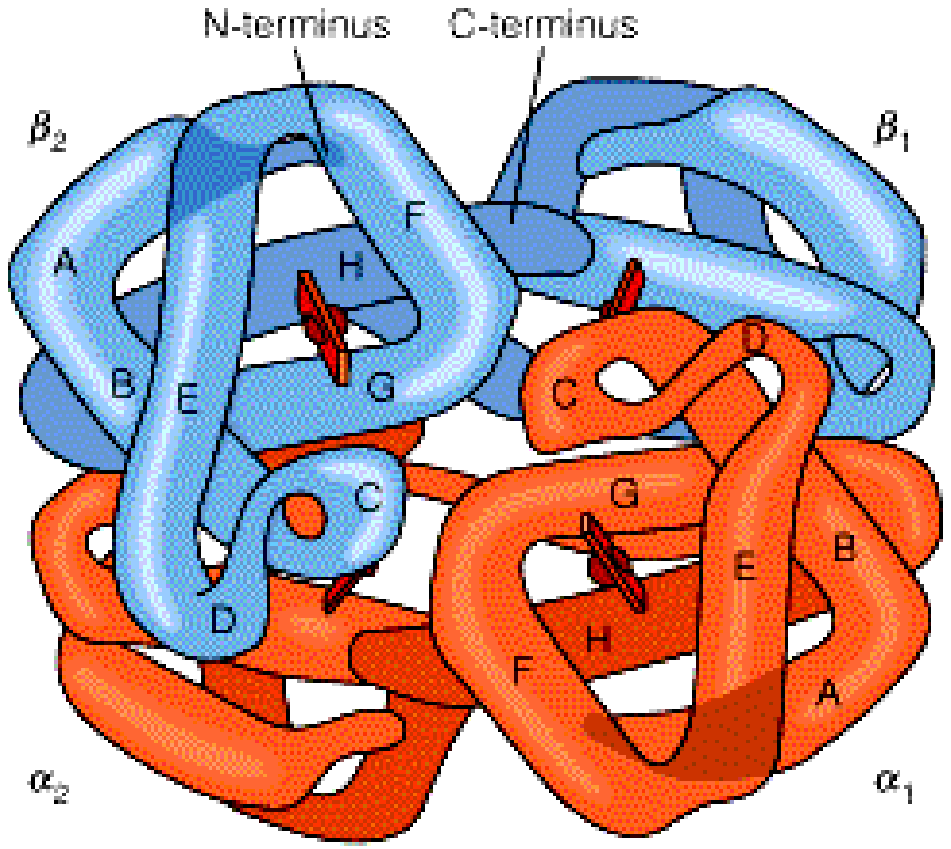
This is myoglobin, as it is composed of only 1 subunit, but the same applies for hemoglobin.

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

Structure of hemoglobin

- Hb is a globular protein, with Multiple subunits (2α + 2β)
  - Each Hb has **4** subunits, a tetramer, each subunit contains one heme group, resulting in a total of four oxygen-binding sites per hemoglobin molecule.
- α polypeptide = 141 amino acids (**Last aa Arg141**).
- **β** polypeptide = 146 amino acids (**Last aa His146**).

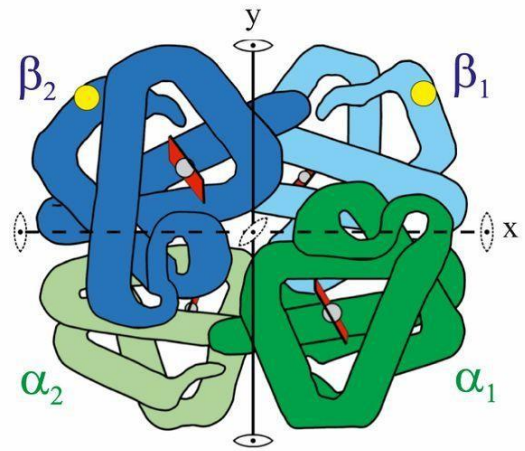
  The first amino acid in both is valine.
- You don't have to memorize the specific amino acids or their sequence numbers. Just remember that **histidine** and **arginine** residues near the C-terminus are **positively charged**, and these positive charges play an important role in **electrostatic interactions** that help **stabilize** the protein's **structure**.
- Positive cooperativity towards oxygen. The last Oxygen has 100-250 fold stronger propensity for binding to hemoglobin than the first hemoglobin. This occurs because the binding of the first oxygen induces conformational changes in hemoglobin that increase its affinity for subsequent oxygen molecules.
- Regulated by allosteric effectors – lecture 2.



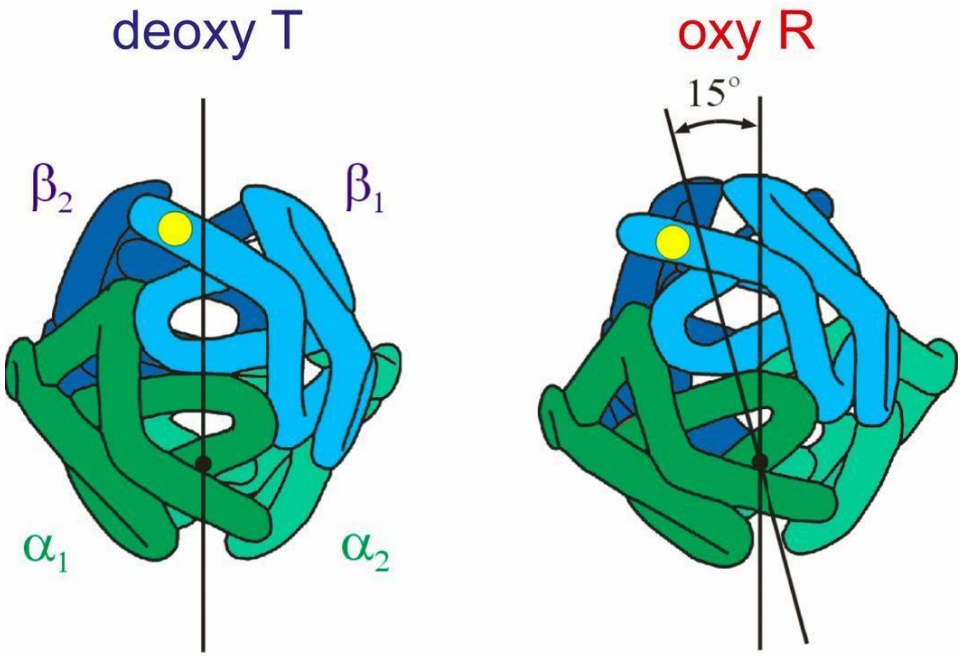
Hemoglobin

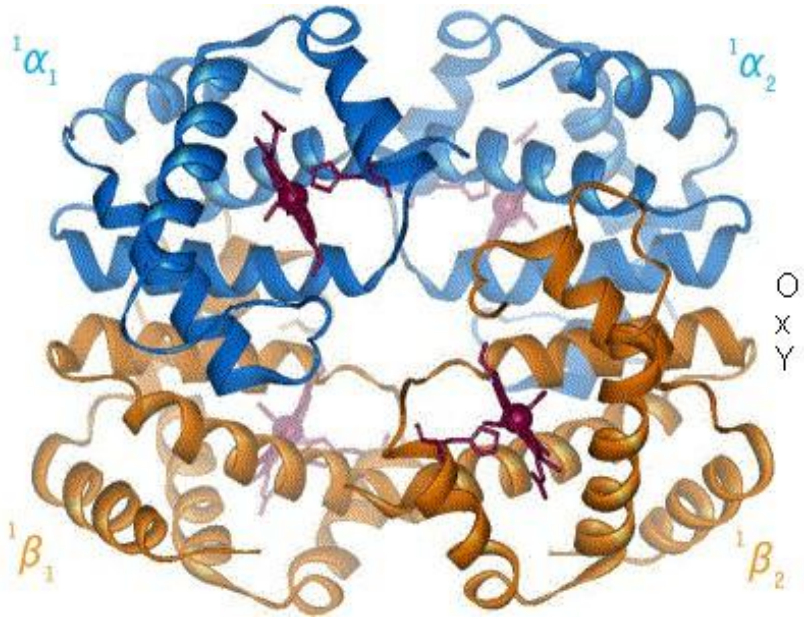
Explanation next slide

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.





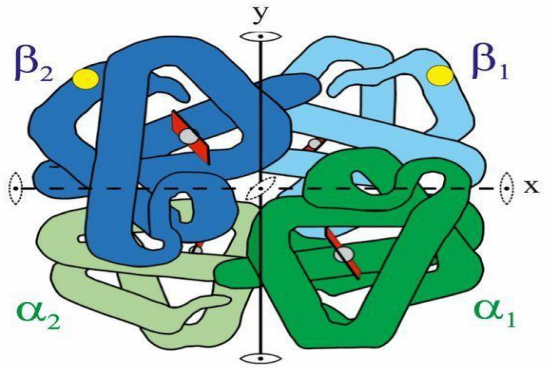
Structural change of hemoglobin – Explanation

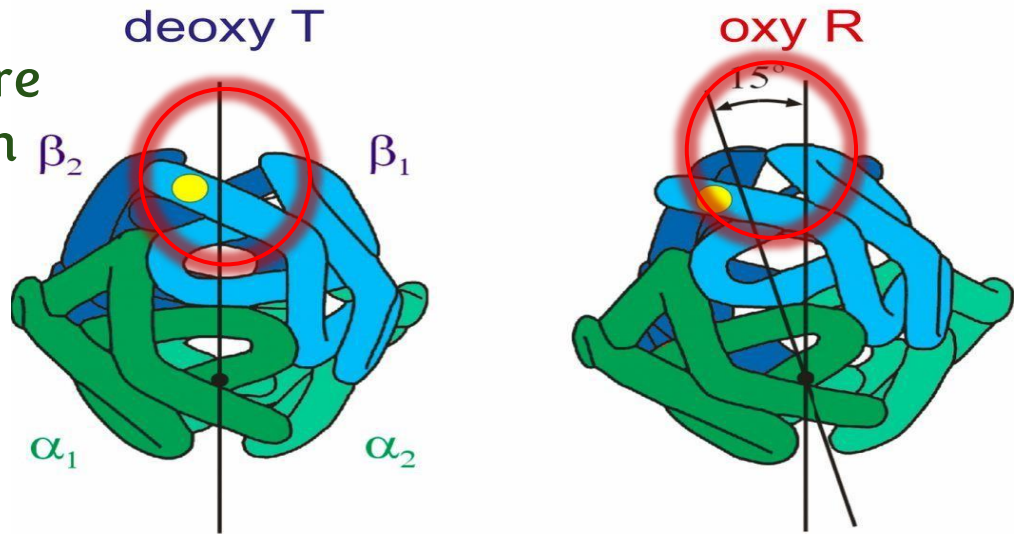
- The representation of hemoglobin as two α and two β subunits is a simplified model, but we will use it for clarity.

❖ Hemoglobin exists in **two** main conformational states:

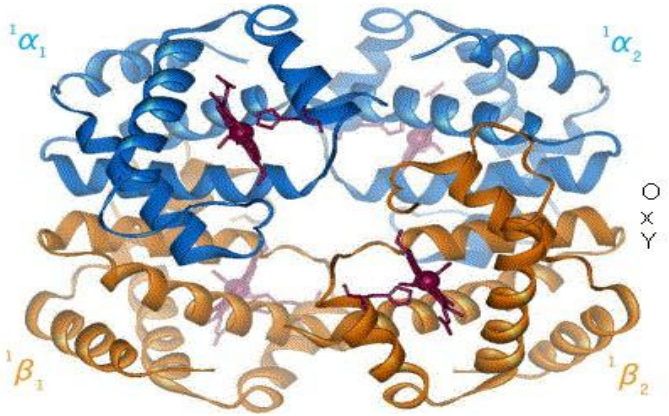
- **Tense (T) state**: the three-dimensional structure is more **open** at the surface, with low affinity.
- **Relaxed (R) state:** the structure is more compact or **closed** at the surface with high affinity.

➢ However, these surface conformational differences are not directly responsible for oxygen binding, as oxygen binds to the heme groups located at the core of the molecule.



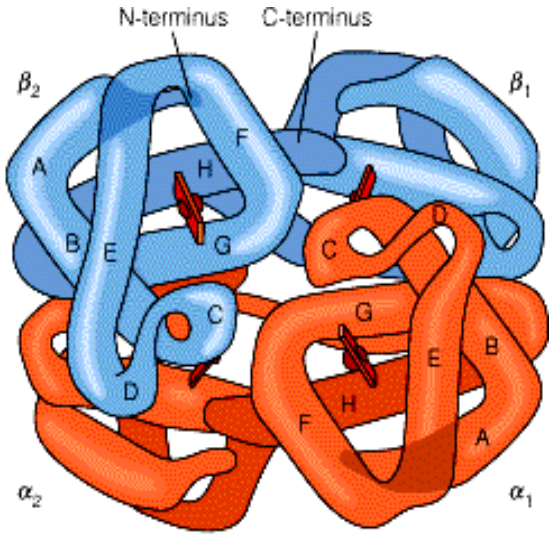


To see structural changes of hemoglobin, click on its structure.

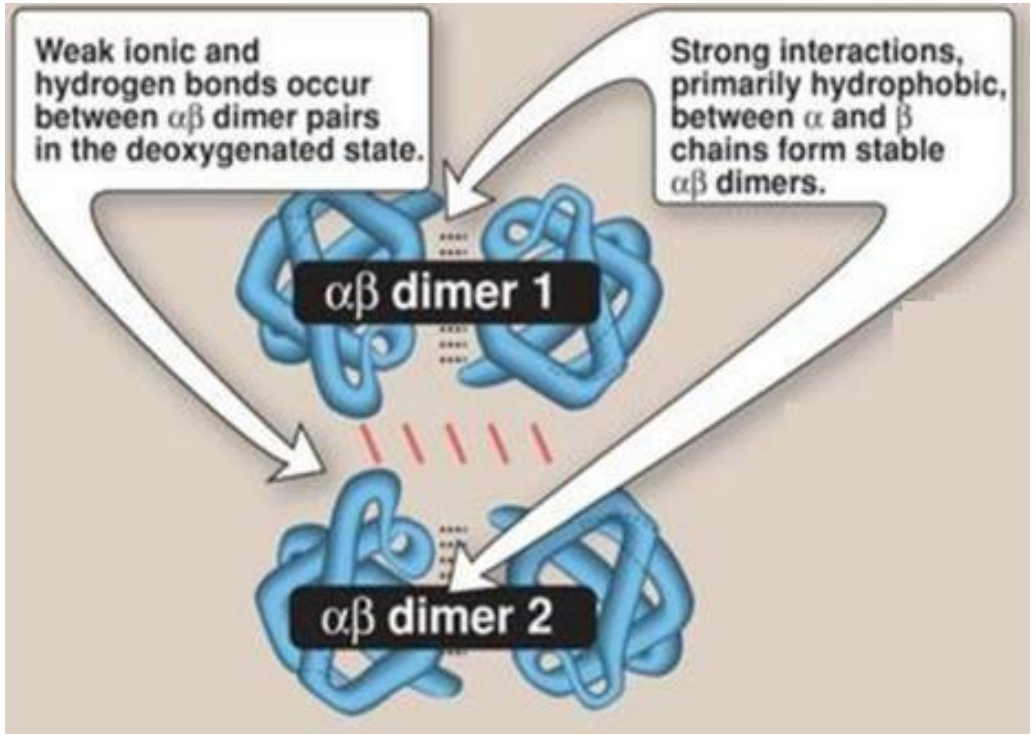


How are the subunits bound?

- A dimer of dimers (I made up this term)
  - $(\alpha–\beta)_2$, each $(\alpha–\beta)$ dimer is stabilized by **hydrophobic** interactions. In addition to some **electrostatic interactions** (such as those involving arginine residues) and hydrogen bonds.
  - Note how they interact with each other.



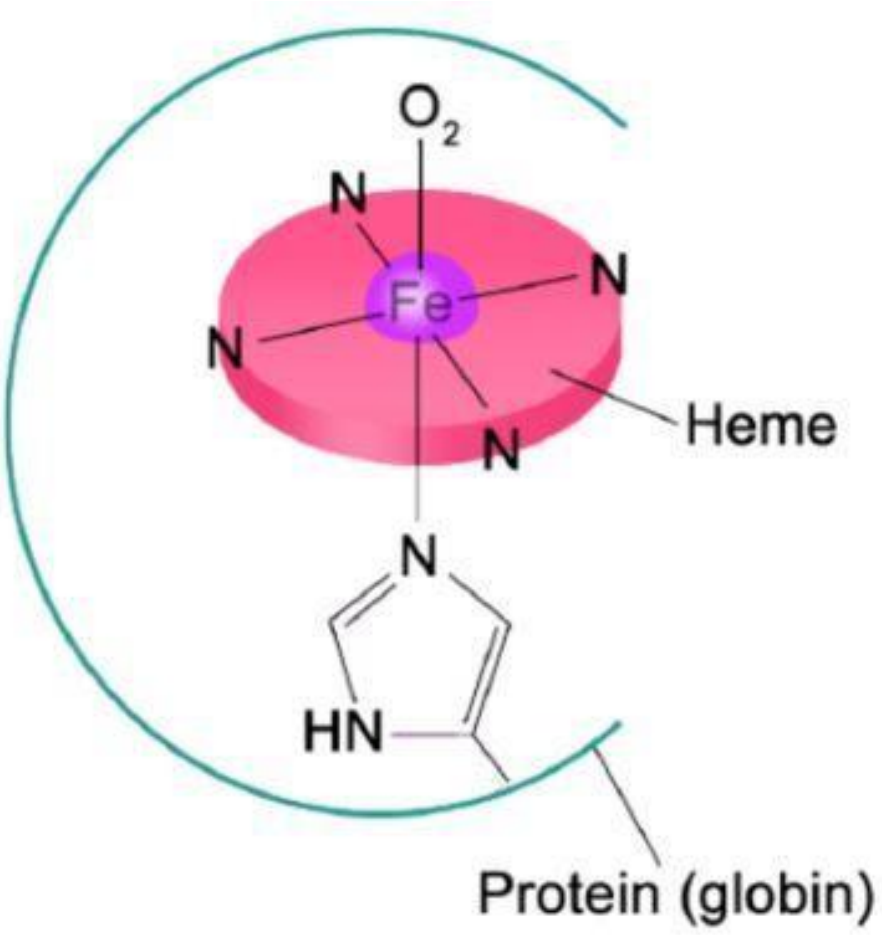
❑ It's important to note that the two α subunits are positioned opposite each other and therefore **interact only weakly**. This arrangement differs from what is shown in the left figure (see slide 20).



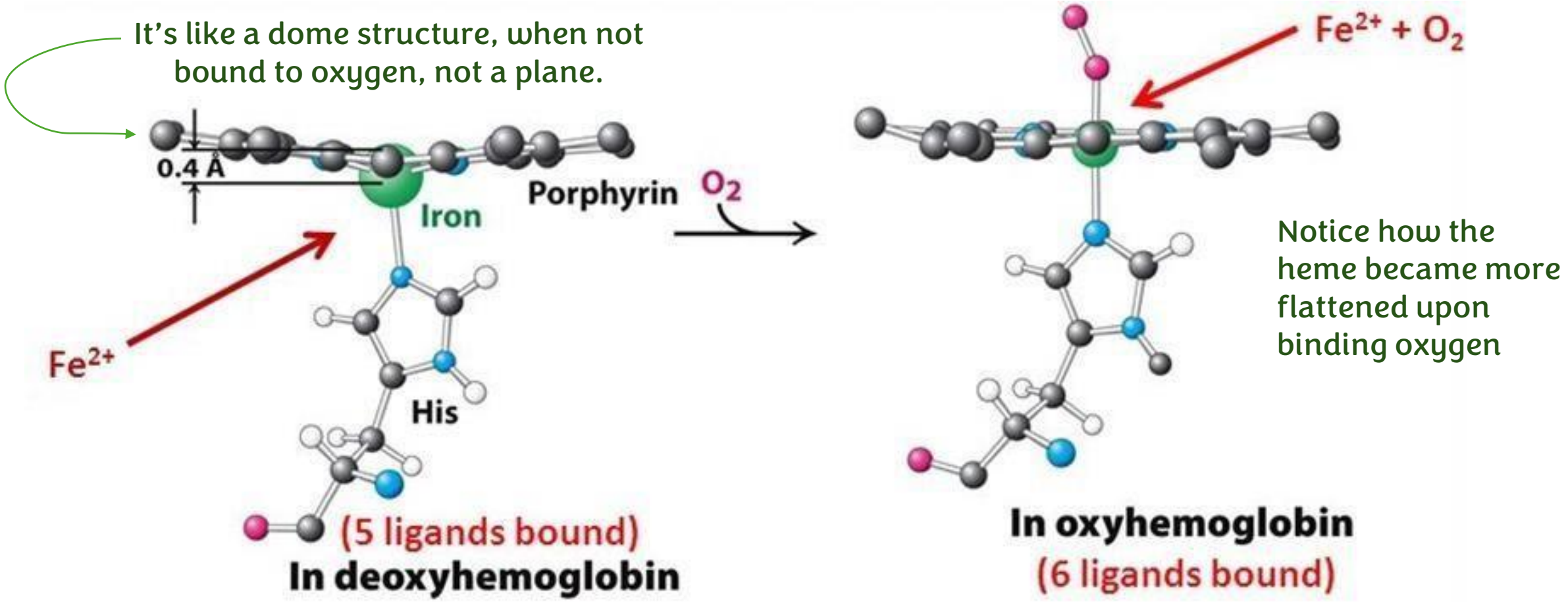
2- Structural changes upon binding Oxygen

Fe^{+2} attachments

- Fe^{+2} makes 5 bonds initially.
  - 4 with the protoporphyrin and the 5th with the globin protein through the **proximal Histidine (His8)**, it is a covalent bond (This is a very important bond, as it is **responsible** for the conformational changes that **increase hemoglobin's affinity for oxygen**).
  - Proximal histidine is at position 8 but the doctor said it's at position 9.
- In the presence of Oxygen, Fe^{+2} makes a 6th bond with Oxygen.

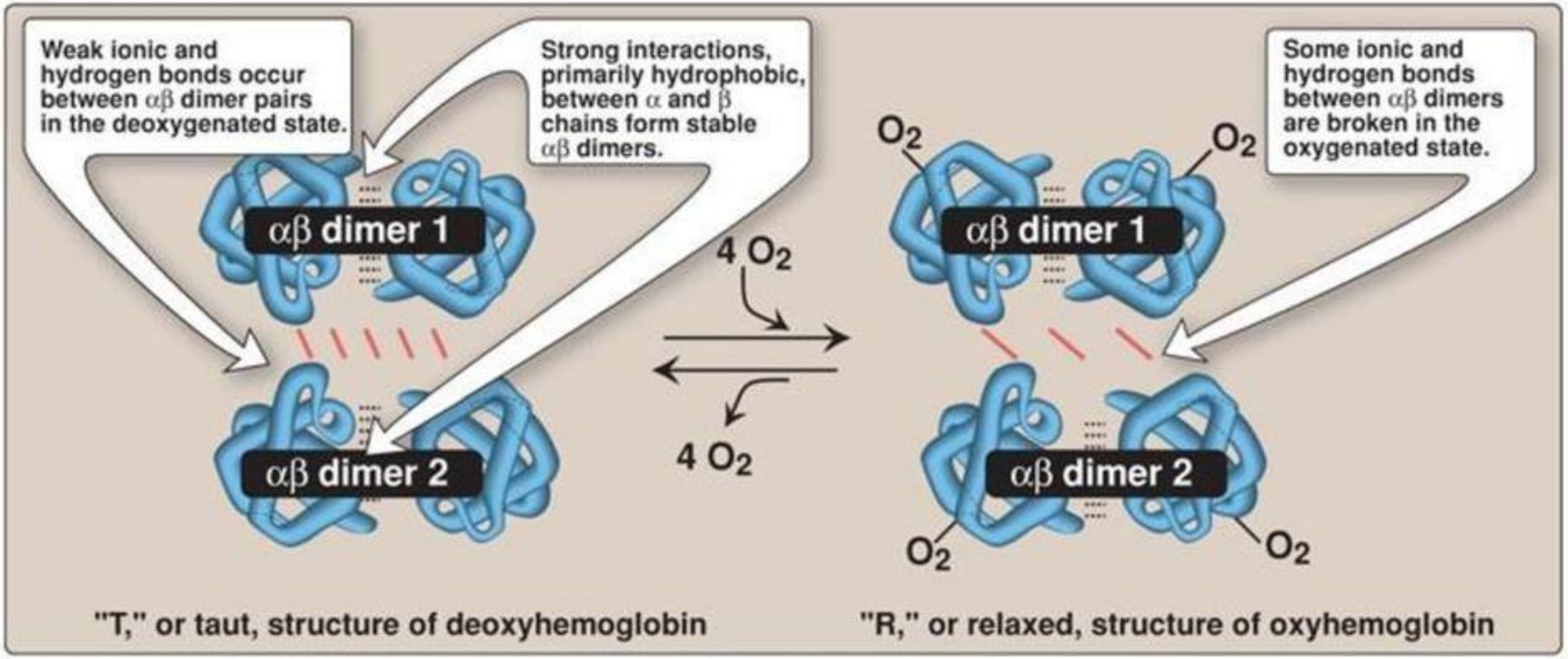


Structural amplification change



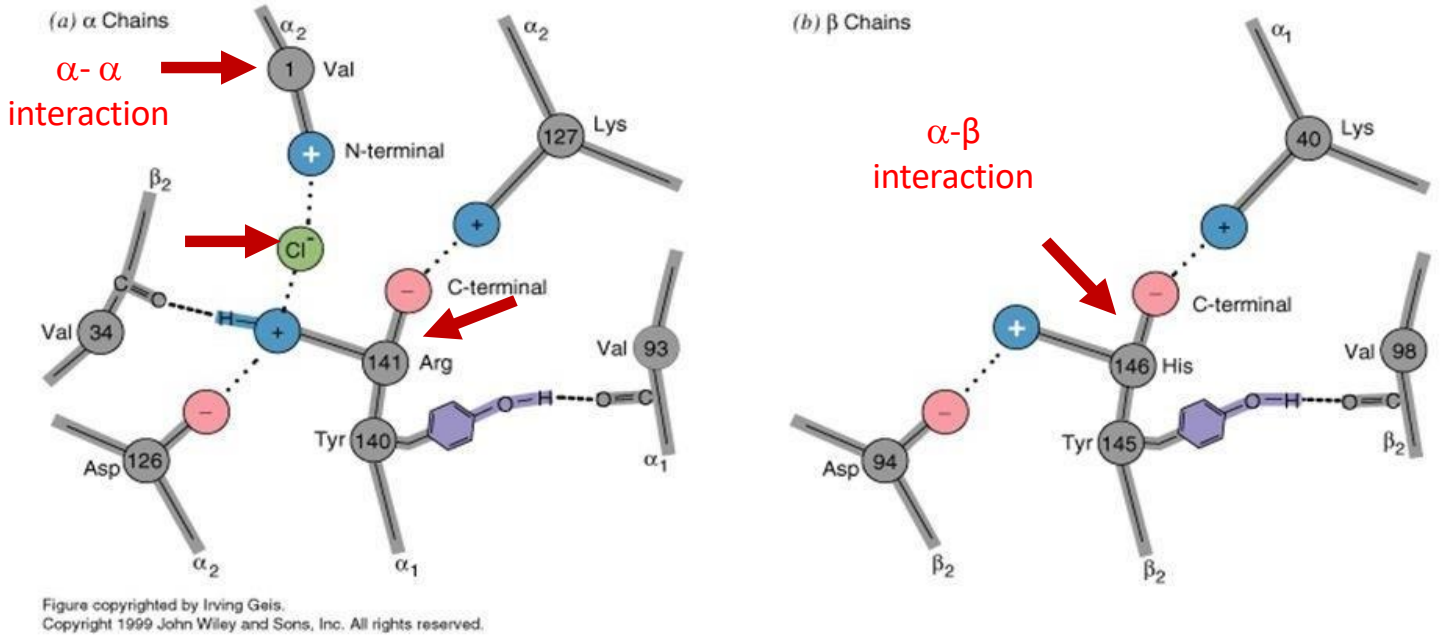
- The small move of Iron upon binding to Oxygen and the pull on the proximal His a.a. propagates through the protein and causes breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.
- It causes a change in the tertiary structure of individual hemoglobin subunits, about 15 Angstrom as a whole, allowing them to bind to Oxygen more readily; increased affinity by 100-250 folds.

Broken electrostatic interactions and H-bonds



The broken bonds

- When no ligand is bound, the T (tense) form is more stable than the R (relaxed) form, however **Oxygen stabilizes the R state.**
- **Electrostatic interactions** especially at the C termini and hydrogen bonds **stabilize the T-form of hemoglobin**.

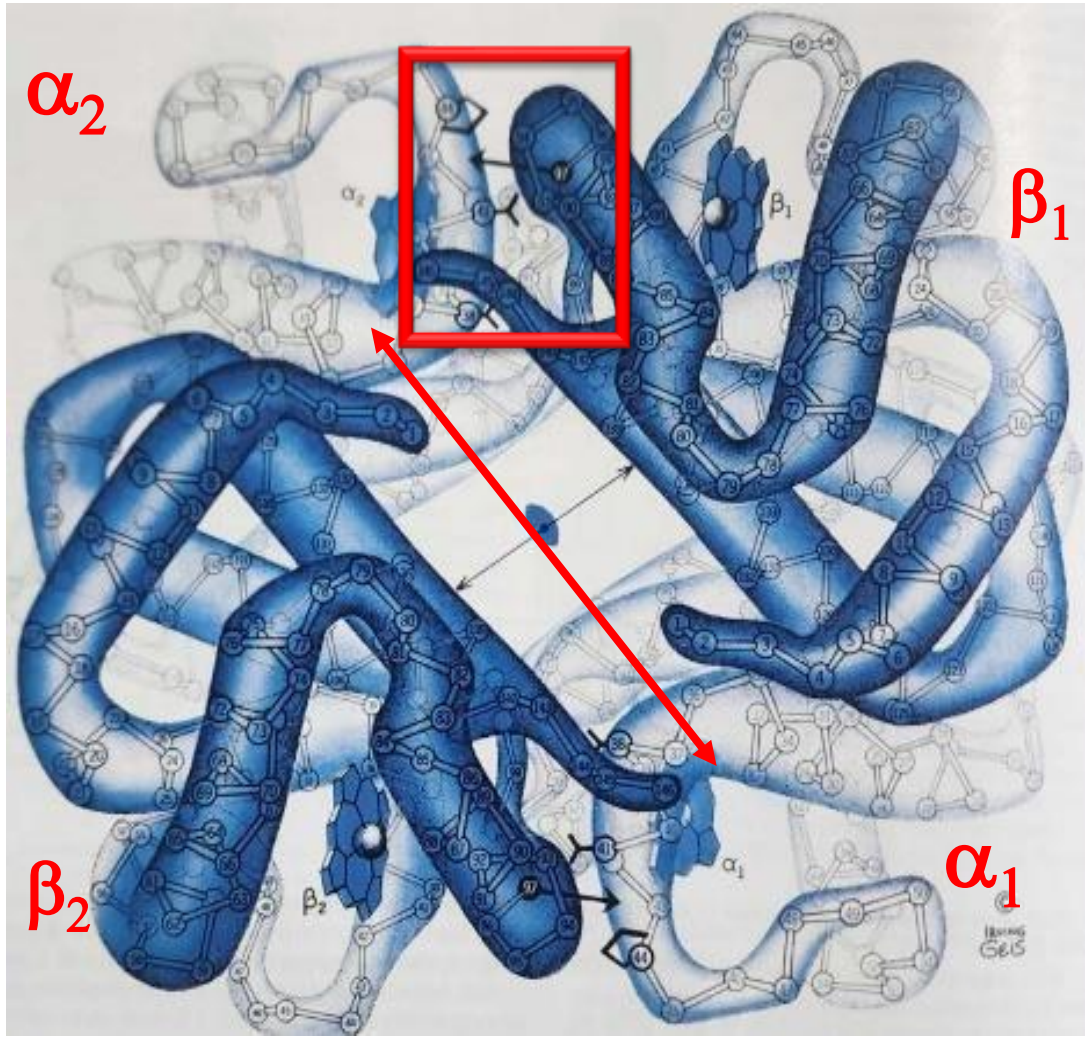


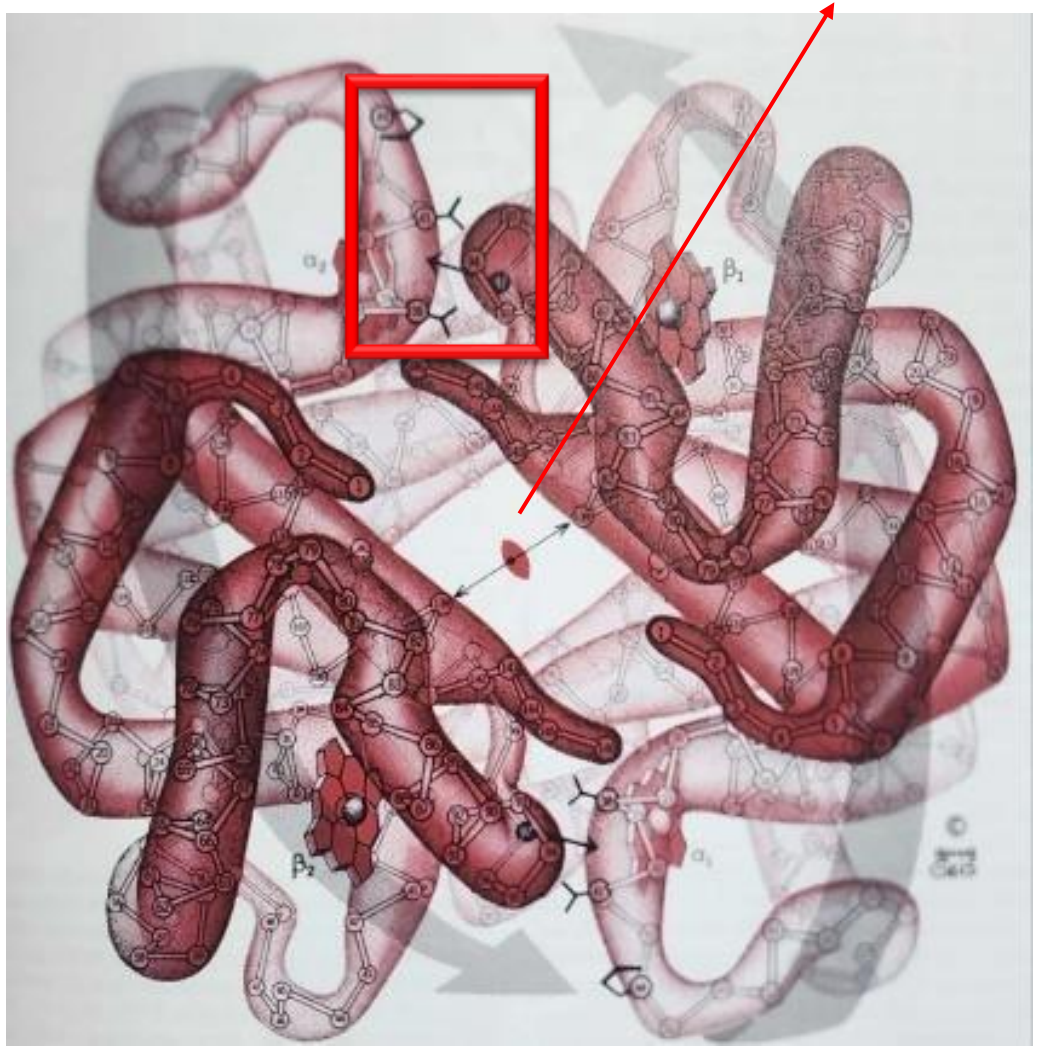
- When Oxygen binds, it causes the breakage of the electrostatic interaction and the Hydrogen bonds causing a **sliding** movement in the polypeptides.

Notice how α-subunits are far from each other, so weak ionic bonds.

Reformation of H bonds

Notice how the central area shrunk when in the R state.





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

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

Red boxes: observe interaction alterations when changing states(slide 23).

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.**

- The binding of the first oxygen induces a conformational change in hemoglobin that **flattens the heme and alters the other binding sites**, making them more ready to bind additional oxygen molecules.

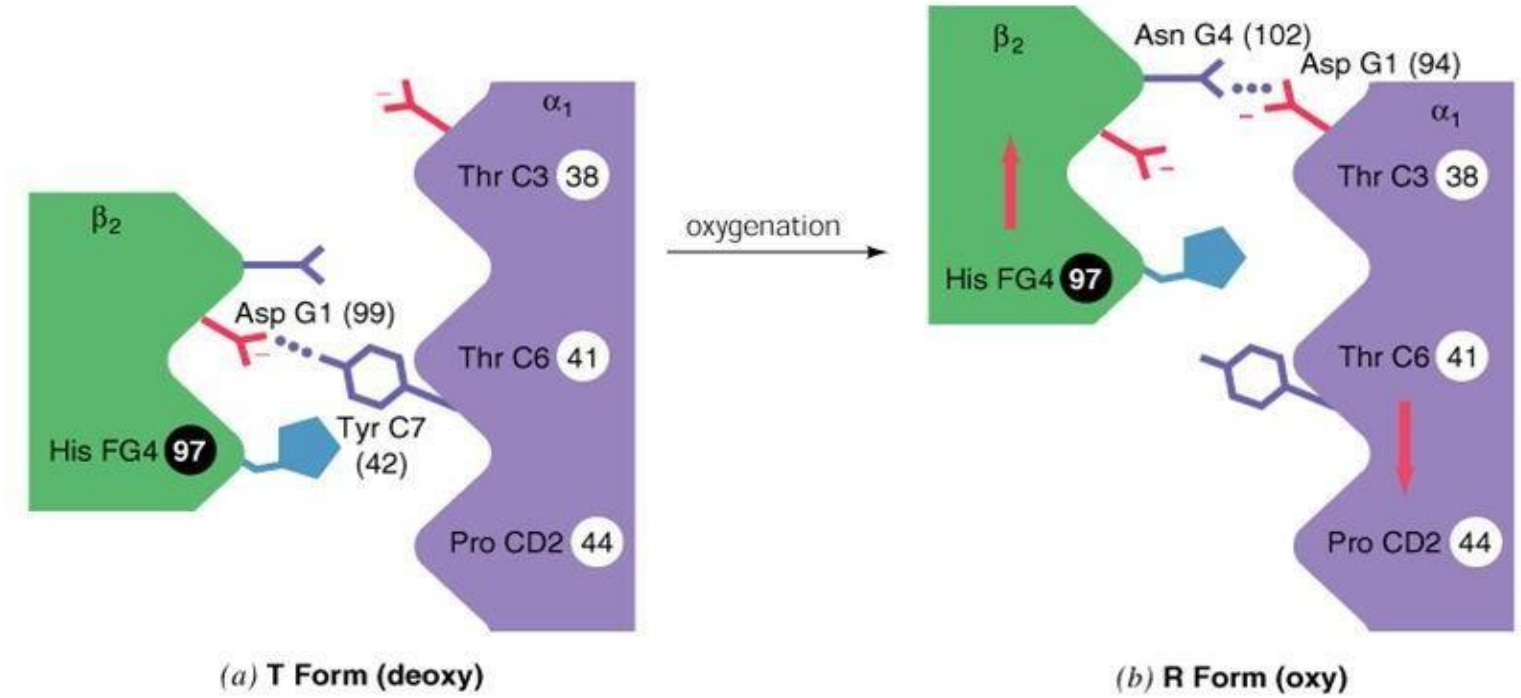
Reformation of H bonds

- 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.

Reformation of H 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)

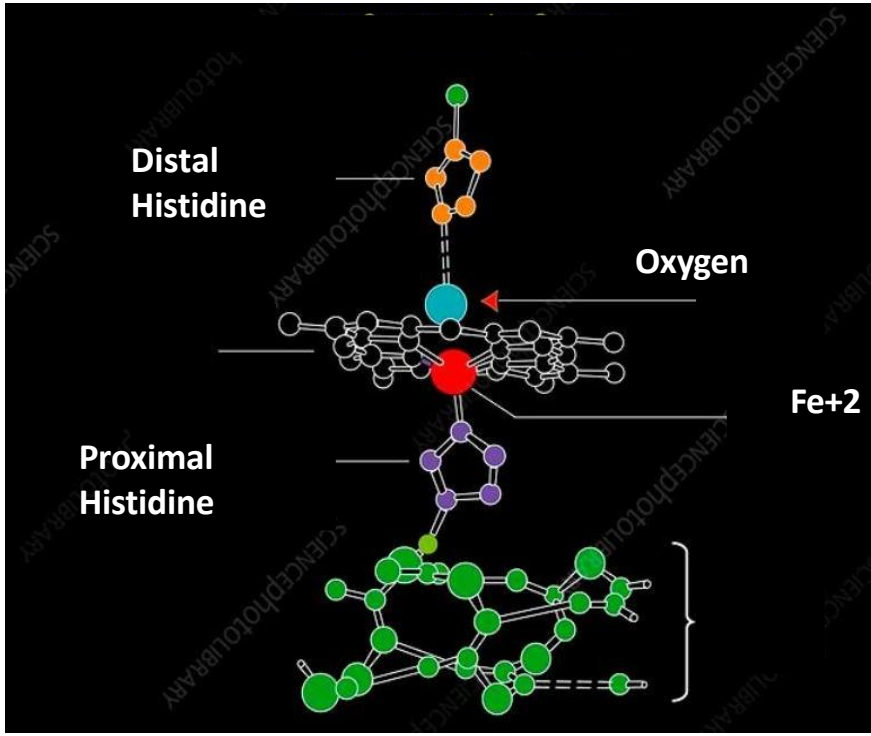


(a) T Form (deoxy)

(b) R Form (oxy)

Last piece of information about the structure

- There is another Histidine called the distal Histidine that has several functions:
  1. It works as a **gate keeper** that allows the passage of **Oxygen only**, through electrostatic interactions.
  2. 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).
- ✓ To summarize, there are **three important histidine residues** in hemoglobin:
  1. **C-terminal histidine:** involved in **electrostatic interactions** that help stabilize the protein.
  2. **Proximal histidine:** directly bonded to Fe^{2+} and responsible for the **conformational change** that increases oxygen affinity.
  3. **Distal histidine:** acts as a **gatekeeper** for oxygen entry and helps prevent oxidation of Fe^{2+} to Fe^{3+}.



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.*

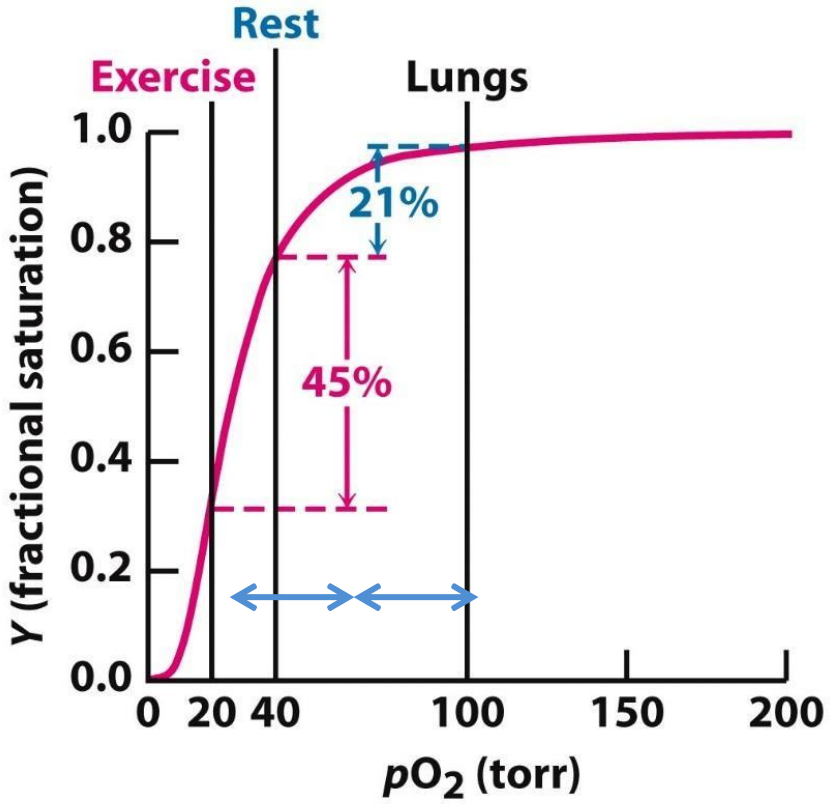


Figure 7.10
Biochemistry, Seventh Edition

See explanation next slide

Explaining the **Oxygen saturation curve**

- The X-axis represents oxygen pressure in torr, and the Y-axis represents hemoglobin saturation as a fraction.
- The curve has an S-shaped pattern, known as **sigmoidal**.
  - This means that at low pO_2, hemoglobin has some saturation, but a small increase in oxygen pressure leads to a large rise in saturation due to **positive cooperativity**.
- In general, the higher the oxygen pressure, the greater the fraction of hemoglobin that is saturated.

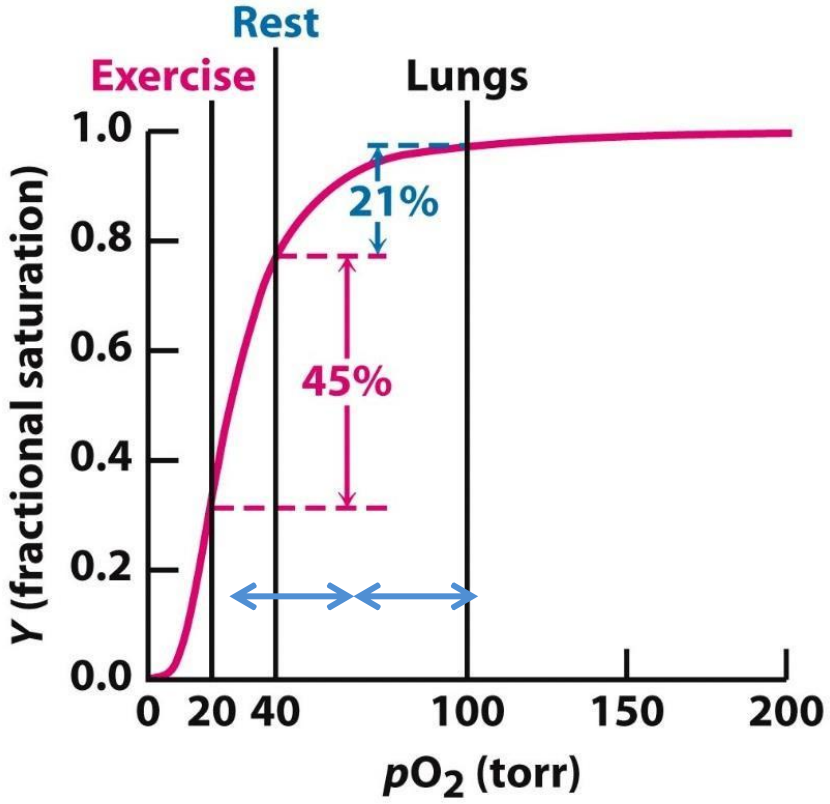


Figure 7.10
***Biochemistry*, Seventh Edition**

Summarizing the previous 2 slides

- In summary, hemoglobin is nearly **fully** saturated in the **lungs**, where the pO2 is 100 torr. As it moves toward **resting** tissues, where pO2 is around 40 torr, hemoglobin saturation remains high at about **77%**, dropping only by roughly 21% because cells are consuming less oxygen. During **exercise**, when pO2 falls to around 20 torr, hemoglobin saturation can drop as low as **37%**, reflecting the increased oxygen demand and active exchange with CO_2, so more oxygen is released to the tissues.

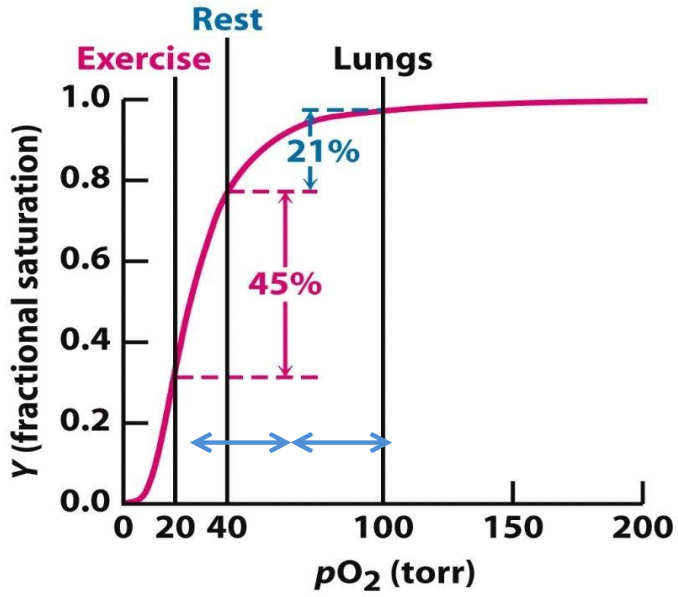
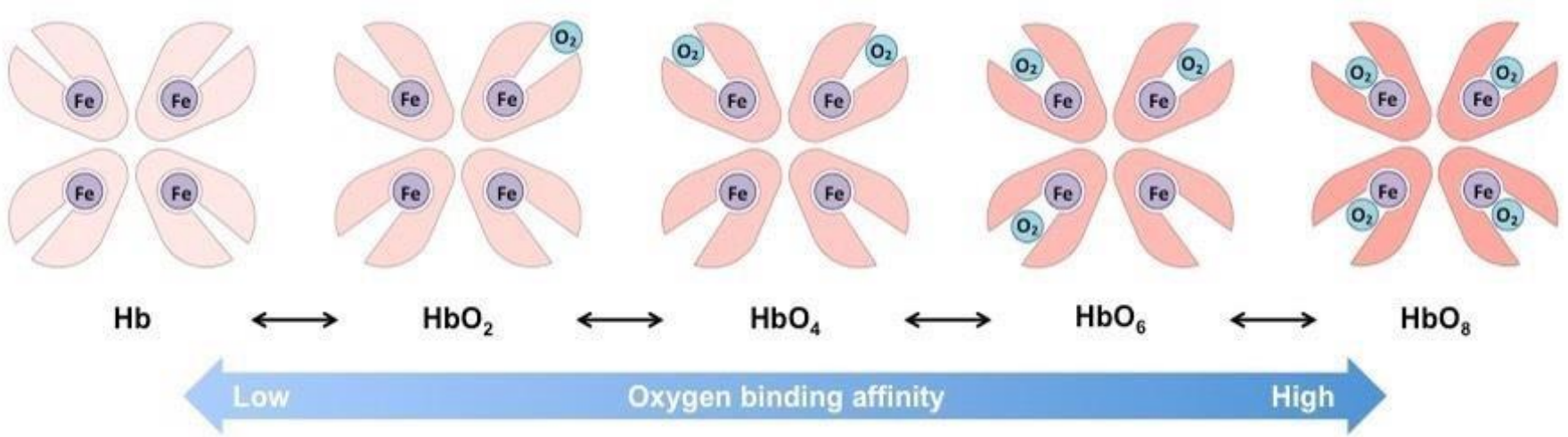


Figure 7.10
Biochemistry, Seventh Edition

Don't memorize the exact numbers; Focus on understanding the trends and making estimates.

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

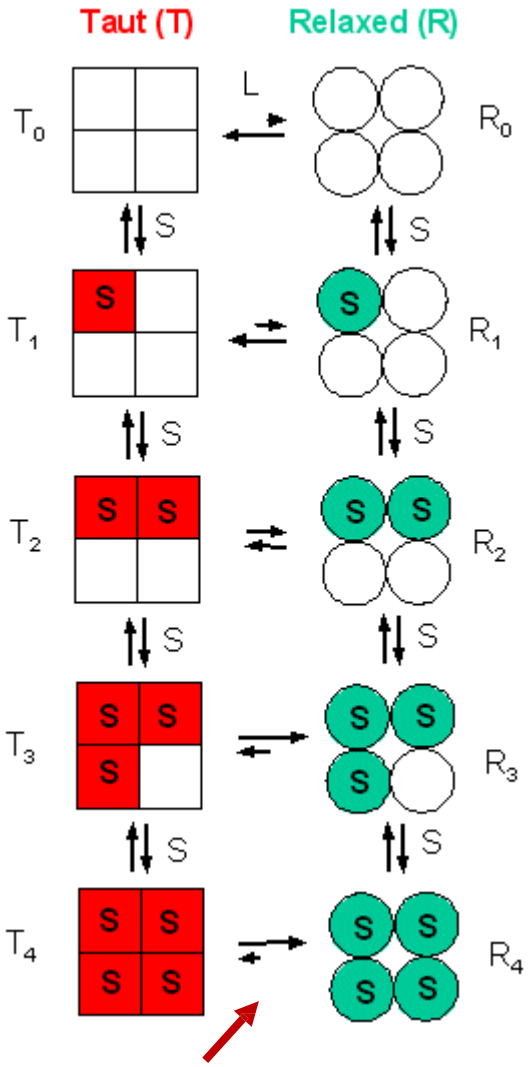
- Simply, cooperativity means that the binding of one molecule makes it easier for additional molecules to bind.
  - For example, when one oxygen binds to hemoglobin, it increases the affinity for the next oxygen, and so on.

❖ 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.

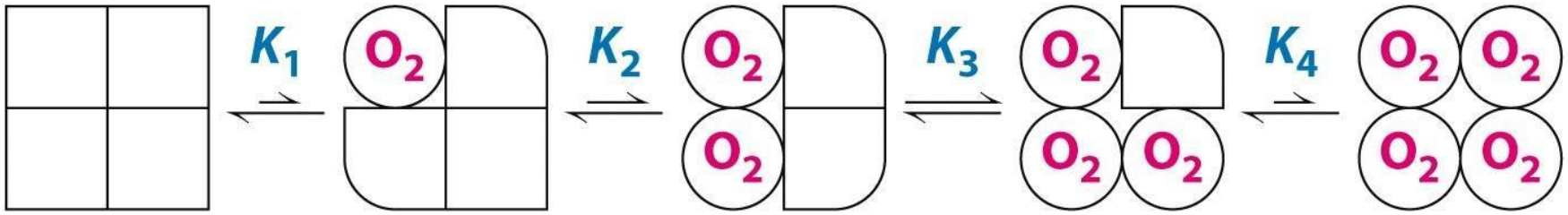
The concerted model (MWC model)

- The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.
- When hemoglobin is **not** bound to oxygen, its structure exists in equilibrium between the two states but preferring the T state.
- Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.
- As hemoglobin binds oxygen molecules, the equilibrium gradually shifts toward the R state. With the binding of a few oxygen molecules, the balance begins to favor the R state, and when fully saturated with oxygen, the equilibrium is strongly shifted toward the R state.
- **This demonstrates that oxygen binding promotes a transition from the T state to the R state.**



Note direction of arrows

The sequential, induced fit, or KNF model



- In the **sequential model**, hemoglobin undergoes **gradual** structural changes.
- 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.
- When a single oxygen molecule binds, it slightly shifts the structure of the nearby subunits from the T state toward the R state. As more oxygen molecules bind, these shifts continue, and when all four oxygen molecules are bound, hemoglobin is fully in the R state.
- This process creates multiple states (intermediate structures) as hemoglobin progresses toward full oxygen saturation.

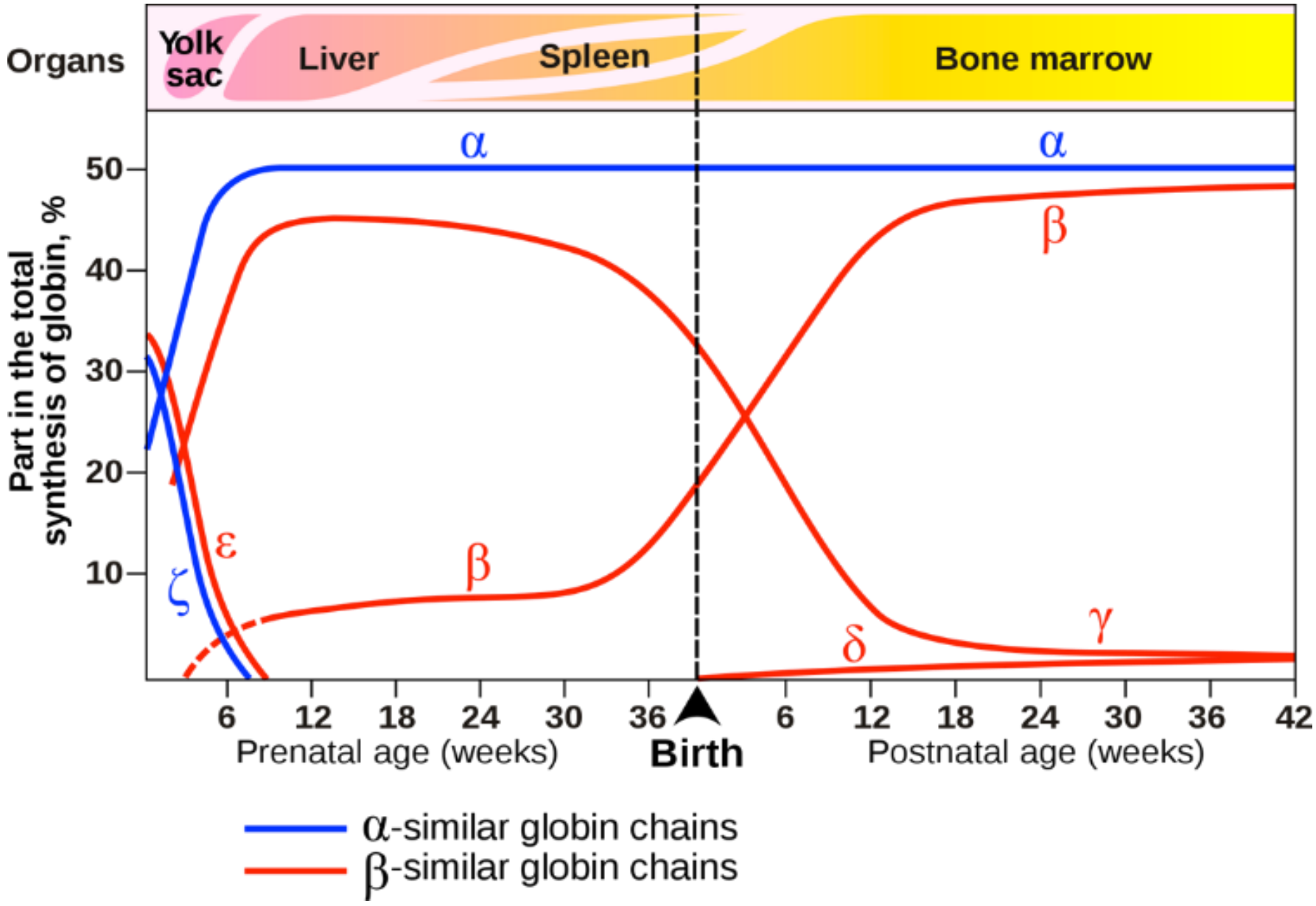
The sequential, induced fit, or KNF model

- Binding of an oxygen molecule causes shifts in hemoglobin's three-dimensional structure by exerting strain on the other subunits, altering their binding sites and thereby increasing their affinity for oxygen.
- Now, the question is 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**.

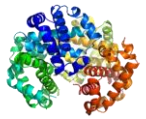
4- Types of Hemoglobin

See next slide...

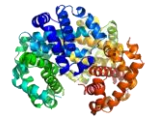
Developmental transition of hemoglobins



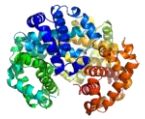
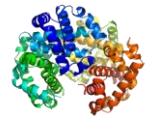
Alpha	Α α
Beta	Β β
Gamma	Γ γ
Delta	Δ δ
Epsilon	Ε ε
Zeta	Ζ ζ



Summarizing types of hemoglobin(1)



- Hemoglobin can be classified into three main types based on age and globin chain composition: embryonic, fetal, and adult.
- The predominant adult hemoglobin (HbA) consists of two α and two β subunits.
  - The α-globin subunits are synthesized early in development, replacing the ζ subunits that are expressed during the embryonic stage, and they persist throughout life. In contrast, β-globin synthesis begins later, around 6 to 12 weeks of gestation, gradually replacing the ε subunits of embryonic hemoglobin.
    - Thus, the ζ and ε subunits are transient, as they are replaced by α and β subunits, respectively.
- ζ and ε subunits Have the highest oxygen binding affinity.
- The γ subunits are predominantly expressed during fetal life, forming fetal hemoglobin (HbF; $\alpha_2\gamma_2$).
    - HbF has a higher affinity for oxygen than maternal HbA, facilitating the transfer of oxygen from maternal to fetal blood.

 Summarizing types of hemoglobin(2) 

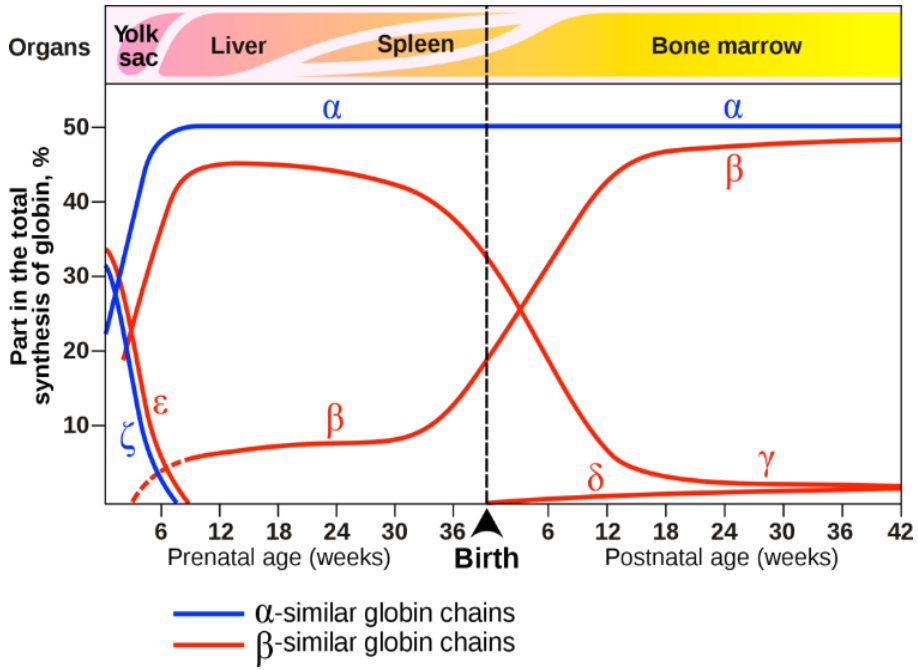
- After birth, the synthesis of δ subunits commences, forming $\alpha_2\delta_2$, which persists into adulthood but constitutes only a small fraction of total hemoglobin.

❖ Based on the previously mentioned information, hemoglobin can be classified as follows:

- **Adult Hemoglobin (HbA):**
  - Main forms: HbA1 ($\alpha_2\beta_2$) – the predominant type, and HbA2 ($\alpha_2\delta_2$) – a minor form. γ chains may persist in small amounts in adults, forming ($\alpha_2\gamma_2$) - HbF traces.
  - Site of synthesis: Bone marrow.
- **Fetal Hemoglobin (HbF):**
  - Composition: $\alpha_2\gamma_2$ (predominant) and a small amount of $\alpha_2\beta_2$ may appear late in gestation.
  - Site of synthesis: Liver and spleen.
- **Embryonic Hemoglobin (HbE):**
  - Possible forms include $\zeta_2\varepsilon_2$ (major), $\alpha_2\varepsilon_2$, $\zeta_2\gamma 2_2$, and $\zeta_2\beta_2$.
  - Site of synthesis: Yolk sac.

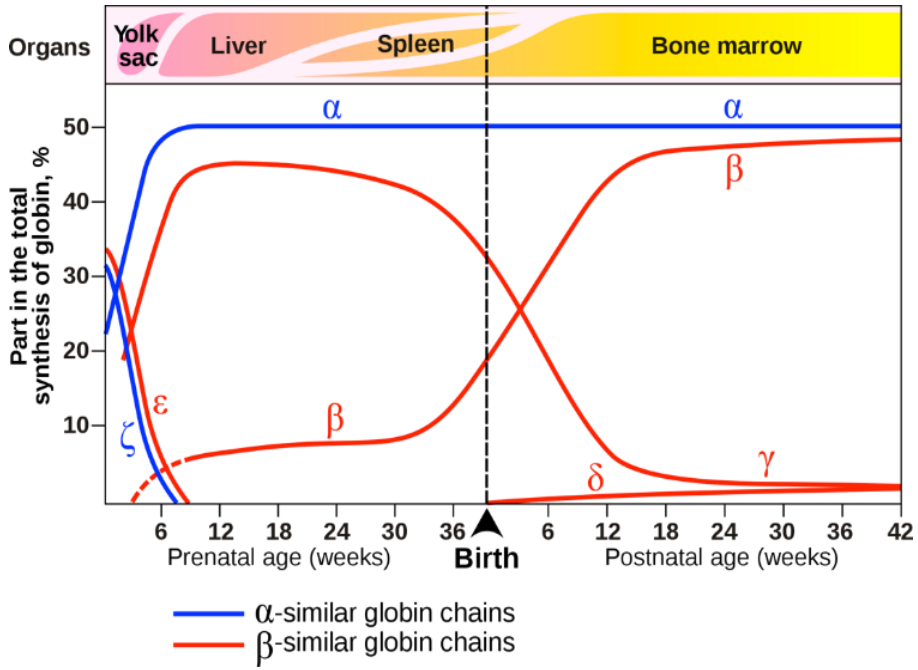
The embryonic stage

- 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

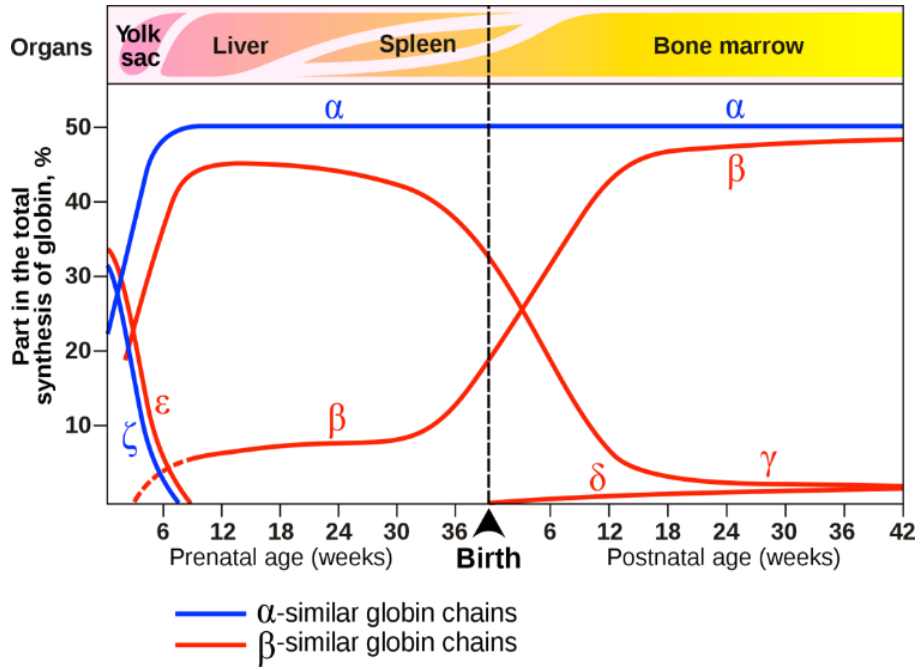
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines dramatically and fetal hemoglobin synthesis starts from the **liver.**
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides ($\alpha 2 \gamma 2$)
- **The gene expression of the α polypeptides is active 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.

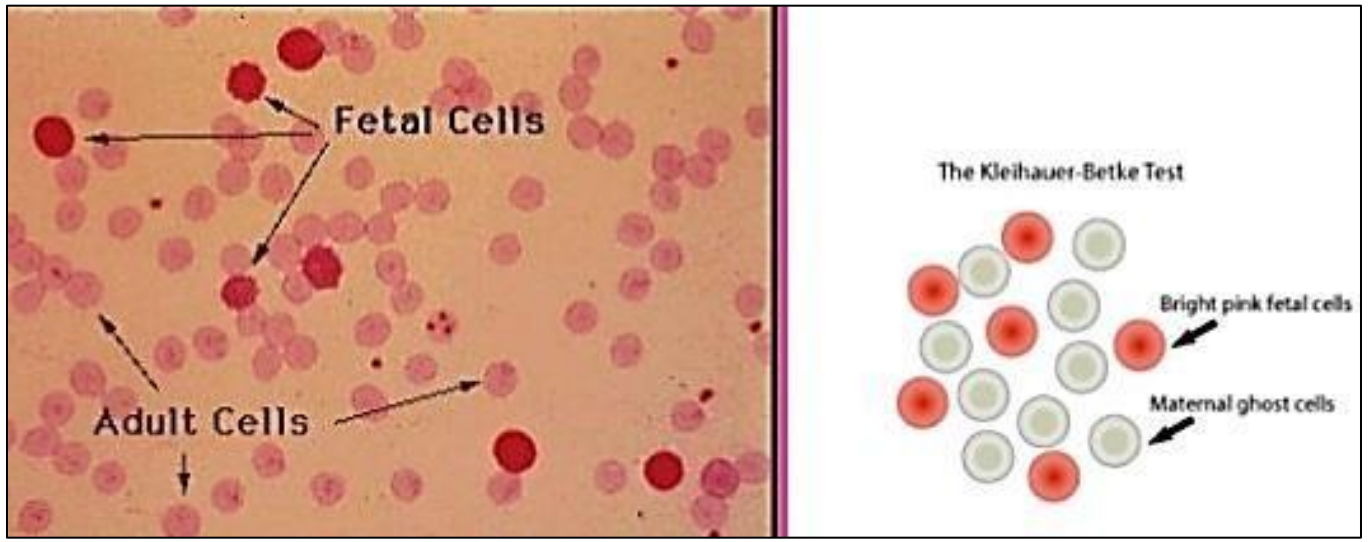
  At birth, synthesis of both γ and β chains occurs in the bone marrow.
- The major hemoglobin is HbA1 (a tetramer of 2 α and 2 β chains).
  - A minor adult hemoglobin, HbA2, is a tetramer of 2 α chains and 2 delta (δ) chains.



Explanation next slide

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)



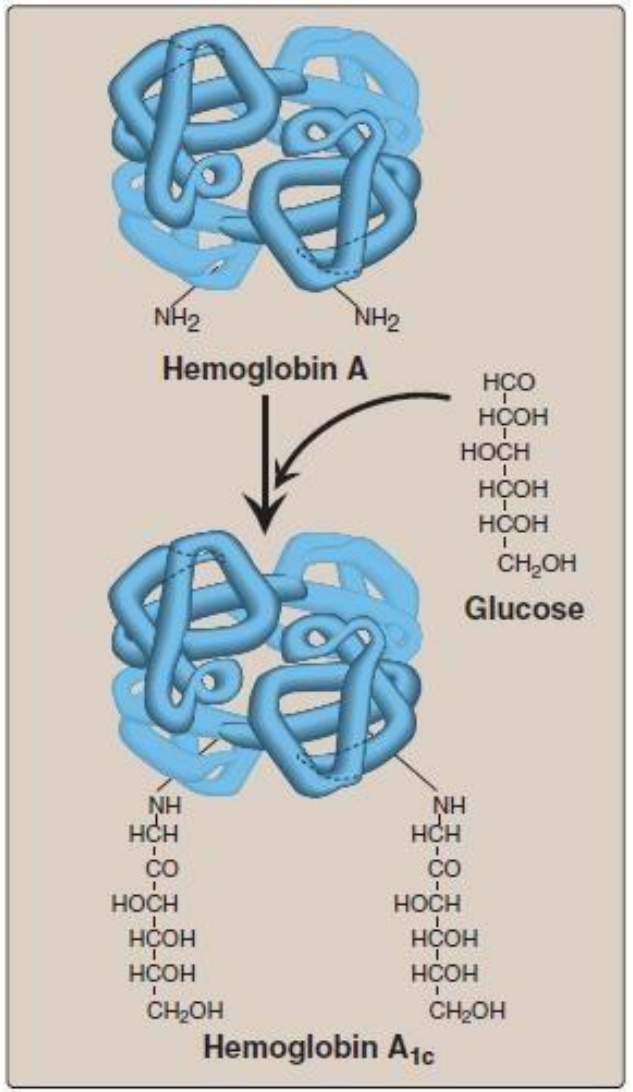
Keilhauer Betke test Explained

- The principle of the test is based on the difference in hemoglobin types:
  - **Maternal blood** contains hemoglobin with α and β chains.
  - **Fetal blood** contains hemoglobin with α and γ chains.
- When an acid is applied, adult hemoglobin (αβ) is more easily eluted (washed away), while fetal hemoglobin (αγ) remains stable.
- After acid treatment and staining, **fetal red blood cells retain the dye**, appearing more distinct under the microscope.
  - ➢ This result indicates the presence of fetal blood in the maternal circulation. Sometimes, fetal anemia can reveal such a leak.
- This process is **different from Rh incompatibility**, though both involve interactions between maternal and fetal blood.

5- Hemoglobin for detecting Diabetes

Adult hemoglobins and diabetes

- Under high levels of glucose, HbA1 can be glycosylated non-enzymatically with a hexose in a process called **glycation** 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.



Side note: It should be referred to as glycation rather than glycosylation, since glycosylation involves the enzymatic addition of sugars to proteins or peptides, whereas in this case, the attachment of glucose to hemoglobin occurs **non-enzymatically**. However, the original slides use the term *glycosylation*, even though the lecturer clarified that the correct process is **glycation**.

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).

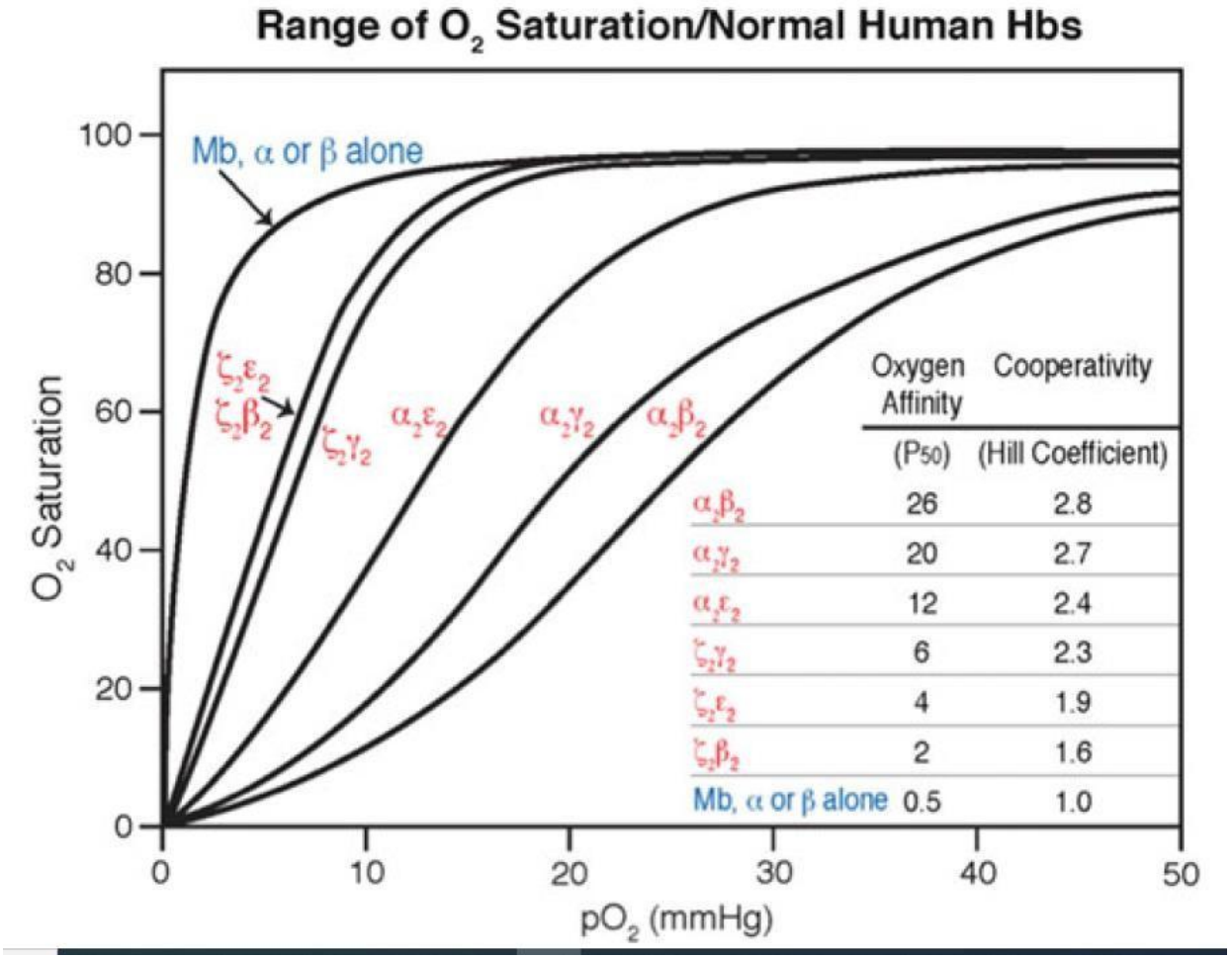
Table

	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	≥ 6.5%	> 125 mg/dL	≥ 200 mg/dL

Memorize

Different Hemoglobin types binding to Oxygen

- As the curve goes to the left, more affinity to oxygen is observed.
  - When oxygen levels (pO2) fall, hemoglobin with high affinity continues to hold oxygen tightly and releases it only at very low oxygen pressures.
- Among the hemoglobin types, the α and β chains of adult hemoglobin (HbA) release oxygen most easily compared to other forms.



I'll add doctor's Mamoun previous explanation in the following slide.

Explanation of the previous slide

- The oxygen saturation curve shifts to the right as newer forms of hemoglobin replace the preceding ones. Along this trend, the affinity of hemoglobin for oxygen decreases, the p50 increases, and higher oxygen pressure is needed to achieve the same saturation levels.
    - This explains why pregnant women often feel short of breath—they need to supply enough oxygen to the developing embryo, which has a stronger affinity for oxygen than the mother.
- During the embryonic stage, embryonic hemoglobin binds oxygen with very high affinity, meeting the demands of rapid metabolism and cell division, both of which require high oxygen levels.
- The general order of hemoglobin types from highest to lowest oxygen affinity is: embryonic hemoglobin, fetal hemoglobin, and adult hemoglobin.

6- The Genetics of Globin synthesis

🧬 Just before diving into hemoglobin genetics

- All body cells have the **same DNA**, whether they are skin, bone, or hair cells.
- Specialization of cells is controlled by the activation or suppression of specific genes.
  - The activation and deactivation of genes are regulated by **time** (age) and **developmental stage**.
    - During fetal life, specific genes are turned on, while others are turned off. As the individual grows, fetal genes are turned off, and adult genes are turned on.

❖ Gene expression is precisely programmed and time-specific.

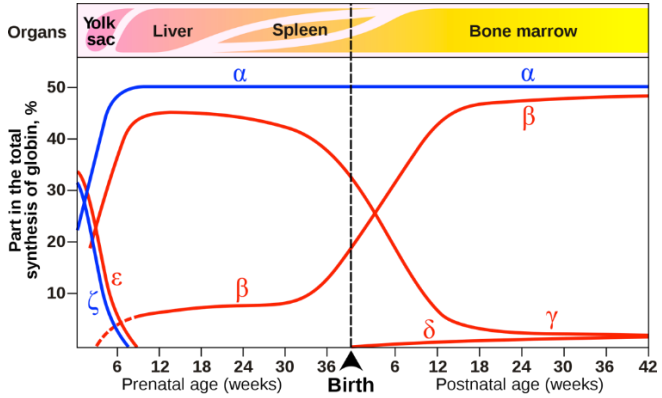
1. Each gene has a **promoter:**
   - The promoter is the site where transcription factors and RNA polymerase bind to initiate transcription.
2. Gene transcription is influenced not only by promoters but also by **enhancers**.
   - Enhancers are **DNA sequences that increase the efficiency of gene transcription.**
   - Enhancers bind to proteins known as **activators**, which interact with promoters to activate transcription.
3. Additional regulatory elements include **silencers** and **DNA methylation**.
   - DNA methylation and silencers are part of epigenetic regulation.

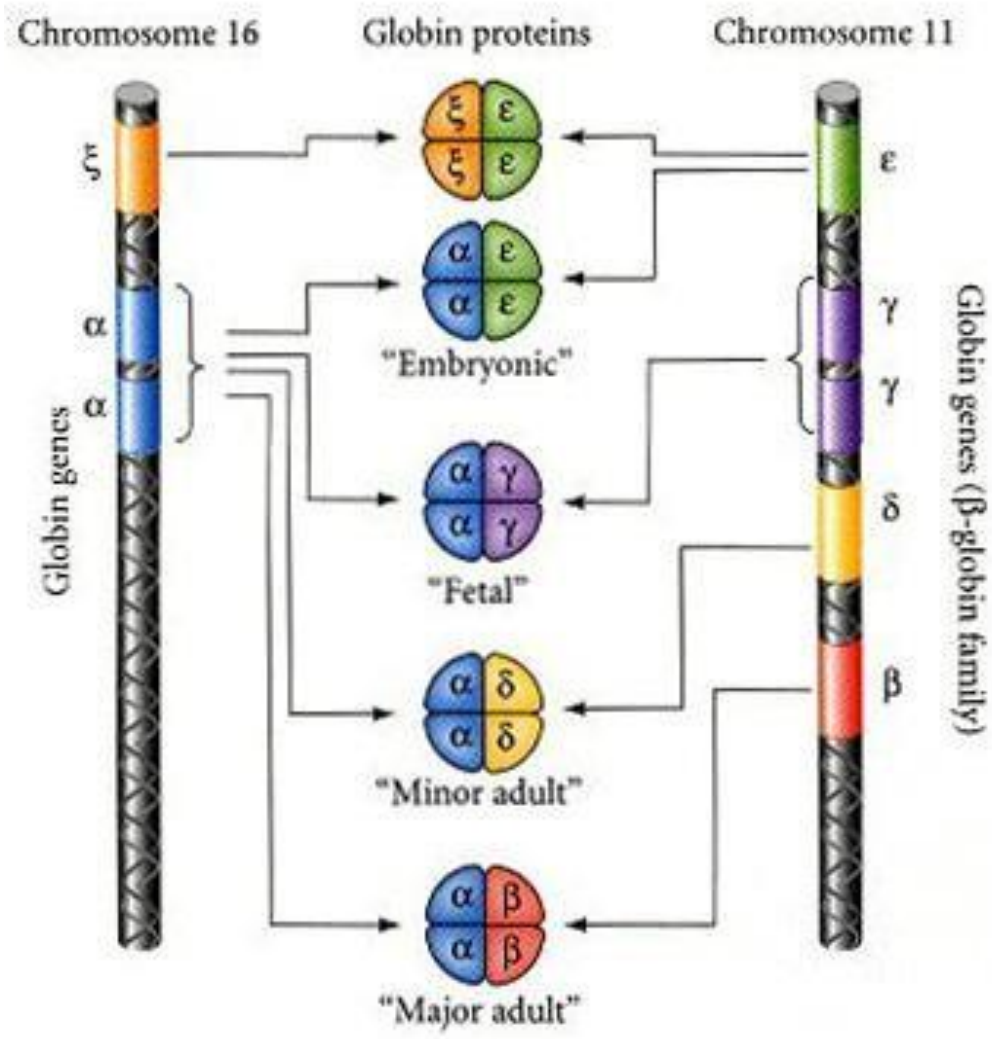
➢ **The regulation of gene expression in cells is highly complex.**

- **Epigenetic mechanisms** control gene expression without changing the DNA sequence itself.

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.*





The genes - Explanation

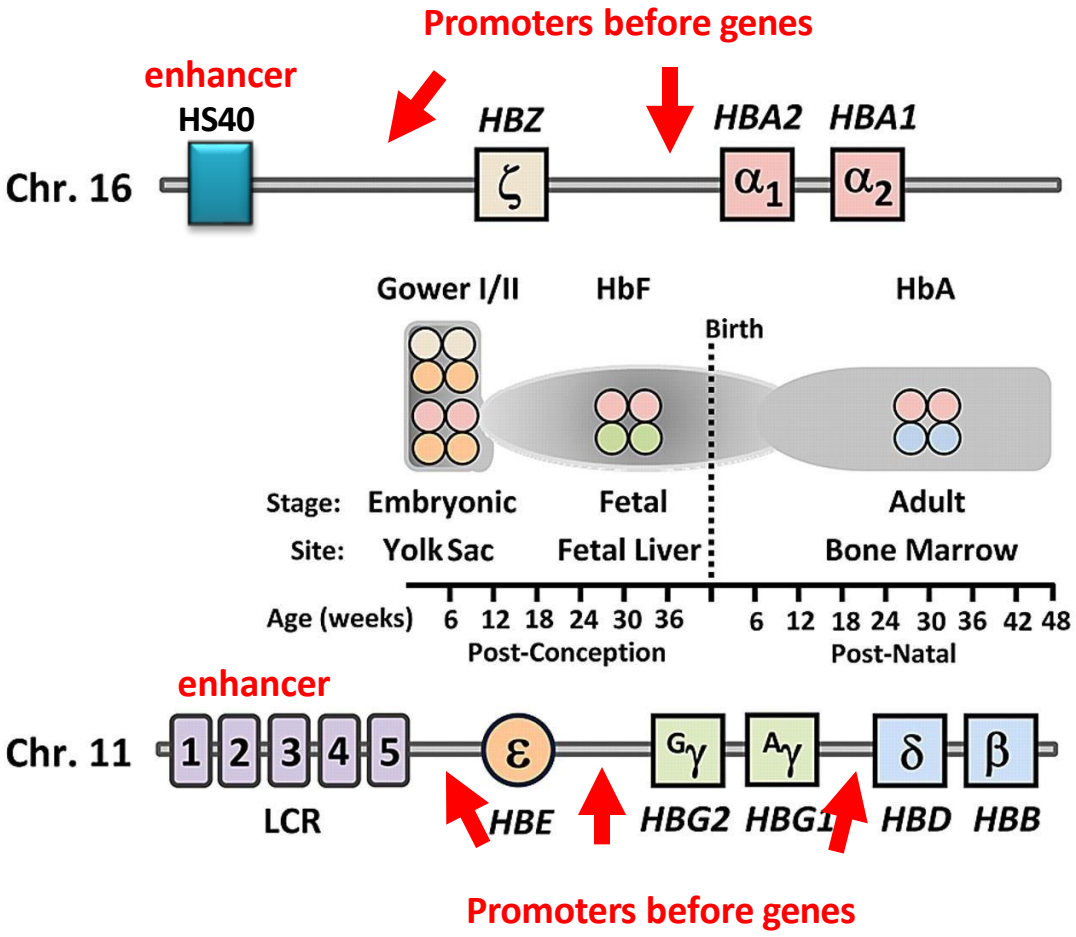
- **Globin genes** responsible for hemoglobin synthesis are found on **chromosome 16 and chromosome 11.**
  - Chromosome 16 contains the ζ and α globin genes.
  - Chromosome 11 contains the ε, γ, δ, and β globin genes.

❖ **Globin gene expression changes according to developmental stage:**

- ζ and ε genes are expressed together in the early embryonic stage.
- α and ε genes are expressed together in the late embryonic stage.
- α and γ genes are expressed together during the fetal stage to form fetal hemoglobin (HbF).
- α and δ genes are expressed together in the adult stage to form hemoglobin A2 (HbA_2).
- α and β genes are expressed together in the adult stage to form hemoglobin A (HbA).

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).



Locus structure - Explanation

- Each globin gene has its own promoter region.
- As said before, regulation is also done by **enhancers**:
  - On chromosome 16, enhancer **HS40** regulates the ζ and α genes.
  - On chromosome 11, the **locus control region** (LCR) acts as an enhancer for ε, γ, δ, and β genes.

❖ **Function of the LCR as an enhancer:**

- During the fetal stage, the LCR interacts with the γ -globin promoter to activate γ -globin transcription.
- After birth, the LCR shifts to interact with the β -globin promoter to activate β -globin transcription.

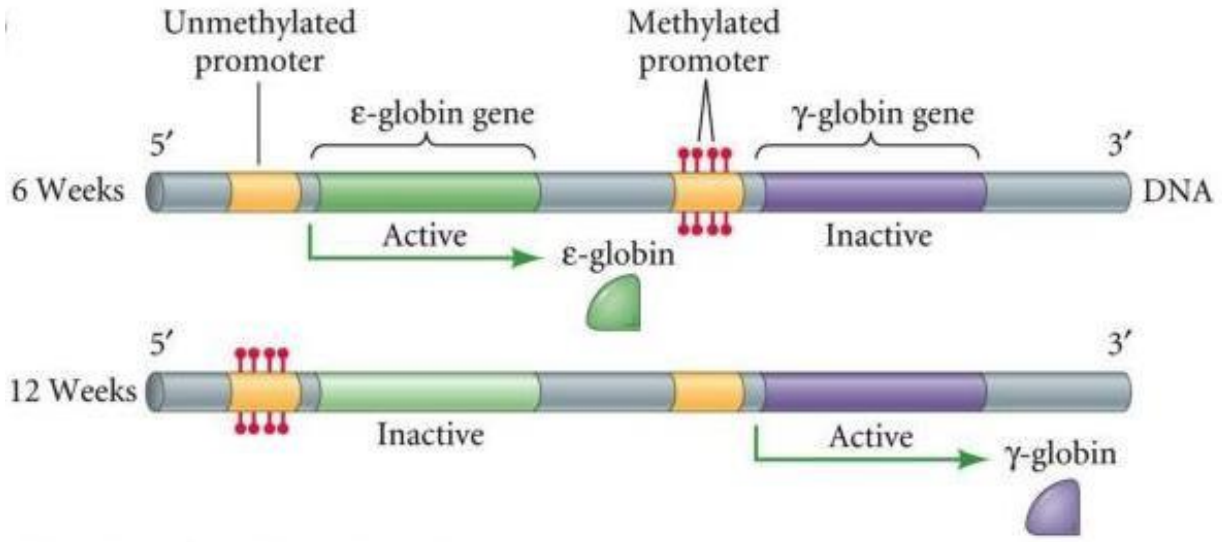
- Thus, **enhancer-promoter interactions change over time**, ensuring proper globin gene switching through development.

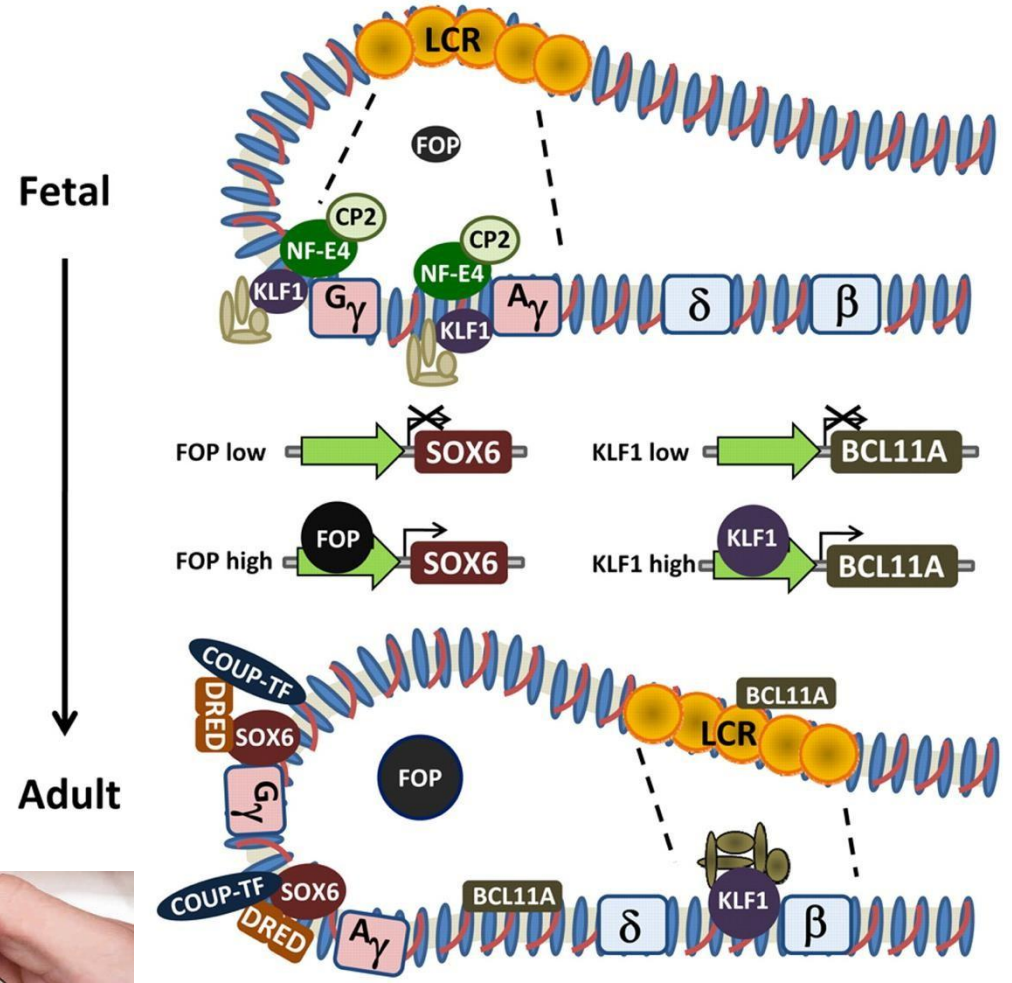
To understand gene expression, regulators and enhancers: see the last resource slide 59

Explained before.

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.).





Article | Published: 12 October 2022

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

Ruopeng Feng, Thiyagaraj Mayuranathan, Peng Huang, Phillip A. Doerfler, Yichao Li, Yu Yao, Jingjing Zhang, Lance E. Palmer, Kalin Mayberry, Georgios E. Christakopoulos, Peng Xu, Chunliang Li, Yong Cheng, Gerd A. Blobel, M. Celeste Simon & Mitchell J. Weiss

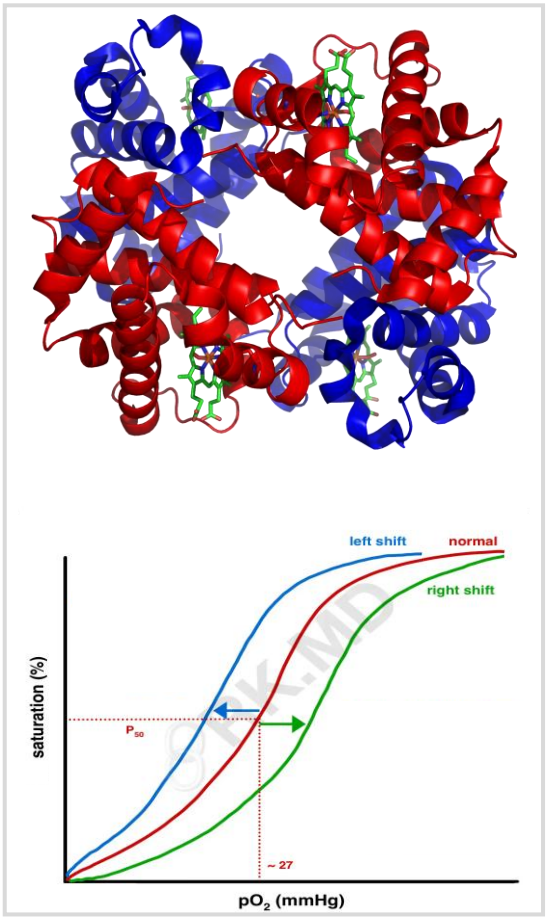
- **CRISPR-Cas9** technology has been used experimentally to convert **β-globin expression to γ-globin expression.**
  - ➢ This approach is being investigated for the treatment of thalassemia by reactivating fetal hemoglobin (HbF).

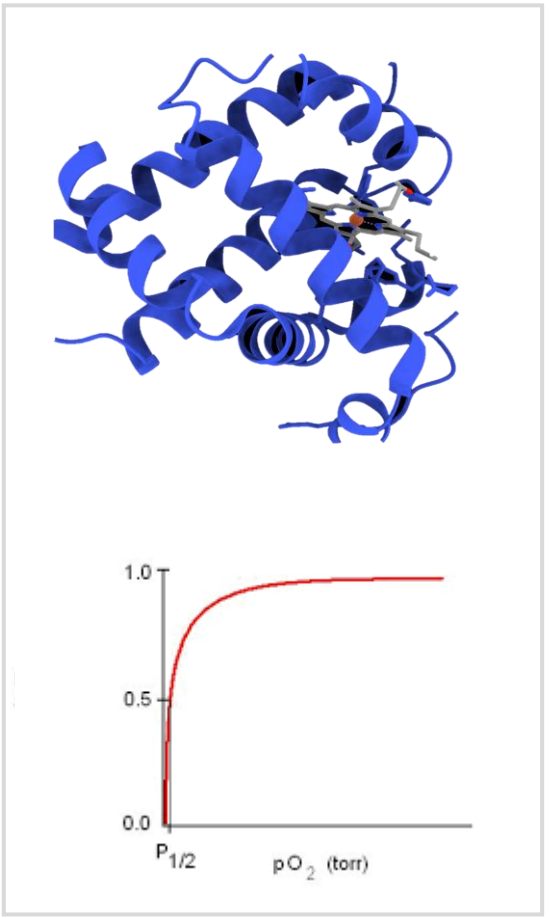
Abstract

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.

Biochemistry Quiz 1

I bind oxygen depending on how much is already bound, letting me pick it up efficiently and release it where it's needed. My curve shows this dynamic behavior. Who am I?





External Resources

- The summary was created using modified Slide 20 from the Biochemistry final material: [Link].
- Marks' Basic Medical Biochemistry, Fifth edition, p. 109-112. (Hard copy, not PDF)
- 2022 lecture: [Link].
- Hemoglobin Biochemistry, Neural academy on YT: [Link].
- Transcription & Enhancers, Maxanim Gentaur Ltd. on YT: [Link]

Special thanks to AI

For any feedback, scan the code or click on

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	14	… (such as those involving arginine residues (**arg146**))…	(such as those involving arginine residues)…
	37	After birth, the synthesis of δ subunits commences, forming $\alpha_2\gamma_2$…	After birth, the synthesis of δ subunits commences, forming $\alpha_2\delta_2$…
V1 → V2			