بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 6

Globular Proteins pt.2

Written by : Shahd Thamer
Rand Alkhateeb

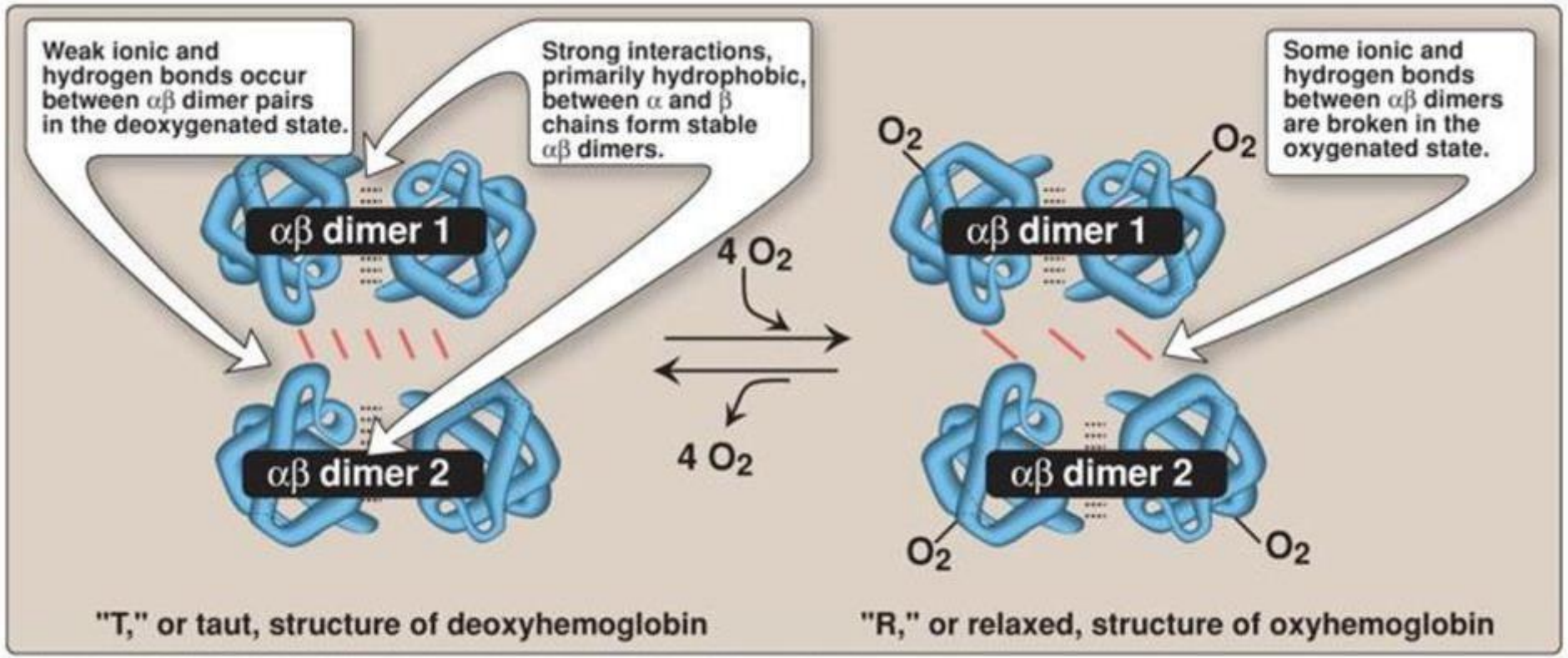
Reviewed by : Lamar Khorma

Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Electrostatic interactions are broken

Electrostatic interactions are broken in the R state.



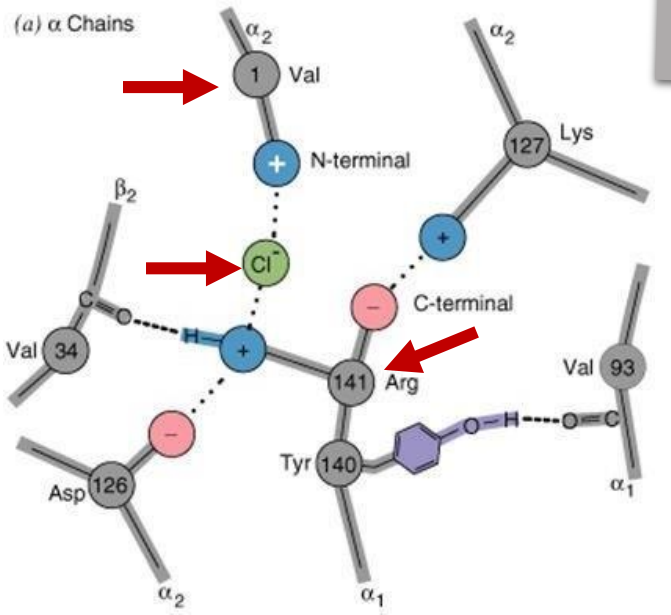
The broken interactions

These numbers are to help you visualize what happens

- Electrostatic interactions and hydrogen bonds that stabilize the T-form of hemoglobin are broken upon movement of polypeptides.

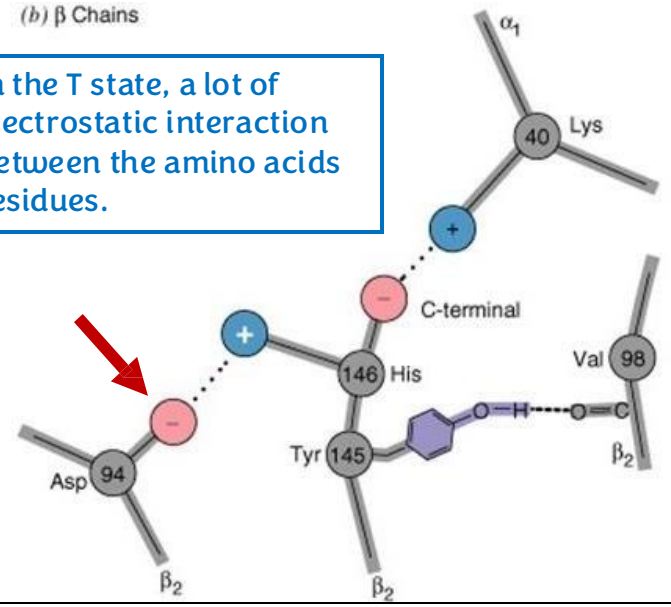
N^+ of Val1 (α2) …….Cl^- ……. R^+ of Arg141 (α1) ……. C=O of Val34 (β2) & Asp126 (α2)
R^+ of Lys127 (α2) ……. C^- of Arg141 (α1)
R^+ of His146 (β2) ……. R^- of Asp94 (β2) On the same chain

Do not memorize



Recall: α subunit has 141 amino acid
First one is valine which is positively charged and interacts with Cl- by electrostatic interactions, the same chloride ion does electrostatic interactions with Arginine (141, last amino acid in α chain).

Do not memorize



In the T state, a lot of electrostatic interaction between the amino acids residues.

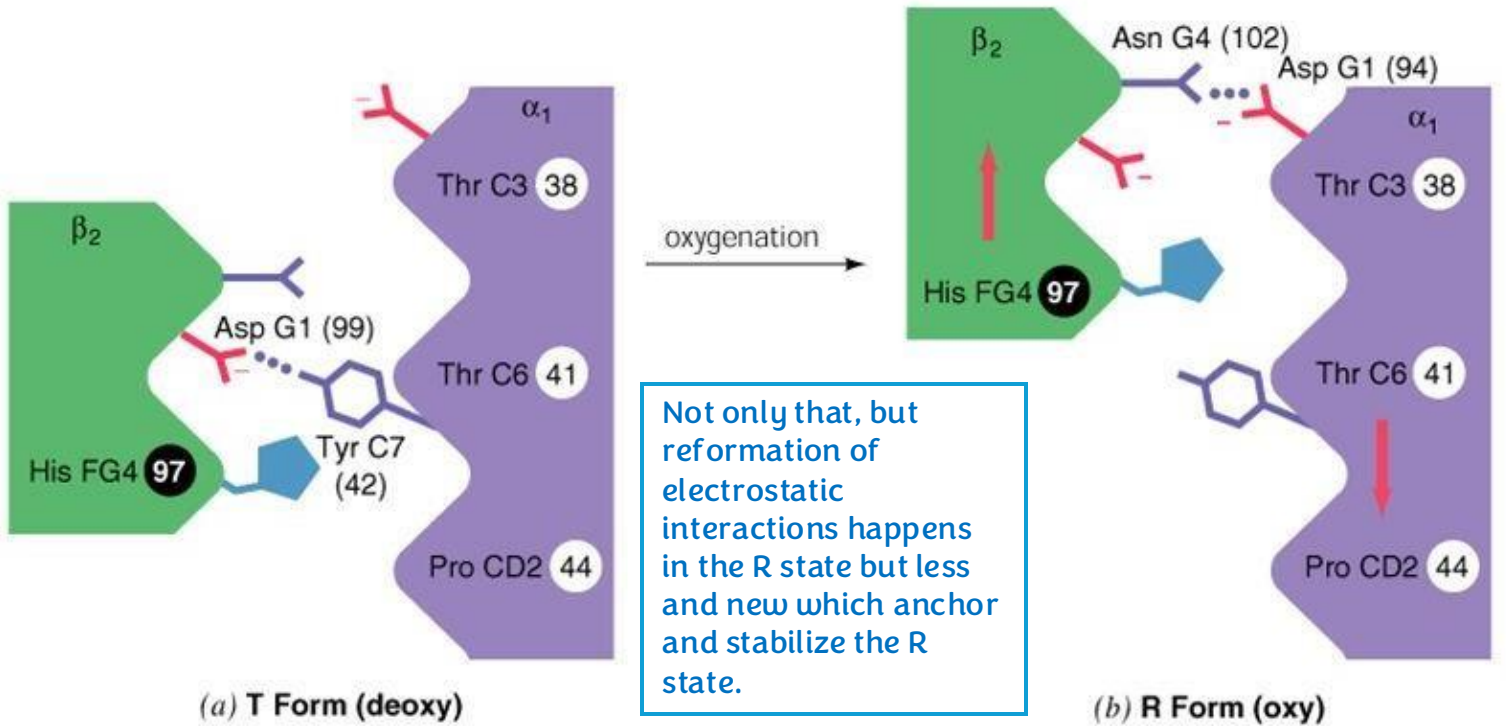
C^- of His146 (β2) ……. Lys127 (α2)

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 O2 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.

These electrostatic interactions break down when sliding happens, amino acid residues get apart. So the electrostatic interactions that formed and stabilized the T state are broken and the molecule transforms to the relaxed state, which increases the affinity of the molecule for oxygen

The electrostatic interactions in the T state stabilize the structure, when oxygen binds-> the sliding happens and the electrostatic interactions are broken.



(a) T Form (deoxy)

(b) R Form (oxy)

Not only that, but reformation of electrostatic interactions happens in the R state but less and new which anchor and stabilize the R state.

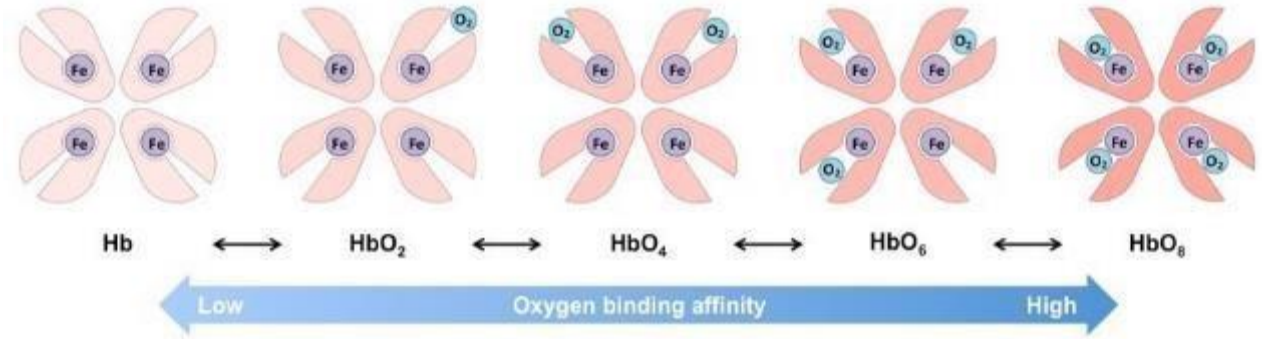
Do not memorize

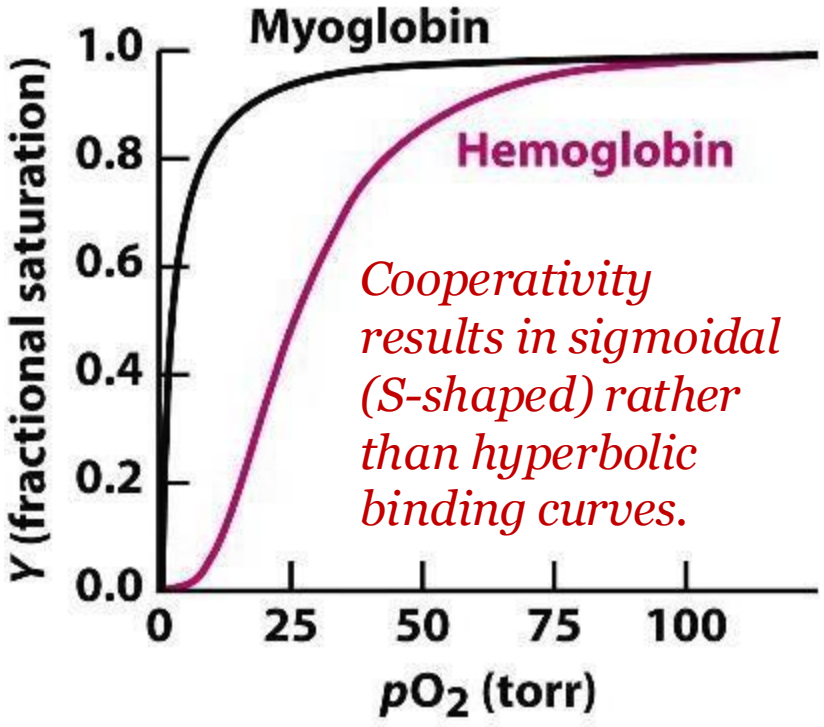
Note that you don't need to memorize all the amino acids but some of them the doc emphasized on them:
His146 in β chain (last amino acid)
Val1 Arg 141 in α chain.
Other than that is not required.

Binding is cooperative

This is a specific feature for allosteric proteins, cooperativity: binding of one oxygen to one heme group makes it easier for a second oxygen to bind and so on.

- Conformational changes lead to cooperativity among binding sites.
- Binding of the first O_2 breaks some salt bridges with the other chains increasing the affinity of the binding of a second molecule.
- Binding of the second O_2 molecule breaks more salt bridges increasing the affinity towards binding of a third O2 even more, and so on.
- Binding is cooperative.
- Oxygen is a homotropic effector (the allosteric modulator is the substrate itself).





Cooperativity results in sigmoidal (S-shaped) rather than hyperbolic binding curves.

That's why the shape of the curve is sigmoidal because we have high affinity and low affinity state
As the oxygen increases the affinity increases as well, why?
Because we have cooperativity.

So when we say we have "allosteric enzymes" :
They have cooperativity
They have two structures (T=less active and R=more active)
Meaning also that binding of a substrate to the first subunit makes it easier for another substrate to bind.

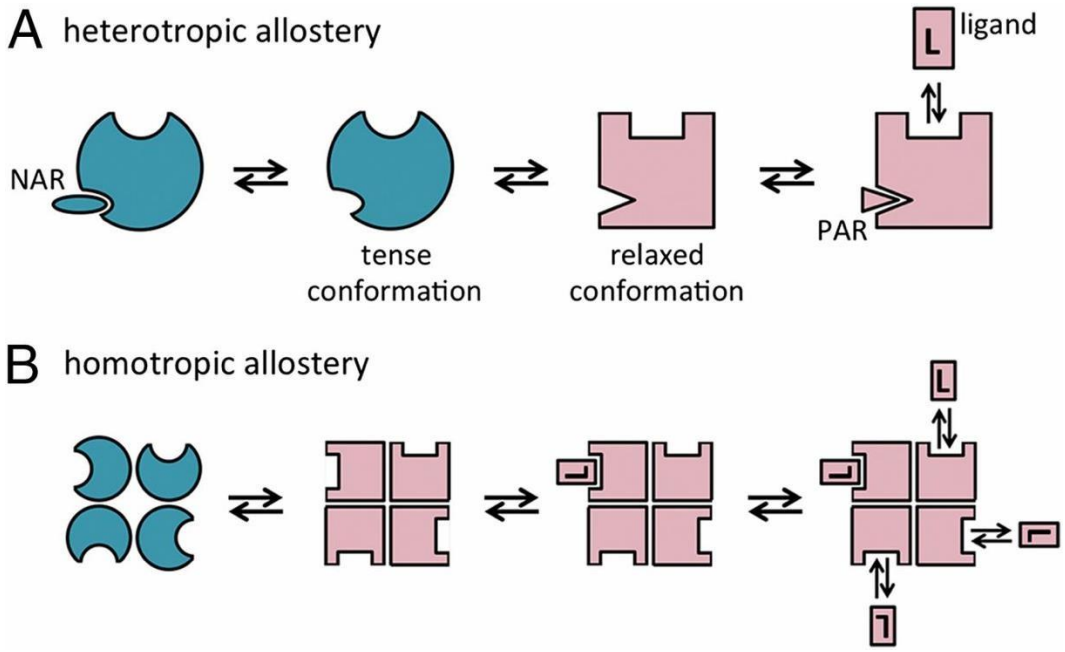
Oxygen is a homotropic effector; affects the binding of a second oxygen (same molecule -homo-)

While heterotropic effectors affect the binding of a different type of molecule -hetero-, like CO2 affects the binding of oxygen

Some terminologies

Regulate the affinity of the hemoglobin molecule, affect the allostery and the structure (high to low affinity or vice versa)

- Homotropic allosteric regulator/effector: effector and ligand regulated by the effector are the same molecule (e.g., O_2 binding affects subsequent O_2 binding).
- Heterotropic allosteric regulator: effector and ligand are different molecules (e.g., H^+ or BPG binding affects O_2 binding).
- Positive allosteric interaction: effector binding increases affinity for ligand.
- Negative allosteric interaction: effector binding decreases affinity for ligand.



The Hill constant (coefficient)

This equation informs us if the protein is allosteric or not.

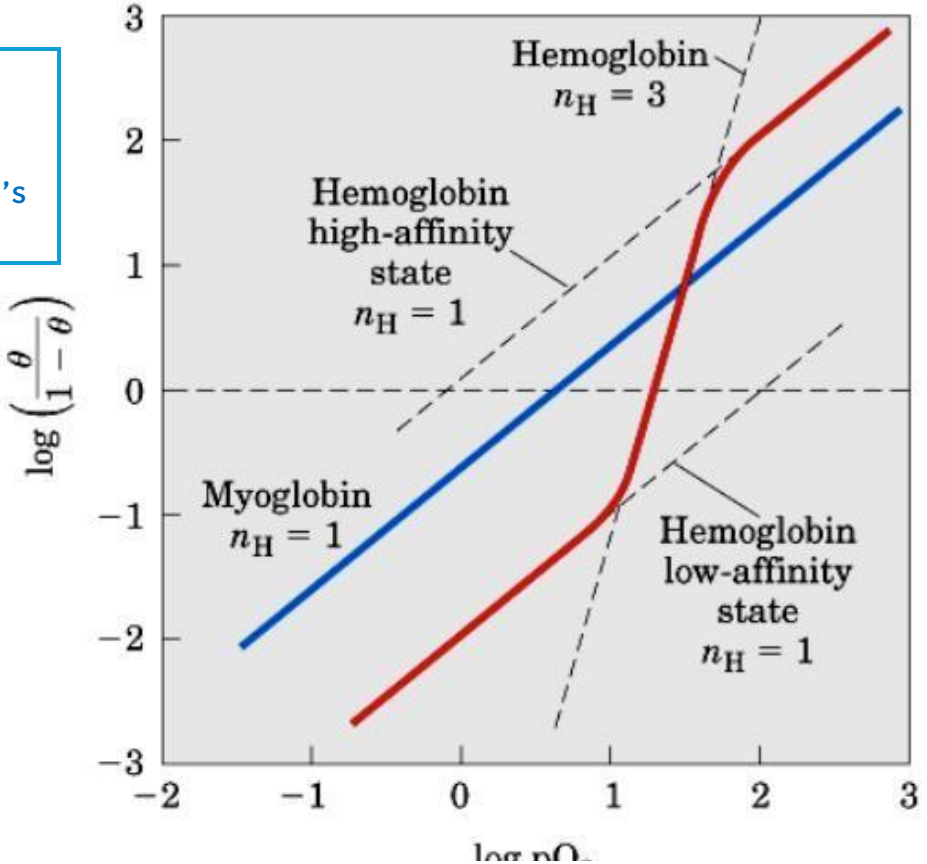
Do not memorize

- The Hill plot is drawn based on an equation (*you do not have to know it*).
- **n = Hill constant -** determined graphically by the Hill plot
- n is the slope at the midpoint of binding of log (Y/1-Y) vs. log of pO_2.
  - 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.*

N: the Hill's coefficient. (The doc said mistakenly that it's y or θ but it's n).

We can have an allosteric protein that has a value of n=4, which means that the allostery is highly affected. While a value of n=1.5 means that it is an allosteric protein but has a low degree of allostery

For example, myoglobin will have a value of n=1, no allostery) no change in the structure). Hemoglobin has a value of n=2.6 which means that it is an aollosteric protein and the effect of oxygen binding is positive allostery "higher affinity" . This value does NOT indicate the number of oxygen molecules to transform the hemoglobin from low affinity to high affinity



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

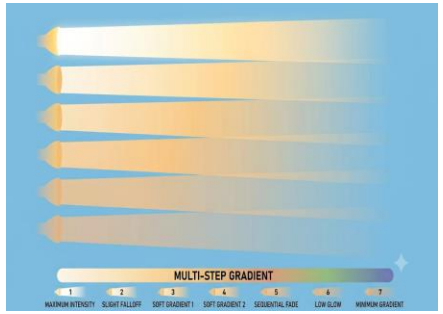
Y or θ is the fraction of oxygen-bound Hb

→ Y = mX + b (linear plot)

Cooperativity models

- Two models of cooperativity that could explain the observed data
  - Concerted model – all subunits undergo the conformational change Simultaneously **“rough structure”**
    - There are only two states, R and T.
  - Sequential model – the subunits undergo the conformational change one at a time. **“Has sequence in affinity”**
    - There are multiple states between full T and full R.

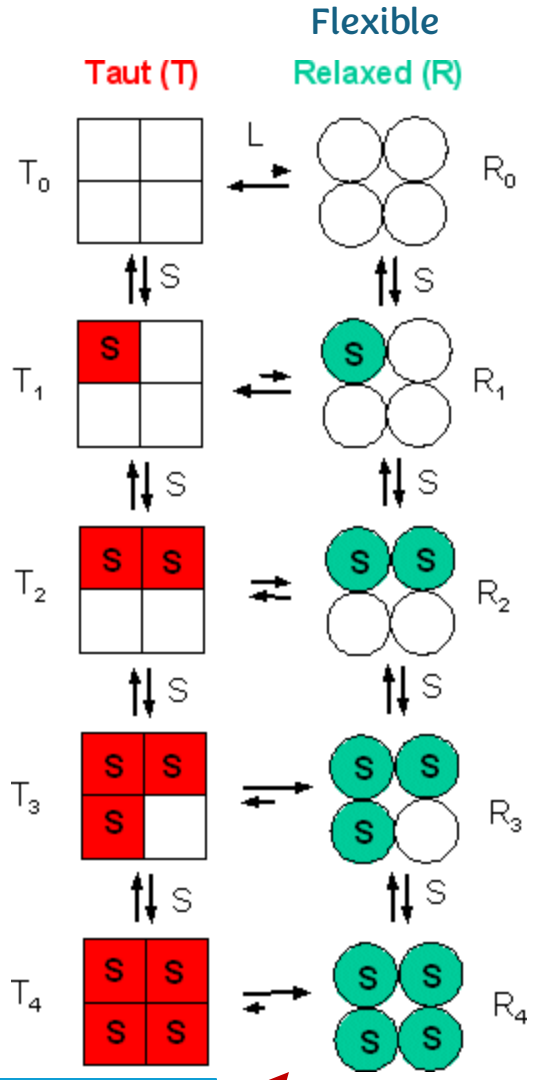
Let’s take an example of a light in a room you can turn it on or off.
But there are other lights which have a modulator that can control the “intensity” of light, light travels in several degrees of intensity.



So here the question pops up, this allosteric protein works like an on off light (R state or T state)?
Or is there structures that are intermediate?
Scientists discovered that hemoglobin undergoes only 2 structures either T or R state, while other scientists found out hemoglobin has intermediates in affinity, until now there is no inevitable answer because experiments show that the two models are true.

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.
- This allows unoccupied subunits to adopt the high affinity R-state.



If we don't have oxygen at all and looked at hemoglobin molecules,
10% will be in R state
90% will be in T state
Even if we don't have oxygen.
When we add more oxygen,
30% R state, 70% T state
(The oxygen changed the equilibrium more to the R state but the T state is still the dominant)

Adding more oxygen increases the probability of having R state molecules and changes the equilibrium more to the R state.

When oxygen binds to one of the polypeptides makes the probability of the hemoglobin molecule being in the R state higher

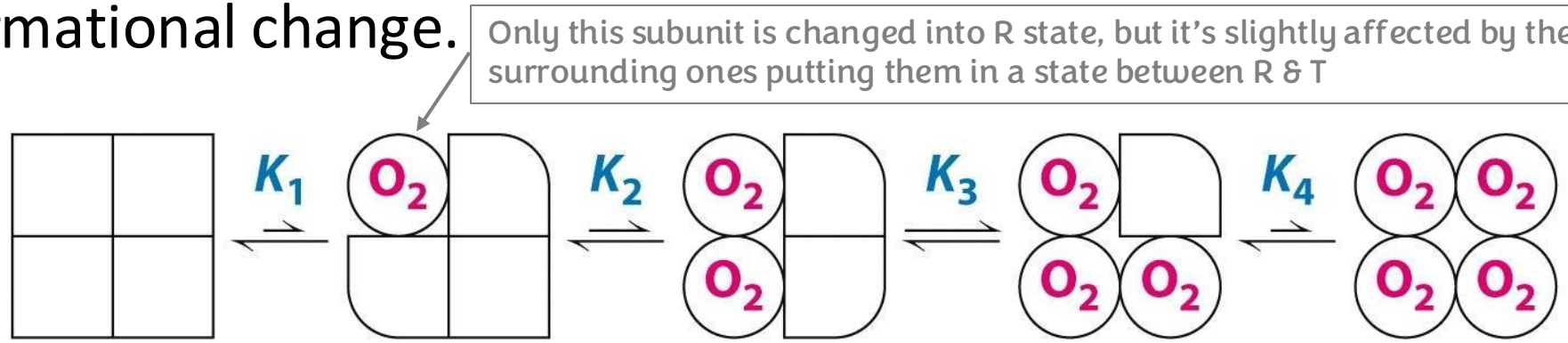
So basically the concerted model claims that hemoglobin exists either in T or R state and oxygen changes the equilibrium making the hemoglobin has higher probability in being in the R state.

At the end –binding the all 4 polypeptide the Oxygen- the equilibrium towards the R state become 90% in the R state and 10% in the T state

Note direction of arrows

The sequential, induced fit, or KNF model

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



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

Here, oxygen changes one of the polypeptides making it in the R state and slightly changes the polypeptides around it (closer to R state) and so on. Just like when you rotate someone's finger, you keep rotating it until it's utterly rotated with intermediate levels

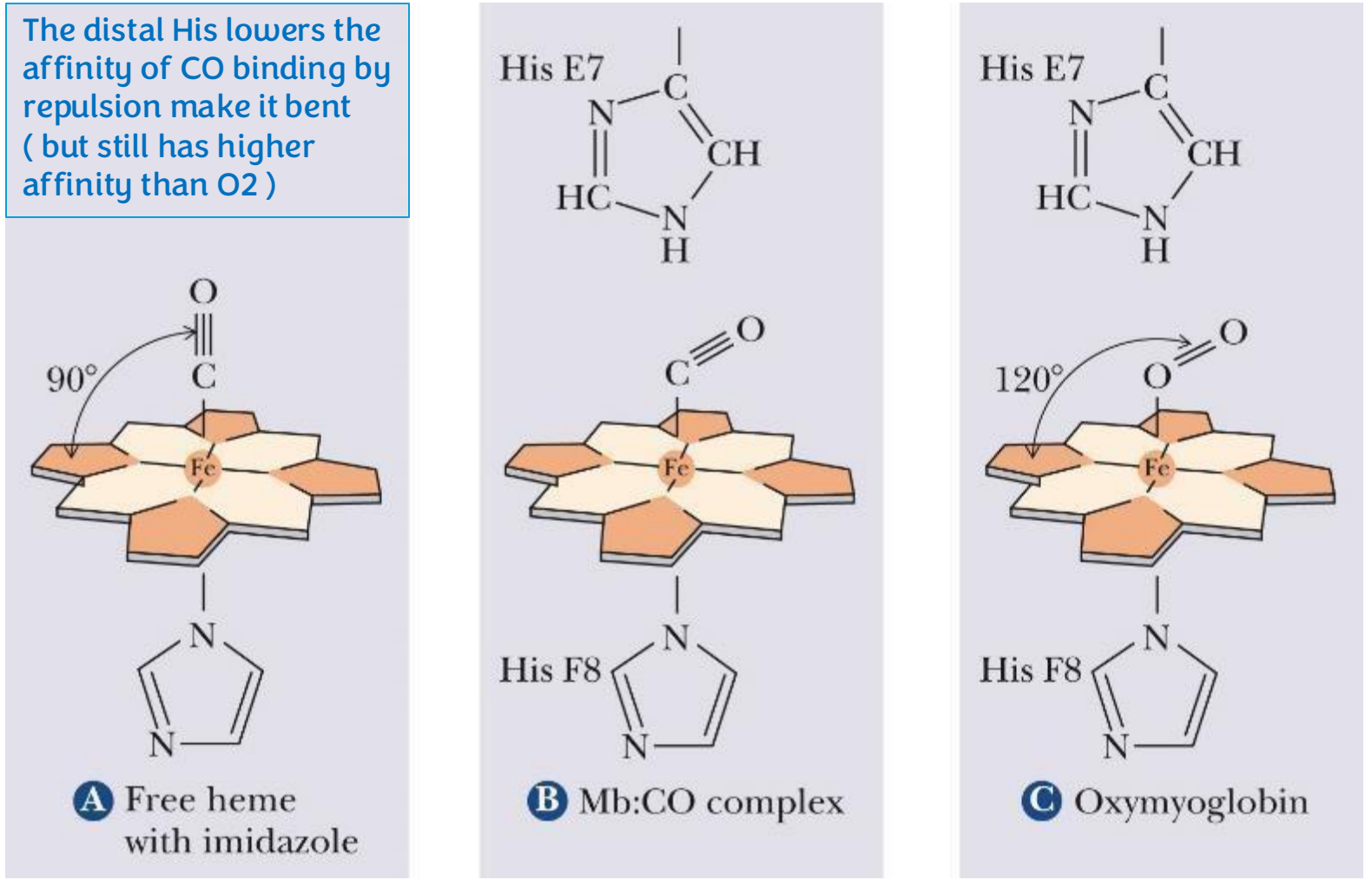
Another significance of distal histidine

It is the gate keeper, organizing the entry of gases to the heme, only allowing O2 to enter (& CO because they look alike

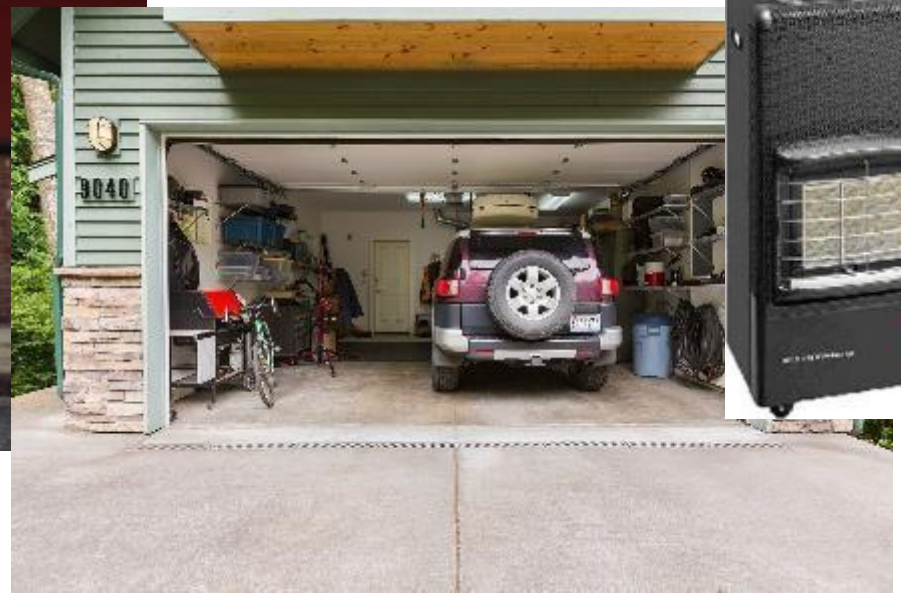
- CO prefers straight bonding, but O_2 prefers bent bonding.
- CO binds to free heme with higher affinity (thousands folds more) than O_2.
- The affinity of CO to myoglobin-bound heme is only 250 times more than O_2.
- Yet, CO occupies 1% of hemoglobin, but 99% if distal His does not exist.

Because of the distal His & the low presence of CO in the surrounding air, we don't have high levels of CO in our blood (which will kill us if we had 🙂)

The distal His lowers the affinity of CO binding by repulsion make it bent (but still has higher affinity than O2)



Accidents



وفاة أب وأم وابنهما إختناقاً بـ "صوبة غاز"

وفاة مواطن وزوجته اختناقا بصوبة الغاز في بيرين

وفاة 3 اشخاص من عائلة واحدة اختناقا بسبب صوبة غاز في الموقر

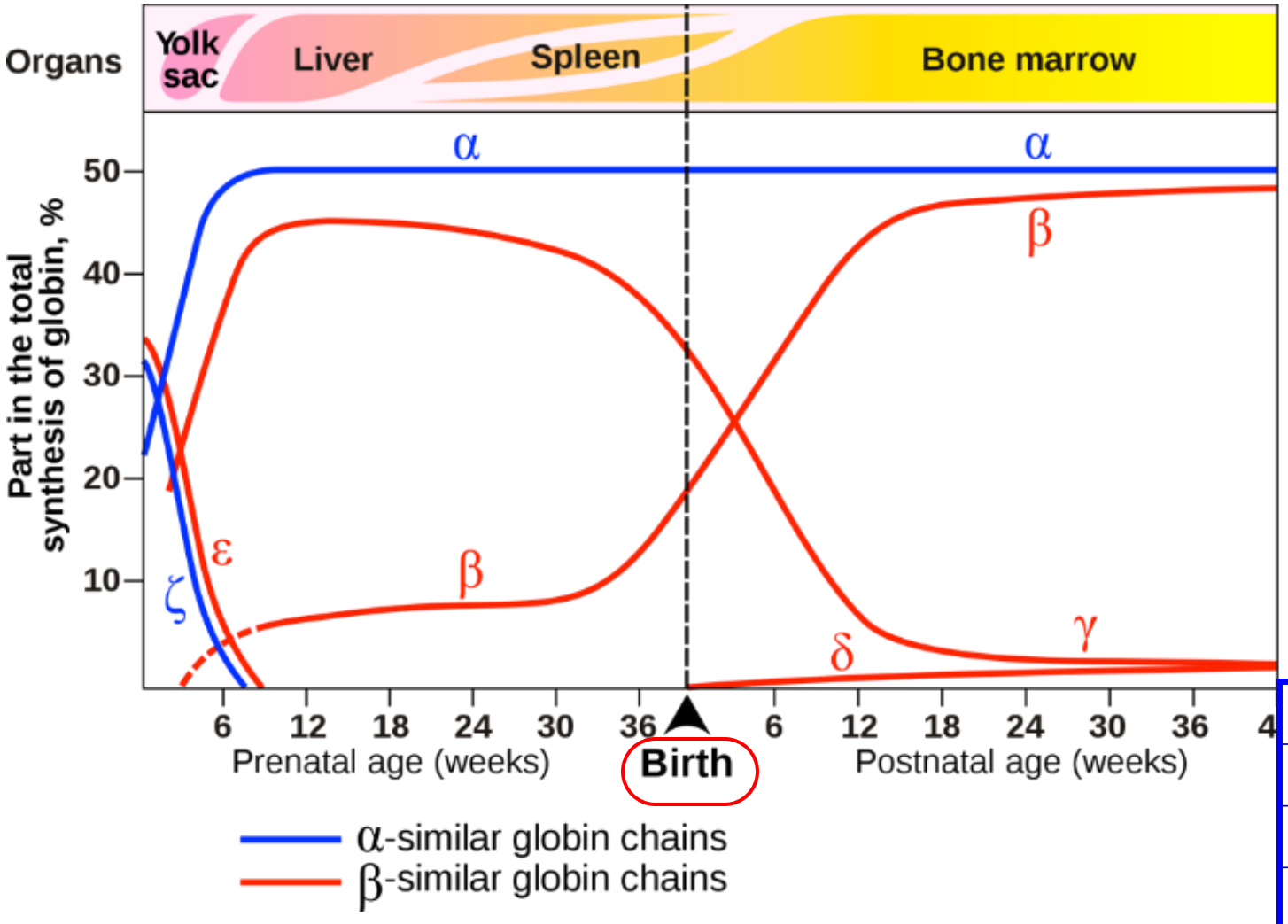
24 وفاة اختناقاً بالصوبات منذ بداية الشتاء.. و«الدفاع المدنى» تحذّر

It is not only one hemoglobin

It’s not a single molecule that exists in our body, there are several hemoglobin that are changing depends on development stage.

Developmental transition of hemoglobins

- embryonic stage: epsilon & zeta are present
- fetal stage: alpha & gamma increase while the previous two decrease
- before birth: beta start to increase while gamma decrease
- at birth: 60% alpha & gamma (fetal hemoglobin), 40% alpha & beta (adult hemoglobin)
- adult stage : mainly alpha & beta, delta increase after birth but present in very low levels
- It starts from fertilization

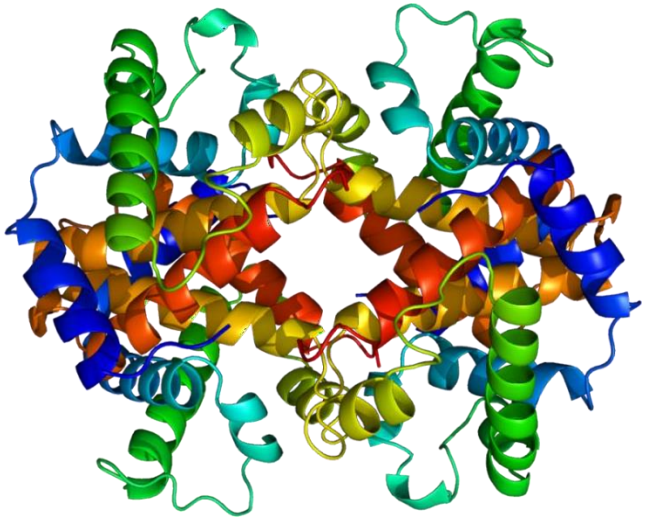


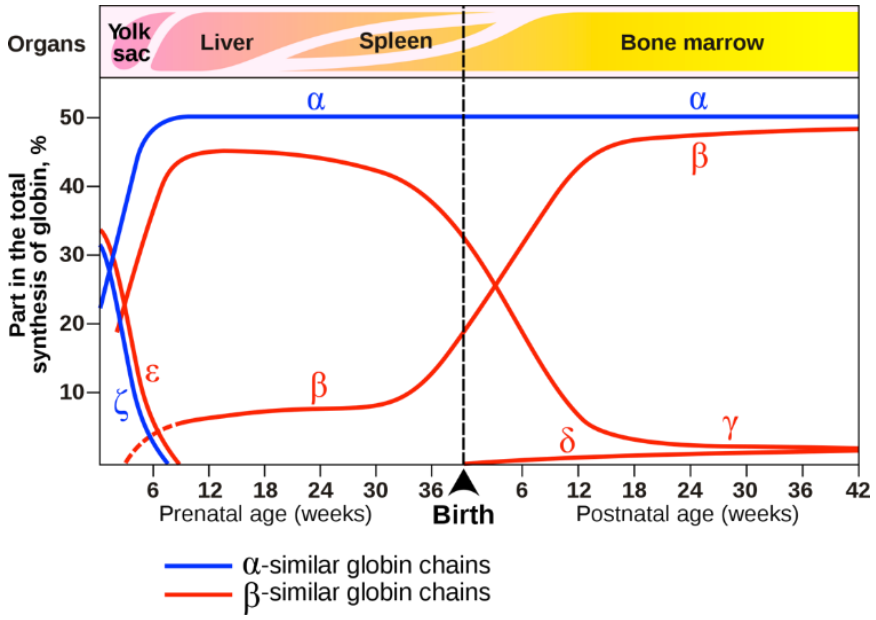
Alpha	Α α
Beta	Β β
Gamma	Γ γ
Delta	Δ δ
Epsilon	Ε ε
Zeta	Ζ ζ

The embryonic stage

~ 8 weeks

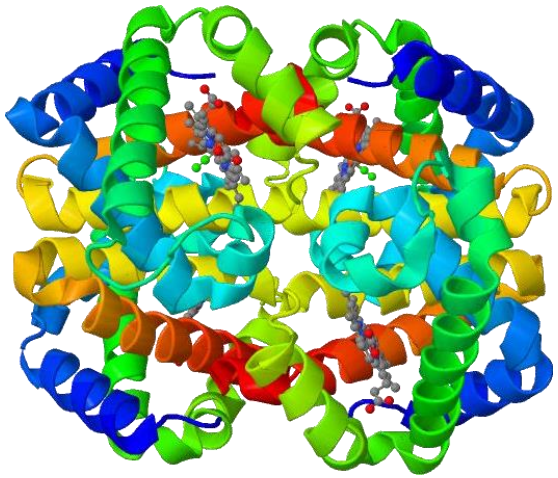
- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1) is a tetramer composed of 2 zeta (ξ) chains and 2 epsilon (ε) chains.
- Other forms exist (*do not memorize*): HbE Gower 2 (α2ε2), HbE Portland 1 (ζ2γ2), HbE Portland 2 (ζ2β2).

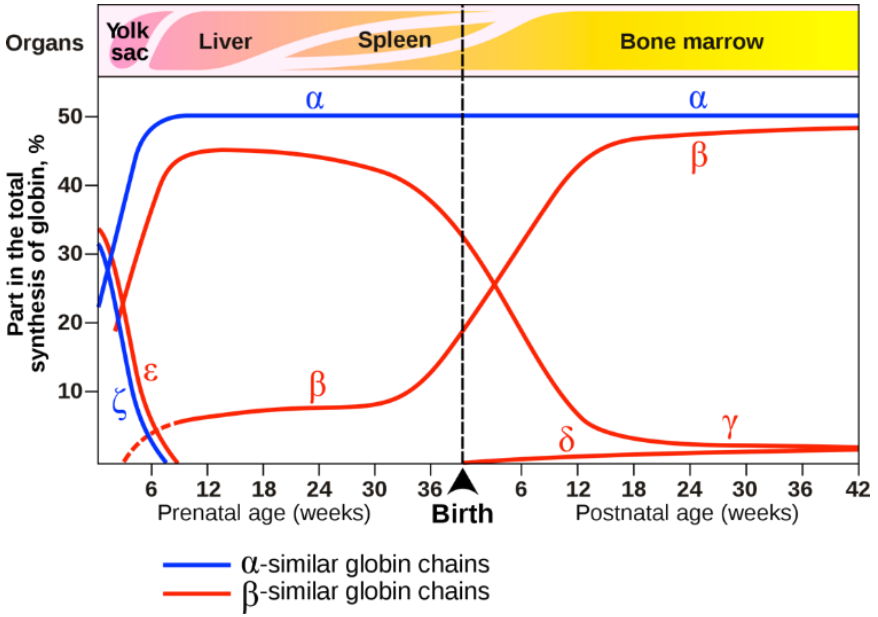




The fetal stage

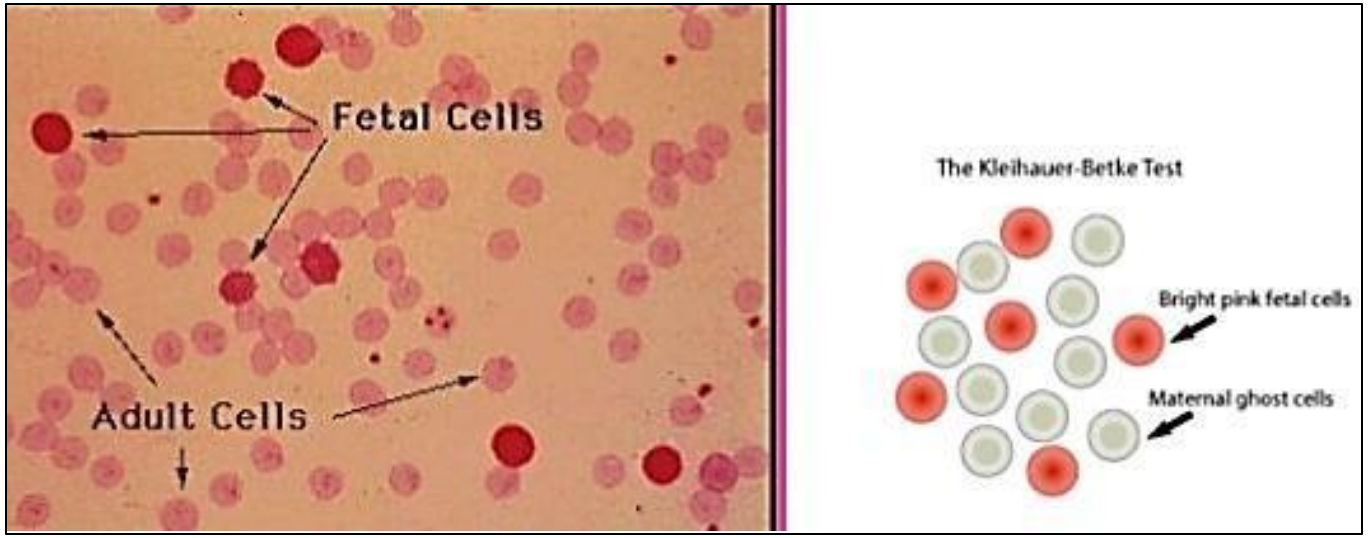
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines and fetal hemoglobin synthesis starts.
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides ($\alpha 2 \gamma 2$)
- The gene expression of the α polypeptides is active throughout life.

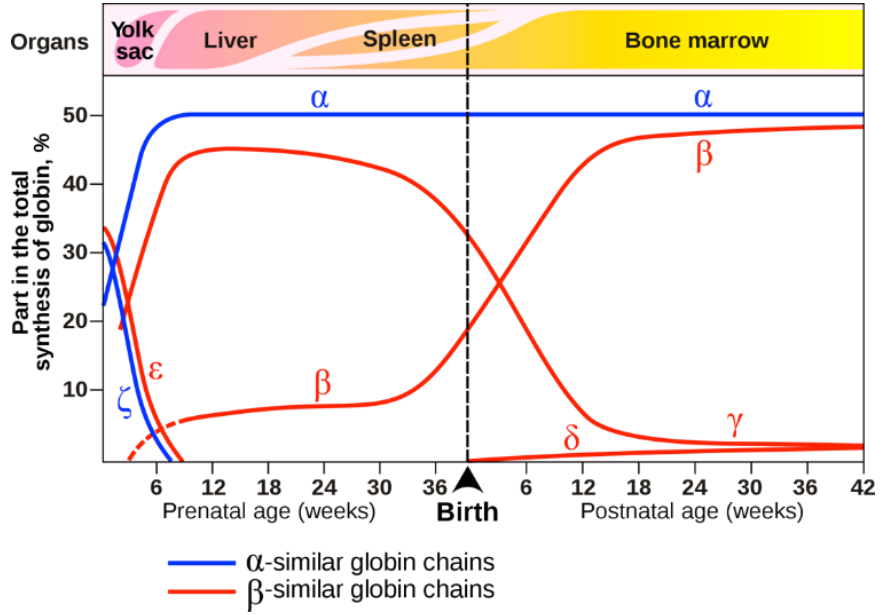




The adult stage

- Shortly before birth, there is a gradual switch to adult β-globin.
- Still, **HbF** ($\alpha_2\gamma_2$) 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.

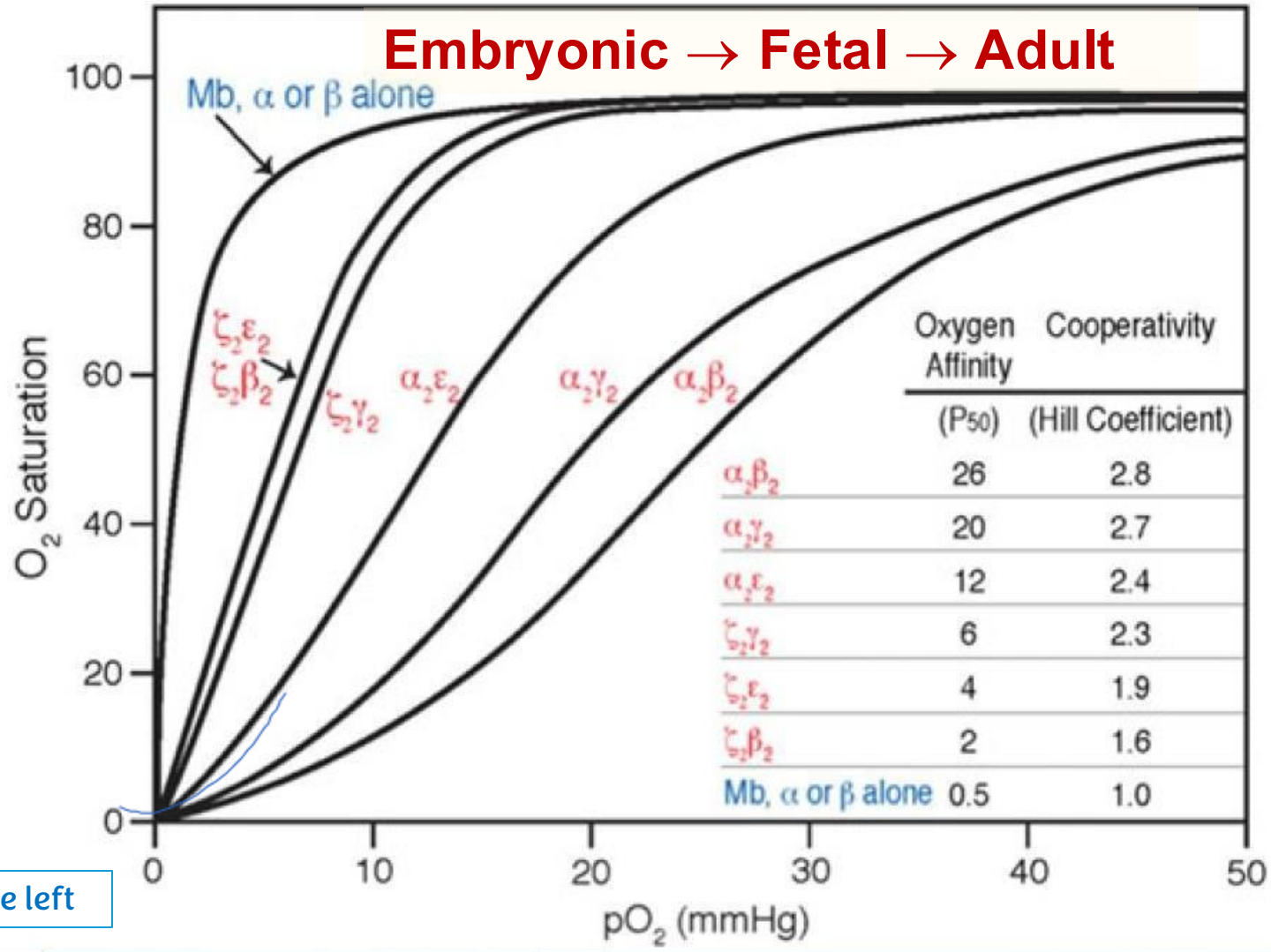




Do not memorize

Range of O_2 Saturation/Normal Human Hbs

Embryonic → Fetal → Adult



	Oxygen Affinity	Cooperativity
	(P_{50})	(Hill Coefficient)
$\alpha_2\beta_2$	26	2.8
$\alpha_2\gamma_2$	20	2.7
$\alpha_2\varepsilon_2$	12	2.4
$\zeta_2\gamma_2$	6	2.3
$\zeta_2\varepsilon_2$	4	1.9
$\zeta_2\beta_2$	2	1.6
Mb, α or β alone	0.5	1.0

Note that the HbF has higher affinity to O2 than HbA

The curve shifts to the left

P50% becomes lower → the affinity becomes higher

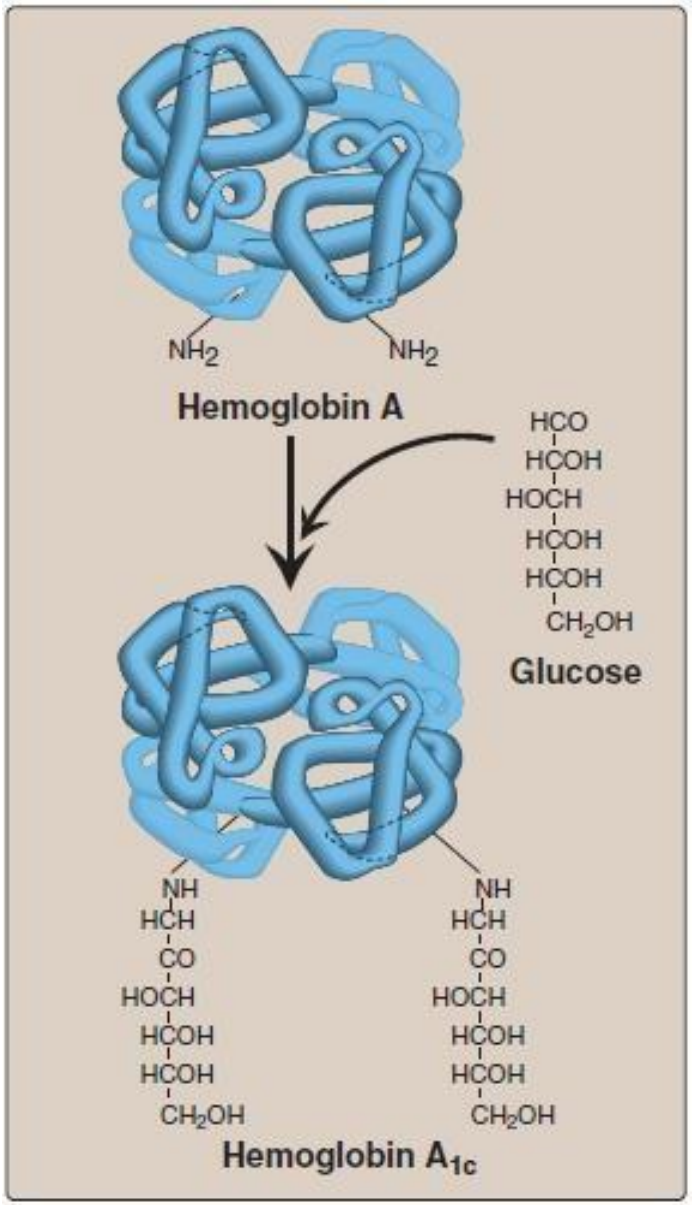
Adult hemoglobins

Glycated = add sugar to the hemoglobin spontaneously (non-enzymatically
Glycosylation = add sugar to the protein enzymatically

- HbA1 can be glycated non-enzymatically with glucose 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.

يستخدم لفحص مستوى السكّر التراكمي
So it's a perfect indicator for diabetes
In diabetes, the glycated hemoglobin more than 5.5%

The increase of the glucose in blood, the increase of glycated hemoglobin (HbA1c).
We have 5.5% of hemoglobin in blood is glycated, it can be a bit greater or lower than 5.5%



Advantages of HbA1c testing

Indicates the body's ability to control glucose levels

- Blood fasting glucose level is the concentration of glucose in blood at a single point in time when fasting for a few hours. (over short period of time)
- **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). (over long period of time)

  Shows whether these levels change with prescripted drug, diet, etc..

  - HbA1c can be expressed as a percentage (DCCT unit, used in the US) or as a value
  - in mmol/mol (IFCC unit).

Table

Memories red columns

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

Test yourself

Quiz!

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)

رسالة من الفريق العلمي:

«معالي الأمور، والطموحات الكبرى في العلم والتعليم والإصلاح والتغيير والنهضة بالأمة، لا تكشف وجهها لك حتى تمسح العرق عن جبينك بيد ترتعش من العناء»

-الشيخ إبراهيم السكران |مسلكيات ١٢