بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture 20

Globular proteins (pt.2)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

اللهم استعملنا لنصرة دينك

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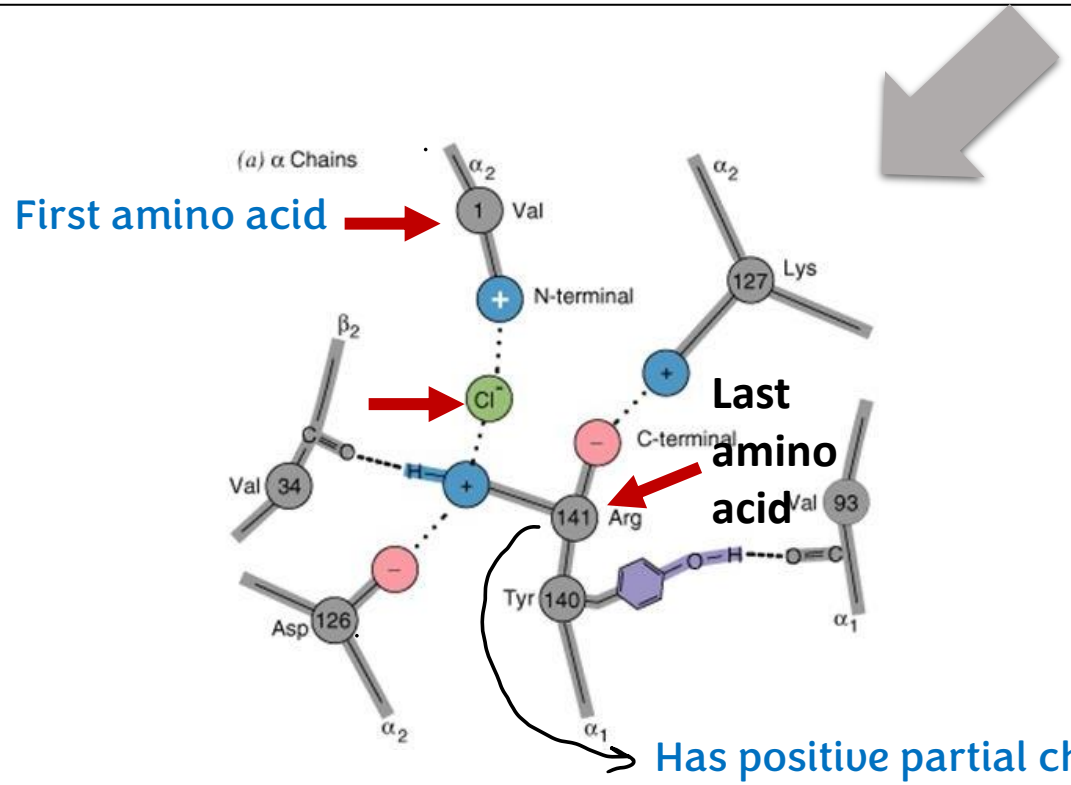
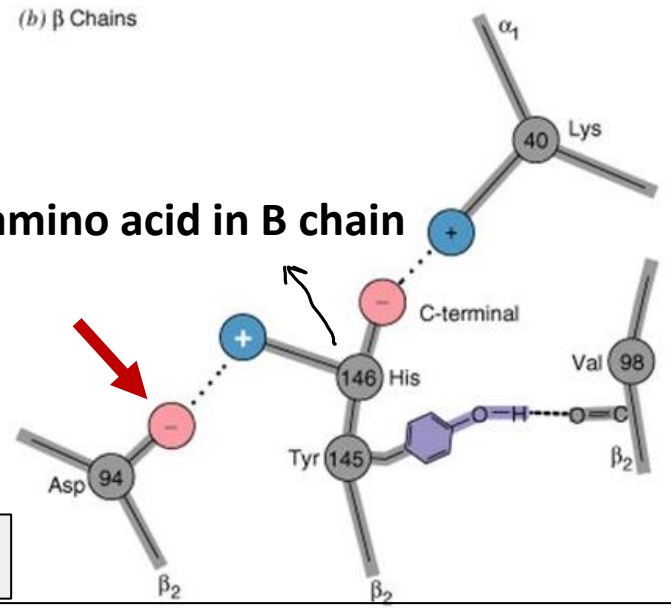
The broken interactions

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

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)

Do not memorize





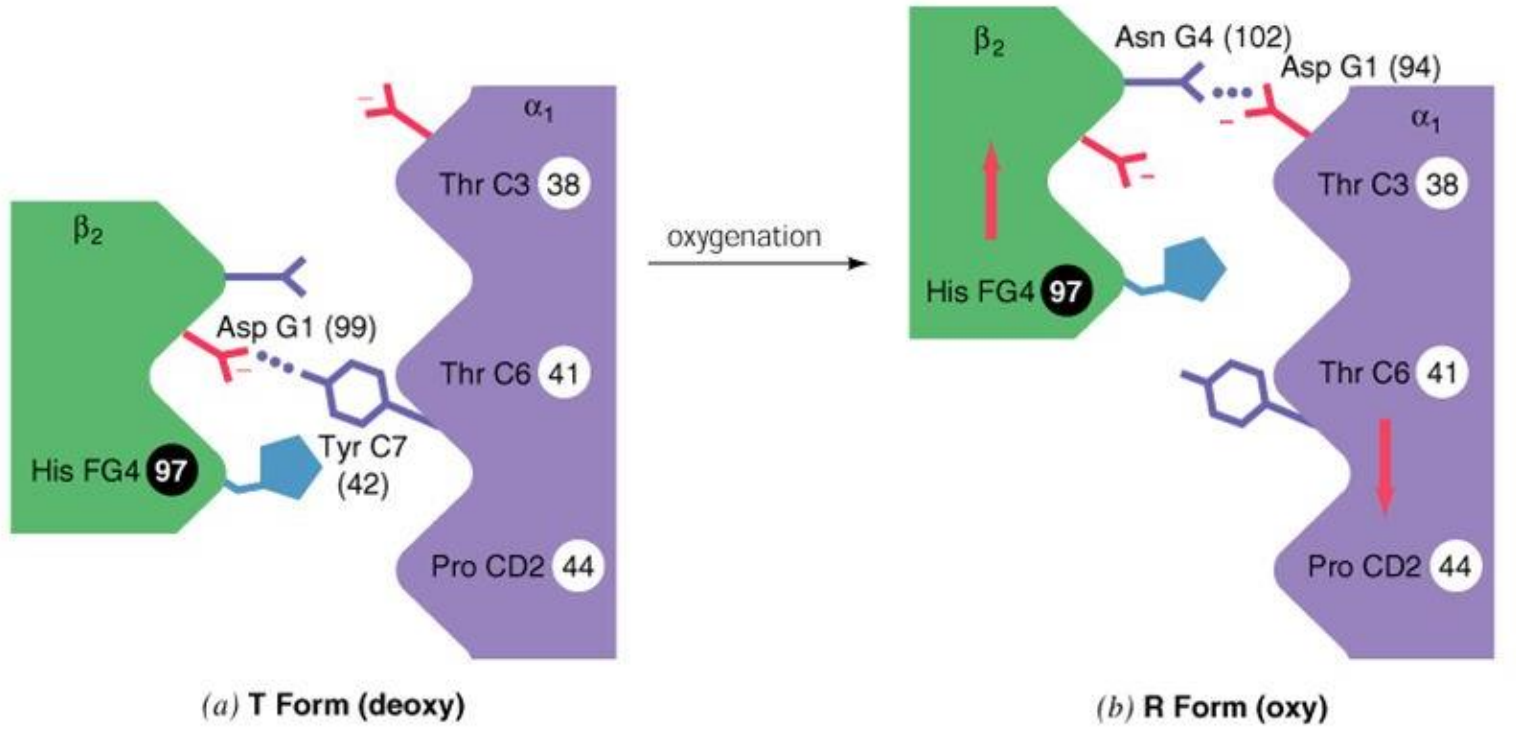
Do not memorize

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 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.

Do not memorize

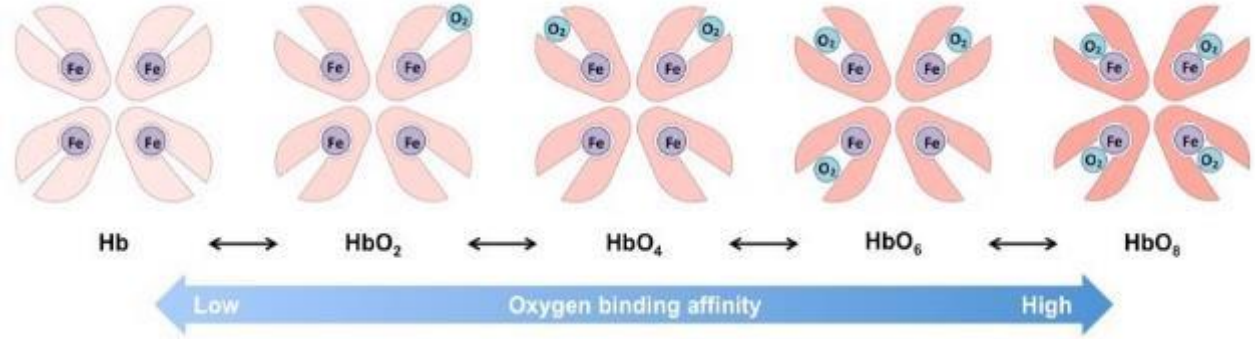


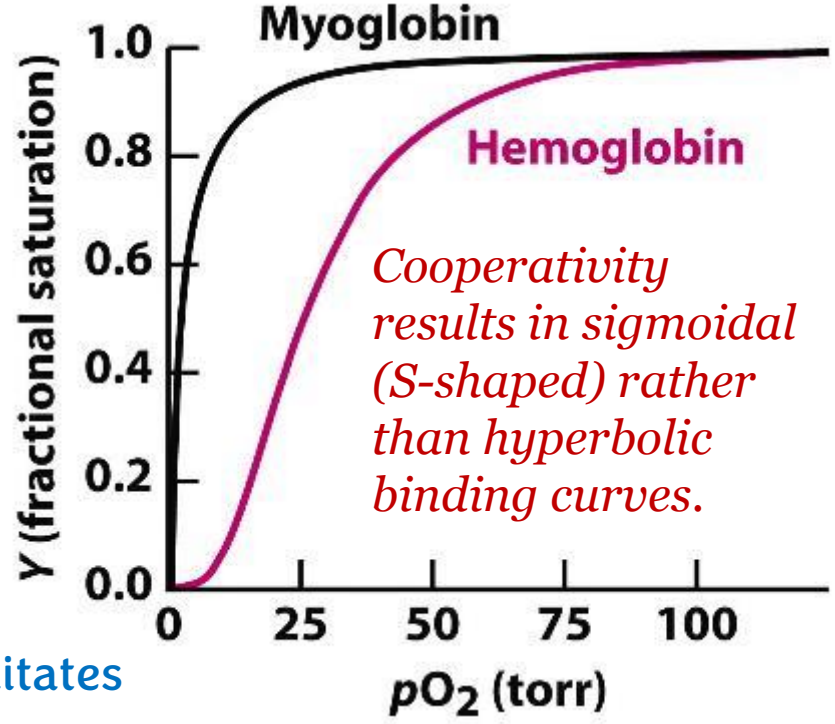
(a) T Form (deoxy) (b) R Form (oxy)

Binding is cooperative

For every oxygen (O_2) bound to the hemoglobin molecule, electrostatic interactions are weakened further.

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



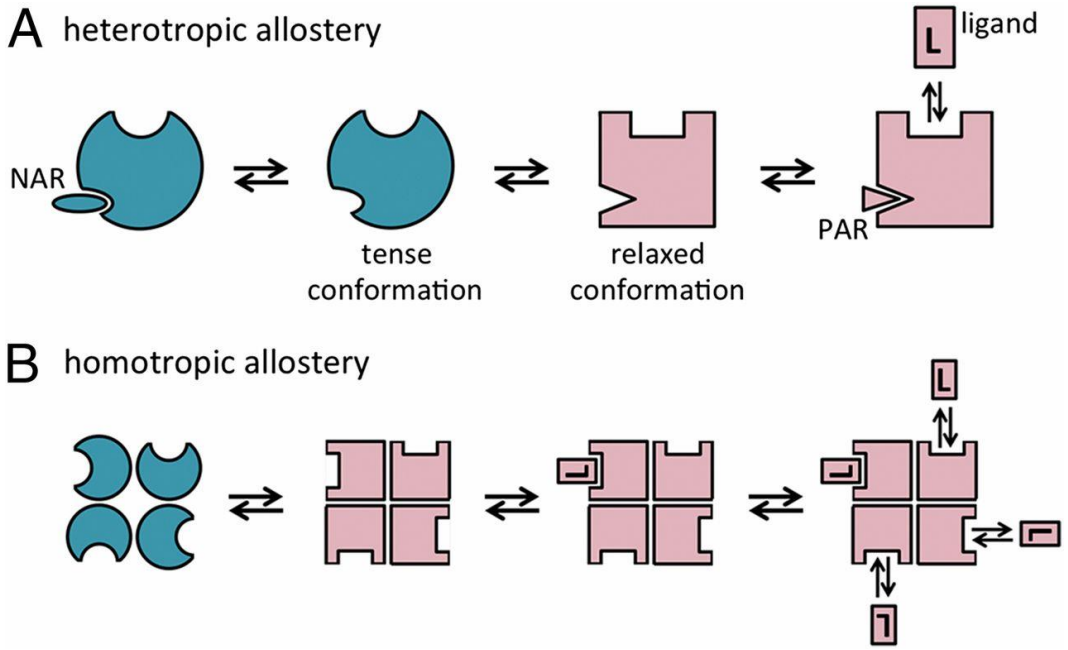


Cooperativity results in sigmoidal (S-shaped) rather than hyperbolic binding curves.

Every oxygen that binds to hemoglobin facilitates another oxygen binding to it.

Some terminologies

- 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.



Cooperativity models

Scientists made two models to explain the change in hemoglobin structure from T to R state and vice versa:

- The concerted model, which proposes that hemoglobin undergoes a sudden structural change, is like an on-off situation, lacking any intermediate structures.
- The sequential model which says that the hemoglobin gradually change its structure, so we will see an intermediate structures between T and R states.

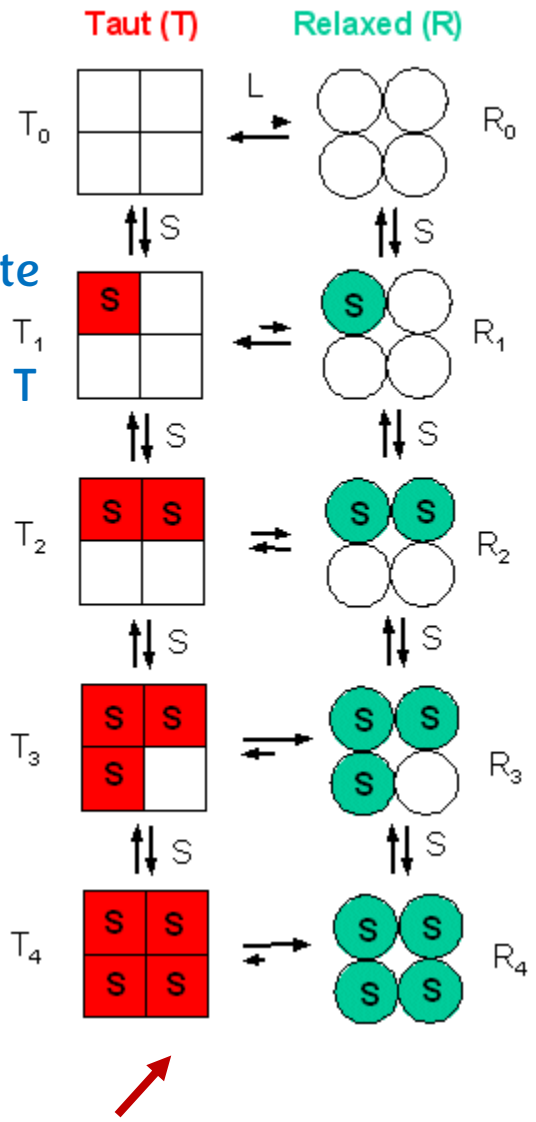
- Two models of cooperativity that could explain the observed data
  - Concerted model – all subunits undergo the conformational change simultaneously
    - There are only two states, R and T.
  - Sequential model – the subunits undergo the conformational change one at a time.
    - There are multiple states between full T and full R.

The concerted model (MWC model)

In this model the hemoglobin exists in two forms, either in the T state or in the R state.

When the hemoglobin is not bound to oxygen its structure exists in equilibrium between the two states, but with the equilibrium more shifted towards the T state. (95% in the T state and 5% in the R state).

When hemoglobin binds to one oxygen molecule the equilibrium will be 75% in the T state and 25% in the R state. When it binds two oxygen molecules, the equilibrium shifts to 50%-50%, and when it binds four oxygen molecules, the equilibrium shifts to 5% in the T state and 95% in the R state. **Notice that when hemoglobin binds to oxygen the equilibrium will be shifted more towards the R state.**



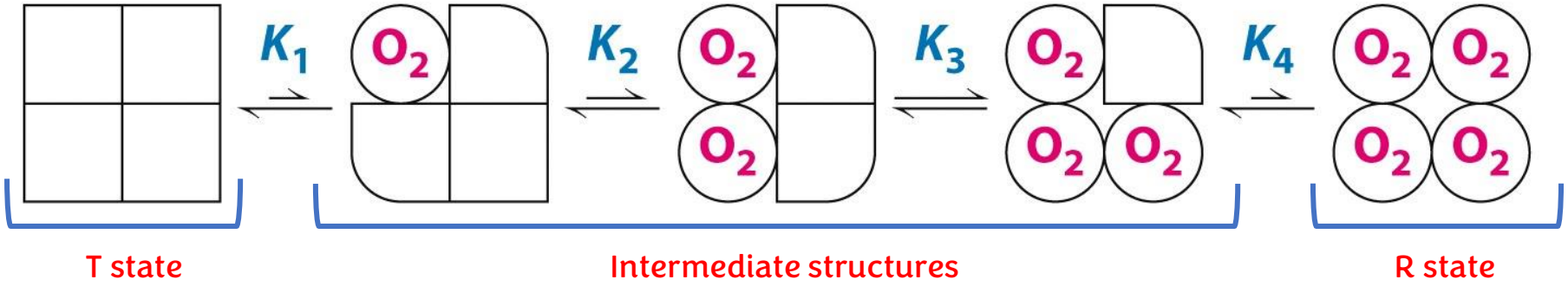
- The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.
- Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.
- This allows unoccupied subunits to adopt the high affinity R-state.

Note direction of arrows

The sequential, induced fit, or KNF model

In the sequential model, hemoglobin gradually changes its structure. The binding of one oxygen molecule will only change the structure of the bound subunit (from T to R) and slightly change the structure of the nearby subunit. This gives us 3 intermediate structures until hemoglobin becomes fully saturated with oxygen.

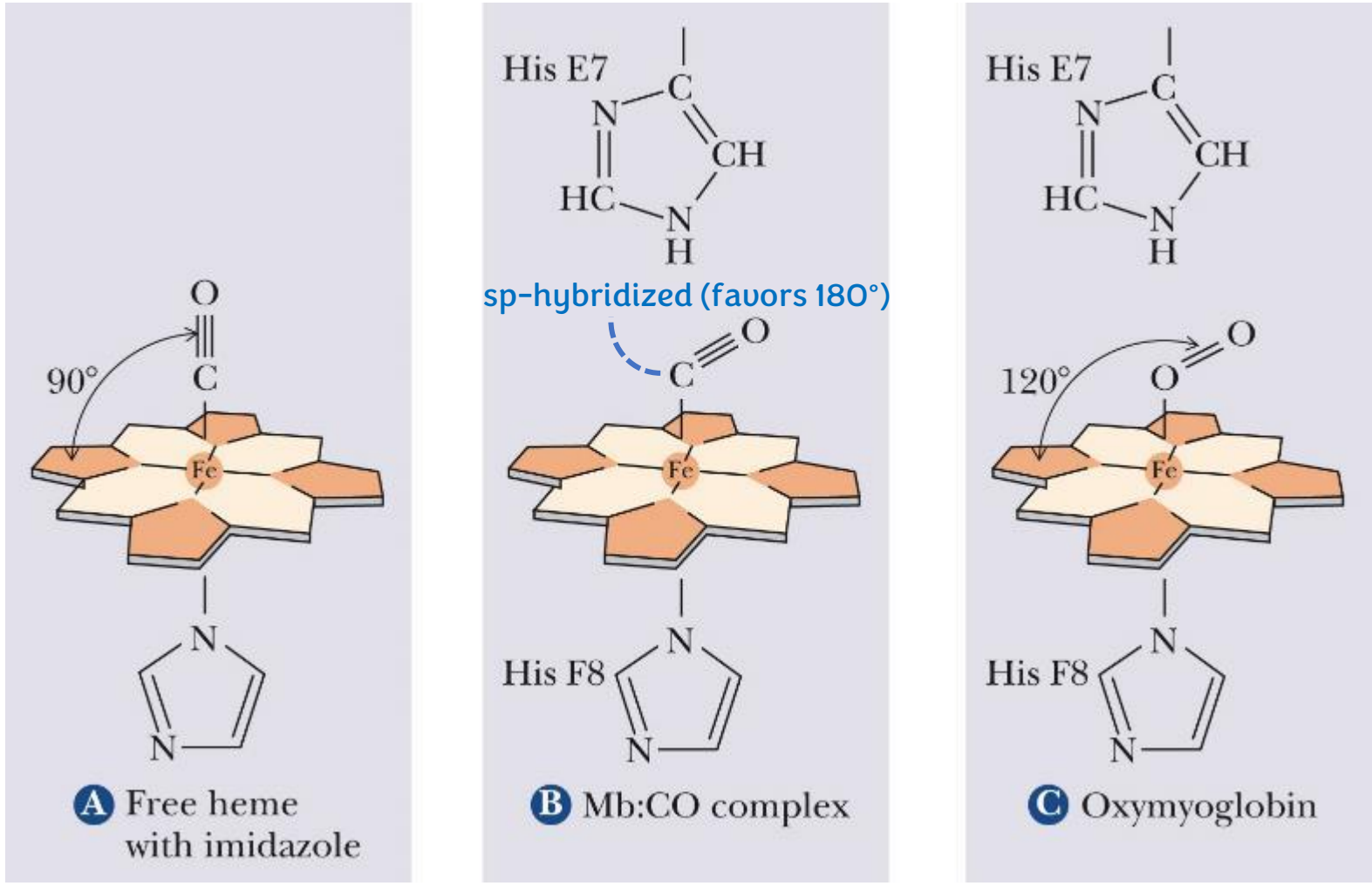
- The subunits go through conformational changes independently of each other, but they make the other subunits more likely to change, by reducing the energy needed for subsequent subunits to undergo the same conformational change.



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

Another significance of distal histidine

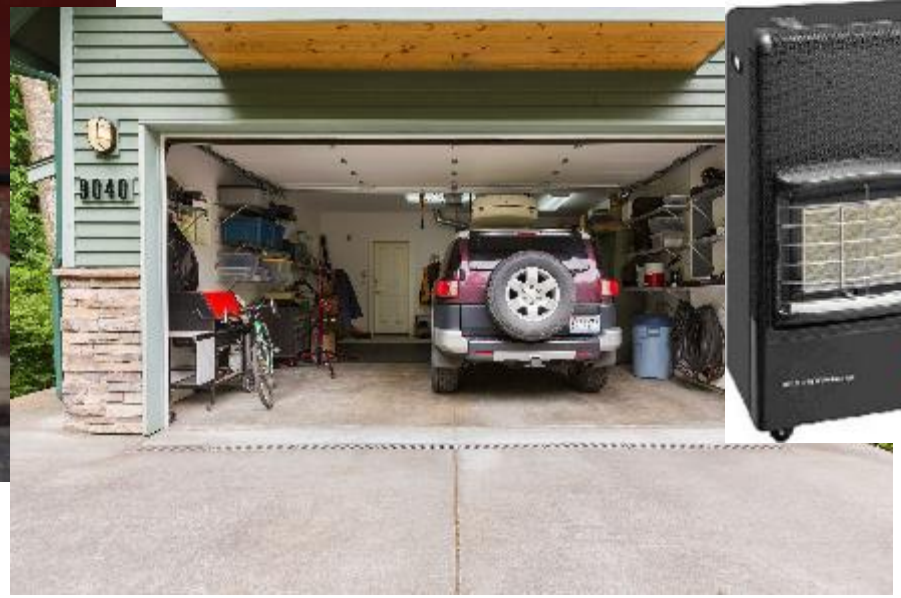
- CO prefers straight bonding, but O_2 prefers bent bonding.
- CO binds to free heme with higher affinity (thousands folds more) than O_2.
- The affinity of CO to myoglobin-bound heme is only 250 times more than O_2.
- Yet, CO occupies 1% of hemoglobin, but 99% if distal His does not exist.



The ratio of bound O_2 is higher than bound CO in globin-bound heme although the affinity is 250:1 in favor of CO. The reason behind that is the relatively huge abundance of oxygen in comparison to carbon monoxide.

Accidents

Carbon monoxide (CO) can displace oxygen (O_2) in binding to hemoglobin, potentially leading to death if CO levels become excessive. This can occur in environments with high CO concentrations, which can be caused by factors like (صوبة الغاز).



وفاة أب وأم وابنهما إختناقاً بـ "صوبة غاز"

وفاة مواطن وزوجته اختناقا بصوبة الغاز في بيرين

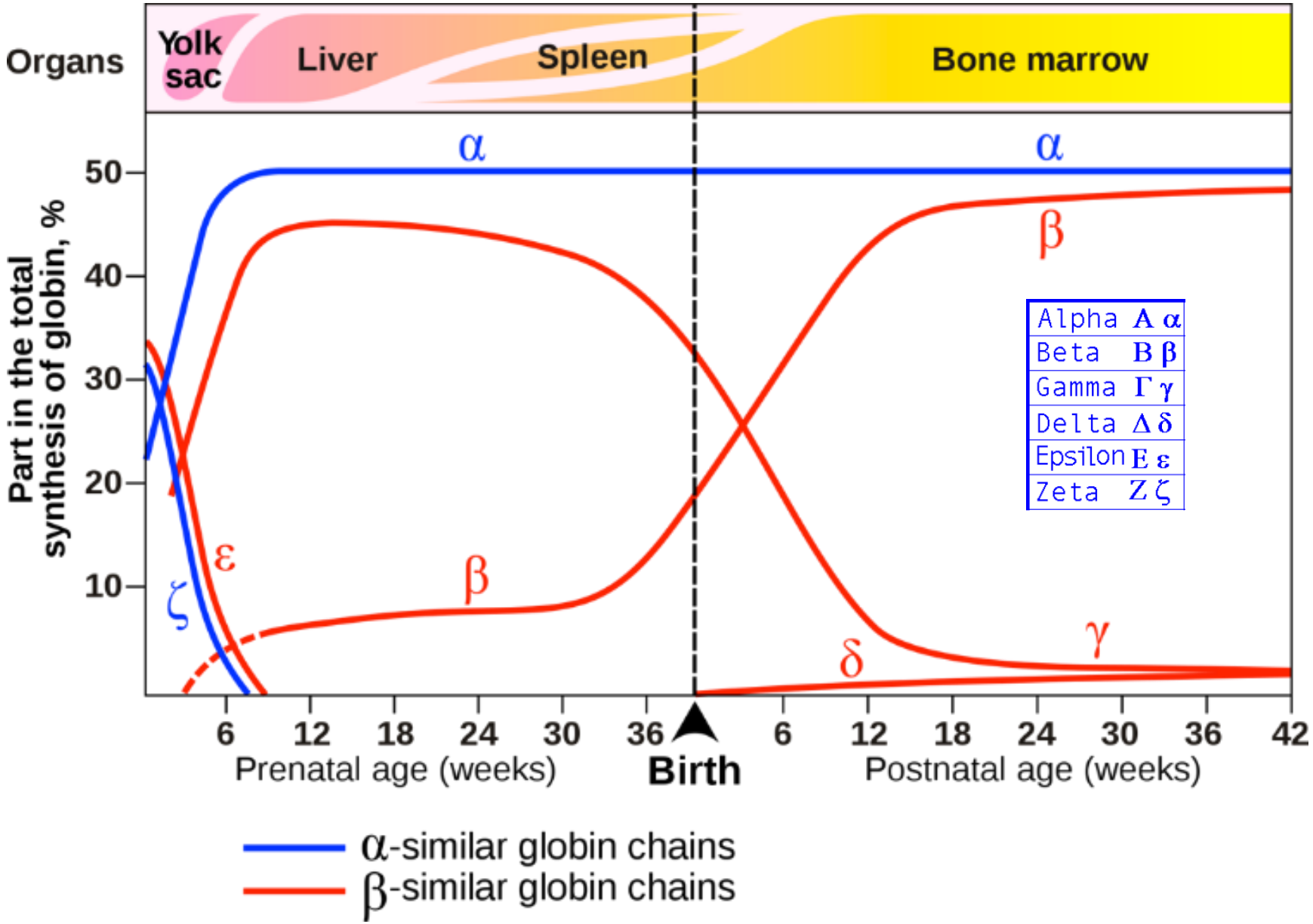
وفاة 3 اشخاص من عائلة واحدة اختناقا بسبب صوبة غاز في الموقر

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

It is not only one hemoglobin

There are more types of hemoglobin including $\alpha_2\beta_2$.

Developmental transition of hemoglobins



Some notes about the graph:

The zeta and epsilon chains are the precursors of the alpha and beta chains, respectively.

After the early prenatal stages, the alpha chain remains, while the zeta chain is permanently eliminated.

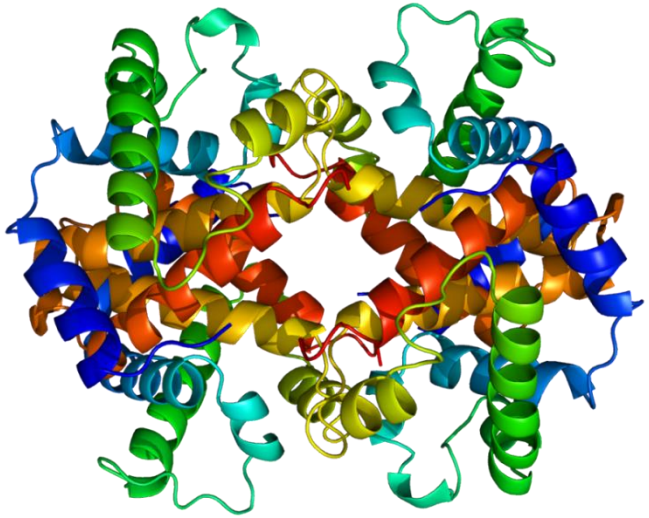
In contrast, the beta chain can be substituted by the gamma and delta chains in adults, but the beta form predominates.

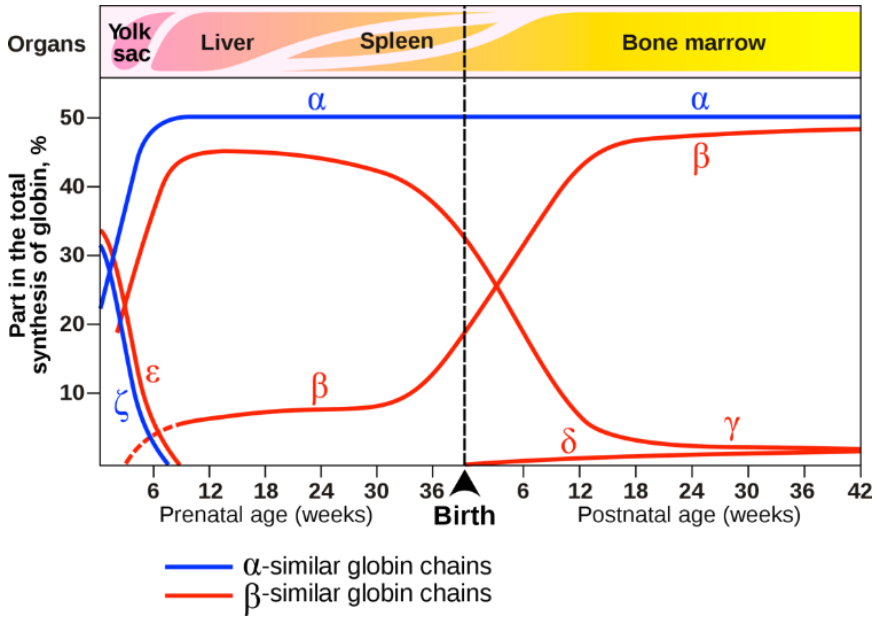
See the graph for relative proportions.

The embryonic stage

HbE Gower 1:
E stands for Embryonic stage

- Hemoglobin synthesis starts 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).

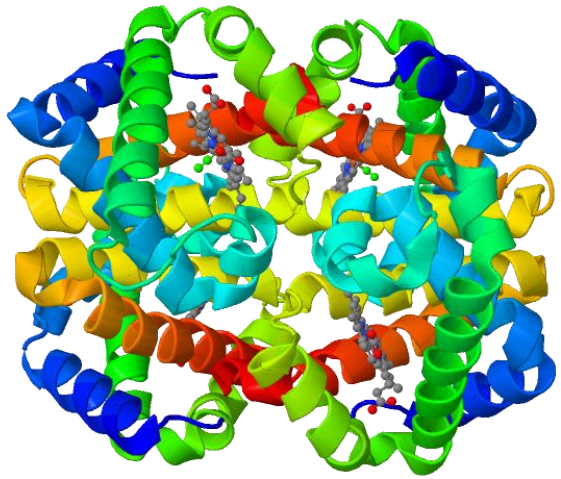


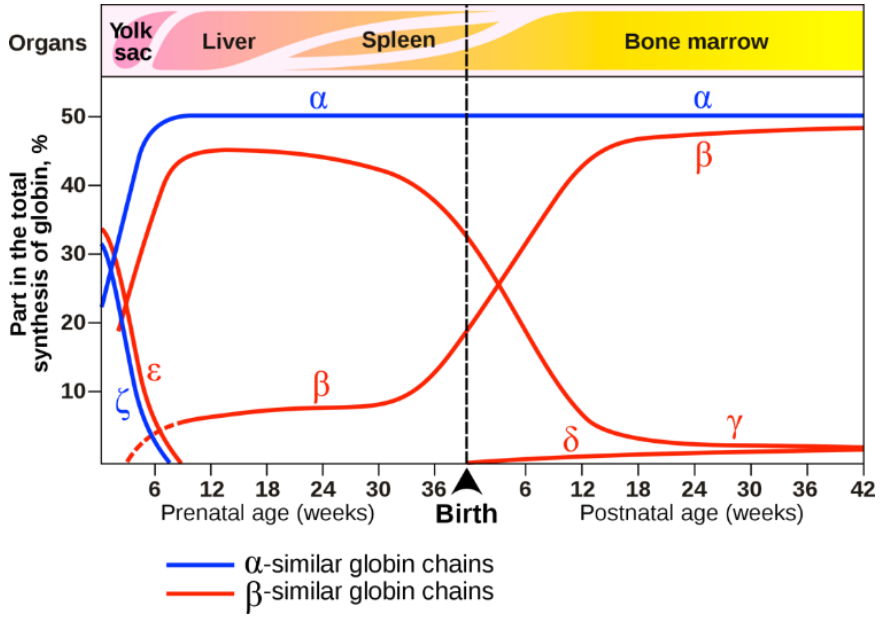


HbF:
F stands for Fetal stage

The fetal stage

- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines and fetal hemoglobin synthesis starts.
- Fetal hemoglobin (HbF) consists of **two α polypeptides and two gamma (γ) polypeptides (α2γ2)** A tetramer.
- The gene expression of the α polypeptides **is active throughout life.**





The adult stage

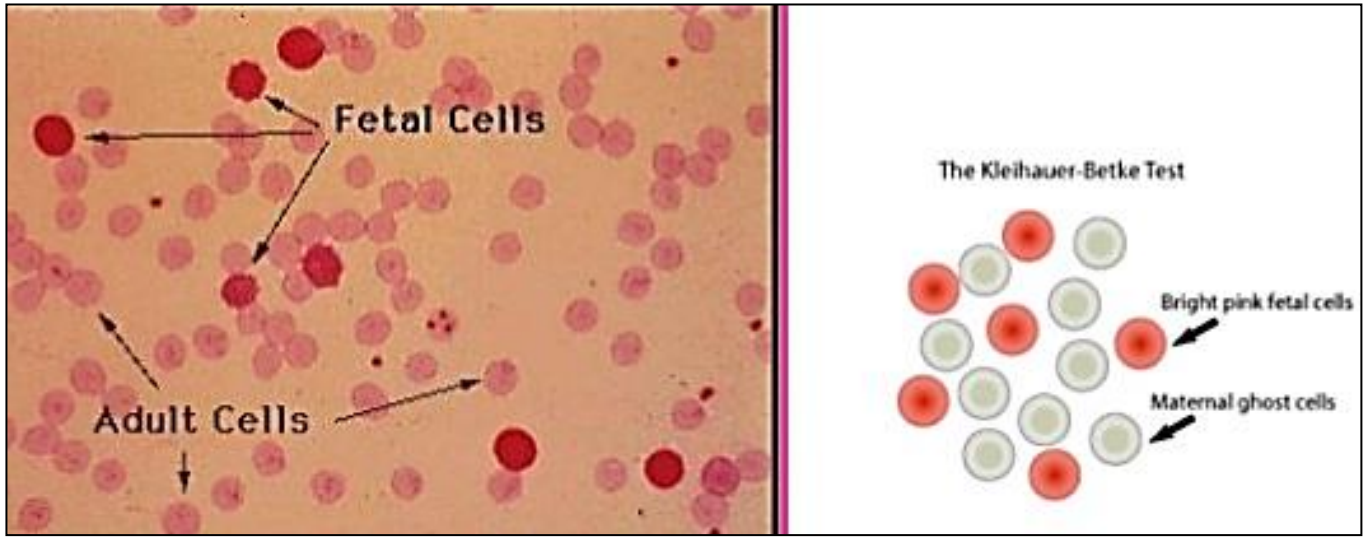
Shortly before birth, there is a gradual switch to adult β-globin.

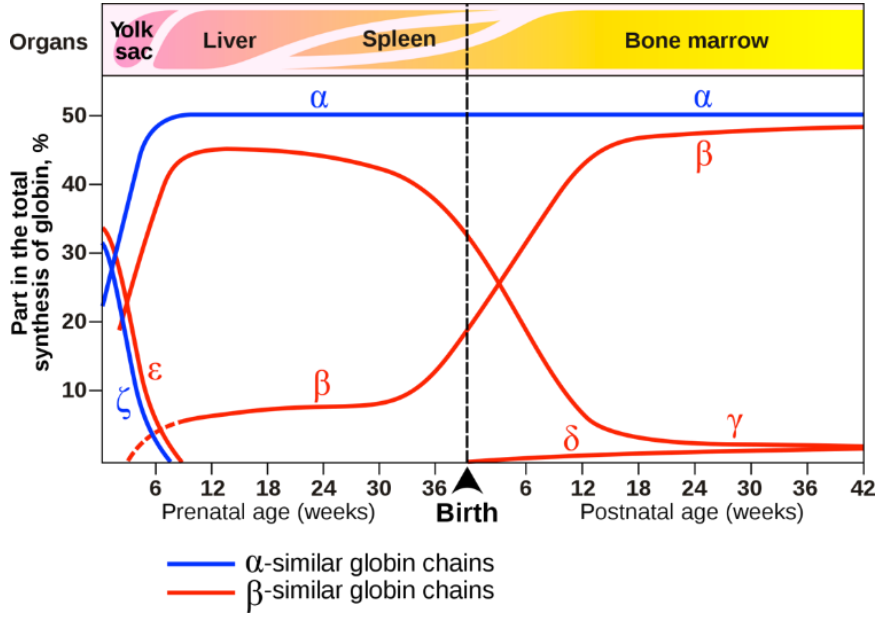
Still, HbF makes up 60% of the hemoglobin at birth, but 1% in adults.

At birth, synthesis of both γ and β chains occurs in the bone marrow.

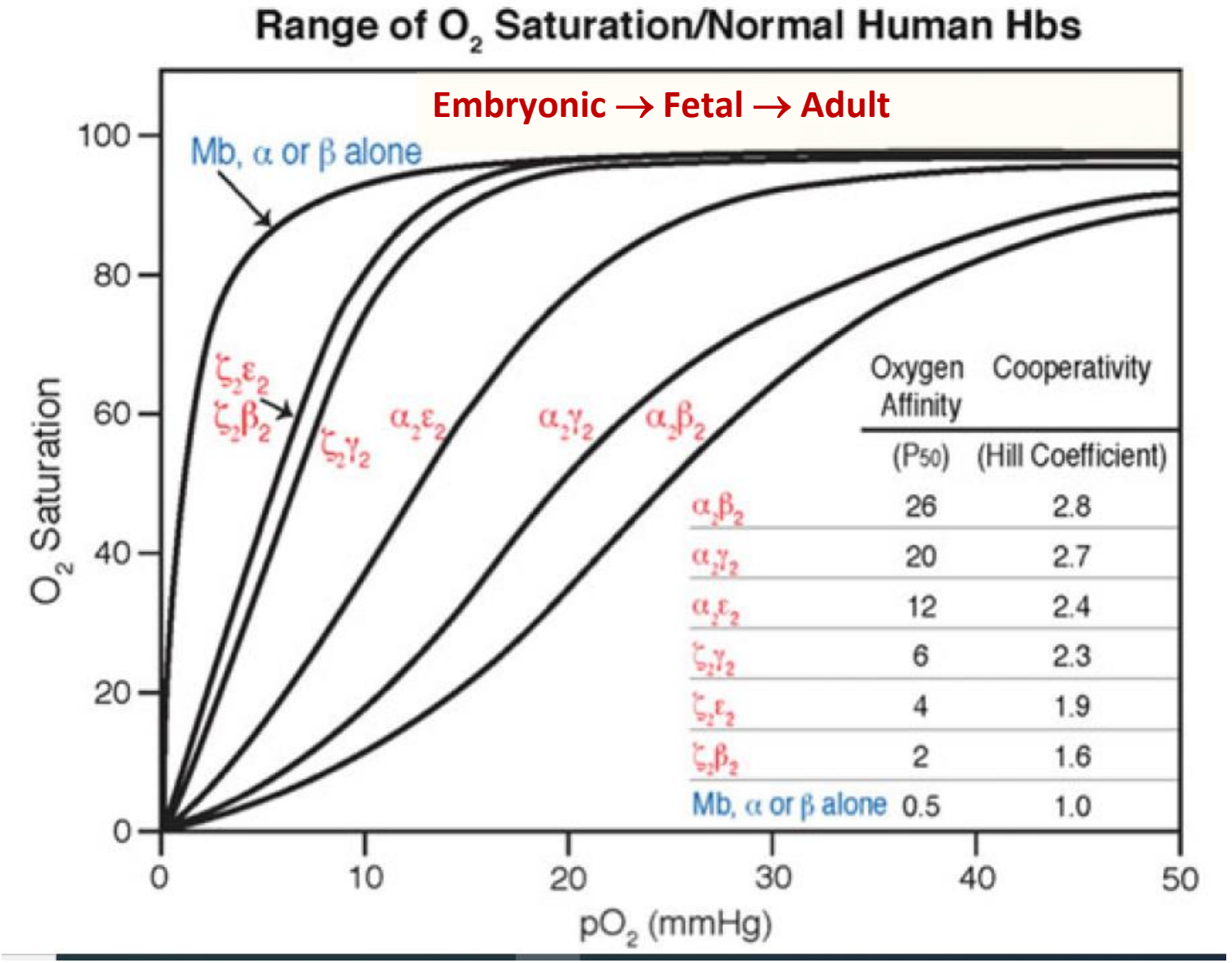
The **major** adult hemoglobin is HbA1 (a tetramer of 2 α and 2 β chains).

A **minor** adult hemoglobin, HbA2, is a tetramer of 2 α chains and 2 delta (δ) chains.





The O_2 saturation curve shifts to the right as "newer" forms take over preceding ones.



	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

Regarding the trends in the graph (going from left to right):

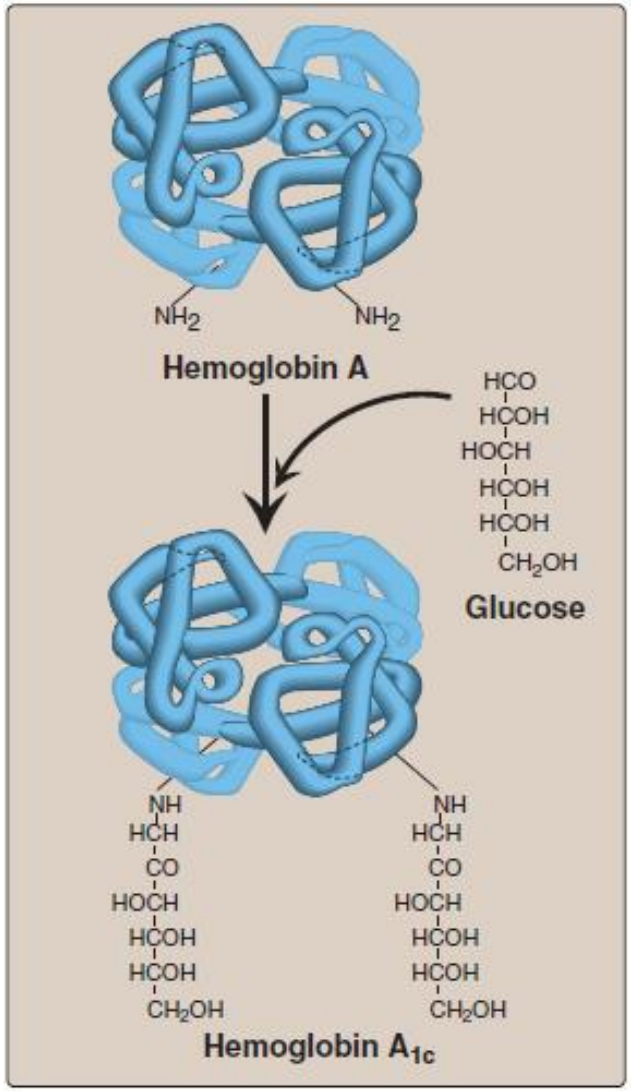
- Affinity of Hb forms to O_2 decreases.
- The p50 of Hb forms increases.
- More pO_2 is needed to reach the same saturation values.

This is why pregnant women are always gasping for air. They need to get enough oxygen to satisfy the needs of the growing embryo, which has greater affinity to oxygen than the adult woman.

Adult hemoglobins

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

There is a direct positive relation between the concentration of glucose in blood and the amount of HbA1c (the higher the blood sugar, the higher HbA1c), and this helps in developing a long-term measure for high glucose levels (see next slide).



Advantages of HbA1c testing

We have 2 main measures for glucose levels:

1. Fasting blood glucose level: The concentration of glucose in blood at a single point in time after fasting for a few hours.

If the blood glucose level after fasting is high, the reason is usually the incapability of cells to take in glucose (probably diabetes).

because it needs a longer time to change considerably

2. **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).

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

Table Normal fasting blood glucose levels are in the range of 90-100 and up to 120 (or 110 recently) mg/dL

Fasting Glucose Level | Glycosylated Hb

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

Information in red boxes are important (not as exact numbers, but you must be familiar with different categories)

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

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2			
V2 → V3			

Additional Resources Used:

رسالة من الفريق العلمي:

“You are nothing” ✨

~ Dr. Mamoun Ahram

الله يبارك فيه وبعلمه ❤️