بسم الله الرحمن الرحيم

BioChemistry | FINAL 5

Globular proteins pt.2

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Notes from the previous Lecture

When we talk about globular proteins, the best examples are hemoproteins. They have a heme group which is large micro cyclic molecule (it is organic and could be synthesized in our body) includes Iron in the middle .

Hydrophobic pocket of myoglobin indicates stabilization the heme by keeping it in reduced state .

Distal histidine works as a gate. As a result,

it stabilizes the oxygen bonding and makes it angular .

Hemes differ in structure and could be differentiated by spectroscopic features .

Notes from the previous Lecture

Why do we need hemoglobin ?

To maximize the saturation of oxygen inside the blood

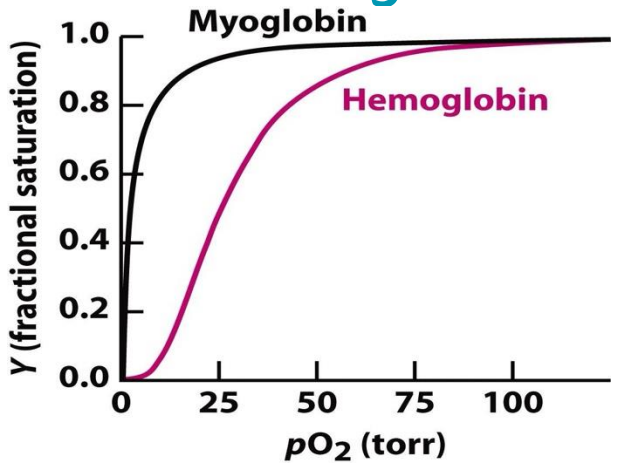
Hemoglobin adopts a cooperative behavior which will give a sigmoidal plot while the myoglobin curve is hyperbolic.

The sigmoidal curve tells that there is a cooperative behavior;

this means that the protein you are dealing with is an allosteric protein (which is a protein that includes more than one subunit and the binding of material (A for example) at subunit one affecting

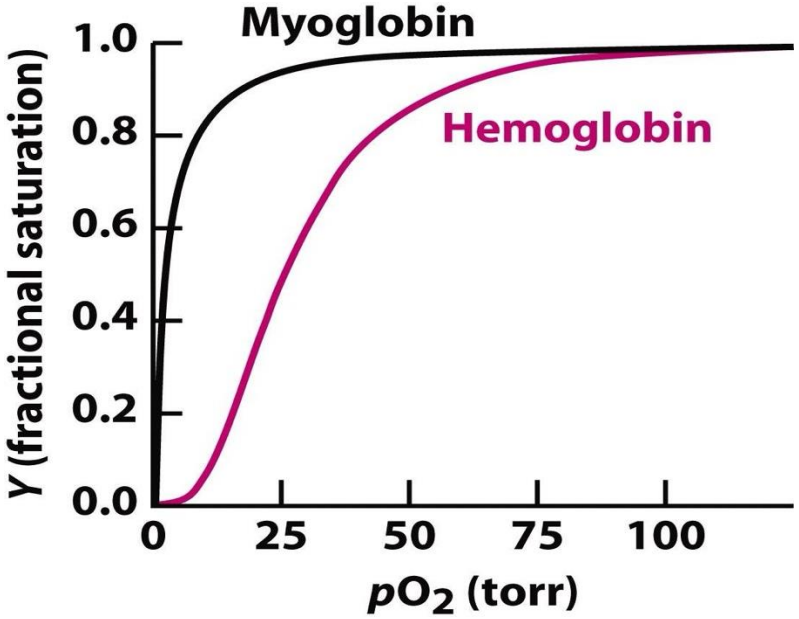
the behavior of other subunit and we call the

material (A) the effecter.



Notes from the previous Lecture

It could be a homotropic effector if it is the same with the material that will bind to another subunit, and heterotopic effecter if they were different so in hemoglobin oxygen is homotropic effecter.



Notes from the previous Lecture

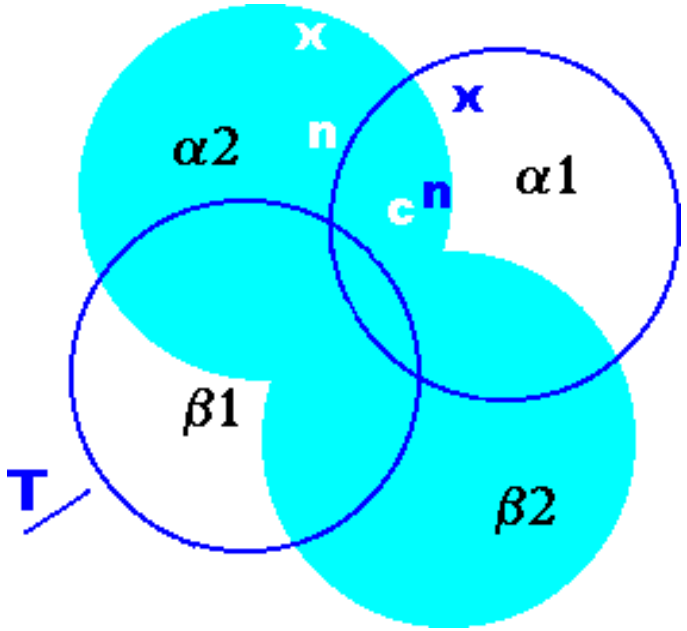
We have other modifiers that are heterotopic.

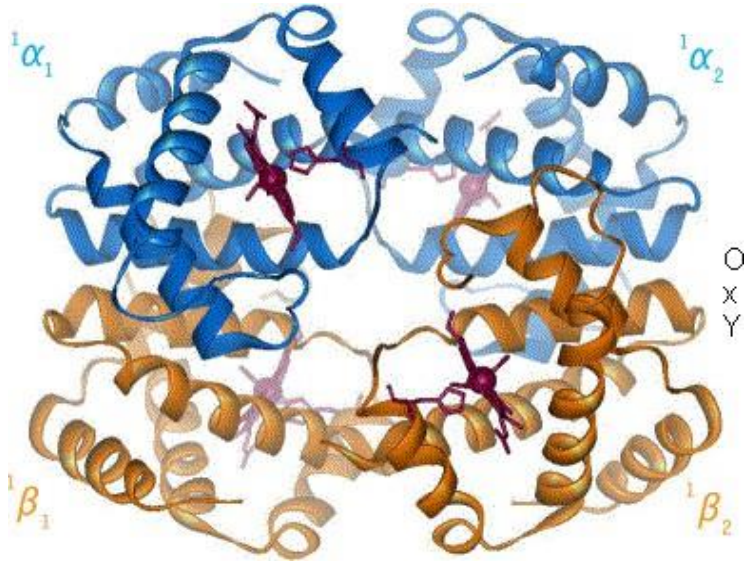
Is the sigmoidal plot good or bad ?

Clinically we agreed upon it is good because at high oxygen levels lungs will be fully saturated and tissues will start releasing oxygen and that's what I need and when oxygen level is low saturation of hemoglobin gets back low so fluctuation doesn't affect you.

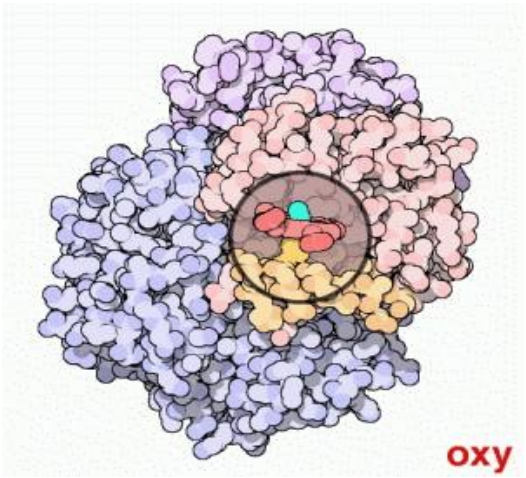
Hemoglobin is an allosteric protein that exists in two conformational states: the T (tense) state, which has low affinity for oxygen, and the R (relaxed) state, which has high affinity. While many allosteric proteins are enzymes, hemoglobin is a classic non-enzymatic example

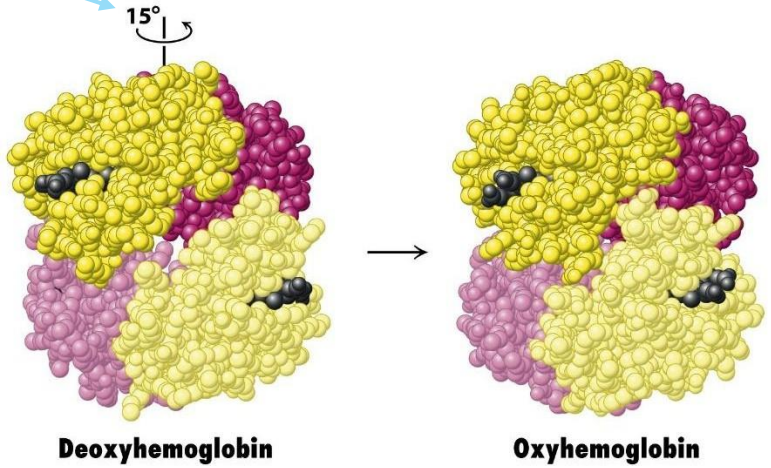
DEOXYHEMOGLOBIN & OXYHEMOGLOBIN



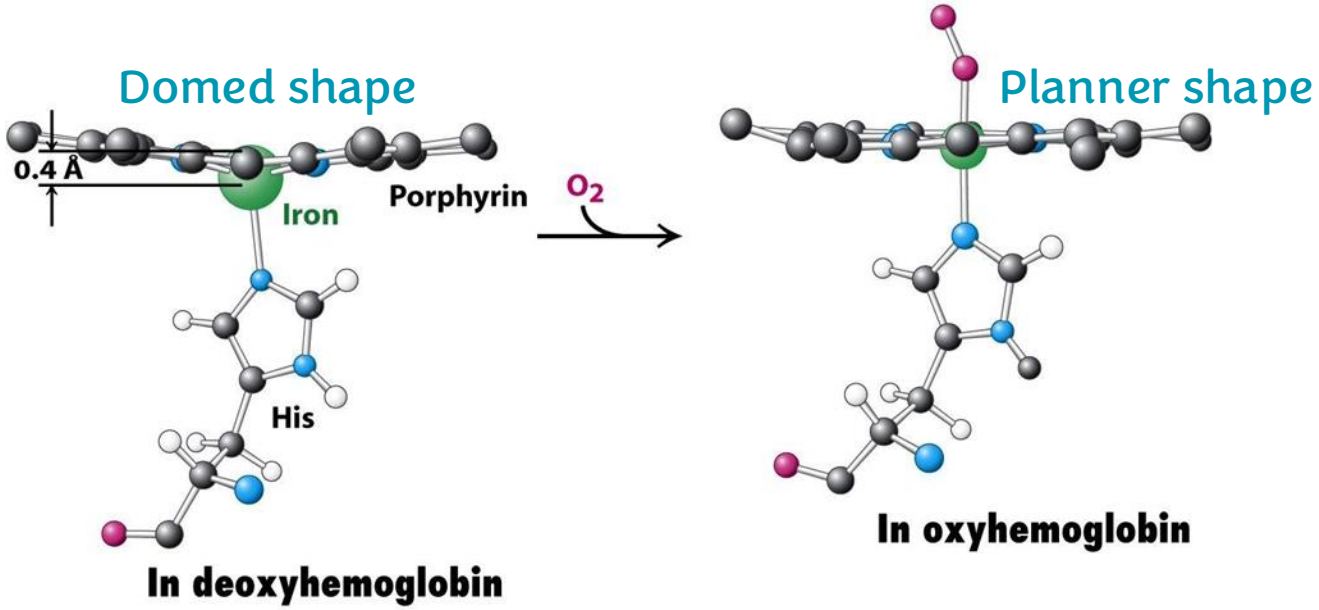


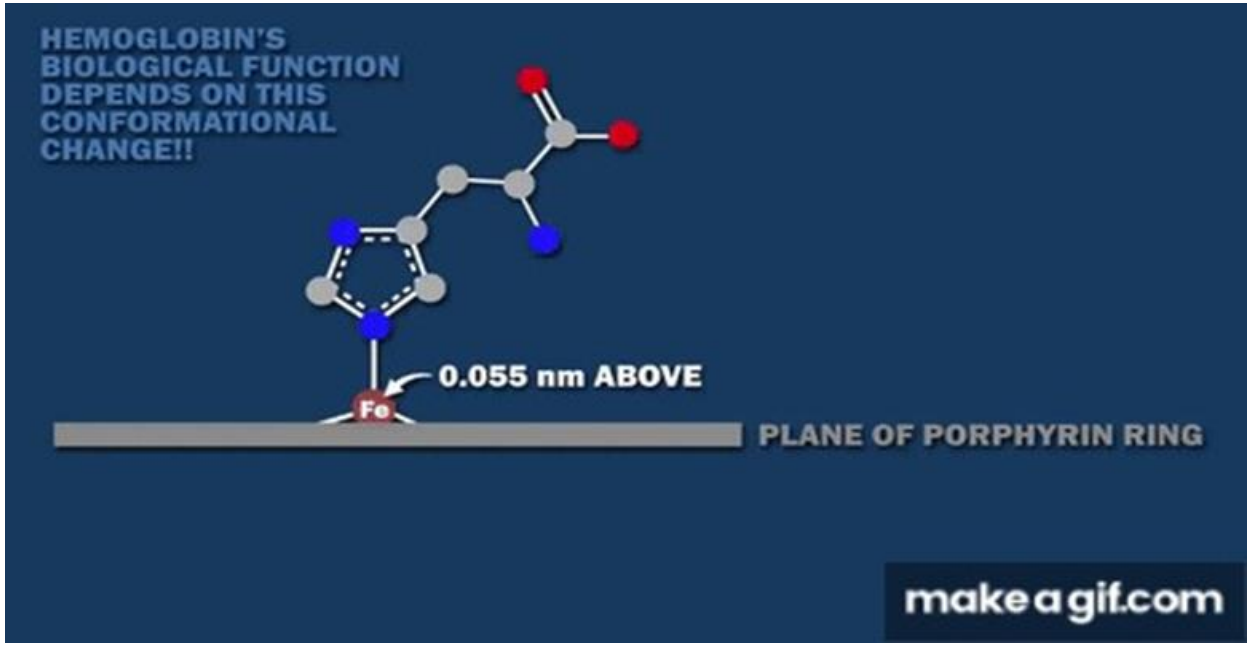
Rotation by **15 degrees** because of the decrease in bonding.
when the bonding increases every thing will turn back as it was

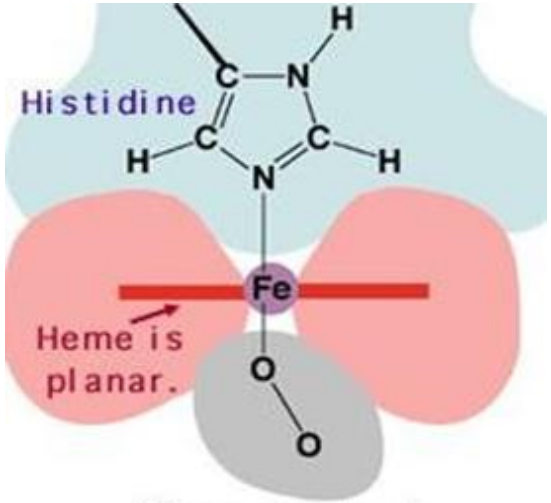


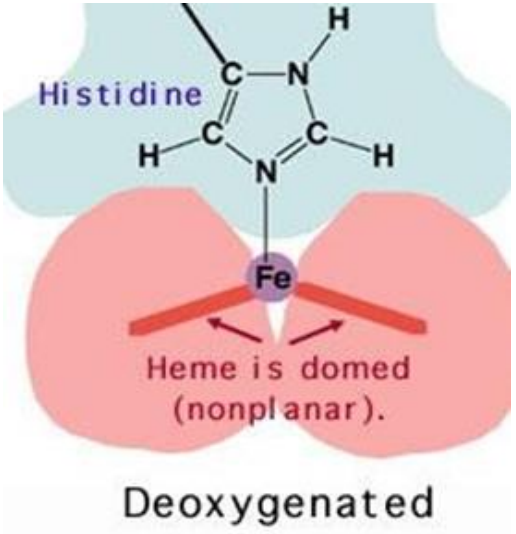


AFFECTS HEMOGLOBIN FUNCTION BUT NOT MYOGLOBIN



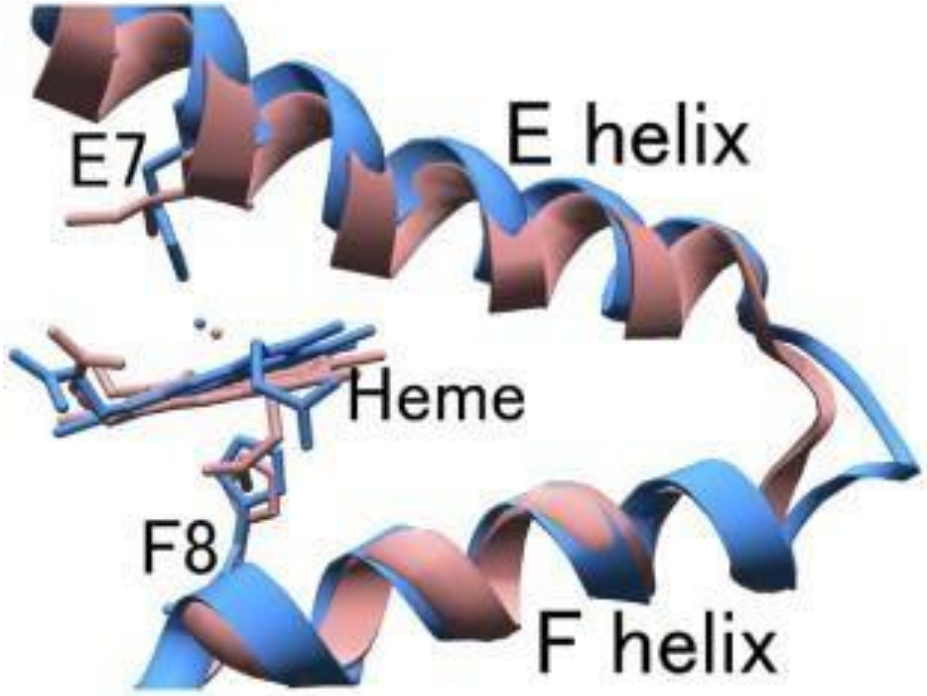


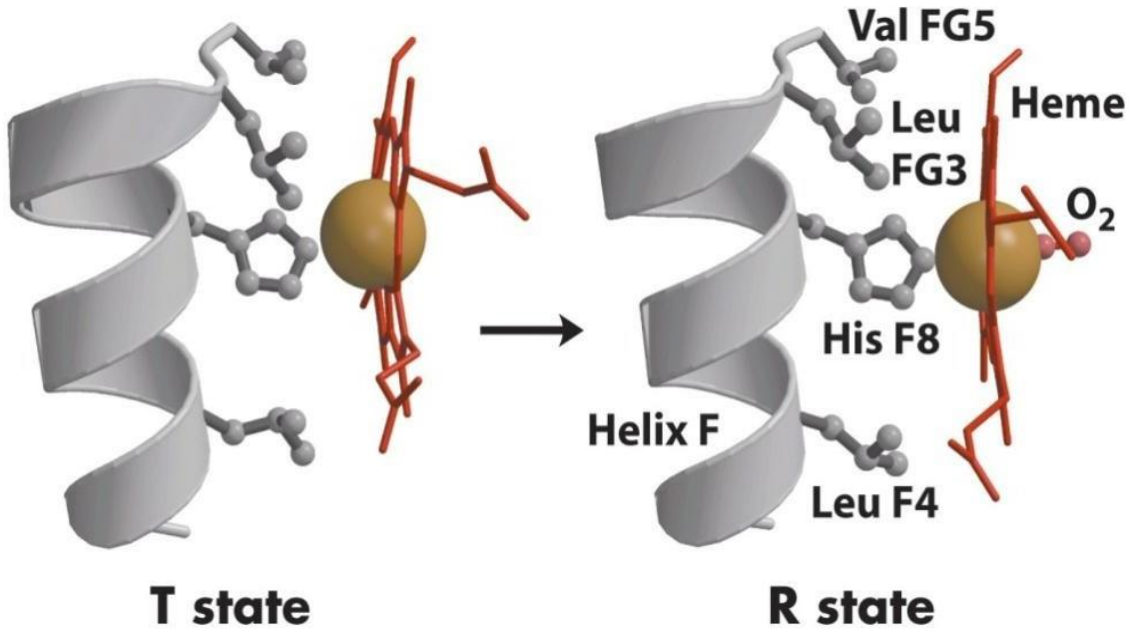




As you see the oxy-deoxy hemoglobin differs in shape from each other ,**but why ??**
The structure of heme is planner and when it gets inside the protein it gets attached with proximal histidine which will engage the iron toward histidine as a result the heme will be angular(domed) , in case of binding to oxygen it will pull it above by 0.4 A so the heme is planner again , this will pull the histidine and other amino acids that binds to it like dominos .

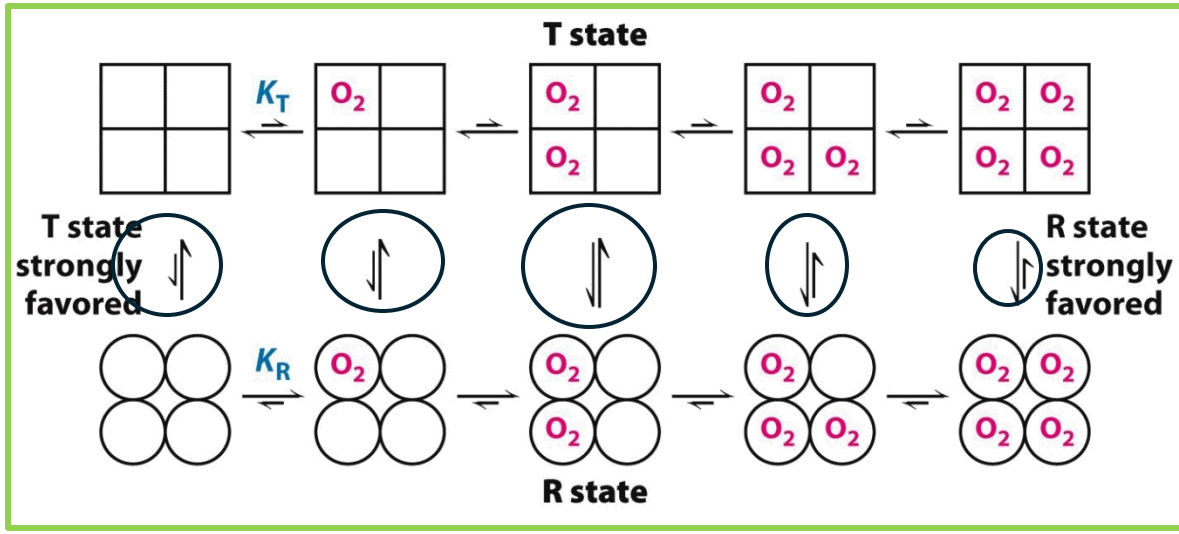
HOW DOES THE SWITCH OCCUR?

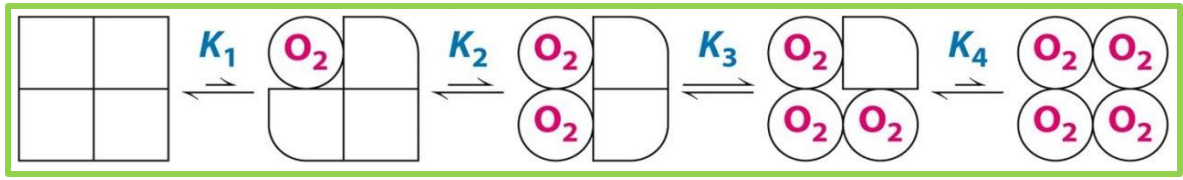




THE MECHANISM OF THE SWITCH

- Do all the chains transform at one stage (a quaternary change) or each chain by itself (a tertiary change)?
- Two models: Concerted vs. Sequential
- Concerted:
  - In the absence of O2 (T is favored)
  - In the presence of O2 (R is favored)
  - Does not depend on the number of O2 bound to tetramer
- Sequential:





Some allosteric protein concerted and some sequential and some are both, and with regard to hemoglobin some adopt sequential and some are concerted

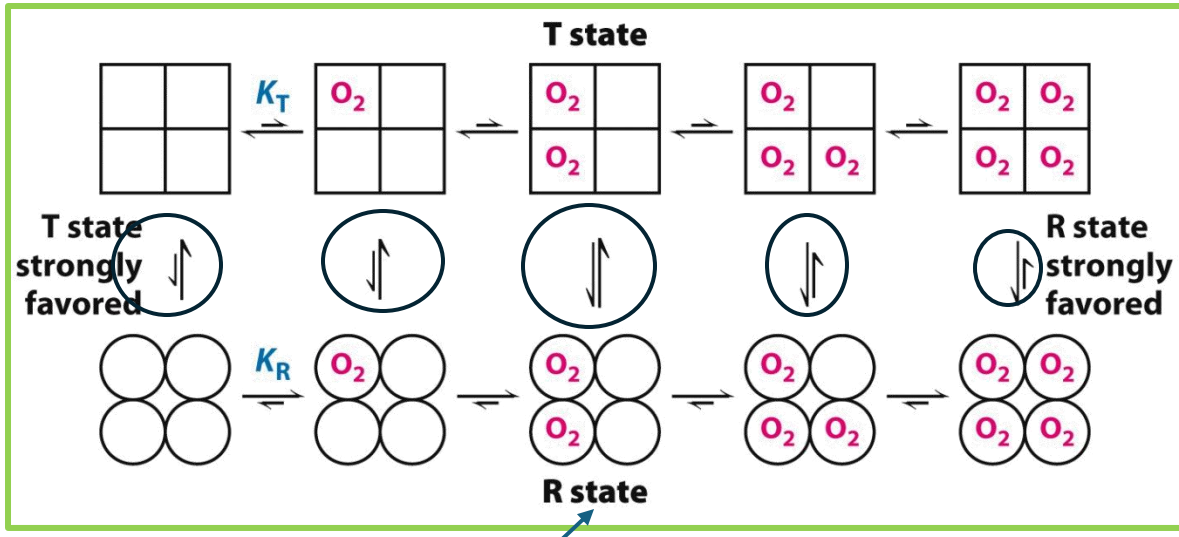
THE MECHANISM OF THE SWITCH

circle is high affinity
square is low affinity the arrows are meaningful

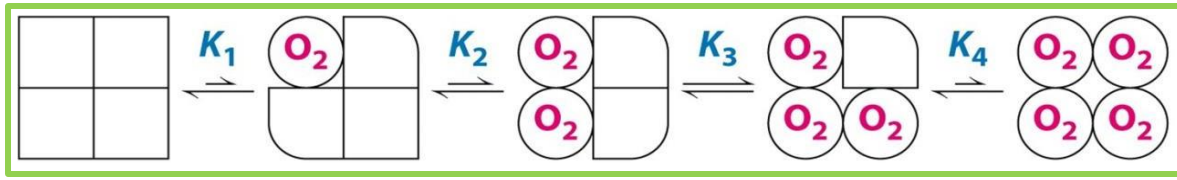
Sequential model :subunit by subunit converting and all of them in equilibrium depending on partial pressure of oxygen

Some allosteric protein concerted and some sequential and some are both, and with regard to hemoglobin some adopt sequential and some are concerted

Concerted model : all subunit converted at same time from low affinity state to high affinity state



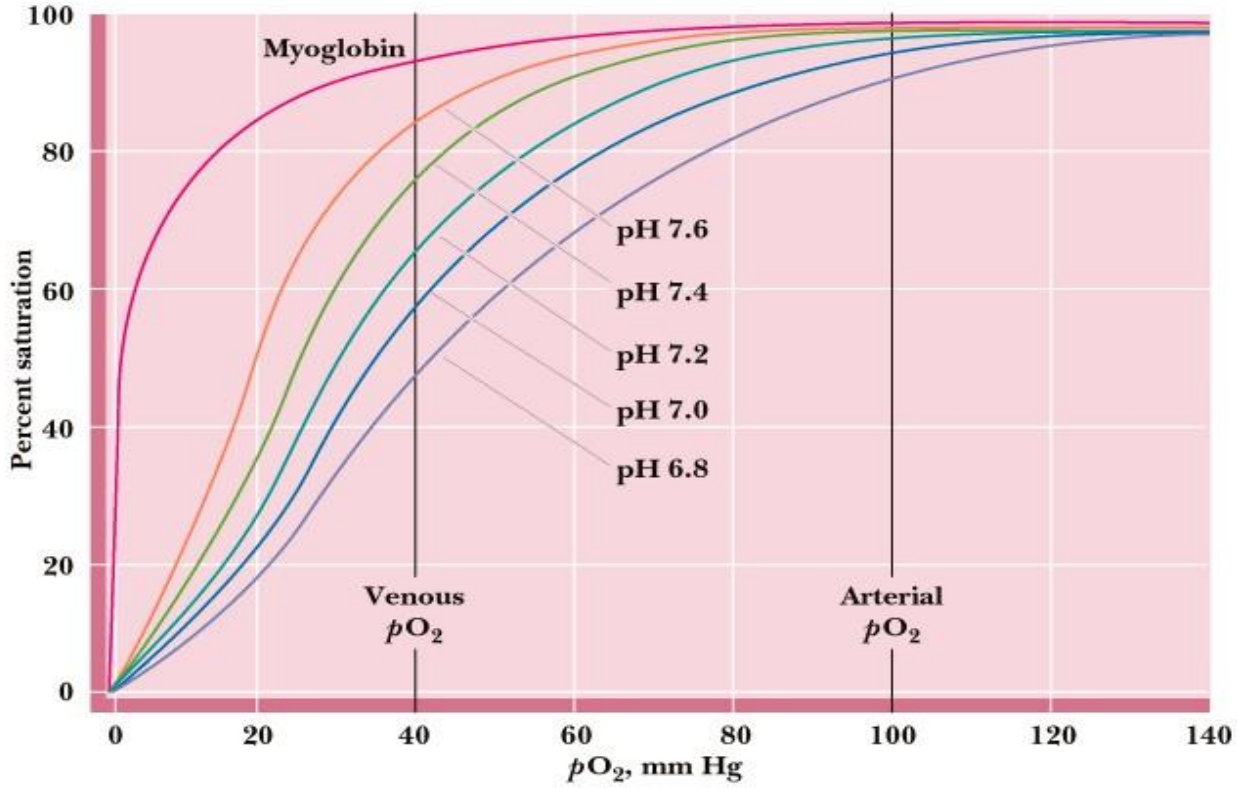
The same concentration of
(T and R) because it's half oxygenated

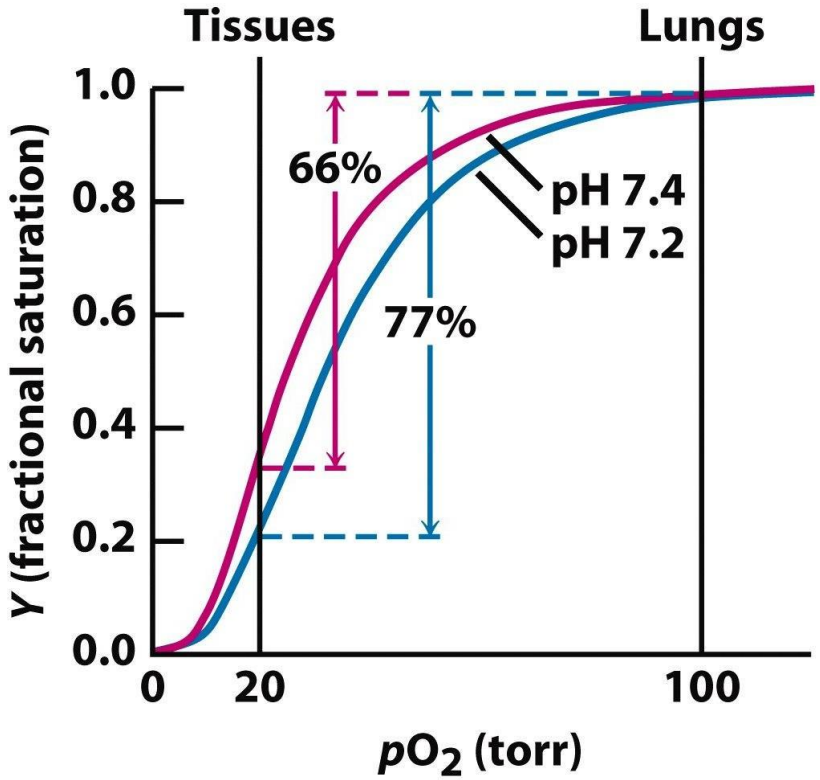


HEMOGLOBIN IS AN ALLOSTERIC PROTEIN

- Allosteric effectors (modulators) bind to a protein at a site separate from the functional binding site (modulators may be activators or inhibitors)
- Oxygen binding and release from Hb are regulated by allosteric interactions
- Hemoglobin cooperativity behaves as a mix of the above two models
- Homotropic vs. heterotropic effectros
- The major heterotropic effectors: hydrogen ion (H+), carbon dioxide (CO2), and red-cell 2,3-bisphosphoglycerate (2,3-BPG)

Hydrogen ion affects the PH, look at the chart in next slide it represents the partial pressure of oxygen and the percent of saturation in different PH values ,any allosteric enzyme or protein as it comes will have a sigmoidal plot and it will never be hyperbolic because they cooperate with each other, but modifiers could make it less or more hyperbolic(more or less sigmoidal) but it will never ever be hyperbolic by nature.





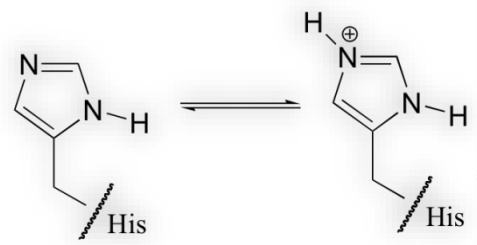
BOHR EFFECT

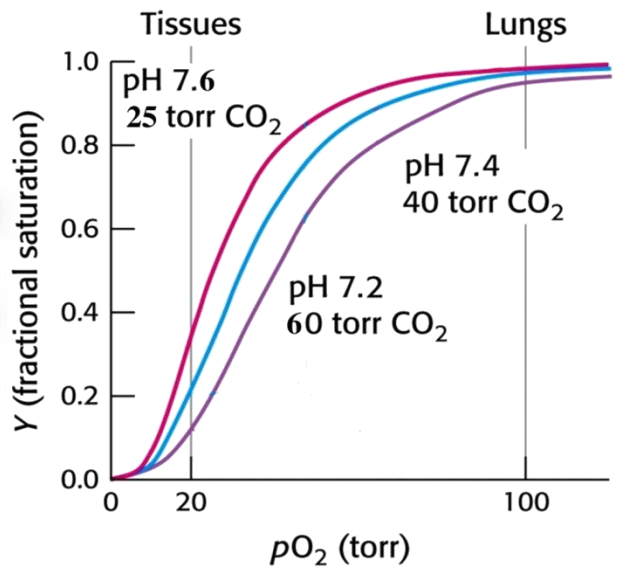
- Binding of protons favors loss of O_2
- So; Lowering the pH decreases the affinity of oxygen for Hb
- Is this physiologically relevant? (lung vs. tissues)

T state has less affinity towards O2

MECHANISM OF THE BOHR EFFECT

- CO2 and H+ help in favoring and stabilizing the T-form
- pH: More protons will protonate His allowing for more salt bridges which help in forming and stabilizing the T-form (O_2 release)
- CO_2:
  - Metabolism produces CO_2 in tissues (pH)
  - CO_2 binds the N-terminus (pH & salt bridges)

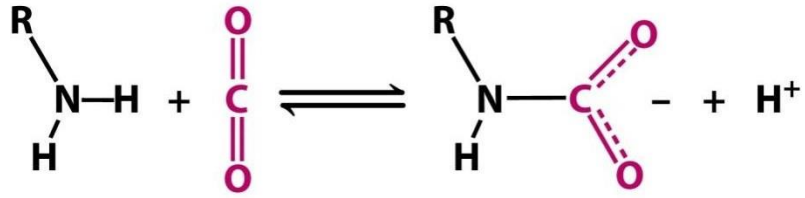




$$CO_{2(aq)} \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

(H_2O participates in the first step)

pK_a of H_2CO_3 is 6.35



N terminus

Carbamate Ion

Each hemoglobin has 4 N-termini

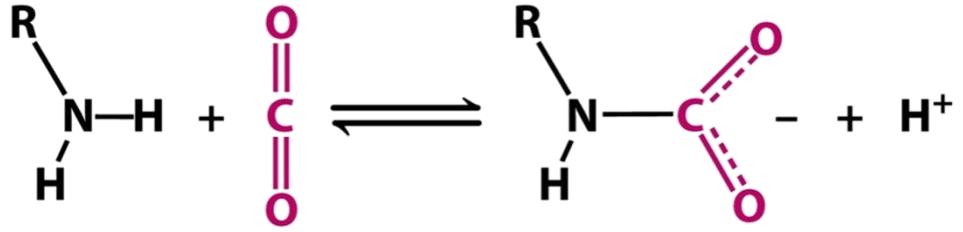
By decreasing the pH value you increase the T state by increasing the ionic bonds found between dimers
why don't you protonate the other amino acids, which are negatively charged ? it is low enough to protonate histidine but at the same time it is not low enough to protonate the negatively charged amino acids , since it's in the range of 6.8 and the side chains of the amino acid PKa = 4-4.5

And at the same time, you are increasing protonation of Histidine and making **much more** ionic interactions

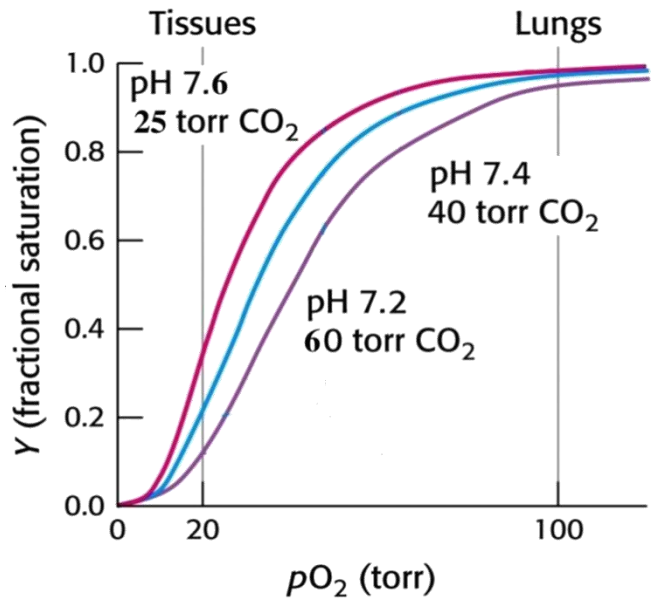
Doctor's note : When the pH decreases more of the Histidine side chains will be protonated. In comparison, the side chain carboxylic group has a pKa of 4, so the negative charge will not be as affected by the change in pH. More of the positive charge and the same concentration of negative charge will lead to more formation of salt bridges leading which favors the T state(low affinity to O2)

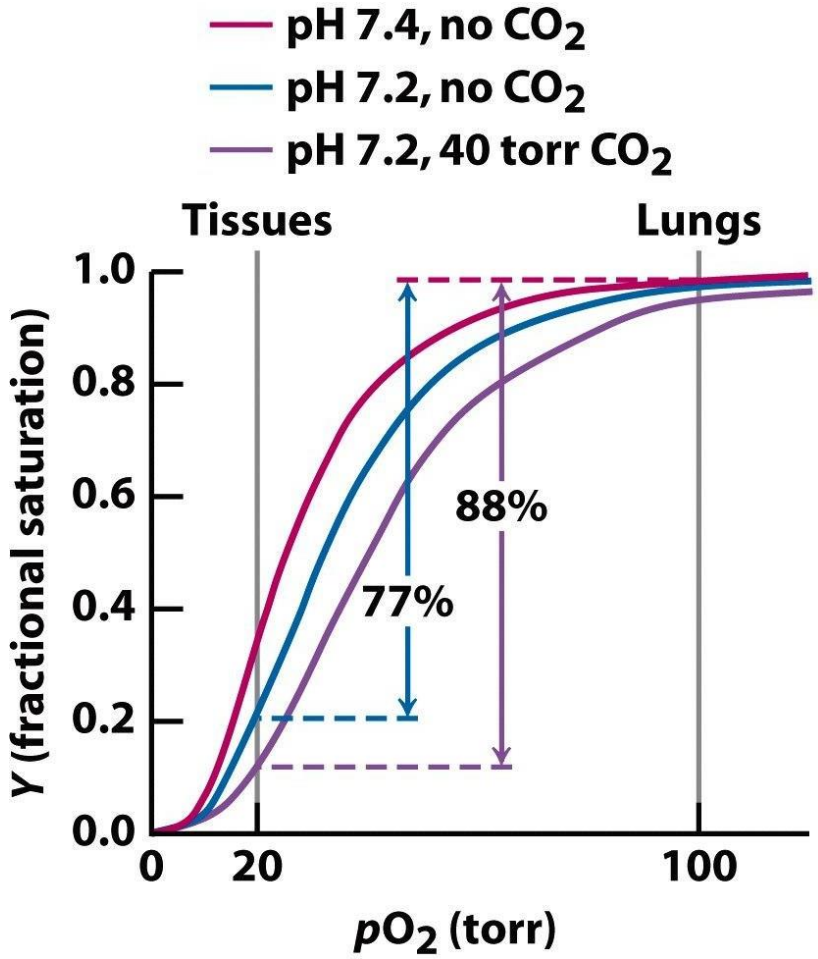
Does CO has an effect on hemoglobin? Yes, it binds to the same site as O2

IS THERE A REAL CO2 EFFECT OTHER THAN THE PH?



Carbamate

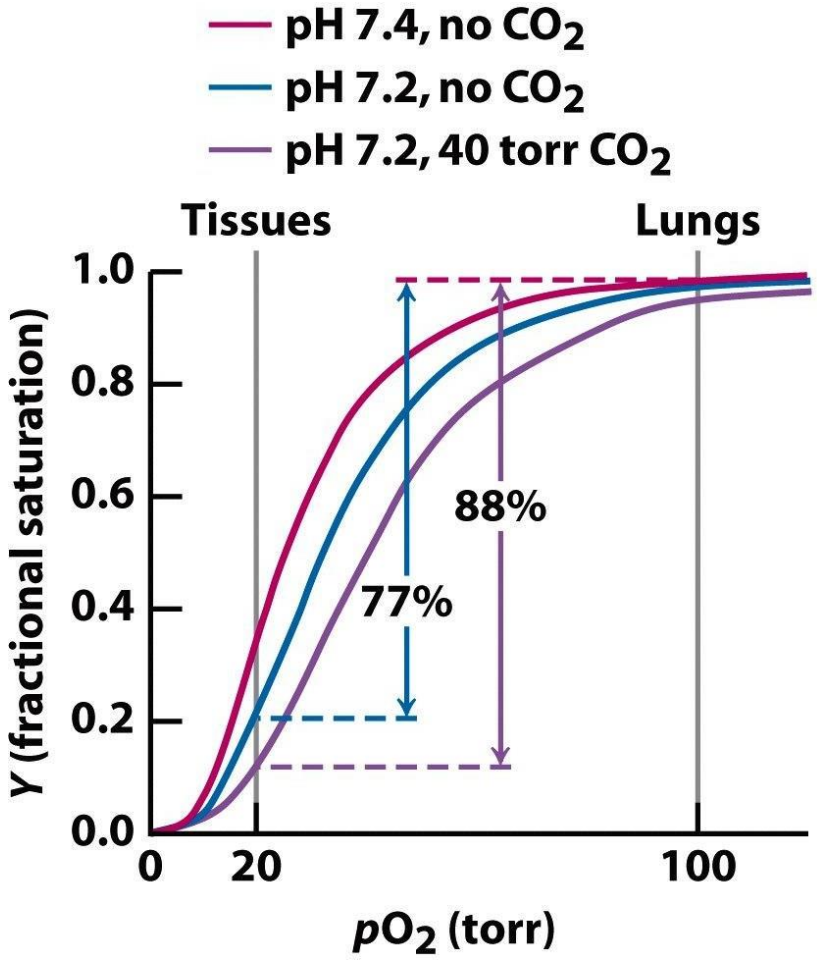




$$CO_2 + H_20 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

CO2's effect is indirect, how?
when it gets coupled with water it produces H2CO3, which also produces HCO3- and H+ (remember the midterm material)
Also,
The pH in tissues is lower than in the lungs, why?
Because CO2 is produced from Kreps Cycle in cells

It also can be direct, How?
By doing some tests
Put the hemoglobin in a solution and change the concentration of CO2 BUT keep the pH value constant

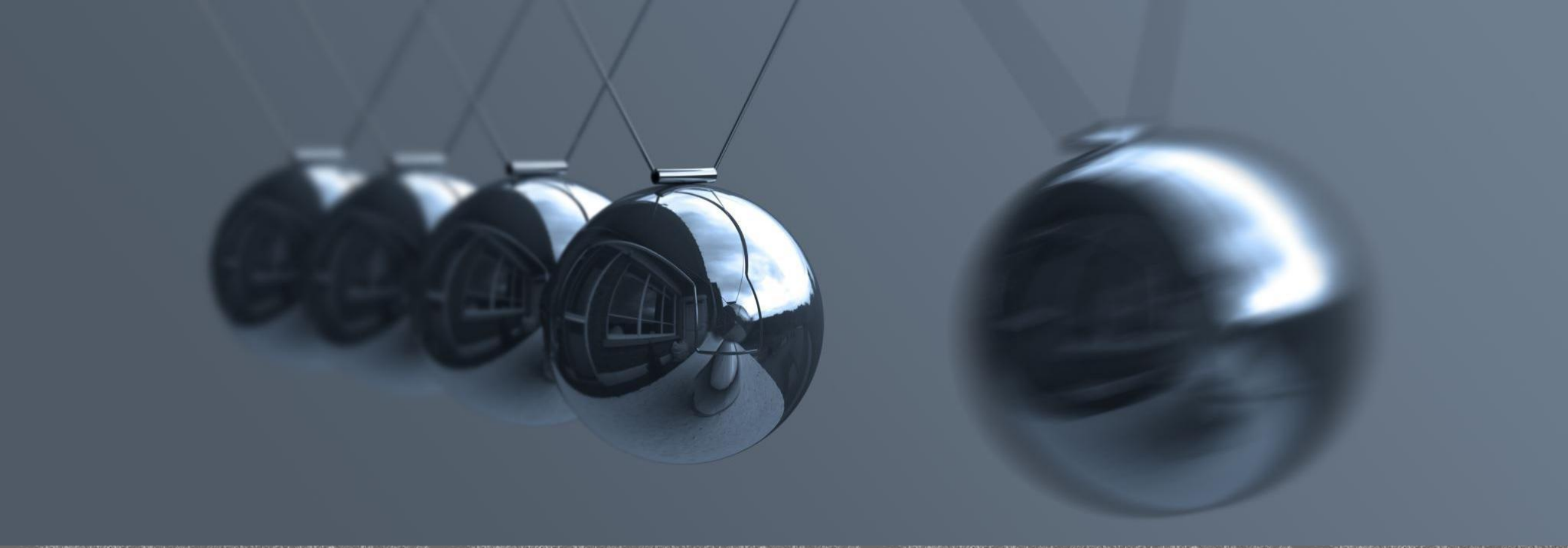


PH is constant, the difference here is the partial pressure of CO2

The curve shifted to the right= it becomes more sigmoidal

It has lower affinity towards oxygen

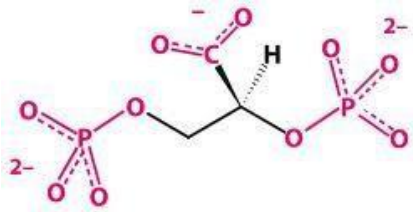
It favors the T state



OTHER ALLOSTERIC EFFECTORS

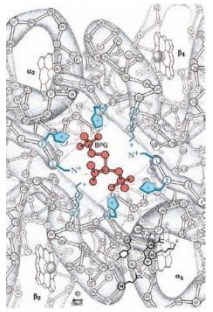
*Other than H+ and CO2

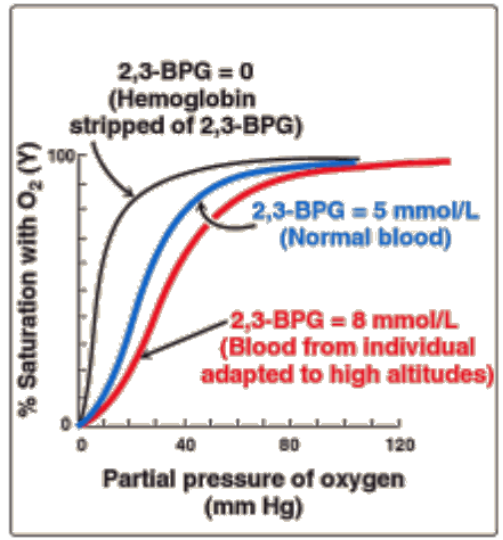
2,3-BISPHOSPHOGLYCERATE (BPG)

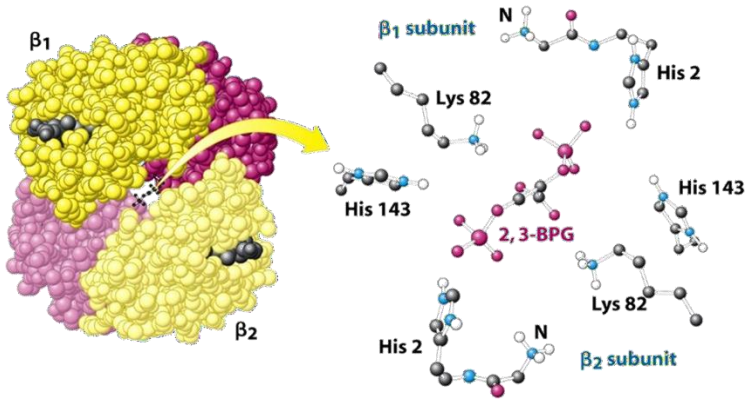


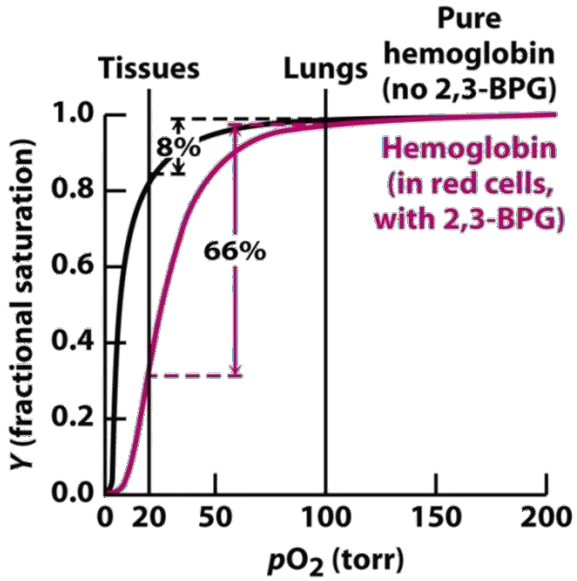
2,3-Bisphosphoglycerate
(2,3-BPG)

- Is a glycolytic intermediate, negatively charged
- Reduce the oxygen affinity of hemoglobin
- Binds in the central cavity (1BPG/Hb)
- Binding creates salt bridges which favor the deoxy form. Physiological relevance?
- Conc. of BPG increases at high altitudes, emphysema, & chronic anemia
- Consideration on transfusion









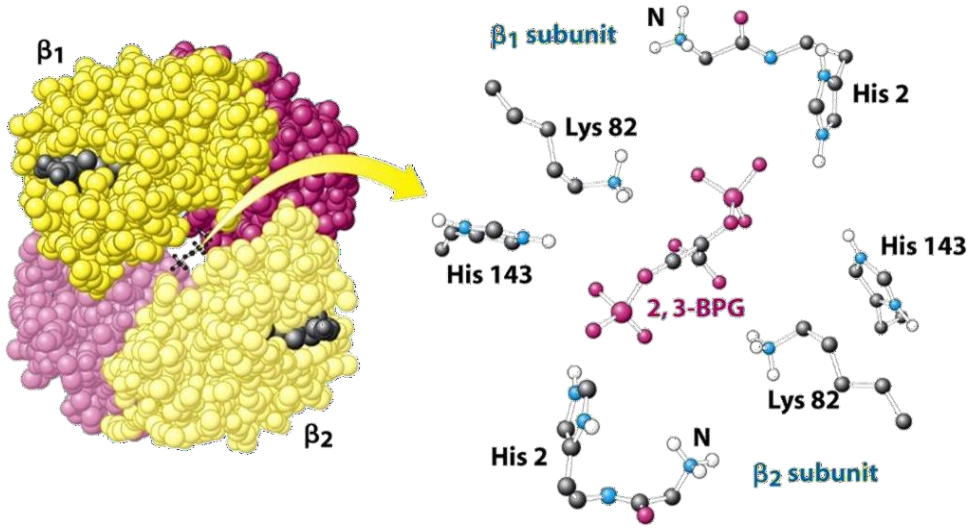
2,3 BISPHOSPHOGLYCERATE (BPG)

In glycolysis, we have 10 steps in which one of them produces BPG which is an intermediate that transforms into 3-phosphoglycerate

Bis = 2 , so it has 2 phosphate groups on carbon # 2 & 3
Glycerate = 3 carbons
Ate = acidic in its nature

BPG is highly negative, why ?
It has 3 carbons, one which is acidic in its nature and the other 2 carbons have phosphate groups attached

BPG binds in the central cavity, favoring the T state, lowering the oxygen affinity of hemoglobin



The numbers resemble the position of amino acids in the poly peptide chain

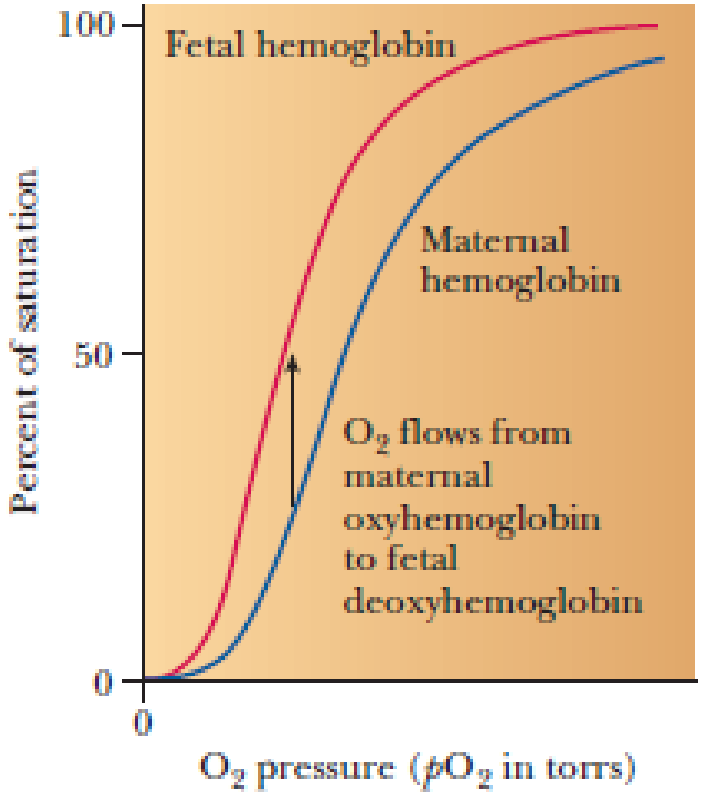
Side chains of amino acids should be polar since they are facing water (Histidine and Lysine)

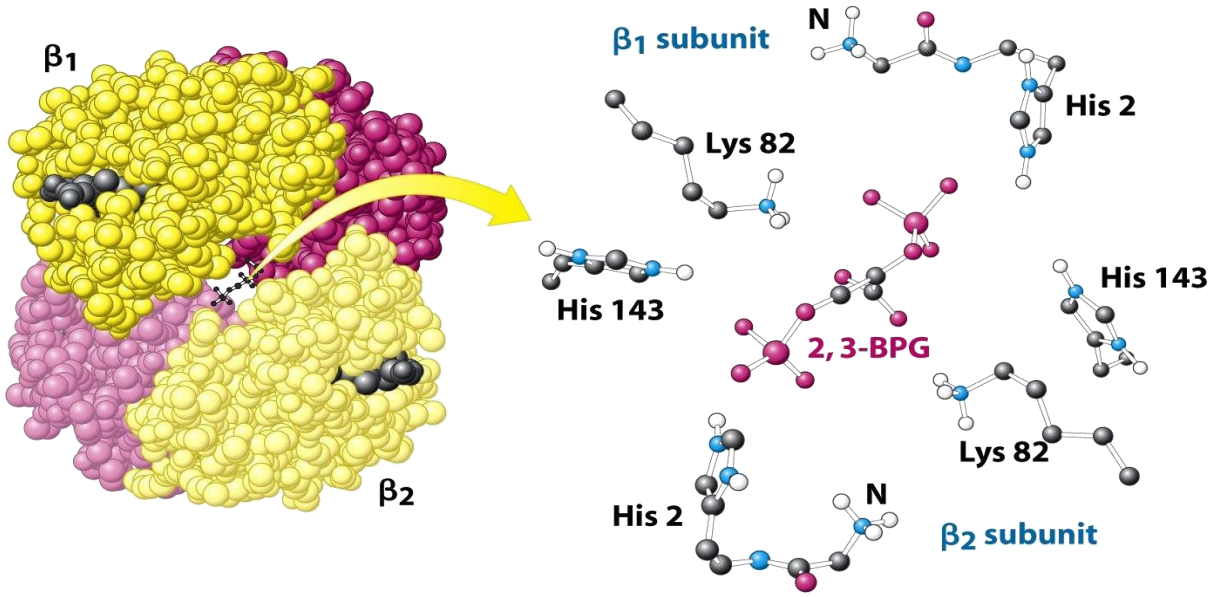
BPG is found in all cells across your body, even higher in RBC's
Why? Kreps cycle doesn't occur in RBC's only glycolysis, which lowers the affinity of O2 towards hemoglobin

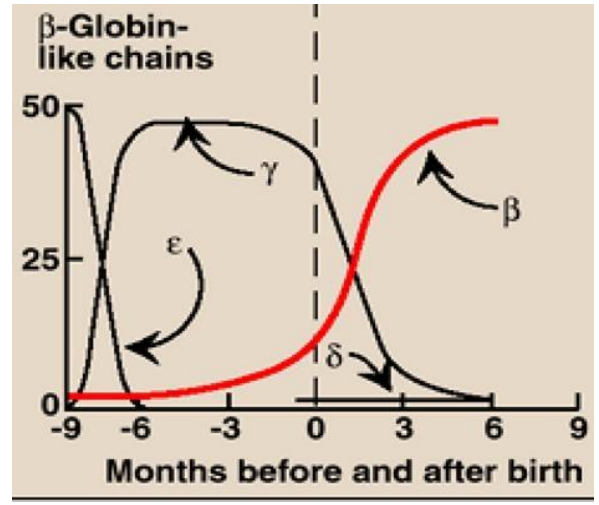
Can myoglobin take O2 from hemoglobin ? Yes, since its affinity to O2 is higher
Can hemoglobin take O2 from another hemoglobin ? Yes, in case of pregnancy (next slides)

FETAL HEMOGLOBIN

- Fetal Hb has higher affinity towards oxygen
  - α2γ2 A2β2 in adults
  - Adult hemoglobin, βHis143-BPG salt bridge, fetal hemoglobin, the γ –Ser instead of His)

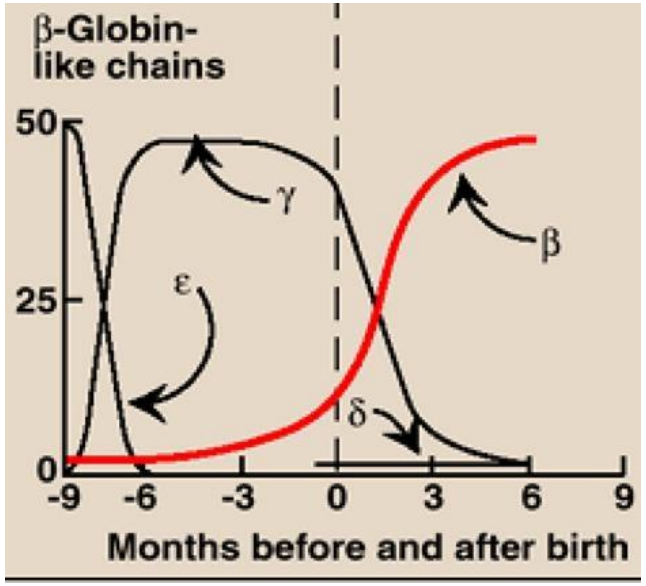






Hemoglobin can take O2 from another Hb if the structure differs
Structure is different = Affinity is different

How does the γ subunit have more affinity towards Oxygen ?
The amino acid His is replaced by Ser
And so the γ subunit doesn't have a lot of positive charges to bind with BPG
Which makes its affinity towards Oxygen higher

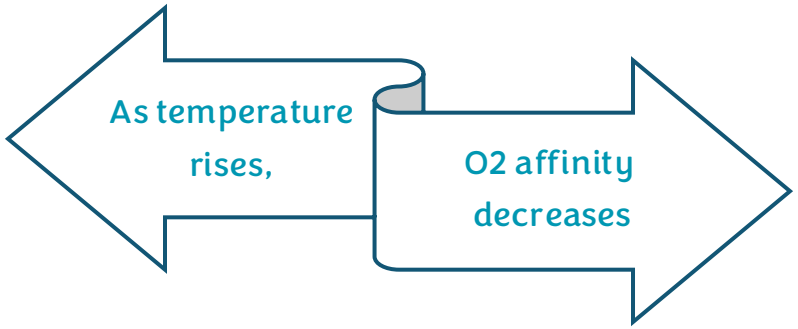


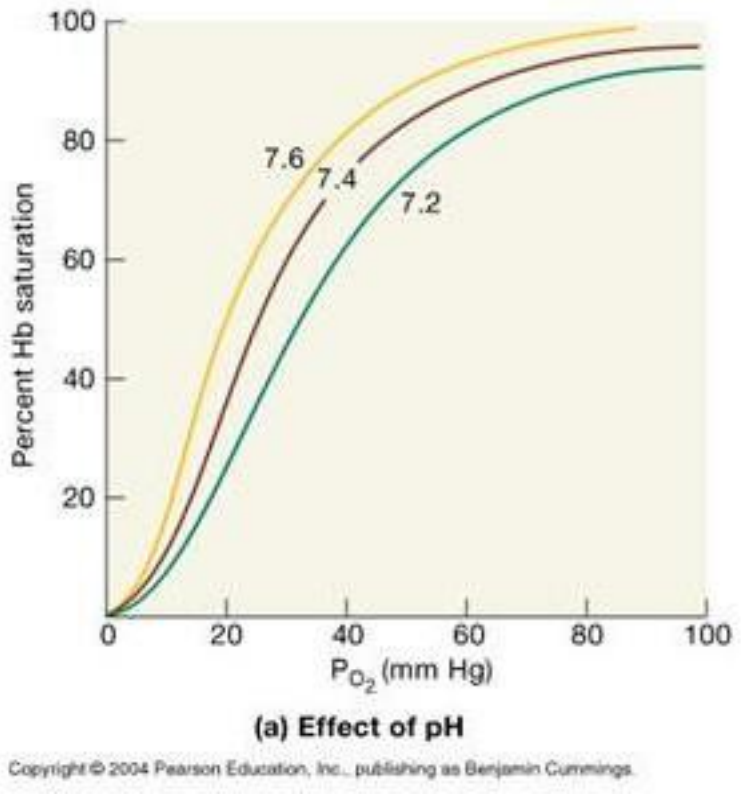
At birth, there is an underproduction of γ subunits and an overproduction of β subunits

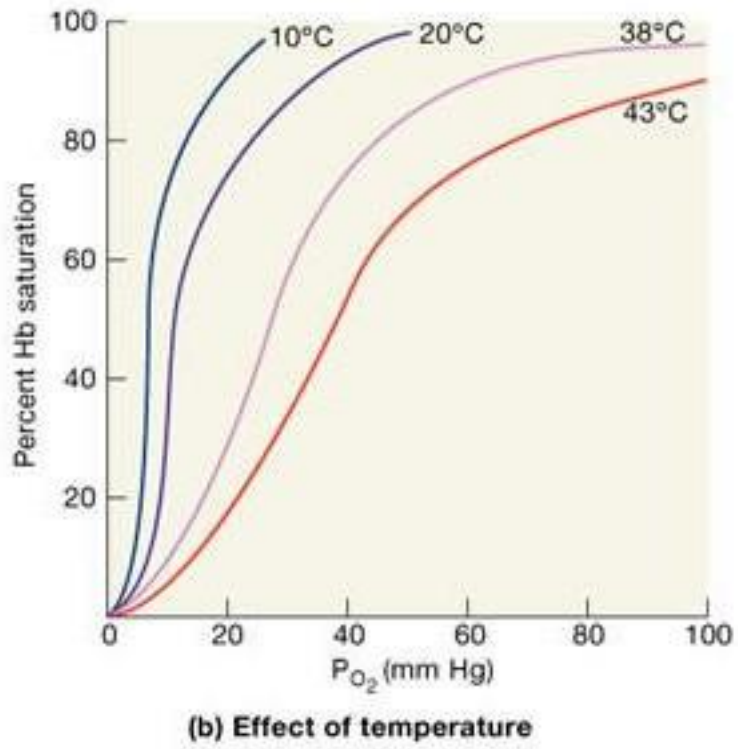
Doctor's note :
You cannot donate blood directly to a patient, the blood should be tested and screened first
In the blood collection and storage facility (blood center),
BPG will degrade overtime, that’s why blood units get evaluated before given to patients

EFFECT OF TEMPERATURE

- As temperature rises, O2 affinity decreases
- Temperature affects both myoglobin and hemoglobin







Since all types of hemoglobin contain alpha subunits, playing with it will cause drastic changes :

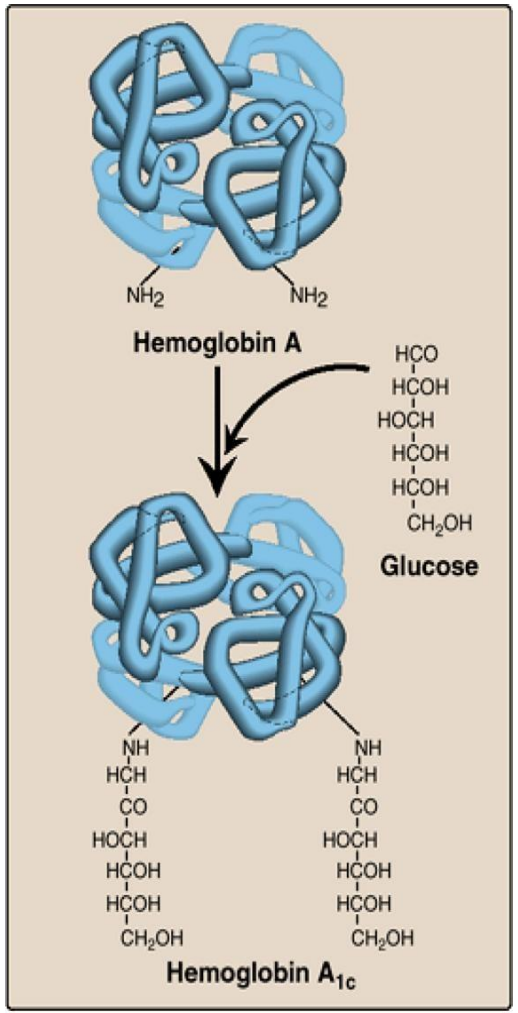
MINOR HEMOGLOBINS

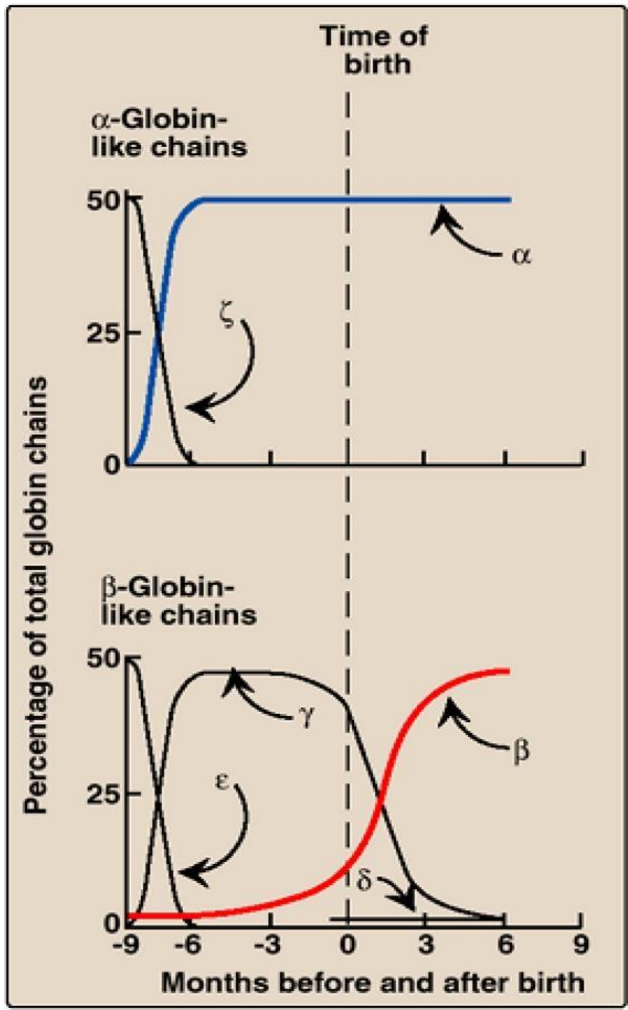
- Hemoglobin A_2 (Hb A_2)
- Hemoglobin A_{1c} (HbA_{1c})
- HbA

If someone's HbA1c is more than 6% that means they are in the prediabetic range

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3–9%

We don't know much about it

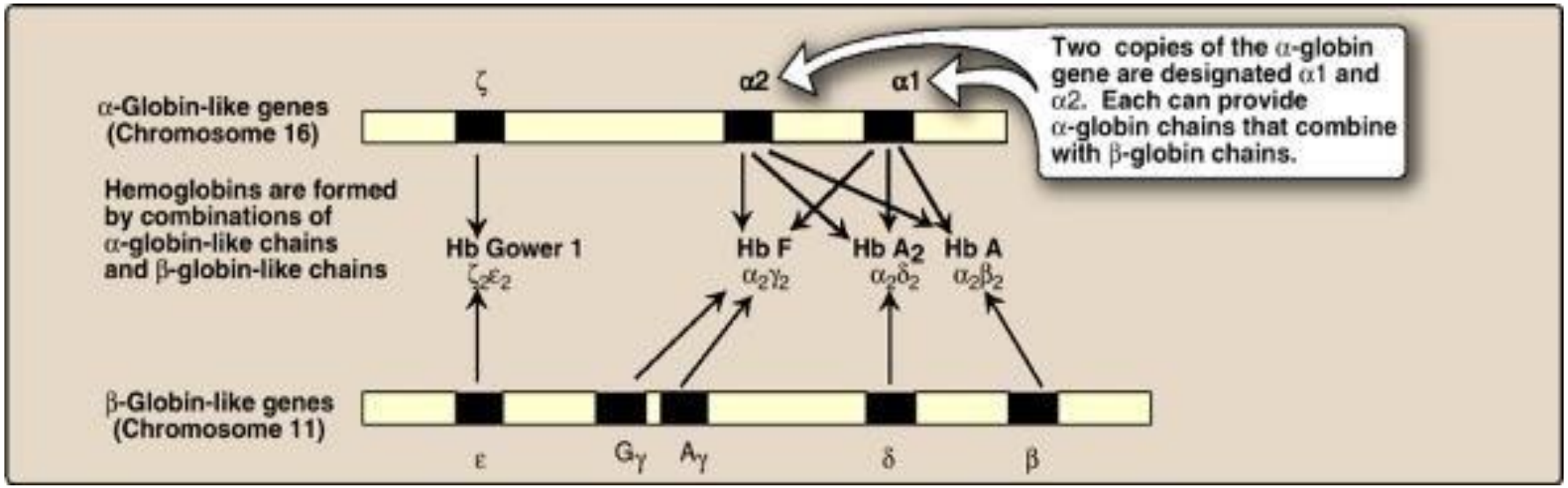




THE GLOBIN GENES

2 genes = 4 alleles

- α-Gene family: located on chromosome 16 & contains 2 genes for the α-globin chains plus others (remains on throughout life)
- β-Gene family: located on chromosome 11 & contains a single gene for the β-globin chain. Also contains 2γ genes and the δ gene



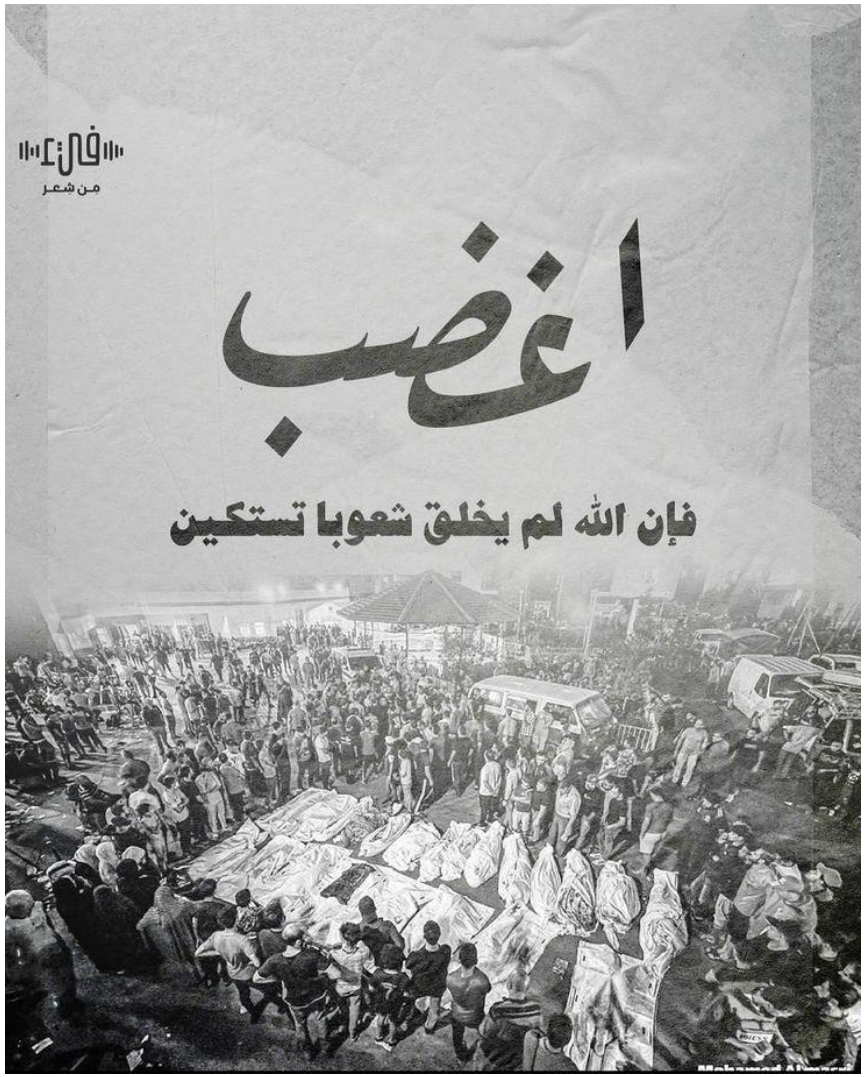
For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slide 27	The whole slide changed	When the pH decreases more of the Histidine side chains will be protonated. In comparison, the side chain carboxylic group has a pKa of 4, so the negative charge will not be as affected by the change in pH. More of the positive charge and the same concentration of negative charge will lead to more formation of salt bridges which favors the T state(low affinity to O2)
	Slide 5	By decreasing the pH value you increase the T state by breaking down the ionic bonds	By decreasing the pH value you increase the T state by increasing the ionic bonds
	Slide 15	Last paragraph changed	Last paragraph changed
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:



الاستعمار قد رسم الحدود ونحن من قدّسها
لا تفصِلُنا حدودٌ رُسِمت على الخريطة ثمّ طال الزمن فانحفرت في قلوب المسلمين المُوحّدين فصار زوالُ هذه الحدود من القَلوب أصعب من زوالِها على أرضِ الواقع.
لن تتحرّر الأمّة وتنهضَ إلّا إذا أبَدنا حدود سايكس-بيكو من قلوبِنا

المسلمُ أخي.. المسلمُ أخي سوريًّا كان أو فلسطينيًّا، أردنيًّا عراقيًّا سودانيًّا هنديًّا باكستانيًّا يمنيًّا جزائريًّا خليجيًّا أم أجنبيًّا أعجميًّا وهلُمّ جرى
فكما أنّ أفراحي وأخي واحدة؛ فإنّ **همومُنا كذلك واحدة**
من تخلّف عن النصرة فقد خذل، من ألِفَ مُصاب أخيه فقد خان
كلّ ديارِ المسلمين دياري وكلُّ همّ المسلمين همّي
وحسبُنا الله ونِعم الوكيل