Hemoglobin
An overview and more

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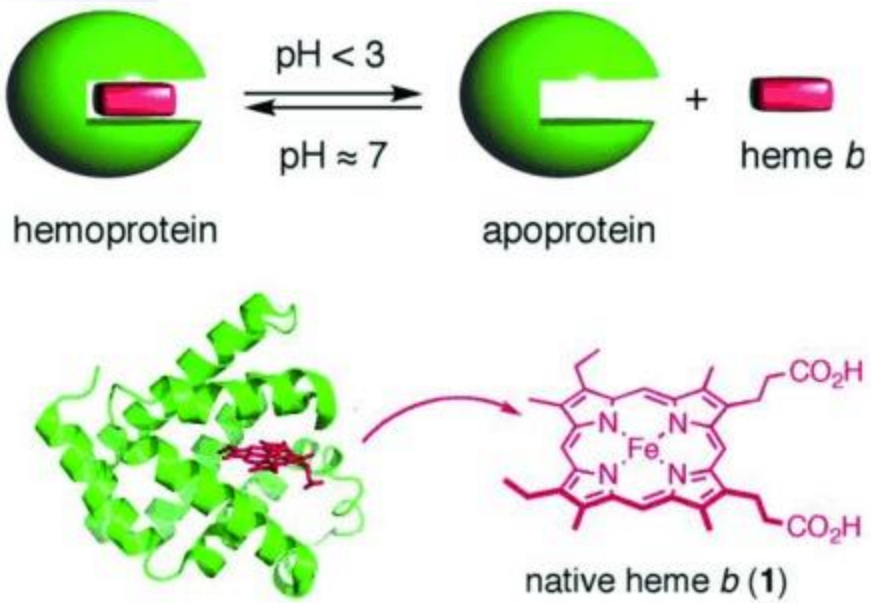
Hematopoietic-lymphatic system

Resources

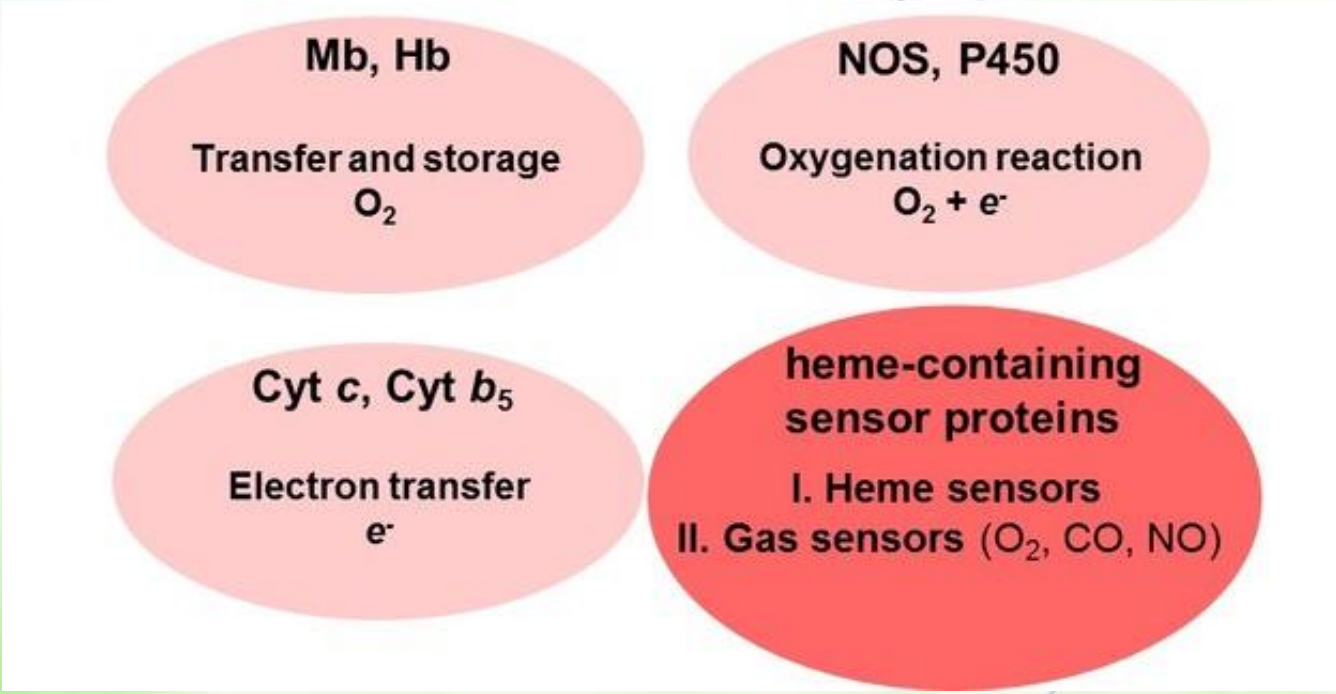
- This lecture
- Myoglobin/Hemoglobin O2 Binding and Allosteric Properties of Hemoglobin (http://home.sandiego.edu/~josephprovost/Chem331%20Lect%207_8%20Myo%20Hemoglobin.pdf)
- Lecture 3: Cooperative behaviour of hemoglobin (https://www.chem.uwec.edu/chem452_f12/pages/lecture_materials/unit_III/lecture-3/overheads/Chem452-lecture_3-part_1-overheads.pdf)

Hemoproteins

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

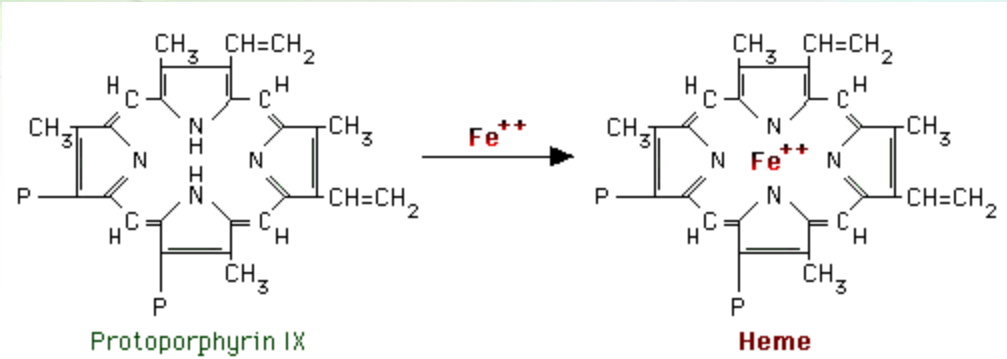


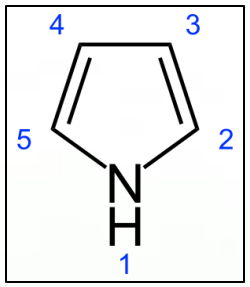
A prosthetic group *is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.*

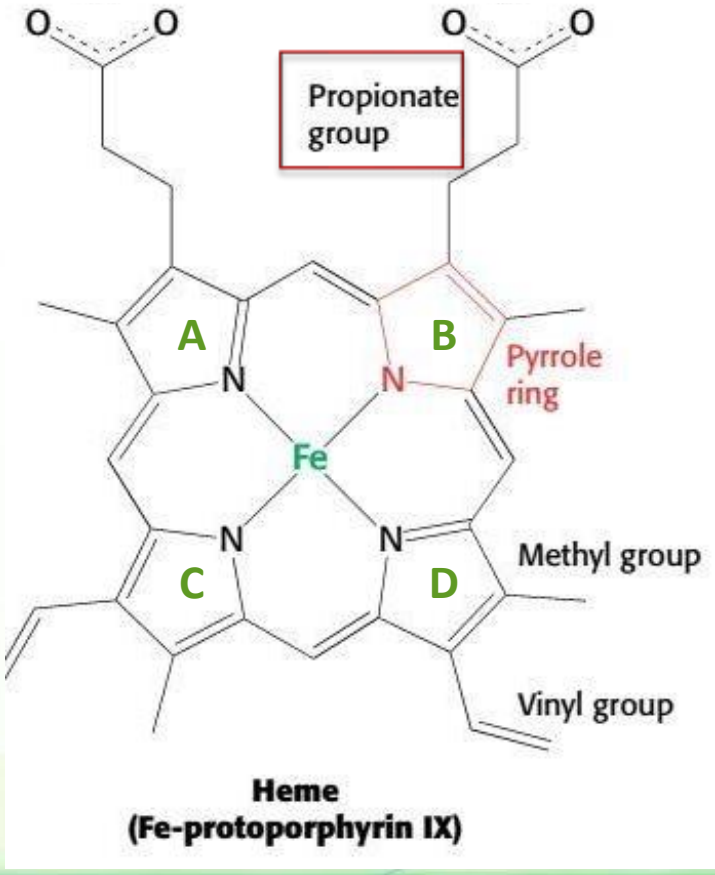


Heme structure

- It is a complex of protoporphyrin IX + iron (Fe^{2+}).
- The porphyrin is planar and consists of four rings (designated A-D) called pyrrole rings.
- Each pyrrole can bind two substituents.
- Two rings have a propionate group each.
- *Note: the molecule is hydrophobic.*
- Fe has six coordinates of binding.

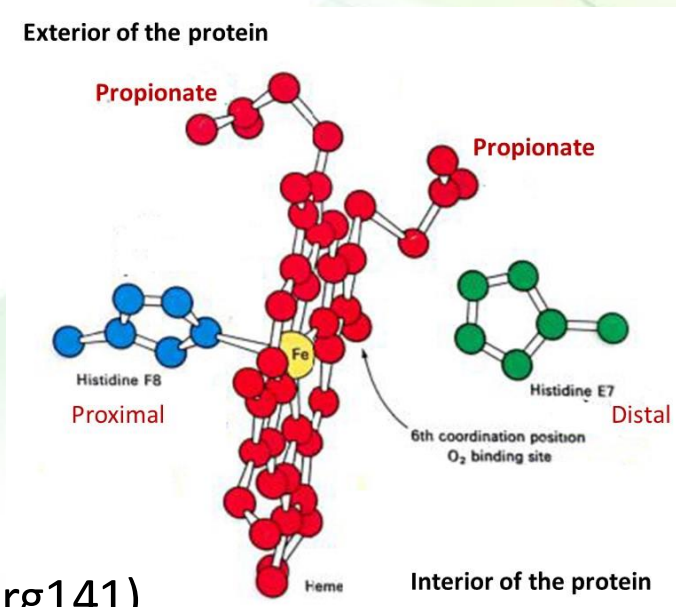


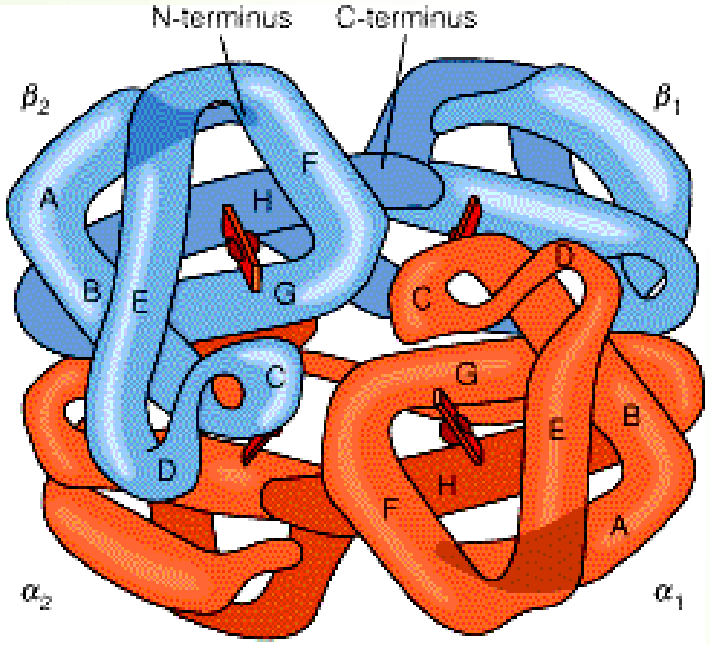


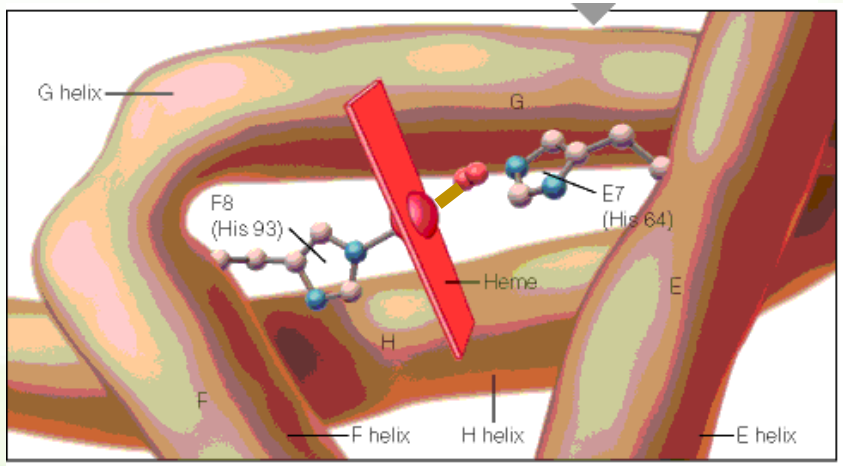


Structure of hemoglobin

- Hb is a globular protein.
  - Typical amino acid distribution
    - Positions of two histidine residues
      - Proximal and distal
- It is an allosteric protein.
  - Multiple subunits (2α + 2β)
    - α polypeptide = 141 amino acids (Arg141)
    - β polypeptide = 146 amino acids (His146)
    - The first amino acid in both is valine.
  - Altered structure depending on bound molecules
  - Positive cooperativity towards oxygen
  - Regulated by allosteric effectors

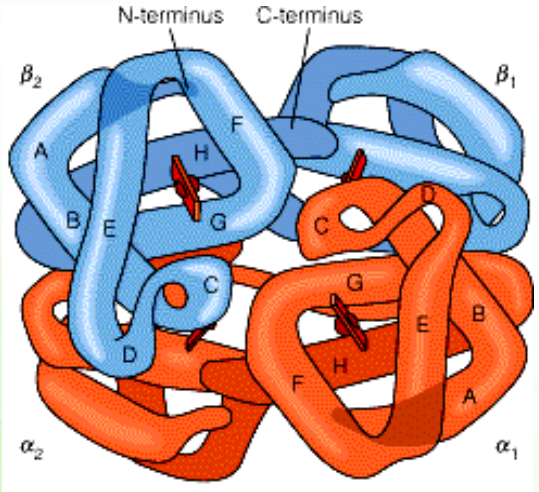


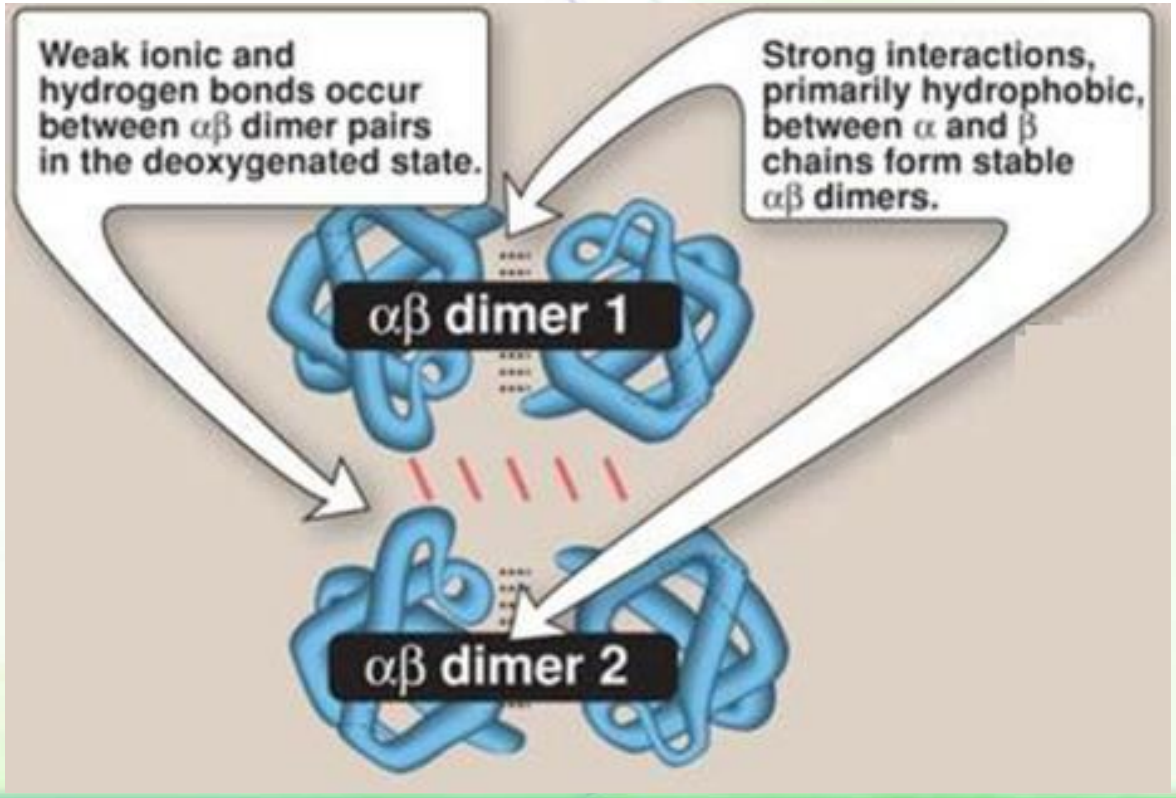




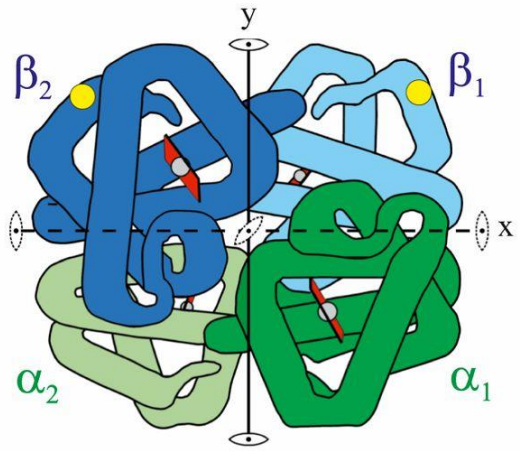
How are the subunits bound?

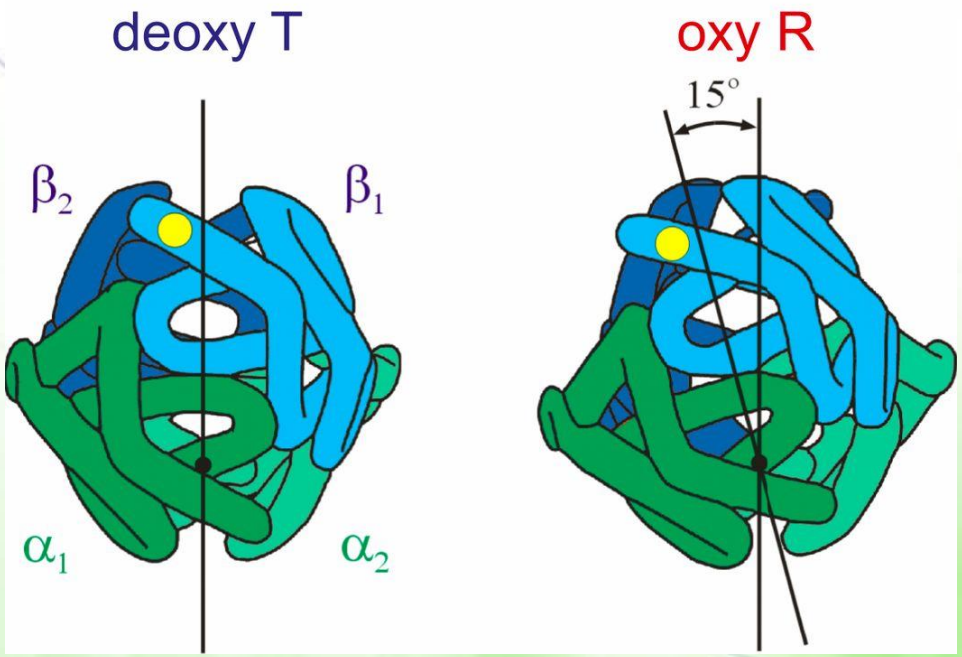
- A dimer of dimers (I made up this term)
  - (α-β)2
  - Note how they interact with each other.

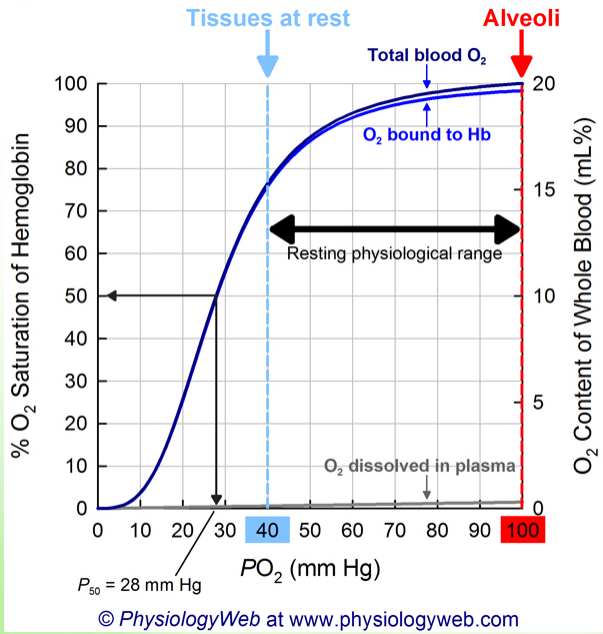


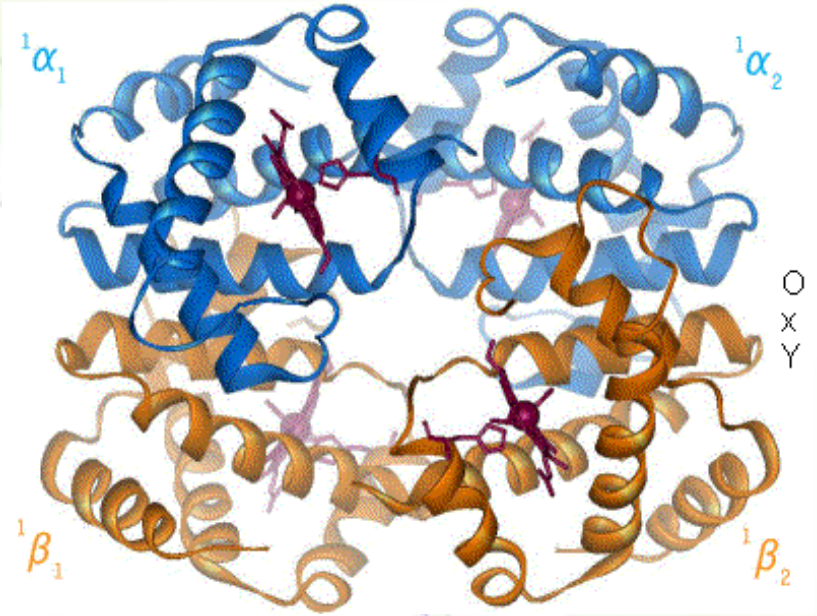


Structural change of hemoglobin



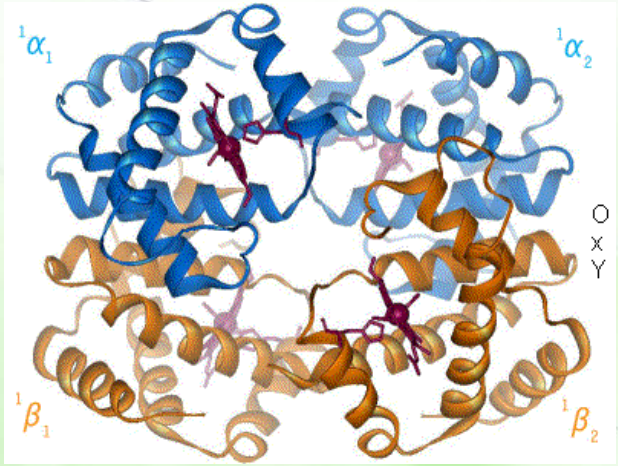


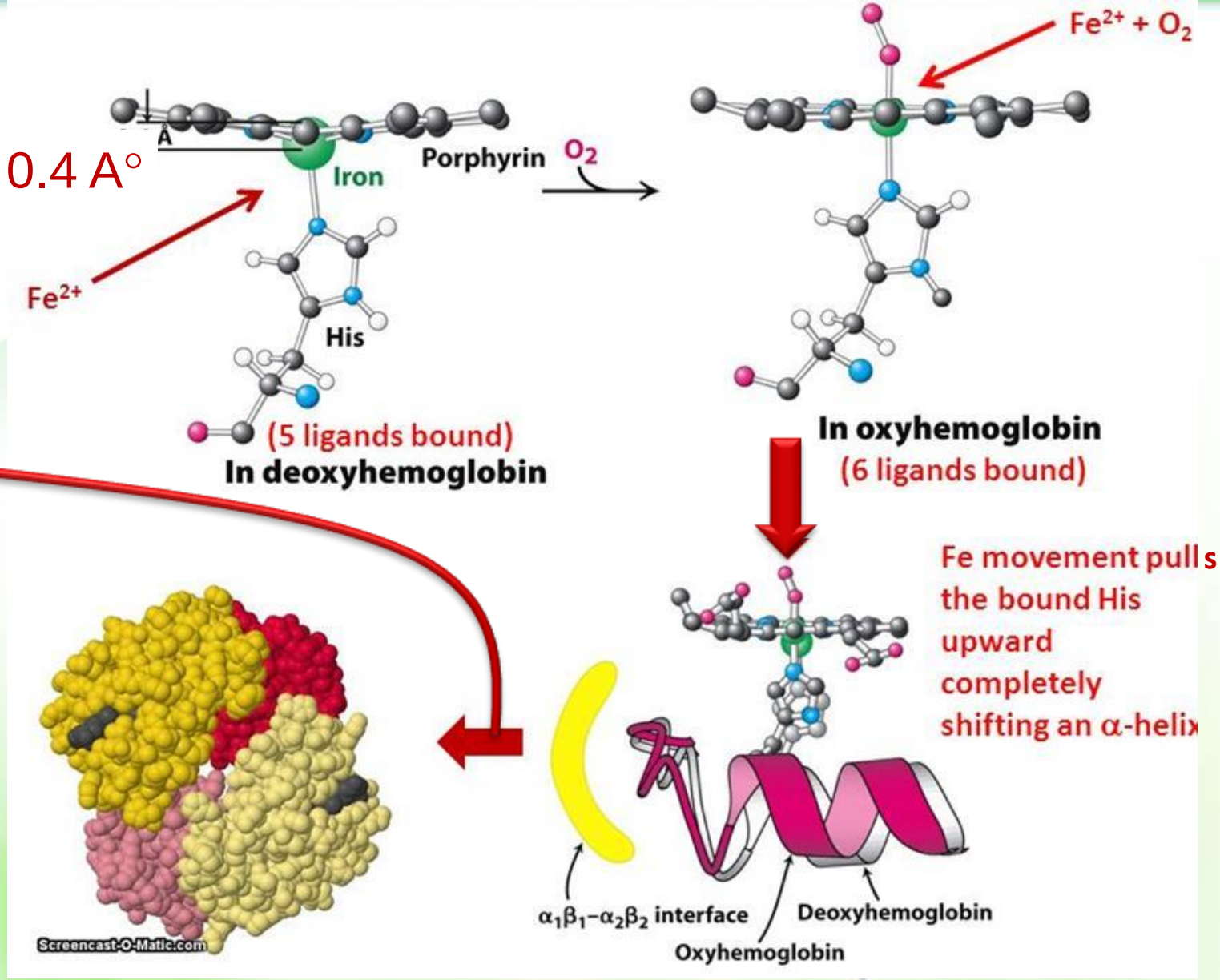




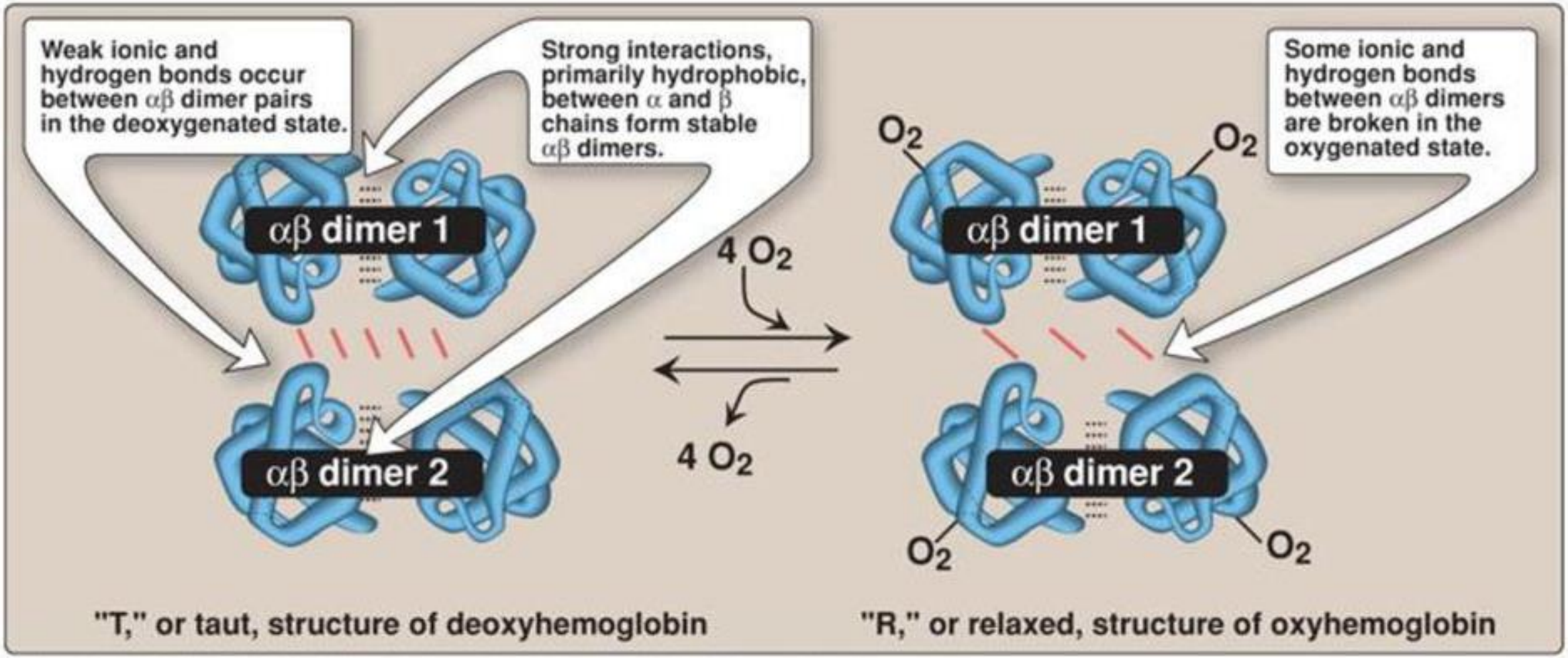
Structural amplification change

- Changes in tertiary structure of individual hemoglobin subunits
- Breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.



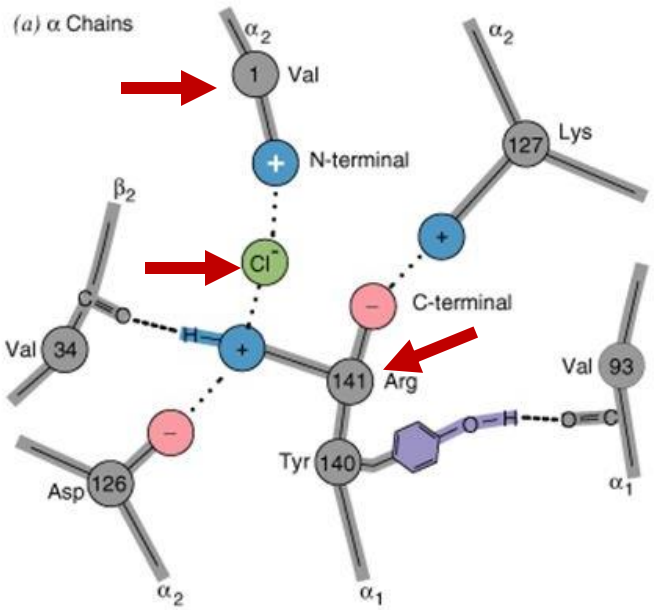


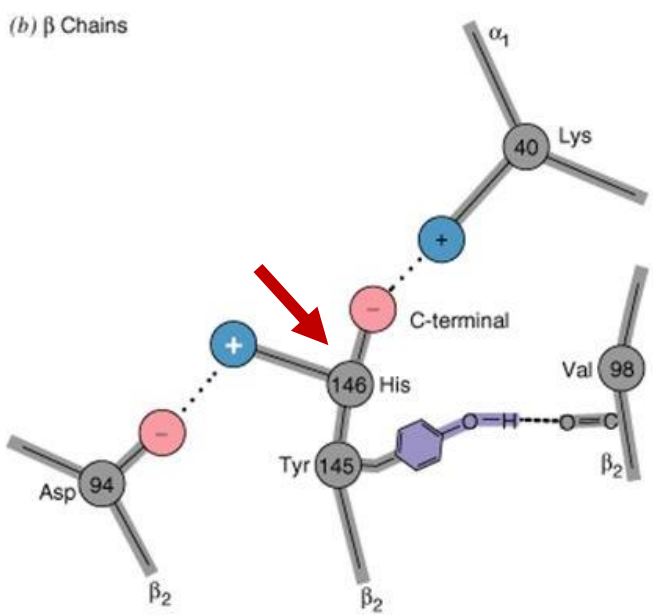
Broken electrostatic interactions and H-bonds

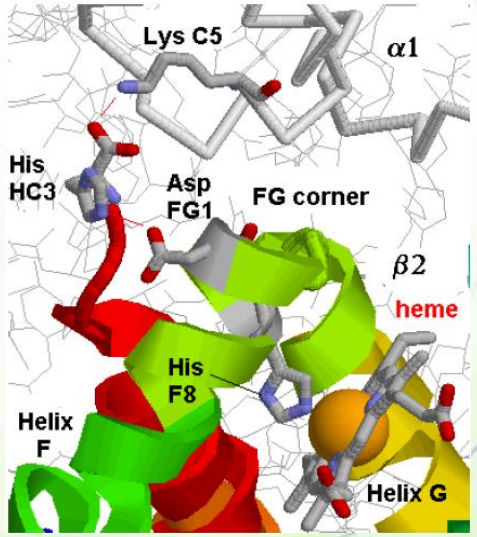


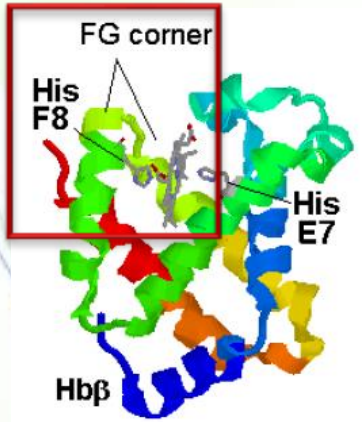
The broken interactions

- Electrostatic interactions and hydrogen bonds that stabilize the T-form of hemoglobin are broken upon movement of polypeptides.
  - Note the groups, the protonation status, and the allosteric effectors



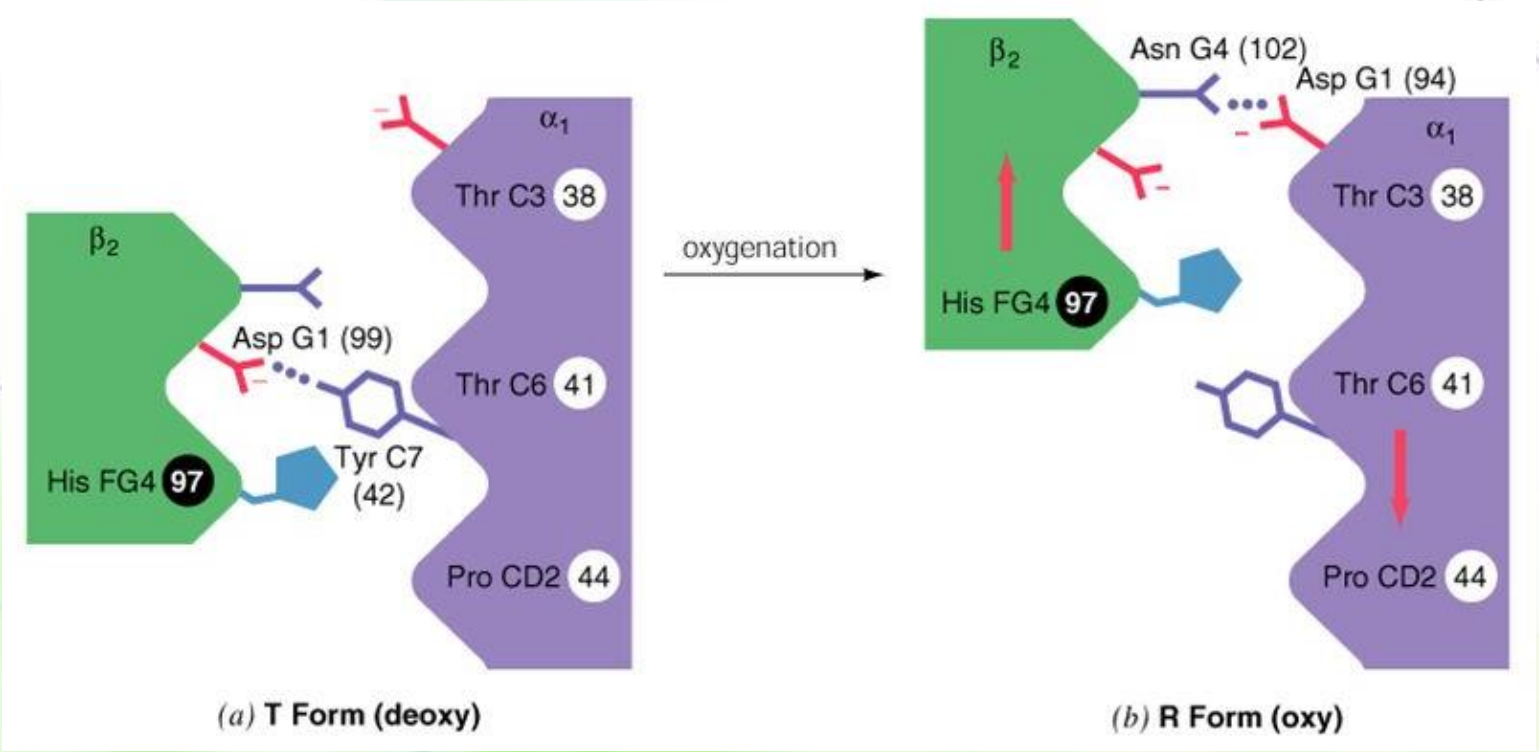




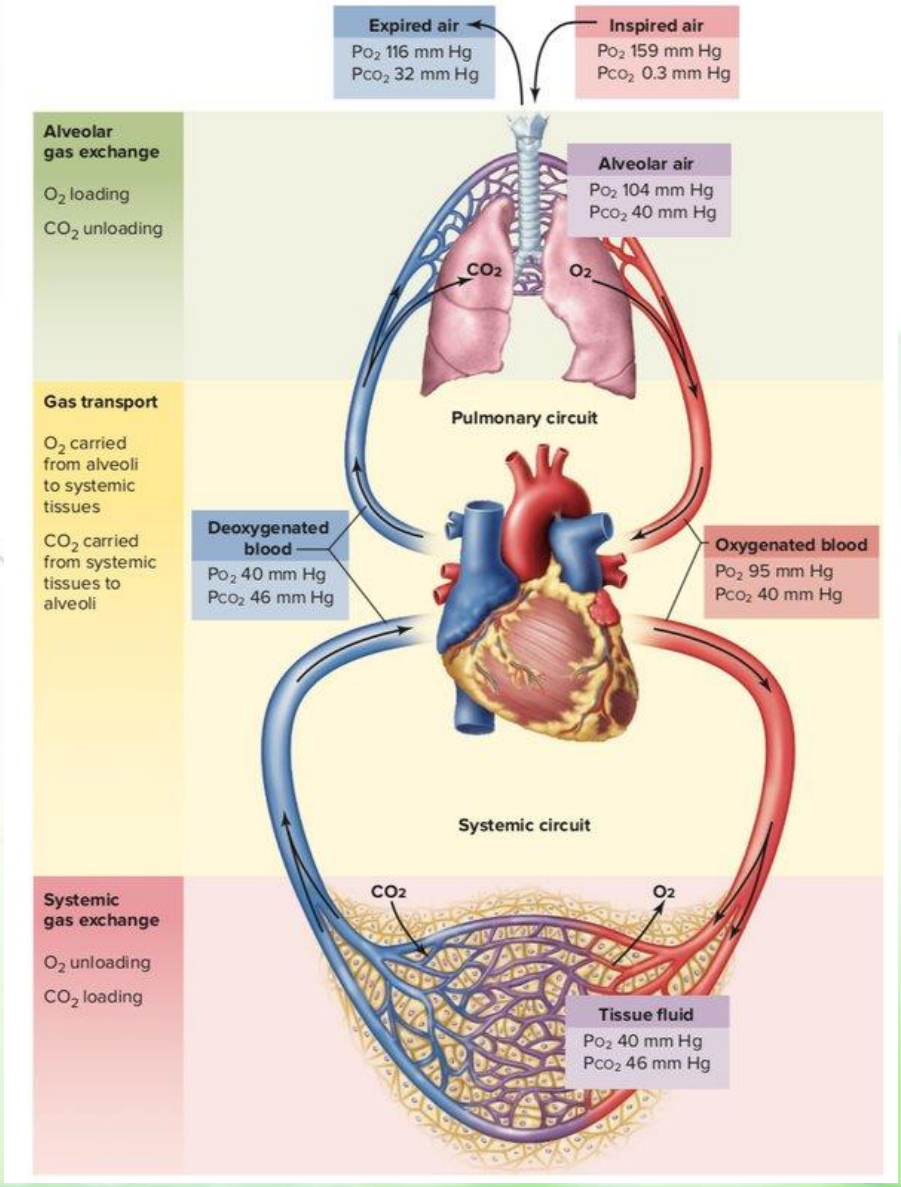


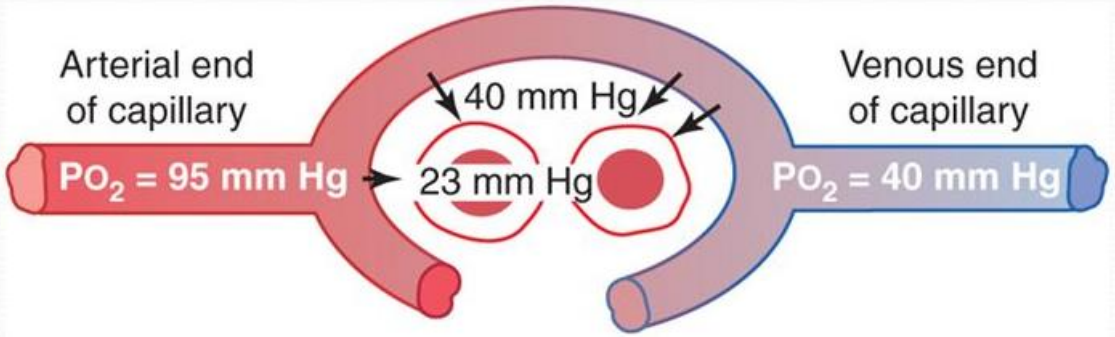
Reformation of hydrogen bonds

- 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.



Oxygen distribution in blood versus tissues





Oxygen saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
  - It is allosteric.
- At 100 mm Hg, hemoglobin is 97% saturated (oxyhemoglobin).
- As the oxygen pressure falls, oxygen is released to the cells.
- *Note: at high altitude (~5000 m), alveolar pO2 = 75 mmHg.*

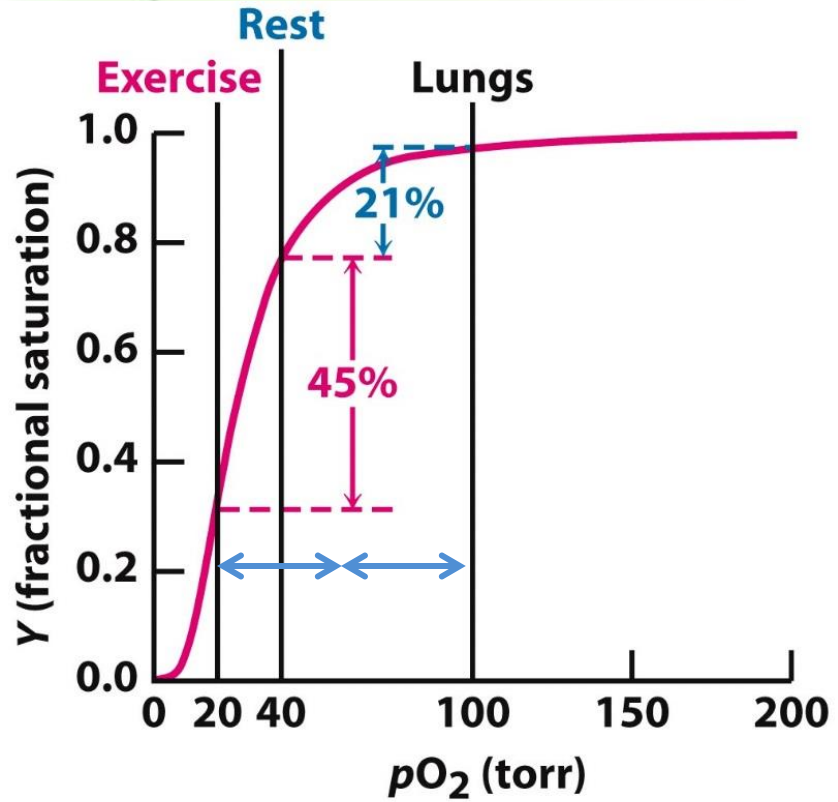
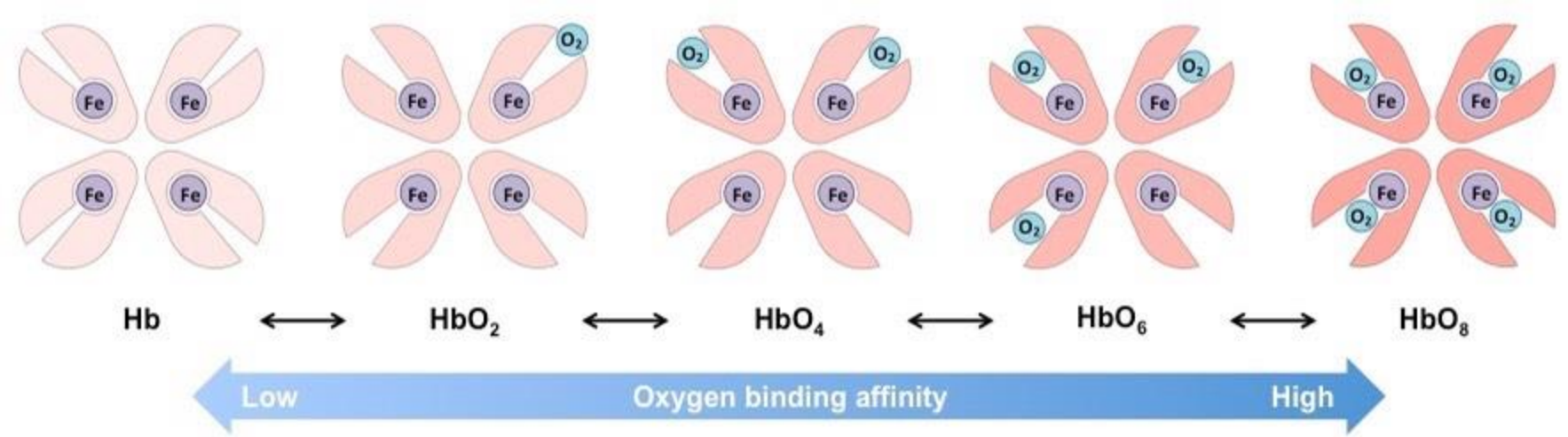


Figure 7.10
Biochemistry, Seventh Edition

Positive cooperativity



- 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)**.

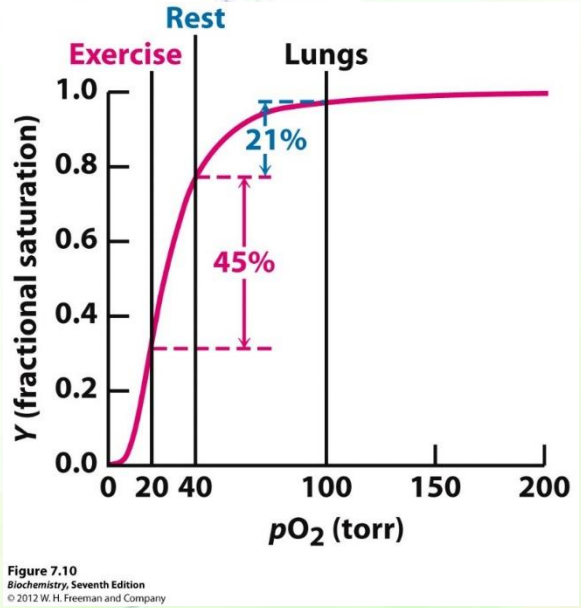


Figure 7.10
Biochemistry, Seventh Edition

The Hill constant (coefficient)

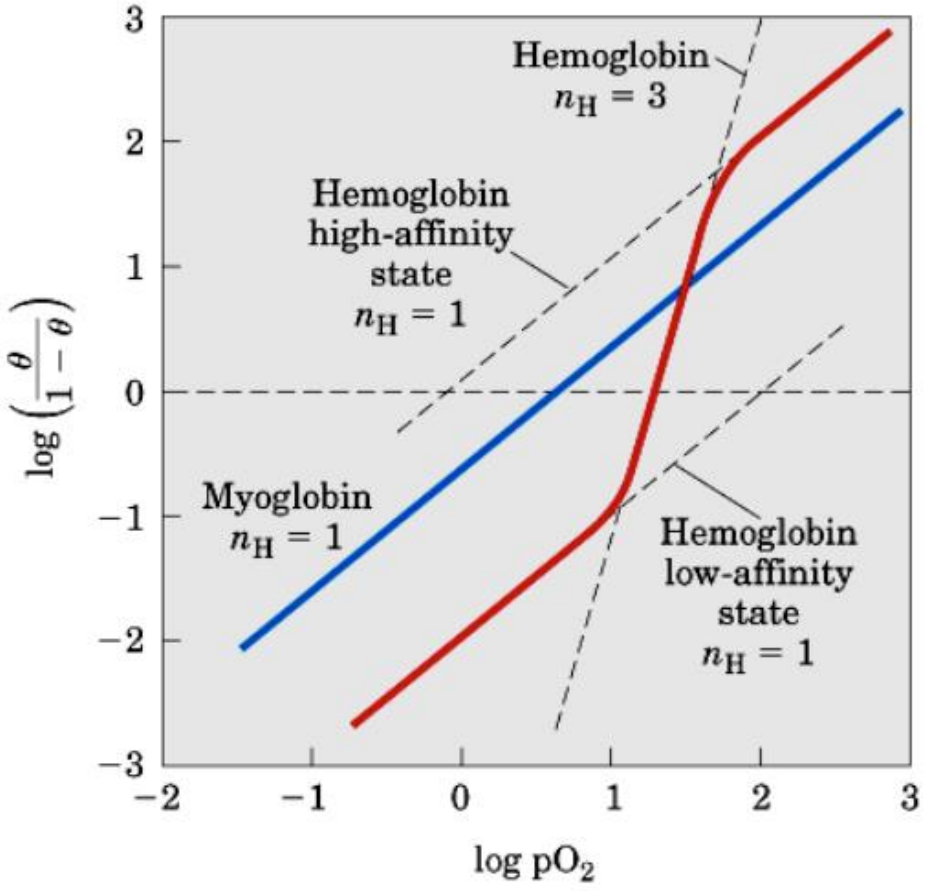
- The Hill plot is drawn based on an equation (you do not have to know it).
- n = Hill constant - determined graphically from the hill plot
- n is the slope at the midpoint of binding of log (Y/1-Y) vs. log of pO_2.
  - if n = 1 then non cooperativity
  - if n < 1 then negative cooperativity
  - if n >1 then positive cooperativity

- *The slope reflects the degree of cooperativity, not the number of binding sites.*

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

Y or θ is the fraction of oxygen-bound Hb

→ Y = mX + b (linear plot)

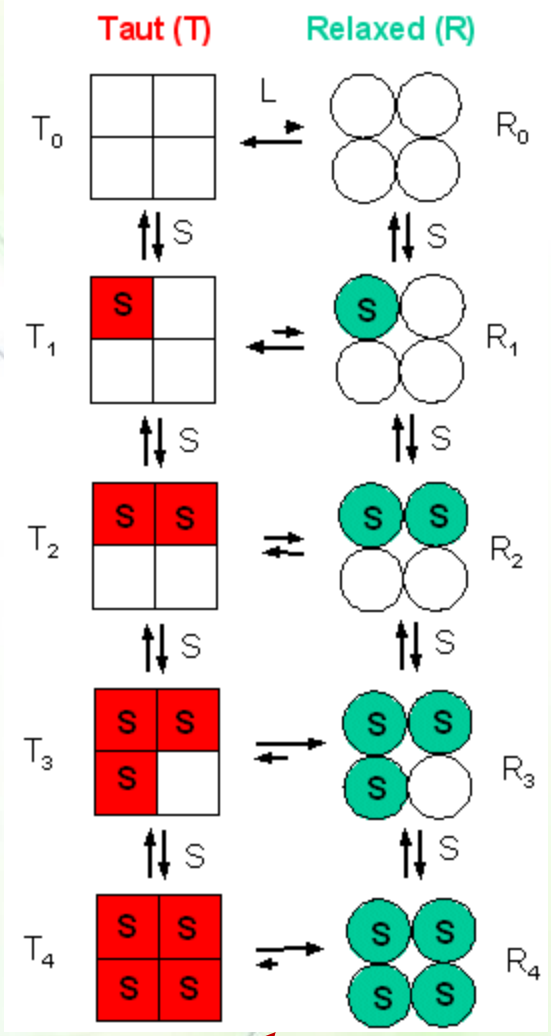


Cooperativity models

- Two models of cooperativity that could explain the observed data
  - Concerted model – all subunits undergo the conformational change simultaneously
    - 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)

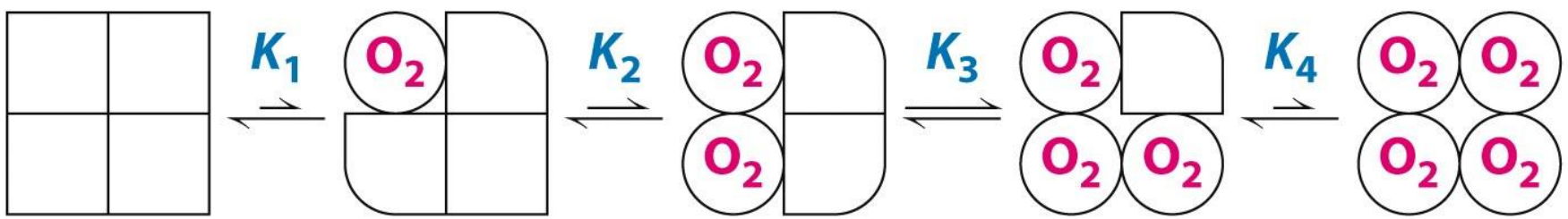
- The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.
- Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.
- This allows unoccupied subunits to adopt the high affinity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



Note direction of arrows

The sequential, induced fit, or KNF model

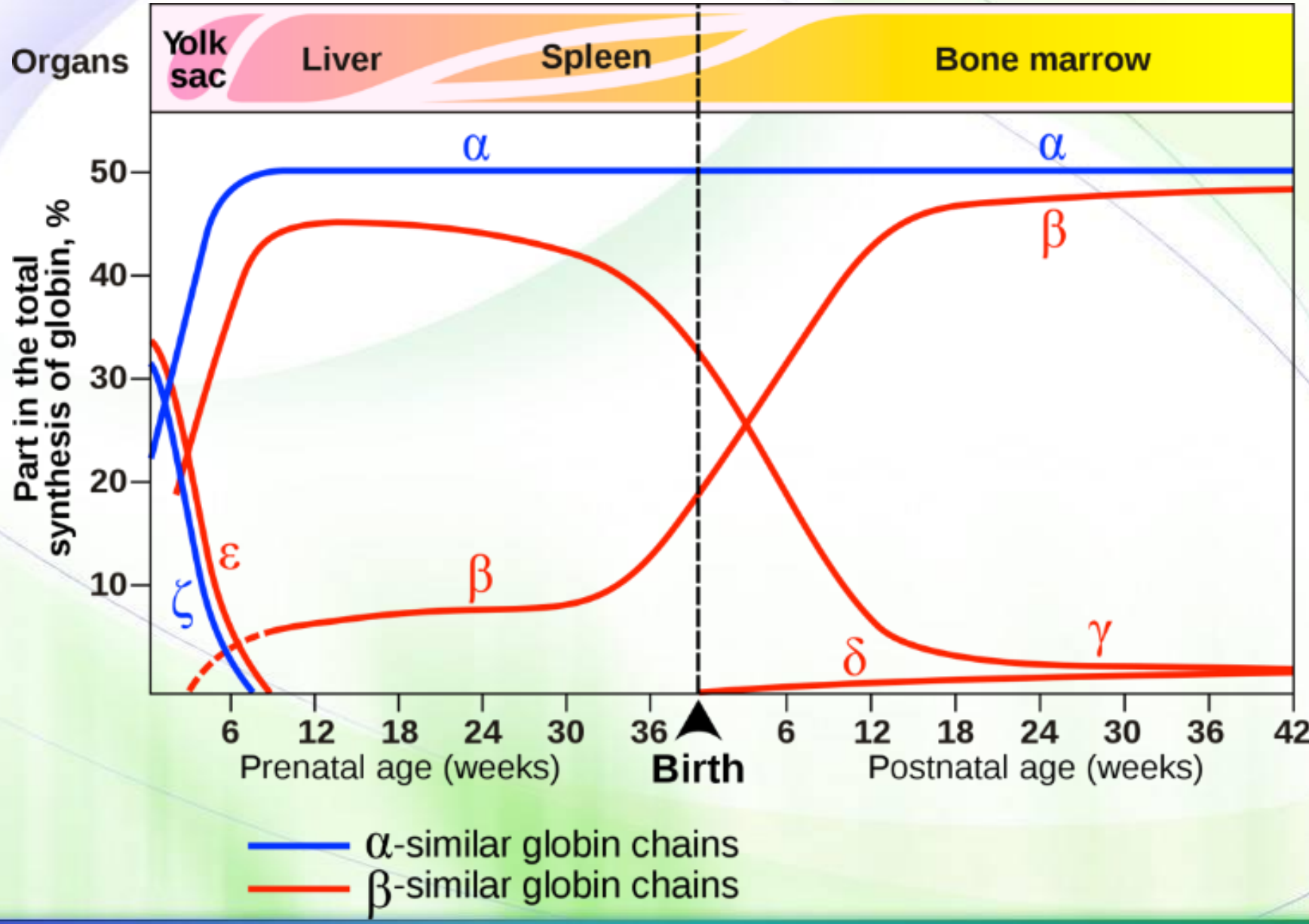
- The subunits go through conformational changes independently of each other, but they make the other subunits more likely to change, by reducing the energy needed for subsequent subunits to undergo the same conformational change.



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

It is not only one hemoglobin

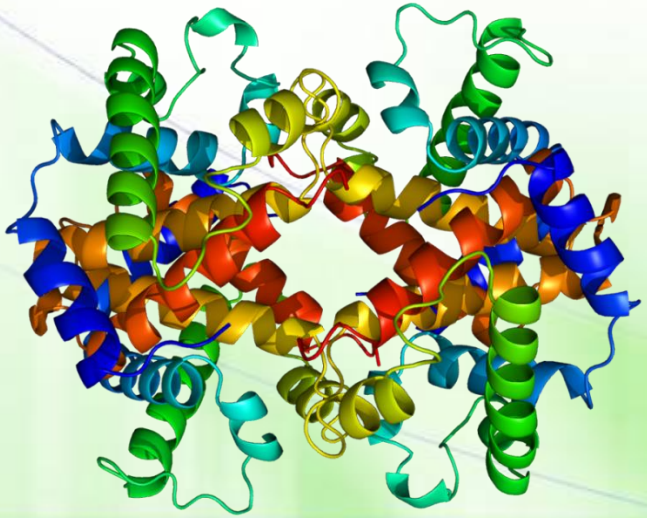
Developmental transition of hemoglobins

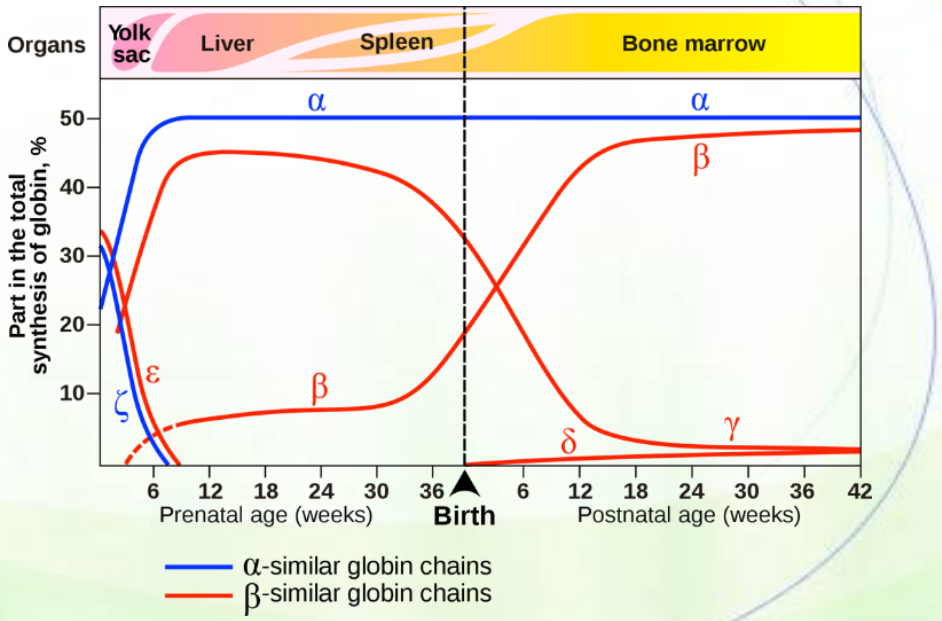


Alpha	Α α
Beta	Β β
Gamma	Γ γ
Delta	Δ δ
Epsilon	Ε ε
Zeta	Ζ ζ

The embryonic stage

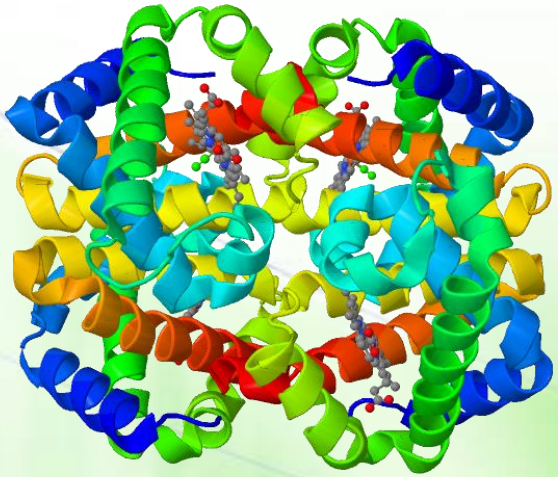
- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1) is a tetramer composed of 2 zeta (ξ) chains and 2 epsilon (ε) chains
- Other forms exist: HbE Gower 2 (α2ε2), HbE Portland 1 (ζ2γ2), HbE Portland 2 (ζ2β2).

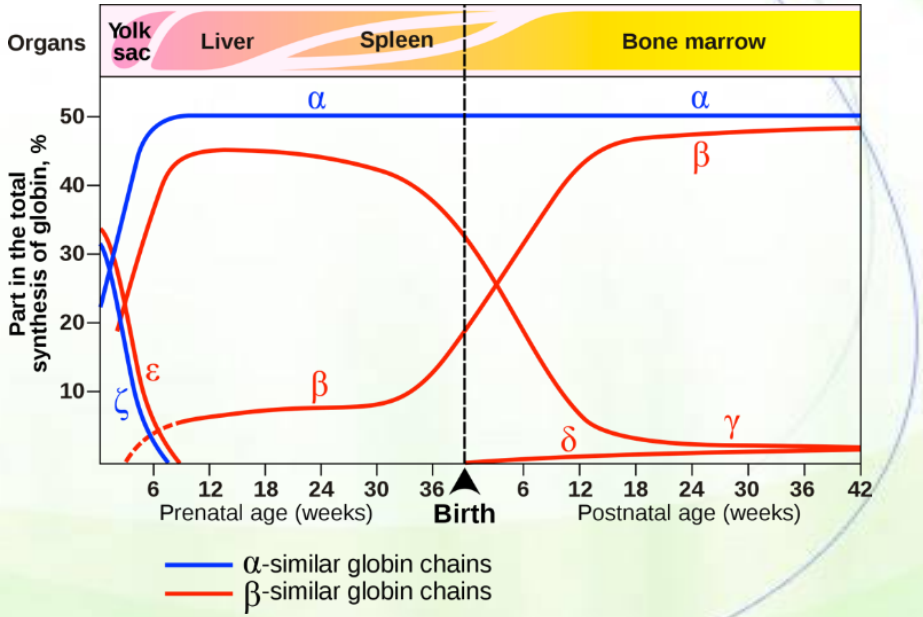




The fetal stage

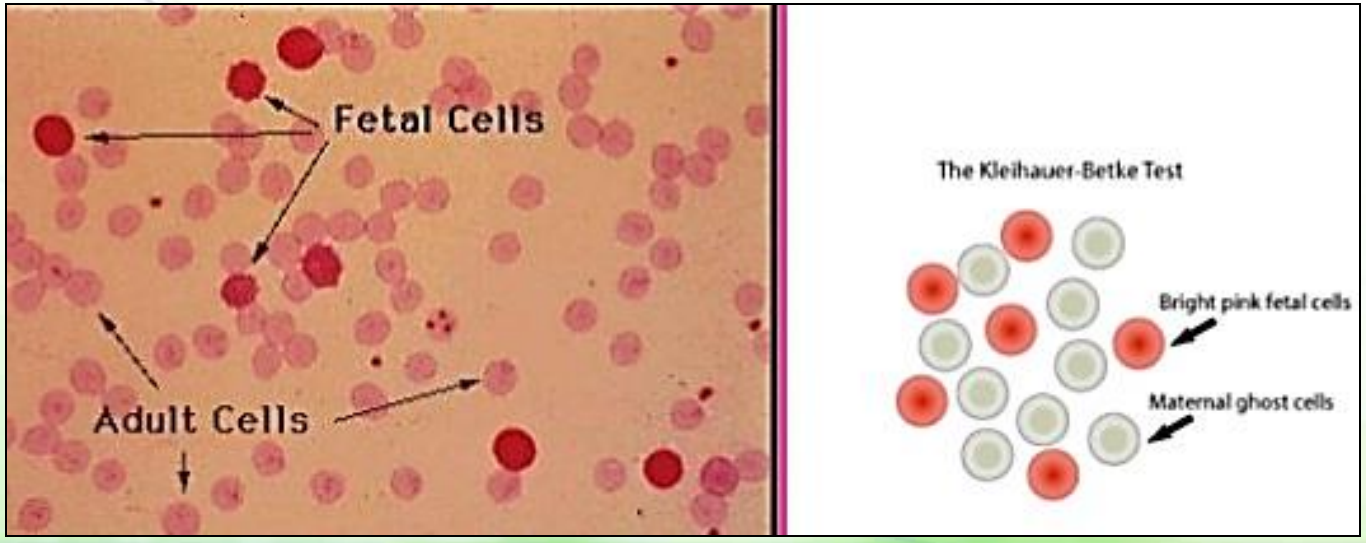
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines dramatically and fetal hemoglobin synthesis starts from the liver.
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides ($\alpha 2 \gamma 2$)
- The gene expression of the α polypeptides is active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

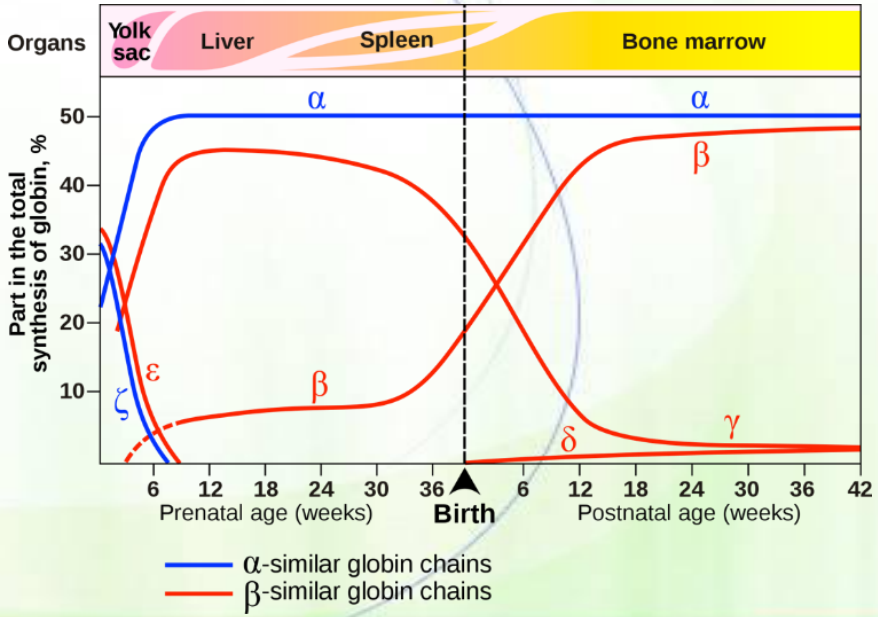




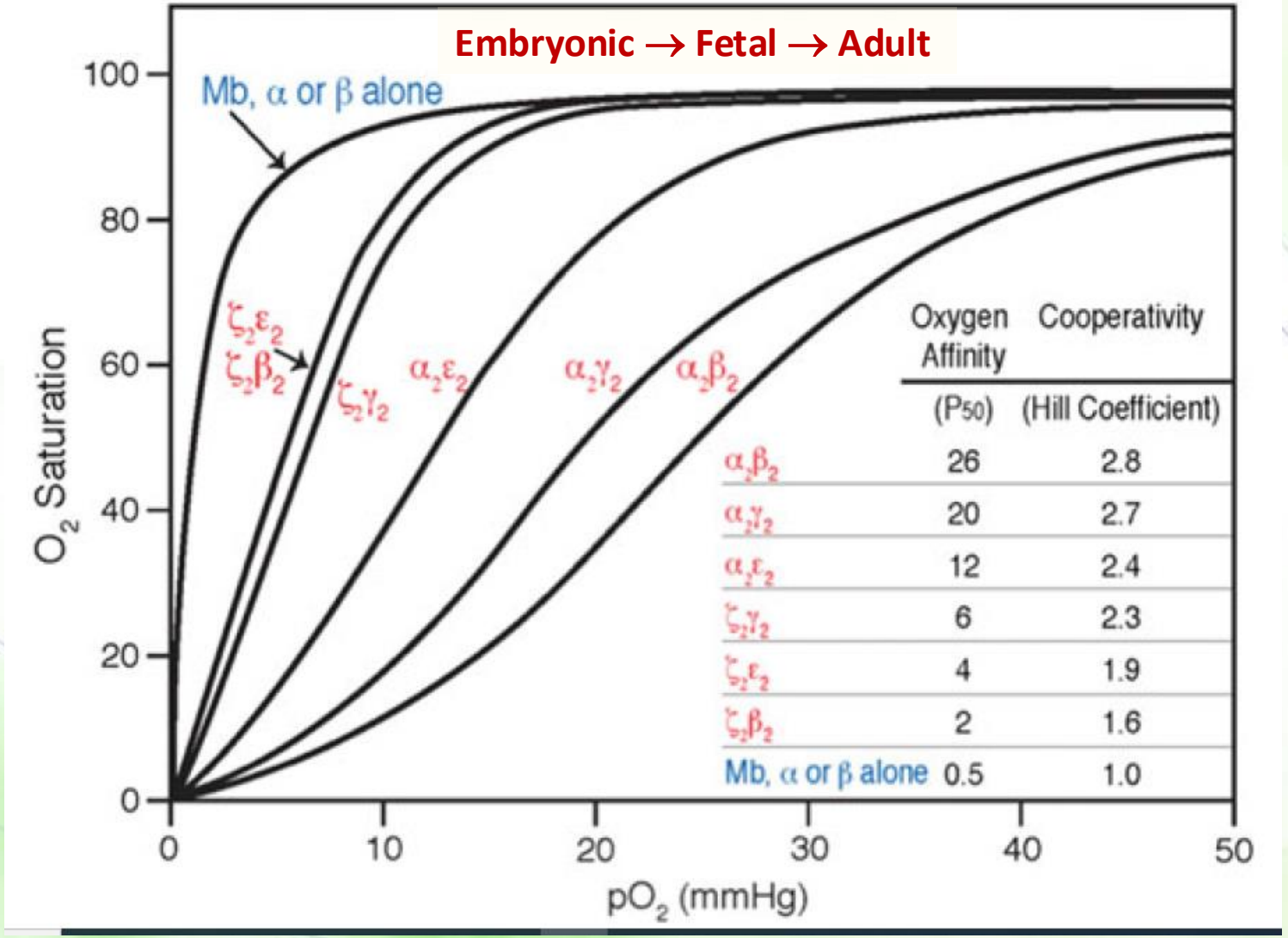
The adult stage

- 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.





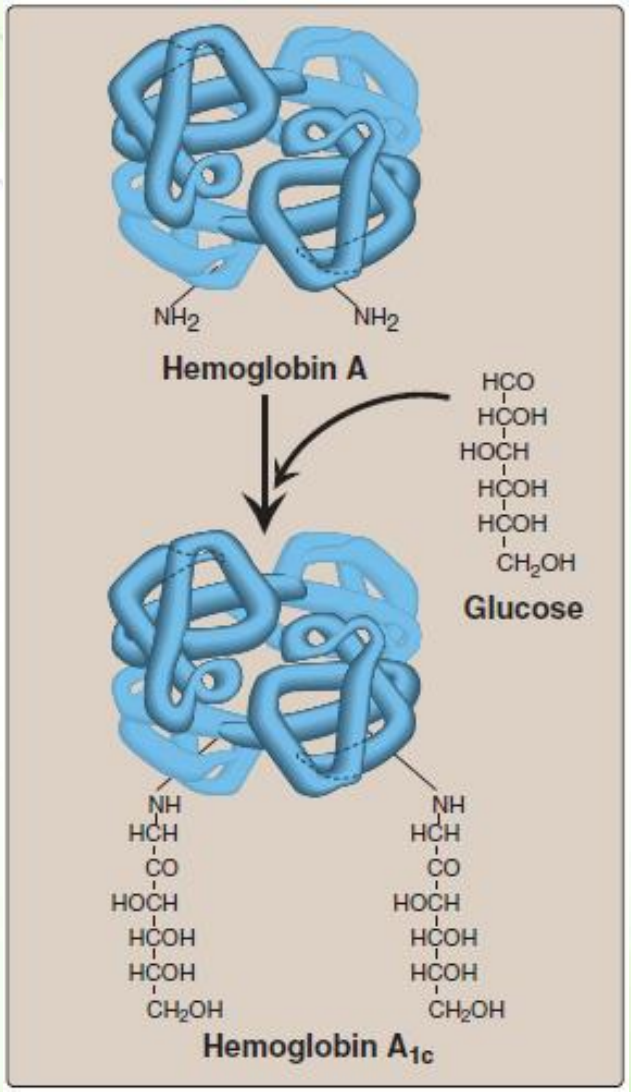
Range of O_2 Saturation/Normal Human Hbs



	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

Adult hemoglobins

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



Advantages of HbA1c testing

- Blood fasting glucose level is the concentration of glucose in blood at a single point in time when fasting for a few hours.
- **HbA1c** level provides a longer-term trend, similar to an average, of how high blood sugar levels have been over a period of time (2-3 months).
- HbA1c can be expressed as a percentage (DCCT unit, used in the US) or as a value in mmol/mol (IFCC unit).

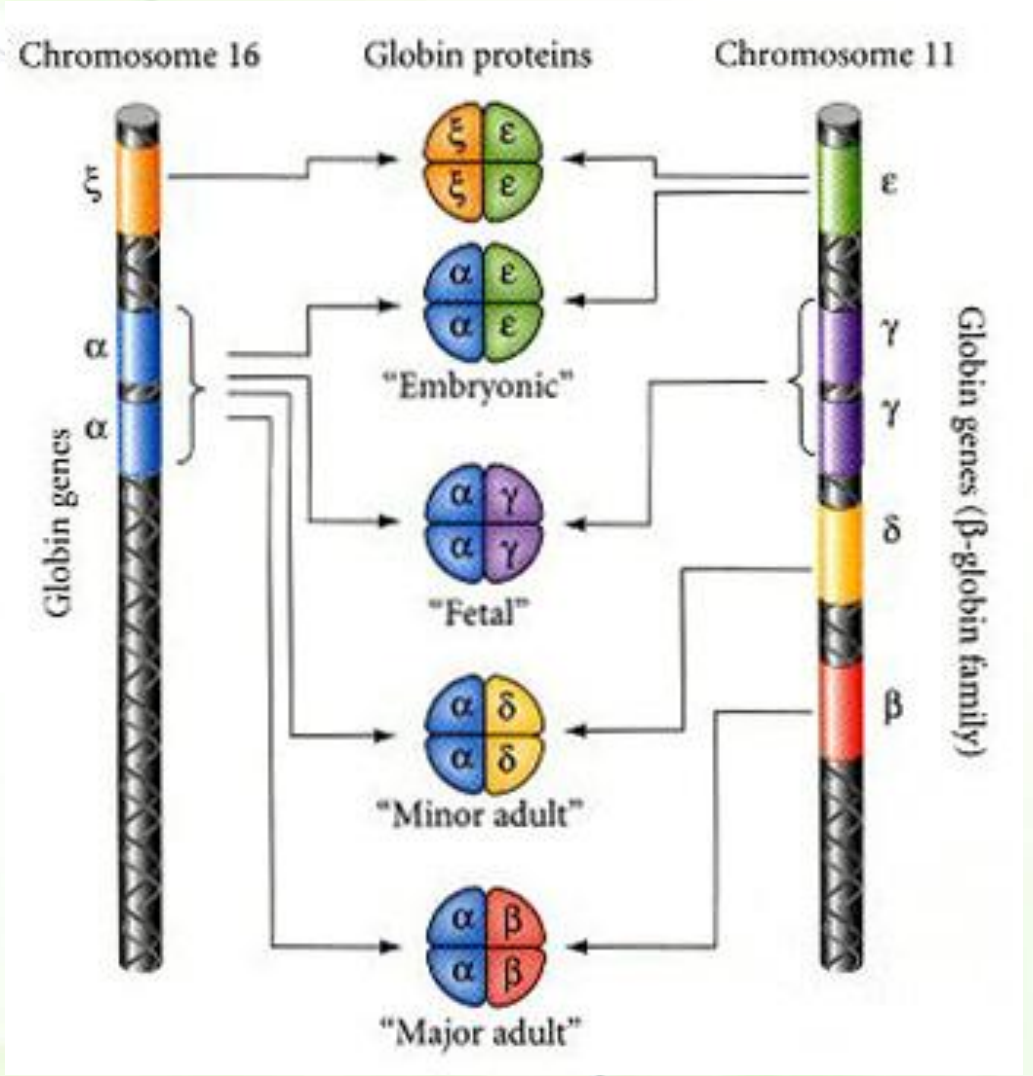
Table

	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

Genetics of globin synthesis

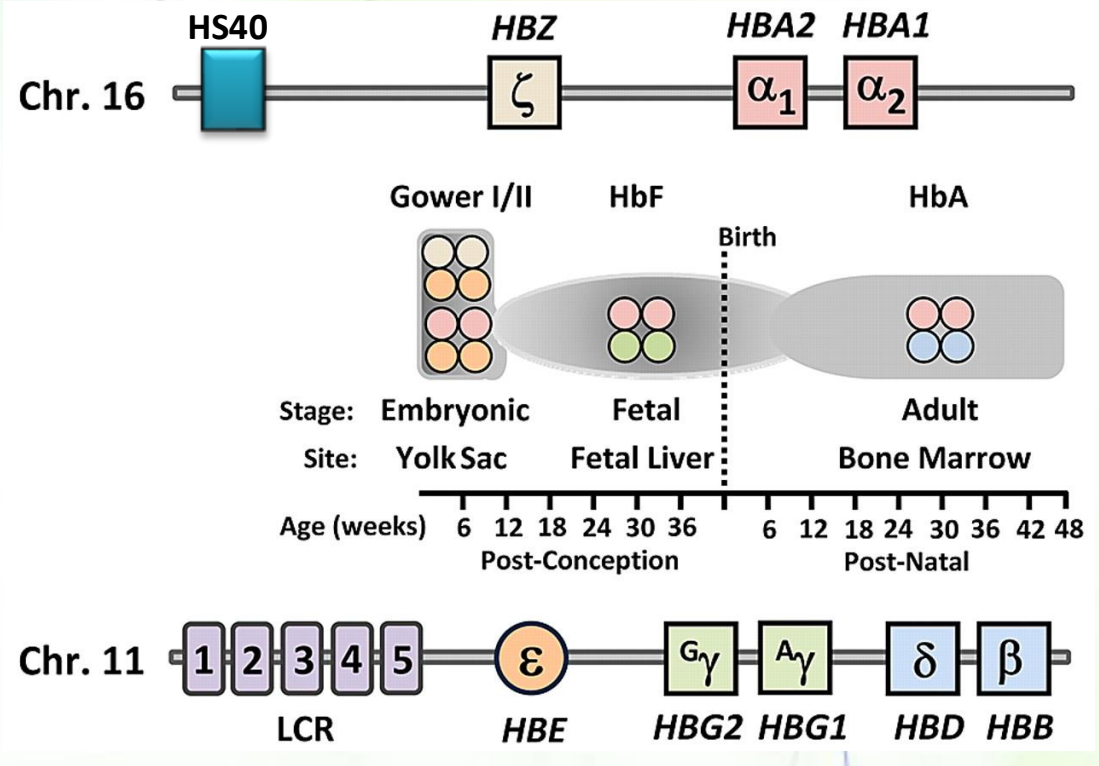
The genes

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



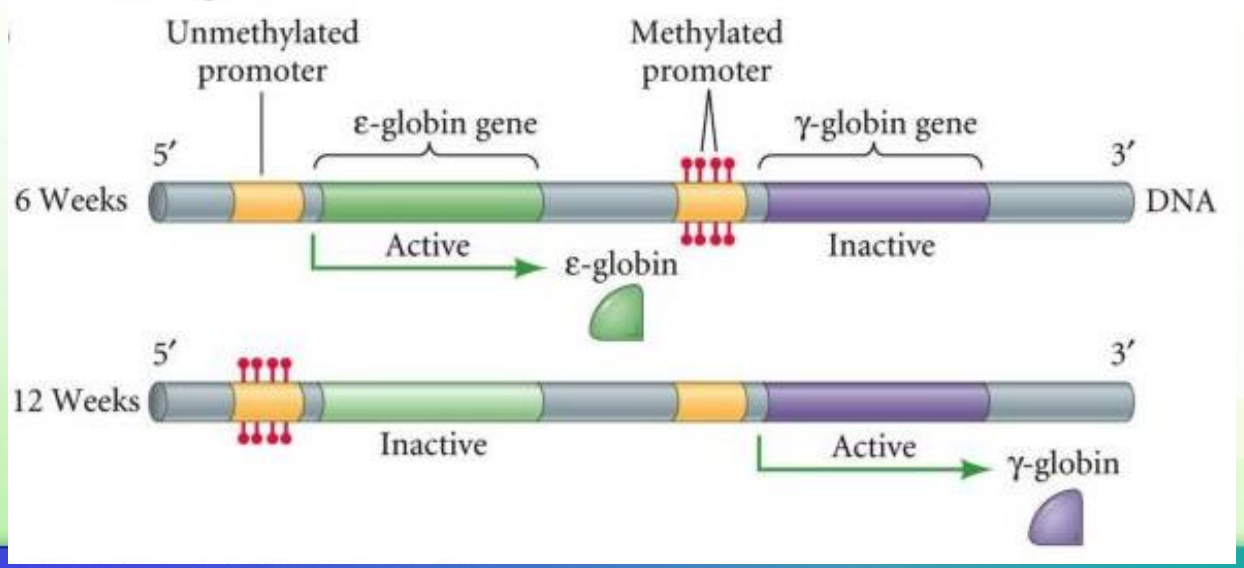
Locus structure

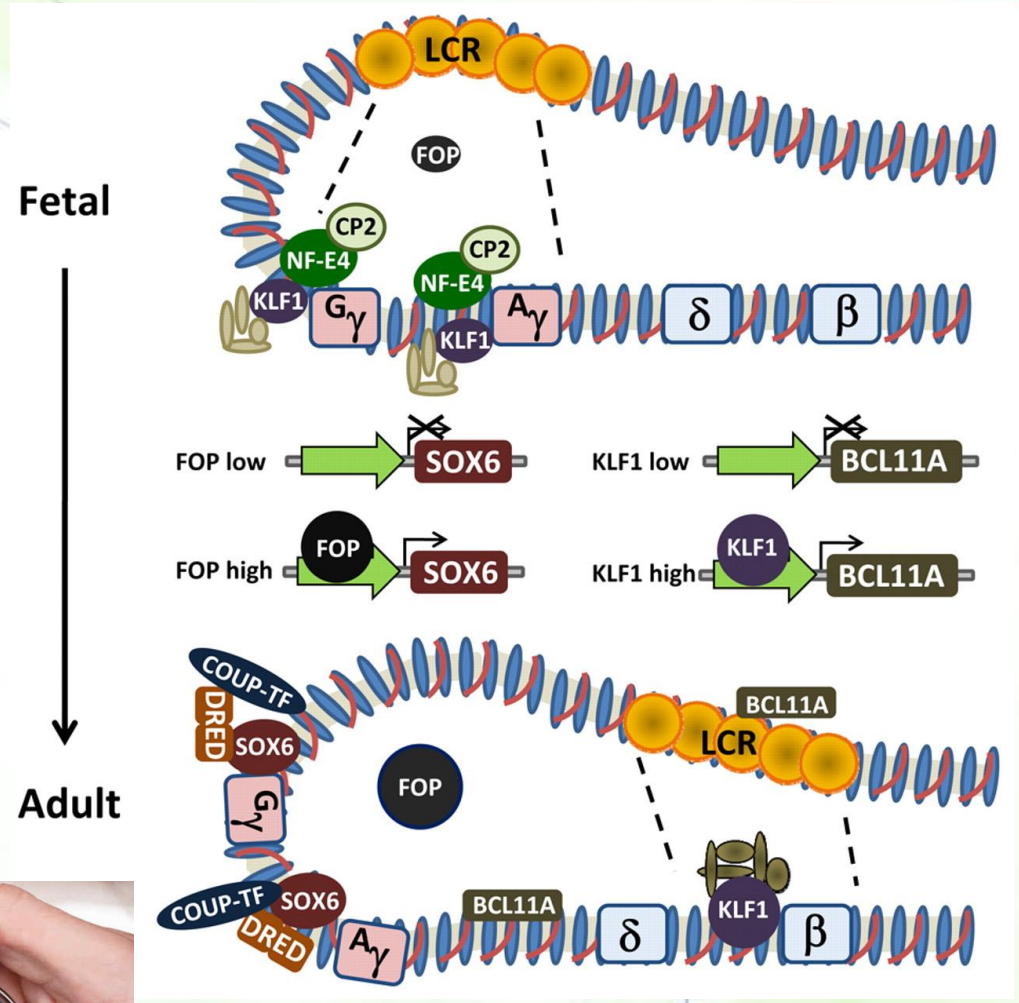
- Each gene has its promoter and regulatory sequences (activators, silencers).
- The α gene cluster is controlled by the HS40 region.
- The β-globin cluster is controlled by a master enhancer called locus control region (LCR).



The mechanism of regulation

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





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

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Abstract

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