Hemoglobin
An overview and more

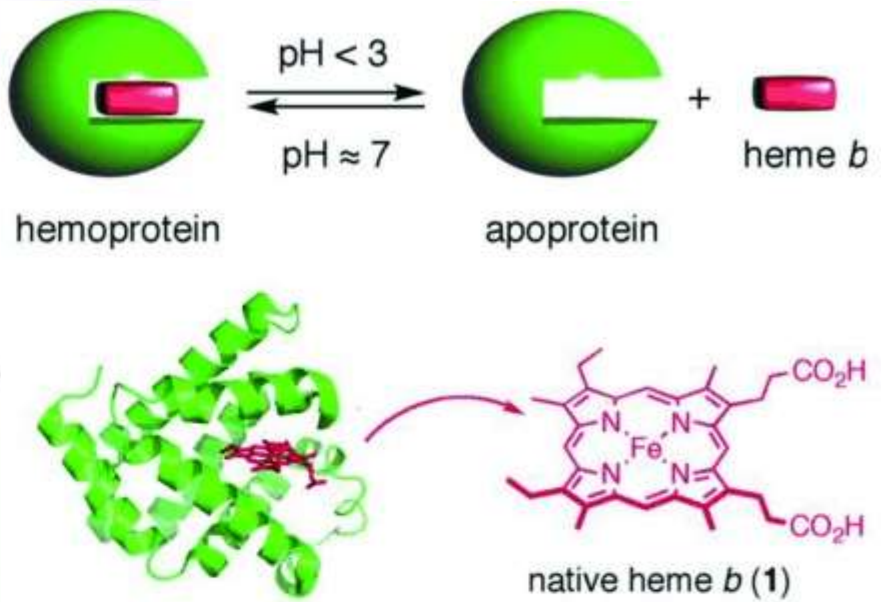
Prof. Mamoun Ahram
Hematopoietic-lymphatic system

Resources

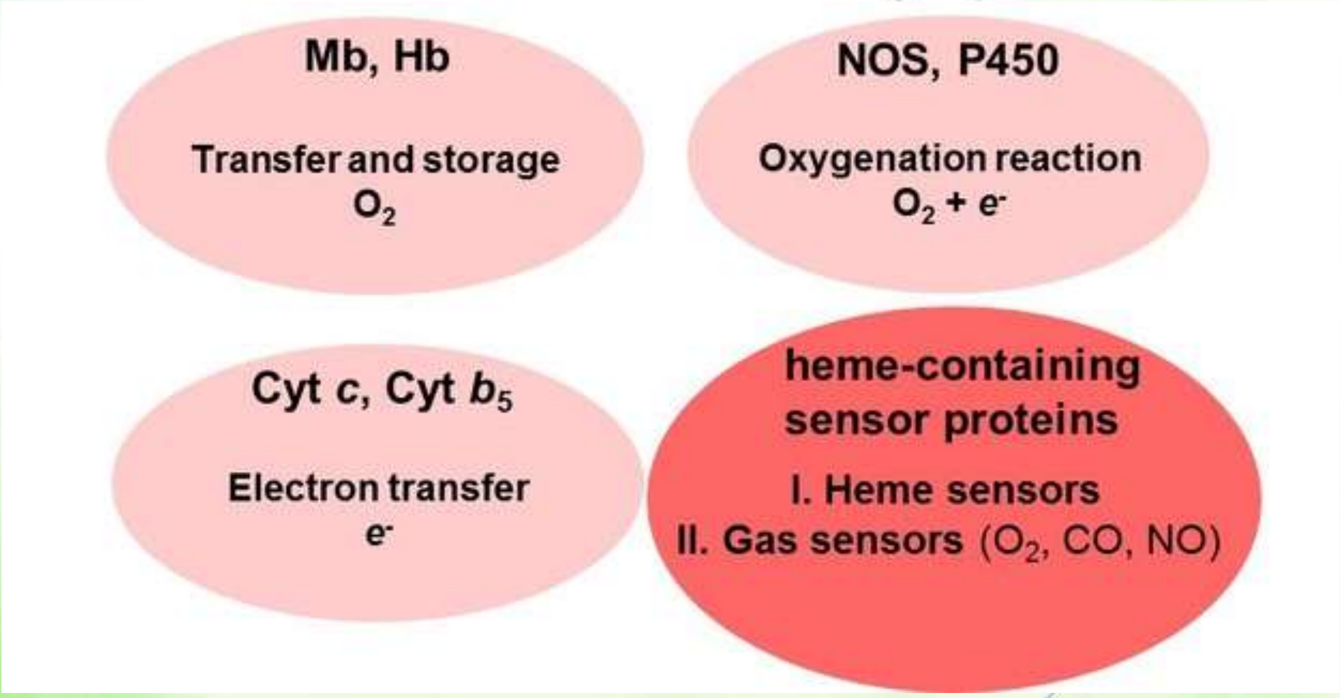
- This lecture
- Myoglobin/Hemoglobin O2 Binding and Allosteric Properties of Hemoglobin (http://home.sandiego.edu/~josephprovost/Chem331%20Lect%207_8%20Myo%20Hemoglobin.pdf)
- Lecture 3: Cooperative behaviour of hemoglobin (https://www.chem.uwec.edu/chem452_f12/pages/lecture_materials/unit_III/lecture-3/overheads/Chem452-lecture_3-part_1-overheads.pdf)

Hemoproteins

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

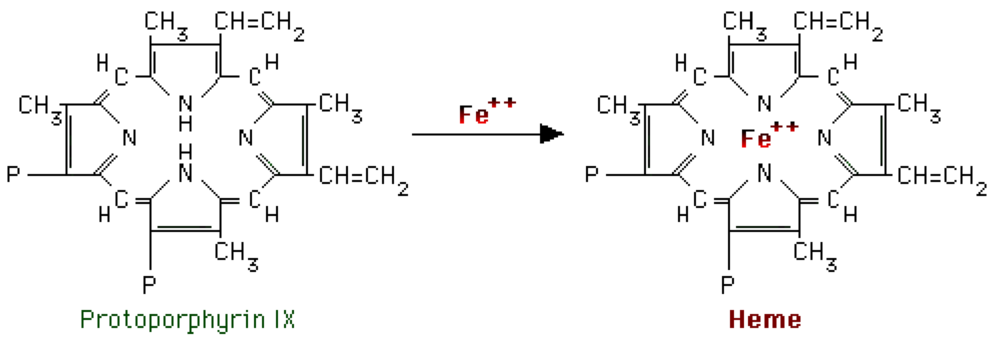


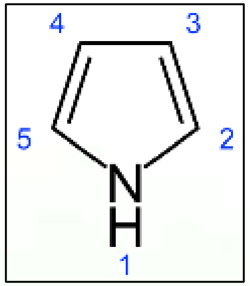
***A prosthetic group** is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.*

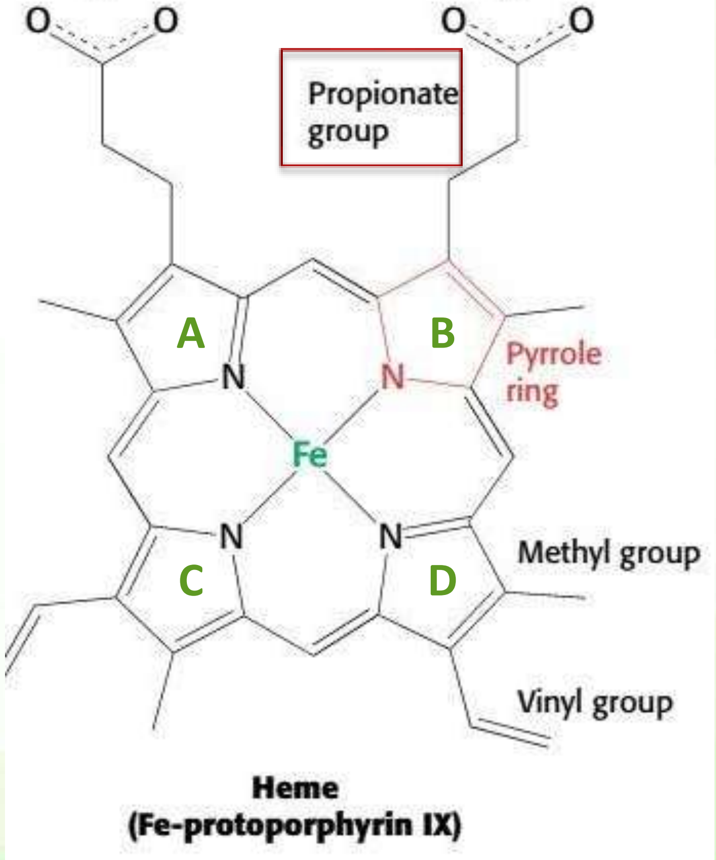


Heme structure

- 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.

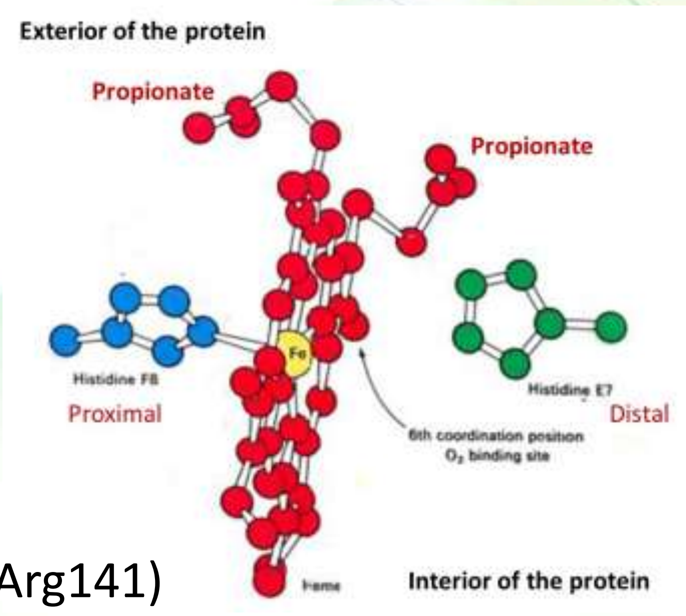


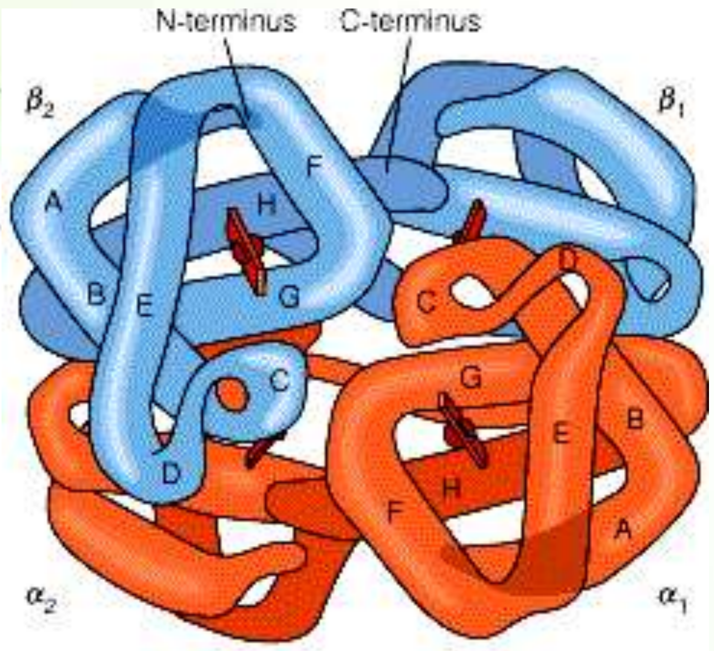


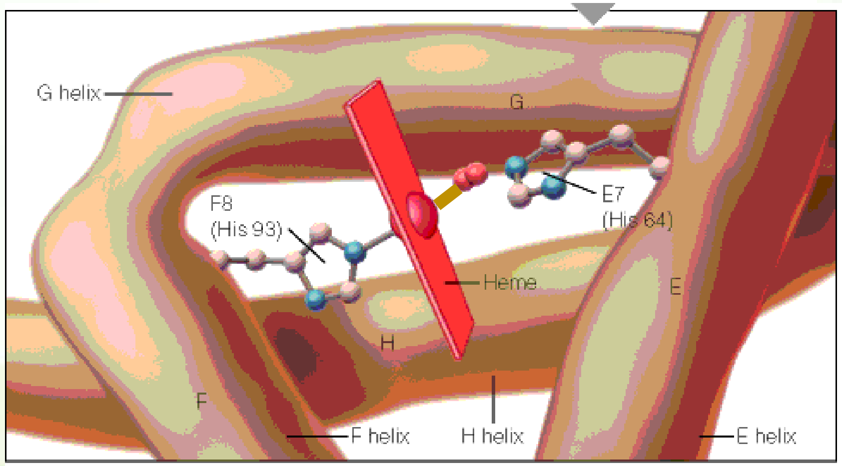


Structure of hemoglobin

- 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

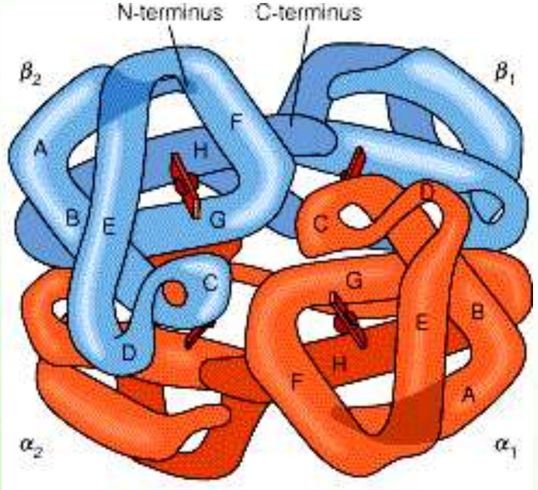


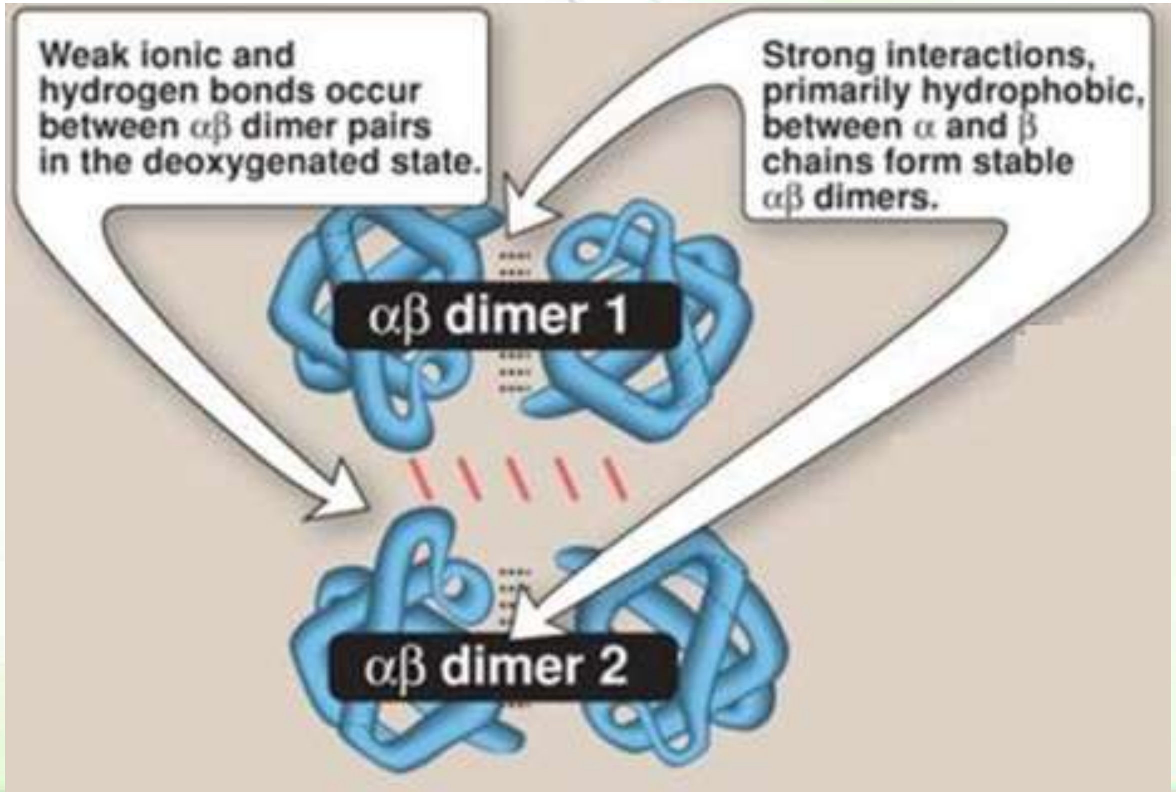




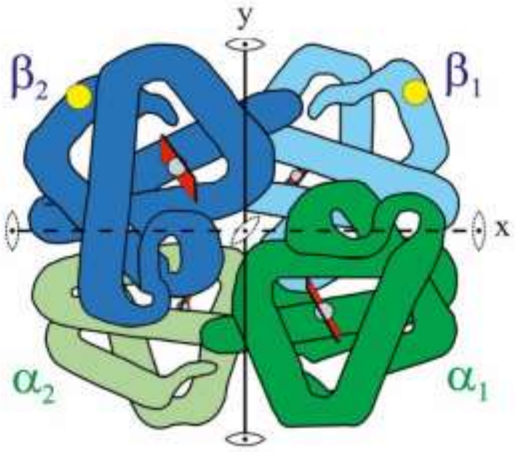
How are the subunits bound?

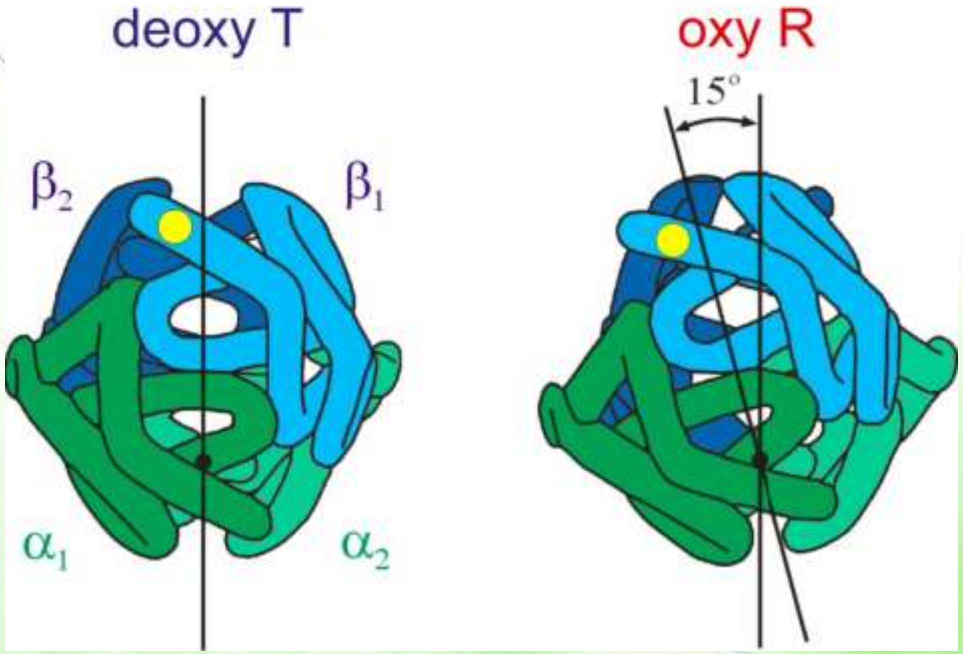
- A dimer of dimers (I made up this term)
  - (α-β)2
  - Note how they interact with each other.

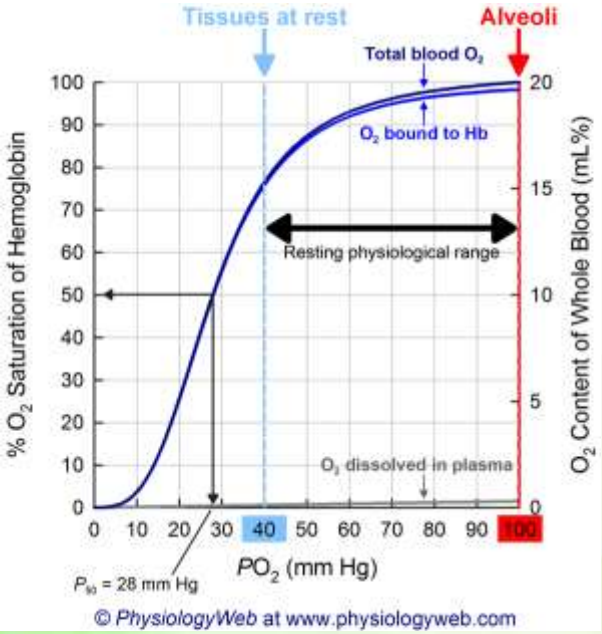


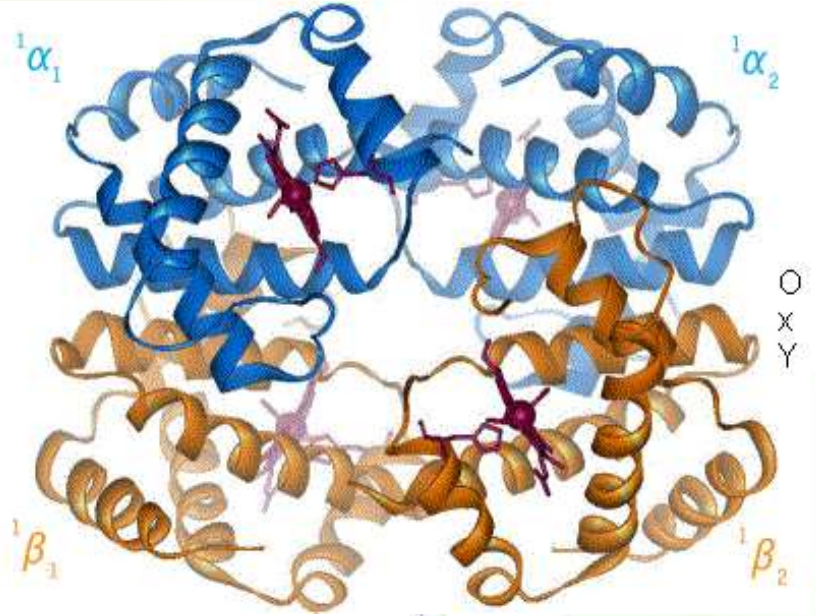


Structural change of hemoglobin

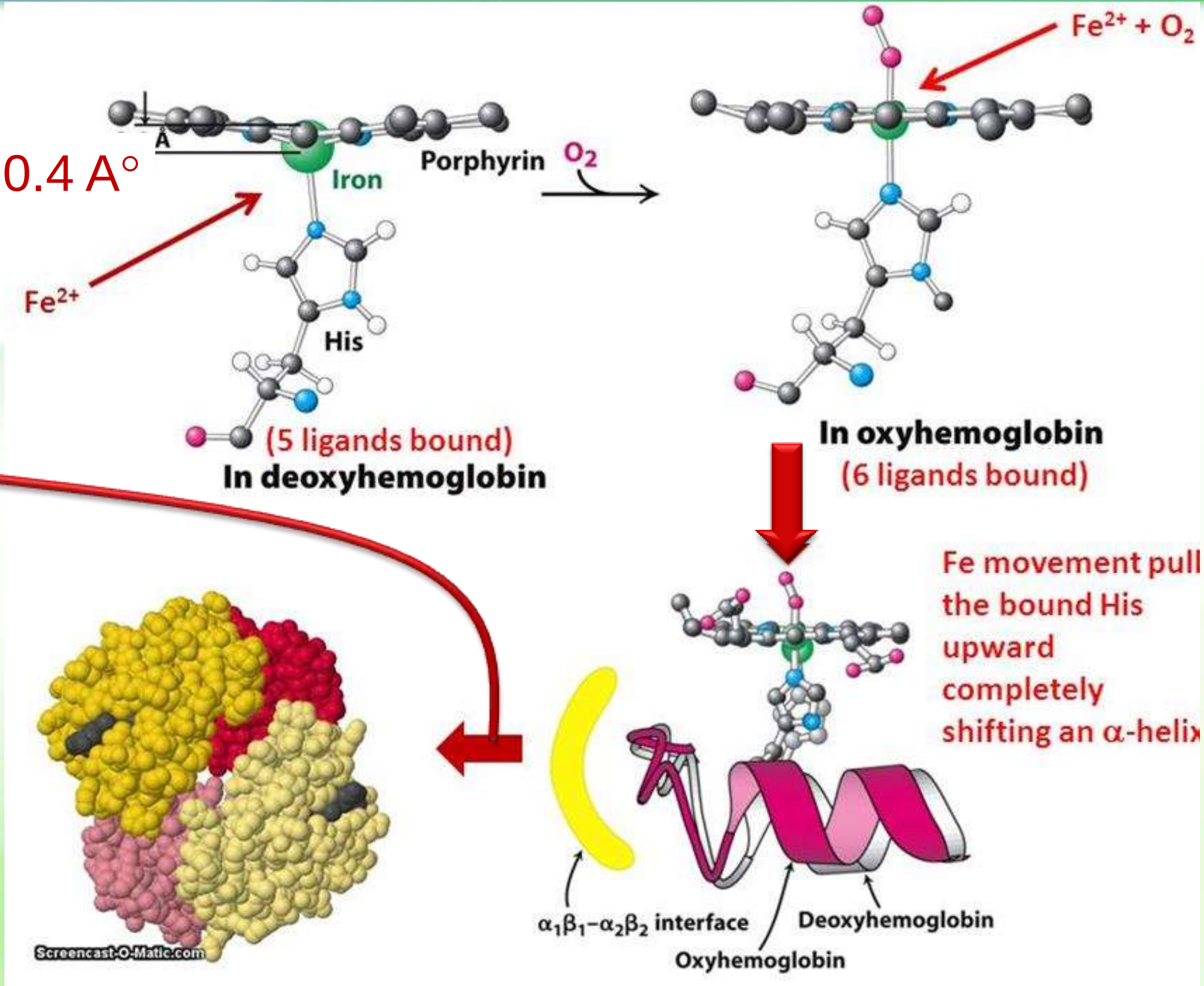




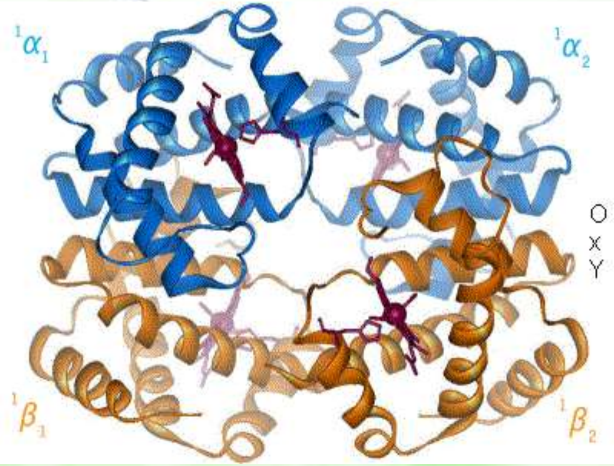




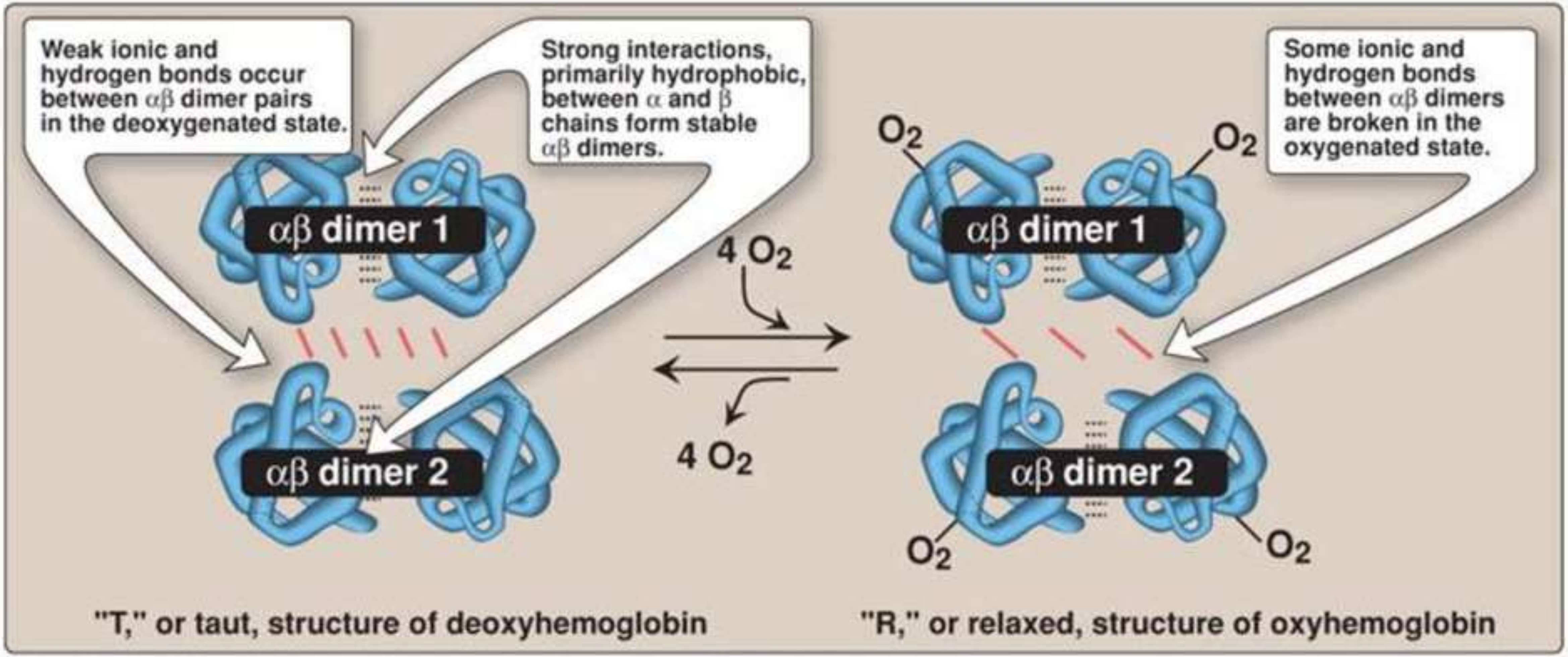
Structural amplification change



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

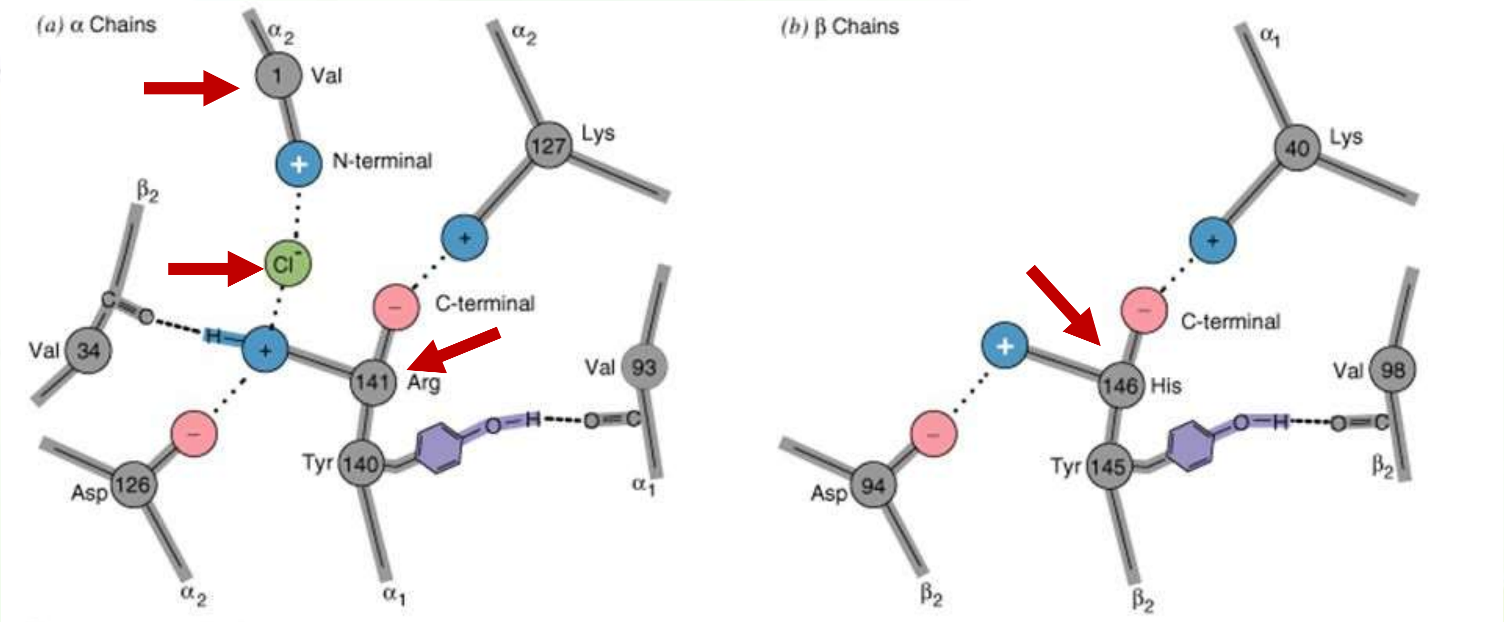


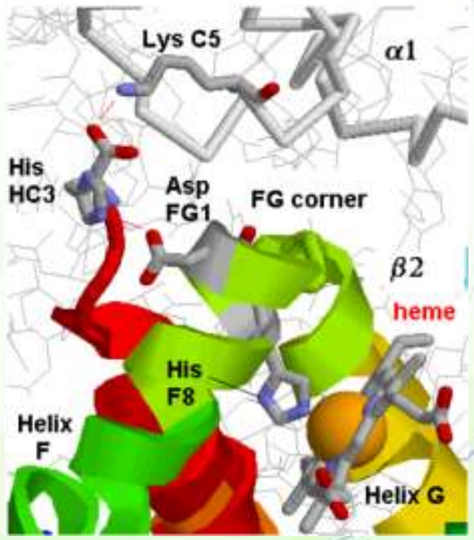
Electrostatic interactions are broken

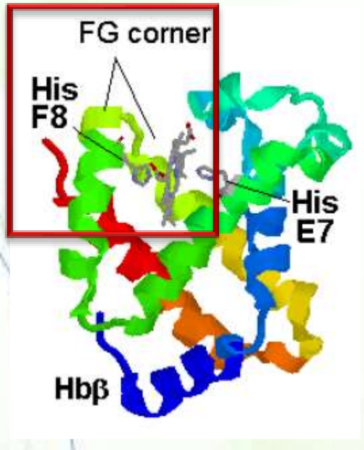


The broken electrostatic interactions

- Electrostatic interactions and hydrogen bonds (at the C-termini of the alpha and beta chains) that stabilize the T-form of hemoglobin are broken upon movement of the alpha-helix.
    - Note the groups, the protonation status, and the allosteric effectors

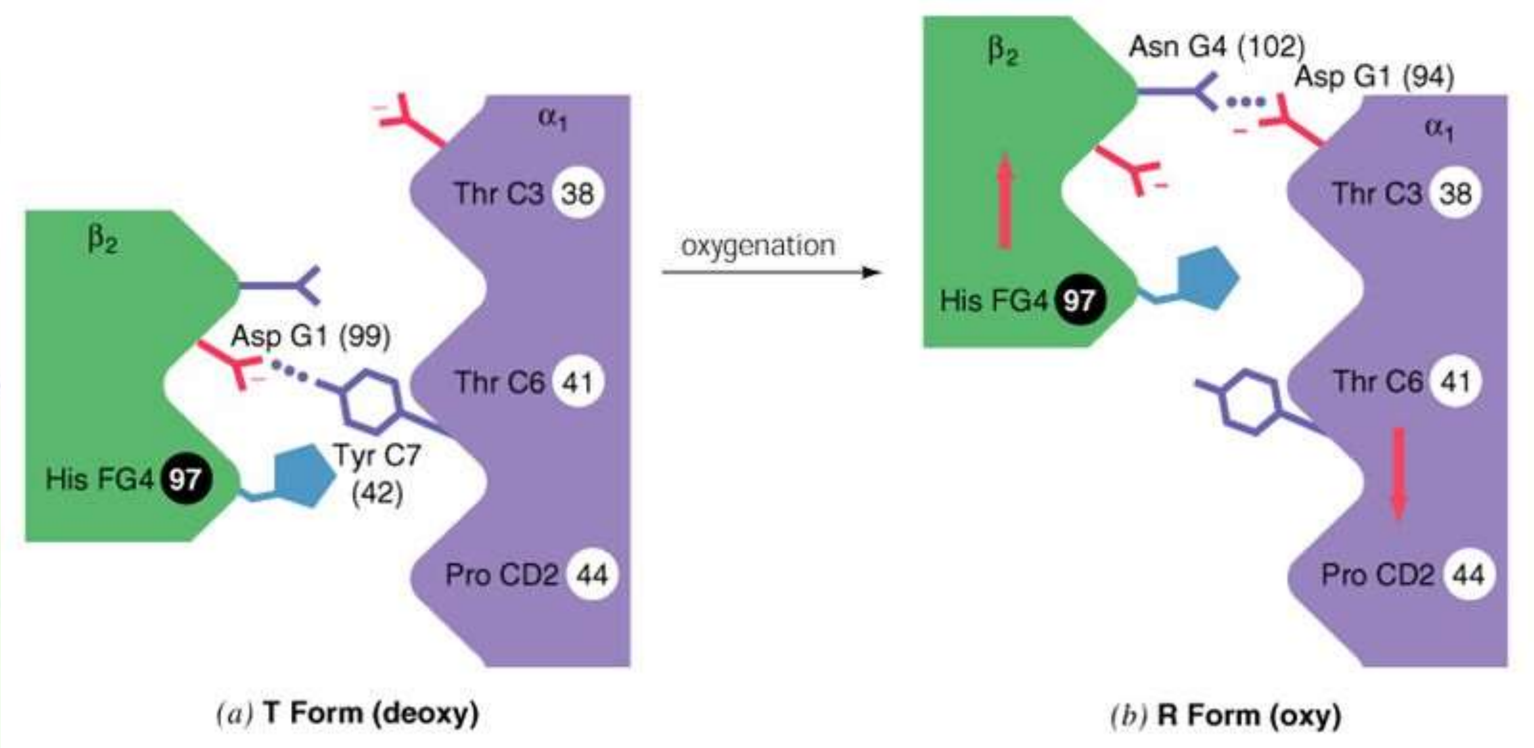




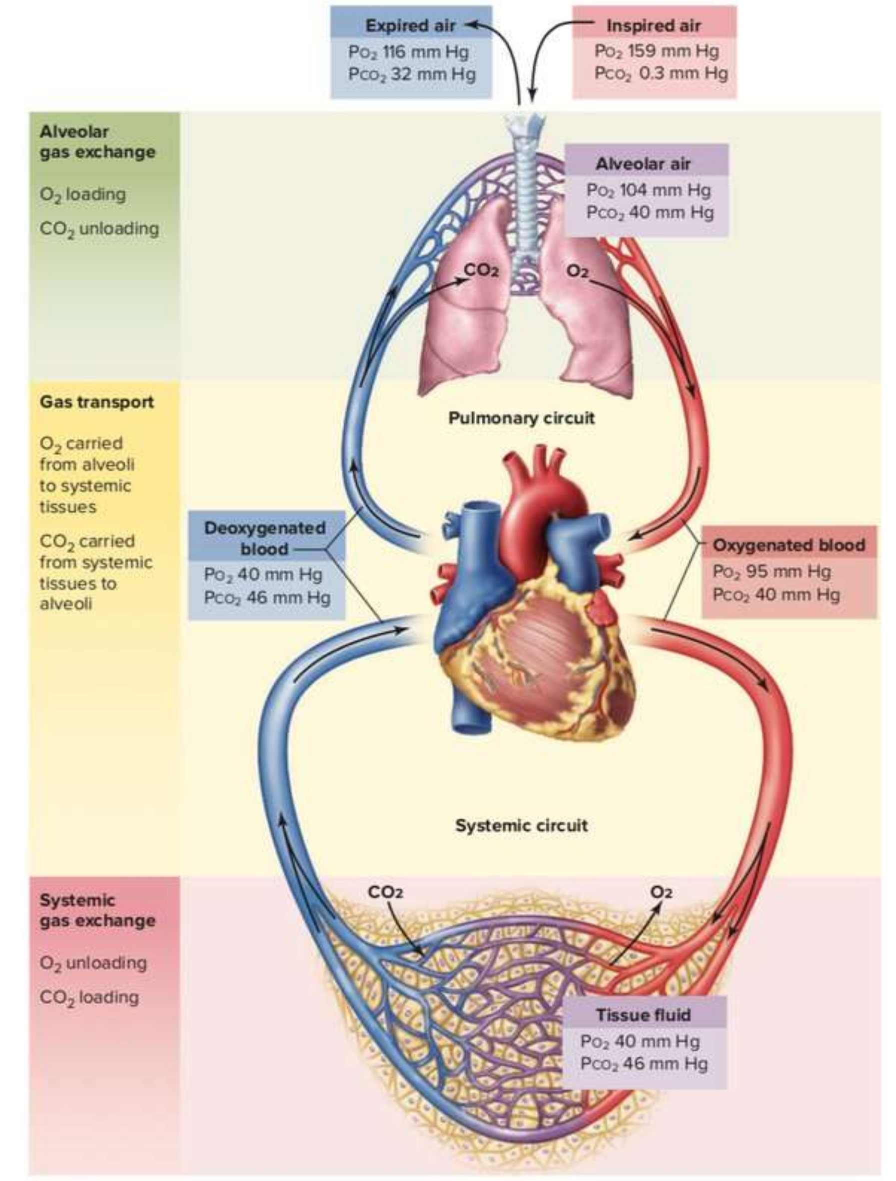


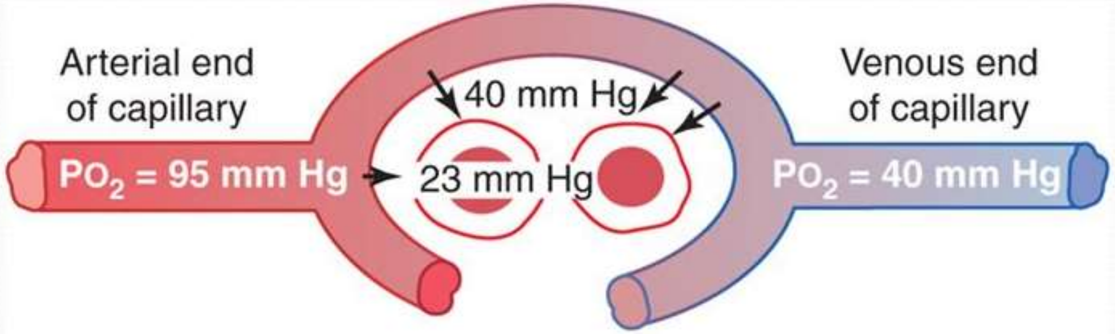
And reformation of hydrogen bonds

- T-state hemoglobin (deoxyhemoglobin) is stabilized by a hydrogen bond between Asp G1 (99) of β 2 with Thr C6 (41) of α1.
- When O_2 binds, the α1 surface slides and a hydrogen bond is formed between Asp G4 (94) of α chain with Asn G4 (102) of β 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.*

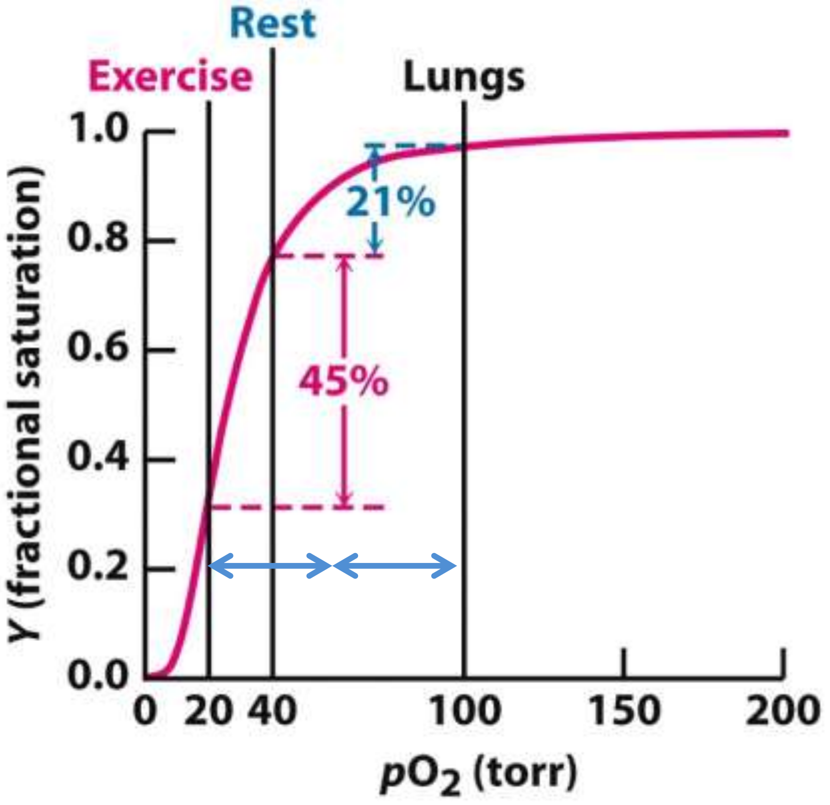
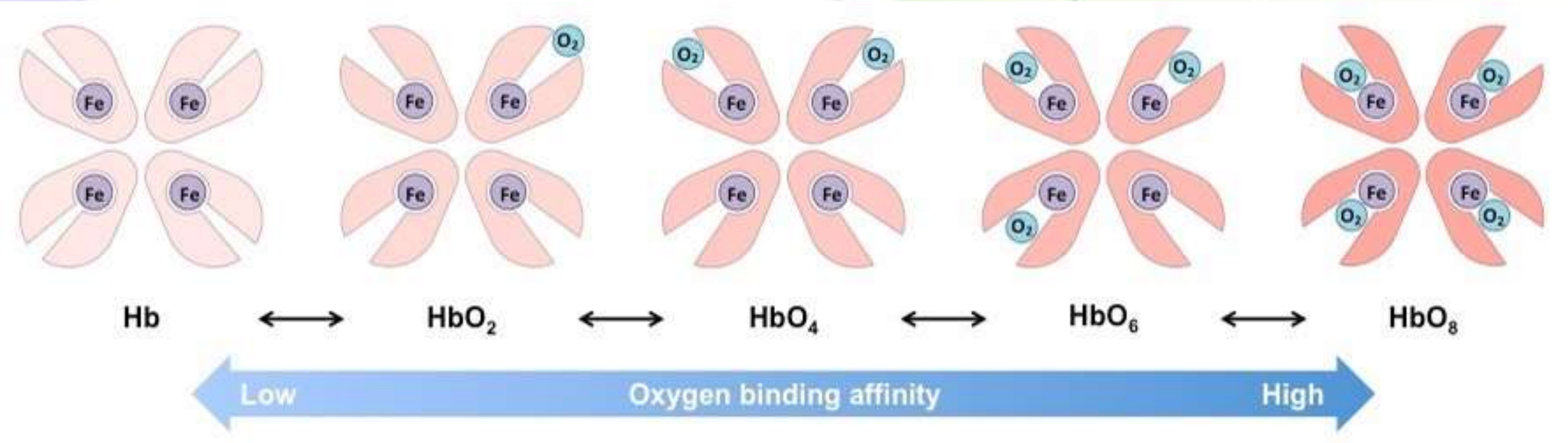


Figure 7.10
Biochemistry, Seventh Edition

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)**.

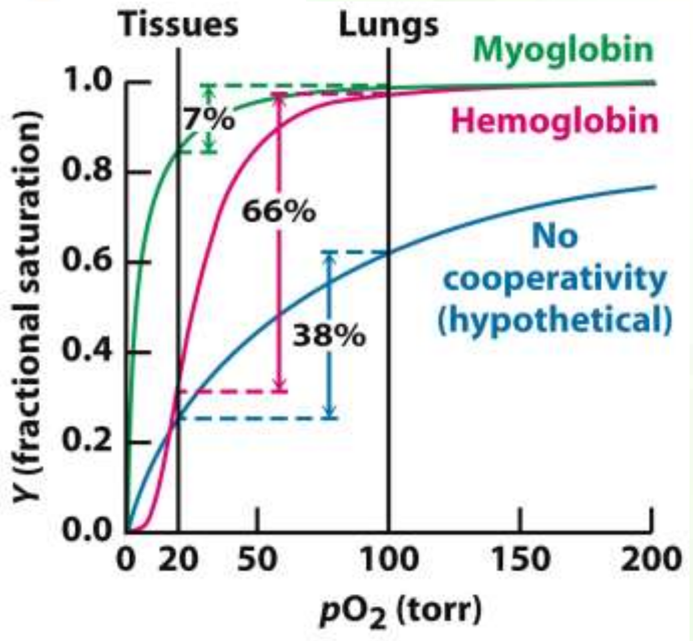
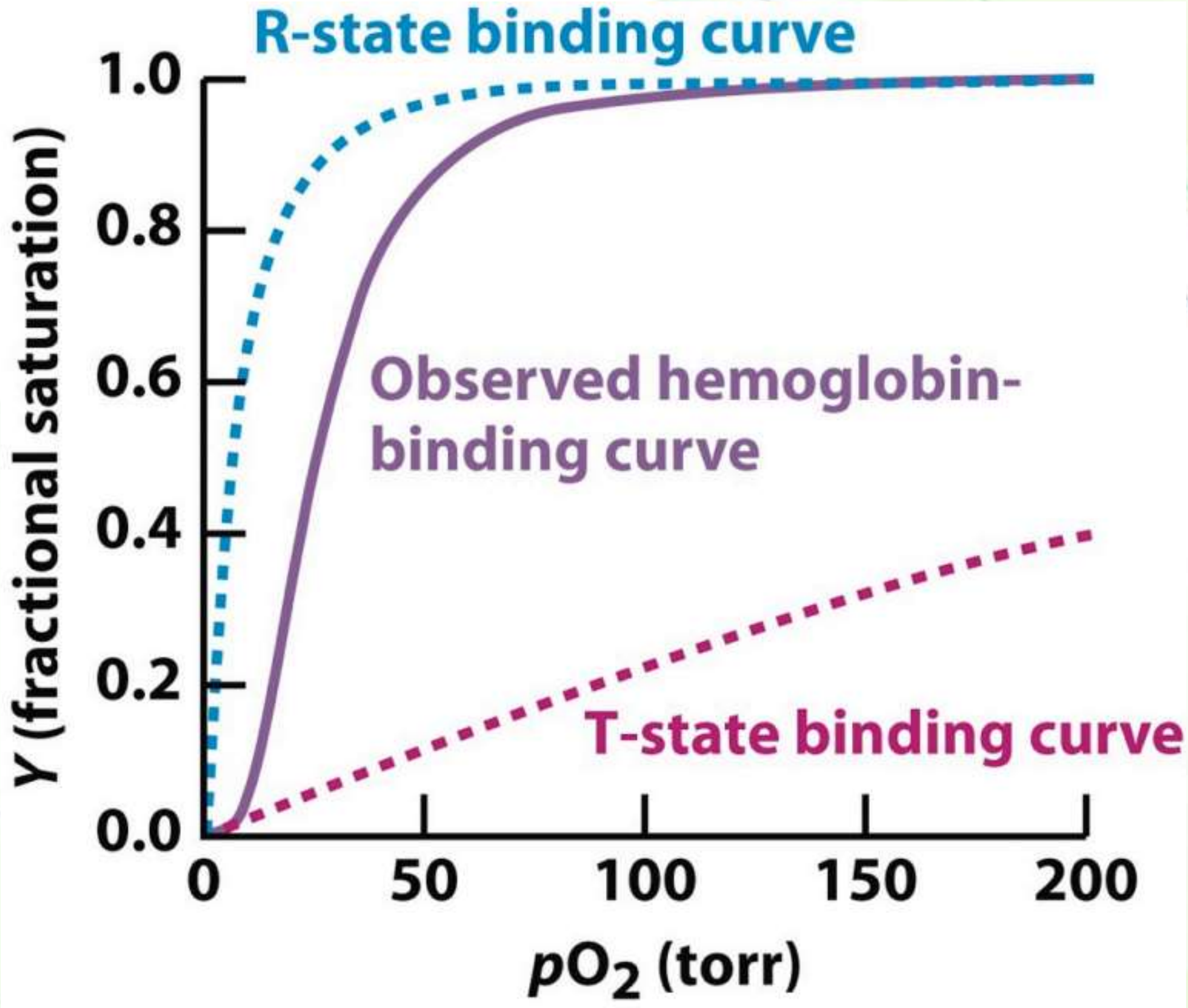


Figure 7.9
Biochemistry, Seventh Edition



R-state binding curve
Observed hemoglobin-binding curve
T-state binding curve
Y (fractional saturation)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
pO2 (torr)

The Hill constant (coefficient)

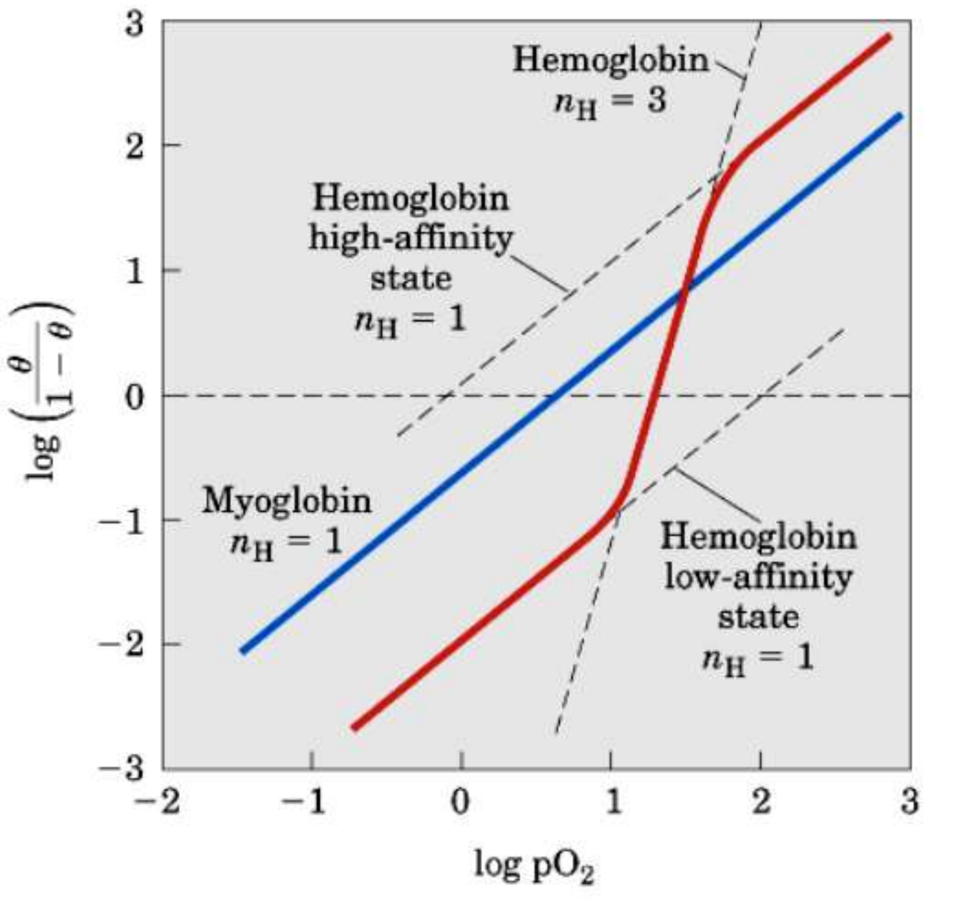
- The Hill plot is drawn based on an equation (you do not have to know it).
- n = Hill constant - determined graphically by the - hill plot
- n is the slope at midpoint of binding of log (Y/1-Y) vs log of pO2
  - if n = 1 then non cooperativity
  - if n < 1 then negative cooperativity
  - if n >1 then 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

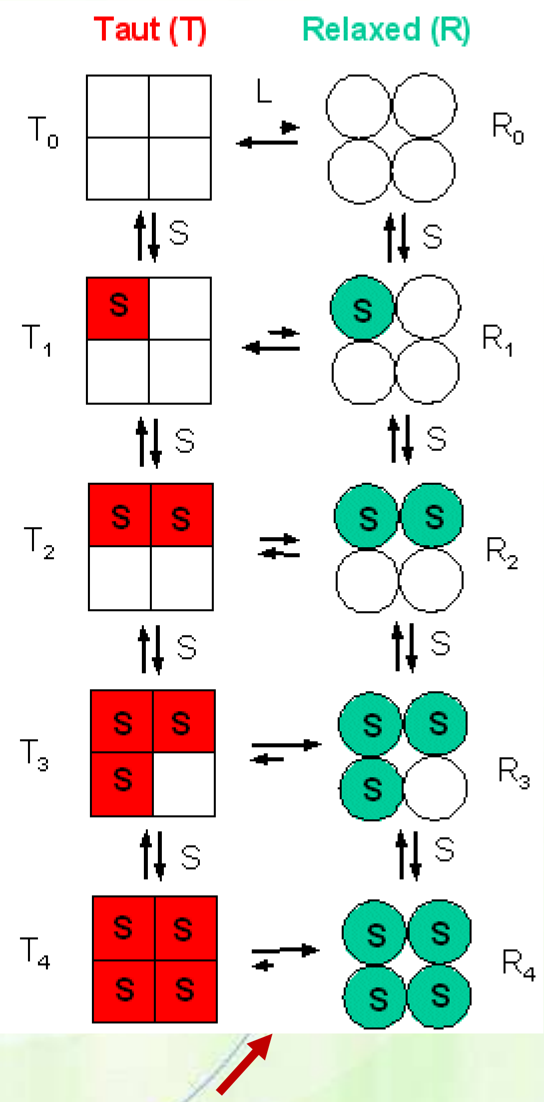
- Two models of cooperativity that could explain the observed data

  (1) Concerted model – all subunits undergo the conformational change simultaneously

  (2) Sequential model – subunit undergo the conformational change one at a time

The concerted model (MWC model)

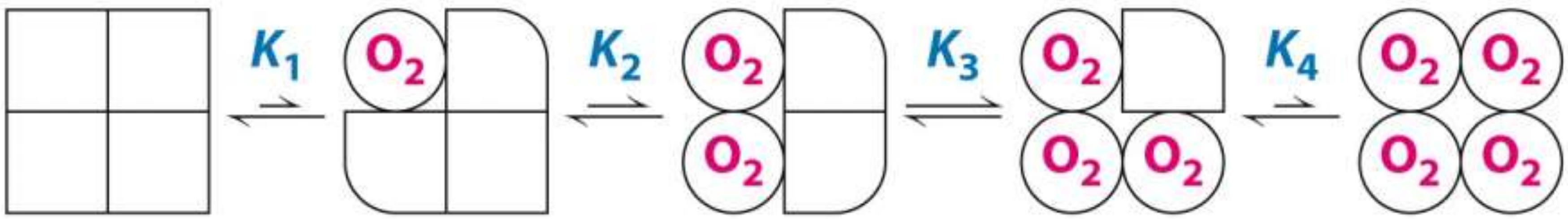
- The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.
- Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.
- This allows unoccupied subunits to adopt the high affinity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



Note direction of arrows

The sequential, induced fit, or KNF model

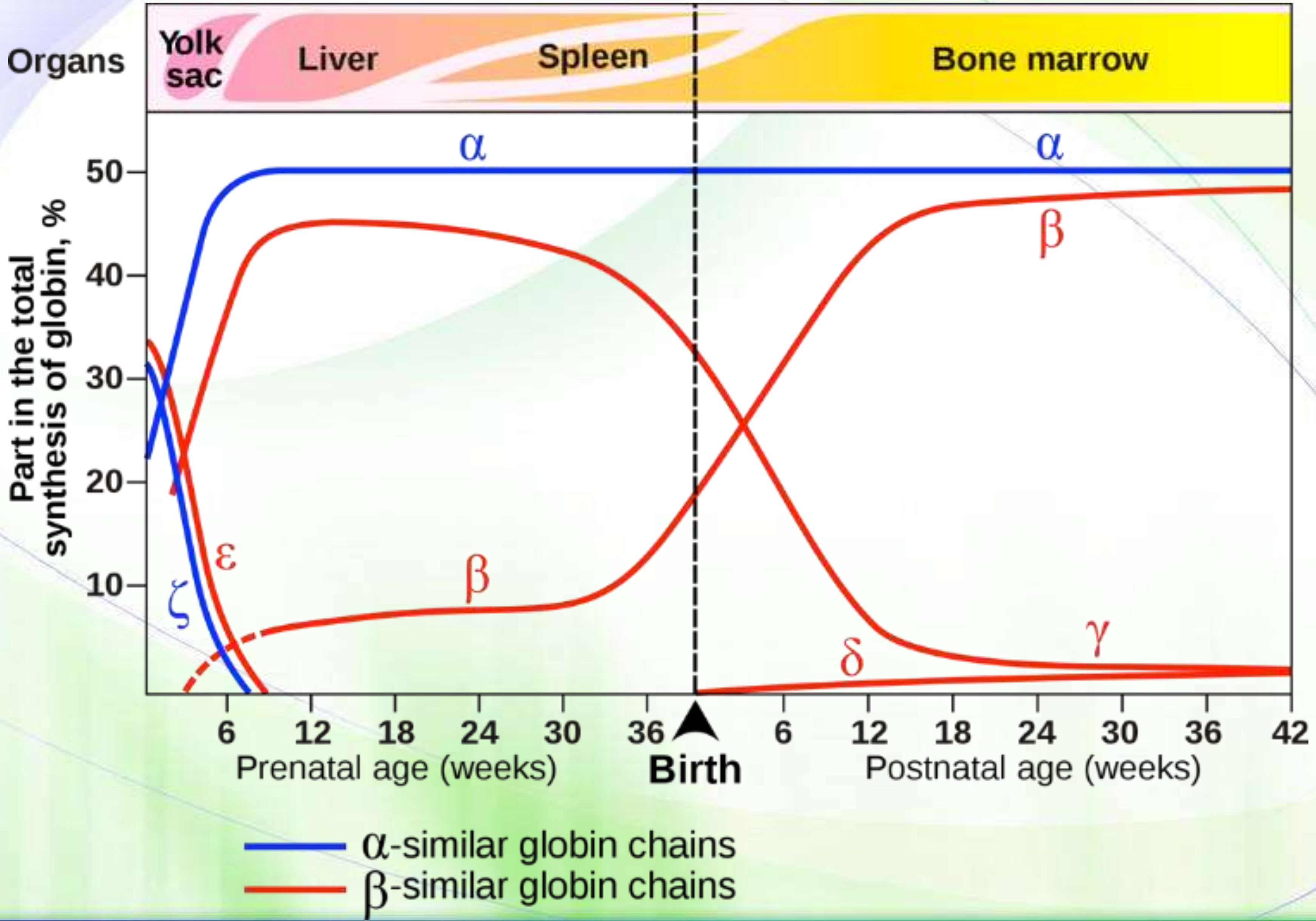
- The subunits go through conformational changes independently of each other, but they make the other subunits more likely to change, by reducing the energy needed for subsequent subunits to undergo the same conformational change.



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

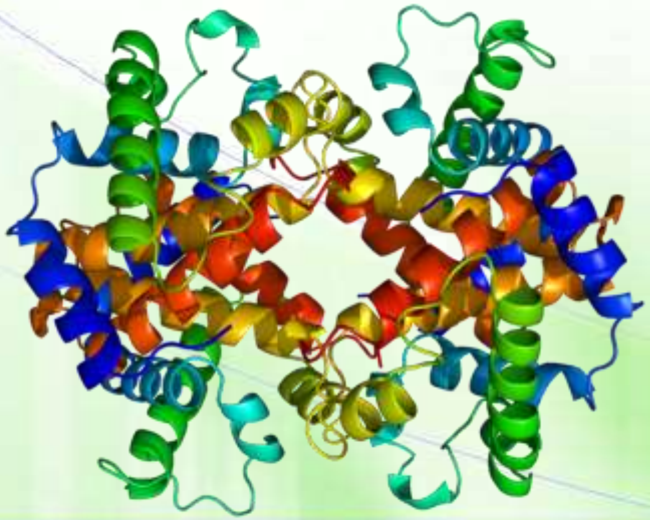
It is not only one hemoglobin

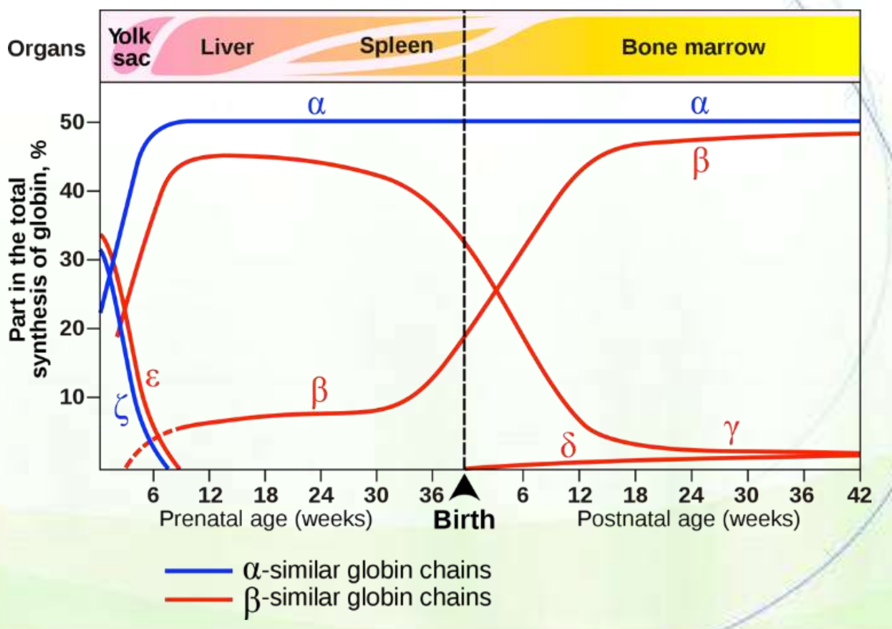
Developmental transition of hemoglobins



The embryonic stage

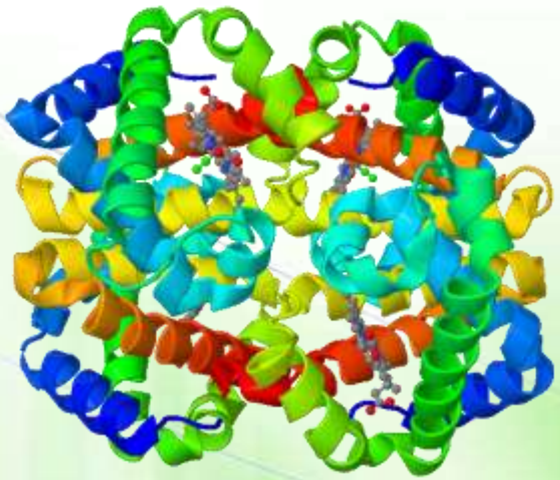
- Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac.
- The major hemoglobin (HbE Gower 1) is a tetramer composed of 2 zeta (ξ) chains and 2 epsilon (ε) chains
- Other forms exist: HbE Gower 2 (α2ε2), HbE Portland 1 (ζ2γ2), HbE Portland 2 (ζ2β2).

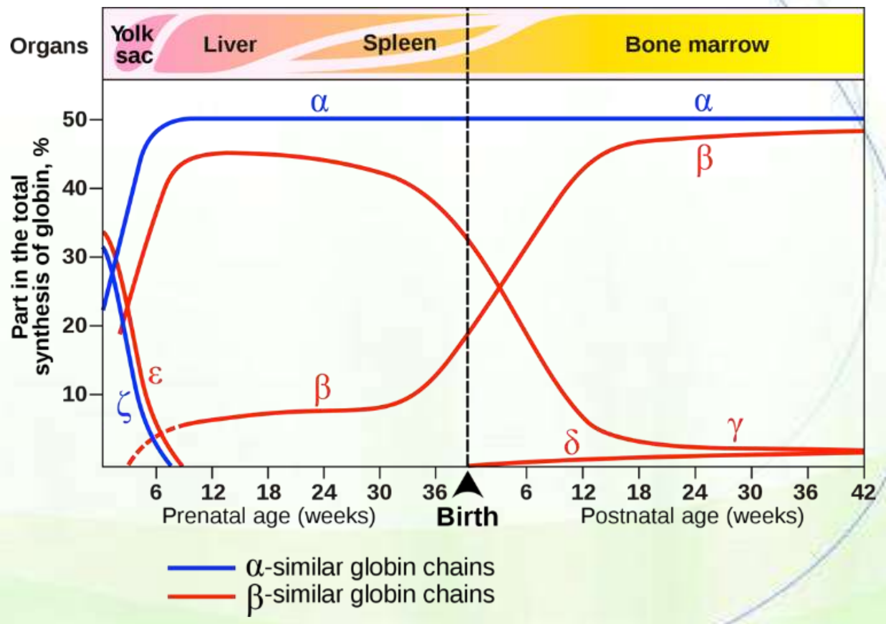




Beginning of fetal stage

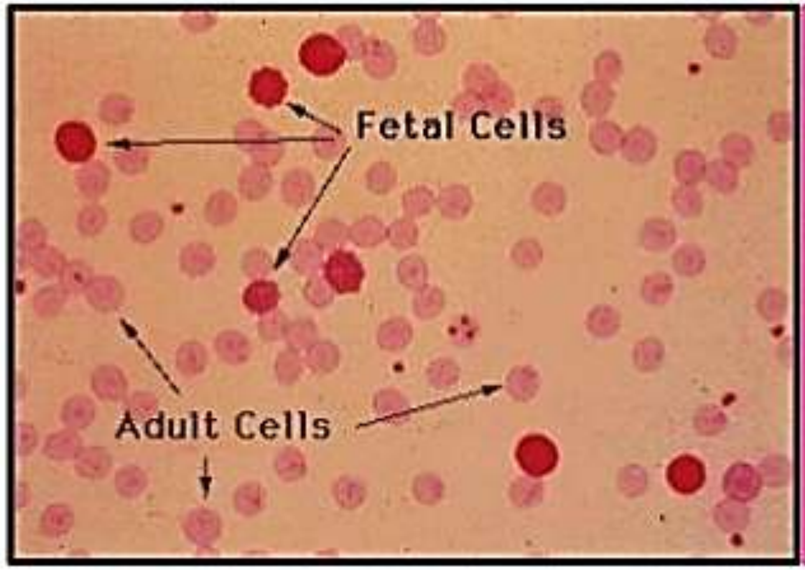
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines dramatically and fetal hemoglobin synthesis starts from the liver.
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides (α2γ2)
- The α polypeptides remain on throughout life.

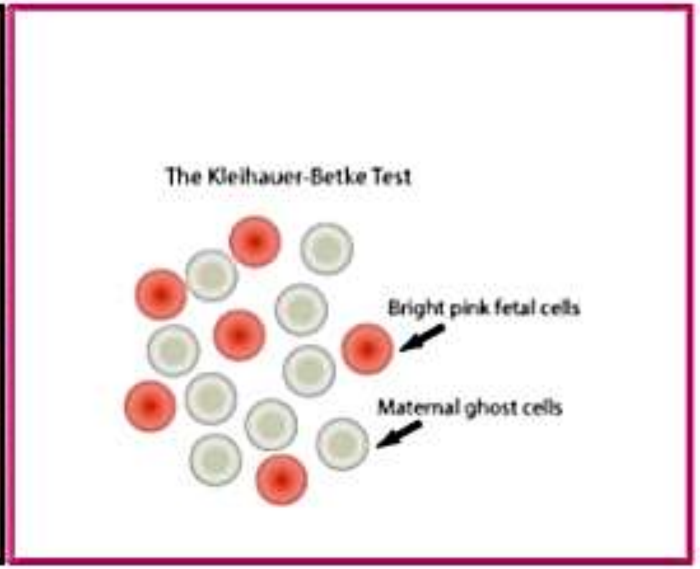


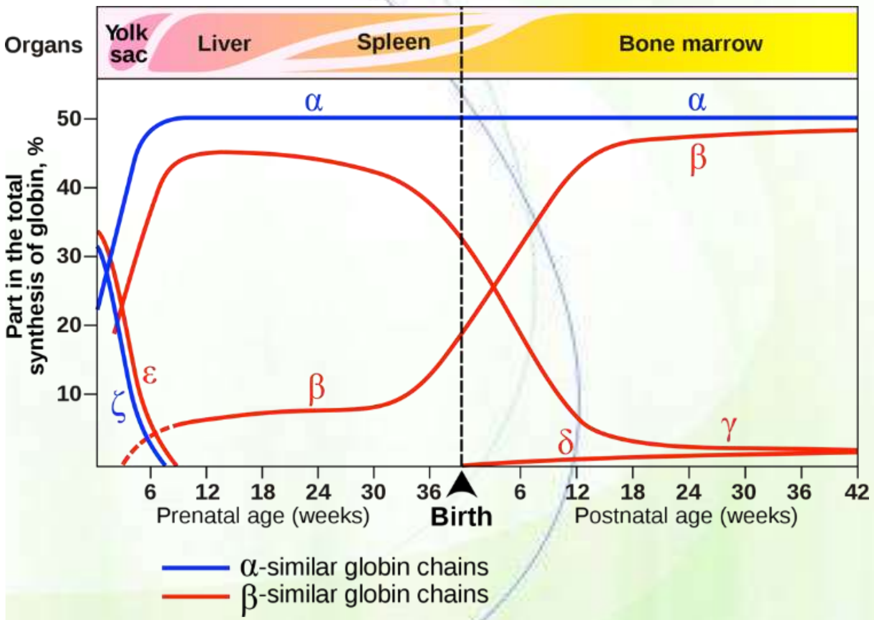


Beginning of adult stage

- 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.

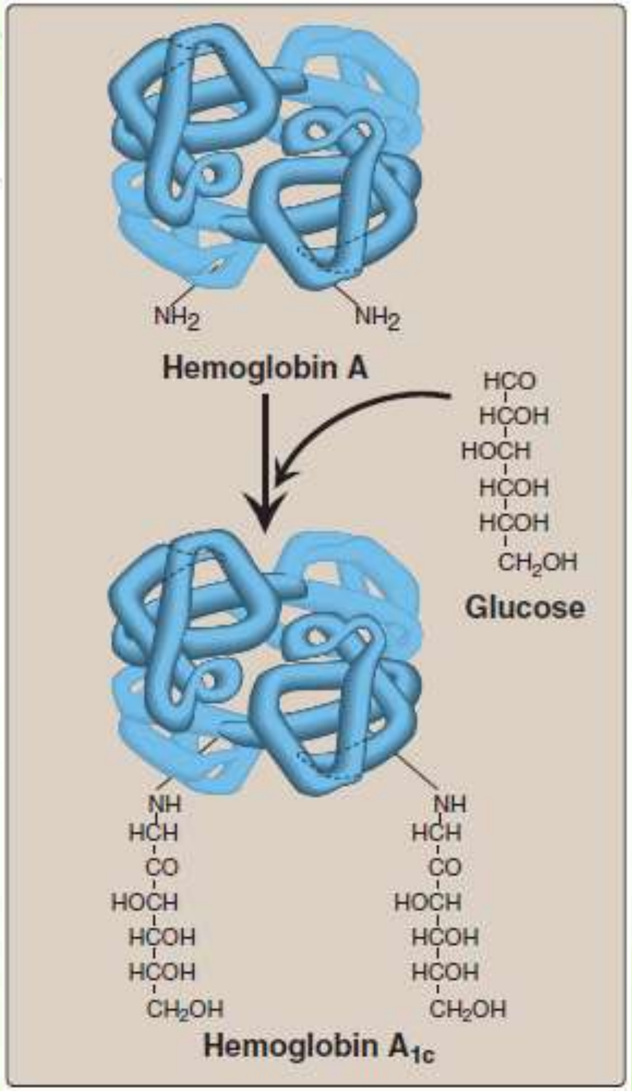






Adult hemoglobins

- 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.
- HbA can be glycosylated with a hexose and is designated as HbAc.
  - 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 your blood at a single point in time, i.e. the very moment of the test.
- **HbA1c** 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). ***IFCC is new and used in Europe***.
- Limitations of HbA1c test:
  - It does not capture short-term variations in blood glucose, exposure to hypoglycemia and hyperglycemia, or the impact of blood glucose variations on individuals' quality of life.

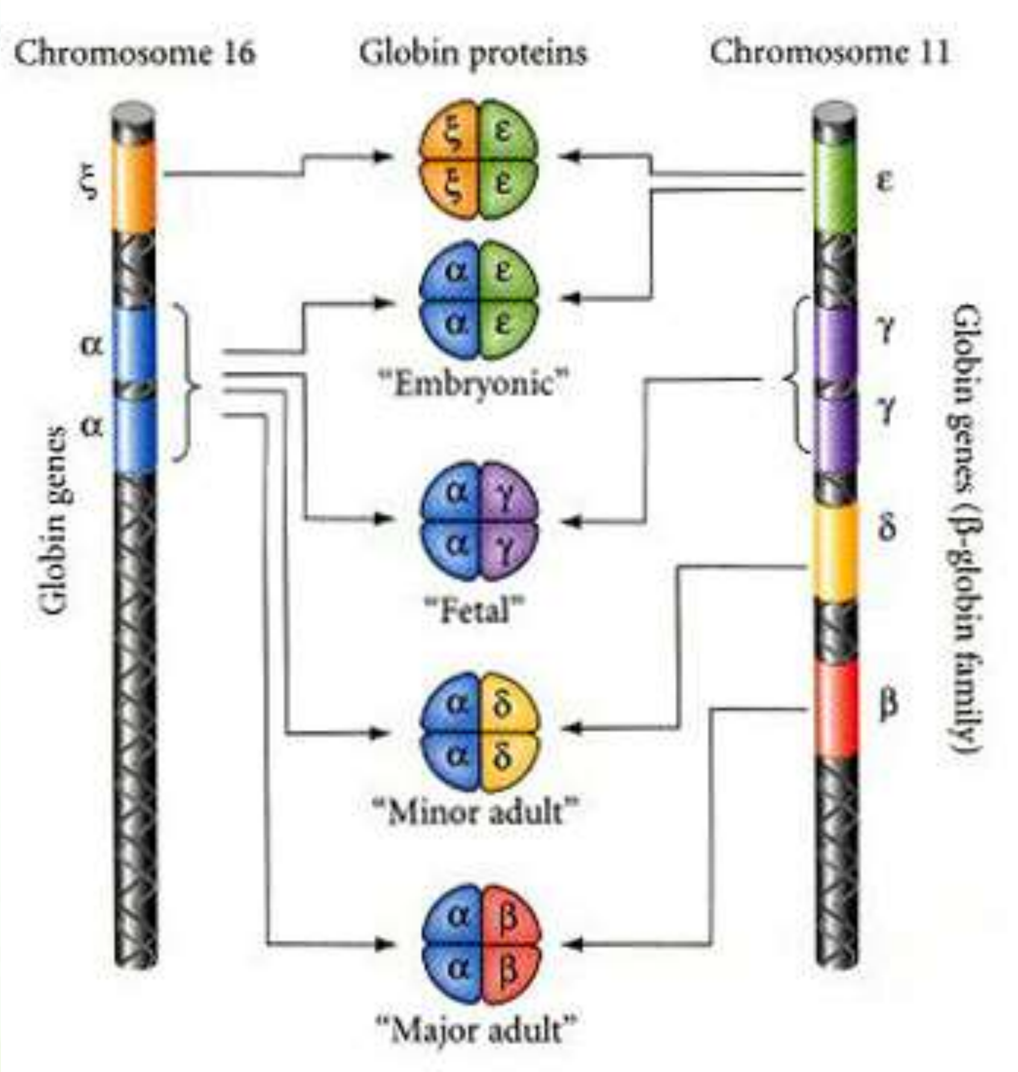
Table

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

Genetics of globin synthesis

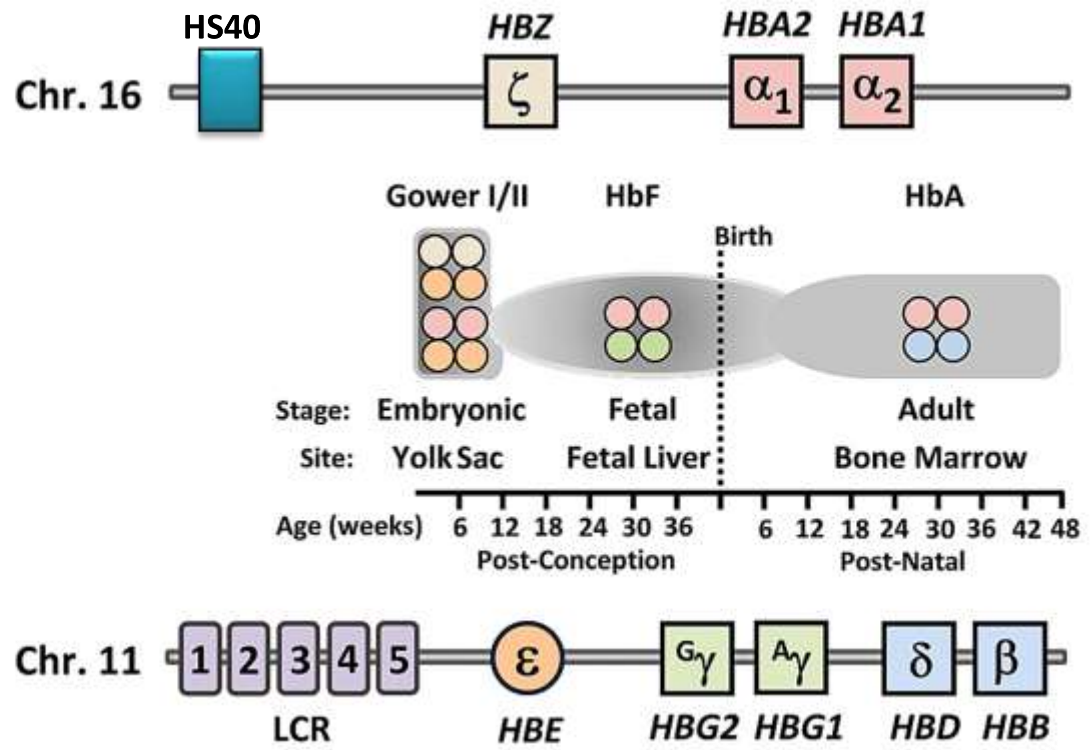
The genes

- The α gene cluster contains two α genes ($\alpha 1$ $\alpha 2$) and ζ (zeta) gene.
- The β gene cluster contains β gene in addition to ε (epsilon) gene, two γ (gamma) genes, and δ (delta) gene.
- The gene order parallels order of expression.
- 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 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), and chromatin looping.
- Note: treatment!!