Hemoglobin
An overview and more

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Hematopoietic-lymphatic system
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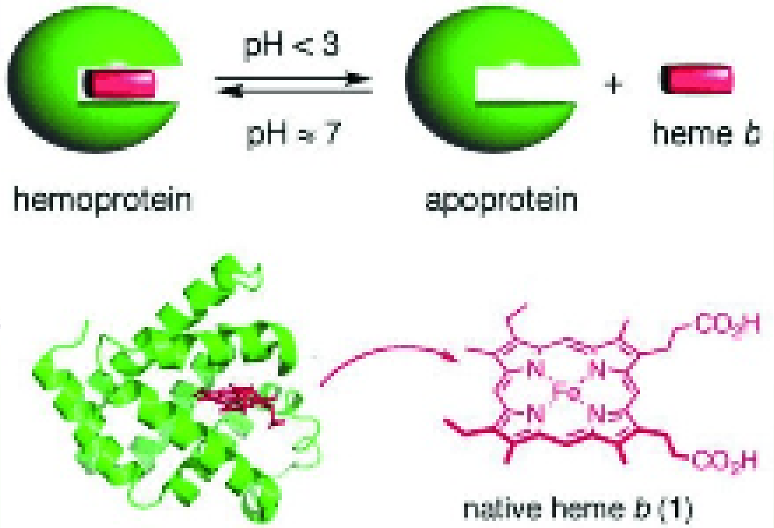
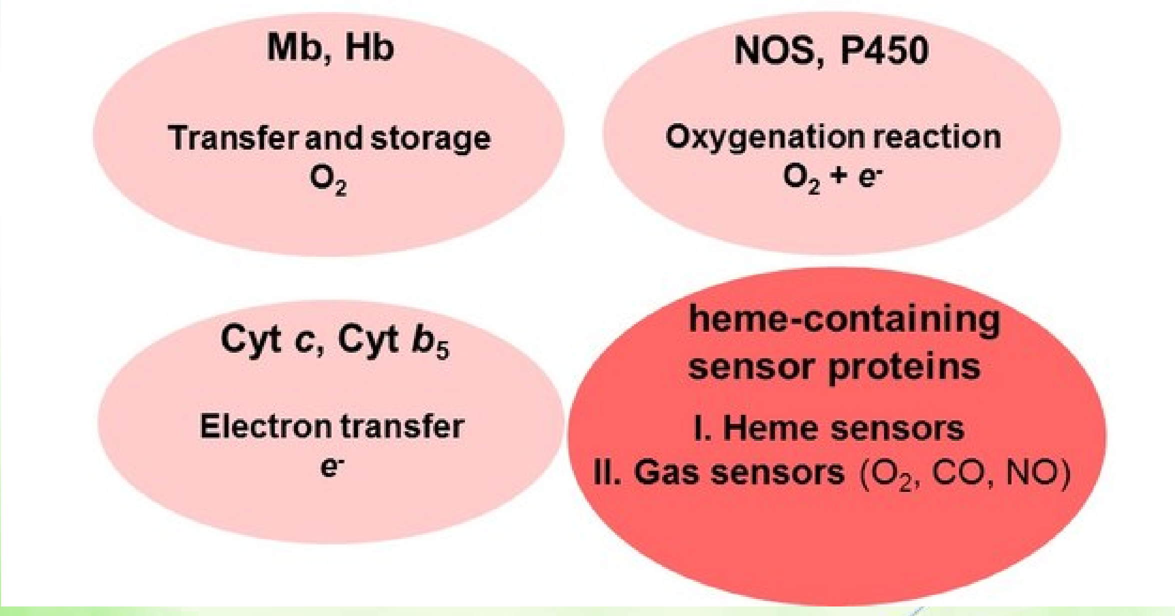
Hemoproteins

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

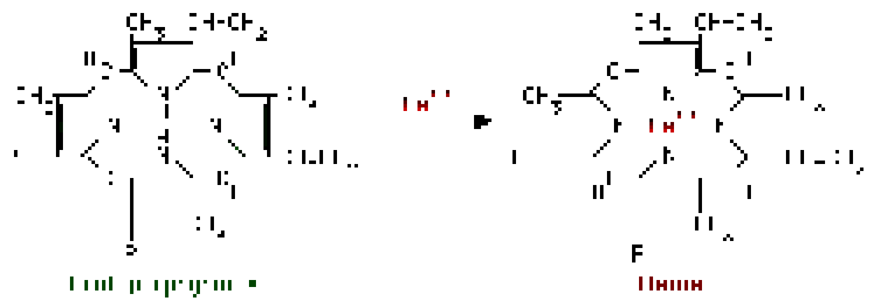


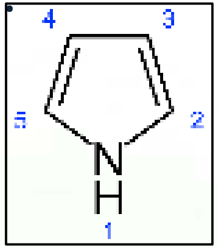
A ***prosthetic group*** *is a tightly bound, specific non-polypeptide unit required for the biological function of some proteins. The prosthetic group may be organic (such as a vitamin, sugar, or lipid) or inorganic (such as a metal ion), but is not composed of amino acids.*

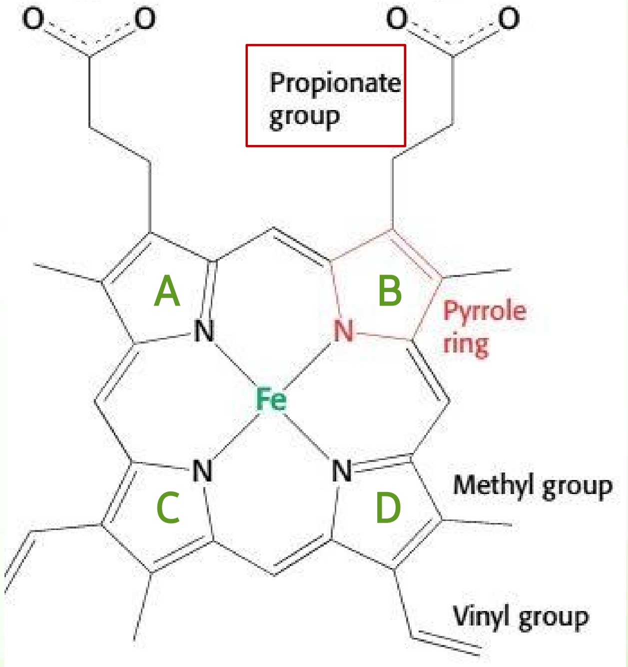


Heme structure

- 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.



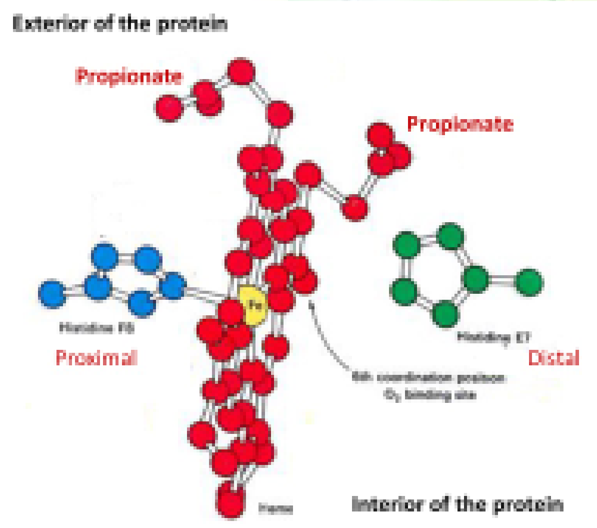


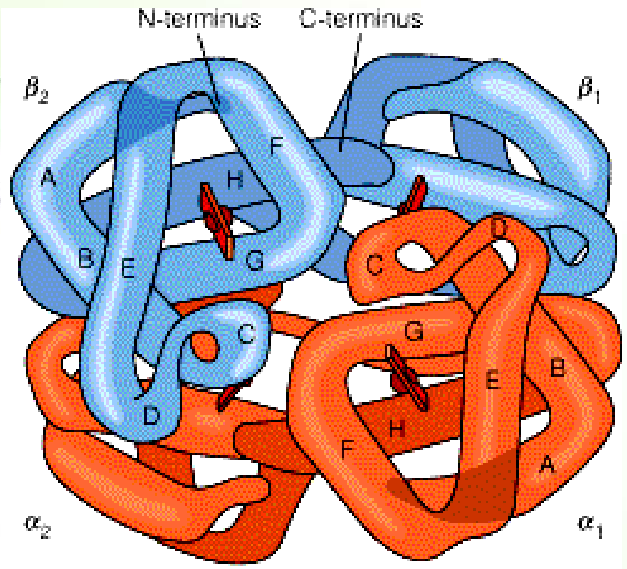


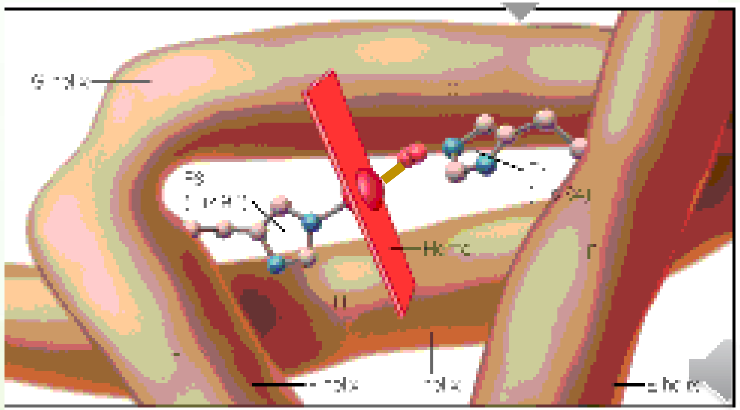
Heme
(Fe-protoporphyrin IX)

Structure of hemoglobin

- Hb is a globular protein.
  - Amino acid distribution
    - Positions of two histidine residu
      - Proximal and distal
- It is an allosteric protein.
  - Multiple subunits ($2\alpha + 2\beta$)
    - α polypeptide = 141 amino acids
    - β polypeptide = 146 amino acids
  - Altered structure depending on bound molecules
  - Positive cooperativity towards oxygen
  - Regulated by allosteric effectors

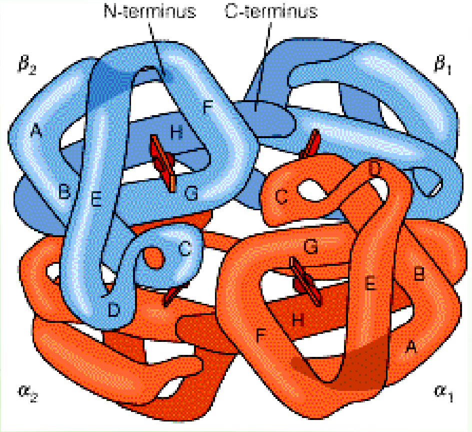


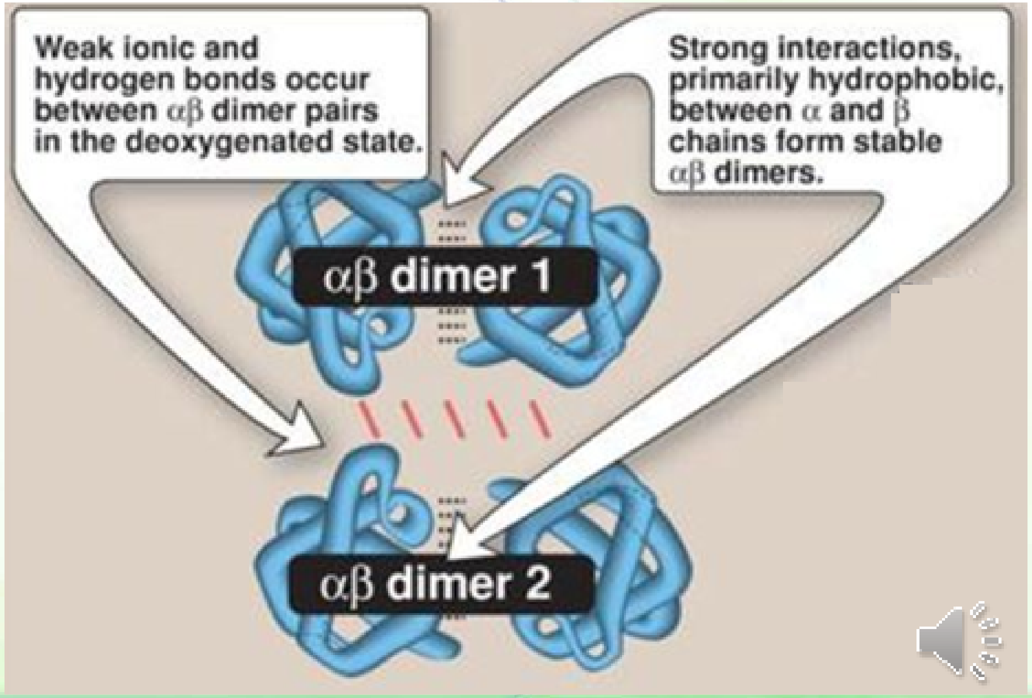




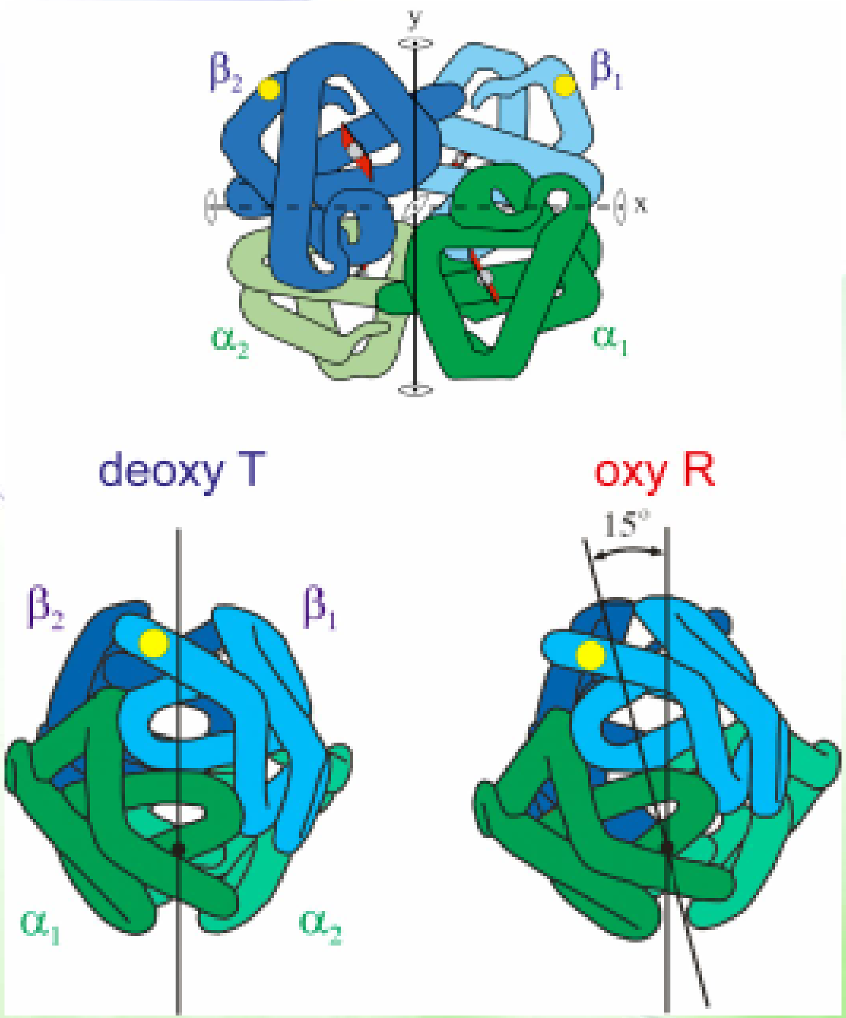
How are the subunits bound?

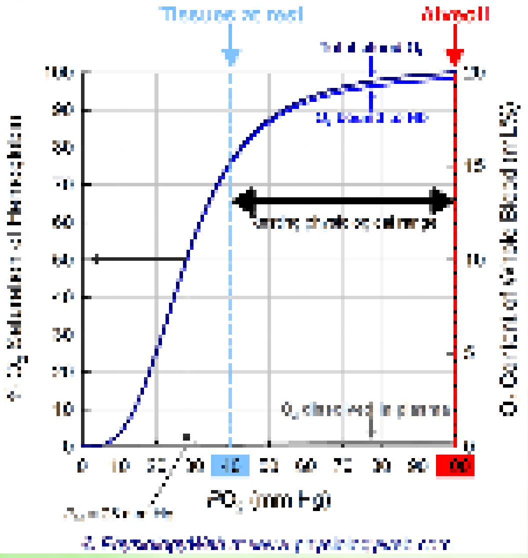
- A dimer of dimers (I made up this term)
  - $(\alpha\text{-}\beta)2$
  - Note how they interact with each other.

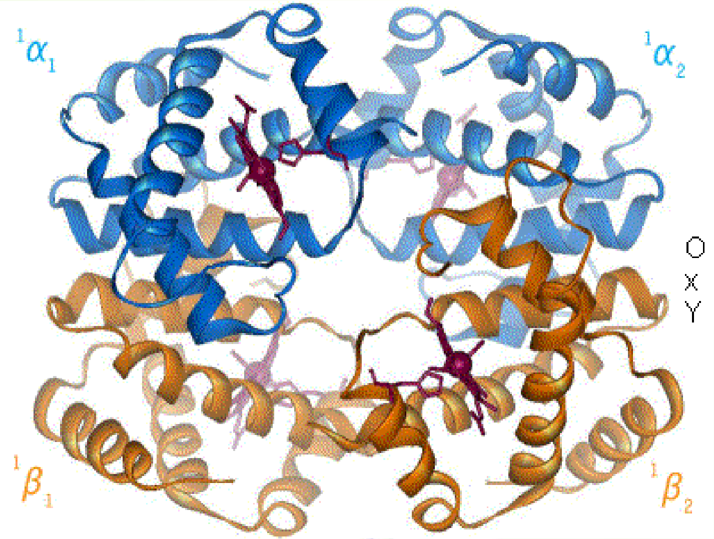




Structural change of hemoglobin

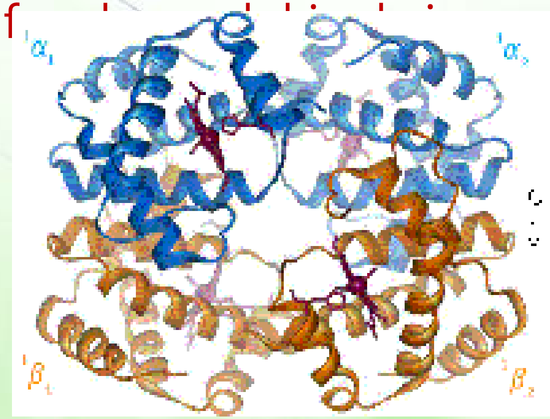


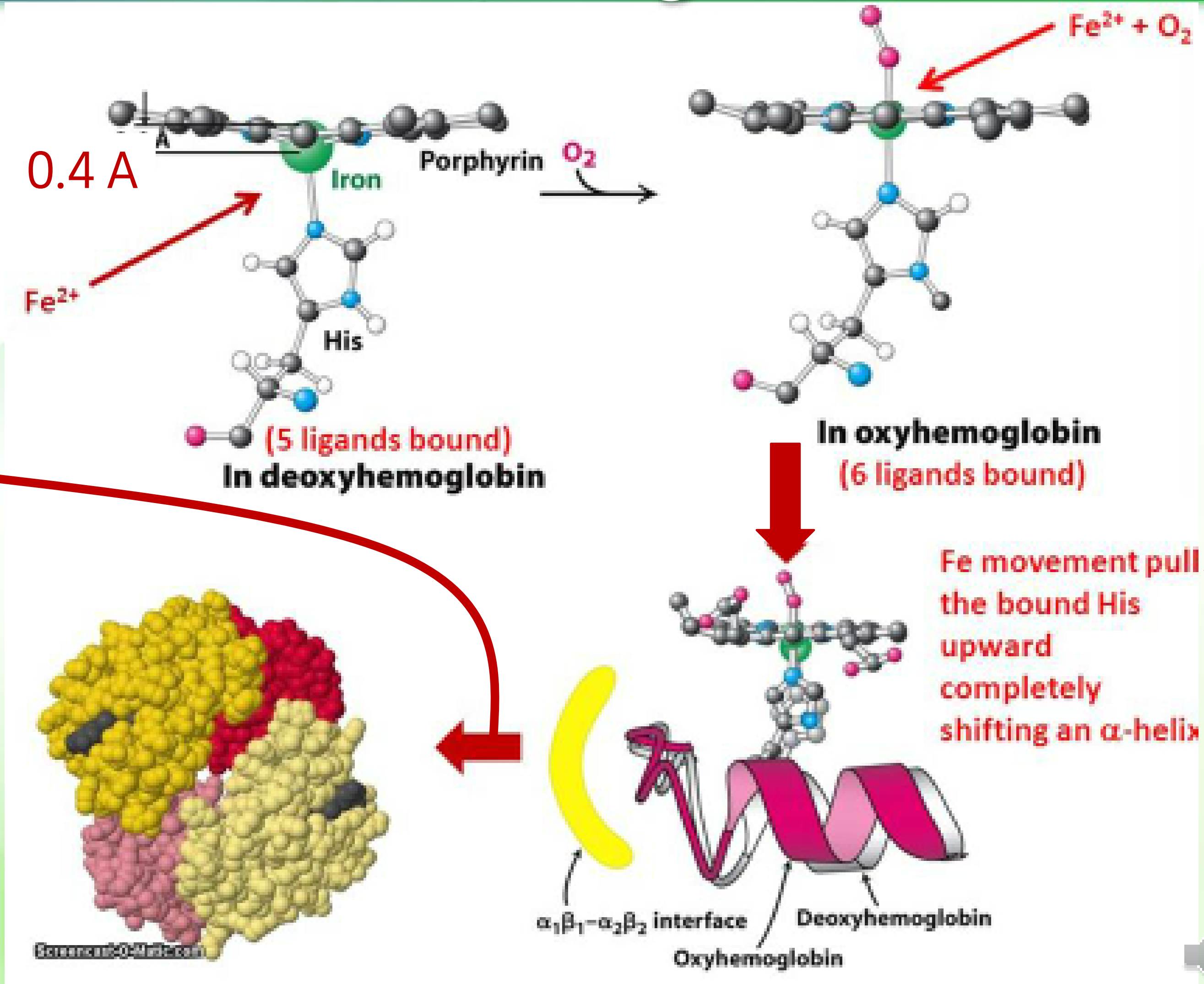




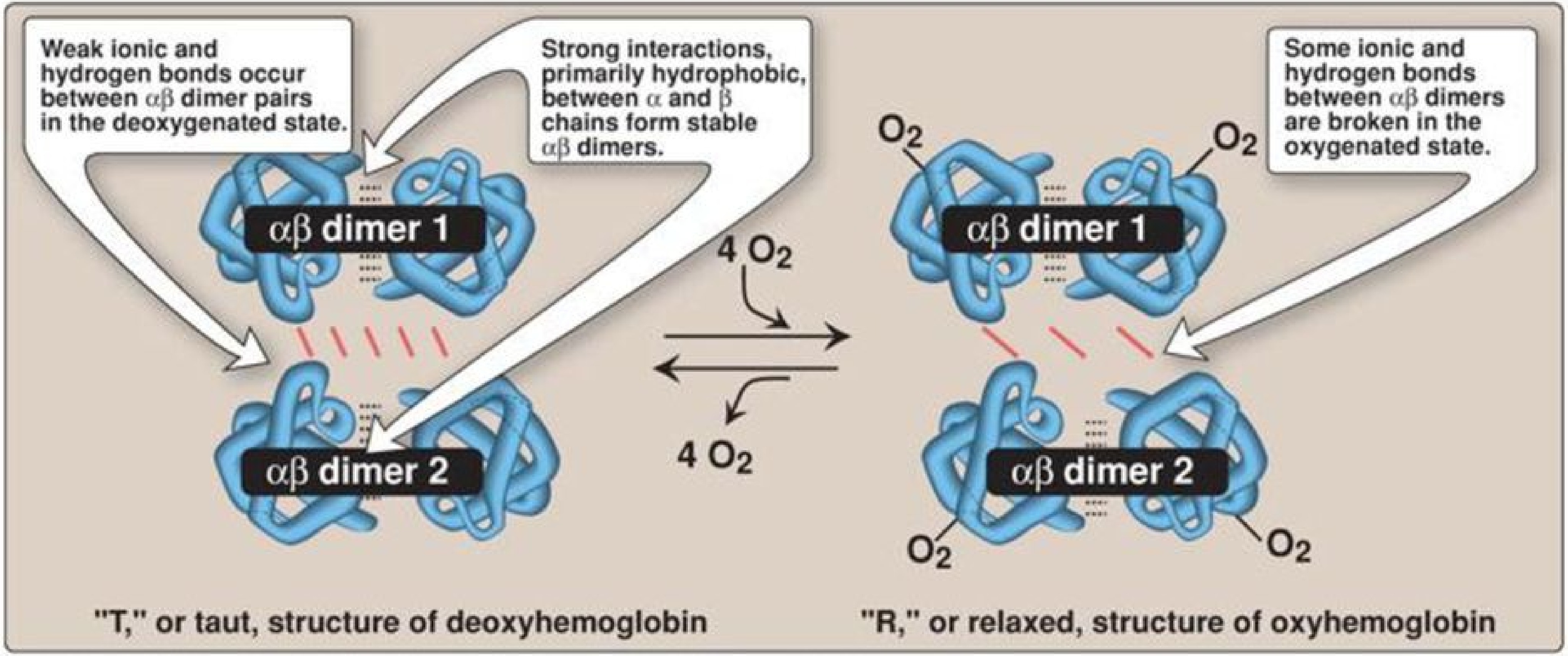
Structural amplification change

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



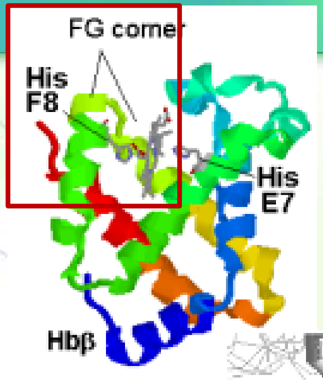


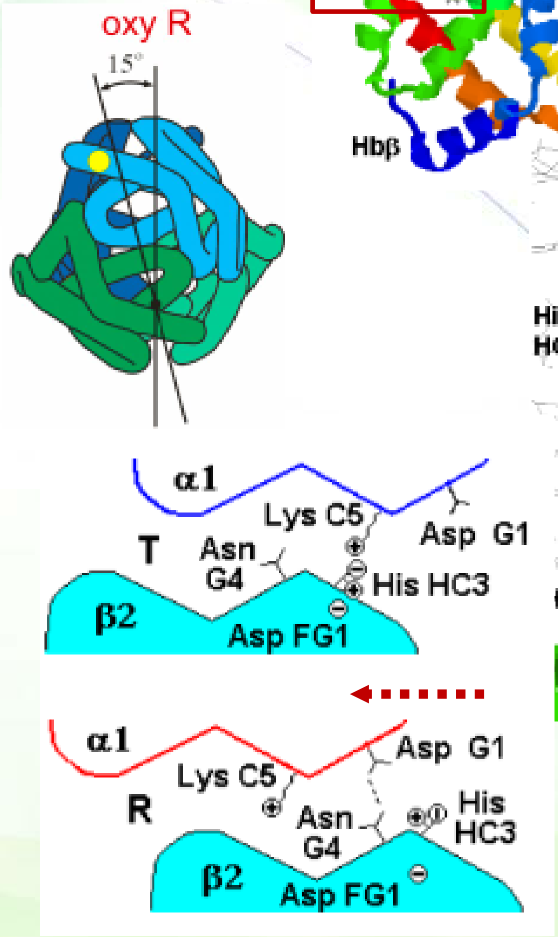
Electrostatic interactions are broken

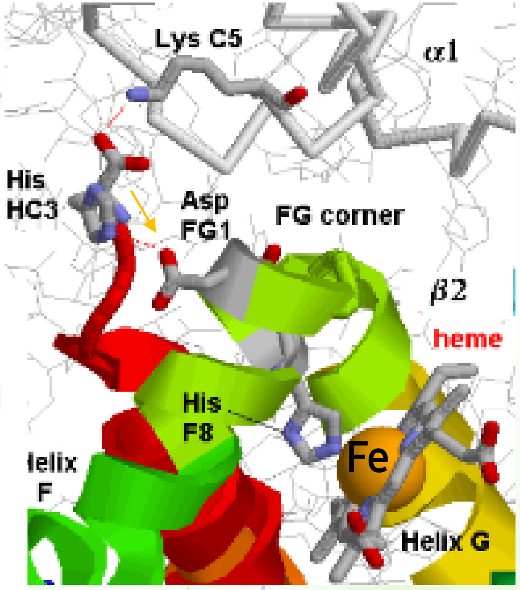


Let's zoom in…

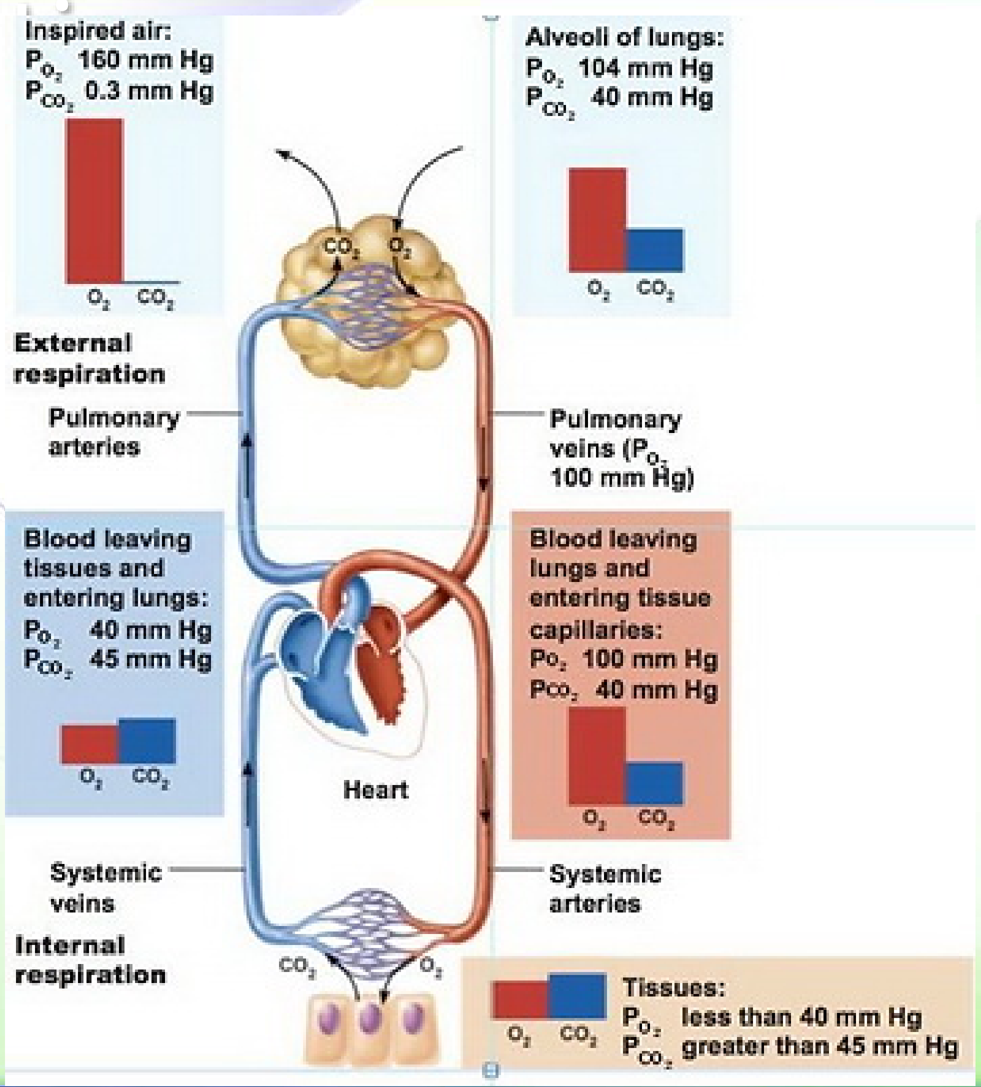
- In the T-state, The C-terminal His146 amino acid of the β-globin forms two ion pairs :
  - Its C-terminal carboxylate group ion-pairs to Lys C5 of the opposite α-globin, β2 to α1 or β1 to α2
  - its side chain ion pairs to the Asp FG1 of the same β-globin.
- When O_2 binds, the α1β1 globin rotates 15° relative to axis of deoxy α2β2, the α1 surface slides and His146 relocates and loses contact with Lys C5 and Asp FG1.
- A hydrogen bond is formed between Asp of α chin with Asn of β chain stabilizing the R form of hemoglobin.

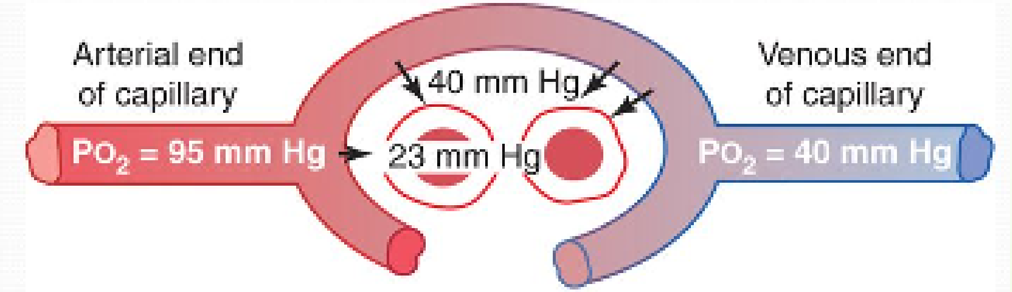






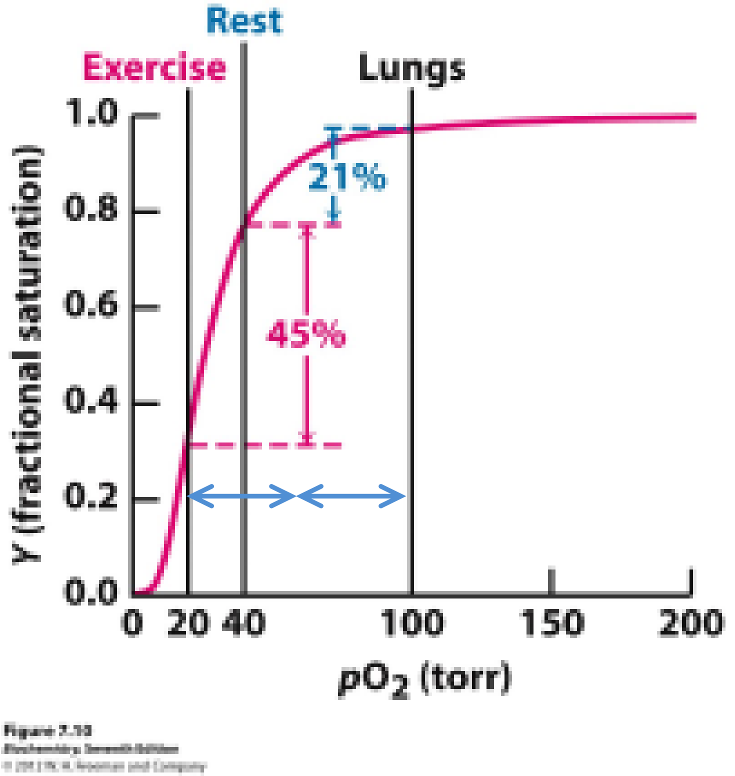
Oxygen distribution in blood versus





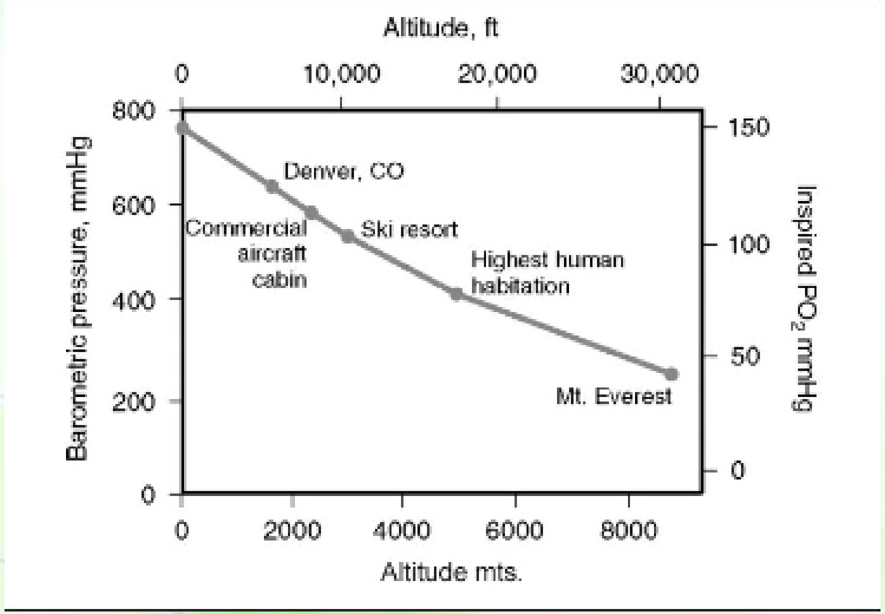
Oxygen saturation curve

- The saturation curve of hemoglobin binding to O_2 has a sigmoidal shape.
  - It is allosteric.
- At 100 mm Hg, hemoglobin is 95-98% saturated (oxyhemoglobin).
- As the oxygen pressure falls, oxygen is released to the cells.
- *Note: at high altitude (~5000 m), alveolar pO2 = 75 mmHg.*

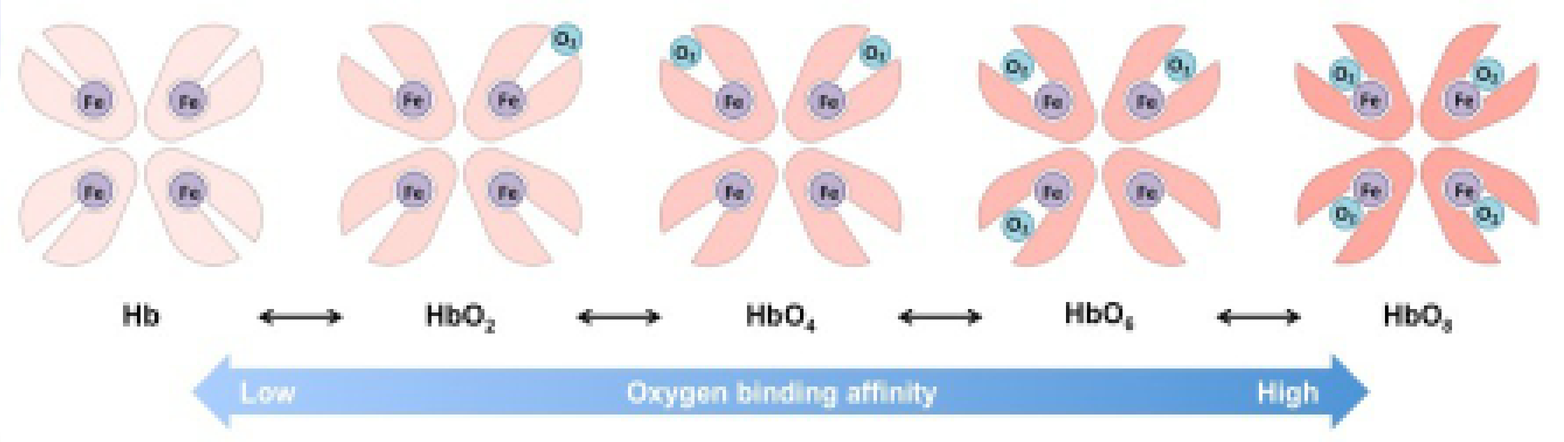


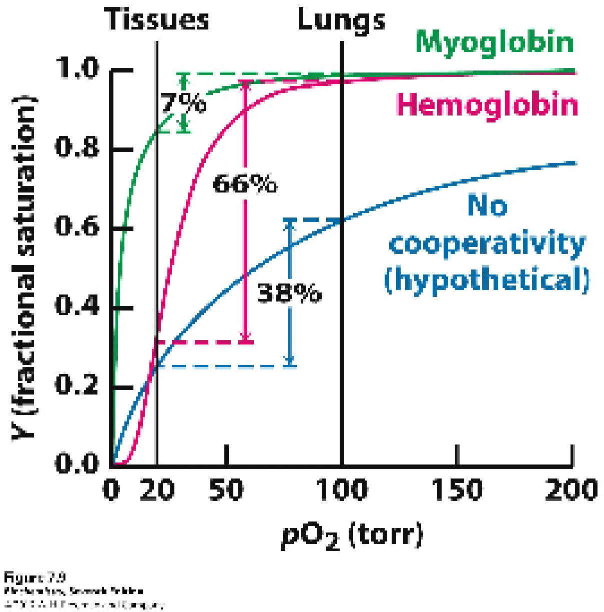
pO_2 at different altitudes

Altitude (feet)	Atmospheric Pressure (mm/Hg)	PAO_2 (mm/Hg)	PVO_2 (mm/Hg)	Pressure Differential (mm/Hg)	Blood Saturation (%)
Sea Level	760	100	40	60	98
10,000	523	60	31	29	87
18,000	380	38	26	12	72
22,000	321	30	22	8	60
25,000	282	7	4	3	9
35,000	179	0	0	0	0



Positive cooperativity



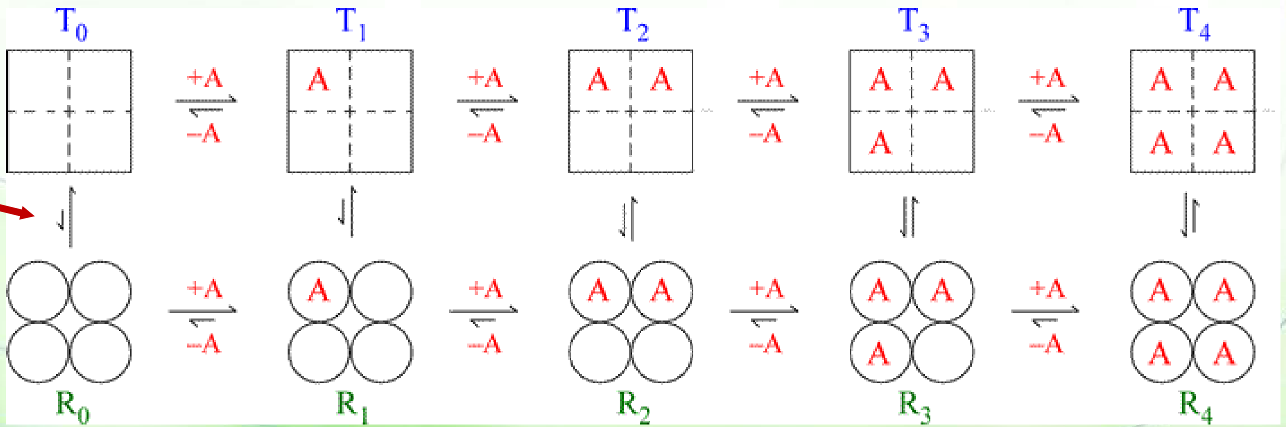


The concerted model (MWC model)

Most accurate

- The protein exists in two states in equilibrium: T (taut, tense) state with low affinity and R (relaxed) state with high affinity.
- 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 (to be discussed)**.

Note direction of arrows



The sequential, induced fit, or KNF model

Less accurate

- 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.
- Ligand binding may also result in negative cooperativity.
  - The MWC model only suggests only positive cooperativity.

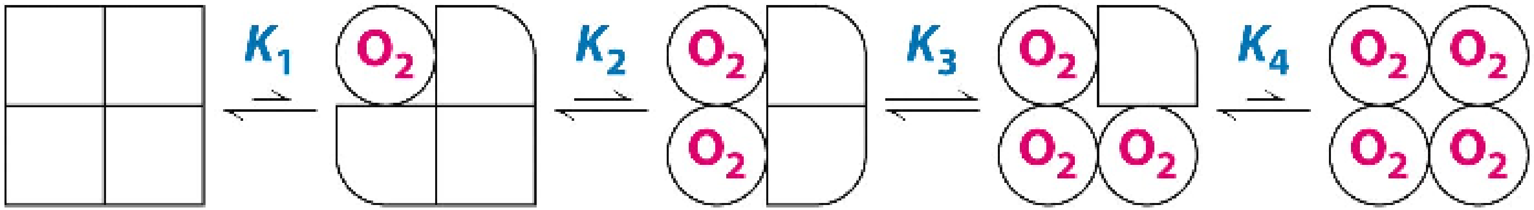
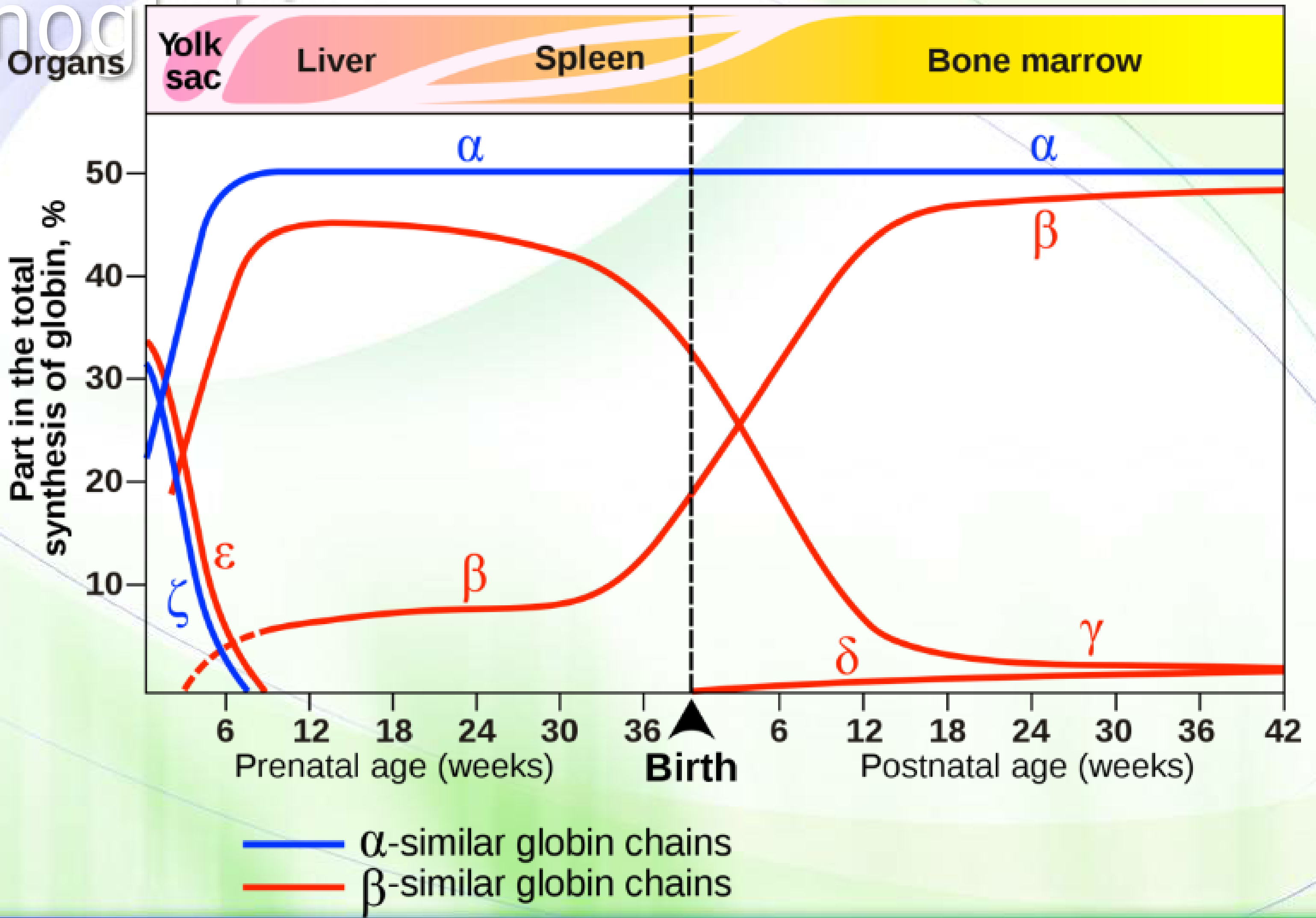


Figure 7.14
Biochemistry, **Seventh Edition**

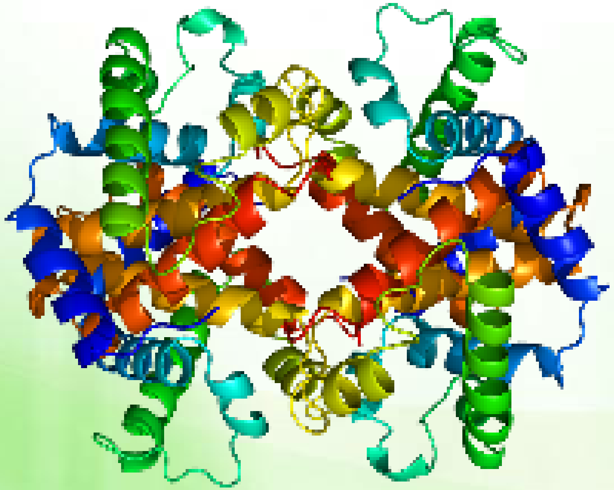
It is not only one hemoglobin

Developmental transition of hemoglobin



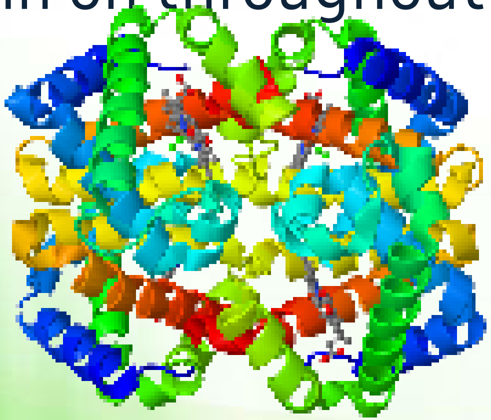
The embryonic stage

- 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 ($\alpha 2\varepsilon 2$), HbE Portland 1 ($\zeta 2\gamma 2$), HbE Portland 2 ($\zeta 2\beta 2$).



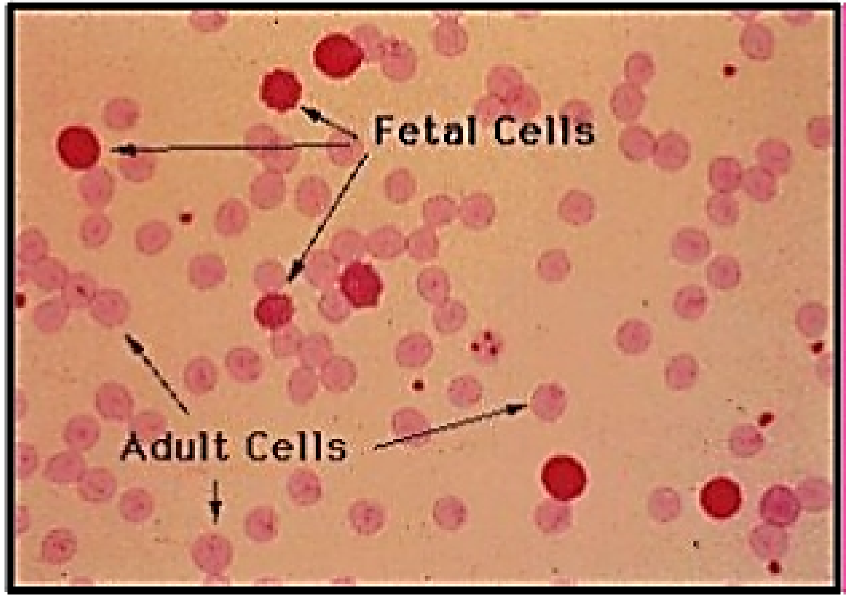
Beginning of fetal stage

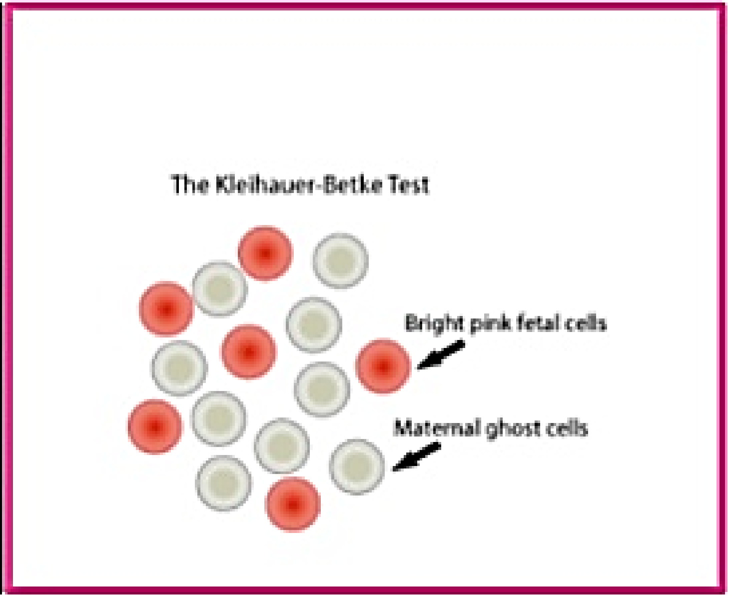
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines dramatically and fetal hemoglobin synthesis starts from the liver.
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides ($\alpha 2\gamma 2$)
- The α polypeptides remain on throughout life.



Beginning of adult stage

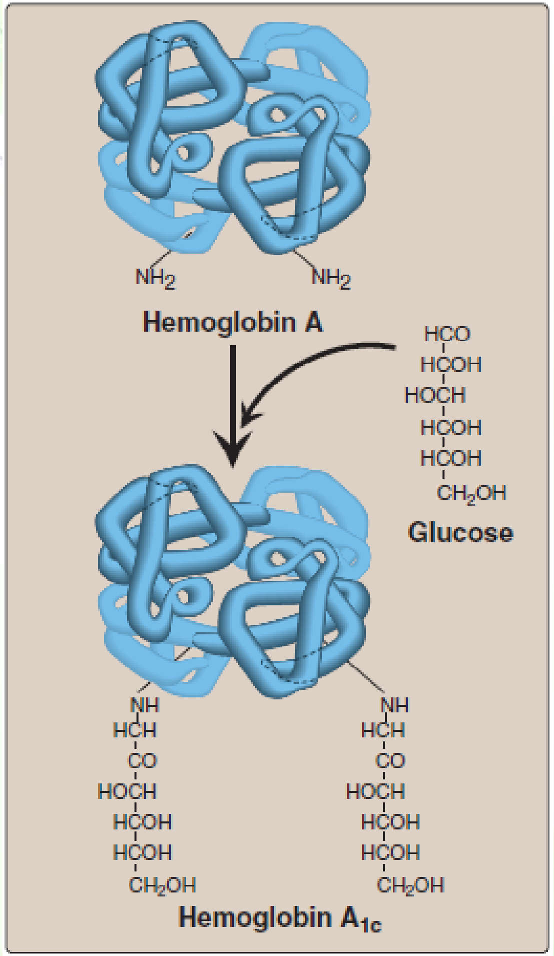
- Shortly before birth, there is a gradual switch to adult β-globin.
- Still, HbF makes up 60% of the hemoglobin at birth, but 1% of adults.
- At birth, synthesis of both γ and β chains occurs in the bone marrow.





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 your blood sugar levels have been over a period of time (2-3 months).
- HbA1c can be expressed as a percentage (DCCT unit) or as a value in mmol/mol (IFCC unit). *IFCC is new*.
- 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.

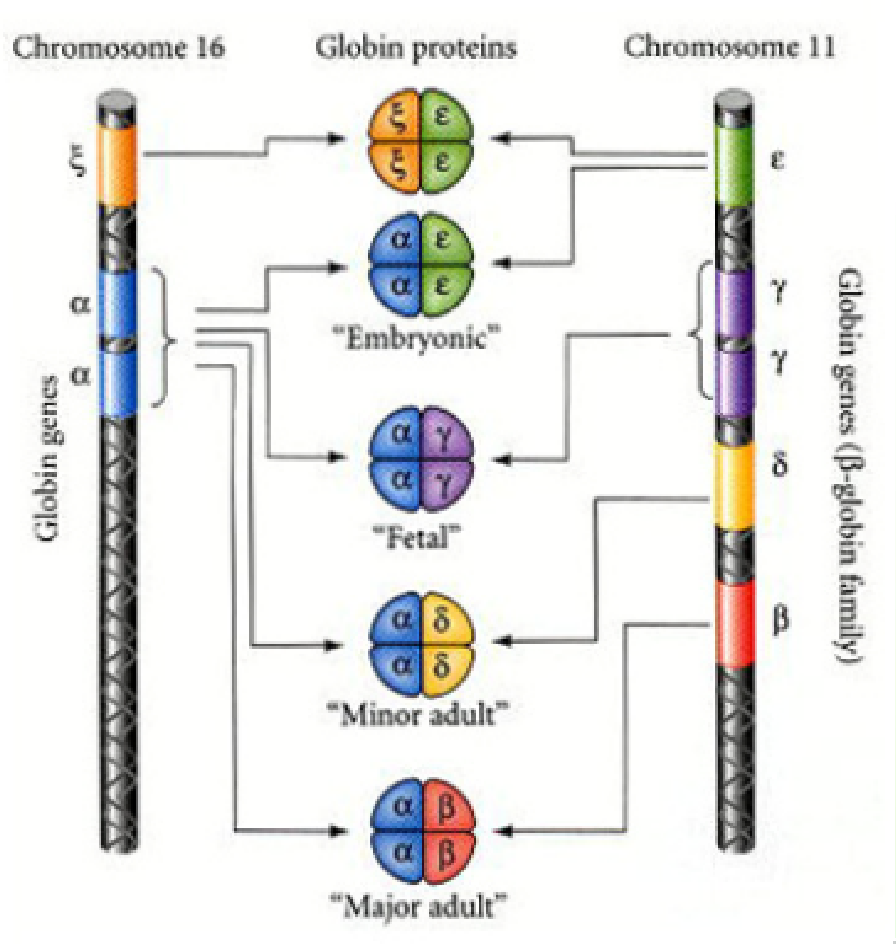
Table

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

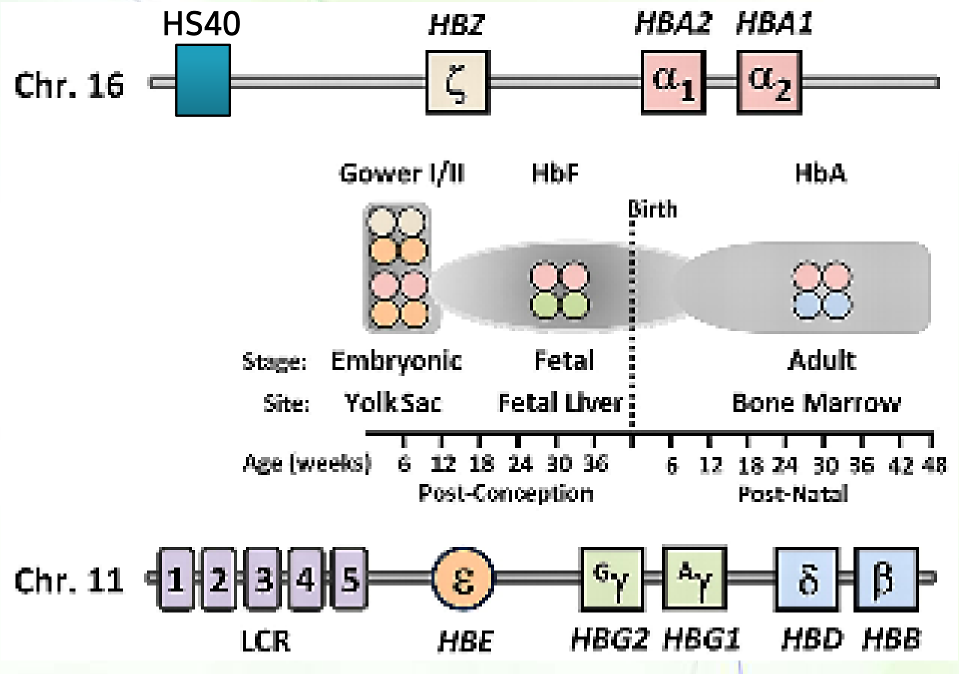
The genes

- The α gene cluster contains two α genes (α1 α2) 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!!