Hemoglobin
An overview and more

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Blood module
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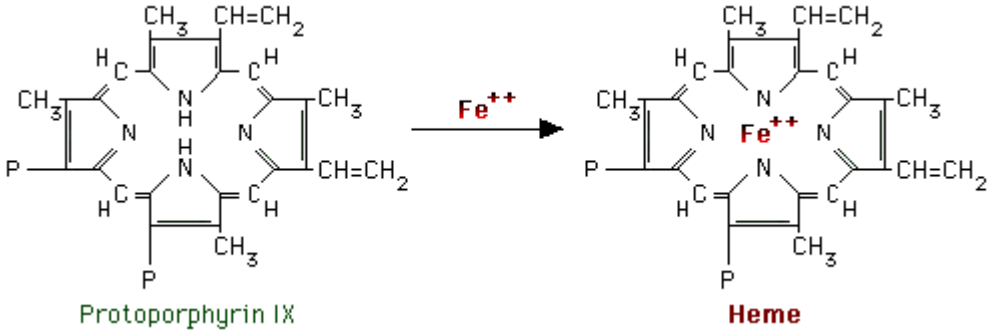
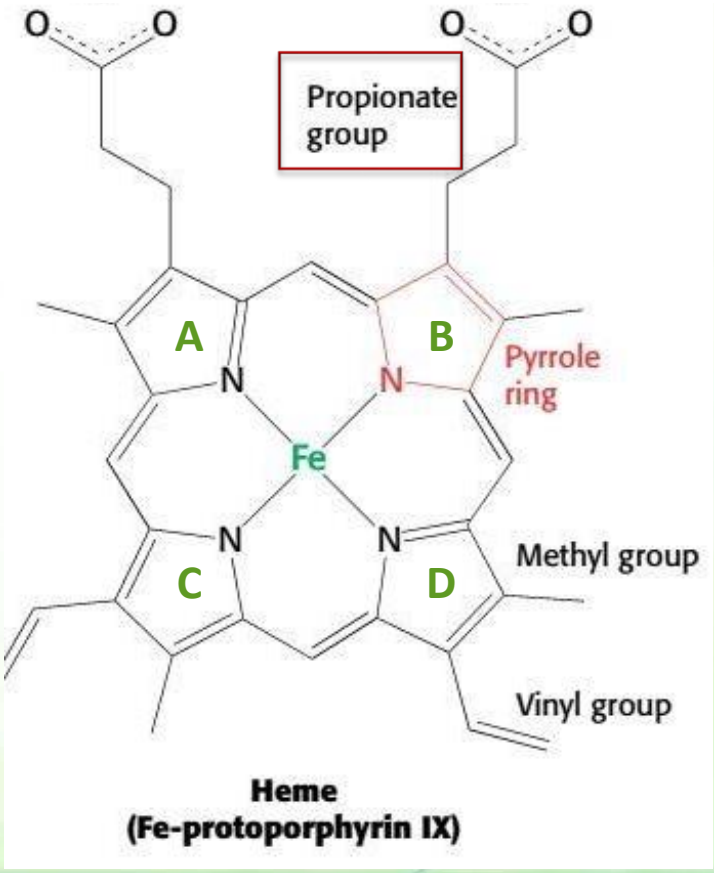
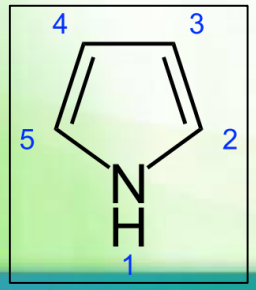
Heme structure

- 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.

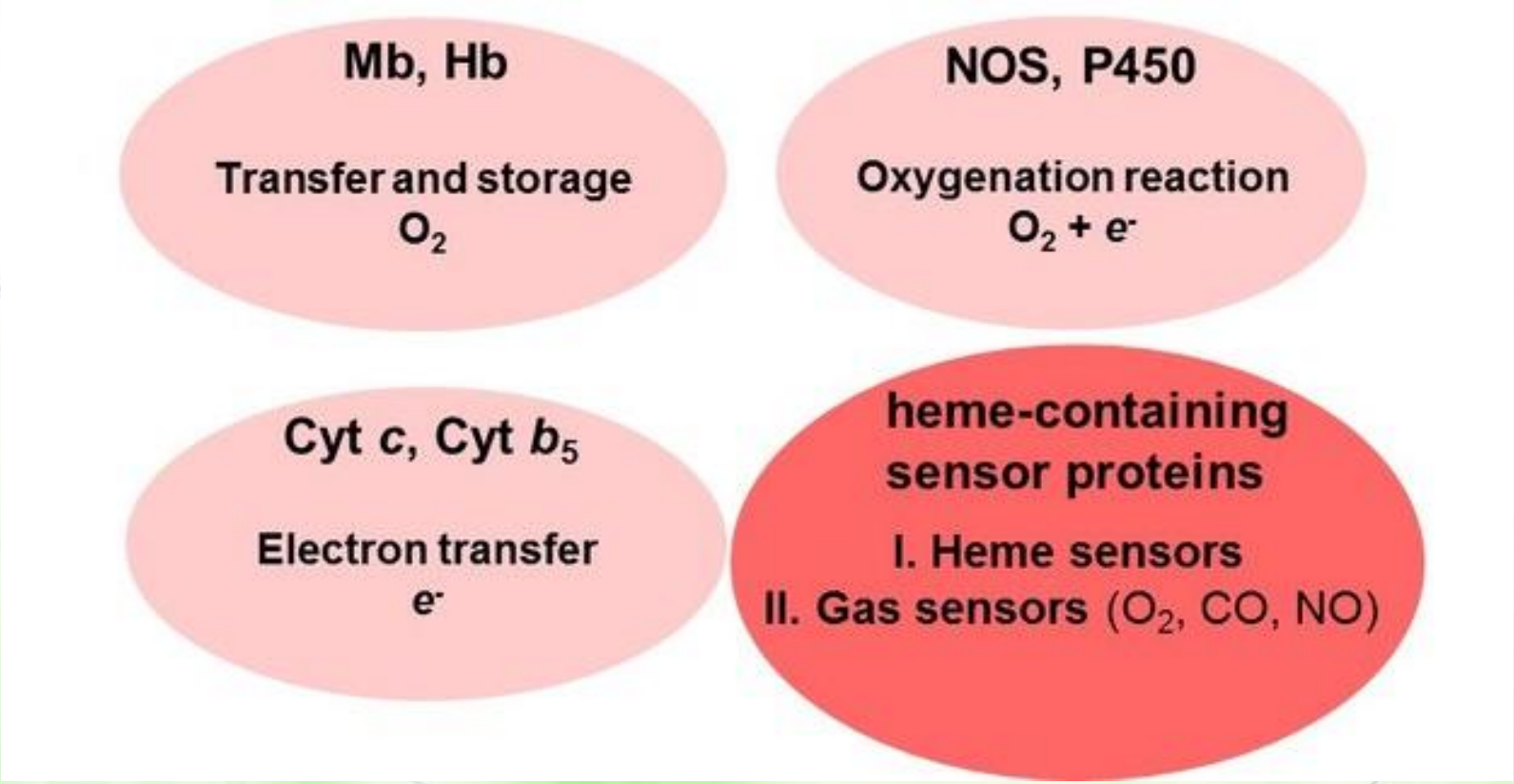






Hemoproteins

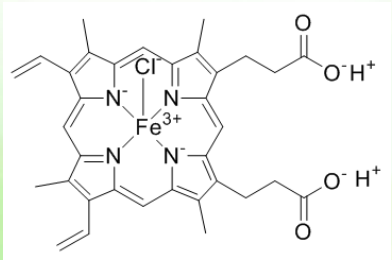
- Many proteins have heme as a prosthetic group called hemoproteins.

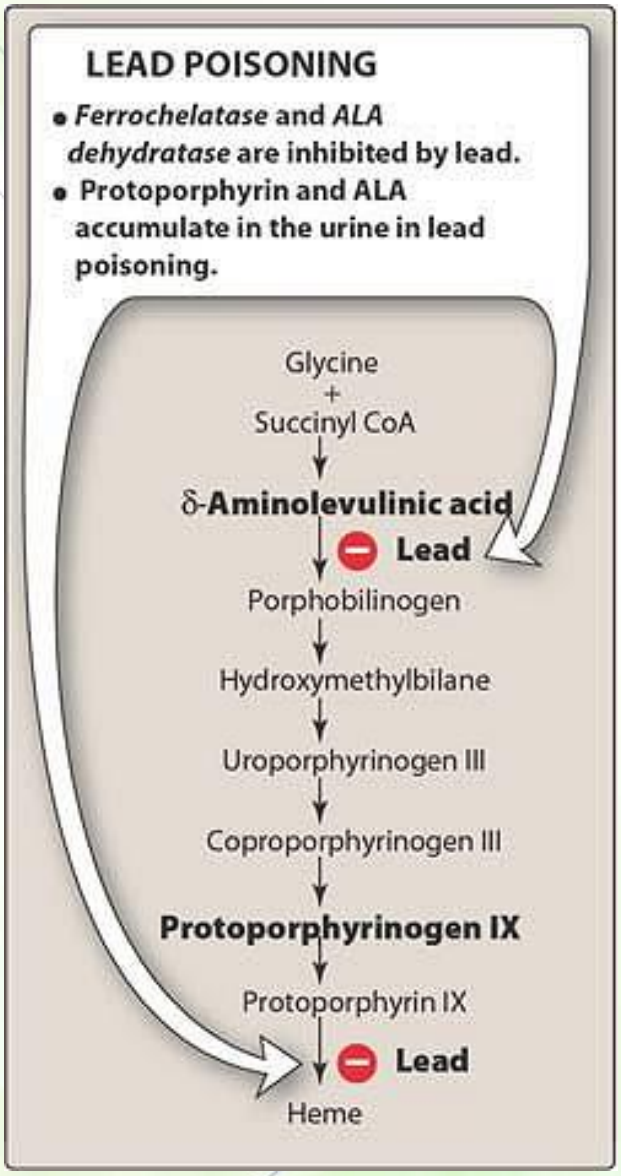


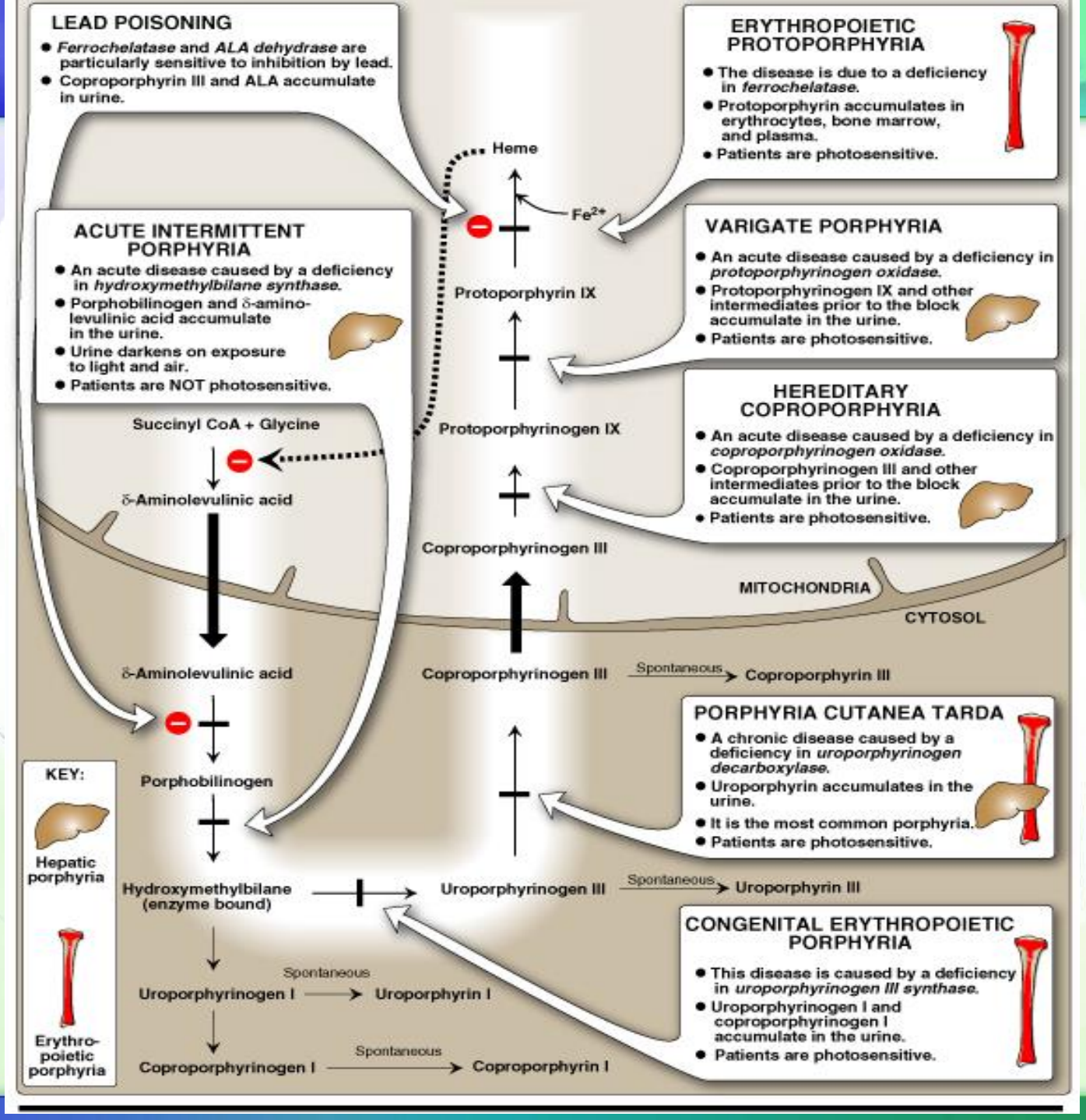
Synthesis of heme

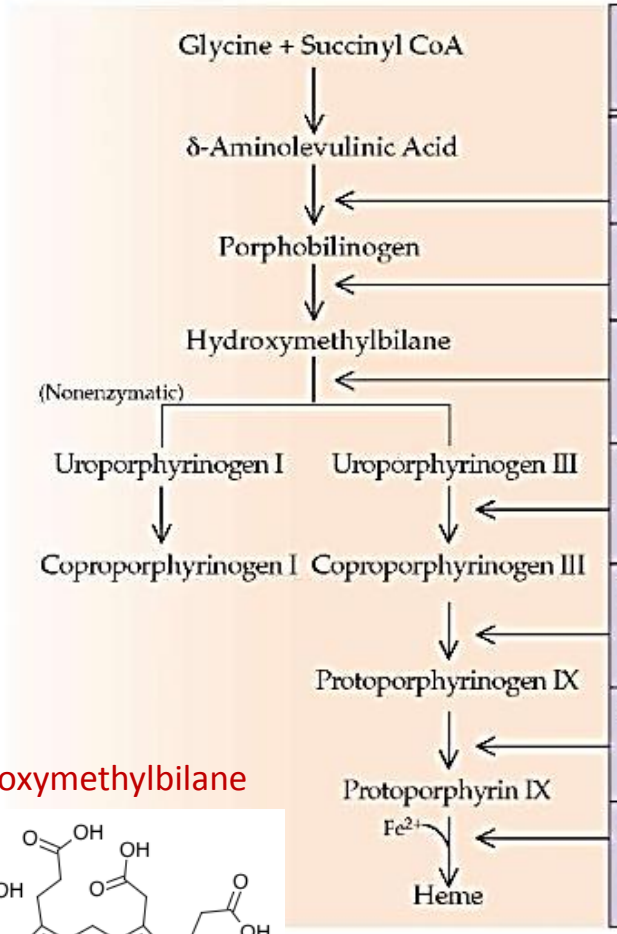
- Liver and erythroid tissues are main sites of synthesis.
- The first reaction is the rate limiting and committed step.
- It requires vitamin B6 (pyrodoxal phosphate).
- It is regulated by hemin.

- The last reaction is spontaneous, but can be catalyzed by ferrochelatase.



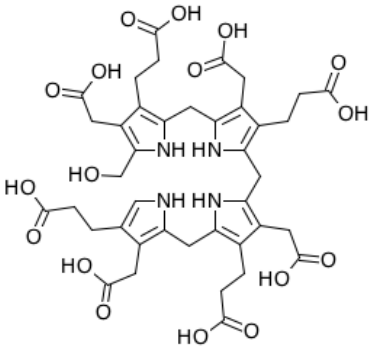



LEAD POISONING
Ferrochelatase and ALA dehydrase are particularly sensitive to inhibition by lead.
Coproporphyrin III and ALA accumulate in urine.
ERYTHROPOIETIC PROTOPORPHYRIA
The disease is due to a deficiency in ferrochelatase.
Protoporphyrin accumulates in erythrocytes, bone marrow, and plasma.
Patients are photosensitive.
Heme
Fe2+
ACUTE INTERMITTENT PORPHYRIA
An acute disease caused by a deficiency in hydroxymethylbilane synthase.
Porphobilinogen and δ-amino-levulinic acid accumulate in the urine.
Urine darkens on exposure to light and air.
Patients are NOT photosensitive.
Protoporphyrin IX
VARIGATE PORPHYRIA
An acute disease caused by a deficiency in protoporphyrinogen oxidase.
Protoporphyrinogen IX and other intermediates prior to the block accumulate in the urine.
Patients are photosensitive.
Protoporphyrinogen IX
HEREDITARY COPROPORPHYRIA
An acute disease caused by a deficiency in coproporphyrinogen oxidase.
Coproporphyrinogen III and other intermediates prior to the block accumulate in the urine.
Patients are photosensitive.
Succinyl CoA + Glycine
δ-Aminolevulinic acid
Coproporphyrinogen III
MITOCHONDRIA
CYTOSOL
δ-Aminolevulinic acid
Coproporphyrinogen III
Spontaneous
Coproporphyrin III
PORPHYRIA CUTANEA TARDA
A chronic disease caused by a deficiency in uroporphyrinogen decarboxylase.
Uroporphyrin accumulates in the urine.
It is the most common porphyria.
Patients are photosensitive.
KEY:
Hepatic porphyria
Erythro-poietic porphyria
Porphobilinogen
Hydroxymethylbilane (enzyme bound)
Uroporphyrinogen III
Spontaneous
Uroporphyrin III
CONGENITAL ERYTHROPOIETIC PORPHYRIA
This disease is caused by a deficiency in uroporphyrinogen III synthase.
Uroporphyrinogen I and coproporphyrinogen I accumulate in the urine.
Patients are photosensitive.
Spontaneous
Uroporphyrinogen I
Uroporphyrin I
Spontaneous
Coproporphyrinogen I
Coproporphyrin I



Enzyme	Disease	Major Symptoms	Accumulation Products
δ -Aminolevulinate Dehydratase	ALAD Deficiency Porphyria	Neurovisceral	Urinary ALA
Porphobilinogen Deaminase	Acute Intermittent Porphyria	Neurovisceral	Urinary ALA and PBG
Uroporphyrinogen III Cosynthase	Congenital Erythropoietic Porphyria	Photosensitivity	Erythrocyte and Urinary Uroporphyrin I and Coproporphyrin I
Uroporphyrinogen Decarboxylase	Porphyria Cutanea Tarda; Hepatoerythro-poietic Porphyria	Photosensitivity	Urinary 7-Carboxylate Porphyrin; Fecal Isocoproporphyrin
Coproporphyrinogen Oxidase	Hereditary Coproporphyria	Neurovisceral and Photosensitivity	Urinary ALA, PBG, and Coproporphyrin; Fecal Coproporphyrin
Protoporphyrinogen Oxidase	Variegate Porphyria	Neurovisceral and Photosensitivity	Urinary ALA and PBG; Fecal Protoporphyrin
Ferrochelatase	Erythropoietic Protoporphyria	Photosensitivity	Erythrocyte, Plasma, and Fecal Protoporphyrin

Hydroxymethylbilane



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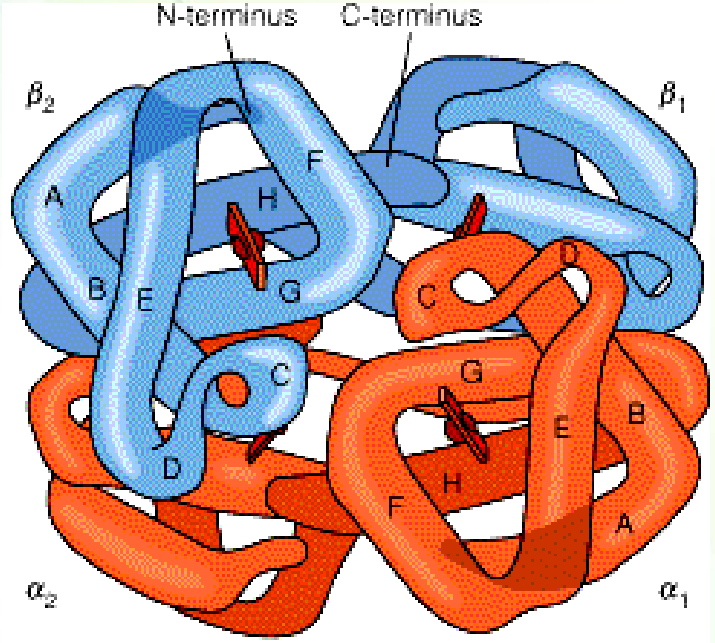
Treatment

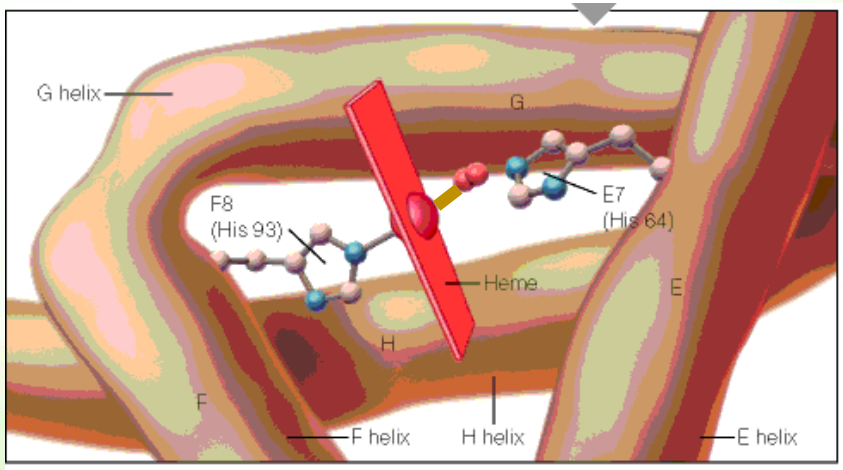
- Intravenous injection of hemin and glucose
- Protection from sunlight
- Ingestion of beta-carotene.

Hemoglobin

Structure of hemoglobin

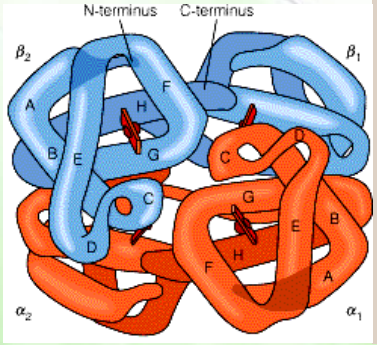
- Hb is a globular protein.
  - Amino acid distribution
    - Positions of two histidine residues (proximal and distal)
- It is an allosteric protein.
  - Multiple subunit (2α + 2β)
  - Altered structure depending on bound molecules
  - Positive cooperativity towards oxygen
  - Regulated by allosteric effectors

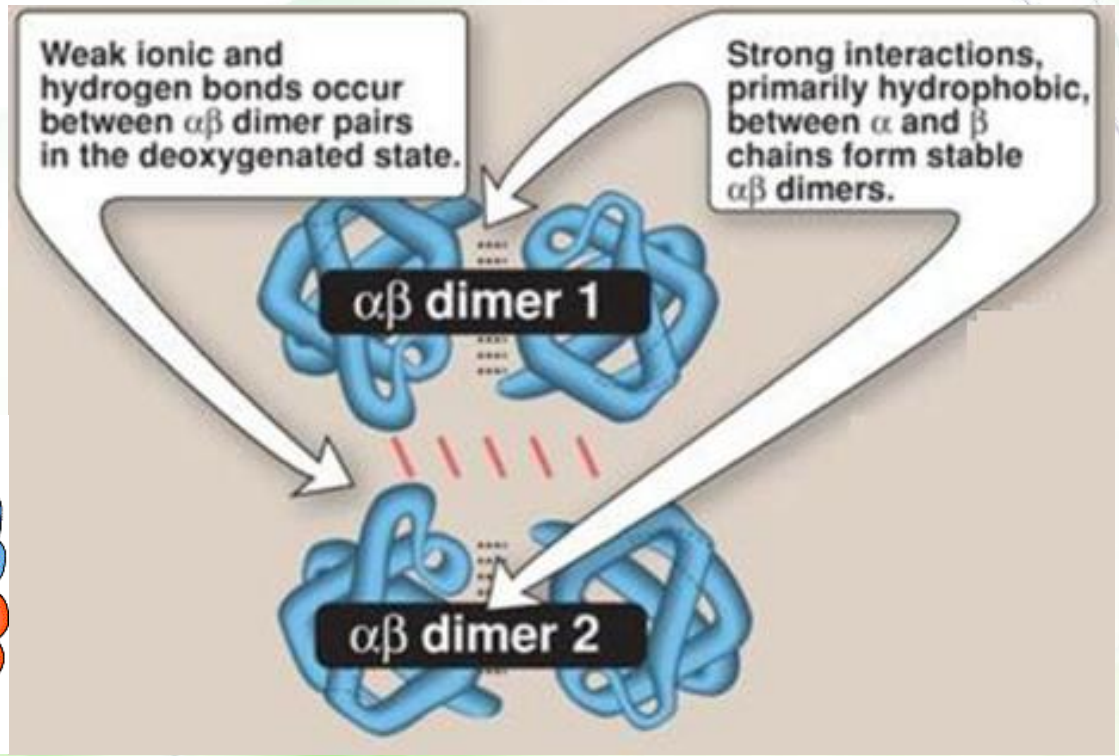




How are the subunits bound?

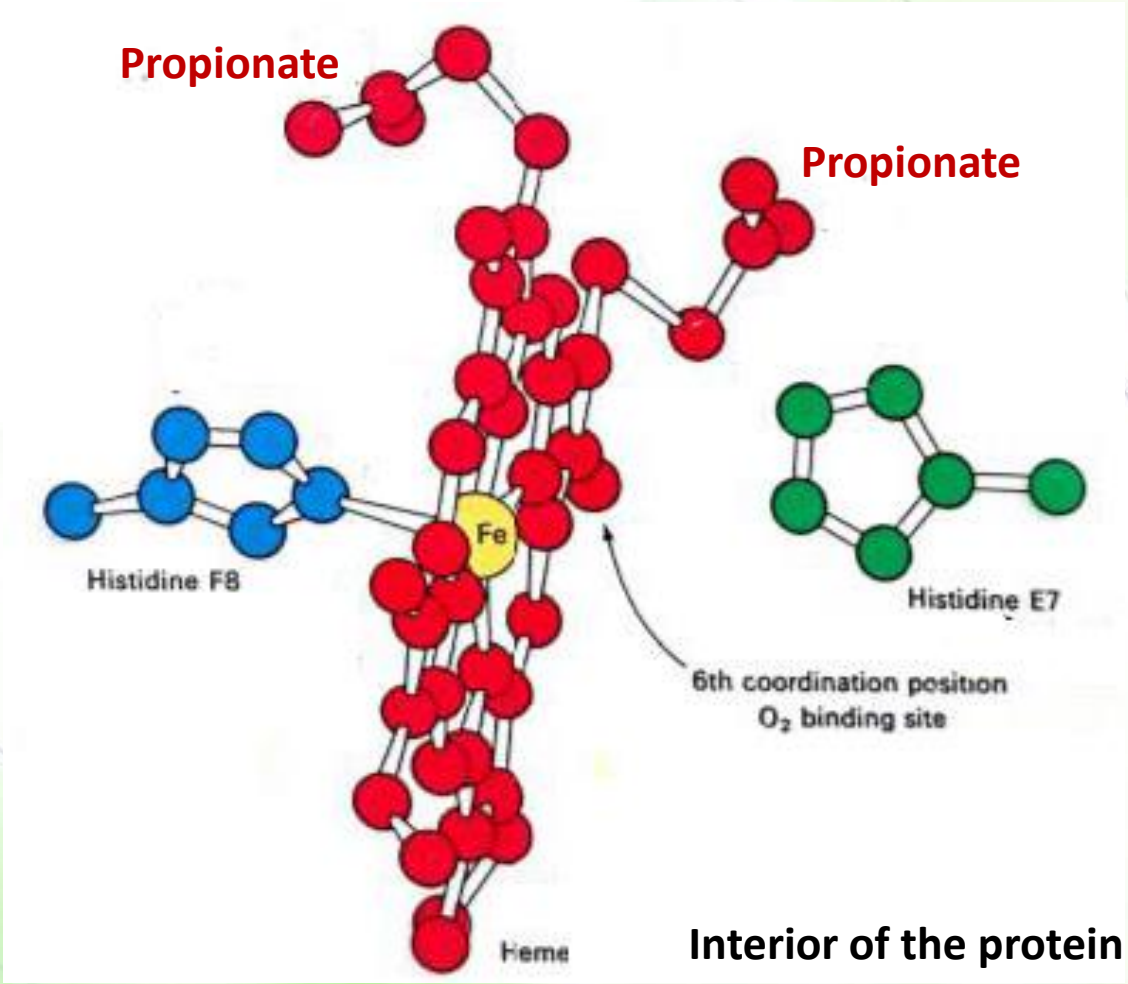
- A dimer of a dimer (*I made up this term*)
  - $(\alpha\text{-}\beta)_2$
  - Note how they interact with each other.





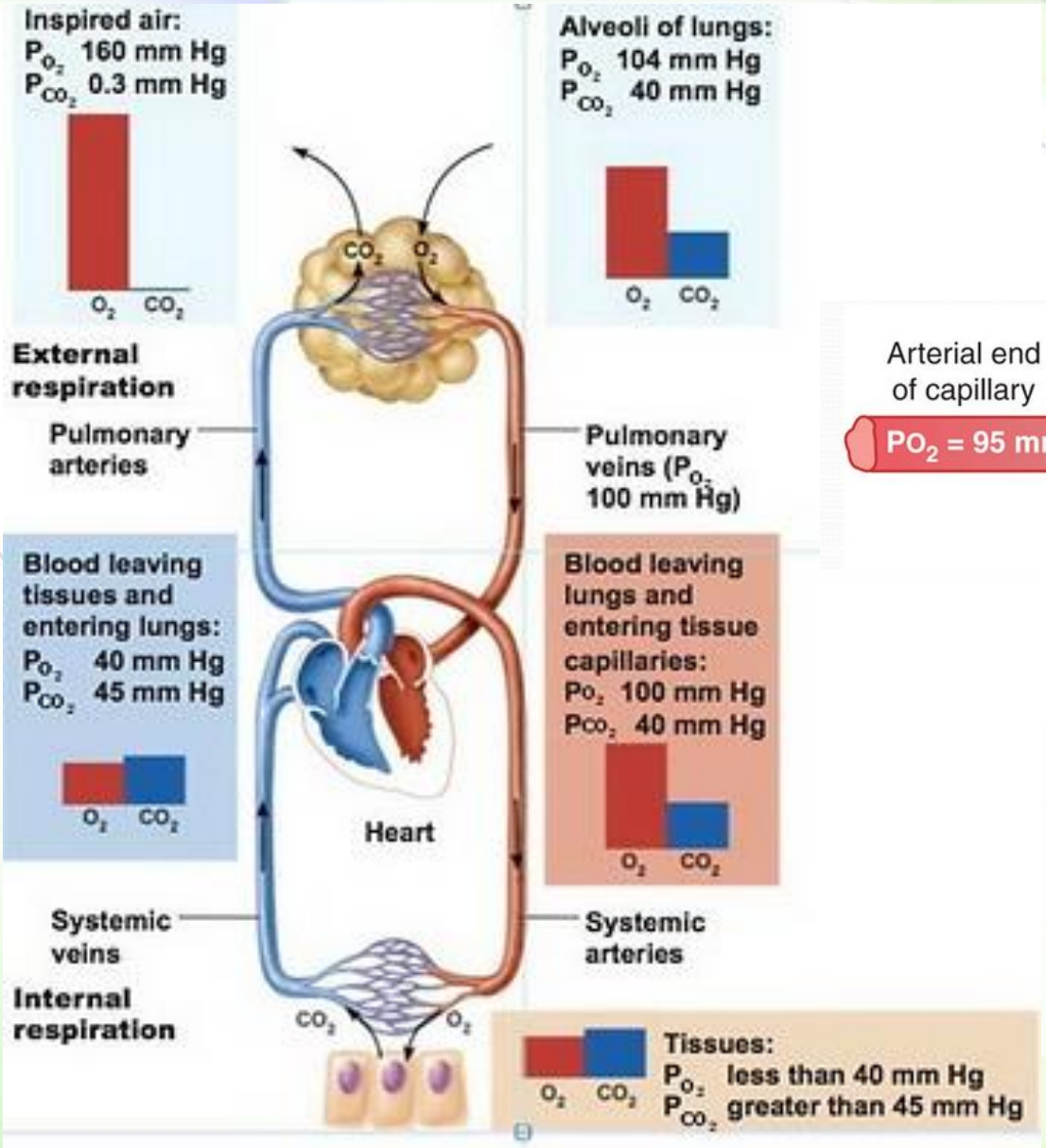
Heme binding to hemoglobin

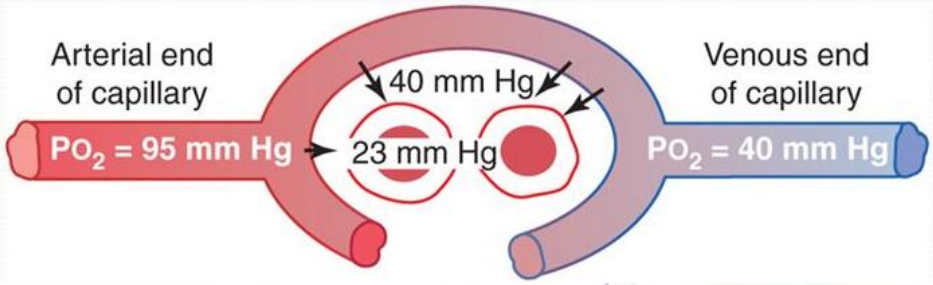
Exterior of the protein



Interior of the protein

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 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.*

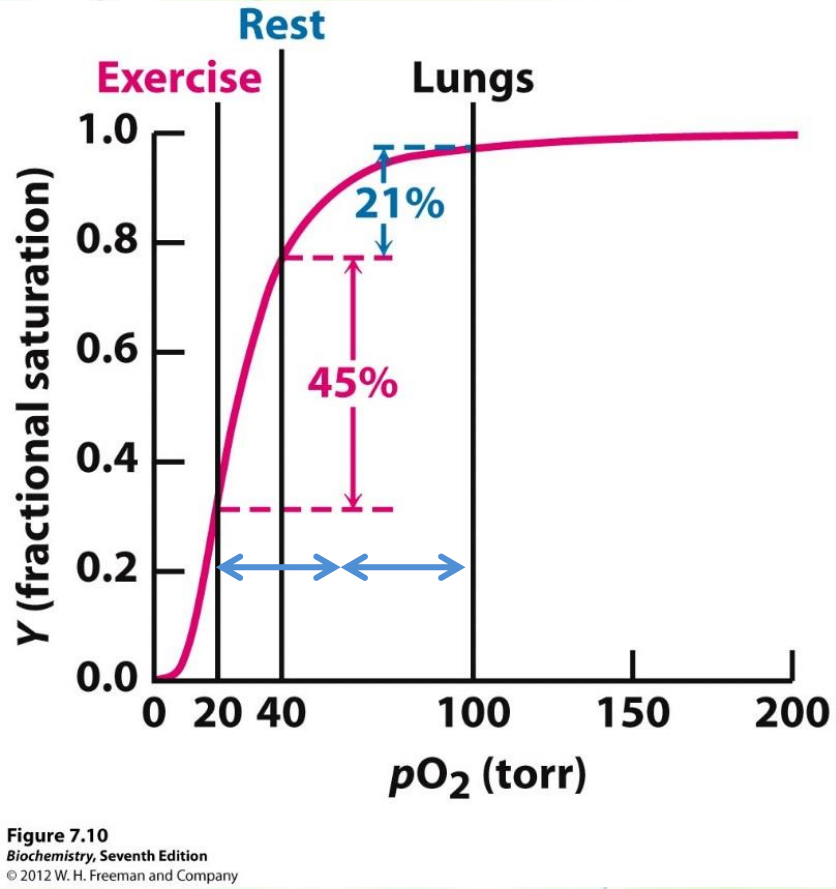
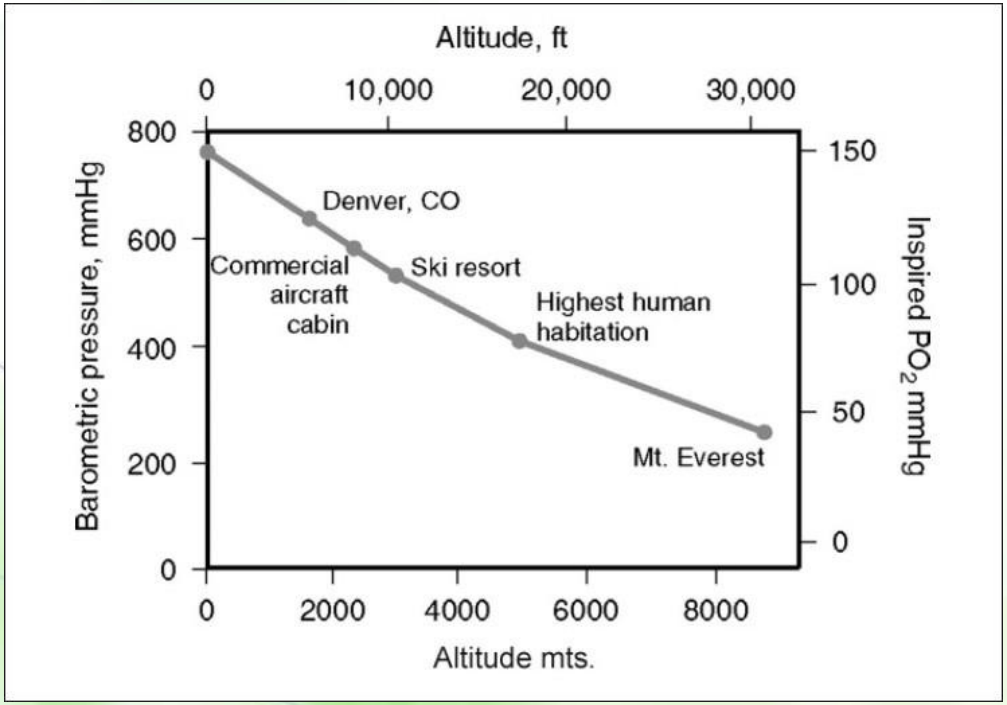


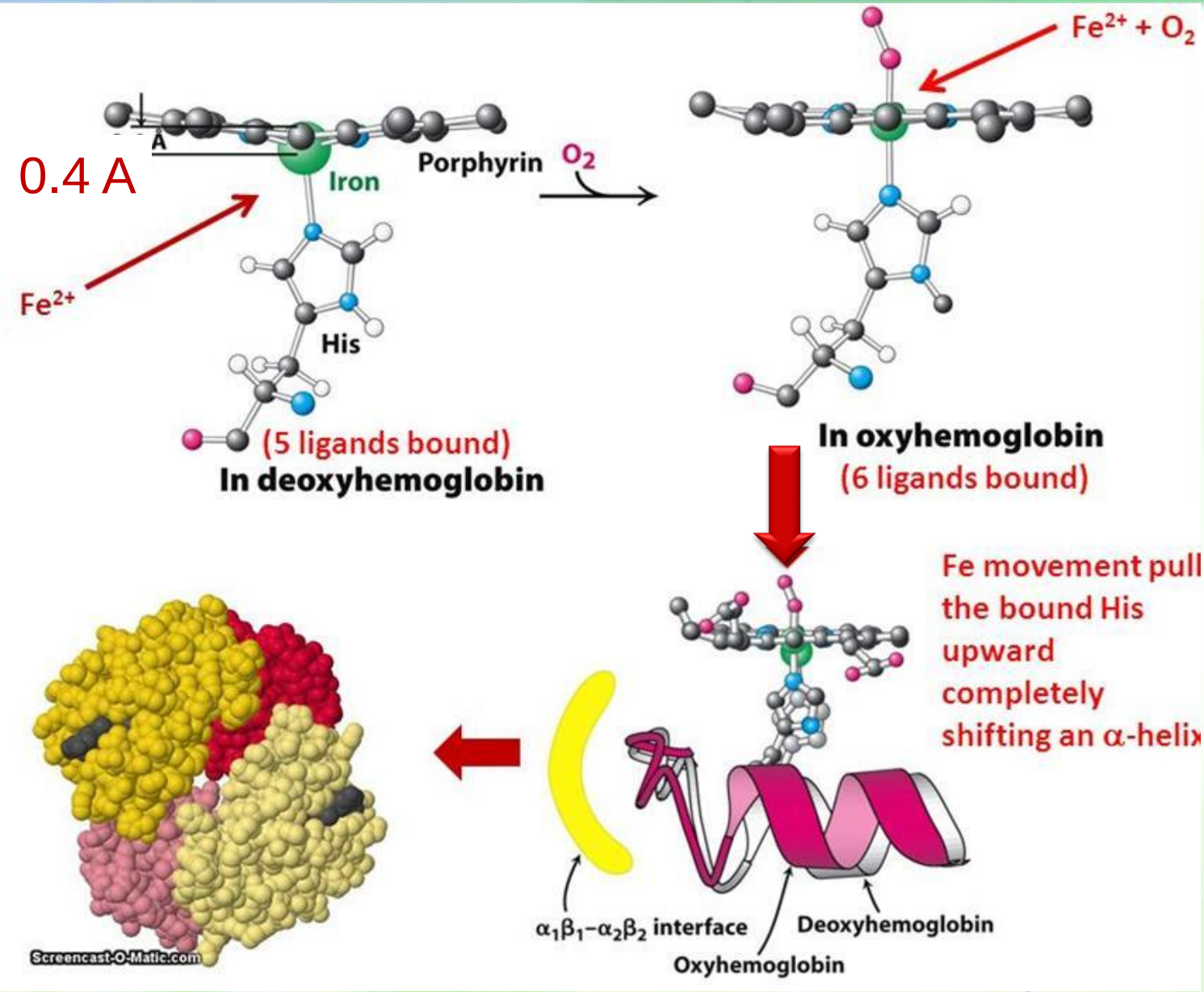
Figure 7.10
Biochemistry, Seventh Edition

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



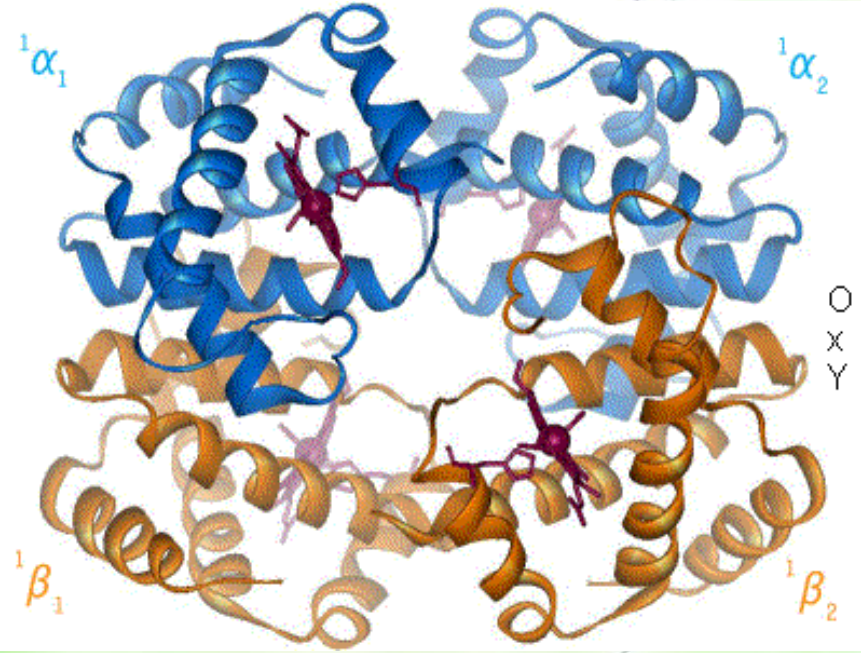
Structural amplification change



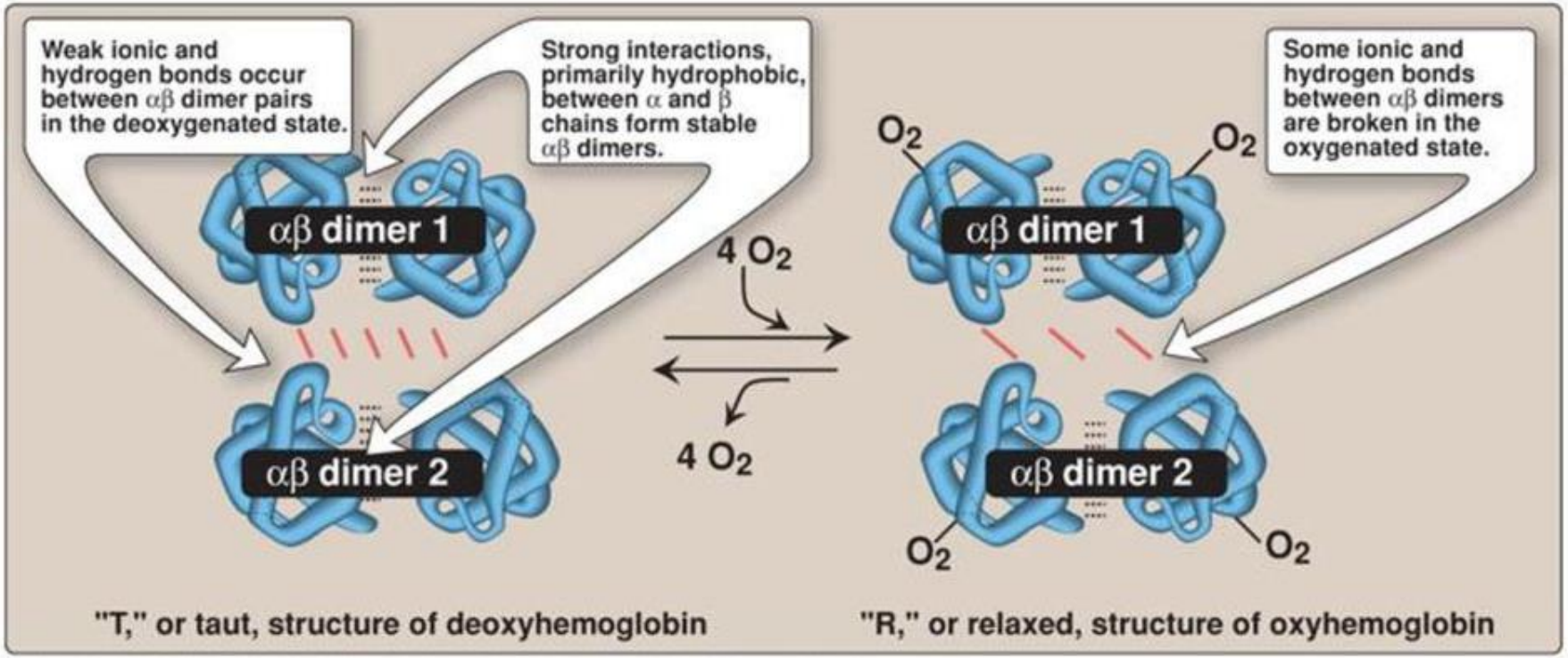
How does the structure change? (2)

- This movement triggers
  - changes in tertiary structure of individual hemoglobin subunits
  - breakage of the electrostatic bonds at the other oxygen-free hemoglobin chains.

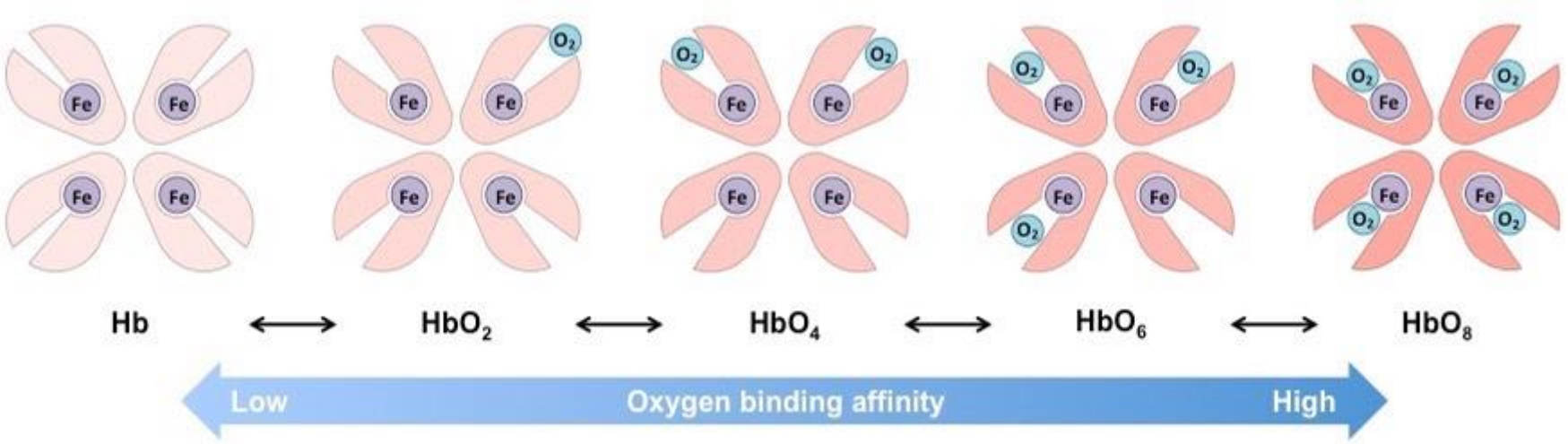
In myoglobin, movement of the helix does not affect the function of the protein.



Electrostatic interactions are broken



Positive cooperativity



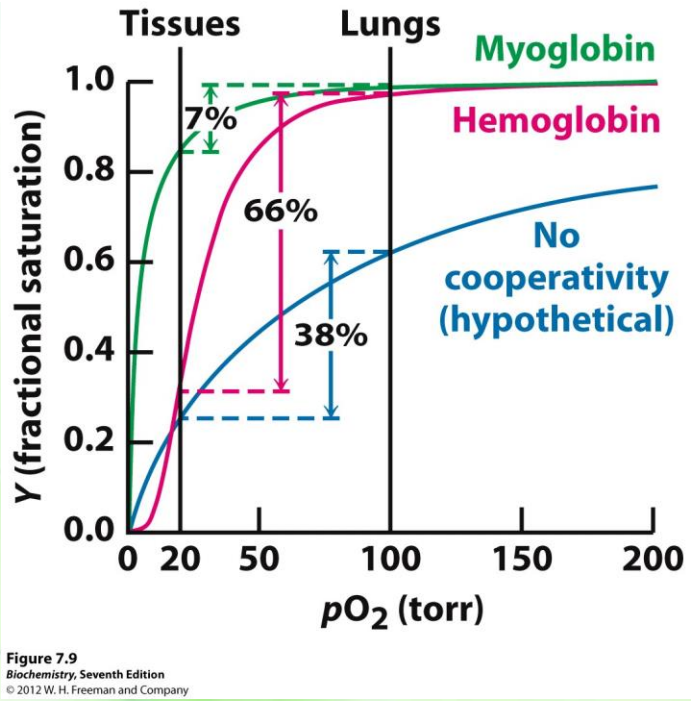


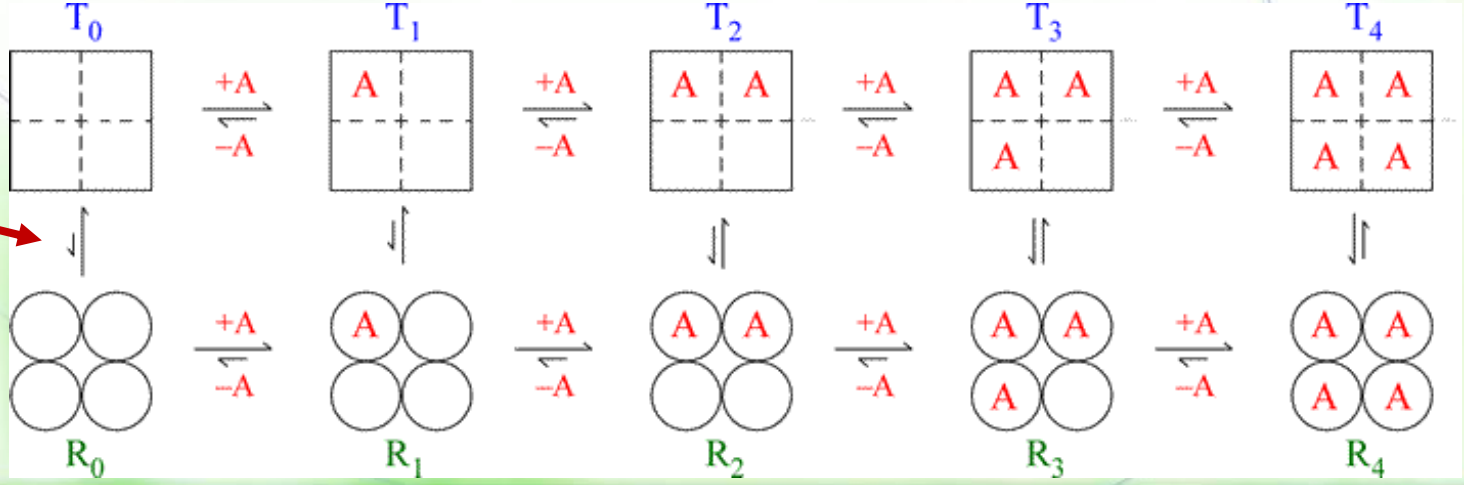
Figure 7.9
Biochemistry, Seventh Edition

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.

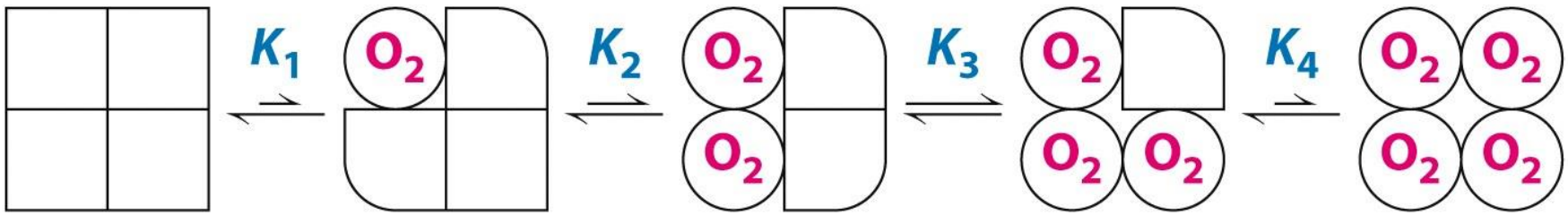
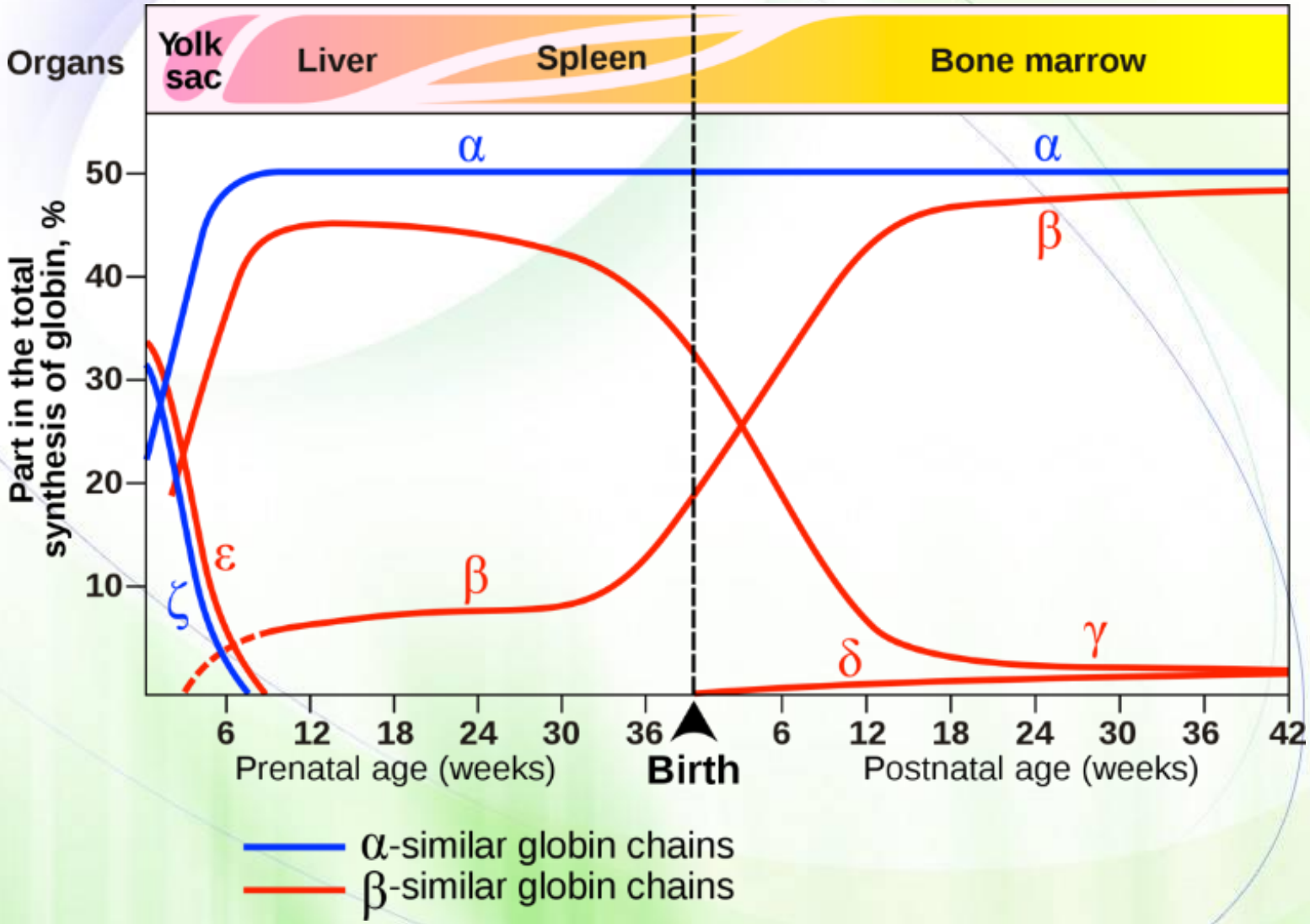


Figure 7.14
***Biochemistry*, Seventh Edition**

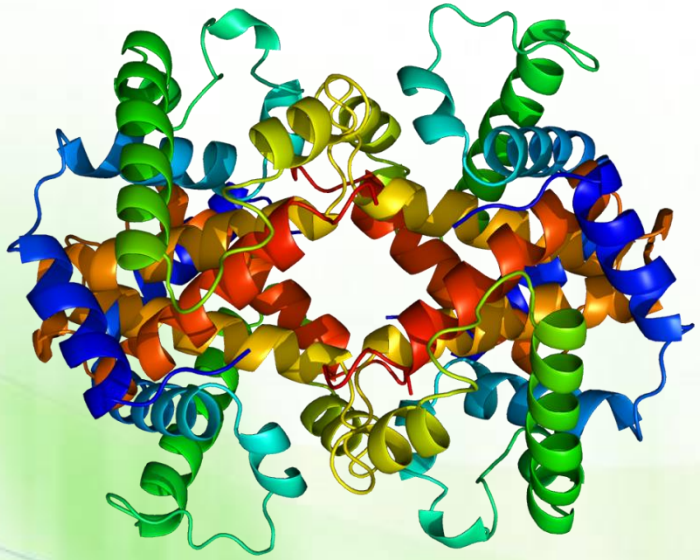
It is not only one hemoglobin

Developmental transition of hemoglobins



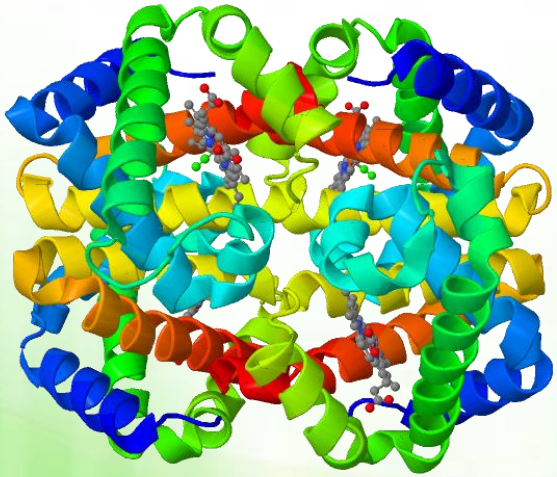
The embryonic stage

- 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 ($\alpha_2\varepsilon_2$), HbE Portland 1 ($\zeta_2\gamma_2$), HbE Portland 2 ($\zeta_2\beta$).



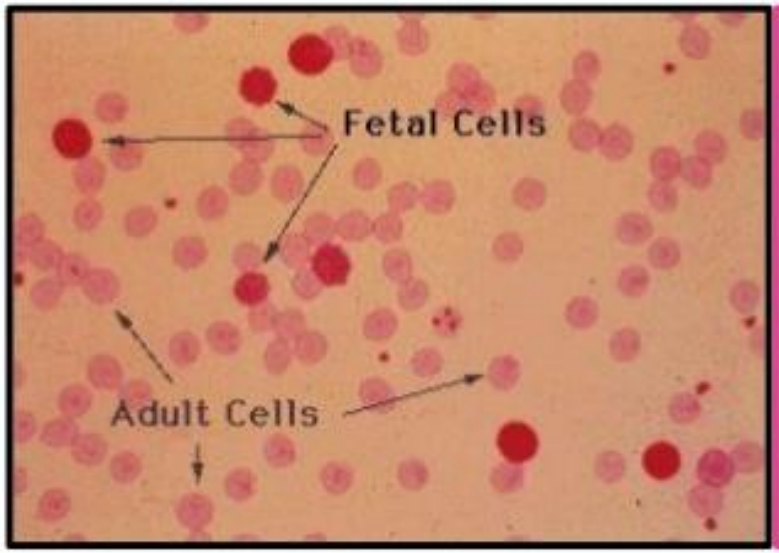
Beginning of fetal stage

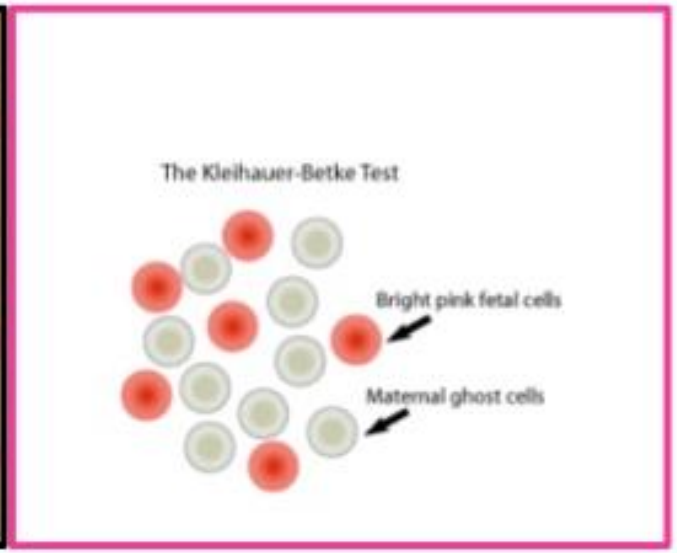
- By 6-8 weeks of gestation, the expression of embryonic hemoglobin declines dramatically and fetal hemoglobin synthesis starts from the liver.
- Fetal hemoglobin consists of two α polypeptides and two gamma (γ) polypeptides ($\alpha 2 \gamma 2$)
- The α polypeptides remain on throughout life.



Beginning of adult stage

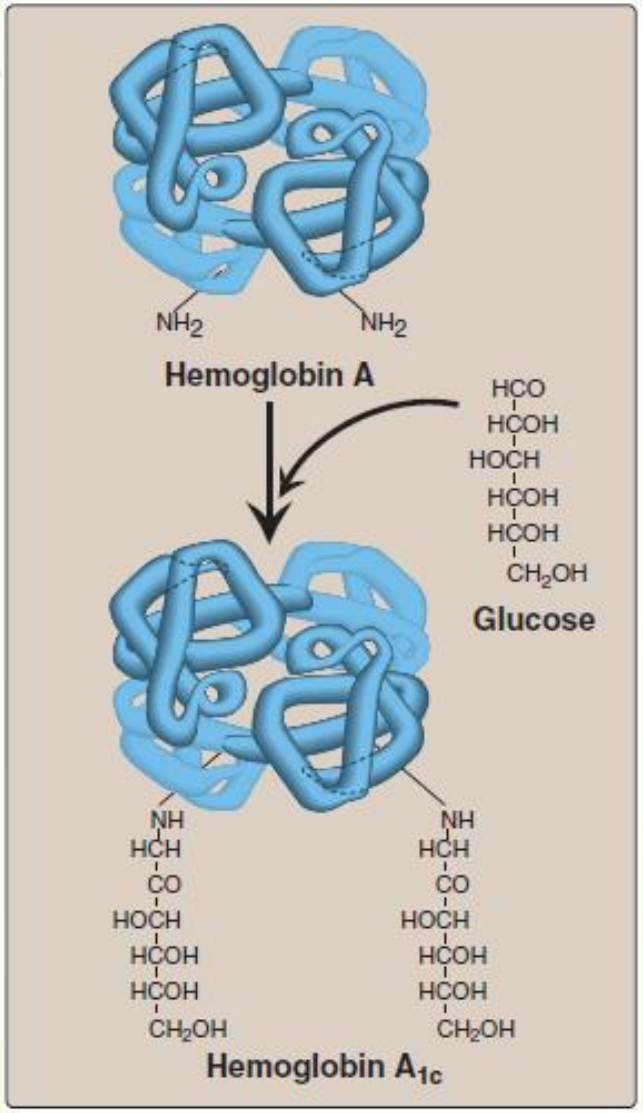
- Shortly before birth, there is a gradual switch from to adult β-globin.
- Still, HbF makes up 60% of the hemoglobin at birth, but 1% of adults.
- At birth, both synthesis of γ and β chains occurs in the bone marrow.





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 HbA_c.
  - The major form (HbA_{1c}) is attached to glucose attached to valines of β chains.
  - HbA_{1c} is present at higher levels in patients with diabetes mellitus.



Advantages of HbA1c testing

- **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).
- **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 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.

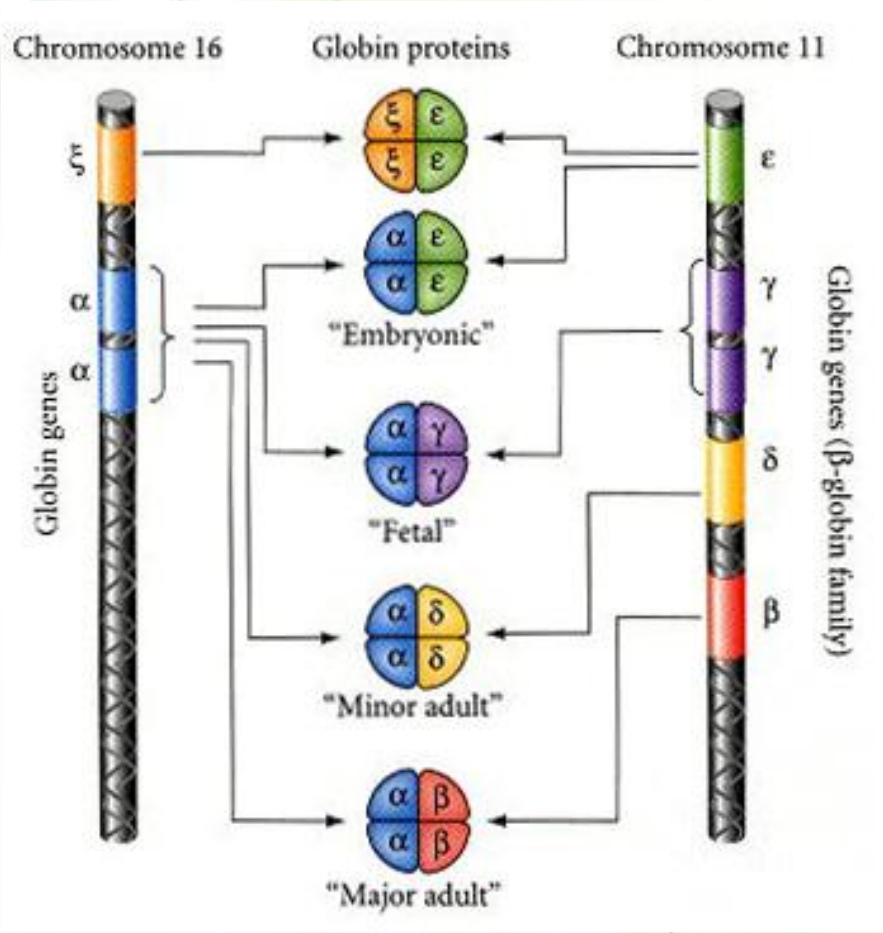
Table

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

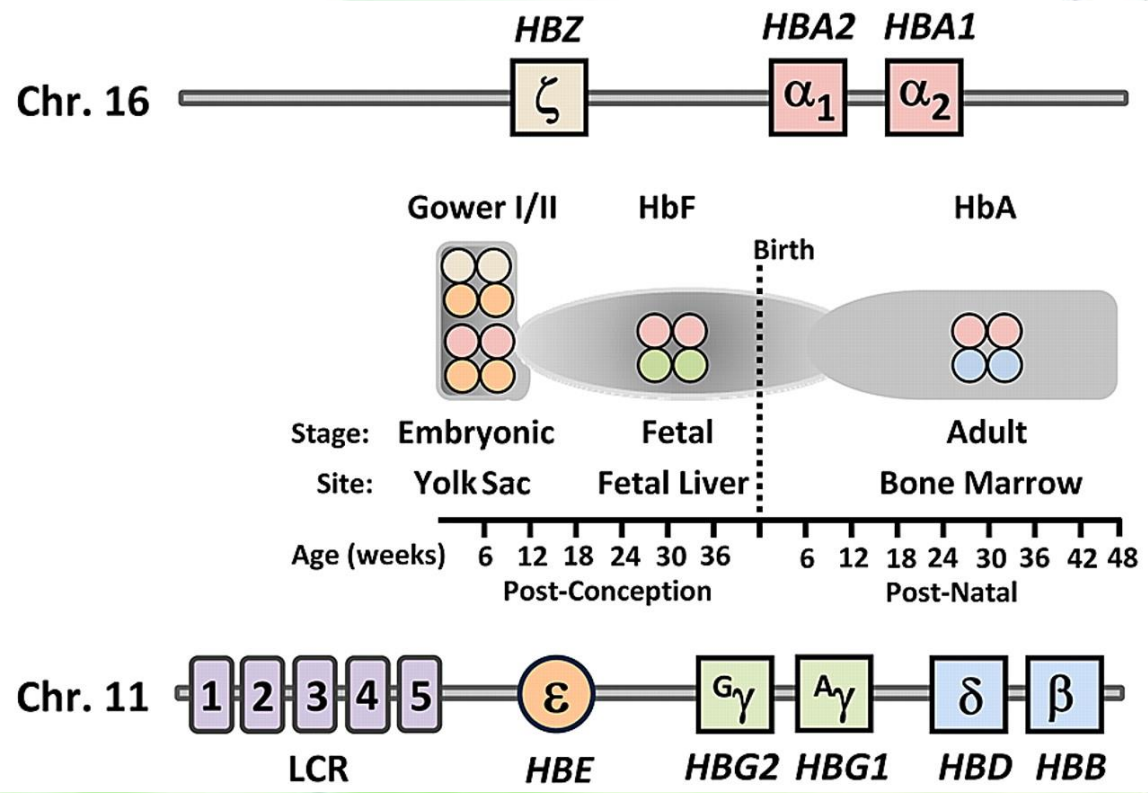
The genes

- The α gene cluster contains two α genes ($\alpha 1$ $\alpha 2$) and ξ gene.
- The β gene cluster contains β gene in addition to ε gene, two γ genes, and δ 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 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!!