Chemistry and Functions of Hemoproteins

Chemistry And Functions Of Hemoglobin and Myoglobin

Synopsis

- What are **Hemoproteins?**
- What is **Hemoglobin**?
- **Structure** of Hemoglobin
- **Functions** of Hemoglobin
- **ODC** and **Factors** affecting it
- Normal **Hb Variants**
- Hemoglobin **Derivatives**

INTRODUCTION

Hemoproteins

What Are Hemoproteins?

- Hemoproteins are **Conjugated Proteins**
- With **Heme** as a **Prosthetic group in their structures.**

- **Hemoproteins** are **Globular Proteins**

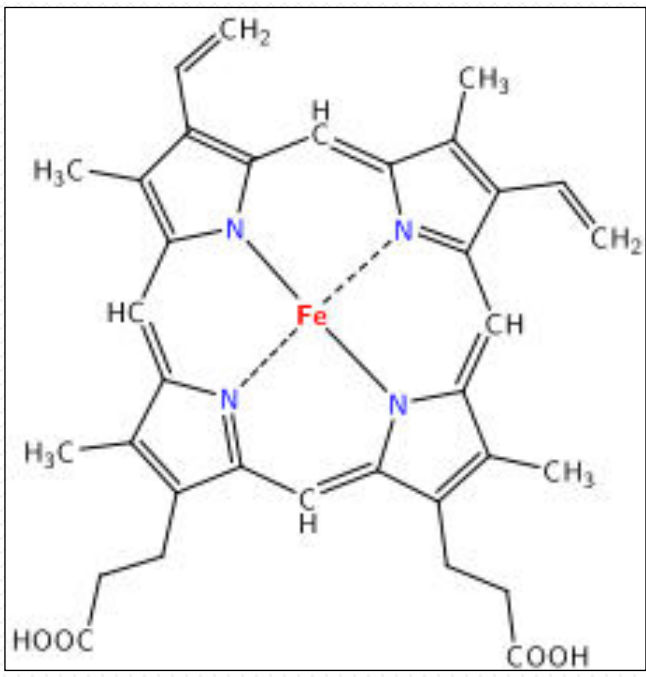
(Whose Axial ratio less than 10)

Examples Of HEME CONTAINING PROTEINS AND ENZYMES Of Human Body

Human Body Hemoproteins
Compounds with Heme group

Heme Containing Proteins

1. Hemoglobin (Hb)
2. Myoglobin (Mb)
3. Cytochromes (ETC Components)



Heme Containing Enzymes

1. Catalase
2. Peroxidase
3. Tryptophan Dioxygenase/ Tryptophan Pyrrolase

- **Hemoproteins are vital for human body**

Study Of Hemoglobin

What Is Hemoglobin?

- **Hemoglobin(Hb)** is a **major Hemoprotein** of Human body.

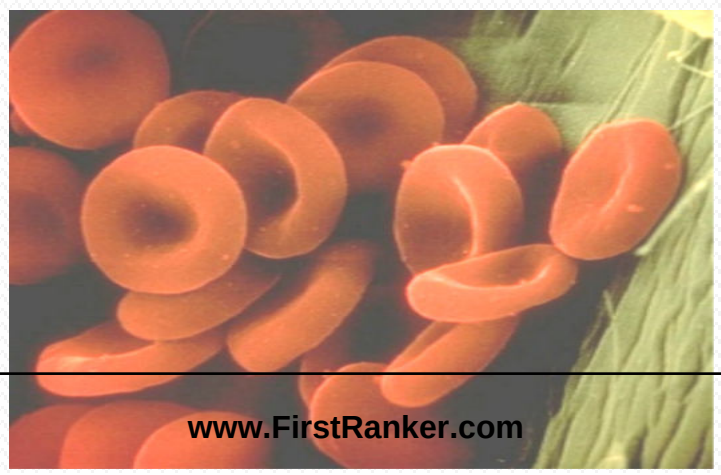
- **Hemoglobin Chemically is:**
  - **Conjugated Protein**

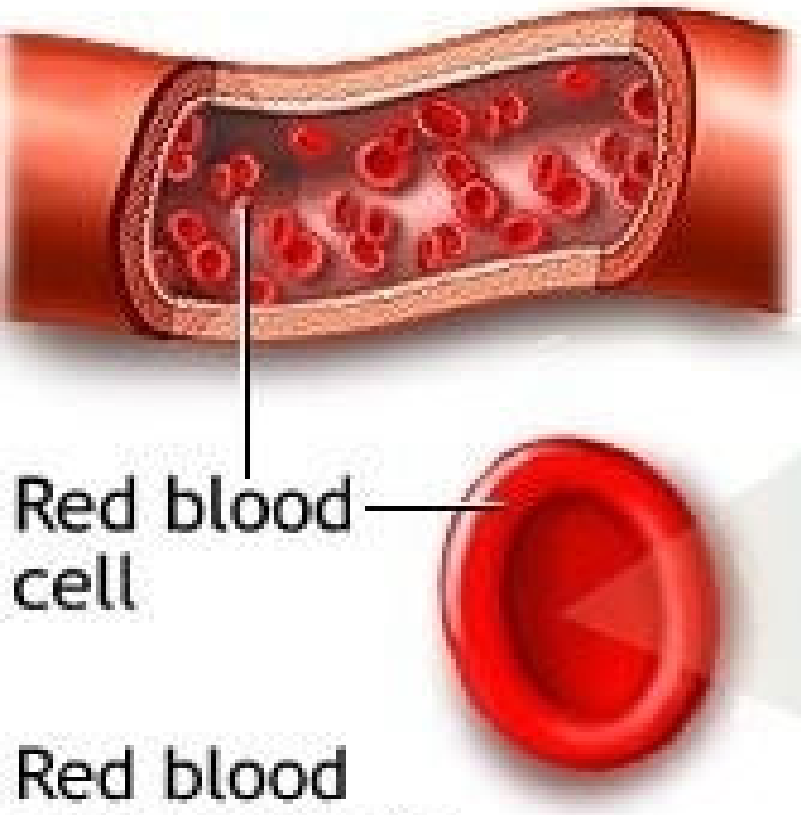
- **In Hemoglobin**
  - **Heme** is a **Prosthetic group**
  - **Globin** is a **Protein part**

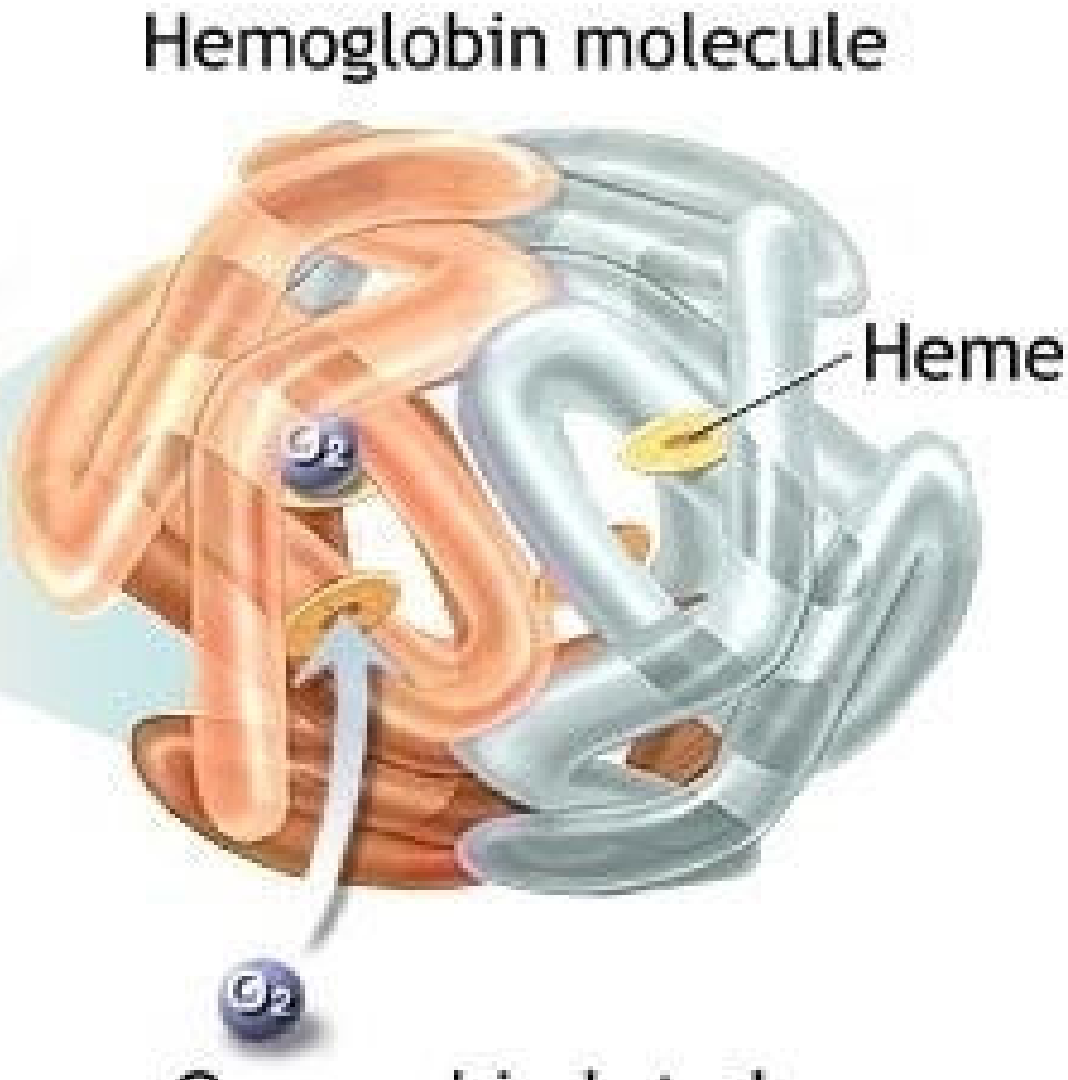
(Hemoglobin = Heme + Globin)

- Hemoglobin(Hb) is **Red color pigment**
- **Location Of Hemoglobin-**

  Inside Red blood cells/Erythrocytes of blood.







- **Amount Of Hemoglobin-**
  - Each RBC has approx **250-300 million Hb molecules**
  - **In 25 x 10^{12}** Erythrocytes **-750 gm of Hb**

- **Hemoglobin In RBCs Occupies:**

- **33%** of the **RBC volume (1/3)**

- **90-95%** of the **dry weight of RBC is by Hb.**

- **Normal concentration of Hemoglobin in the Human Blood:**

Adult Males-
13.5–17.5 gm/dL

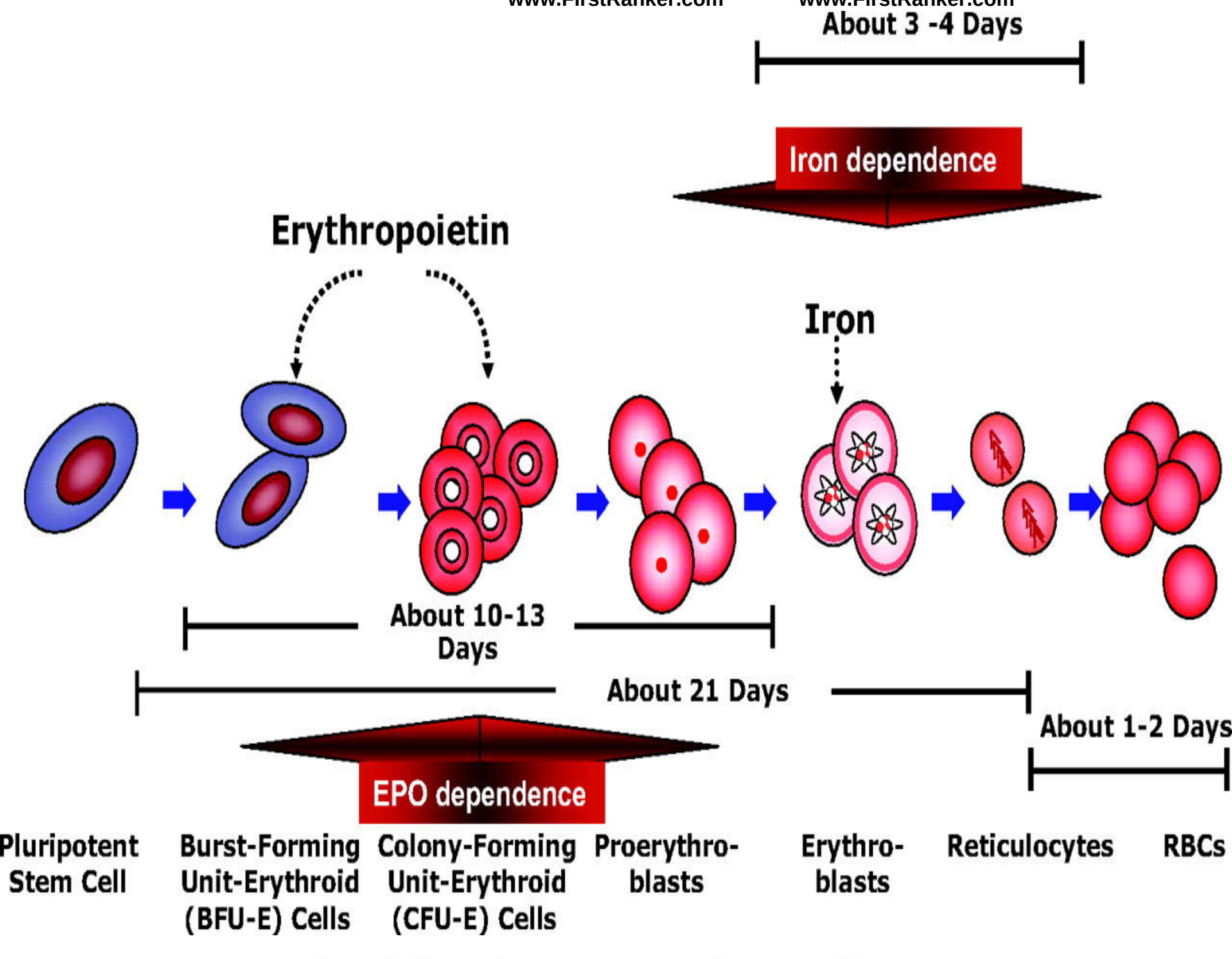
Adult Females-
12.5–16.5 gm/dL

- **Hemoglobin Biosynthesis-**

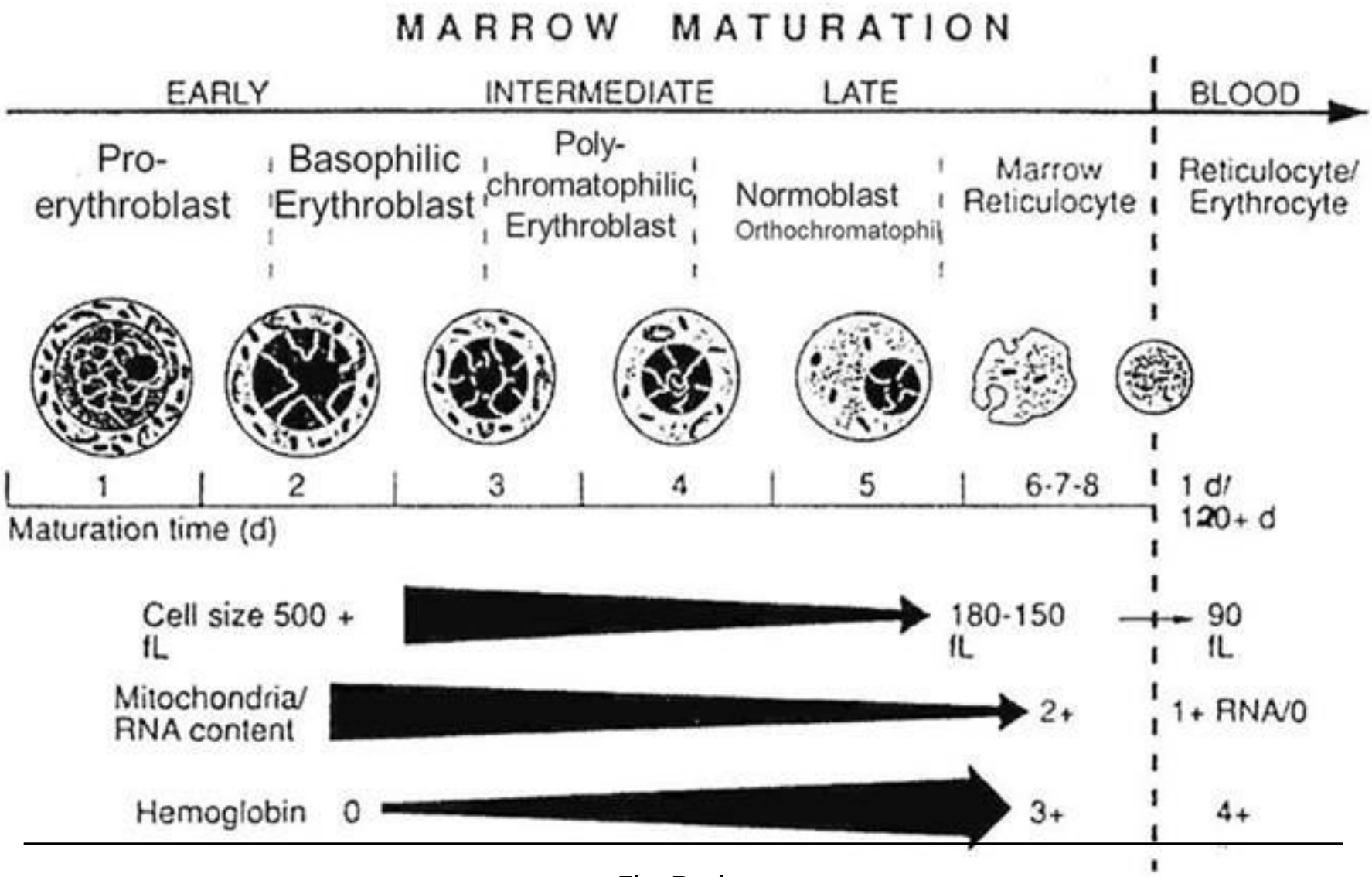
6.25 gm/day is the amount of Hb biosynthesized during stages of **Erythropoiesis in bone marrow**.

- **Synthesis of Hb** begins in **Proerythroblast:**

- **65% at Erythroblast stage**
- **35% at Reticulocyte stage**



Recognizable Stages of Erythropoiesis



Hemoglobin Function

- **Hb is associated to Respiration Mechanism**
  - Hb is a **characteristic of Aerobic life** very important for survival.
  - Hb brings **exchange of Gases-**: **O_2 and CO_2**

Terminologies of Hemoglobin

- **Hemoprotein** -Heme is a prosthetic group
- **Chromoprotein** - Red in color
- **Metalloprotein** - Metal Iron (Fe) present
- **Respiratory Protein-** Connected to Respiration process and Respiratory Chain(Electron Transport Chain)
- **Oxygen Binding Protein**-Binds with molecular Oxygen and transports it.

HISTORICAL ASPECTS Of Hemoglobin

- Hemoglobin **due to its red color,** has been **of interest since antiquity.**

- **Hemoglobin was a :**
  - **First Protein to be crystallized - 1849.**
  - First Protein whose **Mass accurately measured.**
  - **Mol weight of Hb-67,000 Daltons.**

- First proteins to have **X-ray Diffraction structure determined.**
- First protein to be **studied by Ultracentrifugation.**
- First protein to show that a **point mutation can cause problems.**

STRUCTURE OF HEMOGLOBIN

- Two parts of Hemoglobin
  - **Heme**-Prosthetic group
  - **Globin**-Protein part

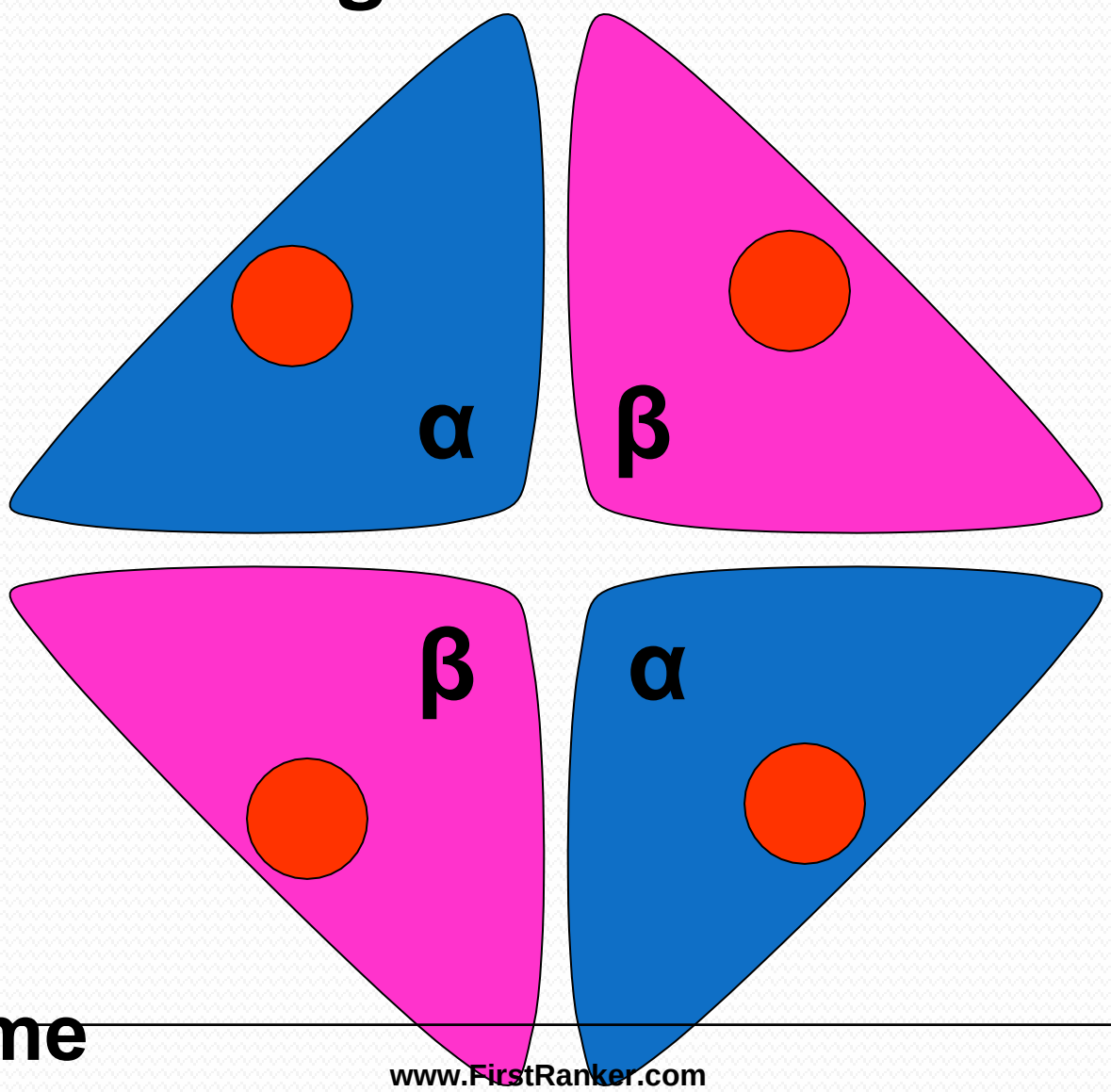
Hemoglobin Structurally

- **Tetrameric**-contain 4 subunits.
- **Quaternary level** of structural organization.
- **Allosteric, Complex, Compact**
- **Spheroidal= 64 x 55 x 50**
- **Globular Protein**

- Hb of adults (**Hb A**) is a **Tetramer with**
- 4 Polypeptide subunits /4 **Globin subunits**
- Consisting of **2 α-** and **2 β-Globin chains**

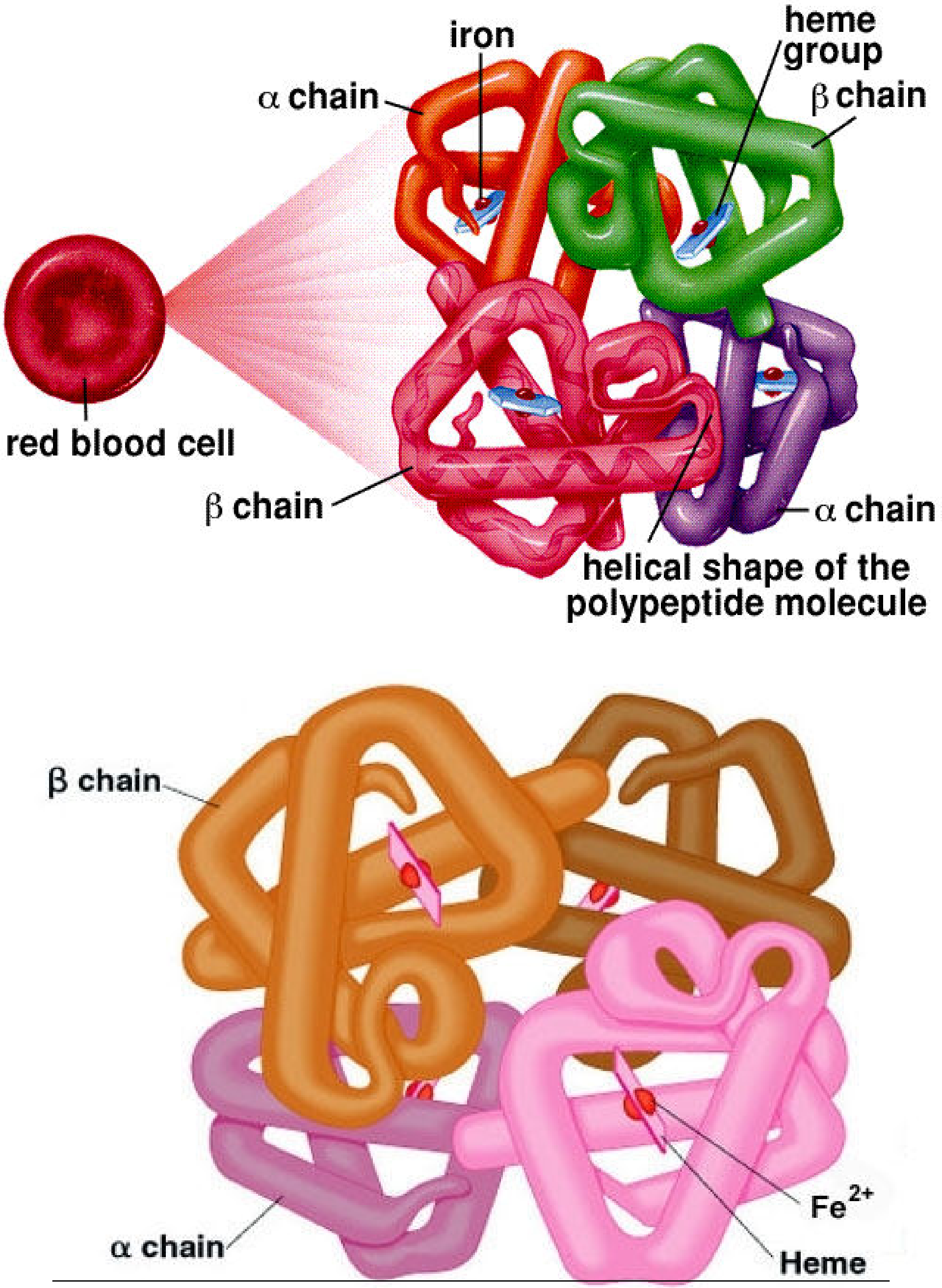
- **Each Subunit of Hemoglobin contains:**
- **1 Globin Chain** and **1 Heme group with a central Fe^{2+} ion** (Ferrous ion)

Hemoglobin Structure



Heme

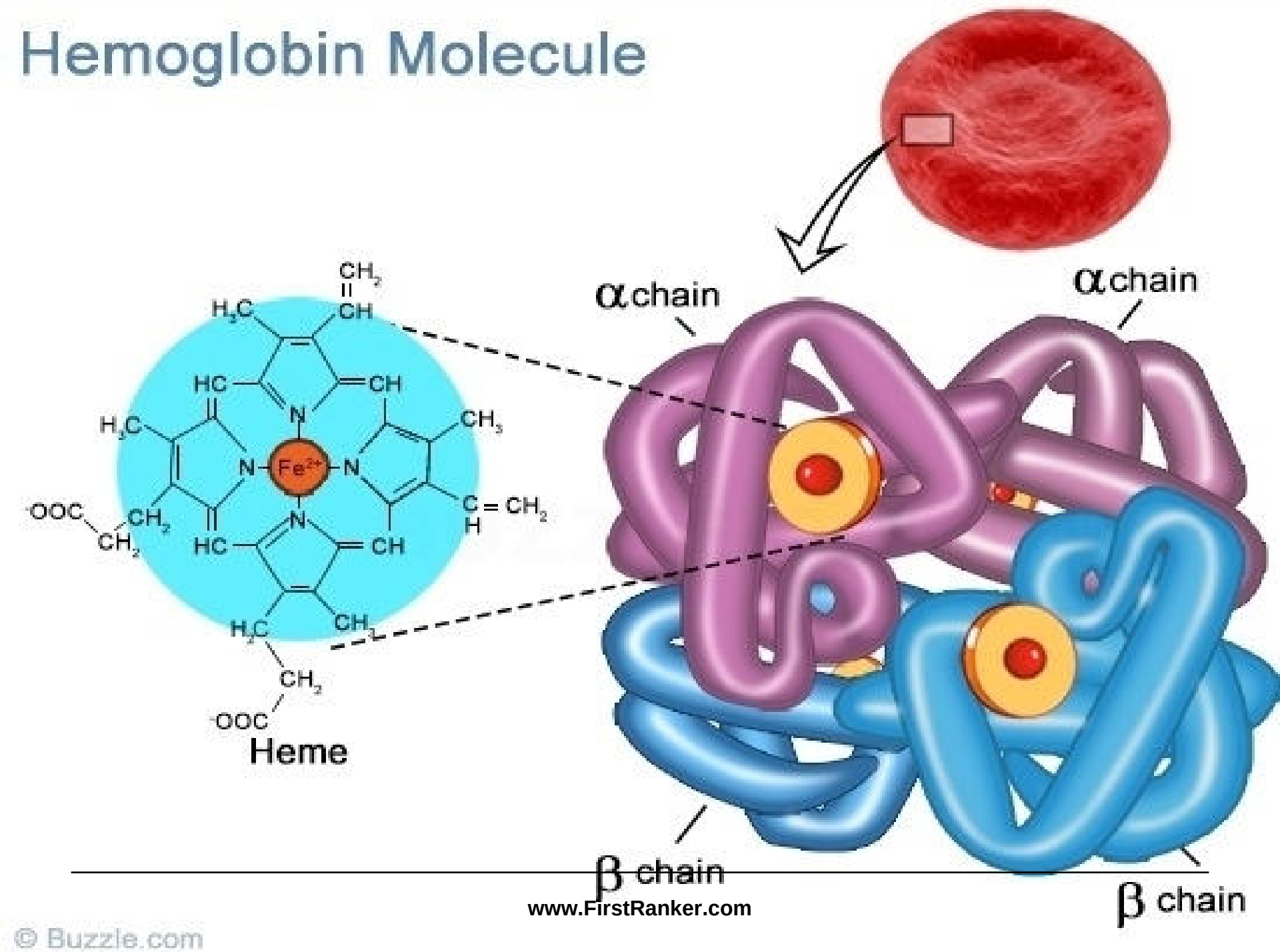
Hemoglobin Molecule

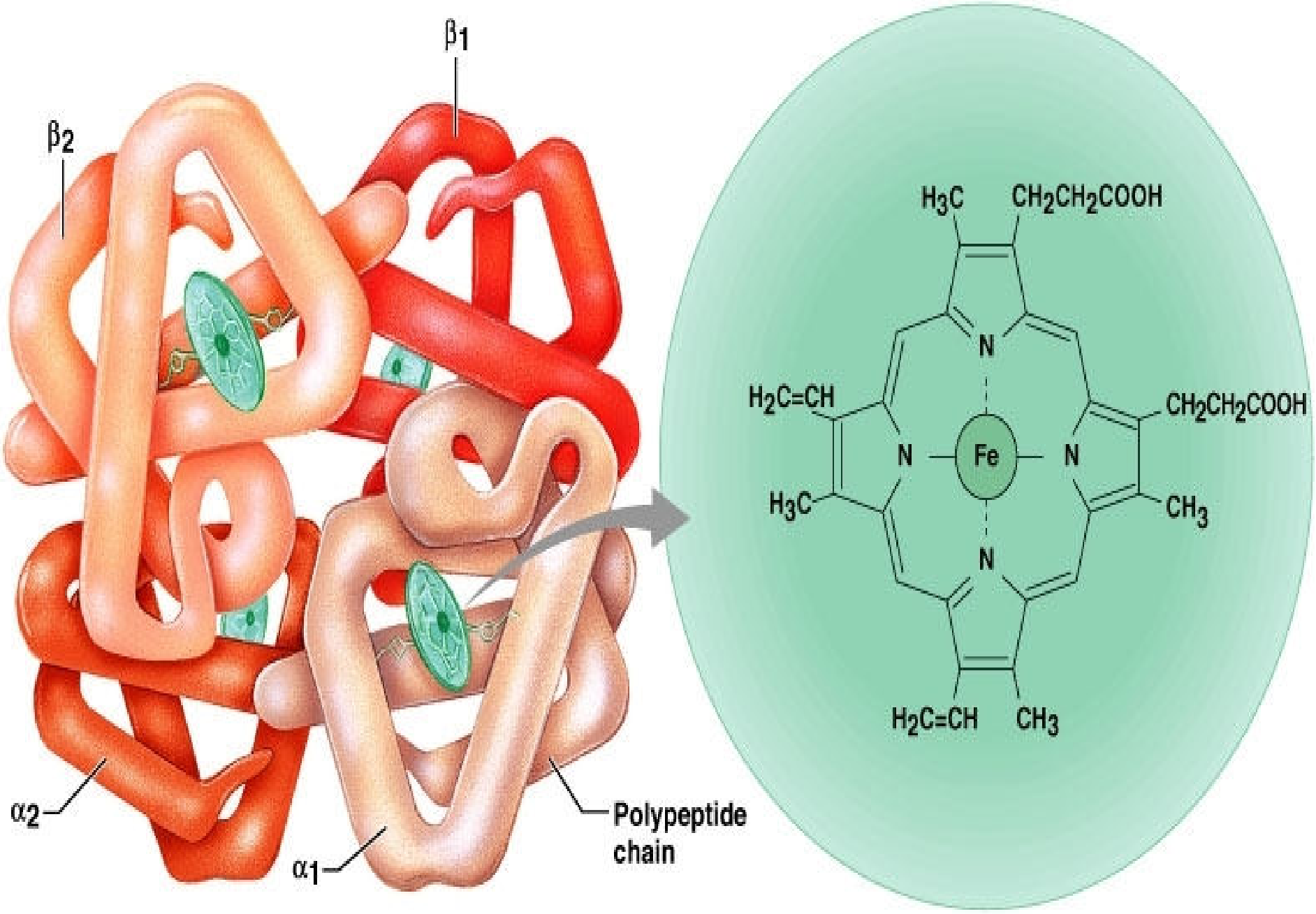


- **One Hemoglobin molecule- 4 Subunits**
  - **One Subunit**- 1 Polypeptide Globin chain and 1 Heme moiety
  - **Four Subunits-** 4 Globin Polypeptide chains+ 4 Heme moieties.

- **1 Heme** binds with **1 Oxygen molecule**
- **4 Heme** binds with **4 Oxygen molecule**
- **1 OxyHb = 4 Globin+4Heme+4Oxygen**

- In Hb 4 polypeptide chains are visualized as **two identical dimers, (αβ)1 and (αβ)2.**





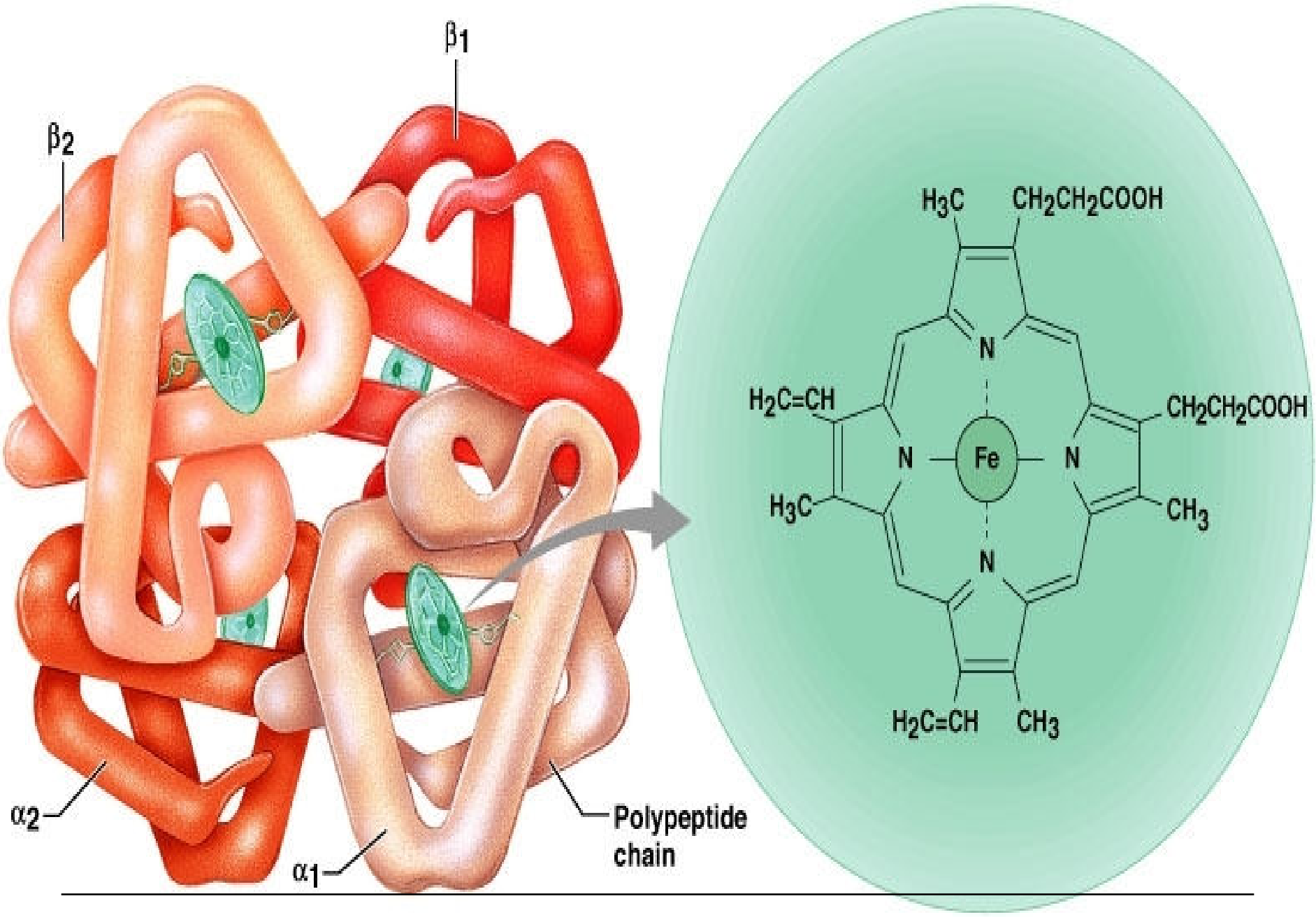
(a) Hemoglobin (b) Iron-containing heme group

- **Two dimers are linked** to each other by **weak polar bonds-** movement at the interface of these two occurs more freely.

- Two **polypeptide chains** within a dimer are **held together** tightly by:
- **Ionic bonds** and **Hydrophobic interactions**, which prevent their movement relative to each other.

❖ **Thus Hb with Quartenary structure is in native conformation.**

Significance of 4 Hb Subunits



(a) Hemoglobin (b) Iron-containing heme group

- **$\alpha_1\beta_1$ and $\alpha_2\beta_2$:**
  confirms stability of the molecule.
- **$\alpha_1\beta_2$ and $\alpha_2\beta_1$:**
  confirms solubility of the molecule.
- **$\alpha_1\alpha_2$ and $\beta_1\beta_2$:**
  permit oxygenation and deoxygenation.

- **α2-β2 or α1-β1** interface has **35 amino acid residues contact**.
- **α1-β2 and α2-β1** have **19 amino acid residue contact.**

- **Hemoglobin has**
- **Outer** relatively **Hydrophillic surface** (Composed of polar a.a **/Provides Solubility**)
- **Interior Hydrophobic** (Made of non polar a.a /insoluble a .a- **Influences Folding**)

STRUCTURE /CHEMISTRY OF HEME

What Is Heme?

- **Prosthetic group of** Hemoproteins
- **Red color pigment**
- **Located interiorly** in **hydrophobic Heme pocket** present in **Globin subunit of Hb.**
- **Metalloporphyrin**

- Chemically Heme is a **Ferroprotoporphyrin.**

Heme Is- Ferroprotophoryin-IX

- Protoporphyrin IX ring + Ferrous (Fe^{++})

Structure Of Heme

- **Structure Of Protoporphyrin IX-**
- **Cyclic substituted Tetrapyrrole ring structure** (I,II,III and IV Tetrapyrrole).

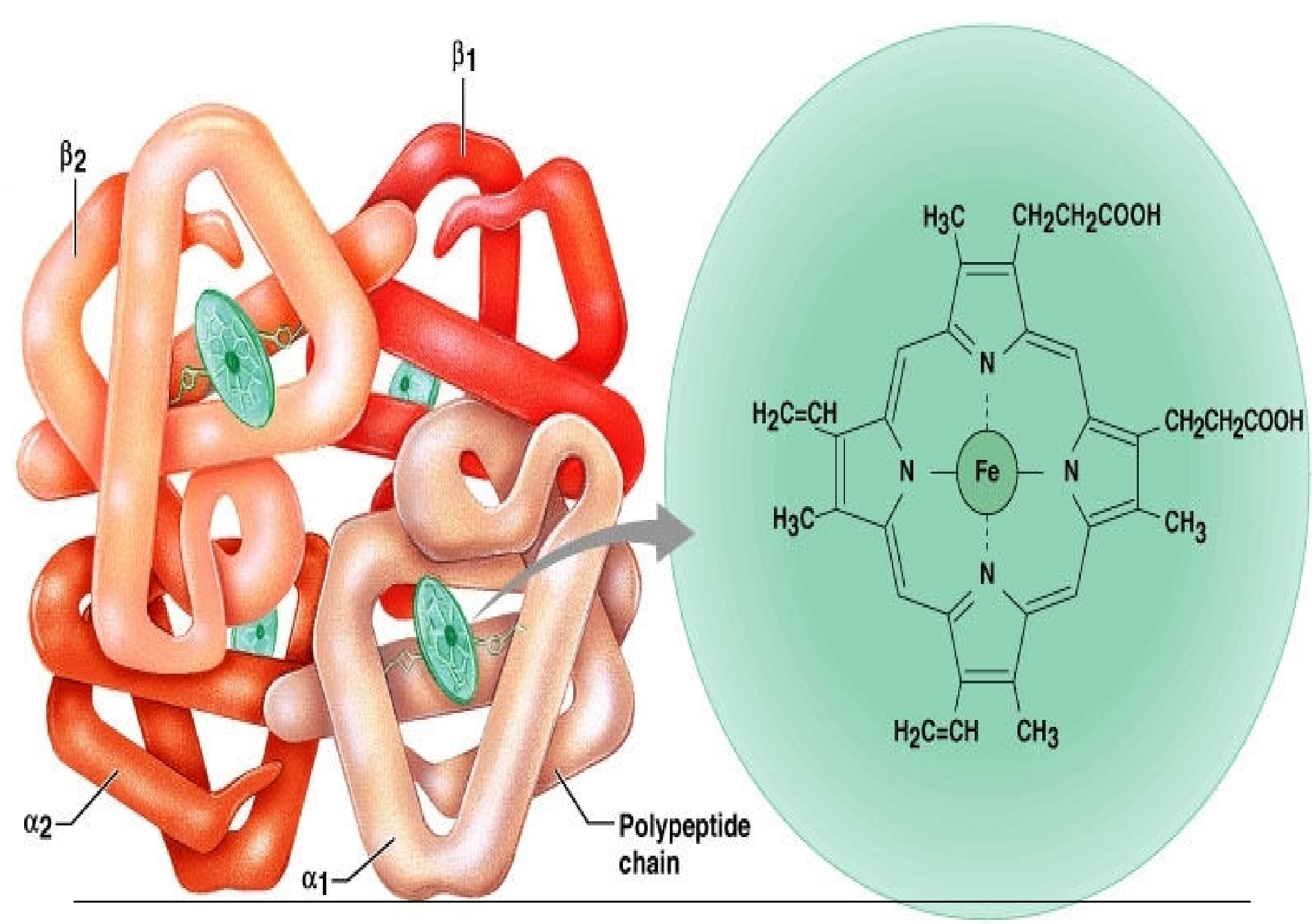
- Tetrapyrrole rings has **substituted groups** in **systematic** manner- **MV,MV,MP,PM**

(M=Methyl ,V=Vinyl, P=Propionyl)

- In Protoporphyrin ring ,Four substituted Pyrrole rings are linked by- **4 Methenyl bridges**

Planar network of **conjugated double bonds of Heme absorbs visible light** →and give **red color to Heme.**

Structure of Heme



(a) Hemoglobin (b) Iron-containing heme group

Iron in Heme

- Functional form Iron in Heme is-
  - **Ferrous form(Fe^{++})**
  - **Reduced state**

- **Fe^{++} located centrally** in Protoporphyrin ring system.

- **Fe of Heme is Hexavalent.**

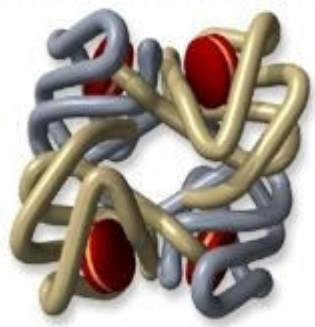
- Fe of Heme forms **6 coordinated bonds to satisfy its six valencies:**
  - **4 bonds linked** with each **Nitrogen of 4 Pyrrole rings.**
  - **5th bond linked** with **Proximal Histidine (F8)** of Globin chain (α Globin=87, β Globin=92).
  - **6th bond is** with **Oxygen**.

- **Binding of Iron in Hemoglobin-**
- **Fe^{++} is bound to :**
  - **4 Nitrogen of Protoporphyrin ring**
  - **Globin chain (Nitrogen of Proximal His)**
  - **Oxygen**

- Iron content of Hb - **3.4 mg / gm of Hb**

Role Of Iron in Hemoglobin

- The Mineral, **Iron, plays indirectly** an important role in the body's
- **Delivery and use of Oxygen by working Muscles.**



- **Iron helps in binding Oxygen** to **Hemoglobin,**
- **Oxygen get bound to Hb** then travels in the blood stream to reach each and every cell of the body.

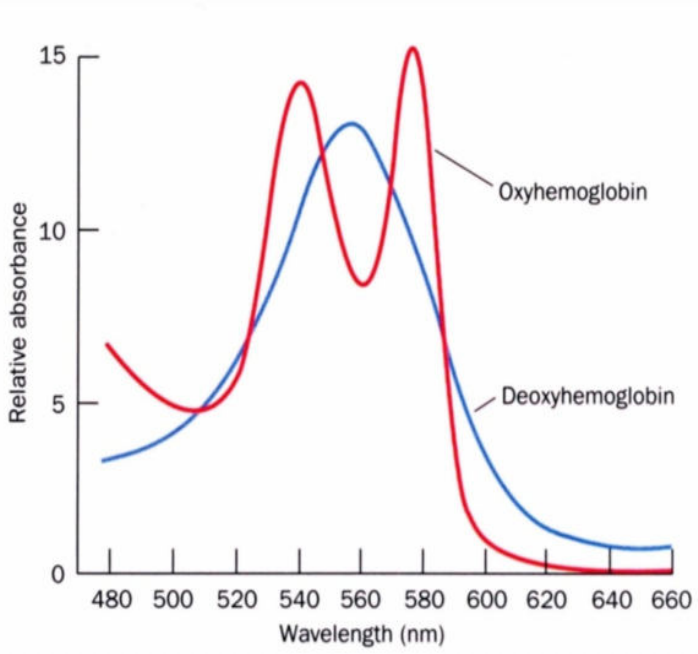
- **Required** amount of Oxygen, delivery to cells
- **Increases** the body's **ability to perform work.**

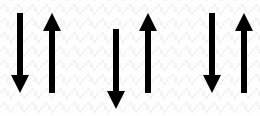
- **Iron supports Aerobic Exercise**
- It has been postulated that a **lack of Iron in the body :**
  - **Reduces Aerobic capacity**
  - **Impair endurance performance of exercise.**

- **Iron of Heme gives red color**

The visible absorption spectra for Hemoglobin

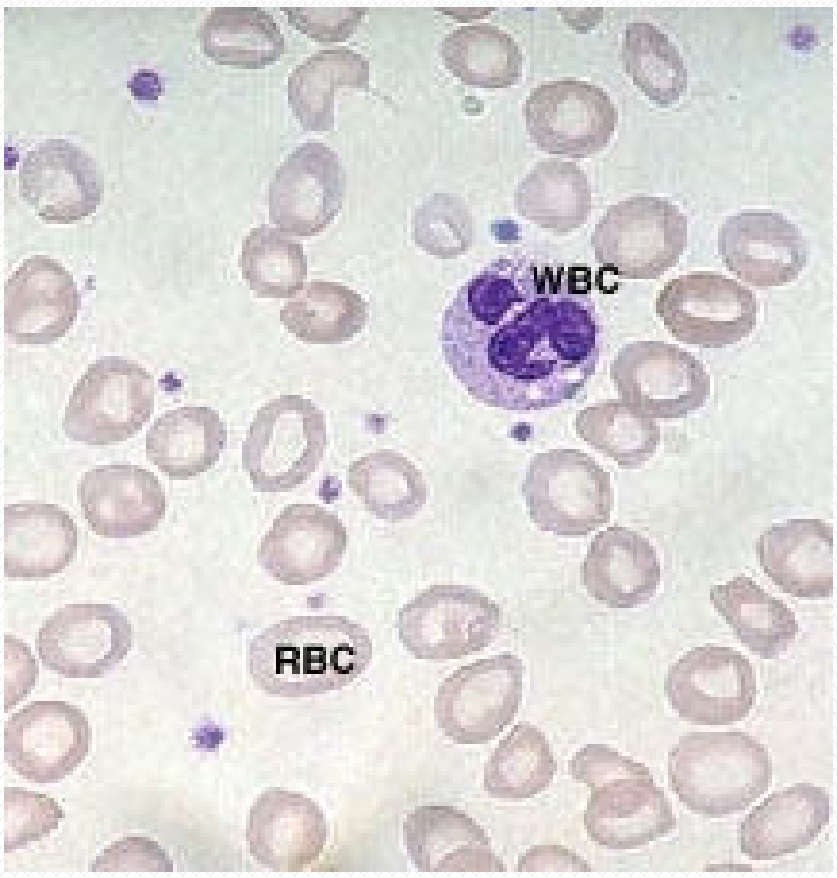
The red color arises from the **differences between the energy levels of the d orbitals** around the Ferrous atom.





Dietary Iron Deficiency

- Features of Iron deficient red blood cells
  - **Low number of red blood cells**
  - **Hollow and blanched red cells**



- **Iron deficiency is related to**
  - **Iron Deficiency Anemia**
- Low dietary Iron
- Low Heme and Hb formation
- Low Oxygen transport and release at tissues and cells
- Low cellular respiration
- Low ETC operation in cells
- Low ATP production in cells
- Low cellular activity

Structure of Globin

Globin Subunits

Adult Hemoglobin has 4 Polypeptide chains

2α and 2β (identical pair).

Alpha Globin chains-

Composition- 141 amino acids

Molecular. Wt = 15,126 Daltons

Biosynthesis-Expression of α Globin gene on 16th Chromosome.

Beta Globin chains-

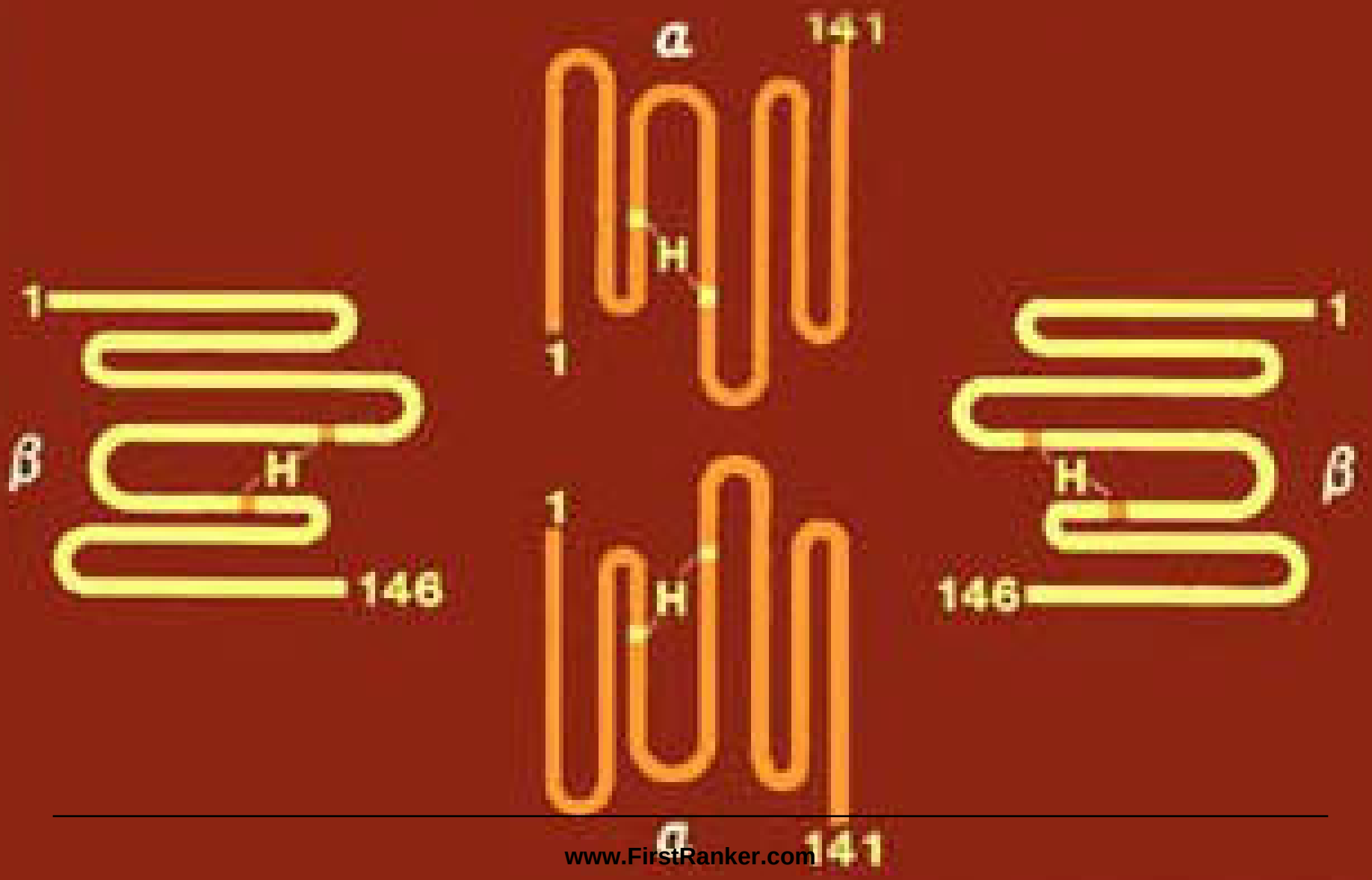
Composition- 146 amino acids

Molecular. Wt =15,866 Daltons

Biosynthesis- Expression of β Globin gene on 11th chromosome.

- **In Hemoglobin –**
- **2α (282 amino acid** residues)
- **2β (292 amino acid** residues)

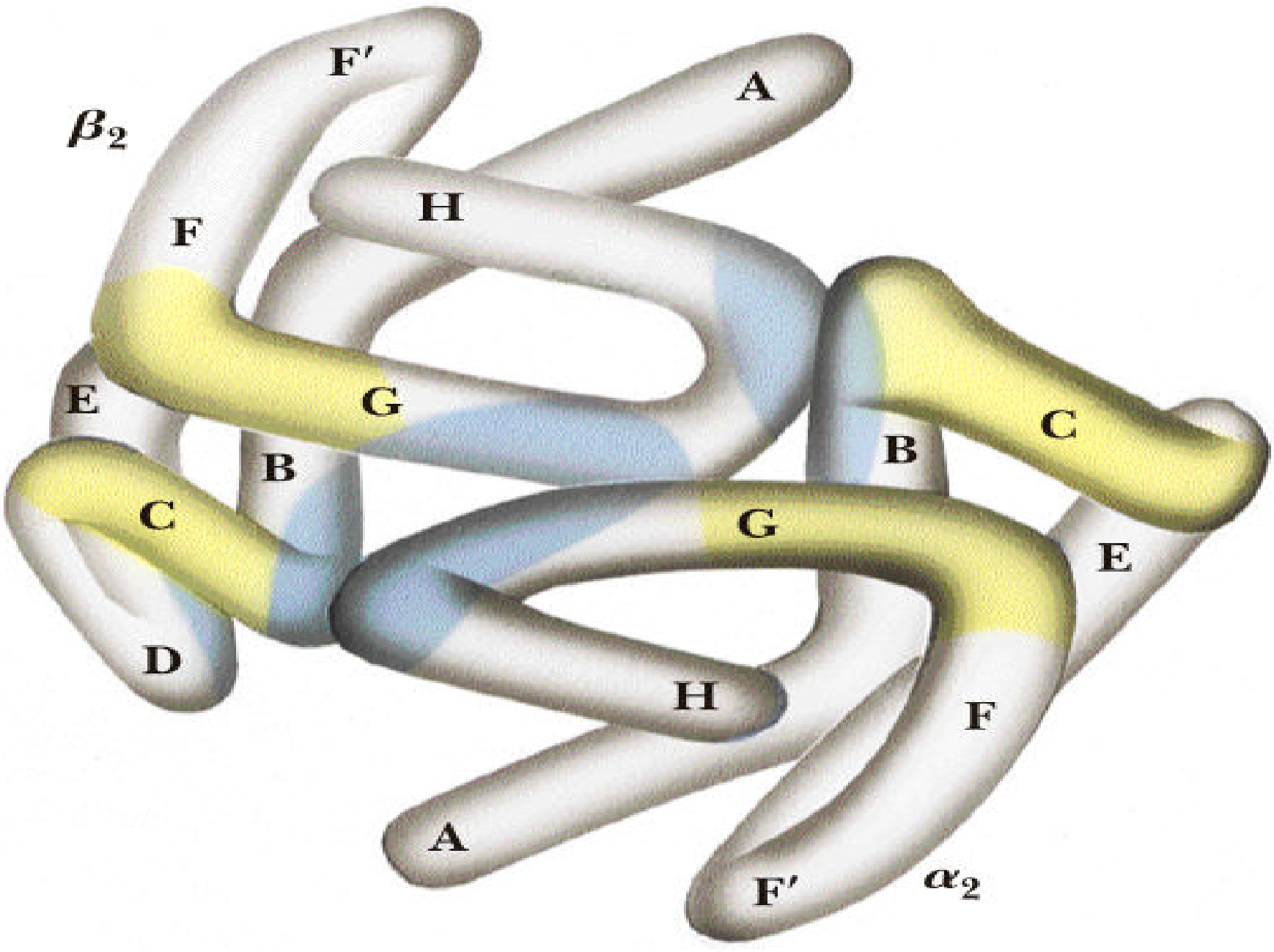
- **Total 574 amino acids are** present in 1 Hemoglobin molecule.



- Each linear Globin Polypeptide chain folds

- To form **3 dimensional Tertiary structure subunit.**

- **Polypeptide chain has 8 Helices** named as A, B, C, …H.



Heme Pocket

- Heme Pocket is a **crevice/ hollow hydrophobic area**
- Formed in the **interior of Globin subunits**
- To **locate the Heme moiety** in it.

- The **Heme pocket** is surrounded by **E , F and H helices** but not with **A , B, C ,D and G.**
- Heme group is **tucked between**

  E and F helices of Globin subunit.

- Amino acids in Globin chain are **identified by**
- **The helix name** and **position of a.a in that helix.**
  - **E7 His (Distal His)**
  - **F8 His (Proximal His)**

- **Distal Histidine-E7** (α 58 ,β 63)
- **Proximal Histidine -F8** (α 87 ,β 92)

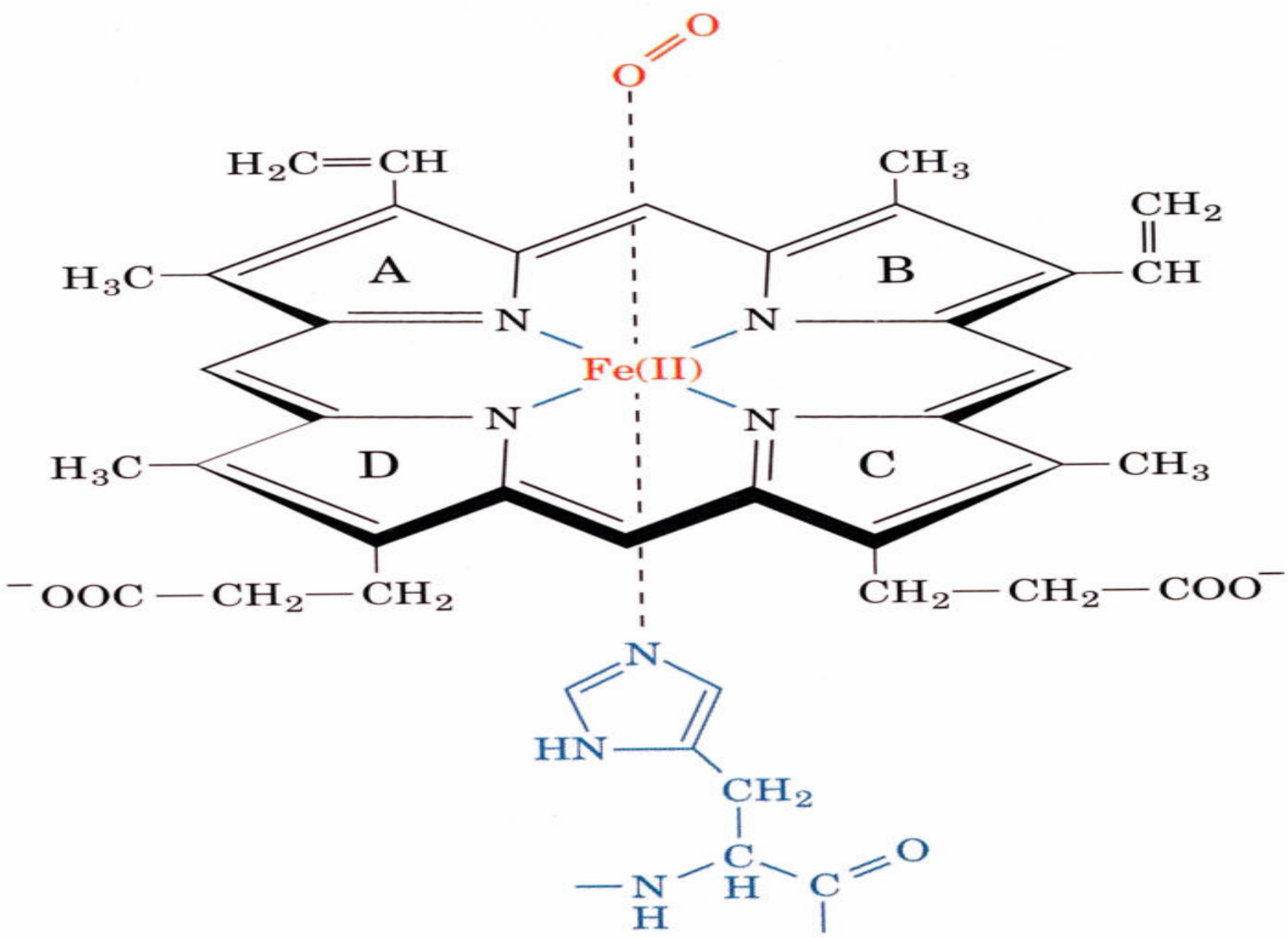
- **Fe^{++}** of Heme is linked to **Proximal Histidine** (F8)
- **O_2** is linked to **Distal Histidine(E7).**

- **Proximal and Distal His are present in Heme pocket**
- In which Heme residue lies and facilitates Oxygen binding.

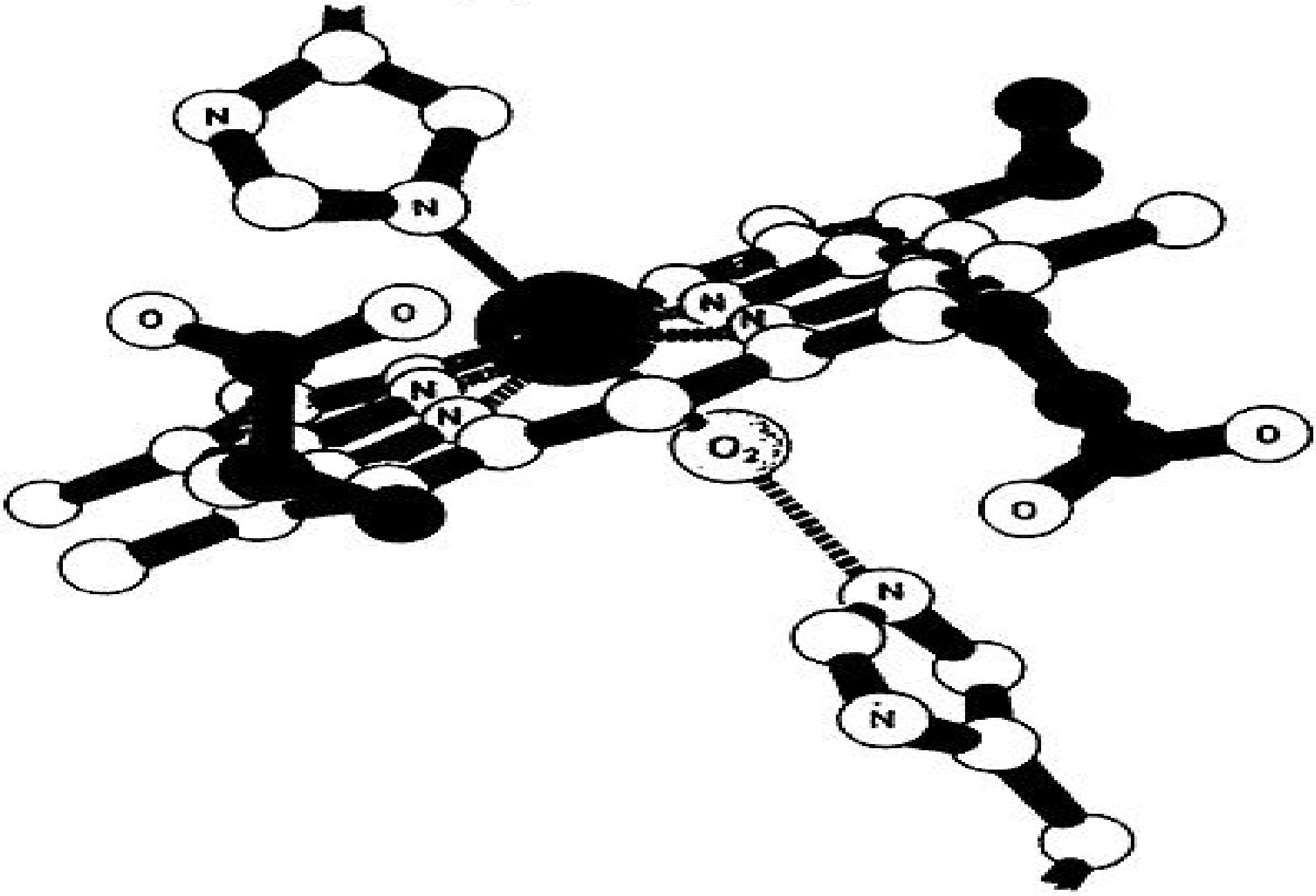
- **Linking of Divalent O2 :**

1. **Fe^{++} of Heme**
2. **Nitrogen of Imidazole group of Distal Histidine of Globin chain(α 58, β 63)**

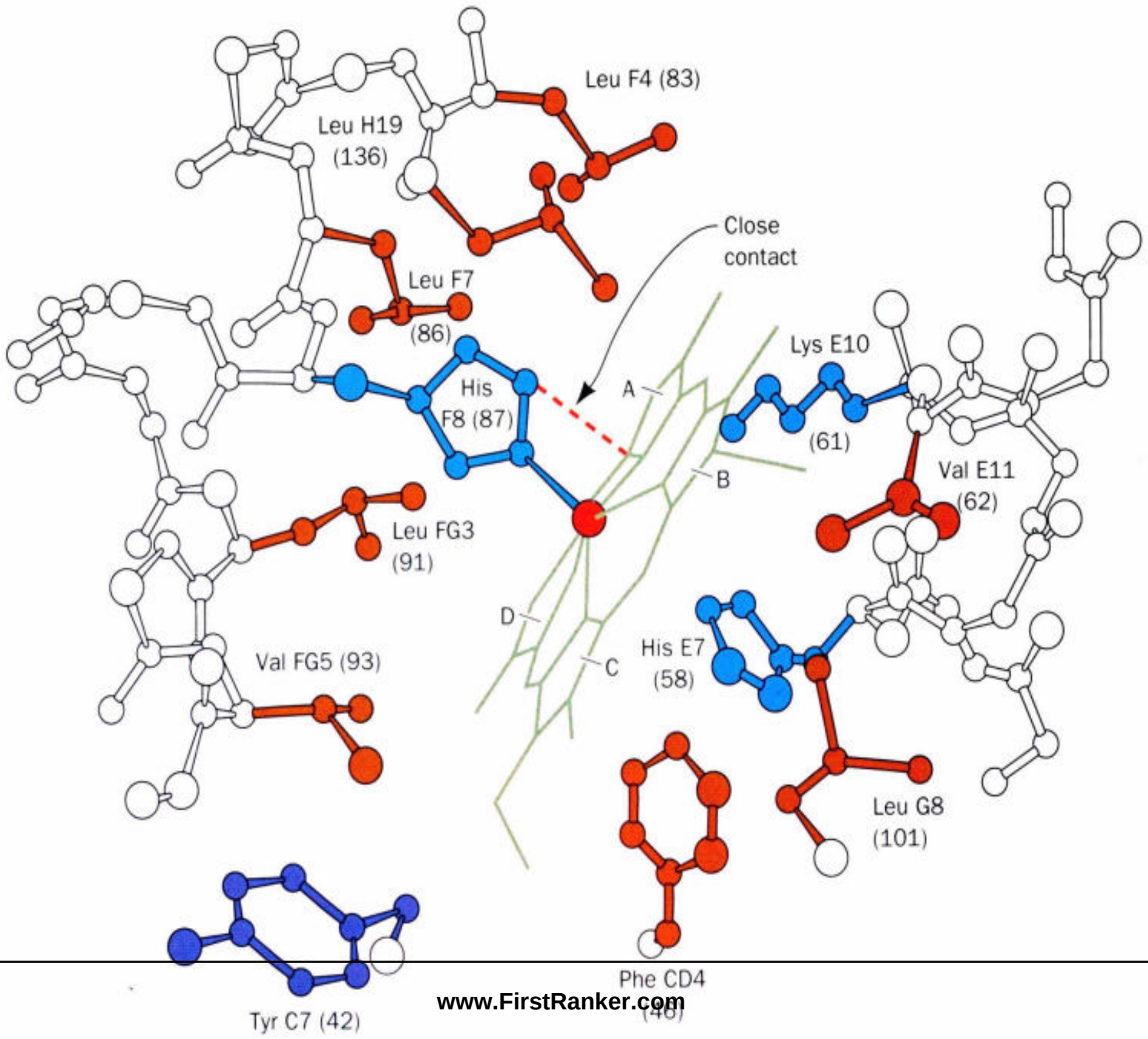
- **Thus to attain stability Oxygen is bound to both Heme and Globin .**



Proximal His (F8)



Distal His (E7)



FUNCTIONS OF HEMOGLOBIN

- **Hemoglobin** has important role in **Respiration mechanism-**
  - **Hb Majorly Transports-**
    Oxygen (97% -100%)
  - **Hb Minorly Transports –**
    Carbon dioxide (15% -25 %)

- **Deoxy Hemoglobin Transports-Protons(H^+)**

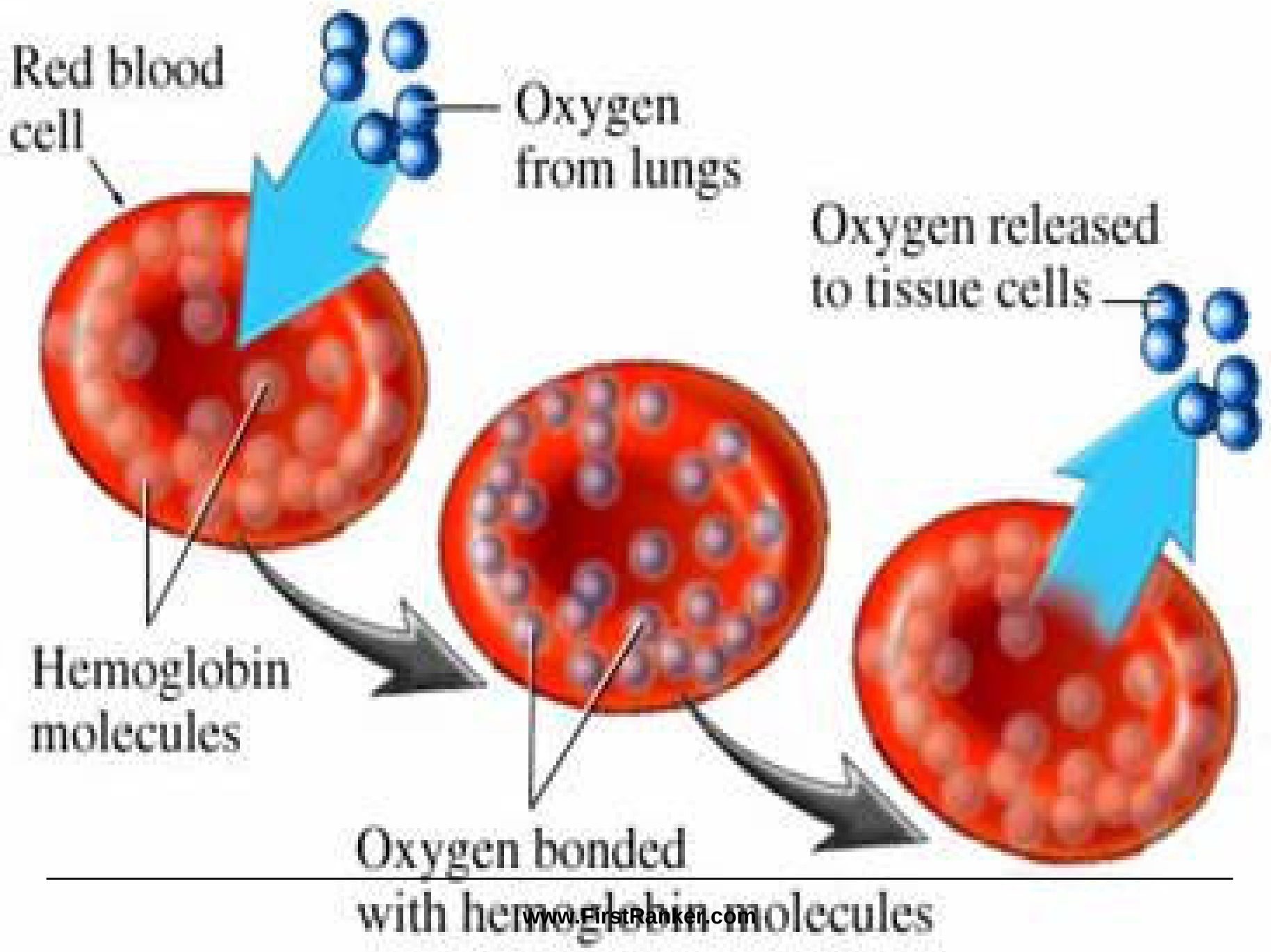
- This is also termed as **Haldane effect**

- **Hemoglobin Plays Role as Buffer-**
- **(Hb/Hb-H^+) in the Erythrocytes**
- Resists change in pH
- **Imidazole group** of amino acid **Histidine** of Hb molecule –
- **Participates** in **buffering mechanism** of Hb.

Role Of Hemoglobin in Respiration

- Since **Hemoglobin** has important **role in respiration** mechanism, it is termed as **Respiratory Protein**.

- **Respiratory Protein** Hb serves in transport and **exchange of gases** **(O_2 and CO_2) between lungs and tissues**.



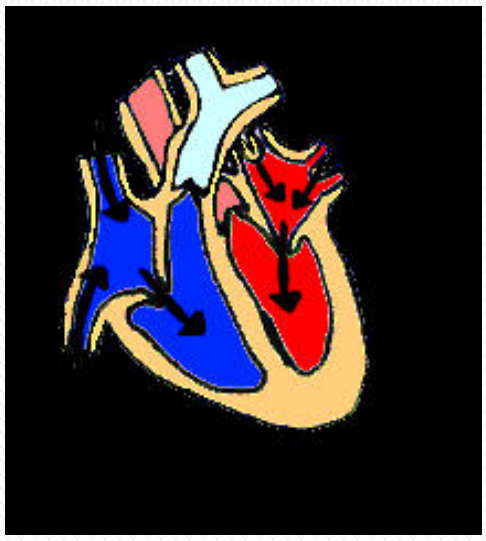
How Significant Is The Presence of Hemoglobin To Human Body?

Why Naturally There Is Presence Of Hemoglobin In the Living Bodies?

Presence of Hb in blood Gives less load to Heart

- Body cells requires approx. **500 gm/day** of **molecular Oxygen**.
- **Molecular Oxygen** is **sparingly soluble** in body fluids.
- This **limits the Oxygen transport in blood** ~~**< 30 gm /day**~~.

- In fact if the body had to **depend upon dissolved Oxygen in the plasma** to supply Oxygen to the cells.



  - The **Heart would** have to **pump 140 liters per minute**.

  - Instead **of normally 4 liters per minute.**

- **Hemoglobin a Polar, Oxygen binding Protein/Oxygen carrying Protein of blood.**

- Increases the binding and **effective transportation of Oxygen through blood.**

- **Presence of Hb** in blood **facilitates** the blood
- To **dissolve** approx **70 times more Oxygen** than the plasma without Hb can do.

- Total Hb present in each RBC
- Carry approx. More than **1 billion Oxygen molecules.**

- Thus to accomplish the following functions Red blood cells has Hemoglobin (Hb):
  - **Transfer of O_2 from lungs to tissue**
  - **Transfer of CO_2 from tissue to lungs**

- Hemoglobin **serve as a vehicle** for **transporting the Oxygen**
- Through blood **to reach each and every cell.**

- Oxygen transported by Hb and reached to every cell is **used up in Mitochondrial ETC**

  (Respiratory Chain/Cellular respiration)

- To **generate ATP**

  (Oxidative Phosphorylation)

SALIENT FEATURES OF OXYGENATION AND DEOXYGENATION OF HEMOGLOBIN

Oxygenation/Loading of Oxygen

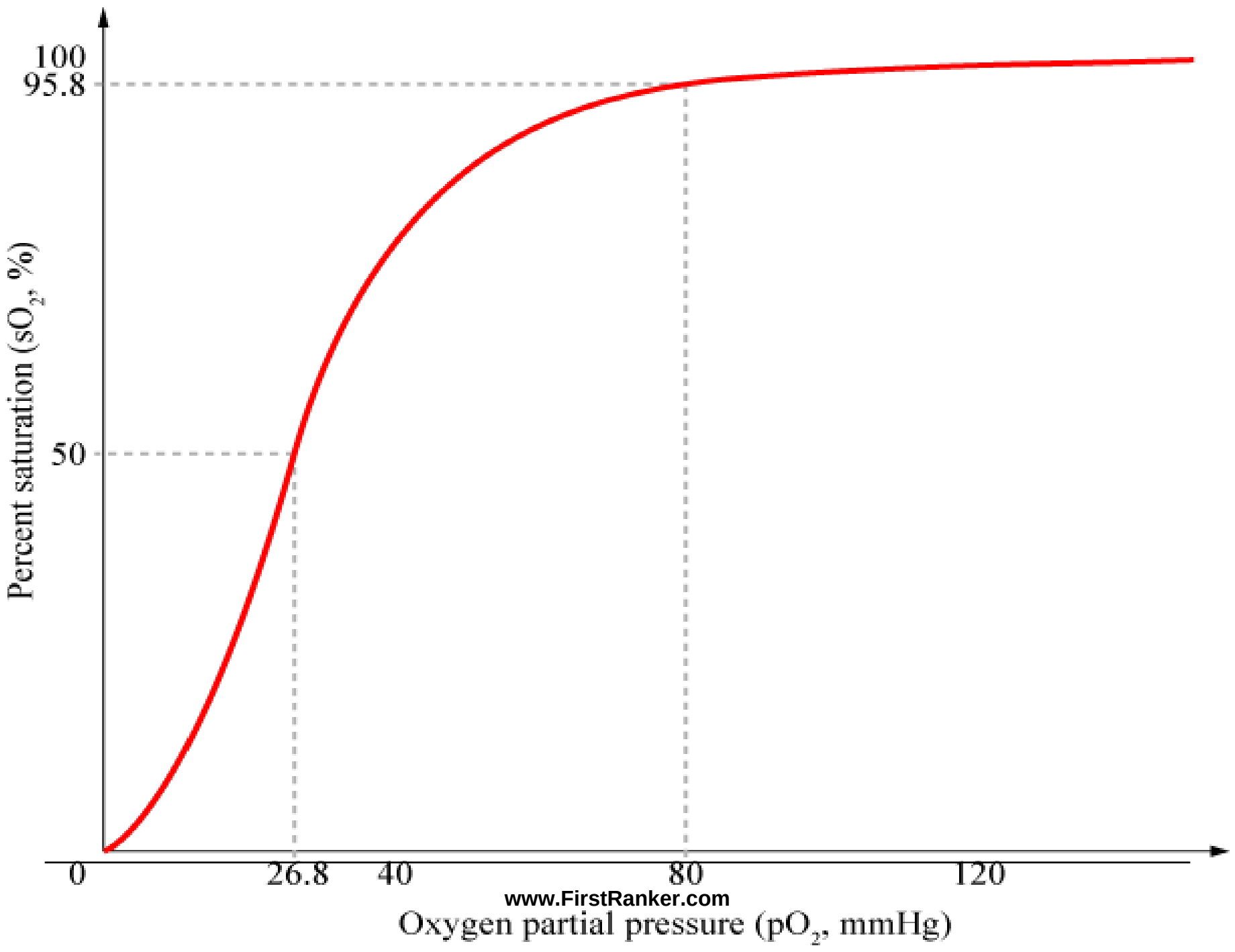
- Hemoglobin gets Oxygenated
  - At Lungs
  - At increased pO_2 concentration (100-120 mm Hg)
  - At decreased pCO_2

Saturation Of Oxygen By Hb

- **Normal ranges of pO2**
- **100-120 mm Hg in arterial blood at Lungs**
- **35-40 mm Hg in venous blood at tissues.**

- **Hemoglobin is 97 % saturated** with Oxygen when it leaves the **Lungs-(Arterial Blood-Oxy Hb).**
- Under resting conditions Hb is about **75% saturated with Oxygen** when it returns-**(Venous blood- Deoxy Hb).**

Pulse Oximeter Is An Instrument That Measures The Percentage Hb Fully Saturated With Oxygen In Arterial Blood



- Thus the **degree of saturation with Oxygen is related to:**
  - **Oxygen tension (pO_2)**
  - **Oxygen requirement for metabolic use at cellular level**

- **Features of Oxygenation of Hb**
- **Oxygen binds with Hb** to form **HbO_2**
  - Oxygen links to **Ferrous** form of Iron, of Heme
  - **Non enzymatically, loosely and reversibly.**

- During oxygenation **One Hb molecule with 4 Heme can bind to four O2 molecules.**

- **Binding of Oxygen to Heme of Hb subunits:**
  - **Is weakly at low pO2**
  - **Is tightly at high pO2**

Rate Of Hb Oxygenation:

- Less than 0.01 sec is required for Hb Oxygenation.

- During Oxygenation **Ferrous** of Heme is **not oxidized to Ferric.**

- **Oxygenation of Hemoglobin** causes
- Considerable **structural conformational change in Globin subunits.**

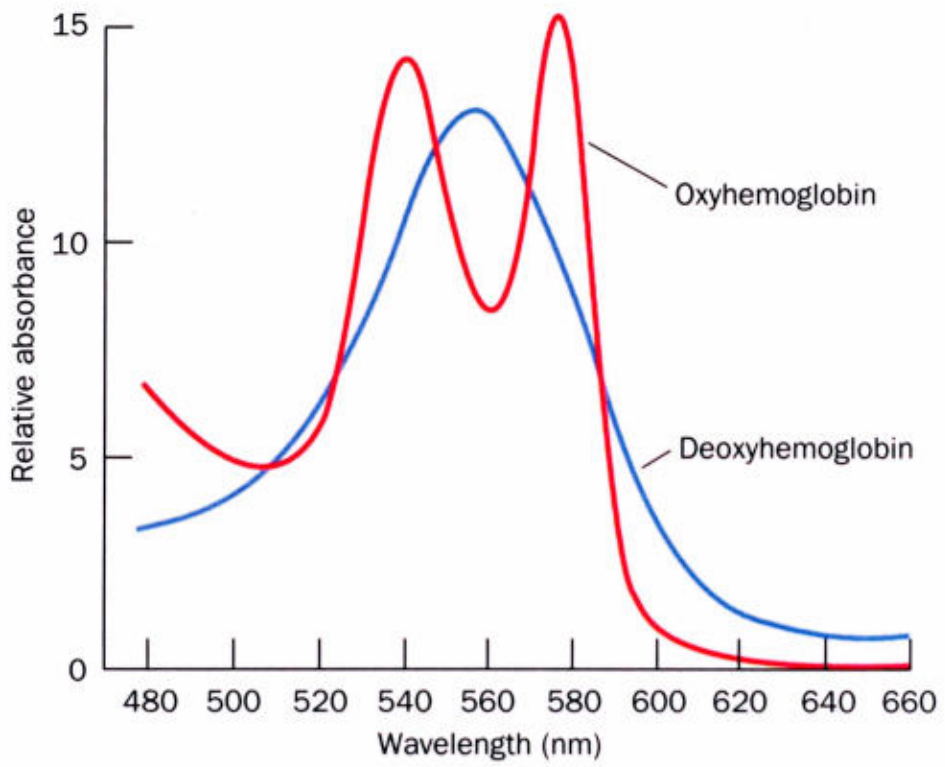
Binding of Oxygen to Hb rearranges the electronic distribution and alters the d orbital energy.

This causes a difference in the absorption spectra.

Bluish for Deoxy Hb

Reddish for Oxy Hb

Measuring the absorption at 578 nm allows an easy method to determine the percent of Oxygen bound to Hemoglobin.



- **Ferric form of Iron** is **non functional form** and cant bind with Oxygen.

Deoxygenation/Unloading or Offloading of Oxygen

- **Hemoglobin gets Deoxygenated**
  - **At Tissues**
  - **With Increased pCO_2**
  - **Decreased pO_2 levels (40 mm Hg)**

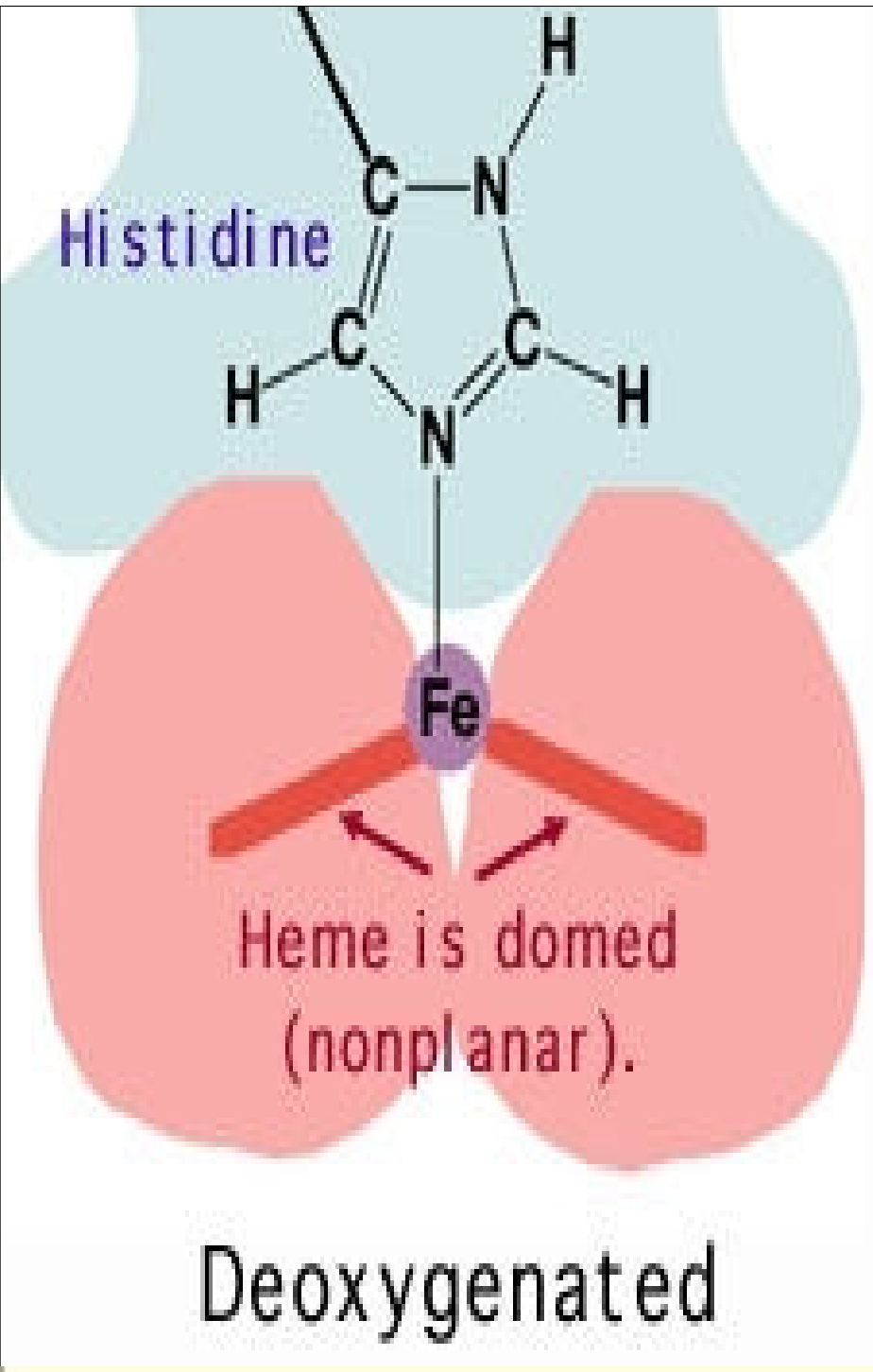
- Deoxy Hb has **2,3-Bis Phospho Glycerate** (23BPG) within it located centrally

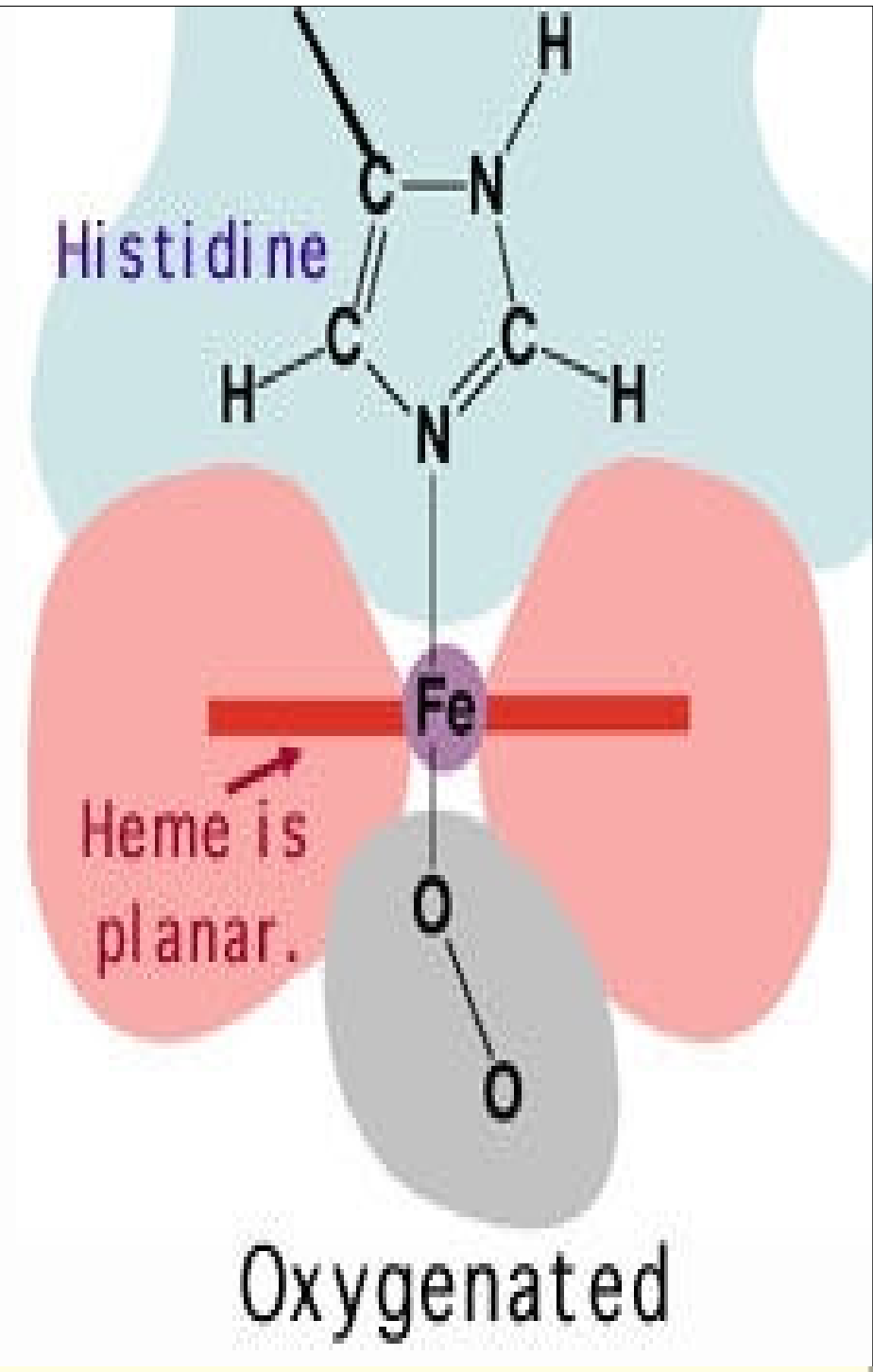
- 2,3-BPG is pushed out of the Deoxy Hb molecule during oxygenation

- **Globin chains move closer** when Hb is **Oxygenated**.

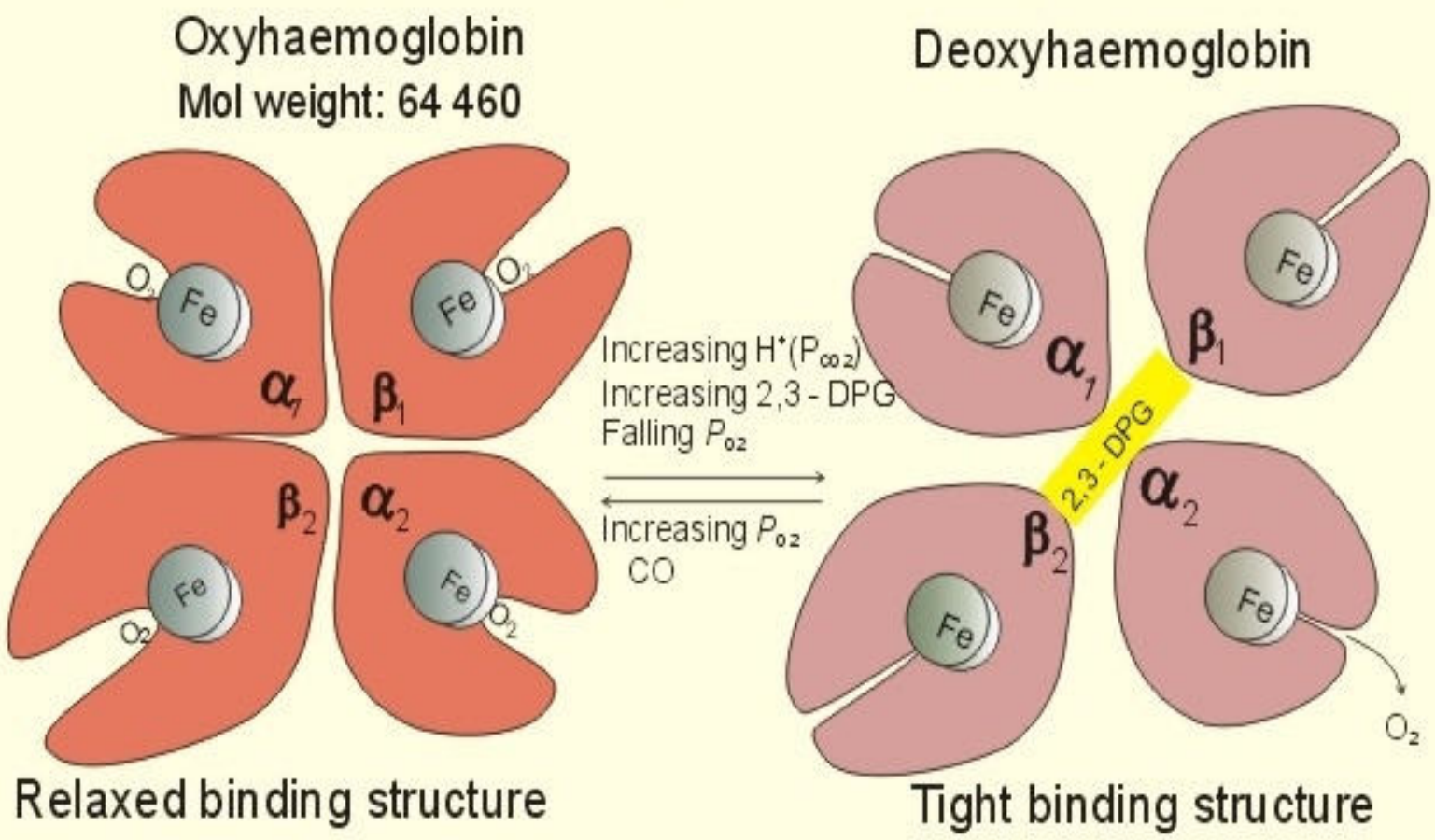
- Globin **chains are pulled apart** when Hb is **deoxygenated**

- This **permits entry of 2,3-BPG** resulting in unloading of Oxygen





Oxygen Binding and Unloading



Normal oxygen binding capacity (20 kPa): 1.34 ml STPD g^{-1} (theoretical: 1.39)

One mol of gas has a STPD volume of 22.4 l. Thus, 1 g of haemoglobin in theory binds: (1/64 460) * 4 * 22 400 ml STPD g^{-1}) = 1.39 ml O_2 g^{-1}.

Arterialized blood contains: 1.34 * 149 (g l^{-1}) = 200 ml O_2 STPD l^{-1}.

Fig. 8-3

KMc

- When **Hb** is fully saturated with Oxygen
- Each **gram** of Hemoglobin is bound with **1.34 ml** of **Oxygen**.

COOPERATIVE BINDING MECHANISM OF OXYGEN WITH HEMOGLOBIN

Oxygen Binds To Hemoglobin with Cooperative Mechanism

Positive Allosteric Effect Of Hemoglobin

- Hb is an **Allosteric Oxygen binder with cooperative mechanism**.

- **Cooperative binding mechanism is due** to **Tetrameric structure** of Hb.

- **Oxygen binding at the four sites to the Heme** of Hemoglobin **does not happen simultaneously**.

- The **binding of the first O2 to one** subunit of Hb.
- **Enhances the binding of futher O2 molecules** to remaining subunits of Hb **with greater affinities.**

- When **Oxygen binds effectively** with **one subunit**

- There **increases** the Oxygen **affinities** for **remaining adjacent subunits**, this is called **positive cooperativity.**

- **Fourth Oxygen molecule** binds to fourth subunit of Hb
- **300 times rapidly** and **tightly** as that of first Oxygen bound to first subunit.

- Thus Hemoglobin is a **remarkable molecular machine**
- That **uses motion** and **small structural changes** to regulate this action.

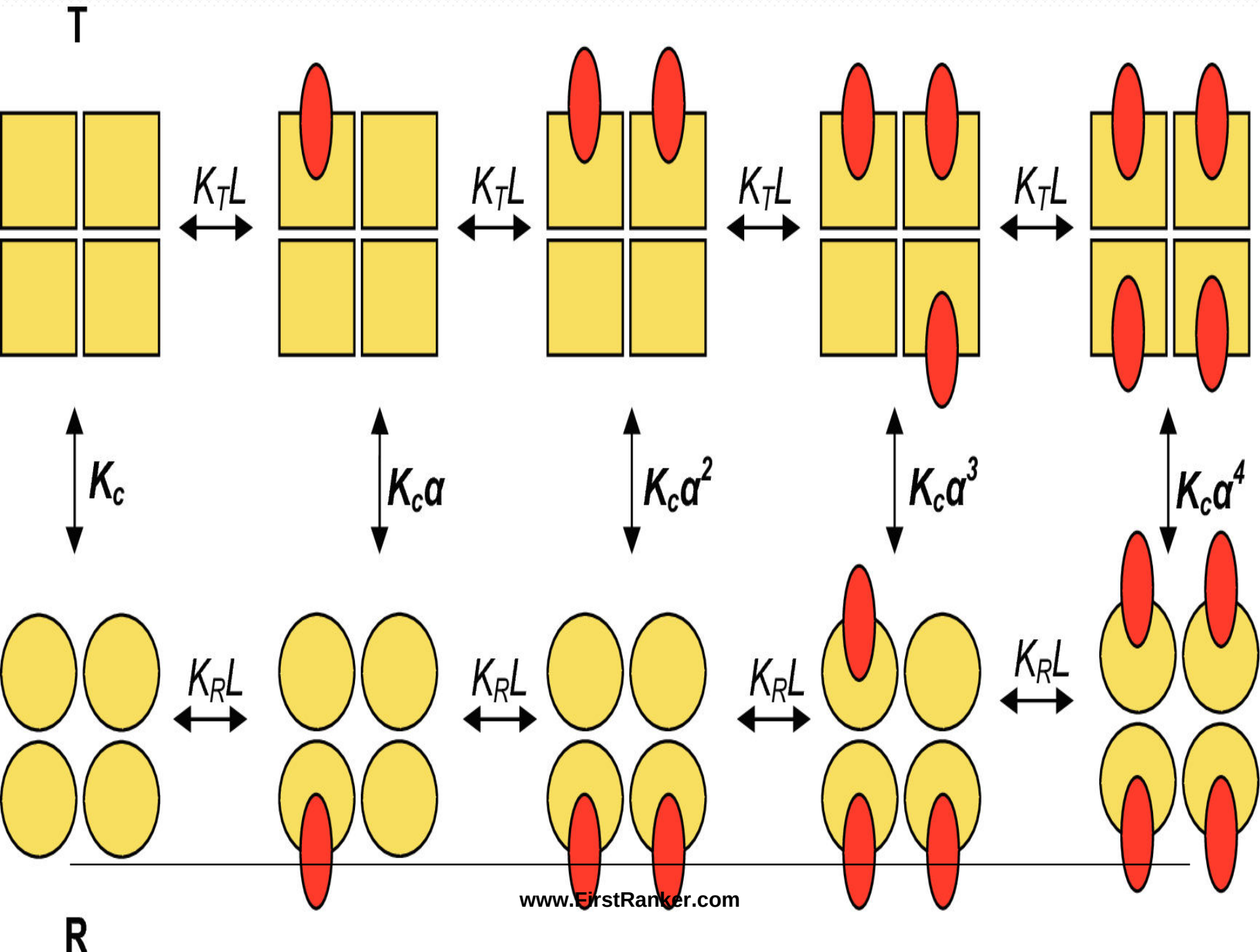
- When a **First Oxygen binds** to Fe in Heme of Hb,
- The **Heme Fe is drawn into the plane of the Porphyrin ring.**
- This initiates a **series of small conformational changes** that are **transmitted to adjacent Globin** subunits.

- **Oxygen ligand binding information** is **transmitted from one subunit of Hb to another.**

- **During Deoxygenation** Hemoglobin **releases its bound Oxygen.**
- As soon as the first Oxygen molecule drops off, the Hemoglobin starts changing its shape.

- This prompts the remaining three Oxygen molecules to be quickly released.

- In this **positive cooperative way**
- Hemoglobin picks up the **largest possible load** of **Oxygen** in the lungs,
- And delivers the Oxygen **where and when** needed.



T AND R FORMS OF Hemoglobin

- During loading and unloading of Oxygen by Hb there occurs **considerable amount of Allosteric movement**.

- This is **due to the Oligomeric /Tetrameric Structure** of the Hb molecule.

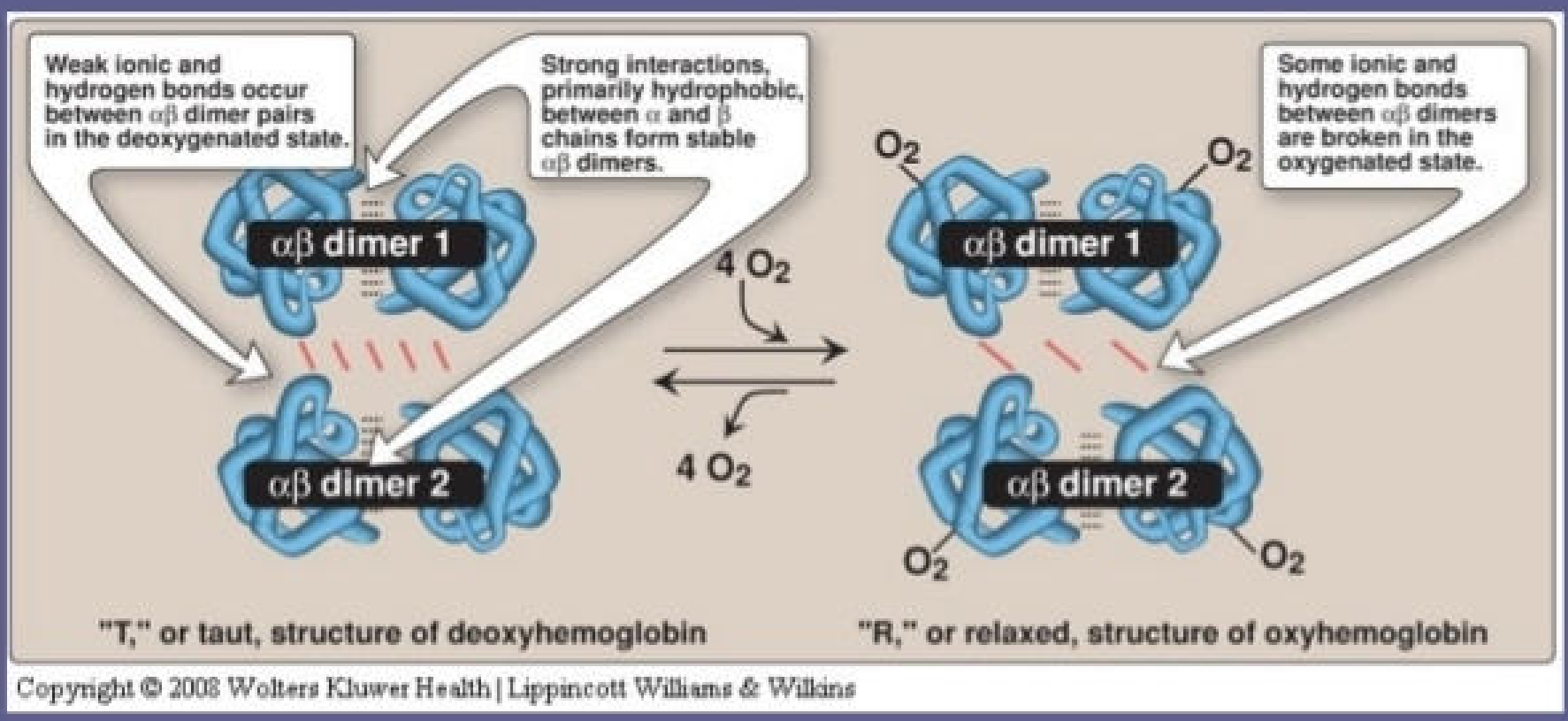
Models for Allosteric Behavior

- **Monod, Wyman, Changeux** (MWC) Model:

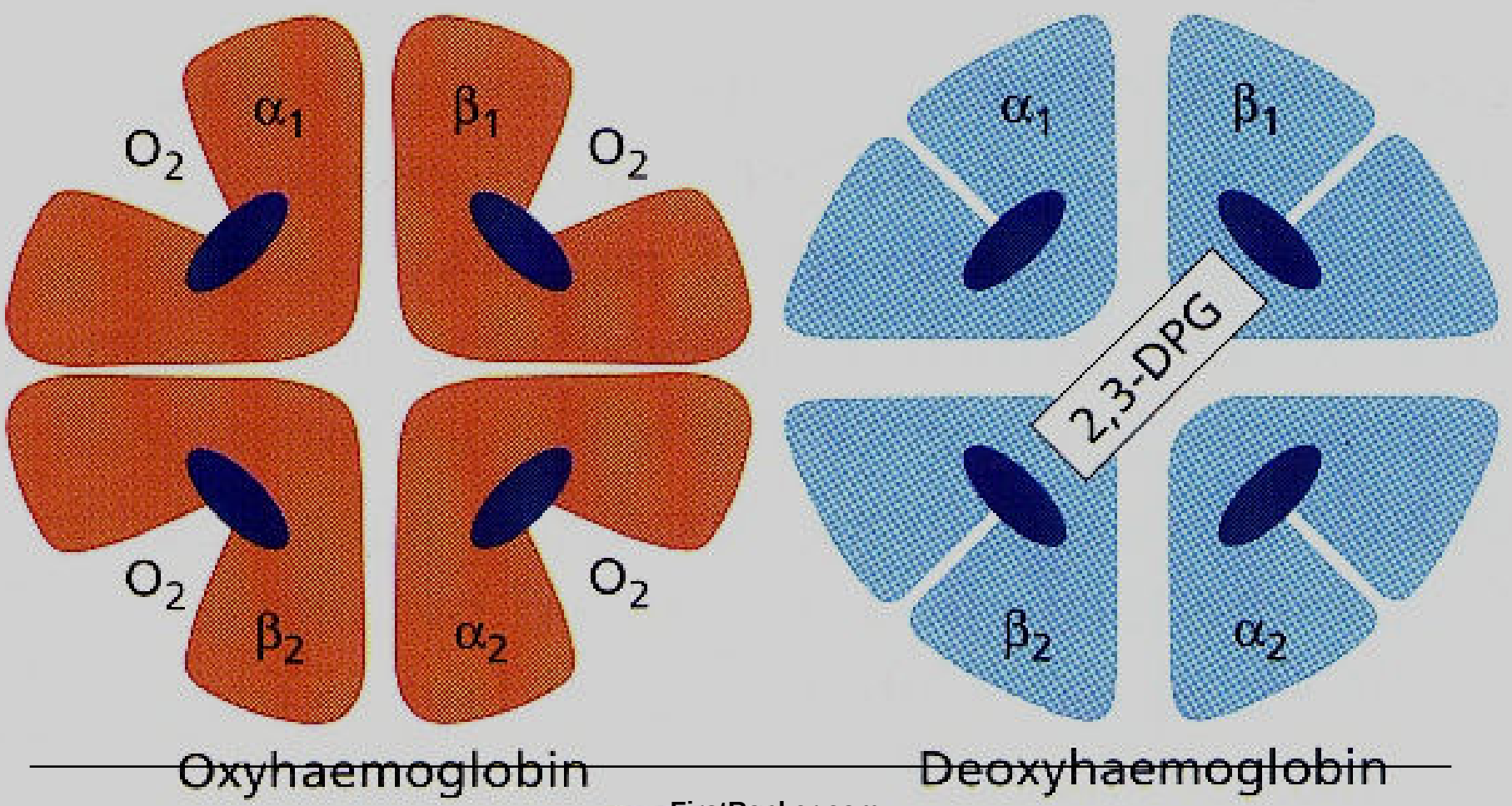
  Allosteric Proteins can exist in two states:

  - **R (Relaxed) State – Oxy Hb**
  - **T (Taut/Tensed) – Deoxy Hb**

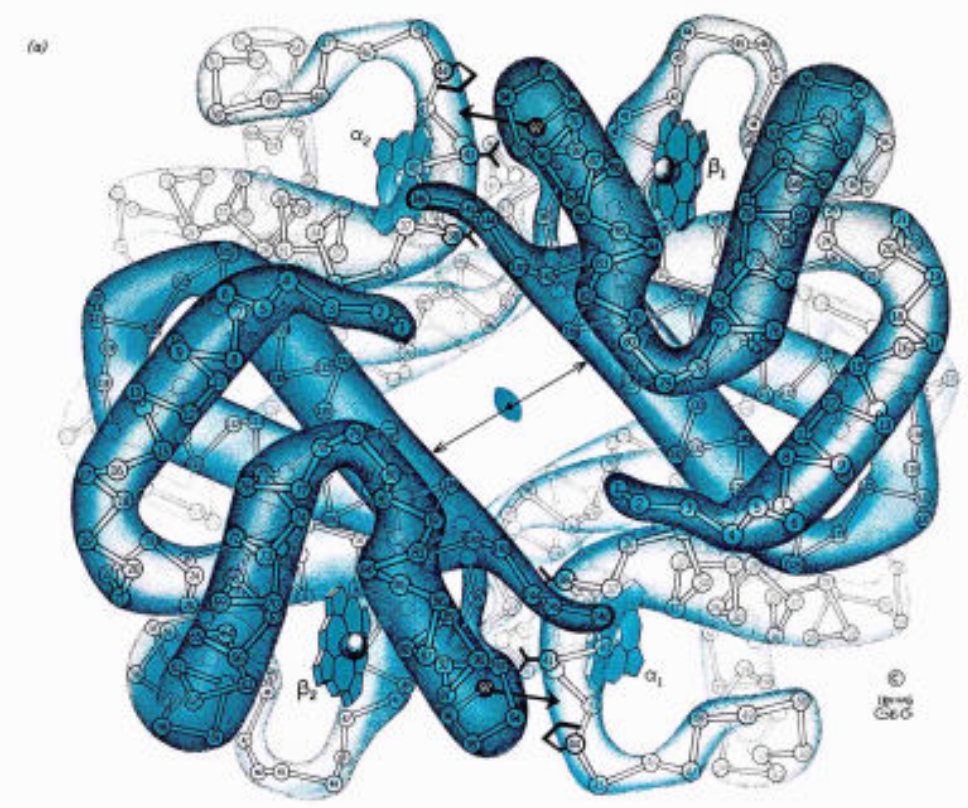
HbA structure



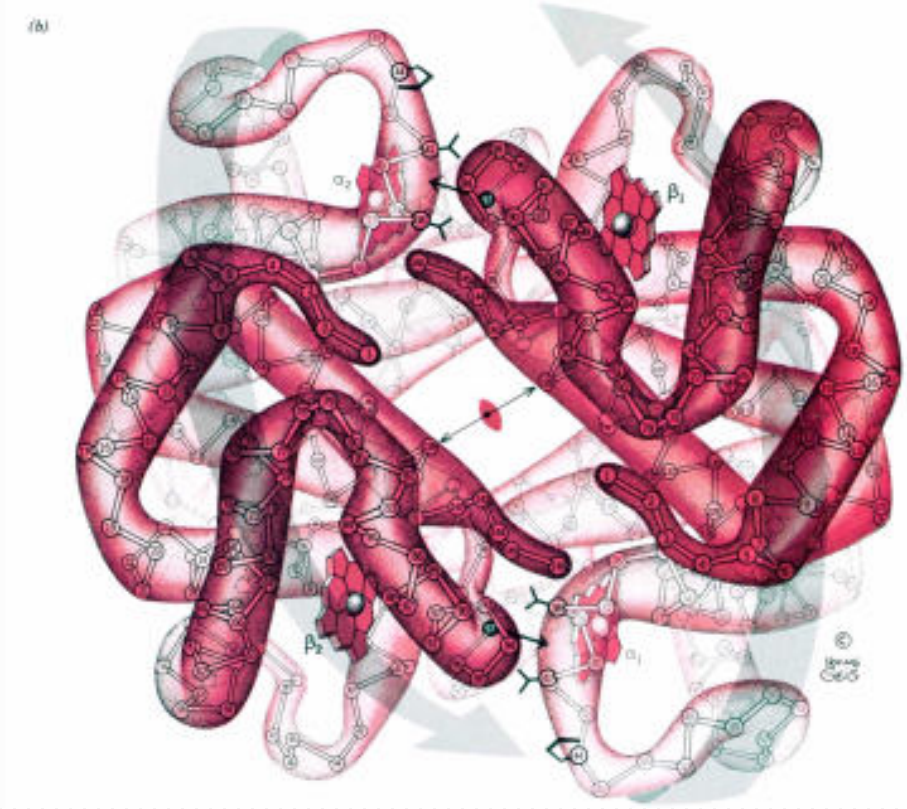
Oxy & Deoxyhemoglobin



Quaternary structure of Deoxy and Oxy Hemoglobin



T-state



R-state

- The conformation of the **Deoxy state of Hb** is called the **T state**
- The conformation of the **Oxy state of Hb** is called the **R state**

T form of Hb

- **Deoxygenated Hemoglobin is T form or Tensed/Taut** form of Hemoglobin conformation.

- **T form /Tensed/Taut form** of Hb has:
  - **Centrally 2,3 BPG**
  - **Hydrogen instead of Oxygen**
  - **CO_2**

  (Illustration Man with Three Tasks)
- These moieties are held together by:
  - **Eight salt bridges/ non covalent** interactions.

- **Thus T form is more constrained form.**

- T form predominates in the absence of O2.

- **T form has lower affinity for Oxygen** in low pO2 environment.

- At the **center** in **T form** of Hb there occupies **2,3BPG molecule which stabilizes the Deoxy state of** Hb.

- Hb has **more affinity for 2,3 BPG when pO2 is low.**

- Hence **R form(OxyHb) at low pO2**
- Gets attracted towards **2,3 BPG**
- Binds with it and looses its Oxygen at Tissues.

R form of Hb

- Oxygenated Hemoglobin is a **R form** or **relaxed form** of Hemoglobin conformation.

- During Oxygenation **salt bridges** of **T form** are **broken**
- **T** conformation is transformed to **R form**.
- **R form is less constrained.**

- **R form** has **higher affinity for Oxygen** in high pO_2 environment.

- At the **Lungs** where **pO2 is high**
- **T form(Deoxy Hb)** has now **higher affinity for O_2, than 2,3 BPG**
- **Hence T form binds with Oxygen, extruding 2,3BPG and get transformed to R**

- In **R form** of Hb
  - **Only Oxygen is bound**

- **R form has No linkage of**
  - **2,3BPG molecule**
  - **Protons**
  - **CO_2**

$$T \leftrightarrow R$$

$$Hb + \uparrow pO_2 \leftrightarrow HbO_2$$

Deoxy-Hb **Lungs** **Oxy-Hb**

- **Increase of** partial pressure of Oxygen (**pO2**)
- Causes the conversion of **T-form to R-form of Hb**.

- **Transformation Of**
- **T to R** form of Hb is at **Lungs**
- **R to T** form of Hb is at **Tissues**
  - **Directly depends upon pO2 concentrations in the environment of body and cells.**

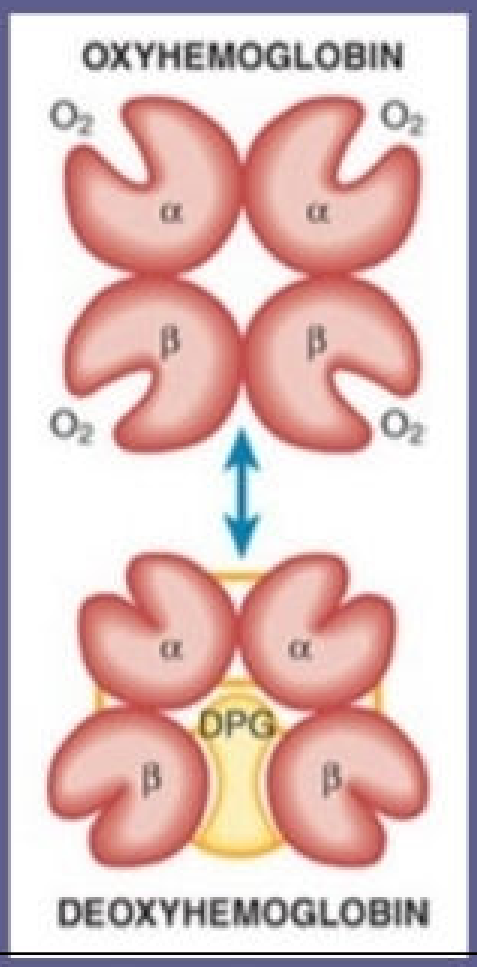
T-form of Hb

_The deoxy form of Hb

_Tout form

_The movement of dimers is constrained

_Low-oxygen-affinity form



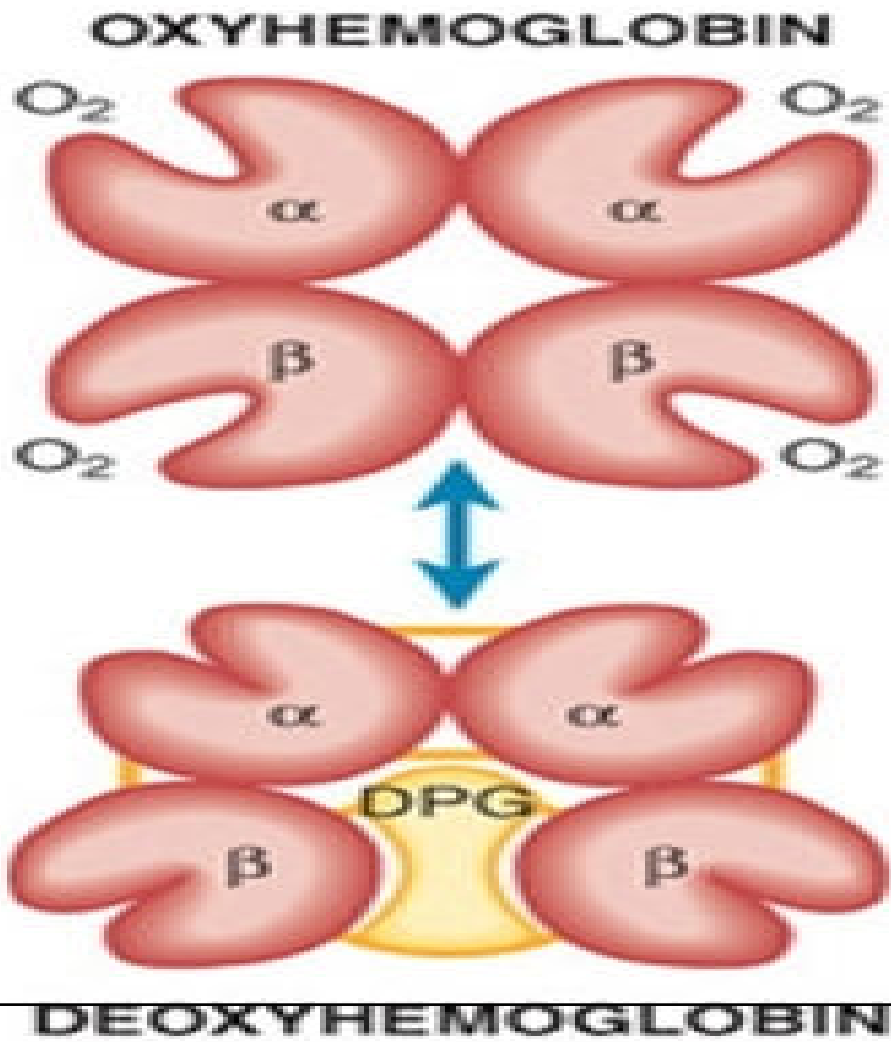
- The conformational changes of Hb from **T** to **R** form and **vice a versa** are known as **"Respiratory movement".**

- **O2 binds** much tighter to **R** than to **T**.

- **R form** of Hb(OxyHb) is more negatively charged.

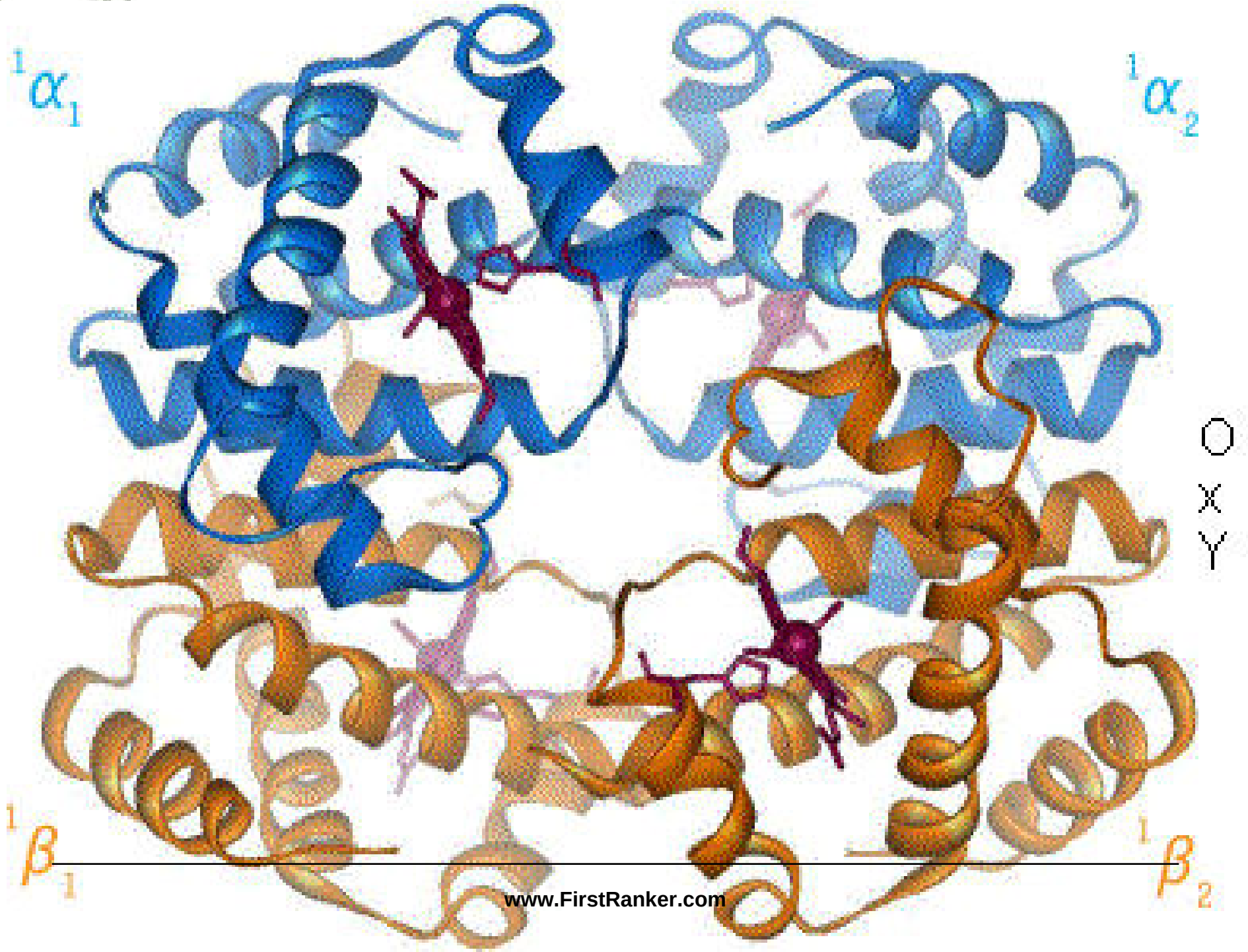
- **T-form** (Tense/Taut) has a **much lower oxygen affinity** than the **R-form**.

Oxy versus Deoxy Hemoglobin

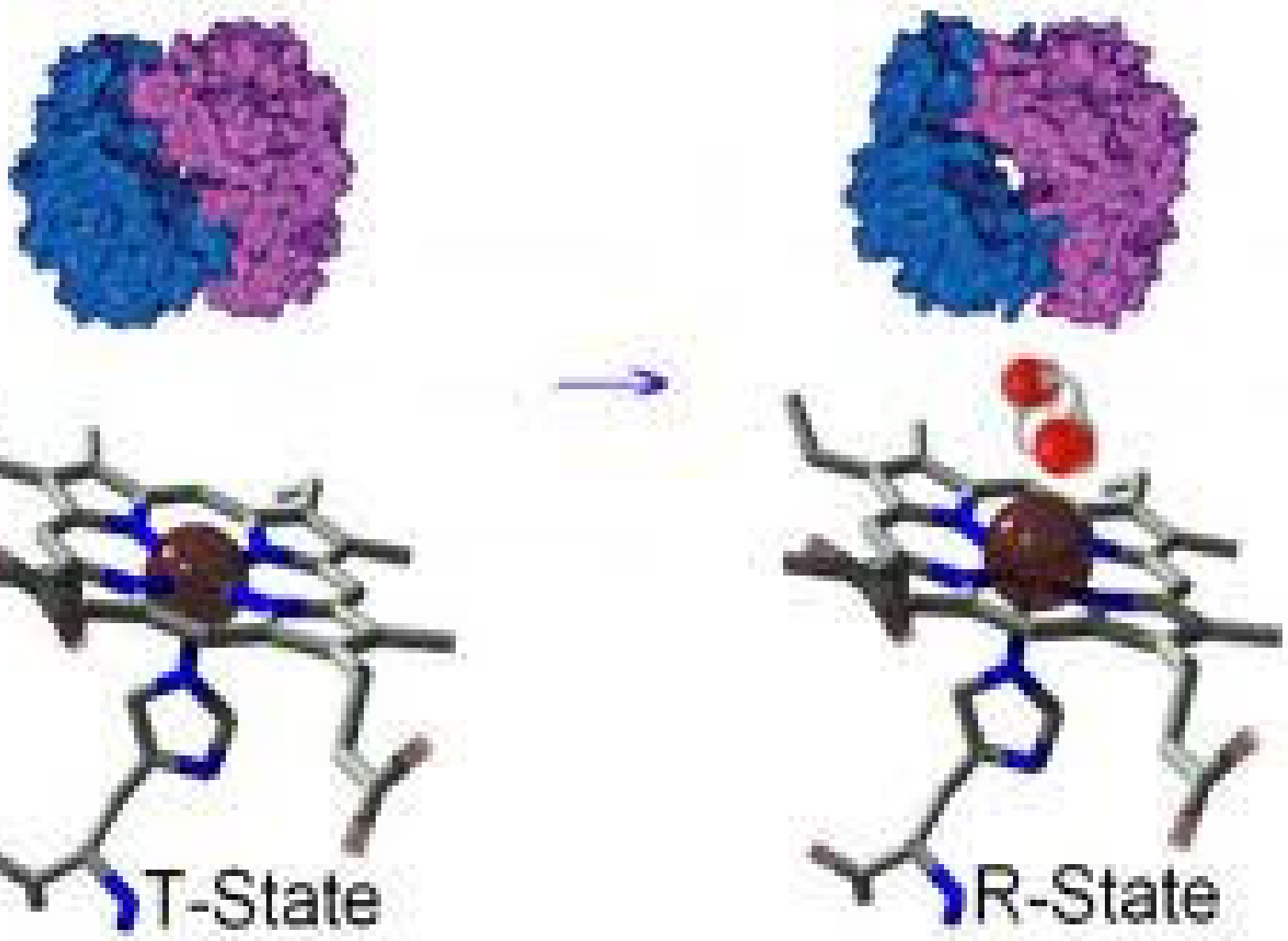


T and R forms of hemoglobin

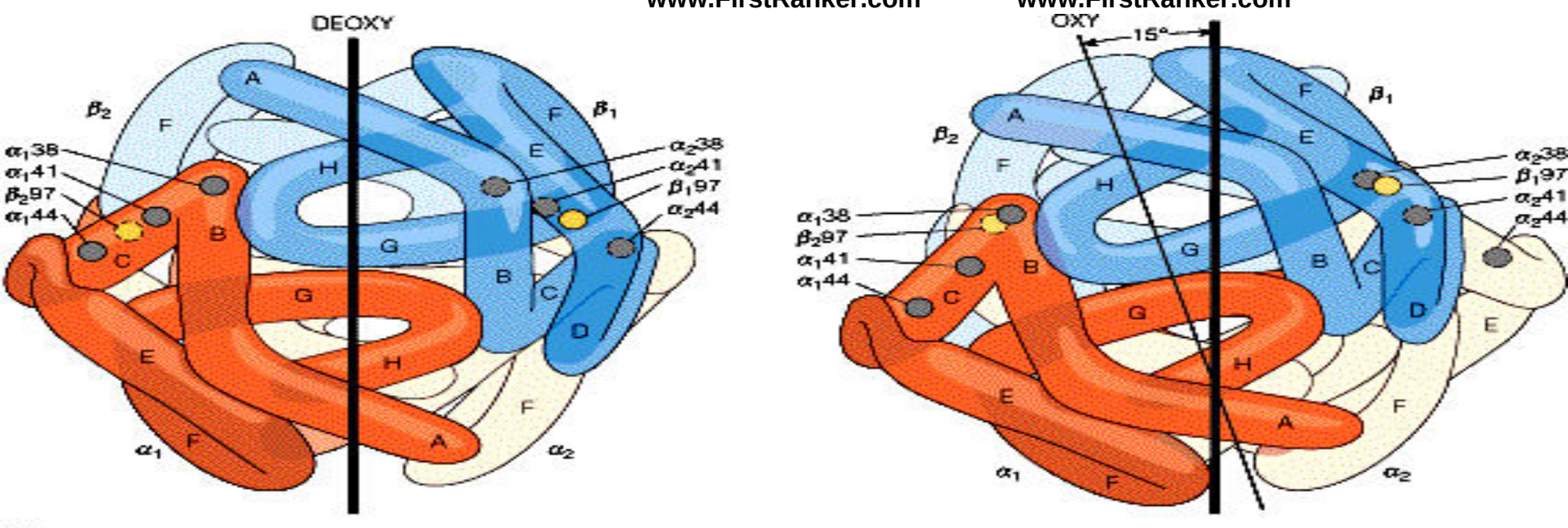
- The four subunits ($\alpha_2\beta_2$) of hemoglobin are held together by weak forces.
- The relative position of these subunits is different in oxyhemoglobin compared to deoxyhemoglobin.
- T-form of Hb:
- The deoxy form of Hb exists in T or taut (tense) form.
- The H & ionic bonds limit the movement of monomers.
- The T-form of Hb has low oxygen affinity.



T→R Conformational Transition

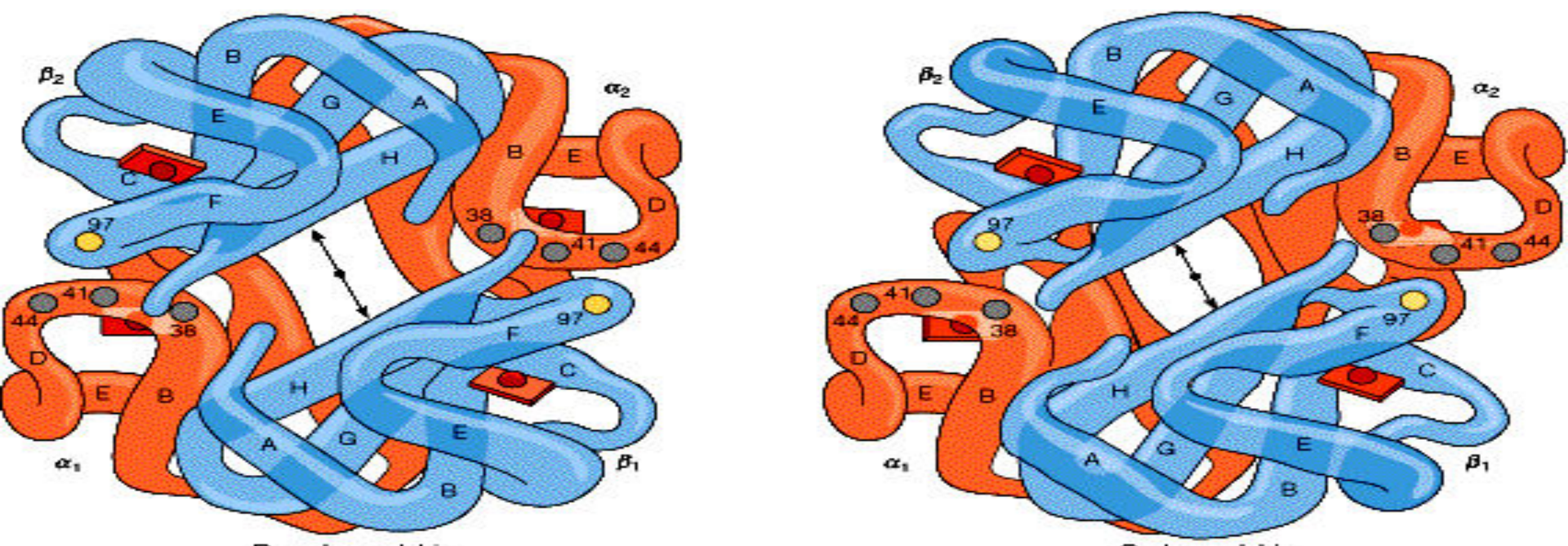


Oxygenation rotates the α1β1 dimer in relation to α2β2 dimer about 15°



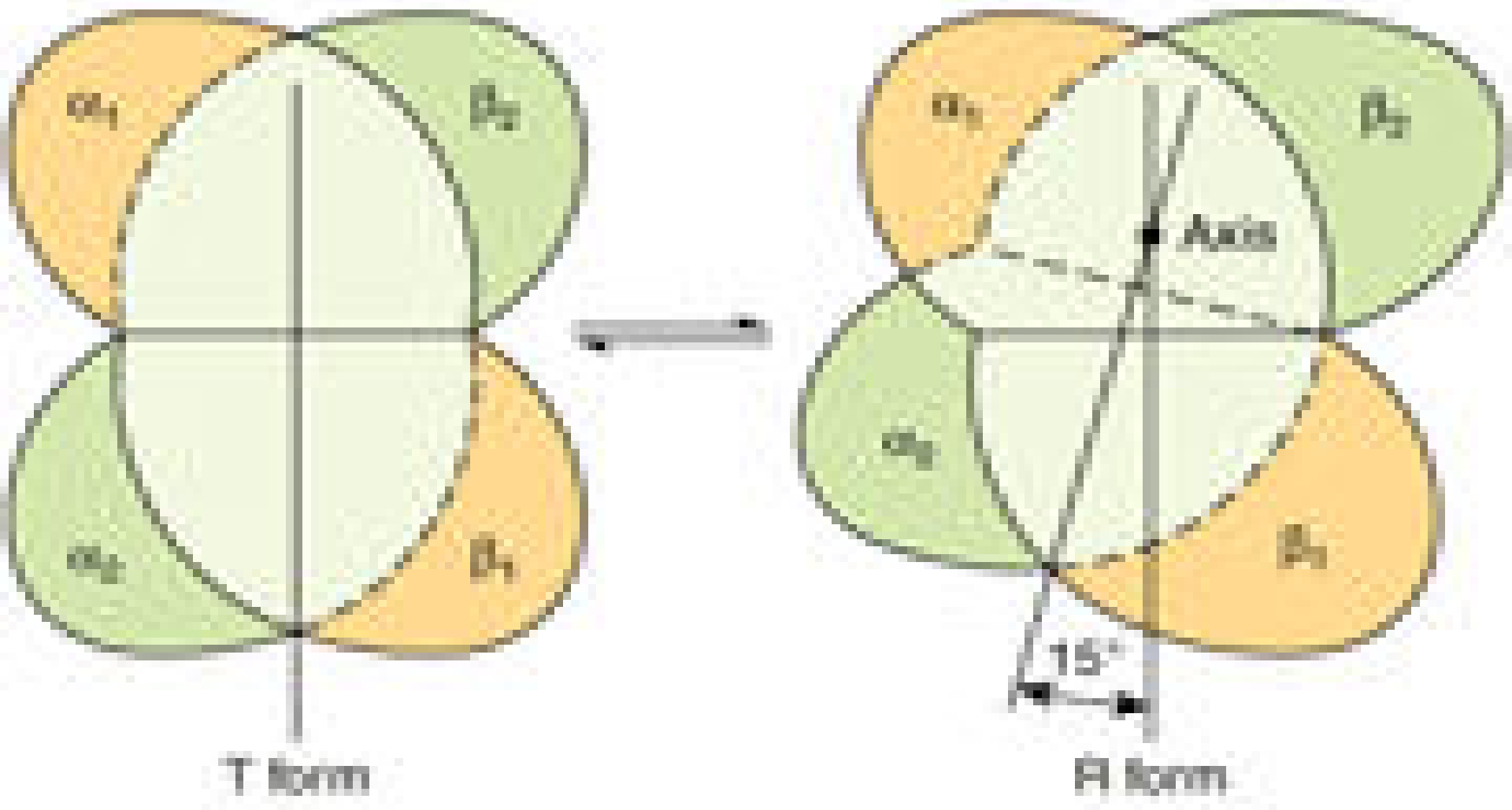
(a)

quaternary changes in Hb upon oxygenation

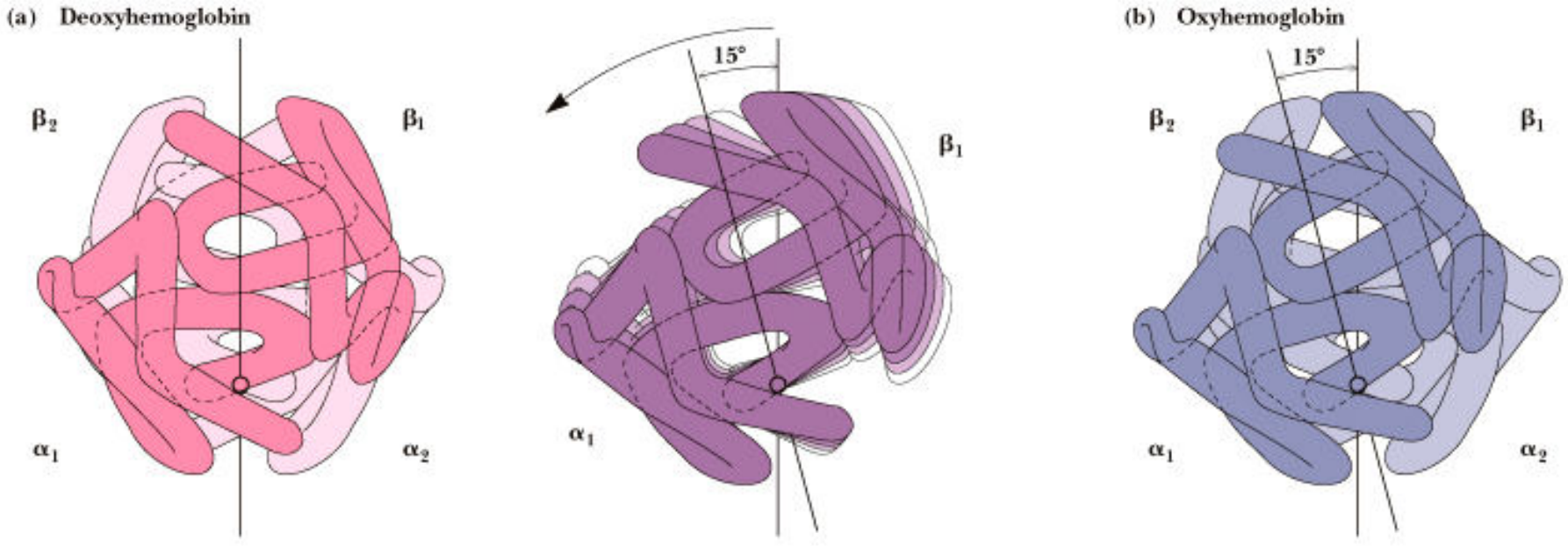


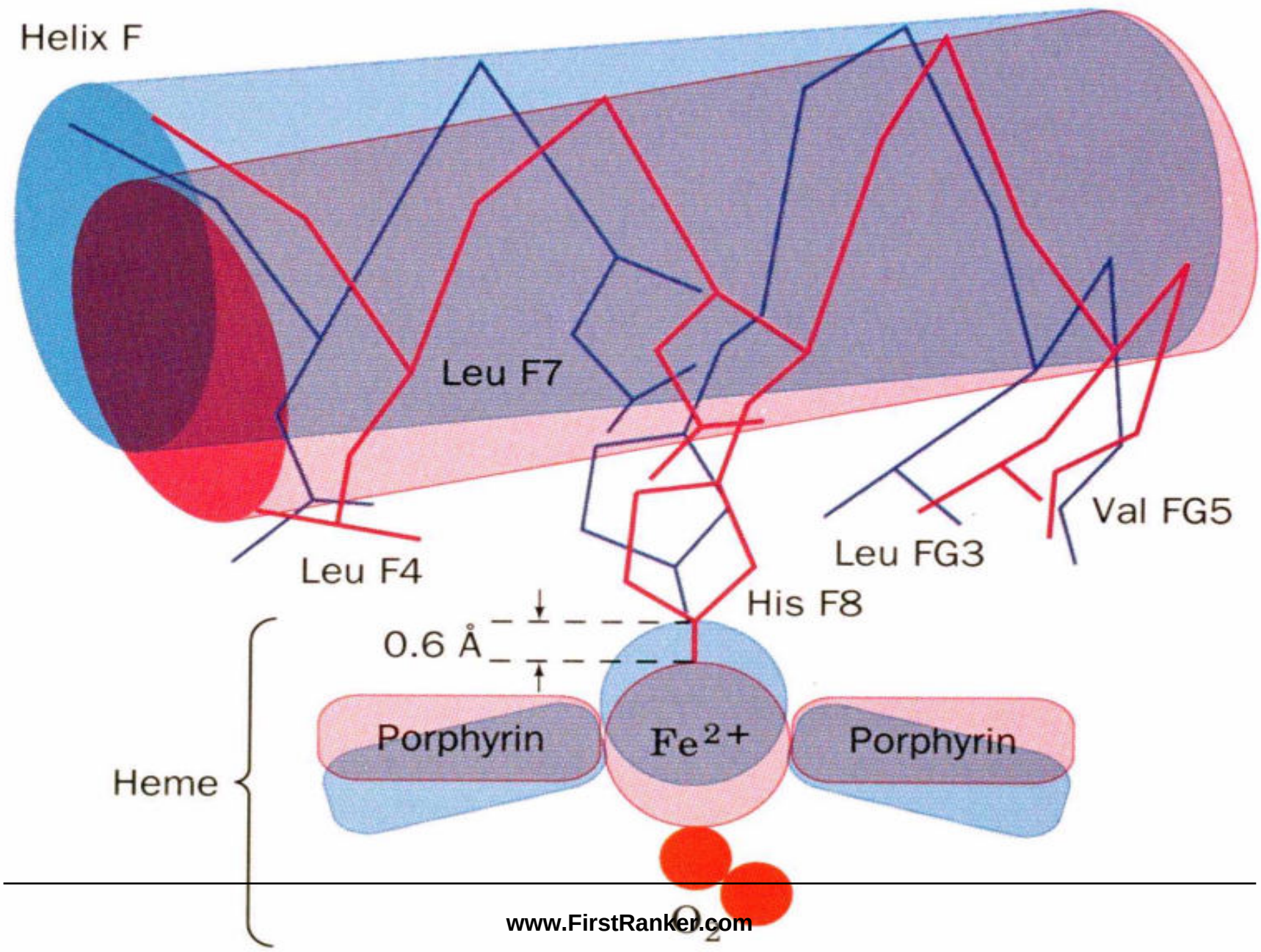
Deoxyhemoglobin Oxyhemoglobin

(b) http://www.aw.com/mathews/ch07/fi7p12.htm



Garrett & Grisham: Biochemistry, 2/e
Figure 15.31





T Form of Hb	R Form Of Hb
Deoxy Hb is in T form binds with CO_2,H^+ and 2,3BPG	**Oxy Hb is in R form binds only with Oxygen**
T form has **8 salt bridges** linked in between the dimer subunits	**Salt bridges are broken** in between the dimer subunits during oxygenation of Hb.
More constrained form	**Less constrained form**
2,3 BPG is centrally located in T form of Hb	2,3 BPG is extruded out from R form of Hb
T form has **low affinity for Oxygen**	R form has **higher affinity for Oxygen**
T form of Hb **predominates** in low pO_2	R form of Hb **predominates** at high pO_2

Illustration

- **Lungs – Class Room**
- **Tissues/Cells– House Environment**
- **Oxygen- Study/Knowledge**
- **Hemoglobin- Student**
- **pO_2 –Teacher**
- **Increased pO_2- Knowledgeable and Skilled Teacher**
- **Decreased pO_2-Poor knowledge and Skill**
- **T form of Hb- Student at House with Dance,Sport,Internet**
- **R form of Hb- Student at Class Room with Study**
- **Oxygenation- Grasping of Knowledge**
- **Deoxygenation- Revision /practice of Knowledge**
- **Metabolic Condition-Examination**

Significance of Tetrameric Allosteric Structure

- Hb being **Tetrameric, Allosteric protein facilitates**
  - **Cooperative binding mechanism** of Oxygen.
  - **Enhances the efficiency** of Hb as an **Oxygen transporter**

- Hb **rapidly bind with oxygen in lungs** where pO2 is high (100 mm Hg)
- Hb **liberate Oxygen at tissue capillaries** where pO2 is low (40mm Hg)

4 Factors Affecting (Allosteric Effectors) Loading and Unloading of Oxygen At Lungs and Tissues

1. pO2 Concentration
2. pCO2 Concentration
3. pH (H^+ Ion Concentration)
4. 2,3 BisPhosphoGlycerate (2,3BPG/2,3DPG)
5. Glucose Concentration

pO2 Concentration

- **At lungs pO2 concentration is high pO2 =100-120 mmHg /torr**
- This **favors oxygenation** and loading of oxygen by DeoxyHb.
- **DeoxyHb (T form)** transformed to form **OxyHb (R form).**

- **At Tissues pO_2 concentration is low 35-40 mmHg /torr**
- This favors deoxygenation and unloading of oxygen by **OxyHb**
- **OxyHb(R form)** transformed to form **Deoxyhb (T form).**

pCO2 And pH

- **At tissues** due to **active metabolism**
- There is **high concentration of pCO_2 and H^+ ion concentration**

(Low pH values).

- **Increased pCO_2 and low pH** at tissues
- Favors the **OxyHb to loose affinity for Oxygen**,
- Which in turn **help in unloading/off loading of oxygen** at tissues

(R form changes to T form).

The Bohr's Effect

Relation of Hemoglobin between pCO_2, pO_2 and pH

- Described by **Danish Physiologist Christian Bohr In 1904**

- The **Bohr effect** is a physiological phenomenon which states that:
- ***Hemoglobin's Oxygen binding affinity is inversely related for both acidity and concentration of Carbon dioxide.***

- Thus The **effect of pCO_2 and pH** on **OxyHb** is known as **Bohr's effect**.

- **Bohr effect facilitates release of Oxygen/Unloading Of Oxygen.**

- Since the tissues are relatively **rich in Carbon dioxide**, the **pH is lower** than in arterial blood;

- **Bohr effect is a manifestation of**

- The **acid-base equilibrium of Hemoglobin.**

- $CO_2 + H_2O \xrightarrow{CA} H_2CO_3 \xrightarrow{CA} H^+ + HCO_3^-$

- **Hydration of CO_2** in tissues and extremities leads to **Proton production**.
- These **Protons are taken up by Hb** after Oxygen released at tissues to Lungs.
- The **Protons transported by Hb are released at the lungs.**

- **Binding of protons to Hb diminishes Oxygen binding to Hb.**
- **Binding of Oxygen to Hb diminishes Proton binding to Hb.**

- **As the Proton(H^+) concentration increases**

- **Affinity of** Hemoglobin towards **Oxygen is reduced**.

- **At acidic pH** (More H^+ ion concentration)-**Favors unloading of Oxygen from OxyHb**

- **At alkaline pH**(Less H^+ ion concentration)-**Favors loading of oxygen to Deoxyhb**.

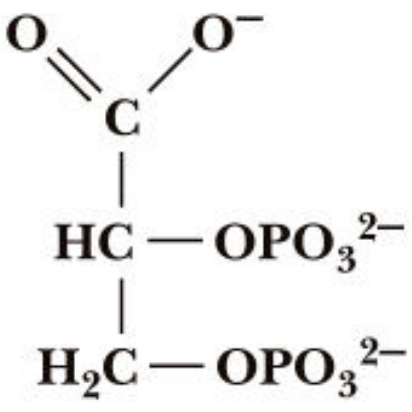
- At **lungs low pCO2** and low **H^+.**
- **Favors oxygenation** or loading of Hb by O2.
- **Deoxyhb transports H^+(protons) from tissues to lungs.**
- **On oxygenation of Deoxyhb, the protons are liberated at lungs.**

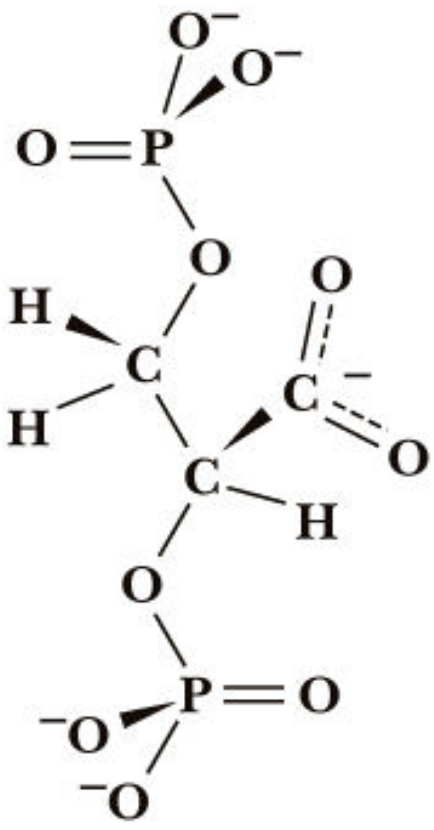
Effect Of 2,3BPG on Loading and Offloading Of Oxygen by Hb

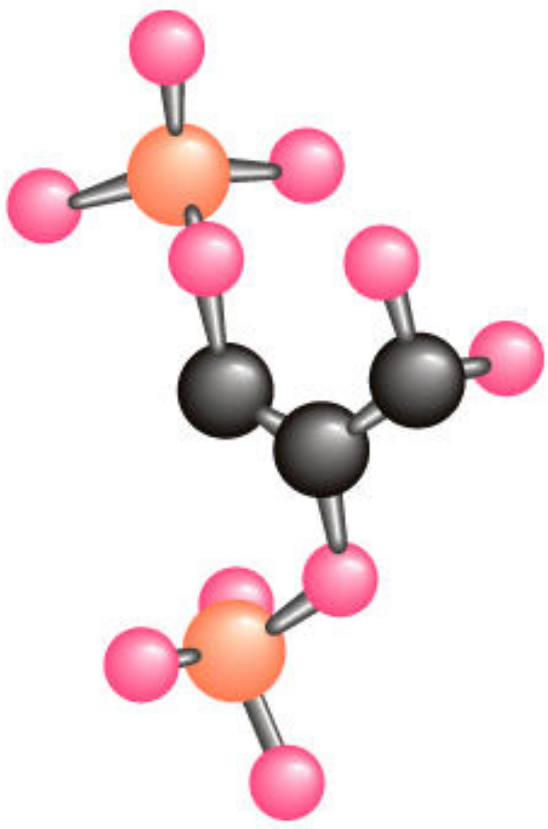
2,3 Bis Phospho Glycerate

- **2,3 Bis Phospho Glycerate** (2,3BPG/2,3DPG) is an **intermediate** of **Rapaport Leubering cycle**
- Related to **Glycolysis inside mature Erythrocytes.**

Garrett & Grisham: Biochemistry, 2/e
Figure 15.36







- **2,3BPG is impermeable to RBC membrane.**
- Glucose metabolism in Erythrocytes **increases the concentration of 2,3BPG**.

The "Inside" Story……

- **Where does 2,3-BPG bind ?**
  - "Inside"
  - In the **central cavity of Hb molecule.**
- **What is special about 2,3-BPG ?**
  - Negative charges interact with **2 Lys, 4 His, 2 N-termini of Globin**.

- **At low pO2, 2,3BPG has high affinity for adult Hb**.

- **Increased 2,3BPG levels** –favors Oxygen unloading by Hb.

- **Decreased 2,3BPG levels** -favors Oxygen loading by Hb.

- The **T form of Hb** has **2,3 BPG centrally located**

- **Which lowers the affinity for Oxygen**.

- As the partial pressure of Oxygen increases(pO2),
- The 2,3, BPG is extruded out, and the Hemoglobin resumes its original state, known as the "**Relaxed**" or "**R**" **form**,
- R form has a **high affinity for Oxygen**.

Conditions Of High levels of 2,3BPG

- During conditions of **cellular deprivation of Oxygen**.
- 2,3BPG levels in Erythrocytes are increased

Conditions Of High levels of 2,3BPG

- Hypoxia
- At high Altitudes
- Severe Anemia
- Lung Diseases
- Cardiac disease -Anoxia
- Blood loss

2,3 BPG levels in Hypoxia

- **2,3BPG levels increases** in hypoxia and at high altitudes.

- Changes in 2,3-BPG levels play an **important role in adaptation to hypoxia.**

- In hypoxic conditions pO2 is low and
- **2,3 BPG levels are high**
- **Due to affected metabolism of Glucose in RBC's.**

- **Increased 2,3-BPG levels** in red cells
- Decreases Oxygen affinity
- **Facilitates unloading of Oxygen to tissues**.

- Increased 2,3-BPG also plays a role in **adaptation to exercise.**

Conditions of Low 2,3BPG levels

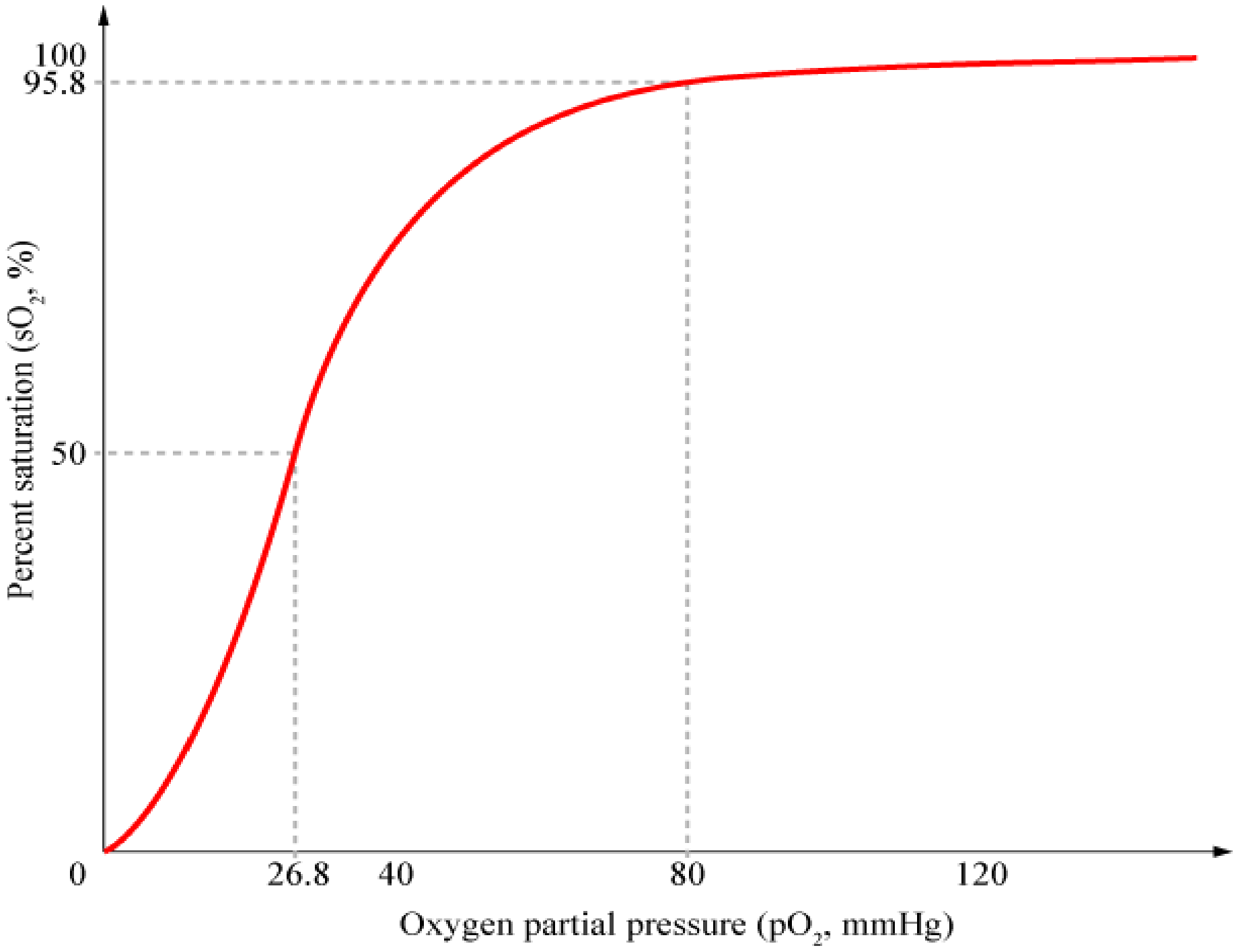
- **Prolonged starvation**
- **Erythrocyte disorders** reduces the levels of 2.3BPG.
- Low 2,3 BPG **reduces low unloading of oxygen at tissue level.**

- However formation of **2,3-BPG is not very essential to life.**
- An individual who **lacked the enzymes necessary for 2,3-BPG synthesis** (Rapaport Leubering) was perfectly well except for mild **Polycythemia.**

- The **increased Oxygen affinity of stored blood is accounted (Blood Banks)**
- **Due to reduced levels of 2,3-BPG.**

- **Inosine addition** to stored blood in blood bank
  - **Increases the 2,3BPG levels** in it
  - This **favors unloading Oxygen on blood transfusion**.

OXYGEN DISSOCIATION CURVE OF HEMOGLOBIN (ODC)



- **Oxygen Dissociation Curve (ODC)** of Hemoglobin

- **ODC describes the relation** between

- **Partial pressure of Oxygen (pO_2)** and **percent saturation of Oxygen by Hb.**

ODC for Tetrameric ,Allosteric Hb molecule is sigmoid shaped (S shaped)

Sigmoid Shaped ODC Curve Due To

Positive Allosteric Effect

Cooperative Binding Mechanism Of Oxygen With Hb

p50 Of ODC

- **P50 is that pO2 value**
- Where the **Hb is 50 percent saturated with Oxygen.**

P50 is 50% saturation of Hb at pO2 of 27 mm Hg.

- In ODC of Hb
- P50 for Adult Hb is 27 mm.Hg/torr

- ODC depicts

- O2 carrying capacity of Hb at different pO2

Salient Features Of ODC of Hb

- **Oxygen Dissociation Curve Depicts:**
- **Oxygen uptake and release by Hemoglobin.**

- **ODC Describes**
- **The fractional saturation of Heme groups of Hemoglobin with Oxygen at various Oxygen partial pressures.**

- Normally the partial O_2 pressure in the **Lungs** is **100 mm.Hg** and the Hb **is 100 %** saturated with O_2.

- **In Tissues** the partial oxygen pressure is **40mm.Hg** and the Hb is **75% saturated** with O_2.

- **100 - 75 = 25%** of the **O_2 is released by OxyHb** and **delivered to the tissues.**

Percent Saturation Of Hb At Different pO2

pO2 in torr	Percent Saturation Of Hb
100 in Alveoli	97 %
40 in resting muscles	64 – 75 %
20 in working muscles	20%
10 in vigorously exercising muscles	10%

- The sigmoid shape of the ODC curve shows that:
  - With a **small drop in partial O_2 tension (pO2).**
  - A **significant amount** of **O_2 release/offloading by OxyHb** will occur.

- It is to be noted that the **OxyHb** reaching to **tissues**
- Does **not releases** its **Oxygen completely** at **one instance.**

- Instead the release of Oxygen by OxyHb at tissues is
- **As per the cellular need for the Oxygen .**

- This **regulated way of Oxygen release** by OxyHb at tissue level
- May **prevent from generation of oxygen derived free radicals (Reactive Oxygen Species: ROS)**
- Which **in turn protect the peroxidation of cellular biomolecules** by action of ROS.

FACTORS AFFECTING ODC
OR
ALLOSTERIC MODULATORS of ODC

The characteristics of normal ODC depends upon following factors:

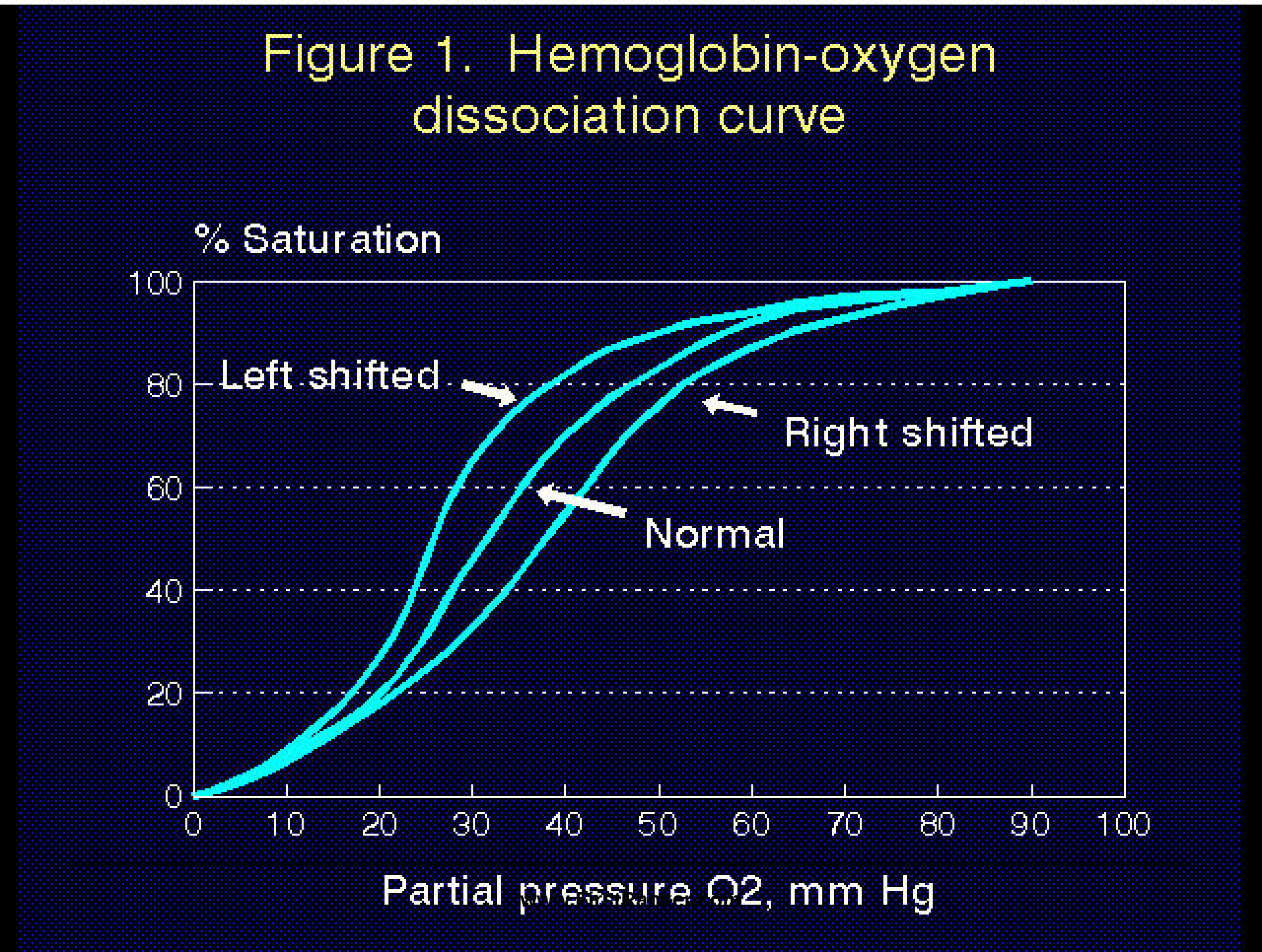
- **Hemoglobin Structure**
- **Environment within the Erythrocyte**

The environment Of RBCs depends upon:

- pO_2
- pCO_2
- pH(H^+ concentration)
- 2,3-Bisphosphoglycerate
- Glucose Concentration
- Metabolic Condition
- Temperature

- Increased
- H^+,pCO_2, 2,3BPG,Temperature
  - **Causes unloading of Oxygen from OxyHb.**
  - They are **Negative Allosteric effectors** of ODC.

Types And Conditions Of ODC Shifts



Right Shift of ODC With Its Conditions

- If the Oxygen Dissociation Curve is shifted towards Right

❖ Oxygen is unloaded by OxyHb

- Oxygen affinity is decreased by Hb
- Oxygen is not linked and not retained in the Hb structure

Conditions Which Shift ODC To Right Hand Side

Low Oxygen Affinity/Easy Oxygen Delivery/Easy Unloading/ Prompt Offloading of Oxygen

- High pCO_2 (Increased Metabolic States)
- High H^+ (Acidosis)
- High 2,3-BPG: Hypoxic , Anoxic Conditions
- Exercise
- High body temperature : (Fever)

- Anemia : Hb S (low pO2)

Mnemonic for Factors causing Right Shift of ODC :

CADET

- C – CO_2
- A – Acid (H^+)
- D– 2,3-BPG /2,3 DPG
- E – Exercise
- T – Temperature

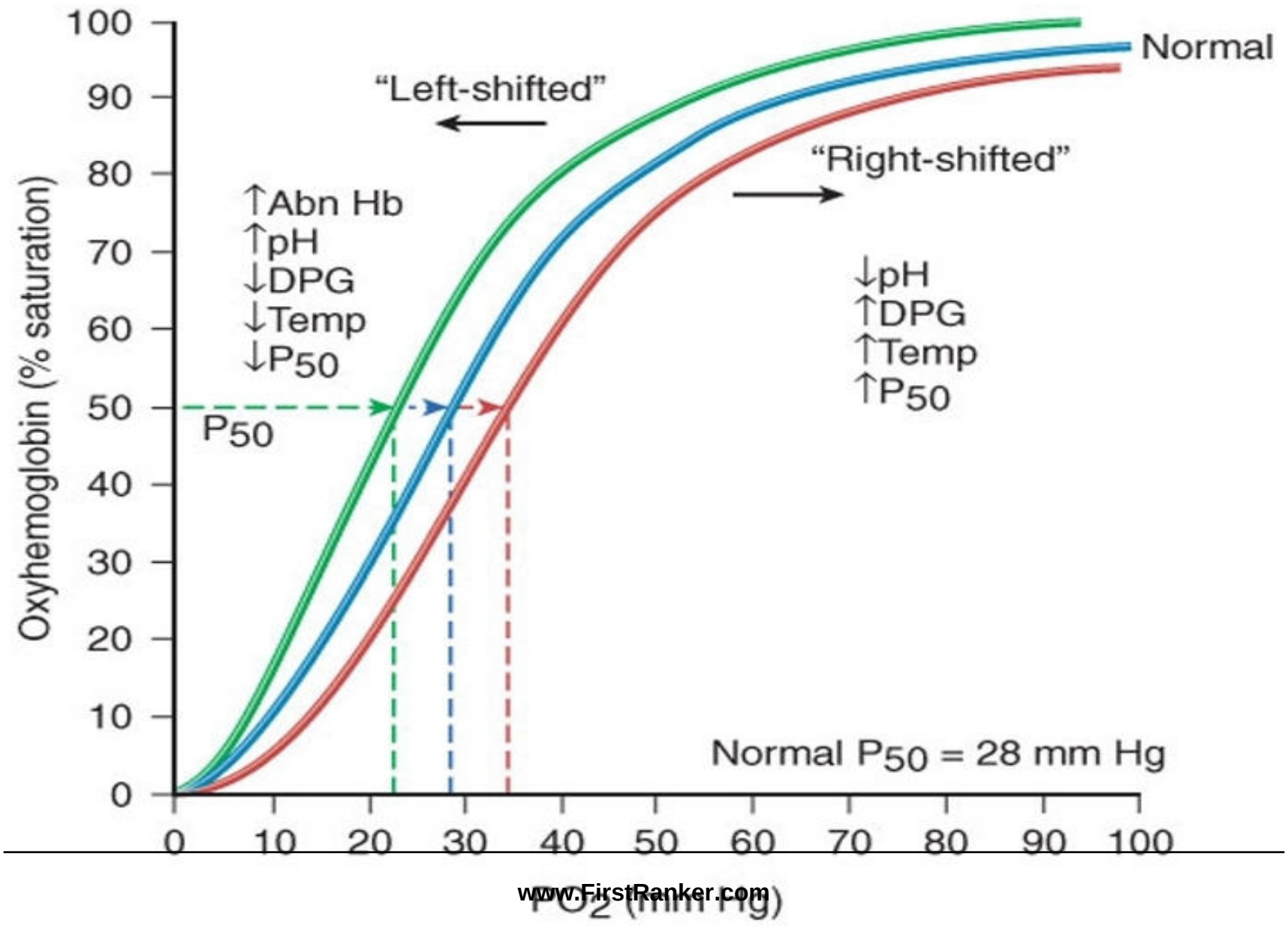
Left Shift of ODC With Its Conditions

- If the Oxygen Dissociation Curve is shifted towards Left
- Oxygen is not unloaded by OxyHb
- Oxygen affinity is increased by Hb
- Oxygen is linked and retained in the Hb structure

Conditions Which Shift ODC To Left Hand Side

High affinity for Oxygen/ Low oxygen Delivery/poor unloading of Oxygen

- High pO2
- In Alkalosis (Low H^+ high $HCO3^-$)
- Low 2,3-BPG
- HbF
- Increased Methb and Carboxyhb



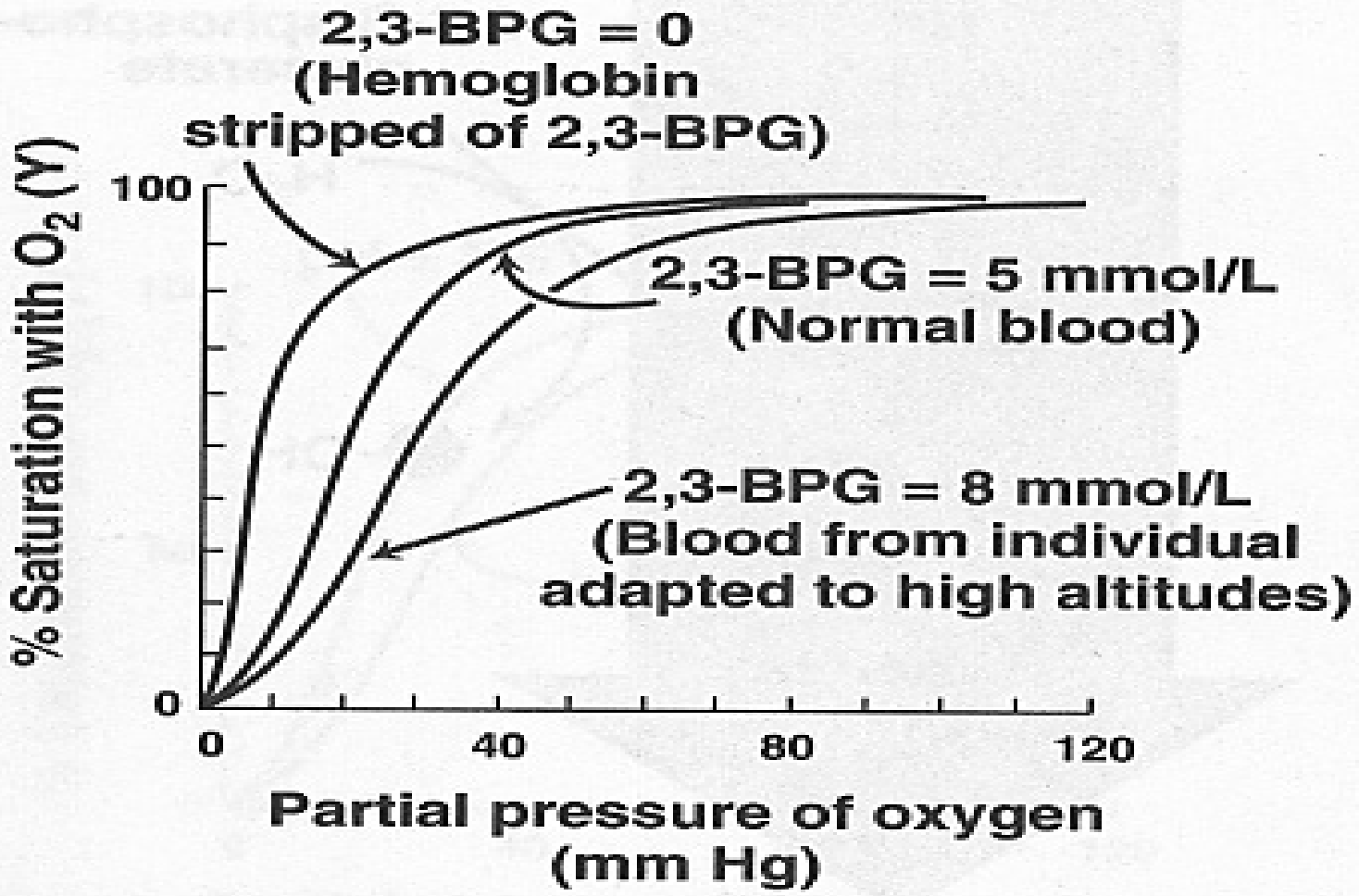
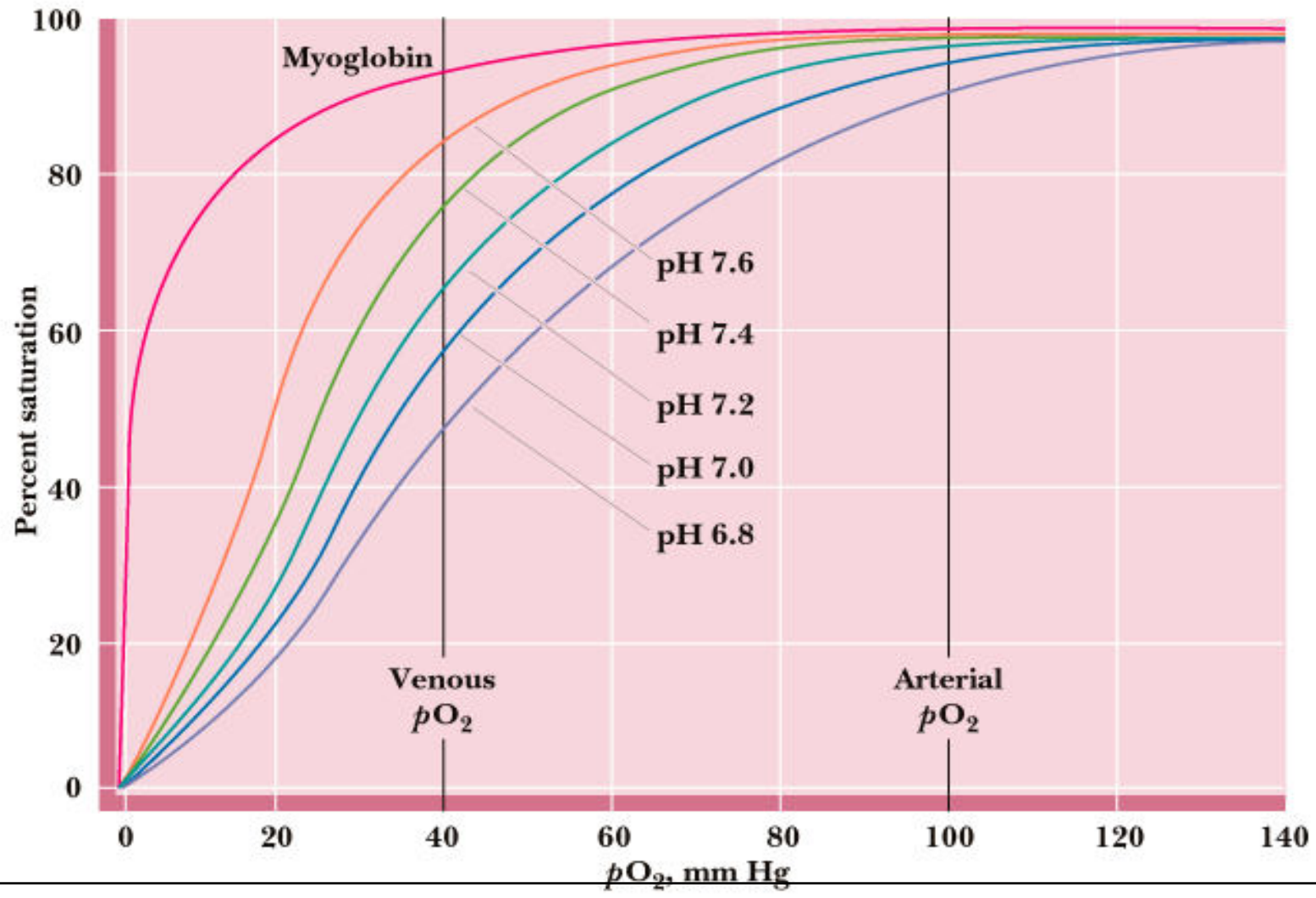
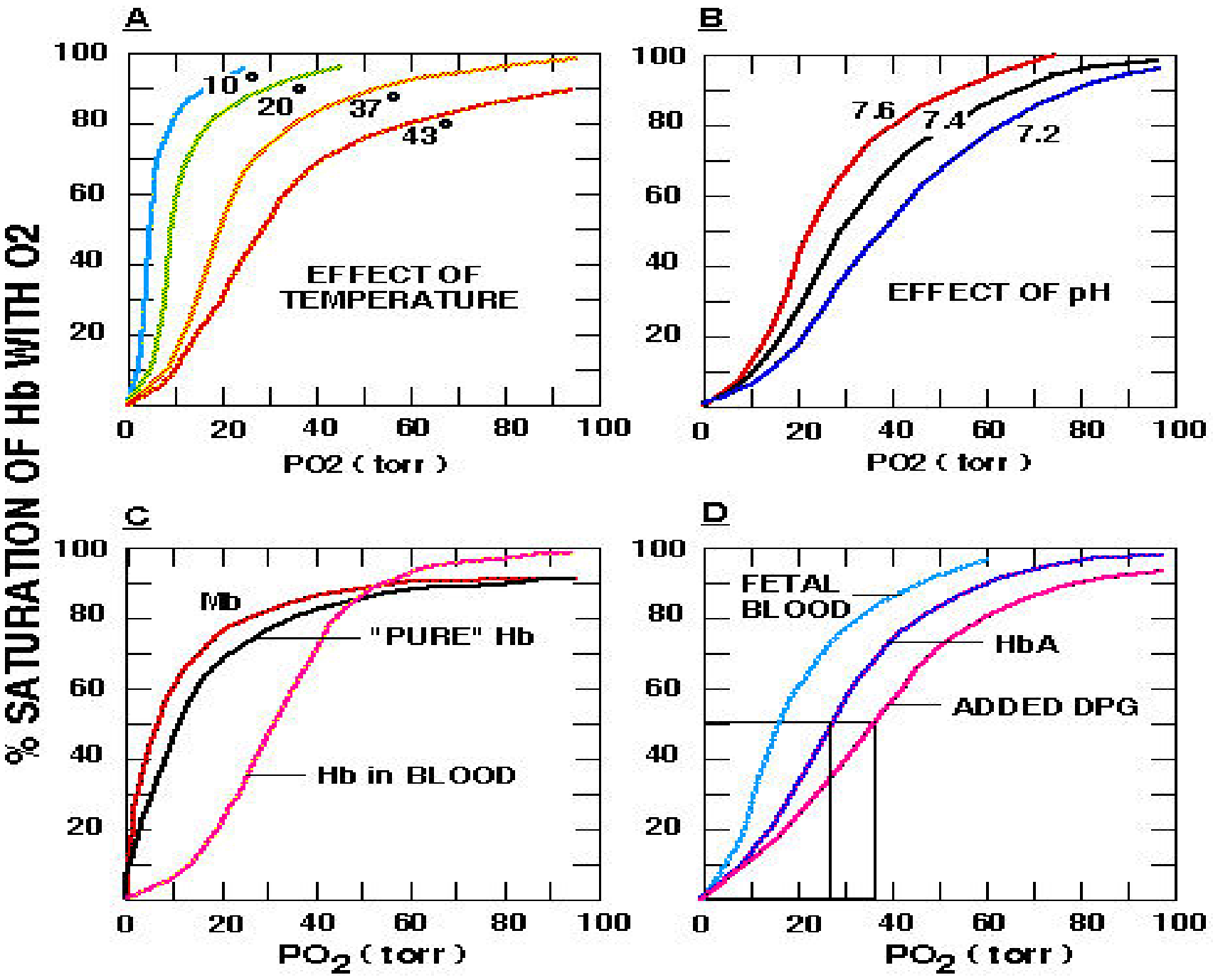


Figure 3.12
Effect of 2,3-BPG on the oxygen affinity of hemoglobin.

Garrett & Grisham: Biochemistry, 2/e
Figure 15.34

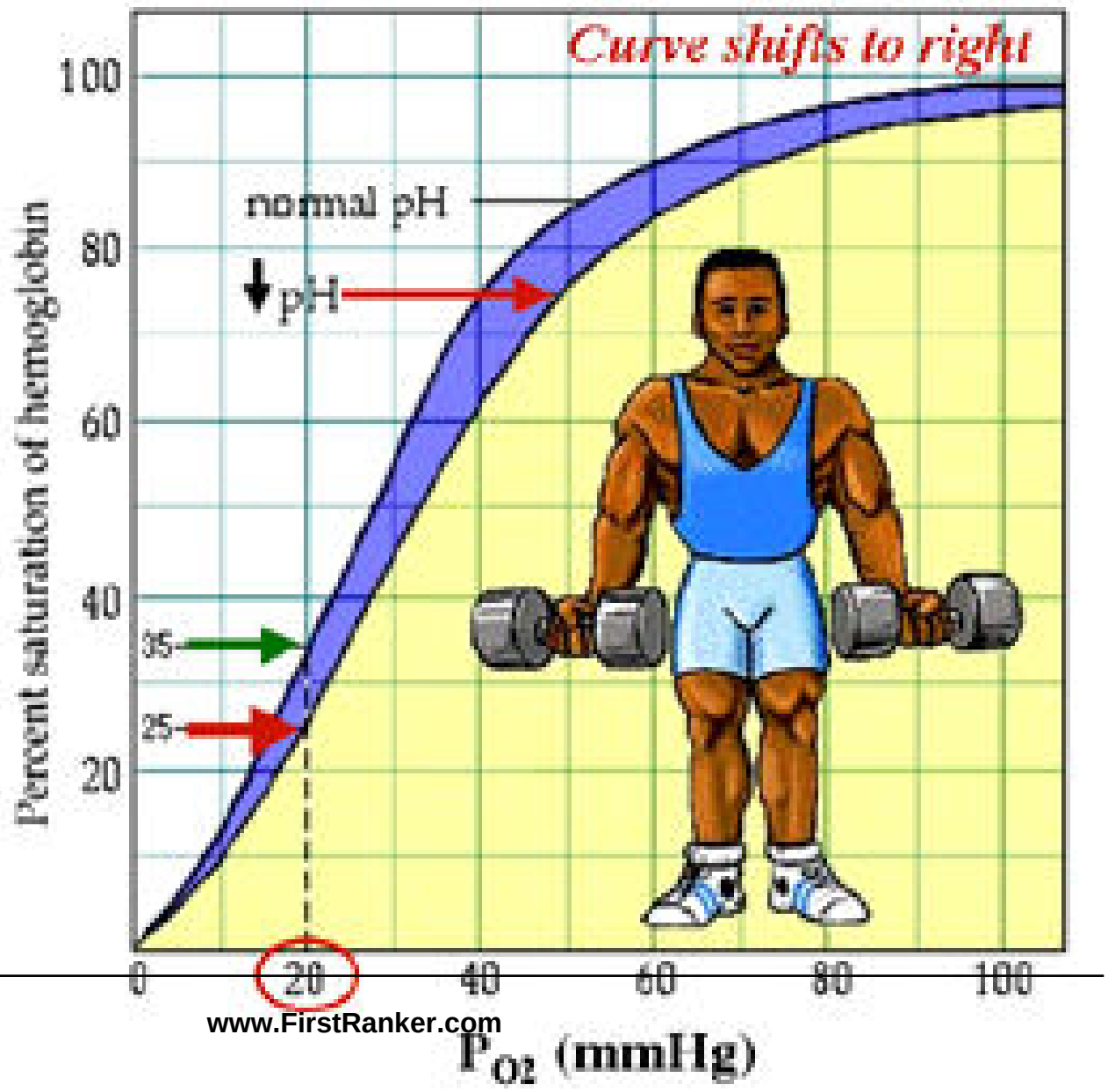




Oxygen-hemoglobin Dissociation: Exercise

Factors shifting curve to right

- ↓ pH
- ↑ Temperature
- ↑ P_{CO2}
- ↑ 2,3 BPG



Transport Of CO2 and H^+

- About **75 - 80%** of tissue **Carbon dioxide** is processed and transported **in the form of HCO_3^- (Bicarbonate ions)**

- **Carbon dioxide** formed during **metabolism** in tissues is out in plasma

- Then it **diffuses freely into the Erythrocytes**

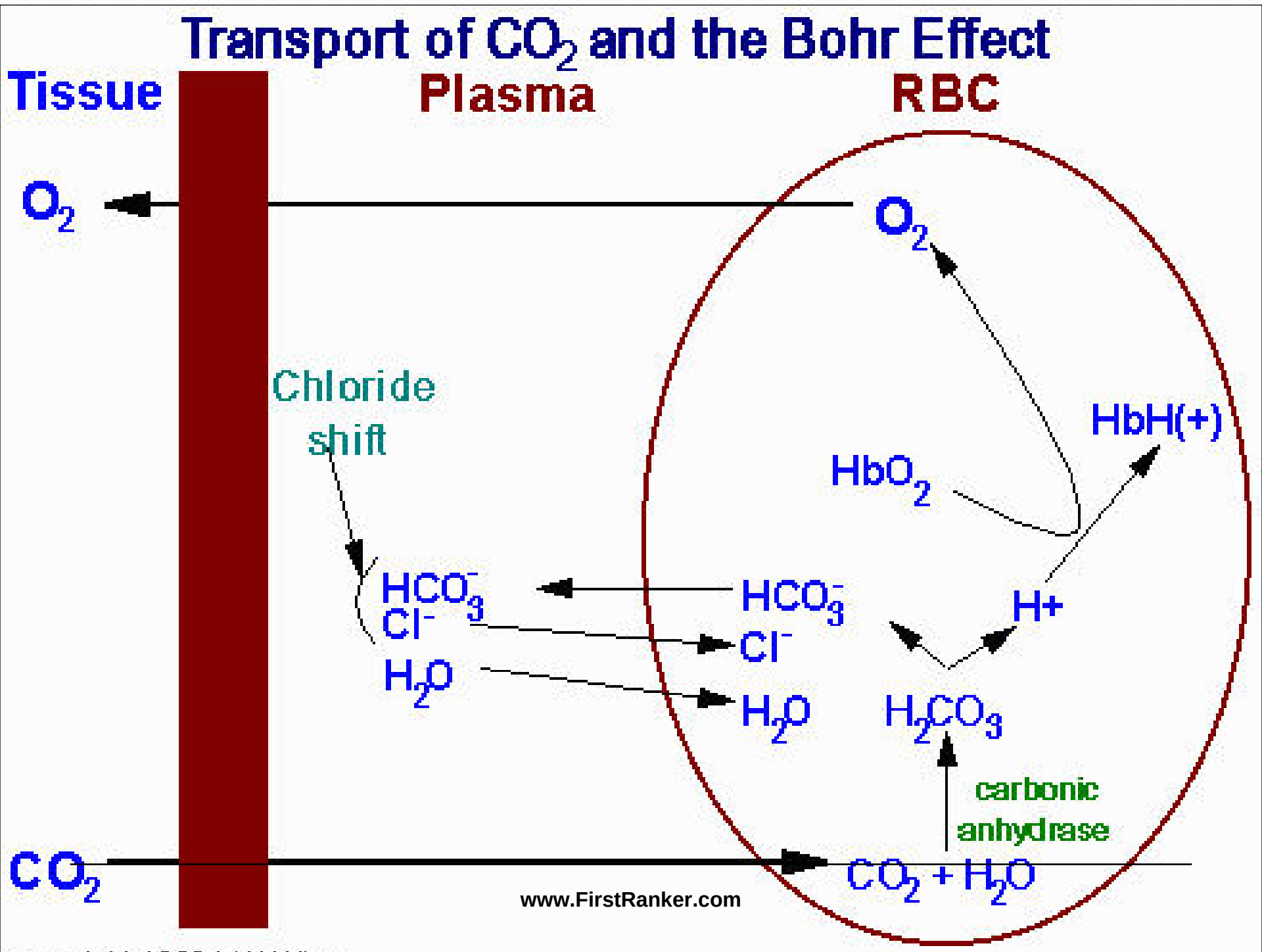
- In **aqueous solutions, carbon dioxide** undergoes a pair of reactions
- biocatalyzed by enzyme **Carbonic Anhydrase** (CA).

Reaction 1

$$CO_2 + H_2O \Longrightarrow H_2CO_3 \text{ (Carbonic Acid)}$$

Reaction 2

$$H_2CO_3 \Longrightarrow H^+ + HCO_3^- \text{ (Bicarbonate ions)}$$



- Where the presence of an enzyme **Carbonic Anhydrase** facilitates **reaction 1.**

- The H^+ liberated **in reaction 2** are accepted by **deoxygenated Hemoglobin, and transported**

- The **bicarbonate formed** in this sequence of reactions
- **Diffuses freely** across the red cell membrane and a portion is **exchanged with plasma Cl^-**,
- A **phenomenon called the "Chloride shift."**

- The bicarbonate ions are carried in plasma to the lungs
- **Where excretion of CO_2** occurs in the expired air.

Hb Minorly Transports CO_2

- 15 – 20% of CO2 is Transported by Hb.

- Transport of **Carbon dioxide** by Hb, is **unlike that of Oxygen**

- **CO_2** does **not bind to Heme/Fe^{++} of Hb**

- **CO_2 is linked to Globin part** of Hb and transported.

- **CO_2 is bound** to the
- To Deoxygenated Hemoglobin
- In **β Globin chains**
- At **N-terminal Amino groups** of **Valine residue**
- To form **Carbaminohemoglobin**

- **2 molecules of CO_2** are linked to **1 Hemoglobin**
- Transported **through blood from tissues to Lungs and expired out.**

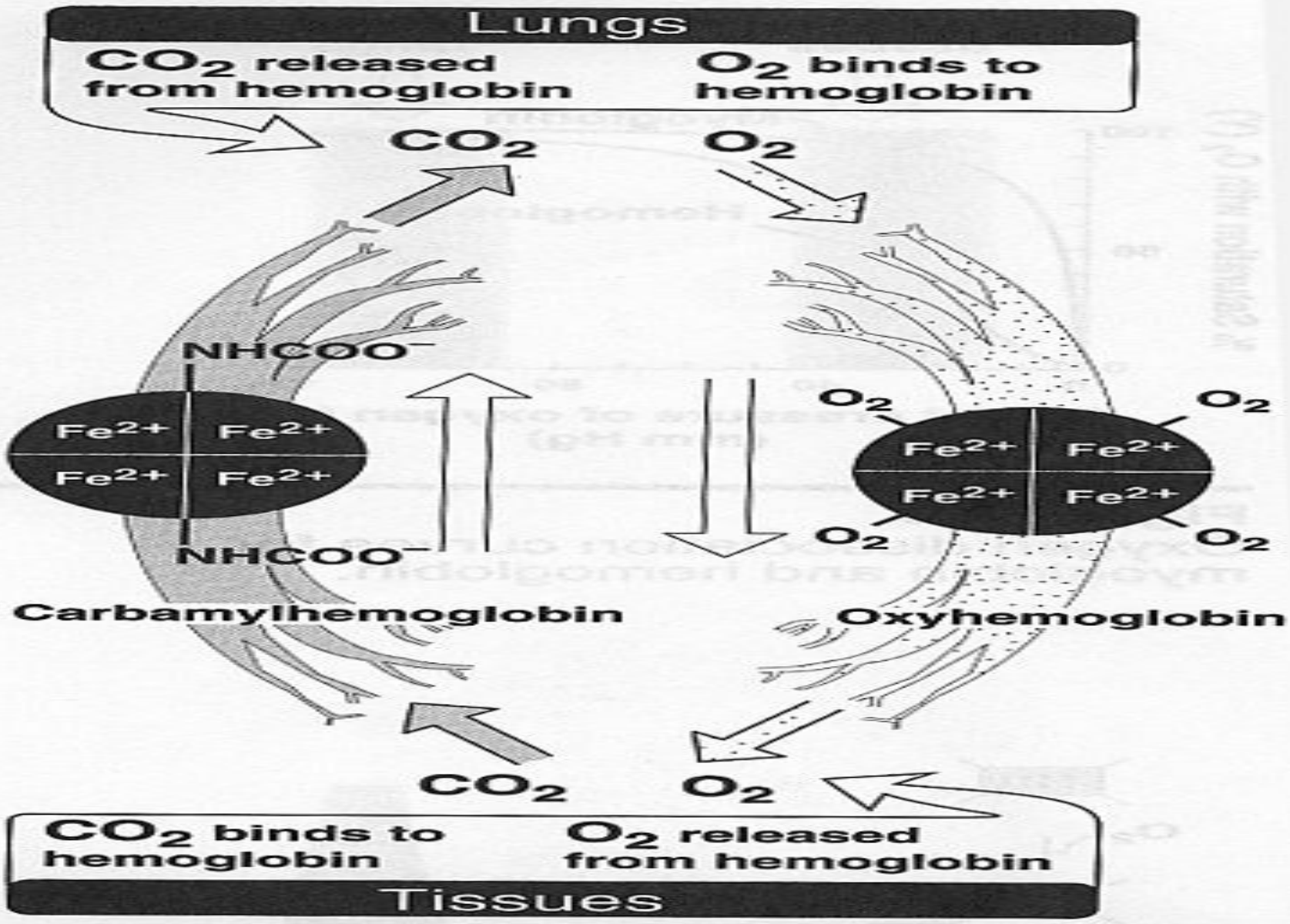


Figure 3.8
Transport of oxygen and CO_2 by hemoglobin.

- **5% of CO_2** is carried in **free, dissolved form** through blood.

- **Thus Deoxy Hb** carries:
  - **CO_2** and **Protons** from Tissues to Lungs.

- At Lungs as Oxygen gets bound to Deoxyhb
- The CO_2 and H^+ comes off of Deoxyhb and expired out of Lungs.

At Lungs	At Tissue level
Respired air – pO2 is high –90-100 mm Hg	**Metabolism** pO2 is low-40mm Hg pCO2 is high. pH low (H^+ high),2,3BPG high.
Hb is oxygenated to OxyHb (R Form) Cooperative binding mechanism of O2 to Hb	OxyHb is dissociated to release oxygen at tissue level./O2 is unloaded. OxyHb is deoxygenated
'T' form is transformed to 'R' form.	**R form is transformed to T form.**
O2 binds to Fe $^{++}$ of Heme non enzymatically loosely and reversibly.	**O2** released by Hb at tissue level is **utilized** for **Biological Oxidation and process(ETC).**
4 O2 to 1 Hb 1.34 ml O2/gm of Hb transported	15-25% of Co2 is transported to lungs by Hb forming Carbaminohemoglobin and expired out through lungs.
O2 is directly linked to Fe $^{++}$ of Heme and distal His of α 58 a.a and β 63 a.a of Globin.	CO2 is not linked to Fe $^{++}$ of Heme But linked to **amino groups of Val residue.of β Globin subunits**

NORMAL HB VARIANTS

- **Normal Hb variants** are **type of Hemoglobins**
- Present in **different physiological phases** of human life.

- **Role of Normal Hb Variant :**
  - **To rightly fit** for that particular **physiological phase of life**
  - **Transport and Deliver Oxygen as per need** and **maintains normal cellular**

Examples of Normal Hb Variants Of Human Body

Globin Chain Synthesis, starts at 3rd week of gestation.

- **Embryonic Stage/Embryonic Hb -**
  - Hemoglobin Gower I ($\zeta_2\varepsilon_2$)
  - Hemoglobin Gower II ($\alpha_2\varepsilon_2$)
  - ~~Hemoglobin Portland ($\zeta_2\gamma_2$)~~

Fetal Stage:

- Major Hb : Hb F ($\alpha_2\gamma_2$)
- Minor Hb :HbA1($\alpha_2\beta_2$)

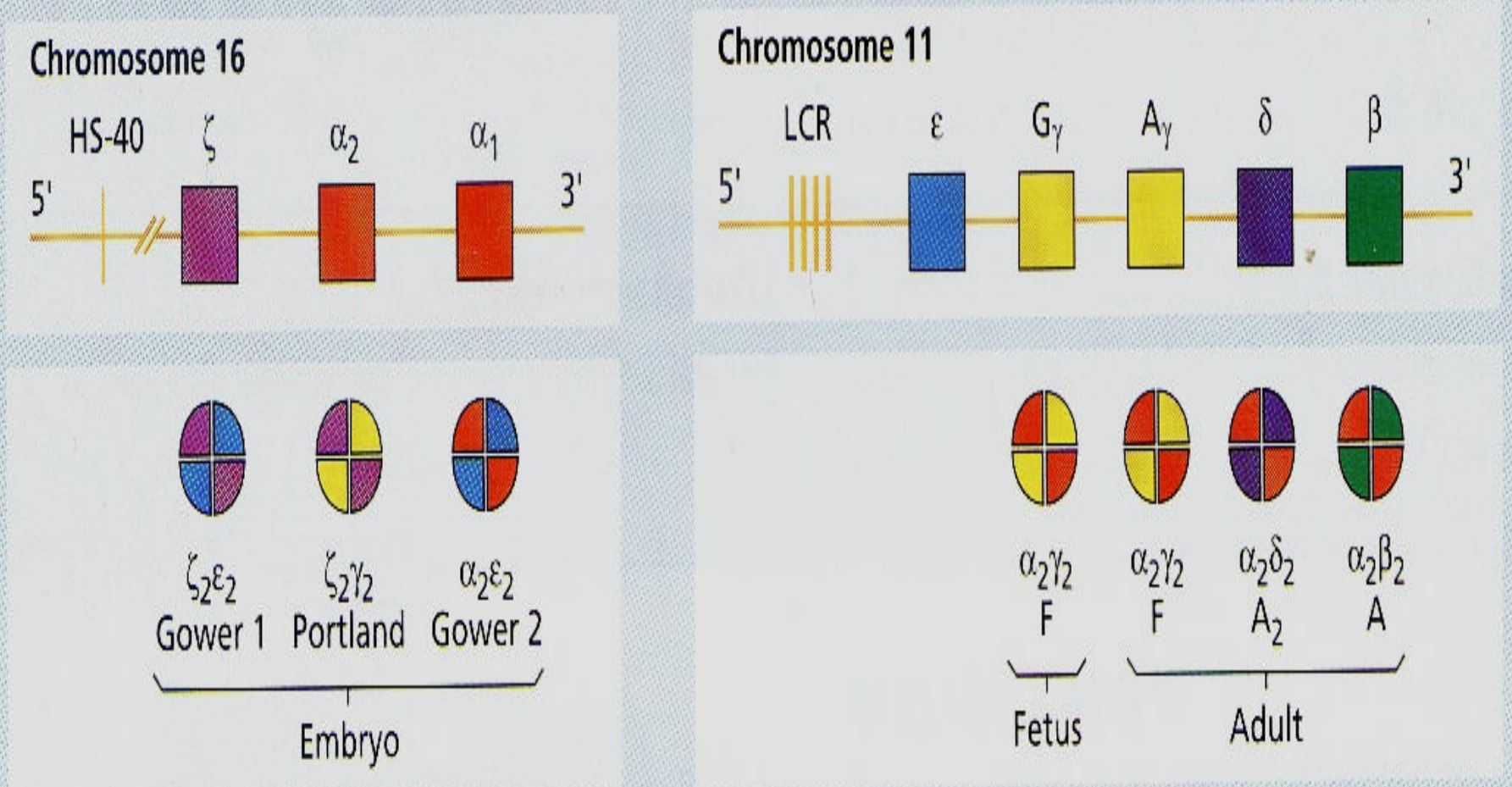
Adult Stage:

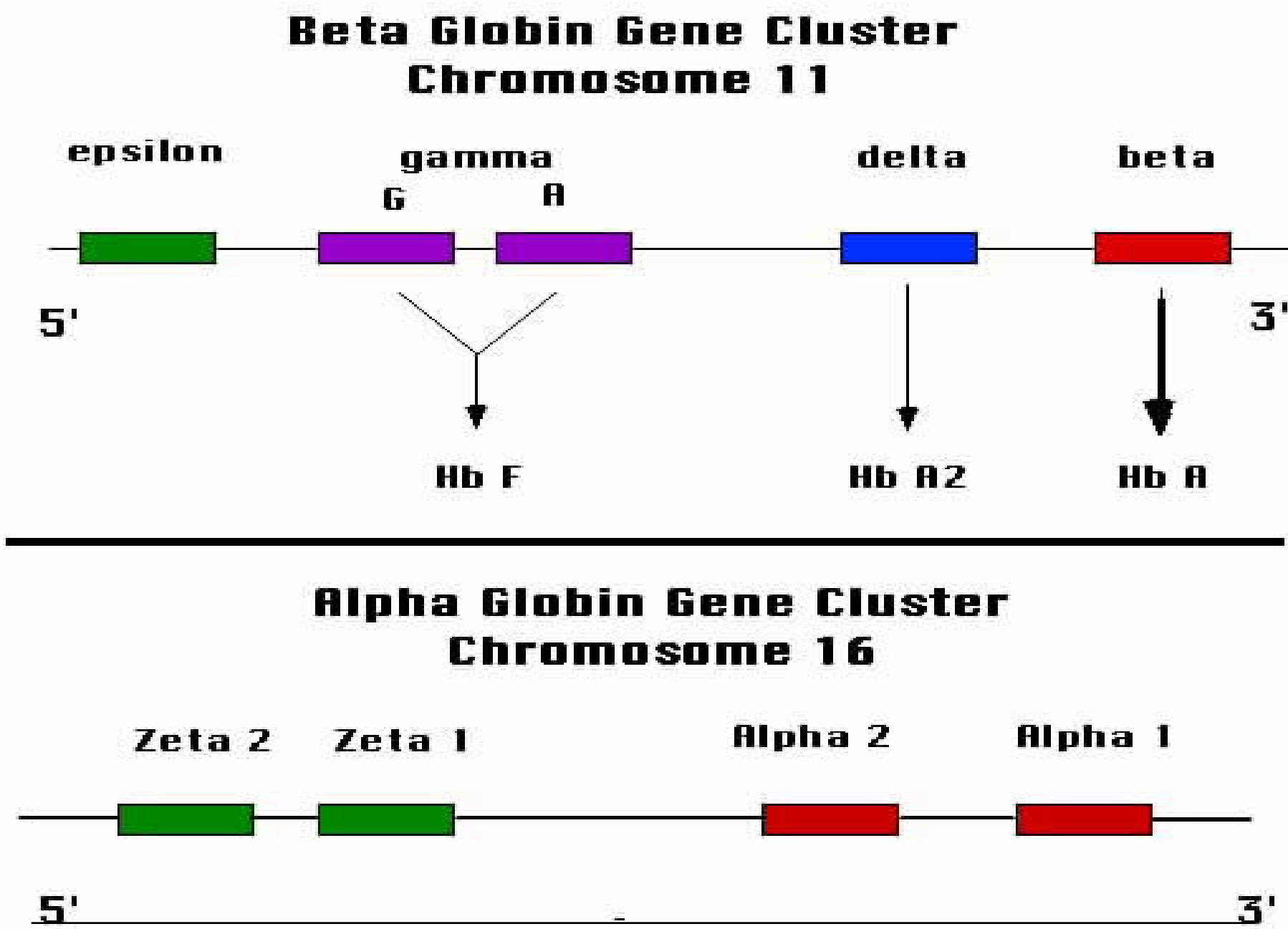
- **Major Hb : Hb A1 ($\alpha_2\beta_2$)**
- **Minor Hb :**
  - Hb A2 ($\alpha_2\delta_2$)
  - Hb A3 (In old RBC's)
  - Hb F ($\alpha_2\gamma_2$)
  - Glycosylated Hb/Hb A1c

- All Globin polypeptide chains are **homologous** which **arise from same ancestral Genes.**
- **Beta Polypeptide chain**-146 a.a
- **Gamma chain**-146 a.a
  (differ in 39 a.a from β chain)
- **Delta chain** -146 a.a
  (differ in 10 a.a from β chain)

GLOBIN GENES
Of
Normal Hb Variants

Globin Gene Clusters





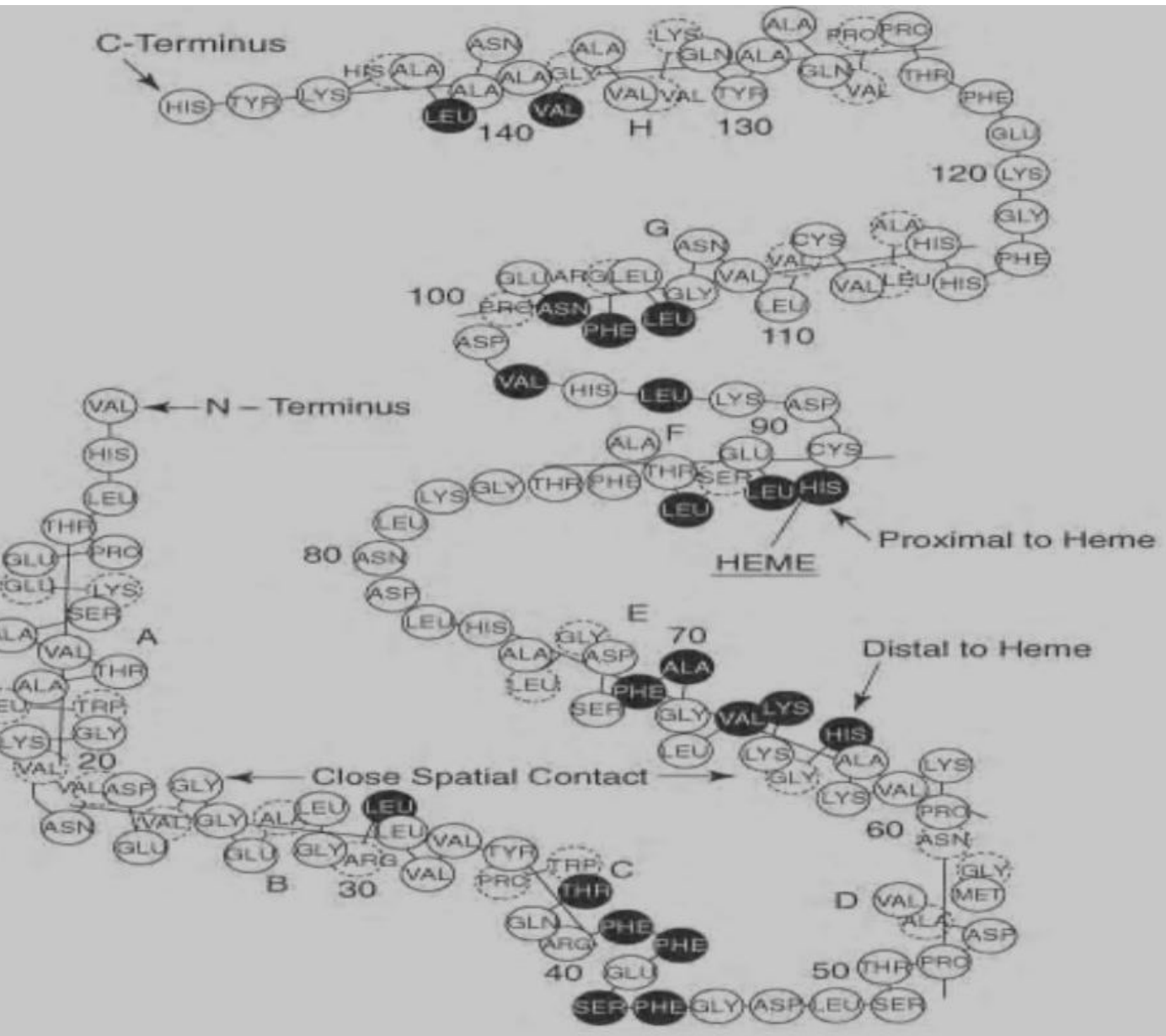
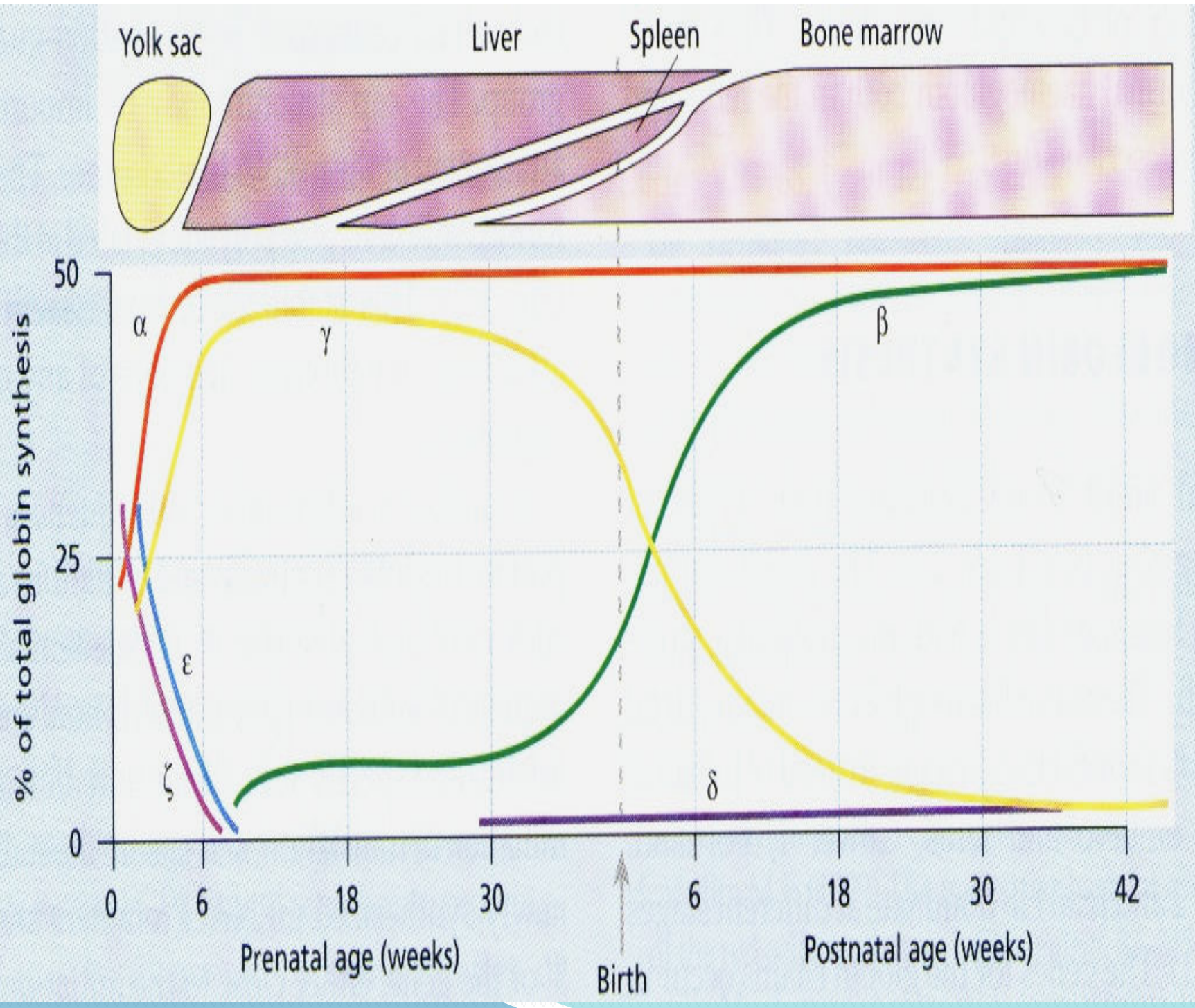


Figure 28-1 The β-globin chain, showing helical and nonhelical segments. The helical segments are labeled A through H, and nonhelical segments are designated NA for residues between the N terminus and the A helix, CD for residues between the C and D helices, and so forth. *(From Huisman THJ, Schroeder WA: New Aspects of the Structure, Function, and Synthesis of Hemoglobin. Boca Raton, FL, CRC Press, 1971.)*

GLOBIN CHAINS In Different Stages Of Human life



Fetal Hemoglobin (HbF)

- **HbF** is a normal **Hb variant of fetal life**
- **Hb F Predominates:**
  - **Fetus**
  - **New born infants**

- **Fetal Hb (Hb F)**
- Globin part has : **2 α** and **2 γ** subunits.

- **γ Globin** chain **differs from β Globin** chain in **39 amino acid residues**

- **Histidine residue at 143 position** of β Globin chain of **Hb A** is **replaced** with **Serine a neutral amino acid In Hb F** .

Biosynthesis Of Hb F

- Expression of following Globin genes will produce α and γ Globin chains to form HbF:
  - α Globin Gene located on **16 Chromosome**
  - γ Globin Gene located on **11 Chromosome**

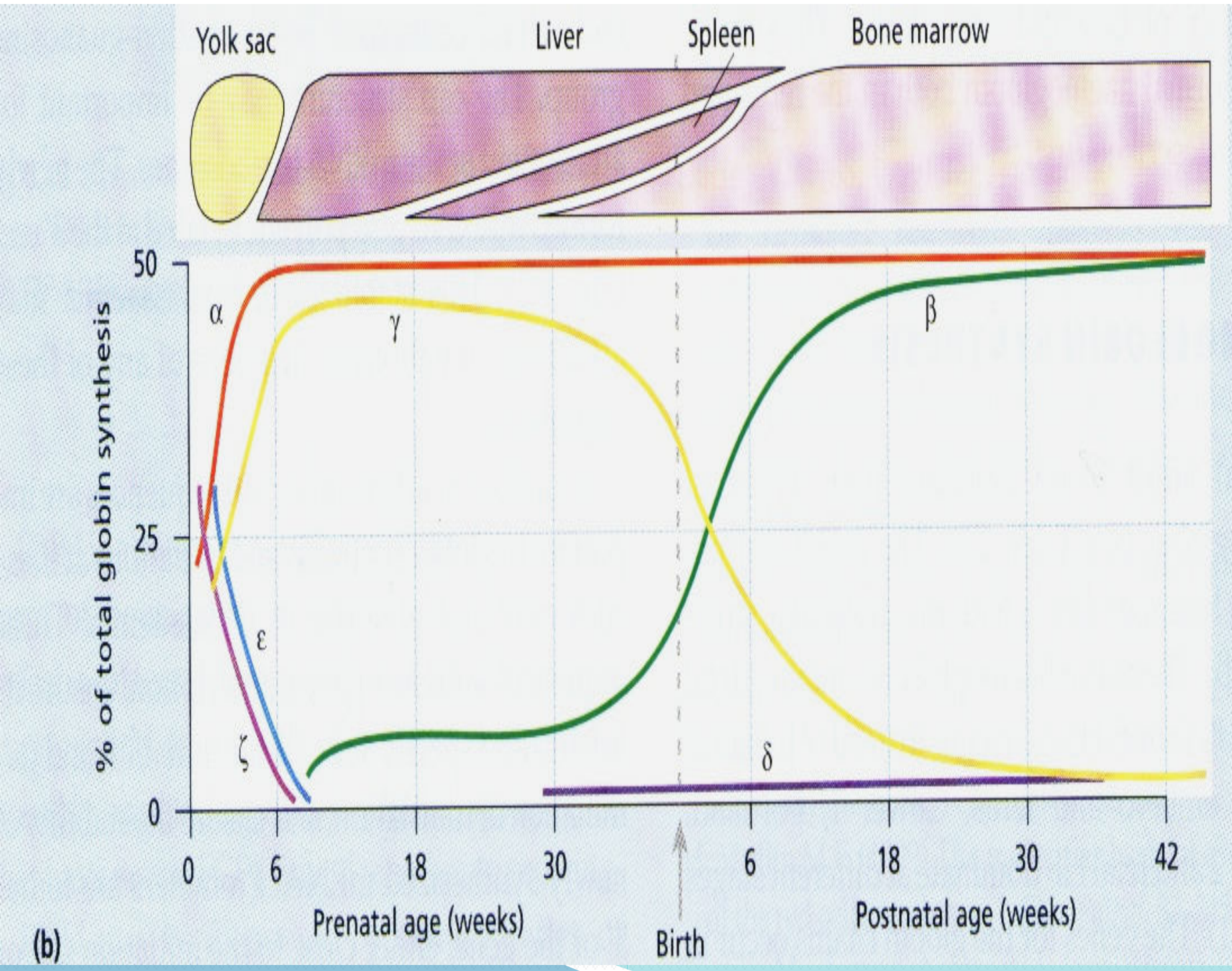
- **Hb F biosynthesis** starts **by 7th week** of gestation.

- In Fetus **Hb F predominates** during
- **Second and Third trimester** of gestation

- **At birth** in newborn infants.

- After birth there is **rapid post natal decline in HbF levels.**

- **Within 4 months after birth HbF** is almost **completely replaced by Hb A.**



Function and Features Of HbF

- HbF functions in loading and unloading of Oxygen in Fetus and new born infants.

- **Hb F has a high affinity for O2 than HbA1.**
- Hb F has **low affinity for 2,3 BPG**

- **HbF binds with O_2 at lower pO_2 concentrations** than Hb A1.

- **P 50 for Hb F** is **20 torr.**

- HbF has **low Oxygen releasing/unloading** capacity.
- Thus **ODC for HbF** is shifted **towards Left.**

Significance Of Hb F in Fetal Stage

- The fetus is circulated with **maternal blood**
- Which has **comparative low pO2** as that of Lungs.

- **Hb F** having **high Oxygen affinity**

- Gets **oxygenated at low pO2 of maternal blood.**

- This makes **more efficient trans placental transfer of Oxygen** from maternal blood to fetal HbF.

- **Thus Hb F in fetal phase rightly fits for this state:**
- Since there is a **low metabolic activities in fetal cells** and requirement of low Oxygen.
- Thus **low release of Oxygen by HbF suffice** in this condition.

High levels of Hb F In Adults Is Abnormal

- Normally **HbF in adults is less than 1 %**
- **HbF more than 1% in adult hood is abnormal.**

- 15-20% of HbF is found in patient of **Sickle Cell disease.**

(Defect in β Globin Genes)

- More higher percent of HbF is noted in individuals suffering from **β Thalassemia.**

(Defect in β Globin synthesis)

- **High levels of HbF** in Adults will have **low release of Oxygen** at tissue levels:
- Where the metabolic state and requirement of Oxygen is high.
- Thus **HbF does not fit in adult life.**

P50 values for Hb A and HbF

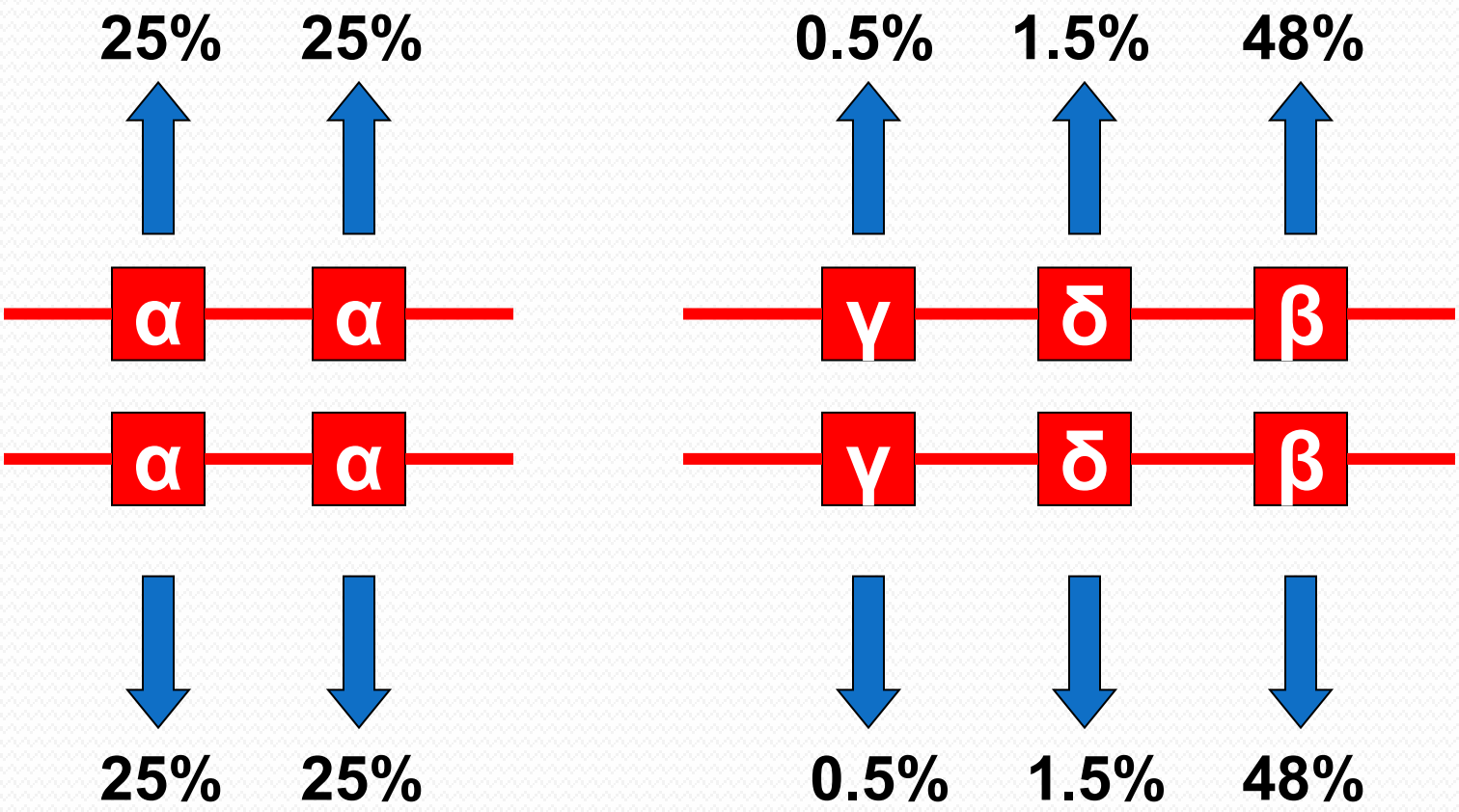
- **P50 for Hb A= 27 mm.Hg**
- **P50 for Hb F= 20 mm.Hg**

Adult Hemoglobins

Adult Hemoglobin Forms

	Hb A_1	Hb A_2	Hb F
Globin chain combinations	$\alpha_2\beta_2$	$\alpha_2\delta_2$	$\alpha_2\gamma_2$
Normal %	96-98 %	1.5-3.2 %	0.5-0.8 %

Globin chain synthesis in adults



Chromosome 16 Chromosome 11

Hb A1

- HbA1 is the **major form of Hb in adults** and in children over 7 months.
- **Globin** has **2 α and 2 β** subunits.

Hb A2

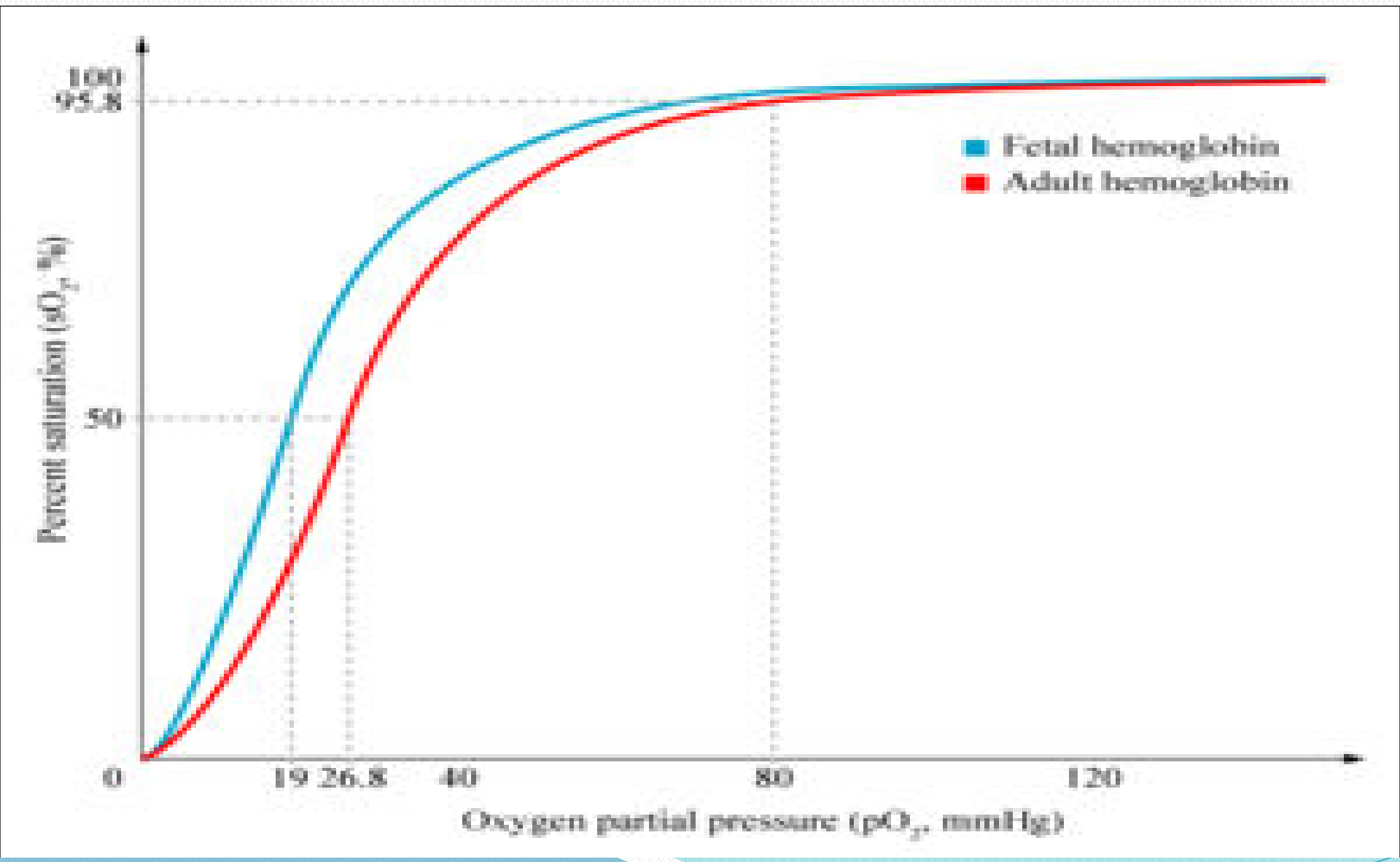
- HbA2 has **2 α and 2 δ** Globin subunits.
- **Hb A2** is a **minor form** of Hb in adults.
- Hb A2 is **2 – 3%** of a total adult Hb.

Hb A3

- HbA3-**altered form of HbA1** found in **old RBC's.**
- Approx 3-10 %.

Hb A1	Hb F
Predominant after 1 year of birth and adults.	Predominant in fetus and new born infants.
Globin chain-α2 β2	Globin chain-α2 γ2
Less affinity towards O2 and more affinity towards 2.3 BPG at tissues	More affinity towards O2 and less affinity towards 2.3 BPG at tissues.
P50 is 27 mm.Hg Unloading power of oxygen at tissue level is high.	**P50 20 mm.Hg** Unloading power of oxygen at tissue level is low.
HbA1 denatured by alkali	Hb F resistant to alkali denaturation.

ODC of Fetal Hb F vs. Adult Hb

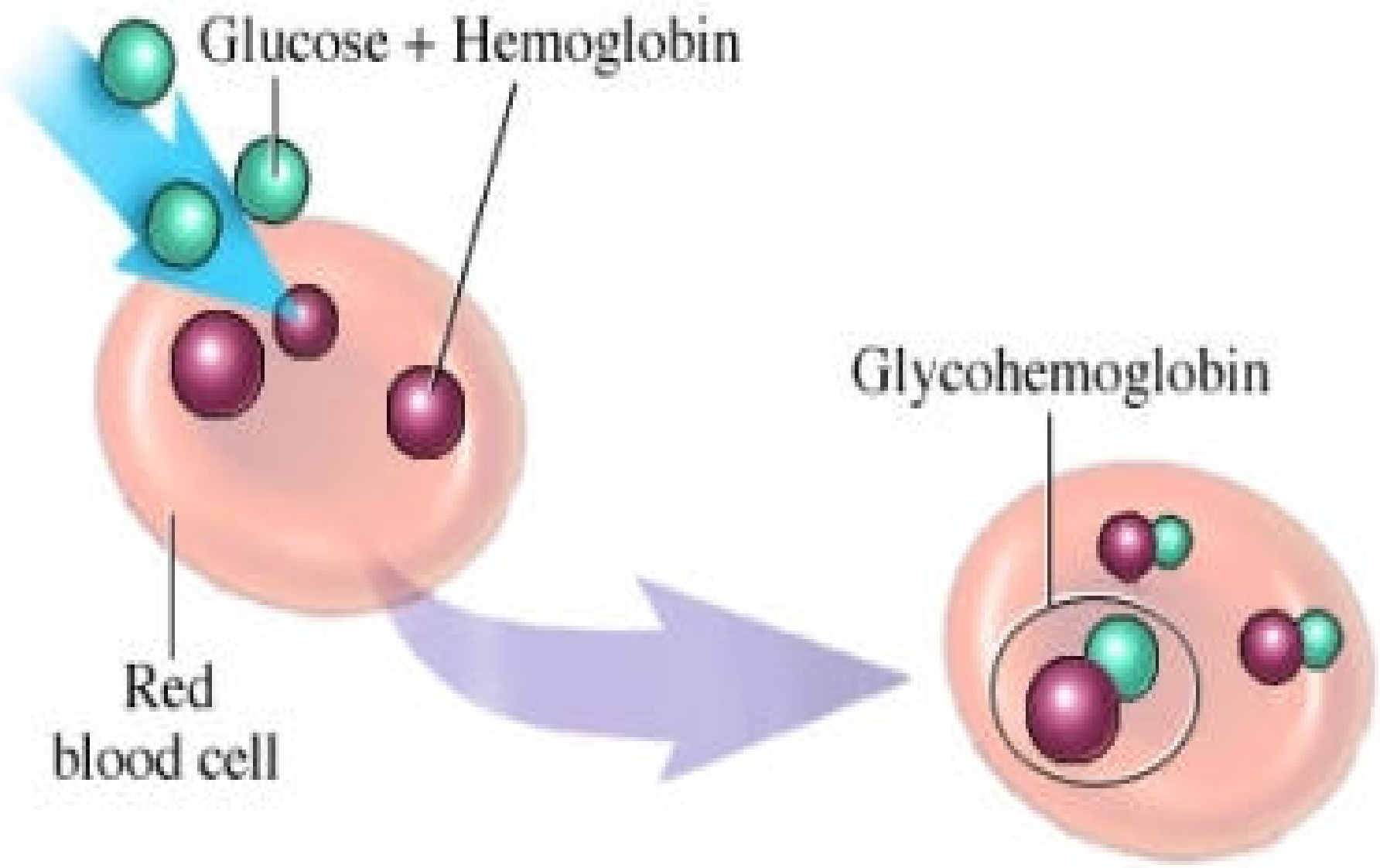


GLYCOSYLATED HEMOGLOBIN (HbA1c)

- Hb undergoes **spontaneous glycosylation** with Glucose present in Blood/RBCs.

- The **extent of glycosylation with Hb** depends on the **plasma concentration of Glucose.**

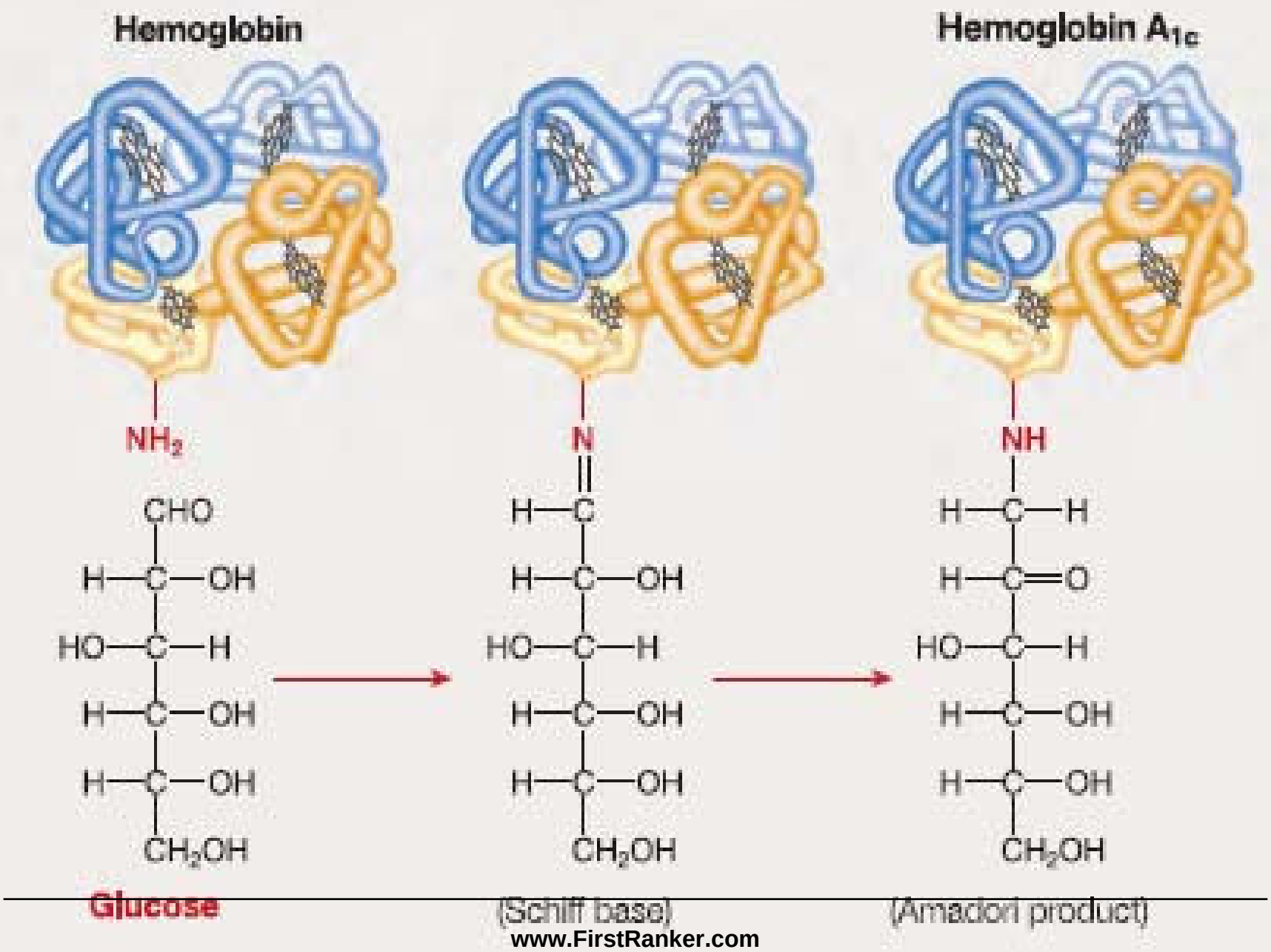
- Once Hb is glycated it remains till the **life span of RBC** (120 days).



Site Of Linkage Of Glucose To Hemoglobin

- **Glucose is linked** to **Globin part** of Hemoglobin to Amino acids:
  - **Valine** (terminal a.a) of β Globin chain and
  - **Lysine** € amino group

- Later the linked Glucose is transformed to **1-Deoxy Fructose.**



Significance of Estimation of Blood Glycosylated Hemoglobin

- Glycosylated Hb (HbA1c) in **normal healthy** adults is **less than 5%**

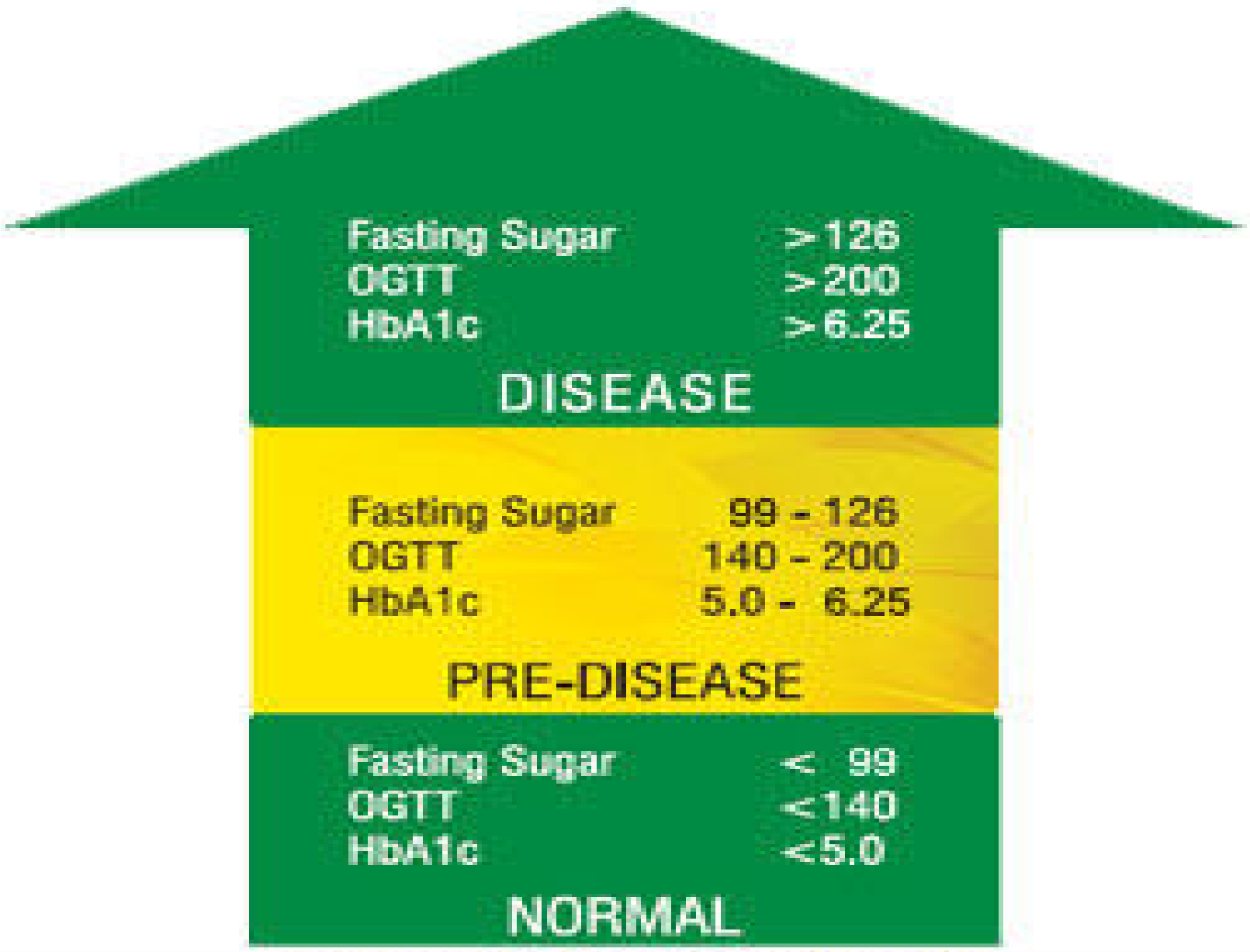
- In **Diabetes mellitus** the HbA1c is **more than 5%**

WHO Criteria for Diabetes Mellitus

HbA1c > 6.5%

- Levels of Glycated Hb gives idea of
- Blood Glucose levels of a person in **last 3-4 months back.**

- Thus estimation of Glycosylated Hb from blood specimens in clinical Biochemistry laboratory:
  - Gives **Index of Glucose Control** in **patients of known Diabetes mellitus.**



A1C Chart

Average Daily Blood Sugar	A1C Level
135	6%
170	7%
205	8%
240	9%
275	10%
310	11%
345	12%

Table 1. Chart showing how average daily average plasma blood glucose levels compare to 2-3 month A1C test results.

- As the blood Glucose levels increases
- The percentage of Glycosylated Hb increases

High Levels Of Glycosylated Hb Decreases Oxygen Transport to Tissues

- Increased Glycosylated Hb **increases its affinity** for Oxygen.
  - **Prevent** release/**unloading** of **Oxygen at tissues**
  - **Induces hypoxia** in extreme cases

- Increased Glycosylated Hb
  - **Decreases Oxygen saturation with Hb.**
- Increased Glycosylated Hb
  - **Decreases Oxygen release at tissues**

Risk Of High Levels Of Glycosylated Hb In Patients Of Diabetes Mellitus

- **Diabetes mellitus Patients**

Glycated hemoglobin of 6.5% -
Less risk for development of **Diabetic complications.**

Glycated hemoglobin of 12 %-
High risk for development of **Diabetic complications.**

HEMOGLOBIN DERIVATIVES

- Hemoglobin interacts with **chemical agents** to form **Hb derivatives.**

- During formation of Hb derivatives **mostly Fe^{+2} part of Hb is involved.**

NORMAL HB DERIVATIVES

- **Normal Hb derivatives** are **physiological and functional forms of Hb.**

- **Examples** of **Normal Hb derivatives.**

  - **OxyHb- Hb** Bound to O_2
  - **Reduced Hb- Hb** Bound to H^+

ABNORMAL HB DERIVATIVES or Dyshemoglobins

- **Abnormal Hb Derivatives** are **Acquired ones**:
- Abnormal Hb derivatives are **formed:**
  - When blood **interacts with Chemical pollutants/Drugs which has affinity for Hb.**

- Abnormal Hb derivatives has **Heme Iron** linked to **other chemical compounds instead of O_2**

OR

- **Hb is in a state where Oxygen may not get linked to Heme.**

Examples of Abnormal Hb Derivatives

1. **Carboxyhemoglobin-** CO linked to Fe^{+2} of Hb
2. **Methemoglobin**-Fe^{+2} of Heme transformed to Fe^{+3}
3. **Cyanmethemoglobin**-CN linked to Methb
4. **Sulfhemoglobin-**H_2S interacted with Hb,
5. Sulfur linked to Fe^{+2} of Hb
6. **Hematin-** Ferriprotoporphyrin.
7. **Hemin-** Hematin Chloride.
8. **Hemochromogen -** Heme with denatured Globin.
9. ~~**Cathemoglobin - Hematin with denatured**~~ Globin.

Consequences of Abnormal Hb Derivatives/ Dyshemoglobins

Dyshemoglobin Causes Cyanosis

(Low Oxygen Saturation By Hb)

- Dyshemoglobins in acquired states **affect normal structure and function** of Hb.
- Dyshemoglobins **are non functional forms of Hb.**
- Dyshemoglobins **affects Oxygen transportation** from lungs to tissues.

CARBOXYHEMOGLOBIN
or
Carbon Monoxide Poisoning

- **Carbon Monoxide** (CO) is a **colorless ,odorless, toxic gas**
- Present in atmosphere as **chemical pollutant.**

Sources of CO

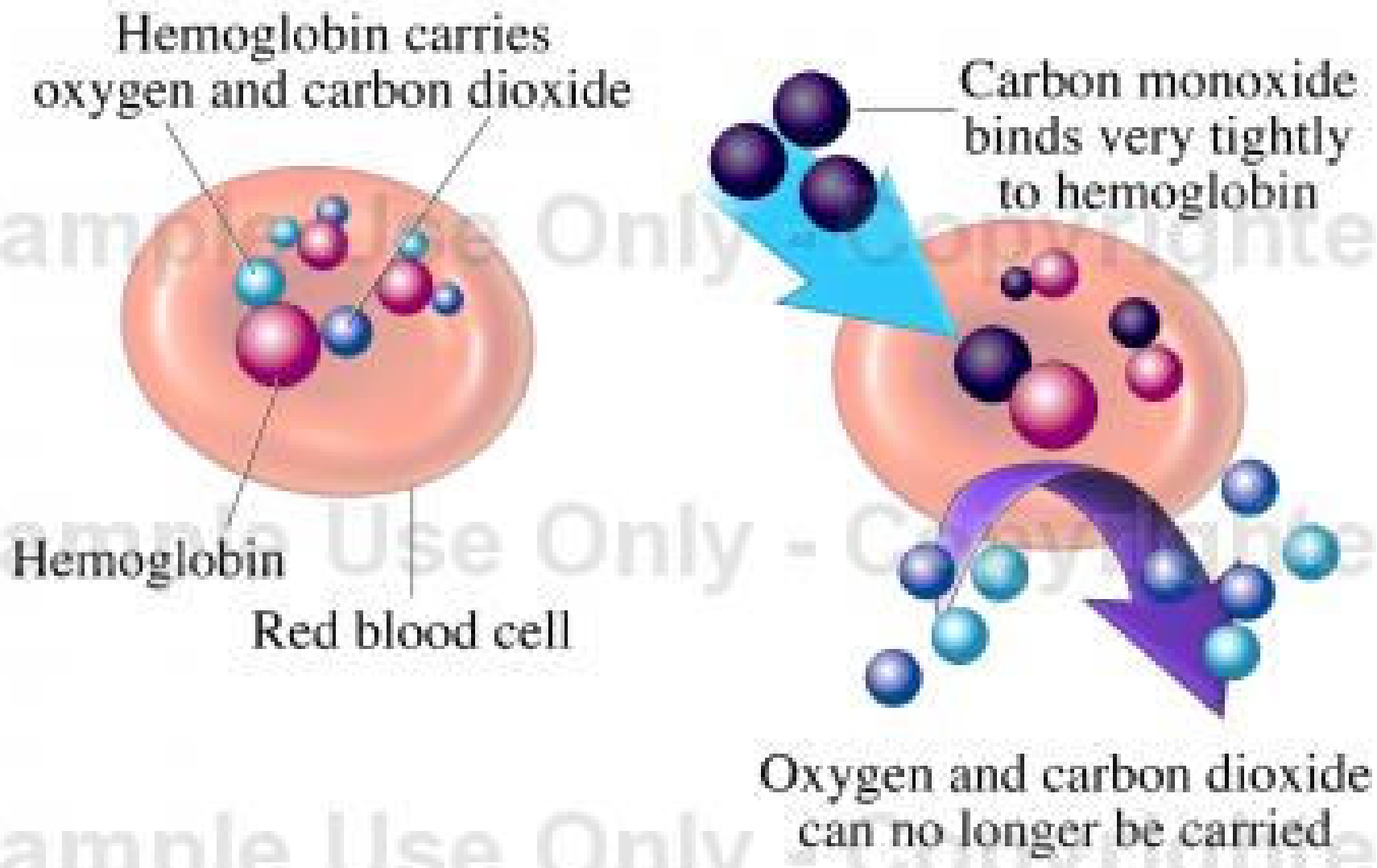
- Product of **incomplete combustion** of **fuel by vehicles**.
- Byproduct of **Coal mines.**
- **Cigarette Smoking** (more than 4%).
- Endogenous normal **metabolism-Heme catabolism**(Heme Oxygenase step)

- **CO has 200 times more affinity for Hb than O2.**

- CO readily links to Fe^{+2} of Hb and form- **Carboxyhb (Pink colour).**

- CarboxyHb has no place for binding O2.

- CarboxyHb reduces transportation and delivery of O2 by Hb.



- CarboxyHb **delivers CO at tissues instead of O_2.**

- **CO released in cells** is **inhibitor** of **Cytochrome oxidase** in **ETC.**

CarboxyHb Toxicity

- **Toxicity due to CarboxyHb is noted** when concentration is **more than 20%** in blood.
- Concentration **more than 40 -60% of Carboxyhb** in body may **lead to death**.

- **Symptoms of CarboxyHb Toxicity:**
  - **Nausea**
  - **Vomiting**
  - **Headache**
  - **Breathlessness**
  - **Irritability**
  - **Fatigue**

Investigation for CarboxyHb

- Study of blood sample using **Hand Spectroscope.**
- Characteristic **bands at 527 and 580 nm in green region** of visible spectrum confirms presence of CarboxyHb.

Management and Treatment Of CO Poisoning

- Carbon monoxide poisoning may be reverted
- By increasing **high concentrations of O_2**

- Cyanotic cases of Carboxyhb treated by administration of **oxygen mask/Oxygen cylinder.**

- **Oxygen under high pressure** is **helpful in managing severe cases of CO toxicity.**

- Increased pO_2 favors replacement of CO by O_2 to form OxyHb transport and deliver to tissues and support the metabolic function.

METHEMOGLOBIN

- **Methemoglobin** (MetHb) is an **abnormal Hemoglobin derivative.**

- **Methemoglobin has Hematin/ Heme Iron in Ferric** (Fe^{+3}) **state.**

- **Hematin** is **Ferriprotoporphyrin**

- **Hematin +Globin = Methemoglobin**

- MetHb has **defect in Heme** with **normal Globin** part.

- MetHb has **non functional Iron**- which cannot bind with O2 and transport it.

- **Methemoglobin is non functional oxidized form of Hemoglobin.**

- **Fe^{+3}** of Hb gets **coordinated with water instead of Oxygen at the sixth position.**

Formation of Methemoglobin OR Causes Of Methemoglobinemia

- **Normally** about **1%** of **Methemoglobin** is produced in **blood circulation**.
- **Abnormal high levels of blood Methemoglobin is-**

Methemoglobinemia

- **Causes for Methemoglobinemia:**
  - **Acquired Cause :**
    - Increases above 2% can occur with the **ingestion of strong oxidant drugs**

- When blood is exposed **to Oxidant Drugs** ,Hb interacts with it and **Fe^{+2} of Heme truly gets oxidized to Fe^{+3}.**
  - **Potassium Ferricyanide**
  - **Nitrites**
  - **Chlorates**
  - **Antipyrins**
  - **Sulfa Drugs (Sulfonamides)**
  - **Aniline Dyes**

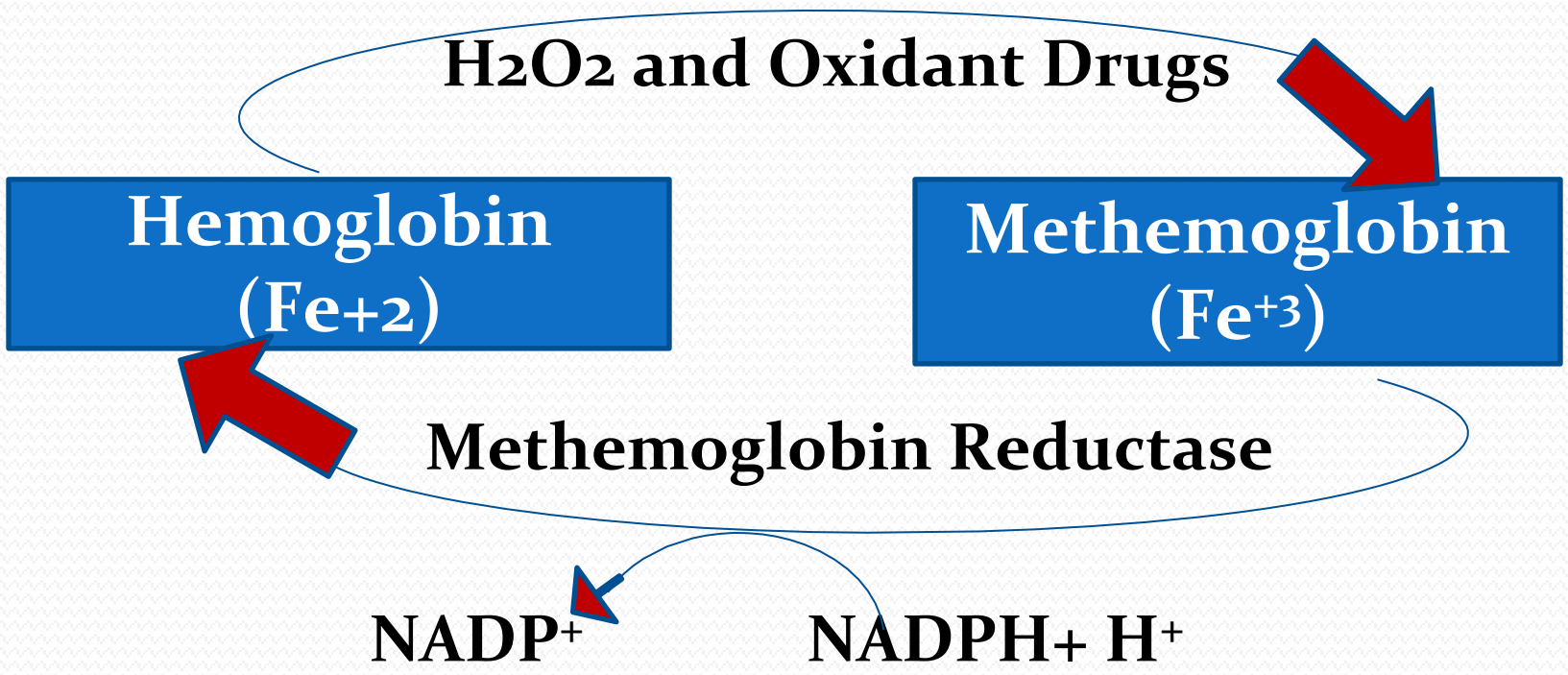
Conversion Of Methemoglobin To Hemoglobin

- Reducing agents converts Methemoglobin to functional Hb.
  - **Glutathione**
  - **Ascorbic acid**

- **Enzymes** converts Methemoglobin back to Hemoglobin:

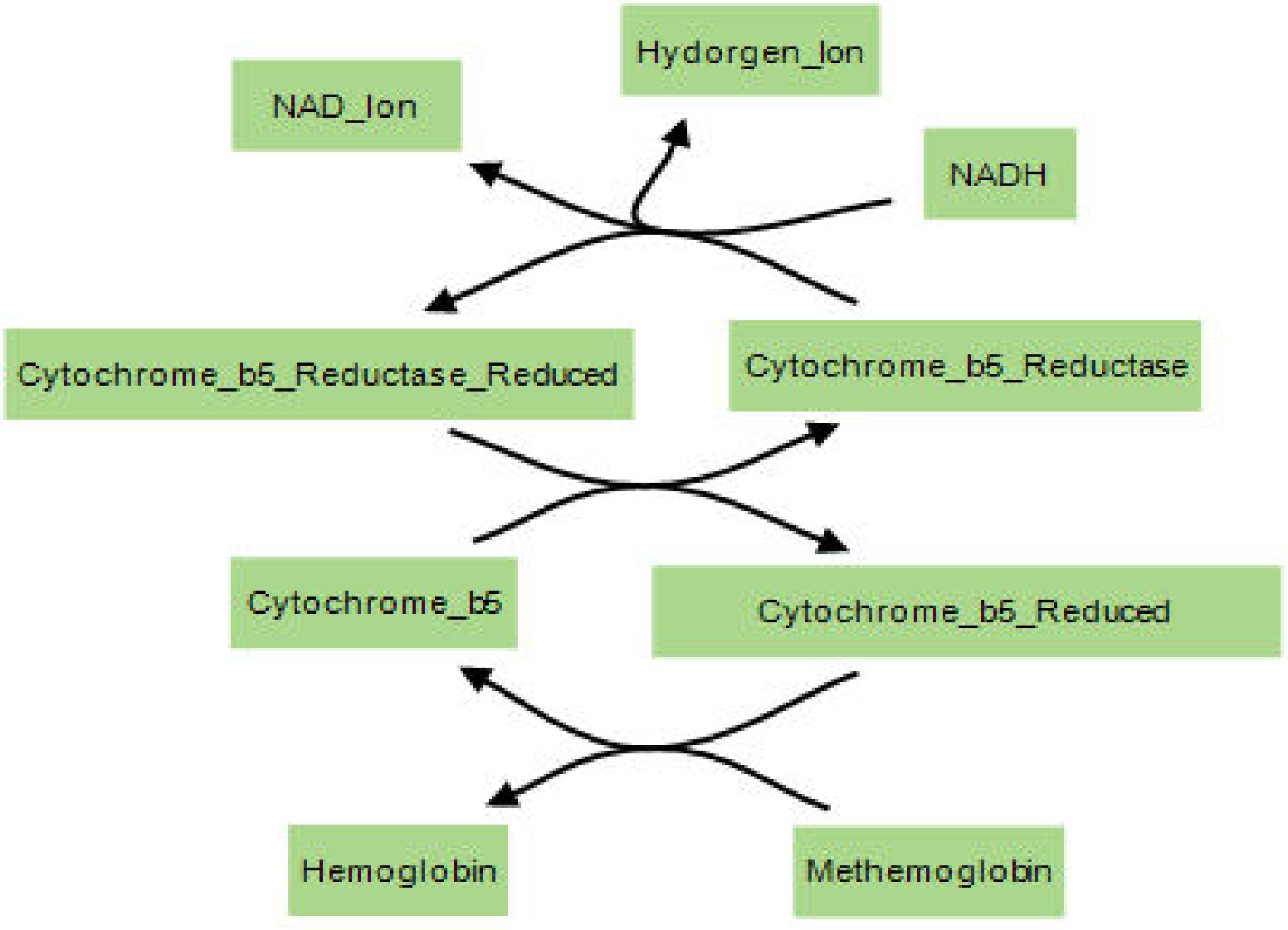
- **Methemoglobin Reductase**
- **Cytochrome b5 Reductase**

Conversion Of Methemoglobin To Hemoglobin is $NADPH+H^+$ Dependent



- The **source of NADPH+H$^+$** for the use of Methemoglobin reductase activity is **Pentose Phosphate Pathway(HMP Shunt).**

- **Defect in HMP Shunt affects** the **conversion of**
- **Methemoglobin to Hemoglobin** due to **devoid** of **NADPH+H$^+$**



Congenital Causes of Methemoglobinemia

- As a result of deficiencies of
  - **Methemoglobin Reductase**
  - **G6PD enzyme of HMP shunt**

Familial Methemoglobinemia

- **Inherited deficiency** of Enzyme **Methemoglobin Reductase** in the body
- Causes **Familial Methemoglobinemia.**

- **G6PD deficiency** of **HMP shunt** reduces generation of **$NADPH+H^+$**
- Which in turn affects **Methemoglobin Reductase** activity also **leads to Methemoglobinemia.**

- **Methemoglobin Reductase in absence of NADPH+H$^+$**
- Does **not convert Methemoglobin** back **to Hemoglobin**.
- Methemoglobin levels in blood gradually increases to **Methemoglobinemia.**

Consequences Of Methemoglobinemia

- **Methemoglobin** is **brown colored pigment.**

- Hence **Methemoglobinemia** – termed as :

Chocolate Cyanosis

Toxic Effects Of Methemoglobin

- Methb has Fe^{+3} which is **non functional**
- Does not bind and **transport O_2 to tissues**.
- Instead binds with water.

- **10-20%** of Methemoglobin- **Mild Cyanosis**.
- **50-60%** of Methemoglobin- **Severe Cyanosis**, Cardiopulmonary Symptoms-Tachycardia, Depression.
- **More than 60%** of Methemoglobin- **Unconsciousness and death.**

Investigation Of MetHb

- Study of Blood Sample using **Hand Spectroscope.**
- Performing **Schumm's Test (Spectroscopy)**

Management Of Methemoglobinemia

- Oral administration of reducing agents
  - **Ascorbic acid**
  - **Methylene Blue**

- Dried blood and old meat have brown color.

- Butchers uses Ascorbic acid to reduce Methemoglobin to make the meat look fresh!!

Sulfhemoglobin

- Sulfhemoglobin - occurs when the sulfur content of the blood increases due to
- **Ingestion of sulfur containing drugs**
- In **chronic constipation**(Gut bacteria acts on unexcreted material **produces H_2S**)

- **Sulfhemoglobin is greenish compound** where sulfur is **covalently attached** to **Porphyrin ring** (Not to Iron).

- **Sulfhemoglobin cannot bind with Oxygen.**

- **Unlike** the formation of **Carboxyhb and Methb,**

- The **formation of Sulfhb** is an **irreversible change of Hb**.

Drugs producing Sulfhemoglobin:

- Dapsone (Leprosy treating drug)
- Phenacetin
- Acetanilide
- Sulfanilamides
- These drugs produce Methb too

MYOGLOBIN

- **Myoglobin (Mb)-**

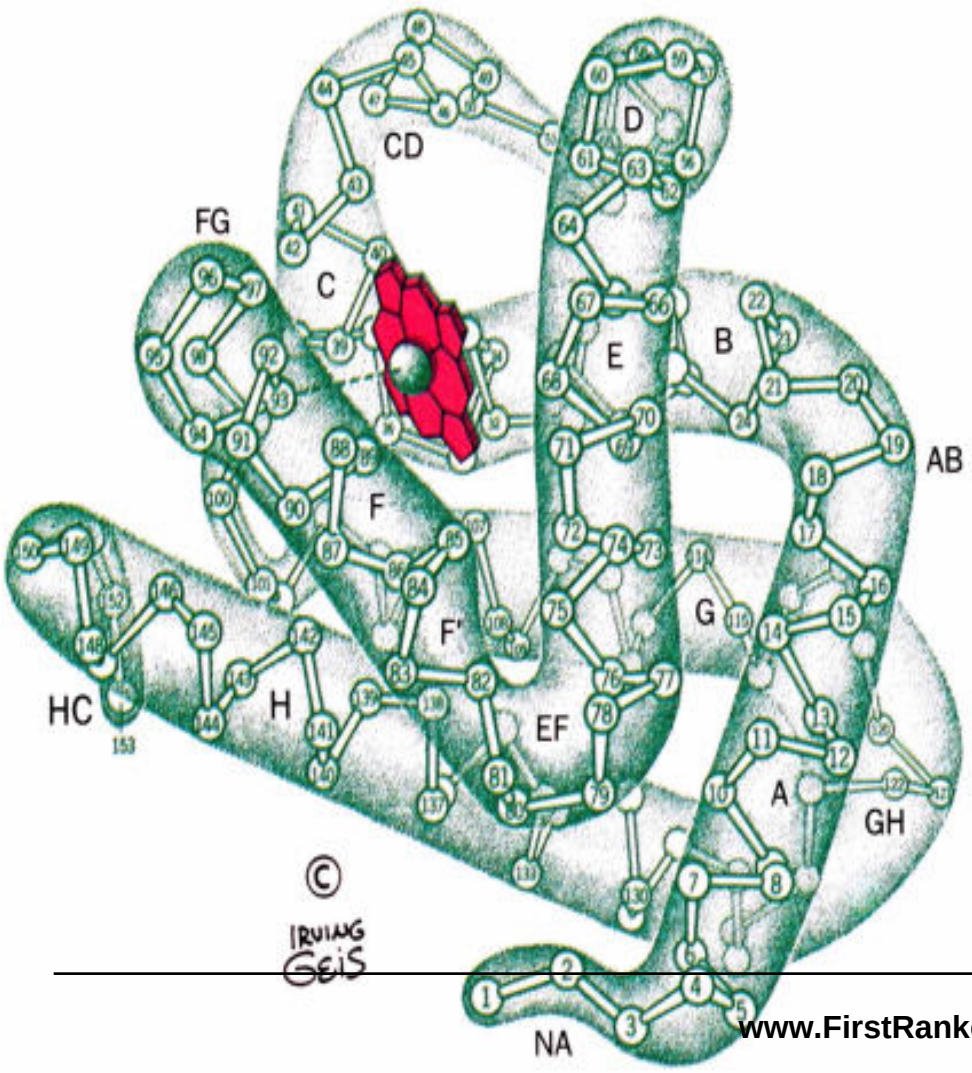
- **Mb is a Hemoprotein of red skeletal muscles.**

- **Primarily occur in Cardiac muscles.**

Structure Of Myoglobin

- **Myoglobin** is a **Monomeric unit**

- **Mb is composed one Globin chain and one Heme moiety.**

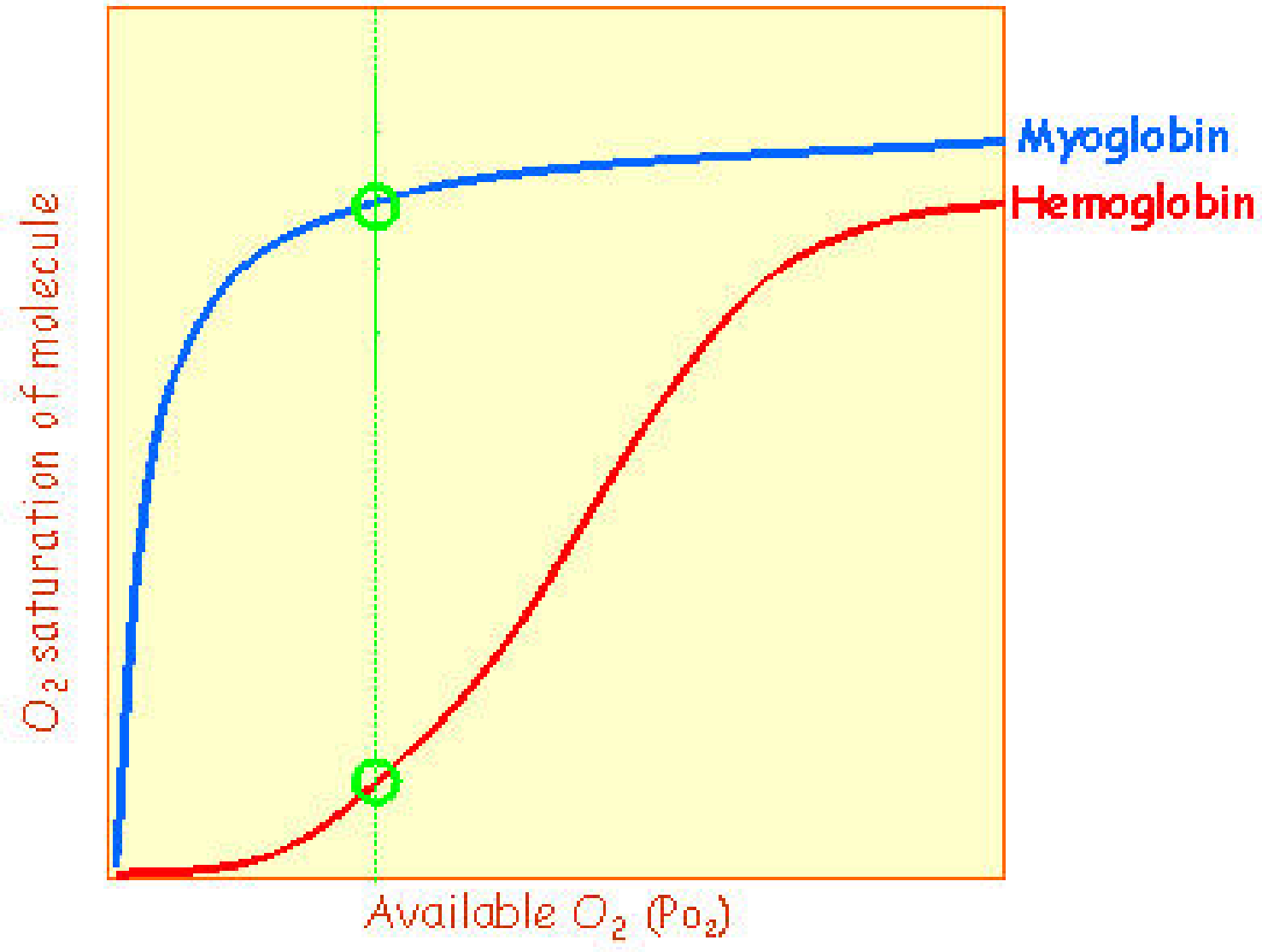


- **Mb is Spheroidal ,globular molecule 44 x 44 x 25**
- **Mb: Mol Wt 17,200 Daltons.**
- **Myoglobin is rich in alpha helix.**

- **Globin part of Myoglobin** is composed of **single polypeptide chain**
- **Composed of 153 amino acid residues.**

- **Myoglobin contains 1 Heme group which binds with 1 O2 .**
- **Iron in Mb is Fe^{2+}** (Ferrous ion) the functional form that binds Oxygen.
- **Oxygen binds as the sixth ligand to Fe** (MbO_2)

- **Myoglobin** has **very low p50 value 2-3 torr/mm.Hg.**
- **ODC** of Myoglobin is **simple hyperbolic curve.**



- In comparison to Hemoglobin A and HbF **Myoglobin has high affinity for Oxygen.**
- Hb F and Mb has low p50 values as compared to p50 value of Hb A1.
- With low p50 ,more Oxygen binds at low pO2.

Function Of Myoglobin

- Myoglobin is found in cytosol of **skeletal and Heart muscles.**

- **Myoglobin facilitates rapidly Respiring muscle tissue**

- The rate of O_2 diffusion from capillaries to tissue is slow because of the solubility of Oxygen.
- **Myoglobin increases the solubility of Oxygen**.
- Myoglobin **facilitates Oxygen diffusion**.

- **Myoglobin in Muscle Cells is a:**
  - **Oxygen storing Hemoprotein**
  - **Reservoir of Oxygen**

- **Myoglobin does not allow Oxygen to remain in free state:**

- Oxygen diffused in muscle cells is used up in Oxidative phosphorylation.
- If Oxygen remained unused in the cells it immediately binds with Mb to form MbO_2

- MbO_2 releases/unloads O_2 when required.
- **MbO_2 unloads oxygen** at **extreme conditions**
- When **pO_2** of cellular level reaches to **5 mm Hg.**

- Myoglobin releases Oxygen in rapidly respiring cells.
- The released O2 is used up in Oxidative Phosphorylation.

- Mb present within muscle cells comes out in blood after damage to muscle cells.
- Mb is found **abnormally in blood and urine of MI cases**.
- **Thus elevated Myoglobin levels in blood/urine** is **a marker of Myocardial damage**.

- **Metmyoglobin does not bind to Oxygen.**
- Since oxidation of Fe^{+2} yields Fe^{+3} -Ferric iron (non functional form)

Differentiate Between Hb and Mb

S.No	Hemoglobin (Hb)	Myoglobin (Mb)
1.	Hb is **Oxygen transport protein in RBCs of blood**.	Mb is **Oxygen storing protein in muscles**.
2.	**Tetrameric** has four Heme and **binds with 4O2**	**Monomeric** has one Heme and **binds with 1 O2.**
3.	**Oxygenated at Lungs**	**Oxygenated at Muscle Cell Cytosol.**
4.	HbO2 unloads oxygen at tissues when pO2 is at 40 mmHg. **P50 for HbA1 is 27 torr.**	MbO2 unloads oxygen at cell cytosol when pO2 is at 5 mmHg. To rapidly respiring cells **P50 for Mb is 2 torr.**
5.	**ODC is sigmoid shaped**	**ODC is hyperbolic shaped.**
6.	**Hb has 574 amino acids. Mol .wt-67,000 Daltons.**	**Mb has 153 amino acids. Mol wt-17,200 Daltons.**

Cytochromes

- Cytochromes – **Hemoprotein**.

- **Cytochromes** are **components of ETC**
- **Who** bring **Oxidative phosphorylation** and **generates ATP** .

- **Cytochrome P450-** Involved in **Drug detoxification.**

Catalase and Peroxidases

- **Enzymes present richly in Peroxisomes of cells.**

- Catalase and Peroxidase are of Enzyme Class- **Hydroperoxidases.**

- **Glutathione Peroxidase (R.B.C)**
- **Leucocytes Peroxidase (W.B.C)**

Catalase and Peroxidase Detoxify H_2O_2

- **Substrate for Catalase and Peroxidase is H_2O_2 which** detoxify it.
- Catalase and Peroxidase decomposes $2H_2O_2$ to 2 H_2O and O_2.

- **Role of Catalase and Peroxidase**

- **Prevents accumulation of H_2O_2 (Toxic free radical)** in cells .

- Prevents **Peroxidation of membrane lipids** and **protect cellular lysis.**

Tryptophan Dioxygenase

- **Tryptophan Dioxygenase/Tryptophan Pyrrolase** involved in Tryptophan catabolism.

Deficiency of Tryptophan Dioxygenase

- Accumulates Tryptophan without its breakdown to **liberate Acetyl-CoA** (Ketogenic precursor) and **Alanine** (Glucogenic precursor).

- Deficiency of **Tryptophan Dioxygenase**
- Blocks **Kynurenine Pathway** for the biosynthesis of **Niacin from Tryptophan**.

Effect Of Cyanide and Carbon Monoxide on Hemoproteins

- **CN and CO** disrupts physiological function of HemoProteins.
- Thus **CN inhibits** the function of **Hb ,Mb, Cytochromes**.

- **Impaired activity** of these **Hemoproteins**
- Badly **affects Oxygen metabolism and ATP generation.**
- More affected cells are **Nervous system,**

Erythrocytes

Questions Of Hb Chemistry

1. Structure Of Hemoglobin
2. Heme Structure
3. Globin Structure
4. Functions of Hemoglobin/Biomedical Importance of Hemoglobin.
5. Salient features of Hemoglobin Oxygenation and Deoxygenation.
6. Allosteric Effectors of Loading and Unloading of Oxygen by Hemoglobin.
7. 2,3 BPG and its role in Hb.
8. ODC of Hemoglobin and factors affecting it
9. CO_2 Transportation in human body

10. Normal Hb Variants
11. Glycosylated Hb and its significance.
12. Hemoglobin Derivatives
13. Dyshemoglobins/Abnormal Hb derivatives
14. CarboxyHemoglobin
15 . Methemoglobin
16. Types of Hemoproteins

Differentiate between following:

1. Hb A and Hb F
2. Hemoglobin and Myoglobin
3. T form and R form of Hb
4. Hb at Lungs and Hb at Tissues/ Oxygenation of Hb and Deoxygenation of Hb.