Dioxygen storage and transport

PG Third Semester

Bioinorganic Chemistry-III

Lecture 5, 6 & 7

Bapan Saha

Assistant Professor, Chemistry

Handique Girls' College

Guwahati-01

Contents

- Oxygen transport proteins
- Structures of Heme proteins – Mb and Hb
- Functions of Heme proteins – Mb and Hb
- Co-operative and non co-operative binding of dioxygen
- Bohr Effect
- Hill coefficient
- Dioxygen toxicity
- Synthetic dioxygen carrier
- Nature of heme-oxygen binding

Books/References used and suggested

- Bioinorganic Chemistry by Bertini, Gray, Lippard and Valentine
- Inorganic Biochemistry by Cowan
- Bioinorganic Chemistry by A. K. Das
- Environmental Chemistry by A. K. De
- Oxford Chemistry Primer by Fenton

Dioxygen in Living Organism

- Dioxygen is produced in biosphere through photolysis of water during photosynthesis in cyanobacteria, green algae and plants.
- Most organisms require dioxygen to survive.
- Mostly consumed in the terminal (or primary) step of oxidative phosphorylation.

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

- Some small animals and plants with large surface-to-volume ratio, an adequate supply of dioxygen can be obtained from diffusion across cell membranes.
- For other organisms (scorpions to whales), diffusion does not supply sufficient dioxygen for respiration.
- Dioxygen carrier protein is required for higher organisms.
- The carrier must bind and release dioxygen at a rapid rate.

Dioxygen transport proteins

General features of dioxygen carrier proteins: Three components

- First, the active site (dioxygen binding site), a complex either of Cu or Fe (hemoglobin, hemerythrin, hemocyanin etc.)
- Second, the dioxygen carrier protein (facilitates sequestration of dioxygen), (lungs, gills etc.)
- Third , the delivery system (blood plasma, heart etc.)

Metalloprotien	Active site (deoxy)	Color change (deoxy to oxy)	MW (D)	Subunits	MW(av)/ subunit
Hemoglobin (vertebrate)	Heme Fe II	Purple to red	64,000 (Human)	4	16,000
Hemoglobin (invertebrate)	Heme Fe II	Purple to red	Upto 3.3×10^6	192	17,000
Erythrocruorin					
Chlorocruorin	Chloroheme Fe II	Purple to green	Upto 3.1×10^6	192	15,000
Hemocyanin					
Mollusc	Cu I….Cu I	Colorless to blue	$\sim 9 \times 10^6$	160 (mollusc)	52,700
Arthropod			$\sim 9 \times 10^5$	12 (arthropod)	76,600
Hemerythrins	Fe II….Fe II	Colorless to burgundy	108,000	8	13,500

Where hemoglobin is found ?

- Hb is the iron containing dioxygen transport metalloprotein in the RBC of all vertebrates (possible exception, the Antarctic fish *Cyclostomata*).
- Hb is found in the tissues of some invertebrates (including some insect larvae), molluscs, almost all annelid worms (erythrocruorins (Er) in arthropods and chlorocruorins (Ch) in some annelid).
- Hb is found in some plants (e.g., leghemoglobin in the nitrogen-fixing nodules of legumes).
- Some organisms (the clam *Scapharca equivalvis*) also feature a dimeric hemoglobin.

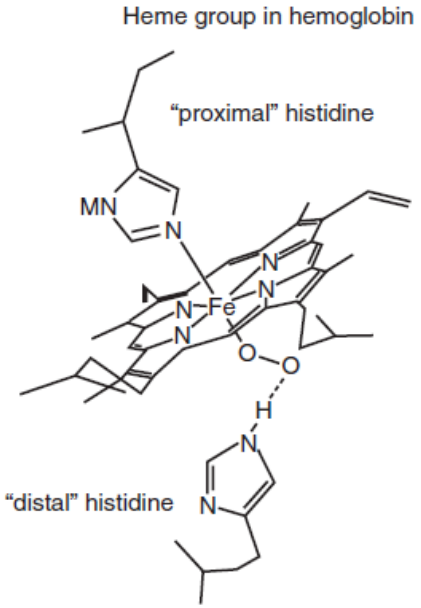
Molluscs - Hemocyanin

Sipunculan - Hemerythrin

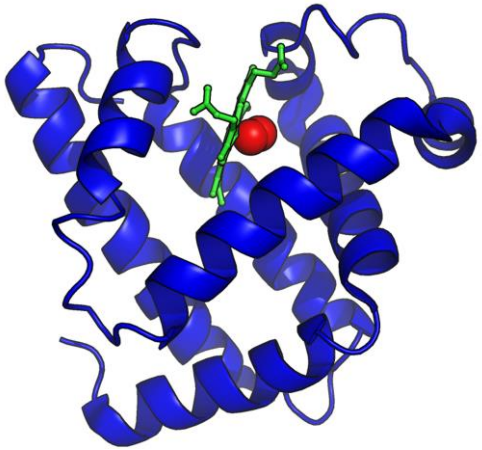
Marine worm - Chlorocurin

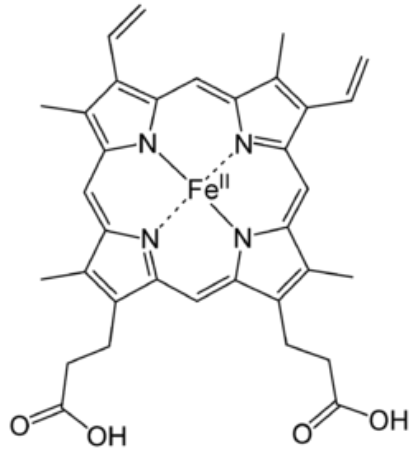
Annelids - Erythocruorin

Myoglobin and its structure



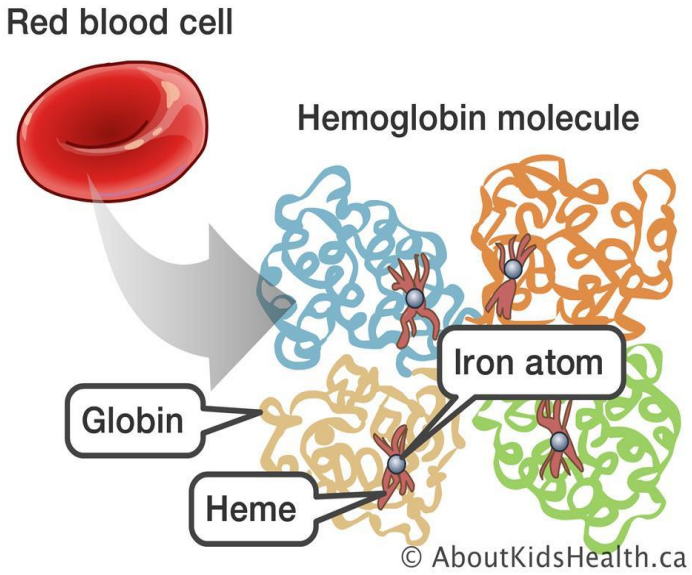
- Mb (MW ≈ 17000 D) is found in cytosol within the cell. Present in muscles and bone marrow
- It is a single heme group surrounded by protein chain.
- Heme unit is a Fe (II) porphyrin ring (tetrapyrrole ring) with high spin Fe (II) (d^6).
- 153 amino acid residues in protein chain folded about single heme group.
- Fifth co-ordination site of Fe(II) is occupied by N-atom from imidazole side chain of histidine segment.
- Eight side chains (four methyl, two vinyl and two propionic acid)
- High spin Fe(II) ($t_{2g}^4 e_g^2$) has ionic radius ≈ 92 pm and is large enough to get fit in the hole of porphyrin ring, deoxy form is pseudo-octahedral (out of the plane).
- Fe(II) is low spin ($t_{2g}^6 e_g^0$) in oxy form and has ionic radius ≈ 75 pm. Fe(II) drops into the hole in the porphyrin ring (F-O-O angle ~ 115°).

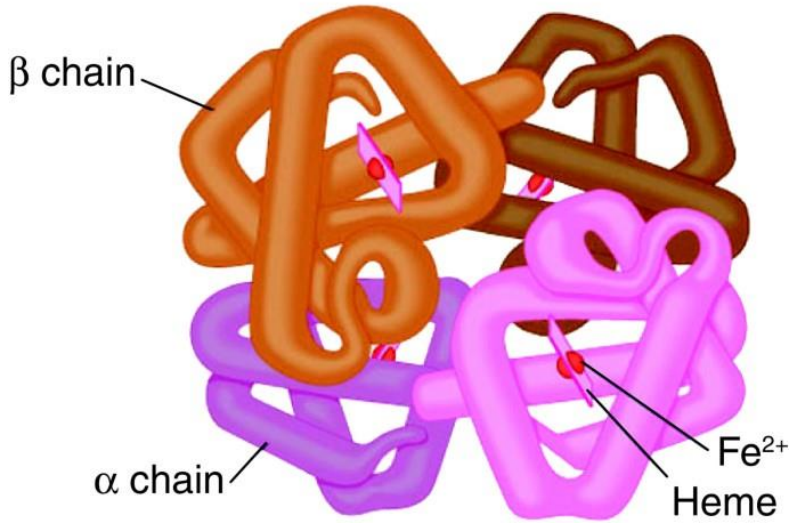


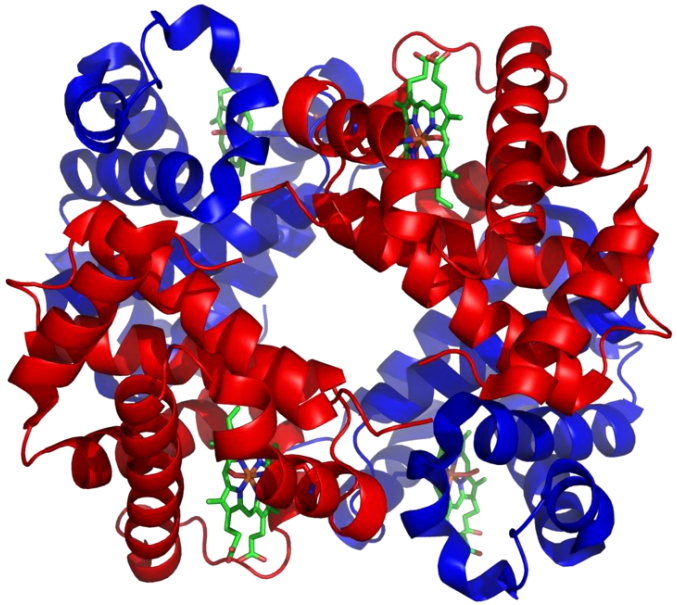


Hemoglobin

- Discovered by Hunefeld in 1840.
- Max Perutz (1959) determined the molecular structure of hemoglobin (XRD).
- Resulted in his sharing of Noble prize with John Kendrew (1962), for their studies of the structures of globular proteins.
- Hb is considered as an approximate tetramer Mb, MW around 68000 D
- Hb has a quaternary structure, characteristic of many multi-subunit globular proteins.
- Most of the amino acids in hemoglobin form alpha helices, and are connected by short non-helical segments (loops)



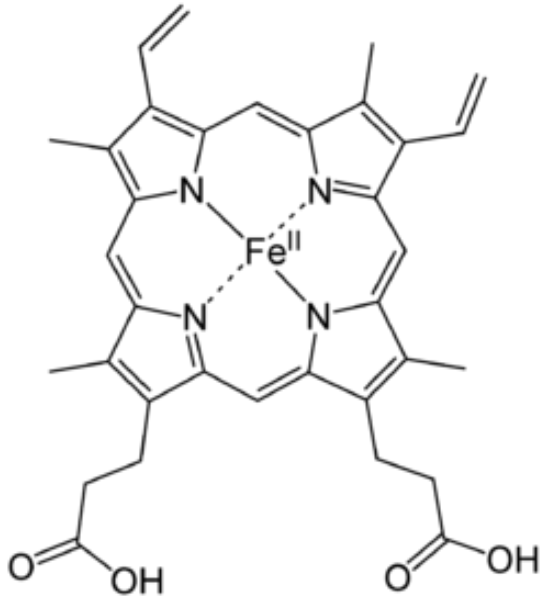




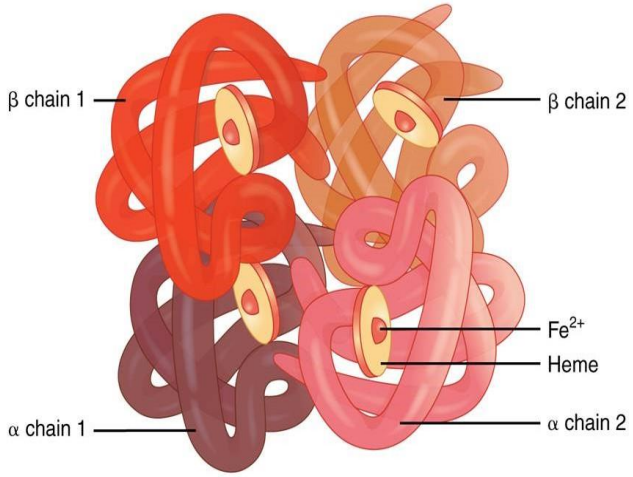
Quaternary structure of hemoglobin

Structure of Hemoglobin

- Hb structure is **heme** and **globin**.
- Hb is an approximate tetramer Mb (four heme groups).
- Heme group is an Fe(II) porphyrin complex (tetra pyrrole unit).
- Fe(II) ion coordinates to the four nitrogen atoms of four pyrrole rings.
- The fifth co-ordination site of Fe(II) is occupied by an imidazole N-atom of from a histidine segment (proximal histidine) below the porphyrin ring.
- Pyrrole units are connected at the α-carbon by methylidene bridges
- Eight side chains (4-methyl, 2-vinyl, 2-propionic acid) are present at the tetrapyrrole moiety.
- Globin is made up of four polypeptide chains.
- Two alpha (α) and two beta (β) chains with 141 (α) and 146 (β) amino acids respectively ($\alpha_2\beta_2$).
- Fe(II) is high spin ($t_{2g}^4 e_g^2$) and resides out of the porphyrin plane.
- In deoxy form, weakly bonded H_2O is proposed to fill the sixth co-ordination site forming a distorted octahedron.



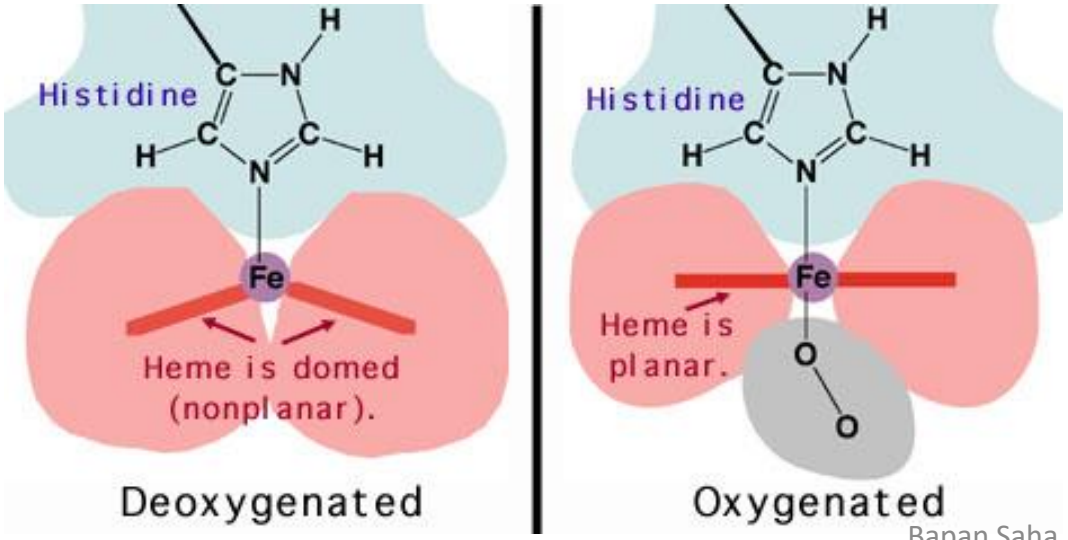
Heme unit

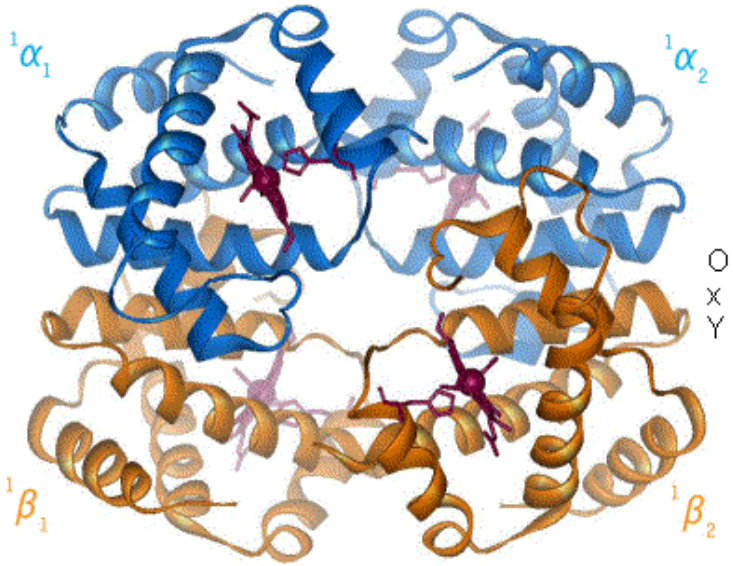


Globin part

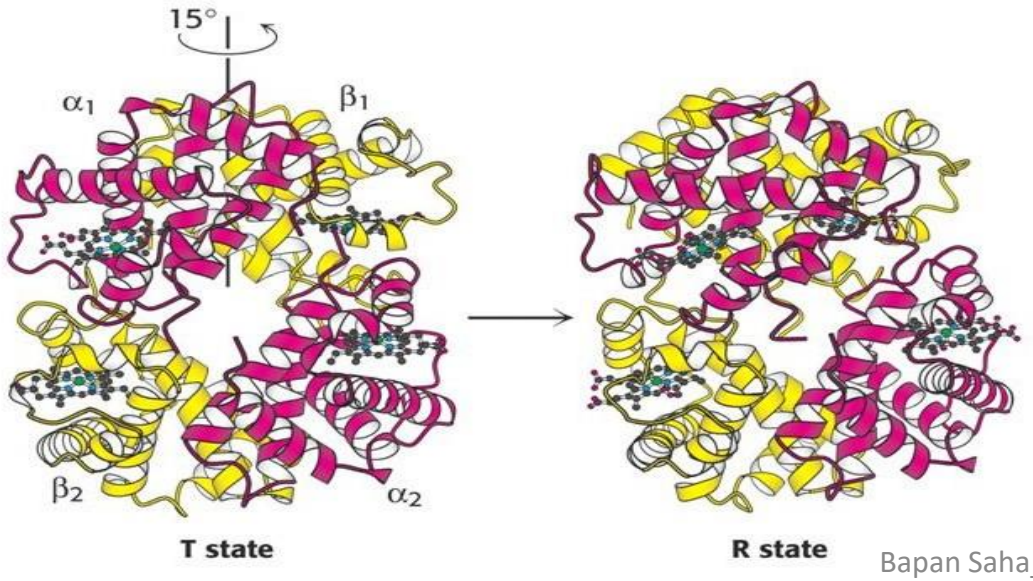
Hemoglobin binding to dioxygen-Conformation Change

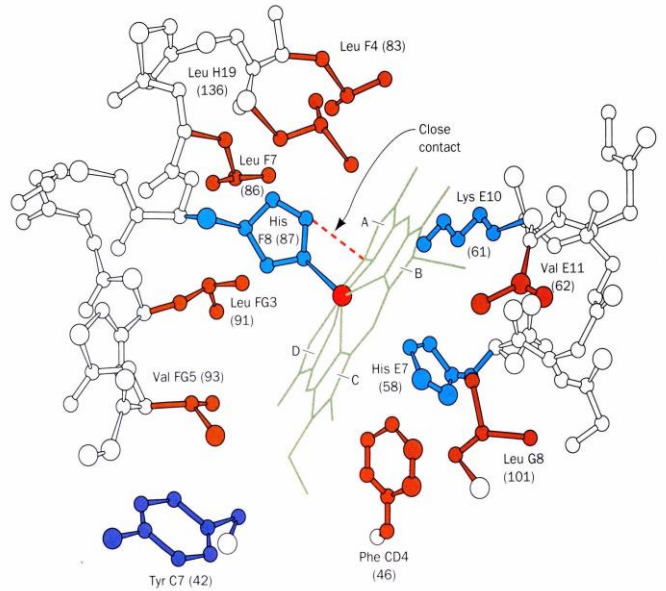
- Sixth position of Hb is reversibly coordinated by O_2 via coordinate covalent bond, resulting in an octahedral arrangement.
- Conformations of the deoxy and oxy-Hb are called the T (tensed) and R (relaxed) state respectively.
- Dioxygen binds in an "end-on bent" geometry where one oxygen atom binds to Fe and the other protrudes at an angle (Fe-O-O angle about 153°).
- One αβ half of the molecule rotates around 15° relative to other half, change in the quaternary structure.
- Upon oxygenation, two of the heme groups move about 100 pm towards each other while the two others separated by about 700 pm.





- Fe(II) is about 0.6 Å out of the heme plane in the deoxy state and upon oxygenation, it is pulled back into the heme plane.
- This motion is particularly important for Hb because it pulls on the proximal histidine ligand and helix F moves.
- Binding of dioxygen on one heme is more difficult, but its binding causes a shift in the α_1 and β_2 contacts and moves the distal His E7 and Val E11 away from the oxygen's path to the Fe on the other subunits.
- This process increases the affinity of heme toward oxygen.
- The T state with reduced oxygen affinity will be changed to the R state without binding oxygen because the other subunits switch upon oxygen binding.





Physiology of Hemoglobin and Myoglobin

- Hb carries O_2 from lungs to tissues and gets transferred to Mb (stored) for metabolic processes.
- To be thermodynamically favourable, Mb must have greater affinity for O_2 than Hb in the tissues/cell.
- Equilibrium constant for Mb-O_2 complexation is simple. If total amount of Mb ([Mb]+ [Mb-O_2] is held constant and O_2 concentration is varied, hyperbolic curve is obtained (in cell Mb is largely oxygenated).
- Equilibrium constant for $(Hb\text{-}O_2)_4$ complexation is complex and the exponent 2.8 arises from cooperative binding. This results in a sigmoid curve for oxygenation of Hb.
- Binding of O_2 by Hb is also pH dependent and at higher pH it is favorable (Bohr effect).

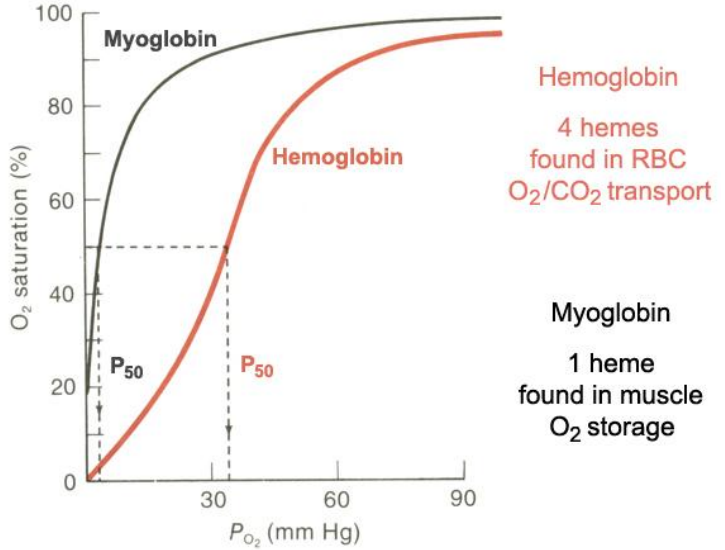
Lungs: $Hb + 4O_2 \longrightarrow Hb(O_2)_4$

Tissues: $Hb(O_2)_4 + 4Mb \longrightarrow 4Mb(O_2) + Hb$

Equilibrium constants for $Mb(O_2)$ & $Hb(O_2)_4$ complexation are

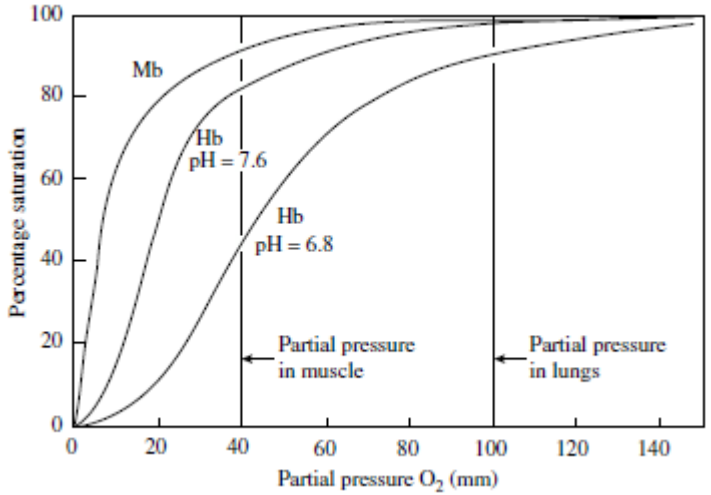
$$K_{Mb} = [Mb(O_2)] / [Mb][O_2]$$

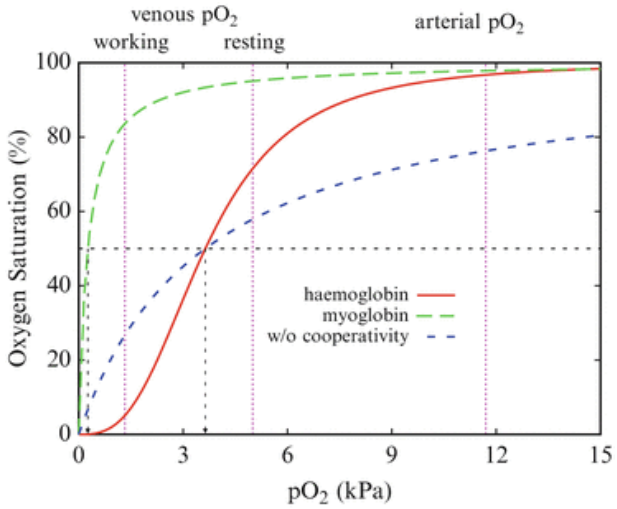
$$K_{Hb} = [Hb(O_2)_4] / [Hb][O_2]^n \qquad n < 4 , n \sim 2.8.$$



Cooperativity of Hemoglobin

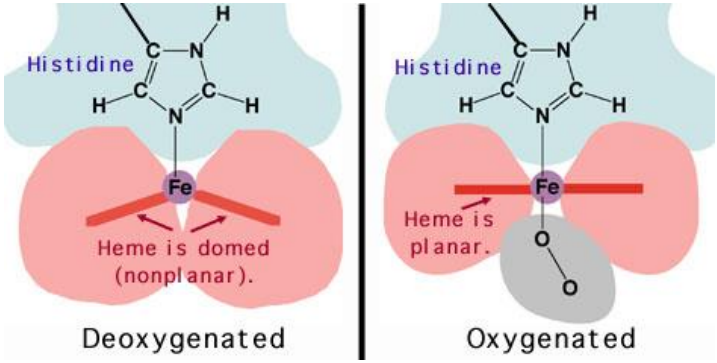
- The binding of four O_2 molecules to hemoglobin is interdependent.
- **Cooperativity**: Presence of several bound O_2 molecules favors further oxygenation and conversely, if only one O_2 molecule is present, it dissociates more readily than a highly oxygenated species.
- At low O_2 concentrations Hb is less oxygenated (tends to release) and at high O_2 concentrations Hb is oxygenated to larger extent.
- The Hb-O_2 binding constant depends on oxygen partial pressure.
- Cooperativity allows the oxy-Hb to carry the maximum amount of O_2 to the tissues and then allows the deoxy-Hb to release the maximum amount of O_2 into the tissues.
- Results in sigmoidal behavior for oxygenation of Hb. Mb upon oxygenation yields a hyperbolic curve.

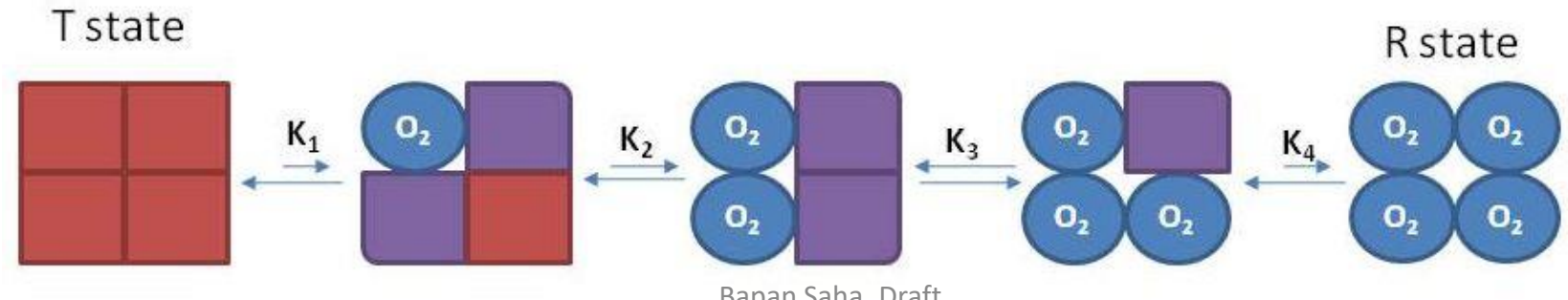




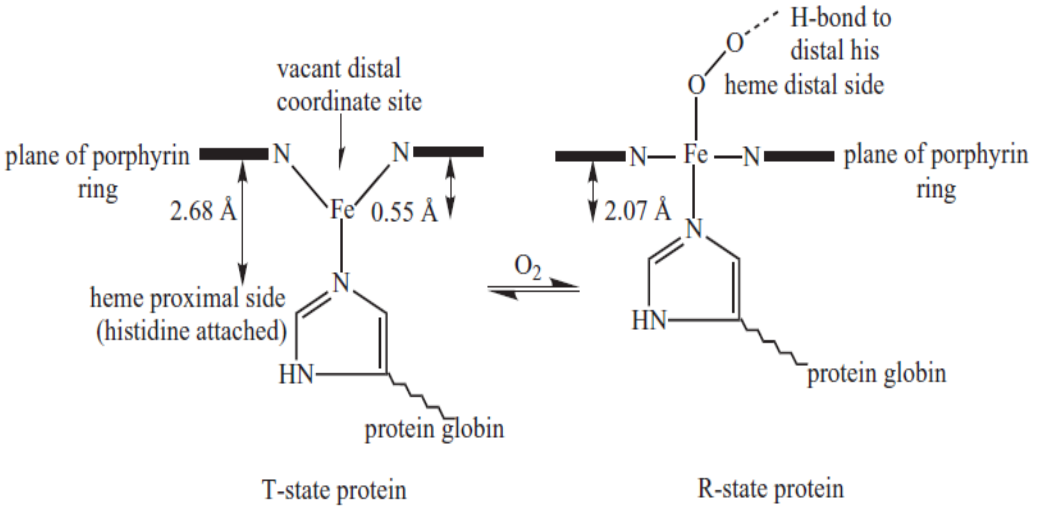
Trigger action - mechanism of co-operativity

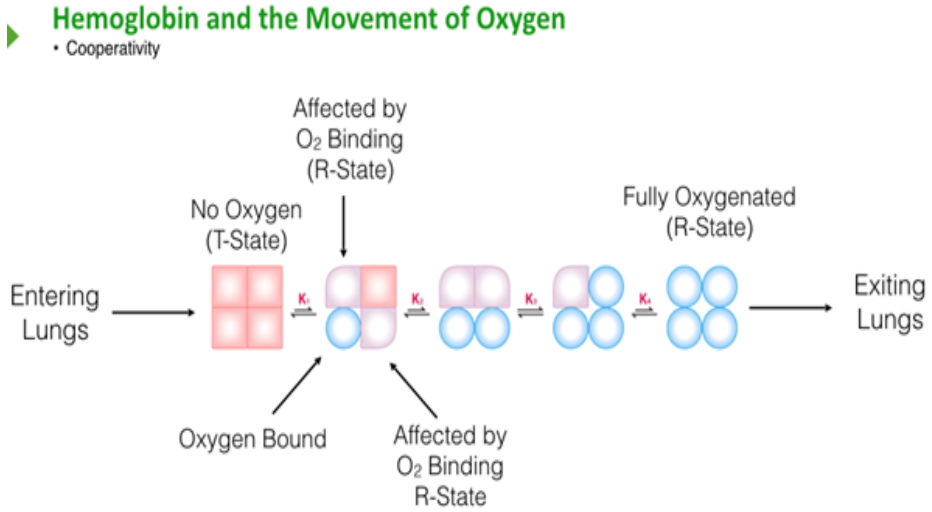
- Hb can exist in two different forms: T-state (deoxy) & R-state (oxy)
- T-state has a much lower O_2 affinity than the R-state (almost equal to isolated α and β chains).
- Increasing the partial pressure of O_2 causes the conversion of T-state to R-state.
- O_2 binding at the four heme sites in Hb is not independent and simultaneous.
- Binding of 1st O_2 molecule is difficult but 2nd, 3rd & 4th gets progressively easier and easier.
- As the body circulates, the O_2 level drops and Hb releases its bound O_2 into the tissues.
- The key or trigger in the Perutz mechanism is the high spin Fe(II) atom in deoxy-heme.
- The Fe(II) is forced to sit above the porphyrin plane with Fe-N distance of about 206 pm.
- The heme group is domed upward towards the proximal histidine.





- Upon oxygenation, the Fe(II) is low spin and smaller, move towards the porphyrin plane with Fe-N distance of 198 pm (attributed to steric interactions between histidine, globin chain and heme unit).
- This results considerable strain on the oxy-heme and associated tertiary structure of the globin within the T-state, discouraging the addition of first O_2 and pushes the last O_2 off in the tissue.
- Addition of second O_2 molecule takes place with similar results.
- Addition of third O_2 molecule results in interconversion to R state and Fe(II) moves into porphyrin hole.
- This allows the fourth heme to bind the last O_2 molecule without any constrains.





Thermodynamic factors

- Hb must bind and release dioxygen at a rapid rate. For the process (M-oxygen carrier),

$$M + O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MO_2 \qquad \text{.....(1)}$$

$$K_c = [MO_2]/[M][O_2] \qquad \text{.....(2)}$$

- The solvent dependent quantity $[O_2]$ in equation (2) can be replaced by solvent independent quantity $P(O_2)$, the partial pressure of dioxygen. The new equilibrium for the process (1) is given by,

$$K_p = [MO_2]/[M]\ P(O_2) \qquad \text{.....(3)}$$

- The affinity can thus be conveniently expressed as the partial pressure of dioxygen required for half-saturation of the species M, $P_{1/2}(O_2)$. Under such conditions, $[M] = [MO_2]$ and we have

$$P_{1/2}(O_2) = 1/K_p \qquad \text{.....(4)}$$

where $P_{1/2}(O_2)$ is given in torr or mm Hg. The dioxygen affinity is composed of enthalpic ΔH and entropic ΔS components, with

$$\Delta G^\circ = - RT \ln K = \Delta H^\circ - T\Delta S^\circ \qquad \text{……..(5)}$$

Within a family of oxygen carriers the values of ΔH° and ΔS° are usually similar. Large deviations are therefore indicative of a change in nature of the oxygen binding process.

Non-Cooperative Dioxygen Binding

- If the oxygen-binding sites M are mutually independent and non-interacting, the plot of concentration of species MO_2 as a function of the partial pressure of O_2 is analogous to Langmuir isotherm. The plot of the fractional saturation of dioxygen binding sites (θ) versus $P(O_2)$ is hyperbolic curve labeled "non-cooperative" (Fig. A) where,

$$\theta = [MO_2]/([M] + [MO_2]) = K_p P(O_2)/(1 + K_p P(O_2)) \quad .....(6)$$

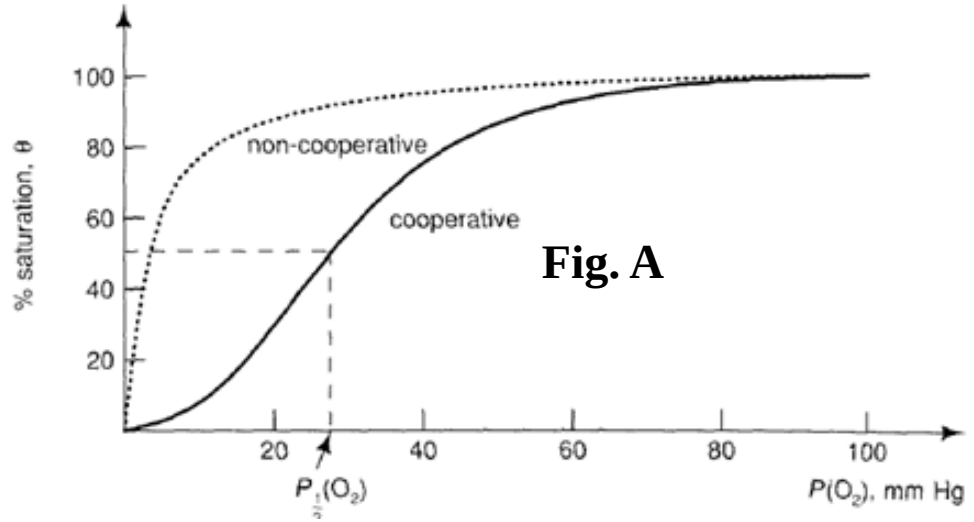


Fig. A

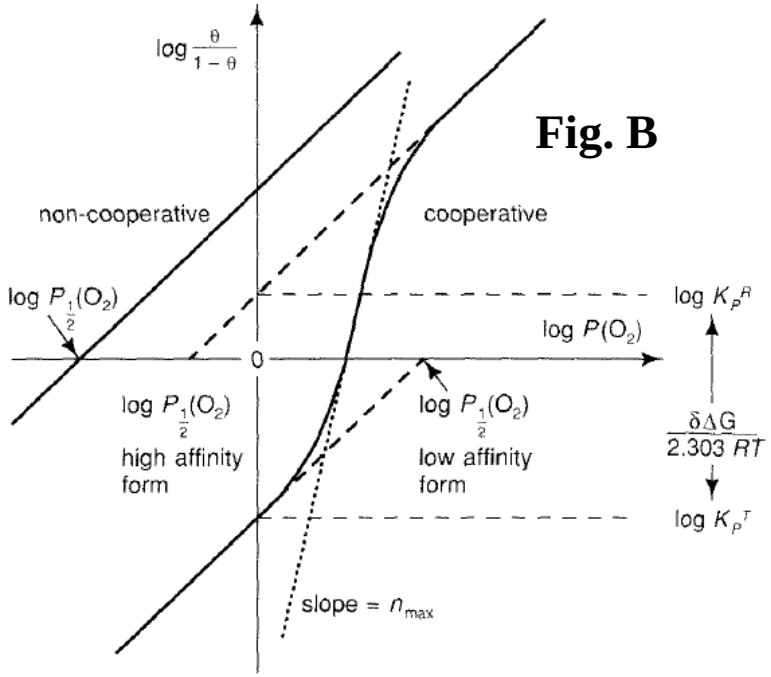


Fig. B

- Alternatively, plot of log (θ/(1 - θ)) vs log $P(O_2)$, called as Hill plot, gives a straight line with a slope of unity and an intercept of -log $P_{1/2}(O_2)$ (Fig B). (n_H = 1, for Mb, non-cooperative)
- A differential form is shown as the dotted line (Fig. C).
- Such binding, where the dioxygen sites are independent of each other, is termed non cooperative

Cooperative Dioxygen Binding

- Dioxygen binding in Hb is interdependent. The binding or release of dioxygen at one site may affect the affinity and kinetics of ligand binding or release at a neighboring site (sigmoidal curve, Fig. A). Thus, the dioxygen binding is co-operative in nature.

Degree of co-operativity can be characterized in a number of ways:

- By means of a Hill plot of $\log(\theta/(1-\theta))$ versus $\log P(O_2)$, the limiting slopes (which should be unity) at high O_2 pressure and low O_2 pressure may be extrapolated as shown in Fig. B to $\log(\theta/(1 - \theta)) = 0$, where $\theta = 0.5$

Two limiting values for $P_{1/2}(O_2)$ are obtained:

1. $P_{1/2}(O_2)$ characterizing the regime of high partial pressure of dioxygen, where the O_2 affinity is high (positive cooperativity).
2. $P_{1/2}(O_2)$ value characterizes the regime of low partial pressure of dioxygen, where affinity is relatively low.

   The difference in affinities can be converted into a difference between the free-energy change upon O_2 binding in the low-affinity state (K_p^T) and the high-affinity state (K_p^R).

$$\delta\Delta G^\circ = - RT \ln (K_p^T / K_p^R) \qquad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

- A second way to characterize cooperativity involves fitting the oxygen-binding data at intermediate saturation ($0.2 < \theta < 0.8$), that is, about the inflection point in a Hill plot - to the Hill equation

$$\theta/(1- \theta) = K_p P^n(O_2)$$

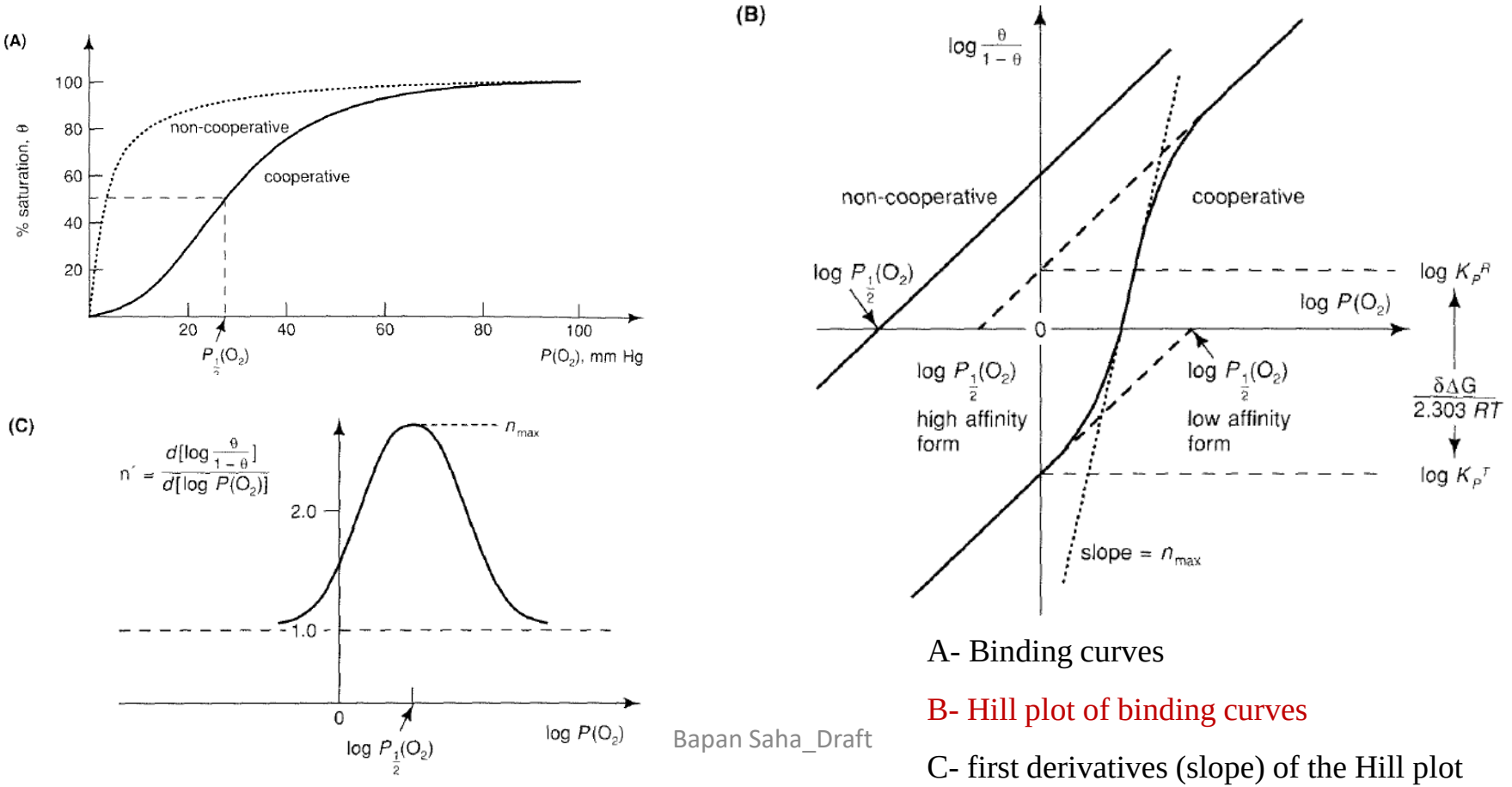
$$\log (\theta/(1 - \theta)) = - \log(P_{1/2}(O_2) + n \log(P(O_2)) \qquad \ldots\ldots\ldots\ldots\ldots\ldots(8)$$

- The Hill coefficient (n) is an empirical coefficient and has value of unity for non-cooperative binding.
- Any number greater than unity indicates positive cooperativity (For Hb, n_H (max slope)= 3.0-3.5, $n_H > n$). The fit is only approximate, since the Hill plot is only approximately linear about the inflection point (Fig B).
- Intercepts with broken black line at the 0 value for $\log (\theta/(1 - \theta))$ indicate $P_{1/2}(O_2)$ and so O_2 binding affinity (lower $P_{1/2}(O_2)$ = higher affinity
- Hb high affinity O_2 binding $\log P_{1/2}(O_2)$ = upper asymptote intercept
- Hb low affinity O_2 binding $\log P_{1/2}(O_2)$ = lower asymptote intercept
- A more precise value of n may be obtained by plotting the slope in the Hill plot (n') as a function of $\log P(O_2)$ (Fig. C).

$$n' = d[\log (\theta/(1 - \theta))]/d[\log (P_{1/2}(O_2)] \qquad \ldots\ldots..(9)$$

Non Cooperative and Cooperative Dioxygen Binding Plots

- The maximum value of n' is taken as the Hill coefficient n.
- The maximum in this first-derivative plot of the binding curve will occur at $P_{1/2}(O_2)$ only if the Hill plot is symmetric about its inflection point.
- For tetrameric hemoglobin, a maximum Hill coefficient of around 3 and for hemocyanin (n) may be as high as 9.



A- Binding curves

B- Hill plot of binding curves

C- first derivatives (slope) of the Hill plot

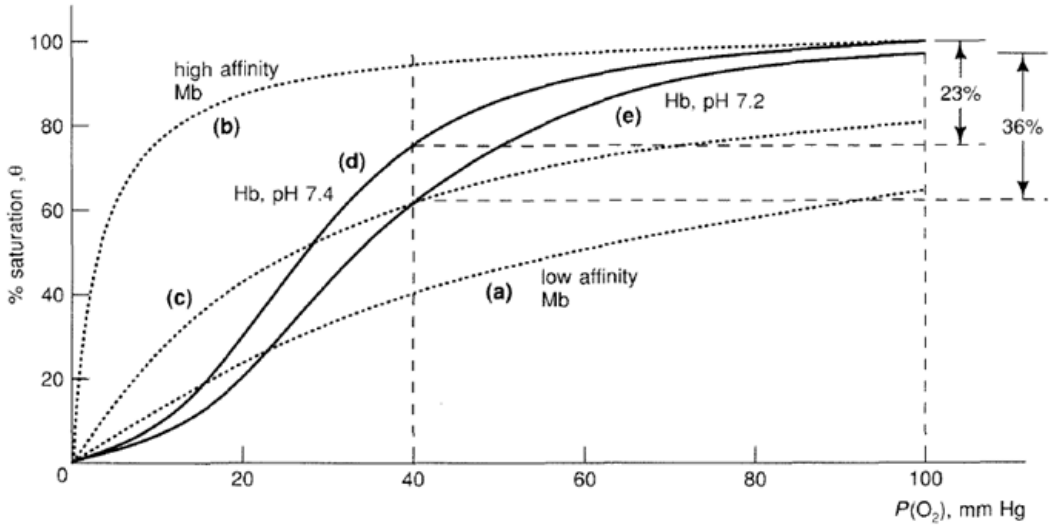
Benefits of Cooperative Ligand Binding

- With cooperative binding, the problem of inefficient and inflexible O_2 delivery disappears. If hemoglobin bound O_2 non-cooperatively, then the hyperbolic binding curve (c) in Figure D would represent the O_2 binding. Instead, the observed binding follows curve (d).
- Since the partial pressure of dioxygen in the lungs and arterial blood of vertebrates is around 100 Torr, but in the tissues and venous blood it is around 40 Torr, then at these pressures a typical myoglobin ($P_{1/2}(O_2)$ = 1 Torr) remains effectively saturated.
- On the other hand, about 25 % of the available dioxygen can be delivered, even in the absence of myoglobin. With venous blood remaining 75 percent oxygenated, hemoglobin has substantial capacity to deliver more O_2 at times of exertion or stress when $P(O_2)$ in the tissues falls below 40 Torr.
- The net result is that whole blood, which contains about 15 g of hemoglobin per 100 ml, can carry the equivalent of 20 ml of O_2 (at 760 Torr) per 100 ml, whereas blood plasma (no hemoglobin) has a carrying capacity of only 0.3 ml of O_2 per 100 ml.

Allosteric effectors

- The molecule/ligand whose co-ordination (presence) with a center influences the binding/release of other ligand (same/different) at that site is known as allosteric effector.
- The cooperative interaction where binding of one molecule of a substance influences the binding of next molecule of the same kind is described as homotropic allosteric interaction.
- A heterotropic allosteric interaction occurs when both the two interacting ligands are different.
- Oxygen binding in vivo is significantly modulated by allosteric effectors (both homo and hetero) through the interaction with the protein.
- For Hb, natural allosteric effectors are H^+, CO_2 and 2,3-diphosphoglycerate (2,3-DPG or BPG, present in RBC).
- Increasing concentrations of H^+ and CO_2 progressively lower the affinity of deoxy-Hb towards O_2, thereby enhancing the release of coordinated O_2 (curve e).
- When O_2 consumption outpaces O_2 delivery, glucose is incompletely oxidized to lactic acid (instead of CO_2). The lactic acid produced lowers the pH, and O_2 release from oxy-Hb is stimulated (curve e) - Bohr effect (here the allosteric effector is H^+). Thus in an activity where O_2 is needed much, due to Bohr effect oxy-Hb releases O_2.

- 2,3-DPG shows a heterotropic allosteric effect due to which O_2 affinity of Hb decreases with increase in its concentration and thereby stimulating the release of O_2.
- 2,3-DPG is part of a subtle mechanism by which O_2 is transferred from mother to fetus across the placenta. The subunits comprising fetal Hb and adult Hb are slightly different.
- In absence of allosteric effectors, the oxygen binding curves are identical.
- However, 2,3-DPG binds less strongly to fetal Hb than to adult Hb. Thus, fetal Hb has a slightly higher affinity for O_2, thereby enabling O_2 to be extracted from mother's Hb.



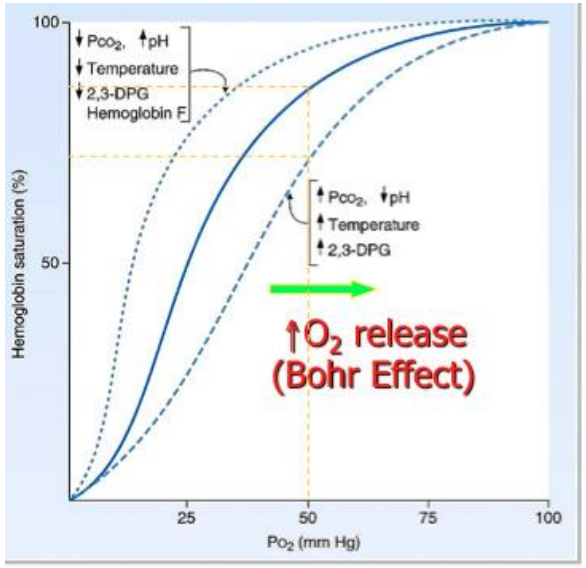
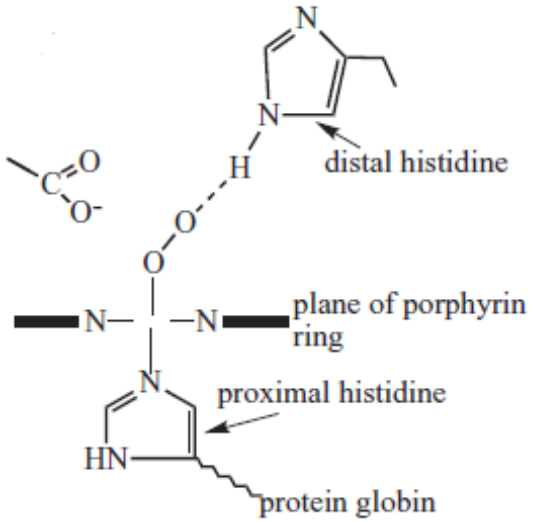
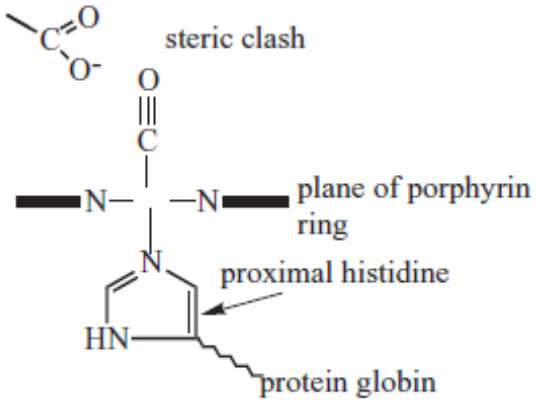


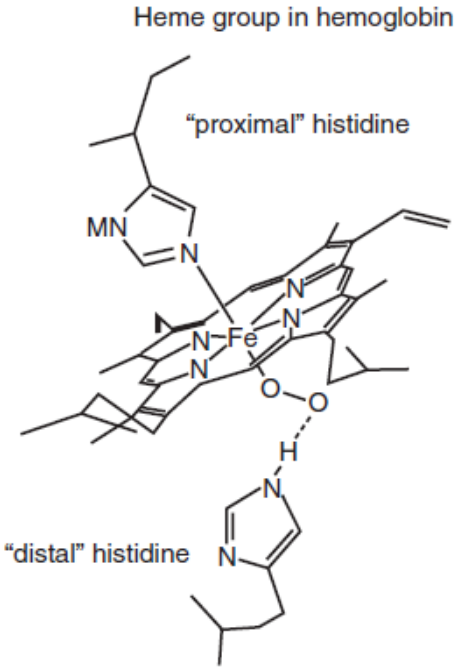
Figure D: Physiological benefit of cooperativity and heterotropic allosteric effectors

Role of distal and proximal histidine

- Proximal histidine residue binds the fifth coordination site via its imidazole moiety and distal histidine resides in the region of sixth coordination site (does not coordinate to Fe(II))
- The imidazole group from proximal histidine (F8) residue acts as a good σ-donor to facilitate the Fe(II) to act as better π-donor, making O_2 a better π-acid ligand to induce spin pairing at Fe.
- CO is a powerful poison to Hb and Mb (binds CO strongly)
- The globin protein drastically reduces the CO affinity. The presence of imidazole moiety from distal histidine (E7) residue in the region of sixth coordination site does not allow CO to form linear Fe-CO bond. The bent Fe-CO bond is very weak (for O_2, angular binding is normal)

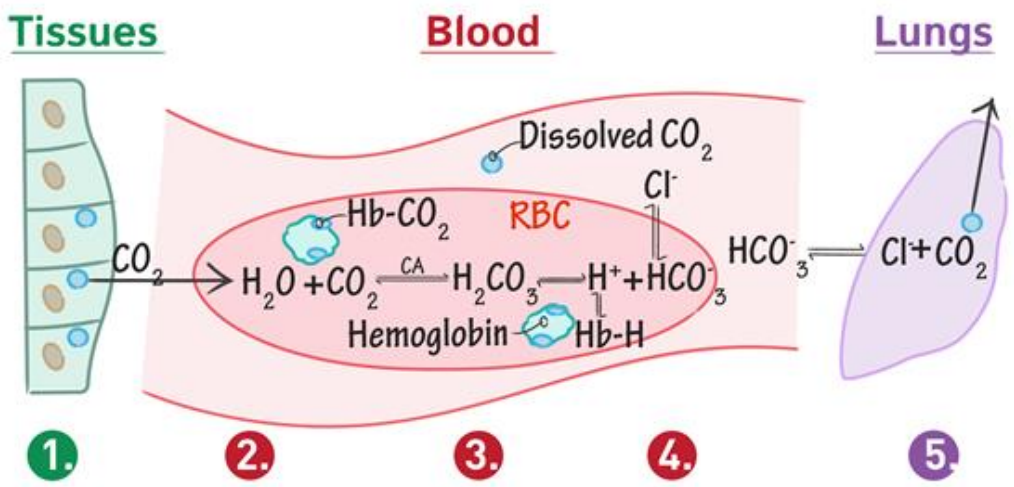


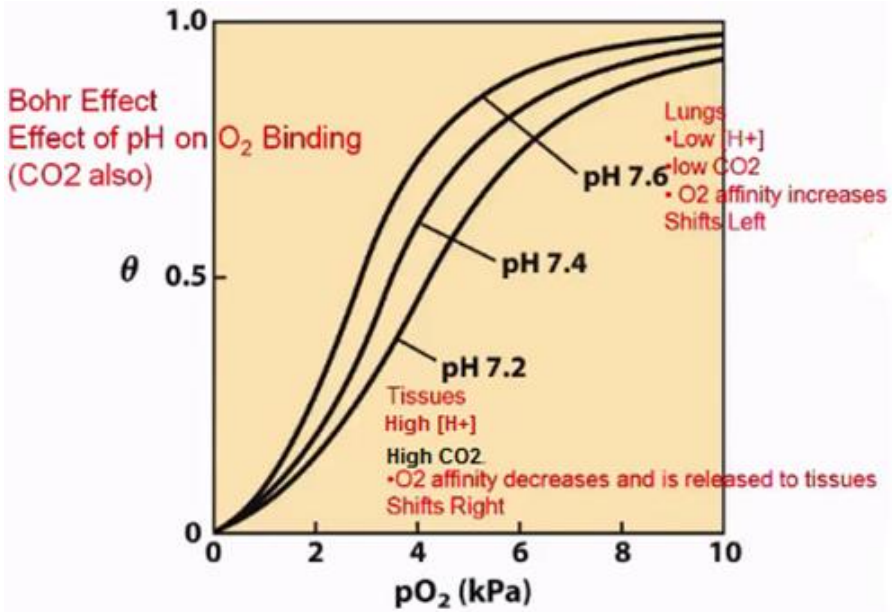




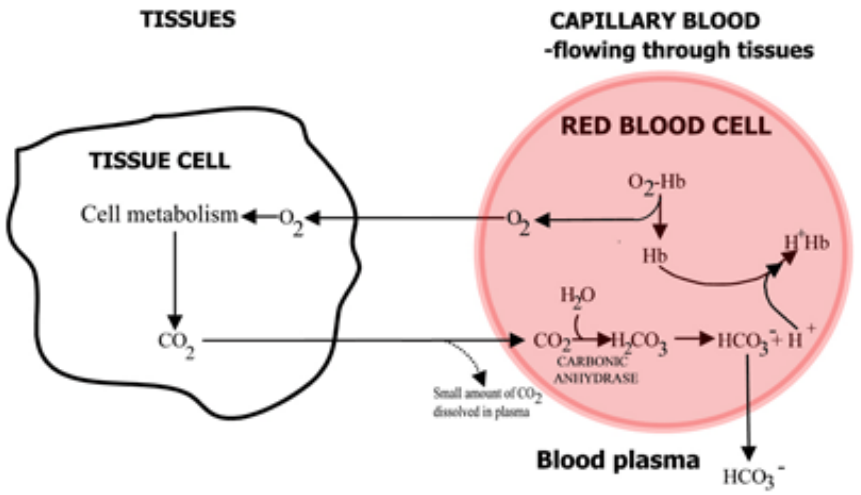
Bohr effect- effect of pH

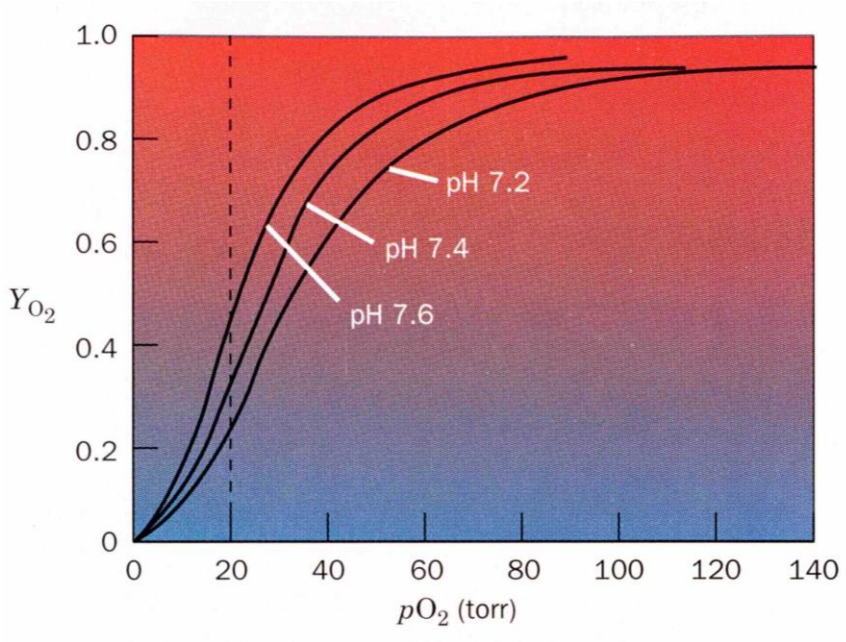
- Hb binds one proton (H^+) for every two O_2 molecules released.
- This favors the conversion of CO_2 (tissue metabolite) into HCO_3^- ion promoting its transport back to the lungs.
- Similarly, the production of CO_2 from cell respiration and of lactic acid from anaerobic metabolism favors the release of O_2 to the tissues.
- Beneficial at the tissue level where lower pH decreases O_2 affinity and promotes O_2 release.
- As the pH increases the P_{50} value decreases, indicating an increase in O_2 binding and vice versa.
- **Root Effect:** A very large Bohr effect, where O_2 affinity decreases sharply with pH.





- The concentration of H^+ ions can alter the affinity of Hb towards oxygen. This is because hemoglobin in the T-state has a higher affinity for H^+ ions than it does for oxygen.
- As pH goes down ([H^+] goes up), Hb enters the T state and its affinity for oxygen goes down. More oxygen is needed to achieve maximum percentage saturation (Bohr effect).
- It allows oxygen to dissociate at tissues with a lower pH: a good indicator of rate of cellular respiration.
- Lower the pH, the more the dissociation curve shifts to right.
- **Origin of Bohr effect**: When hemoglobin is in T state the N-terminal groups of α-subunits and C-terminal of β- subunits are protonated, allowing the ionic interaction with nearby carboxyl groups. This interaction help to hold the hemoglobin in T state. Thus, oxygen affinity of hemoglobin decreases. At low pH oxygen binding affinity decreases.





Uptake of CO_2 by hemoglobin

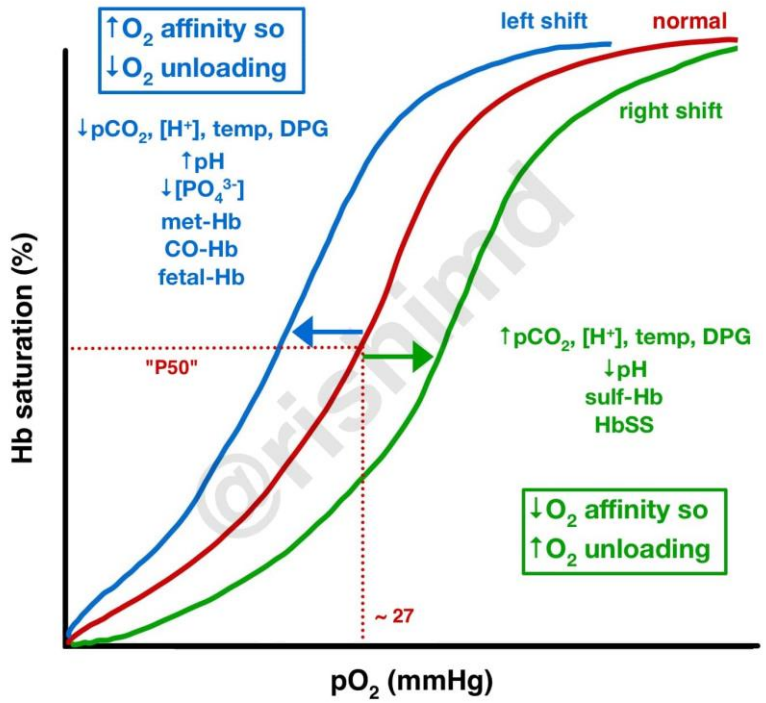
- In RBC, the enzyme carbonic anhydrase catalyzes the conversion of dissolved CO_2 to H_2CO_3, which rapidly dissociates to HCO_3^- and a free H^+.

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

- Histidine residues in Hb can accept H^+ and act as buffers.
- Deoxygenated Hb is a better proton acceptors than the oxygenated form.
- Stabilization of the produced H^+ will shift the reaction to the right, thus the enhanced affinity of deoxyhemoglobin for protons and thereby enhancing the synthesis of bicarbonate and increasing the capacity of deoxygenated blood for CO_2.

Oxygen-Hemoglobin dissociation curve

- It is a plot of Hb saturation vs O_2 partial pressure and provides some insight of how blood carries and release O_2.
- Strength with which O_2 binds to Hb is effected by several factors. These shift the curve.
- **Blood pH:** Lowering of blood pH reduces the affinity of Hb for O_2, more O_2 is delivered.
- **CO_2 concentration**: Higher the CO_2 concentration in tissue, less the is the affinity of Hb for O_2, the more O_2 is released.
- **Blood Temperature**: Increased blood temperature reduces the affinity Hb for O_2. Higher temperatures mean oxygen has more kinetic energy, making it more likely to dissociate.
- **2,3-DPG** (2,3-diphosphoglycerate): It decreases the affinity of hemoglobin for oxygen. At high altitudes, 2,3-DPG level is elevated to decrease the O_2 affinity and thereby facilitating its release in tissues.

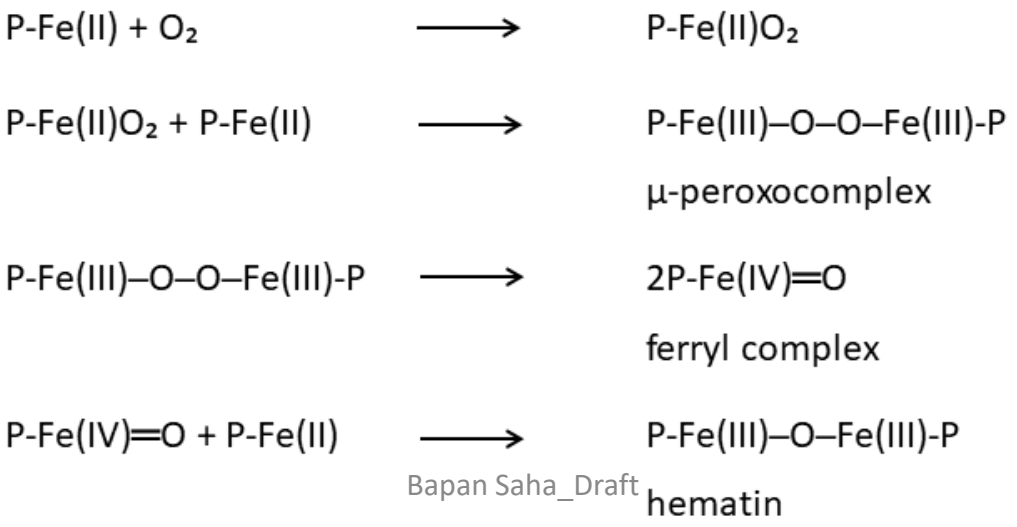


Rightward shift indicates decreased affinity, easier of release O_2.

Leftward shift indicates increased affinity of Hb to bind O_2.

Dioxygen Toxicity

- Dioxygen toxicity takes place due to irreversible binding of O_2.
- Free heme in aqueous solution is immediately converted to a new μ-oxodimer, hematin.
- The first step is the binding of O_2 molecule, as in hemoglobin
- The bound O_2 can co-ordinated to a second heme, forming a μ-peroxo complex.
- Cleavage of the peroxo complex gives two molecules of ferryl complex with the Fe atom in +4 OS.
- Finally, attack of the ferryl complex on another heme results in the formation of hematin.
- Formation of μ-peroxo complex (dimerization) is less favorable at low temperatures (<-40°C) or by sterically preventing the bimolecular contact of an Fe^{III}-O_2^{I-} moiety with an Fe(II) moiety.



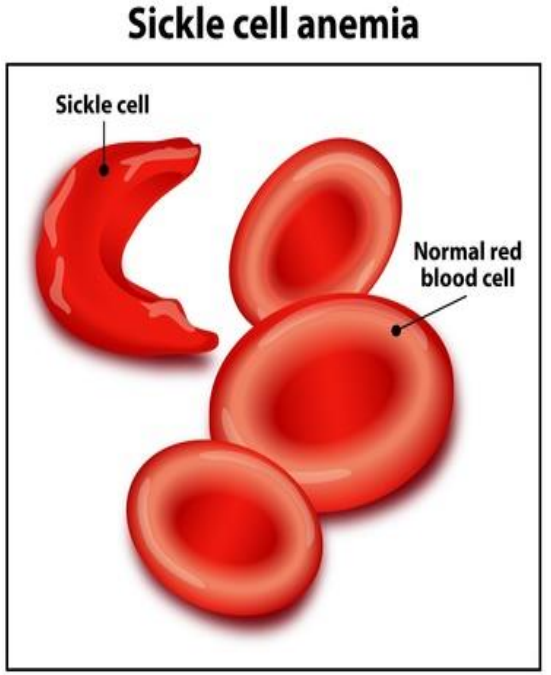
Function of globin

- Histidine, F8 binds to the proximal side and the oxygen binds to the distal side.
- Heme without globin interacts with oxygen and Fe(II) is oxidized to Fe(III), no longer binds oxygen.
- Globin introduces steric hindrance on one side of the heme plane thereby preventing dioxygen toxicity. The globin acts to (a) modulate oxygen binding affinity & (b) make reversible oxygen binding possible.
- Globin weakens the interaction of CO with the heme and stabilizing the binding of dioxygen by distal histidine (E7) residues.
- The protonation and deprotonation sites in globin chain are important in maintaining the biological pH and CO_2 transport.
- Moreover, improper sequencing of amino acids results in several genetic disorder.

Anemia

Sickle-cell anemia – abnormal hemoglobin (due to defects in globin chain)

- Sickle-cell anemia (Hb) is the most common form of abnormal hemoglobin
- The hydrophilic glutamic acid at β-6 position is replaced by hydrophobic valine.
- Causes a distortion of cell into sickle shape.
- Hemoglobin polymerizes and precipitates – severe deformation in RBC



Cooly's anemia

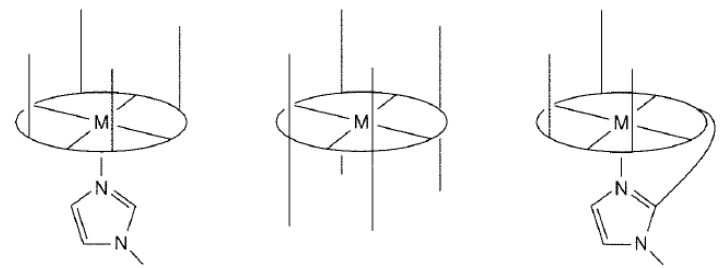
- Genetic blood disease, results from insufficient production of β-chains

Thalassemia

- α and β chains are wrongly produced.
- α-thalassemia is caused by an erratic synthesis or total absence of α-globulin chains of Hb
- β-thalassemia is caused from the defect in the β-globulin chains of Hb

Synthetic Dioxygen Carrier

- Nature prevents the irreversible binding of dioxygen and protects Hb from hematin formation.
- Steric hindrance provided by the globin part of the molecule prevents one oxoheme from attacking another heme. The embedded heme then binds dioxygen reversibly.
- Some simple compounds to mimic the natural dioxygen carrier have been synthesized.
- The model compounds contain the basic iron-porphyrin unit but attempt to simulate the globin protein in a simpler way.
- Like natural systems, importance of steric hindrance and hydrophobic environment has been illustrated in different models and synthetic dioxygen carriers.
- In model compounds, the steric hindrance has been introduced in different possible ways.
- All these compounds can bind dioxygen reversibly and illustrate the role of bulky globin unit.

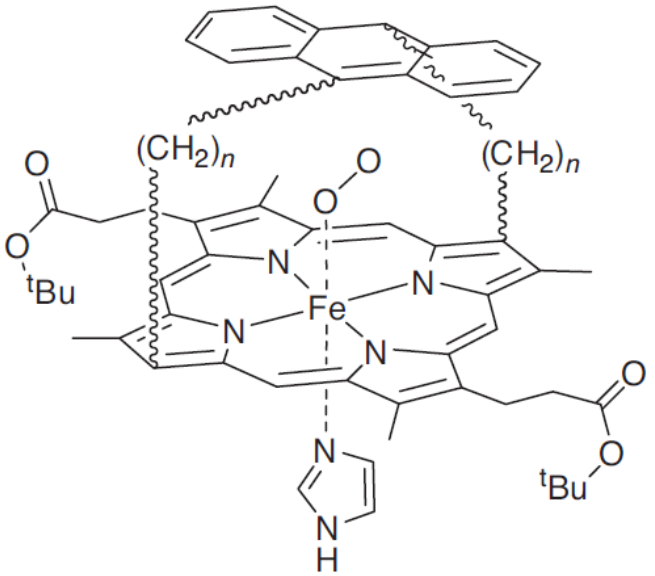


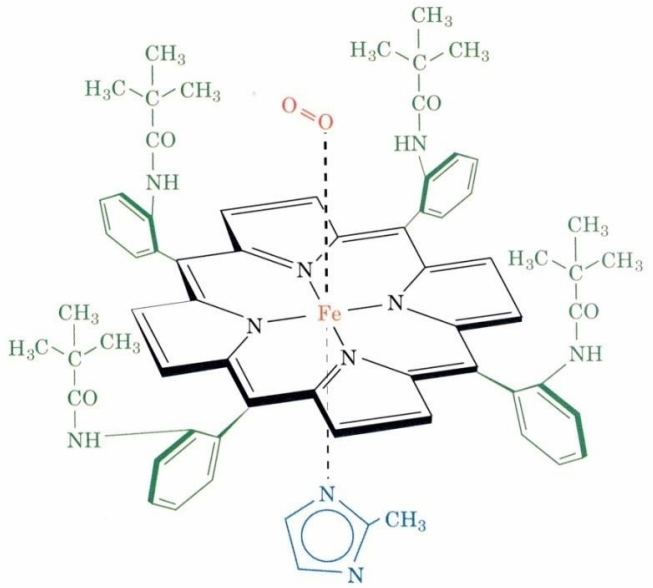
protection on one side: excess base required

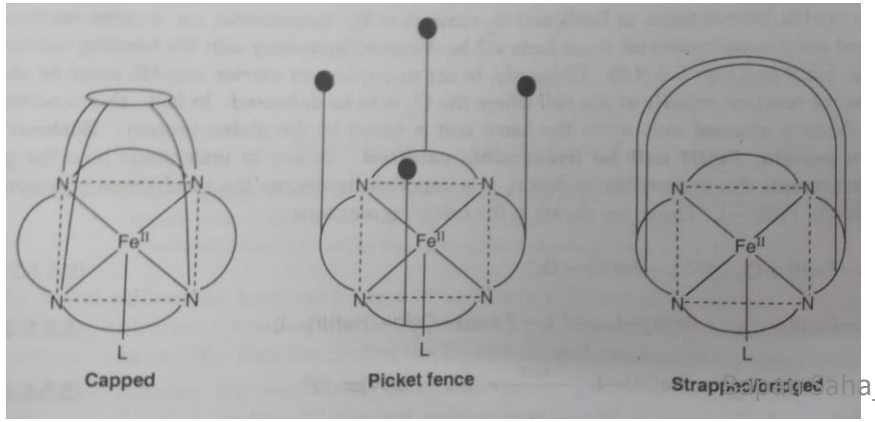
protection on both sides

protection on one side: stoichiometric control with chelated base

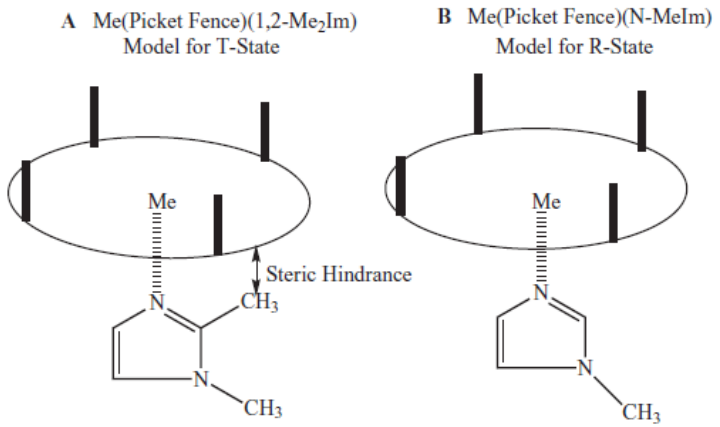
- **Picket fence model:** Three of four large groups are projected on one side of the porphyrin plane and the other side is kept unhindered for imidazole. These bulky group create a fence on one side of the plane to prevent the formation of a binuclear complex to be produced in the irreversible oxidation.
- **Strapped/bridged model:** One chain extends one side of the porphyrin ring leaving room for dioxygen in the same side.
- **Roofed/capped model:** The chains make a complete enclosure on one side of the ring providing sterically hindered binding sites even to the dioxygen molecules.



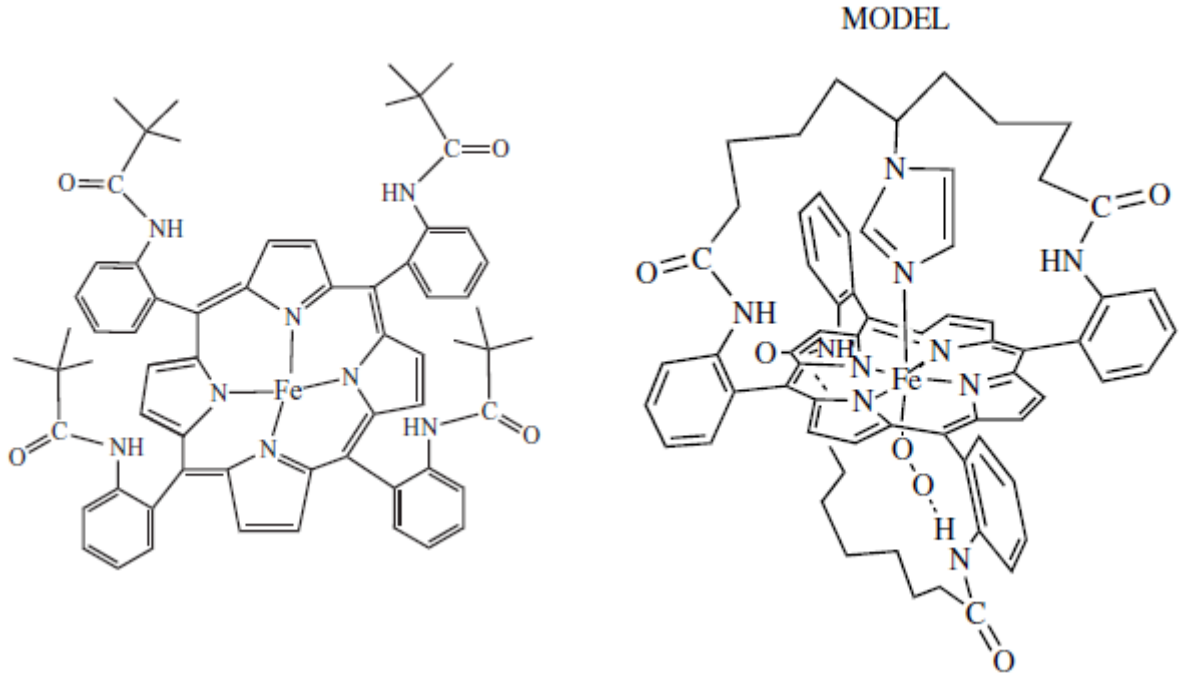




- In summary, researchers found a number of methods for avoiding μ–oxo dimer formation and preserving a five - coordinate Fe(II) in iron containing model compounds, through:
- Modifying the imidazole to preserve the T – state
- Modifying the porphyrin to sterically prevent addition of a large sixth ligand. The most well - known version of these is the “picket-fence” porphyrin illustrated.
- Attaching the five - coordinate system to a rigid support, reducing its mobility and ability to add a sixth ligand.



Picket-fence porphyrin models. (A) Model for T-state and (B) model for R-state.



Haldane effect

- The Haldane effect relates the number of proton released with a change in saturation of binding site (θ) at constant pH
- In alveolar capillaries of lungs, the high concentration of O_2 unloads H^+ and CO_2 from hemoglobin through oxygenation. The release of proton stabilizes the deoxy form through the formation of salt bridge interaction. This property is the Haldane effect.

$$H^+ + HbO_2 \rightleftharpoons H^+Hb + O_2$$

- The significance of this equation lies in realizing that oxygenation of Hb promotes dissociation of H^+ from Hb, which shifts the bicarbonate buffer equilibrium towards CO_2 formation; therefore, CO_2 is released from RBCs.

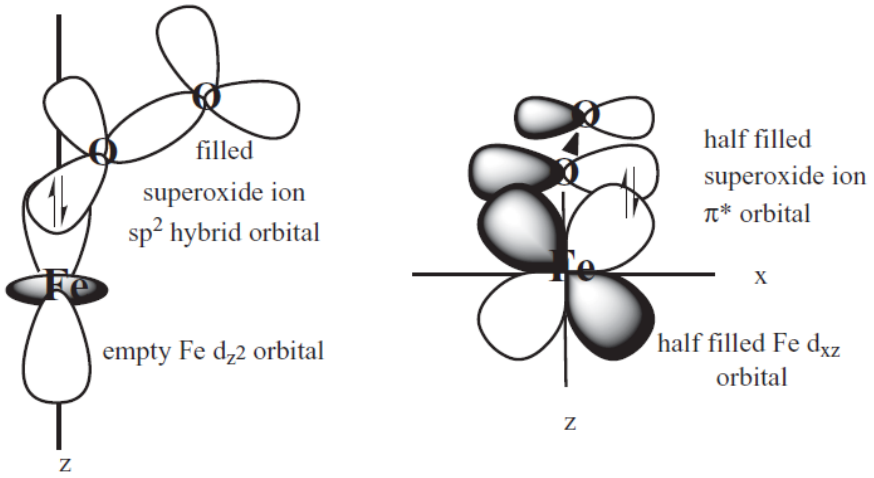
$$CO_2 + H_2O \longrightarrow H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

- In the oxygen-rich capillaries of the lung, this property causes the displacement of carbon dioxide to plasma as low-oxygen blood enters the alveolus and is vital for alveolar gas exchange.

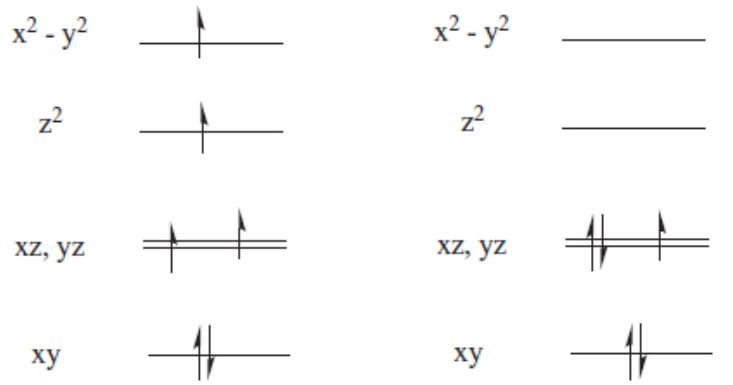
Nature of Heme-Dioxygen bonding

- Dioxygen can bind to metal in several ways, three are known in biology viz. superoxo (oxyHb), peroxide (oxyhemocyanin) and hydroperoxo (oxyhemerythrin).
- The geometry is a function of the metal, its oxidation state and associated ligands.
- In binding, oxygen temporarily and reversibly oxidizes Fe(II) to Fe(III) while oxygen temporarily turns into the superoxide, O_2^- ion
- O_2 acts as a one electron acceptor leading to low spin Fe(III) with t2g5 configuration and O_2^- ion assuming octahedral geometry.
- The unpaired t2g5 electron of Fe(III) undergoes antiferromagnetic coupling with the unpaired electron of antibonding π^* orbital of O_2^- ion giving rise to diamagnetic properties.
- During oxygenation, O_2 makes a bent bond with metal center viz. one σ-type interaction between metal dz2 orbital and π^* orbital of O_2 and another π-type interaction between dx2-y2 orbital and orthogonal π^* orbital of O_2. The order of the O-O bond is about 1.5

- In oxyhemoglobin, the stretching frequency for O-O bond appears at 1106 cm^{-1} with O-O separation is around 1.30 Å. This is significantly close to the O-O stretching of 1097 cm^{-1} in O_2^- ion
- Although such O-O separations and vibrations are consistent with coordinated peroxide or superoxide moieties, the net amount of charge is transferred onto the dioxygen ligand from the metal.
- The nearest formal oxidation state of iron in Hb-O_2 is the +3 state, with oxygen in the −1 state (as superoxide O_2^-).



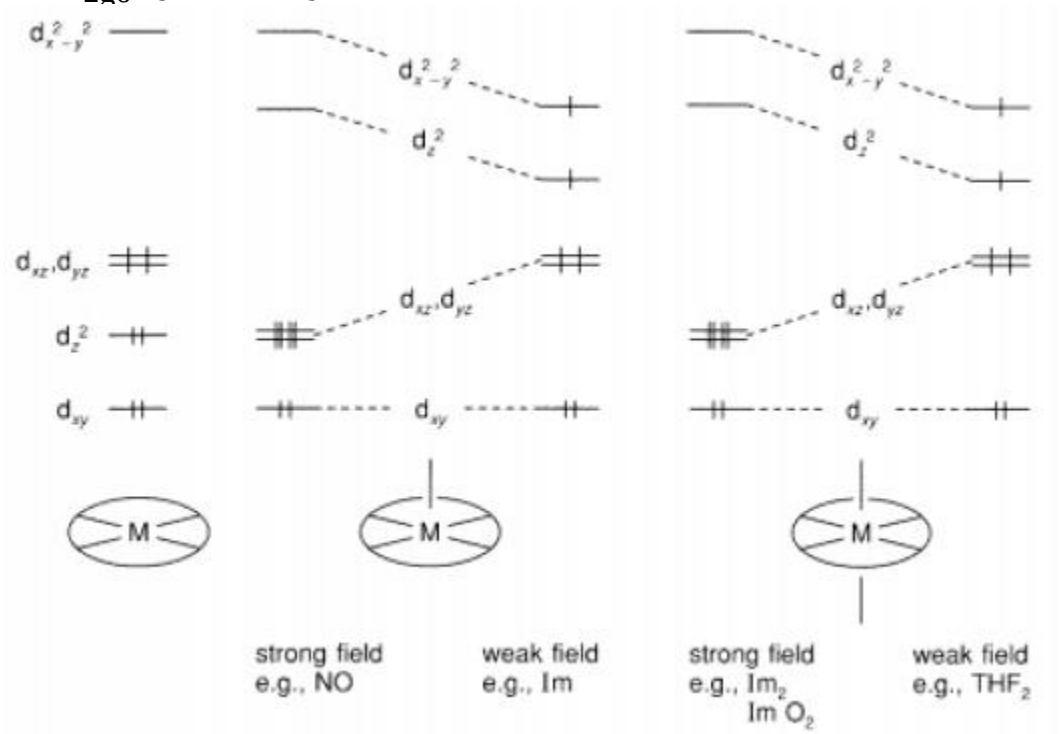
Bonding of the O_2^- ion to Fe(III) in Mb or Hb.



- Iron porphyrins may be octahedral (two axial ligands), square pyramidal (one axial ligand), or square planar (no axial ligand). Metal d orbitals, now having partial porphyrin π* character, are split as shown

- Radius of HS Fe(II)(S=2)/Fe(III)(S=5/2) metal is much greater the LS Fe(II)(S=0)/Fe(III)(S=1/2). This difference influences Fe-$N_{porphyrin}$ separations, porphyrin conformation, and the displacement of the iron center with respect to the porphyrin plane.

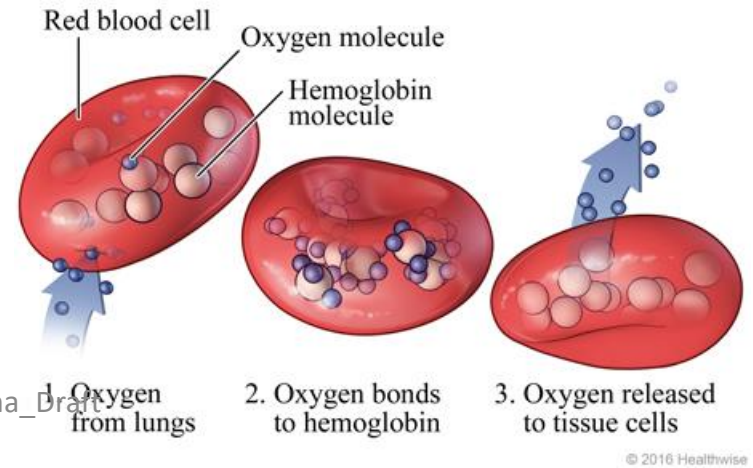
- For iron(II)-porphyrins, a pair of imidazole or imidazole and CO ligands occupy the axial position giving diamagnetic complexes (S = 0) with approximate t_{2g6} symmetry.



Hemoglobin vs Myoglobin

Hemoglobin	Myoglobin
Four polypeptide chain (MW=64000 D)	Single polypeptide chain (MW=16.7 D)
Exhibits cooperative binding	Does not exhibit cooperative binding
Low affinity for oxygen, depends on oxygen concentration	High affinity for oxygen, does not depend on oxygen concentration
Found in blood stream	Found in muscle
Takes oxygen from lungs and transports to the rest of the body	Stores oxygen in the muscle cells and releases it when needed
Hb A (primarily), Hb A2 and Hb F are the types found in humans	A single type is found in the cells

Function of Hemoglobin




THANK YOU