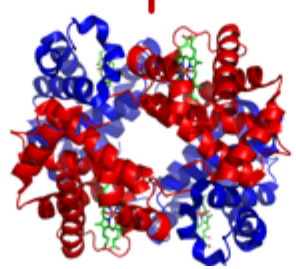
BIOINORGANIC CHEMISTRY

Types of O_2 Carriers

- Oxygen Carriers
  - Hb Based
    - Recombinant Hb
    - Polymeric Hb
    - Encapsulated
      - Nanocapsules
      - Liposome Encapsulated Hemoglobin (LEHb)
    - Conjugated Hb
  - Non-Hb Based
    - Perfluorocarbons



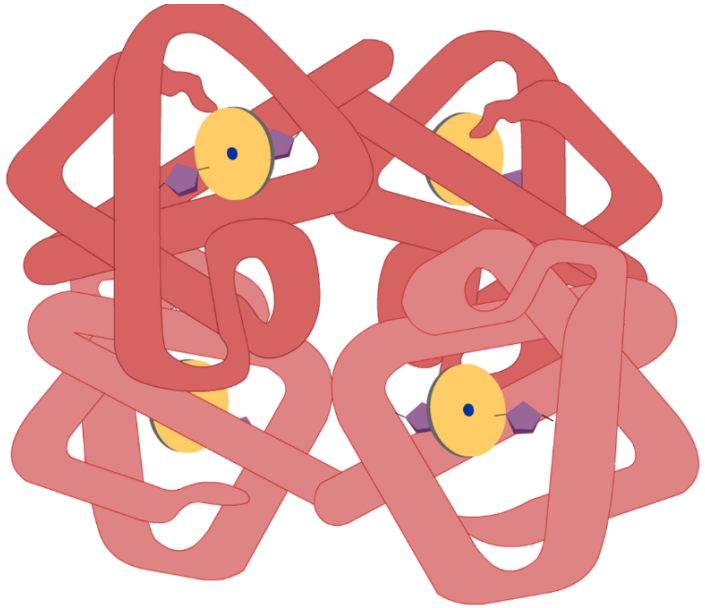
Biochemistry of Iron

- Iron is an essential bioelement for most forms of life, from bacteria to mammals.
- Its importance lies in its ability to mediate electron transfer.
- In the ferrous state, iron acts as an electron donor, while in the ferric state it acts as an acceptor.
- Iron plays a vital role in the catalysis of enzymatic reactions that involve electron transfer (reduction and oxidation, redox).
- Proteins contain iron as part of different cofactors, such as iron-sulfur clusters (Fe-S) and heme groups, both of which are assembled in mitochondria.

The most important iron containing biomolecules are Hemoglobin and Myoglobin

Hemoglobin

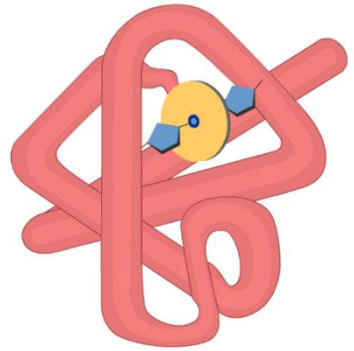
- Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates.
- Hemoglobin in the blood carries oxygen from the lungs or gills to the rest of the body (i.e. the tissues).
- There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism.
- A healthy individual has "12 to 16" grams of haemoglobin in every 100 ml of blood.



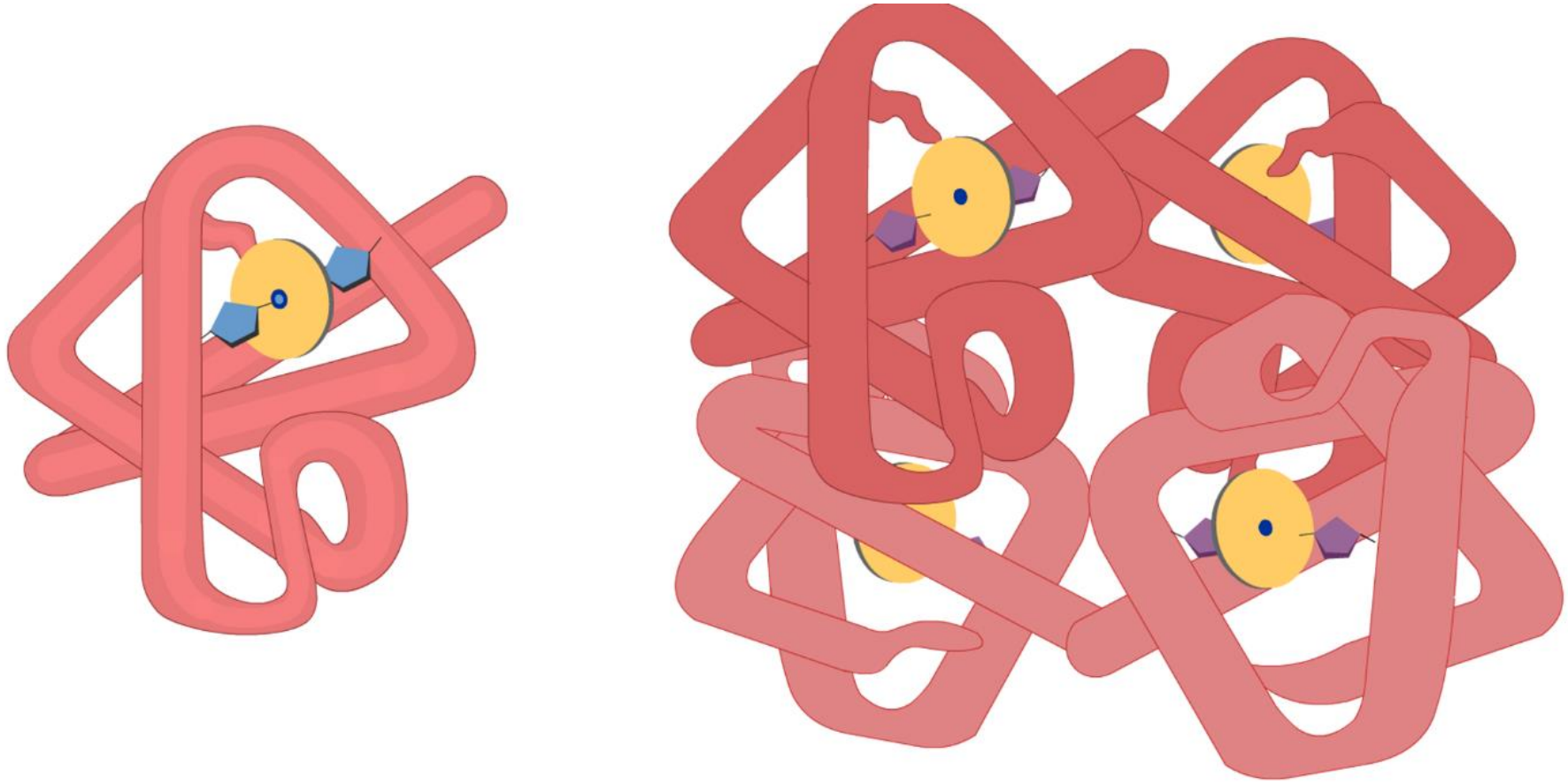
Hemoglobin

Myoglobin

- Myoglobin (symbol Mb or MB) is an iron- and oxygen-binding protein found in the muscle tissue of vertebrates in general and in almost all mammals.
- It is not related to hemoglobin, which is the iron- and oxygen-binding protein in blood, specifically in the red blood cells.
- In humans, myoglobin is only found in the bloodstream after muscle injury.
- Myoglobin is the primary oxygen-carrying pigment of muscle tissues.
- High concentrations of myoglobin in muscle cells allow organisms to hold their breath for a longer period of time.
- Myoglobin is found in Type I muscle, Type II A and Type II B, but most texts consider myoglobin not to be found in smooth muscle.
- Molecular mass is 17000 which is ¼ times that of Hb
- Hb is a tetramer of Mb



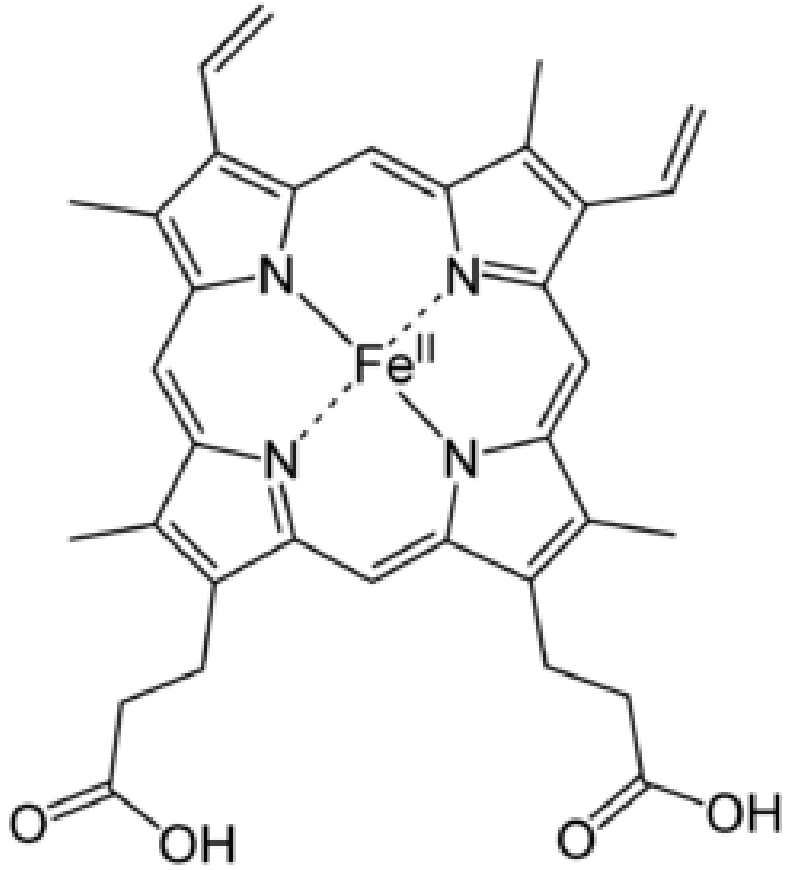
Both Hemoglobin and Myoglobin contains Protein part and Non-protein part



Myoglobin

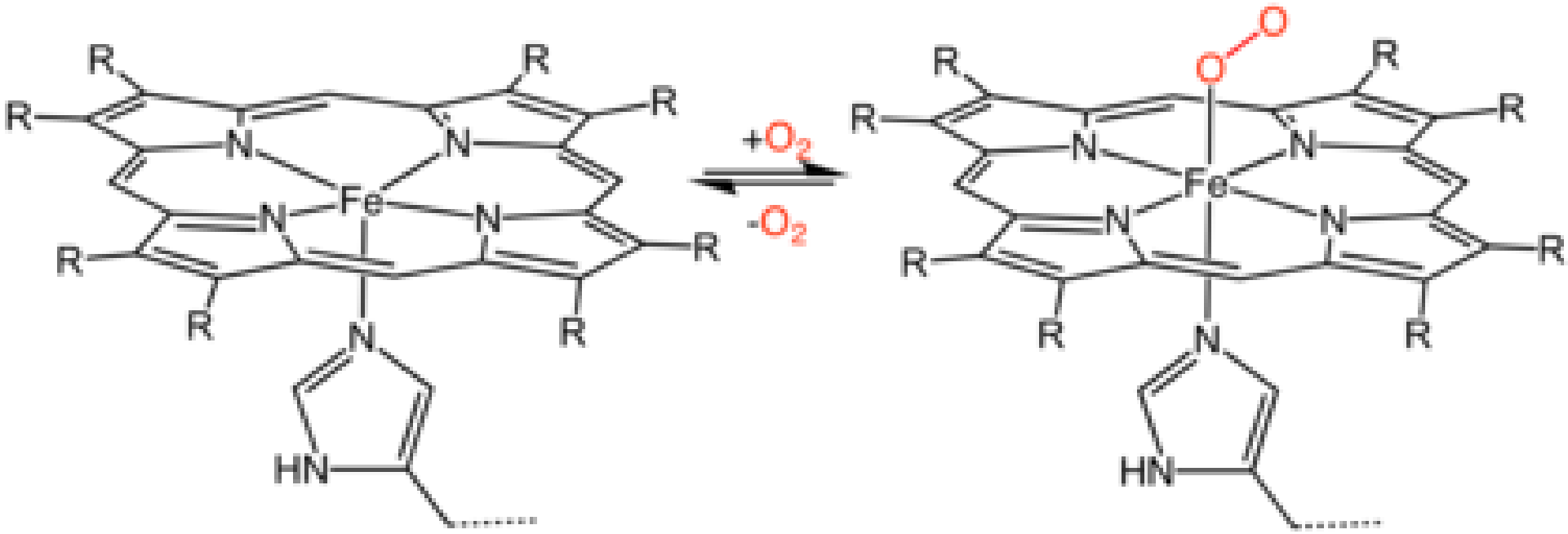
Hemoglobin

Non-protein part



- Heme or haem is a coordination complex "consisting of an iron ion coordinated to a porphyrin acting as a tetradentate ligand, and to one or two axial ligands.
- Many porphyrin-containing metalloproteins have heme as their prosthetic group; these are known as hemoproteins.
- Hemes are most commonly recognized as components of hemoglobin, the red pigment in blood
- Also found in a number of other biologically important hemoproteins such as myoglobin, cytochromes, catalases, heme peroxidase, endothelial nitric oxide synthase.

Non-protein part



- Free heme is very sensitive to O_2 and can bind with O_2 to form oxyheme Fe(II)-O_2 complex
- In presence of water it changes to Fe(III)-Protoporphyrin complex called hemin or hematin which useless for oxygen transport
- Fe(II)-O_2 binding is reversible
- Fe(III)-O_2 binding is irreversible

Protein part

- 4 polypeptide chains, ie 2 alpha and 2 beta chains
- Alpha chain has 141 amino acids and beta chain has 146 amino acids, Totally 574 amino acids
- Associated with each polypeptide chain, there is a heme part. In Hb, 4 heme groups and 4 protein chain
- Function of the peptide chain is to stabilize the heme in Hba nd Mb
- Protein chain folded about the heme reduced the access of H_2O to iron and produces a hydrophobic environment which prevents the irreversible oxidation of Fe (II)

Myoglobin

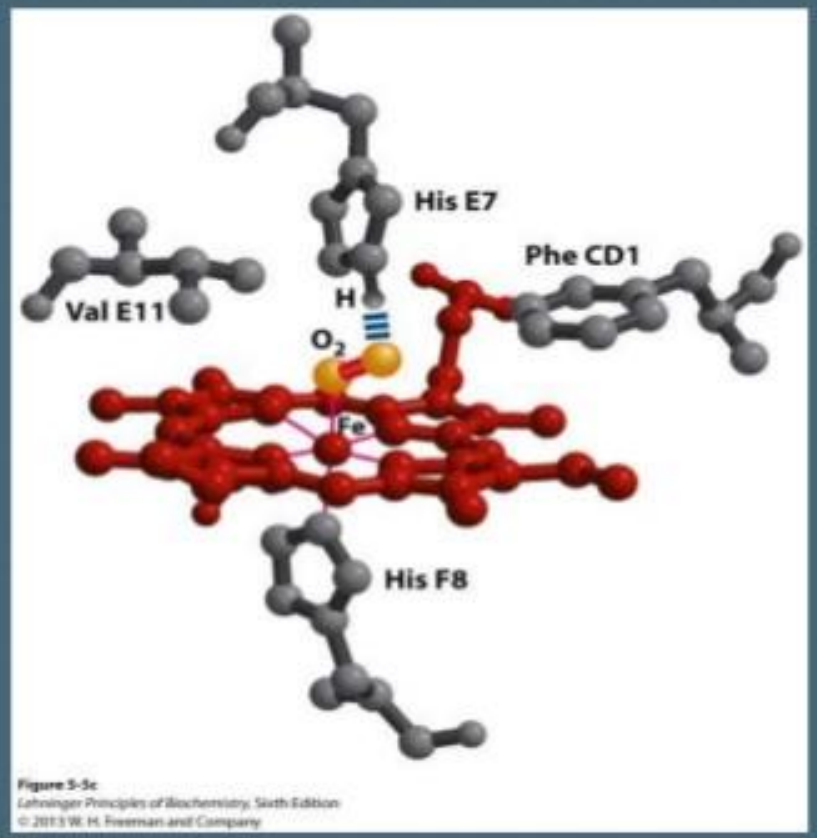


Figure 5-5c
Lehninger Principles of Biochemistry, Sixth Edition
© 2013 W. H. Freeman and Company

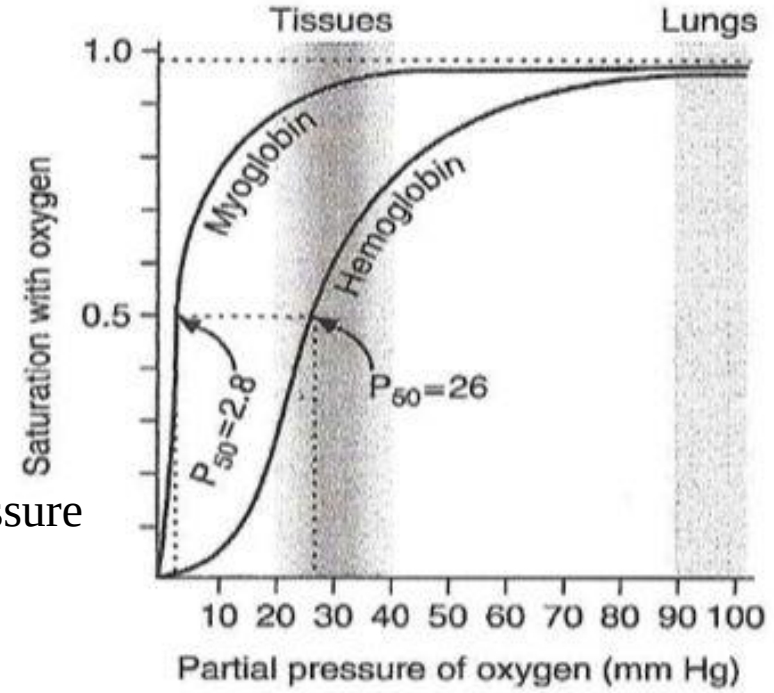
- α-helical secondary structure
- Contains one heme group in its internal hydrophobic cleft and therefore can bind **one** oxygen molecule, forming **oxymyoglobin**
- Oxygen binds to ferrous iron atom on heme group. This binding is **stabilized** by hydrophobic interactions and a nitrogen atom derived from **histidine**
- Transports oxygen from capillaries to mitochondria in working tissue

- Both are iron containing proteins.
- While haemoglobin is present inside red blood cells and serves to deliver oxygen to tissues, myoglobin is present mostly in muscles to supply them with oxygen in strenous exercise or stress when demand is more.
- Hb has lesser affinity for oxygen as compared to myoglobin so that it ($Hb.O_2$) can dissociate and release oxygen bound to it.
- Hemoglobin and Myoglobin, both are oxygen binding proteins however, they differ largely in their function
- Myoglobin is a monomeric protein and binds molecular oxygen and carry to muscle tissues.
- Muscle cells use myoglobin to exchange oxygen during active respiration.
- Myoglobin consists of 8 right handed α-helices and each protein molecule contains one heme prosthetic group and each heme residue contains one central coordinately bound iron atom.
- Oxygen is bound directly to the iron atom of the heme prosthetic group.
- It transport and store oxygen. Binds oxygen more tightly and easily.

- **Hemoglobin** is a **tetrameric protein** and binds molecular oxygen on RBCs.
- Being a tetramer it binds **four oxygen** molecules and distribute them throughout the whole body.
- It serves to deliver oxygen needed for cellular metabolism and removes the resulting waste product, carbon dioxide from the body tissues.
- **Human hemoglobin is composed of two α (alpha) and two β (beta) subunits.**
- **Each α-subunit has 144 residues, and each β-subunit has 146 residues.**
- Structural characteristics of both α (alpha) and β (beta) subunits are similar to myoglobin.
- 4 sub units of Hb are linked with one another via salt bridges present between the 4 polypeptide chains
- These salt bridges are formed due to the electrostatic interaction between NH_3^+ and COO^- groups present in the polypeptide chain
- Due to the presence of these salt bridges, Hb is under strain.
- Free Hb is called deooxy Hb and oxygentated Hb is called oxy Hb.
- Deoxy Hb is n a tensed form and oxy Hb is in relaxed form

	Myoglobin	Hemoglobin
Primary structure	153 amino acids	141 (α-chain) and 146 (β-chain) amino acids
Secondary structure	Eight α-helixes	Eight α-helixes for each α-chain and β-chain
Tertiary structure	Folding of α-helixes	Folding of α-helixes
Quaternary structure	None (monomer)	Tetramer of two identical αβ-dimers
Function	O_2 storage	O_2 delivery

Oxygen transport mechanism in hemoglobin



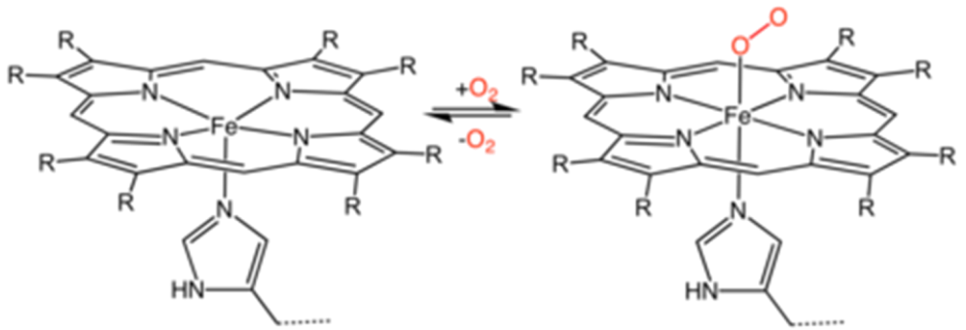
- Oxygen uptake of Hb and Mb are different
- Mb takes up O_2 in a 1 : 1 ration with Fe^{2+}, but in Hb the 4 heme units are not independent
- From the plot it is very clear that Mb gets saturated with O_2 at very low partial pressure
- % saturation of Hb increases with partial pressure of oxygen
- Hb has strong affinity towards O_2 in lungs (O_2 partial pressure is high)
- Hb has little affinity in tissue (O_2 partial pressure is high)
- Mg has strong affinity towards O_2 in the tissue even if the partial pressure is slow
- Hb gets saturated with O_2 in the lungs
- When oxygenated Hb reaches the tissue, it release the O_2 and it will be taken up by Mb

PERUTZ MECHANISM

- Hb has two conformational states - the deoxy or T state and the oxy or R state.
- In deoxy haemoglobin quaternary structure interactions are constrained → T-state [Tensed state\ taut]
- When O_2 binds, it relaxes the quaternary structure → R-State [Relaxed state]
  - [Relaxed coformation due to interaction]

Oxygen transport mechanism in hemoglobin

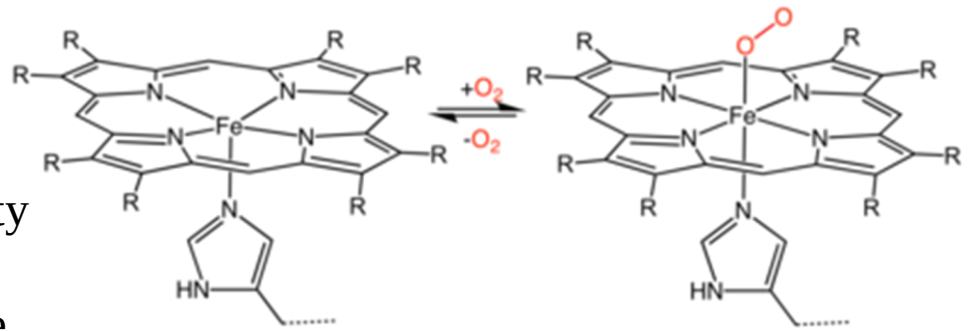
- Phenomenon where addition of O_2 to one heme group of Hb facilitates the addition of oxygen to other heme group is called co-operativity effect



- Co-operativity can be explained by Perutz mechanism
- Significant changes occur in Hb during its oxygenation
- When heme is not bound with O_2, Fe is not in the plane of porphyrin ring.
- It will be slightly raised from the plane.
- But when bound to O_2, the Fe comes to same plane of porphyrin
- On oxygenation in Hb, two heme groups move about 100 pm towards each other, two others separate about 700 pm
- In Hb, the heme group is dome shaped having the iron atom about 0.5 A^0 out of porphyrin plane and the Fe-N bond of histidine reside of poly peptide chain about 8^0 off the perpendicular plane

Oxygen transport mechanism in hemoglobin

- When O_2 molecule binds to Fe atom of a heme group through its vacant sixth coordination site, iron atom becomes low spin, becomes smaller in radius
- Bond length of Fe-N bond decreases from 218 pm to 200 pm
- Low spin iron moves towards the porphyrin plane and just fits in the cavity
- This pulls the coordinated histidine to move about 0.5Å towards the plane
- Whole polypeptide chain moves apart
- Drastic change in the tertiary structure of polypeptide chain ie some polypeptide salt bridge breaks
- Presence of salt bridge introduces strain in Hb molecules, rupture of these salt bridges relaxes Hb
- Conformational changes increases the crevice size of the remaining heme groups
- Sixth coordination sites of Fe atoms in the other heme group becomes more approachable to the attacking O_2 molecules
- The above explanation of cooperativity effect is called trigger mechanism
- Fe-O2 bond length is bent to minimize steric repulsion



Oxygen transport mechanism in hemoglobin (Co-operativity effect)

- When a substrate binds to one enzymatic subunit, the rest of the subunits are stimulated and become active. Ligands can either have positive cooperativity, negative cooperativity, or non-cooperativity.
- The sigmoidal shape of hemoglobin's oxygen-dissociation curve results from cooperative binding of oxygen to hemoglobin.
- An example of positive cooperativity is the binding of oxygen to hemoglobin.
- One oxygen molecule can bind to the ferrous iron of a heme molecule in each of the four chains of a hemoglobin molecule.
- Deoxy-hemoglobin has a relatively low affinity for oxygen, but when one molecule binds to a single heme, the oxygen affinity increases, allowing the second molecule to bind more easily, and the third and fourth even more easily.
- The oxygen affinity of 3-oxy-hemoglobin is ~300 times greater than that of deoxy-hemoglobin.
- This behavior leads the affinity curve of hemoglobin to be sigmoidal, rather than hyperbolic as with the monomeric myoglobin.
- By the same process, the ability for hemoglobin to lose oxygen increases as fewer oxygen molecules are bound

- It transport **oxygen. Concentration of hemoglobin is high in RBCs.**
- **Binds oxygen loosely and with difficulty.**
- Hemoglobin in its deoxygenated state has a **low affinity for oxygen** compared to myoglobin.
- When oxygen is bound to the first subunit of hemoglobin it leads to subtle changes to the quaternary structure of the protein.
- This in turn makes it easier for a subsequent molecule of oxygen to bind to the next subunit.
- Thus, with the initial oxygen binding to a subunit, the remaining unbound subunits become more receptive to oxygen.
- This phenomenon is called an allosteric (through space) interaction/ cooperativity and is clearly illustrated in the sigmoidal curve for oxygen binding to hemoglobin at neutral pH.

Bohr effect

- The Bohr effect is a physiological phenomenon first described in 1904 by the Danish physiologist Christian Bohr:
- Hemoglobin's oxygen binding affinity (see oxygen–haemoglobin dissociation curve) is inversely related both to acidity and to the concentration of carbon dioxide.
- Since carbon dioxide reacts with water to form carbonic acid, an increase in CO_2 results in a decrease in blood pH resulting in hemoglobin proteins releasing their load of oxygen.
- Conversely, a decrease in carbon dioxide provokes an increase in pH, which results in hemoglobin picking up more oxygen
- The Bohr effect increases the efficiency of oxygen transportation through the blood.
- After hemoglobin binds to oxygen in the lungs due to the high oxygen concentrations, the Bohr effect facilitates its release in the tissues, particularly those tissues in most need of oxygen.
- When a tissue's metabolic rate increases, so does its carbon dioxide waste production.
- When released into the bloodstream, carbon dioxide forms bicarbonate and protons

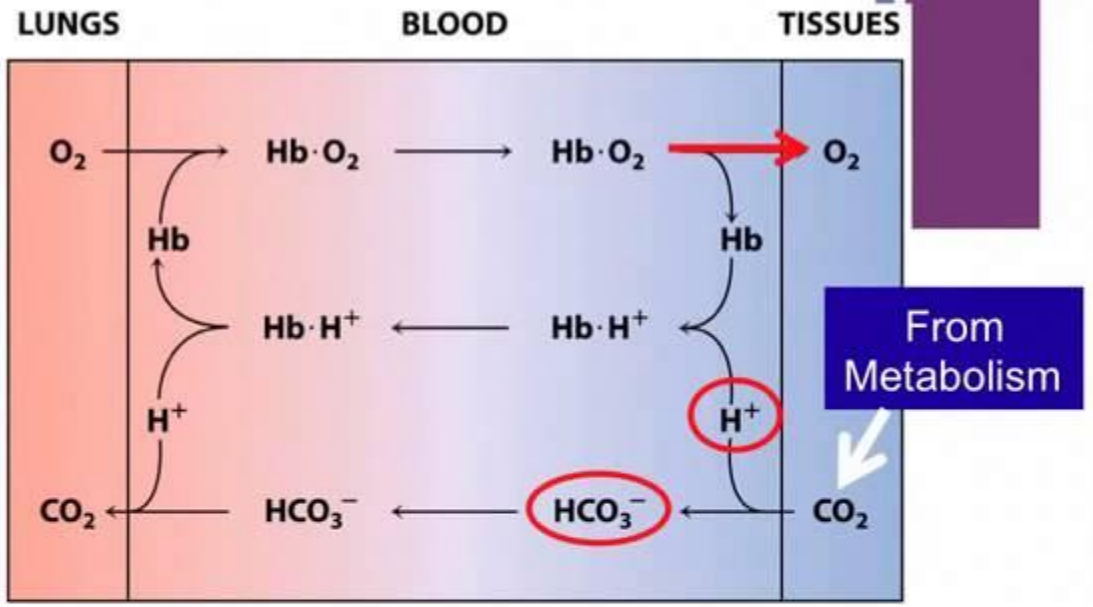
Bohr effect

- Although this reaction usually proceeds very slowly, the enzyme carbonic anhydrase (which is present in red blood cells) drastically speeds up the conversion to bicarbonate and protons.
- This causes the pH of the blood to decrease, which promotes the dissociation of oxygen from haemoglobin, and allows the surrounding tissues to obtain enough oxygen to meet their demands.
- In areas where oxygen concentration is high, such as the lungs, binding of oxygen causes haemoglobin to release protons, which recombine with bicarbonate to eliminate carbon dioxide during exhalation.
- These opposing protonation and deprotonation reactions occur at an equal rate, resulting in little overall change in blood pH.
- The Bohr effect enables the body to adapt to changing conditions and makes it possible to supply extra oxygen to tissues that need it the most.

Bohr effect

- For example, when muscles are undergoing strenuous activity, they require large amounts of oxygen to conduct cellular respiration, which generates CO_2 (and therefore HCO_3^- and H^+) as byproducts.
- These waste products lower the pH of the blood, which increases oxygen delivery to the active muscles.
- Carbon dioxide is not the only molecule that can trigger the Bohr effect.
- If muscle cells aren't receiving enough oxygen for cellular respiration, they resort to lactic acid fermentation, which releases lactic acid as a byproduct.
- This increases the acidity of the blood far more than CO_2 alone, which reflects the cells' even greater need for oxygen.
- In fact, under anaerobic conditions, muscles generate lactic acid so quickly that pH of the blood passing through the muscles will drop to around 7.2, which causes haemoglobin to begin releasing roughly 10% more oxygen.

Regulating O_2 Binding to Hb



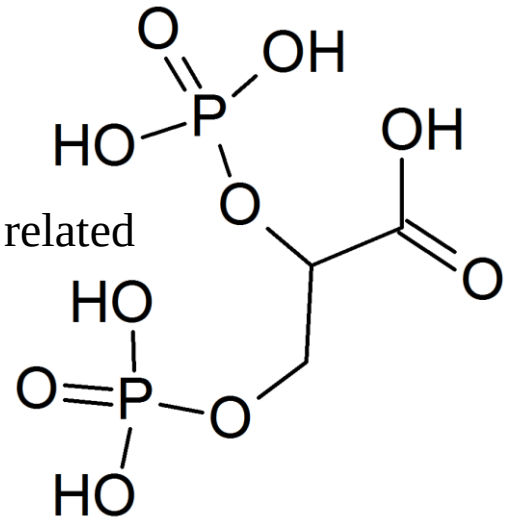
- Bohr effect
  - What happens in **tissues**?
    - They use O_2 in respiration and produce CO_2
    - CO_2 diffuses into red blood cells (RBCs have Hb)
    - Carbonic anhydrase converts to bicarbonate and produces H^+

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

    - Low pH induces Hb to release O_2
    - Net effect: release O_2 in tissues, RBCs takes up CO_2 (Hb binds some)

Phosphate effect

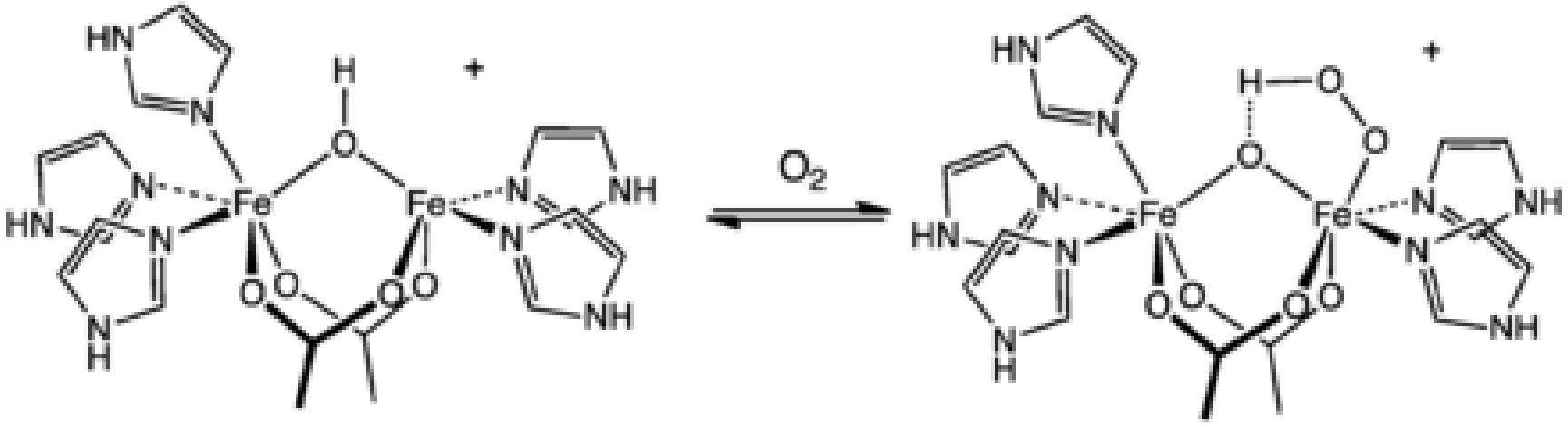
- Deoxy Hb has greater affinity for DPG, DPG will keep the beta chain apart and favours the O_2 release which stabilize the deoxy form



- In Working tissues, DPG concentration is very high and it favours the transfer of O_2 to Mb
- When DPG is added to Hb, its O_2 affinity decreases, Binding of DPG and binding of O_2 are inversely related
- 2,3-diphosphoglyceric acid (conjugate base 2,3-diphosphoglycerate) (2,3-DPG)
- 2,3-BPG is present in human red blood cells (RBC; erythrocyte) at approximately 5 mmol/L.
- It binds with greater affinity to deoxygenated hemoglobin (e.g. when the red blood cell is near respiring tissue) than it does to oxygenated hemoglobin (e.g., in the lungs) due to spatial changes
- 2,3-BPG (with an estimated size of about 9 angstroms) fits in the deoxygenated hemoglobin configuration (11 angstroms), but not as well in the oxygenated (5 angstroms).
- It interacts with deoxygenated hemoglobin beta subunits by decreasing their affinity for oxygen, so it allosterically promotes the release of the remaining oxygen molecules bound to the hemoglobin,
- Enhancing the ability of RBCs to release oxygen near tissues that need it most. 2,3-BPG is thus an allosteric effector.

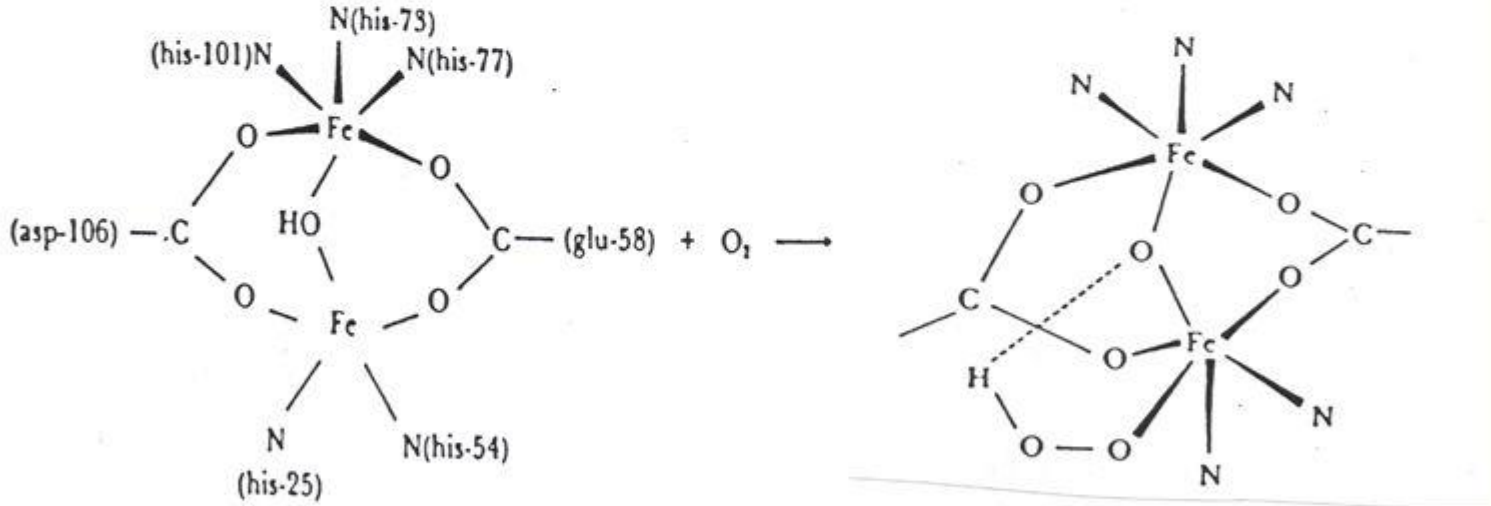
Hemerythrin

- Hemerythrin is an non-heme oligomeric protein responsible for oxygen (O_2) transport in the marine invertebrate phyla of sipunculids, priapulids, brachiopods, and in a single annelid worm genus, Magelona.
- Hemerythrin is essentially colorless when deoxygenated, but turn a violet-pink in the oxygenated state.
- Hemerythrin does not, as the name might suggest, contain a heme.
- Recent evidence has revealed hemerythrin to be a multi-functional protein



Hemerythrin II.

In resting state it contains hidroxo and carboxylate bridged Fe^{II}-ions. One of the Fe^{II} ions is unsaturated coordinatively and thus behaves as O_2 binding site. The Fe^{II} ions are oxidised to Fe^{III} the peroxo group is stabilised by hydrogen-bonding too. (The reversibility is not complete.)



Explanation

- In a great variety of worms the oxygen- carrying molecules are iron-bearing proteins , but they donot contain porphyrins. They are all presumably similar in chemical nature and are called hemerythrins. The mos studied one is that which is derived from saltwater worm Goldfingia gouldii. It has a molecular weight of 108,000 but consists of eight identical subunits. Each unit consists of two iron atoms.

- Each subunit consists of 113 aminoacids arranged in four nearly parallel helical segments having 30 to 40 Angstrom lenth. The iron atoms are held within these 4 segments . The 2 iron atoms are close together. In the case of aquametherythrin , which contans two iron atoms (in +3 state)

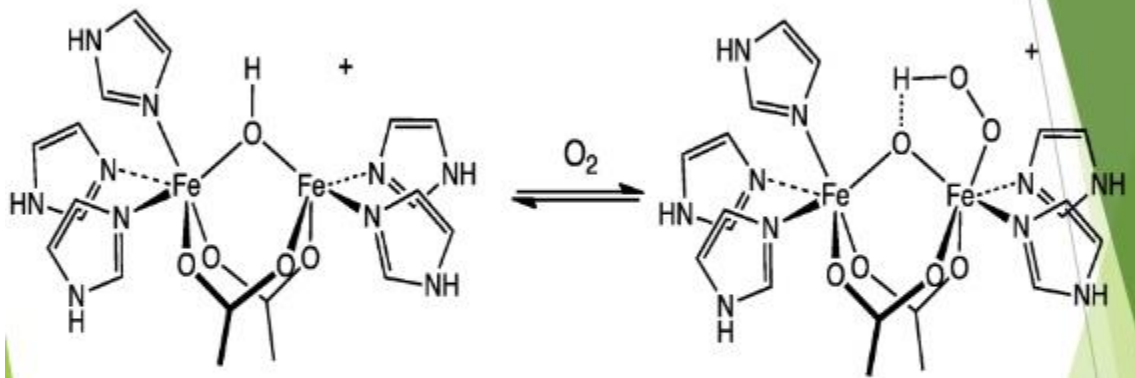
Oxygen affinity

- The oxygen affinity of this particular hemerythrine is not ph sensitive , though others are. Hemerythrine bind oxygen 5 to 10 times more strongly than hemoglobin and myoglobin. Each subunits can bind one oxygen molecule, thus the ratio of iron to oxygen is 2:1 .

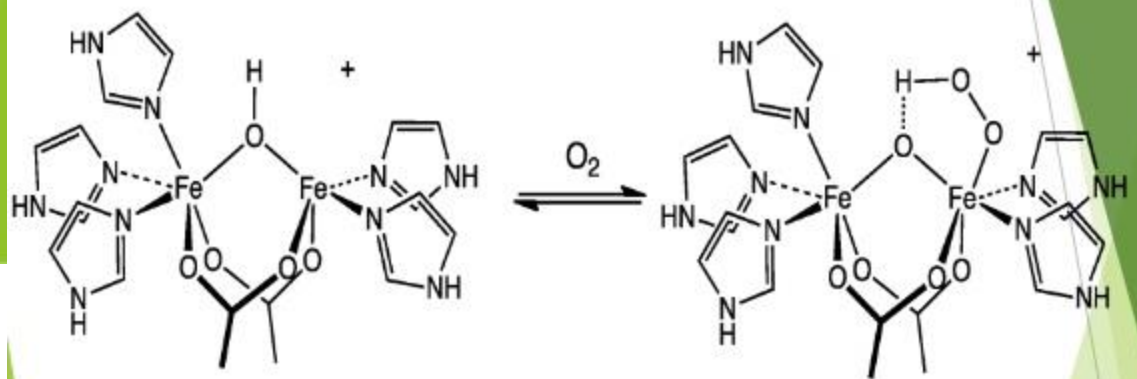
Oxygen binding mechanism

The mechanism of dioxygen binding is unusual. Most O2 carriers operate via formation of dioxygen complexes, but hemerythrin holds the O2 as a hydroperoxide. The site that binds O2 consists of a pair of iron centres. The iron atoms are bound to the protein through the carboxylate side chains of a glutamate and aspartates as well as through five histidine residues.

The uptake of O2 by hemerythrin is accompanied by two-electron oxidation of the diferrous centre to produce a hydroperoxide (OOH−) complex. The binding of O2 is roughly described in this diagram:



Deoxyhemerythrin contains two high-spin ferrous ions bridged by hydroxyl group (A). One iron is hexacoordinate and another is pentacoordinate. A hydroxyl group serves as a bridging ligand but also functions as a proton donor to the O2 substrate. This proton-transfer result in the formation of a single oxygen atom bridge in oxy- and methemerythrin. O2 binds to the pentacoordinate Fe2+ centre at the vacant coordination site (B). Then electrons are transferred from the ferrous ions to generate the binuclear ferric (Fe3+,Fe3+) centre with bound peroxide



- Hemerythrin affinity for carbon monoxide (CO) is actually lower than its affinity for O2, unlike hemoglobin which has a very high affinity for CO. Hemerythrin's low affinity for CO poisoning reflects the role of hydrogen-bonding in the binding of O2, a pathway mode that is incompatible with CO complexes which usually do not engage in hydrogen bonding.

Similarities of Hemerythrin with hemoglobin and myoglobin

- Like Hb and Mb, hemerthyrin contains Fe(II) which binds O_2 reversibly- but when oxidized to met hemerythrin it does not bind O_2
- In deoxy form, one co-ordination site is vacant
- There is monomeric form analogues to Mb in the tissues.
- An octameric form for molecular weight 1000000 transports O_2
- Similar to Hb, it consists of 4 chains-2 alpha and 2 beta - each of which is very similar to the single chain of Mb

Differences of Hemerythrin with hemoglobin and myoglobin

- Quaternary structure of Hb is stable, but octameric form of hemerythrin appears to be in dissociative eqm with monomeric units in solution
- Both the monomer and octomer have two Fe(II) atoms at the O_2 binding site
- Reaction takes place via a redox reaction to form Fe(III) and the peroxide
- Fe(III) atoms are at different environment (Mossbauer data)
- This indicates that O_2^{2-} coordinating one Fe atom or each Fe atom having different ligands
- Hemerythrin shows little or no cooperativity
- About O_2 binding – Substrate O_2 enters the sixth coordination site on the lower Fe atom and become reduced to a hydroperoxide group which is held by a hydrogen bond to the binding Oxygen

Comparison of the various oxygen transport proteins

Property	hemoglobin	hemerythrin	hemocyanin
Metal ion	Fe^{II}	Fe^{II}	Cu^{I}
Number of subunits	4	8	10 – 100
M	65.000	108.000	450.000 – 10 000 000
M:O_2 ratio	1:1	2:1	2:1
Colour (deoxy)	purply-red	colourless	colourless
Colour (oxy)	bright red	violet-pink	blue
Metal bindig site	porphin	protein	protein