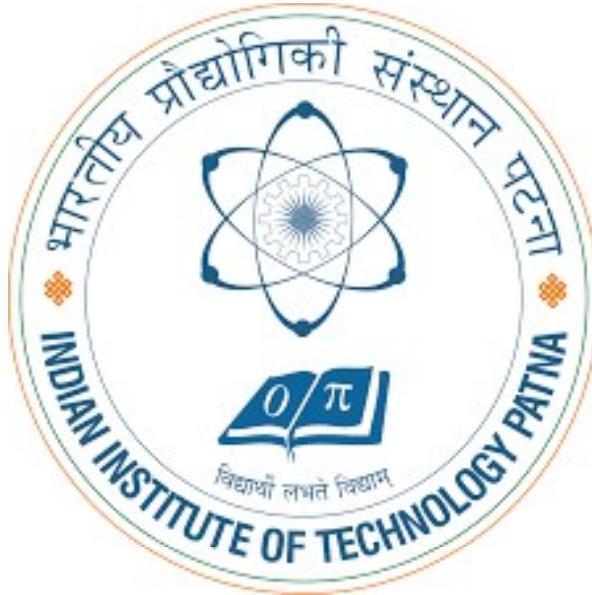


CH103: Introductory Chemistry



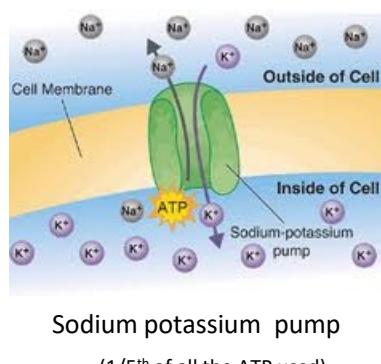
Bioinorganic chemistry: Trace elements in biology, heme and non-heme oxygen carriers, haemoglobin and myoglobin

Bioinorganic chemistry

Study of Inorganic elements in the living systems

11
Na
22.98

19
K
39.09



20
Ca
40.08

12
Mg
24.31



26
Fe
55.85

Hemoglobin
Myoglobin
Cytochromes
Ferredoxin

27
Co
58.94

Vit B12

29
Cu
63.55

Hemocyanin

30
Zn
65.38

Carbonic anhydrase
Carboxypeptidase

Bioinorganic chemistry

- 40 different elements present in human body.
- H,C,O and N contains 99 % of the atoms of living body.
- Next abundant are Mo, Mn, Fe, Co, Cu, Zn, F, and I. They are present in trace amounts and are known as essential trace elements.
- Fe, F and Zn are present in gm quantities and rest are in mg quantities.
- Ultratrace elements are Li, Si, V, Cr, Se, Br, Sn, and W.

Bioinorganic chemistry

- Essential Metals: The living system can not survive without it and eventually dies- Na, K, Mg, Ca, Mn, Fe, Co, Cu, Zn, Mo
- Beneficial Metals: The life process gets hampered but cannot lead to death- Li, V, Cr, Ni, Sn, W

Na	100 g	Mn	15 mg
K	200 g	Fe	5-7 g
Mg	35 g	Co	1.5 mg
Ca	1500	Ni	5 mg
V	15 mg	Cu	200-300 mg
Cr	2 mg	Zn	2-3 g
		Mo	10 mg

~ 70 Kg person

Bioinorganic chemistry

$10^{-8} \text{ mol dm}^{-3}$	Fe	Zn	Cu	Mo	Co	Cr	V	Mn	Zn
Sea -2	0.005	8	1	10	0.7	0.4	4	0.7	0.5
Human Plasma	2200	100	1600	1000	0.002	5.5	18	11	4.5

$10^{-8} \text{ mol dm}^{-3}$	Na^+	Mg^+	Ca^+	K^+	HPO_4^{2-}	Cl^-	SO_4^{2-}
Sea	470	50	10	10	0.001	55	28
Human Plasma	140	1	3	4	1	100	1

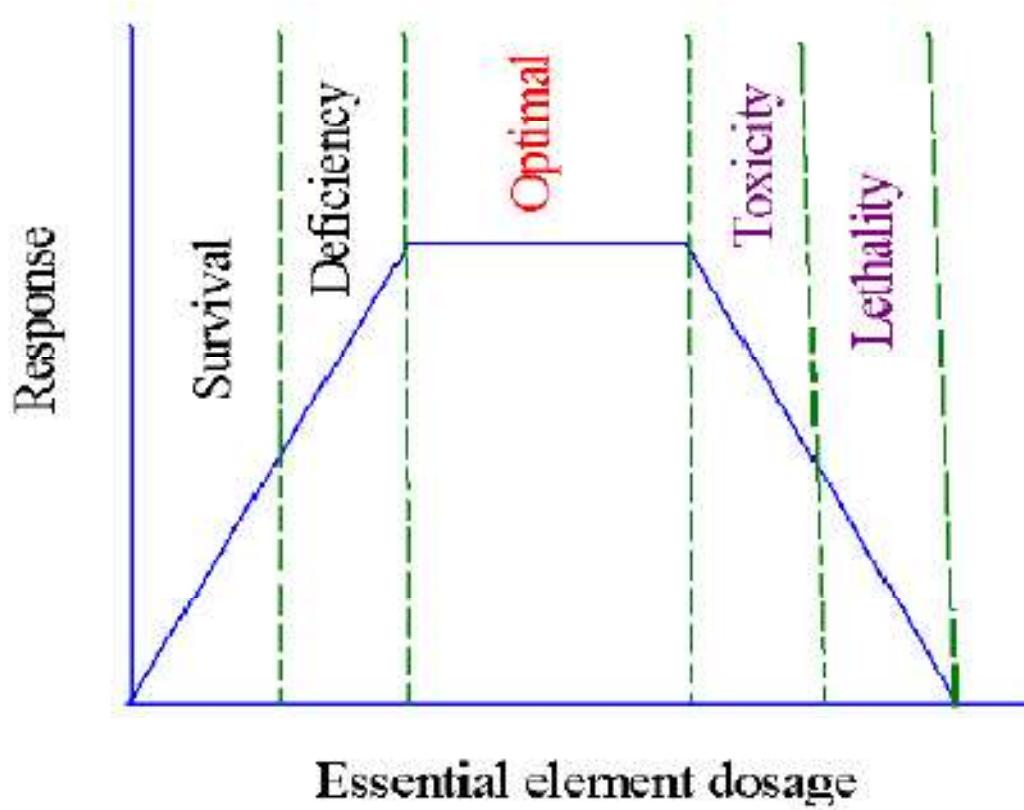
Bioinorganic chemistry

Important roles *metals* play in biochemistry

Metal	Function
Na, K	Charge carrier, osmotic balance
Mg, Zn	Structural, hydrolase, isomerase
Ca	Structural, charge carrier
V, Mo	Nitrogen fixation, oxidase
Mn	Photosynthesis, structural, oxidase
Fe, Cu	Dioxygen transport and storage, electron transfer, oxidase
Ni	hydrogenase, hydrolase

Bioinorganic chemistry

Every essential element follows a dose-response curve



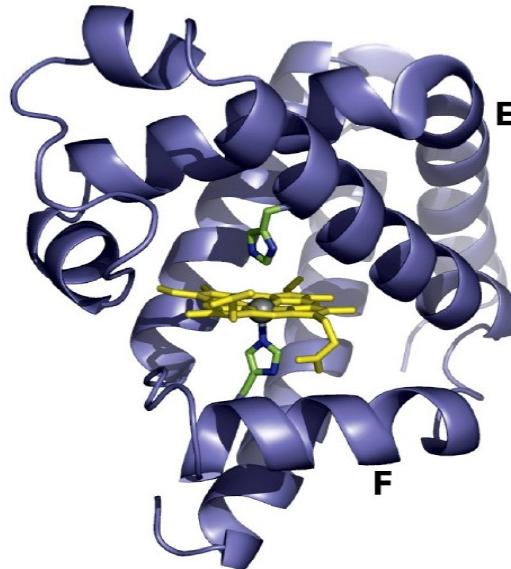
Bioinorganic chemistry

Structure of a metallo-protein : A metal complex perspective

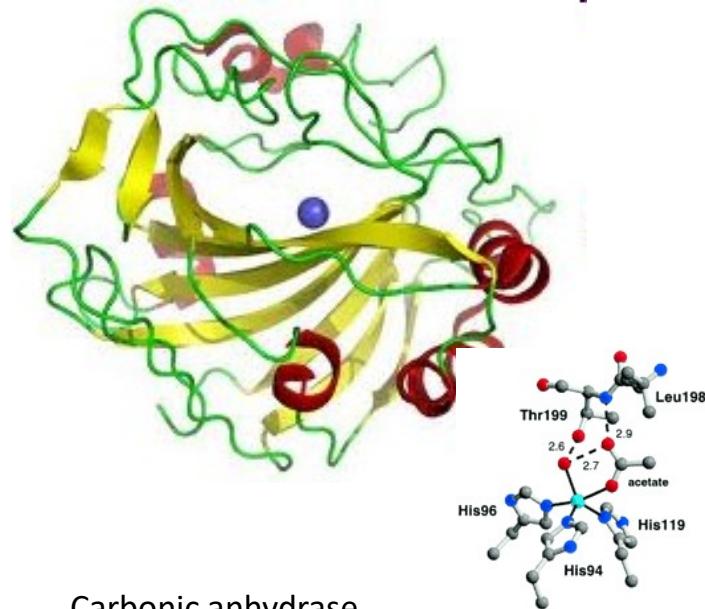
Spiral - α helix form of protein Tape - β Pleated sheet form of protein

Prosthetic groups – A metal complex positioned in a crevice. Some of the ligands for this complex or some times all of the ligands are provided by the side groups of the amino acid units.

The geometry around the metal and bond distances and angles are decided by the protein unit



Myoglobin

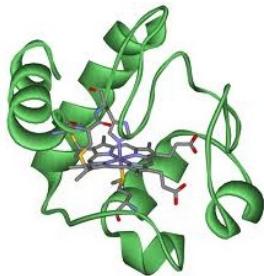


Carbonic anhydrase

Bioinorganic chemistry

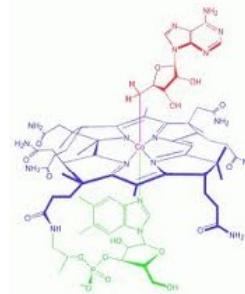
Metalloenzymes and Oxygen carriers =
Protein + Cofactor

A **cofactor** is a non-protein chemical compound that is bound to a protein and is required for the protein's biological activity. These proteins are commonly **enzymes**. Cofactors are either organic or inorganic. They can also be classified depending on how tightly they bind to an enzyme, with loosely-bound or protein-free cofactors termed **coenzymes** and **tightly-bound cofactors termed prosthetic groups**.

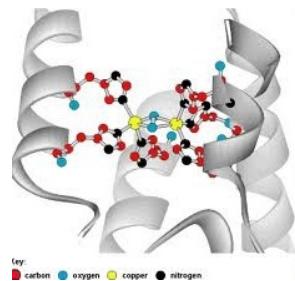


Cytochrome C

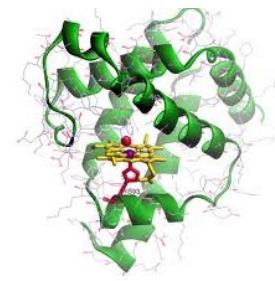
Porphyrins with different metals at its centre are a common prosthetic group in bioinorganic chemistry



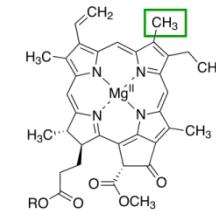
Coenzyme B12



Hemocyanin



Myoglobin

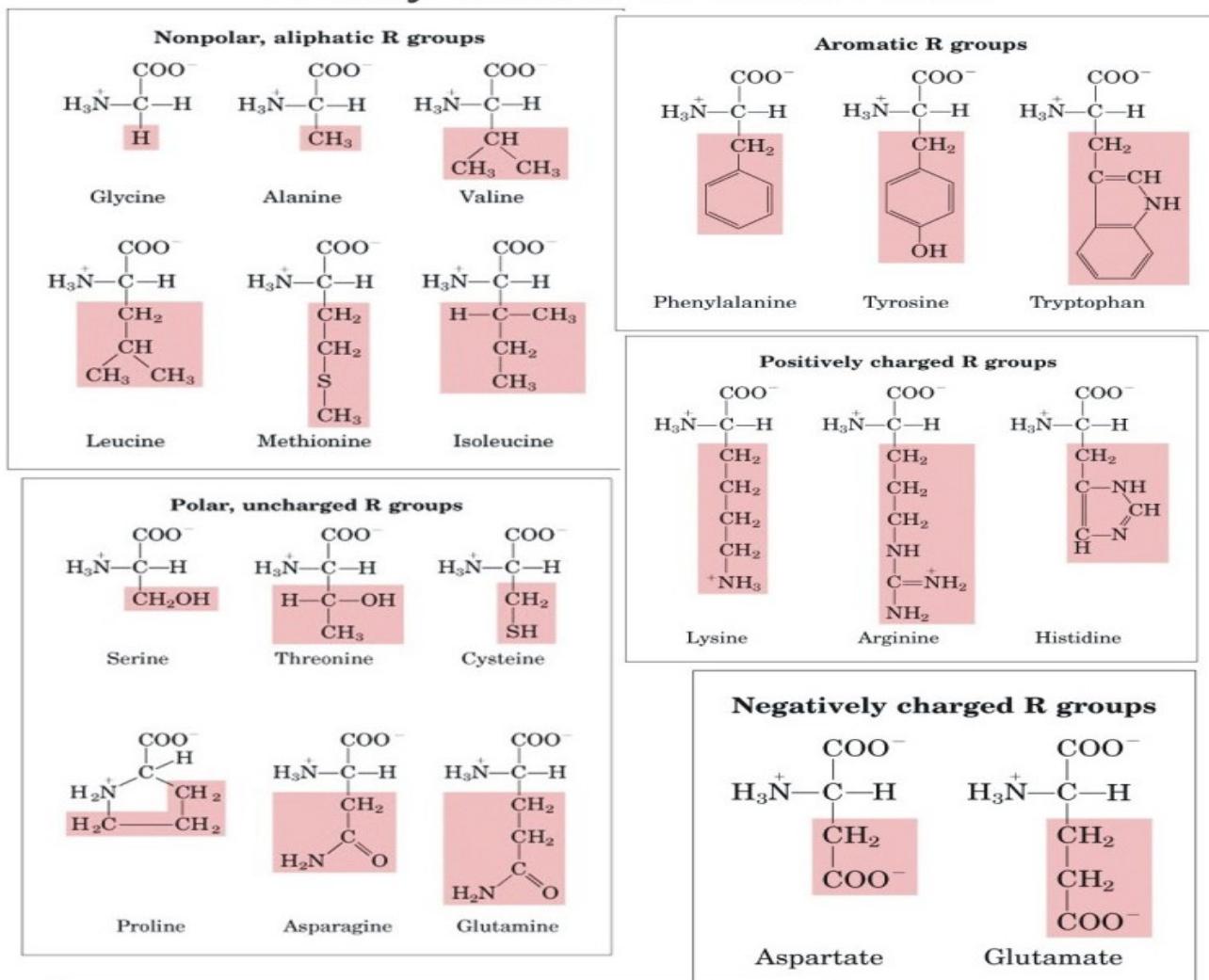


Chlorophyll

Bioinorganic chemistry

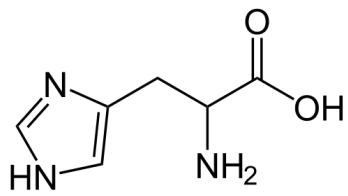
Proteins –consists of different amino acids in a specific sequence connected by the peptide bond –

Twenty standard Amino Acids

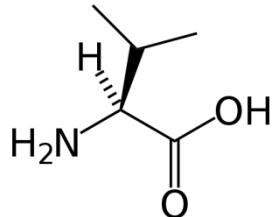


Bioinorganic chemistry

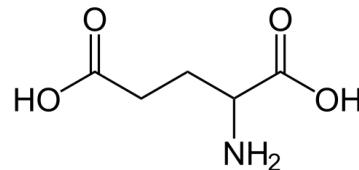
A few important amino acids relevant to the present course



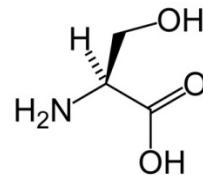
HISTIDINE This amino acid has a pKa of 6.5. This means that, at physiologically relevant pH values, relatively small shifts in pH will change its average charge. Below a pH of 6, the **imidazole** ring is mostly protonated.



VALINE is a branched-chain amino acid having a **hydrophobic isopropyl R group**. In sickle-cell disease, valine substitutes for the hydrophilic amino acid glutamic acid in hemoglobin. Valine is **hydrophobic**.



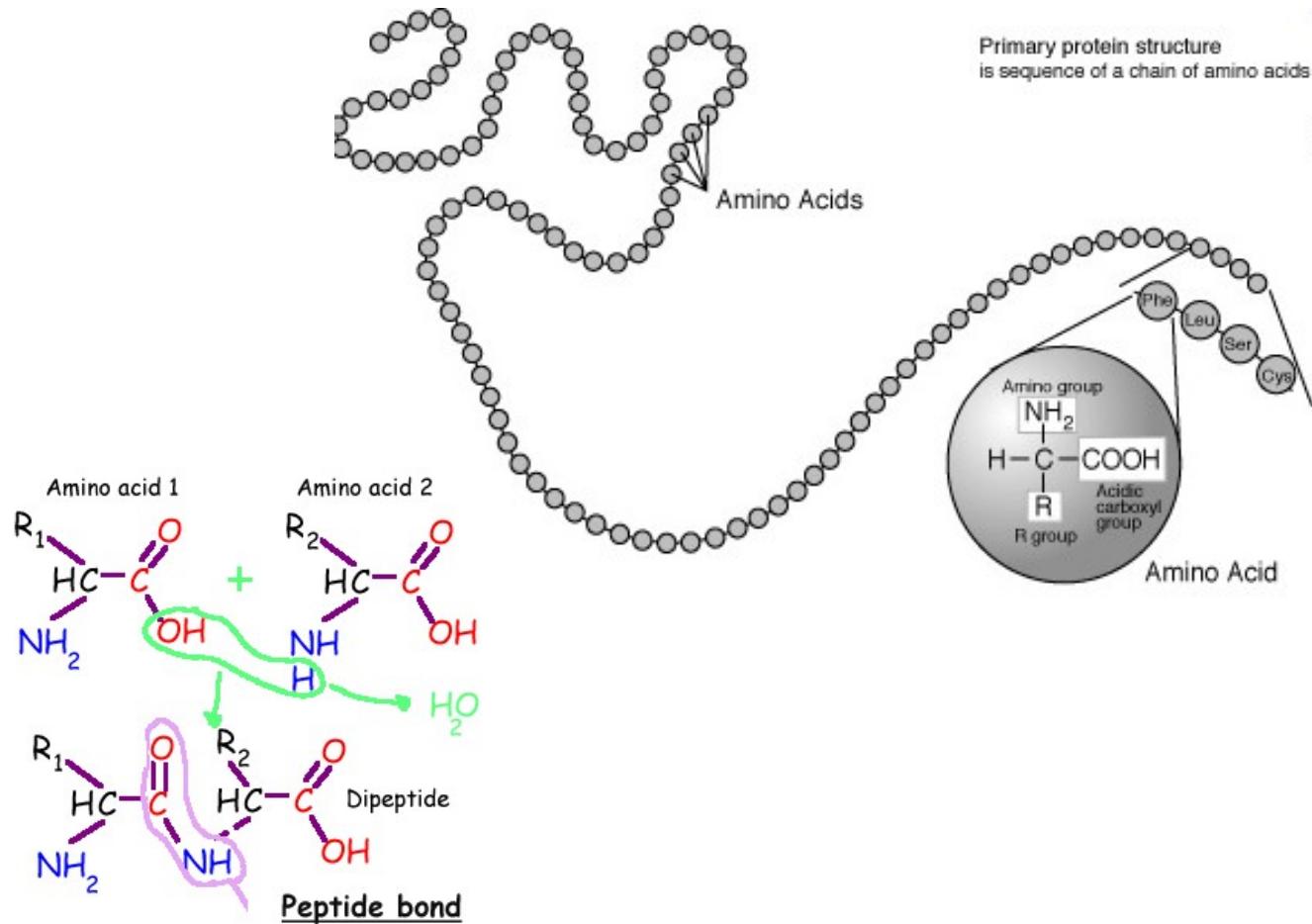
GLUTAMIC ACID has carboxylic acid functional group which is **hydrophilic**, has pKa of 4.1 and exists in its negatively charged deprotonated carboxylate form at physiological pH ranging from 7.35 to 7.45.



SERINE Serine is an amino acid having a CH_2OH side group. By virtue of the hydroxyl group, serine is classified as a **polar amino acid**. Serine was first obtained from silk protein, a particularly rich source, in 1865.

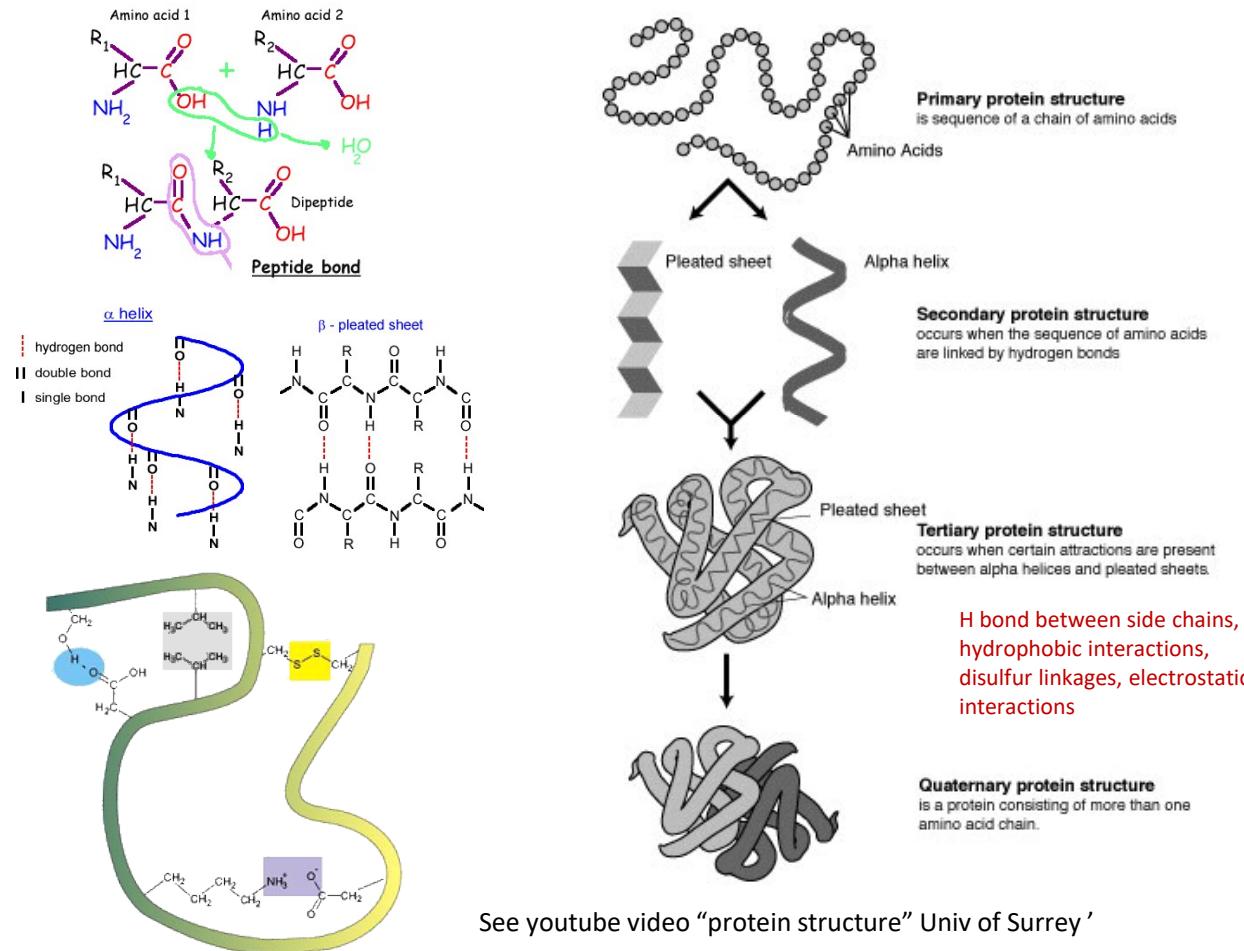
Bioinorganic chemistry

The primary structure of a protein



Bioinorganic chemistry

The four levels of protein structure



Bioinorganic chemistry

Oxygen Transport Proteins

Heme Protein

1. Hemoglobin
2. Myoglobin

Nonheme Protein

3. Hemerythrin
4. Hemocyanin.

Bioinorganic chemistry

Oxygen Transport Proteins

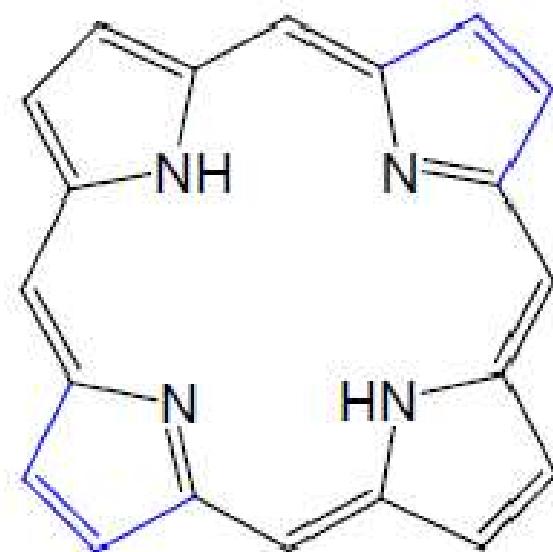
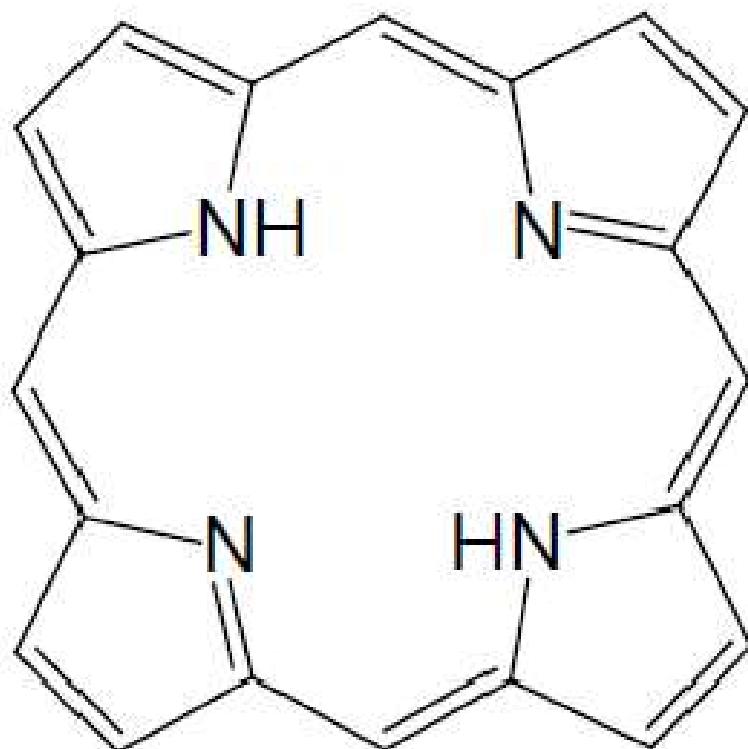
Some Properties of Oxygen Transport Proteins

O ₂ carrier:	Myoglobin	Hemoglobin	Hemerythrin	Hemocyanin
Source:	Higher animals, some invertebrates	Higher animals, some invertebrates	invertebrates	Arthropods, mollusks
Metal:	Fe	Fe	Fe	Cu
Metal:bound O ₂ stoichiometry (ligands):	Fe:O ₂ (heme, histidine)	Fe:O ₂ (heme, histidine)	2 Fe:O ₂ (nonheme, protein side chains)	2 Cu:O ₂ (nonheme, protein side chains)
Metal ox state in deoxy form/ <i>d</i> electrons (color):	II/ <i>d</i> ⁶ (red-purple, violet)	II/ <i>d</i> ⁶ (red-purple, violet)	III/ <i>d</i> ⁶ (colorless)	IV/ <i>d</i> ¹⁰ (colorless)
Metal ox state in oxy form/ <i>d</i> electrons (color):	II/ <i>d</i> ⁶ –O ₂ or III/ <i>d</i> ⁵ –O ₂ [–] (red)	II/ <i>d</i> ⁶ –O ₂ or III/ <i>d</i> ⁵ –O ₂ [–] (red)	III/ <i>d</i> ⁵ (burgundy)	II/ <i>d</i> ⁹ (blue)
Approximate molecular weight (kDa):	17	65	108	400 to 2 × 10 ⁴
Number of subunits:	1	4 (some species have up to 10)	8	Many

Bioinorganic chemistry

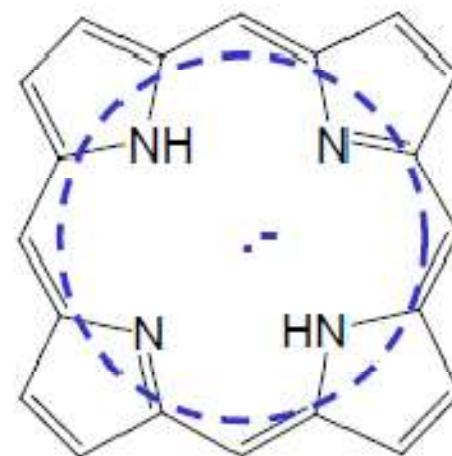
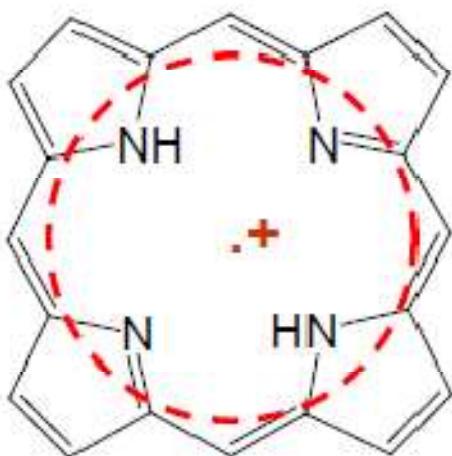
Heme and porphyrins

11 double bonds. 22 π electrons. Fully conjugated.
18 π electrons only are necessary to ensure delocalization



Bioinorganic chemistry

A rich redox-chemistry

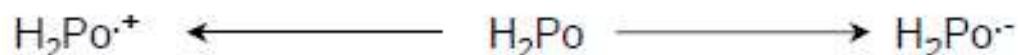


Delocalized radical

The formation of radical species is reversible.

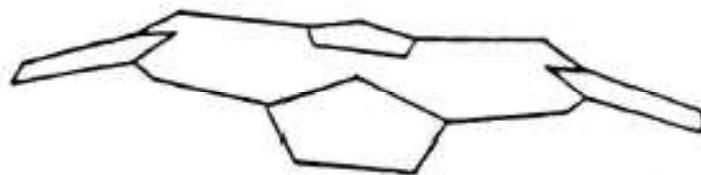
cation radical <= oxydation

réduction => anion radical

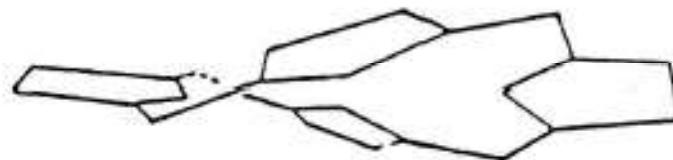


Bioinorganic chemistry

Porphyrins can be highly **distorted**



Domed conformation



Ruffled conformation



Saddle shaped conformation

Hemoglobin and Myoglobin

Myoglobin is the oxygen *storage* protein

- - High affinity for O₂
- - Major physiological role is to facilitate oxygen transport in rapidly respiring muscle.

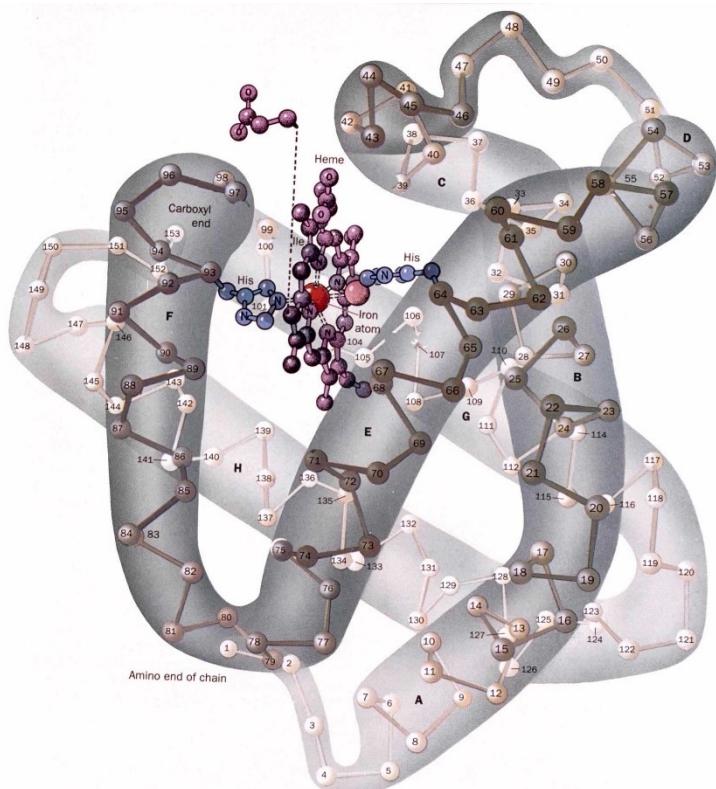
Hemoglobin is used for *transport* of oxygen from the lungs, gills, or skin of an animal to its capillaries.

- - Lower affinity for O₂ than myoglobin
- - Also for removing CO₂ from tissues
- - CO₂ is a major product of metabolite oxidation

Myoglobin Structure

Myoglobin is composed of a single polypeptide chain with 153 amino acid residues. It measures 45x35x25 angstroms with about 70% alpha-helix content.

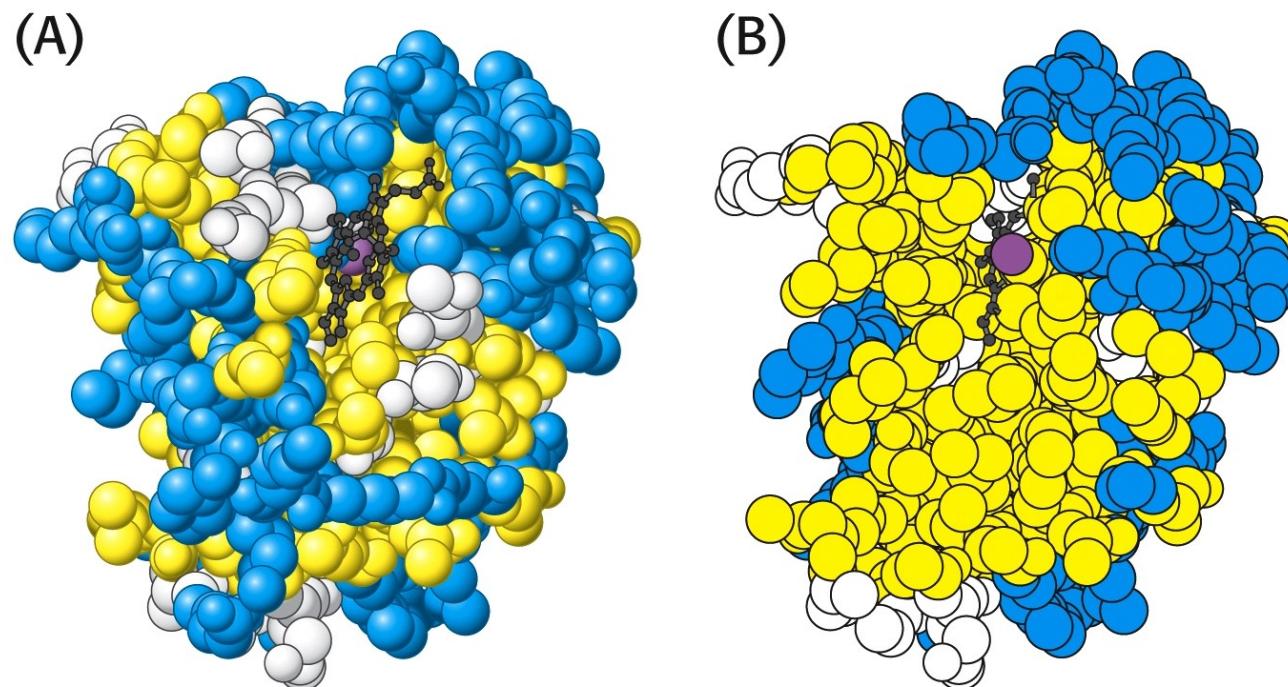
Each myoglobin molecule contains a prosthetic (helper) group: a Protoporphyrin IX and a central iron atom collectively called “heme.”:



Helices are named A, B, C, ...F. The heme pocket is surrounded by E and F but not B, C, G, also H is near the heme.

Myoglobin Structure

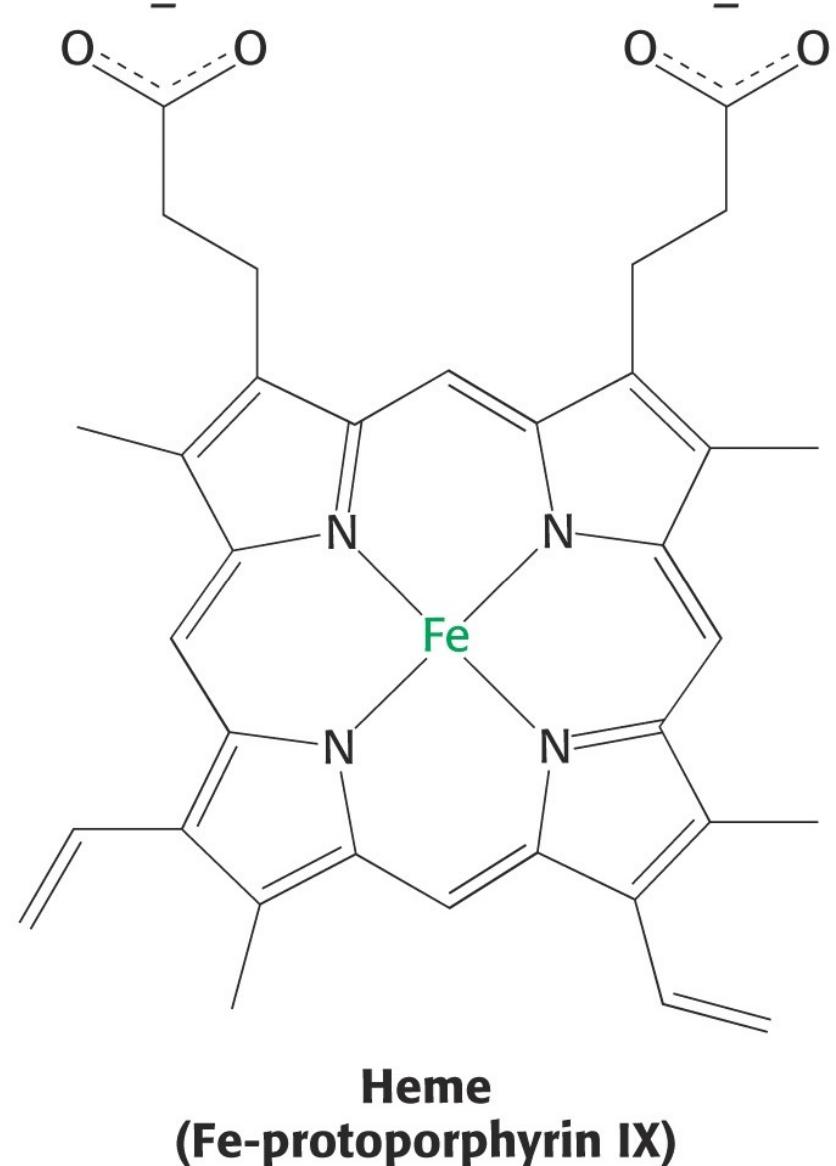
As with most water-soluble proteins, its polar amino acids are located on the external surface of the protein, to maximize interactions with water. Non-polar amino acids are located almost entirely on the interior of the protein, leaving very little space inside.(Blue = charged amino acids; Yellow=hydrophobic amino acids.)



Myoglobin Structure

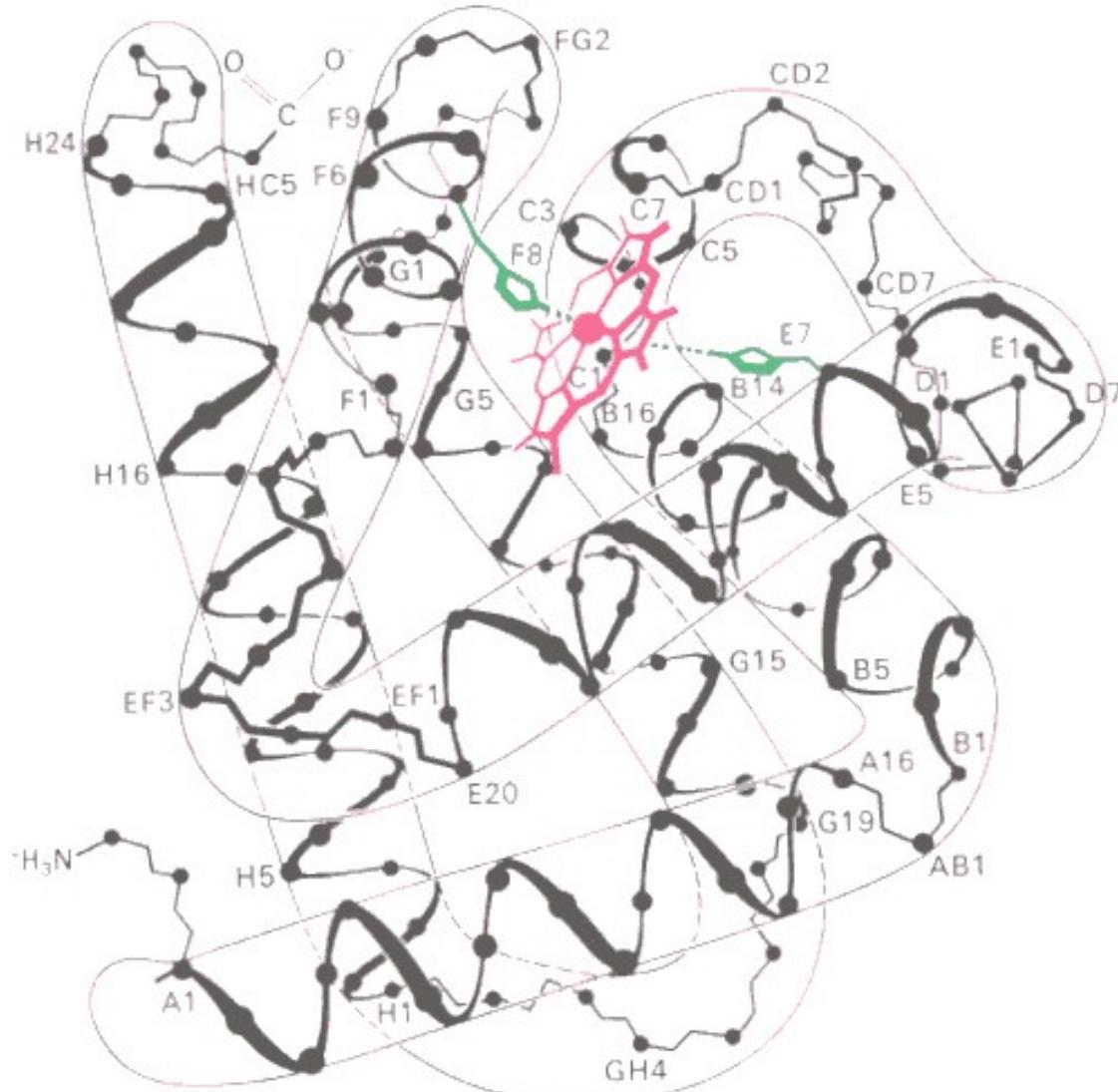
The heme group is held in place by hydrophobic interactions to the non-polar interior region of the protein. It is not attached by any covalent linkages. (In fact, it may be removed, leaving the “**apoprotein**” behind.)

An iron ion fits perfectly into the center of the protoporphyrin, chelated by four nitrogen atoms of a **tetrapyrrole** ring system.



Myoglobin Structure

Since iron ions are hexadentate, each has **six coordination sites**. One of these two other sites forms a coordinate covalent bond to a nitrogen atom in histidine F8 (proximal). Another histidine (*E*7, distal) is close to the sixth coordination position.

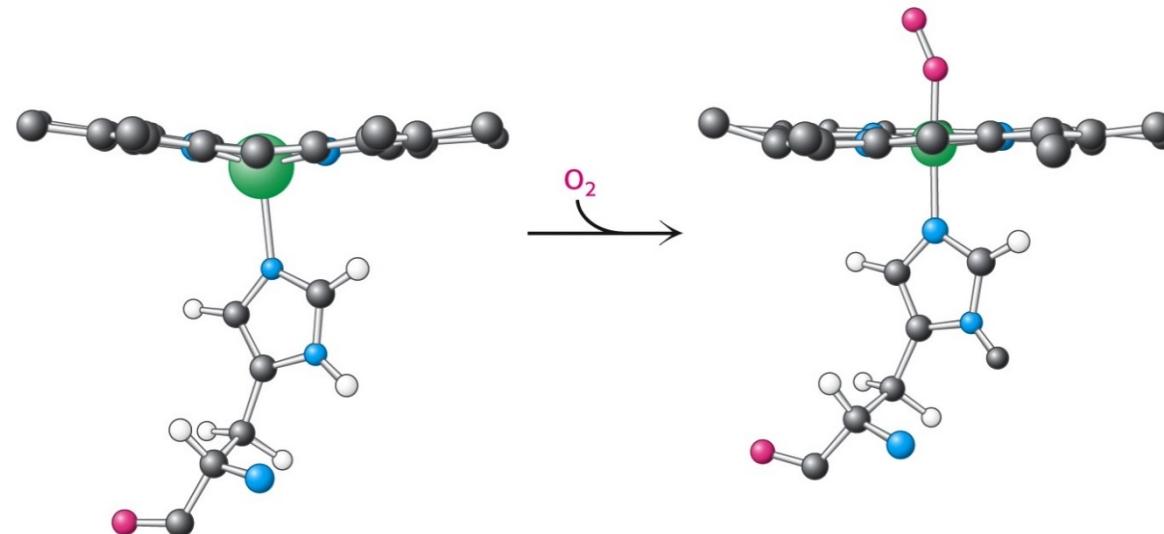


Myoglobin Structure

The iron ion is the binding site for oxygen molecules. The iron ion often converts between the free Fe^{2+} (ferrous ion) state and the bound Fe^{3+} (ferric ion) state.

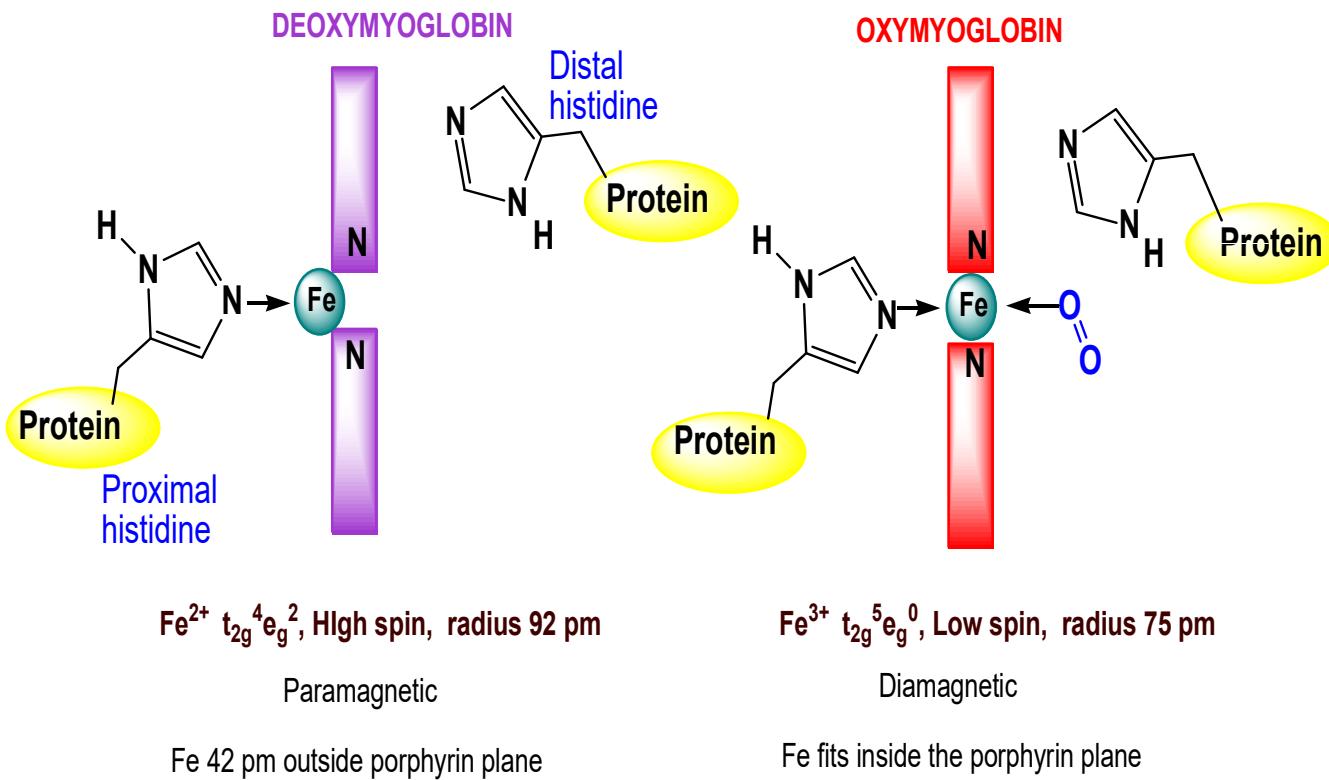
In the unbound state, the iron atom is slightly proximal (above) the plane of the protoporphyrin. As oxygen binds to the distal side of the ring, it pulls the iron atom about 0.29 angstrom closer to the plane of the ring.

Although this distance is small, the movement is amplified, causing significant shifts throughout the tertiary structure of the protein.



Myoglobin Structure

Changes at the active site during oxygenation of Myoglobin



Myoglobin Structure

The position of the distal histidine (E7) prevents O₂ from binding too strongly to the iron atom.

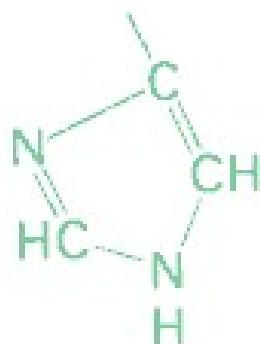
Maximal binding strength is achieved when the three atoms [Fe-O=O] form a linear sequence. However, the distal histidine prevents this from occurring, and the diatomic oxygen binds in a “bent” configuration.

Carbon monoxide also binds to the iron atom in myoglobin. In fact, it will displace oxygen and form a much tighter bond than oxygen, due to its more polar bond.

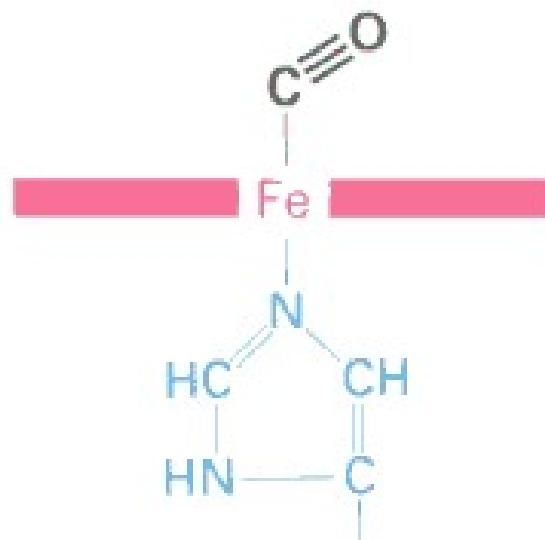
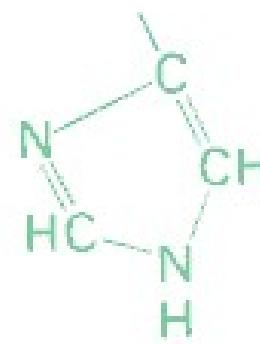
Even low concentrations of CO can displace O₂. This explains how even low concentrations of CO can cause asphyxiation in the presence of O₂!

Myoglobin Structure

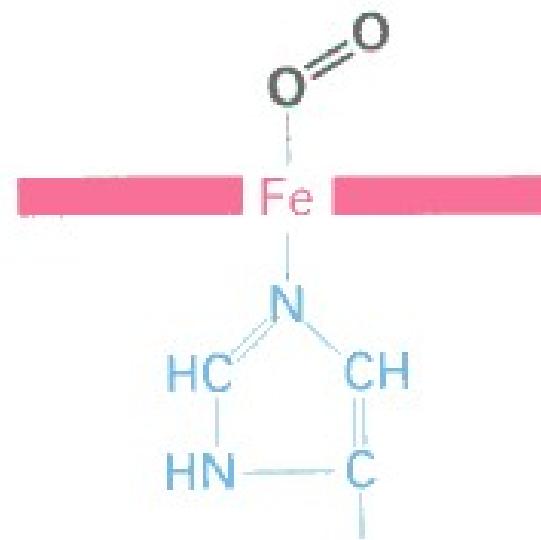
Fortunately, CO also binds in a “bent” configuration. This weakens the attraction, such that eventually the CO will dissociate over time, allowing recovery.



His
E7



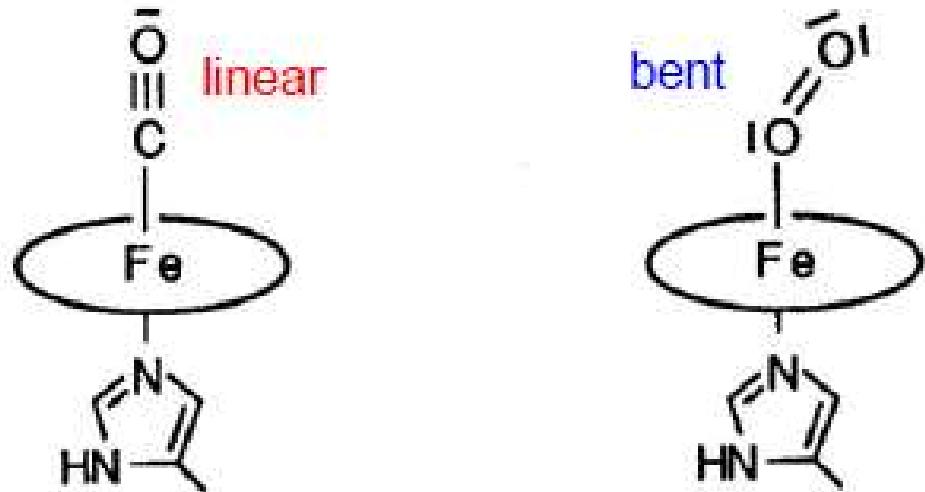
His
F8



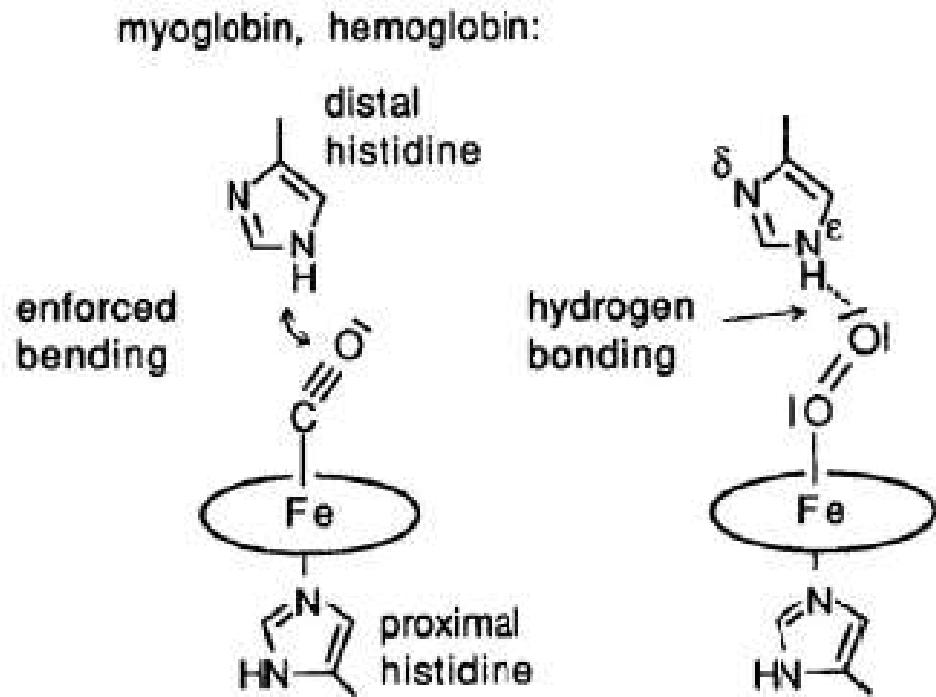
The role of the distal His:

free heme complexes

Distal His forces ligands to bend: this is energetically unfavourable for binding of CO
Because of this, small amounts of CO in air can be tolerated!



Distal His also plays a role as a proton shuttle (to avoid attack of protons on bound O₂)

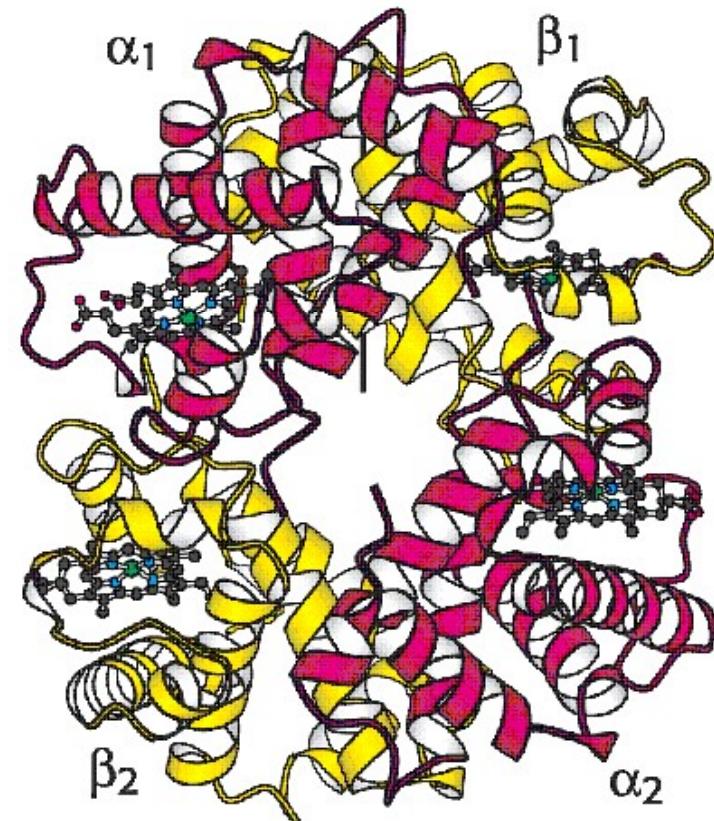
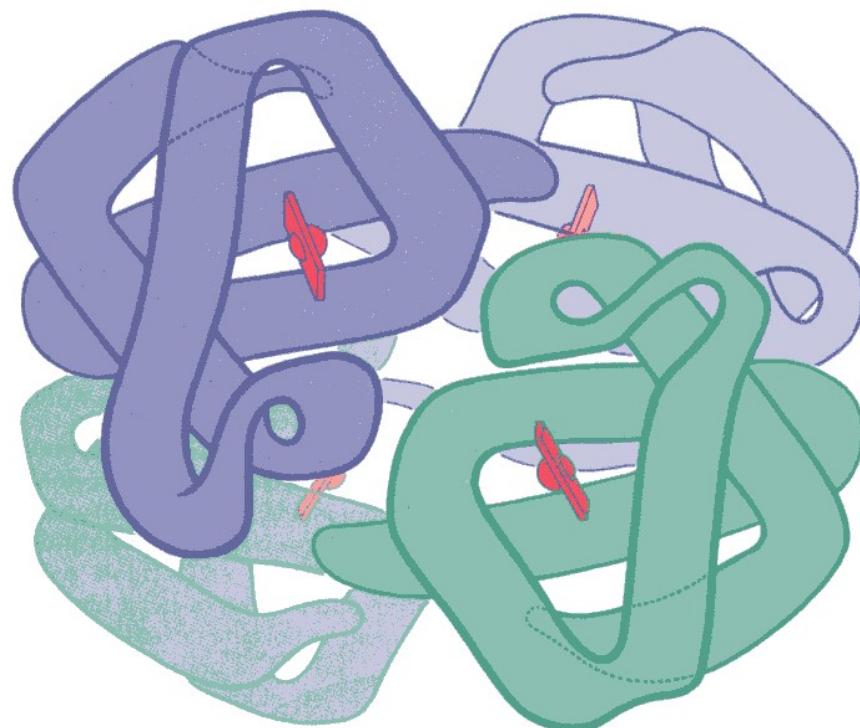


Hemoglobin Structure

- Hemoglobin is a much more complex molecule than myoglobin.
- The protein is nearly spherical with a 55 angstrom diameter and molecular mass of 64.45 kD.
- It is a tetrahedron containing:
 - 4 protein subunits,*
 - 4 protoporphyrins, and*
 - 4 iron atoms.*
- Each hemoglobin molecule can transport four oxygen molecules (one per Fe atom).

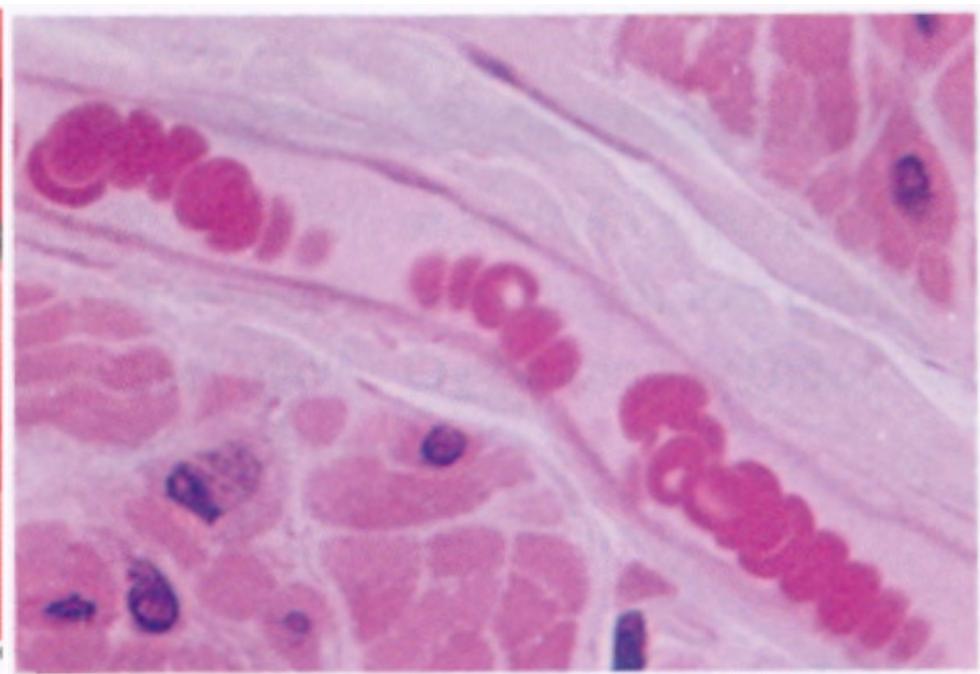
Hemoglobin Structure

The two alpha subunits have 141 amino acids, while the two beta subunits contain 146 residues.



Hemoglobin – Tetrameric Structure

Hemoglobin is located in erythrocytes, where it greatly increases oxygen solubility, facilitating as much as 68 times higher oxygen concentrations than in water alone.



Human erythrocytes. These red blood cells, or erythrocytes, are shown moving in a capillary. Each erythrocyte contains about 300 million hemoglobin molecules.

O_2 affinity is a crucial point:

O_2 must be taken from atmosphere,

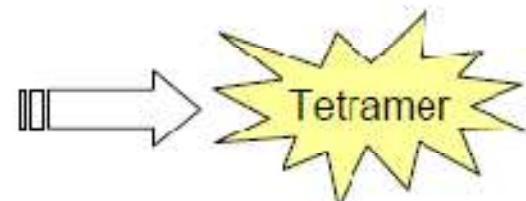
Transported and

Delivered in due place in an *efficient way!*

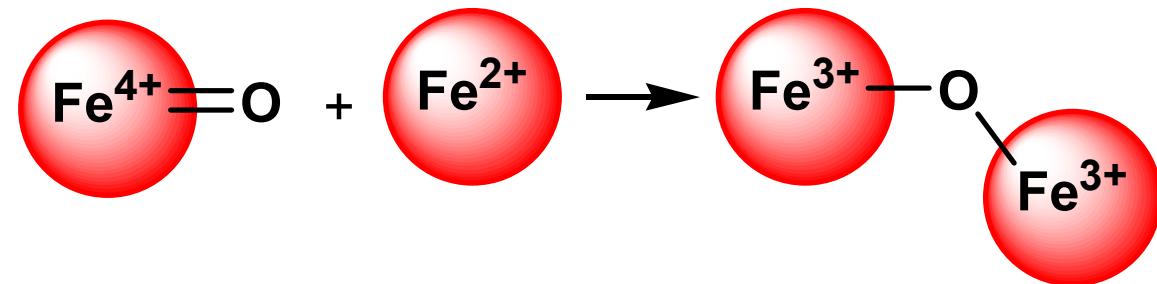
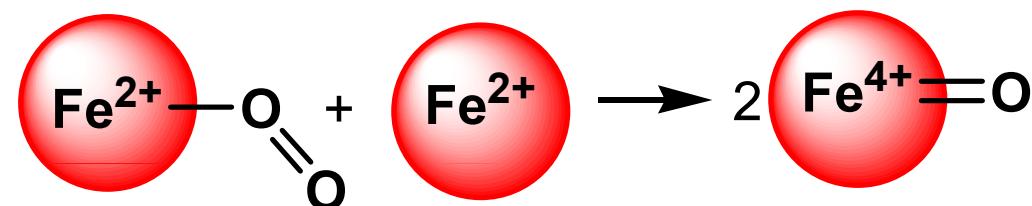
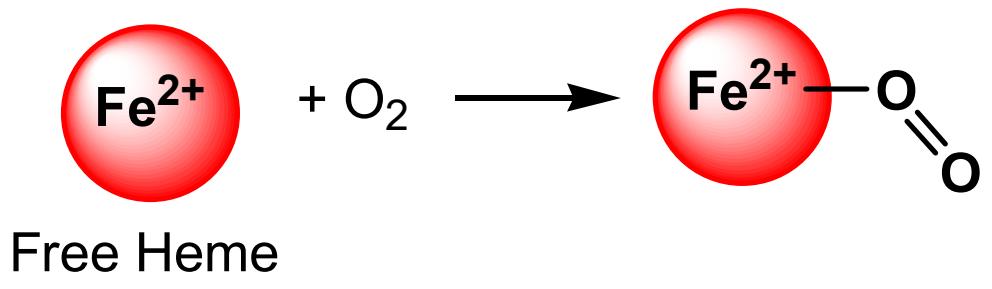
Differences between Hemoglobin and Myoglobin

Hb: high affinity in lungs
low affinity in muscles

Mb: high affinity in muscles



Oxygen Binding



Hemoglobin

When Fe(II) goes to Fe(III), oxidized, it produces methemoglobin which is brown and coordinated with water in the sixth position. Dried blood and old meat have this brown color.

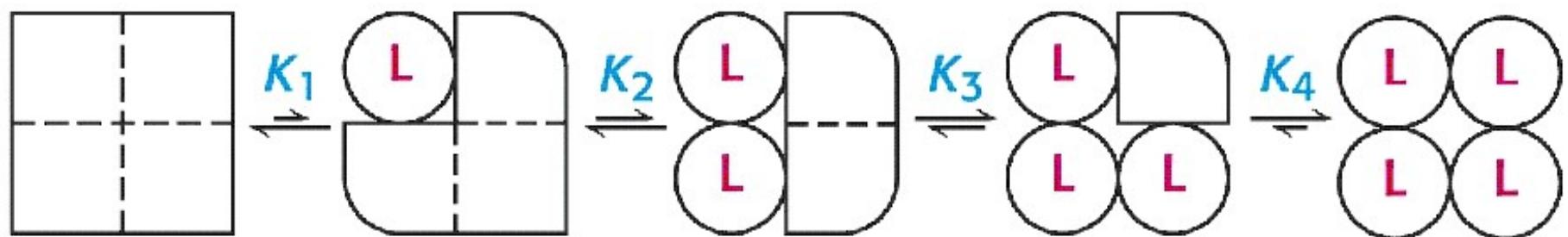
Butchers use ascorbic acid to reduce methemoglobin to make the meat look fresh!!

There is an enzyme methemoglobin reductase that converts methemoglobin to regular hemoglobin.

Hemoglobin - Oxygen Transport

- Oxygen binds to hemoglobin on each of the four iron atoms.
- This occurs sequentially, with the affinity of each the four sites changing as the sites become occupied with oxygen.

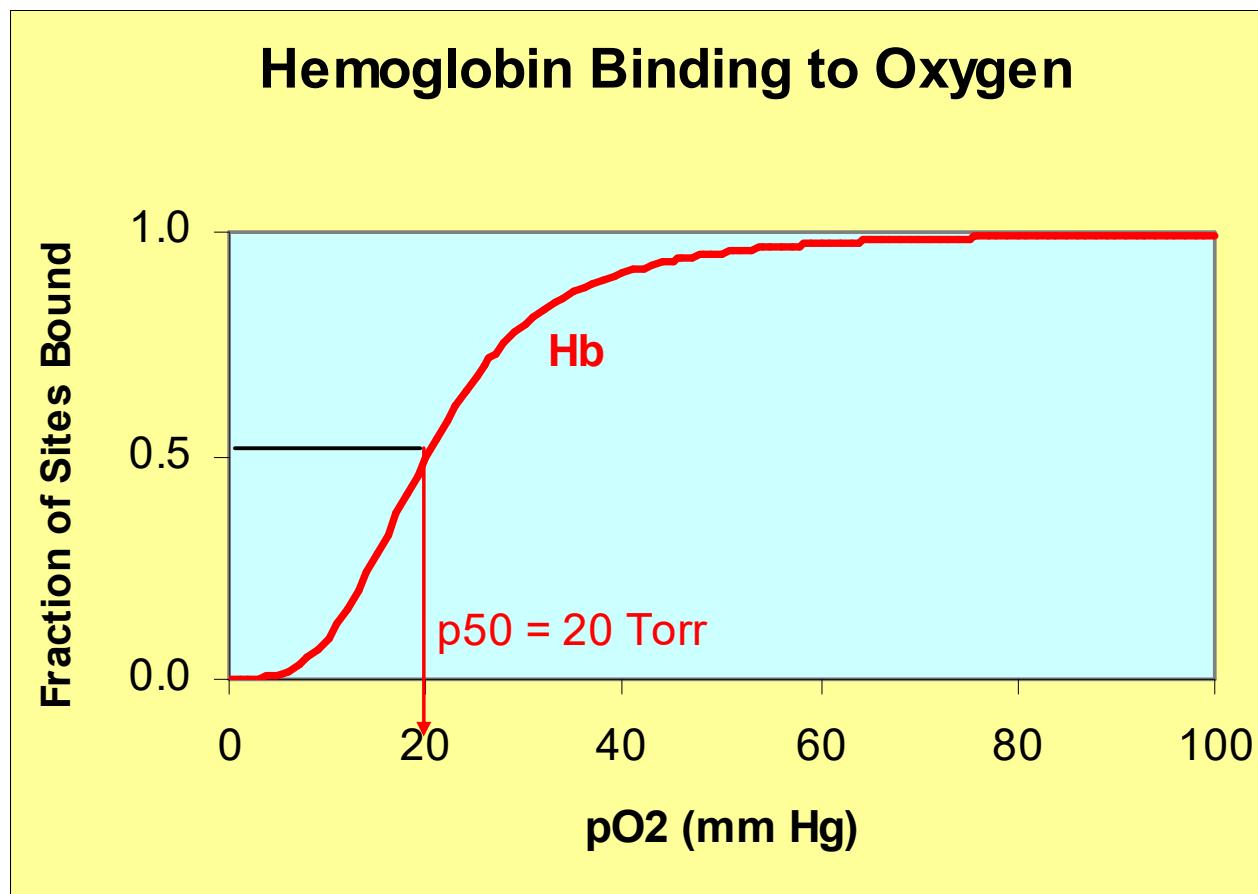
L = “*ligand*” of molecular oxygen (O_2).



The hemoglobin molecule exhibits lower affinity for the first molecule of oxygen to bind. Its affinity increases as subsequent oxygen molecules bind.

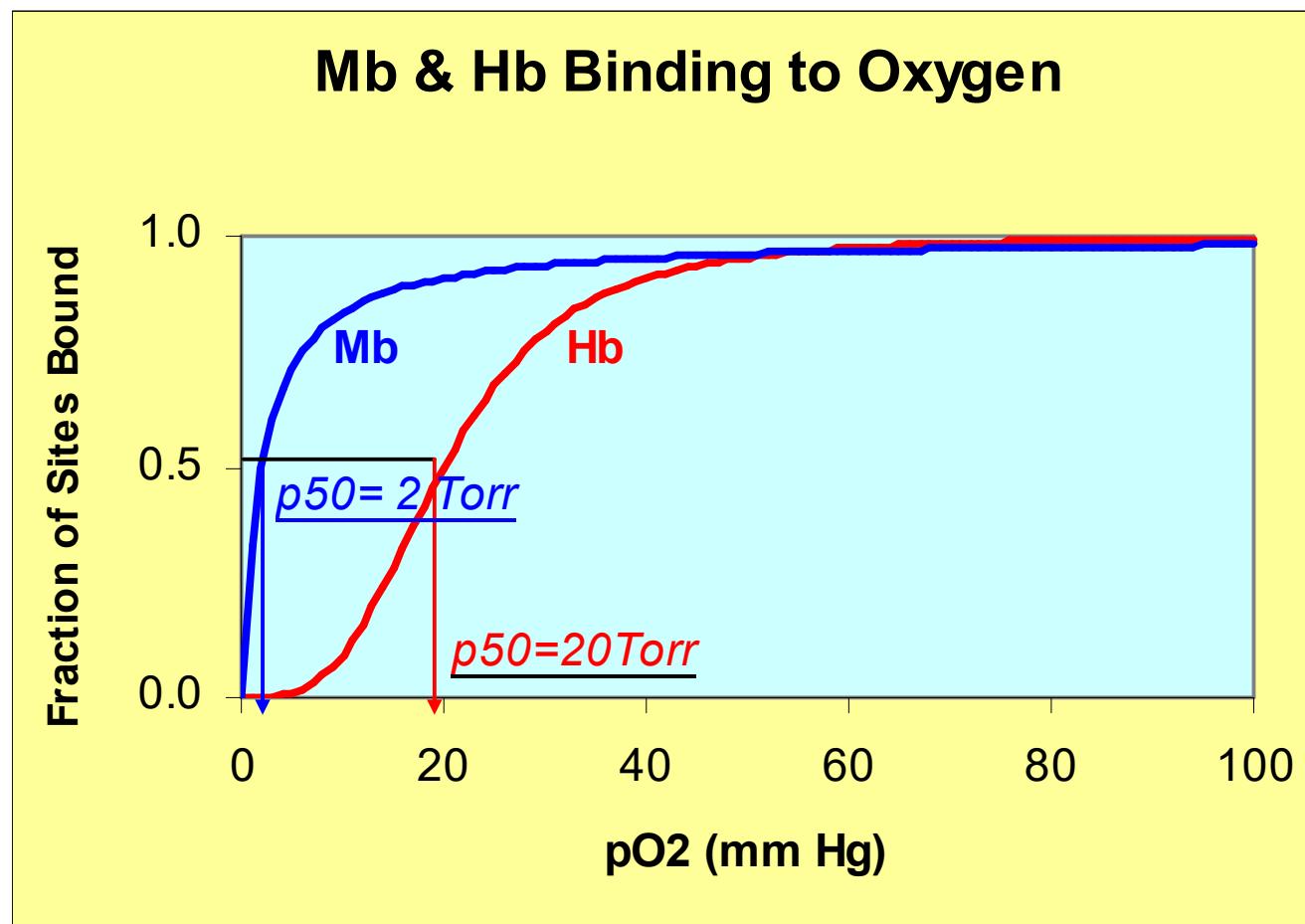
Hemoglobin - Oxygen Transport

- Plotting oxygen binding to hemoglobin at various oxygen concentrations shows this change in affinity:



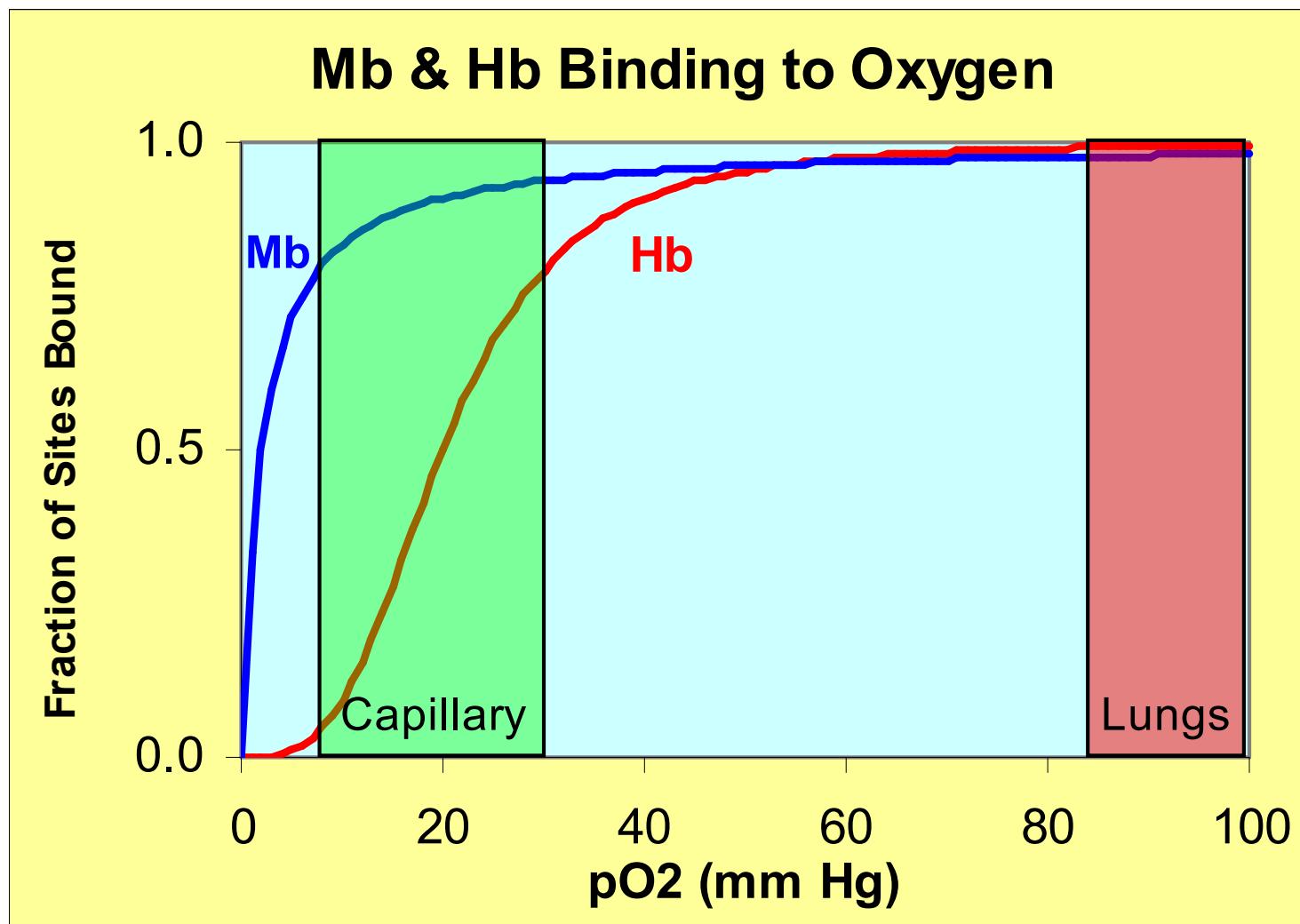
Hemoglobin - Oxygen Transport

- When the binding curve for myoglobin is compared to hemoglobin, a distinctly different binding profile is observed:

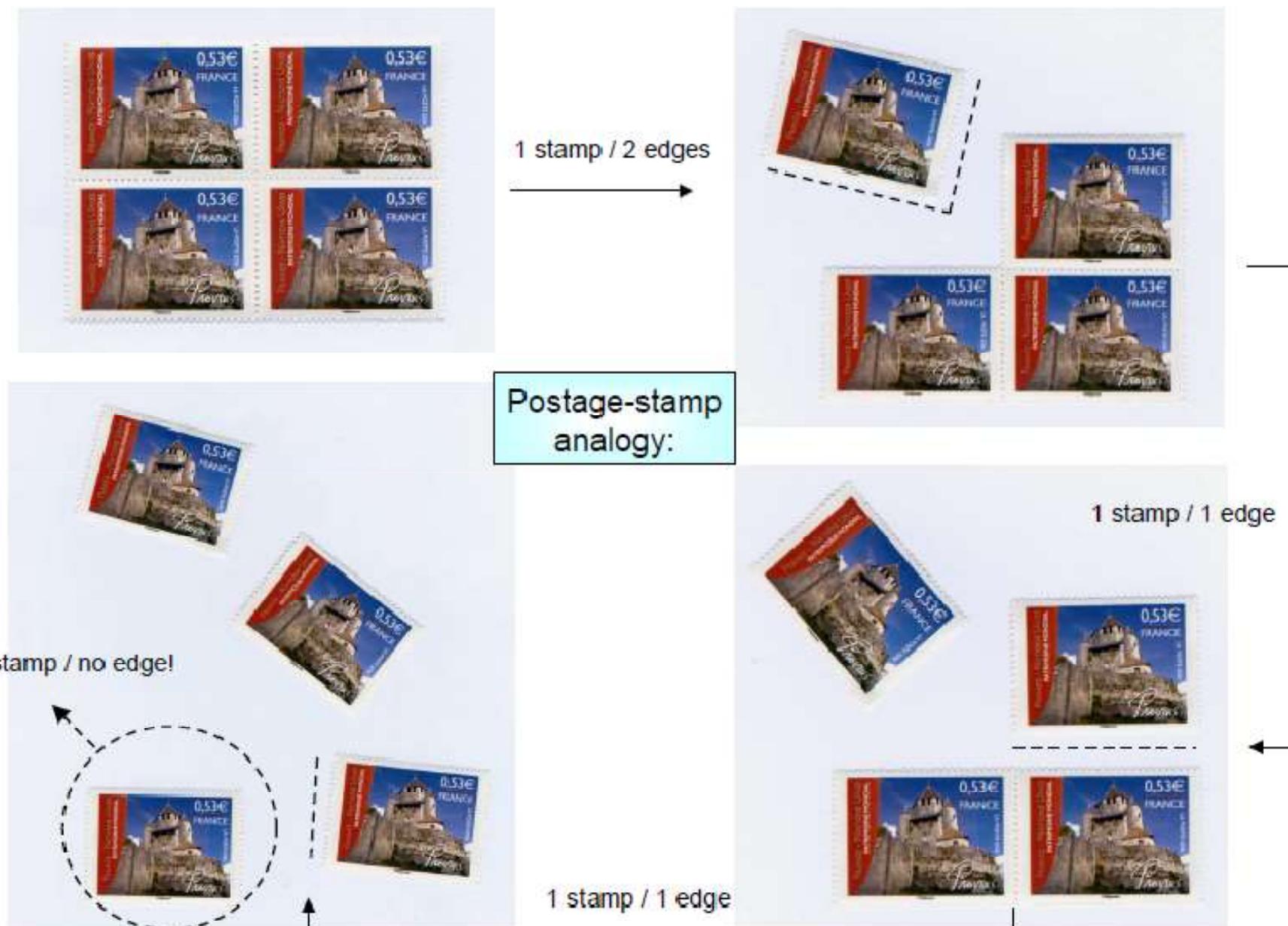


Oxygen Binding: Myoglobin vs. Hemoglobin

At lower concentrations of oxygen (as in the capillary), myoglobin has higher affinity for oxygen than does hemoglobin:



Hemoglobin – Oxygen Binding “Cooperativity”



Hemoglobin – Oxygen Binding “Cooperativity”

- When the first O_2 molecule binds to one of the four heme groups a number of structural changes occur:
 - The movement of the Fe atom into the heme plane also draws in the F8 [proximal] histidine, leveraging a big change in its subunit.
 - The alpha and beta groups rotate $\sim 15^\circ$ with respect to one another, disrupting non-covalent linkages between its neighboring subunits.
 - The open “channel” in the center of the subunits becomes much smaller, bringing the beta chains much closer than before
- These structural changes increase affinity for oxygen in the remaining three subunits.

Hemoglobin – Oxygen Transport

- Transport of oxygen by both myoglobin and hemoglobin can be modeled mathematically.
- This relatively simple algebraic formula is:

$$Y = \frac{pO_2^n}{pO_2^n + p50^n}$$

Where

Y = fraction of heme sites bound to oxygen,

pO_2 = partial pressure of oxygen,

$P50$ = parital pressure of oxygen at which 50% of sites are bound

N = cooperativity coefficient (*Hill coefficient*)

Hemoglobin – Oxygen Transport

- Oxygen Binding In the Pulmonary System:

Assume that $p50 = 35 \text{ Torr}$ in the alveolar capillaries: $pO_2 = 100 \text{ Torr}$:

$$Y_{alv} = \frac{100^3}{100^3 + 35^3} = \underline{\underline{0.985}}$$

- Oxygen Binding in the Peripheral Tissues:

Assume that $p50 = 35 \text{ Torr}$ in the capillaries and $pO_2 = 20 \text{ Torr}$:

$$Y_{alv} = \frac{20^3}{20^3 + 35^3} = \underline{\underline{0.157}}$$

Difference: (“*Delta Y*”) = $0.985 - 0.157 = \underline{\underline{0.828}}$

This means that 82.8% of the hemoglobin sites transported oxygen to the tissues.

Myoglobin – Oxygen Transport

What if myoglobin were utilized to transport oxygen? (n=1)

- Oxygen Binding In the Pulmonary System:

Assume that $p_{50} = 35$ Torr in the alveolar capillaries: $pO_2 = 100$ Torr:

$$Y_{alv} = \frac{100^1}{100^1 + 35^1} = \underline{0.741}$$

- Oxygen Binding in the Peripheral Tissues:

Assume that $p_{50} = 35$ Torr in the capillaries and $pO_2 = 20$ Torr:

$$Y_{alv} = \frac{20^1}{20^1 + 35^1} = \underline{0.364}$$

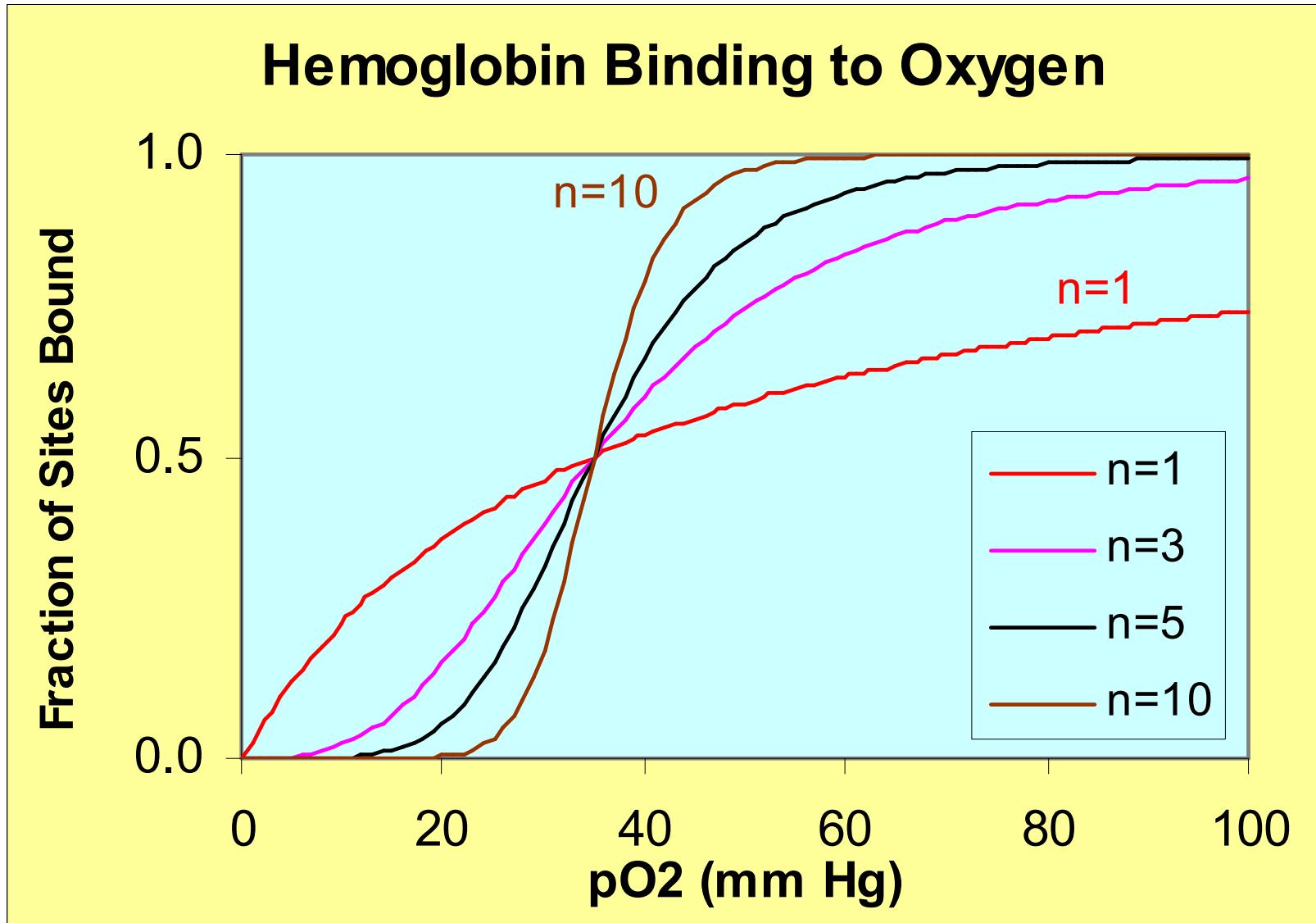
Difference: (“*Delta Y*”) = $0.741 - 0.364 = \underline{0.377}$

In this case only 37.7% of the myoglobin sites transported oxygen to the tissues!

Hemoglobin – Oxygen Binding “Cooperativity”

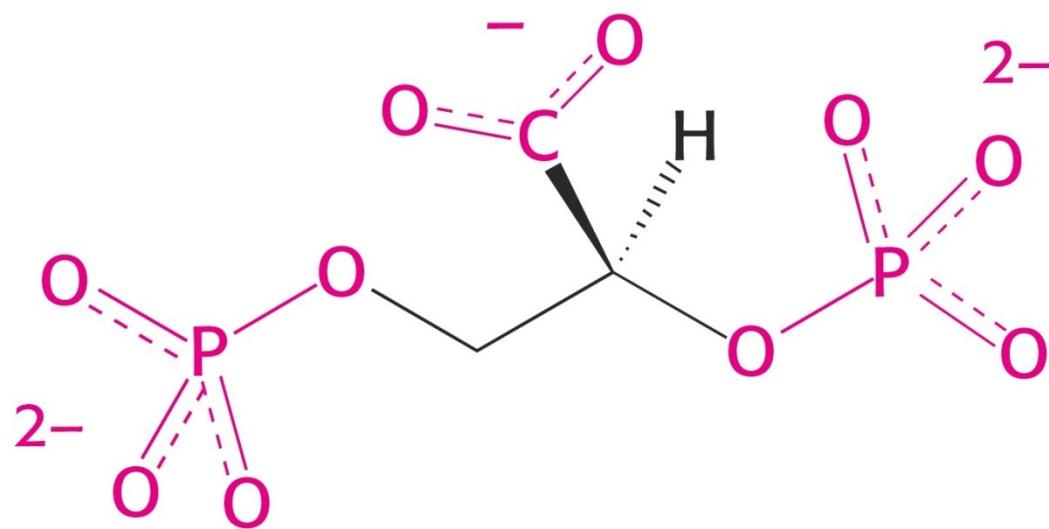
- The key to the dramatic difference in oxygen transport efficiency is hemoglobin’s **“cooperativity.”**
- The exponent in the prior equation is often called the **“Hill Cooperativity Coefficient.”**
- As “n” changes, so does the sigmoidal shape of the oxygen binding curve.

Hemoglobin – Oxygen Binding “Cooperativity”



Hemoglobin – Oxygen Binding “Cooperativity”

- A small, highly polar molecule, 2,3-bisphosphoglycerate is responsible for much of the cooperativity in Hb.

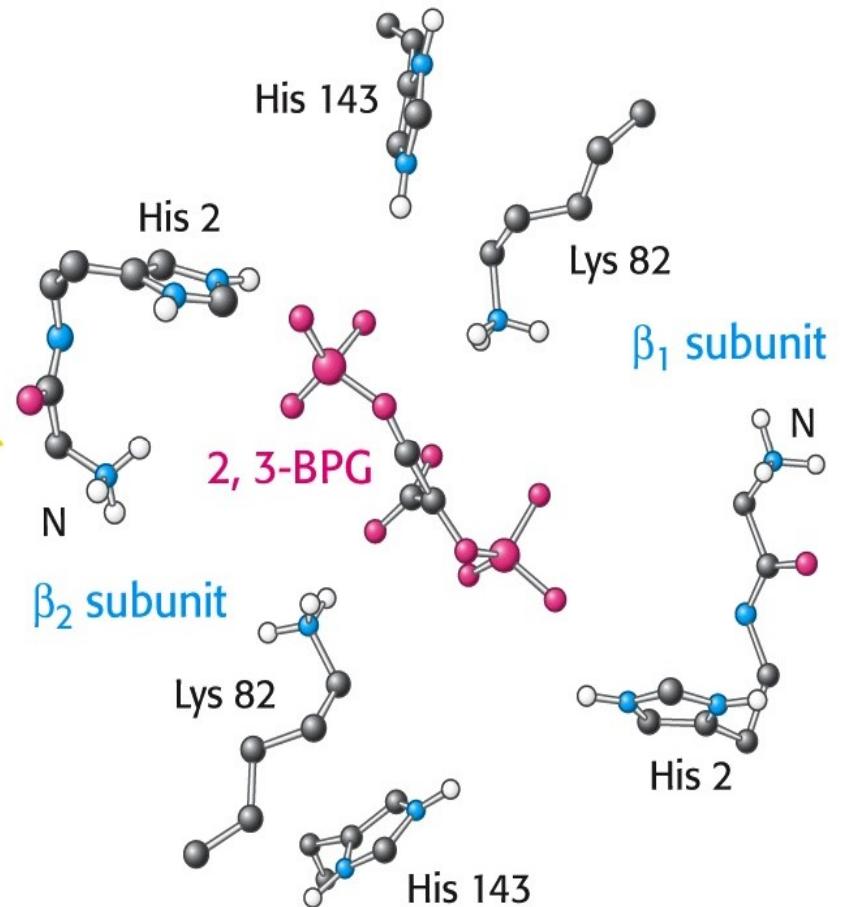
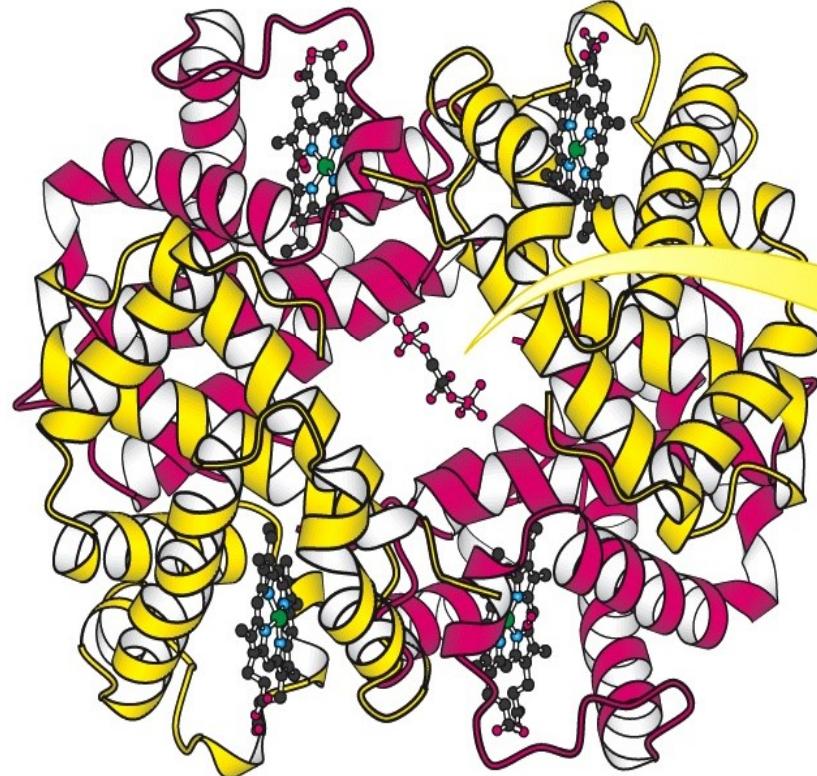


**2,3-Bisphosphoglycerate
(2,3-BPG)**

Hemoglobin – Oxygen Binding “Cooperativity”

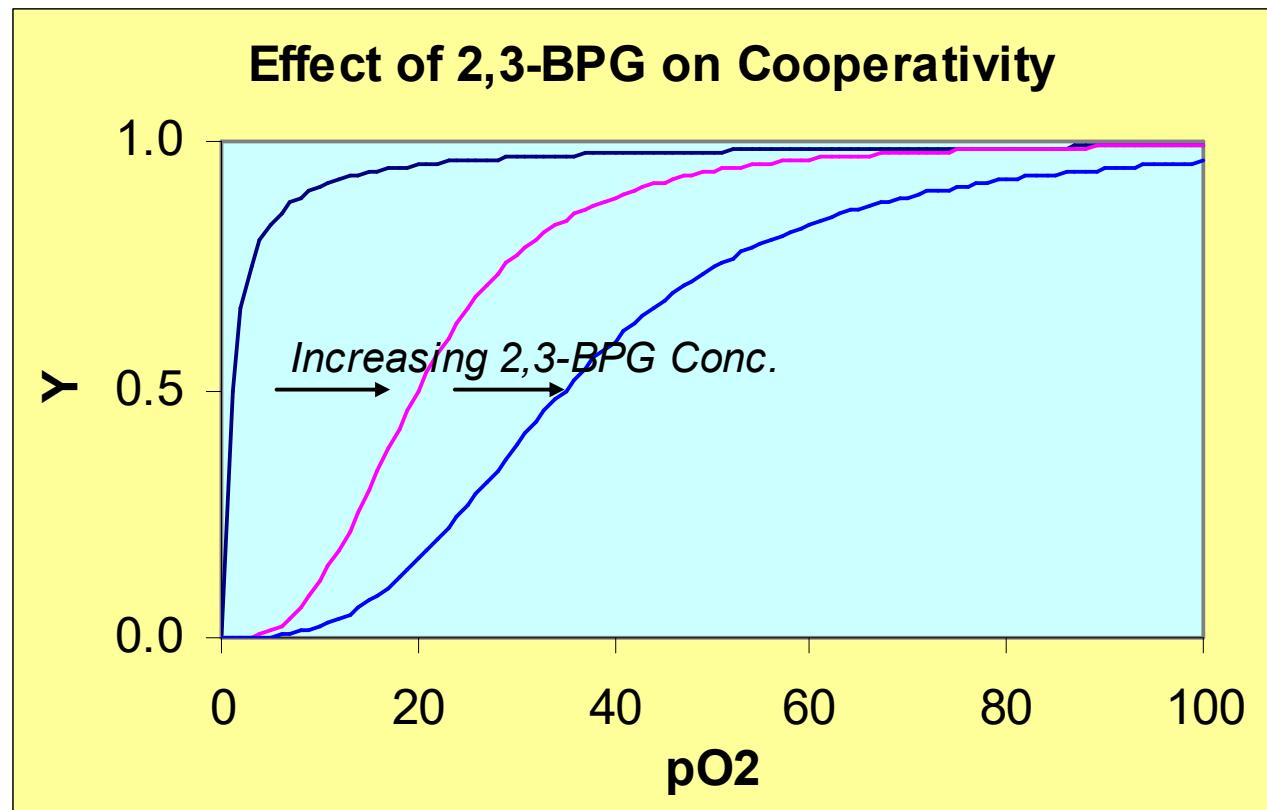
- 2,3-BPG binds inside the cavity between the four subunits of Hb.
- 2,3-BPG binds only to deoxygenated Hb, when there is room in the cavity.
- When one or more O_2 molecules are bound, 2,3-BPG can not fit into the smaller cavity.
- Therefore, the binding of O_2 and 2,3-BPG is mutually exclusive (only one or the other). Both can not bind at the same time.

Hemoglobin – Oxygen Binding “Cooperativity”



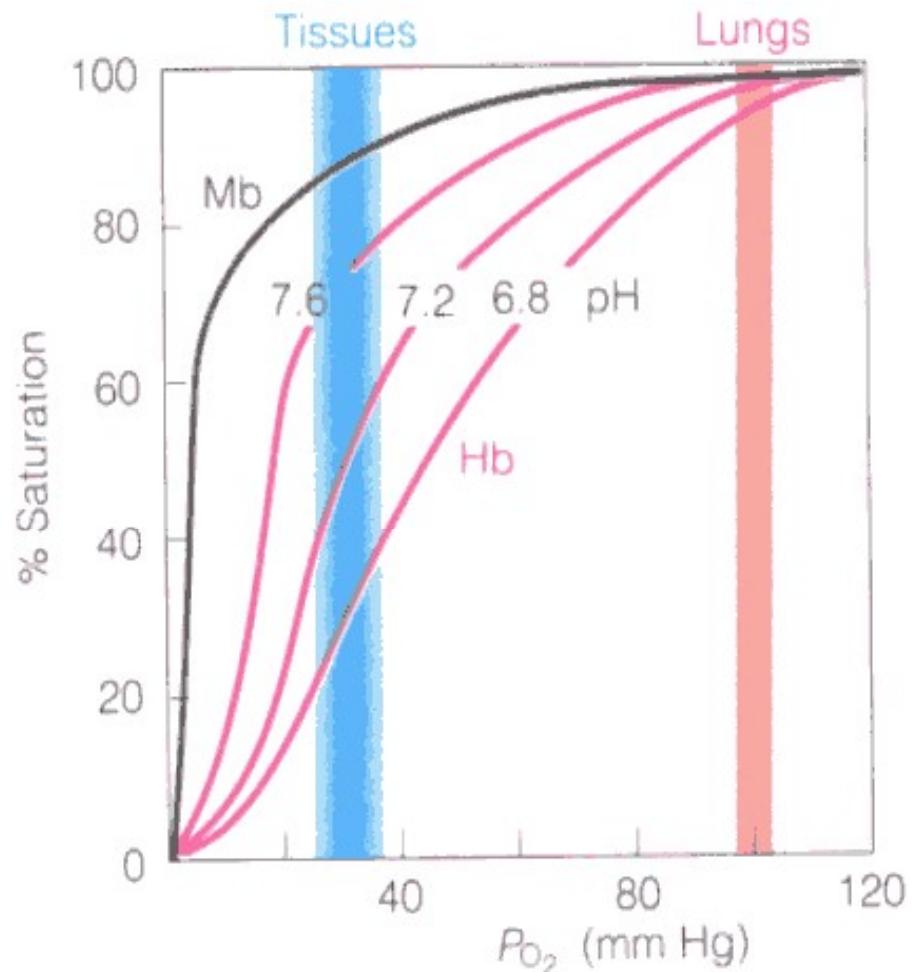
Hemoglobin – Oxygen Binding “Cooperativity”

- Increasing the concentration of 2,3-BPG shifts the plot of oxygen binding to Hb. This increases the dissociation of O₂ in the peripheral tissues.



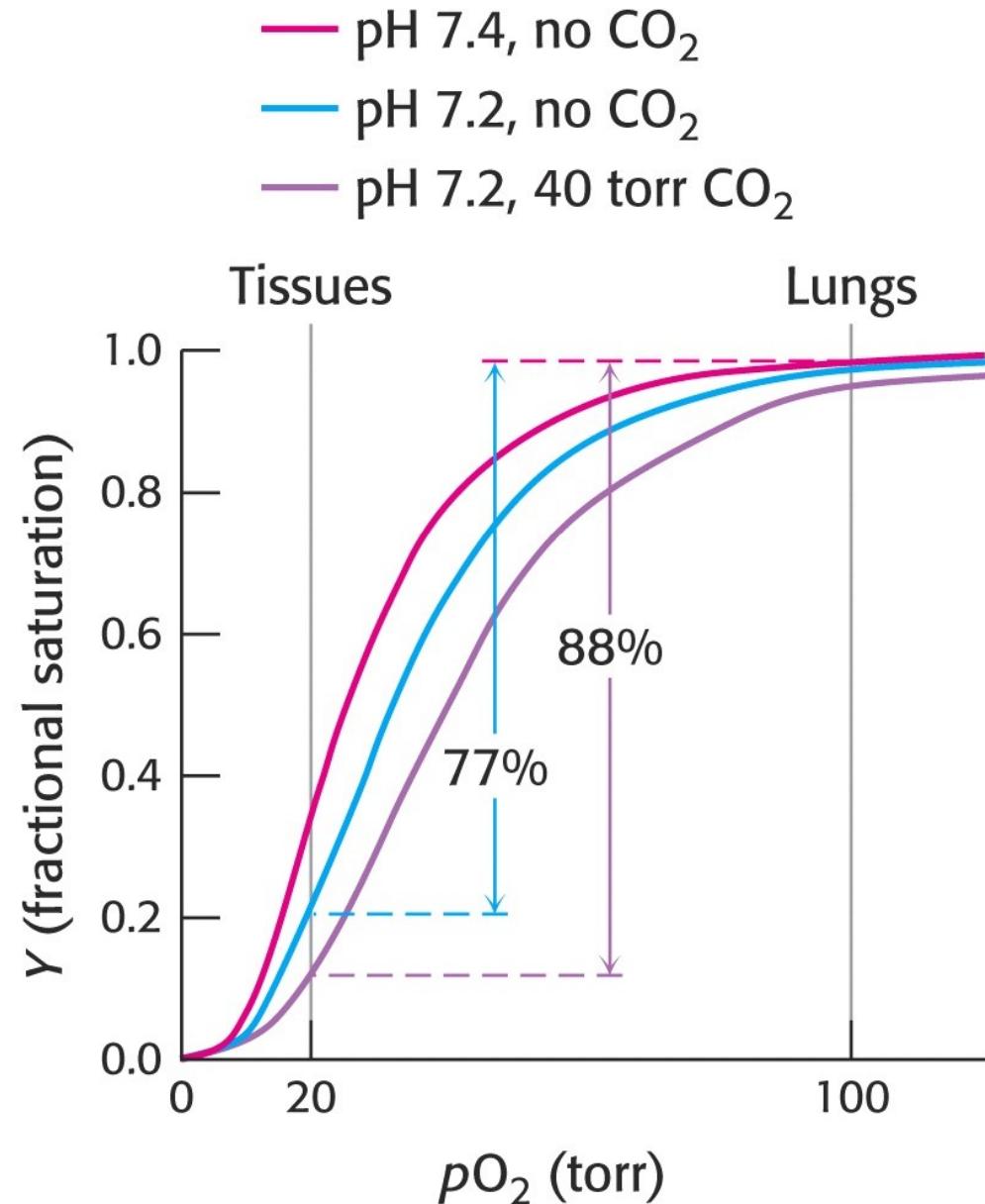
Hemoglobin – Oxygen Binding “Cooperativity”

- pH of the blood also affects oxygen affinity for Hb.
- Lower pH decreases oxygen affinity.
- This automatically releases oxygen in peripheral tissues where active respiration has produced increased levels of carbon dioxide, resulting in lower pH caused by carbonic acid: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3$
- This phenomenon is often called the “Bohr Effect.”



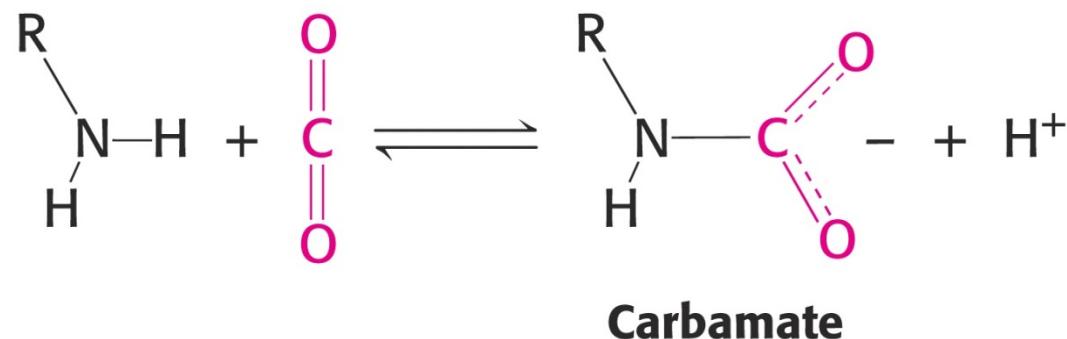
Hemoglobin – Oxygen Binding “Cooperativity”

- The result of the Bohr Effect is to deliver more total oxygen between the lungs and the peripheral tissues at lower pH:



Hemoglobin – Oxygen Binding “Cooperativity”

- In addition to its role in oxygen transport, hemoglobin also transports CO₂ from the tissues back to the lungs for disposal.
- CO₂ is not transported by the heme group. Rather, it binds to the terminal amino groups by forming “carbamates.”
- In the alveolae, the equilibrium shifts and the carbamates revert to free amines, releasing CO₂.



Hemoglobin – Sickle Cell Anemia

- A slight change in amino acid composition causes “*Sickle Cell Anemia*.”
- This genetic disease drastically impedes oxygen transport and is manifested as distorted “*sickled*” erythrocyte shapes:
- In Hemoglobin “S,” valine is substituted for glutamate at position #6 of the beta chains. This $\beta 6$ location is at the surface of the protein:



Oxygen Transport Proteins

In the biological world, 4 main systems:

Some Properties of Oxygen Transport Proteins

O ₂ carrier:	Myoglobin	Hemoglobin	Hemerythrin	Hemocyanin
Source:	Higher animals, some invertebrates	Higher animals, some invertebrates	invertebrates	Arthropods, mollusks
Metal:	Fe	Fe	Fe	Cu
Metal:bound O ₂ stoichiometry (ligands):	Fe:O ₂ (heme, histidine)	Fe:O ₂ (heme, histidine)	2 Fe:O ₂ (nonheme, protein side chains)	2 Cu:O ₂ (nonheme, protein side chains)
Metal ox state in deoxy form/ <i>d</i> electrons (color):	II/ <i>d</i> ⁶ (red-purple, violet)	II/ <i>d</i> ⁶ (red-purple, violet)	III/ <i>d</i> ⁵ (colorless)	IV/ <i>d</i> ¹⁰ (colorless)
Metal ox state in oxy form/ <i>d</i> electrons (color):	II/ <i>d</i> ⁶ –O ₂ or III/ <i>d</i> ⁵ –O ₂ [–] (red)	II/ <i>d</i> ⁶ –O ₂ or III/ <i>d</i> ⁵ –O ₂ [–] (red)	III/ <i>d</i> ⁵ (burgundy)	II/ <i>d</i> ⁹ (blue)
Approximate molecular weight (kDa):	17	65	108	400 to 2 × 10 ⁴
Number of subunits:	1	4 (some species have up to 10)	8	Many

Hemocyanin

Function:

Oxygen transporter of several groups of invertebrates
(*e.g. octopus, lobster, snails, spiders, etc.*)

Structure: complex protein → M ~ 450 000 - 9 000 000
It consists of 10 - 100 subunits. M ~ 50-70 kDa/subunit

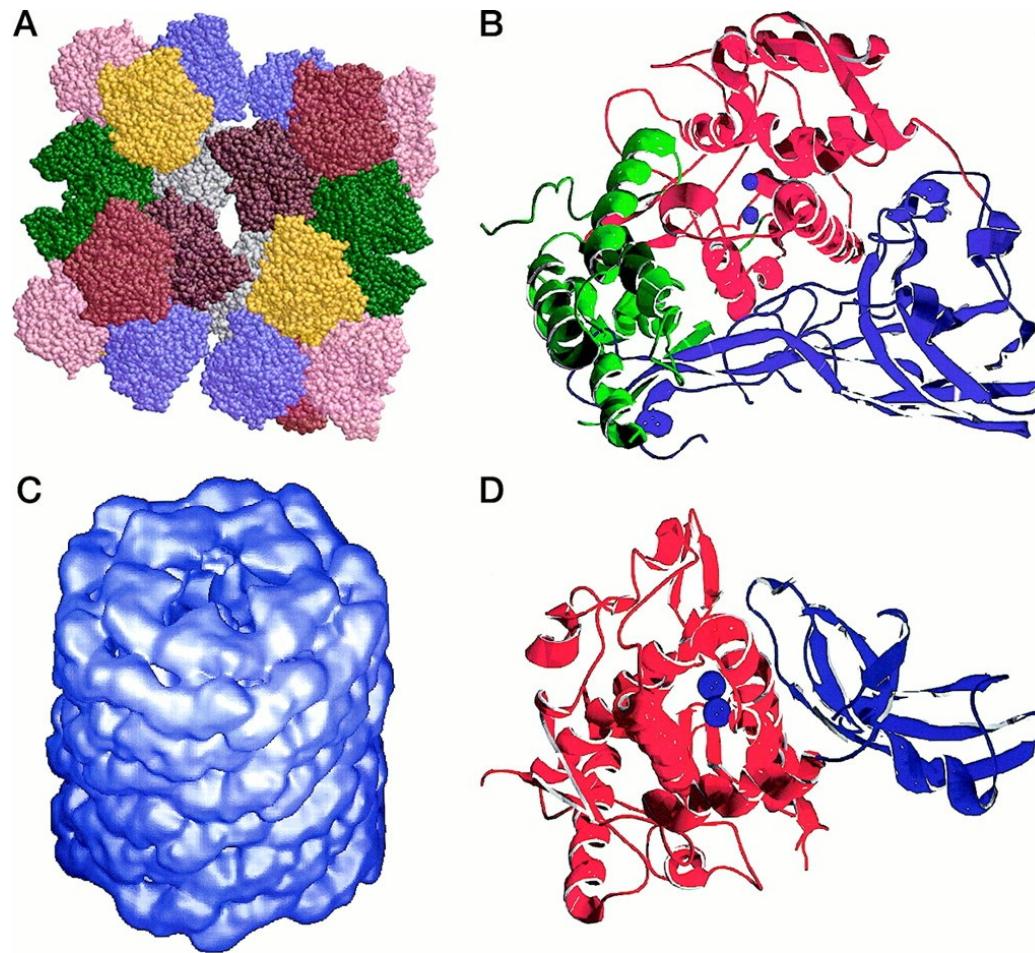
Copper content: 2 copper/subunit

Mechanism of Oxygen binding:

[Cu(I)]₂ + O₂ ⇌
deoxy: Cu(I),
Colourless, diamagnetic

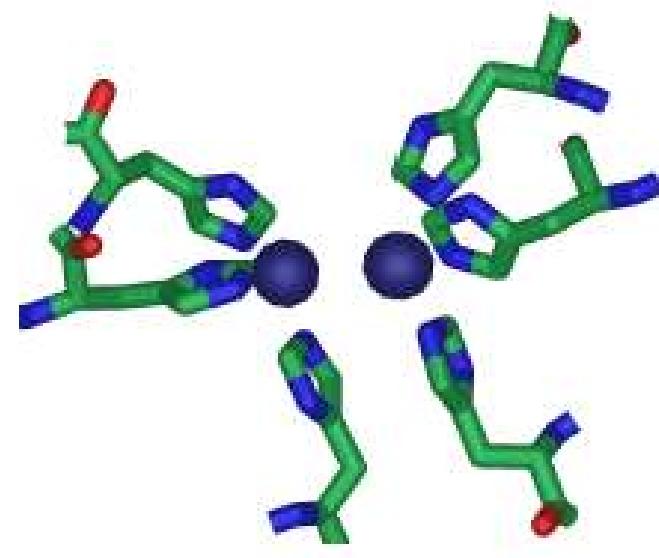
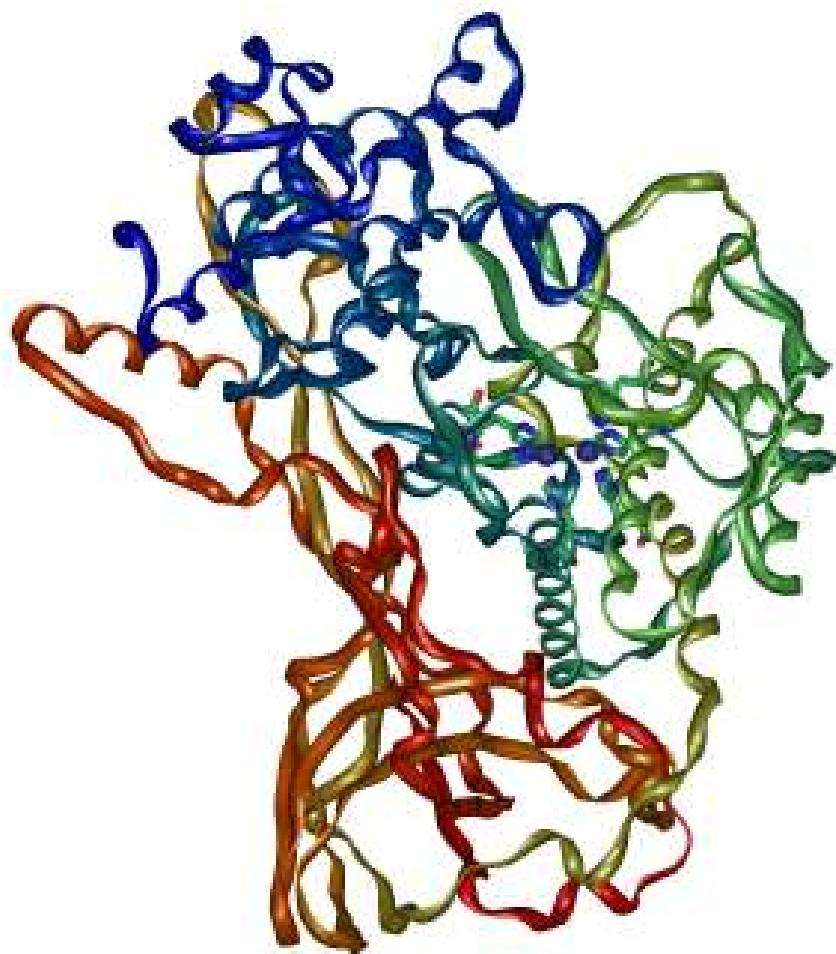
[Cu(II)-O₂²⁻-Cu(II)]
oxy: Cu(II)-peroxo
blue/green, diamagnetic

Hemocyanin structures



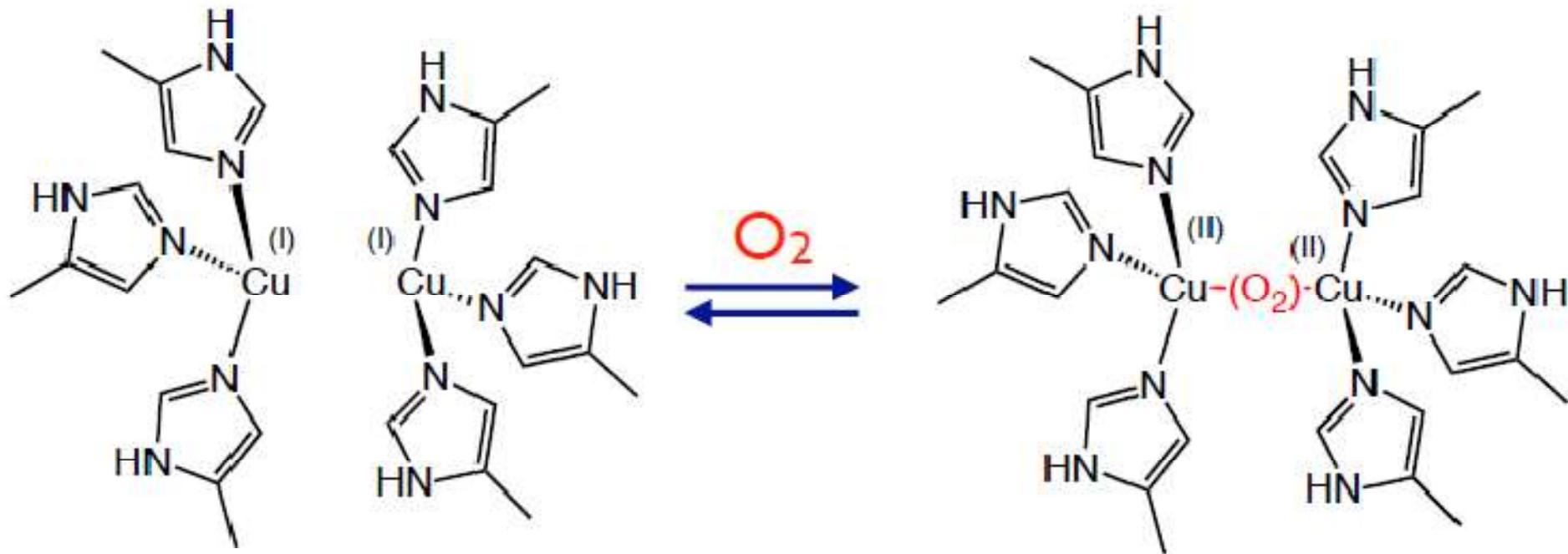
A. 24mer from *Eurypelma* (a tarantula) B. Single subunit from *Limulus* (horseshoe crab) C. 20 x 8mer from *Haliotis* (Abalone) (each individual polypeptide is an 8-fold repeat) d. C-terminal subunit from *Octopus*.

Hc active site



$$d_{\text{Cu-Cu}} = 3.8 \text{ \AA}$$

Oxy form of Hemocyanin (oxy-Hc)

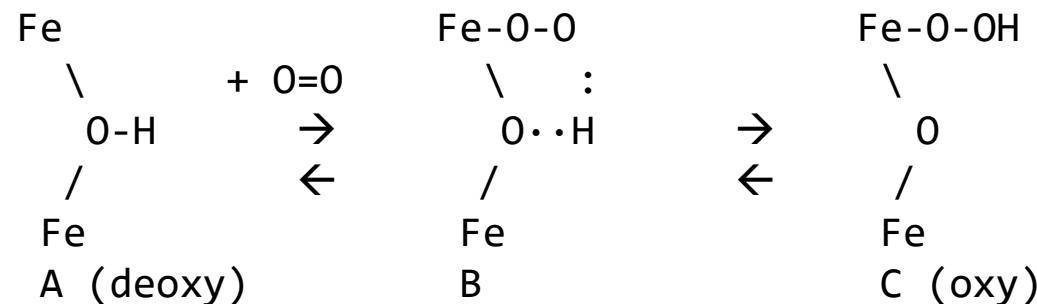


X-ray: $d_{Cu-Cu} = 3.8 \text{ \AA}$
EPR: Silent

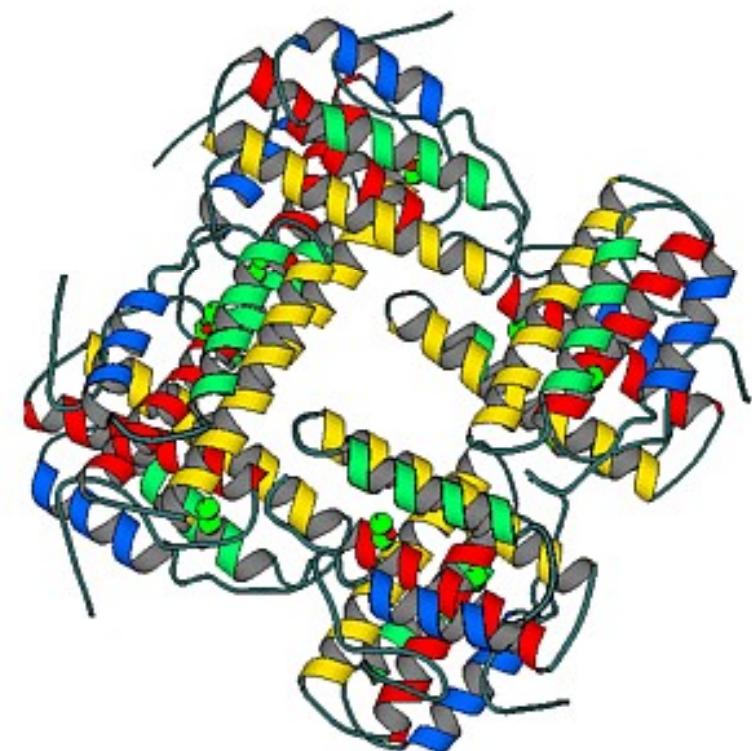
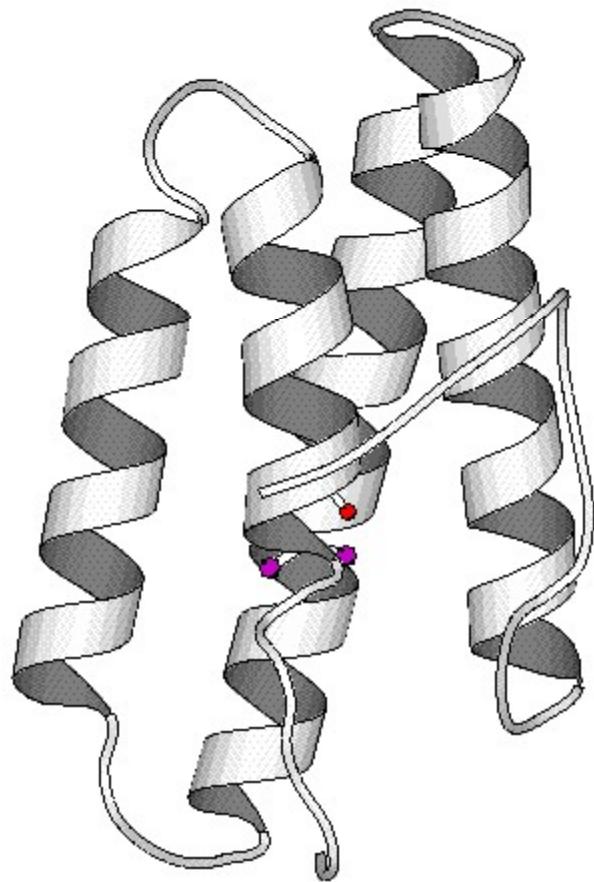
EXAFS: $d_{Cu-Cu} = 3.67 \text{ \AA}$
Raman: $\nu(O-O) = 749 \text{ cm}^{-1}$
UV-vis.: 345 nm ($\varepsilon = 20\,000 \text{ M}^{-1} \text{ cm}^{-1}$)
550 nm ($\varepsilon = 1000 \text{ M}^{-1} \text{ cm}^{-1}$)
EPR: Silent

Hemerythrin

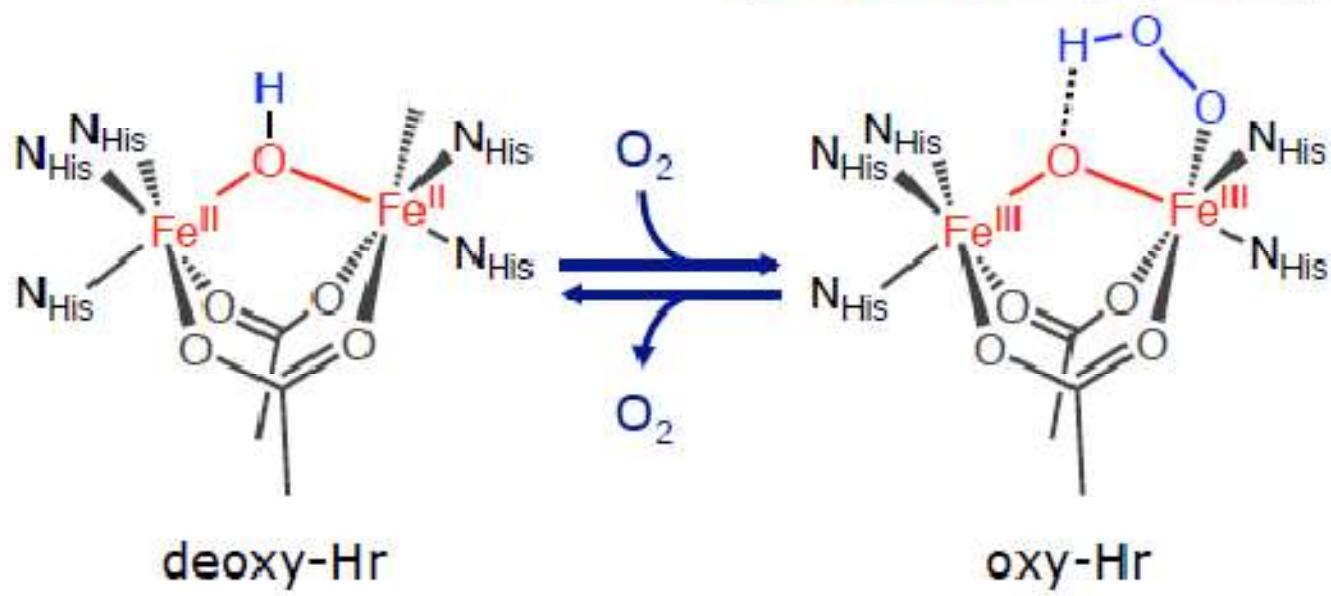
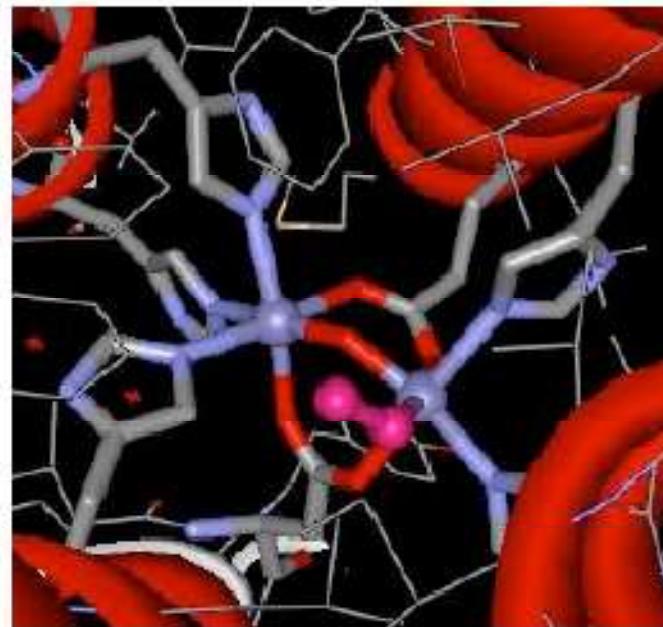
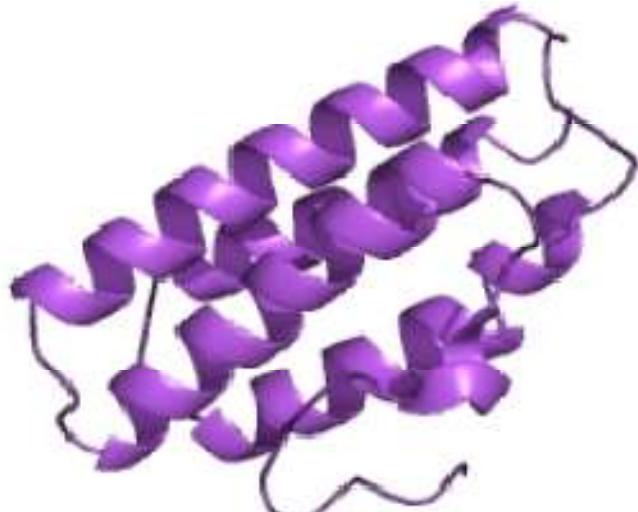
- Sipunculids, brachiopods, priapulids, bacteria
- Binuclear iron center
- $\text{Fe(II)} \rightarrow \text{Fe(III)}$
- 13-14 kDa monomers
 - Each monomer has 2 Fe, binds 1 O_2
- Form (most often) octamers
- Not cooperative



Hemerythrin

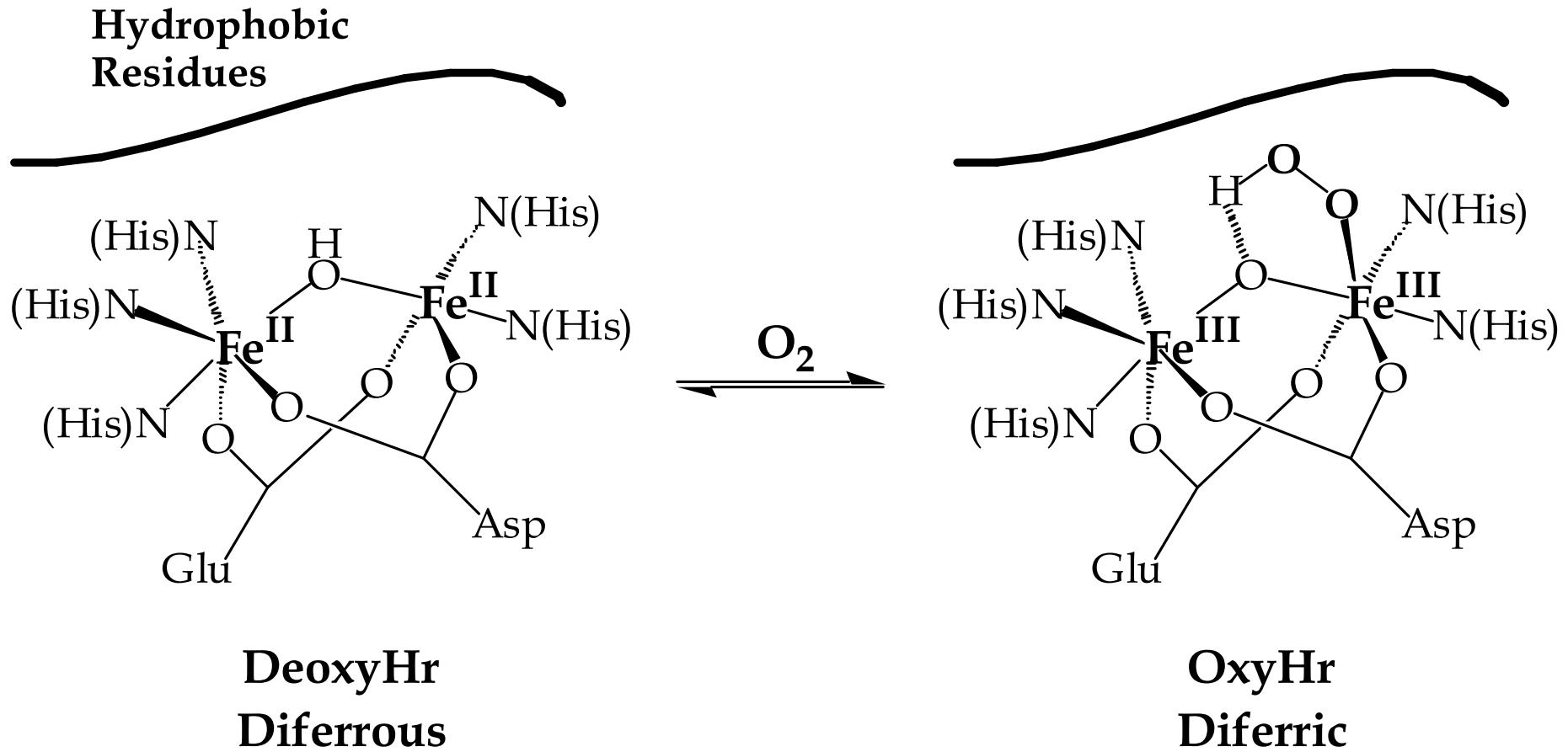


Hemerythrin (Hr)



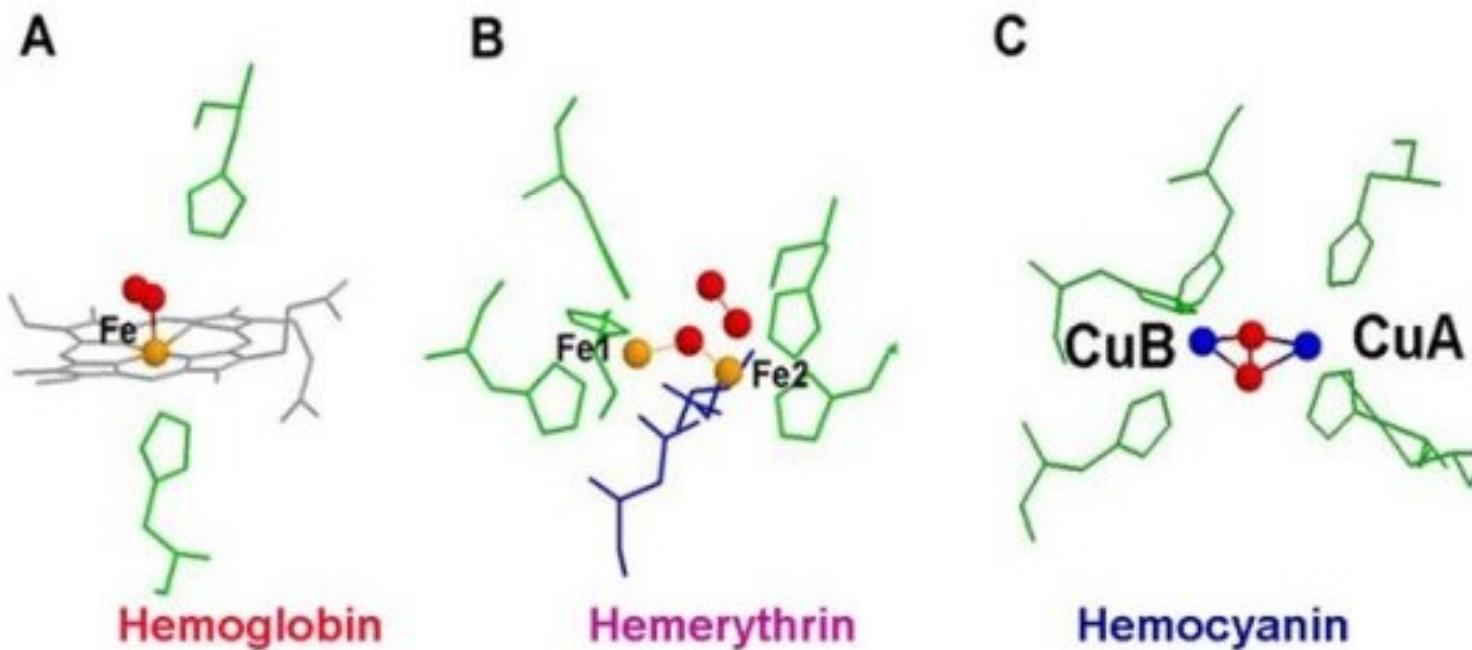
Back

Chemistry at the Active Site of Hemerythrin (Hr)



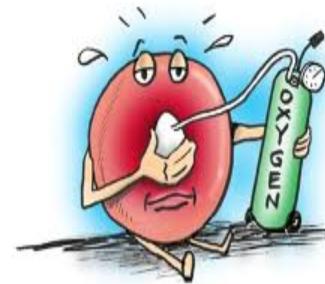
Note proton-coupled electron transfer
Evidence for proton transfer comes from resonance Raman work

O₂ Binding Sites



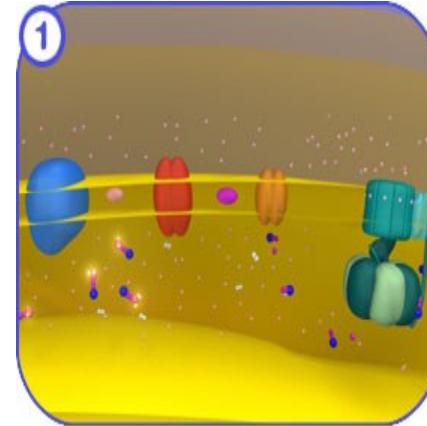
Why do we need oxygen or why do we breathe?

Oxygen is required for efficiently converting glucose to energy and generate ATP (Oxidative phosphorylation). In the presence of oxygen 18 times more energy is released from the oxidation of glucose



What happens to oxygen in our body and where does it happen?

Oxygen gets converted to water. It happens on the inner membrane of the mitochondrion exactly at the last stage of electron transport chain (**on cytochrome c oxidase**)



How exactly does oxygen change to water ?

Protons present inside the mitochondrion along with the electrons of the correct potential (generated during oxidation of food and supplied through the electron transport chain) react with O₂ and convert it to water generating a proton gradient

What does this reaction result in?

The proton gradient generated during the electron transport chain and conversion of O₂ to water drives the **molecular machine** called ATP synthase which makes ATP from ADP and P_i (Inorganic phosphate). ATP is the universal currency of energy in living

Youtube: ATP synthase molecular machines