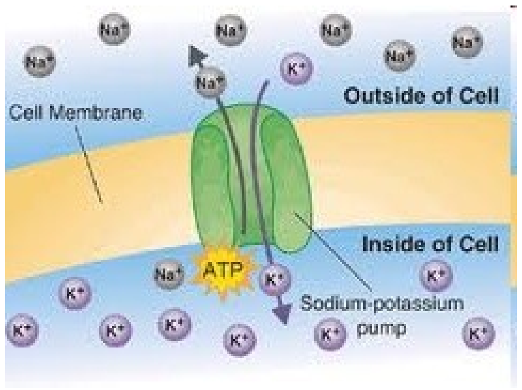
Bio Inorganic chemistry

Study of Inorganic elements in the living systems

11
Na
22.98

19
K
39.09



Sodium potassium pump

(1/5th of all the ATP used)

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

Important roles *metals* play in biochemistry

1. Regulatory Action — Sodium potassium channels and pump

   Na, K — *Nerve signals and impulses, action potential muscle contraction*

2. Structural Role — Calcium in bones, teeth

   Ca, Mg — *provide strength and rigidity*

3. *Electron transfer agents* — Cytochromes: redox intermediates

   Fe^{2+}/Fe^{3+} — *membrane-bound proteins that contain heme groups and carry out electron transport in Oxidative phosphorylation*

4. *Metalloenzymes* — Carbonic anhydrase, Carboxypeptidase

   Zn — *biocatalysts, CO_2 to HCO_3^-, protein digestion*

5. *Oxygen carriers and storage* — Hemoglobin, Myoglobin, Hemocyanin

   Fe, Cu — *18 times more energy from glucose in presence of O_2*

6. Metallo coenzymes — Vitamin B 12

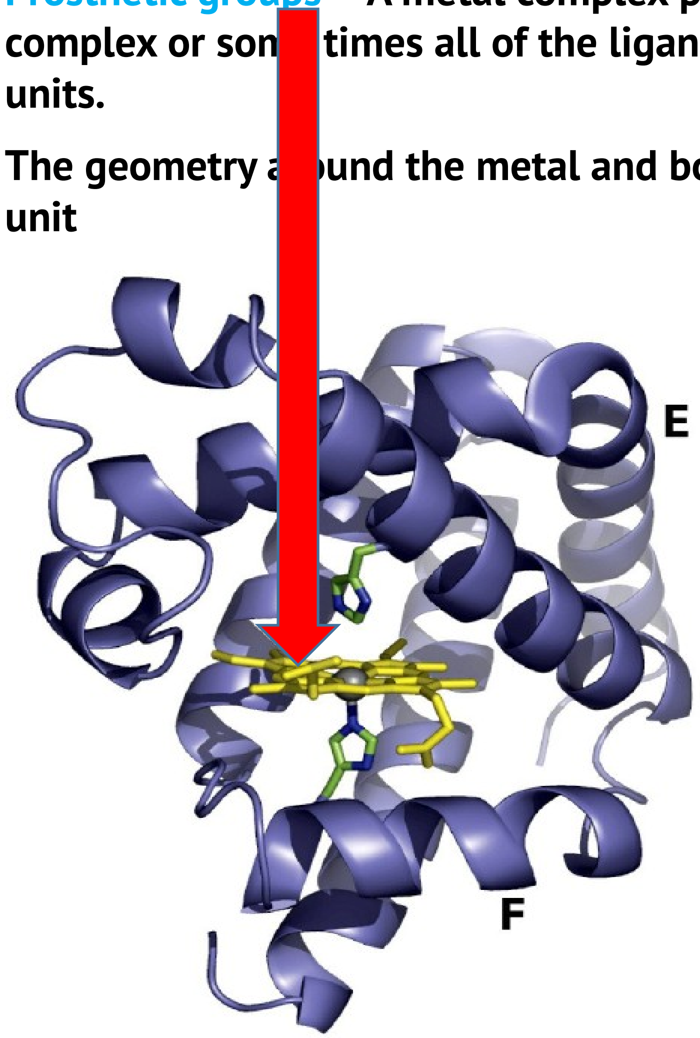
   Co — *biomethylation*

Structure of a metallo-protein : A metal complex perspective

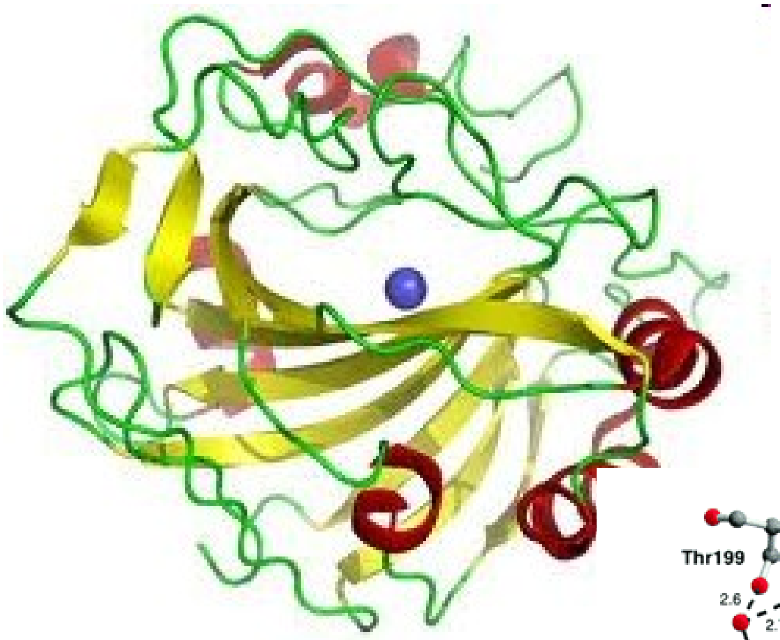
Spiral - α helix form of protein   Tape - β Pleated sheet form of protein

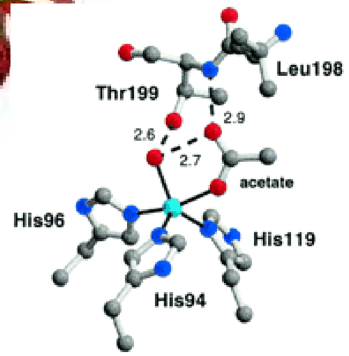
Prosthetic groups – A metal complex positioned in a crevice. Some of the ligands for this complex or sometimes all of the ligands are provided by the side groups of the amino acid units.

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



Myoglobin

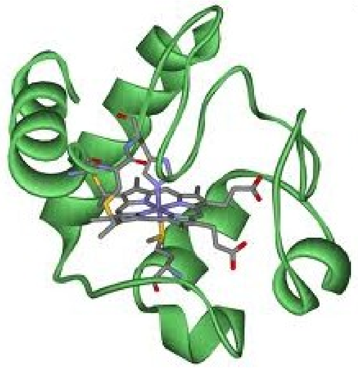




Carbonic anhydrase

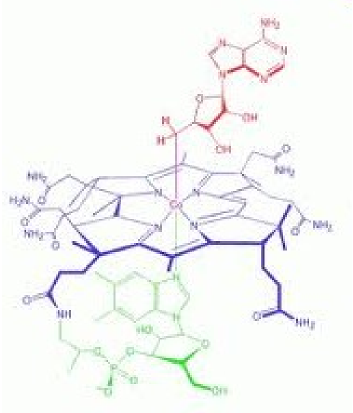
Metalloenzymes and Oxygen carriers = Protein + Cofactor

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.**

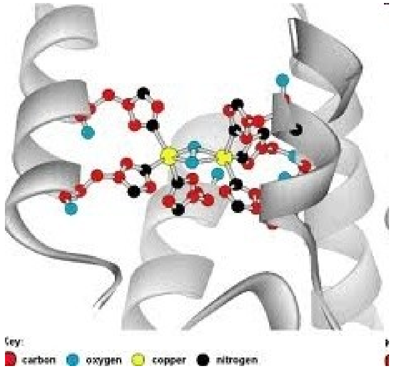


Cytochrome C

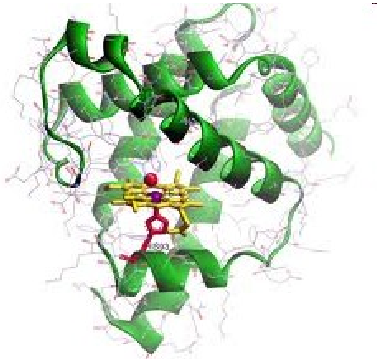
Porphyrins with different metals at its centre are a common prosthetic group in bioinorganic chemistry



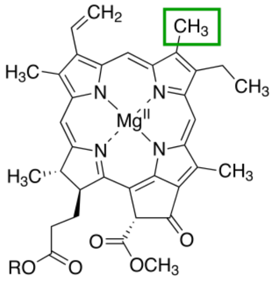
Coenzyme B12



Hemocyanin

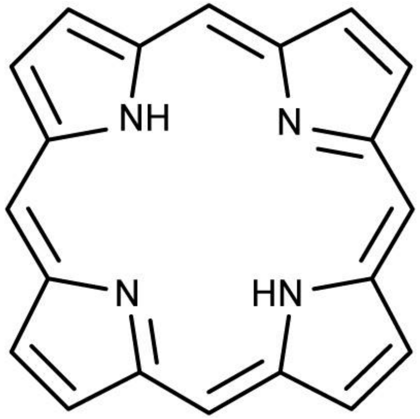


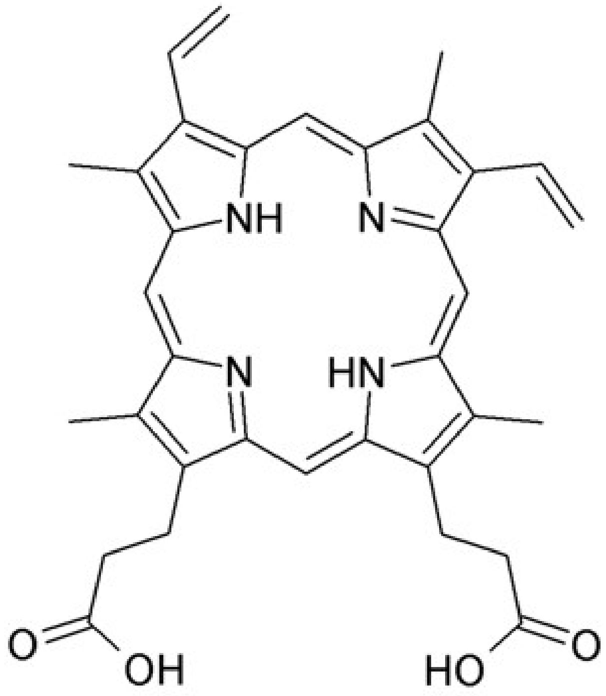
Myoglobin

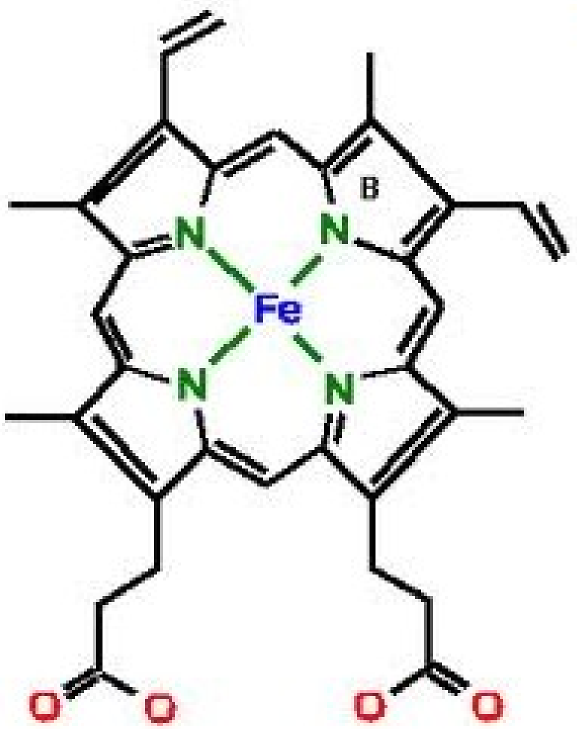


Chlorophyll

Protoporphyrin IX and Heme

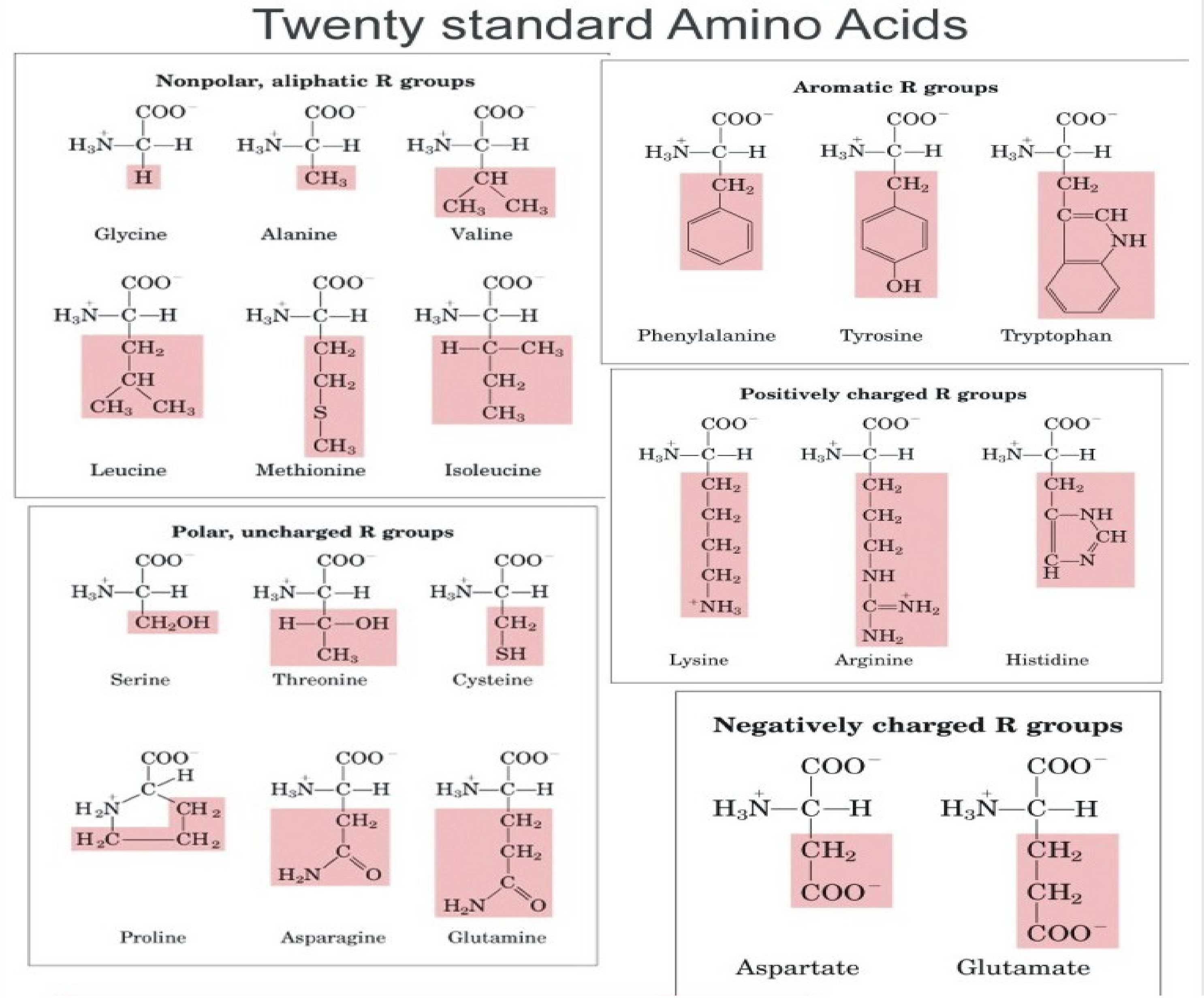




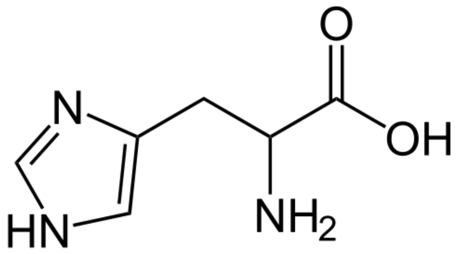


15 different ways to arrange the substituents around the porphyrin. Only one isomer protopophyrin IX is found in the living system. Porphyrins are planar and aromatic

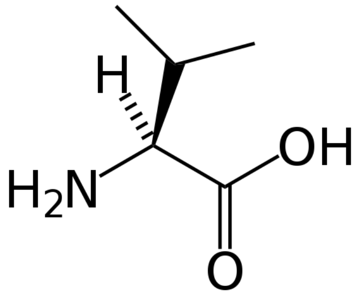
Proteins – consists of different amino acids in a specific sequence connected by the peptide bond –



A few important amino acids relevant to the present course

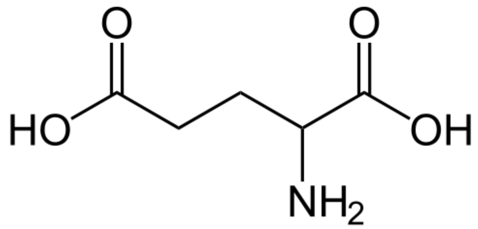


HISTDINE This amino acid has a pKa of 6.5. This means that, at physiologically relevant pH values, relatively small shifts in pH will change its average charge. Below a pH of 6, the imidazole ring is mostly protonated.

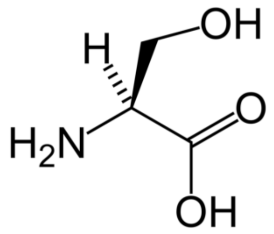


VALINE is a branched-chain amino acid having a hydrophobic isopropyl R group. In sickle-cell disease, valine substitutes for the hydrophilic amino acid glutamic acid in hemoglobin.Valine is

hydrophobic

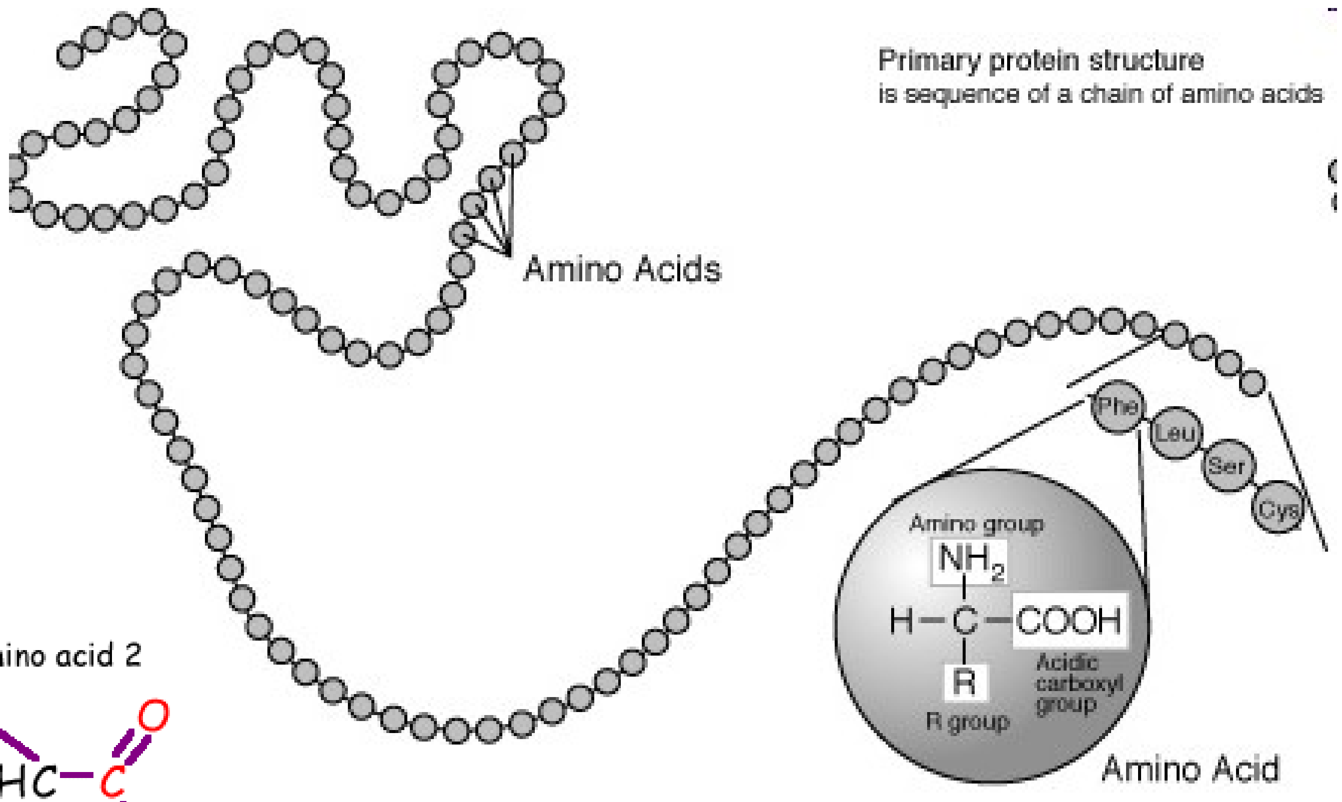


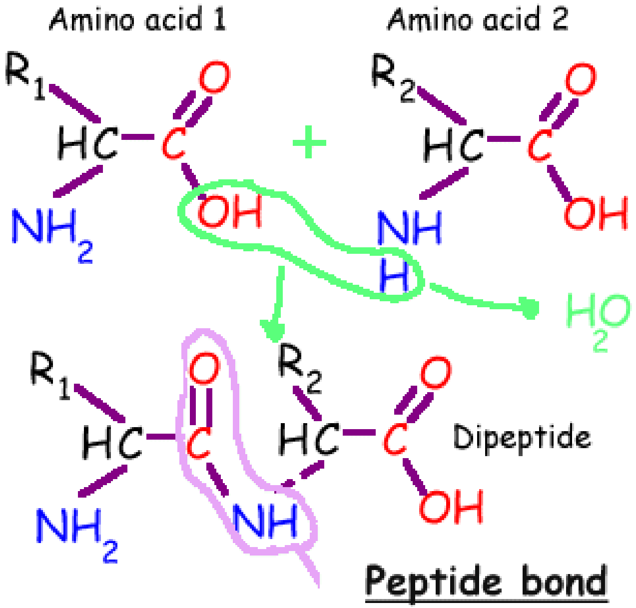
GLUTAMIC ACID has carboxylic acid functional group which is hydrophilic, has pKa of 4.1 and exists in its negatively charged deprotonated carboxylate form at physiological pH ranging from 7.35 to 7.45.



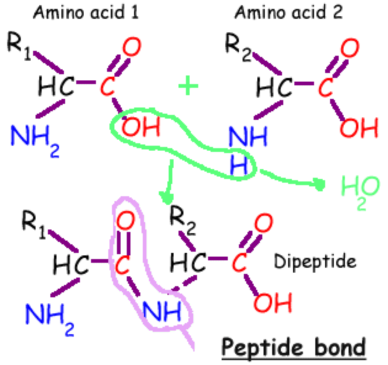
SERINE Serine is an amino acid having a CH_2OH side group. By virtue of the hydroxyl group, serine is classified as a polar amino acid. Serine was first obtained from silk protein, a particularly rich source, in 1865.

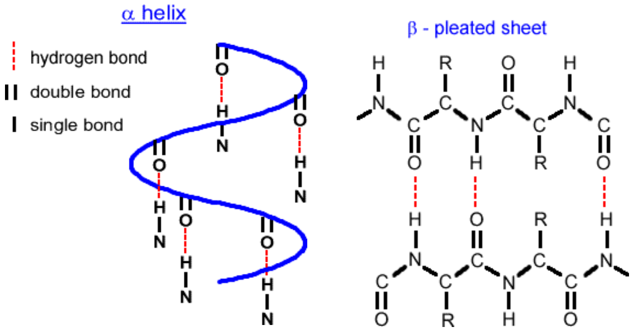
The primary structure of a protein

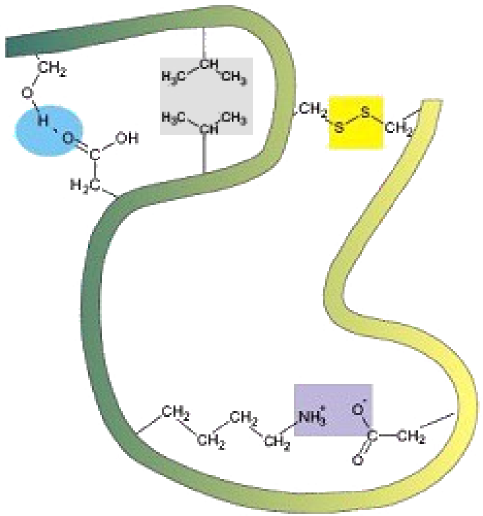


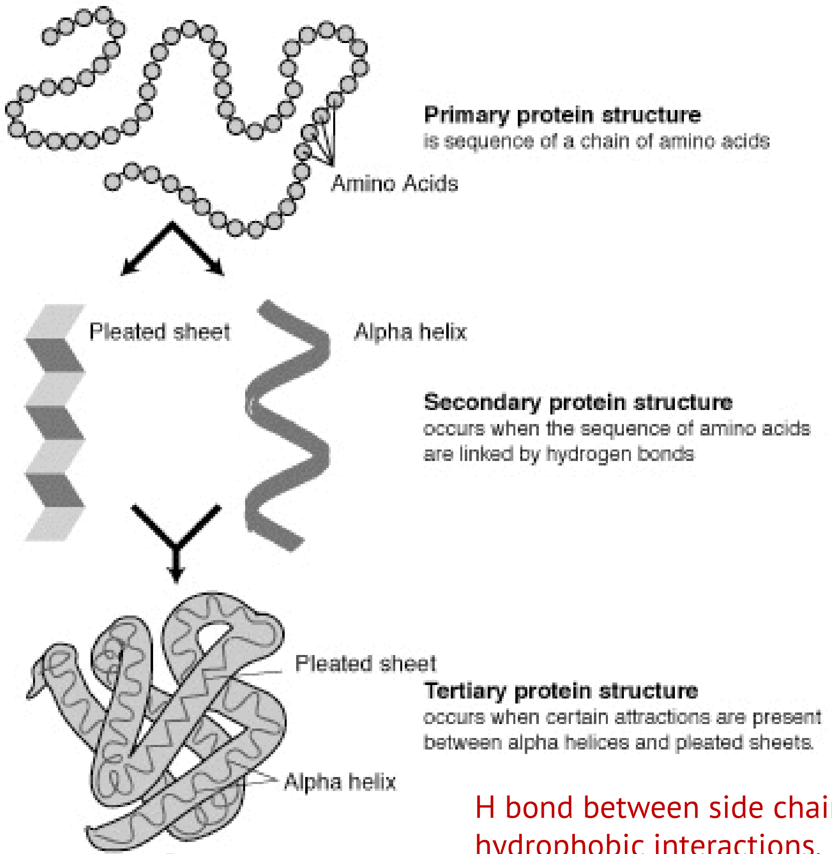


The four levels of protein structure

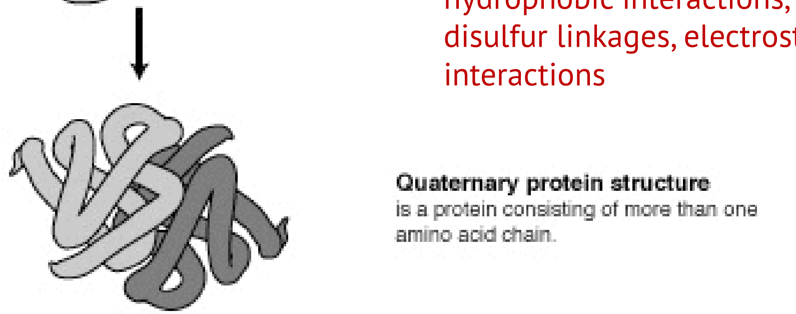






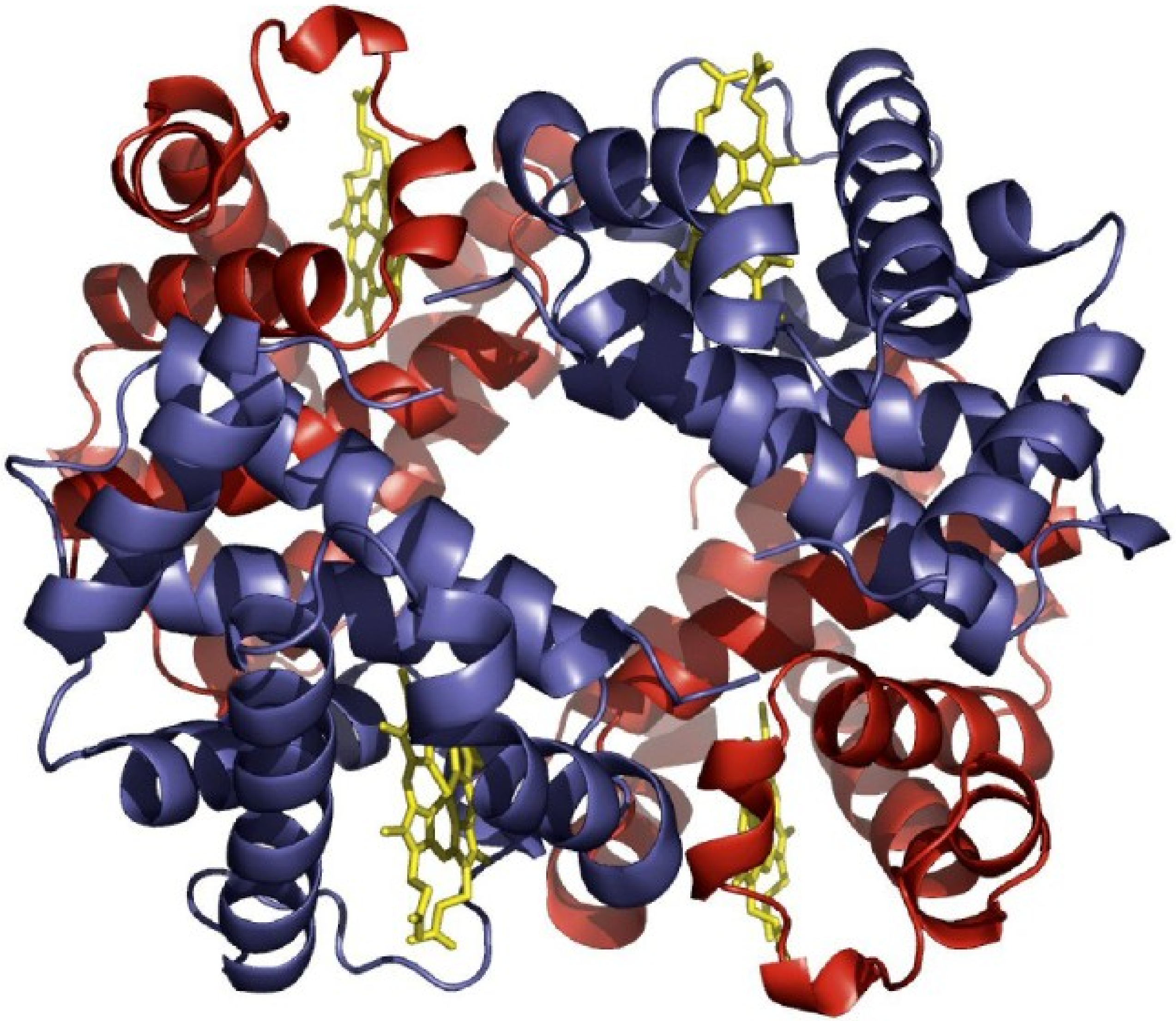


H bond between side chains,
hydrophobic interactions,
disulfur linkages, electrostatic
interactions



See youtube video “protein structure” Univ of Surrey’

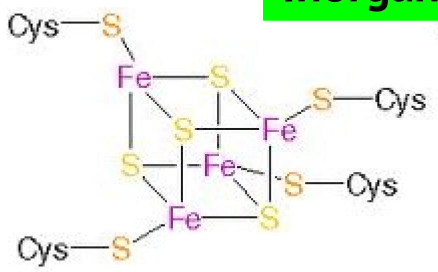
Hemoglobin- a quaternary structure of a protein

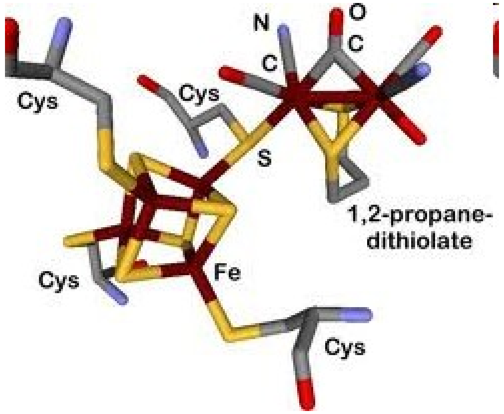


4 units

Each unit has a prosthetic group (heme) embedded in a crevice and partly coordinated by histidine units

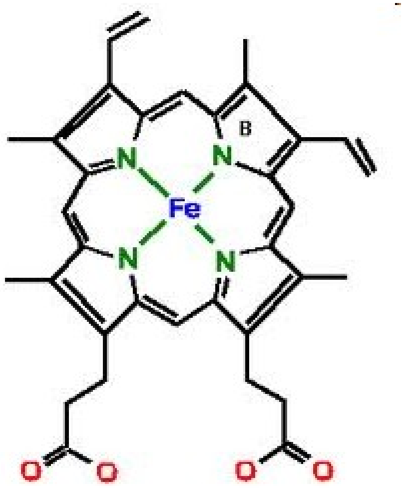
Inorganic Active site / Prosthetic group

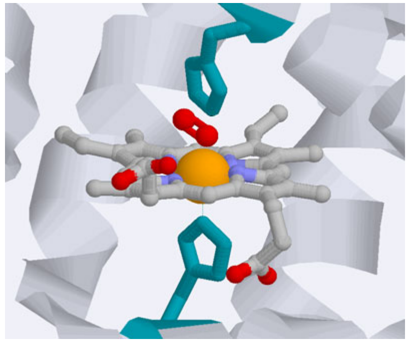




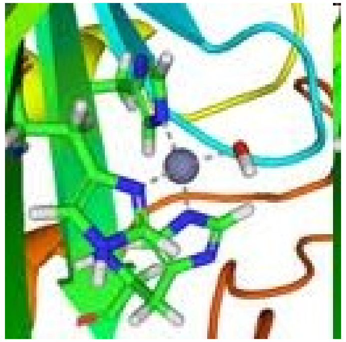
Ferredoxin (e transfer)

In molecular biology the **active site (prosthetic group)** is part of an enzyme where substrates bind and undergo a chemical reaction. It can perform its function **only when it is associated with the protein unit**

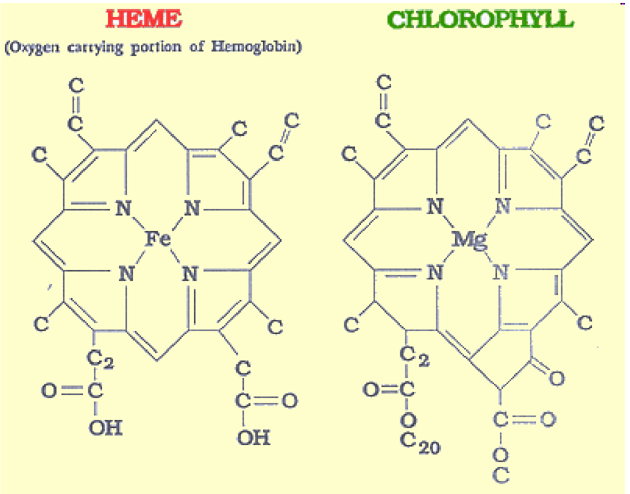


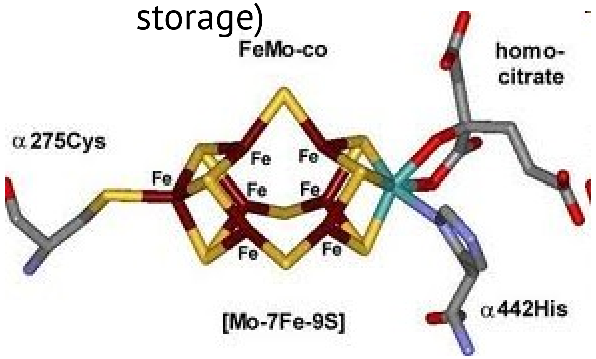


Heme in Myoglobin (O_2 storage)



Carbonic anhydrase Enzyme)



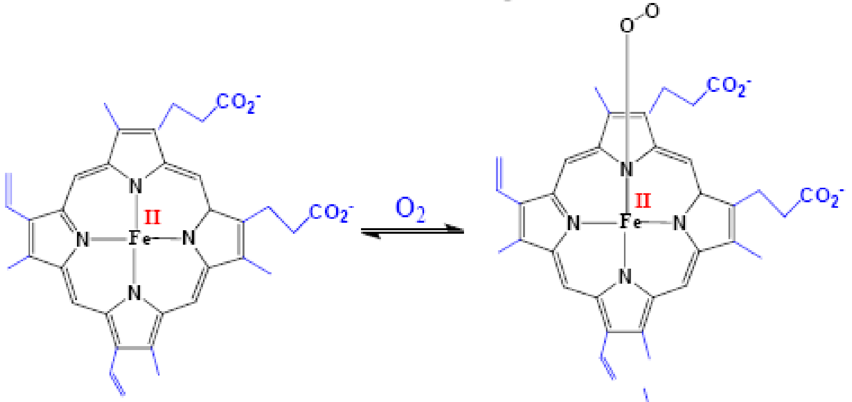


Nitrogen Fixation

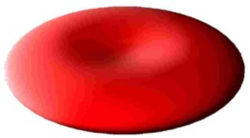
Inorganic Prosthetic group of three well known oxygen carriers

Active-Sites of Enzymes

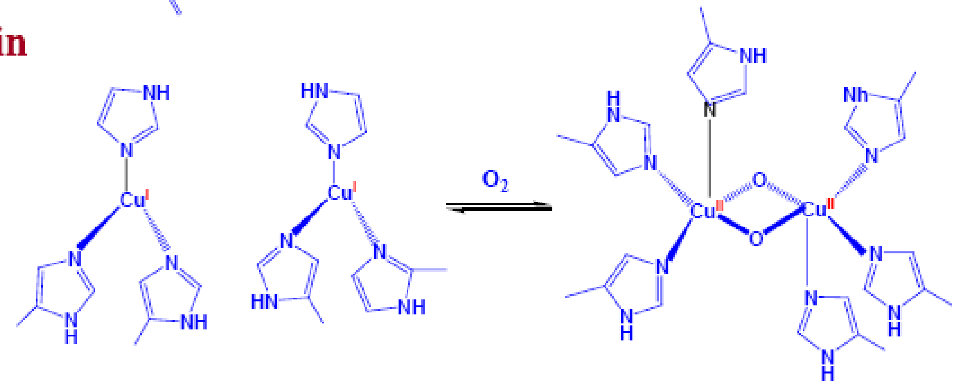
Hemoglobin



Present in Vertebrates

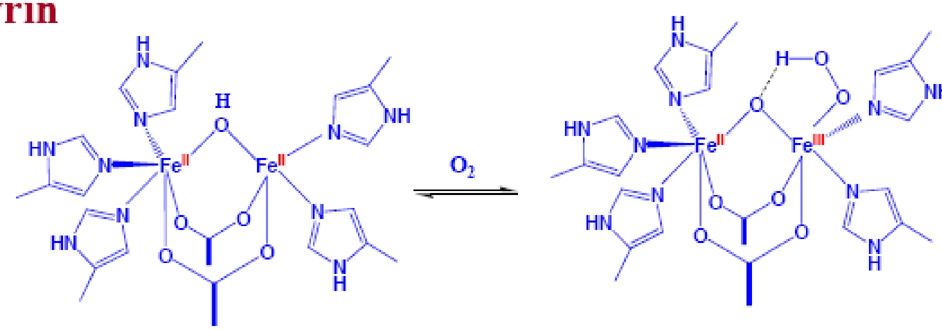


Hemocyanin



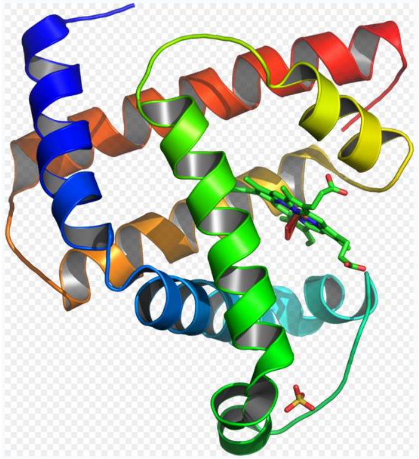
Present in molluscs

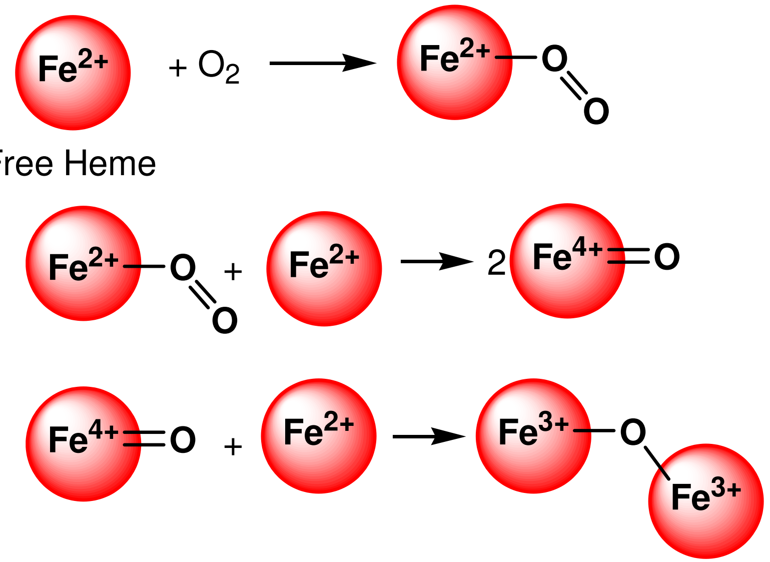
Hemerythyrin



Present in some sea worms

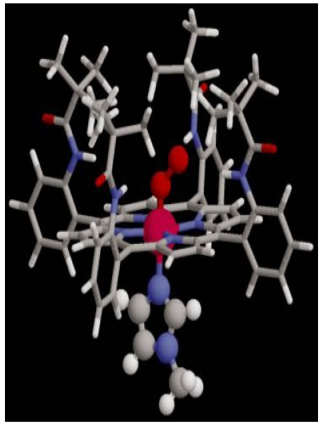
Can the prosthetic unit part of a metalloprotein perform its normal function without the protein unit around it ?





Reversible binding of O_2 is possible on when protein unit is present around the heme unit

picket fence porphyrin



Oxygen : A few Questions

Why do we need oxygen or why do we breathe?

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

What does this reaction produce and how?

How exactly is oxygen carried around and stored in the body?

How exactly is CO_2 removed from the body?

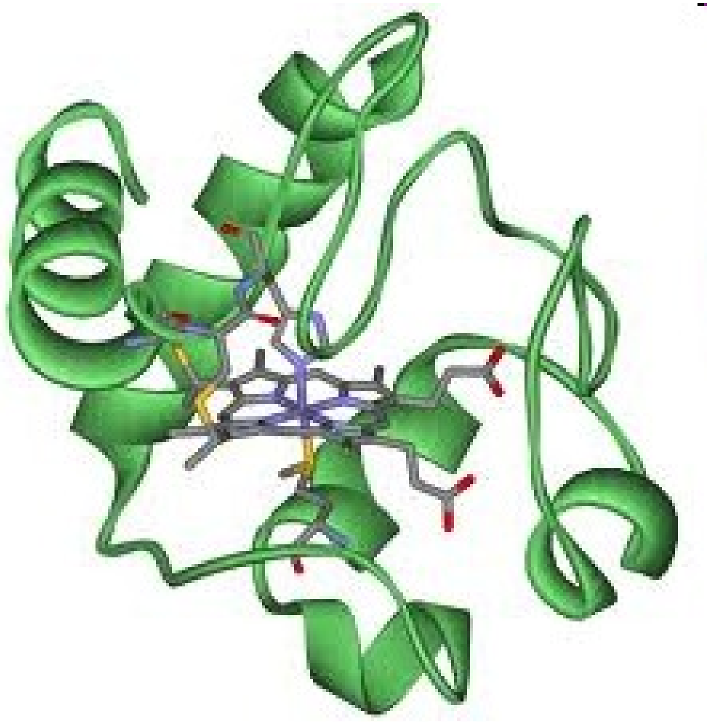
Electron transfer agents **Cytochromes: redox intermediates**

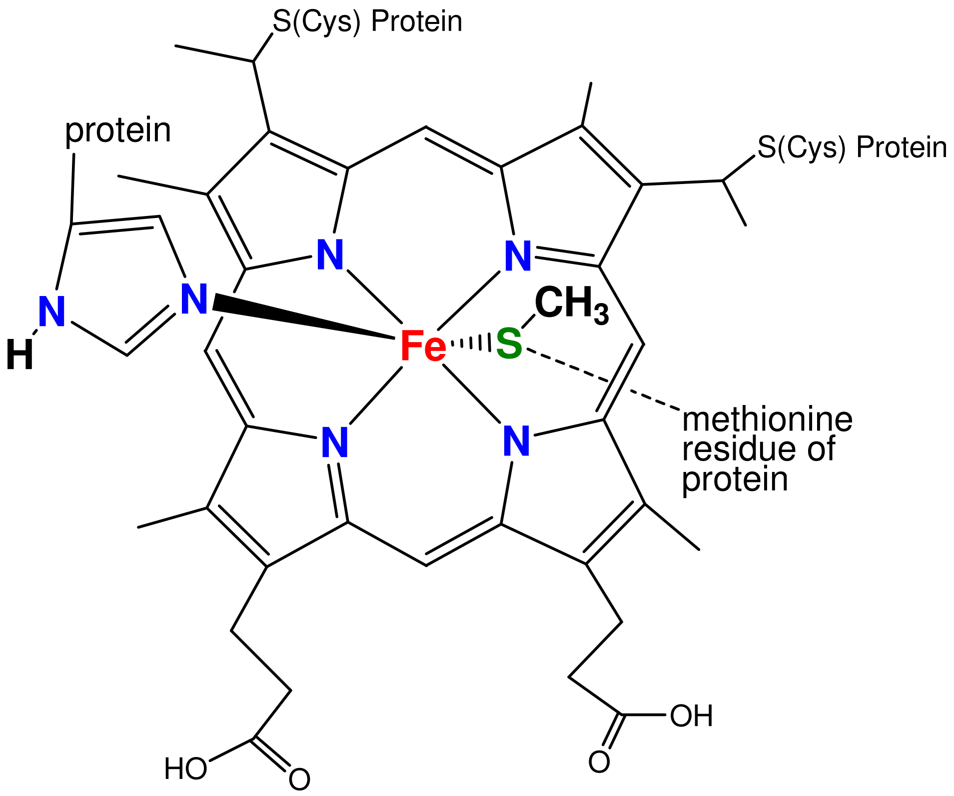
Fe^{2+}/Fe^{3+} ***membrane-bound proteins that contain heme groups and carry out electron transport in Oxidative phosphorylation***

Cytochromes are, in general, membrane-bound (i.e. inner mitochondrial membrane) heme proteins containing heme groups and are primarily responsible for the generation of ATP via electron transport.

They are found bound on the inner mitochondrial membrane either as monomeric proteins (e.g., cytochrome c) or as subunits of bigger enzymatic complexes that catalyze redox reactions. These heme proteins are classified on the basis of the position of their lowest energy absorption band in the reduced state, as cytochromes *a* (605 nm), *b* (~565 nm), and *c* (550 nm).

Electron transfer agents; e.g. Cytochrome C



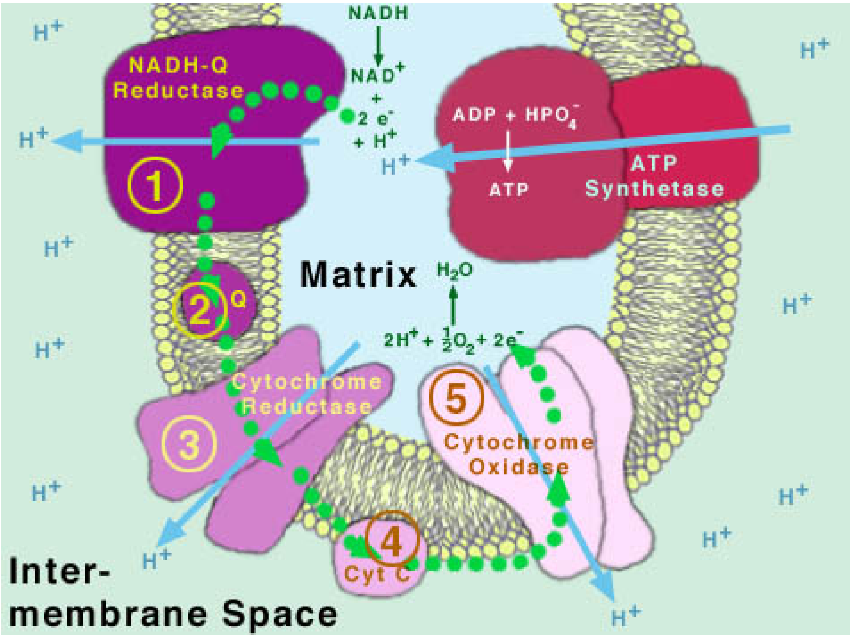


Glycolysis + Oxidative phosphorylation: How food is converted into energy

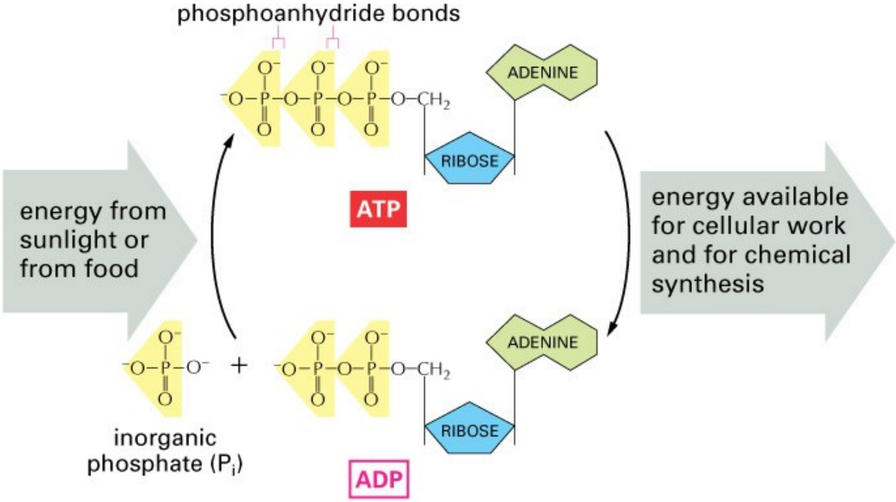
$$\text{Glucose} + 36\ ADP + 36\ Pi + 36\ H^+ + 6\ O_2 \longrightarrow 6\ CO_2 + 36\ ATP + 42\ H_2O$$

Glucose gives 18 times more energy when oxidized

$$ATP + H_2O \longrightarrow ADP + Pi + H^+ + \text{energy} \qquad \Delta G^0 = -7.3\ \text{kCal/mole}$$

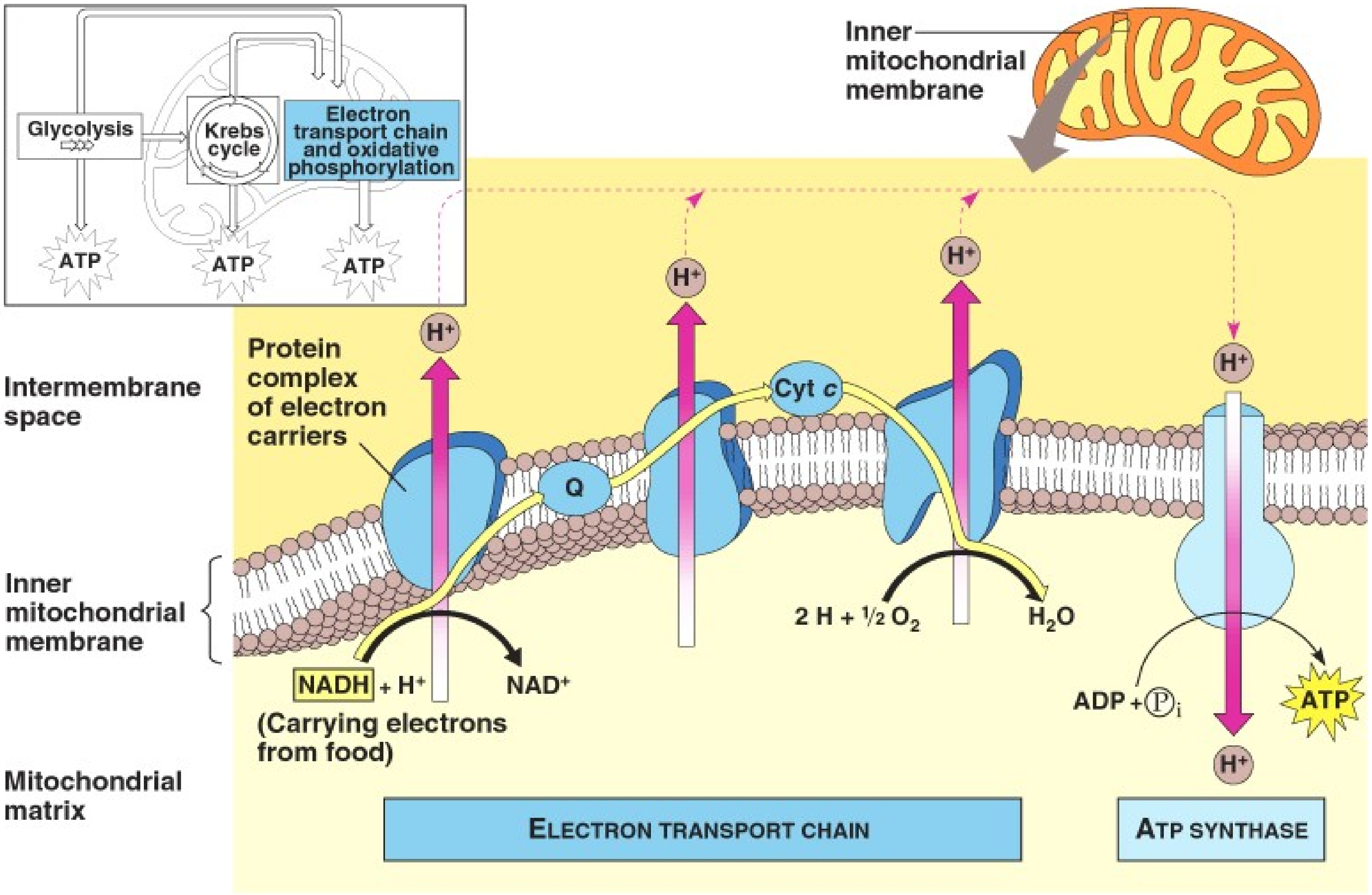


Different forms of Cytochromes (except Cytochrome P-450) are involved in the electron transfer process leading to ATP synthesis and conversion of O_2 to H_2O

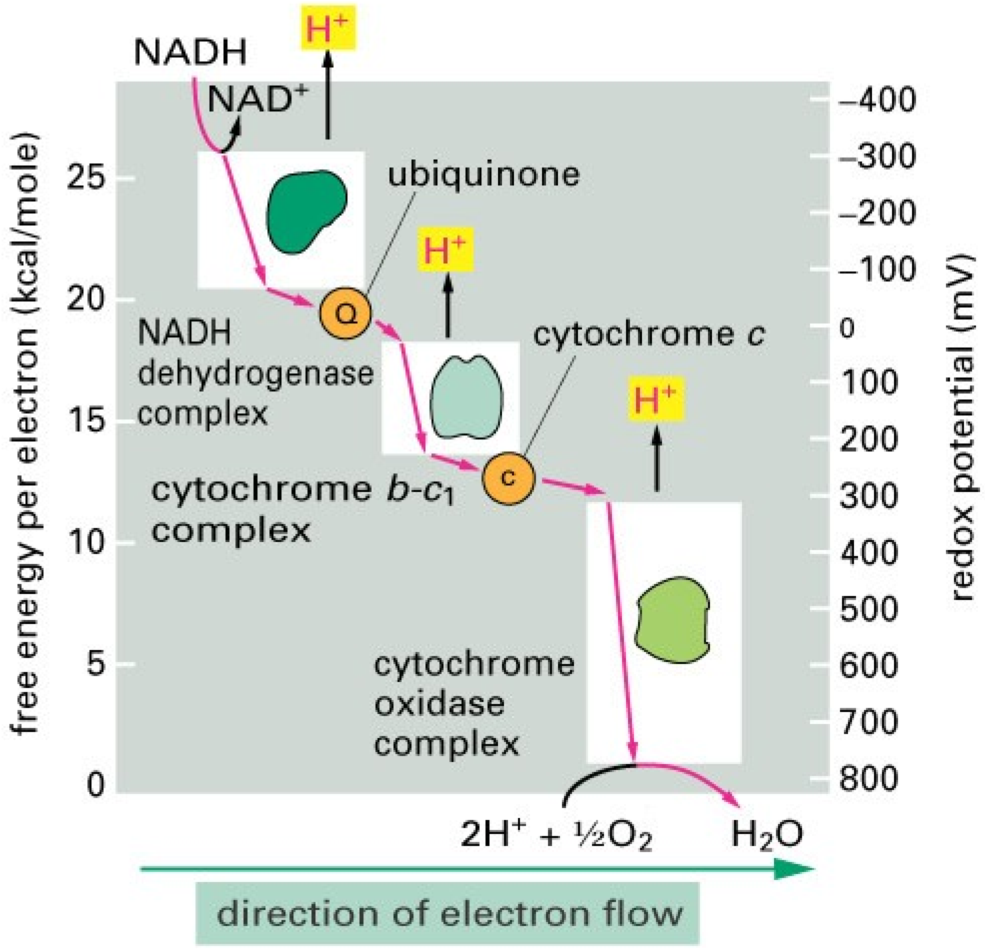


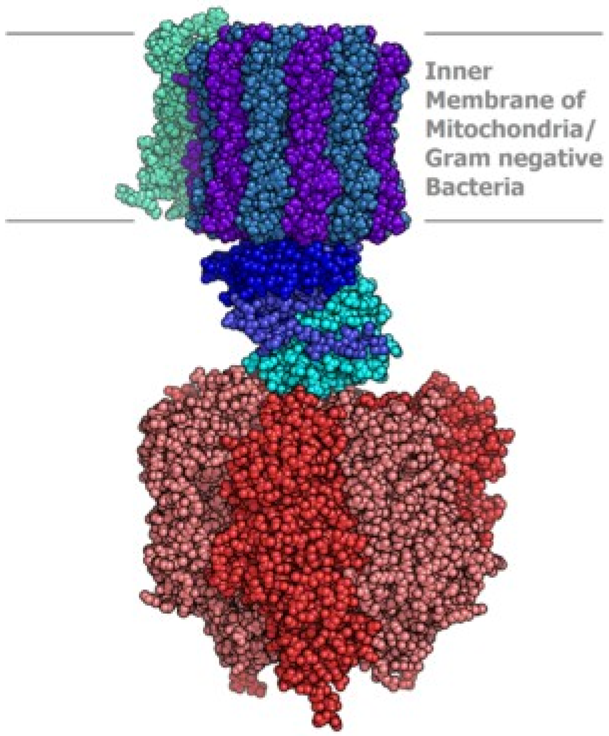
ATP : Universal currency for energy in living systems

See youtube video 'cellular respiration (electron transfer chain)'



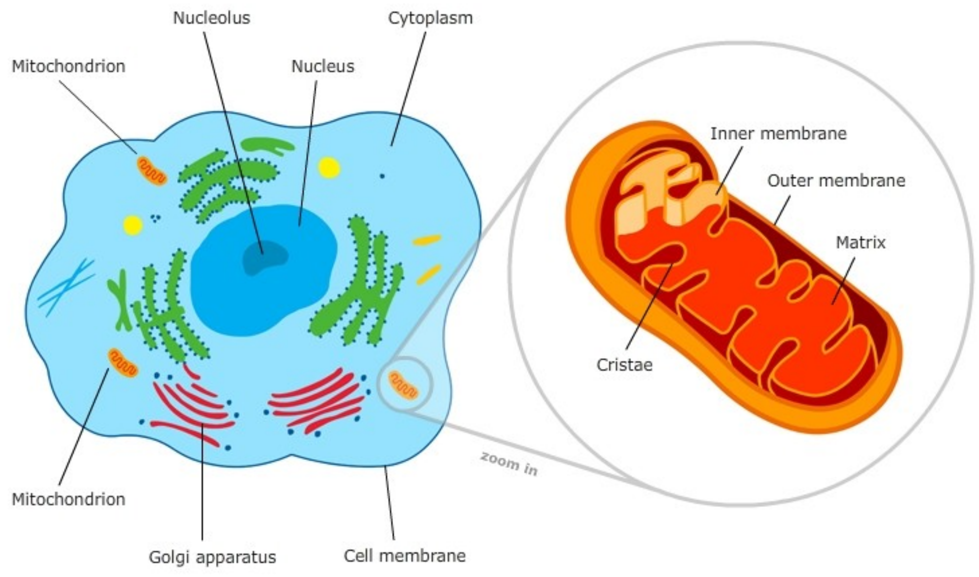
Glycolysis
Krebs cycle
Electron transport chain and oxidative phosphorylation
ATP
ATP
ATP
Inner mitochondrial membrane
Intermembrane space
Protein complex of electron carriers
H+
H+
H+
H+
Q
Cyt c
Inner mitochondrial membrane
NADH + H+
NAD+
(Carrying electrons from food)
2 H + 1/2 O2
H2O
ADP + Pi
ATP
H+
Mitochondrial matrix
ELECTRON TRANSPORT CHAIN
ATP SYNTHASE

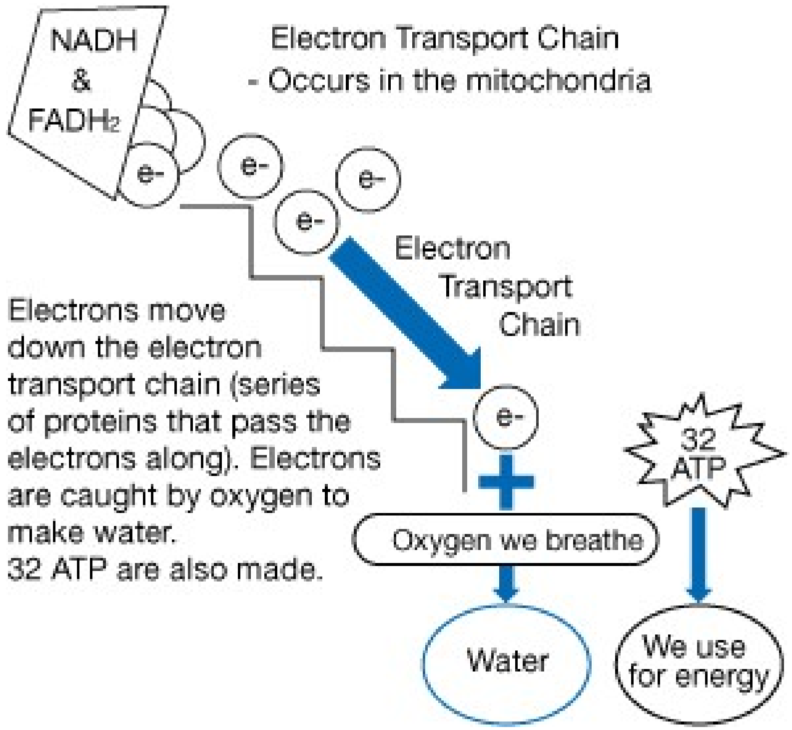


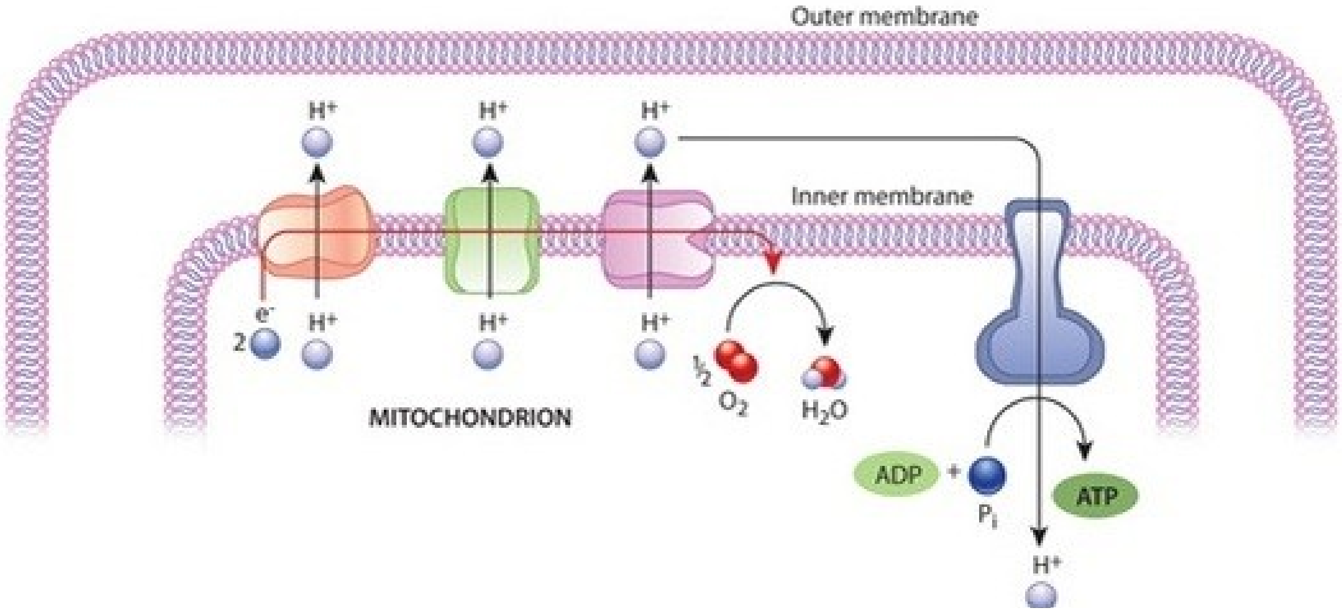


Actual structure of ATP synthase unit (a molecular machine!)

Cytochromes a and a_3	Cytochrome c oxidase with electrons delivered to complex by soluble cytochrome c (hence the name)
Cytochromes b and c_1	Cytochrome c reductase


Nucleolus
Cytoplasm
Mitochondrion
Nucleus
Inner membrane
Outer membrane
Matrix
Cristae
zoom in
Mitochondrion
Golgi apparatus
Cell membrane


NADH
&
$FADH_2$
Electron Transport Chain
- Occurs in the mitochondria
e-
e-
e-
e-
Electron
Transport
Chain
Electrons move down the electron transport chain (series of proteins that pass the electrons along). Electrons are caught by oxygen to make water.
32 ATP are also made.
e-
+
32 ATP
Oxygen we breathe
Water
We use for energy

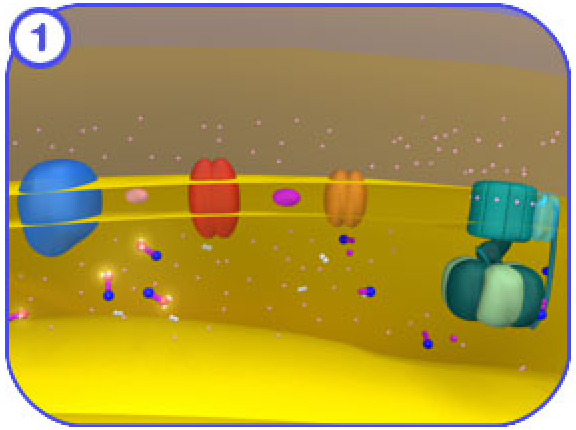

Outer membrane
H+
H+
H+
Inner membrane
e-
2
H+
H+
H+
½ O_2
H_2O
MITOCHONDRION
ADP
+
P_i
ATP
H+

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 cytochorme 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 O_2 and convert it to water generating a proton gradient

What does this reaction result in?

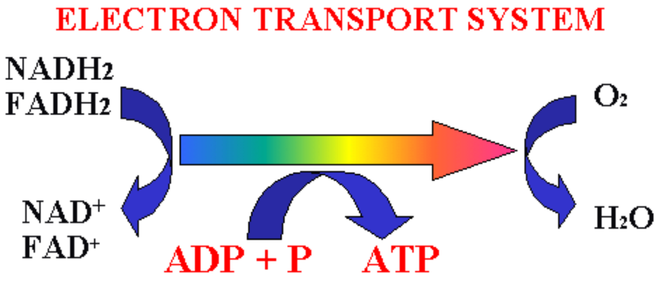
The proton gradient generated during the electron transport chain and conversion of O_2 to water drives the **molecular machine** called ATP synthase which makes ATP from ADP and P_i (Inorganic phosphate). ATP is the universal currency of energy in living systems

Su…gar goes to ATP…. !

How exactly is oxygen carried around and stored in the body?

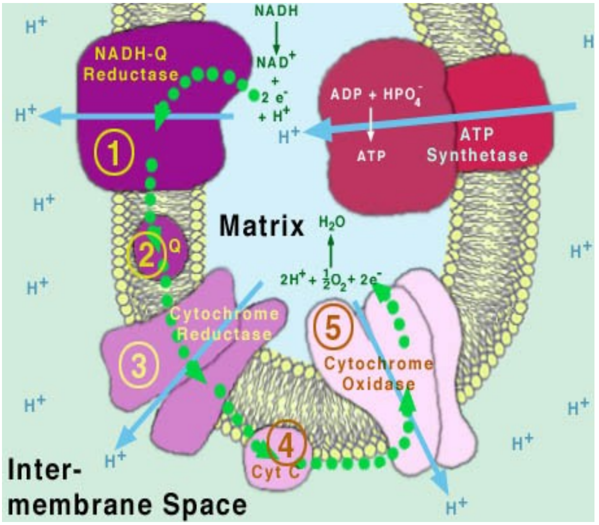
Youtube: ATP synthase molecular machines

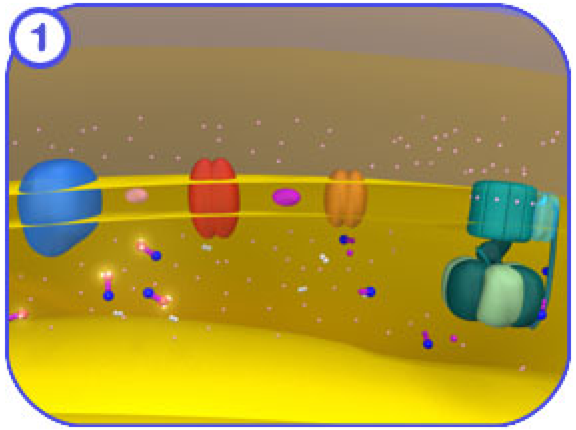
Cytochromes in the electron transport chain



Cytochrome reductase (or b): Complex unit having 2 different hexacoordinated hemes and an Fe-S cluster

Function :electron transfer and proton gradient generation





Cytochrome c: Unit having one hexacoordinated heme

Function: electron transfer

Ageing is related to generation of oxygen based free radicals which degrade mitochondrial membranes

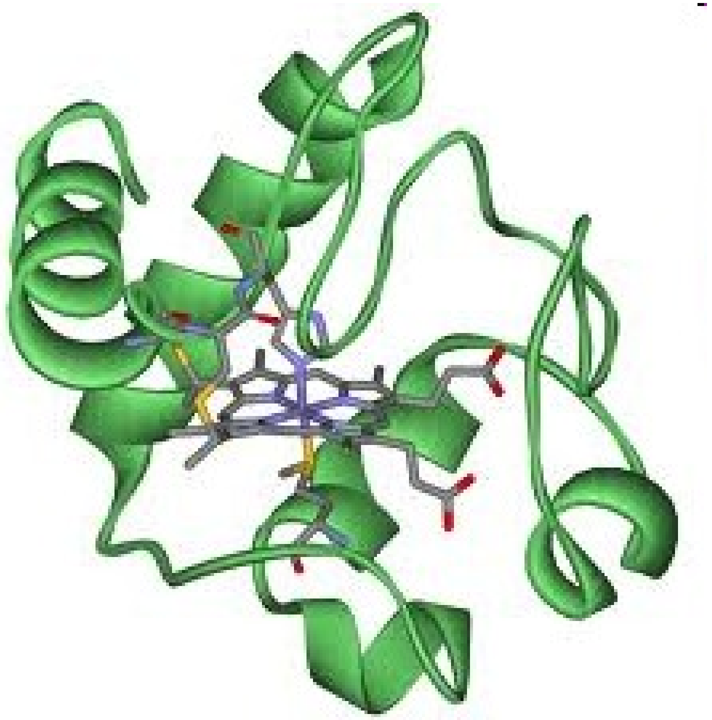
Cytochrome c oxidase: Unit has pentacoordinated heme and tricoordinated Cu

Function: electron transfer to O_2 converting it to water

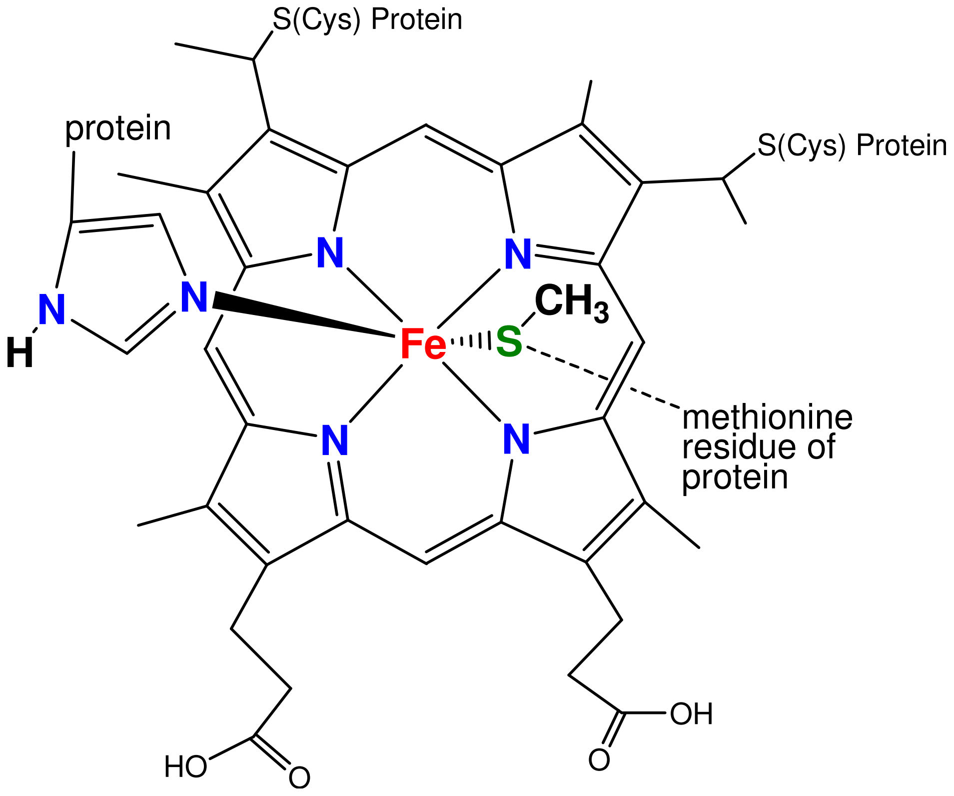
+ proton gradient generation

Redox intermediates in electron transfer

Cytochrome C



Protein chain has 103 amino acid units in some fish, 104 units in terrestrial vertebrates and 112 in plants



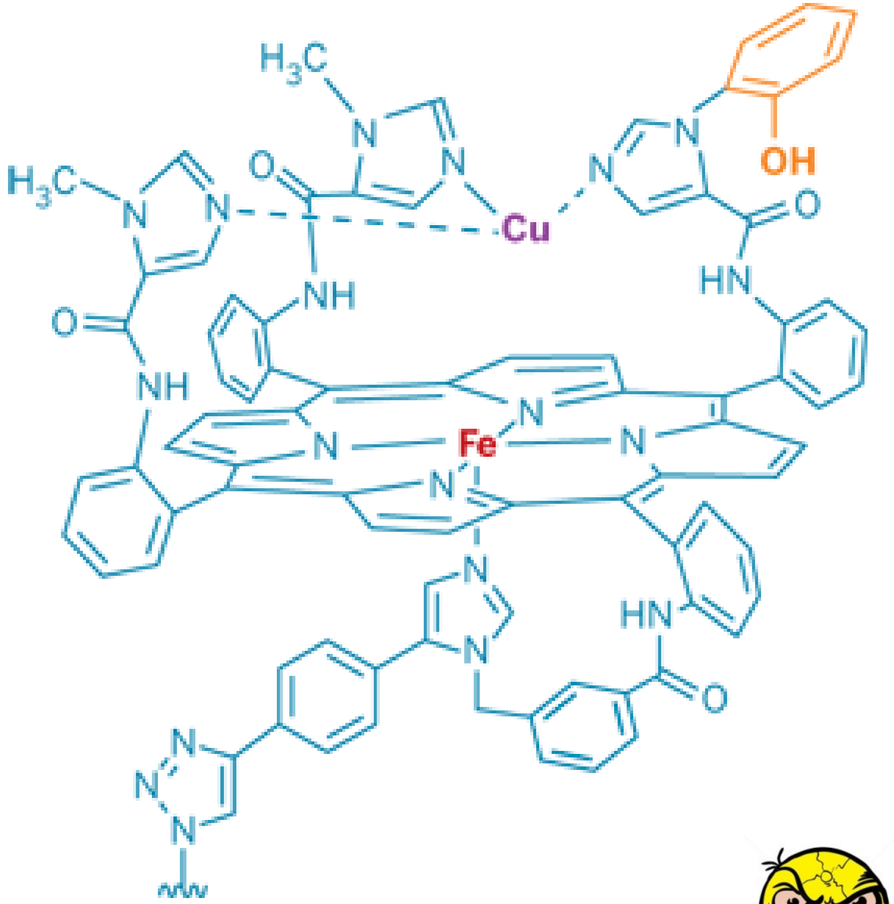
The most structurally well understood cytochrome. The heme active site is hexa coordinated with N from a histidine residue and S from a methionine residue. Present in photosynthesis and respiration chains- **one of the oldest chemicals present in biological processes**

Active site of Cytochrome c oxidase

The last enzyme in the respiratory electron transport chain and is located in the mitochondrial membrane. It receives an electron from each of four cytochrome c molecules, and transfers them to one oxygen molecule, converting molecular oxygen to two molecules of water.

The Fe is pentacoordinated and binds O_2 (along with the Cu) before reducing it.

This is also the site which CN^- binds during cyanide poisoning [stabilizing the Fe^{3+} state and preventing its redox (Fe^{2+}/ Fe^{3+}) activity] (Cyanide is a very strong ligand)



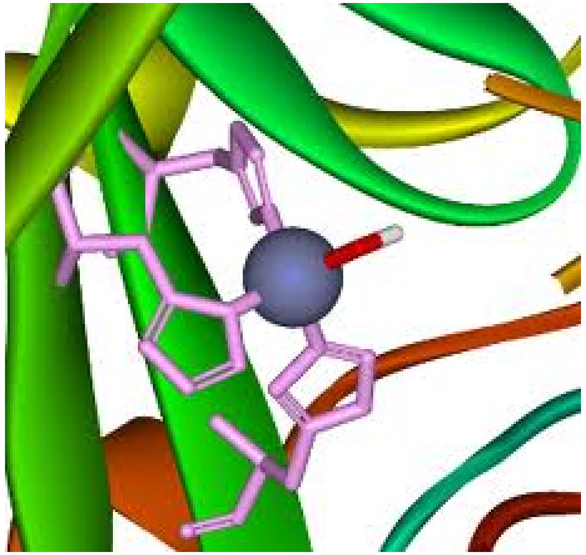
Youtube video: A metabolic poison How cyanide disrupts ATP synthesis

Cyanide

Summary reaction:
$4\ Fe^{2+}$cytochrome + $8\ H^+_{in}$ + O_2 → $4\ Fe^{3+}$cytochrome + $2\ H_2O$ + $4\ H^+_{out}$

Metalloenzymes: Carbonic Anhydrase

A single polypeptide chain (M = 29,000) complexed to one Zn^{2+} ion. The zinc prosthetic group in the enzyme is coordinated in three positions by histidine side-chains. The fourth coordination position is occupied by water. This causes polarisation of the hydrogen-oxygen bond, making the oxygen slightly more negative, thereby weakening the bond. A fourth histidine is placed close to the substrate of water and accepts a proton. This leaves a hydroxide attached to the zinc. The reaction catalyzed by carbonic anhydrase is given below which occurs 5000 times faster in presence of the enzyme:



$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} HCO_3^- + H^+$$

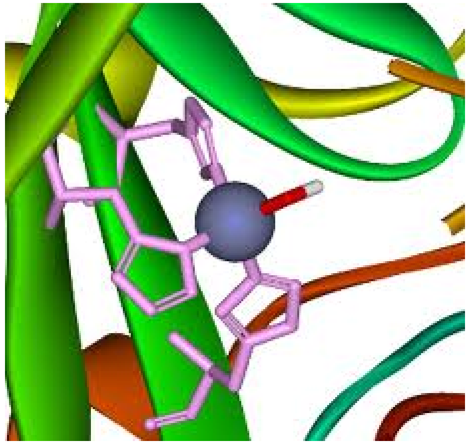
in tissues- high CO_2 concentration)

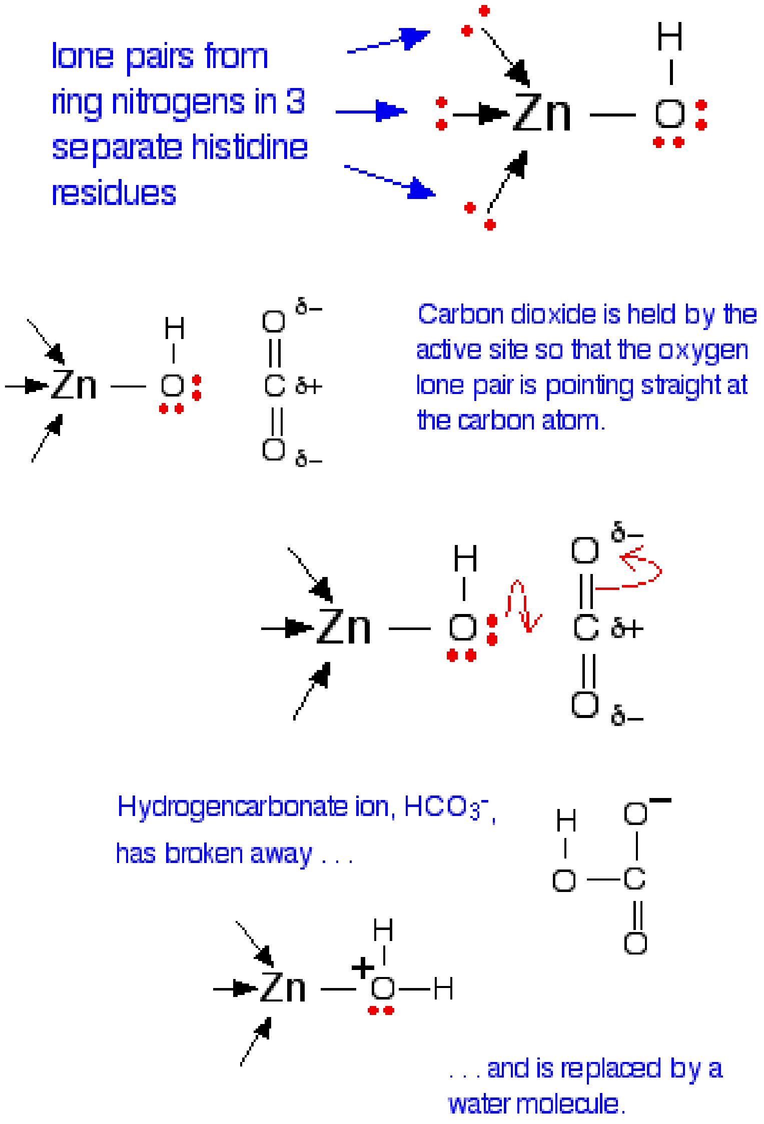
Carbonic anhydrase has one of the highest overall rates of reactions of any enzymes. This is expressed in terms of turnover number of a catalyst (number of substrate molecules converted per molecule of the enzyme per second; same as TOF in organometallic catalysis). For human carbonic anhydrase it is **400,000 to 600,000 per second**.

Most efficient catalytic reaction known so far !!

Reaction increases acidity in the tissues

Metalloenzymes: Carbonic Anhydrase





Why Zinc?

A good Lewis Acid

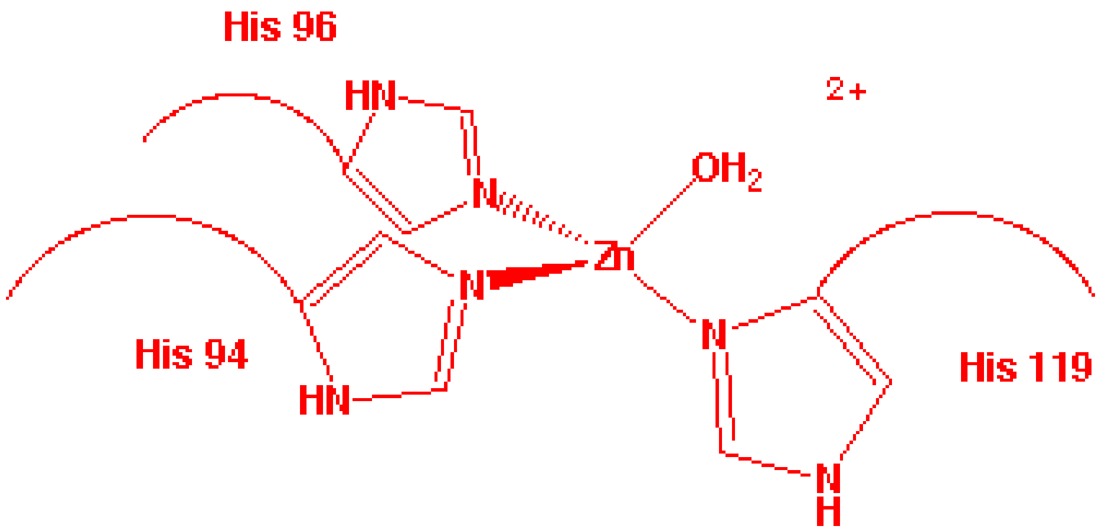
Only one stable oxidation state

Complexes are labile than other divalent metals

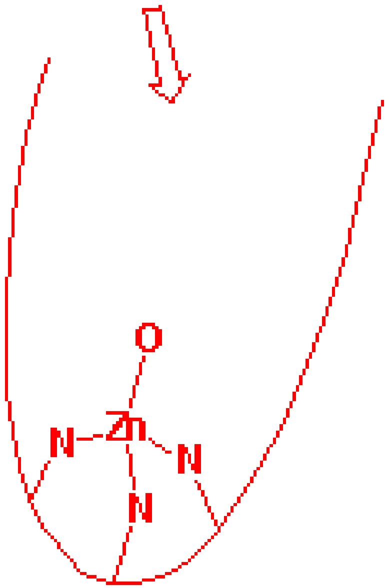
Favors tetrahedral geometry

The active site also contains specificity pocket for carbon dioxide, bringing it close to the hydroxide group. This allows the electron rich hydroxide to attack the carbon dioxide, forming bicarbonate

The zinc coordination sphere of the "resting" enzyme



The enzyme "cleft"

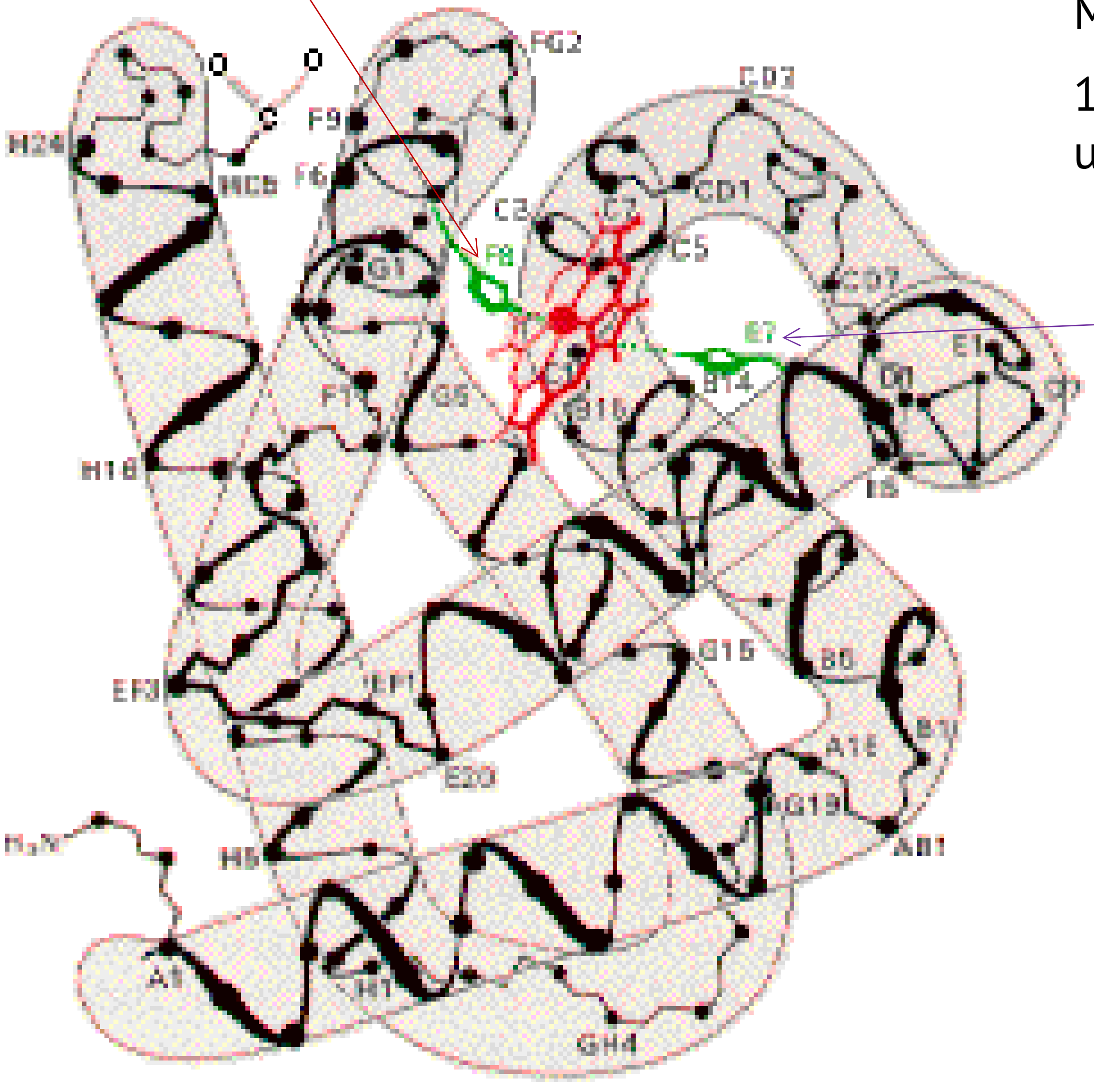


Carbonic anhydrase increases acidity in the vicinity..With enough carbonic anhydrase enzymes present, therefore, carbon dioxide can cause a decrease in the pH of the solution due to all the protons produced from its reaction with water.

Watch Youtube video carboanhydrase

Dioxygen storage in the tissues: Myoglobin

Proximal histidine

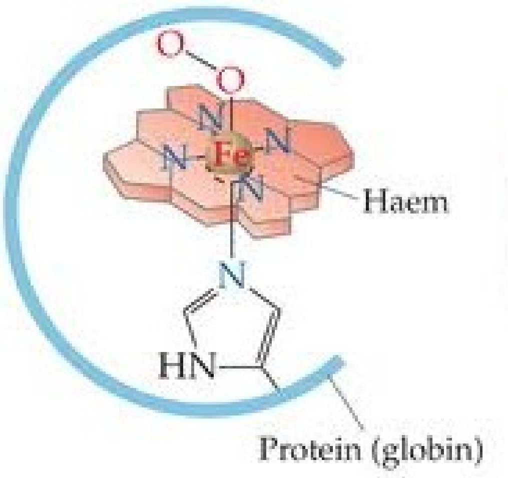


Eight α helixes (~75%),

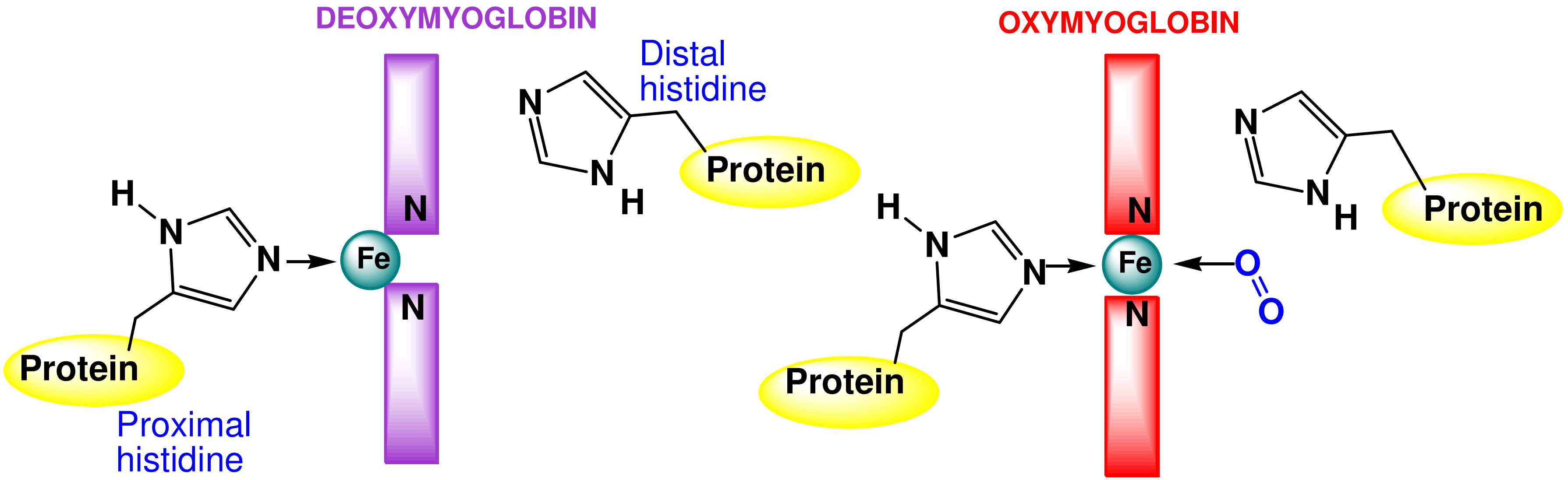
M. Wt. ~17,000

153 amino acid units Single heme unit pentacoordinated (deoxy)

Distal histidine



Changes at the active site during oxygenation of Myoglobin



Fe^{2+} $t_{2g}^4e_g^2$, HIgh spin, radius 92 pm

Paramagnetic

Fe 42 pm outside porphyrin plane

Fe^{3+} $t_{2g}^5e_g^0$, Low spin, radius 75 pm

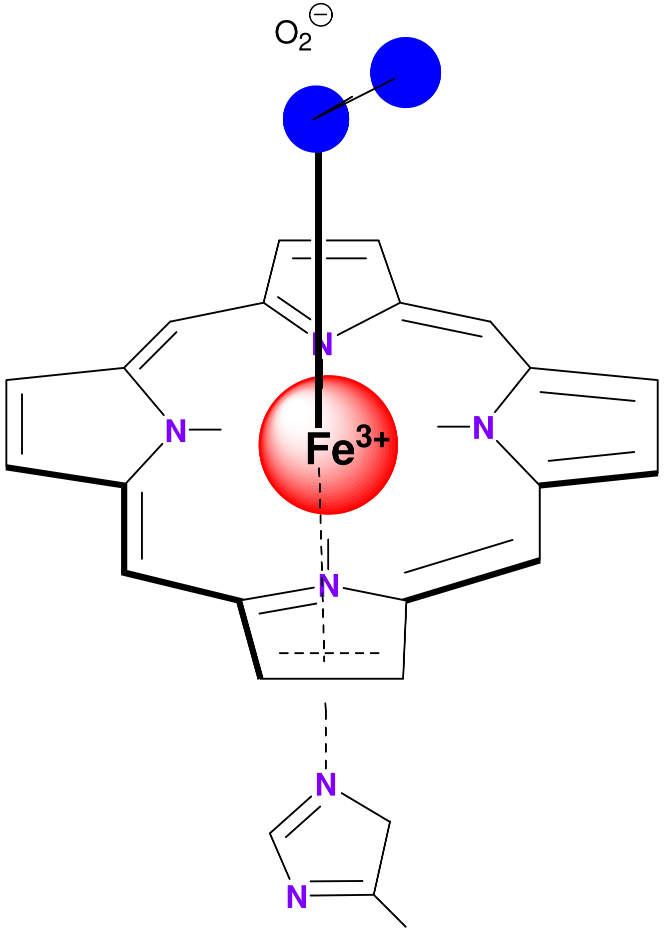
Diamagnetic

Fe fits inside the porphyrin plane

Role of distal histidine: Makes O_2 to bind in a bent fashion and makes it difficult for CO to bind in a linear fashion.

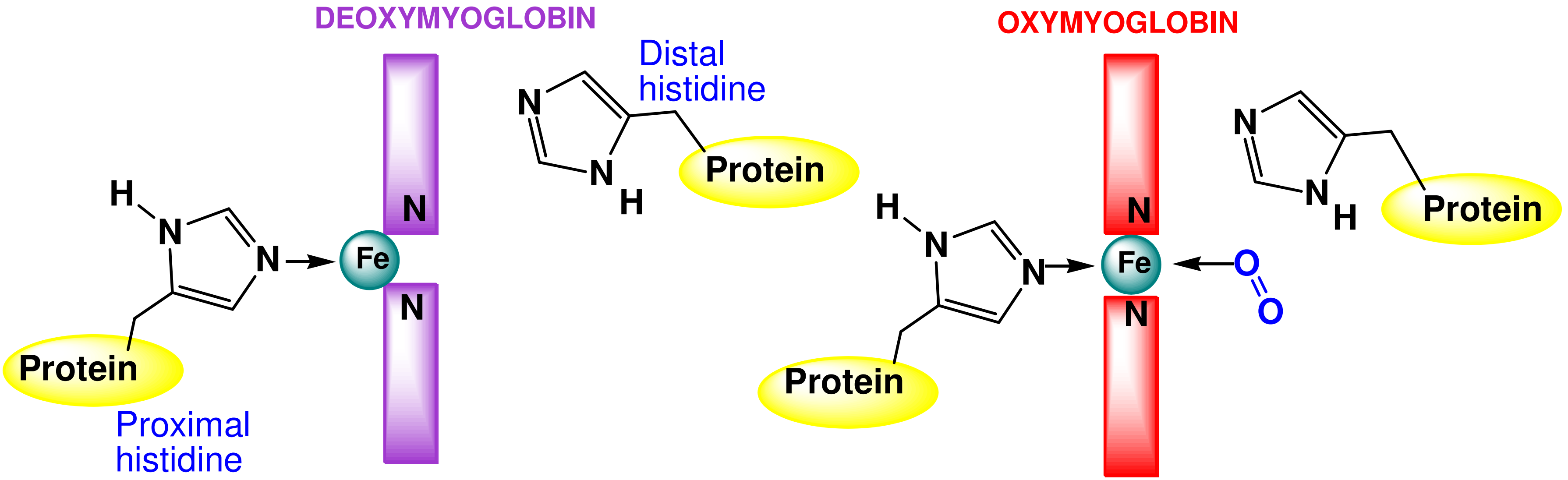
An isolated heme binds CO 25000 times as strongly as O_2 in solution. In the living system binding affinity for oxygen is reduced considerably. For CO to bind strongly, it has to bind linearly which is made difficult by distal histidine

Oxymyoglobin and oxyhemoglobin: Evidence for Fe^{3+} O_2-



$\nu_{O\text{-}O}$ of oxyhemoglobin, 1107 cm^{-1}
is closer to the
$\nu_{O\text{-}O}$ O_2^- value of 1145 cm^{-1} than
$\nu_{O\text{-}O}$ O_2 value of 1550 cm^{-1}

This difference suggests the formation of O_2^- which is a spin ½ ion in combination with low spin Fe^{3+} which is also spin ½ and these two spins can pair by what is well known as ***antiferromagnetic coupling*** and will be diamagnetic



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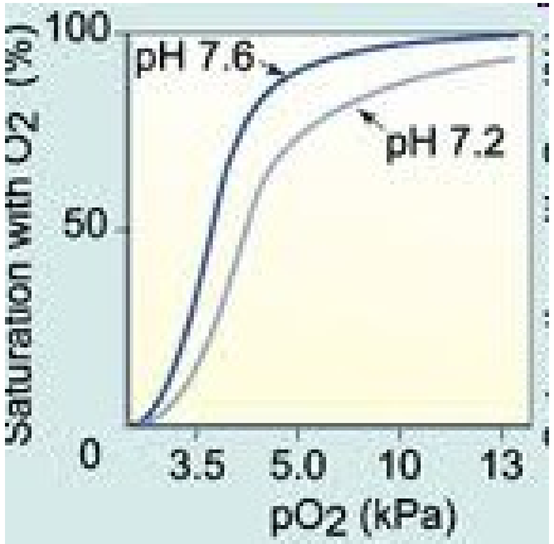
Basics of oxygenation remains same for Hb and Mb. But there are some differences in the way the four units get oxygenated. This begins with pulling of the proximal histidine when Fe gets inside the plane of the porphyrin ring upon oxygen binding

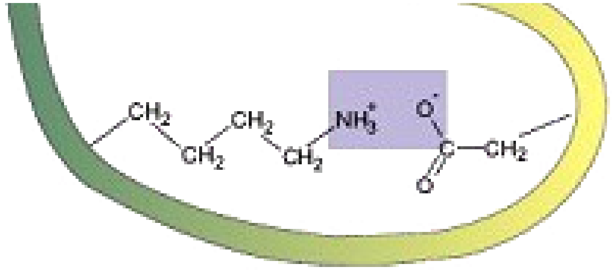
The Bohr Effect

Christian Bohr, father of Niels Bohr discovered this effect. An increase in concentration of protons and/or carbon dioxide will reduce the oxygen affinity of hemoglobin



The chemical basis for the Bohr effect is due to the **formation of two salt bridges of the quaternary structure**. One of the salt bridges is formed by the interaction between Histidine 146 and Lysine 40. This connection will help to orient the histidine residue to also interact in another salt bridge formation with the negatively charged aspartate 94. The second bridge is formed with the aid of an additional proton on the histidine residue.

Below a pH of 6, the imidazole ring of histidine is mostly protonated thus favoring salt bridge formation

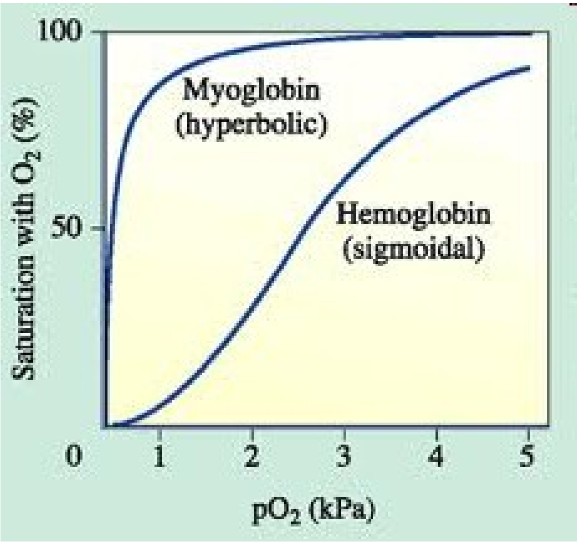


A salt bridge (weak electrostatic interaction)

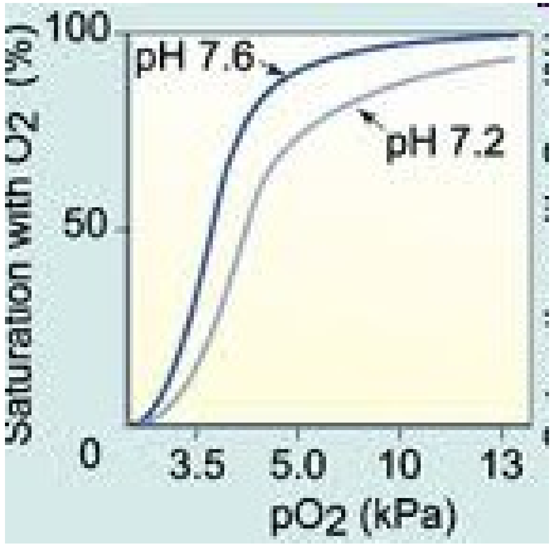
Hemoglobin; An allosteric protein

An allosteric protein does not have fixed properties. Its functional characteristics of are regulated by specific molecule present in its environment. Hemoglobin is an allosteric protein while Myoglobin is not.

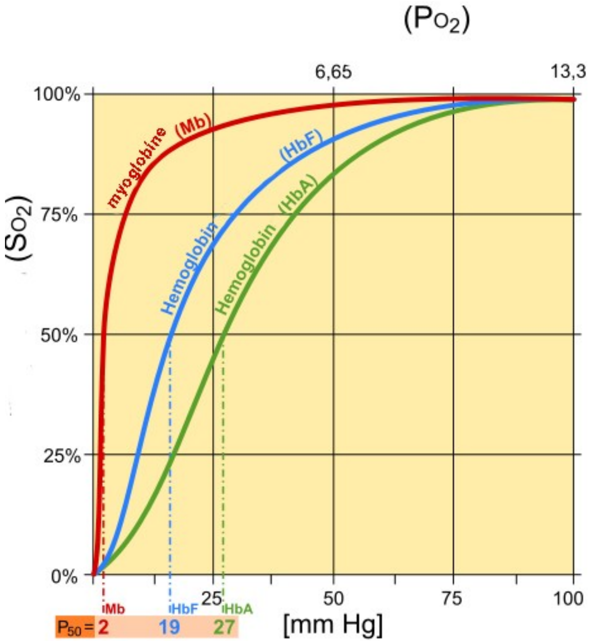
Function of Hemoglobin in the living system is regulated by oxygen partial pressure, H+ concentration and 2, 3 biphosphoglycerate presence (BPG)



O_2



Bohr Effect (effect of H+ on Hb)



2,3-BPG (saturation of O2 on Hb-F)