

**Fundamentals of Protein Chemistry**  
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**Module - 08**  
**Motor Proteins and Metalloproteins**  
**Lecture - 39**  
**Metalloproteins – II**

We continue our discussion on metalloproteins. In the previous lecture we looked at metalloproteins, understood their coordination and which metals were involved in specific biochemical processes in the body. In the course of the lecture we looked at the role of magnesium, manganese, copper and zinc in some specific examples.

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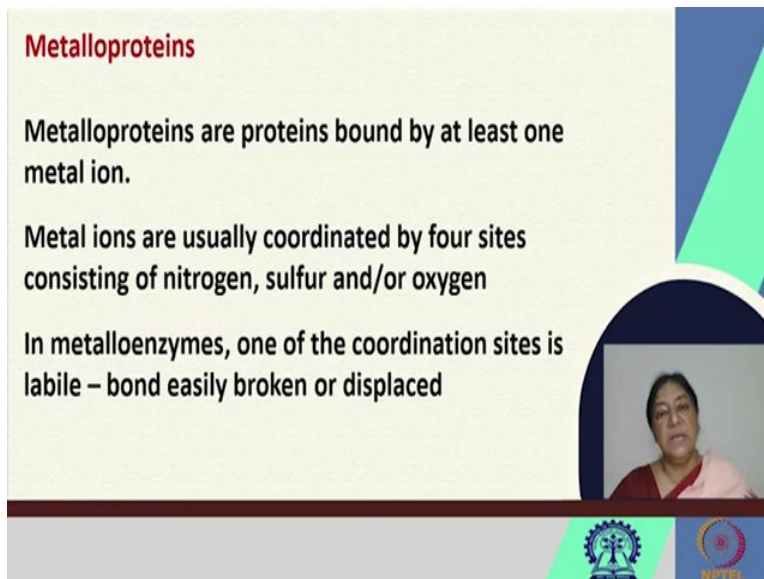
**CONCEPTS COVERED**

- Iron-sulfur proteins
- Catalase and Peroxidase
- Nitrogenase
- Ferritin

The slide features a video inset of Prof. Swagata Dasgupta in the bottom right corner. At the bottom of the slide, there are logos for IIT Kharagpur and NPTEL.

In this lecture we will be looking at iron sulfur proteins, it will be focused on iron. This will include catalase, peroxidase, nitrogenase and also ferritin.

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**Metalloproteins**

**Metalloproteins are proteins bound by at least one metal ion.**

**Metal ions are usually coordinated by four sites consisting of nitrogen, sulfur and/or oxygen**

**In metalloenzymes, one of the coordination sites is labile – bond easily broken or displaced**

The slide features a video inset of a woman in the bottom right corner. At the bottom of the slide, there are two logos: the IIT Bombay logo on the left and the NPTEL logo on the right.

Metalloproteins in general as we saw in the previous lecture, are proteins that are bound by at least one metal ion and we looked at the abundance of the different types of metal ions that are present in proteins. The coordination occurs through the nitrogen as we saw mostly from the imidazole of histidine residues, the sulfur from the sulfur containing amino acids cysteine or methionine and oxygen that could be part of the carbonyl oxygen atoms, could be from the carboxylic acid side chains or also water.

In metalloenzymes in general one of the coordination sites is labile, making one of the bonds that could be easily broken or displaced for a specific enzymatic reaction to occur.

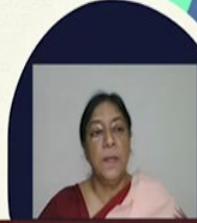


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**Iron:** d-block transition element : -2 and 6+

Exists as - ferrous ( $\text{Fe}^{2+}$ ), ferric ( $\text{Fe}^{3+}$ ), or ferryl ( $\text{Fe}^{4+}$ )  
 $\text{Fe}^{3+}/\text{Fe}^{2+}$  form common

Can bind to a large number of ligands/proteins  
 - participates in many biochemical pathways  
 For example:

- electron transfer reactions in mitochondria
- binding and transport of oxygen
- regulation of cell differentiation and growth

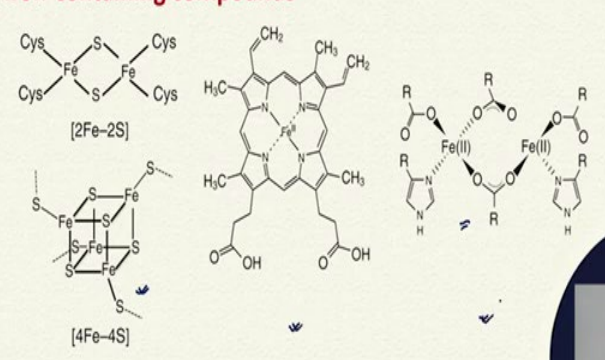




We will look at iron in a bit more detail. This is what is called a d block transition metal and it has a large number of possible oxidation states. In the body it exists as the ferrous, that is the  $\text{Fe}^{2+}$ , the ferric  $\text{Fe}^{3+}$  or ferryl  $\text{Fe}^{4+}$ ; and the  $\text{Fe}^{3+}/\text{Fe}^{2+}$  combination is the most common.




Iron can bind to a large number of ligands and proteins and as a result, participates in many biochemical pathways. For example, we have seen iron in some of the enzymatic reactions that we looked at. We will also be considering hemoglobin myoglobin, where we have iron as a transport protein; where we will have the binding and the transport of oxygen and see how it is involved in the regulation of cell differentiation and growth.

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**Iron-containing compounds**



Iron-sulfur cluster ✓      Heme ✓      Oxo - di - iron example


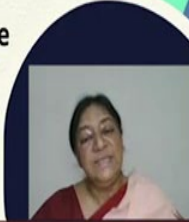
The iron containing compounds that we see in the body are the iron sulfur cluster, a very interesting type of iron containing compound present in a number of proteins. The heme that is

present as we know in hemoglobin and myoglobin and an oxo-di-iron example that is shown, where we have the coordination with the oxo and in this case we have the iron sulfur, in this case we have the heme and in this case we have an oxo-di-iron.

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### Iron-Sulfur proteins

- Occurs extensively in all living organisms
- Participates in electron transfer processes
- Rubredoxins (Rd) – one iron centre
- Ferredoxins (Fd) – multiple iron centres
- Fe bound to S either from Cys or inorganic sulfide
- Coordination number of iron is always 4
- All have more or less tetrahedral geometry




The iron sulfur proteins occur extensively in living organisms. This participates in electron transfer processes. We have an example called the rubredoxins. These rubredoxins have one iron centre and ferredoxins have multiple iron centres. The iron is bound to sulfur either in the form of an inorganic sulfide or in the sulfur from cysteine and the coordination number in this case is 4 and all of them have more or less tetrahedral geometry in their structural aspects.



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### Rubredoxins (Rd)

- Small iron containing proteins
- Present in sulfur metabolizing bacteria
- Simple iron-sulfur protein having single iron per molecule
- Takes part in one e<sup>-</sup> transfer



PDB ID: 2PVX




If we look at the rubredoxins. This [refer to slide] is an example of a rubredoxin protein. It is a small iron containing protein and it is present in sulfur metabolizing bacteria. The simple iron sulfur protein has a single iron per molecule of the protein it takes part in one electron transfer.


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**Rubredoxins (Rd)**

- This is a single iron tetra thiolate protein
- Unlike other iron-sulfur proteins, it does not have inorganic sulfur
- Fe is coordinated by 4S from Cys
- Iron exist as Fe(II) and Fe(III)
- Shows a – ve redox potential



The diagram illustrates the active site of a rubredoxin protein. It shows a central iron atom (Fe) coordinated to four sulfur atoms (S), each of which is bonded to a cysteine residue (Cys). The diagram is labeled 'Active site'.



The slide also features a small inset video frame showing a woman speaking. At the bottom of the slide, there are logos for a university and a center named 'SIPTEL'.


This is the active site where the iron sulfur is obtained, where we have the sulfurs that are coordinated from the cysteine residues present in the protein, that coordinate the iron. So the single iron tetra thiolate protein. The thiolate indicates that this is from the sulfur atom that we see in the cysteine.

Unlike other iron sulfur proteins, rubredoxin does not have an inorganic sulfur. We will see examples where inorganic sulfur atoms are present. It is coordinated by the 4 sulfur from cysteine residues and it exists as  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ , being involved in a specific process showing a negative redox potential.

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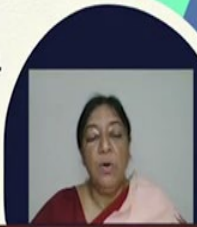

**Ferredoxins (Fd)**

- $\text{Fe}_2\text{S}_2$  or [2Fe-2S] protein
- Usually present in plant (spinach) - referred to as plant type Fd
- Also found in mammals and bacteria



Crystal structure of human ferredoxin-1 (FDX1) in complex with iron-sulfur cluster

PDB ID: 3P1M

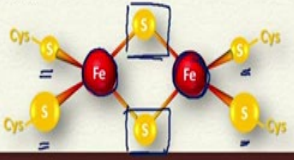





Ferredoxins that are multiple iron centers, have an indication of the presence of an iron sulfur cluster. In this case their representation is in this manner where we have 2Fe-2S or written as  $\text{Fe}_2\text{S}_2$  protein, indicating the type of the cluster that is present on the iron sulfur cage, as it is sometimes called, present in the protein. This is called the plant type ferredoxins, present in the spinach plant and also found in mammals and bacteria.

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**Ferredoxins (Fd)**

- $2\text{S}^{2-}$  bridges
- Core structure  $\{\text{Fe}_2(\mu_2\text{-S})_2\}$
- Oxidized  $\text{Fe}^{3+}-\text{Fe}^{3+}$  form reduced to  $\text{Fe}^{2+}-\text{Fe}^{3+}$  form by one  $e^-$  transfer
- $E^0 \sim -0.15 \text{ V to } -0.45 \text{ V}$

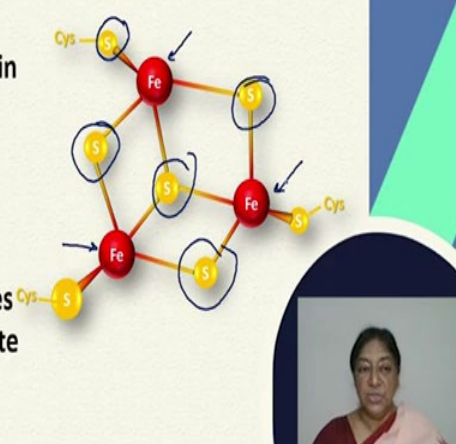
If we look [refer to slide] at the specific structural aspects of this protein, these are the two iron atoms that are present. These are coordinated with the sulfur from the 2 cysteine amino acids present in the polypeptide chain of the protein in each case. But also we have a coordination with an inorganic sulfur.

So this is where we see the specific 2 sulfide bridges. The core structure is represented as  $\{Fe_2(\mu_2-S_2)\}$ . The oxidized  $Fe^{3+} - Fe^{3+}$  form is reduced to an  $Fe^{2+} - Fe^{3+}$  form, from a one electron transfer and the EMF is about 0.15 V to -0.45 V.

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**Ferredoxins (Fd)**

- $Fe_3S_4$  or [3Fe-4S] protein
- $Fe^{3+}$  in oxidized form
- 2  $Fe^{3+}$  and one  $Fe^{2+}$  in reduced form
- Present in enzyme aconitase that catalyzes the conversion of citrate to isocitrate



The diagram illustrates the [3Fe-4S] ferredoxin core structure. It features three iron atoms (Fe) in red spheres and four sulfur atoms (S) in yellow spheres. Two sulfur atoms are bridged between the iron atoms, forming a  $\mu_2-S_2$  core. The iron atoms are also coordinated to terminal sulfur atoms, some of which are linked to cysteine (Cys) residues. A small inset video shows a woman speaking.

The ferredoxins structure can also have a  $Fe_3S_4$  type of structure, the representation would be 3Fe-4S in its nomenclature, where again we see the iron sulfur cage that could have the sulfur from the cysteine residue or the inorganic sulfurs, that are associated here in this specific type of 3Fe-4S protein.

Here [refer to slide],  $Fe^{3+}$  in its oxidized form is seen and we have 2  $Fe^{3+}$  and 1  $Fe^{2+}$  in the reduced form. So there are 3 atoms of iron that we see here,  $Fe^{3+}$  originally in its oxidized form, all of them and in the reduced form we have 2  $Fe^{3+}$  and 1  $Fe^{2+}$ . This is present in the enzyme aconitase; this enzyme is involved in the catalysis of the conversion of citrate to isocitrate.

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### Ferredoxins (Fd)

- $\text{Fe}_4\text{S}_4$  or [4Fe-4S] protein
- High potential Iron-sulfur protein
- Distorted cubic structure
- Present in nitrogenase and hydrogenase

PDB ID: 1JM1

We saw the rubredoxins are the one that have a single iron centre, while ferredoxins have multiple iron centres. We saw an example of 2 an example of 3; this is where we have 4 iron centres. So, this is a 4Fe-4S protein. It is a high potential iron sulfur protein, forming a distorted cubic structure because of the coordination and considering that the polypeptide chain is involved in the coordination. This is present in a protein called nitrogenase and hydrogenase.

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### Rieske protein

2 His to one Fe

Components of cytochrome bc1 complexes and cytochrome b6f complexes

PDB ID: 1JM1

The rieske protein is another protein that has the iron sulfur cage, where we have the combination of 2 histidines coordinated to an iron and we have 2 inorganic sulfur. And these are components of cytochrome bc1 complexes and cytochrome b6f complexes; very important aspects of electron transfer.

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**Hemerythrin (Hr)**

- Oligomeric protein that participates in oxygen transport
- It is a non-heme iron containing protein
- Found in few marine invertebrates

PDB ID: 1HMD

In the protein hemerythrin, we have the structure as an oligomeric protein that participates in oxygen transport. It is a non-heme iron containing protein and it is also found in a few marine invertebrates.

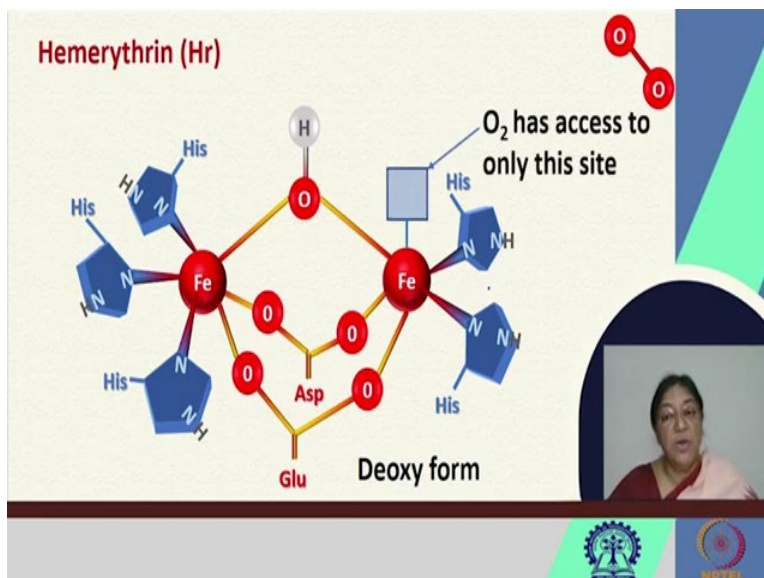
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**Hemerythrin (Hr)**

- Mostly occurs as an octamer
- Some tetrameric, trimeric and dimeric forms are also found
- All monomers contain 4 helix bundles
- Each monomer has a di-iron site

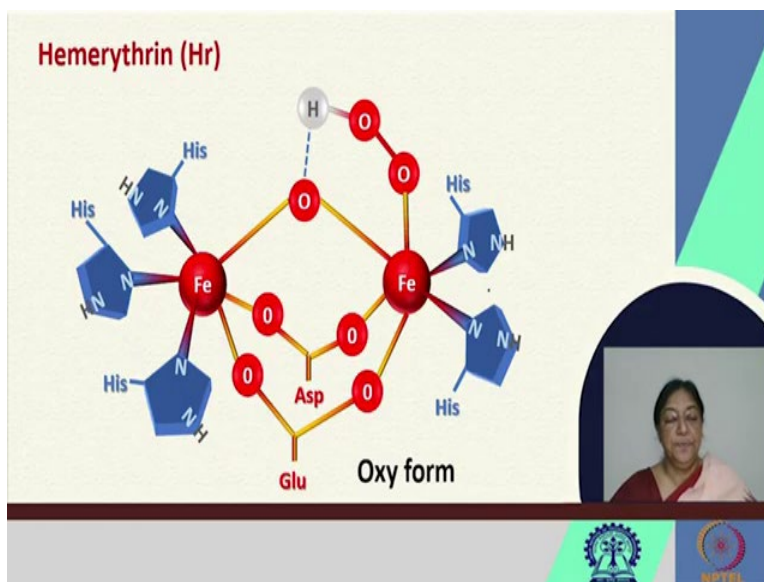
This structure occurs mostly as an octamer and some tetrameric, trimeric and also dimeric forms are also available. Here [refer to slide] we see the monomers that have a 4 helix bundle to them, as indicated by the 4 helices that are observed here; the 4 helices that form the 4 helix bundle. Each monomer has a di-iron site.

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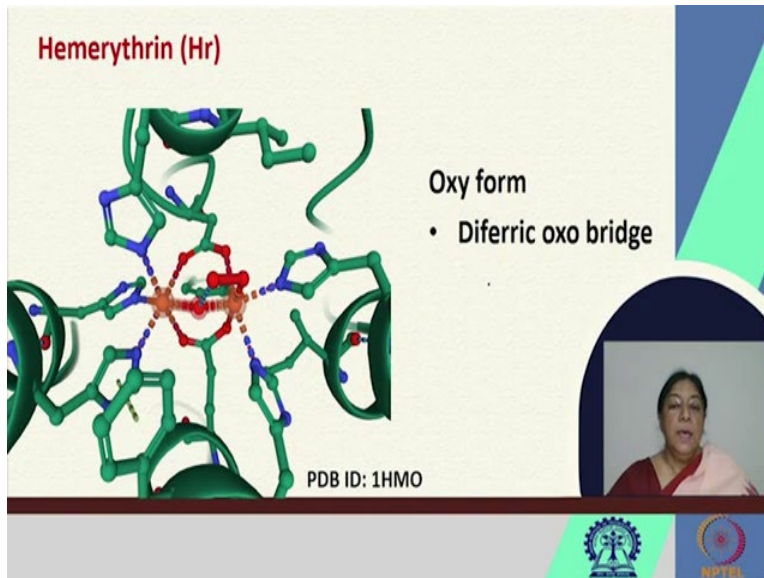
In this case we have the di-iron site, the coordination is through the histidine. So, we have now a glutamic acid come into the picture [refer to slide], where we have the specific coordination with the aspartic acid, glutamic acid and the histidine moieties in hemerythrin, but there is no heme as seen in the other cases. Where we have the deoxy form in this manner and once we have the oxygen bound form, the site available on the iron is where oxygen binds.

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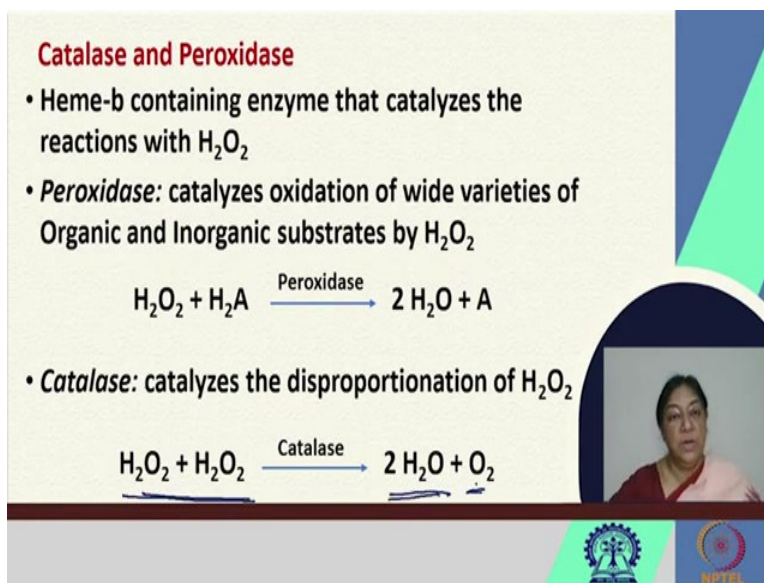


We have the formation of a specific coordination site present on iron and we will have bound oxygen to it. The oxy form has a diferric oxo bridge, as it is called. This is an example of hemerythrin.

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For catalase and peroxidase, these are specific proteins that are heme binding proteins; the heme-b containing enzyme that catalyzes the reactions with hydrogen peroxide. Peroxidase catalyzes the oxidation of a large variety of organic and inorganic substrates by  $\text{H}_2\text{O}_2$ . And this [refer to slide] is an example of the reaction of peroxidase. Catalase on the other hand, catalyzes the disproportionation of  $\text{H}_2\text{O}_2$  in a specific type of reaction, where we notice the disproportionation to  $\text{H}_2$  and  $\text{O}_2$ ; an oxidation and a reduction.

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### Catalase and Peroxidase


- The function of this enzyme is to prevent any potentially dangerous oxidant build up
  - Cytochrome c peroxidase
  - Horseradish peroxidase
  - Human erythrocyte catalase



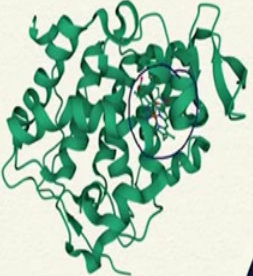
The function of the catalase and the peroxidase enzymes, is to prevent any potential dangerous oxidant buildup and these are specific examples of such type of enzymes, the cytochrome c peroxidase, horseradish peroxidase and human erythrocyte catalase.

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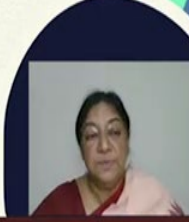
### Catalase and Peroxidase



PDB ID: 1QQW  
**Human erythrocyte catalase**



PDB ID: 2PCB  
**Cytochrome c peroxidase**



This [refer to slide] is the structure of the human erythrocyte catalase and this is the structure of the cytochrome c peroxidase, where we can see the specific bound iron atoms here.

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**Catalase and Peroxidase**

- Active site cavity contains His and in some case Asp or Arg
- Side chain ideally situated to interact with  $\text{H}_2\text{O}_2$  when bound to Fe
- Facilitates cleavage of the O–O bond

In the heme bound form, we have the  $\text{Fe}^{3+}$  bound in this form, there is a specific coordination with the 2 oxygen atoms here. The active site cavity contains histidine and in some cases even aspartic acid or arginine. The side chains are ideally situated in a manner that would interact with the hydrogen peroxide that was bound. So here we have the oxygen of the hydrogen peroxide bound to Fe.

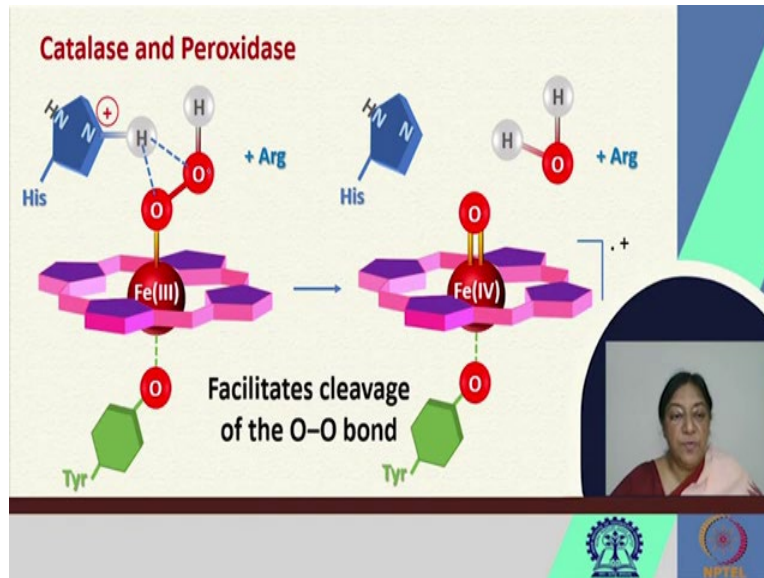
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**Catalase and Peroxidase**

- Arg residues (Arg72, Arg112 and Arg365) form a salt bridge with the carboxylate group of heme
- Helps to keep the heme group in position as well as increases the redox potential
- One Arg is involved in binding of the cofactor NADPH

This facilitates the cleavage of this specific bond. The arginine residues involved here, are arginine 72, 112 and 365. They form a specific salt bridge with the carboxylate group of heme and this helps to keep the heme group in position, in addition to increasing the redox potential. One arginine is also involved in the binding of the cofactor NADPH.

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So the reaction is such that we have the formation of water.

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**Nitrogenase**

- Nitrogen is essential for amino acid synthesis and nucleic acid synthesis
- Atmospheric  $N_2$  is inert and thus can not be used directly for biosynthesis
- Conversion to usable form like  $NH_3$  is essential
- Industrial production of  $NH_3$  requires high pressure and high temperature
- Nitrogenase does the same at normal conditions

We now look at nitrogenase. In nitrogenase, this is a protein involved in the conversion of the nitrogen to a usable form such as ammonia. Nitrogen is essential for the amino acid synthesis and nucleic acid synthesis. The atmospheric nitrogen in this case is inert and it cannot be used directly.



So, it has to be converted to a usable form such as ammonia, to be utilized for the production of amino acid. Now when we look at this from an industrial point of view, it requires extremely high pressure and high temperature conditions for the conversion to occur. However nitrogenase can perform this at normal conditions.

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**Nitrogenase**

- Catalyzes the reduction of dinitrogen to ammonia
- Plays an important role in nitrogen fixation
- These enzymes are produced by certain bacteria like Azotobacter

PDB ID: 1G21





The protein itself catalyzes, therefore the reduction of dinitrogen to ammonia and it plays a very important role in nitrogen fixation. These enzymes are produced by certain bacteria like azotobacter and they occur in three forms the molybdenum form, the vanadium form and the iron form.

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**Nitrogenase**

- Occurs in three forms  
– Mo, V and Fe forms
- Molybdenum nitrogenase, found in legume-associated rhizobia is the most extensively studied and characterized

PDB ID: 1G21





Molybdenum nitrogen is found in legume associated rhizobia and is the most extensively studied and characterized.

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## Nitrogenase

- Nitrogenase is a complex enzyme
- Consists of two types of protein –
  - ✓ Fe – protein
  - ✓ FeMo protein

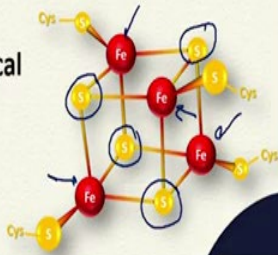





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## Nitrogenase

### Fe – protein:

- Homodimer – dimer of identical subunits which contains one  $[Fe_4S_4]$  cluster
- $[Fe_4S_4]$  cluster lies at the interface between 2 identical subunits
- Redox potential depends on presence or absence of ATP

So, this is a complex enzyme that contains basically two types of proteins; one is the Fe protein and the other is the FeMo protein. In the Fe protein it is a homodimer, that means it is a dimer of identical subunits, which contains one  $Fe_4S_4$  cluster.


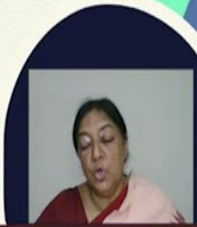
Here [refer to slide] we have an  $Fe_4S_4$  cluster. We see the coordination where we have the 4 sulfide atoms here, the 4 iron atoms in these positions that form the cube. So the cluster is formed by the 4 Fe and the 4 S, that is what forms the cube. This lies in an interface between the 2 identical subunits. This is in the interface between the 2 identical subunits and the redox potential depends upon the presence or the absence of ATP.

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**Nitrogenase Fe – protein**

- Function – to transfer electrons from a reducing agent, such as ferredoxin or flavodoxin to the FeMo protein
- Electron transfer requires an input of chemical energy that comes from the attachment and hydrolysis of ATP
- ATP hydrolysis also causes a change in the conformation
- This conformational change facilitates easier electron transfer by bringing the Fe protein and MoFe protein closer together





So when we look at the Fe protein, its function is to transfer electrons from a reducing agent such as ferredoxin or flavodoxin, to the FeMo type protein. This electron transfer requires an input of chemical energy, that comes from the attachment and hydrolysis of ATP. The ATP hydrolysis results in a change in the conformation and following this change in conformation, this facilitates the electron transfer by bringing the Fe protein and the FeMo protein closer to each other.

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**Nitrogenase**

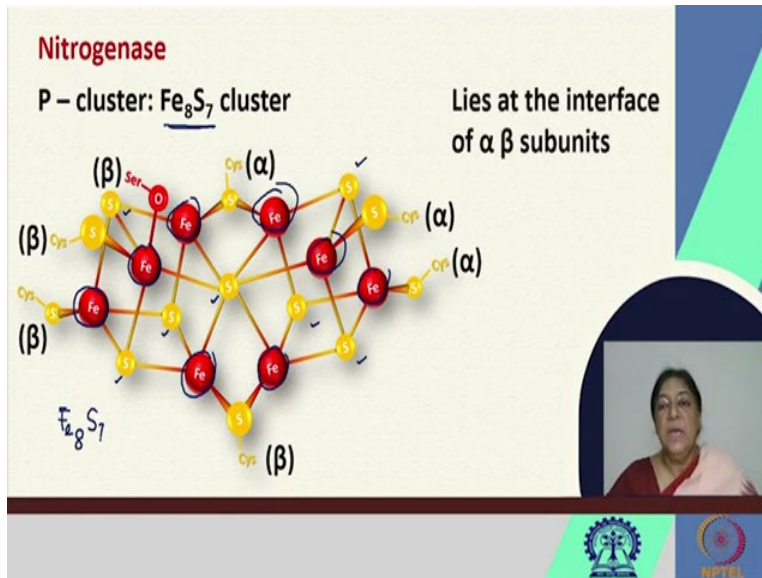
**FeMo protein:**

- It is a heterotetramer consisting of two  $\alpha$  subunits and two  $\beta$  subunits
- It contains –
  - ✓ two iron-sulfur clusters, called P-clusters that lies at the interface between the  $\alpha$  and  $\beta$  subunits
  - ✓ and two FeMo cofactors present within the  $\alpha$  subunits



This [refer to slide] is the FeMo protein details. It is a heterotetramer that contains two  $\alpha$  subunits and two  $\beta$  subunits. This contains two iron-sulfur clusters that are called P-clusters, that again lie between the interface of the  $\alpha$  and the  $\beta$  subunits and the two FeMo cofactors are present within these  $\alpha$  subunits.

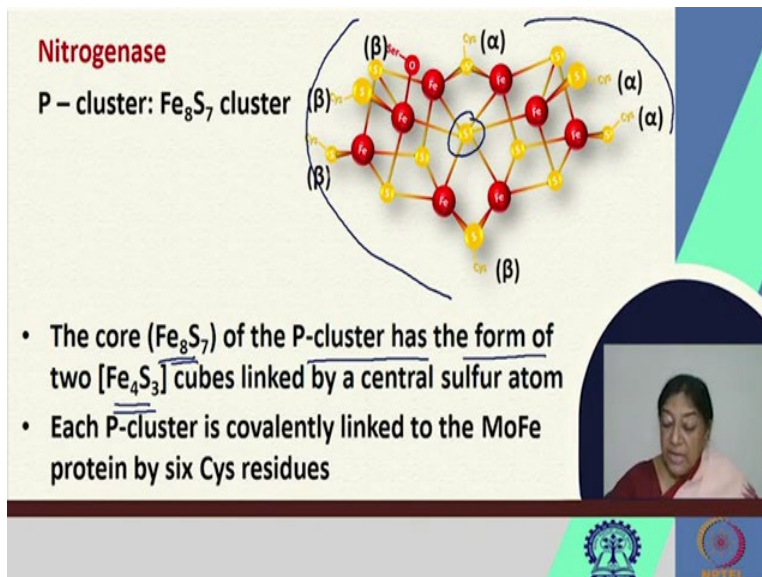
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When we look at the P cluster, it is a cluster of  $\text{Fe}_8\text{S}_7$ . When we consider this cluster, we have the  $\alpha$  and the  $\beta$  and then the P cluster. If we count the number of irons we have 8; so this is an  $\text{Fe}_8$ .

Following this, we look at the number of sulfur atoms that are not associated with the polypeptide chain. We have 7; so this is  $\text{S}_7$ . If we look at the other sulfurs we will see that they are associated with the cysteine residues that are part of the polypeptide chain. This lies at the interface of the  $\alpha$  and the  $\beta$  subunits.

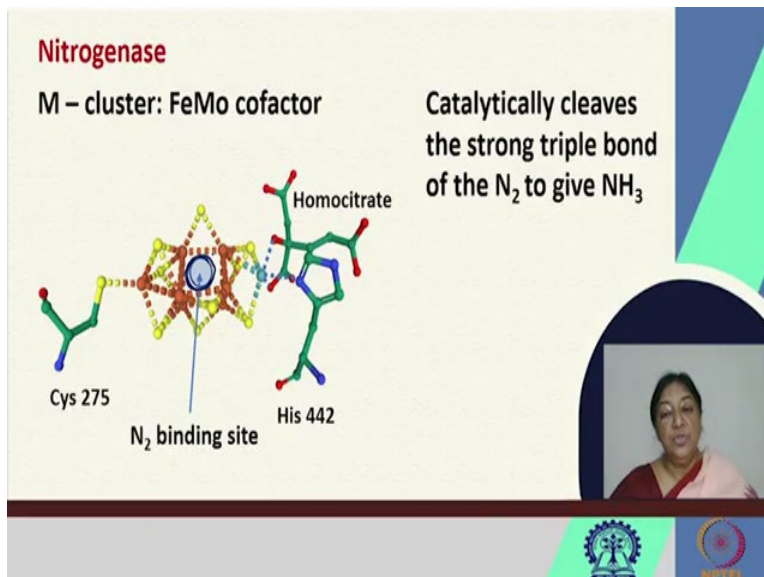
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So this [refer to slide] P cluster, the  $\text{Fe}_8\text{S}_7$  cluster. We have the  $\alpha$  sub unit, this is where we have the  $\beta$  subunit and this is at the interface of the clusters. The core  $\text{Fe}_8\text{S}_7$  of the P cluster has a form of 2  $\text{Fe}_4\text{S}_3$  cubes that are linked by this central sulfur atom. Each P cluster is covalently linked to

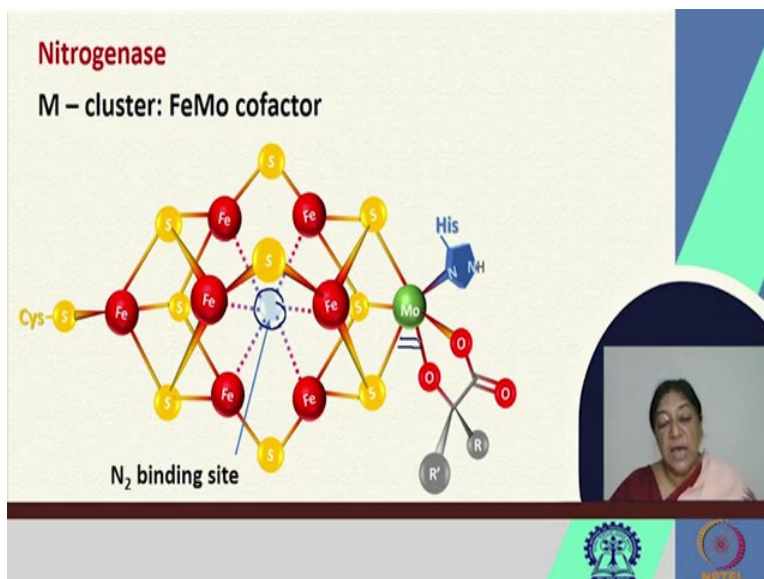
the MoFe protein by 6 cysteine residues as we can see, in the specific structure of the cluster that is shown.

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When we look [refer to slide] at the FeMo cofactor, there is the specific coordination associated with this binding site and the accommodation of the nitrogen is extremely important in the nitrogen fixation study or the biological nitrogen fixation associated with the nitrogenase enzyme. This has the capability of catalytically cleaving the strong triple bond of nitrogen, to result in the formation of ammonia.

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If we look [refer to slide] at the M cluster that is associated with the FeMo cofactor, in this case we are looking at the nitrogen binding site present here and we see the association of the molybdenum that is coordinated not only to a histidine, but to specific side chains and carbonyl atoms associated with the polypeptide chain.

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**Nitrogenase**

**M – cluster: FeMo cofactor**

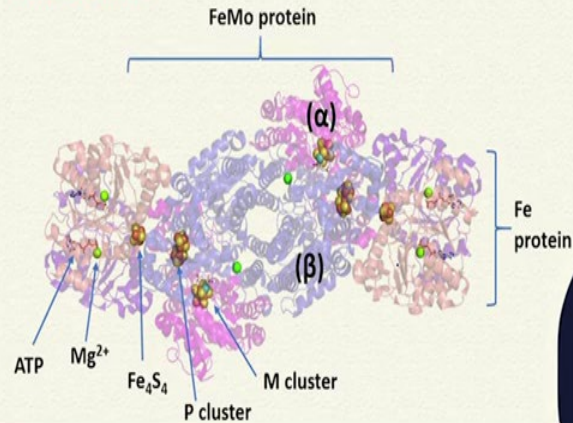
- Each FeMo cofactor ( $\text{Fe}_7\text{MoS}_9\text{C}$ ) consists of two non-identical clusters –
  - $[\text{Fe}_4\text{S}_3]$  and  $[\text{MoFe}_3\text{S}_3]$
  - They are linked by three sulfide ions
- Each FeMo cofactor is covalently linked to the  $\alpha$  subunit of the protein by one Cys residue and one His residue



So, each FeMo contains 2 non identical clusters that have  $\text{Fe}_4\text{S}_3$  and  $\text{MoFe}_3\text{S}_3$ ; one of the iron atoms being replaced by the molybdenum. They are linked by 3 sulfide ions and each FeMo cofactor is then covalently linked to the  $\alpha$  subunit of the protein by one cysteine residue and one histidine residue.

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**Nitrogenase**



FeMo protein

$(\alpha)$

$(\beta)$

Fe protein



ATP

$\text{Mg}^{2+}$

$\text{Fe}_4\text{S}_4$

P cluster

M cluster





So we have the specific orientation of these methodologies; the compounds, the M cluster, the P cluster, the Fe<sub>4</sub>S<sub>4</sub> and the α and the β subunits. Where we will see the Fe protein, the FeMo protein and specific iron sulfur cages associated with the proteins.

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**Nitrogenase**

**FeMo protein:**

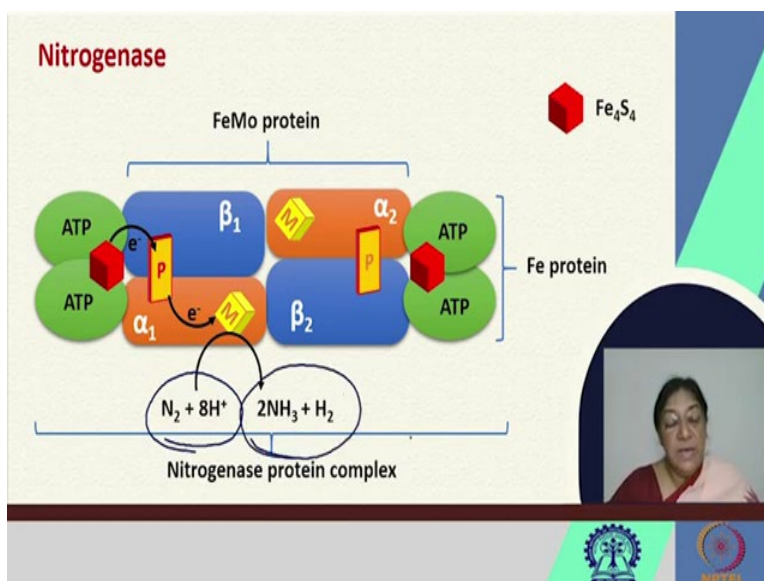
- Electrons from the Fe protein goes to the MoFe protein at the P-clusters
- Then electron transfer to the FeMo cofactors occurs
- Each of the FeMo cofactors acts as a site for nitrogen fixation
- The N<sub>2</sub> binding happens in the central cavity of the cofactor



So the electrons from the Fe protein go to the MoFe protein at the P-clusters and this is followed by electron transfer and we have these cofactors acting as sites for the nitrogen fixation. The nitrogen binding as we saw happens in the central cavity of the cofactor.

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**Nitrogenase**



FeMo protein

Fe<sub>4</sub>S<sub>4</sub>

ATP

ATP

ATP

ATP

β<sub>1</sub>

α<sub>2</sub>

α<sub>1</sub>



β<sub>2</sub>

Fe protein

N<sub>2</sub> + 8H<sup>+</sup>

2NH<sub>3</sub> + H<sub>2</sub>

Nitrogenase protein complex




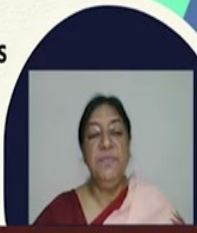
Here [refer to slide], we see a cartoon representation of the Fe protein, where we have the Fe protein, the FeMo protein and we have the whole nitrogenase protein complex, an important

complex, involved in nitrogen fixation. This is where we have our nitrogen fixation. Any  $N_2$  go to  $2NH_3$ , in this protein complex.

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**Transferrin**

- Mediates the transport of iron through blood plasma
- Acts as an antimicrobial agent by scavenging iron
- Binds iron tightly but reversibly
- Generally found in biological fluids of vertebrates eg. - tears, saliva, milk (lactoferrin)

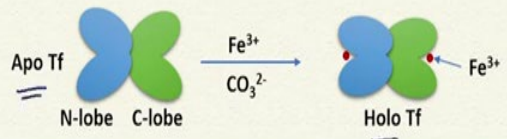


Another very important iron containing protein associated with iron storage and transport is transferrin. Transferrin mediates the transport of iron through the blood plasma. It acts as an antimicrobial agent as well, to scavenging the iron that is present. It binds iron tightly but reversibly, so that it can be utilized when required and it is generally found in biological fluids.



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**Transferrin**

- Contains 2 similar domains (N-lobe and C-lobe)
- Each domain comprised of two sub-domains
- Each domain binds one  $Fe(III)$  and one  $CO_3^{2-}$  ion
- Domains show a hinge like bend to clamp down upon the iron – carbonate unit



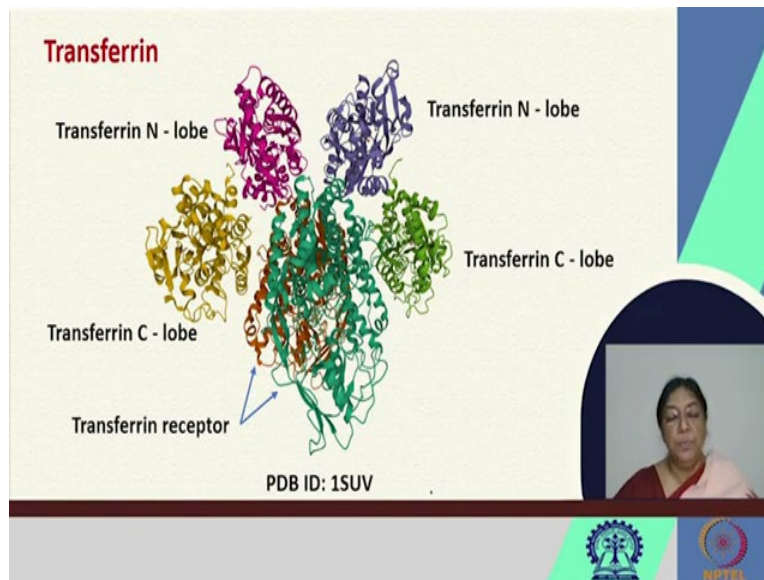
The diagram illustrates the binding of iron and carbonate ions to transferrin. On the left, Apo Tf is shown with two lobes, N-lobe (blue) and C-lobe (green). An arrow labeled with  $Fe^{3+}$  and  $CO_3^{2-}$  points to the right, where Holo Tf is shown. The Holo Tf structure shows the N-lobe and C-lobe clamped together, with red dots representing  $Fe^{3+}$  ions and blue dots representing  $CO_3^{2-}$  ions bound within the structure.



The transferrin contains 2 similar domains; the N lobe and the C lobe and these again are comprised of two sub-domains that binds an  $Fe^{3+}$  and a carbonate ion. The domain shows a hinge

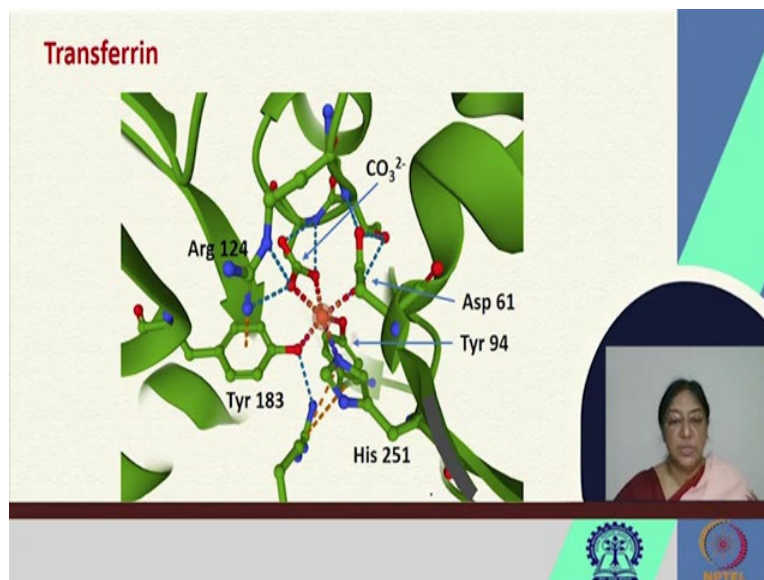
like bending type of capability, to clamp down on the iron carbon unit. So this [refer to slide] is how it clamps down on the iron carbon unit, where we have the apo form of transferrin and we have the holo form of transferrin.

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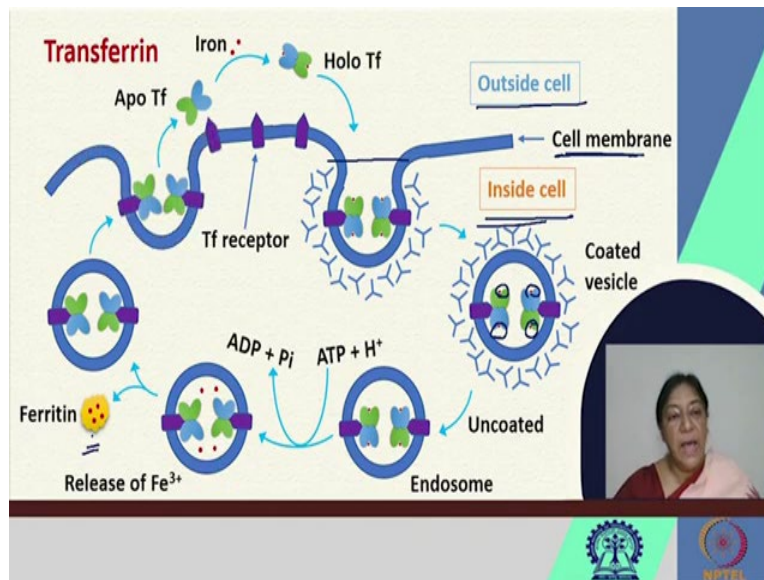
This [refer to slide] is the transferrin example where we have the N lobe and we have the C lobe and the specific transferrin receptor, where it is going to bind to the iron for the specific transport.

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The transport therefore occurs in a fashion where we have the coordination, we have the carbonate ion here and the coordination of the iron occurs through the tyrosine and the histidine moieties present here.

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The apo Tf now with the iron that is going to form the holo Tf, has a specific transferrin receptor. This transferrin receptor then forms inside the cells on the transferrin receptor that is present on the cell and we have the cell membrane.

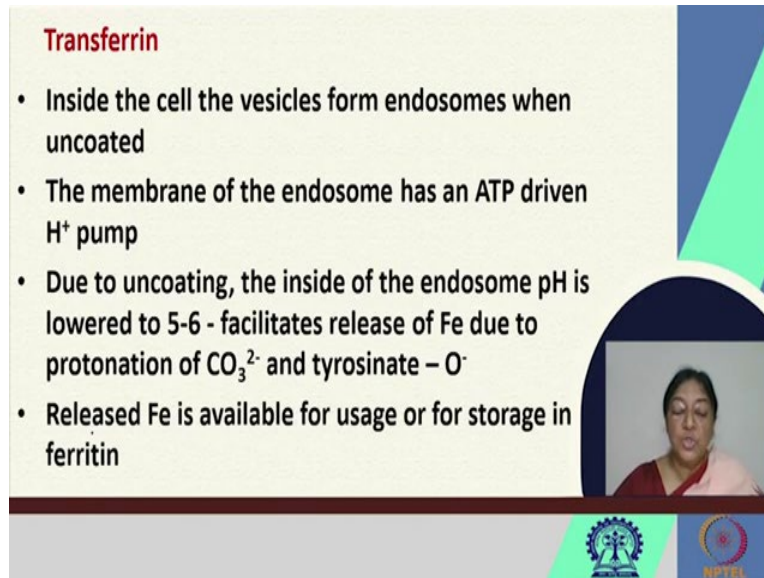
What is formed in this case, is a specific coated vesicle that has the iron bound to it inside. This [refer to slide] is where we have the iron bound, the red dots indicate the iron bound. It is a coated vesicle that is cleaved off here forming a coated vesicle inside the cell. Once this is uncoated it forms what is called an endosome, that with the help of an ATP proton pump then releases the iron. Once this iron is released it is taken up by ferritin, which is the storage protein and we again have our apo Tf ready to bind iron again.

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### Transferrin

- Inside the cell the vesicles form endosomes when uncoated
- The membrane of the endosome has an ATP driven  $H^+$  pump
- Due to uncoating, the inside of the endosome pH is lowered to 5-6 - facilitates release of Fe due to protonation of  $CO_3^{2-}$  and tyrosinate  $-O^-$
- Released Fe is available for usage or for storage in ferritin



So inside the cell, the vesicles form endosomes when they are uncoated. The membrane of the endosomes has a specific ATP driven proton pump and due to the uncoating the inside of the endosome pH is lowered to about 5 or 6 and this facilitates the release of iron due to the protonation of the carbonate and the tyrosinate  $O^-$ . The released Fe is now available for storage in ferritin.

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### Ferritin

- Ferritins are metalloproteins that can contain unusually large amount of metal (iron) and releases it in a controlled fashion
- Storage of iron in an inert but accessible form
- Produced by almost all living organisms, including archaea, bacteria, algae, higher plants and also animals




So what is ferritin? Ferritins are metalloproteins that can contain unusually large amounts of metal that is the iron and release it in a very controlled fashion. The storage of iron in an inert, but accessible form and it is produced by almost all living organisms.

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### Ferritin

- Ferritin is a hollow globular protein comprised of 24 subunits with a molecular weight of 474 kDa
- Inside the ferritin shell, iron ions form crystallites together with phosphate and hydroxide ions
- In animal ferritin, iron is stored as oxy-hydroxide  $[\text{Fe}^{\text{III}}(\text{O})(\text{OH})]$


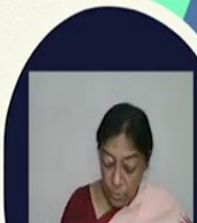


This is a hollow globular protein a 24 mer protein; that means it has 24 subunits with a molecular weight of 474 kDa. Inside the ferritin shell, iron ions form crystallites together with the phosphate and hydroxide ions, where it is stored in an oxy hydroxide form.

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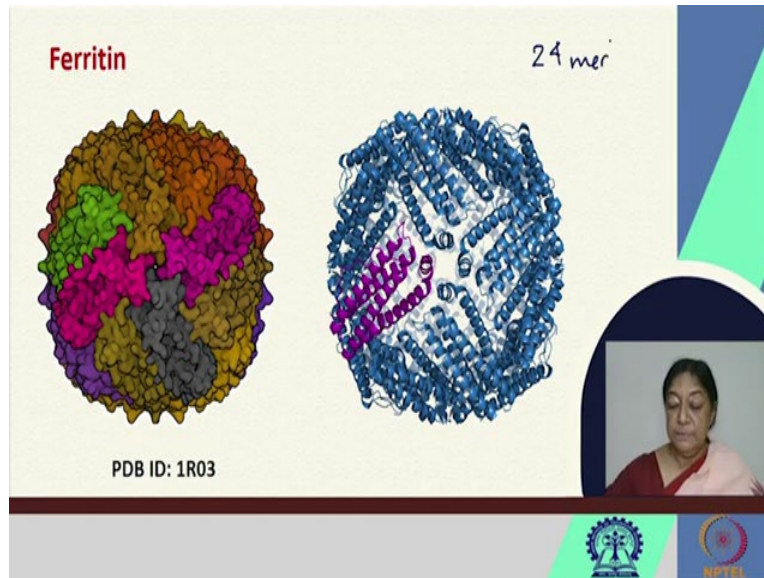
### Apoferritin

- Apoferritin is Ferritin without the iron
- It is generally present in the mucosa membrane of the intestine and the liver
- It performs an important biological function in the binding and storing of iron
- This is achieved by combining with a ferric hydroxide–phosphate compound to form ferritin



Apoferritin on the other hand is ferritin without the iron. This is generally present in the mucosa membrane of the intestine and the liver. It performs a very important biological function in the binding and the storing of the iron and this is achieved by combining with ferric hydroxide phosphate compound to form our ferritin.

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**Ferritin test**

Determines total iron storage capacity

- Diagnosis for iron deficiency or iron overload

Normal range for ferritin in blood serum:

Females: 12 to 150 ng/mL

Males: 12 to 300 ng/mL

High levels: liver disease, rheumatoid arthritis...

Low levels: iron-deficiency anemia

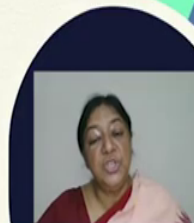
This [refer to slide] is the structure of the 24 mer ferritin that we see. The ferritin test is used nowadays to determine the total iron storage capacity. So apart from hemoglobin tests that are now done to determine the amount of hemoglobin in the body, that being an iron transport protein as well, this is an iron storage capacity protein.

So the diagnosis for this is tested for iron deficiency or iron overload. The normal range for ferritin in blood serum is given for females and males and if we have a high level this could lead to liver disease and rheumatoid arthritis among others and low levels could lead to iron deficiency anemia.

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### Two -site immunoradiometric assay

- Blood serum is mixed with antibody (antihuman ferritin) conjugated plastic beads that act as a solid phase. Ferritin present in the serum will bind with the solid phase.
- The insoluble antihuman ferritin complex is treated with radio labelled ( $^{125}\text{I}$ ) antihuman ferritin.
- Solid phase is then washed and counted in a gamma counter and the ferritin concentration determined using calibration curve.



The typical test is called a two side immunoradiometric assay. We will not go into the details of this, this is beyond the course. But just to give you some idea that the blood serum is mixed with that specific antibody, that is an antihuman ferritin and these are conjugated on plastic beads that act as a solid phase.

The ferritin that is present in the serum will bind with this. The insoluble antihuman ferritin complex is then treated with a radio labelled antihuman ferritin and the solid phase is just washed off and then this is checked with the  $\gamma$  counter, to check the amount of ferritin concentration using a calibration curve, that can be indicative of whether there is iron deficiency or iron overload in the body.

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### Summary

- Importance of metal ions in biochemical pathways
- Metal coordination and electron transfer



So our discussion on metalloproteins has touched upon the importance of metal ions in biochemical pathways. We have looked at specific metal ions in this aspect and specific aspects of metal coordination and electron transfer.

(Refer Slide Time: 31:35)

**REFERENCES**

- Shriver and Atkins Inorganic Chemistry
- Metalloproteins: Methods and Protocols. Editors: Fontecilla-Camps, Juan C., Nicolet, Yvain (Eds.) 2014
- doi: 10.1016/j.cub.2013.05.033

The slide features a dark blue header with the word 'REFERENCES' in white. The main content area is light beige. A small video inset in the bottom right shows a woman with dark hair wearing a red and white sari. At the bottom, there are two logos: a circular emblem on the left and the 'SIPTEL' logo on the right.

These [refer to slide] are the references.

Thank you.