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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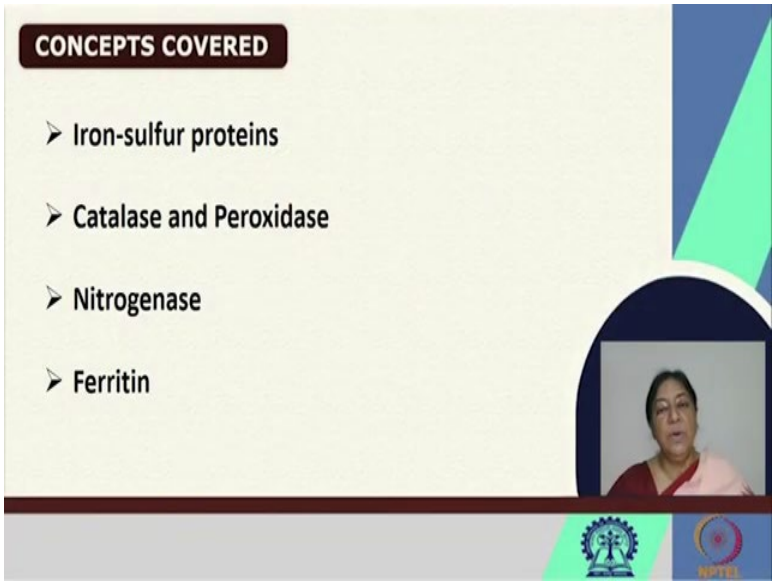

In this lecture we will be looking at iron sulfur proteins, it will be focused on iron. This will include catalase, peroxidase, nitrogenase and also ferritin.

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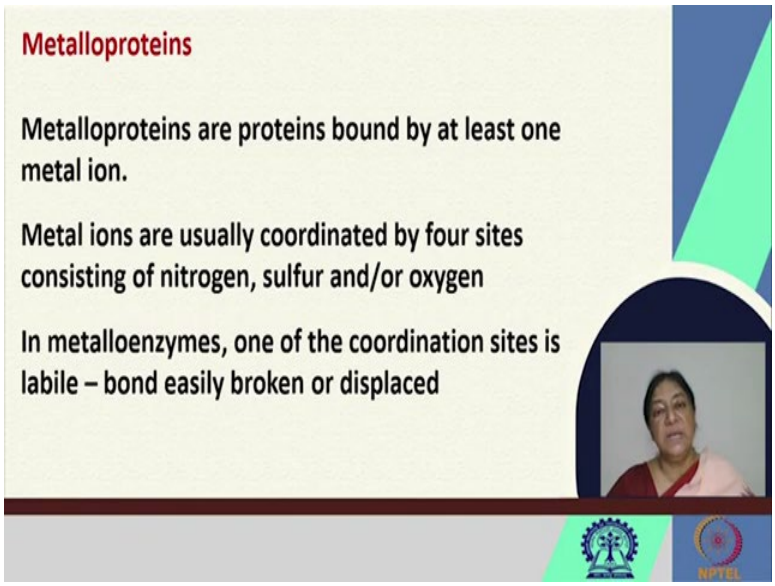

Metalloproteins in general as we saw in the previous lecture, are proteins that are bound by at least one metal ion and we looked at the abundance of the different types of metal ions that are present in proteins. The coordination occurs through the nitrogen as we saw mostly from the imidazole of histidine 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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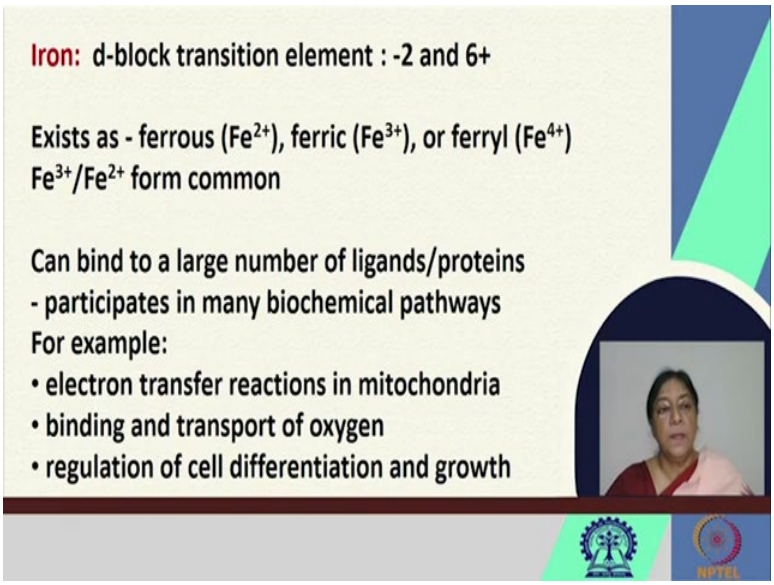

We will looking at iron in a bit more detail. This is what is called a d block transition metal and it has a large number of possible oxidation states. In the body it exists as the ferrous, that is the Fe^{2+}, the ferric Fe^{3+} or ferryl Fe^{4+}; and the Fe^{3+}/Fe^{2+} combination is the most common.

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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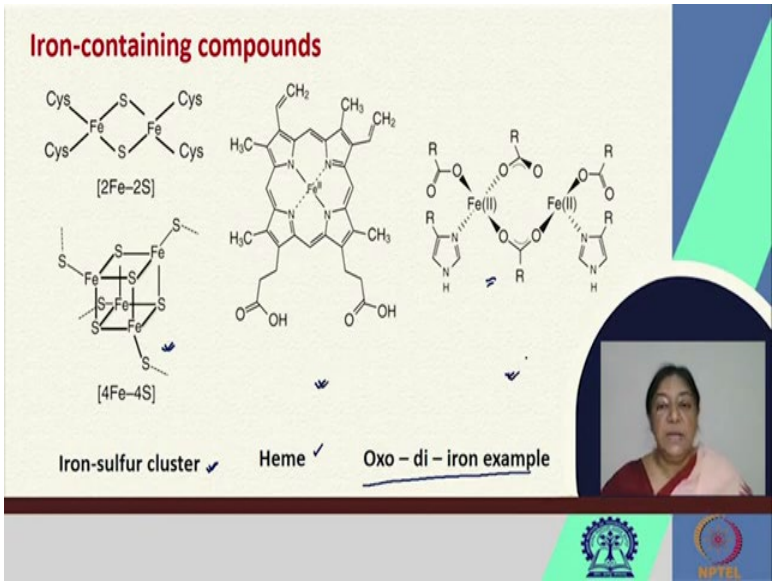

The iron containing compounds that we see in the body are the iron sulfur cluster, a very interesting type of iron containing compound present in a number of proteins. The heme that is

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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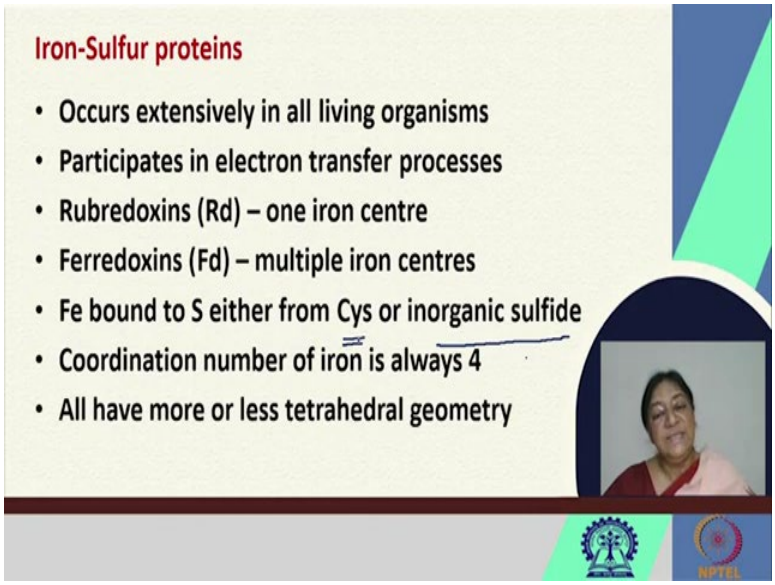

The iron sulfur proteins occur extensively in living organisms. This participates in electron transfer processes. We have an example called the rubredoxins. These rubredoxins have one iron centre and ferredoxins have multiple iron centres. The iron is bound to sulfur either in the form of an inorganic 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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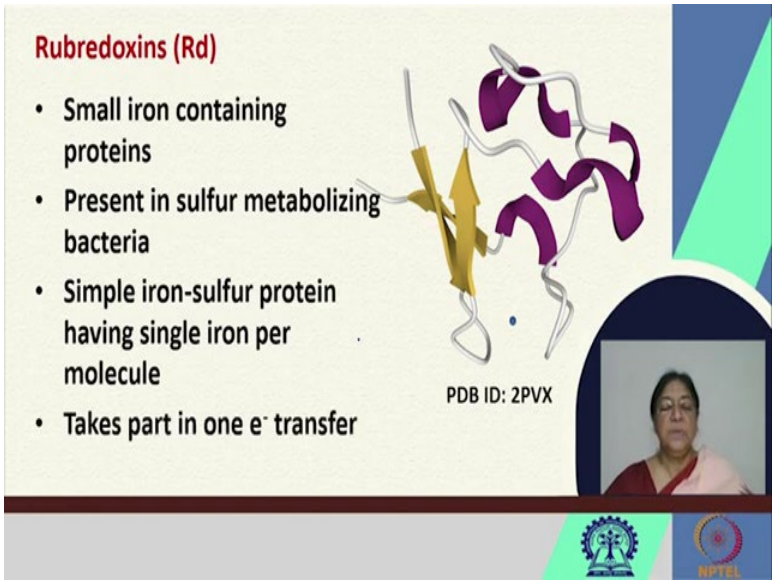

If we look at the rubredoxins. This [refer to slide] is an example of a rubredoxin protein. It is a small iron containing protein and it is present in sulfur metabolizing bacteria. The simple iron sulfur protein has a single iron per molecule of the protein it takes part in one electron transfer.

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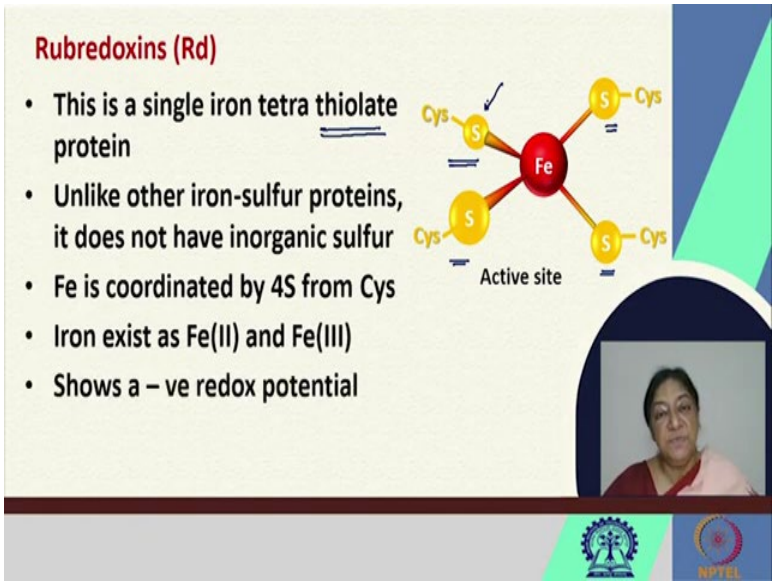

This is the active site where the iron sulfur is obtained, where we have the sulfurs that are coordinated from the cysteine residues present in the protein, that coordinate the iron. So the single iron tetra thiolate protein. The thiolate indicates that this is from the sulfur atom that we see in the 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 Fe^{2+} and Fe^{3+}, being involved in a specific process showing a negative redox potential.

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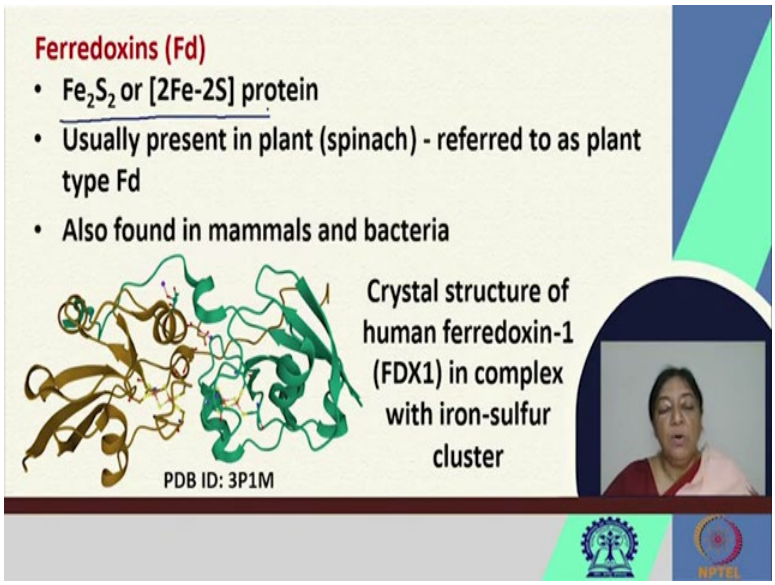

Ferredoxins that are multiple iron centers, have an indication of the presence of an iron sulfur cluster. In this case their representation is in this manner where we have 2Fe-2S or written as Fe_2S_2 protein, indicating the type of the cluster that is present on the iron sulfur cage, as it is sometimes 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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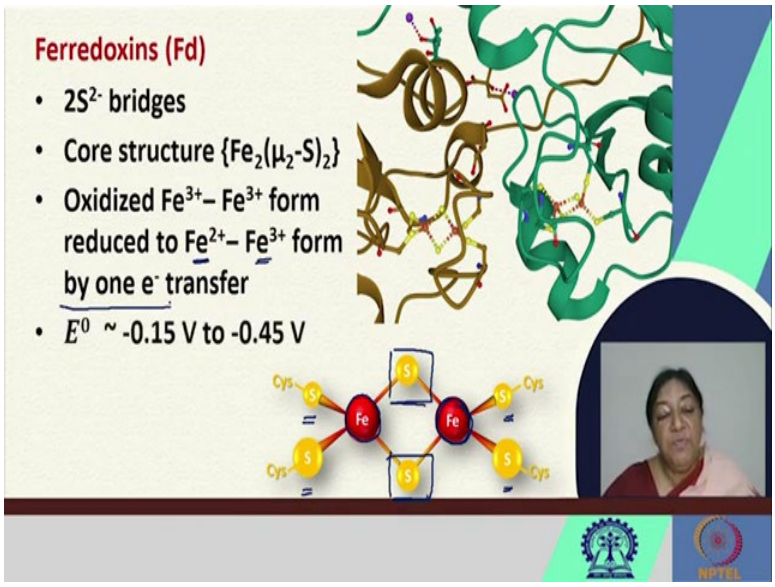

If we look [refer to slide] at the specific structural aspects of this protein, these are the two iron atoms that are present. These are coordinated with the sulfur from the 2 cysteine amino acids present in the polypeptide chain of the protein in each case. But also we have a coordination with an inorganic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

So this is where we see the specific 2 sulfide bridges. The core structure is represented as $\{Fe_2(\mu_2\text{-}S_2)\}$. 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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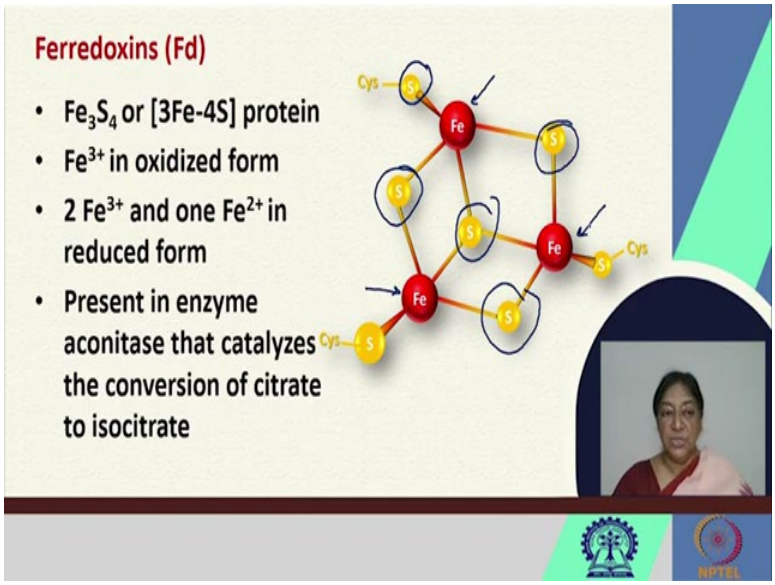

The ferredoxins structure can also have a Fe_3S_4 type of structure, the representation would be 3Fe-4S in it is nomenclature, where again we see the iron sulfur cage that could have the sulfur from the cysteine residue or the inorganic sulfurs, that are associated here in this specific type of 3Fe-4S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

Here [refer to slide], Fe^{3+} in it's 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 it is 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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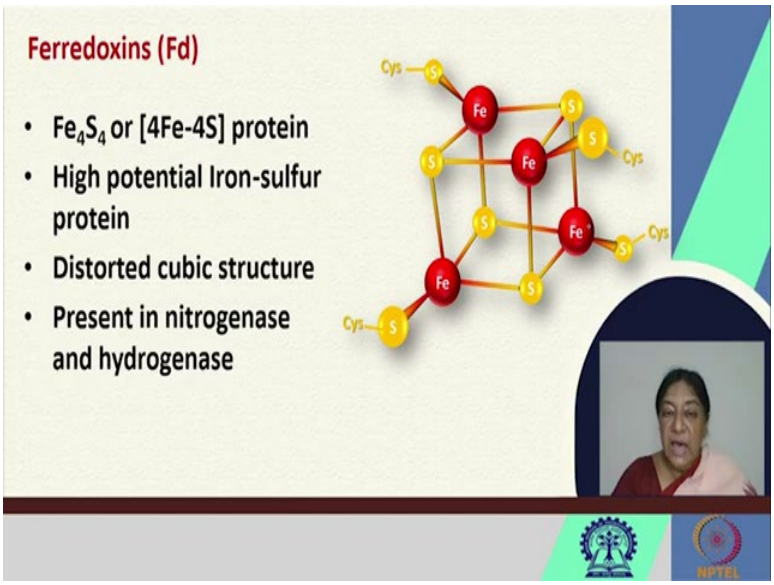

We saw the rubredoxins are the one that have a single iron centre, while ferredoxins have multiple iron centres. We saw an example of 2 an example of 3; this is where we have 4 iron centres. So, this is a 4Fe-4S protein. It is a high potential iron sulfur protein, forming a distorted cubic structure 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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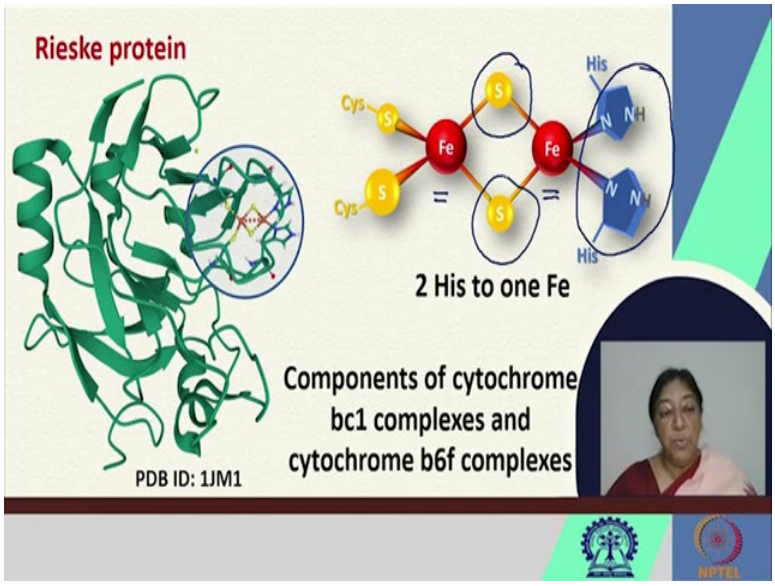

The rieske protein is another protein that has the iron sulfur cage, where we have the combination of 2 histidines coordinated to an iron and we have 2 inorganic sulfur. And these are components of cytochrome bc1 complexes and cytochrome b6f complexes; very important aspects of electron transfer.

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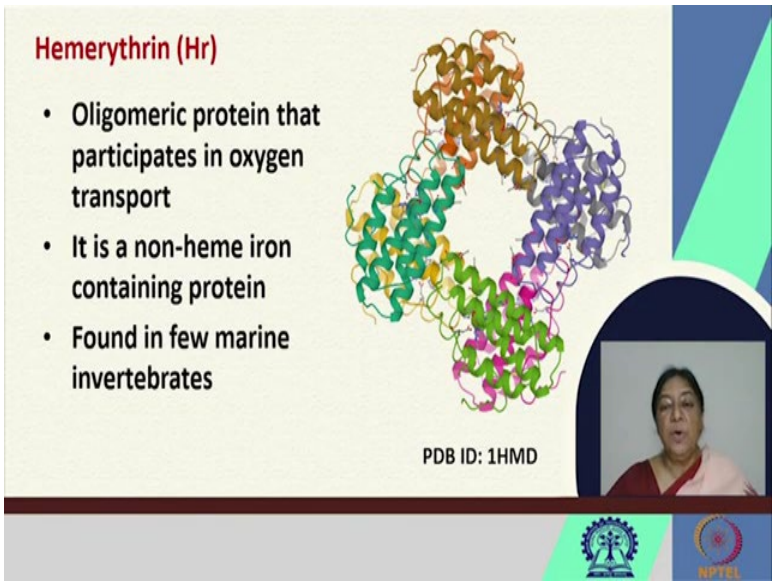

In the protein hemerythrin, we have the structure as an oligomeric protein that participates in oxygen transport. It is a non-heme iron containing protein and it is also found in a few marine invertebrates.

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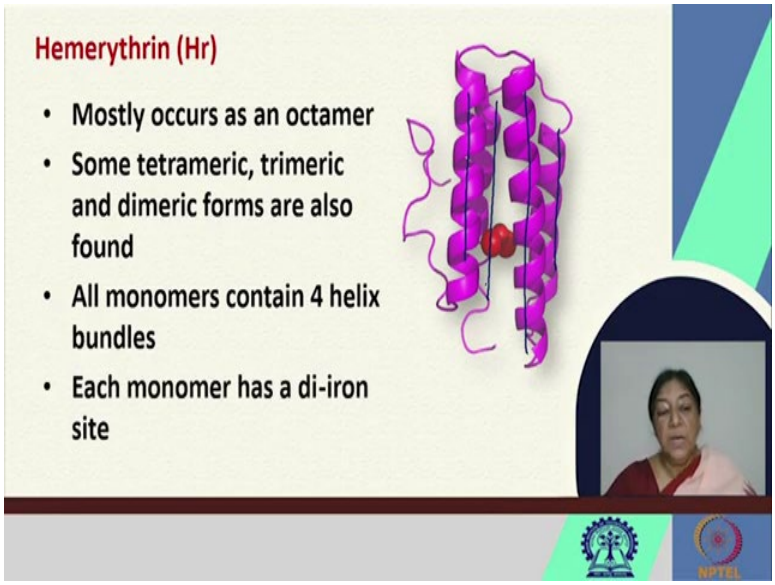

This structure occurs mostly as an octamer and some tetrameric, trimeric and also dimeric forms are also available. Here [refer to slide] we see the monomers that have a 4 helix bundle to them, as indicated by the 4 helices that are observed here; the 4 helices that form the 4 helix bundle. Each monomer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

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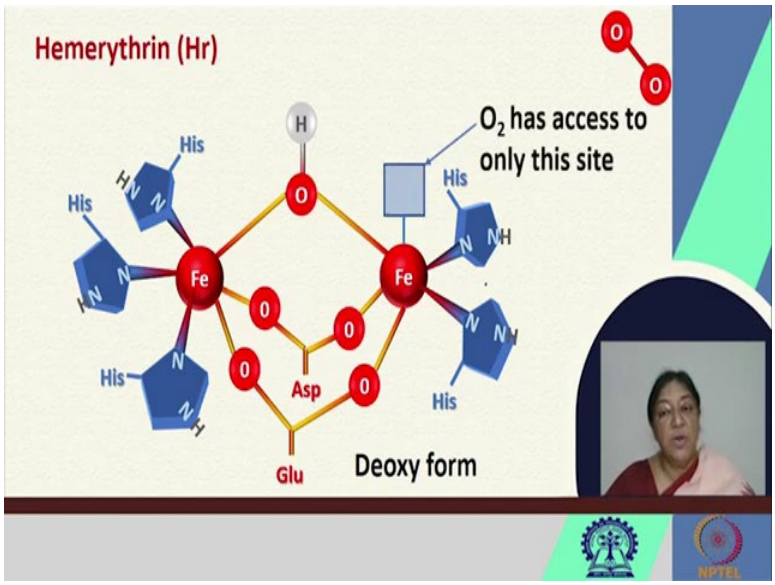

In this case we have the di-iron site, the coordination is through the histidine. So, we have now a glutamic acid come into the picture [refer to slide], where we have the specific coordination with the aspartic acid, glutamic acid and the histidine moieties in hemerythrin, but there is no heme as seen 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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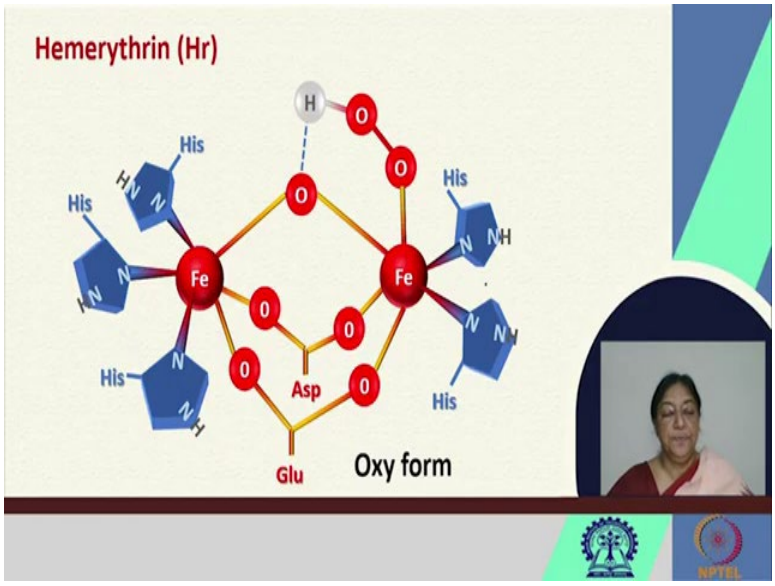

We have the formation of a specific coordination site present on iron and we will have bound oxygen to it. The oxy form has a diferric oxo bridge, as it is called. This is an example of hemerythrin.

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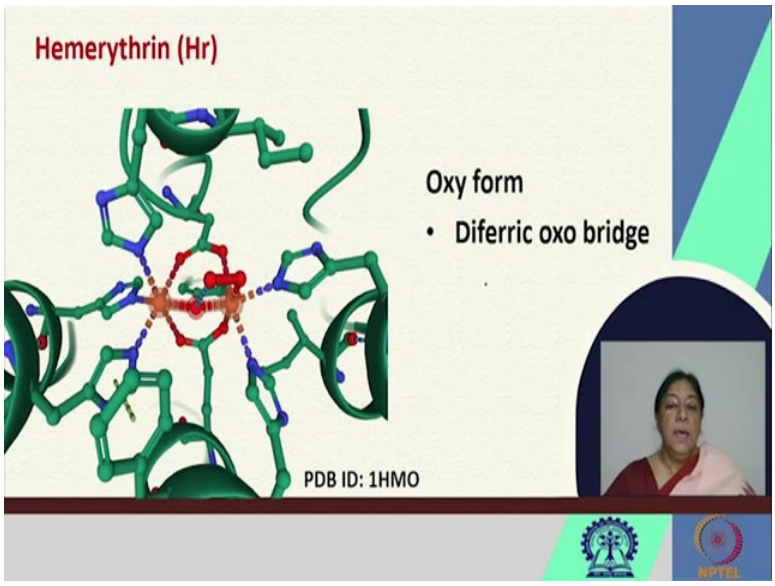

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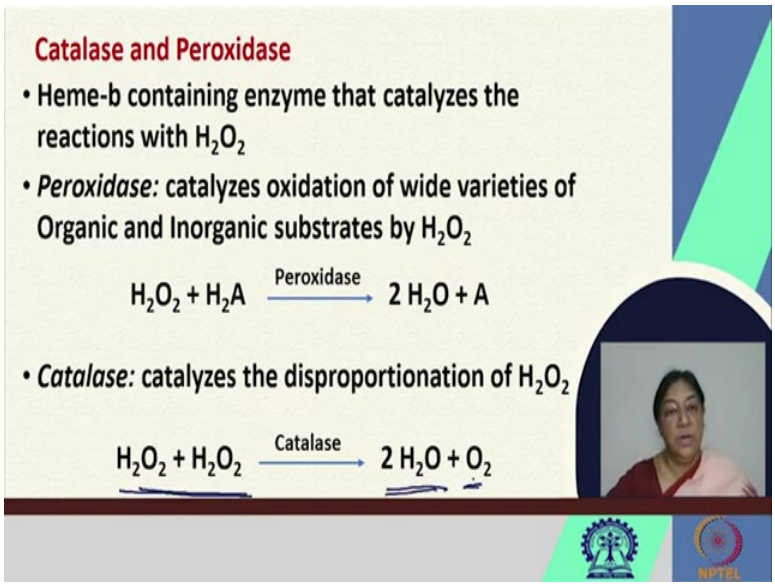

For catalase and peroxidase, these are specific proteins that are heme binding proteins; the heme-b containing enzyme that catalyzes the reactions with hydrogen peroxide. Peroxidase catalyzes the oxidation of a large variety of organic and inorganic substrates by H_2O_2. And this [refer to slide] is an example of the reaction of peroxidase. Catalase on the other hand, catalyzes the disproportionation of H_2O_2 in a specific type of reaction, where we notice the disproportionation to H_2 and O_2; an oxidation and a reduction.

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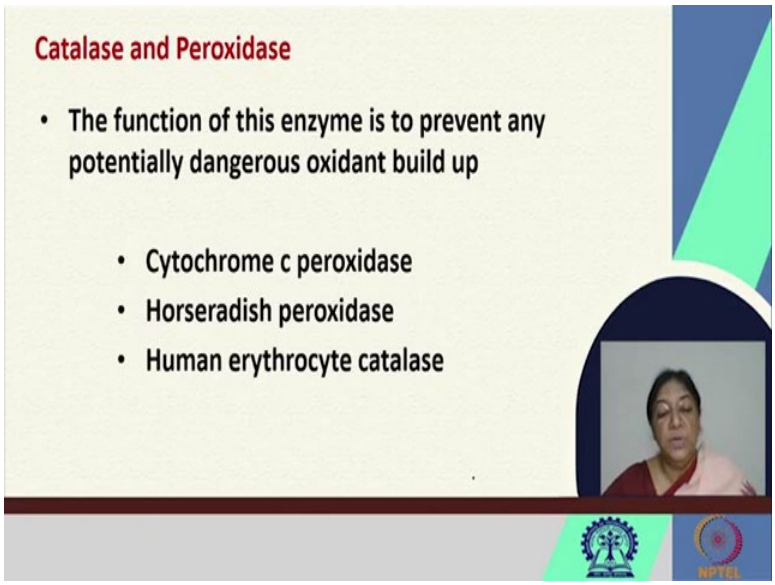

The function of the catalase and the peroxidase enzymes, is to prevent any potential dangerous oxidant buildup and these are specific examples of such type of enzymes, the cytochrome c peroxidase, horseradish peroxidase and human erythrocyte catalase.

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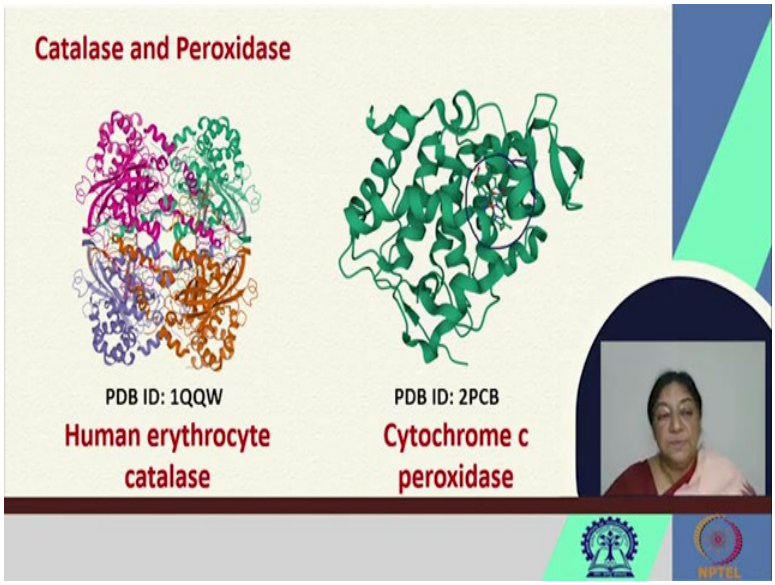

This [refer to slide] is the structure of the human erythrocyte catalase and this is the structure of the cytochrome c peroxidase, where we can see the specific bound iron atoms here.

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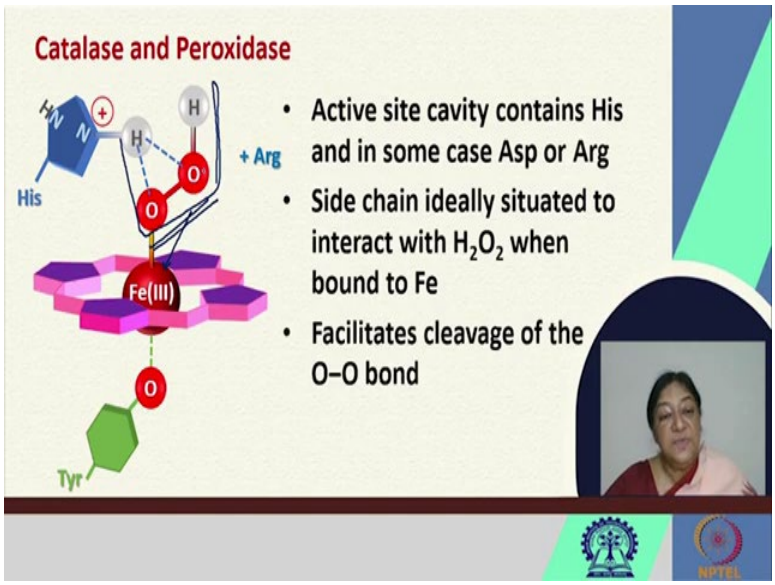

In the heme bound form, we have the Fe^{3+} bound in this form, there is a specific coordination with the 2 oxygen atoms here. The active site cavity contains histidine and in some cases even aspartic acid or arginine. The side chains are ideally situated in a manner that would interact with the hydrogen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

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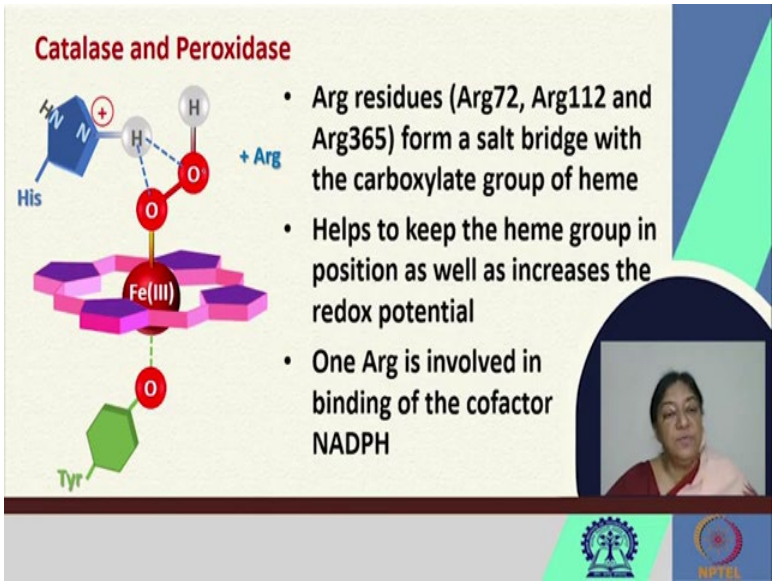

This facilitates the cleavage of this specific bond. The arginine residues involved here, are arginine 72, 112 and 365. They form a specific salt bridge with the carboxylate group of heme and this helps to keep the heme group in position, in addition to increasing the redox potential. One arginine is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

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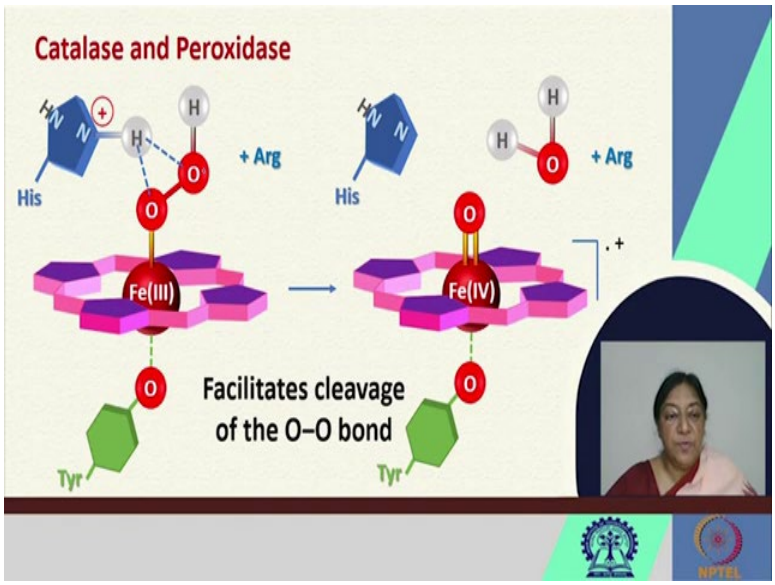

So the reaction is such that we have the formation of water.

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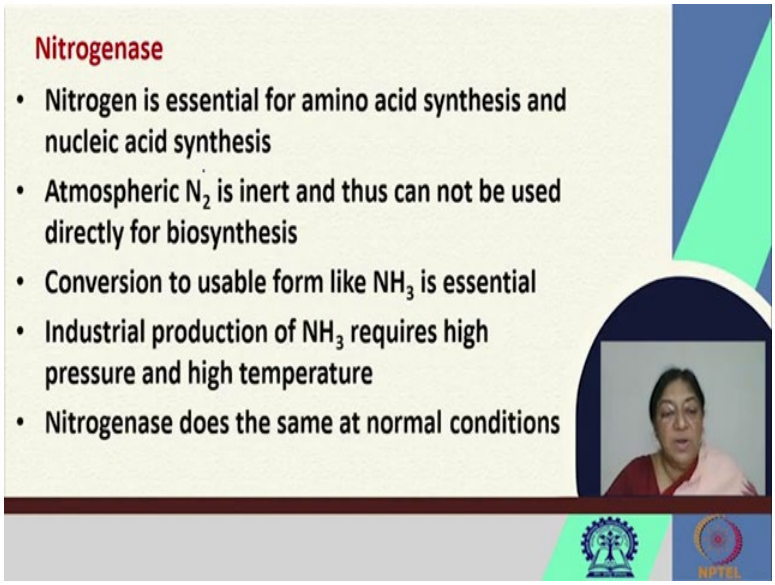

We now look at nitrogenase. In nitrogenase, this is a protein involved in the conversion of the nitrogen to a usable form such as ammonia. Nitrogen is essential for the amino acid synthesis and nucleic acid synthesis. The atmospheric nitrogen in this case is inert and it cannot be used directly.

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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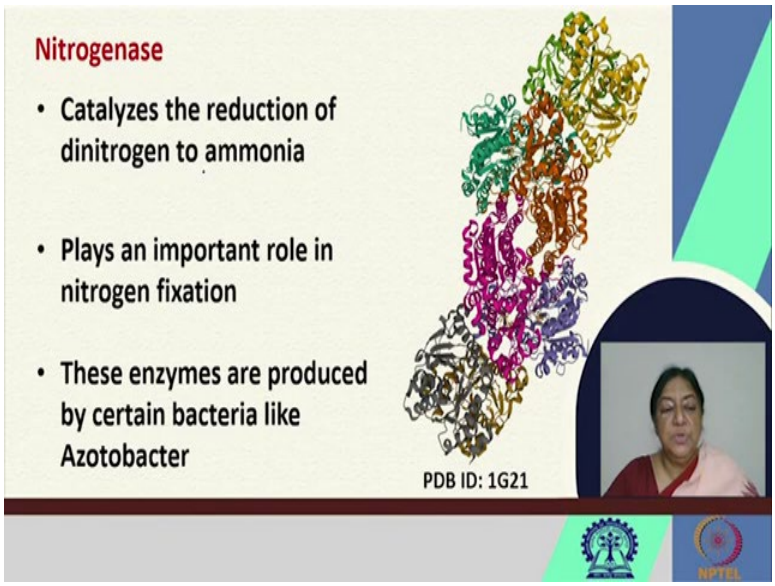

The protein itself catalyzes, therefore the reduction of dinitrogen to ammonia and it plays a very important role in nitrogen fixation. These enzymes are produced by certain bacteria like azotobacter and they occur in three forms the molybdenum form, the vanadium form and the iron form.

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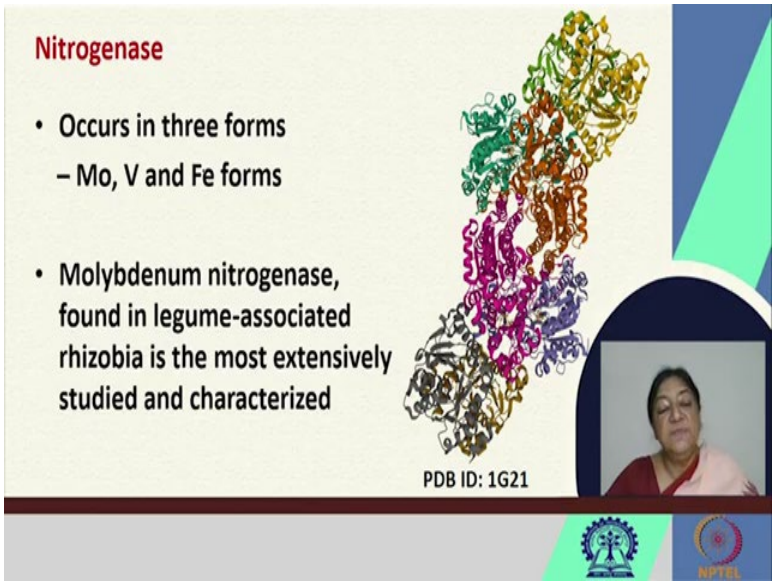

Molybdenum nitrogen is found in legume associated rhizobia and is the most extensively studied and characterized.

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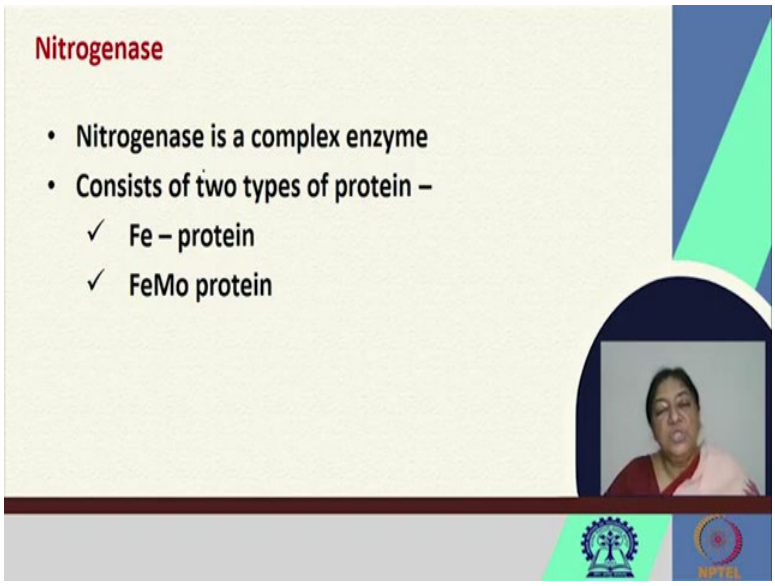

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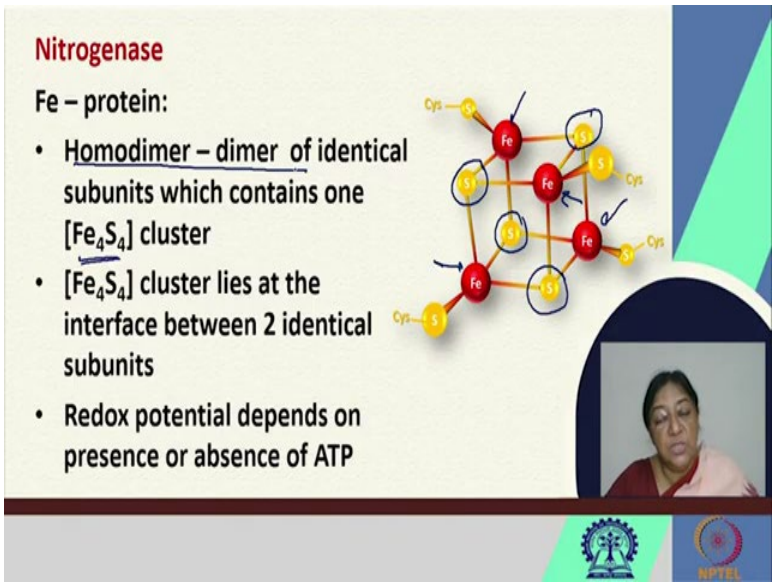

So, this is a complex enzyme that contains basically two types of proteins; one is the Fe protein and the other is the FeMo protein. In the Fe protein it is a homodimer, that means it is a dimer of identical subunits, which contains one Fe_4S_4 cluster.

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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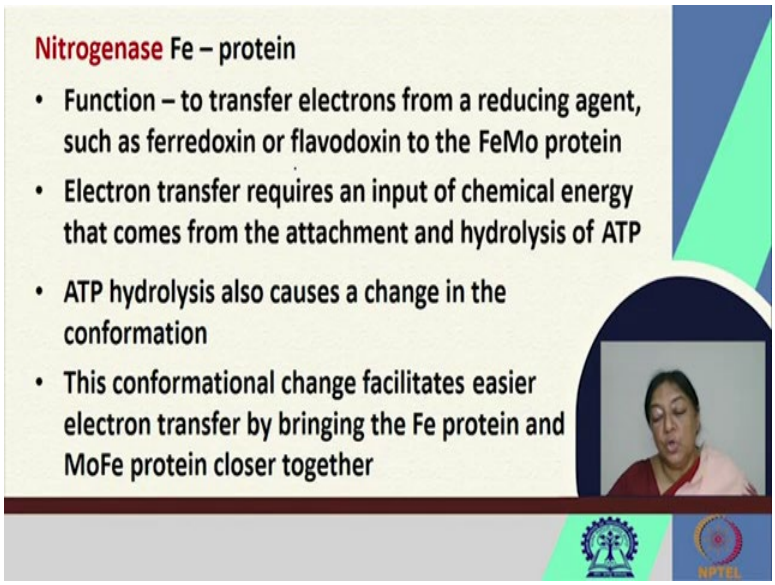

So when we look at the Fe protein, its function is to transfer electrons from a reducing agent such as ferredoxin or flavodoxin, to the FeMo type protein. This electron transfer requires an input of chemical energy, that comes from the attachment and hydrolysis of ATP. The ATP hydrolysis results in a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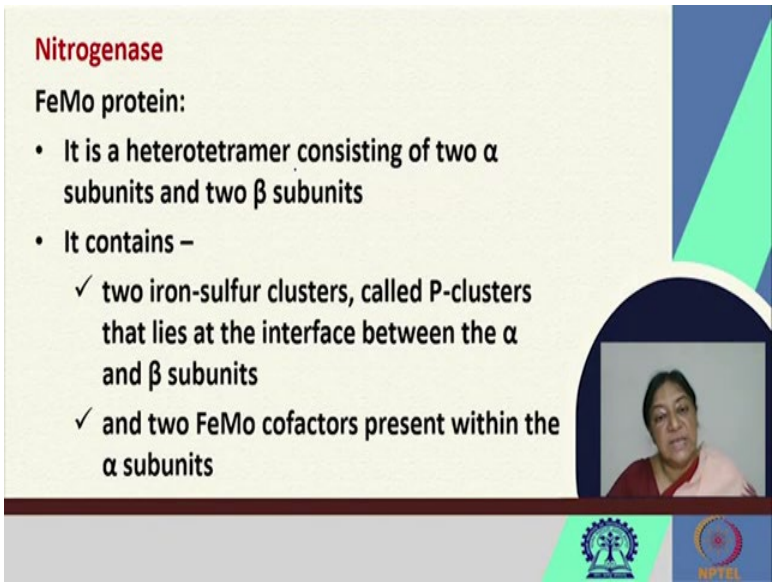

This [refer to slide] is the FeMo protein details. It is a heterotetramer that contains two α subunits and two β subunits. This contains two iron-sulfur clusters that are called P-clusters, that again lie between the interface of the α and the β subunits and the two FeMo cofactors are present within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

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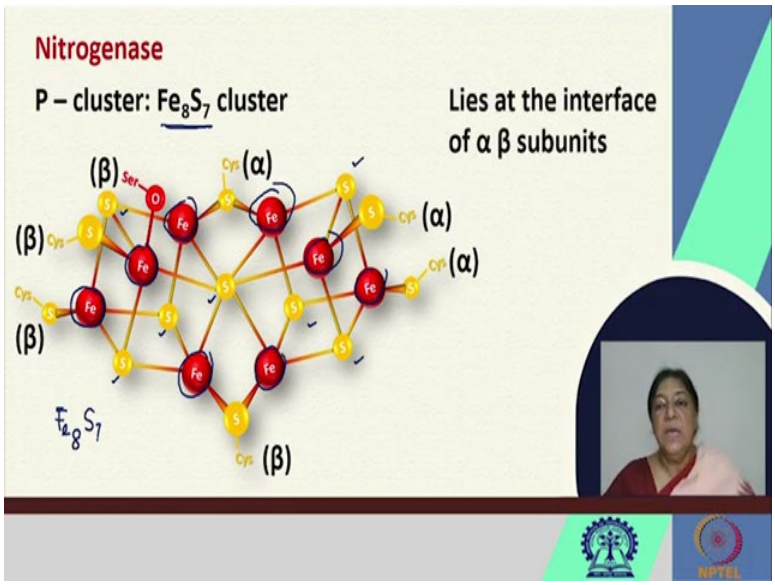

When we look at the P cluster, it is a cluster of Fe_8S_7. When we consider this cluster, we have the α and the β and then the P cluster. If we count the number of irons we have 8; so this is an Fe_8.

Following this, we look at the number of sulfur atoms that are not associated with the polypeptide chain. We have 7; so this is 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 α and the β subunits.

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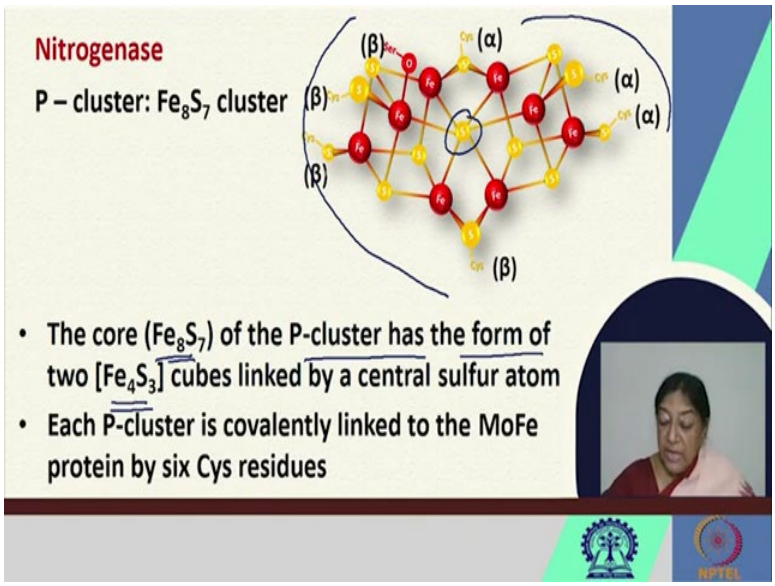

So this [refer to slide] P cluster, the Fe_8S_7 cluster. We have the α sub unit, this is where we have the β subunit and this is at the interface of the clusters. The core Fe_8S_7 of the P cluster has a form of 2 Fe_4S_3 cubes that are linked by this central sulfur atom. Each P cluster is covalently 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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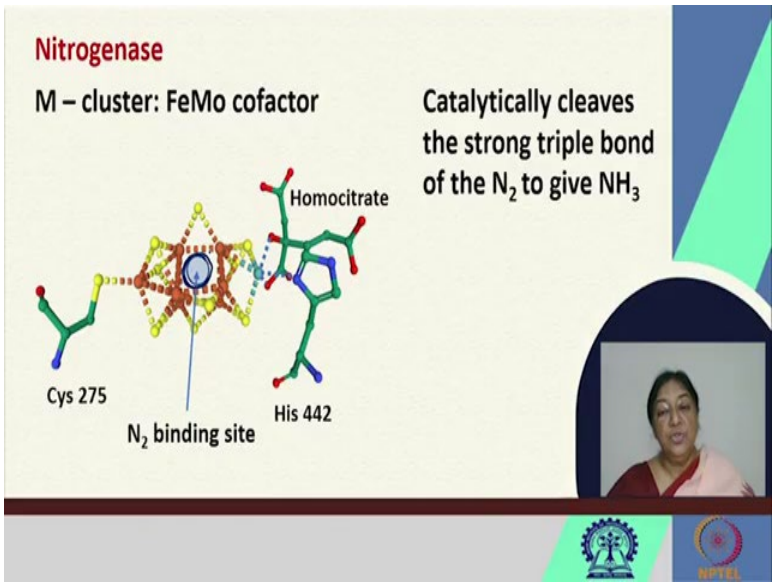

When we look [refer to slide] at the FeMo cofactor, there is the specific coordination associated with this binding site and the accommodation of the nitrogen is extremely important in the nitrogen fixation study or the biological nitrogen fixation associated with the nitrogenase enzyme. This has the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

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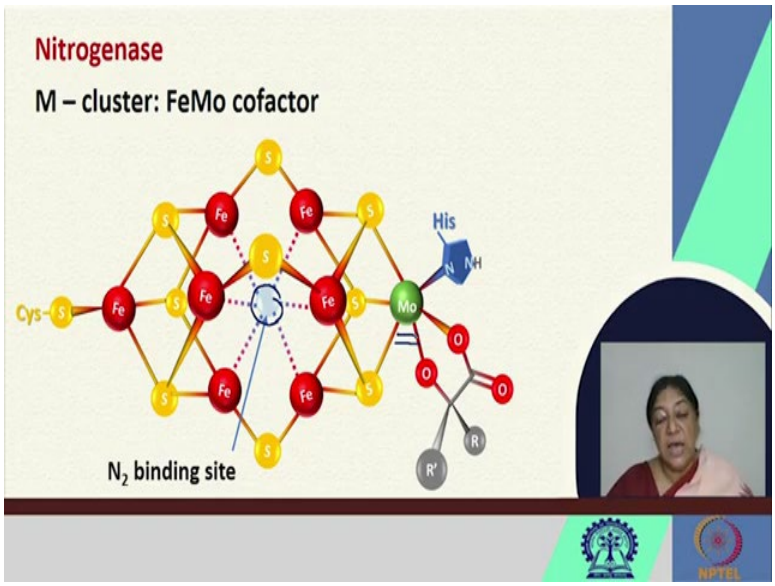

If we look [refer to slide] at the M cluster that is associated with the FeMo cofactor, in this case we are looking at the nitrogen binding site present here and we see the association of the molybdenum that is coordinated not only to a histidine, but to specific side chains and carbonyl atoms associated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

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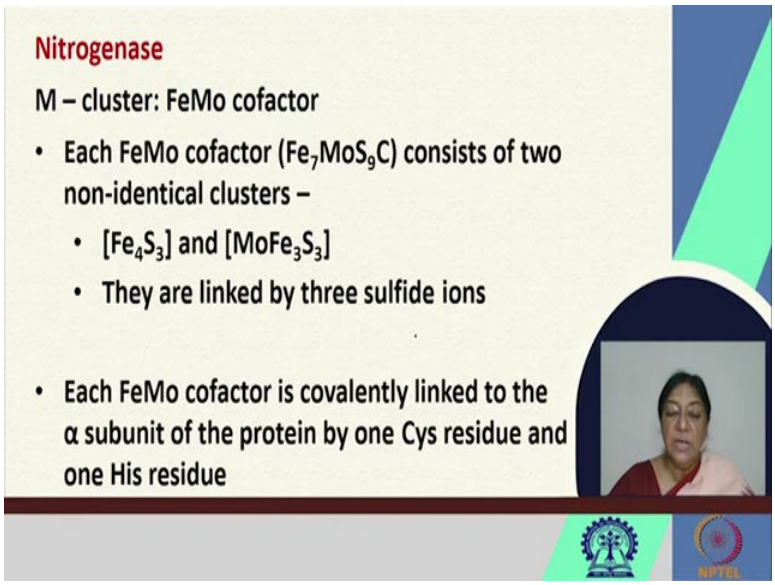

So, each FeMo contains 2 non identical clusters that have Fe_4S_3 and $MoFe_3S_3$; one of the iron atoms being replaced by the molybdenum. They are linked by 3 sulfide ions and each FeMo cofactor is then covalently linked to the α subunit of the protein by one cysteine residue and one histidine residue.

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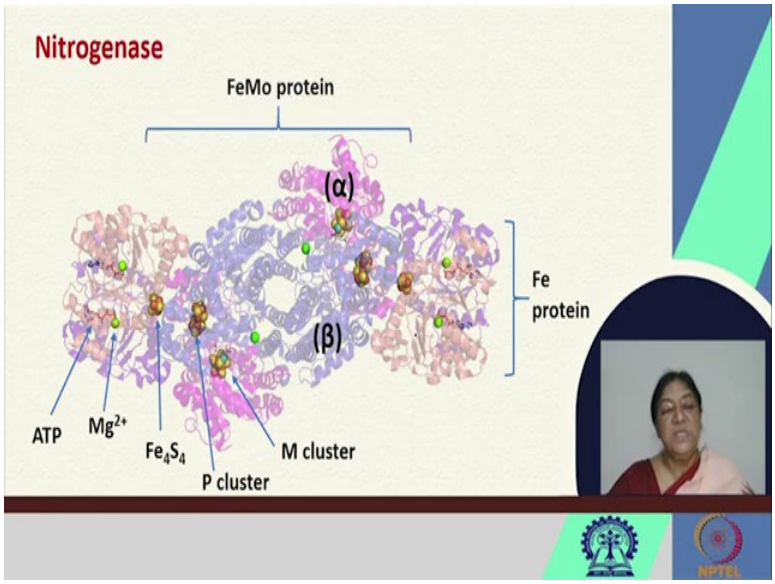

So we have the specific orientation of these methodologies; the compounds, the M cluster, the P cluster, the Fe_4S_4 and the α and the β subunits. Where we will see the Fe protein, the FeMo protein and specific iron sulfur cages associated with the proteins.

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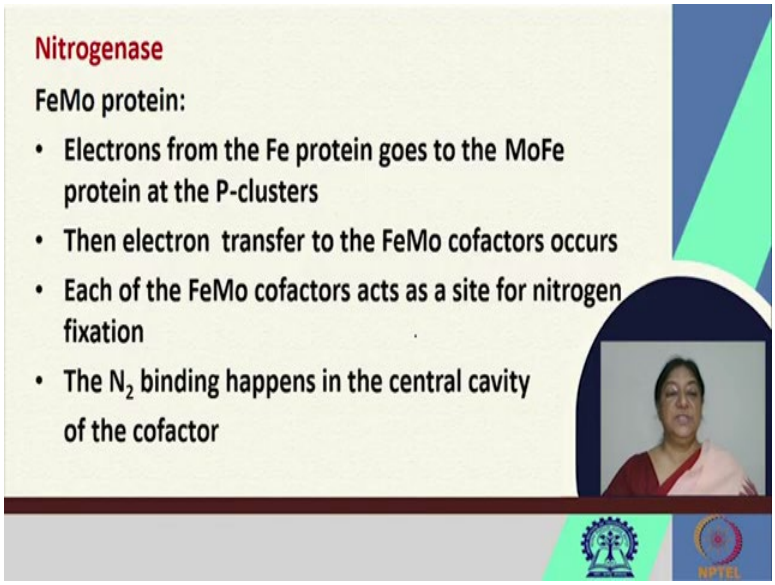

So the electrons from the Fe protein go to the MoFe protein at the P- clusters and this is followed by electron transfer and we have these cofactors acting as sites for the nitrogen fixation. The nitrogen binding as we saw happens in the central cavity of the cofactor.

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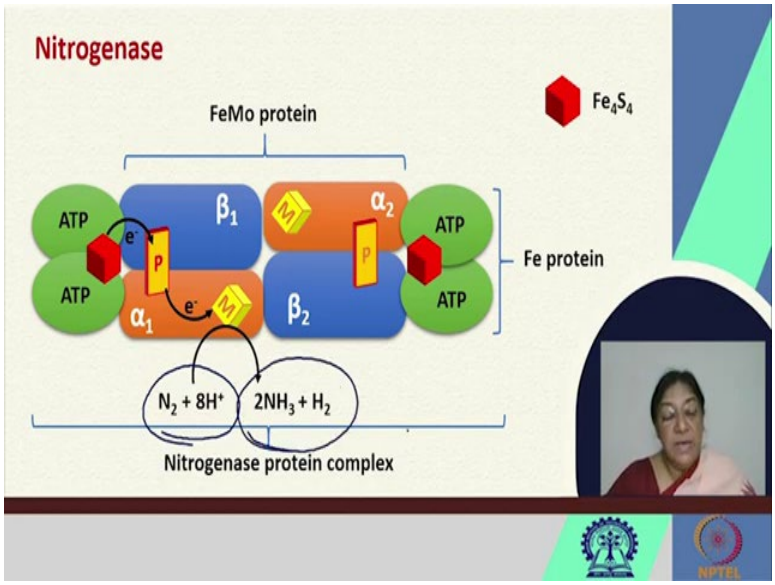

Here [refer to slide], we see a cartoon representation of the Fe protein, where we have the Fe protein, the FeMo protein and we have the whole nitrogenase protein complex, an important

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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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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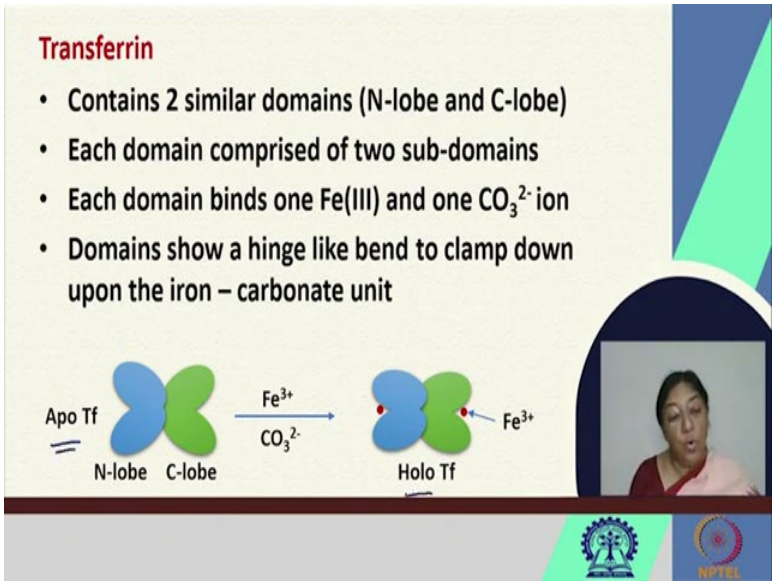

The transferrin contains 2 similar domains; the N lobe and the C lobe and these again are comprised of two sub-domains that binds an Fe^{3+} and a carbonate ion. The domain shows a hinge

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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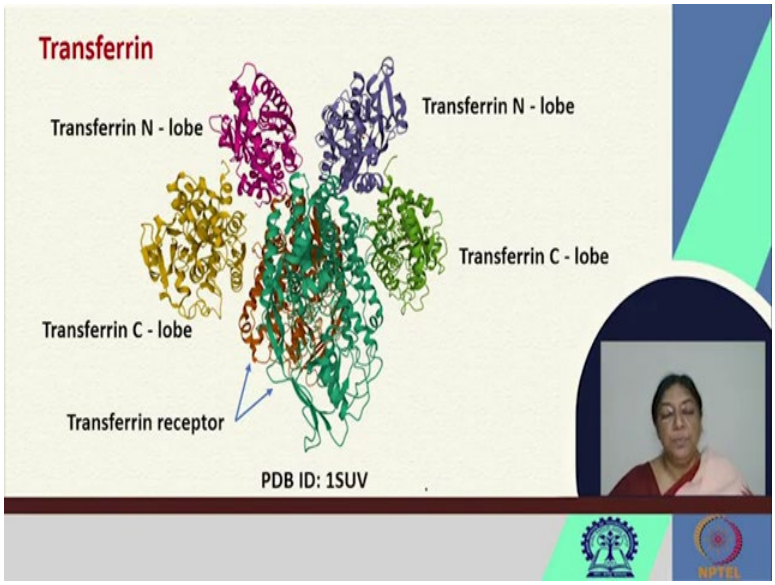

This [refer to slide] is the transferrin example where we have the N lobe and we have the C lobe and the specific transferrin receptor, where it is going to bind to the iron for the specific transport.

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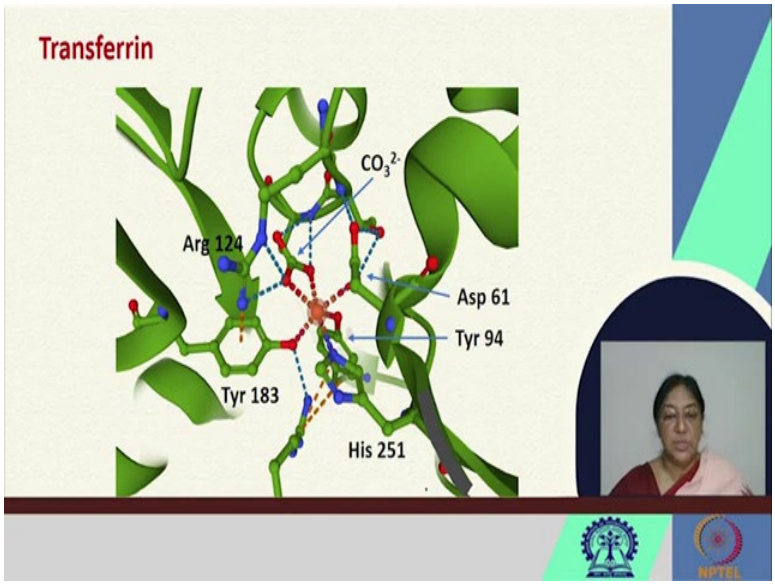

The transport therefore occurs in a fashion where we have the coordination, we have the carbonate ion here and the coordination of the iron occurs through the tyrosine and the histidine moieties present here.

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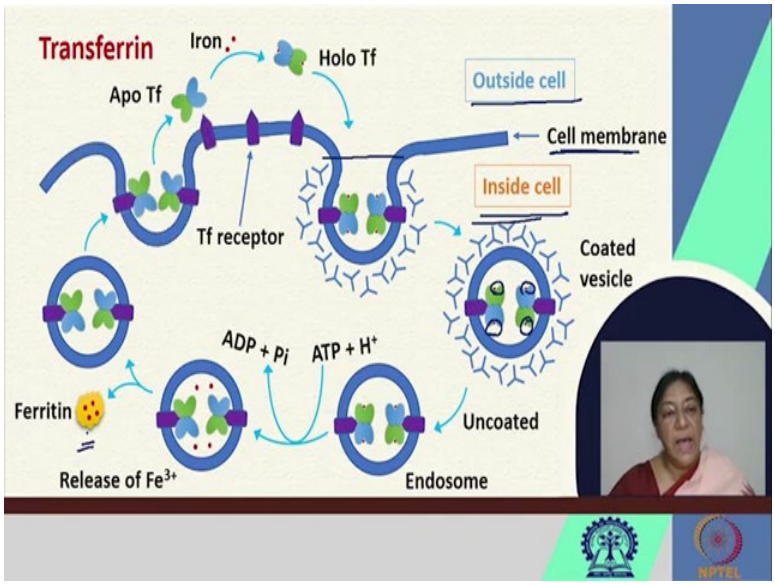

The apo Tf now with the iron that is going to form the holo Tf, has a specific transferrin receptor. This transferrin receptor then forms inside the cells on the transferrin receptor that is present on the cell and we have the cell membrane.

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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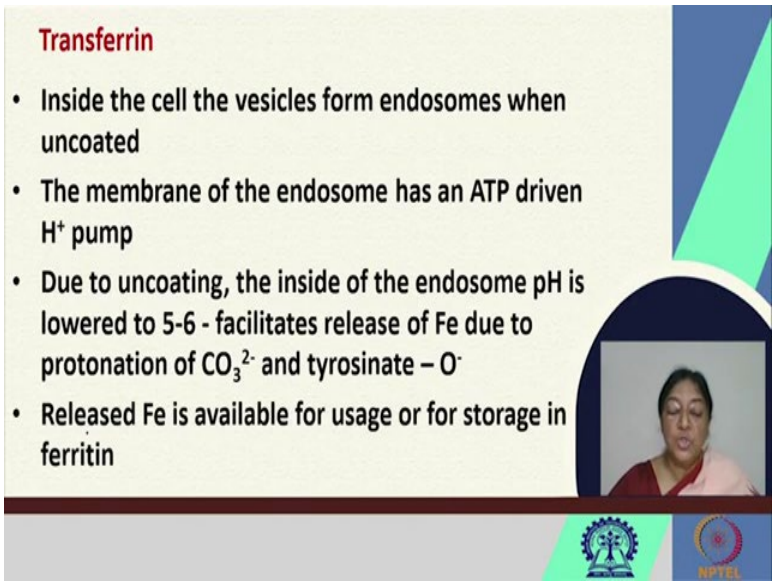

So inside the cel,l the vesicles form endosomes when they are uncoated. The membrane of the endosomes has a specific ATP driven proton pump and due to the uncoating the inside of the endosome pH is lowered to about 5 or 6 and this facilitates the release of iron due to the protonation of the carbonate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

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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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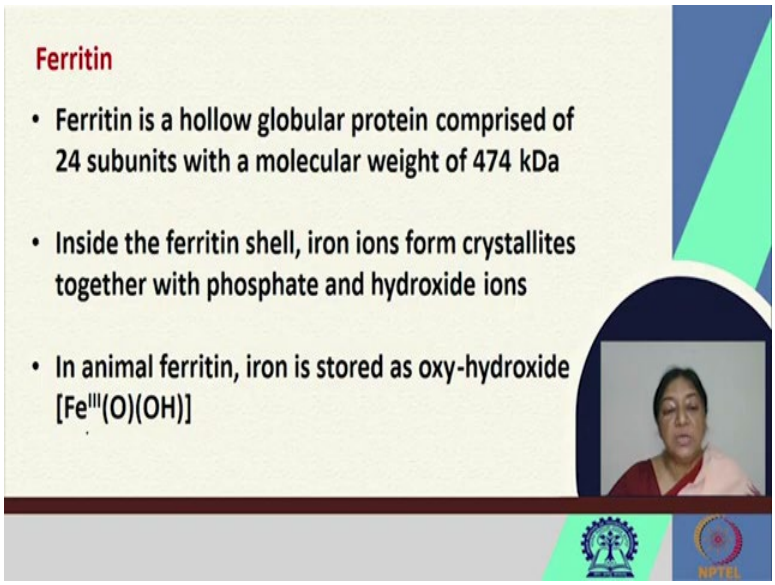

This is a hollow globular protein a 24 mer protein; that means it has 24 subunits with a molecular weight of 474 kDa. Inside the ferritin shell, iron ions form crystallites together with the phosphate and hydroxide ions, where it is stored in an oxy hydroxide form.

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Apoferritin 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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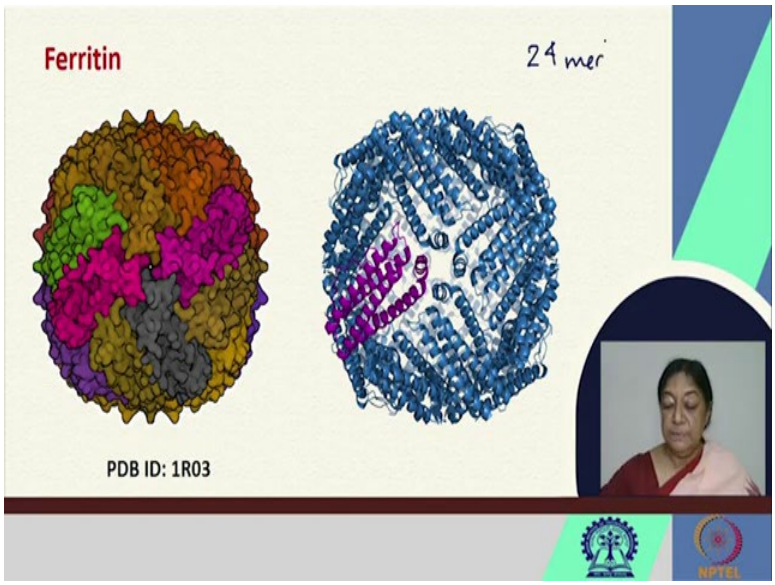

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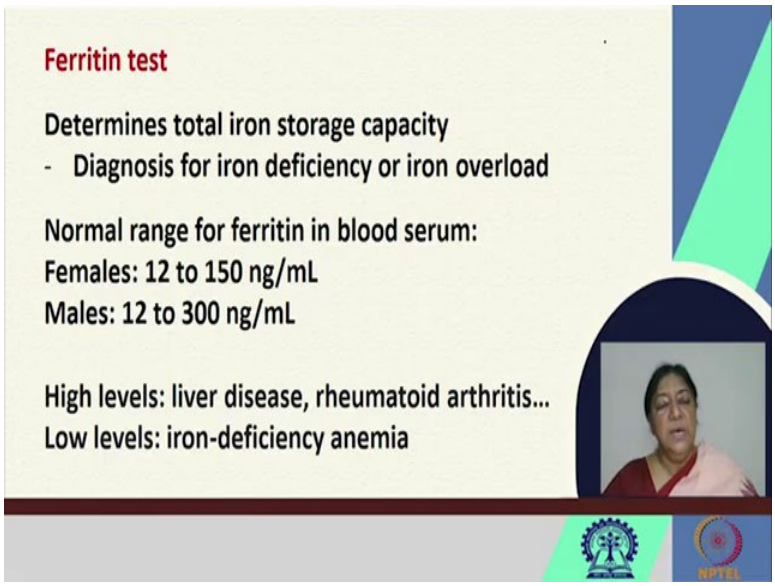

This [refer to slide] is the structure of the 24 mer ferritin that we see. The ferritin test is used nowadays to determine the total iron storage capacity. So apart from hemoglobin tests that are now done to determine the amount of hemoglobin in the body, that being an iron transport protein as well,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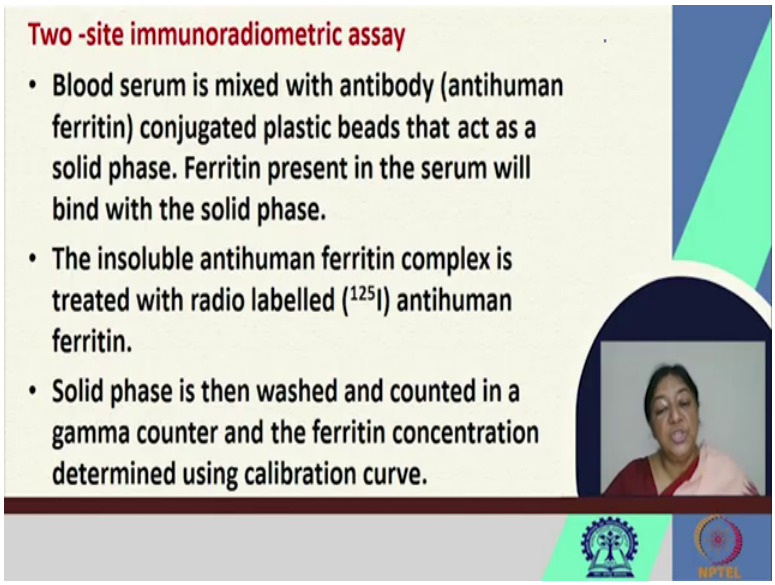

The typical test is called a two side immunoradiometric assay. We will not go into the details of this, this is beyond the course. But just to give you some idea that the blood serum is mixed with that specific antibody, that is an antihuman ferritin and these are conjugated on plastic beads that act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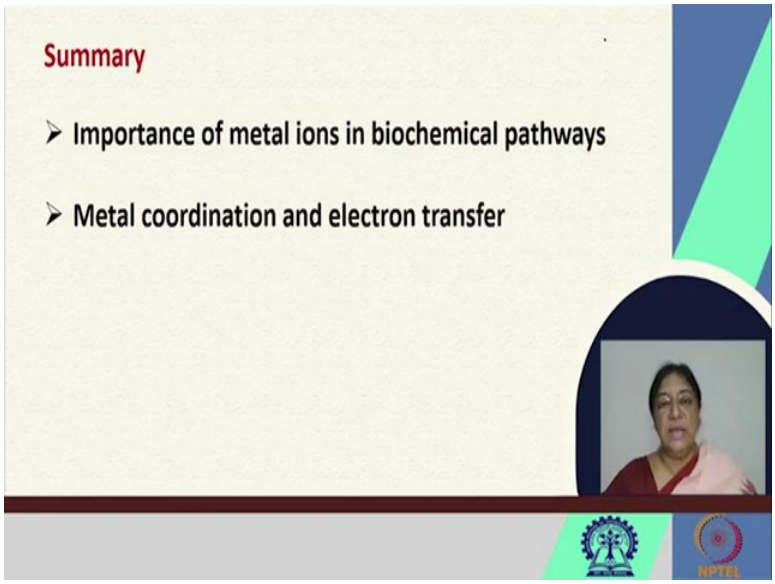

So our discussion on metalloproteins has touched upon the importance of metal ions in biochemical pathways. We have looked at specific metal ions in this aspect and specific aspects of metal coordination and electron transfer.

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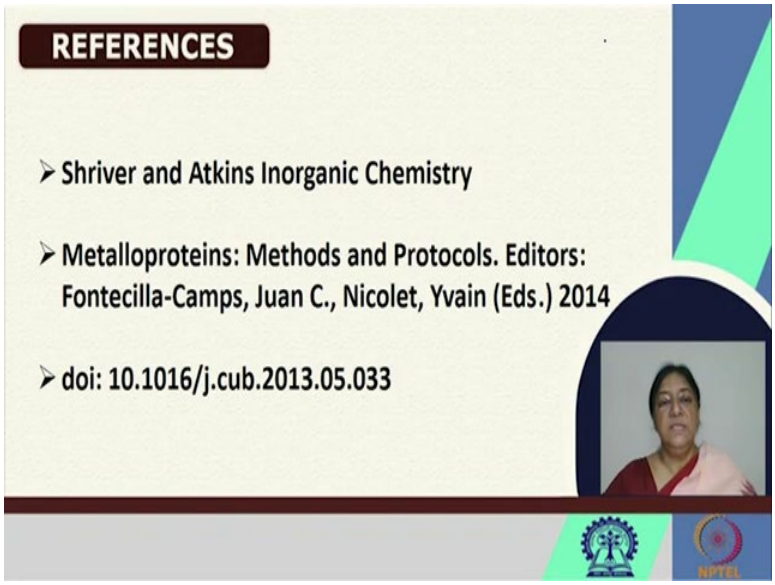

These [refer to slide] are the references.

Thank you.