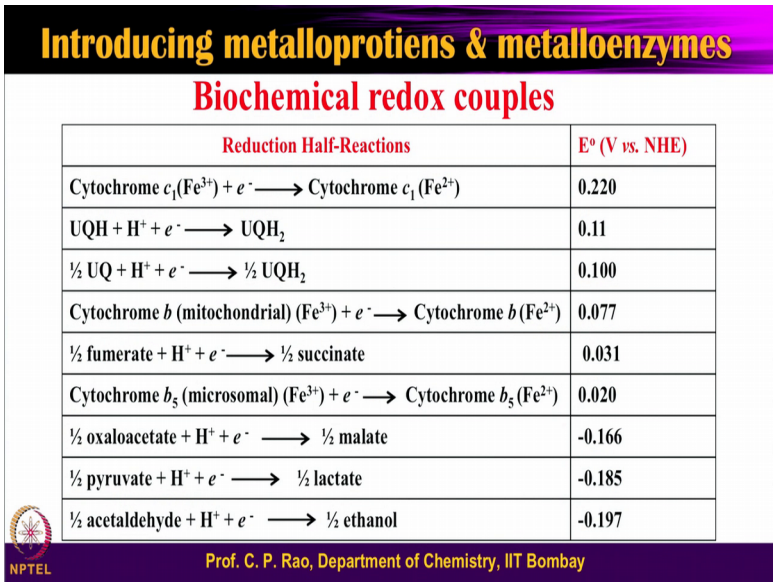
Inorganic Chemistry of Life Principles & Properties
Prof. C. P. Rao
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 28
Role of Iron in Life - Perspective of electron transfer proteins

Good morning, welcome you to the next class on the Inorganic Chemistry of Life Principles and Perspectives. In the just previous class we were looking at the electron transfer, general principles phenomena and when it comes to the proteins.

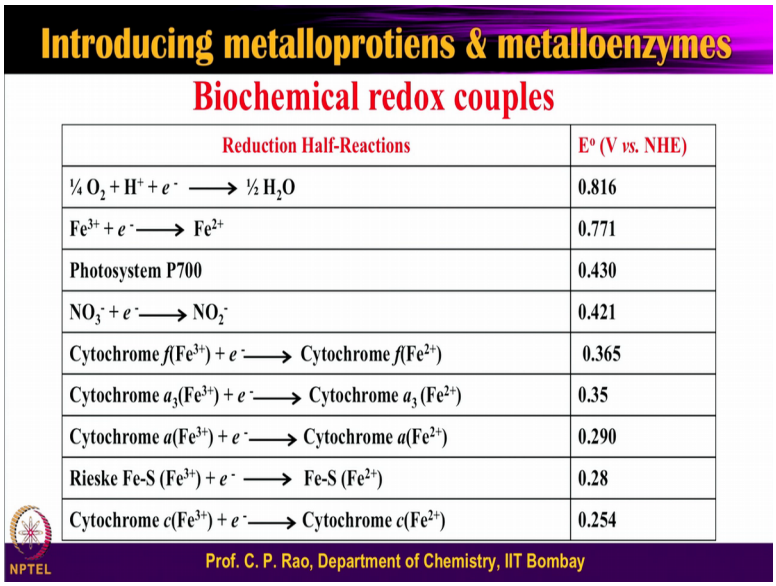
And towards the end we looked at the redox potential there are lot of enzymes, having different redox potentials because what is bound to the metal centre is changing as well as the protein changes; these things change either if it is a hemeprotein or non hemeprotein. And these ranges is quite a lot, and this range is compatible with the range of the potentials that are found for various reactions that happen in the biological systems.

(Refer Slide Time: 01:02)



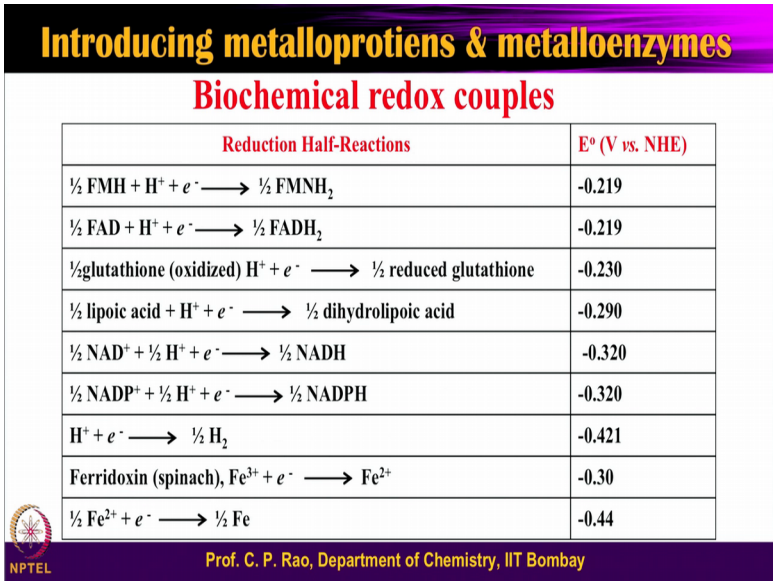
And these I have shown through the couple of slides here.

(Refer Slide Time: 01:03)



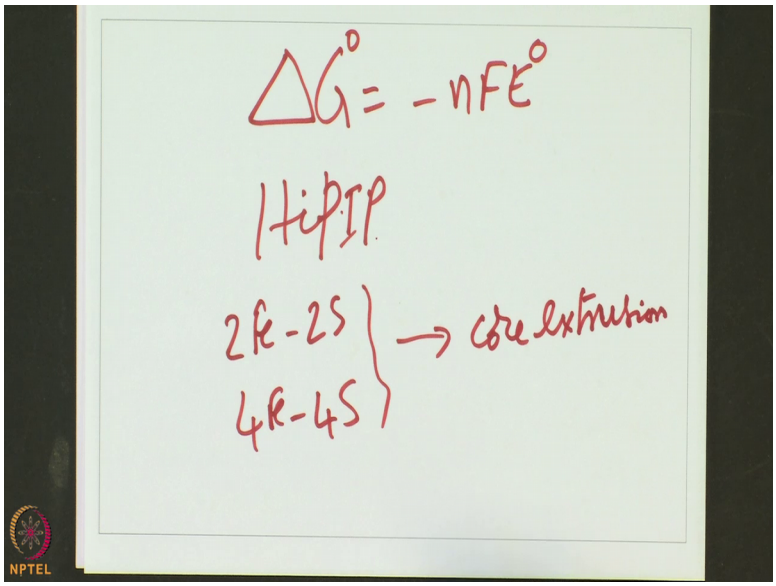
Some reactions that take place, these are all mostly the oxygen kind of reaction all iron enzymes so, other kinds of reactions that occur.

(Refer Slide Time: 01:13)



So, these all span somewhere minus around one old difference. So, which is reasonably well covered by all the enzymes, and you know how to understand this particular redox potentials, and I will explain you as this.

(Refer Slide Time: 01:35)



So, this should be understood from the delta G 0 is minus nFE naught and if the E naught that is redox potential is minus, then will be power plus; that means, reduction is not favourable. The E naught is plus, then the delta G is minus and the redox reduction is favourable. So, the more the positive reduction potential, the more there that it will be in the reduced form. The more the negative potential is the more its tendency to be in the oxidized form.

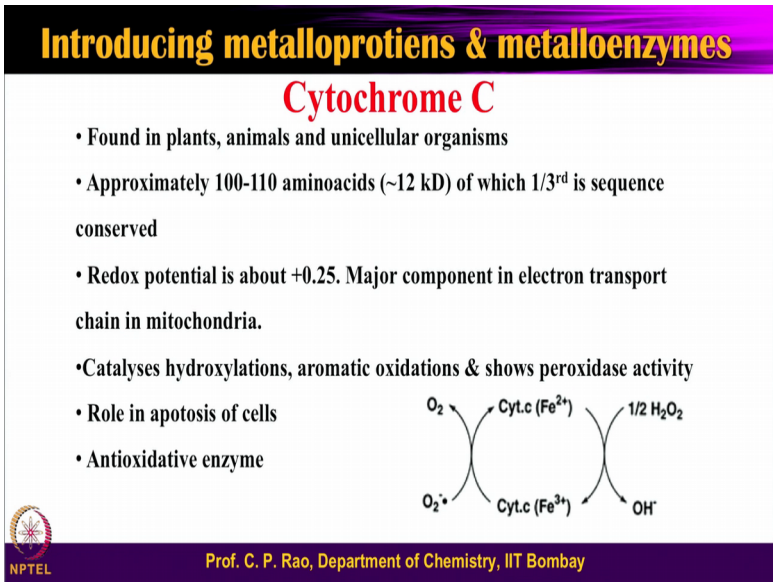
Now, you understand. So, there are two forms for a redox thing, one is in the reduced form other in the oxidized form. So, relatively speaking which one has more positive more in the reduced form, the other one will be in there more in the oxidized form.

So, if you understand this trick the entire thing can be followed. So, what is the trick? The del E naught potential if it is more positive, that will tend to be more in the reduce; that means, it will give away the it will take the electron in the reduced form it will be very happy, and if it is a negative potential it tends to be in the more in the oxidized process; that means, it will give away the electron ok.

So, the donor property or acceptor property of centre a metal ion centre in a protein, depends upon its redox potential. So, the more positive, it will be happy to be in the more reduced form more negative in form, it will be more happy to be in the oxidized form. So that means, an enzyme can go from a reduced form to oxidized form, back to reduce from an oxidized form.

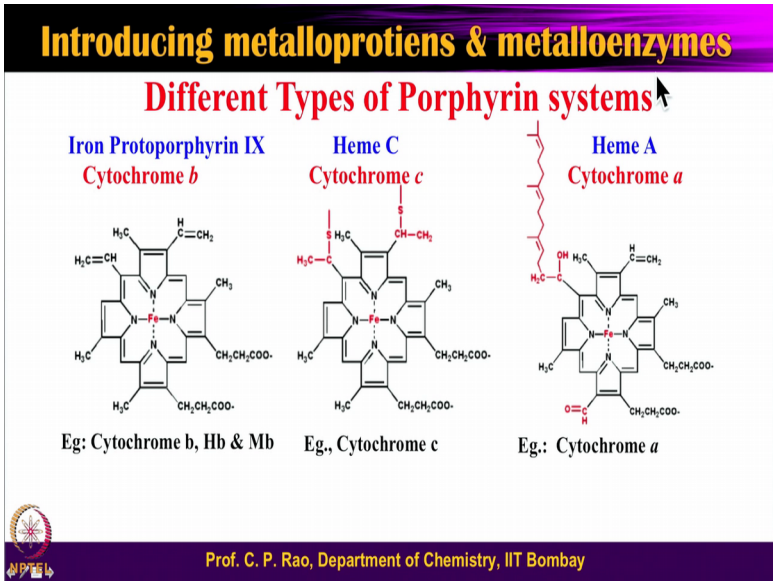
So, in the process it can change one electron transform. This as I promised you in the previous class, we will be looking at some example. Let us look at as an example of Cytochrome C.

(Refer Slide Time: 03:19)



We have already seen what is the cytochrome C earlier I will also be showing again in the next maybe you can see back that one.

(Refer Slide Time: 03:30)



So, cytochrome C is the one which is shown over there, here you can see that this is the one it is connected through thioether linkages with the protein and of course, it has the

both the protein acid groups etcetera etcetera. So, this will be the part in the cytochrome C and the remaining protein will be there which I will show in a while ok.

This is found in plant, this is found in animals, this is found in cellular small unicellular systems etcetera, it has about 100 to 120 amino acids with about 12 kilo Dalton, it is a small relatively small protein. Interesting and important parameter of this is about one third of its amino acids if you look at across the various species, there is a sequence homology.

So, at least one-third of them is the sequence homology, and you should not forget that those residues which are close in the park symmetry of the heme that conserved in almost all the things and reduction potentials are around plus 0.25 plus 3 etcetera and these are involved in the electron transport chain in mitochondria.

So, these are involved in the electron transport chain, they are also involved in a variety of reactions like hydroxylation reaction again involved through the electron transfer. It does not do the final hydroxylation, but it is involved in the process of an allowing the electron transfer taking place, aromatic oxidations peroxidase activity many of these kind of things.

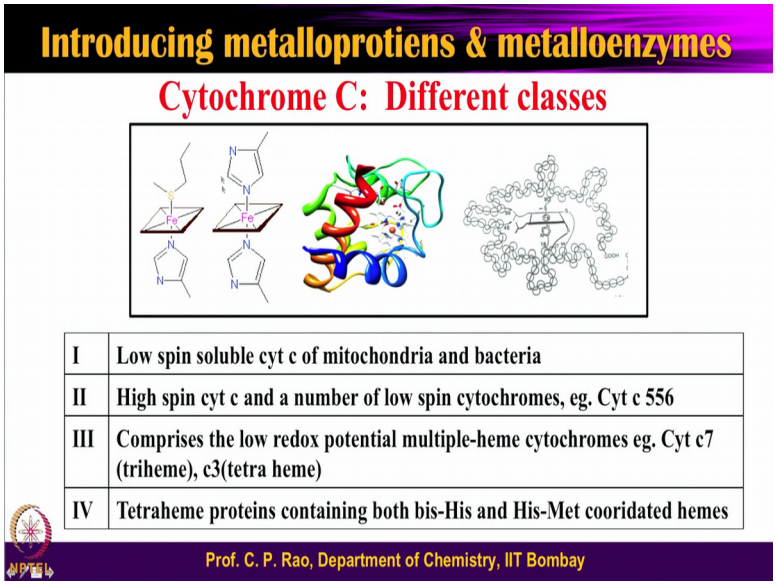
They are involved in apoptosis process, and they are involved anti oxidative. That what is anti oxidative? I mentioned earlier day lectures that the O 2 minus or other radicals other species ROS reactive oxygen species. So, removing those reactive oxygen species is on anti oxidative activity. So, anti oxidative activity also for example, O 2 minus should be removed and the radical should be removed and make into O 2.

So, O 2 is danger free and O 2 minus dot is a highly dangerous a toxic. So, therefore, cytochrome C in its oxidized from F e 3 plus means oxidized form. Will take up this electron and go to Fe 2 plus cytochrome C Fe 2 plus; that means, the enzyme is reduced the O 2 minus is oxidized O 2.

So, oxidized enzyme is reduced to reduce the enzyme. And in the process of this returning that will give that this will iron 2 plus its oxidized; that means, it gives away an electron and that electron will be given to this and that will breakdown the peroxide into OH minus ok. So, the peroxide is less is more toxic than OH minus O 2 minus dot is much more much more the toxic.

So, it converts into the less toxic form so that. But the role of this cytochrome is involved in the electron transform. So, basically in this kind of a systems.

(Refer Slide Time: 06:47)



Now, as you can see that there are different kinds of a classes of cytochrome C are there. Cytochrome C if you see in the enzyme in the heme there are the iron is connected with the 4 the pyrrole rings this the 4 nitrogens and that is what is shown by this the tetragon of this.

Then you have one more coordination fifth and one more coordination is sixth; actually this you can call it as fifth, this you can call it as sixth and this is coming from the histidine and this is coming from the methionine, in some cases both will be histidine. So, there are different kinds of cytochromes where you have both the histidines or histidine and methionine and the these are all influenced in the iron center in terms of the iron spin state, is the iron center in terms of the iron spin state.

So, if you have a low spin state soluble cytochrome C, this is found in mitochondria bacteria; high spin cytochrome C and a number of low spin cytochromes they are found in this cytochrome C 556. What is 556? Is the absorption maximum wavelength 556 nanometer.

So, now I explained you I will not explain later on cytochrome C. I have shown you and the number that is coming next to that is the absorption wavelength. Then you have type

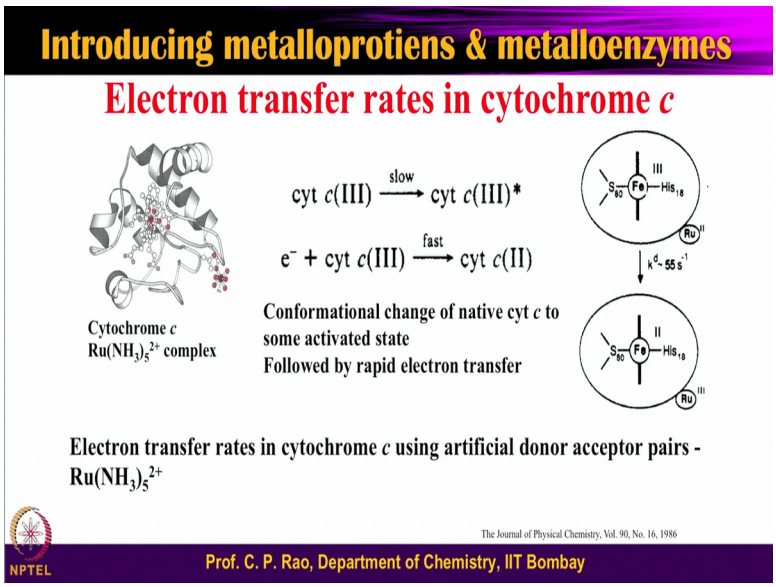
3 has comprises of the low redox potential multi heme cytochrome, example cytochrome C 7, triheme cytochrome C 3 which is tetra heme.

So, there are 3 hemes are involved there are 4 hemes are invoved is not 1is not 2, there 3, there 4. So, this is another kind of thing. And the tetra means containing both histidine and histidine methionine coordinated hemes and these come under the different class.

So, class 1, class 2, class 3, class 4 and so many kinds of cytochrome C are involved they are all cytochrome C family it is called cytochrome C family. And just simple cytochrome C structures shown over there a lot of protein structure this side, protein structure this side and here is a heme and that is shown much more here and you can see this heme is reasonably well exposed to the exterior.

So, therefore, it can interact with the exterior, which you can interact with another enzyme, which can act as a acceptor therefore, can give away the electron get oxidized and reduced all these kinds of things can happen, here both reduction oxidation can happen.

(Refer Slide Time: 09:28)



How the electron transfer occurs? As you can see that this example has been worked out by using ruthenium ammonia complexes people have also studied with everything imperial to restrict within ammonia complexes; they can be used and you have the enzyme cytochrome C in the oxidized form, we will get into these slowly into the

activated form. And this activated form in the presence of course, this one and it forms a complex go to the activated form is ready for electron transfer, and the electron transfer going into this one will give the iron 2 C 2 means iron 2.

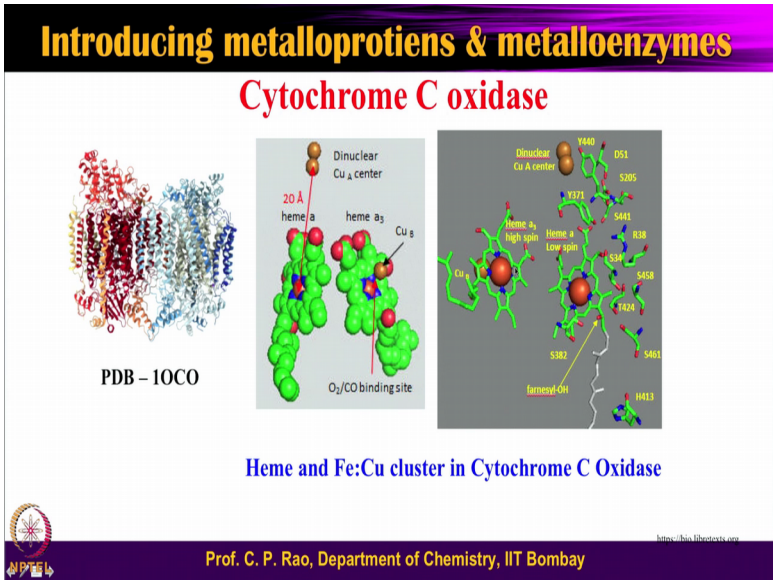
So, this needs goes through conformational changes of native cytochrome C to the activated complex. So, that activated complex will allow the electron transfer at a very fasted. So, what is the slow means, it is the rate determining step. So, rate determining step here is the formation the complex and the complex converted into the transition state or activated complex.

So, conversion into the activated complex is the redetermining step so; that means, it is the reorganization energy that is the basically is favourite. So, once that is activated then the electron transfer goes very fast, the kd is around 55 second inverse.

So, though these are the kinds of things that we are looking at. So, these are capable of depending upon the partner, this can act either as a donor or as an acceptor because these are all depends on the potential. So, all that I want to demonstrate to is, you have a the ground state complex and this complex has to be converted into an activated complex therefore, energies involved; Then of course, connected to the medium between the donor and acceptor.

Now, let us say assume that the cytochrome C has gone from cytochrome C in the iron 3 oxidation state to a cytochrome C in the iron 2 oxidation state, how will it return back? Let us explain over here and this thing is accepted done by the cytochrome C oxidase.

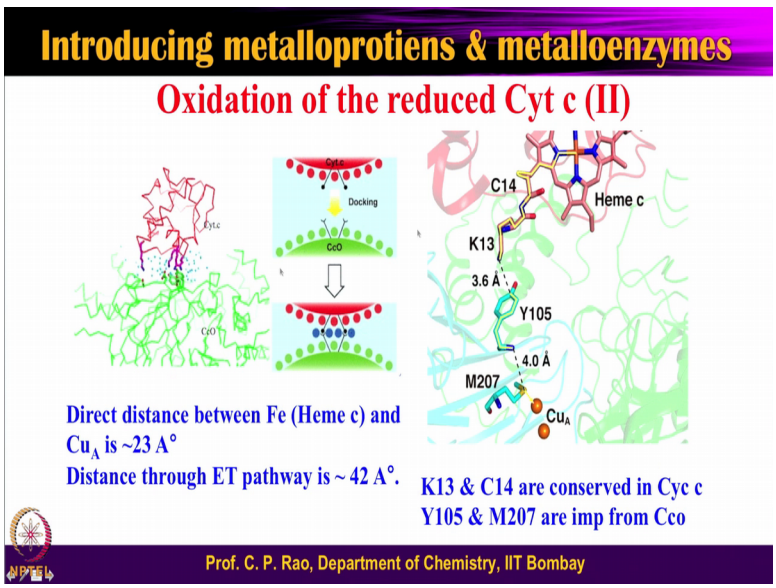
(Refer Slide Time: 11:42)



This is an enzyme called cytochrome C oxidase and this enzyme for biological inorganic chemists this portion is important or this portion either is one and the same. And this is the enzyme structure. So, this cytochrome C oxidase is a bit complex protein, having the 2 copper center copping having a heme a, having a heme a 3 and having si copper b center, the whole thing is surrounded by the protein here you can see that.

Now, how does the electron transfer finally, take place between the cytochrome C and cytochrome C oxidase. Let us assume cytochrome C is reduced form, it has to be oxidized under is what is done in this particular case; and let us x let us look at to explain this.

(Refer Slide Time: 12:37)



So, this is coming from the cytochrome C, and this is coming from the cytochrome C oxidase and these two have a docking in this region.

So, you assume that the cytochrome C docks with the cytochrome C oxidase and forms a kind of a complex you see that. This region is the region where conductivity should work and that is exactly the region of this. There are many details of this where the water is involved etcetera, I will not go into details this is coming from the cytochrome C this part is coming from the cytochrome C oxidise, you see the 2 coppers. And there are some residues like the K 3 and C 14 16 and lysine. 16 14 number and lysine 13.

These two which are coming from the cytochrome C are extremely important. Similarly the methane in 207 and tryptophan 105, coming from these cytochrome C oxidase are also equally important. So, this is the region if you take this center and to this center, and this distance is around 23 angstroms. But if you go through this particular path this is called electron transfer path and this is around 40, 42 2 angstroms and that is where.

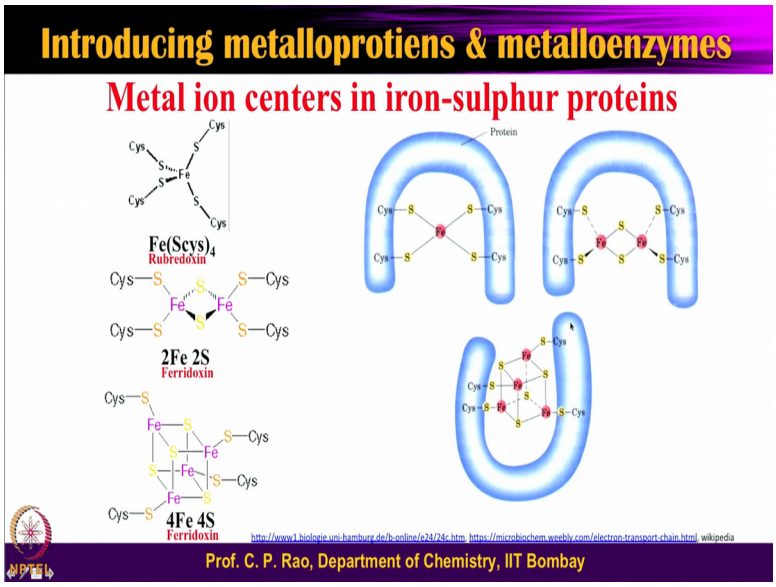
So, these are important how do we know these are important? You can mutated these residue any of this residue or both these residue; similarly any of this residues or both of these residues, and the mutated protein will not do the electron transfer between the cytochrome C and cytochrome C oxidase; that means, these residues are absolutely important.

They are present in the corresponding in that and; that means, this region is absolutely important therefore, the water present in this the medium that is present in this is absolutely important. We will not go into details because that will come into purview of physical chemistry a lot, which I am not getting into the details of this. But as an inorganic chemistry we need to know this center, we need to know this center, and what are the as the residues are involved.

So, now you understand how an electron transfer is taking place between the cytochrome C and cytochrome C oxidase. In this case between this region to the copper center, then it will go to the A 3 then you will go to the A etcetera etcetera and then finally, to the reaction center.

So, we not worried into that part of it, but (Refer Time: 15:02). So, this is one example I have given you the heme containing let us look at one example for the non heme containing.

(Refer Slide Time: 15:06)



So, non heme containing electron transfer proteins of iron are called iron sulphur proteins. If you call iron sulphur protein or non heme as one on the same and there are several classes of which important ones I am going to explain in this course, there is 3 types there are more types are there.

So, one is there is just 1 iron and 4 cysteines are bonded as you can see here, Fe Scy cysteine 4 times these are called rubredoxin it is called rubredoxin. Then you have another case one iron 2 cysteines, another iron with 2 cysteines the 2 irons are by 2 sulphide grooves this is called ferridoxin, but is called 2 iron 2 sulphur ferridoxin. This is called rubredoxin or one iron sulphur protein 2 iron 2 sulphur protein, this is another case which is 4 irons.

So, each of the iron is connected to one cysteine whereas, cysteine is coming from. The cysteine is coming from the protein it is not a cysteine just simple amino acid, which coming from the protein. So, protein wraps around. So, like that it is shown over there a protein wraps around, protein wraps around and you can see the protein wraps around.

So, all the 4 cysteines are coming from here, all the 4 cysteines are coming from here, all the 4 cysteines are coming from here, this is for 1 iron iron sulphur protein this is 2 iron 2 sulphur protein, this is 4 iron 4 sulphur protein. So, this is called rubredoxin, this is called ferridoxin to iron sulphur this is ferridoxin 4 iron 4 sulphur.

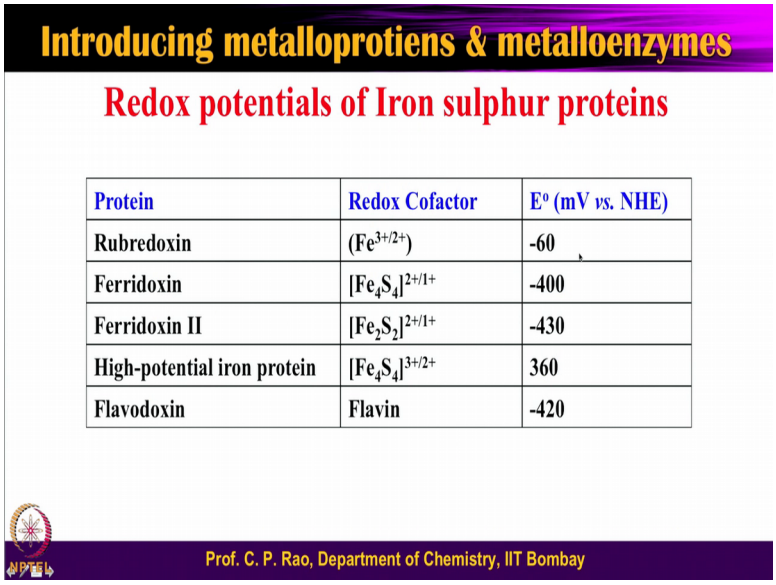
There are some more classes, but we do not need we just these are sufficient. There are proteins having different kinds of protein electron transfer, sometimes more than one of the same type, they are also there, but with some little modifications. So, therefore, a redox potentials will differ. Why nature has chosen so many? Because to control the redox potentials in various ways ok.

So, I hope you understand 1 iron iron sulphur protein, 2 iron 2 sulphur protein, 4 iron 4 sulphur protein ok. So, in all these there are two types of sulphur linkages, one is from the cysteine oil sulphur it would have been SH, which is called thiol, proton is lost then this is called thiolato, thiolato bridge.

And this is called S 2 minus sulphido. How do one differentiate these ones? Just take this protein add some acid mineral acid and if you get hydrogen sulphide smell; that means, you have a sulphide S 2 minus, which is also known as inorganic sulphur it is called organic sulphur. Cysteine oil sulphur is called organic sulphur, the bridge sulphur here is called the inorganic sulphide.

And this upon mineral acid addition like HCl something will get H 2 S and then from the smell itself you can fake find out. In this case if you add mineral acid nothing will happen, no false smell will come whereas, in this case in this case you will come you understand that.

(Refer Slide Time: 18:07)



Let us look at more closely such proteins rubredoxin minus 60 ferridioxins. So, minus 400, ferridoxin to a different type and to Fe 2 S 2 minus 4 thirty there is something called high potential iron sulphur protein or iron protein high potential iron sulphur protein or high protein this is called the Hi pip.

So, high potential iron protein or iron sulphur protein. So, this has got hi positive potential plus 360 whereas, these are having minus values minus 400, minus 430 whereas, plus 360. And flavodoxin it is a flavin not a iron sulphur protein minus 420. So, here itself you can see the iron sulphur proteins can show as positive as plus 360 as negative as minus 430 difference is 800 millivolts as you can see that a large amount of millivolt kind of thing.

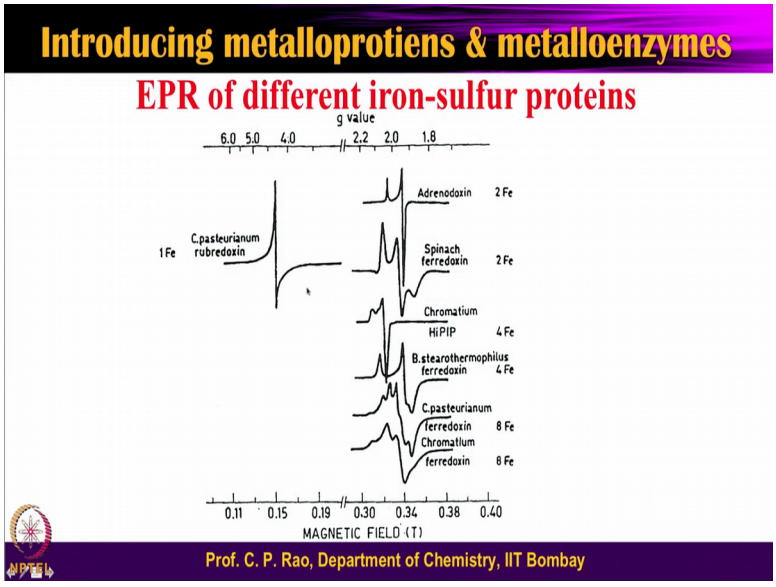
So, the iron sulphur proteins are very nice to study and in fact, you can take an iron sulphur protein and do, like to find out whether it is a 2 iron 2 sulphur protein or 4 iron 4 sulphur protein how do we find out? Do a core extrusion. So, how will you do? Because all of these are associated with the thiol.

So, you use excess thiol, you take the protein and add excess thiol from exterior outside and it will take the core out and the protein becomes the apoprotein and you can check that absorption spectrum and you can compare with the standard one and you can identify by using the absorption spectrum, you can identify whether it is a protein has got iron with 4 thiolates or 2 iron 2 sulphur protein, a 4 iron 4 sulphur protein or whether it is

a rubredoxin, but this is a ferridoxin of 2 irons and type, is the ferridoxin of 4 iron type you can absolutely find out from the absorption spectrum of these enzymes.

How by adding excess thiol to the protein and extracting the core and looking at the absorption spectrum; and comparing with the (Refer Time: 20:29). Similarly, you can also look at their EPR spectra.

(Refer Slide Time: 20:34)

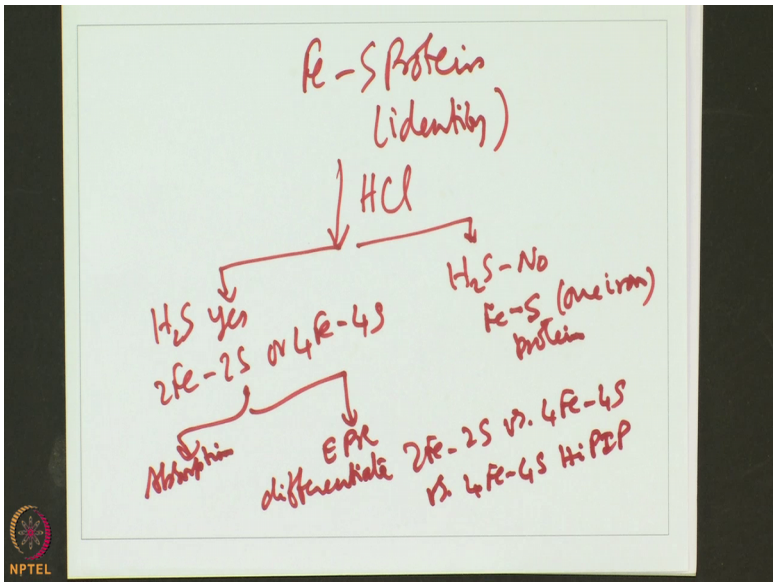


See the EPR spectra of the iron one iron protein see looks very nicely around between 4 to 5 is the g value. Now, if you look at the 2 iron 2 sulphur protein you can see these are the things and if you go to 4 iron 4 sulphur this one, this one, this one and this 3 were very similar and intermediate to this 2 iron and 4 iron is the iron high pip.

So that means, if you look at the absorption spectrum, if you look the EPR you can identify whether it is a one iron system or 2 iron 2 sulphur system or 4 iron 4 sulphur system or 4 iron 4 sulphur with Hi PIP system, all this can be very well identified from the absorption spectrum from the EPR spectra, it is very interesting very simple proteins.

So, you can just do the core extrusion and then compare with this. So, if you have iron sulphur proteins, first thing is you can do a test are the mineral acid and you can find out whether it is inorganic sulphide, is there or not. So, if it is there now the next question is that, whether it has any iron 2 or iron 4 sulphur. So, how will you do that? They take let us say a protein mix.

(Refer Slide Time: 21:55)

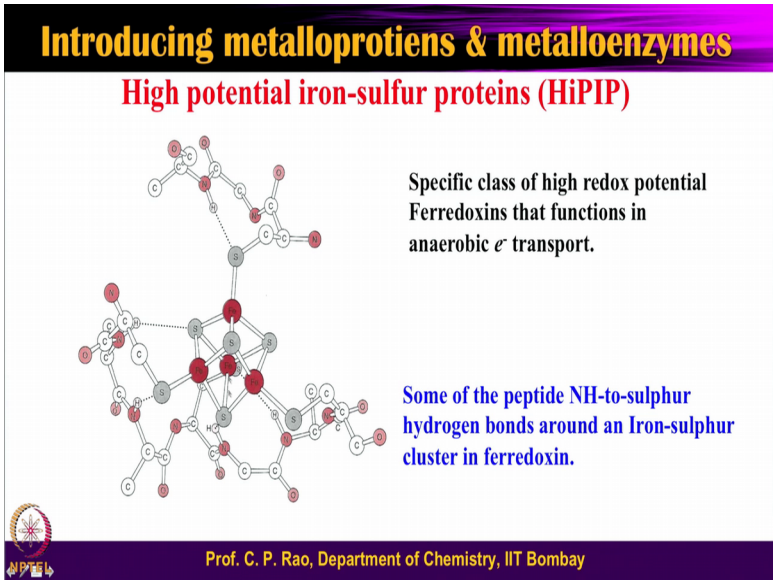


So, then say iron sulphur protein; Iron sulphur protein and you want to identify which one is this. So, first is HCl and then the H 2 S yes H 2 S no.

So, this means this is iron sulphur, one iron protein and if it is yes, it could be 2 iron 2 sulphur or 4 iron 4 sulphur. So, you can measure absorption, you can measure EPR from this you can differentiate differentiate 2 iron, 2 sulphur versus 4 iron 4 sulphur versus 4 iron 4 sulphur which is the Hi PIP. So, this all these can be identified very nicely very well, this kind of things.

So, having seen is we talked about the iron sulphur protein, which are having a negative redox potential Hi PIP proteins having a positive 400 360, why are these having the positive redox potential.

(Refer Slide Time: 23:26)

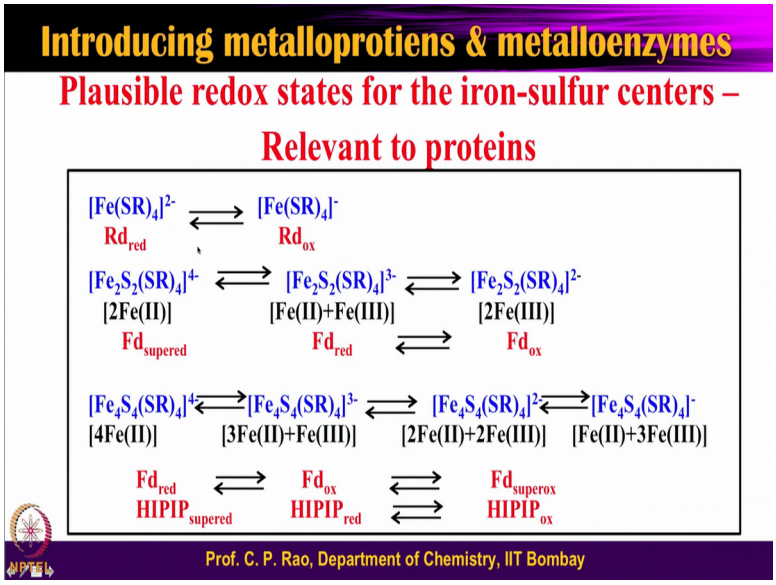


See the example over here this iron sulphur protein and the protein is surrounding they are buried number 1. There are Hi PIP iron proteins are buried the iron sulphur cluster is buried inside, and second thing is these things are stabilized by the NHS hydrogen bonds you see NHS hydrogen bonds NHS hydrogen bond so many.

So, these the sulphur the the thiolate sulfur as well as the inorganic sulphides they are stabilized by the hydrogen bonding, they are stabilized by the hydrogen bonding. So, they are interior. So, the two reasons one is there inside the protein, they stabilized by a large number of hydrogen bonds with the cysteines sulphur with the inorganic sulphur therefore, there redox potentials are very different.

Hence, boon in disguise iron sulphur proteins will go from minus 400 to plus 400 even more range of the redox potentials. Now, let us look at the redox characteristics they redox characteristics.

(Refer Slide Time: 24:34)



So, I told you 1 iron protein 1 iron means there is only 1 iron center. So, that iron center can iron can undergo what iron 3 iron 2 that is what. So, you take iron 2 into 3 4 thiolates, then you have a 2 minus, because 4 minus 2 plus 2 minus and then 1 minus; that means, iron is 3.

So, it can only have one type of one possible redox, reduced protein will go to oxidize oxidative protein will go to reduce because 1 iron a for 1. Now, come to the 2 irons. So, when you have 2 irons, one both the irons can be in 2 oxidation state or both the iron can be in 3 oxidation state or both one iron can be in 2 other iron can be in 3 these are the three possibilities, 2 iron 2 sulphur ok

So, you take thiolate has 1 minus, sulphide has 2 minus, iron accordingly you can calculate. 4 minus and 4 minus 8 minus out of that 4 minus is there. So, 4 plus they 2 irons; So, 2 plus. So, the 2 irons 2 plus and this is now 3 minus means, 1 iron into 1 iron 3 and this is 2 minus which is both the iron 3. So, both iron 2, 1 iron 2 1 iron 3 and the both iron 3s.

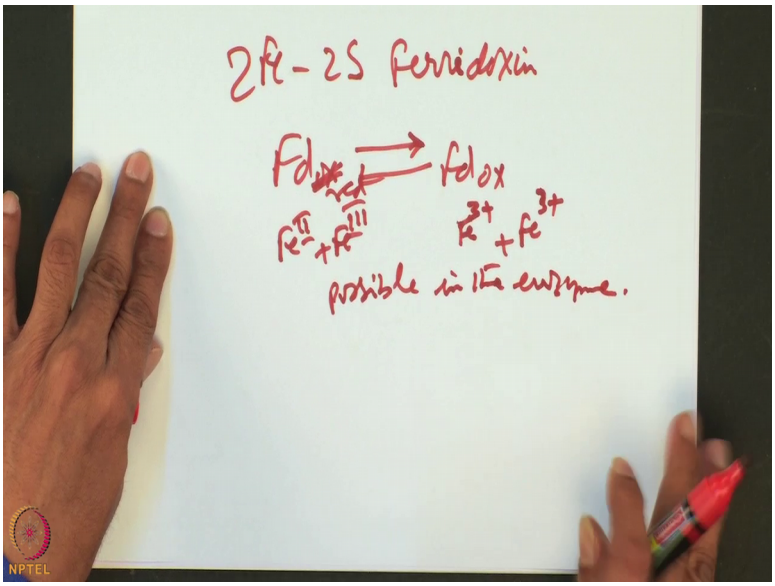
So, this is called super reduced, this is called reduced, is called oxidised. The protein can never undergo between the reduce to this super reduced, it can only undergo between reduced and oxidized in canners of di Fe 2 S 2. So, three possibilities as far as the complexes are concerned, but the enzymes show only these two and enzymes shows only these two that is 1 iron 2, 1 iron 3 or both iron threes, but it will never show both iron 2.

So, ferridoxins will not show 2 iron ferridoxin will never show super reduced are both the iron 2.

Similarly, when you have 4 irons there are so many possibilities. All 4 in 2 plus, all 4 in 3 plus or 2 of them 3 plus 1 2 plus etcetera etcetera etcetera. All these possibilities are there; of which the most favoured are shown over there and the this is 4 iron 2s, 3 iron 2s and 1 iron 3, 2 iron 2 and 1 2 iron 3s, 1 iron 2 and 3 iron 3s of course, you can have all 4 iron 3s, but never found even in small complexes. But in small complexes these four are being formed, but in protein if you see in the regular these things ferridoxin.

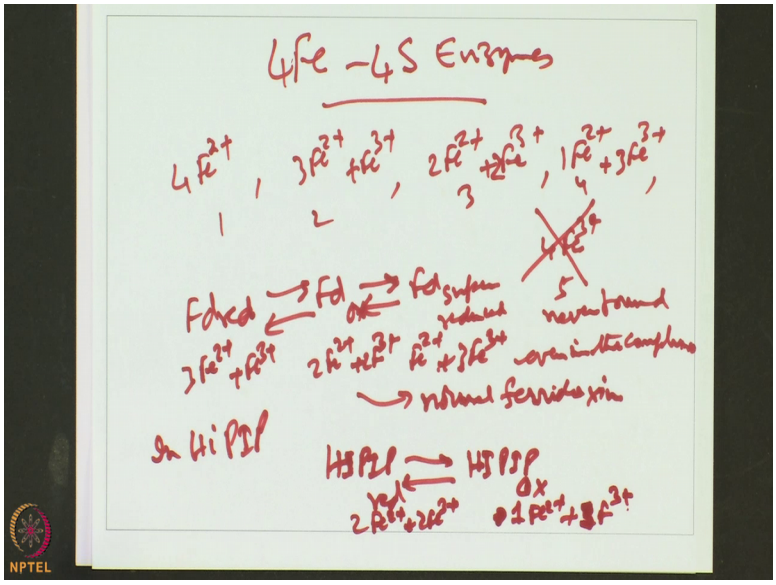
You can find between iron reduced which is this one to oxidize, which is this one and super oxidise and regular ferridoxins regular 4 and 4 sulphur ferridoxins let us see 2 iron 2 sulphur ferridoxin.

(Refer Slide Time: 27:34)



So, you have F d oxidized to F d oxidized to sorry reduce to oxidized. So, this is 1 iron 2 plus 1 iron 3 and this is iron 3 plus plus iron 3 plus, both iron 3s 1 iron 2 one iron 3 this is only possible in enzyme. And now if you take 4 iron 4 sulphur case so, many possibilities are there of which the 4 iron 4 sulphur enzymes ok.

(Refer Slide Time: 28:24)

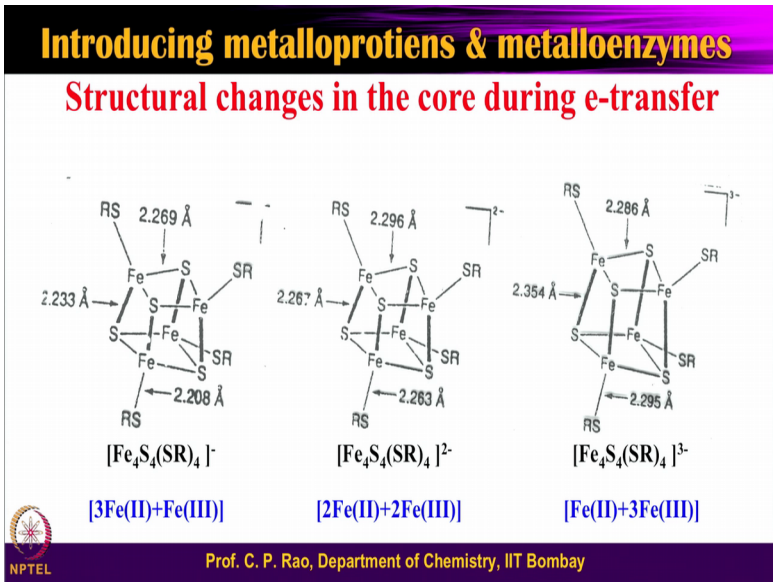


4 iron 2s 3 iron 2s plus iron 3 2 iron 2s plus 2 iron 3s or 1 iron 2 plus, 3 iron 3s and 4 iron 3 plus.

This is never formed; only these 1, 2, 3, 4, 5 is never found even in complexes only these four are found in the complexes, and of which the F d reduced, F d oxidized, F d super reduced are found in the proteins for the normal proteins. So, this is what? 3 iron 2 plus plus iron 3, 2 iron 2 plus plus 2 iron 3 plus and 1 iron 2 plus plus 3 iron 3 plus. So, this is in normal ferridoxins and in Hi PIP and this is the other way.

So, it is the super reduced reduced to oxidized. So, Hi PIP reduced to Hi PIP oxidized ok. So, that is 2 iron 2 plus plus 2 iron 3 plus going to 1 iron 2 plus plus 1 iron 3 3 iron 3 plus. So, only these are the two forms are possible in these systems ok.

(Refer Slide Time: 30:56)



So, if we try to look at these try to understand what happens at these, from small molecules it has been formed, the 3 iron 2 iron 3, 2 iron 2 2 iron 3, 1 iron 2 1 3. When you look at the crystal structures from small molecule; this iron sulphur distance versus thiolate distance; you go to this 2.233 because 2.267 becomes 2.354. Look at this distance also similar way increases.

You look at this 2.208, 2.268 2.295. So, what is happening? As you keep oxidizing one iron from here to here, one more iron from here to here these are basically expanding. So, therefore, you have an expansion, when is oxidized and it will contract when it is reduced oxidized reduced. So, that will bring accordingly conformational changes in the protein that is bound and that is what is involved in the electron transfer process.

So, in the electron transfer process of these iron sulphur proteins, there is a reduction oxidation takes place during the period when it oxidizes it will expand it will reduce it will contract. So, expansion contraction of these course will basically involve in the protein conformational changes, and such protein conformation changes are basically responsible for the protein electron transfer.

So, this with this I closed the electron transfer part and then I will going to the enzymes in the next class. So, I have explained the basic principles of electron transfer and some examples of the heme, non-heme, iron center.

Thank you very much.