Metals in Biology
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Lecture – 11
Hemerythrin and azidohemerythrin

Hello, welcome back. Today we will discuss Hemerythrin. So, we are discussing Metals in Biology.

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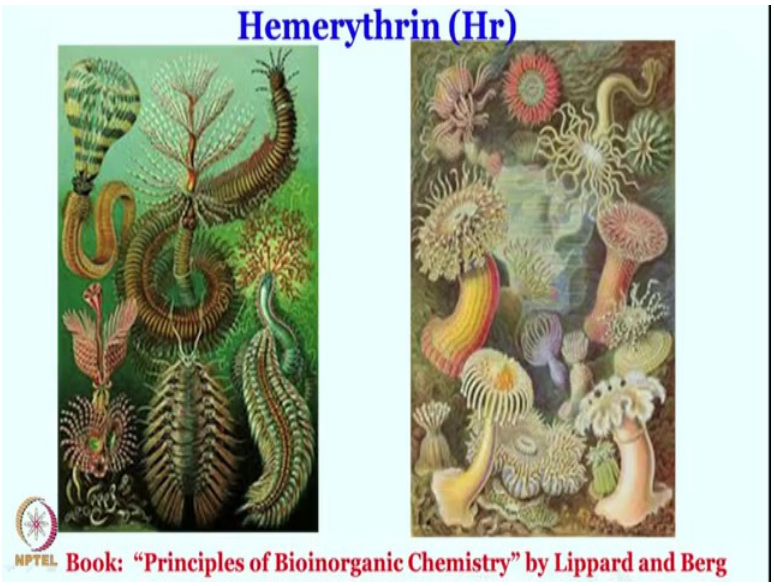

The book to follow is the "Principles of Bioinorganic Chemistry" by Lippard and Berg. Well, it is a fascinating enzyme hemerythrin, its a metalloenzyme; that means, metals are there. Well in mammals for example, in human we have blood. Blood is red due to hemoglobin, but there are many other species in the world which does not have hemoglobin, but still they are aerobic species. They inhale air and they are air dependent.

They are must be some other enzyme which is responsible for carrying the oxygen in different parts of their body. The job this is done in the invertebrates or marine invertebrates by hemerythrin. This hemerythrin are having 2 iron centers.

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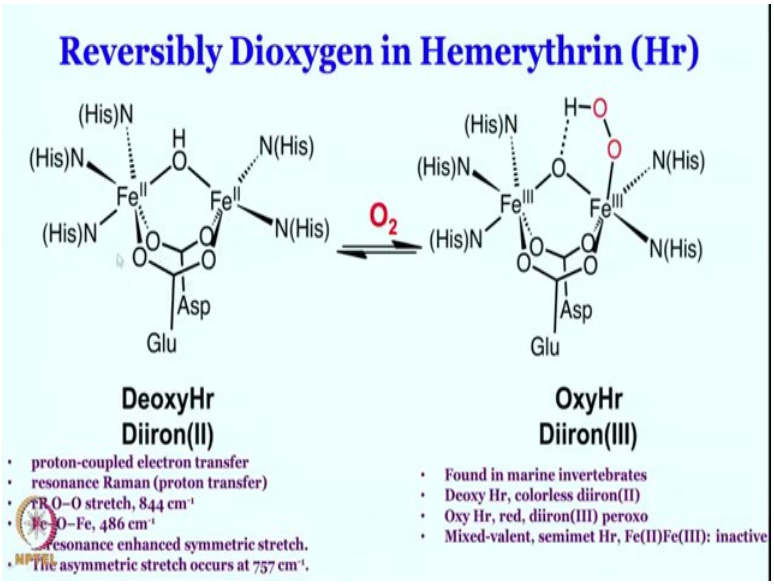

As you can see this is a reversible Dioxygen binding in Hemerythrin. This is the reduced form of hemerythrin also known as deoxy hemerythrin, iron centers are in plus 2 oxidation state. Each of the iron is having 3 histidine or sorry 3 histidine in one of them and on other one, we are having 2 histidines.

So, it is a Di-iron center that is responsible for oxygen carrying in those species, where we do not have hemoglobin. Not for all of them, but for mainly those which are marine invertebrates. Those are the species looks like, this is something like the species which looks like these are having these hemerythrin.

Now, as you have seen 3 nitrogen or the histidine coordinations are there on iron; 2 histidine coordinations are there on this other iron. They are linked by aspartate and glutamate. These are bridging carboxylate linkage. In addition to aspartate and glutamate, they are also having hydroxy bridging between them. This is crystallo graphically characterized intermediate. So, it is very clear that this is what we have in the deoxy hemerythrin. Upon binding with oxygen because they are oxygen carrier, a new species is form where oxygen is reduced doubly; that means, each of the iron will provide one electron and will form a peroxo species.

So, oxygen is 2 minus, 1 minus is bound over there and another minus is picking up the proton from this hydroxo overall it is forming diiron III hydroperoxo species bridged by a mu oxo species now so, that is quite fascinating. Well, here during these oxygen

binding and electron transfer process, a proton coupled electron transfer happened. So, this overall transfer of this proton happened in a PCET mechanism by PCET mechanism, one can utilize the resonance Raman data to characterize these fully oxidized oxy hemerythrin diiron species.

Resonance Raman data shows that this oxygen-oxygen stretch is around 844 wave number. We will see in the next slide what this mean, I can tell you in advance that this means that this is a hydroperoxo species. This is a peroxo oxygen-oxygen stretch that is around this 844 wave number region. Resonance Raman also says that these iron oxo iron stretch, these iron oxo iron symmetrical stretch is around 486 wave number and the asymmetric stretch is around 757 wave number for this iron oxo iron.

Well, as I mention these are found in marine invertebrates and this is the deoxy form of the hemerythrin which is colorless these are diiron II species bridged by glutamate and aspartate and hydroxy completely colorless species. Oxy hemerythrin upon binding with oxygen. The oxy hemerythrin is forming these are having both the iron in +3 oxidation state and this species is red in color. This was a colorless species turning into red upon oxygen binding. Or one can assume that there is another intermediate that can be there or one can perhaps characterize them that could be a iron II iron III intermediate which is basically called the semimet hemerythrin which is inactive.

I hope it is crystal clear for you guys that hemerythrin is the oxygen transport protein for a number of species such as those marine invertebrates, they do not have hemoglobin like us that their blood is not really made up of hemoglobin.

Now, these are the species which will carry oxygen and most importantly just like every oxygen transporting protein, the oxygen binding has to be reversible they should be; they should be easily subtling between deoxy form and the oxy form. This is quite intriguing that during oxygen binding, it gets reduced doubly to peroxo not only that a proton coupled electron transfer process is involved. This such a compound is really fascinating, as you may have notice one side is having 3 in the histidine, another side have 2 histidine. Therefore, the oxygen binding site is open or available only on this iron center not on the other iron center.

This means that nature has really designed it perfectly so that everything remains as much constant as possible, but only this subtle oxygen transport or oxygen binding can

happen at one of the iron center. Let us look at the resonance Raman data for the various oxygen derived species.

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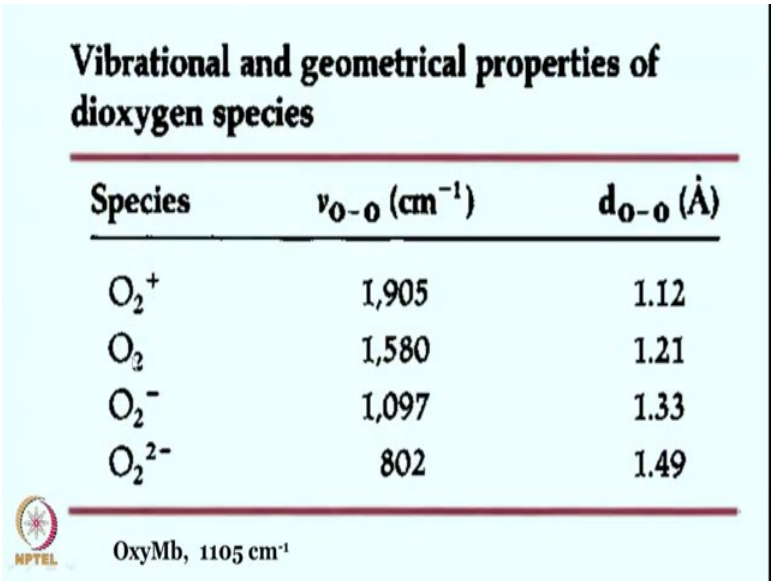

If it is oxygen O2 the from air, this oxygen-oxygen stretch would be at 1580 wave number. If it is oxidized form of oxygen, where oxygen-oxygen bond is stronger; then, we have a increase in the oxygen-oxygen stretch from 1580 to 1905 wave number.

Similarly, there is a change in the oxygen-oxygen distance from 1.21 angstrom to 1.12 angstrom, but if oxygen is reduced by 1 electron which is known as superoxide, this oxygen-oxygen stretch goes down to around 1097 wave number and the oxygen-oxygen distance increases because now it is reduced by 1 electron. If it is reduced by 2 electrons from this molecular oxygen, if it is reduced by 2 electron, this species is called peroxide peroxo or peroxide, this is the superoxo.

Now, this peroxide species, will have a further decrease in oxygen-oxygen stretch around 800 wave number. Of course, these value may vary slightly, but as you can see nearly 300 wave number difference is there, even if they are varying depending on the legend on the metal center. Still it will be very characteristic to a particular species whether this is a superoxo, peroxo. These are almost a finger print spectral features that can be seen in these oxygen bound compounds. As you have notice that the oxygen-oxygen stretch goes up significantly to 1.49 angstrom ok.

Remember what we have seen in oxyhemerythrin case that oxygen stretch was 844 wave number ok, so, 844 wave number is the oxygen-oxygen stretch. Now, if you try to match that that is falling in these region that this is a peroxide in nature right and more importantly, as you will see that oxymyoglobin has this oxygen bound in an superoxo format, but you see that superoxo stretching is matching quite well with that of those expected. So, 1097 and observe is 1105 that is fantastic, I mean these are quite interesting.

So, these can serve as a very readily available tool to confidently identify any reactive intermediate such as those we have seen in hemerythrin, its oxymyoglobin, hemoglobin and even haemocyanin and so on. Many different oxygen containing species can be characterized to very easily by such resonance Raman spectral data. Moving on well, there is earlier studies which shows that there is this crystal structure.

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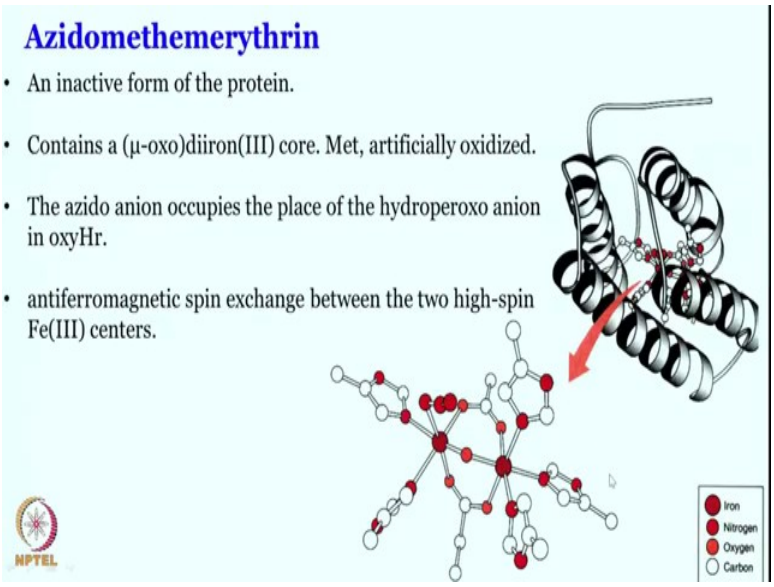

That is I believe somewhere in 1980's, I forgot to put the reference here. So, this is azidomethemerythrin rythrin, so, azidomethemerythrin.

Now, in this case ah, in the last case as you see that 2 histidine was there and the vacant coordination site was binding oxygen, but before those structure were known. This structure was first reported with an azido binding group and later on these sort of species or similar species with acetonitrile or OTf other species are also known. So, this vacant coordination site can be easily probed and these 3 histidine as you can see are definitely

there on another one and these aspartate and glutamate binding as well as the hydroxy binding is there.

This is an inactive form of the protein. So, this protein crystal structure, this contains a mu-oxo diiron III core. So, this is the mu-oxo diiron III core and this is artificially of course, oxidize the azido ion; this is the azido ion occupies the place of hydroperoxo anion in oxy hemerythrin as we have mentioned earlier that this is the site is now actually bound by this azido. The hydroperoxo is not there that is why azido it is bound with azido anion and these are the early crystal structure which is clearly showing the characteristic core of these diiron center. There is antiferromagnetic spin exchange between the 2 iron center the 2 high spin iron 3 center in this case are exchanging their spin through this oxo bridge right.

So, these are early studies, but quite informative about the relative orientation, relative binding and the core of the hemerythrin ok. There are number of studies that is done towards mimicking such azido metmyohemerythrin structure.

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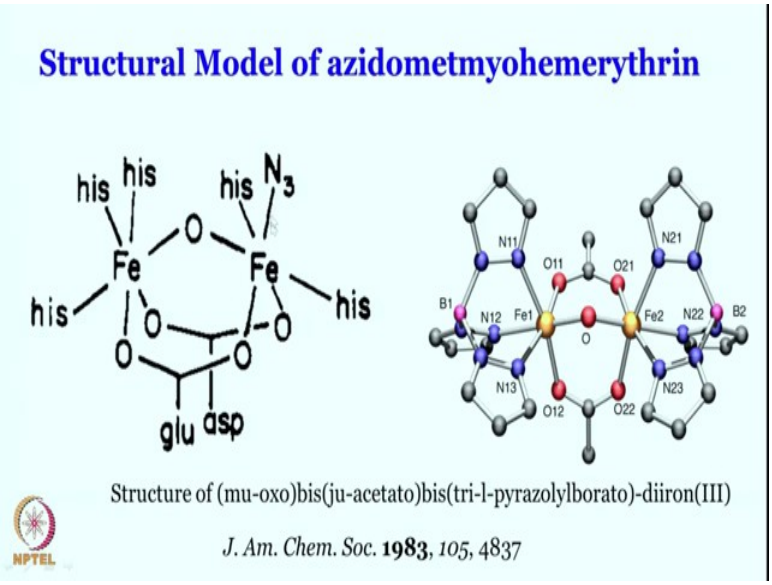

So, this is from the this 1983 report as you can see clearly 3 histidine on one iron 2 histidine on another iron as well as the azido group. Now, there are and then the bis mu oxo bridging is between their mu-oxo bridging is there not bis mu-oxo bridging single mu-oxo bridging is there between the 2 iron center right. Now to mimic such species,

scientists or the synthetic chemist has worked long and quite successfully now the model of these species has been reported.

This is an earlier attempt where trispyrazolylborate ligand system has been used for synthesizing this diiron based system and as you can see these are the acetate bound intermediate bridge between the two iron center as well as the oxo bridge is there. But one thing that is missing is the vacant coordination site. Both the iron center are having 3 iron center. Once again, this is a model study right synthetic study; this is not really the enzyme.

This is a synthetic study which clearly shows that each irons are having 3 nitrogen and both of them, but as you remember in the enzyme on one side there is only 2 histidine or the 2 nitrogen ok. These sort of mimicking studies are quite exciting ok. Because although in this case it is not the correct mimic, but these initial attempts less the pathway for the future generation studies which takes quite a lot of inspiration and then, subsequently were able to exactly match this sort of structure or the enzyme deoxy even the oxy hemerythrin structure. We will see those soon.

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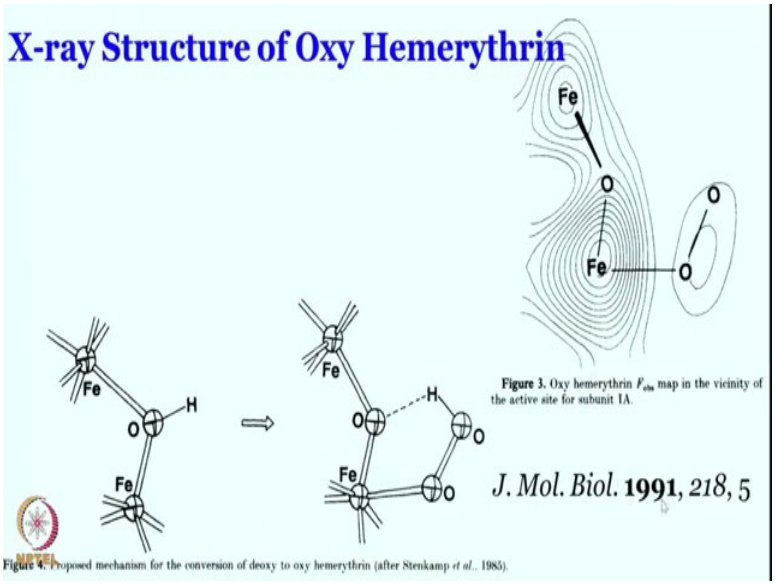

This is a X-ray structure of oxy hemerythrin back in 1991. This is clearly shows that these irons 2 iron center in the deoxy form, the hydroxo bridging is there during oxygen reaction or oxygenation leads to these hydroperoxo intermediate and these as you can see the plot clearly shows that this hydroperoxo is bound over there and I must tell you that

these hemerythrin or hemerythrin is readily crystallizable. Almost easily it is one of those enzyme which can be crystallized quite easily and it was quite amazing to get these crystal structure even with the oxygen bound form right.

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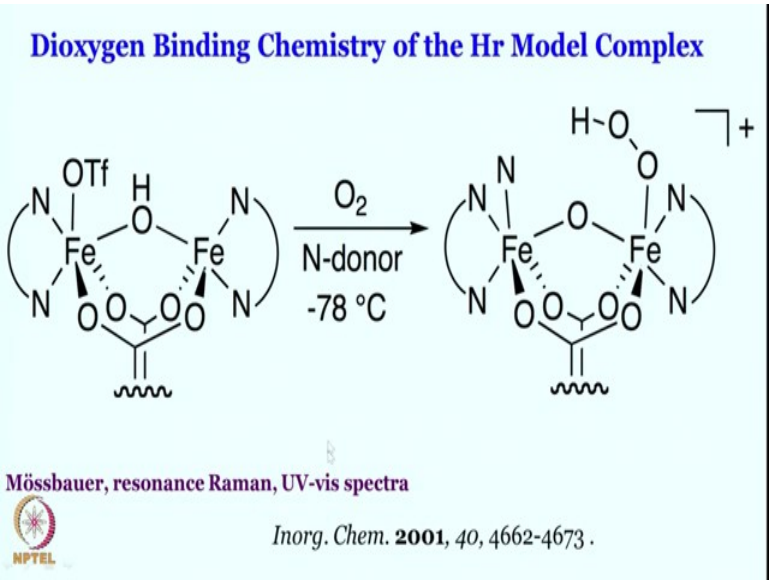

Well, a number of studies has been done this is one study by Professor Steve Lippard group himself and in these cases this dioxygen binding chemistry of hemerythrin modeling is done. One of the thing that we mention in the last case that although this trispyrazolylborato mimicking was quite good and the early stage of those mimicking those are effective mimic, but one two many ligand were there instead of 3 histidine and 2 histidine motif, it was 3 histidine 3 histidine motif or 3 nitrogen and 3 nitrogen motif. You must know that it is not essential to mimic exactly the histidine, you can perhaps play with pyridine, you can add pyridine or imidazole I mean you know all these mimicking studies with essentially deal with the same heteroatom.

So, histidine has nitrogen coordination with iron; synthetic chemist will take any ligand that has nitrogen coordination. It could be aromatic I mean it could be pyridine nitrogen, it could be aliphatic nitrogen, it could be any let say imidazole nitrogen which mimics quite well the histidine. It is not limited to just the histidine any nitrogen containing ligand is fine.

In these cases a series of such compound been synthesized and it is quite interesting to note that its instead of 2 3 histidine now, it has taken the researcher have taken 2 nitrogen

on each of the iron and one of them is having OTf and subsequently this OTf coordination can be let say displaced by another nitrogen coordination such as imidazole or suitable pyridine coordination or even azido coordination overall any solvent or an extra monodentate ligand can be ligated while displacing this OTf.

But quite interestingly the oxygen chemistry done at low temperature shows that the hydroperoxo species can form in the synthetic complex ok. So, synthetic modeling gives an opportunity to better understand the enzyme itself what happens to the synthetic chemistry modeling, if it is perfect; then, it is going to perfectly match those data of the natural enzyme.

So, that is to be able to mimic and to be able to perform the chemistry in greater detail and in absolutely complete detail is quite exciting for synthetic chemist because this sort of it sort of understanding and ability can lead to a great catalyst synthesis synthesis and it can have implication in understanding the process as well as if there is any deficiency that can be deficiency of the enzyme study that can be kind of kind of overcome by these synthetic mimic studies.

In these cases although enzyme studies are quite good and known, but a number of cases enzyme studies will not be known a lot of metalloenzyme studies are not that very easy. Therefore, these sort of synthetic studies are quite exciting. You must be very excited to note that humans afford to understand and mimic these hemerythrin was quite successful ok, if you look at the data of these compound which is a iron hydroperoxo species, all the spectroscopic data including Mossbauer resonance Raman, UV vis spectra matches almost as close as one can get; perhaps can think of getting right.

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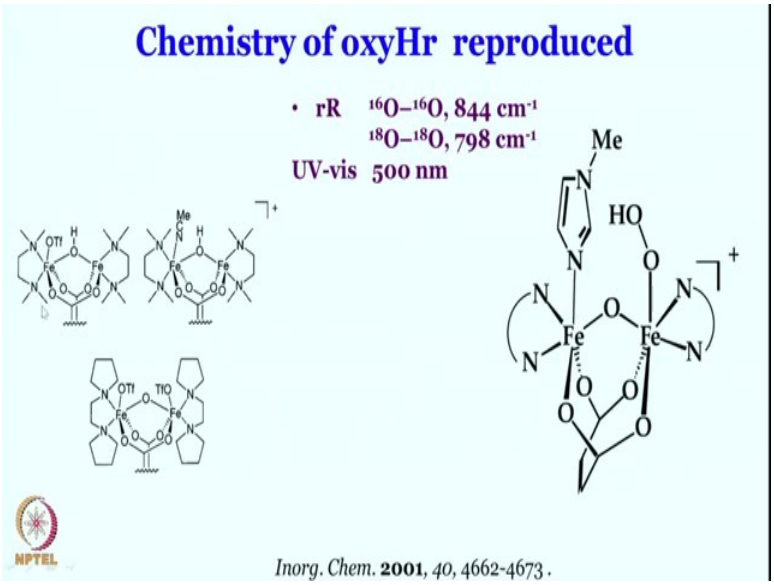

So, that is fascinating and these are some of those examples that we were talking that these are the bidentate ligand system different easily preferable, bidentate ligand system can be employed. These are some of the representative example and these were done quite beautifully as you have seen the oxygen-oxygen stretch remember it was 842 is I believe, now it is 844 wave number or exactly 844 wave number previously and not only that it is also possible to label the oxygen.

So, it was normal oxygen and then the O 18 labeled oxygen this shifts resonance Raman spectra data shifts from 844 wave number to 798 wave number that is quite fascinating. UV visible spectra is quite characteristic of what has been seen in case of the enzyme itself. So, this is so one such compound where these sort of ligand and then, is N methyl imidazole is attach and the hydroperoxo species is also formed. This is once again the report by Professor Steve Lippard's group.

So, I hope you are able to get some sense that these mimic, synthetic mimic are going to be quite useful in understanding these enzymes and the greater details and the ability to understand almost every aspects of a metalloenzyme which has been perfected by nature, I think is quite remarkable ok.

So, let us try to overview this class what we have discussed so far. We have seen that these hemerythrin is part of a marine invertebrates and these are the diiron center completely bridged species with bridged by multiple ligand as you can see; one

aspartate, one glutamate and one hydroxide. This is an unsymmetrical diiron core, one side is 3 histidine, another side is 2 histidine; it reacts with oxygen and binds oxygen at this vaccine site of the second iron center and form a peroxo but not just any peroxo it is a hydroperoxo while formation it is undergoing a proton coupled electron transfer right.

So, this is really clear I hope and this oxygen-oxygen stretch is 844 wave number exactly the number also matched by the synthetic analogues or synthetic mimic where 3 nitrogen not need not be necessarily histidine, but 3 nitrogen from the ligand is mimicking not only on this side of the enzyme core, but also the left hand side with 2 nitrogen and 1 hydroperoxo along with these oxo bridge. This sort of mimic this sort of structure is completely mimic well of course, it there has been a continuous effort in understanding this enzyme over decades.

Now, we are in a position when we believe that we understood in really great detail um, but initial studies back in 70's and 80's where quite preliminary I would say which has been matured over the years and decades. Quite interestingly, I hope you also noted that these resonance Raman is quite exciting tool for these sort of species or this sort of intermediate characterization because you must be understanding that these intermediates are very very sensitive.

These are often most often these are only low temperature stable intermediate, you will not be able to see these intermediate at room temperature and that is quite phenomenal to mimic I would say and then, thanks to these spectroscopic technique which is now taken as a standard in characterizing these species with or without the crystal structure.

Now, these oxygen oxygen stretch are invariably going to dictate the assignment of the right species. For example, as you have seen if it is getting reduced the stretching goes down as well as the length of the oxygen-oxygen bond goes up, but for that you have to have the crystal structure right that is quite not feasible in a lot of cases. And therefore, this sort of this sort of studies are quite important just to have a resonance Raman structure or resonance Raman assign spectra will tell you what is there in reality.

Of course, getting a crystal would be perfect, but life is not that rosy all the time; most often for these sort of species you have to rely on spectroscopic techniques such as low temperature UV visible spectra and resonance Raman and if possible let say low temperature a EPR and NMR, if it is feasible or Mossbauer's or in addition to that for

iron for example, iron centers this is the Mossbauer spectra will be quite amazing to characterize specifically to tell what sort of spin state is there; what is the electronic configuration and most importantly there are other spectroscopic technique in addition to the X-ray crystallography if X-ray is there that is fantastic if it is not available also the XXAPS XSAPS these sort of different spectroscopic technique can be use to further characterize these intermediate with almost 100 percent confidence for these sort of species.

And those are very sensitive strategy and of course, mind you that these are also very expensive studies, but nonetheless those can be now routinely done. Thanks to the development over the last few decades which has made these possible made possible characterization of these species in greater detail. We have seen the azido bound hemerythrin and the mimicking aspect of these how and what people have done; some of it there are many studies that has been done we did not get into the detail to keep it simple.

So, there were initial attempt which were almost as good, but then that was not mimicking the vacant coordination site, iterative designing then has address that issue. This is the crystal structure showing exactly what we have seen in the earlier drawing. This is the phenomenal studies by Lippard group which exactly mimic what we have seen in the enzyme. With this, let me conclude then that we are able to able to see this these spectral features as well as the human efforts to understand these enzymes in greater detail. So, keep studying hemerythrin and other related topics and we will get back to you soon in the next class.

Thank you.