Metals in Biology
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Lecture – 37
Summary of Dioxygen reactivity in Iron

Hello welcome back, I will today try to summarize what we have learned in terms of the iron chemistry ok. Iron as you have discussed at the very early stage is one of the most abundance metal on earth and it is no wonder, biological system has taken up iron and therefore, had utilized for doing various important chemistry, like these are the redox chemistry these are going to be exciting chemistry, where both the iron oxidation state as well as for example, oxygen oxidation state will vary.

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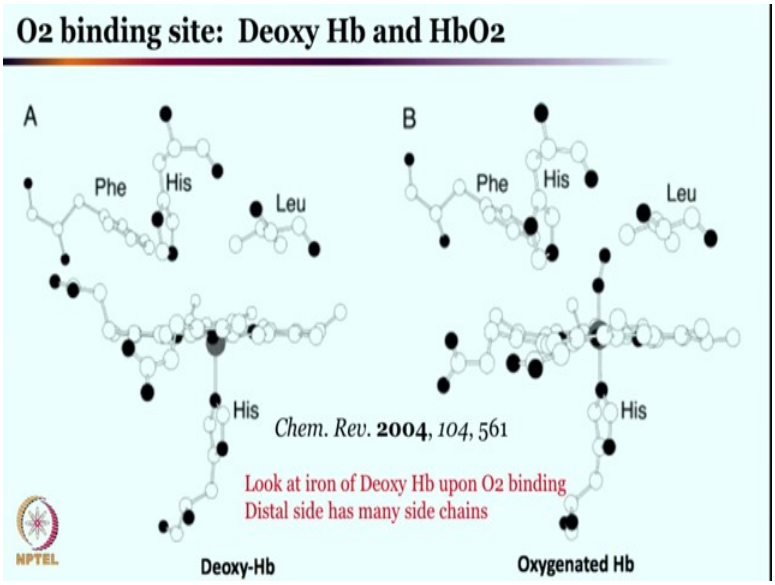

We have seen first of all the oxygen transport by iron right, we have seen in case of hemoglobin, we have these iron 2+ high spin high spin species which is outside the plane and bound with histidine, it push in it is pushed in when iron III oxo species is formed or iron III sorry super oxo species which formed. This is the reason why we are alive right. This is beautiful chemistry supported by these beautiful side chains and this chemistry we have seen many a times.

So, iron can act as the metal for the porphyrin center or protoporphyrin nine center in case of hemoglobin and myoglobin for keeping all of us alive and can transport oxygen

from lungs to different part of the body, and this is why our blood is also red right. We have learned that and the intricacy of these chemistry.

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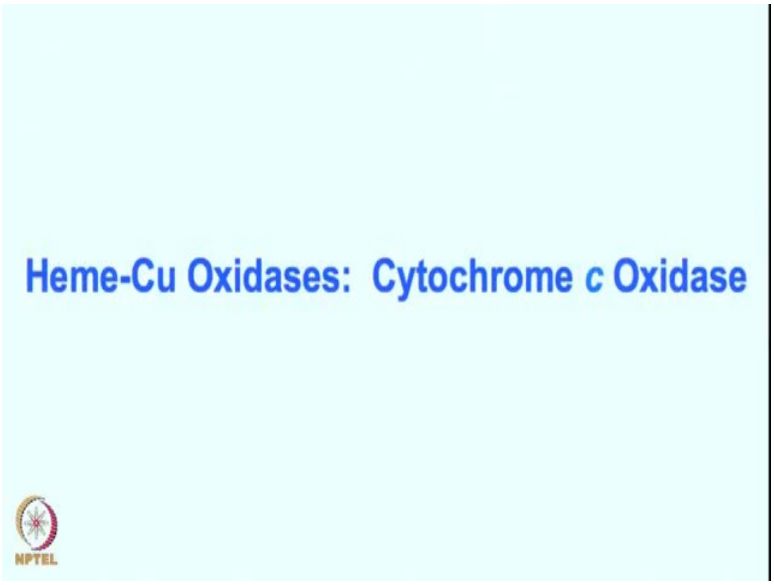

We have also learned the heme copper oxidases. Once again the same iron chemistry, but with a twist of adding copper into the mix.

So, you have the copper chemistry and the iron chemistry individually. In the last class we have summarized how the copper chemistry is and today let us say in this part we will see how iron and copper can come to a compromised situation and can do wonder for the biological system.

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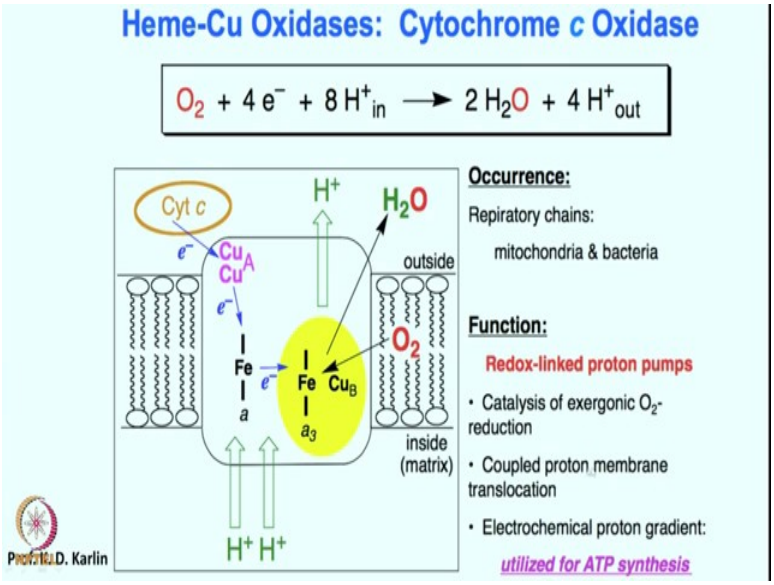

For instance the Cytochrome C Oxidase which is nothing, but Heme copper Oxidase, it can convert oxygen into water, a very very difficult transformation, if you look at it requires 4 electron 4 proton overall to convert it into water by doing so, it is participating in the proton gradient creation and overall this is a very important transformation in nature ok.

Cytochrome C oxidase has quite a few active species or quite a few metal centers that is involved of course, there is a iron copper center which is the one who will briefly discuss will not discuss about this cytochrome C center, which is the heme center supported by the axial ligands such as cysteine and histidine on both the side on one side histidine another side cysteine. So, this is just the electron transfer side, there is another die copper center which is responsible for just the electron transfer.

Overall it is the hopping of electron from cytochrome C to copper center to the iron center and all the way to the heme copper oxidase center this center. This is where the oxygen is taken up to convert to be converted into the water, these are all membrane bound protein as you have seen from the membrane here ok.

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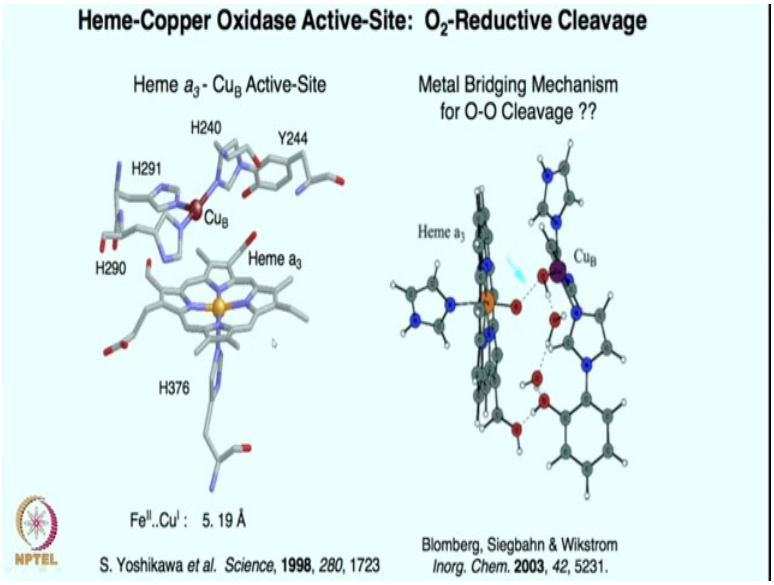

So, the active site in that heme copper oxidase or cytochrome C oxidase main active site of course, there are other metal as you have seen is this porphyrin iron center as well as this copper center, this is similar to what you have seen let us say for one of the copper center of hemocyanin or tyrosinese 3 histidine is there, but of course, in hemocyanin tyrosinase you have 2 copper centers. Here one copper center with 3 histidine, but with a twist of adding this phenol the tyrosine histidine cross link phenol being upended right over there, this is the heme center where you have the iron center iron from the axial side there is this histidine.

So, proximal side is histidine and the distal side is opened for oxygen coordination, some crystal structures are there which is suggestive of some intermediate, but these are highly questionable.

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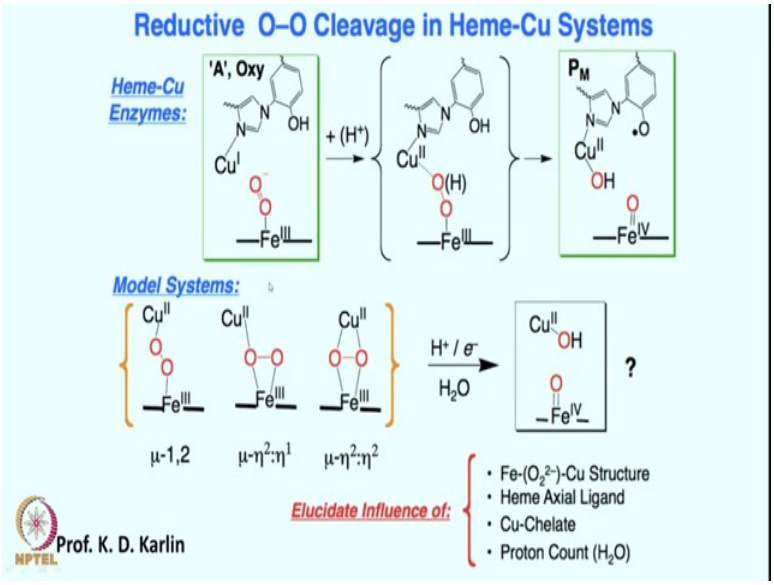

In any case the proposed mechanism is such that iron will fast from the iron super oxo species just like what we were discussing earlier just like you have seen in case of the hemoglobin myoglobin with the histidine coordination, it will bind with the oxygen to give the iron III super oxo. Iron would have been outside the plane now pulled in with the histidine also moved in, in any case this is going to be the iron III super oxo species the very fast formed intermediate.

Characterization of such intermediate in the synthetic studies is done, but most importantly it will immediately almost immediately react with copper 1 to give rise to a peroxo intermediate, this peroxo intermediate could be the iron III O O copper II or it could be a iron III hydro peroxo intermediate all of these species will add up in reducing oxygen by multiple electrons overall forming the water upon oxygen oxygen cleavage in this peroxo species. We would get the copper two hydroxo species in presence of this phenoxy radical formation via the hydrogen atom abstraction of through the species will also get high valent iron oxo intermediate formation.

We have seen the nature of these iron copper species, it could be an end on bound geometry, it could be side on end on bound geometry or it could be side on and side on bound geometry. But most likely if it is a tetra dented ligand, then this is the species forming, if it is a tridentate ligand this is the species is forming perhaps one can rule out such sort of species formation. Since we in the enzyme have a tridentate ligand. So, this

is the species likely to be forming in the enzyme. We have seen the crystal structure in synthetic setup for such intermediate where iron III which having a side on geometry and end on geometry for the copper case.

Finally, we were able to see that in synthetic studies both these side on bound as well as these side on end on and side on side on bound geometry can be interrogated further by a proton and electron to give the oxygen oxygen cleavage which is nothing, but forming water because the protonation of these intermediates should give the rise to the water formation.

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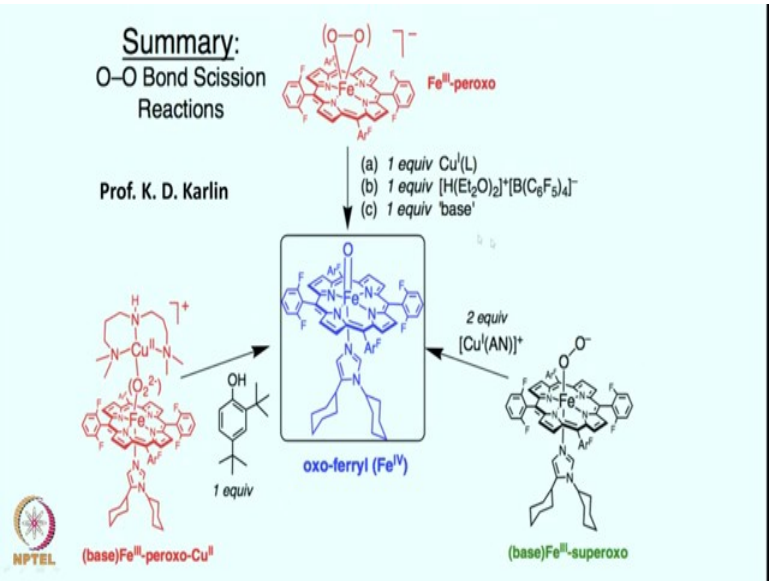

This is fantastic, we have seen in the summary for these you know synthetic model studies, that these species is characteristic of each of these species are very characteristic. They have now very very fingerprint like identification in terms of the UV visible spectra, resonance Raman spectra, excerpts different other fast spectroscopic and sensitive spectroscopic technique can be utilized for characterizing the so, form intermediate.

So, the synthetic understanding it so far such that we can follow each and every step of what is happening in case of oxygen. So, we can start with oxygen, reduce it by one electron or to give the super oxo, another electron transfer to give the peroxo species most likely this is the peroxo species formation and then the protonation and further electron transformation, electron transfer cleaves the oxygen oxygen bond.

Well to summarize the synthetic efforts what has been done so far is iron copper peroxo species has been characterized and synthesized and characterized and upon adding hydrogen atom in the form of proton and electron it was possible to detect these iron oxo species formation, which is definitely indicating that oxygen oxygen bond of oxygen dioxygen molecule is clipped to form the species right.

One can also independently start from these iron III peroxo species where oxygen is reduced twice, one electron from iron another from outside as a electron source to give the iron III peroxo species. These iron III peroxo species then can react with a equivalent of copper 1 as well as proton and base this can gives rise to the species. Just to remind you if the base is not there which is this one, this n cyclohexyl dicyclohexyl imidazole this reaction does not work. Of course, if proton is missing this reaction does not work. If copper one is missing once again this has no relevance in terms of the iron copper heme peroxidase chemistry right.

So, one can again reach out it from a different angle also by synthesizing the axially ligated iron III super oxo species, one can then also interrogate such species towards the 2 equivalent of copper AN, where one equivalent will act as a electron transfer reagent to make it the iron III peroxo, another equivalent will react with it to further take you to the formation of this oxo species.

So, to summarize the heme copper oxidase of course, you have seen the stepwise formation and stepwise understanding in the slides when we were discussing originally, but here to simply summarize that we understand that enzyme such as hemoglobin and myoglobin can reversible bind oxygen, does not do any other oxygenation chemistry or any other advantageous chemistry or adventitious chemistry.

But in case of heme copper oxidases as you have seen this is quite fascinating where oxygen itself is converted to water, but by taking advantage of both the heme iron center and copper chemistry together. So, it is combining the best of the two world of one of iron chemistry, another of copper chemistry putting them together such a very difficult transformation such as oxygen to water molecule conversion can be possible and we have seen that this has a real implication in terms of our biological transformations ok.

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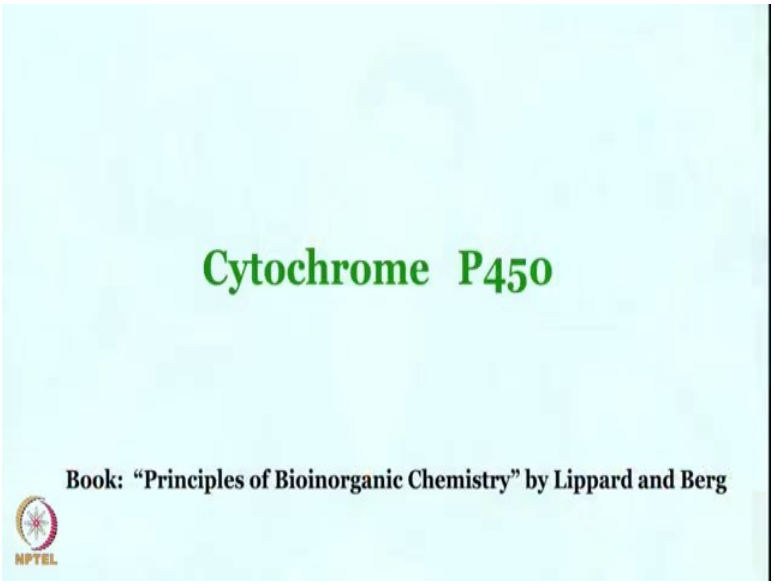

Now, moving the gear in summarizing this; so, we will see the cytochrome P450 right. This rings the bell right, we have discussed this cytochrome P450, once again this is a heme enzyme of course, here no copper is involved just the heme center. But not here oxygen is going to be converted to water; oxygen will be utilized to do the oxygenized chemistry; that means, if you have aliphatic substrate, if you have many other substrate the cytochrome P450 can do the reaction on the organic substrate.

Cytochrome P450 is a is the best synthetic organic chemist ever almost the reactions the synthetic chemist cant perhaps dream of doing efficiently can be done relentlessly smoothly by cytochrome P450 right.

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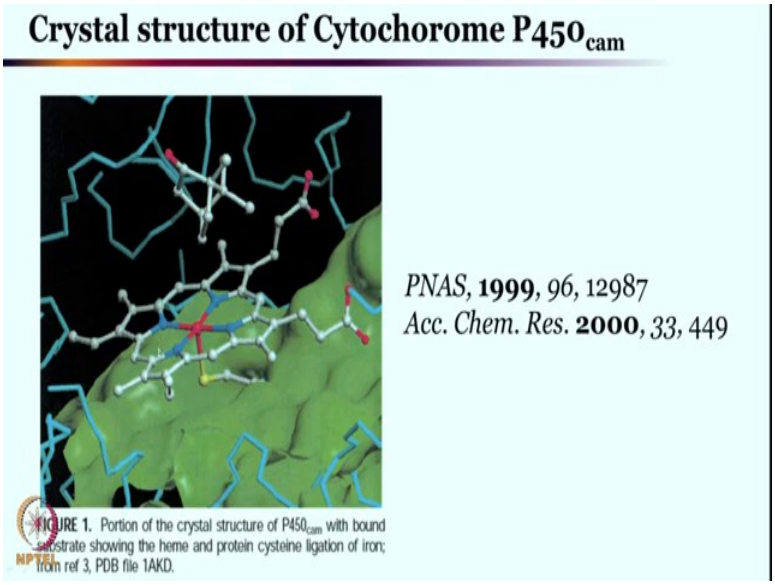

Cytochrome P450 is crystal structure, this is the CAM 4 bound crystal structure, this is the heme site, but one notable change here from the previous cases of hemoglobin, myoglobin and cytochrome c oxidase is this cysteine thiolate binding right. Cysteine thiolate binding is different previously it was the histidine coordination, now this binding can be extremely efficient or extremely important both for the oxygen oxygen bond cleavage as well as your important iron high valent oxo intermediate stabilization right.

So, this iron high valent oxo intermediate upon stabilizing of course, upon forming and little bit stabilizing it can abstract hydrogen atom from the substrate and subsequently it can hydroxylate the aliphatic substrate right.

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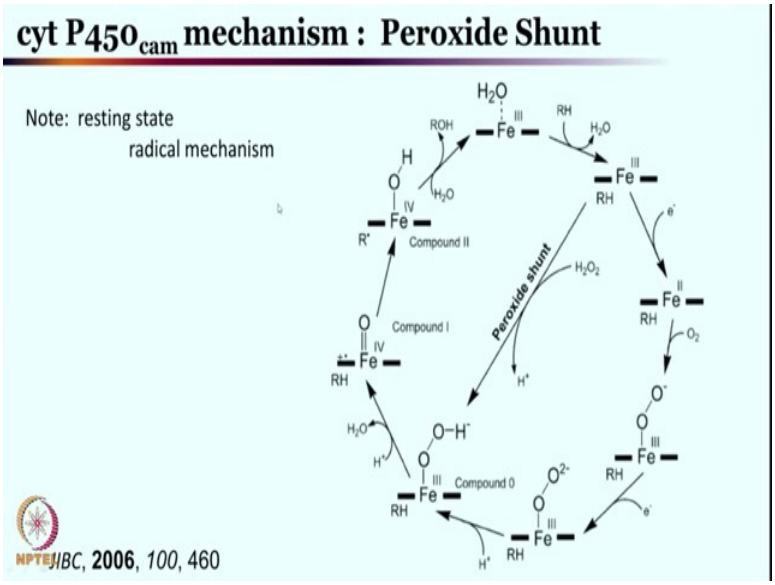

So, the reaction mechanism to some very quickly, we have seen the ion III species which would be outside the plane although this their drawing does not reflect, this is the resting state of the enzyme organic substrate, you know orient itself in front of the active site oxygen binding as well as activation leads to super oxo. Another electron transfer through this you know iron IV cluster and the big chain of event that gives rise to the iron III peroxo species formation protonation gives rise to the iron III hydro peroxo species formation.

Remember none of these species formation are have are happening in case of the hemoglobin and myoglobin. But in case of cytochrome c oxidase just like cytochrome P450, cytochrome c oxidase may have some similarity in these steps, we have similar perhaps intermediate in cytochrome c oxidase also, but none of these intermediates are fully characterized in those cytochrome c oxidase cases. But here we have informations that these species are called these compound 0 or iron III hydro peroxo species which can undergo the cleavage of the O H bond to give the iron IV oxo radical cation.

So, essentially iron V oxo which can abstract hydrogen atom from RH to give the iron IV hydro oxo. Well you have seen also the catalase activity and the peroxidase activity, if this pathway is not forming or this is not the desired pathway oxygen is missing or the electron transfer is not sufficient in presence of the hydrogen peroxide as an intermediate or as an active species, we can utilize these iron III species to shuttle between this iron

III and iron III hydro peroxo. This is known as peroxide shunt, but this is the mechanism you have seen for the peroxidases right.

So, the peroxidase chemistry essentially involve the formation of these iron III hydro peroxo species starting from the iron III aqua complex from directly from their it forms this intermediate and from there on it can go on to give rise to the water molecule right and in presence of still it would require two H dot from the substrate, but overall it can it can give you from hydrogen peroxide to two equivalent of water, for alkyl hydro peroxo to one equivalent of alcohol and one equivalent of water.

Of course, there is formation of these high valent iron oxo intermediate, which can then further in presence of an electron and proton like H dot can give rise to this intermediate and then finally, back to that intermediate.

So, this catalytic cycle is also true for the peroxidase right that we have seen and for the cattle age we have seen that, it is just the settling between these iron III intermediate and iron for IV oxo radical intermediate these two intermediate shuttles. Here just the H2O2 is taking part and H2O2 is converted to one equivalent of water and one equivalent of oxygen.

If enough H2O2 is not present then the formation of this intermediate can be questioned or can be possible, but in presence of the reductant such as NADPH or so, even if enough H2O2 is not present still these intermediate remain valid no other intermediate is possible in those cases as well right.

So, this is quite interesting, they are quite interlinked as you can see both the cytochrome c oxidase cytochrome P450 catalase peroxidase all are playing very simple game of game of high valent iron oxo species formation right. So, we have seen in cytochrome C oxidase, we are seeing in cytochrome P450 we have seen in the catalase, we have seen in the peroxidase all of them are essentially for being high valent iron oxo intermediates right in different form.

Now, as you have seen in case of cytochrome C oxidase, there is a iron copper center these are only heme center ok.

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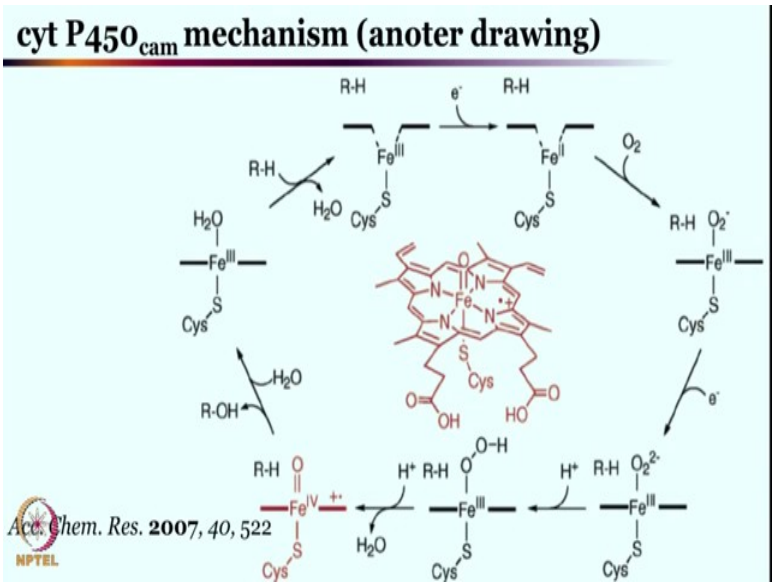

The same diagram we have seen one more time in the of the iron being outside the plain, how iron is pushed in overall giving rise to this sort of intermediate which is quite fascinating overall to follow that this oxygen then react as well to give you the steps that we have just discussed. So, this is the same intermediate we have discussed right.

So far its you have to study a little bit because otherwise it gets complicated or confusing because these are related and these are similar yet distinct, they are not similar or same. They are similar, but not the same, there are certain differences you need to understand. So, the peroxidase chemistry catalyzed chemistry and cytochrome P450 chemistry is very similar. You should you should read it together understand the difference and cytochrome c oxidase is little different of course, you have seen hemoglobin myoglobin is completely different they are kind of the no hassle enzyme, it is just do it just dodge one activity and that is oxygen transport right.

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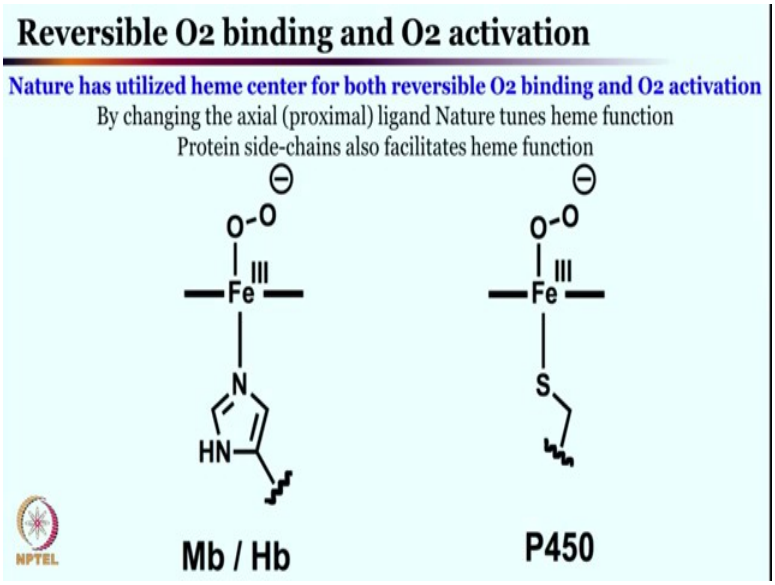

So, that is quite interesting for all of us as to remember. Moving on what we have understood also clearly is this hemoglobin and myoglobin utilizes histidine and cytochrome P450 type of enzyme utilizes this cysteine thiolate, wherever the high valent iron oxo spices formation and their stabilization is required these cysteine intermediate is called upon. Nature has utilized heme center both for the reversible oxygen binding and oxygen activation and subsequent reaction by changing the nature of the axial ligand from histidine to the cysteine we have seen that nature tools its reactivity.

Protein side chain of course, plays a key role, this shot of cysteine thyroid bridging helps you in overall hydrogen bonding or the proton conduit to form if the, to form the hydro peroxide intermediate and then the oxygen cleavage becomes easier because of this big push that is coming out from the thiolate. So, the thiolate is negatively charged ligand effectively, it pushes the electron from the thiolate to the iron to all the way to the O O and therefore, the oxygen oxygen cleavage becomes easier.

Overall I would also say that these irons high valent oxo species once it is from that is this high valent iron IV oxyradical cation that is iron V essentially can also be stabilized quite nicely by this thiolate cross linking or thiolate linking. So, therefore, it is essential to understand the nature motive of making these almost similar thing but but really distinct thing by subtle changes nature did not play too much with these species right alright. So, that is about the heme chemistry.

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Let us say next look at the non heme chemistry. Non heme chemistry as you have seen are yet another avenue right. This is once again very very fascinating right. So, so far we have seen at least one porphyrin is there we have seen hemoglobin, myoglobin cytochrome c oxidase cytochrome P450 catalyzed peroxidase all of these cases we have at least one heme center. Now, we want to get rid of the heme center and we want to put the simple ligand such as histidine right.

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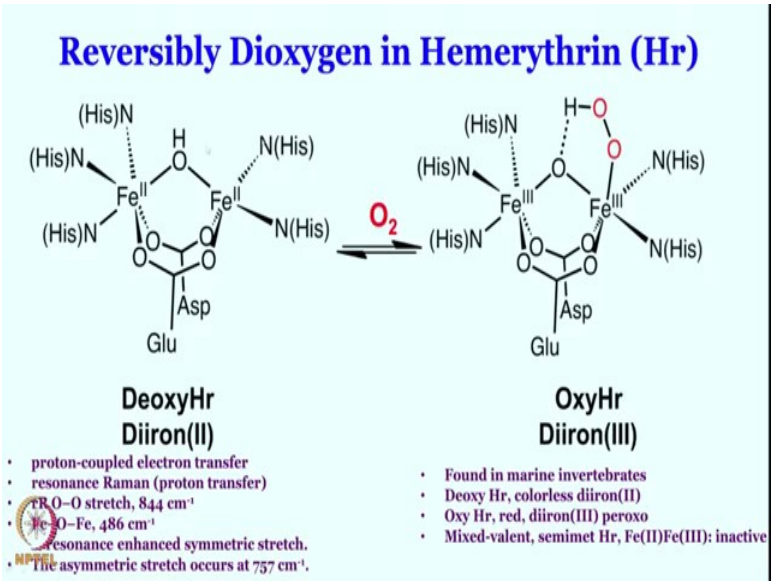

So, we have all already seen in one case of just histidine that is, but this is an unsymmetrical case as you know, 3 histidine 2 histidine, but this is the hemerythrin case which is not doing any sensitive chemistry I would say in terms of the substrate oxygenation or oxidases, this different type of chemistry. But this is a pretty important reaction that it binds with iron center upon binding also it ends up transferring electron, that as we have summarized earlier and this oxygen is really binding is really reversible.

So, whenever it is required from this species, it can remove the oxygen and comes back to the species right. So, this is important for marine invertebrates for their life, but there is no organic substrate to be functionalized in these cases and these are well lit, well built intermediate you see 2 iron centered breezed by glutamate and has parted and hydroxyl and is histidine ligand and histidine ligand unsymmetrical ligand these are beautiful right. But we do not we have seen earlier and the spectroscopic features and everything, but let us look at some of the chemistry that can be done by these non heme iron center. Of course, not exactly these center, but these are known heme right because the porphyrin ring is not there.

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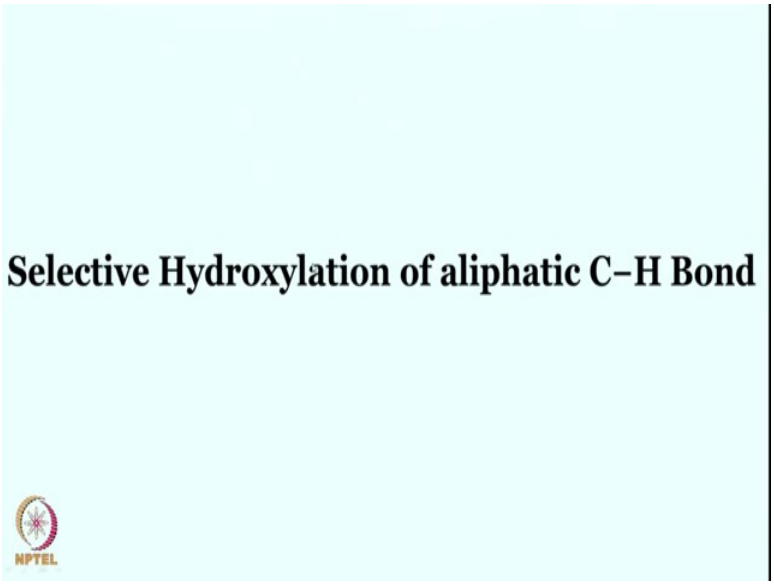

So, these are known him center if you look at further, let us look at the some of the hydroxylation chemistry that we have seen some time back. So, these hydroxylation chemistry is super important from the synthetic perspective as well of course, from the

enzyme perspective it is very very important, but it is also very exciting from the synthetic perspective.

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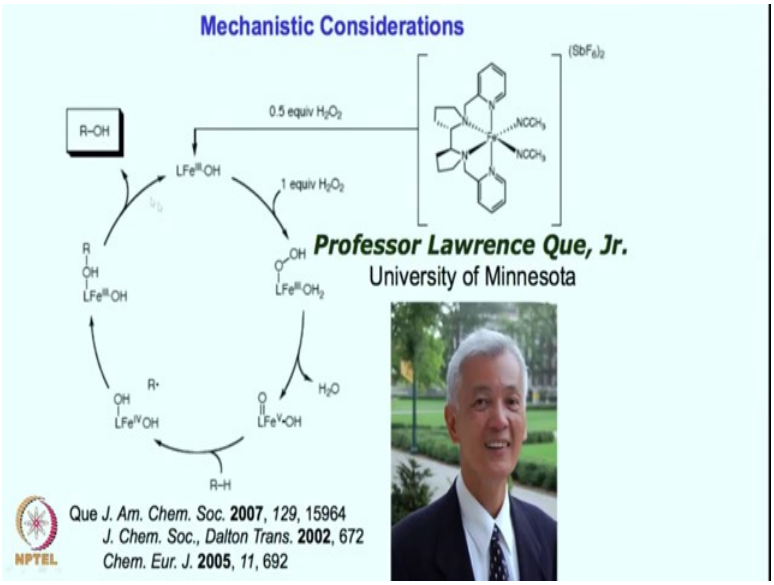

This mechanistic understanding goes as simple, that for the synthetic studies as well this iron III hydroxide is forming, it can react with hydrogen peroxide to give you the iron III hydro peroxo species. These iron III p hydro peroxide species these are all synthetic studies I am talking at this point right now.

These iron III hydroxyl peroxo species can undergo oxygen oxygen bond cleavage to form iron V oxo hydroxo intermediate right. Iron V oxo hydroxo intermediate is formed, these iron V oxo hydroxo intermediate is a super reactive intermediate can abstract hydrogen atom from RH to give you R dot and iron IV dihydroxy intermediate. These rebound between RR dot and OH would give rise to the ROH intermediate right.

So, this is quite phenomenal you have seen such shorts of high valent iron oxo species formation in case of cytochrome P450, just a moment ago we were discussing cytochrome P450 also has essentially iron V oxo, which is iron IV oxo radical cation which can do quite a beautiful lot of chemistry with the porphyrin ring, but these are non porphyrin ring or non heme ligand system which are also capable of doing these chemistry right.

So, we these are similar chemistry what we have seen in cytochrome p 450, but these are non heme iron chemistry which is also quite exciting chemistry that that we know of right.

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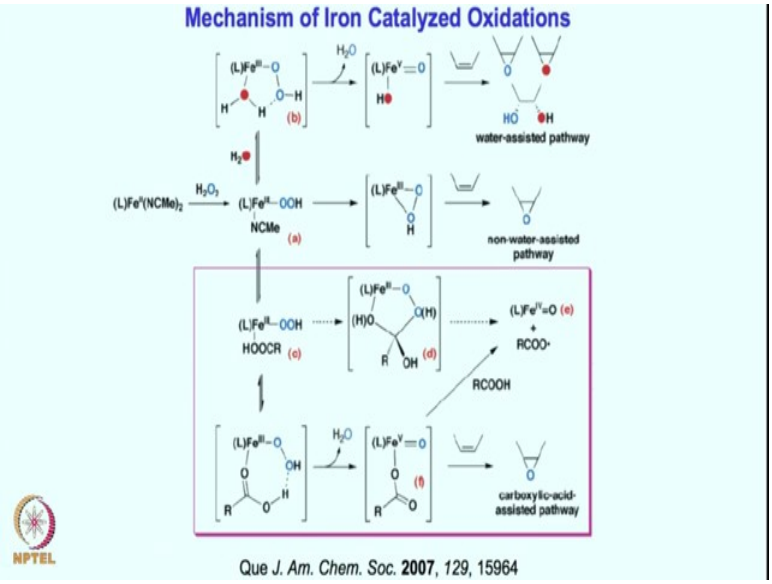

Now, this cleavage of the oxygen oxygen bond in iron III hydroperoxo can be influenced by water or by the carboxylic acid. Water can assist in this ring formation mode where the oxygen oxygen bond cleavage becomes feasible and confirm really iron oxo hydroxo intermediate, which can then undergo the epoxy and or the syst hydroxylation product formation.

In case of this hydro peroxo species as you know so far that this hydro peroxo intermediate can also undergo oxygen oxygen cleavage with the help of the hydrogen bonding from let us say acetic acid. This acetic acid hydrogen bonding weakens or makes it easier to break the oxygen oxygen bond to make the iron V oxo along with the carboxylate linkage. This carboxylate linkage linked iron V oxo once again can react with with the olefin to give the epoxy resin product, there could be quite interesting beautiful chemistry coming out of these as well.

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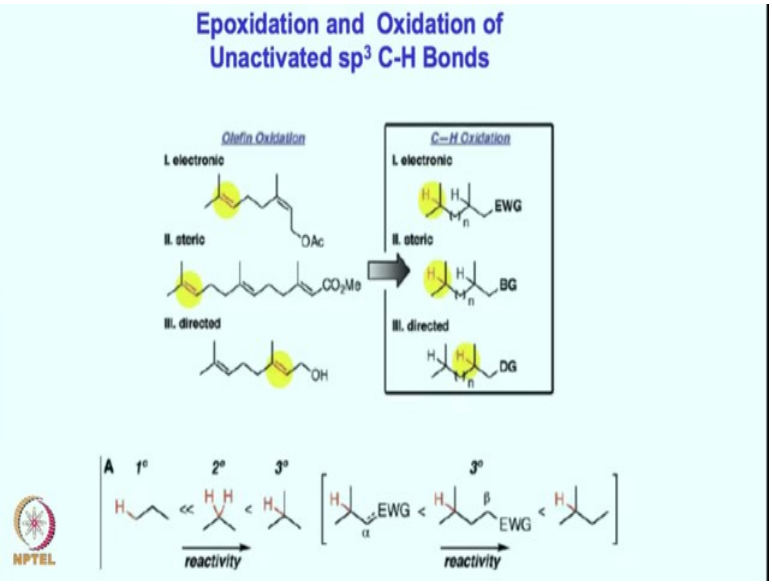

So, these are synthetic chemistry, but most importantly this sort of synthetic chemistry can be utilized in predictably selective hydroxylation chemistry right. We can have predictably selective chemistry originating from these non heme iron synthetic compound or we have this reactivity of these iron oxo hydroxo or high valent iron oxo intermediates are quite predictable in this nature, where tertiary is found to be more reactive than secondary then the primary centre right. If you can actually reverse this reactivity mode with the suitable electron withdrawing group, once you have an electron withdrawing group present that molecule becomes less reactive in this case no electron withdrawing group is there. Therefore, it is more reactive this tertiary center this tertiary center is very less reactive since electron withdrawing group is there.

In this case this starts at the center is very close to an electron withdrawing group therefore, this is not that reactive as well. In fact, in such a case maybe tragedy is not reactive too much compared to even the secondary one. So, these short of selectivity in case of olefin epoxidation can also be extended both in terms of the directing group, sterically demanding group and as well as the electron richness can be monitored and can be predicted for a given organic substrate. So, depending on the substrate complex the substrate is predict some for the hydroxylation site becomes much more easier right.

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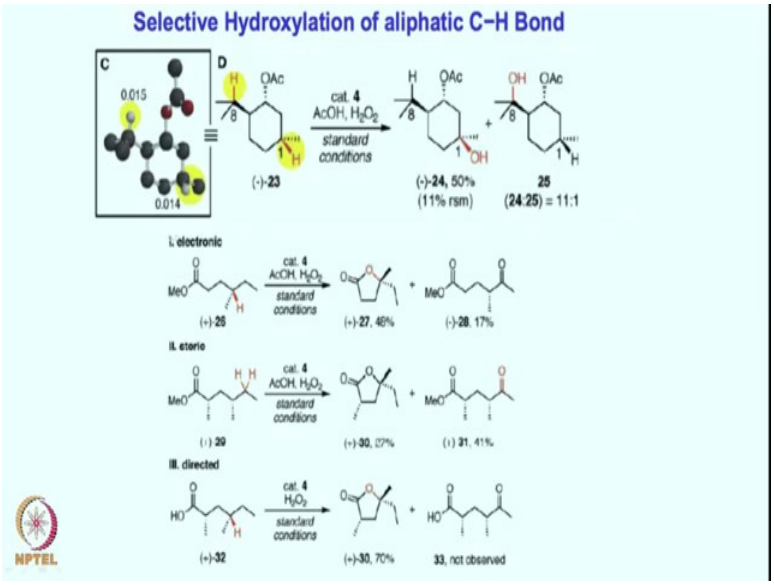

We have seen that for example, if you are looking at this compound, well you have a primary center, you have a secondary center, second tertiary center secondary center of course, primary center. But primary are not reactive, secondary are not reactive too much, tertiary is going to react and that is what you see. If you introduce a steric bulk so, this becomes not so, prominent at this point because it is sterically hydroxylation is sterically hindered.

And therefore, even the secondary center is getting reacted over here of course, if you have a if you have a secondary site or primary site the product will be ketone nor the hydroxo product, because the upon first hydroxylation further oxidation becomes inevitable and very very facile and of giving this product.

Now, this sort of intermediate we have seen this is the site of the reaction because these sites are deactivated by this electron withdrawing ester group. You can override these sort of bias of electron steric bias if you have a directing group instead of making it a star if you make acid.

Then this acid is now going to direct this C-H bond to give the corresponding hydroxylated product that is fascinating. In this molecule as you can see you have one tertiary center another tertiary center, another tertiary center, these center is sterically encountered or a hinder this is also not too reactive due to the electron deficiency and

this is the site of where the reaction is happening and selectively this is the site where reaction is happening.

It is important to also note that, there are going to be other product there is other product formation, but those are not too much compared to the major product formation. So, we can perhaps put those on the site, but nonetheless they are forming right.

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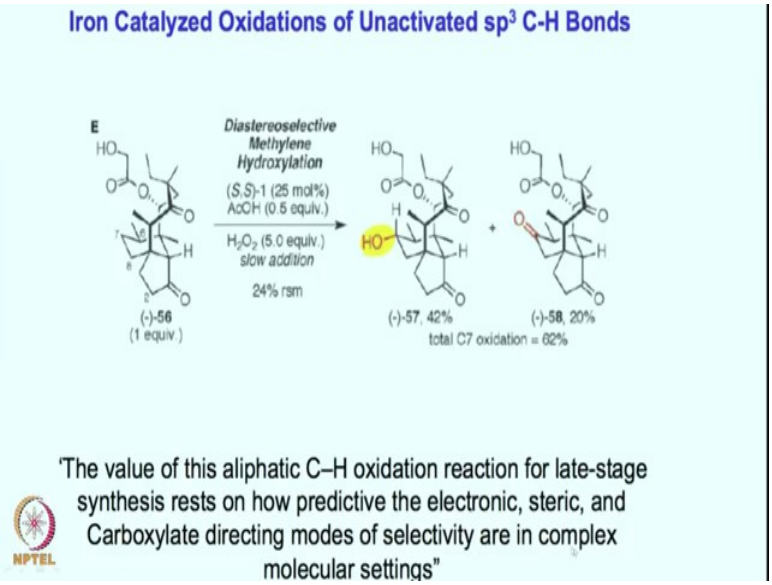

So, more the complex substrate, you have better it is for its predictably selective functionalization in this case. Out of all different CH bond that is present in here selectively this hydroxylation can be possible in this case, even the keto formation becomes difficult because of the steric hindrance. So, this is a highly engineered substrate, highly complex substrate and the prediction becomes much more easier.

So, in this type of chemistry just let me conclude by saying that the value of this aliphatic CH hydroxylation or CH oxidation reaction for late stage synthesis rates on how predictive the electronic static and carboxyl and directing modes of selectivity are in complex molecular setting. In other words more complex the substrate is better it is for the for the active species to do the chemistry selectively with this will come back again.

Thank you very much.