Metals in Biology
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Lecture – 38
Summary of Fe-O2 chemistry

Hello welcome back, we are nearing to the final few classes of this course and that is Metals in Biology. We are trying to summarize little bit quickly so, that you can study these for exam purpose relatively easily. What we have seen in case of the iron oxygen intermediates that, they are very capable of doing oxygenation chemistry some of them are oxygenases some of them are oxidizes right.

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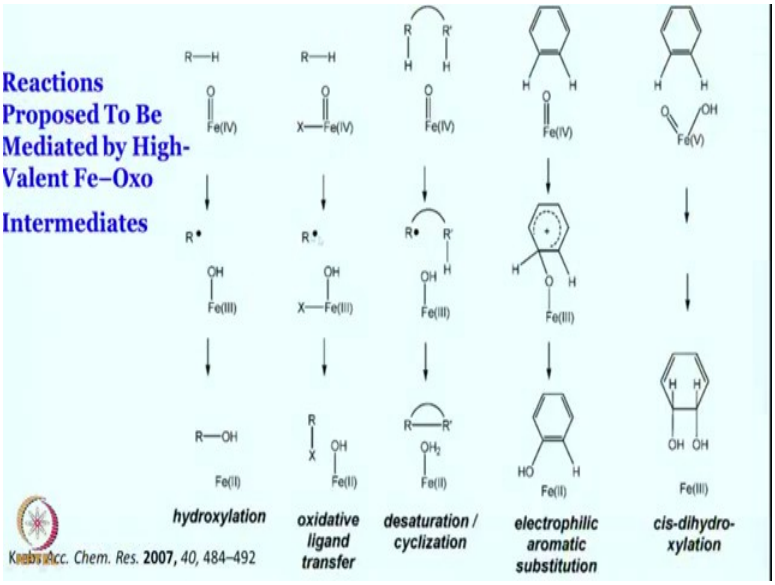

But more importantly almost every organic substrate can be functionalized by utilizing the iron oxygen chemistry. This chemistry can be between the two iron center between the oxo species is bridged in between or it could be as fascinating as those the terminal oxo species or these porphyrin oxo based species.

There are non heme oxo based species as we have also seen, but these species are also known to be very very effective. So, you have seen these many different types of reaction hydroxylation oxidative ligand transfer, desaturation cyclization electrophilic aromatic substitution and cis dihydroxylation reaction; all these reactions you are hopefully now familiar with not most of them are going via the radical mechanism as

you can see in there. This one where arene activation is involve or arene oxygenation is involve, it is going through an electrophilic aromatic substitution reaction. The cis dihydroxylation can be mediated by these oxo hydroxo species to form this from this in extremely exciting products.

All these cases this is this oxo radical formation and corresponding the CH bond activation and the radical formation is key and that is what we have seen over the many many different high valent iron oxo intermediate cases.

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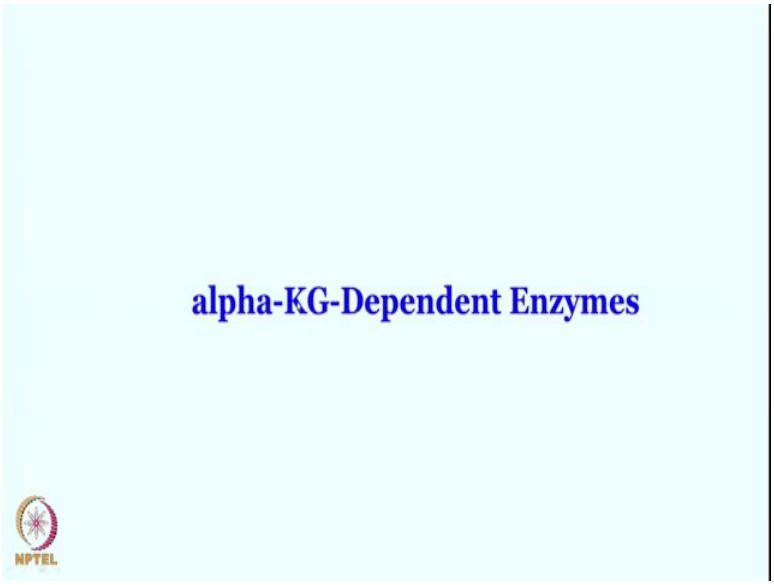

One of the exciting species you perhaps would remember as well that the alpha ketoglutarate dependent enzyme are capable of doing both the oxygenation chemistry as well as the halogenation chemistry depending on the slight changes they have, in their coordination motif, but nonetheless they will be having this alpha ketoglutarate as an internal substrate for their activity.

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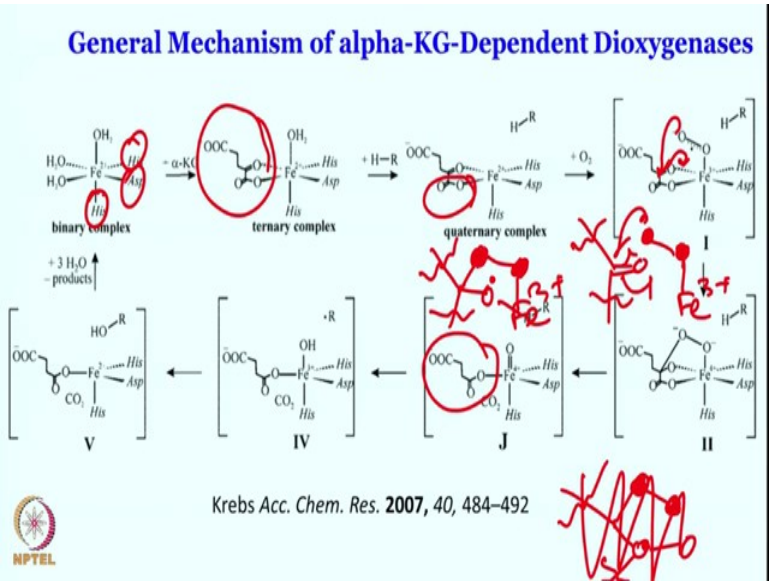

So, what you have seen earlier with the alpha ketoglutarate dependent enzyme is simply it binds with the non heme iron center and oxygen activation that takes place at the iron center before that the substrate will orient itself, get it ready, get set go and then oxygen will start a gets activated and the iron center it fast from a iron III super oxo which immediately is trapped or attacked attacking the keto moiety of the alpha ketoglutarate, the alpha keto center is getting reacted with forming an alkyl peroxo like intermediate, which can then undergo oxygen oxygen bond cleavage to give rise to these iron IV oxo species.

These are reactive intermediate and the substrate is sitting right close to it therefore the oxo will obstruct hydrogen atom to form the hydroxo and they are radical right this R radical and hydroxo radical can combine with each other to give the ROH once again this water molecule goes out alpha ketoglutarate comes in RH takes it position, get set perfectly oxygen comes in forms the iron III super oxo iron II plus was there it forms iron III plus it formed a super oxo this super oxo attacked the alpha keto center to form these cyclic nice intermediate where it is now iron IV thanks to the nucleophilic attack on this keto center which is then getting O minus by transferring one electron from the iron center oxidizing is to iron IV plus from iron III plus right.

Now, these iron IV plus alkyl peroxo type of intermediate can undergo the oxygen oxygen bond cleavage wherever whatever way you want to see it with the simultaneous

expulsion of the CO2 moiety it gives rise to the succinate bind the iron IV oxo intermediate which is the real active species for obstructing hydrogen atom from the sp3 C-H bond right over here this is nothing, but you can see that iron III O dot this O dot and this RH will form the bond or from this iron III OH bond and RR dot radical will be generated during the process. So, I am sure you are having a clear understanding among these different OH and O bond.

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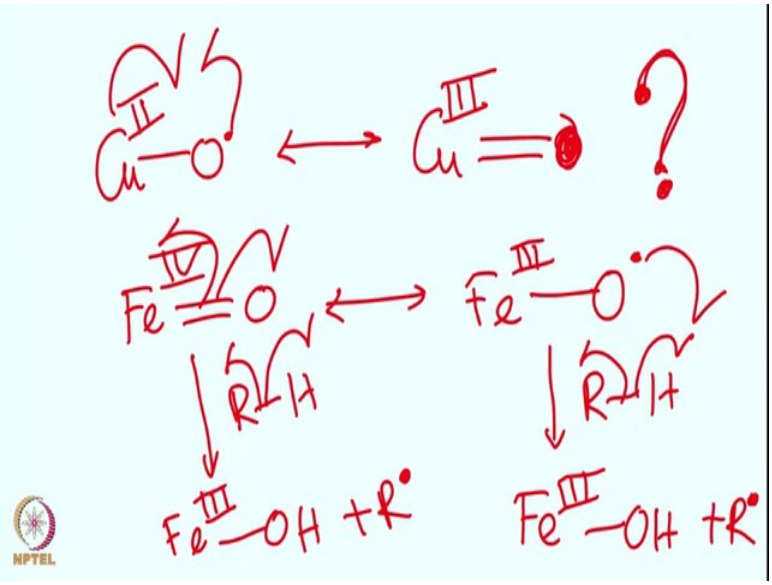

For instance if you have seen earlier this Cupryl species for example,. So, we are saying that Cupryl is nothing, but Cu II O dot this is exactly same as Cu III double bond O because this will give electron that will give electron to form that. Similarly for example, if we are talking about iron IV oxo species, this is also nothing, but going to be iron III these are same species in different form one can write iron III O dot.

Now, this as you have seen in the last slide these iron IV oxo was reacting with RH to give you what? To give you iron III OH. How that is happening? Because this iron for oxo is nothing, but iron III O dot and if you are giving RH to this, this is forming R dot this is forming H dot and these two bonds. So, overall you get iron III O H plus R dot hopefully this is clear. On the other hand over here if you want to draw it is nothing, but this bondage breaking and then that is giving and that is forming the R dot. So, that is R dot.

In any case whether you want to think that iron IV oxo is a double bond O iron or it is the iron III o dot all leads to the exactly same product iron III hydroxo R dot iron III hydroxo R dot. So, this is the pattern of the radical formation you can you can say the same thing for a copper oxo intermediate or a iron IV oxo intermediate although these species in Cupryl species in the in the enzymatic setup although propose are still doubtful in the synthetic setup. Recently some evidences are coming, but still one need to further develop this chemistry in the synthetic setup.

So, that they are relevant can be understood in enzymatic setup ok. So, overall I hope you are able to understand this mechanism. So, you are forming the succinate from this alpha ketoglutarate you are getting rid of this carbon dioxide moiety from here and I hope you understood that how it is attacking over there, and this is O minus H forming and then these O overall is get oxidizing or so, overall oxidizing iron III plus let me draw that step again. So, this is here a oxygen oxygen radical is attacking a keto center, now this keto center over there will form a radical overall it would be forming OOCO let us say these are oxygen level maybe it would be easier to understand.

So, that is there, these are there this is iron center now this. So, I am redrawing that over here. So, this is now attacking and this keto moieties are drawn over there this oxygen oxygen iron is giving rise to a carbon center and O dot right this is iron III plus this is remain iron III plus and then this iron III plus transfer one electron from here to give 2 oxygen which becomes iron IV and O minus that is what I am trying to explain. Hope this electron transfer does not throw you off too much and you can count these electron step wise without much of a problem. Let me draw that one more time very clearly for you and that is very simple process I hope it is not getting complicated for you.

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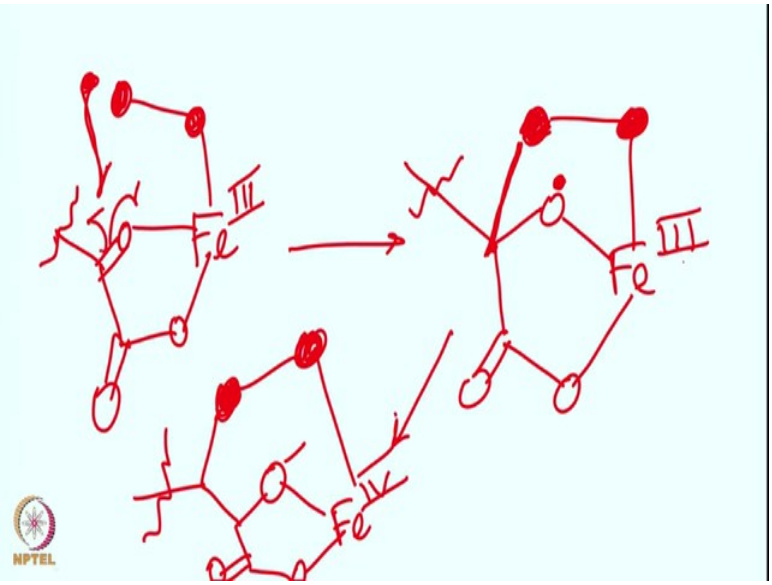

So, you start with iron III super oxo iron III super oxo ok. You have a keto center attached with it with you, this is the one which is going to form the carbon dioxide in any case I am not drawing the rest of it. So, this intermediate goes on to attack this center right. So, this pi bond will cleave to give you this oxygen iron III remaining part remain constant right. So, that is what you see right. So, this is what you see along with the (Refer Time: 10:12) bond O dot over there.

So, this O dot over there is attacking and forming this radical and that radical forming in this bond right. This is the new bond form and this is the radical that is coming over there this radical and then pick up one more electron from this iron III overall to give you this center as iron IV and this oxygen becomes O minus along with formation of this ox alkyl peroxo intermediate right. I hope you got it correct without any trouble in understanding right. So, this radical attack at the carbon center and that oxygen radical form one electron transfer from that center to give rise to iron IV and this carboxylic.

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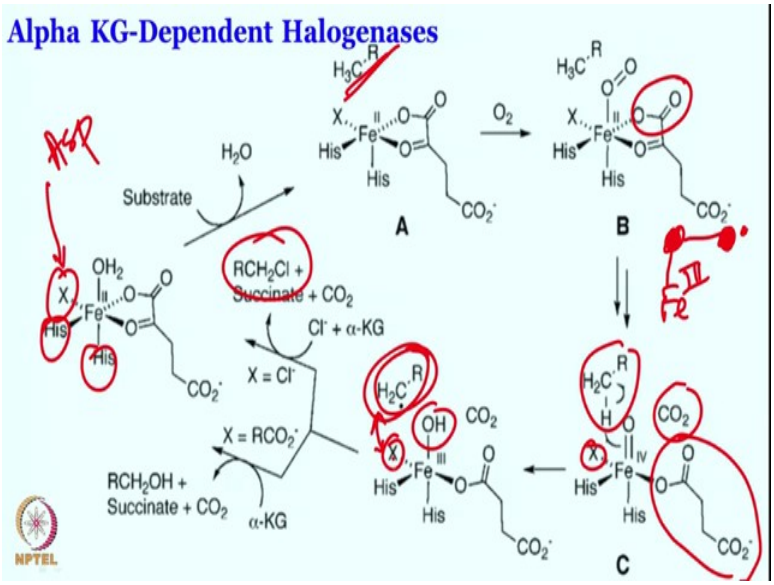

Anyway let us move on what we have seen that these this alpha ketoglutarate can form iron oxide intermediate very interestingly and very simply then that can go on to react with the substrate to give you the substrate hydroxylation product. The twist here is if you do not have that you know 2 histidine 1 1 1 1 carboxylate intermediate 2 histidine and 1 aspartate intermediate the facial triad that the facial triad so, called facial triad intermediate if you do not have, 2 histidine and one aspartate these 3 moiety if you do not have then you are you are in for in quite exciting part and that is just 2 histidine just 2 histidine and the and 1 X the halogen.

So, this was aspated in the last case now this is halogen this halogen will then be reactive with the radical center that is getting generated at iron; for instance if you are bringing the alpha ketoglutarate of course, at alpha ketoglutarate in substrate is sitting right over there iron two center is there reacts with oxygen binds with it and then form the iron III super oxo intermediate, iron III super oxo intermediate right iron III super oxo some intermediate then that attack on the carbonyl center of this and subsequently it from the iron IV oxo with the succinate, this is upon decarboxylation of this alpha ketoglutarate unit.

Now, this iron IV oxo unit as you can see over here iron IV oxo unit over here then can abstract hydrogen atom just like what you have seen in the hydroxylation chemistry, but only difference is you have the halogen over there. Now it can abstract hydrogen atom

from the substrate to give the substrate radical intermediate along with the formation of O H. New there is a competition between X and O H where OH losses because this radical is close to this X, and more importantly the reduction potential is helping out as well. Overall, this x selectively transfer what they are no hydroxylated product is found.

So, RCH2X is form in this case RCH2Cl is formed if x equals chloride and if we if you have you know other things not present or you have difficulties, then hydroxylation product may be formed. So, these alpha ketoglutarate dependent enzymes are I guess quite exciting not only they are capable of doing the hydroxylation chemistry, they can also do the halogenase chemistry right.

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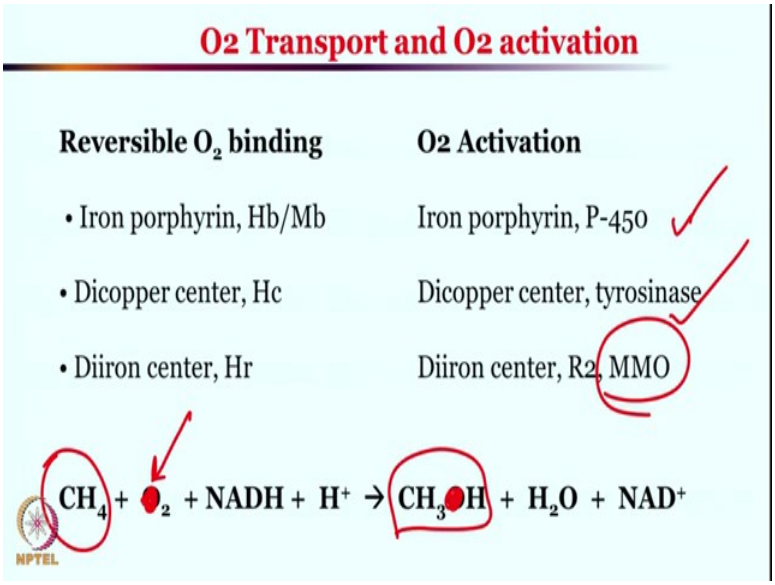

We have seen that as well. So, what we have seen so, far that not only reversible dioxygen binding oxygen activation is also fascinating right and more fascinating I would say and more challenging more opportunities and more substrate scope can be involved.

Iron porphyrin cytochrome P450 chemistry we have discussed already and summarize it, dicopper center tyrosinase also we have discussed we have discussed in the last few classes about MMOs, I will briefly mention these again. So, the main reaction we are trying to study is in case of MMO, these are going to be a non-heme iron center once again dont mix between the heme and non-heme iron center. These are going to be the non heme iron center methane is going to be converted into methanol with the help of the

oxygen if you are having the leveled oxygen this is going to be leveled over there right that is correct.

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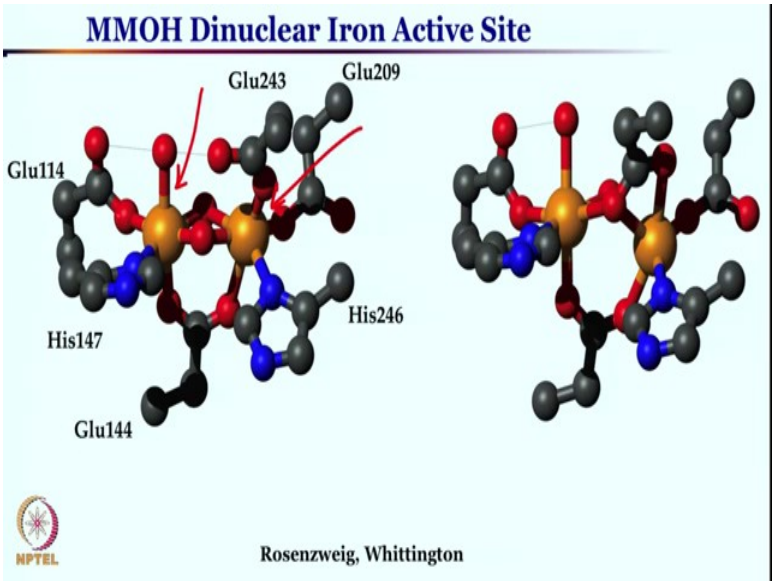

So, these are fascinating enzyme, as you have seen there are going to be two iron center and these both the iron centers are quite exciting over there, these are unsymmetrical as you see the coordination environment around this site is completely different compared to that one which is which is quite fascinating you have one glutamate here, you have two glutamate over there right.

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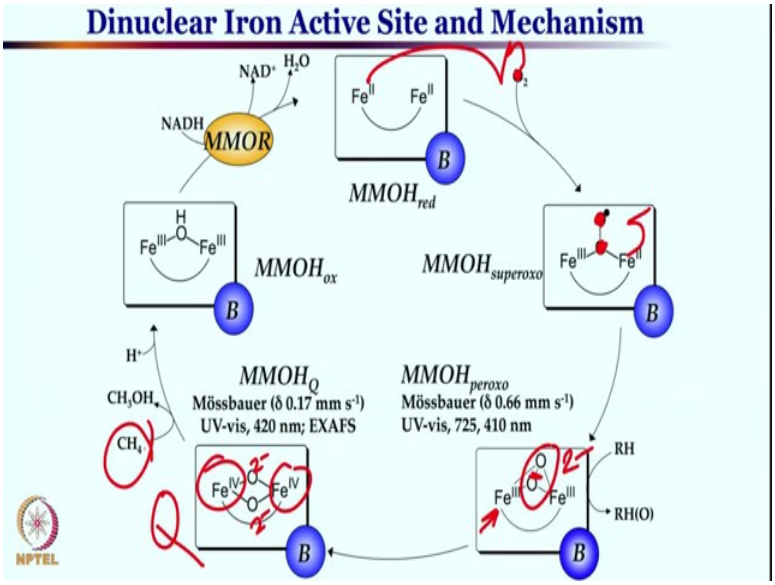

Now, if you have seen the earlier mechanism once again the oxygen comes into the picture and earlier mechanism suggests that this oxygen will follow up all the way through to first of all transfer one electron one electron from here and one electron from there to form the superoxo intermediate. This superoxo intermediate can again transfer one electron from there to give you the iron III 2 minus this is the peroxo intermediate from there on this oxygen oxygen bond can be cleaved to give you the bismuoxo.

Where oxides are 2 OO 2 minus O 2 minus iron IV and iron IV, it was iron III this oxygen oxygen bond cleavage gives you iron IV iron IV 2 minus 2 minus. Now, the methanol comes and react with this Q intermediate, this is the intermediate Q which is responsible for the methane activation to give you the methanol product.

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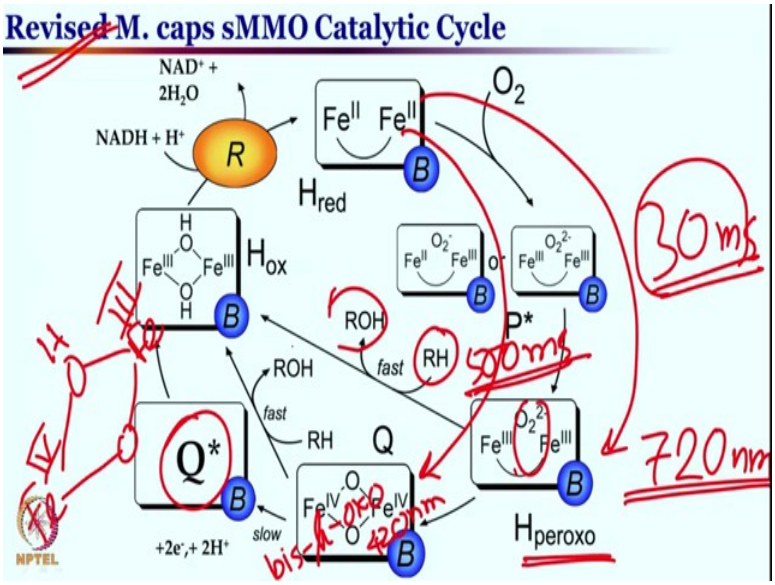

There is a slight change or the revised mechanism subsequently this revised mechanisms tells that this iron IV oxo or iron IV dioxo intermediate will be capable of forming a new intermediate perhaps as you have seen, this new intermediate is going to be iron IV hydroxo oxo iron III intermediate.

Although further characterizing this intermediate is not that very easy, but more importantly I believe that that this peroxo intermediate can also be reacting with the methane substrate to give you the methanol along with the formation of di iron III dihydroxo intermediate. This peroxo and this bismuoxo this is bismuoxo muoxo intermediates are quite exciting intermediate for these species. As you have as we have

noticed there that these intermediates are quite exciting if you are following overall if you are overall following if you are overall following up to here, this is a 30 milliseconds right 30 millisecond time frame.

If you are following overall from here to here, this is a 500 millisecond time frame right. So, this is forming this intermediate is forming if you are following for 30 millisecond, if you are mixing oxygen with this reduced species within 30 millisecond or right at 30 millisecond you can follow up this one, what happens to this what is the decomposition product and this has a characteristic band around 720 nanometer. If you are mixing these species with oxygen and for waiting for 500 milliseconds this one you form and this is having a very characteristic band at 420 nanometers right.

These species are the one you can follow and then react it with methanol a methane to give the methanol product right.

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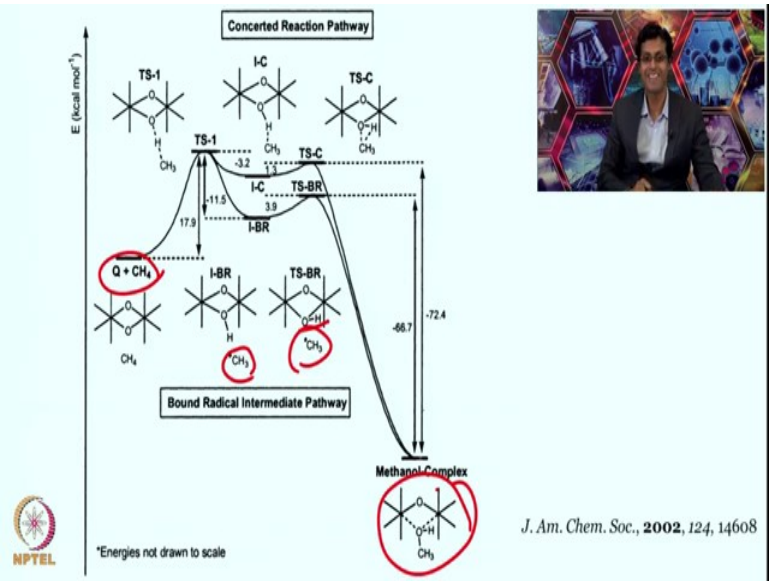

So, overall what you have seen previously is the decay of these sep 420 peak or 720 peak can be rationalize can be followed quite easily to give the give the detail kinetic studies. The detailed studies shows that it is actually more exciting than what we thought Q the intermediate Q as you have seen the bis mu oxo intermediate can react with methane to give you a bond making and bond forming step over here, where we have seen a oxo hydroxo species is generated.

This oxo hydroxo intermediate can either go through a radical intermediate or through a concerted intermediate; concerted intermediate is more challenging or more energy demanding. Therefore, we end up getting a more preferred pathway where this radical species are formed upon this hydroxo, electron rearrangement we get hydroxo radical this hydroxo radical and the CH3 radical combines to give you the product. Of course, the alternative pathway is little bit more energy demanding therefore, you do not perhaps follow this intermediate that very clearly right.

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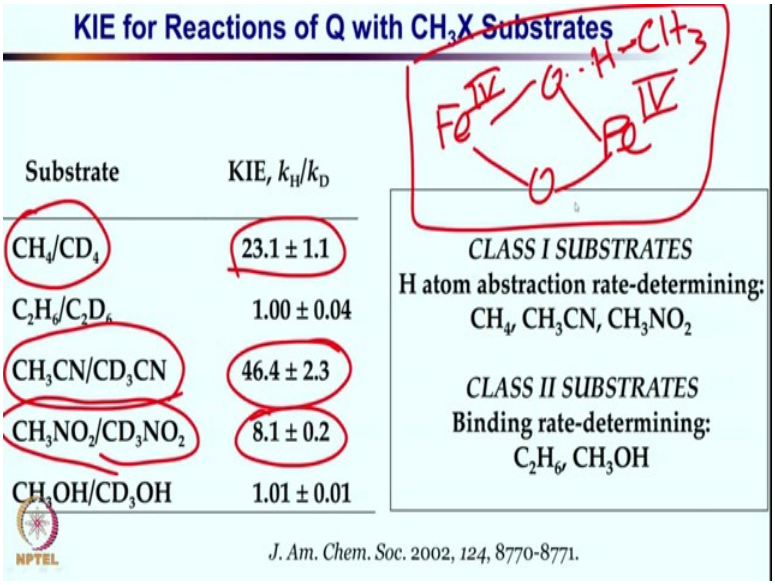

So, this is a quick summary for these methane monooxygenase, you have seen also two types of substrate over there one substrate is the methane and corresponding other substrate which will have the very high kinetic isotope effect right. So, these are having very high kinetic isotope effect as you can see over here, these are these highlighted ones right now are known as the class one substrate, where methane approach could be could we could we it could be interesting and simple.

But more importantly the C-H activation is the rate limiting step. So, the diffusion of methane towards this Q intermediate. So, if you are looking and looking at the Q intermediate which is nothing, but iron IV oxo iron IV intermediate, this intermediate is approach of this with respect to this CH4 is very simple nothing no problem happens over there.

And this approach overall approach of CH4 with respect to bismuoxo is not the rate limiting, but this activation of this bond is rate limiting for this case of CH4 and CD4. And similarly as you have seen in case of acetonitrile and in case of nitromethane this approach is not problematic, but CH activation is the most damaging or most difficult step. On the other hand for the substrate like substrate like this ethane and the methanol there this approach of the substrate towards this active site is the one which is critical or most challenging and therefore, we will end up get getting the diffusion control process.

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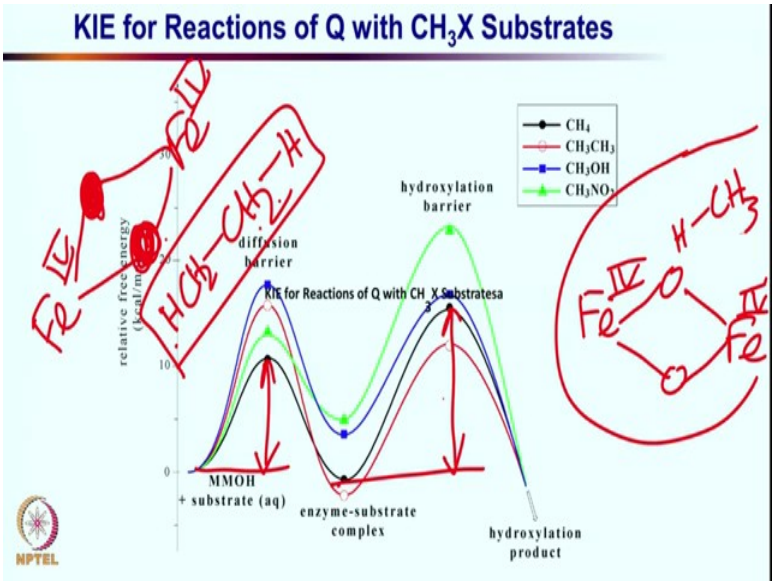

If you are remembering the previous drawing carefully, you will note that the for methane this is the black curve this path is the diffusion of methane towards. So, towards the iron IV oxo species is not really problematic.

So, this diffusion or pre orientation of the methane is happening quite easily this can happen quite easily, but this is not so, easy if you have approach of ethane and that is how the substrate specificity perhaps is obtained. See this is the same intermediate as this, but if you have a HCH2CH2; that means, CH2H; that means, the ethane this approach of this into towards this is not that very easy.

And this diffusion control is its becoming a diffusion step is becoming the rate determining step. As you can see from the m methane case this is not very high or alternatively, but the other step that is the C-H activation step is much bigger and much

stronger more importantly for methane bond dissociation and energy is around 105 kcal per mole.

For example, for ethane it would be 98 to 99 kcal per mole which makes a huge difference that C-H bond dissociation becomes easier and this is also perhaps the one of the reason why ethane C-H activation will not be problematic one, but reorientation or approach of the substrate diffusing the substrate with respect to the iron IV oxo intermediate will be critical as you see there.

So, for the methane case this is shorter this is taller for methane case this is taller this is shorter that is the summary from the MMO.

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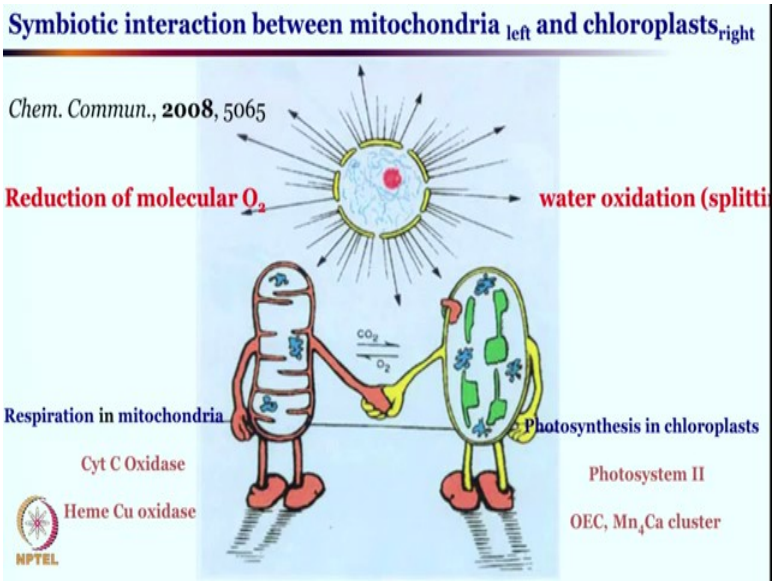

So, the substrate dependent chemistry we have seen. A exciting part we have we were seeing the 4 manganese 1 calcium cluster I think the first thing that should come into mind that these are microscopic reverse of the cytochrome cytochrome C oxidase which is heme copper oxidase, which is responsible for converting oxygen into water, but in the photo system you are converting water into oxidant.

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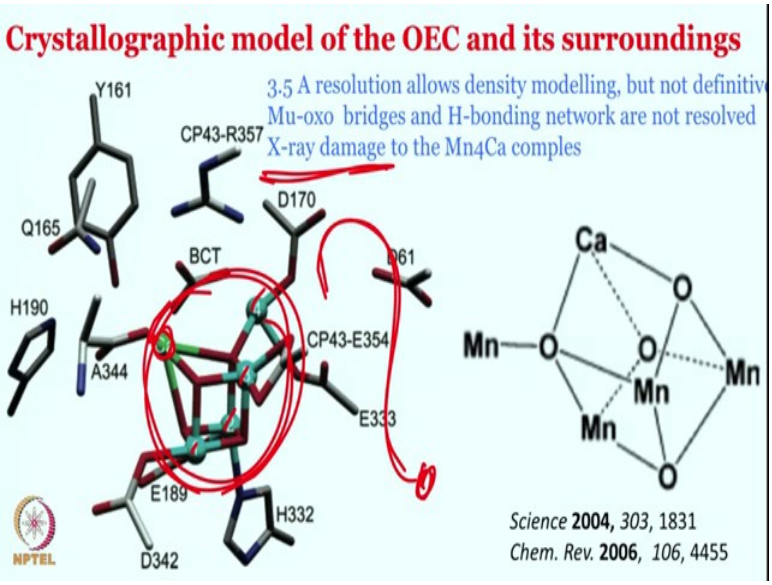

And the crystal structure as you have seen the gigantic crystal structure have many caveats in it many problem in it, but nonetheless this is the four calcium 1 2 3 4 four calcium four manganese center and one calcium center that is over there this can be redrawn like that, but although this crystal structure is known, but still this is completely questionable, the X-ray damage is the major reason why people do not believe that this is the structure for the oxygen evolving complex.

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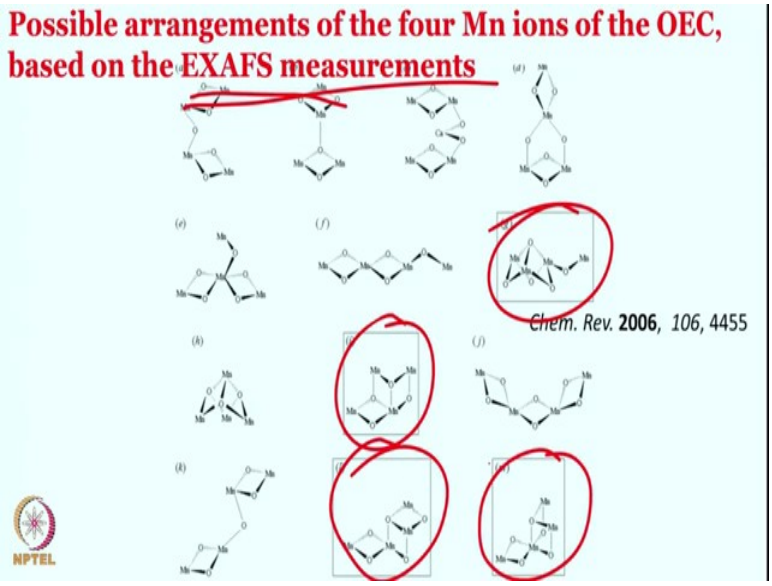

So, what is the structure well nobody knows still now these are the these are the major structure that is proposed and believed to be still active, but nonetheless although some accepts data are there, but still people are debating on these some of these structure perhaps can be ruled out, but perhaps can still be existing in the in the in discussion. But, I think for time being we can or for since this debate is still on we cannot really rule out any of these structure completely, but these are the structure people believe are mainly happening.

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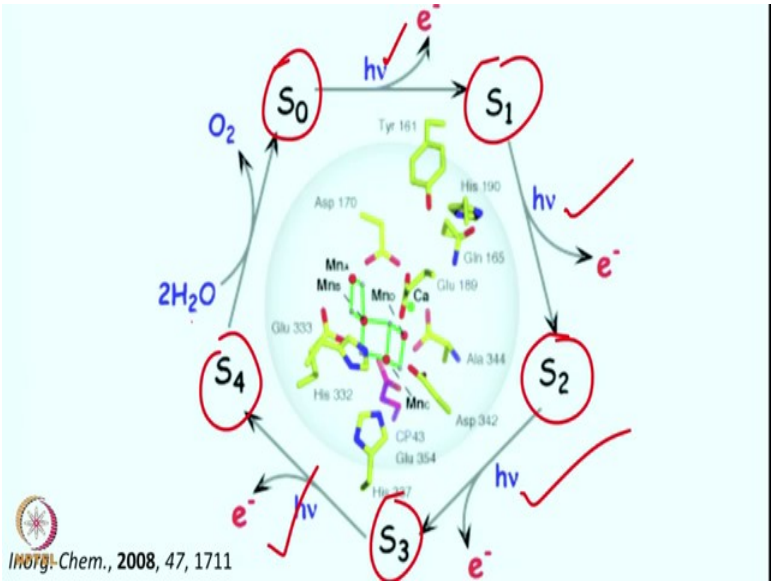

So, we have seen S 0 oxidation state we have seen S0 oxidation state S0 S1 S2 S3 and S4 oxidation state. In each of next steps in oxidation happening oxidation happening oxidation happening and in the final step oxygen oxygen bond formation is taking place.

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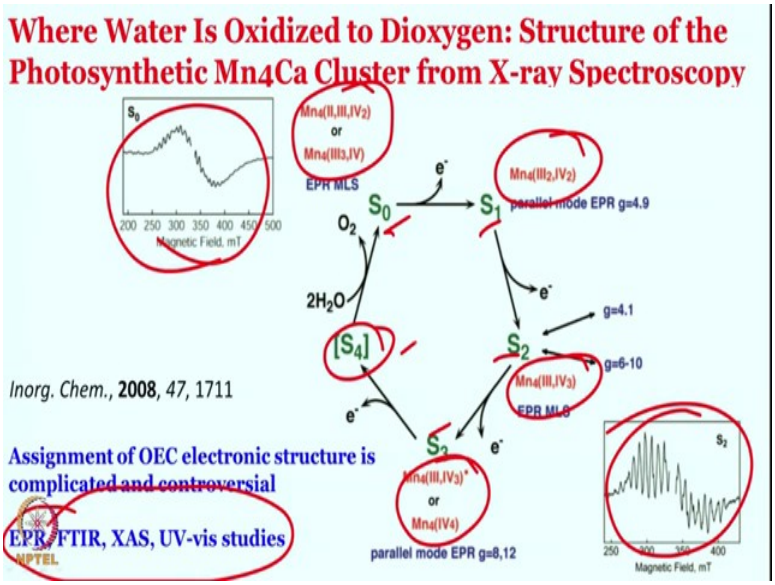

You have seen that the oxidation state dilemma between these two these species are still debatable; this is quite fixed, this is quite fixed, this is still debatable. And finally, this is the fully oxidized form there are multiple line spectrum in the EPR which is helping us in assigning some of the spectra always this simulation of this band as well as the experimental observation has to be quite critically done otherwise the conclusion can be problematic.

Different experimental studies are done to understand these intermediate in metals that are better respect or better clarity, but still their problem exists in these cases.

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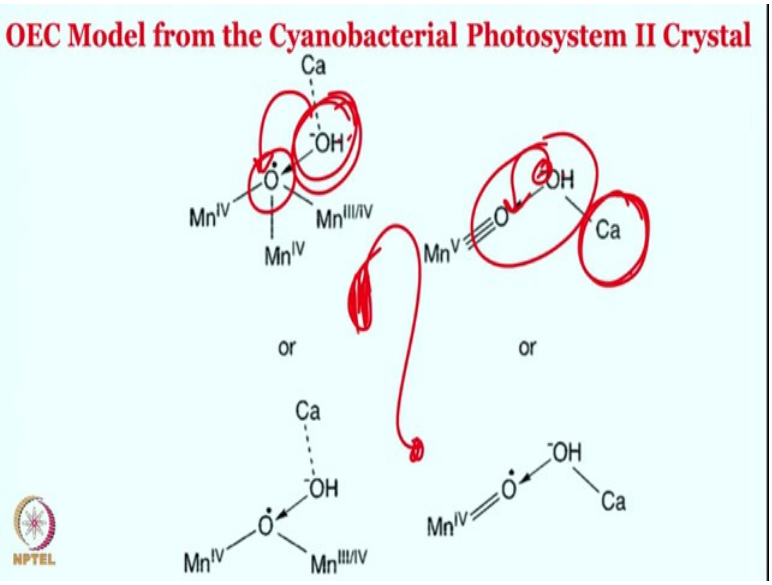

The oxygen-oxygen bond forming process as you would imagine that that is the most important step and that is that is that is that is the key point of discussion over here. Some believe that it is the calcium hydroxyl which is I think it is quite reasonable because calcium is the Lewis acid, it activates the water molecule to makes its hydroxide and this hydroxide and then can attack on this oxy center, but in other believes that calcium is not really part of, but anyway that debate for another day.

This again calcium hydroxide either this hydroxo can attack on these breathes manganese oxo or the terminal manganese oxide still that is questionable, I think we can we can we can perhaps leave as it is these questions are very difficult to solve at this point and we can move on because in the literature so, far no clear understanding is obtained ok.

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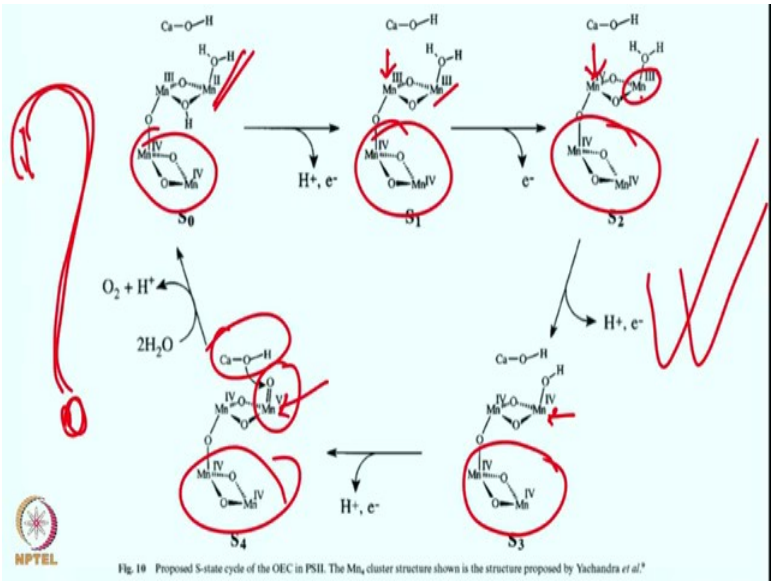

Fig. 10 Proposed S-state cycle of the OEC in PSII. The Mn_4 cluster structure shown is the structure proposed by Yachandra *et al.*[9]

So, at the end what we have in the picture is, I think if anything you would like to remember I think this is the case you should remember these next two slides you should remember. Although this is questionable, but still this is the thing you should remember what is happening over here? The core over they are these center remained constant this is one of the proposal and this is something I believe is most likely closer to be the truth, but again nobody has seen the real active site.

So, what is happening over here is manganese aqua molecule is right over there and it is in manganese 2 + this is center, it is getting oxidized to manganese 2 to manganese 3, and then this manganese 3 is oxidized to manganese 4. Subsequently this manganese three aqua complex further gets oxidized to manage 4 hydroxo and finally, to manganese 5 oxo which is the real electrophile we are looking at where calcium hydroxo can come and attack. If calcium hydroxo is attacking on this manganese 5 oxo the oxygen oxygen bond bondage form I think job is done.

Well again this is remaining a question mark over there, but this is the one if you have to remember anything for the manganese 4 calcium structure because it is so, complicated you have to remember something to better understand it there are caveats, there are problems. But, I think this is something you should try to remember if the manganese 4 calcium oxo structure is drawn for the oxygen oxygen bond formation I think this is the structure to be drawn ok.

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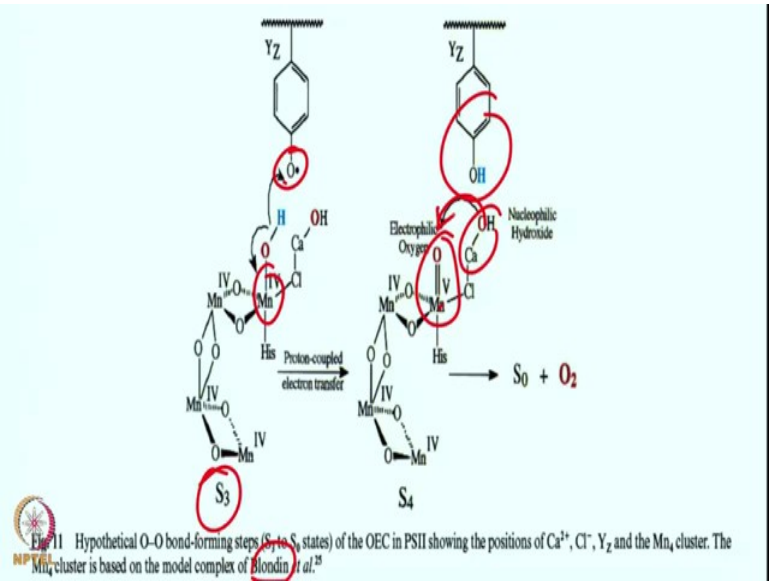

Fig. 11 Hypothetical O-O bond-forming steps (S_3 to S_0 states) of the OEC in PSII showing the positions of Ca^{2+}, Cl^-, Y_Z and the Mn_4 cluster. The Mn_4 cluster is based on the model complex of Blondin *et al.*[25]

Let us moving on, there is another exciting twist in this business and that is the formation of the oxo radical from the tyrosine unit, which is proposed in some of the cases in case of blondin that this is the oxidized, which is responsible for hydrogen atom abstraction for this manganese hydroxo species in S3.

I think this is quite fascinating most likely to be the truth or true, but who knows what is happening, but overall this phenol is forming as you can seen over here and manganese V oxo is formed and calcium hydroxide is risely coupled with the manganese center where this attack can takes place and oxygen oxygen bond can be formed.

Well, I hope you are able to make some sort of sense about this complex structure but you can read always more there are many references given a great chemical reviews and other reviews are available and, you are free to study all those, please do understand that these remains still complicated and many questions are still there which need to be addressed over the decades to come ok. With this we will see you soon in the final few classes very very soon ok.

Thank you.