Metals in Biology
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Lecture - 30
Dinuclear Iron active sites for CH 4 to CH 4 OH conversion & its mechanism

Hello. Welcome back to Metals in Biology. We will continue discussing on methane monooxygenase, what a fantastic enzyme we have in our hand well.

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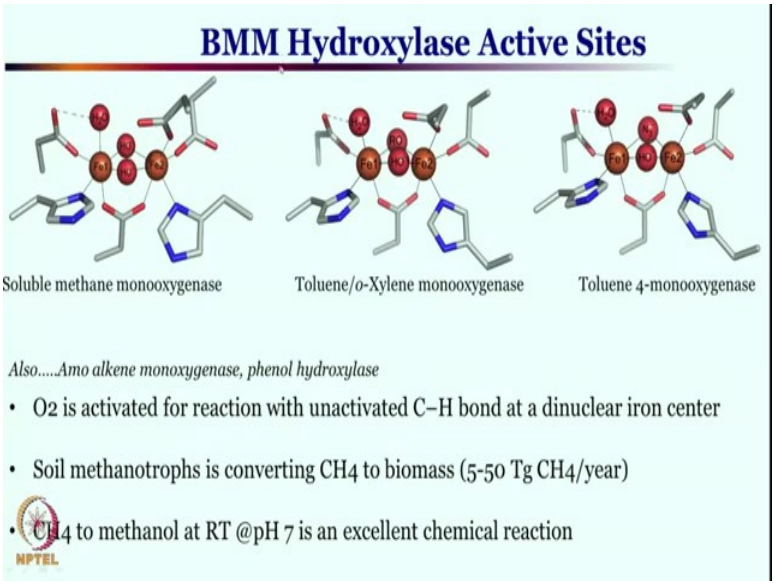

This is a class of the bacterial multi component monooxygenase which is executing hydroxylase activity and you have seen in the last class, we have this soluble methane monooxygenase that can convert methane into methanol 2 iron centers bridged by 2 hydroxy units. You have histidine histidine carboxylate linkage another carboxylate in mono coordination carboxylate here in mono coordination.

Water molecule is there completely unsymmetrical sites with respect to these 2 iron center much similar to what we have seen in terms of the hemerythrin of course, the ligands are different, but unsymmetrical dinuclear coupled by the ligand right. So, we can have the similar BMM hydroxylase in toluene case, if you use toluene as a substrate. These are the enzyme to go for its hydroxylation chemistry ok; certain difference are there in terms of bridging overall almost exactly same as you have seen in here in the sMMO.

In case of toluene 2,4-hydroxo toluene synthesis this is the active site and once again very little difference are there with respect to the bridging ligand, almost similar everything is as close as possible with respect to these other structures. There are other BMM a hydroxylases such as, Amo alkene monooxygenase, phenol hydroxylase. These structure as well activities are nothing, but oxygenation chemistry such as alkene to epoxy phenol to catechol and so on. All these cases molecular oxygen is activated at the metal center, at the iron center and subsequently it is been utilized for unactivated C-H bond functionalization ok.

By doing so, for example this active site can convert greenhouse gas such as methane into methanol, which is a big part of the catalytic cycle of the carbon cycle ok. By doing so, a huge amount of methane can be converted to biomass over all biomass generation is really controlled or really have great implication in through this methane monooxygenase enzyme ok.

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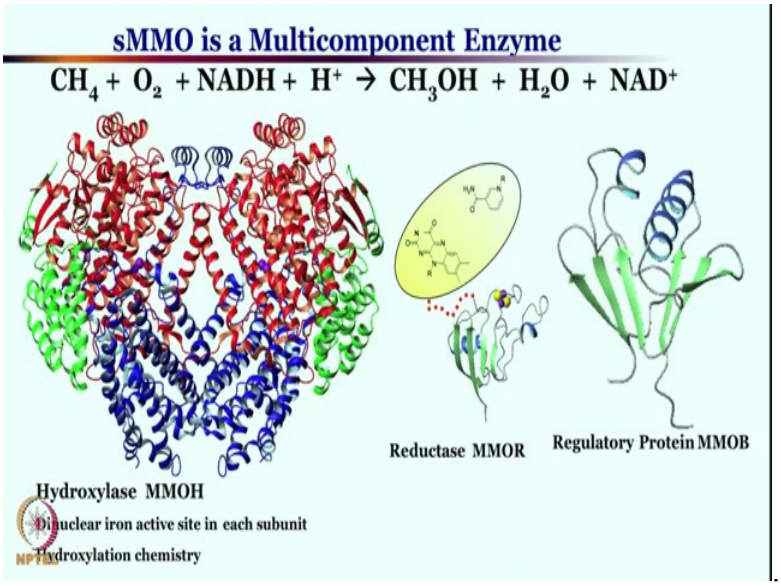

These are multi component enzyme meaning that many different components are there for its complete reactivity. So, the reaction over here is methane to methanol formation, all these components are required then and the nature has designed it perfectly. It is a huge enzyme that we have seen in the last class as well. Major chemistry that is happening over here, once again is the hydroxylation of methane to methanol.

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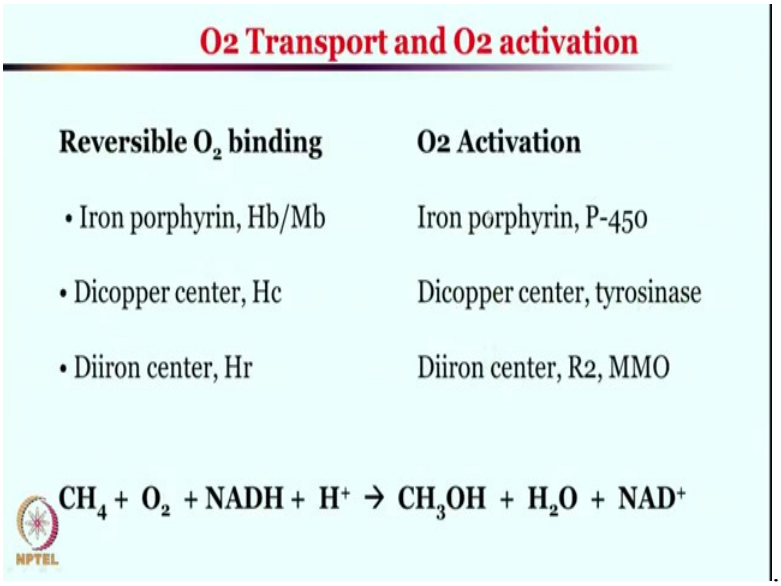

We have seen the similarity between the oxygen binding and oxygen activation, how almost exactly same enzyme is used both for oxygen binding and oxygen activation. In case of oxygen binding it is just binds with oxygen and does nothing else almost. Of course, some chemistry is happening, but not really substrate related chemistry it is just redox chemistry happening with oxygen or involving oxygen in case of all these enzymes. But exactly same intermediate or active species are utilized in a different sense by slight variation of the ligand perhaps and in presence of organic substrate to do wonderful transformation perhaps, the best reaction a synthetic chemist can expect something like this is the most challenging reaction even today that remained kind of unsolved in a catalytic manner.

If the such reaction can be done in synthetic laboratory in a true catalytic sense, I think this is one of the biggest discovery of the century would be. So, this reaction remained catalytically not so viable industrically industrially not so, viable in synthetic setup. But in enzyme, but in biological system it is done really routinely and very easily these are the perhaps one of the most fascinating reaction that one can see in the biological setup ok.

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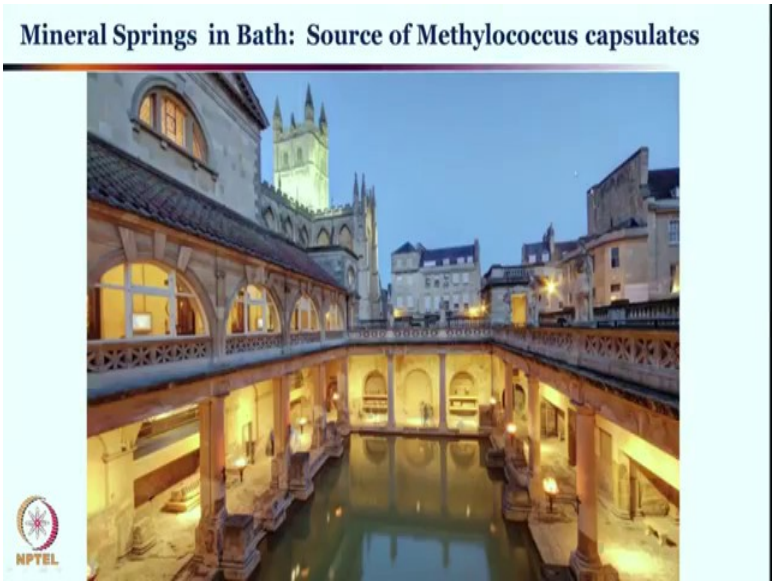

The these bacterial are grown in this nice atmosphere; in this mineral spring in the spring water.

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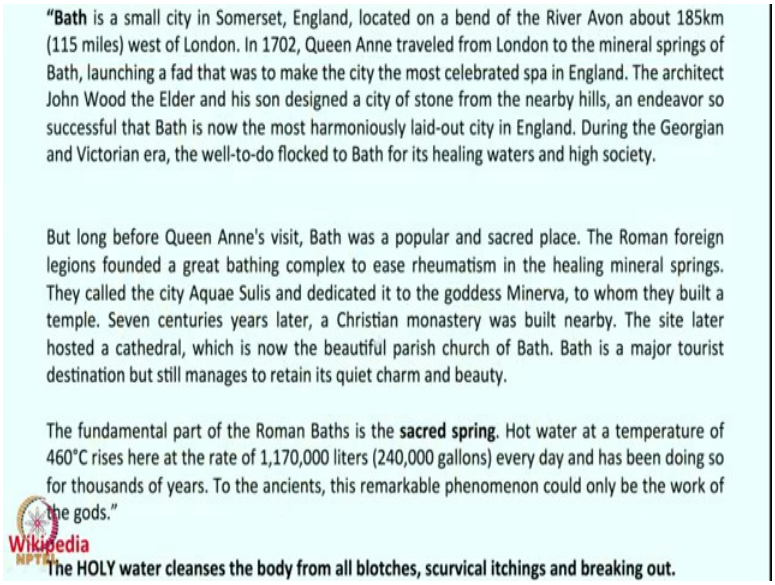

You can, you we have discussed briefly these many places including the bath England that is the the sacred having the sacred spring. This is being the house of generating these methylococcus bacteria.

The water is so good, because of this bacteria that it can cleanses the body from all blotches scurvical itching and breaking out which is something comparable to holy water

right. So, water can solve many problems and that is nothing, but due to the presence of this methylococcus bacteria and other bacteria that can be generated under this hot spring water that is then used as a great destination for the tourist.

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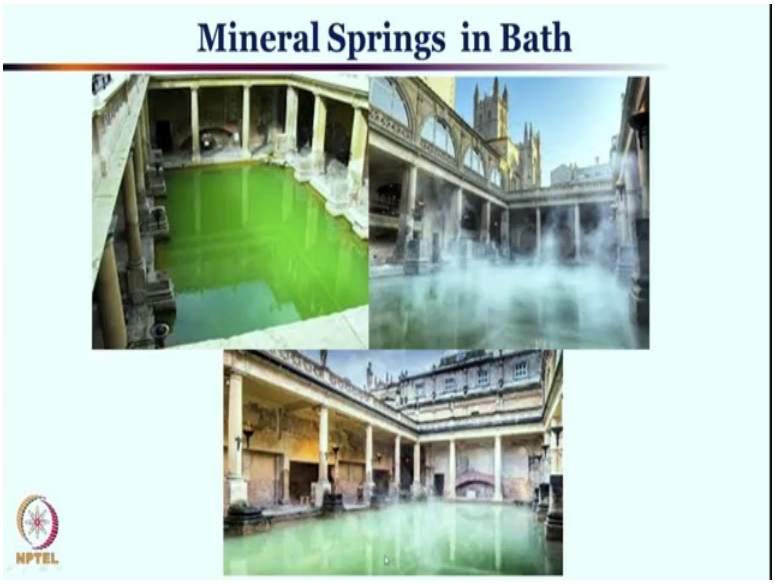

So, this is the as you can see these pictures are really beautiful and if you have time, do visit there ok.

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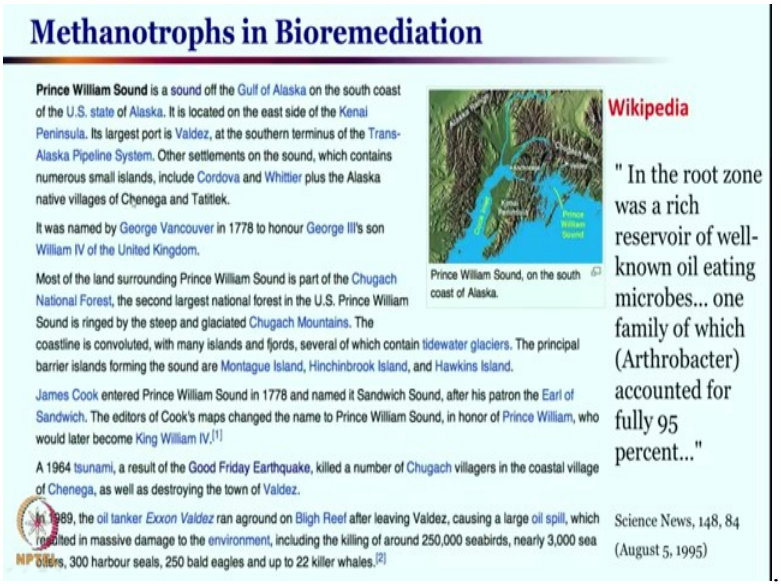

So, not only for enjoyment, for restoring natural beauty also these methylococcus bacteria which is responsible for converting methane to methanol is used to for bioremediation. So, when as we discussed the Exxon mishap happened in 1989. The water was really polluted and the thick oil layer was all over this huge area of this Prince William Sound. Now by growing this bacteria, this oil can be degraded and this oil eating microbes which is nothing, but this methylo methylococcus one can restore the beauty of Prince William Sound and the nearby area.

So, these enzymes or these bacteria which is producing these enzymes not only capable of converting methane to methanol and also, not only perhaps the reason for the hot spring popularity. These can also contribute to the bio remediation and the long lasting effect of these transformations are quite interesting.

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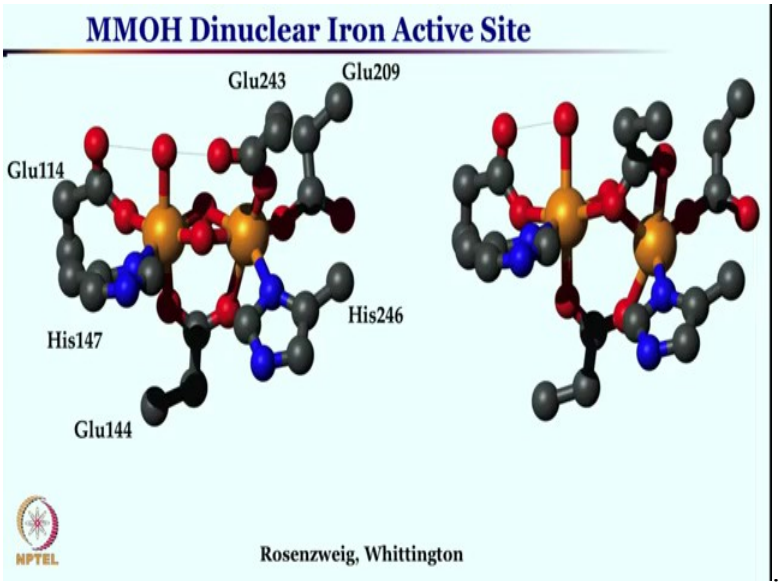

If you zoom down at the active site since the crystal structures are known of these enzymes. So, the you see what we were showing in the first slide, where two iron centers are there bridged by the glutamate and one histidine on each of the iron center and two glutamate on one iron center one glutamate, on the another iron center and the remaining site is occupied by the water molecule.

So, although these are unsymmetrical iron center, but these are fascinating centers which can activate the methane right. We will see that the active site right over here is responsible for converting methane to methanol today ok.

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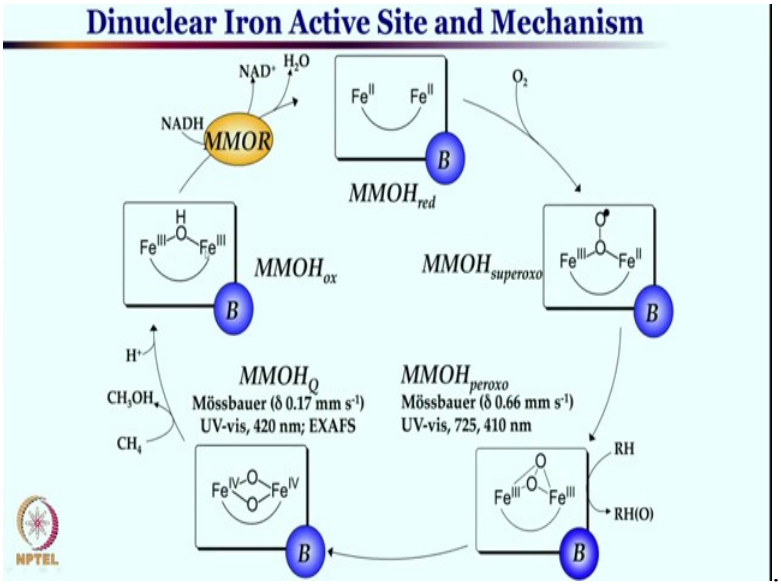

The active site that we have discussed this is the proposed mechanism, it stays there for a little bit, but more importantly this has been now revised. So, this is the this is the abbreviated form of this active core, so this active core reacts with oxygen to give rise to a mixed balanced situation iron III, iron II superoxo species which can then be reduced. The superoxo moiety can then be reduced by another electron from one of the iron center to give iron III, iron III diperoxo and this is the species which is responsible in some cases been proposed that to give the substrate hydroxylation. Indeed it is also proposed that this species will undergo formation of the oxygen, this species can also undergo oxygen bond cleavage to form these iron IV iron IV dioxo species. So, this species can undergo oxygen oxygen bond cleavage to give the iron IV iron IV dioxo species.

This dioxo species can react with methane and this is believed to be the true active species, which is reacting with methane to give the methanol. As you see this is the peroxo intermediate, this is the bismuoxo intermediate; these two species are having completely different oxidation state; and therefore, their spectral behaviors are completely different it can be characterized confidently.

So, this two species MMH OH peroxo and MMOH oxo, this bismuoxo are completely characterizable by different spectroscopic technique and subsequently this hydrogen atom abstraction perhaps from this methane will give rise to the hydroxo bridge species.

So, this bismuoxo will be converted to hydroxo and then methane will be converted to methanol that we will see in a moment.

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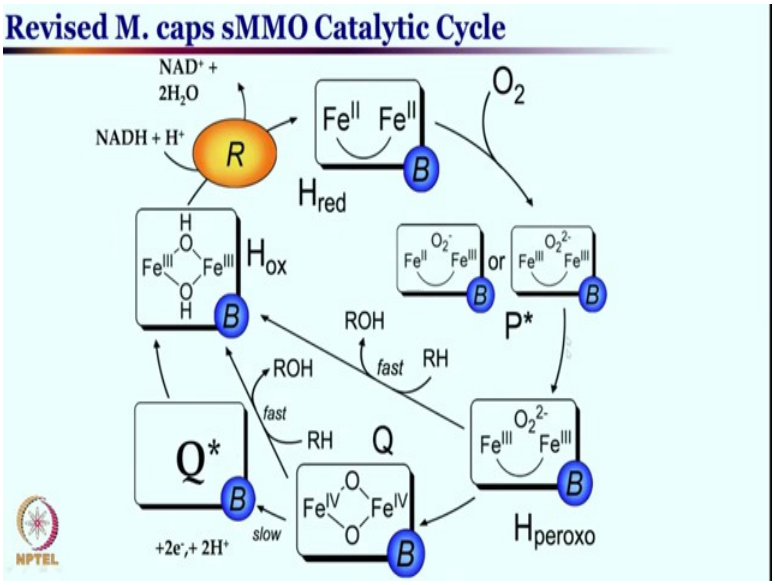

But there is a twist there is a revised mechanism, where it is supposed that or it is proposed that this iron IV iron IV dioxo intermediate is giving a new intermediate which is quite natural. Perhaps a oxo hydroxy which is not completely characterized and this species is the one which is responsible for the for the real hydroxylation chemistry.

We will see this step in more closely. So, the revised version of this enzyme is this peroxo, can react with the substrate to give the same dihydroxo intermediate along with that while the oxo is forming; this oxo can also react via a new intermediate that is oxo hydroxy perhaps and in that intermediate which can then go to form the die hydro so intermediate. So, once again just to summarize this part, so we start with the completely reduced form diiron species, reacting with oxygen to give the iron III superoxo species iron III iron II mixed valent super oxo species.

This then can form the peroxo species upon reducing this oxygen moiety further to the peroxo level. So, superoxo to peroxo level and then we have this iron III iron III peroxo intermediate from their own substrate can react directly to give the kind of the tool to give the dihydroxy iron III species. Alternatively this spieces can undergo oxygen bond cleavage to give the iron for bismioxo intermediate which then can react to an oxo

hydroxo intermediate while reacting with methane to give the iron III iron III dihydroxo intermediate right.

So, so, far I hope there is no issues let us look at this step little more carefully what happens and how can we perhaps interfere or get idea about these intermediate how reactive they are and how fast they are ok? So, we are going to look at just this step over here ok.

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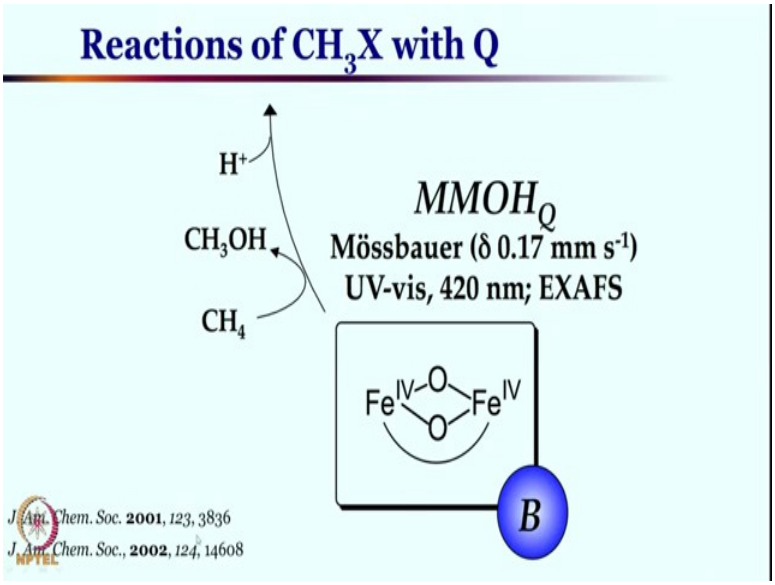

Let us let us look at and draw it separately. So, these are by the work by professor (Refer Time: 12:17) Lippards group all these some of these slides and presentations are also from the book as well as the online version of material that is available out there. In the internet so, this iron IV iron IV dioxo intermediate, we are looking at it has very characteristic spectrum and these are very unique for this sort of intermediate.

What we are trying to look here is, would react with methane to get the methanol perhaps because that is the overall activity. And how do we really kind of know that this is this species is forming and then that is reacting ok. So, the question in hand is we would like to probe the step where methane is reacting with methanol and that we say it is this, but then how we generate this species in isolation? It is going to be very reactive intermediate indeed these are so, reactive intermediate, you have fraction of second millisecond to react right we will see that.

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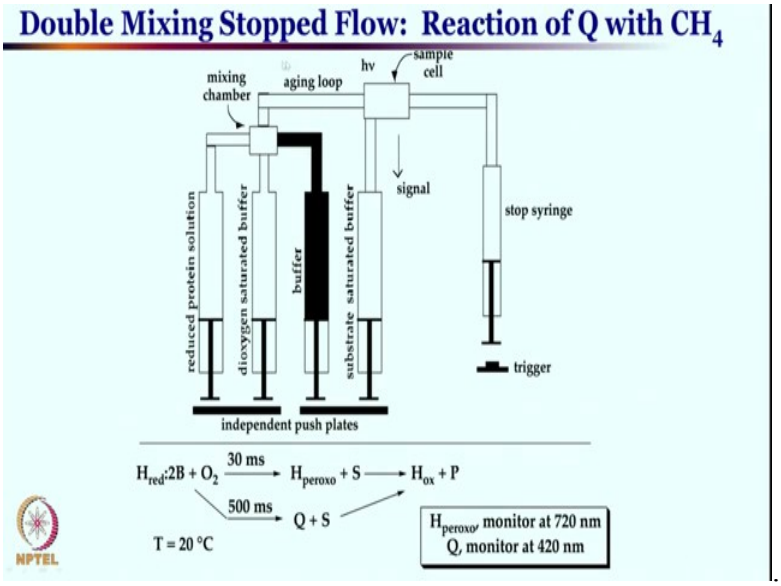

Now, a special technique that has been utilized for kind of trapping or forming this intermediate therefore, perhaps one can think of studying this step. This is nonetheless not going to be a easy reaction set up. So, this is what is double mixing stopped low kinetic studies? So, where this intermediate Q is this is intermediate Q is getting reacted or reacted with methane to give the methanol product ok. So, what we are doing here we are taking here reduced protein solution.

So, that is iron II, iron II solution this is the dioxygen saturated buffer and there is another buffer which is looked into it but here we are taking the saturated solution buffer. So, look the reduced species and oxygen are together this is one push plate and this is another push plate where the substrate; that means, methane in this case and the buffer extra buffer are added together. So, these are independent push plates. So, what we are trying to tell is, this whole reaction we are trying to follow and we will try to see the intermediate by following this by UV visible spectra, but this lifetime of these intermediates are very short, not necessarily every intermediate we will be able to see.

What we will see in a moment that if we are studying right after 30 millisecond and started recording spectra, this is the species that is tracked. If we are letting the reaction go for 500 millisecond, this is the species that we can track. So, both these species are having a very distinct UV visible spectra. So, from there the let us say the decomposition of the UV visible spectra will tell us which peak of course, the you know formation as

well as the decomposition of these two species spectra, this is happening at 30 millisecond and up to all the way here is happening up to 500 millisecond.

So, depending on when we started recording and which band we are following? We will be able to get a clear idea of its reactivity pattern because these are very very characteristic peak in these gives the characteristic peak in UV visible study ok. Let us look at that once again so if you are starting with the reduced compound reacting with oxygen. So, this is the reduced compound this is the oxygen, so this is where you are starting the intermediate formation right over here, but most importantly substrate is given on a different loop.

So, when substrate is given that is going to be determining what is happening or which intermediate is getting interrupted by the substrate? So, if we are letting this reaction go for 30 millisecond and then, adding this then introducing this two solution; that means, we are we are trying to follow the peroxo and the substrate reactor; let us say peroxo plus methane reaction. So, after 30 milliseconds if this is pushed, so we are then looking at the peroxo reacting with methane ok. If after 500 millisecond, we are looking at then, we are looking at that intermediate Q formation and the reaction of substrate let me try to clear out one more time.

So, depending on the time at what times point we are introducing the substrate because that we can control. So, we can program it in such a way so that after pushing it 30 millisecond within 30 millisecond this will pushed in. So, these are so, fast and so accurate and these are very expensive instrument. So, you do not have to worry that there is any too much problem into there. But after mixing these together you will be then following this UV visible spectra, let us say in these cases and see if what is going on over there right.

So, if you are letting it go for 500 millisecond this reaction let these reduced reaction reduced iron species reacting with dioxygen for 500 millisecond and then, pushing your you know button for your substrate, then you are then studying the intermediate Q and the reaction the of that with the substrate. So, once again this is 30 millisecond and then you are introducing substrate then you are following this step. If you are allowing it for 500 millisecond then this species is form and after that then, you are following this

process one of these process or both of these processes where bismuoxo is reacting with the substrate methane right.

So, if you do that you will be able to follow these or monitor these because, at peroxo species has a characteristic band at 720 nanometer, these peroxo intermediate has a very characteristic band at 720 nanometer; and this intermediate has characteristic band at 420 nanometer. So, by following these two bands you can follow either the peroxo species or the intermediate Q.

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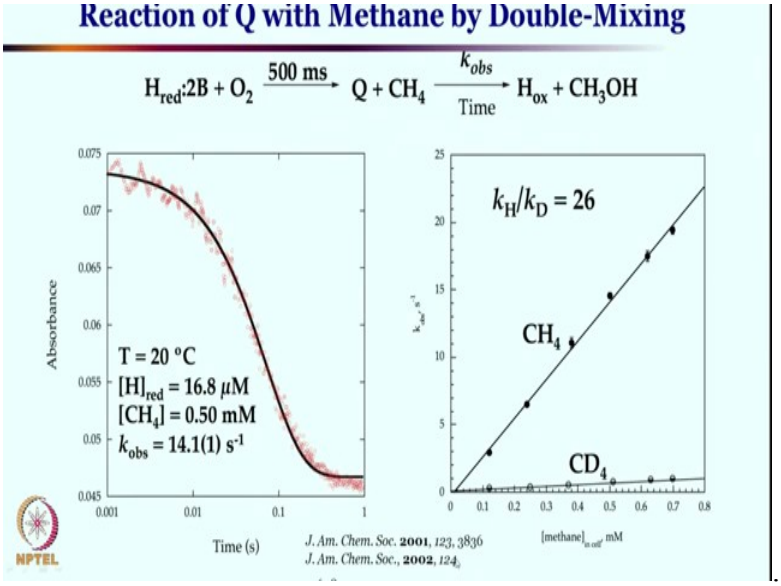

Let us look at what has been done over here, if this is showing that this iron II, iron II reduce species reacted with oxygen; that means, we are talking about here iron II, iron II reacted with oxygen and getting mixed over here to give the bismuoxo intermediate right.

So, to give this intermediate we are following, so if these two are allowed for 500 millisecond this Q intermediate is following and right over there this methane is added after 500 milliseconds; this is pushed methane is added. Now you are following the band for the Q that is at 420 nanometer what you see you see that absorbance is going down in this particular fashion; and from there with respect to methane, you can get the k abs calculation with respect to the concentration of methane. So, you can do a series of experiment and you can follow this plot, where k abs versus methane plot will be straight line as you see over there.

So, this is not one experiment you have to take many different concentration of methane and by varying different concentration of methane you will be able to draw this plot. So, what we are doing over here, we are taking different concentration of methane over here and studying it independently. So, one study with done with one concentration, you vary the concentration, then again you study you vary the concentration, then again you study multiple studies are done by keeping these constant or exactly same you are just changing over here.

In the methane concentration and mixing this Q intermediate after 500 millisecond with this methane substrate; under that situation what we see that this plot can be generated and replacing methane with the deuterium over here CD4 we can have a plot right over here the CH4 by CD4 plot gives you the kH by kD value of 26. So, this is the plot we are looking at and overall methane in forming methanol and this H ox intermediate that intermediate is generated into the process ok.

So, what we are trying to say over here right now then, we have taken different concentration of CH4 and we have also taken different concentration of CD4 and we have studied this reaction. From the different concentration of CH4 this is the plot we obtain and from different concentration of CD4 this is the plot we obtained where did we vary CH4 and CD4. So, we have varied the CH4 and CD4 over here one time CH4 particular concentration, next time different concentration of CH4 and then series of experiment like that and subsequently taking CD4 and different concentration of CD4 we are able to get this plot and that gives the kinetic isotope effect value of 26 which is really fantastic value very high value.

Of course indicating some sort of tunneling is also involved, but this is definitely kinetic high kinetic isotope effect value. So, C-H bond dissociation is involved or activation is involved into the rate determining step and that is the most difficult and crucial step for the methane to methanol formation. These kinetic isotope effect studies are pretty informative in particular when the values are so high; this is got to be the C-H bond activation is the most difficult step and most critical step over there ok. So, then based on that let us look at the mechanism what could be the mechanism that is following in these cases ok.

There are many different type of mechanism one can think of these are the most plausible two mechanism are plotted this is from this reference.

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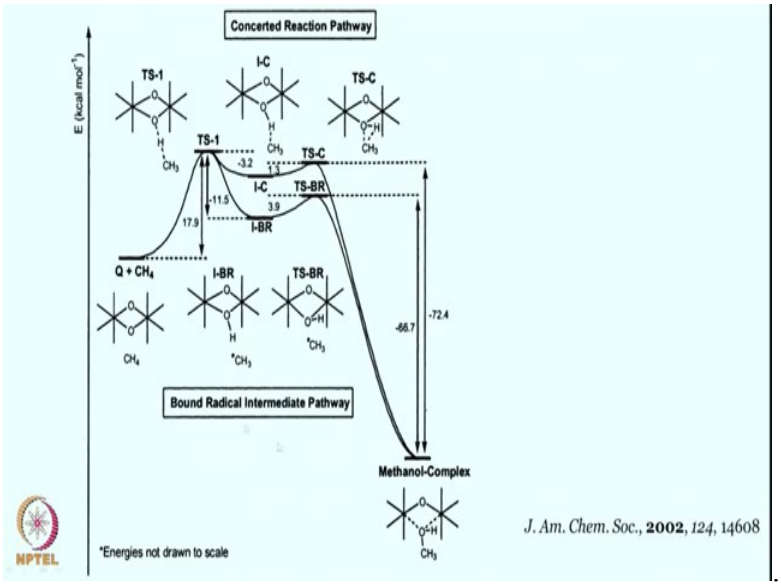

So, what is drawn over here is a schematic version of this dye iron center bridged by these 2 oxo methane is right over here right. So, this methane is right over here this is reacting with this bismuoxo species fantastic. So, the first step would be the activation of C-H bond, there is nothing wrong I think that is going to be the common step and this is the most difficult step and this is where we see that kinetic isotope effect value this high kinetic effect value of 26 is absorbed. So, that is the, this step. So, this is the transition state one which would look like CH3 and H bond C-H bond is breaking ok. So, this is the bond breaking step of the C-H bond and as well as the formation of trying to form the OH bond right formation of the OH bond is going on C-H bond is breaking and OH bond is forming and that is the transition state.

That is the common transition state originating from CH4 from there on what we can see that there could be two different pathways one would involve a concerted mechanism; that means, the bond making and breaking are happening simultaneously without generating any sort of radical intermediate. So, as you can see over here. So, C-H bond is broken OH is forms CH3 is over there and then simultaneously bond formation is going on. So, this is called concerted reaction pathway together all these things are happening. So, no separate radical intermediate type is happening. So, C-H bond is broken OH bond

is formed and then OH is also simultaneously binding with the CH3 to give you overall CH3, OH right.

So, in this first pathway what you see that this muoxo intermediate is reacting with methane. So, this is the intermediate Q reacting with methane breaking the C-H bond of the CH4 one of the C-H bond of CH4 and then it is going this h is going towards oxygen to form the OH bond fantastic. Now this OH bond formation happening while C-H bond is breaking in the concerted pathway this OH bond formation; as well as the new CH3, O bond formation CO bond formation are happening also simultaneously. So, these are the corresponding transition state for these concerted reaction pathway; as you can see this is going to be a higher energy pathway nonetheless will give the same product that is methanol as you can see over here.

So, this is the methanol bound intermediate from there on methanol will be dissociated. Alternatively another mechanism could be from this intermediate it can go on to form this transition state of course, CH bond breaking and OH bond formation is going on, but during this formation of OH there is an intermediate involved that is O OH. So, you can say that Q star intermediate that perhaps we are proposing over there. So, this is the Q star intermediate forming along with the formation of CH3 radical right. The CH3 radical subsequently will react with OH two and to give you the CH3 OH along that pathway of course, OH radical and CH3 radical will interact to give this intermediate. We will come back to this in the next class.

So, what we will see here is in the next class how these intermediates are forming and how they are approaching. The computational details you will be looking at this is from this paper by professor Lippard's group(Refer Time: 27:33). Keep studying methane to methanol methane monooxygenase or bacterial multi component monooxygenase these are a huge group of group of enzymes these are similar enzymes, but doing fascinating chemistry. From methane to methanol benzene or toluene to hydroxy toluene and phenol to catechol olefin to epoxide, there are many different reactions is non heme iron centers are doing. We will start looking at the reaction mechanism in little detail in the next class keep studying.