Metals in Biology
Prof. Debabrata Maiti
Department of Chemistry
Indian Institute of Technology, Bombay

Lecture – 16
Copper – Oxygen chemistry – Part III Reactivity summary

Hello, welcome back to today's class on Copper Oxygen chemistry. In the last class we have seen, how a mononuclear copper oxygen chemistry can react with organic substrate to give beautiful chemistry. Let us try to see, this synthetic chemistry how they are related or relevant to the enzyme chemistry right. So, inorganic compounds, inorganic laboratory and the enzyme, how they are merging with each other well.

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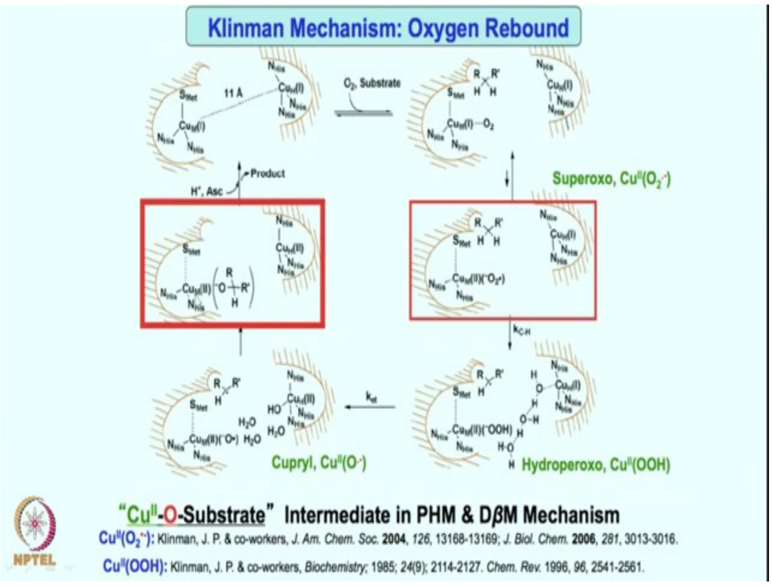

Klinman Mechanism: Oxygen Rebound

"Cu^{II}-O-Substrate" Intermediate in PHM & DβM Mechanism

$Cu^{II}(O_2^{\cdot-})$: Klinman, J. P. & co-workers, *J. Am. Chem. Soc.* **2004**, *126*, 13168-13169; *J. Biol. Chem.* **2006**, *281*, 3013-3016.

$Cu^{II}(OOH)$: Klinman, J. P. & co-workers, *Biochemistry*; 1985; *24*(9); 2114-2127. *Chem. Rev.* 1996, *96*, 2541-2561.

As we were discussing in enzymes, there are these intermediate which is copper alkoxo intermediate. In the last class you can see that this copper alkoxo intermediate was forming from the ligand itself. So, ligand is part of the ligand is acting as substrate.

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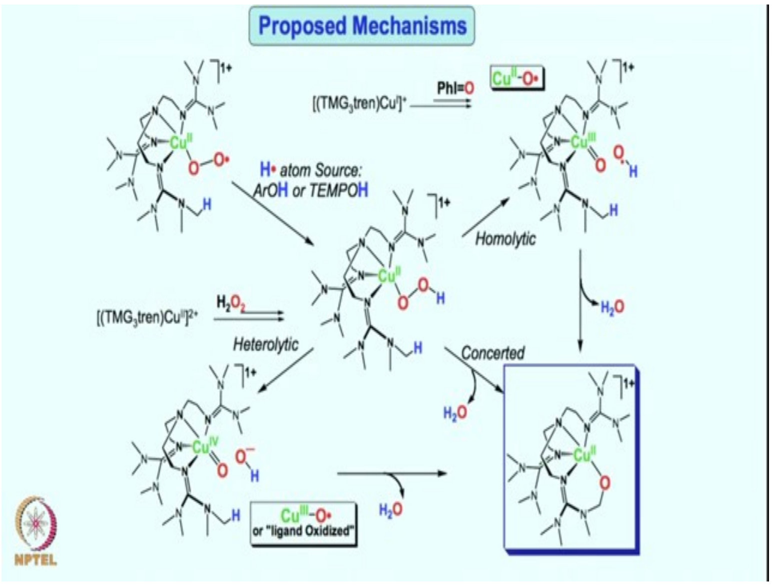
Proposed Mechanisms

If you look at this in a overall reactivity pattern, then show the copper mononuclear superoxo species will react with oxygen to give the copper oxygen intermediate, which is superoxo fully characterized can abstract hydrogen atom from the organic substrate to give perhaps the copper hydro peroxo intermediate and subsequently oxygen, oxygen bond cleavage or the mechanism could be hemolytic, heterolytic or concerted, but overall this water molecule can go out to give you this copper alkoxo intermediate right.

Of course, the mechanism this detail can vary, but the take home message simply over here is this synthetic, mononuclear, copper, oxygen species are indeed capable of doing chemistry similar to what has been observed in enzyme. So, the conclusion I would say is it is possible that these copper superoxo species are the real active species in PHM and DBM, but not definite. It is possible that these are the reactive intermediate, as you have seen in enzyme there is a crystal structure, which is exactly what is found in synthetic set up with a suitable ligand the end-on copper superoxo species.

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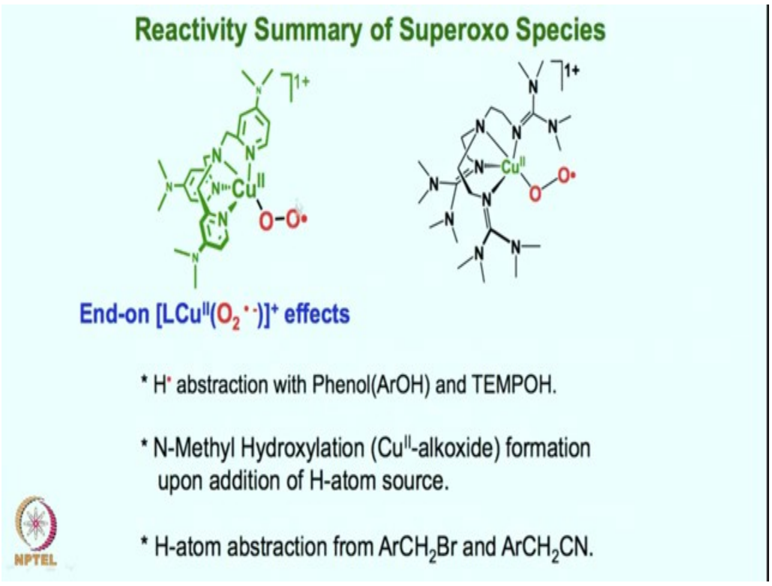

Reactivity Summary of Superoxo Species

End-on $[LCu^{II}(O_2^{\bullet -})]^+$ effects

- $H^{\bullet}$ abstraction with Phenol(ArOH) and TEMPOH.
- N-Methyl Hydroxylation (Cu^{II}-alkoxide) formation upon addition of H-atom source.
- H-atom abstraction from $ArCH_2Br$ and $ArCH_2CN$.

Let us look at then the summary what we have seen so far with these two copper superoxo species, is they are capable of doing this chemistry, which is parallel to what is seen in the enzyme. Now, let us try to look at the other species right, other species that we have not seen so far too much is the hydroperoxo intermediate.

As I mentioned that these copper superoxo species can abstract a hydrogen atom from the substrate or a suitable H donor and can form a copper to hydro peroxo intermediate. But of course, another proton and electron can do the job, but those formations are not that very reliable so far, although some reports are known.

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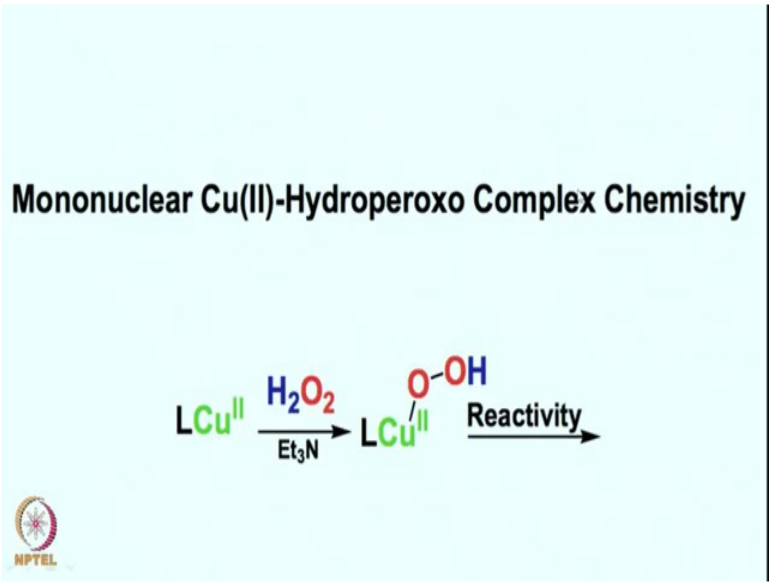

Mononuclear Cu(II)-Hydroperoxo Complex Chemistry

$$LCu^{II} \xrightarrow[Et_3N]{H_2O_2} LCu^{II}\text{-}O\text{-}OH \xrightarrow{\text{Reactivity}}$$

But mainly to form the copper hydro peroxo species chemist has relied on this reaction of ligand copper two with hydrogen peroxide in presence of base which can give give these copper II hydroperoxo intermediate right. So, ligand copper II hydrogen peroxide and base, just acid base simple type of chemistry to give the copper hydro peroxo species we are interested in knowing the reactivity ok.

Now, if we understand the how this species reacts with substrate, this can also then add to the value of our understanding where what perhaps is happening in those enzymes PHM and DBM, whether such mononuclear species just like copper superoxo are capable of being the doing the substrate hydroxylation chemistry. Well, before proposing copper superoxo as the real active species.

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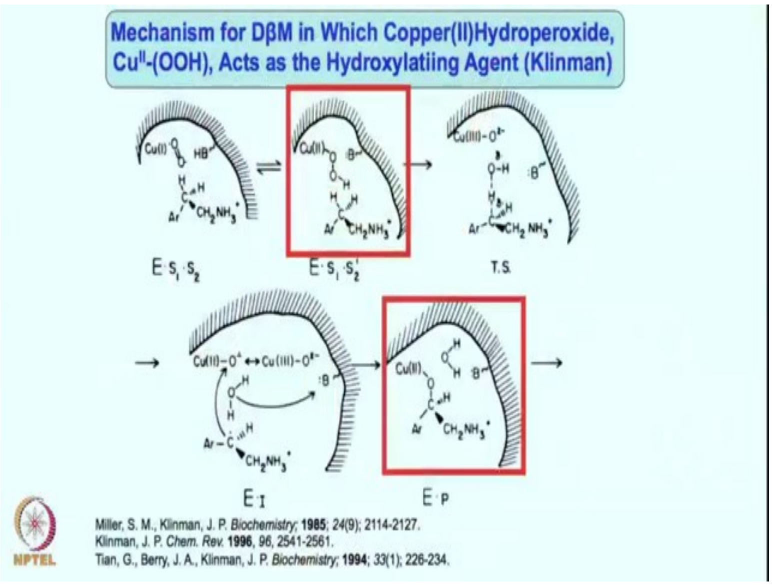

Mechanism for DβM in Which Copper(II)Hydroperoxide, Cu^{II}-(OOH), Acts as the Hydroxylatiing Agent (Klinman)

Miller, S. M., Klinman, J. P. *Biochemistry,* **1985**; 24(9); 2114-2127.
Klinman, J. P. *Chem. Rev.* **1996**, 96, 2541-2561.
Tian, G., Berry, J. A., Klinman, J. P. *Biochemistry,* **1994**; 33(1); 226-234.

The same group were studying quite a lot and suggested that it is the copper hydro peroxo that is the real species in doing the substrate hydroxylation. The mechanism can be followed quite easily as you were discussing, there is possibility of forming these intermediate and the question is can we synthetically show just like what we were discussing in the mononuclear end on copper superoxo species, can we show that these hydroperoxo species, once formed they are also capable of doing substrate hydroxylation chemistry.

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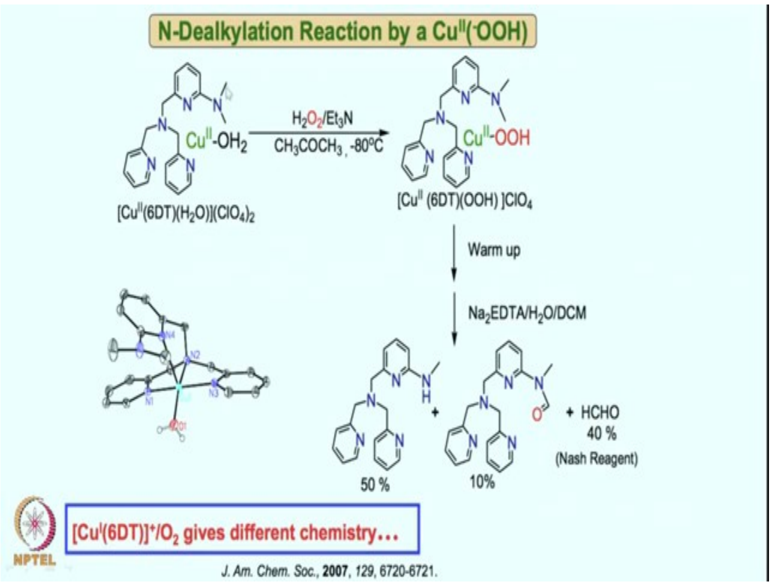

N-Dealkylation Reaction by a $Cu^{II}(^{-}OOH)$

$[Cu^{I}(6DT)]^{+}/O_2$ gives different chemistry...

J. Am. Chem. Soc., **2007**, 129, 6720-6721.

Let us take a special case, where we have the organic substrate within the ligand backbone. This is the same tetradentate ligand, ligand or similar ligand what we were discussing for copper, this is TMPA best ligand appended with this dimethyl amine. Now, once a copper two hydroperoxo species is formed by utilizing this hydrogen peroxide and triethylamine, what it is found that it is indeed can do chemistry at this substrate that is the N-Dealkylation chemistry is happening, where upon oxygen-oxygen bond cleavage and hydroxo radicals can abstract the hydrogen atom and can then undergo beautiful chemistry.

That is essentially saying that if your organic substrate is placed correctly or very close to the active species perhaps, then copper hydro peroxo species also is capable of doing the substrate hydroxylation chemistry that is quite powerful I would say. Well, the orientation of the organic substrate has to be perfect right. As you have seen, the organic substrate is right over there and the hydroperoxo species is generated right over here and then they can react.

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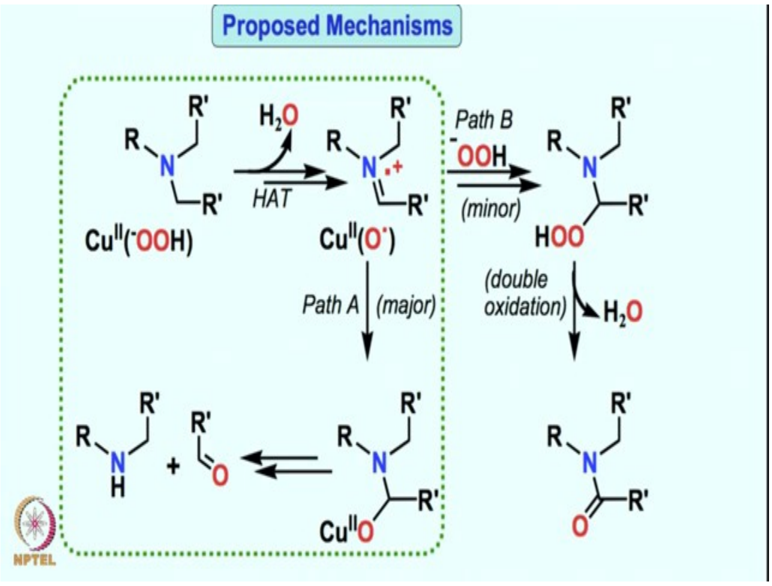

Proposed Mechanisms

So, let us look at briefly the mechanism of it. So, the hydro peroxo species is forming substrate is sitting of course, ligand is attached with the substrate and overall a iminium radical cation formation and Cupryl intermediate formation can give the substrate hydroxylation intermediate, subsequently N-Dealkylation chemistry can go on.

Now, overall them what we have seen and this is quite interesting to note that both a copper superoxo and a copper hydroperoxo are capable of doing the substrate hydroxylation chemistry. Now, where that leaves us in terms of the mechanism, that we were seeing earlier. Well, if you recall the enzyme mechanism, well let us go back to the enzyme mechanism, where we believe that this is the copper superoxo intermediate in the first case that is abstracting hydrogen atom and giving the substrate radical and forming hydro peroxo intermediate.

Now, we have seen this mechanism how it perhaps can be making all sense that copper superoxo is doing the chemistry and abstracting the hydrogen atom and then oxygen-oxygen cleavage and giving the product, but imagine that this step is not happening over here or the substrate is not getting undergoing hydroxylation or the abstraction right over here. The electron, one more electron can come from this copper center to this superoxo intermediate to give a peroxo and upon protonation a copper hydroperoxo species can form.

Of course, this substrate will stay as it is and then this hydroperoxo then can abstract hydrogen atom right that is quite powerful going to be as well. So, this leaves us an opportunity to further understand the substrate, but none of the synthetic chemistry studied so far have taken the dopamine or C Terminus backbone and show that they can do the chemistry that remain quite interesting to note, right.

So, as we are trying to say that we have in hand both the possibilities that is both copper superoxo and copper II hydroperoxo if given a suitable atmosphere can undergo the chemistry that enzyme is looking for or enzyme is capable of doing ok. What is still really not known so far is how one can perhaps synthesise and stabilize the cupryl intermediate that is copper II O dot intermediate and this intermediate then therefore, can be responsible subsequently for those substrate hydroxylation chemistry right, well.

Let us go back to the debate again and that debate is let me get back to few more few slides quickly, where we were earlier discussing and that discussion should be centered around this square is.

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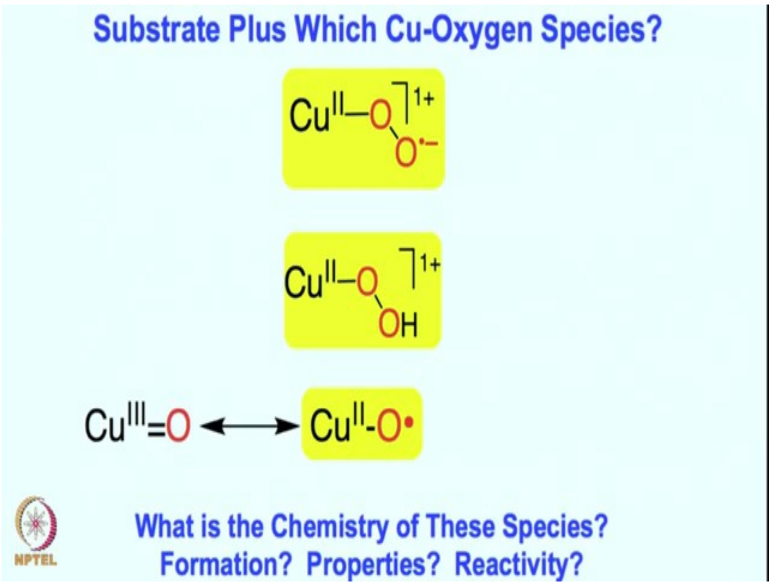

Substrate Plus Which Cu-Oxygen Species?

$[Cu^{II}-O-O^{\bullet -}]^{1+}$

$[Cu^{II}-O-OH]^{1+}$

$Cu^{III}=O \longleftrightarrow Cu^{II}-O^{\bullet}$

What is the Chemistry of These Species?
Formation? Properties? Reactivity?

Whether copper superoxo species is doing the chemistry in enzyme or copper hydroperoxo species doing the chemistry in enzyme or cupryl is doing the chemistry in enzyme. To sum up the debate, let us first acknowledge the fact that the first intermediate as you have seen over here is the only intermediate crystallographically characterized in enzyme. There has been proposition of formation of this intermediate as well as that intermediate the hydro peroxo as well as cupryl, but not much characterization has been done in terms of enzyme.

In synthetic studies so far, this copper superoxo been synthesized, but not too many are known the reactivity pattern is turning out to be interesting, but none of them has really shown the reactivity with the real substrate that enzyme is using. We have also seen, how copper hydroperoxo species can be generated, but most importantly that is not how exactly actually in nature, this copper hydroperoxo species is getting generated.

In synthetic setup often, it is the copper II compound that is reacting reacted with hydrogen peroxide, but in enzyme it is the copper I oxygen at chemistry subsequently a hydrogen atom on the superoxo is giving the hydroperoxo species. So, those studies are still need to be done in a in a quite emphatic manner. These studies are ongoing, this is a developing field, but overall with a suitable copper hydroperoxo species we have seen that if the substrate is juxtaposed, our position really perfectly with respect to the reactive species it is indeed possible to do the substrate hydroxylation chemistry right.

So, the synthetic studies didn't really conclude so far, on either of these compound that whether this is the species or that is the species. This remains still a debate whereas, perhaps it is more likely that this is the real intermediate which is doing the substrate hydroxylation chemistry, but one still cannot rule out this one, but what is happening with this cu pril, which is similar to what is known for let us say iron IV oxo.

So, Cu II O dot is nothing but Cu III double bond O the cupryl, this is equivalent to iron IV oxo and iron IV oxo is known to be quite reactive, but this cupryl intermediate so far are not that very easy or it is not turning out to be that very easy to stabilize. Therefore, the reactivity pattern of these complexes are not really well understood.

So, well perhaps one day we will see that it is clearly, it will perhaps be clearly proven that this is the real intermediate in the enzyme. Well, so far it still remains an elusive species, but given an opportunity computational studies shows that this can be really more reactive compared to these other species and therefore, one can still think of having this as a real species and in PHM and DBM. Well, it is also interesting to note that one must do the synthetic chemistry studies in inorganic laboratory to try to synthesize this complex, stabilize them, study the reactivity of them, and then try to corroborate the reactivity of the enzyme copper oxygen chemistry with the known copper oxygen chemistry in the laboratory.

This compression and contrasting will give rise to an opportunity to better understand these intermediate. Essentially one must note that the chemistry that happens in synthetic laboratory is not going to be different in any way compared to the enzyme, but the great thing about the enzyme is it can precisely control the chemistry, which control the type of control we can talk about in enzyme, it is not really possible in synthetic setup and that is because in enzyme the site or the chemistry happening at a one particular site and everything is well developed over there.

It if nature needs one metal and one substrate, it has designed the metalloenzyme exactly for that, but in synthetic setup imagine of doing that is that is going to be really difficult ok. So, setting up the system perfectly is really done in nature, but in synthetic set up every possibilities that one can think of are happening that as I was discussing there are possibility of many different copper oxygen species formation, the moment you have a ligand copper complex. You are opening a pandoras box, I would say and that means, you are competing with many different possibilities and stabilizing one of those intermediate out of many possibilities and doing the chemistry cleanly so that other species are not forming or interfering its really challenging. And this is where, you know synthetic studies which involves these ligand copper complex are going to be quite interesting and it is a ever developing field.

But the similar thing one can think of for almost every you know metal oxygen chemistry, it could be iron, it could be manganese, it could be cobalt or nickel, it could be any other metal oxygen chemistry that one can think of these complexities arises in each and every cases. We cannot run away from these opportunities or the possibilities of forming many different species starting from one single point and that is a ligand can make the metal center reactive so that it can react with oxygen, but the danger in the process is it is not limited to one metal, one oxygen; another metal that is present over there did not react yet with oxygen can come in and can play spoil sport in some cases.

So, a mononuclear species although first formed then a dinuclear species formation possibility arises and things gets complicated and therefore, why hopefully we are now able to understand that why these mono nuclear species synthesis, characterization and study is going to be challenging. Despite the challenges we have seen that some of the species are formed and they are reactivity studies has been carried out, overall still it is a open field to understand that whether we have a particular mono nuclear species over the other one at least in enzyme that is doing the chemistry.

In synthetic setup, it is at points it’s very difficult, but when we have a very well defined intermediate; it is then perhaps not that very difficult to study its reactivity pattern and can have some sort of conclusion that can have bearing on the enzyme at all as well. In the subsequent class we will discuss, how these reactive intermediate are also relevant in many other substrate oxygen chemistry.

For example, this is definitely the type of chemistry we are trying to discuss are not limited with one particular metal. In the next class we will see, how the iron oxygen chemistry or iron reactive intermediates chemistry can be actually utilized in a real synthetic setup, where the substrate hydroxylation can be carried out little more reliably compared to let us say copper oxygen chemistry that has been known so far.

This mononuclear copper oxygen chemistry is kind of I would say, little more sensitive compared to the iron oxygen chemistry or iron reactive intermediates chemistry. This chemistry as you will see in the next class, are capable of delivering the hydroxylated product which are of synthetic value. So, far copper oxygen chemistry is so sensitive that extracting the mononuclear copper oxygen chemistry for substrate hydroxylation has not then been very easy ok. Relatively iron oxygen intermediate or iron mononuclear intermediate are stabilized and can be studied for doing excellent chemistry. Those are thus going to be the topic for the next class.

And we will see how these species are generated and the multiple ways perhaps they can also be generated and then can be reacted subsequently once it is formed, then they can prepare or for the substrate hydroxylation chemistry. So, we will end here today for the copper oxygen chemistry which we have seen very clearly is quite interesting also it is quite challenging and complicated a clear answer in terms of PHM, DBM mechanism reaction mechanism for substrate hydroxylation.

Still remained a debate and I hope some of you will be excited about this debate and try to solve this, because this is really exciting if you look at this is happening in our brain, this chemistry is happening in our brain and that is important to understand it has direct relevance in many different diseases, many different problems associated with the metal oxygen compounds right.

So, I hope you will keep studying on this topic in greater detail and try to understand what is known so far and what remained in the field to understand so that you perhaps may be getting excited about such chemistry and think about the research problem that can originate from such topic, till then, keep studying, talk to you soon.

Thank you.