Metals in Biology

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

Lecture – 12
Dioxygen reactivity in copper

Hello welcome. Today, we will discuss Dioxygen reactivity in copper. In other words, if you have a ligand copper complex, how it is going to react with oxygen. We have seen many copper enzymes are present in nature. These metalloenzymes containing copper can effectively promote different oxidation, oxygenation chemistry.

Today, we will see the basis of these chemistry. Essentially, we will try to learn what happens in laboratory if we are trying to synthesize these copper complex. Is it going to be reactive as what is seen in the enzyme or is it going to react differently? There are many challenges that is associated in synthetic laboratory reactions compared to the enzyme.

In enzyme we have a completely defined atmosphere where let us say one copper will react with one oxygen or two coppers will react with two oxygen. But such a well defined system cannot be predicted that very easily for a synthetic chemist. Synthetic chemist whenever they are trying to understand the copper oxygen chemistry, iron oxygen chemistry, manganese oxygen chemistry, nickel whatever chemistry we are trying to understand, we usually run into the problems of over reactivity.

Sometime they are not reacting, but often they are uncontrollably reactive. So, once need to really understand how to tune the reactivity and get a desired product so that a desired reaction can be powerful. Of course, all the copper oxygen chemistry products are not going to be equally reactive. Their reactivity pattern is expected to be different. Those are the patterns we will try to learn today.

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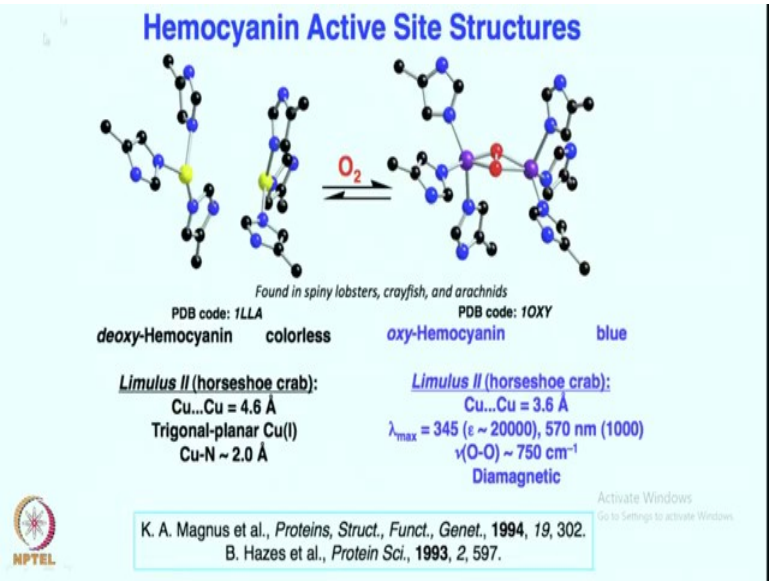

This is a beautiful enzyme right; Hemocyanin. You have 2 copper centers here; 3 histidine on each of the copper center. It is found in spiny lobsters, crayfish, arachnids. This is the oxygen carrier protein just like we what have in our blood, hemoglobin for oxygen carrying purpose; in these lobsters, crayfish and arachnids, these are the centers where oxygen binding occurs and oxygen can be carried out reversibly.

Oxygen can be delivered reversibly at different part of these species. Each of them are tri dented copper center as you can see and this is the form is called deoxy-Hemocyanin. This is a colorless compound. These are all copper I complexes. The distance between the 2 copper centers are 4.6 angstrom. Each of them are trigonal, planar geometry and copper nitrogen distance is overall nearly 2 angstrom. Once it reacts with oxygen, the species that is formed in the process is called oxy-Hemocyanin which is blue in color. This compound is blue in color.

It has a very characteristic UV visible band at 340 nanometer which is a very strong band and there is another characteristic peak at 570 nanometer which is also quite strong, but nothing like 345. If you look at the resonance Raman spectra the oxygen-oxygen stretch is arising at 750 wave number which is quite exciting for other purpose, I will come back to that in a moment.

Now, this complex is going to be diamagnetic meaning that these both of these coppers are now oxidized to copper 2. Each of the copper has given 1 electron to the oxygen

center to make it copper 2 copper 2 peroxomoiety. This dicopper peroxo species is the one which is going to be going to be having a copper-copper distance of 3.6 angstrom; for 4.6 angstrom the distance shortened to 3.6 angstrom. Almost it is breathing right. It was far apart oxygen brings them closer, relatively closer to each other right.

This oxy-Hemocyanin is giving blue color and this is very characteristic of the short of speak these short of species both the UV visible spectra, resonance Raman spectra, EVR spectra which shows the diamagnetic structure and also the also the crystal structure clearly showing that it is a side on bound peroxo species. We will come to that in a moment, but two copper is binding with both the oxygen equivalently. So, these are very distinct copper oxygen species formation.

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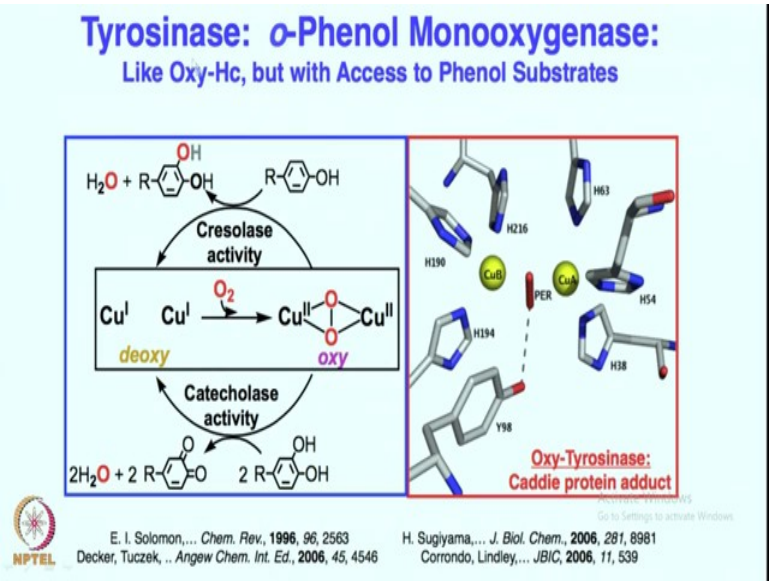

Now, if you look at another enzyme tyrosinase, it has exactly similar structure. Well, in this case the previous case of hemocyanin, there is no organic substrate involved in the whole process. Only job it does its binds with oxygen carries it and release it wherever it is required. So, it is a completely reversible process. Oxygen is getting reduced, but it gets back to its original form when it is need to be delivered right.

So, this is not involved in too much of a chemistry, its almost oxygen binding chemistry. Of course, electron transfer is occurring, but no organic substrate is getting reacted with this. Now, with tyrosinase we will see what happens with such a molecule or for such a molecule, if an organic substrate is hanging close to this active species. Is this active

species going to react with the organic substrate? Answer is yes and that is what we find in tyrosinase right.

This is another enzyme a very very interesting compound; we see our active site metallo enzyme. Once again, just like in hemocyanin, we have two copper I center which are in deoxy form it reacts with oxygen to give dicopper peroxo intermediate in and side on bound geometry. So, both the oxygen atoms are bound with both the copper and their equidistance from each other ok. So, this distance and all these distances are equal to each other.

Now, if we are placing the same species as we have seen in the hemocyanin, this is the species we have seen in the hemocyanin. Now, if we are having a phenol, then this reactive species can be active with respect to this one and can oxygenate phenol to form the catechol. So, if you are saying this is the copper center, that is the copper center; these are the 3 histidine. These are the 3 histidine and the phenol is sitting over here. It is very close to this copper peroxo intermediate over here and then, it is getting overall oxygenated at the ortho position of the phenol to give the catechol. If one is using the catechol as a substrate, these catechol can react with this further, with this peroxo intermediate to give the quinone inter product.

So, this activity is called phenol to catechol formation is called Cresolase activity and this catechol to quinone formation is called the Catecholase activity. Overall tyrosinase is a fascinating enzyme which can which can do really beautiful and simple chemistry by utilizing the copper oxygen species reactive copper oxygen species in the enzyme. Now, how or what has been really known in terms of the copper oxygen chemistry from the synthetic laboratory. These are the studies or the results from the enzyme, but as we understand that the chemistry for synthetic compound as well as the enzymatic compound are not going to differ right.

Since, these are the compounds forming; can we prepare such compound in our laboratory and see that if similar reactivity is found. Actually its other way around first the synthetic studies has been done reactivity pattern and all other studies has been done and subsequently, enzyme studies suggested and definitely later on proved that similar chemistry is happening, what is known in the synthetic chemistry. Well, that is I think is

another exciting fact to know that that the enzyme studies understanding has directly been influenced by the understanding of the synthetic chemistry.

And always almost always the understanding of the synthetic chemistry set up is going to be much more in detail compared to the enzyme and that is precisely because we can controlled on the synthetic chemistry compound rather easily controlling the reactivity, understanding the reactivity in enzyme setup in greater detail is always going to be challenging compared to the synthetic ones. Let us look at the overall copper oxygen species that one can think of; this is by no means complete list just to give an overview.

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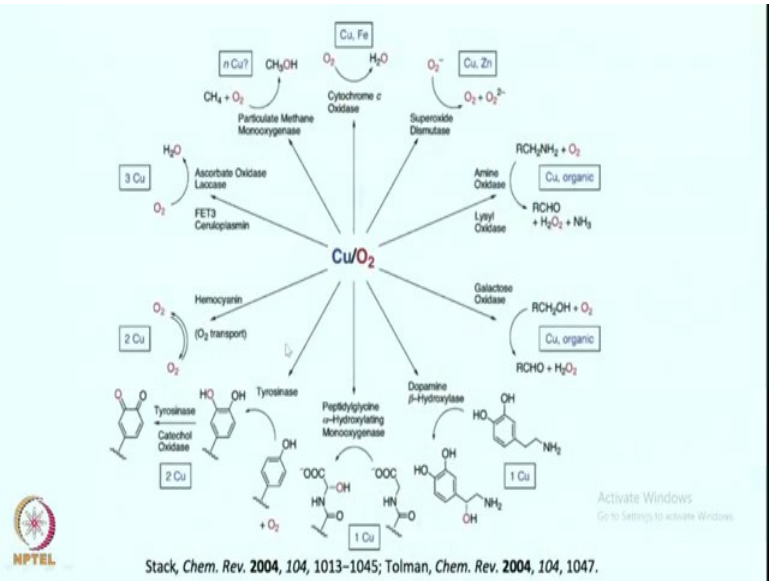

We will we have just seen this copper oxygen species giving tyrosinase activity, catecholase and the crystal is activity. This is the one we were seeing right now, this activity we have seen in the last slide. Of course, there is the oxygen activation or oxygen carrying, oxygen transport is happening. (Refer Time: 12:10) the hemocyanin these two we have seen right now. We will be seeing quite a lot of these two PHM and DBM in maybe 4-5 class.

So, we will discuss about these two enzyme separately, their reaction mechanism, these are very important enzyme. These are having although a dinuclear copper side, but a mononuclear copper oxygen species are responsible for these hydroxylation chemistry. As you see here, alpha position getting hydroxylated and this is going to be quite exciting to learn these mechanism of action for these mononuclear copper oxygen

chemistry presumably that mononuclear copper superoxo chemistry for these two, we will discuss that later.

Now, one of the thing which we have also learned or you know will be quite interesting to know is the Cytochrome C-oxidase chemistry which is a beautiful chemistry. We will not be discussing too much of the particulate methane monooxygenase which convert methane to methanol, we will learn this chemistry by utilizing iron right di-iron species. Amine oxidase and galactose oxidase are quite interesting on their own rights; galactose oxidase mechanism we will see briefly and we might will not be discussing too much of this one ok.

There is another enzyme which is fascinating to which converts superoxide into oxygen and peroxide. So, dismutation type of reaction happens. So, this enzyme is called Copper Zinc SOD superoxide dismutase right and then, there is ascorbate oxidase which will again briefly mentioned who can convert oxygen to water where 3 copper center or multiple copper centers are involved in the process.

Overall either a single copper site as you can see single copper site or a dicopper site like these are responsible for carrying out many reactions as you can see some of these over here. We will discuss some of them in future for this course, but we will focus today on how the copper is reacting with oxygen in synthetic setup ok. So, all these are some way or the other connected, but we need to understand them separately.

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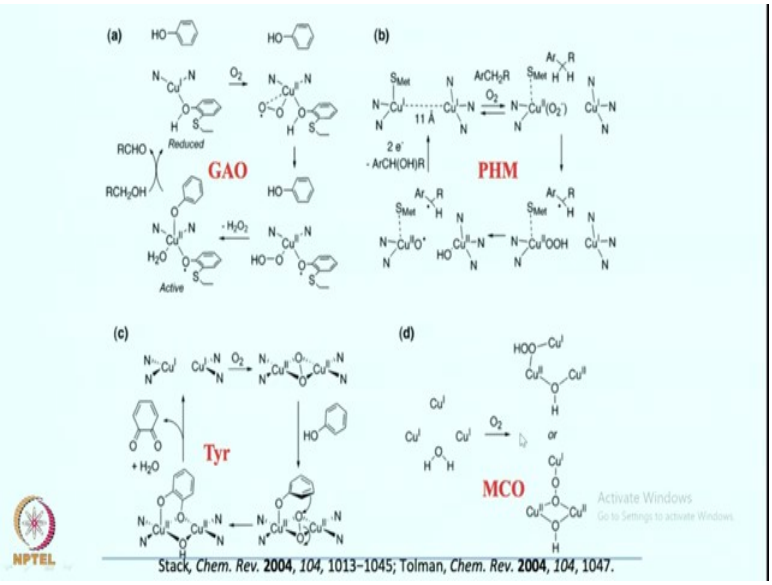

Let us briefly look at the galactose oxidase. It converts essentially alcohol to aldehyde ok. This alcohol is converted to aldehyde by galactose oxidase, it has 2 histidine and these cross linked tyrosine moiety is there and this essentially it is a phenol to histidine and phenol moiety is there and we have oxygen reaction with this copper center to give this copper to superoxo intermediate. One electron transfer to the oxygen to give the copper to super (Refer Time: 15:20) intermediate this copper 2 superoxo (Refer Time: 15:22) intermediate, then abstract hydrogen atom from this phenol to give this phenoxy radical and copper to hydroperoxo intermediate.

Subsequently, oxygen bond cleavage and formation of water takes place or in these case particularly hydrogen peroxide goes out upon protonation of this hydro peroxo species to give rise to a phenoxy radical intermediate oxygen-oxygen bond cleavage is a possibility. But did not happen in these case and it then, then underwent this phenoxy radical formation that has happened over here this phenoxy radical and copper II, now bound with phenolate can modulate the reactivity of the alcohol.

For example, by utilizing this phenoxy radical, one can think of or it can be shown in the enzyme setup that we can do these chemistry quite efficiently. This is the PHM chemistry that we will discuss in next after next 4-5 class. I guess yeah approximately 4 classes, we will we will discuss that after 4 more classes, we will discuss that multi copper oxidase has at least 3 copper presence in there. The oxygen binding is not 100 percent understood, but this is going to be predict; this is has been predicted to be one of these two and among many other possibilities that is out there.

Now, these dicopper iron site in tyrosinase is the one which we were discussing earlier. So, we will not be focusing too much on this right now. In the last slide, we have seen we will see this one again. We have seen little bit of this one before, but well maybe we can discuss briefly. There is this 2 copper center bound by 2 histidine or you know it could be 2 histidine, it could be even tridentate ligand system. But overall we can see that oxygen can react to give you the copper peroxo species, if you want to go back for example, tyrosinase you see the 3 histidine there, 3 histidine is there.

So, more correct drawing would be with the 3 histidine. Anyway, this is to show the plane of the molecule and we find that a peroxo species is formed during this reactivity of 2 copper 1 and 1 oxygen. Now phenol is ligated with the copper center and position in

such a way so that an electrophilic aromatic substitution reaction can go on and that is what is happening over here.

A electrophilic aromatic substitution reaction giving rise to the oxygenation of the ortho position of the phenol that is in turn is very exciting; that means, that we are going to form the benzoquinone directly from this intermediate, that is fascinating. We will we might we will discuss some of these or similar reaction mechanism little later. So, two copper centers, they form the peroxo, peroxo participate in oxygenating the phenol substrate.

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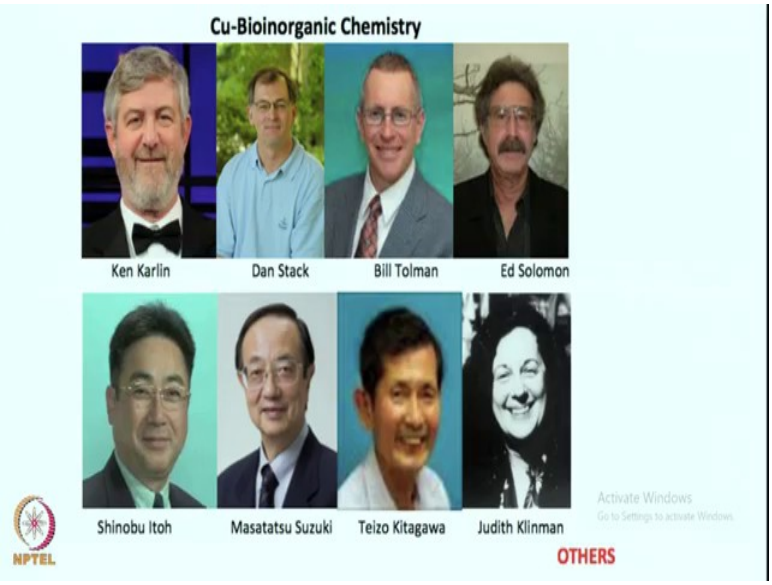

There are quite a few names; quite a few big groups that has been that has been involved all over the world for solving and understanding these copper oxygen chemistry.

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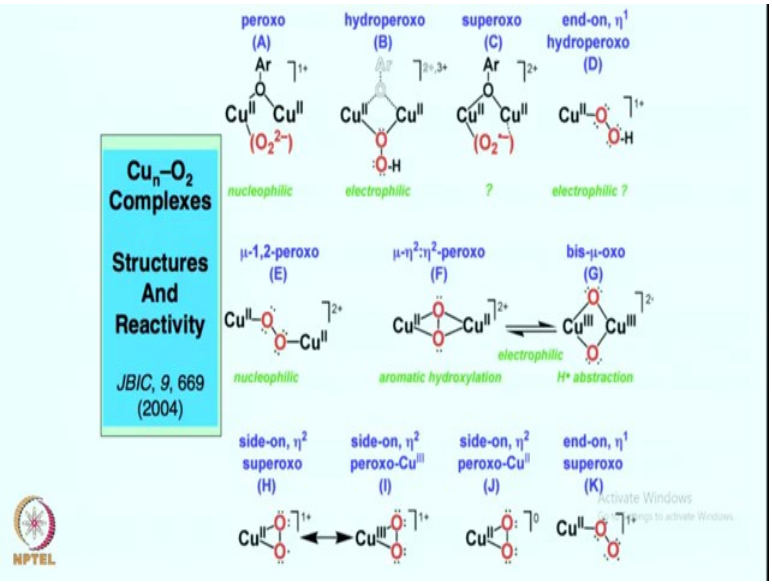

What became increasingly clear by these studies is this is far more than simple. This is indeed very very complicated to understand what goes on. Upon decades of studies shows that the ligand is controlling the geometry around the copper center. If a tetradentate ligand is present its slightly that if this end on peroxo species is going to be the reactive intermediate. If a bidentate or tridentate ligands are present as you have seen earlier a side on peroxo species is formed.

So, both of these are the peroxo species and both of these copper centers and these copper centers are going to be copper II plus, but the way they are bound with the metal center is differing. So, in this case they are bound in an side on geometry; in this case they are bound in an end on geometry. This is going to be nucleophilic in nature, these are going to be electrophilic in nature or these centers are quite interesting and aromatic hydroxylation chemistry as I was mentioning in the last slide can also be feasible.

So, these are going to give you nucleophilic reactivity ok. So, these are delta minus over here and these are delta plus over here. So, a protonation perhaps would be possible over here and a protonation will not be possible over here. These are nucleophilic as you can see, these are electrophilic both of them are electrophilic in nature.

Now, these two copper centers are in +2 oxidation states. If a suitable ligand exists usually with the bidentate ligand, what we see that even this oxygen-oxygen bond of the peroxo can be cleaved to give you copper III copper III bis mu oxo intermediate which

can abstract again the hydrogen atom. So, all this is telling that when we are taking a suitable ligand copper complex and reacting it with oxygen, there is possibility of formation of many different types of copper oxygen chemistry.

The species that is going to be formed is going to be dictated by the ligand for the metal center. If it is a tetradentate ligand present over there we can expect an end on geometry as is in here. This is called end on peroxo, dicopper peroxo or mu 1 2 peroxo. If it is a tridentate or bidentate ligand usually for the tridentate one, we get the side on peroxo species. These are similar species, but the reactivity pattern is completely different.

If you have usually bidentate ligand and suitable solvent and depending on the ligand, we can also in addition to this peroxo, we can also get this bis mu oxo intermediate. These two species can be in equilibrium. In fact, these and that can also be in equilibrium. Let's not get into too much of the details, but overall what we realize that this bis mu oxo species can be formed upon cleaving the oxygen-oxygen bond and both the copper centers are now oxidized to copper 3+ right.

Now, this is nucleophilic; these are electrophilic in nature; these are all dinuclear species right. There is indeed a possibility of forming the mono nuclear species. In fact, while forming these dinuclear species, first from intermediates are these mononuclear species; depending on the ligand, static and electronic properties, we can expect one of these species is forming as a first step. These reaction, these species formations while reacting with ligand copper 1 complex and oxygen, these are very very fast.

In fact, these are also very very unstable these species need low temperature such as -80, -90, -120 where so where it would be possible to perhaps detect it and study to some extent. Compared to that these are I would say these are still thermodynamically preferred product, but kinetic formation or the first formation of this species is definitely there which a lot of studies has shown that in recent years.

But overall these are going to be much more reactive intermediate and these are the one which is known as mono nuclear copper oxygen species. This is going to be an end on superoxo species. This is going to be a side on peroxo species. So, this is going to be depending on the charge. So, this is going to be the side on peroxo species. This is going to be side on eta 2 peroxo with copper 3 and this is going to be obviously, the side on superoxo species. So, end on superoxo as you see only one end of the oxygen is bound

with copper, the other one is not. In this case both the oxygens are bound with copper equally.

So, this is going to be the side on superoxo species, we will see this slide in next 4-5 class again, where we will discuss these mononuclear copper oxygen chemistry. But overall just to tell you that there is possibility of forming many different types of copper oxygen species. Once we are reacting them under laboratory setup. Perhaps in enzyme we have we have very we have very important formation of only one species, but in case of in case of synthetic setup, we have very limited option of controlling these reactivity pattern.

So, all of these species are likely the control is going to be with the temperature solvent, but more importantly ligand. So, that we have briefly seen. Let us look at little bit of their reactivity pattern.

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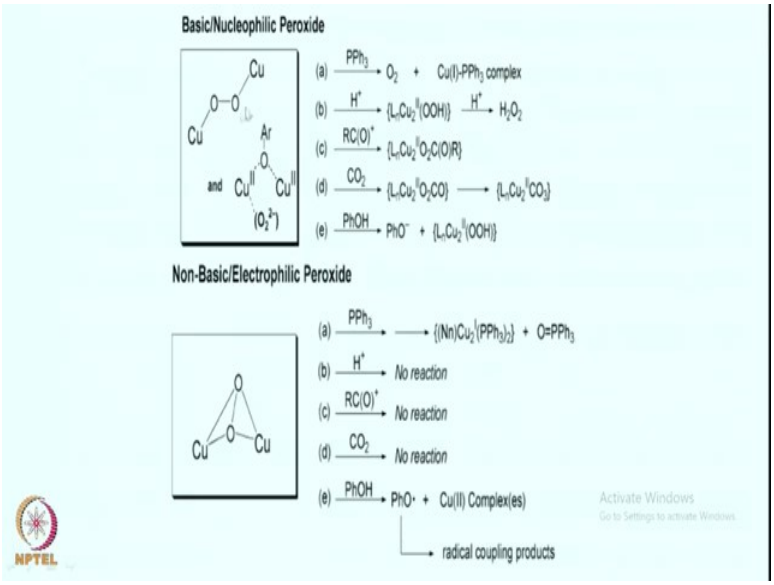

So, as I was trying to mention that these end on peroxo; this is the end on peroxo. These are going to be nucleophilic in nature; that means, these are delta - delta + ah. On the other hand, these are going to be side on peroxo in nature and they are going to be electrophilic in nature. It is going to be delta plus delta + delta - delta -.

So, as expected if we are bringing a cation such as H plus protonation, it would be possible to protonate because this is delta - delta - to give the hydrogen peroxide overall;

if you are bringing an acyl moiety, acyl moiety can also react with this because this is minus delta - This is a cation. It's going to react with this. If we are reacting with carbon dioxide that is also a feasible reaction can give you this carbonate species finally and overall this is quite interesting.

Even the phenol can be deprotonated to give the give the phenolate intermediate. On the other hand, this side on bound copper oxygens intermediate which is known as the side on peroxo cannot react with proton. Because this is delta + this is delta + this is a cation this cannot react with proton, it cannot react with acyl equivalent; it cannot react with CO3 because once again these are delta plus delta plus no reaction.

But once we are reacting with phenol, we get the phenoxy molecule from these species and from there on once a phenoxy radical is generated radical-radical coupling can go on and copper II complex can form.

So, overall in this class therefore, we have seen that different type of copper oxygen species can form, if the ligand copper complex is reacted with oxygen and their reactivity pattern is also varying as you have seen end on peroxo species are nucleophilic in nature and side on peroxo species is electrophilic in nature.

You keep studying these different complexes and their reactivity pattern. We will discuss from here in the next class and we will try to see the reactivity of the copper ligand complexes in the reduced form to give the oxygenated product oxygen chemistry and their reactivity pattern of the species in the next class as well see you soon.