Metals in Biology
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Lecture – 13
Copper- O 2 intermediates

Hi there. How are you guys doing? Hopefully things are going great. We have discussed in the last class, how copper oxygen chemistry can be taken to a level where we can understand the species formed in greater details. The complexity of the reactions gives rise to the main product formation, it could be mono nuclear species, it could be di nuclear of course; it could be multi nuclear as well which we did not discuss too much.

In the last class, we have seen that these dinuclear peroxo species either in an end on geometry or in a side on geometry can be formed and their reactivity patterns are different. This species is nucleophilic in nature the end on one and this is side on in nature. Both the species are formed from ligand copper I complex. Yet another species could be this bis mu oxo l where oxygen oxygen bond is cleaved.

So, arising from ligand copper 1 and molecular oxygen, now we have an oxide not only one; two of them coming from two of the oxygen atoms.

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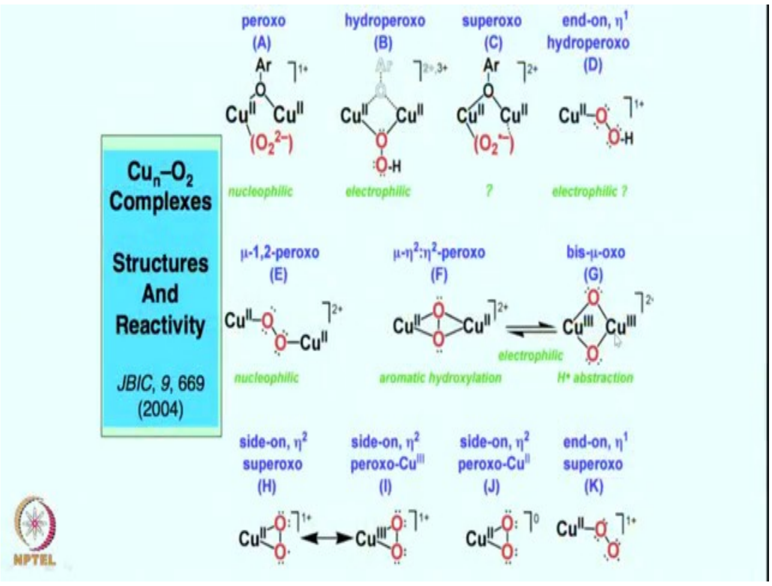

Cu_n–O_2 Complexes

Structures And Reactivity

JBIC, *9*, 669 (2004)

These coppers are in +3 oxidation state now. Of course, there are mononuclear species which we will be discussing in four to five class separately.

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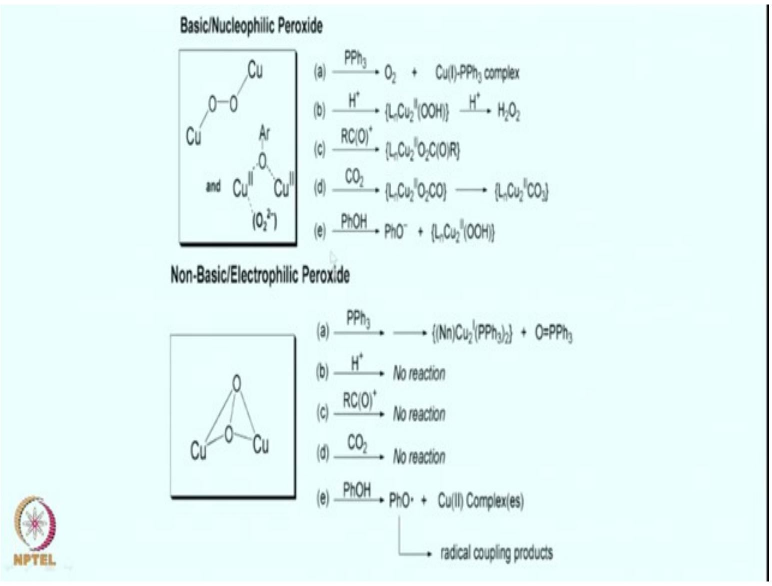

Basic/Nucleophilic Peroxide

(a) $\xrightarrow{PPh_3} O_2$ + Cu(I)-PPh$_3$ complex

(b) $\xrightarrow{H^+} \{L_nCu_2^{II}(OOH)\} \xrightarrow{H^+} H_2O_2$

(c) $\xrightarrow{RC(O)^+} \{L_nCu_2^{II}O_2C(O)R\}$

(d) $\xrightarrow{CO_2} \{L_nCu_2^{II}O_2CO\} \longrightarrow \{L_nCu_2^{II}CO_3\}$

(e) $\xrightarrow{PhOH} PhO^- + \{L_nCu_2^{II}(OOH)\}$

Non-Basic/Electrophilic Peroxide

(a) $\xrightarrow{PPh_3} \longrightarrow \{(Nn)Cu_2^{I}(PPh_3)_2\}$ + O=PPh$_3$

(b) $\xrightarrow{H^+}$ *No reaction*

(c) $\xrightarrow{RC(O)^+}$ *No reaction*

(d) $\xrightarrow{CO_2}$ *No reaction*

(e) $\xrightarrow{PhOH}$ PhO· + Cu(II) Complex(es)

→ radical coupling products

Now, as we have discussed these species are nucleophilic, this end on bound peroxo and side on bound peroxo are electrophilic.

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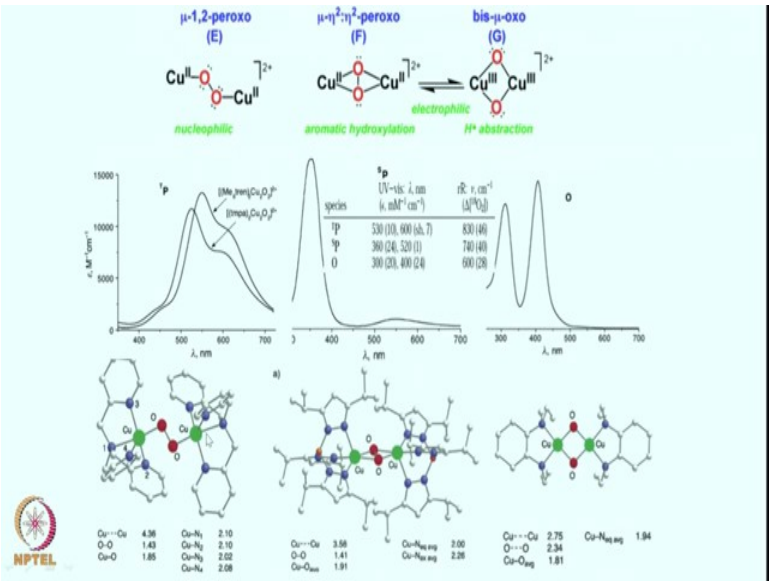

species	UV-vis: λ, nm (ε, mM^{-1} cm^{-1})	rR: ν, cm^{-1} (Δ[^{18}O$_2$])
^{T}P	530 (10), 600 (sh, 7)	830 (46)
^{S}P	360 (24), 520 (1)	740 (40)
O	300 (20), 400 (24)	600 (28)

Cu···Cu	4.36	Cu-N$_1$	2.10
O-O	1.43	Cu-N$_2$	2.10
Cu-O	1.85	Cu-N$_3$	2.02
		Cu-N$_4$	2.08

Cu···Cu	3.56	Cu-N$_{eq\ avg}$	2.00
O-O	1.41	Cu-N$_{ax\ avg}$	2.26
Cu-O$_{avg}$	1.91		

Cu···Cu	2.75	Cu-N$_{eq\ avg}$	1.94
O···O	2.34		
Cu-O$_{avg}$	1.81		

Well today, we will see their crystal structure. These are crystallographically characterized intermediate and as you can clearly see a tetradentate ligand known as TPA or TMPA can support the copper center so, that this end on bound geometry can be formed.

As you can see each of the copper is bound with one of the oxygen atom only this distance copper oxygen distance and this copper oxygen distance are completely different. On the other hand, this copper oxygen distance and this copper oxygen distance are exactly the same and these two copper centers are bound with this oxygen moiety in an side on fashion. Both these one and this one are peroxo unit; that means, oxygen is reduced by two electrons one from each of the copper. Copper I, we started with after giving rise to 1 electron each it is copper II, copper II, oxygen II minus.

So, this is the formulation where overall we can have plus 2 charge in the complex because this is 2 - this is 2 + this is 2 + overall 2 + species here. Over here similarly, we have a neutral ligand and therefore, we have copper II + copper II + and oxygen 2 - that is peroxo overall this is a 2 + compound. Similarly this species or this species even can be in equilibrium with bis mu oxo where the oxygen bond is broken now this is oxide oxygen 2 - oxygen 2 - copper III + copper III + total 6 + and 4 - total gives in rise to the overall 2 + charge for this complex.

These two species are electrophilic in nature. We have seen the reactivity pattern of these species reactivity pattern of the species is quite similar to what we have seen for the side on peroxo species. If you look at these, these are only differing in the ligand. Ligand could be let us say 4 coordinated, tetradentate or 3 coordinated or 2 coordinated, but the type of species that is formed are completely different. Roughly saying that this is 4 coordinated this is 3 coordinated this is 2 coordinated species. The ligand varying as you can see in the crystal structure. This is a bis coordinated, its copper center giving you bis mu oxo intermediate. These are tris pyrozelol borate type of ligand giving you side on bound geometry.

So, this is end on, this is side on, this is bis mu oxo without any oxygen bond there. These species can also have very characteristic UV visible spectra as you can see over here across the ligand not only this ligand, you can have very many different type of tetradentate ligand. They will give a similar peak pattern with little bit shift in this in this peak position, but overall this is a very characteristic species and can be taken as a standard to detect the such intermediate.

Remind you all these intermediates are unstable intermediate although they are dimer thermodynamically stable compared to the mononuclear species, but still these are completely unstable intermediate. If you take this ligand copper I complex of these or any of these, then and react with oxygen at room temperature. Most often you do not see anything, it is just the decomposition product which is arising from any of these intermediate.

These are true intermediate and very reactive intermediate often a let us say di nuclear copper dihydroxy instead of oxo or other reactive and stable further stable intermediate is generated which can be considered as the decomposition product. None of these product you might will be able to see at room temperature, you have to lower down the temperature and the stability need to be monitored through the spectroscopic technique or the colour changes, the colour changes are white also quite distinct in nature. As we discussed, this is purple in colour this is really blue in colour and these are giving rise to the quite intense band in the irreversible spectra.

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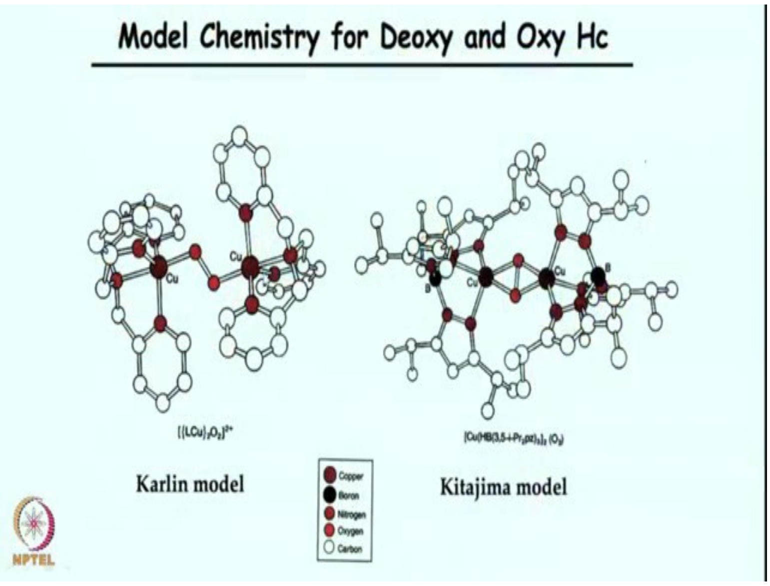

Model Chemistry for Deoxy and Oxy Hc

$[(LCu)_2O_2]^{2+}$

Karlin model

$[Cu(HB(3,5\text{-}iPr_2pz)_3)]_2(O_2)$

Kitajima model

Here is the crystal structure as we were showing earlier also, this is the first ever crystallographically characterized copper oxygen intermediate, that is the same structure as we have seen in the last slide.

This is the first sideon bound peroxo crystal structure, just to tell you that this crystal structure was known much before the oxy hemocyanin structure was known. So, the inorganic chemists were able to synthesize and characterize by spectroscopic technique including UV visible spectra Raman resonance Raman spectra and was confident enough to tell that this is exactly what is happening in hemocyanin oxy hemocyanin case, because the spectral feature for the oxy hemocyanin was known, but the crystal structure was not known. Synthetic chemists indeed have succeeded in many instance in predicting the structure in advance and appropriately correctly for many of the reactive intermediates, where crystal structure in the enzyme was obtained much later than the synthetic studies.

So, these are really a wonderful moment for the synthetic chemist to corroborate, what chemists can do and what nature can do. Nature is obviously, very designed very efficiently every active site. But synthetic chemists can try to learn from them even in some cases can go ahead a step where synthetic chemist understanding can be employed, can be seen as a fast step forward to understand the metalloenzymes all right. So, this is the model by Kitajima, this is the Carlins model.

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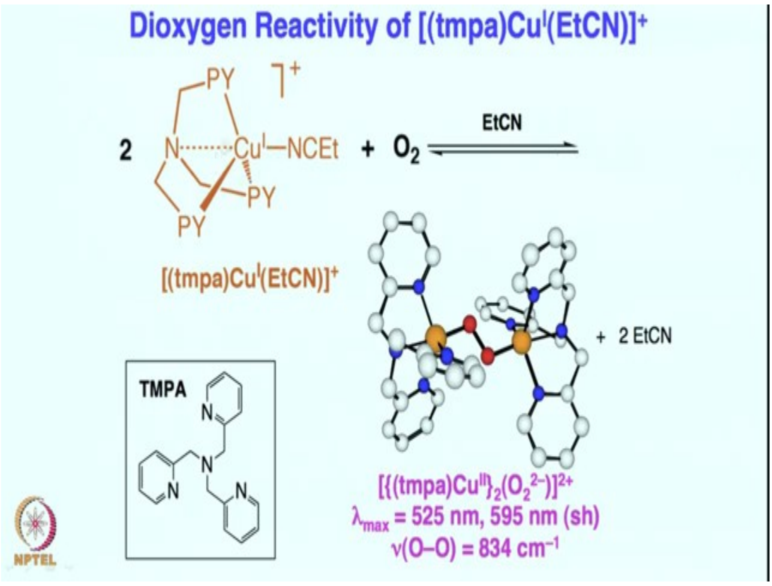

Dioxygen Reactivity of $[(tmpa)Cu^{I}(EtCN)]^{+}$

2 $[(tmpa)Cu^{I}(EtCN)]^{+}$ + O_2 $\xrightarrow{EtCN}$ $[\{(tmpa)Cu^{II}\}_2(O_2^{2-})]^{2+}$ + 2 EtCN

TMPA

λ_{max} = 525 nm, 595 nm (sh)

$\nu(O\text{–}O)$ = 834 cm^{-1}

So, let us look at this tetradentate ligand system. This is the TMPA or also known as TPA in iron chemistry literature. So, this TMPA copper species which is yellowish or rather light yellow color reacts with oxygen, to give this end on peroxo species and there is direct formation of this species while we are trying to follow it at room at low temperature; for example, propionitrile minus 80 degree C and or so.

So, if one is taking this ligand copper I complex reacting with oxygen directly this intermediate is forming which is purple in colour.

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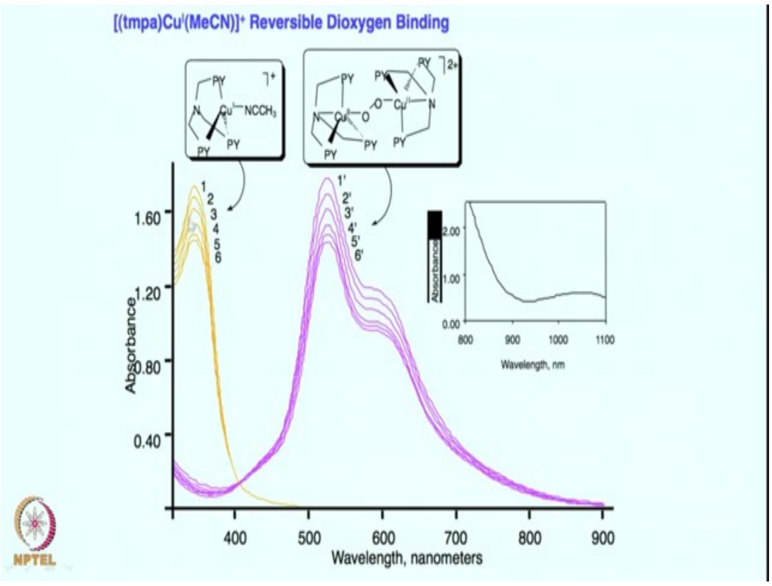

$[(tmpa)Cu^{I}(MeCN)]^{+}$ Reversible Dioxygen Binding

So, the disappearance of this ligand copper I complex and only the decomposition of these species can be seen right. So, this is quite interesting. So, one species disappears another species forms completely and very very efficiently and cleanly. It is a very clear reaction irreversible spectra is quite beautiful as well as the colour is quite exciting for this complex, this is approximately the colour of the corresponding species.

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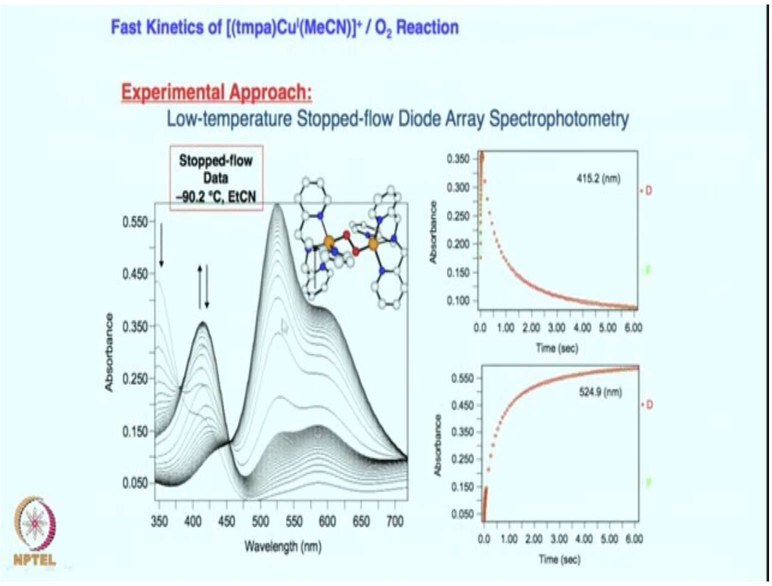

Fast Kinetics of $[(tmpa)Cu^{I}(MeCN)]^{+} / O_2$ Reaction

Experimental Approach:

Low-temperature Stopped-flow Diode Array Spectrophotometry

Now, if you study this intermediate by low temperature stop flow diode array spectrophotometry, there is a completely different story to tell. Of course, the copper I is disappearing, but before formation of this end on bound peroxo a completely new intermediate is generated from this which can be considered as fast formed intermediate while reacting ligand copper I with the oxygen.

So, we are trying to say that the ligand copper I complex reacts with oxygen to give an intermediate, which cannot be detected even at - 80 - 90 degree centigrade by irreversible spectroscopy. But the same compound when or same reaction when studied under the first reaction condition or first spectroscopic technique such as stop flow diode array spectrophotometry, it can be seen that a new species is formed and disappearing at the same time to gives rise to this little bit stable; this end on peroxo species at low temperature.

So, that you can see this is the disappearance of 450 nanometer band this one. So, the formation of this band cannot be followed properly. The disappearance can be followed formation is too fast right; formation of this band is too fast, but over few seconds actually essentially it is gone and you can see nearly within 1 second everything is gone and that is why in normal spectroscopic technique, the moment mixing is happening we cannot detect this because this is too fast for normal spectroscopic technique. Here it is a first spectroscopic technique also shows that not only disappearance of this peak, you we can see the formation of this peak this characteristic peak for the peroxo species as is shown over here within 1 second literally majority of the species is formed.

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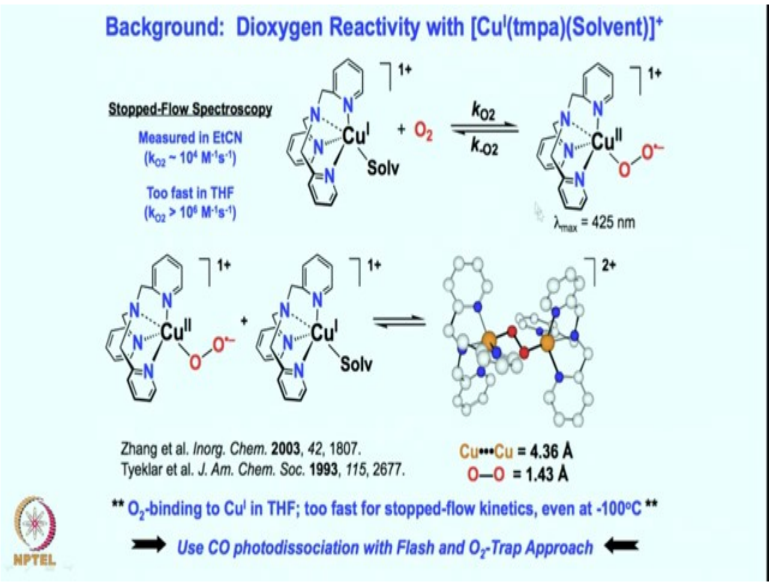

Background: Dioxygen Reactivity with $[Cu^I(tmpa)(Solvent)]^+$

Stopped-Flow Spectroscopy

Measured in EtCN
($k_{O2} \sim 10^4\ M^{-1}s^{-1}$)

Too fast in THF
($k_{O2} > 10^6\ M^{-1}s^{-1}$)

Zhang et al. *Inorg. Chem.* **2003**, *42*, 1807.
Tyeklar et al. *J. Am. Chem. Soc.* **1993**, *115*, 2677.

Cu•••Cu = 4.36 Å
O—O = 1.43 Å

** O_2-binding to Cu^I in THF; too fast for stopped-flow kinetics, even at -100°C **

➡ *Use CO photodissociation with Flash and O_2-Trap Approach* ⬅

So, what essentially then we are trying to tell that, these ligand copper I complexes reacts with oxygen to give ligand copper II peroxo intermediate like this. This ligand copper II superoxo intermediate, then can react with another equivalent of copper to give this peroxo species which is crystallographically characterized. We will discuss this in the context of copper II peroxo intermediate later on in next 4-5 class, but most importantly to note that this is the first formed intermediate which is green in color if it is synthesized separately, this is quite challenging to stabilize. Because this is really unstable intermediate and it was quite exciting to see that this can be stabilized in a suitable ligand backbone.

And this copper complex is always there because first from intermediate will not be able to react with all of them or some of the copper I will be immediately over there, that can react subsequently to give this end on bound peroxo intermediate. So, what we have just learned then, this ligand copper I complex are capable of reacting with oxygen 1 is to 1 geometry 1 is to 1 stoichiometry and then they give a first form intermediate which is very reactive immediately reacts with another one to give the end on peroxo geometry. Now let us look at the sum of the reactions that can be promoted by these copper oxygen chemistry.

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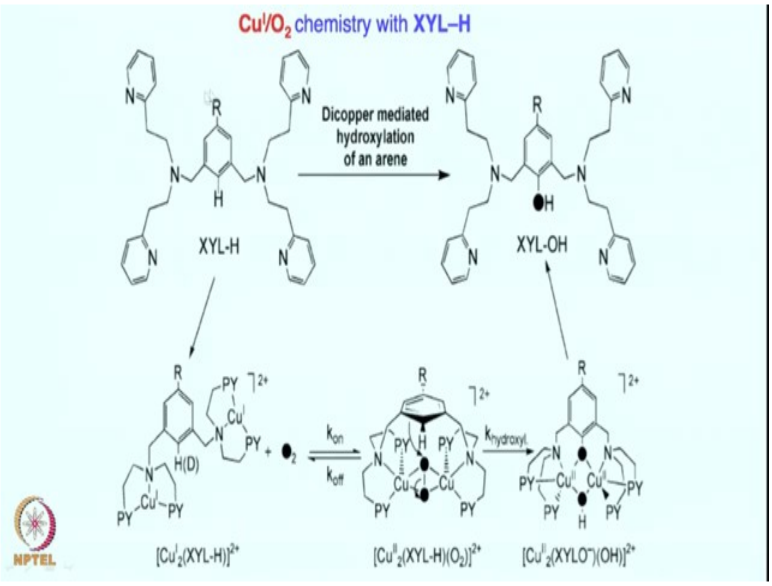
Cu^{I}/O_2 chemistry with XYL–H

Let us say you have one half is having a benzene ring in between these two ligands. We have seen two different ligands from two for two different copper center and they are bridged by the oxygen.

So, of course, they are going to bridge by oxygen what if we place a tridentate ligand and another tridentate ligand bridged by benzene ring. Well of course, since it is a tridentate ligand one can expect to have a side on bound copper peroxo species. This is now intramolecular setup. So, tridentate ligand here, tridentate ligand there; you will give an intramolecular copper copper peroxo intermediate. Now the benzene ring is actually positioned right what we see in nature that substrate as a substrate is positioned perfectly in front of the active site. Due to this what will happen that since we know that these are going to be delta + and this is nucleophilic in nature as long as electron it is not electron deficient, this is nucleophilic in nature. So, electrophilic aromatic substitution type of reaction mechanism can pick up. So, the olefin or these pi electron attacks.

So, electrophilic aromatic substitution reaction takes place, it is a oxy hydroxy or phenoxy hydroxy intermediate heat generated as you can see the by the arrow and this intermediate is quite exciting essentially, then it is allowing us after demetallation to get the hydroxylation. So, this is a benzene ring hydroxylation by the copper copper catalyzed or copper mediated dinuclear peroxo species side on peroxo species right. So, we started with the benzene appended with two ligand or two arms, where each of the side can have one of the copper centers. Now these two copper center giving rise to this peroxo intermediate which can then react to give such a reactive intermediate and then the demetallation will give a clear cut compound as we see over here.

So, net this benzene hydroxylation essentially benzene to phenol transformation has happened during this process which is quite exciting.

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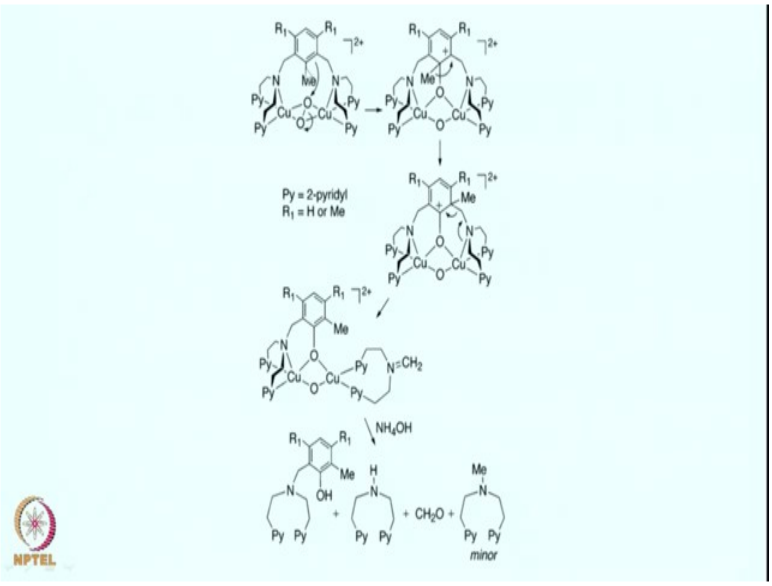

Well one can think of blocking that site where the oxygenation was going on by placing let us say methyl group like exactly similar chemistry, having a benzene ring, but placing a methyl group at that that head position or the middle position which is exciting going to be again. What is going to happen at that center if it is it is blocked by a methyl group? Of course, the similar electrophilic aromatic type of substitution reaction carries and follows, ensures and subsequently methyl sub methyl this transfer or shift happens at the at the alpha position or at this position, which gives rise to a very exciting intermediate as you can see over there, subsequent rearrangement gives rise to the phenol product, but now the methyl is shifted from this position to there.

So, as you can see of course, ligand is not getting NB alkylated in the process, it got chopped off from the system, but quite interestingly as you can see this is turning out to be quite a quite a phenomenal compound as you do see that methyl shift as well as hydroxylation happened. So, both over here methyl shift and hydroxylation happen and

NB alkylation chemistry or yeah you know, we are seeing that this carbon carbon bond also is getting cleaved.

So, overall as you can see here at least 3 different things has happened. The hydroxylation methyl transfer as well as carbon-carbon bond cleavage by the copper oxygen chemistry. This is phenomenal; I mean quite if quite a exciting reaction as you can see a product distribution can be also be found and each of the product can be fully characterized by for by column chromatography and these reactions showcase the power or the strength of the copper oxygen chemistry to promote various oxygenation chemistry, rearrangement chemistry as well as the cc bond cleavage chemistry in a suitable situation here is here is the compound that is generated.

If you remember that we are we are generating this oxygenated compound right, if this oxygenated compound is now having a copper I complex to start with one can synthesize this phenol containing copper I copper I species from here and then try to do the oxygen chemistry.

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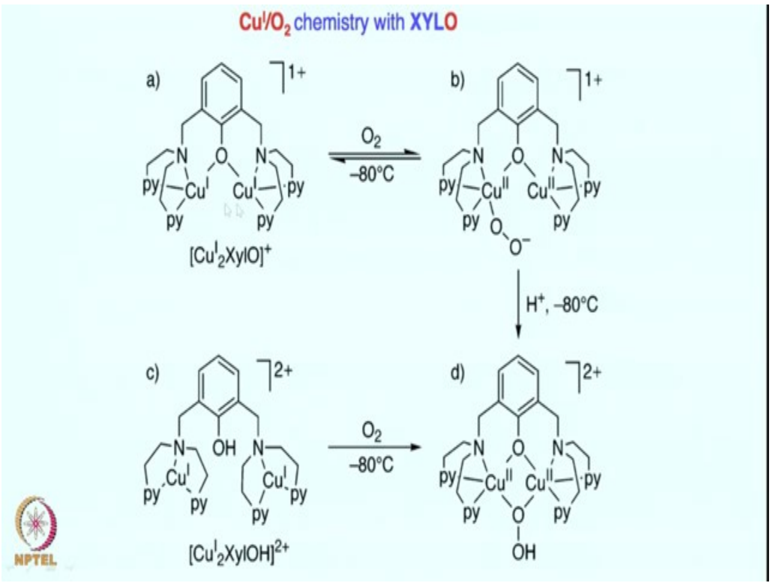
Cu^I/O_2 chemistry with XYLO

So, what we are saying is this intermediate, this is now a phenol it can be deprotonated and overall it could be singly charged this is a phenolate O- copper 1 copper one. So, overall 1 + if 1 is interested in reacting such a species with oxygen what will happen? Of course, this is a tridentate ligand, there is a tridentate ligand and there is a bridged or bridging phenol of phenolate. Now this bridging phenolate can react with oxygen to give the peroxo intermediate, this was a copper I that was a copper I. So, one one electron each of these copper gives on the oxygen to give copper II copper II peroxo intermediate. This intermediate can be protonated and this there could be a bridged dicopper oxo bridgeD you know phenoxy, phenoxy bridgeD hydro peroxo intermediate like this as upon protonation and subsequently this same species can also be generated by starting from copper I copper I phenol and reacting with oxygen. In this case, proton equivalent can be generated or provided by the phenol instead of phenoxy.

So, if it is phenoxy proton can be provided later on to give the this copper dicopper oxo hydro peroxo intermediate. If we are starting from a hydroxo species a side on peroxo and the subsequent hydrogen atom abstraction, chemistry can gives rise to the overall a quite interesting copper hydro peroxo bridged hydroperoxo intermediate.

All these chemistry are done at very low temperature, at room temperature none of these intermediate can be stabilized and same these can go to give further decomposition product. Well we can go on and discuss many other reactivity of the copper oxygen species, will be limiting our discussion in figuring out only few example.

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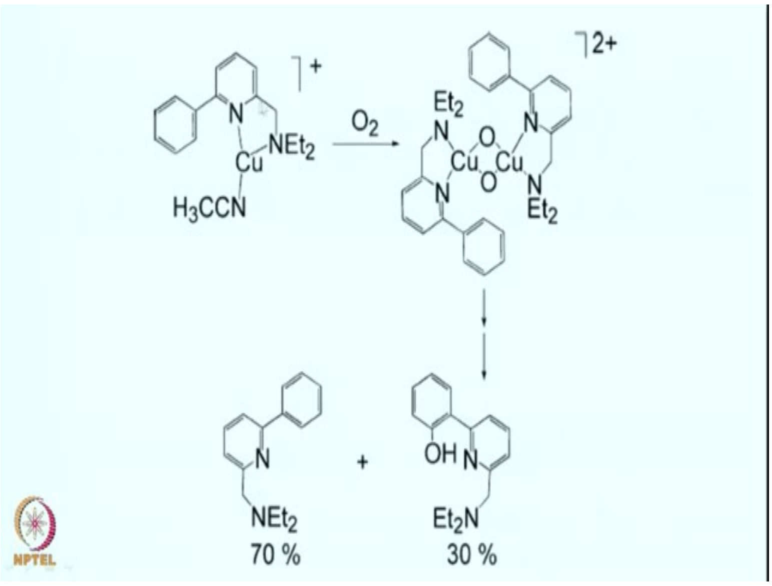

For example, for instance we can have a bidentate ligand as you can see over here this is a bidentate ligand appended with a phenyl ring over here. Now this can be reacting this can be reacting with oxygen to give since this is a bis or bis coordinating or bidentate ligand, it will give a copper III copper III bis mu oxo intermediate the ligands are bidentate. So, here is one ligand, here is another ligand; here is one copper there is another copper and bis mu oxo this is of 2 - oxygen 2 - this is also oxygen 2 - copper III + copper III +. As we have discussed earlier that these are the species which are going to be really negatively charged right now a electrophilic aromatic substitution type of reaction appended which is right here appended and this position can gives rise to the can give rise to the hydroxylation At this position right which is quite exciting, which is quite phenomenal that ortho hydroxylation of this appended phenyl ring can be done to give to give the product as we see over here of course, a lot of product can remain unreacted. that is quite interesting, but more importantly very recent studies also shows that this is not necessarily capable necessarily the side on peroxo that is involved in the oxygenation chemistry of tyrosinase, this has a really clear cut and long standing implication where we initially thought or it is usually always thought that is the side on peroxo that is doing the chemistry.

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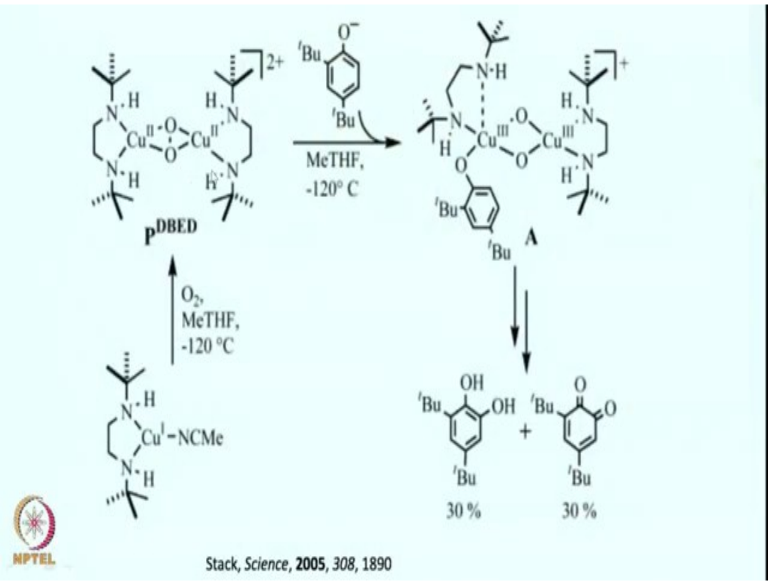

Stack, *Science*, **2005**, *308*, 1890

In clay in case of in case of hemocyanin and in case of course, hemocyanin we have side on peroxo, but the oxygenation chemistry in tyrosinase also have the same species and therefore, it is this species that is responsible for oxygenation of the phenol right. But recent studies shows that with this particular ligand there is a clear equilibrium shift from a side on peroxo to bismuoxo species, once this phenolate is added. If it is a phenol addition, then perhaps this is the species that is responsible for the oxygenation chemistry of the phenol.

Once this phenolate is added to this system then this equilibrium shifts as we have mentioned earlier there is always an equilibrium between this peroxo and the bis mu oxo. This shift of reaction mechanism or reaction intermediate from an side on to a bis mu oxo species which is promoted by the organic substrate is quite exciting what it is turning out to be fact that this phenolate is binding with one of the copper center and then overall this bismuoxo species is the one which is the true active species for oxygenating.

So, one can then perhaps think of the tyrosinase chemistry is promoted by most likely this sort of copper III copper III bis mu oxo intermediate as opposed to the familiar belief that this is a side on peroxo, that is going to do the chemistry. So, once again there is a little bit debate here that is whether a side on peroxo is that true active species for the phenol hydroxylation in the tyrosinase activity or it is rather the phenolate bound copper intermediate which is a bismuoxo intermediate and that is the one which is responsible for the chemistry that is seen at the ortho position of the phenol; or this still remains wide open, but what interests us all that; that there is a possibility that both or either of them can promote such hydroxylation chemistry. So, we have seen that that these species are quite reactive. Let us see one or two more example and conclude this reactivity pattern.

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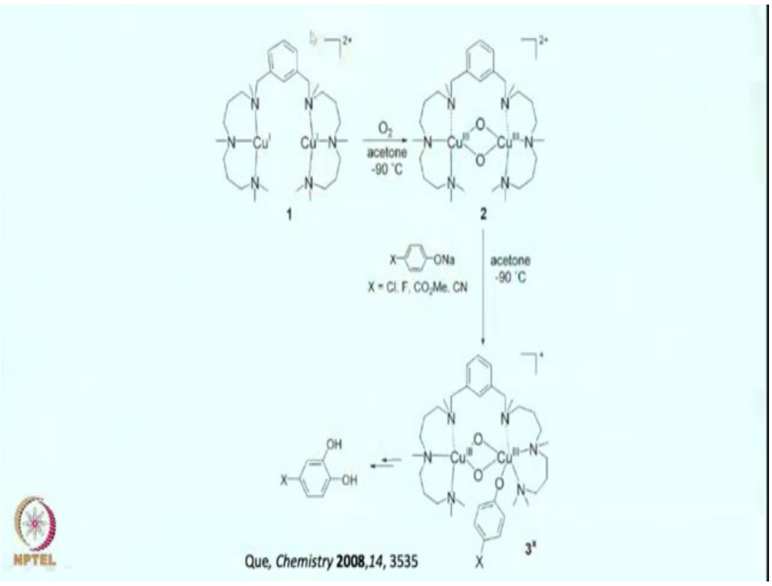

Que, *Chemistry* **2008**, *14*, 3535

Here you can see once again a bridged benzene ring appended with a tridentate ligand, this is another tridentate ligand the same tridentate ligand on both the side oxygen is there and in these cases, this short of aliphatics side chain or aliphatic ligand is capable of promoting bismuoxo species. These are not side on peroxo species, these are directly bismuoxo species with that tridentate ligand. Remember tridentate ligand usually prefer to give the side on peroxo but in this case the completely aliphatic moiety is giving the bismuoxo species and that is that is fantastic. This is completely characterizable from there a phenolate once again is thrown into the system and it is shown that phenolate can also promote a similar chemistry in these cases where oxygenation happened at the ortho position of the phenol.

So, overall I hope and we what we discussed today and hope you understand clearly that these copper oxygen chemistry are quite exciting right. The nuclear chemistry wise there is at least 3 measures players; end on peroxo, side on peroxo and the bismuoxo all these 3 spaces are capable of doing beautiful chemistry. End on peroxo is nucleophilic in nature and the side on peroxo and bismuoxo are electrophilic in nature. We have seen beautiful chemistry including the benzene ring hydroxylation chemistry by utilizing these intermediates and it has direct relevant absolutely direct relevance on what goes on in the tyrosinase where you have seen that phenol or the benzene ring containing substrate phenol containing substrate. It is appended right next to the side on peroxo geometry.

Whereas, in hemocyanin case, there is no organic substrate to react despite having a reactive side on peroxo still we see that a reversible binding is facilitated for the oxygen transport. But the same oxygen transport intermediate can be utilized further for promoting substrate hydroxylation chemistry. I think this is quite a remarkable to understand that these chemistry are quite interesting a same species exactly same species in one hand can be used as oxygen transport agent by introducing a suitable organic substrate close to the active site nature has chosen in places to react it with the organic substrate to give the beautiful chemistry.

So, I think you know such a precision and such a beautiful delivery of the target and carry out the proper function by carrying these reactions are quite phenomenal and that is why perhaps nature has always an edge over synthetic chemists, where in synthetic chemists can showcase the strength and the details of the structure. Whereas, biological studies can be little difficult at points to understand it in greater detail what chemists can offer. We will continue discussing on that, do keep studying and in the in that in the subsequent code classes will be discussing the mononuclear copper oxygen chemistry, not right now. After 3-4 classes, we will be discussing that and then we will come back to some other iron based chemistry as well.

Thank you. Keep watching, keep studying. Good luck.