Metals in Biology
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Lecture - 20
Nitrous Oxide reductase and its model complex

Hello, welcome to Metals in Biology in today's class, we will discuss Nitrous Oxide reductase and its model complex. Nitrous oxide as you may know is a greenhouse gas, it is also known as laughing gas, it has many detrimental effects on ours nature, but also it is one of those gas, which is involved in the nitrogen cycle.

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As you know, the denitrification process involves nitrite to NO to N_2O and finally, nitrogen formation.

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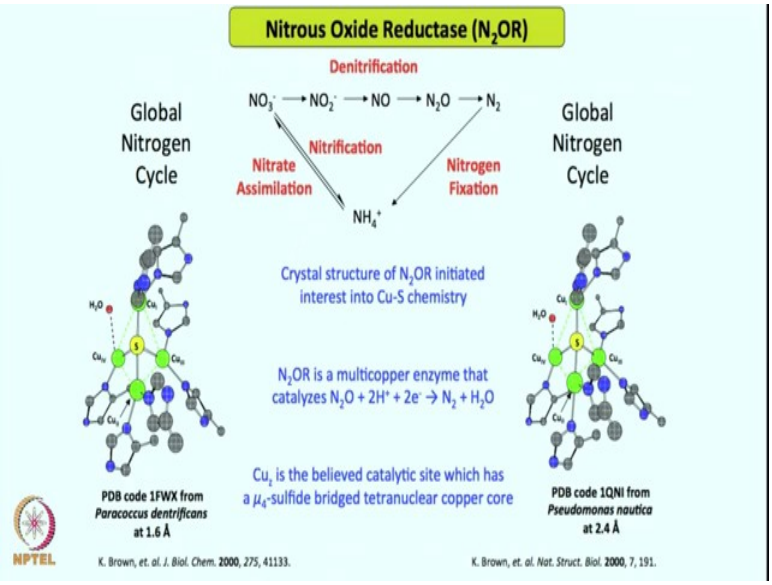

From nitrate this whole process starts and there is of course nitrate assimilation and nitrogen fixation. Overall global nitrogen cycle is linked with nitrous oxide.

The process by which nitrous oxide is converted to nitrogen is our major focus today. So, here as you can see nitrous oxide is going to nitrogen with the help of this enzyme known as nitrous oxide reductase also known as copper Z side or copper Z side. Copper g is the believed catalytic cycle, which has a u4sulfide bridged tetranuclear copper core. As you can see 4 copper centers are bridged with this sulfide. Of course, this another center which was not clear at the beginning I will come back to that later is initially believed to be water molecule.

Each of the copper centers are associated with ligands, as you may be able to see here, that this fourth copper center is associated with only one histidine on the other hand, all other copper centers are having two histidines on them. This site once again is the center, where this nitrous oxide is converted to nitrogen, it is a multi copper enzyme as you have seen; 4 copper centers are there that catalyzes nitrous oxide +2 proton + 2 electron going to nitrogen and water.

As you clearly can see that, this is having a copper sulfide structure or copper sulfide motif, the core of this is still unknown in the literature from synthetic community. The enzymatic study so far are not very clear in terms of suggesting a clean pathway by which this nitrous oxide is getting activated and then, but of course, first it has to bind

activated and then, it should be converted to nitrogen. The mechanism of action of such copper Z side is not clear till date.

Let us look at some of the proposed mechanism, there are number of studies mainly with the computational works that suggest that a pathway involving nitrous oxide binding happens between the two copper centers. Of course, in addition to the copper Z side there is another center known as copper A, which is responsible for transferring electron that is required for the nitrous oxide reduction to convert it into nitrogen.

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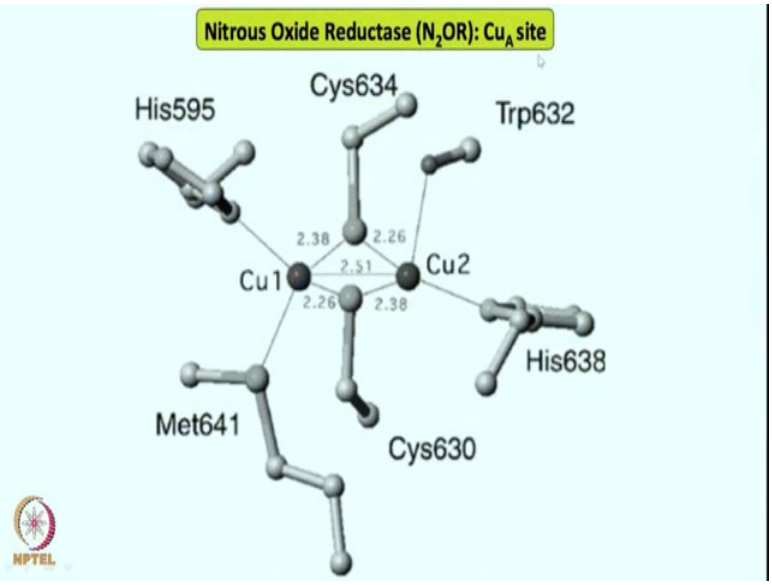

Once again, this nitrous oxide requires these electrons in the whole process to occur and those electron transfer site can be considered as this dicopper thiolate or cysteine bridged dicopper center which can act as electron transfer site. So, this side as you have seen earlier that these are copper A site ok. This does not participate in nitrous oxide reduction at all, this just provides electron does not participate directly, but provides electron for the Nitrous Oxide to dinitrogen.

Let us look at the mechanism, by which perhaps nitrous oxide can be activated and then, subsequently with the help of 2 proton and 2 electron can be converted to nitrogen and the complete catalytic cycle can be drawn.

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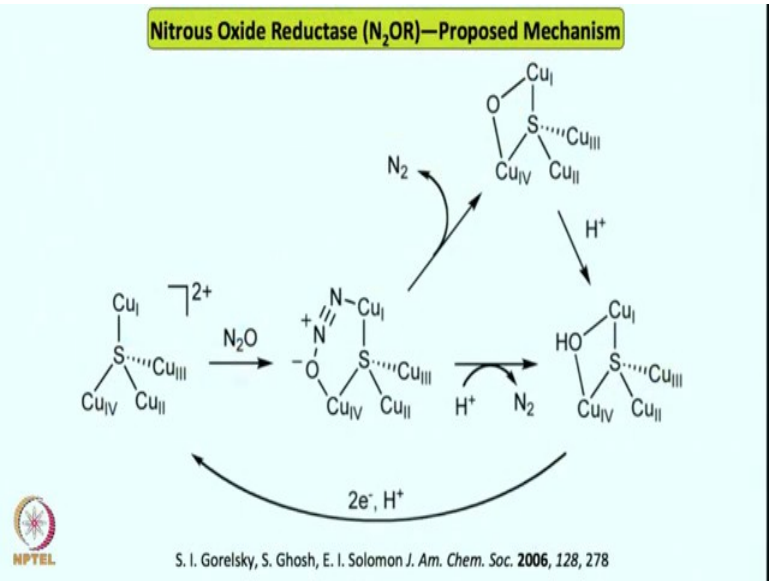

So, here is a putative mechanism of course, this can be still debated and especially the fact that the structure be need to be further modified at this point. In any case, if we assume that these 4 copper centers are bound with copper which is very clear from the crystal structure that is the case, that 4 copper centers are bound with copper this is a sulfide S2- sulfide and nitrous oxide is binding between these two copper center. As you can see it has been proposed that this nitrogen is bound with copper 1 and this copper 4 is bound with oxide, these are computational studies some experimental studies are also done. Overall this is the mode of binding that is suggested for Nitrous Oxide.

Once this is bound, one can exclude nitrogen from here to get this copper oxo copper bridged, from here on a protonation and O subsequent this 2 electron transfer can lead to the overall completion of catalytic cycle. Of course, this protonation can directly happen simultaneously when nitrogen is going out to form this intermediate and subsequently it can be converted to the starting point.

So, overall this Nitrous Oxide will bind between 2 copper center, copper 1 and copper 4, subsequently activation will take place and then, removal of nitrogen will give rise to this copper oxo copper core which can undergo protonation or a direct protonation will lead to the nitrogen formation from which 2 electron and 1 proton can lead to the regeneration of the active site.

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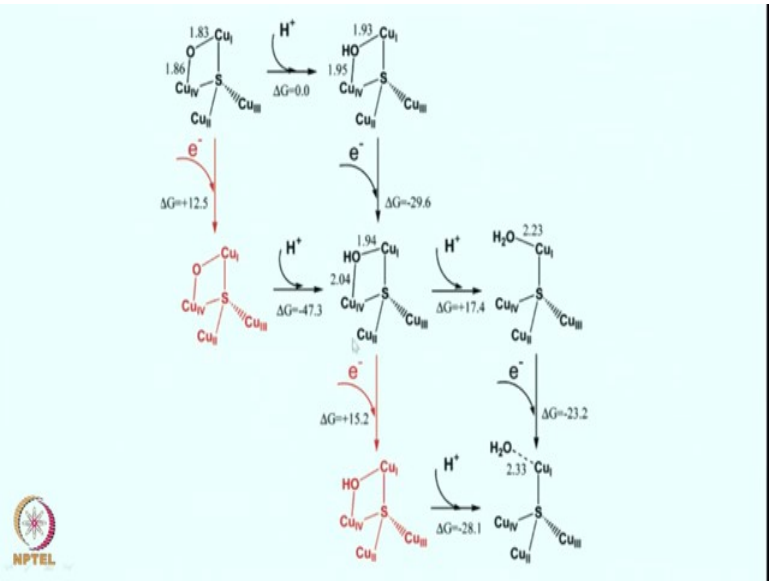

Well this proton and electron steps can be further degraded, which is more convenient which steps occur first as you can see one of these intermediate that we were talking over there can be taken up. So, we are talking about this intermediate. This intermediate putative intermediate can be taken up and a proton transfer first subsequently an electron transfer can be proposed to generate this intermediate hydroxo bound intermediate, subsequently another proton and another electron will give rise to the reduced form of the nitrous oxide reductase.

As you can see that this perhaps is the preferred pathway, the other pathway involved are little more energy demanding perhaps therefore, this ET PT ET PT electron transfer, proton transfer these pathway perhaps is not favourable and likely not to be happening. So, proton transfer, electron transfer, proton transfer, electron transfer perhaps is the pathway that is happening this is what computational studies are suggesting ok.

So, overall as you have seen that nitrous oxide is binding between 2 copper center and the whole process still would require 2 proton and 2 electron, but the catalytic cycle is able to stepwise take up this proton and electron and then, the whole catalytic cycle can be converted, the whole can be completed, the complete catalytic cycle would require regeneration of the catalyst, which can be done as well and nitrous oxide can be reduced to nitrogen which is looking fantastic. But the problem is such for synthetic chemists such a copper g site or 4 copper 1 sulfide structure is quite a dream.

So, this copper sulfide structure has actually excited chemist synthetic chemist biinorganic chemist to look into the copper sulfur compound synthesis and try to see if such a compound, which is found in nitrous oxide reductase can be synthesized in laboratory. Till that, a great synthetic model that that mimics the structural as well as the functional activities of the nitrous oxide reductase is unknown, but studies are on going towards understanding and better representing the mechanism of such reaction.

In order to understand, the copper sulfur chemistry various research groups have invested heavily on this. We will discuss some of the ligand copper complexes that is capable of reacting with sulfur or sulfur analogues to give the copper sulfur containing molecules.

Let us look at some of those efforts which tries to mimic the activity of nitrous oxide reductase. But I must tell you that those mimic has not been that great yet. So, efforts are still on. Let us look at the initial studies or preliminary studies that has been done till date to solve or to understand the copper sulfur chemistry.

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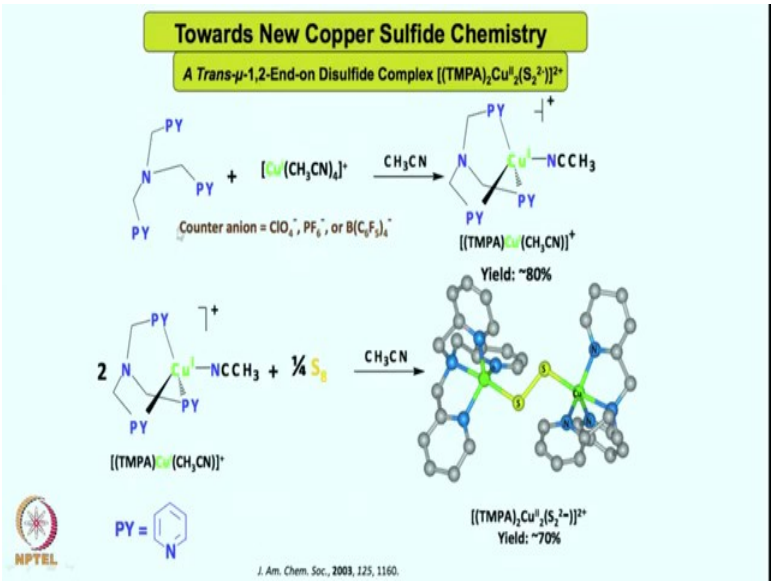

Well, the first slide over here shows that 4 nitrogen centers or this famous TPA, TMPA ligand based will react with copper to give copper I (TMPA) complex, we have seen this earlier in the context of copper oxygen chemistry and copper mononuclear oxygen chemistry, copper dinuclear oxygen chemistry. So, this copper complex can excitingly be reacted with elemental sulfur, which is quite fantastic just like oxygen the sulfur can be reacted and just like oxygen and end on bound disulfide can be generated.

And if you have remember that, if these tetradentate ligands are reacting with oxygen it was forming dicopper peroxo intermediate where oxygen was reduced by 2 electrons. Here, the sulfur moiety is essentially replacing oxygen atoms and then, we are seeing that this sulfur 2 sulfurs are 2-this is copper II + this is copper II + overall this is a very nice compound which gives rise to the similarity between the reactivities of sulfur and the oxygen with this tetradentate ligand system, that is quite exciting to note.

But one of the problem happens during this reaction and that is associated with the fact that this is not a pure compound. This is not the only product that is forming from these reaction.

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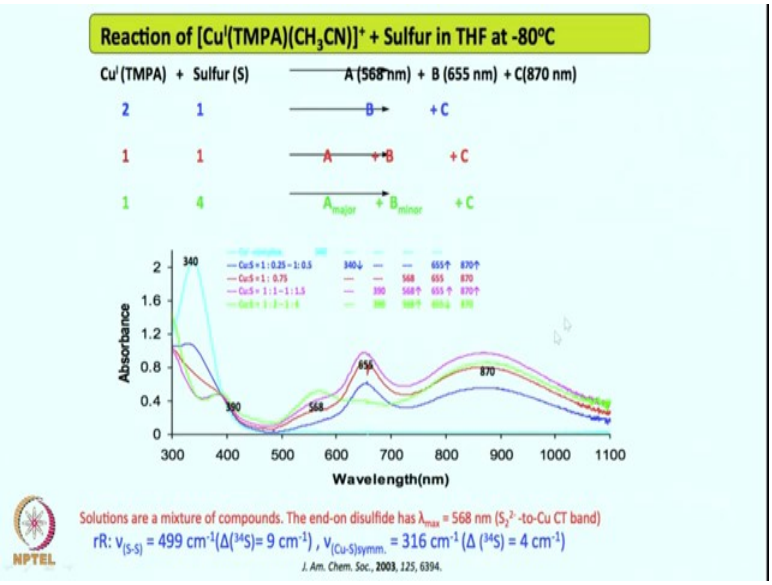

They are indeed at least 2 more product or even perhaps much more than total 3 compounds are present. So, depending on the amount of copper and sulfur added these products ratios are varying. So, this is one product, this is the end on disulfide product. This is the peak associated with that, this is another compound, which remained unidentified till date. This is perhaps another compound which is also remain unknown till date for the nature of these compounds.

So, if you are reacting with this ligand copper I complex unlike the oxygen reaction which can give clearly only 1 product end unbound dicopper di or dicopper peroxo species. In this case the sulfur reaction gives rise to at least 3 compounds, where we are clearly seeing that one of them is this crystallographically characterized in non bound

disulfide complex. So, no matter what has been done to modify this ligand system, to prevent the other product formation or to gives rise to only one product formation, that remains unsuccessful.

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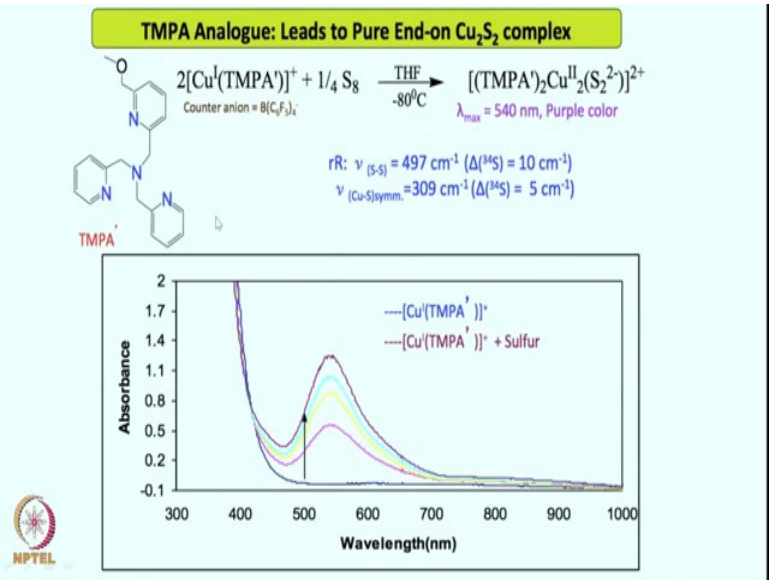

Subsequent studies shows that, if a substituent on this pyridine ring is placed in only one arm not in every arm at only 1 arm at this 6 position, then it is indeed possible to prevent multiple product formation and exclusively form one product that is that end on disulfide compound.

So, this is where the ligand designing and understanding of the ligand becomes extremely crucial. For instance, if these dimethoxy or methylene methoxy units are put on every arm of this pyridine, there is no reaction absolutely with sulfur. If two of them are also substituted two of the pyridines are substituted with any substituent at this position once again 6 position this once again does not react. Any change of further ligand understanding or ligand design will either lead to multiple product formation or no reactivity with elemental sulfur, which is S8.

So, overall the monosubstituted pyridine was essential to give rise to this end on disulfide bound complex, which is having a clear UV-Vis spectra at 540 nanometer and this is purple in colour, this is quite interesting and it gives only one product from the mixture of compounds, from the same compounds or same ligand without this substituent and we can now gave with this substituent we can get a clearly only one

product. That is quite interesting, but this also suggests that the ligand designing has a crucial role to play in preventing or in promoting the desired product that one is looking for. So, the other 2 compounds that was forming from these reactions are till unknown.

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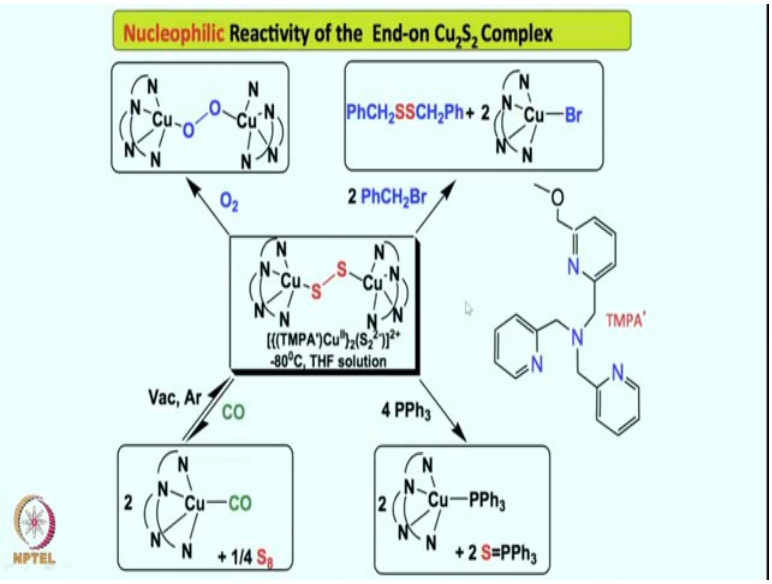

What if this pure compound end-on disulfide compound is reacted with different reagent for example, if one is bubbling oxygen through this it is the end-on di end on peroxo species form indicating that these reactions are reversible in nature indeed. Copper sulfur binding is turning out to be quite reversible, I must note that over here, this is upon addition of more and more sulfur or with respect to time this peaks goes up. But if we warm up this compound it goes back to the copper I. So, this reaction is completely reversible with respect to temperature. So, the cooling down gives rise to this product formation, warming up loses this sulfide unit. So, it is a completely reversible reaction just like what we have seen in the oxygen transport protein.

So, with respect to with the ligand copper complex the oxygen binding is reversible although the peroxo or related compounds are forming over there, but oxygen transport remained completely reversible. Similarly, sulfur binding is completely reversible, which can be also manifested by the fact that the oxygen bubbling gives rise to this species which is an end-on bound crystallographically characterized intermediate. This intermediate is also crystallographically characterized and this is also a definitely crystallographically characterized.

Therefore this also shows that oxygen binding is stronger than the sulfide binding for the cases with the track tetradentate ligand system. If benzyl bromide is reacted with this copper disulfide compound di bibenzyl bisulfide or benzyl disulfide dibenzyl disulfide is formed. This type of product formation indicates that your sulfur is having a negative charge right.

If it is having negative charge then only it can attack on a positively charged carbon center over here and that is how this product perhaps is forming. So, like this end on peroxo this disulfides are nucleophilic in nature. So, this is a nucleophilic center on delta minus charge is there and that is why it is attacking on benzyl bromide.

One can also purge carbon monoxide through this to give the copper I CO complex, this is a copper II copper II this will be a copper I, CO complex this is also copper II and copper II complex. During this benzyl bromide reaction one can expect to get copper II bromide which is also crystallographically characterized. With reaction of triphenylphosphine this copper phosphine complexes are formed along with the formation of triphenyl phosphene sulfide, 4 equivalent of this is used 2 equivalent of copper 2 equivalent of triphenyl phosphene sulfide can be generated from such reactions. That is all about the end on disulfide complex.

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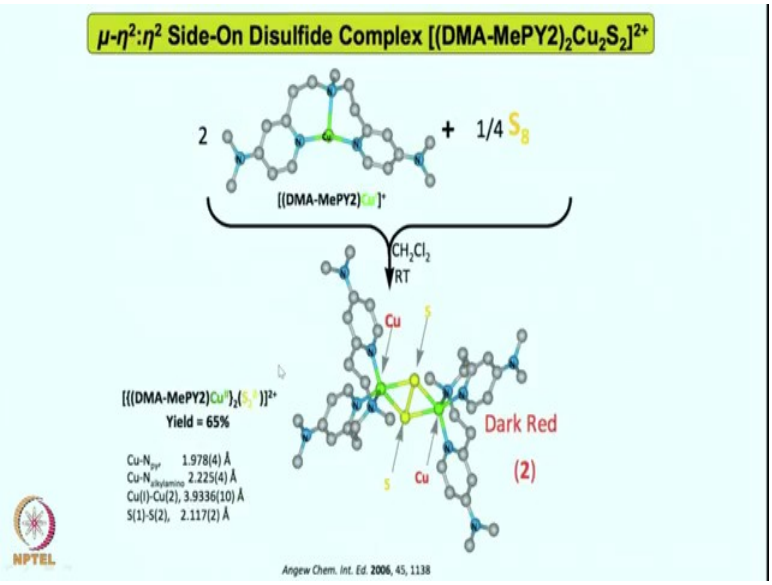

Let us look at what happens, when a tetradentate ligand is reacted with a sulfur that is what we have seen. Now, what happened if a tridentate ligand such as this one dimethyl

aminoMePY2 ligand, this is the ligand crystallographically characterized starting material. If such an ligand metal complex this is the copper, this is a nitrogen pyridine nitrogen, this is pyridine nitrogen this is the other aliphatic nitrogen.

Now, if such a compound is reacted with elemental sulfur as we can see now, that a dicopper disulfide is forming. Now, the bridging mode is side on previously, as you have seen the crystal structure it is end-on in nature, but with a tridentate ligand system it is gives rise to a side-on disulfide just similar to what you have seen for the tridentate ligand and oxygen reactivity. These reactions are occurring at room temperature, it does not require indeed at low temperature reactions are sluggish, these reactions are occurring at room temperature and this gives rise to this diamond beautiful diamond core for the dicopper disulfide complex. You can see the crystallographically characterized intermediate different bond length and this is quite interesting right.

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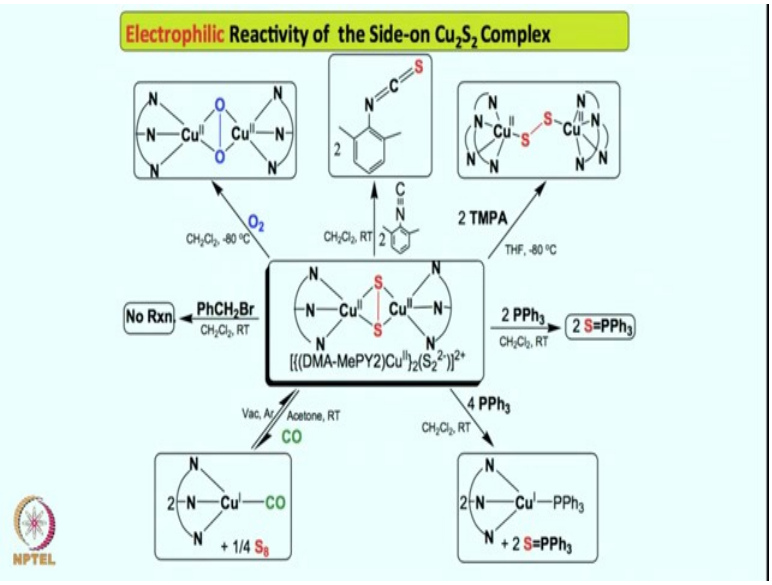

Well, if you try to see the reactivity pattern for this reaction well this does not disappoint you, it is actually turning out to be quite similar to what we have seen for the side-on bound peroxo species ok. So, this is the side-on bound peroxo, if you take the side-on bound disulfide this is side on bound just like this, each of the sulfides are equidistant from the copper, each of these copper right. And this intermediate once reacted with oxygen at low temperature it is capable of providing this side-on bound peroxo species. Once again that reinforces the fact that these compounds are not that stable compared to

this dicopper peroxo species and more importantly this copper sulfur binding are reversible in nature.

One interesting reagent in this case is this is a phenyl isocyanide, if you are reacting with phenyl isocyanide this C- will be able to pick up this sulfur, because the sulfur is positive. So, this is a electrophilic mode of reactivity ok. So, this is a delta+ a nucleophile will be able to pick up this sulfur to give rise to this phenyl isothiocyanate complex. PH and CS compound can be generated from ArNC.

Similarly, for the end on disulfide cases the previous one if you are reacting this one with isocyanide you do not get any reaction ok, that is also quite interesting and quite insightful that these sulfur or these sulfides are nucleophilic in nature and nucleophilic sulfur will not react with another nucleophilic center. Well, it is also possible to exchange the ligand. So, this is a tridentate ligand. tridentate ligand can be exchanged with a tetradentate ligand quite interestingly this side-on bound disulfide now will give end-on bound disulfide, that is I would say quite fascinating, not only oxygen ligand exchange the ligand for the copper can also be exchanged and sulfur can be transferred to isocyanide to give isothiocyanate. Again carbon monoxide can be displacing this sulfur unit just like what we have seen in case of the end-on disulfide.

Phosphene reactions are interesting, if you see only two equivalent of phosphene is added then we will be able to see that then we will be able to see that 2 equivalent of phosphine sulfide is getting generated. If 4 equivalent is reacted the reaction is similar to what we have seen in case of the end on disulfide.

Quite surprising not surprisingly I would say benzyl bromide as you have seen that this reagent reacting with benzyl bromide to give bibenzyl bisulfide, but in this case there is no reaction. Once again, reinforcing the fact that these disulfides are electrophilic quite interestingly as you can see these benzyl bromide reactions and isocyanide reactions are quite clear, that the reactivity pattern for these disulfide end-on disulfide versus side-on disulfides are completely different.

So, side-on disulfides are electrophilic in nature, that is this one this reaction demonstrate that no product formation also demonstrate that, end on disulfide that is this one is nucleophilic in nature. So, there is a delta minus here, delta plus here this delta minus

will react with benzyl bromide and therefore, we will see that bibenzyl Bisulfide will be forming on the other hand these sulfide will not be able to react with isocyanide right.

Overall, this is what settles then that side on disulfides are looking like, electrophilic in nature. And the end on disulfides are nucleophilic in nature there are many other copper sulfur complexes since then has been reported.

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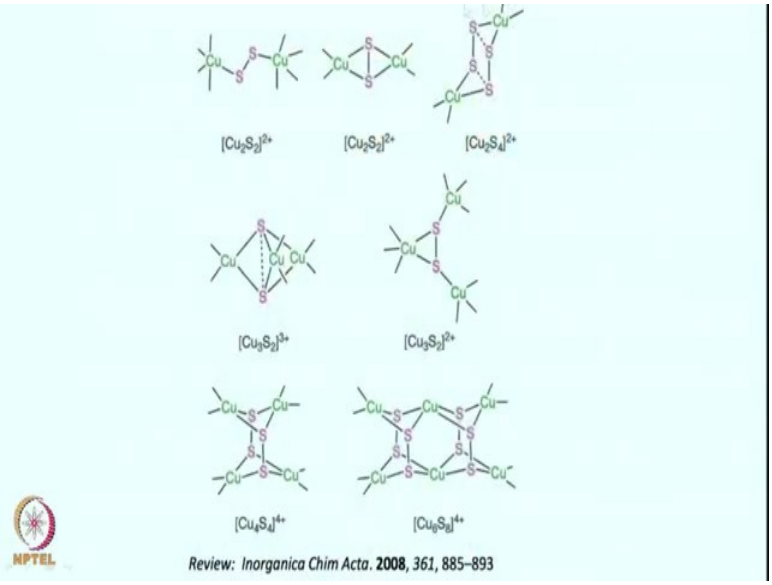

And it is quite interesting to note that, these are the really beautiful type of compound, that one can get, the reactivity pattern and some of their reactivity towards nitrous oxide has also been studied to show that some of these could be useful intermediate to understand the nitrous oxide reductase chemistry. Will not discuss too much about all these different different thing that.

So, these different copper sulfide, copper sulfur chemistry that has been known are also quite interesting, all these compounds gives rise to the new and exciting modes of binding with copper and sulfide. Overall these are the different compounds that has been reported so far much more are coming, but one thing to notice that, none of these actually exactly represent 4 copper 1 sulfur motif ok. These, these compounds remain quite exciting their reactivity patterns are quite important on interesting as well, but perhaps what is most interesting is there is a new twist in this copper G chemistry and that is the questionable ligand that was over there.

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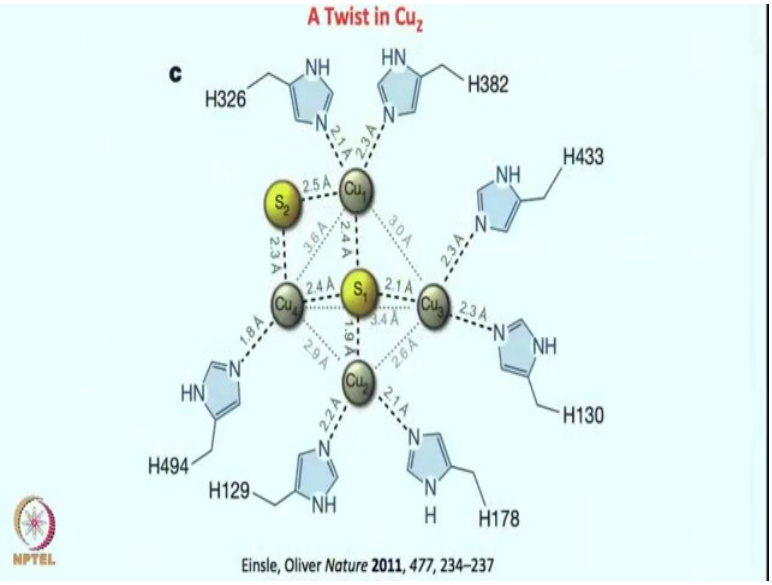

Now, this ligand is turning out to be not what aqua or the hydroxo moiety, but it is a sulfur. So, a second sulfur unit has been identified previously, this was the core structure proposed 4 copper 1 sulfide. Now this new report suggests that, that there is a second sulfide that is present and bridge between copper 1 and copper 4.

So, the mechanistic studies that we have proposed or we have seen previously in the literature, that need to be perhaps reconsider and this now, then settles that this is if you look at this is a dicopper disulfide bridged in one sense and in other sense, that 2 copper centers are also attached. Overall 4 copper disulfide not 4 copper 1 sulfide unit. As you have also clearly seen each of the coppers are having 2 histidine over here and all the way here, but this copper is having one histidine.

So, likely that this is one of the site for the nitrous oxide binding, but the detail mechanism perhaps need to be reconsidered, overall this remains a puzzle in the, synthetic chemistry as well as the understanding of the biological mechanism of this nitrous oxide reductase. With this let us wait for the next class.

So, in this class I hope we are able to discuss in brief the state of the art nitrous oxide reduction chemistry. Nitrous oxide is getting reduced to nitrogen, but the mechanism can be questioned, it is now believed or now clearly shown that it is 4 copper and 2 sulfide, that is involved into the nitrous oxide reductase therefore, the earlier structure is now refined and the mechanism that has been suggested earlier need to be reconsidered.

But most importantly, this structure or this enzyme structure has given rise to the enough interest of synthetic chemists to study the copper sulfide chemistry, which has given many different complexes 2 of those reactivity. We have seen end-on disulfide is nucleophilic in nature and side-on disulfide is electrophilic in nature right and of course, none of these are capable actually to convert nitrous oxide to nitrogen.

So, therefore, much more studies are required, much more rigorous and long term studies are required to mimic and catalyze catalyzed nitrous oxide to nitrogen by a synthetic model complex alright. keep studying, we will see you in the next class.

Thank you.