

**Inorganic Chemistry of Life Principles & Properties**  
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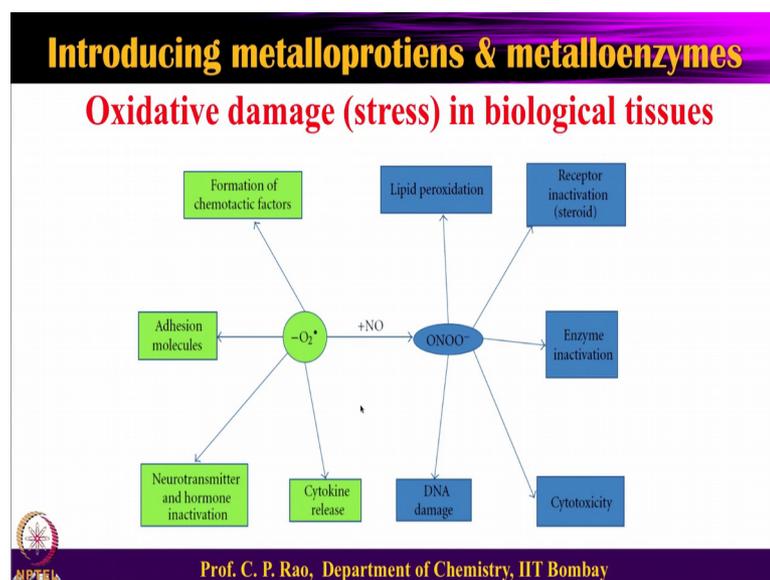
**Lecture - 23**  
**Enzymes based on manganese in life**

Welcome you all to the next class on Inorganic Chemistry of Life. In the previous, class we have been looking at the manganese based enzymes, I said the manganese based enzymes based on 1 manganese, there 2 based on 2 manganese and 3 manganese and towards the end of the class we were trying to look at the corresponding reactions, reactions the superoxide going from superoxide radical, superoxide dismutation of the radical to the hydrogen peroxide and the catalase disproportionate in the hydrogen peroxide to water and oxygen.

And we also looking at the photosystem where the carbon dioxide is converted to the carbohydrate and the water is oxidized to oxygen. And we looked at 2 other reactions as well one is the ribonucleotide reductase; where ribonucleotides deoxyribonucleotides are synthesized from the ribonucleotides by the removal of the OH group from these 2 prime position and replacing it with a hydrogen.

So, that is it and one another example we have looked at was the case of the dioxygenase I talked to you that dioxygen is will be taught to you more detailly under copper, under iron ok.

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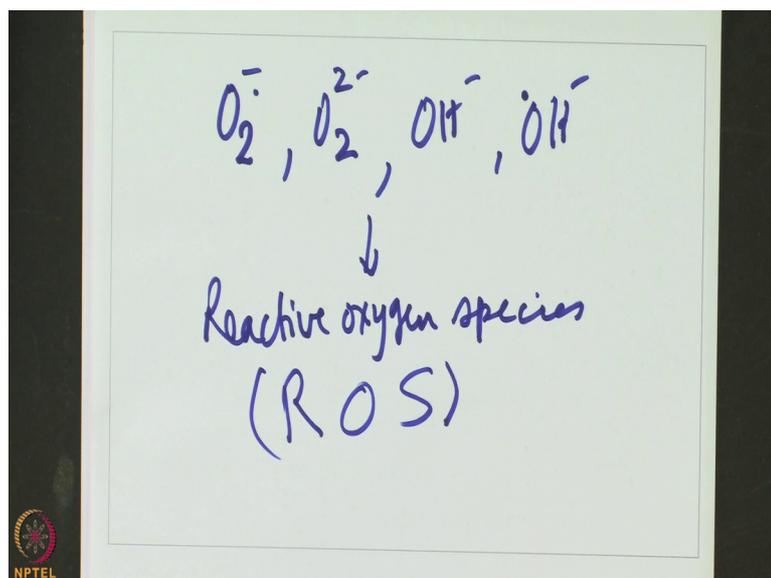


So, let us see in this class; let us look at what is the kind of a problem that one would expect, what kind of a damage aspects one would expect with the with the  $O_2^-$  superoxide radical.

So, I am sure you must have; everyone must have heard something called oxidative stress. So, oxidative stress is nothing, but the formation of oxygen based radicals, oxygen based anionic species which are in turn involved in reacting with the tissue and spoiling the tissue cell or organ and that is what is referred as the oxidative damage. As you can see here  $O_2^-$  which is superoxide radical can combine with a nitrosyl group and can affect the lipids, can affect cells, can affect various kinds of things in the biological system and all this leads to the damage of the biological tissue.

So, therefore, all this is referred as reactive oxygen species; most of the times these are referred as a ROS and some people will talk; call this as ROS. So, I would prefer not to use the word ROS, but I would use the word ROS; Reactive Oxygen Species.

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So, this reactive oxygen species is an important terminology  $O_2$  minus dot  $O_2^{2-}$  minus  $OH$  minus  $OH$  minus or  $OH$  dot. So, all these kinds of things are called the Reactive Oxygen Species which is written as ROS ok.

So, I will be referring to ROS at different stages, so ROS is a reactive oxygen species which cause damage to the tissue cells and tissue; therefore, which is referred in the in the form of oxidative stress.

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## Introducing metalloproteins & metalloenzymes

### Manganese Superoxide Dismutase (Mn-SOD)

- This protein transforms toxic [superoxide](#), a by product of the [mitochondrial electron transport chain](#), into [hydrogen peroxide](#) and diatomic [oxygen](#).
- This function allows SOD to clear mitochondrial [reactive oxygen species](#) (ROS) and, as a result, confer protection against cell death.
- It plays an antiapoptotic role against [oxidative stress](#), ionizing [radiation](#), and [inflammatory cytokines](#).
- It encodes a mitochondrial protein that forms a [homotetramer](#) and binds one manganese ion per subunit.
- This protein binds to the superoxide by products of [oxidative phosphorylation](#) and converts them to [hydrogen peroxide](#) and [diatomic oxygen](#).

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Now let us look at the enzyme which indeed does take care of this particular O<sub>2</sub><sup>-</sup> radicals and try to disproportionate these ones. So, that particular enzyme is called the superoxide dismutase it is referred as superoxide dismutase.

Since it is a manganese containing we call it is a manganese superoxide dismutase of course, this is very vital in the cells because it will; it will protect the cells from the reactive oxygen species and thereby it will protect the cells from the apoptotic cell death; so, it will protect from that. So, that is in other words against oxidative stress ok. So, all these things and this enzyme is encodes the mitochondrial protein and it forms a homotetramer and binds one manganese per subunit there are 4 manganese for 4 for the total protein.

And this protein binds the superoxide of course, by the product of the oxidative phosphorylation and converts the converts the superoxide into the hydrogen peroxide as we have seen already and the dioxygen O<sub>2</sub>. So; that means, partially it is making the harmful O<sub>2</sub><sup>-</sup> to O<sub>2</sub> which is harmless, H<sub>2</sub>O<sub>2</sub> which is partially harmful that we will see in a while.

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**Introducing metalloproteins & metalloenzymes**

**Reactions & Proposed mechanism for Mn-SOD**

**First superoxide ion is oxidized and the second superoxide ion is reduced.  
 In the first step manganese oxidation state is reduced by one and the second step the same is regained.**

$Mn^{III}SOD_{OH} + O_2^- + H^+ \rightarrow Mn^{II}SOD_{H_2O} + O_2$   
 $Mn^{II}SOD_{H_2O} + O_2^- + H^+ \rightarrow Mn^{III}SOD_{OH} + H_2O_2$

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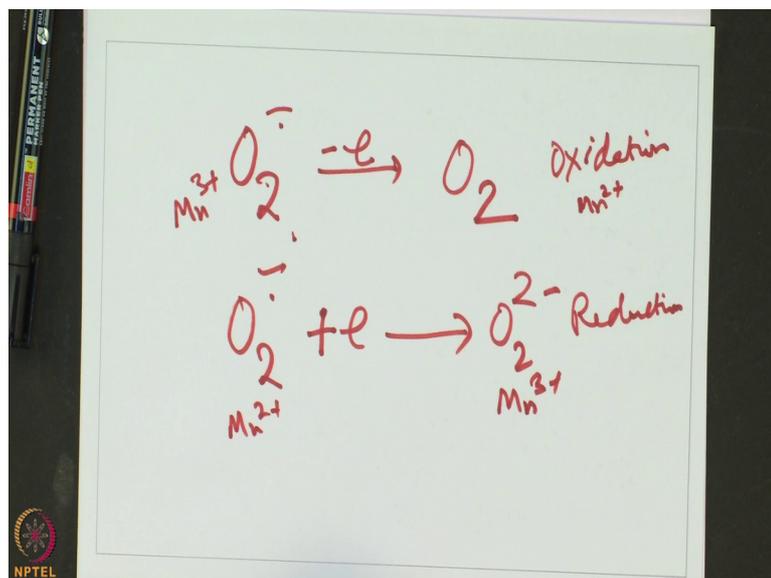
Let us look at the kind of reaction that happens in this particular case and you see that the enzyme is shown over there. So, this is what one of the units of the total tetrameric protein; so, this is called the monomer of the protein.

So, 4 such things will join together and form the total protein and the 4 manganese centers therefore, you have reactivity happening at each station. Now the manganese superoxide is an enzyme and here we are showing one subunit and the manganese is surrounded by these groups which you can see very clearly over there; one histidine, other histidine, then the other histidine oxo or hydroxygen species; which is suspended or connected through the secondary force interactions coming from the protein this is also protein.

So, this is the kind of a situation; so, what will happen when you add to this enzyme 1 mole of a  $O_2^-$ ? So, the manganese in this state as the manganese 3 plus and the manganese 3 plus when the  $O_2^-$  is added and the  $O_2^-$  is oxidized means the electron should be going to the manganese. So, the manganese 3 plus when the electron comes to the manganese center; it becomes manganese 2 plus.

So, manganese 3 plus is reduced,  $O_2^-$  is oxidized all that we would say. So, now this is the  $O_2^-$  which is the oxidized,  $O_2^-$  oxidized goes to will not stay; it will go, escape away. And now; so now you have an enzyme in the manganese II state and this manganese II state enzyme is again is reactive towards the one more or captures one more mole of  $O_2^-$  and this will react with the manganese center and to get the oxidized and get self reduced. When the  $O_2^-$  is reduced, what will happen?  $O_2^-$  is reduced it will become  $O_2^{2-}$ .

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So, in the first case; stage you have  $O_2^-$  and this will give away electron ok. So, therefore, will give  $O_2$ ; in the second step  $O_2^-$  is added and to this you are basically taking the electron to this. So, therefore, and that will give to  $O_2^{2-}$  kind of thing (Refer Time: 07:54). So, you have oxidation part and you have a reduction part.

So, this means the manganese 3 here manganese 3 plus will go to manganese 2 plus and then manganese 2 plus will go to again manganese 3 plus ok. So, it will go to the manganese 3 plus, so the manganese 2 plus will go to this. So, this  $O_2^-$  is oxidized, manganese is reduced,  $O_2^-$  is reduced manganese is oxidized.

All that you have to understand is very nicely, very simply it is a redox reaction; very simply it is a redox reaction nothing more than that no need to worry at all; so very simple to remember understand the whole thing. So, first mole the  $O_2^-$  gets oxidized to  $O_2$  manganese 3 gets reduced to manganese 2 and then second  $O_2^-$  will reduce; that means, reduced form of  $O_2^-$  is  $O_2^{2-}$  which is peroxo kind of species and this will improve the proton will go as a hydrogen peroxide and go back to the manganese III.

So, the same thing is shown over there nothing more; manganese III SOD,  $O_2^-$  plus H plus manganese 2 SOD and this converts to water and oxygen is last. Now, manganese II form of SOD with one more mole of the  $O_2^-$  will go to the manganese III  $O_2$  ok. So, first superoxide ion is oxidized and the second superoxide ion is reduced; in the first step the manganese oxidation state is reduced by 1 and then second state the it is regained.

So, I hope you understand the whole thing; now so, in this particular enzyme what have you created? You created or generated  $O_2$  plus  $H_2O_2$ . So, so  $O_2$  is harmless  $H_2O_2$  is there, this has to be taken care.

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## Introducing metalloproteins & metalloenzymes

### Manganese catalase

Catalase is a common **enzyme** found in nearly all living organisms exposed to oxygen (such as **bacteria**, plants, and animals)

It **catalyzes** the decomposition of **hydrogen peroxide** to **water** and **oxygen**

It is a very important enzyme in protecting the cell from **oxidative damage** by **reactive oxygen species** (ROS)

Catalase has one of the highest **turnover numbers** of all enzymes; one catalase molecule can convert millions of hydrogen peroxide molecules to water and oxygen each second



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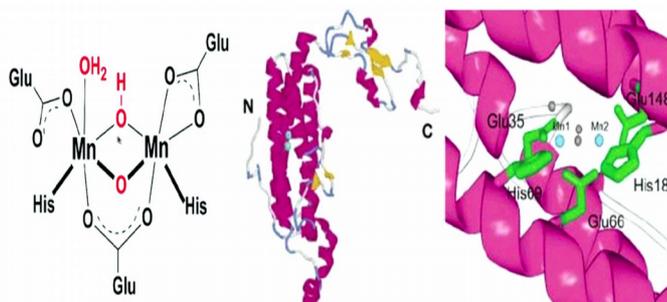
And this is taken care by an enzyme called catalase; enzyme since it is manganese is present, manganese catalase enzyme; since this is also saves the cells from dying. So, it is also oxidative stress reliever works ROS reactive oxygen species. And if you look at the catalyzes of this one; has a huge amount of turnover is done by these enzymes and so as you can see that it converts and finally, it convert to the one the water and the O<sub>2</sub>.

So, they both are harmless ok. So, let us look at the reactive center present in the manganese catalase.

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## Introducing metalloproteins & metalloenzymes

### Active site of manganese catalase



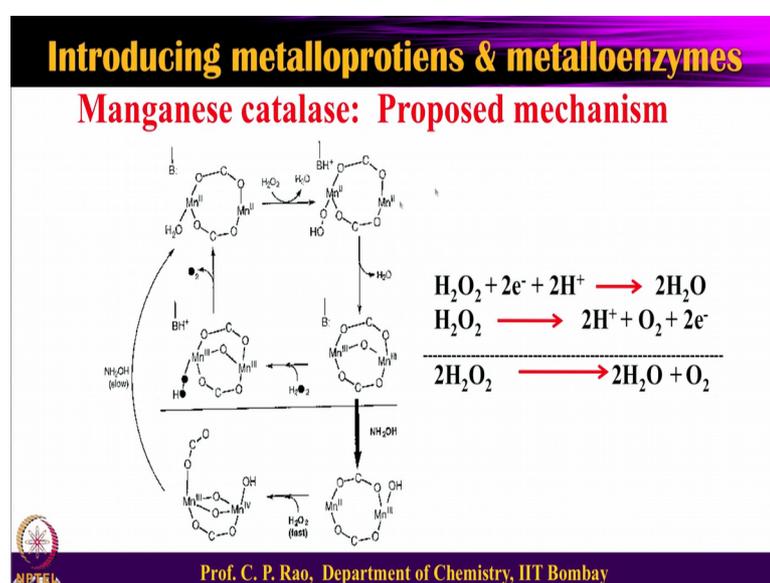
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Manganese catalase has a slightly different kind of a center than what the manganese superoxide dismutase has got. Manganese superoxide dismutase has got is a monomial manganese center, manganese catalase has got dimanganese center.

Now, look at the dimanganese center; the protein is shown over here this is one of the subunits of this and if you just focus a bit on this side, you can see one manganese ion here another manganese ion here; is labeled as manganese 1, labeled as manganese 2 and surrounded by some groups which are directly bonded, some groups which are is directly bonded and that is what you can see here. You can see here one of the histidine is bonded and the glutamic is bonded; one of the histidine is bonded one of the glutamic is bonded also is connecting these 2 manganese is a bridge to glutamic.

So, it is a di nuclear a perfect bridge di nuclear a complex as you can see that at one stage it has a bridging of this OH as well during the reactivity of this ok. So, this you understand the active site; the metal site we are studying the biological inorganic chemistry and inorganic chemistry of life. So, we are interested at the metal center and therefore, we are looking at the things happening at the metal center.

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Now, let me draw your attention to the following aspect of it ok. So, what is this enzyme? The manganese catalase and you have the 2 manganese centers here and the manganese II and manganese II and you have a the bridging etcetera. And this will take

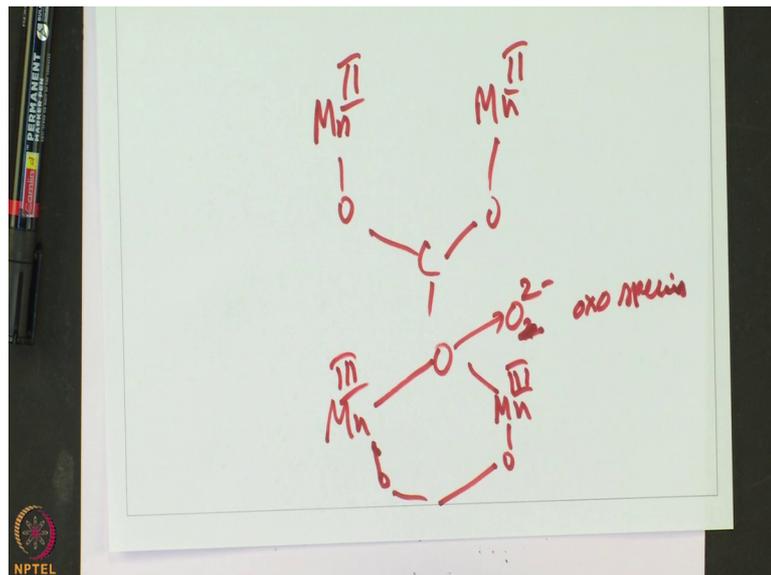
up the hydrogen peroxide and there is a water present water will be displaced nothing to worry.

So, hydrogen peroxide is forming into this and remove this water will lead to the manganese center to be oxidized. You see that the manganese center is oxidized, manganese center is II plus II, plus II will go to the manganese center in the plus a; this B is the one which is the base which is nothing, but a carboxylic group in the protein side chain of the one of the protein moiety is; when it takes of the proton becomes a BH plus and that is what is says. So, that is called general base reaction and that proton is given away and that will go into the water.

So, therefore, you have the and this stage where the manganese III manganese III dimanganese III, like in the previous case it was manganese III to manganese II, here it is manganese II to manganese III. It is a reverse kind of a reaction, this manganese III now manganese dimanganese III will take up another  $H_2O_2$  molecule and this  $H_2O_2$  molecule it is again interacting with this and that leads to the removal of the  $O_2$  moiety, out of this and then leaves the water molecule in this.

And you see that there is a simple carboxylate bridge here, but when it comes to the dimanganese 3; it demands O bridge that is called  $O^{2-}$  bridge.

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So, it is the manganese II the manganese is manganese II to manganese II, you have only carboxylate bridge, but when it goes to the manganese III to manganese III, so you have one that is ok and this is what is what we remove is O<sub>2</sub> minus.

So, this is oxo species; this oxo species comes when we have a manganese III kind of thing. And that is what you see in the structure over here this is a bridging and this bridging will again fall down and this goes as a water, as you can see in this little performance or O<sub>2</sub> is. So, the first case in the first step the water is coming out; in the second step O<sub>2</sub> is coming out.

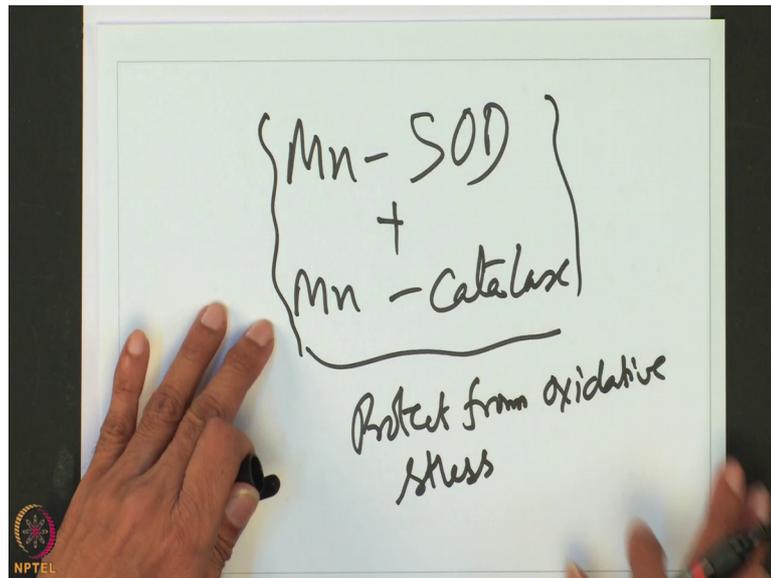
So, therefore, H<sub>2</sub>O<sub>2</sub> is converted into the water and the O<sub>2</sub>, you can see the reaction here H<sub>2</sub>O<sub>2</sub> plus 2 electrons plus 2 protons giving to H<sub>2</sub>O O H<sub>2</sub>O<sub>2</sub> giving 2 H plus O<sub>2</sub> plus 2 e. The total reaction is 2 H<sub>2</sub>O<sub>2</sub> go into water and oxygen and this is what thing is what is happening understand that. So, this process is reduction process and this process is an oxidation process.

So, in the previous case the first step is oxidation; second step is reduction, with respect to the metal point of view first step is the reduction; second step is oxidation here it is the other way round. So, it goes from the II to III and III to II there are III to II and II to III; so, that is the kind of differences. So, these are some extra you know reactions in presence of the ammonia or ammonium hydroxide, you can try to trap this particular species and you can take out and H<sub>2</sub>O<sub>2</sub> and you can get the bridging on to.

So, this part of the cycle is not very important if you understand this 4 things is mostly good enough ok; going dimanganese II to dimanganese the peroxo bridging to the dimanganese III and then losing out the water and the next hydrogen peroxide binding will take back to the manganese II and the O<sub>2</sub> kind of thing.

So, now you understand together when you add the first part is the superoxide is dismutase to the; to the O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> by the catalase the H<sub>2</sub>O<sub>2</sub> is dismutase it to the water and O<sub>2</sub>.

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So, therefore enzymes of manganese SOD plus manganese catalase. So, will protect from oxidative stress is together. So, these this is an important aspect that one need to understand in this ok. Let us go to the next enzyme the last one in the manganese series is the bacterial photosynthetic apparatus.

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**Introducing metalloproteins & metalloenzymes**

**Bacterial Photosynthetic Apparatus**

**Green Plant Photosynthesis**

<http://employees.csbsju.edu/hjakubowski/classes/ch331/oxphos/olphotosynthesis.html>

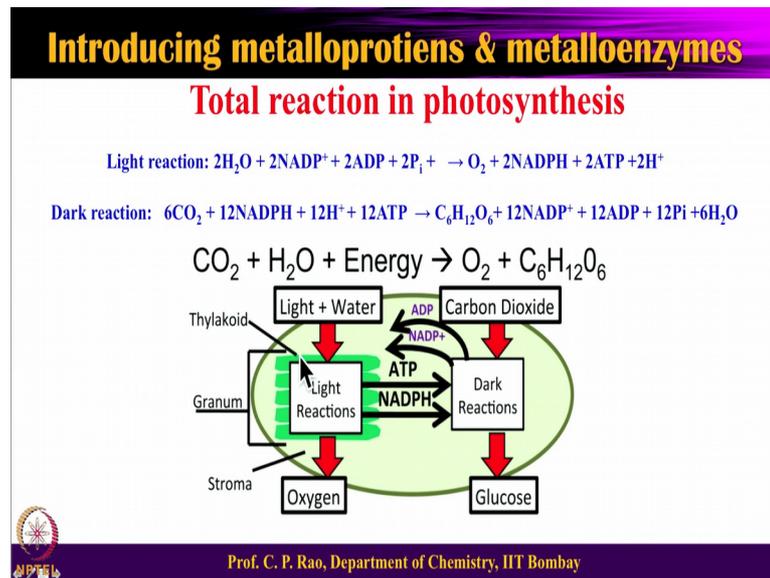
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It is a very huge one and I will show you on the next slide the reactions etcetera. All that we are interested in this is the (Refer Time: 17:19) as an inorganic chemist. So, we are

interested in this species where this particular region where the oxygen is being evolved.

And that is where is shown over there the water going to O<sub>2</sub> and this is where you have the manganese cluster and this is where comes under the photosystem 2. Rest of things are not important for biological point of a where, what kind of proteins are there and this is a membrane etcetera; so, green plant photosynthesis.

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Let us look at the kind of reactions that happen and you know very well in the photosystem; there are 2 parts of the reaction, one is the light driven reaction, other is the dark reaction.

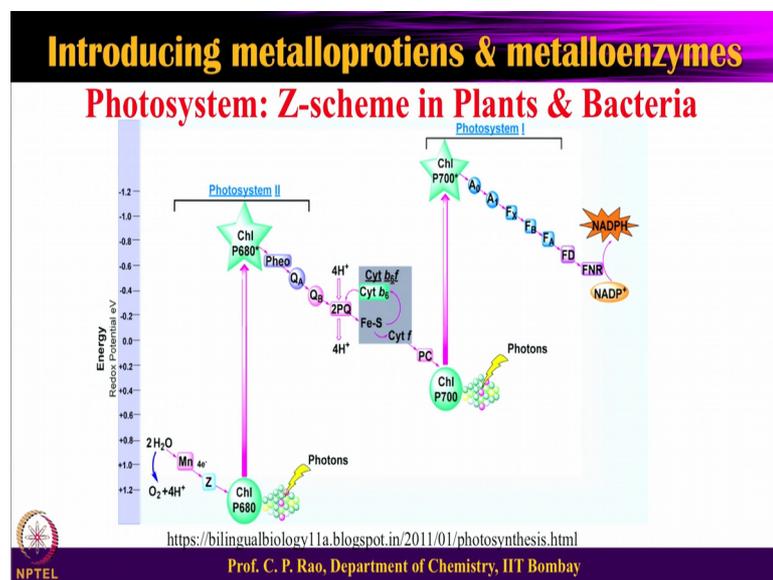
So; that means, there is a light and there is a lapse dark; there is a light, there is a lapse which is a dark. So, light and dark stimuli in the light what is happens is that the light is necessary to oxidize the water to the O<sub>2</sub>; of course, NADP and of course, in the process that ATP synthesis takes place.

So, the NADP gets reduced and water gets oxidized to O<sub>2</sub>; the second part is the dark part which we are not going to be concerned in this particular biological inorganic chemistry, but just to see the reaction; the CO<sub>2</sub> is converted to the C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> which is carbohydrate therefore, you have a reduction occurring in this.

And so, this whole thing is by the reduced, so NADPH 12 protons the 2 electron reduced. So, therefore, you have the 12 ATPs is converted; so you have a lot of ATP consumed in the second reaction ATP synthesized in the first reaction, but; however, we are concerned with the first reaction. The same thing is shown over here; there is a light driven reaction and the dark driven reaction.

So, the light reaction the light plus water is converted to oxygen and then carbon dioxide is converted into the glucose ok.

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So, let us look at how this particular thing is seen in this enzyme; enzyme has got 2 parts one is photosystem II and other is photosystem I, we are not at all involved in explaining the photosystem I, but we are involved in explaining the photosystem II.

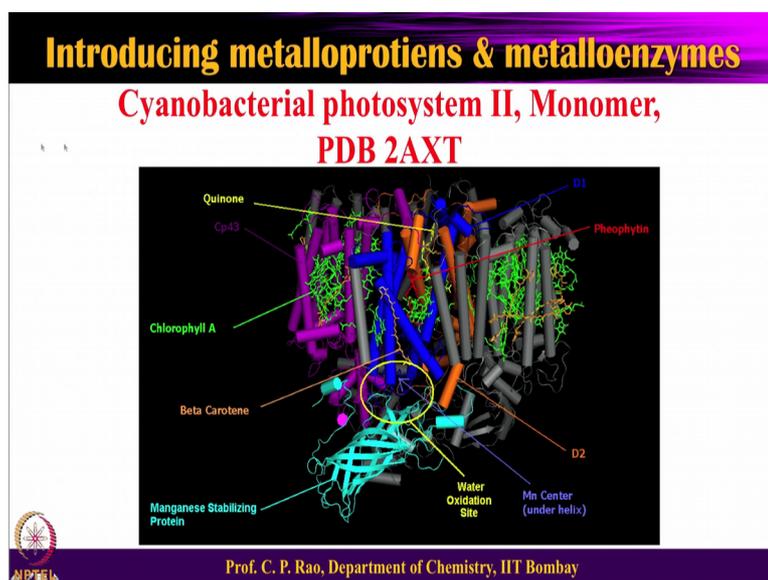
And that to in the photosystem II at this stage you have the manganese cluster, we will be looking at this as an important event. You see there are different reactions happening I will explain you first look at this particular thing in form of energy, given in the form of electron volts, you can see is also can be converted into the into the potential.

So, if you look at this particular center is the one which is involved in the oxidation of the water. So, when you oxidize the water; obviously, you are releasing the electrons and these electrons are carried over here and this is the antenna where it can collect the photons and that will take the excited state and that this excited state the positive charge

and the negatively charge electron will separate and this separated electron will pass through all of these.

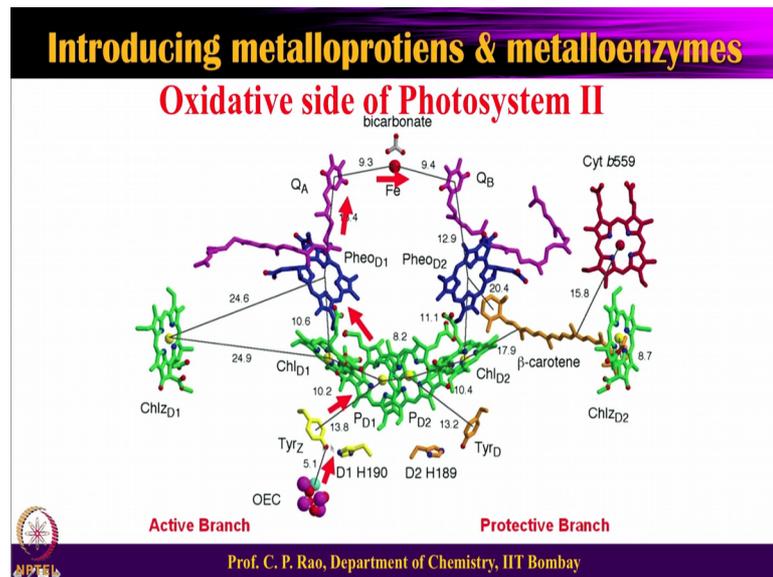
And this is called a funnel and these are different proteins which is not a part of this story now, there are different iron electron transport proteins. I will be explaining electron transport proteins when I come to the iron story and when I come to the copper story also and this will go here and this part will go into the photosystem I.

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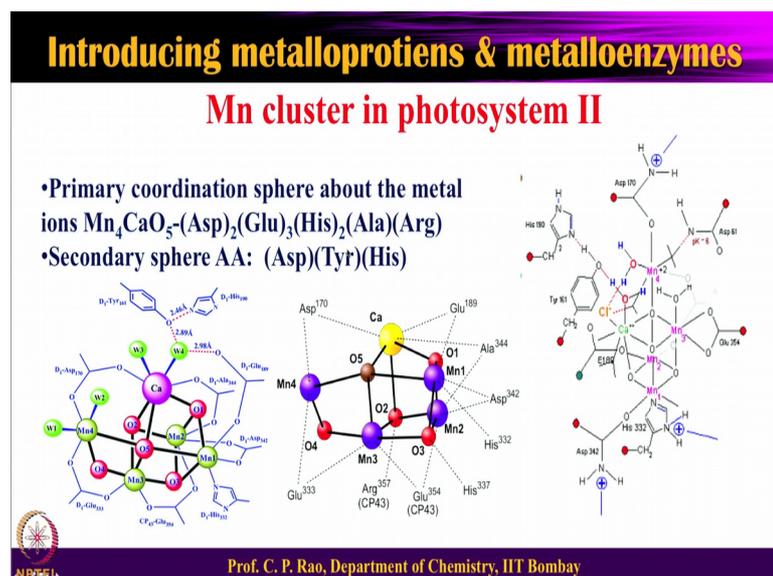
So, let us focus at this particular thing how what happens in this? So, before going to that just not to frighten you with this is the kind of a photosystem cyanobacterial photosystem II huge protein, we are interested in this particular region where circled; where the tetranuclear manganese cluster is involved. So, that is where the water oxidation happens and that is what we are going to look at in the thing.

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Now, so I talked to you that when the light impinged on the source and the oxidation of the water occurring; releasing the electrons, the electrons will go through this kind of a funnel. So, you can see these arrows are showing the direction of these ones and all these groups collect the light and there light harvesting. So, you have a coupled light harvesting plus the electron transporting and beyond this it will go to the to the photosystem I, we are not interested in that and that is how the electron transport.

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Now, let us see very close to the manganese center; as I told you earlier is a manganese tetranuclear cluster, is a tetranuclear manganese cluster along with calcium. Now there are 5 ions, you can see here the purple ones is manganese 1, manganese 2, manganese 3 and the manganese 4 and calcium. So, manganese 1 2 3 and calcium form a kind of a distorted cubane structure where the manganese 4 is protruding outside.

So, you can sit leisurely and go through the; what are the connections with the protein is on we shown here, what are the secondary interactions are also here. So, this dot dot dot lines are shown through the secondary interactions. So, all these species the manganese is stabilized by the coordinations as well as the oxo species are stabilized by the all the red ones are the oxo species; by lot of secondary interactions which are shown by the dot dot dot kind of thing.

So, you by looking at this you will get; so, the important aspect in this is the role of this manganese cluster. In fact, the manganese 1 2 3 is not directly involved in oxidizing the water to O<sub>2</sub>, but it is the manganese 4 and the calcium these are involved, but all other manganese are important because there is a lot of electron redox processes happening.

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**Photosystem II: Oxygen evolving complex**

$2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$

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And there the manganese 1 2 3 will play a role. So, you can also see how this particular light harvesting system and this is where actually the manganese 4 cluster and then water converting into this. So, we are not interested in the rest of the part, as you can see here

the cluster the cubane and the manganese outside converting. So, therefore, it is this manganese and the calcium which are important in this.

Now, look at the kind of a steps that we have; as I told you it is a light and dark and light and dark and light and dark. So, the original state of the enzyme is called S 0 and after one pulse of light it is called S 1, next pulse of light is called S 2, next pulse of light S 3, next pulse of light S 4 and then go back to this.

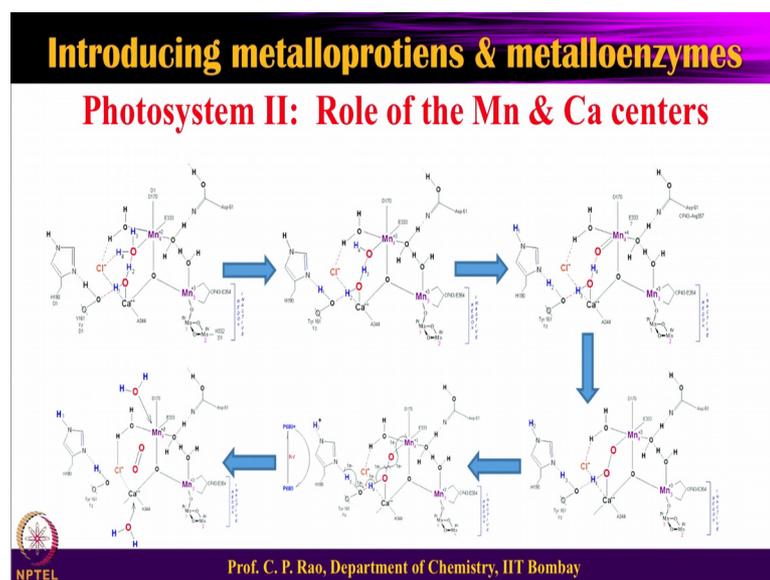
So, as a result of this; what are you seeing here? Manganese oxidation states are changing. So, what is in each case? 1 electron is coming out, 1 proton; 1 electron, 1 electron, 1 proton, 1 electron, 1 proton; you count all of these 4 electrons and 4 protons you can see and force there is a water entry into this and that is. So, the total reaction is 2 waters converting into O<sub>2</sub> plus 4 H plus 4 electron and that is what is done by these ones, but actual water binding and conversion into O<sub>2</sub> is done between the calcium and one of the fourth manganese; not with other things, but all other manganese are also absolutely important.

So, III of the manganese III plus I manganese IV plus what it means is and then one oxidation taken place therefore, 2 3 2 or in 3 plus 2 or in 4 plus and then one more electron. So, 1 3 plus 3 of the 4 plus and at this stage either all 4 plus not very clear 1 3 plus 3 or the or 4 plus, but there could be a radical also of the underling inside you do not know exactly.

So, the well understood regions are S 0, S 1 and S 2; in fact, the other states are not. In fact, in the literature if you go and see; a lot of small molecular complexes are already being synthesized and studied very well for S 0 system, S 1 system, S 2 with a tetranuclear manganese complex is not known, but in this course I am not taking up all those details in this.

So we understand that totally all this 8 electron, 4 electrons and 4 protons happening through these oxidation states.

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So, as I told you one of important aspect in this is that the all the 4 the manganese ions are not directly involved with the O<sub>2</sub> or water, but all the manganese ions are important in the electron transfer, as you have seen in the previous the cycle that is shown there.

But the only one of the manganese and the calcium center are involved in water activation and this is further supported by one of the anion support; this anion will be favoring this hydrogen bonding system and favors. So, the water binds from the manganese and from the calcium center and the conversion of this water into the manganese oxo species and that is where the oxidative state occurs.

And this oxidized manganese IV is reactive therefore, it reacts with one of the neighboring waters and for the O-O bond, which you can call this as a peroxo bond kind of a equal into peroxo bond. And this peroxo bond is further activated by breaking up and to give the O<sub>2</sub>. So, there are other kinds of mechanisms in the literature; we are not taking up, this does not mean other mechanisms do not occur why other mechanisms are not there?

Because we do not know as I told you in the previous slide; the S<sub>3</sub> state is not well understood and S<sub>4</sub> state of course is not understood because this goes very fast. S<sub>0</sub> is very well known, S<sub>1</sub> is very well known, S<sub>2</sub> is very well known therefore, there could be more than one different kind of a mechanism that is the reason why we are saying all this.

So, though there are 4 manganese it is only 1 manganese ion which is directly involved in binding to oxygen in converting the sorry binding to water and converting into O peroxide and then to O<sub>2</sub> ok. All other manganese are important for in the electron transfer funnel in this thing. So, so overall what we have seen?

Overall what we have seen in the manganese story; manganese has large number of oxidation states, but we see that the important things are 2 plus, 3 plus, 4 plus very rarely maybe 5 plus not so, much so, the 5 plus and beyond under 2 oxidative in nature and below 2 plus are to reduce if in nature therefore, these are not; there are variety of oxidative redox kind of enzymes are there.

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**Introducing metalloproteins & metalloenzymes**

**Conclusions**

- Manganese has been involved in number of enzymes like SOD, catalase, dioxygenase and in photosystemII
- Mn in SOD enzyme decompose O<sub>2</sub><sup>•-</sup> (can cause oxidative stress) to O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> and itself shuffles between Mn(II) and Mn(III)
- Enzyme catalase catalyze the decomposition of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub>
- In photosystemII four Manganese are present and those are interacting with the calcium through oxygen linkage.

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But I have tried to give you only a few of those examples and the ribonucleotide reductase, the superoxide dismutase the catalase, the photosystem and dioxygenase of which 3 cases I have explained the mechanism that is superoxide dismutase which converts the superoxide into the into the hydrogen peroxide plus O<sub>2</sub>. And this is further taken up by this hydrogen peroxide is further taken up by catalase and catalase is a dinuclear manganese system and this will convert the H<sub>2</sub>O<sub>2</sub> into O<sub>2</sub> and plus H<sub>2</sub>O.

So, overall SOD and catalase together will save the cells, will save the tissue, will save the body from the oxidative stress or reactive oxygen species ROS. So, therefore it is important I have explained that; the mechanism in one case is going from 3 to oxidation

3 plus to 2 plus in the other case and reversal, in the other case 2 plus to 3 plus and the reversal; the one is mononuclear other is dineutral.

Then the other enzyme that I have explained you is a not an enzyme; a part of the enzyme in the photosystem II, where the tetranuclear ion manganese cluster is involved.

Though the tetranuclear manganese cluster is involved; it is associate with another calcium and calcium plus 3 manganese will form a distorted cubane structure and the fourth oxygen which is standing outside or sitting outside is one which is important in that binding to water; converting to manganese oxo species, converting the this into the O<sub>2</sub> peroxy species; then further converting into O<sub>2</sub>.

So, but all the other 3 of manganese are also important; as you can see in the manganese are going through the 3 plus 2 4 plus and etcetera that we have seen through S<sub>0</sub> to S<sub>1</sub> to S<sub>2</sub> to S<sub>3</sub> to S<sub>4</sub> back to S<sub>0</sub> and I told you S<sub>1</sub>, S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub> are very well understood S<sub>3</sub> and S<sub>4</sub> is not much understood; S<sub>1</sub>, S<sub>0</sub>, S<sub>1</sub>, S<sub>2</sub> cases; there are a large number of moral complexes are built. And I think we will probably stop the story of manganese at this stage after explaining these 3 enzyme mechanisms. Then we will go into the next class in the iron enzymes.

Thank you very much.