Bioinorganic Chemistry
Prof. S. P. Rath
Department of Chemistry
Indian Institute of Technology, Kanpur

Lecture – 04
Metals in Biology: Nature's Selection [Part-3 of 4]

So, we have been discussing the role of Metals in Biology. In the previous lecture, we focused on the concentration range on which the metal ions are most active.

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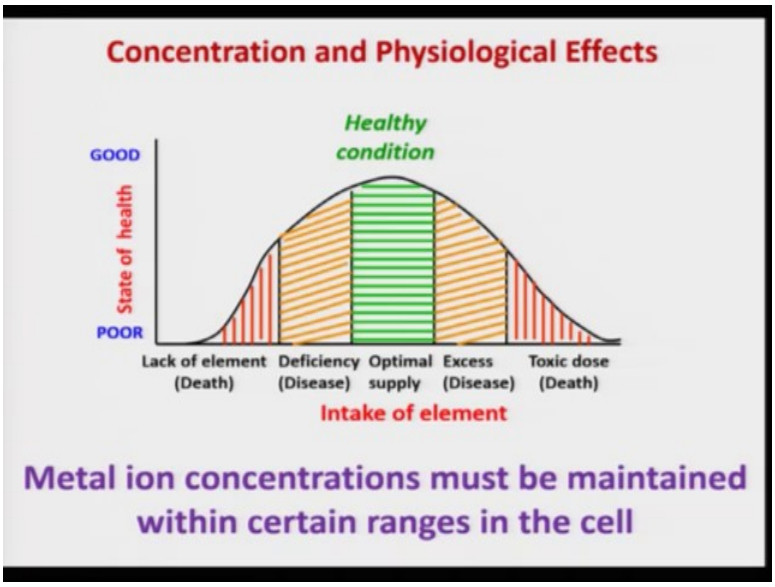

So, metal ion concentration must be maintained within certain range in a cell. If you have more or less concentration both will causes diseases and eventually it can causes death.

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Now, here I have shown some of these effects of metal deficiency in humans. As you can see that it creates lots of problem.

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And if you have these problems you have to go to the doctor, and doctor will then prescribe lots of medicine to supplement those metal deficiency.

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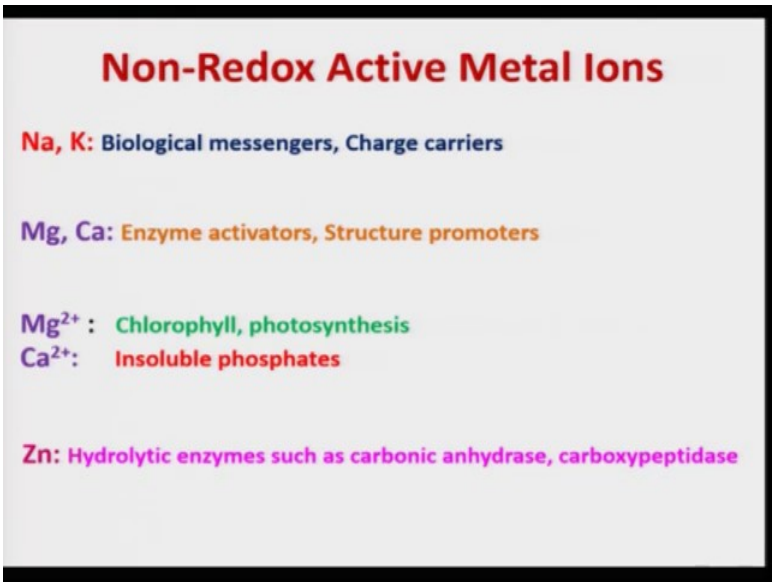

Now, we also have discussed in my last lecture about that non-redox active metal ions and their role in the biology. We now discuss in this lecture the utilization of redox active metal ions.

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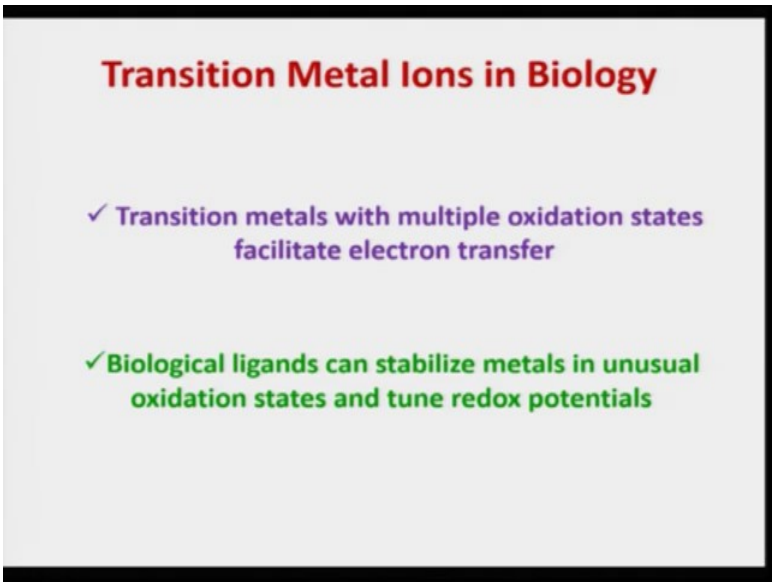

Now, transition metal ions in biology. What transition metals are doing? Transition metals with multiple oxidation states facilitates electron transfer. Biological ligands can stabilize metals in unusual oxidation states and tune their redox potential.

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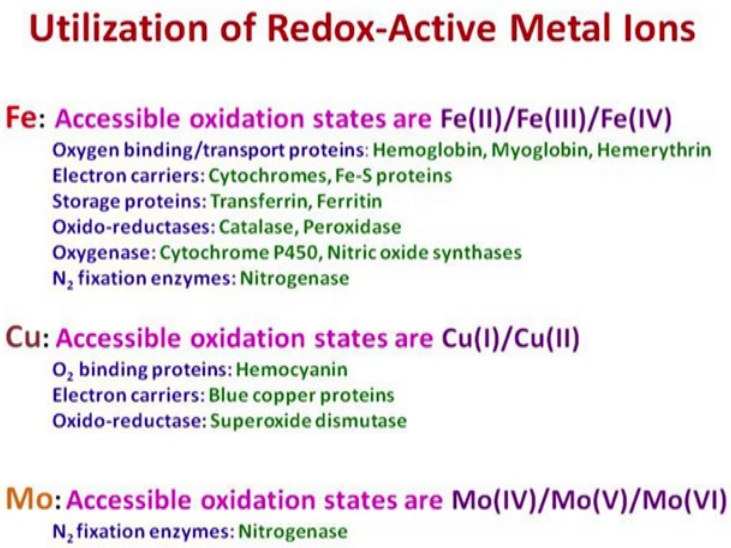

Now, some of these transition elements are shown over here along with their utilization. For example, iron accessible oxygen states are Fe(II), Fe(III) or Fe(IV), and it found in large number of proteins, enzymes starting from oxygen binding and transport protein hemoglobin, myoglobin, hemerythrin. Storage proteins like transferrin, ferritin. Oxido-reductase: catalase, peroxidase. Oxygenase: Cytochrome P450. Nitrogen fixing enzymes: nitrogenase. So, lots of enzymes and proteins are having this iron, where - they use different stable oxidation states during their catalytic process.

Similarly, copper; copper accessible oxygen state in biological system is Cu(I) and Cu(II), and they are also found in large number of proteins and enzymes such as hemocyanin, blue copper protein, superoxide dismutase. Molybdenum, accessible oxygen states are Mo(IV)/Mo(V)/Mo(VI) and found in nitrogenase, where the aerial nitrogen fix. We will discuss in more details thing.

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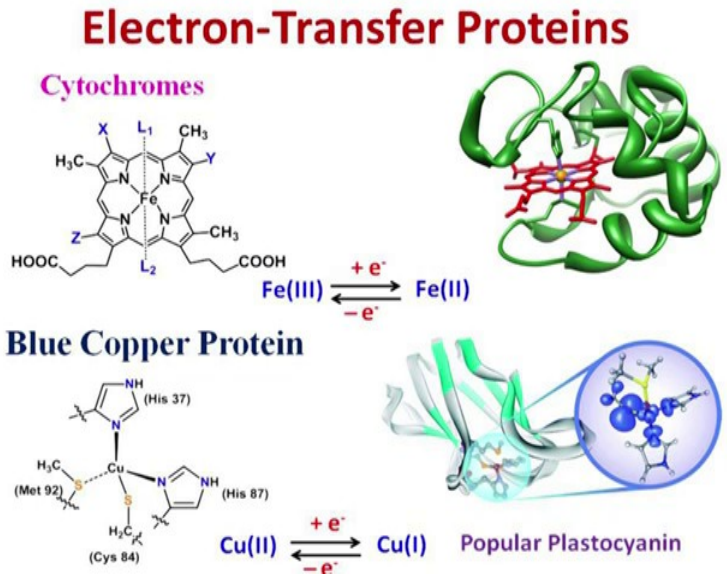

Some of these electron transfer proteins, I have displayed over here. For example, cytochromes you can see that is a Heme protein with two different axial ligand, but it is responsible for electron transfer like Fe(III) convert to Fe(II) and Fe(II) convert to Fe(III). Blue copper protein is a copper containing protein, which is responsible for electron transfer like Cu(II) converting to Cu(I) and Cu(I) releases electron converted to Cu(II).

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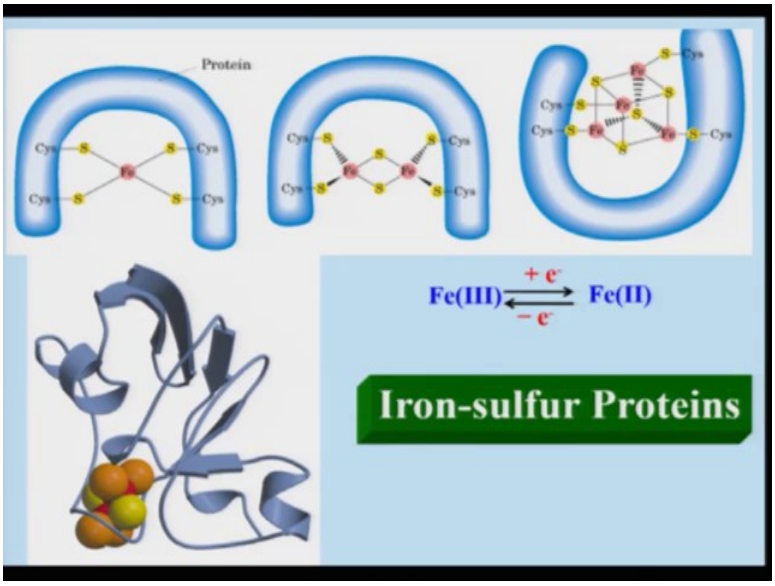

There are also many iron sulfur clusters or iron sulfur proteins are known in biology as some of them are displayed over here, one iron center, two iron center, three iron center, even four iron centers are also present.

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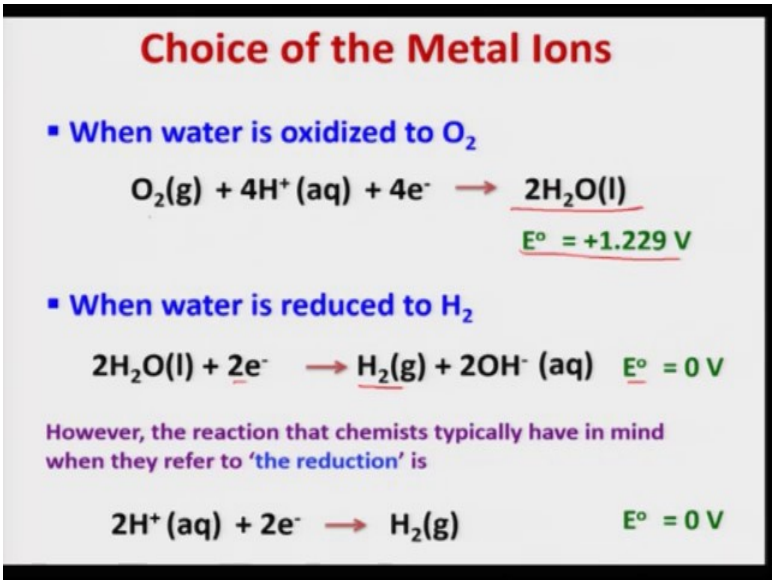


Now, question is that how nature has selected some of these metal ions in its active site for its redox activity? Now, in order to understand that, we need to know that what kind of metal ion should be chosen in the biological system.

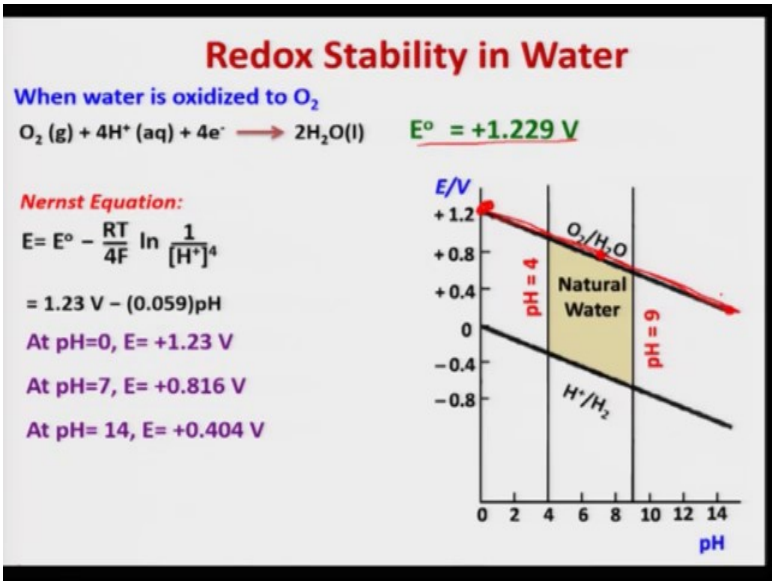
As you all know in biology we have water as a medium, so any redox couple can be used which does not oxidized water to oxygen. And the potential which is required to oxidize water is +1.229 Volt. The reaction is shown over here:

with a redox potential of +1.229 Volt, and if any redox couple have potential more than that it will oxidized water to oxygen. We will discuss it soon.

Now, also this water should not be reduced to hydrogen. So, then the system cannot survive. For example, as you can see that this water with two electron gives rise to hydrogen gas with a E^0 values of 0 Volt. Now, the reaction that chemists typically have in mind when they refer to the reduction is:

$$2H^+(aq) + 2e^- \rightarrow H_2(g)$$

So, any redox couple which can reduce water to hydrogen is not suitable in the biological system. (Refer Slide Time: 06:39)



Now, let us look at little bit carefully on the water oxidation process. Like, oxygen you know this is:

$$O_2(g) + 4H^+(aq) + 4e^- \rightarrow 2H_2O(l)$$

Its E^0 value of 1.229 Volt. Now, if you use Nernst equation, you can relate this potential with the pH, and as you can see that once you change this pH, you can also vary the formal potential of this couple. And this is indeed is happening at pH-0, the potential is +1.23 over here, around pH-7, it is around 0.816 and at pH-14 this is around +0.404, and you can have a straight line like this. This is the barrier about this water is supposed to oxidized to oxygen.

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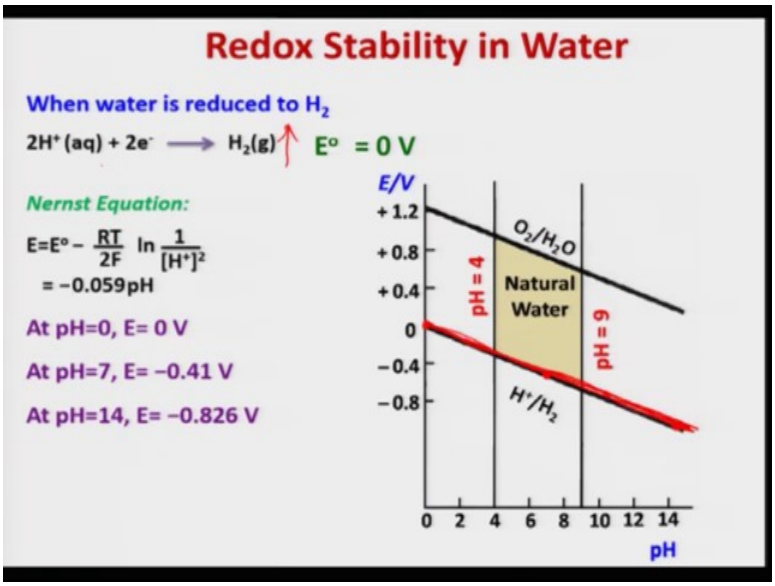

Now, similarly let us look at the reduction of water. Now,

$$2H^+(aq) + 2e^- \rightarrow H_2(g)$$

And E^0 is 0 Volt, and if we correlate it with the pH, the redox potential will be E=-0.059 multiplied by pH. And once you vary your pH, you can also change your redox potential. For example, at pH-0 the potential is 0, at pH-7 the potential is -0.41 Volt, which is nearly here and at pH-14 it is -0.826 Volt. It is like decent.

Again, if I connect this three line, you get a line below that the water will be reduced to hydrogen. So, it is supposed to release hydrogen gas if one redox couple is below these lines, which is shown over here. Now, there is another point which one should keep in mind before looking at the redox stability in water that is called Over potential.

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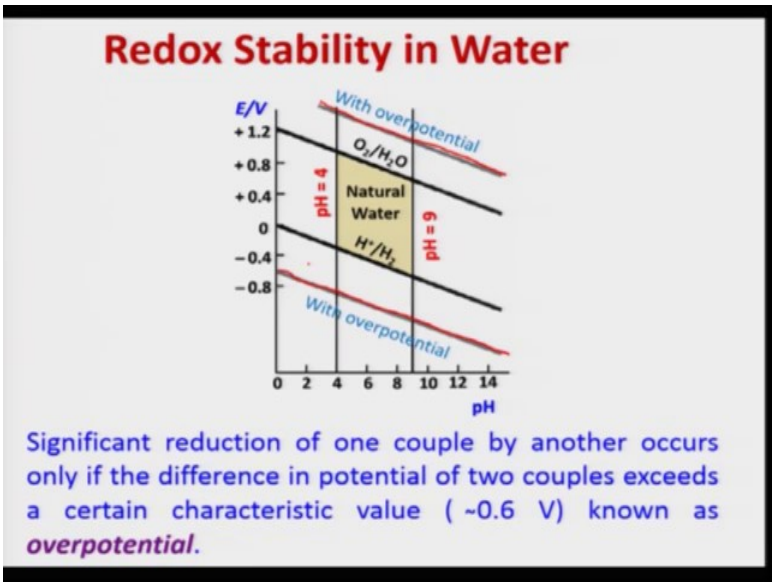

Significant reduction of one couple by another occurs only if the difference in potential of two couple exceeds to a certain characteristic value which is known as over potential. Mostly, in this kind of system, it is around 600 millivolt or we can say that 0.6 Volt, and you see that this is what the line with the over potential for the oxidation side and also for the reduction side, you can have.

And as you can see that the shaded portion is the redox stability in water. Any redox couple which has a potential within this shaded region is stable in the water medium we have chosen here redox potential between pH-4 to pH-9 because mostly in biology, pH varies between these two limits. So, the shaded portion is the redox stability in water.

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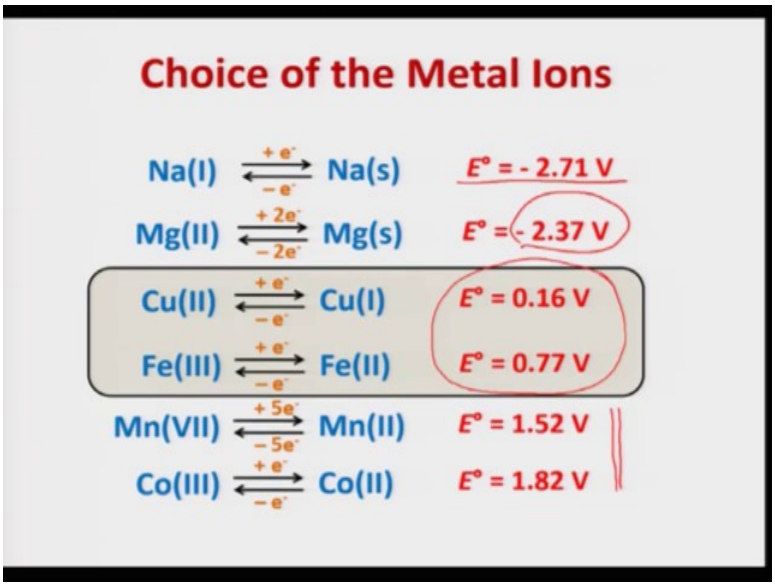

Now, if this is true, then what kind of metal ions nature should select? And some of these potentials are shown over here. Na^+ to Na, this potential is high negative -2.71 volt and that is the reason why nature has never used sodium as a redox active center. Mg(II) to Mg, the E^0 value is very large negative and thus not utilized by nature.

And if you look at copper and iron their potentials are within that range which is called stability field of water and this potential are neither too oxidizing nor too reducing and nature has mostly chosen these metal ions for doing this redox activity in the biological system. However, this manganese, cobalt their potential also a large positive, too oxidizing in nature and nature has not utilized these couples as well.

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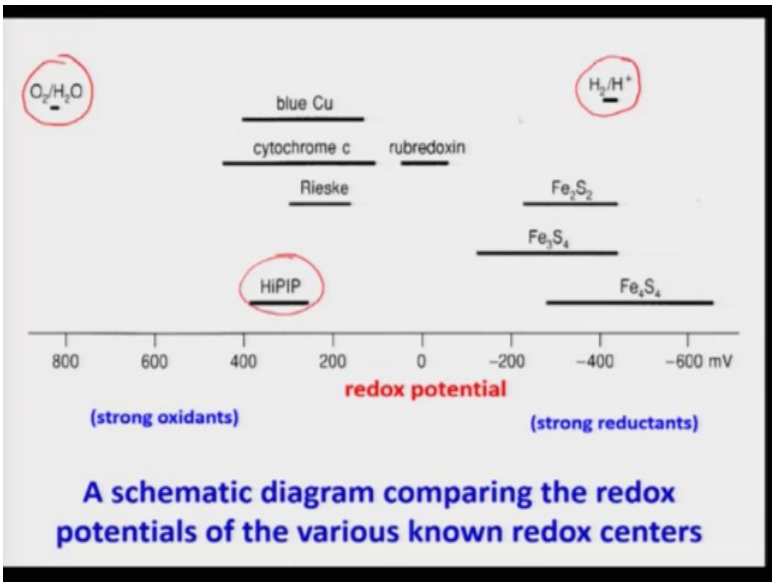

Now, here I have shown a schematic diagram comparing the redox potential of various known redox centers in biology. As one can see that there are two limits, one is in the positive side this is what is the upper limit. If one cross that limit then water will oxidized to oxygen. However, in the negative side also that there is a limit where water would be reduced to hydrogen and all this redox couple are between these two extreme reactions. And if you see that in the positive side, this blue copper which is a copper containing proteins responsible for electron transfer. Cytochromes, rubredoxin, these are all iron containing proteins. And also HiPIP you say also iron sulfur clusters.

In the negative side, you can see that mostly Fe_2S_2, Fe_3S_4 or Fe_4S_4 is there and they are the source of strong reducing agents in the biological system. So, as you can see that both this redox centers are either copper or iron, and no other metal ions are chosen for this kind of activity.

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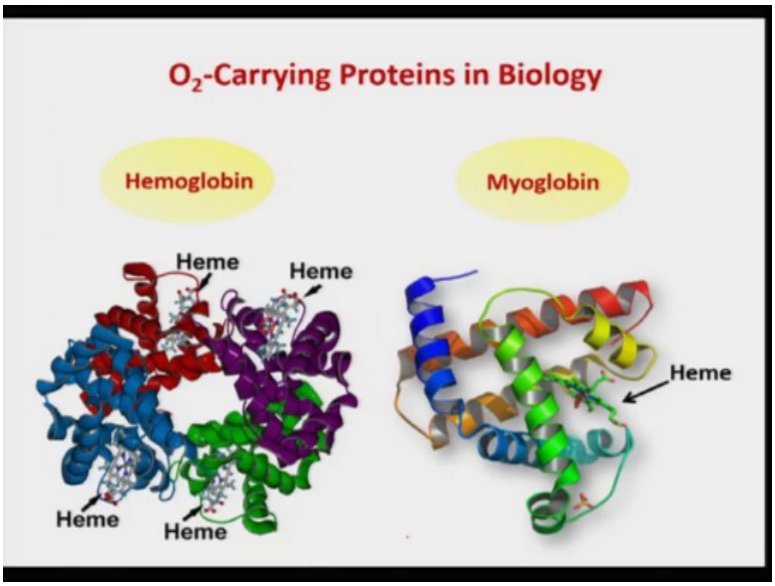

I will now show few examples to tell you how this metal is so important for their activity. For example, oxygen carrying proteins, like you know that hemoglobin and myoglobin. Hemoglobin is a tetrameric unit, myoglobin is a monomeric unit. We will discuss in details although in subsequent lectures.

But the important point I like to emphasize over here is that you see that that heme unit are so small and inside the heme centers this metal is there. But most of the part is covered by the proteins, large amount of proteins which are wrapping the molecules. In myoglobin this is monomer, still large amount of proteins are wrapping this molecules and dioxygen only binds with this iron, although iron is present only in very small amount.

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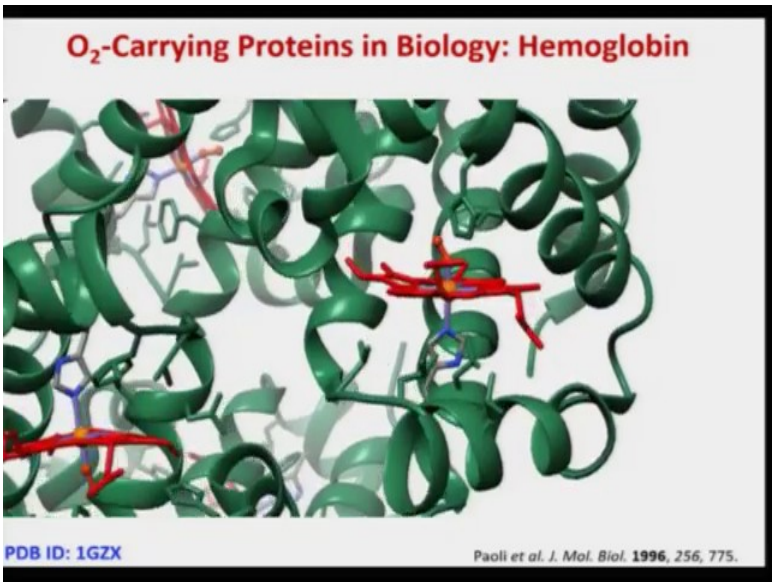

So, here as you can see that protein structure of this hemoglobin, so whenever here in the oxy form you can see that that iron binds this dioxygen.

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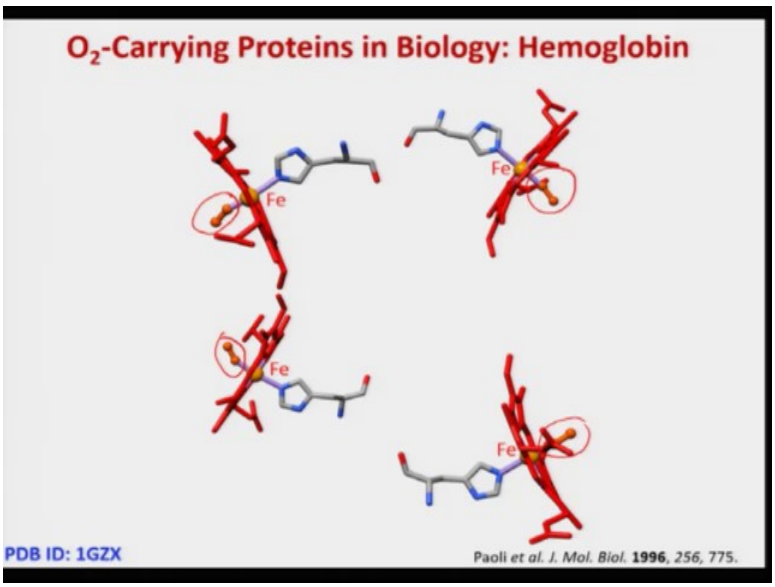

When you remove the protein, you see that this dioxygen it binds in one unit, another unit is a dioxygen, dioxygen and dioxygen. So, one heme unit binds only one oxygen. And since it is a tetramer, so four dioxygen unit binds to that.

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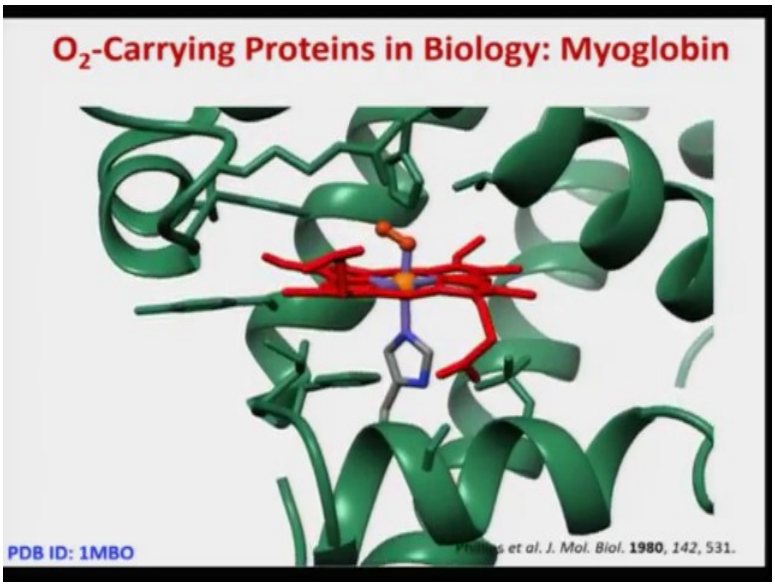

In myoglobin, however, this is monomeric unit and so one unit binds to dioxygen as you can see. But the important thing is that although the iron is present in very small amount they are only responsible for carrying dioxygen.

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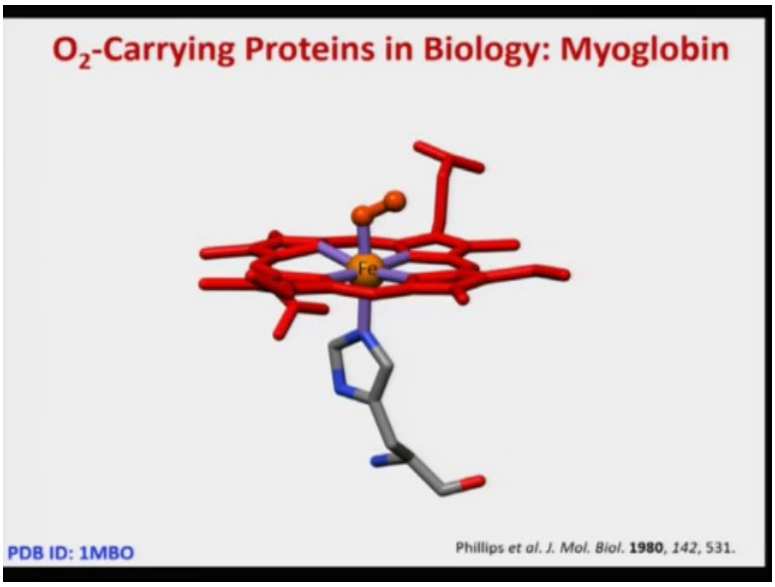

Another example is cytochrome P450 which I will also discuss in subsequent lectures in details.

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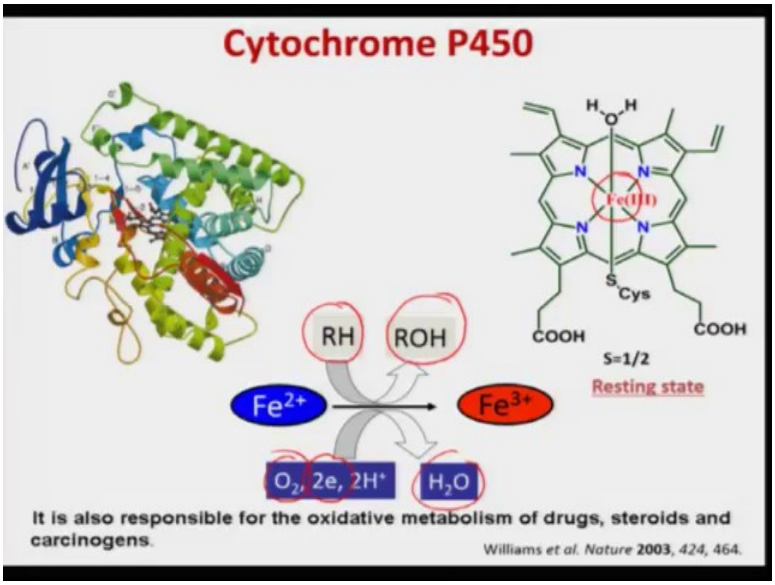

What this cytochrome P450 is doing? This is doing a very important job in our day-to-day life. It is responsible for oxidative metabolism of drugs steroids and carcinogens. What is happening that this RH converted to water soluble ROH and in presence of oxygen and electron which eventually converts to water. We will discuss in details in subsequent lectures. However, this iron which is present in the active site is playing the key role during this transformations.

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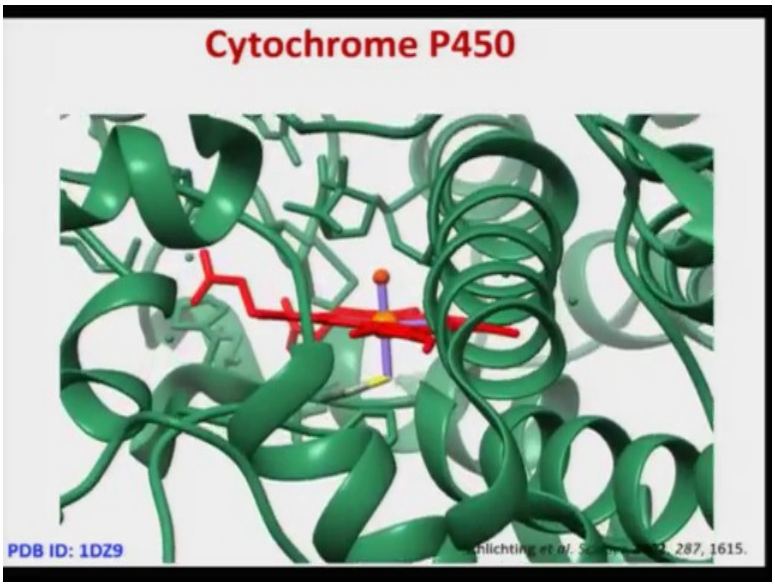

So, cytochrome P450 the structure is shown over here. As you can see there are huge protein chains wrapping this Heme centers, and this iron is actually responsible for its activity.

However, then question is what proteins are doing? Of course, protein has some role, proteins actually select the substrate that which substrate to come close, which substrate will convert and all other things which we will discuss in details.

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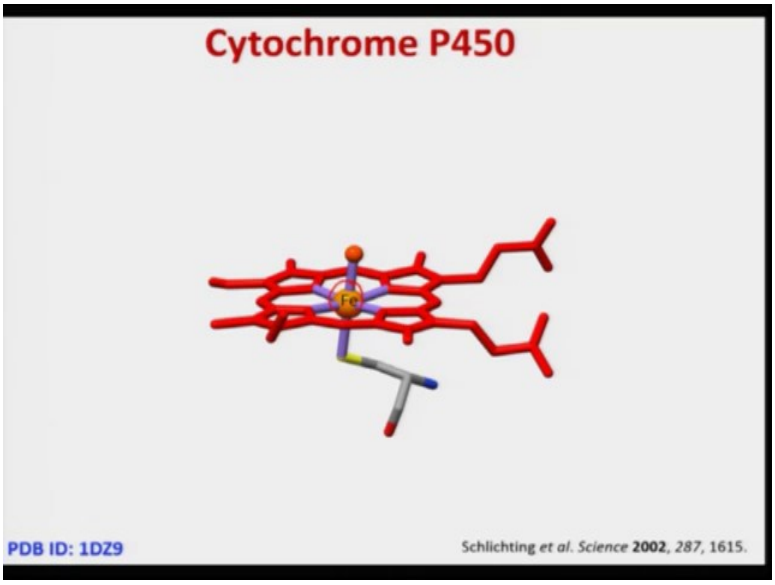

So, once you remove this protein you see that heme unit and iron is sitting inside the core of this porphyrin ring which is actually playing the key role during this transformation process.

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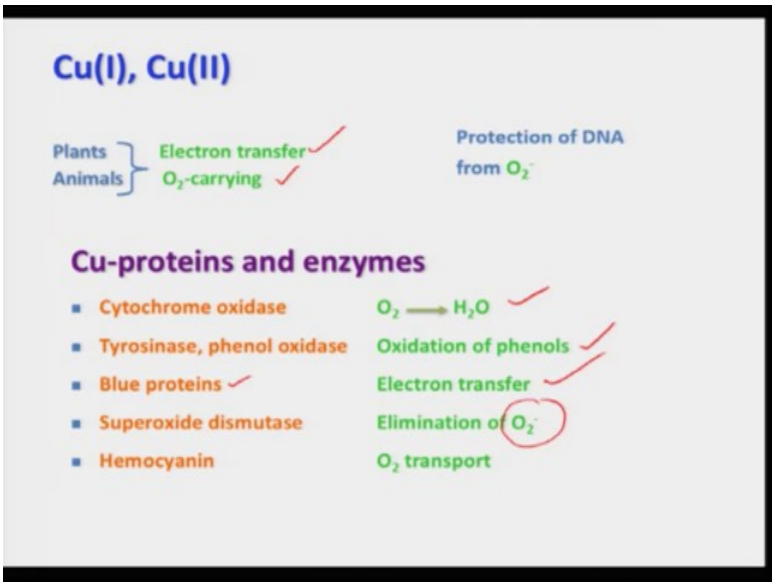

Coming to copper, as I have said that large number of copper containing proteins and enzymes are there which are responsible for various biological activity. They are responsible for electron transfer. They are also responsible for oxygen carrying in the body, and some of this enzymatic activity like cytochrome oxidase converts dioxygen to water which is a source of all sorts of energy. We will discuss in details. Tyrosinase, phenol oxidase: oxidation of phenol, we will discuss shortly. Blue copper protein, actually responsible for electron transfer. Superoxide dismutase is a elimination of biological poison which is superoxides. Hemocyanin, which is responsible for oxygen transport for some lower form of life.

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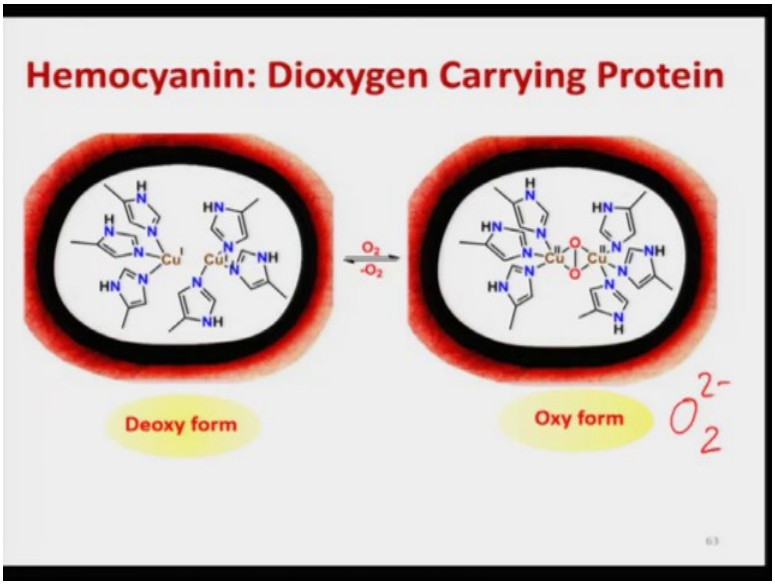

Say hemocyanin is shown over here, and you can see that there are two copper center Cu(I), Cu(I) and these histidines ligated to the copper and one dioxygen binds. It binds into peroxides as a peroxides O_2^{2-}, we will discuss in details. And it binds in a reversible fashion. So, the important point is that that copper ion is responsible for this dioxygen binding. So, we will now discuss why do potatoes and apples turns brown.

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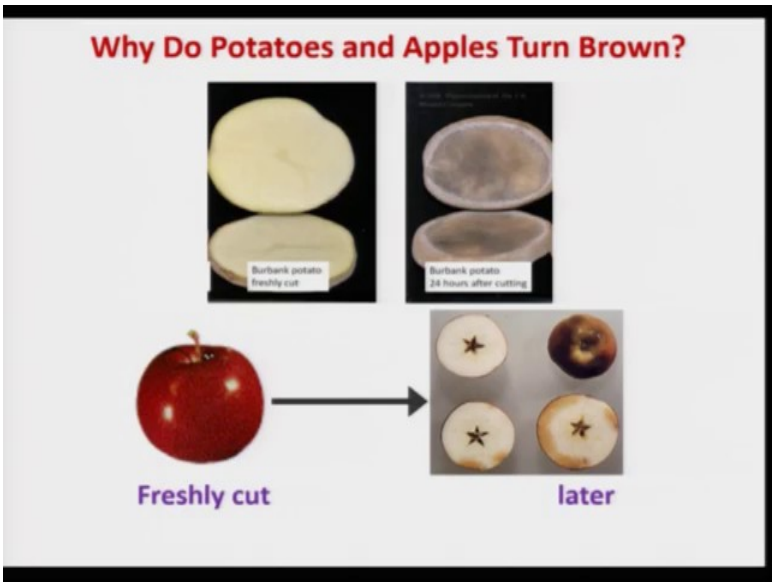

You must have seen that you cut potato and keep it for several hours and you will see that the browning on the surface of this potato and apple. And this browning is very important because it protects that insects and other to destroy the surface and once you need it you can cut that brown portion and it would be as fresh as it was at the beginning. Now, who does these things? The tyrosinase is responsible for such browning on the surface.

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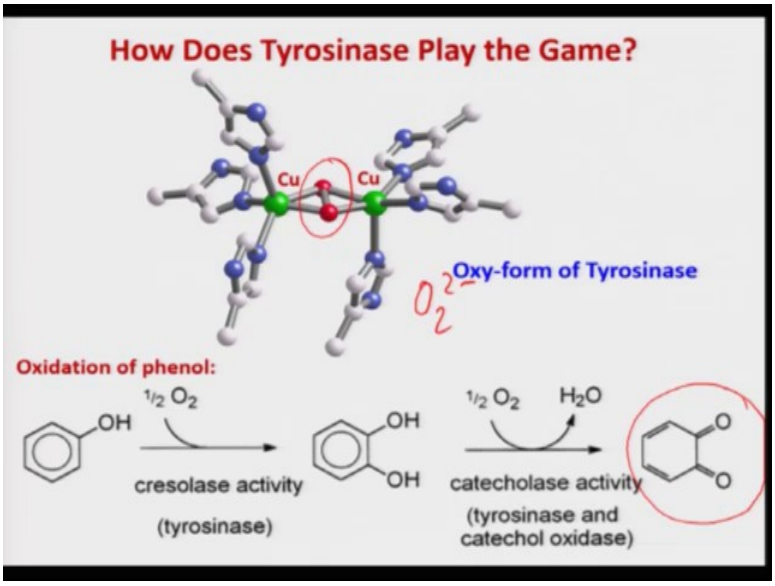

Now, let us talk about tyrosinase. How does tyrosinase play this game? So, this oxy form of the tyrosinase is shown over here, as you can see that there are two copper center and a center is ligated with three histidine and in between as you can see that this is we will show that it is O_2^{2-}, and this tyrosinase actually oxidize phenol from monophenol to diphenol and from diphenol to quinone and this quinone is actually brown which is responsible for browning at the surface.

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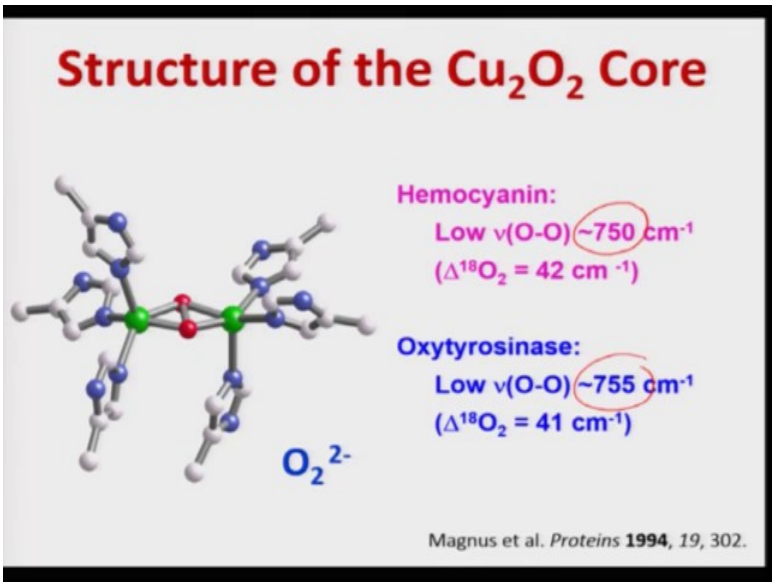

Now, the structure of Cu_2O_2 core is shown over here and you can see this O-O stretch which very similar to those observed in hemocyanin. In hemocyanin, it was 750 cm^{-1}, in oxy-tyrosinase it is around 755 cm^{-1}. And which suggests that it is the O_2^{2-} peroxides state.

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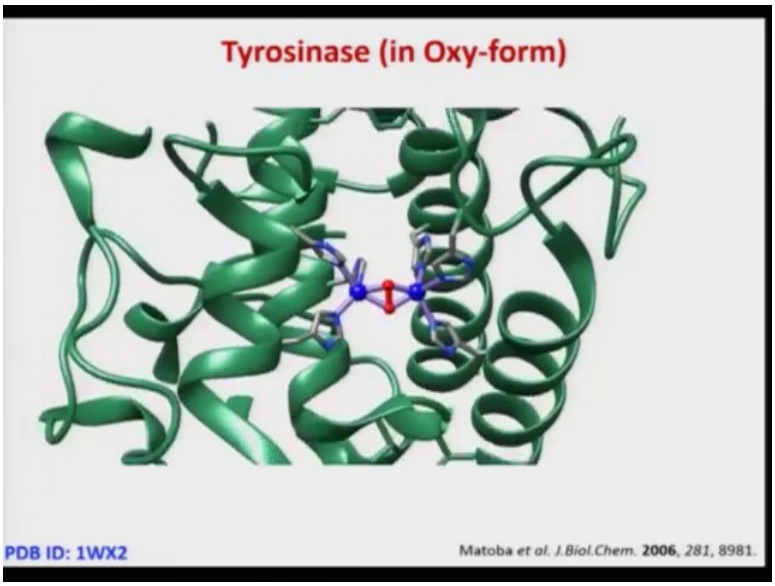

And I will now show you the X-ray structure of tyrosinase in the oxy form. As you can see that a huge proteins is wrapping this dicopper unit and responsible for its specificity.

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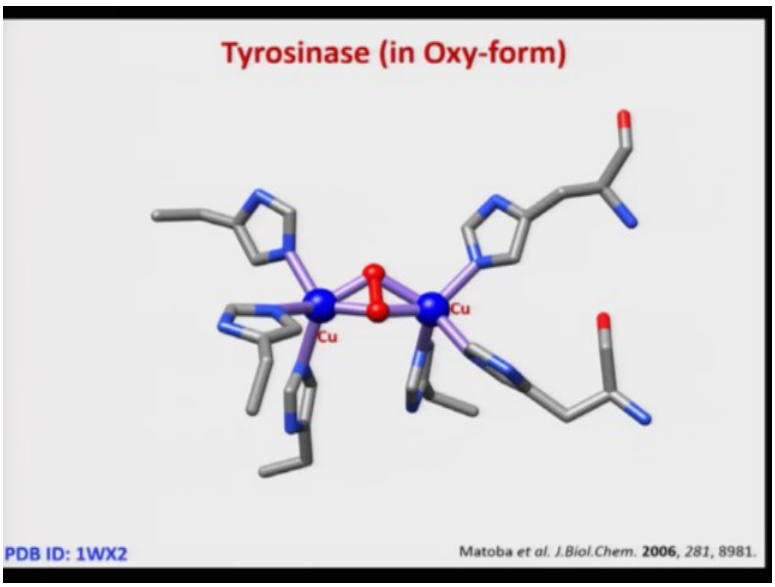

Now, once you remove this protein, you will see this dicopper unit shown over here, where all these activities is happening. I will show you soon the mechanism how tyrosinase works. Now, this dicopper units is bridged with this peroxides as I have shown in my previous slide.

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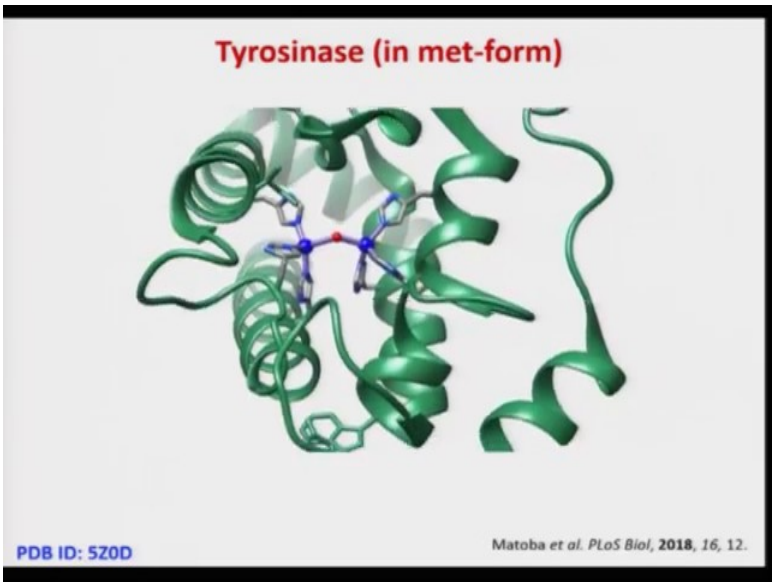

Now, this tyrosinase in met-form is also shown over here, the X-ray structure is displayed and as you can see here this the dicopper unit is sitting inside the huge protein chains and if you remove this protein chain you will see the dicopper unit very clearly which is bridged by the hydroxyl group.

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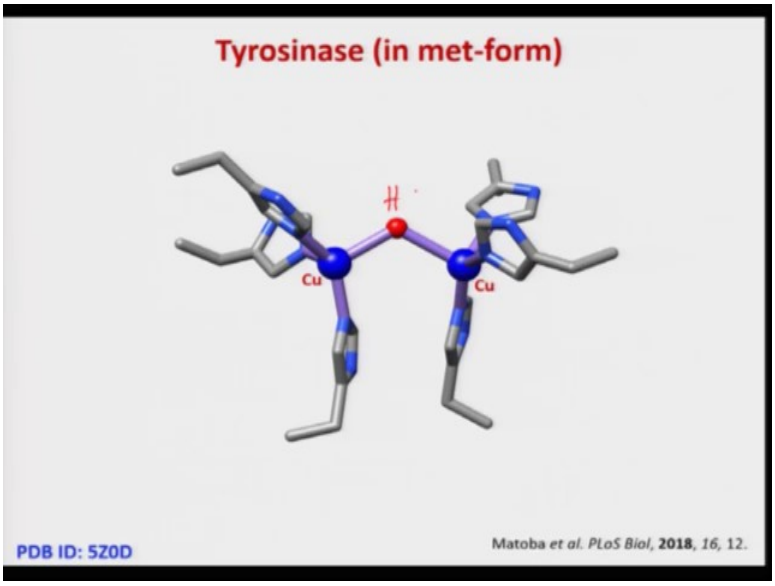

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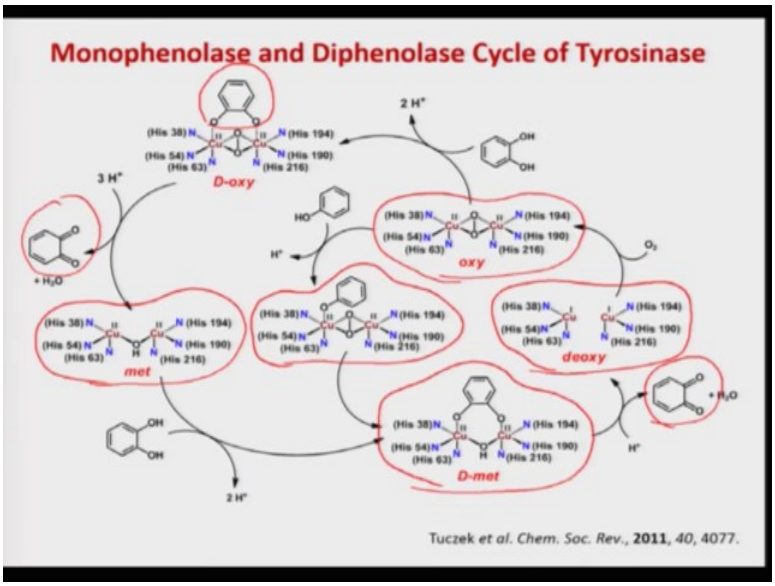

Now, I will be showing that how tyrosinase convert monophenol to diphenol and then quinone. So, let us see how it works. So, this is what is the deoxy form of the tyrosinase. As you can see that two copper unit here in copper 1 state. And when dioygen comes and binds it becomes an oxy form, so copper 1 becomes copper 2 getting oxidized. However, dioxygen getting reduced to O_2^{2-} peroxo form in oxy-tyrosinase. And there are two possibilities, one is that monophenol can binds or diphenol can binds. So, if monophenol binds to one copper center, this is the structure if it monophenol binds to one copper center, then it converts to diphenoxylate form which eventually getting oxidized to quinone and water.

Now, if diphenol binds to the both the copper center, it bridges, as you can see that between two copper center and then this diphenol oxidized to quinone. And then this is this becomes the met-dicopper unit where this is two copper center is bridged by hydroxyl group.

And once again, the one more catechol or diphenol unit comes in binds to this and as you can see this is it is a met-form, it is in Cu(II), two copper centers and then this is also diphenoxylate and this diphenoxylate will be oxidized to quinone and reduced to deoxy form, where Cu(II) goes back to Cu(I) center. And now it is it ready to take one oxygen and goes back to the oxy form and starts the catalytic cycle once again. So, this is the

way a monophenol converts to diphenol and then quinone which is responsible for the browning of the surface in potato and apple.

So, what we have discussed today. In this lecture we have discussed the role of redox active metal ions in biological system. Transition metals with multiple oxidation states facilitates electron transfer, and as discussed today mostly iron and copper proteins are utilized for electron transfer and dioxygen binding and activation. Although, the metal ions are present in extremely small quantities and sounded by large number of protein chains, all the activities are performed only at the metal center which clearly justify the crucial role played by the metal ions in biology.

In my next lecture, I will discuss coenzyme B_{12} dependent enzymes and highlight their role in catalyzing a large varieties of chemical transformations in biology. We will look at the various design principle used and nature selection of the cobalt and chlorine macrocycle there in.

Thank you.