Bioinorganic Chemistry
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Lecture - 06
Design Principles Used in Chemical Biology: Some Noteworthy Examples

Hello everybody and welcome back to the short course of Bioinorganic Chemistry. We have discussed so far, the importance of inorganic elements in biological system and also how these elements are being selected out of so many elements present in the periodic table. Although, the metal ions are present in extremely small quantities and surrounded by a huge protein chains. The biological activities are performed only at the metal centers which clearly justify the crucial role played by those metal ions.

I also have showcased; how our lives affected directly out of such elements and causes various diseases. Enzymes are catalysts that greatly enhanced the rate of a specific reaction by a factor as much as 10^{12} making side reactions unimportant. The yields are often cent percent with very high efficiency and stereo specificity. This indeed is looking like a magic as compared to our day to day experiences in the laboratory.

It would be indeed great to see, how such miracles are indeed possible in biology without violating any basic principles of science. I will now, illustrate with some examples, how the beautiful design principles make all these differences in biology. The natures design in controlling some of the key chemical processes would be highlighted here, in my next four lectures, which are extremely important for our life. Today, I will showcase one such Noteworthy Example.

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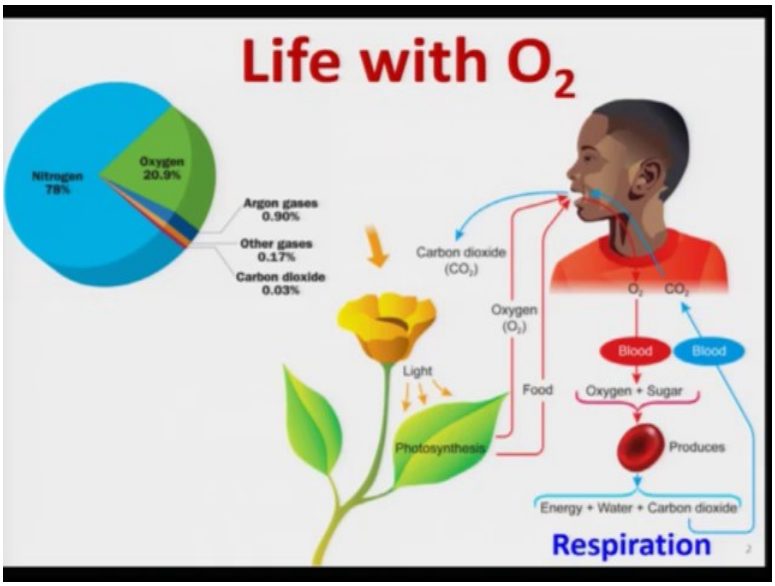

If we look at environment particularly here, what we find? We find that nitrogen is around 78 percent and oxygen is 20.9 percent, huge amount of oxygen which we inhale through blood and this oxygen is responsible for the production of energy and releases the carbon dioxide, which through blood again goes out of our body. So, oxygen is so important for our survival in this earth.

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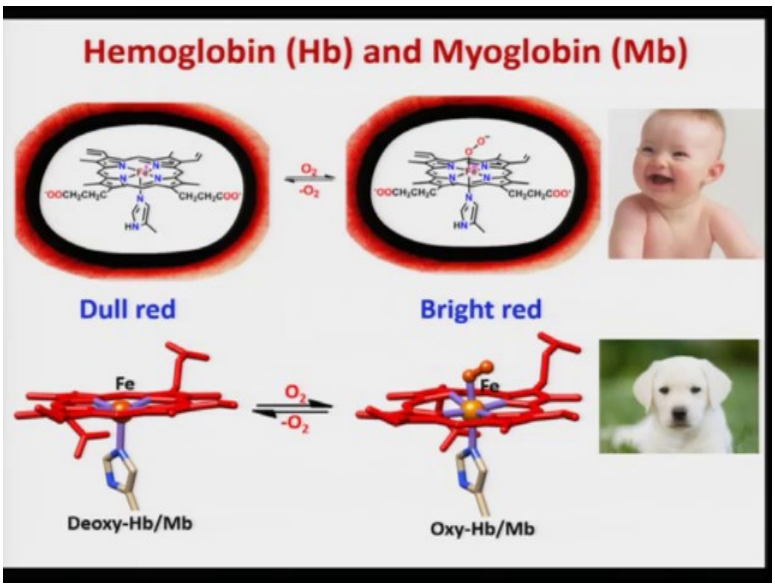

The proteins which are responsible for dioxygen transport and storage in our body is myoglobin and hemoglobin. The monomeric unit is shown over here, as you can see that this is the deoxy form and when oxygen binds it becomes oxy form.

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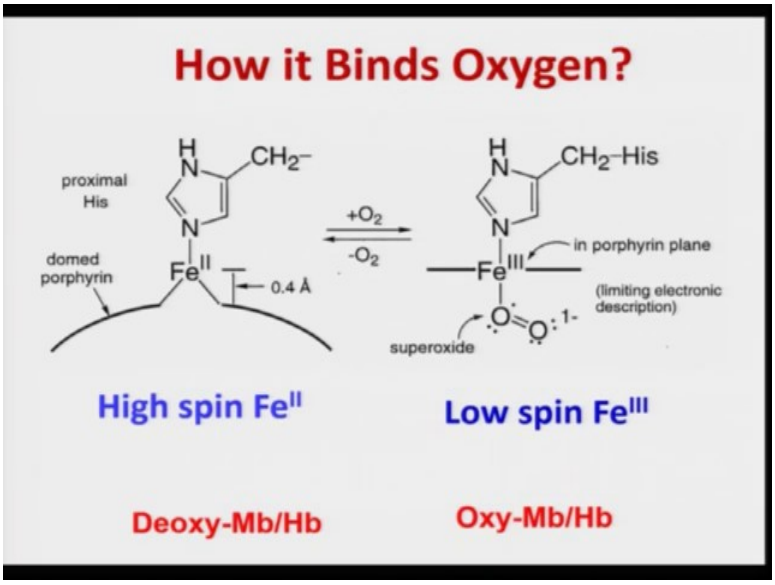

So, what is exactly happening during this transformation, this Fe(II) in deoxy form will convert to Fe(III); that means, Fe(II) is oxidized to Fe(III) whereas dioxygen is reduced to superoxides O_2^-, and there is a huge structural change occurring during such transformations, which we will discuss probably after a week or so in details. However, what you can see here this transformation is completely reversible.

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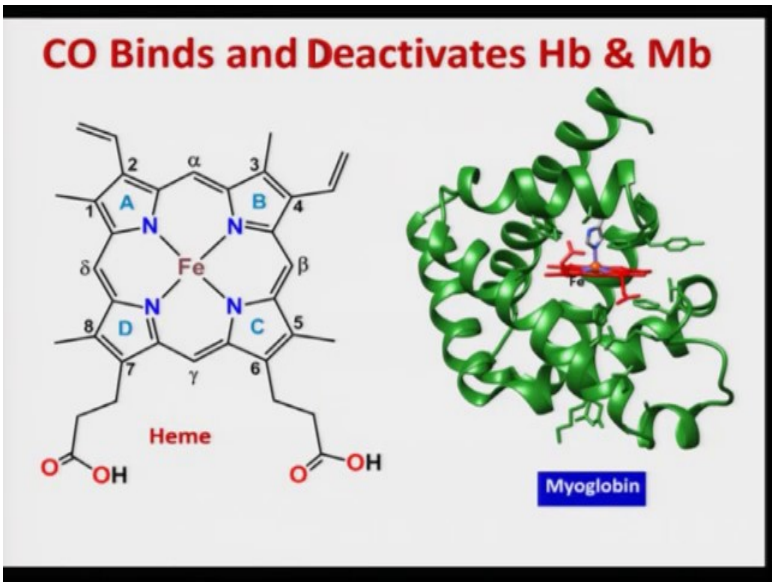

Now, carbon monoxide also, can binds and can deactivate hemoglobin and myoglobin and as you all know that the binding affinity of free heme group for carbon monoxide is about 25000 times that for oxygen whereas, the binding affinity of myoglobin for carbon monoxide is only 250 times greater than that of dioxygen.

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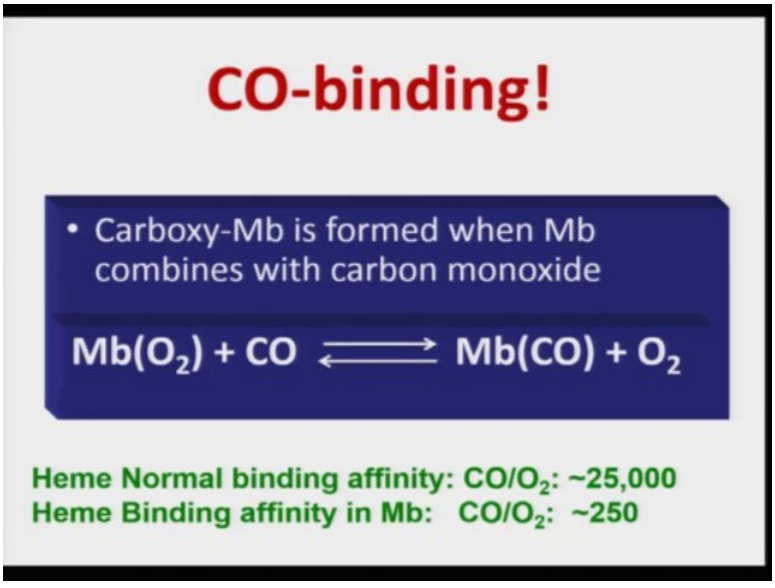

So, carbon monoxides this binding affinity is much more so, it immediately replaced dioxygen and forms that carboxy myoglobin.

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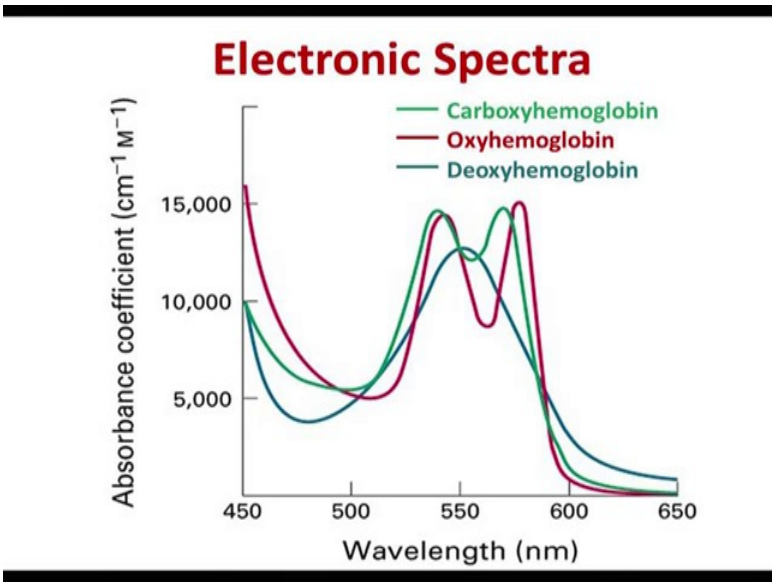

Now, once this carboxy myoglobin is formed, you can detect very easily by spectral change, you can see that the green one is that carboxy hemoglobin and which is very distinct features as compared to oxyhemoglobin and deoxyhemoglobin.

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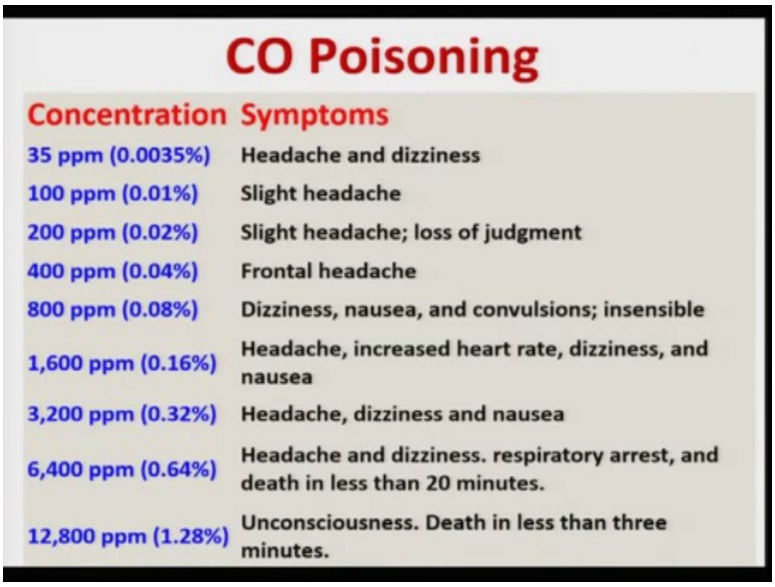

This carbon monoxides is just like a poison in our body and I will show you here that how this concentration of carbon monoxide actually, responsible for various symptoms including the death. For example, this if you have like 35 ppm, it will give you an headache and dizziness; 100 ppm concentration, you know it will give also slight

headache; 200 ppm is also headache and loss of judgment; 400 ppm, frontal headache; 800 ppm, dizziness and insensible features; 1600 ppm, approximately 0.16 percent, it causes headache increased heart rate dizziness and other related symptoms. If we increase to 3200 ppm, just 0.32 percent, you also have huge headache and dizziness and nausea.

Now, if you increase this concentration to 6400 ppm, it is approximately 0.64 percent, it causes headache, dizziness and respiratory arrest and indeed death in less than 20 minutes. If you increase the concentration further like 12800 ppm, it just one 0.28 percent, it will give immediate unconsciousness and death in less than 3 minutes. So, you can see yourself, how carbon monoxides are poisonous for us.

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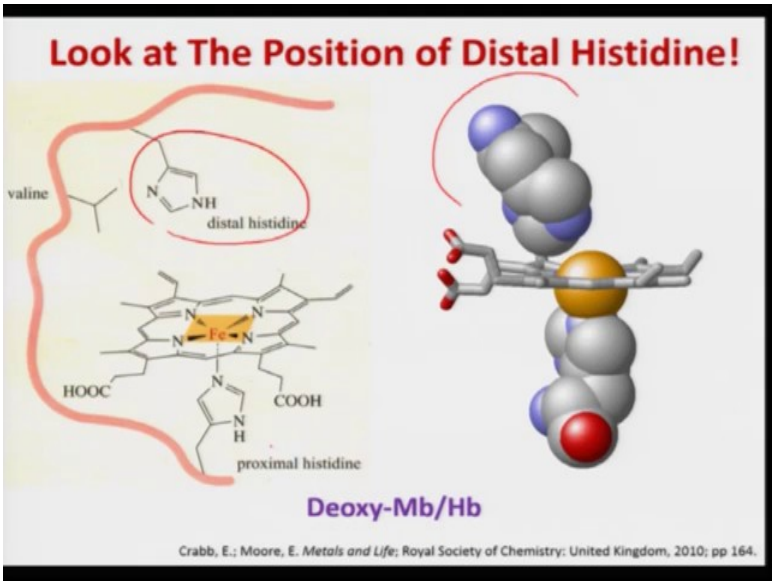

Now, let us look at the positions of the distal histidine in hemoglobin and myoglobin. This distal histidine is shown over here and you know the space filling model also is shown that, this iron is ligated with four nitrogen of the heme center and fifth position is a histidine which is called proximal histidine; however, the sixth position it is an empty and distal histidine which is sitting above this iron, but not directly ligated to iron and you can see that this is like a gate. We are going to discuss soon that how this distal histidine indeed responsible for discriminating oxygen from carbon monoxides.

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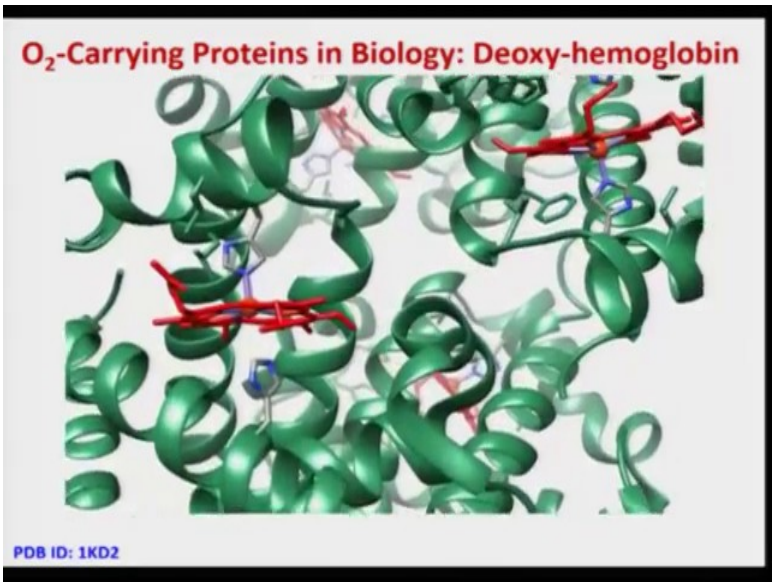

So, look at the X-ray structure of deoxyhemoglobin here. As one can see that, this distal histidine is just sitting above this iron center and it is not ligated directly to the iron, but it is playing a very crucial role, which I am going to show you now.

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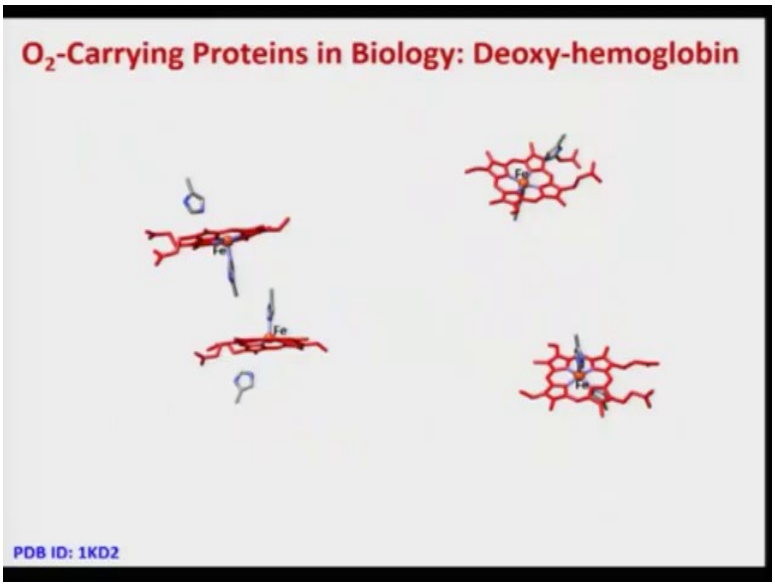

You will see that this is heme center and this is distal histidine and you see that six position is not coordinated.

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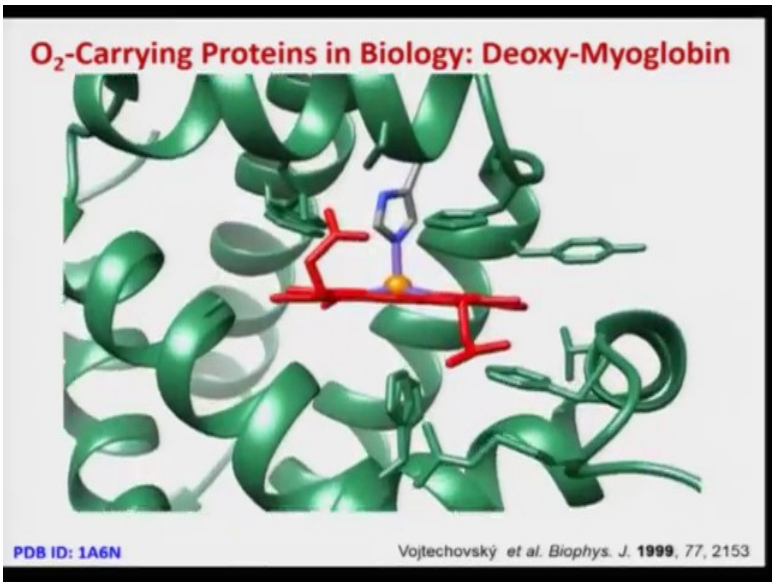

Now, let us look at the myoglobin also. Here also, you see that the just above this iron center there is an distal histidine and which are actually responsible for discriminating carbon monoxide and oxygen. This iron center actually binds dioxygen.

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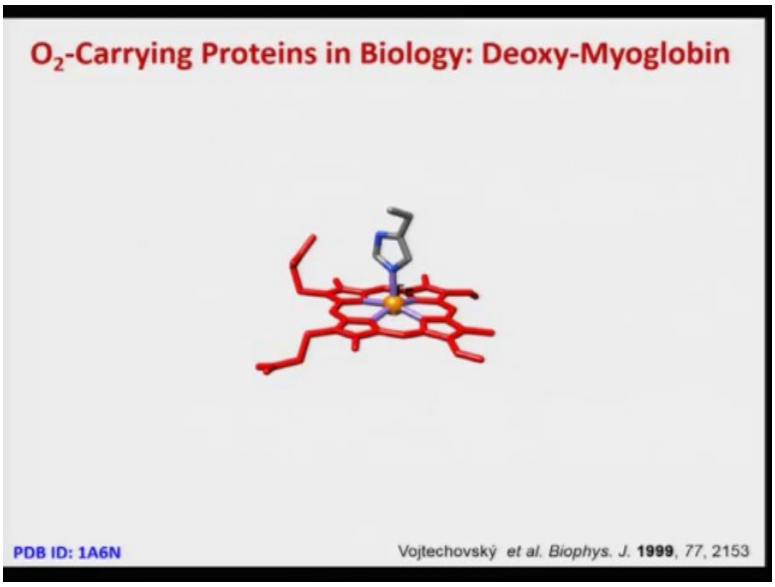

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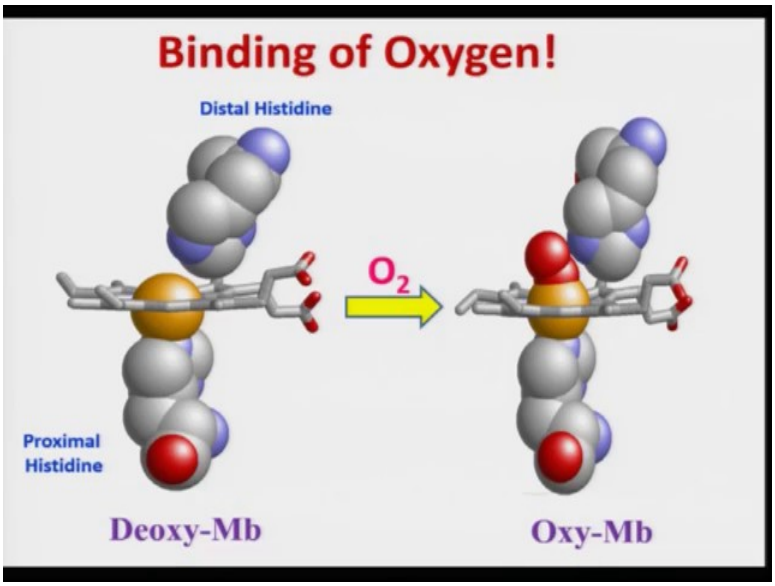

Now, this binding of dioxygen is shown here, as one can see that this distal histidine in deoxy hemoglobin or myoglobin. The space filling model clearly shows that it is just above this iron center although, it is not ligated to the iron center, because of it's distance.

Now, once dioxygen binds as one can see that dioxygen, which I have already said that it forms superoxides, it just able to fit perfectly, because dioxygen binds in an angular fashion to hemoglobin and myoglobin and distal histidine indeed facilitates also going through this hydrogen bonding. So, let us stabilize this dioxygen binding to these heme centers. We will discuss in details in the subsequent lectures.

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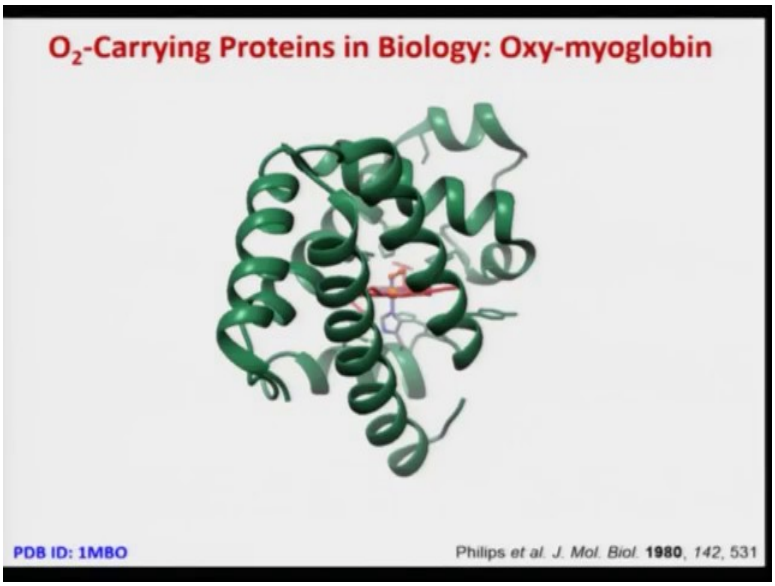

However, if you look at what happens after dioxygen binding? Again, this is the X-ray structure of oxy myoglobin, huge protein chain surrounded the heme center and one can see that this distal histidine is just allowing dioxygen to binds in angular fashion you, if we remove this protein chains, you see this dioxygen binds angularly to iron and this is what is happening in case of oxymyoglobin.

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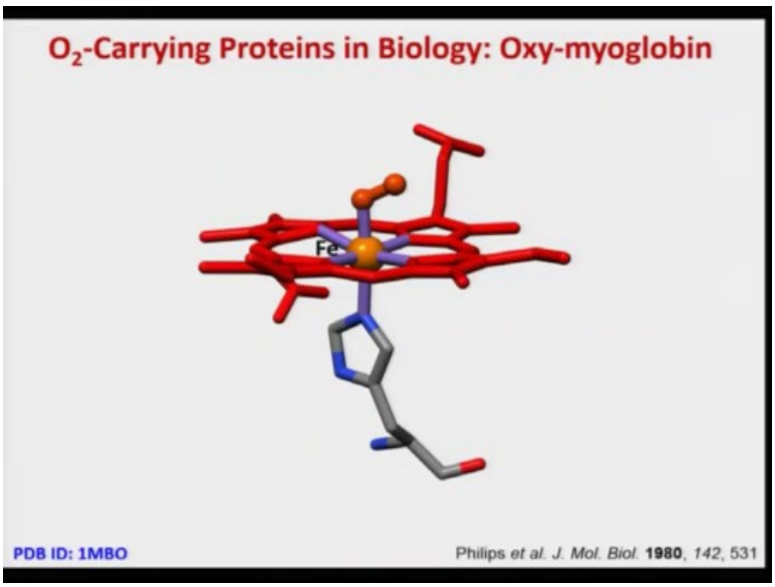

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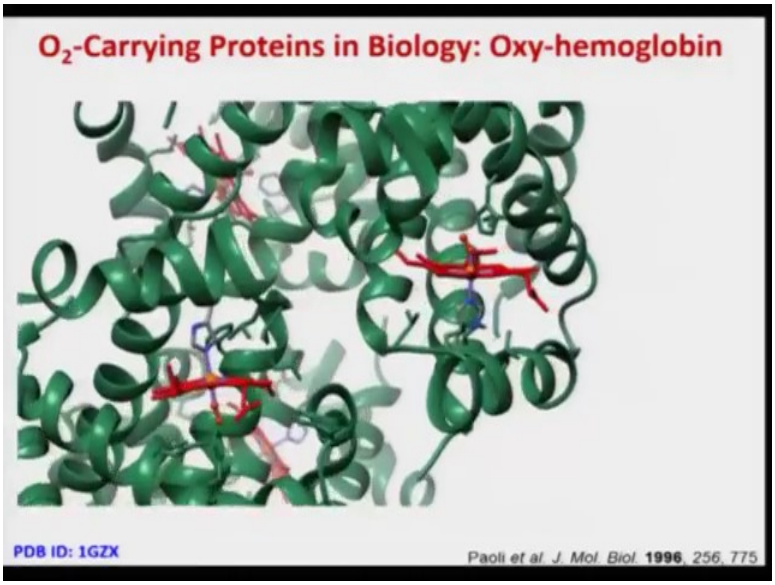

Similar things also happen with oxyhemoglobin. This is the X-ray structure of oxyhemoglobin, as you can see that there are four heme center; however, huge protein chains which are covering this hemes centers.

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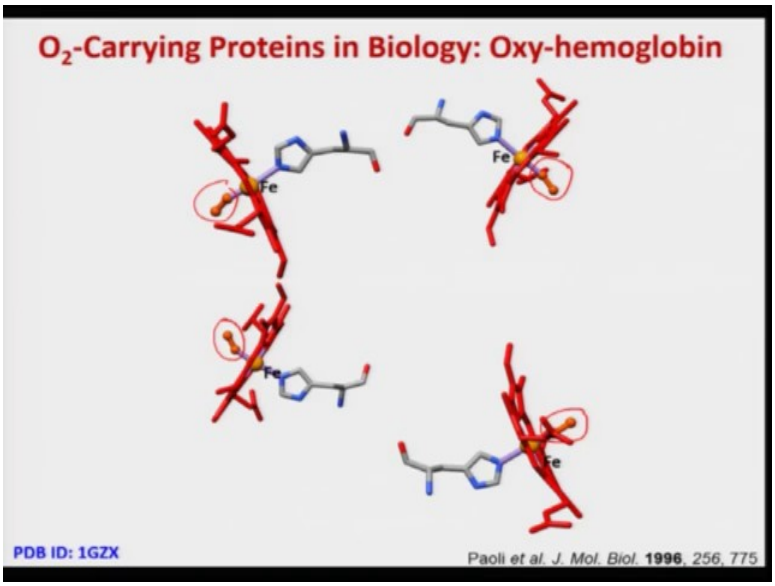

Now, once you remove this protein chains you see that four heme center, where dioxygen binds and you know this is all these dioxygen binds perfectly and dioxygen each angular in nature. So, dioxygen binds in a angular fashion in this way.

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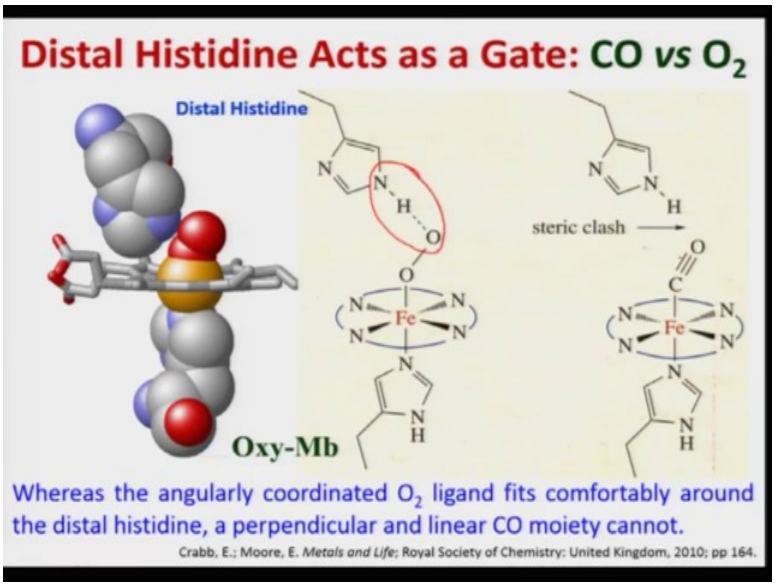

So, distal histidine acts as a gate. This is the oxy myoglobin as I have just said, the distal histidine also facilitates this angular binding of dioxygen by forming an hydrogen bond N-H-O hydrogen bond, which further stabilize this oxygen binding on this iron. In contrast carbon monoxide, which suppose to bind in a linear fashion due to the steric crowding or steric hindrance from this distal histidine.

So, this carbon monoxide is now forced to bind in an angular fashion. So, it is interesting to see whereas the angularly coordinated dioxygen ligand fits comfortably around the distal histidine, a perpendicular and linear carbon monoxide moiety cannot, it is forced to bind in a angular fashion.

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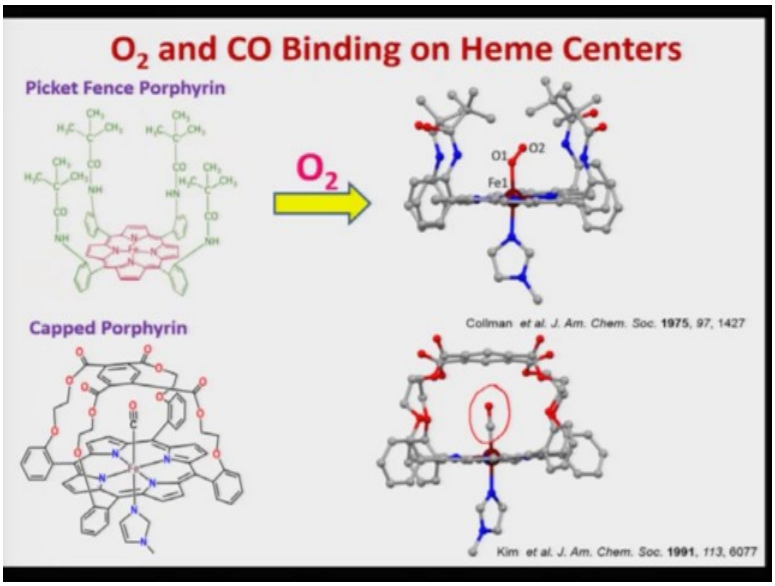

If you look at the literature, there are many such molecules which are structurally characterized. Also, two such molecule is shown here, where in one like picket fence porphyrin, where dioxygen binds, it binds in a angular fashion.

There is another example kept porphyrin, where this iron center also binds carbon monoxide, but in a perfectly linear fashion. So, carbon monoxides bind linearly whereas, dioxygen binds in a angular fashion and if you look at the literature, this is what is the case many structures are being published already. I am showing just two example to impress you that this indeed is the fact.

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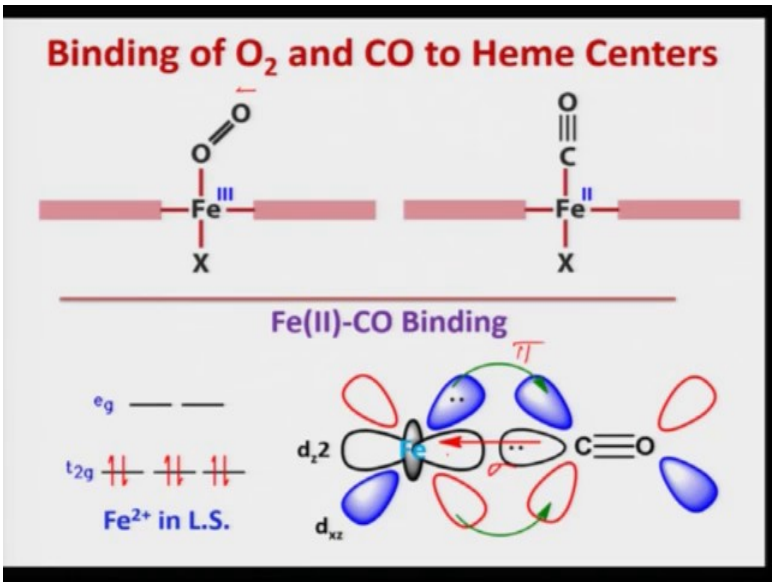

Now, let us look at this binding a little bit more carefully. So, as I have said that iron centers bind dioxygen and then iron it would be Fe(III) and this would be O_2^- superoxide's; so Fe(III) superoxide's. However, carbon monoxides binds to iron center. So, this iron remains Fe(II) and carbon monoxide is a neutral ligand. Now, let us look at closely the binding of iron carbon monoxide. So, as you know carbon monoxide is a strong field ligand.

So, this Fe(II) would be in low spin. So, this is t_{2g}^6. So, all this d_{xy}, d_{yz} and d_{xz} are filled completely in a low spin complex; however, if you look at this iron CO bonding carefully, what you see as you all know that metal carbonyl bond is well studied here, this is the σ bond and this is what is the π. So, this is the filled σ orbital overlap with empty

d_{z^2} orbital of iron and increases the electron density around iron and as a result of that this iron d_{xz} orbital, which is indeed filled, they give back this electron density to the empty π* orbital.

So, it is an give and take policy, we call it as a synergic effect. So, carbon monoxide and iron, it if it is a linear that orbital overlap, would be very good and that is the reason why carbon monoxide always binds in a linear fashion. As I have already said that carbon monoxides always prefer to bind in a linear fashion and this indeed happens in free heme, where there is no protein around this molecule.

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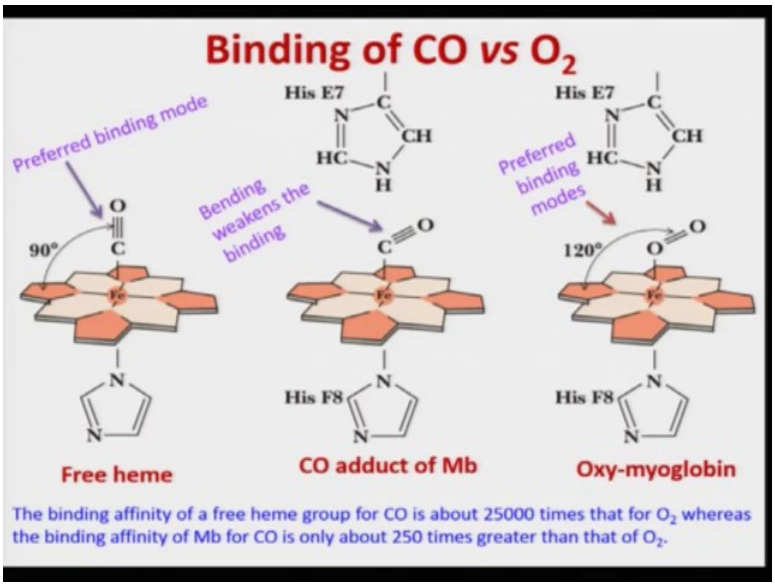

In contrast carbon monoxide in myoglobin and hemoglobin binds in a angular fashion, because of the distal histidine and thereby, bonding becomes weak. In contrast, dioxygen always prefers to bind in a angular fashion, indeed this angular fashion is facilitated by this hydrogen bonding. The binding affinity of a free heme group for carbon monoxide is about 25000 times that for O_2 whereas, the binding affinity of myoglobin for carbon monoxide is only about 250 times greater than that of oxygen, because of this angular binding and this is a weak binding mode for carbon monoxide. *Le Châtellier's* principle states that, if stress is applied to a system at equilibrium; the equilibrium will see it in the direction that will remove the trace.

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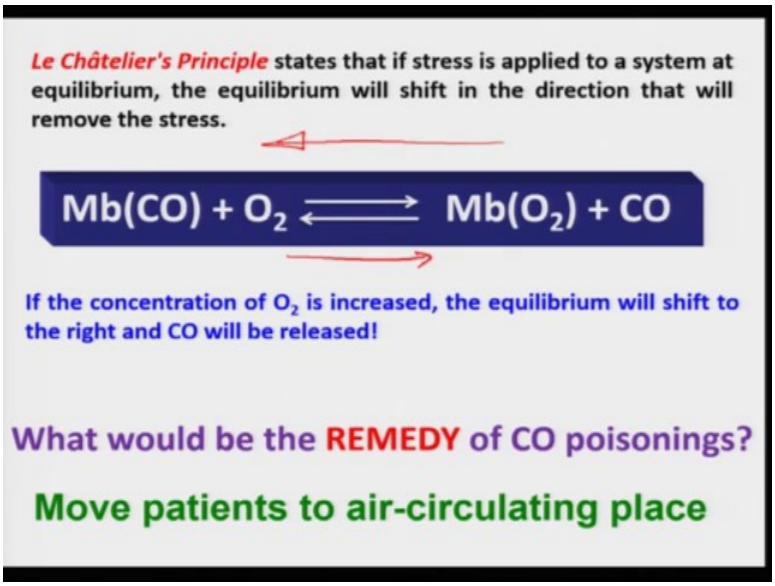

What would happen, if we add oxygen and this oxygen can in principle also replace carbon monoxide and forms oxy myoglobin? So, they are in equilibrium. As I have said that carbon monoxide binds only 250 times stronger to iron center of hemoglobin and myoglobin than the oxygen; however, if the concentration of dioxygen is increased then what would happen? The equilibrium would be shifted to the right direction and carbon monoxide can be released like this otherwise, normally carbon monoxide binds strongly to iron center.

So, equilibrium would be shifted to the left. So, what would be the remedy of carbon monoxide poisoning? Is simply you move the patient immediately to air circulating place where concentration of oxygen is much more so the equilibrium would be shifted from left to right and the patient would be get rid of carbon monoxide poisoning.

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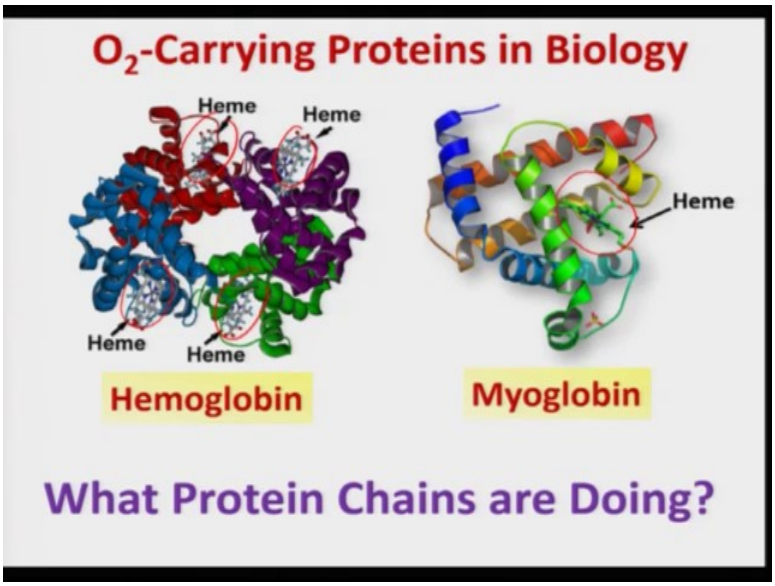

Now, coming back to oxygen carrying proteins in biology. I have shown these two molecules, which we have in our body, one is hemoglobin another one is myoglobin. Hemoglobin is tetrameric unit, as you can see that there are four heme centers one, two, threee, four and myoglobin is one heme unit; however, interesting to note that there is a huge protein chains in both hemoglobin and myoglobin, which are covering this entire heme unit.

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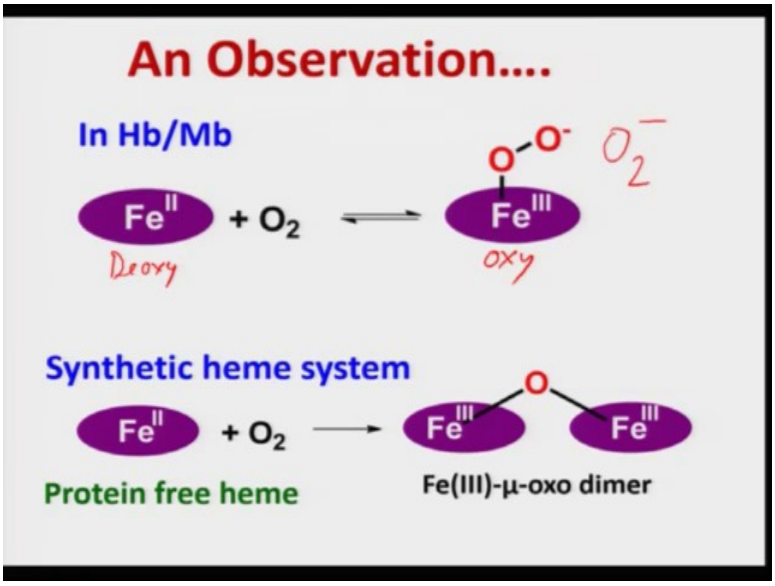

Now, question is that what this protein chains are doing? Now, in order to answer to this, I will show you an observation. In hemoglobin and myoglobin oxygen binds reversibly. So, Fe(II) binds oxygen convert to Fe(III) and dioxygen convert to superoxide O_2^-, superoxide and this molecule, the oxyhemoglobin and myoglobin releases oxygen and goes back to the deoxy form.

So, this is the deoxy form and this is the Oxy form. So, the oxygen Oxy is completely reversible in our body. In contrast if you have a synthetic heme unit it is a protein free heme. Like if you make some molecule in the laboratory what happens? It also react with dioxygen, but completely irreversible way it immediately forms this Fe(III)-μ-oxo dimer, which is very stable.

So, you see the difference in hemoglobin and myoglobin this heme center carries dioxygen reversibly; however, this same heme centers, without the protein chain converts to Fe(III)-μ-oxo dimer so; that means, it does not bind dioxygen reversibly.

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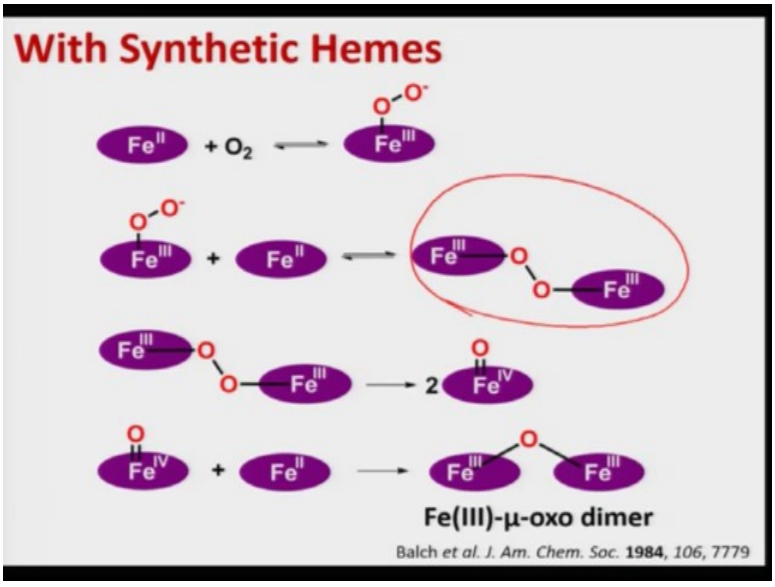

In synthetic heme also this Fe(II) centre binds dioxygen forms this Fe(III)-O_2^- as we have seen in case of hemoglobin and myoglobin, but then this Fe(III)-O_2^- immediately react with another Fe(II) center forms, this peroxide bridge complex and this peroxide bridge complex eventually, convert to Fe(III)-μ-oxo dimer. Thus, synthetic heme center cannot bind dioxygen in a reversible fashion and it immediately reacts with dioxygen gives rise

to Fe(III)-μ-oxo dimers. We will be discussing all these details in our subsequent lectures.

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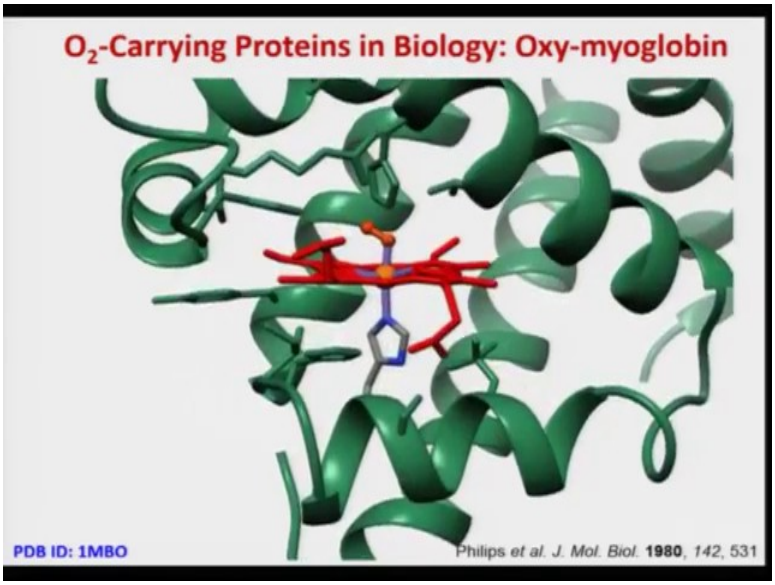

Now, this is the X-ray structure of Oxymyoglobin, as one can see that this iron center bind dioxygen in angular fashion and this huge protein chains are actually wrapping this heme unit.

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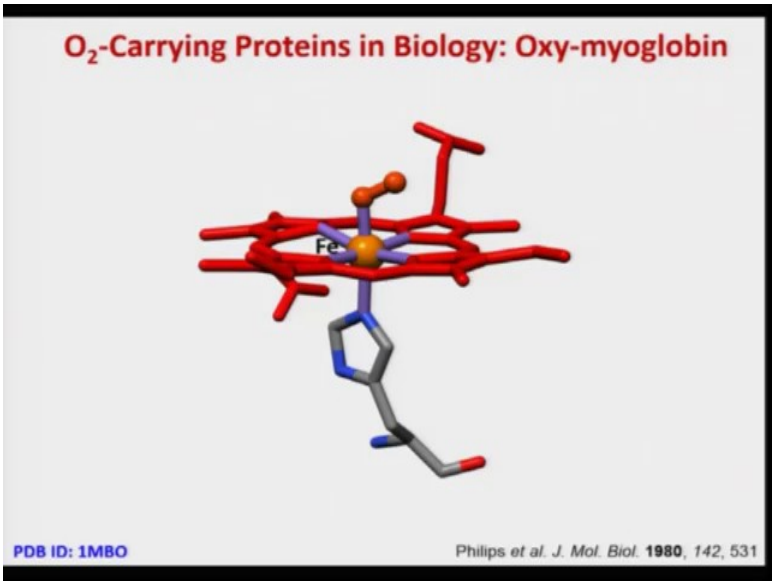

And once, you remove this protein chain, then you see that dioxygen binds very nicely to iron and this coordination is there.

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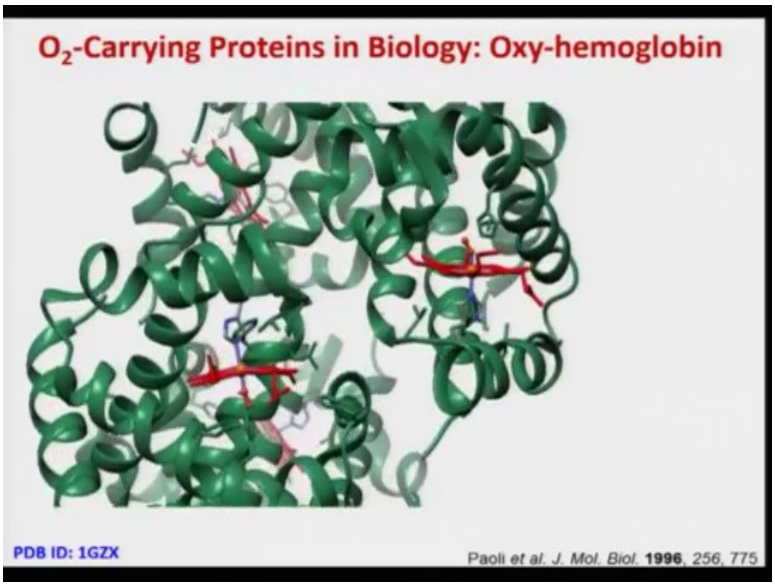

Similarly, X-ray structure of oxyhemoglobin is shown over here, as one can see that all four heme center or iron centers also binds dioxygen is a angular fashion and once, I removed this protein then this heme centers you can see, but please note that, there is a huge protein chains which are actually surrounding this heme unit.

So, this two heme center cannot come close to each other. So, that that μ-peroxo species can formed and once you remove this protein chain, you can see yourself that iron center binds dioxygen comfortably.

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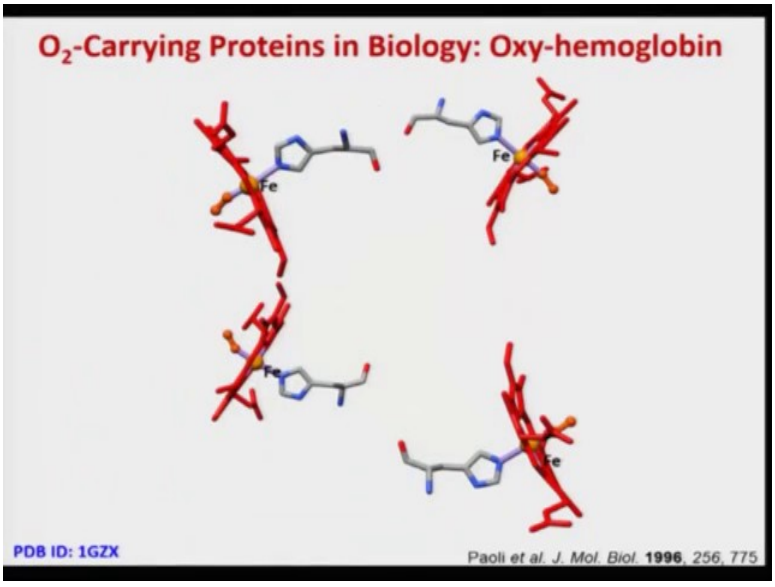

So, what is happening? With globin chain, as we have just seen in hemoglobin and myoglobin that iron center binds dioxygen comfortably and in a reversible fashion.

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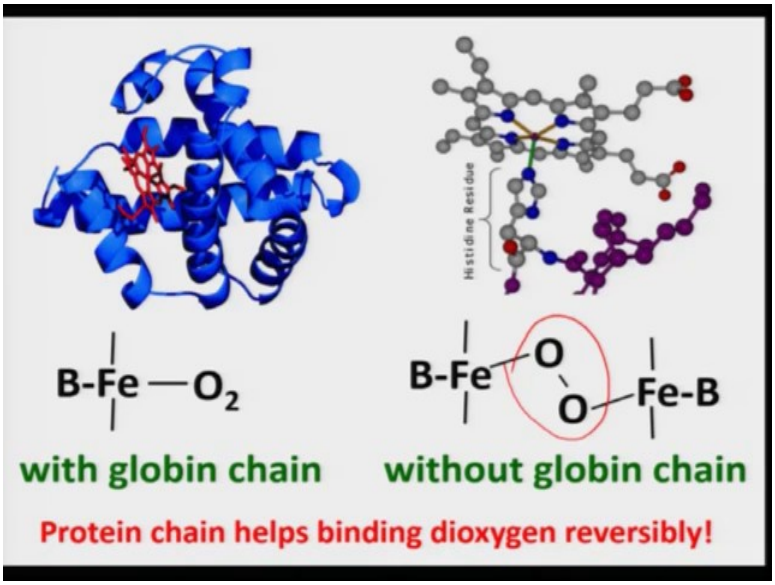

In contrast, if we remove this globin chain or protein chain, then what is happening that this peroxo bridge species is being formed, which eventually responsible for the formation of stable μ-oxo dimer. Indeed, protein chains helps the binding of dioxygen in a reversible fashion in hemoglobin and myoglobin.

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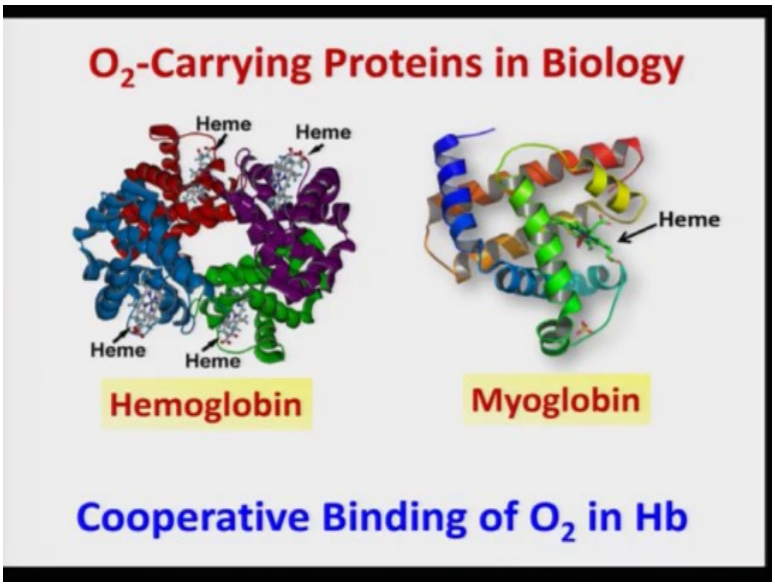

Now, coming back to this oxygen carrying proteins in biology, as I have shown here the hemoglobin and myoglobin and what this protein chains are doing; is only separating these two heme centers so that they cannot come close to form μ-oxo dimer. Let us see, so it also helps the cooperative binding of dioxygen in hemoglobin.

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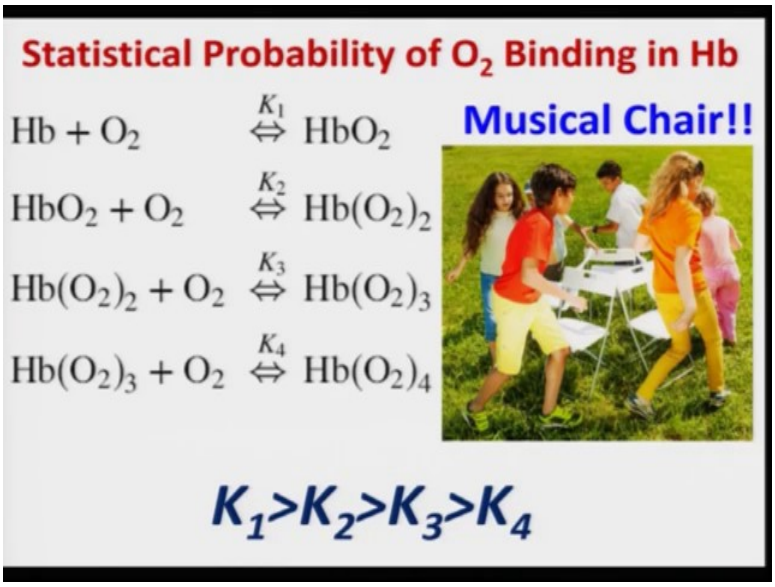

This is what is the statistical probability of dioxygen binding in hemoglobin. As I have said already that is hemoglobin tetramer; there are 4 heme centers. Now, the one heme centers bind dioxygen is a K_1, then the next oxygen binds and then third, then fourth and this successive oxygen binding constants are; K_1, K_2, K_3, K_4 and if you look at the probability, the K_1 is greater than K_2, it greater than K_3, is greater than K_4.

So, this is what one can get out of just statistical probability and one can easily see this. If you look at the musical chair, in the musical chair what is happening that if you have like four chairs now, if you occupy one chair then the next person can only occupy one out of these three and next to next can occupy one out of just two and the last one can occupy the only one remaining chair. So, this is what you observe in the musical chair. What is happening in this cooperative binding of dioxygen in hemoglobin?

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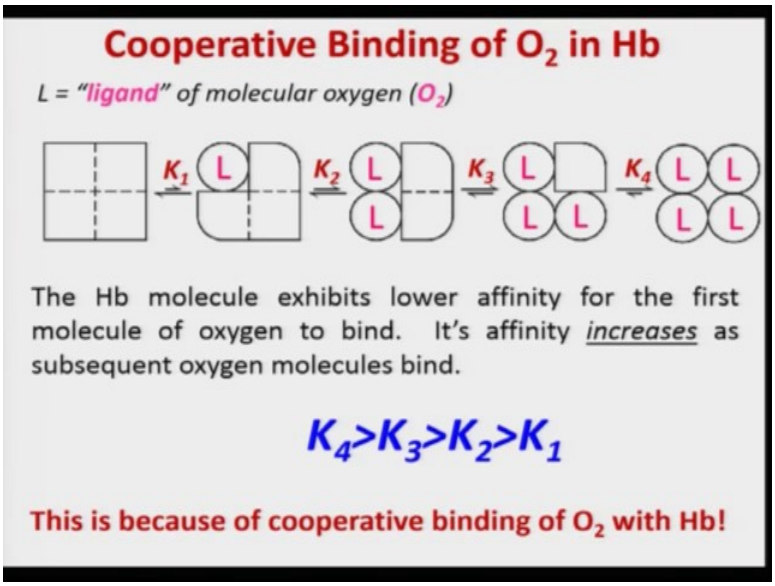

So, if the L is the molecular oxygen now, this is a tetramer so four chairs are open. Now, when one L comes in so this is actually K_1, there is a lot of structural change, then the next oxygen K_2, then the third oxygen, then the fourth oxygen.

So, what is exactly happening? In case of hemoglobin the molecule exhibits lower affinity for the first molecule of oxygen to bind, then its affinity increases as subsequent oxygen molecules bind to iron centers and as a result of that, the order of success finding constant is completely reverse. So, K_4 is more than the K_3, which is more than the K_2 and then which is more than the K_1. The order is completely reverse as compared to the statistical probability, we have just seen in my previous slide.

So, this is, because of cooperative binding of oxygen with hemoglobin, which is possible, because of this tetrameric nature. Indeed, such cooperativity is responsible for smooth transfer of oxygen from hemoglobin to myoglobin in our body. We will discuss these in details in our subsequent lectures. In summary, we have discussed so far the importance of design in discriminating oxygen and carbon monoxide just by using distal histidine as a gatekeeper.

I also have illustrated, how protein chains are actually responsible for reversible dioxygen binding in hemoglobin and myoglobin. I have also shown the cooperative binding of oxygen in hemoglobin, which would be discussed at length, after a week. In my next lecture, I will show case how protein chains indeed direct completely different

reactivity in a large variety of enzymes although, they are all going through a nearly the same reactive intermediates.

Thank you very much.