Fundamentals of Protein Chemistry
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Module - 08
Motor Proteins and Metalloproteins
Lecture - 40
Myoglobin and Hemoglobin

This last lecture of module 8, is going to be on the functions of myoglobin and hemoglobin; one of the most important proteins that are present in our body involved in oxygen binding.

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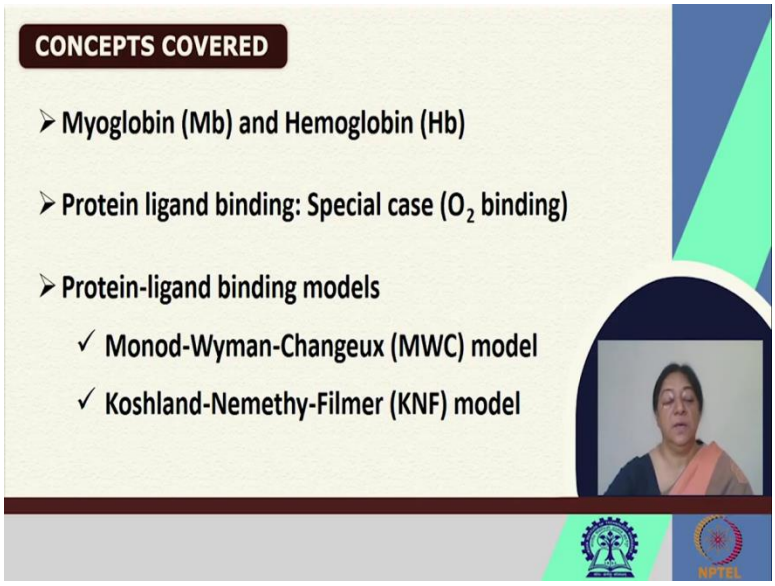

When we look at the specific topics related to myoglobin and hemoglobin, it is a special case of protein ligand binding where we are looking at oxygen binding to 2 different proteins.

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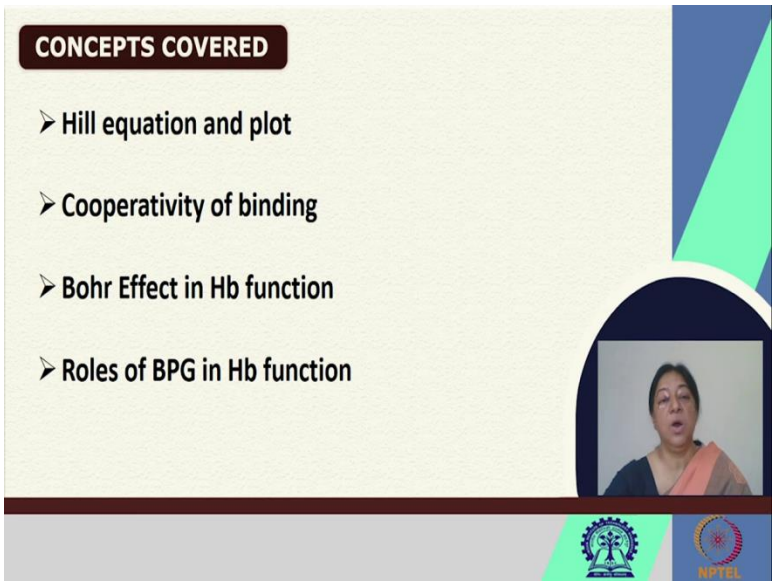

We will be looking at the different models and concepts related to what we mean by cooperativity, the Bohr effect and the roles of specific binding characteristics of some unique molecules, to hemoglobin.

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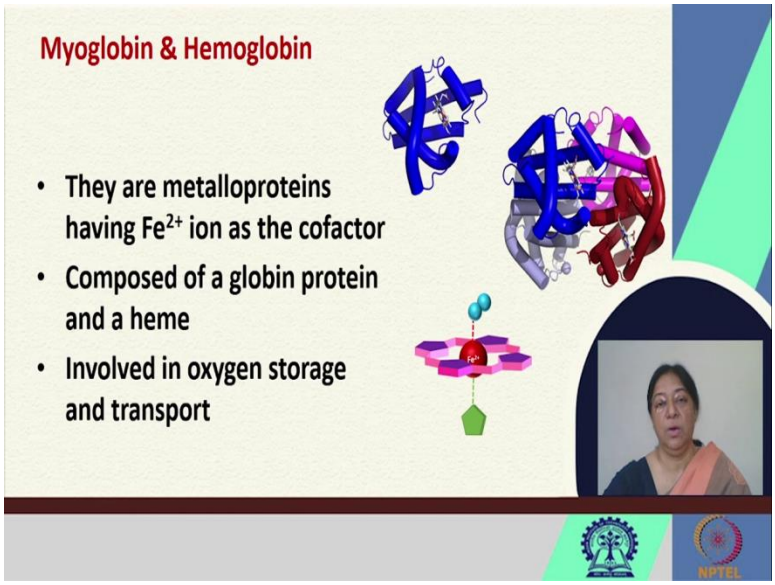

When we look at the structures of myoglobin and hemoglobin and the functionalities, we see that they are two very similar proteins in the sense that they have the globin domains. They are metalloproteins, belonging to this module where we discussed different metalloproteins. This being a specific example with the ferrous ion, the Fe^{2+} ion as the cofactor, it is composed of a globin protein and a he and involved very importantly in oxygen storage and oxygen transport.

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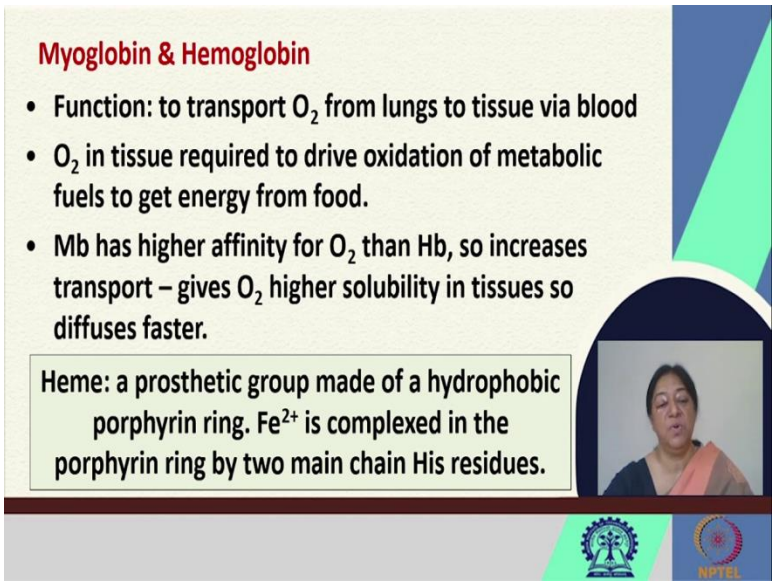

The function to transport oxygen from the lungs to the tissue via blood and oxygen in the tissue required to drive the oxidation of the metabolic fuels to get energy from food. Myoglobin, we will see has a higher affinity for oxygen compared to hemoglobin. So it increases it is transport capability, gives oxygen higher solubility in tissues and diffuses faster.

We will see the specific binding curves, that we had looked at in the protein ligand binding lectures and see how they can be associated with what we mean by a storage protein and a transport protein. The heme is the prosthetic group present, that is made up of hydrophobic porphyrin ring and Fe^{2+} is complexed in this porphyrin ring, by 2 main chain histidine residues.

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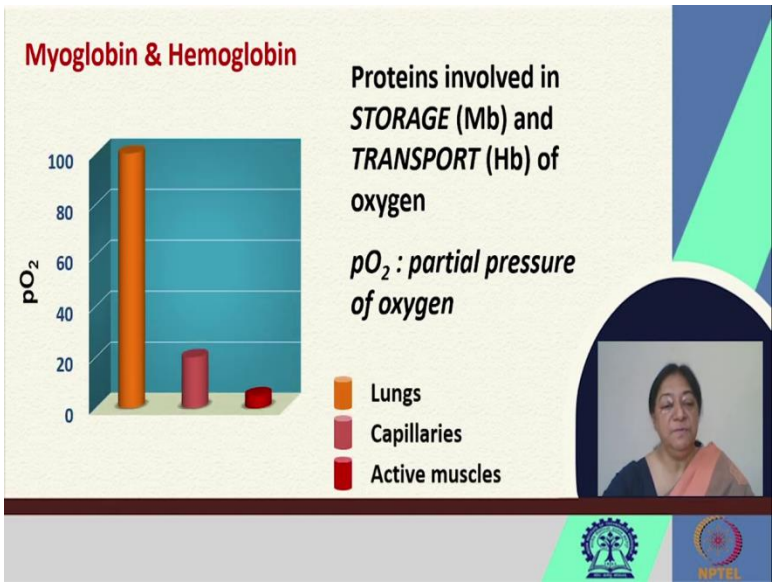

The proteins involved in storage is myoglobin and for transport of oxygen is hemoglobin. In the graph shown here [refer to slide], we are looking at the specific partial pressure of oxygen and its

percentage. The partial pressure given here in millimeter, where we have its location in the lungs, in the capillaries and in the active muscles.

So we see that in the lungs we have the maximum amount of oxygen, followed by the capillaries and then the active muscles.

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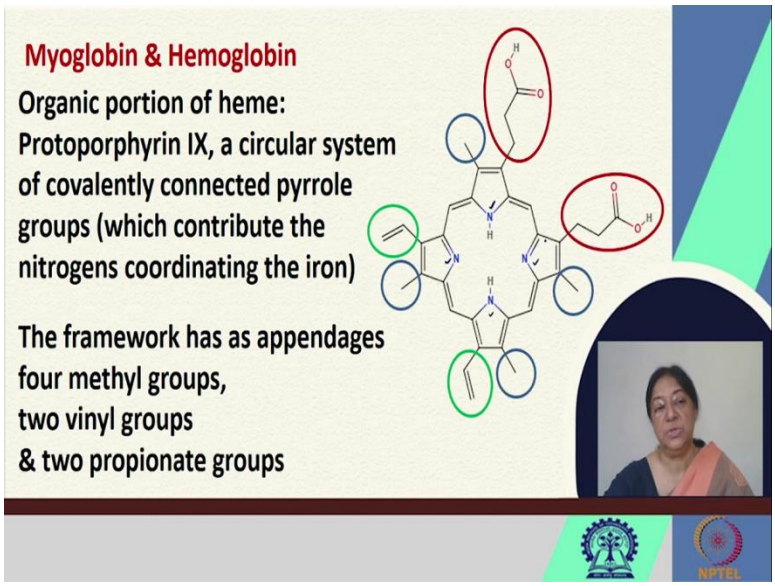

The organic portion of the heme, that is the protoporphyrin IX, is a circular system that has a covalently connected pyrrole groups which contribute to the nitrogens that coordinate the iron. So, these [refer to slide] are the nitrogens of the pyrrole groups that coordinate the iron and the framework in this case has specific appendages. These are four methyl groups, then we have two vinyl groups and two propionate groups.

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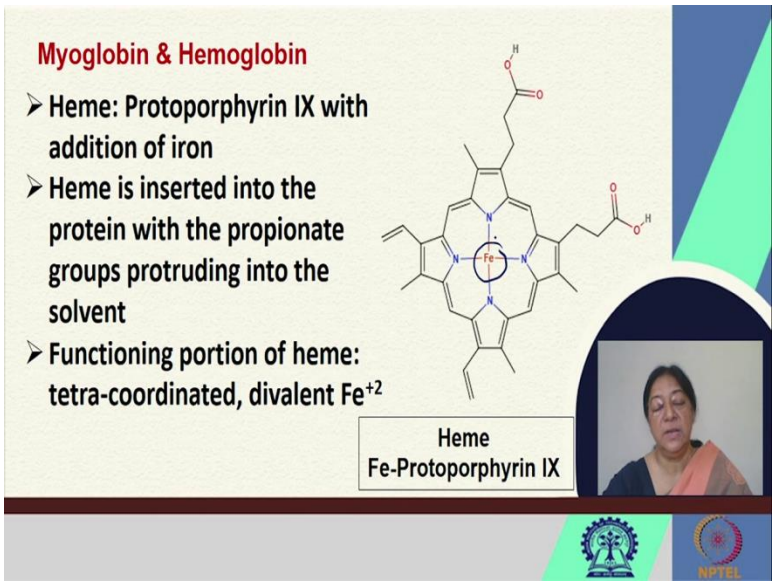

Together this then binds the iron to form what is called heme. So heme is iron bound to protoporphyrin IX in the manner shown where the coordination is with the nitrogen atoms. Heme is inserted into the protein with the propionate groups, that protrude out into the solvent and the functioning portion of the heme is the tetra coordinated divalent Fe^{2+}.

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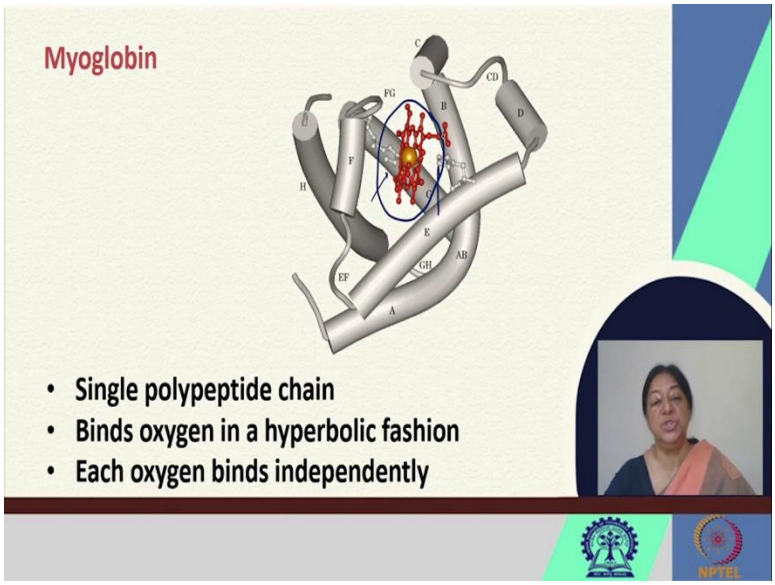

The myoglobin protein has a single polypeptide chain and it binds oxygen in a hyperbolic fashion and each oxygen in this case binds independently. It is comprised of several helices as we can see them [refer to slide] marked in this figure. Here is our heme moiety and the iron present here, as shown as this golden sphere, has the specific coordination with the histidine residues that are present in myoglobin.

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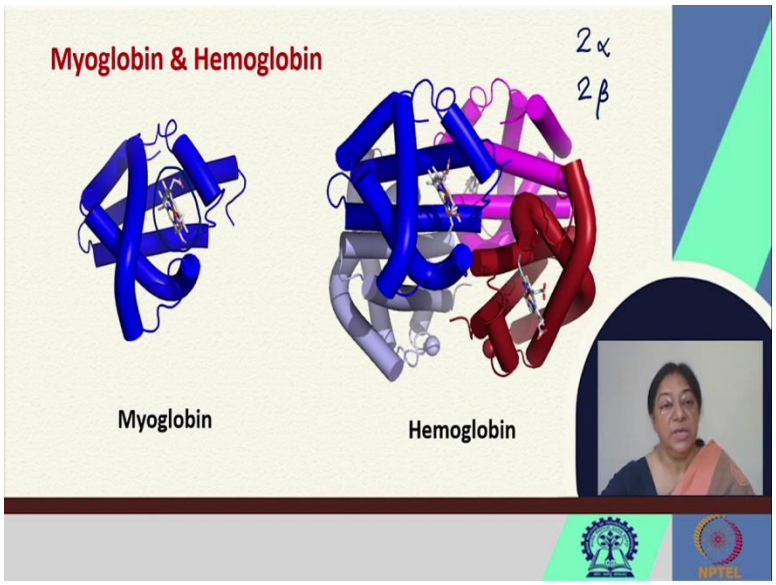

[Refer to slide] this is the structure of myoglobin; the single polypeptide chain that has a globin domain with the heme attached. The hemoglobin has, as we have seen before also, 2α and 2β subunits in a tetrameric form and each of these globin domains has a heme bound to it, in the structure of hemoglobin shown here with the 4 subunits of hemoglobin.

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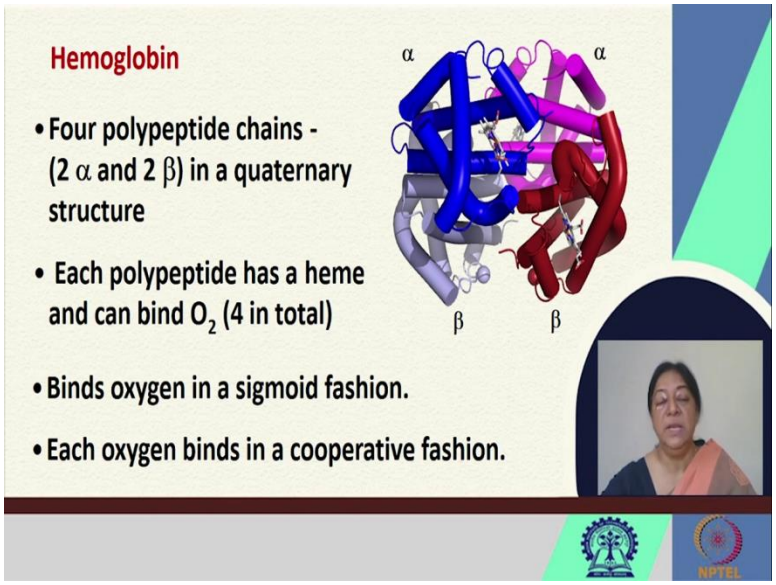

The 2α and 2β subunits have four polypeptide chains in the quaternary structure of hemoglobin and each of these can bind an oxygen. Which means that because of the 4 subunits there are 4 heme moieties and each of them can bind an oxygen. The binding in this case is a sigmoidal fashion binding and each oxygen will bind in a cooperative fashion.

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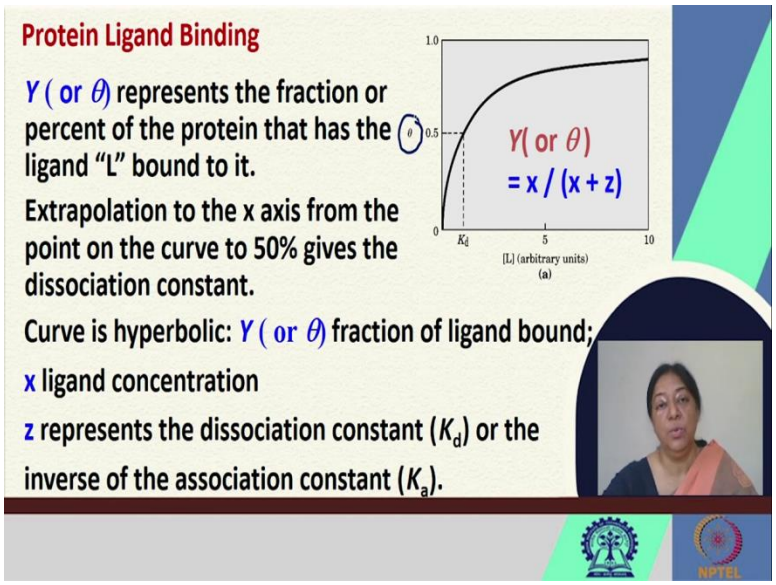

If we go back to our protein ligand binding curves, we will understand that there is a fraction of the ligand that is bound. In this case our ligand is the oxygen molecule and we had understood how we can look at or estimate the affinity for a specific ligand to a protein. In this case, we are looking at the ligand oxygen and the binding is to myoglobin and hemoglobin.

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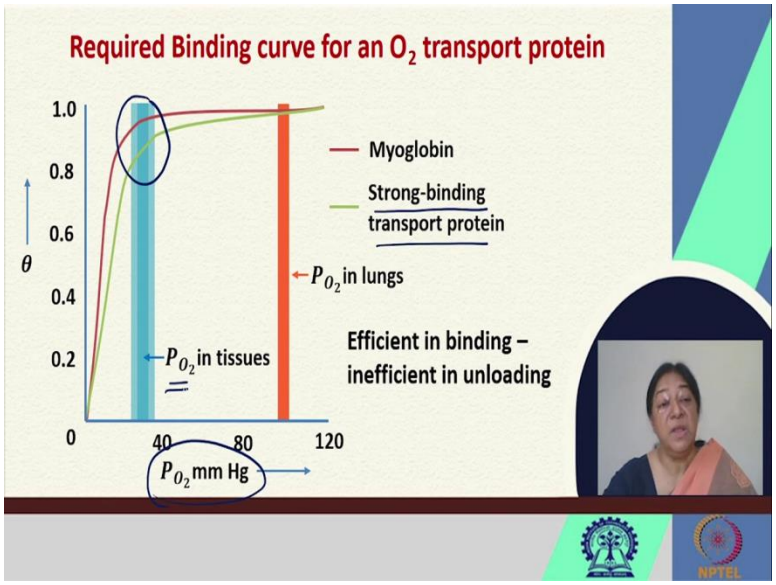

If we want a required binding curve for a transport protein, the best way for a transport protein to bind would be like myoglobin, where we would have a strong binding transport protein. Which means that if we reach the oxygen saturation level or the protein is saturated at a lower oxygen level, it means that there is strong binding. The hyperbolic fashion curve for the myoglobin, shows the strong binding.

However, this [refer to slide] is the indication of the partial pressure of oxygen in the tissues and this is the partial pressure of oxygen in the lungs. This is efficient in binding, but when there is a lower concentration of oxygen in the tissues, it will not unload the oxygen because it has a very strong affinity or a strong binding capacity. So this is inefficient in loading, unloading, but efficient in binding.

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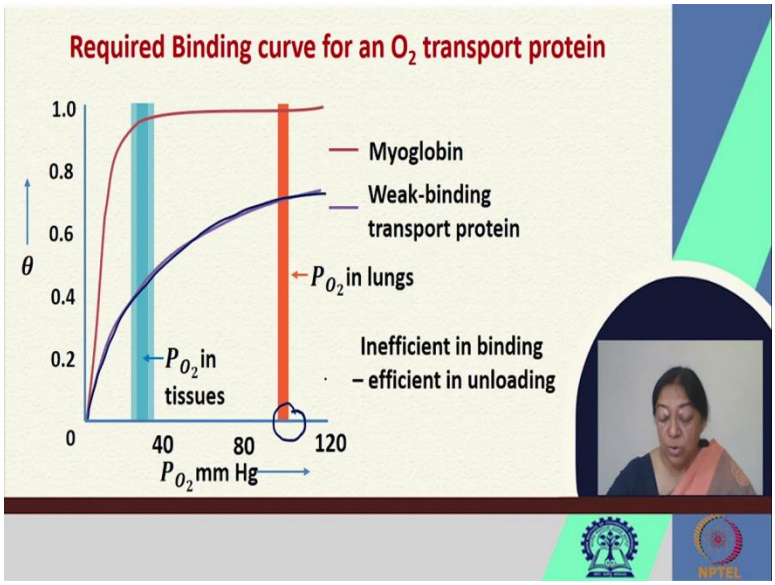

What we need is, we need a weak binding transport protein. But in this case what happens is, it does not bind to the full maximum capacity where we have a higher partial pressure of oxygen. However, it is a transport protein that could transport the oxygen. This would not be as efficient in binding, but would efficiently unload its cargo, in this case which is oxygen.

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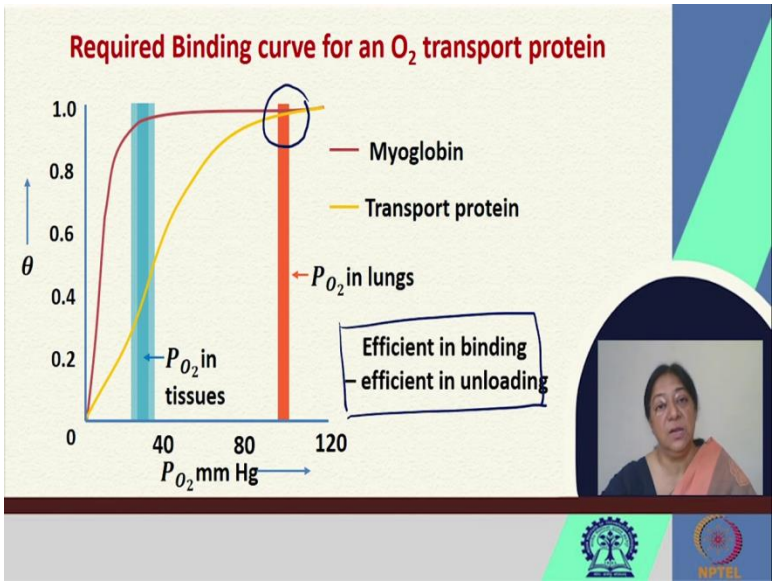

So what we need is, we need a proper transport protein that would be efficient in binding and also efficient in unloading the oxygen. This is the case where we have an efficiency in binding, indicating where the partial pressure of oxygen is high, it would have a high affinity for it, but when the partial pressure is low, it would be efficient in unloading as well.

This would be our ideal transport protein and for a storage protein we would want to bind it efficiently, faster and at a lower concentration of oxygen.

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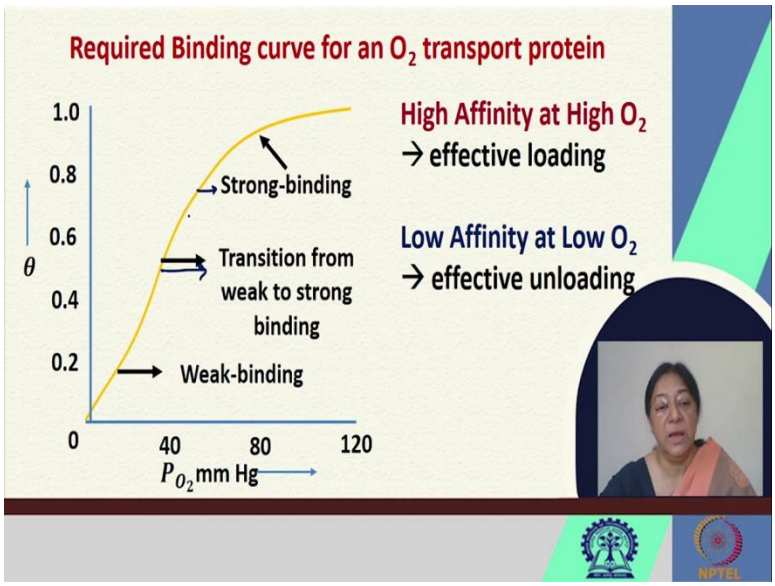

So, when we are looking at a required binding curve for an oxygen transport protein, we would have weak binding relatively at lower concentrations or lower partial pressure values for oxygen. Then there would be a transition from a weak to a strong binding.

So there would be high affinity at high oxygen levels, indicating very effective loading and there would be low affinity at low oxygen levels, indicating effective unloading. We would have the weak binding initially, the transition from weak to strong binding, which would result in effective loading at high affinity, effective unloading at low affinity of oxygen and ideal transport protein.

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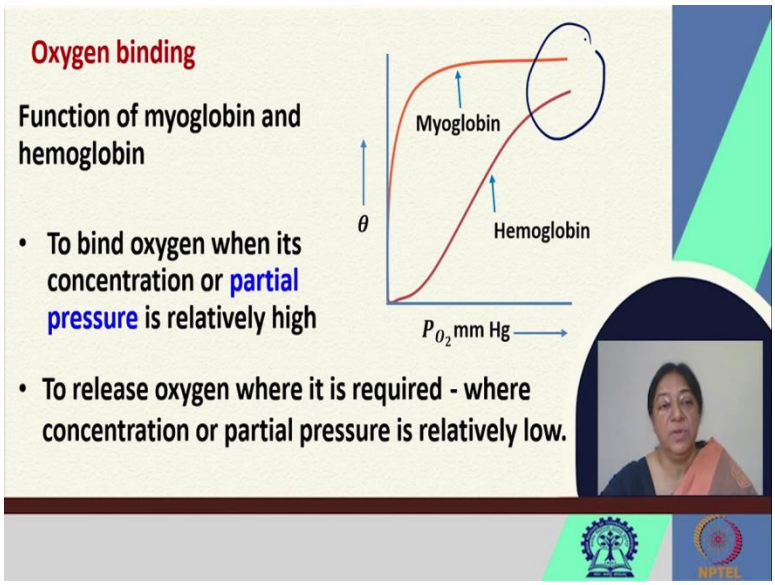

We have myoglobin that shows the hyperbolic fashion curve and we have hemoglobin that shows the sigmoidal fashion curve. The function of myoglobin and hemoglobin is to bind oxygen when it is partial. When its concentration or partial pressure is high, both of them will bind very efficiently.

But to release oxygen where it is required, the concentration of the partial pressure is relatively low, and hemoglobin will release the oxygen at a lower concentration of the oxygen levels.

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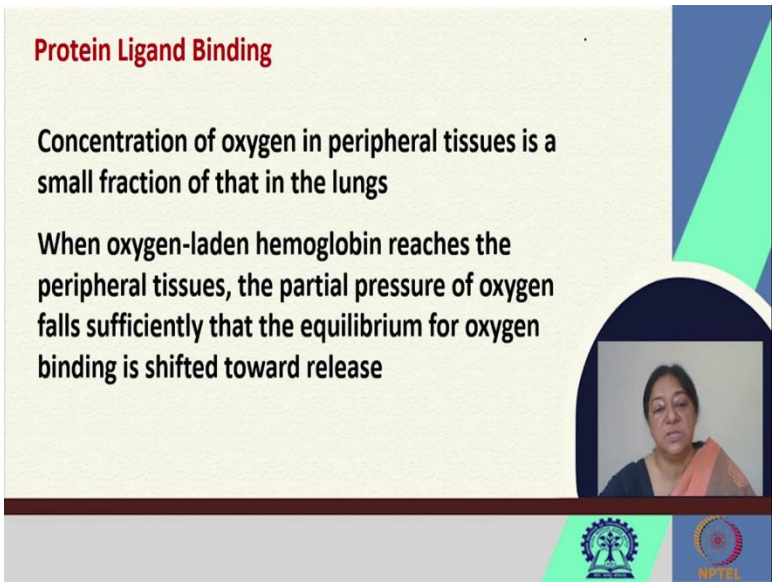

If we look at the protein ligand binding cases, the concentration of oxygen in peripheral tissues is a small fraction of what is there in the lungs. So when oxygen-laden hemoglobin reaches the peripheral tissues, the drop in partial pressure of oxygen falls to such a level, that the equilibrium for binding is shifted towards the release of the oxygen.

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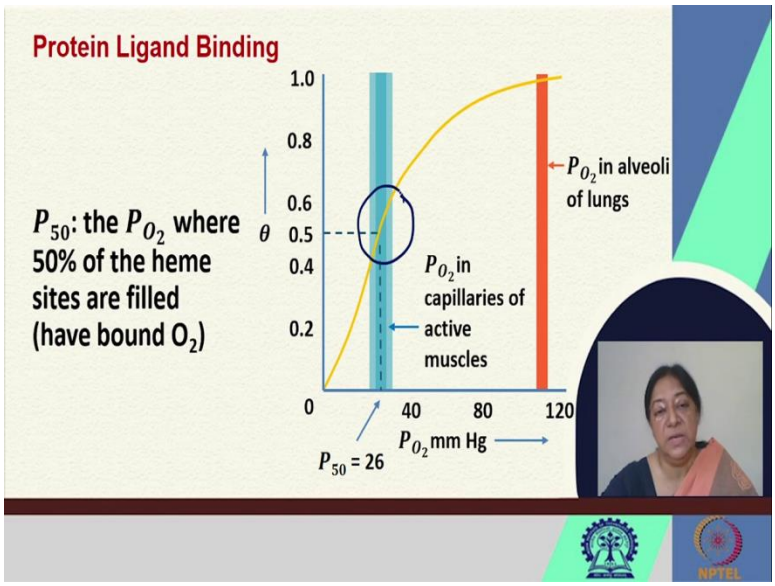

The protein ligand binding can be studied by a specific curve. We have the oxygen levels in the capillary, the active muscles and those in the lungs. As the hemoglobin travels with its cargo of oxygen from the lungs to the active muscles, it will release the oxygen at this position to be able to account for the functionality or for the activities that are required for the muscle.

The p_{50} is the partial pressure of oxygen, where 50% of the heme sites are filled, indicating that there is bound oxygen in this specific hemoglobin or myoglobin sites.

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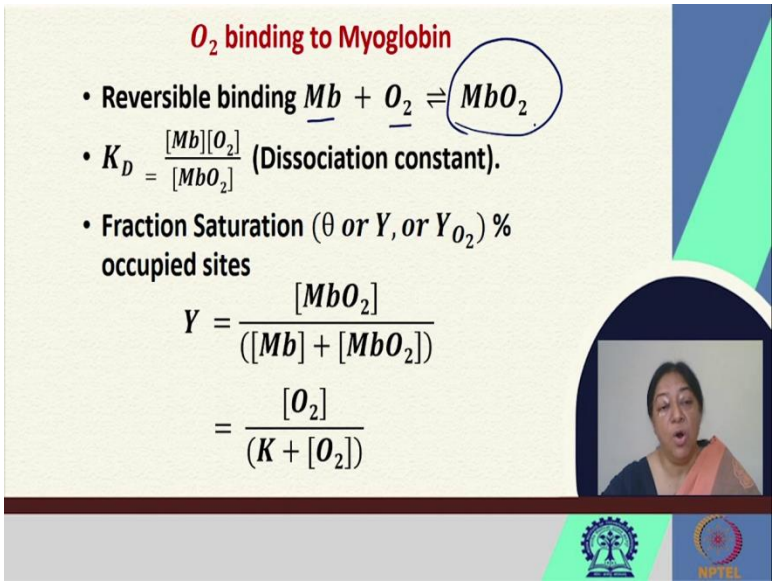

If we look at the protein ligand binding equilibria associated with this, we have a reversible binding where we have the myoglobin bound to the oxygen. To form this myoglobin oxygen

complex, the protein ligand complex has been associated with a dissociation constant, like we looked at in the protein ligand binding lectures.

So, we have a specific fraction that is associated with the occupied sites and from that, given the pressure of oxygen, we can find out the fraction of sites that are occupied.

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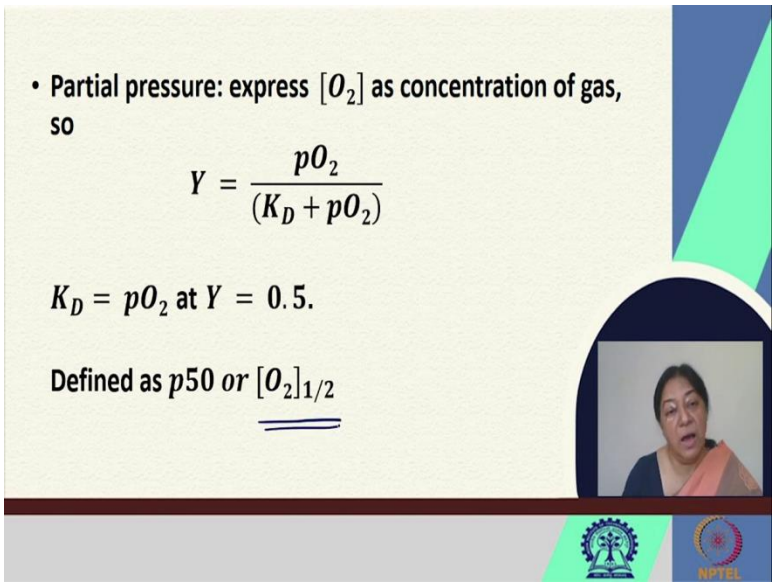

The partial pressure, that is the O_2 concentration given here, can be expressed by this fraction given: $Y = pO_2 / (K_D + pO_2)$ and we can get the fraction that is occupied, half of the sites occupied, which is defined as the p_{50} or the O_2 at half.

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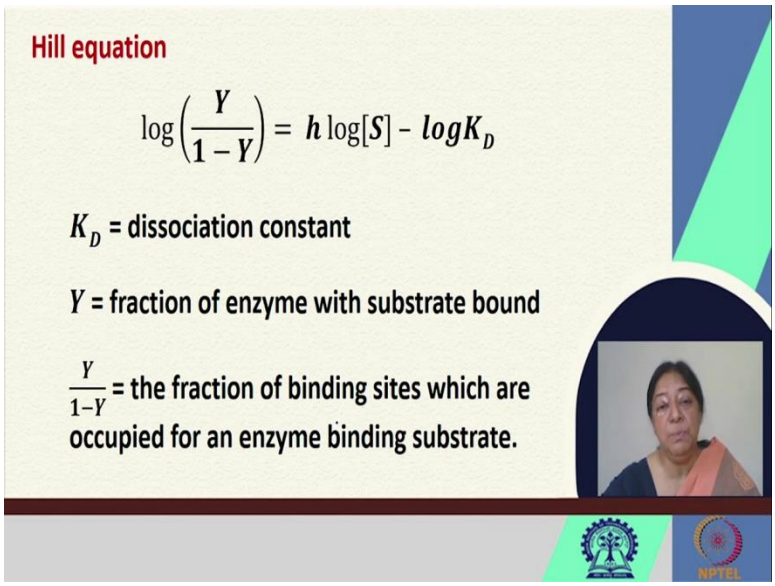

When we look at a specific example now, the hill plot as we had seen before, we can find out the fraction of the enzyme with the substrate bound, that can be plotted with the substrate concentration in this case oxygen, to give us our hill equation: log (Y/1-Y) = h log[S] - logK_D our hill plot that will give us an indication of whether there is cooperativity or not.

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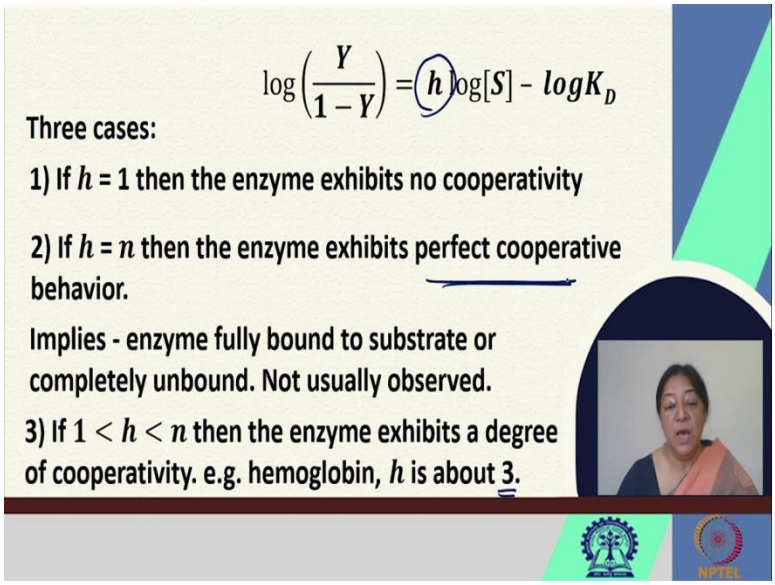

So there are three cases, that when we have the h equal to 1 in our expression, there is no cooperativity. When we have complete cooperativity or we can have a specific intermediate, when the enzyme exhibits a specific degree of cooperativity. In this case hemoglobin has this cooperativity and the equation that we show here has a value where the h value is about 3.

There are 4 such sites. We do not have perfect cooperativity, but we have a degree of cooperativity indicating that, when one oxygen molecule is bound to one of the subunits of hemoglobin, it facilitates the binding of the other oxygen molecules to the other subunits, giving us a specific degree of cooperativity.

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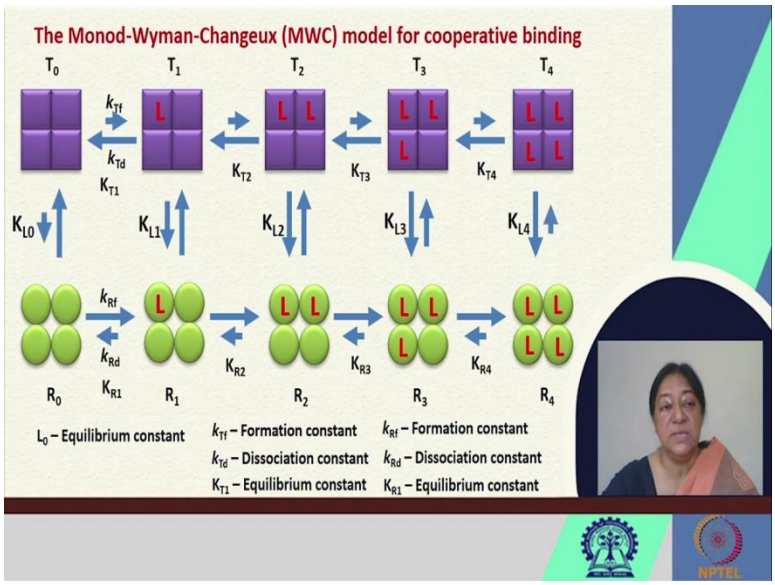

There are 2 models for this cooperative binding. One is the Monod-Wyman-Changeux model, the MWC model for cooperative binding. That gives us what is called a tense state and a relax state. There is an equilibrium between these 2 states and we have now an equilibrium constant associated with this. We have the variations where we have the ligand bound specific association constants, formation constants, and dissociation constant, associated with the equilibria that arise because of the oxygen binding.

If we look [refer to slide] at all the 4 cases, we have the 4 ligands of the 4 oxygen molecules that can bind to the different states and we can have an all or a none state, where we would have a tensed under relax state for the oxygen binding.

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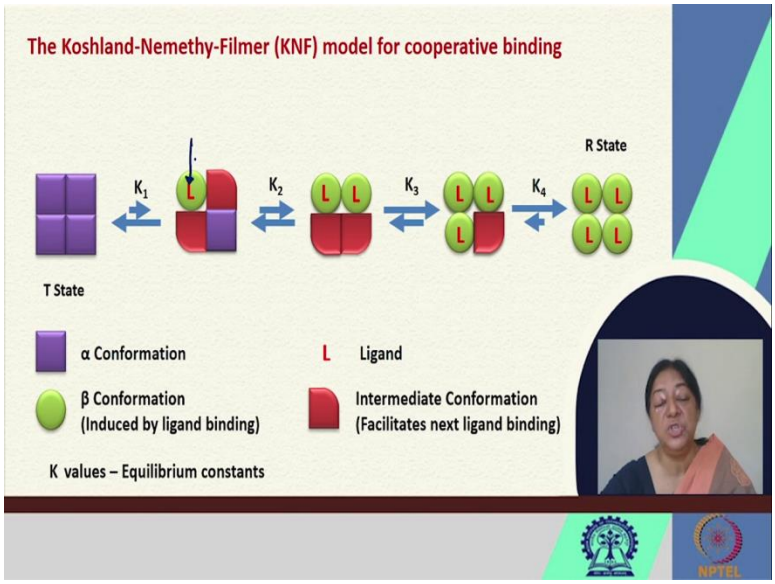

In the other model that is considered the KNF model, the Koshland-Nemethy-Filmer model for cooperative binding. Here also we have a similar situation, where we have an α conformation that is associated with the ligand binding and the β conformation that is induced in the α conformation for each of the subunits on ligand binding. And there is an intermediate confirmation for the other ones that are close to where the ligand has bound in this case, that facilitates the next ligand binding in this model of cooperativity.

Again we have the specific association, the equilibrium constants associated with the ligand binding and the cooperativity that allows the next ligand molecule to bound. Given the change in the confirmation of the specific binding site or the specific domain of the binding, that is going to allow us to get to the tense state, to the relax state on binding. But here we see that every unit has this conformational chain, as it is binding not in an all or none state.

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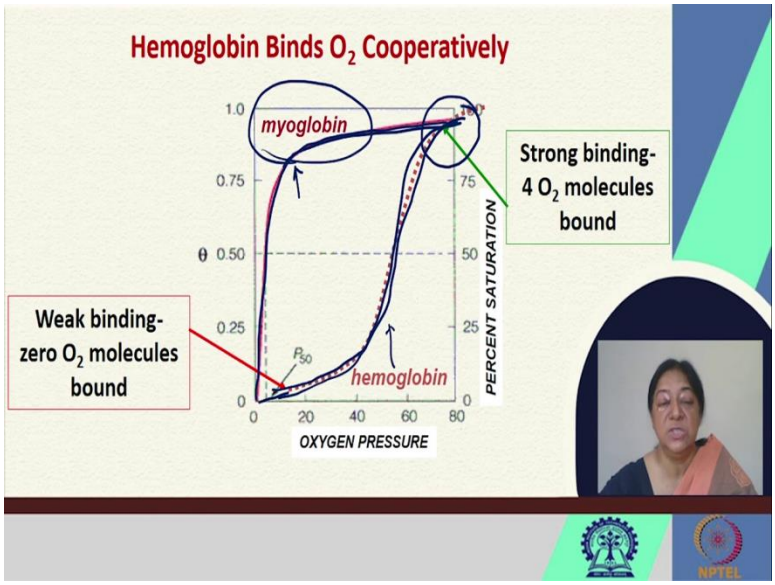

So we looked at hemoglobin binding oxygen in a cooperative manner. We have the hyperbolic fashion curve for myoglobin and the sigmoidal fashion curve for hemoglobin and we understand the reason why this is extremely necessary for the myoglobin to be a very good storage protein and hemoglobin to be a very good transport protein; where both of them will have strong binding at high values of the partial pressure of oxygen.

But as we go near the tissues, myoglobin will still retain its affinity for oxygen, but hemoglobin would release its cargo and then would gradually get back to a situation where, if it came back to the lungs with higher oxygen concentrations it would bind 4 oxygen molecules and release it near the muscles, where the partial pressure of oxygen is low.

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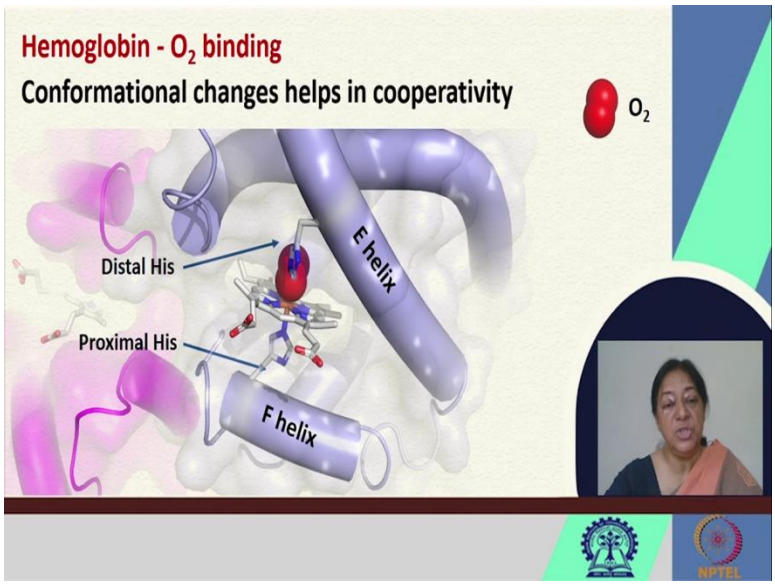

If we now look [refer to slide] at a cartoon representation of hemoglobin oxygen binding, we have our specific subunit where we have one heme moiety and the histidine residues; the proximal histidine residue and the distal histidine residue. We have the 2 helices, the E helix and the F helix and oxygen. On oxygen binding, we have a conformational change that occurs when oxygen is bound. There is a movement of the helices, which results in a conformational change that now helps in cooperativity of the binding.

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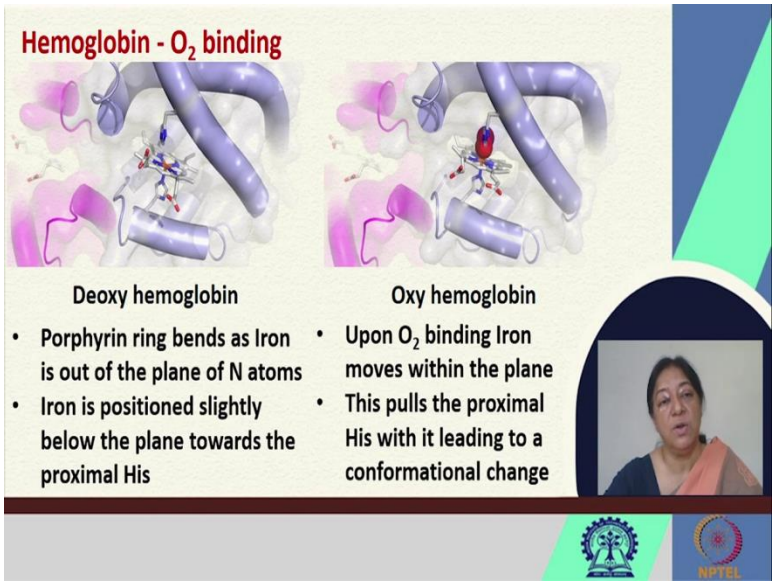

The 2 components therefore, deoxy hemoglobin and oxy hemoglobin have special characteristics. The porphyrin ring bends as the iron is out of plane of the N atoms for deoxy hemoglobin and the iron is positioned slightly below the plane towards the proximal histidine. On binding of oxygen, the iron moves within the plane and in the process, it pulls the proximal histidine with it, that results in this conformational change on oxygen binding.

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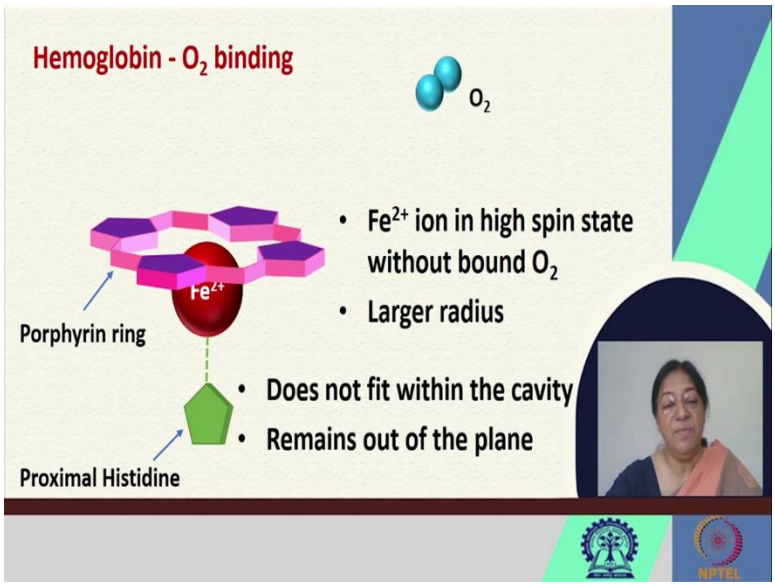

So, if we look at the porphyrin ring that is present when we have Fe^{2+} in the high spin state without bound oxygen, it has a larger radius. It is associated with the proximal histidine at this point and it does not fit within the cavity and so it remains out of the plane.

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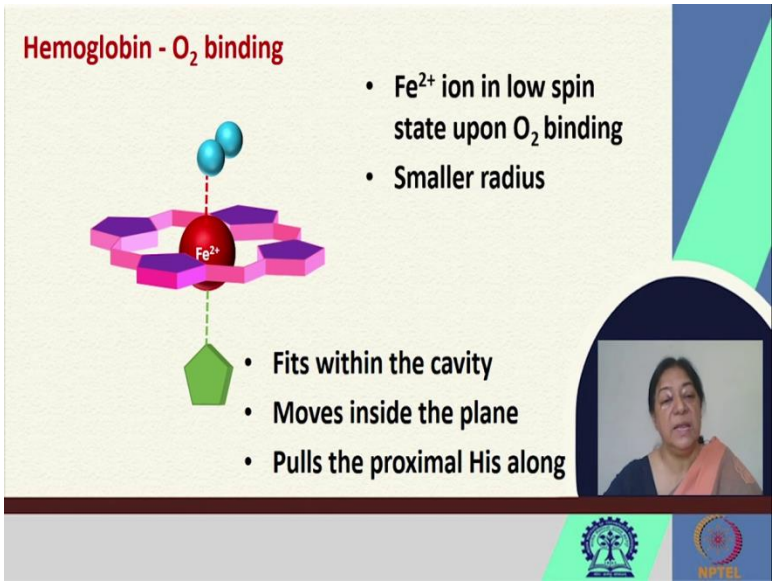

However, on the binding of oxygen what happens, it fits within the cavity. It moves inside the plane and as a result it pulls the proximal histidine along with it, because Fe^{2+} now goes to the low spin state upon oxygen binding, has a smaller radius and now can fit in the ring.

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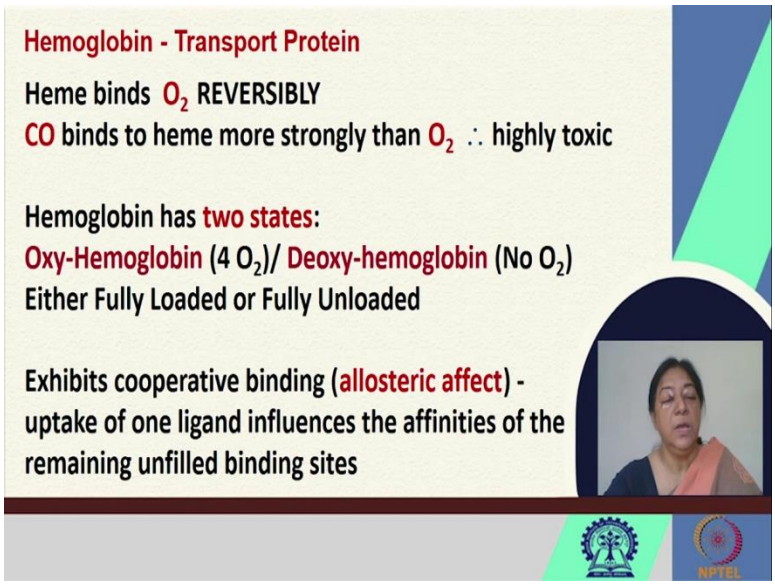

So we looked at hemoglobin as a transport protein. We found that it binds oxygen reversibly. We will be looking at an example where we will show carbon monoxide binding to hemoglobin and see how and why it is highly toxic. Hemoglobin has two states; an oxy hemoglobin state and a deoxy hemoglobin state, where it is either fully loaded with oxygen in the 4 of the subunits or fully unloaded.

It exhibits a cooperative binding, an allosteric affect where the uptake of one ligand, that is the uptake of one oxygen, is going to influence the affinity of the binding for the remaining unfilled binding sites of the heme moieties of the other subunit.

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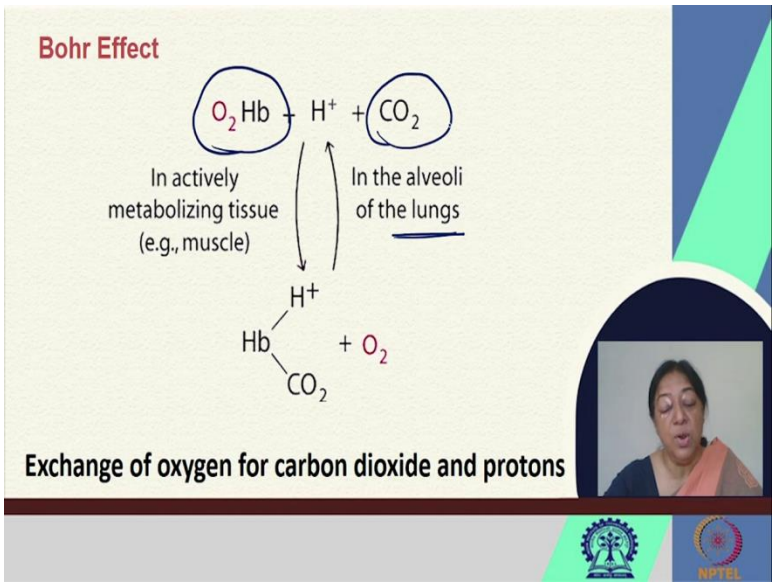

There is another effect called the Bohr effect which amounts to an exchange of oxygen for carbon dioxide and proteins. This is essential because we have the oxy hemoglobin where

oxygen is bound to hemoglobin in the lungs, where there is a high content. But in the lungs due to respiration, there is a formation of carbon dioxide.

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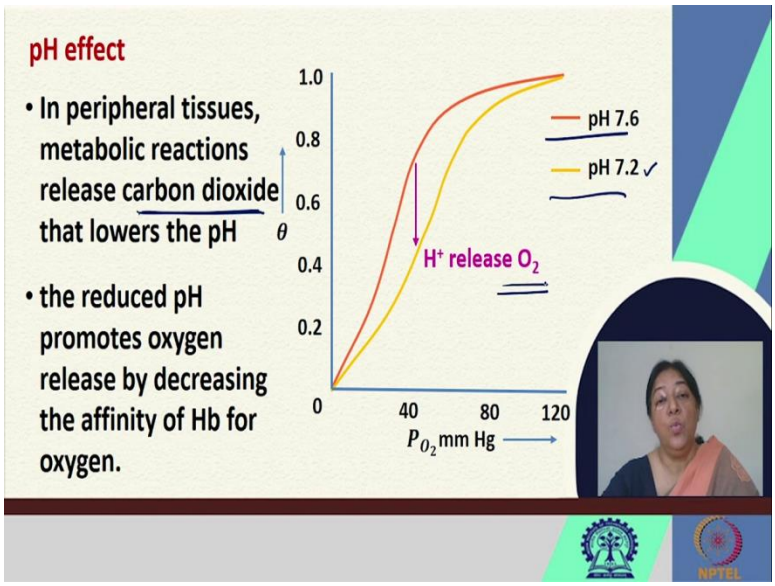

There is a bound case of carbon dioxide bound to hemoglobin and the proton effect. This pH effect found in peripheral tissues has a metabolic reaction that results in the release of carbon dioxide. So what happens in peripheral tissues, is because of several metabolic reactions, there is the release of carbon dioxide as it remains in a bicarbonate form, lowers the pH. Now, the reduced pH promotes oxygen release in a sense of the hemoglobin because it decreases the affinity of hemoglobin for oxygen.

So the lower pH, that is pH 7.2, is shown here [refer to slide]. We know the physiological pH is 7.4. So if the pH drops by just 0.2 units to 7.2, this pH is enough to decrease the affinity of hemoglobin for oxygen and so this would release oxygen at the peripheral tissues, where there are metabolic reactions going on.

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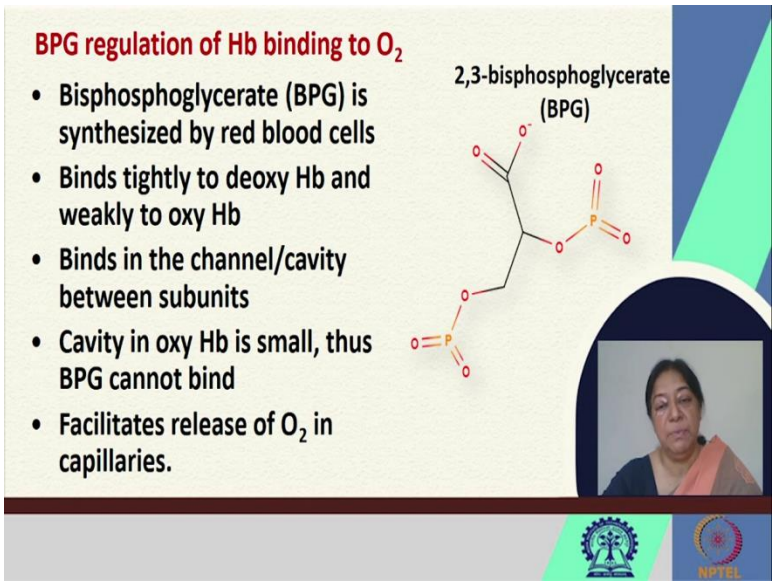

Another very interesting aspect is the bisphosphoglycerate regulation of hemoglobin binding to oxygen.

Bisphosphoglycerate is synthesized by red blood cells. It binds very tightly to deoxy hemoglobin and weakly to oxy hemoglobin. The location is between the channel or the cavity between the subunits of hemoglobin. We know that hemoglobin has 4 subunits and BPG binds between these subunits. The cavity in oxy hemoglobin being small, it is unable to bind BPG to it, but deoxy hemoglobin can and this facilitates release of oxygen in the capillaries.

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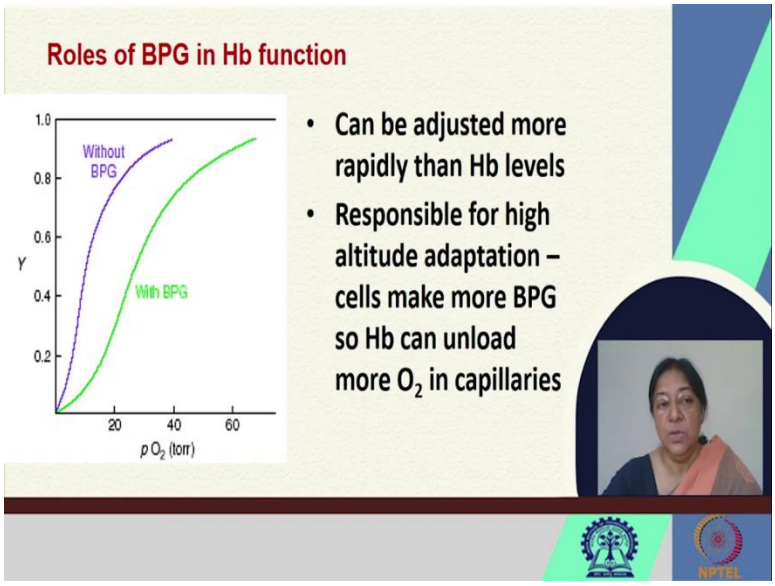

But there is another use for BPG because it can be adjusted more rapidly than hemoglobin levels, this is responsible for high altitude adaptation. At that point in time, when BPG is synthesized at a greater degree in the body, the cells make more BPG. So what happens is, BPG binds to the

deoxy hemoglobin and thus the hemoglobin with bound oxygen can unload more of the oxygen available to it.

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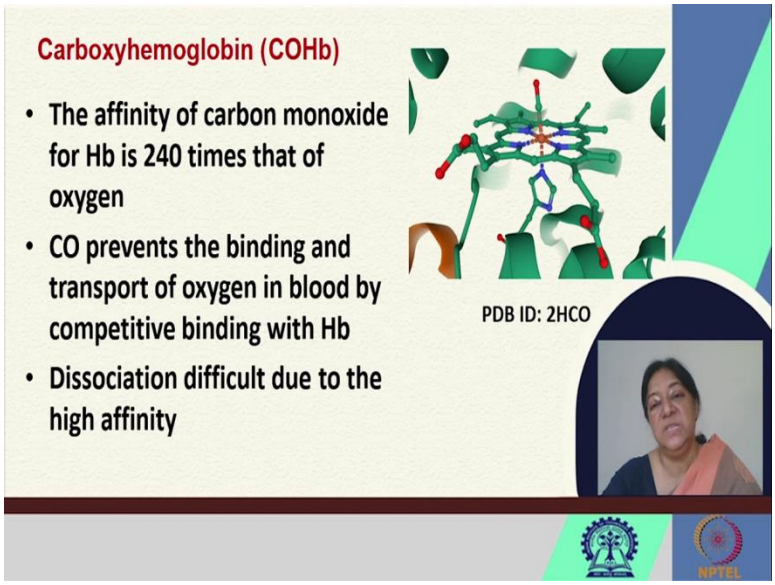

In carboxyhemoglobin, where we have carbon monoxide bound to hemoglobin, the affinity of carbon or monoxide for hemoglobin is 240 times more than that of oxygen. So, carbon monoxide prevents the binding and transport of oxygen in the blood by competitive binding with the hemoglobin molecule and because of this high affinity, dissociation of CO from hemoglobin is very difficult.

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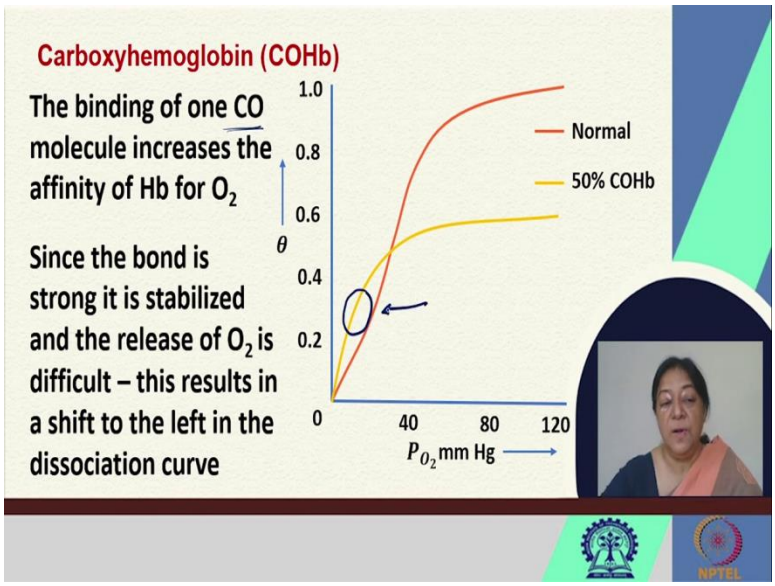

So what happens in this case is, there is a normal transport protein profile as we can see in the graph here [refer to slide] and there is one that has 50% carbon monoxide Hb. The binding of one of these CO molecules interestingly, increases the affinity of hemoglobin for oxygen. But because the bond is stabilized due to the presence of the carbon monoxide, it is not released. So this amounts in a shift to the left of this curve, the dissociation curve.

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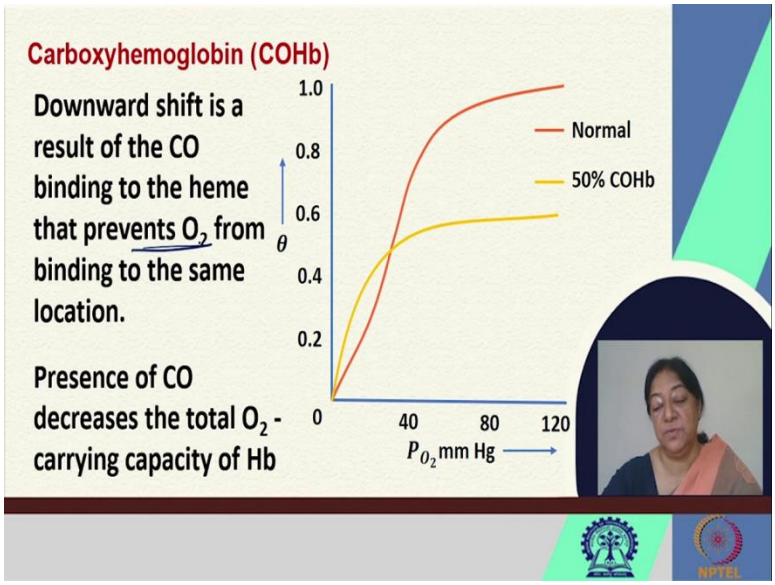

This shift prevents oxygen unloading in the peripheral tissues, where the concentration is low, making the oxygen concentration much lower in the tissues than normal. As a result, there is also a downward shift because there is the prevention of oxygen binding to the same location. So the presence of CO decreases the total oxygen carrying capacity of hemoglobin, resulting in the CO poisoning as it is known.

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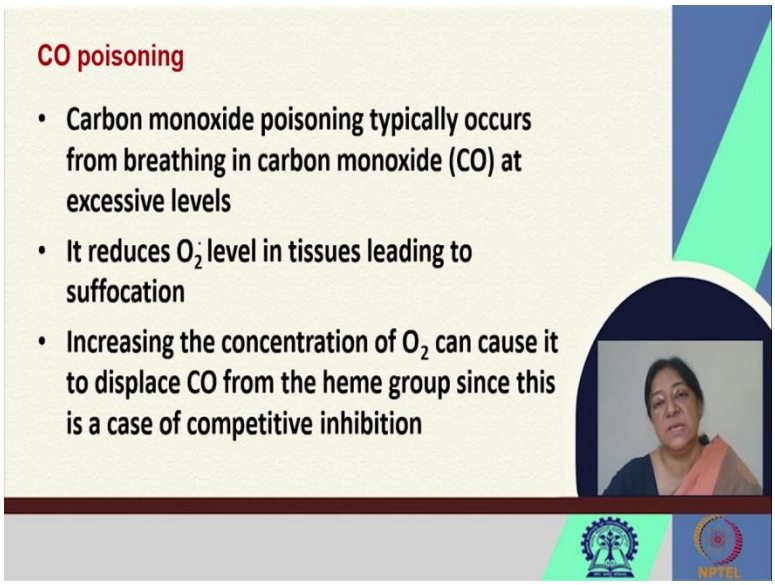

Carbon monoxide poisoning typically occurs from breathing in carbon monoxide at excessive levels. This reduces the oxygen level in tissues because the oxygen is not unloaded in the tissues where the CO has bound and this binding of the CO has resulted in the hemoglobin oxygen stabilization of the bond, which prevents its release. But increasing the concentration of O_2 can cause it to displace the carbon monoxide from the heme group, since this is a case of competitive inhibition.

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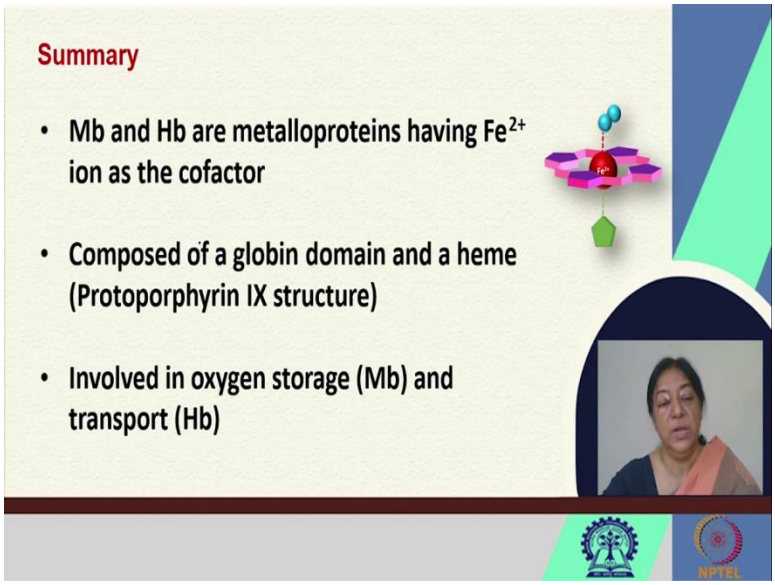

In summary, we looked at the myoglobin and hemoglobin metalloproteins that have Fe^{2+} as the cofactor. This is composed of a globin domain and a heme, that is the protoporphyrin IX structure with the Fe^{2+} ion attached to it. This is involved in oxygen storage, as we saw in myoglobin and in transport, as in hemoglobin.

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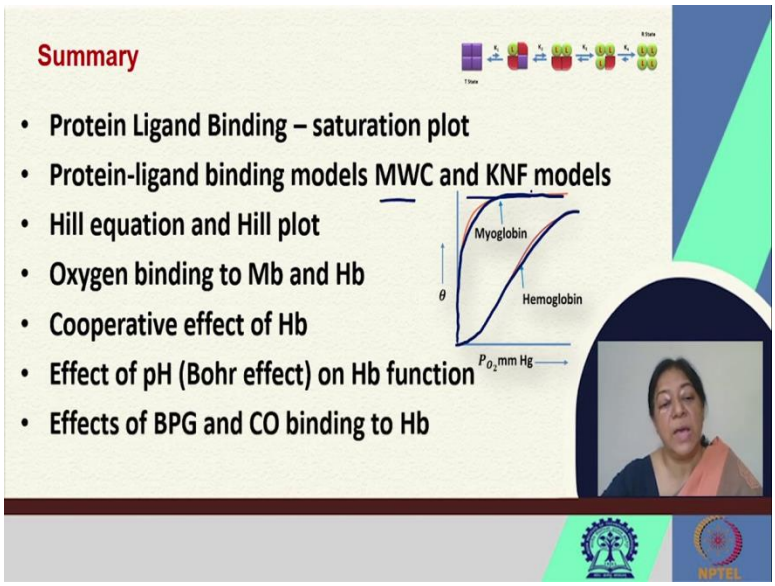

We looked at the specific types of curves associated with their binding and we found out that this protein ligand binding in the saturation plot, has a specific mode where we have a hyperbolic type binding for myoglobin, it being a storage protein and for hemoglobin we have a sigmoidal curve, it being a transport protein.

In the protein ligand binding models we looked at two different types of models, the MWC and the KNF model. The hill equation and the hill plot for oxygen binding to the proteins, indicated that there was a cooperativity associated with oxygen binding to hemoglobin and we looked at the effect of pH on the functioning of hemoglobin. In addition, we had a look at the effects of BPG and CO binding to hemoglobin and how this is monitored.

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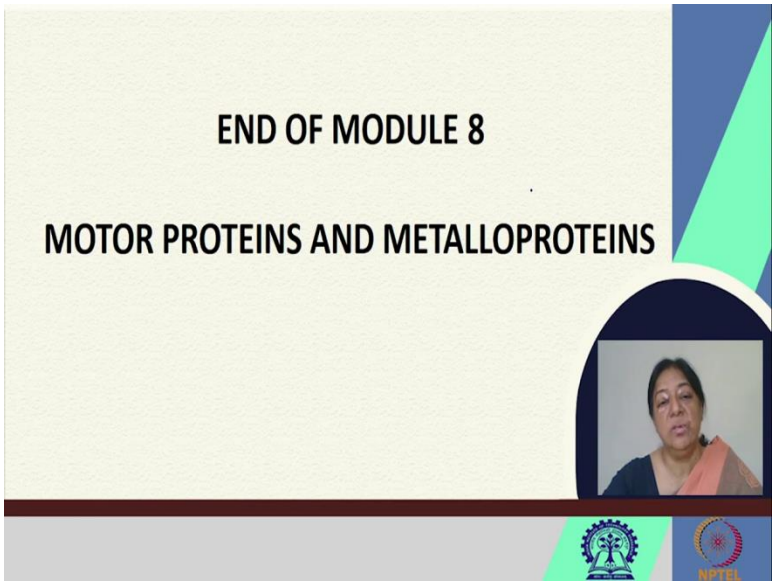

This is the end of module 8, where we spoke about motor proteins, metalloproteins and of myoglobin and hemoglobin.

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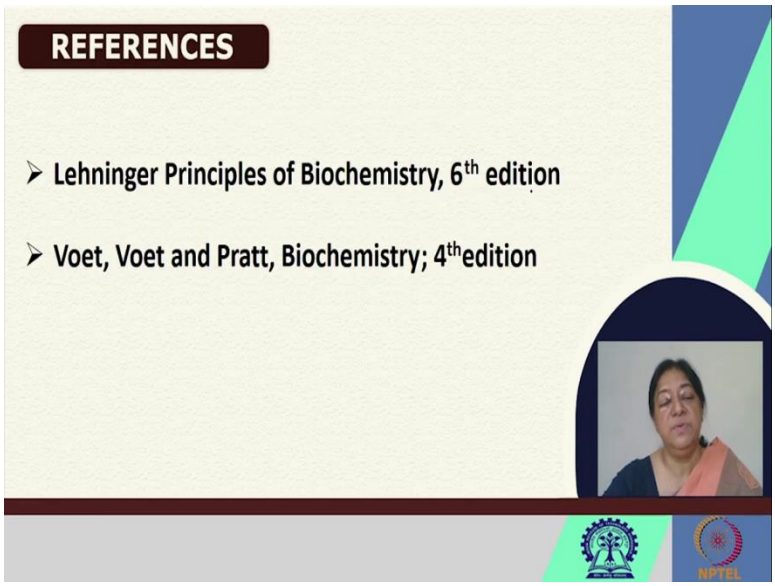

These [refer to slide] are the references.

Thank you.