Introduction to Complex Biological Systems
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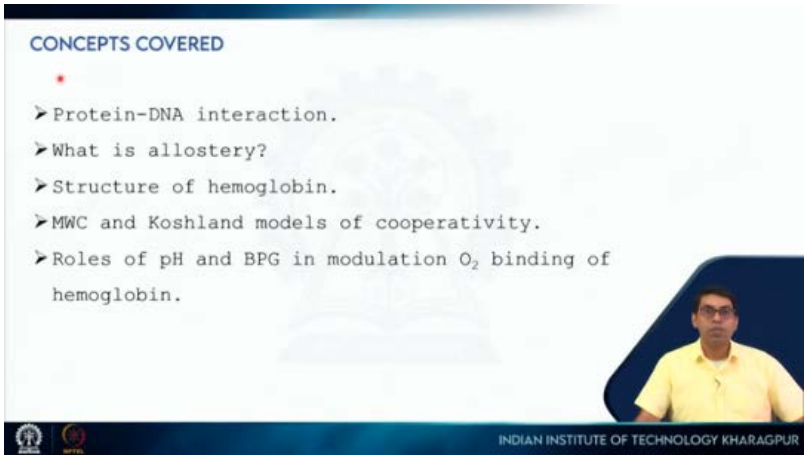
Lecture 14
Allosteric regulation of proteins, e.g. haemoglobin

Welcome back. So in this lecture, I am going to finish up on the protein-ligand interaction, and I will talk about the allostery of proteins. So I will complete the discussion on protein-DNA interactions. So that will complete the discussion on protein-ligand interactions.

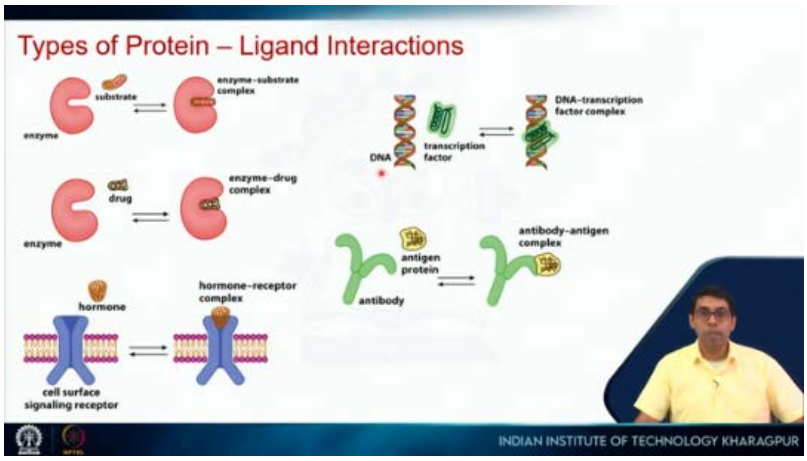


Then I will define what allostery is. As an example, I will discuss the structure of hemoglobin. I will also discuss models of cooperativity and the roles that pH and BPG play in the modulation of oxygen binding to hemoglobin. So we have already seen this in the previous lecture slide that there are different types of protein-ligand interactions. It can be a protein-small molecule interaction.

It can be a protein-protein interaction, like in the case of hormones and cell receptors, or it can be a protein-DNA interaction. So we are going to focus mostly on this. Protein-antigen interaction, or antibody-antigen interaction, is also a protein-protein interaction. So today, I am going to focus on these interactions where a protein interacts or binds to DNA specifically. So it can also bind to DNA non-specifically, but we are going to talk about specific binding.

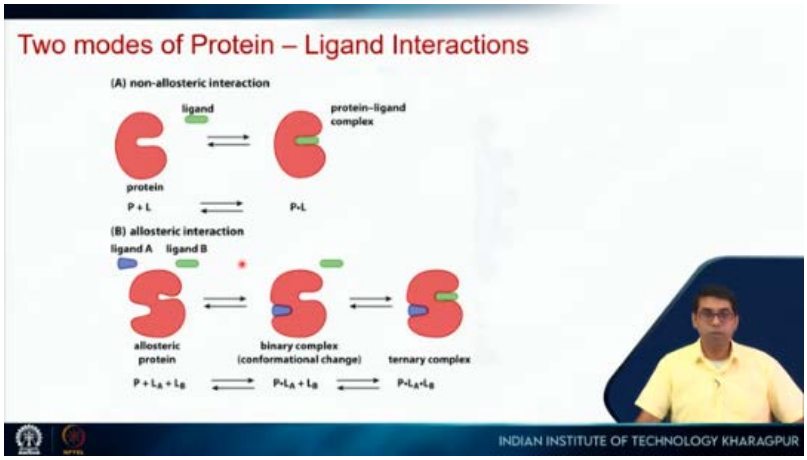
And what I mean by specific binding is that the protein identifies a particular sequence of nucleotides on the DNA and binds to that sequence. So these are the two types of protein-

ligand interactions that we have discussed. So I am going to finish up on this where a protein is there, and it binds the ligand, and the protein-ligand complex is formed. And then we also discussed two types of modes of interaction. One is the lock-and-key model, where the ligand fits perfectly to the binding site, or the induced-fit model, where the ligand loosely binds and then that results in a structural change in the protein, and then the ligand binds more tightly.



But both will come under this type of interaction. The second type of interaction, which I am going to focus on mostly today, is allosteric interaction. In this case, there are two molecules. One molecule binds to a particular site of the protein, and this type of molecule here is called ligand A, but we will call this a modulator. So the modulator will bind here, and that binding will result in a change in the shape of the binding site of the protein, which is at a distance.

And then the protein will bind the ligand B, which we will call the ligand. So, this is the ligand, and this is the modulator. So, the modulator binding results in a change in the shape of the binding site for this ligand, and then the ligand will bind. So, this will be a positive modulator. We will also see a negative modulator where the ligand can bind, but when the modulator comes and binds, the ligand cannot bind. So, we will see examples of this.



Let us get back to this non-allosteric interaction. So, this is just a basic refresher on the structure of DNA. You have already seen this. So, DNA is a double helix, having a double helix structure where two strands form complementary interactions with each other. So, that is the backbone of the DNA, and the bases are forming hydrogen bonds with each other.

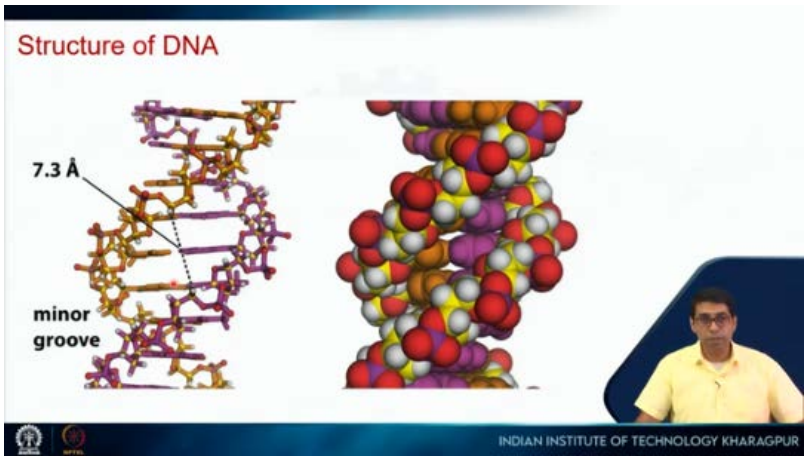
So, we have AT and GC pairs inside. If you look at the structure of the DNA, even though it is helical, it is not very symmetric. So what I mean by that is if we look at the distance between the two strands, it turns out that the distances are of two types. One is, so in this case, you see the distance is 15 angstroms. So, this creates a groove.

So if we have this sphere, we represent each atom as a sphere instead of a ball and stick, then you can clearly see the formation of a groove. You see this is a groove, right? And this groove is called the major groove. If we look at the other side, this one or this one, which is shown here in more detail, you see here the distance is smaller that is 7.3 angstrom. And you can see that the two strands are very close to each other, and the gap between them is much smaller.

So this one is called the minor groove. So there is a major groove. So this will be the major groove, and this will be the minor groove. In the case of the major groove, the distance between the two strands is 15 angstrom and in the case of the minor groove, it is 7.3 angstrom.

So the distance is actually almost half, actually less than half, than that of the major groove. Another interesting aspect of DNA structure is, if we look at the backbone, the backbone is formed of phosphate and sugar, right? So they are exactly the same for all the nucleotides. The difference comes from the base, the nitrogenous base, which is either A, T, G, or C, and those nitrogenous bases are here. Now, if DNA has to bind specifically to a particular sequence, it has to make contacts with the bases. If it only interacts with the backbone, then the backbone is exactly the same for all nucleotides, right?

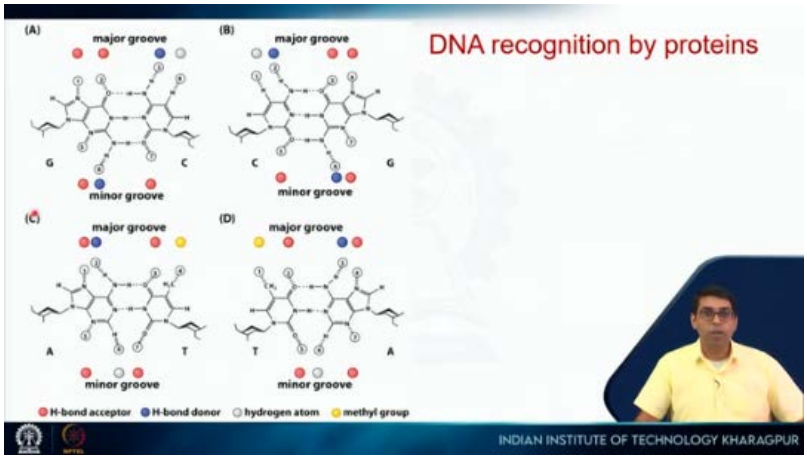
So, it has to somehow interact with these nitrogenous bases and we will see that the majority of these interactions are either hydrogen bonds or hydrophobic interactions. So, if we focus on this AT and GC pair, the GC pair, GC or CG, is shown on the top, and AT or TA is shown at the bottom. So, if you focus on this structure, you can see that there is the sugar, and then there is this nitrogenous base that is your G. For the complementary strand, this is the sugar, and then there is the nitrogenous base, which is C. So, G and C form three hydrogen bonds like this. Similarly, you can have the reverse, which is C and G, right? This part of the base forms the major group, and this other side forms the minor group. If we look at the functional groups which are exposed, that is the side chains of proteins of amino acids on the proteins can interact with then we will see these are the functional groups which are available in the major group.



So this is a red hydrogen bond acceptor. This is also a hydrogen bond acceptor, which means that *NH* or *OH* type of groups from amino acid side chains or even the backbone can interact with this. This is a hydrogen bond donor, so this can interact with oxygen or

C double bond *O* kind of groups on the protein side chains or the backbone. This is just hydrogen, so it can form some sort of hydrophobic interactions. The same is true for this.

On the minor group side, we see again two hydrogen bond acceptors and one hydrogen bond donor. So, it turns out that in the case of minor groups, we have fewer functional groups plus, the gap is also smaller so there is not much space for amino acid side chains to interact, which means that most of the interactions that we see between protein and DNA are through the major group, but that does not mean that we do not see any interaction with the minor group, so it is also there. Similarly, for the AT pair, there is a hydrogen bond acceptor, hydrogen bond donor, hydrogen bond acceptor, and also we have this methyl group, which can form very good hydrophobic interactions with hydrophobic residues of amino acid side chains. And this is actually true.

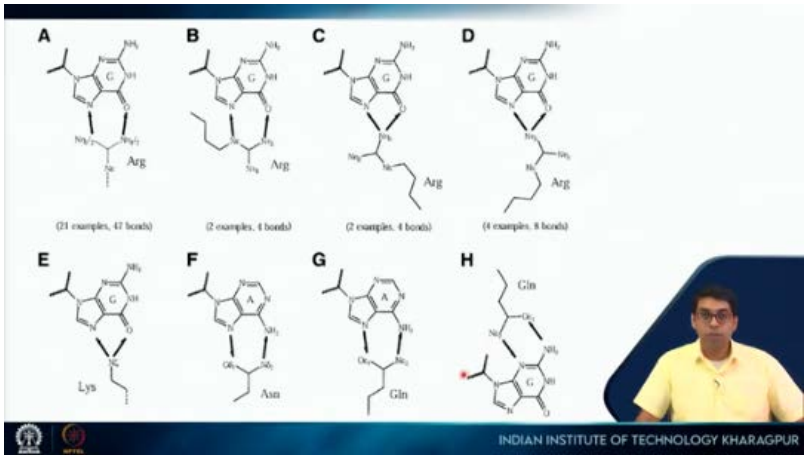


So we do see some of those interactions shown here. So on top, what we see are the nitrogenous bases, and at the bottom, we see various side chains. So what we see here is the interaction of guanine. With the arginine side chain, so the arginine side chain can form hydrogen bonds with the guanine, and it turns out that they form very nice pairs of hydrogen bonds. So this is the guanidinium moiety of the arginine side chain, and it can, so this is the hydrogen bond donor, so the *H* is here, and these are the hydrogen bond acceptors. So we can have a different orientation of the side chain.

We can have one NH_2 group forming hydrogen bonds with both. So there are two hydrogens. So each of those can act as a hydrogen bond donor and similarly shown here. So guanine arginine interaction is very common, and this type of hydrogen bond is seen

quite often. So it means that if you have arginine in the binding site of a protein or a transcription factor,

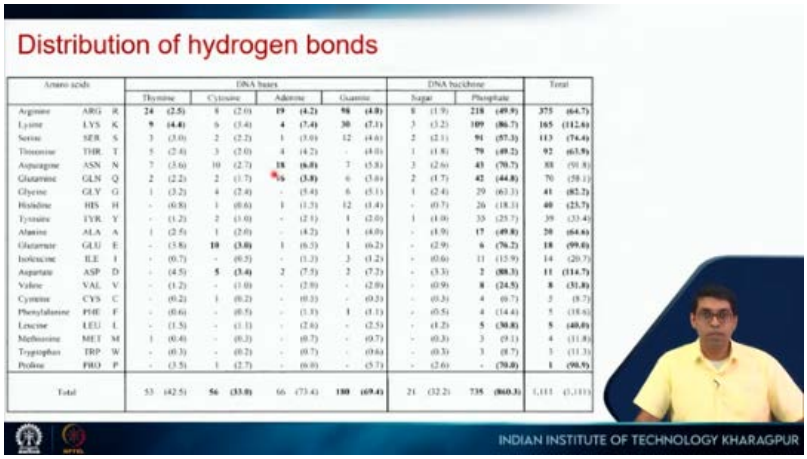
then, you can guess correctly that this will most probably form hydrogen bonds with guanine on the DNA in the major group. Similarly, lysine can form hydrogen bonds with guanine, asparagine can form a pair of hydrogen bonds with adenine, glutamine can also form a pair of hydrogen bonds with adenine, and glutamine can form a pair of hydrogen bonds with guanine. So, all these hydrogen bonds are seen quite often in various protein-DNA structures that have been solved. This table summarizes some of such interactions. So, I am not going to go through all of these; you can pause the video here and look at it for yourself.



I will just highlight one or two interactions. So, as I said, arginine and guanine. So, this is arginine, and its interaction with all four bases is shown here, and these are all hydrogen bonds. So, for the number of proteins, several proteins were studied here, and arginine side chains were found to interact, the 24 hydrogen bonds between arginine and thymine, 8 between arginine and cytosine, 19 between arginine and adenine and 98 between arginine and guanine.

So, you can see that there is an overwhelming number of hydrogen bonds between arginine and guanine. So, this is most probably the most common interaction that is seen. Similarly, if we look at glutamine, you can see that glutamine forms hydrogen bonds very efficiently with adenine. So, glutamine and asparagine both. So, the side chains are very similar, only the distance varies.

So, glutamine and asparagine both form nice hydrogen bonds with adenine. So, how does this look structurally? Structurally, it looks something like this, where we have a helix. So, this type of helix is called a DNA recognition helix. So, this helix will fit nicely into the major groove.

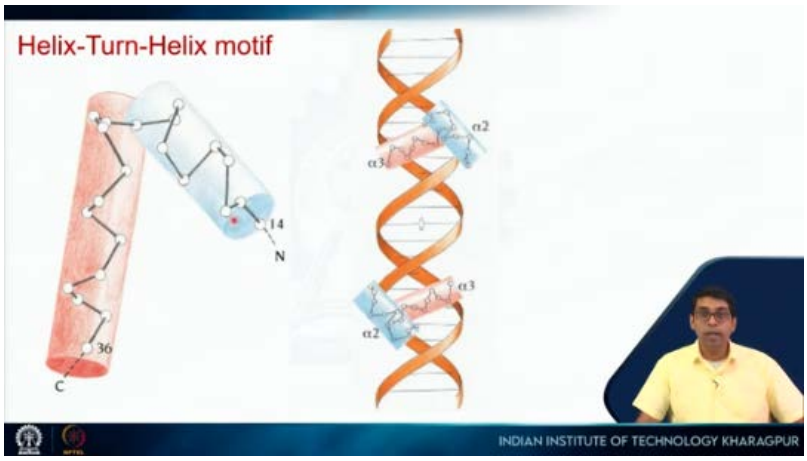


So it turns out that the size of the major group and the sort of the radius of the helix fits so well that the side chains that are coming out of this helix can nicely interact with the bases as well as the phosphate and sugar groups on the DNA. So, a helix will insert itself like this, and it can interact with the DNA. So, it can interact with the sugar and the phosphate group, which will give it nonspecific interactions, but of course contribute to the binding affinity. And the side chains, some of the side chains will also interact with the nitrogenous bases, which will give it the specificity towards that particular sequence.

So another thing that I would like to point out here is this particular motif called the helix-turn-helix motif. So what are motifs? Motifs are something that are found in proteins, in several proteins. So we have talked about the hierarchy of protein structure. So I talked about primary structure, secondary structure, tertiary structure, and then also quaternary structure.

So, secondary structure is where we have these helices and beta strands, and then quaternary structure is where all these secondary structures come together to form the three-dimensional folded form. Motifs are something that is in between the secondary structure and tertiary structure. So, for example, this one is a helix. So, this is one helix, then there is a turn followed by another helix. So, this is called a helix-turn-helix motif.

Now, this particular motif is found in many structures. So, this one by itself will not be able to form a stable structure. So, this will be a part of a structural structure, but this helix-turn-helix motif is found in many proteins, especially those that bind DNA. And this motif will bind DNA like this, where this second helix of the helix-turn-helix motif turns out to be the DNA recognition helix, and it will insert itself into the DNA major group. So, we will see one example.

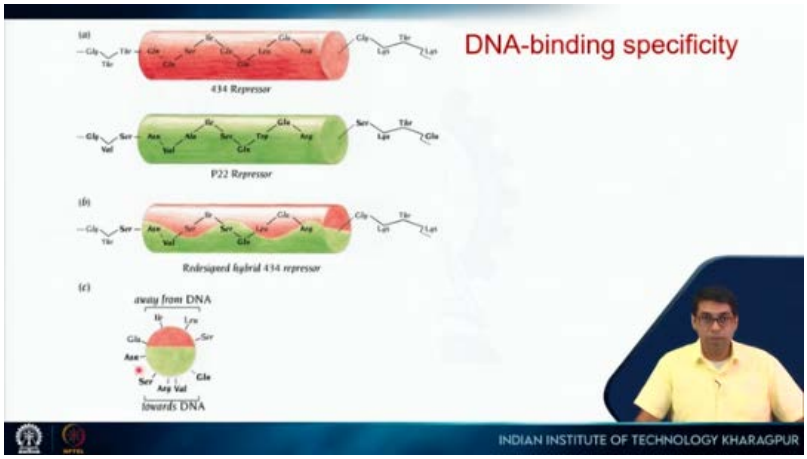


This helix and helix are found in these two proteins. So, one is the 434 repressor. So, this is the lac repressor that we have discussed before and the P22 repressor. Now, if you see, this is the helix. So, the DNA binds to this side.

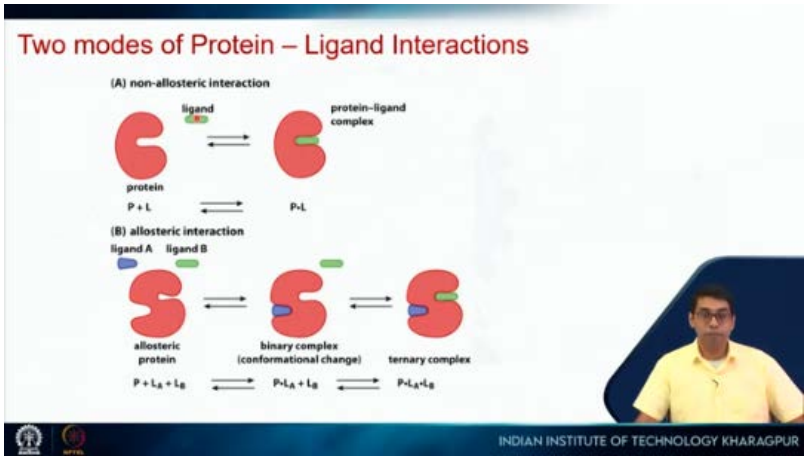
So, the residues which are on this side of the helix, the residues which are on this side of the helix, they will interact with the DNA. And you can see that these residues are different. So, in this case, it is valine and here it is glutamine, here it is glutamine, here it is glutamine, and then some other residues are different. So, depending on the residues which are on this half of the helix,

they will interact with the DNA, and those interactions will determine what sequence this helix will recognize. Which means that we can engineer these proteins by changing these residues which are on this side of the helix that interact with the DNA. This is exactly what was done. So, a redesigned hybrid 434 repressor was formed where these wild-type residues were replaced by these residues. So, you can see that this is changed by this valine, this is here, and then some of the other residues like this glutamine are also changed by serine.

And right, like so, now when these changes have been made, so this asparagine is here instead of glutamine. So, when these changes were made, the overall tertiary structure is that of the 434 repressor, but only these few amino acids were changed, which are coming from the P22 repressor, and now this hybrid structure will bind the DNA. For the P22 repressor, not that of the 434 repressor. So, we have changed, or this was done. So, these residues have been changed, which are towards the DNA, and that changes the specificity of the DNA binding for this particular protein. So, that concludes our non-allosteric interaction.



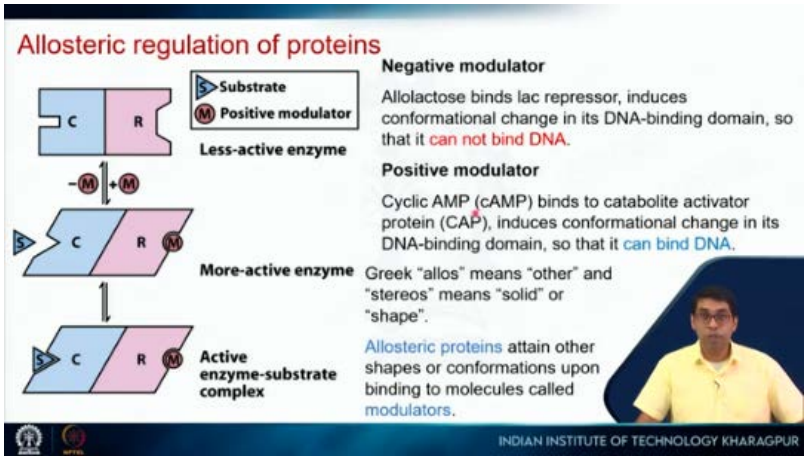
So, we have seen how small molecules interact with proteins and how DNA interacts with proteins. They all have their unique principles, and we can understand those principles and use them to modulate these types of interactions according to our needs and benefits. The second one that I am going to talk about is allosteric interaction. So, in allosteric interaction, the protein will have two binding sites. In one site, the modulator will bind, and once it binds, it changes the shape of the protein. It can change the whole shape or it can change only a particular part of the protein.



So, shape or conformation and once this change happens, now the substrate can bind. So, allostery comes from Greek words: 'allos', which means 'other,' and 'stereos', which means 'solid' or 'shape.' So, allosteric proteins attain another shape. So, this other shape or conformation occurs upon binding to molecules, which we call modulators.

Now, we have already seen some examples. So, we have seen the example of the lac repressor. Allolactose is a modulator that binds to the lac repressor. It induces a change in the protein so that it cannot bind to DNA. So, allolactose is a negative modulator because it results in the unbinding of DNA.

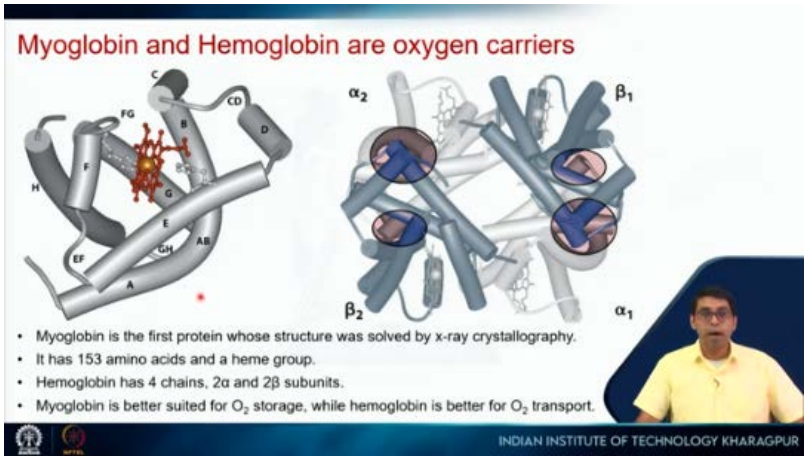
So, when it binds to the protein, the substrate cannot bind, or the DNA cannot bind. There is another reverse example of this where cyclic AMP binds to the catabolite activator protein and induces a conformational change in its DNA binding so that now it can bind DNA. So, this is the reverse of the lac repressor and allolactose. So, in this case, cyclic AMP is a positive modulator.



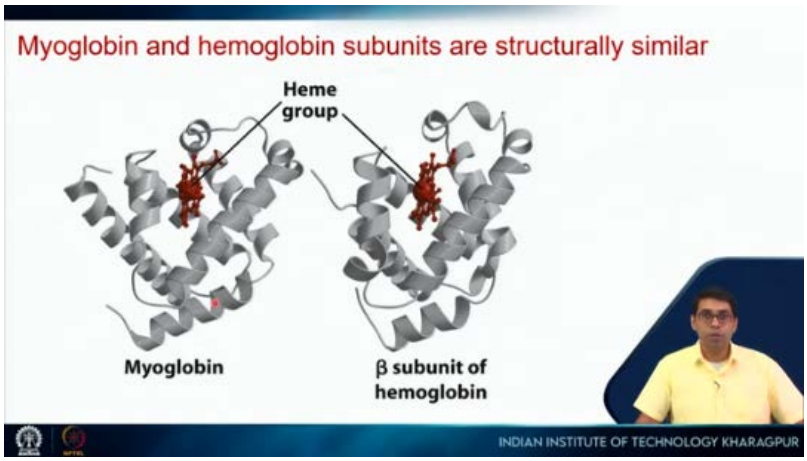
So, let us look at hemoglobin because I am going to talk about allostery, and hemoglobin is a classical example of a protein that shows not only allostery but also another important phenomenon called cooperativity. So, here are two proteins: the one shown on the left is myoglobin. It is a single polypeptide chain, and in its binding site, it has this heme group which contains a porphyrin ring and a ferrous ion, and this is the one that will bind oxygen. Hemoglobin has the exact same structure as myoglobin. Additionally, it has four subunits.

Instead of just one polypeptide chain, there are four polypeptide chains, and each of them carries this heme group. So, hemoglobin: each of them can carry this heme group. So, hemoglobin can bind four molecules of oxygen. It turns out that myoglobin is actually the first protein whose structure was solved by X-ray crystallography. It is not a very big protein; it has only 153 amino acids and the heme group.

Hemoglobin has four chains, and their structures are very similar to that of myoglobin, and it turns out that there are two alpha chains and two beta chains. So, these two sequences are the same, and these two sequences are the same. Myoglobin is better suited for the storage of oxygen, whereas hemoglobin is better suited for the transport of oxygen, and we will see what we mean by that in the next few slides.

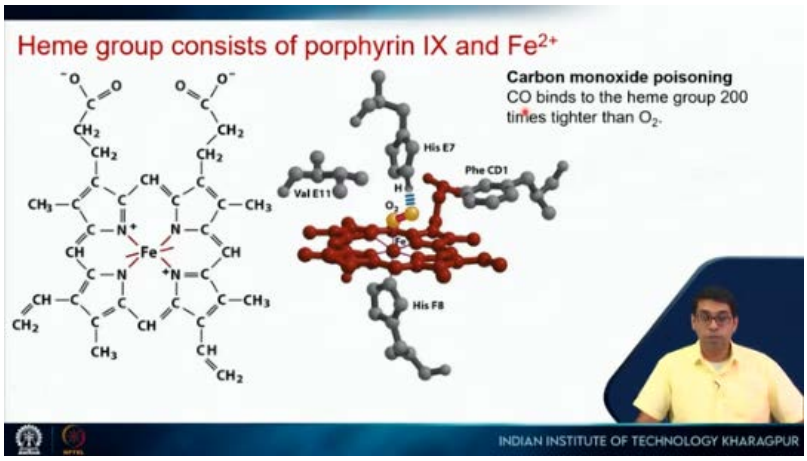


So, if we compare the structure of myoglobin and one of the subunits, let us say the beta subunit of hemoglobin, then you will see that they look exactly the same. So, we have very similar alpha helices in the same positions, and the heme group also binds in the same site.



So, this is the heme group that consists of two parts, one is a porphyrin 9 moiety. So, porphyrin, depending on these groups that are present in these sites, can be classified as different numbers. So, the one we are talking about is porphyrin 9, and there is a ferrous iron, or the ferrous ion, at the center. This ferrous ion forms an octahedral geometry, so it coordinates with these four nitrogens, then at the bottom, there is this proximal histidine, so this histidine is on helix F that also forms this coordination, so this will be a nitrogen atom on the histidine, and the sixth coordination is formed by the bound oxygen. There is also another histidine, which is called the distal histidine, which is on the E helix of this protein. So, together, this forms the binding site of the protein.

Now there is something that is very interesting. So, oxygen is something that hemoglobin carries from our lungs to our tissues. It turns out that carbon monoxide, which has a very similar structure and shape as oxygen, binds to the heme group 200 times tighter than oxygen and this is something that is very problematic because carbon monoxide, if it binds this way, then it acts as an inhibitor of the heme group. So, it will prevent the hemoglobin from binding further oxygen, which means that the number of available hemoglobin molecules will decrease if there is carbon monoxide present. So, that will result in a lower supply of oxygen, and that is carbon monoxide poisoning, which is very dangerous because carbon monoxide is a colorless and odorless gas. So, in less ventilated places where you have some source of carbon dioxide, maybe from a fire or a diesel generator that is running, it can create a substantial amount of carbon monoxide that a person can breathe, and that will result in the inhibition of hemoglobin. So, let us look at some facts about hemoglobin. It turns out that the solubility of oxygen is very low in aqueous solutions.



So, it is around 7.7 milligrams per liter in water at 35 degrees centigrade, very close to our body temperature, and 1 atmospheric pressure. So, which means that since we need to carry oxygen over large distances from our lungs to different body parts, we need to increase the solubility, and that is where hemoglobin comes in. Red blood cells, which are also called erythrocytes, carry hemoglobin in such a way that one-third of the weight of these cells actually comes from hemoglobin. So, they are tightly packed with hemoglobin, and these cells survive for roughly four months, 120 days.

Erythrocytes are very interesting cells. We will discuss cells in more detail in next week's lecture, but I just wanted to point out a few important or interesting facts about these red blood cells. So, they are formed from precursor stem cells called hemocytoblasts, and once they are formed, of course, they will need all the cell organelles to produce hemoglobin. So, they produce a large amount of hemoglobin. Once that is done, then they don't need the DNA, and they don't need all the other machinery which is needed for protein production, so all of these organelles are extruded out. They are all thrown out, the nucleus, mitochondria, endoplasmic, and the majority of the cell packed with hemoglobin. This is something that will be important, and we will see this again and again in the next few slides.

The partial pressure of oxygen in our lungs is 13.3 kilopascal and in our tissues is 4 kilopascals. So, there is of course less partial pressure in tissues compared to the lungs. Now, we will see curves like this. So, theta is a term which is saturation, right? So, saturation means that theta goal has a value of 0 to 1.

So, if it is 0, it means that hemoglobin has no oxygen bound to it. If it is 1, it means that hemoglobin is completely saturated with oxygen, and we can express theta in terms of this. Now, here the expression is written in such a way that we are assuming that one molecule of hemoglobin binds only one molecule of oxygen. So, this will be more for myoglobin, not hemoglobin. We have to modify this expression for hemoglobin.

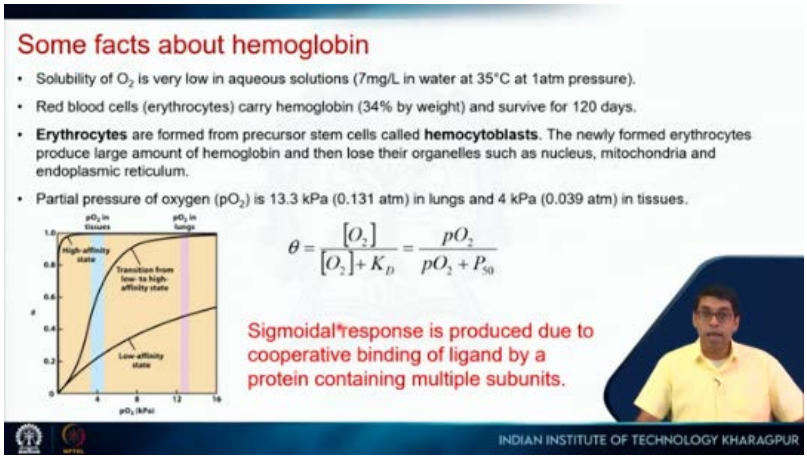
So, the concentration of oxygen or the ligand divided by the concentration of the ligand plus K_D. We can replace this concentration of the ligand with the partial pressure of oxygen, and we can replace K_D with P50, which is the partial pressure at which 50% of the molecules are bound to oxygen, or theta equals 0.5. Now, if we have this, if my K_D or P50 is low, then my binding curve will look something like this, and if my K_D or P50 is high, then it will look like this. Now, this is where this is the partial pressure of oxygen in lungs, and this is the partial pressure in tissues. If the affinity is low, then our theta will be somewhere here. So, let us say 0.5. So, 0.5 or 50% of myoglobin-like molecules will bind oxygen. And then, when it goes to tissues, it will be around 20%. So, 50% minus 20%, only 30% of myoglobin will release oxygen, right?

So the release of oxygen that we get is only 30%. On the other hand, if it is binding very tightly, let us say this is 99% and this is 98%, then the release of oxygen is only 1%, even though it is binding oxygen, but it is not releasing it. So, that is why myoglobin is a very poor oxygen carrier. We do not want that. We want something different, and that is where hemoglobin comes in.

Its binding curve does not look like this, which is a hyperbola. It looks like this sigmoid curve. So in the lungs, it binds oxygen, which is, let us say, around 98% saturation. And in the tissues, it comes down to, let us say, 50%. So 98% minus 50%, that is 48% of unloading of oxygen.

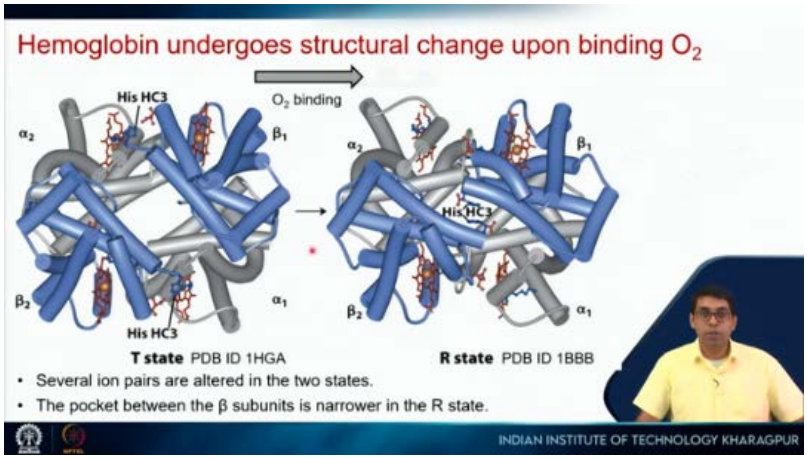
So, almost 50%, as if 50% of hemoglobins are carrying oxygen between lungs and tissues. So, this is much more efficient than these two. So, how do we get that? This results from a particular phenomenon called cooperative binding of ligand, and cooperative binding of ligand arises when there is allostery between multiple subunits of a protein. So just allostery in a single polypeptide chain will not work.

You need multiple subunits and you need allostery between them so that there is cooperative binding. And when all of these things happen together, we get a sigmoidal response like this. So let us look at the hemoglobin structure again. And it turns out that hemoglobin actually has two different conformations. So this is one conformation which is referred to as the T state or the tense state.



And this is another conformation which is referred to as the R state or the relaxed state. Hemoglobin has a lower affinity for oxygen in the T state and a higher affinity for oxygen in the R state. Also, oxygen binding shifts the equilibrium from the T state to the R state. So, what is the difference between these two? There are all these different ion pairs, or these are actually electrostatic interactions between different amino acid side chains.

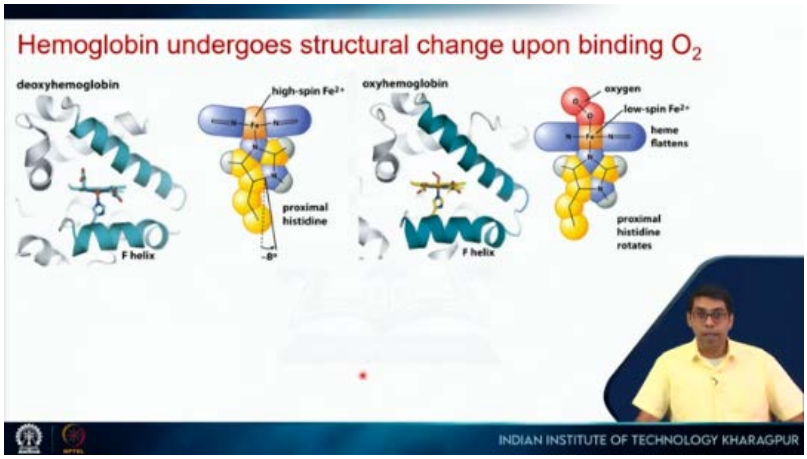
So, these are present in the T state but not in the R state. Also, you can see that this central portion is different. So, structurally they are quite different, significantly different from each other. So, how do we get this structural difference? It turns out that it is triggered by the binding of oxygen to the heme group. So, in the absence of oxygen, this is the deoxyhemoglobin; the porphyrin ring looks like this.



It has a slightly bent structure and then this proximal histidine is interacting with the iron. So, in this case, the iron is in a high-spin state. When oxygen binds, it changes the iron to the low-spin state and this heme group flattens. So, you see it is slightly bent here, it flattens, and that results in a tug on the proximal histidine, which rotates, and this small change results in a change in the helix F, which results in bigger changes in the rest of the protein.

So, all these ion pairs are changed, and the protein actually changes its conformation. Another important interesting fact happens that it goes from the T state to the R state, so the neighboring subunits also go from the T state to the R state, which means that they can now bind oxygen more tightly and this is where cooperativity comes in. Once one molecule of oxygen binds to a particular subunit, the next oxygen will bind tighter, the third one will

bind even tighter, and the fourth one will bind even tighter. That is why we see this sigmoidal curve where it zooms up as more and more oxygen binds, and then, of course, it reaches saturation. So, to understand that, we have to plot, and the cooperativity can be plotted nicely by something called a Hill plot.



So, before I do that, let us introduce two more terms in terms of allostery and cooperativity. So, we saw that in the case of the lac repressor and cap protein, there is a modulator which results in the change in DNA binding affinity. So, it is either allolactose for the lac repressor or it is cyclic AMP for cap. So, here the modulator is different from the ligand, and this type of interaction is called heterotropic interaction, but in the case of hemoglobin, the modulator and the ligand are both oxygen.

So, the binding of oxygen to one subunit results in tighter binding of oxygen to the second subunit. So, here the ligand and the allosteric modulator are the same. In this case, the interaction is called a homotropic interaction. We can represent the binding of hemoglobin to multiple oxygen molecules like this.

$$K_D = \frac{[P][L]^n}{[PL_n]} \qquad \theta = \frac{[L]^n}{[L]^n + K_D} \qquad \frac{\theta}{1-\theta} = \frac{[L]^n}{K_D}$$

$$log\ log\ \left(\frac{\theta}{1-\theta}\right) = nlog[L] - log\ log\ K_D$$

$$log\ log\ \left(\frac{\theta}{1-\theta}\right) = nlog\ pO_2\ - nlog\ P_{50}^n$$

So, in this case, *n* will be 4. So, hemoglobin plus 4 molecules of oxygen gives you hemoglobin oxygen 4. So, O_2 4 complex. You can also divide it into 4 equations. So,

hemoglobin binds 1 oxygen, it will be *PL*, then another oxygen will be *PL2*, then another oxygen will be *PL3*, then another oxygen will be *PL4*. So, if we do that, our K_D gets modified like this, where instead of having just *L*, we have L to the power N, because we have N number of ligands here.

So, it will be $P\ x\ L\ x\ L\ x\ L\ x\ L$. So, L repeated 4 times. And, then this will also modify our saturation equation like this, where we replace *L* with L^n and this is K_D. So, we can replace. So, now if I subtract *1* from this and then divide it by $(1-\theta)$, I can change this equation to look something like this. So, θ by $(1-\theta)$ equals to L^n divided by K_D. If I take the log on both sides, then the left-hand side will be log of θ by $(1-\theta)$, and the right-hand side will be log of this.

So, it means the log of L^n. So, *n* comes in the front. So, $nlog[L] - log\ log\ K_D$. Now, what we can do is we can replace this with the partial pressure of oxygen (pO_2) and we can replace this with P_{50}. So, if we do that, then the equation looks like this and again K_D will be P_{50}^n. So, we get an *n* in the front. Now, if I plot the log of this versus the partial pressure of oxygen, this is a straight line equation.

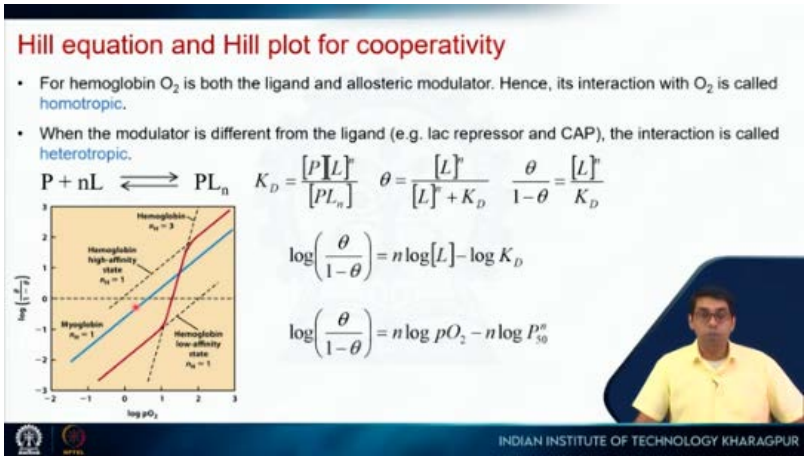
And remember that it is always easier to extrapolate straight line data. So that is why people will always linearize equations like this. So, if we plot this versus the partial pressure of oxygen, it will look something like this. So, what is this? Now, you see I mentioned that hemoglobin has two different conformations: T state and R state.

In the T state, it binds oxygen weakly and in the R state, it binds oxygen tightly. So, the T state is the one where the binding of oxygen will shift slowly towards the R state. So, this is my $log\theta$ by $(1-\theta)$. So, the binding of oxygen and this is my partial pressure of oxygen, and both are in the log.

So, at lower concentrations of oxygen, hemoglobin is in the T state. So, we will get a curve like this. So, this straight line represents the binding of oxygen to the T state. When the partial pressure is more, then the affinity increases. So, it shifts, and this straight line represents the binding of hemoglobin.

Binding of oxygen to hemoglobin in the R state. But then what actually happens is initially most all the hemoglobin is in the T state and oxygen is binding. So, it will follow this curve and then as more and more hemoglobin or more subunits get converted to the R state, it will depart from this and start moving towards this affinity. Once it reaches this, then of course, it will follow this line. So we have two different slopes so this slope is *1*, and then there is this slope which has a Hill coefficient of *3*.

So this Hill coefficient of more than *1* represents cooperativity. Which means that once oxygen starts binding, it will shift the equilibrium towards the R state, which means that the next molecules of oxygen will bind hemoglobin with tighter affinity. So, in plots like this, whenever we get a slope that is more than 1, we say that there is cooperativity between different subunits. So, how do we explain that? There are two models that were proposed in the 1960s.



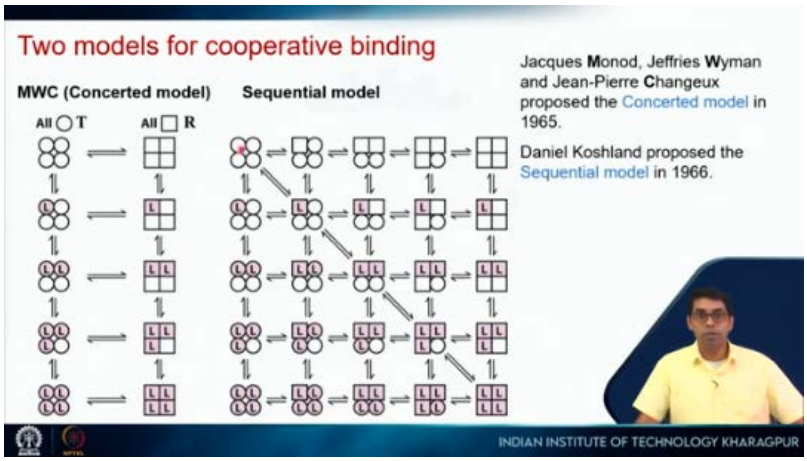
So, one is called the concerted model, and the other one is called the sequential model. And these were proposed by two groups of scientists. So, the concerted model was proposed by Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux in 1965. The next year, Daniel Koshland and his group members, his co-workers, proposed the sequential model. So, let us look at this.

The concerted model states that all the subunits are either in the T state, which binds oxygen weakly, or in the R state, which binds oxygen tightly. And then we have equilibrium between all these different states. So, you can see there are K_D values here and there are equilibrium constants here. So, as more and more oxygen binds, the equilibrium will be

shifted towards this right-hand side. So, fewer molecules of hemoglobin will be in this state, and more molecules of hemoglobin will be in this state.

So, in the free state, all will be in the T state, and when we reach saturation, all of them will be in the R state. The sequential model says that all the subunits can be either in the T state or in the R state. So, the number of possibilities is, of course, much more. So, again, in the free state, all of them are in the T state, and when all the subunits are bound to oxygen, it will completely shift towards the R state, and there are all these different multiple paths by which it can start from here and reach here. But, overall, what happens is, as this T state gets converted to the R state, your affinity will keep on increasing.

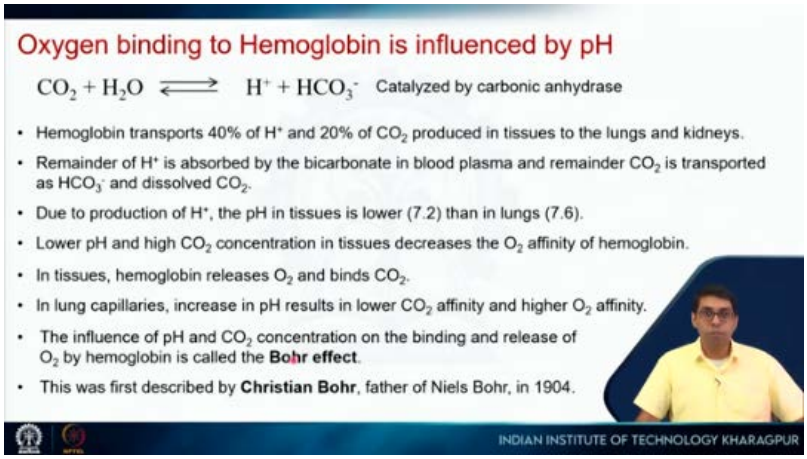
So, the equilibrium will keep on shifting from the left-hand side towards the right-hand side. So, there is more to oxygen binding to hemoglobin. It turns out that hemoglobin also carries back carbon dioxide from the tissues to the lungs. So, in the tissues, carbon dioxide is formed, and it can react with water to form bicarbonate ions and protons. And this reaction is actually catalyzed by an enzyme called carbonic anhydrase.



And this carbonic anhydrase is present in a high concentration in the erythrocytes, or the red blood cells. So, hemoglobin transports 40% of protons and 20% of carbon dioxide produced in the tissues to the lungs. So, it is important to note that the solubility of carbon dioxide is much higher in water than that of oxygen. So, the remainder of the protons that are produced is absorbed as bicarbonate in blood plasma, and it is carried through that remainder of carbon dioxide, which is transported as bicarbonate. So, 70% of it will be like this, and 20% will be carbon dioxide and 7% will be dissolved carbon dioxide. So, 20% is

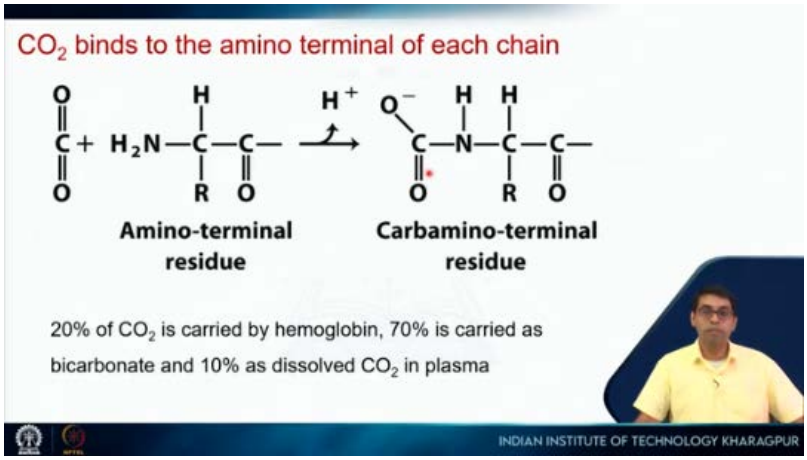
carried by hemoglobin. Now, because of the production of these hydrogen ions, the pH in the tissues is actually lower than that in the lungs that is 7.2 versus 7.6, and it turns out that this lower pH and high concentration of carbon dioxide decreases the oxygen affinity of hemoglobin in the tissues compared to that in the lungs. That is what I am saying here. So, this influence of pH and carbon dioxide on hemoglobin binding to oxygen is called the Bohr effect.

So, the Bohr effect was first described by Christian Bohr in 1904, and he turns out to be the father of the famous physicist Niels Bohr. So, how does hemoglobin carry carbon dioxide? So, carbon dioxide is a much bigger molecule. It is not going to bind the heme group. It actually binds or reacts with the amino terminal of the polypeptide chains.



So, this is the free NH_2 group of the polypeptide chain, the N-terminus. Carbon dioxide can react with it to form this type of carbamino terminal. Okay. Now, this results in the changing of these ion pair interactions. So, it actually stabilizes the T state.

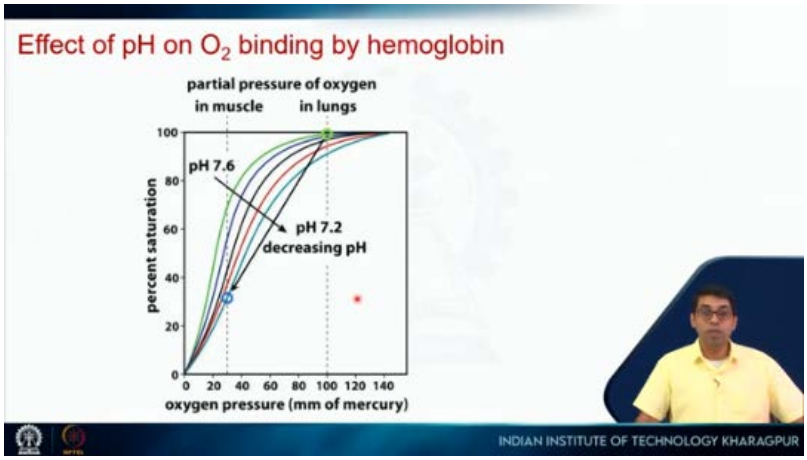
So, if it stabilizes the T state, what happens? The affinity of oxygen binding will decrease. So, let us look at this. This is the pH dependence of hemoglobin. So, this is the same plot.



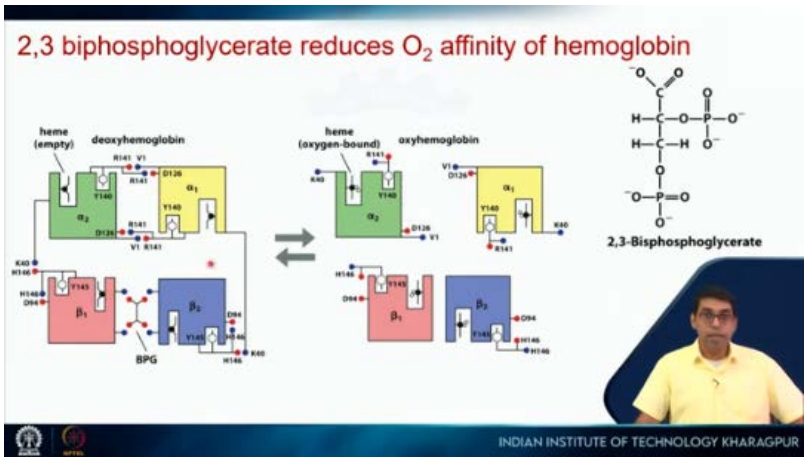
Percent saturation you can think of it as theta, partial pressure of oxygen. So, in the lungs, this is the partial pressure, and in the tissues, this is the partial pressure. In the lungs, the pH is 7.6. So, the curve will look something like this, the one on the extreme left. As pH decreases from lungs to tissues, the curve will shift.

So, it will be this right-hand one in the muscle. So, in the lungs, the pH is high, and the pressure is high. So, this will be the saturation. So, almost 100%, let's say 98% or 99%. In the tissues, the partial pressure has decreased, and because of pH and carbon dioxide, this has also shifted.

So, now it is here. So, you can see that 98% minus 30%, so that is almost 70% of oxygen offloading happens between the lungs and the muscles because of this effect, because of the Bohr effect. So, there is another molecule called 2,3-biphosphoglycerate or BPG, which also modulates the binding of oxygen to hemoglobin. So, it turns out that BPG interacts with the two beta subunits like this.

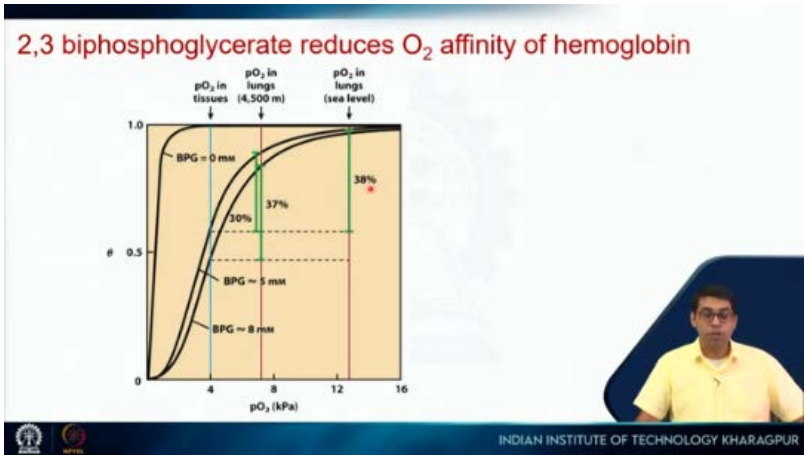


So, you have these negative charges. So, these negative charges will form ion pair interactions with the positive side chains of the beta subunits. And it also stabilizes the T state compared to the R state, which means that the presence of BPG reduces the oxygen affinity of hemoglobin. So, what is the effect of that? The effect of this is seen when we are in a condition of low oxygen availability or low partial pressure of oxygen.



So, one example will be that if you go mountain climbing, where the partial pressure of oxygen is less. So, this is the partial pressure of oxygen at sea level. This is the partial pressure of oxygen when you are at a height of 4500 meters and this is the partial pressure in the tissues, which remains the same. BPG, it turns out that the amount of BPG is changed between these two conditions. Right. So, when there is less BPG, oxygen binds by this curve, which is shown on the left. So, we have less BPG under normal conditions. So, oxygen binds here and then it releases in the tissues.

So, you will see these numbers are changing between plots, but the idea is the same. So, let us say 30% of oxygen is released. If the same curve persists, at a height, the partial pressure has decreased, so it binds here, and then the tissue is the same, so it releases here. So, this is only 30%. So, instead of 38%, only 30% of oxygen is released, which means that the available oxygen will be less. But if the curve shifts to this one on the right-hand side because of a higher concentration of BPG, then a lesser amount of hemoglobin will bind oxygen, but the release will be greater, and you can see that this number is 37%, which is almost the same as this. So, even though the hemoglobin is less saturated, the offloading is the same, which means that the oxygen availability becomes almost the same due to the increased concentration of BPG.



So you can read about all of these in these different books. So, I have suggested several books. You can go through them and read the details about protein-ligand interactions, hemoglobin, and aldosterone. Thank you.

REFERENCES

Following books may be referred to

- Lehninger Principles of Biochemistry, 4th Edition
- How Proteins Work (Mike Williamson)
- Introduction to protein structure (Carl Branden & John Tooze)
- Biochemistry (Lubert Stryer)
- The Molecules of Life: Physical and Chemical Properties