Fundamentals of Protein Chemistry
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Module - 11
Protein Macromolecule Interactions II
Lecture - 51
Protein Protein Interactions - 1

In our 2nd module of protein macromolecule interactions, we will be looking at protein-protein interactions, protein peptide interactions a discussion on chaperone proteins and what we call IDPs: intrinsically disordered proteins. In the last module we looked at protein carbohydrate and protein nucleic acid interactions.

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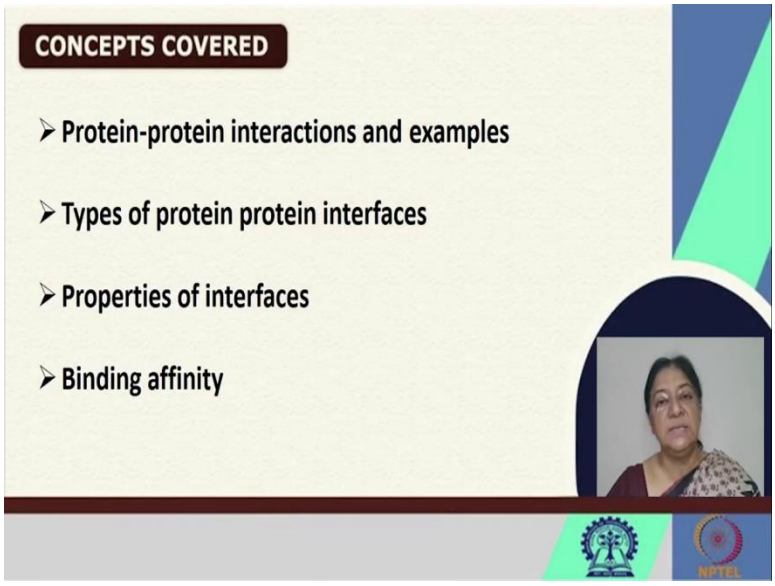

In each of these cases we are looking at a ligand interacting with the protein. The ligand in this case is the carbohydrate, the nucleic acid and in the next two lectures we will be looking at proteins as the ligands.

The interactions of proteins are extremely important in several aspects of biochemical processes. What we are going to look at is the types of protein-protein interfaces, the properties of these interfaces and specific binding affinities related to the protein ligand interactions that we had studied before.

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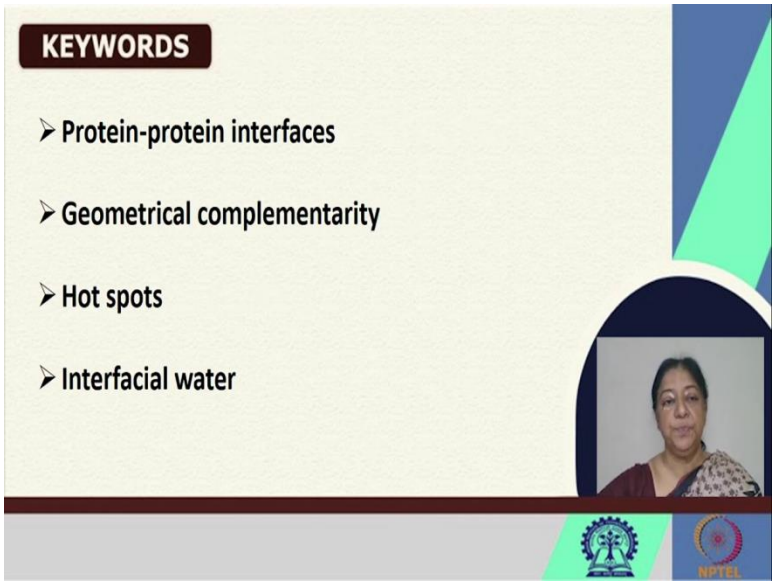

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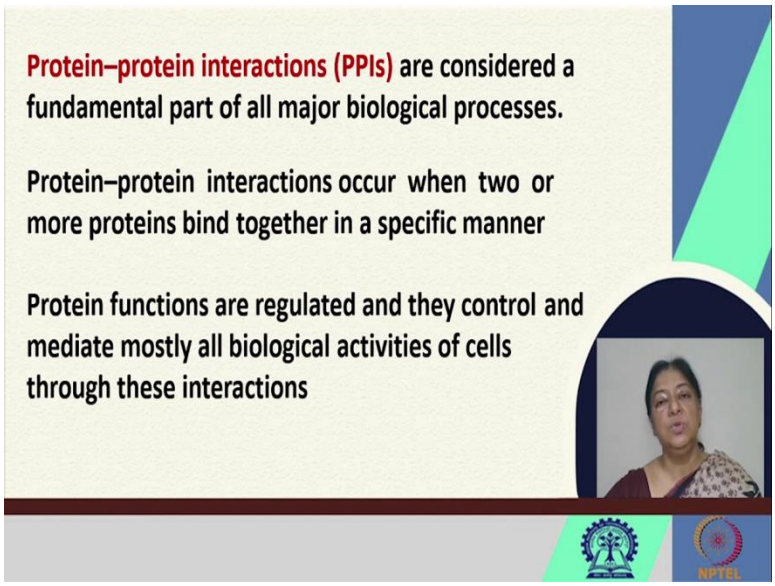

In an understanding of what we mean by protein-protein interactions that are commonly called PPIs, these are a fundamental part of all major biological processes. They occur when two or more proteins bind together in a specific manner and there are many functions that are regulated mediated and even controlled by this complex formation almost all biological activities of cells occur through these interactions.

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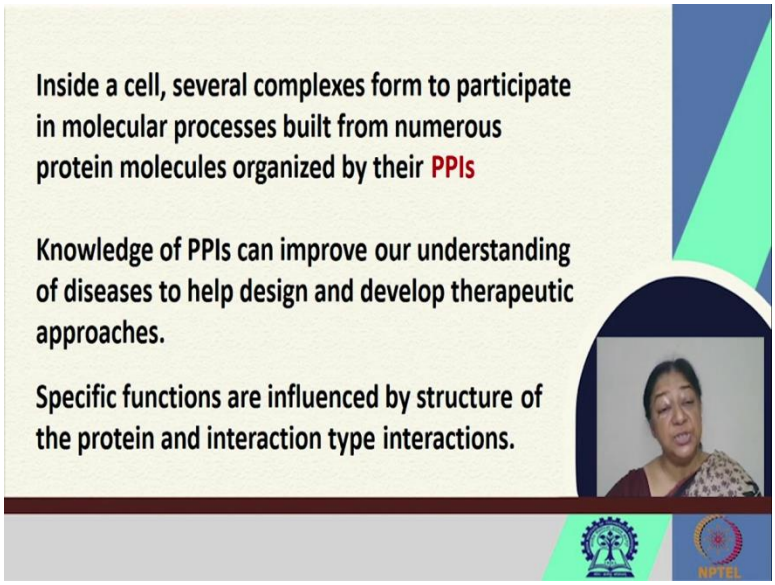

Inside the cell we have several complexes that are formed to participate in molecular processes as we have seen, that are built up through these PPIs. So, a knowledge of these PPIs can actually improve our understanding of diseases to help design and develop therapeutic approaches and we will see how this specific topic can be approached from an understanding of the protein-protein interactions. The specific functions therefore, are influenced by the structure of the protein and the interaction type interactions that occur.

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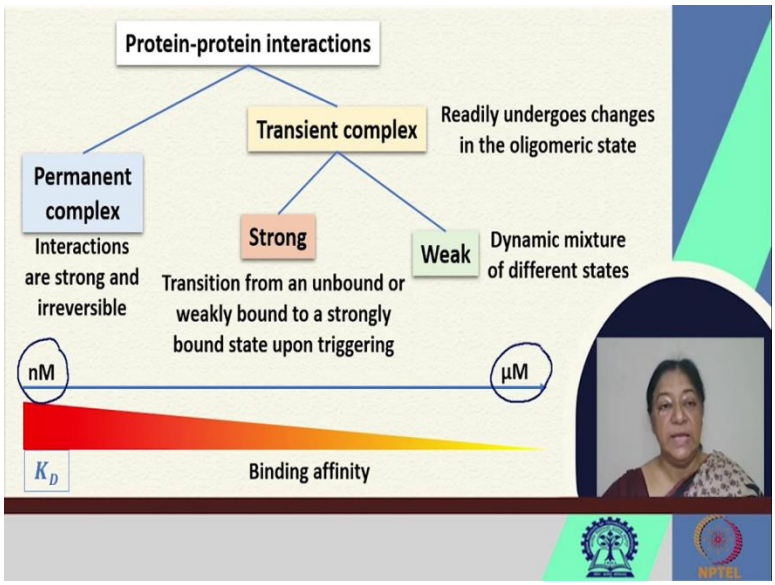

So if we look at protein-protein interactions in general, they can be of two types. We can have permanent complexes, where the interactions are strong and irreversible. For example, where we have monomeric subunits of a protein forming an oligomeric unit; where the interactions are strong they are irreversible in a way and they form a permanent complex.

On the other hand we could have what is called a transient complex, where there is a specific change in the oligomeric state of the protein or it could just interact for the specific reaction to occur.

This can be of the weak type, where we could have a dynamic mixture of different states or we could have a strong type, where there is a transition from an unbound or a weakly bound to a strongly bound state upon triggering; that could be the binding of a small molecule, a metal or a cofactor that is going to trigger a specific reaction that requires a protein protein complex to be formed.

If we look at the affinity or rather the dissociation constant values that we had studied previously in protein ligand interactions, we realize that the binding affinity is going to be very strong for a permanent complex, as reflected in the values of the K_D constant. We know that a smaller K_D indicates a tighter binding and the weak are of the order of micromolar values for the K_D.

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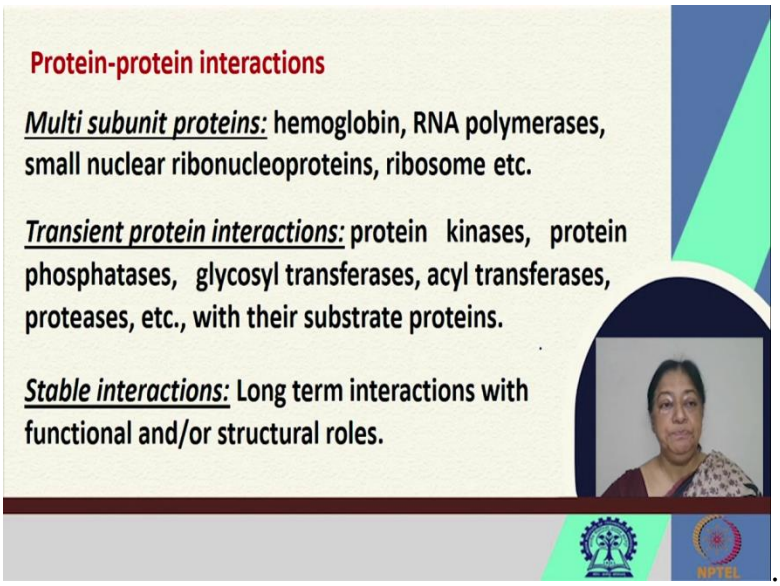

So, the protein-protein interactions can occur in multi subunit proteins say for example, hemoglobin, ribosome, RNA polymerases. We have transient protein interactions such as protein kinases, phosphatases and what they do is they interact in a very transient manner, with the specific protein that it has to complex with and then performs the particular reaction or the specific biochemical process that has to occur and that would be a transient type of interactions.

There are some stable interactions as well that are long term interactions, that have specific functional or structural roles.

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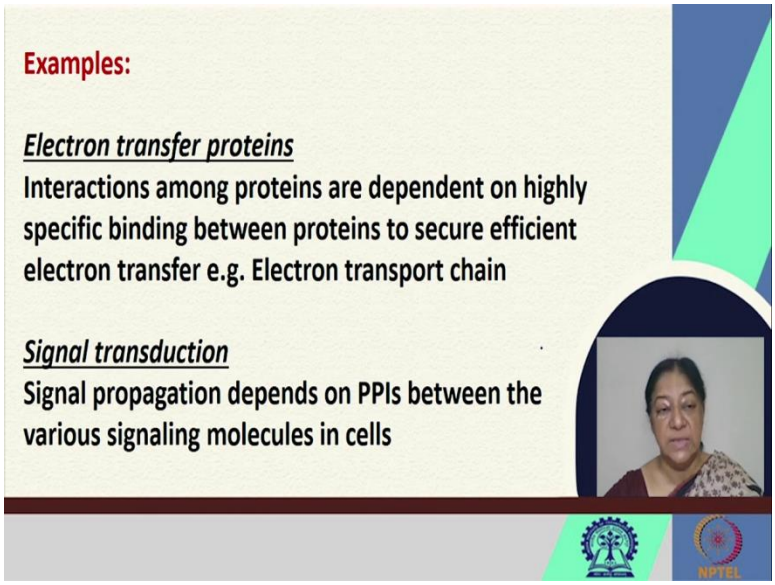

The examples that we have looked at or we have studied in the process of this course, is for example electron transfer proteins. Here the interactions among the proteins are very dependent on a highly specific binding between the proteins, so that there is efficient electron transfer in the

electron transport chain. For signal transduction where we need signal propagation, this also depends highly on the protein-protein interactions between the various signaling molecules in the cells.

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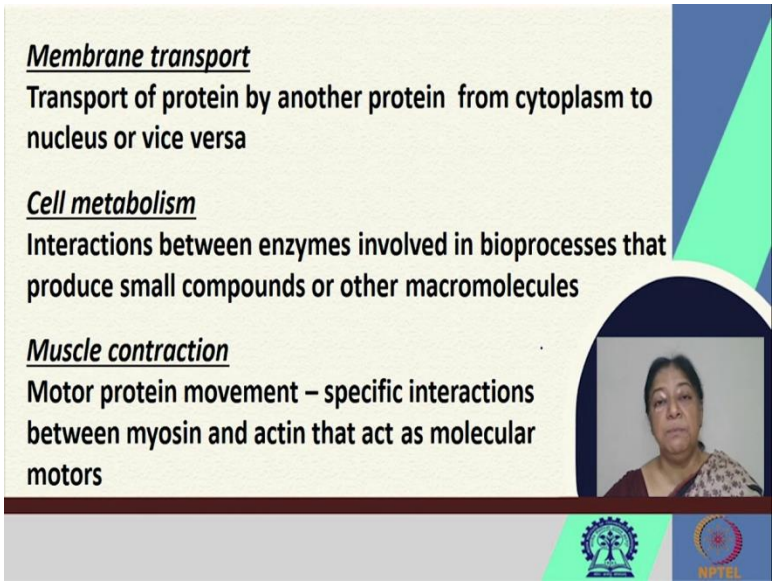

In addition we have membrane transport, the transport of proteins by another protein from the cytoplasm to the nucleus or vice versa, where we have a specific integral membrane protein or a peripheral membrane protein, that would take part in this type of transport. Then there is cell metabolism, where we have the interactions between enzymes that are involved in bioprocesses that produce the small compounds or other macromolecules in a specific enzymatic type of reaction.

Then muscle contraction, where we have the motor protein involvement for example, the myosin and the actin that have to act together in the molecular motor action.

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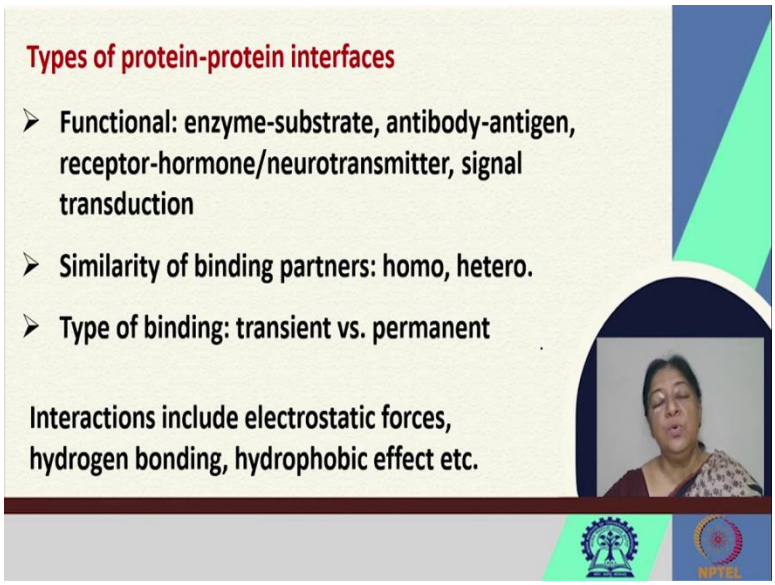

So, the types of protein-protein interfaces that we have here can be of a functional type. They could be enzyme-substrate, antibody-antigen, receptor-hormone or signal transduction type. There can be a similarity of the binding partners, they could be similar which could be in a homotype or a heterotype and the type of binding can be transient or permanent.

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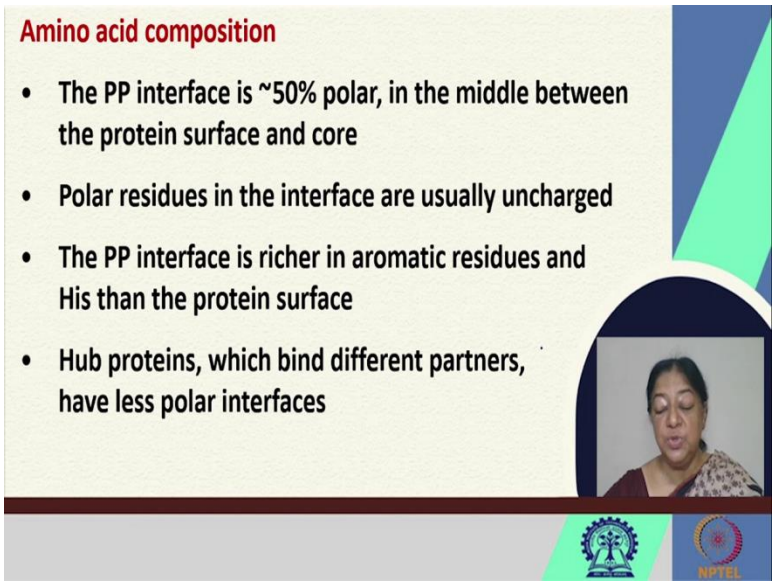

So the interactions that occur in these specific types of protein-protein interfaces, they could include electrostatic forces, hydrogen bonding, hydrophobic effect, similar to what we see in the specific types of interactions in tertiary structure; the non covalent types of interactions. The amino acid composition that is seen at the protein-protein interface is 50% polar in nature approximately, in the middle between the protein surface and the core.

The polar residues in the interface are usually uncharged in this case and we see that they are richer in aromatic residues and histidine, than the protein surface. We have something called hub proteins, where there is a binding with different partners.

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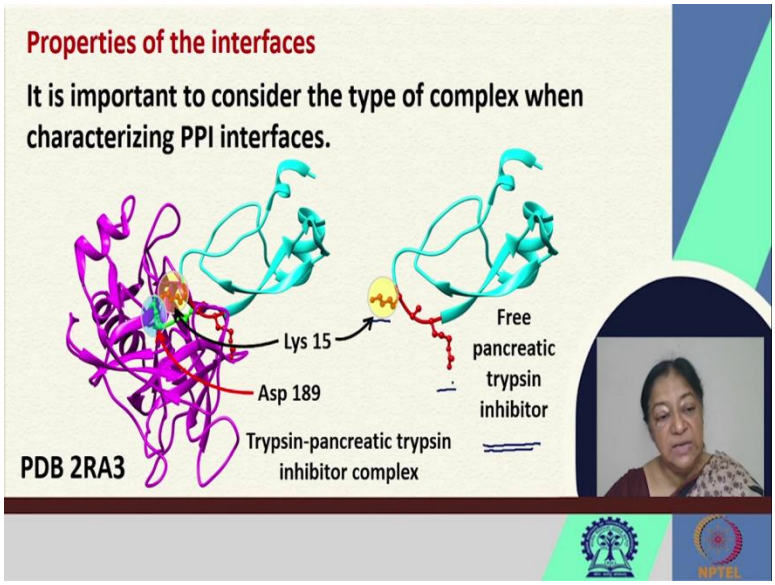

If we look at the properties of the interface, it is important to consider the type of complex when we are characterizing this protein-protein interaction interface. For example, if we are looking at a trypsin-pancreatic trypsin inhibitor complex, then there are specific residues that are involved in the interaction.

So we have the free pancreatic trypsin inhibitor here [refer to slide]. These are the specific residues that are involved in the interaction. The characteristics of this residues is such, that they would have an interacting partner in the partner protein that it is interacting with. In this case we have a lysine which has an electrostatic interaction with an aspartic acid of the protein, then we look at the specific types of interactions.

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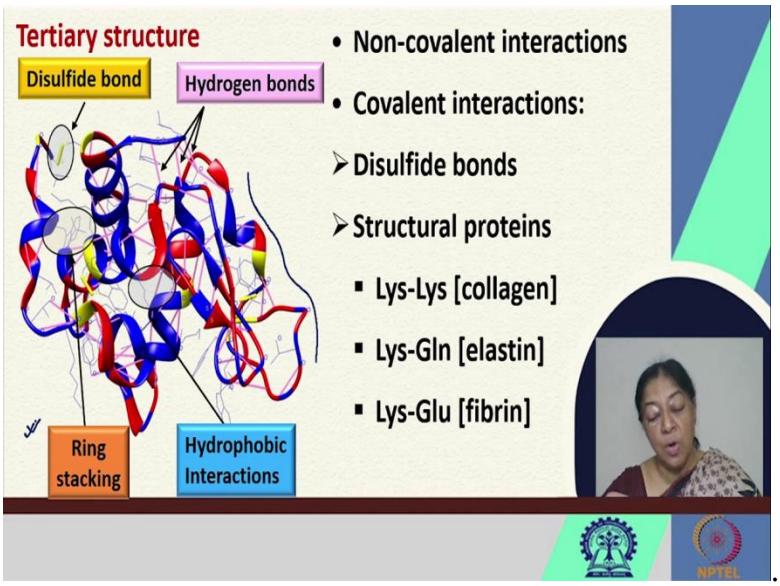

These types of interactions as we saw, could be an electrostatic type of interaction which we usually see on the surface of a protein and they could be of a hydrogen bonding type. Disulfide bonds on the other hand connect two parts of a protein together, could be of an inter molecular type and we have ring stacking, that we would see for the aromatic interface.

So, the properties that we see for a non-covalent tertiary structure interaction and the covalent interactions that we observe, are similar to what we can see in the different types of proteins where we have a homo complex or a hetero complex.

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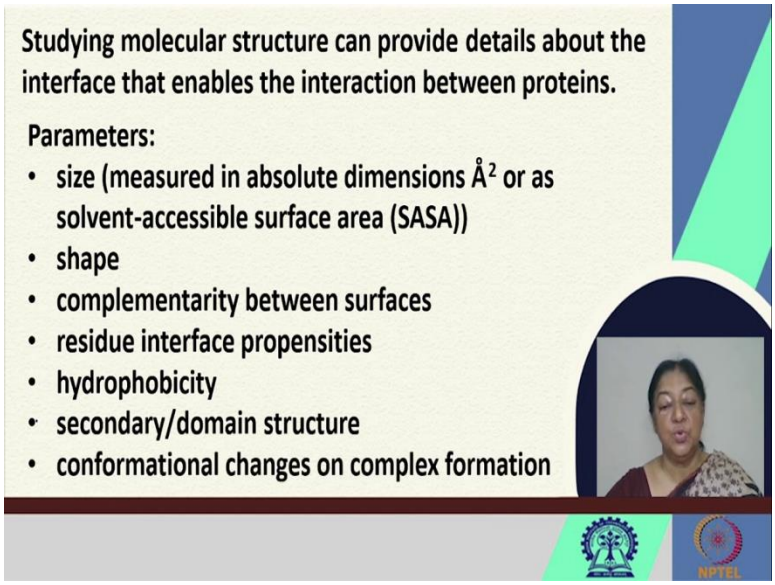

When we study this molecular structure, we can get details about the interface and this study enables us to understand the interaction between the proteins.

The parameters here are the size, what is the solvent accessible surface, how much of the protein molecule is accessible for interaction with another molecule, the shape of the molecule, the complementarity between the surfaces; we need a geometric complementarity as well as a chemical complementarity, the residue interface propensities, the hydrophobicity and the secondary and domain structure that is also going to be important in an understanding of what triggers a protein-protein interaction to occur and the conformational changes that might occur due to complex formation.

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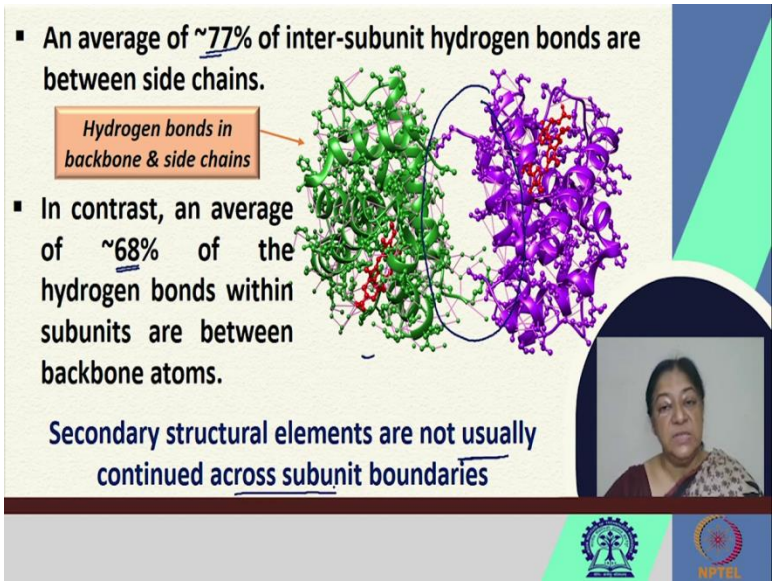

What we have is an average of 77% of inter subunit hydrogen bonds that are found between side chains. However, an average of about 68% of the hydrogen bonds are found within subunits, are between the backbone atoms.

So if we look at the hydrogen bonds in the backbone and the side chains, we have approximately a 77% that are inter subunit type. In contrast we have 68% that are within the backbone atoms here. The secondary structure elements that are considered here are usually not continued over subunit boundaries. So they are confined within the specific subunit that they occur in.

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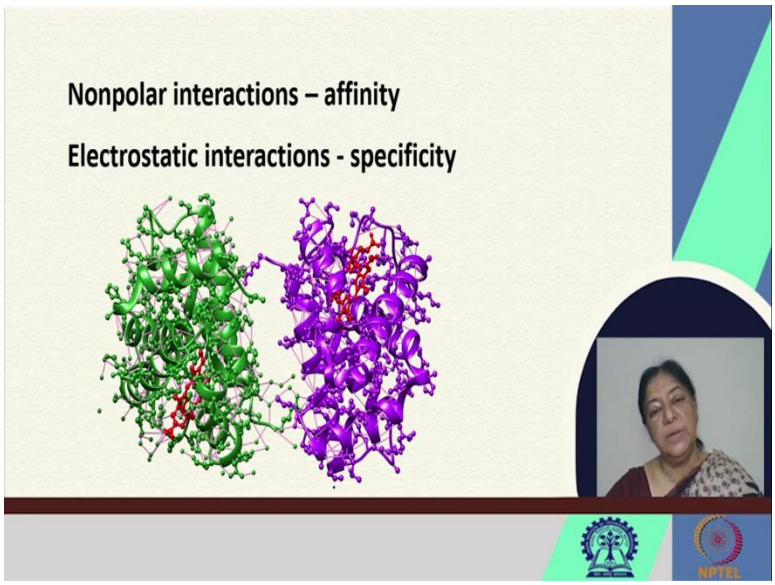

So when we are looking at the nonpolar interactions, we have the affinity. The electrostatic interactions give bring about some specificity and both of these are important in the complex formation that we see, that are going to bring these two protein molecules together.

So the nonpolar interactions are going to get the affinity and the specificity, both of which are important in bringing of the formation of a protein-protein complex, that is going to be a result of protein-protein interactions.

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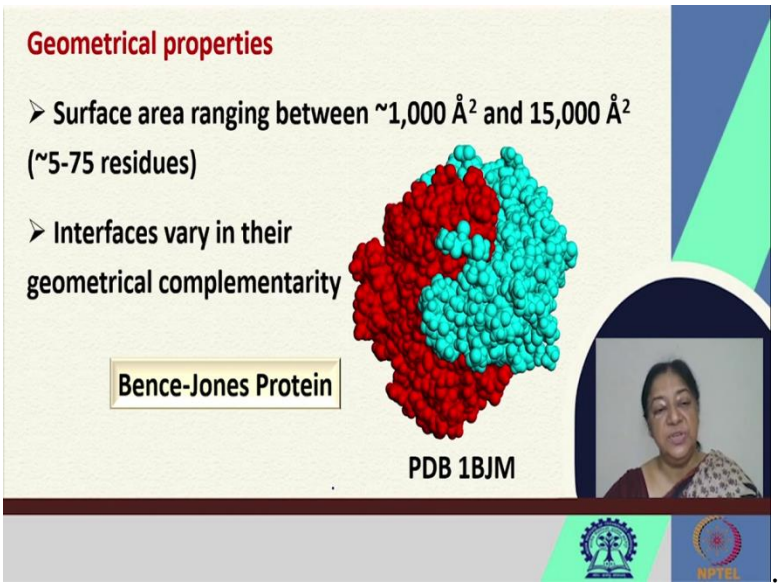

The geometrical properties that we have to look at is, the specific types of surface area. The surface area can range between 1000 $Å^2$ to even 15000 $Å^2$, covering 5 to 75 residues in the protein and the interfaces can vary in the geometric complementarity.

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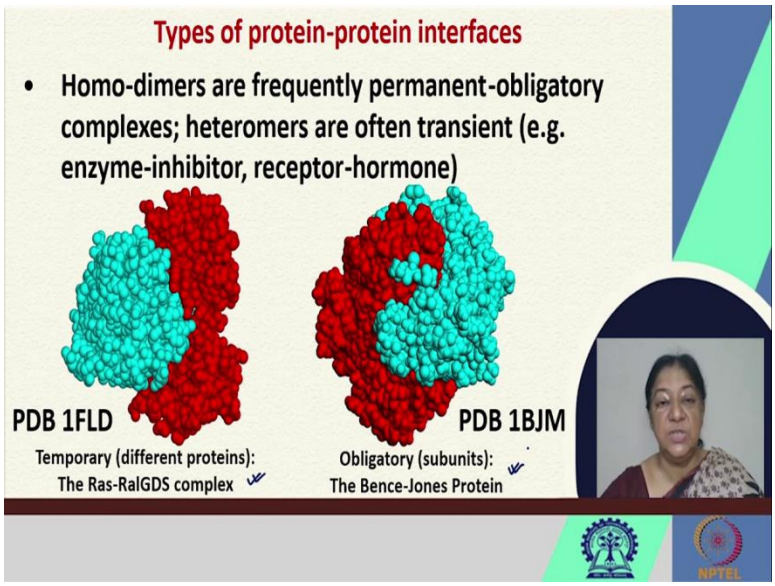

If we look at the specific types, they can be homo dimmers. These are frequently permanent-obligatory complexes; that means they have an obligation to interact with each other and heteromers are often transient. For example, if we are looking at an enzyme inhibitor type or a receptor hormone type of complex.

If we look at the specific type of temporary and permanent complexes that occur, the types of protein interfaces are going to depend upon the interactions present and the chemical complementarity that we look at. So, we can have an example of a temporary complex where we have different types of units. We can also have an obligatory one that is a subunit type, that are obligated to stay together in the dimeric form of the protein.

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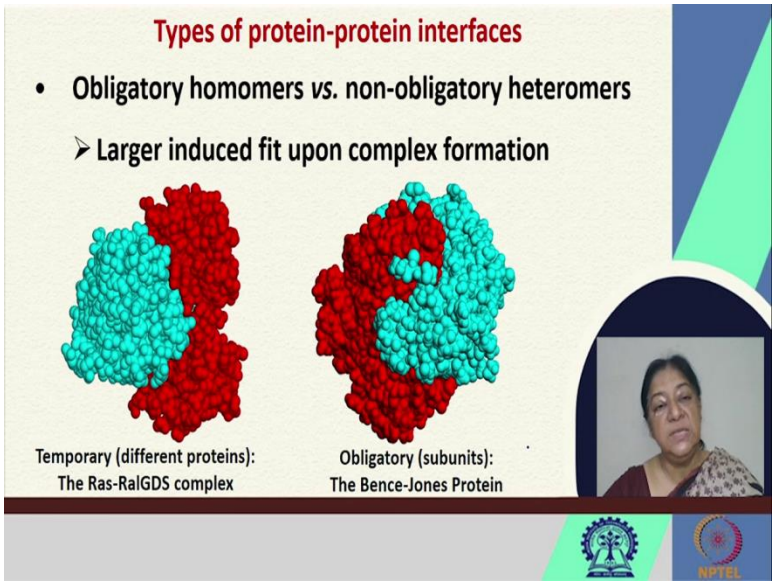

When we look at obligatory homomers versus non-obligatory homomers, the interfaces tend to be larger, more hydrophobic, more complementary and have a greater affinity because they are obligated to stay together to form the stronger complex that we see in dimeric proteins or in specific oligomeric proteins when we look at the specific induced fit upon complex formation, this means that the molecules adapt to interact with the other subunit, so that they form this complex.

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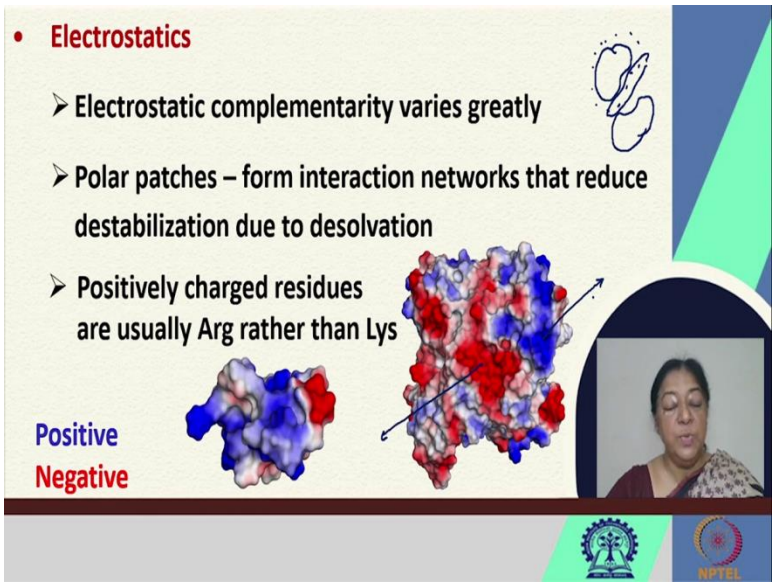

When we look at the electrostatics of the interactions, the electrostatic complementarity can vary greatly. We have polar patches on the surface, they form interaction networks that reduce the destabilization due to desolvation. We have to remember that when we have the protein molecule, the protein molecule is surrounded by the water molecules that solvate the protein molecule.

Now if we have another protein molecule that is going to come to interact with this, the interface water residues are destabilized due to the desolvation process, when we have the complex formation. So the interaction network could involve polar patches. We will also see how we have some water molecules that can also be present in the interface.

They have positively charged residues that are comparatively more arginine than lysine and we have the complementarity of the surfaces here [refer to slide] that would result in a strong interaction. These are the negative patches and these are the positive patches. Depending upon the structural electrostatic complementarity, we can have a complex formation. So we have the positive and negative parts here.

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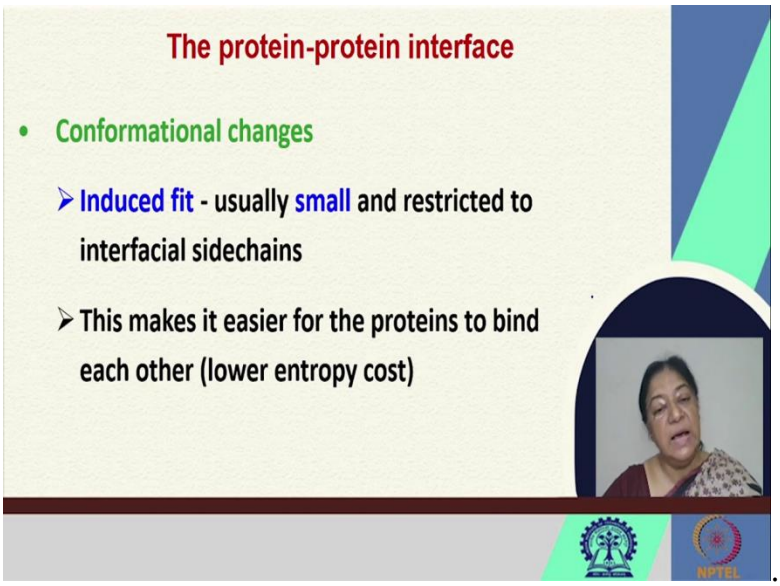

The protein-protein interface can also undergo conformational changes. The induced fit that occurs is usually small and this is restricted to the interfacial side chains that accommodate to form the complex formation. So what happens is this makes it easier for the two proteins that are coming together, to bind at a lower entropy cost.

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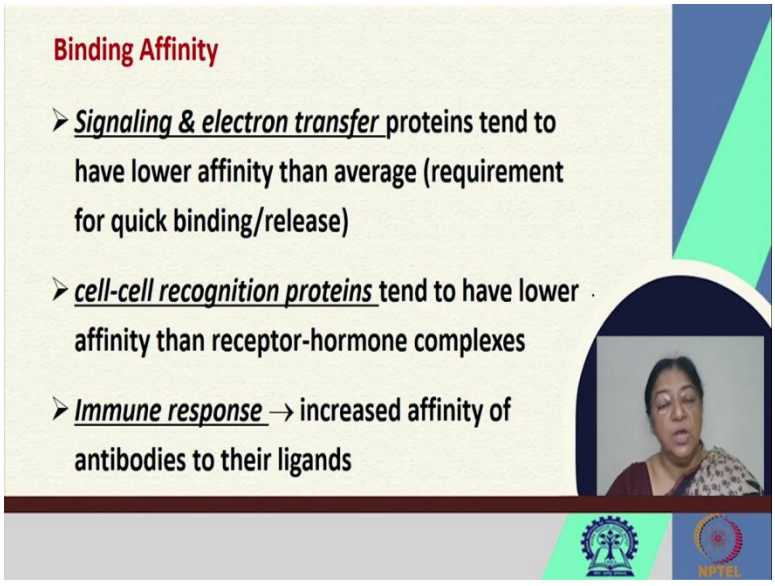

The binding affinity that we see in the specific types of examples given here[refer to slide], are for signaling and electron transfer. In this case proteins tend to have a lower affinity than average because it is required for quick binding and release, so you do not want too tight a complex to form. So the affinity is not that high.

The cell-cell recognition process also has to have lower affinity than the receptor hormone complexes and the immune response however, has increased affinity of the antibodies to their specific ligands or the antibody antigen interactions.

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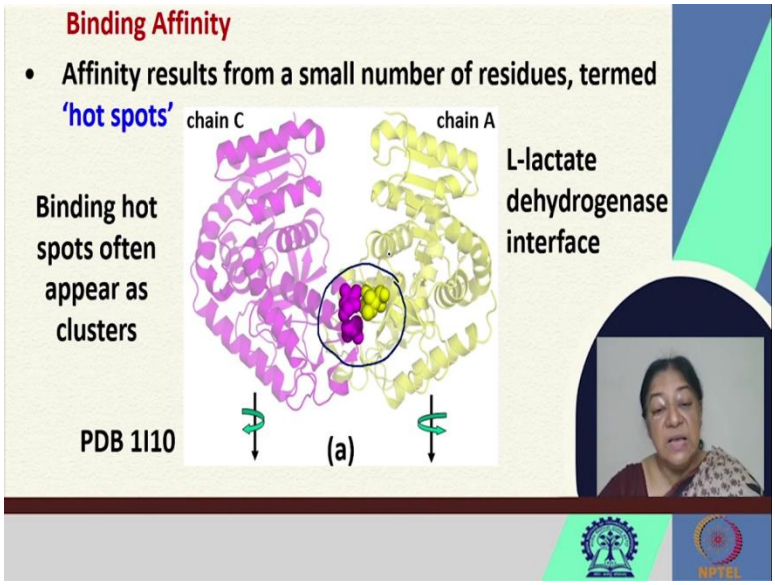

So the affinity that we see, results from a small number of residues that are confined on the surface that are termed hot spots. These hot spots are involved in the specific interaction that is going to result in the interface residues interacting with each other, to form the protein protein complex, in the protein-protein interaction.

The binding hotspot that we see in this [refer to slide] particular example where we have the lactate dehydrogenase interface, indicates that in chain A and chain C, there are specific binding hotspots that actually appeared as clusters on the surface.

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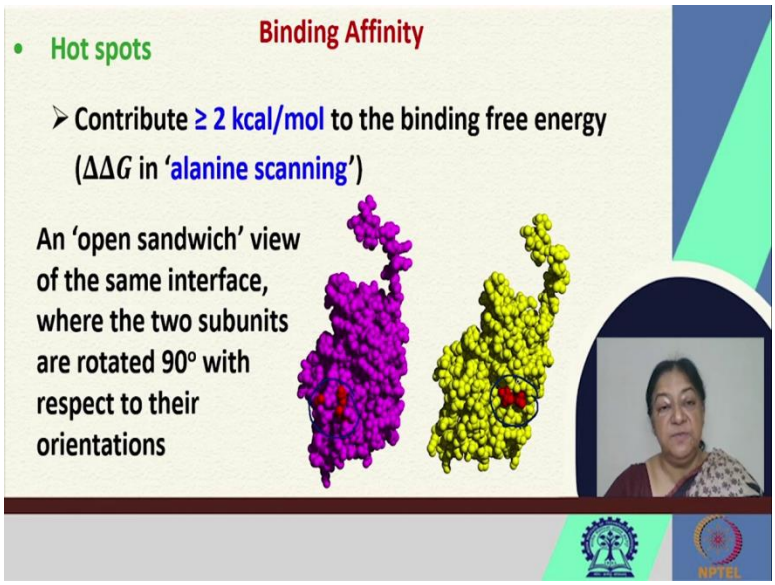

These hot spots contribute to the overall ΔΔG of the binding free energy and if we look [refer to slide] at the two complexes in this form, where we have an open sandwich view of the same interface, where the two subunits are just rotated 90° to each other. So this is where we have the interacting hotspot, that is important in bringing these two chains together to form the specific complex.

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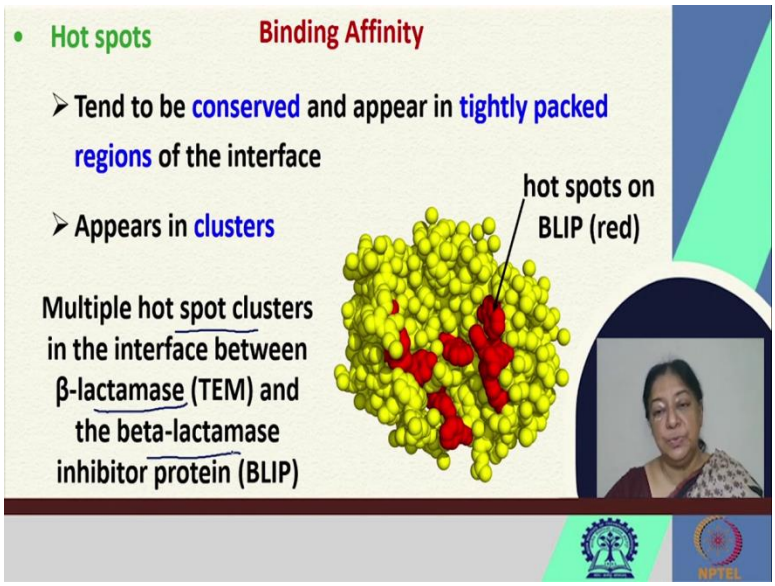

When we look at these hot spots, they tend to be conserved in nature and they appear in tightly packed regions of the interface. They appear in clusters and they have specific hotspot clusters and specific proteins that are the interface, that are the result of this specific interaction to occur. Wherein this [refer to slide] case, this is an example of a hot spot cluster between the β-lactamase and the β-lactamase inhibitor protein.

These hot spots would result in a strong interaction that would hold the complex together. Again it depends upon the type of affinity whether it is low affinity or high affinity, would depend upon the type of interaction or the type of specific process that it goes through.

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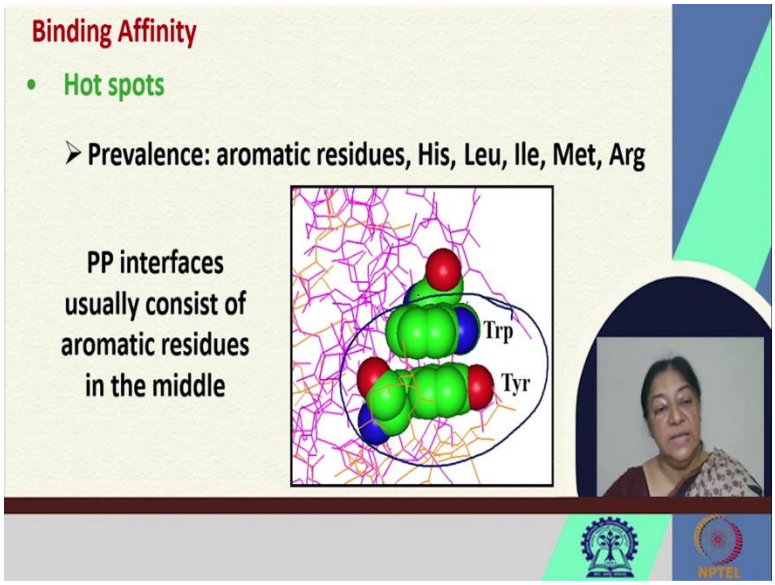

So we have these hot spots of prevalence, of aromatic residues as well as histidine leucine, isoleucine, methionine and arginine. We have the PP interfaces the protein protein interface, where we have aromatic residues, where we can have an involvement of stacking interactions in the middle of this interface.

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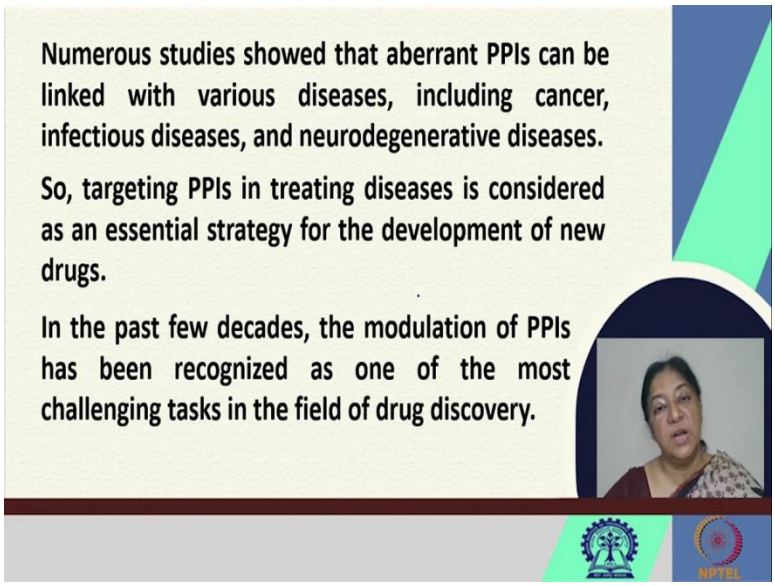

So these numerous studies show that aberrant protein-protein interactions can be linked with various diseases because we understand that the protein protein complex formation is involved in many biological processes; whether its signal transaction or cell cell addition or even motor protein myosin acting interactions. These PPIs in the treatment of diseases, is considered an essential strategy for the development of new drugs and there can be modulation of these PPIs that can be recognized for drug discovery.

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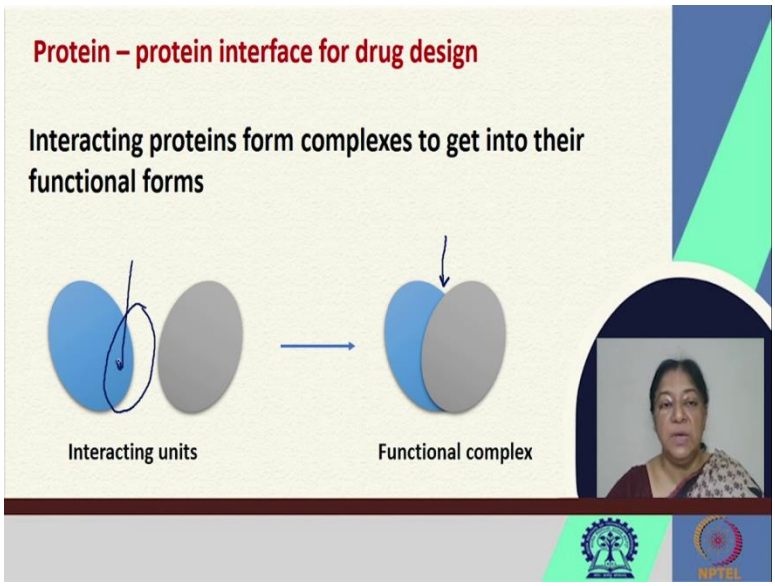

So, if we look at the protein protein interface for drug design we know that there are interacting units and the interacting units form specific complexes to get to their functional complexes or their functional forms. So this [refer to slide] is where we lose a specific surface area. We can find out what the specific interface residues are, the hot spots that are going to be involved in the interaction and we can target the specific points to develop drugs for inhibition of the protein protein complex formation, that would be required to inhibit a specific biochemical, biological process.

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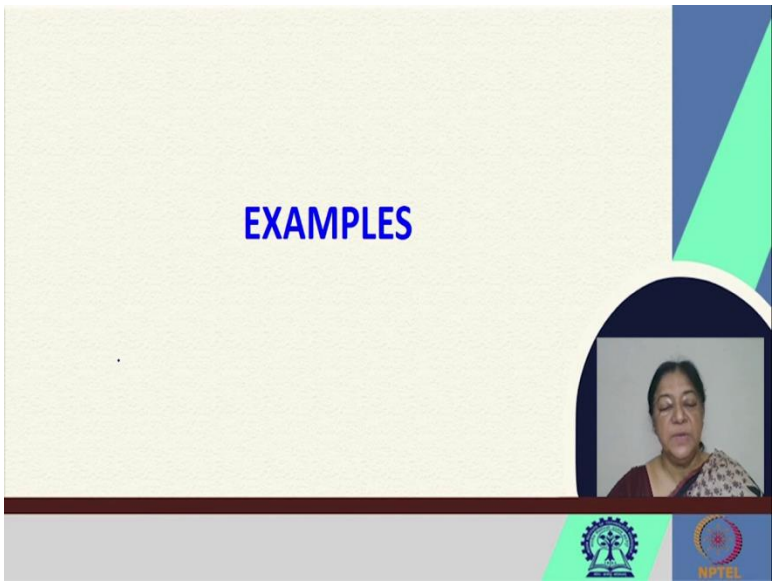

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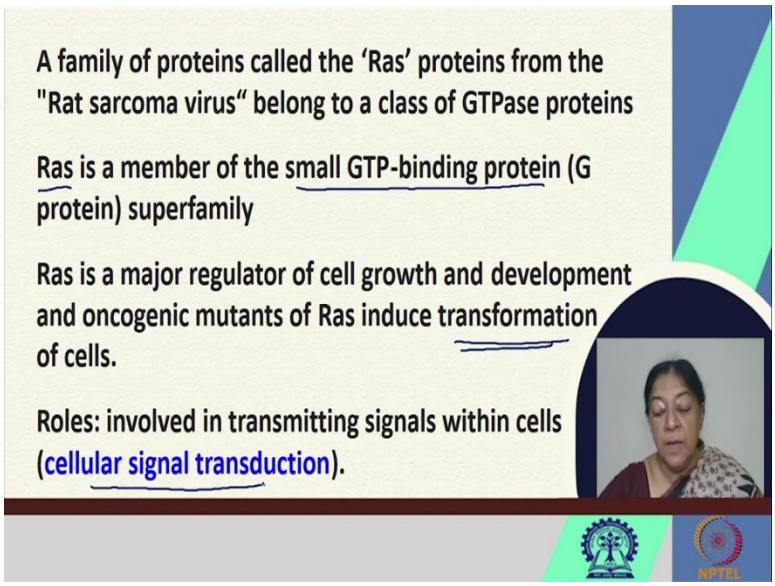

If we look at some specific examples, we have a family of proteins called the Ras proteins. They are from the Rat sarcoma virus. They belong to a class of GTPase proteins. So, this Ras is a member of the small GTP binding proteins of the G protein superfamily. This protein is a major regulator of cell growth and development and oncogenic mutants of Ras, they induce specific transformations in cells. The roles of this specific protein are in transmitting signals within the cells, in what we call cellular signal transduction.

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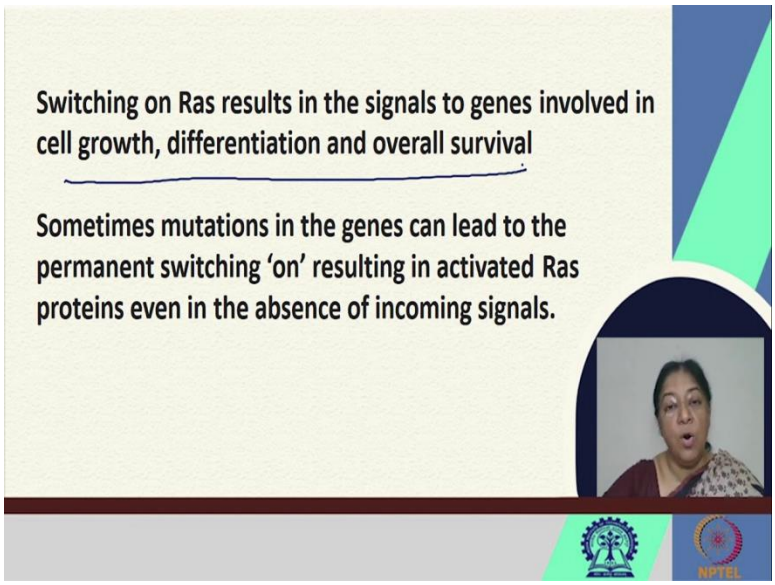

The switching on of this Ras protein, results in the signals to genes that are involved in cell growth, differentiation and overall survival. Now what happens is, if there are mutations in these genes, this can lead to a permanent switching on, that is going to affect the cell growth and differentiation. So even in the absence of incoming signals, if these mutations results in an on signal all the time then the process is going to continue, that is going to be detrimental to health.

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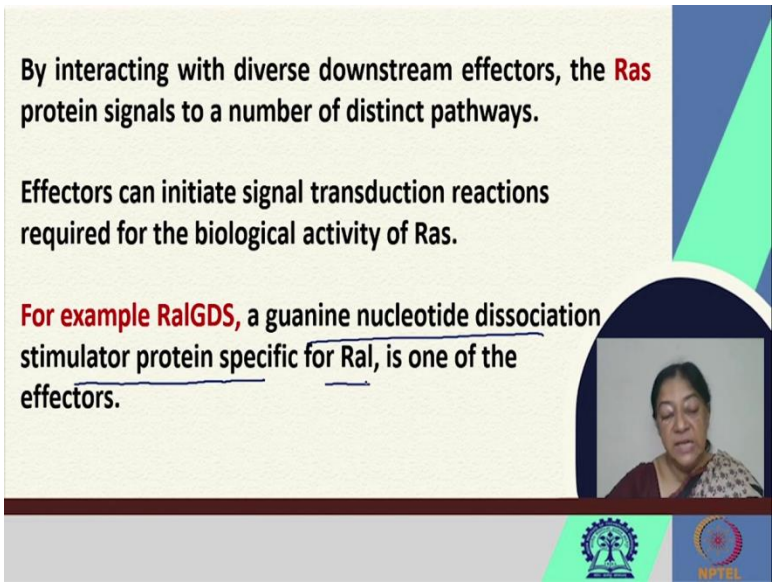

Now what happens is, if these can interact with specific downstream effectors, the Ras protein signals are involved in a number of distinct pathways. The effectors initiate signal transduction reactions that are required for the biological activity of the Ras. We will just look at an example of an interface, guanine nucleotide dissociation stimulator protein, specific for Ral is one of the effectors.

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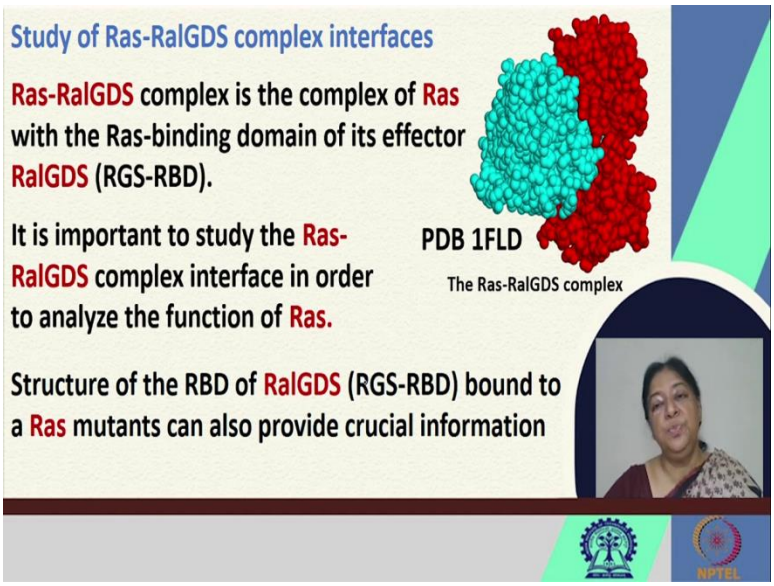

So the study of these complex interfaces, is going to give us some information about how they can be targeted for design of any drugs. When we are looking at the Ras binding domain of its effector, we are looking at the interface.

The complex interface gives us an important platform to study and to analyze the function of the protein and to know where to target it, to stop any aberrant protein-protein interactions, that is going to lead to disease. The structure of these bound complexes, bound to the mutants even, can also provide crucial information required for the development of drugs against this Ras protein.

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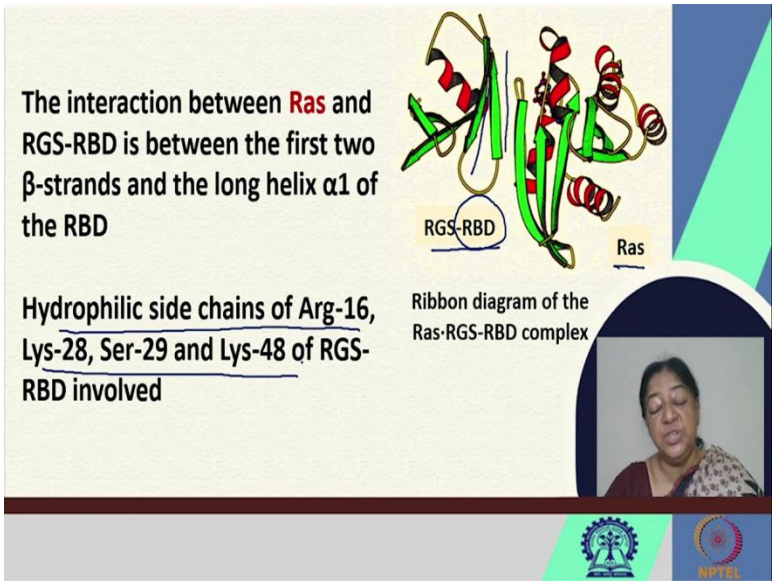

So if we look at this specific interface, where we have the Ras protein and the RGS-RBD protein type the interaction between the Ras and the RGS RBD is between the first two β strands and the long α helix of the RBD. So, here [refer to slide] we have the β strands and we have the Ras binding domain, where we have the interactions.

What we see is they have hydrophilic side chains that are involved in the interactions. So, we understand that the surface properties of the individual proteins as they were in solution, would still have the hydrophilic surface or the hydrophilic side chains on the surface of these two proteins, but they are also involved in the interaction between these two proteins.

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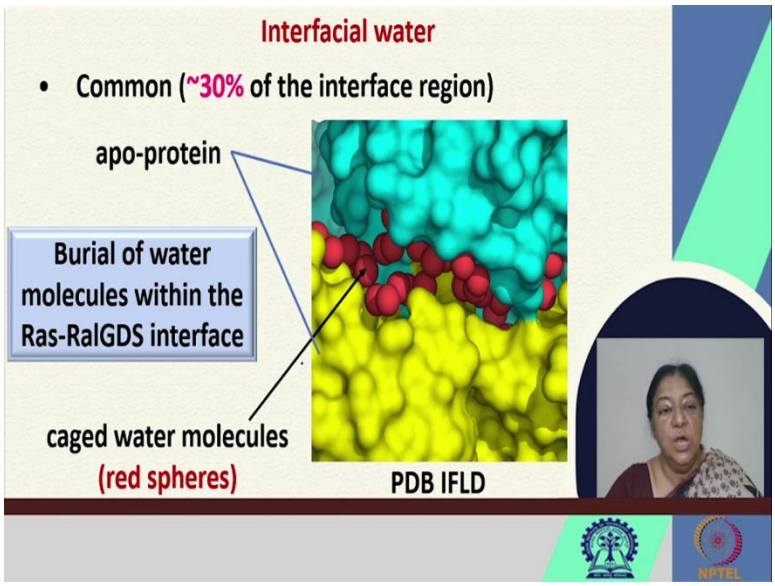

This means that there is going to be some interfacial water because about 30%of the interface region can be involved with water. So, what we see [refer to slide] is because there are hydrophilic residues on the surface that would be solvated, the interfacial water also remains when we have the caged water molecules or the red spheres that are trapped in the protein-protein interface.

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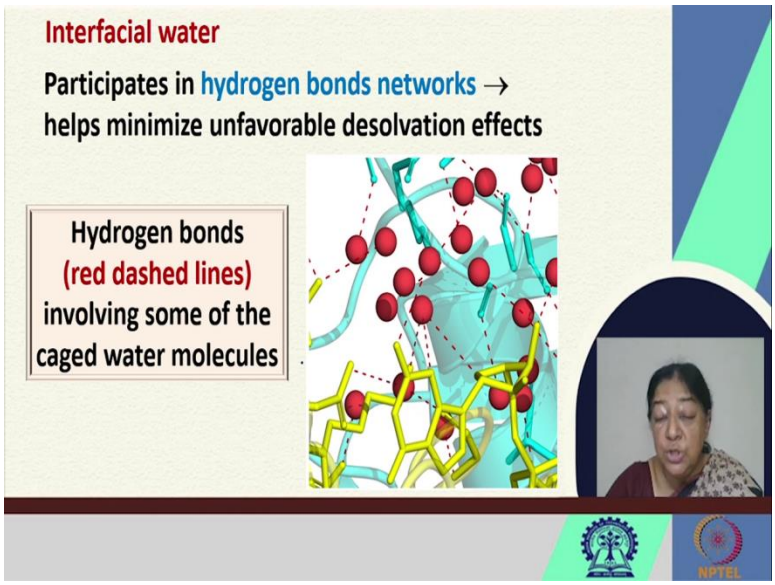

So we have an apo-protein, where we have this specific interfacial water. This participates in hydrogen bonding networks and helps minimize unfavorable desolvation effects. This is where we have a lot of interaction, where we have hydrogen bonds that involve some of the caged water molecules that are also very important in the protein-protein interaction that occurs.

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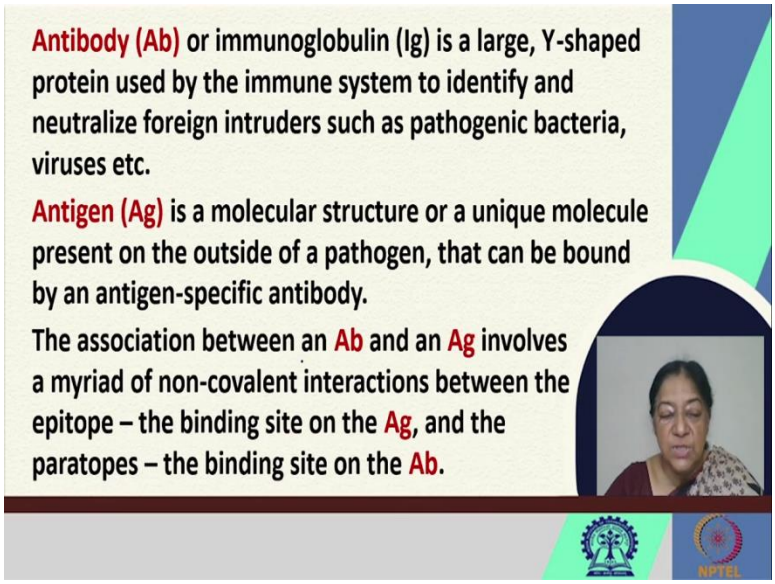

Another very important example is the antibody-antigen type of example. The antibody we know is a large Y-shaped protein that is used for the immune system to identify and neutralize foreign intruders, be they bacteria or viruses and we have the antigen, that is a molecular structure or a unique molecule present on the outside of the pathogen, that can be bound by an antigen specific antibody and this association is very important.

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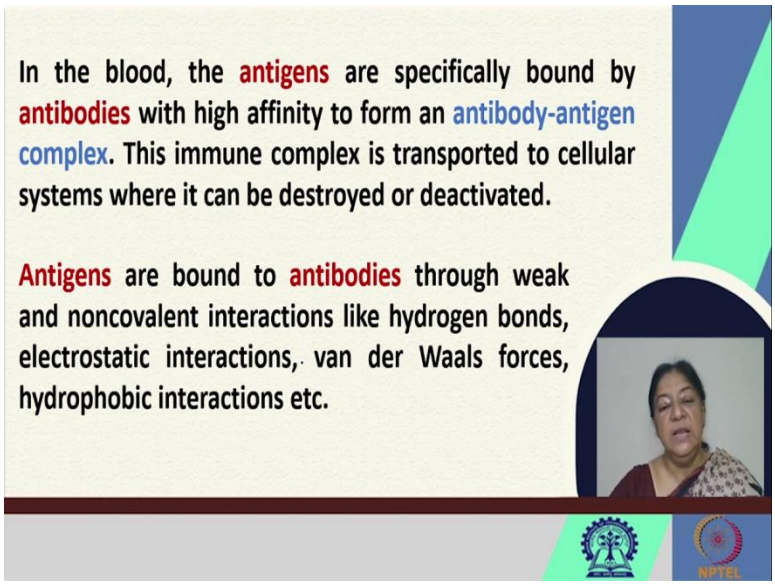

If we look at an overall structure of the antibody what we have is, we have in the blood the antigens that are specifically bound to antibodies in very high affinity complexes, to form the antibody-antigen complex.

This immune complex is transported to the cellular systems, where it can be destroyed or deactivated. These antigens are bound to antibodies through weak and noncovalent interactions like we saw for the previous cases hydrogen bonds, electrostatic interactions, van der Waals forces and hydrophobic interactions.

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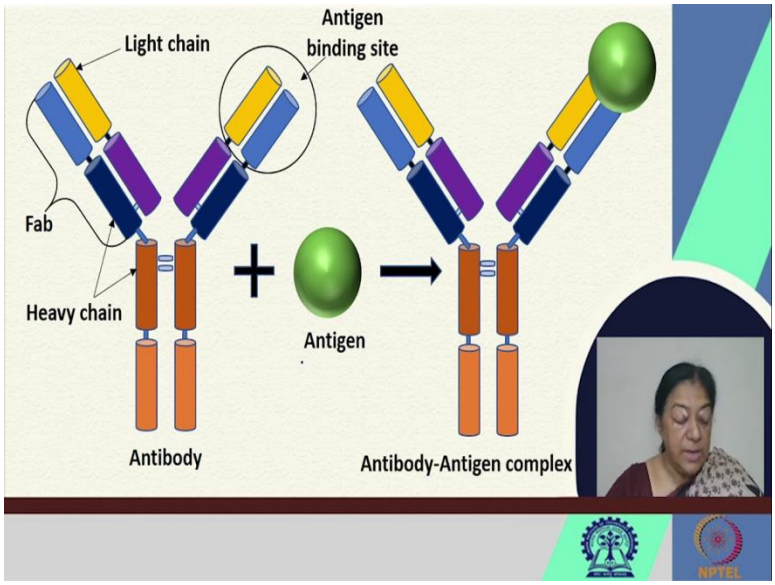

But these are extremely important interactions in the protein factor. So we have the antibody, we have the light chain, we have what is called an Fab domain, a heavy chain and we have an antigen binding site. These are very specific in nature with strong high and very high binding

affinity. So when we have the antigen, we have a specific antigen binding site that is going to bind to the antibody. Now there are lots of variations that can occur in an antibody-antigen complex.

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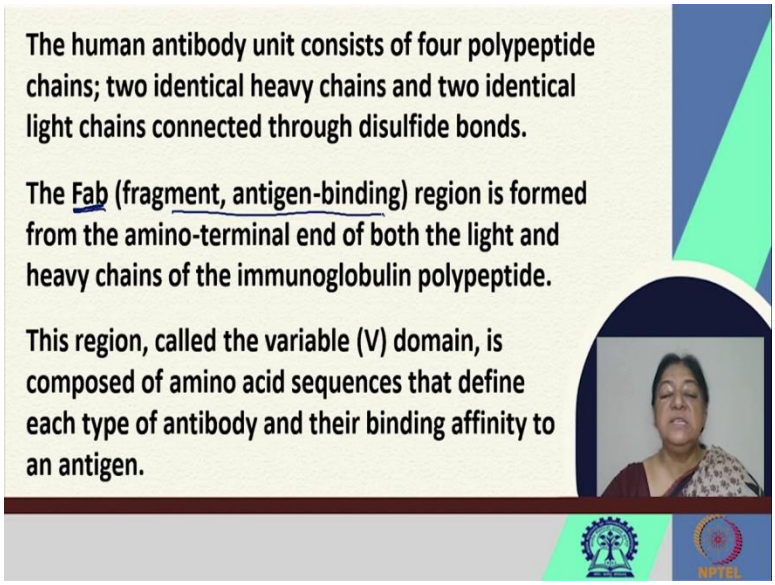

So, the human antibody consists of four polypeptide chains. There are two identical heavy chains and two identical light chains, that connect through disulfide bonds. We have the Fab, that is the antigen binding fragment, that is formed from the amino terminal as we saw in the previous figure. This region is called the variable domain and it is composed of amino acid sequences that define each type of antibody and the specific binding to an antigen.

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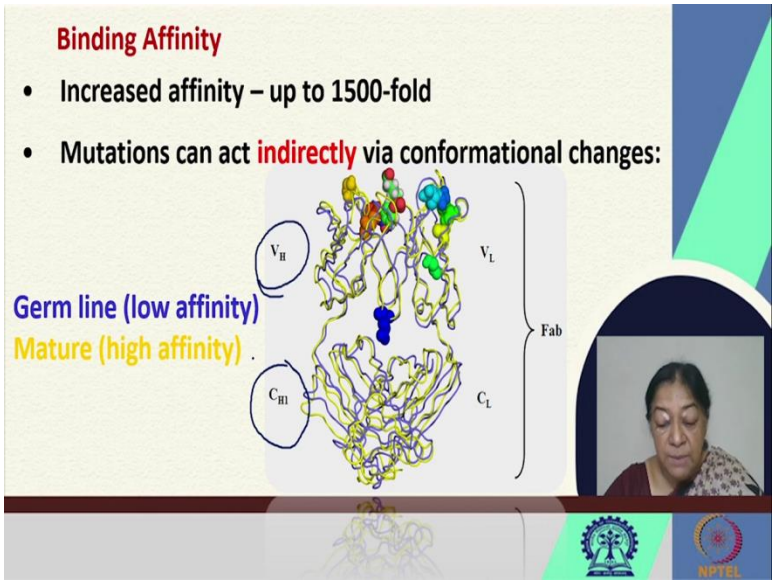

So when we look at the binding affinity, we can have increased affinity up to 1500-fold and mutations can actually indirectly, via their conformational changes, can affect the binding. So we can have a high affinity binding or a low affinity binding, depending upon what happens in the variable and the constant regions in their interactions, in the antibody, in the binding domains that we see.

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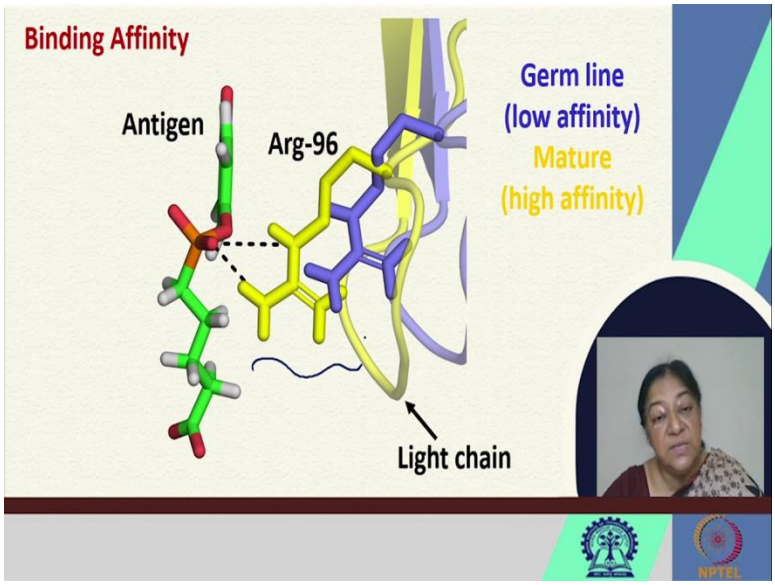

So if we look [refer to slide] at the specific light chain binding here where we have an arginine 96, we can see that there is the antigen that interacts with the antibody and there is a specific movement, where we would have a low affinity type and a high affinity type, that is going to have the specific interaction between the side chain in this specific case.

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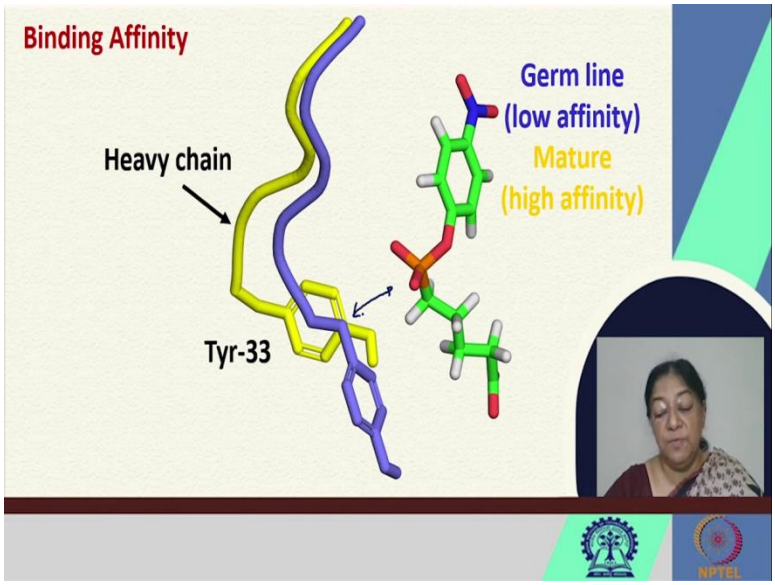

It is going to be a specific heavy chain, that is going to again interact with its specific partner in a high affinity set. So there is going to be a movement of these chains for a facilitated protein-protein interaction.

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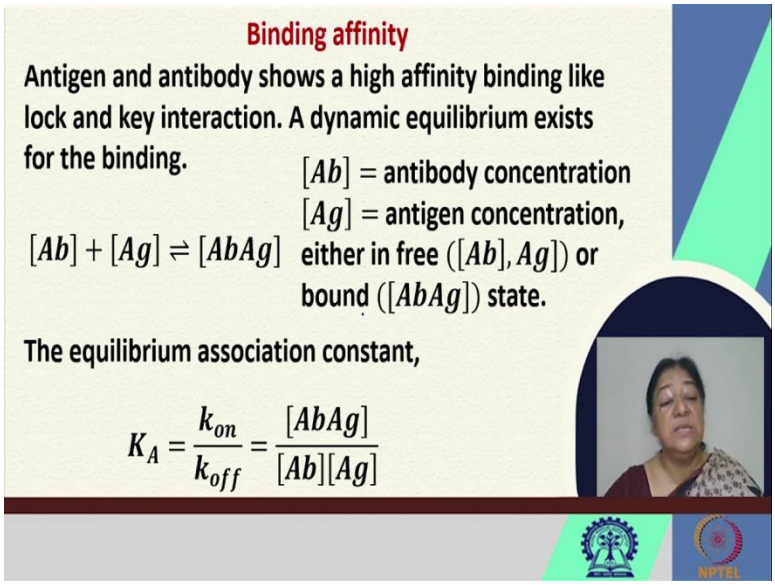

If we look at the binding affinity, antigens and antibodies show very high binding affinity, like a lock and key interaction and we have a dynamic equilibrium similarly to, what we saw in a protein ligand binding. Here [refer to slide] we have an antibody-antigen interaction.

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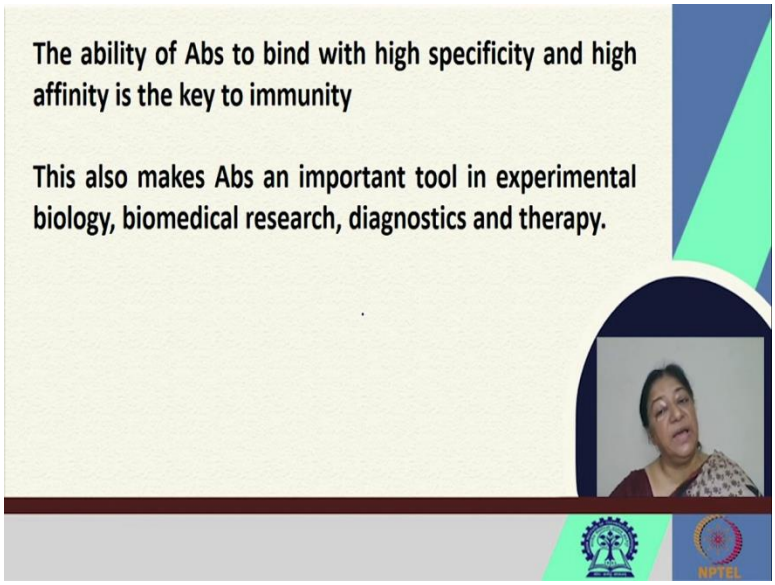

Again we can have an equilibrium association constant that from the ratio of k_{on} and k_{off}, is going to give us an association constant from which we can understand the specificity and the affinity

of this and this makes it an important tool in experimental biology and research for diagnostics and therapy.

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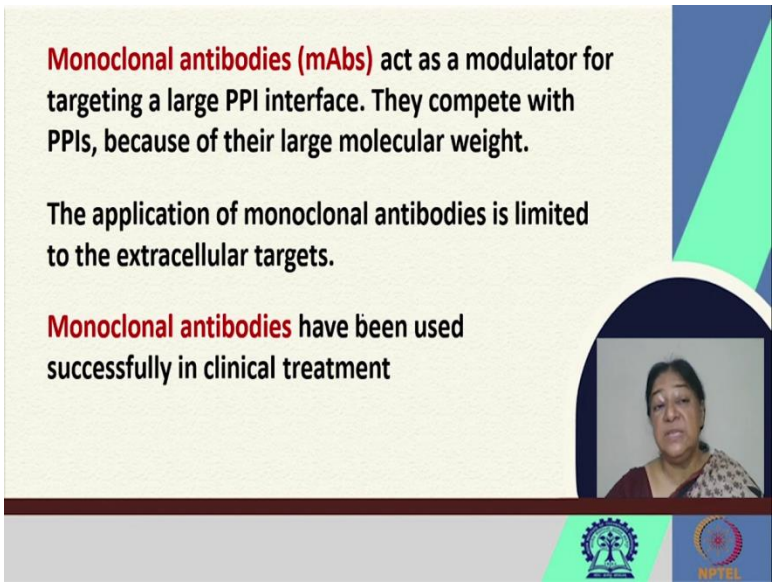

This is where we have monoclonal antibodies, that act as modulators for targeting these protein-protein interfaces. They compete with PPIs, because of their large molecular weight and the application of these monoclonal antibodies, is limited to the extracellular targets. What we have here is some specific type of antibodies that have actually been used in clinical treatment.

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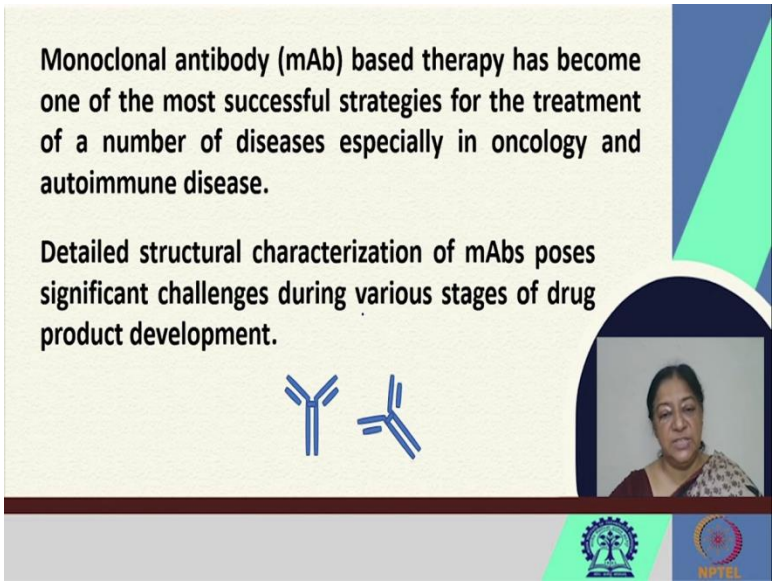

In this case, this is used in specific types of diseases where the detailed structural characterization of the monoclonal antibodies is required, for specific types of interactions with antigens.

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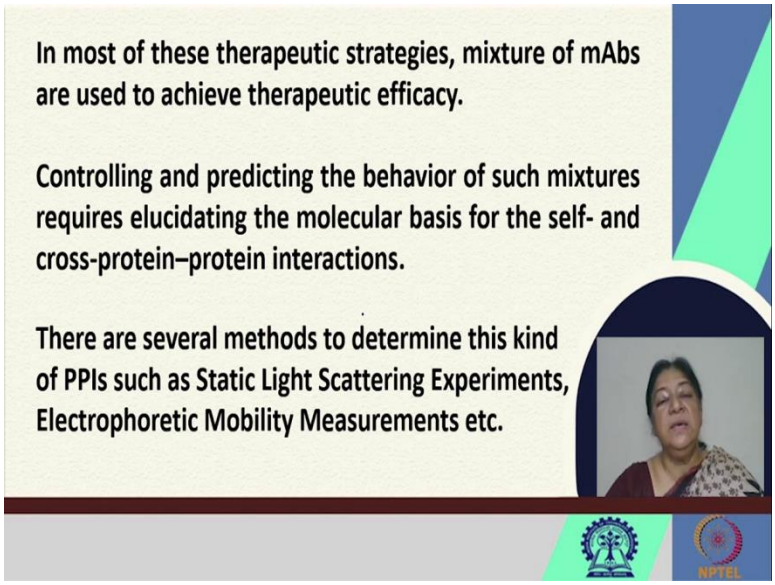

What we have in this therapeutic strategy mixture of antibodies that are going to actually interact with specific antigens and what happens is they have protein-protein interactions that occur and these can be studied through several methods.

So the idea here is, that if we have a foreign body an antigen, we have different types of antibodies, that are formed, that are designed to interact or bind with these antigens in the forming of the antigen-antibody complex, that then can be destroyed.

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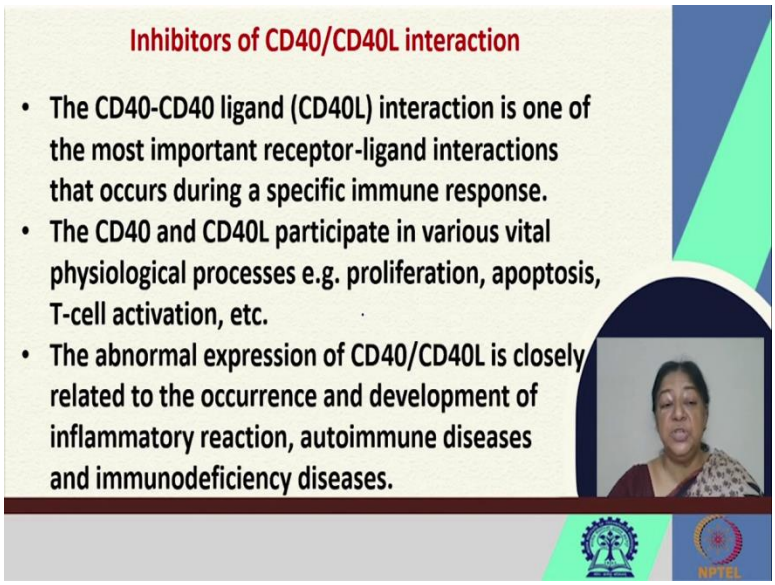

For example, if we look at an inhibitor of a CD40 CD40L interaction. This is one of the most important receptor ligand interactions that occurs due to a specific immune response. They participate in several reactions in physiological processes and the abnormal expression is related to many diseases.

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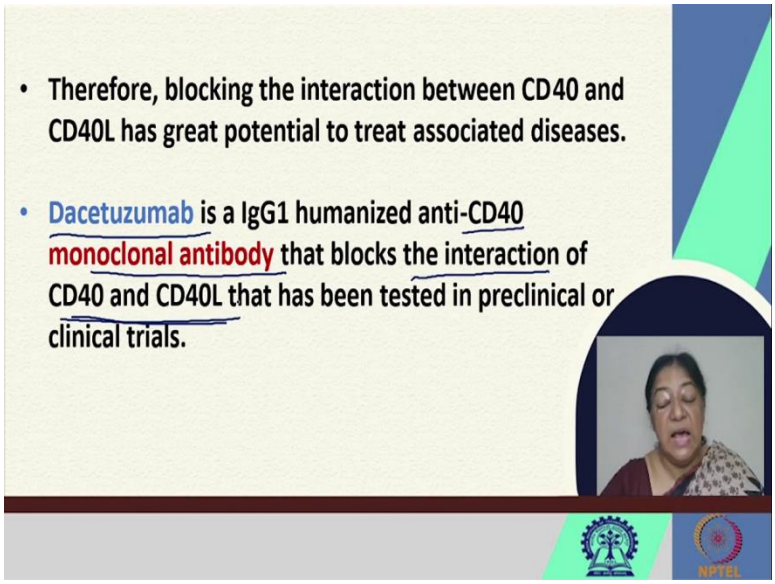

So the idea here is, that if we block the interaction between the CD40 and the CD40L, it can treat the associated diseases. There is a specific type of anti-CD40 monoclonal antibody that actually blocks the interaction of this CD40 to CD40L and this has been tested in clinical trials.

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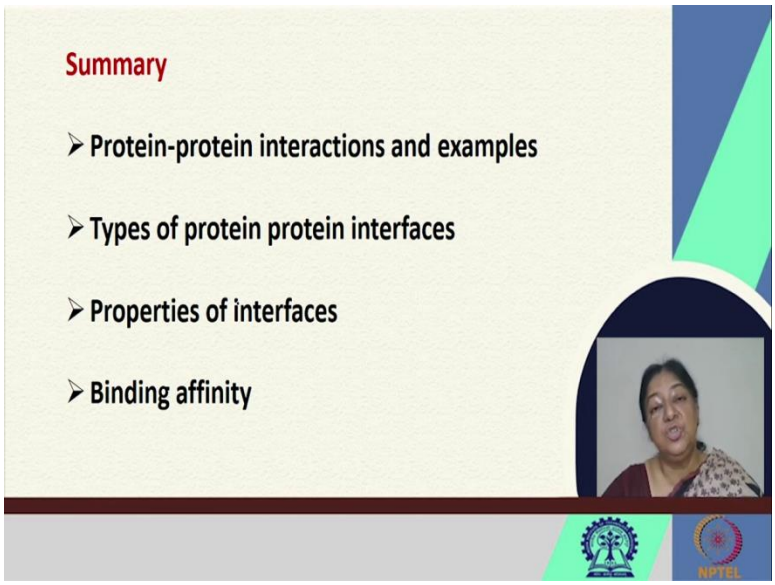

So what we have looked at is, we have looked at the types of interfaces, the protein-protein interfaces, what comprise the interfaces, what residues are likely to be in the interface, what accessible surface area we might lose and in addition to the geometric complementarity and the chemical complementarity, the types of water molecules that may be present that are trapped in the hydrophilic surfaces of the protein-protein complexes that nevertheless are required; that could be of a permanent type or a transient type.

So depending upon the type of protein-protein interface, we would have specific hot spots that would result in a strong affinity that would be required for specific types of protein-protein complexes.

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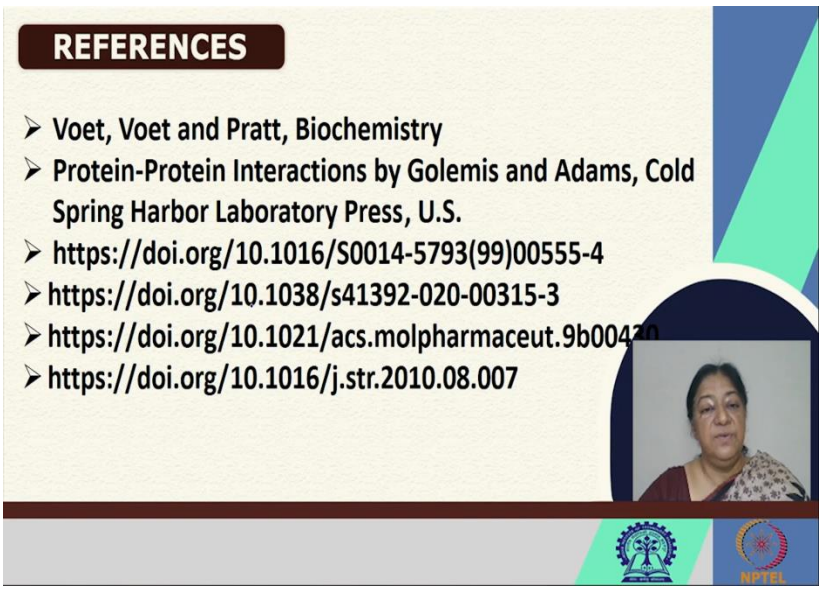

These [refer to slide] are the references.

Thank you.