Fundamentals of Protein Chemistry
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Module - 11
Protein Macromolecule Interactions II
Lecture - 52
Protein Protein Interactions - II

We continue our discussion on protein-protein interactions, where we have looked at the geometric and the chemical complementarity of these protein-protein interactions in the previous lecture.

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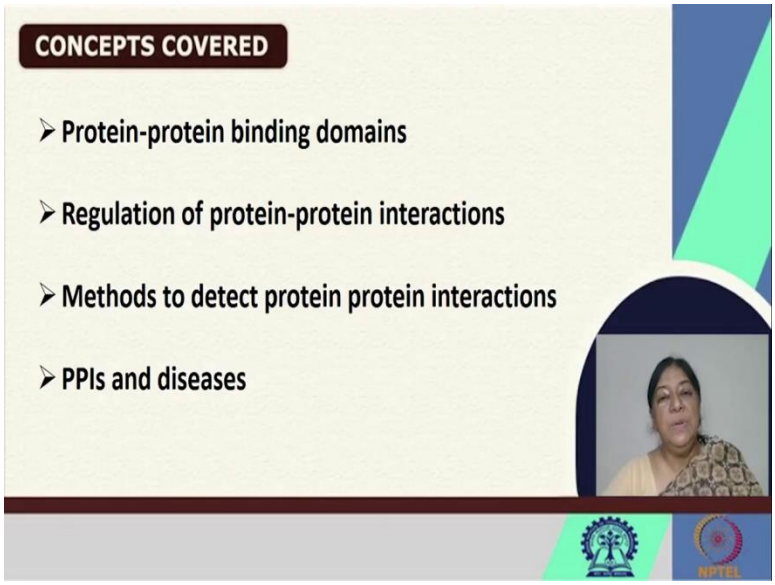

In this lecture we will be looking at protein-protein domains, the regulation of these protein-protein interactions, a specific method to detect these protein interactions and how the PPIs and diseases are related.

In the identification of the domains, in the understanding of how a protein-protein interaction is going to take place, we realize that the recognition is the most important. In the previous lecture we looked at the interfaces, the specific amino acids that may occupy the interface, the loss in the surface area on the interactions and how there may be induced fit.

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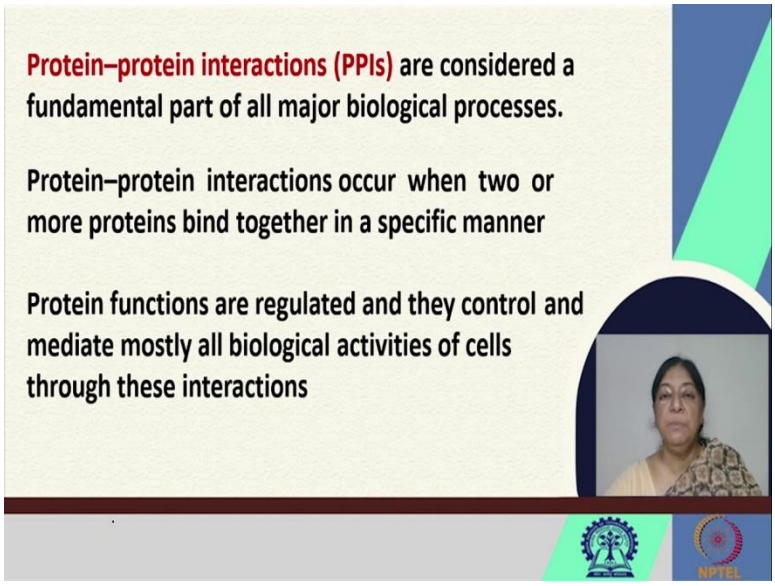

When we look at the specific protein-protein interactions, we realize their importance in all biological processes and we have these interactions when two or more of them will bind in a specific manner, with high or low affinity; depending on the specific biological activity that they are involved in.

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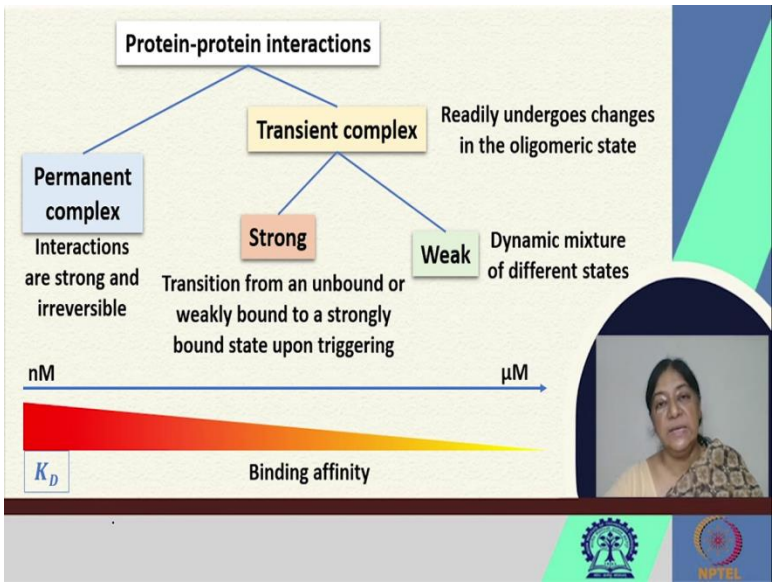

So for the protein-protein interactions we can have a permanent complex where the interactions are strong and irreversible, like in specific subunit proteins where we have the quaternary structure involved which is a permanent complex of the monomeric units.

We can have transient complex where we have either a weak type, depending upon the specific conditions, the specific reaction that has to occur and also a strong type, where we can have a transition from an unbound weakly bound to a strongly bound state that is triggering on the action of any cofactor or ATP hydrolysis. If we look at the specific dissociation constants associated with this, we see that the binding affinity for the permanent complexes are much smaller, the K_D values are smaller, indicating a tighter complex.

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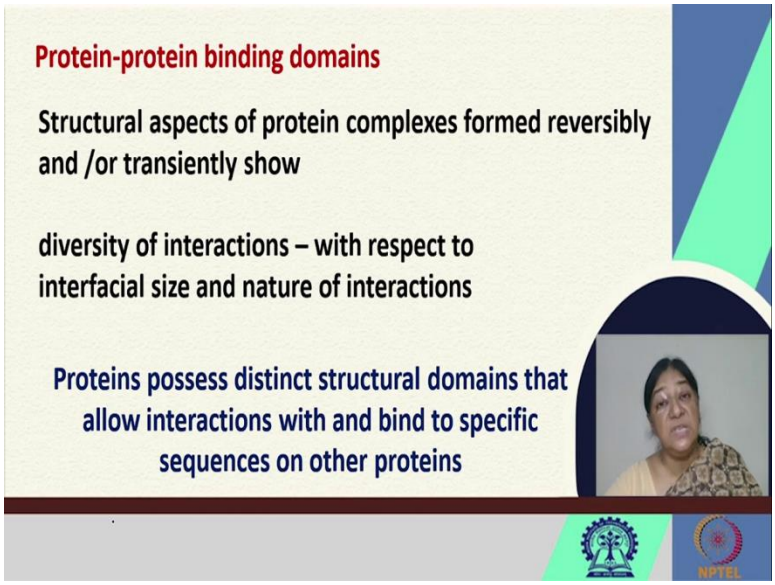

When we consider the protein-protein binding domains, we try to understand what kind of residues are involved in these interactions and whether that are common motifs that are involved in the recognition where we have protein-protein interactions.

The structural aspects of the protein complexes formed are reversibly or even transiently show a diversity of interactions, with respect to the interfacial size, as well as the nature of the interactions. So they possess these distinct structural domains, that allow interactions with specific sequences of other proteins.

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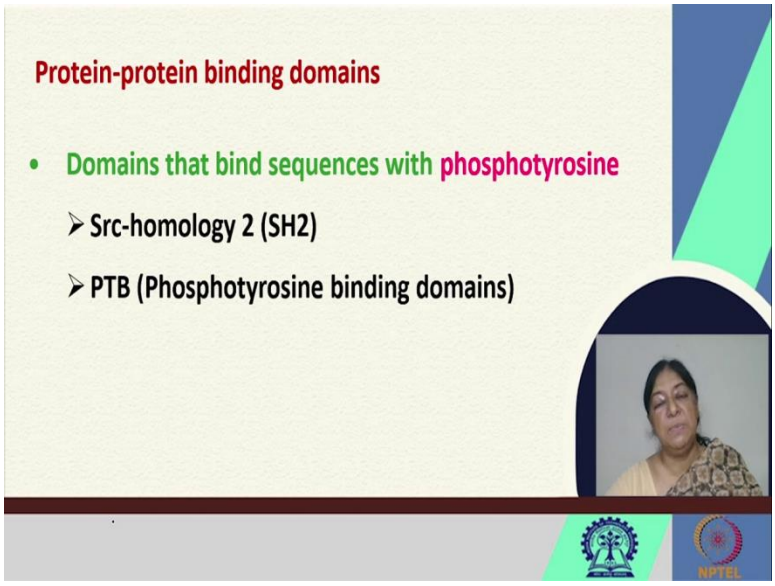

If we look at the protein-protein binding domains, there are domains that bind sequences with phosphotyrosine. So, this one example is the Src homology 2 the SH2 type or a phosphotyrosine binding domains that may be present on specific proteins.

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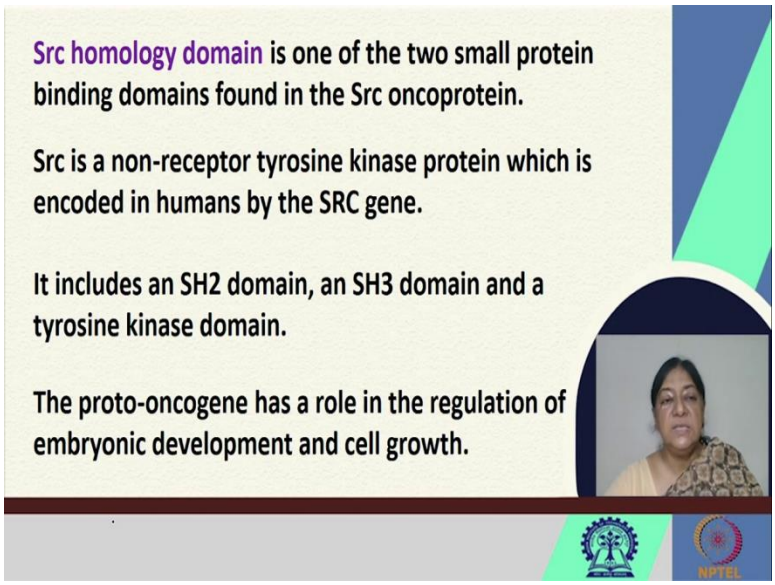

The Src homology domain is one of the two small protein binding domains, that are found in the Src oncoprotein. Now Src is a non-receptor tyrosine kinase protein, which is encoded in humans by the Src gene. It includes an SH2 domain, an SH3 domain and a tyrosine kinase domain. The proto-oncogene has an important role in the regulation of embryonic development as well as cell growth.

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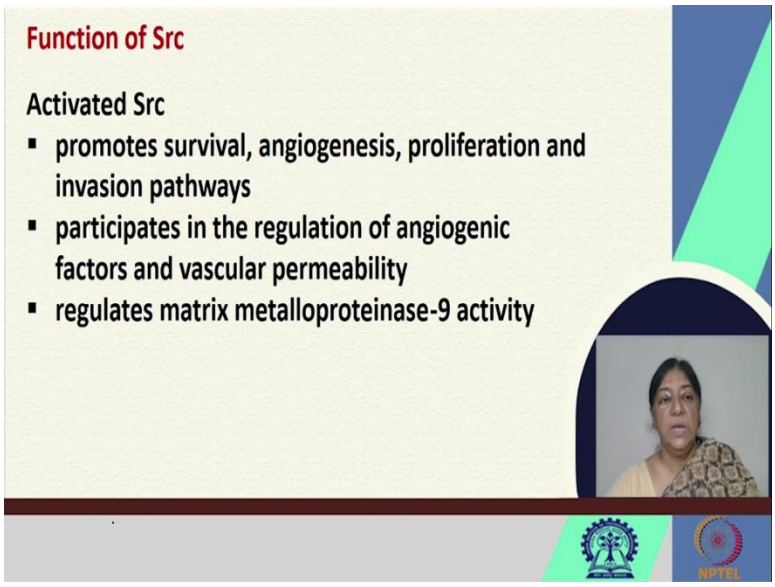

So the function of the Src, is when it is activated it promotes survival, angiogenesis, proliferation and invasion pathways. It participates in the regulation of angiogenic factors and vascular permeability and regulates the activities of many proteins including the mmp-9 activity.

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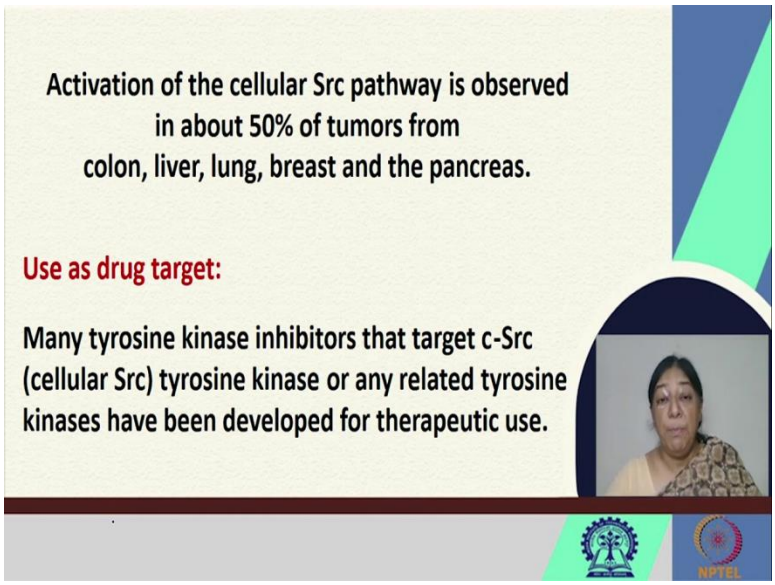

So the activation of the cellular Src pathway is observed in many tumors, as such it is used as a drug target. Many tyrosine kinase inhibitors that target the cellulose Src tyrosine kinase or any related tyrosine kinases have been developed for therapeutic use.

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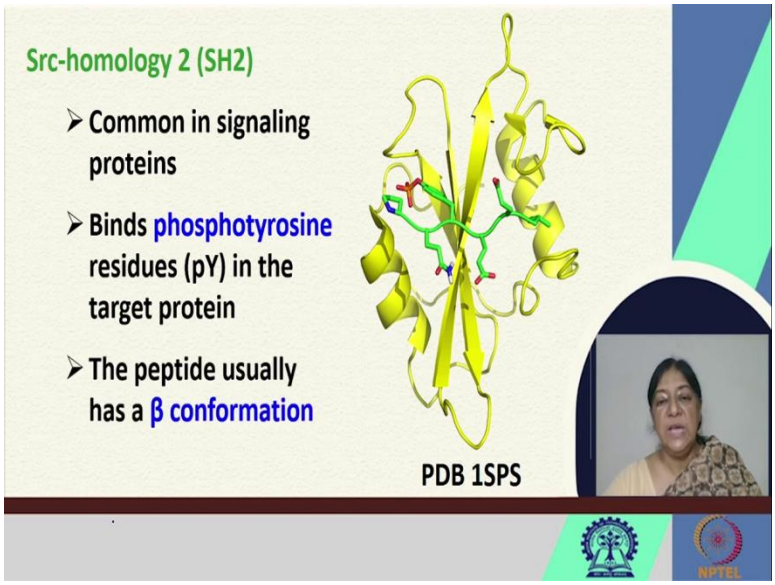

If we look at the SH2 protein and the recognition it has, it is very common in signaling proteins and it binds the phosphotyrosine residues in the target protein. The peptide usually has a β conformation.

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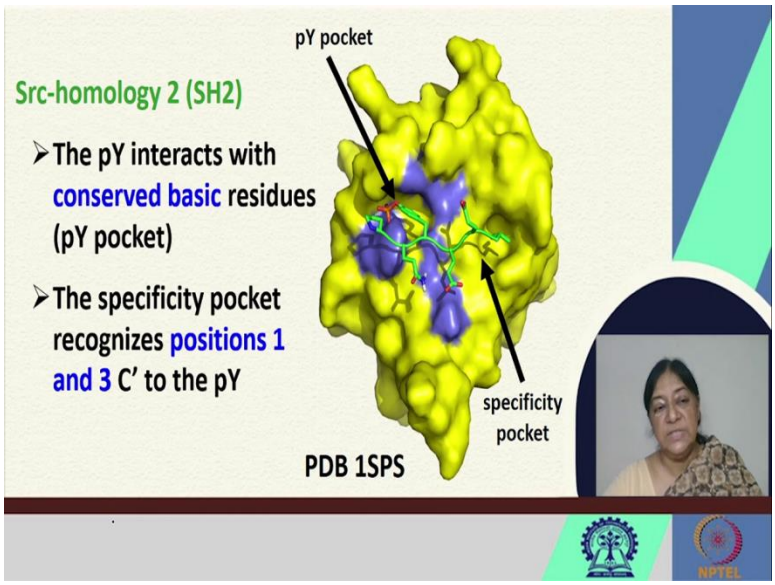

Now, the importance of this [refer to slide] protein is in the pocket or the cleft that is seen in the specific receptor protein. There is a pY pocket, that is the phosphotyrosine pocket and we have a specificity pocket, related to the geometric and the chemical complementarity that is required for the strong affinity. So the pY interacts with conserved basic residues and the specificity pocket recognizes specific positions of the receptor protein.

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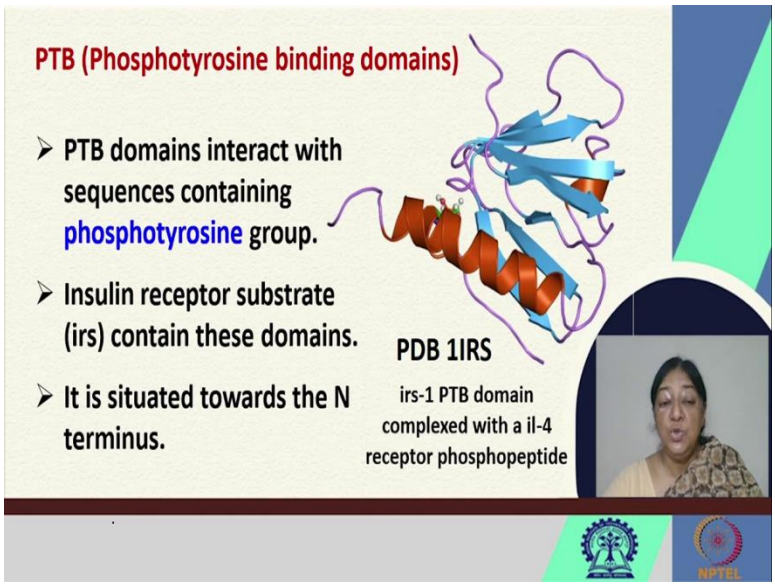

If we look at other phosphotyrosine binding domains, the importance of these domains lies in their geometry, in their overall structure and in the residues that are required for the specific recognition. So in the PTB domains they interact with sequences again that contain the phosphotyrosine group and one such is the insulin receptor substrate, the IRS that contains these domains. This is situated towards the N terminus.

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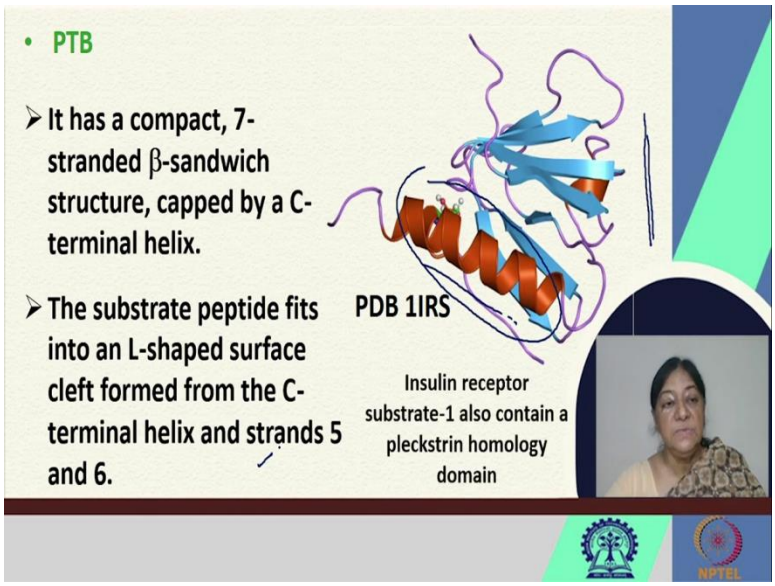

The PTB, as we can see [refer to slide] it has a compact 7 stranded β-sandwich structure that is capped by a C terminal helix and the substrate peptides fit into the L shaped surface cleft, that is formed by this C terminal helix and the strands 5 and 6.

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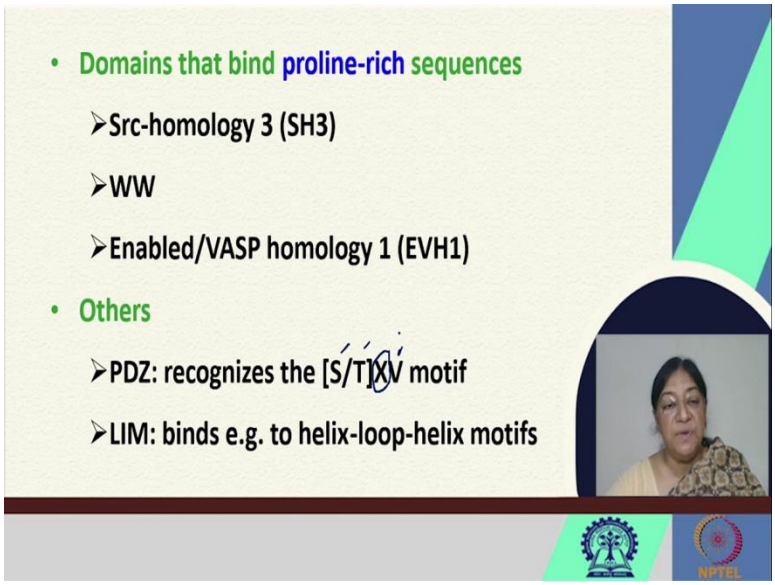

If we look at the other types of domains, there are domains that can bind proline rich sequences. This is the Src-homology 3, the SH3 type domain, a WW domain and the enabled VASP for a EVH1 domain and there are others such as the PDZ domain, that recognizes as we can see serine, threonine, any amino acid and a valine motif.

We also have the LIM domain that binds to helix-loop-helix motifs. So the specific recognition sites have been defined by these domains.

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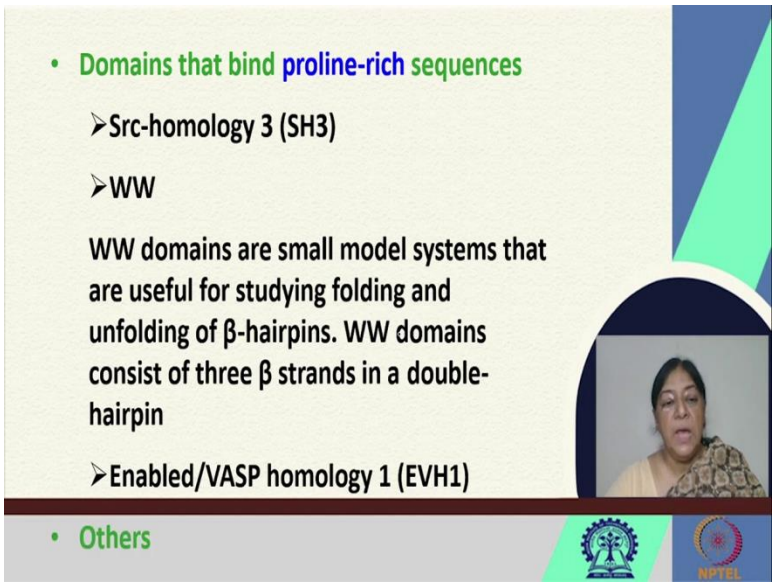

The domains that bind proline rich sequences, an example is these WW domains. The WW domains are small model systems that are useful for studying folding and unfolding of β-hairpins and they consist of 3 β strands in a double hairpin and we also have the enabled VASP homology 1 (EVH1).

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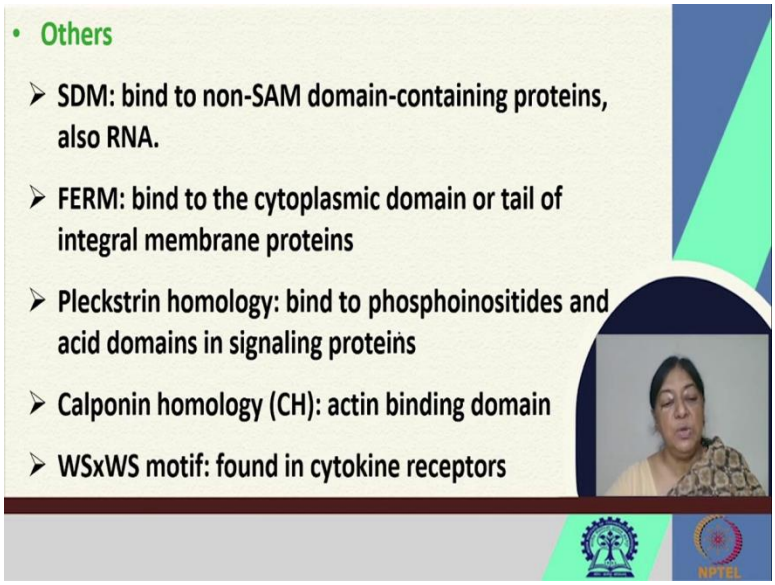

Now when we look at the other types that we have. We have the SDM, this binds to a non-SAM domain containing protein also RNA, the FERM type that binds to the cytoplasmic domain or the

pleckstrin homology type that binds to phosphoinositides and acid domains in signaling proteins and a CH type, a calponin homology actin binding type.

But what we need to recognize or understand that there is specific recognition involved in the protein-protein interactions for the specificity and the high affinity that is associated with the specific processes, specific biological action of each of these domains. Then we have another the WS and WS motif found in cytokine receptors.

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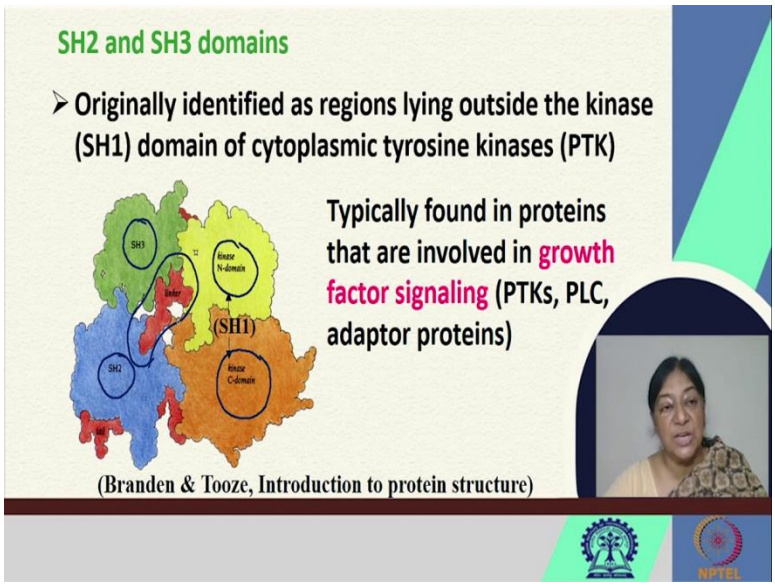

So, when we look [refer to slide] at the SH2 and SH3 domains, they are originally identified as regions lying outside the kinase domain of the cytoplasmic tyrosine kinase. So if you look at the overall structure that are typically found in proteins that are involved in growth factor signaling, we have the SH2 and SH3 domains here and here is the kinase N domain and the kinase C domain. These are connected through a linker. This structure is important as we can see, in the formation of this specific complex for the necessary action to occur.

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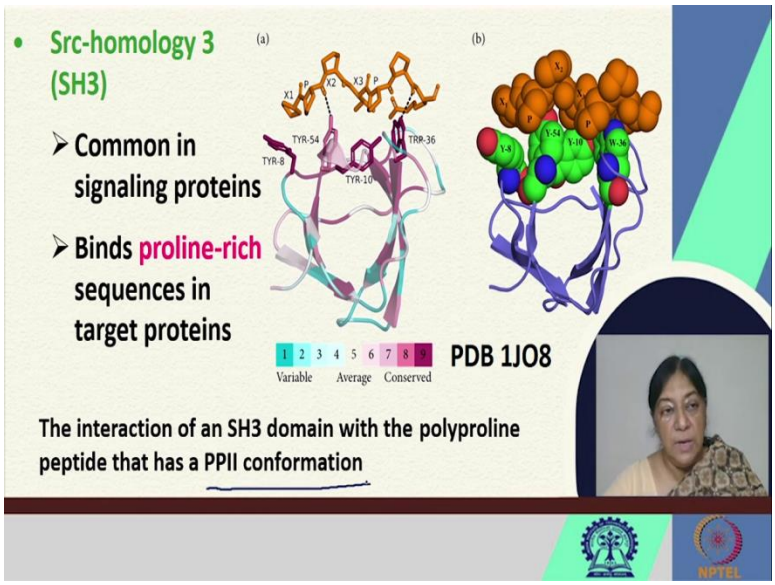

If we look at the SH3 domain, that is a domain that recognizes the polyproline peptide. This is common again in signaling proteins and it binds proline rich sequences in target proteins and we see that it binds polyprolines in a PPII conformation.

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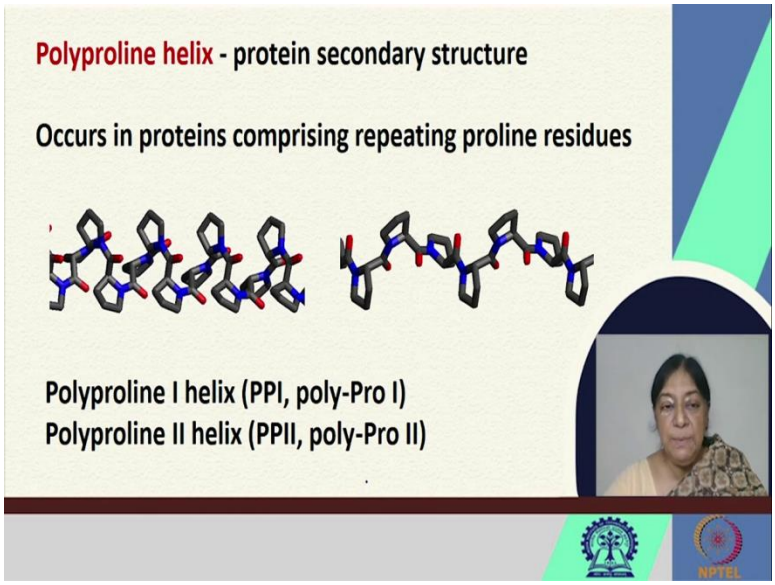

If we try and see what the polyproline helix means, this is an adaptation of a specific type of protein secondary structure involved with a polyproline type of residue involvement, where we have proteins that are comprised of repeating proline residues. So, we have the polyproline I type and the polyproline II type.

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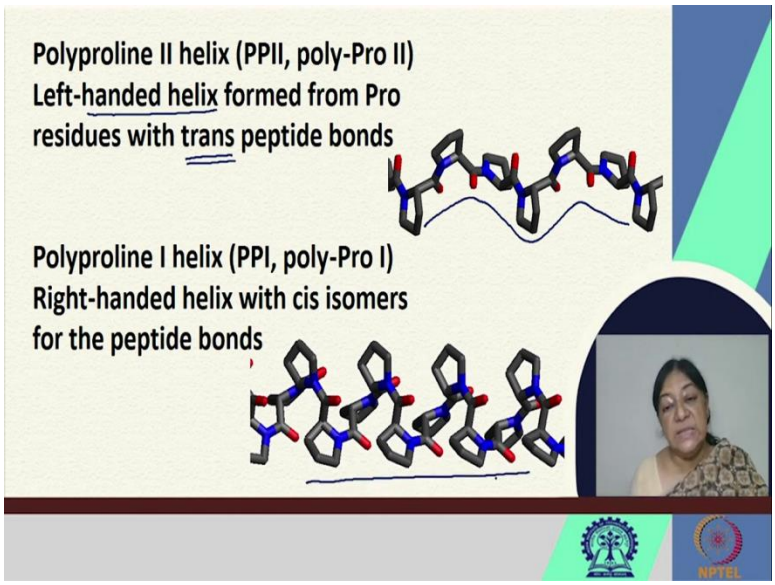

When we look at the polyproline II, the PPII types of helix we will see that they have a specific characteristic. These are left handed helices that are formed mainly from trans peptide bonds and they appear in this [refer to slide] fashion.

This is what is recognized by these specific types of domains that recognize a polyproline PPII type helix. For the polyproline I type helix, the PPI, we have right handed helices with cis isomers for the peptide bonds. The polyproline helix in this type appears in this [refer to slide] fashion.

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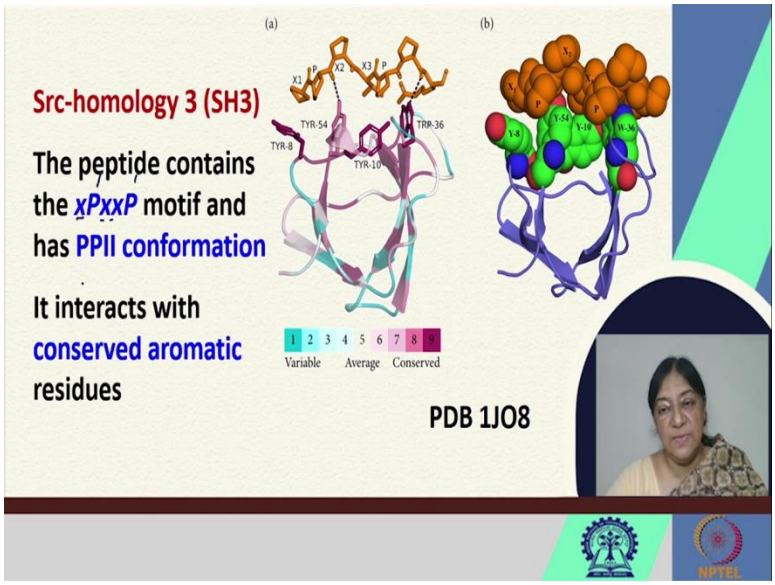

If we look [refer to slide] at the SH3 type of domain, this peptide contains the *xPxxP* motif and has the PPII conformation. It interacts with conserved aromatic residues that are there in the receptor.

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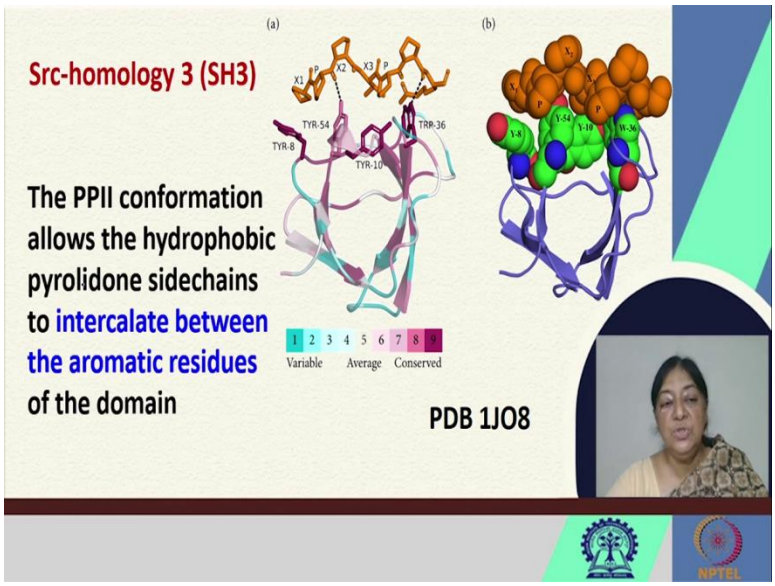

The PPII conformation allows the hydrophobic pyrrolidone sidechains to interconnect between the aromatic residues of the domain.

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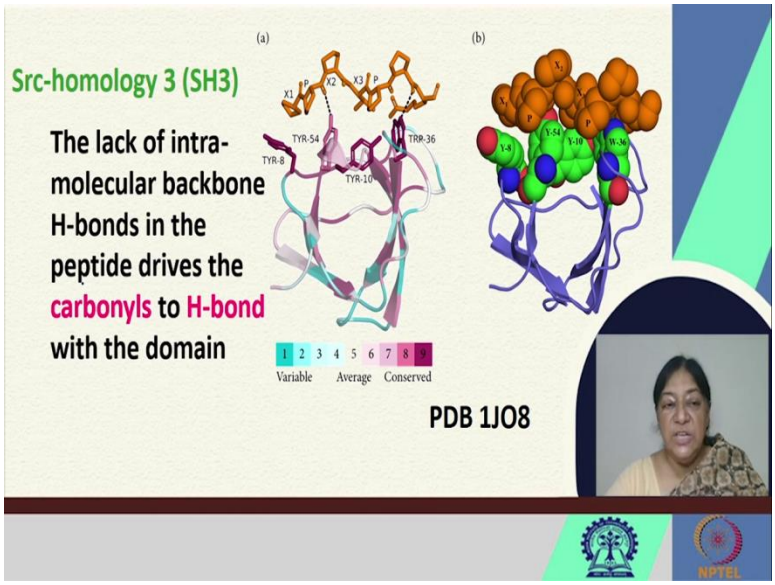

As such, there is a lack of intramolecular backbone hydrogen bonds in the peptide and this drives the carbonyls to hydrogen bond with the domain, creating an affinity site for the specific recognition required.

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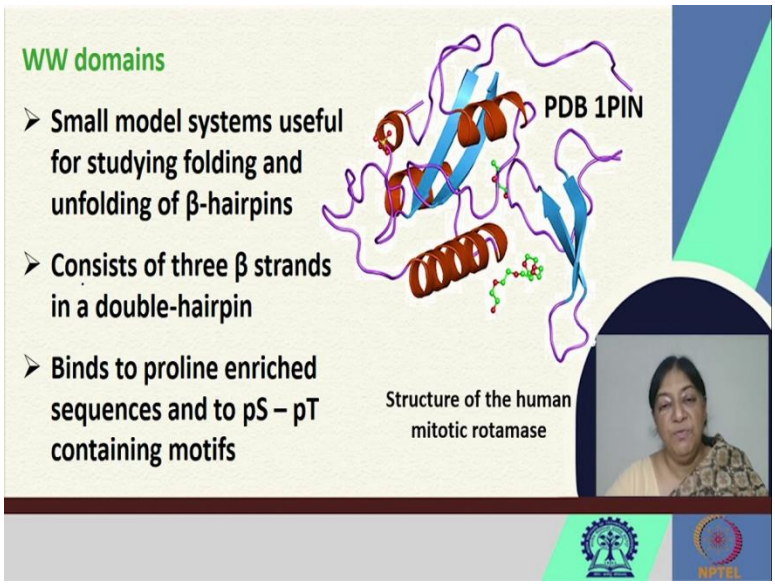

The WW domains are small model systems, that are useful for studying folding and unfolding of β-hairpins. These consist of three β strands in a double hairpin mode and again bind to proline enriched sequence and also to phosphoserine and phosphothreonine containing motifs. So, we have a specific structure associated with this for the human mitotic rotamase, which has the WW domain for the recognition in a protein-protein interaction specifics.

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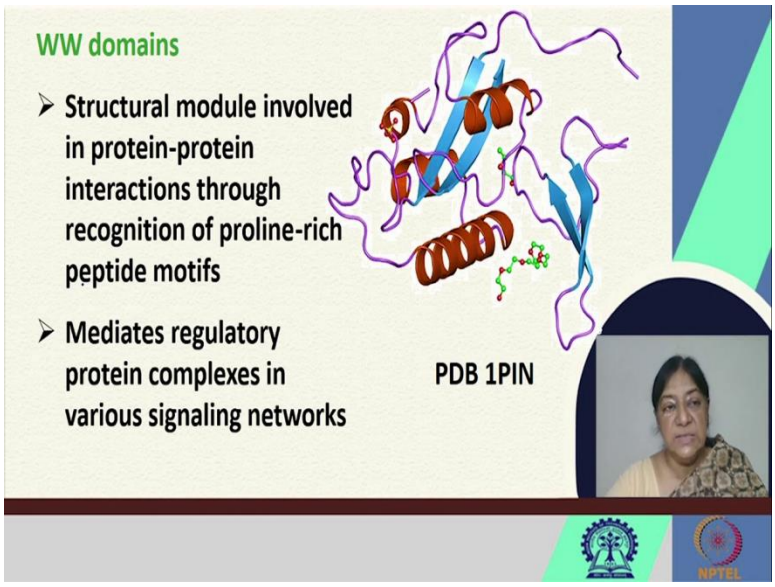

The structural module involved in the protein-protein interactions, is again through the recognition of proline rich peptide motifs and this mediates regulatory protein complexes in various signaling networks. So it is important in the signaling.

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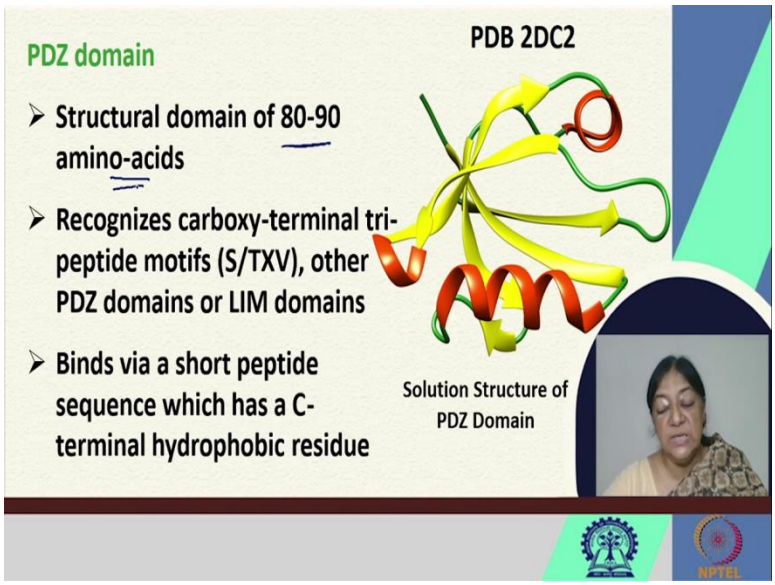

The PDZ domain is another domain that is involved in recognizing the carboxy terminal tripeptide motifs and also other PDZ domains or LIM domains. Thus, this structural domain consists of 80 to 90 amino acids in the recognition site. This binds via short peptide sequence, which has a C terminal hydrophobic residue, that is required for the recognition.

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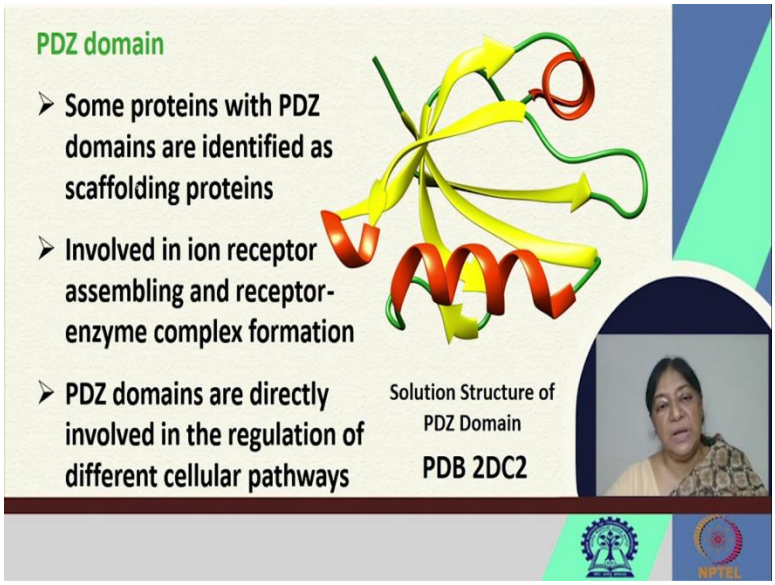

Some proteins with this PDZ domains are identified as scaffolding proteins and they are involved in iron receptor assembling and receptor enzyme complex formation and these PDZ domains are also directly involved in the regulation of different cellular pathways. So, we realize the importance of the types of residues involved in this recognition.

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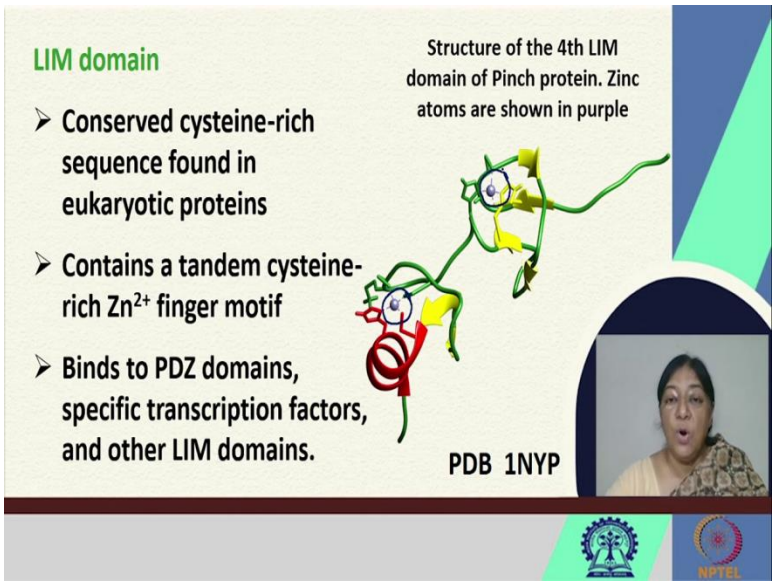

In the last domain that we are going to look at is the LIM domain. There are several other domains that are also known, but we will look at these specific ones. This has a conserved cysteine rich sequence, that is found in eukaryotic proteins. It contains a tandem cysteine rich zinc finger motif, where we see the zinc attached to this specific LIM site. This binds to PDZ domains, specific transcription factors and also other LIM domains.

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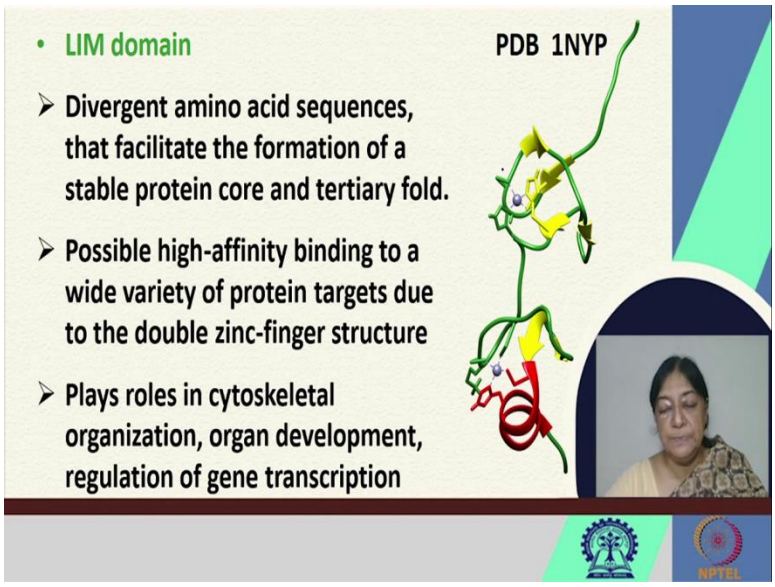

In this specific helix-loop-helix recognition, there are divergent amino acid sequences that facilitate the confirmation of a stable protein core and a tertiary fold that is required for the recognition. Then there is possible high affinity binding to the wide variety of protein targets, due to the double zinc finger structure observed. It plays a very important role in cytoskeletal organization, organ development and the regulation of gene transcription.

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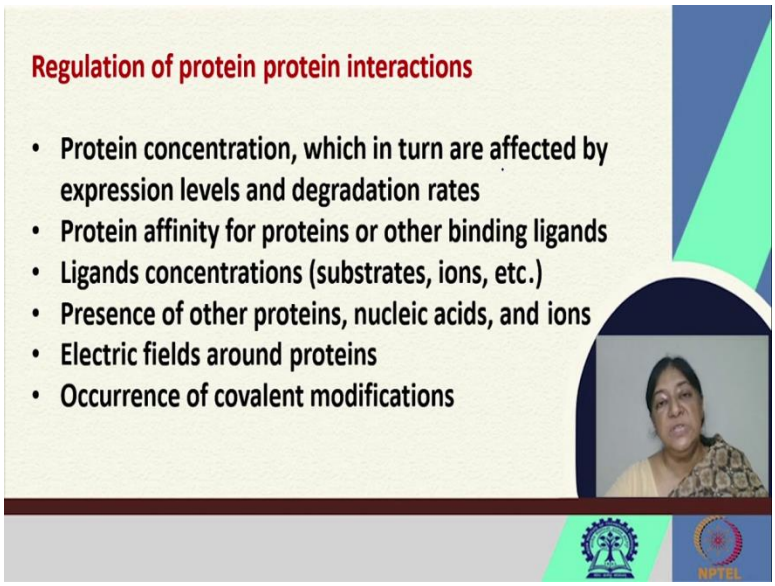

A look at these different types of protein-protein interactions means, there has to be a regulation of the protein-protein interactions. This can be regulated by the protein concentration, which in turn is affected by the expression levels and also the degradation rates. The specific protein affinity for other proteins or other binding ligands. The ligand concentrations, the substrates or the ions. The presence of other proteins, nucleic acids and ions and also the electric fields around the proteins.

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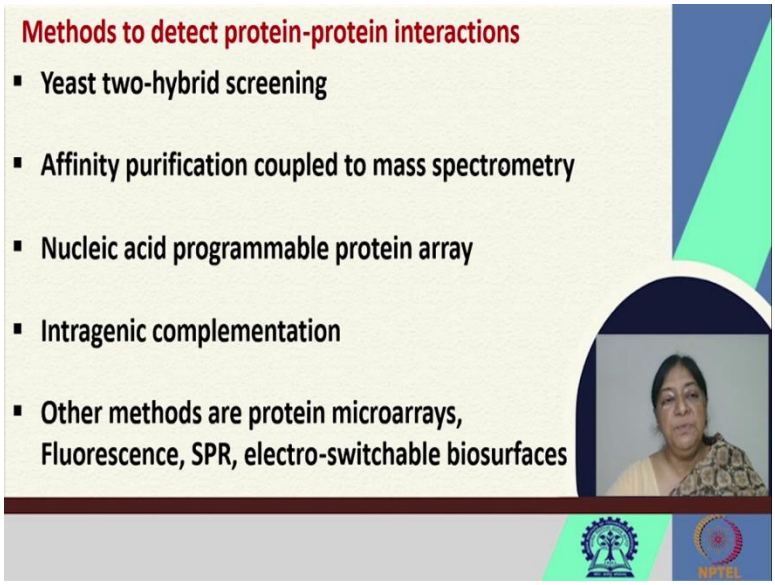

So, there are several factors that are going to regulate or affect the protein-protein interactions that are necessary to be understood and there is the occurrence of covalent modifications, that

may break or possibly interrupt the protein-protein interaction site, affected in a manner that it would not be able to perform its specific biological function.

There are several methods that are required to detect these protein-protein interactions. If we know that a specific protein-protein is interacting, there are several tests that can tell us whether there is a specific protein-protein interaction. There is the yeast two-hybrid screening, known as the Y2H test; the affinity purification that can be coupled with mass spectrometry to check for specific affinity of two proteins and we have different protein array methods and other methods including fluorescence SPR and electro-switchable biosurfaces.

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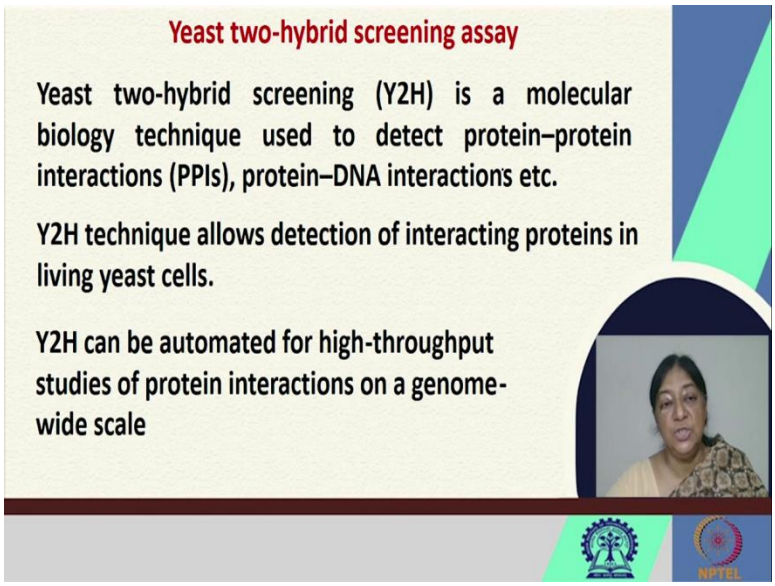

We will briefly be looking at the Y2H screening. In this screening assay, the yeast two-hybrid screening assay, this is a molecular biology technique that can be used to detect protein-protein interactions and protein DNA interactions. It allows the detection of interacting proteins in living yeast cells and it can also be automated for high throughput studies to understand protein interactions on a genome wide scale.

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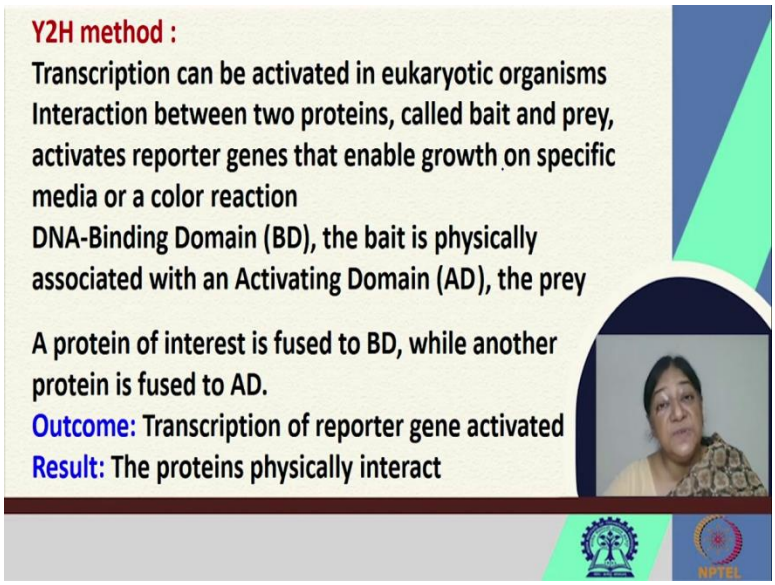

This method is used because there is a transcription that can be activated in eukaryotic organisms. This is taken into consideration and there are the interactions between two proteins, the bait and the prey, that activates the reported genes that enable the growth that could be on a specific media or a specific colored reaction, to indicate that there has been a protein-protein interaction involved.

There is a DNA binding domain, the BD which is the bait. If it is physically associated with an activative domain the prey, then a protein of interest is fused to the BD, while another is fused to the AD. If these interact with each other, there is the transcription of the reporter gene that is activated. So the result indicates that these proteins, physically interact with each other.

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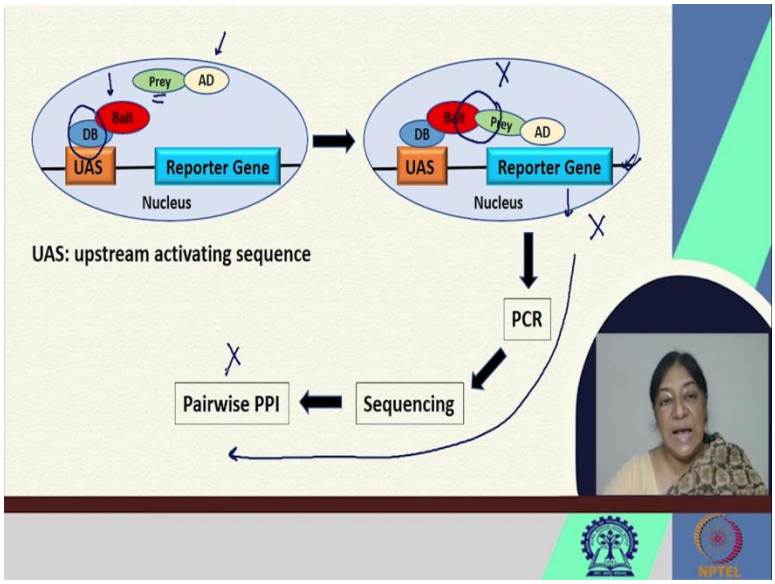

So just for a cartoon representation [refer to slide] a schematic, we have the DNA binding domain where we have the bait attached, we have the activating domain where we have the prey attached. If there is an interaction between these two proteins, then the reporter gene is activated and as such we will see a pair wise protein-protein interaction. However, if there is no interaction between the bait and the prey, then this reported gene will not be activated and there will be no pair wise protein-protein interaction involved.

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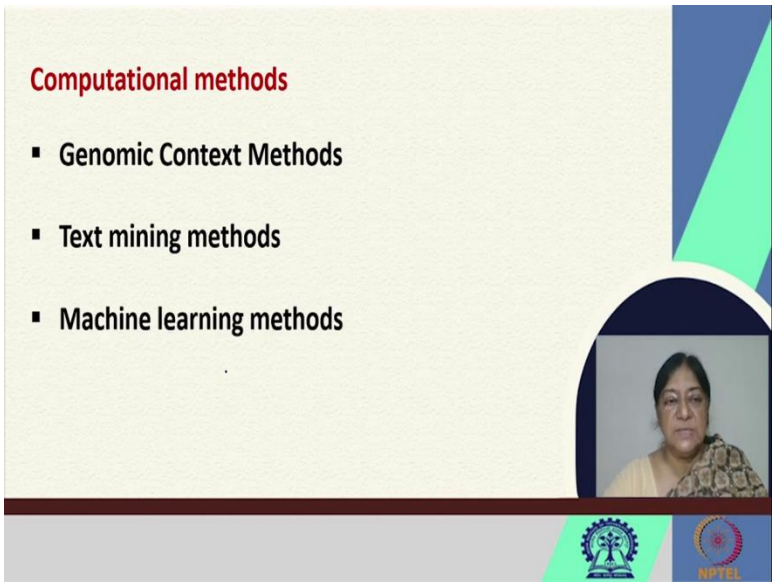

This means with this assay possible, there are the possibilities to get a large number of protein-protein interactions. So specific computational methods have been developed to look at these protein-protein interactions from different points of view, where we can look at the genomic context methods, the text mining methods or machine learning methods.

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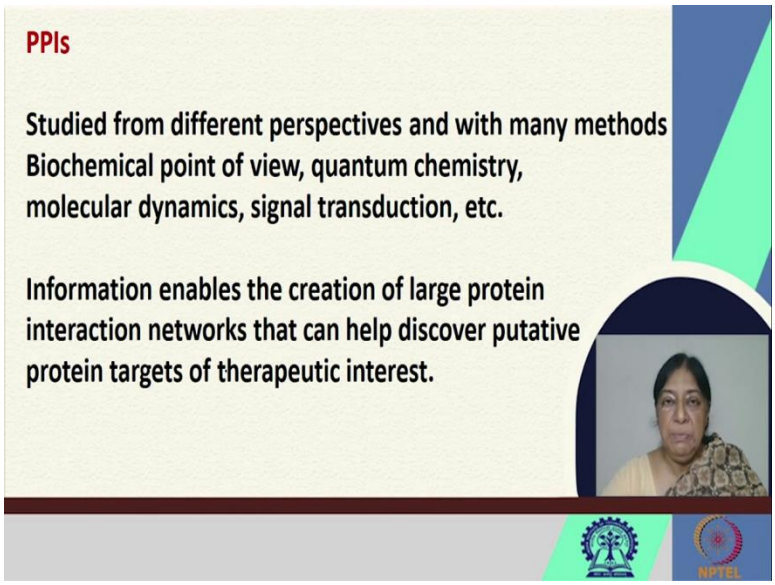

In an understanding of the PPIs that can be looked at from different perspectives, from a biochemical point of view, looking at a molecular dynamics or signal transduction, there is a lot of information available that of the creation of large protein interaction networks that can actually help discover other putative protein targets that may be of therapeutic interest.

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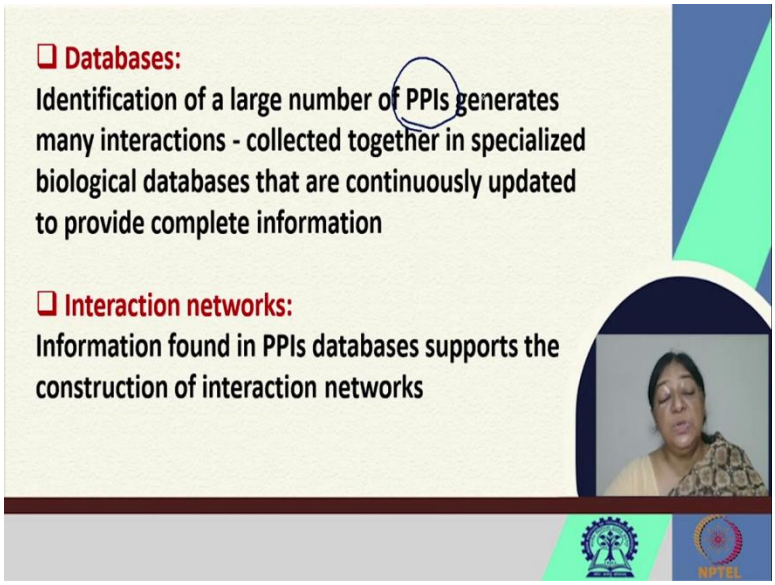

This is done by looking at specific databases. These databases are generated from the information of specific protein-protein interactions that are collated and collected from biological databases, that are continuously updated to provide complete information. There are specific interaction networks that are possible, where construction of these interaction networks gives us an idea about how the interactions occur and which proteins can interact with which other proteins.

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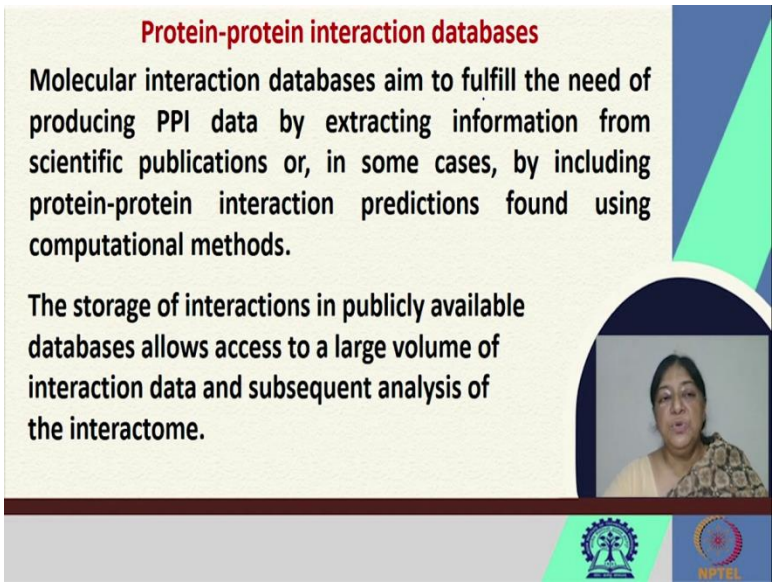

Based on that, there are protein-protein interaction databases that are obtained from extracting information from scientific publications. Other protein-protein interaction predictions can be found using these computational methods. So, we have the storage of these interactions in publicly available databases, that allow access to a large volume of interaction data that can give us insights into protein-protein interactions.

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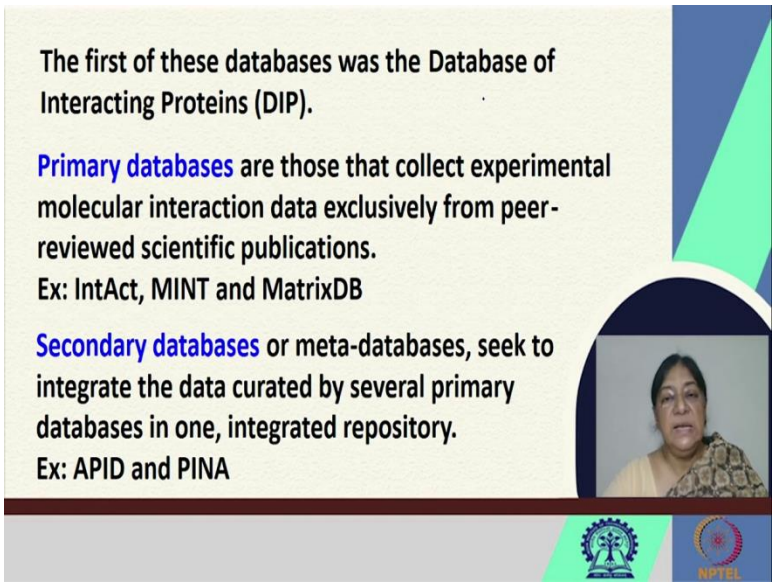

To look at this, there is the database of interacting proteins. There is a primary database which is thus the collection of experimental molecular interaction data, exclusively from peer reviewed scientific publications. So protein-protein interactions reported will get a place in this matrix DB

or the primary database. The secondary database is a meta-database that integrates the data that has been curated from the primary database into an integrated repository.

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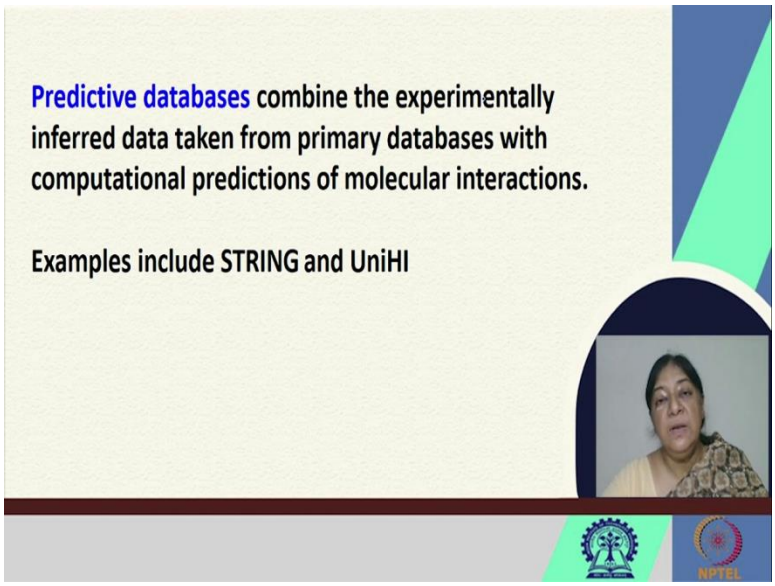

Then we have the predictive database, that then combines the experimentally inferred data taken from the primary database with computational predictions of possible molecular interactions. Now, this is important in identification of specific networks and see whether we have interactions between proteins, between networks that were not known or cannot be determined experimentally.

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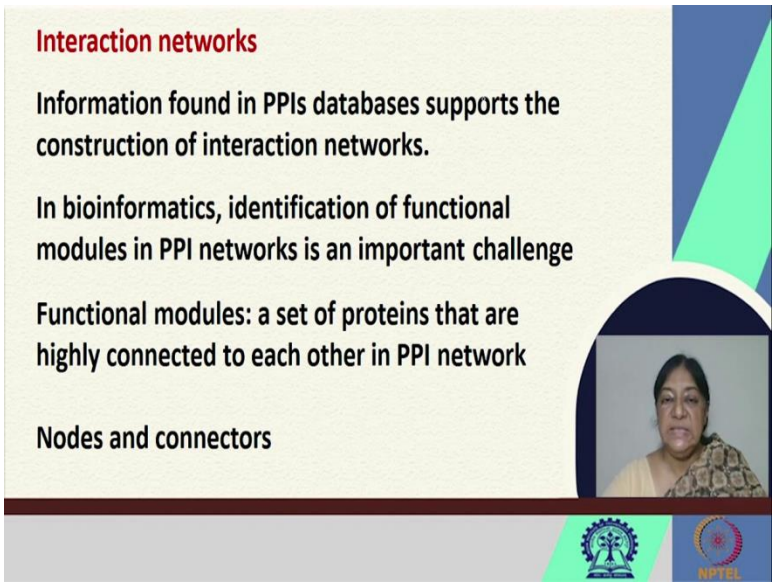

The information found in the PPI databases, supports the construction of these interaction networks and we have functional modules of the PPI networks that help us try to connect different proteins together, that are involved say in a specific process or involved in a specific disease because we realize that having the specific biological process or any biochemical reaction, is a cascade of effects that require a large number of proteins in the cascade reactions that take place, to interact with each other for the specific process to occur.

In this case we have what are called nodes and connectors, that connect these different proteins.

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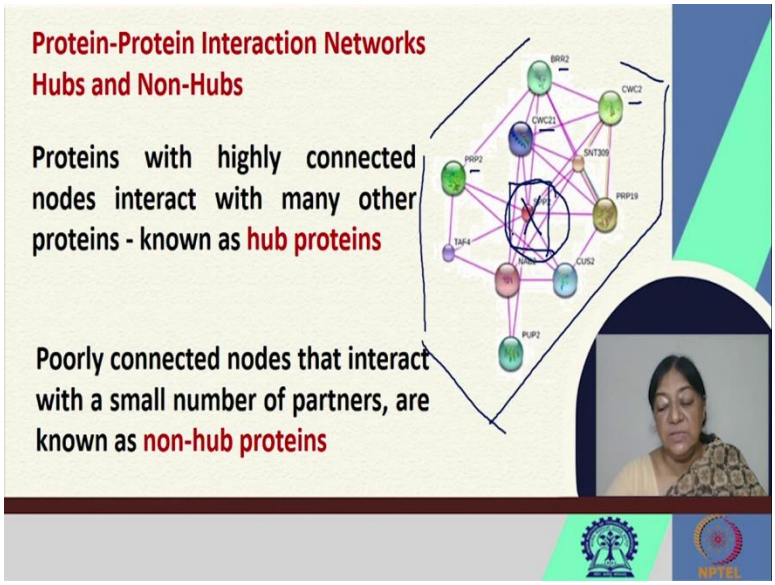

In the protein-protein interaction networks, we have what are called hub proteins and non-hub proteins. Proteins with highly connected nodes that interact with other proteins are known as the hub proteins and the non-hub proteins are those that are poorly connected, that interact with only a small number of partners.

For example this [refer to slide] interaction network shows us that this particular protein has a larger network. So this would be a hub protein that has highly connected nodes, that are going to tell us that if we are going to target a network that is involved in a manner where this set of proteins are involved in a specific reaction, then it would be good to target this specific protein to see if we can inhibit its action, which would mean that we would in turn inhibit its interaction with the other set of proteins, that it is associated with.

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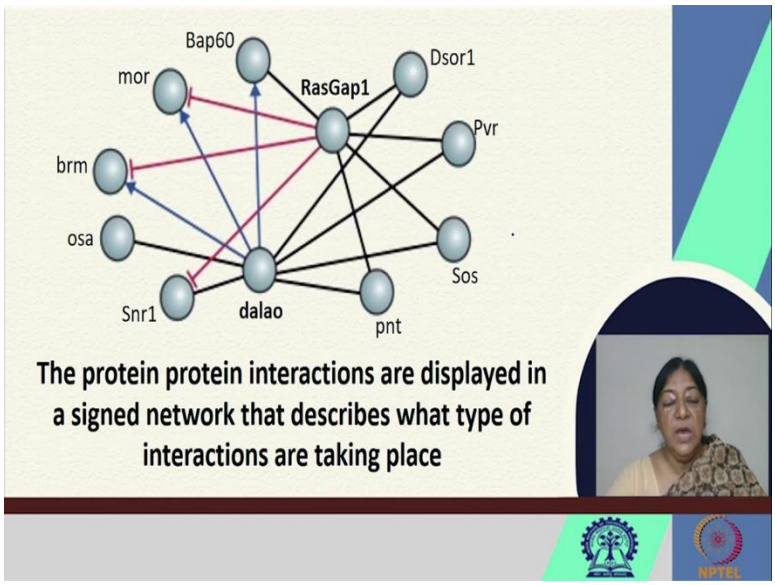

So similarly the protein-protein interactions can be displayed [refer to slide] in this network, that describe what types of interactions are actually taking place.

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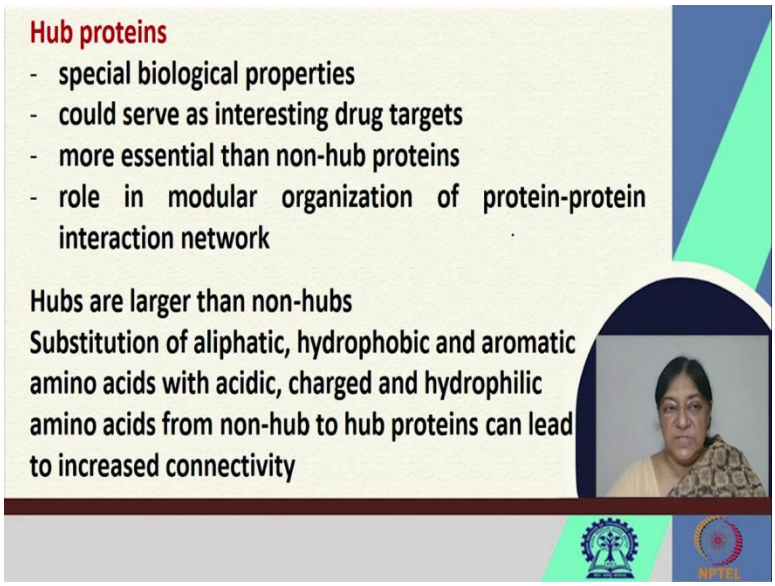

So, we can look at these hub proteins that have special biological properties and they could serve as interesting drug targets because they seem to be more essential than the non-hub proteins and they have a large role in modular organization of the protein-protein interaction network. The hubs are larger than non-hubs and the substitution can change the connectivity.

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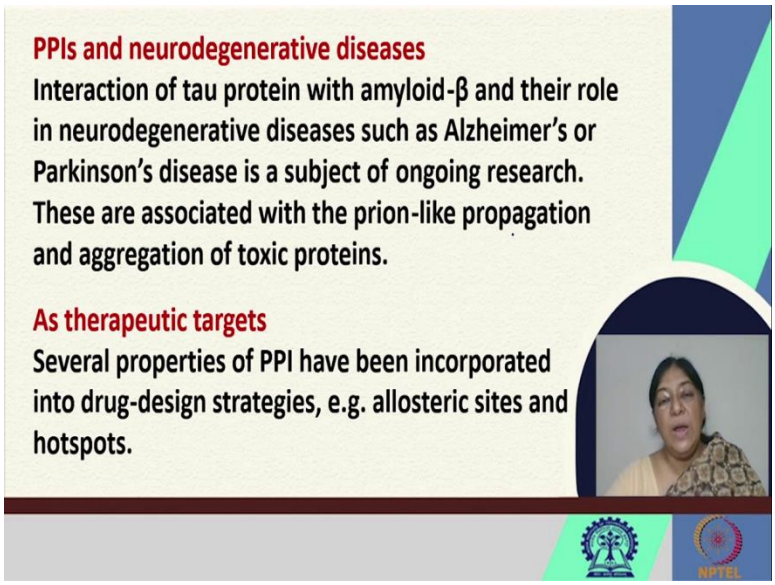

When we look at PPIs and neurodegenerative diseases for example, we can look at the interaction of the tau protein with the amyloid-β and their specific role in neurodegenerative diseases, such as the Alzheimer's or the Parkinson's disease. This is a subject of ongoing research and these are associated with prion like propagation and aggregation of toxic proteins.

So they can be used as therapeutic targets. Several properties of the PPI have been incorporated into drug design strategies, where we look at the specific hotspots, the conserved regions that are required for the interactions and allosteric sites that might affect the protein-protein interaction per se.

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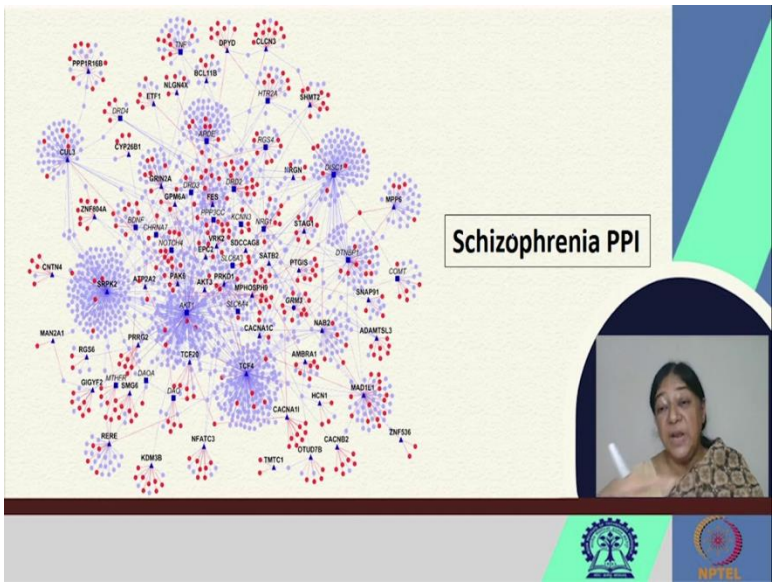

So if we look [refer to slide] at an example of a schizophrenia PPI, this is what the PPI network looks like. So we can see the location of the specific hub proteins, but we realize the whole

cascade of interactions and how large this interaction network is, that tells us about the protein-protein interactions involved in this specific disease. These protein-protein interactions can give us an idea of which specific proteins to target in an attempt to combat disease.

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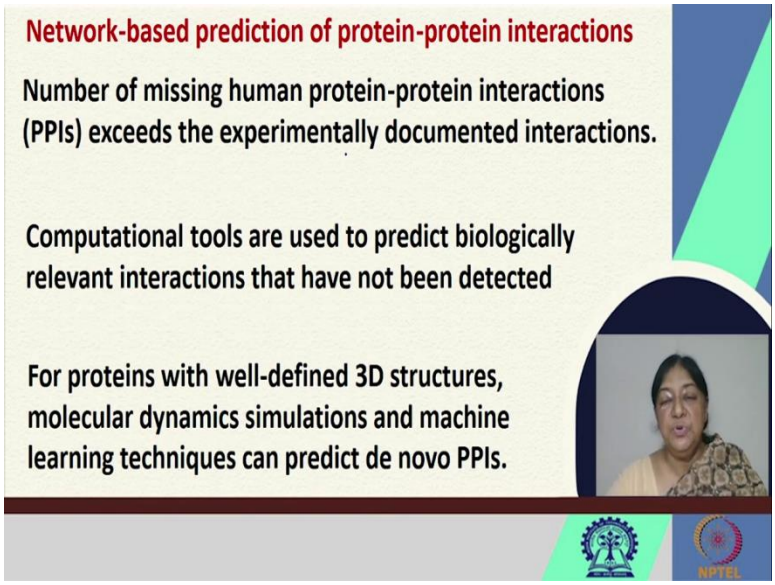

So, the network based prediction of protein-protein interactions thus is extremely important because the number of missing human protein-protein interactions, far exceeds the experimentally documented interactions. These computation tools are used to predict biological relevant interactions that have yet to be detected. For proteins with well defined 3D structures, there are MD simulations that may be possible and machine learning techniques that can predict de novo protein-protein interactions.

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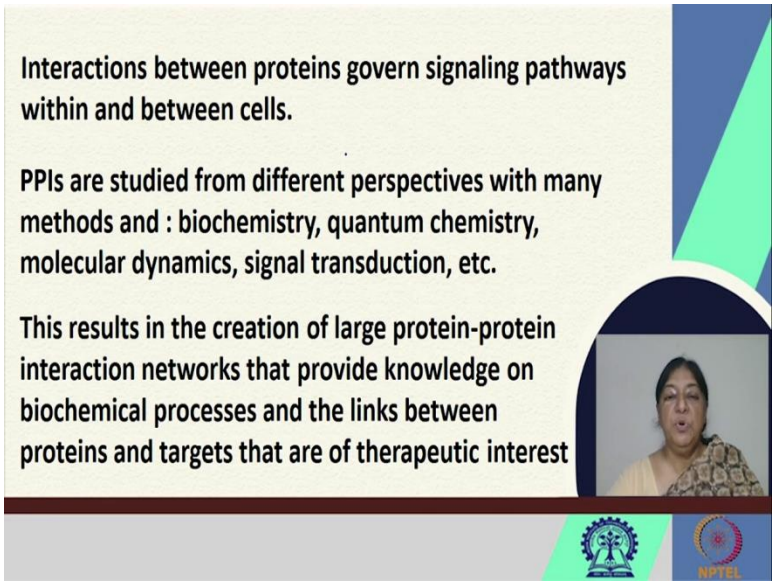

The interactions between proteins that govern signaling pathways between and within cells, can be studied from different perspectives and we see the results. This results in a creation of large protein-protein interaction networks, that provide us with knowledge on the biochemical process and the links between proteins and targets that are of therapeutic interest.

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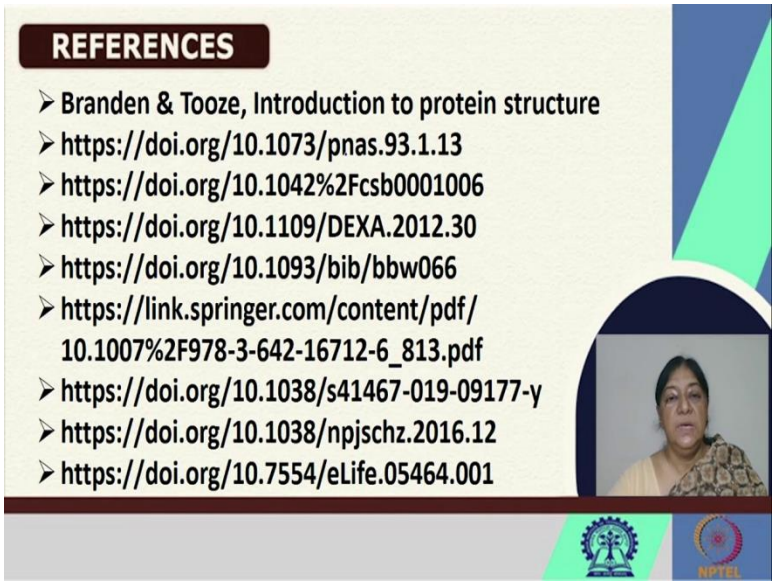

These [refer to slide] are the references.

Thank you.