Interactomics Basics and Applications
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Lecture – 02
Introduction to Interactomics

Welcome to the MOOC course on Interactomics. Proteins are dynamic molecules which interact with a wide variety of biomolecules such as lipids, nucleic acid and various a small drug inhibitors, metabolites and many other biomolecules to provide different type of significant information for physiological action. Proteins also interact with one another to form the large complexes. These complexes regulate various fundamental processes such as signal transduction and gene regulation. Edited understanding of protein interactions provides an opportunity to understand the protein functional behavior.

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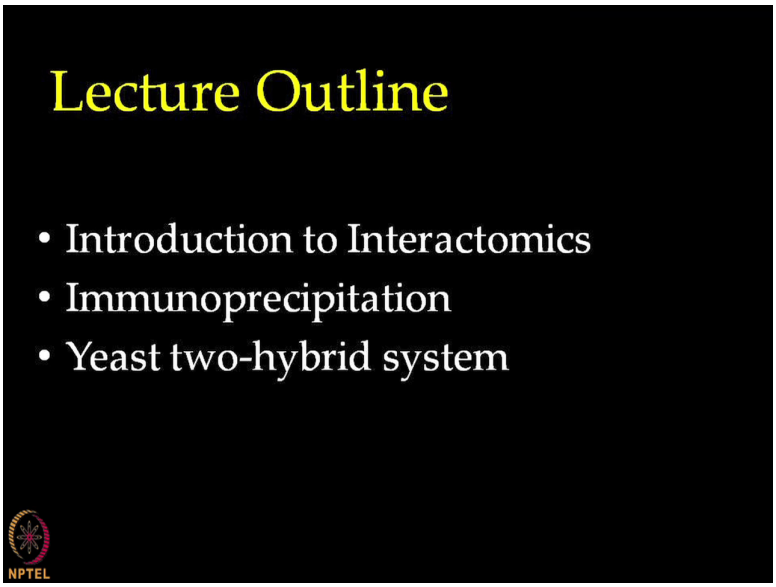

Today in this lecture I will describe what is interactomics, and a historic perspective of the conversional techniques involved in studying interactions which includes yeast two-hybrid and immunoprecipitation techniques. Let us first start with interactomics. Interactomic comprises the study of interactions and their consequences between various proteins and other cellular components. The network of all such interactions known as interactome, which aims to provide better understanding of genome and proteome functions.

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Interactions can have different kinds of impacts; it could be positive as well as negative. The one which is shown here in the slide shows the negative impact. Biology has evolved several mechanisms that regulate interactions such as posttranslational modifications, and presence or absence of activated or inhibitor molecules.

Interactions can also be modulated by altering the expression level of proteins. Deliberant interactions such as a one shown in the slide can lead to the disregulation of cellular functions and ultimately diseases such as cancer may happen. Proteins interact with the variety of biomolecules, the interaction of proteins with another protein or other biomolecules help to perform these functions.

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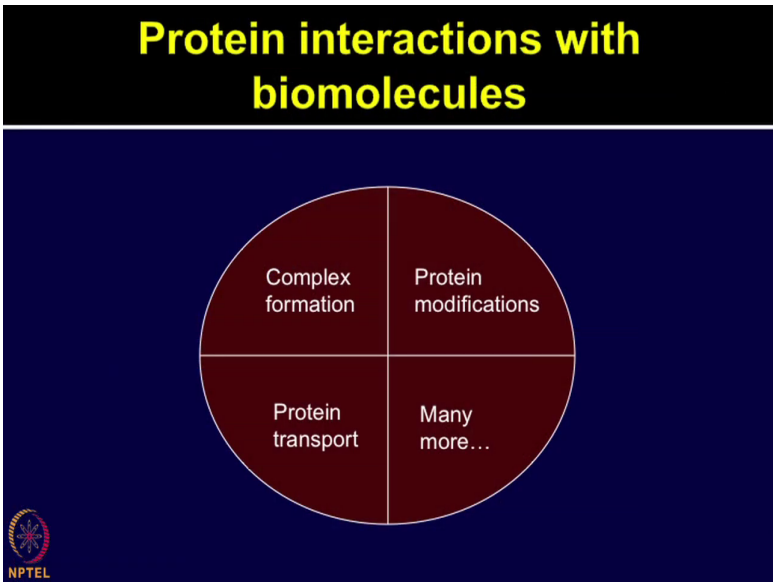

These proteins after interactions form complexes that modify the proteins help in protein transportation as well as many properties in which they are involved.

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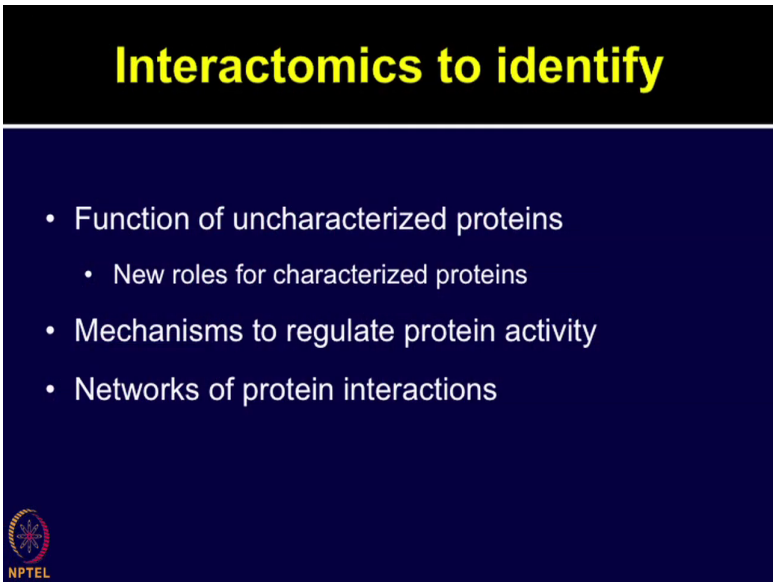

The field of interactomics aims to identify the function of uncharacterized proteins, so that one can assign the new role of various proteins. The mechanism to regulate protein activity can also be understood by studying the interactions. The network of protein interactions provide very valuable information for the processes such as signal transduction. And various type of pathways in which these proteins can be involved.

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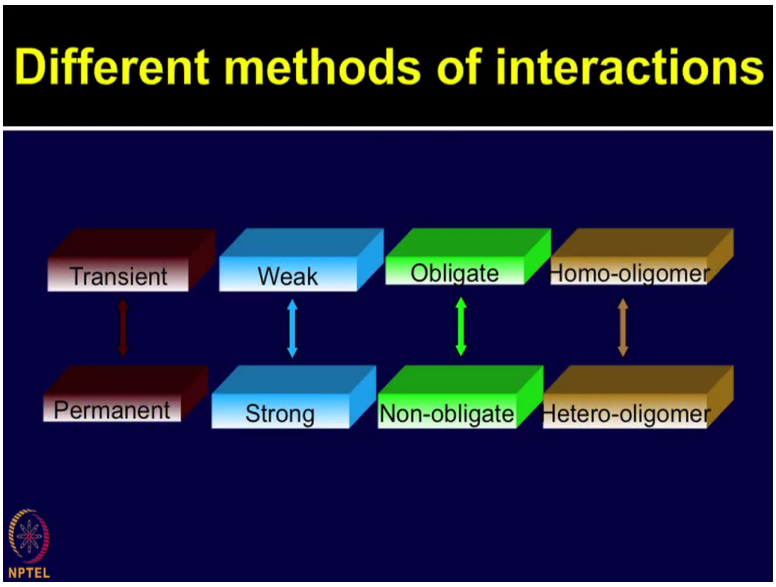

The different methods of studying interactions, because interaction occurs in various ways. There are few interactions which are very transient that will be very short duration where as other interactions could be permanent which may alter the activity.

Few interactions are very weak, whereas other interactions could be very strong interactions can also be obligate or non obligate they can form homo oligomers as well as different kinds of oligomeric units, which could combine to form the hetero oligomer. It just gives you glimpse of the complexities involved in the interactions, because is studying the interaction require various type of technologies just because of the shear diversity of the interactions as well as different type of complexities involved.

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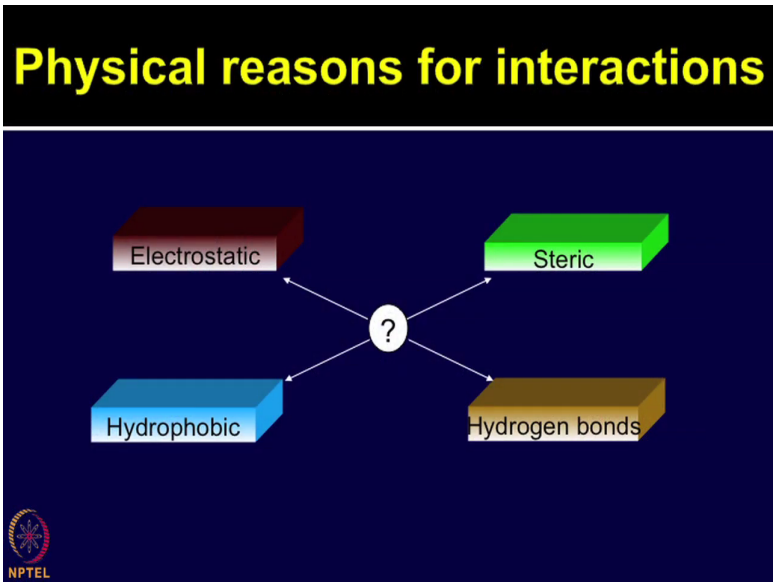

So, what are the physical reasons for these interactions; it could be electrostatic, hydrophobic, hysteric or hydrogen bond etcetera.

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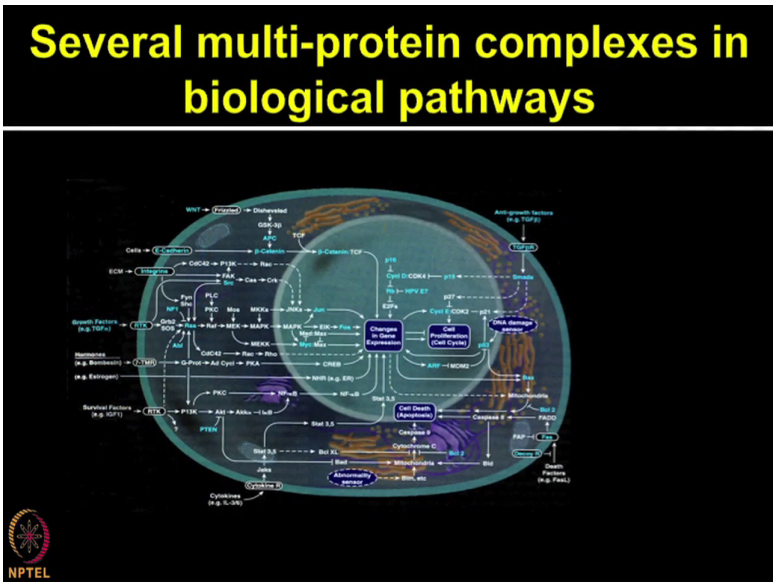

Protein-protein interactions have various potential effects. Few effects are described in the slide such as catalysis, protein inactivation, altered kinetics, alteration of substrate a specificity for substrate binding, new binding site information etcetera. These are just a few examples of potential effects of protein-protein interactions, but they alter magnitude of effects which ultimately lead to either positive effects or this regulation which may result in various diseases.

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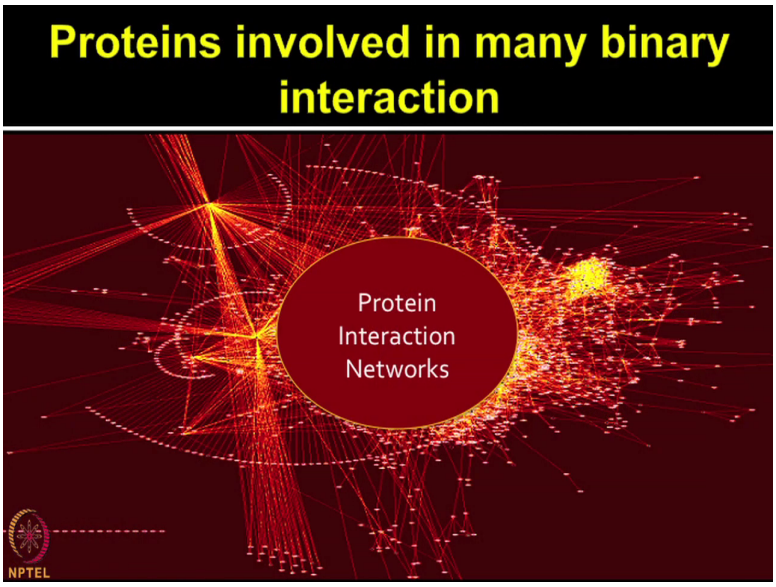

Still proteins are involved in many binary interactions and understanding of the underlying biochemistry of proteins. And biological interaction is critical element for the development of novel therapeutics and diagnostics.

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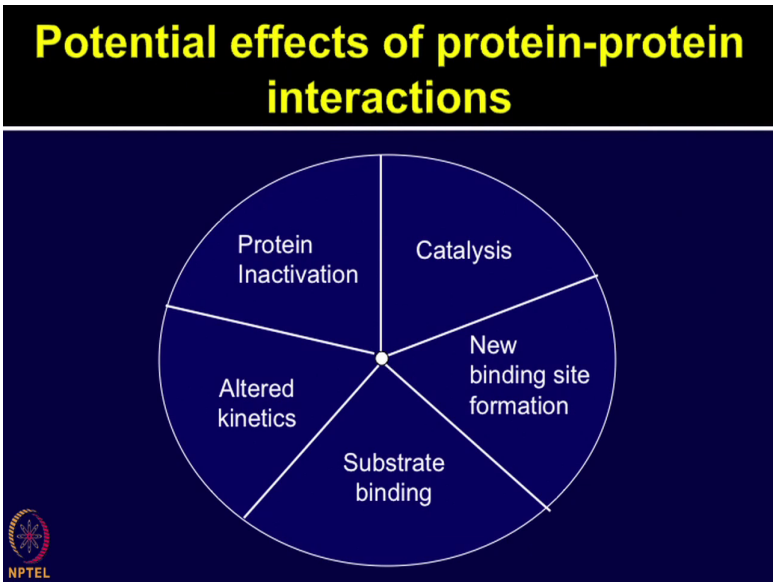

Protein-protein interactions have various potential effects, some of these effects are described in the slide. They alter multitude of effects which ultimately lead to either positive effects or this regulation which may result into various diseases.

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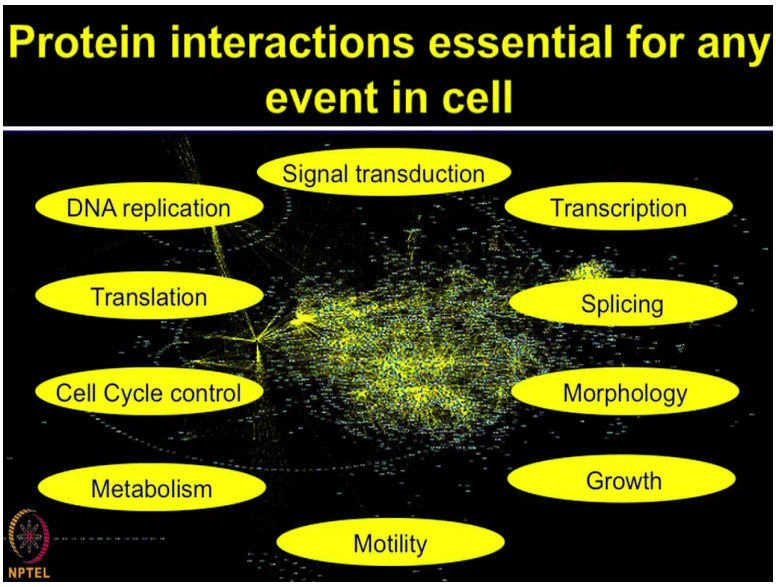

So, protein interactions are very essential for any cellular mechanism, where to discuss about signal transduction, DNA replication, translation, cell cycle control, how the metabolic processes are govern the mortality, how the growth and morphology are altered is splicing, transportation etcetera. I am sure if you think about your biological questions of interest, you will be able to add many more examples here how protein interactions are essential for the activity of the cell.

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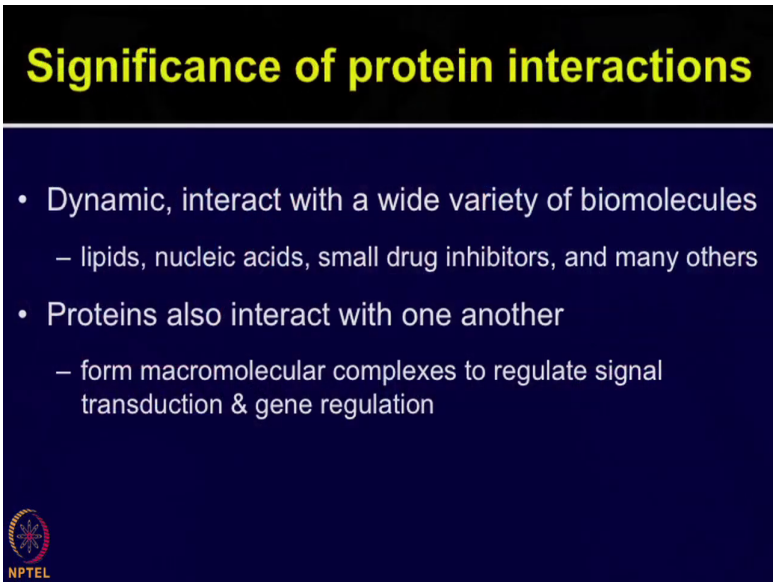

So, what is the significance of histidine protein interactions? These are very dynamic which interact with a wide variety of biomolecules as I discussed previously such as lipids, nucleic acids, a small drug inhibitors and many other biomolecules. Proteins also interact with one another to form the large complexes which regulates signal transduction and gene regulation. Let me now describe you few concepts involved in the interactomics field by showing with this animation.

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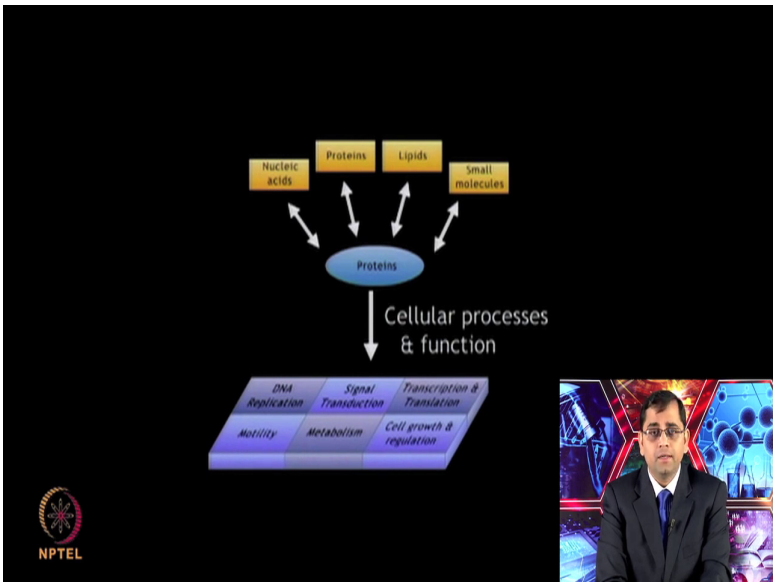

Interaction studies of protein with various biomolecules help in deciphering and understanding a functions of various proteins in the complex network of cellular pathways. Protein interacts with other biomolecules such as nucleic acids, lipids, hormones, etcetera. We execute a multitude of function in living organisms such as signal transduction, growth and regulation and metabolism. These are few examples, there are many other cellular processes and functions in which these are also involved.

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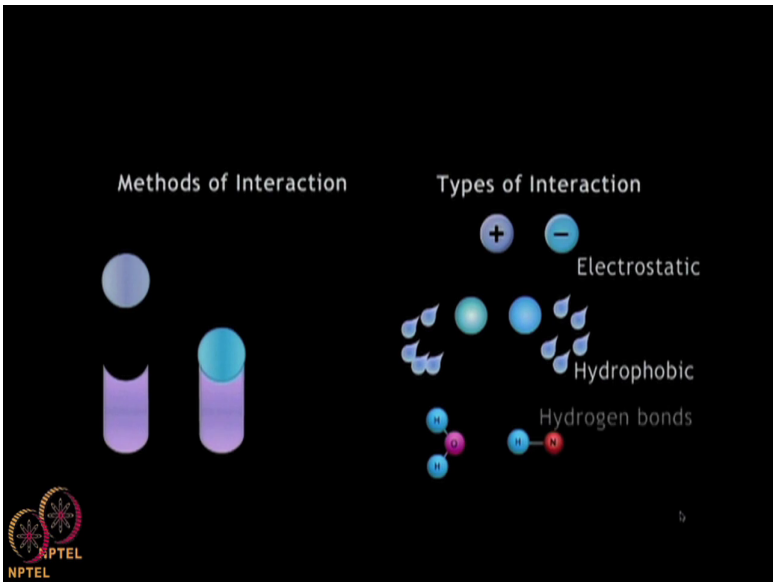

Protein interaction with other biomolecule can be of several different types. They may be weak or a strong, obligate or non obligate, transient or permanent. The physical basis for these interactions include electrostatic, hydrophobic, hysteric interaction, hydrogen bond etcetera.

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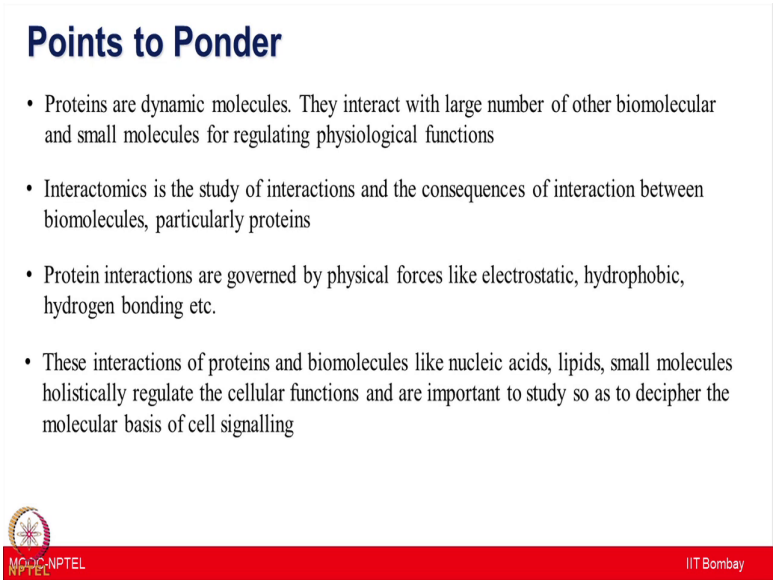

So, now, let us talk about different methods to study the protein-protein interaction. Understanding the protein-protein interactions provides important clues to the function of proteins, the ratification of interactions with known proteins may suggest the functional role played by a novel protein.

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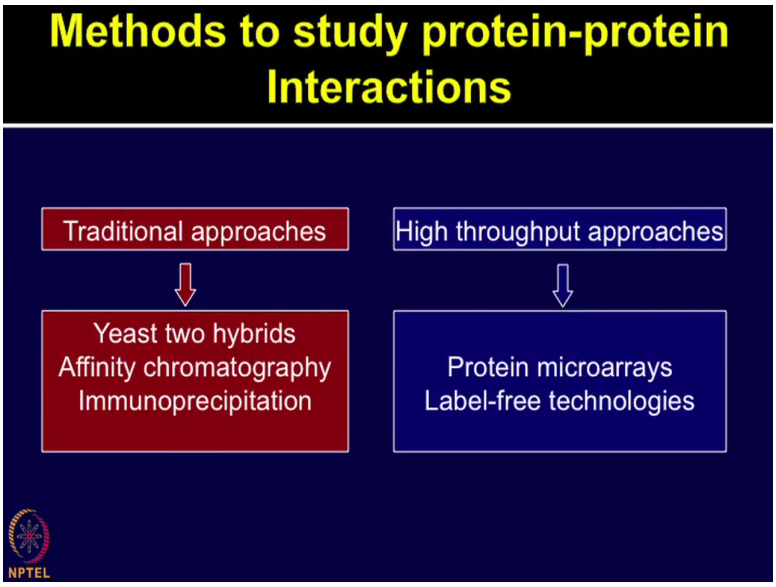

There are two broadways of a studying this protein-protein interaction; one is a traditional approach and then there are few high throughput approaches. The traditional approaches reliably on yeast two hybrid, affinity chromatography and immunoprecipitation.

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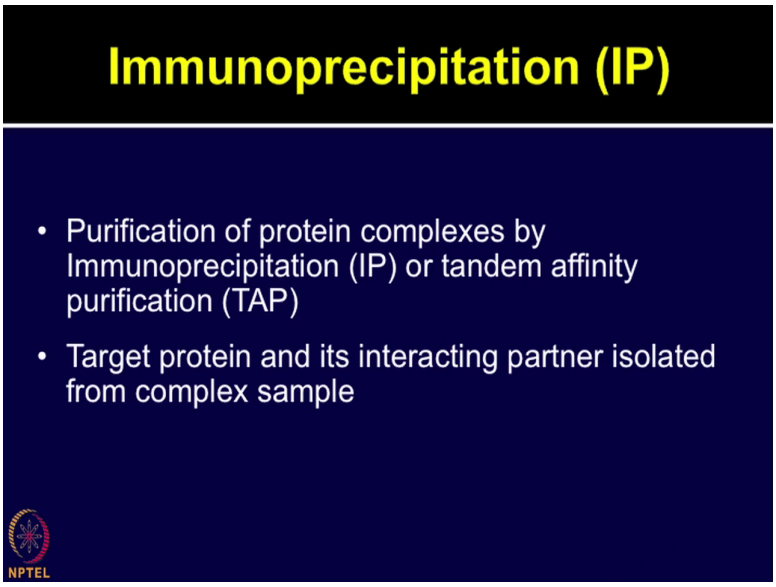

The more recent studies include high throughput approaches such as protein microarrays and different type of label free biosensors. The traditional way of studying protein-protein interactions involved yeast two hybrid an immunoprecipitation. These are too widely recognized technologies which have been used to map the protein-protein interactions on a large scale. These are yeast two hybrid also known as YTH and IP with MS also known as IPMS technique.

Both of these approaches helping to identify thousands of novel interactors in different organisms including human, c elegance, drosophila etcetera. Let us first talk about immunoprecipitation technique. IP or tandem affinity purification also known as TAP is a technique use to purify the protein complexes and study the protein-protein interactions.

Depending on the protein that need to be purified different types of tags could be attached to the (Refer Time: 11:33) proteins.

Through the immunoprecipication method the purification of protein complexes by IP or tandem affinity purification method is perform. The target proteins and its interacting partners or isolated from a given complex sample. And then by using different kinds of tags these proteins can be isolated from a mixture and then further processed, which I described in the next slide.

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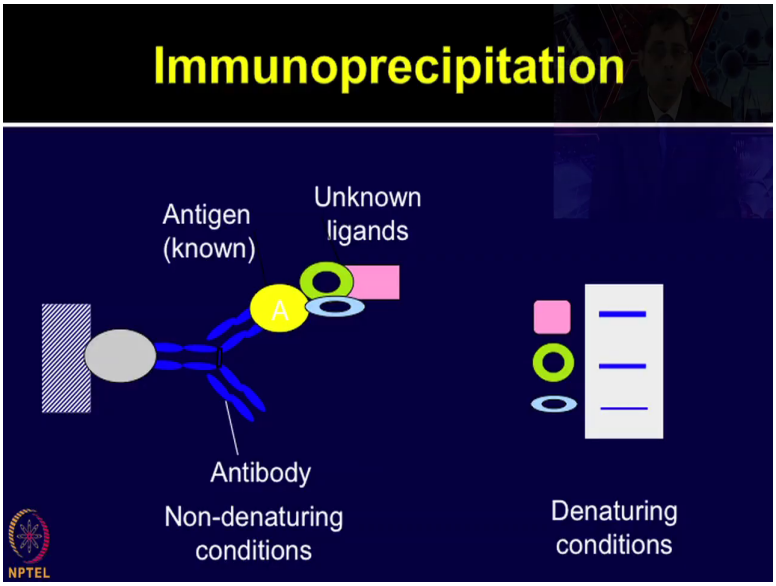

In IP method, the antibody is specific to the bait is attaché to the whole cell extract which found the complex. Remember this establish performed in the negative or not denaturing conditions. The protein-protein complex is immobilized on protein A or protein G (Refer Time: 12:27). Protein complex is evaluated and further analyzed on a lgs page gel. As you

can see on the slide it shows that antibody is used which binds to the antigens and the unknown ligand and this complex is separated on lgs page gel. This condition is done under denaturing condition, whereas the first step was performed under non denaturing conditions.

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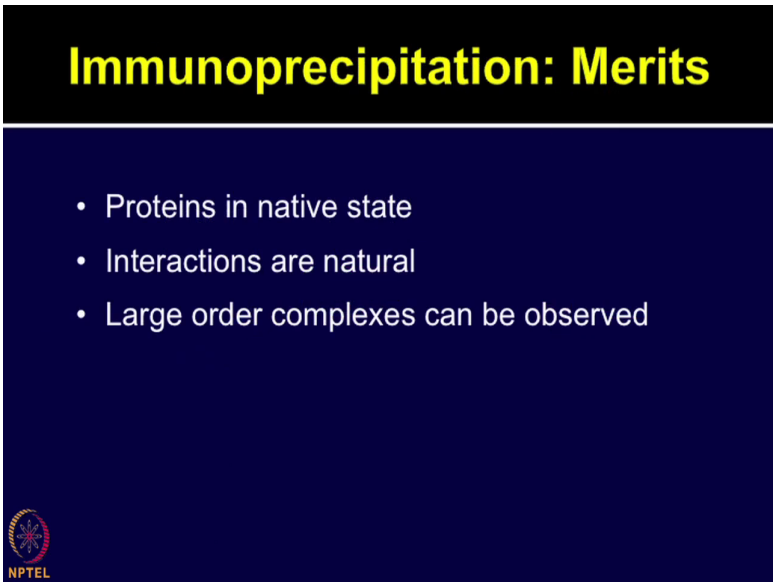

What are the merits of using IP method? In this method proteins are isolated in the native state. Why native state is important? The native state will allow the native complexes to be formed. It also allows the formation of posttranslational modifications. It is essential to perform these steps in the native or non denaturing conditions. Interactions by using the IP method are natural and by using these methods large order complexes can be observed, because the native state will promote the native complex formation.

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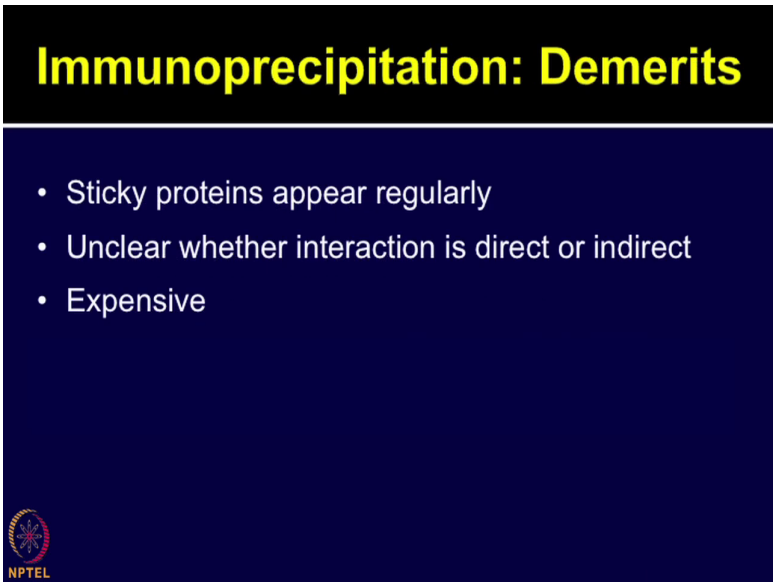

What are the different demerits of using IP method? It has been reported that frequently a sticky proteins may be picked up the proteins which are not a specific, they could also be eluted out because they are sticky on the other protein surface. It means few proteins that are a specifically interacting with target of interest will be isolated along with non a specific a sticky proteins.

It is unclear whether this interaction is direct or indirect, because the proteins which are directly interacting will bind along with those indirectly bound interacting proteins will also be eluted. This method is also quite expensive, because it needs a good equipment setup, and different type of analytical and computational analysis.

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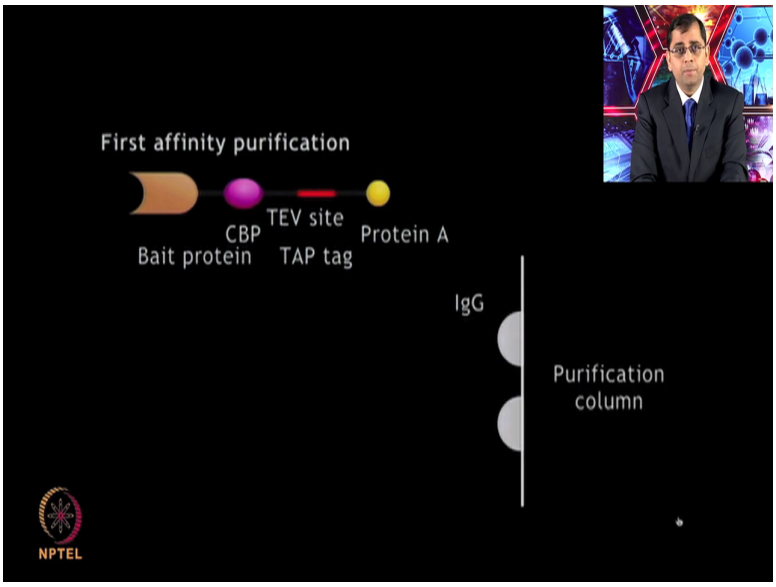

So, let us discuss the immunoprecipitation method how the mechanism occurs in more detail by showing you this animation. In immunoprecipitation, the protein of interest with fuse with the tap tag which contains a calmodulin binding peptide a TEV cleave site and protein A. Depending upon the proteins to be studied this tag can be modified. A tag is then bound to a column to affinity interactions which is the protein A and IgG. A protein mixture whose interactions with a breed protein are to be studied is then added.

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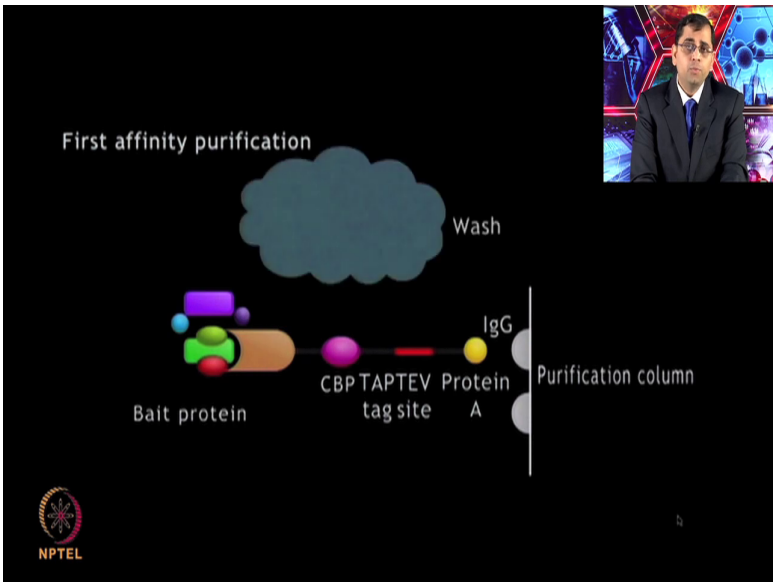

Some of the proteins for the complex with the bait protein to a specific binding interactions. The remaining unbound protein are then washed away. This is followed by cleavage at the TEV site.

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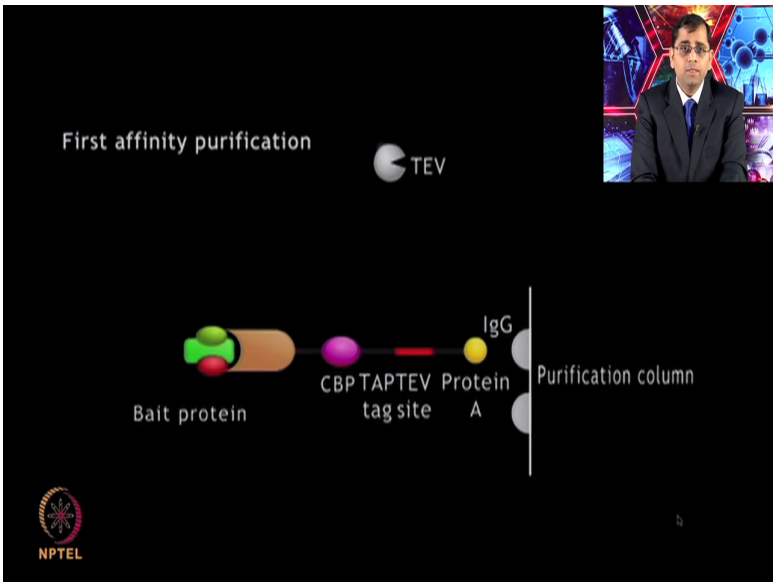

But the TEV protease to release only the protein complex bound to CBP. These reactions constitute the first affinity step.

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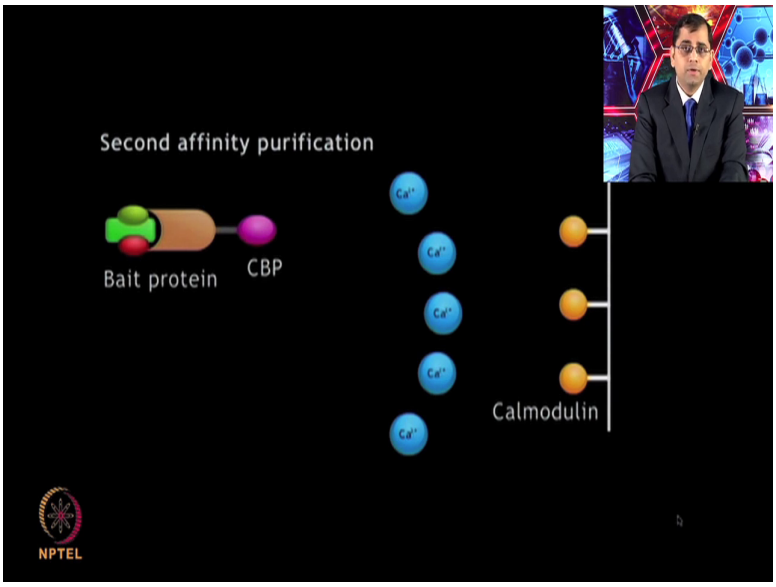

In the second affinity purification step, the bait (Refer Time: 16:13) complex is bound by other CBP domain to a calmodulin functionalized column in the presence of calcium ions. The column is washed to remove any other unwanted contaminants after which accurative agent is added which complex the calcium ions.

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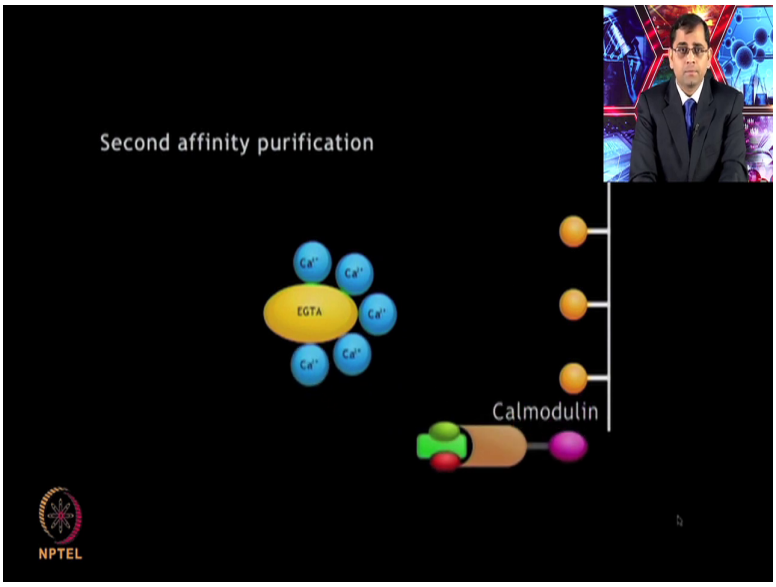

While these are removed the CVP calmodulin attraction is weak end and leads to the release of purified protein complex.

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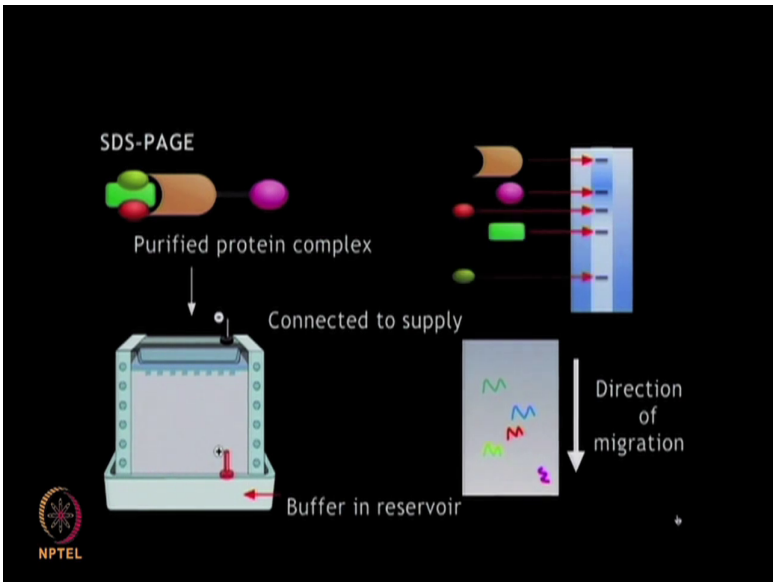

When the protein complex has been purified, the component of the complex are separated by electrophoresis under reducing condition. DLS gel is then analyzed and the protein components evaluated, thereby providing and understanding about the interaction with the bait protein of interest protein interaction.

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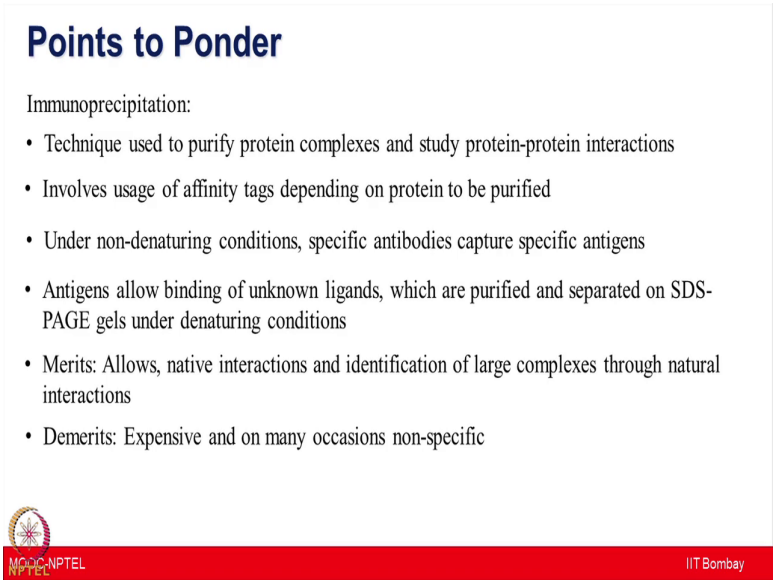

So, we have discussed the traditional ways of a studying protein-protein interactions. First one was immunoprecipitation. Now, let us to move on to the next method which is yeast two hybrid. In yeast two hybrid, two types of proteins are involved; a bait protein and a prey protein. Bait protein is protein of interest whose interaction is to be studied.

The bait protein is fused with the binding domain of the transcriptional activator by inserting it and expressing along with the binding domain in a suitable manner. The prey protein is a protein whose interactions with the bait needs to be determined and that is also known as prey protein. The prey protein is fused with the activation domain of the transcriptional activator. So, the successful interactions of the bait and the prey protein activates the transcription of reported genes.

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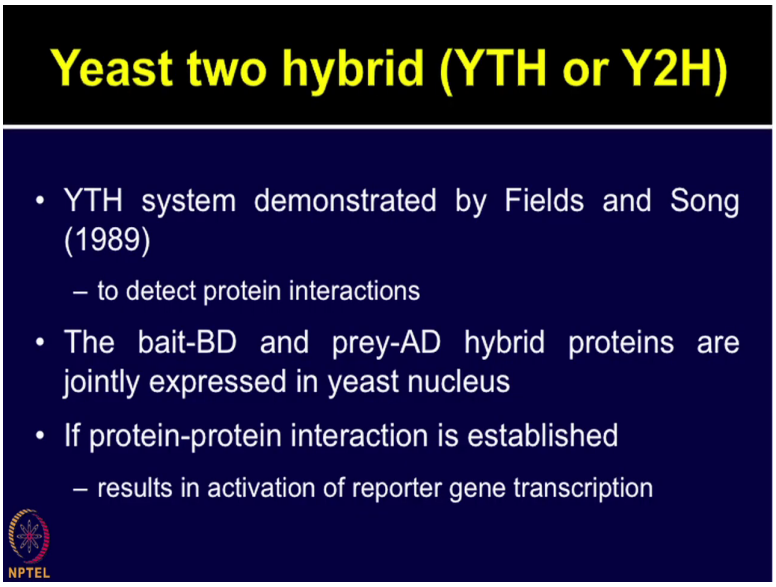

Yeast two hybrid system was first demonstrated by scientist fields and song in 1989 for studying the protein interactions. Since then this approach has been widely used in different organisms for different type of biological questions to be addressed.

In general yeast two hybrid system uses the bait binding protein and the prey activation domain. These hybrid proteins are jointly expressed in the yeast nucleus. If the protein interactions established between the bait and the prey which are coupled with BD and AD, then transcription will occur.

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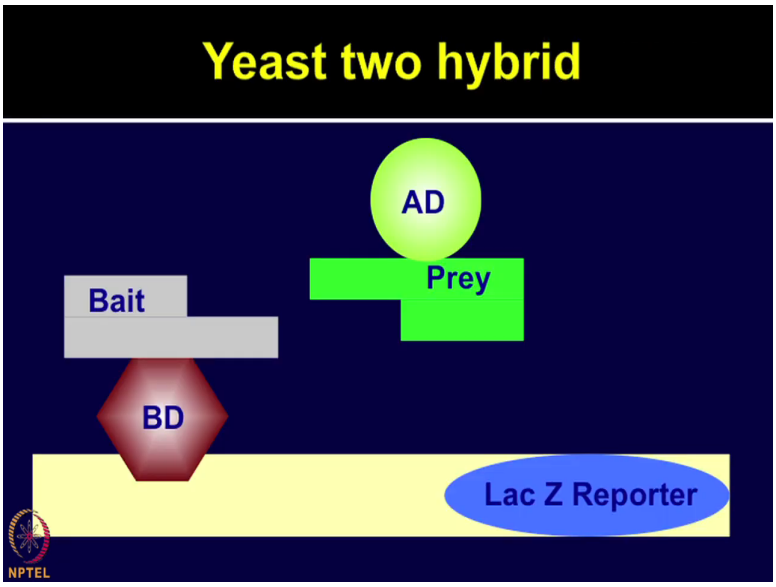

As you can see the slide, the transcriptional activation for the two hybrid system consists of two protein domains. One is DNA binding domain which remains bound to the promoter region and is fused with suitable bait protein whose interaction with another protein is required for the study. The activation domain is other domain of the transcriptional activated which is fused with the prey protein. This activation domain when bound to the DNA binding domain forms the functional transcriptional be activated and brings the expression of reporter genes.

As you can see in the slide the bait protein binds with DNA binding domain, and the part of the protein or the prey with transcribe activator domain or AD domain. While the BD binds with DNA while the ad activates the transcription when both of these are associated the bait with the BD, and prey with AD when they bind together the transcription event occurs.

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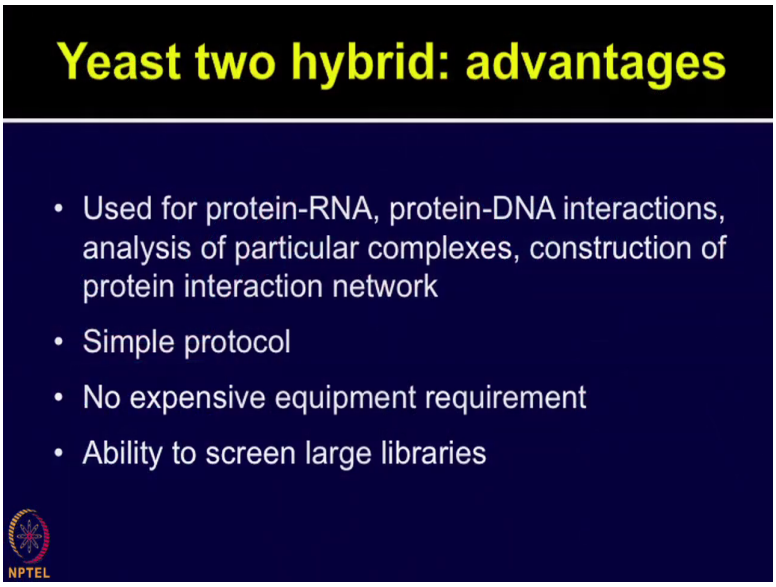

What are the different advantages of using yeast two hybrid system? As I mentioned since 1989, when it was shown first time studying the protein interactions using yeast two hybrid. It has been used for various type of interactions including protein RNA, protein DNA, analysis of particular complexes as well as a studying the large protein interaction networks. The protocols for yeast two hybrid is quite simple. Unlike the other approaches there is no requirement for heavy instrumentation here. The method also allows to screen the large libraries very rapidly.

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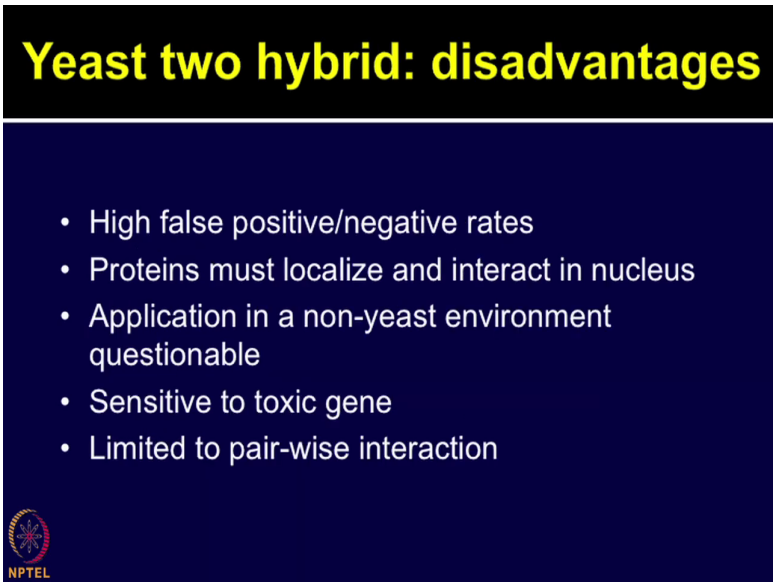

What a different disadvantages of using yeast two hybrid system? It provides very high positive and negative rates. So, high false positive rate has been one of the major limitations of this approach. The protein must localize and interact in the nucleus. So, that is the limitation of the approach, because it is restricted to the binary interactions or if you compare with immunoprecipitation the complexes cannot be formed. So, application in a non yeast environment is also questionable because this system mostly used here is yeast. The protein folding if you are aiming for studying the mammalian system is not guaranteed. It is also not quite sensitive to the toxic genes.

Finally it is limited to the pair-wise interactions. If you have fair idea about the molecules which are potentially interacting then you can use the pair-wise study by using yeast two hybrid. But also if you just want to generate a list of potential interactors which may or may

not be true then those can be quickly screened by using different libraries by using yeast two hybrid method.

Once you have established the list of potential intractors, then you can use different type of validation approaches for real establishing how many of these proteins are interacting well and how many of these are false positive. So, yeast two hybrid screening remains as a novel biological techniques which can be used for screening and the discovery of protein-protein, and protein other bio molecular interactions. Let me show you how yeast two hybrid words in the following animations.

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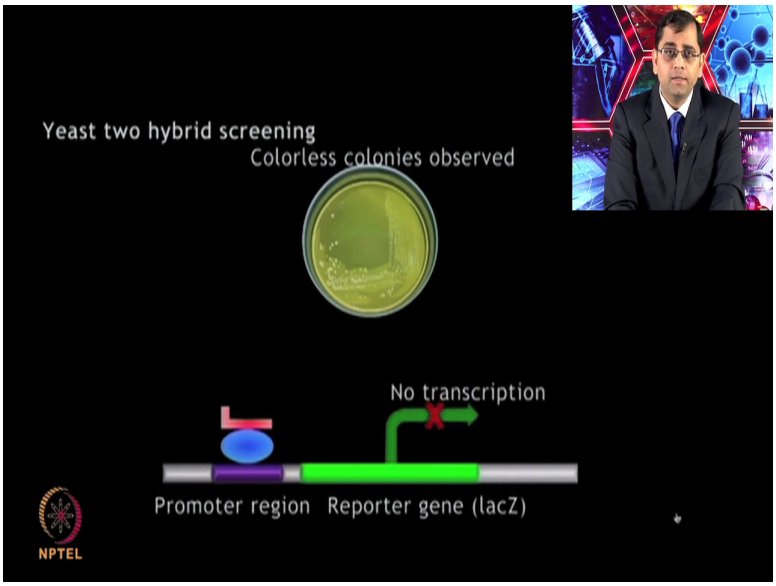

We will now talk about yeast two hybrid. In the yeast two hybrid screening, the binding of the transcriptional activated protein composed of binding domain and activation domain to the promoter region. It essential for expression of the corresponding reporter gene located

downstream of the promoter. The binding domain is fused with the bait protein, while the activation domain is fused with the prey protein.

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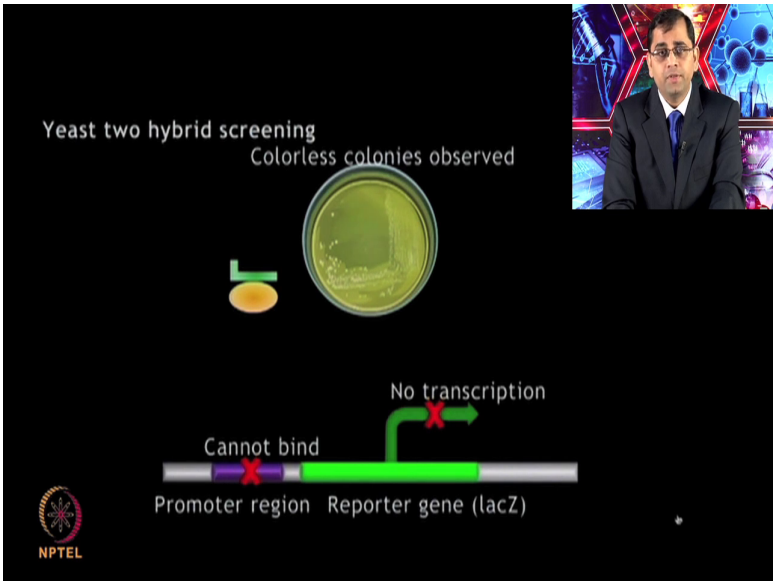

Binding of either one of the fusion protein to the promoter is not sufficient to bring about transcription of the gene. Yeast two hybrid screening protocol uses this interaction as the basis for a screening protein interaction. When the beat protein bound with the binding domain interacts with prey protein fused with the activation domain.

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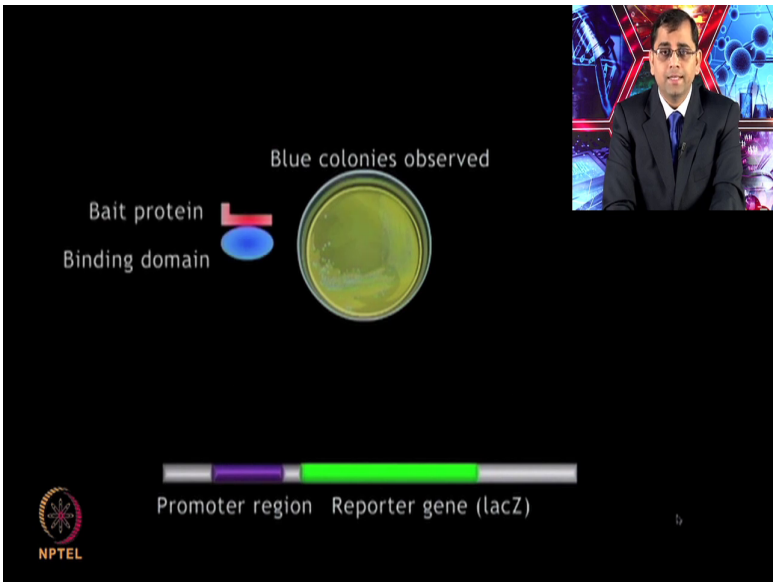

There will be expression of reporter gene which can be easily detected. Lac Z, is a commonly used reporter gene most protein products which are electricity cleave is substrate x gal resulting in blue color colonies.

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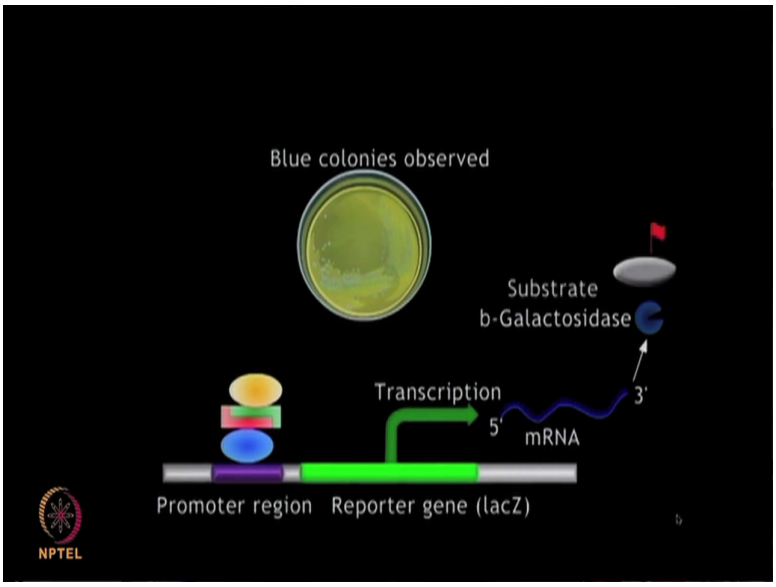

Let me show you yeast two hybrid concept, apply then one of the studies by showing this interaction.

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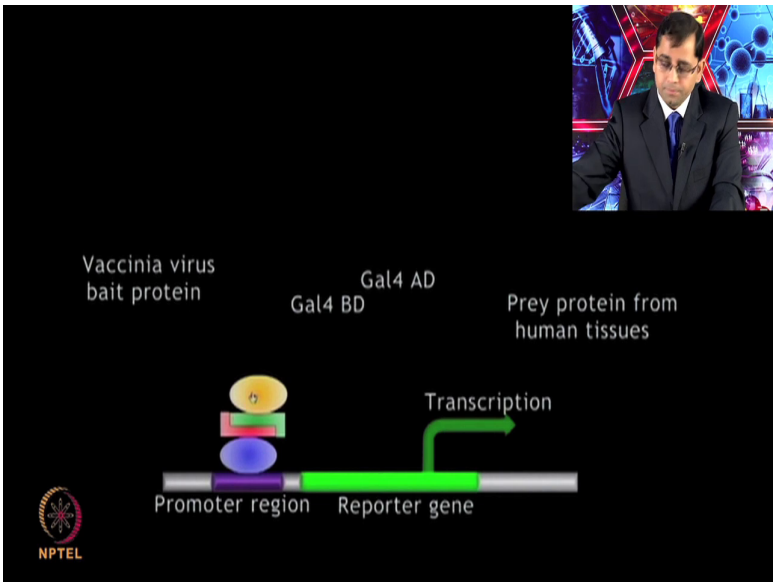

The study the protein-protein interaction between the bacteria virus and human protein; in this study authors used a virus bait proteins fused with a c terminals of gal four binding domain BD while the prey human proteins were fused with gal 4 activation domain of the transcriptional activator. These interaction were further validated by other techniques which gave the successful validation rate of 63 percent.

Let me show you by this interaction how this yeast two hybrid screening can be used. If you drag and drop the components such as the transcription will be carried out. You can see the first gal 4 domain adhere to the promoter region, and now as soon as the activation domain binds their transcription events happen.

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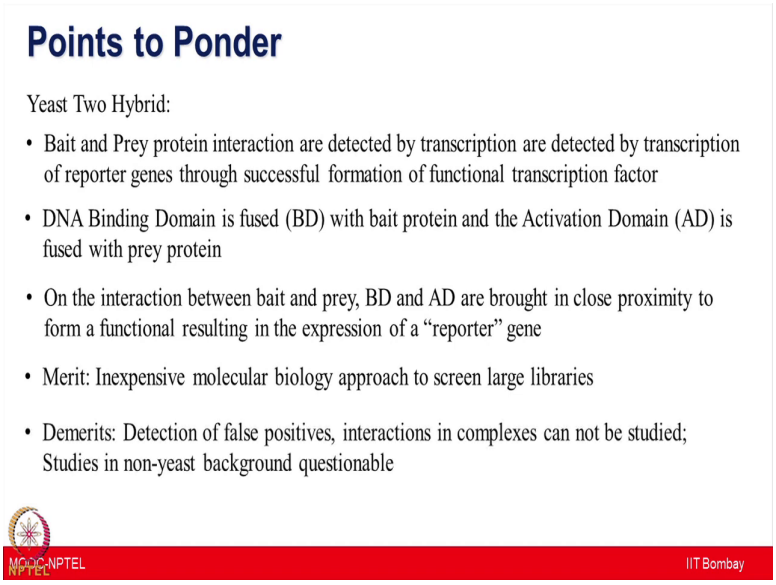

So, in summary today we talked about significance of a studying interactions in the field known as interactomics. We discussed different types of traditional approaches which have been used for a studying the protein interactions such as yeast two hybrid and immunoprecipitation assays.

In the next lecture, we will discuss a variety of platforms currently being used for a studying the proteins in the high throughput manner by using the exciting field of protein microarrays, I will describe to you how different content such as antibodies, verified proteins peptides, or even cell free expression could be perform to make the protein microarrays.

Thank you.