Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-1.
Introduction to Interactomics.

Welcome to the MOOC NPTEL course on Interactomics. Proteins are dynamic molecules which interact with wide variety of bio-molecules such as lipids, nucleic acids as well as various small drug inhabitants, metabolites and other bio-molecules to provide different type of significant information for physiological actions. Proteins also interact with one another to form larger complexes. These complexes regulate various fundamental processes such as signal transduction and gene regulation.

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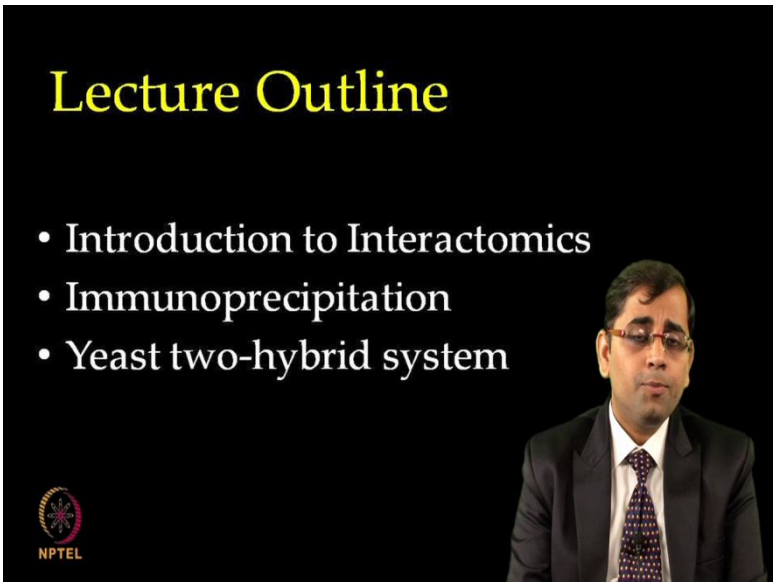

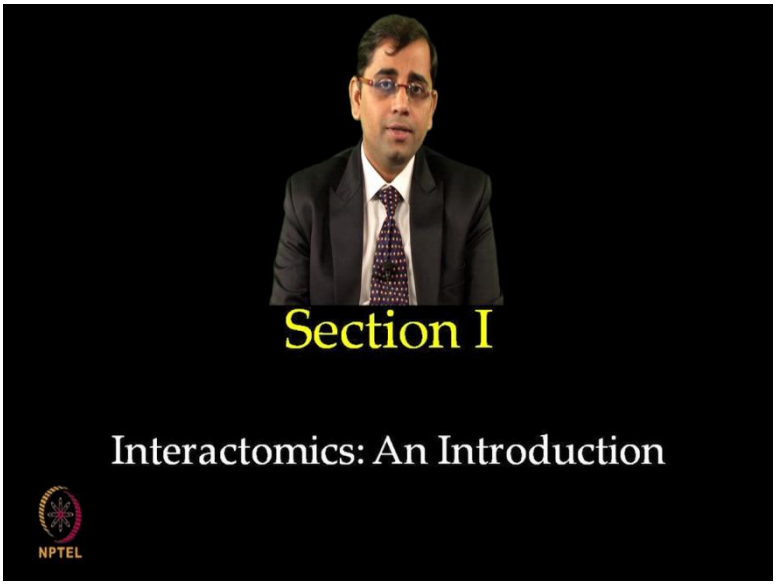

A detailed understanding of protein interactions provide an opportunity to understand protein functional behavior. In the first lecture of this module, we will begin by understanding the basics of emerging discipline of Interactomics and provide a historic perspective of the conventional techniques involved in the studying protein interactions. This broadly includes yeast two-hybrid and immunoprecipitation techniques.

So, first let us begin our journey to understand Interactomics. Interactomics comprises the study of interactions and their consequences between various proteins and other cellular components. The network of all such interactions is known as interactom. If one simply breaks down the interacting molecules in a cell which essentially are DNA and proteins, so, we can obtain the better understanding of genome and proteome functions.

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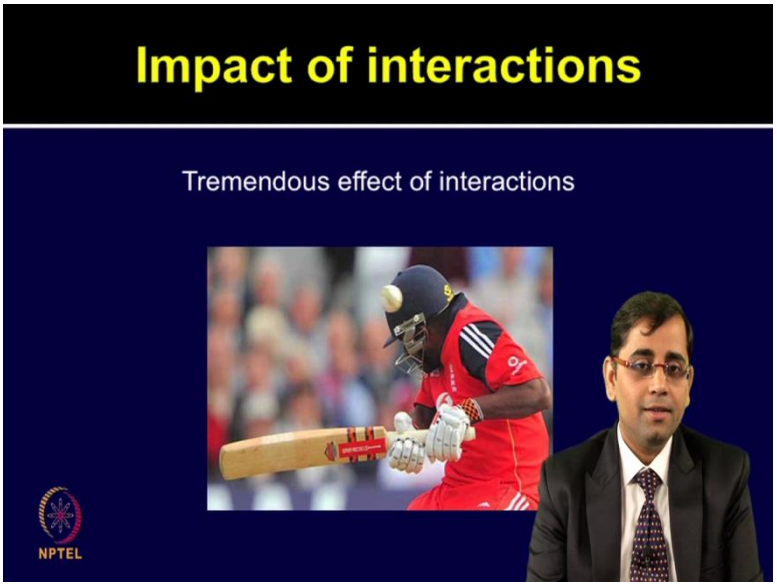

Interactions may have different type of impacts, it could be positive as well as negative, as we can see in this slide, there is tremendous effect of interaction but it always not positive. Any kind of interaction good or bad would have a resulting impact, while this slide shows an effect of one such interaction, the processes in the cell are way too complex and protein interactions in general impact every single cellular function. The complex biology of living systems comprise of several mechanisms that regulate these interactions. Regulatory mechanisms like post translation modifications and presence or absence of activator or inetrpretor molecules are few examples of such evolved mechanisms.

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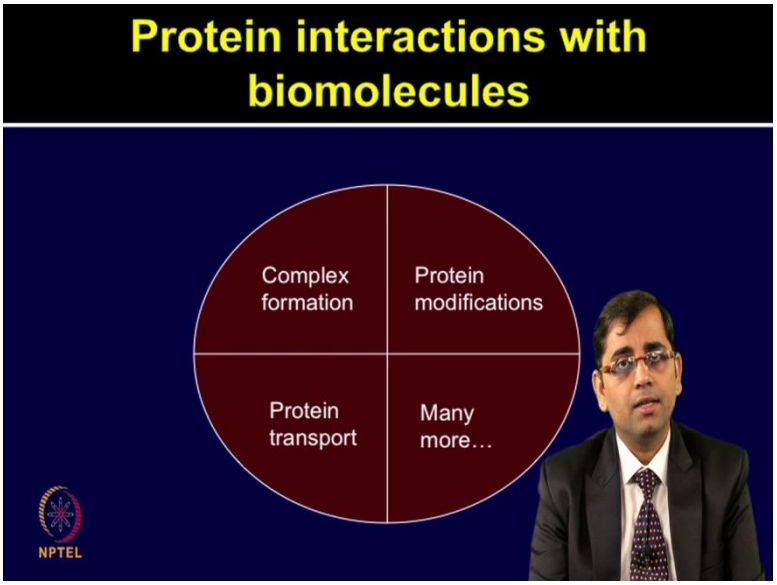

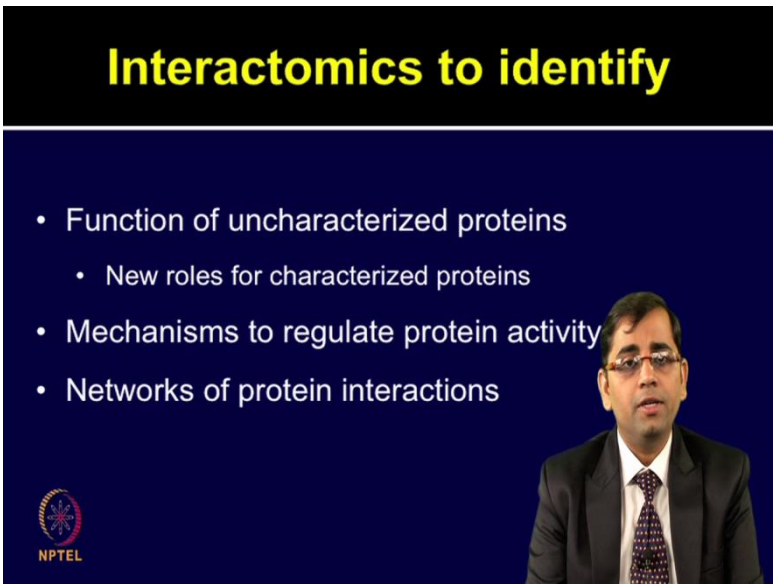

Interactions can also be modulated by altering the special levels of proteins. Proteins interact with variety of molecules and these interactions are key to proper functioning of a given cell.

Proteins affect interactions, form complexes, which modifies the proteins and help in protein transportation and affect the various functions in which they are involved. 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 valuable information for crucial life processes, like signal transduction and bio-chemical pathways in which they are involved. The physical and chemical nature of these interactions are varied. There are few interactions which are very transient, that is they would last for very short duration while there could be other interactions which could be permanent. Few interactions are weak whereas other interactions could be very strong. These characteristics significantly affect the activity of proteins and the signals which they trigger.

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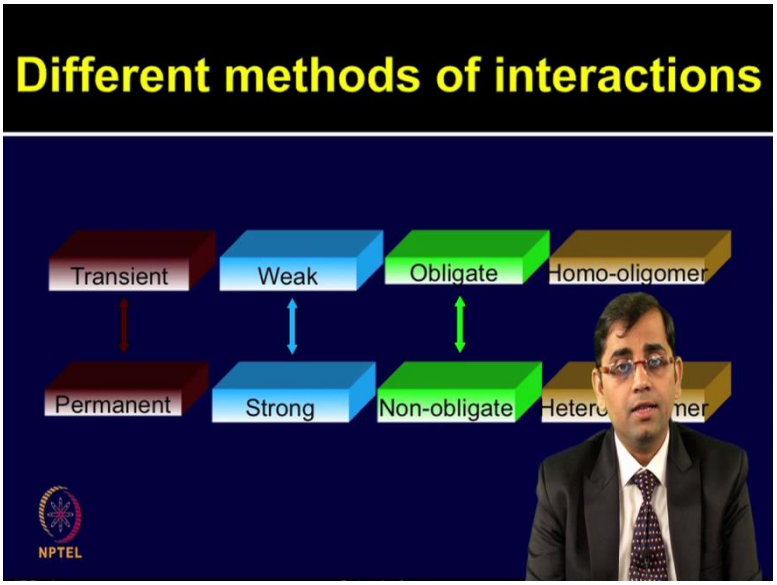

Interactions can also be obligate or non-obligate, they can form homo-oligomers as well as different kind of oligomeric units to combine and form hetro-oligomer. So, from this discussion you have got a glimpse of the complexities involved in protein interactions a study in this molecular interactions thus necessitates various technologies primarily because of the diversity and complexity involved in these interactions.

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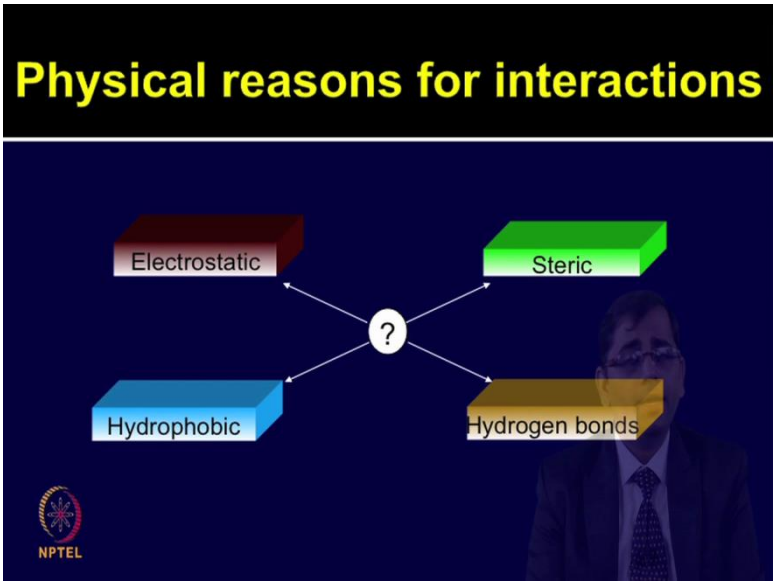

Let us look at the various physical forces and their implications resulting in protein interactions, these are mainly electrostatic, hydrophobic, steric or hydrogen bonds. Proteins form multi-protein complexes and they are involved in various biological pathways just like the one shown figure here. By studying the interactions one can understand the complexities of the biological pathways in which these proteins are involved.

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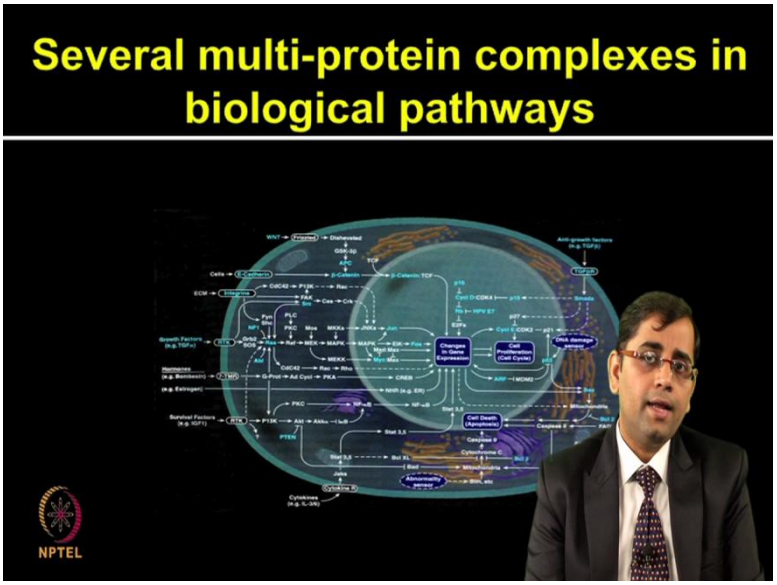

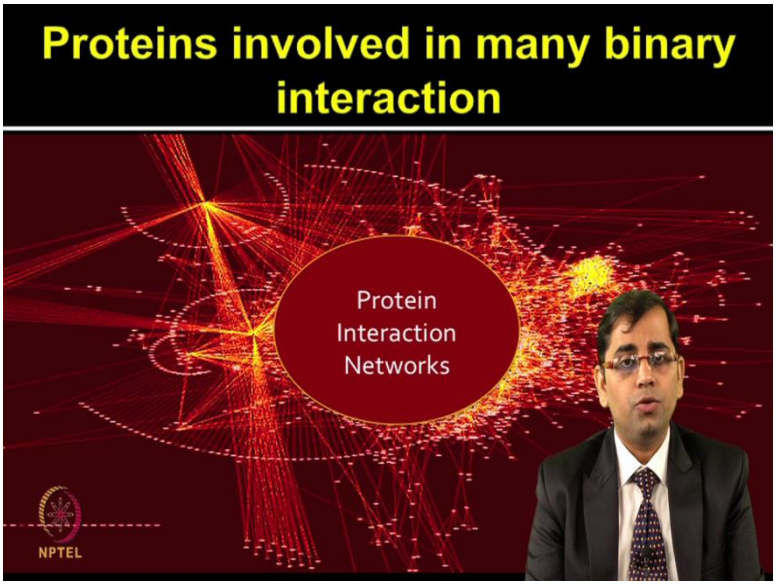

So, these protein interactions studies ultimately help in obtaining quantitative information as well as crucial signaling cascades improving the existing information on these varying diagrams, Since proteins are involved in mainly bindery interactions an understanding of the underlying bio-chemistry of proteins and biological interactionses a critical elements for the development of novel therapeutics and diagnostics.

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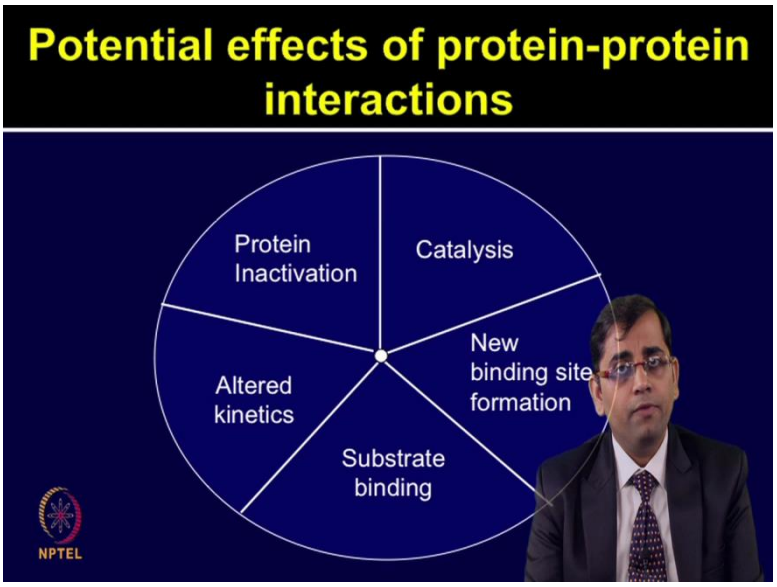

Like discussed earlier protein-protein interactions have various potential affects you can have a look at few common effects described in this slide, catalysis, protein inactivation, altered kinetics, substrate specificity, new binding site formation are few such examples but these interactions impact multitude of downstream effects. If the interactions are normally regulated, the system is healthy however any aberration in these processes may lead to

disregulation which may ultimately result in various disease manifestations. Therefore, protein interactions are very essential for any cellular mechanisms.

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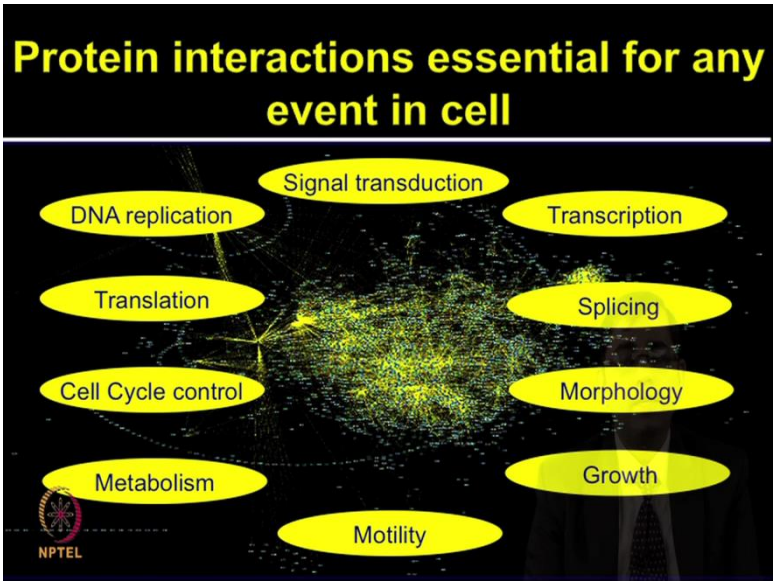

Several examples are illustrated in this slide and by the end of course you would be able to list many more examples here and understand more deeply as to how protein interactions are essential for the activity of cell.

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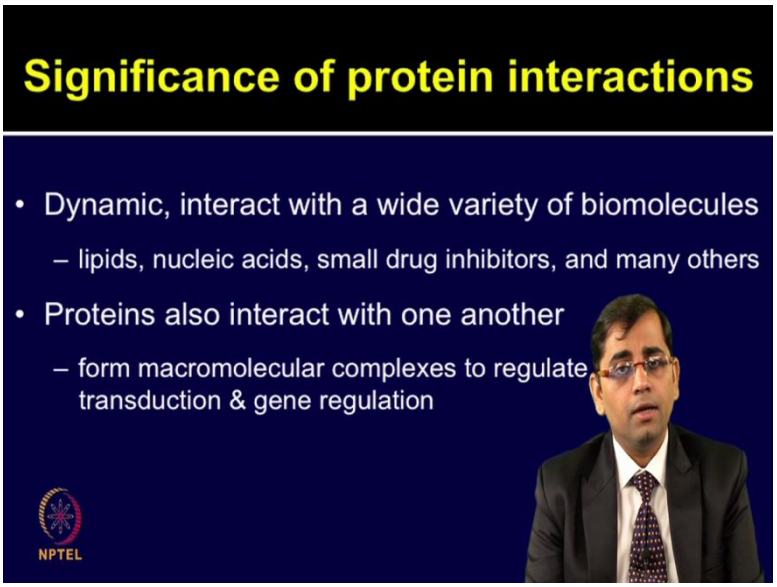

So, what is the significance of the studying protein interactions? Proteins are dynamic and interact with various bio-molecules and regulate several functions including signal

transduction and gene regulation. Now that you understand what the domain of Interactomics is all about, let me show you an animation to illustrate various aspects of Interactomics.

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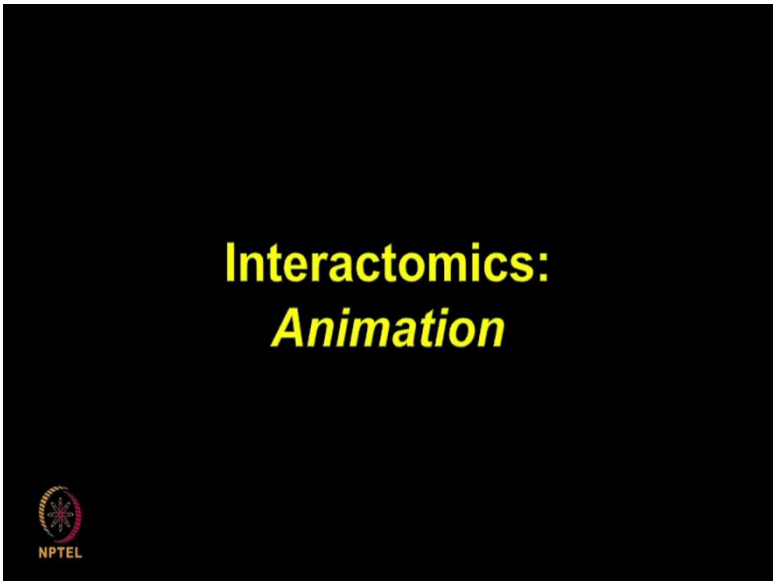

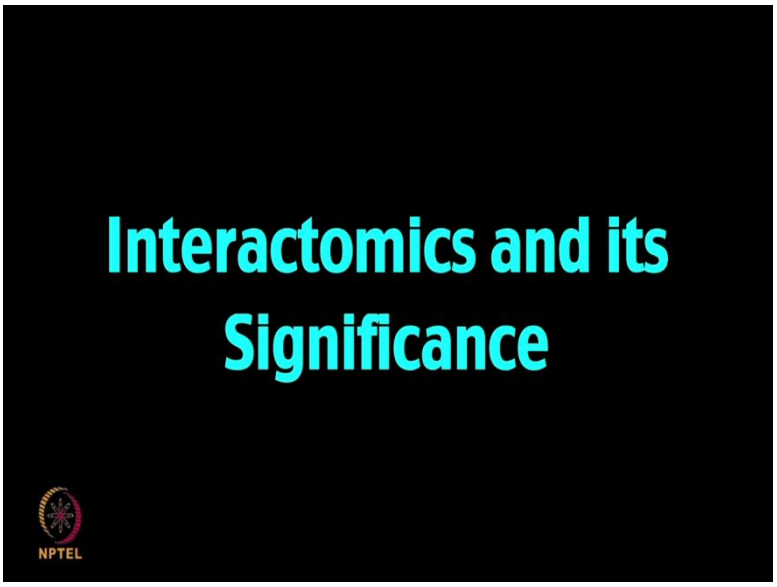

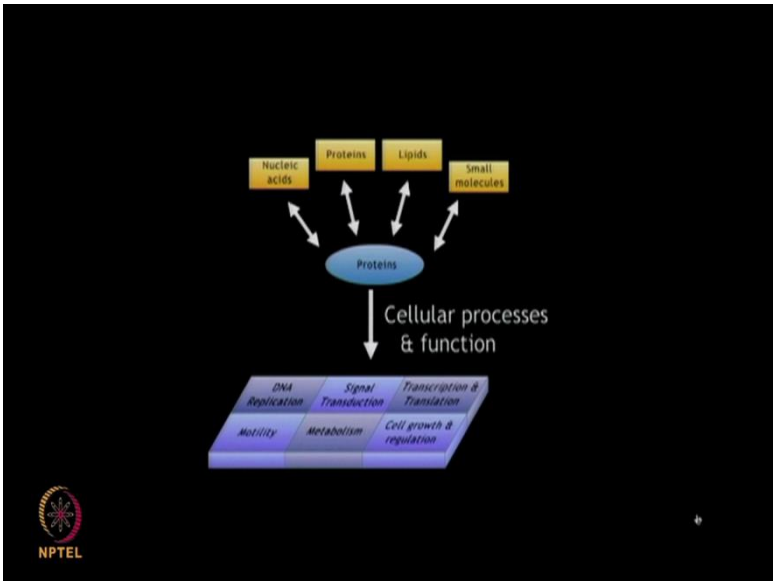

Let discuss, the Interactomics and it is significance in this animation. Interaction studies of proteins with various bio-molecules help to decipher and understand the function of various proteins in the complex network of cellular pathways. Proteins interact with other bio-molecules such as nucleic acids, lipids, hormones etc.etc. to execute a multitude of functions important living organisms such as signal transduction, growth and regulation and metabolism these are few examples clubbed any other cellular processes and functions in which these are also involved.

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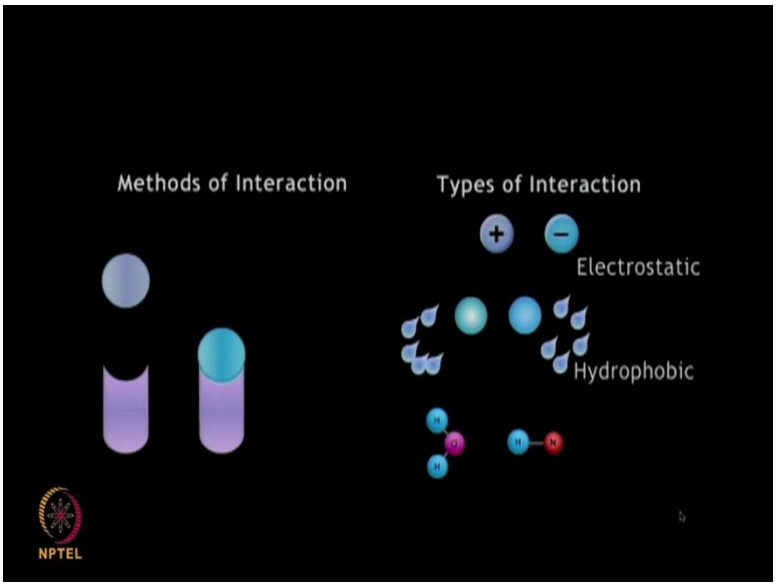

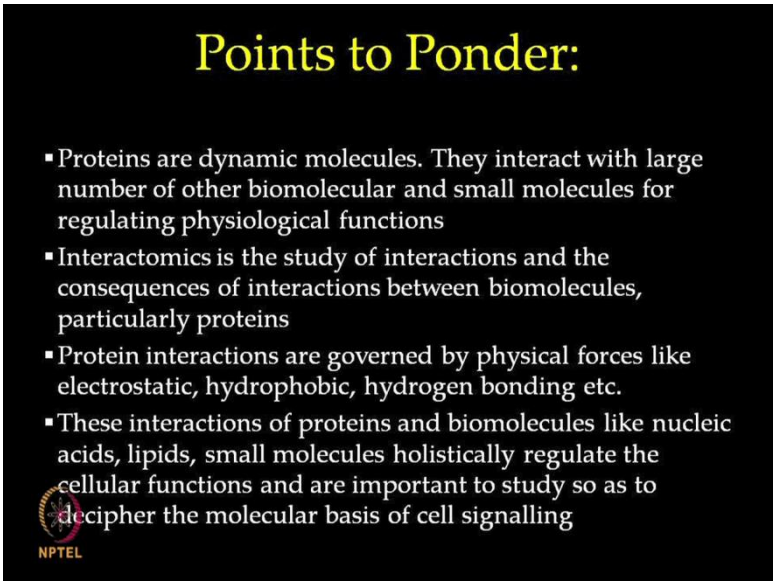

Protein interactions with other bio-molecule can be have several different types. They may be weak or strong, obligate or non-obligate, transient or permanent. The physical bases for these interactions include electrostatic, hydrophobic, steric interactions, hydrogen bonds etc.etc.

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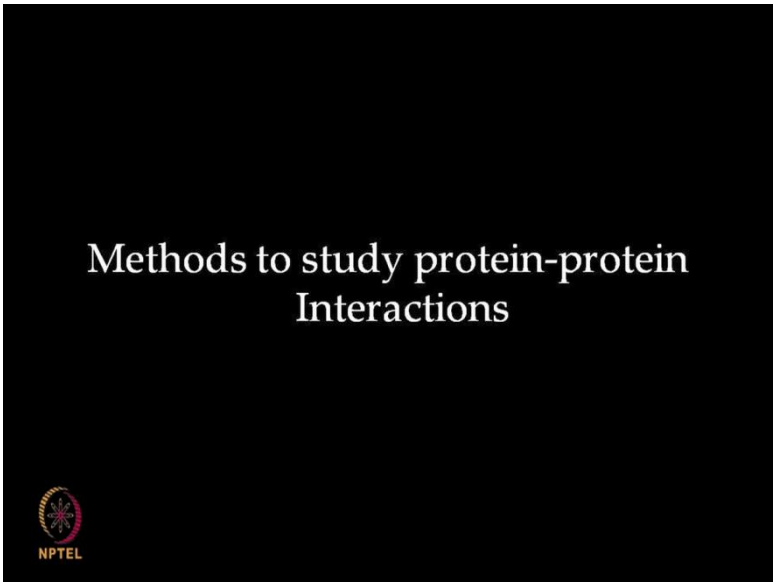

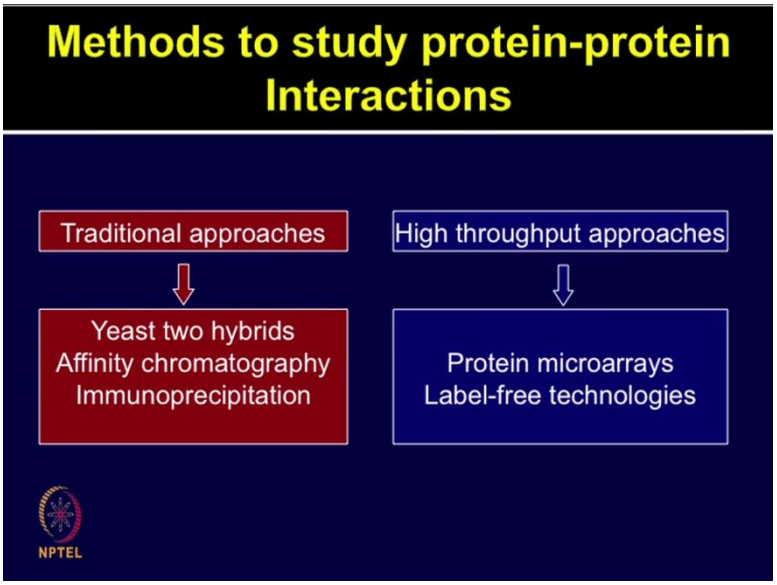

Let us now move on to different methods used to study protein-protein interactions. Understanding these protein-protein interactions provide important clues to the function of proteins. Identification of interactions with known proteins may suggest thei functional role played by a novel protein. There are two different ways of a studying these protein-protein interactions, the traditional approaches which are very popular and used as classical experiments to establish any protein interactions. However nowadays, there are quite a few high throughput approaches emerging, which are making their mark in the Interactomics field.

The traditional approaches like yeast two-hybrid, affinity chromatography and immunoprecipitation and more recent approaches include protein microarrays and different types of label-free technologies. We will deal all the traditional approaches in this lecture. The traditional ways of studying protein-protein interactions involve yeast two-hybrid and immunoprecipitation. These are two widely recognized technologies which have been used to map protein-protein interactions on a large scale, both of these approaches have been used to identify thousands of novel interactions in different organisms such as human, C elegans, drosophila etc.

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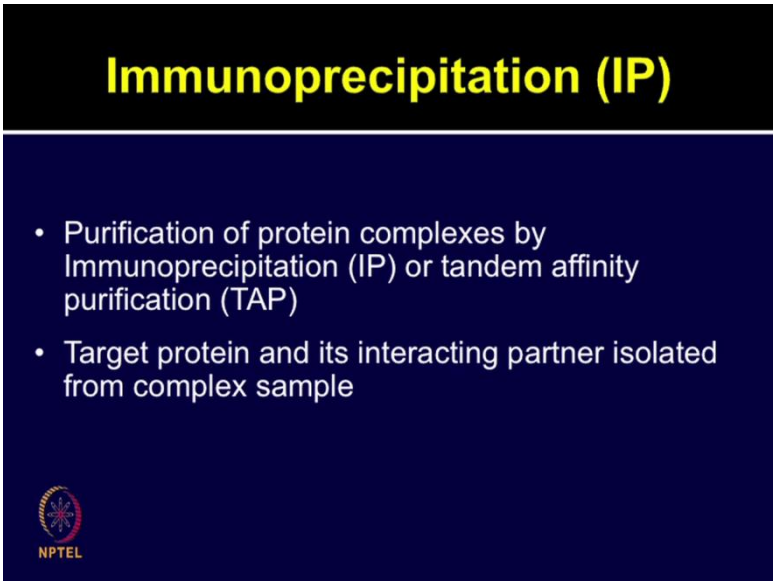

Let us now talk about, immunoprecipitation technique. Immunoprecipitation or Tandem affinity purification also known as TAP is a technique used to purify protein complexes and study protein-protein interactions. Depending on the protein that need to be purified, different types of tags can be attached to the bead protein. By using immunoprecipitation method the purification of protein complexes by IP or tandem affinity purification is performed. Target proteins and its interacting partners are isolated from the given complex sample and then by using different tags, these proteins can be isolated from a mixture and further processed.

Let me describe this process in the coming slides. In immunoprecipitation method, the antibody is specific to the bead is attached to the whole (())(13:36) which forms the complex, remember this step is performed in the native or non-denaturing conditions. The protein-protein complex is immobilized on protein A or protein G sepharose beads is protein complexes eluted and further analyzed on SDS-PAGE gels , as you can see on this slide, it shows that the antibody is used which binds with the antigen and unknown ligands. This complex can be separated on SDS-PAGE gel. This is done under denaturing conditions as a gaze of first step which was performed in non-denaturing conditions. What are the methods of using IP or immunoprecipitation?

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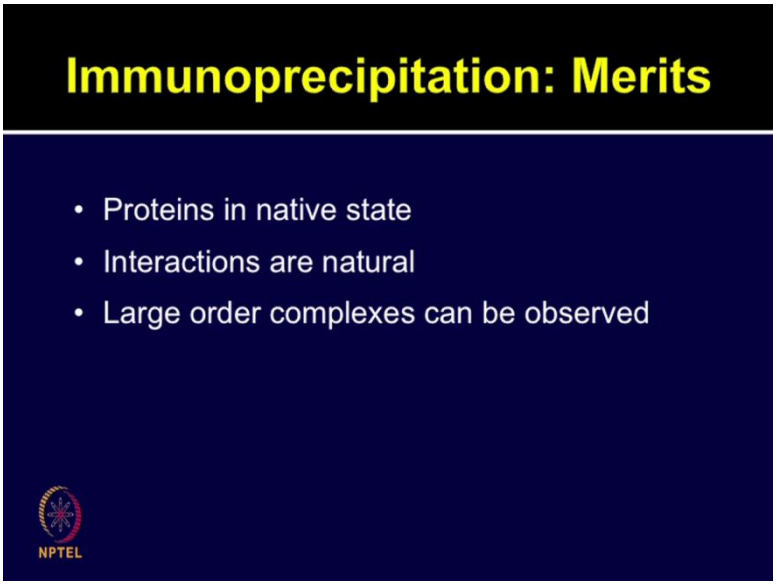

In this method, proteins are isolated in native state. Why is native state so important, because the native state allows native complexes to be formed. It also allows the formation of post-translational modification. It is essential to perform these steps in native or non-denaturing conditions. Interactions by using IP method are natural and by using this method large complexes can be formed because the native state will promote the native complex formation. What are demerits of immunoprecipitation?

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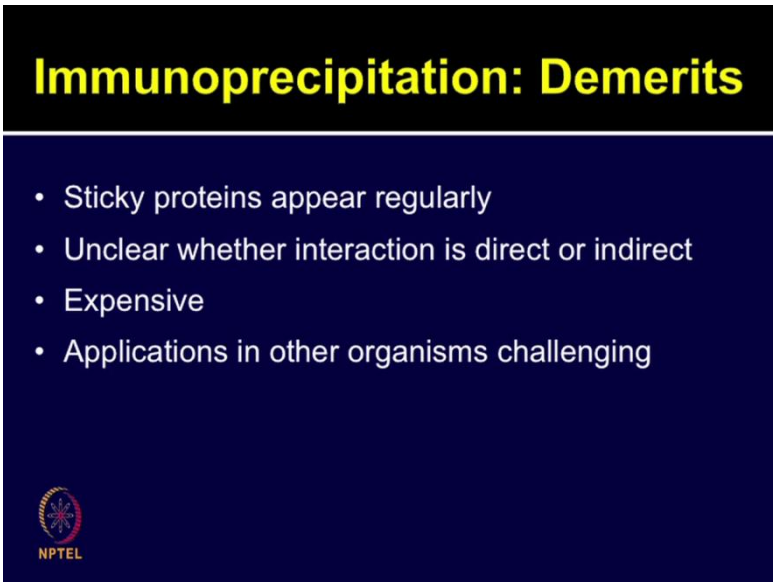

It has been reported that frequently sticky proteins may be picked up. Proteins that are non-specific could also be (()eluted because there is sticky on other protein surface. It means few

proteins that are specifically interacting with target of interest will be isolated along with sticky proteins. It is unclear whether this interaction is direct or indirect because the proteins which is directly interacting will be bound and along with those proteins indirectly bound proteins may also be eluted. This method is also quite expensive because it needs a good equipment setup, different type of analytical and computational analysis. Applications in organisms other than yeast have been challenging but more people have applied this method to different types of organisms.

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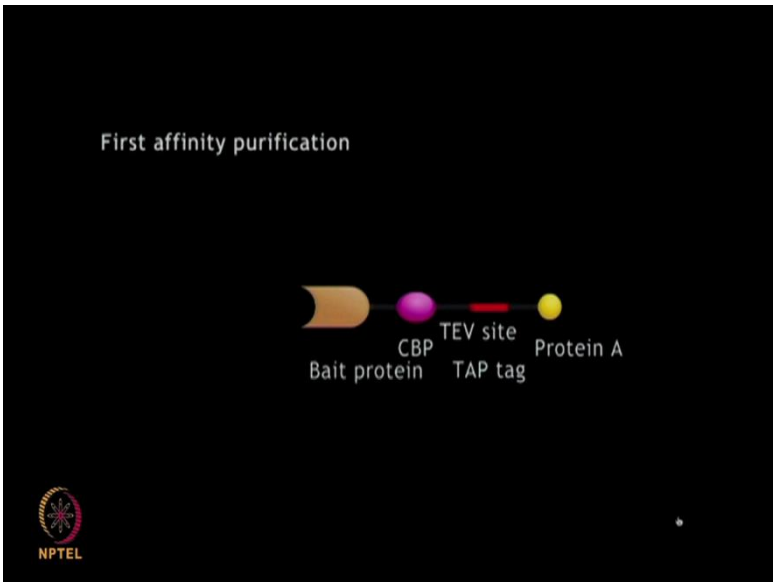
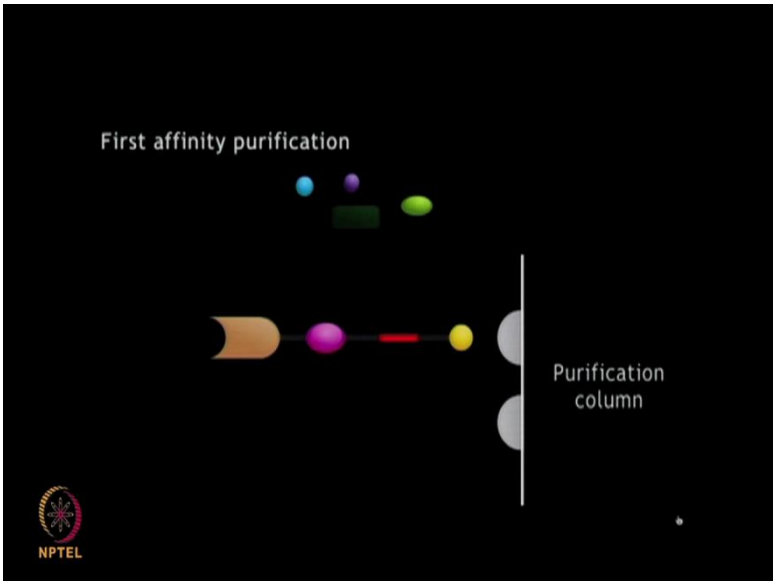
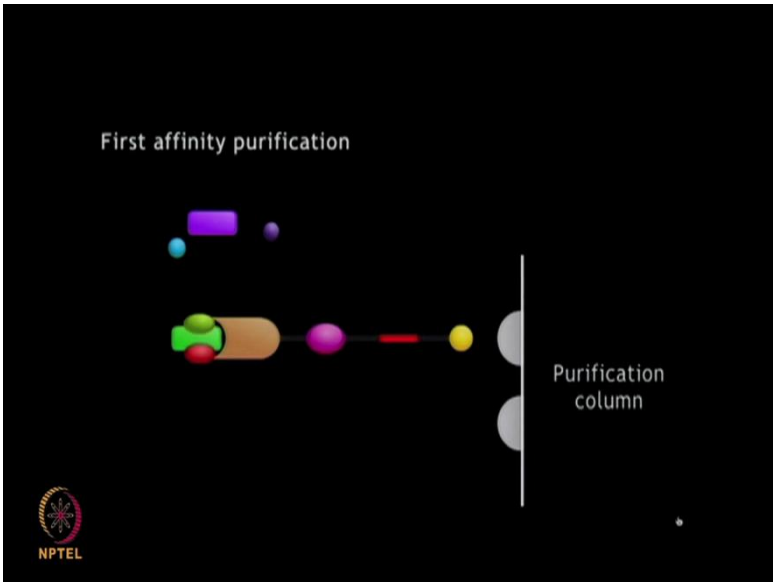



First affinity purification
Purification column
NPTEL


First affinity purification
Purification column
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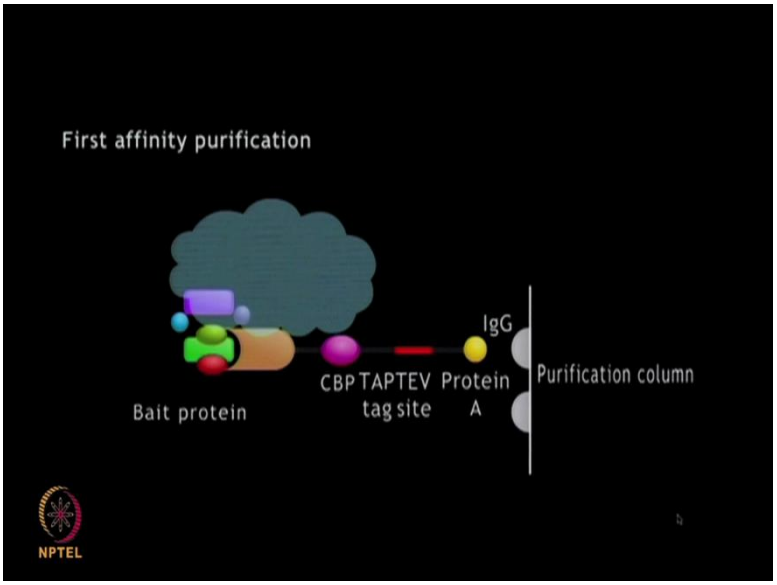
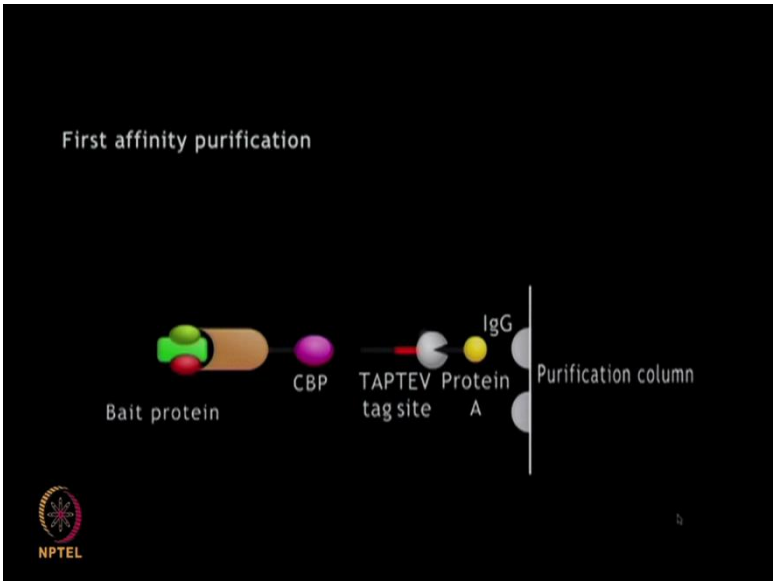
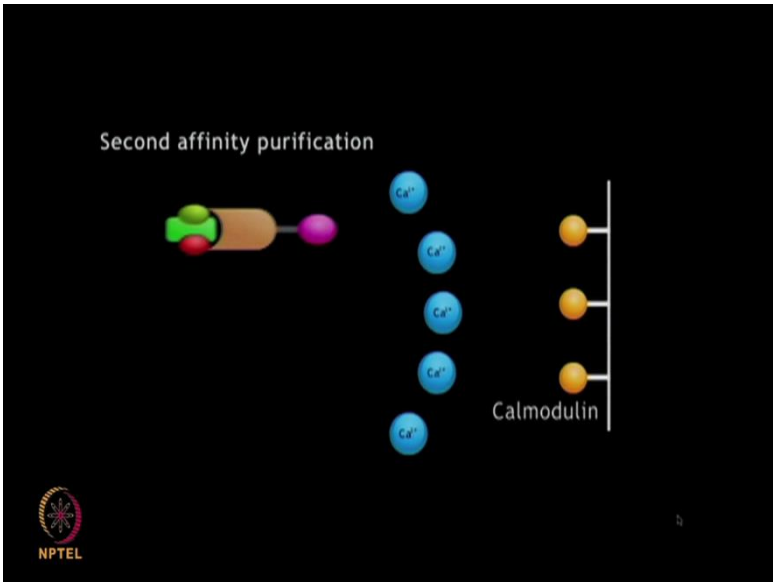
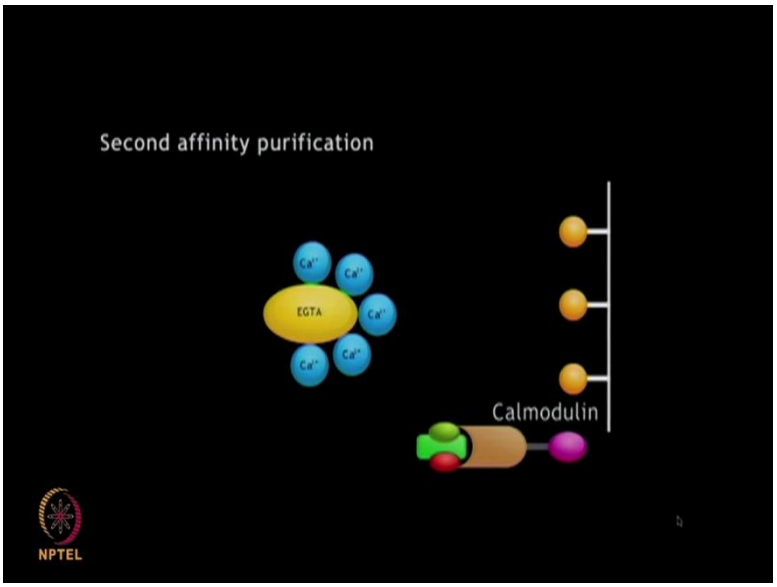
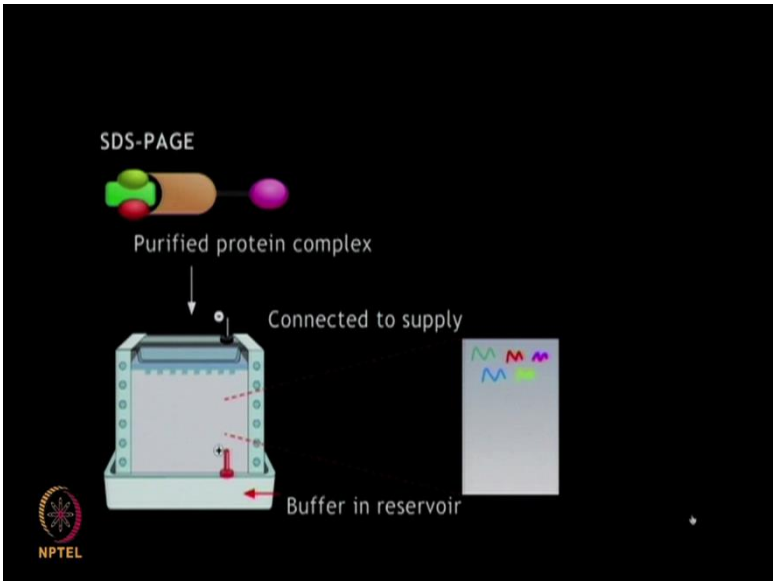
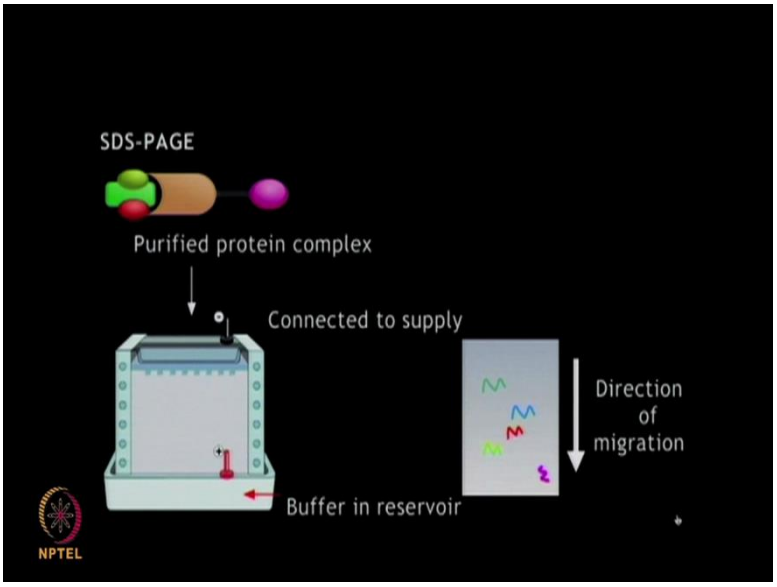

First affinity purification
IgG
Purification column
Bait protein
CBP
TAP tag
TEV site
Protein A
NPTEL


First affinity purification
IgG
CBP
TAPTEV tag site
Protein A
Purification column
Bait protein
NPTEL


Second affinity purification
Calmodulin
NPTEL


Second affinity purification
EGTA
Calmodulin
NPTEL





Points to Ponder:

Immunoprecipitation:

- Technique used to purify protein complexes and study protein-protein interactions
- Involves usage of affinity tags depending on protein to be purified
- Under non denaturing conditions, specific antibodies capture specific antigens.
- Antigens allow binding of unknown ligands, which are purified and separated on SDS PAGE gels under denaturing conditions
- Merits: Allows, native interactions and identification of large complexes through natural interactions
- Demerits: Expensive and on many occasions non specific

NPTEL

So, let us go through this animation where we can go over the steps involved in immunoprecipitation method in more detail. In immunoprecipitation, the protein of interest is fused with a TAP tag which contains a calmodulin binding peptide, a TEV cleavage site and protein A. Depending upon the proteins to be studied, this tag can be modified. The tag is then bound to the column through affinity interactions between the protein A and IgG, the protein mixture whose interactions with the bait protein are to be studied will get added. Some of the proteins form a complex with the bait through the specific binding interactions. The remaining unbound proteins are washed away which is followed by the cleavage at the TEV site by using enzyme TEV (())(17:51) to list only the protein complex bound to the calmodulin binding protein. These reactions constitute the first affinity step.

Now, let us talk about the second affinity purification, in which the bait prey complex is bound where the 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 a (())(18:28) agent is added, which complexes the calcium ions. Once these are removed, the CBP calmodulin interaction is weakened and leads to release of purified protein complexes.

Once the protein complex has been purified, the components of the complex can be separated by using electrophoresis under reducing conditions. The SDS gel is then analyzed and the protein components can be evaluated to provide an understanding about the interactions, as you can see different type of bands are visible.

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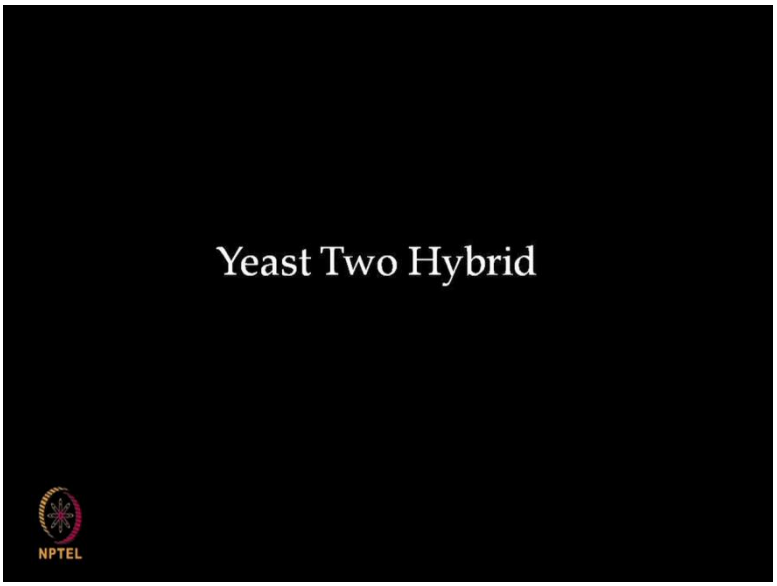

So, we have discussed, the traditional ways of studying protein-protein interactions, we have covered the first technique, which is immunoprecipitation. Now, let us move on to the next approach, yeast two-hybrid. In yeast two-hybrid, two types of proteins are involved, a bait protein and prey protein. Bait protein is the 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 need to be determined. The bait protein is fused with the activation domain of the transcriptional activator.

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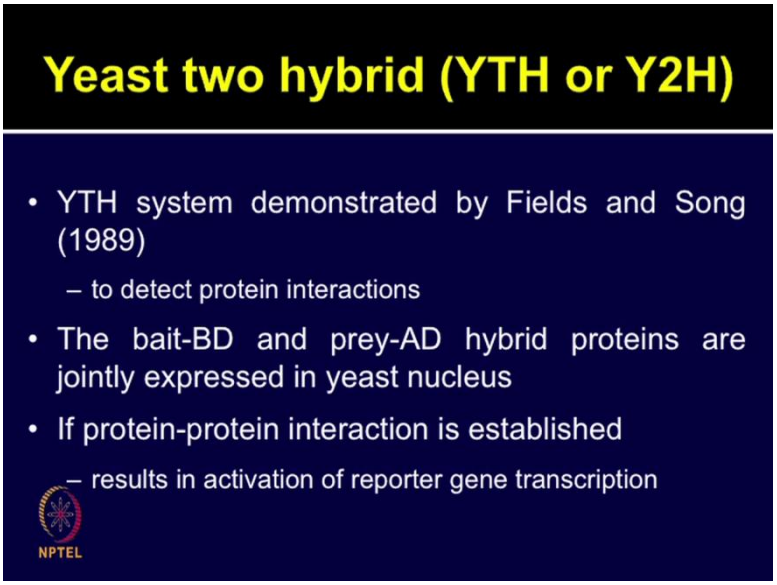

So, the successful interaction of the bait and prey protein activates the transcription of reporter genes. The yeast two-hybrid system was first demonstrated by scientist Fields and Song in 1989 for studying interactions. Since then this approach has been widely used in different organisms for various types of biological questions. In general, the yeast two-hybrid system uses bait binding domain and prey activation domain. These hybrid proteins are jointly expressed in yeast nucleus. If protein interaction is establish between the bait and the prey, which are coupled with BD and AD domains then transcription will occur.

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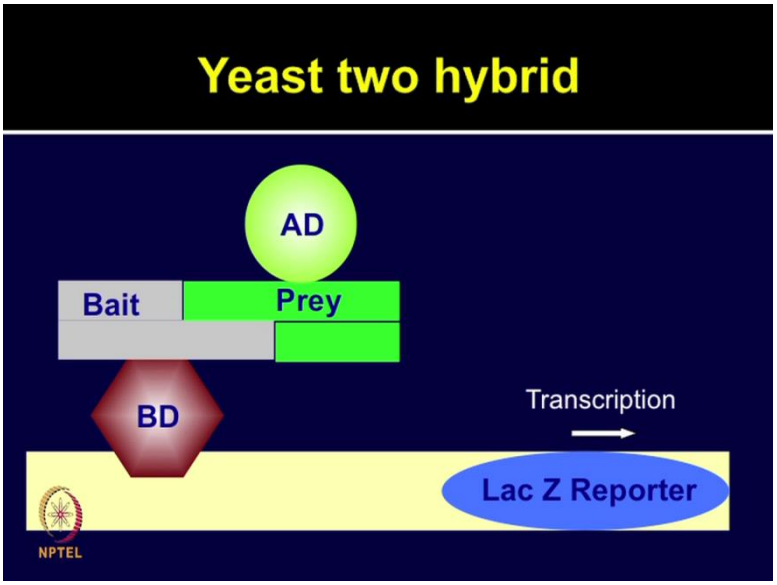

As you can see in this slide, the transcriptional activation for the two hybrid system consists of two protein domains. One is DNA binding domain, which remains bound to the promoter region and is fused with a suitable bait protein whose interaction with another protein is required for a study. AD is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

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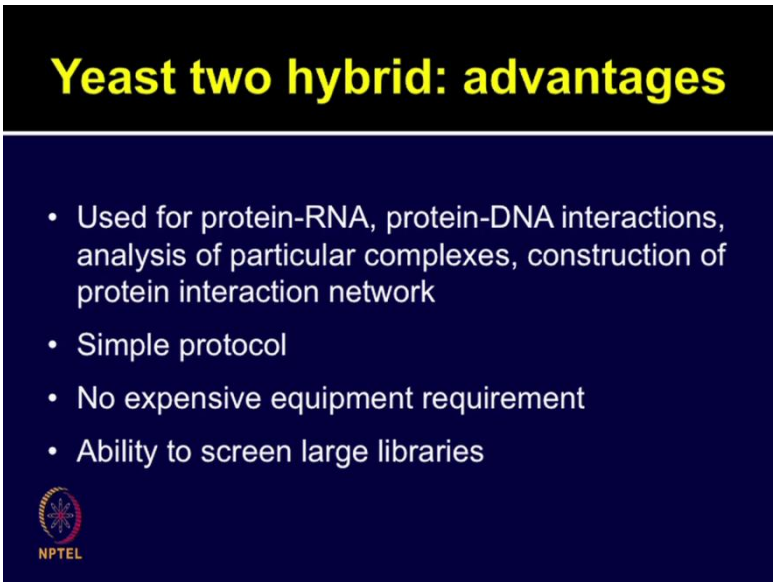

So, what are the different advantages of using yeast two-hybrid system? As I mentioned since its inception in 1989 when it was established as a method for studying the protein

interactions, it has been used for various types of applications to study interaction between protein-RNA, protein-DNA, protein-protein and to analyse various complexes for studying large protein interaction networks. The protocol for yeast two-hybrid is quite simple. Unlike other approaches, there is no requirement for heavy instrumentation here; this method also allows screening of large libraries. What are the different disadvantages of using yeast two-hybrid system?

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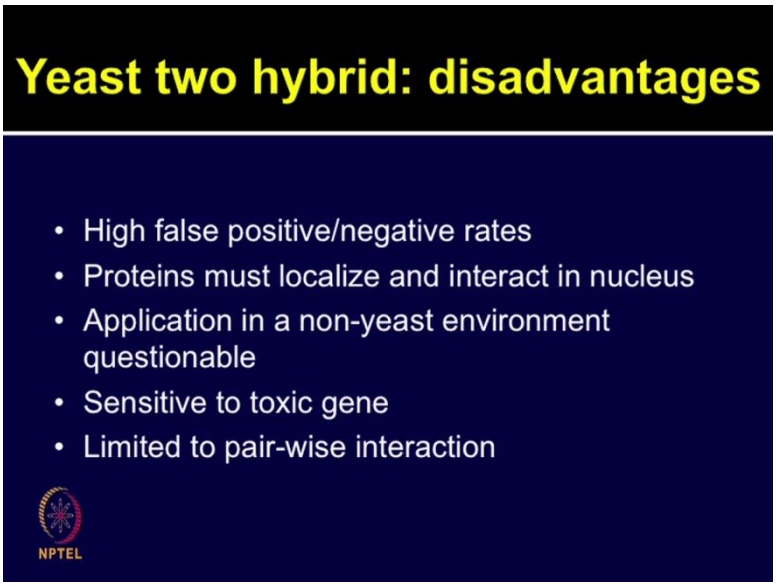

It provides very high false positive and negative rates. The high false positive rates have been one of the major limitations of this approach. The proteins must localize and interact in the nucleus. So, that is a limitation of this approach because it is restricted to the bindery interactions as against a technique like immunoprecipitation where multi-protein complexes can be studied, also application in a non-yeast environment is questionable because a system was developed and standardized in yeast. Protein folding as in mammalian systems is not guaranteed considering the background is that of yeast.

The system is also quite sensitive to the toxic genes. So, finally it is limited to pair-wise interactions if you have fair idea about the molecules which are potentially interacting then you can use pair-wise study by using yeast two-hybrid if you just want to generate a list of potential interactors, one can be quickly used yeast-two hybrid to screen various (())(24:47) libraries and once you establish the list of potential interactors, then you can use different methods for validation to re-establish how many of these proteins interacting well and how many of them are false positives.

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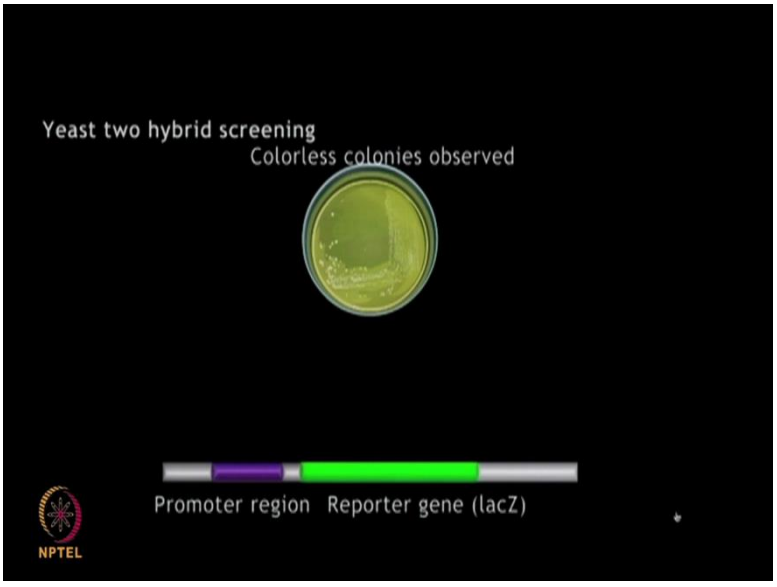
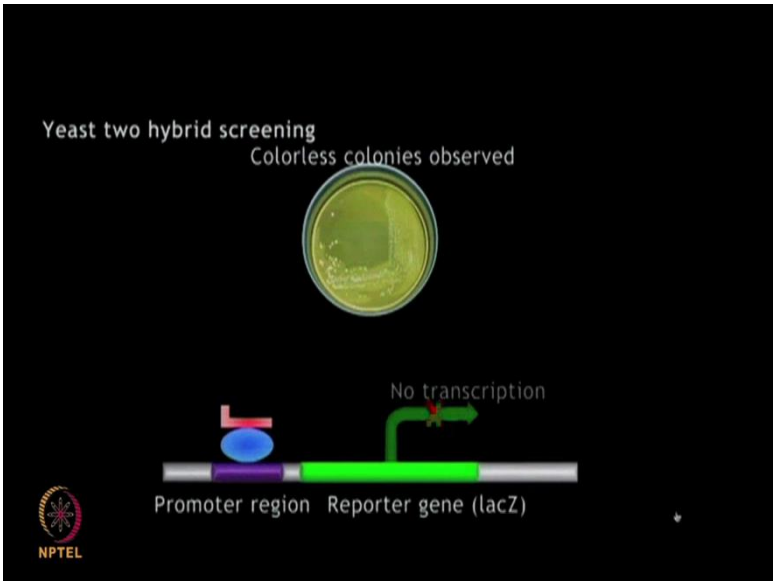
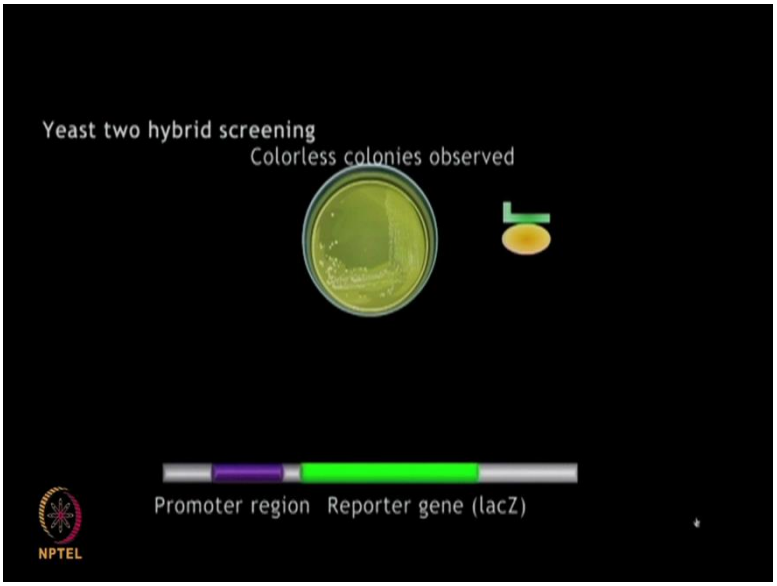
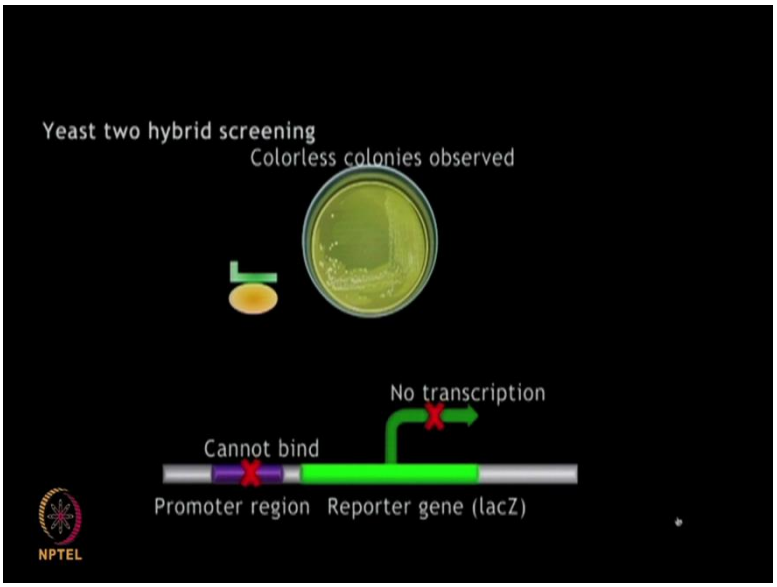
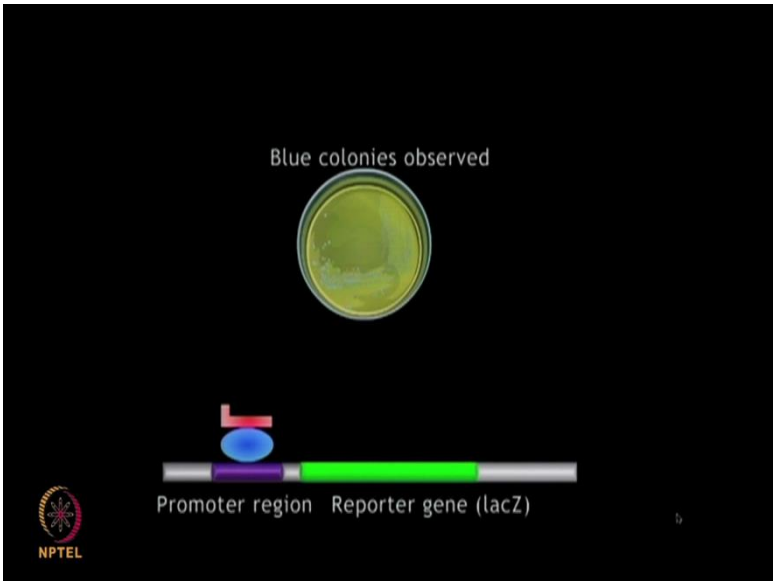
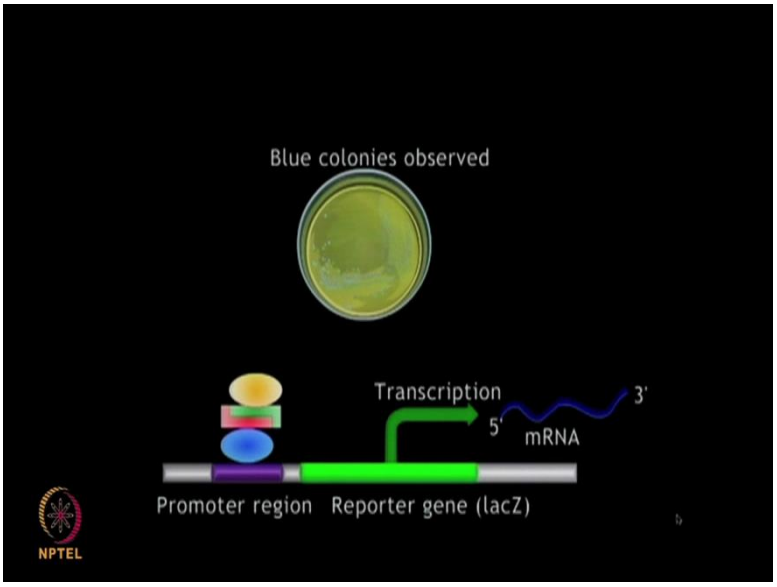


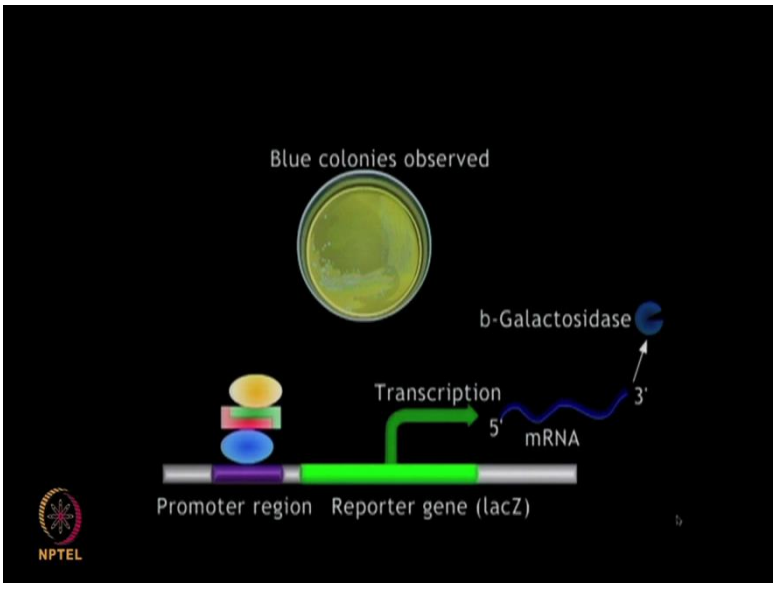

Yeast two hybrid screening
Colorless colonies observed
No transcription
Promoter region
Reporter gene (lacZ)
NPTEL


Yeast two hybrid screening
Colorless colonies observed
Promoter region
Reporter gene (lacZ)
NPTEL


Yeast two hybrid screening
Colorless colonies observed
No transcription
Cannot bind
Promoter region
Reporter gene (lacZ)
NPTEL


Blue colonies observed
Promoter region
Reporter gene (lacZ)
NPTEL


Blue colonies observed
Transcription
5'
3'
mRNA
Promoter region
Reporter gene (lacZ)
NPTEL


Blue colonies observed
b-Galactosidase
Transcription
5'
3'
mRNA
Promoter region
Reporter gene (lacZ)
NPTEL

So, the yeast two-hybrid screening remains as a classical biological technique which has been used for screening and the discovery of protein-protein and protein other bio-molecular interactions. Let us now, go through an animation to understand the working of yeast two-hybrid system. We will now talk about yeast two-hybrid. In yeast two-hybrid screening the binding of transcriptional activator protein composed of binding domain and activation domain to the promoter region. This is 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 with the prey protein. Binding of either one of the fused proteins to the promoter, it is not sufficient to bring about transcription of the gene. The two hybrid screening protocol uses this interaction as the basis for screening protein-protein interactions. When the bait protein bound with the binding domain interacts with the prey protein fuse with the activation domain, there will be expression of the reporter gene which can easily be detected. The Lac Z is commonly used reporter gene whose protein product beta galactosidase leaves the substrate X gal, resulting in the blue color colonies.

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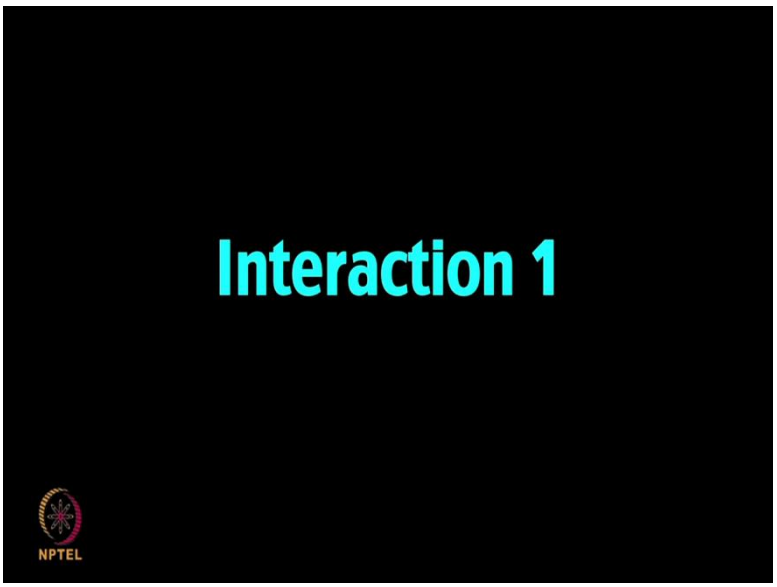
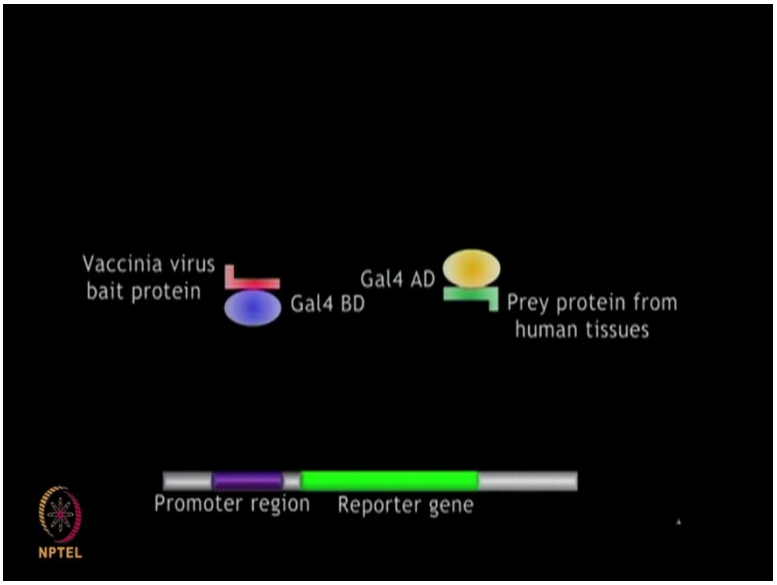
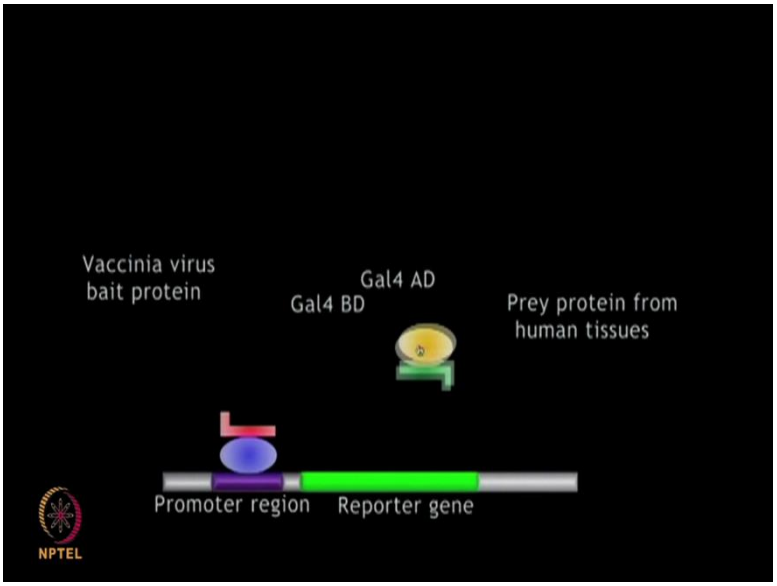
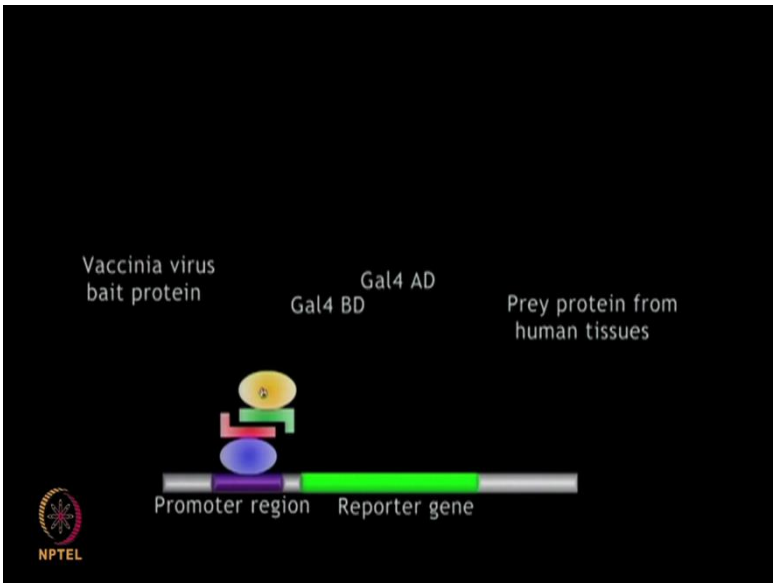
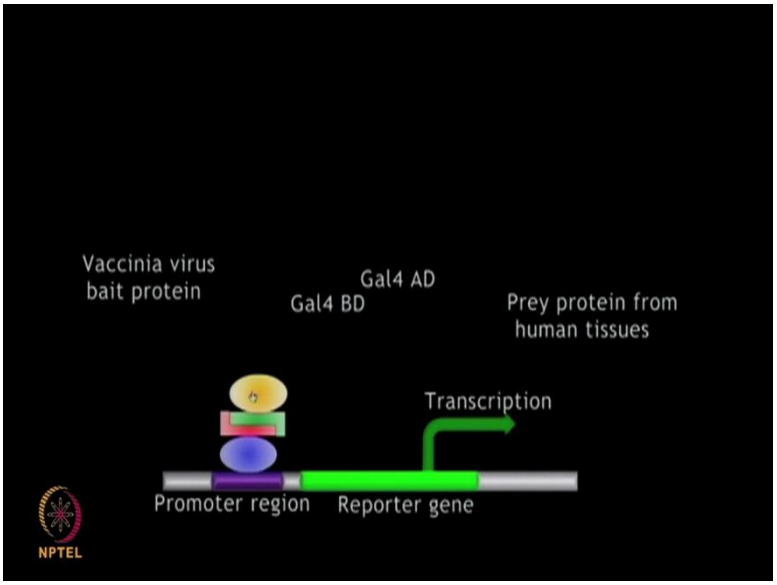



Vaccinia virus
bait protein
Gal4 BD
Gal4 AD
Prey protein from
human tissues
Promoter region
Reporter gene
NPTEL


Vaccinia virus
bait protein
Gal4 AD
Gal4 BD
Prey protein from
human tissues
Promoter region
Reporter gene
NPTEL


Vaccinia virus
bait protein
Gal4 AD
Gal4 BD
Prey protein from
human tissues
Promoter region
Reporter gene
NPTEL



Let me show you the yeast two hybrid concept applied in one of the studies by showing this interaction. The yeast two-hybrid screening approach was used by (())(27:37) 2009 to study the protein-protein interactions between the vaccinia virus and human proteins. The virus bait proteins were fused with the C (())(27:51) terminus of Gal4 binding domain while the prey human proteins were fused with the Gal4 activation domain of the transcriptional activator. These interactions were further validated by other techniques which provided successful validation rate of 63 percent.

So, let me show you by this interaction how this yeast two-hybrid screening can be used. So, let us drag and drop the component so that the transcription will be carried out, as you can see the first Gal4 binding domain adhere to the promoter region and now, as shown as the activation domain bind there the transcription event happens.

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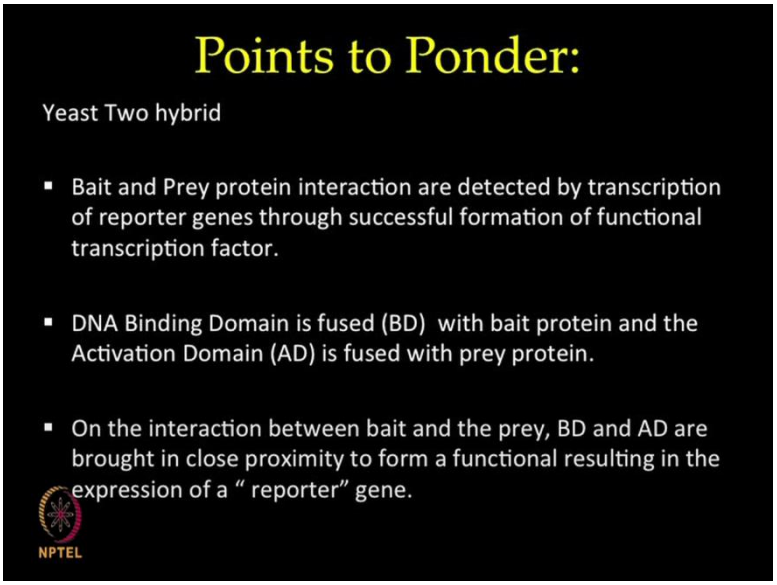

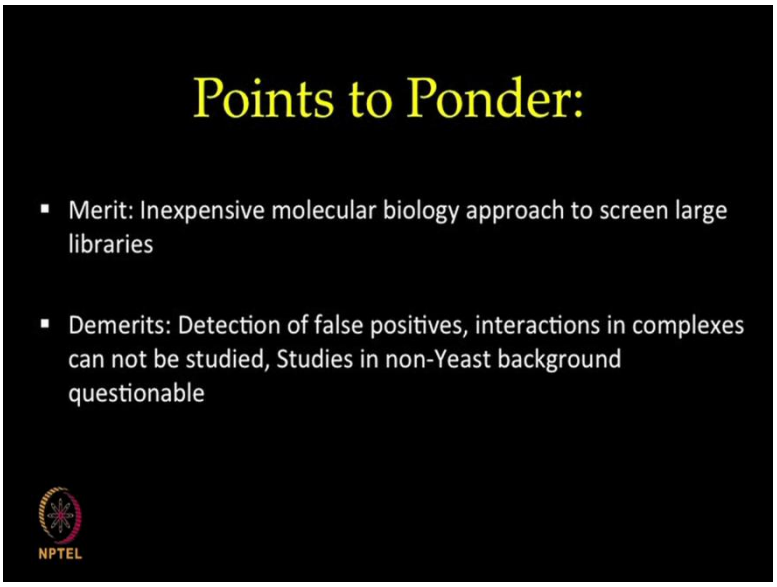

So, this brings us to the end of first lecture into this module, today we discussed the significance of a studying interactions in the field known as Interactomics. We discussed different types of traditional methods, which have been used for a studying these protein interactions namely immunoprecipitation assay and yeast two-hybrid. In the next lecture we will discuss, a variety of platforms currently being used for a studying protein interactions in high throughput manners. Thank you.

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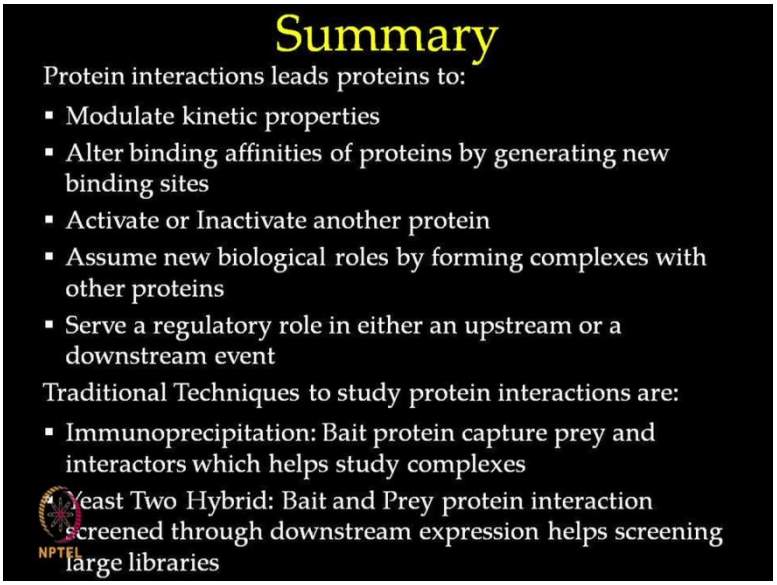

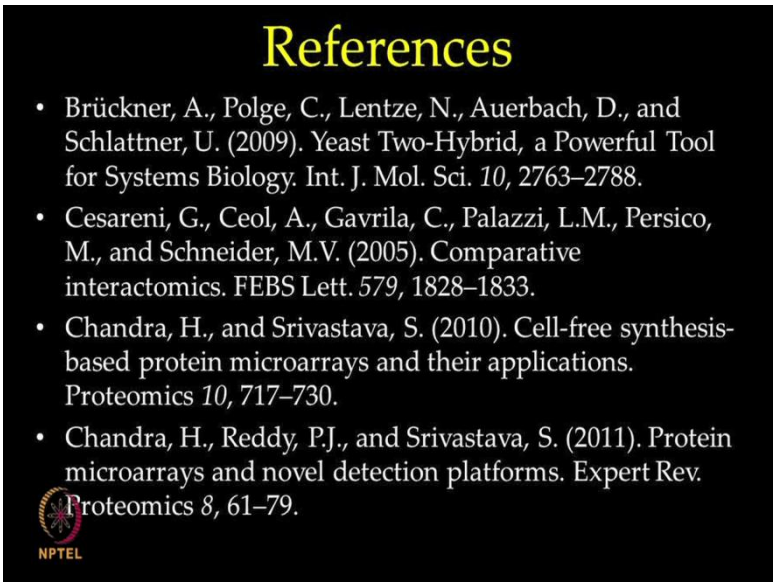

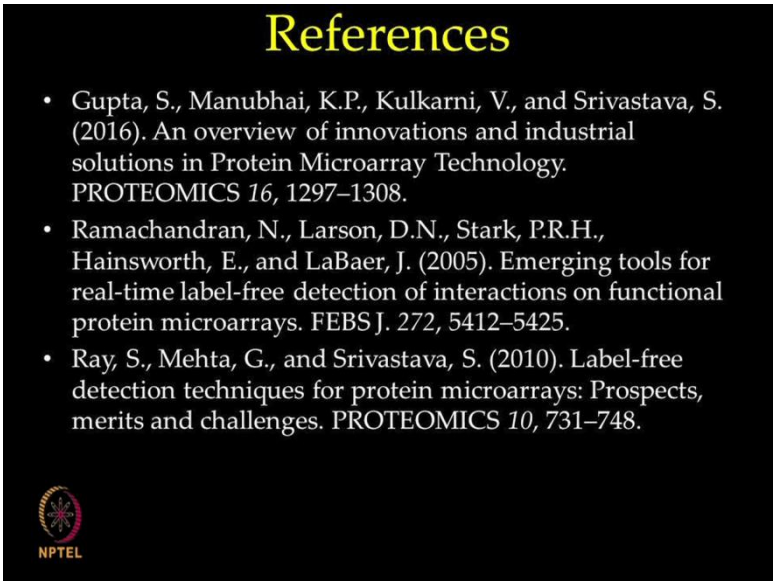

References

- Reddy, P.J., Sadhu, S., Ray, S., and Srivastava, S. (2012). Cancer Biomarker Detection by Surface Plasmon Resonance Biosensors. Clin. Lab. Med. *32*, 47–72.
- CSHL Press Classical protocol (2005). Identification of associated proteins by coimmunoprecipitation. Nat. Methods *2*, 475–476.