Proteomics: Principles and Techniques
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Lecture No. # 26
Interactomics: Yeast Two-Hybrid
Immunoprecipitation Protein Microarrays

Welcome to the proteomics course. Today, we will talk about interactomics, which is studying interactions of proteins and other biomolecules. We will discuss various type of techniques such as yeast two-hybrid, immunoprecipitation and protein microarrays. So, as you know the proteins are dynamic molecules which interacts with a wide variety of biomolecules such as lipids, nucleic acids as well as various small drug inhibitors metabolites, and many other biomolecules to provide different type of significant information for physiological actions.

Proteins also interacts with one another to form larger complexes, these complexes regulate various fundamental processes such as signal transduction, and gene regulation. A detailed understanding of protein interactions provides an opportunity to understand the protein functional behaviour.

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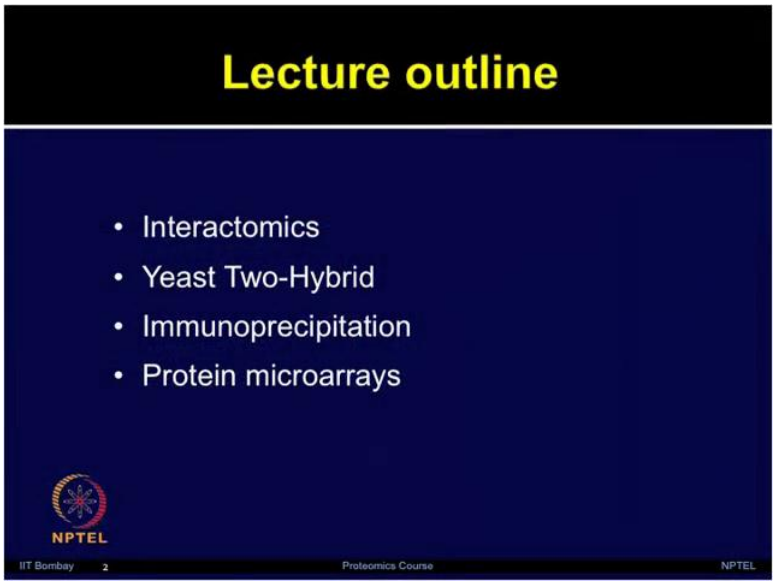

So, in today's lecture we will discuss about what is interactomics, different ways of studying interactions such as yeast two-hybrid, immunoprecipitation and then we will move on to the high-throughput approaches such as protein microarrays. So, let us first

start with the interactomics. So, interactomics comprises the study of interactions and their consequences between various proteins as well as other cellular components. The network of all such interactions known as interactome which aims to provide a better understanding of genome and proteome functions.

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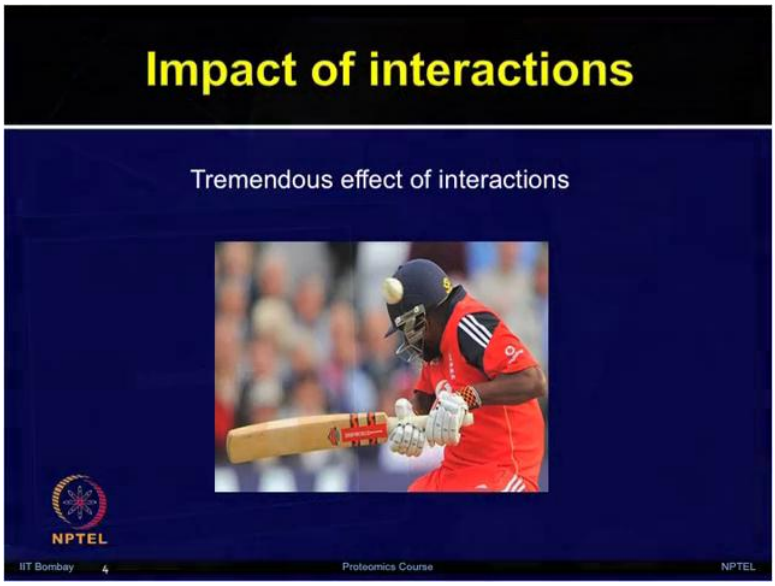

So, interactions can have different type of impacts it could be positive as well as negative. The one such as shown in the slide shows the negative impact. So, biology has evolved several mechanisms that regulate interactions including a variety of post translational modifications and the presence or absence of an activator or inhibitor molecule. The interactions can also be modulated by altering the expression levels of proteins. The aberrant interactions such as one shown in the slide can lead to the dis regulation of cellular functions and ultimately diseases such as cancer may happen. So, proteins interact with variety of biomolecules. The interaction of proteins with other proteins as well as other biomolecules help to execute their functions.

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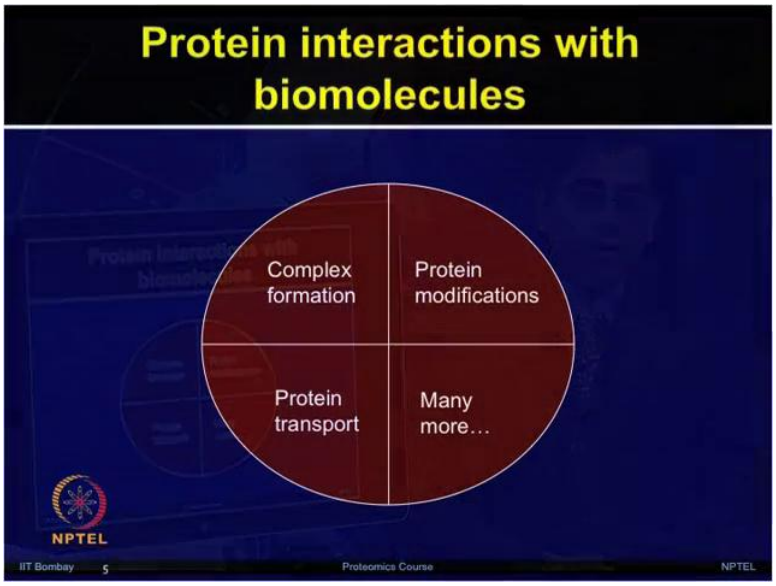

And these proteins after interactions form complexes, they modify the proteins. They help in protein transportation as well as there are many more properties in which they are involved. So, interactomics aims to characterize the function of un characterized proteins.

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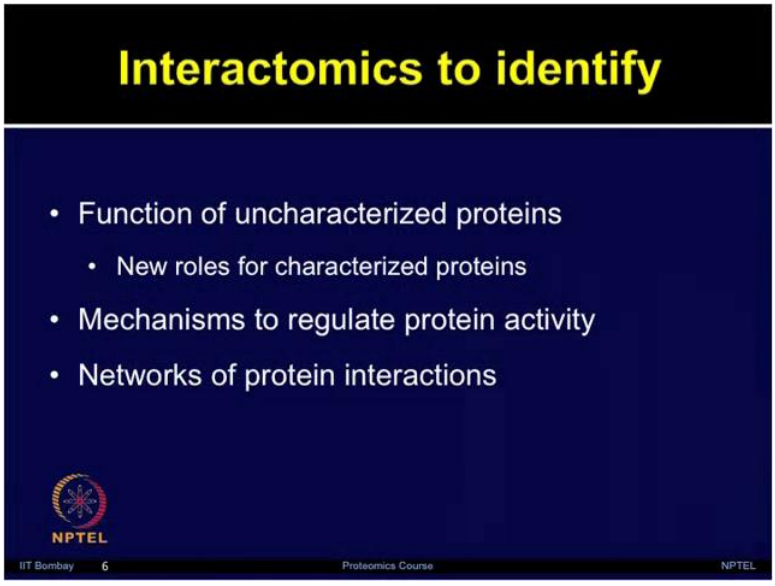

So, that one can assign the new role of various proteins. The mechanism to regulate the protein activity can also be understood by studying the interactions. And network of protein interactions provide very valuable information's for the biological processes such

as signal transductions and different type of pathways in which these proteins could be involved.

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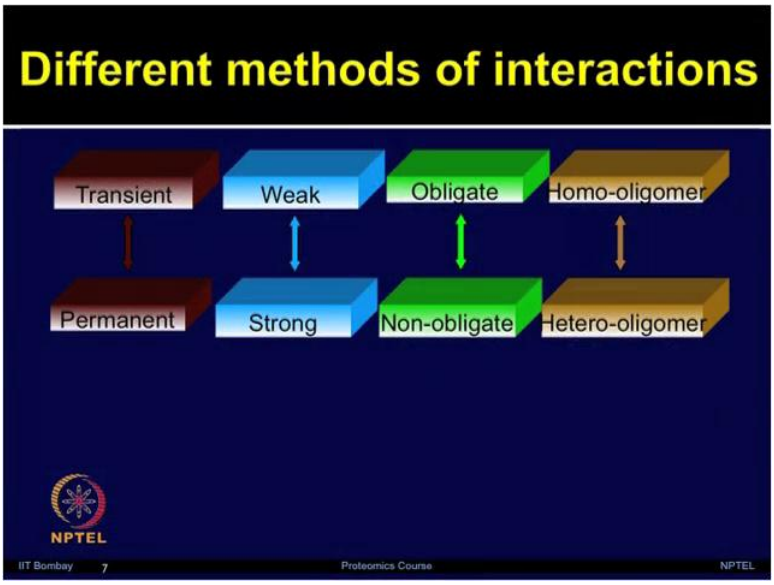

So, there are different methods of studying interactions because interactions occur in various ways. There are few interactions which are very transient that will be for very very short duration whereas, other interactions could be permanent which may alter the activity. Few interactions are very weak whereas, other interactions could be very strong. Interactions could also be obligate as well as non-obligate, they can form homo-oligomers as well as different type of oligomeric units can combine and form the hetero-oligomers.

So, this gives you a glimpse of the complexities involved in the interactions because studying the interactions require various type of technologies just because of the diversity of interactions as well as different type of complexities involved.

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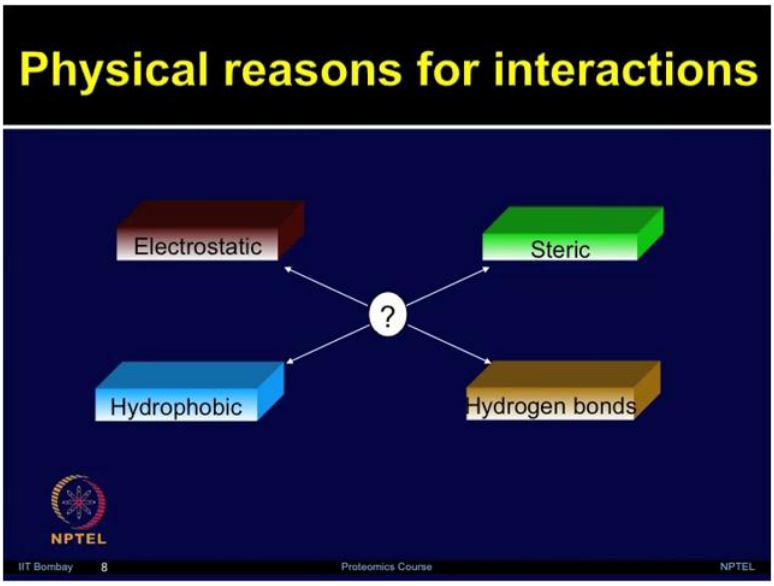

So, what are the physical reasons for these interactions? It could be electrostatic hydrophobic hysteric hydrogen bonds etc. Earlier in our modules when we talked about proteins and its different properties we discussed some of these physical reasons.

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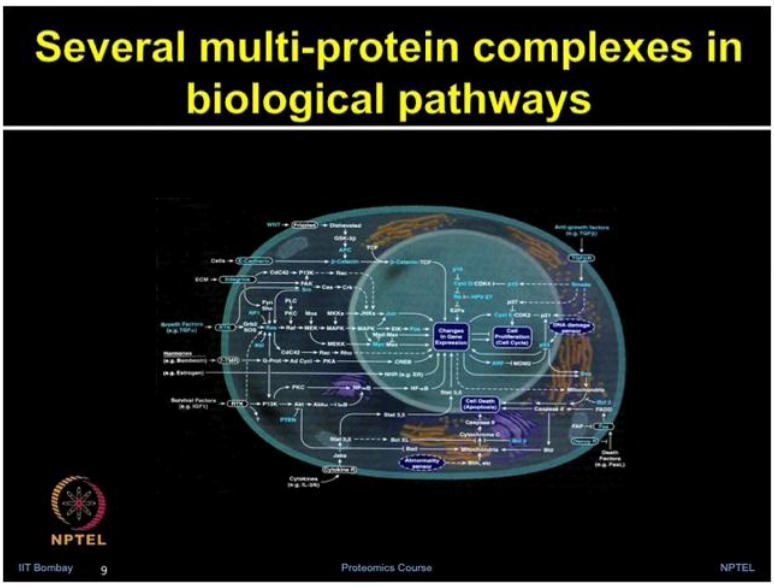

So, these proteins could be involved in the various biological pathways and form multiprotein complexes such as one shown in the figure here. And by studying the interactions one can slowly start getting the information for the complex biological pathways in which these proteins could be involved. So, these protein interaction studies

ultimately helps to develop the protein interaction networks and the varying diagrams for different type of quantitative information.

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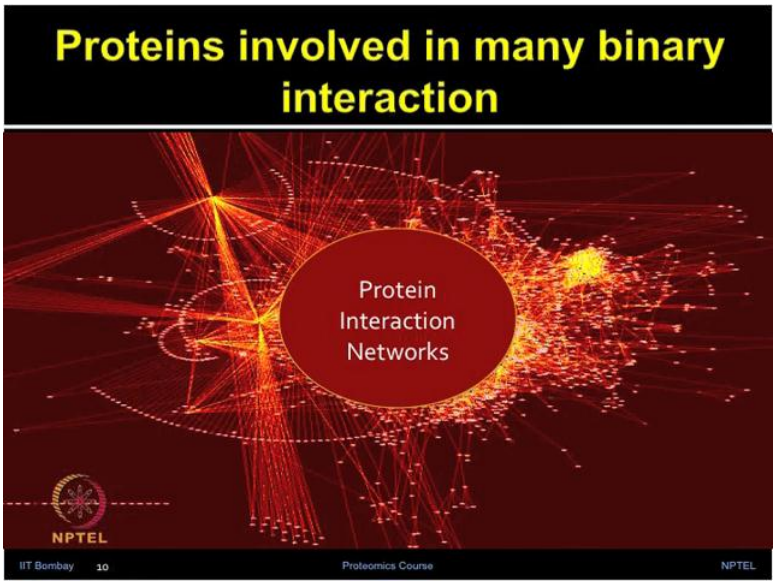

Since proteins are involved in the many binary interactions. So, an understanding of the underlying biochemistry of proteins and biomolecular interactions is a critical element for the development of novel therapy tricks and diagnostics.

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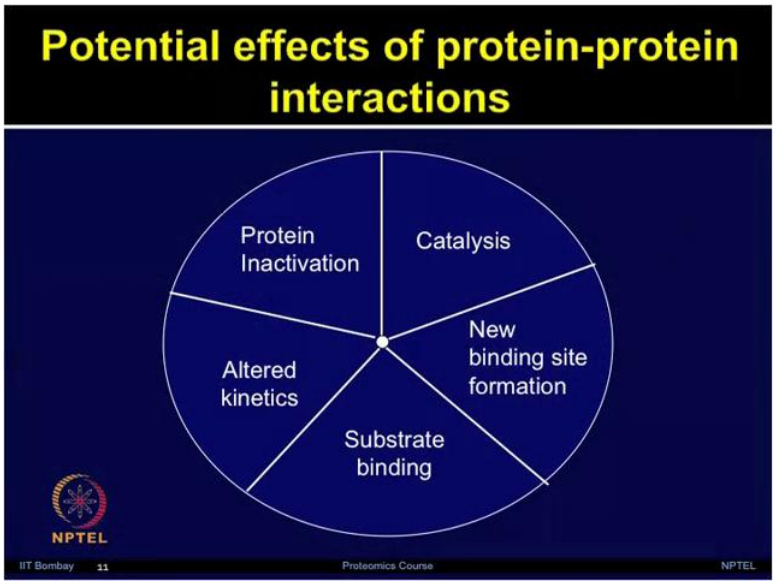

The protein-protein interactions have various potential effects, few effects are described in a slide such as catalysis, protein inactivation, altered kinetics, alteration of substrate

specificity for the substrates binding and new binding site information. So, these are just few examples of potential effects of protein-protein interactions, but they actually altered multitude of effects which ultimately lead to the either positive effects or dis regulation which may result into various diseases.

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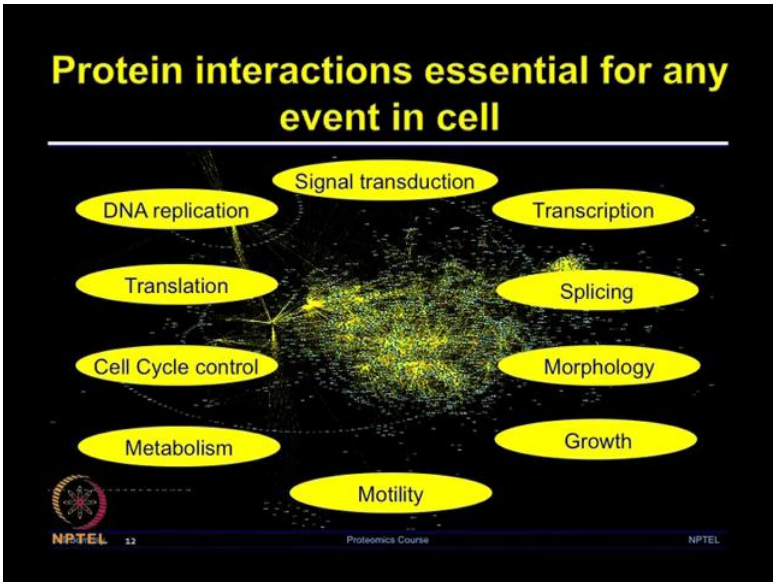

So, protein interactions are very essential for any cellular mechanism whether we talk about signal transduction, DNA replication, translation, cell cycle control, how the metabolic process are governed, the mortality, how the growth and morphology are altered, splicing, transcription, etc. I am sure you will be able to add many more examples here how different type of protein interactions are essential for the activity of cell.

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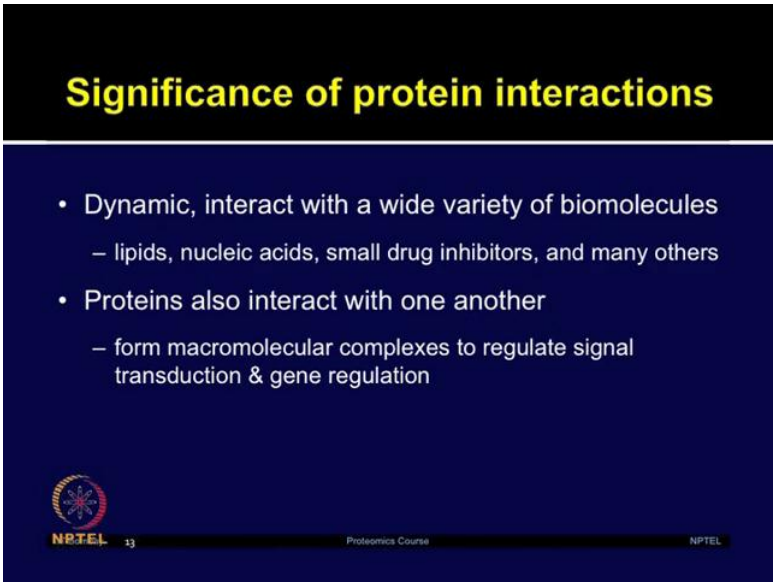

So, what is the significance of studying the protein interactions? These are very dynamic, which interact with the wide variety of biomolecules, as we discussed previously such as the biomolecules lipid nucleic acids small drug inhibitors and many other type of biomolecules. Proteins also interact with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

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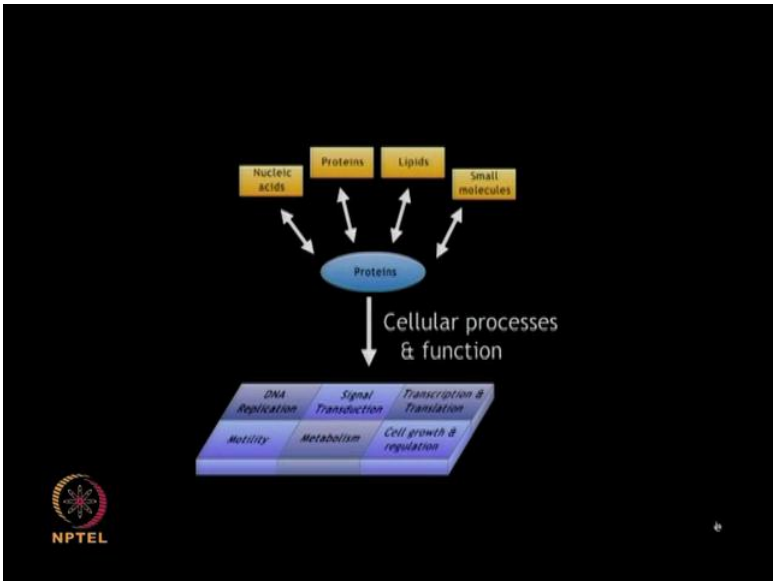

So, let us discuss the interactomics and its significance in this animation. Interaction studies of proteins with various biomolecules help to decipher and understand the function of various proteins in the complex network of cellular pathways. Proteins interact with other biomolecules such as nucleic acids, lipids, hormones, etc to execute a multitude of functions 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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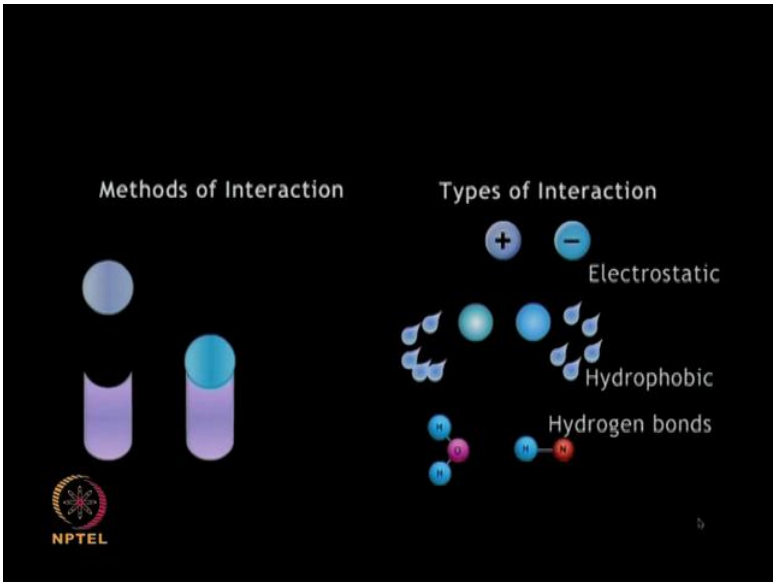

Protein interactions with other biomolecules can be of several different types. They may be weak or strong, obligate or non-obligate, transient or permanent. The physical basis for these interactions include electrostatic, hydrophobic, hysteric interactions, hydrogen bonds etc.

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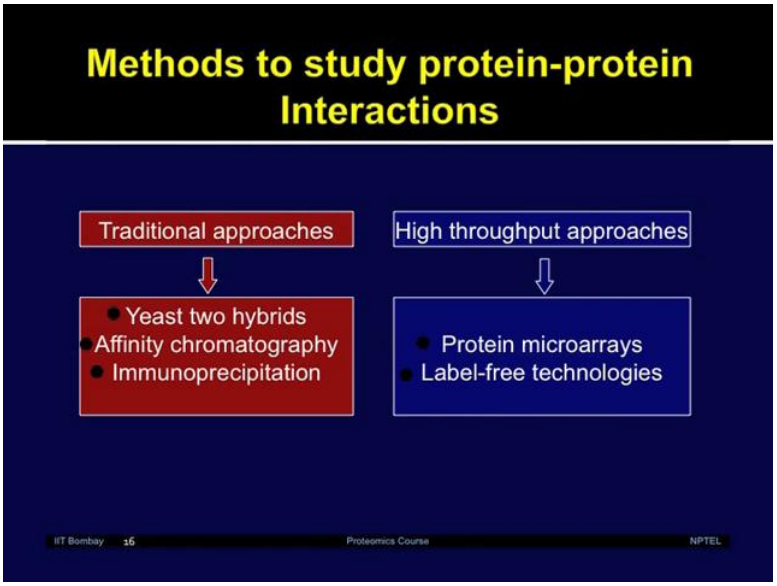

So, now let us talk about what are different methods to study the protein-protein interactions. Understanding the protein-protein interaction provides important clues to the function of proteins. The identification of interactions with known proteins may suggest the functional role played by a novel protein. So, there are two different ways of studying these protein-protein interactions, one is a traditional approach and then there are few high-throughput approaches.

The traditional approach has heavily used is two-hybrid, affinity chromatography and immunoprecipitation. The more recent approaches include the high-throughput technologies such as protein microarrays and different type of label-free technologies. The label-free technologies will be discussed in more detail in the subsequent lectures. So, as different type of protein microarray platforms will also be elaborated in the following lecture, but today we will focus more on the three major approaches yeast two-hybrids, immunoprecipitations and conventional protein microarrays as I mentioned there are many type of protein microarray platforms which will be discussed in the following lectures.

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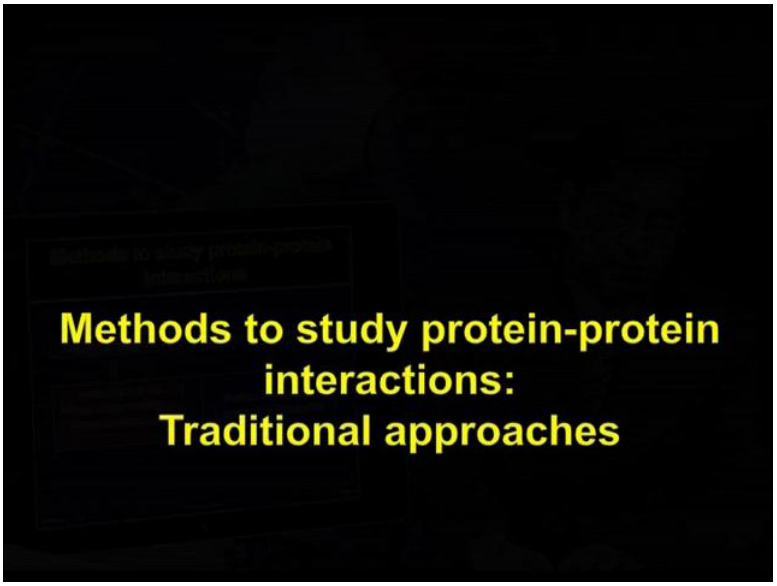

So, the traditional ways of studying the protein-protein interactions involve yeast two-hybrids and immunoprecipitations. These are two widely recognised technologies which have been used to map the protein-protein interactions at large scale. These are yeast two-hybrid also known as YTH and immunoprecipitation with mass spectrometry identification, also known as IPMS. So, both of these approaches is two-hybrid and immunoprecipitation have been used to identify 1000's of novel interactions in different organisms including human c elegance drosophila etcetera.

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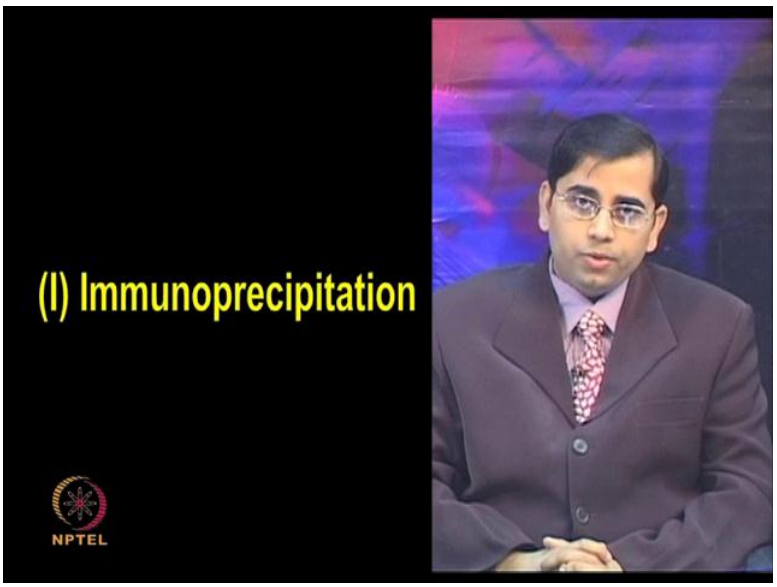

So, let us talk about immunoprecipitation technique. Immunoprecipitation or tandem affinity purification also known as tap is a technique which is used to purify protein complexes and study their protein-protein interactions, depending on the protein that needs to be purified different type of taps can be attached to the bait protein.

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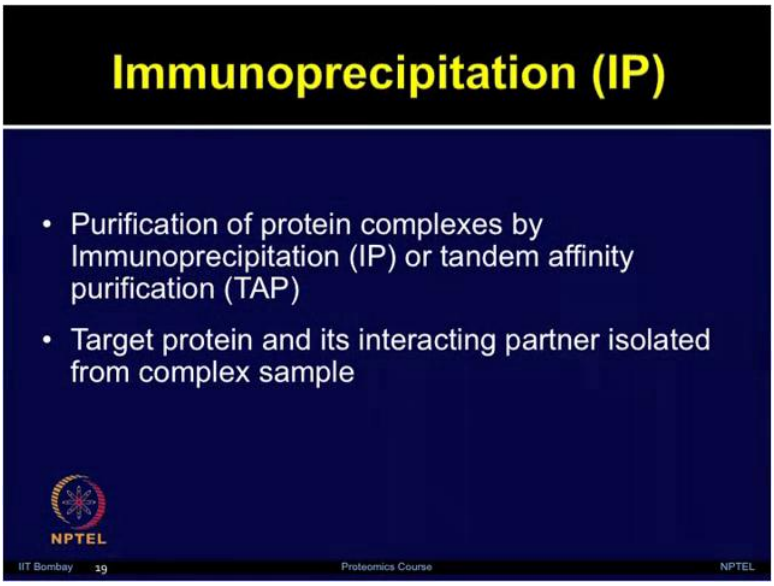

So, the immunoprecipitation method the purification of protein complexes by immunoprecipitation or tandem affinity purification methods is performed. The target proteins and its interacting partners are isolated from a given complex sample and then by using different type of taps, these proteins can be isolated from the mixture and then further processed.

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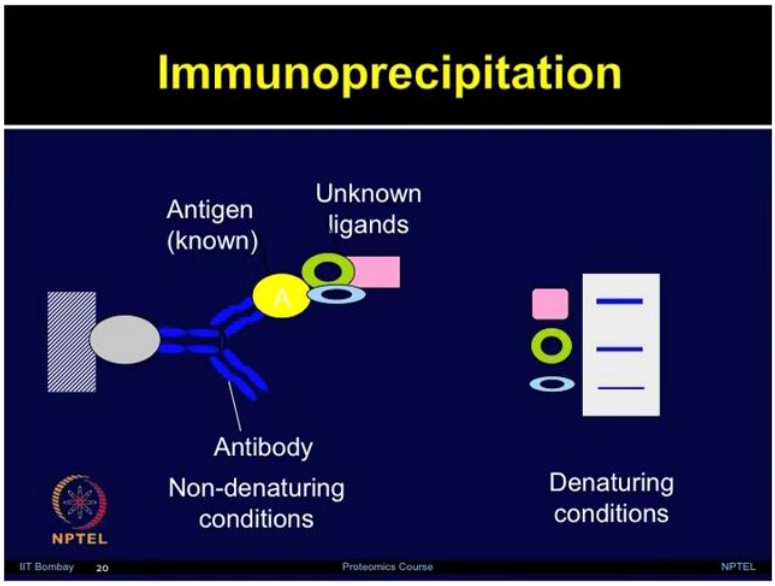

Which I will describe in the next slide. In the immunoprecipitation method the antibody is specific to the bait is attached to the whole cell extract which forms the complex. And remember this step is performed in the native or the non-denaturing conditions the protein-protein complex is immobilised on protein a or protein g sepharose bait protein complexes eluted and then further analyzed on the SDS-page gel.

As you can see on the slide it shows that antibody is used which is binding with the antigen as well as the unknown ligands and then this complex mixture is separated on the SDS-page gel as shown on the right panel. Now, this condition will be denaturing condition whereas, the first step was the non-denaturing condition.

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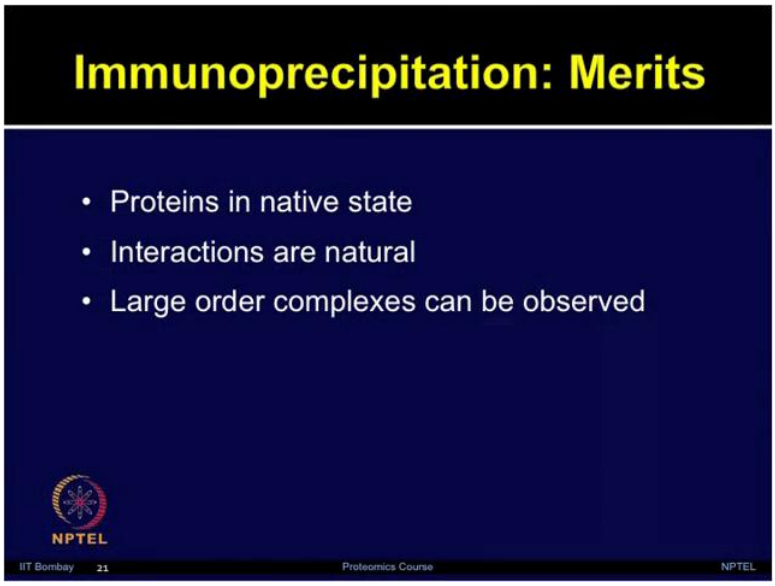

What are different advantages or merits of using immunoprecipitation method? In this method proteins are isolated in the native state. Now, why this native state is important. So, the native state will allow native complexes to be formed it also allow to form the post translation modification. So, that is why it is essential to perform these steps in the native or non-denaturing conditions. Interactions by using immunoprecipitation method are natural and by using this method large order complexes can be observed because the native state will promote the native complex per formation.

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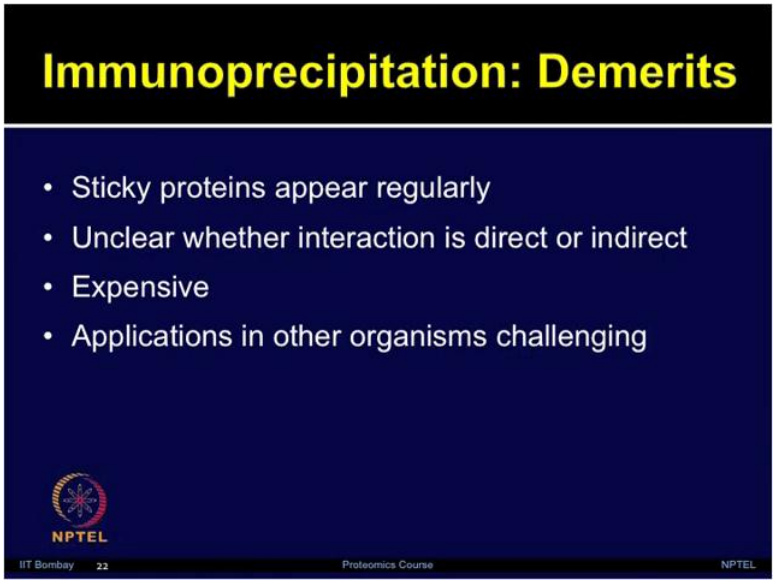

What are different demerits of using IP method? It has been reported the frequent occurrence occurrence of sticky proteins. Many proteins which may not be specific for that interaction could also be eluted just because they are sticky on the other protein surface. So, these proteins could also provide us the non-specific results, it means few proteins which are specific specifically interacting with the target of interest will be isolated as well as there are some sticky proteins which are adhere to the interacting proteins will also be isolated. It is unclear whether this interaction is direct or indirect because the proteins which are directly interacting those will be bind as well as those which are indirectly also binding to the interacting proteins those will also be eluted.

Now, this method is quite expensive because it needs for both good equipment set up as well as different type of analytical and computational analysis applications in other organisms other than yeast have been challenging, but now people have applied this method for different type of organisms. So, let us describe the immunoprecipitation method how this mechanism occur in more detail by showing you this animation.

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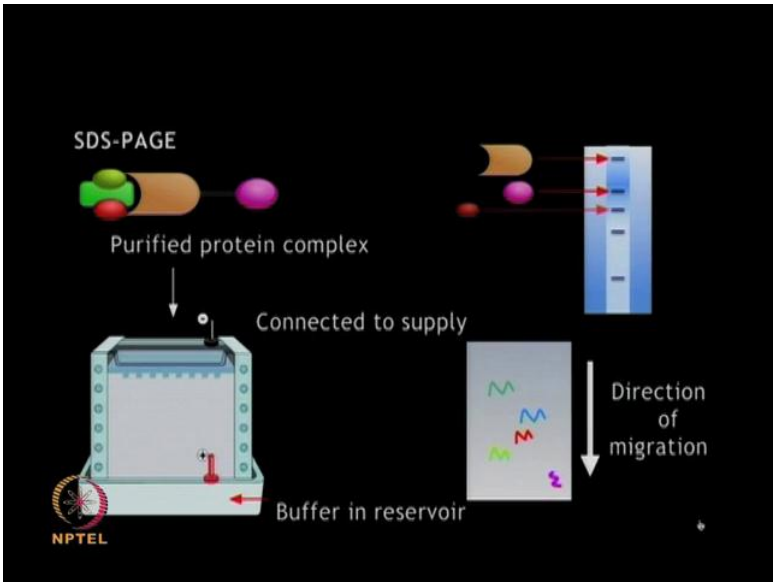

In immunoprecipitation the protein of interest is fused with a tap tag which contains a calmodulin binding peptide, A TEV cleavage site and protein a depending upon the proteins to be studied this tag can be modified. The tag is then bound to the column through affinity interactions between the protein A and I g G. The protein mixture whose interactions with the bait protein are to be studied is then added, some other proteins

form a complex with the bait through this specific binding interactions, the remaining unbound proteins are washed away.

This is followed by the cleavage at the TEV site by using enzyme TEV protease to release 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 by the CBP domain to a calmodulin functionalised column in the presence of calcium ions. The column is washed to remove any other unwanted contaminants after which a kinativ agent is added which complexes the calcium ions, once these are removed the CBP calmodulin interaction is weaken 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. So, we have discussed the traditional ways of studying the protein-protein interaction. First one was immunoprecipitation.

Now, let us move on to the next approach yeast two-hybrid. In yeast two-hybrid there are two different type of proteins 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 also known as BD of the transcriptional activator by inserting and expressing it along with the binding domain in suitable manner. The prey protein is a protein whose interaction with the bait needs to be determined and that is also known as prey protein. The prey protein is fused with activation domain known as AD of the transcriptional activator. So, successful interaction of the bait and the prey protein activates the promoter region which in turn activates the transcription of reporter genes.

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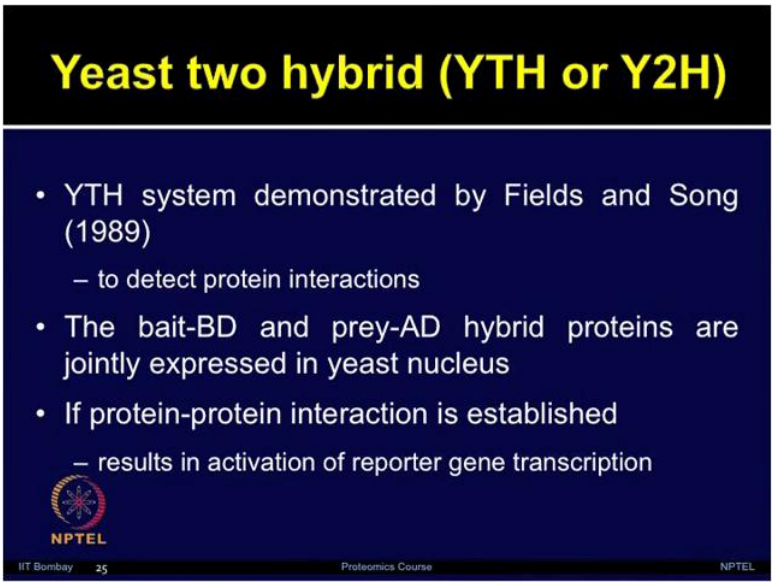

So, yeast two system is two-hybrid was first demonstrated by scientist Fields and Song in 1989 for studying the protein interactions. Since then this approach have been widely used in different organism for various type of biological questions. In general the yeast two-hybrid approach uses the bait binding domain and prey activation domain, these hybrid proteins are jointly expressed in the yeast nucleus. If the protein interaction established between the bait and the prey which are coupled with the binding domain and the activation domain then the transcription will occur.

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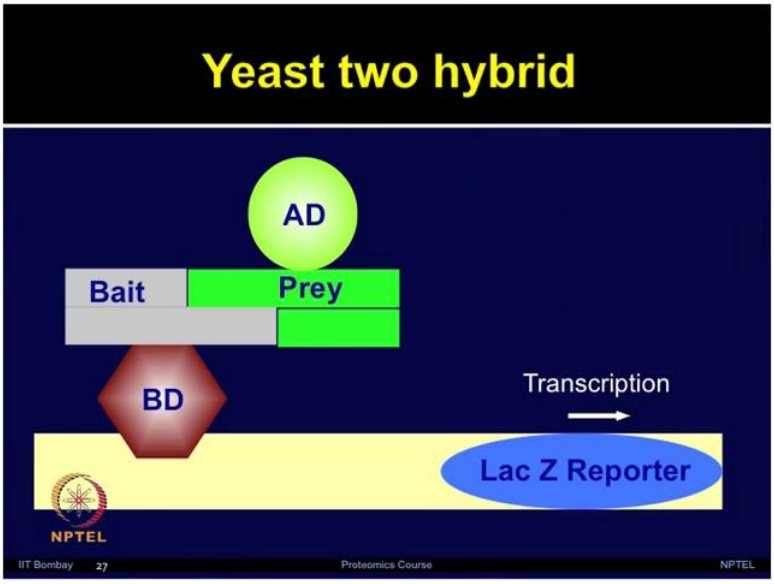

So, as you can see in the slide the transcriptional activation for the two-hybrid system consist of two protein domains one is DNA binding domain which remains bound to the promoter region of the DNA and is fused with a suitable bait protein whose interaction with another protein is required for study. The activation domain this is other protein domain of transcriptional activator which is fused with the prey protein.

This activation domain when bound to DNA binding domain forms the functional transcriptional activator and brings the expression of reporter genes. So, as shown in the slide the bait protein binds with the DNA binding domain BD and the perturb protein or the prey with transcribe activator domain or AD. Once the BD domain binds with the DNA while the activator domain AD activates the transcription when both of these are associated. So, as you can see in the slide the bait with the BD domain and prey with the AD domain when they bind together the transcription event occurs.

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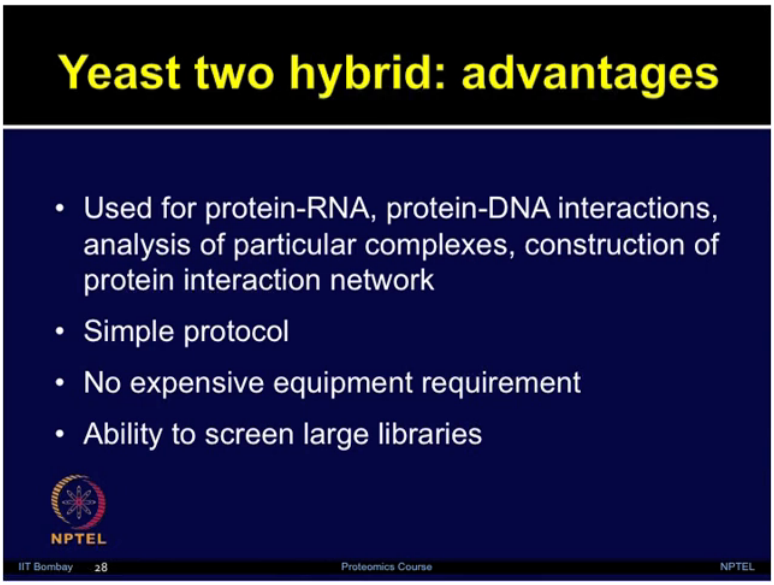

What are different advantages of using yeast two-hybrid system? As I mentioned since nineteen eighty nine when it was shown first time for studying the protein interactions it has been used for various type of applications including protein RNA, protein DNA analysis of particular complexes as well as studying the large protein interaction networks the protocol for these two-hybrid is quite simple unlike the other approaches there is no requirement for heavy instrumentation here. This method also allows to screen large libraries.

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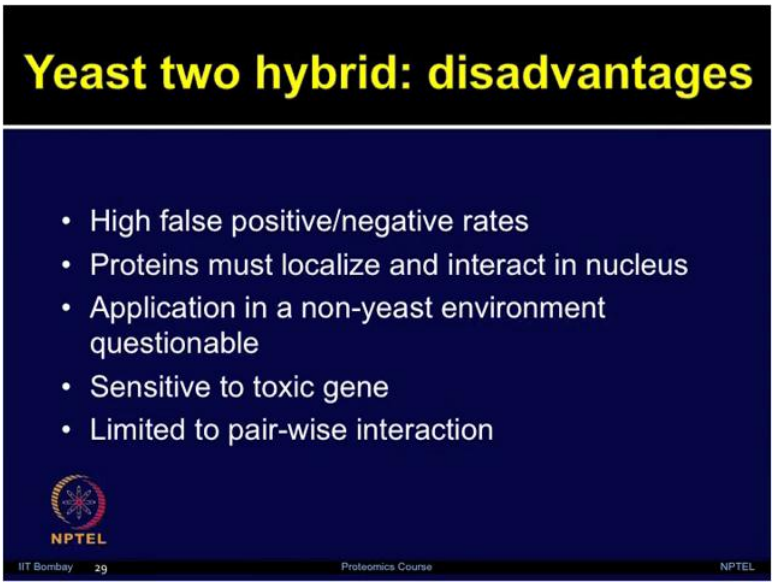

What are different disadvantages of using yeast two-hybrid system? It provides very high false positive and negative rates. So, high false positive rate that has been rather major limitation of this approach. The proteins must localize and interact in the nucleus. So, that is the limitation of the approach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

So, if you have good idea or 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 want to just generate a list of potential interactions which may or may not be true then those could be quickly screened by using different libraries by using yeast two-hybrid method. Now once you have established a list of various potential interactions then you can use different type of validation approaches for re-establishing how many of those proteins are interacting well and how many of those are false positives. So, yeast two-hybrid screening remained as the novel molecular biology technique which has been used for screening and discovery of protein-protein and protein other biomolecular interactions.

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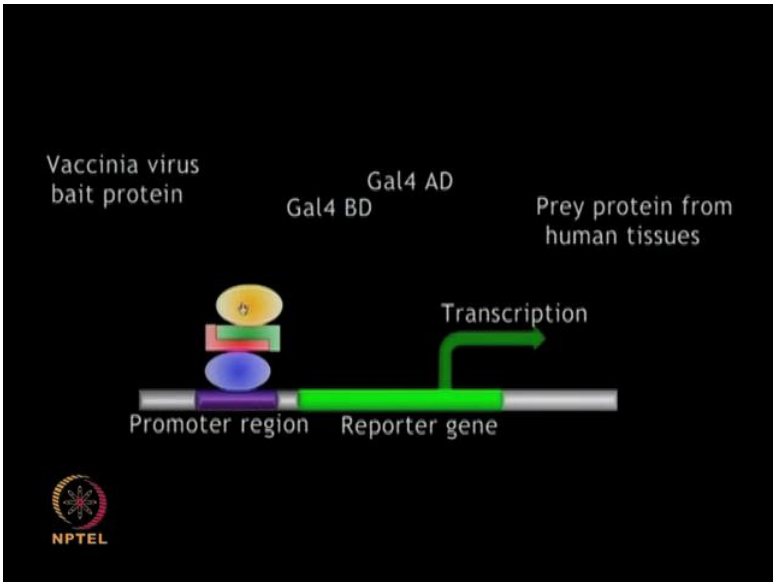

So, let me show you how yeast two-hybrid works in following animation. We will now talk about yeast two-hybrid. In yeast two-hybrid screening the binding of transcriptional activator protein composed of binding domain and activation domain to the promoter region. This is essential for expression of the corresponding reporter gene located downstream of the promoter. The binding domain is fused with the bed protein while the activation domain it is fused with the prey protein. Binding of either one of the fusion 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 bases for screening protein-protein interactions. When the bed 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 lacZ is commonly used reporter gene whose protein product beta galactosidase cleaves this substrate x-gal resulting in the blue color calonies.

Let me show you the yeast two-hybrid concept applied in one of the studies by showing this interaction. These yeast two-hybrid screening approach was used by Jang et al 2009 to study the protein-protein interactions between the vaccinia virus and human proteins. The virus biat proteins were fused with the c terminus of gal four binding domain while the prey human proteins were fused with the gal four 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 gal four binding domain adhere to the promoter region and now as soon as the activation domain binds there the transcription event happens.

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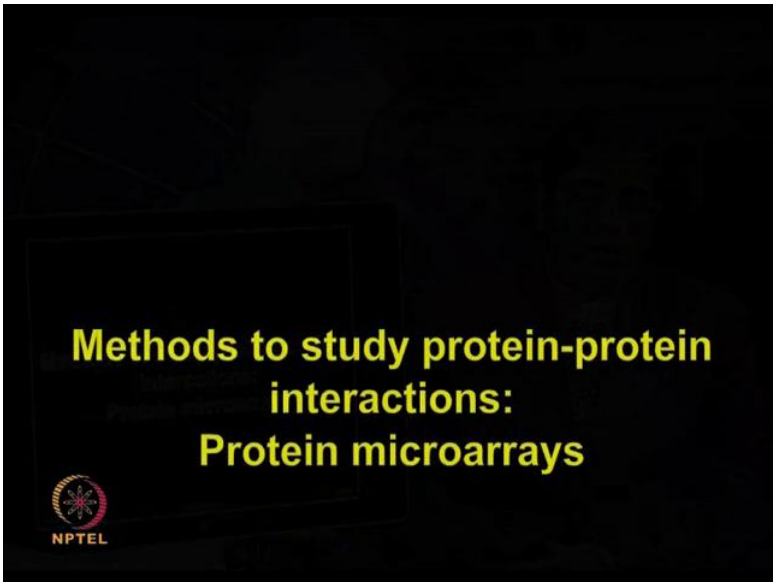

After discussing about different type of traditional method which have been used for studying protein-protein interactions now let us talk about some of the high-throughput approaches which have also been use for studying protein interactions. Though many interactions are discovered by using yeast two-hybrid and immunoprecipitation studies the reports for high pulse fast positive rate as well as the poor reproducibility of some of the earlier discussed method have been a limitation, aside from these technical issues both methods immunoprecipitation and yeast two-hybrids are primarily end point assays that occurs in a close system inside the cells. So, modulating the experimental conditions and different type of parameters becomes very challenging. Protein microarrays address some of these limitations such as providing an open system that enable the monitoring effect of various type of modifications.

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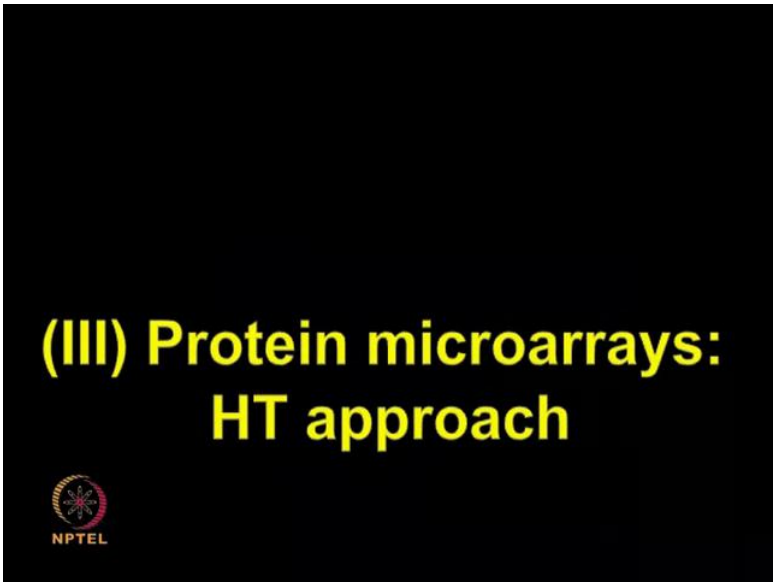

Let us first discuss some of the high-throughput approaches different type of protein microarray platforms. So, just we have been discussing that high-throughput genomic and proteomic projects they capture data at the scale of an entire organism and incorporate data into relational data bases from which insight into various biological system organization of physiological networks can be derived. Different type of hypothesis can be made based on these large data set. The genomic era has fostered the development of many new methods such as sequencing s m p as well as generation of DNA microarrays the success of DNA microarrays at the time when most of the genes were sequenced, it was almost year 2000 and till 2003 when we had availability of all the genes sequences at that time DNA microarray technology reached to its maximum potential because it was very easy to screen 1000s of genome, 1000s of gene and full genome of a any given organism such as human for which almost 30,000 genes were already available. So, by using DNA microarrays scientist have shown their potential of the high-throughput genomic technologies. The success of genomic technologies such as DNA microarrays have motivated the development of protein microarrays.

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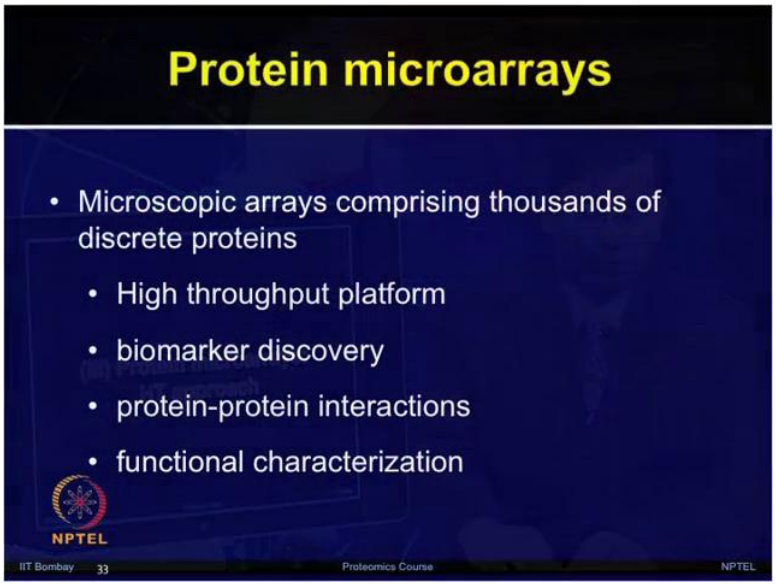

So, protein microarrays, these are microscopic arrays which comprises 1000s of discrete proteins now the concepts of protein microarrays has estered a great deal of excitement in the proteomics community because it can be applied for several applications such as high-throughput platform, biomolecular discovery, protein-protein interactions, functional characterization of different proteins, identification of different substrates, drug inhibitor studies, etc. So, both the protein microarray technology is fully realized it promises to enable the study of a broad variety of protein features at an unprecedented piece and scale.

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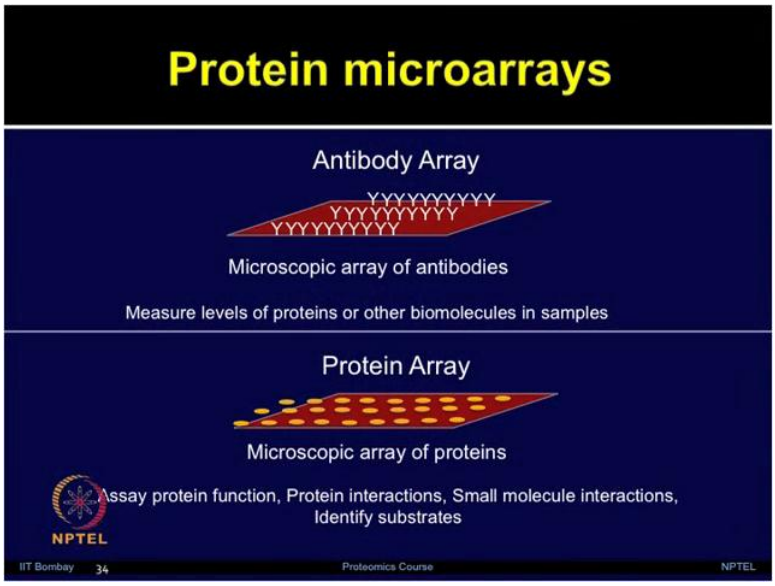

In the protein microarrays, fall into two general broad classes antibody arrays as well as test protein arrays. As shown in the slide the antibody array is an abundance based method which is intended to a form the users or the investigators how much of each protein is present in a sample or to identify the proteins whose abundant is differentially expressed in one sample as compared to the other sample.

So, antibody arrays they print 1000s of antibodies on the chip surface and then it has been used for measuring the level of proteins or other biomolecules in different samples to compare the control versus experimental conditions for protein expression. Now, in the test protein arrays the proteins are spotted as oppose to the antibodies which was the earlier case. Now, this is done by using procedures that preserve the activity of proteins on the array surface. The goal of these test array proteins is to perform functional studies. So, that different type of functions can be assigned, different type of biological questions related to the protein activity and its function can be studies on using these test protein arrays. So, these have been used for assaying the protein function, protein interactions, studying about a small molecule interactions as well as identification of substrates.

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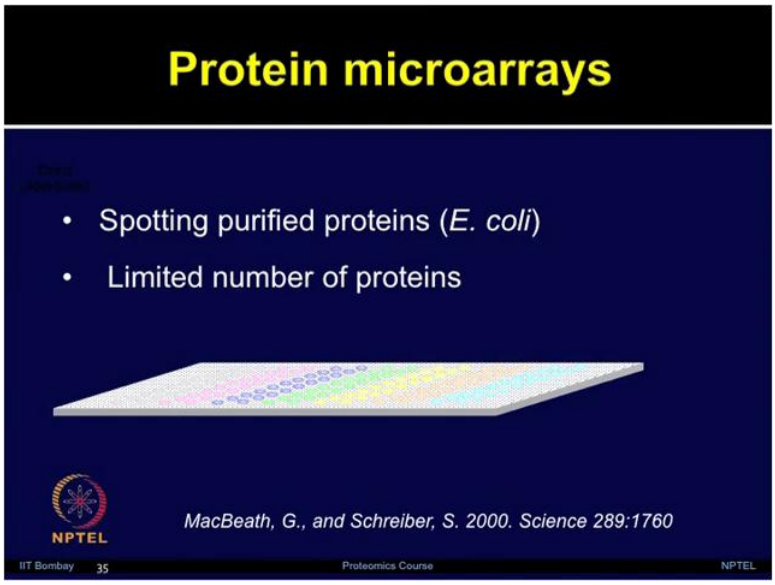

As compared to the DNA microarrays which have shown its promises and potential in various biological application there are relatively few studies published in the protein microarrays. The protein microarrays still remains very challenging just because the generation of the content the protein is very challenging.

So, Gavin Macbeth at Harvard he first demonstrated the feasibility of printing the protein on the chip surface in high density array similar to the DNA microarrays. So, in 2000 at the time when DNA microarrays have reached to its maximum potential. The proteomic community was still wondering whether similar type of platform can also be used for yeast ring the protease, can we study protease at a scale by using the protein microarrays. So, Gavin Macbeth first showed the concept that proteins can be printed in the high-throughput platform on the chip surface, but he used very limited number of proteins. So, the limitation of this work was it was performed on the relatively small set of proteins which reflects that difficulty of generating the proteins we do not have an analogue of PCR which could just amplify the proteins. So, protein content generation was one of the major challenge, but theoretically this concept was shown almost in year 2,000. The E-coli verified proteins were spotted on the chip surface, but very few proteins were printed.

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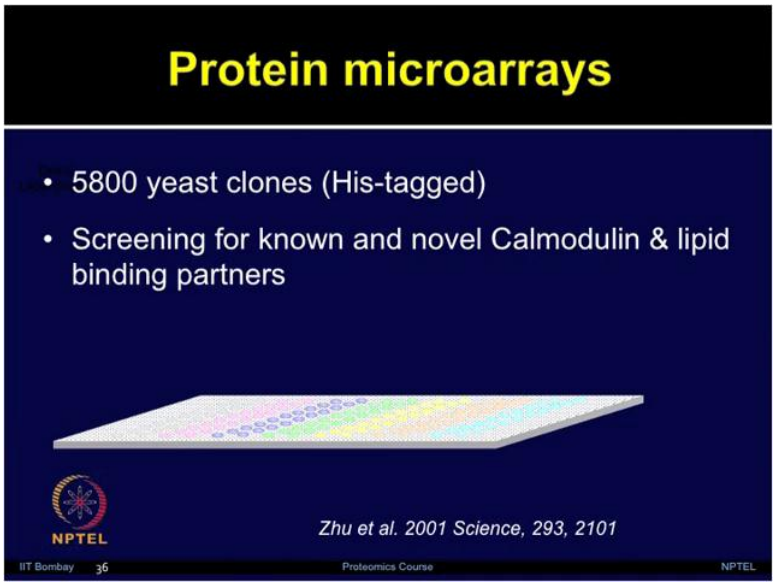

Now, success of this study motivated other scientists to start doing the protein microarrays. So, other group in Mich Schneider's lab started doing the yeast proteome chips and they used 5800 yeast clones which were histidine tagged, to screen for the known and novel calmodulin and lipid binding protease. Now, this was a full scale yeast protein array and it showed in 2001 the potential of these chip surface, these chip technologies for using protein interactions and different type of other functional applications.

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So, the different type of platforms which are available for studying the proteins by using protein microarrays. Let us have a quick look on some of these available platforms.

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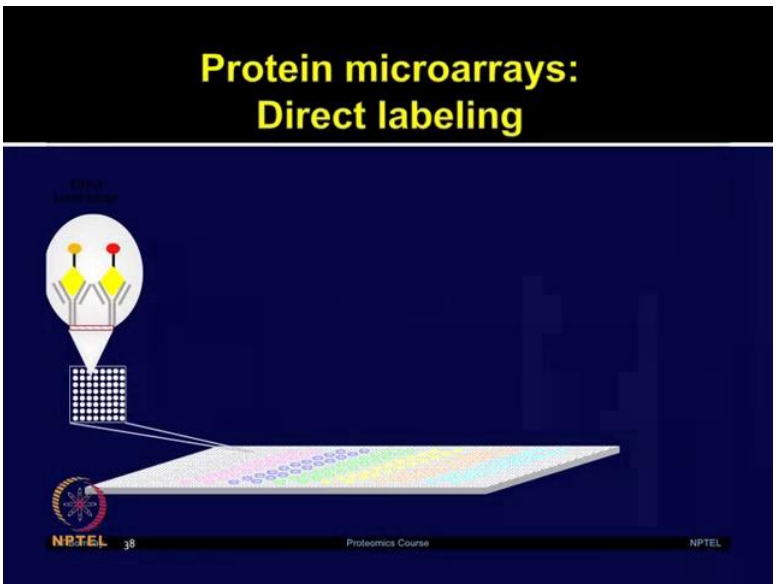

So, antibody have been used to print on the chip surface for various proteomic applications in various type of orientation the one shown here show the direct labeling, where the targets proteins are labeled with the florescence or other tag which allows detection after it is captured by the antibody immobilized on the array surface.

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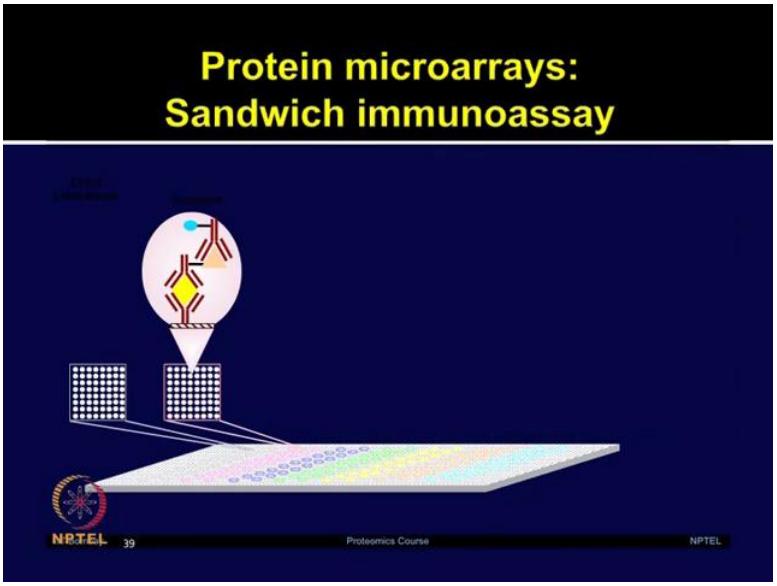

Now, other approach is sandwich immunoassay. In which the target protein is captured by an antibody followed by detection with labeled secondary antibody as shown in this slide.

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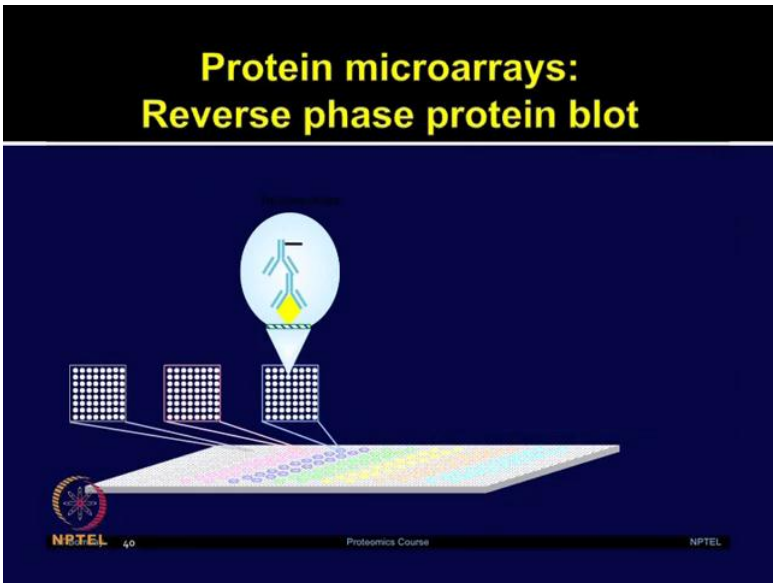

The reverse phase protein blot. In this method the complex mixtures such as cell lysates are printed and probed with the specific detection labels, all these three methods the directing labelling, sandwich immunoassays, and reverse phase protein blot, rely on antibodies obtaining good quality of antibodies and obtaining antibodies at the human

proteomic scale or any proteomic scale remains very challenging. So, people have started exploring different methods of printing proteins on the chip surface for different type of applications.

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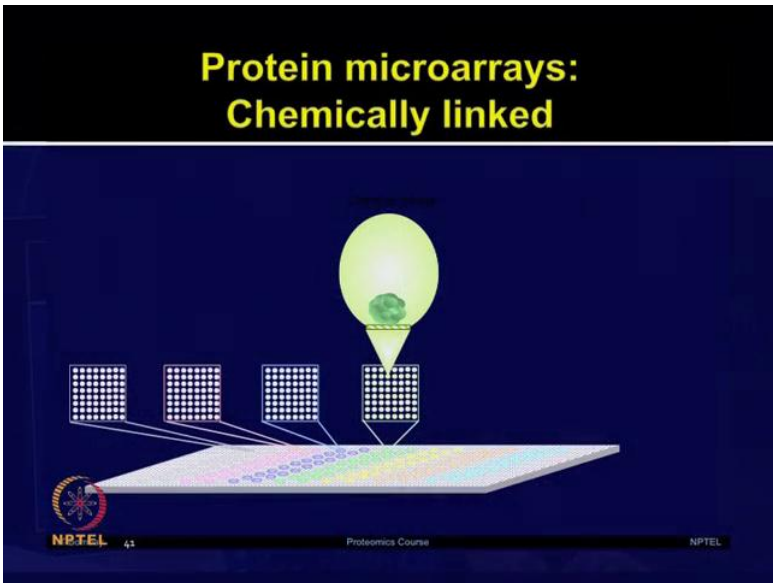

So, very conventional or most widely used method for printing the proteins involve the chemical linkage. The purified proteins are immobilized on the functionalized glass light and it could be used for various applications. If one can purify large number of proteins then this could very ideal approach of printing the proteins on chip surface and studying the different type of interactions.

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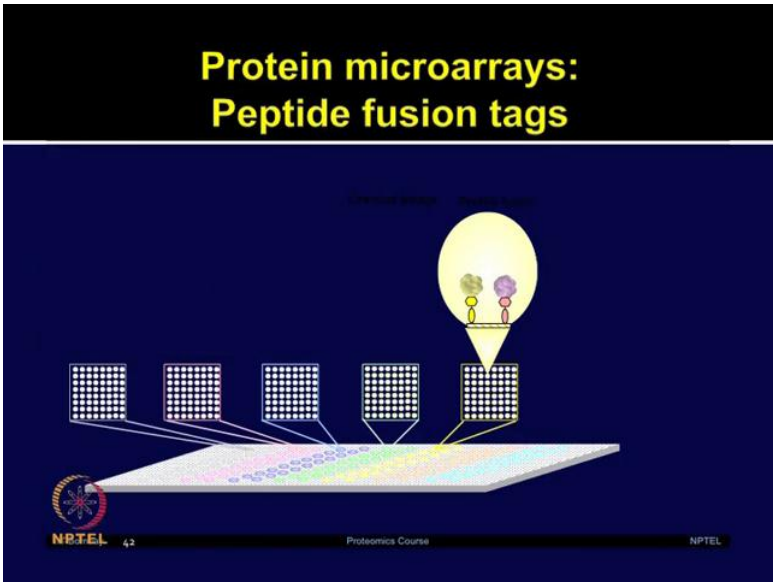

Peptides fusion tags which is another approach because peptides can be synthesized artificially. So, proteins fuse to the GST 6 histidine tag and spotted on the nickel coated slides have also been used for doing the protein microarrays.

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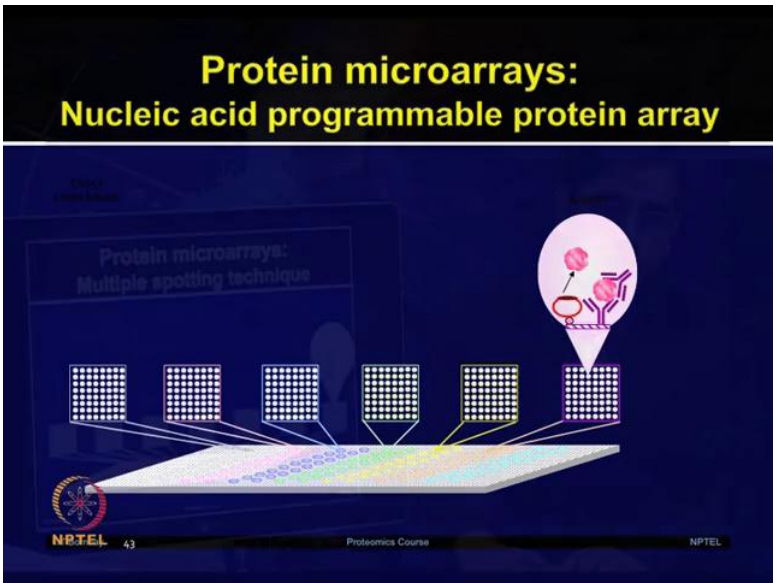

Now, due to the challenges involved in purifying the proteins or synthesizing the peptides, scientists have also explored the ways to eliminate the protein purification process. The George Schreiber at Harvard his group developed nucleic acid programmable protein arrays method in which the c DNA containing GST tags are

printed on the array surface along with capture and (( )) GST antibody protein is expressed by using the self re-expression system and captured by the antibody.

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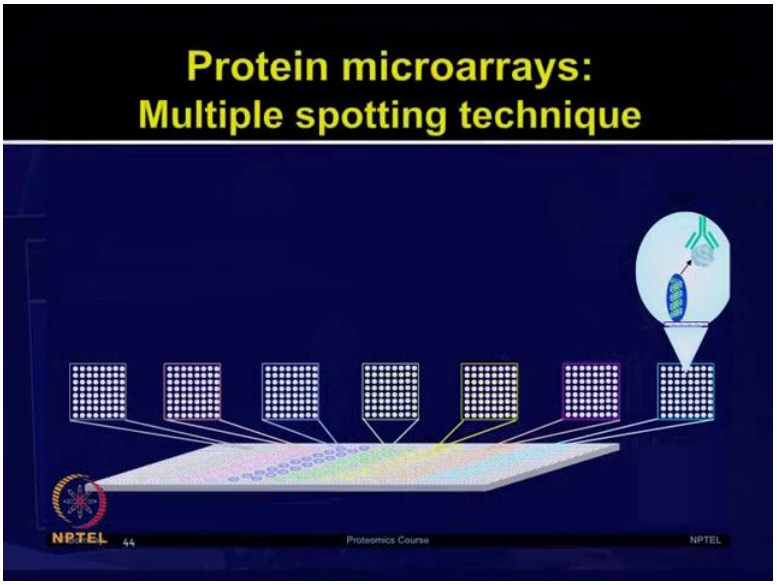

Now, another self re-expression based method try to overcome some of the previously used methods limitations and it try to print the self re-expression system as well as the c DNA on the chip surface by using multiple spotting technique. So, missed or multiple spotting technique involves self re-expression in situ expression of the unpurified PCR products and the cell free lysates are printed on the top of this spot. So, that both in vitro transcription and the translation can be performed on the chip surface.

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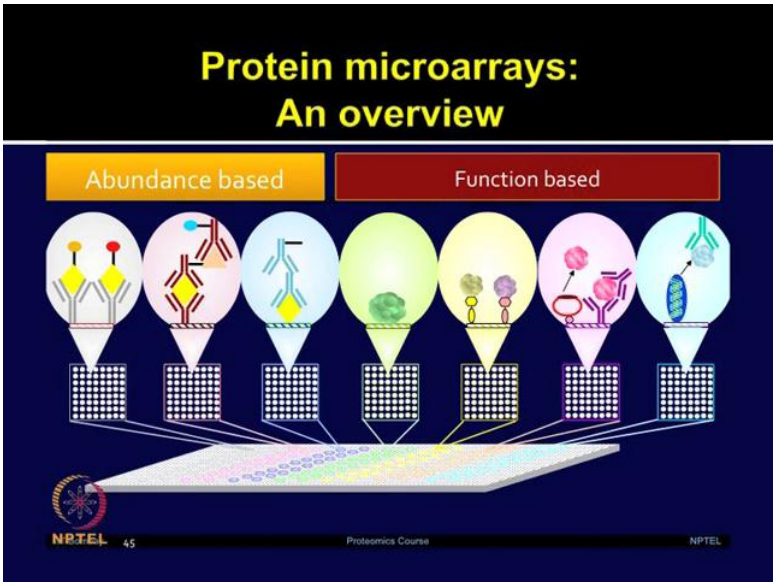

So, we have seen the different type of microarray platforms available, as you can see this overview slide they are different type of abundance based as well as function based protein microarrays available. We discussed about direct labelling, sandwich immunoassays, reverse phase protein blots as shown in the abundance based methods and then we talked about chemically linked, peptide fusion, nucleic acid programmable protein arrays and multiple spotting techniques in the function based methods.

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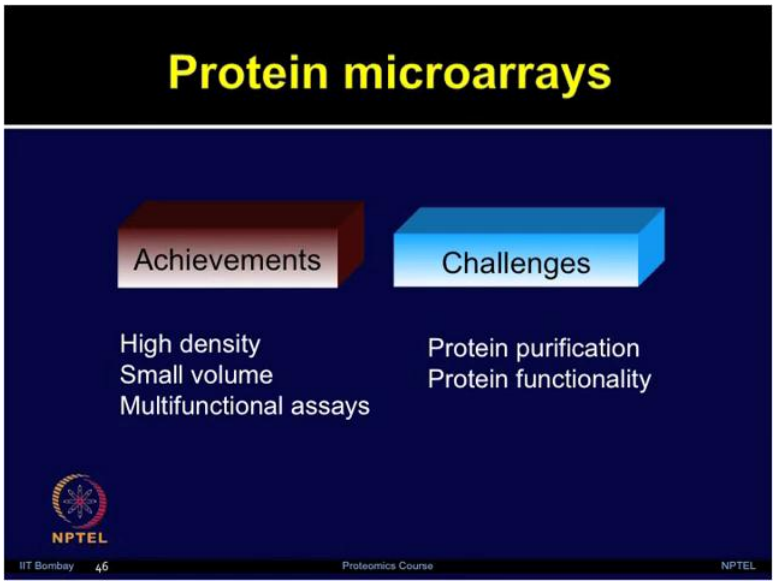

Protein microarrays have provided a high density high-throughput platform which is one of the major achievement of using this technology, very small volume of clinical samples or biological samples or pharmaceutical samples can be used on these array surfaces and multi functional assays can be performed. However, there is still various limitations and challenges of using the protein microarrays which includes generating the protein content it is purification as well as keeping it functional stable on the bench or in the shelf surface on the shelf life.

So, development of functional protein microarrays on which 1000s of discrete proteins are printed at high spatial density offers a novel tool to interrogate the protein function in high-through put manner. In this animation I will discuss different type of features different type of processes involved in the protein microarrays.

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Before we talk about protein microarrays how they are generated. Let us discuss the need for protein microarrays.

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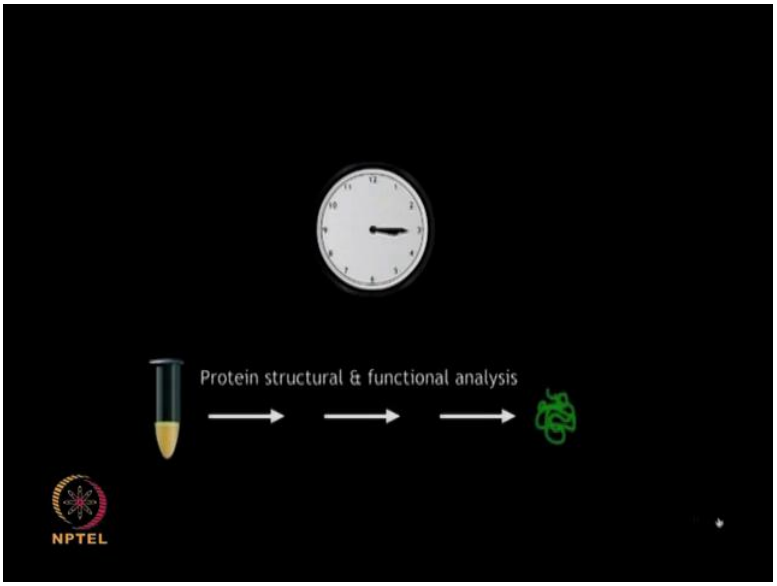

The functional analysis of proteins is a time consuming process which requires many steps analysis of a single protein at a time would be tedious and laborious procedure. Analysis of several protein samples will undoubtedly take longer time if they are run once at a time.

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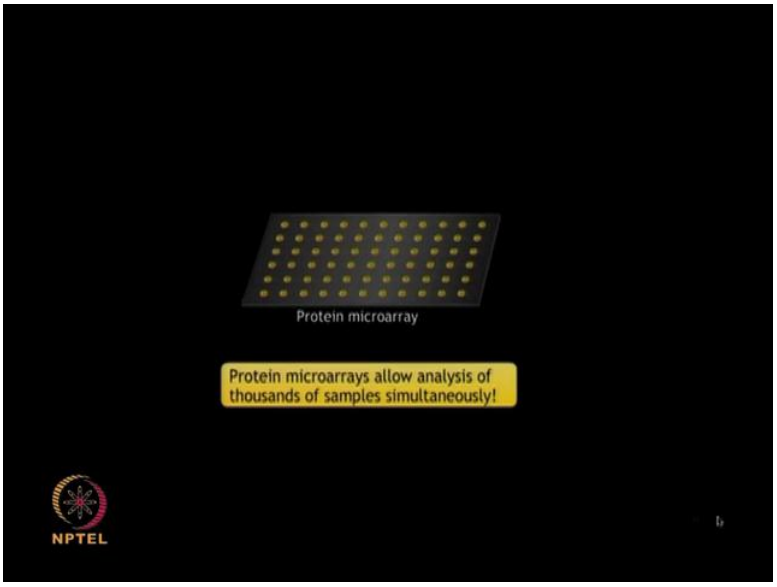

So, protein microarrays have successfully overcome this hurdle by allowing analysis of several sample simultaneously.

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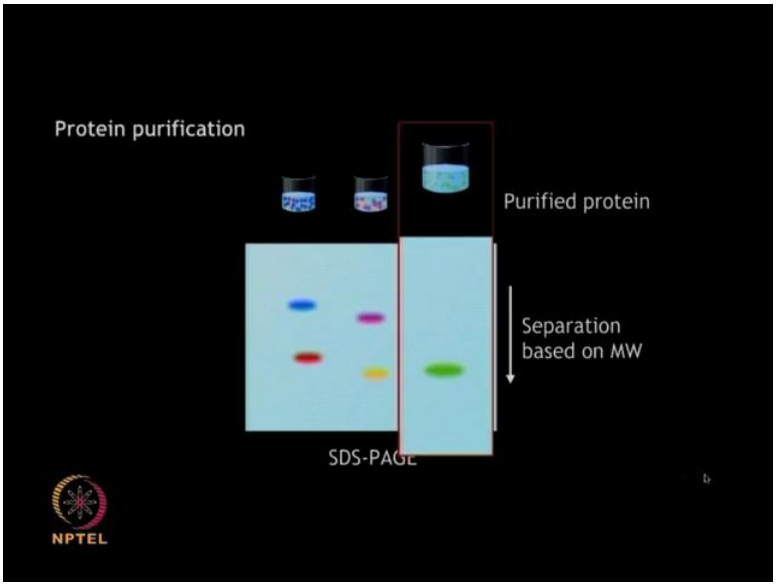

How to express the proteins and purify? The gene coding for the protein of interest is expressed in a suitable heterologous host system such as E-coli by using expression vectors like plasmids. The whole cell machinery is used for transcription and translation which results in a mixture of proteins consisting of the target proteins along with other host proteins. Still the protein of interest is expressed along with other proteins native to the host it is essential to purify the target proteins before it can be used for protein microarrays application.

So, this can be achieved by using chromatography procedures to obtain pure target proteins. The unwanted proteins are first yielded out and then the specific proteins can be purified and eluted. The protein purity can be tested on the SDS-page gels. Tags like histidine six are often fused with the protein of interest.

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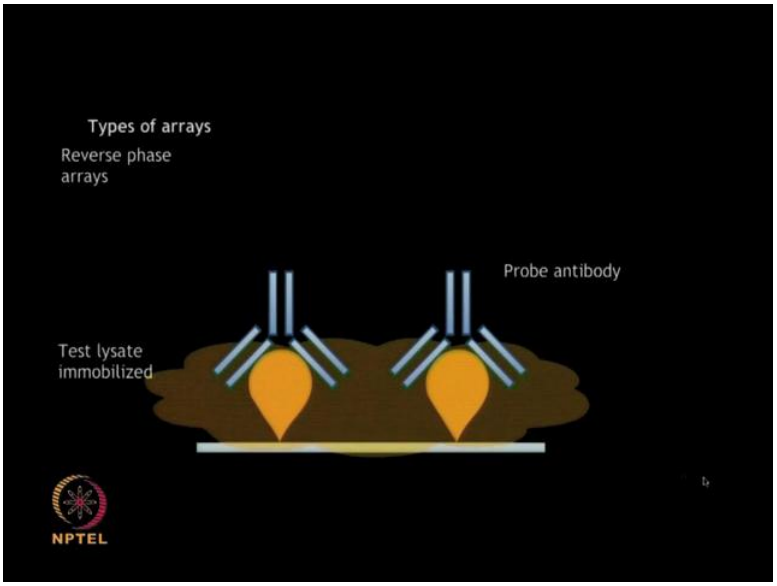

The array functionalization the array surface is functionalized with the suitable chemical reagent that will react with groups present on the protein surface. The aldehyde silane derivatizations are commonly used as interact well with the amino groups present on the protein surface which results in the fun capture of the proteins. The protein solution is printed on to the array surface in extremely small volumes by means of robotic printing device that has small pins attached to it for printing purpose. The slides are kept for a suitable duration following the printing a strip, to allow capture of the proteins on to the array surface. The unreacted sites are quenched by a blocking solution such as b s a which also prevents any non specific protein binding in subsequent strips.

The two different type of protein arrays which are commonly used. The forward protein arrays and the reverse protein arrays. In forward phase arrays the analyte of interest such as an antibody or aptamer is bound to the array surface and then probed by the lysate which may contain the antigen of interest. Where as in reverse phase arrays the test silver lysate is immobilized on the array surface and then probed by using detection antibodies specific to the target of interest.

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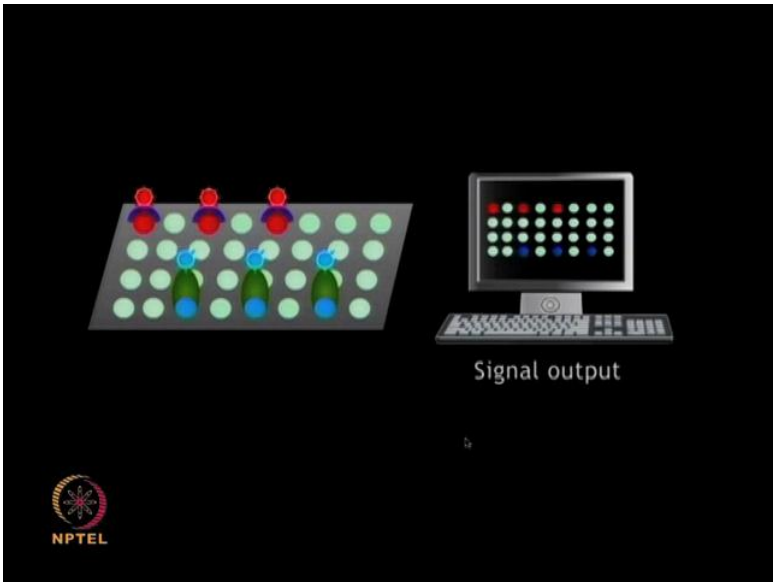

Protein detection and analysis. In direct labeling detection techniques all the target proteins are labeled with the fluorescent or radioactive tag that facilitates easy detection upon binding to the immobilized capture antibody on the array surface. In the sandwich assay, a fluorescent tagged secondary antibody that recognizes a different epitope on the target antigen binds to it and detected by means of fluorescence. The protein microarrays are then scanned in a microarray scanner that allows detection of the fluorescently labelled proteins or antibodies the output from the scanner is received by the software's from which data can be further analyzed. There are certain well characterized proteins can be printed on a array as shown in this animation.

Now, a proof of concept array is shown here where well characterized proteins are printed on the array surface along with their corresponding query molecule shown on the left side labeled with different fluorescent dyes. Now, by using this interactivity lets match the protein interacting pairs such as Jun and Fos p 53 and MDM 2 by dragging the query to the correct protein on the array surface in order to see the signal output. From the array surface there are both p 53 and Fos proteins present. Now if we drag the Jun protein it should interact with the Fos protein as you can see by this interactions here now m d m two proteins interact with p 53 protein well this interaction established then these signals can be detected by using scanner.

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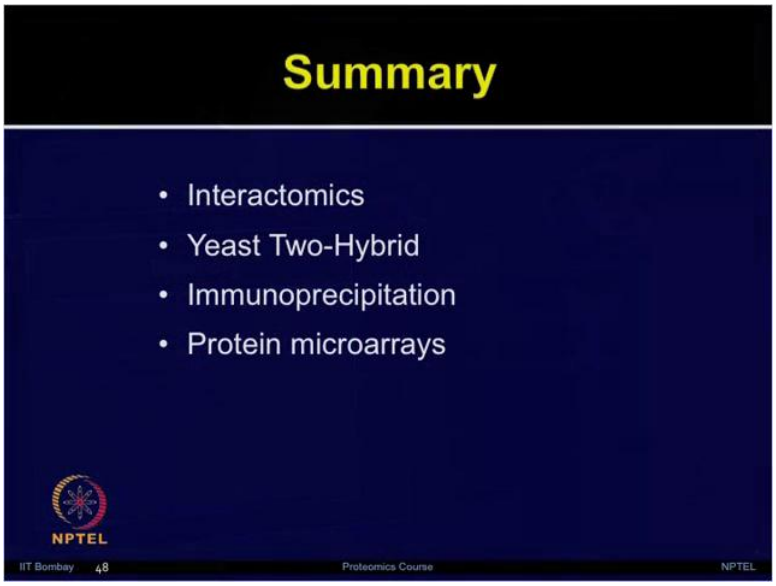

So, in summary today we discussed the significance of studying interactions in the field known as interactomics. We have discussed different type of traditional methods which have been used for studying these protein interactions such as yeast two-hybrid, and immunoprecipitation. Then I have shown you variety of platforms currently being used for studying proteins in the high-throughput manners by using protein microarrays. There are different type of advancement happened in the protein microarray field such as use of self re-expression based methods as well as coupling the protein microarrays with the label free detection systems. I will discuss some of these advancement, and these techniques in the following subsequent lectures. Thank you.