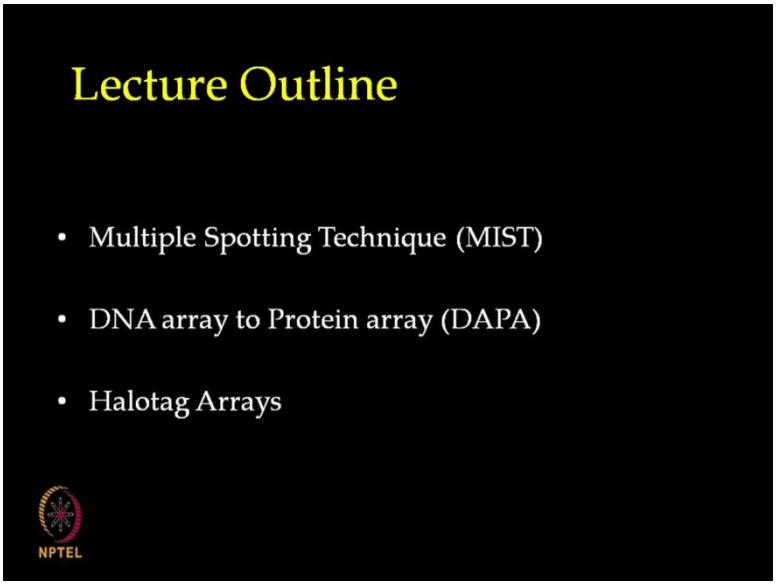
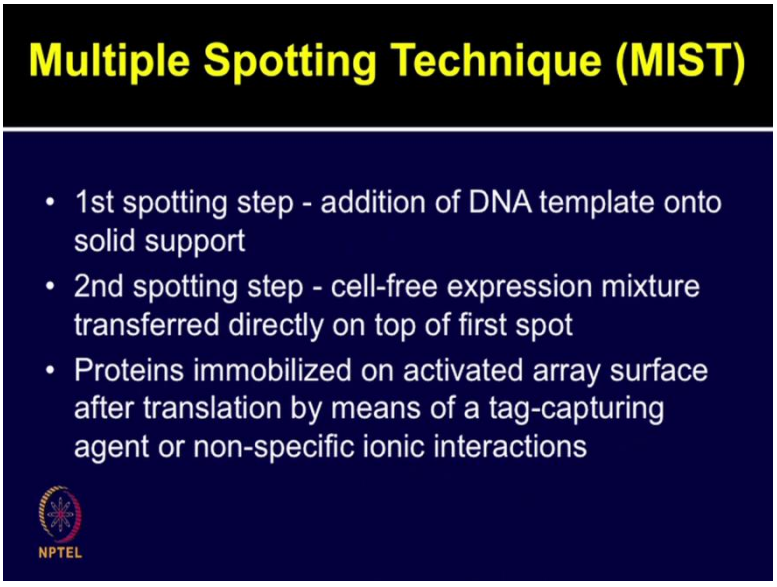
Interactomics Protein Arrays and Label-Free Biosensors
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay
Module 06
Lecture 28
Cell-free Synthesis Based Protein Microarrays MIST DAPA and Halotag Arrays

(Refer Slide Time: 0:45)



Welcome to MOOC intractomics course. In our previous lecture, we discussed about 2 cell-free expression systems. In our previous lecture, we discussed about two cell-free expression protein microarrays namely, Protein in-situ arrays and Nucleic acid programmable protein arrays, PISA and NAPPA.

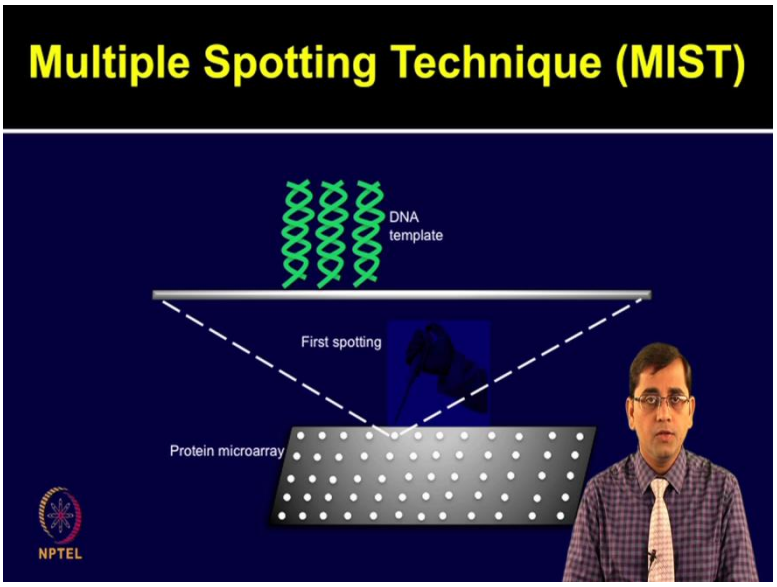
(Refer Slide Time: 1:11)

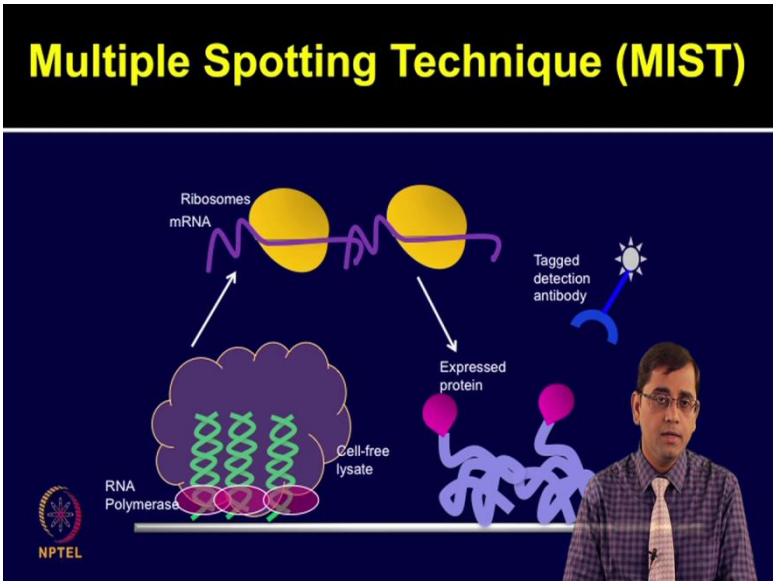


Today we will talk about multiple spotting techniques. This is another approach which facilitates generation of high density protein microarrays by using cell-free expression system. In MIST approach both DNA and cell-free expression system are printed on chip surface. Two rounds of spotting is performed and in first spotting step addition of DNA template to the microarray solid support is performed and during second spotting cell-free expression mixture is transferred directly on top of the first spot which contains DNA.

The aim is to print DNA as well as cell-free expression system so that after incubation protein can be directly synthesized on the same feature and one does not need to add or perform a separate step of cell-free expression system. The proteins which are immobilized on the activated array surface after translation by means of a tag capturing agent or non-specific ion interaction.

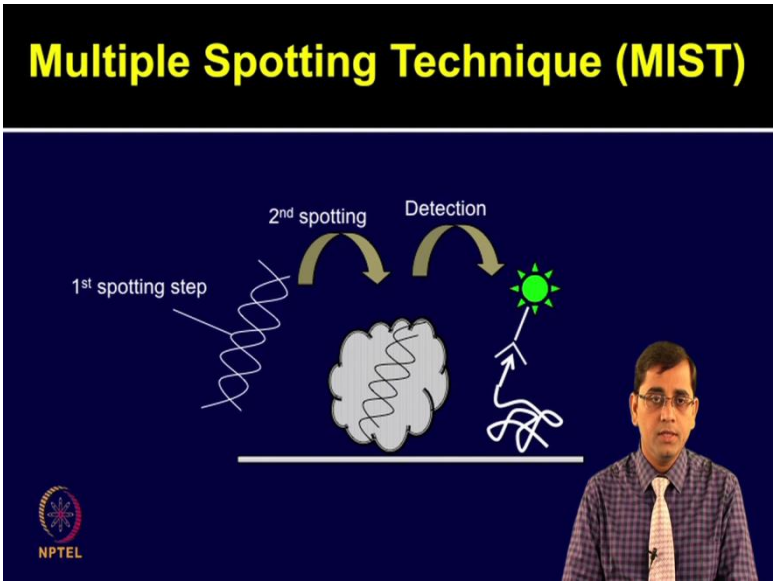
(Refer Slide Time: 2:15)





In the multiple spotting technique or MIST, the first spotting step as shown in the slide involves addition of DNA template on to microarray support, after first spotting is performed with cell-free expression mixture is directly transferred on top of the first spot. In this way where two printing step are involved on top of each DNA template cell-free expression system is also printed, after incubation both transcription and translation processes happen and proteins are synthesized which can be detected by (())(2:51) antibodies.

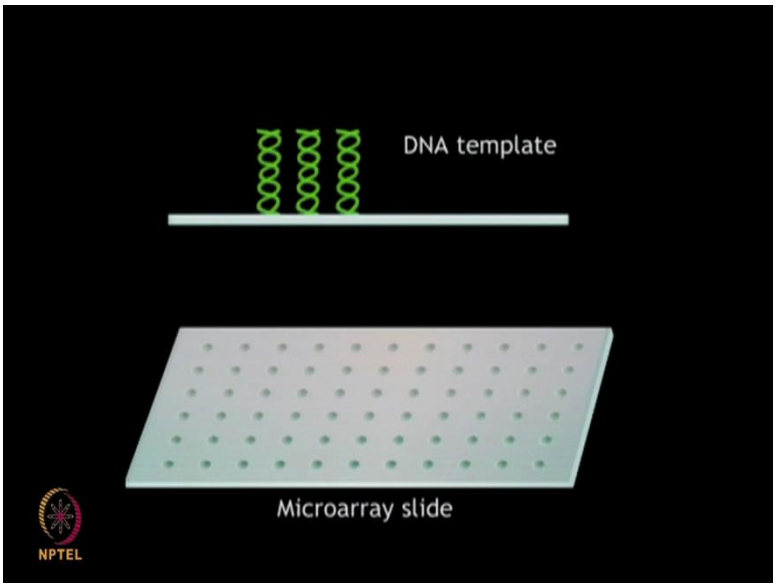
(Refer Slide Time: 2:58)



Overall in MIST technique, the DNA template is spotted in the first step followed by the cell-free lysate in the step, which is directly added on top of the first part. The expressed protein is detected by using fluorescently tagged antibodies. The inventors of MIST technology reported that even 35 femto gram of PCR product was sufficient for expression and detection

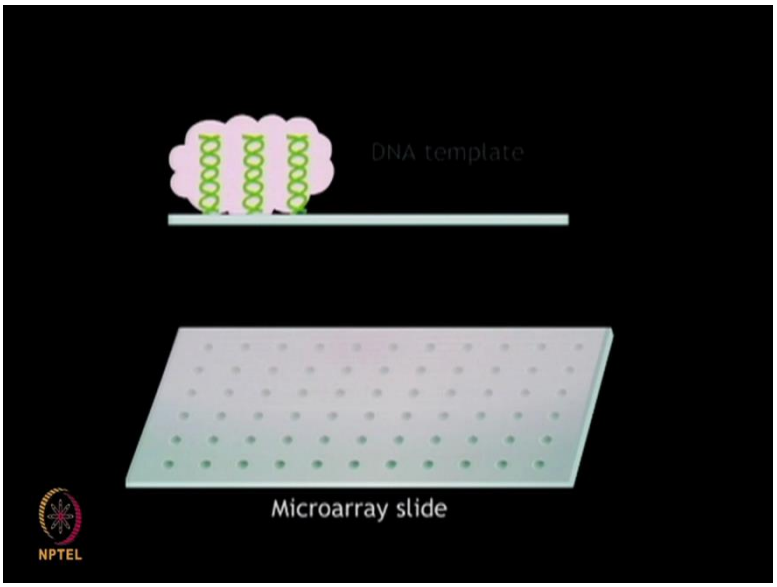
of wild type green fluorescent protein. The high density arrays containing 13000 spots per slide can be achieved by using MIST technology. Let us now discuss the working principle of MIST by showing this animation, Multiple spotting technique or MIST.

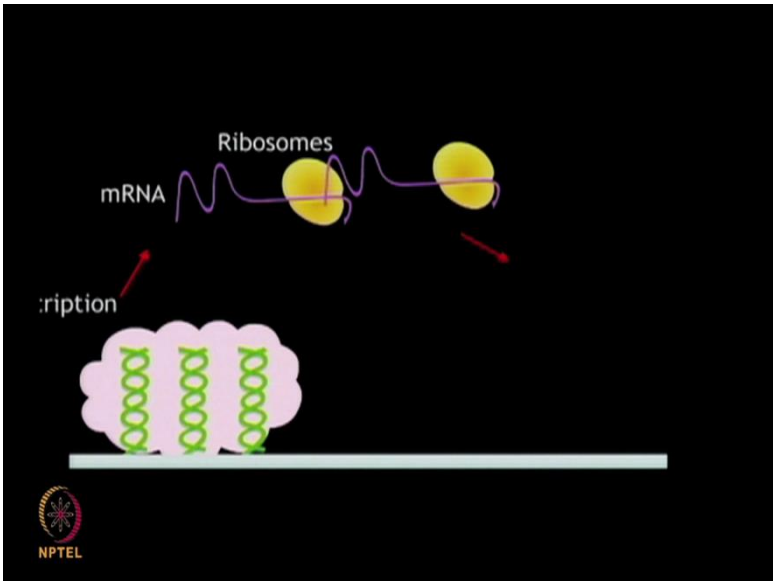
(Refer Slide Time: 4:19)

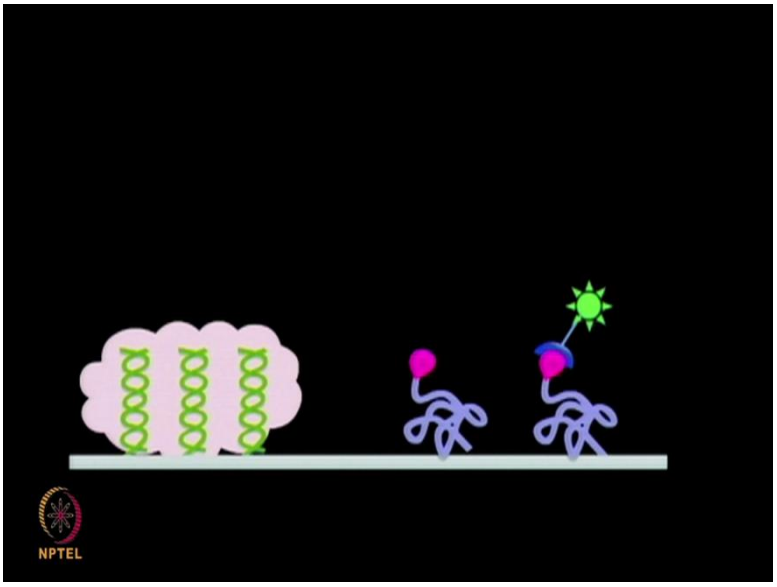


In this technique, the first spotting step of the multiple spotting is capable of producing high density arrays. It involves addition of template DNA onto the solid array support. The template DNA can even be in the form of unpurified PCR product, which is one of the major advantage of using this MIST technique.

(Refer Slide Time: 4:35)

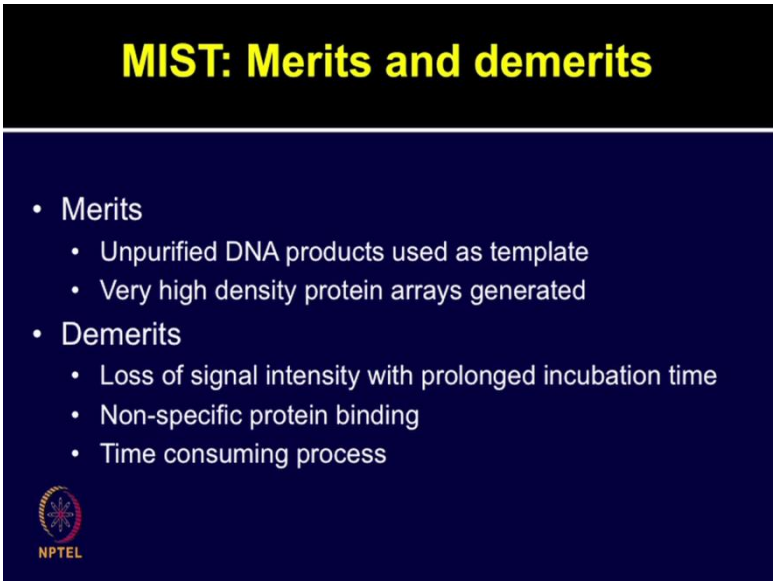






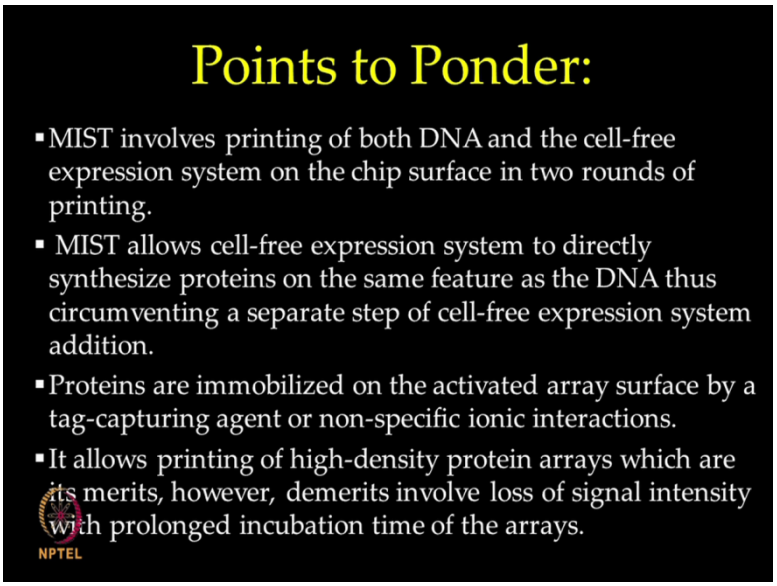
The second spotting step involves addition of cell-free lysate directly on top of the first spot. The transcription and translation can begin only after the second spotting step. The protein expressed from the template DNA binds to the array surface by means of non-specific interactions which is one of the drawbacks of this procedure. A detection antibody is specific to the protein of interest is added which indicates that protein expression levels by using suitable fluorophore.

(Refer Slide Time: 5:27)

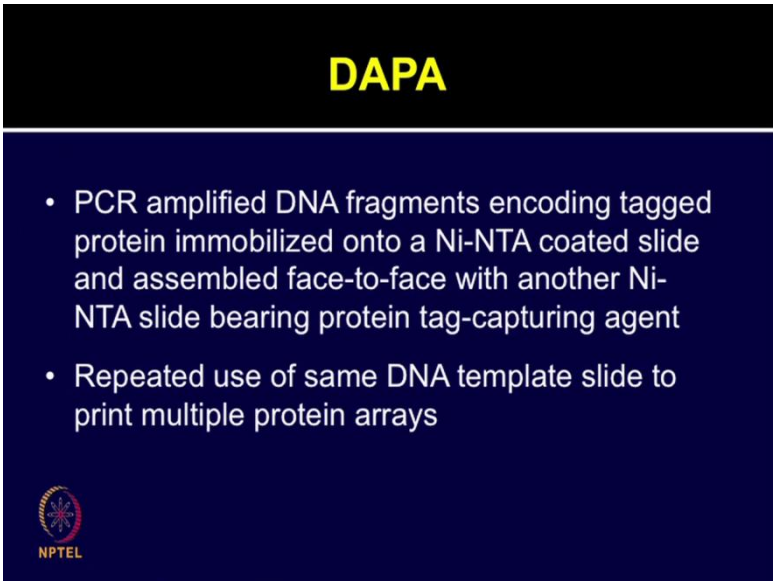


There are various merits and demerits of using MIST technology. It involves unpurified DNA products that can be used as template source which is not the case in NAPPA. In this method, very high density protein arrays can be generated because spot chemistry is not very complicated. The limitation of using this technique is that there is loss of signal intensity with prolonged incubation time of the arrays. Since, in this case even the cell-free expression systems are printed on top of the arrays, the stability could be one of the major issues. The non-specific protein binding as well as the overall process is more time consuming. These are some of the limitations of MIST technique.

(Refer Slide Time: 6:37)

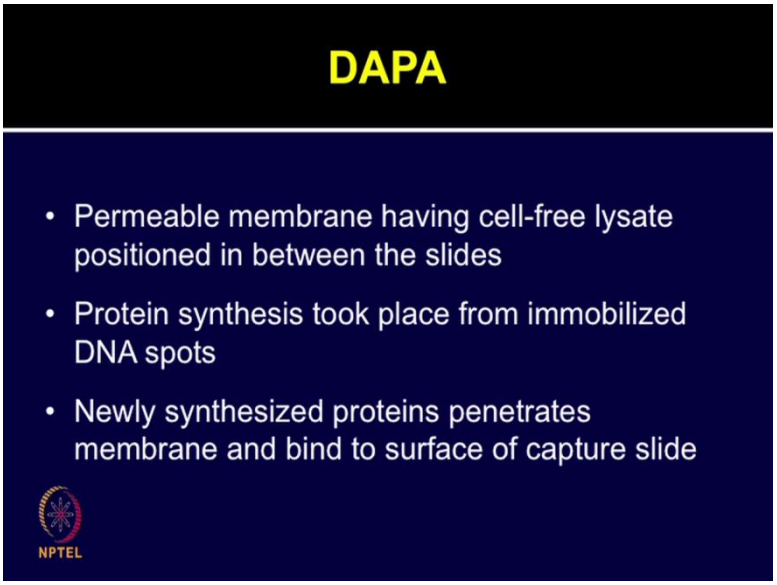


(Refer Slide Time: 7:11)



Let us now discuss DNA array to protein arrays or DAPA. The DAPA technique makes possible, the repeated use of DNA template site for printing multiple rounds of protein arrays. So, some same DNA template multiple proteins slides can be produced. In DAPA, the PCR amplified DNA fragments which encode tagged proteins immobilized onto a nickel-NTA coated slide and assembled face to face with another nickel-NTA slide bearing protein tag capturing agent. The repeated use of same DNA template can be performed here and multiple protein arrays can be generated by using DAPA method.

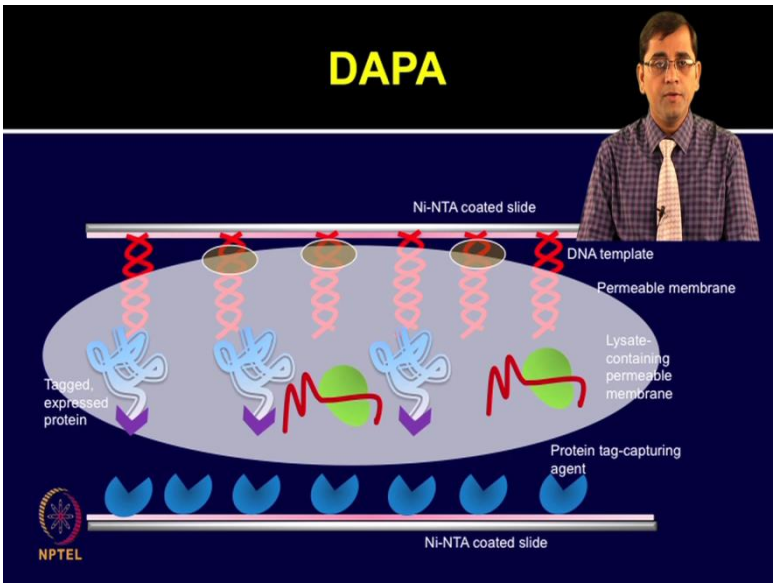
(Refer Slide Time: 7:38)



In DAPA method, the permeable membrane which contains cell-free lysate, which is positioned between two slides for these proteins to be defused. Protein synthesis takes place

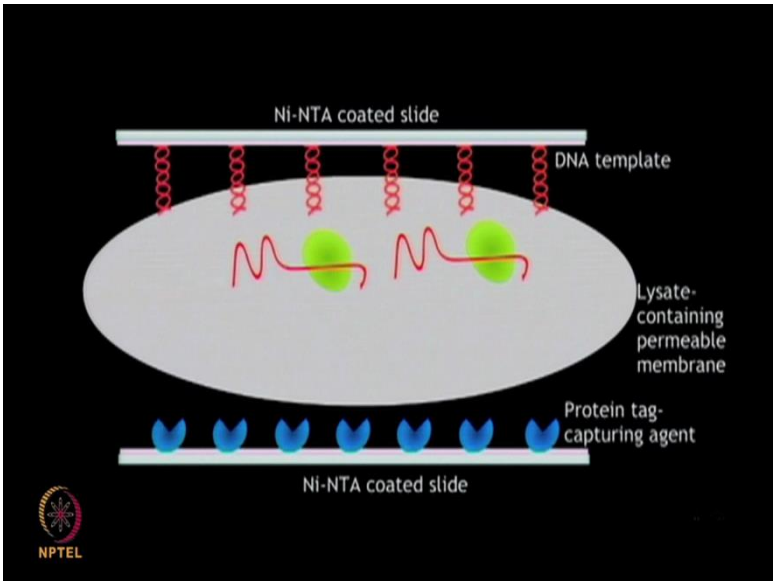
on this membrane and then the synthesized protein defuse from the membrane and then move on to the other slide for capture. The newly synthesized protein penetrates the membrane and bind to the surface of capture slide that can be seen in this slide.

(Refer Slide Time: 8:10)



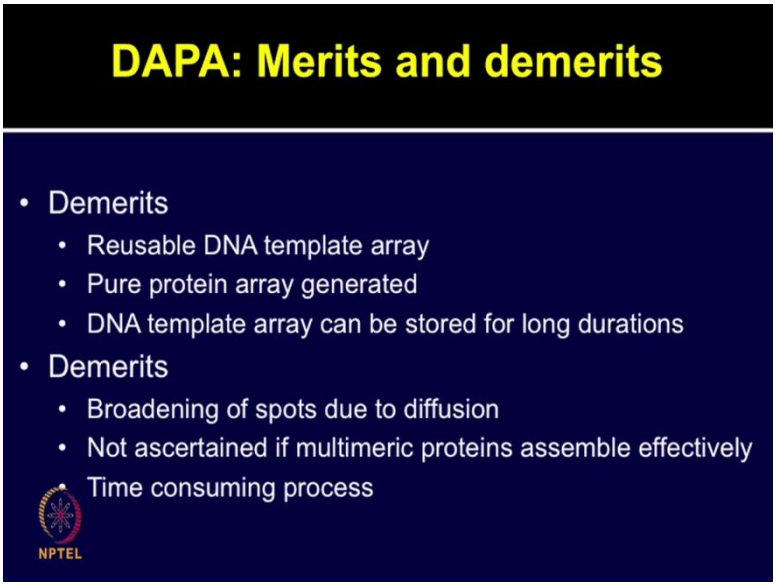
In DAPA, the PCR amplified DNA fragment encoding the tag proteins are immobilized on to a nickel-NTA coated slide and assembled face to face with another nickel-NTA containing protein tag capture agent. In between these two slides, a permeable membrane containing cell-free lysate is placed. The protein synthesis takes place from the immobilized DNA spots. The newly synthesized proteins can penetrate this membrane and bind to the surface of capture slide. In DAPA approach, the investigators produced an array of double hexa-histidine tag GFP and data was found to be comparable with existing protein array technologies. With DAPA, it is possible to use same DNA template repeatedly to print multiple protein arrays. It has been shown that one can use this template for printing almost 20 arrays.

(Refer Slide Time: 9:45)



So, let me now show you the working principle of DAPA in this animation. DNA array to protein array known as DAPA. In DAPA, the slides bearing the DNA template and the protein tag capturing agent are assembled face to face with a lysate containing permeable membrane placed in between. The expressed protein slowly penetrates the membrane and gets immobilized on the slide surface through its capture agent. The DNA template array can be reused several times by using this method.

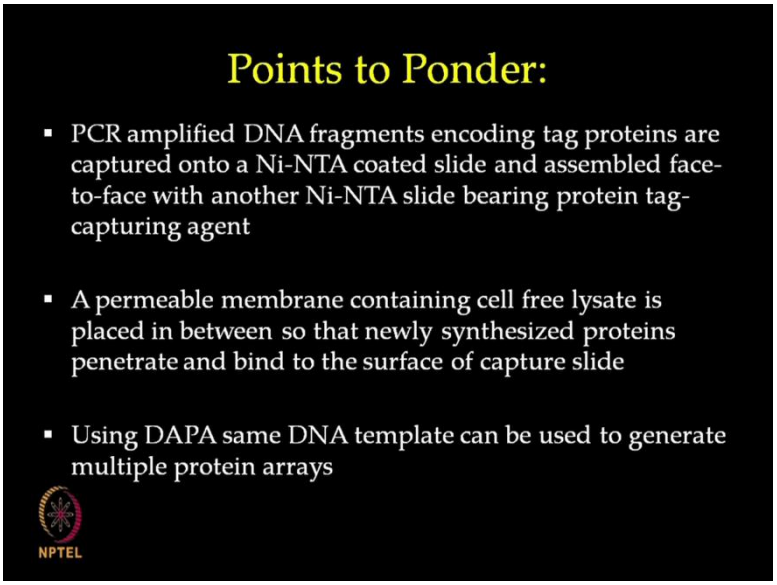
(Refer Slide Time: 10:07)

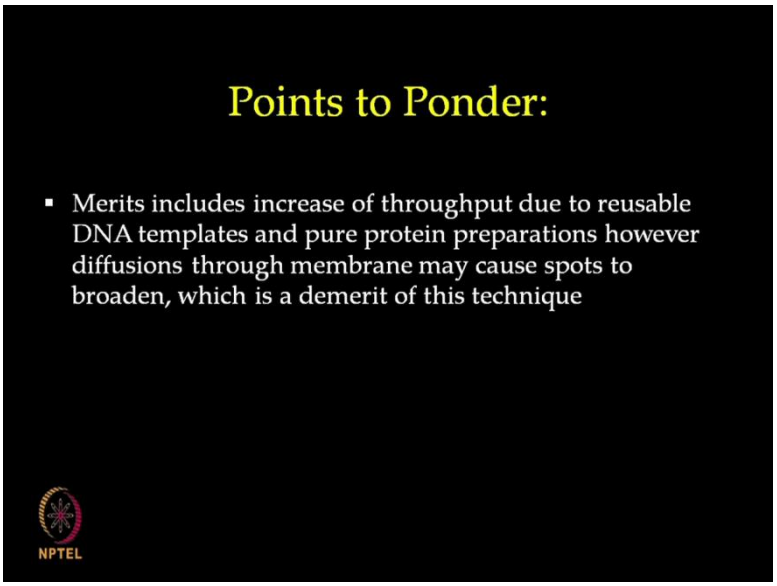


DAPA method provides few advantages as compared to the previously described methods; one could get pure protein because the protein is defused from the membrane. The reusable DNA template which is able to print multiple chips by using this chemistry and of the source

template which is DNA that array can be stored at room temperature for long duration, when there is a need for making the protein array one can use the membranes with the lysate and then followed by generation of multiple protein arrays however, there are certain limitations of using DAPA method including the broadening of a spots due to diffusion, it is not ascertained if multimeric protein assemble effectively, it is also time consuming process.

(Refer Slide Time: 11:14)

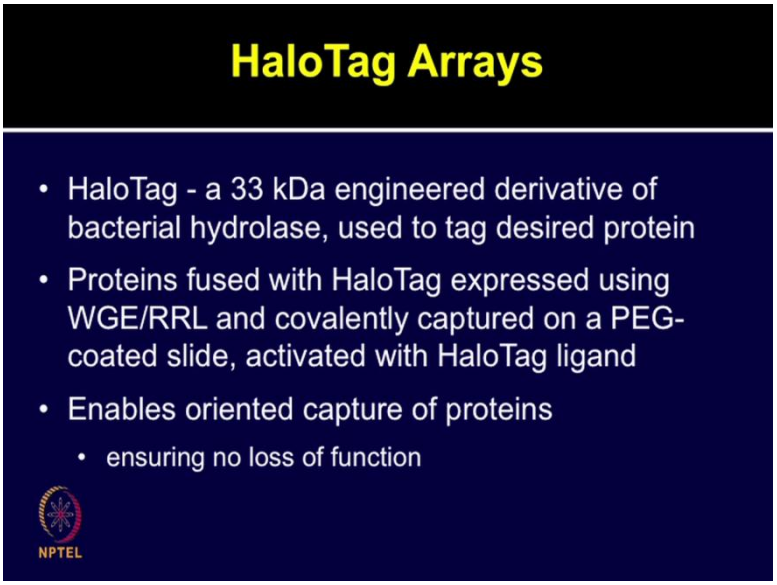


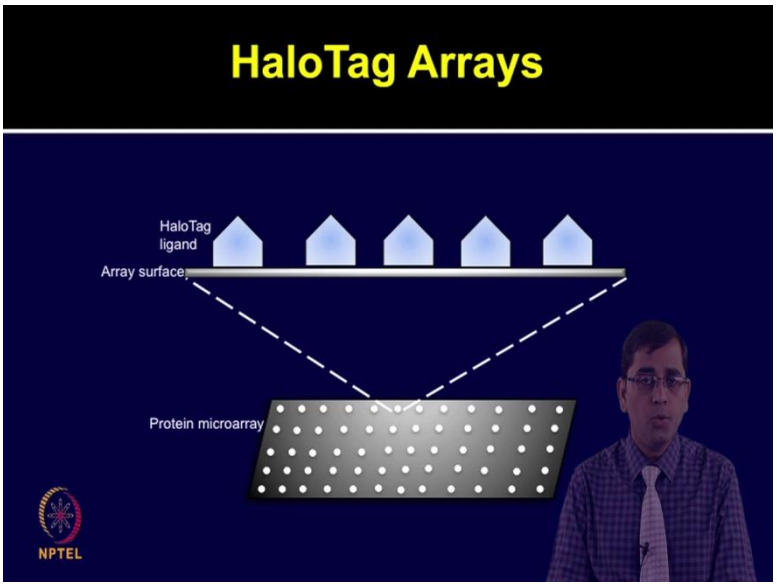


Let us move on to latest technology which is halotag arrays. The halo link protein array systems have been developed by Promega Company which combines few technologies together to create protein microarrays. First of all it uses cell-free expression transcription and translation system. It uses halotag which is mutated hydrolase protein that forms the covalent bond with the halotag ligands. Thirdly, it uses ploy-ethylene glycol coated glass slides

activated with halotag ligand for specific capture of proteins which are expressed by using cell-free expression system.

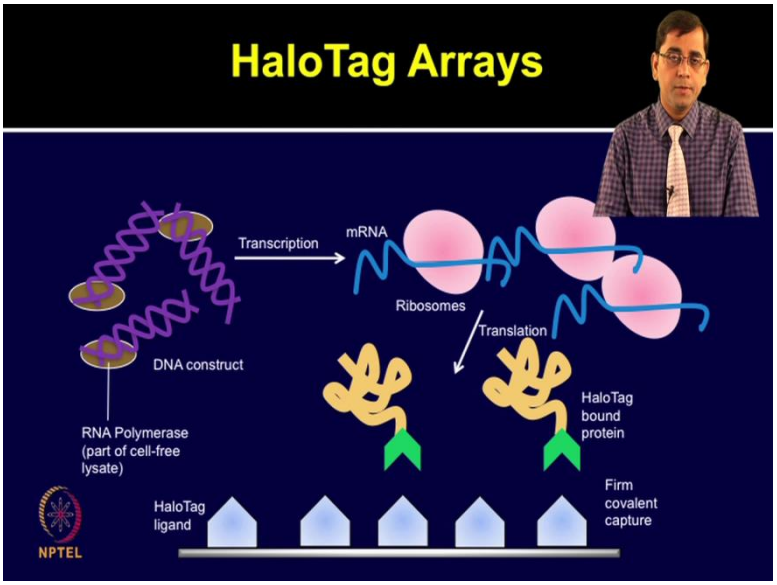
(Refer Slide Time: 12:26)





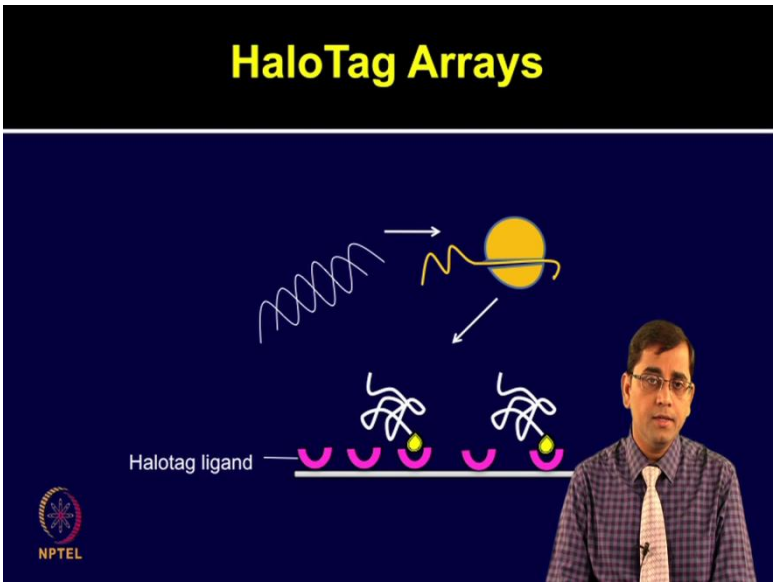
The halotag is a 33 kilo Dalton engineered derivative of bacterial hydrolase which is used to tag desired proteins. The proteins which are fused with halotag are expressed by using wheat germ extract expression system or rabbit reticulocyte lysate and covalently captured onto PEG containing slides. These are then activated with halotag ligands; these halotag arrays achieve oriented capture of proteins and thereby ensures no loss of function or minimal loss of function as shown in this slide the poly-ethylene glycol coated slide can be activated using halotag ligands.

(Refer Slide Time: 13:14)



The proteins are fused with halotag are expressed by using cell-free expression system and are covalently captured on poly-ethylene glycol coated glass slides. So, halotag method enables oriented capture of proteins.

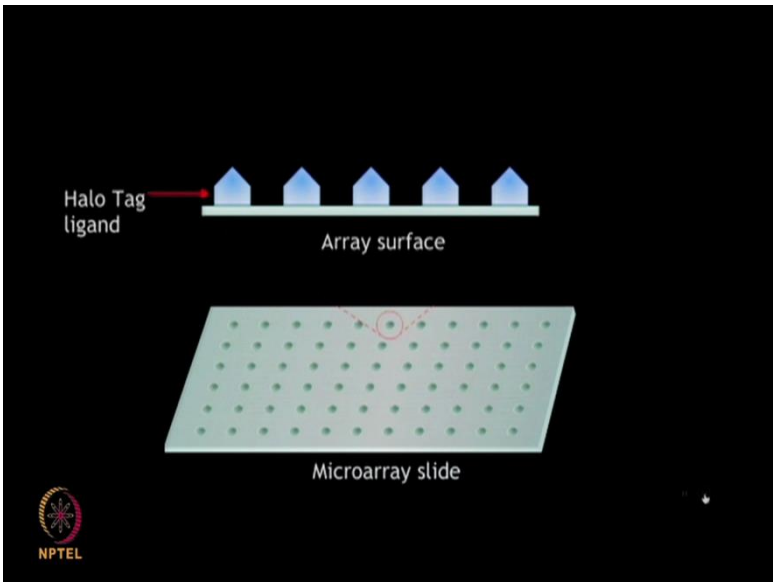
(Refer Slide Time: 13:34)

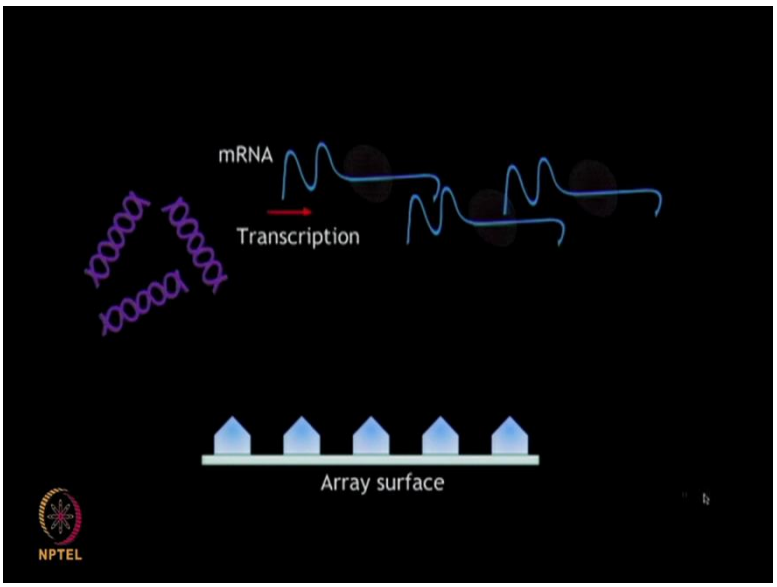


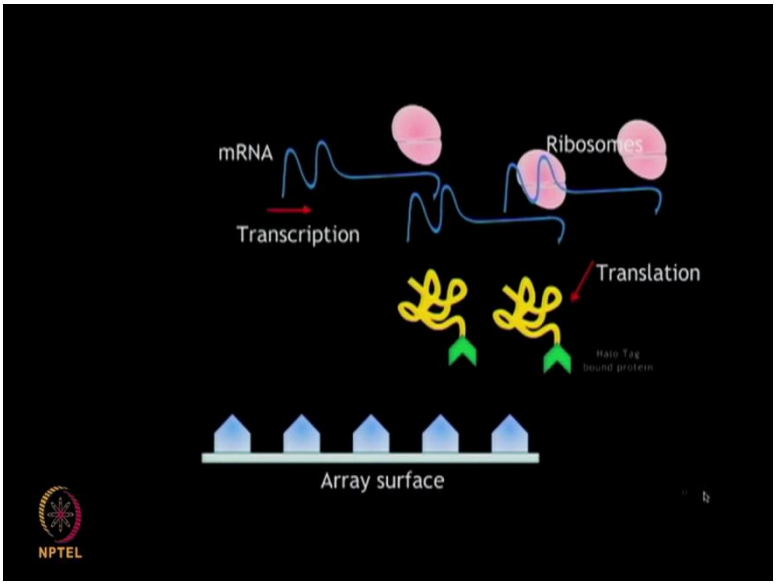
In a nut shell (())(13:29) the halotag fused protein is expressed as can be seen here and covalently captured on PEG-coated slide and activation is performed by using halotag ligand. This provides very strong covalent interaction and minimizes loss of synthesized protein which usually occurs in other protein microarray based methods.

In protein arrays, one need to perform several washing steps if the proteins or the molecules are bound on a surface with a very strong interaction, then there will be minimal loss from the surface which can be achieved in this case by using halotag system. So in halotag arrays a capture chemistry which is based on binding of halotag protein with synthetic ligand that enables covalent and oriented capture of proteins on solid surface directly from the cell-free expression based system. This method not only overcomes the limitation of protein purification, but also overcomes several other limitations which are commonly observed in any protein microarray technology. Here, let us discuss the working principle of halotag arrays with this animation.

(Refer Slide Time: 15:10)

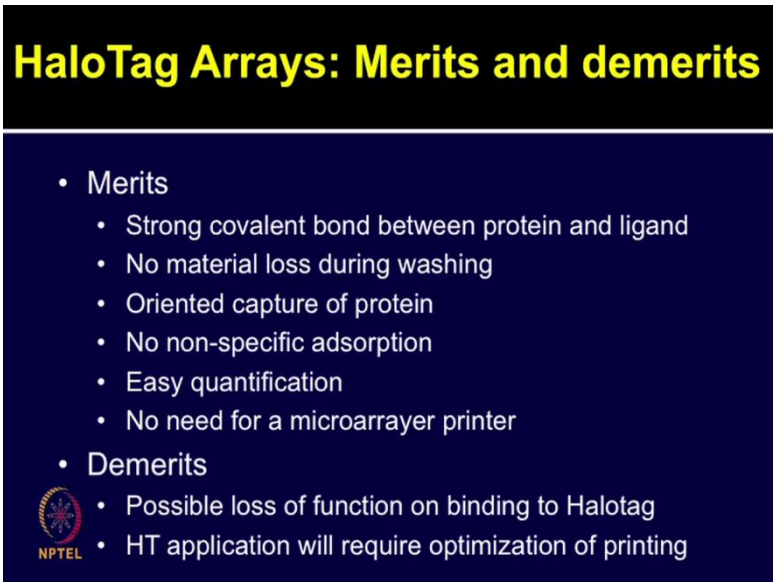






Halotag technique. In halotag technique, the slide is activated with the halotag ligand which captures the express protein through firm covalent interaction, which prevents any material loss and ensures oriented capture of the protein. The halotag fused protein is expressed by using lysate such as rabbit reticulocyte lysate RRL or wheat germ expression system and synthesized protein is covalently captured onto the array surface through the halotag ligand. The specific interaction ensures the oriented capture of protein and prevents any possible functional loss.

(Refer Slide Time: 16:09)

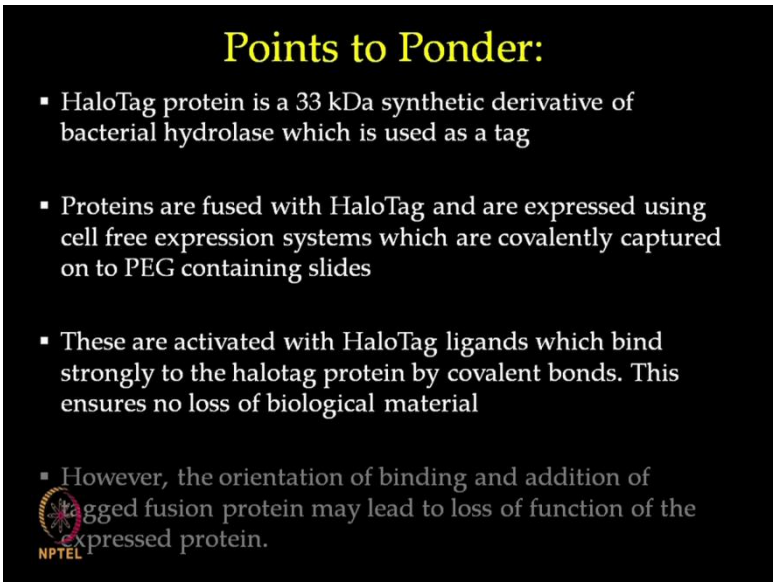


Halotag arrays have various advantages such as the strong covalent binding between the protein and the ligand, no material loss occurs during washing steps because of very strong covalent interaction. The proteins are captured oriented and there is no non-specific

adsobstion due to PEG-coating. The quantification is easy and one do not need the microarrayer printer to print the proteins on this chip because the commercial kit of halotag arrays provide a gas kit, which can be used for printing the arrays however, there are certain limitations of using halotag arrays. It has not been being shown that is system cannot be used for high density arrays, also few spots using the commercial gas kits have been shown.

So, applicability of this technology for high density protein microarrays is yet to be seen. High density arrays in theory are possible, but one need to ensure that even at the high density these array function properly then there is possibility of loss of function on binding to the halotag. These types of quality control checks have already been performed, but more biological questions need to address however more biological questions need to address on these arrays before confidently applying these technology for the clinical applications.

(Refer Slide Time: 17:59)



In summary, protein microarrays offer a range of diverse applications and are being adopted extensively for clinical and other biological applications. The cell-free expression systems facilitate synthesis of several proteins in single reaction and produce proteins on demand and eliminate concerns of storage and protein stability. Several cell-free systems have been used developed in the past decade and in the last two lectures we have discussed the working principles, merits and demerits or some of these most promising cell-free expression based protein microarray systems. In the next lecture, we will focus on one of the technology nucleic acid programmable protein arrays or NAPPA and try to understand how to make the arrays and its application for various biological questions Thank you.

(Refer Slide Time: 19:23)