

Interactomics Protein Arrays and Label-Free Biosensors

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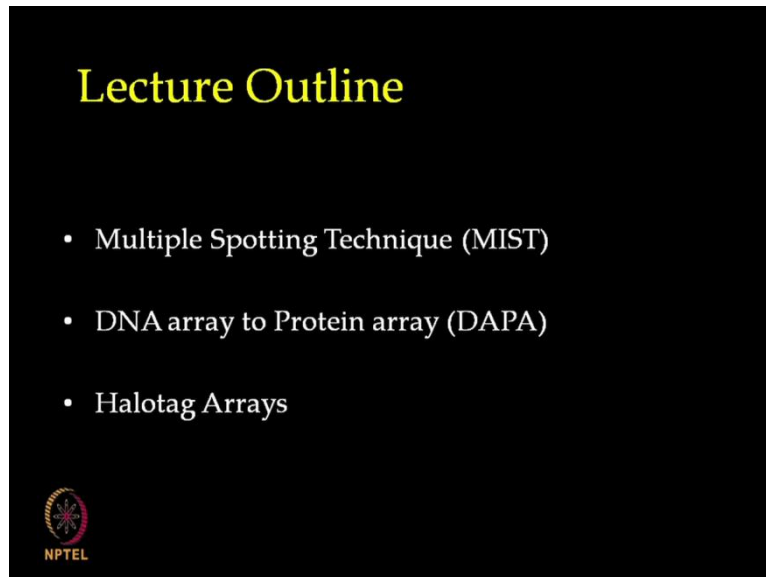
Indian Institute of Technology Bombay

Module 06

Lecture 28


Cell-free Synthesis Based Protein Microarrays MIST DAPA and Halotag Arrays

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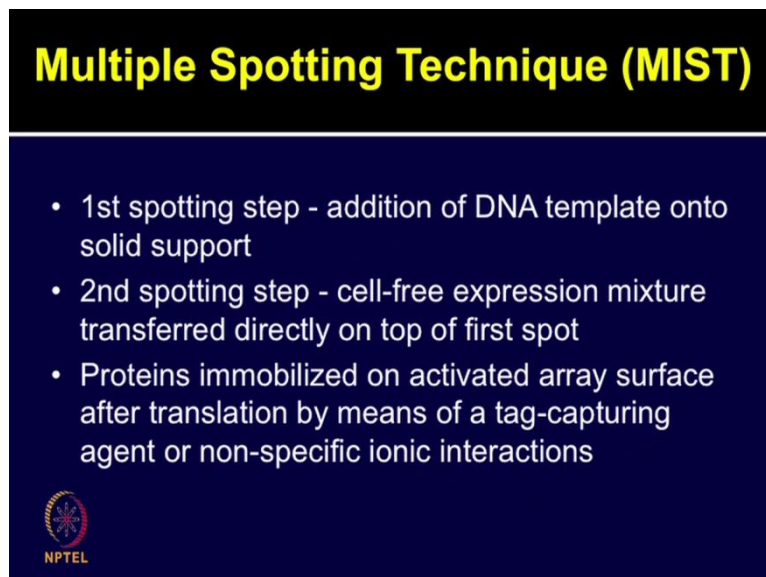
Lecture Outline

- Multiple Spotting Technique (MIST)
- DNA array to Protein array (DAPA)
- Halotag Arrays

 NPTEL


Welcome to MOOC interactomics 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.

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Multiple Spotting Technique (MIST)

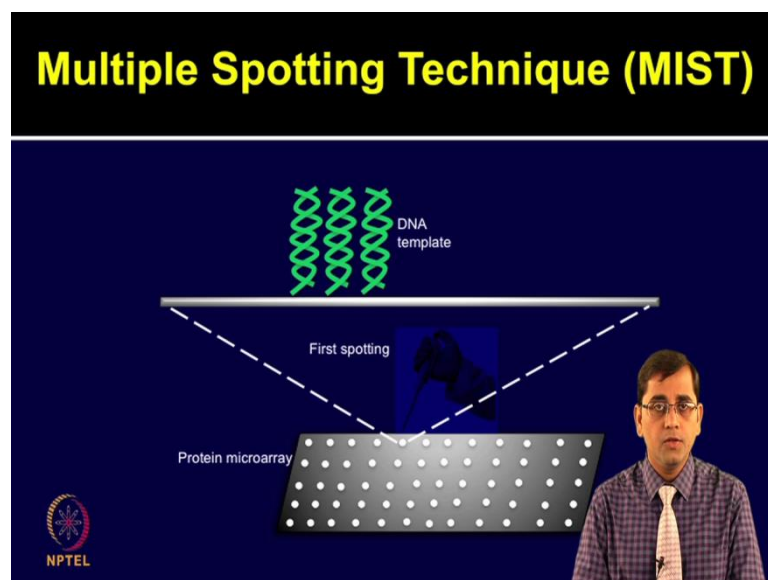
- 1st spotting step - addition of DNA template onto solid support
- 2nd spotting step - cell-free expression mixture transferred directly on top of first spot
- Proteins immobilized on activated array surface after translation by means of a tag-capturing agent or non-specific ionic interactions

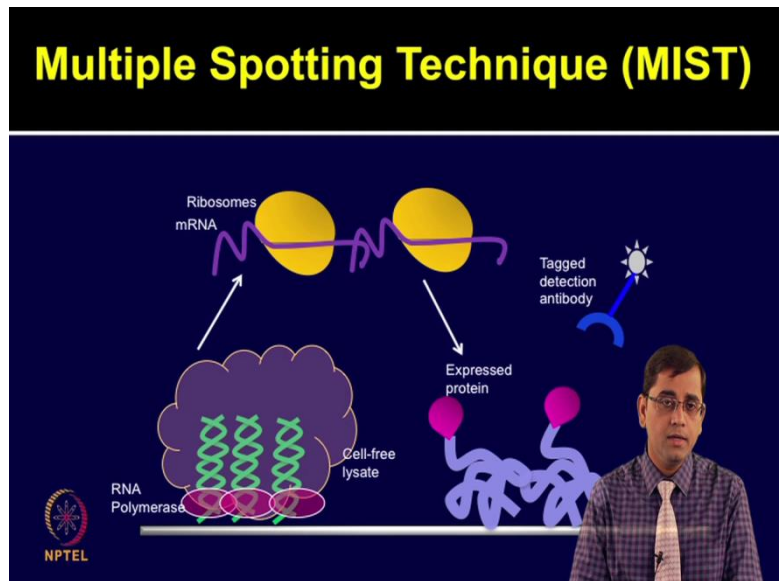
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Today we will talk about multiple spotting techniques. This is another approach which facilitates generation of high density protein microarrays by using cell-free expression system. In MIST approach both DNA and cell-free expression system are printed 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.

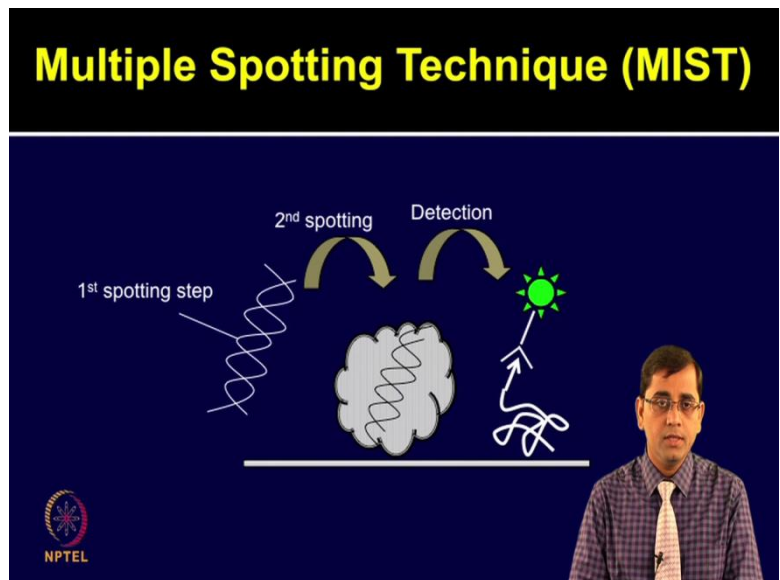
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In the multiple spotting technique or MIST, the first spotting step as shown in the slide involves addition of DNA template on to microarray support, after first spotting is performed with cell-free expression mixture is directly transferred on top 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.

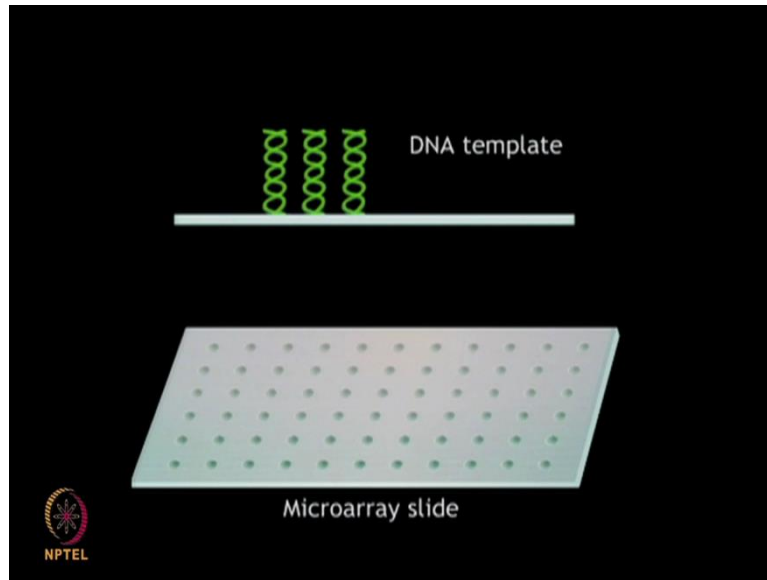
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Overall in MIST technique, the DNA template is spotted in the first step followed by the cell-free lysate in the step, which is directly added on top of the first part. The expressed protein is detected by using fluorescently tagged antibodies. The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

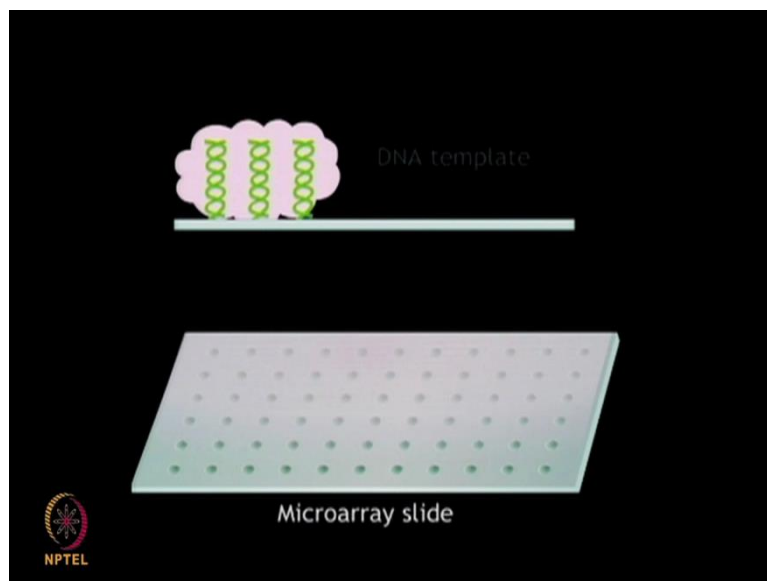
of wild type green fluorescent protein. The high density arrays containing 13000 spots per slide can be achieved by using MIST technology. Let us now discuss the working principle of MIST by showing this animation, Multiple spotting technique or MIST.

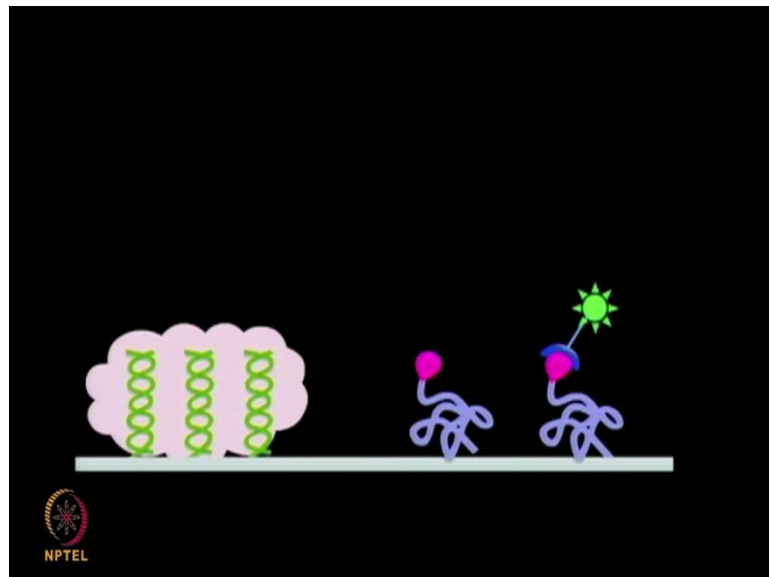
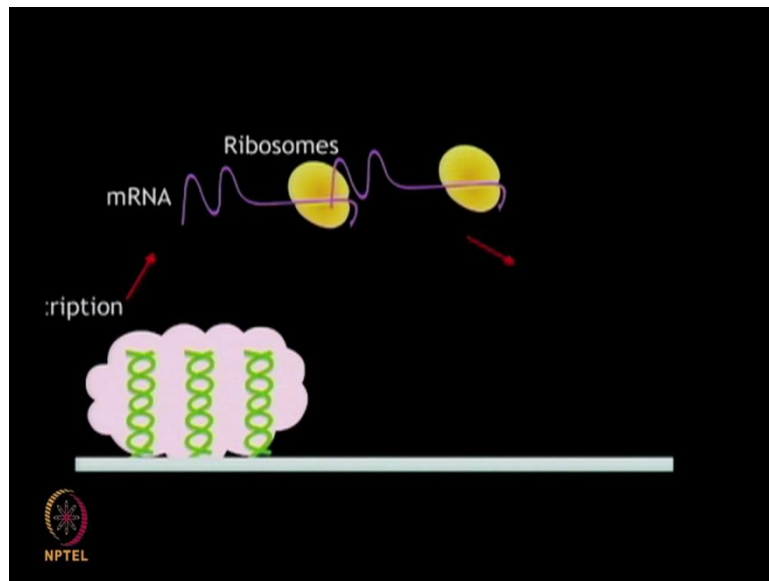
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In this technique, the first spotting step of the multiple spotting is capable of producing high density arrays. It involves addition of template DNA onto the solid array support. The template DNA can even be in the form of unpurified PCR product,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

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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.

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MIST: Merits and demerits

- Merits
 - Unpurified DNA products used as template
 - Very high density protein arrays generated
- Demerits
 - Loss of signal intensity with prolonged incubation time
 - Non-specific protein binding
 - Time consuming process




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.

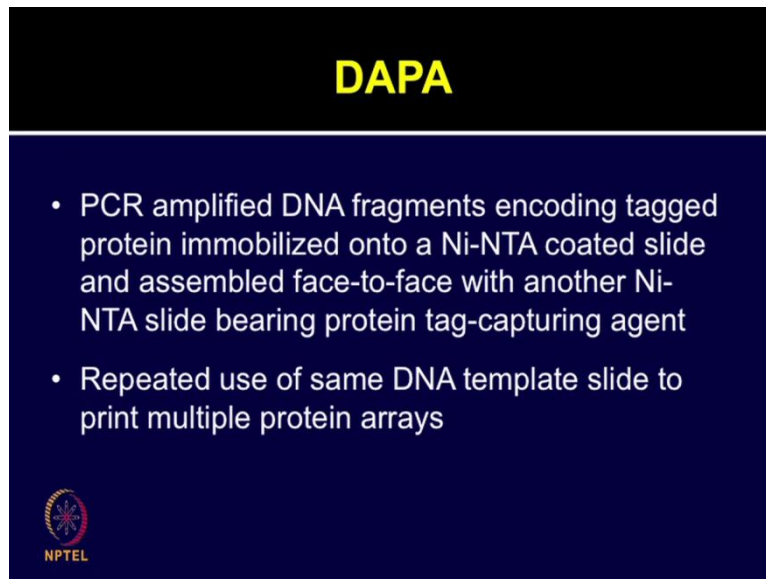
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Points to Ponder:

- MIST involves printing of both DNA and the cell-free expression system on the chip surface in two rounds of printing.
- MIST allows cell-free expression system to directly synthesize proteins on the same feature as the DNA thus circumventing a separate step of cell-free expression system addition.
- Proteins are immobilized on the activated array surface by a tag-capturing agent or non-specific ionic interactions.
- It allows printing of high-density protein arrays which are its merits, however, demerits involve loss of signal intensity with prolonged incubation time of the arrays.




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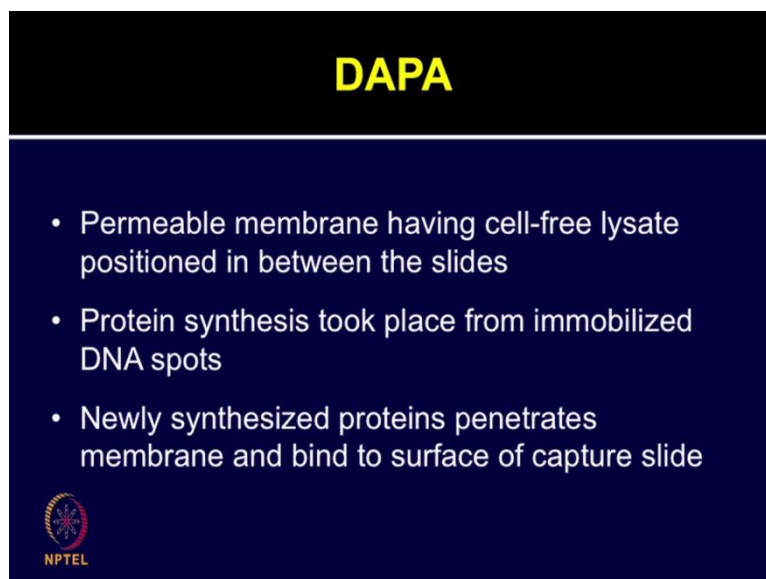
DAPA

- PCR amplified DNA fragments encoding tagged protein immobilized onto a Ni-NTA coated slide and assembled face-to-face with another Ni-NTA slide bearing protein tag-capturing agent
- Repeated use of same DNA template slide to print multiple protein arrays




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.

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DAPA

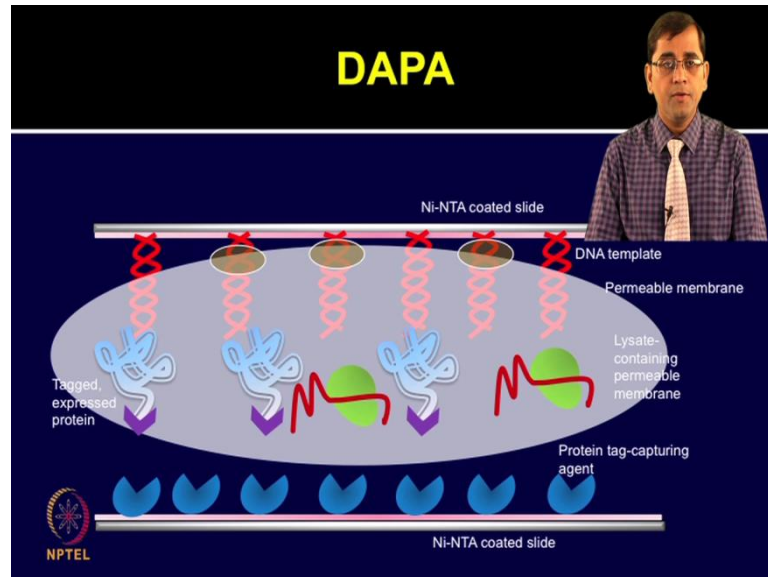
- Permeable membrane having cell-free lysate positioned in between the slides
- Protein synthesis took place from immobilized DNA spots
- Newly synthesized proteins penetrates membrane and bind to surface of capture slide



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

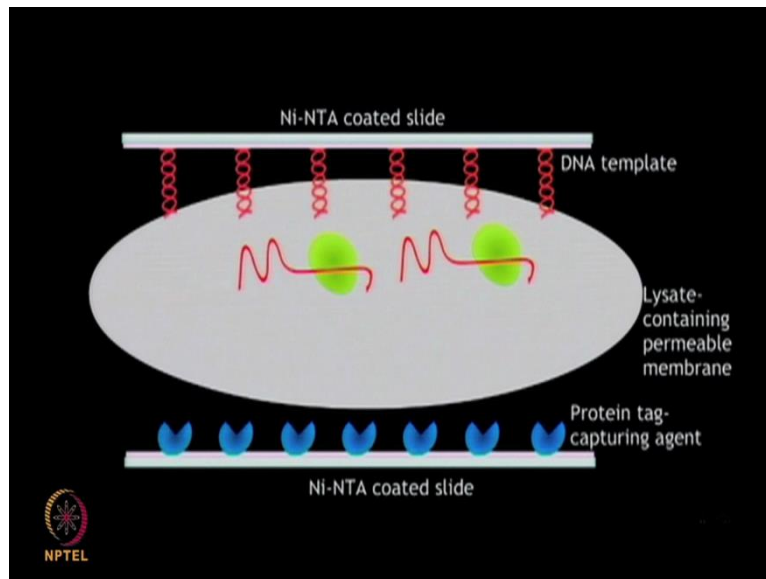
on this membrane and then the synthesized protein diffuse from the membrane and then move on to the other slide for capture. The newly synthesized protein penetrates the membrane and bind to the surface of capture slide that can be seen in this slide.

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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 hexahistidine 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.

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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.

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DAPA: Merits and demerits

- Demerits
 - Reusable DNA template array
 - Pure protein array generated
 - DNA template array can be stored for long durations
- Demerits
 - Broadening of spots due to diffusion
 - Not ascertained if multimeric proteins assemble effectively

Time consuming process




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.

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Points to Ponder:

- PCR amplified DNA fragments encoding tag proteins are captured onto a Ni-NTA coated slide and assembled face-to-face with another Ni-NTA slide bearing protein tag-capturing agent
- A permeable membrane containing cell free lysate is placed in between so that newly synthesized proteins penetrate and bind to the surface of capture slide
- Using DAPA same DNA template can be used to generate multiple protein arrays



Points to Ponder:

- Merits includes increase of throughput due to reusable DNA templates and pure protein preparations however diffusions through membrane may cause spots to broaden, which is a demerit of this technique




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 poly-ethylene glycol coated glass slides

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


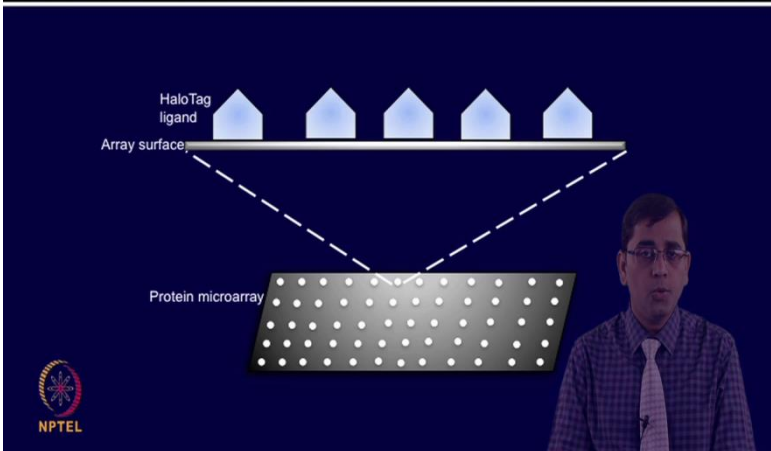
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HaloTag Arrays

- HaloTag - a 33 kDa engineered derivative of bacterial hydrolase, used to tag desired protein
- Proteins fused with HaloTag expressed using WGE/RRL and covalently captured on a PEG-coated slide, activated with HaloTag ligand
- Enables oriented capture of proteins
 - ensuring no loss of function

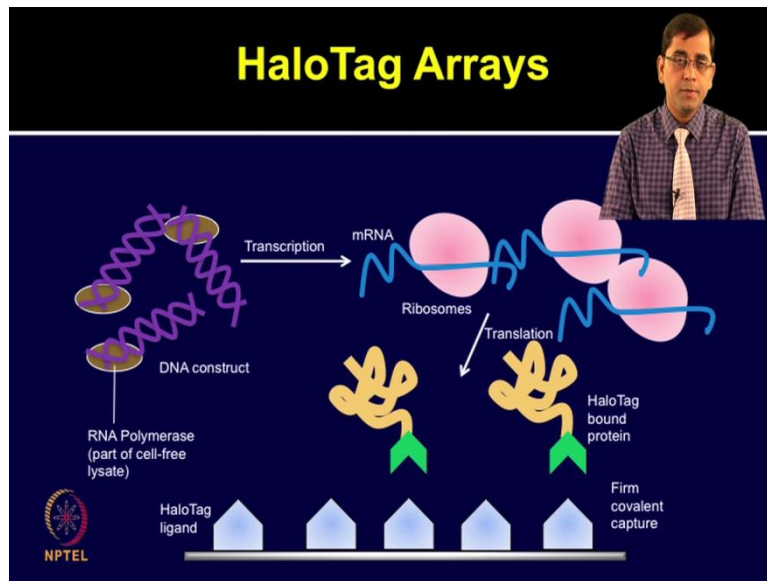


HaloTag Arrays



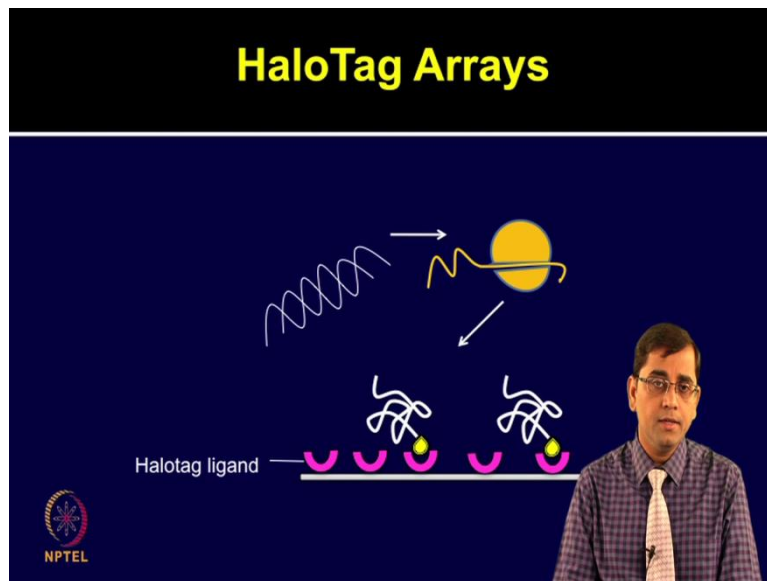
The halotag is a 33 kilo Dalton engineered derivative of bacterial hydrolase which is used to tag desired proteins. The proteins which are fused with halotag are expressed by using wheat germ extract expression system or rabbit reticulocyte lysate and covalently captured onto PEG containing slides. These 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.

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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.

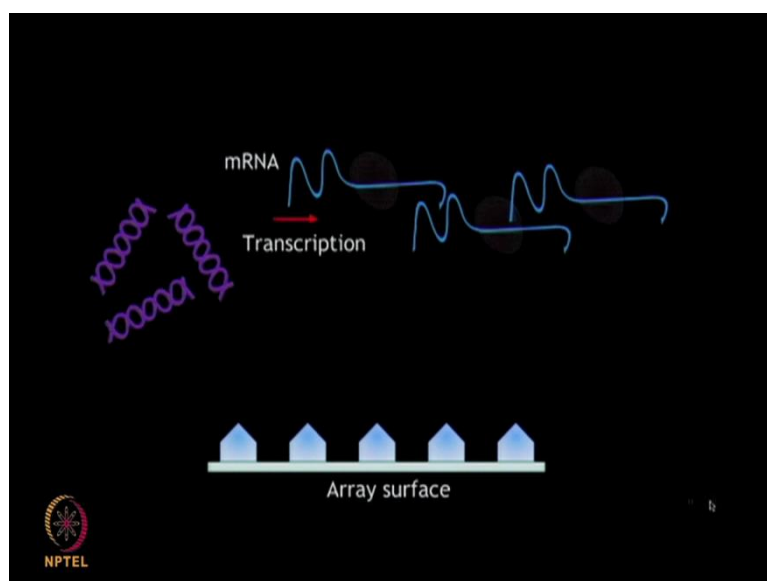
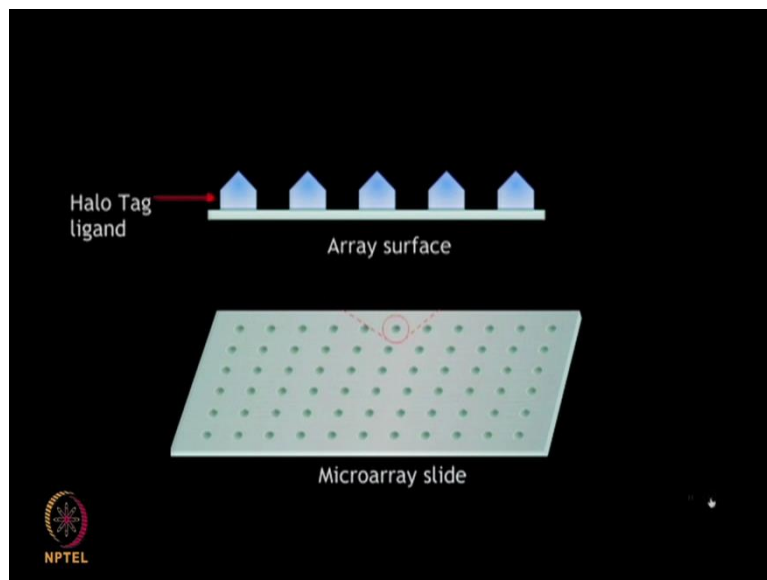
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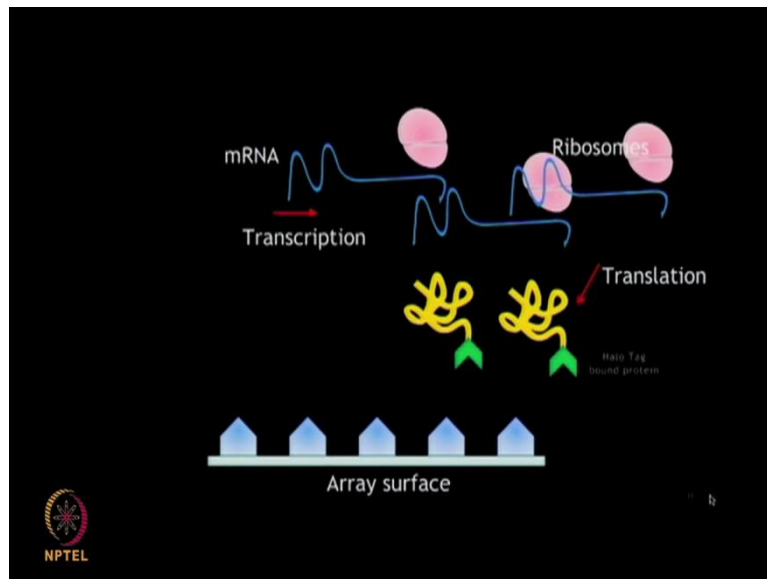


In a nut shell (())(13:29) the halotag fused protein is expressed as can be seen here and covalently captured on PEG-coated slide and activation is performed by using halotag ligand. This provides very strong covalent interaction and minimizes loss of synthesized protein which usually occurs in other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

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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.

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HaloTag Arrays: Merits and demerits

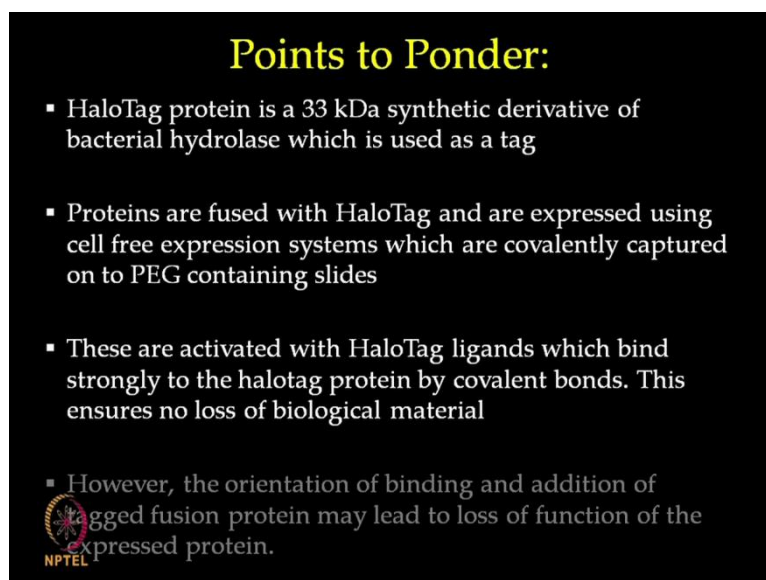
- Merits
 - Strong covalent bond between protein and ligand
 - No material loss during washing
 - Oriented capture of protein
 - No non-specific adsorption
 - Easy quantification
 - No need for a microarrayer printer
- Demerits
 - Possible loss of function on binding to Halotag
 - HT application will require optimization of printing

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

adsorption due to PEG-coating. The quantification is easy and one does not need the microarray printer to print the proteins on this chip because the commercial kit of halotag arrays provides a gas kit, which can be used for printing the arrays. However, there are certain limitations of using halotag arrays. It has not been shown that this 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 needs to ensure that even at the high density these arrays 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 be addressed. However, more biological questions need to be addressed on these arrays before confidently applying this technology for the clinical applications.

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Points to Ponder:

- HaloTag protein is a 33 kDa synthetic derivative of bacterial hydrolase which is used as a tag
- Proteins are fused with HaloTag and are expressed using cell free expression systems which are covalently captured on to PEG containing slides
- These are activated with HaloTag ligands which bind strongly to the halotag protein by covalent bonds. This ensures no loss of biological material
- However, the orientation of binding and addition of tagged fusion protein may lead to loss of function of the expressed protein.

NPTEL

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 of 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.

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Summary

- Cell free expression based protein arrays provide a huge advantage over traditional microarrays:

-They allow increase of throughput, ease of fabrication, reduction of overall costs and circumvents tedious traditional protein production and purification procedures

-Due to availability of CFES in a number of systems (prokaryotic as well as eukaryotic (plant and animal), it is possible to get near native configuration of proteins.

- MIST, DAPA and Halotag arrays utilize these principles to generate cost effective arrays which can allow functional proteomic studies. These arrays can be generated on demand and hence can be stored easily.



References

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