Bioengineering: An Interface with Biology and Medicine
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Lecture – 38
Protein Interactions & Systems Biology

Welcome back to the session of interactomics. In the last lecture, we discussed about different traditional methods to study protein-protein interactions like yeast 2 hybrid immunoprecipitation. We moved on to talk in more detail about latest high throughput technologies like protein microarrays, how it can be used? Today, we are going to talk in the same continuation about interactomics.

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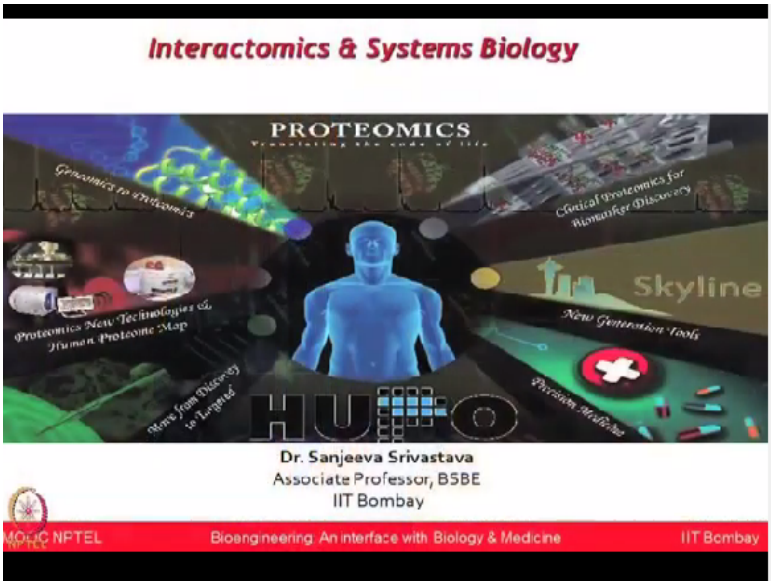

Then I am going to give you an overview of some new tools which can be used like label-free biosensors, especially surface plasmon resonance paired with biosensors, how they can be used to study biomolecular interactions.

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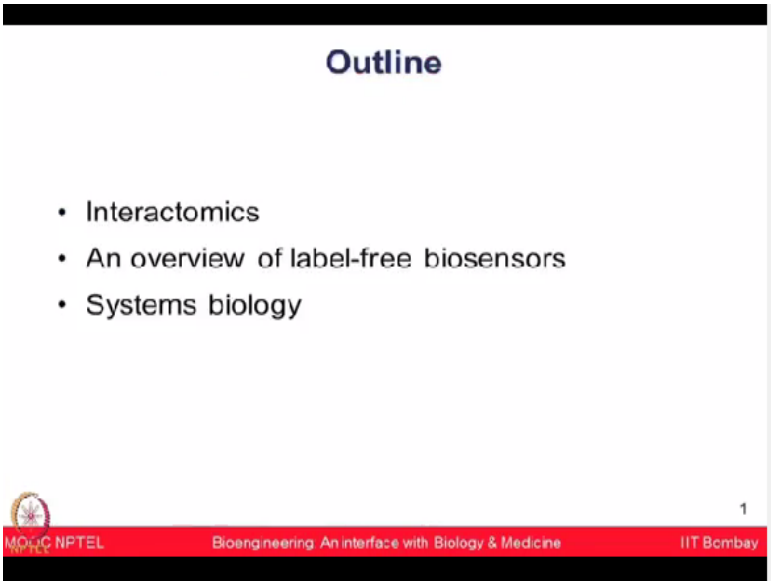

And then finally we will briefly talk about systems biology approach, the new emerging field in looking at a high throughput data.

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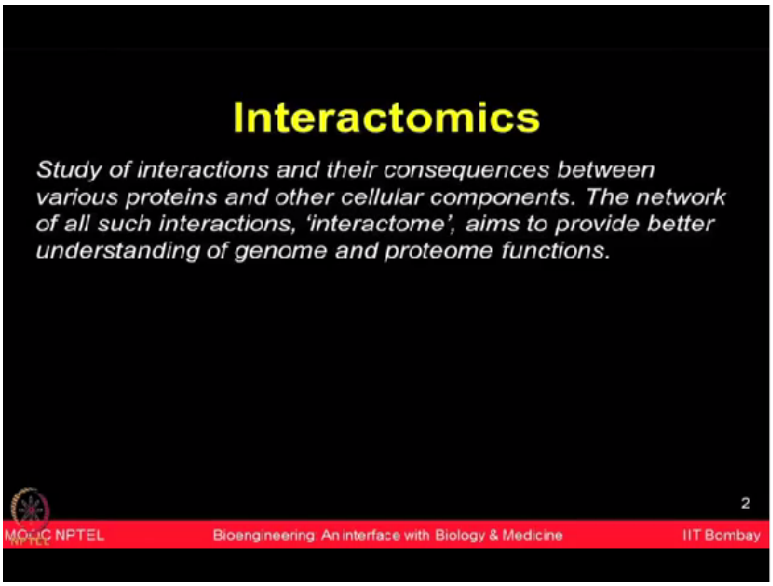

Interactomics is a field which is aimed to study the interactions and their consequences between different proteins and other cellular components. The network of all such interactions, which is interactome aims to provide better understanding of the genome and the proteome functions.

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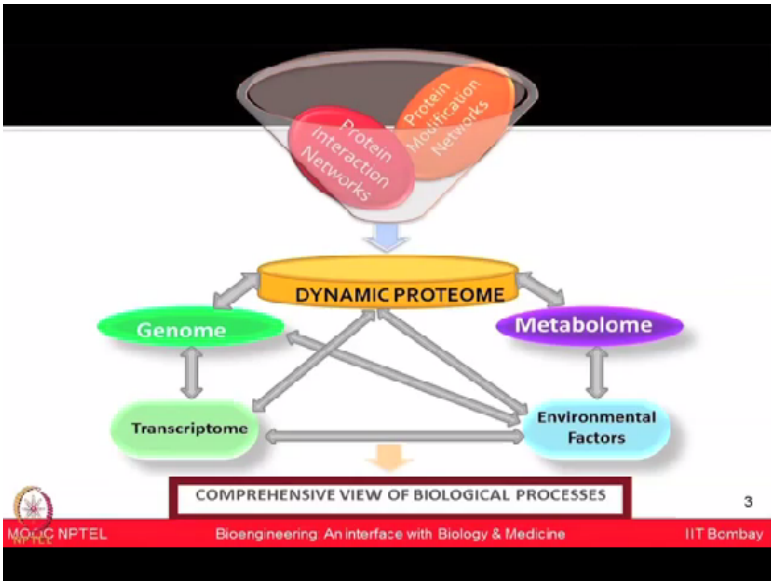

So I had shown in the slide the proteome is very dynamic and if you look at the connection with other biomolecule, is very intricate. For example, it is connected with the genome, metabolome, transcriptome and the various environmental factors. The flux is a fall of them are actually governing the dynamic proteome and to study proteome, you have to look into the protein interaction networks as well as the protein modifications which are happening like different type of PTMs for example phosphorylation, etc.

So all of these are, you know, the complex processes involved in governing any physiological systems.

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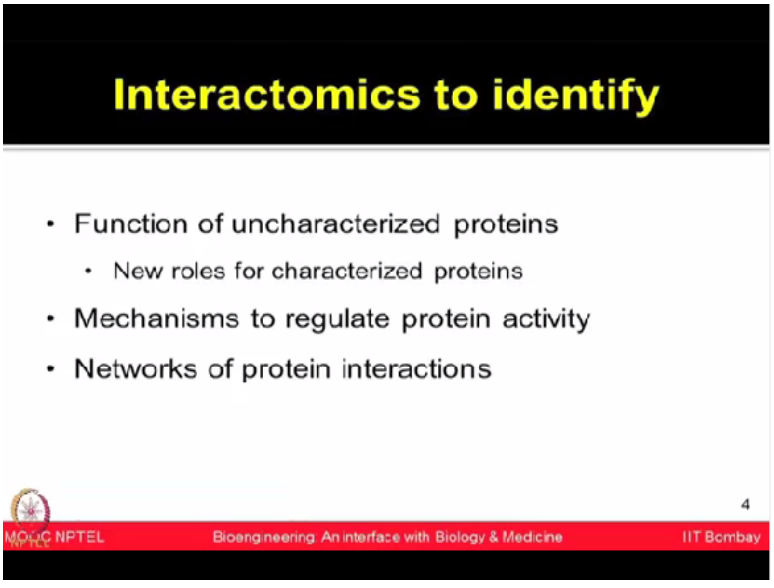

So interactomics aims to identify the function of uncharacterized proteins, trying to define the new roles for the characterized proteins. What are the mechanisms to regulate the protein activity and what are the possible networks of these protein interactions. So some of these are the major goals and to do that, you know, people look for different type of interactions which are sometimes interactions could be very transient just for, you know, fraction of seconds or could be permanent which could be very strong interactions.

So interaction could also be divided into the weak interactions or the strong interactions.

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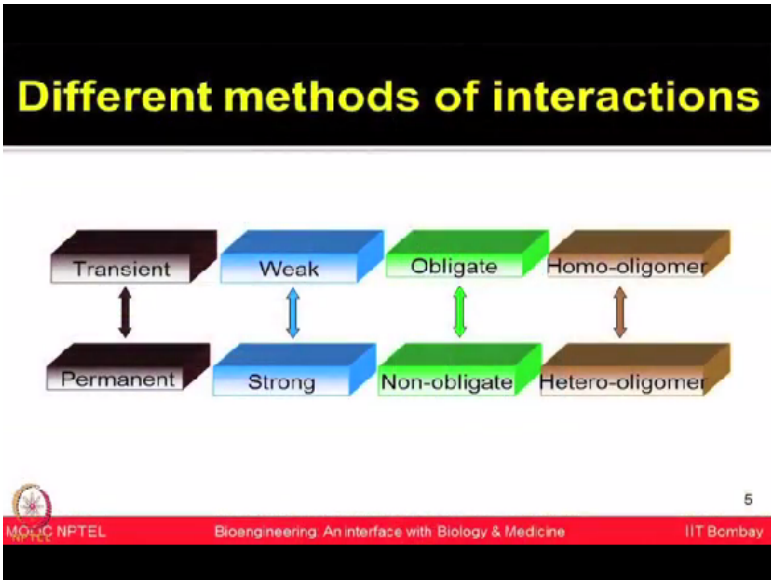

It could be obligate or it could be even non-obligate. It could be homo-oligomer or multiple units can come together as a hetero-oligomers. So different ways of, you know, interactions may happen. And what could be the physical reasons for such interactions to happen?

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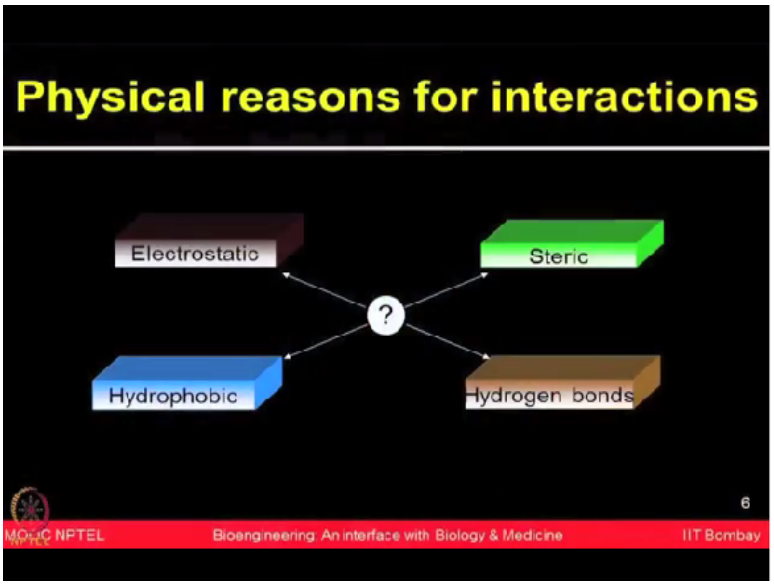

So for example, you know, electrostatic, steric, hydrophobic, hydrogen bonds, all of these are actually involved in these kind of interactions. And again studying and knowing that, you know, different methods of interactions, what are the physical reasons for them and then how to use technologies to study interactions becomes very crucial.

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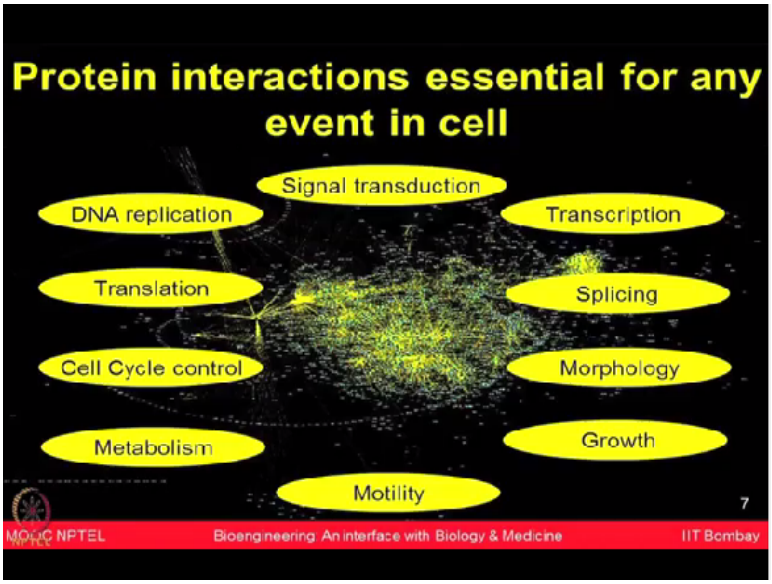

So protein interactions as we have been discussing that it is quite, you know, crucial for any event which is happening in the cell, whether you term as a signal transduction, look at a DNA transcription, translation, even replication process, cell cycle control, various type of metabolic hubs, as well as, you know, the morphology and the splicing, the growth of individuals, motility, many of these things are, you know, involved. Many of these biological events actually depends

on these biomolecular interaction events especially protein-protein interactions to happen.

So we have discussed that, you know, there are 2 broad categories to study the protein interactions and some of the latest technologies which includes protein microarrays and label-free biosensors are what are discussing in more detail to really illustrate all of you that in which way now technologies are really able to help us, the biologists, to find out the information in much more dynamic and much more high throughput and probably much more precise manner as well.

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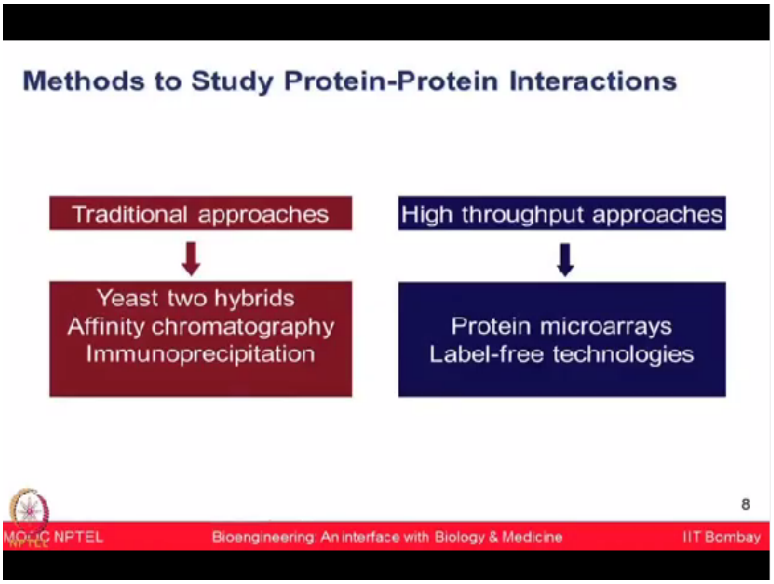

So today, I am going to talk to you about label-free biosensors.

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The label-free techniques, how they can be used to study biomolecular interactions.

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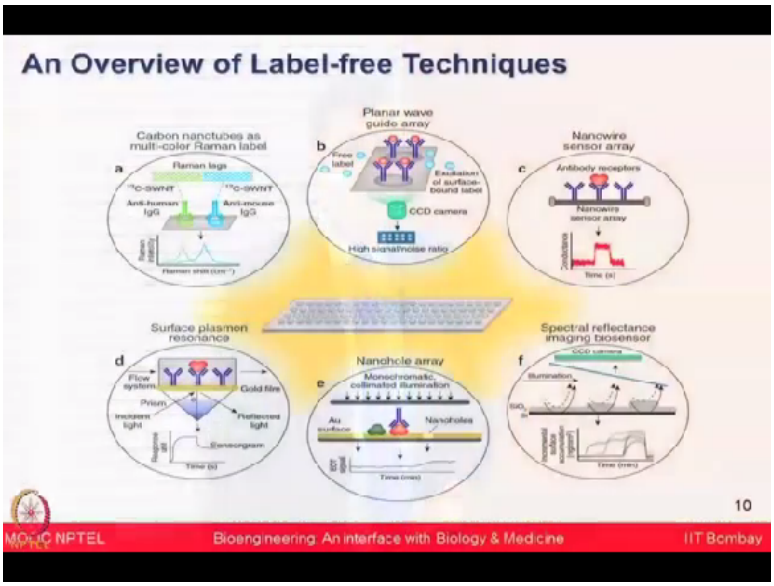

So here I am providing you an overview of label-free techniques and the image shows at least, you know, the 6 of the popular platforms, which could be, you know, not only label-free but also could be linked to the even microarray based platforms.

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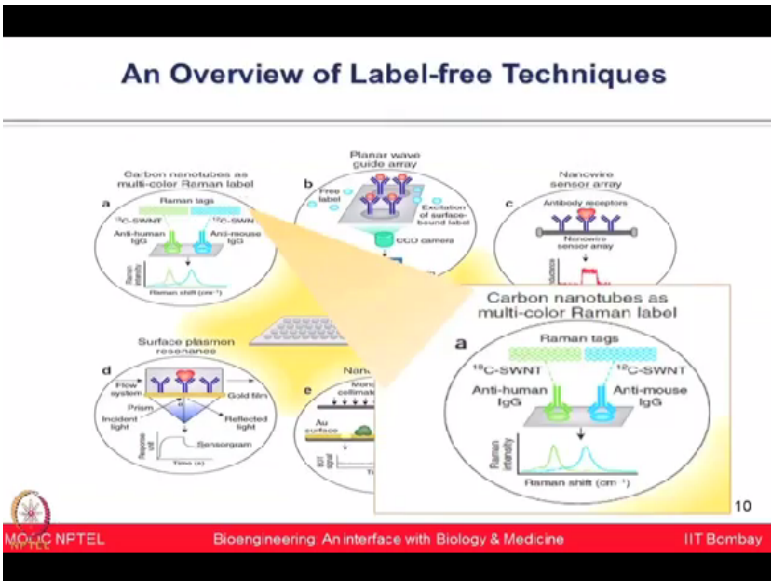

Among them the shown is carbon nanotubes as multicolour Raman labels.

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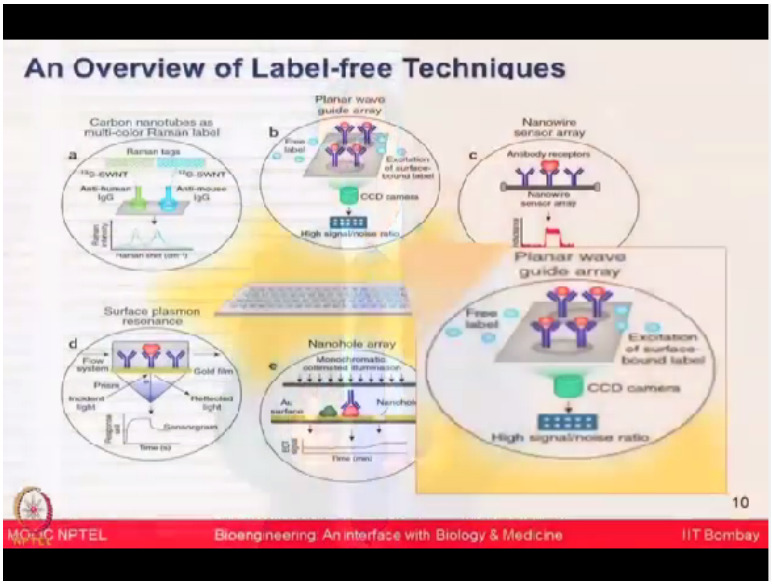

The B panel shows to you is the planar wave guide arrays.

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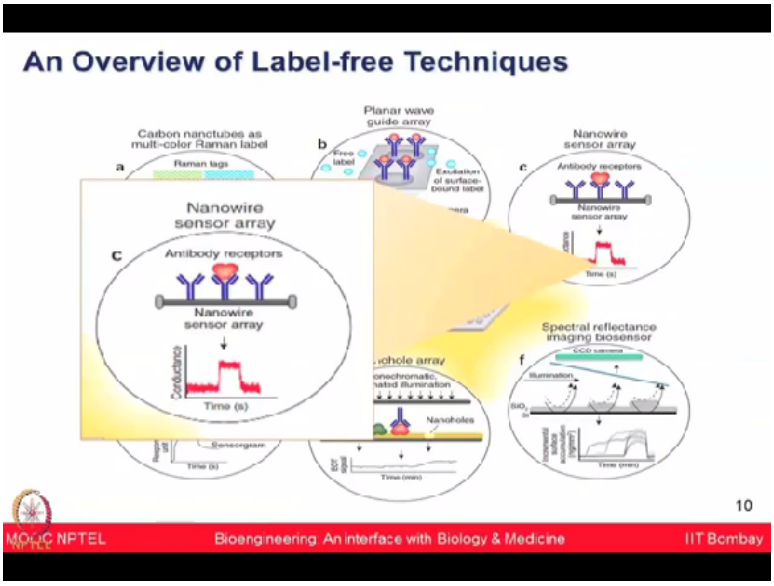

The C panel is about nanowire sensor arrays.

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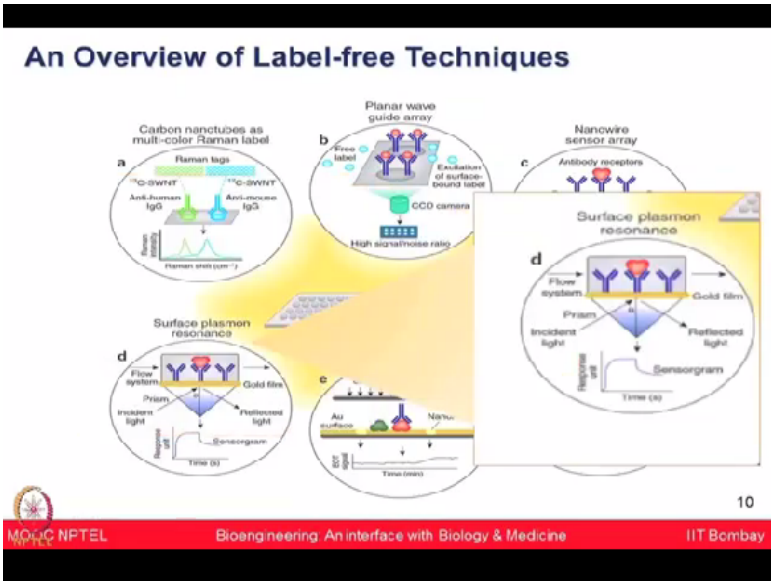

D panel about surface plasmon resonance.

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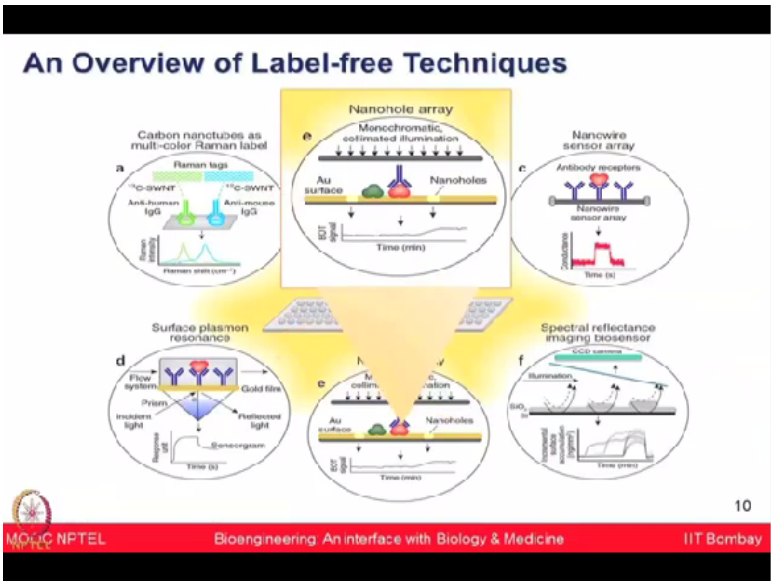

E panel on the nanohole arrays.

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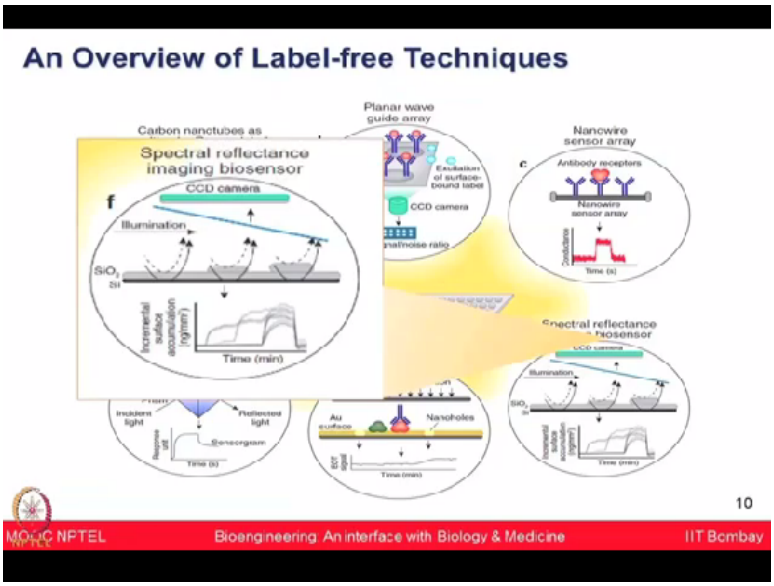

And F panel on spectral reflectance imaging biosensors. So all of these are different circular shown to you is essentially based on some physical principles involved in looking at the biomolecular interactions. For example, you know, when an antibodies are mobilized on the nanotube and if a protein binds to it, what is the change in the conductor which can happen or what is the change in the reflectance in a medium, what is the change happens looking into the interference properties.

So some of these properties have been used to look at the molecular interactions in the label-free

manner. So here you are not adding the Cy3, Cy5 or fluorescence labels the way we have done in the microarray technology but rather we are looking at how 2 biomolecules could interact together and can be measured that is there any change in the physical properties of them happening which could be measured using different type of physical based principles and the technologies which are associated with them.

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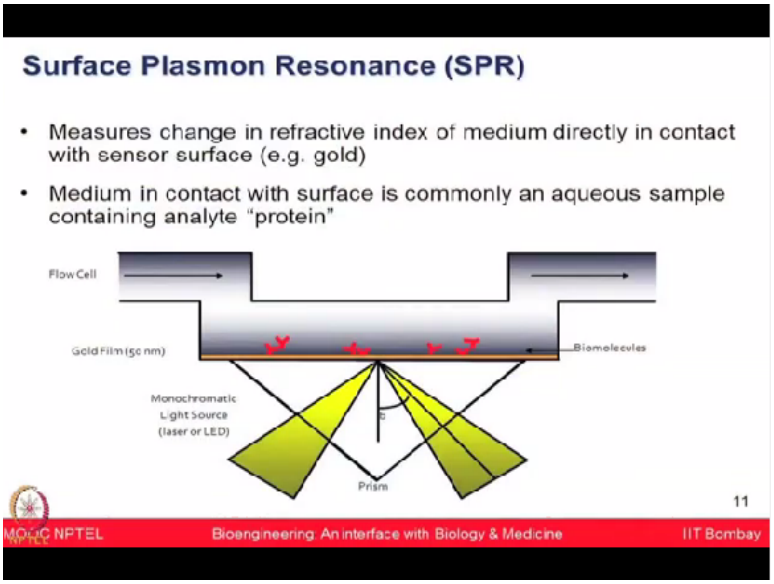

One of the platforms which is quite popular in the field is surface plasmon resonance or SPR. SPR measures changes in the refractive index of the medium which is directly in the contact with the gold surface and as you are measuring the percentage of refractivity change happening or how much, you know, the change in the refractance is there, based on those measurements, you can monitor that whether a binding event is happening or not which could be measured in the form of sensorgram which measures changes in the SPR signal versus time.

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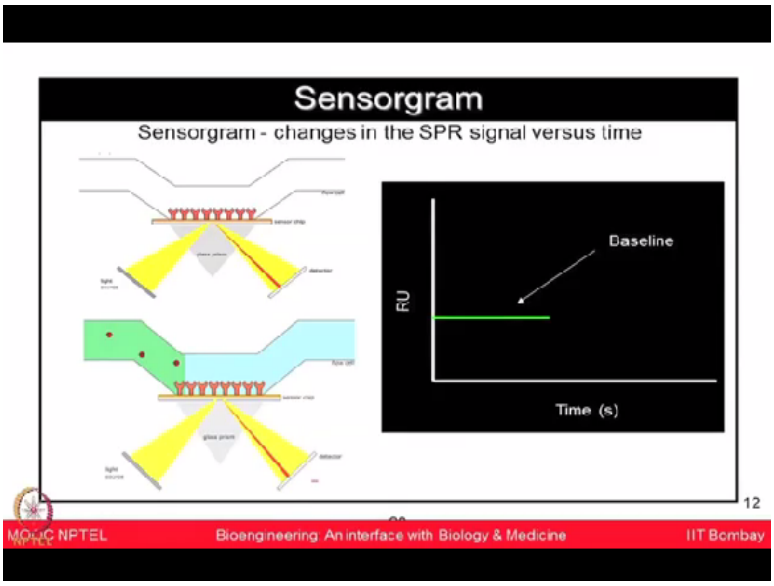

So for example, as you can see in the plot, on the x-axis, we have the time scale. On the y-axis, we have response units and then we are looking at from the baseline initially when there is no interaction happening, the stable baseline is there. And as soon as the 2 molecules start interacting, from those molecules you can see now the association rate can be seen in the green curve if you are seeing. Now the binding can be seen.

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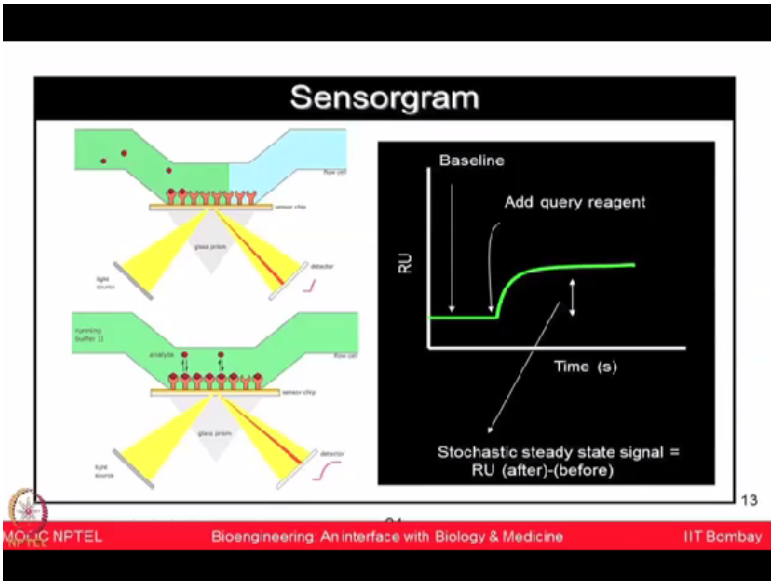

After some time, these binding gets, you know, stabilized. You can see stochastic steady state signal which is RU after and before. And then as the binding progresses, you are continuing your experiment.

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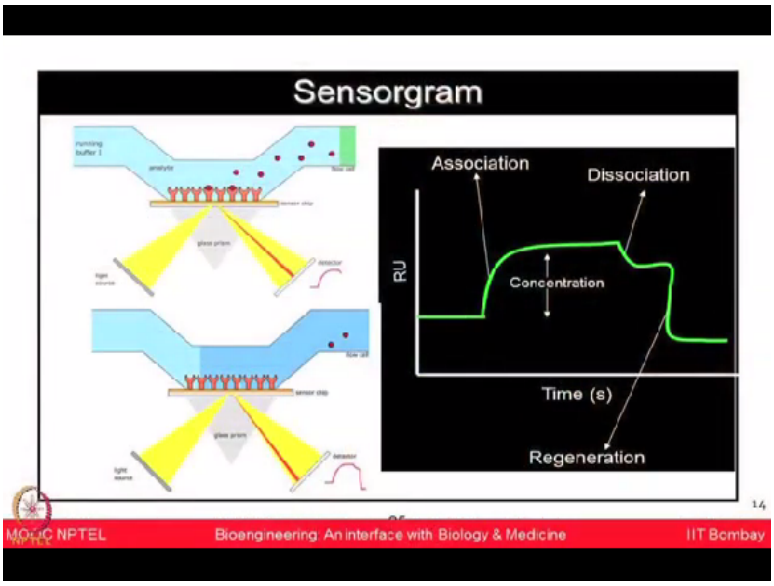

You can, you know, if you are floating more of the buffer and interaction is not very strong, then slowly the molecules will start dissociating out and that is you are off rate. The molecules are getting dissociated.

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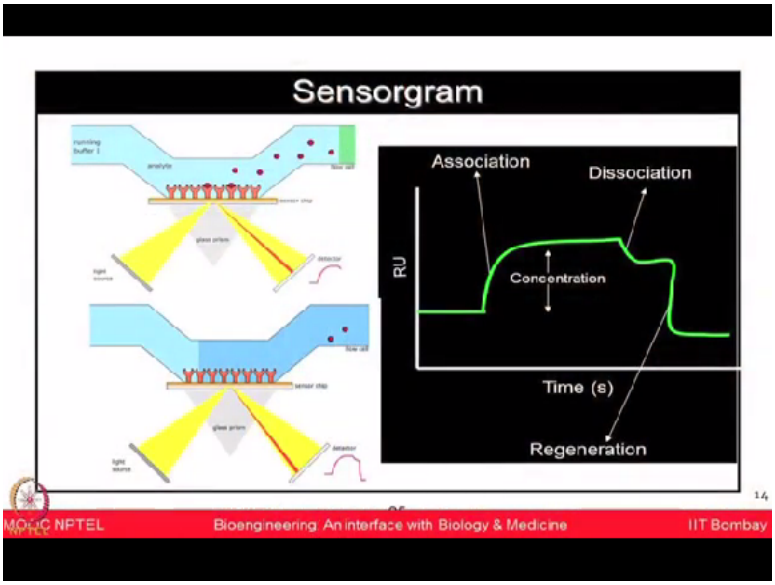

And then finally, you can use some mild assets to regenerate the chip and now that is known as the regeneration process. So in this manner, you can see the sensorgram which involves the stable baseline, the association or the on rate, the dissociation or the off rate and then coming back to the regeneration to make the chip which can be again used back for the, these applications.

However, you know, the currently available SPR platforms can maximal use may be 4 channels or the 4 interactions to study simultaneously whereas think about biological problems and we want to study even may be 100 if not 1000s of these kind of phenomenon to happen simultaneously.

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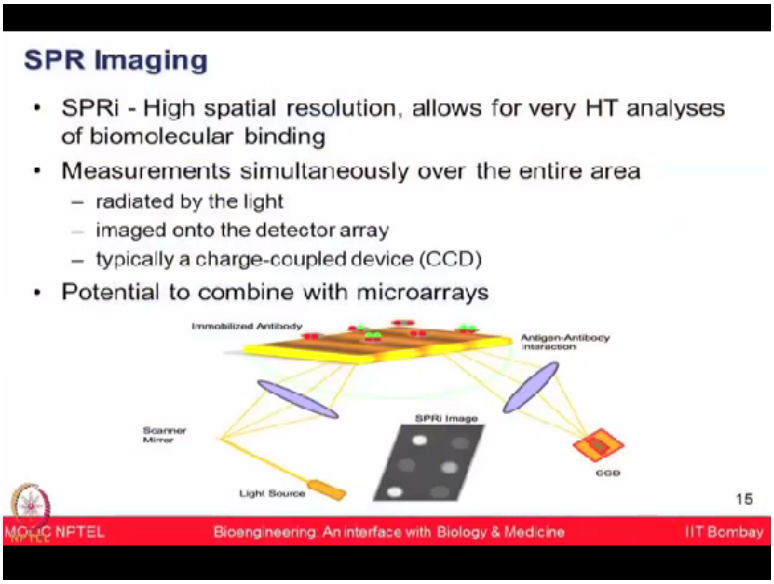

So SPR imaging is a new platform which has come forward which has ability to combine the power of both SPR and the microarray based platforms where intention is to look into the high spatial resolution which could allow for the high throughput analysis of the biomolecular binding.

Here you are measuring the simultaneously the entire area of the chip surface rather than looking at the very specialized, localized area what you measure in the SPR and to do that, the whole chip surface is radiated by the light. Then you are imaging on to the detector arrays, and then you are linking that with the CCD device to capture the whole image.

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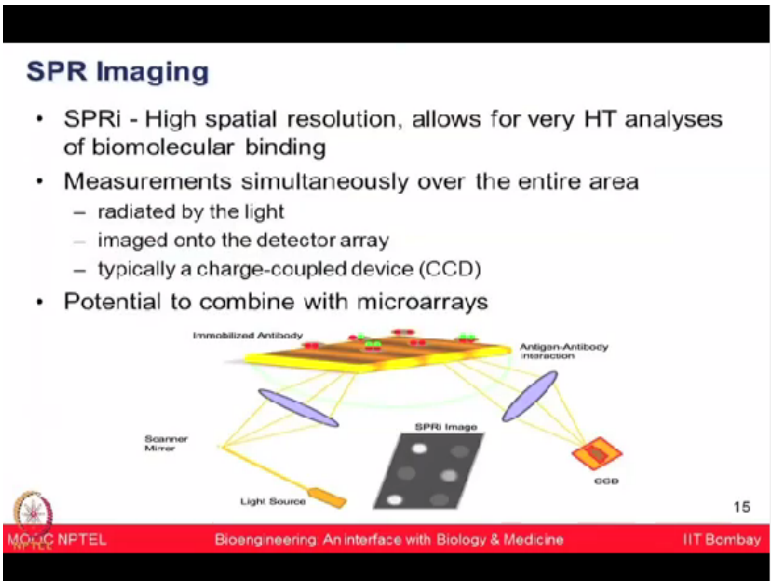

So this platform has shown lot of potential to combine with the microarrays.

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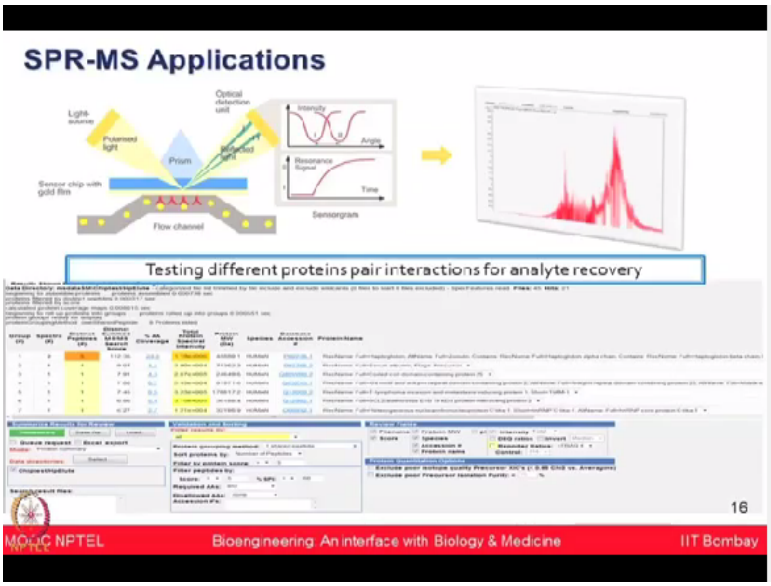

SPR could also be combined with another powerful proteomics platform which is mass spectrometers. For example, you are looking at, you know, how 2 molecules are interacting and from your experiment, you can see there is, you know, binding event is happening but you do not know, you know, this molecule which is bound what this protein is, right. So now to identify those proteins, one could elute them out and then analyze them using mass spectrometers. So now you can say that protein X binding is happening because of the interaction with protein Y.

And now you can identify those using mass spectrometers. So this is a new application which is

SPR image space application. So what I want to emphasize you that in which way technology is, integration of technologies, more robust platforms which are coming forward, are really helping us to build these kind of, you know, newer approaches which can be so relevant to study these biological phenomenons.

So while we have, you know, studied at least, you know, in some detail the basics of the label-free technologies, we are going to provide you some more detail understanding of one of the label-free biosensor platform which is surface plasmon resonance.

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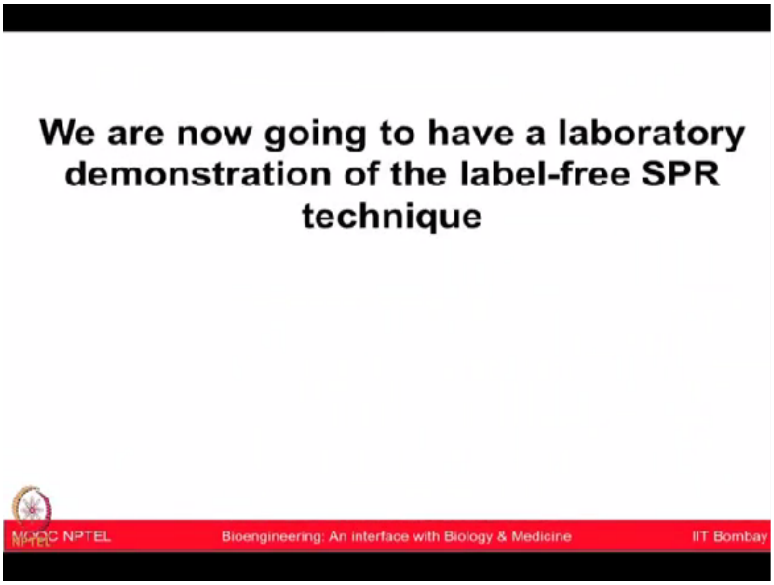

So now let us have the laboratory demonstration for the SPR. (Video Starts 09:56 - Video Ends 30:18)

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Hello. I am Dr. Vaishali Kerekatte. I am a scientist in Dr. Sanjeeva Srivastava's lab at IIT, Bombay. Today I will be talking to you and showing you surface plasmon resonance. So now let me show you the sensor chip that we use for immobilizing the ligand. There are many types of sensor chips depending on what kind of molecule you want to immobilize and study. So typically for proteins, there is a type of chip, if you want to immobilize a lipid molecule like liposomes, there is a different kind of chip.

Today, the experiment, the binding experiment we are going to conduct is going to be studying beta 2-Microglobulin antibody which is the ligand and beta 2-Microglobulin protein as the analyte which will be passed over the ligand. So we are going to immobilize anti beta 2-Microglobulin antibody on this sensor chip. This is a CM5 sensor chip which is a very versatile chip that is used. Let me quickly show you the surface so that you can have an idea of…

So this is a plastic casing and inside it is the chip and this is the gold surface on which the ligand will be immobilized. So this is housed inside and this is the entire thing is then put in the machine. Now these are various sample racks. So depending on how many samples you have, what is the volume of your samples, you can use any of these sample racks. So in these racks, depending on how much volume of reactants you are using, there are different kinds of vials that can be used.

So you have these kind, you have these kind. So this takes more volume than this one. And they fit into these, into the rack very nicely. And after you close it, this entire thing will go into the machine. This is the Biacore T200 machine. This is where we conduct our SPR experiments. Let me show you the different parts of the machine. So this is where the sensor chip goes, this compartment.

So you control it through the monitor. If you say eject chip, it takes about a minute for it to eject. So you will see it coming out and then you insert your sensor chip. This is the sample rack compartment. This is where your sample rack goes in with all your different tubes. Now if you see on the left side, this is where you have a bottle with your running buffer. So this is the buffer that goes through the machine as well as the one that flows over your sensor chip. This is also typically the buffer that is used for your binding reactions.

So you have to make sure that this buffer matches exactly with the buffer that you use for diluting your ligands or diluting your analytes, etc. Then on the right side, you can see there are 2 bottles here. One is just plain water which is used for flushing out and this is the trash bottle where all the waste comes out. Okay, so this is where, this is the compartment where your chip goes in.

You can see any kind of chip that you can enter into this compartment. So typically when the machine is on standby, we put in the maintenance chip. So I am just going to close it and say dock chip for right now. This software that we control the machine through is called the Biacore T200 control software. There is another software here which is called the Biacore T200 evaluation software.

So that is where you get the output of your experiment in the form of sensorgrams and once we are finished with the experiment, I will show you how to extract the sensorgram and how to interpret them. Okay, so now our chip is docking. Now we can go start the experiment. I will show you how to set it up and then how to put the samples in and start the binding experiment, okay.

So now we are going to remove the maintenance chip and we will be adding our CM5 biosensor chip that I showed you before. So we insert it here and we are going to dock it. Here we will say, so this is the step of docking of the chip. An SPR experiment has 3 major steps. The first is the immobilization step. This is where you immobilize your ligand on to the chip surface. The second step is the interaction step where you are actually measuring the interaction between your ligand and your analyte.

And the third step of an SPR experiment is regeneration. This is where after your analyte has bound, you want to make sure to remove every molecule of analyte that is bound to the ligand so that you make the chip surface available for the next round of experiments. So your ligand stays bound but the regeneration removes every analyte molecule that is bound in the previous experiment.

So now in the first step, we are going to do the immobilization step. We use something called as amine coupling in which you are using EDC and NHS to activate your chip surface. The chip surface has a dextran matrix to which there are carboxymethyl groups. So your protein is going to attach to this through amine groups. So in this step, you are activating a surface, adding your ligand and then washing off the excess and then blocking the parts of the surface that are not bound with your ligand.

So this is the first part of the experiment which is the immobilization step. So when we start our immobilization experiment, we will be controlling everything through the control software. We open the wizard that is there for immobilization. So open new wizard template, click on immobilization, okay. So this window opens up. Here each sensor chip has 4 channels on it. They are called as flow channels and typically per experiment you use 2 flow channels.

One is called the reference flow channel. The other is called a experimental one. So what happens is in your reference flow channel, you do not put any ligand. It is either a dummy ligand or you just put buffer through but it goes through all the steps that are involved in immobilization. The reason being is the reference flow cell allows you to measure any kind of non-specific binding that might be happening and then in your second flow cell is where you

include the ligand.

So the flow cell 1 goes through every single step that your flow cell 2 will undergo except that it will not have any ligand on it. You sample compartment temperature is also 25 degrees. In this machine, you can control the temperature from 4 degrees to 40 degrees. So depending on what conditions are optimal for your binding experiment, you can choose the temperature. Then you click next.

So this gives you the rack positions. So basically it tells you these are the different types of reactants that you will have to put into the machine and this tells you where to put it in the rack. So if you see the rack positions, this exactly corresponds to the rack here. So for example, this is how you place it. So for example, this position is A1 which is shown here. So this one corresponds exactly to this one.

So as you can see B1 is the description of what you need to put in B1 is over here. So B1 is EDC-NHS, those are the activating reagents. B3 is empty because these are being very reactive compounds. They will mix inside the machine, the machine will take in the reagents from each of these tubes and then mix it here. So you need to keep an empty tube. Ethanolamine is the reagent that is used to block the sides that have not been occupied by your ligand.

So 1, 2, 3, 4; B1, B2, B3 and B4 are for your flow cell 1 which is the reference flow cell. The same thing gets repeated for your flow cell 2. The EDC, NHS, empty tube, ethanolamine. Here there is one extra tube which is your ligand which is the anti beta 2-Microglobulin antibody that is your ligand. So I am going to place these tubes into each of these corresponding positions. So you have EDC which goes in to B1.

You have NHS which goes into B2. You have empty which goes into B3. You have ethanolamine which goes into B4. So now we are finished with the B row. Now we start with the C. You get EDC, you have NHS, empty, ethanolamine and your ligand, okay. Then you close the rack and this is now ready for inserting into the sample compartment rack. So what you do is then you click on next and place your…

So now we have placed the rack on the stand and we are going to insert it into the machine. Once the insertion of the sample rack is over, you click on next. So then it gives you a run protocol and you have to follow all this. At this point you can check whether you have inserted the correct sensor chip, whether all the samples and reagents are in the rack. So you get this window where you get your run protocol.

Here you make sure that the, at this stage you make sure that the correct sensor chip is docked. Make sure that all the samples and reagents that you want are loaded correctly. Then you place the buffer, make sure that the correct buffer is in your left hand side tray. Make sure that there is enough buffer for the entire run. Also that the caps are tight and there are no bubbles that will be taken up by the tube.

Also if your waste bottle is full, make sure to empty that. Make sure there is fresh water in this right hand side bottle. And then you will click on start. So that starts your immobilization reaction. Okay, so now we have finished the immobilization step of this binding reaction. Let me show you what it looks like. So now at the end of the immobilization step of the SPR reaction, we open the Biacore T200 evaluation software and I have opened the file, the output is a sensorgram.

So you click on all sensorgrams. So as you can see, this is, let me show you first the reference cell. So this is the sensorgram from the reference cell. Here as you can see, this is the step where the EDC-NHS is acting and this is the ethanolamine step where the unbound sights are being blocked. If you go to our experimental flow cell where we have added the ligand, as you can see here, this is the EDC+NHS.

Then the ligand is being bound to your surface and then as you put the ethanolamine, your unbound sights are being blocked. So you can see the difference between your experimental cell and your reference cell where there was no ligand being bound. So that concludes the immobilization step. Your ligand is now bound to your sensor chip surface. Now we will go to the next step which is the binding reaction. In this step, we will be passing the analyte over the

ligand, over the chip surface which has a ligand on it.

Now we will start with the binding experiment. Once again we work with the control software. We are going to open a new wizard template, binding analysis. Okay, so if you remember from our immobilization, we have used flow paths 1 and 2. So we will be doing a 2-1, that means whatever is measured in your reference flow cell, that is flow cell 1, will be subtracted from your flow cell 2 to give you a specific binding reaction.

Chip type is of course CM5. So you will be flowing your sample which is your analyte which is the beta 2-Microglobulin protein and after each sample flow, you will have a regeneration step. You click next. We will not be doing any conditioning or startup cycles here. So the main thing to remember in a binding reaction is that you just want the yes, no answer. Is your analyte binding to your ligand or not.

So we are giving very simple instructions here. We give a very standard flow rate. We do not need to do any extra steps. We just want a yes or no answer to whether binding is happening or not. So we give a contact time of 180 seconds that is enough for your ligand to bind to your analyte. Your flow rate is 10 microliters/minute. Then your regeneration solution, in this case, it is a low pH solution, glycine HCl, so the contact time of your regeneration solution on the chip will be 30 seconds, flow rate of 10 microliters/minutes and a 5 seconds stabilization period.

Then you click on next. So your beta 2-Microglobulin protein will be in 2 different concentrations. So you can check it under low concentration and under high concentrations and each of these will be done in duplicates. So the low 1 and low 2, high 1 and high 2. And then since we have already primed during the immobilization step, we will not be doing that. The temperature settings are the same.

Then you go to next. So again you get the rack positions here and it tells you in which position to put each of your reaction mixture. So the first one is your glycine HCl which is the regeneration solution, goes into A1. So I am going to place the glycine in the A1 position. Then in your B1 is the beta 2-Microglobulin protein high. So this is high 1 which is in B2. Then your, sorry your B1.

Your B2 will get high 2. So I will place that in B2. B3 has low 1 and B4 has low 2. Close the sample compartment. Click on eject rack. Okay. Click on next. Once again you get the run protocol. Basically make sure all your checks are in place. Your samples and reagents are loaded correctly.

Make sure your buffer is placed correctly in the left hand side tray. It gives you an estimated run time. So this entire binding reaction will take about 26 minutes. And then you hit on start which will start the binding reaction. Once again once your binding reaction is over, you open the Biacore evaluation software and you can open your sensorgram through this software. So let us open the file.

Click on all sensorgrams and here you can see the lower 2 lines are the low concentration of your analyte and these 2 are the high concentration of your analyte. So as you can see initially, this initial part is where the buffer is being flown over your sensor chip. As you start, as the analyte starts flowing over your chip, you see an increased binding.

At some point, you stop the analyte and you start the buffer and it starts going down and then you have your regeneration solution which gets it back to baseline and then again your buffer is flowing through. So as you can see the low concentrations are giving a low binding whereas the high concentrations are giving a high binding and the duplicates are showing repeatability. So basically the binding experiment has worked.

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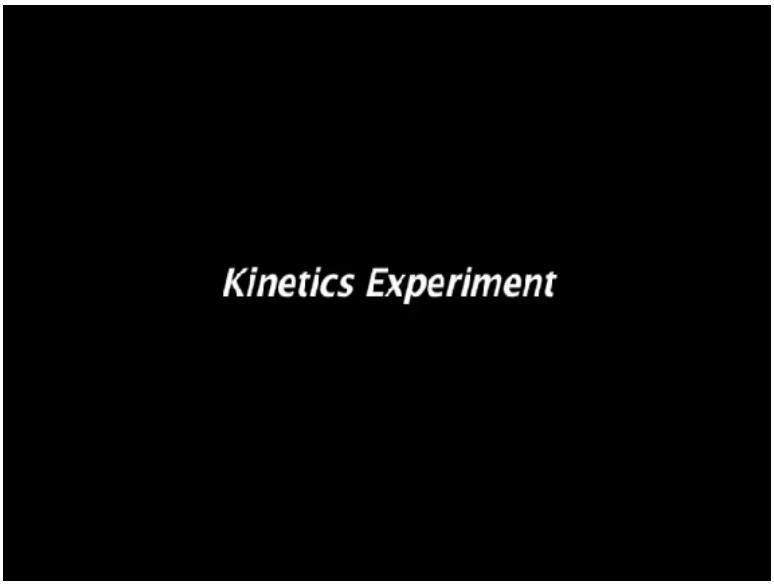

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Let me also show you a kinetics experiment which we have not performed but I will show you a typical sensorgram that you get from a kinetics experiment. So what we saw just now was a very basic yes, no kind of experiment where you are asking the question is my analyte binding to the ligand or not? In a kinetics experiment, you get much more information. So it is an experiment where you can find out the association constants, the dissociation constants, the affinity constants.

Basically you have a concentration range of analytes that you will pass over your ligands and

you can calculate the various constants based on the sensorgram curves that you get. So let me just show you, so click on all sensorgrams. Surface bound because we are measuring kinetics on the surface of the chip, okay. So here what we have done is, we have used a range of analytes. We have used 0, 2 nanomolar, 4 nanomolar, 8 nanomolar, 16 nanomolar and 32 nanomolar of analytes that are passing over our sensor surface.

So if you see these curves, this is the 0 concentration, 2 nanomolar, 4 nanomolar, 8 nanomolar which was carried out in duplicates, 16 nanomolar and 32 nanomolar. So as you can see, you know, here you have the buffer. At this point, at 0, you have started passing your analyte over the ligand. So you can see an increase in the association of your analyte with the ligand. At this point, you stop passing ligand and only buffer is passing.

So you can measure the dissociation of your analyte from the ligand, okay. And then you have regeneration. So if I click next, let me just go back for a minute. So here again you are using 2-1. So in SPR it is very important to do something called as double referencing where you use reference subtraction as well as blank subtraction. Blank subtraction means you always have a 0 concentration which is subtracted from each of your measurements and reference subtraction means you are subtracting the reference flow cell from your experimental flow cell.

Both these ensure that the signal that you are measuring in your experiment is very specific to your ligand-analyte interaction and not due to some kind of nonspecific binding that is being, which is brought about by any kind of molecule in your buffer, okay. So here you have blank subtracted sensorgram. So you see that the 0 has disappeared. Then you click on kinetics, okay. Now here you are going to be fitting to a 1:1 binding model.

So you click on fit. It goes through a bunch of iterations until you get the best fit, okay. And then this is the output. So if you see these tabs here, there are 4 tabs, a quality control, report, residuals and parameters. So in your quality control, you see all the green tick marks which means that all the QC checks have passed. So kinetic constants are within your instrument specs. The kinetic constants are uniquely determined and there is no significant bulk contribution.

If you go the report, you can see that it has calculated your association constant, you dissociation constant and your affinity constant. Your chi square value should be less than 1 which it is showing. Your U value should be less than 20 which again it is showing. So these are pretty good results here.

Your residuals should be within these green lines here. That means you can except this data. If it lies beyond these 2 red lines that means you cannot except this data and you have to go back to optimizing your experiment, seeing what went wrong. But here there seems to be a very good fit. This what is sensorgram from a kinetics experiment looks like.

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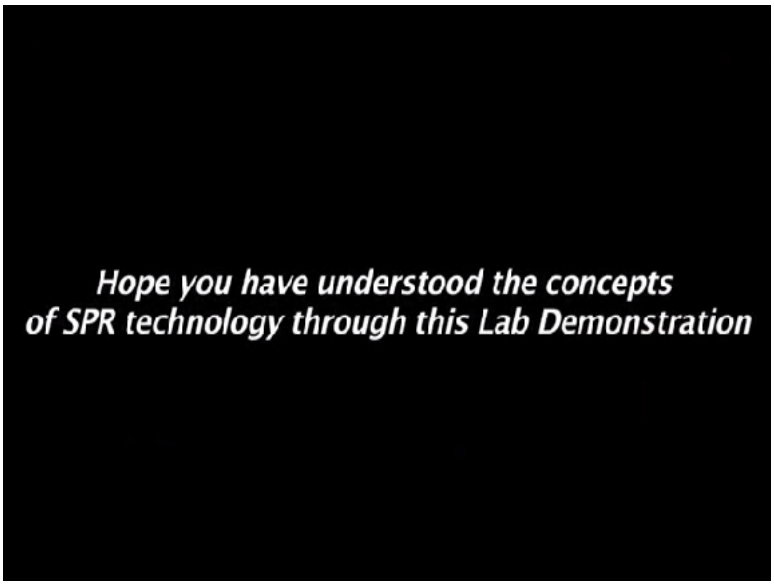

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And all of these omics technologies, you know, which is kind of high throughput data generation technology which generates, you know, big data in a very short time and, you know, in a very short span of duration, aims towards looking at the entire systems. But that is somehow not possible if you are looking at only one part of the picture. If you are looking at only the genes or looking at only the RNA or the proteins. You may not able to get the entire picture of what is happening in the physiological system.

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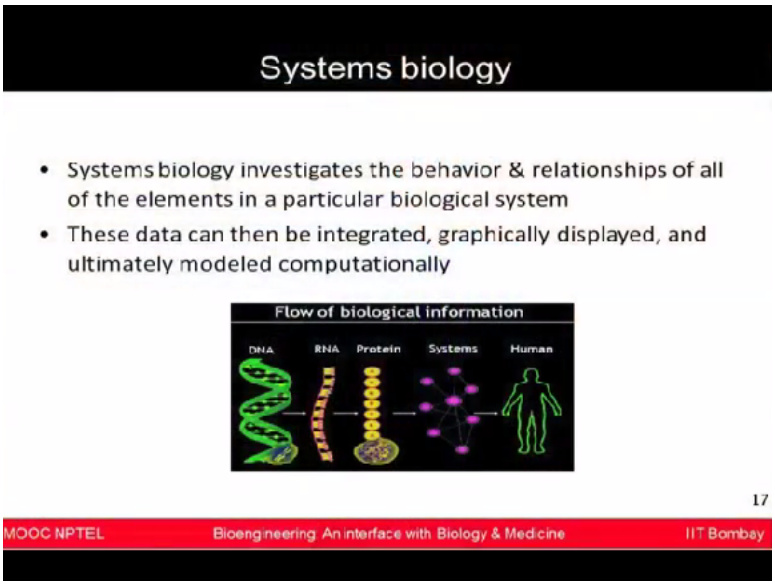

So system biology is a field which investigates the behaviour and relationship of all the elements of a particular biological system and looks for integration of the data which could be then eventually computationally modeled and then finally you can display using formation to enhance

understanding of a complex physiological systems.

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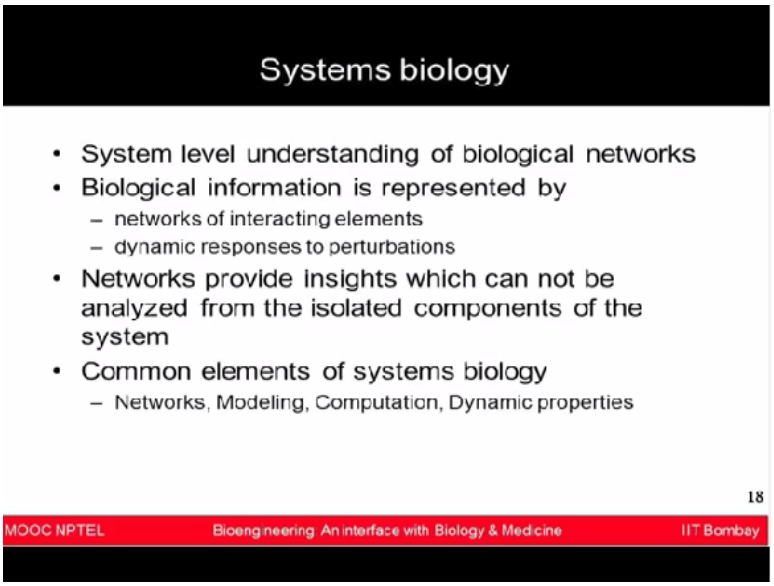

A systems biology aims to provide the systems level understanding of biological networks, the biological information which you obtain could be represented in the form of the networks which are, you know, for the interacting elements, interacting proteins, another biomolecules and how they are dynamic in response to various perturbations which are happening.

So these networks could provide the insights which cannot be analyzed from the individual isolated components of the system and therefore, you have to pay attention to various components which are equally important for the systems biology field. Looking at the networks, looking at the models, doing the computational analysis and the dynamic property analysis. Broadly there are 2 major approaches which people have used to look at the systems based data.

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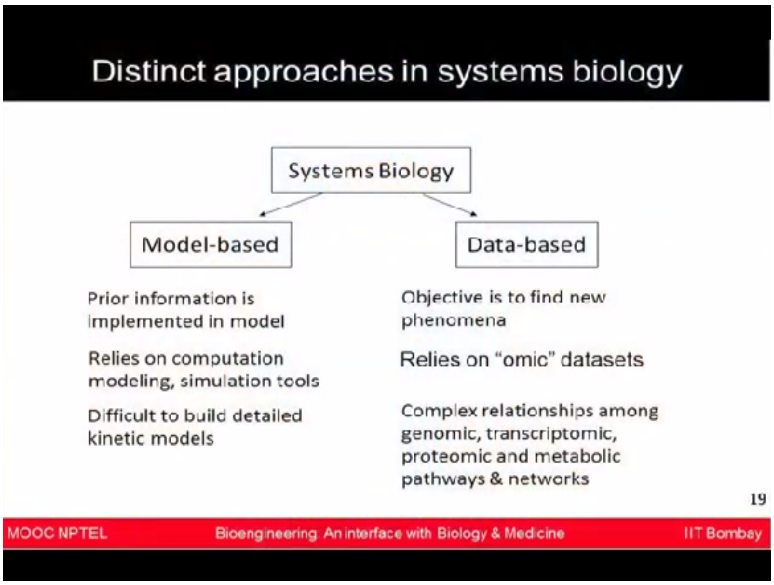

A model based or data-based analysis. In the model based, the prior information is implemented in the model itself whereas in the data based approach of system biology, the objective is to find out what is the new phenomenon there. In model based systems biology relies on computational modeling. For example, you know, different simulation tools whereas data based relies on the omic datasets.

The model based field is difficult to built detailed kinetic models whereas data based, you are having, you know, various informations from the similar data, so you can look into the complex relationship among genomic, transcriptomic, various type of other, you know, proteomic or metabolic networks, various pathways, the networks which could be derived from the scene.

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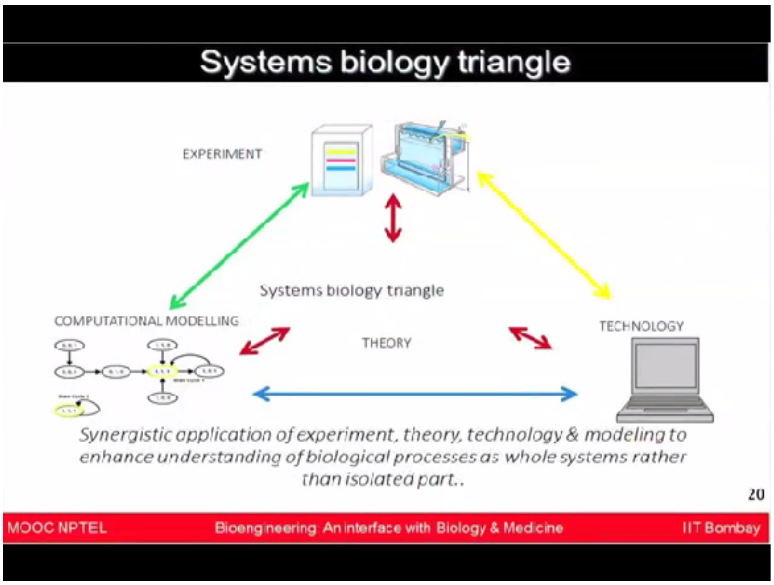

A systems biology triangle involves majorly 3 aspects. One is the experimental part where data is being generated. Second is computational modeling where, you know, you are purposing some models. Third is the integration with the technologies. So all these 3 are the, you know, important components of systems biology which is known as systems biology triangle. The synergistic application of experiment, theory, technology and modeling to enhance our understanding of biological processes as a whole system rather than looking them at the isolated level.

So what is important here that, you know, we want to understand the whole system and if you are looking at systems, probably, you know, the systems properties, it is not, you know, just addition of looking at the DNA+RNA+protein and going to give you, you know, a 1+1+1=3 but rather these individual component information when you analyze in the complex network form, you will find that, you know, probably a new system same properties are emerging which is giving you better clue, the better understanding of the system itself.

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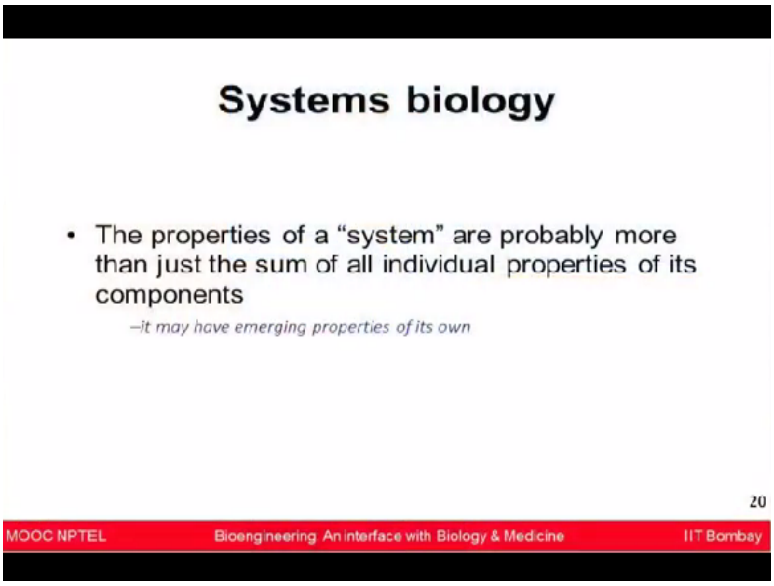

So that is what, you know, the power of this new field of systems network and systems biology.

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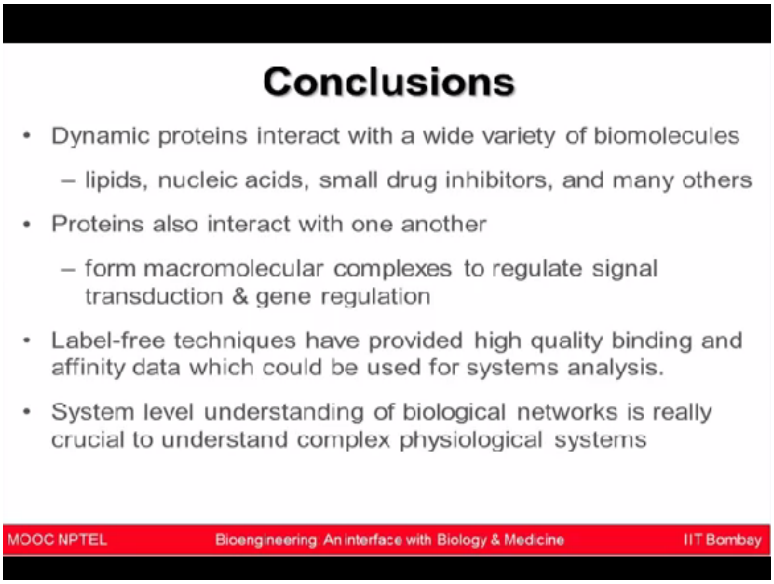

In conclusion, we have discussed today the dynamic protein molecules which interact with variety of other biomolecules for in the field of interactomics. These interactions could be studied using the label based platform which we talked in the last class, especially the protein microarrays.

Or it can also be studied in the label-free manner which is looking at the surface plasmon resonance or many other physical principles which could measure the protein interactions in the label-free format. The systems level understanding is really crucial to provide the understanding

of the complex physiological systems.

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