Interactomics: Basics and Applications

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Lecture – 28
An Overview of Label-free Technologies

In the next lecture, I will focus upon the Label-free Detection Platforms and their Applications. In today's lecture, I will walk you through various label-free detection techniques available and their advantages over the label based detection platforms to study the molecular interactions and their kinetics.

There is always the debate whether we want very accurate quantification using labels or labels are going to affect the properties of biomolecule and we should use the innate property and you have the label-free quantification. So, we will have these discussions in this lecture. Further we will discuss about a few label-free platforms in detail and their interpretation of the sensorgrams obtained. So, let us continue with my lecture which I delivered in this workshop.

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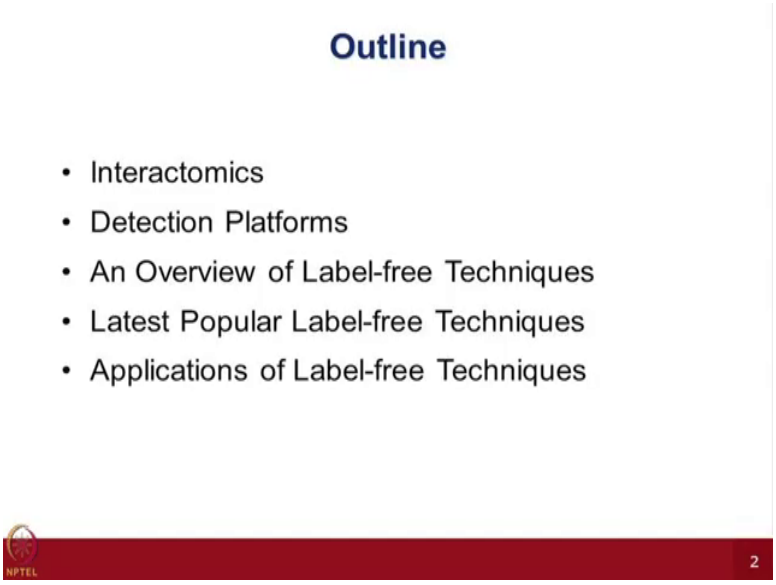

Let me start with the outline what I am going to talk. A couple of things which you have been hearing already the field of interactomics. I will not take much time on that. But then I will move to comparing the detection platforms which is because you are you know hearing lot of lecture some Josh on protein microarrays. You have heard many of these speakers on reverse phase arrays and peptide arrays, all of those requires some level of you know label based detection system.

And yesterday you also started getting exposed to some of the label-free platforms. So, brief comparison of those two type of detection platforms. Some of the overview of the label-free technologies. There are many in fact, you know you just got, some exposure of SPR study surface plasma resonance, but there are you know many emerging technologies which are already in have a started making some impact but many of them are still on the testing phase.

Some of the latest popular you know label-free technology platforms as you go along in the course, and then some of the you know brief overview of applications. I will not have too many case studies, but I will at least give you the flavor of what all things can be done because, our intention with this course is that many of you can start planning your experiment and think about you know how broadly these technologies could be use for everybody.

Because they are not biased for any kind of sample, they are not biased for any specific project. Depending on your objectives I think you can very much modulate these things for your own research.

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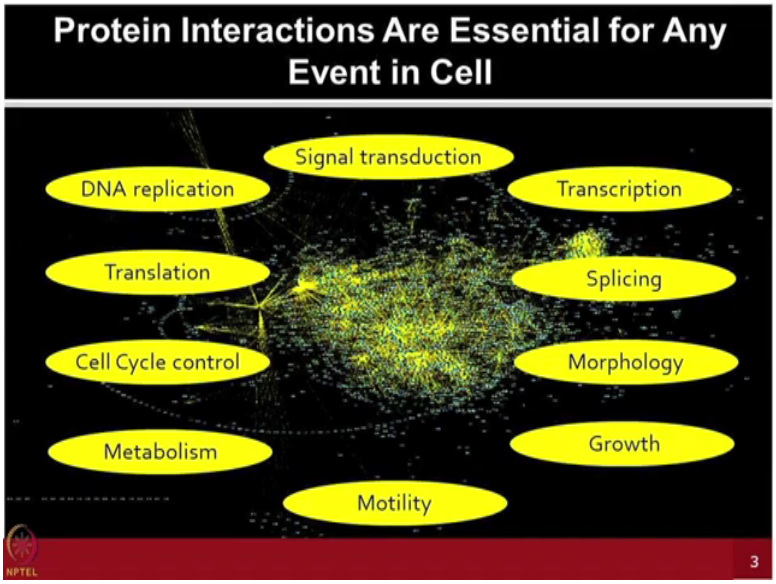

So, I am sure by now you are convinced that you know these kind of technologies are very useful to study the entire interactome which is a involved in many of the important activities whether you talk about signal transaction, to splicing morphology growth, metabolism,

translation, DNA replication. These are all pretty relevant for any kind of physiological processes which we want to study.

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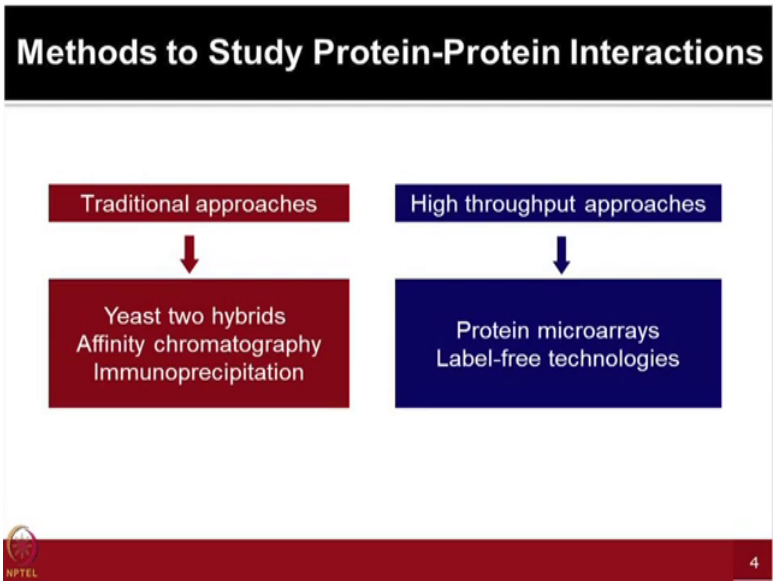

So, therefore, many type of protein interaction methods have come forward. The traditional approaches includes yeast two hybrid and different type of IP methods, and the latest technologies you are now happy and convinced that you know protein microarrays are you know emerging as one of the solid platforms including cell free expression based arrays, which includes NAPPA arrays. And then we have couple of label-free platforms the biosensors which are equally crucial as well.

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So, let us talk about detection platforms and their comparisons.

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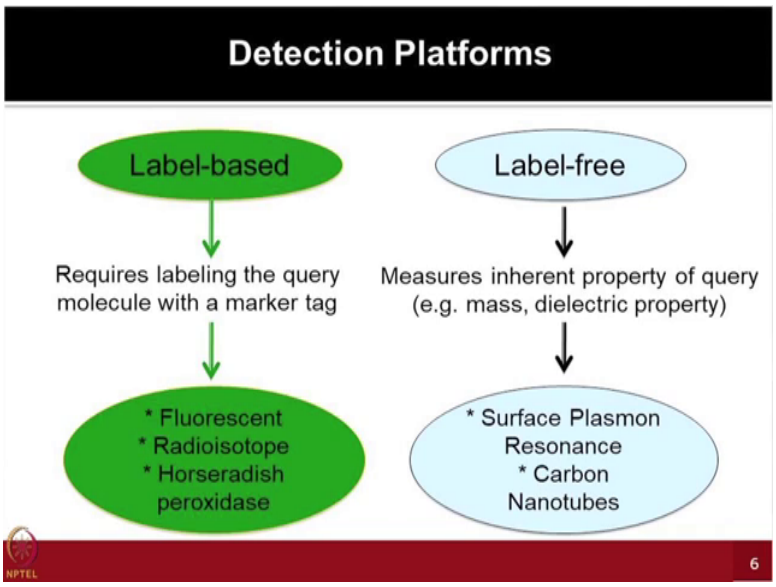

So, you want to detect the signal after doing the assay, right and that is where whatever you have hypothesized at the end only looking at the dots or looking at the curve you know that your experiment has worked or not. So, therefore, you need to have some robust detection platforms.

And to do that either you are using some sort of label to follow your experiment and then you are trying to measure those labels either with the fluorescence based methods or chemiluminescence based methods sometime, even radioactivity based assays can be very powerful.

So, you require some sort of labels which could be used in the marker to follow your experiments or you can also think about label-free approach because whenever you are labeling a protein irrespective of what chemistry you use you are adding something from

outside on a given molecule. And that is going to definitely in some way affect the overall structure, overall binding and it may result into certain artifacts.

So, for many kind of you know critical drug discovery platforms, it is you know it becomes more apparent that you probably do not want to change the narrative structure of the molecules and you want to study them in their own you know natural environment as much as possible without adding any sort of extra label on top of them.

As a result many type of label-free platforms have emerged which essentially aims to look for the properties of the molecule itself. If two molecules are binding what kind of mass is getting changed, what kind of dielectric properties are getting changed, do we see some sort of percentage reflectivity change because of the binding intensity, can we see some sort of interference change..

Many of the physical principles are being used to look at the molecules and molecular interactions, and can we measure those properties. So, as I mentioned the label based the read outs could be many of these fluorescent, radioisotope, HRP based systems. For label-free you got little exposure of SPR, even many of the nanotechnology based platforms like carbon nanotubes, etcetera are also equally powerful and they have been used for doing these kind of measurement as well.

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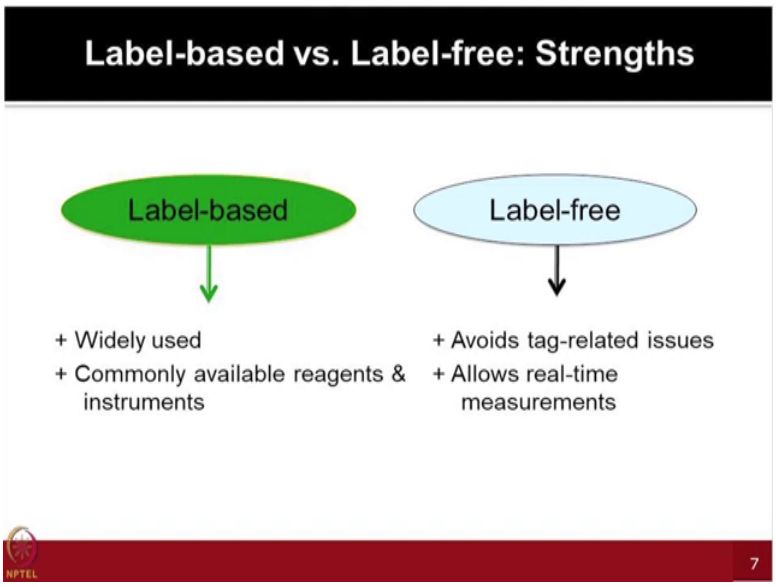

Label based, one of the major advantage here is that these technologies are available. The kind of you know every lab, every center will have those scanners, so that you have easy readouts, you can definitely do the scanning. I am sure in any given building you will have some of these instruments available.

Reagents are easily available, even if you do not have a HRP, you do not have psi 3 or any of the dyes I am sure you can borrow from somebody in the neighboring lab and you can do the experiments. But you cannot do these kind of experiments with the label-free kind of approaches when the technologies are very specific.

However, the label-free platforms are avoiding the tag related issues which you observe in case of label based and most importantly you are actually monitoring the biomolecular

interactions in the real time manner. And that is pretty powerful because all the protein micro technologies are great.

But it is like you know takes whole day time to do the experiment sometime even you know longer if you are doing a blocking and all and at the end of the day then only you are relying on the scanner to show you that you know your spots are lighting up or not, or you see some sort of you know huge background, you see you know your controls did not work out and you are equally you know hugely disappointed. But you had literally no control on as the experiment progressed whole day.

In this case here, if binding is not working you will you know after you have watched just for 20 minutes time you stop it and now you will bring the solution, change the concentration, change the temperature, do different pH scouting. So, you have many ways of planning to modulate experiment you are not going to just wait for something to happen ten hours time and then you say that, it is happen or not happen.

More importantly that you know in case of microarray or other kind of label based platforms you are only measuring the signal at the end which gives you an idea it happened or did not happen. So, you have a positive signal or a negative signal. So, an interaction happened or a biomarker is present you can just say qualitatively it is showing you signal.

And of course, you know that you have many different measures of having different controls which could be used to do the connotation as well. But in this case when you are talking about some of the level free platforms you have even ability to say that you know yes binding is happening.

But what can be on rate, what can be off rate, what can be overall decision constant for these and how my kinetics is actually getting changed. So, these are the added information on top of what you would only obtain in the microarray based or any kind of label based platform. So, that is added advantage of the label-free technologies.

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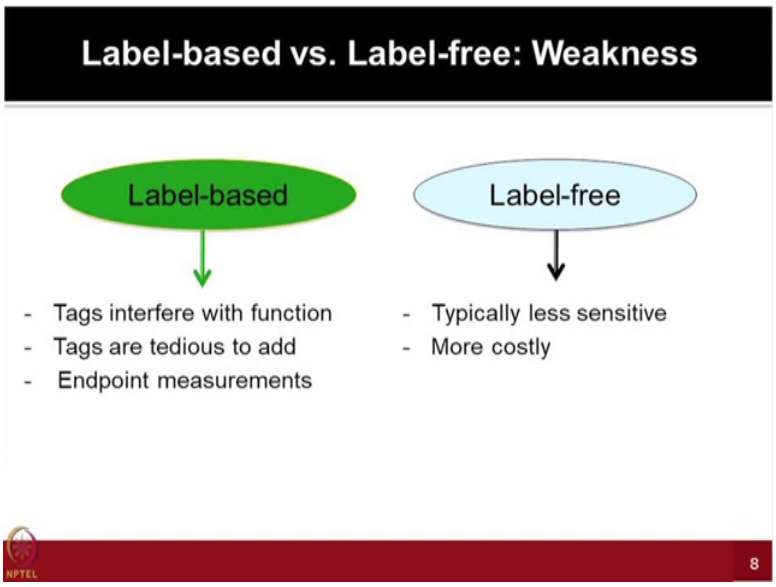

So, label based because of the tax you know there might interfere with the function, you may have certain kind of issues which can give you artifacts and this is endpoint measurement because you are not having control on how the assay progresses.

Label-free technology having lot of advantages, but there is still very specialized many of them are not available in many labs. You will not find those biosensors available everywhere. They are sometime more costly and again you still have to ensure that all the signal which you measure is coming because of the actual binding and not because of the bulk effect, not because of something else which is happening and you are seeing an artefactual response.

So, many of the things that is still under the kind of you know more in fancy, more early developmental stage which needs to be tested out well.

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So, now just to give you a feel that you know we are covering 2 or 3 label-free technology within this course but there are many which are actually under development. And there are lot of physics people who are actually you know contributing towards this way of making many type of biosensors, where they are involving different type of physical principles how they could be used to measure molecular interactions.

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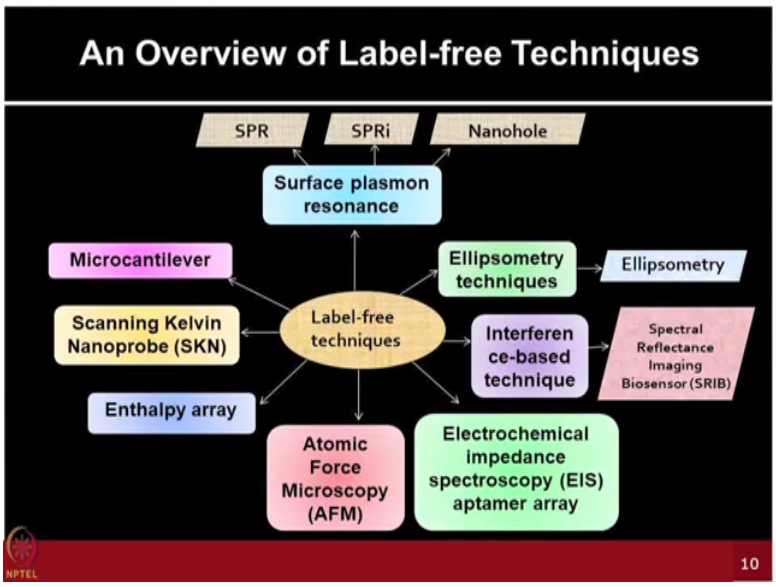

So, you know so many, I am sure you know you can find out SPR just one among them. One of the label-free platform which can give rise to multiple type of even technology platforms including Surface Plasmon Resonance which we talked briefly, Surface Plasmon Resonance imaging base platform, SPRI or nanohole arrays. All these 3 depends on one of the principle which is SPR.

Then there are many platforms which are emerging based on the ellipsometry, we have interference based methods, we have Electrochemical Impedance Spectroscopy, Atomic Force Microscopy, Enthalpy Arrays, a Scanning Kelvin Nanoprobes, Micro Cantilever. So, I am sure you appreciate there are many technologies which are under development right now, there all looking at different type of physical principles which could be used for looking at the biomolecular interactions in the label-free manner.

So, while we cannot talk everything right now, but at least some of them I will talk, I will give you some examples of those. But idea here is that you know many times when a physical scientist work on these kind of biosensors, their objective is to only show that two molecules are interacting and by looking at interference or conductance change or you know reflectance change, you can measure a binding.

And then they stop over there because they would have taken very standard protein sets you know, they will take BSA, anti-BSA or they will take you know if you tell know there are 5 more interesting proteins to test out they will take those highly abundant protein and the robust antibodies to test out those assays.

And this things are usually not good, because when you want to try out your actual experiment which is depending on many (Refer Time: 10:50) low abundant proteins and anti-bodies was not so great for them then I think you know your assays do not work as robust as you would have seen the you know proof of concept level.

So, many of these technologies are a still under proof of concept level which needs to show that what could be done at the actual biological sample level. Therefore, what all I have shown here not everything has reached to biological lab and not everything is actually holding up to the biological experiment, because biological samples they have lot of low abundant proteins, you will not have that strong analyze to test out, all right.

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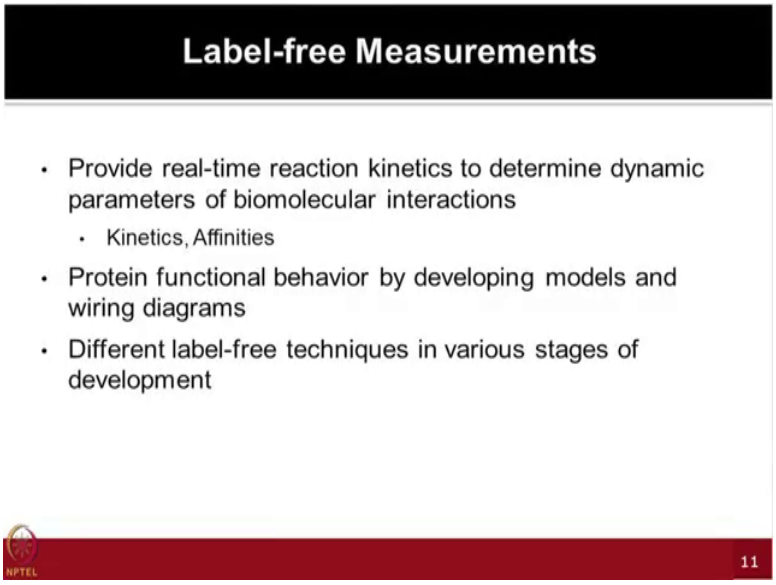

So, all the label-free measurements irrespective of which platform we talk there all measuring the biomolecular properties, in its pretty much kind of without changing anything on top of them and its adding the information which is providing you either kinetics information or you are looking at the affinities. These are added information what one could obtain along with you know just yes no answer which you can obtain from other binding experiments.

You know more importantly, the new area which are emerging which is essentially system biology area, the intention is can be generate lot of these values which is on rate, off rate for different type of binding interactions which are happening. And now develop those as a model and wiring diagrams which could be used to predict many unknowns because how many actual experiment can you do for so many you know proteins.

So, let us say you know for a given protein wild type condition and there is many mutant forms which you want to test their affinity set is now with a drug or with other molecule, now you can build let say from 50 of their mutants you can develop some of the values which can give you the some models.

And now can those models be used to predict the behavior of another unknown mutant. So, that kind of stuff is going to really happen if we have the available information or at each level of on rate of binding, off rate of binding, what is the k d value and then you build the models and that is (Refer Time: 12:41) lot of system biologist have a started working in this area and are going to contribute much more heavily towards these directions.

So, different type of label-free techniques as I mentioned are in various developmental phases. Of course, Biacore is pretty leading in that way because you have already over you know 20 plus years of the technology is Enfield available. But many technologies you know which are a still just emerging including some of the bio-layer interferometry based methods have really come off very forward fast even a mystery technologies are emerging. So, those could be good platforms to be used.

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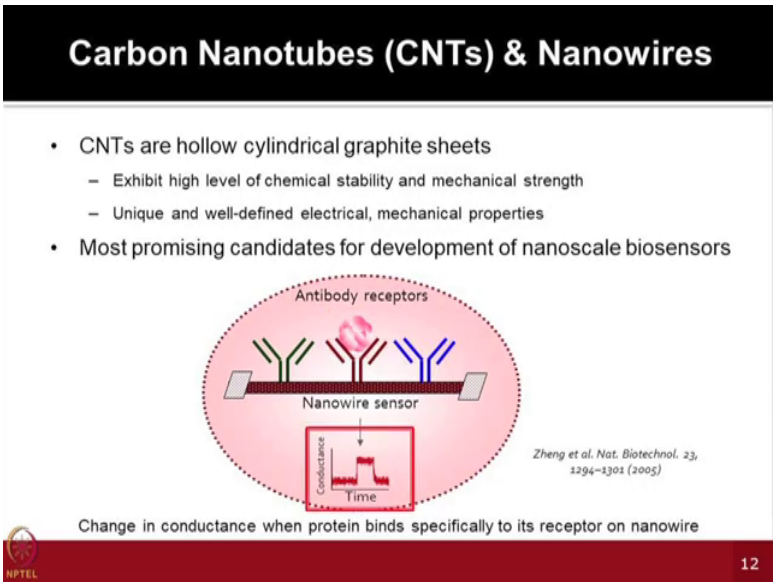

I am just showing you very briefly some of the principals involved of a various type of label-free technologies. One of them is showing you here now these kind of nanowires nanowire sensors on which antibodies are immobilized. If a protein binds a specifically to this empty body then you will see change in the conductance happening and this is recorded here over the time period.

Simple technologies, simple concept can have so much utility clinical wise. Imagine that you know when lot of SARs issues were happening different type of viruses people are getting affected. That time you know immediately on lot of you know metal detectors on the airports, these type of platforms were used to detect it with the patients or the individuals who are coming are they carrying these kind of antigens possible and then they were quarantined and all kind of these things were happening on the airports.

So, just you know immediately this papers were published lot of you know applications has started emerging out of them. Simple concept you are measuring antibody antigen interactions looking at the conductance change and if that can give you some idea that you know some individuals are carrying those antigens which are you know going to give rise to certain viral disease. Then probably you can do much more specific test on those.

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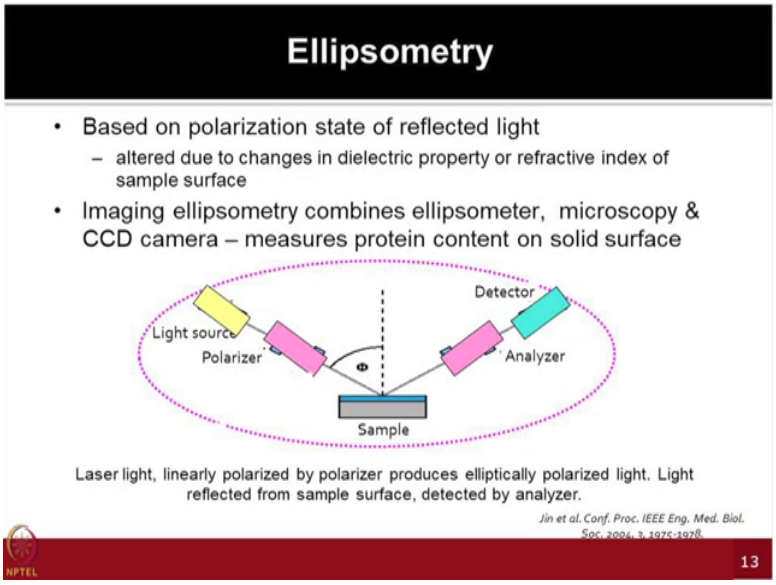

So, another platform which is you know which has shown some promise because of the low cost and that ellipsometry based platforms, where you are measuring how much polarization change you can see as a result of binding. And you know again its very a simple setup which involves a microscopy and CCD camera and it ideally measures the protein contents which is not unlike the other platforms which are requiring lot more costly setup, this platform is very cost effective method.

Although, it has not shown as many biological application as we would have seen from other platform, but this was a robust thing to show that in cost effective manner you can do label-free technologies.

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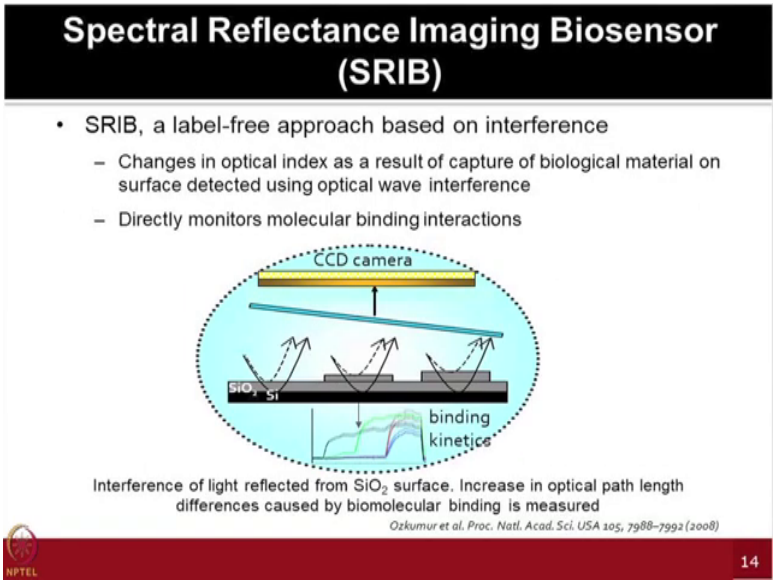

One more thing which you know we have seen some good promise of this paper based on a spectral reflectance imaging biosensor or SRIB, where you have you know different layers of silicon and silicon dioxide. And by varying the you know concentration of this, if you have now the protein printed in different amount you can see their change in the binding happening.

And this particular concept was again you know published in PNAs which shows how beautifully just looking at the change in the interference you can see that how much material is printed in different quantity and can be measured in the label-free manner.

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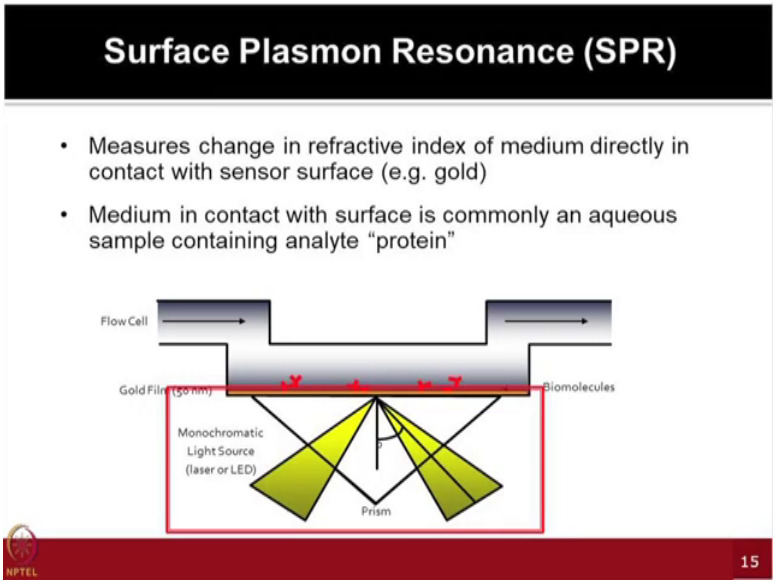

Then comes the SPR technology which is Surface Plasmon Resonance, which you are exposed already on a prism and on a gold slide. You have certain antibodies printed for example, some protein molecules are flowing here, and as you see the binding is happening by changing the you know percentage reflectivity you can measure those particular things in the real time manner.

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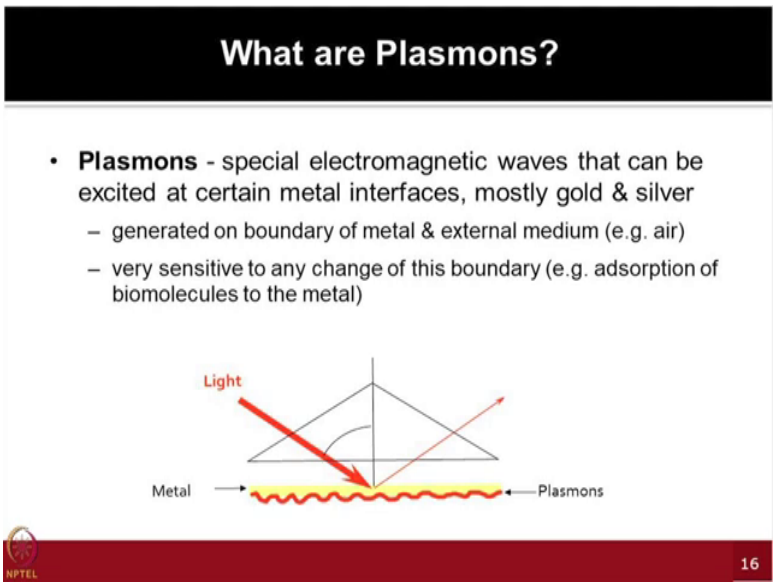

And what you are actually measuring, this kind of Plasmon's which are on the interface of the gold surface when these you know electrons are being generated. And those you are measuring throughout your you know reaction and by changing the solution then you are seeing what is the SPR angle being changed from one to other condition and those SPR angle is being used to monitor your entire binding reactions. And that you measure in the terminology which is used as a SPR sensorgram.

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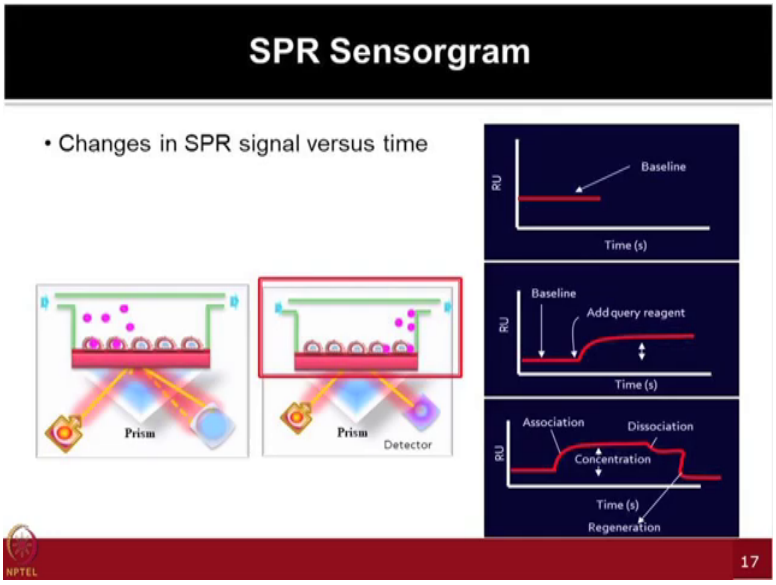

These sensorgrams could be used to monitor your entire binding activities for the reactions. For example, initially you when you are just you know having the molecules coming in and start to bind with antibody for example, you can start seeing there is an initially baseline there which is stable..

As the reactions are happening there is some binding happening you can see an on rate happening association of these molecules, and then after some time they will you know get saturated. You are still injecting your buffer and then ideally they should start washing off and then you will see you know off rate going in and now the you know you are doing these experiments on the noble metals which are very costly essentially gold you are using.

So, you want to regenerate, you want to reuse the same slide again and again at this couple of times. So, then you are trying to do regeneration and you are using some mild assets to chop

off your this binding of molecules without disturbing your you know printed molecules, and then this chip can be used after you know again you have to wash off with the buffer, couple of rounds and the same chip could be used again for next round of interactions.

So, in this way you know you can reduce the cost of the experiment, otherwise the gold slide and all these chips which we are buying are way too costly.

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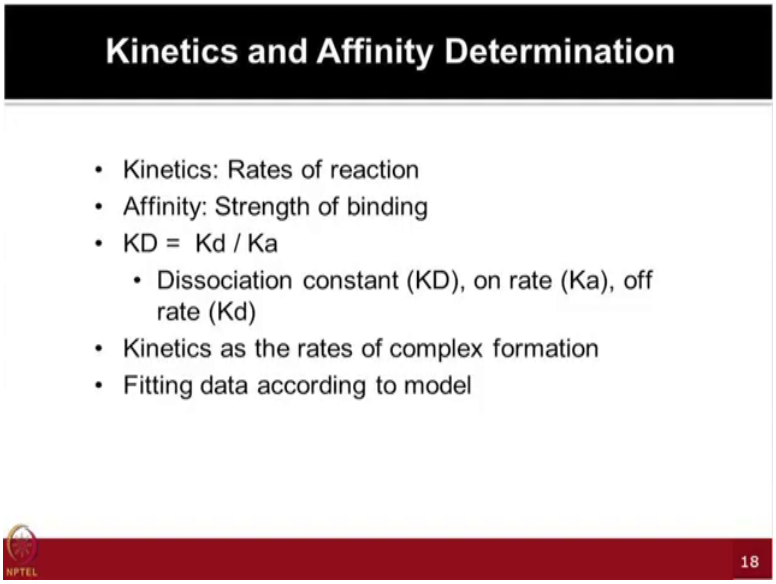

So, but what its offering you? Of course, its offering you much more powerful information as we talked you are getting the kinetics or the rate of reaction how you know you can monitor in the label-free and the real time manner. You are looking at both on rate and off rate of these reaction to happen unlike the label based methods where you are just talking, a binding is happening or not happening. Here you are saying that, binding is happening, but probably the

on rate was very fast or off rate was you know very slow. So, those kind of information you can obtain.

And many of these informations having lot of utility in the pharma sector and that is where you know even we see that you know more than academic labs you know Biacore and SPR and many of the label-free technologies are much heavily used by the pharma sector who are drug discovery and drug you know drug designing and drug testing kind of you know experiments. They have lot more utility for you know using these platforms because it is just having binding information is not sufficient, how the binding is happening on rate and off rate differences are much more crucial to know over there.

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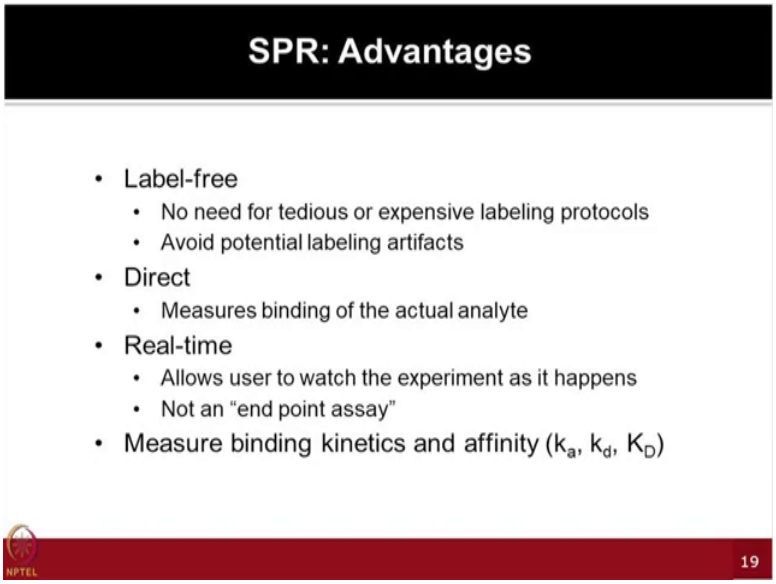

So, advantage of SPR I sure you are all convinced that you know you have lot of promise here, you are not doing any sort of extra thing on your molecules which you want to test for

interaction. So, there is no labeling which you are applying. You are directly measuring the biomolecular interactions using the physical properties, and then you have full control on how the reactions are progressing and as a result you can actually modulate the conditions to make it work.

Many times we are trying out you know different type of concentrations of different antibodies, then we are doing the first initial screening or you know you can use different type of flow rates to even try out. So, many things you can play with it in that in the initial round of experiments to find out what can be the best condition for your binding to be observed. And now once you finalize those condition then you can apply the same on your full experiment with many of the molecules of interest, all right.

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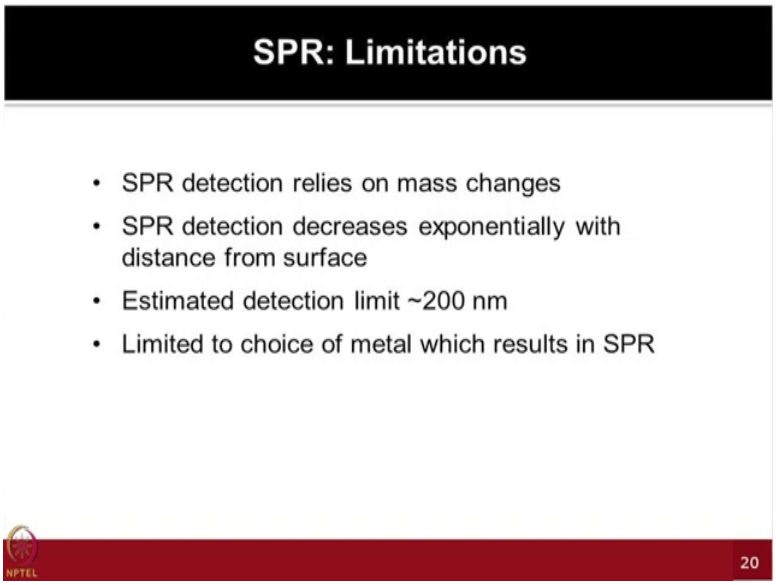

So, I think we have talked that you know there is lot of merit for using these things. But of course, there are limitations as well because everything what you are relying looking at the mass changes, and sometime this could also come from the bulk effect that is may not be the actual binding happening from the molecules.

So, when you are immobilizing the molecules depending on the size of those detection also you know going to get less or more sensitive because exponentially as you know the distance is further from the chip surface, you are going to see the loss in the signal and the detection limit estimated is around 200 nanometers in this cases. Limited to the choice of you know metal which you are using because that is a gold metal which is useful for doing these kind of experiments.

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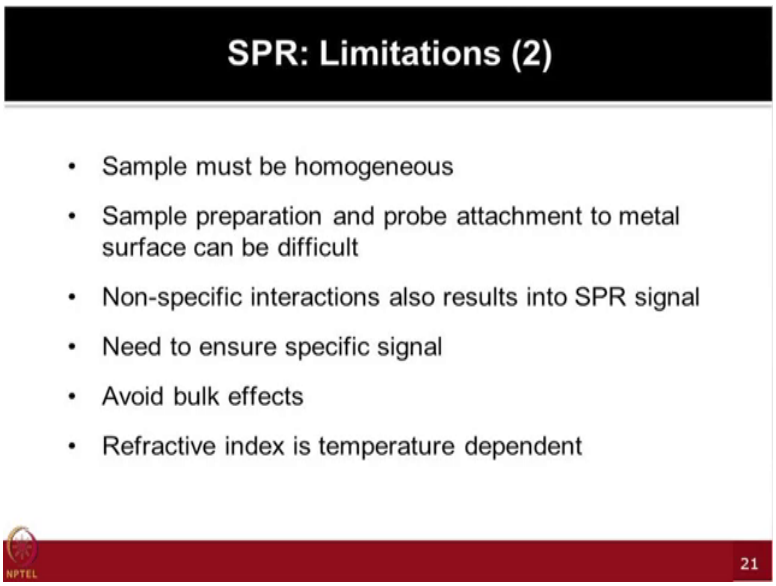

Your samples has to be really clean, it has to be homogeneous, you have to do the you know proper removal of all the gas etcetera just so that you know bubble is blocking your columns, there is nothing as getting clogged as your you know flow rate is changing for measuring the binding.

So, sample preparation and these you know probe attachments could be difficult, but I am sure these are some of the generic thing which is applied for any kind of you know the chip based assays which you have to follow. You have to ensure that you know your washing and your all kind of cleanup steps are well performed, otherwise you will have many nonspecific interaction which may result.

To ensure that you are getting a specific signal its good idea to have lot of positive controls and you can vary the concentrations of those and see you know tell what is smallest lowest concentration you can still measure those signals. And those can be used to follow your various you know, not everything you can do on one chip if you have to test many of your molecules.

So, then those kind of you know one flow cell let us say I am sure you are aware of the different flow cells involved in the by a core experiment for example. So, one flow cell you can just block it which can be used for you know your qc checks for many experiments and then those could be and you can vary the other flow cell for testing your unknowns.

So, therefore, you will have a still full control even as you are going along, the chip has not gone bad and your you know the molecules are for positive controls are behaving same way. So, of course, you know many of these things in the field of label-free technologies you have to avoid ensure that the binding what you are seeing is actually real binding, it is not artifacts, it is not and sometime you will see a reaction is happening on the temperature dependent manner.

So, you have to also you know play with those. I think fortunately now some of these instrument and technologies have now to give you the range from 4 degrees to you know you

can vary till 37 to 50 degrees even to try your different conditions. Of course you know we are (Refer Time: 22:04) looking in this course about high throughput platforms.

Intention is I am sure you know with Josh talk you are pretty much you know convinced that if you can have the chip which can screen 10000 proteins. Of course, you know you are going to talk very quality data high quality data, you are reducing, your time, you are reducing the cost everything and so much variation which comes by doing you know too many experiments on if you had 1000 protein and 1 chip versus 10000 and 1 chip, right.

Same thing is applicable here as well on the SPR based platforms where if you are doing only 4 maximum interactions or 4 maximum testing of the you know binding on the Biacore trainer platform. What is if you can do 1000s on a different platform? And that is where the concept of SPR imaging became very powerful with hope that can we build the platform which can do the high throughput interaction studies using the same principle what we have used for SPR.

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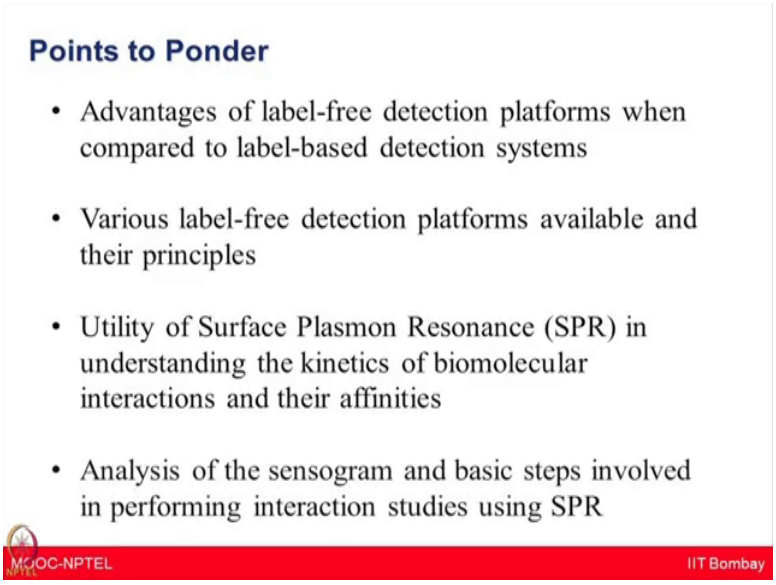

So, I hope after attending my today's lecture you are now able to appreciate the utility of using label-free detection techniques to define the biomolecular interactions and analyzing their binding and kinetics. Although, there are many label-free detection platforms currently available, but most of them are a still under infancy and they have shown the utility only at the proof of concept level.

It means most of these new technologies and bio-sensor platforms they have shown the experimental evidences that these principles were only with the known pair of robust antigen and antibody based detection. However, when you have to test out the actual biological samples and the biomolecules which are in the physiological context they are very very subtle amount very low concentration, then one need to think about how to use technology platforms robustly.

In this light one of the technology based on the SPR and the Biacore Technology have over the period from long time showed its utility. Also a new advancement in the area biolayer interferometry technology has also shown lot of promise. Nevertheless, SPR is one of the most acceptable platform to study the biomolecular interactions whose principles and data analysis was explained.

In this lecture, I hope now by looking at a sensorgram, looking at these binding curves you can now make sense of what has happened in this particular experiment, especially for the interaction kinetics. We will continue our discussions on latest advancement in this area and also how to integrate different technology platforms with existing label-free biosensors in the next lecture. See you then.

Thank you.