NATIONAL PROGRAMME ON TECHNOLOGY ENHANCED LEARNING (NPTEL)

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Applications of Interactomics using Genomics and Proteomics technologies

Course Introduction by
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MOOC-NPTEL

Applications of Interactomics using Genomics and Proteomics Technologies

Lecture-26
Biomolecular interactions using Bio-Layer Interferometry (BLI) – Part I

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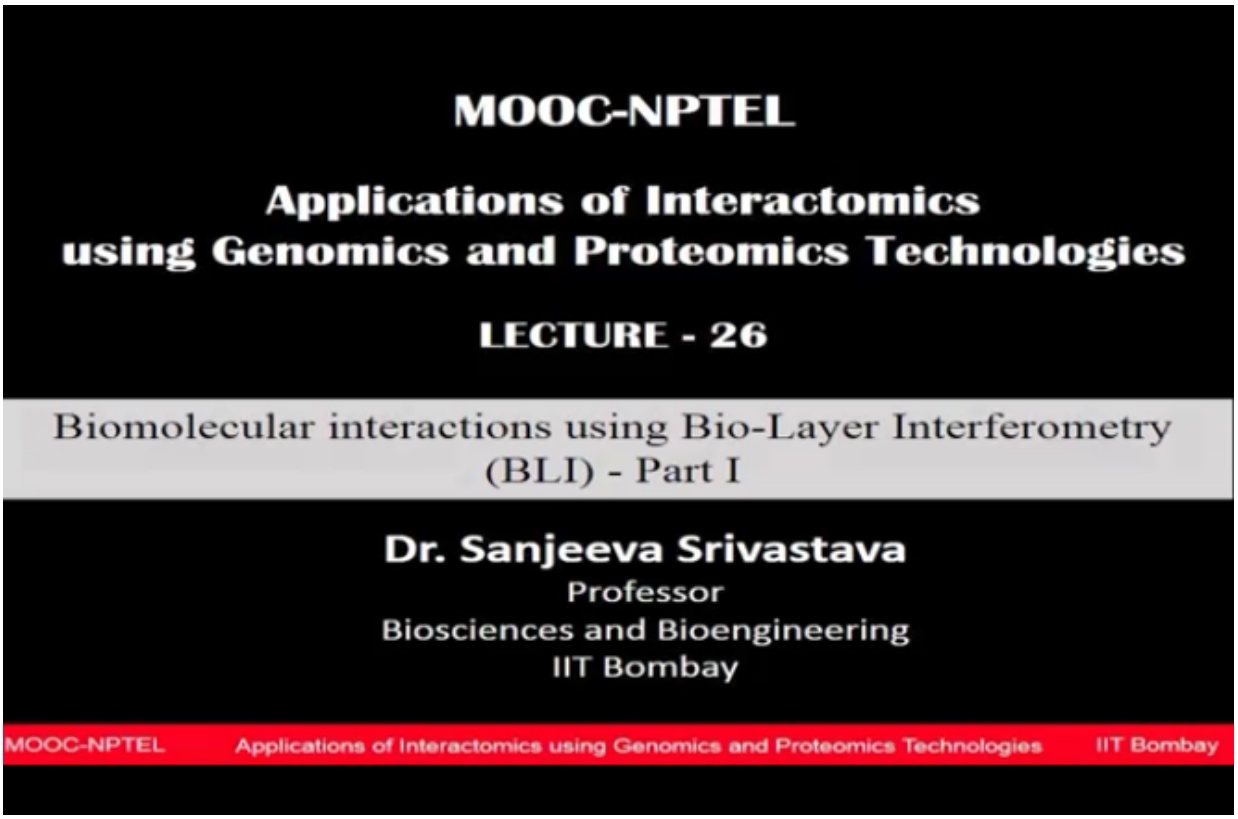

Dr. Sanjeeva Srivastava:- Welcome to MOOC course on Applications of Interactomics using Genomics and Proteomics Technologies. Today's lecture will be delivered by Mr. Susheel Vaidya, who is an Application Scientist ForteBio, Pall Life Sciences. He has worked in proteomics, lipidomics, a small molecule characterization using mass spectrometry SPLC, NLC MS based method development for biomolecules and a small molecules.

He has thorough understanding about the technical strategy planning in analysis of bio similar characterization. Within next two lectures Mr. Vaidya will have interaction with his about novel label-free biosensors, especially he will talk to you about bio-layer interferometry based platform through a lecture and a demonstration sessions. So let me welcome Mr. Vaidya for his lecture on BLI technology.

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Mr. Susheelendra Vaidya: I'm Susheelendra Vaidya, I'm Application Scientist for the Pall ForteBio base instruments. I'll take care of this applications parts in the entire India where exactly the installation your, the support the training as well as the product promotions, this is the alternate technology where exactly the bio-layer interferometry, the interference based technology, the SPR is the one the Surface Plasmon Resonance, but this is the technology why that's a very different and as well as the high-throughput platform.

So how this technology will be helpful in your the interactomics kind of workflows, and I will go through the some, what the technology we had the informations, how the principle behind this, how you can use this technology in your applications, so I'll go through that, as you know that from the morning onwards we are discussing why the bio-molecular interactions are very important, if you look at in our body systems or most of the living organisms
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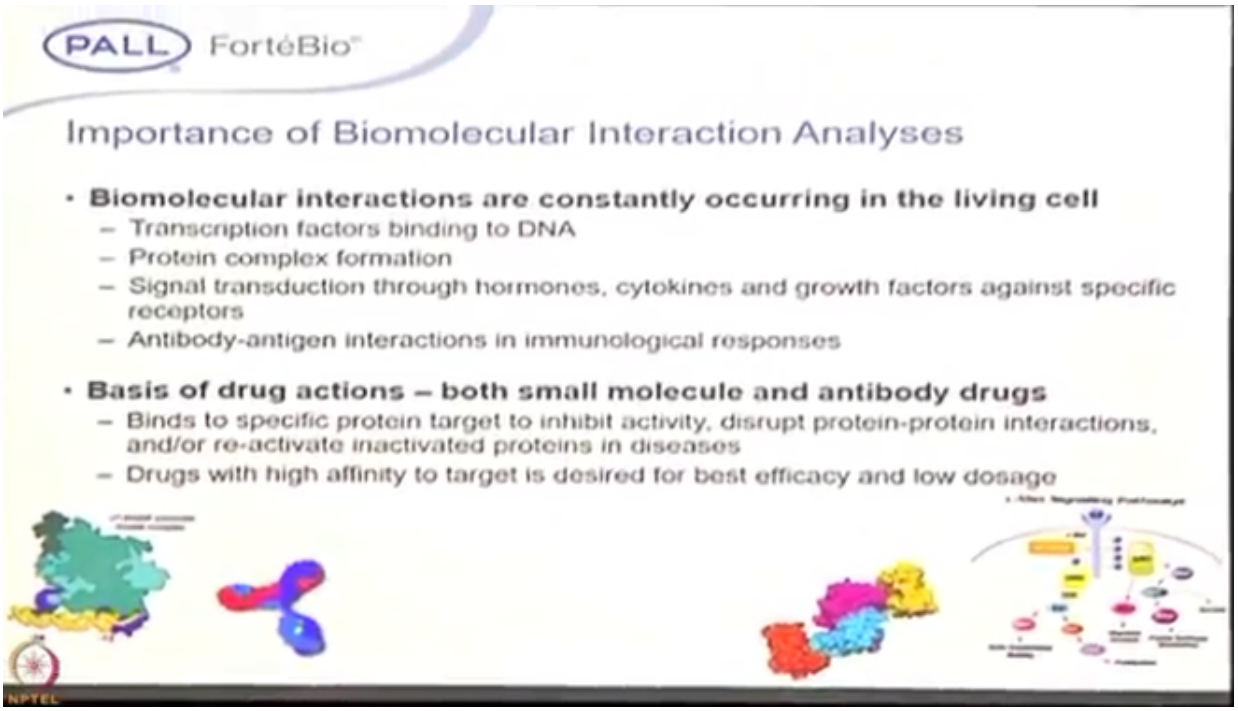

whatever is happening in the systems all through like a transcription factors binding to the DNA or the protein complex formations, or in terms of the signal transduction where exactly the hormones and the growth factors and all, what interactions happen those things, and as well as the immune response such that the antigen antibody interactions, all this works on based on the interactions.

And even when it comes to the drug discovery where exactly we want to understand the mechanism of the interactions, how the drugs is binding to the particular target, when we are discovering such kind of a molecules, we have looked at the affinity, so when it comes to the doors I can give you a simple example, any drug if you take it like a some drugs you had take it like a thrice set, three times a day, some drugs you have to take it a once in a week, some drugs you have to take it in a like a once a day, so how all these dose actually decides?

So these doses are depends upon the interaction platforms, if you'll say you have a target when it is any molecule you have discord when it is bind, so how the affinity is? How strong is the affinity? If it is that affinity is the strong then you required a less amount of hydro that, if it is you required a more dose then you had to break like a, the action will be like a, it is clearing from your body very fast then you required a more doses, so how these will be helpful in your _3:47_ areas.

So in this actually when you look at all the systems interaction is the very important phenomena, so we need to understand, we need to characterize it, so if you look at what are all the conventional technologies people are in routine they are using, the ELISA based platform square exactly people are using for the screening of the interactions, so when it comes to the limitations of this ELISA is like that more it is a time consuming and you required a manpower more on to develop the assays and then you had to screen it further and you have to select the right candidate for that.

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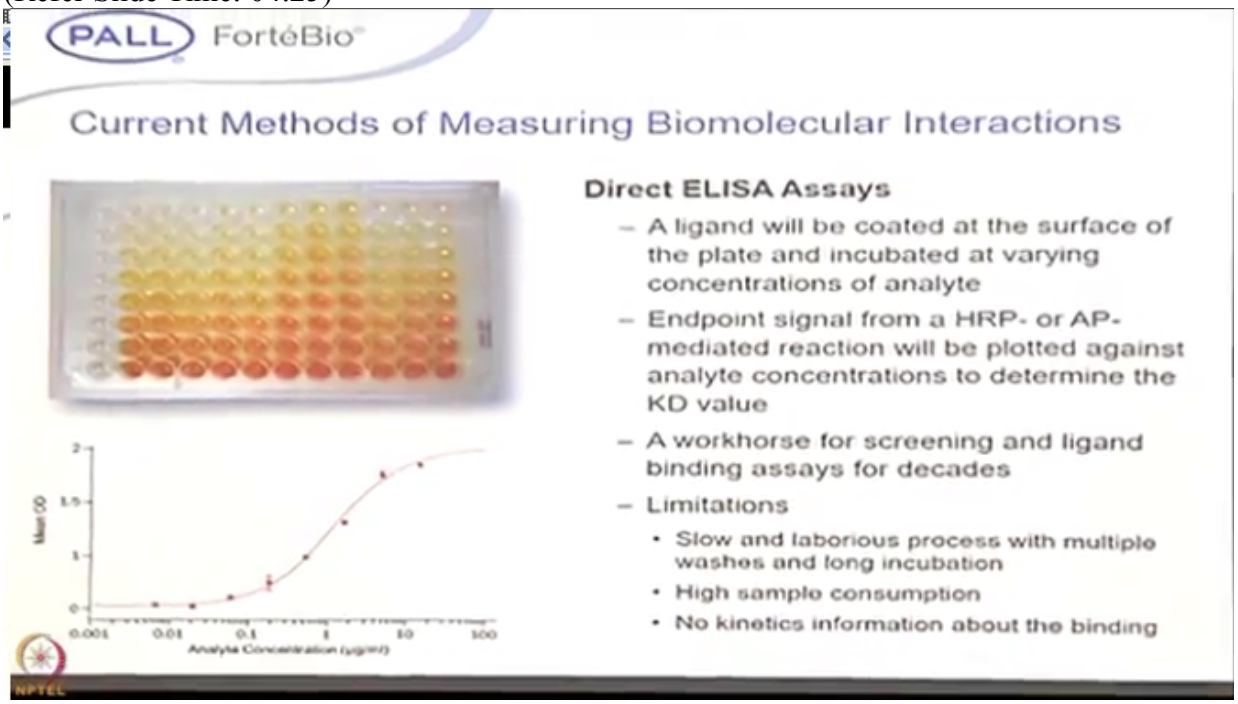

When it comes to this it takes a long, long time the limitation, and the reagent and the consumption of the reagents also more, and it is a more laborious. The other technology if I look at is the ITC, the Isothermal Calorimetry based titration, where exactly the interactions when you can decide this part with the interaction as well as where exactly the parameters like delta S, where, when any reactions happens in the interactions where there maybe heat released in the medium or heat absorbed in the medium, so those are the parameters you can find out, but the word of the limitation with the ELISA and the ITC is like that you can determine based on the concentration versus the response, then you can plot where you can study, you can read the study state, but this will provide you that the KD value, what we call it as KD is nothing but the affinity constant, but it will not provide you the kinetics parameters, like on rates and the off rates, this is the limitations with the ELISA or the ITC based technologies, so why people are moving to the label free platforms is where you can get, this is the difficult example when there is a ligands say suppose the compound A and the protein A and the protein B, this forms a complex and this is the forward direction,

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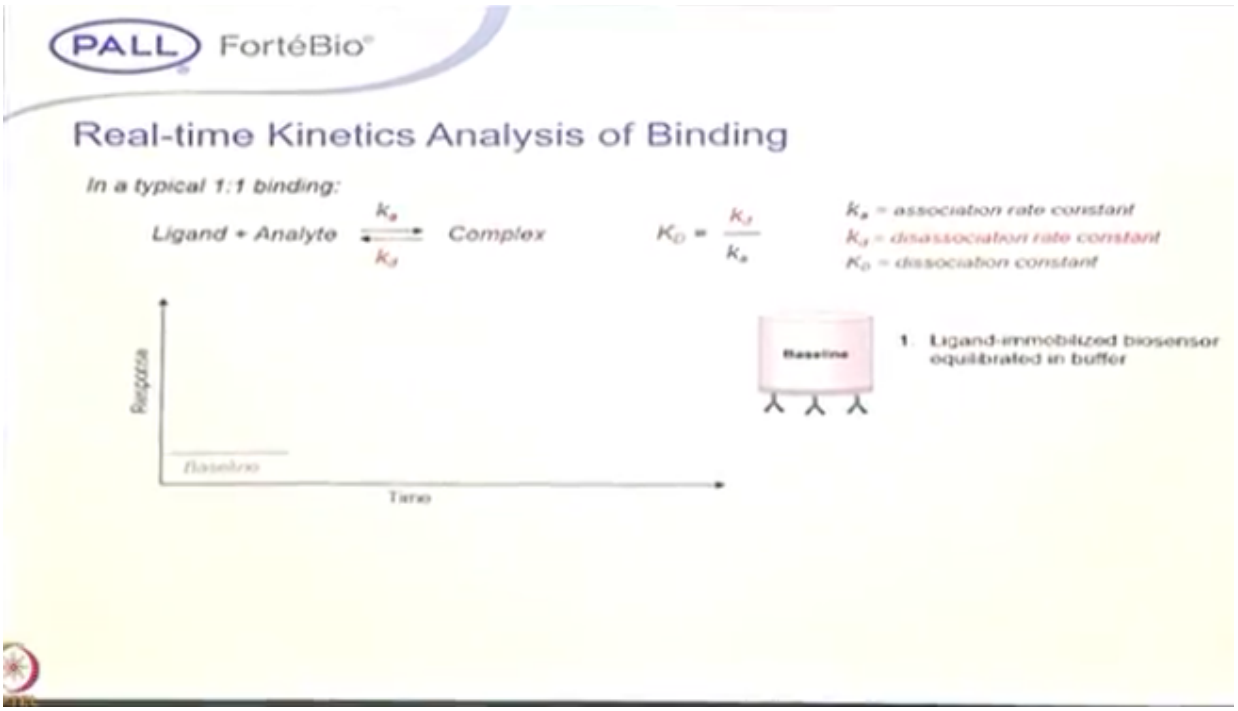

when the complex formation the finally it will disassociates to the once again A+B, so these label free interaction platforms are applicable to the reversible kind of reactions.

So if you look at when any molecule binding say, suppose ligand A it is binding to the analyte, when you see this is the response,

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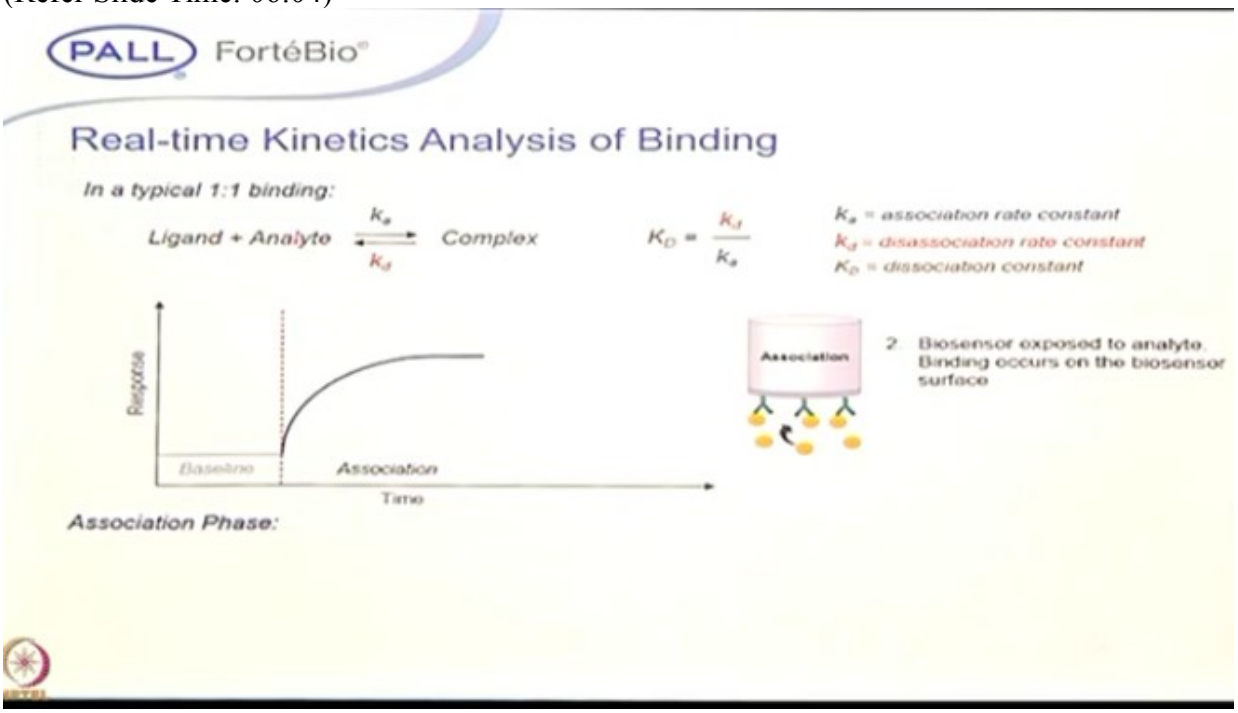

this phase we call it as association phase, when the same complex when it dissociates back, so the complex will be dissociates then you can see the dissociation, so the real time interactions

will provides the both on rates and the off rates, this is the kinetic information, so none of the any other techniques like ITC or the Elisa it will give this information, so that's why this is an very important tool for the on rate and off rate determinations.

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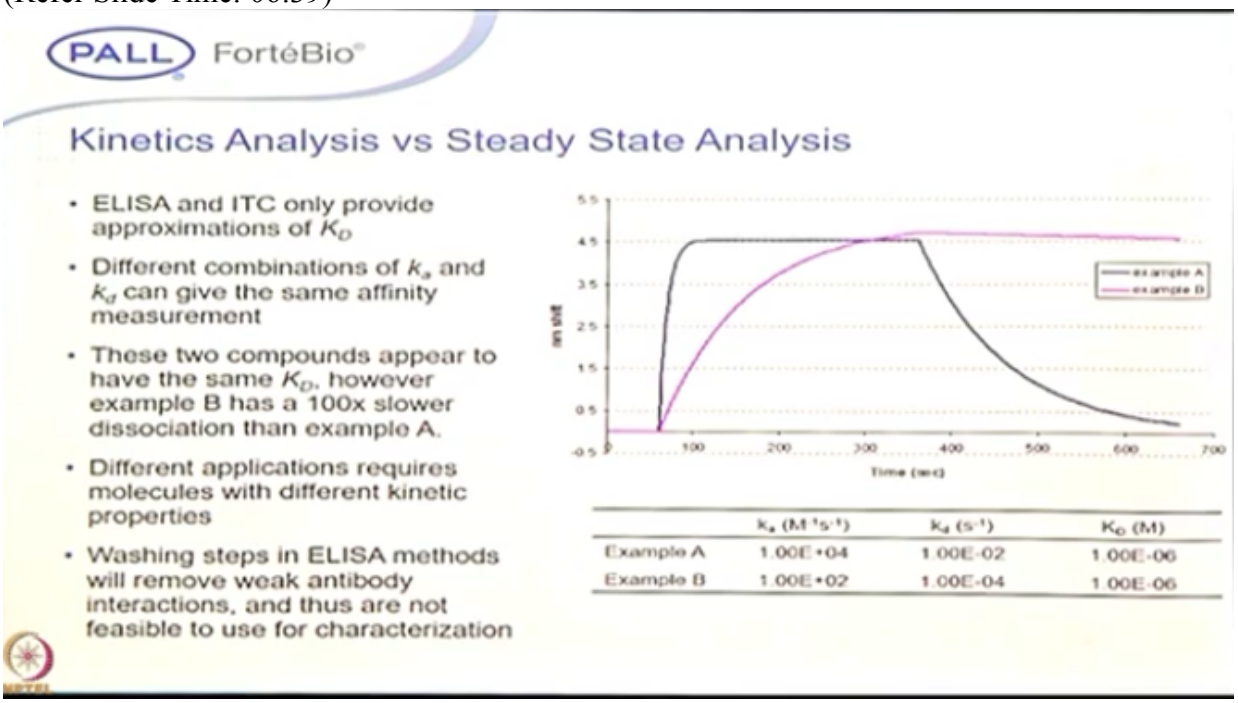

So if I look at the one of the example then vent comes to the Elisa based, based on the study state analysis if you look at the KD parameters for this, from the Elisa it looks like both are same, when you performed on the label free platform if you look at the kinetic constant parameters, see this example the blue trace if you look at it is dissociating very fast, when it comes to the pink one the dissociation is very slow, okay, so why this is important is like the based on this the rate of the dissociations we can able to see the differentiation between the, when you are selecting the right candidate.

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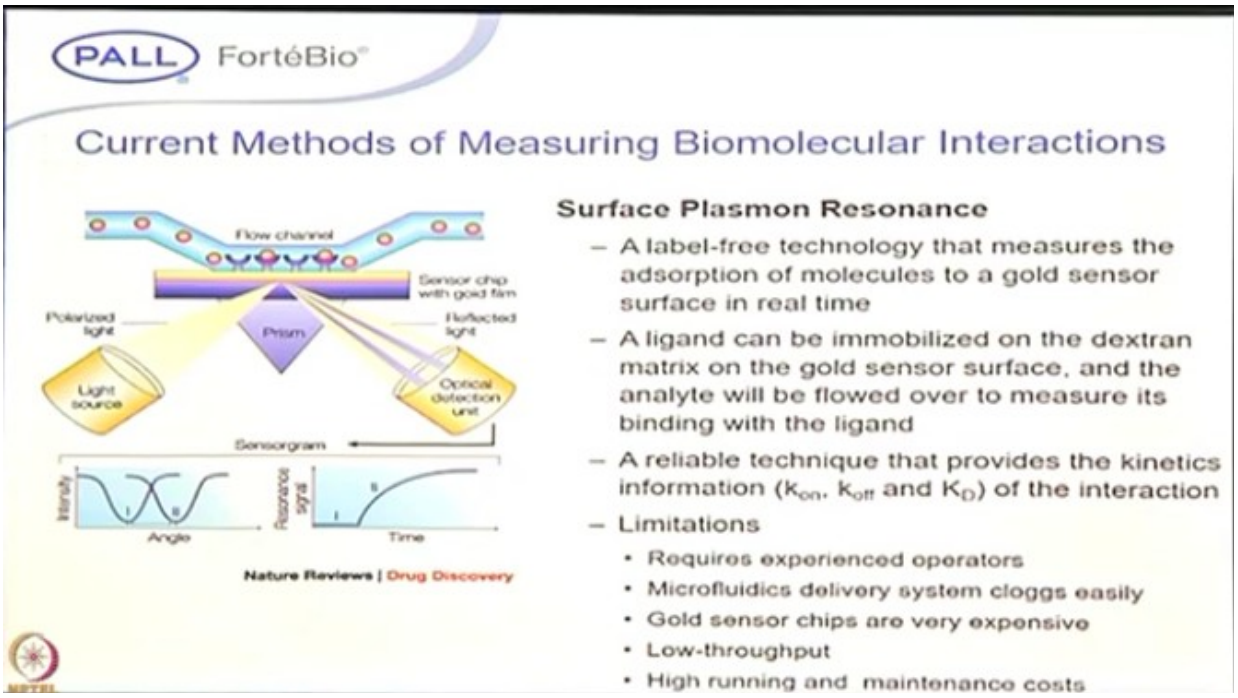

The SPR technologies, the one the real time label free interaction systems where exactly on the gold chip you have a matrix to that you are quoting one of the protein of interest and then you pass the analyte through the fluidic systems and then you can see the interaction change with respect to the angle.

So when it comes to the limitation, when the SPR when we are doing you required a like a dedicated operator for this, and apart from that the time matrix part for the initialization and then get to the data it is like a TDS, it's almost like a day it will take, so when you are doing such kind of a interaction you required a more patience and to get the data it's a more time taking and all, but when and apart from that one of the important is the microfluidics delivery systems, when you are working with any kind of samples which are like a cell culture based or if the sample from the body fluids and all that you have so much cell debris or any other impurities and all.

There may be a possibility of that there is, they clock the system, the fluidic system, so that is a very expensive in case of the SPR, if it is something at clogged it is a very, the flow cell is a very hairy like structure typically, if it is clogs then we have to replace the assembly of that, that is the very expensive and the maintenance cost will be more.

But when it comes to the other technologies, the BLI technology there is a more advantage I'll discuss on that, and apart from that low-throughput, actually here it is in the SPR techniques you have a channel, you have to inject one concentration over the other, serial dilutions you have to inject over the surface, but when it comes to the BLI technology it's a very high throughput, that's why the limitations comes into that, when you are deciding the right candidates in case of the screening experiments it is the BLI's the more advantage in that.

So when it comes to the BLI technology I'm going to talk on this, this is the ForteBio is the parent company they invented this technology in the year 2003 onwards and for the first system

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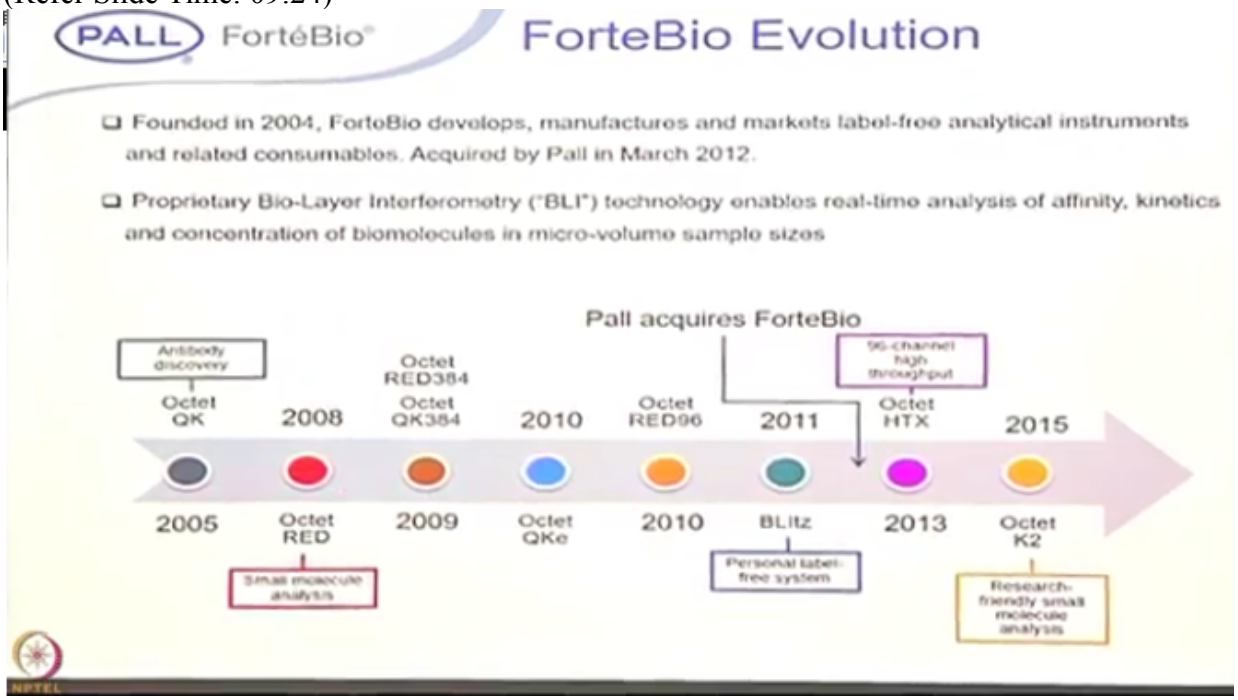

if you look at the 2005 it is coming to the market, we call it as Octet QK, and over a period of year we have different instruments coming to the market based on the throughput and all that. So with this I can start what exactly the principle behind,

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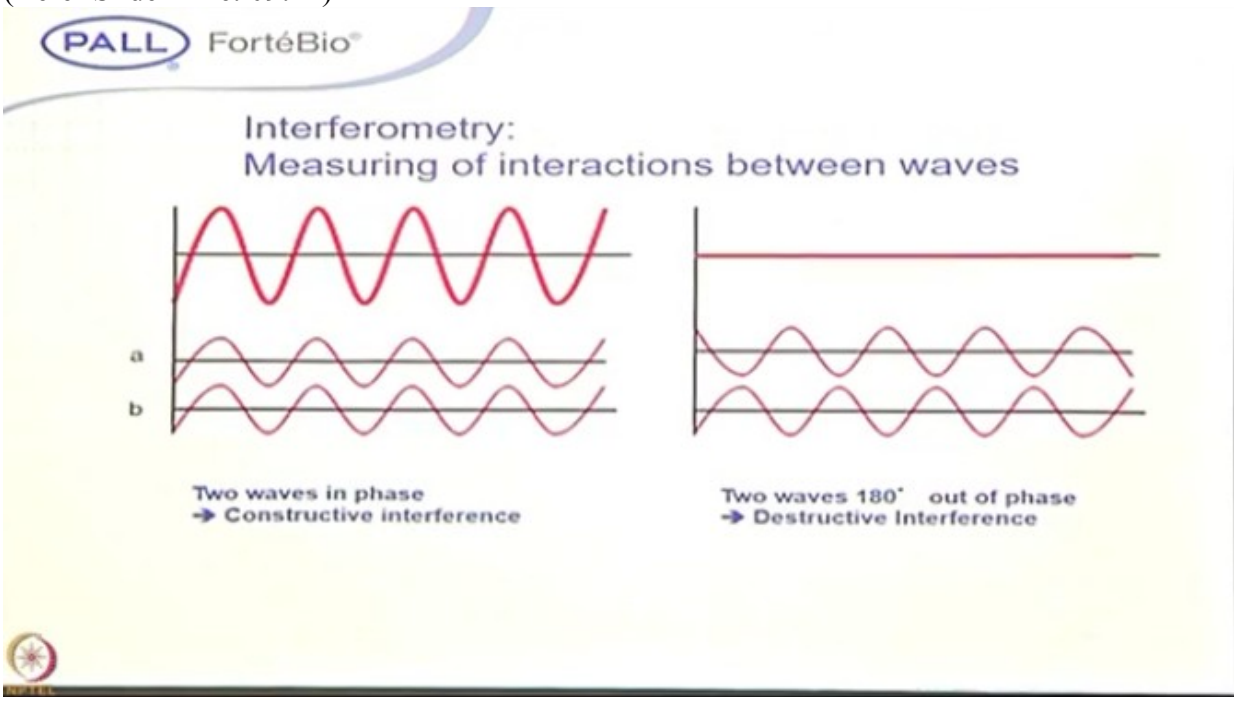

if you take it most of the interaction systems or any analytical techniques all are based on the light, light is the except mass spectrometry rest of the analytical if you take it analytical any all are light based, as you know that light have a property, the wave property is the one, and the

interference when it words the BLI comes, the bio-layer interferometry, but the interference patterns makes the signal pattern here.

As I mentioned it works on the wave property, as you know that when the light passes through the fiber optic, there is a some kind of a matrix you have obstruction, the wave it is forward hitting that matrix and get reverse back, so what happens is this is exactly the forward way, this is the reverse way, when it both forward and the reverse wave when it is super imposed together you can see there is an amplitude getting increased, this is we call it as the constructive interference.

And the same way this is the forward wave, this is the reverse wave, when it is, this is the forward and the reverse wave when its opposite together at 180 degree, then what happens is that signal get cancelled here, so this is we call it as the destructive interference.

So constructive interference and the destructive interference makes the signal pattern, so how we are doing that? So we are using a biosensor here, as in the SPR they uses the chips,
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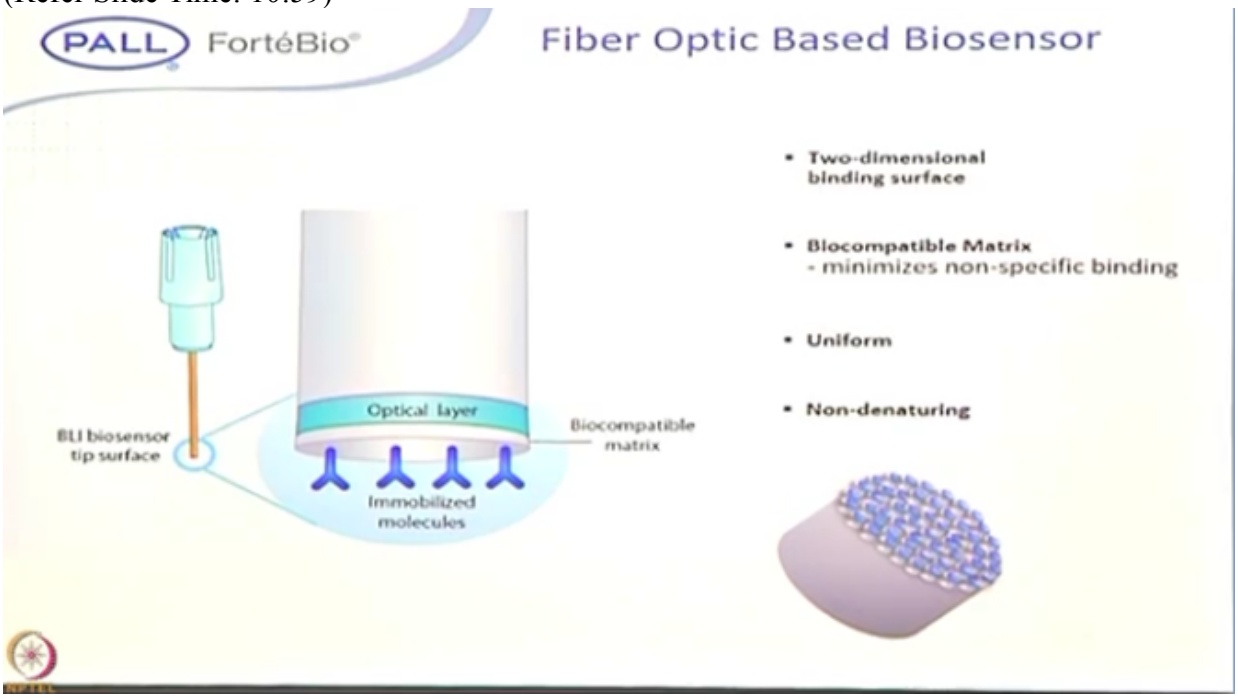

the similar way we are using the biosensor here, the biosensor if you look at this is the sensor typically it looks like a needle, this is made up of a plastic and this is your glass capillary which is nothing but the fiber optic.

At the tip of this fiber optic we are quoting the biocompatible layer, it is a optic layer, to that optical layer you are attaching a one of the protein of your interest, say suppose you have a two binding partners, one of the protein you have to attach on this, it is nothing but the solution solid interface, solution-solution interfaces are the I can say that giving example ITC, ELISA you can see as a solid solution interface as a, because one of the thing you are quoting on the plate, so these label feedback form also like a solution solid interfaces.

So one of the protein we are immobilizing over here, and then dip into the well containing a corresponding binding partner, so this sensor is a 2 dimensional binding surface, the matrix what we have quoted at the tip is the biocompatible, it is inert in nature you can work with any kind of physiological systems, buffers or the you can work from the PH ranges from 2 to 10, depending upon the application so most of your bio-molecular interactions happens at the physiological PH, and it is a uniform when we manufacture the sensor tip surface it's uniform across the lots and we test and then we releases, and whatever we have the quoted material is a non-denaturing, it will not interfere with your interaction system.

So if you take the diameter of this, the optic fiber is just only 600 micrometer, you required a very less amount of your sample immobilized on the sensor surface, so if you look at how this exactly the instrumentation inside on the bio-layer interferometry,
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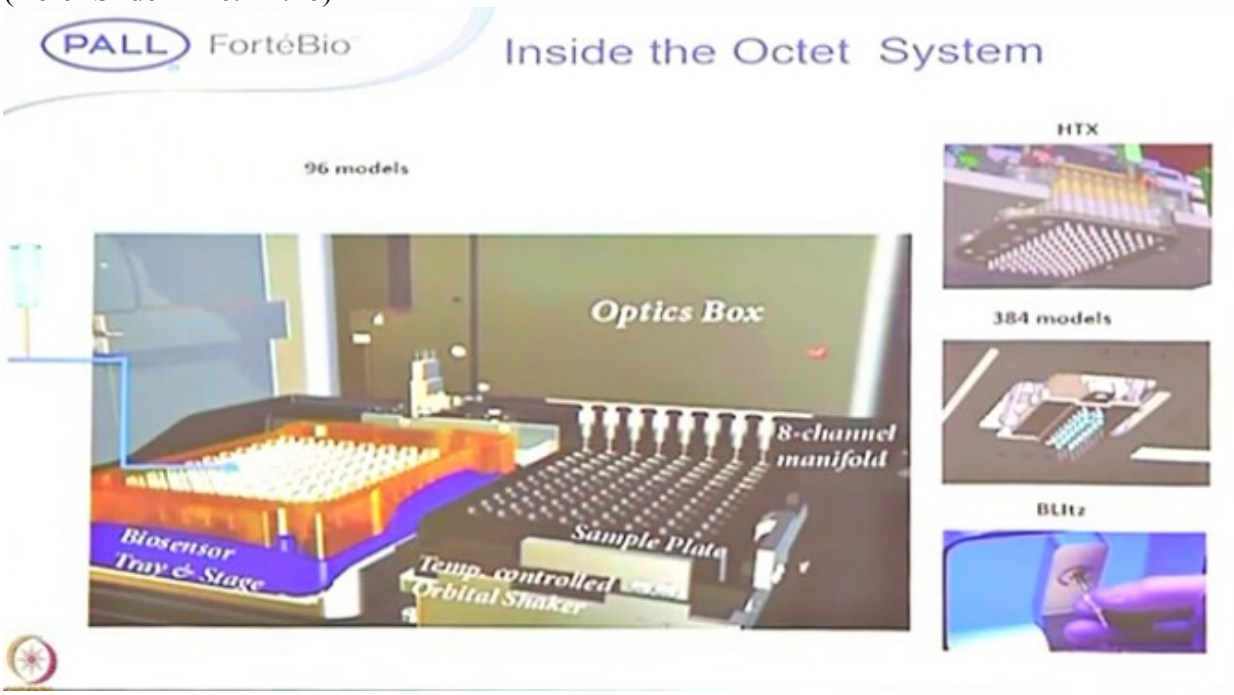

the octet platforms, this is the spectrophotometer here and it is connected with the robotic arm and these are the sensor tray, if you look at this is the 96 well plate form, so the robotic arm pick up the sensor and dip into your 96 well plate format, so this the sample plate we have a orbital shaker and the temperature control.

What is the difference between the SPR and the BLI's? We don't have any microfluidics here, just it works on the dip and rate, it's just pick up the sensor and dip into the well, so everything happen the reaction, whatever happening at the tip of the sensor, so in case of the microfluidic devices like SPR, the flow assessed in the binding, but here we have a orbital shaker which assist in the binding, so when it comes to the high-throughput we have a different channels like in SPR if you take it there is a 4 channel, 2 channel instruments, so where in exactly the 4 channel means you can pass the three analytes and one acts as a reference, and here we have a instrument, we have a 8 channel here, 8 interactions you can measure simultaneously that's why where the throughput comes we have a 96 channel here, 96 interactions you can perform in one goal, and we have a 16 channels, 16 interactions we can perform in one goal, we have a single

channel also, 2 channel and even we have a 2 channel auto meters instruments, so how exactly, what the principle behind this?

As I mentioned it's work on the interference based, okay, so the sensor, this is the sensor compartment, the sensor actually pick up the robotic arm pick up the sensor and dip into the well containing first buffer, and I'll show you in the subsequence at how it work, okay.

So what exactly the principle behind is? As I mentioned it's interference based, we are passing a light, it's just a white light, light get reflected back, if you look at you can see the reflections coming from the one internal layer, and one from your the ligandant, just I take it's I had immobilized one of the protein of interest from the sensor surface,
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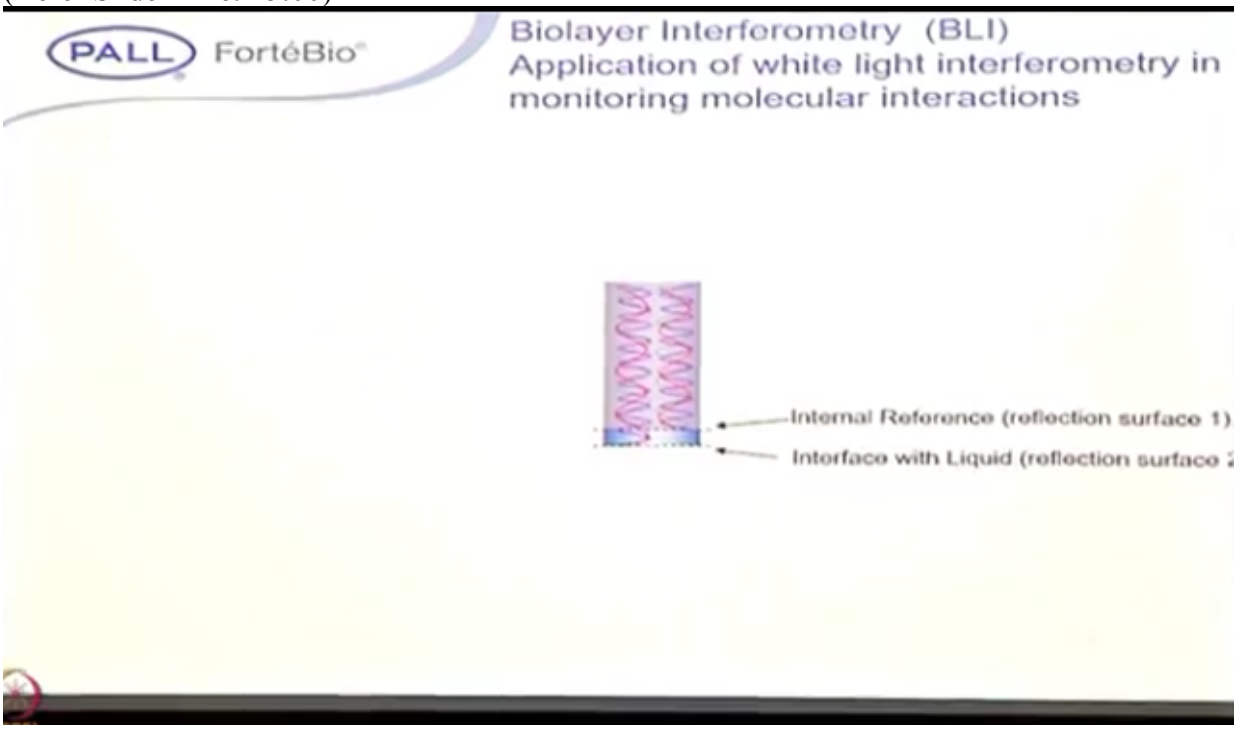

then I dip into the well containing buffer, if you look at all this what exactly it is amplitudes, different amplitudes we have, constructive interference, destructive interference,
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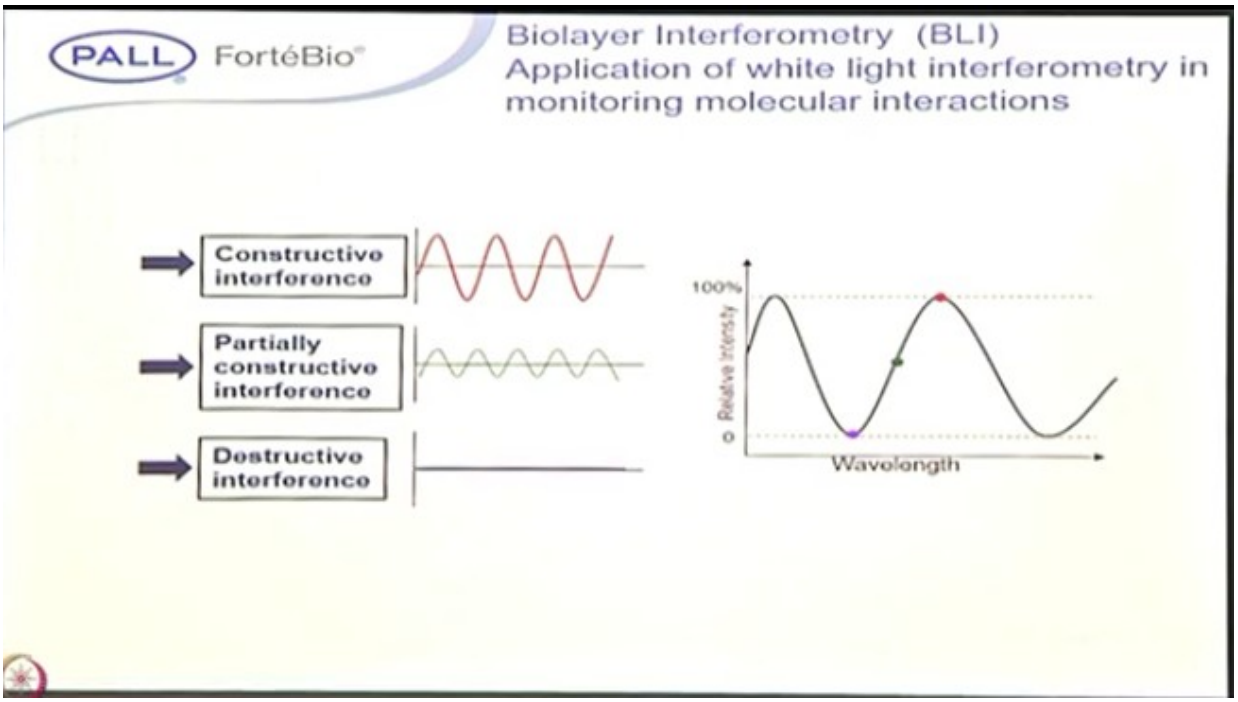

this is what we exactly we plot, relative intensity versus the wave length, so what happens? Say the one which have a higher amplitude where exactly the waves get superimposed, then you can see the amplitude getting waves where exactly there is a partially superimposed you can see this kind of, and there is a destructive interference there is this completely signal get cancelled.

So what happens? When the same sensor, when you dip into the corresponding binding partner, now you can see that, earlier was just only light reflecting from the blue coloured layer, now you can see there is a one more orange layer, if you see this now right which is reflecting from the orange layer.

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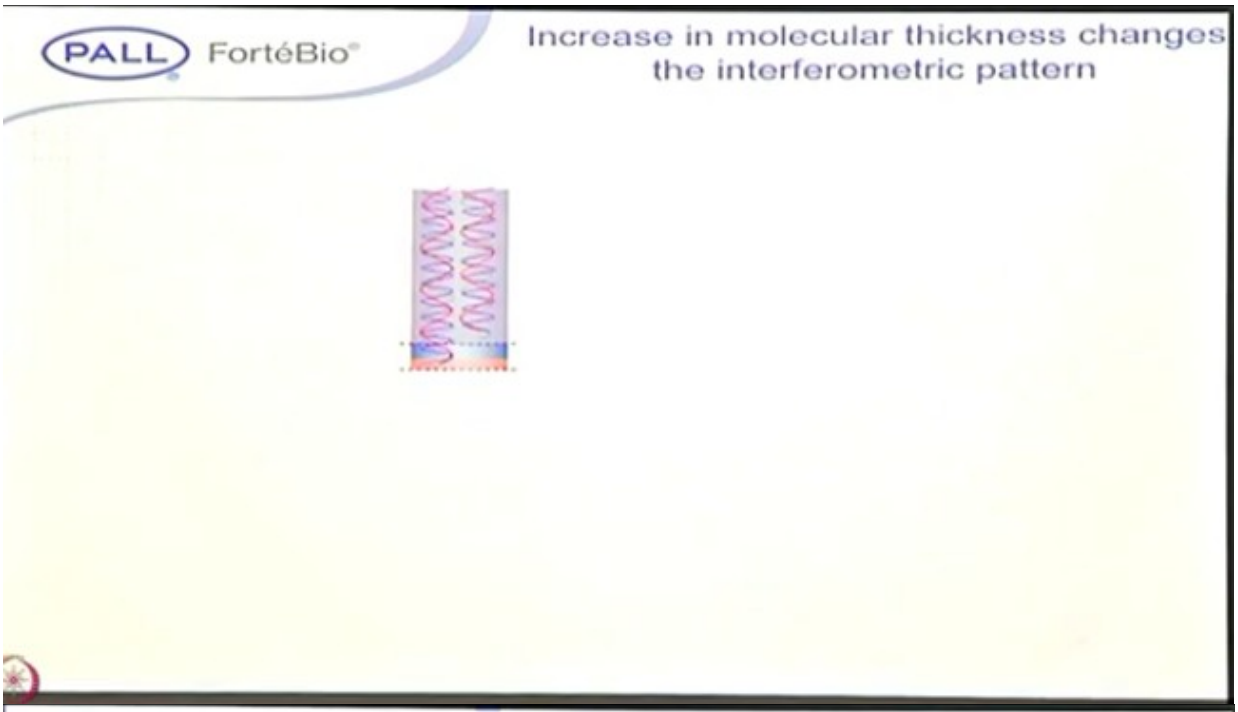

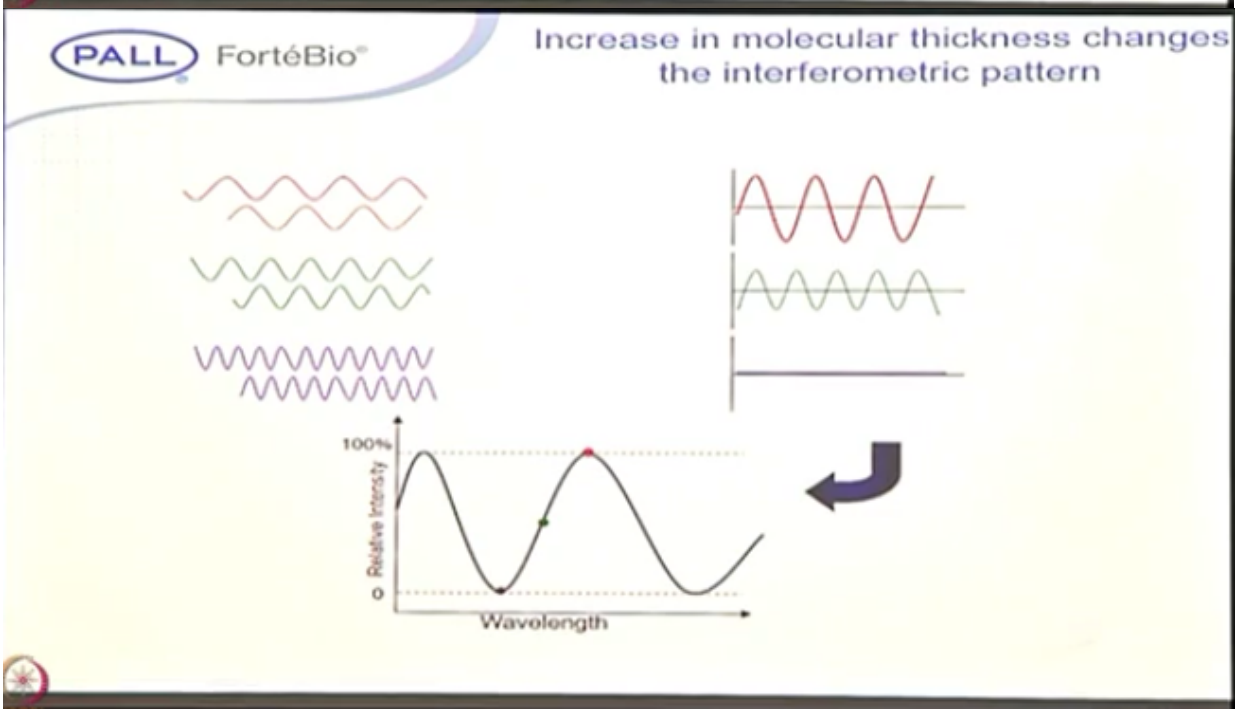

Once again if you look at all these waves are there, earlier was the plot like this due to the molecule starts binding to the sensor surface you can see there is a shift in the interference pattern, this is what exactly as the molecule binds to the sensor surface you can see the shape, so what happens exactly on the tip? The molecules bind to the sensor surface it forms a bio-layer, its depends upon the thickness of the bio-layer, more the molecules bind to the sensor surface sorry back, more the molecules bind to the sensor surface you can see the relatively shift in your spectrum to reversible ride, this is what exactly the principle, so using this phenomenon as the more molecule you can bind you can see the corresponding relatively shifts

towards the right, so you can do the quantitation using this phenomenon, not only the kinetics you can determine the quantitation, so in real what happens? Molecules bind to the sensor surface you can see the real time picture as the molecules binds,
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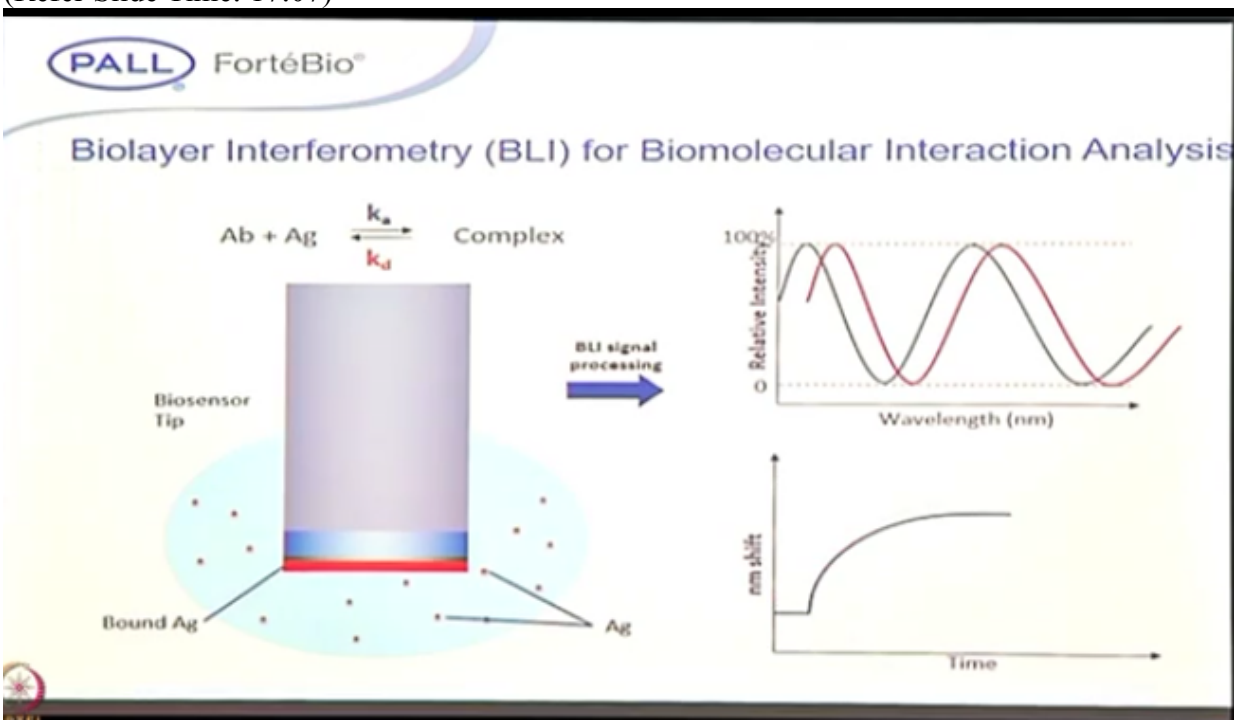

then it leads to the equilibrium, then the same sensor when you wash off the bound molecule you can see the dissociation, this is what exactly the real time you can look it, the background is this one, the molecules biosensor surface, then you make a bio-layer and relatively shifts and once you washed off then it will comeback, so what exactly information you can get from this?
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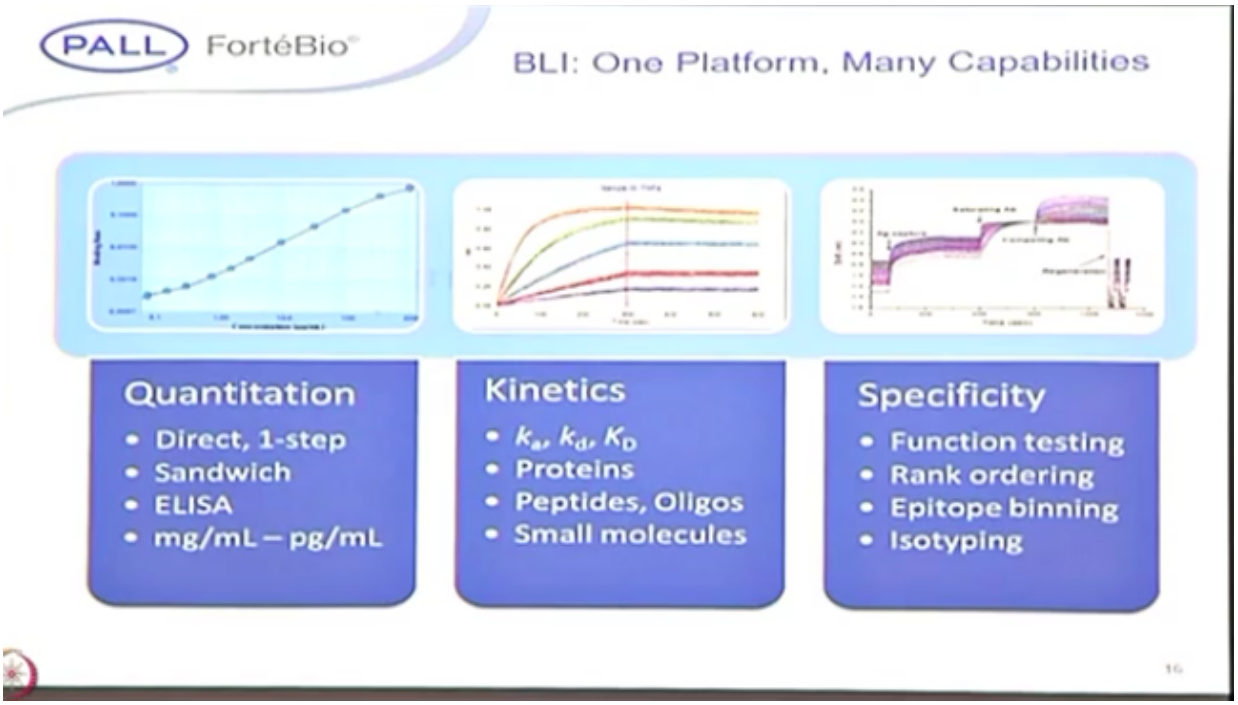

So as I mentioned the one based on the shift you can determine the concentrations, you can do the direct one step kind of binding, sandwich, if you want to convert ELISA platform on to the instrumentation you can convert it, so depending upon you can determine the micromolar concentration you can go as low as the nanogram or the MG per melto picogram per ML, so the quantitation you can determine and the kinetics. The kinetics as I mentioned on rates, off rate and the offendy constant you can determine irrespective of whatever the biomolecule is maybe a protein to protein, protein to small molecules, protein DNA, protein RNA and all that.

Apart from the specificities where exactly you can do the functional testings, functional testing with respective FC gamma interactions where the people are doing in the drug discovery, the rank ordering when you are doing the hybridoma screenings with a large map platforms where you can provide the, which is the best antibody will bind to the target based on you can give the rank ordering on the off rate of the molecules.

And the epitope meaning we have a dedicated software for the epitope meaning you can do the screening of your epitope where exactly your molecules finds that, and the isotyping also subtyping of your the IgG's. So we have lot of applications on this, I'll go through later on those applications, see one of the important parameter is most of the people you, you people are doing the quantitations based on the like bad photosis or the total protein content.

When you say suppose you have a protein in a kind of a matrix where exactly you're expressing protein of interest in a cell culture or the, you want to determine in the patient samples, or how much my protein of interest which is there or not, so you can easily because it's works on the dip and rate, there is a no such kind of say even though if it is cell debris are there, impurities are there, anything is there, it is purely an affinity based interactions you can easily quantitate using this phenomenon, so that's why the advantage is like that you can go with any kind of sample matrix, it's need not like the you had to go for the purified one, when it comes to the SPR you have to go with the more purified samples.

And the second advantage is like that you can go with a PH ranges easily like 2 to 10 depending upon the application you can screen quickly because it's have a high throughput 8 channel, you can quickly screen the samples which PH is the favor for the binding.

So these are the platforms we have, this are the 16 channel instrument, this is the 2 channel instrument, this is the 8 channel instrument, and this is the 96 channel instrument depending upon the high-throughput what we required you can choose the instrument in that,
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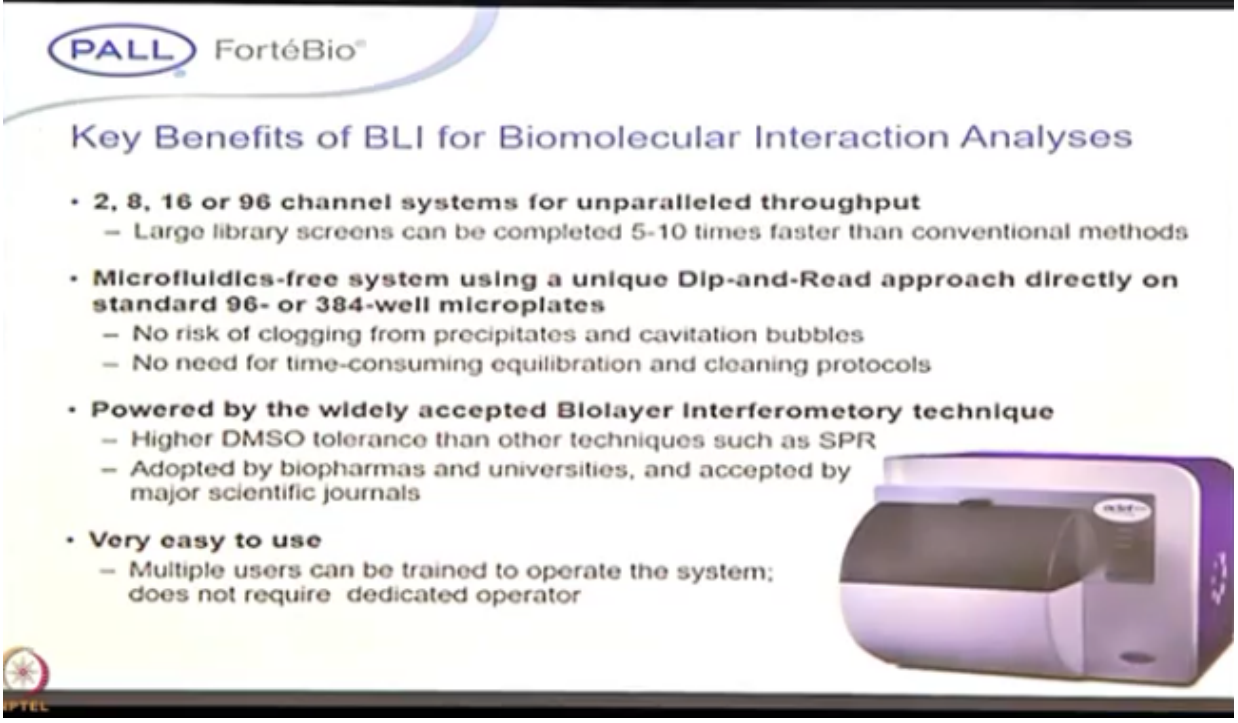

and the more advantages like that as I mentioned there is a no clogging, its work on the dip and rate, and the high throughput you can save your time, you can quickly screen the experiments, and the good thing is like that when it comes to the interactions where exactly the samples have a DMSO or the glycerol.

When it comes to SPR, presence of the DMSO and the glycerol sometimes you can see the bulk effects, because SPR is a very sensitivity to those changes, but with dip and rate you can easily go with the such kind of, there is a no interference from the glycerol or the DMSO in that, and the good advantage is like that is typically the software is so user friendly, just if I train for half an hour you can start your experiments, no dedicated operator required for this, typically the programming is like your ELISA plate reader so what all are the ranges, what kind of molecules we can go?

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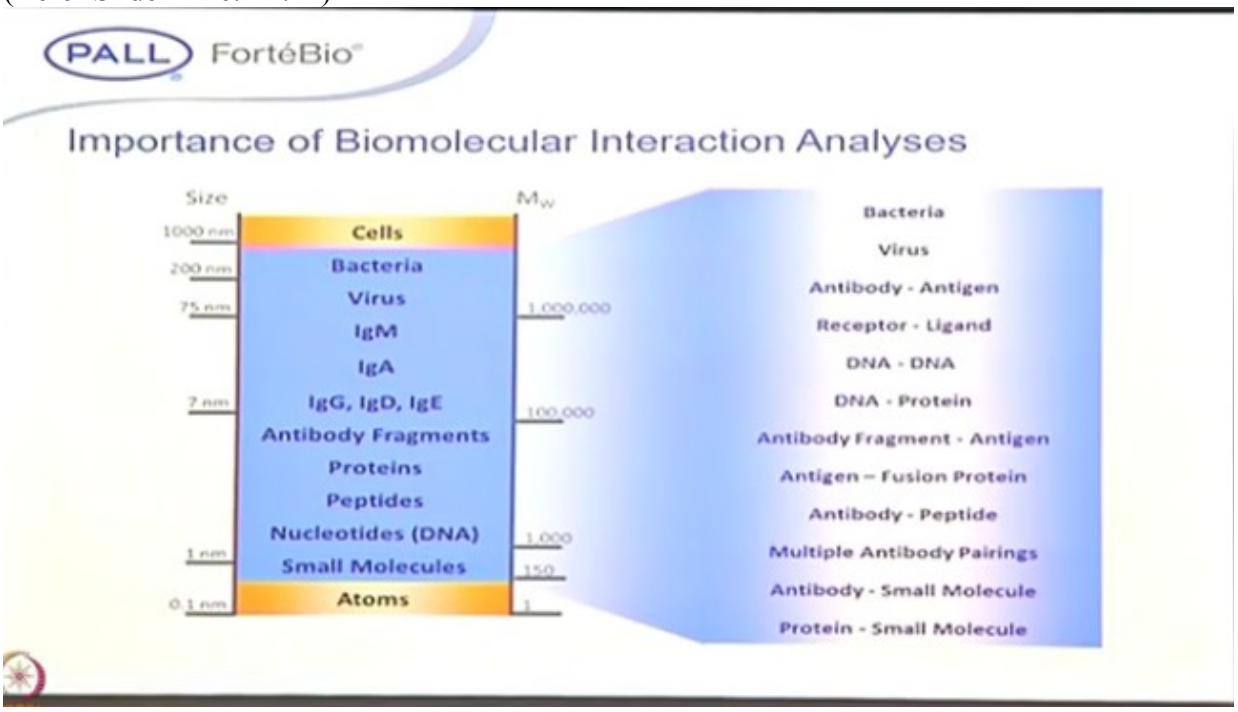

Except cells and atoms we can go in the blue areas, all these biomolecules we can go with the interactions, I can show you the cells recently we have one for the publication from the gen map, I'll discuss with those things.

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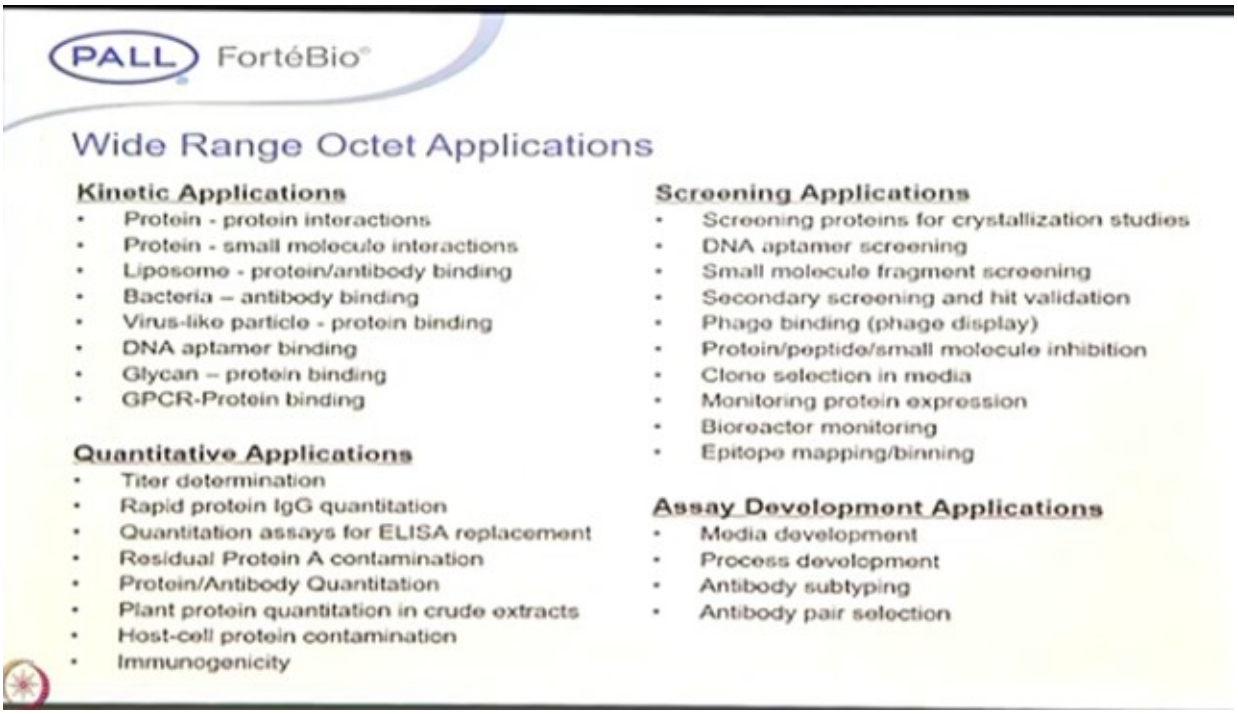

So these are the different applications we have segregated, kinetic applications, quantitatives, screenings, and development, assay developments and all that, we have publications for this, we have more than 1000, 1500 from the past 10 years we crossed all published in impact factor generals, so how exactly it works? If you look at the animation,

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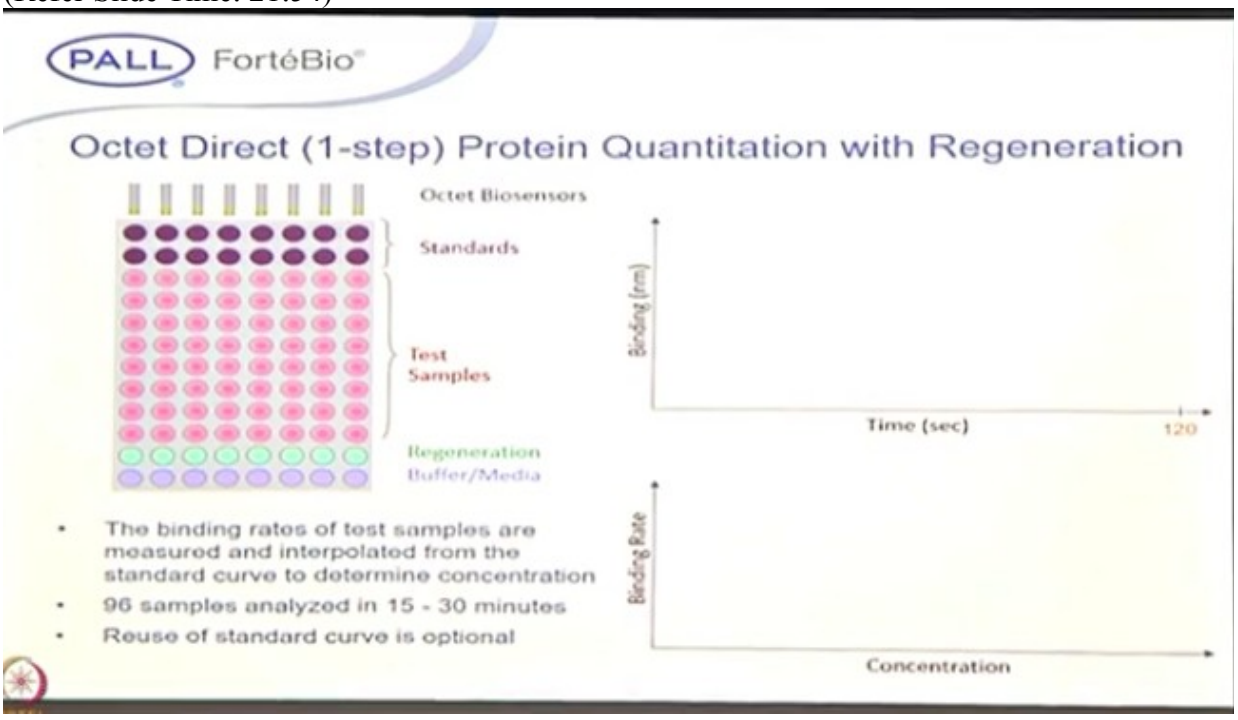

if I say this is the typically protein quantitation, if I take it any affinity based, so suppose you are working with one of the histac protein how much is expressed in your culture, so suppose if

I have a standard in that, if I dilute in the plate, 96 well plates lower to higher dilutions, take a 8 sensors when I dip in this you can get a curse like this, based on the binding rate versus the concentrations,
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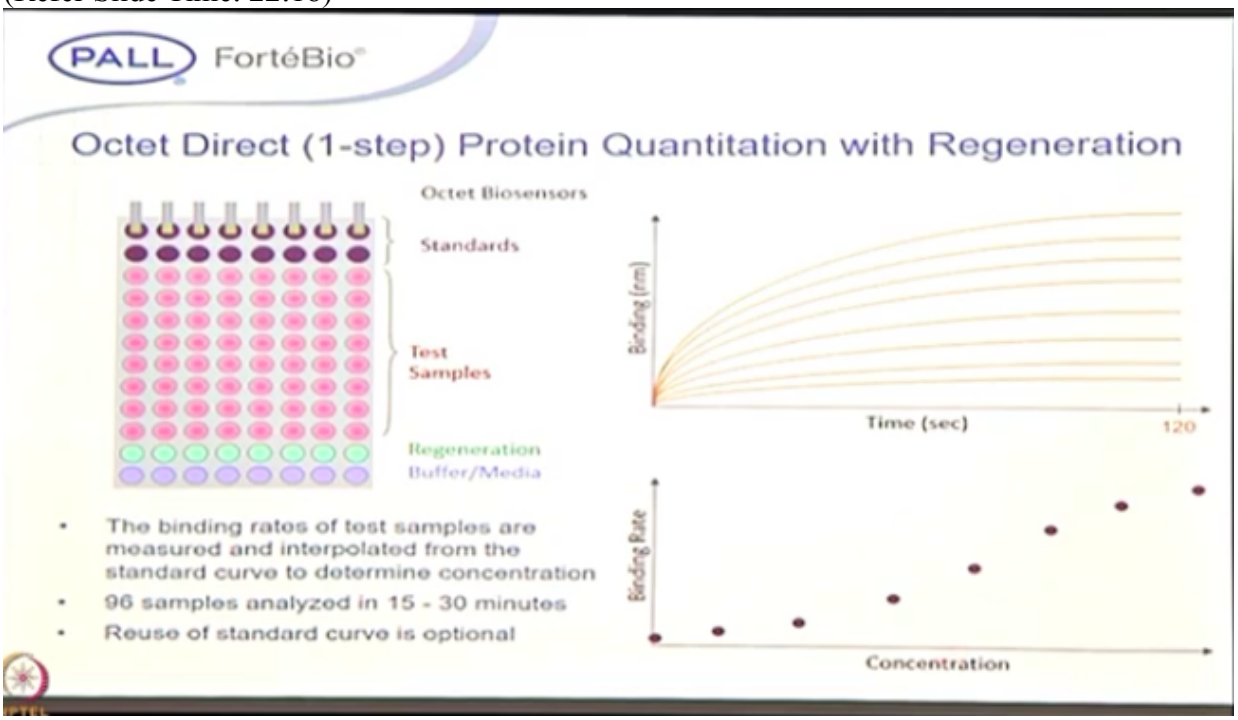

the higher concentration have bigger and the lower ones are this.

When the same sensor if I go for the regeneration, so where the bound analyte get washed off here, and then when it dips in to the unknown solutions where your cells, means your protein is expressed you can easily quantitate using this plot, so very quick experiment in a just in 15 minutes you can determine the concentration of your protein of interest, so how exactly works the kinetics workflows.

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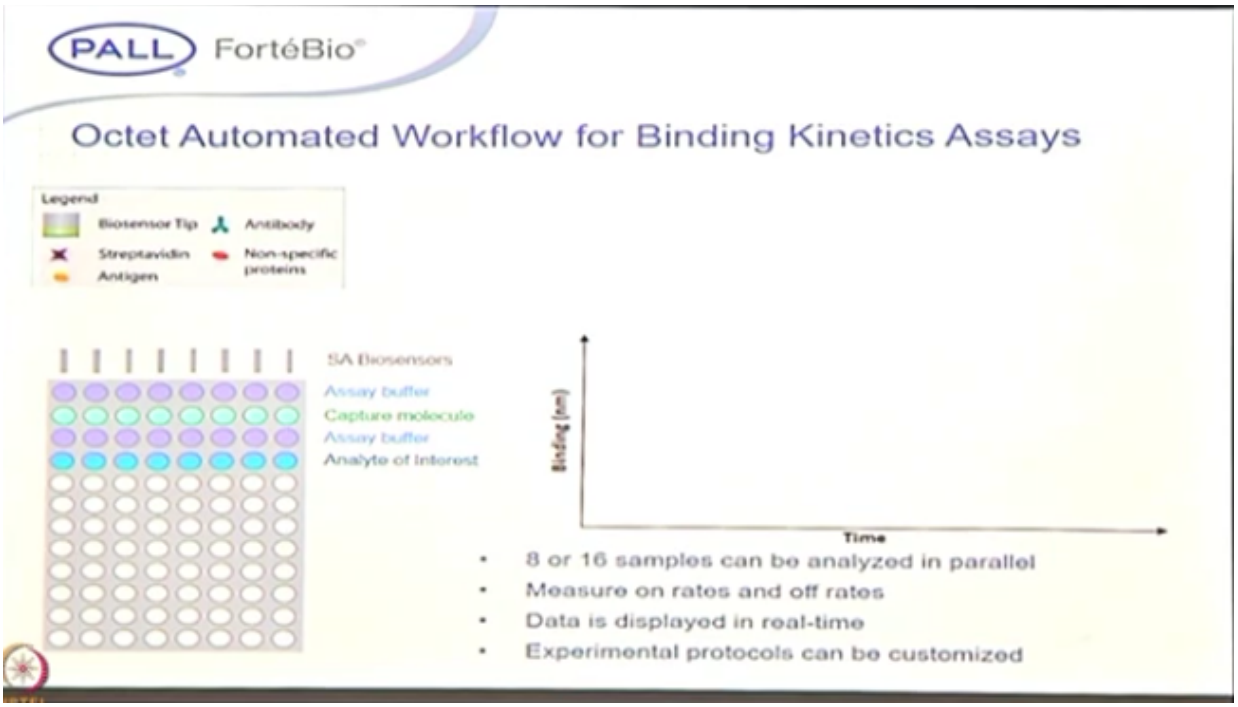

First the sensor will dip into the well containing a buffer, you get a baseline here and next it will comes to dip into the well which have a the loading and then goes back to the well containing a buffer there exactly the unbound material get washed off

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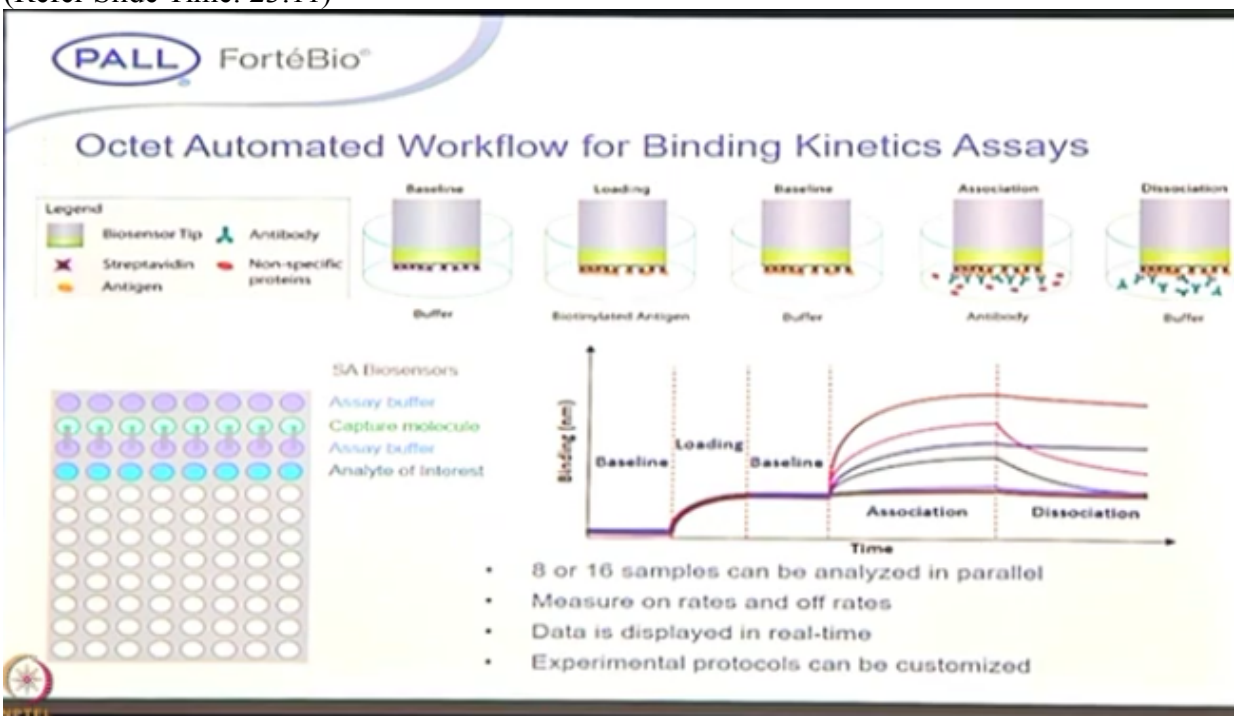

and then corresponding analyte it is binding you can see the signal and it goes back to dissociation.

A very simple, it's just you had to the robotic arm back in forth, just I'll explain once the buffer the sensor, the sensor dip into the well containing a first line of buffer you can get a just a baseline, there is a no any molecule binding to that, that's why you get a baseline, when the same sensor when it move to the next well containing a capture molecule, one of the protein of interest you have to immobilize on the sensor surface you are putting in the second well, then the sensor dip into that well you can see the loading response, as I mention some molecules went to sensor surface you can see the change, right, the phase shipped, so you can see the loading response, when the same sensor move back to the well containing a buffer here just a baseline, there is a no molecules binding, unbound molecules get washed off here, and the sensor next move to well containing a buffer, sorry, the analyte we can see the response, and the same sensor goes back to the well containing a buffer bound analyte get washed off.

Very simple to operate this experiment, you can quickly screen just in a 15 to 20 minutes we can get the kinetics data, so we have a,
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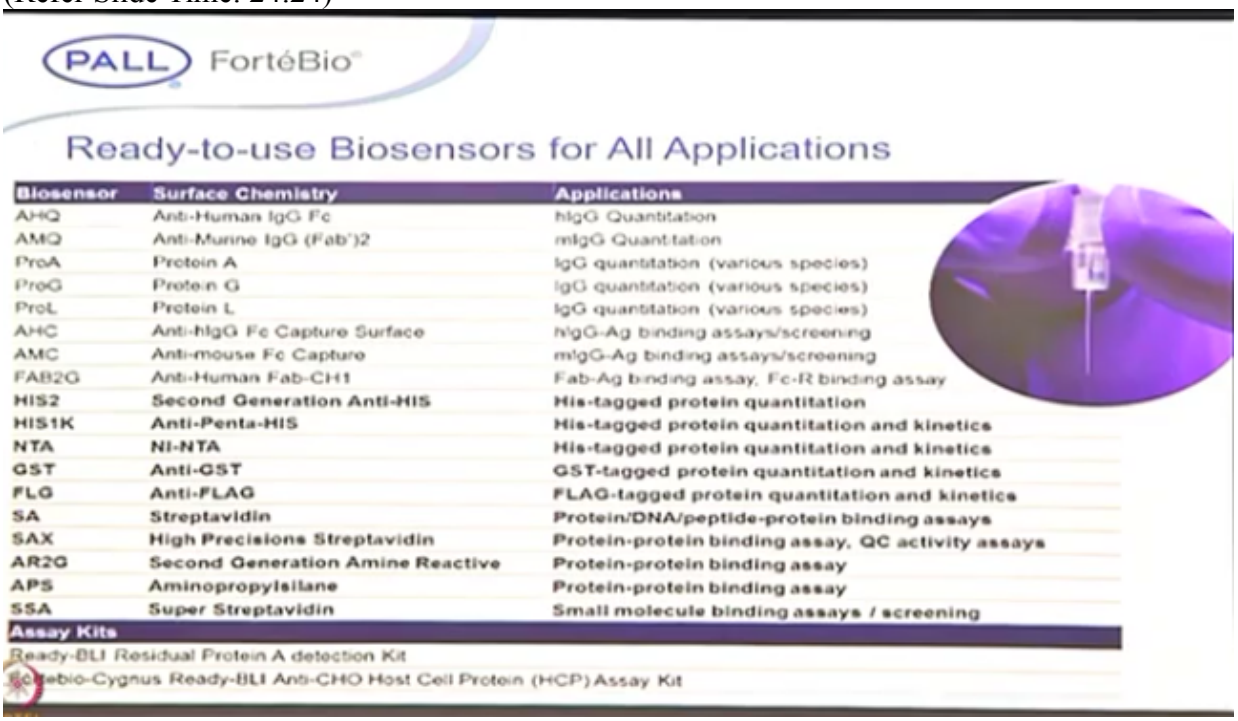

on the shelf we have a different biosensors chemistries with us depending upon your protein of interest we have a tag, or you can choose or if you don't have any tag you can go with the some kind of modifications like biotinylations or the amine coupling you can immobilize, so these are the pre-quoted for the antibody platforms, we have protein A, protein G, protein L, and some FC capture sensors, and for the histac based proteins we have a nickel NTA, we have a Anti-HIS Sensors, we have a GST tag basis, we have Anti-GST sensors, and if there is a no any tag your expressions then you can bark and late your protein of interest, and then you can coupled to the streptavidin sensor, the biotinylated protein.

We have a APS sensors, aminopropylsilane for the hydrophobic interaction, we have proteins more hydrophobic, no need any tag or anything just we can just bind to the protein of interest

on that. And for the super streptavidin we have a, this is a quad based sensors especially for the small molecule protein interactions.

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So we have come to the application side where, how we are going to use the one of the example here is this is one of the group they had,

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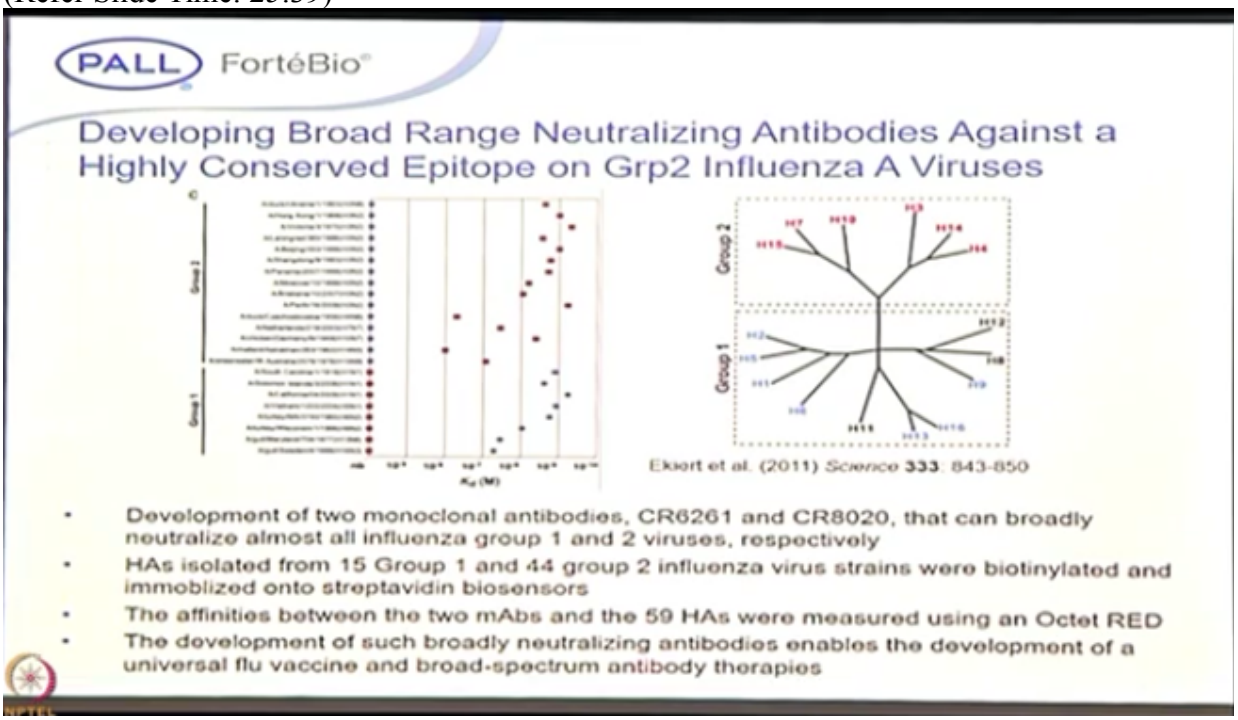

they are interested in developing the vaccine based on the influenza, they identify the two antibodies which are broadly neutralizing capacity to the influenza, in this experiments what the author did is he isolated a 15 group one and the 44 groups different hemagglutinin he had taken from these groups, and then he did a biotinylation, and then he immobilized using the streptavidin sensors all the 59 hemagglutinin to that and then screen with the respective the antibodies, among that if you look at these you can see the binding interactions of the different antibodies to the respective hemagglutinins, from this they had developed the broadly neutralizing of the two antibodies in that, so very quickly we can screen this kind of applications using this.

So next example I can show you is the where, we can understand the proteins structure and the function, here what is the let-7 is one of the important tumors suppression microRNA, so in this what author did is the KSRP is the protein which is binding to the let-7 precursor,
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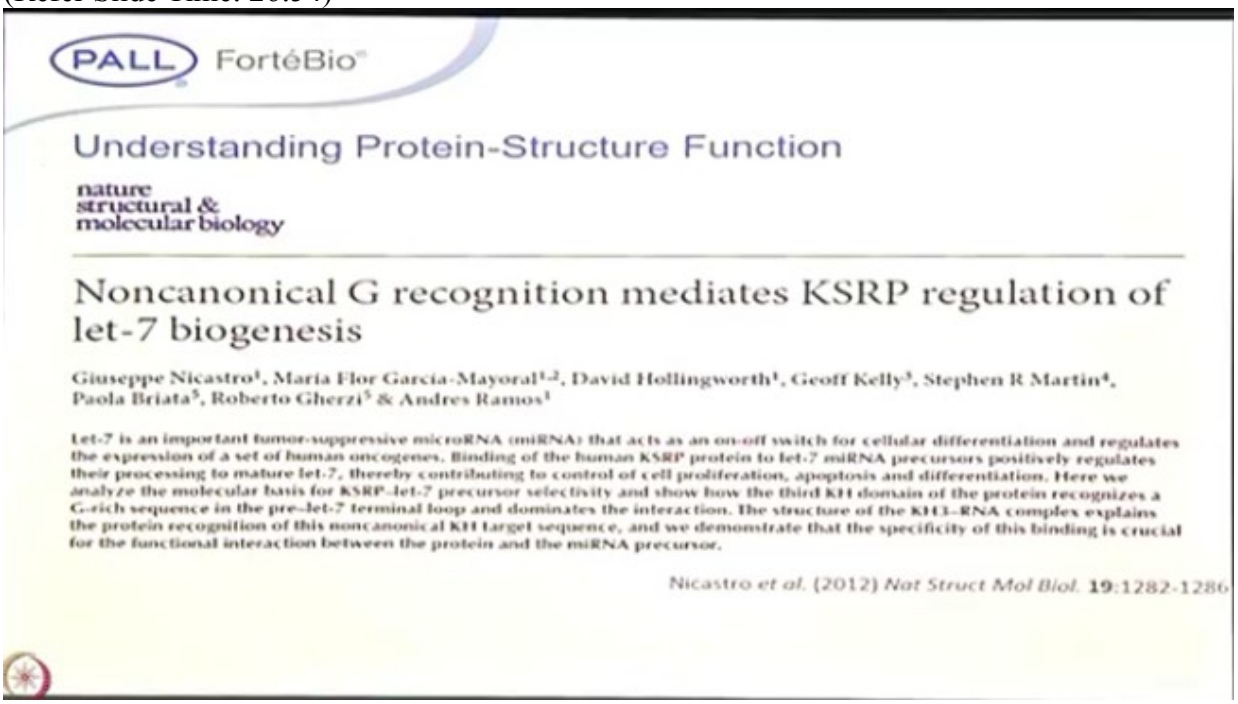

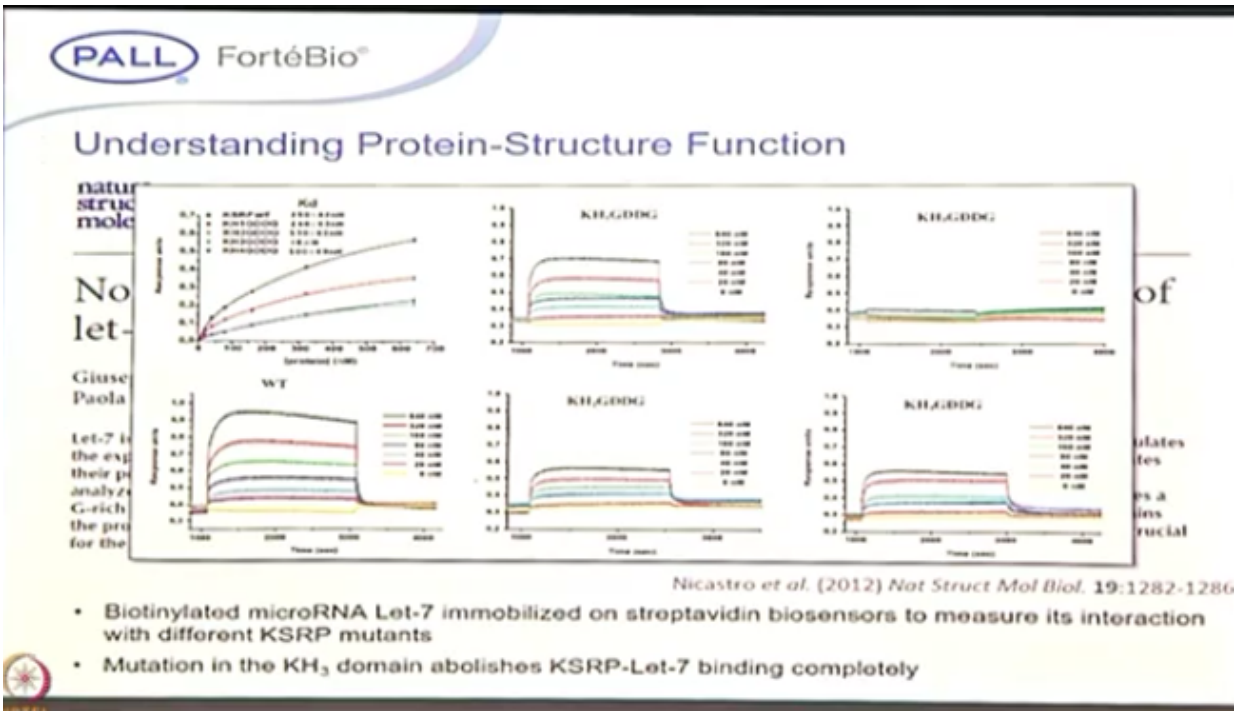

so what happens is here, the KSRP and let-7 is your microRNA which is biotinylated and immobilize on the streptavidin sensor, and this KSRP have a four domains, so among that he did a mutation, in one of the protein, the domain KH3, if he did a mutation it is not binding, so with a wild type he had showed that it is the KSRP binding in the four domains, among the one KH3 if he did a mutation it is not showing a binding, so using this he concluded that with respect to this KH3 domain is the one of the important parameter for the binding of the KSRP protein to the let-7, so it is one of the important, the transcriptional factors for the cell differentiation and very important in case of the recruiting the oncogenes for the, where exactly the cell differentiation happens, if it is a mutation happens in that particular gene there maybe chance of the cursing no mind.

So one more where exactly facilitated binding interactions studies here, so this is one of the example where exactly the fuse system is the one which is important in the cell differentiations c-myc is the important and regulatory gene, so this Cic means the expression of this particular gene is more in case of the cell differentiation,
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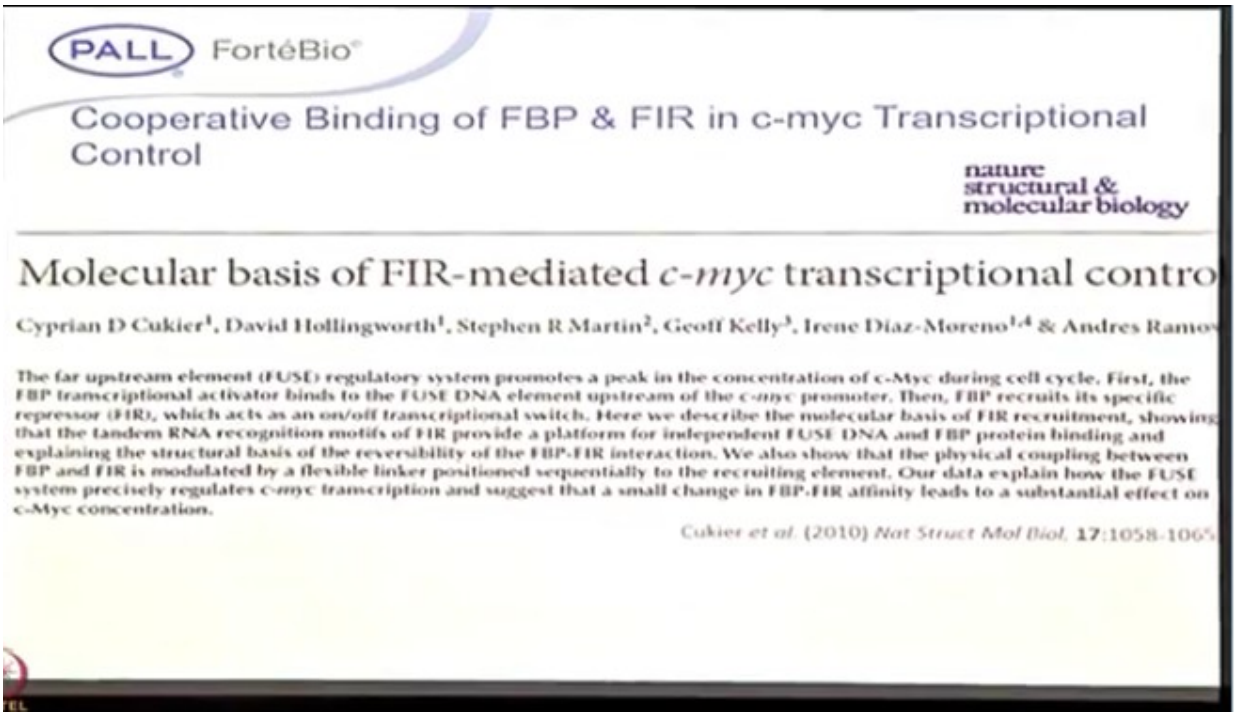

so how this happens, this regulation? So the author concluded using this, there is a called as FBP protein, presence of this FIR only interacting to the FBP protein, so in this experiment what he had done,

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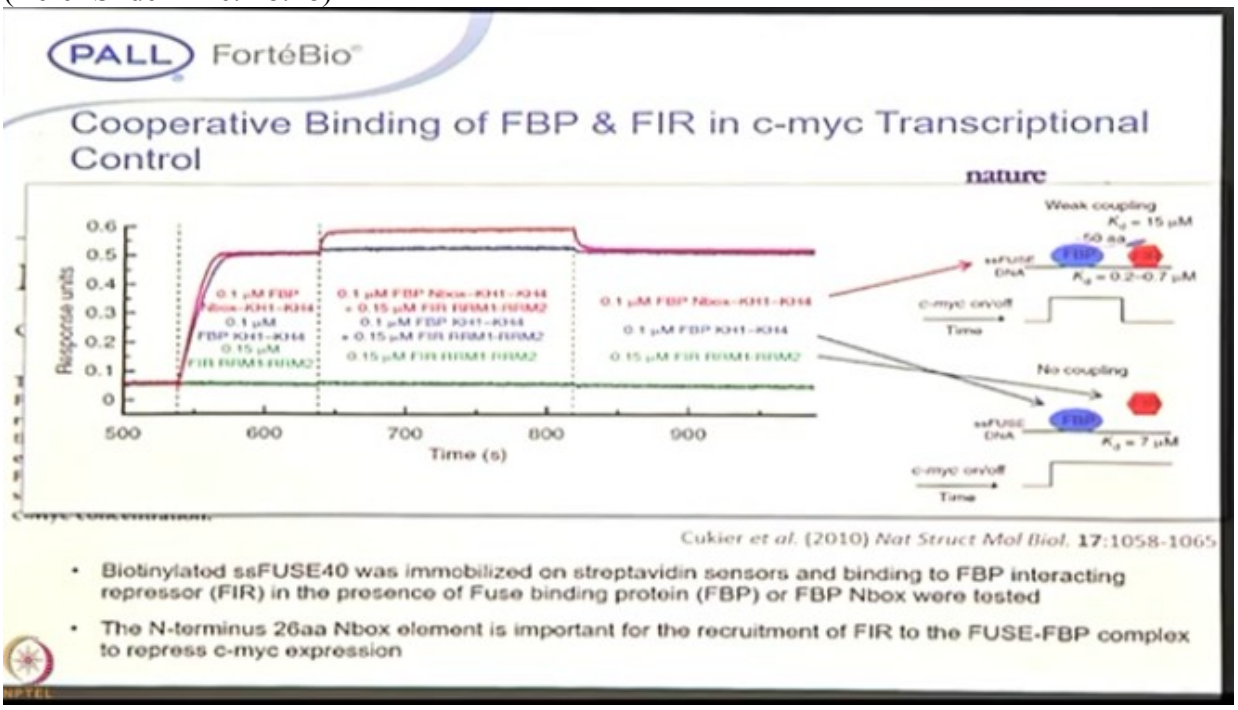

the biotinylated the fuse for the DNA, he immobilize on to streptavidin sensor and then he performed with the binding of the FIR, so in presence of the FBP protein only the FIR is binding without this proteins its not binding, so he concluded like that.

So very important tool when you do the, in case of the basic research, I can quickly screen those, so one more example I'm showing here is the folding and unfolding patterns of the proteins,
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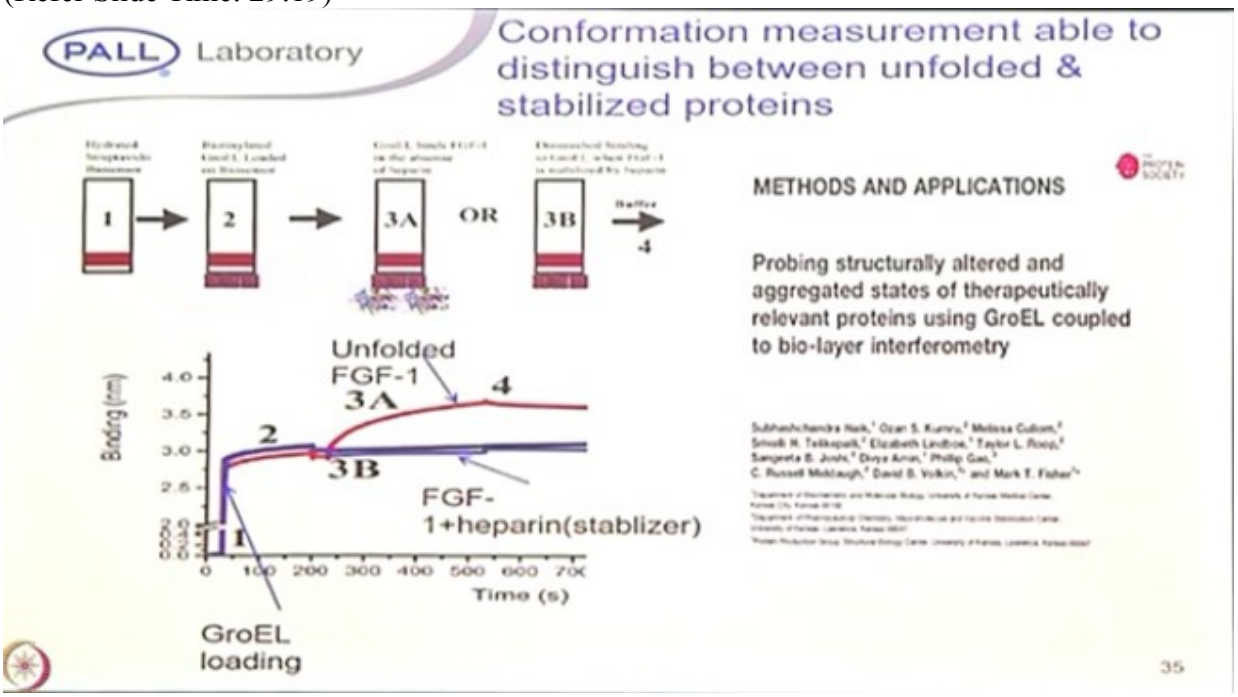

so in this example the sensors where exactly the biotinylated the GroEL is immobilized on the sensor surface, the unfolded protein will be binds very, then you can see the response, the folded protein will not bind, so using the shufferon model you can use for the screening whether the folded or the unfolded pattern can easily study.

And the one more example in case of the diagnostic immunoassays,
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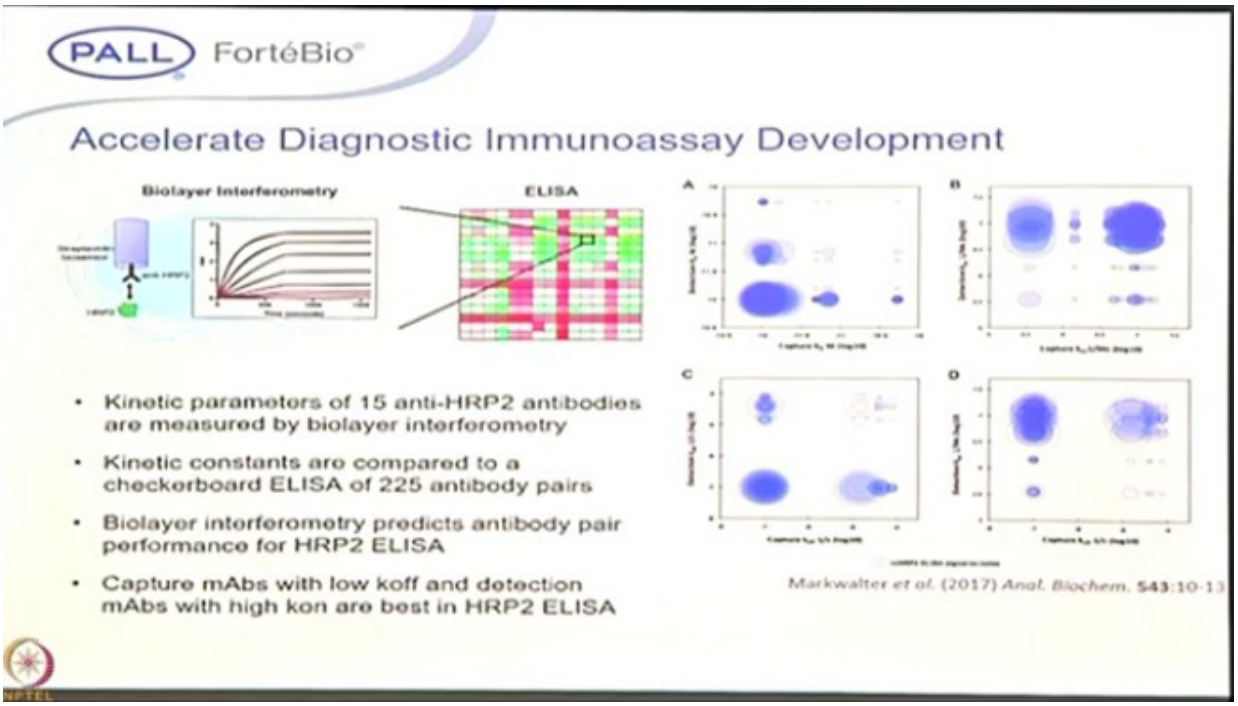

you know giving a example here is like that the kinetics parameter 15 anti-HRP2 antibodies they measure, they compared with the ELISA where they had comparison they had done you can quickly screen which of the pairing, the antibody pairing which have better diagnostic for the, diagnostic applications, when you are developing any ELISA kids or you required a primary antibody and the secondary antibody, you can quickly screen using that, so which are the best ways based on the your the kinetics profiles.

So Aptamers screenings, so Aptamer is one of the area now is picking up comparison to your antibodies,

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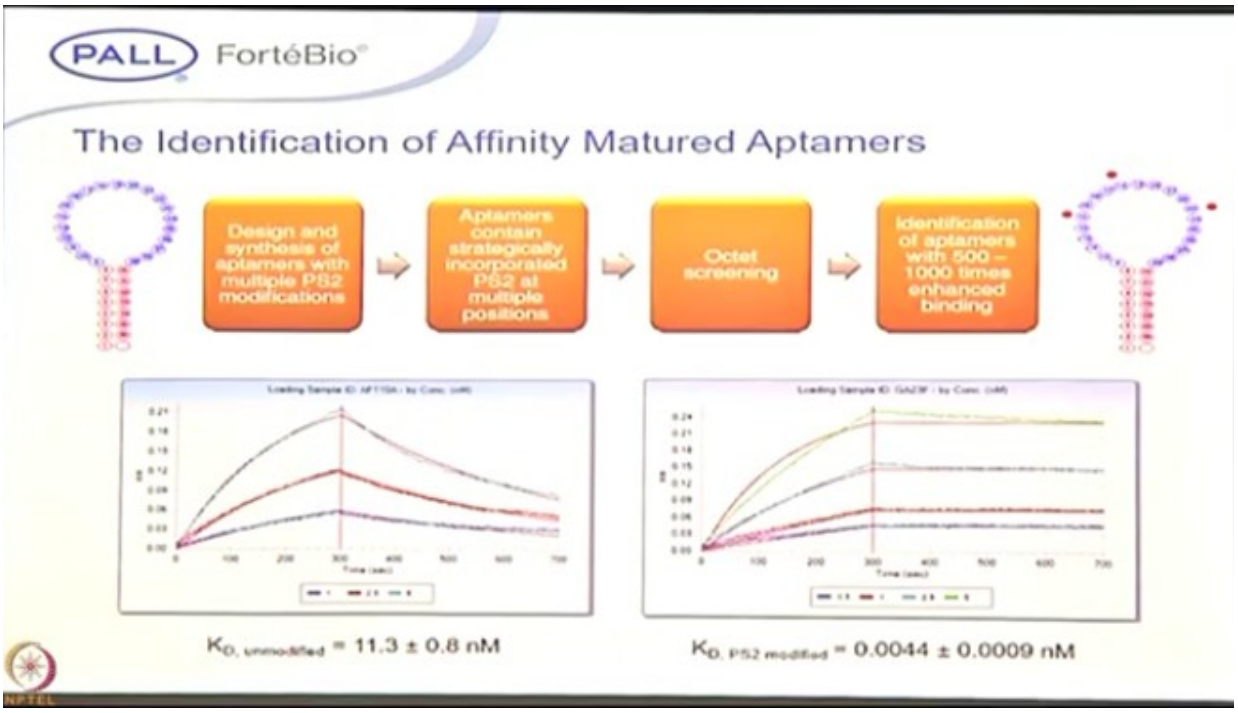

most of the many laboratories in India they are working on the Aptamers, so Aptamers also similar they have a different size and the shape, when it binds to the particular the target you can see the response, so Aptamers also have a like a major application in diagnostic industries, so one of the example you can quickly screen using the Aptamer interaction studies using the BLI technology.

So the one more important where exactly when it comes to drug discovery applications, small molecule protein interactions if you look at with a one concentration different, you can screen as many as compounds as possible and then you can look based on the off rate you can select the right candidates, this is the one of the work from the University of Michigan,
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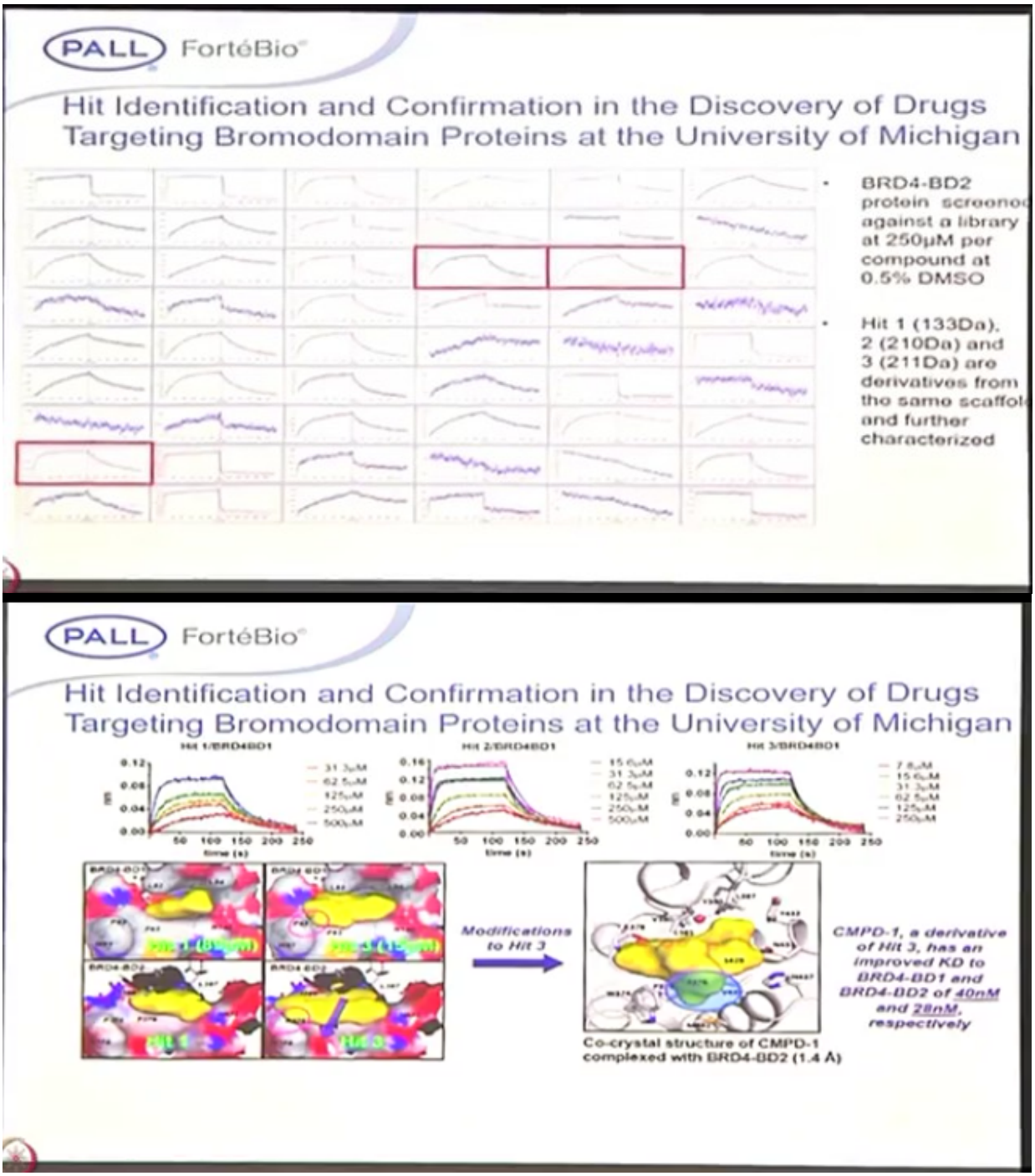

they had done a publication on this, they had developed the three heats with a complete kinetics characterization using this the bromodomain 1 protein and the one more example I can show

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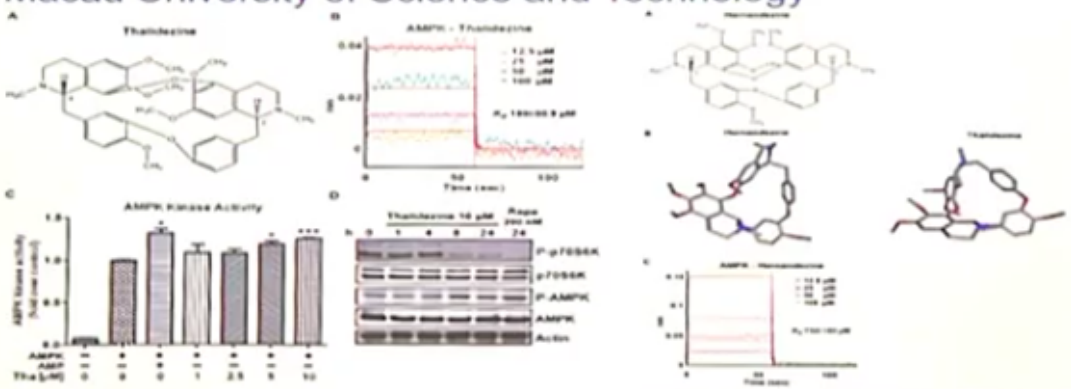
PALL ForteBio

Characterization of an Anti-tumor AMPK Activating Compound at Macau University of Science and Technology



Law *et al.* (2017) *Oncotarget* 8:30077-30091

- Thalidezine, a structural isomer of hernandezine, binds directly with AMPK protein and has a higher affinity than hernandezine as measured by BLI
- Thalidezine induces autophagic cell death in HeLa or apoptosis-resistant DLD-1 BAX-BAK DKO cancer cells

you is the characterization of this AMPK protein to the two compounds Thalidezine and the one more here, so Thalidezine and the structural isomer and the Hernandezine, it's like a, compared to this the thalidezine is the more binding compared to the other platforms, you cn quickly screen using this tool.

And one more the next very important when it comes to the ligand fishing experiments,
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PALL Laboratory

Ligand Fishing

- A technique used for the identification of receptors with unknown specificity
- Describes a quick way to screen crude cell supernatants to identify potentially interesting therapeutics.

Target Groups: Proteomics groups in both pharma and academia

I think it is more relevant when it comes to the interactomic studies, because what is exactly the ligand fishing is, so suppose you don't know what is the protein is interacting to your one of the protein of interest, so suppose you take it the protein of interest mean you can immobilize on the sensor surface and then put it into the cell extracts or a lysates or anything, which is the protein is binding to the sensor surface you can elute it and then you can go for the mass spec studies.

So one of the example I'm showing is the interaction you dip it and then you can elute those the binding combinations, and you can go for the electron microscope or you can go for the mass spec studies, so here one of the example I'm showing is Monitoring Anthrax Toxin Prepore,
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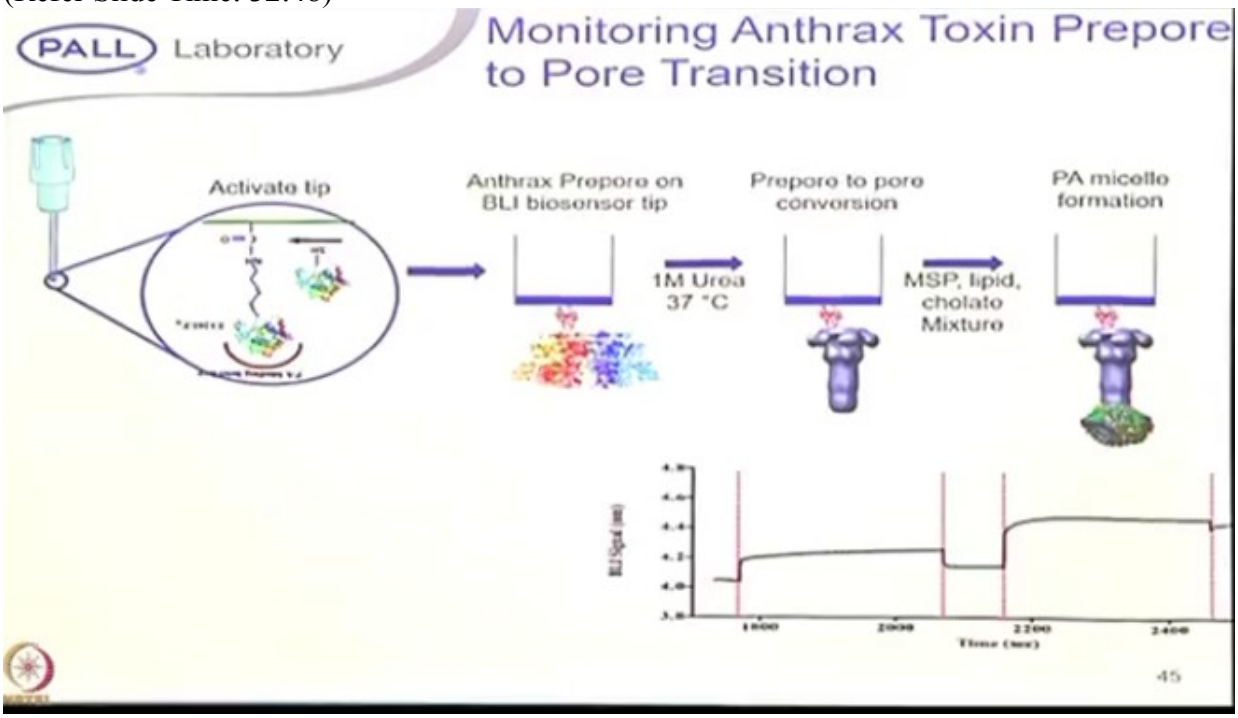

this example they activated that tip and they had immobilize the protein of interest the Anthrax Prepore, and then they had process with 1 molar urea at 37 degree, the prepore to pore conversion happens and when they dip into the lipid cholate mixtures you can see the micelle formation between the pore and the lipid.

So what they had done?
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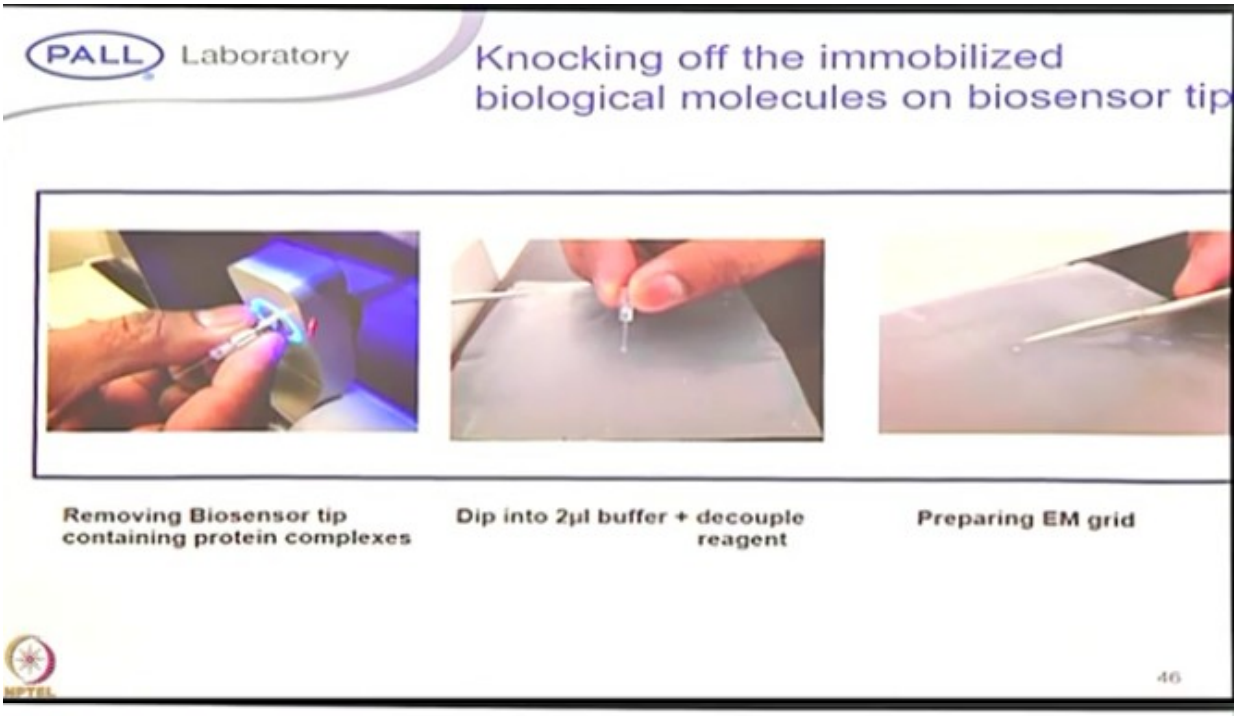

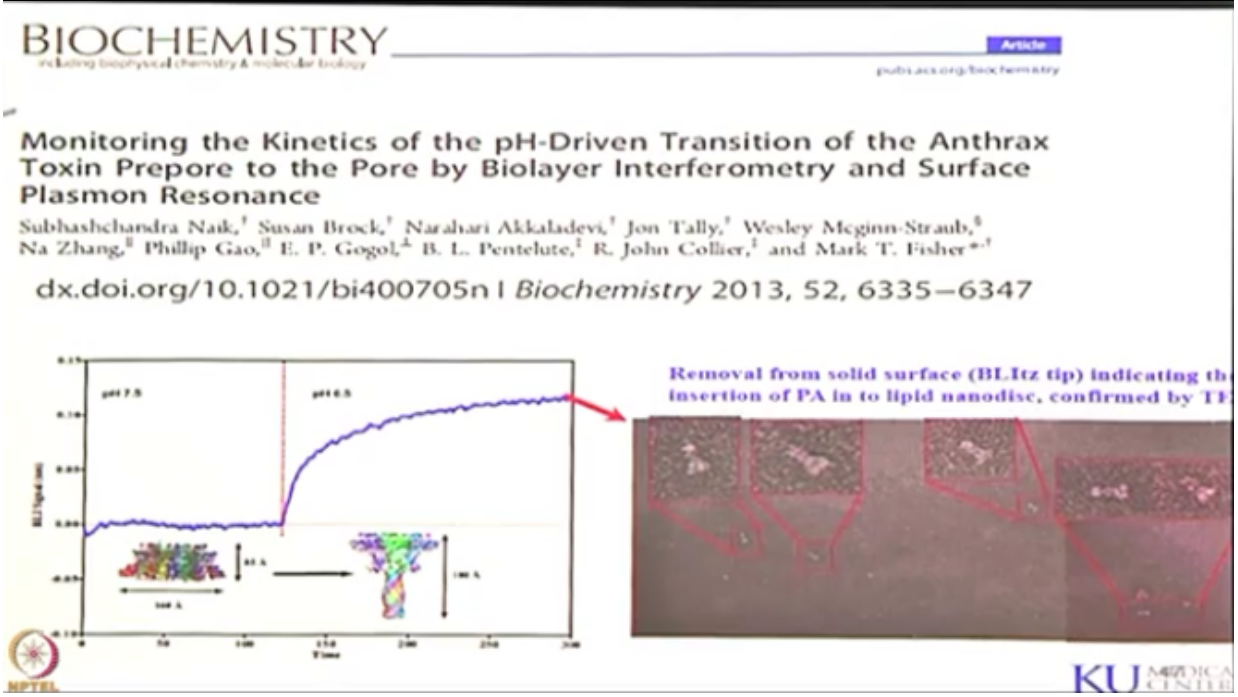

Removing the biosensor tip and they dip in the buffer and decoupling reagent then preparing for the EMI grid you can look at the electron microscope how the complex are there, so you can easily you can perform such kind of experiments, because when it comes to the SPR, when you elute the complex mixture and when it comes to, when you are going to collect that because your sample is more diluted, then you have to concentrated, then you have to perform the further studies, you can enrich in the dip, in the well itself here, so one more example is the, it is the industrial work,

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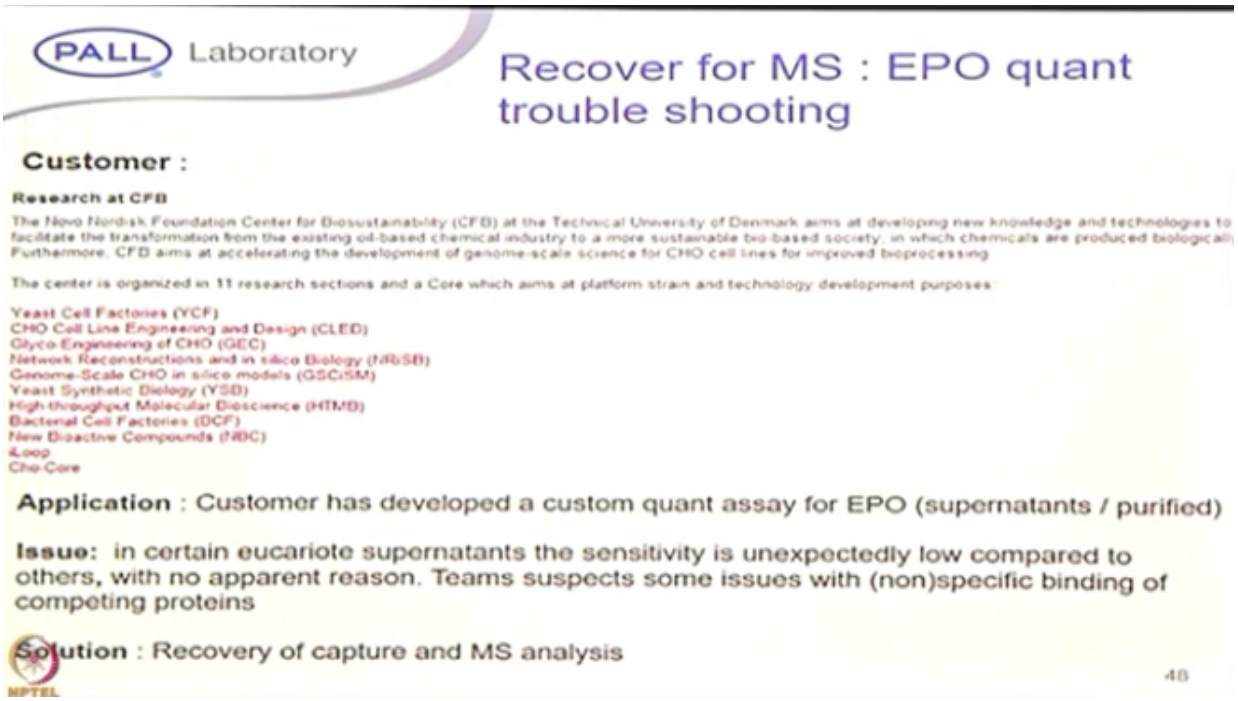

the erythro protein they were expressing the novo nordisk campaigning where exactly happens when they are doing the quantitation they are getting the concentration very less, then they thought that what happening in that,

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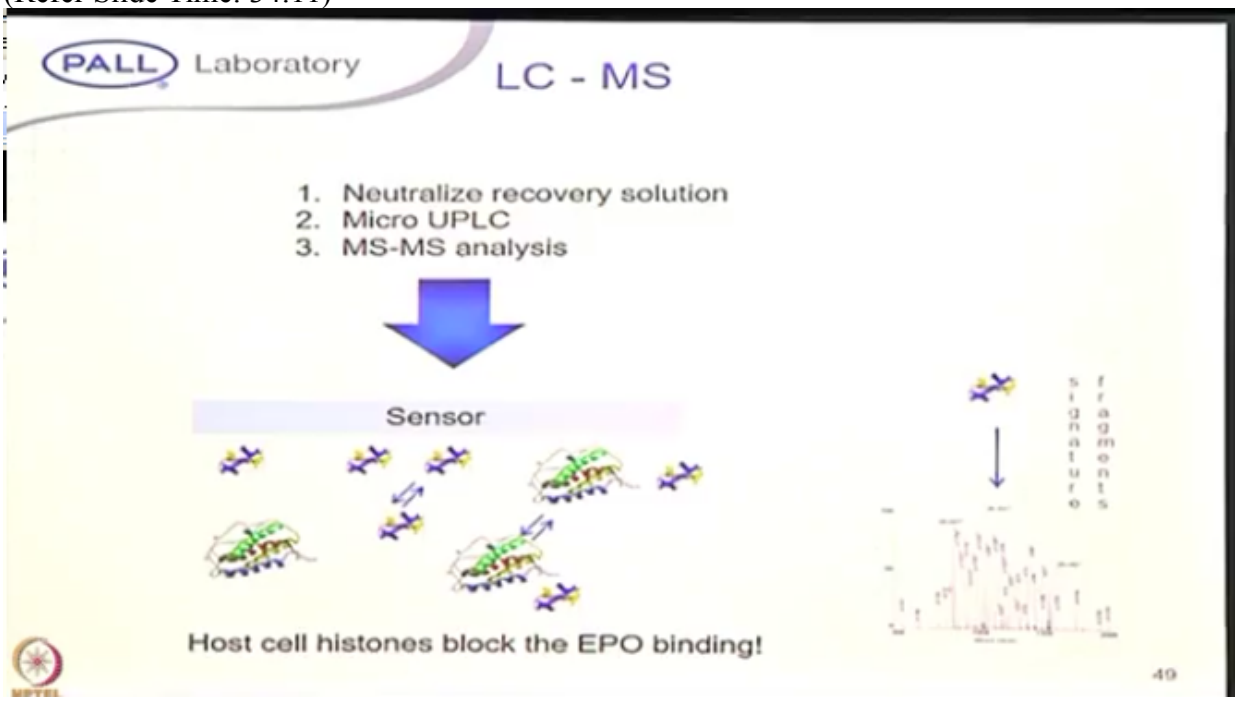

so they identify some host cell histones blocking the EPO binding, so they eluted those mixtures and they performed the mass spec with that, then they identify that the protein of interest is binding to the, it's blocking the binding of the EPO to that.

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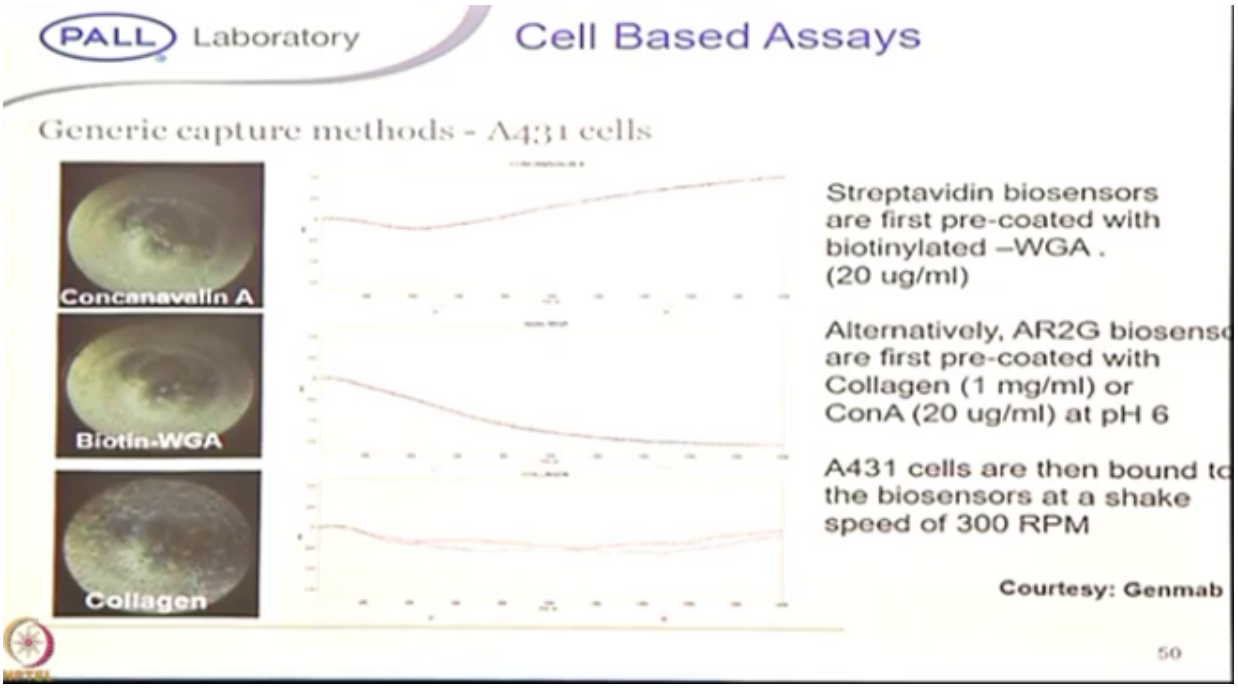

The gen map recently has published this data, they immobilized this cell, the entire cell on the sensor surface, and they had tried a different combinations here, one of the example I can show you is they had taken a collagen here, they immobilize the collagen on the amine coupling sensor, and then the cell which is the A431 cells which is expresses the EGFR and the beta 2 adrenoceptors,

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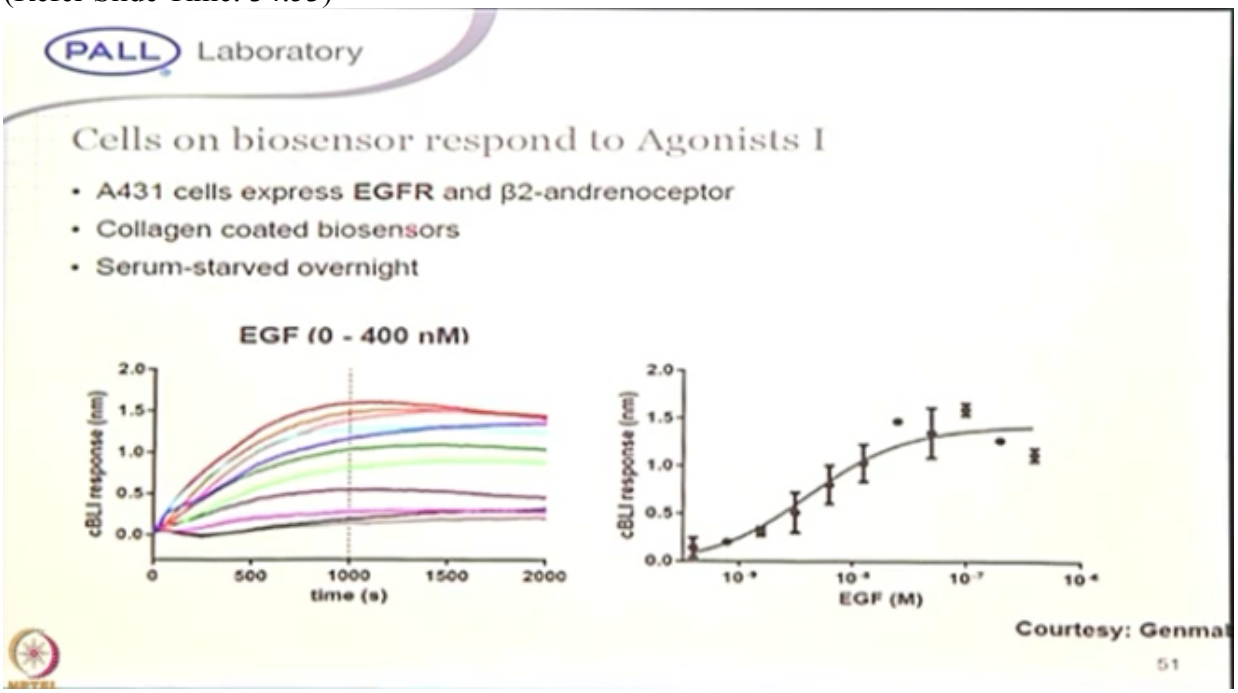

more express receptors they put it the cell on the sensor surface and then they perform the interaction studies with respect to the EGF here, we can see this the binding interaction, it's not exactly the kinetics, it is the orthogonal to your cell based assays, so what happens is say suppose it is a such a cell, it is a big cell you are immobilizing on the sensor surface, then attiny

like some protein is binding to sensor surface you can't see a bio-layer change on the sensor surface, but what happens is when any molecules bind to the respective target on the cell surface we can see in this cell, you can see there is a some events happens, there is acting modulations or any other pathways, so these changes will leads to the signal change on the sensor surface, we call it as a dynamic re-modulation the word.

So using this phenomenon they had done the, the quantitation kind of stuff, the cell based assays orthogonal to that. So here one more example for the beta adrenoceptors they bind the dopamine, if you look at these binding changes here very, very small signals using they had done a correlations, so like that you can even you can as the orthogonal to your cell based assays,

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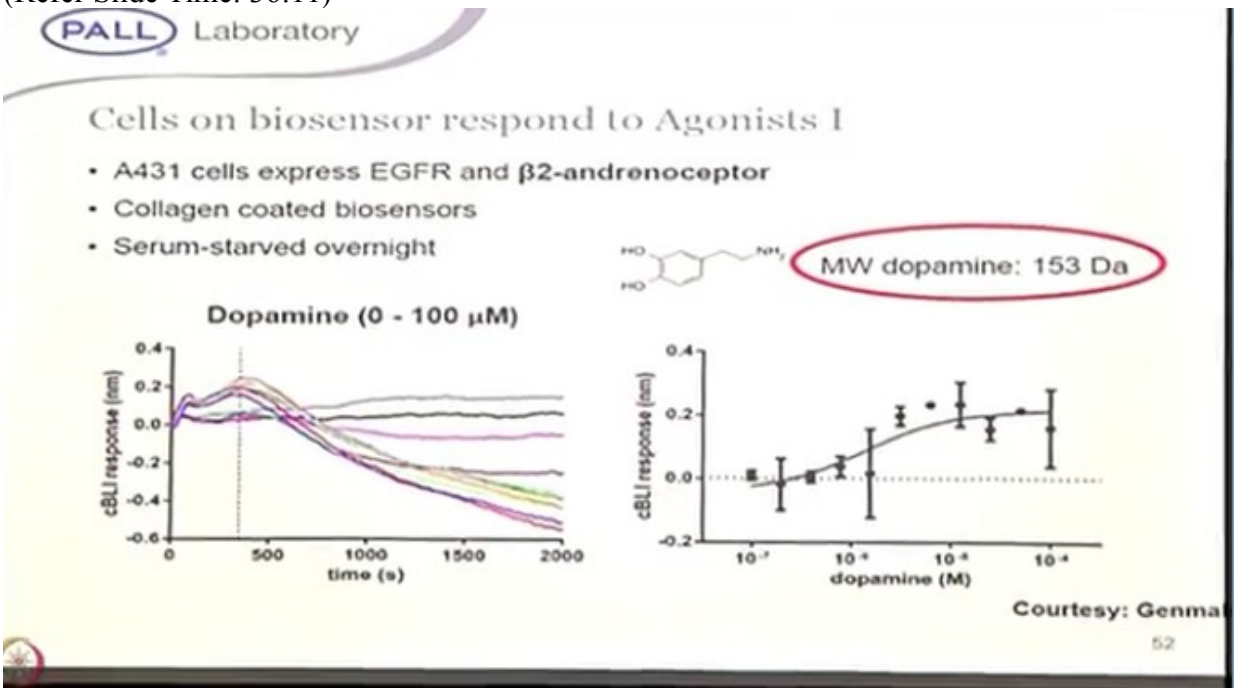

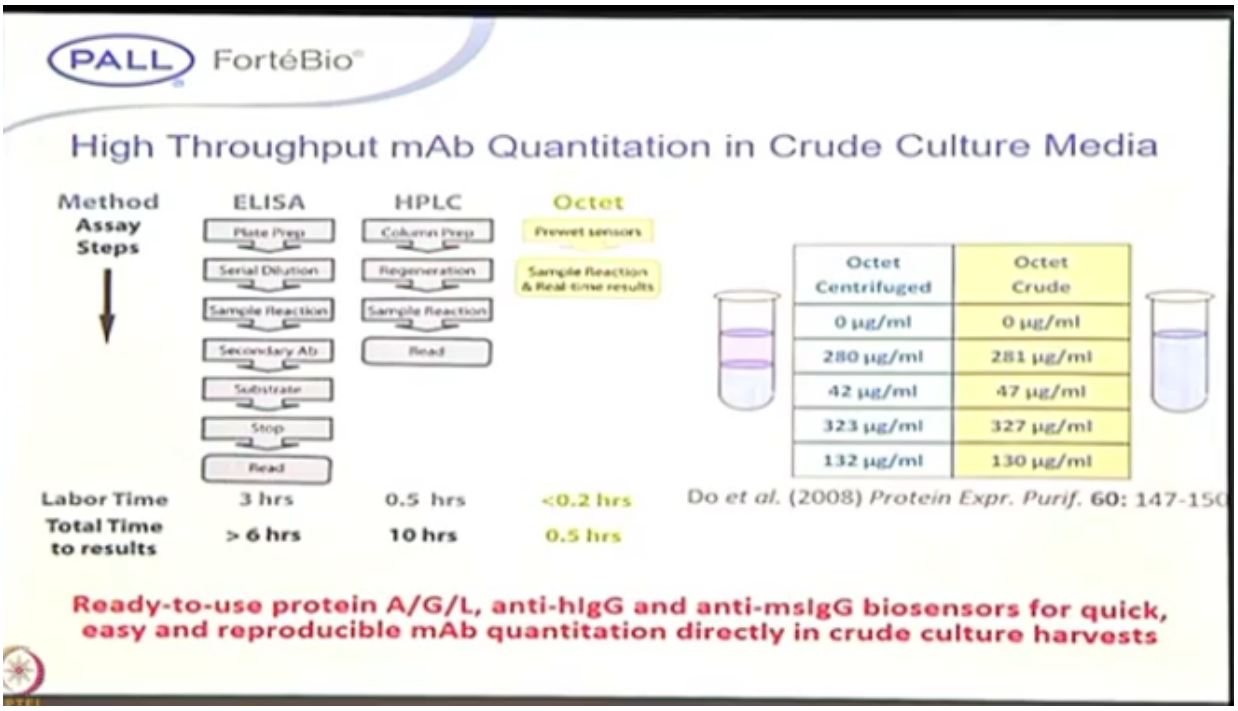

and very important when it comes to the ELISA and the HPLC comparisons with most of the industry people map platforms people are working with, they are using this platform for the title determinations for their antibody productions.

Very quickly the good advantage is like that 96 samples you can screen just in a 40 minutes, or I can say the quantitate the 40 minutes, but in comparison to your protein HPLC or the ELISA based methodologist it will takes a long time, ELISA is roughly takes around 3 to 4 hours but even protein A HPLC for the 96 samples it's like 8 to 10 hours it will take, but you can just in a 40 minutes you can quickly the titrate the protein of interest in this.

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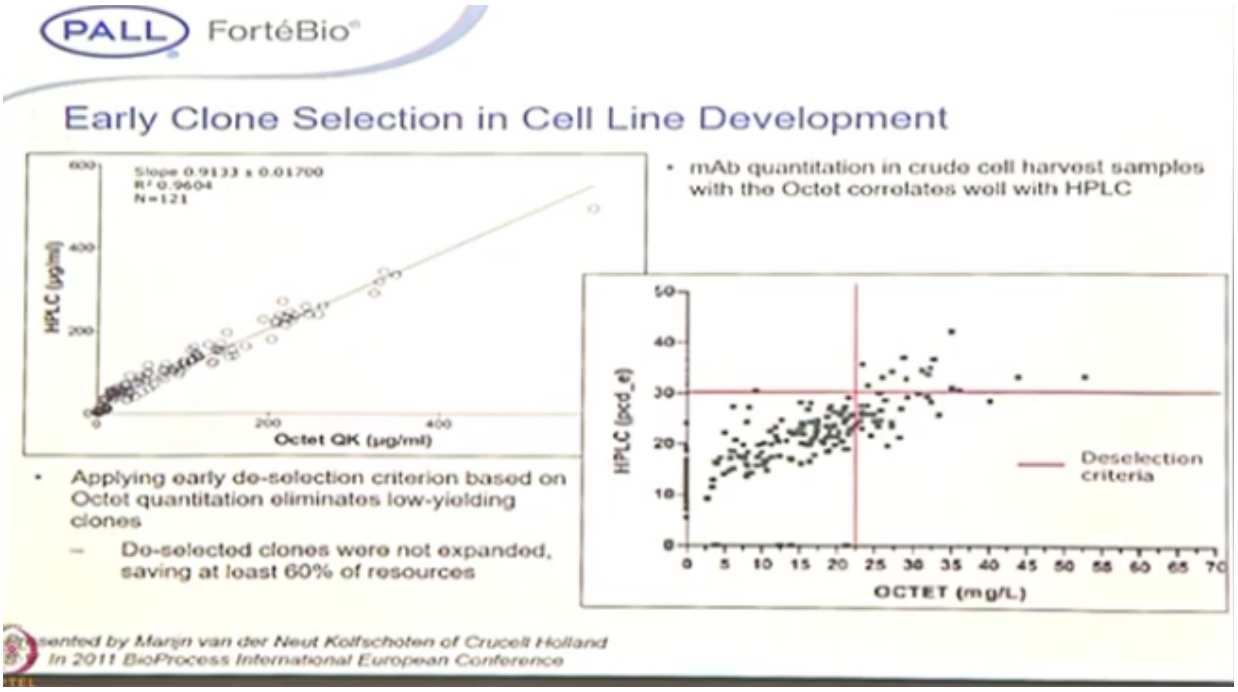

So one of the comparison here they had done the early clone selection in cell line developments, we had done a correlation with respect to the HPLC versus the octet platform, it's on comparable with the other methodologies.

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So these are the systems here we are offering, we have K2 system, this is the two channel instrument, it's a sensitive something like you can go from 150 Dalton as analyte, 150 Dalton you can go to the mega Dalton protein interactions, such as like a sensitivity ways, as on per with the SPR is affinity ranges 10 picomolar to you can go with the 1 millimolar easily, this is

the 8 channel instrument, similar sensitivity, 16 channel it is once again all 3s are similar in this platforms, these are the ones which are lower sensitive compared to the red ones, and this is the one the high-throughput HTX have a 96 channel, so depending upon the applications you can choose the right sensor, you can put into different all applications, either it maybe protein-protein, protein DNA or protein RNA, optimus or cell based assays or the nano particles we have lot of publications in that.

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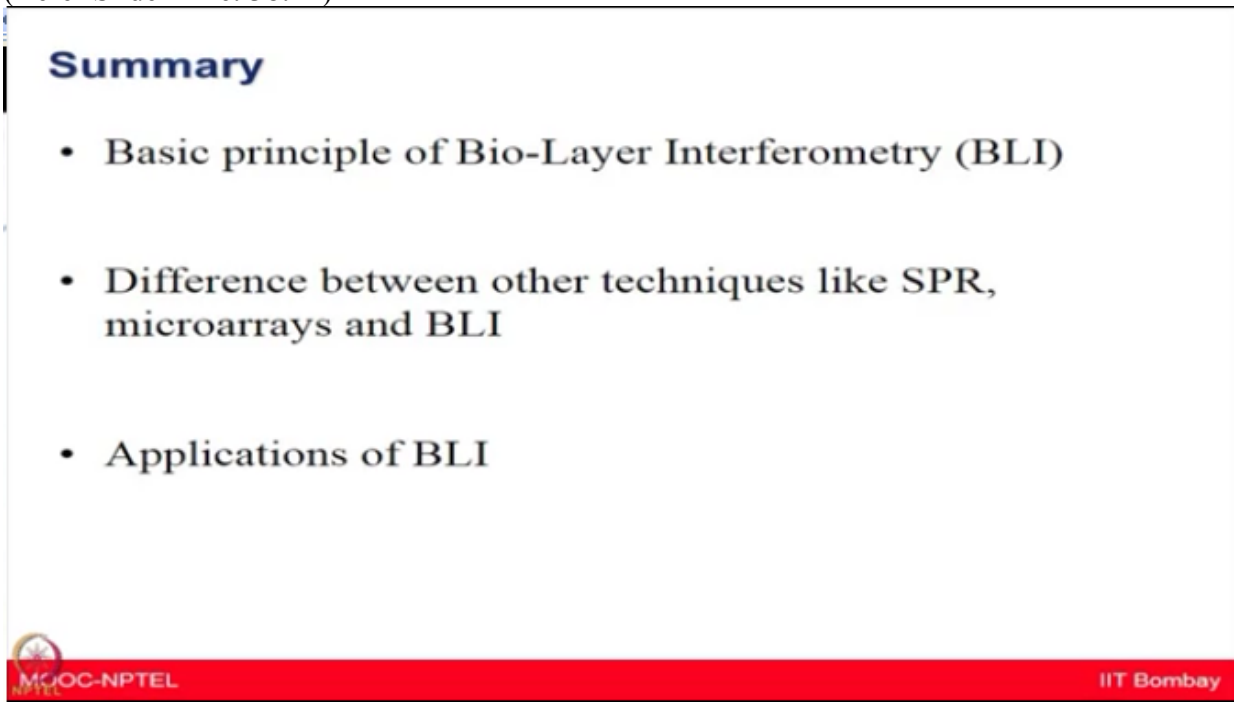

Dr. Sanjeeva Srivastava:- So I hope you got a better understanding about this label-free biosensor, bio-layer interferometry technology BLI, the next lecture will include a demonstration session that was conducted during this workshop, I'm sure you will be now able to understand these concepts much better, and you will also understand how to perform these experiments for your biological samples of interest for your biological problems of interest. Thank you.

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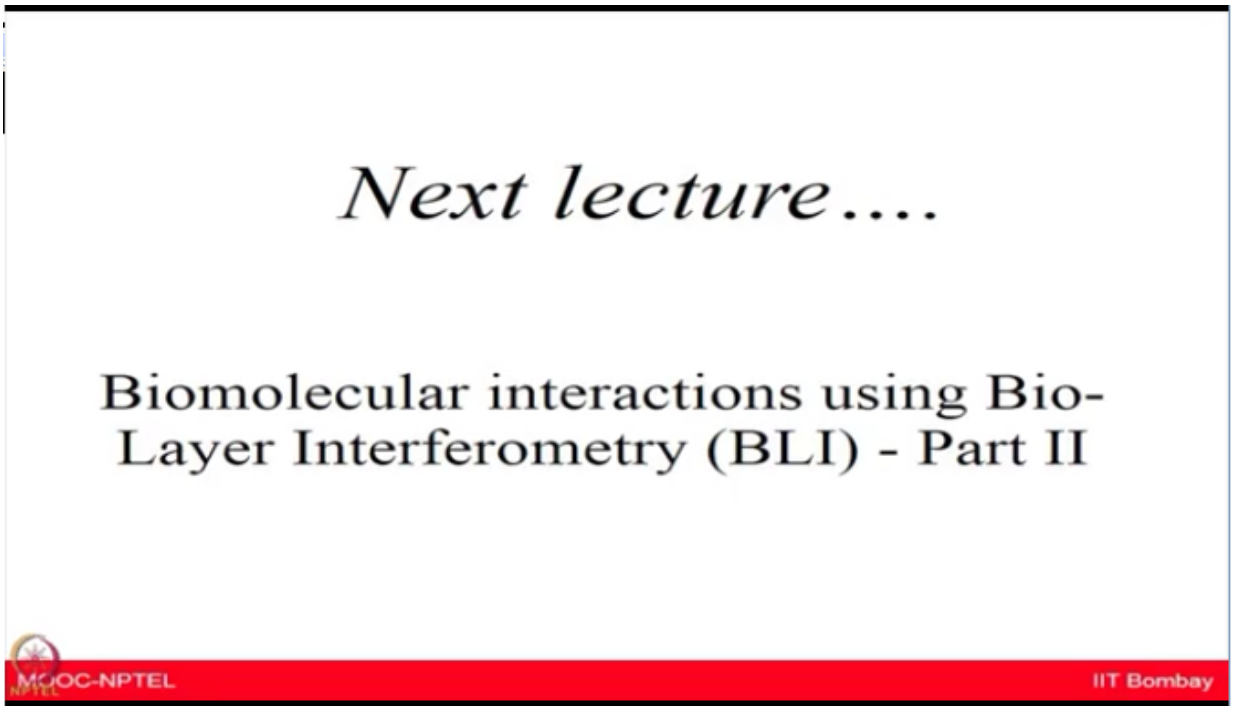

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