Interactomics: Basics and Applications

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Lecture – 39
An Introduction to Bio Layer Interferometry (BLI) and its Applications in Protein Research

Today, we are going to understand another popular label free technology which is based on Bio Layer Interferometry. It is highly useful for a studying biomolecular interactions, we discussed various applications based on surface plasmon resonance SPR. This is another technology platform which is also label free based on different physical principle. BLI is based on simple dip and read system which is useful for measuring interaction between proteins, peptides, nucleic acid a small molecules and lipids.

It is an optical technique that analyze the interference pattern of white light reflected from two surfaces. A layer of immobilized protein on a biosensor and an internal reference layer, any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real time. These biomolecular interactions are measured in a label free environment with the ability to monitor binding a specificity and kinetics.

For example, association and dissociation rate constants and concentration analysis with precision and accuracy. Today, we have Dr. Cao from Pall life sciences with us who will elaborate on the basics of bio layer interferometry technology and its applications in protein research. So, let us welcome Dr. Cao for today's lecture.

Hello, everyone today I am going to give a introduction to bio layer interferometry and its application in protein research field. During the past 10 years further bio has developed a series of systems relying on the bio layer interferometry which is a label free technology and allows you to perform real time analysis of bio molecule interactions and also to perform quantitation of the bio molecules in micro volume sample sizes.

Now, let us have a understanding of the bio layer interferometry or BLI technology. Now have to understand how it works in the real life.

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The core part of the bio layer interferometry or BLI technology lies with the bio sensor. If you take a close look at the bio sensor it actually made by optical fiber in the end. So, at the very end of the bio sensor it is a two dimensional pall compatible matrix.

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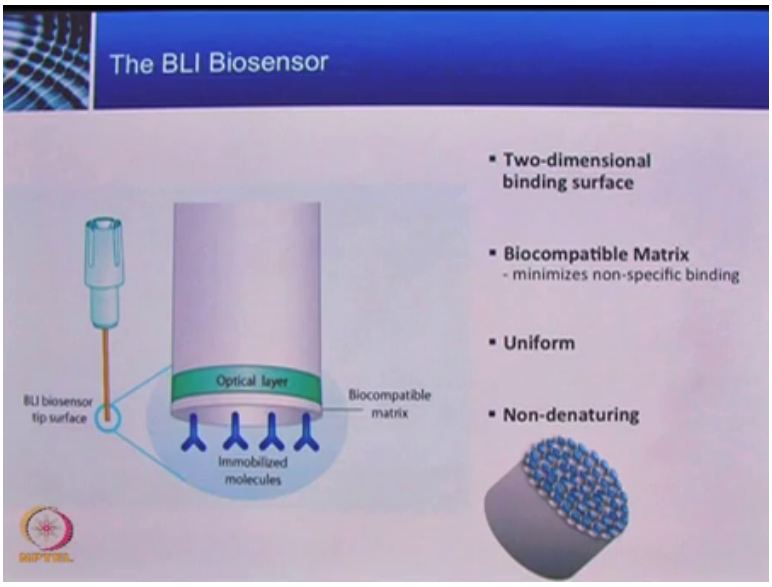

This matrix allows you to immobilize the molecules on the surface to allow further analysis of bio molecule interactions. The benefit of use this patented bio compatible matrix is that its very uniform it minimized in the molecule binding to the membrane and also it is non denaturing. So, what happens you are when we are doing experiments with bio layer interferometry, if we take a close look at the bio sensor we can actually observe two reflection layers?

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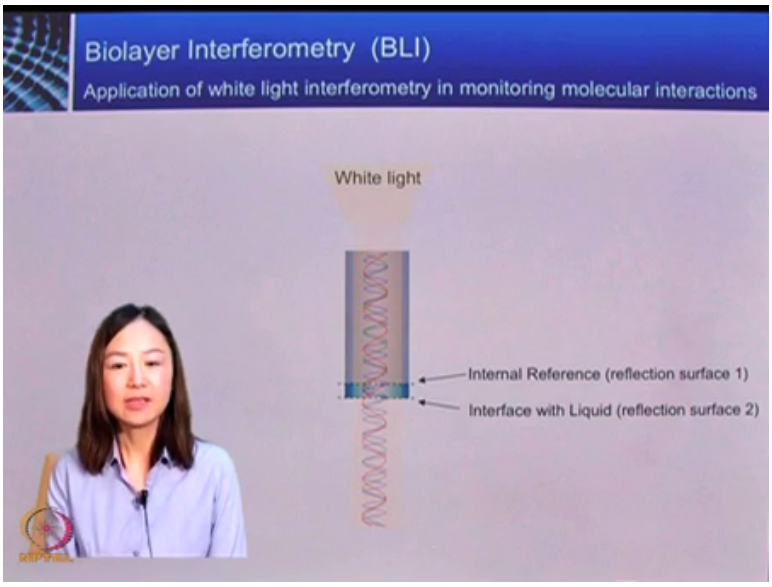

The internal reflection layer is engineered into the bio sensor during the manufacturing process and the second layer is a interface between the tip of the bio sensor and the liquid, when the bio sensor is put into the BLI instruments, the instruments or generate visible lights. These lights work out to the bio sensor and as we know some of the lights will be reflected from the two layers of reflection surfaces.

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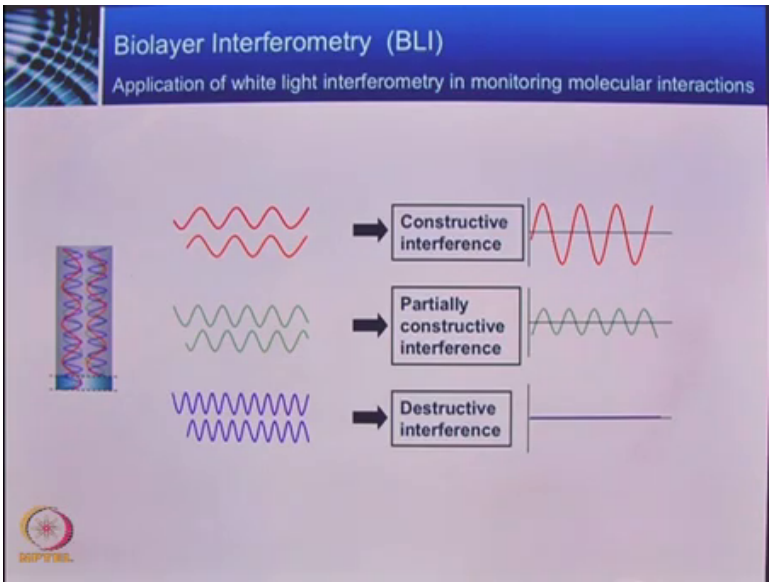

Since the white light has, where lies a different wavelengths still the two reflected wave pings were interact with each other. Sunlight has certain wavelengths will show a constructive interference sound will show a partially constructive interference and a sound will see negative interference or destructive interference.

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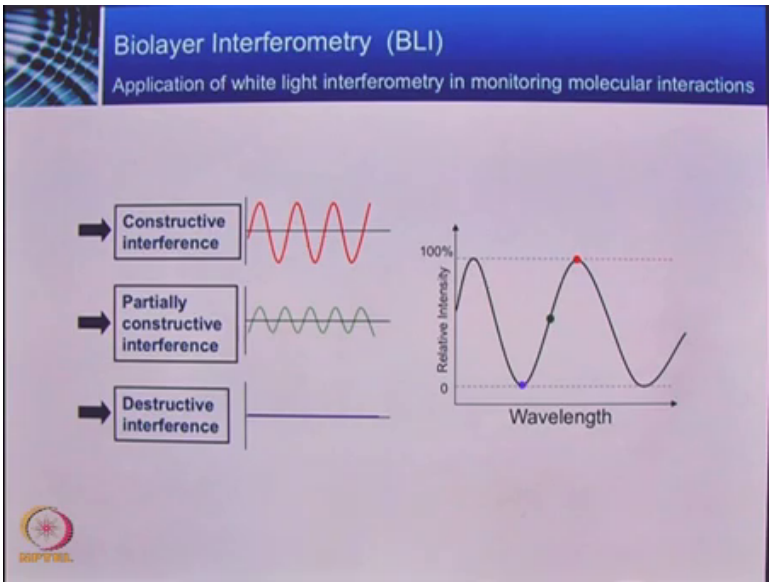

No matter what the system will monitor the reflected tubing's and a plotted the composite or composed two wave lengthses at a this XY plot. Then by plotting the wavelengths or wave pattern of this different waves on this XY plot we can get a original wave pattern.

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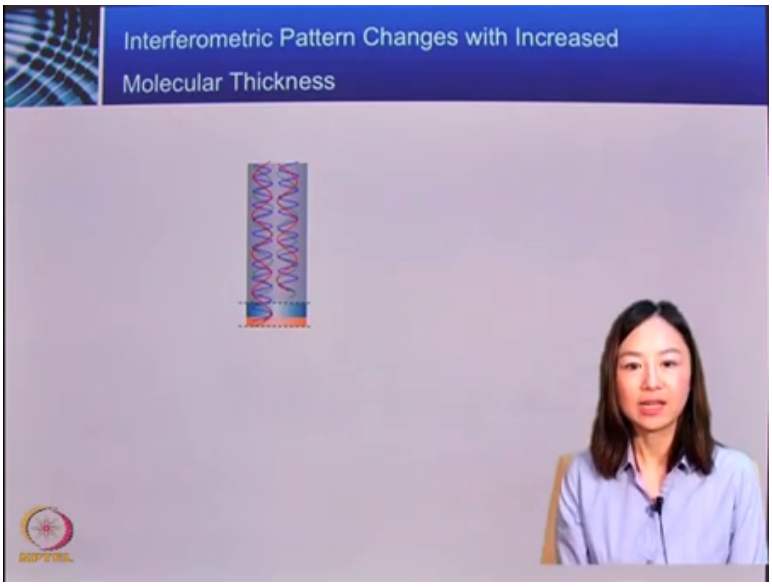

When a interaction happens the molecule were bind to the end of the bio sensor and as a result, we will see the secondary or second reflection surface actually moves down. And, this moving down increased the distance between the two reflection surfaces and this increase will change the interference between the two reflected waves.

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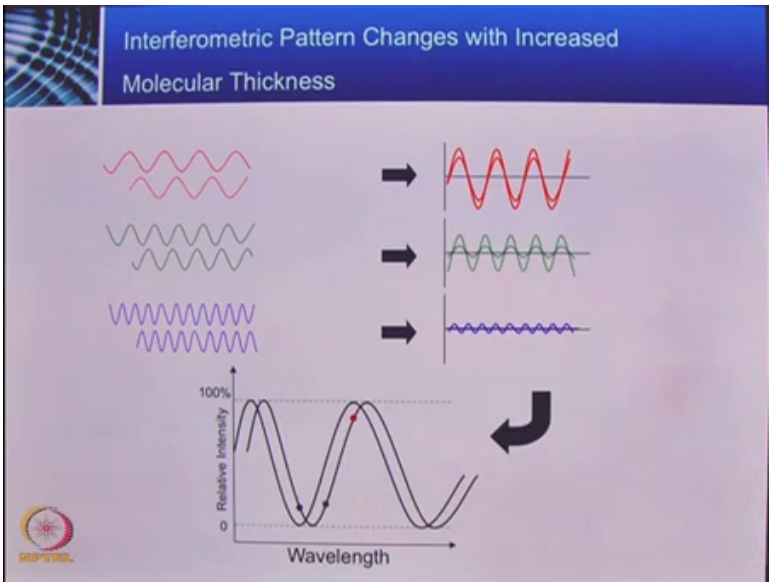

As a result or series originally, we have wave pattern as shown here, but after interaction happens because interference changes. So, we will see a different wave pattern it is kind of shift from the origin of wave pattern. The distance of the shift is proportional to the size of a molecules on the membrane the amount of molecules found in the membrane and also the density of the molecules found on the membrane.

So, the system will monitor this wave; wave pattern shift near real time and from where we can get understanding about the details of the bio molecule interactions.

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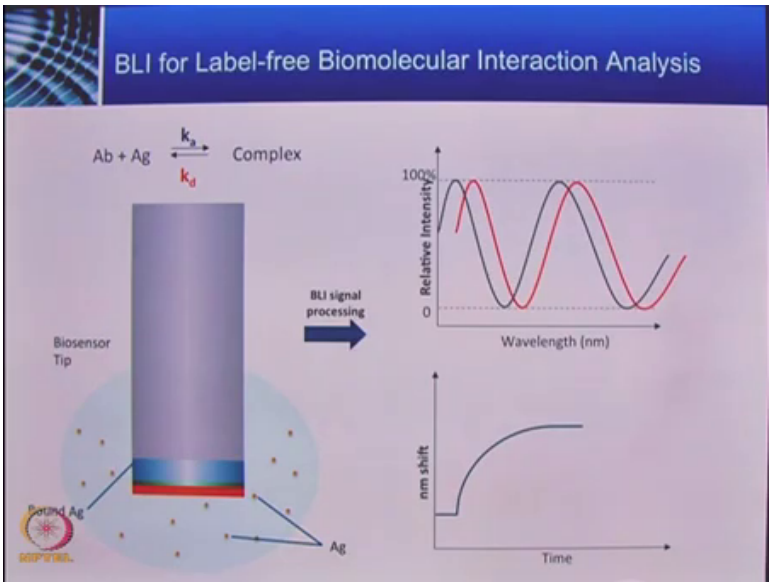

Now, let us look at the example. Well, if we are looking at the antibody antigen interaction as we know the antibody will bind to antigen and form a complex. At the same time the complex will go through a dissociation process and generate the antibody and antigen.

As a result, if we have a bio sensor which already have immobilized antibody on the very end or on the bio matrix or the bio matrix. And, if you we put this bio sensor into the solution contains antigen the antigen will bind to the bio sensor and result a shift of the wave pattern which in turn generate the association curve between the antibody and antigen. Afterwards, if we move to the bio sensor to a buffer solution the antigen will be dissociated from the antibody, then from here we can get a dissociation curve of the antibody antigen interaction.

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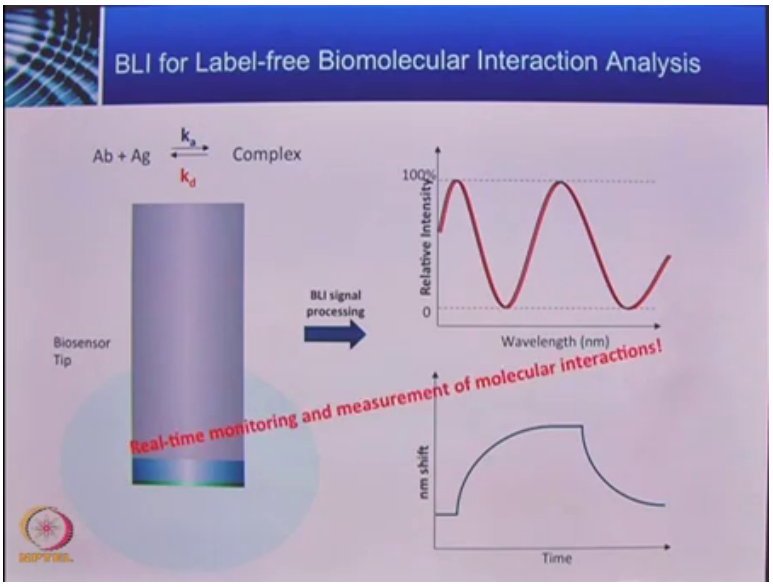

So, this how the system works in the real time because a BLI technology is developed based on this bio sensor which can work with different sample types.

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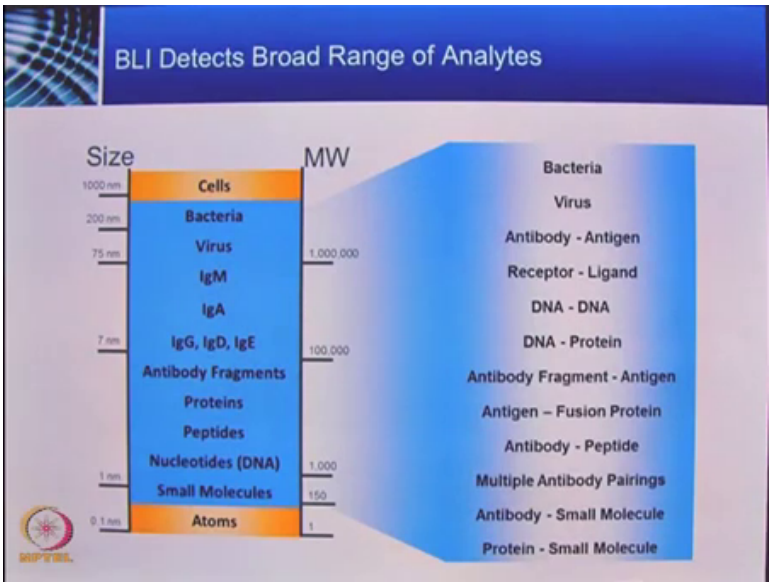

It allowed us to analyze the interactions between bio molecules from molecules down to 1500000 to antibody to recombinant protein, to virus and to bacteria. However, this system may not work directly with the intact mammalian cells because of the size of the cells has exceed the limit of the detection. And as another application, the BLI technology can work with different research purpose.

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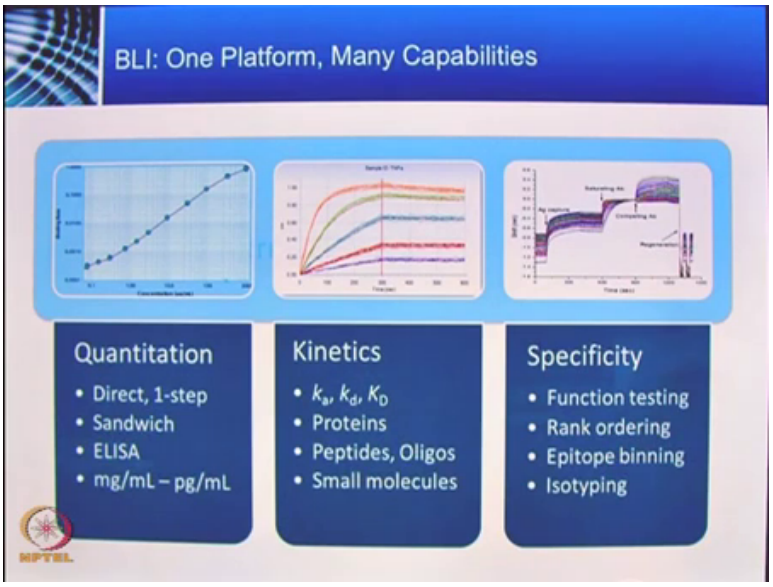

We can use the technology to perform quantitation of the biomolecules. This can be achieved by immobilized a specific antibody or molecule on to the bio sensor and the use it to analyze a concentration of other molecules. It can also be used to analyze the kinetics of the biomolecule interactions for us to get a K on K off K D and in a pharmaceutical industry, it can also used to perform functional testing the epitope beany analysis and also perform isotyping of the antibodies.

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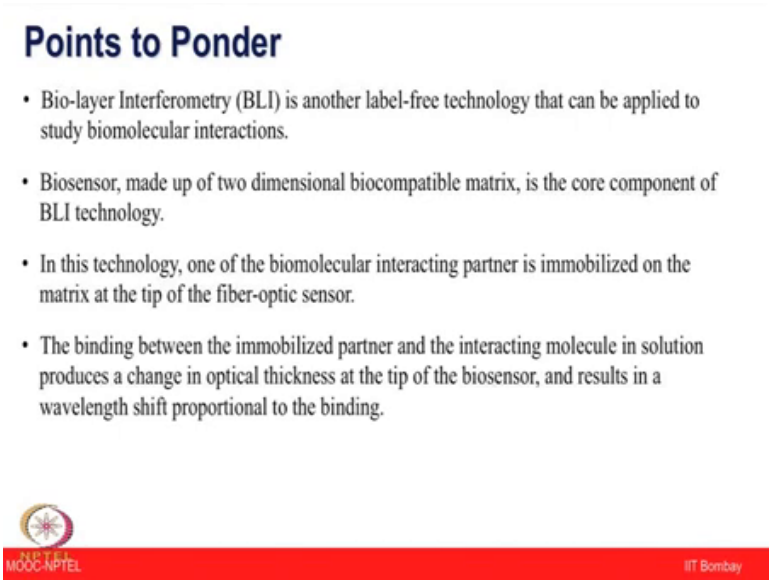

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In the next couple of slides, I will give you some example about the application of the BLI technology in different field. Firstly, let us look at the application of the BLI technology in kinetic application.

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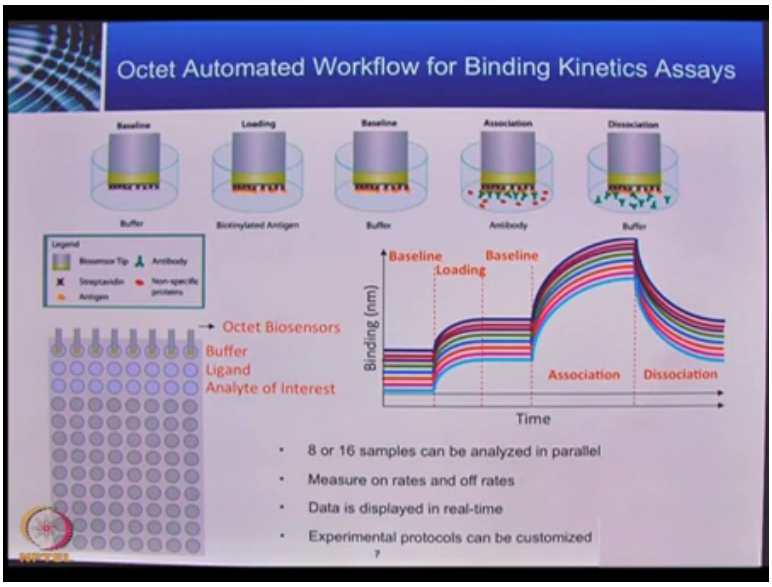

We were doing a kinetic experiment we. firstly, need to coat the bio sensor with a ligand. In this experiment, we are using the bio sensors with streptavidin immobilized on it. So, the ligand has already been biotinylated and we are first put the bio sensor into a buffer to remove the protective agent from the bio sensor. After that the bio sensor is moved into the wells that contains the ligand. The biotinylated ligand will bind to the streptavidin and a generate a loading curve as we can see here. Afterwards, the bio sensor is that moved back to the buffer then we can get the baseline before the real interaction happens. As a first step of the interaction the bio sensor is moved into the wells which contains the analyte of interest.

The analyte of interest will bind to the ligand which has already been immobilized on a bio sensor and from here we can get the association curve between the analyte and it ligand. And as a last step of this analysis the bio sensor is moved back to the wells which contains a

buffer. There in a buffer wells analyte will dissociate from the ligand and we can generate the dissociation curve.

One thing we need to pay attention here is all the interactions the curves are generated in a real time. We can get many details from this analysis. Depends on the system you are using, we are allowed to analyze either 8 samples or 16 samples or even 96 samples at the same time which dramatically speed up the analysis process. Now, let us look at one of the publication which use the bio layer interferometry to analyze a binding between the two proteins.

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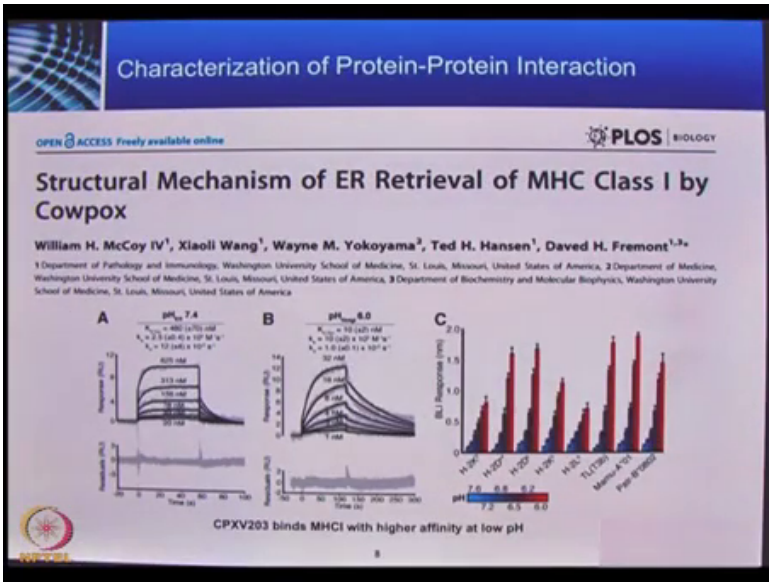

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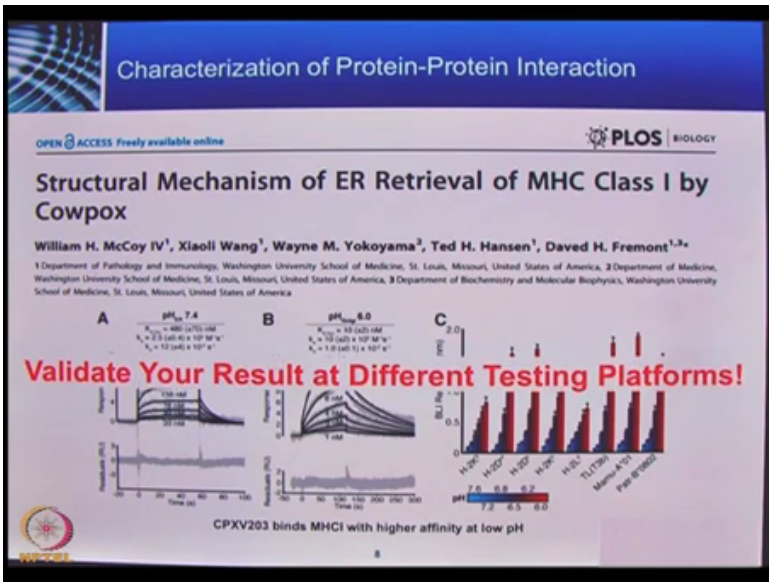

In this particular research, we are studying a binding of the MHC class 1 with another protein called CP XY 2 o 3. So, in this particular study the researchers performed a very stringent analysis between the two molecular interactions. So, they firstly, use the SPR technology which another technology used to analyze bio molecule interactions. Then again the k t value and in another experiment they then performed the analysis in the using the BLI technology and the data show here suppose that the kinetic constants achieved on the BLI technology is the same as a one achieved with SPR technology.

So, the main purpose of this study is to show us that, it is a very important for us to validate our results in different testing platforms. So, this will help us to be sure that results we get is not false positive. I believe these theory or with this kind of thinking will be very useful when you are designing your own experiments. It is another example of the application of the bio

layer interferometry, let us look at quantitative analysis of BLI. When we are doing a quantitative analysis the first step is to generate a standard curve.

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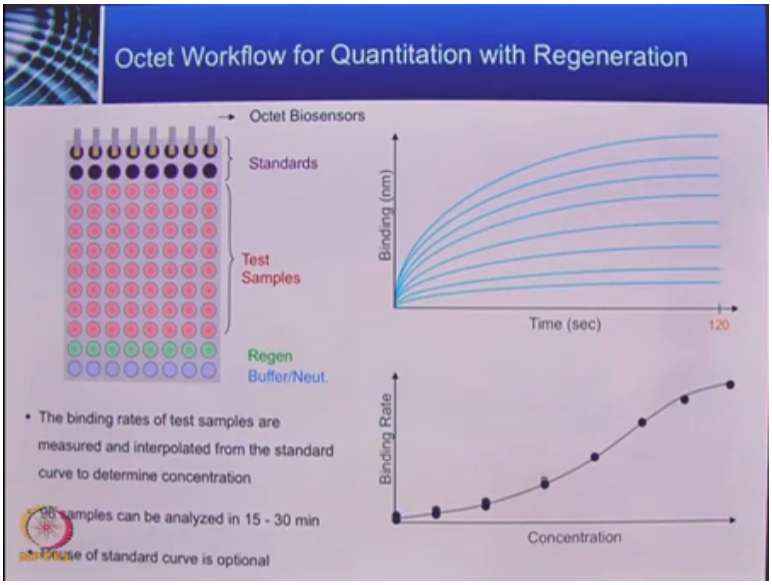

So, during the set up the bio sensors is firstly, dip into the wells which contains the standards. The standard will bind to the bio sensor and a bio calculating a initial binding rate of the molecules to the bio sensor we can get a standard curve. Where is a binding rate as a Y axis and a concentration of the molecules as the X axis.

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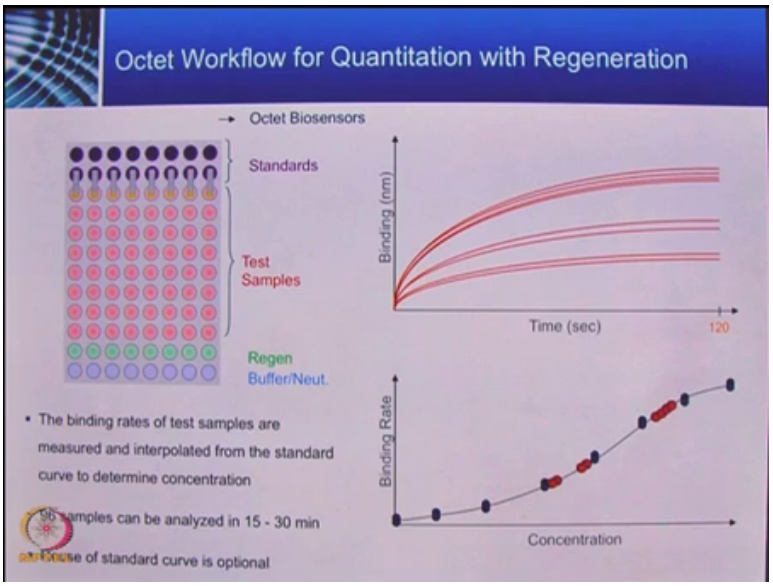

After we get the standard curve, we can then regenerate the bio sensors by putting the bio sensors into the regeneration buffer followed by the normal buffer to neutralize the bio sensor. Afterwards the bio sensors are dipped into the wells, which contains the samples at unknown concentration. From the binding curve, we can also get the initial binding rate of this molecules at unknown concentration and by protein this initial binding rate onto the standard curve we can get to know the concentration of this unknown samples.

One advantage of using BLI technology to analyze the concentration of the molecules is that, it is able to analyze multiple samples at a same time and also it only takes you about 15 to 30 minutes to finish analyzing 96 samples. Depends on the assay you are using you can actually regenerate the bio sensors which means that you only need 16 or 8 bio sensors to analyze the whole 96 well plate or 384 well plates.

So, this dramatically facilitates the application of the BLI technology in the pharmaceutical industry as well as in the academic research field. Here is one of the example which shows

that how we can use BLI technology to detect the protein in a crew sterilized. It in this particular experiment, we are actually analyze the samples were called with a system called BLItz. The BLItz is a single channel system developed using BLI technology.

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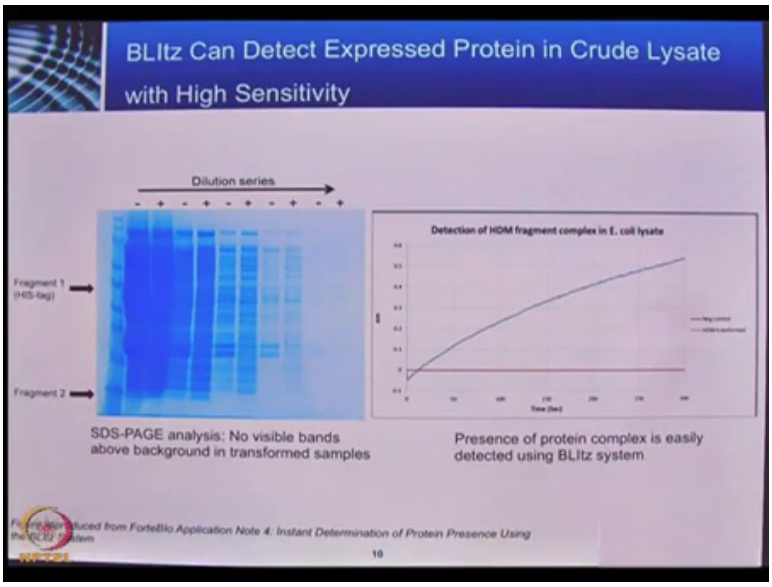

So, in our experiments. we are expressing two molecules or two proteins in E coli. The research have shown that these two proteins will bind to each other and they can form a tight complex in E coli. Between the two proteins one of them has been his tagged. So, we can actually use anti his antibody to detect the particular fragment. We express these two proteins simultaneously E coli and we are on the cell lysate longer SDS page gel.

From this gel, we can see that we actually cannot tell whether the two proteins are expressed in the E coli or not because there are very high background on the SDS page gel. So, how can we do that the researchers in this particular study used BLItz platform to analyze the

concentration the expression of the molecules in the E coli. What they have done is from a cultured E E coli samples they just take out 4 micro liter of the cultured bacteria and then dip this 4 microliter samples onto the BLItz system.

Then we also use a anti his bio sensor attached to the BLItz system and the anti his bio sensor is then dipped into the sample which is all in 4 macro litre. The his type proteins if they are present in a samples they are bind to the his type bio sensor and a generate a binding curve.

So, by looking at the generation of the binding curve we can actually tell where the protein of interest is expressed in the E coli or not. So, we can see here by using only four macro liter samples without any sample purification, we can easily perform the analysis to tell the presence of our protein of interest in the E coli. Which saves really a lot of time from 30 seconds compared with one day of the western blot.

Now, let us look at another application of the BLI technology in a vaccine titer determination. As I mentioned earlier because of the unique feature of the BLI technology, we can actually use the technology to bind to the virus directly without the further sample purificational process.

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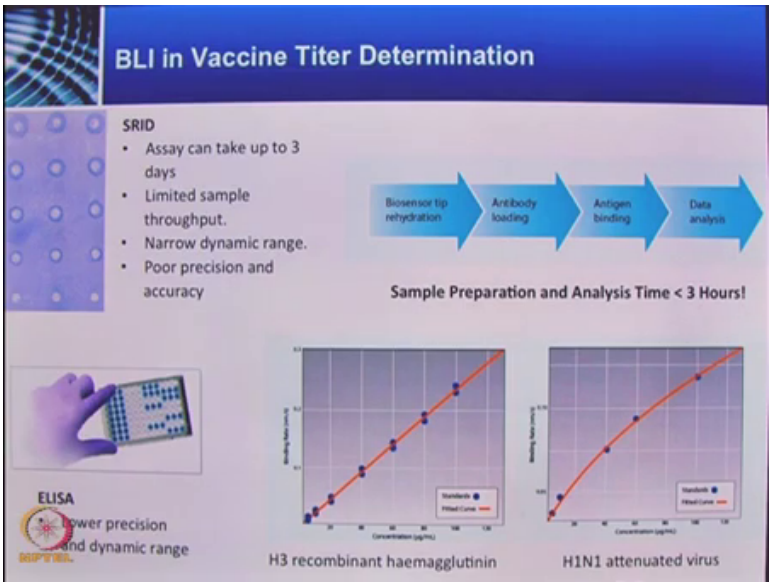

Traditionally, in the vaccine industry where we relying on SRID method to perform the quantitation of the vaccine virus titer. However, this method is very time consuming it takes up to 3 days to analyze the virus titer and also it has a very limited sample throughput with it is able only able to analyze maybe 15 or 16 samples at a same time. Thirdly the sensitivity and accuracy of the SRID method is limited and the interpretation of the final results is actually subject to personal interpretation.

So, this method may not be a very perfect method for you to determine the virus titer in a sample. People have also been using a laser method to analyze the vaccine titer; however, similar to a SRID a laser takes a very long time to finish the whole experiment process and it has very low precision and to determine the titer of the virus and has very limited dynamic

range. To overcome all these shortcomings, the researchers are using the BLI technology to determine the virus titer in the real experiments.

And as we can see here from the sample preparation to the time, we get the final results it only takes about 3 hours for you to finish the whole process. I would like to show you how to use the BLI technology to select the right construct for protein crystallization studies.

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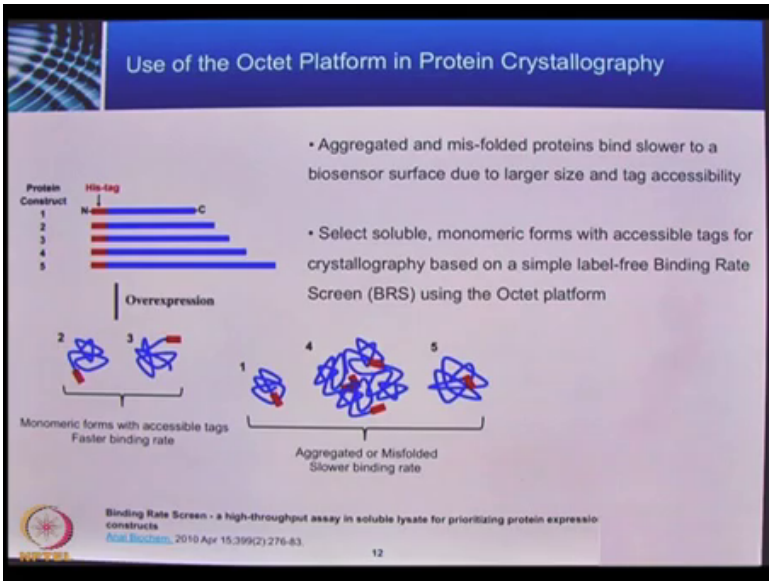

In these experiments so, the researchers would like to crystallize some constructs or different truncations. What they have done is they have attached the his tag together with the protein as a un his tag protein. So, the experiment is carried out based on two assumption. Firstly, if the protein is folded correctly the tag will be exposed on the surface which allows it to point to the bio sensor. On the other hand if the protein is not folded correctly the tag will be buried

inside the protein complex and as a result the mis folded protein are not able to bind to the bio sensor with anti his antibody coated on it also the mis folded protein tends to form aggregates.

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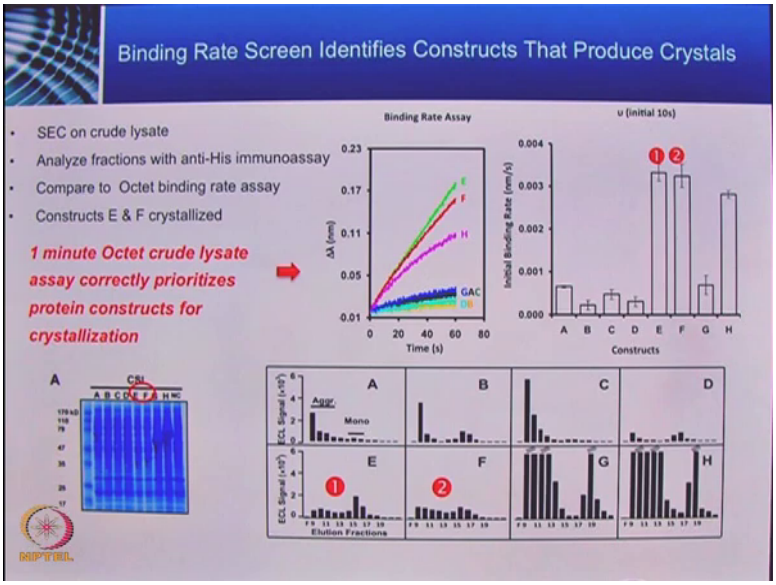

So, in this particular research our researchers. Firstly, perform SEC size exclusion chromatography on a crude cell lysate from there they can get a patent distribution of the different constructs and also do in this by using SEC analysis they can identify which portion is a soluble portion and which portion is the aggregative portion.

As a second step of the study the researchers then express the different constructs in the E coli and use the BLI technology to analyze the binding of the expressed protein to the bio sensors with anti his antibody immobilized on it. So, the researchers measured the initial binding rate of this constructs to the bio sensor and by calculating the initial binding rate they can plot this data on this XY plot as you can see on the upper right corner of the slide.

It is very interesting to notice that the constructs with the highest initial binding rate actually corresponds to the constructs with the highest soluble portion. So, these two constructs is selected for further crystallization study and they have the researchers then get the crystals of these two constructs. The main learning point from this study is that by performing a one minute assay using BLI technology on the octet platform allows you to correctly prioritize the proteins constructs which is suitable for downstream crystallization which helps you to simplify your research process and speed up the process for you to get the crystals for further structural analysis.

Because it has so, wide applications of the BLI technology we have developed a wide range of the bio sensors for you to analyze antibody concentration or interaction or you can use a bio sensors to analyze the his tag proteins or GST tag proteins or if your protein is not tagged or they are not antibodies you can also use other bio sensors such as A R 2 G biosensors which has amine group immobilized on a bio sensor or the streptavidin bio sensors which includes the assay bio sensor, assay assay bio sensor, an assay x bio sensor for you to analyze the streptavidin a biotinylated molecule interaction.

So, as a summary of my lecture, we kind of going to the basic mechanisms of the BLI technology and we also included some of the examples of using BLI technology in different research field which including protein-protein interaction protein quantitation or even protein crystallization.

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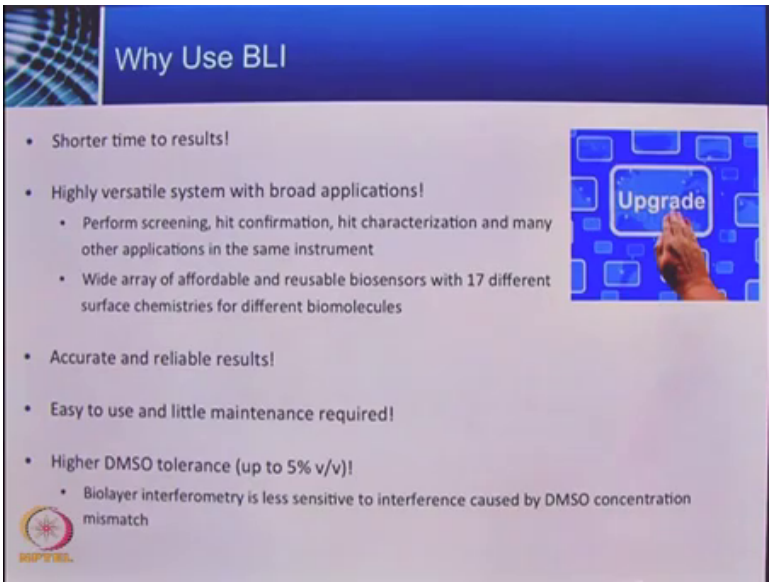

The benefit of using the BLI technology or even the octet platform in your research field is that it allows you to perform your analysis in a short time the platform is very straight forward and it is very easy to use.

Lastly, if you are working with a small molecule samples this system allows you to analyze samples in DMSO up to 5 percent of the concentration. So, here I will wish that going after going through this lecture you have get a better understanding of the BLI technology and also a to understand how this technology will help you in your protein research.

Thank you.

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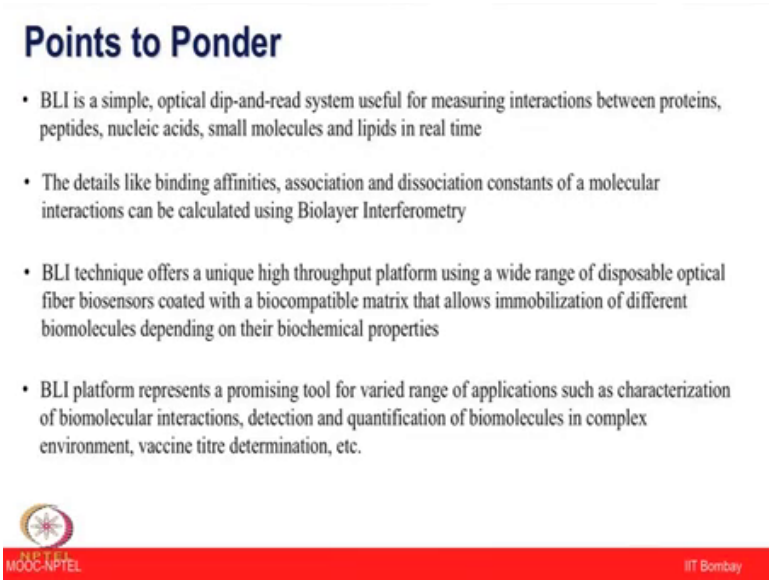

So, today we have learnt about the basics of BLI or Bio Layer Interferometry technology and how it is used for different applications in research in our next lectures. We will discuss and demonstrate in detail about this label free technology BLI to perform the protein-protein interactions.

Thank you.