Proteomics: Principles and Techniques

Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Lecture No.# 37
Surface Plasmon Resonance: Biacore SPR and Data Analysis

Welcome to the proteomics course. Today, we will talk about surface Plasmon resonance. We will have a discussion on bicorn SPR technique and data analysis. The biological systems depend upon the molecular interactions of two or more bimolecular. So, that, they can form the stable complexes for bimolecular interactions the principle of thermodynamics, bimolecular structure and recognition play very crucial role. The identification of interacting protein and protein partners of the known function with those having the uncharacterized role by performing these type of experiment it is possible to understand the biological process of uncharacterized proteins.

The ability to screen large number of proteins simultaneously and rapidly for biochemical activity, interactions of protein and protein, protein lipid, protein nucleic acids, and small molecules require various high throughput instrumentation. So currently, the most popular methods which are use for detection of protein-protein interactions include yeast two hybrid and protein microarrays.

As we have discussed in the previous lectures, that protein microarrays they are one of the very robust techniques for studying protein-protein interactions, but these microarrays require label-based detection system. Often these are fluorescence based detection; however, the label-based detection techniques have certain limitations, such as the tags such as fluorescent tags may interfere with the function, including binding to the integrators and adding them to the queries always not so straight forward. So, there is need for label-free biosensors which can avoid these issues and allow for the real-time measurements.

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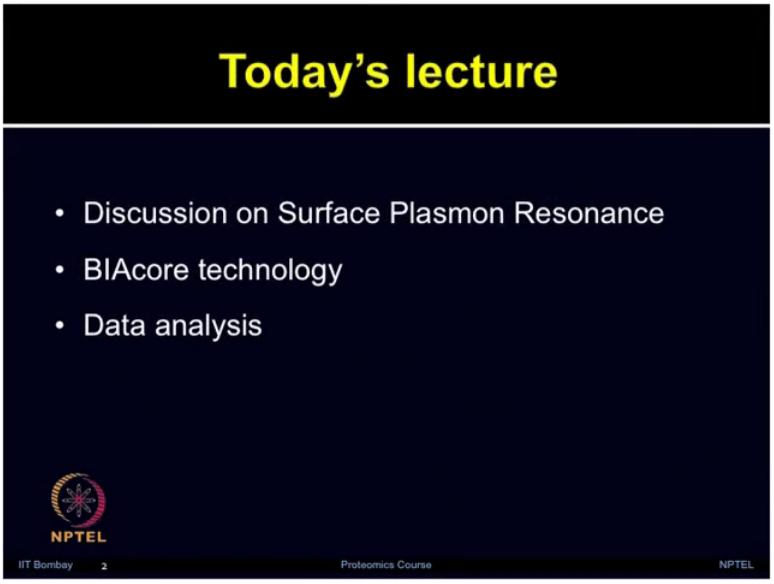

So, in today's lecture, we will have a discussion on a surface Plasmon resonance. And I have invited a guest to discuss about the bicorn technology, as well as how to perform some data analysis using commercial software's. So, now let us discuss about surface Plasmon resonance. This is one of the very efficient and emerging label-free techniques for studying bimolecular interactions. The surface Plasmon resonance biosensors or optical sensors which can exploit the surface Plasmonpolaritons the surface electromagnetic waves that can propagate parallel to a metal or dielectric surface. SPR is used to probe interactions between an analyze in solution and a receptor that are attach to the SPR sensor surface. Binding of molecules in the solution to the surface immobilize receptors changes the refractive index of the medium near the surface.

The change in the refractive index of medium can be monitored in real-time to measure accurately the amount of bound analyze it is affinity for the receptor and the association and dissociation kinetics of the reaction. Over the past decade the SPR biosensor technology has made significant advancement and a large number of SPR sensor platforms. The bimolecular recognition elements and measurement formats have been developed. The major strength of SPR biosensors are their versatility and ease of use, the SPR allows the analyses of receptor ligand interaction for wide range of molecular weights, affinities, and demonstrates the compatibility for small molecules and other chemicals. The SPR biosensors have played a very important role in biological research

into bimolecular and their interactions. And now they are increasingly being use for detection and identification of chemical and biological interactions.

Now, let us move on to the bicorn SPR technology. So, there has been growing interest in commercialization of SPR biosensors, which has lead to a number of systems available in market. The commercial instrument from Pharmacia and then bicorn became available in 1990. Bicornare optical biosensors which can be used to monitor macromolecular interactions in real-time, without need to label the bimolecular. The bicorn is a versatile platform to determine the kinetic rate constant for a variety of interactions. A number of commercial SPR biosensor instruments are available since then; however, the bicorn system is still dominate this field. So, let us now discuss about the bicorn technology with Lilith kishore the business leader research products in g e healthcare life sciences who handle the bicorn system

Hello

Hi

This pleasure to introduce Mr Lalith kishore from g e healthcare to discuss about bicorn surface Plasmon resonance technology. Lalith kishore is business leader research product in g e life sciences and today we will discuss about the surface Plasmon resonance technology and it is various applications in proteomics. So, Lalith welcome to the discussion on SPR technology

Thank you thank you

So, how long have been associated withbicorn SPR technology with g e healthcare.

I have been with g e healthcare for the last12 years and been associated with bicorn for the last four and half years, been associated with proteomics for a slightly longer time for about 7 years now.

So, can you just brief us about your experience of using the biocore technology from last several years my educational background is that I am not a biologist

Though bicorn is a largely biological tool my background is in chemical engineering. So, I am a chemical engineer with MS and management; however, I find very very disuses

for SPR technology these days, I find its use in a biopharmaceuticals, I find its use in basic research, in nanotechnology, in pharmaceutical industry, in Q Clabs. So, basically I think it is my chemistry and chemical engineering background that helps me work with work with bicorn and quite a lot of applications that I see across the country on bicorn technology.

So, you are utilizing your vary background to apply on the different biological problems. Yes yes that is what I think SPR technology also does that actually, uses a very simple technology and applies it to different things in biology and actually gives out the results that are therefore, everyone to see. So, Lalith can you please tell us, how you got interested in working on the surface Plasmon resonance technology.

Yes g e healthcare has been associated with bicorn, as a company for a very long time. In the year 2006 we actually acquired the bicorn. And up until that bicorn was there only in some places in India. So, I got into bicorn in 2006 out of interest in chemical interactions and interaction analysis, and ever since I have been just working on biacore and label-free interaction analysis technologies. So, can you mentioncurrently what are the major applications of SPR in the area of proteomics?

Actually very vast very wide ranging applications of SPR starting from a simple binding analysis or kinetics analysis, analysis of affinity of interactions whether it is protein-protein interactions, protein DNA interactions, protein RNAinteractions, protein small molecule interactions. In drug discovery quality control varied applications of SPR actually we support very large variety of customers from different backgrounds who want to do SPR in their labs. So, definitely you can see probably that SPR application will be very broad in almost all the proteomics laboratory depending upon their experiments and that Question they want to ask…

So, biacore is one of the pioneer in the field of studying the label-free interactions and studying about bimolecular interactions. Can you tell some of the latest advancement what are the major applications by using the biacore technology. Let me start with a video

That shows basically what SPR is and then probably followed by another video of how biological analysis happens on biacore, and then just a few videos shot once on how biacore works. And then I quickly come to the applications of biacore.

So we can...Let me show you the first video.

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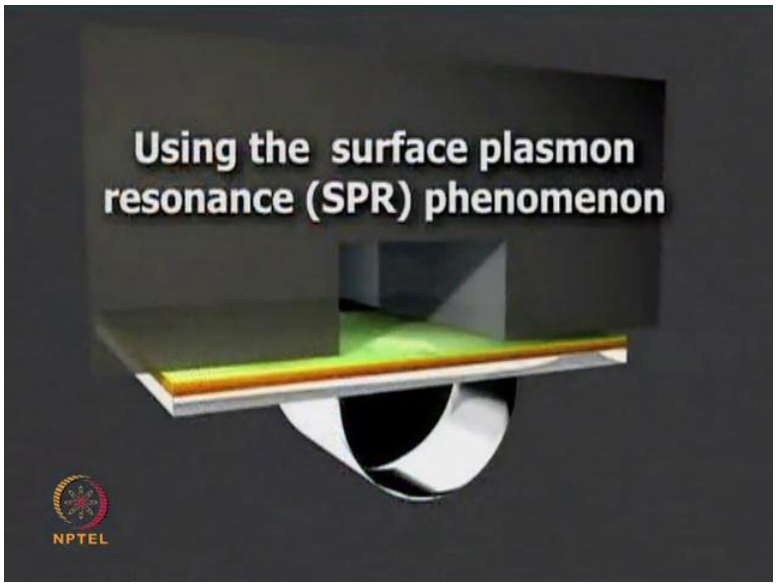

So, what I show here is the basic SPR phenomenon right. This is the SPR chip which you see here, on the top of SPR chip is a gold layer, and on the top of it is a flow cell at the bottom you see a hemispherical prism. So, SPR phenomenon is pretty simple that when you actually have the prism and you shine a laser light through the prism, as you will see shortly. When you shine the laser light through the prism the light reflects at a total angel of total internal reflection. The light reflects at the angle of total internal reflection and evanescent energy waves are created on the top of the chip. And these evanescent energy waves are also called as surface Plasmon's. And this surface Plasmon's are the once which have used to actually study biological interactions. When I go to video two now you will see how biological interactions are studying.

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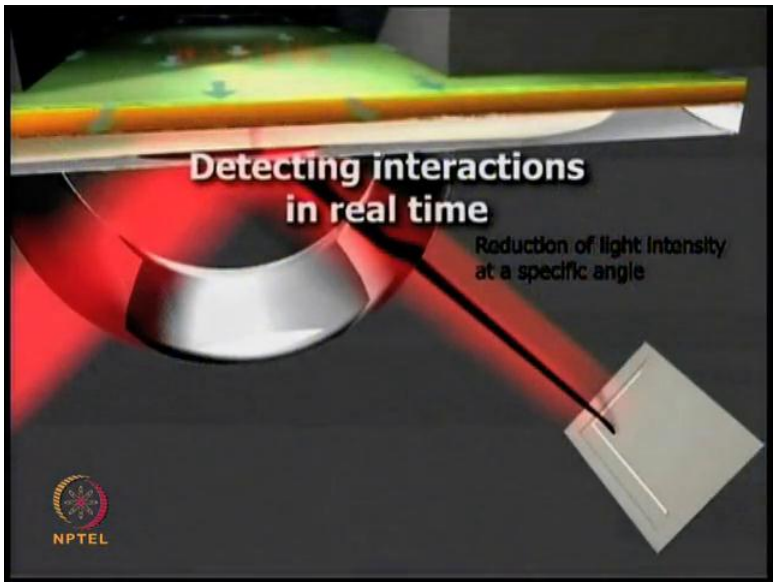

Let us assume that you have an interaction a plus b gives a b. What you do in SPR is you take one of the interact antssay b, and put it on the chip and pass a over it. Let me show you how it happens. You take one of the interactants which is b in this case, and you actually immobilized it on the chip, you can see the molecules getting immobilized right now. And then when the molecules get immobilized there is an increase in mass which changes the refractive index and that is measured in real-time. Now you pass the second interact ant a over it, if binding happens and a b gets formed you see a further increase in mass which is again measure in real-time. You stop the flow of a and start flowing a buffer it comes off in a dissociation and that dissociation is also seen in real-time. So, essential what you are doing with biacore is actually just measuring the amount of mass on surface of the chip. The mass on the surface increases or decreases and that increase or decrease is measured in real-time. This phenomenon is what we apply now to study biological interactions actually, biacore is b i a for biological interaction analysis

So, let me show you a few videos examples, of how biacore can be used to study some in some experiments. This is an example, where a plus b gives a b, if you see the curve and if the curve is existing it means, a b is formed. If you pass a over b and there is no response which means, if you see a flat line then a b is not formed.

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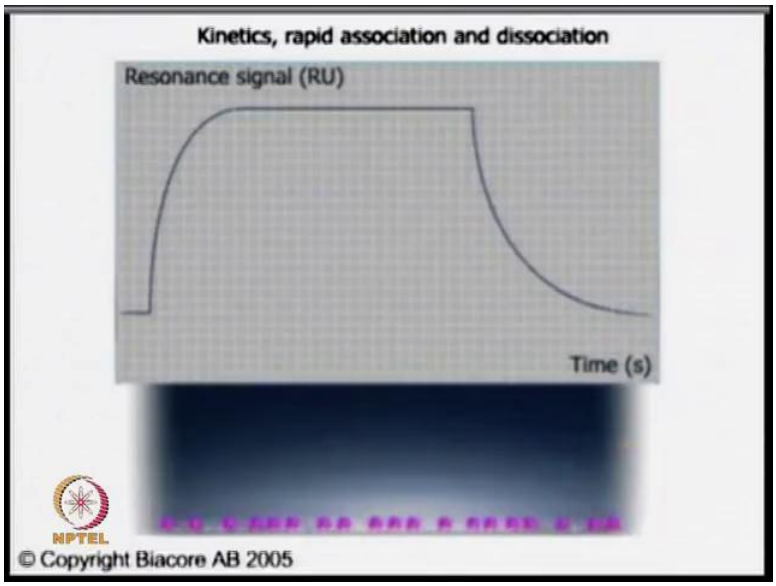

So, it is a very simple example, where you can actually decide whether the interaction is happening or not happening. I will show you another example now of kinetic analysis where, you will see two examples. The first example will be that of an extremely rapid association. So, you see this slope of the curve goes up very fast and comes down extremely fast. So, this is a rapid association and dissociation whereas, if you see the slope slope of this curve that is about to come up it is very slow association and very slow dissociation. So, just by looking at these curves you are able to actually tell if the interaction is fast or if it is slow. So, these are some of the examples of biacore technology at work. I think unlike microarrays were people can detect the interaction, but they cannot tell the nature of the interaction here, the edge is that by looking at this type of kinetics in the curves.

One can also tell about the type of association dissociation and the Overall kinetic analysis.

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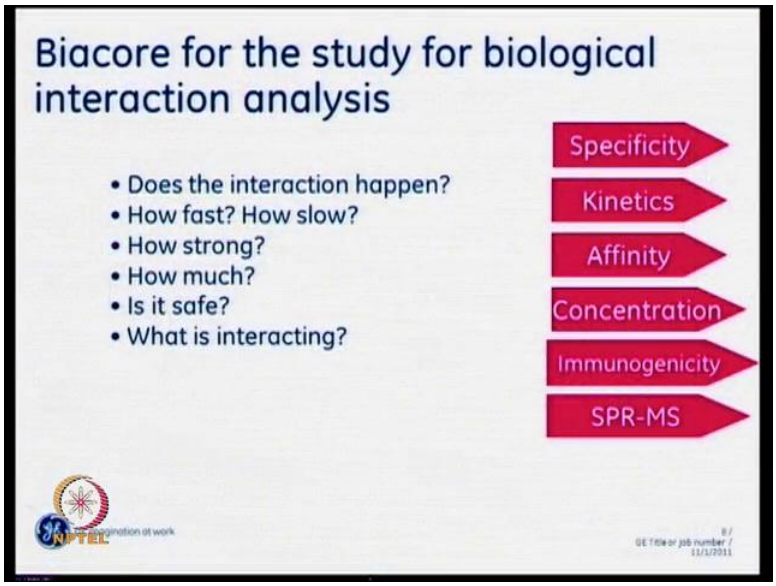

So, and here I have on on myp p t a very simple analysis of different things that can be done with biacore. So, once someone ask me what can be done with biacore, these are the 6 things that can be done with biacore. So, very shortly put these are the 6 applications of biacore. Whenever a plus b forms a b when you are studying an interaction a plus b gives a b. The questions you ask is a first question you ask is does the interaction happen or not which means, is the molecule a b formed or not. The second question you ask is how fast the interaction is or how slow is the dissociation. The third question that you ask is how strong the interaction is. So, what is the affinity of the interaction? The fourth question you ask is how much of the analyze is there Which means, what is the what the concentration of this is analyze. Sometimes in drug industry you ask is this interaction is safe or interaction is not safe.

And sometimes if you have hydrogenanalyze and you see the binding is happening you want to ask what is it that is binding, because there are too many components in this analite. So, what is it specially binding and these are the five different things that you can do with biacore. So, I think you rightly mentioned identifying the very specific interact or is the most challenging aspect of it, yes, because that is why many time people fail and they discover the false interactors. So, I think that is where the SPR has edge over conventional techniques like immunoprecipitation or yeast two hybrids and some other other large screening methods where there is the good chance of identifying the false

positives. Sure sure there is a lot of promiscuous binders in screening experiments. Which will be avoided if you this specificity that biacore gives you?

So, we start with each of these applications in details. The first application which we will talk about is this specificity application. Now some interesting questions that are asked when you when you are doing specificity applications. Is the drug binding to the receptor or not, is the map by identifying a strain or not, or is there any nonspecific binding in the interaction that I am studying, and these questions are very easily answered by biacore.

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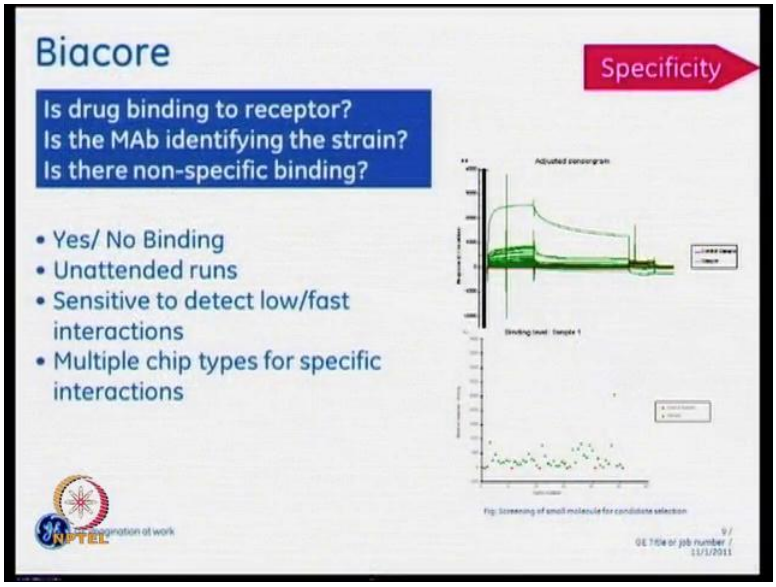

Shown here in my p p t is actually an example, where we are looking for binding and here someone came to ask with about 40 different compounds and they want to see if any of these compounds binds to a receptor. And here we have forty of these experiments done, most of these are not binding or binding at a very base level, but if you look at this presentation. I highlighted one spot here with one molecule that a shown circle in red, this particular molecule is actually binding to the receptor. So, at the end of a very short experiment of looking at the receptor versus candidate binding, you are able to determine which of these candidates is actually binding to the given receptor a very simple example of specificity.

Going on to the next application, so, I will interrupt you here. So, basically they just demonstrate that even if we are very much unaware about the components which could be interacting, this could be a good screening tool right, because if there is a real strong

interaction and specific interaction then probably we can see some out like this if you want to do that first level screening to just quickly find binders. In some kind of a screening experiment like you mentioned then this would be a very good starting point for you to quickly find those binders and take them to the next level. Many times when we have a thirst of identifying some like large drug library right. Or small compounds and then at that time maybe to begin with good straining tool.

Yeah I would also like to point out that this a very small experiment, it barely takes about one minute to this experiment. So, basically per minute you can do one screen and that way you will have a lot of compound screened, you know you know very rapid manner. So, that is the first step in the specificity experiments.

Now, let us assume that you found this candidate and you think that it is a specific binder and now you want to look at the kinetics of the interaction. And everyone understands that kinetics is an extremely important part in drug discovery, in proteomics when you look at interactions one of the important things that you need look at is what is the on rate and off rate of the interaction.

And so, in that sense how fast is this interaction happen and which candidate is kinetically preferred, because if you have two candidates both of them trying to be drugs you should choose the candidates that is kinetically preferred. And in most cases in recent cases actually kinetics is being used to shows similarity of drugs for examples, there is a lot of biosimilars coming out of India. And biosimilars manufacturers actually want to show that their molecule is similar, or equal to the innovators drug then one good way of showing that their similar, is by showing…

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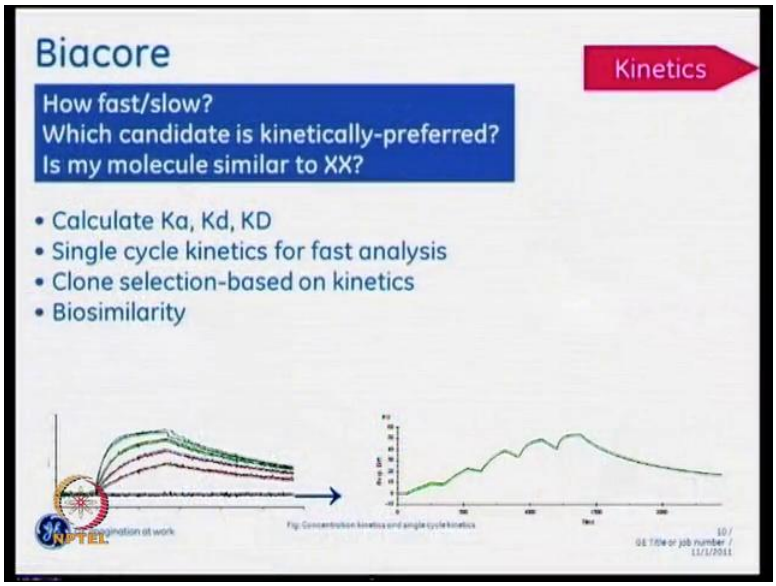

So, that is where again kinetics experiments will be helpful to you experimenters scientists can actually calculate the k on k off, or K a k d and the k capital d which is the affinity of the interaction. They can calculate this and in a very fast way actually understand the interaction a little better than they did before, because in the first instance they only knew whether the interaction was happening or not. Whereas right now they also know that the what the kinetic parameters of this interaction are. And once they know the kinetics of the interaction they come to affinity. So, identifying the on rate off rate and dissociation constant I think this provides a very strong tool and information by… For characterization, For characterization and that is i think the big thirst of all the pharmaceuticals doing by biosimilars.

Anyone who is pursuing biopharmaceuticals or recombinant proteins by biosimilarsor even novel drug discovery people in the small molecule arena. One to actually characterize the interaction in terms the in terms of the on rate the off rate and the affinity of the interaction. And that something that is very well accomplished by by the SPRtechnology. So, when it comes to affinity as you can see here, how strong is the bond. Is the binding strong enough to be physiologically important and this is one very important thing, because more and more drugs are coming out these days which are one dose a day drugs or which are you know fast acting drugs.

And these kind of discoveries depend a lot on kinetics and affinity of the interactions. And that is where biacore can actually come in in a very big way and help people you know genetically or protein engineer their drug. So, that they actually perform better than existing drugs. For a good comparison with the existing to make drugs better Or to discover novel drugs they are actually act better. Both of the cases I think SPR technology can be useful.

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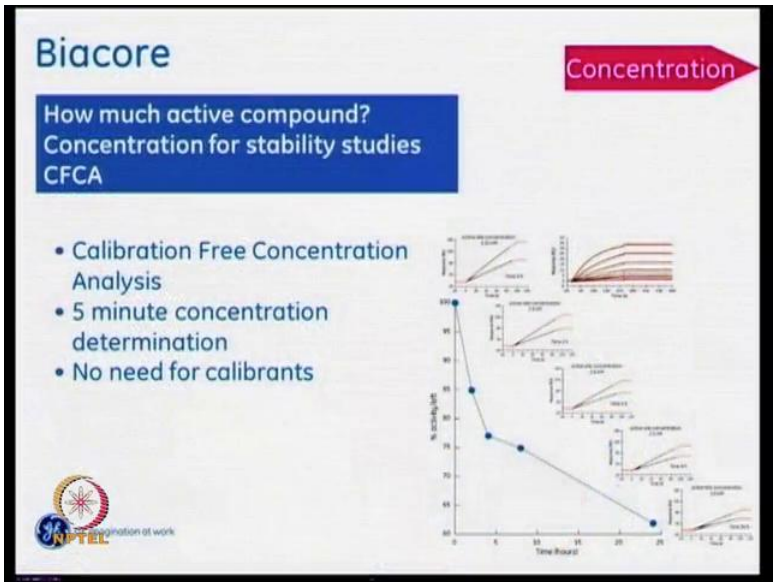

Once you know the affinity and concentration and I always think that concentration is very less studied, but it is very important is, because if you look at concentration analysis across the world for proteins. There is no way that someone can measure active protein concentration without having calibrants in their hands.

Whenever you give a student a protein and say please measure the concentration the first thing that he will ask you is for calibrants. He will ask you for standards and the problem with these standards is that sometimes they are not available, sometimes they are extremely expensive, and being proteins like you know sometimes they are not stable.

They are not stable. So, there is a great need for having a concentration analysis technique that does not need calibrants. And that is where biacore comes in againbiacore does something that is called CFCA which is calibrant free concentration analysis. And. So, within 5 minutes if you have a specific binder for a protein you can actually calculate the concentration of that protein without the need for the calibrants. And since you

already seen in screen one, where we talked about specificity we are talking about specific binding. So, what is measured is not just total protein concentration. What is measured is specific active protein concentration. I think it is very strong application of it, because many times you would like to know how accurately you can determine the protein concentration.

Absolutely especially in quality control and in filling in in biopharmaceuticals again where people need to exactly estimate how much they are actually filling in the final vial. That actually goes to the patient they need more accurate methods of measuring active protein concentration.

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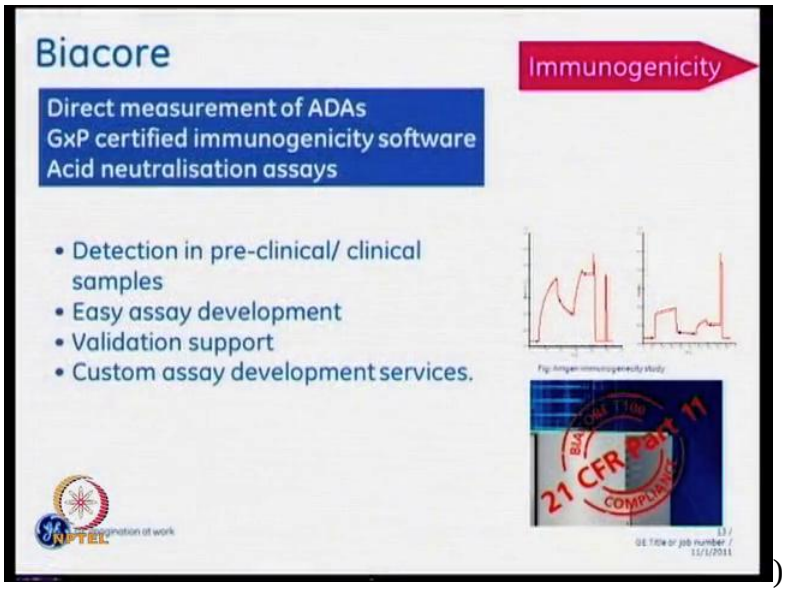

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And that is where I think biacore will play a very big role in letting people estimate that active protein concentration. Now once you seen concentration the next thing is immunogenicity. Worldwide with the increased biopharmaceuticals drugs everywhere needs to be tested for immunogenicity.

Immunogenicity is about direct measurement of antidrug antibodies, in which which should be measured in serum. It is also about bringing a regulatory framework into a system right we talked about only technology and science, but suddenly when it comes to drugs regulators come in.

So, can we actually accurately confidently measure antidrug antibodies in animal sera or human sera, at clinical trial levels? And biacore can actually be used for accurate measurement of antidrug antibodies and for immunogenicity testing of biopharmaceuticals. So, that is another major application of biacore as it comes. The last application of biacore, which if you remember when I talked about in the first slide about specificity. Now if you have a heterogeneous mixture that is flowing over a ligand and something within that mixture is actually bound. And you see a curve and you know that something has bound, but you do not know what it is that is bound. Binding. So, you can use the technique called SPR MS where you can take the bound analyte separate it into a vial and then actually take to a mass spec and identify the protein. So, in addition to doing all that they did before, now you can actually also find out what is it that is bound. So, definitely it is very important, because many times you will not know like what the unknown target is which is interacting or binding right. Yes. So, if you do not if you have this unknown for example, if you have a receptor and if you have cell lysate or if you have some kind of a homogeneous tissue lysate which we you have flowing over the ligand and now you can actually find out what that proteins are. So, it is used in applications like ligand fishing for example, where you have fishing for a ligand. So, those are the kind of examples that…

But in MS you will definitely required protein above certain threshold right. So, how you overcome that issue, because the binding will minimal. And of course…So, one thing is that this is a small interaction happening. So, the amount of protein that you collect may not be sufficient the only way you can overcome it is by actually doing it multiple times collecting enough. So, that you can actually get some kind of an MS response and that is what most of our users do is that they run the same binding as say about 10-20 times.

And collect the bound analyte and then take it to an MS and then get their result. So, in biacore you have a way to collect the flow in the flow cell. And then actually you can accumulate that in multiple multiple runs and then take it. Then, concentrate that and then you do further mass spec. Correct.

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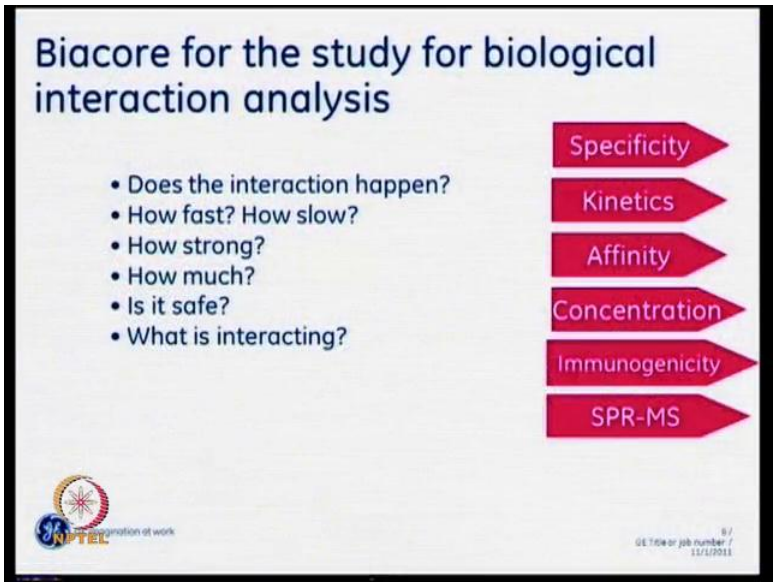

Yes. So, just to summarize again I will just go back to my the first slide that I showed here, which is here. This are the are the six things that biacore can be used for specificity, kinetics, affinity, concentration, immunogenicity, and mass spec analysis. So, that is those are the 6 broad applications of a biacore SPR technology.

That is what very important, because many times a good decision about these products before hand about characterizing these proteins, can save you lot of money lot of efforts down the road, because… Absolutely. If if you can do those experiments in the beginning most often when we actually talk to pharma industry, you know we tell them that biacore or SPR technology is not for success, but more for failure. Only thing is that we say that it is for early failure and it is for cheap failure. So, do not spend too much money on something that does not work, might as well fail early.

So, that is the basic reason why SPR should be used. I think that is very important. So, can you briefly tell us about what are the major instrumentation available from the biacore technology currently for doing the surface Plasmon resonance based experiments?

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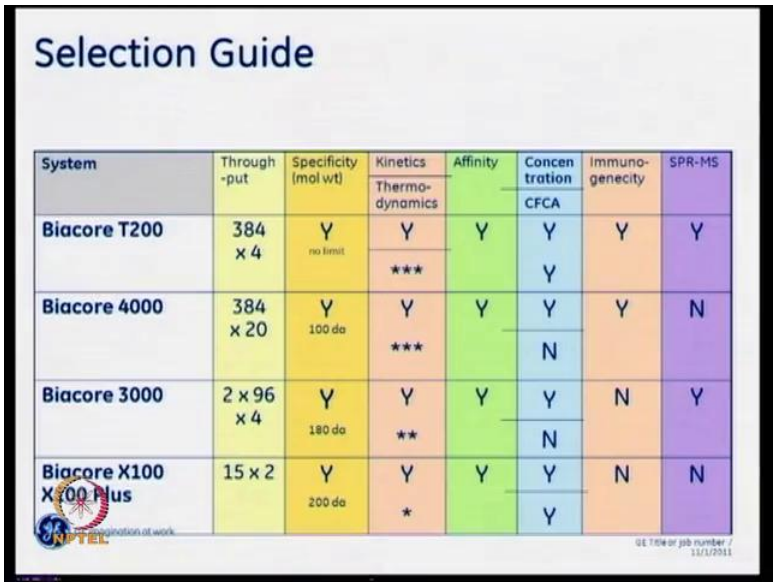

There are basically four different biacore instruments available, there is a very small biacore which is called the x 100.You can see it on pitch it has two flow cells, and it can do some beginning analysis, and then you have the biacore 3000 which is an academic favorite a lot of academicians like it, lot of lot of customers in India who are academics have the biacore 3000. Then we have the latest which is the biacore t 200 very special again, because it has all the things that the biacore 3000 has, but it is the regulatory approved. So, if you are company that works with f d a that works with that works with you know d g c i or some of these regulatory authorities, then I think that we should be using the biacore t 200. The biacore4000 has 20 different immobilization sites on it and biacore4000 can be used with multiple 384 well with robotics.

So, if you are company that has you know extremely high throughput screening, if you are company that does a lot of immunogenicity experiments or if you are doing batch testing or release testing using biacore then you should be using the biacore4000. I must say that most of the customers in India use either the biacore three thousand or use the biacore t 200. So, how easy it is to do these SPR experiment and especially the kinetic analysis by using some software available from biacore.

The the most important thing in a biacore experiment is actually, the experiment design. It is very easy to do the analysis and let me just show you an example of a typical result. And let me show you how the analysis works out. So, if you can see my computer. So, I

am going to open a typical biacore result and here is a biacore result, this is a biacore result where5 samples of different concentrations

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We run over a fixed ligand which was on the chip. And now I am trying to dokinetic analysis. So, if you look at these by results, I select them and I show the results and these are the results. Now this is what are typical results looks like and there is nothing to be worried about, because it looks odd the idea is that your results are all embedded somewhere here. These two big peaks that you see are regenerations. So, the first thing that I do when I do the analysis is actually select the regenerations which are do not need and cut them out.

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So, I cut them out here. So, I say cut and then the rest of the result. So, you want adjust your response access. So, now I have 5 different concentrations 1,2,3,4,5 in different colors. And you have this is the association face, and this is the dissociation face. Now I am going to just baseline this result.

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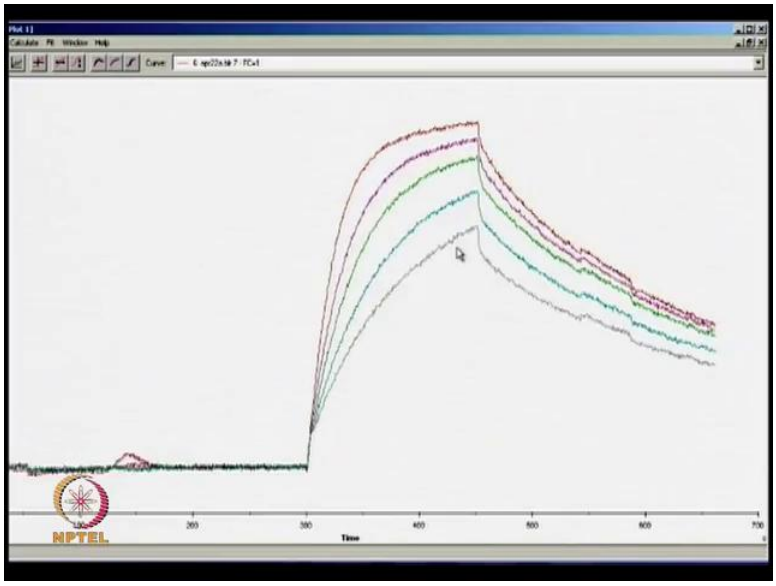

So, I select the baseline here, and then I just go to the adjustment of y axis and I say 0 at the average of selection and then I say add his name. And now all my five results are shown here. Now I am going to do a quick kinetic analysis and it is really extremely

simple to do a kinetic analysis, because all that I do is say calculate. I say kinetics simultaneous k on and k off. And I have already done the cutting and the y transformation. So, I say next and then if I want to I can go and adjust the start and the end time. So, I can actually move this to adjust the start time and the end the time of association and dissociation which I sometimes do, but I think this is pretty well picked by the software already. So, I do not need to do much, I say next and I enter the different concentrations of each of the samples which were run they are entered here now this is very important. These concentrations are known concentrations which which you ran already ,which you are you actually made the concentrations of the analyte and then ran over the ligand.

So, you would do that. Now one of the things that is important here is you choose the model. So, preferably it is always better whenever you do characterization the more you know about your system the better characterization results you get. So, in this case suppose you do know that it is a one to one binding then you would choose that binding, but if you want to you can actually change the the binding model it could be a bivalent analyte it could be a bivalent ligand.

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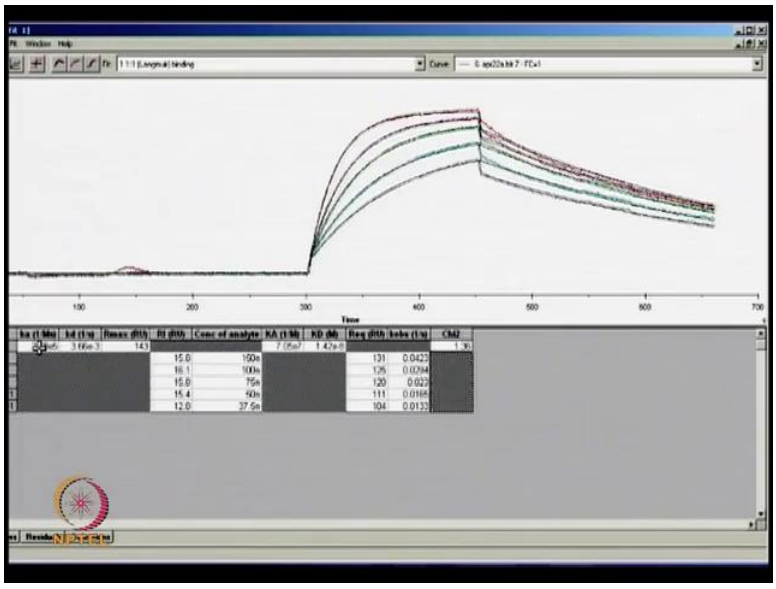

It could be a heterogeneous ligand. So, depending on the model you choose. So, you choose the model that you want to here. And then you say fit and what happens is curves

get fit and there is also thrown up. So, your k on and k off are displayed here. So, it is as simple as that. So, you all you need to do is take a ligand immobilize it on a chip.

Run about 5 different concentrations of your analyte over the chip and this simple analyte and each of these results if you look at the x axis carefully, it is0to 600; that means, each one of these runs the entire run was 10 minutes. And you ran 5 samples. So, it is 5 into 10 minutes. So, 50 minutes with the time taken in between the runs about another 10 to 20 minutes about one hour 10 minutes 1 hour 20 minutes you have the results and you have already characterized your results.

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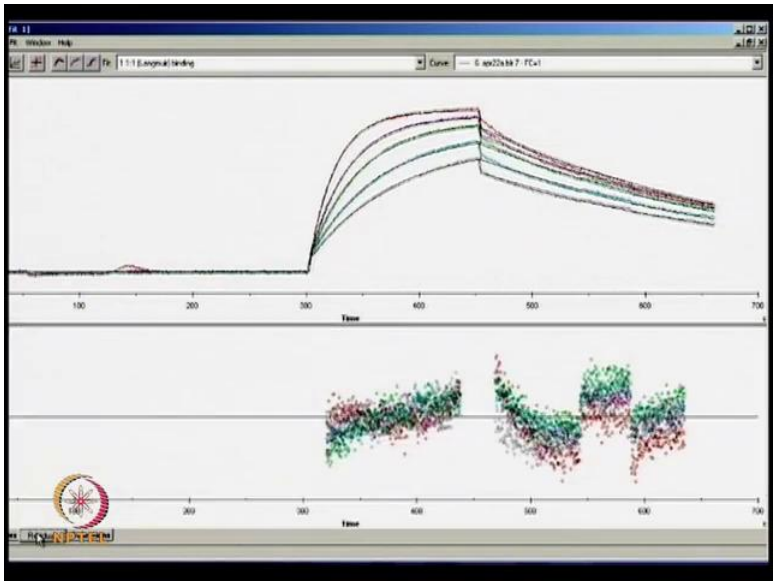

Because you have the k on and k off calculated. And you can also quickly check how good your results are by quickly checking the residuals and you can see the residuals here the chi square values are really between minus 5 to plus 5 percent. So, extremely good. Many times fitting reactions and a very fast analysis that gives you k on and k off I think it is very extremely fast and very good, very easy to use… Interactions. Very easy to use software for your analysis.

So, it is very useful and informative to see the analysis like how easy this to perform the kinetic analysis. Can you give some specific example, of doing kinetic analysis by using biacore system? Let me show you one example, and this again goes back to why kinetics is important. Kinetics is extremely important in biacore analysis and let me show you an

example. So, here is a pitch about three slides on kinetic and affinity analysis using biacore. So, if you look at this slide.

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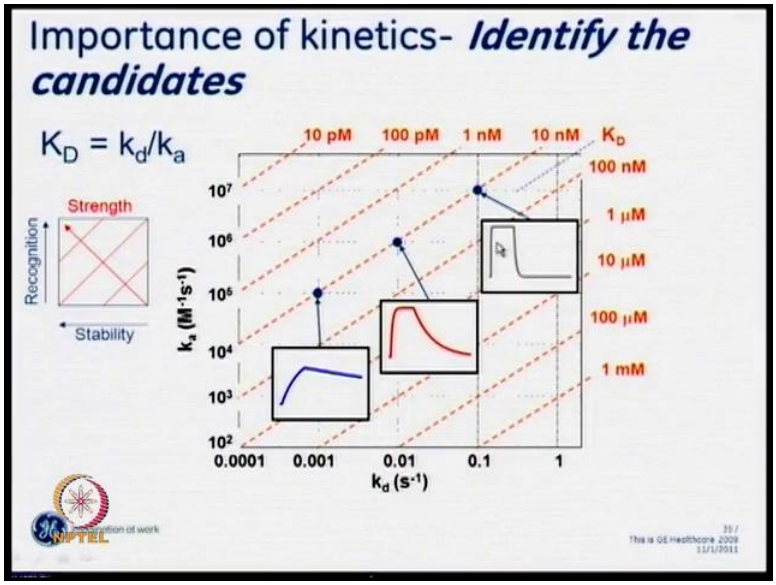

Now, here on this slide you have three interactions which are captured you have interaction one which is captured in blue, interaction two which is captured in red, and interaction three which is captured in black. The important thing about all these three interactions is that all these three interactions have the same affinity. They have varying k on and k off, but they have the same affinity and this is important in drug discovery, because let us say for example, you are looking for pain relief, if you want pain relief you want a drug that acts fast and that stays on for a very long time, but if you want a sleeping pill for example, you want a drug that actually acts slowly, but stays on for only a reasonable amount of time and comes off fast enough.

So, kinetics is very important in choosing a drug candidate now this is an example where if someone chose just on the basis of affinity all these three would have been the same, but since they would make their choice based on kinetics they can actually decide based on thek on and the k off now here is a real life.

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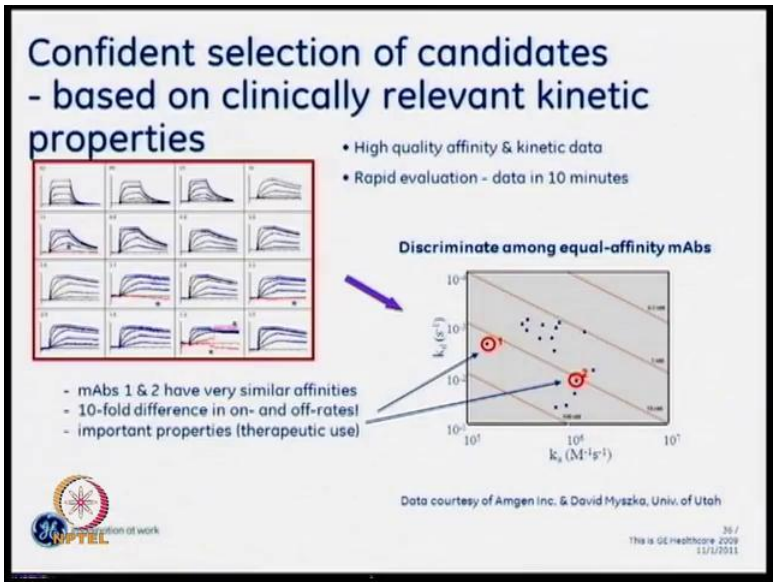

Example in the next slide which I show you which is a publication from Amgen and from Davidmyszka of university Utah. Where actually Amgen uses this data directly to do their clone selection. So, they are doing maps selection of maps selection of clones for maps, and if you look at this say clone number one, and clone number two have similar, affinities, but you know if you look at their on rate and off rate there is a ten folded difference. So, if you look at these two clones for example, which clone should they go for, they should go for the clone which is having more kinetically relevant properties. If they use only affinity for making that choice then there is no choice at all they are both the same. So, this is a live example of where kinetics data is actually being used to capture information regarding k on and k off and then make a more educated decision, knowledgeable decision on which map to go forward with, correct. Now this is very interesting to see this, because you will feel that if you just rely totally on the k d values right if you do not go Individual on the k on and k off. Yes. So, distribute that k d value into k on and k off and make a more knowledgeable eyes.

So, it is very interesting example to appreciate the power of kinetic analysis right. Can you just brief us about what are the major limitations or shortcomings of various surface Plasmon resonance based technologies. Absolutely. So, like any technology this technology should not be viewed as a silver bullet. It does have short comings one of the significant shortcomings being that, if there is any structural differentiation in the protein this would not be able to capture it is mass based sensor. So, any structural changes will

not be captured, there is also a problem that there are situations where you are unable to immobilize the protein that you have on to the chip.

So, you might have to use some capture techniques to do that. There is also there is big question about you know. So, you answer these questions about interaction you answer questions about whether the interaction happens or not how fast how slow how strong how much. You say whether it is safe or not with immunogenicity and you know what is binding, but then sometimes you want to ask the question why is it binding

And why is it binding is answered by structural studies or thermodynamic studies. So, that is where I think biacore can give you a little bit of a direction, but I think you should do more of you know an n n m r study or you should do microcalorimetry and that is what will probably give you more answers on why the interaction is happening. So, further in depth studies will be required on those. Interactions. So, when you have an interaction happening and when it is happen when it is happening very fast.

Now, why is it happening fast is transition state thermodynamics. So, you will have to ask questions in thermodynamics to get those answers. So, sometimes further study is essential and biacore kind stops with these applications. Would you have any final advice take home to the. So, here is here is basically three rules that we have in biacore. The first rule in biacore experiments is biacore technology is extremely easy to understand, biacore technology is extremely easy to analyze, a lot of time must be spent on experiment design. The first thing that I would do if I were working with biacore is spend a lot of time on very carefully considered experiment design. The second thing that I would do if I were conducting a biacore experiment is that I would make sure that I have an extremely pure ligand that I will put on the chip and that is extremely important. The last thing that I would consider and this is true of all experiments and I would agree with me is that it is garbage in garbage out so, make sure your sample preps are correct and remember that this is an analytical instrument, as unlike many other techniques in biotech, this is an analytical instrument it is a mass sensor at the end of the day you could call it in extremely sophisticated weighing machine. So, if you put something on it it will give you the weight, it is as simple as that only thing is you have to do it right. So, I would say that make sure that your sample prep is perfect your experiment design is perfect make sure your ligand is pure.

So, I think you very rightly mentioned that a good experimental design identifying the good ligands working out the chemistry for immobilization. As well as doing the very well sample preparation. All of these are very essential component for doing any successful proteome experiment, and especially the surface Plasmon type of experiments. So, thank you very much Lalith fordiscussing about the biacore technology with us today

Thank you.

And I hope it is informative and useful for my students. Thank you thank you

So, after having a discussion with Lalith kishore on biacore SPR technology. I hope now you are very clear about the instrumentation, the various properties which can be studied by using the biacore system and how quickly one can perform the data analysis. So, conclusion from this whole discussion is that, since the introduction of the biacore SPR instrument. The SPR spectroscopy has become widely used from chemistry and biochemistry to characterize biological surfaces, and monitor the bimolecular binding events. Overall the success of SPR technique is due to the following factors. First the kinetic measurement in real-time that is the major strength of data obtained from the SPR instruments. Second monitoring the adsorption of unlabeled analyzes molecules to the surface. And third its ability to monitor weakly bound interactions due to high surface sensitivity of SPR biosensors.

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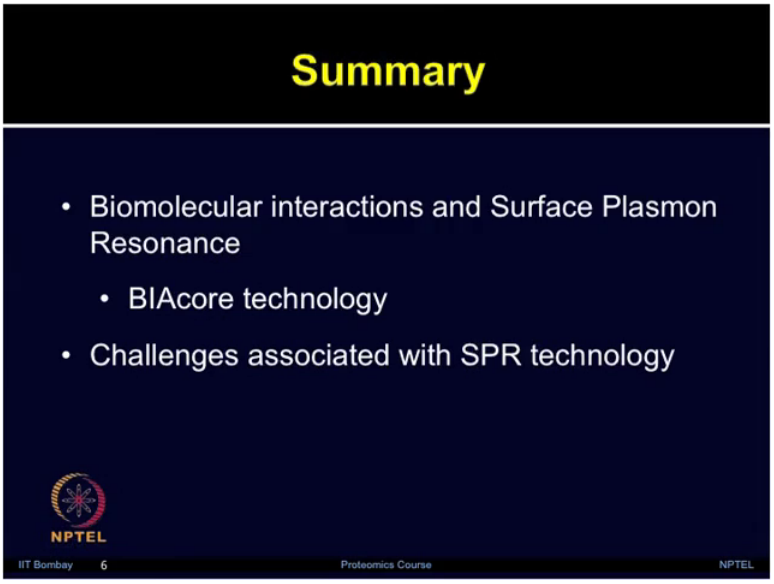

So, in summary in today's lecture, you have learnt about significance of studying the bimolecular interactions, how surface Plasmon resonance technique can be used for studying the bimolecular interaction. A brief introduction of biacore technology and data analysis and then certain issues and challenges which are associated with SPR technology. So, we will continue our discussion on label-free techniques in the subsequent lectures. Thank you.