Interactomics: Protein Arrays and Label-Free Biosensors.
Professor Sanjeeva Srivastava.
Department of Biosciences and Bioengineering.
Indian Institute of Technology, Bombay.
Lecture-13.
SPR: Interactive Session-II.

Welcome to the MOOC Interatomic Course. In our last lecture we studied that major strength of Surface Plasmon Resonance SPR Biosensor is their versatility and ease of use. The SPR Platform allows the ligand analyte interaction for a wide range of molecular weights, and varying affinities and compatibility for small molecules dissolved in organic solvents.

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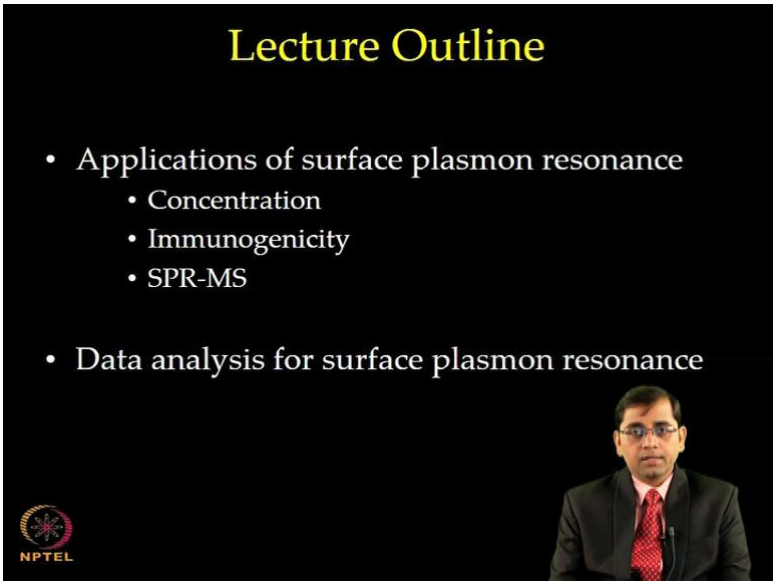

These biosensors play a very important role in biological research to monitor micro molecular interactions in real time without the need of labels. It is one of the reliable platform to determine affinity, kinetics, concentration analysis, and immunogenicity for a variety of interactions which we are going to discuss in today's lecture.

We will also learn a little on how to analyse the data, and perform data analysis using software. Let us now continue our discussion with Mr. Lalit Kishore to discuss where is applications of SPR. So Lalit welcome to the discussion of SPR technology. Thank You.

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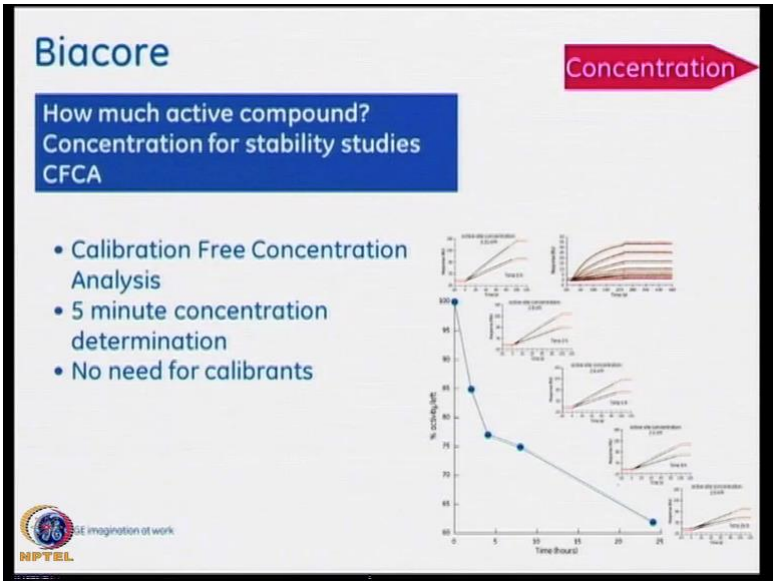

Going on to the next application I always think that concentration is very less studied, but it is very important,is because if you look at concentration analysis across the world for proteins, there is no way that someone can measure active protein concentration without having caliberance in their hands.

Whenever you give a student protein and say please measure the concentration in the first thing he will ask you is for caliberance. Right. He will ask you for standards, and the problem with the standards is that sometimes they are not available, and sometimes they are extremely expensive, and being proteins like you know sometimes they degrade. Not stable. They are not stable. So they is a great need for having a concentration analysis technique that does not need caliberance, and that is where Biacore comes in again. Biacore does something called CFCA which is caliberant free concentration analysis.

So within 5 minutes if you have a specific binder for protein you can actually calculate the concentration of that protein without the need for caliberance. And since you have 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.

That is a 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 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 protein concentration, and that is where Biacore will play a very big role in letting people estimate the active protein concentration.

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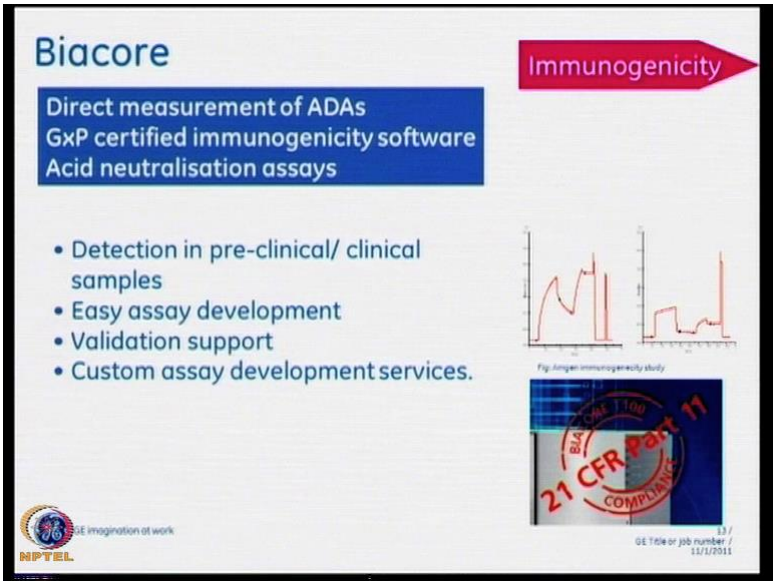

Now once you have seen concentration, the next thing is immunogenicity. Worldwide with increase biopharmaceuticals drugs everywhere need to be tested for immunogenicity. Immunogenicity is about direct measurement of anti-drug antibodies in the which should be measured with serum. It is also about bringing regulatory framework into a system.

Right now we have talked only about the technology and science, but suddenly when it comes to drugs, regulators come in. So can we actually accurately confidently measure anti-drug antibodies in animal sera or human sera at clinical trial levels, and Biacore can actually be used for actual measurement of anti-drug and antibodies, and for immunogenicity testing of biopharmaceuticals. So that is another major application of Biacore as it comes in.

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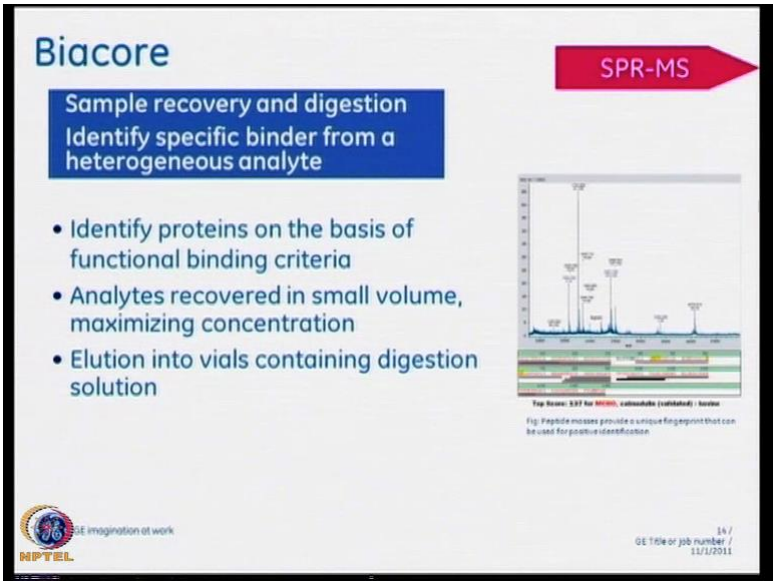

The last application of Biacore which if you remember when I talked about in the first slide about specificity. Now if you have a heterogeneous mixture that is flowing over a ligand, and something that mixture is actually bound. And you see a curve and you know that something has bound but you do not know what it is that is bound.

So you can use a technique called SPR MS where you can take the bound analyte separate it into a vial and then actually take it to a mass spec to identify the protein. So in addition to doing all it did before, now you can actually find out what is it that is bound.

So definitely it is very important many times you will not know what the unknown target is which is interacting or binding that. Yes, so if you have this unknown target for example if you have a receptor, and if you have a cell lysate or if you have some kind of homogeneous tissue lysate which is flowing over the ligand, now you can actually find out what that is. So it is used in applications like ligand fishing for example where you are fishing for a ligand. So those are the kind of examples.

But in MS you will definitely require protein above certain threshold, how you overcome that issue because the binding will be minimal. So one thing is that this is very small interaction happening so the amount of protein 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 MS response. And that is what most of our users do it that they run the same binding as say 10-20 times and collect the bound analyte and then take it to MS and then get their result.

So in Biacore you have way to collect the flow in the flow cell. Yes. Then you actually can accumulate multiple multiple runs, then take it. Then concentrate that, then you do for it. Yes. So just to summarize again I will go back to my first slide that I showed here which is here.

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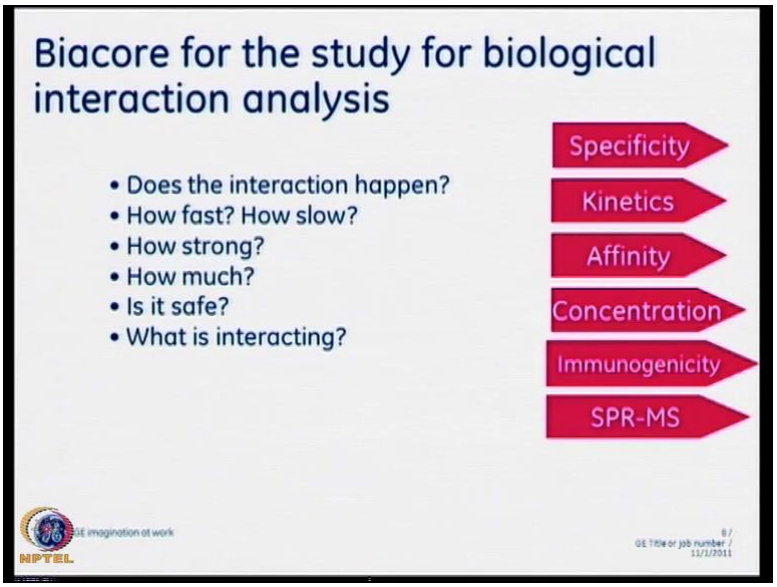

These are the six things Biacore can be used for. Specificity, kinetics, affinity, concentration, immunogenicity, and Mass Spec analysis. So those are the six broad applications of Biacore SPR technology.

That is very important many times ah good decision about these products beforehand about characterizing these proteins can save lot of your money, lot of your efforts down the road because. Absolutely. 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. Right. Only thing that we say is 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. Right. I might as well fail early, that is the basic reason why SPR should be used.

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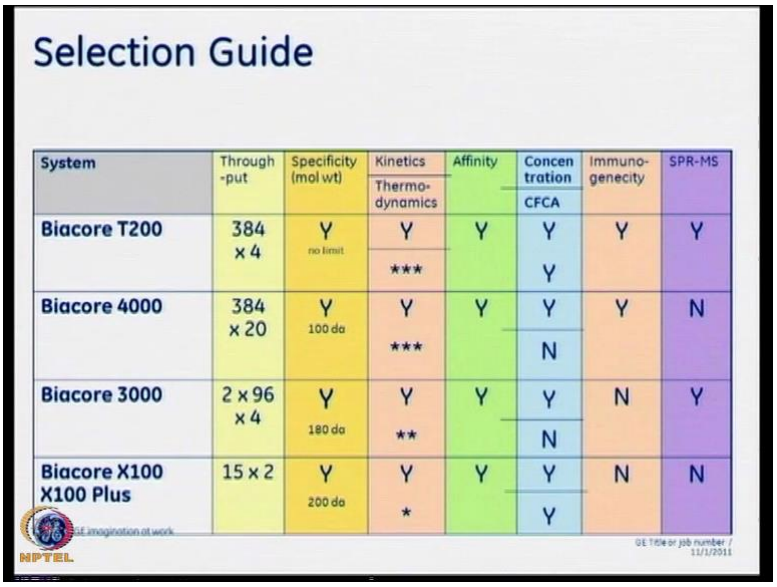

I think that is very important. Can you briefly tell us about what are the major instrumental available from Biacore Technology currently for doing Surface Plasmon Resonance and Space Experiments. there are basically four Biacore instruments available. there is very small Biacore which is called X100. you can see on my page, it has two flow cells, and it can do some beginning analysis, and then you have Biacore 3000 which is an academic favorite, lots of academicians like it. lot of customers in India who have academics have Biacore 3000.

Then we have Biacore which is T200, very special again because it has all the things that Biacore 3000 has but it is regulatory approach. So if you are a company works with FDA, that works with IMIA, works with DGCA, some of these regulatory authorities then I think you should be using Biacore T200. The Biacore 4000 has 20 different immobilization slides on it, and Biacore 4000 can be used with 384 well plates with robotics.

So if you are a company that has extremely high throughput screening, if you are a company that does a lot of immunogenicity experiment or if you are doing batch testing or release testing using Biacore then you should be using Biacore 4000. I must say that most of the customers in India use either Biacore 3000 or use the Biacore T200.

So, how easy is it to do these experiment and specially kinetic analysis by using some of the software available from Biacore. The most important 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. Sure.

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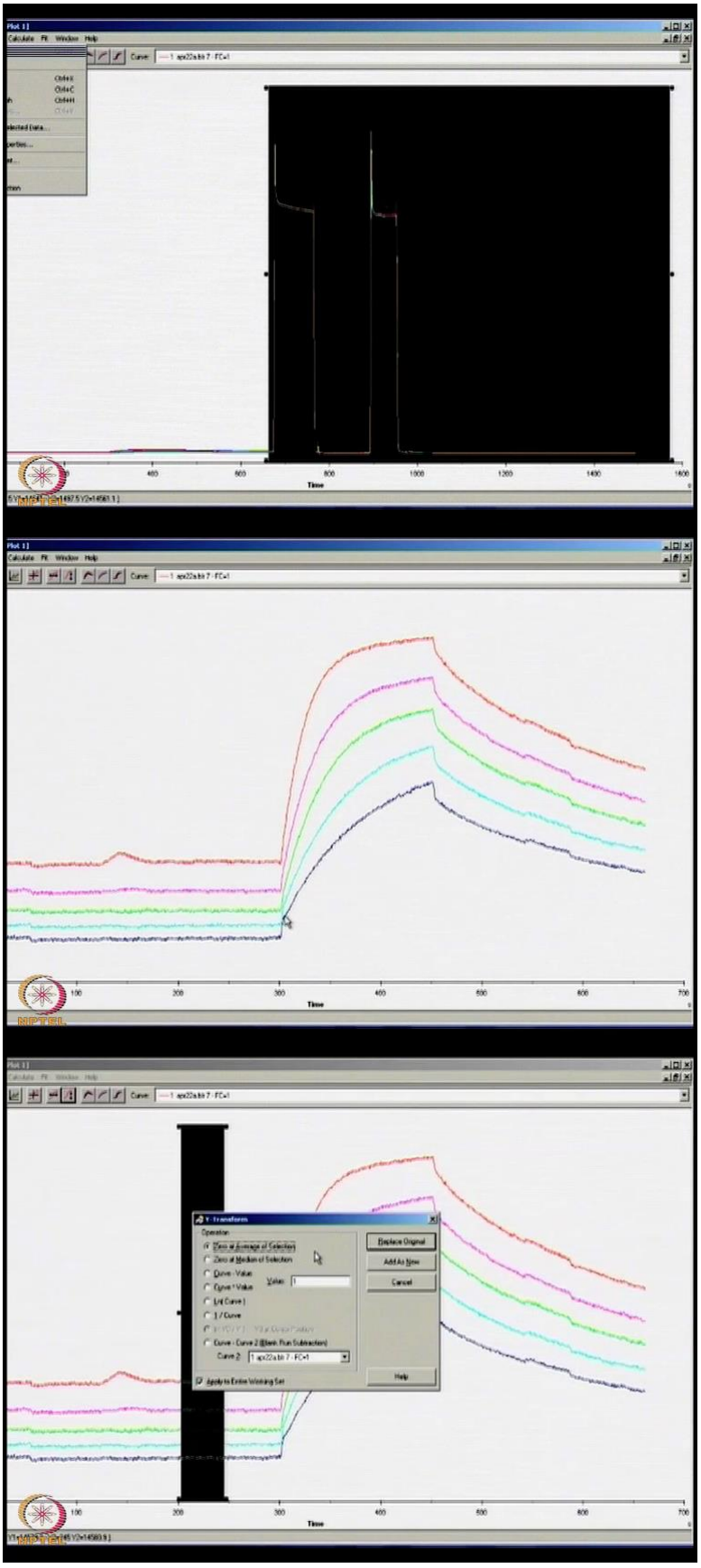
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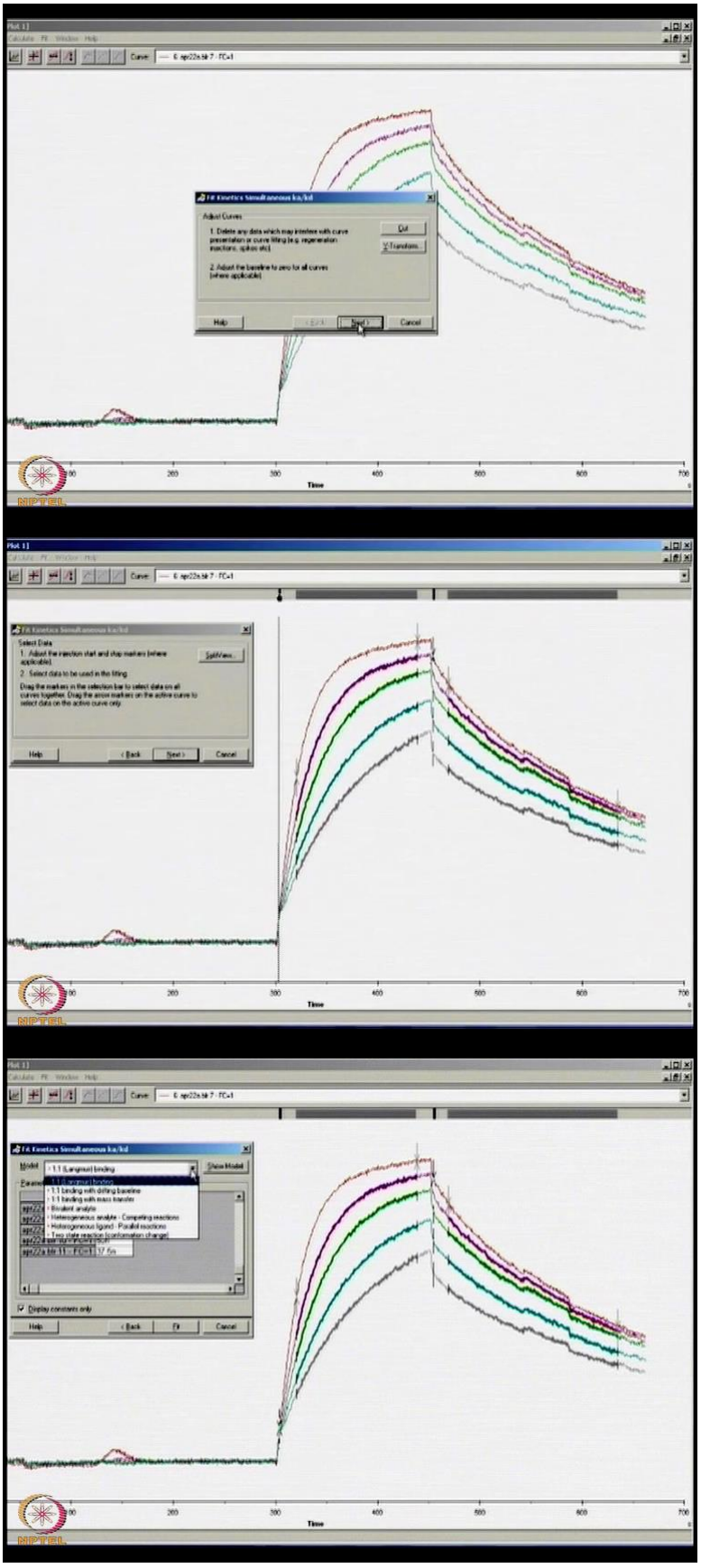

So I am going to open a typical Biacore result, and here is a Biacore result. this is Biacore result where 5 samples of different concentrations were run over a fixed ligand which was on

the chip, and now I am trying to do a Kinetic analysis. So if you have to look these five results, select them, and I show the results, and these are the results.

Now this is what a typical result 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 which you see are regenerations. So the first thing what I do when I do analysis is actually select the regenerations which I don't need and cut them out. So I cut them out here, so I cut, and then rest of the result is here.

This will give you the response actually. So now I have five different concentrations 1, 2, 3, 4, 5 in different colors and this is the association phase, and this is the dissociation phase. Now I am going to just baseline this result, so I will select the baseline here, and then I just go to the adjustment of Y axis, and I set 0 at the average of selection, and then I select add as new. Now all my 5 results are shown here.

Now I am going to do a quick kinetic analysis and it is really extremely simple to do kinetic analysis because all that we do is say calculate, I say kinetics, simultaneous K on and K off and I have already done the cutting and white transformation so I will say next, and then if I want to then I can go and then I can adjust the start and end time.

So I can actually move this and choose the start time and end 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, then I say next, and I enter the different concentrations of each of the samples which were run, then entered here.

Now this is very important here. These concentrations are known concentrations which you ran or you already made the concentration of analyte, and ran them over the ligand. So you would do that. Now one of the things important here is that you would choose the model. So preferably it is always better whenever you do characterization the more you know about the system the better characterization results you get.

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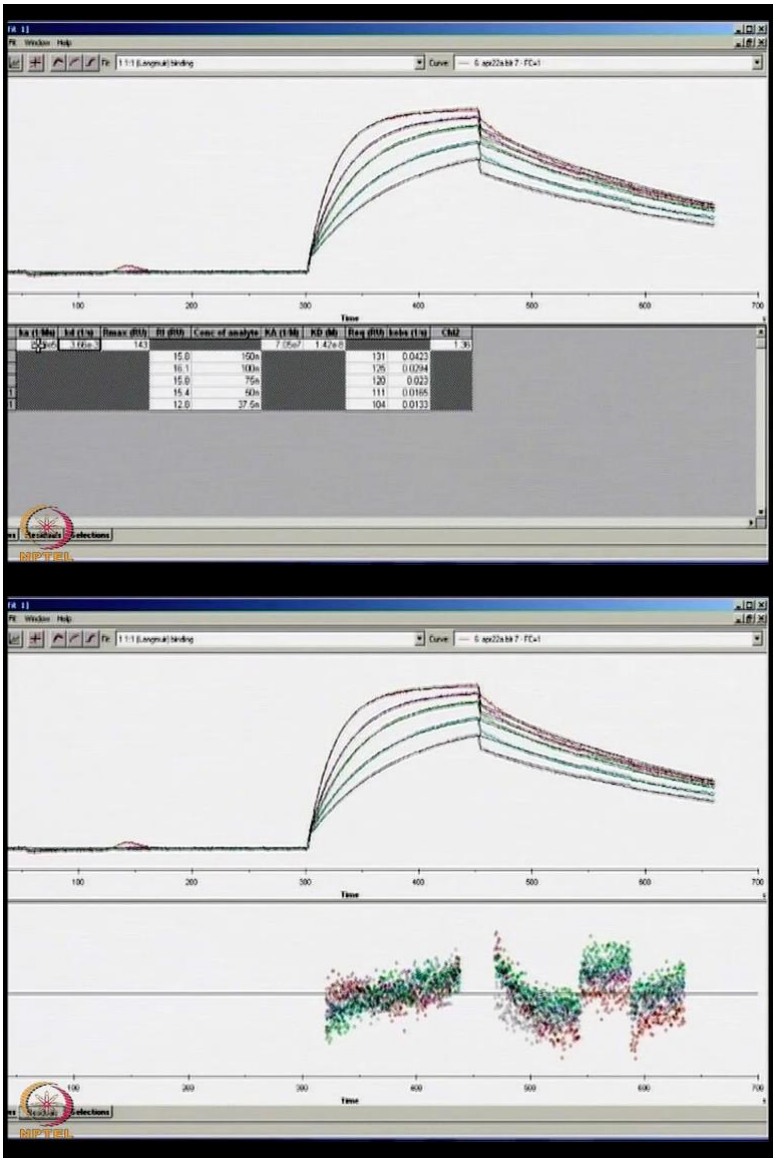

Suppose in this case you do know this is one-to-one binding, then you would use that binding. But if you want to you can actually change the binding model, it could be a bivalent analyte, it could be a bivalent ligand, it could be a heterogeneous ligand, so depending on the model you choose, so you choose the model you want to here. And then you say fit, and then what happens is curves get fit, and the results are thrown up. This is K on, and K off are displayed here.

So it is as simple as that. All you need to do is take a ligand and mobilize it on a chip. Run 5 different concentrations of your analyte over the chip, and this simple analysis, and each of this result if you look at the X carefully it is 0 to 600. That means each of these runs, the entire run, it was 10 minutes. Yes. And you ran five 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 or about 1

hour 10 minutes, 1 hour 20 minutes you have the results, and you have already characterized your results because you have 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. Like Chi square values are really between minus 5 to plus 5. Extremely good when it fit in reactions, and a very fast analysis that gives you K on and K off.

Yes, I think it is very extremely fast and very easy to finalize. Very easy to use, very easy to use software for your analysis. So this was very useful and informative to see the analysis like how easy it is 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 go back to why kinetic is important. Kinetic is extremely important Biacore analysis.

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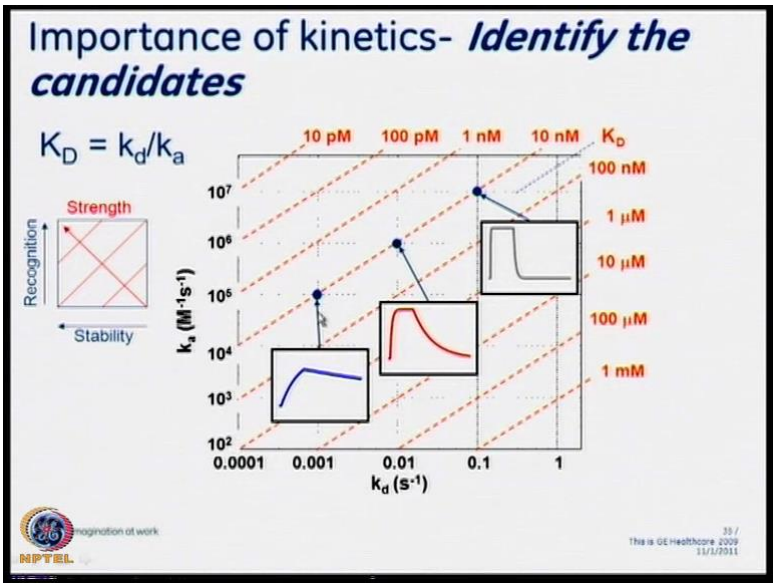

Let me show you an example, so here is a page about three slides on Kinetic and Affinity analysis using Biacore. So, if you look at this slide, now here on this slide you have three interactions which are captured. You have interaction 1 which is captured in blue. You have interaction 2 which is captured in red, and interaction 3 which is captured in Black.

The important things 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. This is important in drug discovery, because let us say for example you were looking for pain relief.

If you want a pain relief then you want a drug that adds 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 only for a reasonable amount of time, and comes out fast enough.

So kinetic is very important in choosing a drug candidate. Now this is an example where someone chose just on the basis of kinetic or 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 K on and K off.

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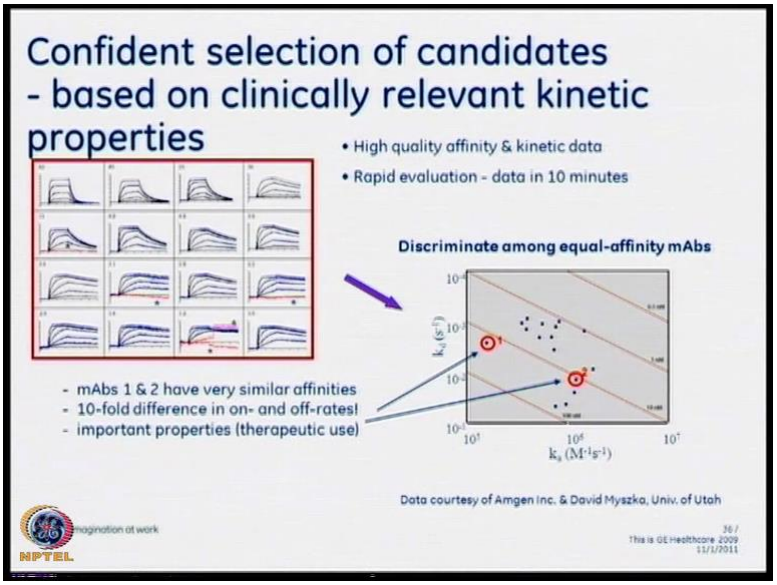

Now here is real life example in the next slide which I show you which is a publication engine, and from Amgen and from David Myszka of University of Utah where actually Amgen uses this data directly to do their cloneclone selection. So they are actually doing maps, selection of maps, selection of clones for maps, and if you look at this clone number 1 , and clone number 2 have similar affinities but if you look at their on rate and their off rate there is a 10 fold difference. If you look at these two clones for example which clone they should go for.

They should go for the clone which is having more kinetic relevant properties. If they use only affinity for making that choice then there is no choice at all, this is same. So this is a live example of where kinetic data is exactly 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.

This is very interesting to see because you will fail if you will just rely on KD value, if you do not go individual on K on, and K off. Yes, so distribute that KD value on K on and K off, and make a more knowledgeable attention.

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 short comings of various Surface Plasmon Resonance based technologies. Absolutely, like any technology this technology should not be viewed as silver bullet. It has some shortcomings ah, one of the significant shortcomings if there is any structural differentiation in the protein this will not be able to capture it. It is a mass based sensor, so any structural changes will not be captured.

Ah 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 this big question, you answer these questions about interactions, you answer the question about whether the interaction happens or not, how fast or slow, how strong, how much, is it safe or not, and you knowwhat is binding, but then sometimes you ask the question why is it binding.

Right! And why is it binding is answered by structural studies or thermodynamic studies. So that is where in think Biacore can give you little bit of a direction but I think you should do more of you know an NMR study or you should do microcalorimetry, and that is what probably give you more answers on why the interaction is happening.

Further in depth studies will be required. Absolutely! When you have an interaction happening and 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 for the study this is essential, and Biacore stops with these applications.

Would you have any final advice take-home to the. Absolutely! So here is basically three rules that we have in Biacore. The first rule in Biacore experiments is Biacore Technology is very easy to understand. Biacore Technology is extremely easy to analyse. 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 very carefully considered experiment design.

The second thing 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 experiment and I am sure that you would agree with me is that it is garbage in garbage out. make sure your sample preps are correct, and remember that this is an analytical instrument.

Unlike many other techniques in biotech, this is an analytical instrument. It is a mass sensor. At the end of the day you can call it an extremely sophisticated weighing machine. So, if you will 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. Ah, 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 have very lightly mentioned that a good experiential design identifying the good ligands working out the chemistry for mobilization. Absolutely. As well as doing very good sample preparation all of these are very essential component for doing any successful protein experiment, and specially the Surface Plasmon Resonance data experiment. Yes. Thank you very much Lalit discussing about Biacore technology with us today. Thank You. And I hope it was useful and informative for our students. Thank You.

Through our discussion on Surface Plasmon Resonance Technology we learnt that there are three major advantages of this approach. First the kinetic measurement in real time is the biggest strength of this method. Second, monitoring the absorption of unlabeled analyte molecule to the immobilized ligand surface, and Third, its ability to detect weakly bound interactions due to high surface sensitivity of SPR.

In summary in last two lectures we learnt about SPR techniques, its applications in different fields of research and a little overview on data analysis. Thank You.

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