Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-12.
SPR: Interactive Session-I.

Welcome to MOOC Interatomic Course. Today we will be discussing about Surface Plasmon Resonance. The biological system depend upon molecular interaction of bio molecules to form stable complexes. The identifications of protein interactions involving proteins with known functions with protein partners to understand the uncharacterised roles can lead to better understanding of these uncharacterised proteins.

The ability to rapidly and simultaneously screen a large number of proteins for studying their bio chemical activity including protein protein, protein lipid, protein nucleic acid, and protein small molecule interactions required very high input instrumentation.

The most popular methods currently being used to detect protein protein interactions include conventional Yeast 2yeast 2 hybrid methods, immune precipitation, and protein microlides. As we know that there are certain limitations associated with labels or tags which may interfere with protein function including their binding to the interactors. Additionally it is always not very straight forward procedure to label the query molecules.

Therefore there is a need for label free biosensors which can avoid such issues, and also allow real time measurement of binding. In today's lecture we will have a discussion on Surface Plasmon Resonance with an invited guest Mr.Lalit Kishore from GE Health Care Life Sciences. We are going to discuss about Biacore SPR technology.

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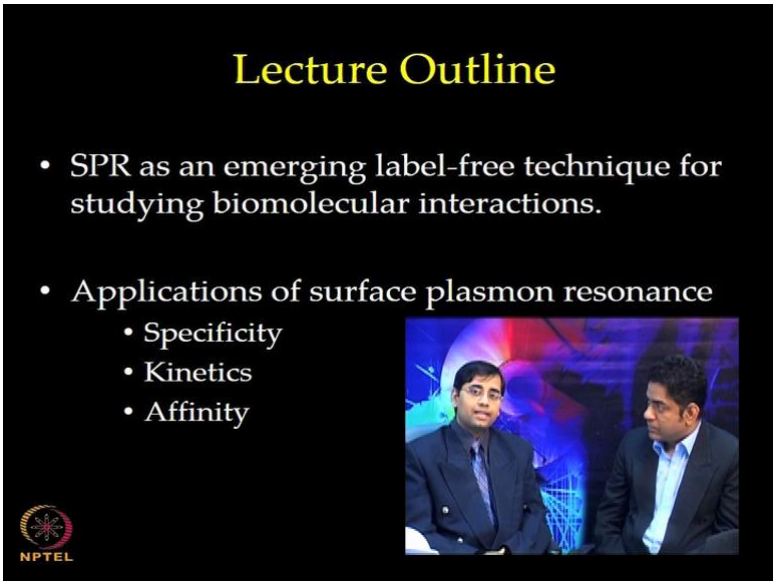

Let us now discuss about SPR as one of the emerging label free technique for studying Bio Molecular Interactions. Today we will discuss about Surface Plasmon Technology, and its various application in Proteomic.

Can you just brief us about your experience of using BIACORE Technology from last several years. my educational background is that I am not a biologist. Though BIACORE is largely a biological tool, my background is in chemical engineering, so I am a chemical engineer in MS in Management. however I find varied uses of SPR Technologies these days.

Ah I find it is used in Bio Pharmaceutical, I find it used in Basic Research, in Nano Technology, in pharmaceutical industry, in QC labs, so basically I think it is my chemistry and chemical engineering background that helps me work with BIACORE. Quite a lot of applications I see across the country and BIACORE technology.

Alright, so you are utilizing your varied background to apply on different biological problems.

Yes, that is what I think SPR technology also does that. Actually it uses very simple technology and applies it to different things in biology and actually gives out result that are there for everyone to see.

Can you please tell us how you got interested in Surface Plasmon Resonance Technology?

Yes, G Healthcare have been associated with BIACORE as a company for a very long time. in the year 2006, we actually acquired BIACORE, and up until that point BIACORE was

there only in some places in India. Ah, so I got into BIACORE in 2006 sheerly out of interest in chemical interactions in interaction analysis. And ever since I have been just working on BIACORE and the label free interactions analysis technologies.

So, can you mention currently what are the major applications of SPR in the area of proteomics?

Ah actually very wide ranging applications of SPR. starting from simple binding analysis or kinetic analysis, analysis of affinity of interactions whether it is protein interactions, protein DNA interactions, protein RNA interactions, protein 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 you can see probably that SPR application will be very broad.

Yes.

Almost in all the proteomics laboratory.

Absolutely.

Depending upon their experiments and questions they want to ask. Yes. So BIACORE is one of the pioneer in the field of studying the label free interactions, and studying about molecule interactions. Can you tell some of the latest advancement, what are the major applications using the BIACORE Technology.

Let me start with a video. Okay. That shows basically what SPR is. Sure. And then probably followed by another video of how biological analysis happens on BIACORE, and then the few videos short ones on how BIACORE works, and then I quickly come to the applications of BIACORE.

Okay, let us begin.

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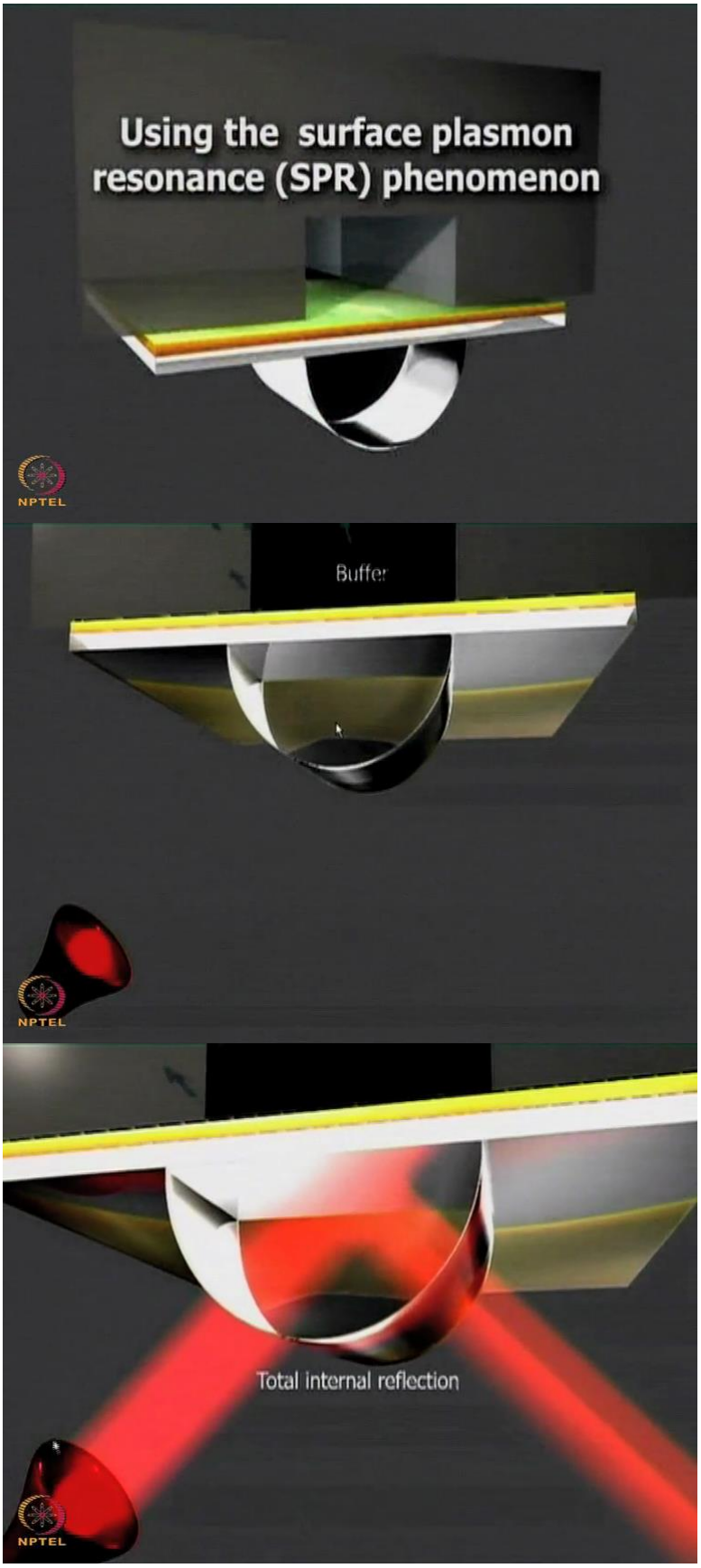

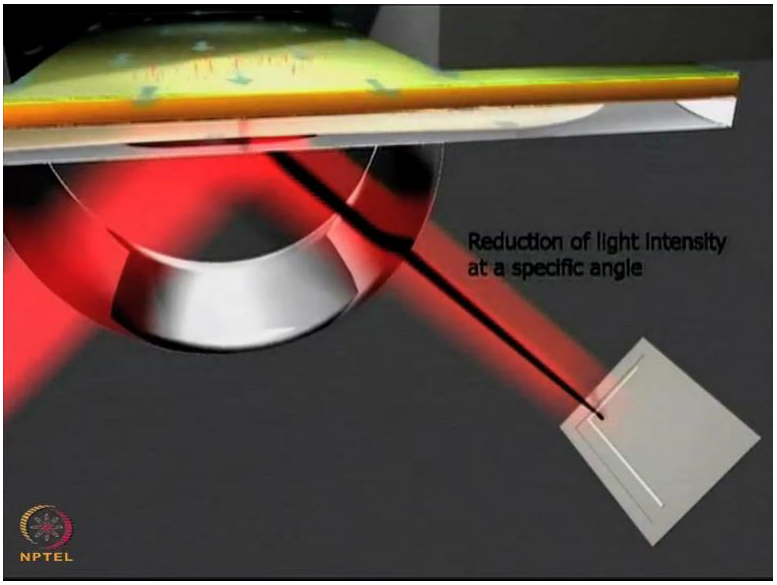

Let me show you the first video. So what I show here is basic SPR Phenomenon, right. Ah, this is SPR chip which we you see here. on the top of this SPR chip is a gold layer, and on top of it is a flow cell. At the bottom you see hemispherical prism. So SPR phenomenon is pretty simple that when you actually have the prism and you shine the laser light vision as you will see shortly. When you shine the laser light through the prism, light reflects at the total angle, angle of total internal reflection.

Ah 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 wave also called Surface Plasmons, and this Surface Plasmons are the ones which are used to actually study biological interaction.

When I go to video 2 now, you will see how biological interactions are studying. Let us assume that you have an interaction A plus B gives AB. What you do in SPR is you take one of the interactants say B, and put it on the chip, and pass A over it. Let me show you how it happens. You take one of the interactions which is B in this case, and you actually immobilize on the chip. You can see molecules getting mobilized right now, and when the molecules get immobilized there is an increase in mass which changes the reflective index, and that is measured in real time.

Now you pass the second interaction A over it, if binding happens and AB gets formed you see a further increase in mass which is again measured in real time. You stop the flow of A, and start flowing a buffer, it comes off in a dissociation, and dissociation is also seen in real time.

So essentially what you are doing in BIACORE is actually just measuring the amount of mass on the surface of chip. The mass on the surface increases or decreases, and that increase or decrease is measured in real time. This phenomenon is what we are applying now to study biological interactions. Actually BIACORE is BIA for biological interaction analysis.

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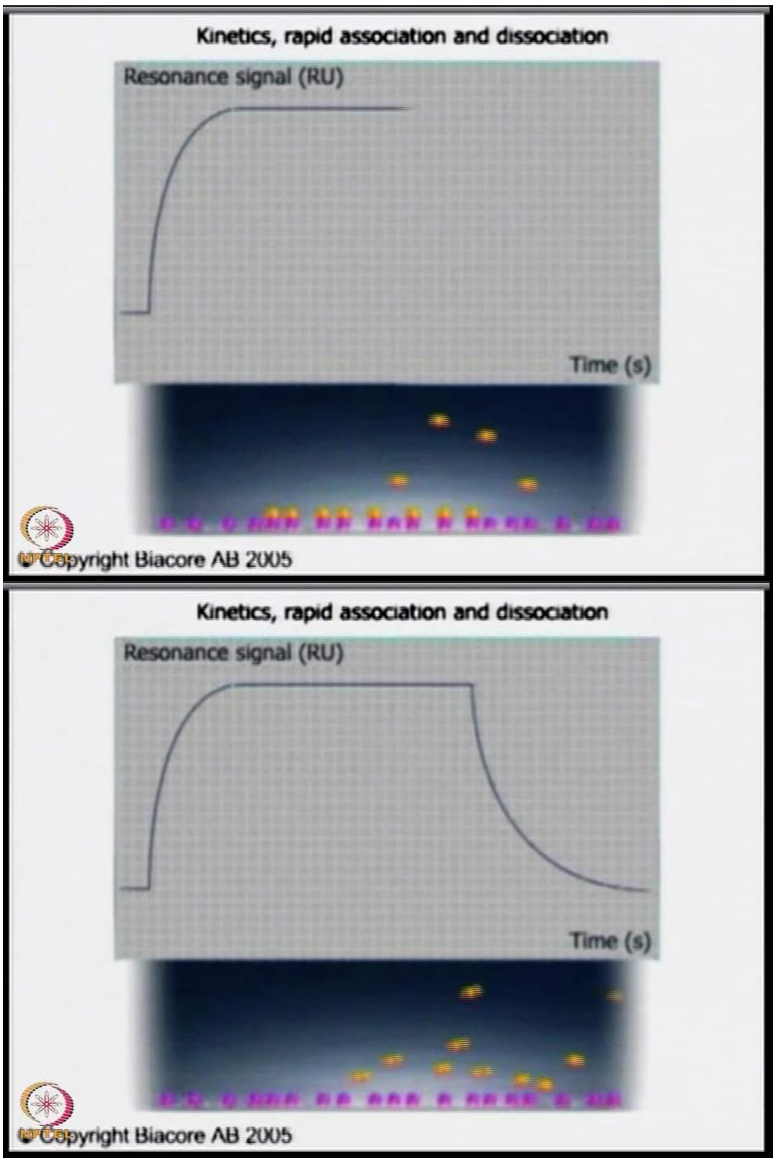

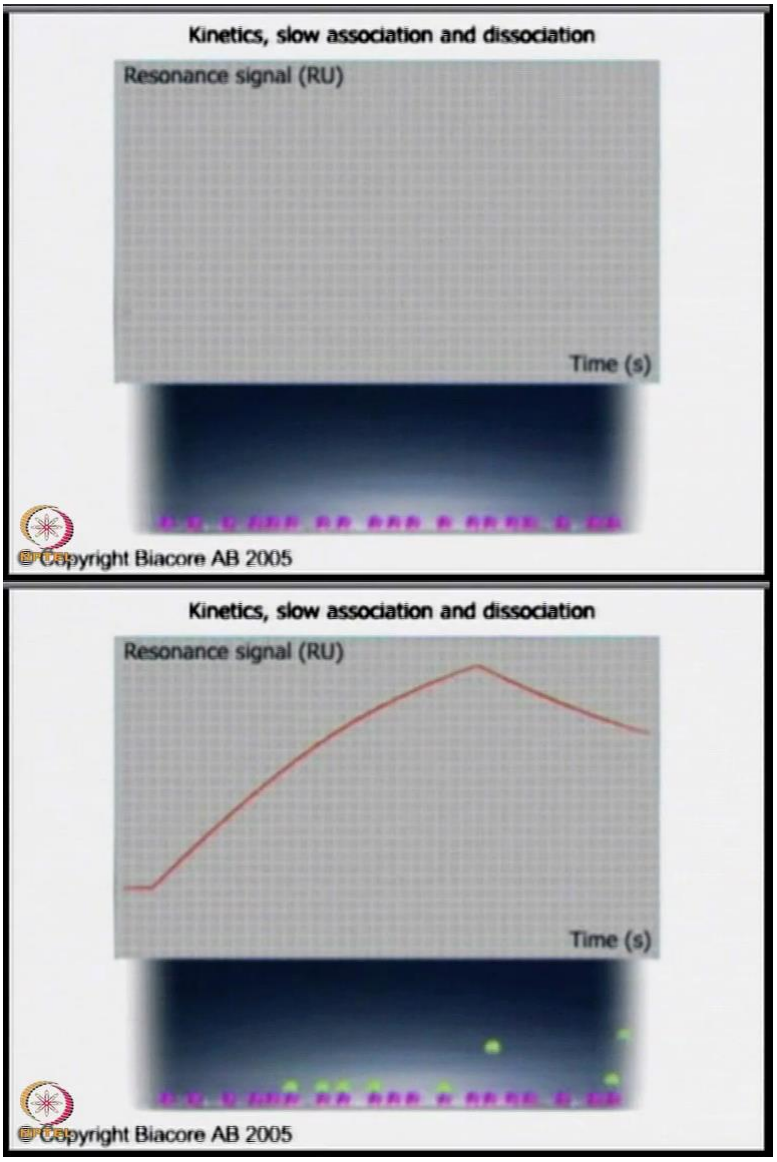

So, let me show you few videos examples of how BIACORE can be used to study some experiments. This is an example where A plus B gives AB. If you see the curve, and if the curve is existing it means AB is formed. If you pass A over B, and there is no response which means if you see a black line, then AB is not found. So, this is 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 curve goes up very fast, and comes down extremely fast. This is a rapid association, and dissociation. Whereas if you see the slope of this curve 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.

Right. So I think unlike microarray where people can detect the interaction but they cannot tell the nature of the interaction. Here the edge is that by looking at the slip of kinetic in the curve you can tell the type of association, association of kinetic analysis.

Yes. And here I have on my ppt a very simple analysis of different things that can be done with Biacore. So when someone ask me what can be done with Biacore, these are the six things that can be done with Biacore. So very shortly put these are the six applications of Biacore.

First whenever A plus B forms AB, when you are studying interaction A plus B gives AB, the questions you ask is A, first question you ask is does the interaction happen or not. Which means is the molecule AB formed or not. The second question you ask is how fast is the interaction, or how slow is the dissociation. The third question that you ask is how strong is the interaction, what is the affinity of the interaction. The fourth question you ask is how much of the analyte is there, which means what is the concentration of this analyte. Sometimes in drug industry you ask, is this interaction the safe interaction or not, and sometime if you have heterogeneous analyte, and you see that the binding is happening, you want to ask what is it that is binding. Because there are too many components in this analyte, so what is it that is specifically binding. And these are the five different things that you can do with Biacore.

I think you have rightly mentioned identifying the very specific interactor is the most challenging aspect of it. Yes. Because that many times people fail, and they discover the fast interactors. Yes. I think that is where SPR has edge over conventional techniques like precipitation or Yeast 2 hybrids and some of the other large screening methods where there is a good chance of identifying the fast positives. Sure, there is a lots of promiscuous binders in screening experiments. Right. Which will be avoided if we have specificity that Biacore gives.

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

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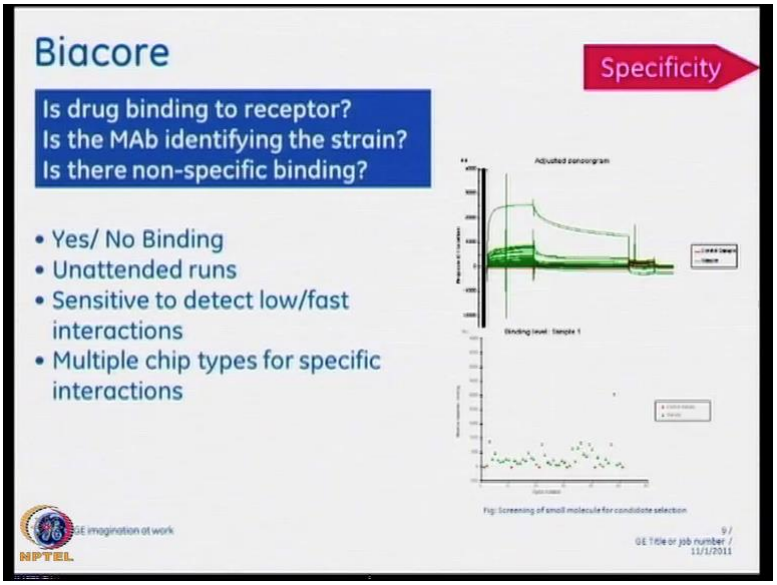

Shown here in my PPT is actually an example where we are looking for binding, and here someone came to us with 40 different compounds, and if they want to see any of the compounds bind to the receptor, and here we have 40 of these experiments done. Most of these are done binding at a very base level, but if you look at this presentation, I have highlighted one spot here with one molecule that shown circled in red.

This particular molecule is actually binding to the receptor. So at the end of 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…

Sorry to interrupt you here, so basically this just demonstrate that even if we are very much unaware about the components which could be interacting, this could be good screening tool. Absolutely. if there is a real strong interaction and specific interaction, then we can probably see some of the (())(13:11) like this.

Absolutely, if you want to do that first level screening then just quickly find binders. Right. in some kind of screening experiment like you have mentioned, then this would be very good starting point for you to quickly find those binders, and take them to the next level.

Right. Many times we have a thirst of identifying some large drug library, right. Yes. Or small compounds, and at that time may be to begin with. Absolutely. Yes, I would also like to

point out that this is very small experiment barely takes about one minute to do this experiment. So basically per minute you can do one screen, and that way you will have lot of components screened in a 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 this is a specific binder, and now you want to look at the kinetics of interaction, and everyone understands that kinetics is an extremely important part in drug discovery. In proteomics when you look at the interactions one of the important things that you need to look at is what is 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 are trying to be drugs, you should choose the candidate that is kinetically preferred. And in most cases in recent cases actually kinetics is being used to show similarity of drugs. For example there is a lot of Biosimilars coming out of India, and Biosimilars, Biosimilar manufacturers actually want to show that their molecule is similar or equal to the innovative drug, then one good way of showing that they are similar is by showing the similarity in kinetics.

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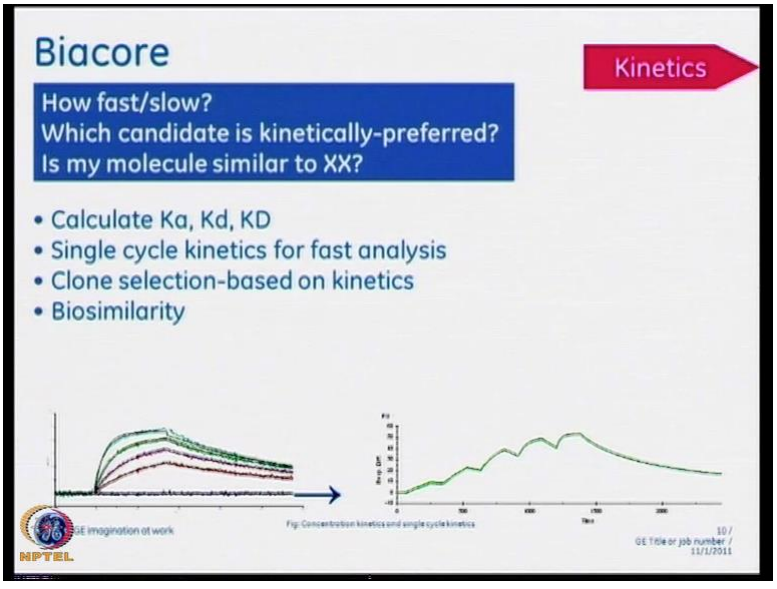

Right. So that is where again kinetics experiments will be helpful to you experimenters, scientists can actually calculate K on or K off or KAKD, K capital which is the affinity of the interaction. They can calculate this in a very fast way actually understand the interaction a little better than they 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 parameter of interaction are. And once they know the kinetics of the interaction, they come to affinity.

So identifying on rate off rate and association constant, I think this provides a very strong tool, and information. For characterization. For characterization, and this is like the big thirst of all the pharmaceutical doing this. Absolutely, anyone who is pursuing biopharmaceuticals or recombinant protein biosimilars , or even normal drug discovery people in the small molecule arena want to actually characterize the interaction in terms of the on rate or off rate affinity of the interaction, and that is something very well accomplished by the SPR technology.

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 day dose drug, or which are you know fast acting drugs, and these kind of discoveries depend a lot on kinetic and affinity of the interaction.

And that is where Biacore can actually come in a very big way and help people genetically protein engineer drug so that they actually perform better than the existing. For a good comparison with existing. Absolutely, to make drugs better or to discover normal drugs that actually act better, both the cases I think SPR Technology can be useful.

As we have learned today SPR Biosensors are optical sensors which are used to probe interactions between alanyte and receptor that are attached to the sensors surface. The binding of molecules in solution to the surface mobilized receptor changes the refractive index of the medium near the surface, and the change in refractive index medium can be monitored in real time to measure the affinity of the query molecule towards its receptors. And the association and dissociation of kinetics can be monitored.

We will continue our discussion on SPR in next lecture with our guest Mr. Lalit Kishore. Thank You.

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