Interactomics: Basics and Applications

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Lecture – 31
Basics of SPR: Surface Chemistry

We have been discussing about the basics of Surface Plasmon Resonance, especially the Surface Chemistry. I hope by now you are familiar with the principle of a SPR. In today's lecture, we will be discussing in depth about the SPR biosensors and surface chemistry. The phenomenon of Surface Plasmon Resonance allows monitoring of the bio-molecular interactions in a label-free manner. The technology has found its way into the practical applications which allows the real time measurement of binding affinities, kinetics as well as the quantitative analysis of substrate unlike in binding interactions.

The real time label-free a non-invasive nature of this technology has made it a key bio-sensing technology in the area of biological research and medical sciences. The SPR instrument comprises of the optical detection system, a micro fluidics and sensor chip surface. The bio-molecular interactions takes place at the sensor surface which plays a crucial role for the immobilization of bio-molecule and the quality of data retrieved.

Today we have invited Dr. Srinivas from GE healthcare to discuss in detail about the features of SPR sensor and their surface chemistry. He will also be discussing about the utility of sensors in studying bio-molecular interactions. So, let us welcome Dr. Srinivas.

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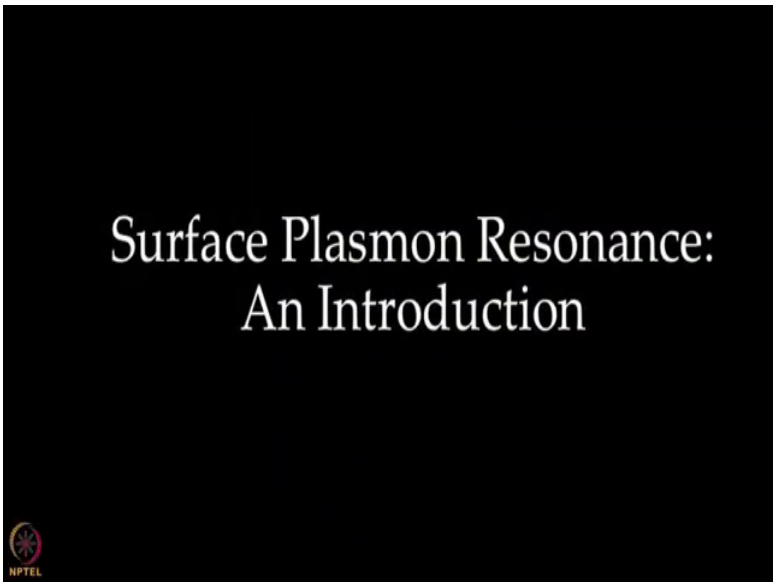

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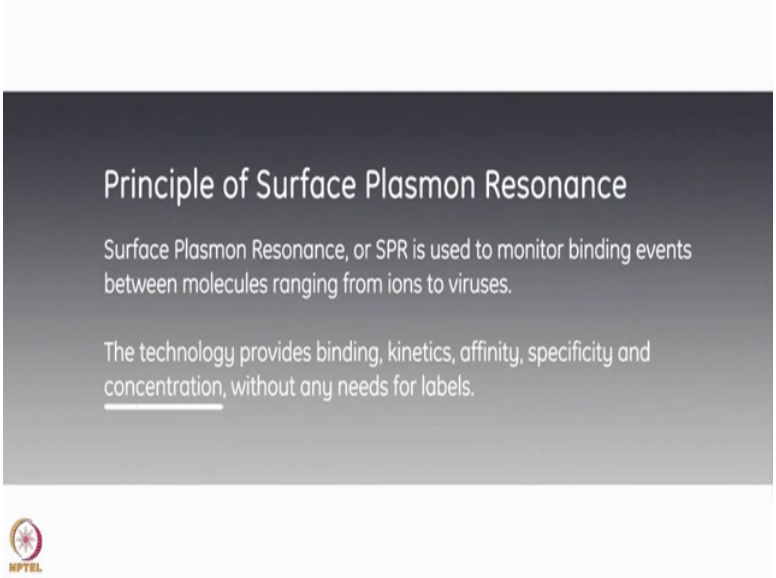

Surface Plasmon Resonance or SPR is used to monitor binding events between molecules ranging from ions to viruses. This techniques allows you to observe binding and measure kinetics, affinity, specificity and concentration without any need for labels.

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Biacore T200 is designed for ease of use and exceptional sensitivity.

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In Biacore systems molecular interactions or monitored under a movable sensor chip by the Surface Plasmon Resonance detector.

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Samples and reagents are held in removable racks and are delivered the chip by micro fluidic system that uses a very low volumes of sample down to a few micro liters.

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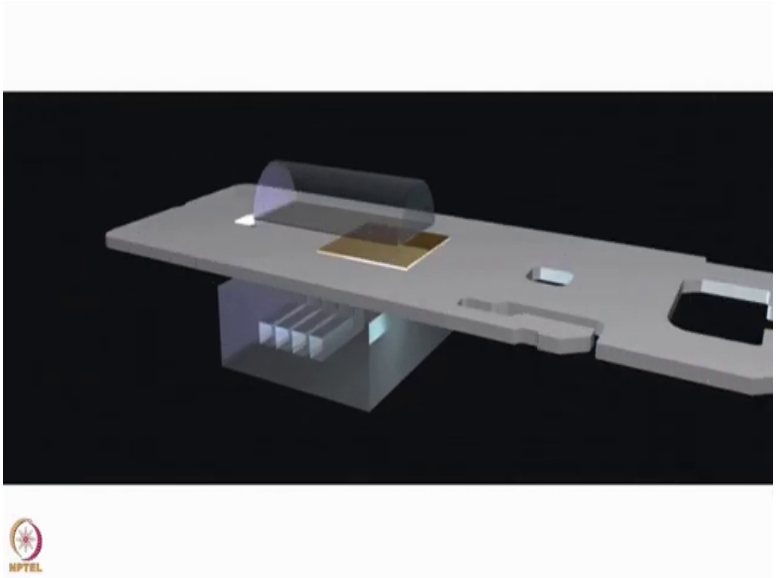

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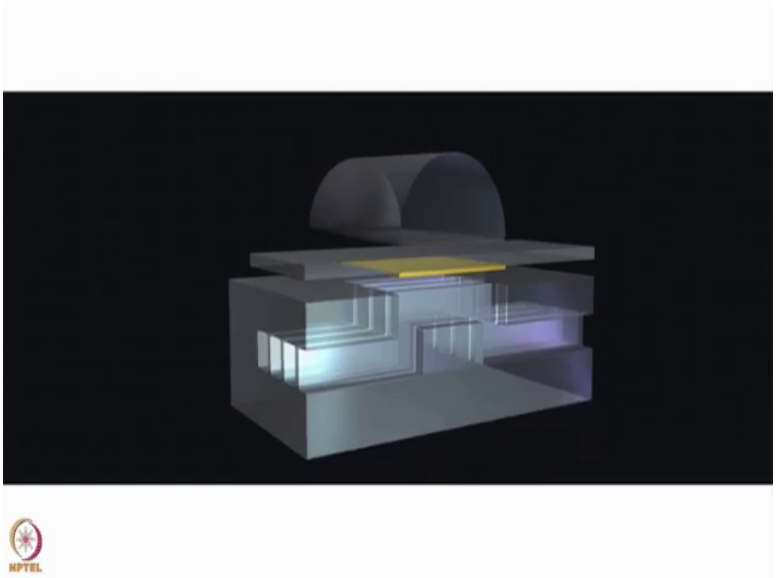

In addition, the micro fluidic systems supplies the sensor chip with buffer from the buffer bottles and delivers waste liquids to the waste bottle.

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The operation of the instrument and the data collection and evaluation is handled by intuitive software. Let us show you how it works.

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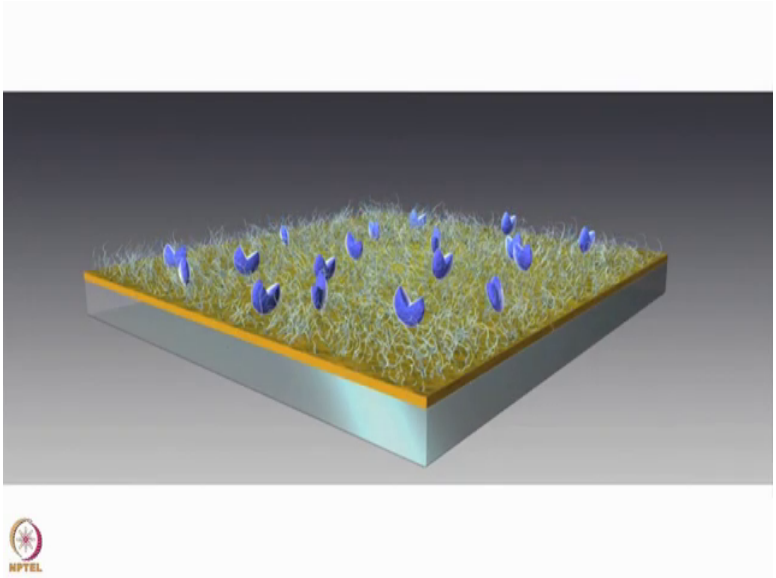

A glass slide coated with a thin gold film creates the sensor surface. For most applications a dextran may discovering the gold film acts as a substrate to which molecules can be attached and provides a hydro silicon environment for the interaction. Other mattresses can be used to attach specific types of molecules. The specificity of the surfaces is determined by the nature of the molecule attached to it.

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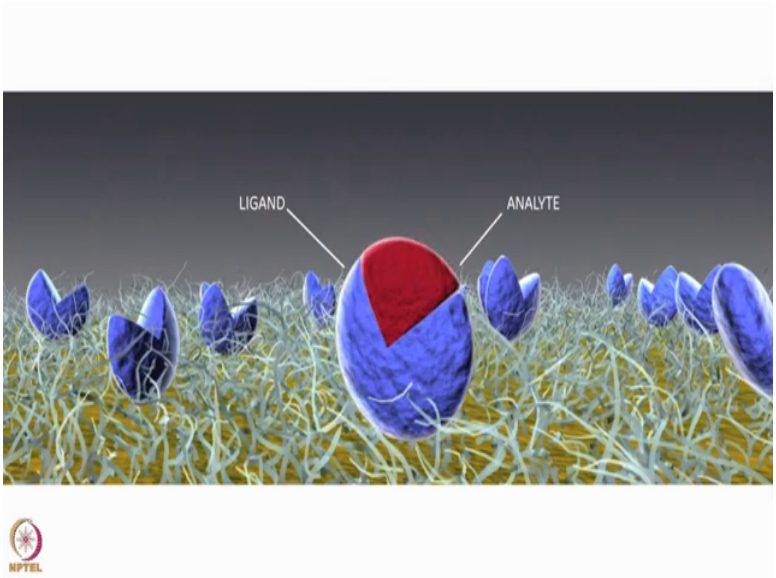

So, one binding partner is attached to the surface of a sensor chip and the other is injected in a continuous flow of solution. Whatever the nature of the molecules involved we called the attached interacting partner the ligand and the partner in solution to the analyte.

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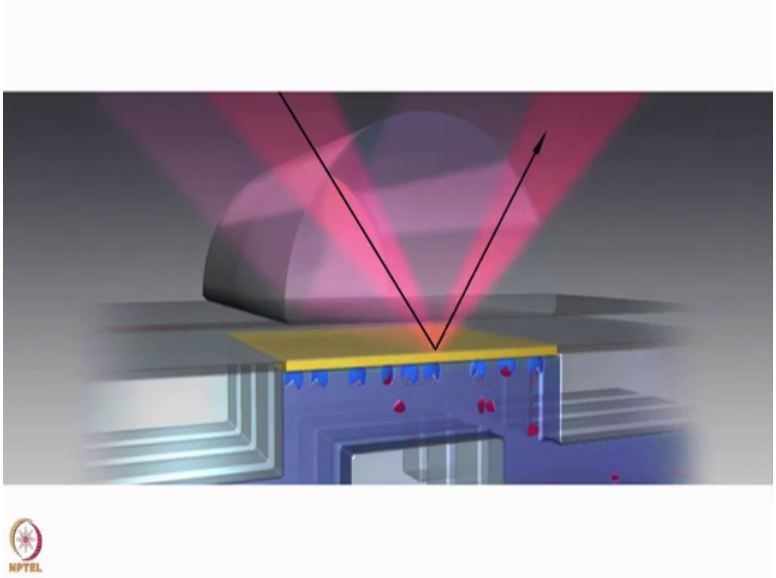

Biacore uses the phenomenon of Surface Plasmon Resonance to detect bio-molecular interactions as they happen. SPR causes a reduction and the intensity of life reflected at a specific angle from the glass side of the sensor surface. As molecules buying to the sensor surface the refractive index close to the surface changes, altering the angle of minimum reflected intensity. The change in SPR angle is proportional to the massive material bound.

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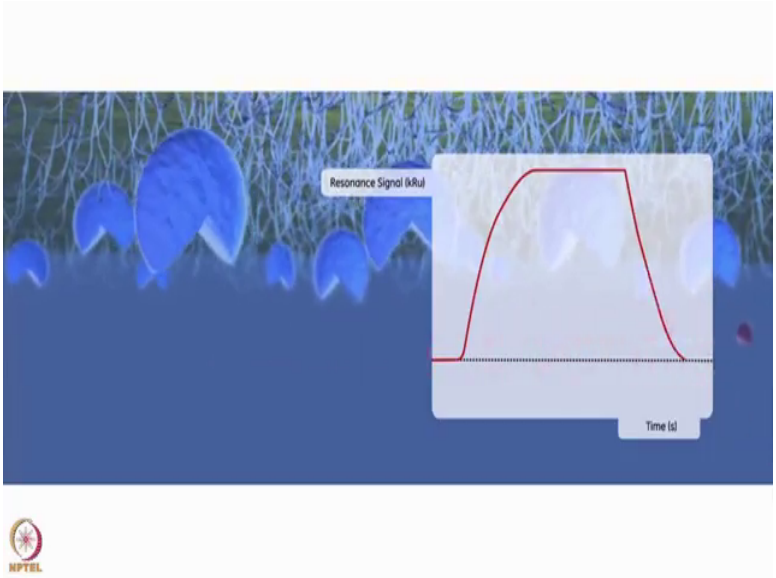

The sensor surface, the micro fluidic system and the SPR detection unit worked together to measure bimolecular interactions. The result from the detection of change in refractive index is displayed as a sensor ground with the binding response on a wide axis is plated against time in the x-axis. Since, light is not penetrate the sample analysis can be performed on colored turbid or opaque samples.

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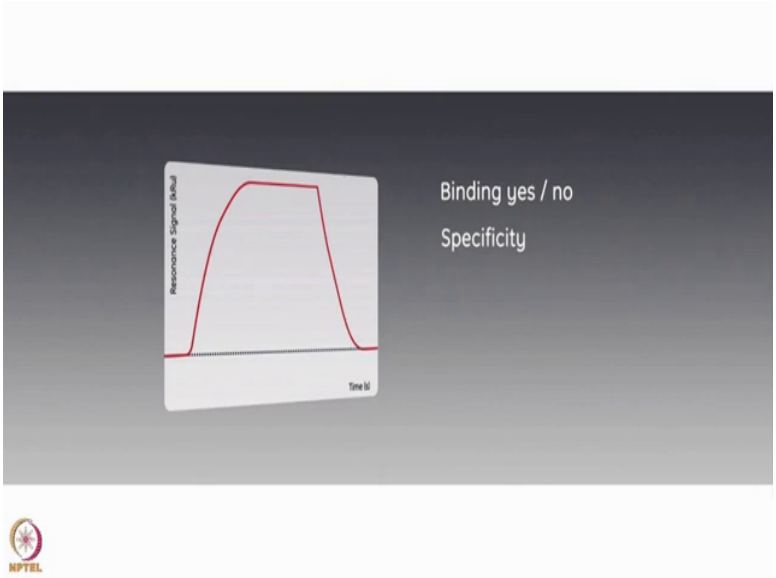

From studying the shape of the sensor ground produced binding yes or no specificity, affinity, kinetics and active binding concentration can be determined. The sensorgram provides real time information about the entire interaction.

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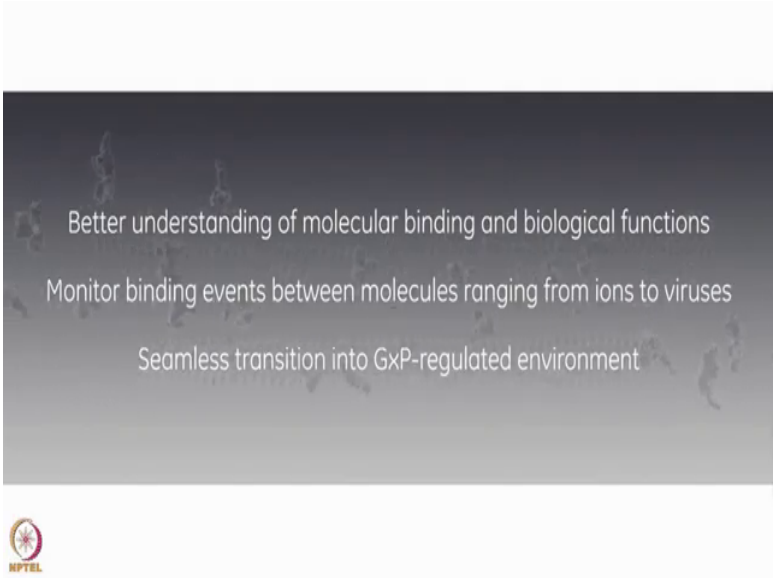

This means that in a single SPR experiment you have never obtained a worth of information about your binding which helps you understand the dynamics of the interaction over to quantify your analyte and all of this without using labels.

Before we start with other sessions, I think we will revise little bit of introductory slides on the Surface Plasmon Resonance. Biacore or a Surface Plasmon Resonance systems are label-free technology this systems are generally used to monitor molecular binding events in real time.

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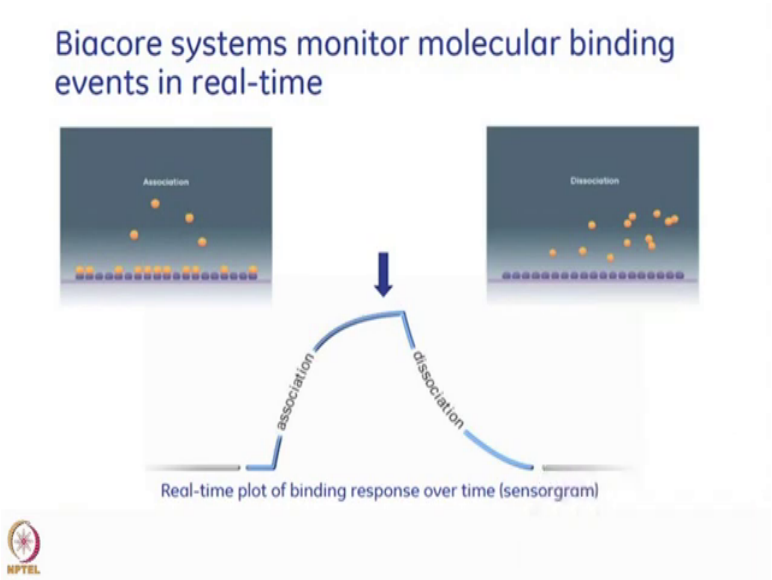

So, basically experiment starts with an immobilization of a ligand on the sensor surface and an analyte is passed over in a solution and which will bind to the ligand. So, here they are two different molecules, one of them is ligand and analyte and the interaction is recorded in a real time and the data that is output on the surface.

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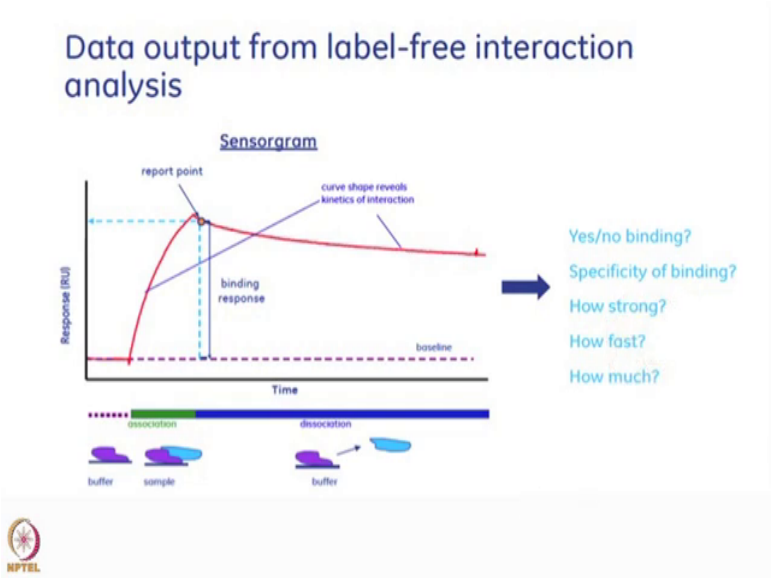

In a sensorgram, you see the binding events which is also called as an association event and there is also a dissociation event, and once the association event is also called as on rate and the dissociation is also called as off rate. So, when you do one on the other the association rate divided by the dissociation rate gives you the equilibrium KD or a capital K capital D. So, this is the output that we get from a Biacore experiment

The sensorgram that is generated from the Biacore or a Surface Plasmon Resonance experiments tend to give us different informations. So, they tend to tell us whether there is binding. So, there will be yes no binding. And once there is binding we will come to know if there is a specific binding or a nonspecific binding.

Once we have determined the binding then we will understand how strong the binding is or how weak the binding is. So, once we have determined this part then probably we will be able

to understand how fast or how slow the molecules are interacting. And once we have come through this stage I think it is very easy for us also to determine what is exact concentration or how much amount of the analyte that is binding.

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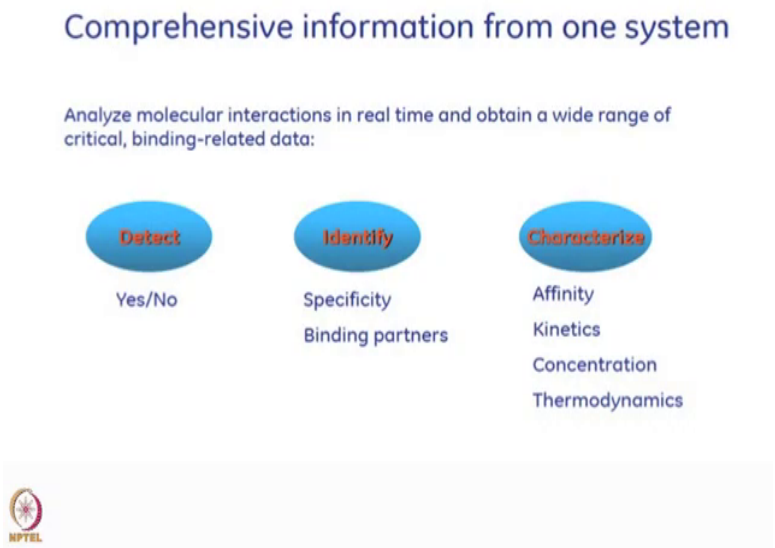

From these kind of an experimentation the comprehensive information that one will generate are detect; that means, yes or no binding, identify the binding partners, identify the specific binding and characterize the binding by different events like affinity which is how tight or how weak the binding is. Kinetics how fast or how slow the binding is. Concentration which is how much amount of our analyte is bound to the ligand. And thermodynamics how kinetic change is over a function of temperature. So, these are the different comprehensive information that is generated from Biacore experiments.

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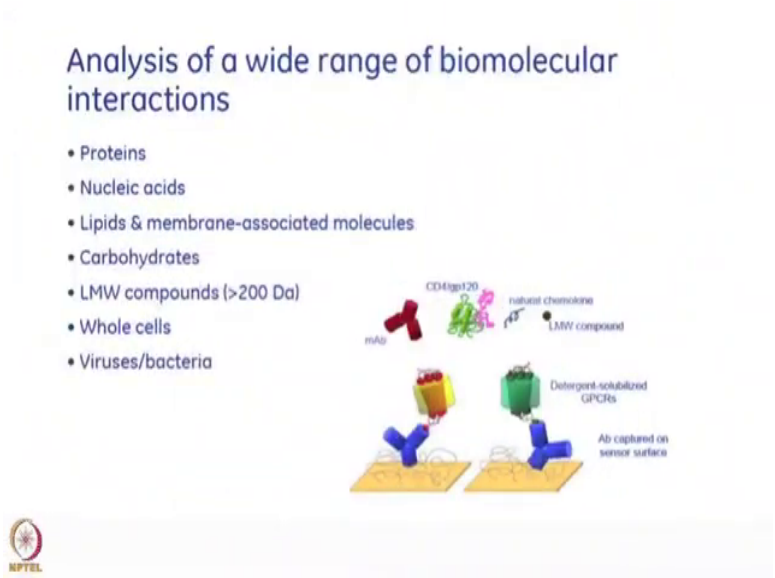

Surface Plasmon resonance will help us to understand a wide range of bio-molecular interactions. Be it be proteins, nucleic acids, lipids, membrane associated molecules, carbohydrates, low molecular weight compounds, the molecular weight of those compounds can be below 200 Daltons, whole cells, viruses, bacteria all of these molecular interaction process can be understood very well using Surface Plasmon Resonance experiments.

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So, the corner stones of any SPR system or a Biacore system is a detection system and a chip system, a micro fluidic system and the software which integrates all of these and generates data. So, in our next sessions and next couple of other sessions we will anyway understand details about sensor chips and the different sensor chips available that the SPR detection system depends upon the in refractive index, and also depend upon the surface concentration and temperature.

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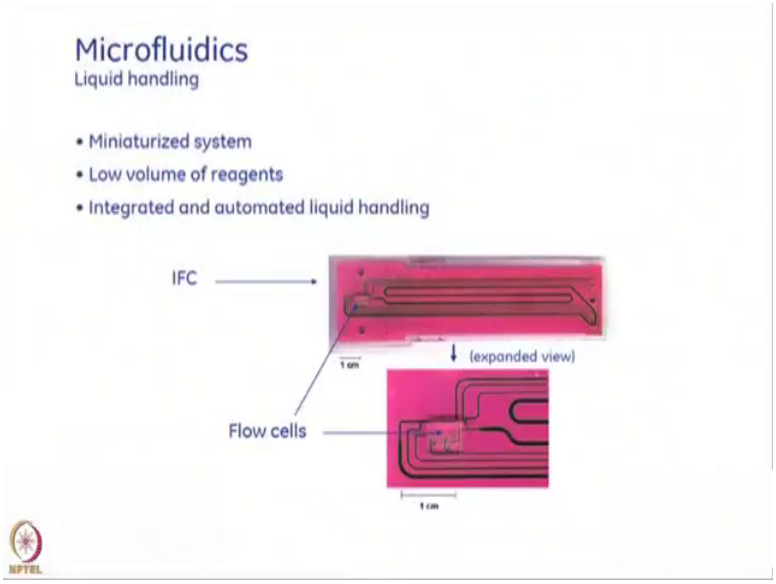

The interactions happen in a miniaturized system called micro fluidics which are the liquid handling part of the system, which contains very low volume of reagents and they are integrated and automated liquid handling systems where the flow cells are actually situated and the actual interaction happens and recorded by the system.

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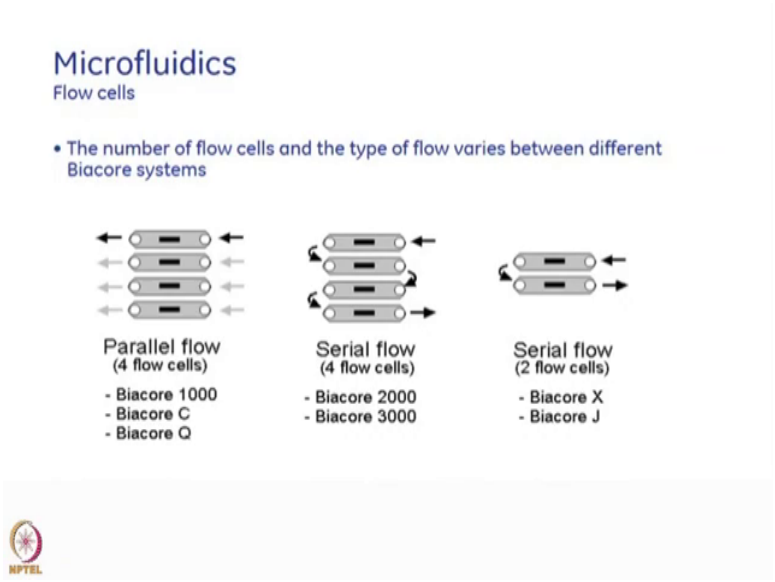

There could be many ways of orientation of the micro fluidics and the flow cells. It could be a parallel way, a serial way or a two cells at a time and different systems can have a different way of the fluidics and also different orientations.

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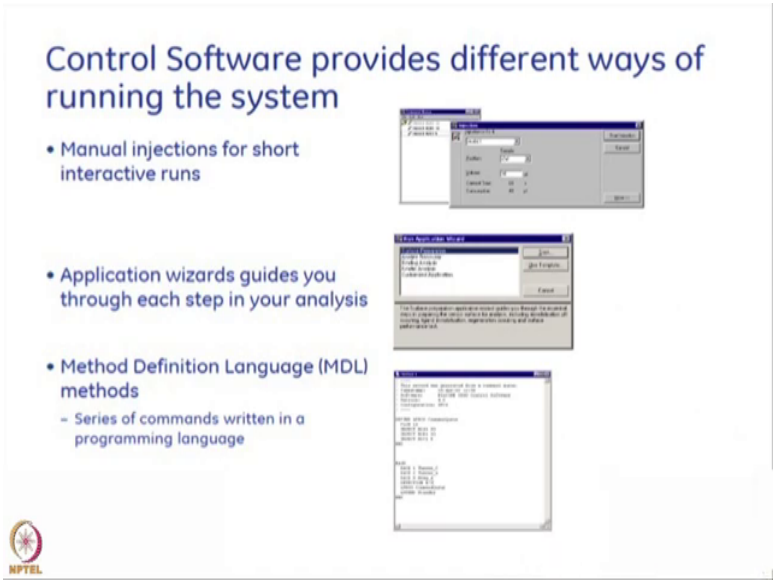

Control software provides different ways of running the system. So, there are events in which we could observe them manually. There are application wizards where we can set up experiments very easily or we could write our own method and record an experiment.

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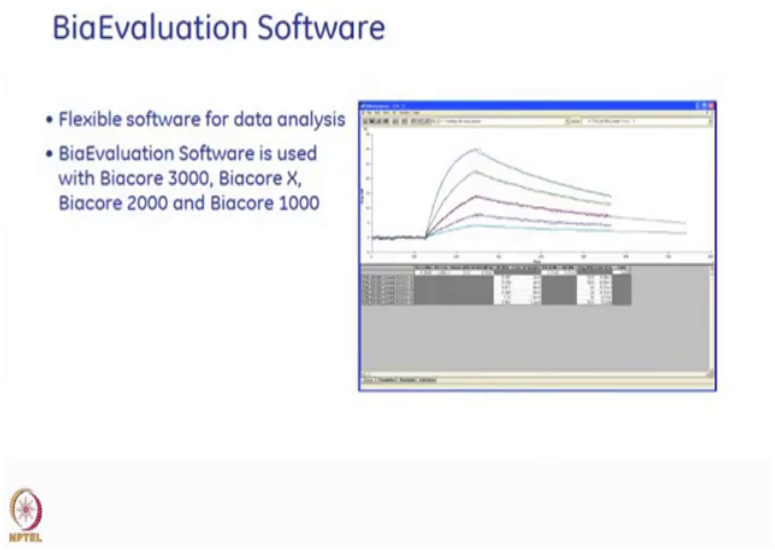

Apart from that there is also a evaluation software that evaluates the data and that data evaluation happens by use of algorithms which fit the data and will give out lot of other parameters for any kinetic event.

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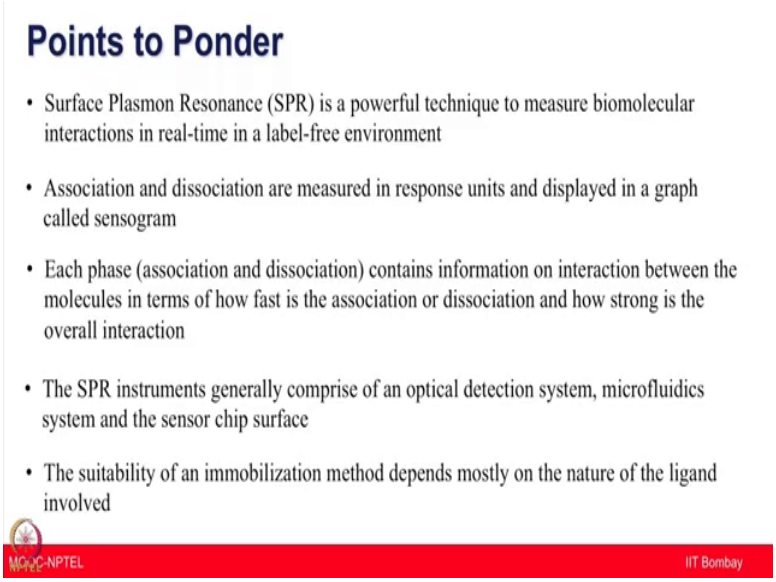

In this session, we will understand the different kind of sensor surfaces and the use of them in immobilization of a ligand.

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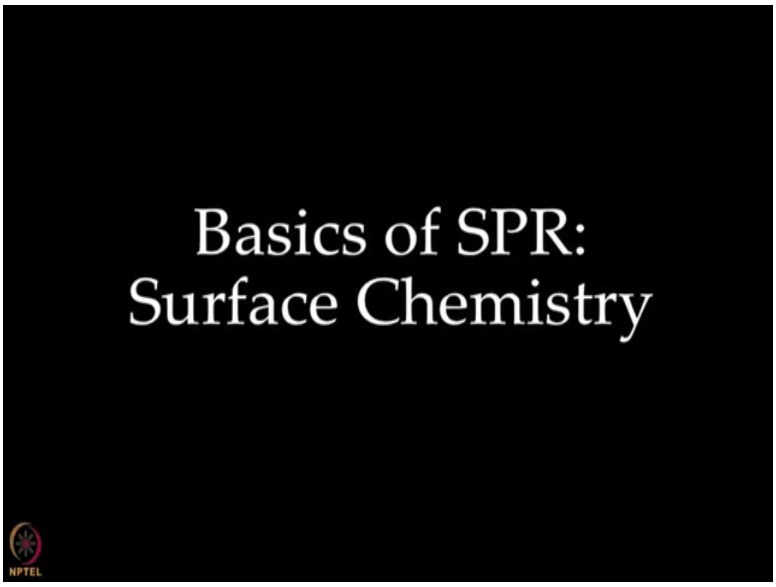

If we know from the basics and from other sessions we have understand about the surface preparation direct immobilization or by capture methods.

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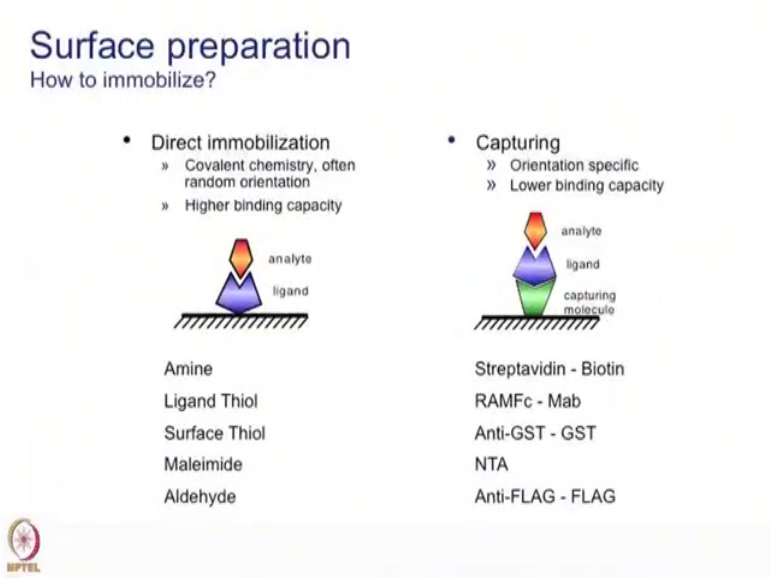

So, direct immobilization is an covalent immobilization which is a permanent immobilization and in this process we immobilized our ligand the choice and on a surface permanently. Other way of doing it would be a capture and so in order to give a different sensors available or different surfaces that are available. And we go into each one of them in little bit detail to understand what they are and what is the major utility of them and how do we design an essay based on these chips.

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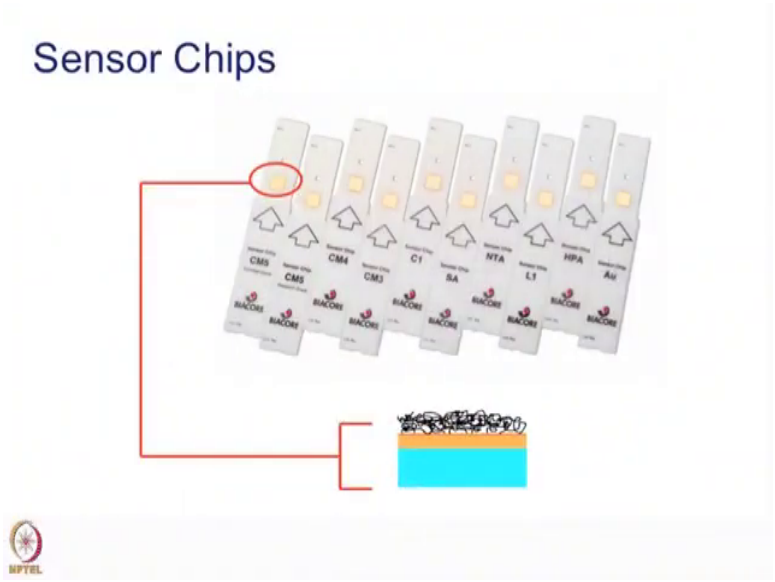

So, the various chips that are available for any SPR experiment to start with they are named as CM 5 chips, CM 4 chips, CM 3, C 1 streptavidin or an SA chip, NTA chip, L 1, HPA and Au or a plain gold surface chip. Each of this chips have a place for an adapter and this adapter actually holds the gold and that gold applies is where the immobilization happens and the adapter actually pushes the gold surface in to the SPR system where actually immobilization happens.

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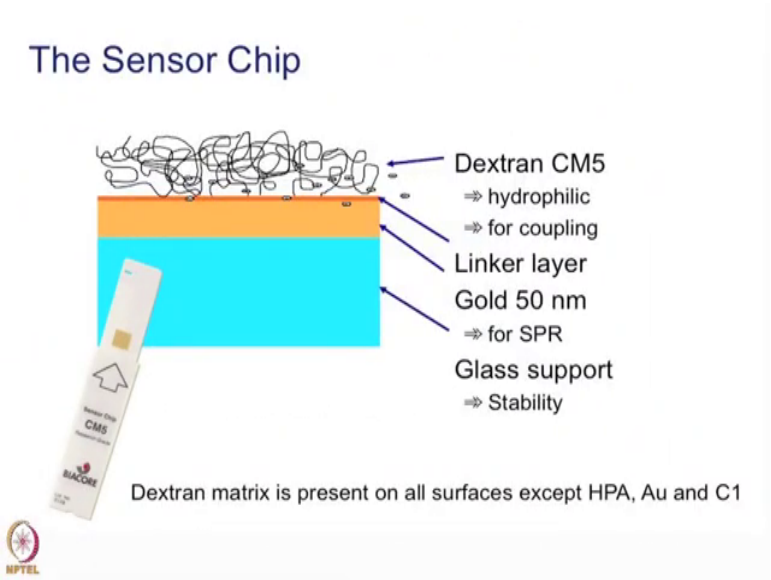

If you look at a particular gold surface on a chip, it contains a layer of dextran and that dextran provides or supports the interaction or immobilization of your ligand on the chip. The dextran is glued to the gold and the gold is present in form of a cassette and that cassette entirely is called as chip. So, looking into this gold surface the major component attached into the gold surface is the dextran and it is very important for us to understands why dextran.

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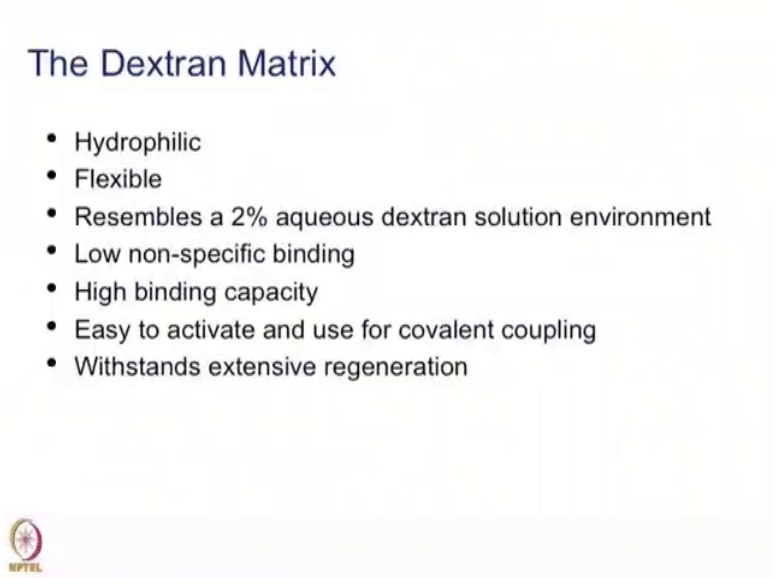

Because dextran is hydrophilic, dextran is flexible, dextran resembles a solution or a aqueous solution 2 percent aqueous solution, and it has a very low non-specific binding and a very high binding capacity, easy to activate or covalent coupling and withstand extensive regeneration.

Now, let us go into the available different chips. To start with we will do the first chip and a universally accepted used chip is a sensor chip CM 5.

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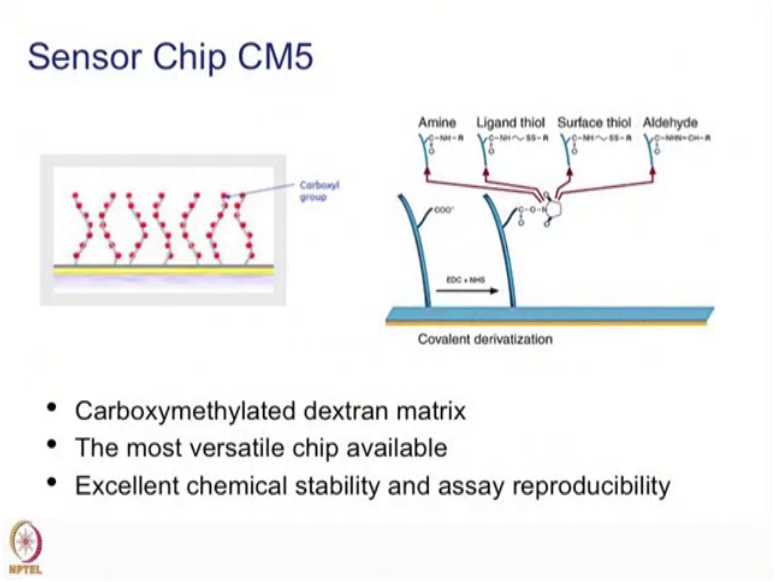

CM stands for carboxymethyl, 5 is certain number in which it shows the amount of branching. So, if you look at this particular slide, the chip actually has some kind of a glue or gold has a glue, the glue actually attaches the dextran to the gold and on the surface of the dextran you see those small areas where which are called carboxymethyl groups which are getting activated and the activation of the carboxymethyl groups helps us to immobilize our ligand by amine coupling or a thiol coupling or a aldehyde coupling.

The reactive sight things on the basic amino acids or amines are generally used to couple to the surface by amine coupling. Thiols are activated and then used to immobilize on the surface in a thiol coupling. Whereas, the carbohydrates that are attached on the glycoproteins are used to do an immobilization and that process is an aldehyde coupling. And as I just said CM 5 is a

very versatile chip, and any of this process or any of these methods can be used to immobilize on a CM 5 chip.

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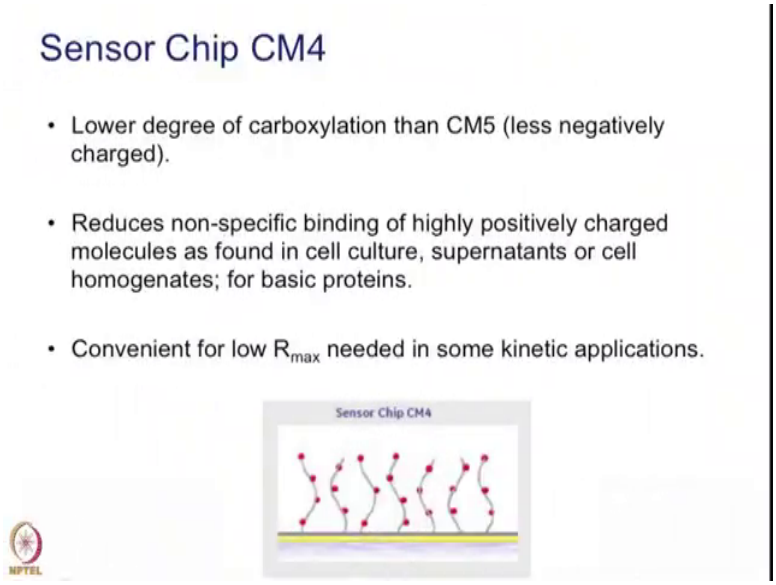

Coming to an another sensor chip called as CM 4. CM 4 has a little less branching and a low charge which is less negatively charged and this particular chips surface is good for low immobilizations or low R max immobilizations, especially cases where you are looking at interactions involving bio-similar

Coming to another chip surface which is called CM 3. Another way of branching of the sensor the carboxymethyl groups on the sensor, here again the matrix is less or little shorter than CM 4 also convenient for low immobilizations and generally good with cell or viruses immobilization or a multi-component complexes.

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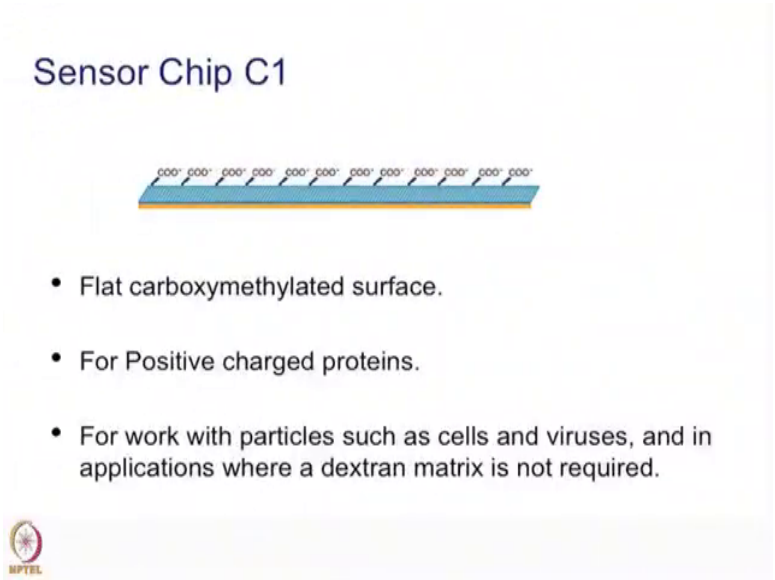

So, coming to an another sensor chip is a C 1 sensor chip. C 1 does not contain any dextran here and it is a flat bed of carboxymethylated surface. Very good with positively charged proteins and can be used again with cells and viruses, for a total immobilization of cell viruses and being used by many available in the literature for immobilization of bacterial cells and millennial cells and useful for studying interactions at cellular level.

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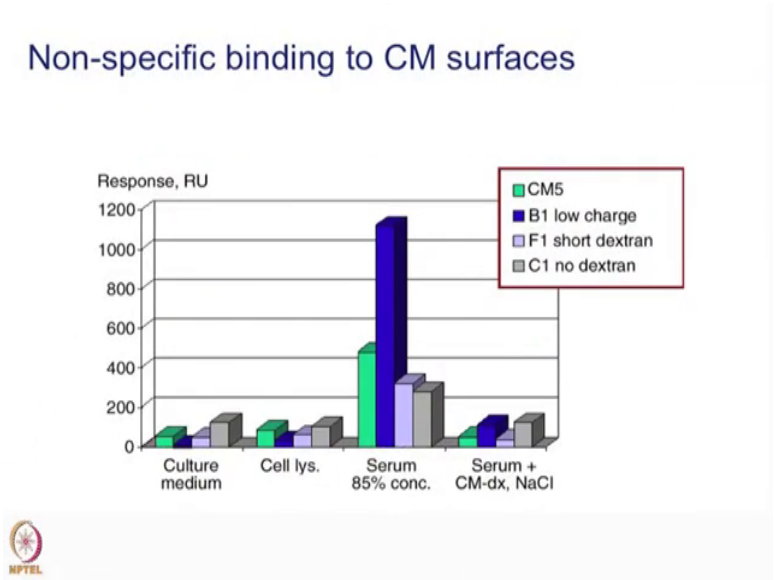

The CM surfaces are very good with non-specific binding which means they have a very little non-specific binding when they are used with culture mediums, when they are used with cell lysates, when they are actually used with serum in a diluted way and in an undiluted way. Also these days, people use these sensor chips for immobilization of cells bacteria and viruses and also for the passage of very complex analytes like blade.

So, another variety or of a chip surface is a streptavidin. Here this is also a CM 5 chip which is pre-immobilized with streptavidin molecules and these streptavidin molecules have a very high binding affinity to biotin and this streptavidin molecules bind to biotinylated proteins peptides, (Refer Time: 19:19) DNA, RNA and will help to immobilize these molecules in any interaction process.

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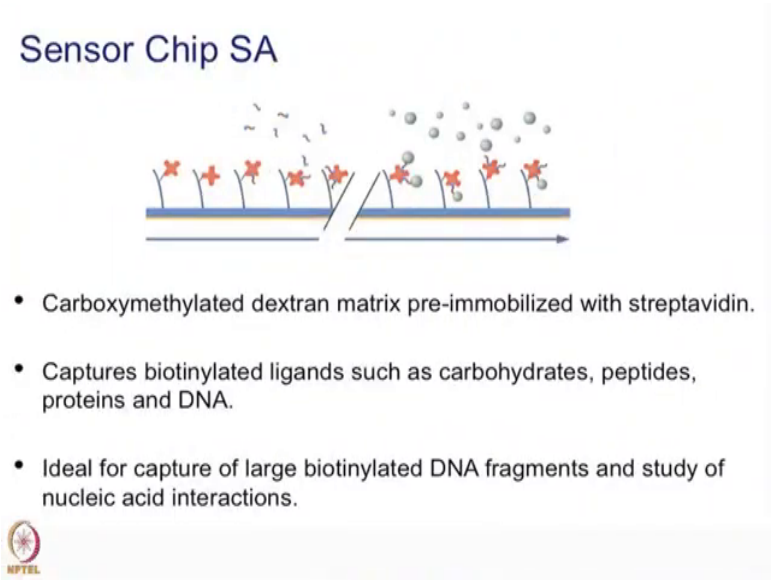

So, ideally literature is available for biotinylated DNA fragments, nucleic acid interactions and other things like peptides have been immobilized on this surface.

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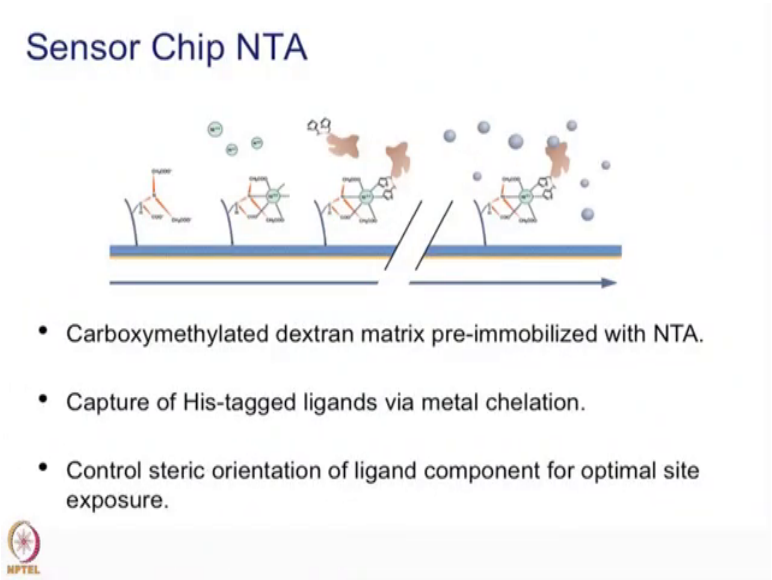

Another variety of chip or a different chips surface is NTA. And this is a complex molecule that is immobilized on the surface of the chip which is a pre-immobilized. And this molecule has an affinity for hexa histidine molecules or tags that are present either at the C terminal or N terminal of a protein.

So, proteins when they are recombinantly expressed with these tags have been used to be captured on NTA surfaces for interactions with other analytes either proteins or complex molecules. These histidine tagged molecules bind to NTA, when the NTA is charged with nickel solutions or copper solutions. And these molecules generally help to control the steric orientation of the ligand for optimal exposure of your ligand.

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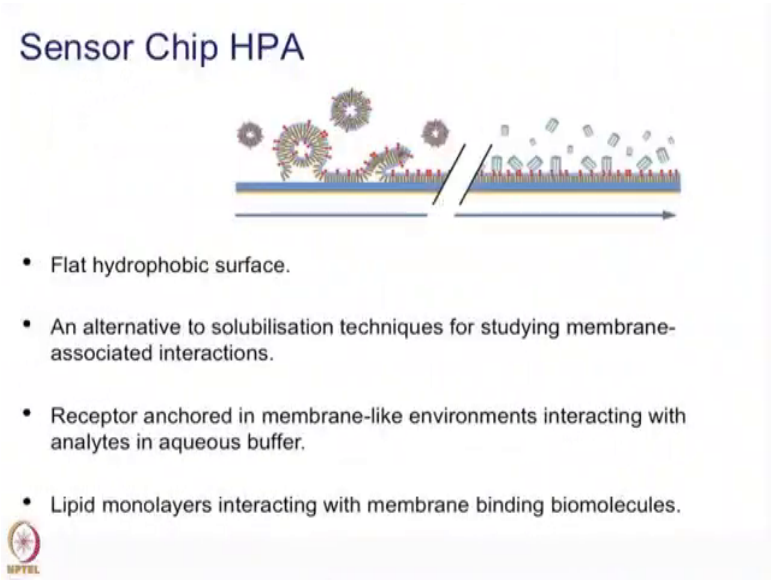

Another very important and a variety of chip surface is HPA surfaces. Are flat hydrophobic surfaces and these surfaces are useful for membrane associated interactions, receptors generally are anchored into a membrane like environments and then they are useful for interaction with analytes. Generally, the lipid monolayers interacting with membrane binding biomolecules kind of a studies are made on the surfaces.

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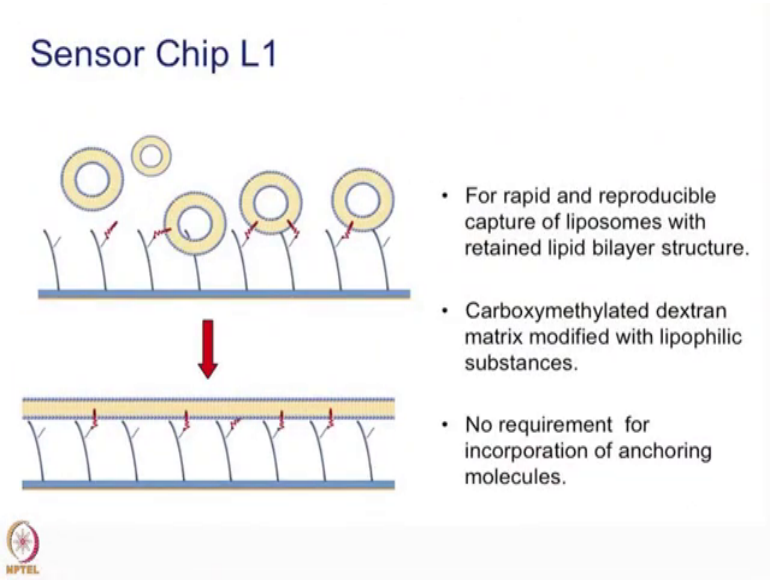

L 1, another surface sensor chip which is also popularly used for studying liposomes, lipid bilayer kind of a molecules and carboxymethylated dextran is modified with lipophilic substances.

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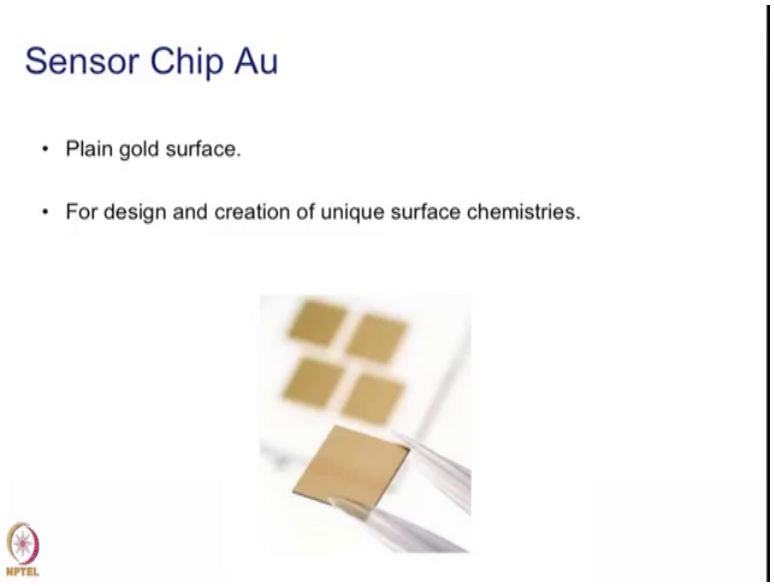

So, another surface is a plain gold surface, where there will not be any dextran and these surfaces are very good with chemists where they can do their own chemistries and useful for interaction. Nowadays, there is also something called the gold plain gold kits that are available and these kits have a portable gold surfaces. The portable gold surfaces can be taken over into a laboratory and you can do the required immobilization of the molecule and can be inserted into the chip cassette and then inserted into the Biacore system for studying the interaction.

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The summary of the different chips as we just went through CM 5, CM 4, CM 3, C 1, SA, NTA, HPA, L 1 are the different kinds of chips that are available for studying the different interactions. So, it is very important for us to identify what are the functional groups that are present on our ligand and how well we could immobilize our molecule on the different variety of a chip.

We are coming to end of this session. So, with this session we have a great understanding of the various chips and the various chemistries that are available. With this we will be able to identify whether we are going for a covalent coupling or a capture method. Once we immobilize our molecule we will go ahead with our regular Biacore experimentation and this particular session helps us to identify the right chip for our experiments.

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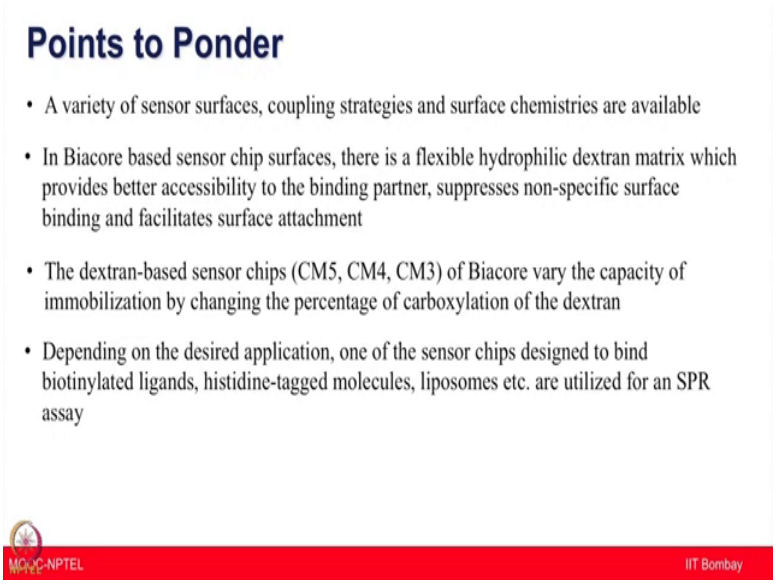

In this lecture, we have discussed about various types of chips available which could be utilized for bio-molecular interactions. Of course, which chip is most suited for which type of application that you have to try out based on some study, some readings, some references and sometime your own practical consideration by trying out different chips. In order to immobilize the ligand on to the sensor surface, it is important for us to identify the functional group present on the ligand and accordingly the sensor surface are selected.

Today's lecture has helped us in identification of the right chip that should be used for the SPR experiment based on desired application. As I mentioned sometime it is very context dependent, you may have to try out more than one chip surface depending on what is the best you want to achieve from that kind of experiment. And some time the binding does not happen

very well, so there you may have to try out different type of surface chemistries to try to achieve the best immobilization.

In the next lecture, we will discuss about the experimental design to perform SPR cells.

Thank you.