Interactomics: Protein Arrays and Label-Free Biosensors.
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Lecture-5.
Basics of SPR: Surface Chemistry.

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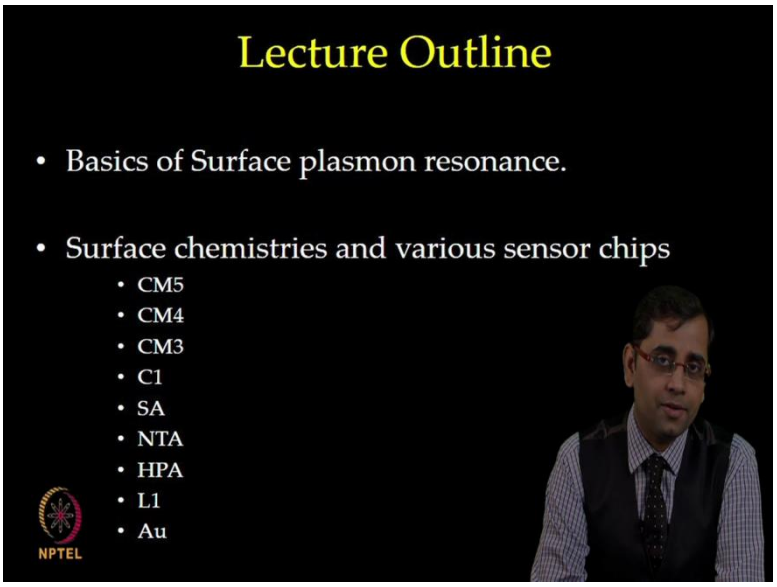

Welcome to MOOC NPTEL course on intractomics, today we are going to talk about basics of surface plasmon resonance SPR with focus on surface chemistry. The phenomenon of surface plasmon resonance has found its way into practical applications in sensitive detectors, capable of detecting bio-molecular interactions, performing thermodynamic studies as well as quantitative analysis of bio-molecules. It has become a key bio-sensing technology in the areas of biological research and medical sciences because of its application in real time label-free and non-invasive nature.

The SPR instrument is comprised of optical detection system, microfluidics and sensor chip surface. The bio-molecular interaction takes place at the sensor surface, which plays a crucial role in performance of the bio-sensor and the quality of data retrieved. In today's lecture we have invited Dr. Srinivas from GE Healthcare.

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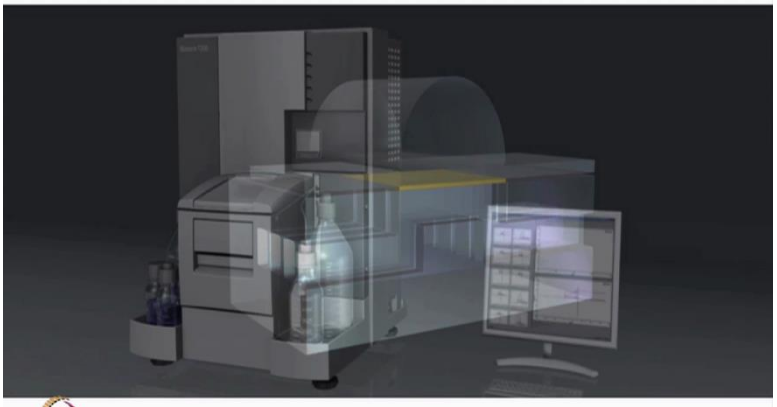

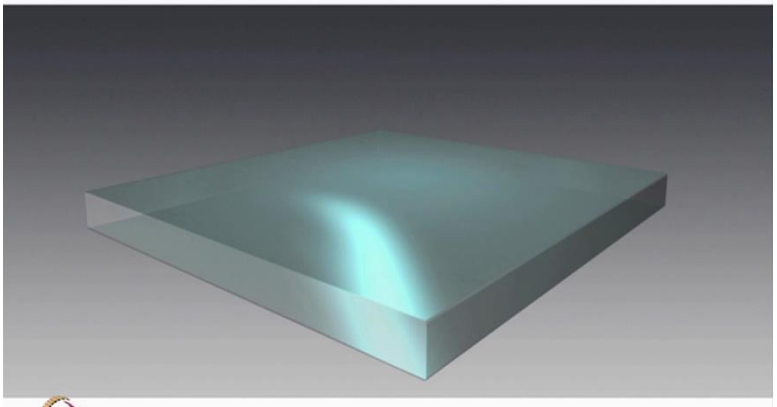

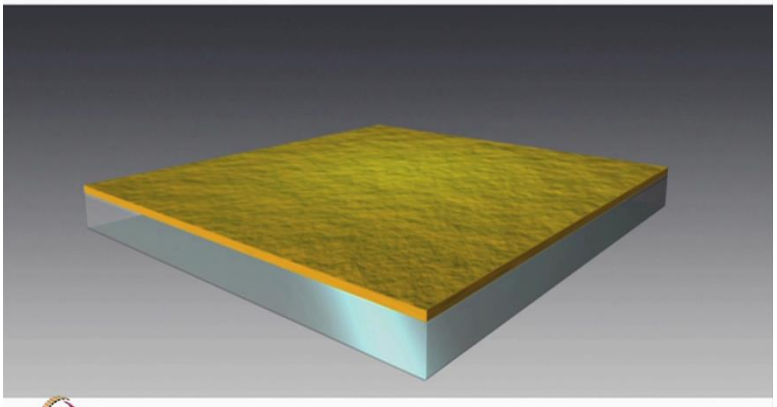

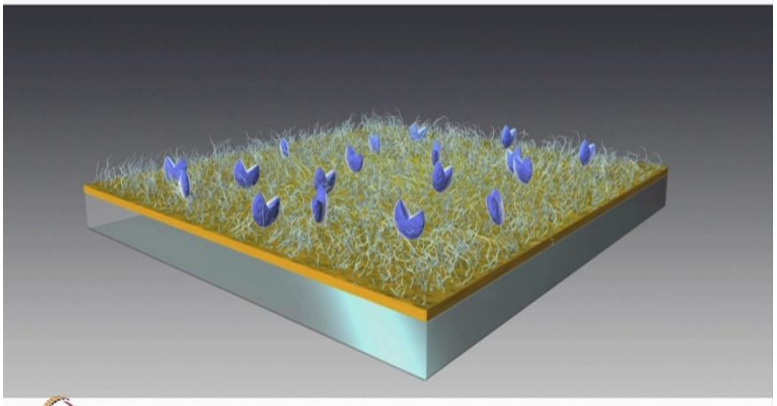

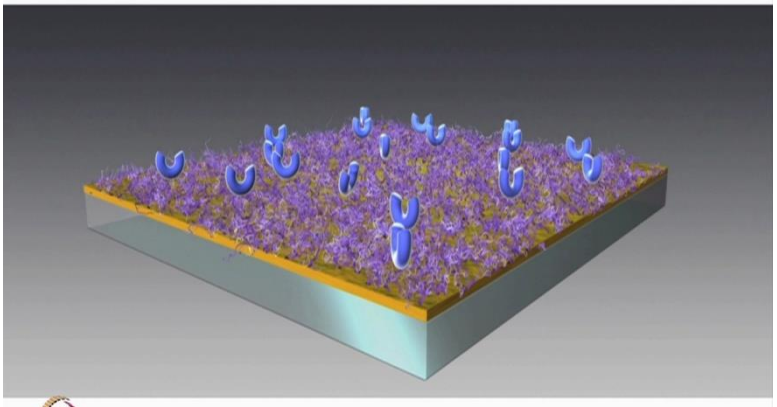

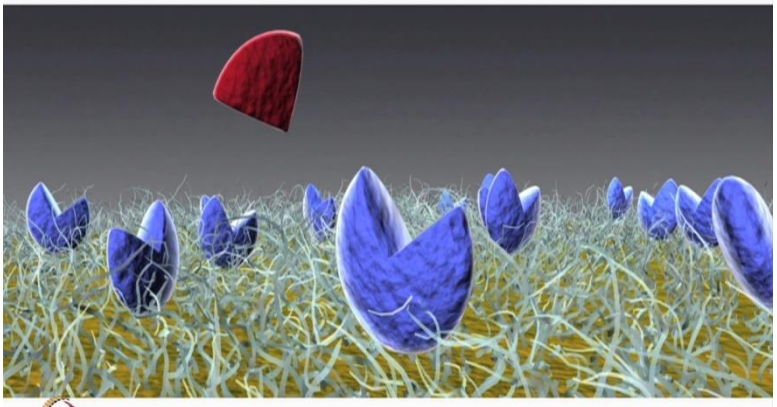

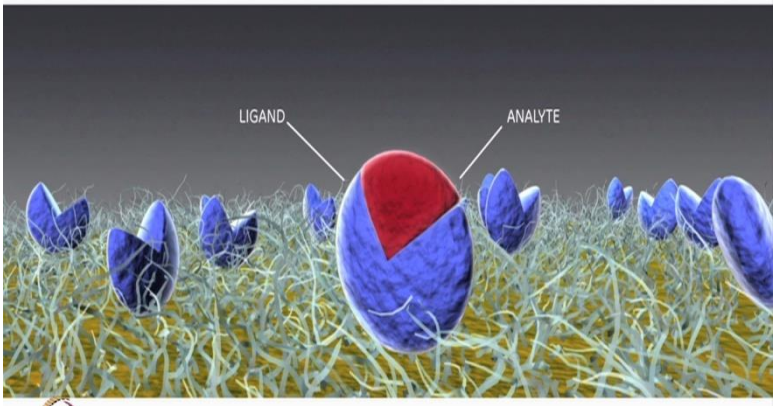
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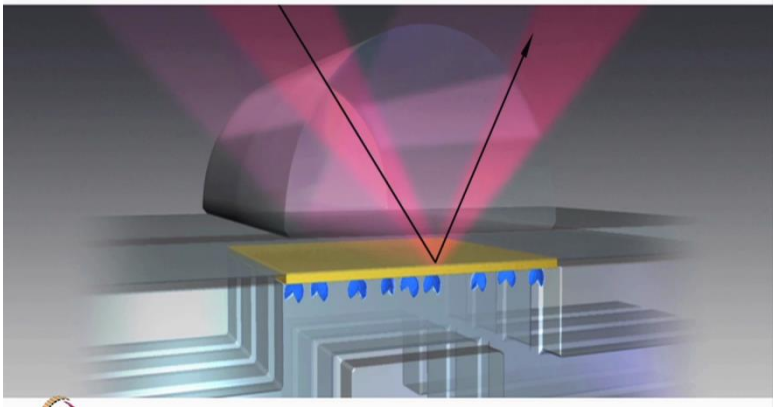

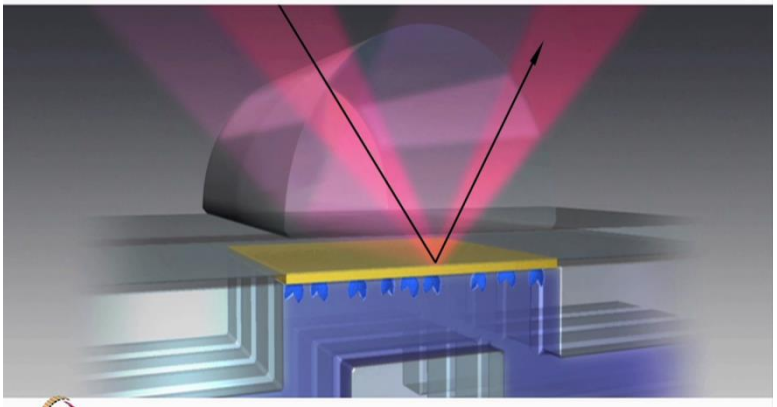

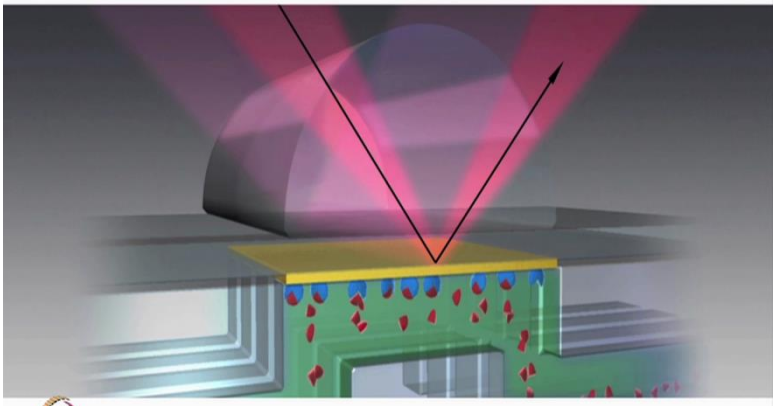

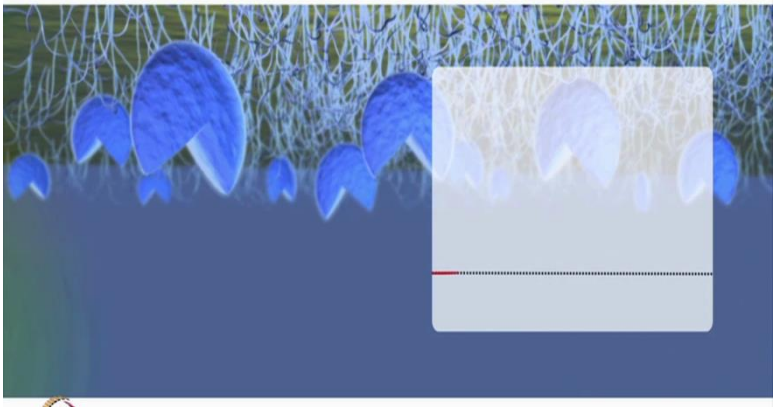

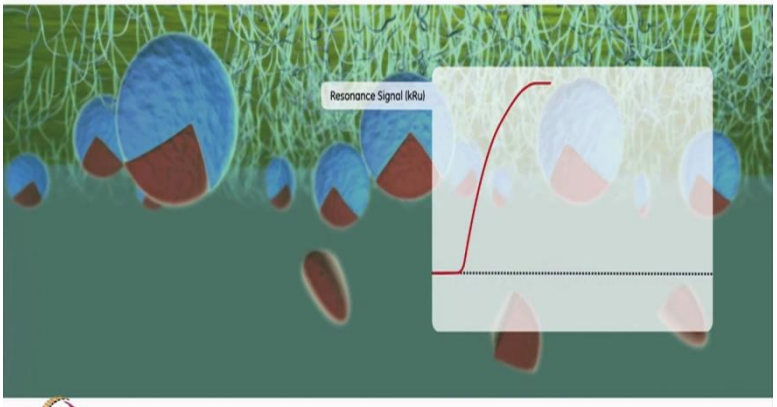
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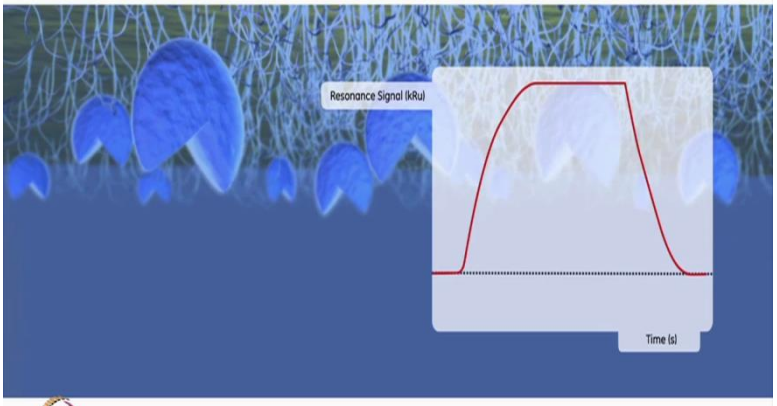
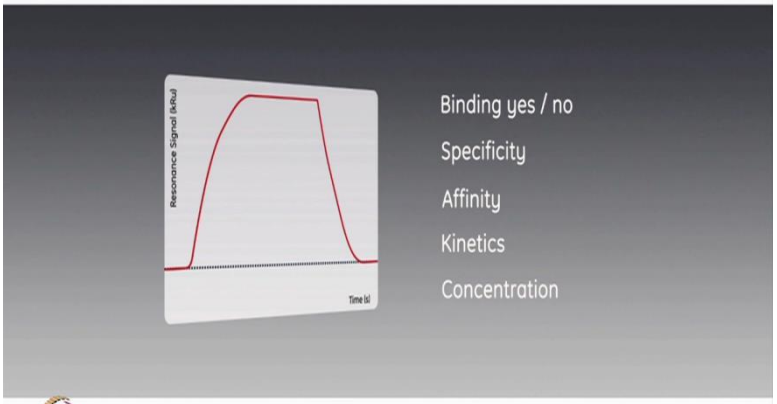
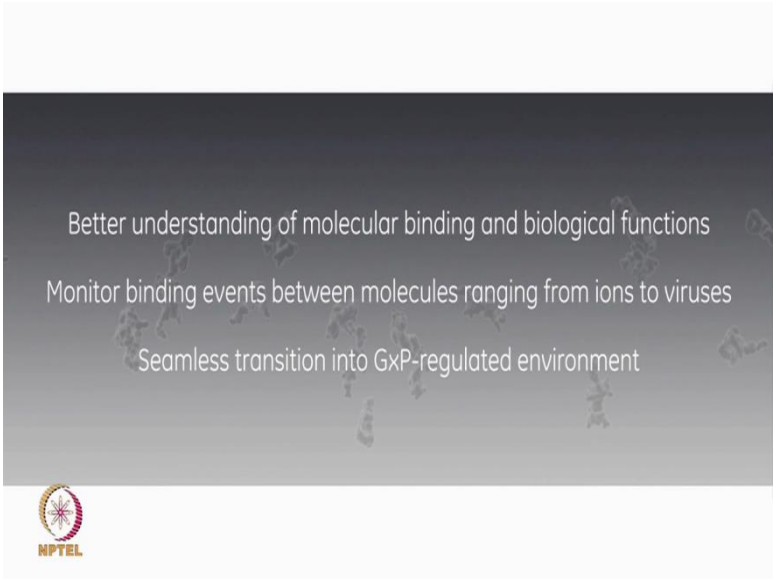

Resonance Signal (kRu)
Time (s)


Resonance Signal (kRu)
Time (s)
Binding yes / no
Specificity
Affinity
Kinetics
Concentration



Welcome Dr. Srinivas, in NPTEL MOOC course on intractomics. Dr. Srinivas will elaborate on some of the features of SPR sensor and their surface chemistries and he will talk to us, how they can be utilized in studying bio-molecular interactions. thank you for the invitation. Surface plasmon resonance or SPR is used to monitor binding events between molecules ranging from ions to viruses. This technique allows you to observe binding, and measure kinetics, affinity, specificity and concentration without any need for labels.

Biacore T200 is designed for ease of use and exceptional sensitivity. In biacore systems, lactulose interactions are monitored on removable sensor chip by the surface plasmon resonance detector. Samples and reagents are held in removable racks and are delivered to the chip via microfluidic system. It uses a very low volumes of samples, down to a few micrometers. In addition, the microfluidic systems applies the sensor chip with buffer from the buffer bottles and delivers its waste liquid to the waste bottles.

The operation of the instrument and the data collection and evaluation is handled by intuitive software. Let us show you, how it works? A glass slide coated with a same gold film, creates the sensor surface. For most applications a Dextranated covering gold film act as a substrate to which molecules can be attached and provides a hydrophilic environment to the interaction. Other mattresses say can be used to attach specific types of molecules.

The specificity of the surface is determined by the nature of the molecule attached to it. So, one binding partner is attached to the surface of the sensor chip and the other is injected in a continuous flow of solution, whatever the nature of the molecules involved we call the attached interacting partner the ligand and the partner in solution the analyte. Biacore uses the

phenomenon of surface plasmon resonance to detect the bio-molecular interactions as they happen.

SPR causes reduction in intensity of light reflected at a specific angle from the glass side of the sensor surface. As molecules bind to the sensor surface the refractive index close to the surface changes altering angle of minimum reflected intensity. The change in the SPR angle is proportional to the mass of material bound.

The sensor surface, the microfluidic system and the SPR detection you need ())(4:51) work together to measure bio-molecular interactions. The result from the detection of change in refractive index is displayed as a sensor gram with the binding response on the y-axis is plotted against time in the x-axis. Since light does not penetrate the sample analysis can be performed on colored, turbid or opaque samples. From studying the shape of the sensor gram produced binding yes or no, specificity, affinity, kinetics and the active binding concentration can be determined.

The sensor gram provides real time information about entire interaction. This means that in a single SPR experiment, you have now obtained a wealth of information about your binding, which helps you understand the dynamics of the interaction or 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 surface Plasmon resonance systems are label-free technology. These systems are generally used to monitor molecular binding events in real time.

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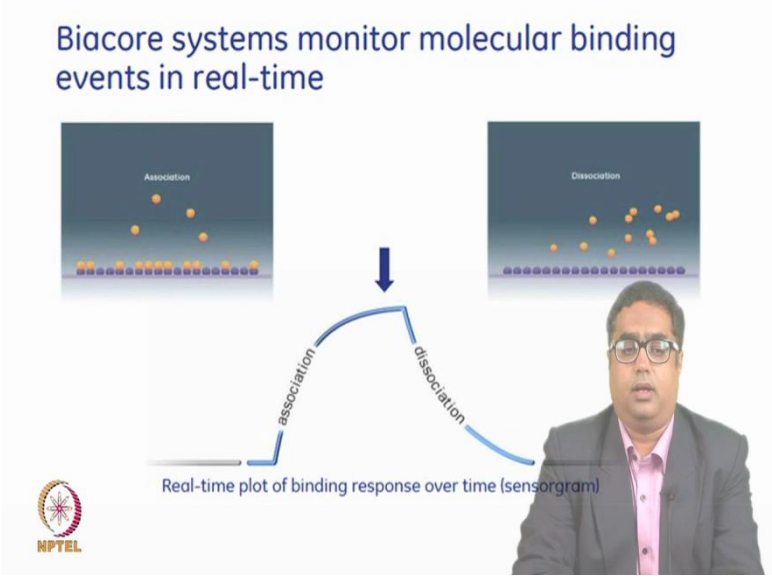

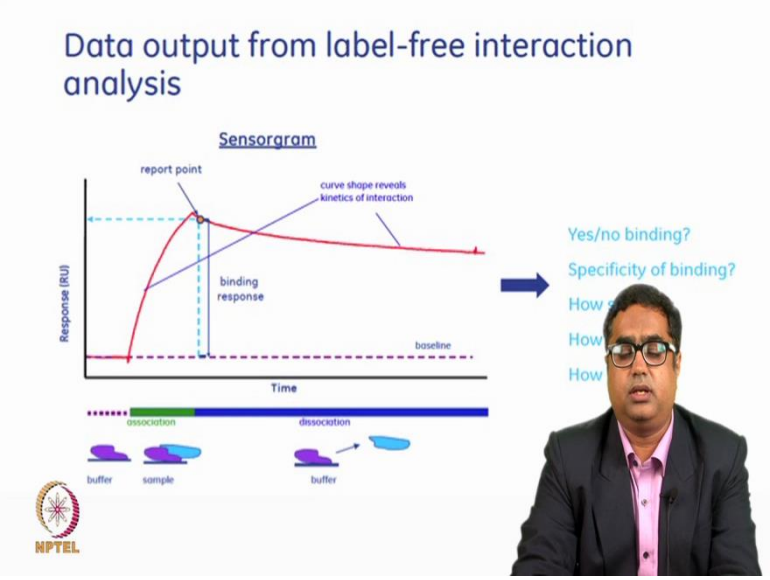

So, basically experiment starts with an immobilization of a, ligand on the sensor surface and an analyte is passed over in a solution and which will bind to the ligand. So, here two different molecules, one of them is ligand and analyte and interaction is recorded in a real time. And the data that is output on the surface in a sensor gram, 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 output that we get from a biacore experiment. The sensor gram that is generated from the biacore or a surface plasmon resonance experiments tend to give us different information. 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 if there is a specific binding or a non-specific binding. Once we have determined the binding then we will understand, how strong the binding is or how weak the binding is?

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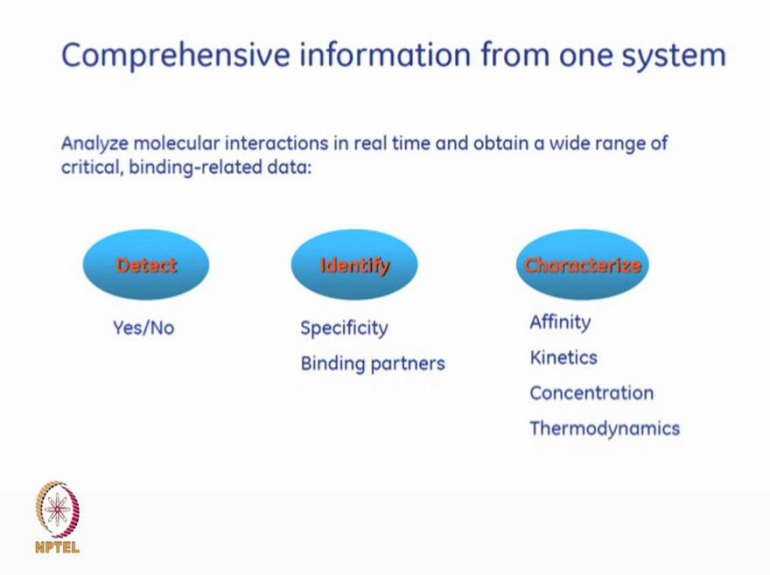

So, once we have determined this part then probably we will be able to understand how fast or how slow the molecules are interacting. And once, we have come through this stage I think, it is very easy for us, also to determine what is a exact concentration or how much amount of analyte that is binding? From these kind of an experiment, the comprehensive information that one will generate are detect that means yes and 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?

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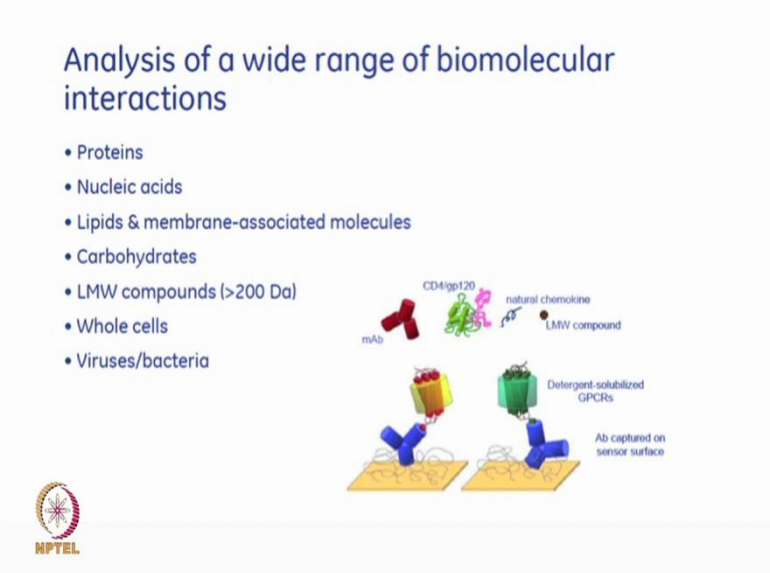

Concentration which is how much amount of our analyte is bound to the ligand. And thermodynamics, how kinetic changes over a function of temperature? So, these are the different comprehensive information that is generated from biacore experiments. Surface plasmon resonance, will help us to understand a wide range of a 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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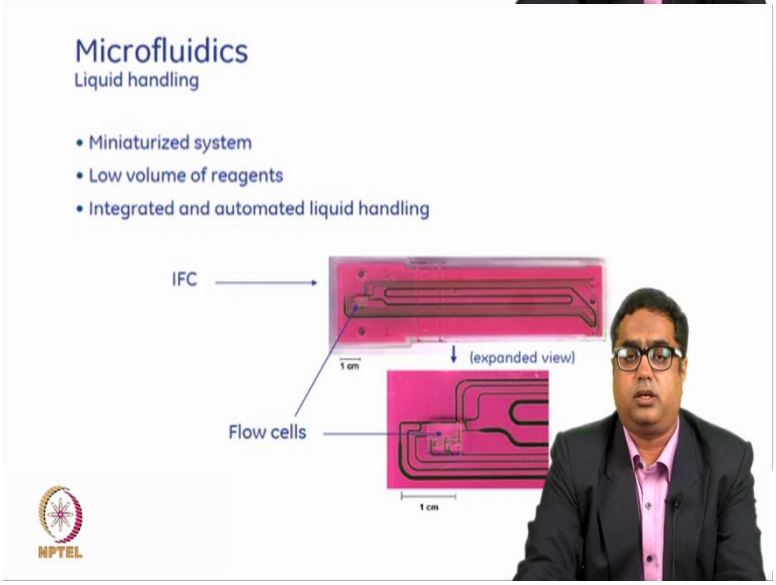

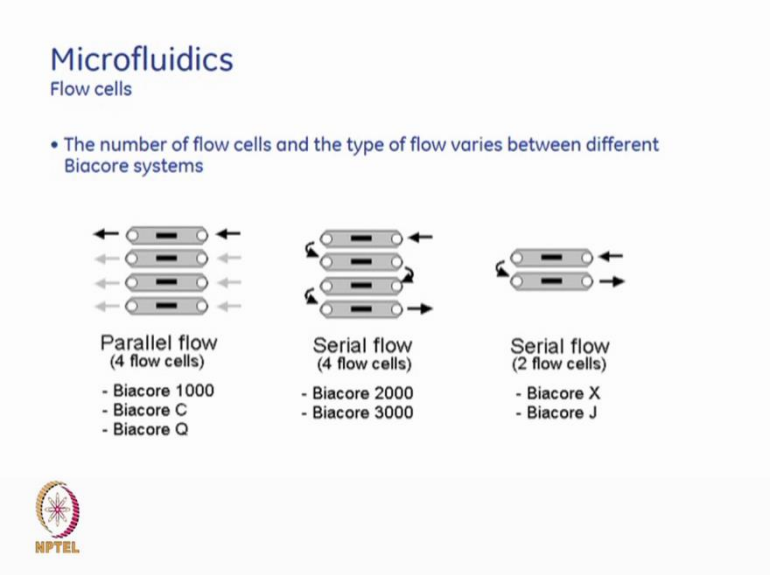

So, the corner stones of an any SPR system or a biacore is a detection system and a chip system, a microfluidic system and the software which integrates all of these and generates data. So, in our next sessions and next couple of other sessions, we will anyway understand details about sensors chips and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

The interactions happens in a miniaturized system called microfluidics 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. There could be many ways of orientation of the microfluidics 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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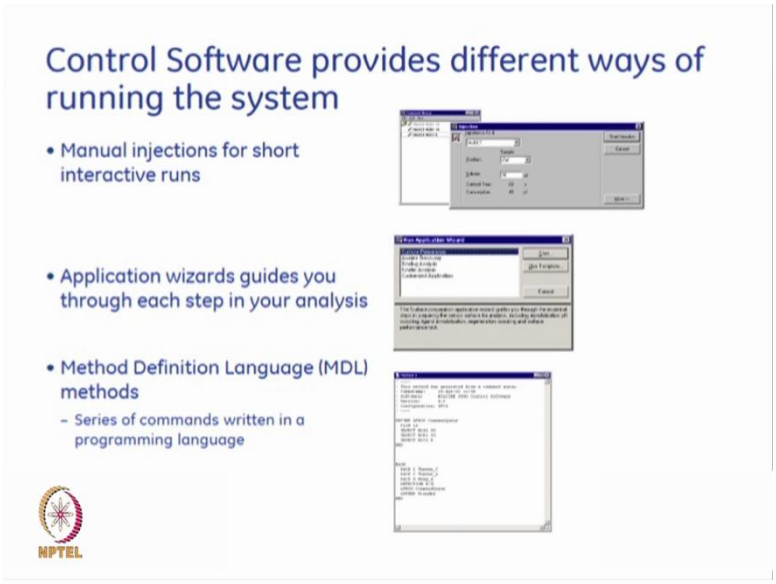

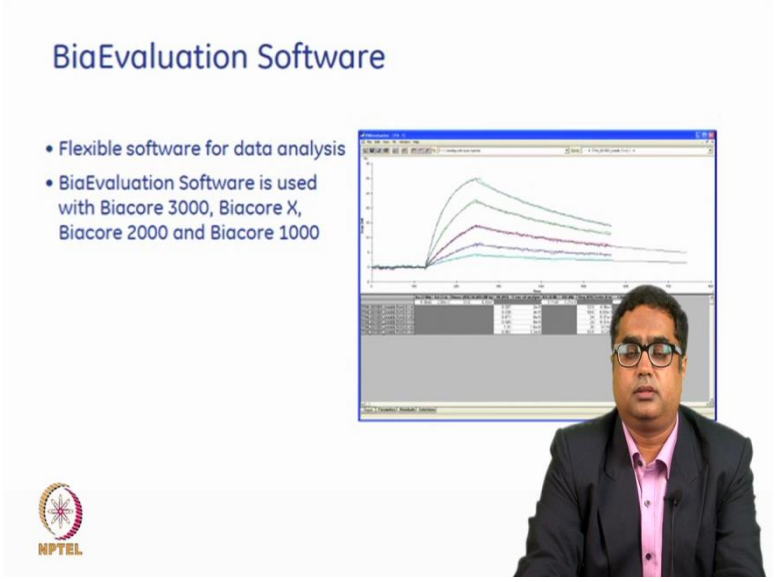

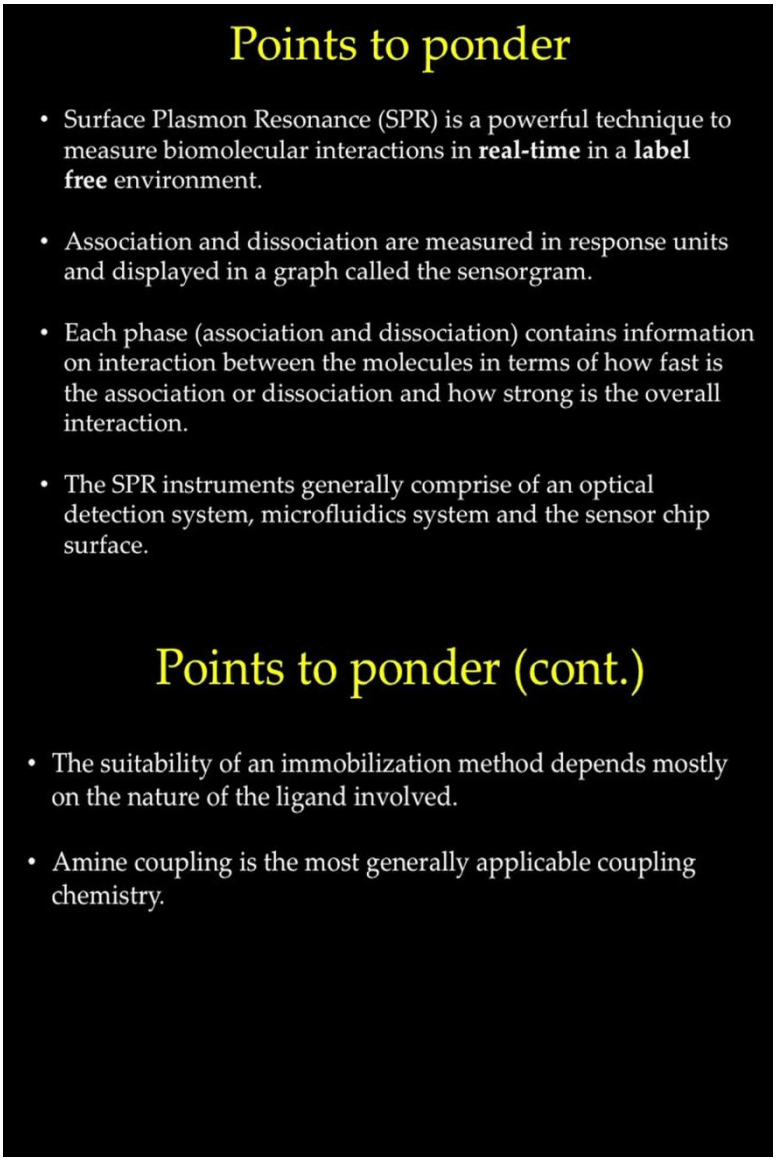

Control software provides different ways of running the system. So, there are events in which, we could observe them manually. There are application wizards where we can setup experiments very easily or we could write our own method and the record an experiment. Apart from that, there is also a evaluation software that evaluates the data and that data evaluation happens by use of algorithms which fit the data and will give out lot of a lot of other parameters for any kinetic event.

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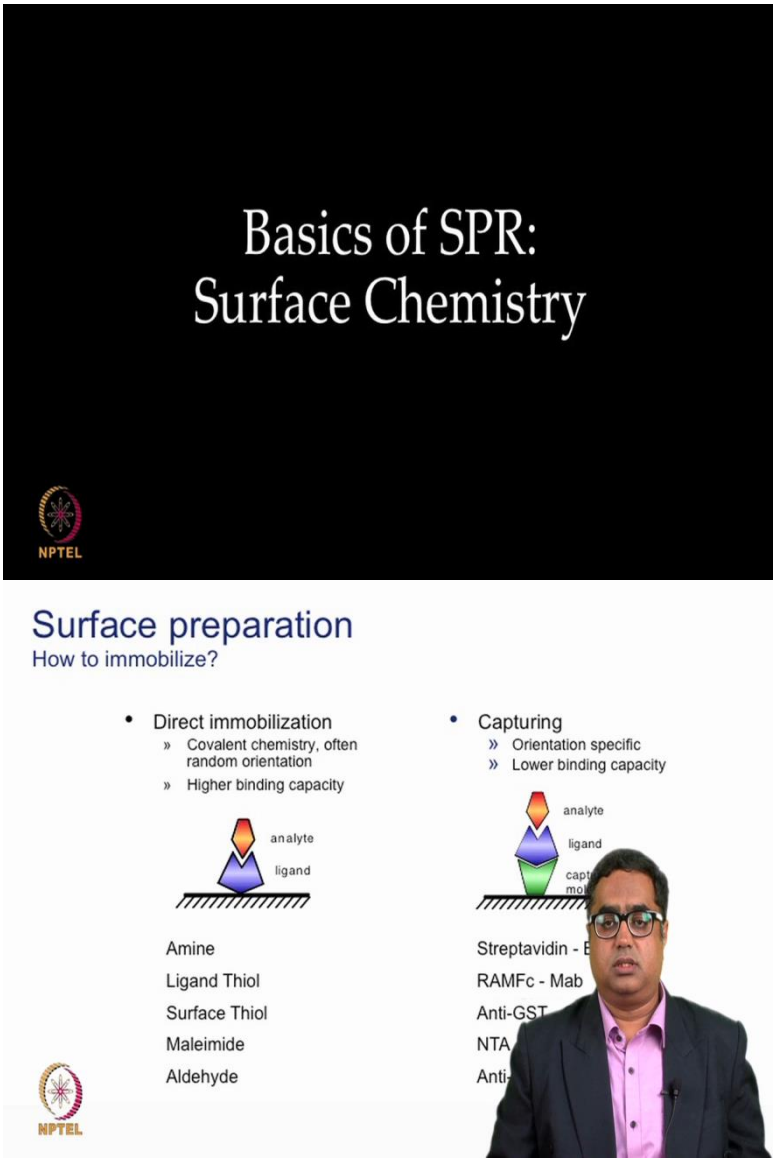

In this session, we will understand the different kind of sensor surfaces and the use of them in immobilization of a ligand, if we know from or the basics and from other sessions, we have understand, about the surface preparation direct immobilization or by capture methods. So, direct immobilization is an equivalent immobilization which is a permanent immobilization and in this process, we immobilize our ligand the choice and on a surface permanently.

uhh 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, a little bit detail to understand what they are and what is the measure utility of them and how do we design an assay based on these chips.

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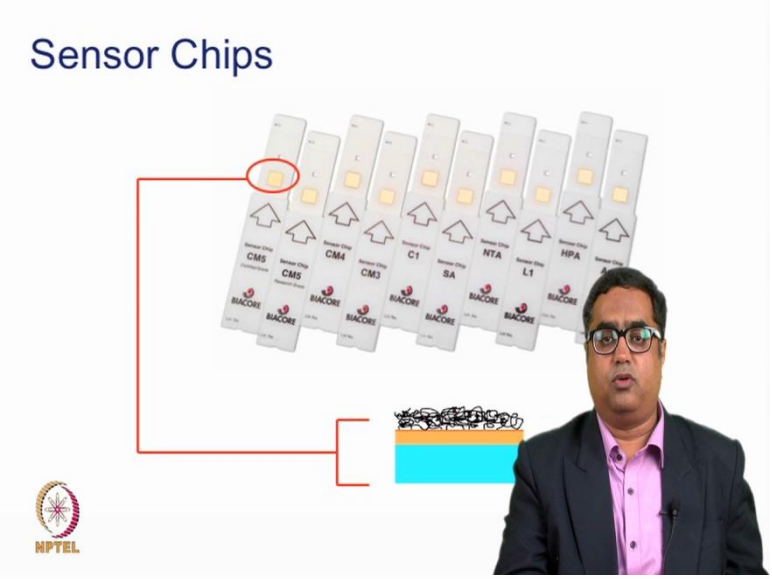

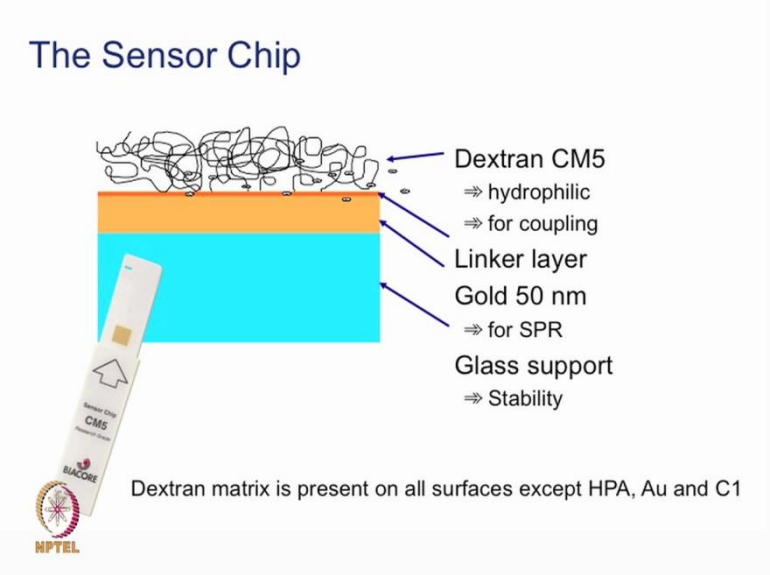

So, the various chips that are available for any SPR experiment to start with, they are named as CM5 chips, CM4 chips, CM3, C1, streptavidin or an assay chip, NTA chip, L1, HPA and Au or plain gold surface chip. Each of these chips have a place, for an adapter and this adapter actually holds the gold and that gold a place is where the immobilization happens and the adapter actually pushes, the gold surface into the SPR system where actual immobilization happens, 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.

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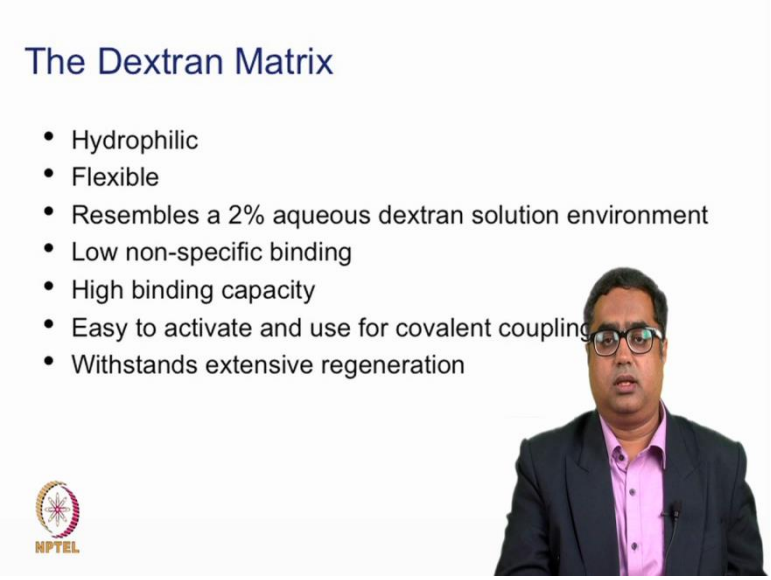

The dextran is glued to the gold and the gold is present in form of a cassette (())(14:15) and that cassette entirely is called as chip. So, looking into this gold surface, the major component attached to the gold surface is the dextran. And it is very important further to understand why dextran because dextran is hydrophilic, dextran is flexible, dextran resembles a solution or a aqueous solution 2 percent 14:46 solution and it has a very low non-specific binding and a very high binding capacity, easy to activate for covalent coupling and withstands extensive regeneration.

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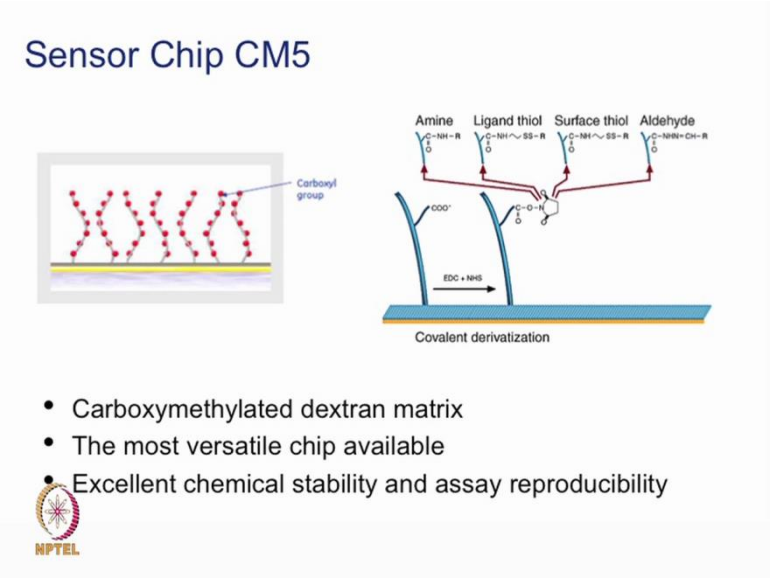

Now, let us go into the available different chips. To start with we will do the first chip and the universally accepted used chip is a, sensor chip CM5. CM stands for carboxymethyl, 5 is certain number in which it shows the amount of branching. So, if you look at this particular slide, the chip actually 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 group, which are getting activated and the activation of the carboxymethyl groups, helps us to immobilize our ligand by amine coupling or thyol coupling or aldehyde coupling.

The reactive side chains on the basic amino acids or amines are generally used to couple to the surface by amine coupling. Thyons are activated and then used to immobilized on the surface in a thyol coupling whereas the carbohydrides that are attached on the glycoproteins are used to do an immobilization and that process is an aldehyde coupling. And as I just said CM5 is a very versatile chip and any of this process or any of these methods can be used to immobilized on a CM5 chip.

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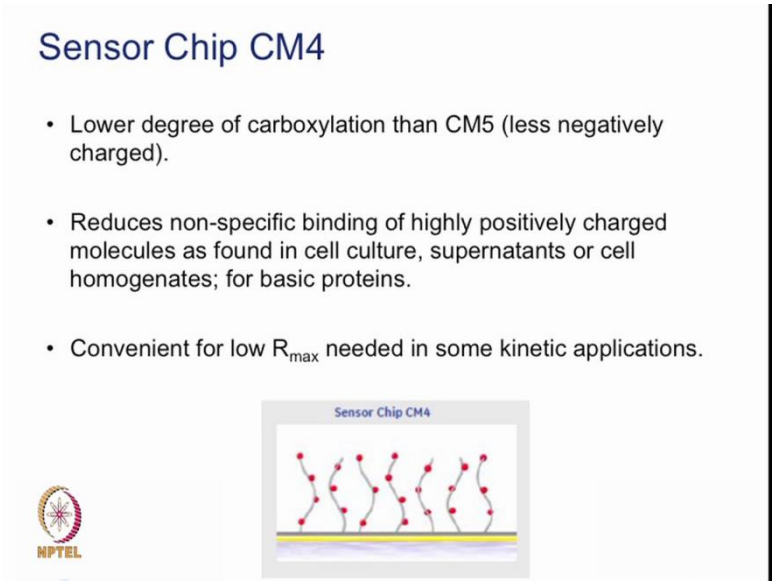

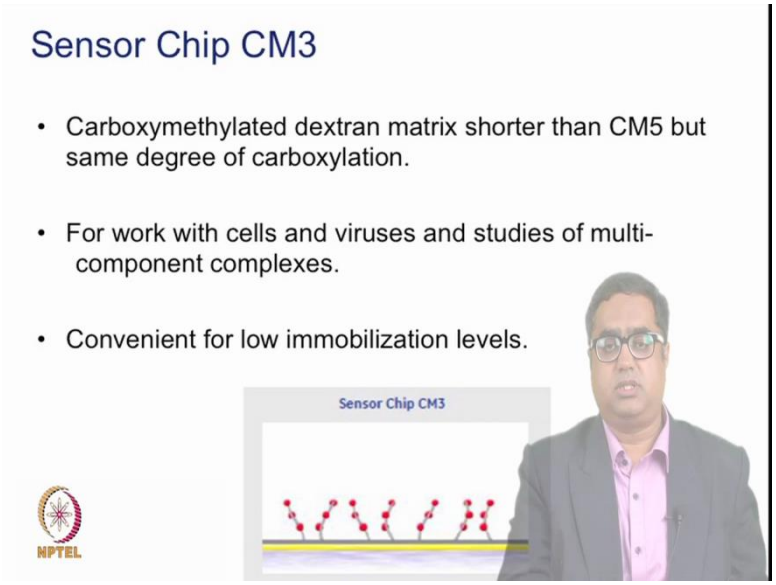

Coming to another sensor chip called as CM4, CM4 has a little less branching and a low charge which is less negatively charged and this particular chip surface is good for low immobilizations or low or max immobilizations especially cases where you are looking at interactions involving bio (())(17:06). Coming to another chip surface which is called CM3, another way of branching of the sensor, the carboxymethyl groups on the sensor. Here, again the matrix is a less or little shorter than CM4 also convenient for low immobilizations and generally good with cell or viruses immobilization or multi component complexes.

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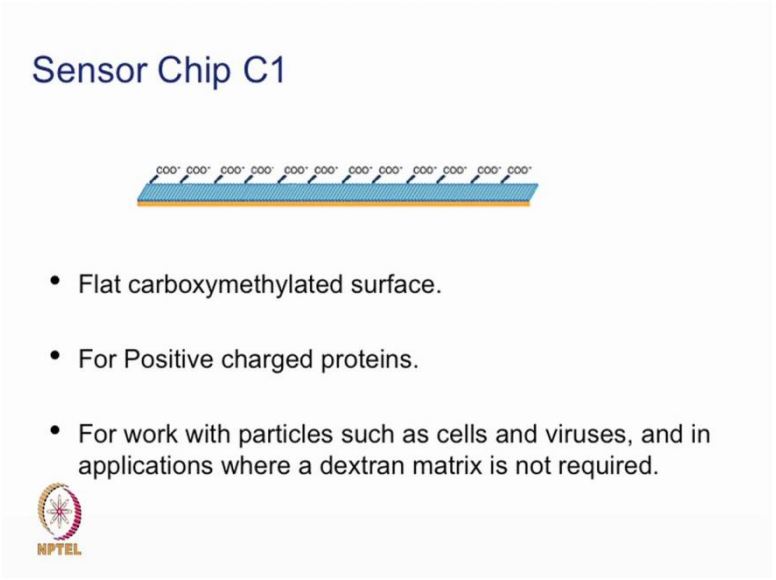

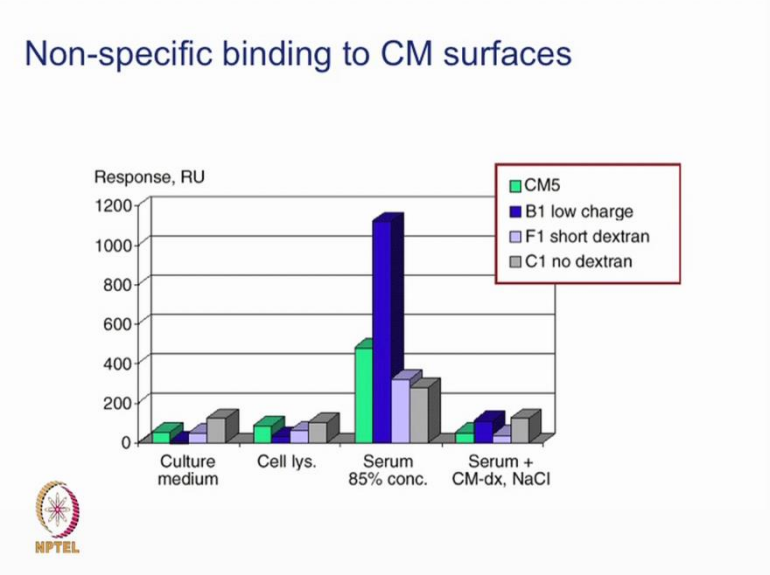

So, coming to another sensor chip is a C1 sensor chip. C1 does not contain any dextran here and it is a flat bed of carboxymethylated surface, very good with positively charged proteins and can be used again with cells and viruses for our total immobilization of cell viruses and being used by many available in the literature for immobilization of bacterial cells and mammalian cells and useful for studying interactions at cellular level.

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 un-diluted 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 (())(18:52).

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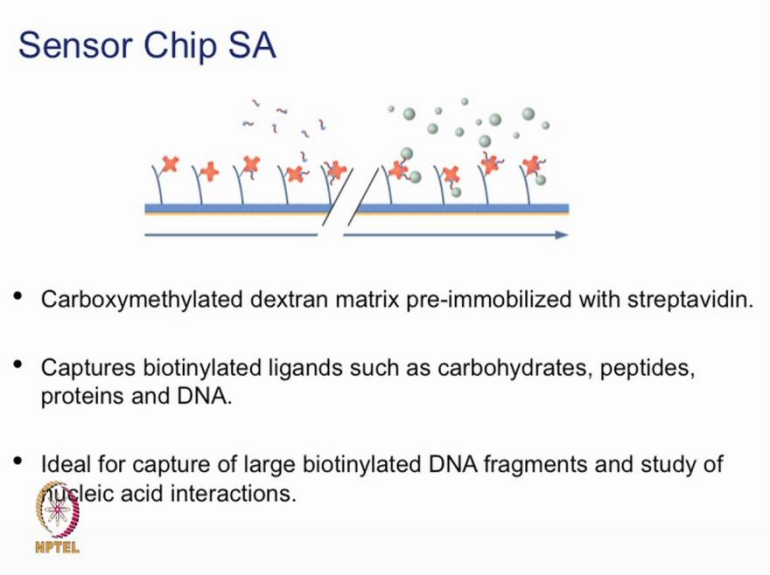

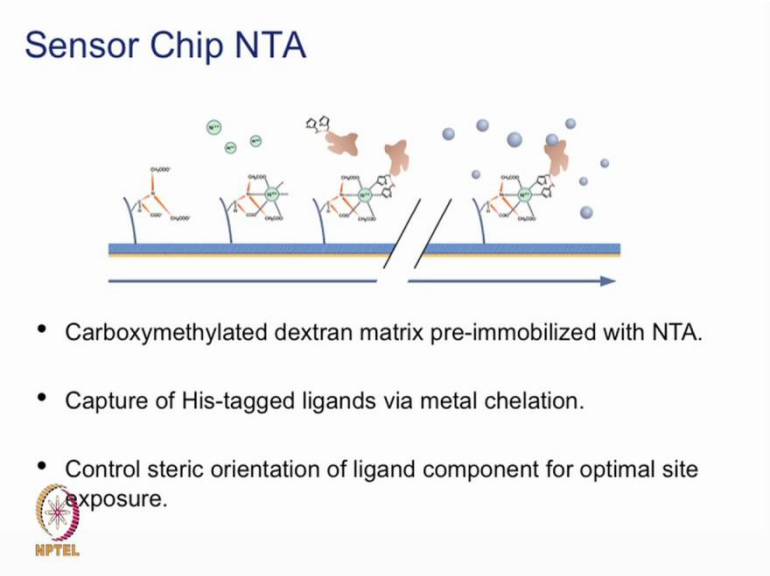

So, another variety or of a chip surface is a streptavidin. Here, this is also a CM5 chip, which is pre-immobilized with a streptavidin molecules and these streptavidin molecules have a very high binding affinity to biotin and these streptavidin molecules bind to biotinylated proteins, peptides, oligos, DNA, RNA and will help to immobilize these molecules in any interaction process.

So, ideally literature is available for biotinylated DNA fragments, nucleic acid interactions and other things like peptides have been immobilized on this surface and other variety of chip or a different chip surface is NTA and this is a complex molecule that is immobilized on the

surface of the chip, which is pre-immobilized and this molecule has an affinity for hexa-histadine molecules or tags that are present either at the C terminal or N terminal of a protein.

So, proteins when they are recombinently 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 in molecules bind 2 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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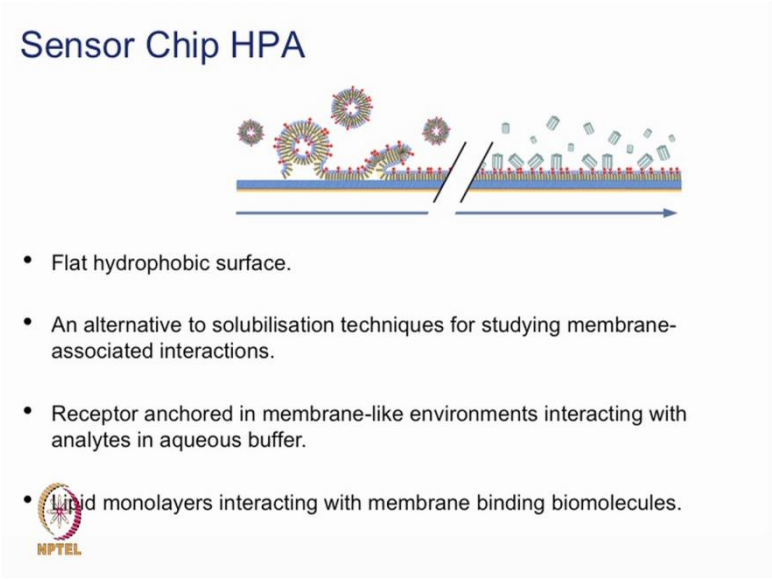

Another very important and variety of chip surface is HPA surfaces are flat hydrophobic surfaces and these surfaces are useful for membrane associated interactions. Receptors generally are anchored into a membrane-like environments and then they are useful for interaction with analytes. Generally, the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

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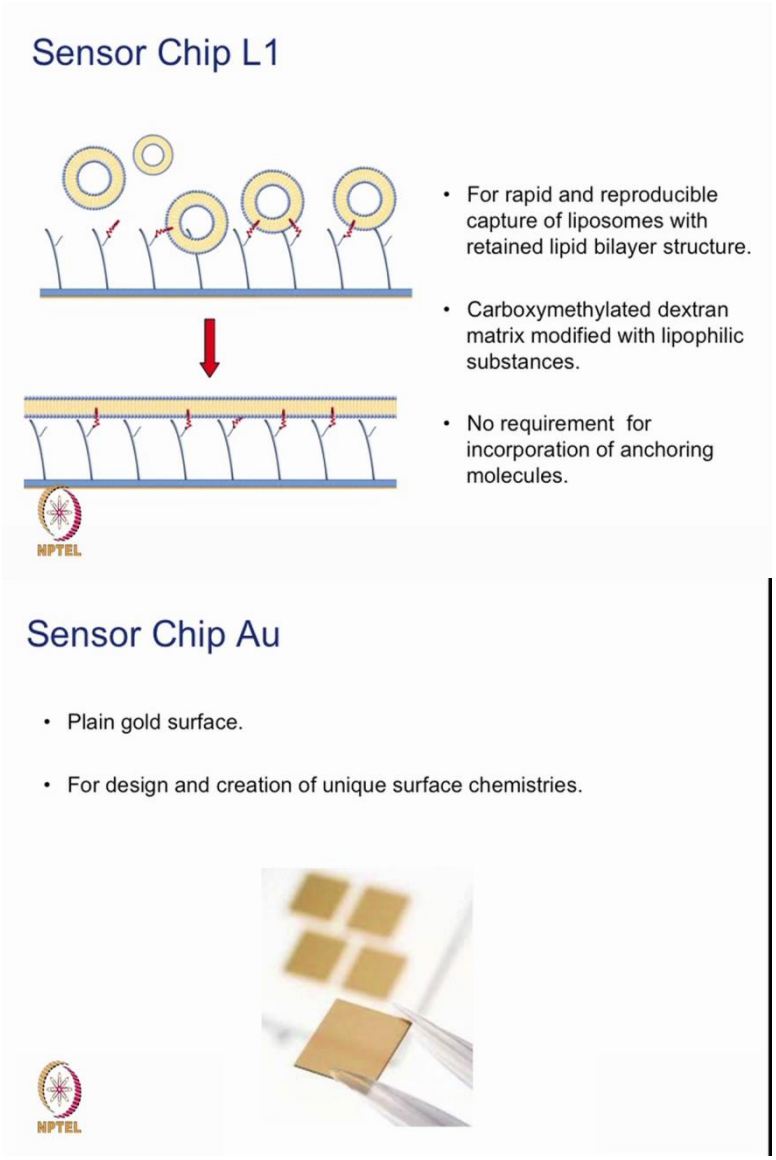

L1 another surface sensor chip, which is also popularly used for studying liposomes, lipid bilayer kind of a molecules and carboxymethylated dextran is modified with lipophilic substances. So, another surface is a plain gold surface where there will not be any dextran and these surfaces are very good 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 surface. 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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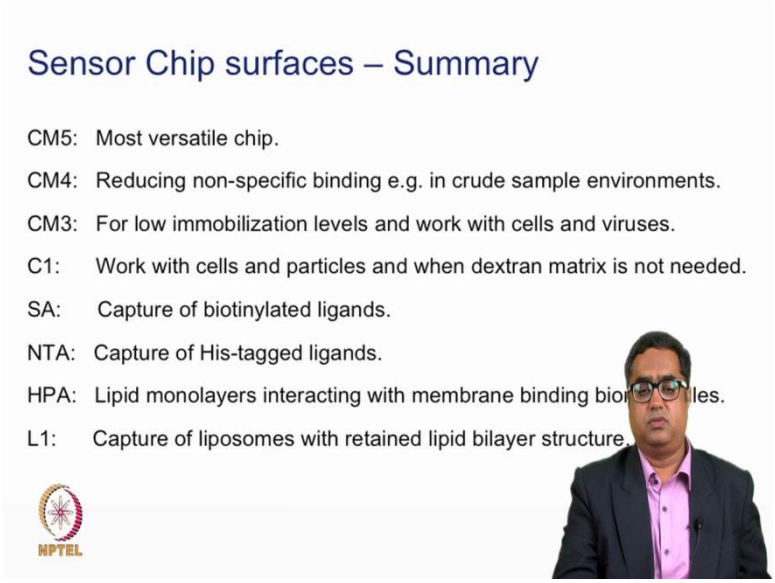

The summary of the different chips as we just went through CM, CM4, CM3,C1, SA, NTA, HPA, L1 are the different kinds of chip that are available for studying the different interactions. So, it is very important for us to identify, what are the functional groups that are present on our ligand and how well we could immobilized our molecule on the different variety of a chip. We are coming to the end of the 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.

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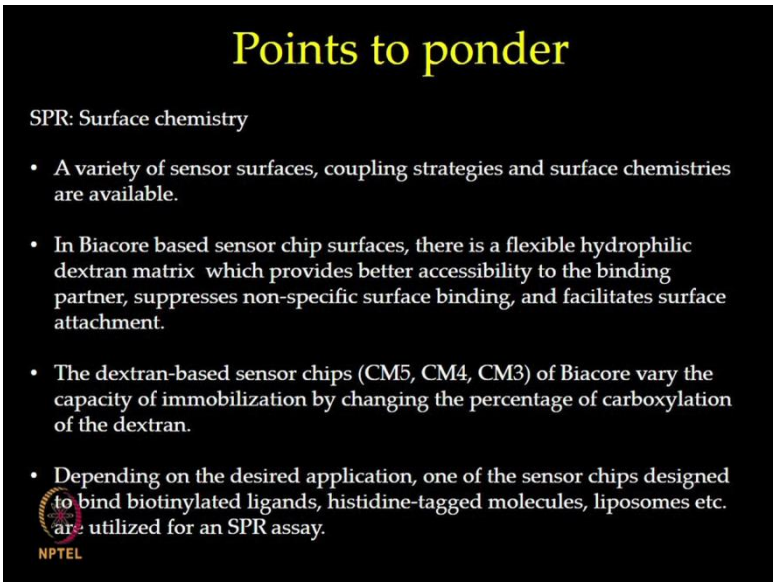

Once we immobilize our molecule, we will go ahead with our regular biacore experimentation and this particular session, helps us to identify the right chip for our experiments. Thank you, Dr. Srinivas for this lecture. So, let us continue our discussion on surface Plasmon resonance assays and experimental design in next class. Thank you.

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