INDIAN INSTITUTE OF TECHNOLOGY ROORKEE

NPTEL

NPTEL ONLINE CERTIFICATION COURSE

Biomedical Nanotechnology

Lec-07
Protein & Glyco Nanotechnology

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Hello everyone I welcome all to the 7th lecture of this course. The 7th lecture is on protein and glyco nanotechnology.

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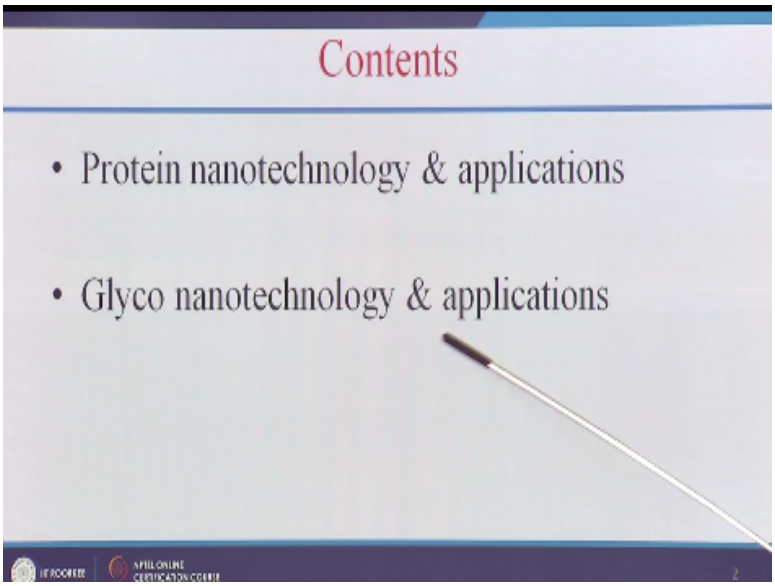

So in this lecture we are going to learn protein nanotechnology and its application, and the other we are going to learn what is glyco nanotechnology and its applications.

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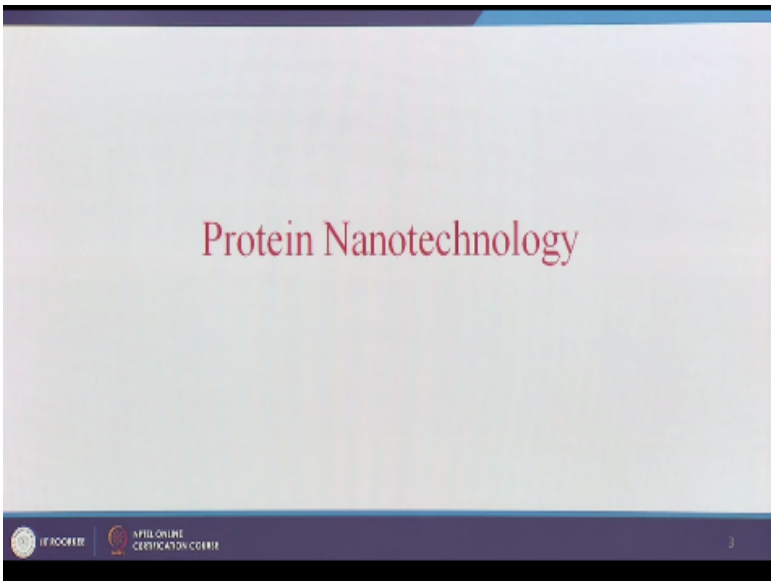

So in the previous lecture we have understood like how we can use DNA for making various nanostructures for various applications. So in this lecture protein nanotechnology we are going to learn how we can use this protein for making various nanostructures for various applications.

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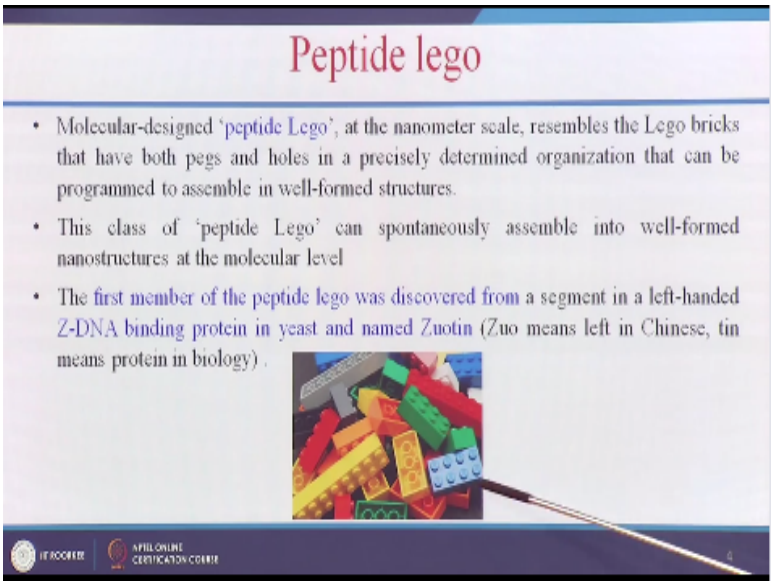

So this lego you might have played in your childhood okay, so using this lego we can build car, home or any kind of building. So similarly we can make a peptide lego, so how we can make a peptide lego using the property of hydrophilic and hydrophobic amino acids. So this hydrophilic and hydrophobic amino acids can assemble in water and it can form like a nanostructures, so that is called as peptide lego.

So the first member of the peptide lego was discovered from a segment in a left-handed Z-DNA binding protein in yeast and it was named as Zuotin okay.

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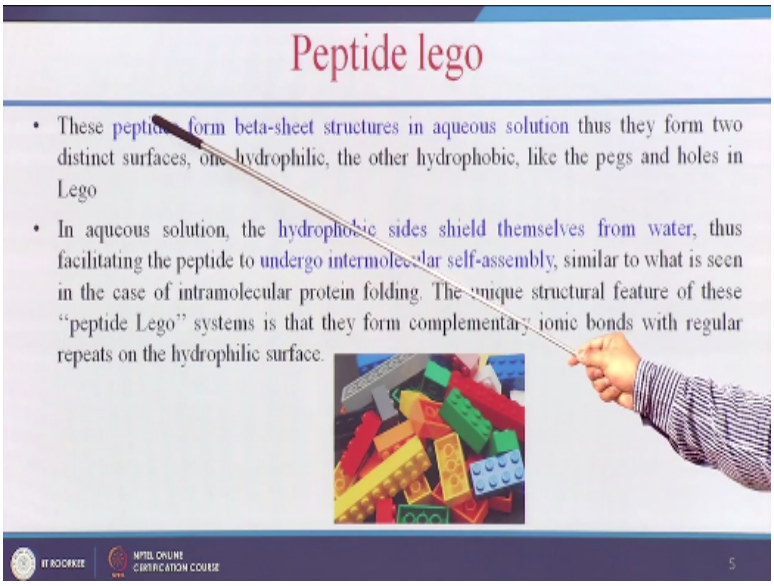

So here these peptides form beta-sheet structures in the aqueous solution, what happens in the aqueous solution, in the aqueous solution this hydrophobic sides shield themselves from water and due to which it undergo a intermolecular self-assembly okay, similar to intermolecular protein folding okay. So this unique structure of this peptide lego is that they form complementary ionic bonds with regular repeats on the hydrophilic surface. So using this peptide we know we can build several kind of structures.

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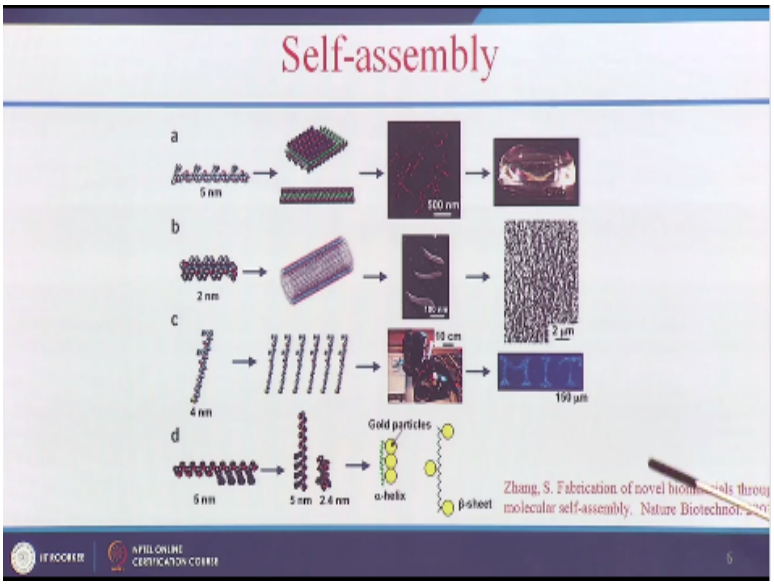

So the first application is we can have a nanofibers scaffold which can be useful for three dimensional cell culture and we can also make nanotubes which is similar to your carbon atoms, but it is a protein based nanotubes that could be useful for that dealing applications. And we can also make protein based ink. So let us see one by one application.

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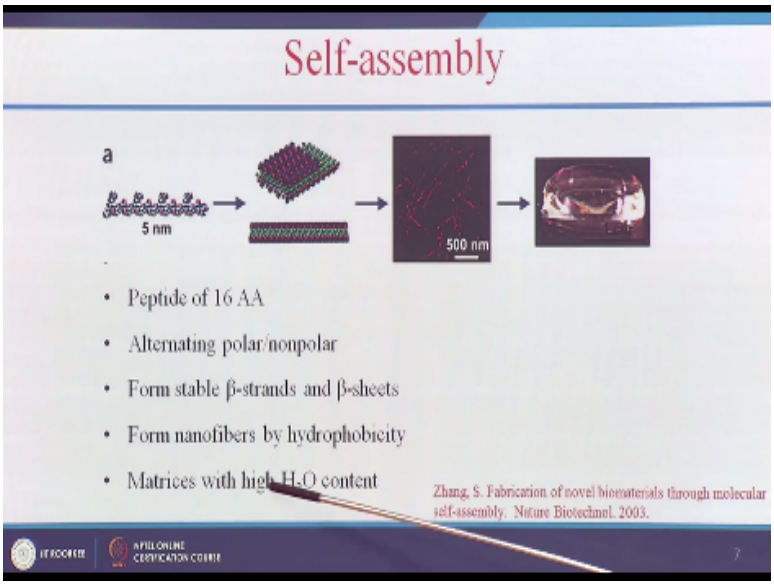

So how we can make this nanofiber based scaffold so here we can use the peptide of 16 amino acids which are having alternating polar and nonpolar, and which forms stable β-trends and β-sheets okay, it forms a nanofibers by hydophobicity and these matrices are with high water content okay.

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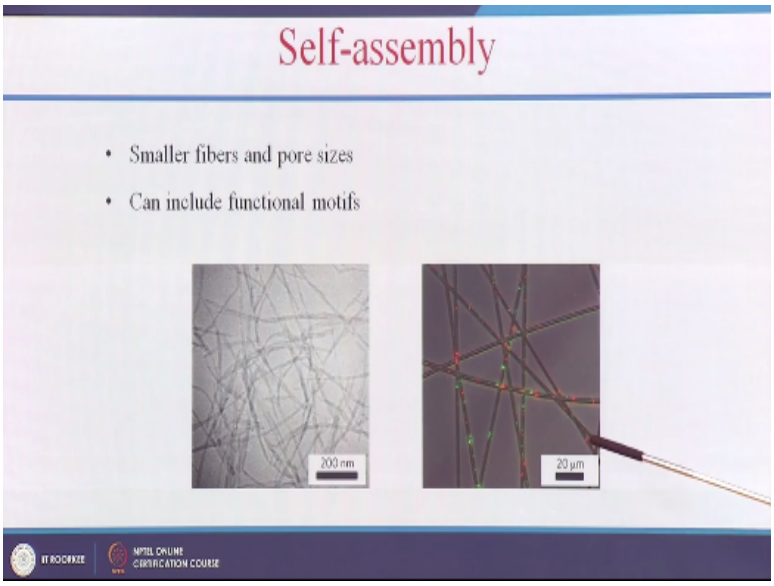

And also these nanofibers are smaller in size and it has a smaller pore size which can exactly meaning like your extra cellular matrix and also we can include the functional motifs. For example, if you want to incorporate GOP that is possible and if you want to load some drugs into these nanofibers that is also possible for therapeutic applications.

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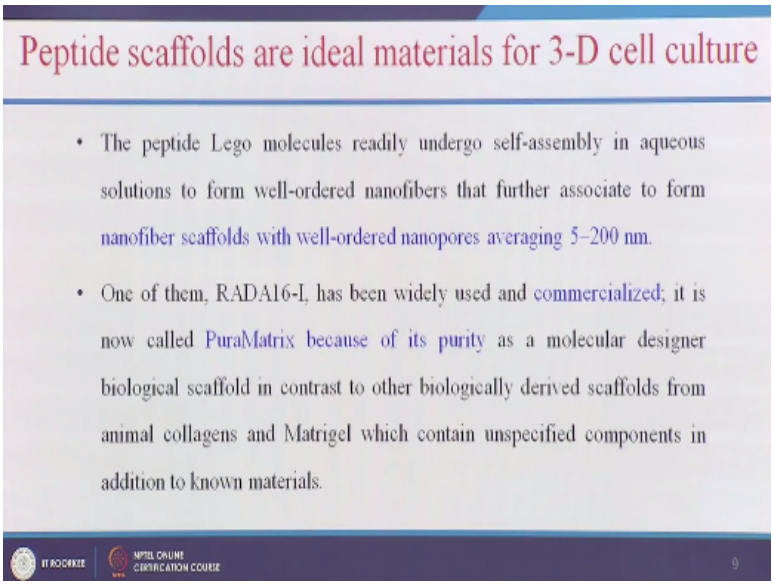

So let us see how this nano scaffold like nanofibers scaffold can be made using this peptides and how it could be useful for three dimensional cell culture okay. So here the peptide lego molecules readily undergoes self-assembly in water and it forms nanofibers. And these nanofibers join together and form the nanofibers scaffold with the pore size of 5-200 nanometer okay. So one of the very good example is RADA16-I and it has been widely used and commercialized in the name of PuraMatrix because of its purity.

And when compared to the other traditional biological scaffold like scaffold collagen and Matrigel so this protein based scaffold have very good porosity okay. And also highly biodegradable and biocompatible.

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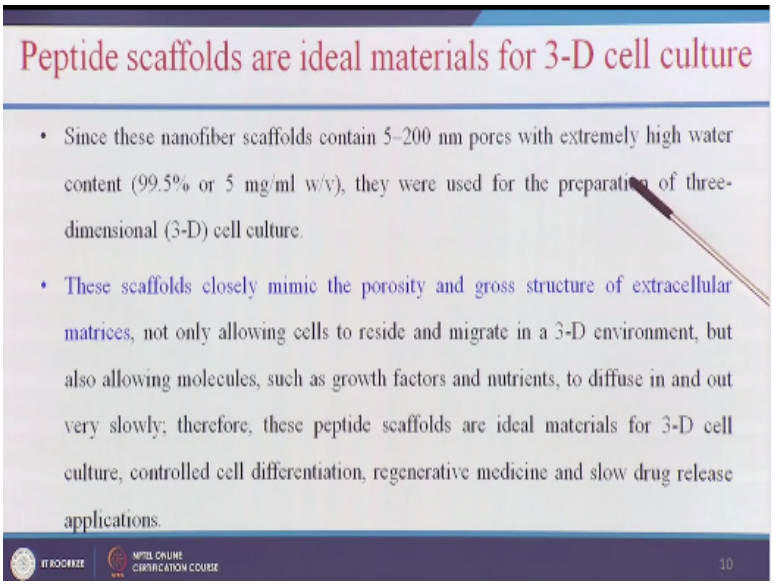

So these nanofiber scaffold contain 5-200 nanometer pores with extremely high water content okay. So they will be used in the preparation of three dimensional cell culture. And these scaffolds closely mimic the porosity and gross structure of your extracellular matrix okay. So it is not only allowing the cells to attach and grow and it is also allowing the growth factors and other nutrients to diffuse in and out very slowly similar to your extracellular matrix okay.

So that is why it will be very useful for controlled cell differentiation and also for application on regenerative medicine and slow drug release application. So let us see why we need three dimensional cell culture and what is three dimensional cell culture.

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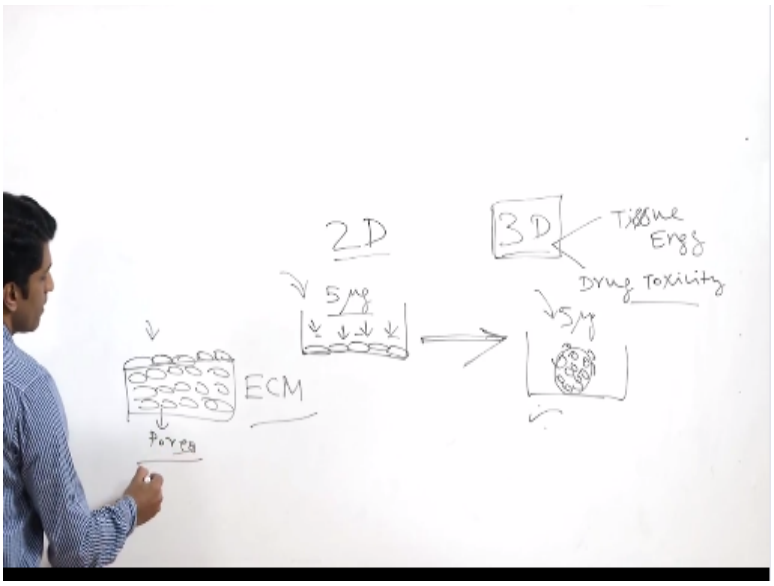

So usually in the lab we grow the cells in two dimensional and in three dimensional can be useful for tissue engineering application and also for studying the toxicity of drugs. So when you study the toxicity of drugs using this two dimensional what happens is all the cells will get equal amount of consideration. For example, if you are using 5mg of drug, so all the cells will be exposed to the particular consideration of drug.

But when you translate these results to your animal model it would not be successful. So the reason is inside your body all the organs are three dimensional okay. So if you make a three dimensional scaffold that can closely mimic your body condition or any of your conditions and we can do the toxicity as well as various other applications. So when you add the same consideration of drug here it is not going to work, because only some of the drug molecules can enter inside and some will attack the cells on the outside.

So that is why this three dimensional scaffold is very useful for studying the toxicity of drugs and also for tissue engineering application. So in case of tissue engineering so if you want to replace the damaged tissue, so you need a scaffold that is support to grow the cells. So this three dimensional scaffold, so will replace your damaged tissue or damaged skin okay. So it will grow and the porosity is very important because it exactly act like your ECM that is extra cellular matrix.

So it terminates the cell to grow nicely on the scaffold as well as it also allows the nutrients to enter through the pores, so that is why exactly the mimic was extracellular matrix okay. So and

another advantage of this protein based scaffold is it is highly biocompatible and biodegradable, so it could be very useful for tissue engineering and so for various other applications.

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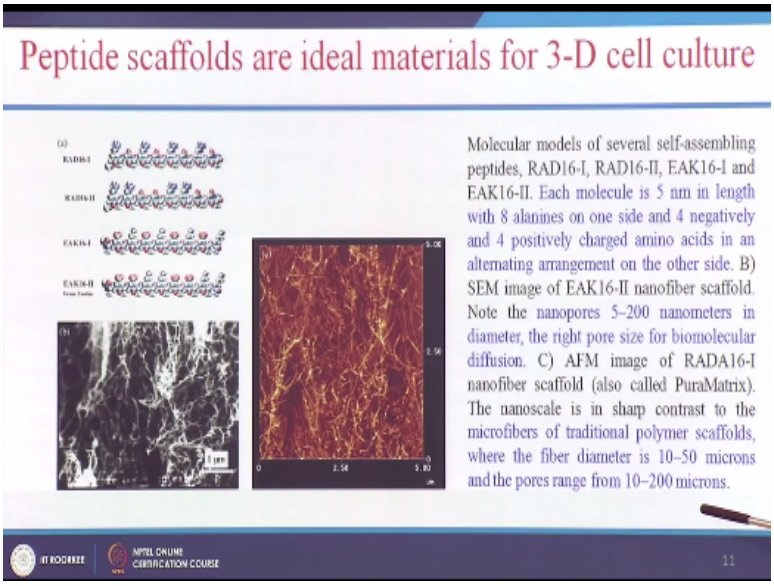

So let us see some of the examples, so these are some of the protein based scaffolds peptide scaffolds okay. So here the each molecule is 5 nanometer in length and with 8 amino acids on one side and 4 negatively and positively charged amino acids in the alternating arrangement on the other side. So that forms the complete nanofiber scaffold. So when you see this scaffold under the scanning out a microscope you can see the porosity is between 5-200 nanometer in diameter okay.

So which is the right pore size for bimolecular diffusion, so when you see the fibers under AFM you can see that porosity is very small like 5-200 nanometers, but in case of microfibers or traditional polymer based scaffolds the fiber diameter will be between 10-50 microns, and the pore size will be ranging between 10-200 microns. So you can see here this pore size is also in a micrometer range and your cell size is on the micrometer range.

So it is not going to close your mimic work body conditions, because your extracellular matrix is in the range of nano network okay. So on the nanofiber network your cells are attaching and growing. So when you make the nanofibers scaffold it will exactly mimic like your body condition.

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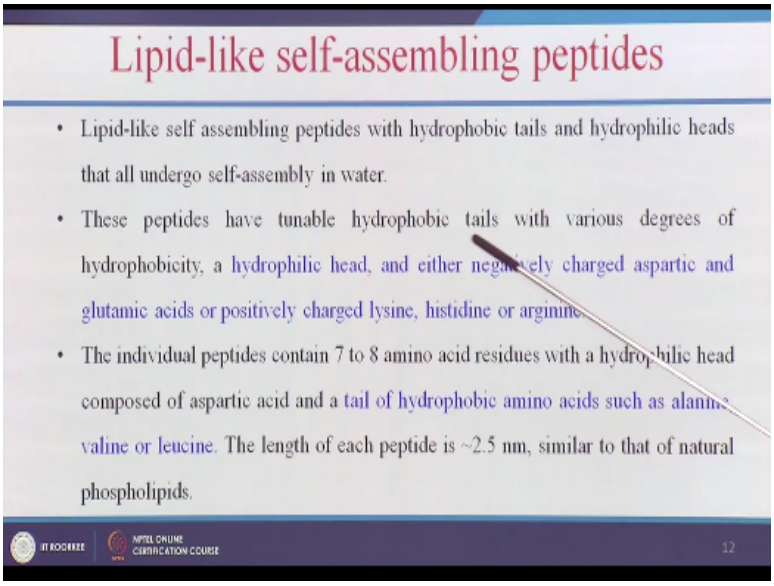

And next one is lipid like self-assembling peptides okay. So here we can make lipid like self-assembling peptides with hydrophobic tails and hydrophilic heads. So which we will undergo self-assembly in water.

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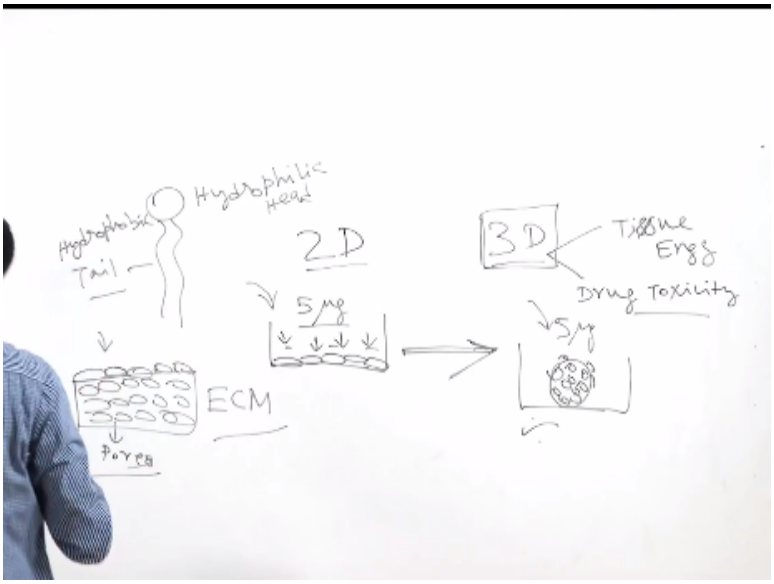

So usually we know that in lipids it have the hydrophilic head and hydrophobic tail. So similarly we can make peptides, so how we can make this using the hydrophilic amino acids and hydrophobic amino acids okay.

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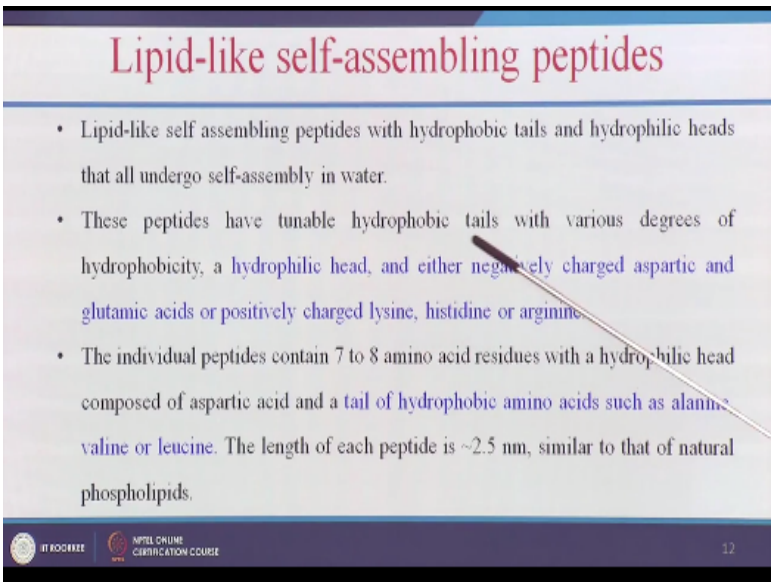

So these peptides have tunable hydrophobic tails with various degrees of hydophobicity and we can also have hydrophilic head. For example, you can use amino acid like aspartic acid, glutamic acid okay these are negatively charged, hydrophilic head and if you want to make partially charged hydrophilic head you can use lysine, histidine or arginine okay.

And these individual peptides contain 7 to 8 amino acid residue okay with the hydrophilic head it can be made up of aspartic acid or whatever mentioned earlier. And the tail can be composed of

hydrophobic amino acids, the hydrophobic amino acids such as alanine, valine or leucine okay. So the length of each peptide is approximately 2.5 nanometer which is similar to your natural phospholipids.

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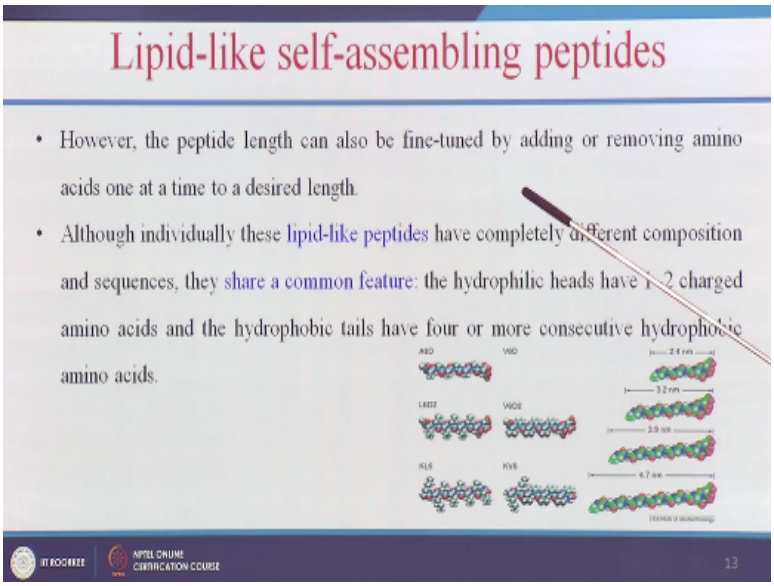

So and again the another advantage of this lipid like self-assembly peptide is we can fine-tune the size of hydrophobic tail by adding the amino acids okay, so we can enlarge the size or we can reduce the size. Although these lipid like peptides have completely different composition okay, so they share a common feature okay, that is it will have like hydrophobic heads like 1 to 2 charged amino acids and the hydrophobic tails which will have four or more hydrophobias amino acids okay ,even though these acids are different but this common this common features.

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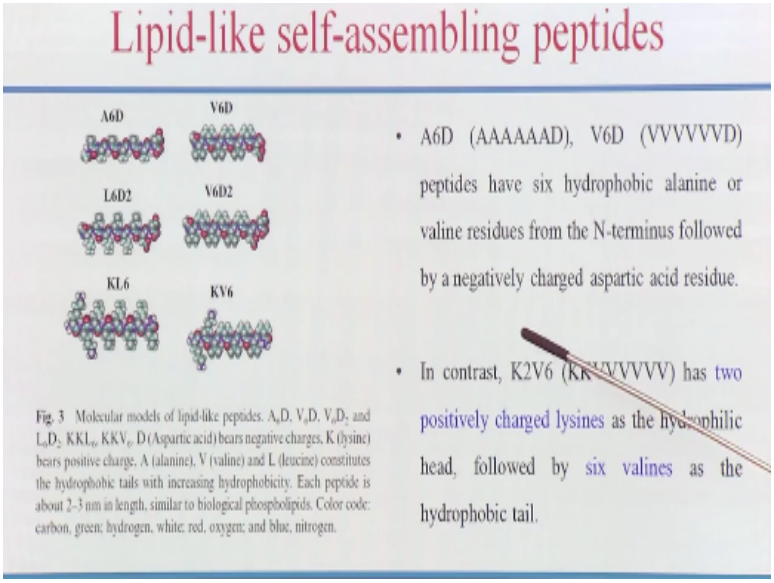

So let us see some examples A6D B^D and the A6D means it has 6 d is your aseptic acid so we can see it is denoted in the particular acid so the k is for the ailment and w and the k2 v6 and that means the 2 K and 6 valley it has 2v positively charged here we have only one head and you are having two positive charge and the hydrophilic head by te 6 valence at the hydrophilic head so we can make this can the peptides and the different kind of heads and the different types of tail by simply changing the ammonia acid position we can extend the size and we can shorten the size.

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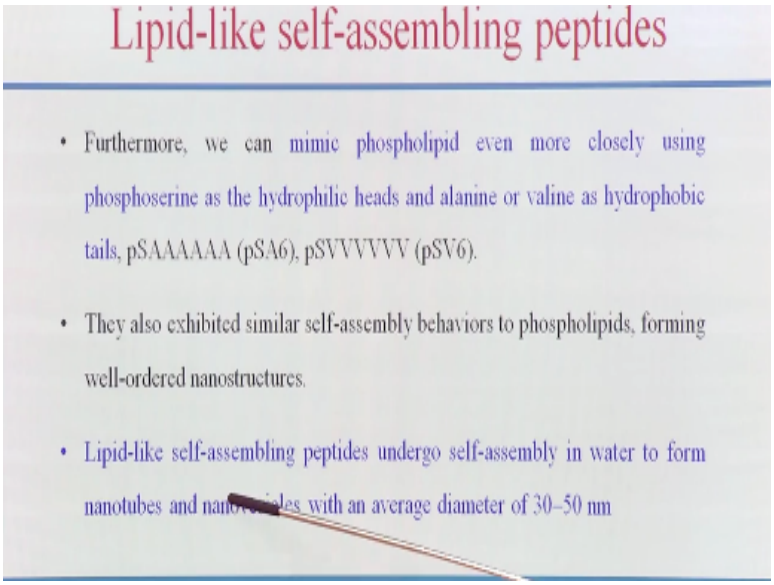

And this is the lipid liquid and we also make self assembling peptides which can mimic your for the faster liquid and the how make the lipid like ;ipid and the mimic like pass liquid and we add some acid called phospherine so if you add this ammonia acid hydrophilic acid and align and the hydrophobic mimic like a phosphoric so this could be the artificial and the cell membrane and the also exuberated and the similar cell behavior to the phosphoric forming well order nano structure okay these lipid like solids and in the peptides and it goes in the self lines and in the water and it can form even the nano tubes as well as average diameter of 32 to 50 mm meter.

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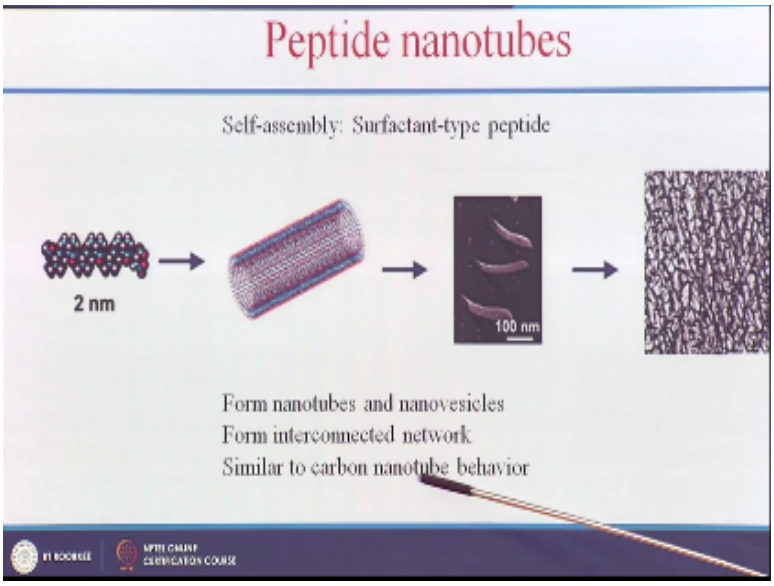

Another example how to make the peptide based nano tubes okay, so using the peptides we can make nano tubes or nano vesicles and which will be similar to your carbon nano tube okay. So here the advantage is it is highly bio nano tube so we can load any kind of drug like and we can use it for theoretical application okay, and also it is bio degradable also, because it is made of protein, it is not going to cause any toxic or induce any responses okay.

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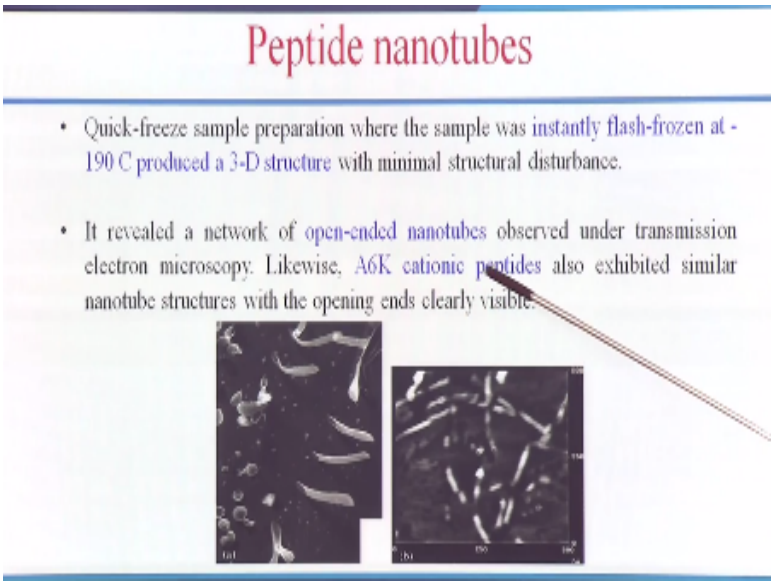

Here you can see here how to make this peptide nano tubes, so how to make it like you can use that quick freeze sample preparation method. So how do you do it? You can instantly flash frozen your sample at – 190 ^{0}c. So which will produce a structural nano tube, so you can have the peptide in your solution and you can immediately flash frozen. So that will assemble and form this kind of nano tubes okay.

So if you see this nano tubes under ultra microscope it will show you open ended nano tubes and again we can also make a cationic peptides using this A6K we can make the cationic peptides. So what is the advantage of having cationic peptides? So it is having like a positive shock, you know that the cells and DNA is a negative charge, so it can easily combine and it can be used for delivering your DNA or for delivering the drugs to the cells.

So this is the advantage and another advantage we can also have a green flow in exposing cationic peptides. So we can easily monitor the nanotubes where it is going? How it is delivering the drugs? So it can also act like your theronastic nano particle or nano tubes okay.

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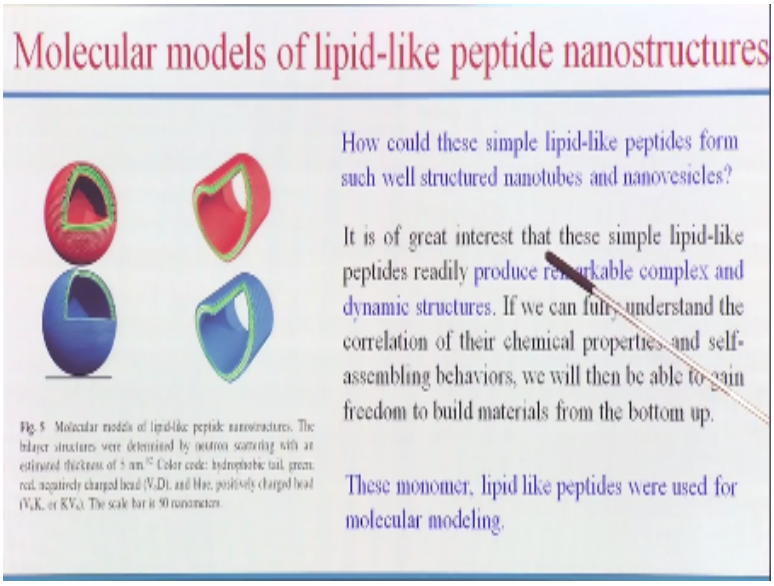

And we can also make structure like spherical and physical like structure, so how could this lipid like peptides can form this kind of nano tube and nano vesicle structure, because still the mechanism is not well understood. But people are trying to understand using this computational modeling as well as various computation tools okay so if you understand how it is forming this kind of remarkable complex and dynamic structures and we can make a various structures and which will have not only biomedical application it will also have application in the other field also okay.

So using the tools like bio informatics and computation biology tools and once you the prepared sequence are floating sequence and followed by that how what kind structure it is forming so we can do the modeling and simulation and we can understand what kind of sequence making what kind of structure okay so based know that you can design your own structures using the simple peptides seed coats.

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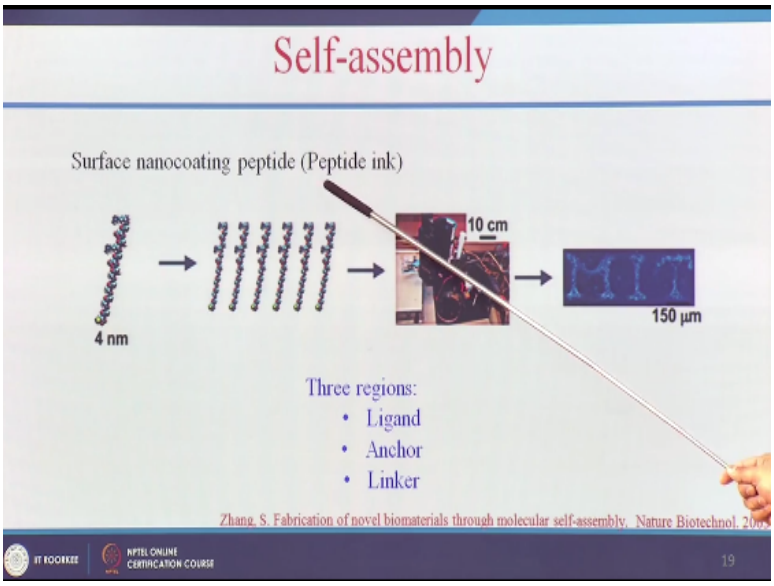

So let us move on to the other application that is peptide ink so we can use that peptides as a ink for writing any obituary structures and when you add the cells it will grow in that particular structure so this peptide ink consists of 3 important regions one is ligand the next one is anchor and third one is linker.

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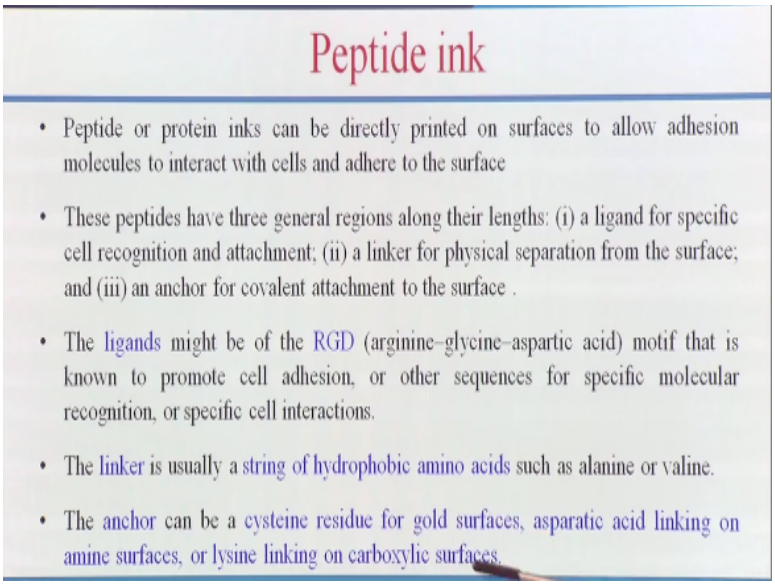

So this peptide or protein inks it can directly printed any surface okay and it will allow the adhesion of molecules to interact with cells and it also adhere to the surface okay and this peptides have 3 regions as I already told you it as a ligand which is for cell specific recognition and also linker for physical separation between the surface and anchor for covalent attachment to the surface.

So here the ligand is usually RGD so in a one of my pervious lecture I already to you what is RGD. RGD is arginine, glycine and aspartic acid so this will promote the attachment cell adhesion okay and the linker is usually made up of hydrophobic amino acids such as alanine or valine and the anchor can be varied so the anchor can be cysteine residue for gold surfaces if you want write it on the gold surface you can use the cysteine and asparadic acid for the amine surfaces and lysine for the catboxylic surfaces.

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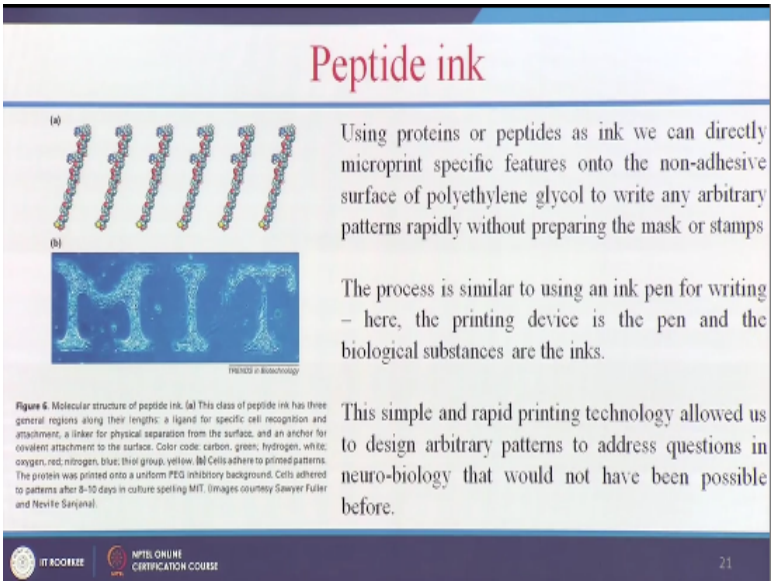

So using this peptide ink or protein ink you can write any kind of arbitrary structures, so nasally if you want to write if you want to make this kind of structures we have to make the mask or stamp. So simple example is you need a stencil kind of thing, so you need a stencil to write the particular alphabet okay. And for every alphabet program if you want to write MIT or if you want IIT.

So you want to make separate separates stamps or mass for writing such kind of structure but in this case the advantage is you can use the proteins or peptide as a ink and pen so you can write any kind of structures and add yourselves it exactly grow on the particular structures okay. So it will grow and what is the advantage and what is the application is like we can understand the various neuro biological questions.

So how the cells communicate and how the cells attach and grow so we can lot of unanswered questions in the neuro biologic can be understand by using this kind of peptide based ink okay. So let me explain one more time the peptide ink what are the three regions in the peptide ink because that is very important.

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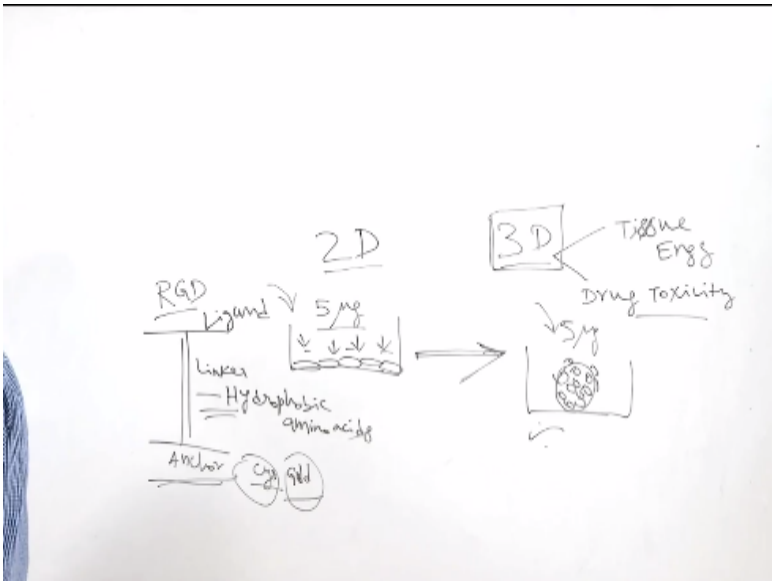

So peptide ink contain three region first one is Ligand okay that is your RGD, and next one is your linker that is made up of hydrophobic amina acids and anchor, so anchor is male is cysteine okay in case of gold surfaces so this peptide ink contain three regions first one is ligand that is RGD so which is useful for cell attachment okay and the linker that is made up of mainly hydrophobic amina acids and the anchor which is holding this peptide ink that is made up of cysteine in case of gold surfaces.

I hope you understood what is the peptide ink and what are the components of the peptide ink, so let us move on to the other application so how we can use this lipid like peptides.

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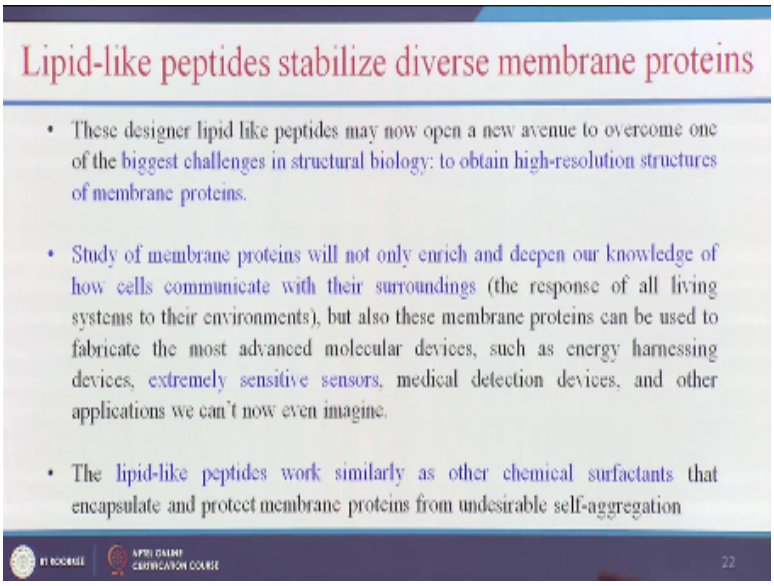

For stabilizing the membrane proteins, so one of the important challenge in the structural biology is to isolate and obtain a high resolution structure of membrane proteins okay. So the study of membrane proteins will not only enrich and deepen our knowledge of how self communicate with their surrounding and it can be also useful for making such kind of affectively sensitive sensor.

For example if you test the hard surface you take out your hand immediately okay, so the membrane protein it is sending the information immediately to the brain and you will take out your hand within a fraction of second. So when you understand this membrane protein thoroughly so we can make extremely sensitive sensors and here we can use the lipid like peptides which work similarly to your chemical surfactants and it will product the membrane proteins from undesirable self aggregation.

So as I told you earlier the main problem in isolating membrane protein is when you isolate this membrane proteins it will self aggregate.

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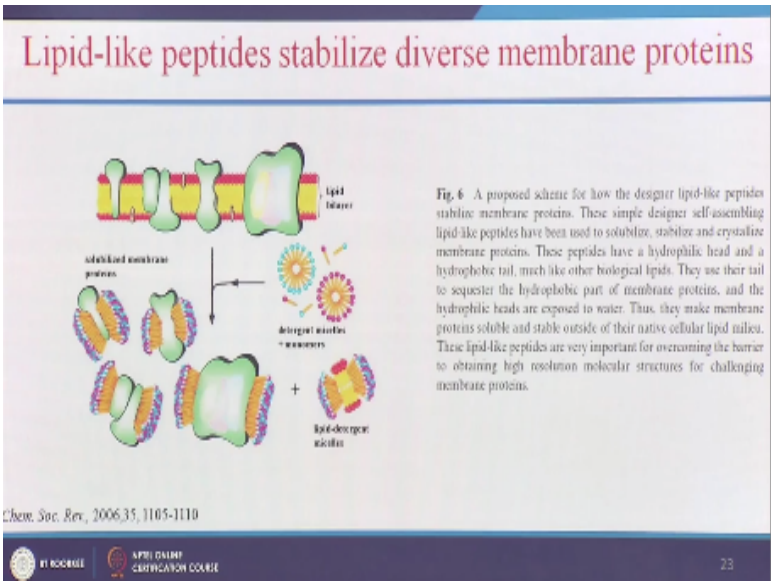

So avoid that we can use the lipid like peptides and it will come and product your membrane proteins and self aggregation so we can isolate and you can study this membrane protein and we can understand and then you can make a extremely sensitive sensors for various application.

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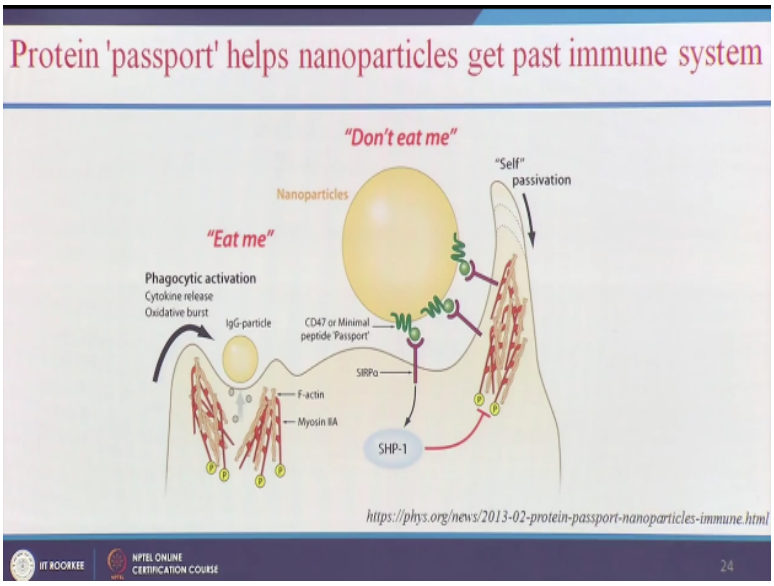

So let us see the another example how we can use the protein at the passport to escape from the immune system, so usually if the nano particle goes inside the body our immune system will come and attack the nano particle, so our nano particle has to escape from the immune system, so that it can reach that target locations and deliver the drug okay. So how we can escape this nano particle from the immune system, see here we can see the example this nano particle is not quartered with protein.

So it is taken it by the immune system immune cells okay and we can make a nano particles which are quarter with the similar to CD 47 protein. So if the nano particle is quarter with the peptide which mimic like was CD 47 it can escape from the immune system and it can deliver the drug to the targeted location.

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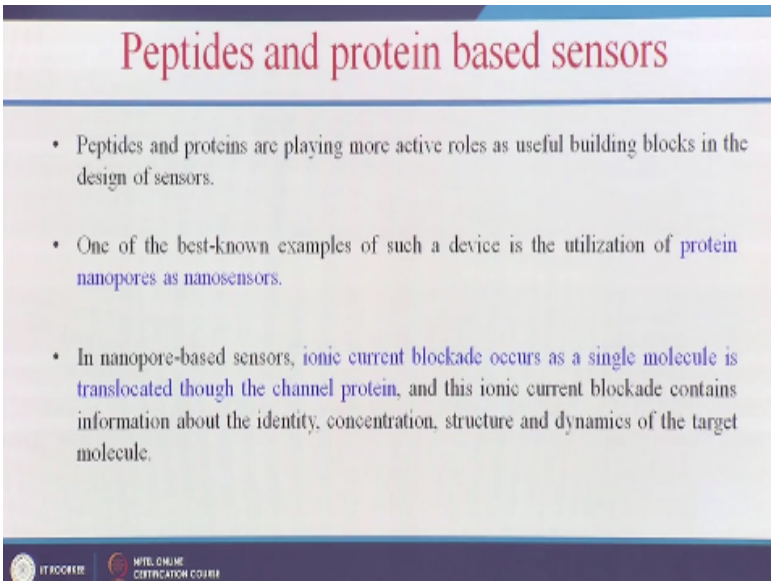

And another example is we can sue the peptide and protein for making sensitive sensors so one of the best known example is we can have a protein nano pores as nano sensors, so here we can sue the nano pore based sensor and the ionic current blockade occurs when a single molecule is trans located though the channel protein.

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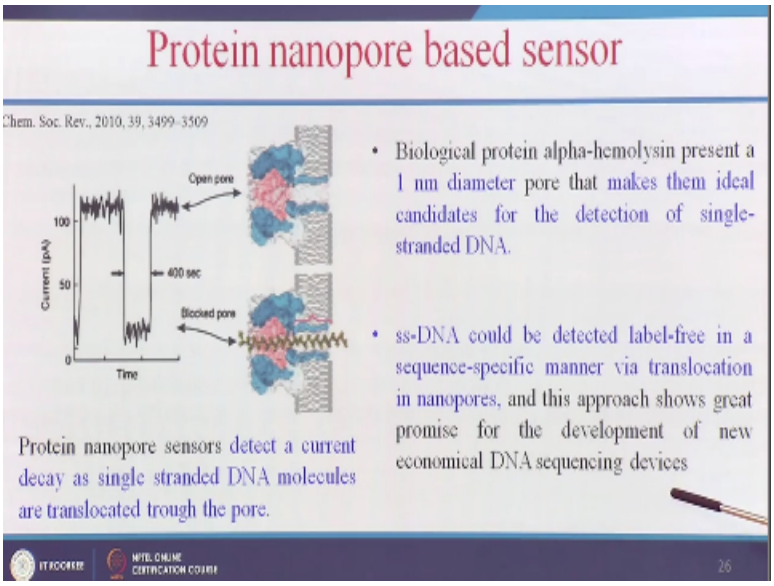

So let us see the example, here we can use the biological protein alpha hemolysin so here the biological protein alpha hemolysin present a 1nm diameter pore which makes them ideal candidates for the direction of single standard DNA. You can see here this is example so this is your alpha hemolysin plus protein bridge is having 1nm diameter pore so we can pass the single standard DNA through this and when the DNA pass through this nano pore there will be a decay in your current there will be a current decay.

So that can be detected and this approach it is showing a great promise for development of economical DNA sequencing device okay.

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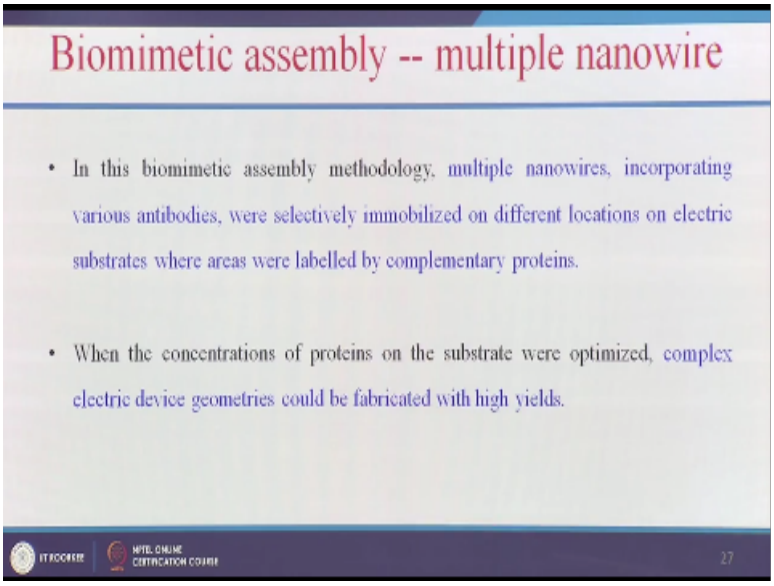

So let us see another example Biomimetic assembly of multiple nano wires so we v\can use the biomimetic assembly methodology and multiple nano wires with various antibodies can be immobilized on the different location on a electric substrates okay. So let us see how it can be made.

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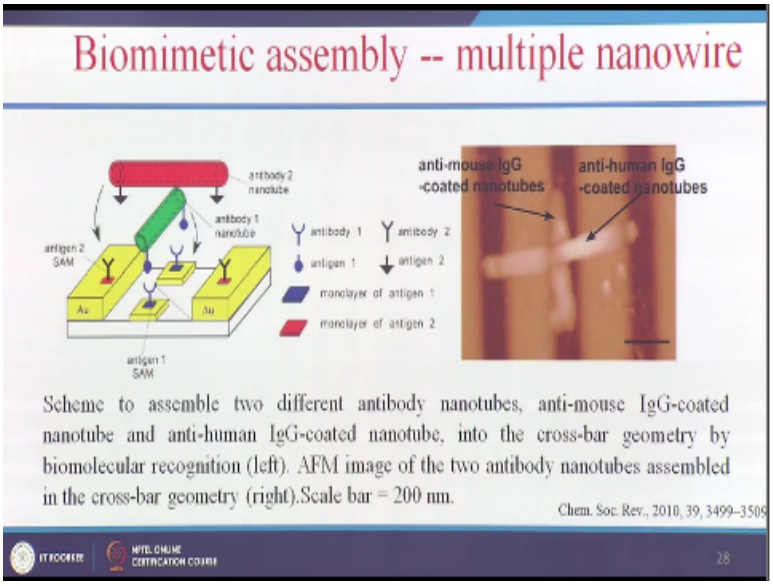

So here we can see here this is the one peptide nano tube and this is another nano tube protein based nano tube and these are having as anti body specially for the various antigen, so this green color will come and bind to this antigen, antigen one and this red color tube will come any bind to the antigen two okay. So when it binds it forms this kind of structure it is under the form we can see here this cross kind of structure, so using this kind of approach we can assemble the peptides and we can make the multiple nano wires. So this will have a very good application in making various electrical devices.

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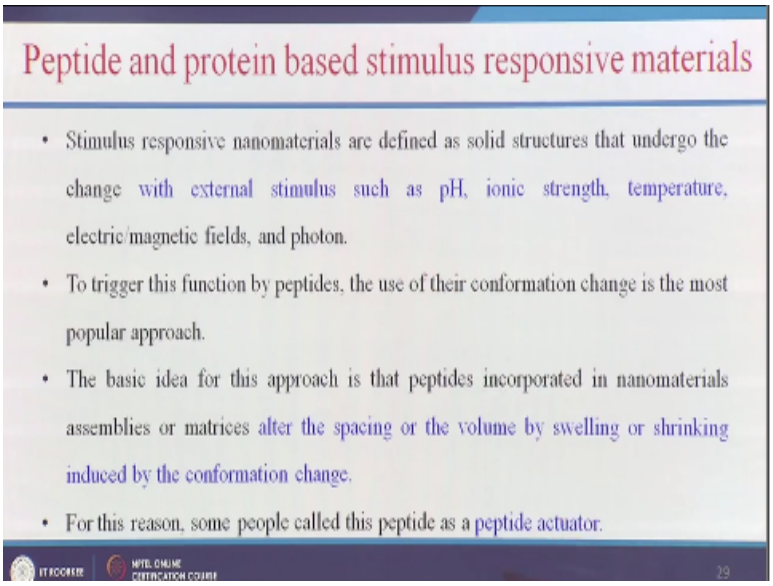

So let me see the another example how we can make protein or peptide based stimulus responsive material okay, so here we can make the stimulus responsive nano materials which are defined as solid structures which are undergo change with a response to external stimuli.

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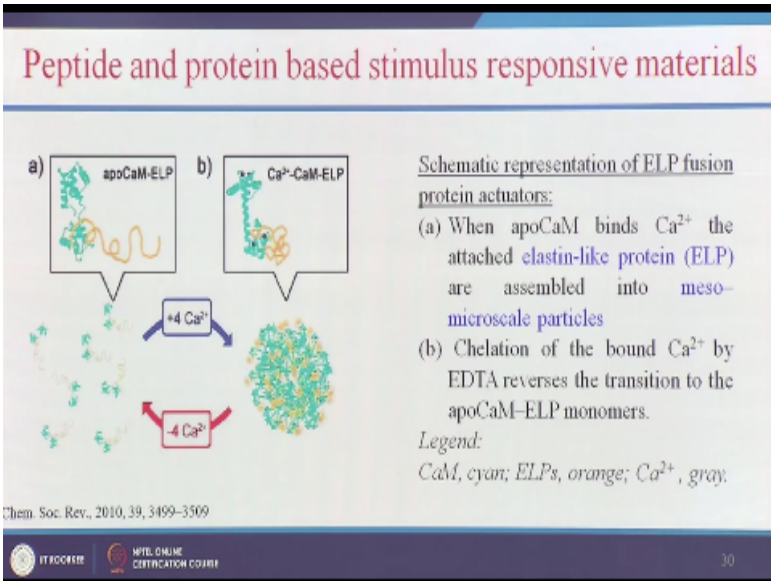

So let us see the example here you can use that apoCaM ELP so ELP is elastin like protein okay so this protein will assemble in to meso micro scale particles in presence of calcium and when you remove the calcium with the help of EDTA which is a chelating agent okay so when re move the calcium it again goes back it to the same apoCaM ELP monomer structure, that means in presence of calcium it is forming meso micro scale particle and when you remove this calcium it is going back to the monomer structure.

So this is when you load this kind of particles with the drug anti based drug or something so with the presence of calcium on the axis of calcium it can be release a drug and which can be useful for a like a stimulus responsive material.

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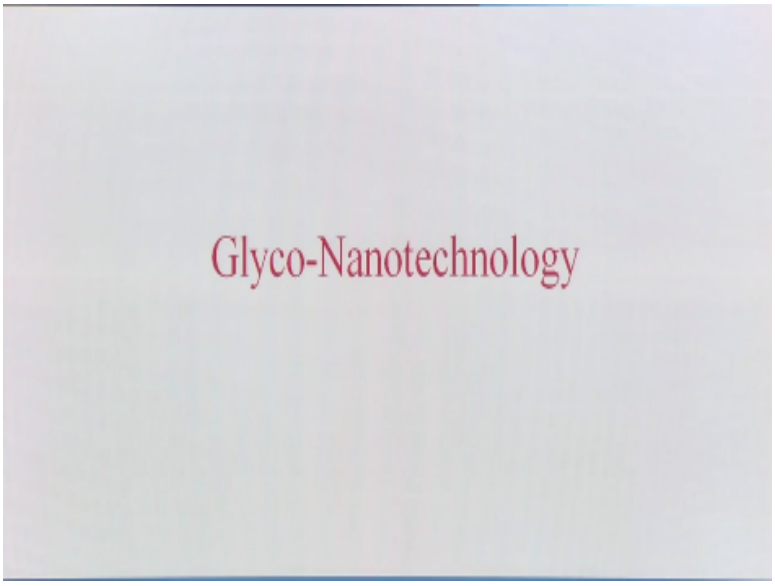

So we are seen several applications of protein based nano materials in the previous slides, so let us see what Glyco Nanotechnology and how we can use carbohydrate that is sure molecule for various nano material applications.

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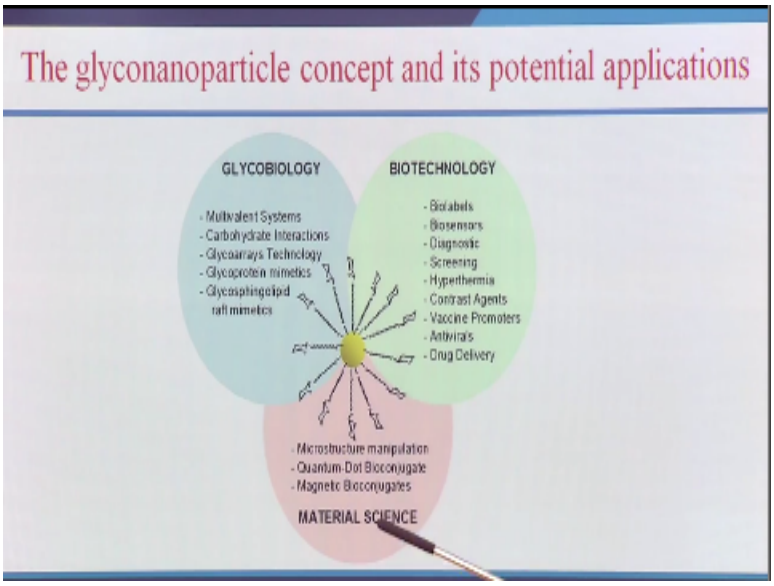

So this Glyco nanotechnology is combination of Glyco biology, biotechnology and material science.

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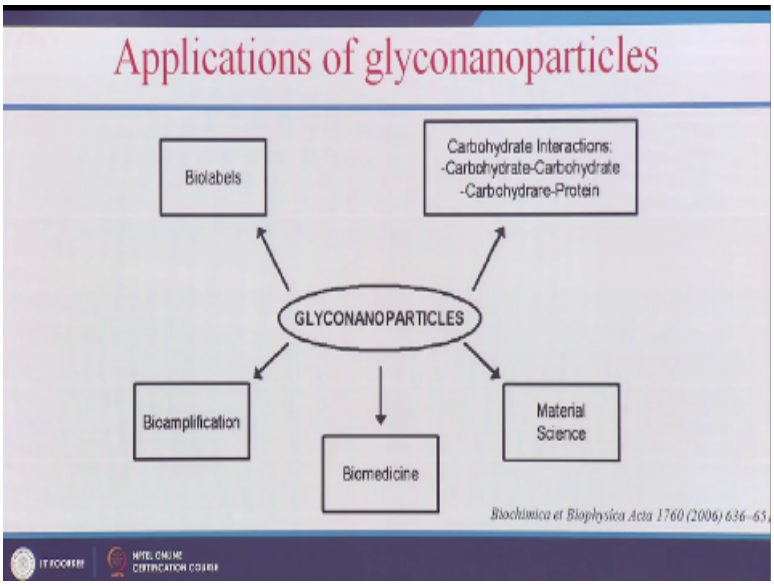

So here we can use that Glyco nano particles for various applications in this lecture we are see the bio labeling application as well as, we are going to understand carbohydrate interactions, how carbohydrate interactions and also carbohydrate protein interaction.

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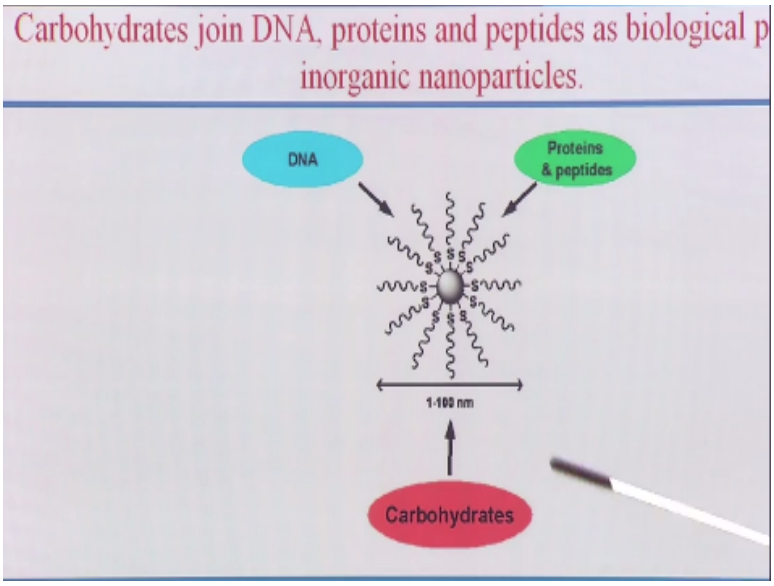

And similarly to DNA and protein we can also use carbohydrates as stabilizing or coordinating in organic nano particles and using that we can make a.

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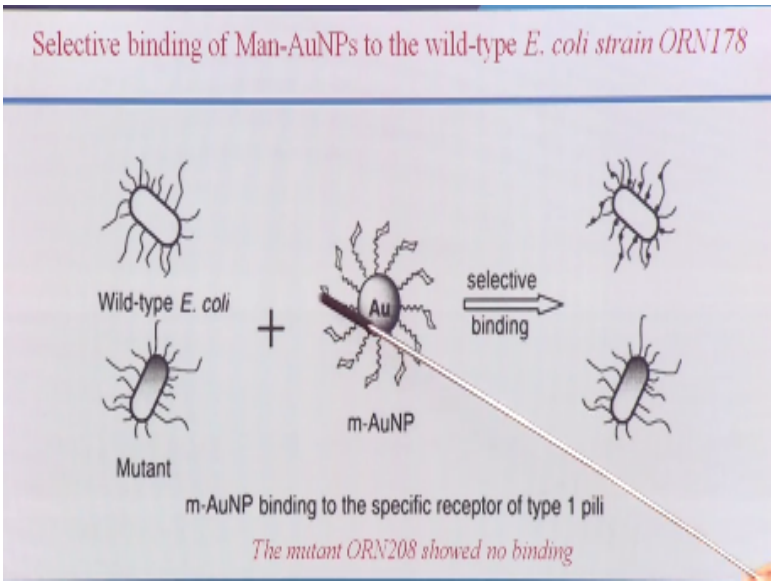

Very good bio sensor, so in this example this goal nano particle is coated with nano sugar. So this nano sugar specific for your wild type bacteria that is e coli bacteria that is wild type and it is not specific for the mutant and when you add this kind of nano particle and we can easily identify whether you are sample containing wild type bacteria or not.

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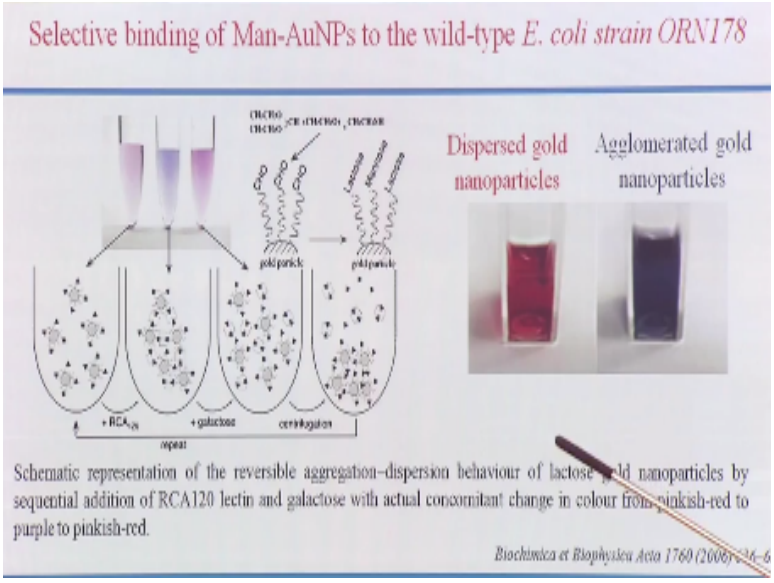

So here you can see the example usually the dispersed gold nanoparticles will be like ruby red color, when the particle aggregate or agglomerate it will form the purple blue color. So using the same principle of spr surface place on essence we can make a bio censors. So here you can see here this goal nano particle will specifically bind to the wild type e coli. So when I add these goal nanoparticles it will bind to the equality bacteria. All the goal nanoparticles will be aggregated.

So when it agglomerated it forms this kind of color change blue color and if there is no wild type bacteria it will be disposed, all the nanoparticles will be disposed, so it will give a this kind of color. So based on the spr we can make a very good bio sensors.

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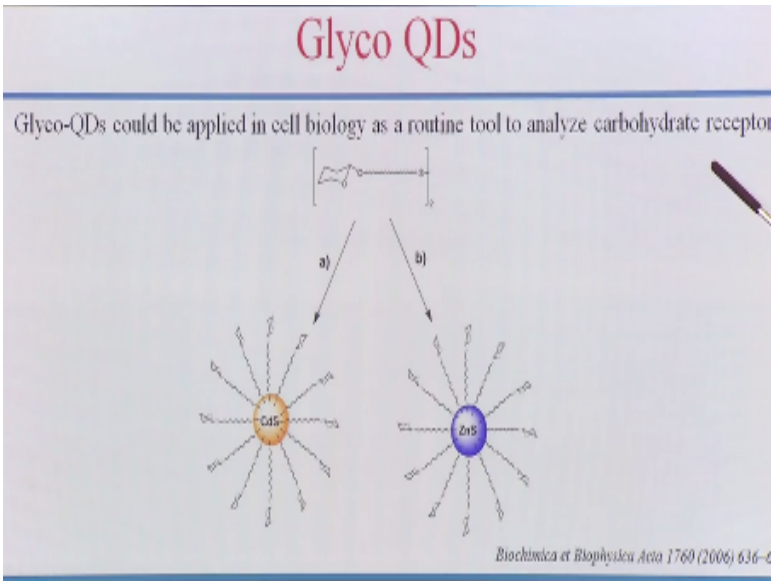

So let us see the another applications glyco based quondam drugs, so the quondam drugs is the semi conductor nano crystal which will give the florescence depends on the size and these condom dot can be quoted with this different sugar molecule. So these glyco quondam drugs could be applied as a routine tool to analyze carbohydrate receptors. So let us see the example.

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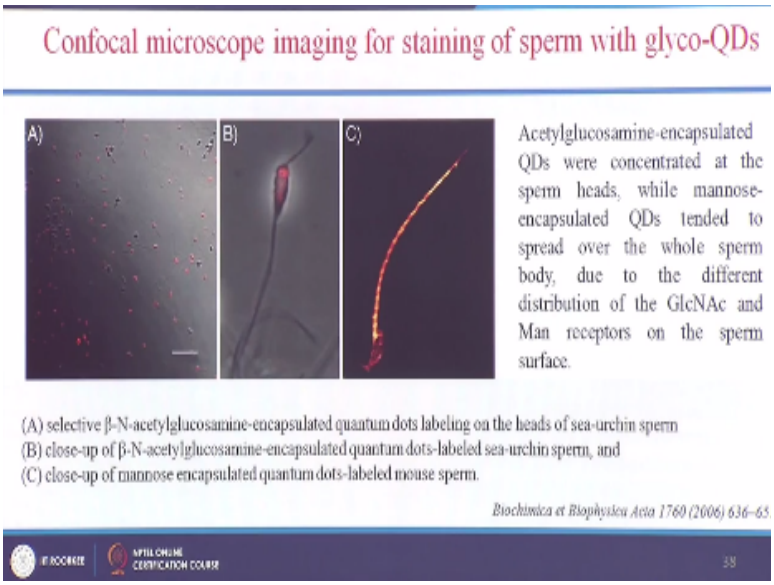

So this is the sperm and this sperm will be having differentiated of sugar receptors on the head region and different kind of sugar receptors on the tale region. So when you use this kind of condom dots quoted with the different sugars we can easily understand and study the different kind of cells okay. So here in this example the acetyl glucosamine encapsulated condom dots it is binding only to the sperm head and when you have the condom dots quoted with nano sugar these are binding to the sperm body.

That means depends on the sugar receptors on the cell this condom dots are binding, so it can be useful for various self specific labeling. So let us move on to other example.

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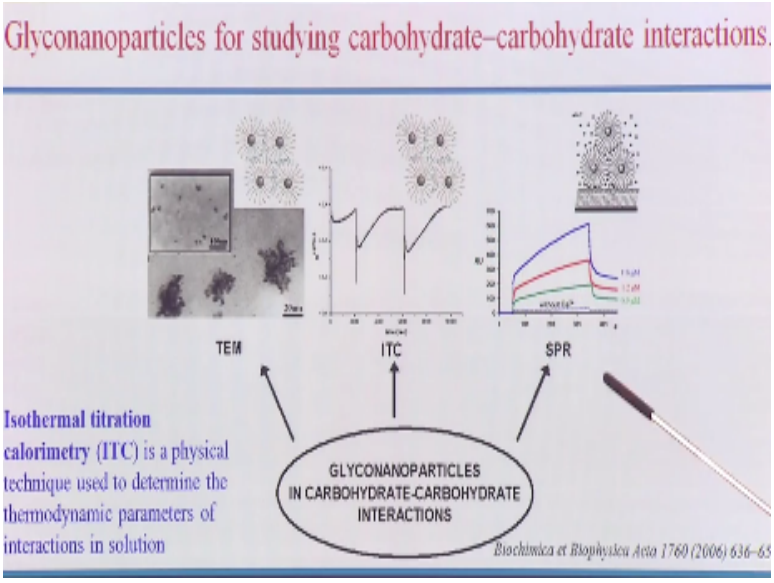

That is how we can study the carbohydrate interactions. So here also you can use the metal nano particles and they are quoted with the sugar molecule, so there is interaction within this sugar that is carbohydrate interactions is there. When you study under this you can see the aggregated particles and then the transmission hydro scope. We can also understand the carbohydrate and carbohydrate interactions by ITC. That is isothermal titration calorimetry, so it is physical technique use to determine the thermodynamics parameter of interaction in solution.

And we can also shed the interactions carbohydrate and carbohydrate interactions by using SPR. I have already told you that if the particles are disposed it will give different SPR, when the particles are carbohydrate and carbohydrate interactions it will give a different SPR and it will also give a different ITC value. When it is disposed it will be different value and when it is agglomerated it will give different kind of ITC value. So let us move on to another example.

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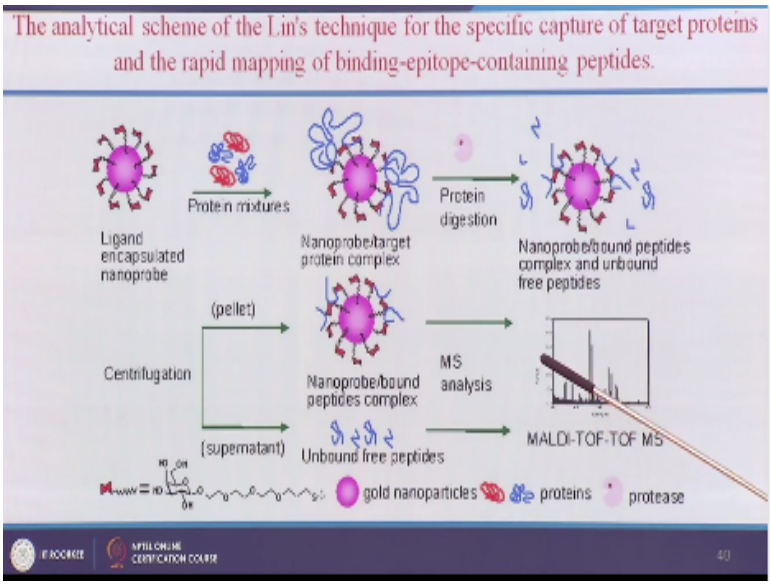

For understanding protein carbohydrate interaction, so here this goal nano particle is quoted with sugar molecule and when you add the protein mixtures the protein will come and bind and when you add the protein it will dig all your unborn proteins and when you do the centrifugation it will bound to the nano particle and when you do the mass spectrophotic analysis you will come to know which part of peptide binding to these to nano particles. So this Lins techniques using this we can understand carbohydrates and protein interaction.

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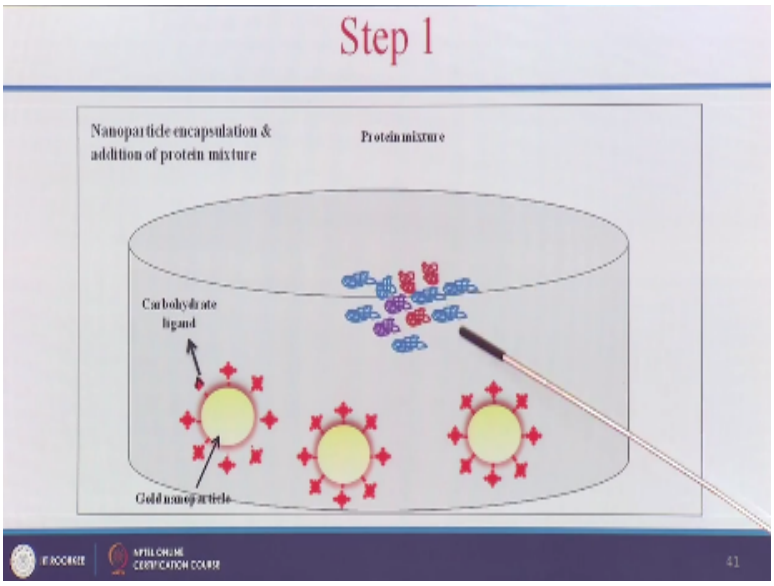

So let us see the same example using a simple animation okay, so here the nanoparticles is goal nano particles and this goal nano particles in the carbohydrate like an, so when you add this protein mixture and you will not understand. Out of this

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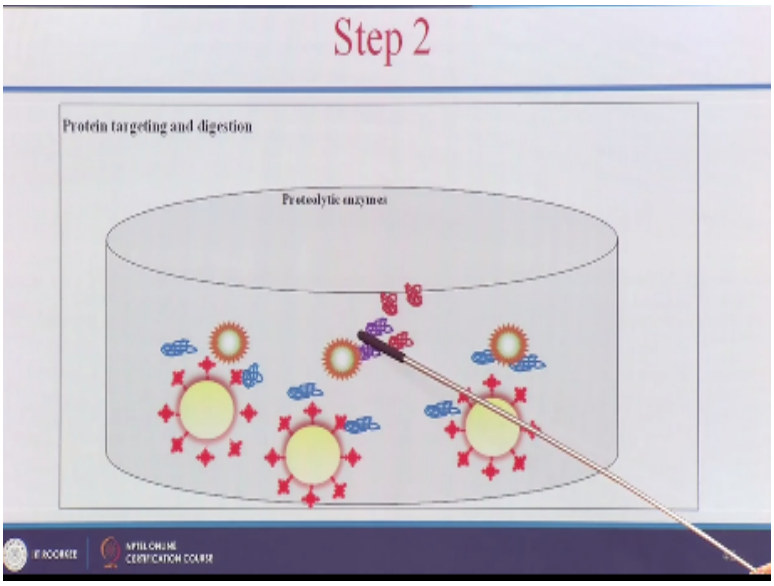

Which is having appetent for binding to these carbohydrate s you can see here out of this protein once it bind to this they can add the photolytic enzymes.

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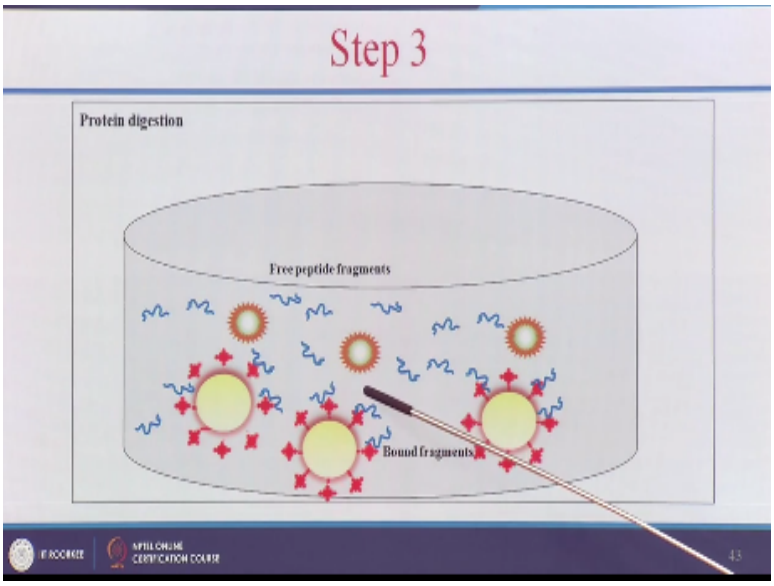

So that will degrade all the protein okay, so the proteins which is bound to these carbohydrates are some other than all other unbound pep tiles will be degrading using the proteins enzymes.

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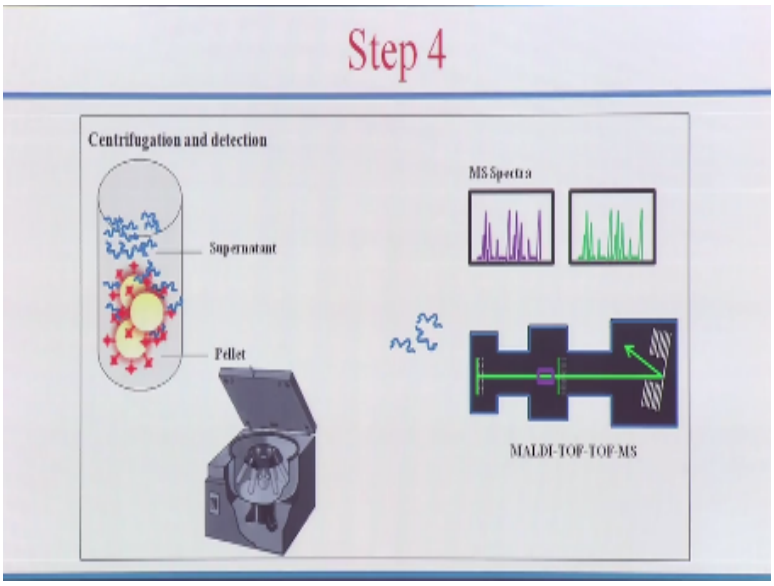

So how do we separate this unbound proteins, we can do the centrifugation, so when you do the centrifugation so the goal nano particles will be settled in and the unbound pep tile will be in superlatives. So we can remove that and we can study under the mass. So based on that you will get the information what kind of pep tile sequence are binding to the particular carbohydrate. So this Linz technique is very important in understanding the carbohydrate and protein interaction.
So let us see the example how we can use.

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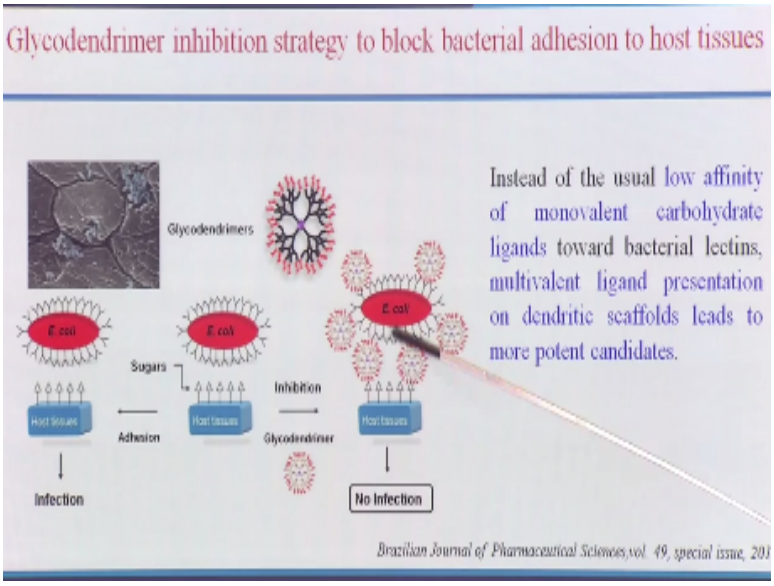

Glyco dendrimer for preventing the bacterial infection, so dendrimer means pre like structure, so here we are going to make sugar, so that will have more potent and will prevent the bacterial infection. You can see here the bacteria normally it will come and bind to the host tissues and it will cause infection that will bind to this equality and will bock the tissue to bind and it will prevent the infection. As the structure having multivalent ligament and it is very efficient in preventing the bacterial infection.

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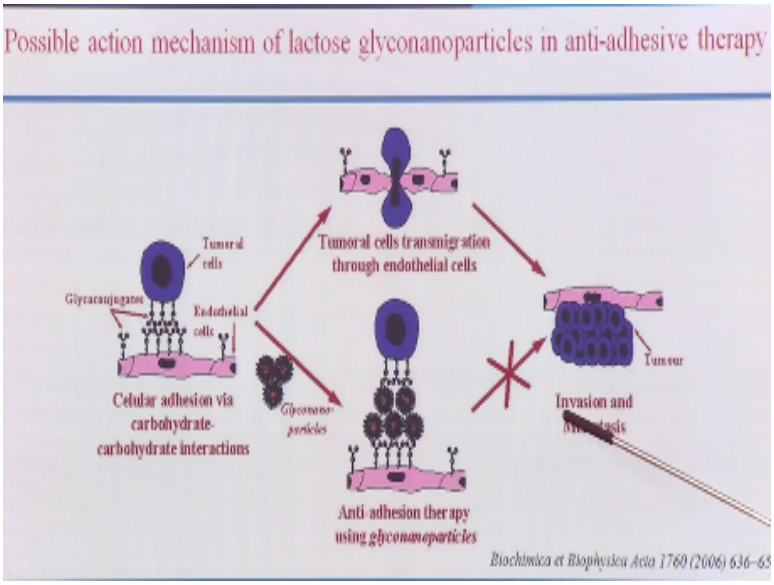

So let us see the example we can also have the lactose glyco nano particle for anti adhesive therapy, so it is useful for cancer therapy. Usually the tumor cell will come and bind to the endothelial cells and it will grow okay, so this step is called as metastasis. So how we can prevent this which can block this attachment the tumor of cell and it will prevent the invasion and metastasis.

As a summary we have learnt what is protein nano technology and under protein nano technology we have learn pep tile logo and using that how can we make nano particle and also we have learnt what is peptize and using this how to make nano tubes, how can we use this pep tiles and we also learn three main components of pepping and also study the applications of protein nano technology.

And under the Glyco nano technology we have learnt how we can use and also for sensing cancer therapy and as well as application carbohydrate and protein interaction. I will end my lecture here thank you all for listening and I will see you in another interesting lecture.

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