Drug Delivery Principles and Engineering
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Lecture – 26
Protein Absorption - II

Hello everyone, welcome to another lecture for Drug Delivery Engineering and Principles. Just a quick recap of what we learned in the last class.

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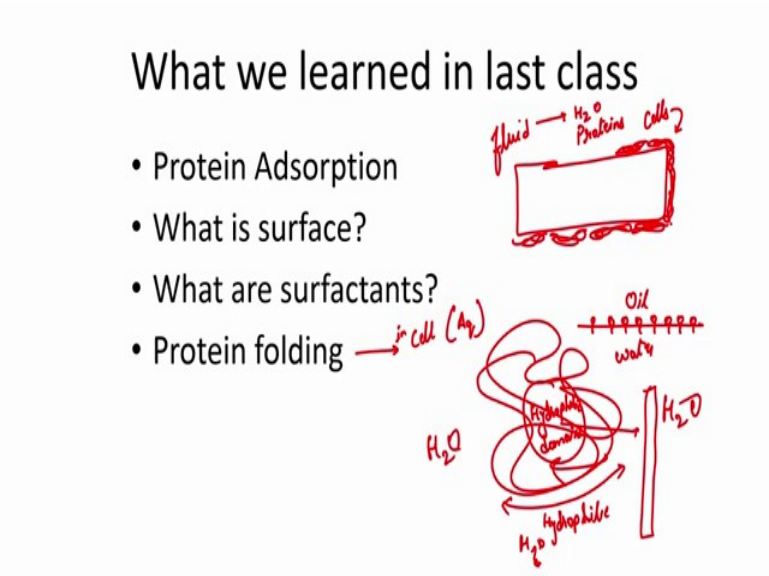

So, were talking about protein adsorption -why we are doing this is because we want to understand what happens when you put any foreign material in the body or in contact with any sort of body fluid. Let us say if this is my body that I am putting in, and the first thing is going to come in contact with of course, the fluid and the major part of the fluid in our body is essentially nothing, but water and then the next major component is proteins.

So, all those proteins that are present in a fluids will start to interact with this particular surface and then depending on how the surface is- whether it is hydrophobic or hydrophilic these proteins will then tend do adsorb on different areas and a different conformation on this surface and only then the cells which are again in the vicinity will come in and they will start interacting with the surface through these adsorbed proteins.

So, the cells actually do not really see the surface very well. What they see is actually the adsorbed protein layer through which they will start interacting.

So, that is why it becomes very important because whatever the cell is seeing is actually nothing, but the absorbed protein. So, we need to study what actually this absorb protein is and how can it be modulated to get that desired function. And so then we talked about in the process of understanding what protein absorption is first is what a surface? So, what defines a surface? How do we know that it is a surface it is an interface between whatever the two medium has been presented to it. We talked about what are surfactants.

So, again what are surfactant? Surfactants are nothing, but molecules that have both hydrophilic and hydrophobic domains and they can phase separate out hydrophilic and hydrophobic domains and essentially let us say if I say this is oil, this is water then these surfactants will essentially line up this interface with the hydrophobic domain going towards the oil phase and the hydrophilic domain going towards the water phase.

And so again this becomes important because what we are saying is proteins are mild surfactants because they contain several amino acids about nearly all proteins with large structures will have about 20 amino acids, all 20 amino acids. And then these 20 amino acids some are hydrophilic, some are hydrophobic even within the structure of the amino acid there are a hydrophilic and hydrophobic domains and because of that these proteins act as mild surfactants and their structure will change depending on what medium they are. So, if they find any hydrophobic domains or hydrophobic surfaces what will happen is the hydrophobic domains will start to come out and interact with those hydrophobic surfaces.

So, that is why we studied surfactants. Then we talked about protein folding which is again a very related thing. So, in natural process let us say if we are talking in cells which is nothing, but an aqueous media. Let's say this is the unfolded protein. So, the protein folding will be in such a way that all these external domains will be hydrophilic and the reason for that is because these external domains will have to interact with the water that is present in the surrounding.

So, they like to interact with the water. So, that is why all these hydrophilic domains will come outside and then all this inner domain will be hydrophobic and again the same

reason that these domains do not really want to interact with the water that is present outside.

So, they want to bury themselves and prevent any sort of interaction happening with the water outside. So, that is why protein folding becomes important because now what we are saying is when this particular proteins now suddenly start seeing a hydrophobic surface, these domains will tend to come out and then these domains will tend to go away and that is why the protein folding will change.

So, that is why we studied protein folding. Just in very very brief terms I mean protein folding it itself is a very complex phenomena in a whole course can be designed on it, but what we talked about is just some general concepts of the way these proteins are going to fold.

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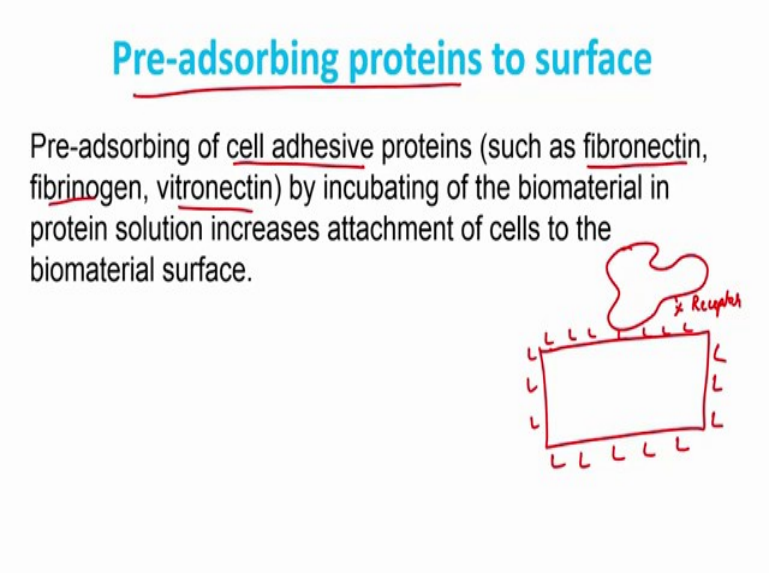

So, having done all that and having established that these surfaces can modulate the protein folding and protein adsorption, another concept that is now being used in the field quite a lot is the pre-adsorption of these proteins to surfaces and why would you need to do that? So, let us say if I have a surface and I want this surface to signal the cells in a certain way. So, I mean if I have a cell that is going to interact with it and I want this particular surface to interact with the cell ligand , x.

So, for that to happen I want to make sure that whatever protein gets coated on the surface has some affinity for this ligand x or this receptor x. So, this is a receptor and let us say I am putting some ligands on the surface.

So, I may or may not put ligands, but essentially I want this surface to interact with the cells through this x. So, one of the strategy the use for that is to pre-adsorb proteins that we know is going to interact with this receptor x and that is one way that I can control the signaling that is going to happen through the surface. See the reason for that is because once I put it in the body or once I put it in contact with the body fluid there are all kinds of proteins in the body fluid, I mean we are talking about thousands and thousands of them and so the probability that let us say a certain protein will come and adsorb to it is fairly low.

So, to prevent it what we do is we first treat it with a solution containing only this ligand L and that will ensure that at least some of the ligand L will remain on the surface and can interact with the cell. So, some of the proteins that are very widely used for this is fibronectin, fibrinogen, vitronectin and these are nothing, but these are proteins that contains a adhesive sites.

And so if I intubate my biomaterial with these proteins for some time what will happen is these proteins will then coat the surface completely and then I can put this implant with cells or in the body. And these proteins may still come off and were going to talk about this in next few slides, but it increases the probability that one of these proteins will now start interacting with this cell receptor x and give the signaling that I want this surface to give.

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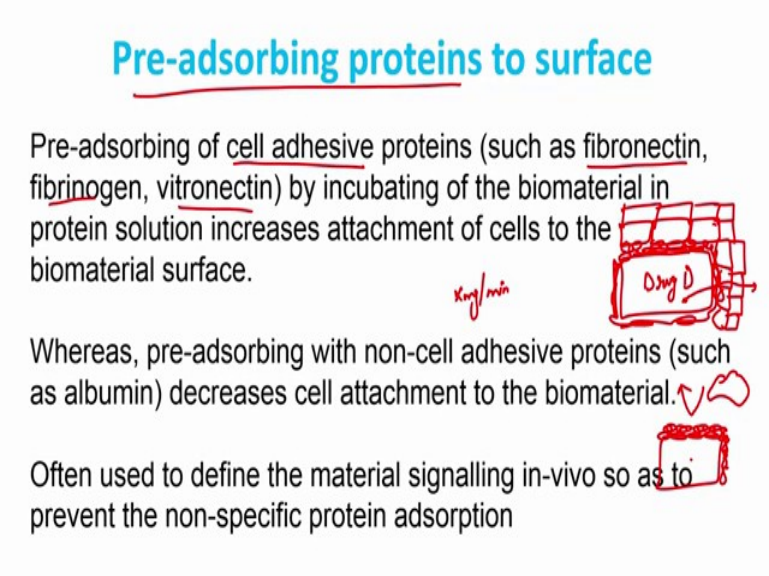

So, whereas, the pre-adsorbing with a non-cell adhesive it can also be used. So, let us say if I want to put an implant which I do not really want to interact with the cell. Let us say I have a implant that is carrying a drug D and all I want to do from this implant is for it to release the drug constantly and not have any sort of unknown proteins coming and absorbing making a layer causing this diffusion of the drug to be difficult from this implant. So, we know that proteins will adsorb to it we cannot avoid it. So, why not let us just absorb something that does not interact with cells.

So, let us say if I directly implanted what will happen? all kind of random proteins will come and essentially adsorb on the surface and once they do that I am not sure that what will that signal to the cell whether they cause a massive cell layer to form on the surface and then that can again lead to cascade of events that can interact with another cell and make a big big layer.

So, that is the last thing I want if I want the drug D to release in the system because now what is happening is not only the drug has to diffuse out from a matrix, but now it has to diffuse through this protein layer and through multiple cell layers which is going to be very challenging and it is something that I cannot control right. Because I initially designed this let us say to release x milligram per minute or something, but now once this sort of cell layer and protein layer has been formed I do not know whether it is going

to release x milligram per minute maybe it is going to come down to X by 2 ,maybe is by x by 4.

So, as a clinician I am very worried now because I do not know how much dose I am giving. So, to prevent that what you can do is you can essentially coat it with a known protein that you know is not going to interact with cells. So, even though you do have a protein layer now, what you do is you prevent the cells from coming in and adsorbing on it. So, that way atleast I know that what is the diffusion of this drug from this matrix through this protein layer and that way I have some good idea as to how much drug is getting released per unit time or per day or whatever it might be.

So, this is one application I am giving you there could be several other applications- maybe the implant is such that we do not want the immune cells to interact, maybe it is carrying cells inside and we do not want immune cells to come in and sort of kill those cells away. So, these are several applications that you can think of in that direction and then several other strategies apart from pre-adsorbing proteins and all of this we are going to talk about in the future classes in this course, but that is just one example that I am giving you.

So, these are things that you can play around with protein adsorption it itself to get some desired result. And then next thing is that often you should define the material signaling in-vivo as well. So, you can prevent non-specific protein adsorption. So, one thing is to prevent the cell and then another thing is to prevent any kind of protein to adsorb on it. So, let us say if some enzymes absorb to my surfaces and they start degrading these surfaces or they start degrading the drug itself. So, again you can control that by pre-adsorbing under proteins and that can somewhat either delay or at least completely abrogate any of this process from happening.

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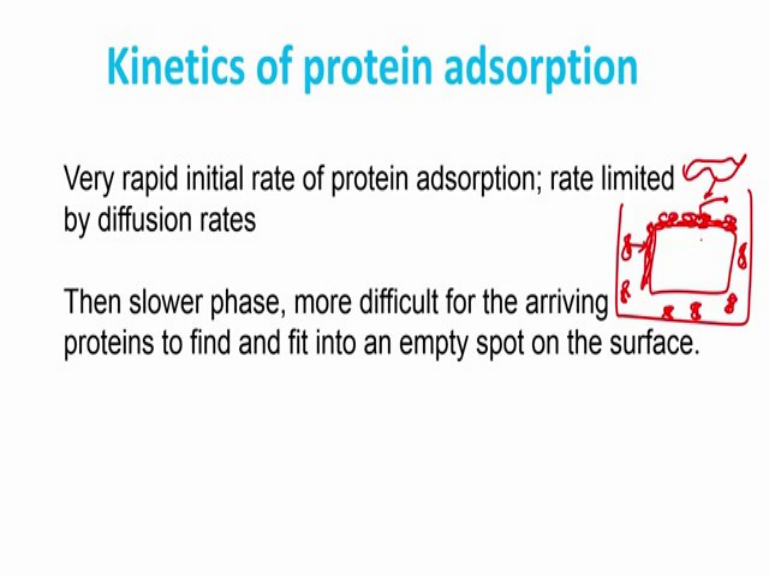

So, let us talk about some kinetics of protein adsorption. So, I initially said in the introduction of this class that the first thing that the implant is going to interact with is water because that is the most abundant fluid around and then the next is going to be proteins and the cells are going to interact through that protein. So, why is that and then the reason for that is that the protein adsorption is a very rapid phenomenon. So, the only thing that limits protein adsorption is the diffusion of the protein from the fluid to the surface of your implant. So, what essentially that means, is if I am putting an implant and I am looking at the implant after a few seconds or few minutes that implant at this point, I believe, would be completely coated with your proteins that are present in the surrounding media.

So, let us say this is your surrounding media, you have proteins. The only limitation of protein adsorption is essentially the diffusion of this protein to reach the surface. Once it reaches the surface we are talking about less than milliseconds for these proteins to adsorb onto the surface. And then what will happen is once proteins have come in and adsorbed, the initial layer of the protein, then let us say another protein is now trying to come - this protein has no space to directly interact with this particular surface it can start interacting with the proteins that are coated on the surface because these proteins may also have some domains which are now getting exposed which was not earlier present, but then this particular protein which is arriving late or the slower phase will have to either remove these proteins from the surface or will not be able to interact the surface

directly and we will have to interact with the already coated layer. So, they will find the empty slot maybe these proteins are large and there is a small protein that can diffuse into these empty slots.

So, those will be able to go in, but then eventually all of the surface will be covered fairly rapidly and it will be very difficult for any further protein to come and interact with it.

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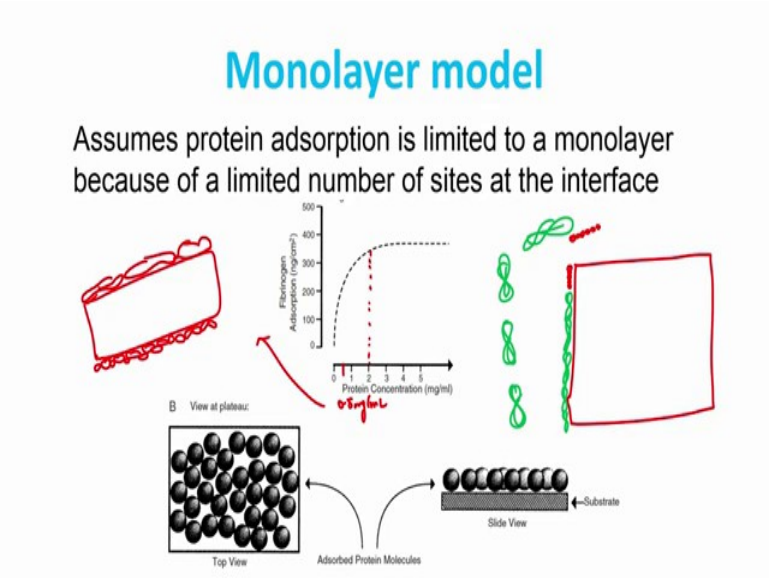

So, then there are several models that are being used to sort of study this protein adsorption. So, one is a monolayer model which is a fairly simple model and what it is it assumes with the protein adsorption is limited to a monolayer. So, what it is saying is let us saying is- if I have a surface. So, what this monolayer model assumes is let us say if there are proteins in the vicinity these proteins will adsorb onto the surface and if there is another protein that needs to come in let us say I have another protein that wants to come and adsorb on the surface, it cannot come and adsorb on top of the surface.

So, this is no and the only thing that can happen is this comes in it, removes one of the protein unit and then goes and start to interact with that empty space that is being created. So, that is a simple monolayer model and if you go by this what you are saying is you have let us say a certain protein concentration being adsorbed. So, as you increase the protein concentration the amount of protein adsorbed is going to increase and we will

come and discuss as to why this will increase and why this will not be constant and just bear with me for a couple of slides.

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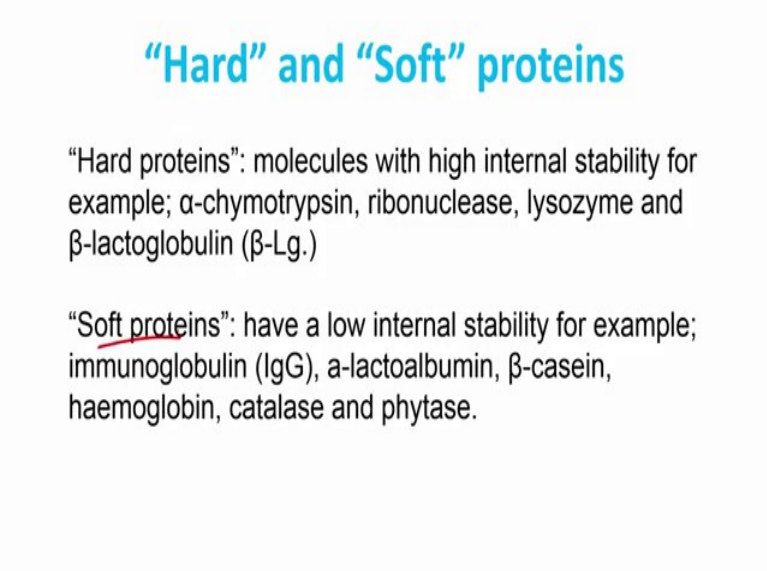

Then there is another concept which is hard and soft proteins. So, what does that mean so; that means, that some of the proteins could be fairly hard meaning they are not very flexible. So, some of these examples are given here ribonuclease, lysozyme and other proteins they are considered to be very hard. So, they have very high internal stability which means that they will not really change the structure a whole lot they will still interact with your surface, but the structure of the protein will still remain and it really is not going to change a whole lot from what the initial structure was. And then the other one is the soft protein and which ,as the name suggests, is nothing, but their very low internal stability which means that if they find a new surface the structure can change quite a lot depending on the contribution of the surface hydrophobicity and hydrophilicity.

So, some of those examples are IgG, beta-casein, haemoglobin and several other examples actually most protein will find are soft proteins and their structures will easily change.

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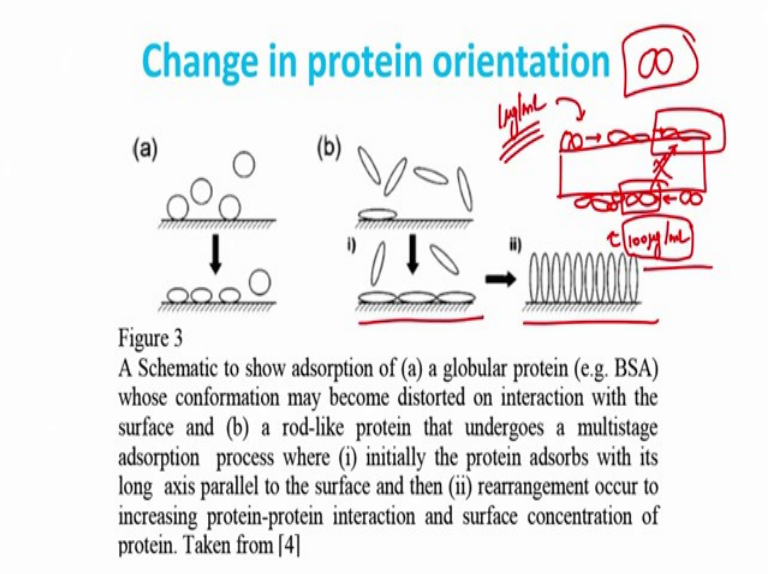

Figure 3
A Schematic to show adsorption of (a) a globular protein (e.g. BSA) whose conformation may become distorted on interaction with the surface and (b) a rod-like protein that undergoes a multistage adsorption process where (i) initially the protein adsorbs with its long axis parallel to the surface and then (ii) rearrangement occur to increasing protein-protein interaction and surface concentration of protein. Taken from [4]

So, next thing that I was initially talking about was this protein orientation and what essentially; that means, is this a few examples here. So, you can have a globular protein essentially meaning some sort of a big large protein with a spherical like structure that comes and there is some change in the structure of this as you can see these spheres are now become more like ellipse and it coats on a surface the other model could be - depending on the concentration that you have used, let us say this ellipse shape protein comes in you can have a configuration where very little amount of protein as adsorbed, but still covers the surface compared to an orientation where a lot of protein has adsorbed and onto the surface. So, what you typically find in the literature is and during the experiments is that this keeps on varying depending on the concentration you are using.

So, let us say if I have a surface and I come with one microgram per ml concentration of a protein and so what will happen is since I am talking about very low concentrations of protein in the solution it is sort of diffusion limitation as to how much protein can come in and adsorb on the surface. What you will find is let us say I have a protein which has this 8 structure. So, it will come it will sort of start interacting with it and what will happen over time is it will start to expand on it because it can find more space. So, this 8 structure gets elongated and elongated.

Hence the structure has changed quite a bit plus a single protein is occupied quite a large space. Now consider a case with the same example where let us instead of 1 microgram

per ml I come in with 100 microgram per ml, now on this particular surface. So, now, what will happen now I have a lot more protein concentration in the surroundings. So, the fusion will not be as limited. So, the proteins will come fairly quickly.

So, let us say a protein gets adsorbed. It is able to change the surface a bit because it still has some more space to which it can absorb to, but by the time it goes to this configuration all the rest of the sites are occupied because th quite a high concentration of protein that is present in the vicinity. So, all of them are coming in and essentially occupying the surface. It cannot really expand do something like this state that is not happening and the same thing will happen if I go at a even higher concentration.

So, what you will find is not only the protein adsorption is dependent on the type of proteins that are present in the surrounding, it is also dependent on the concentration, because the concentration can change the orientation of the protein orientation as well as the structure of the protein. So, if you look at this structure this is different than the structure which is here, even though you use the same protein to start with, but at different concentrations. And then now if I go back to two slides earlier what I was saying. So, let us say if I now have a protein at a concentration of 0.5 mg per ml.

So, the amount of protein that is going to absorb is going to be different than let us say at 2 mg per ml or 3 mg per ml and the reason for that is the proteins that are coming to adsorb on the surface have time to change their orientation and still occupy further space. So, you will have a scenario where what you will get is essentially at lower concentrations you have quite a little protein on a surface whereas, at a higher concentration you may have quite a bit of protein that is getting adsorbed because there is enough protein in the vicinity and there is not enough time for the protein to change it is orientation.

So, that is why you see a curve like this even though it is the same protein ,fibrinogen, that was given to the surface, but depending on the initial concentration you get this sort of increase and then once you reach a saturation concentration where diffusion is no longer a limitation there is enough molecules in the vicinity that will completely saturate the surface, then it does not matter. So, in this particular case this is shown to be more at around 2 mg per ml going to it different for each protein that you are handling with, but

all proteins will typically show a structure like this and this of course, is a assumption with a monolayer model and we will talk about other models as well in this case.

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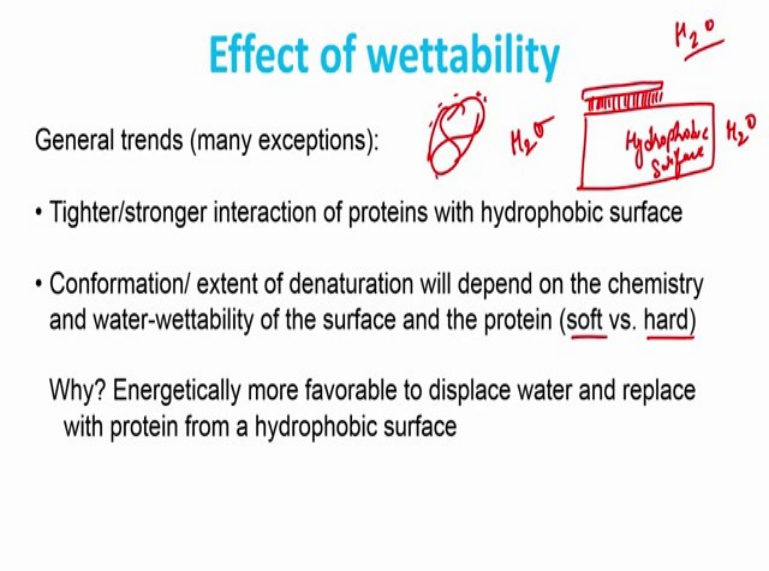

So, what about the effect of wettability? So, the general trends if you assume this model about protein adsorption is that proteins will adsorb quite tightly and strongly to hydrophobic surfaces and that is very obvious. Because let us say if I have a surface which is fairly hydrophobic then the proteins can change the structure quite a bit on this. Let us say this is a protein that has come and started interacting and there is lots of van-der waals interactions that is going to happen between the protein and the surface and there is really no competing force for this hydrophobic surface because everywhere in the surrounding the fluid contains water and that is hydrophilic.

So, these hydrophobic domains will be very happy interacting with this hydrophobic surface and they do not really want to change whatever is the structure because the surrounding is hydrophilic. So, typically what is seen is that very tight and strong interaction of proteins with hydrophobic surfaces. The conformation and the extent of denaturation will also depend on the water wettability again first of all it will depend whether the protein is soft or hard.

So, typically hard proteins will not change the structure, the soft proteins will change the structure, but quite a bit and then the more hydrophobic is the surface the more the change in structure and then again the reason for that is very similar because earlier the

proteins were situated in water and all they are hydrophilic domains were outside whereas, to interact with the hydrophobic surface their structure almost has to go to a complete overhaul where all the inner hydrophobic domains are going to come out and start extracting interacting with the surface.

So, you will see that quite a bit of change that will happen in this structure. And then again as I said it is very energetically favorable for them to displace water from the surface point of view as well right the surface also does not really want to interact with the water. So, surface is also very happy when it comes in contact with these hydrophobic domains. So, we will stop right here in this lecture and we will continue further in the next class.