Introduction to Mechanobiology
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Week - 02
Lecture - 08
Rheology of biopolymer networks

Hello and welcome to our 8th lecture of introduction to mechanobiology. So, in the last few lectures I had discussed how rheology of ECM networks has a huge importance in dictating their mechanical properties, and in shipping the overall responses of the cells. So, one of the important properties of collagen networks or many biopolymer networks is this aspect called stiffening strength stiffening. So, the more you deform the more difficult it is to deform. And I argued that this is an inbuilt property that ensures that these tissues or the networks do not get damaged as a consequence of forces. Because the more stiffed will be it is going to be very difficult to deform it anymore ok.

I will discuss about another procedure another way in which non-linear elasticity can be embedded or incorporated into the function at the single protein level. With that today I will first discuss this protein called fibronectin fibronectin.

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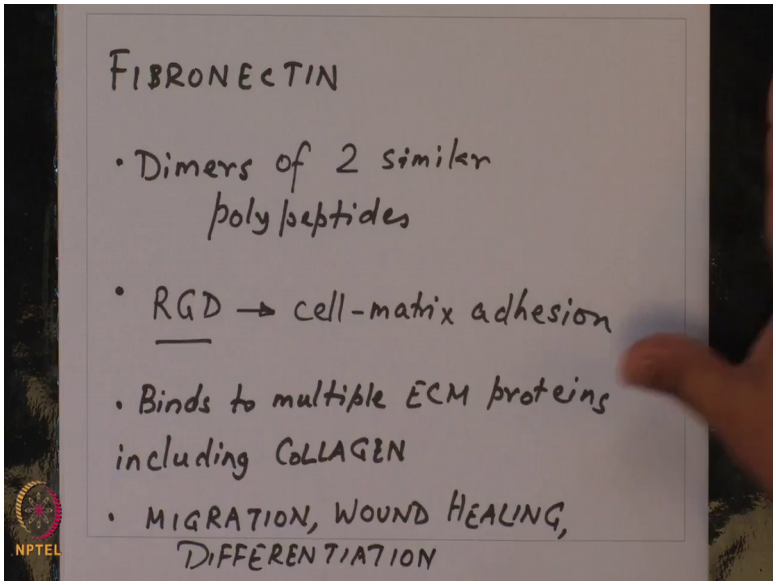

So, fibronectin is one of the most important ECM proteins. It forms dimers of 2 same similar polypeptides. So, notable in this molecule is the sequence of RGD which has

been shown to engage integrins or participate in mediating cell matrix adhesion. So, fibronectin is known to bind to multiple ECM proteins including collagen. It also binds to other fibronectin molecules and proteoglycans ok.

So, it is of key relevance to multiple cellular processes including migration wound healing and differentiation. So, if I were to draw approximately the structure of fibronectin.

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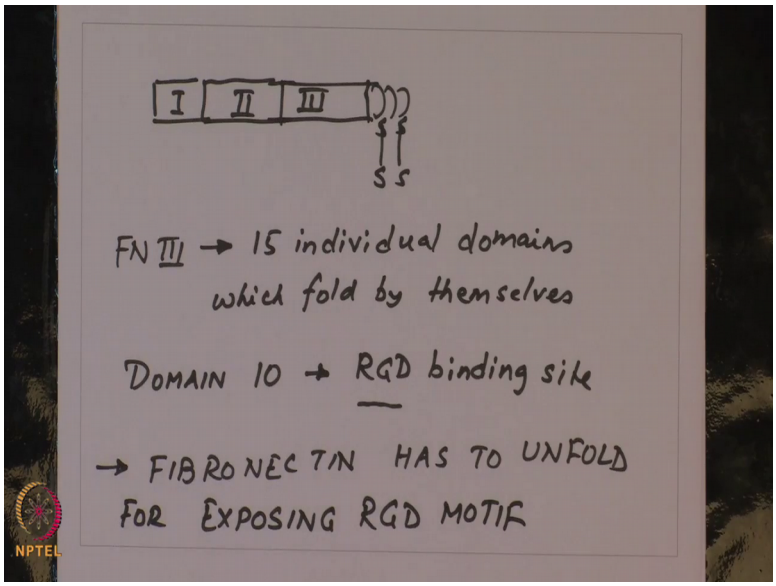

So, you have these 3 modules, 3 modules and there is some extract and the disulfide bonds are actually at the center. And you have the same thing repeating here ok.

So, binding so, many of the binding so, if I particularly look at this module FN 3 it has. So, FN 3 has 15 individual domains which fold by themselves. So, the question here is and of these 15 individual domains one of these so, domain 10 contains the RGD binding site. So, this RGD is actually hidden within this structure. It cannot be recognized by integrins directly. Which means that for this binding site to be exposed it must unfold. So, what this means is fibronectin has to unfold for exposing RGD MOTIF. So, this has to unfold.

Now, you have studied in biochemistry that unfolding can be done using let us say the agents like urea, or by temperature you denature proteins using temperature, but what is it that leads to unfolding in vivo? So, what it turns out it is actually forces exerted by

cells which unfold individual modules and this unfolding exposes cryptic binding sites. So, exposure of cryptic binding sites via unfolding triggers binding of FN 3 modules to other molecules, ok.

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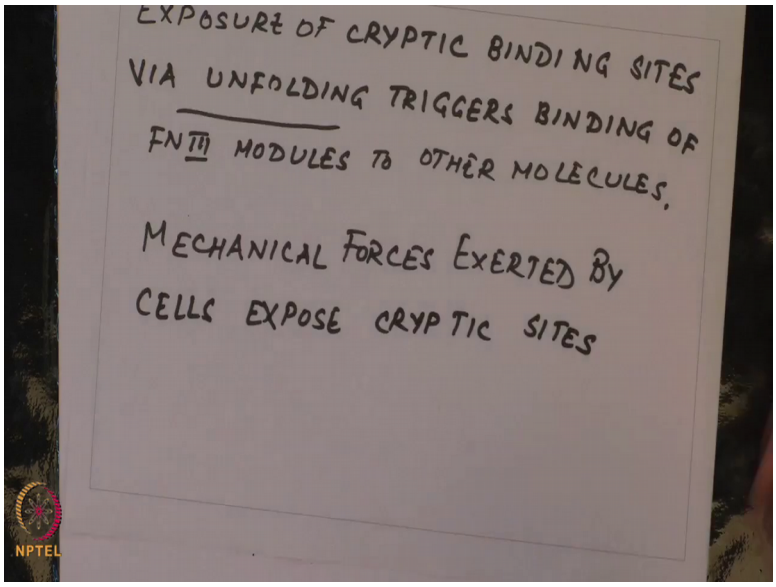

So, it is unfolding right. And how is this unfolding achieved? Via forces, mechanical forces exerted by cells expose cryptic sites. So, this raises a possibility. So, you have FN 3 module.

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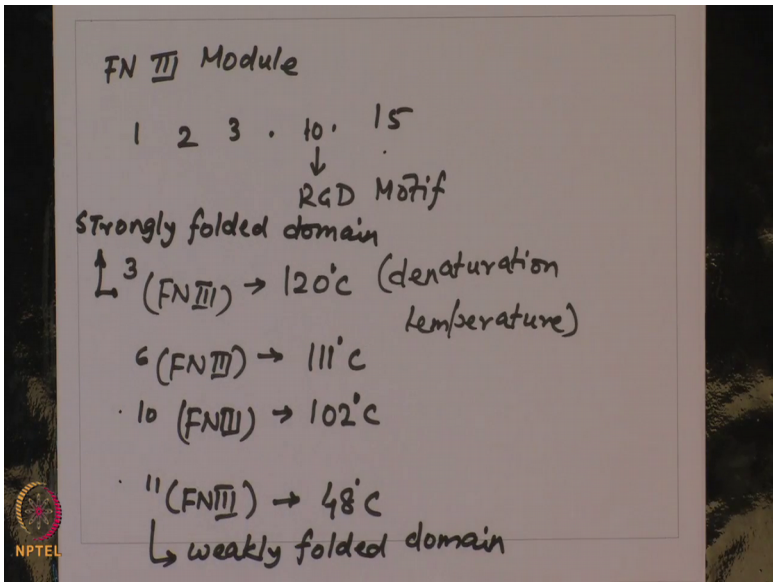

Which has these domains 1 2 3 up to 15. And out of this domain 10 contains the RGD MOTIF ok.

So, for the same molecule imagine you have 3 different types of cells, which have different capability to exert forces. So, depending on the amount it exerts amount of force it exerts it is possible that one or more of the cryptic sites are exposed. So, the magnitude of the force will influence how many of these binding or cryptic sides could expose. And since exposure of cryptic sites opens up this binding of other molecules and activation of signaling transduction signal a signaling cascades, this would mean that the magnitude of force can influence the signaling cascade which gets activated ok.

So, in an attempt to understand how these individual domains are sensitive to forces, experiments were done temperature deviation and experiments were done, and it was found for example, that FN 3. So, your third domain of FN 3 roughly denatures at 120 degree celsius. So, compared to that the 6 domain of FN 3 it denatures at. So, denaturation temperature. The 6 domain of FN 3 is around 100 and 11 degree celsius ok.

Similarly, tenth domain of FN 3 which contains the RGD binding sites unfolds at denatures at 102 degree celsius. So on and So forth and in this way. So, the weakest among the weakest domain is the eleventh domain of FN 3 module, which denatures at forty 8 degree celsius. So, this tells you that there is a gradation of sensitivity of these individual domains to denaturation.

Now, if a domain which unfolds at a higher temperature, this would suggest that this domain is much more stable than any of these other domains. So, this would indicate that domain 3 is among the it is a strong strongly folded domain. And domain 11 is a weakly folded domain. So, one of the challenges of this is of course, temperature denation is denaturation need not necessarily give us a clue of whether the same holds true for in vivo situations, when these molecules are exposed to forces will they unfold in a similar manner.

So, this calls for a technique which we can employ for tracking the unfolding dynamics of the whole molecule and understand what is the strength of individual folded domains.

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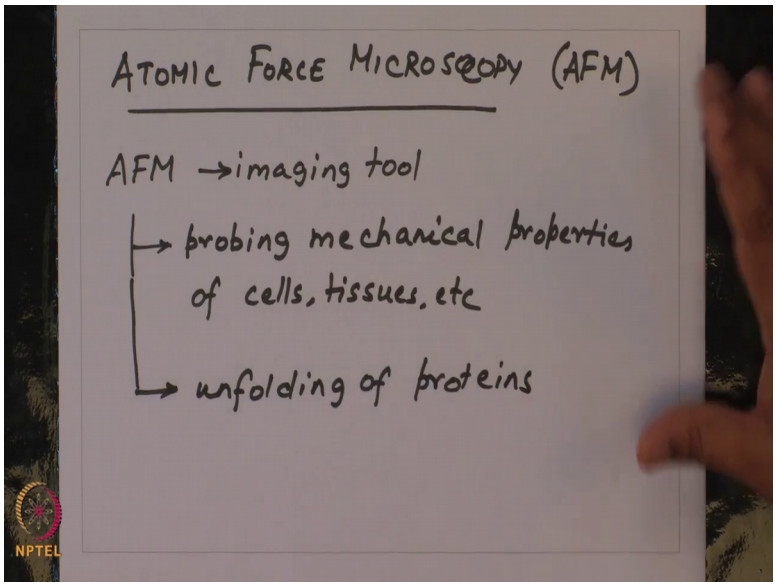

So, one of the techniques which has been employed exhaustively for studying the unfolding dynamics of various proteins is atomic force microscopy. This in short it is referred to as AFM. So, originally AFM was discovered as an imaging tool, it was discovered as an imaging tool; however, in addition to imaging subsequently it has found wider users in other techniques, one of which is for example, probing mechanical properties of cells tissues etcetera ok.

And it has also found use for studying unfolding of proteins. So, let us see how what exactly is an AFM and what does an AFM have. And how would one use an AFM for studying protein unfolding ok.

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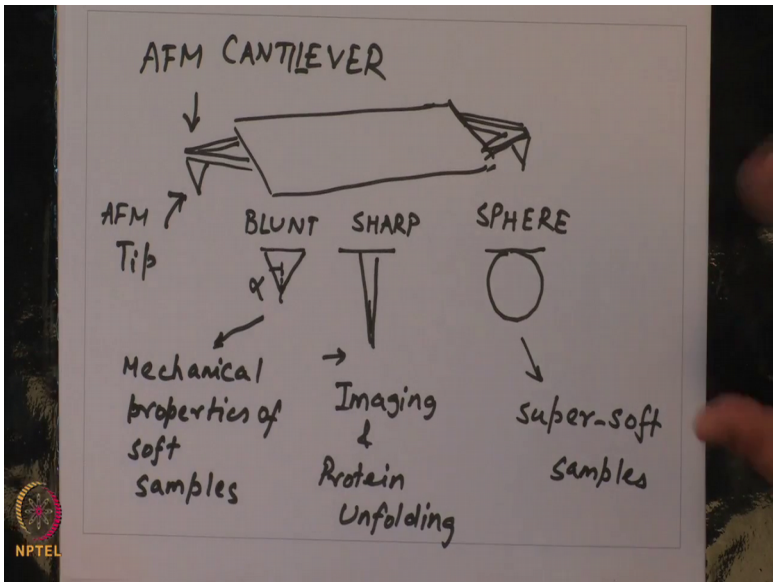

So, if I were to draw an AFM. So, what you have. So, these are tips. So, this is called a AFM cantilever, and this is called an AFM tip it is called an AFM tip. So, you can have various geometries of these tips. So, for example, if you want to image something your tip so, if I were to just draw the tip geometries. You can have tips which are very sharp, you can have this is let us say a blunt tip. You can have a very sharp tip or you can have the tip is nothing but a spherical ball sphere ok.

So, these the tip geometry so, among the other things what you have is the tip geometry. So, this half angle is different in these cases. So, this sharp tip is much preferred for imaging purposes. As well as putting unfolding purposes. This blunt tip is used exhaustively for proving mechanical properties of tissues cells and tissues soft samples while this sphere. So, in comparison to the block blunt tip sphere is preferred for super soft samples. So, based on the overall geometry of the cantilever tip, what you also have is an effective stiffness of this tip ok.

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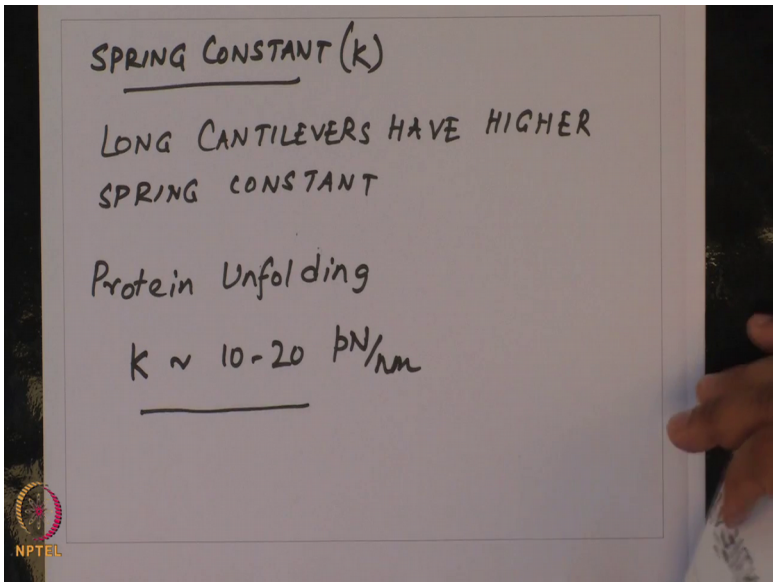

So, you have some spring stiffness spring constant. So, if you are for you can vary the spring constant by varying the length of this cantilever. If your cantilever length is long then the spring constant is high. So, long cantilevers have higher spring constant. So, for probing proteins, for probing proteins or protein unfolding you want. So, k the spring constant k has to be order 10 to 20 piconewton per nanometer, the spring constant has to be of this order, because the forces required for unfolding a protein domain might be of the order of tens of piconewtons.

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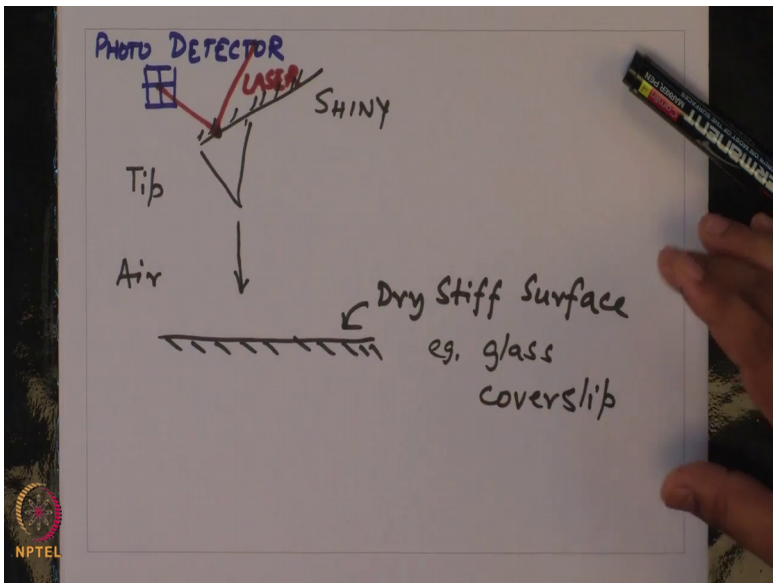

Now, let us see how does the cantilever operate. So, you have you have this is the AFM tip. And what is done so, the backside of this, this is shiny. So, you have a laser which focuses at this point. You have a photo detector and you send a laser light, which focuses on the back of this cantilever and reflects of the back and gets detected by a photodiode photo detector. So, for example let us assume that tip is trying to probe this particular substrate which can be just glass.

So, when the tip is moving through this air let us say this is air, and you have a dry surface, this is a dry stiff surface. Example just glass coverslip in case of biology. So, when the tip is moving through air the tip does not deform right, because it does not touch anything, but as soon as it touches the surface the tip deforms ok.

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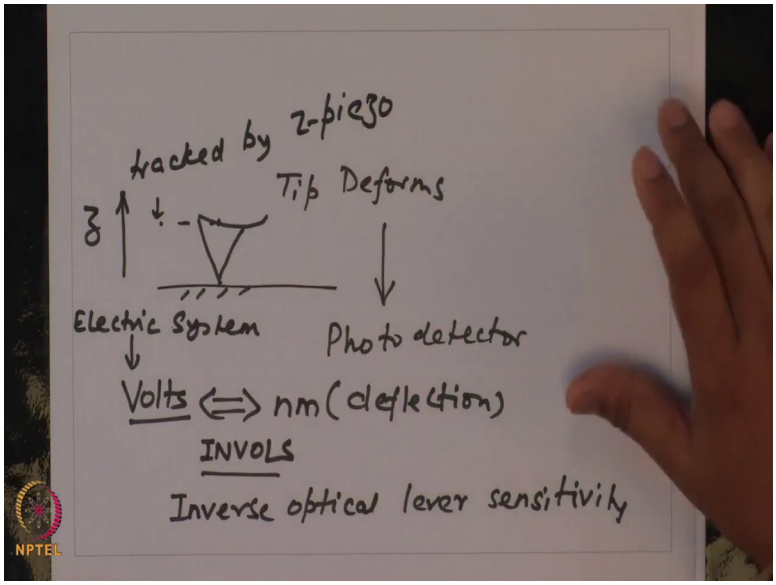

So, when it comes and touches the surface. So, you have deformation of the tip. So, and this deformation is what is tracked by the photo detector. So, any what is detected in the photo detector is in volts ok.

So, you can have to use something called inverse optical lever sensitivity to translate volts to nanometer of deflection. So, the electric system detects everything in volts, in volts and using so, you can calculate something called inverse optical lever sensitivity, this is called invols. So, essentially finding out a way to calibrate volts with nanometer which tracks deflection. And also there is a piezo which tracks the position of the tip. So, you have this z axis the z piezo will track this position tracked by z piezo.

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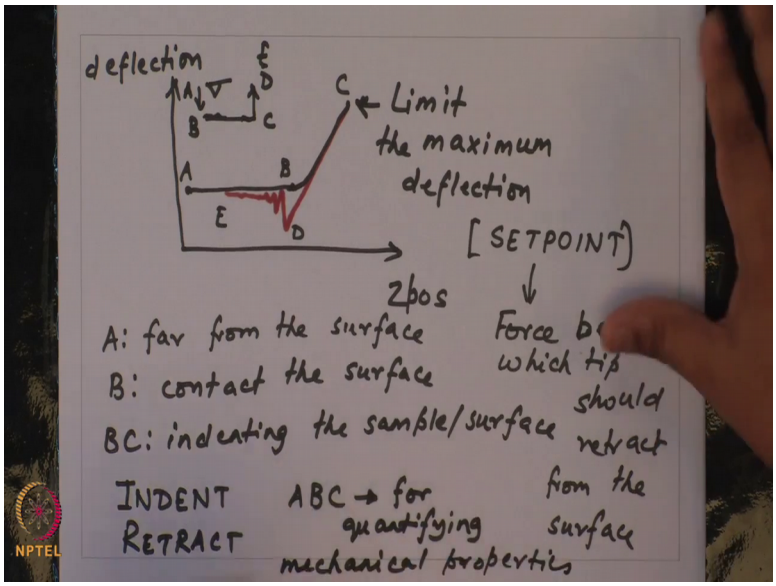

So, if I were to draw a complete cycle, a complete cycle. So, let us say this is z position and this is deflection. So, you generate so, this is a curve that you get as raw data from an AFM experiment. So, let me label some points, at point A you are far from the surface, at point B you contact the surface. So, in B to C you are indenting the sample or surface.

So, the extent of indentation of the sample and the deflection of the tip dictates is dictated by the compliance of the substrate. And after you reach C point so, if you keep on banging your tip against the sample then eventually at some point the tip will break. So, you do not want to do it. So, you would limit the maximum deflection. So, so in AFM jargon it is called as the set point. And this set point you enter in terms. So, set point is nothing but the maximum force. Force beyond which tip should retract from the surface ok.

So, from point C. So, if I were to draw this is indentation. So, from point A to B you are here and from C. So, this So, this is a sample at point C you are going up, C onwards CDE will be points on the way up A and B will be points in the B down. So, this is called the indentation cycle. So, you have 2 parts indent and retract. So, depending on what you want to measure, let us say you want to measured the mechanical properties of the cells, then you want to analyze ABC portion of your data, ABC is for quantifying mechanical properties.

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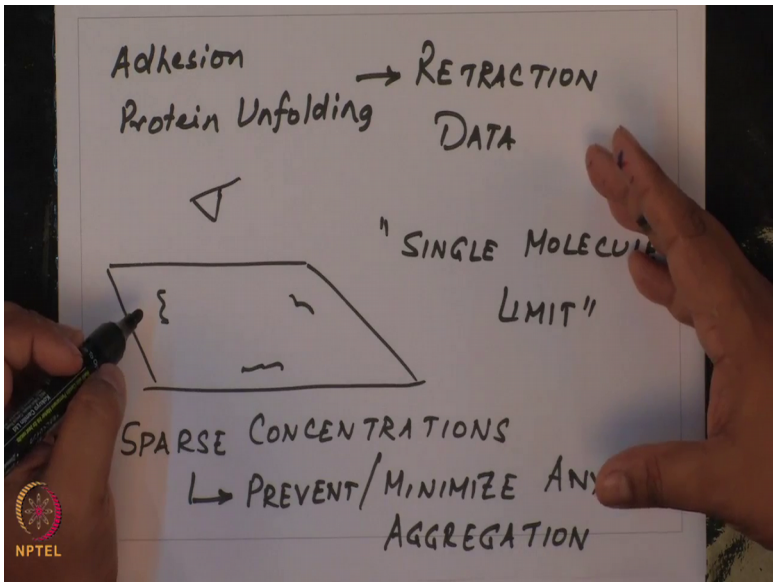

So, for doing addition; so let us say- if I want to quantify addition, all for protein unfolding. So, I analyze the retraction data. I analyze the retraction data for experiments where I want to add quantify addition, or use to quantify protein unfolding. So, that is clear now. So, in our case of protein unfolding, let us say I have a surface, if I were to let us say I draw it like this I have a surface and I have the protein which is anchored at some point. And you have this tip here ok.

So, you want to for protein unfolding studies, you want to operate at sparse concentrations. This is to prevent slash minimize any aggregation. So, you want to operate at the single molecule limit, you want to operate at the single molecule limit ok.

So, we will continue in next class, from trying to understand how does the tip know where the protein is. If I because if I am operating at the single molecule limit I am working with a very dilute specimen. So, how will the tip know where my protein is situated number one. And then what is the signature of an unfolding experiment and how different how can I gain information about unfolding dynamics of individual domains within a protein.

With that I thank you for your attention and we will continue in next class.