Introduction to Mechanobiology
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Week - 03
Lecture - 11
Protein unfolding using AFM

Hello and welcome to a third week lecture of our nptel course introduction to mechanobiology. The last week we had discussed about how non-linear elasticity can be encoded in a single protein, and in that regard we were discussing about this ECM protein called fibronectin. So, fibronectin has 3 modules FN 1 2 3. So, it is a dimer protein homodimer and each of these modules 1 2 3 independently fold.

So, within this folding structure we wanted to ask that how are individual domains within this FN 3 module respond to forces, and does it correlate with our signature, which is here the If you do thermal denaturation what you know is the third domain of FN 3 is among the most stable and it takes nearly 1 20 degrees Celsius for it to denature.

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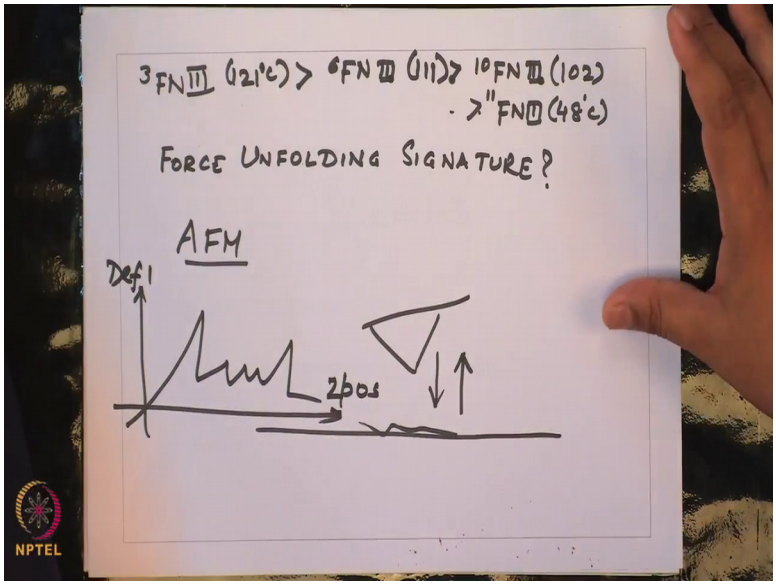

In comparison to the eleventh domain of FN 3 which denatures at 48 degree Celsius.

So, what you have is a vast range of temperature sensitivity, and the question we wish to ask is does the folding unfold does the folding dynamics under forces behave similarly to

that under temperature. And in that regard we had been discussing about AFM and we had shown how what are the different stages in which if you do protein unfolding using An AFM what kind of a signature do you have which is indicative of a protein unfolding event. So, in that regard so, just too briefly recap we have a tip we have a protein and when you pull on it. So, when you indent you form nonspecific bonds of addition with the protein at some random point, and when you try to go up so, you would see in the signature you would have these force like this ok.

So, this is my z position and this is my deflection. So, the question is how do we understand how the folding dynamics or the stability of individual domains within this module.

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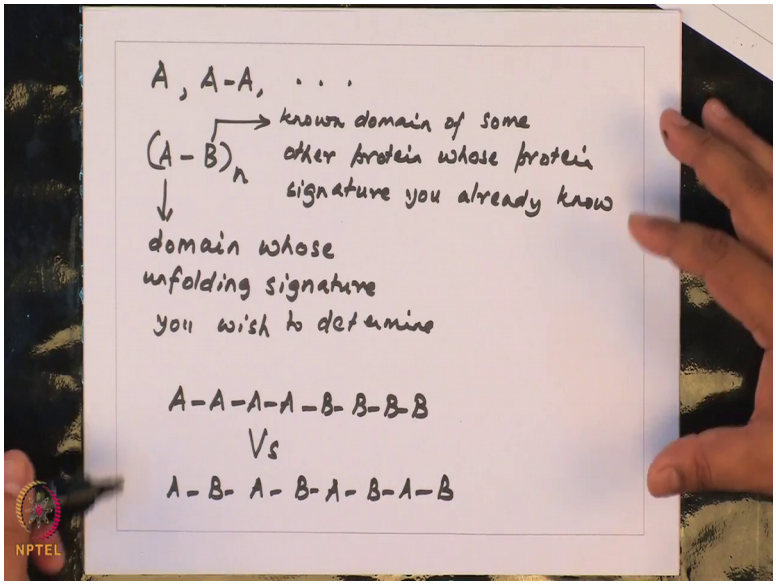

So, that brought us to the question that if you have A A domain A what protein construct would you use, would you use just A A-A or A A or so on and so forth. And what I reasoned was if you do not know how this particular domain unfolds, then it would be wiser to use a combination like AB which is repeated n times ok.

So, A-B so, a is the domain whose unfolding signature you wish to determine. And B is a known domain of some other protein, whose protein signature you already know. So, what we wish to know is when you design your when you engineer your protein, should you have a protein construct which has this structure versus, between these 2 these 2

protein constructs which one would you prefer. So, let us assume you have chosen this particular construct which is a repeated 4 times and followed by B repeated 4 times.

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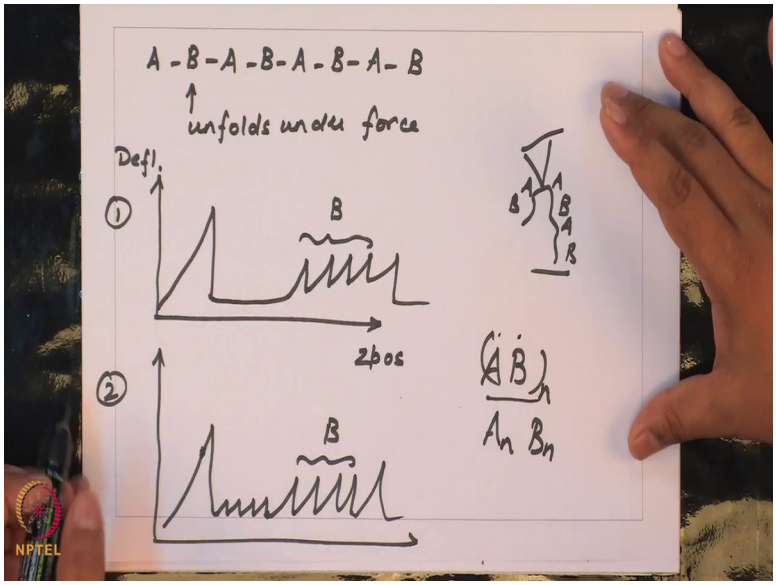

So, in this case and let us say we have done the experiment and this is the output that you get. This is the output you got once. So, you see compared to other curves. So, let me draw another case, compared to this case y for case x you got this long gap extension in between, with almost 0 forces and nothing else. And you have these 2 peaks now you know that these 2 peaks correspond to detachment events. So, they do not really come as a consequence of your domain suggesting that, this long stretch in between is the signature which is representative of your protein construct, but you do not know whether this is really coming from A because B has not contributed.

So, B does indeed on does unfold which means that you should get some unfolding signature. So, in one case x. So, if you pick up the protein if you pick up the protein such that all these B domains are here and all these A domains are here B will not contribute to your signature, suggesting that this long gap of almost 0 force is a consequence of your domain A, but you do not know for sure. Because you have not got10 any contribution from B; however, let us say you have chosen this alternate design and you know that B unfolds under force ok.

So, let me draw, let me draw various representative cases of what you might see. This is one situation, this is another situation. So, what you see here, compared to the previous

case, when you did not see any other signature from B you see some signature of B appearing. Let us assume for the time being these 4 are the corresponding of B. So, you still have 2 cases; so B because wherever your tip touches the protein you are bound to have AB ab sequences in between ok.

So, which means that if you get a peak from B then a peak from a you must have. So, you are guaranteed that you will have signature coming from both A and B. So, this is the advantage of having a repeating unit of AB and then you repeat the structure. So, this is why people prefer AB whole n as opposed to An Bn. Because since you do not have control over where the tip touches the protein wherever your tip touches the protein if you have a, I have a sequence like AB whole n, whatever point the tip pulls the protein you are bound to have both the domains and we pulled up.

So, as a consequence you will get the signature. So, this would suggest that both these cases are 2 possibilities in which A has somehow given your signal ok.

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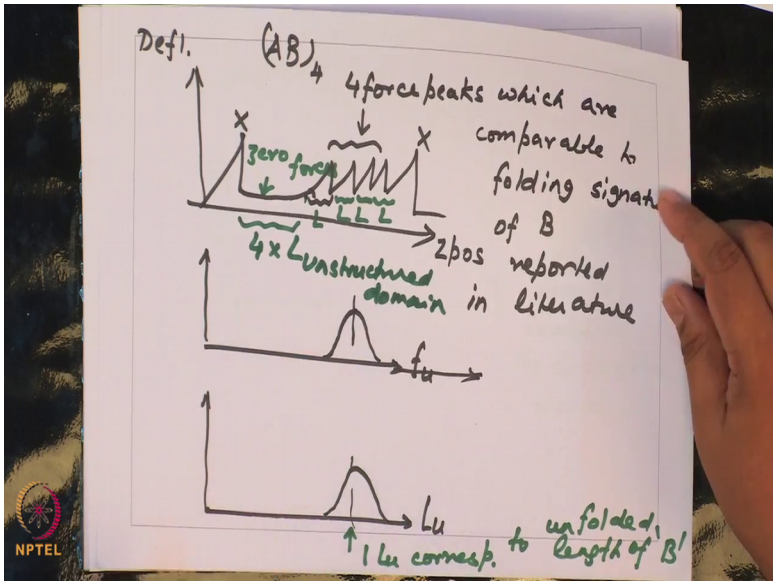

So, let us let us first try to understand what would have happened in this particular case. So, let me redraw this case. So, what do you find disregarding this and the last force, you have you have a protein which is AB whole fourth. So, you have these fourth forces force peaks which are comparable to folding signature of B reported in literature. And you have this long gap ok.

So, for each of these forces what I can plot is the histogram corresponding to the unfolding force f of u and L of u. So, for these peaks let us say I get certain force peak and a certain length peak. So, this is So, each of these lengths L, L, L, L. Will fall in this cystogram and you will have one L u corresponding to unfolded length of B. So, each of these L corresponds to unfolded unfolding of one domain of B. Which suggests So, the absence of any force for this peak.

So, this is almost 0 forces and a long thing. What is it possibly? I would argue this kind of a force signature would suggest that this particular domain A is unstructured.

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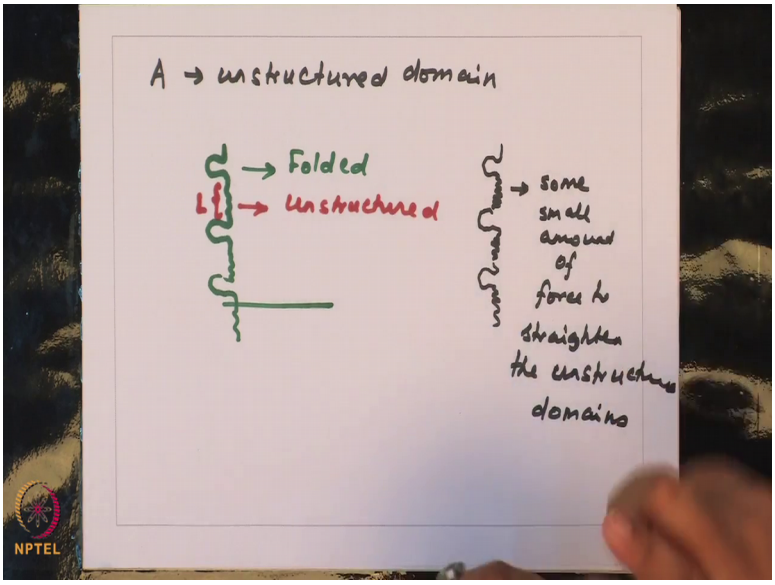

So, let us think of a possibility. So, what I am saying is a is An unstructured domain. And why on what basis do I say it, for the simple reason that if you have let us assume, if I draw a protein like. So, these are folded and these are. So now, let us assume that this length is L ok.

Now because it is unstructured it would not exactly we present like that the protein would not have a configuration exactly like this, but it will have a configuration probably, in other words it will still take little bit of our almost negligible force to straighten this particular domain you would still require some small amount of force to straighten the unstructured domains. So, in that case what this length corresponds to? So, this length corresponds to 4 times length of unstructured domain ok.

So, this long gap of almost 0 force is nothing, but 4 times the length of one unstuck domain of A. So, this is one possible case where you have a domain A, which does not have a structure part same.

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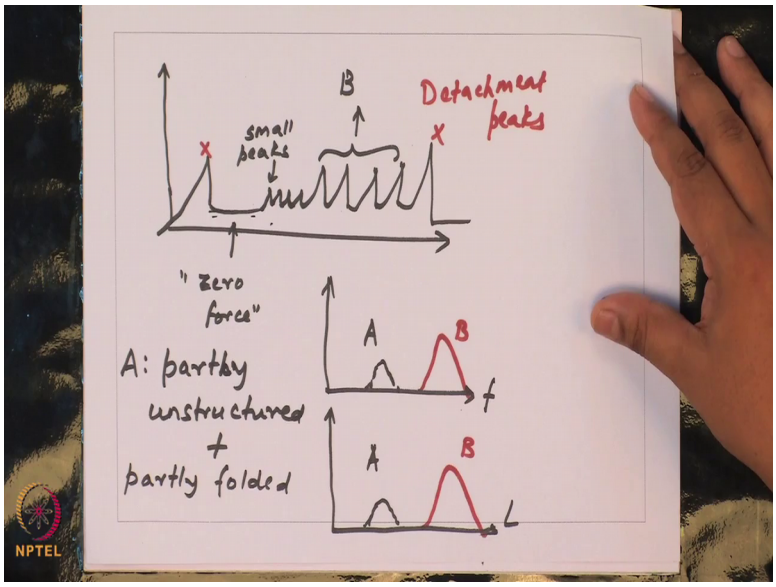

Now, let us take another case. So, once again these are my detachment peaks. So, these are my detachment peaks. I know that these 4 correspond to B. So, folding unfolding of domain B. Now you have a small amount of length again 0 force followed by this small peaks.

So now you have 3 species right. One the unfolding signature of which you know. And then there are 2 additional things. So, once again what you can do is you can plot, the force peak I am not plotting the one for B, but what you have is for these you can get a small force peak and a corresponding length peak. So, this is the force this is the length. So, let me also draw it for B, this is for B. And you have 2 zones one zone of 0 force followed by these 4 peaks. So, this I can ascribe I can assign this force peak and length peak to BA partly, but you still have this small zone where this force is 0 and the domain is getting stretched.

So, what this suggests is this particular a is partly unstructured plus partly folded. So, it suggests that you have 2 zones of a maybe one part of which does fold and that requires a smaller force to unfold, and another part of it which is unstructured. This might be another possibility. For the case for the case let us say A is known to be stable ok.

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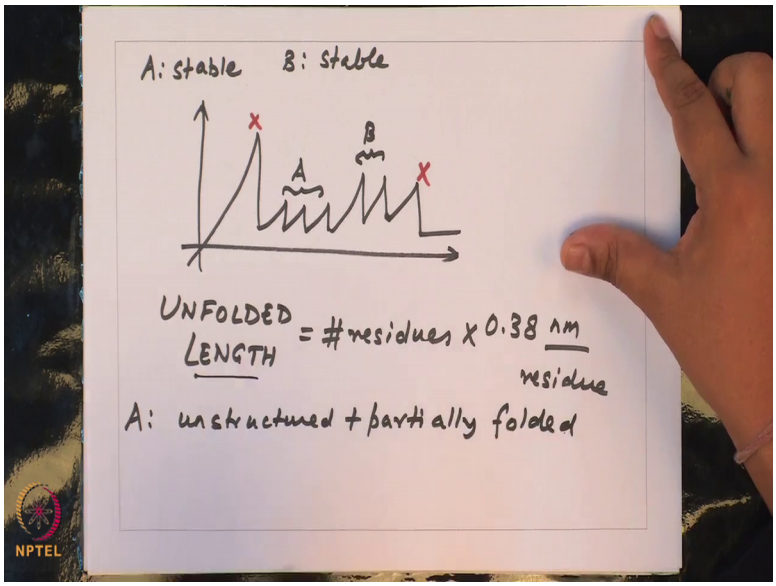

And you have B which is of course stable. In this case I might have a signature ok.

So, the way I have drawn it again, once again I can disregard the last 2 forces. So, I have drawn 2 of B let say and 3 of A, this is one representative curve. So, please note that these 14 pulling experiments have to be done several times. So, as to generate enough statistics, and that is how you will get the Gaussian distribution. So, in this particular case I have drawn these 3 as and 2 Bs. Then I know for sure if I know the protein folding signature of B I know; what is the folding signature of A. And I can confirm it by knowing So, you know that if you have various residues. So, you know that the total unfolded length total number of residues into 0.38 nanometer per residue. This is the rough length of one acid.

So, you know the unfolding length unfolded length, and this formula can also be used for the case where a is unstructured, plus partially folded. Then you can find out now for sure whether in this case your protein a forms these 2 parts, because you can add up this length and this length. So, this case you know that for these 2 put together you must get the overall unfolded length. So, for the case I have drawn here, in this case I have shown 4 peaks small peaks and one unstructured length right.

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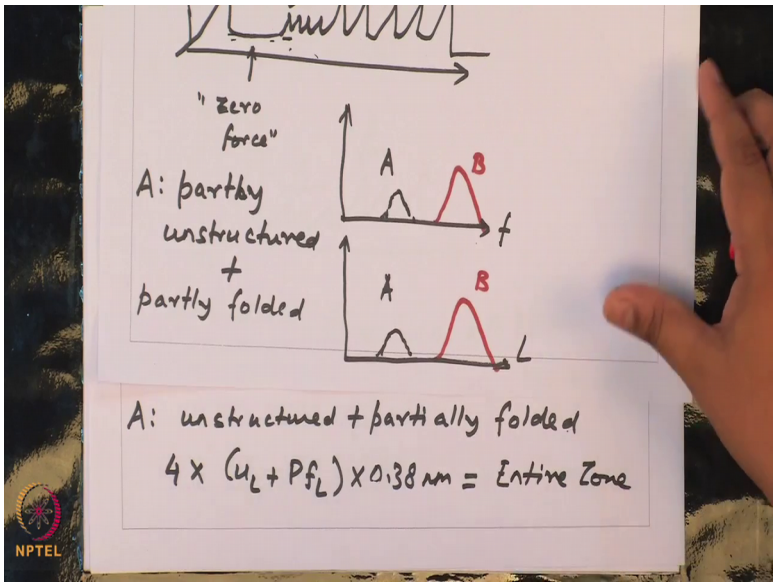

So, you know that 4 time's unstructured length plus partially folded length into 0.38 nanometers should give you the entire length.

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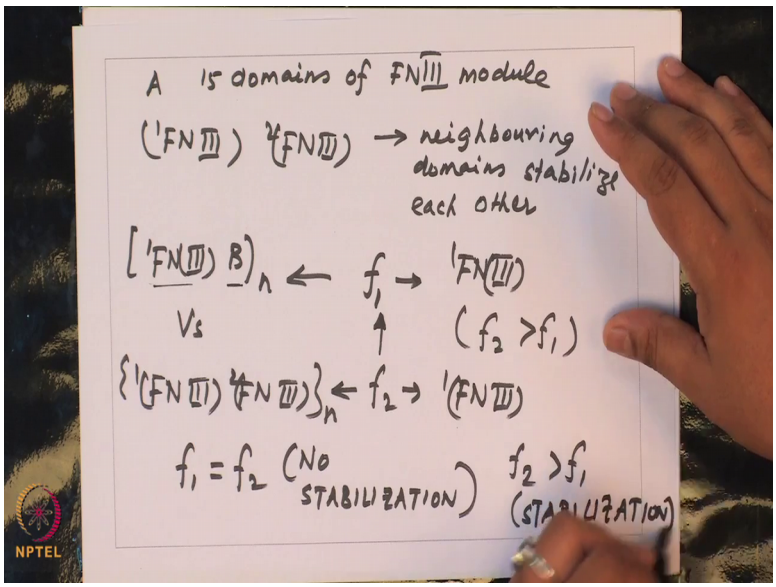

Now, let us think of one possibility let say in the actual domain. So, you had 15 domains of FN 3 module. So, you should have you might have neighbouring interactions between let us say FN 3 1 and FN 3 2. So, neighbours neighbouring domains stabilize each other. In this case what you might see if you have a protein construct which comprises of versus of 2. So, you had one protein construct, where this domain was coupled with this

B which is was unfolding signature you know, versus this case in which 1 and 2 are next to each other and this domain is repeated n times.

So, what you will observe is the force unfolding signature. So, this f 1, let us say corresponds to FN 3 1 domain here. And this f 2 corresponds to FN 3 1 domain from here from this construct. You might see f 2 is greater than f 1. So, if there is a stabilization by neighbouring domain, you might see the f 2 force that you measure to be greater than this f 1 force w, versus if there is no neighbouring interaction then both f 1 equal to f 2 is what we will get, this is no stabilization and f 2 greater than f 1 in the case of stabilization ok.

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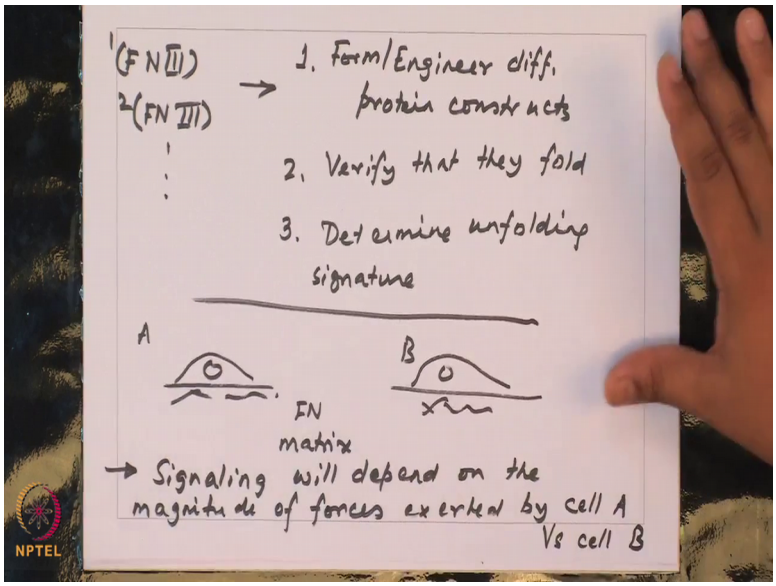

So, using this strategy you can theoretically determine the unfolding force signature of all the domains 1 dot, dot, dot. So, if I were to again write down the strategy form engineer different protein constructs, verify that they fold 3 determine unfolding signature. So, this way you would be in a position to map the forces for each peak. Now let us assume so, you have determined the unfolding signature for all the entire footing.

Now, let us say you have these cells sitting on ECM proteins that says sitting on fibronectin matrix. This is cell type A, this is cell type B. So, the unfolding signature tells you that signaling will depend on the magnitude of forces exerted by cell A versus cell B ok.

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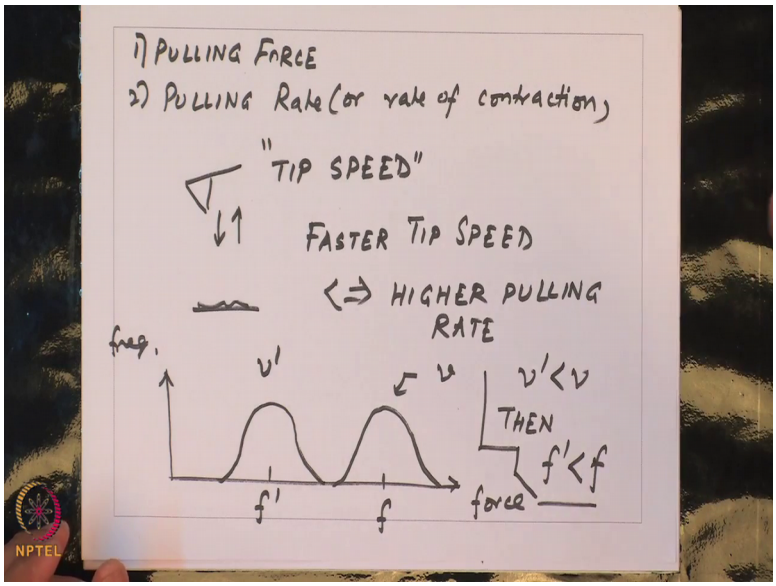

The next question is you know the rate of pulling. So, in addition to pulling so, one is pulling force second is pulling rate or rate of contraction. What would do you expect to see some differences between 2 cell types, which exert pulling forces at completely different rates. And the answer to that is indeed ok.

So, one way of designing that experiment would be here we are protein and what you change what you change is the rate of pulling or the tip speed. So, if you change the tip speed you can simulate. So, faster speed is equivalent to higher pulling rate. So, using this what has been observed is, if I were this is my force and this is my frequency.

Let us say for a given domain this is as a certain tip speed v, what has been observed is for many proteins and many domains, if you pull the protein at a tip speed v prime which is less than v. Then f prime is also less than f. In other words if you pull a proteins slowly it takes less amount of forces to unfold the same protein. This is something very interesting, but it also says that even if the same magnitude of force is exerted the rate at which a protein is pulled also matters.

With that I stop today I stop here today. In the next class will continue from here discuss little bit more about one more aspect about protein unfolding, and then we will go on to study focal adhesions.

Thank you for your attention.