Introduction to Mechanobiology
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Week – 04
Lecture – 16
Cytoskeleton: Actin

Hello and welcome to our lecture of introduction to mechanobiology. So, in the last class we had completed our discussion of focal adhesions and started discussing cytoskeleton the machinery which is required for regulating the structure of the cell its dynamics and for exertion of forces. So, we had started by discussing three different filament systems made of actin, microtubules and intermediate filaments.

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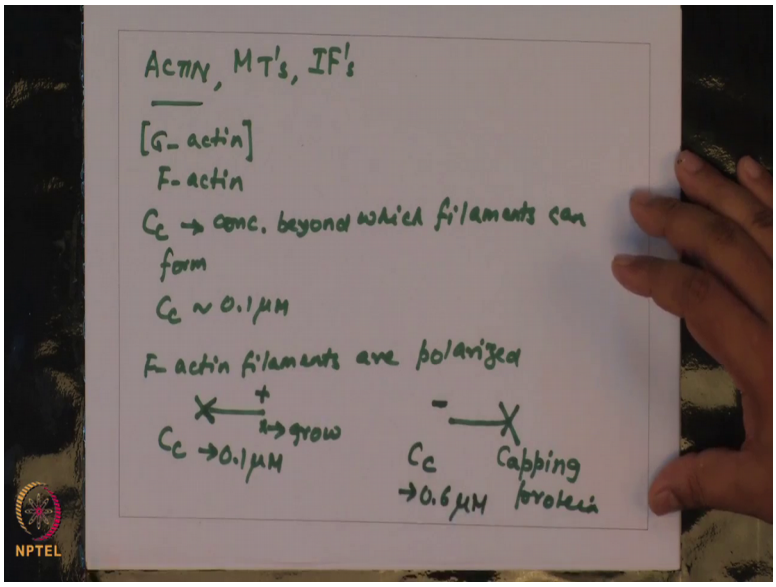

So, these three cytoskeletons collectively dictate the mechanical behavior of the cell and I had started discussing with actin and its polymerization dynamics. So, we have two poles of actin G-actin and F-actin. So, F-actin filaments are formed by polymerization of G-actin and the ability to form filaments actually determined is dictated by the concentration of G-actin. So, below of critical concentration you will not have any filaments. So, C c is the concentration is a critical concentration that is why C c beyond which filaments can form can form and this C c has been estimated to be order 0.1 micro molar.

However careful experiments have shown that these actin filaments tend to be polarized. So, F-actin filaments are polarized. So, what does it mean? Polarized as in if you have a filament it will have a greater tendency to move in one direction and how is it possible. So, what has been found by careful experiments, if you have an existing filament and let us say I block off, I block off one end of the filament and I see at what critical concentration this end can grow, can grow. So, what has been found was it in this end which is called in one end which typically grows faster. So, this is the plus end or the barbed end this critical concentration is 0.1 micro molar, but for the other end. So, if I similarly do an experiment in which I block the barbed end with a capping protein.

So, you typically use a capping protein and you see at what rate this would grow this for the minus end it turns out that C c is 0.6 micro molar, thus at a concentration. So, let us say you have two ends, so concentration of G-actin.

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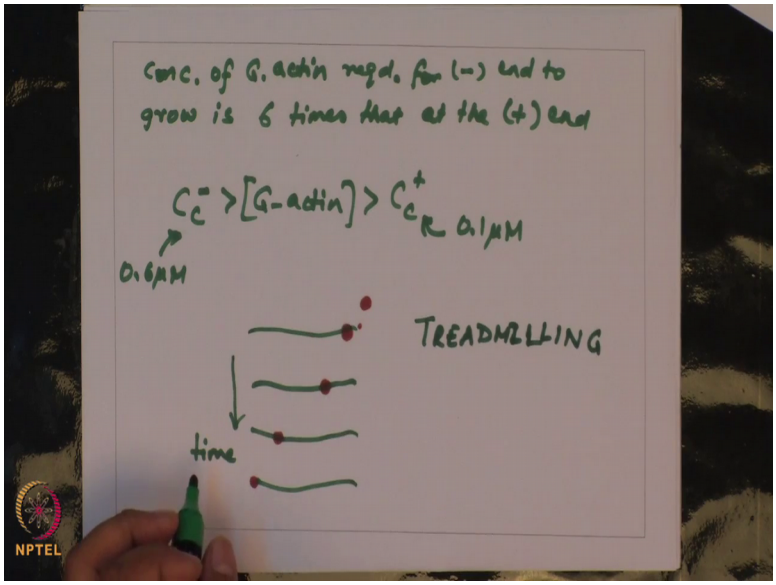

Required for minus end to grow is 6 times that at the plus end this for filament to grow at the plus end you need critical concentration of 0.1 micro molar versus for the filament to grow at the minus end you require 0.6 micrometer. So, you require a 6 fold higher concentration.

So, let us try to find out what will happen if your concentration let us say. So, this value is 0.1 micro molar this value is 0.6 micro molar. So, if you have a filament and you are operating at the monomer concentration which is in between these two ranges what will

happen. So, you have an existing filament and let us say you tagged one of your monomers red. So, this monomer will get added to the filament, because this at this concentration the filament will grow at a much faster rate in the plus end compared to the minus end. So, if you track it as a function of time at sometime you might see that the filament that this red monomer is here and as time goes on it will get pushed back and back and finally, at one time it will come out of the last end.

So, this activity this thing in which something is added as the frontend and gets reached from the minus end this is called tread milling. So, so many interesting aspects of actin what has what is also been observed and demonstrated is that actin let us say you have an existinG-actin filament in the presence of a protein called Arp 2 3 you can have a branching event. So, let us say this is my Arp 2 3 there will be formation of a branch network and this angle is 70 degrees.

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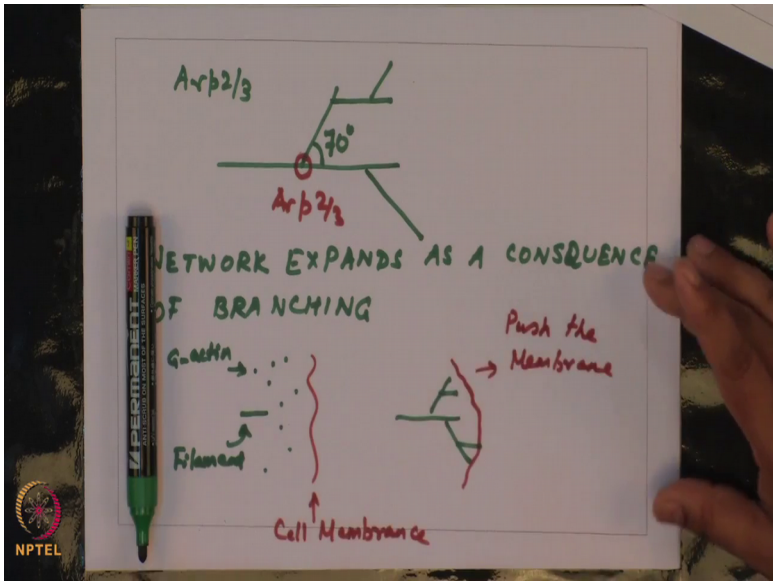

So, as a consequence you can from a single filament in the presence of Arp 2 3 and G-actin you can form a network like this. So, this is expensive. So, network expense as a consequence of branching.

So, imagine you have a thin membrane you be you start with a small filament and you have lots of dots lots. So, these are my G-actin monomers this is G-actin and this is my filament. So, if we have Arp 2 3, what you will see is after some time these filaments will begin to form and they will start exerting force on this membrane. So, this can be my cell

membrane and this growth of this filament network will lead to membrane deformation or rather it will push the membrane; however, if your membrane is stiff let us say instead of having a membrane you are operating with a rigid wall. So, let us say you have a rigid wall, and you have filaments which are polymerized.

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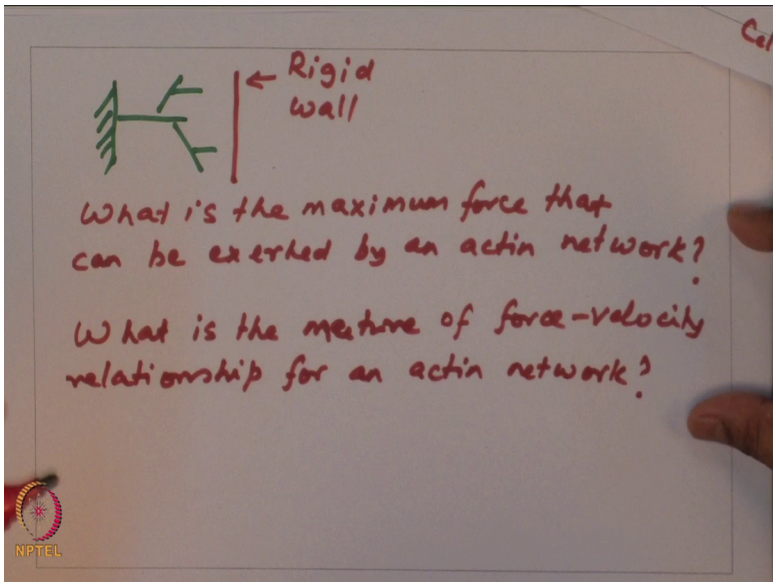

So, this if you have a rigid immovable wall and the backside of your actin network is anchored. So, if you do not anchored then the entire network will flow back; however, your back end is anchored then the network will actually exert a pushing force on the wall. So, one of the most interesting aspects is to find out what is the maximum force that can be exerted by an actin network. The other question which comes is related to it is what is the nature of force velocity relationship for an actin network. So, if you do not restrict this wall the polymerization forces will keep on pushing this wall in this direction; however, imagine if you actually constrain this wall with a certain force f.

So, if the polymerization the effect of polymerization the force generated as a consequence of polymerization is less than this force then probably the wall will remain fixed in its position; however, if the force is greater than this f then the wall will move. So, as a consequence of that you can track at what rate there is a wall move if it is constrained by a force f and it is subjected to polymerization of actin network. So, to answer these two questions I am going to show two approaches one is an experimental one and then one is the computational one.

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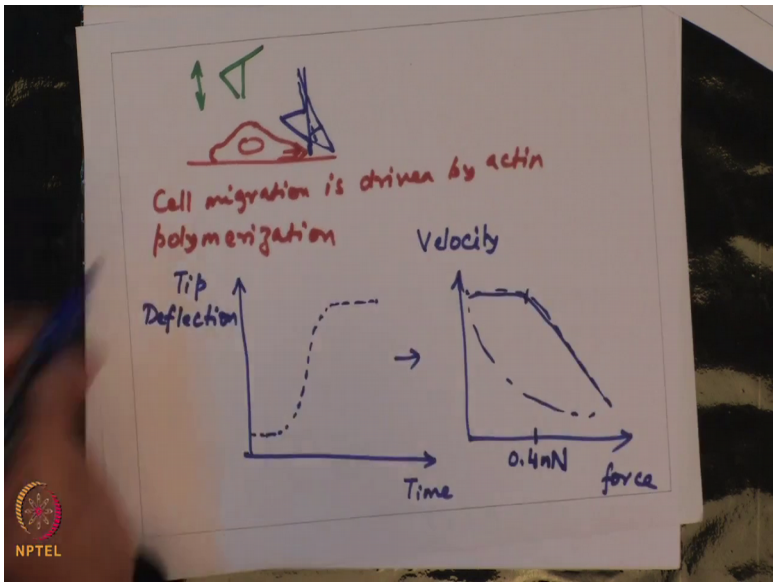

So, experimentally imagine, you have a cell and you setup an a from cantilever. So, in all our protein unfolding experiments, I told you the AFM is moved in this direction, but it is possible to design an experiment in which your AFM cantilever has actually a geometry like this ok

So, what is the advantage of this geometry in this particular experiment when the cell is migrating in this direction you can monitor the deflection of the tip. So, migration is driven by actin polymerization. So, if you put a tip here. So, as if the cell were to push the tip initially the tip will start deforming right, but beyond a certain deflection the tip will. So, as the tip deflects it will try to exert a resisting force on the cell, which means of the cell is subjected to more and more force. So, you can track the displacement as a function of time and what you can get is. So, if I track the deflection of the tip or displacement as a function of time tip deflection. So, you can expect to get a curve ok.

So, from here as of, what you see is as time goes on the cell keeps pushing and pushing which leads to increase of the deflection and once a certain force is reached the cell can push the tip anymore. As a consequence the typical friction plateaus which means the cell stops pushing. So, from here you can generate you can convert this curve into force versus velocity. So, velocity is nothing, but the slope of this curve and force is k times d. So, you can then convert it into a force velocity curve, this curve might look something

like this or something like this. So, experimentally it has been demonstrated by several researchers that you might have a concave fourth philosophy relationship.

So, over a certain magnitude of forces the constant which a cell can migrate at constant velocity, but beyond a certain cutoff the velocity drops significantly. So, this cutoff for fibroblast I think it is of the order of 0.4 nano Newtons. So, this is the maximum force at which the cell can migrate at a constant speed, but this setup is difficult to engineer. So, you cannot use existing or commercially available a AFM, but we have to manufacture design and make your own instrument to be able to do these measurements one alternative in simulations. So, let us (Refer Time: 14:03).

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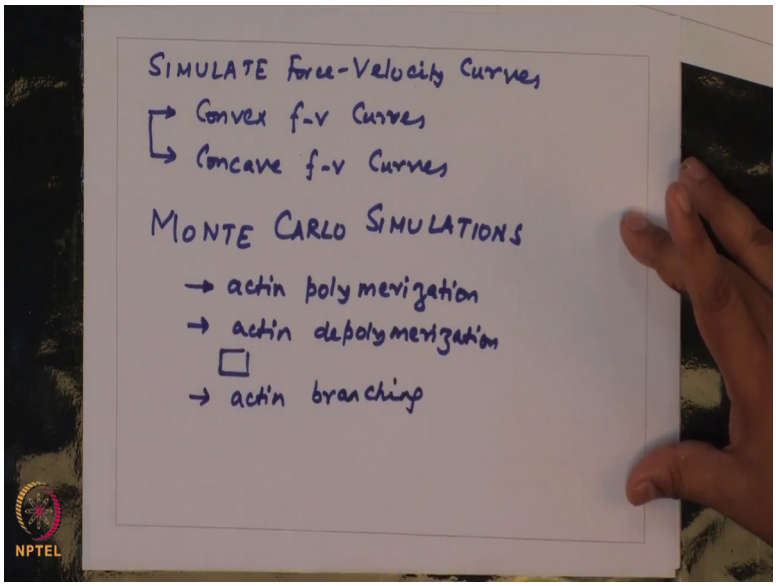

So, I want to simulate force velocity curves. So, and you want to answer that under what circumstances would you get convex f-v curves versus concave f-v curves. So, I will describe one approach where use Monte Carlo simulations. So, how do you set up a Monte Carlo simulation? Let us go step by step imagine. So, as part of the simulation what do I want to do I want to capture the effect of actin polymerization, actin depolymerization, actin branching. So, let me draw how such a model might be set up.

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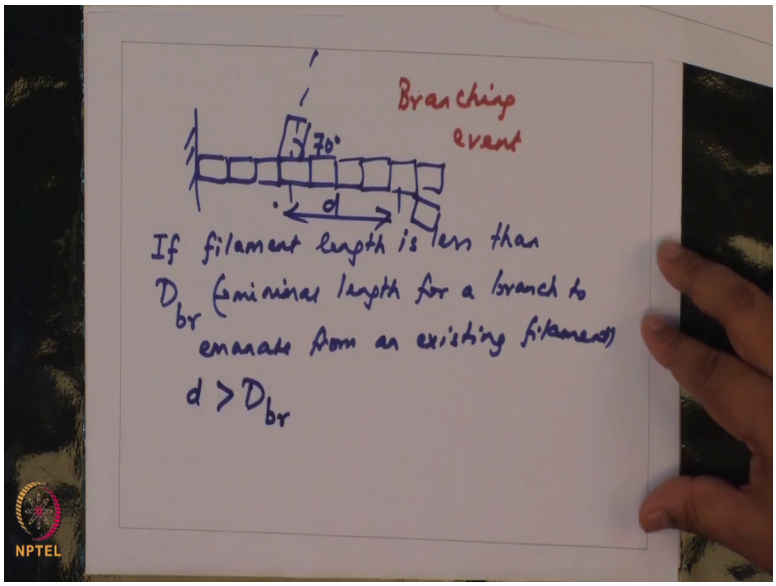

So, imagine you are starting from an actin filament of a certain length, let us say in this case of three monomers and the back wall of the filament is constrained which means that the filaments cannot move back. So, you can prescribe a G-actin concentration and then simulate, so there are three possibilities what can happen here you can have growth of the filament. So, this filament can grow this is one possibility, the second possibility you can have this filament can shrink. So, this is polymerization this signifies depolymerization and you can have one more situation in which let us say if the filament is let us say if your filament is long enough you can add a filament another filament which starts at an angle. So, this angle is 70 degrees. So, this simulates a branching event.

As for the branching to occur there are two constraints. So, one would typically expect that if a filament is too small then a branch is less likely to form. So, for making that in the simulation what you can do is you can say that if filament length is less than some length let us say D br I write this is the minimum length for a branch to emanate from an existing filament. So, till this filament is of length D br and new filament cannot branch from this existing filament what it also ensures, once you have this filament branch this becomes a new filament from here onwards. So, you can have for another branching event to occur let say you have another branching event occurring from here then this distance this distance d has to be greater than D br. So, D br is also the minimum distance between two branching points. So, this is what you can have.

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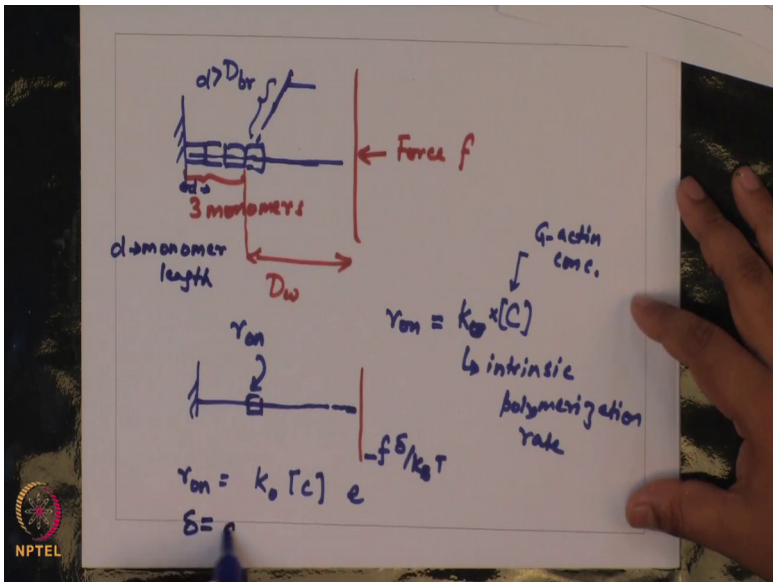

So, second thing is, you want to simulate the growth of this network right. So, you have these filaments which are connected to each other I will just draw like this let us say. So, this is you can have another filament element over here again this length d has to be greater than D br. So, the other thing that you start with is the position of the wall what is the position of the wall at the start of the simulation and what is the force if with which are restraining, so your minimum you are starting with 3 monomers. So, there is a starting distance you can say let us say this distance is D w or D wall. Now let us imagine some scenarios what might happen when the filament polymerizes. So, at the very start when the filament is placed far from the wall you can have a certain rate a monomer can get added at a rate r, r on. So, this are on this r on the rate at which polymerization happens is dictated by two things.

So, you can write the expression for r on as k 0 which is the intrinsic polymerization rate into the G-actin concentration. So, when the filament is far away from the wall this is the rate at which you can add a monomer; however, let us assume a situation in which after as a consequence of subsequent polymerizations the filament is about to touch the wall. So, what will happen in this case your r on you have a force f which is pushing the wall. So, this will be modifying this expression k 0 c and what is typically done is, you have you write down an expression like this where delta. So, let us assume that d is the single monomer length and, you have a dependence e exponential to the power minus f times

delta by K B T. So, K B T has units of energy f is the force delta is the distance the wall has to be moved if you were to add a monomer at that point ok.

So, this delta I am making a distinction between d which is the monomer length and delta. So, for the case of a straight filament if a new monomer is to be added then I must have delta is equal to d; however, if you have a geometry like this. So this is my wall here and I am to add a monomer here. So, this is my d in that case delta is nothing, but d cos theta. So, your theta is 70 degrees because the new filament gets added at an angle of 70 degrees.

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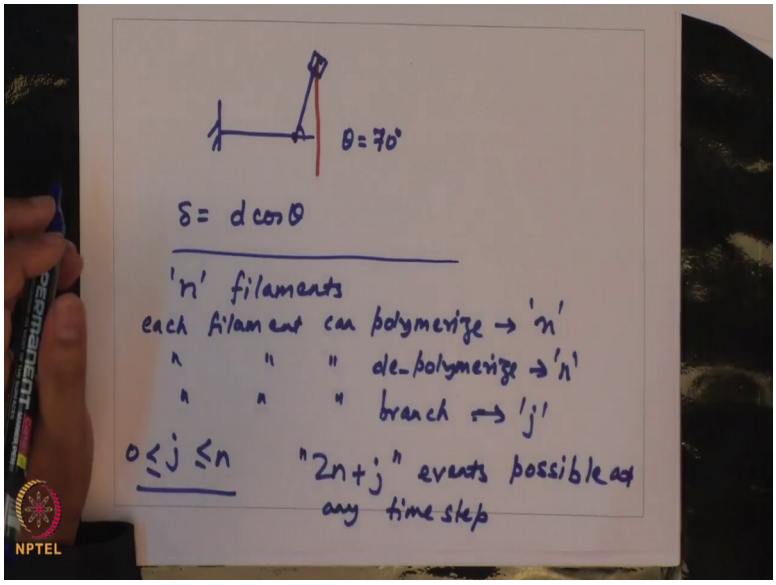

So, delta can be different, but what it says if you look at the functional dependence here that if there is a humongous amount of force which is resting in the wall then the rate of addition is almost close to 0 e to the power minus f delta, but if f is small then probably that wall can be pushed back ok.

So, similarly you can do the same thing for the rate of branching. So, there is given a filament you can have three events right if there are n filaments let us say, n filaments then each filament can polymerize. So, how many possibilities are there n possibilities are there for each filament that exists each filament can depolymerize again there are n possibilities and each filament can branch. So, here it is not necessarily n possibilities, but j possibilities where j is. So, you must have this constraint which is always fulfilled.

Why j is less equal to n because we have the constraint that minimum distance between two branching points minimum distances to branching points has to be greater than D br.

So, you have some intrinsic passing rate kbr, but that will only come to play after you have the distance. So, there are total of 2 n plus j events possible at any time step. So, in Monte Carlo what you do is at any time step you only fire or the only trigger one of the events. So, at any time step you can have a polymerization event, depolymerization event or a branching event. So, you can find out what is the probability so the net rate of polymerization you know, if there are n filaments.

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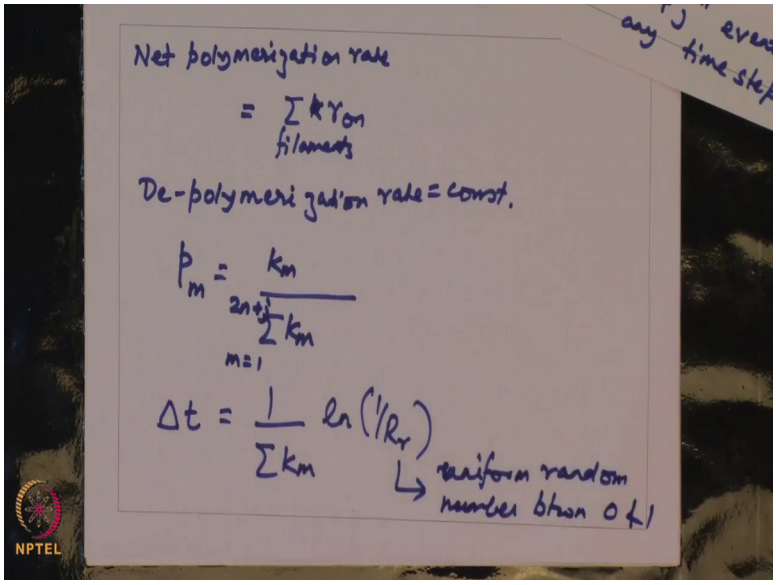

So, net polymerization rate is simply equal to summation of r on for all the filaments. Similarly, you can find net depolymerization rate. So, typically in this simulation the depolymerization rate is constant and you can have the branching rate also. So, in essence you can find out what is the probability of each event this is going to be that particular event by summation of km m equal to 1 to 2 n plus j. So, you can essentially find out the probability of these events and then from here you can find out the delta t at which you are going to trigger that even and this is defined as where r is a uniform random number between 0 and 1. So, I will stop here for today and we will continue this discussion in the next lecture.

Thank you for your attention.