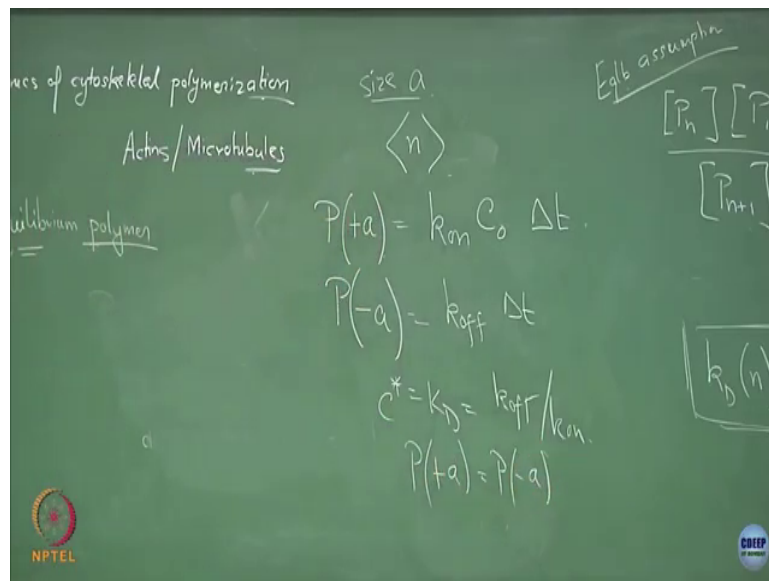


Physics of Biological Systems
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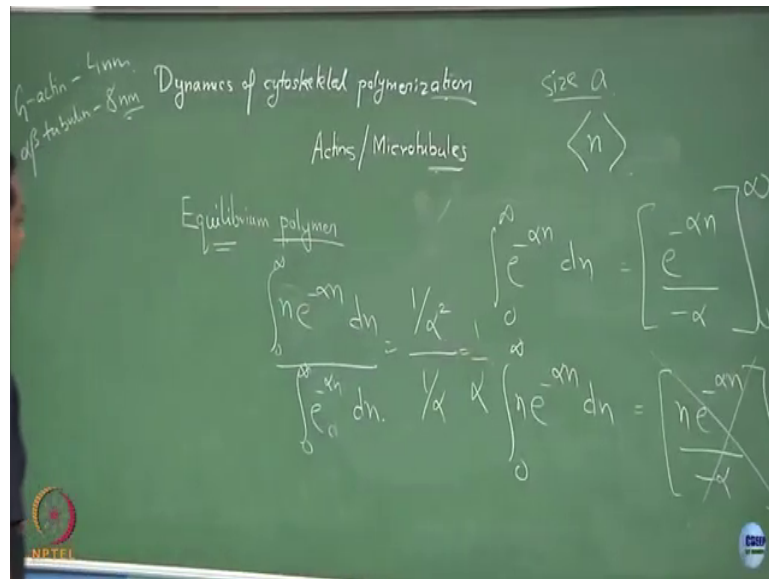
Lecture – 46
Timescale Separation in Enzyme Kinetics

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So, I say that well let I have obtained an, I have obtained some sort of mean length n , right. I can calculate how much time does it take for a microtubule or an actin sort of to reach this mean length, ok. And how do I do that? That why just look at timescales of fluctuations. So, for example, if I were to add a monomer and let us say a monomer has size a , right size a . So, this I did not say.

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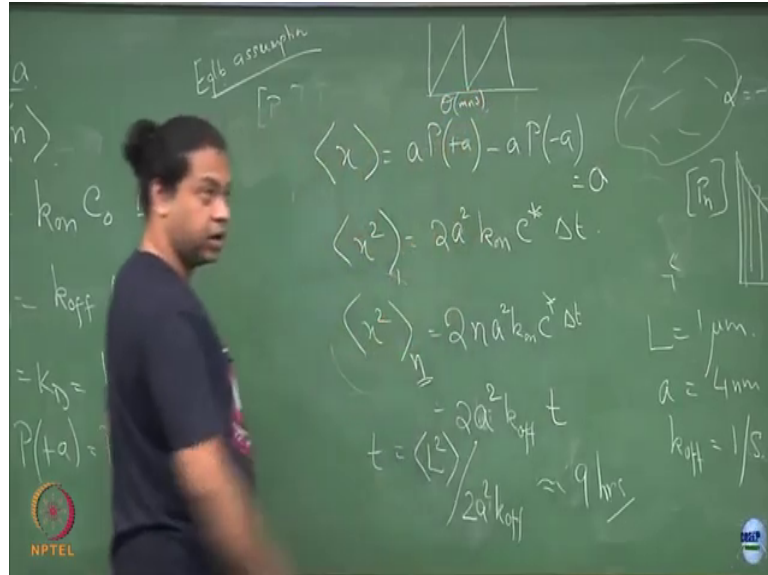


So, for γ actin typically has a size of around 4 nanometers and this tubulin heterodimer this alpha beta tubulin that has a length of around 8 nanometers. So, that is the order we are talking about. So, whenever you add a subunit you increase your length by a . So, this probability of increasing my length by a , is my on rates, so k_{on} , times whatever is the monomer concentration C naught times delta t , right. The rate is k_{on} ; the probability is k_{on} times delta t into the C naught. And the probability of losing a filament and therefore, shrinking your length by a is simply k_{off} to delta t , ok.

And if you are doing this equilibrium model, at equilibrium I have that this C^* or K_D whichever way I write it that is this k_{off} by k_{on} , right. If I put this k_{off} by k_{on} over here, what that means is that at equilibrium P_{n+a} is equal to P_{n-a} , right which is why basically you get that dP/dt is equal to 0, on an average nothing grows or shrinks. But given

that I can now calculate, just like I did for the random walk I can calculate the first couple of moments.

(Refer Slide Time: 02:26)



So, I can calculate what is average x which is a times P of plus a then minus a P of minus a ; which is 0, in this equilibrium approximation because P of a is equal to P of minus a . And you could also calculate what is x square, right. So, that will give me $2 a^2 k$ on C star Δt . This is a^2 of plus a plus a^2 P of minus a , ok.

And if I took, so this is for a single step, this is for a single step. If I took n such steps, so I have a polymer of length n . Let me write small n , then that is nothing, but $2 n a^2 k$ on C star Δt , right. But this n if each step takes the time Δt , $n \Delta t$ is nothing but the total time that you have you have taken to sort of sample this n . So, this is like my $2 t$, $2 a$, let me write $2 a^2 k$ on C star or let me just write $k_{off} t$, ok.

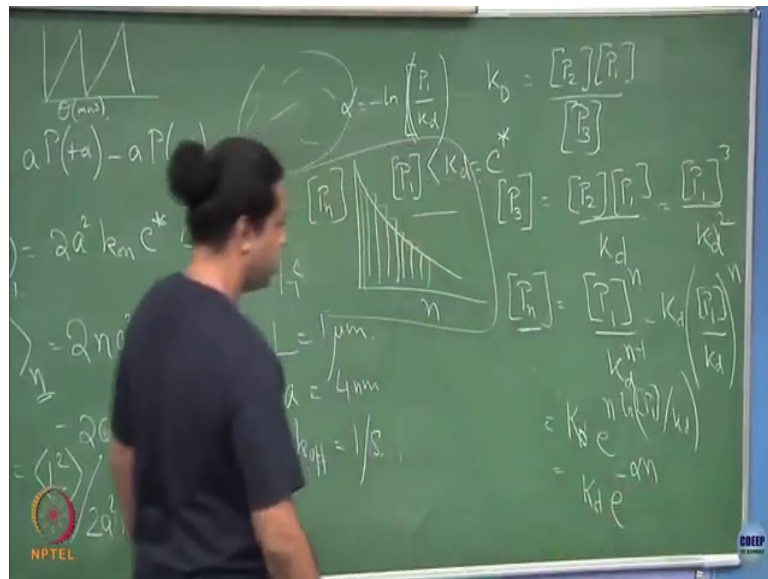
This is the simple sort of random walk that x^2 grows as t . So, it tells me what is typically a time to sort of generate a microtubule or an actin of length L ; that is $2 \frac{L^2}{k}$ of L^2 square by $2 \frac{L^2}{k}$ off; that is my variance. And if I now put in these numbers that my k off I said was 1 per second. My a was, how much? 4 nanometers let us say for actin and I choose a filament that is around 1 micron.

So, if I use these typical numbers again, if I use these typical numbers that let us say my L is looking at a filament which is roughly a micron in length with a subunit that is around 4 nanometers and with the off rate let us say which is 1 per second. I can calculate what time it takes and that time if you put in these numbers comes out roughly around 9 hours, ok.

So, in order to generate sort of mean length of around 1 micron through this sort of a process, through the sort of an equilibrium process it would take around 9 hours; which is simply too long. If you think back to this microtubule dynamical instability plot they grew and shrink shrunk in a matter of minutes.

So, this was of the order of a few minutes, right. And similarly for actin, if you had to wait 9 hours to sort of generate length scales that process would simply not be biologically feasible, so although this sort of an approximation generates the correct distribution which is an exponential. It gets the timescales completely wrong.

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So, the equilibrium model is not good enough to explain the timescales as it should not be in fact, there is no reason to assume that this is in equilibrium. Yes.

Student: (Refer Time: 05:50).

Yes.

Student: Sir, how can expectation that we (Refer Time: 05:55) expectation (Refer Time: 05:57)?

Was 1 upon alpha? Yes.

Student: (Refer Time: 06:05).

Here I do something wrong? So, let us 1 upon 1 minus.

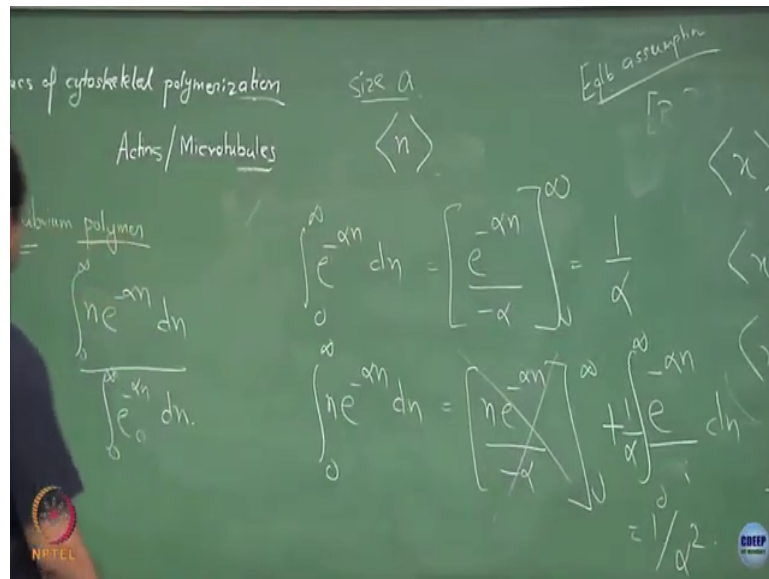
Student: (Refer Time: 06:19).

So, in this in terms of these parameters.

Student: (Refer Time: 06:26).

Let us just do it once. This is this part is fine, right. If the probability distribution is that then this is e to the power of minus α n dn by e to the power of minus α and dn , right, 0 to infinity, 0 to infinity.

(Refer Slide Time: 06:50)



So, let me just do e to the power of minus alpha and dn, 0 to infinity. This is e to the power of minus alpha n by minus alpha, 0 to infinity. So, this is what? 1 upon e to the power of minus infinity is 0; minus e to the power is 1 minus plus 1 upon alpha. That is that and the top part is integral n e to the power of minus alpha n dn 0 to infinity. So, I will do it by parts, first function into integration of the second, 0 to infinity, minus integral of differentiation of the first function into integration of the second.

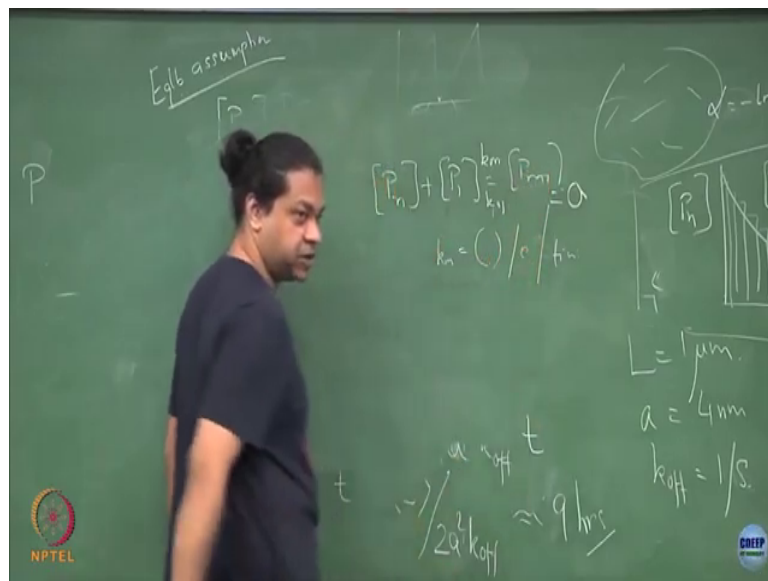
Tell me if I am making a mistake. So, this is this is 0, because infinity will give 0 here and 0 will give 0 there, so that I do not care. This is a plus 1 over alpha of this, and this I know is anyway 1 over alpha. So, this is 1 over alpha square. So, then this is 1 over alpha square by 1 over alpha, so 1 over alpha.

Student: (Refer Time: 08:30) by getting the sum (Refer Time: 08:32).

You are saying the continuum approximation is itself making that, I check that. So, in principle it is true that this is a discrete sum. On the other hand the n is so large that I do not assume that a continuum approximation would make much of a difference. So, maybe one can show that in certain limits that will come back to this 1 over α answers with that one can think about. At least in the continuum approximation I think the 1 over α is correct, ok.

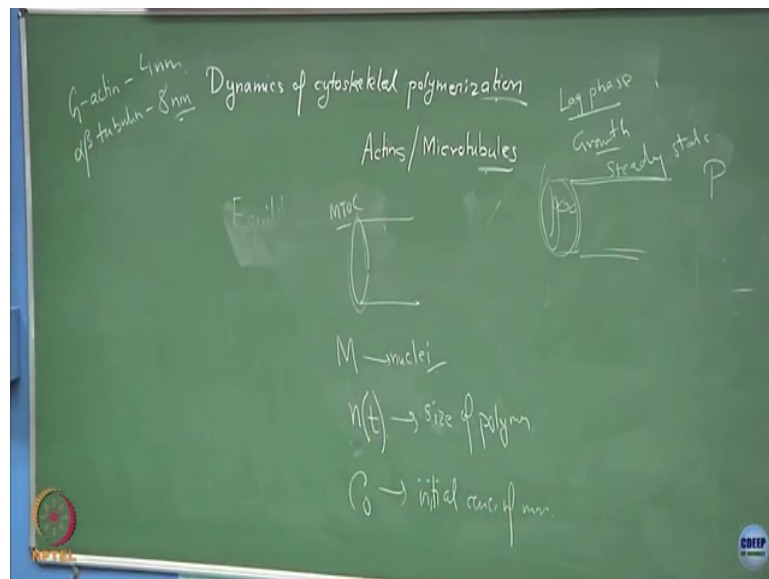
So, let me do a slightly more involved model that takes into account the fact that this concentration of monomers itself is a variable quantity. Yes. The concentration of monomers, right. Because P_n plus P_{n+1} will give you a P_{n+1} , right.

(Refer Slide Time: 09:31)



So, this on rate k_{on} is some number per concentration units per time unit whereas, the off rate is just per time; because you have to have a monomer coming in and meeting this for it to give you the $n + 1$. So, it is it has a constant per concentration unit itself. So, that is the monomer concentration what are you talking about. Yeah, some monomer concentration which I just took a C star.

(Refer Slide Time: 10:23)



So, we will just relax that. So, now, let me say that. So, like I said that often let us say for microtubules you will find that there is this, microtubule organizing center from which the microtubule start to nucleate. If you are doing this experiment in vitro then you will generally have to wait until some of these monomers come together to form a sort of nucleus, a nucleation sort of a n then it starts growing.

So, generally if you were to do in vitro there is a lag phase, there is a lag phase where this sort of nucleus forms. In vivo that is not the case because there is already this microtubule organizing center from which it nucleates. After the lag phase there is a growth phase, where this thing sort of elongates and then there is sort of a steady state where it does not change too much in.

So, let me say that I consider a system that has M nuclei from which these polymers will sort of nucleate, ok. And let me say that the number of number of monomers in the filament is n of t , so this is a function of time and, so this is number or this is the size of the polymer, the size of polymer. And I have some concentration of monomers, some initial concentration of monomers, some initial concentration of monomers which is C_0 . Now, I no longer assume that to be a constant, ok.

(Refer Slide Time: 11:57)

$$\frac{dn}{dt} = k_{on}(C_0(t)) - k_{off}$$

$$= k_{on}\left(C_0 - \frac{Mn(t)}{V}\right) - k_{off}$$

$$\frac{dn}{dt} + \frac{k_{on}M}{V}n = k_{on}C_0 - k_{off}$$

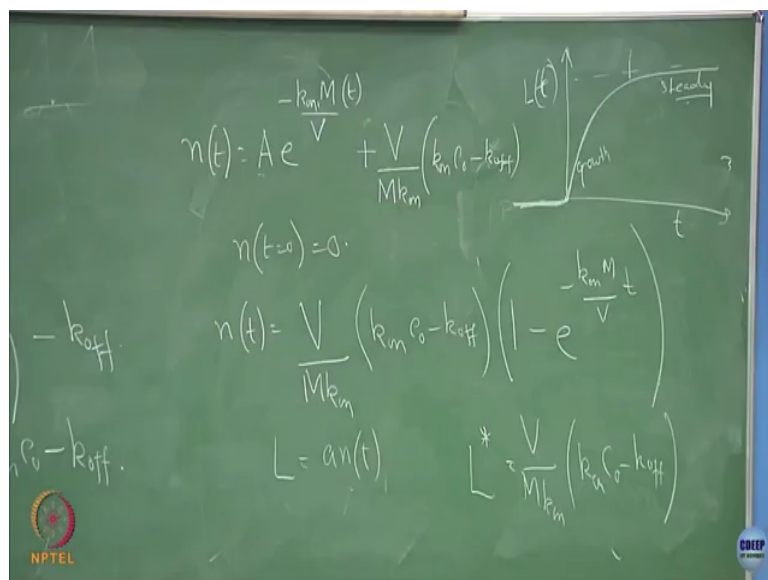
So, now what should I do? So, let me say I want to write dn/dt , right. How does that change? So, there is a k on term, times the concentration, concentration of monomers at time t minus k off term. Let me move all those things out of the way. So, this is essentially what I did even in the equilibrium model, except there I have taken that this C naught is whatever the concentration of monomers presumed to be fixed.

Now, I say well as things polymerize of course, this is going to change, so that is some C naught of t , right. So, how will that depend on this initial concentration? What should I write? So, if I started off with some initial concentration C naught over here after time t how many monomers have I used up? I have used up each polymer uses up n of t , right that is enough that is the length of the filament. There are M nuclei, so there are M polymers that are nucleating.

So, there is M into n of t and this is a concentration unit, so whatever is my reaction chamber I need to subtract the volume of that divided by the volume of that to get the concentration. So, these many polymers remain at time t . If I started off with C naught after time t where I have M filaments each of length n in a volume V , this is the number of monomers that remain to you, and then of course this k off term, ok.

So, now, I have an equation, now I have a differential equation dn/dt , dn/dt plus k on M by V times n is equal to k on C naught minus k off this C naught now is the initial concentration, ok. This is a first order differential equation, you can solve this whatever way we want, I will write down the solution. You can write down in particular solution in a general solution.

(Refer Slide Time: 14:28)



But you can solve this and you can write down what this n of t is going to be. So, this n of t is going to be some constant e to the power of minus k on, k on $M t$ by V plus plus V by $M k$ on and then k on C naught minus k off, right. So, again if you solve this first order differential equation it should be able to show that the complete solution is that this is my n of t , ok.

This is a constant which you need to sort of put in some initial condition. So, for example, if you say that initially at time t equal to 0 this will be 0 , then you can write evaluate your a and then n of t just becomes V by $M k$ on; k on C naught minus k off 1 minus e to the power of minus k on M by $V t$, something. These are all constants this is the only time dependent, 1 minus e to the power of minus some βt , ok.

So, basically if I were to look at how the length of the polymer would behave, in this case if I were to plot L as a function of t versus t . And remember, if n is the number of monomers L is

nothing, but a into n of t, each monomer has length k. So, it is just a into this thing. And this is $1 - e^{-\dots}$ to the power of minus something like this, so that will just go and look something, right. When t is very large, then you will go to this object whatever L star you want to call. So, that is going to be your steady state length, L star is going to be this object V by M k on, k on C naught minus k off.

And this is indeed typically how lengths vary with time. If you neglect the sort of lag phase because I am already starting with nuclei, if you did not start with nuclei you would have you would have a lag phase where nothing was growing and the nuclei were assembly. But then there is a growth phase, so this is called the growth phase and this is the steady, ok. You can do a little better and just even ask that for example, what is this probability to have a filament with n monomers and how does that change with time.

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$$P_n(t) = \frac{N_n}{\sum N_n}$$

$$P_n + P_1 \xrightleftharpoons[k_{off}]{k_{on}} P_{n+1}$$

$$P_{n-1} + P_1 \xrightleftharpoons[k_{off}]{k_{on}} P_n$$

$$\frac{dP_n}{dt} = +k_{off} P_{n+1} - k_{on} P_n P_1 + k_{on} P_{n-1} P_1 - k_{off} P_n$$

So, you could ask what is the probability to have a filament with N monomers that is simply the number of filaments with n monomers, but N n , ok. And you could write down the relevant reactions for this N monomer filament, which is that you can if you have N monomer filament you can add a monomer to it to go to a n plus 1 or you could take a n minus 1 lengths filament and add a monomer to it to go to P_n , right. These are the only two reactions where this P_n will appear. So, you can write this dP_n/dt . And again let us say these are k_{on} and k_{off} .

So, you can write this dP_n/dt , dP_n/dt again, which is plus $k_{off} P_{n+1}$ minus $k_{on} P_n$ plus $k_{on} P_{n-1}$ minus $k_{off} P_n$, right. So, this term and this term represents the flow out of the n length filament, this and this represents the flow into the n length filament. And given this you can ask therefore, how does this average length change with time? How does this average length change with time? What is L ?

(Refer Slide Time: 19:35)

$$L = a n(H)$$

$$\langle L \rangle = \sum_{n=1}^{\infty} a n P_n$$

$$\frac{d\langle L \rangle}{dt} = \sum_{n=1}^{\infty} a n \frac{dP_n}{dt}$$

$$= a k_{on} P_{H-1} - a k_{off} P_1$$

The top graph shows $L(t)$ vs t . The curve starts at the origin and rises, labeled "growth", and then levels off, labeled "steady".
 The bottom graph shows $\frac{d\langle L \rangle}{dt}$ vs t . The curve is a straight line starting from a positive value and crossing the t -axis at a point labeled "d<L>_avg/dt = 0".

In the bottom left corner, there is a chemical reaction: $P_{n-1} + P_1 \xrightarrow{k_{off}} P_n$.
 Logos for NPTEL and CDEEP are visible in the bottom corners of the chalkboard image.

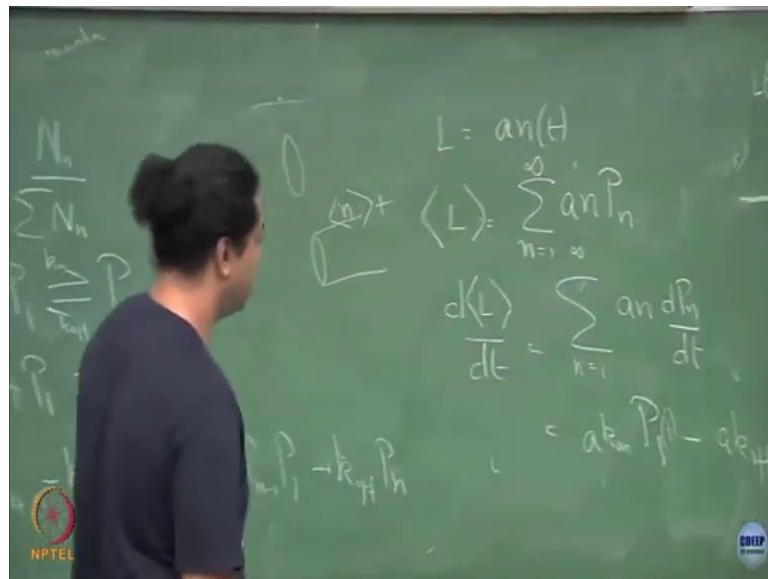
L is nothing but a times n of t, remember. So, average L is nothing but a times n into P of n, right n equal to 1 to infinity. And then you can ask how does this change, dL/dt is nothing, but n equal to 1 to infinity and dP/dt and for dP/dt I know what the time evolution is, that is this equation. So, I can substitute this and I can do this summation. There are lot to do this summation. You can try to do it yourself or you can check the notes. If you do this summation what you will get is, simply that this is a $k_{on} P - k_{off}$ the monomer concentration minus a k_{off} , ok.

So, this is basically what we had started off with, right. $P - 1$ was this $C_{naught} - M_{k_{on}}$ by V . This $P - 1$ is in principle also a function of time. If you say that this $P - 1$ is a function of time this that is the equation we started off with. So, if you plot your dL/dt as a function of concentration of monomers, so dL/dt as a function of the concentration of monomers. Then above this whatever C_{star} which is k_{off}/k_{on} you will have a positive growth, below the C_{star} you will have a negative growth, ok, the C_{star} is k_{off}/k_{on} .

Student: (Refer Time: 21:36).

Yes. So, these are all done in a sort of deterministic, not determine, I am neglecting fluctuations, right.

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So, I am saying that yes I have taken M , but I say that each of them will have n monomers. The way to think about this is that on an average each of them will have the same average n , but plus of course there are fluctuations. So, in principle you should write stochastic differential equations of this and see how important these fluctuations are. So, right now I am not doing that.

Student: (Refer Time: 22:17).

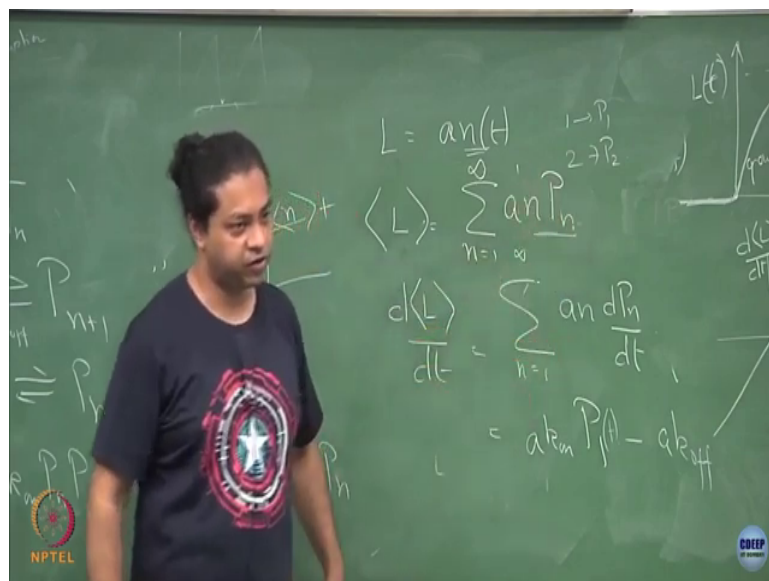
This is the probability to have a filament of length a_n at time t or probability to have more correctly probability to have a , probability that the mean length of the filament will be a_n at time t .

Student: (Refer Time: 22:50).

No, sorry say that once more. We got of expression for the length of the polymer, right.

Student: (Refer Time: 23:04).

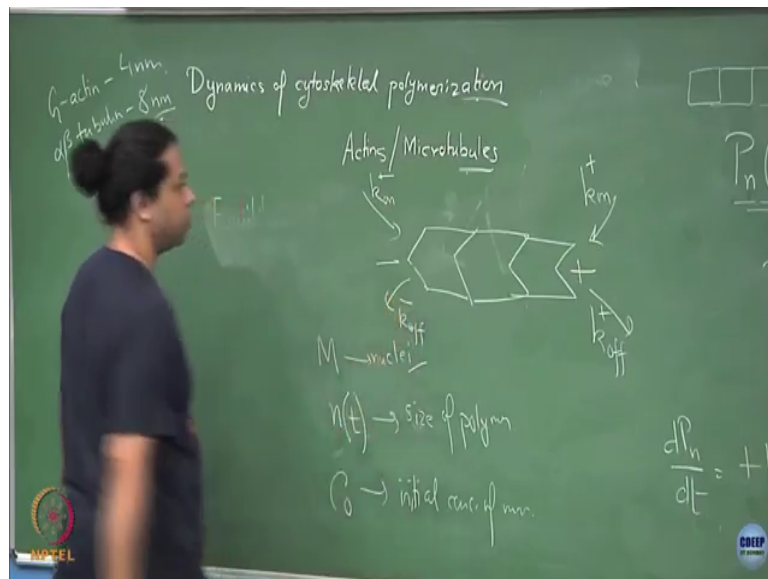
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This one, yes. But then you have this n of t , you can have you have a distribution, right, it is 1 with the probability P_1 , that the mean length of the polymer is 1 with some probability, it is 2 with some probability and so on. So, the average L is going to be whatever $a n$, P_n and there I use this $d P_n dt$, this evolution equation that I already have, ok.

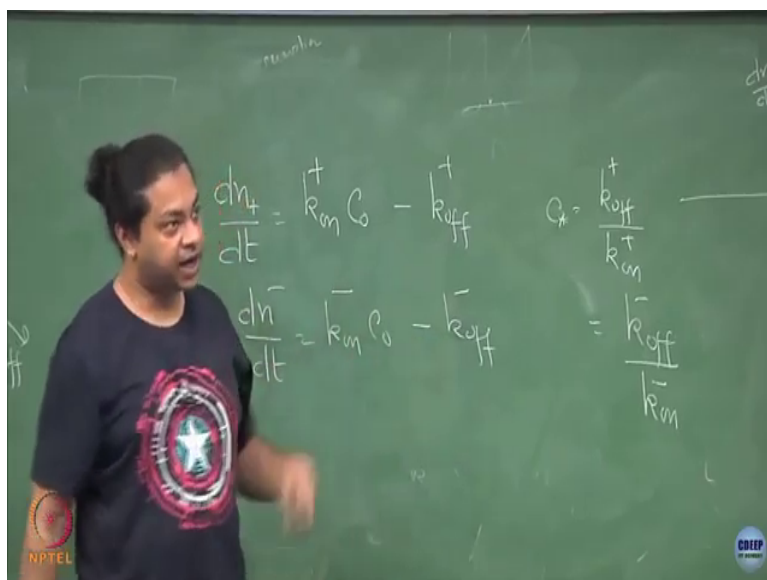
So, at least I have done a little bit now I can do will make it a little bit more complicated. And say, so till now all my filaments that I have been considering are sort of non-polar filaments, there is no differentiation between the plus n and the minus n. So, I can now relax that and say that well. Let me consider a structurally polar filaments.

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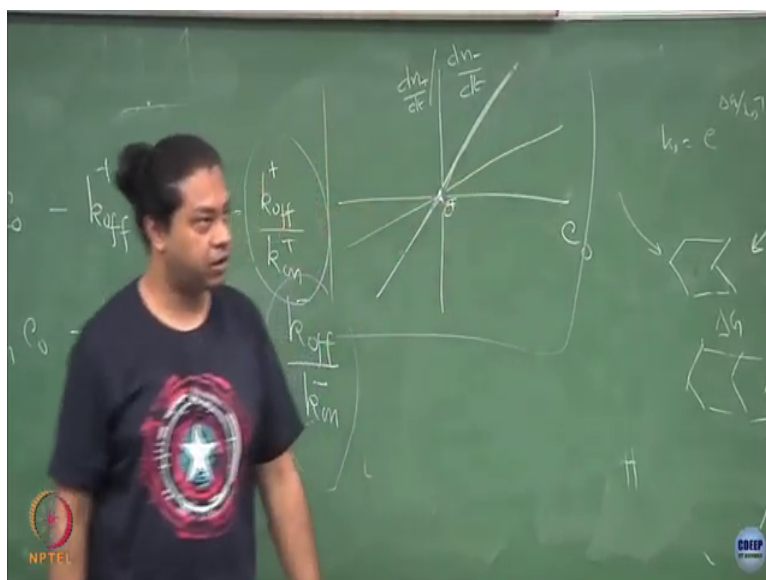
So, let me draw my monomers like this, instead of drawing boxes let me draw a structural asymmetry. So, that one end I will call the plus end, the other the one which looks this skull the barbed end is the pointed end, so one is plus and one is minus. And what does it, is effectively what that means is that what I will say is that I have an association rate which is different at the two ends, so k_{off} and k_{on} plus and k_{on} and k_{off} minus, ok.

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So, what it means is that if write down the rate equations. Let us say for n plus a monomer that is being added on the plus end that is my k on plus times C naught the monomer concentration minus k off plus and at the minus end dn minus dt is k on minus C naught minus k off minus, right. So, basically what this is saying is that what is the rate at which the plus end is growing or shrinking depending on k on and k off, this is the rate at which the minus end is growing or shrinking, ok, dn plus and dn minus, ok.

(Refer Slide Time: 25:43)



And now I can plot these as well. So, let us say again as a function of this monomer concentration C naught; I want to plot dn plus dt and dn minus dt . Remember, this is basically the if I did not have this plus minus this basically the model that we just did which grows if your C naught is greater than C star it shrinks if C naught is less than C star.

What is C star? C star is this $K D$ basically the dissociation constant, so k of plus by k on plus. But in a case like this you can show that this also is necessarily equal to k off minus by k on minus, ok. That these rates themselves can be different, but the ratios of these rates are necessarily the same. Why is that? That is because let us say I have a monomer like this, right and I add a monomer at the plus end, ok. So, I get something this structure.

As opposed to I had this and I add a monomer at the minus end I get the same structure. Once you have this mono structure, there is no way of saying which end you have added the

monomer to, right. It is indistinguishable whether we have added the monomer to this state or this state. Therefore, the free energy difference between these two states ΔG is basically the same regardless of whether you add a monomer here or you add a monomer here. And that free energy difference is just a function of this, so K_D is basically like e to the power of ΔG by $k_B T$, right.

And if the free energy difference is the same regardless of which end you added the monomer to, it means that this dissociation constant of the equilibrium constant must also be the same for both these cases which means that this it is a very nice argument. It says that this regardless of how different these rates are, in a model like this, the ratios of these rates must necessarily be the same, ok. Another way to think about this.

Student: (Refer Time: 28:04).

Yes.

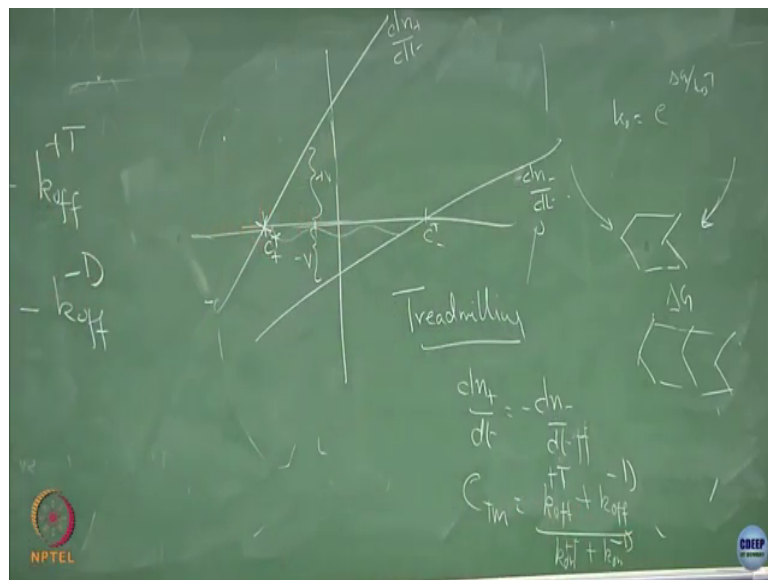
Student: (Refer Time: 28:08).

Why do you say at the minus end this will be greater than that? Realistically, that is what you are imagining. So, then there is a point that this model then says that, let us say that the plus end will grow something, sorry I should pass it through here the plus end will grow like this, the minus end will grow like this. The slopes will be different, but they will intersect at the same C_{star} .

So, when something is growing both ends will grow. If one end is shrinking the other end will also necessarily shrink, which is not a realistic model for example, for treadmill, where one end is growing and another in this shrinking. So, this is a nice argument which actually precisely says what you are saying, that this sort of a model what I have written down cannot be the correct model if I wanted to explain something like treadmilling, where the plus end is growing and the minus end is shrinking, ok.

What do I need to do to do that? What I need to do is to then say that well what is being added here is not what is being taken out here. What is being added here are, these $g T P$ subunits. So, I have a rate plus T . What is being taken out here are these GDP subunits. Once I have this structural asymmetry this argument no longer holds; because now this ΔG can be different, ok. So, once I have these rates that here its being added, with this what is being added is different from what is being taken off what you could have in principle if you were to draw that phase diagram is something like this.

(Refer Slide Time: 29:56)



Again if I plot, so let us say I will have something like this and something like, the two slopes look very similar let me make the two slopes different. Let us here something like this. So, let us say this is my dn plus dt this is my dn minus dt , ok. The slopes are different which is what in the earlier case as well. But the C stars are also different, this is C star plus, this is C star

minus, right. So, you can have a concentration range a concentration of monomers where one end is growing and another end is shrinking.

In fact, you can find exactly a concentration with the velocity of the plus n plus V is exactly equal and opposite to the shrinkage velocity of the minus n ; which is like the perfect treadmilling situation and you can work that out as well. So, the condition for treadmilling if I wanted to do like that, the condition for treadmilling then is that dn plus dt is equal to minus dn minus dt . So, the length of the polymer as a whole remains constant. The plus n grows with some velocity, the minus n shrinks with exactly the opposite velocity.

And then you can calculate at what concentration this will happen. So, if you work this out. So, remember now my equations are these are being at what is being at the plus end are these GTP or ATP subunits, if the minus ends of these GDP or ADP subunits.

Let us say ADP, since this is like treadmilling of actin, in which case you will get a treadmilling concentration of something like k_{off}^{plus} plus k_{off}^{minus} by k_{on}^{plus} plus k_{on}^{minus} . So, the k_{off}^{plus} is a D, now the k_{off}^{minus} is a T, this is a D, the plus is a T this is D, ok.

So, we solve this dn plus equal to minus dn minus. You can solve for this C_{naught} ; which is the concentration of monomers at which you will get perfect treadmilling and a model like this will give you a treadmilling sort of a scenario. And in fact, you can see actually that if you plot the lengths of the growing and the plus end and there is the minus end the curves do look indeed something like this for real actins if you look at experimental curves.