Cellular Biophysics
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Polymerization Dynamics - Part 03

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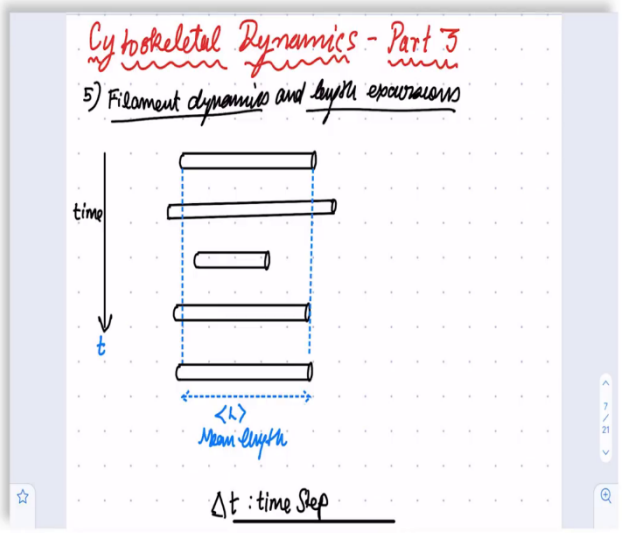

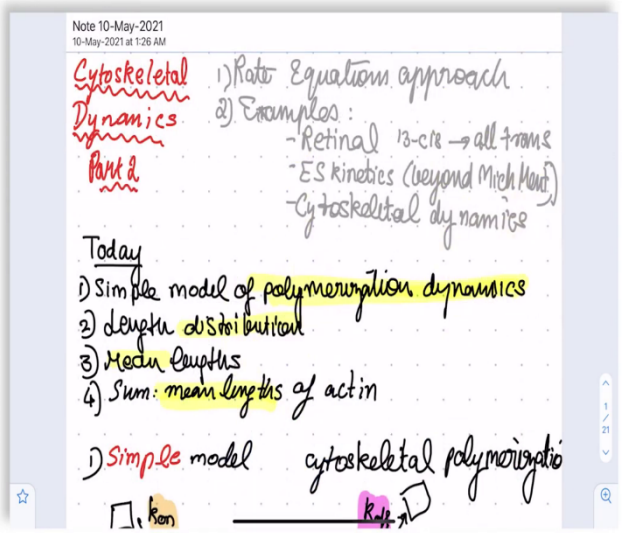

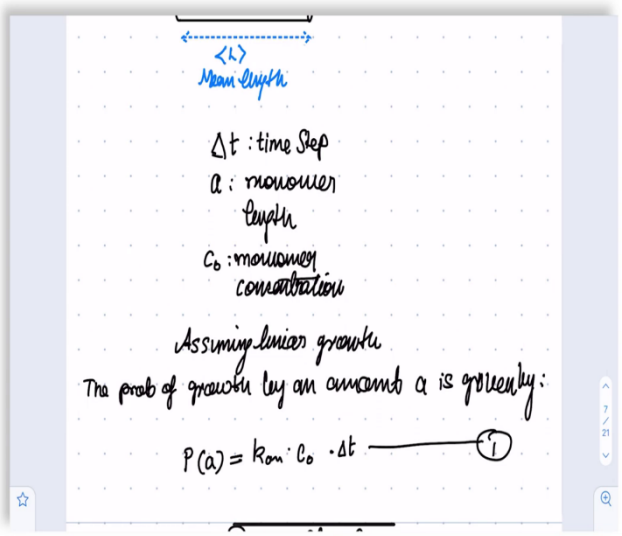

Hi, welcome back. So, this is part 3 of cytoskeletal dynamics. And just to recap, we talked about a simple model, a symmetric model of polymerization dynamics, length distributions which were exponential and the mean lengths 1 by alpha which we then did a small sum to estimate what those mean lengths for action will be. But all these are very static pictures, so for the remaining part, I want to talk to you about dynamics which was the whole theme of this, it was sort of building up towards that.

Filament dynamics, as you can see, can in this particular sense, deviate from some mean length. They may become longer, they may become shorter in time. And that is the premise that we are trying to make sense of by coming up with some arithmetic to make sense of it. So, then the Δ t becomes the time step, a is the monomer length, C_o is the monomer concentration, L averaged is the average length.

Assuming that the growth is linear, there is no branching, the probability of growth by an amount a is given by, obviously the presence of concentration is required, the on rate per micro molar per second and time. So, the left-hand side is the probability of growth and right hand side is that value in terms of typical on rates concentration and time intervals.

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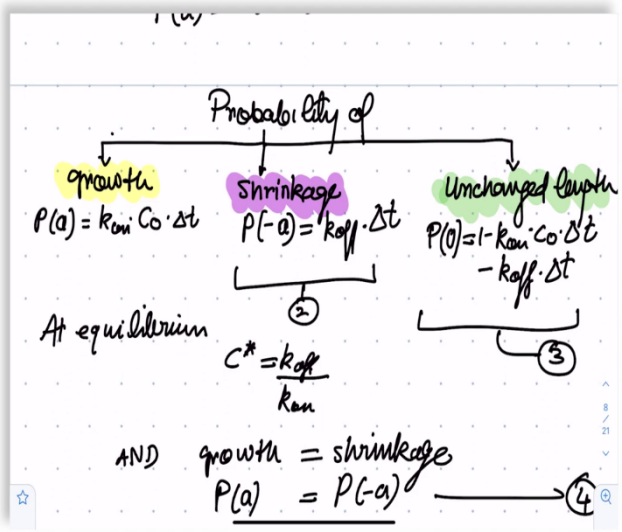

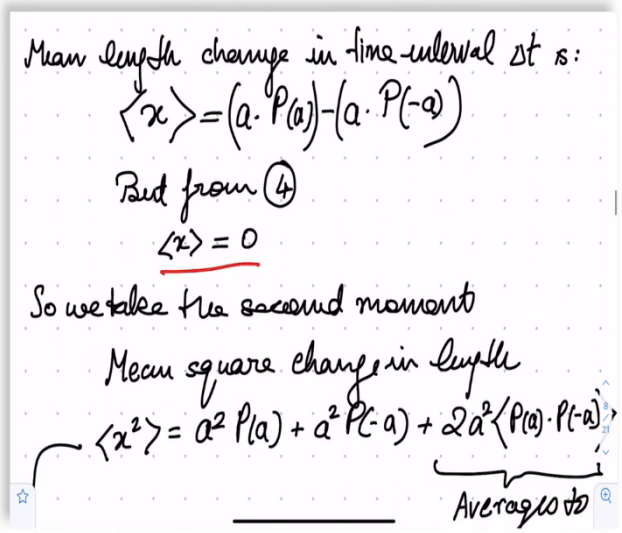

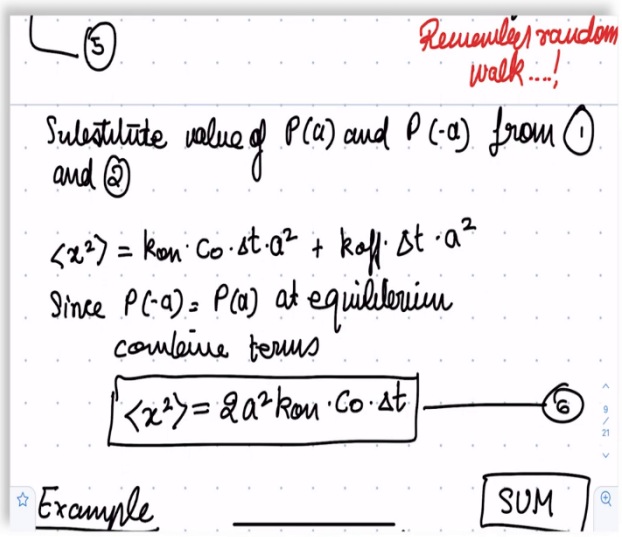

The probability of three possible events however is something that we want to take into consideration, which is that the filament could be growing, in which case the probability is written exactly as we just stated or k on times C_o times Δ t. It could be shrinking, in which case it is given as P of minus a, the removal of a subunit. Or it could just simply be unchanging, and not vary in length at all. And that unchanged length, we denote as P_o is equal to, and now its probability, it should be balanced. So, it is 1 minus the sum of those two, k on time C_o times Δ t and minus k off times Δ t.

So, these are our sort of three questions that we are interested in following. At equilibrium, the ratio of the k off to k on which we also call K_d is also called the c star or the critical concentration. We talked about it last time, about K_d. And at equilibrium also, it is reasonable to assume that the growth and shrinkage are equal, which means that P (a) is equal to P (-a). Then the mean length change in a time interval Δ t becomes x averaged a times P (a) minus a times P (-a).

But remember, we just said that these two are equal, which means that this is basically 0. So, what do we do? We take the second moment and take the square effectively. And so the mean square change in length becomes a square P a times a square P minus a times 2 a square in average P of a times P of minus a. And if you remember how we went about trying to infer the average value of a product of random walk steps going in plus and minus directions, then this product also simply averages to 0.

$$\langle x^2 \rangle = a^2 P(a) + a^2 P(-a) + 2a^2 \langle P(a) P(-a) \rangle$$

And so we can basically deal with only these two terms, a^2 P (a), a^2 P (-a), P (a) and P (-a) are the same, are identical, and therefore we basically end up with a term that combines the rate constants, the k on time C_o times Δ t times a square plus k off times Δ t times a square into either 2 a^2 k on times C_o Δ t or we could have written it as k off Δ t a^2. Either way works.

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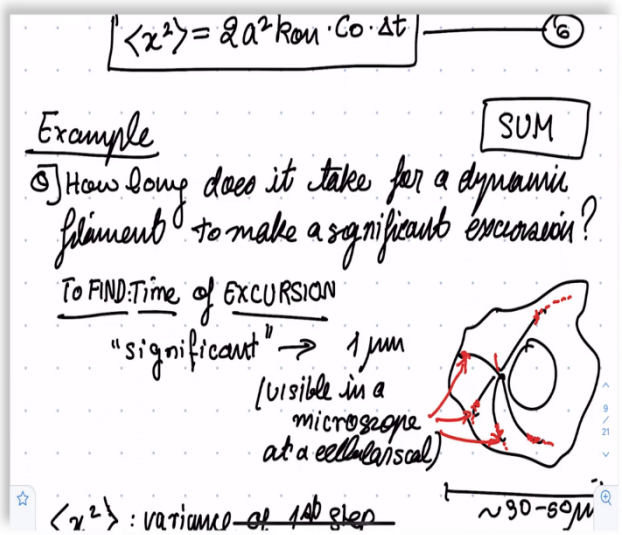

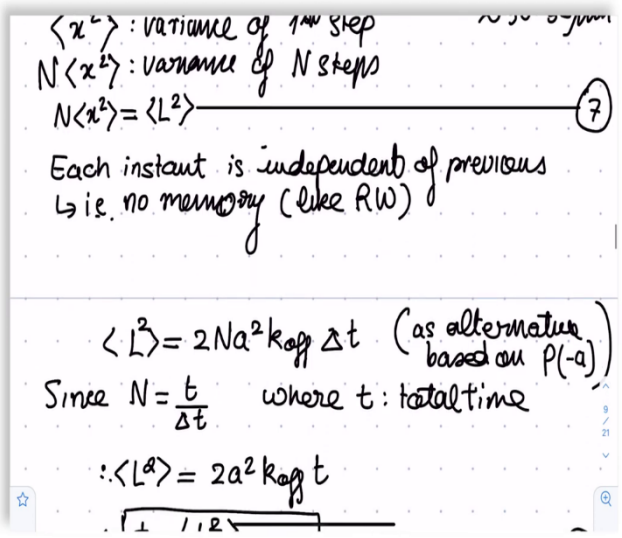

So, let us ask, now with this expression in hand, let us ask how much time does it take for this kind of a dynamical excursion to happen. Remember, we wanted to know how much do they fluctuate. So, we want to find some kind of characteristic time. For this characteristic time, we have to define something practical and so we have asked the question how much time does it take to make a significant change in length, significant as a micron. Why? Because

visible in the microscope and at a cellular scale of a 30 to 50 microns, it makes some sense, it is important. 1 micron is a big deal.

So, we take x^2 as the variance of the first step, N x^2 is the variance of N steps. Therefore N x^2 is equal to L^2. L^2 is now averaged, is our length, each instant is independent of the previous, there is no memory. Just like a random walk. So L^2 is equal to 2 N a^2 k off Δ t. And this is the alternative that I mentioned earlier based on the solution to P (-a) being identical. That is to say, taking the off rate times Δ t.

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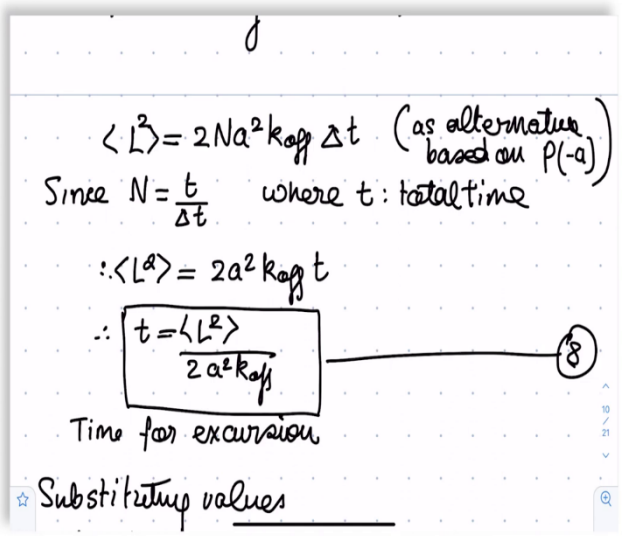

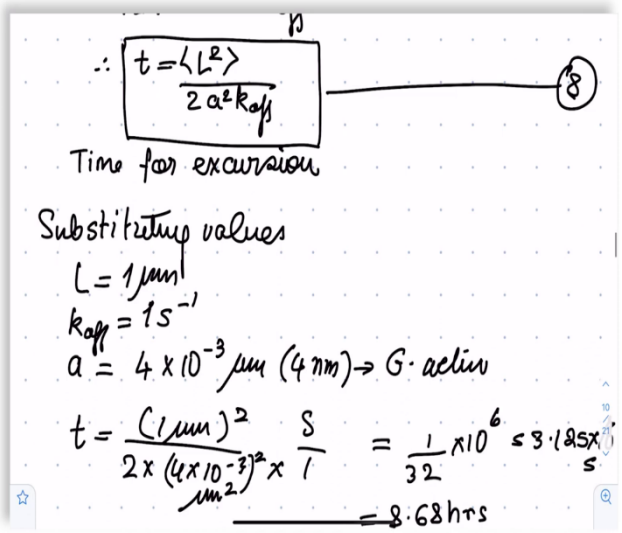

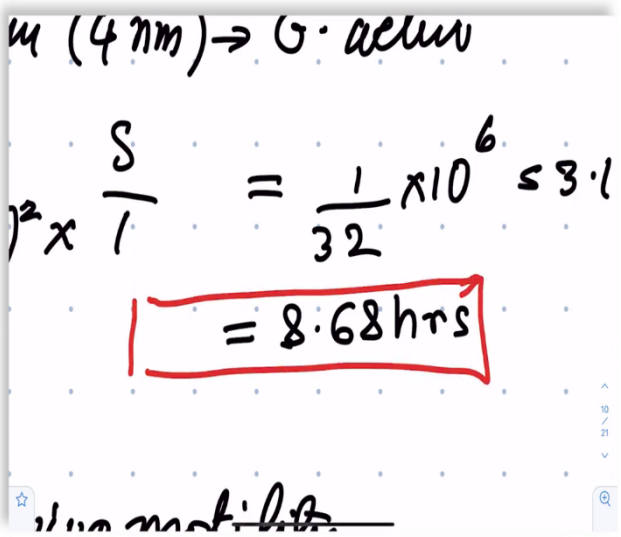

Since n is nothing but t times Δ t, we find that L square is written as 2 a^2 times k off t, which then basically leads us to t time being the average length squared divided by 2 a^2 upon k off. So now, if you want to find the time for excursion, we just need to substitute the values.

We have 1 micron here, k off is 1 second, so 1 in the numerator, 1 in the denominator and then all we have to do, deal with this 1 upon 2 a^2, which effectively gives us this number here which is 1 by 32 into 10^6, which becomes 3.125 into 10^4 seconds, which is actually a very large number in time, which is 2.68 hours. Now, this is surprising. So but, surprising because we do not think something should take this long, 1 micron cannot take 8.68 hours, but in vivo motility, what happens?

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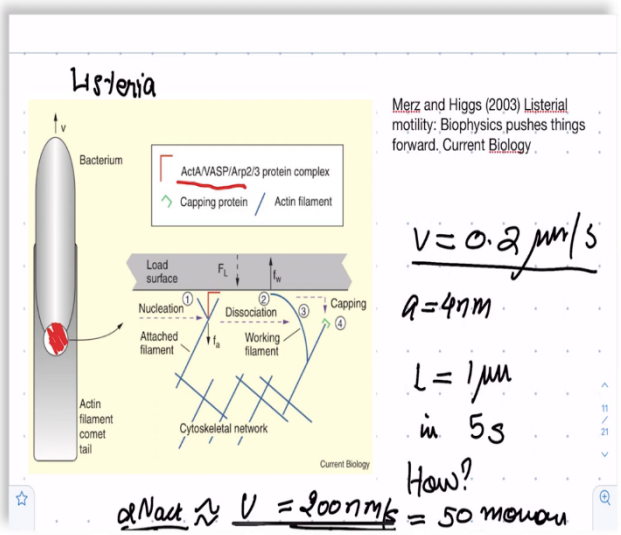

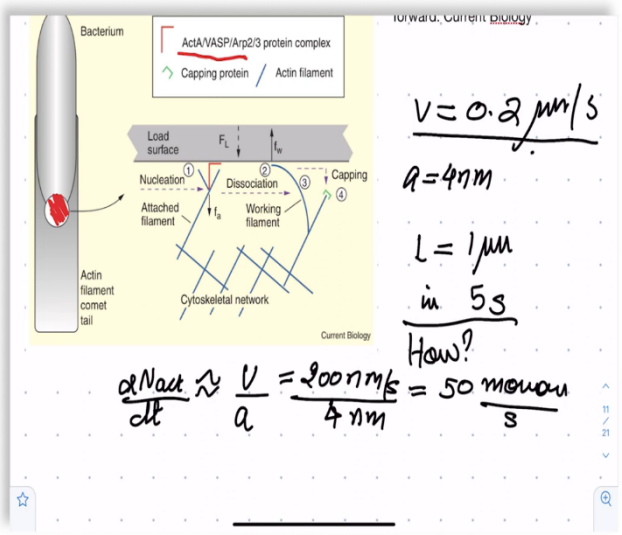

Okay, let us look at typical in vivo motility driven by actin. This is effectively the polymerization rocket of Act a, the protein that, that drives the polymerization of actin at the tail of a bacterium. This is listeria monocytogenes. And if you remember, we did some order of magnitude estimates earlier about cytoskeleton and cytoskeletal dynamics, when I introduced the topic.

And, and this effectively moves at a velocity of 0.2 microns per second. a is 4 nanometres. We take it as the G-actin size, L is 1 micron again, and therefore it means just simply from this, if 0.2 microns take a second, then 1 micron takes 5 seconds. So, we expect that an excursion of 1 micron will take 5 seconds.

Just to know roughly what is the rate of polymerization, we say that if the velocity of movement is about 200 nanometres per second, it is driven, and we can assume in a simplifying sense that it is driven mostly by polymerization. Then dividing it by the nanometre size of the monomer, gives us 50 monomers per second. And if you take 3, then you get 70 monomers per second. Essentially, that is the rate of addition of monomers in a rapidly polymerizing active polymer. That is quite fast.

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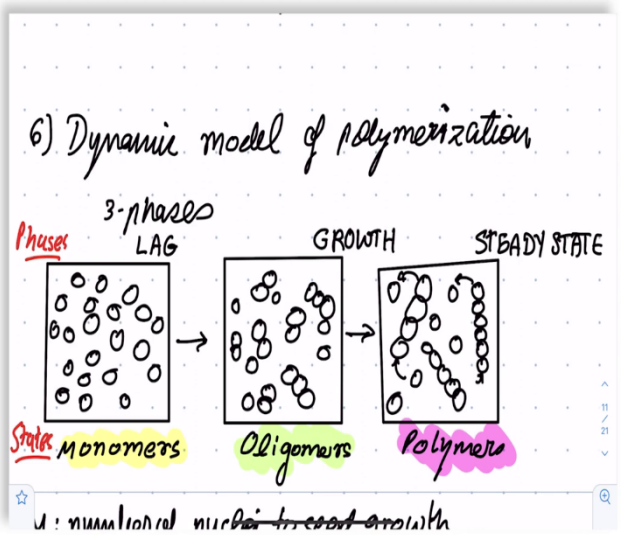

So, we now get to a dynamical model of polymerization, which in fact we try to take a little more into account what is going on at a global sence. So, in some sense we can say that a polymerization process which consists of monomers, oligomers and polymers, it goes through these three phases of lag, rapid growth and achieves a steady state. So, these are the three phases and the three states, and they are represented in this cartoonish fashion with bead-like monomers that are joining form initial nuclei which are the oligomers, and then they then elongate by assembly into polymers.

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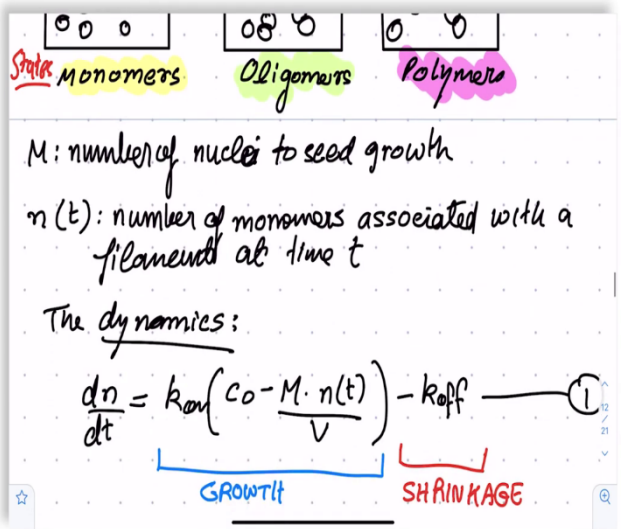

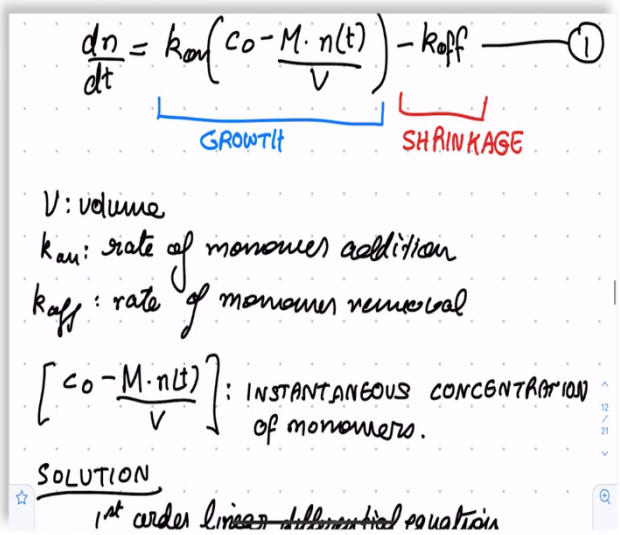

If M is the number of nuclei to seed the growth and n of t is the number of monomers associated with the filament at time t, the dynamics can be given in terms of d n by d t which is a change in number of monomers inside a polymer in a polymeric form, is equal to, and now the growth part k on times c naught minus M times, n times n t upon v minus k off, which is the shrinkage.

$$\frac{dn}{dt} = k_{on}\left(C_o - M\frac{n(t)}{V}\right) - K_{off}$$

So, this is your differential equation form of the dynamics of a filament, this is what we were trying to get at, a dynamical model of polymerization, we need a solution. So, for that we need to take a few more things into account, volume, on rate of monomer addition, off rate of monomer addition and that the instantaneous concentration of monomers is basically the concentration monomers that we started with, minus what became into polymers. This should actually be not n but M.

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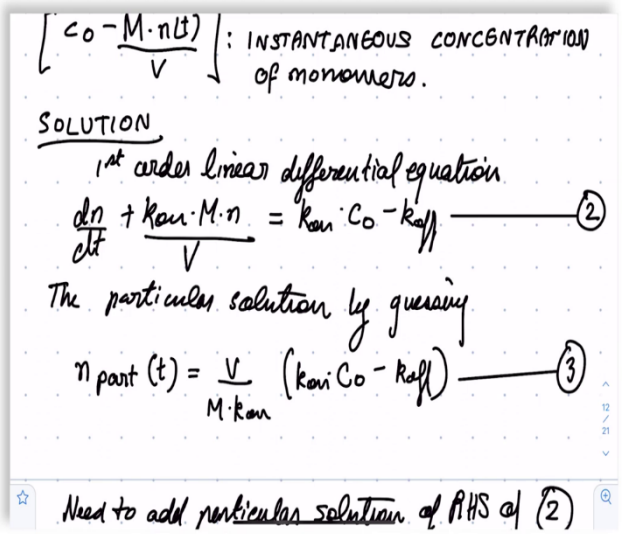

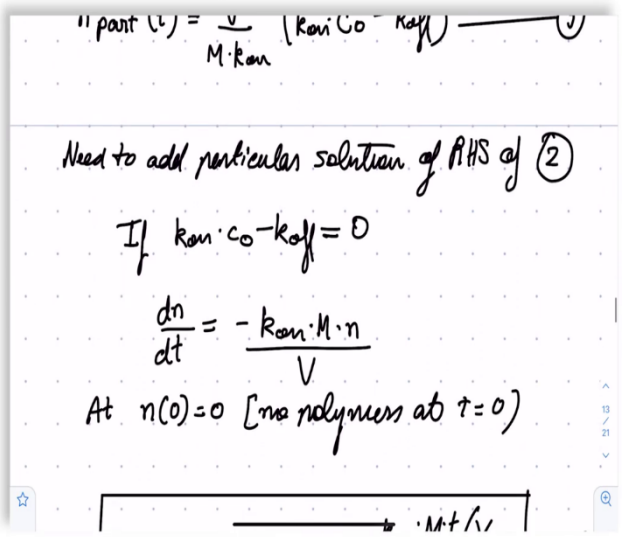

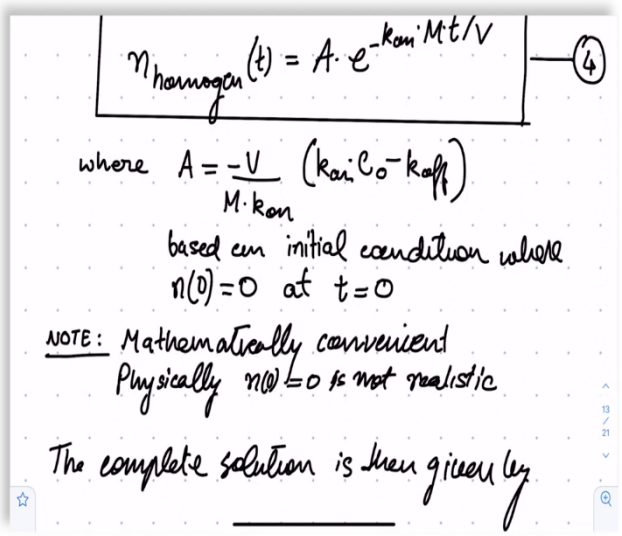

The solution is that of a first order linear differential equation and it gives us d n by d t is, now we take things on one side, and a particular solution by guessing becomes in particular is v times, M times k on up, into the bracket terms which is k on c naught minus k off, but we need to add a particular solution for the right-hand side of 2, which was this guy, this here. And by doing that, we assume that k on times c naught minus k off is 0.

Or in other words the rate of addition and the rate of removal are the same. This is at steady state. In such a case, d n by d t is minus k on times M times n upon v. And at initial conditions, we assume that there are no polymers. And in such a case, the complete solution, the homogeneous solution n of t is A to the power e times minus k on Mt v.

How does this A term show up? We basically again go back to taking the conditions of t is equal to 0 giving n is equal to 0 and then solve for A and get the solution of A is equal to minus v times, divided by M upon k on into in the brackets k on time c naught minus k off. This is a familiar term you have seen, it keeps showing up again and again.

While this is mathematically convenient, physically this may not necessarily always be realistic. And then we need much more complex models, and those are not something we are going to go into right now. There are a series, something like 40 papers in the 80s and 90s which actually dealt with this.

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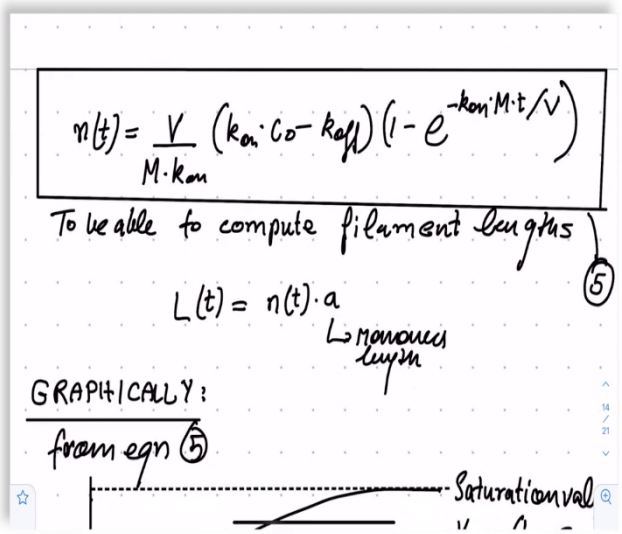

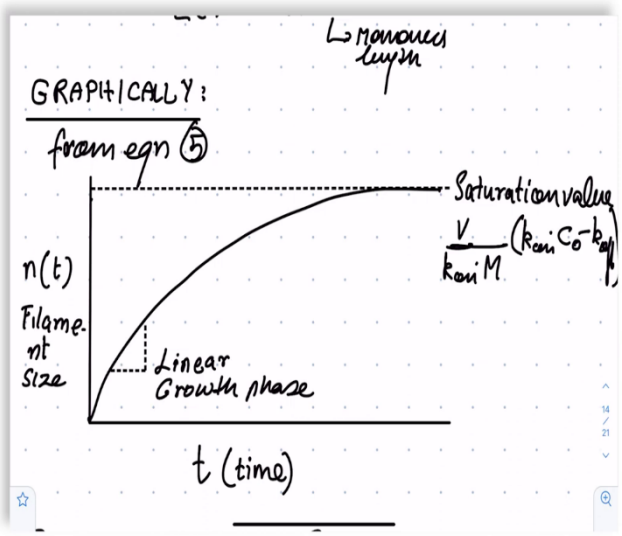

The complete solution is then however given for this system as n of t, is M v upon M k on. And then now, this k on times C_o minus k off and then 1 minus e to the power minus k on times nuclei times time divided by volume the concentration of nuclei, in some senses. To be able to compute filament lengths, we assume that L of t is just simply n t times a. So that we can compare it to experiment.

$$n(t) = \frac{V}{M K_{off}} (K_{on} . C_o - K_{off})(1 - e^{-K_{on} . M . \frac{t}{V}})$$

So, from equation, this equation 5, this so-called complete solution, we can now graph it and get something that looks like a saturation curve. In the initial phases, we have a linear growth

phase and in the saturation phase, we have a value which it converges to which turns out it can be actually written out as v times k on upon M, k on C_o minus k off in brackets.

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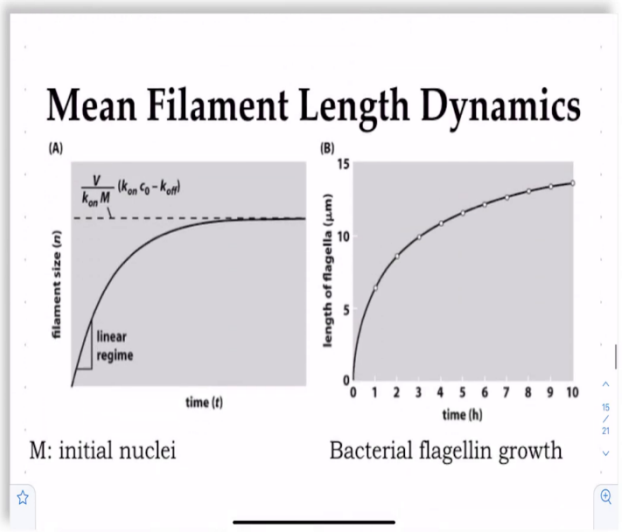

What about real experimental data? Does this, does this ideal graph even compare to anything that we can find? Looks like filament length dynamics from a bacterial flagellar protein called flagellin, when it is plotted over here as a function of time, shows a beautiful match with this theory. And in fact, you can fit it, and using the fit, estimates all the kinetic parameters.

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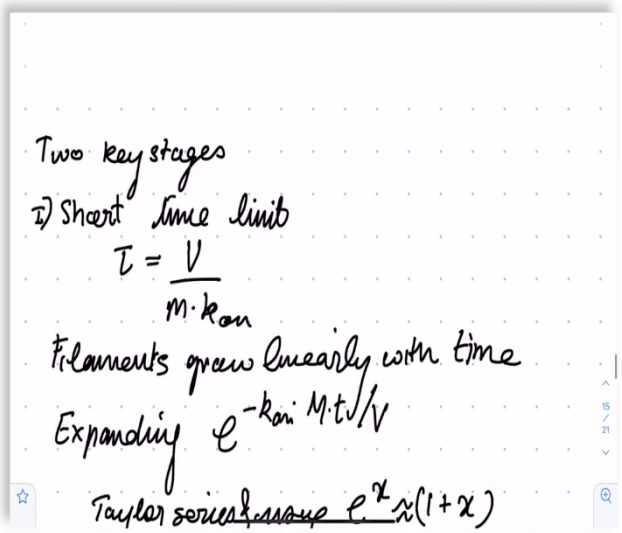

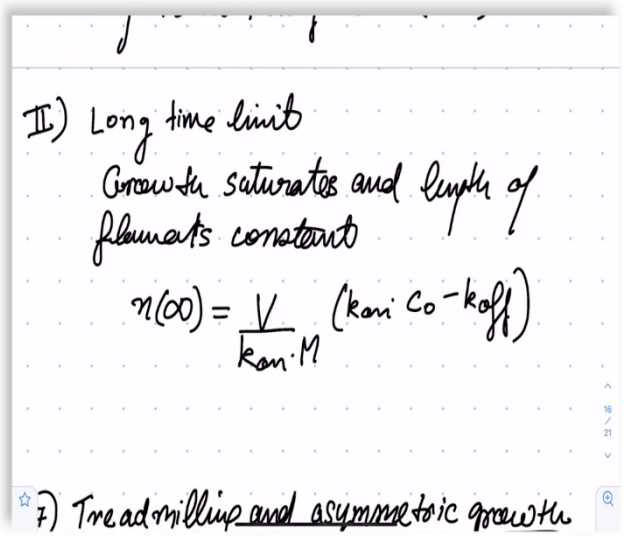

There are two key stages to this growth. The short time limit where tau is v upon M times k on, filaments grow linearly with time and this tau was obtained by expanding linearly this term, this e to the power minus k on times M times v, M time M divided by v and Taylor series expanding.

In the long time limit, the growth saturates and the length of filaments is consistent, is constant, and that value is this num, this expression which we saw earlier, v upon k on times M and k on c naught minus k off. What is the meaning of this? This is sort of telling us that the initial presence of nuclei in the denominator and the difference between the on and off rate, scale the size of the final polymer at which it saturates.

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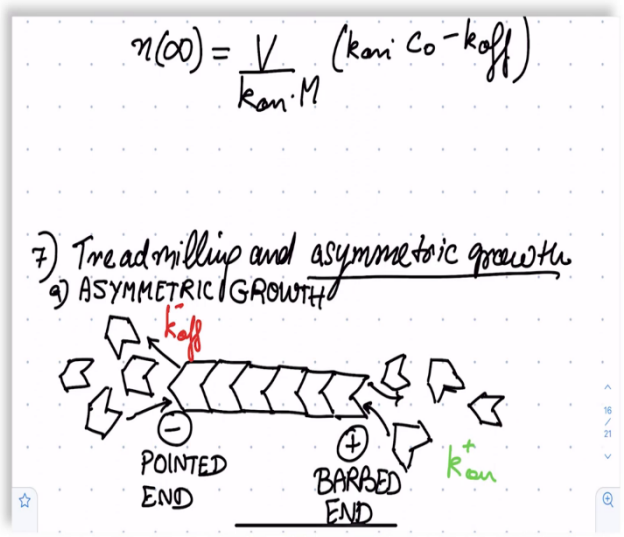

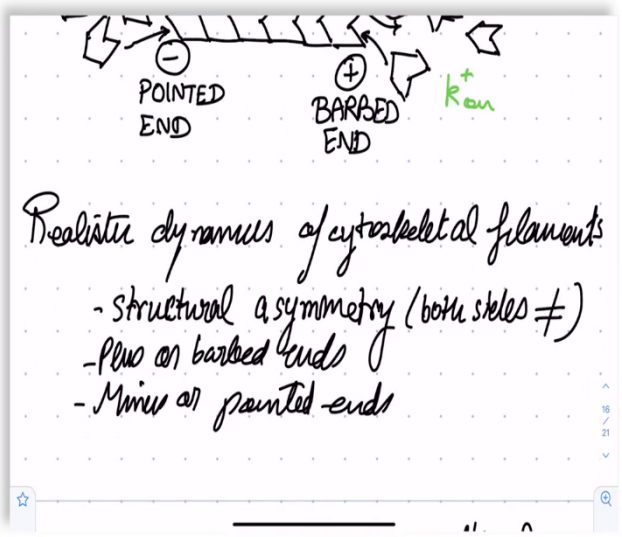

Treadmilling and asymmetric growth are the last topic that we want to cover, because so far as you remember we have been considering a symmetric polymer. We have been assuming that the growth is the same at both ends. But we know from a lot of biology and biochemistry and molecular biology and cytoskeleton dynamics, that this is not the case.

There are so called plus and minus ends. And in actin cases, they are called pointed and barbed ends because how the actin filament looks under the microscope in electron microscopy, to be precise, the nanoscope maybe, it is more precise to say this. The realistic dynamics of cytoskeletal filaments arise due to structural symmetry because both sides, both ends do not grow at the same rate, and they are called obviously, barbed end and minus or pointed end, as we just said.

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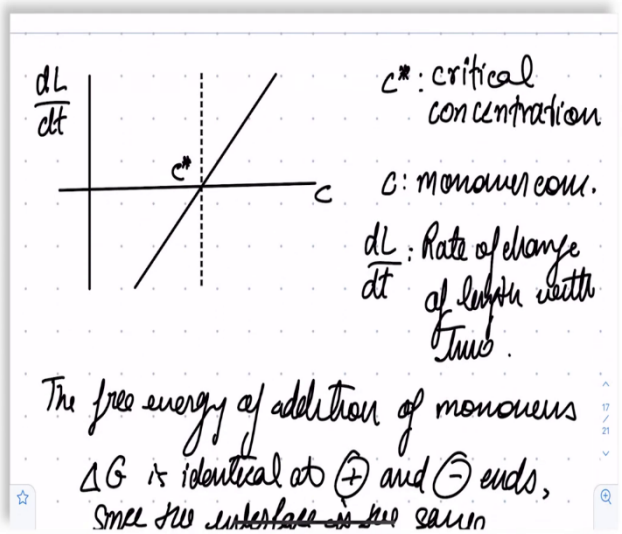

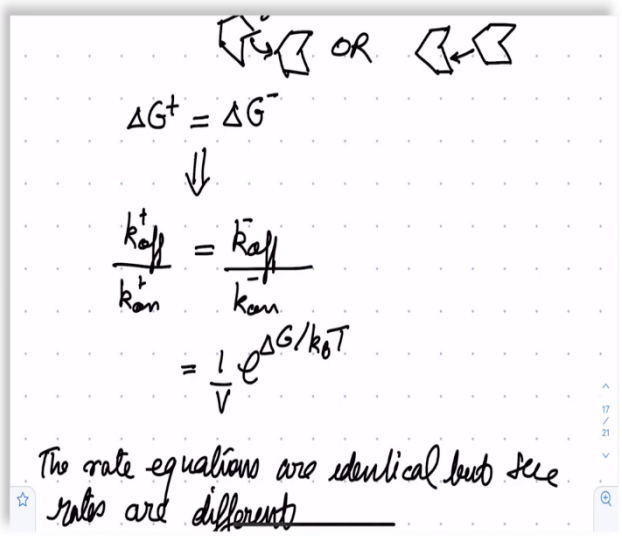

Now in the simplest case, if there is a single filament and it goes at the same rate at both ends, then the curve of the rate of growth d l by d t as a function of monomer concentration, looks something like this. If there is a, and this is the maximum growth rate. The critical concentration is the point at which this line intersects the x-axis.

The dashed line indicates that the exact value, c is the monomer concentration, reality is changing length. The free energy of addition of monomers, if it is identical at both ends, then Δ G plus and Δ G minus are equal and then this ratio is equal and this becomes equal to 1 by v e to the power Δ G k_BT.

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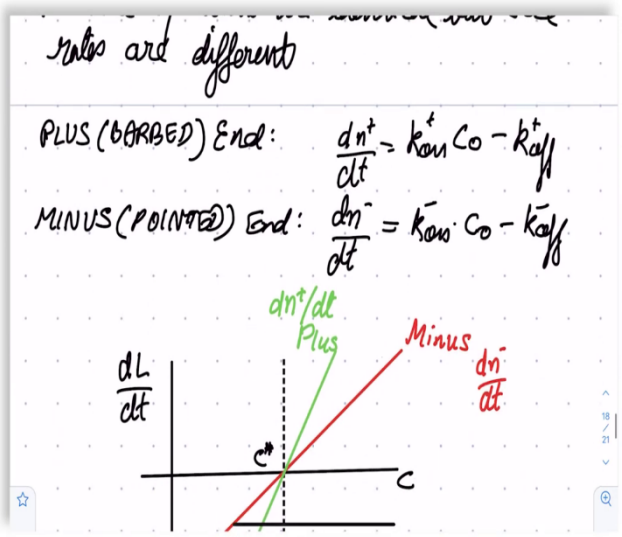

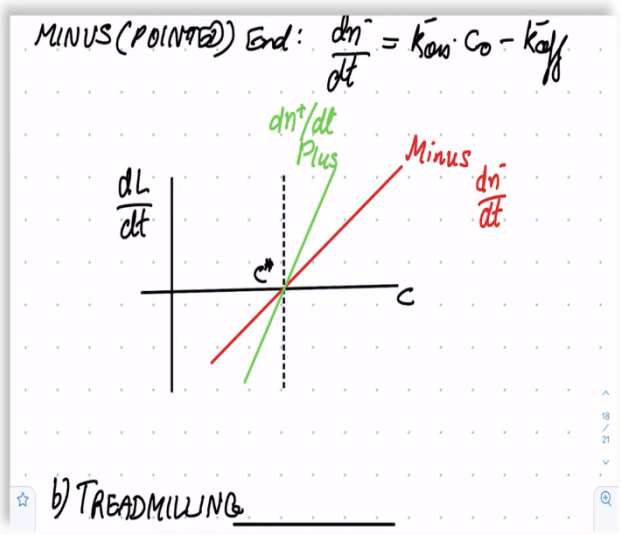

The rate equations are identical but the rates are different, and in such a case you get two rate constants k on and k off, which are not identical to each other and indeed their curves then begin to look like this. They intersect, still at the same c star value, meaning the same critical concentration, but they have different slopes.

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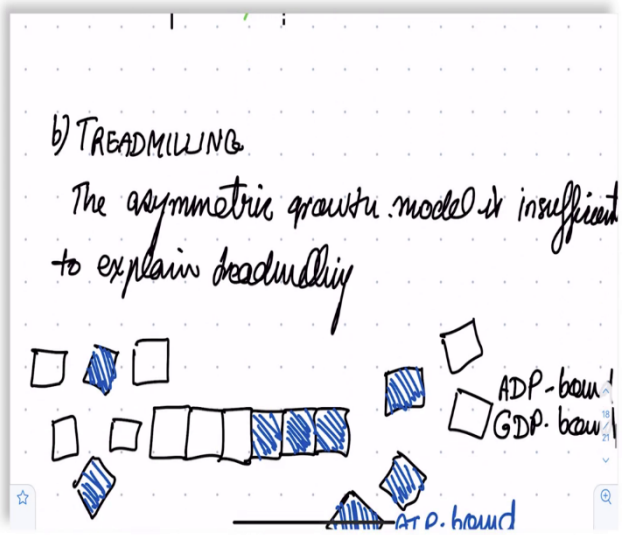

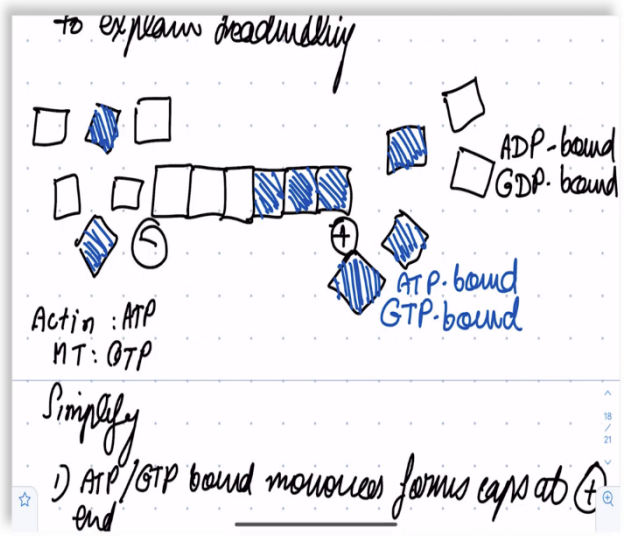

And treadmilling is a special case of this when the asymmetric growth model is insufficient to explain treadmilling, and we also need to invoke not just plus and minus ends but also the fact that there are two states of monomers ATP bound and ADP bound or GTP bound and GDP bound depending on whether you are talking about actin or microtubules. Because as you remember, microtubules are primarily GDP bound or GTPs and actin has an ADP domain.

And they depend on this for their polymer, these high energy phosphate bonds for their polymerization. And this nucleotide specificity is quite acute for instance you cannot replace one with the other. And it is kind of an interesting question how these divergences evolve, but again not for today, please feel free to read more in molecular biology, The Cell and Alberts, there are a few indications of some literature in the past.

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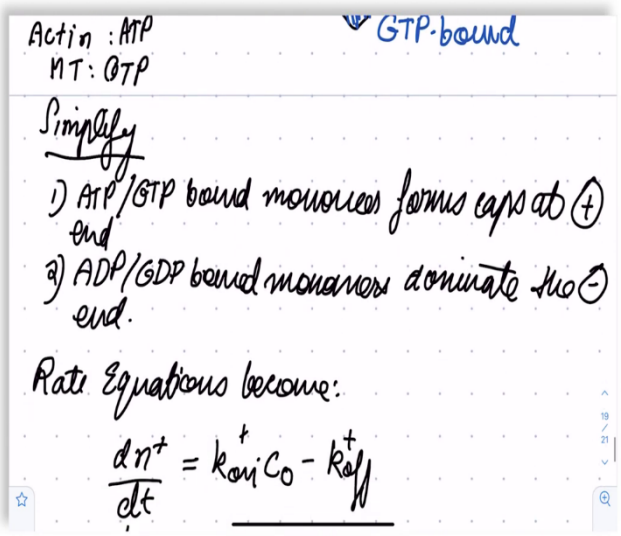

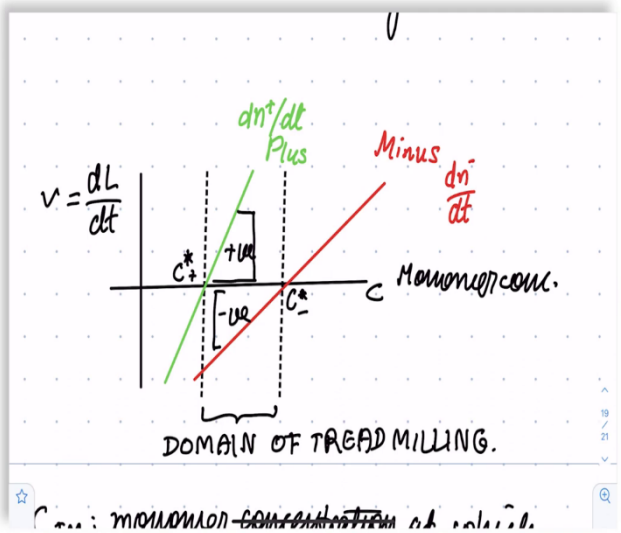

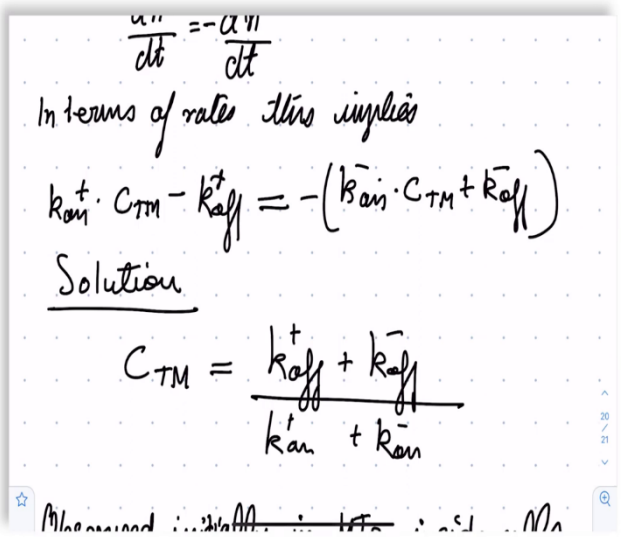

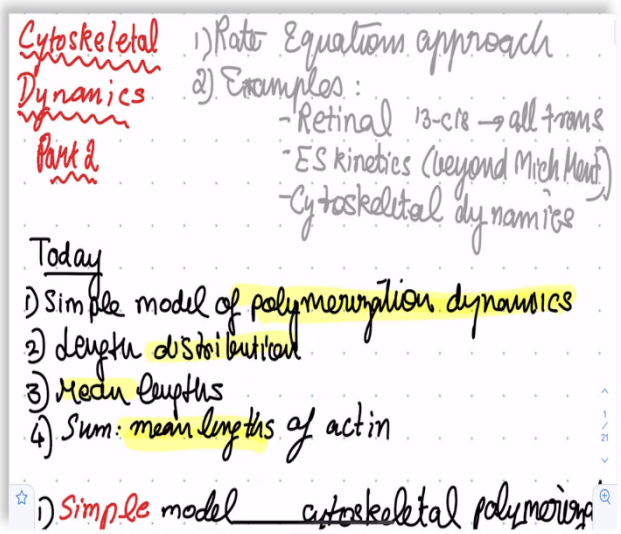

So, if you simplify this, then ATP bound and GDP bond monomers form caps at the plus end as you see here. That means, they are predominantly GTP bound at the plus end and they are predominantly minus end, at the minus end they are predominantly GDP or ADP bound. In such a case then we have the rate equations that then look kind of similar to what we had earlier with no constraints on the ratio of the rates. And in that case there are two c star values.

There are now a critical concentration of the plus and a critical concentration of a minus end. And that, these then have a domain in between, which we call the domain of treadmilling. And that concentration is the monomer concentration at which the d n plus, that is to say, is growth at one end and shrinkage at the other end. And they are equal, but opposite in signs. And in terms of rates, this becomes, by substituting d n plus and d n minus, we get these

terms that this should have a plus here, can be resolved to estimate a solution for critical concentration of, I am sorry, the concentration at which treadmilling will happen, to be the ratio of k offs upon k ons.

And this was initially observed in microtubules and later in actin. So, I will here now. And just to remind you what all we have gone through, we have talked about a simple model of polymerization dynamics, length distribution, medium lengths, sum of median length, I am sorry, the sum that deal an arithmetic to calculate these mean lengths and then we talked a little bit now about filament dynamics and length excursions, where we derived an expression for the extent of the excursion and its relationship to time.

We described a dynamic model of polymerization, we looked at, without really maybe necessarily solving it, we looked at a full solution for the expression where there are dynamics of growth and shrinkage happening at the same time, gives us the full kinetic curve. This looks like experimental data, and then we discussed an asymmetric growth model and the role in treadmilling. Thank you very much.