Cellular Biophysics
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Polymerization Dynamics - Part 02

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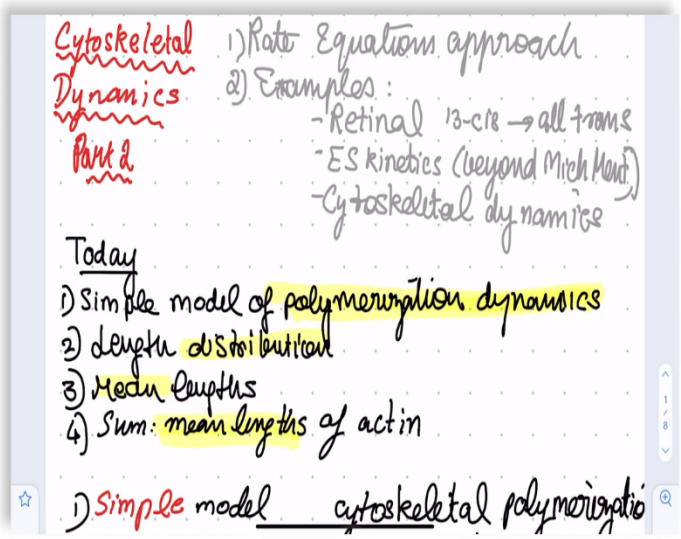

Hi, welcome back. So, we last spoke about the rate equation approach. This should be rate. And took some examples from biological biomolecular systems like the transition from 13-cis to All-trans retinal, which is involved in vision enzyme substrate kinetics beyond Michaelis and Menten, which we did not write down any equations for. And finally, we concluded the cytoskeletal dynamics proves to be a more interesting phenomenon simply because it goes beyond what you have already studied, and something hopefully that might be a little new for you.

So, for today, for this particular module and this called, I am referring to as part-2 of the cytoskeletal dynamics part. We are going to talk about a simple model of polymerization dynamics, which involves assume, making some simplifications with respect to the nature of the polymer, of addition and removal and yet strangely we get some very exciting and useful results from it. And those two results that I am going to discuss for this particular part are the distribution of the lengths and the mean values of the lengths. And we will use the expression that we get from it to actually calculate the mean lengths of actin. So, let us get to it.

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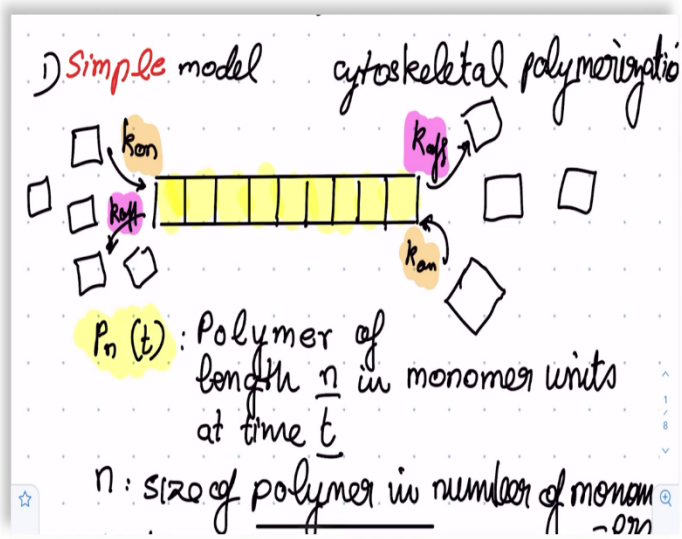

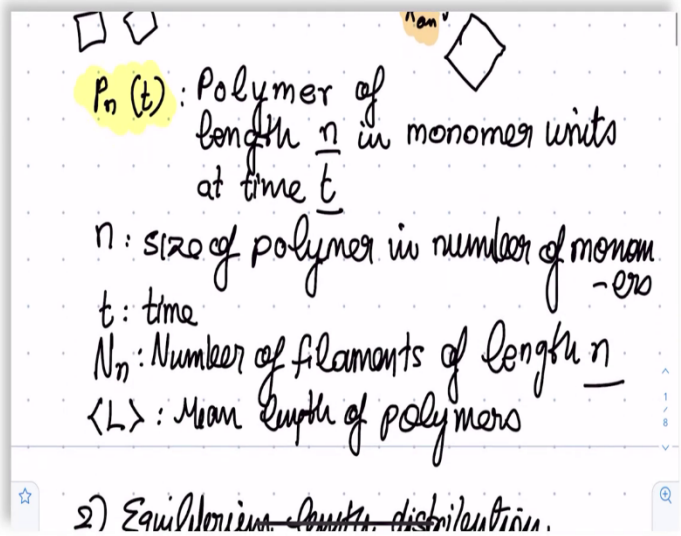

So, what is this simple model that I have been referring to? Now, what you should notice here is that I have modelled my polymer despite what I said earlier like as a line of bricks, these boxes, these boxes are formed into a sort of linear array. They only seem to grow from the ends.

The end growth is itself dependent on two rate constants k_{on} and k_{off}. These k_{on} and k_{off} rates are identical at both ends. In other words, there is no difference between the ends. You can say there is no kinetic polarity. And that this dynamics gives rise to a distribution of polymers, which we are going to try and make some sense of.

So, P n at time t is the polymer of length n, in terms of monomer units at time t, n being the length of the polymer in terms of number of monomers, t being the time. In that case N n is the number of elements of length n, and L averaged is the mean length of the polymers.

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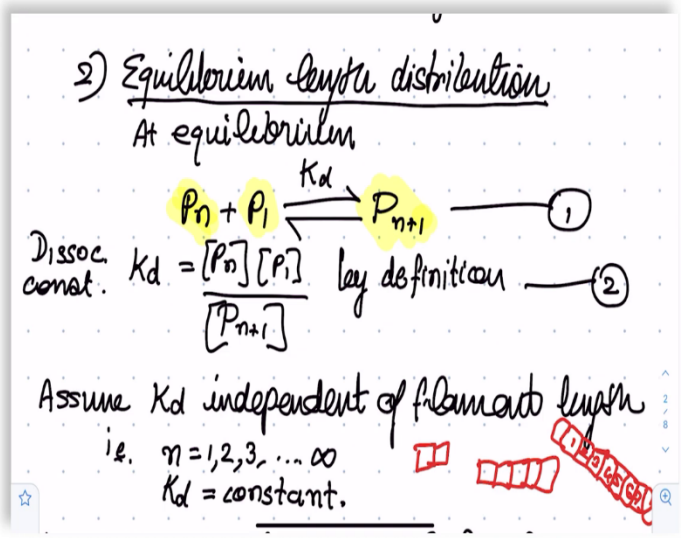

So, what is the equilibrium length distribution like? So, at equilibrium we expect this reaction that is to say for an n sized monomer the addition of, I am sorry, n sized polymer the addition of a monomer results in growth by 1 unit. This is n plus 1. This is reversible and it is governed according to how we have written it by a dissociation constant. This dissociation constant K d, by definition, is the dissociation of P n plus 1.

$$P_n + P_{n+1} \underset{\rightleftharpoons}{\overset{K_d}{}} P_{n+1}$$

$$K_d = \frac{[P_n][P_1]}{[P_{n+1}]}$$

Therefore, it can be defined as P n times P 1 upon P n plus 1. It is by definition. If we assume that K_d does not change no matter whether you have a mono, you have n is equal to, if you have just 2 or 5 or 10 monomers in the filament, it should not matter. This is 1, 2, 3, 4, 5, 6, 7, 8, I am running out of space, 9, 10.

This is another way of saying that the rate constant does not depend, rate constant of growth does not depend on the length of the filament, and we will return to this kind of a question a little later because there are some interesting exceptions to this. But for generality and simplicity, this is a reasonable assumption.

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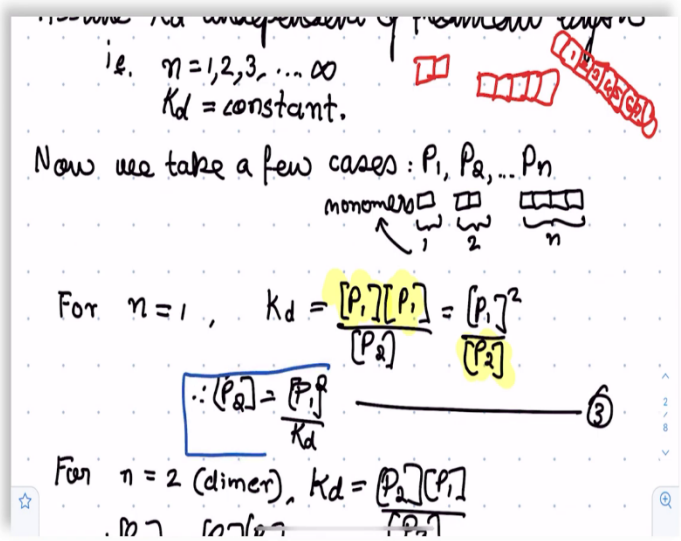

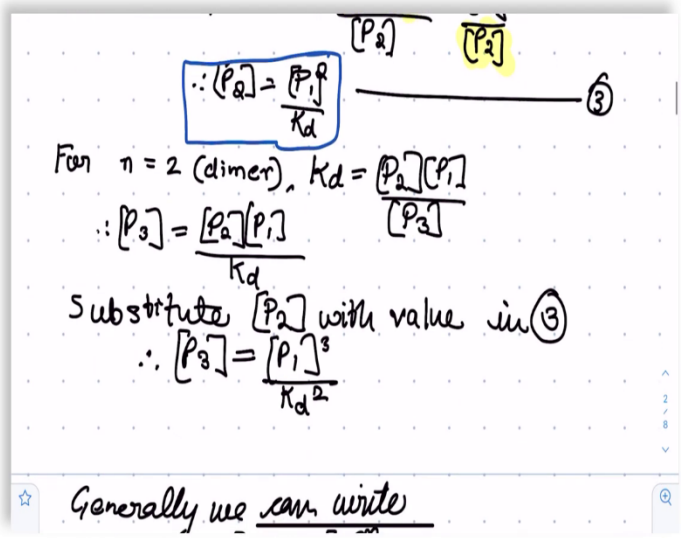

So, we take a few cases. P 1, P 2, P n and we ask what are the expressions that we can determine for their population. So, n 1 gives K_d as P 1 times P 1 upon P 2 which is P 1 square by P 2 which is kind of self-evident to most of you. This was our polymer. These were the two precursors. We can therefore change the signs and write the P 2 in terms of P 1 square by K_d.

So, now you can, get the picture for an n 2, n is equal to 2 dimer, K_d is P 2 times P 1 upon P 3. But if we substitute the value of P 2 in this expression where we have shifted P 3 on the left-hand side, then we get back P 1 P 3 is equal to P 1 cube to the divided by K_d^2. In

other words this, P_1^3 is nothing but the monomer concentration raised to the power which is the same as the size of the number of monomers in the filament.

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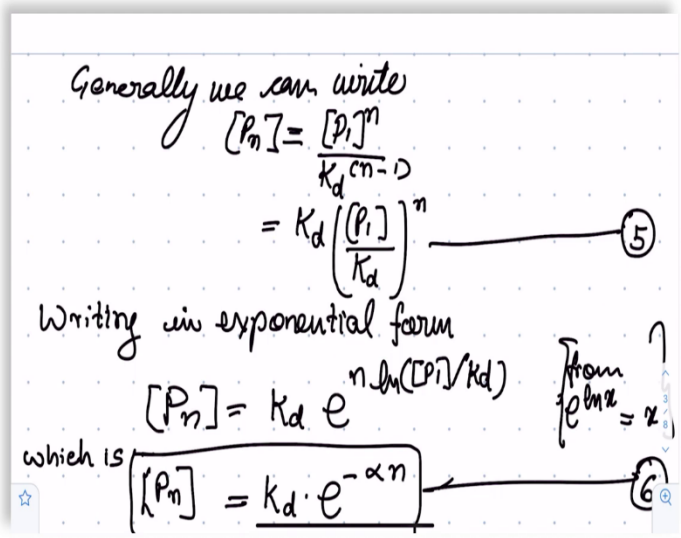

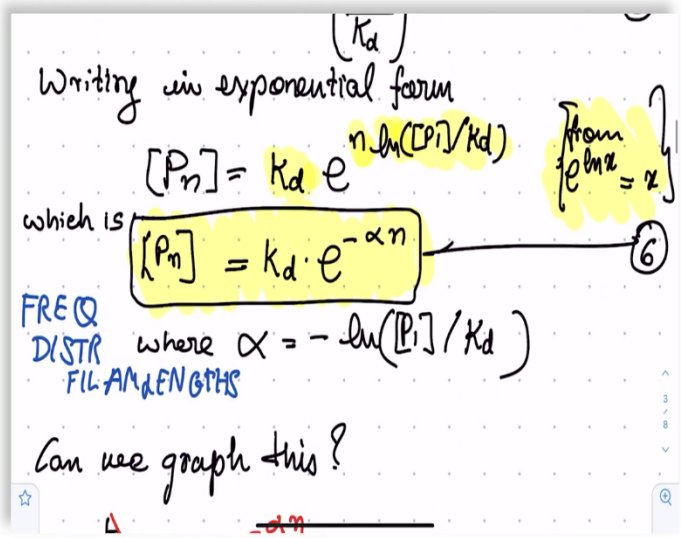

Therefore, it looks like we can generally write P of n concentration meaning how often it is prevalent, if the volume is constant, it's just a number, number of filaments of a certain length is equal to P 1 to the power n upon K_d upon n minus 1. And if you take K_d out because we will see it becomes a little more useful then we get K_d times P_1 upon K_d time to the power n.

Writing this in the exponential form then becomes P_n concentration, meaning to say the number of elements of a certain length n is equal to K_d e to the power n times natural log of P_1 upon K_d. This is coming from this very general idea that e to the power natural log of sum x is equal to x.

$$[P_n] = K_d \cdot e^{-\alpha n}$$

What this allows us to do is number 1, write this in terms of a symbol which we are using here to label this equation number 6 which is P of n is equal to K_d into e to the power minus alpha n. What is alpha? Alpha is minus natural log of P_1 by K_d. We just basically substituted this term here P_1 by K_d natural log by a term alpha. We created a new term.

The negative negative signs cancel out, it becomes plus. But the nice part about this is, just stare at this equation for a while and just think of it that as the x variable in this case, n increases, the number of monomers in the filament or the length of the filament you may say, we see a characteristic dependence which is an exponential decay coming out of this equation number 6 of the number of filaments. In other words, nothing but what we always like to talk about as the frequency distribution of filament lengths.

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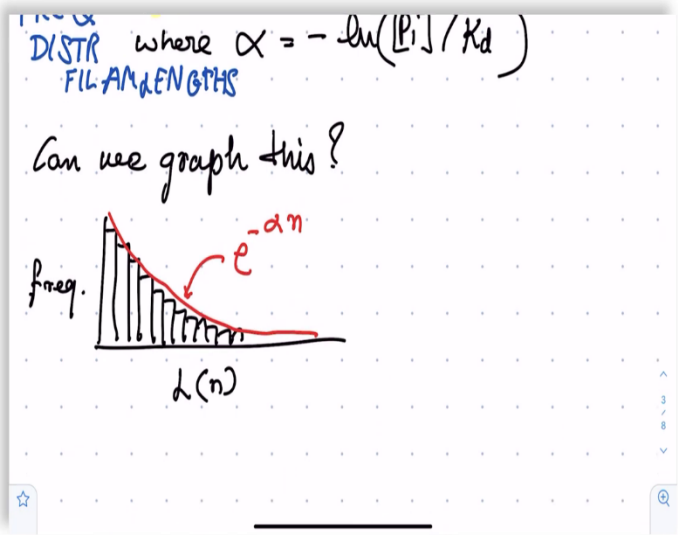

So, can we graph this? Sure. And here it is. So, our graph of the frequency distribution of lengths looks like this. It is, the red line e to the power minus alpha n and alpha is itself given as this expression here, minus natural log of P 1 by K d.

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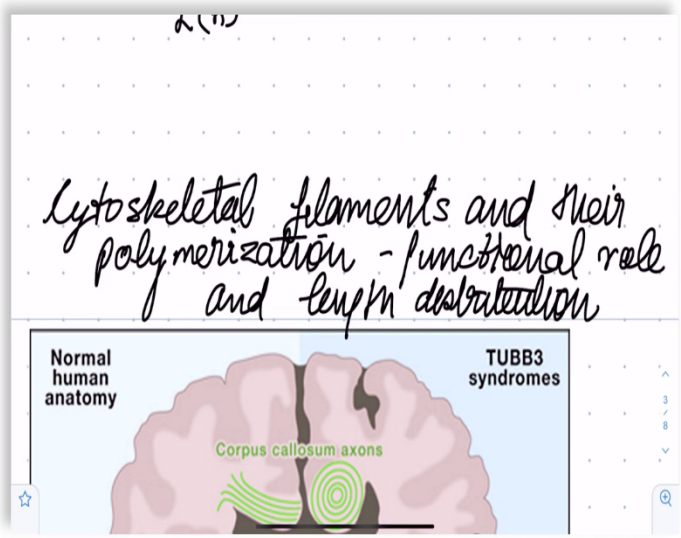

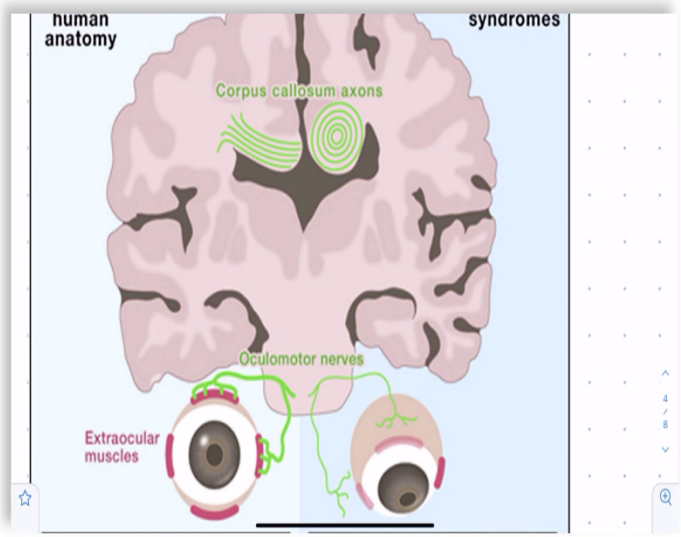

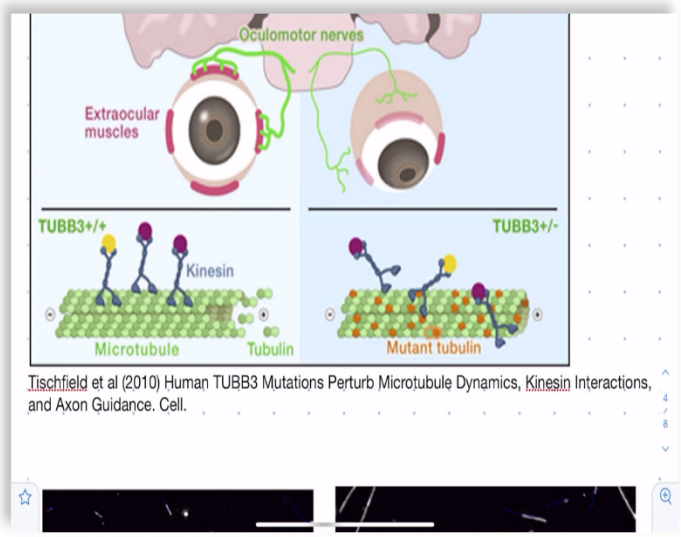

Tischfield et al (2010) Human TUBB3 Mutations Perturb Microtubule Dynamics, Kinesin Interactions, and Axon Guidance. Cell.

So, does this have anything to do with any experimental reality, can we go back and check and so on and so forth because we made a lot of gross simplifications. So, it turns out cytoskeleton filaments and their polymerization have a functional role and their length distribution has functional role. And the most prominent place where this happens is the brain.

And in, particularly in humans, it has been shown that tubulin B, one of the monomers that makes up the tubulin dimer, TUB B3 which is an isomer of tubulin d, when it is missing, results in malformation of corpus callosum axons, and the oculomotor nerves leading to mental defects and vision defects.

And ironically this comes down to the level of the cytoskeletal filaments where the dynamics of the mutant tubulin are altered as compared to the wild type. And the consequences of this are the ones that then seem to appear, to show up in brain defects. What is interesting to note is that not all tubulin Bs were mutated, just TUB B3. And this is also not homozygous but heterozygous. Already, this causes problems.

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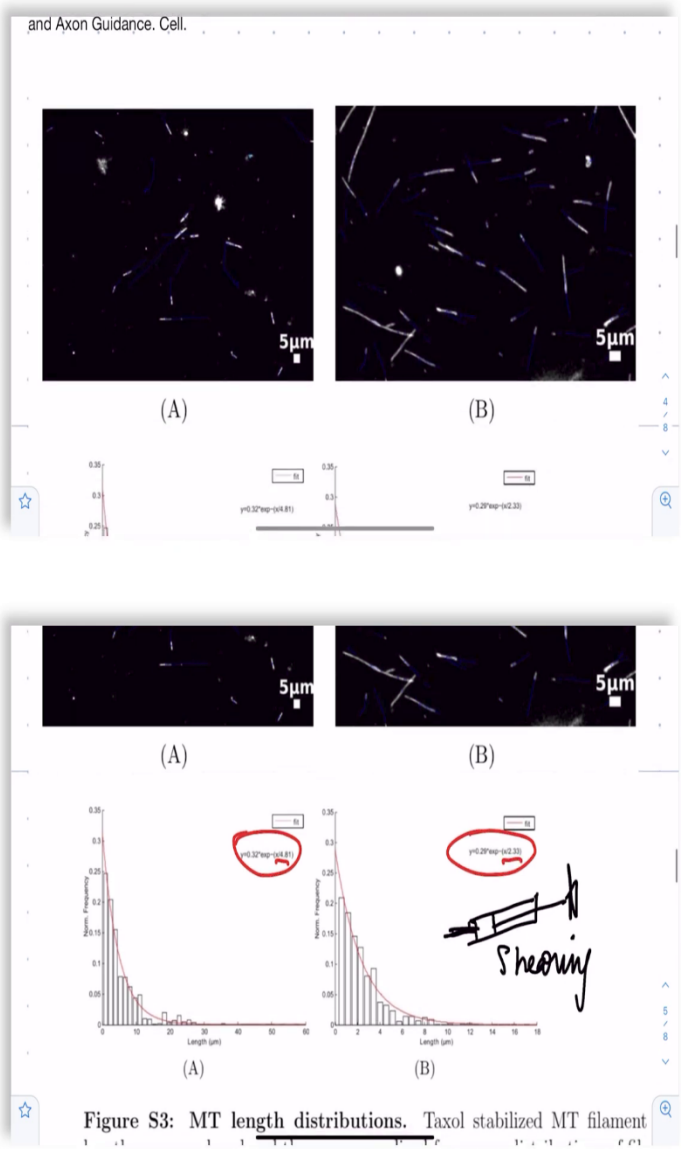

But what about the lengths? So, when in our lab we did some experiments where we assembled tubulin from brains, not human, goats because that is the easiest large mammal that we can get hold of, and analysed the filament lengths made by stabilization, lo and behold, we actually get very nice distributions.

What is the difference between left and right, you may ask? Well, on the left are the filaments that were assembled. And on the right, are the same filaments after passing through a syringe and pumping it up and down by a process called shearing. In other words, we basically broke the filaments.

So, when we do that, we obviously expect a reduction in length. And you see that in the exponential fit with the 1 by lambda in the exponent, indicative of the approximate mean length, that you can get from it. It is 4.81, this is 2.33 after shearing. So, indeed length distributions from experiments seem to fit our theory. What about means?

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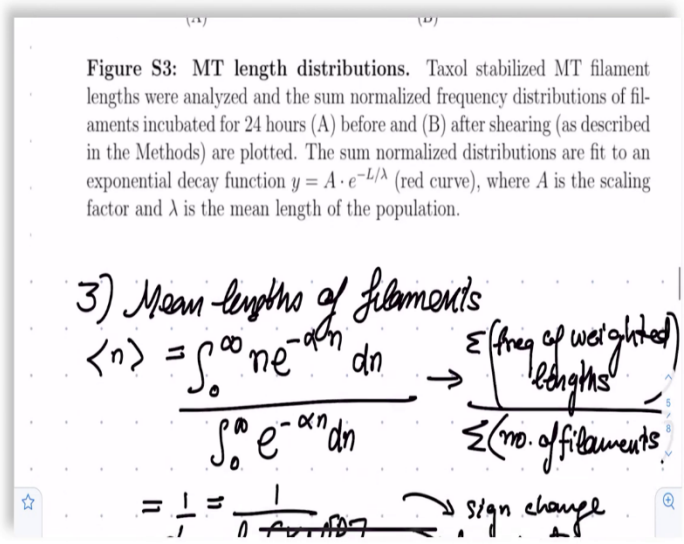

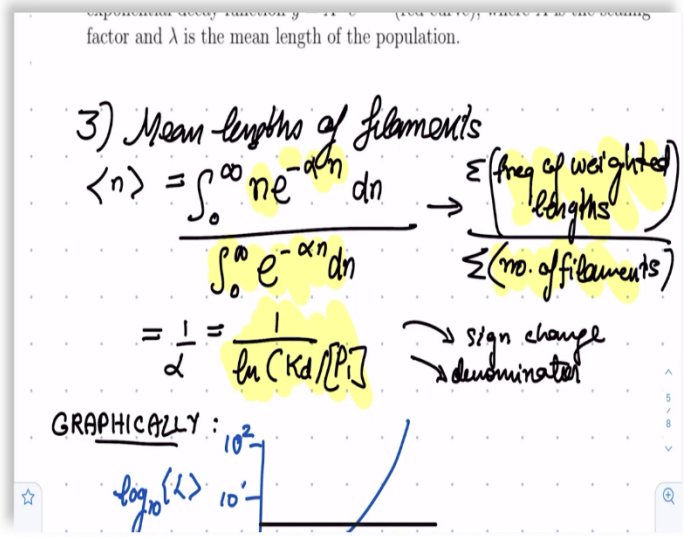

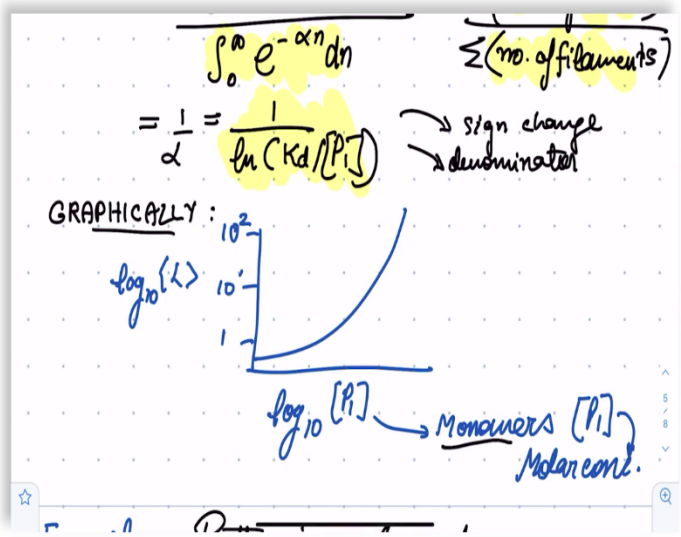

Well, it is fairly straightforward. If you want the mean of something, then you need to take the frequency of the weighted lengths and divide it by the number of filaments. That is what the average means. So, to do that we need to integrate from overall lengths by n, which is the length of that, the weight of that filaments, the weight of that distribution times e to the power minus alpha n, and divide it just simply by the frequency, summed over all lengths.

And this leaves us with this expression here which is 1 upon a natural log of K_d by P_1. Please note that the numerator and denominator in the brackets have shifted a little bit. And graphically, this looks like this. That is to say, as the monomer concentration increases, the mean length increases. This is not very surprising, but how exactly it increases is given by our nice little expression here, which is 1 by alpha. So, can we put in some real numbers and try to make sense of this?

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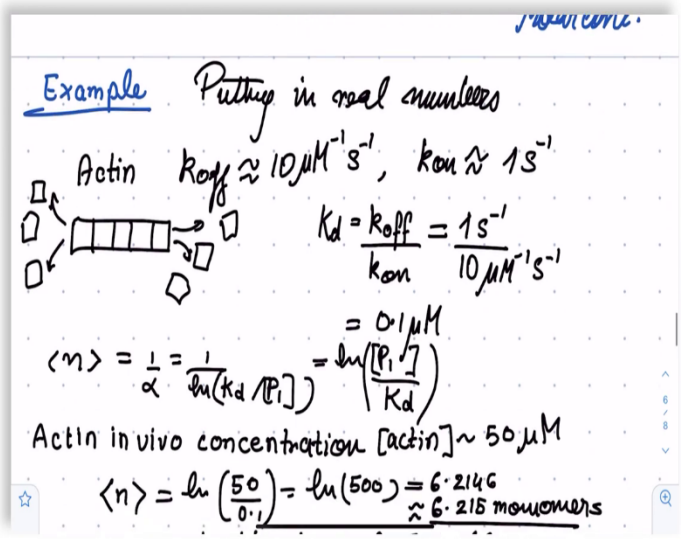

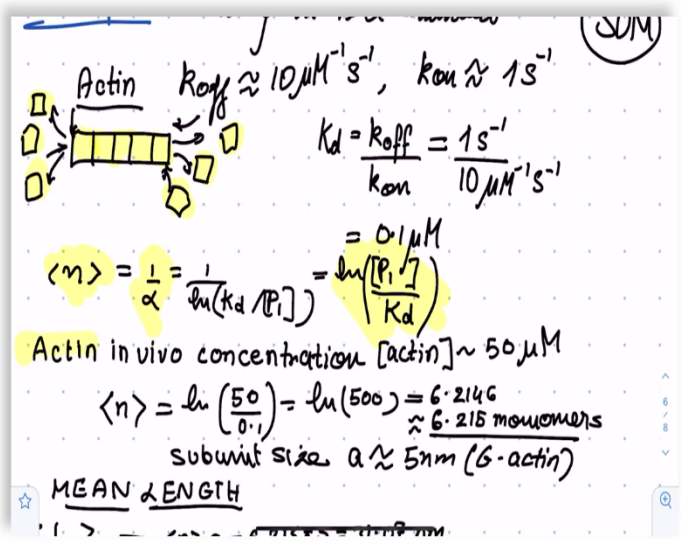

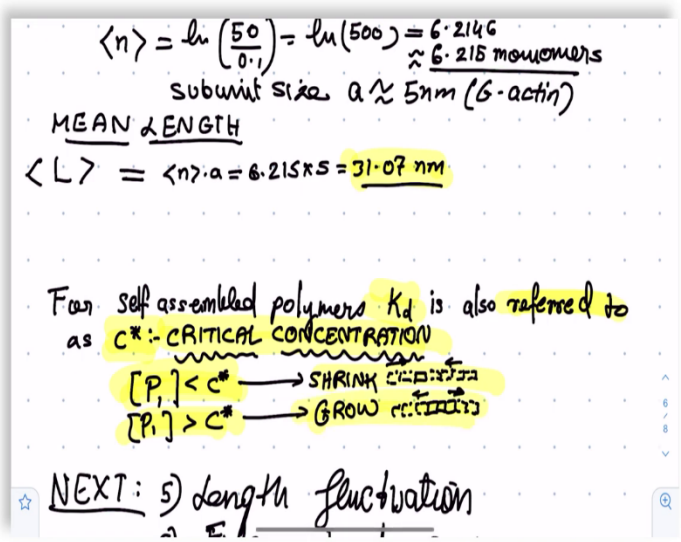

Well, sure. So let us put in some real numbers. And this is the last part of this module which is our sum. So, we are going to take actin, which again we say can behave as a symmetric polymer for this assumption where growth and shrinkage happens at both ends by the addition of, by the addition of monomers into the lattice, and the k off is measured to be 10 per micromolar per second and k on is one per second. This of course means K d is nothing but k off by k on which is 0.1 micromolar in units. Just, please note how we got to that point in terms of the units.

And then substituting into our expression, 1 average of n, that is to say the mean length in terms of monomers is 1 by alpha, we write down K d by P 1 natural log in the denominator, which if we take in the numerator we get natural log of P 1 by K d. So, what is the actin in vivo concentration? Well, reports tell us that in cells like yeast and mammalian cells, it is around 50 micromolar. So when we substitute all the numbers now, we get 50 by 0.1 500 natural log, that is 6.2146 in terms of monomer units.

So, but can we get absolute lengths? Well, subunit size is, of actin is 5 nanometres for G-actin. So, when we now multiply this, we get 31 nanometres as the average length. It is important to note that for so called self-assembled polymers like these, the value of the K_d is also referred to as the critical concentration. This critical concentration we will see, has an interesting role to play. Suffice to say for the moment, we will just simply state

that the monomer concentration, if it is less than the critical concentration, leads to net shrinkage, while if it is greater than the critical concentration leads to net growth.

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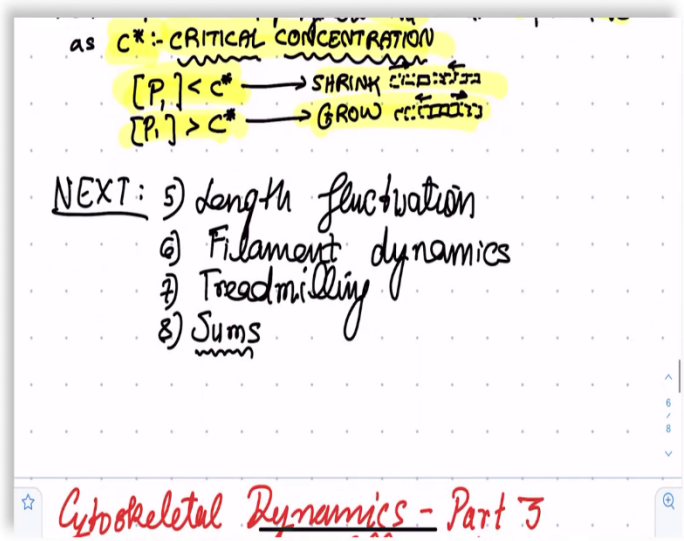

So, for the next part of this module, I am going to talk about length fluctuations, filament dynamics, treadmilling and sums.