Cellular Biophysics
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Polymerization Dynamics - Part 1

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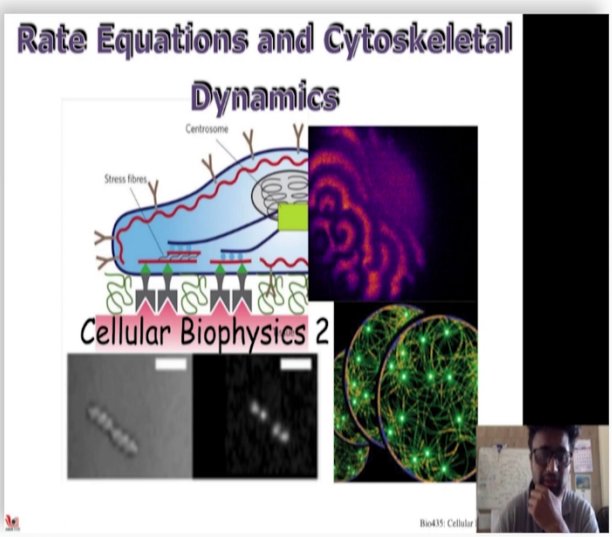

Hi, so as we were talking Rate Equations and Cytoskeletal Dynamics are the theme for this coming section. We will talk about rate equations beyond equilibrium systems of enzyme substrate interactions, Cytoskeletal Dynamics and we will then go on to simple model of polymerization like distribution and how to infer mean filament lengths out of these kind of equations.

Which ironically, we will then finally use to explain what dynamics, how we can encapsulate the dynamics, and using that, we will try and arrive at average expectation times or excursion times, and with such a dynamic model, also try to explain something about a phenomenon that is described initially for microtubules and now even for actin called thread mill.

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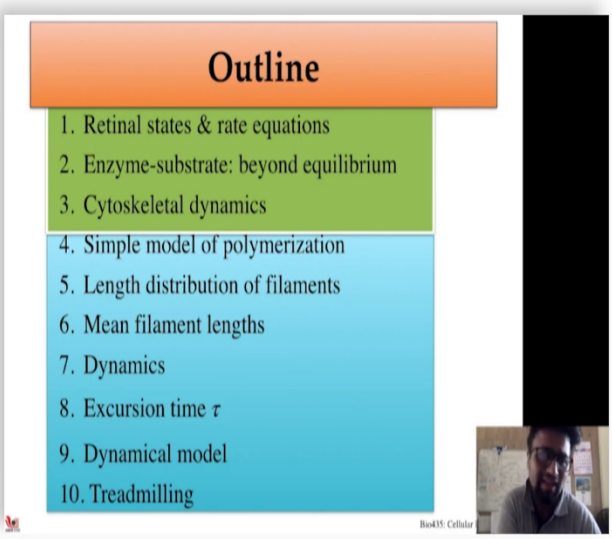

This will sort of lay the foundation for us to talk about molecular motors afterwards. So, let us get to it. What do we mean by rate equations? It is simply this idea that you can discuss in terms of how many, where, when chemical concentrations are speed variables, given high types of molecules and R positions and T times. We assume that the spatial variation of concentration is at a scale much larger than the mean spacing the molecules which are, in other words, this is also sometimes called compartmental, compartment approximation or well mixed approximation.

You will find some of this in your applied bio mathematics in terms of compartment models. And I think those of you who have been following the pandemic from a scientific perspective, as all of you, I hope, should at least try to, as scientists in training, might notice that the susceptible infected recovered or SIR or SIER models that are used to predict what might happen in the coming days to our pandemic are based essentially on the same ideas, mathematically speaking, coupled ordinary differential equations that pool not chemical concentrations but populations of people into categories.

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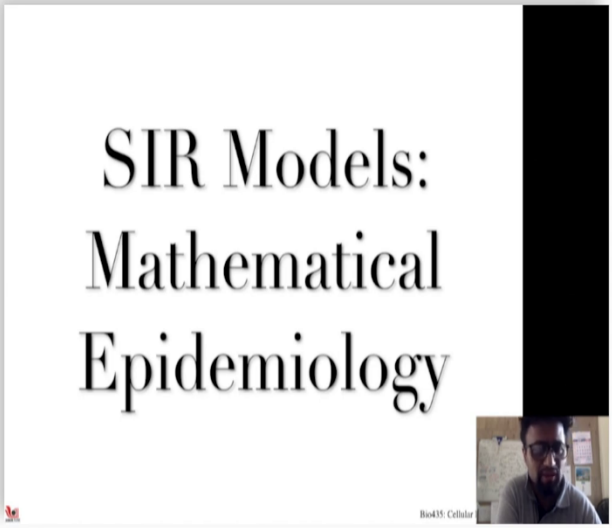

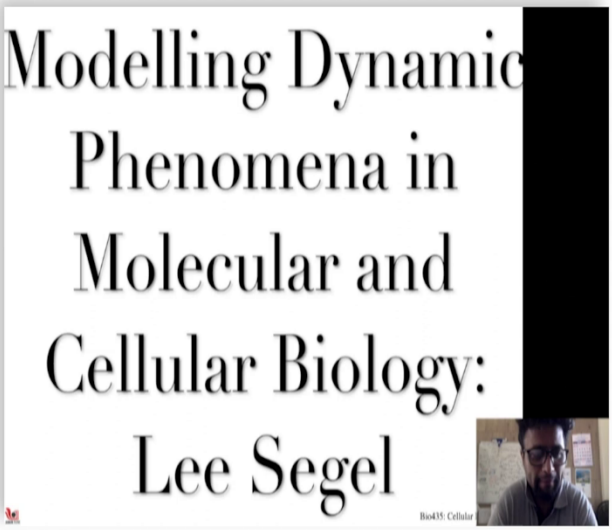

We will call them population I at position R and time T, which then characterizes the susceptible population, infected population and recovered population, for example. But I am digressing. These topics are very exciting, close to my heart, but they are not the topic for our conversation today. Feel free to read. There is a beautiful textbook on the introduction to such modelling by Siegel. It is called mathematical modelling, and computational modelling, I am sorry, in molecular cellular biology.

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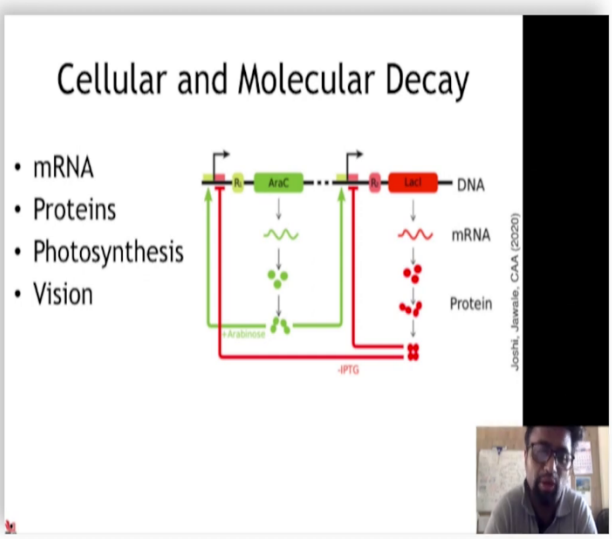

Coming to the most simple process possible, everything decays. mRNA decays, proteins decay, photosynthesis involves decay, vision involves decay, and what you are looking at here is an image from paper that some of your, two of your super and super, super seniors, I do not know how many supers that are are there, power off, wrote with me, to model the gene expression dynamics of an arabinose lactose-inducible promoter system.

This particular gene expression system is a bit unusual. For those of you who want to know more about it, feel free to look at our paper. In essence, we modelled the central dogma. The production of an RNA which is what you see in the squiggly line after the blocks. Those are the DNA, DNA produces RNA, RNA produces protein, protein can dimerize or tetramerize.

And in every state, which is not listed here in this diagram, but is definitely there in our equations, there is a degradation process. So, in a sense you could argue that turnover which is a very popular term used by biochemists, is nothing but an expression of the degree of production and degradation, or in mathematical biology terms, birth and death process, true of every one of us. We just do not want to go too early. So, stay safe, mask up. Sorry, just my plug for the pandemic.

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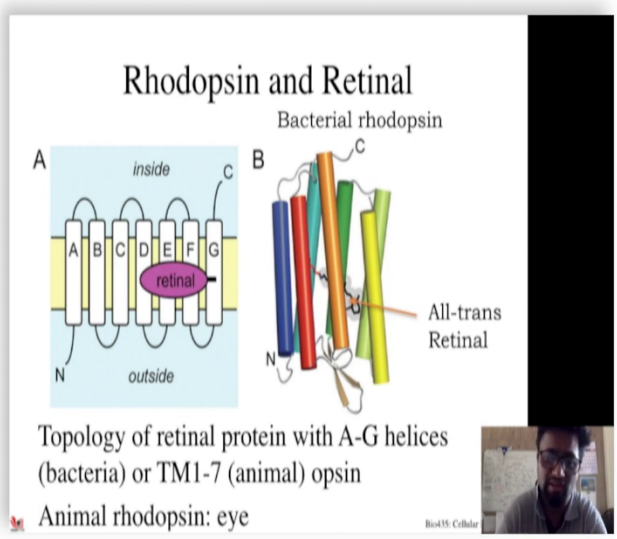

But coming to vision, at the core of it, lies a molecule called Rhodopsin. And at the core of Rhodopsin lies Retinal. So, what you are looking at here is the 7 transmembrane opsin, which in bacteria get referred to as A-G helices, which is how it is labelled here from the paper in ACS chem-bio. As for animals, it is, they call transmembrane domains, what they say.

This, I think should be hardcore evidence for you to remember again of the evolutionary conservation of protein structures and protein sequences, but nevertheless, you should really ask the question, why do bacteria have a protein that is in our eyes or what is the connection. And turns out, for bacteria, this is an energy production mechanism. These are specific purple bacteria, and we will come to that in a minute.

But interestingly enough, at that core, so the protein scaffold is necessary because it translates optical energy, light and photons into nerve conduction signal, because inside this cage that you see on the right hand side of the Rhodopsin molecule with these beautiful rods in the cartoon representation with a little alpha propeller down below, lies the Rhodopsin. And what it is depicted, I am sorry, the retinal, it should be retinal.

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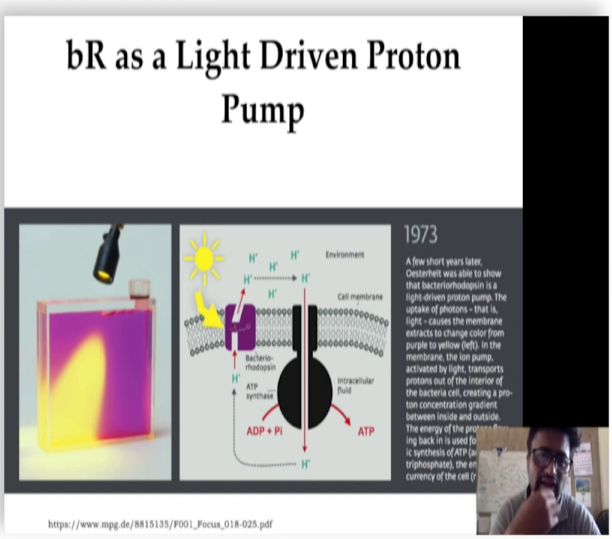

What is depicted is the All-trans state of it. And these are nothing but isomers. Some of the earliest experiments that demonstrated that there is something interesting going on here used a liposome based immobilization of these receptors that is depicted in the right hand side diagram as the purple block, H block, where if you mix these receptors along with ins, that were embedded in membrane vesicles and suspended them in a solution which you see on the left hand side, they formed a nice purple, beautiful purple suspension of colloids.

When you shone light on them with the presence of a pH sensitive dye, which was capable of detecting the presence of H ions, and turning yellow, then that light triggered the efflux of protons from the interior of the vesicle outwards, leading to a beautiful yellow region suddenly lighting up in the bulk. This reaction, representation is taken from the Max Planck website. And this was a key experiment done by Halston Heritant Company.

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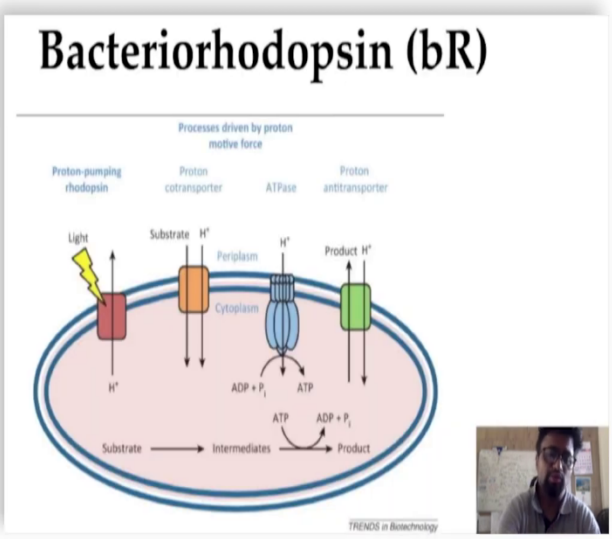

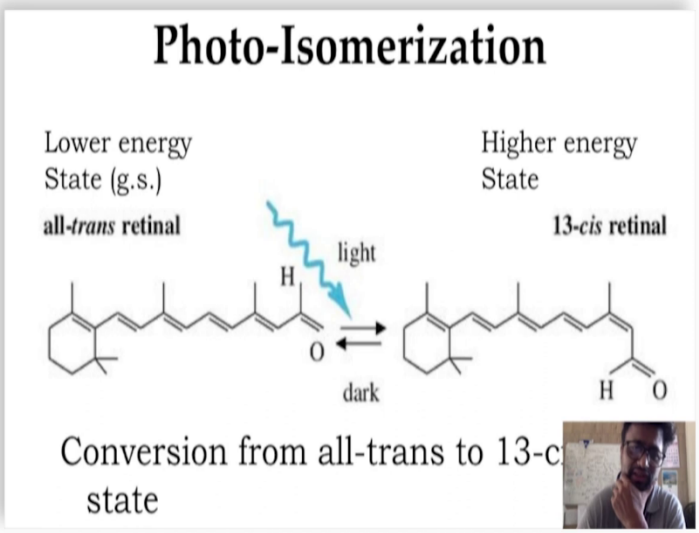

So, indeed, this blue light induced activation of bacterial adoption is essential for converting light energy into ATP, what you would conventionally call photosynthesis, but a much much more trivial more simple version of it compared to the sophisticated and almost mind-boggling machinery that you see in plants.

As I said, at its core is this reaction, this is retinal. So, you are looking at All-trans retinal which is the ground state, lowest energy state, which on the absorbance of photon of light

can be converted to a higher energy state, the 13-cis retinal. And spontaneously, the dark reaction so to speak, it will decay back into All-trans.

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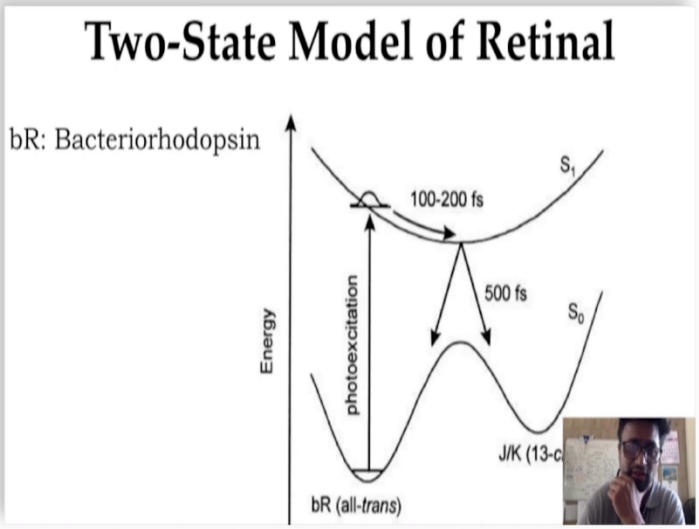

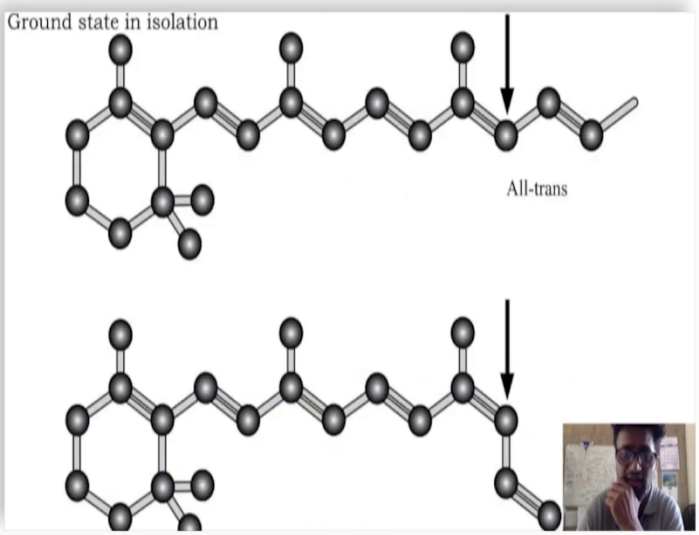

So, this is your Jablonski diagram, depicting just that. All-trans is in the lower energy state, as I just said, and 13-cis is in the higher energy state. And photo excitation triggers this high energy state which is capable of crossing the hump, the energy barrier, and decaying into this, moving into this 13-cis. And spontaneously, 13-cis can go back to All-trans. So, in some senses, now we have two states, and we have a spontaneous rate,

13-cis to All-trans. 13, because of the 13th carbon atom, that is the centre of this, so difference in symmetry.

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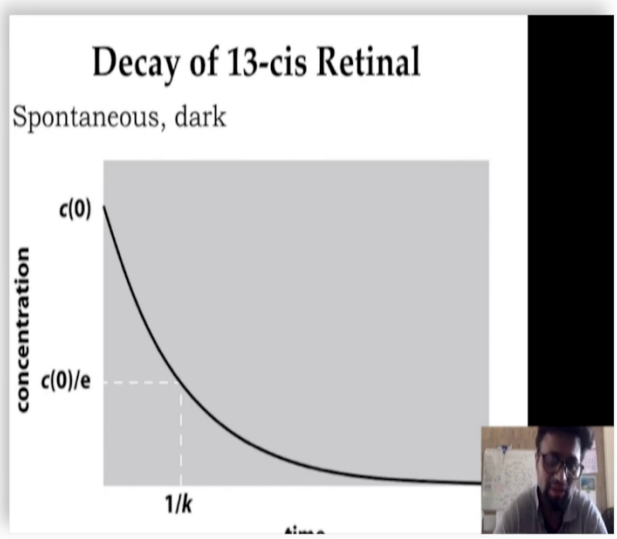

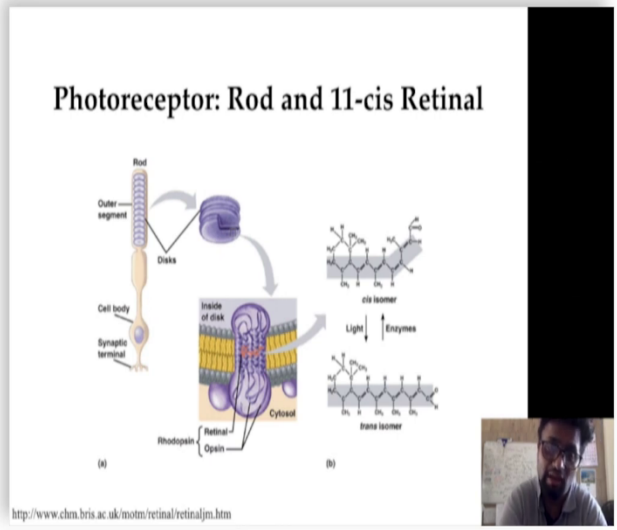

Now, this also means that if you leave 13-cis retinal in the dark, over time its concentration decays. And the decay constant is expressed in terms of this x-axis value in terms of time, as 1 by k. The role of these retinals is in vision in our eyes is to convert that energy that comes from light to transition, absorb and translate it into electrical signal, which is effectively the topic of optical to neuronal transduction in vision, and a very nice topic which I suspect you, on neurobiology course, is covering. The variations of opsins are diverse, in the rods and cones of our eyes, but retinal is common to all of them.

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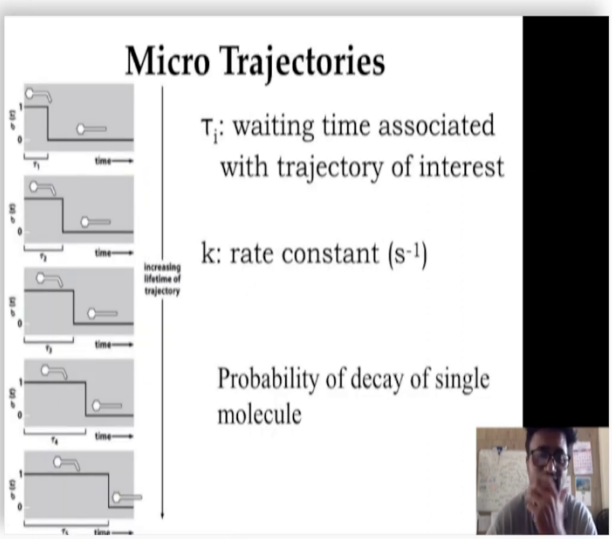

So, in a way one can go back to our statistical idea of many trajectories. So one of the 13-cis goes to All-trans at a certain time tau 1, the next one goes at tau 2, the next one goes at tau 3, tau 4 and tau 5 and so on and so forth. And this is, we can define for each individual, individual micro trajectory as the waiting time associated with the trajectory of interest, with the i_{th} trajectory, where k then becomes the rate constant.

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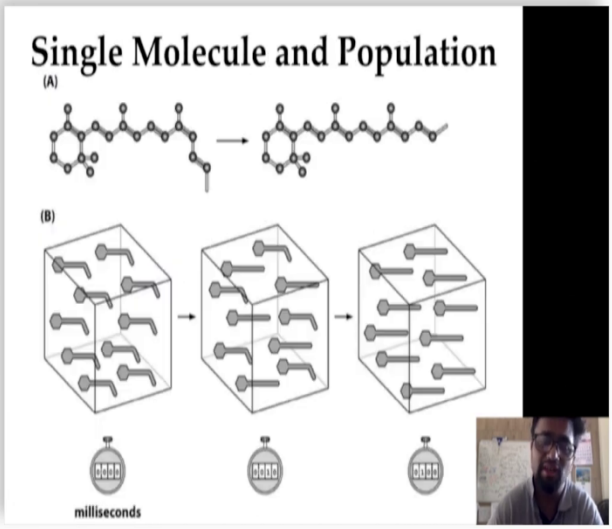
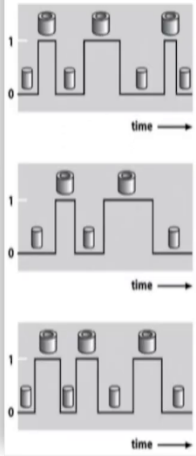


Ion Channel



Trajectories of a two-state model of ion-channels

Switching between states assumed to be instantaneous

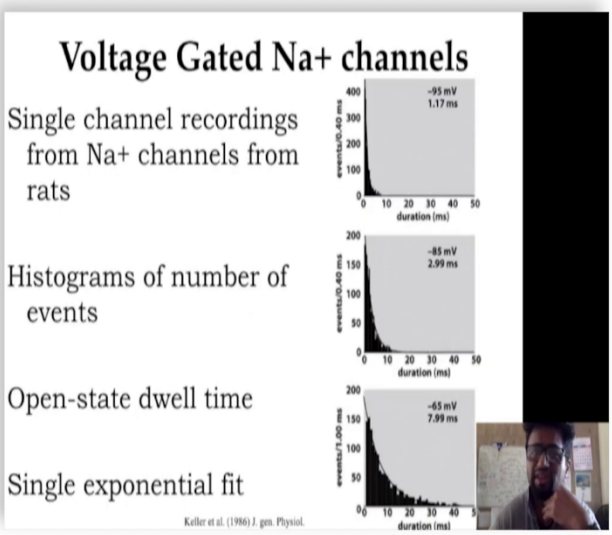
Ion Channel States

O: Open
C: Closed
k_-, k_+: rate constants

$$O \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C$$

N: total no. of channels
N_O: no. of open channels

Calculate $N_O(t)/N$



So, the probability of decay of a single molecule is what we are interested in, and we are really interested in knowing, knowing what happens at a single molecule level, can we get some kind of ensemble idea. And this happens in hundreds of milliseconds. And this is not very different from ion channels which you can also distinguish as being in two states, open and close, and fluctuating between the two, spontaneously switching from the high energy to low energy state, and the dynamic changes in the population will determine whether current will flow out.

So, this open and closed state and k plus k. So, the point I am trying to make is that a, an approach that considers these kind of transition rates is very useful because it is general. So, what you are looking at here are voltage created sodium channels of an events per 40, 0.4 milliseconds. That duration in milliseconds are on the X axis, the frequency distribution effectively of events, in a 40 micro, 400 microsecond window. And under different conditions of potential difference, you see a change in the frequency of the events and their duration.

So, the decay constant derived characteristic time increases from 1.7 milliseconds to 7.9 milliseconds as the potential difference is decreased. Then, the interesting thing to note here is that this is a single exponential fit, and these are so called open state 12 times, that we are looking at in terms of histograms, frequency histograms.

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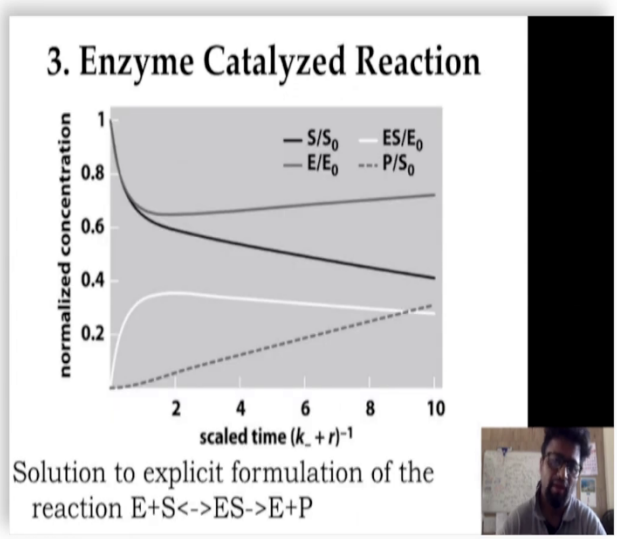

Another place where reaction kinetics and red cones play critical role is in this Enzyme Catalyzed Reactions. We, of course, work for the longest time with the Michaelis menten steady state assumptions, which allow us to write an analytically closed form solution. But the fact of the matter is that there are many situations, especially in Vivo where this may not necessarily be true. The classical idea of enzyme substrate interactions is E plus S gives, it is a reversible interaction producing an ES enzyme substrate complex, which can irreversibly go in one direction to product or go back to E plus S separation.

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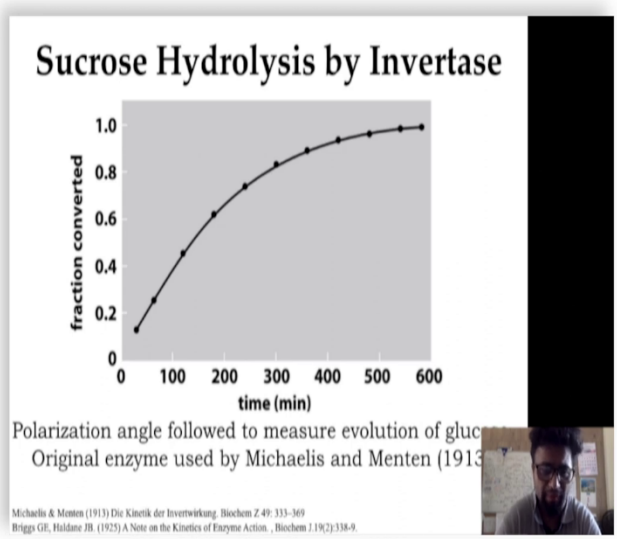

And the original work that was done by Michaelis and Menten, one of the first PhD's in biochemistry, who was a woman, the Kinetik der Invertwirkung. This was work done in Berlin, published in German. I do not think exists in its current, in that form anymore, and led to some very fundamental insights into theory, which in fact, by the way, Michaelis and Menten worked out in terms of the maths in their paper.

Feel free to go back and look at it, there are translations online into English. Though, if you're adept with German, you can try your skills on it. But I do not think you will find the German edition. Anyway, Briggs and Haldane, JB Halden and Briggs, then built on that to highlight the utility of this kind of enzyme kinetic assumptions, and wrote this very landmark paper on enzyme kinetics, the kinetics of enzyme action in a biochemical journal.

So, what we are looking at here is fraction of the substrate converted and it is a function of time which gives you the idea of how much glucose is formed as a function of time, product formation. That product formation itself gives you an initial velocity and this is so called initial velocity approach, where you take the Vi, the Vi gives you for a given concentration of substrate, a value for velocity.

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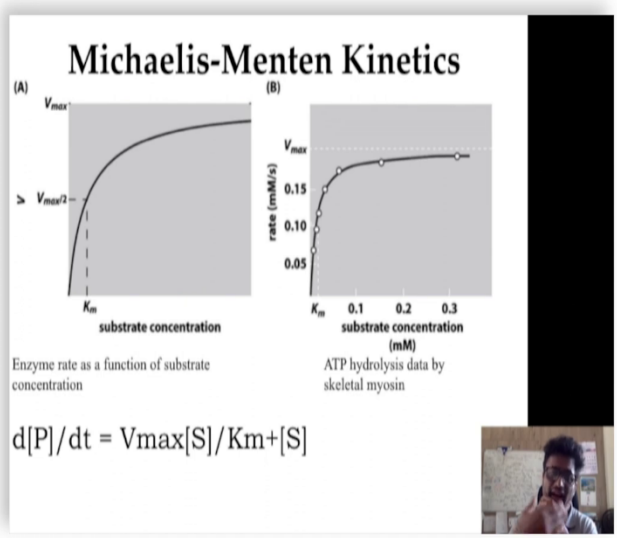

So, you do this reaction for multiple substrate concentrations and you get a V versus S graph which then is fit to the classic Michaelis and Menten equation, which is a saturation function which gives you V max and Km. Those of you do not know what I am talking about, please, please, please, open a biochemistry textbook, the famous one by Lehninger or Stryer or any one of these, and you will find a lot more details.

And this is really what I was talking about, the overall graph that you get from many such individual V versus time. I am sorry, product versus time graphs, where you get a velocity which gives you then the ability to fit this equation. That is to say, V is equal to Vmax times S upon Km plus S, and that gives you the estimate of the maximum velocity, which is the asymptotic value on the Y-axis, and Km which is the half maximum rise constant of the system.

$$\frac{d[P]}{dt} = \frac{v_{max}[S]}{K_m + [S]}$$

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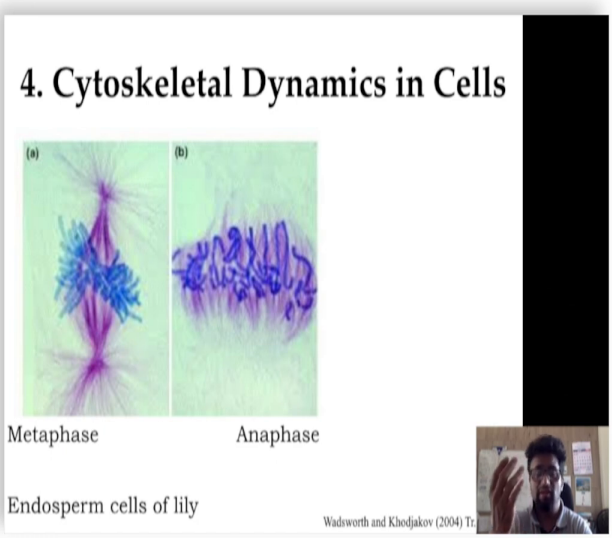

So, suffice to say we now have some kind of an idea of the need for understanding dynamics and using these kind of compartment approaches to make sense of them, but for cytoskeletal dynamics in cells, we may need to add a little bit more theory because unlike just a simple transition from say 13-cis to All-trans, which is an exponential decay, things may be a bit more complex in cytoskeletal, and this is an example of showing you, this is by the way the classic Wadsworth and Khodjakov of image of endosperm cells of lilly.

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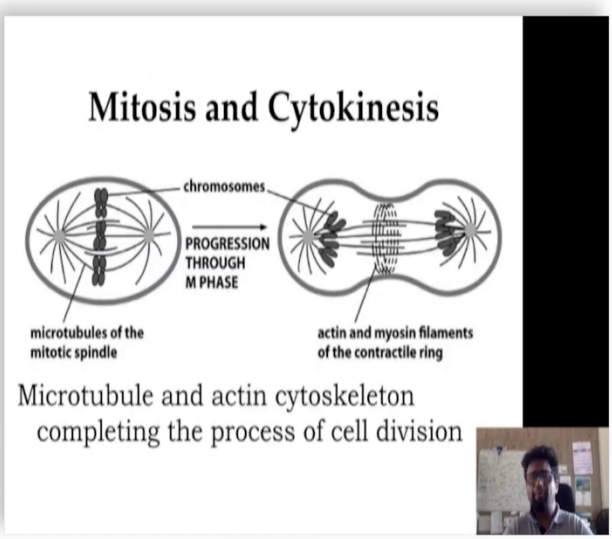

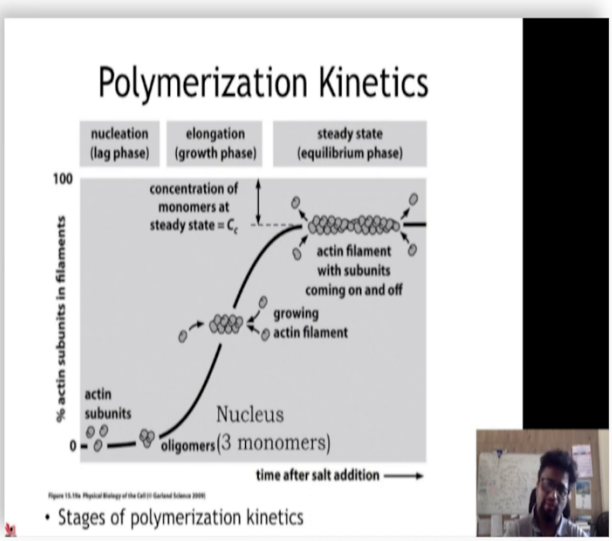

So, mitosis and cytokinesis involves, in fact, the progression of replication of chromosomes through the formation of magnetic spindle, this sort of bipolar structure and this cartoon represents this idea that also contractile ring that forms together with myosin contracts and divides this into two, which is cytokinesis, and this is fundamental to any growth process that we keep discussing every time. If you consider life to be that of replicational division, then this is life.

So, the components that make up this fundamental process of division and growth are effectively satisfied. And what is represented here is in a cartoonish fashion, yet the production, the formation of cytoskeletal filaments of actin So, you will recognize this, hopefully, by the fact that they are just simply two strands, you could say proto filaments one done, bound to each other, staggered by a certain amount.

So they are not just, they are not adding, they are not like two lines of brick, but they are offset. And they are adding in a spiral and they are going that way and what you see is that when actin subunits come together, the initial form of oligomers or higher scale aggregates, sometimes 3, sometimes 8 depending on the system. That in fact, these nuclei are a kinetic bottleneck.

In the sense that the formation of nucleus is essential for the formation of the growing filament. If you do not form those oligomers initially, the later growth does not happen. And this is something that will return to, because this is related to some fundamental kinetic theory of polymerization, which is not of my argument for today.

So, the nucleation lag phase, nucleation is associated with the lag phase something like a bacterial growth because elongation is the growth phase of your bacterial case, and steady state is the analog of the equilibrium case. Having said that, the analogy to the bacterial growth breaks down, because it is not the same thing.

Concentration of monomers left at steady state, when the filaments have reached some kind of dynamic equilibrium with their monomer pool, that concentration of monomers is also called the critical concentration. It is also the concentration which is minimally required for subunits to assemble into oligomers.

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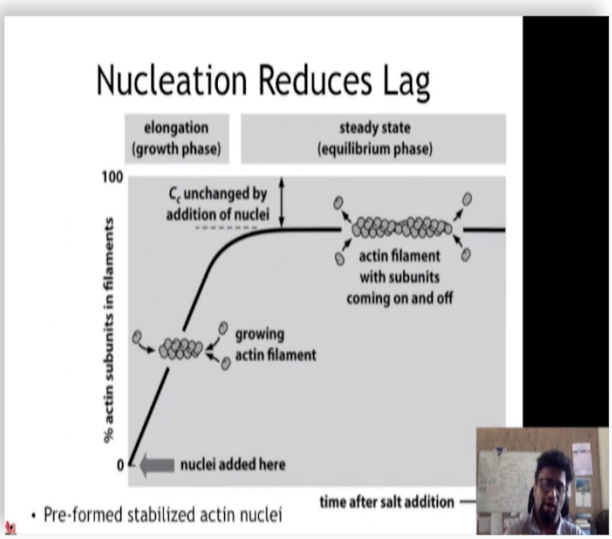

If nucleation is somehow accelerated by adding pre-formed nuclei, then you get rid of the lag phase which is what this graph is showing you and you only get elongation in steady state. Of course, this is all theory, this is the so-called nucleation dependent polymerization theory.

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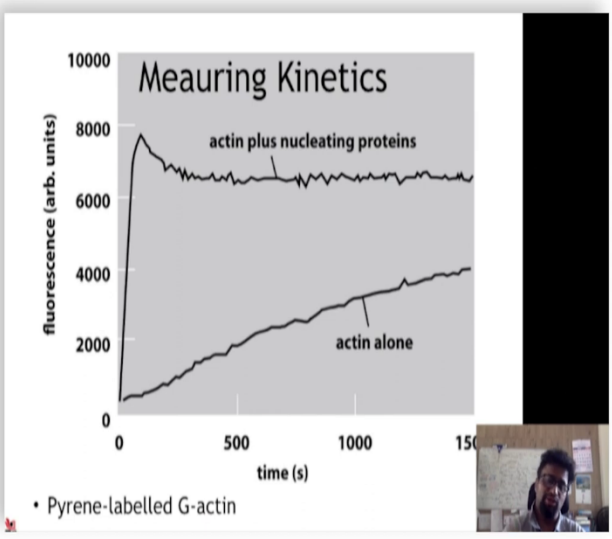

When you actually measure kinetics with, including proteins, you kind of see this theory is validated. These are measurements made with Pyrene labelled G-actin. Actin is, in

conventional lab, biochemistry labs it is purified from muscles. Rabbits are a very popular organism. I do not know why chicken legs are not that often used, but rabbit legs are popular. And the Pyrene labelled G-actin on Y-axis is actually telling you, as far as the polymer is concerned.

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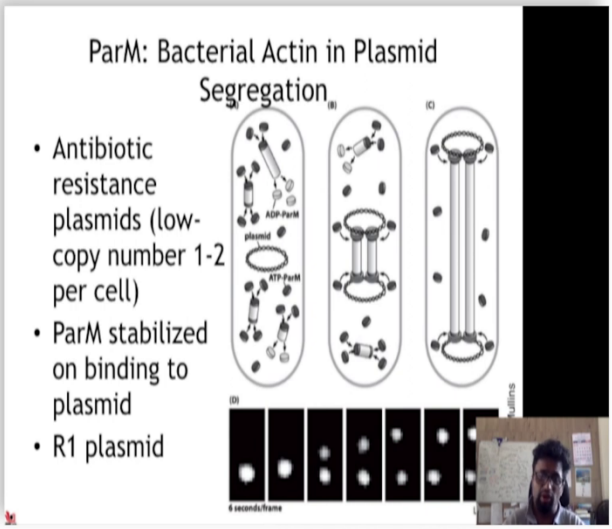

But I remind you about what we spoke about last semester that cytoskeleton is not just restricted to eukaryotes. There are bacterial subsequential elements. And one of the most recently highlighted ones is that which is encoded by the Plasmids Machinery, Plasmid segregation machinery.

What you are looking at here is the low copy number of plasmid segregation machinery that on its own tiny, tiny plasmids genome, carries the genes for cytoskeletal protein called ParM and it is an operon, ParM RC. The ParM forms a, is capable of forming actin-like filaments by the addition of ATP bound ParM molecules.

This is the dark, sort of carrom stripes, carrom coins that you can see. And these carrom coins keep adding and growing, adding and removing. This is a cartoon representation of course, such that the two plasmids are caught, captured by a pair of ParM filaments. They then elongate and they grow and they separate.

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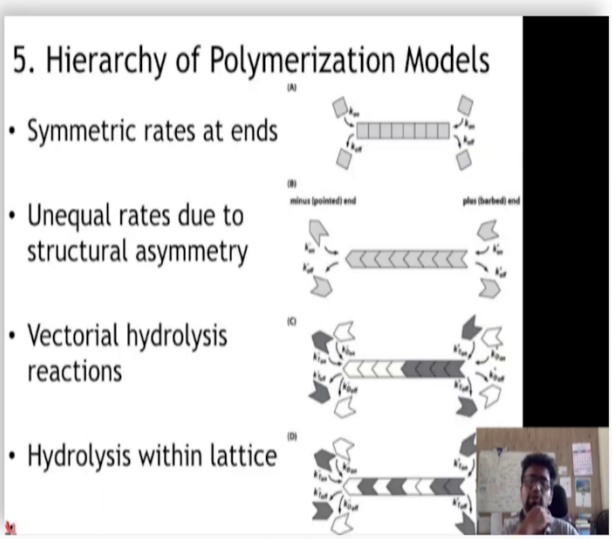

If we want to develop a theoretical model, we need to consider that there are many, many details that we are probably missing. And so we kind of think of the models of polymerization in terms of a hierarchy of complexity. Meaning to say, many levels by which we can divide them into, one is sort of where you have a symmetric rate of growth, there is one of these square bricks that are getting added and moved.

They are both added at both ends. Ends, remember, are important. They do not grow unlike oligomorphic growth where you grow anywhere by aggregation. This is a actin because there have been a high energy triphosphate found, either GTP or GDP, and they end, add on both the ends.

In the simplest case, of course we assume no branching but as you are well aware, actin in the presence of molecules like R23 can form structures, and more recently, kind of analogues to that have been found for tubulin like (()) , but when that K on and K off rate is defined, then we have actually quite neat equations that come out of it, and we will see in the later section, that this makes a lot of sense.

But on the other hand, we do know that filaments have minus ends and plus ends. Minus ends being those, they have nothing to do with charge, they are just simply relating to the

fact that they grow more slowly or they are more likely to decay, the minus ends and plus ends are more likely to grow.

In actin's case, this term is called a pointed end, minus end is called a pointed end, slow growing end. And the barbed end is called, I am sorry, the plus end or the positive end is called as barbed end. This has to do with how the actin filament looks under electron microscopy at its plus and minus ends.

Now given this, you can also understand that we know that there are triphosphate and diphosphate ATP and ADP or GTP and GDP bound filament, monomers, which means that you have potentially, for every on rate, at both ends, yet 2 additional rates of the GDP or ADP bound monomer adding or the triphosphate bond monomer adding, as well as its corresponding removal.

So, 4 rates on the left and 4 rates on the right. We went from 2 rates on the left and 2 rates, 2 rates at the 2 ends, and that came, that was an expansion of the further simplified idea of symmetric rates. Indeed, the dark and light segments of the lattice that you see here are indicator of the fact that there is a difference in the monomers in terms of GTP and GDP bound states, and there is also hypothesis within the lattice which could be directional or it could be random.

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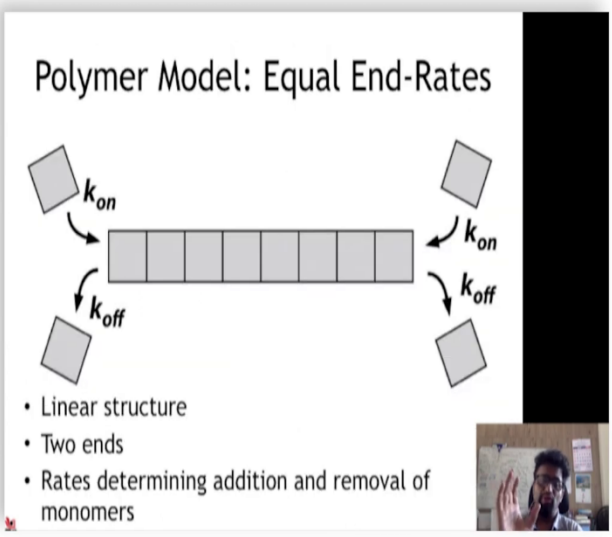

So, in the next section, where you must have derived this polymer model of equal end right, and see how useful it is. Thank you.