Computational Systems Biology
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Lecture 36
Introduction to Dynamic Modelling

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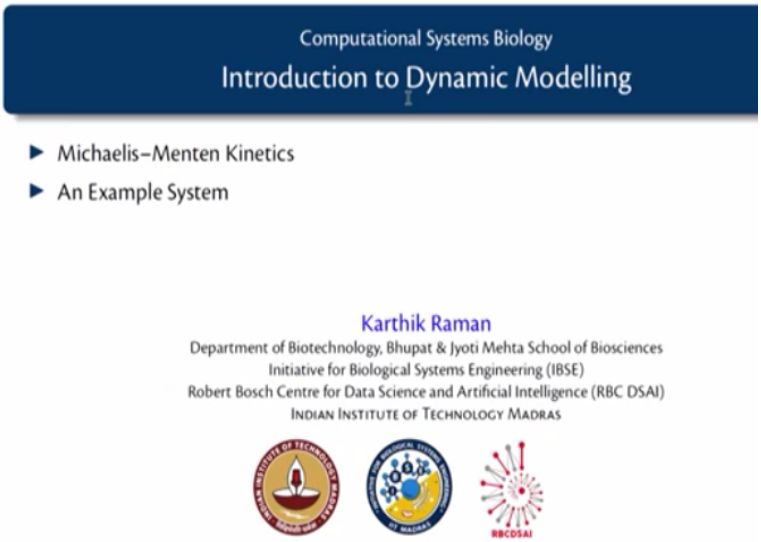

In the previous video, we saw an introduction to dynamic modeling. We will now move on to the most classic example of a biochemical network model, which is Michaelis-Menten kinetics and we will also look at another example system in this video. So let us look at the classic Michaelis-Menten formulation. So Michaelis-Menten formulation is to study the initial rate of an enzyme catalyst reaction. So what is the wiring diagram.

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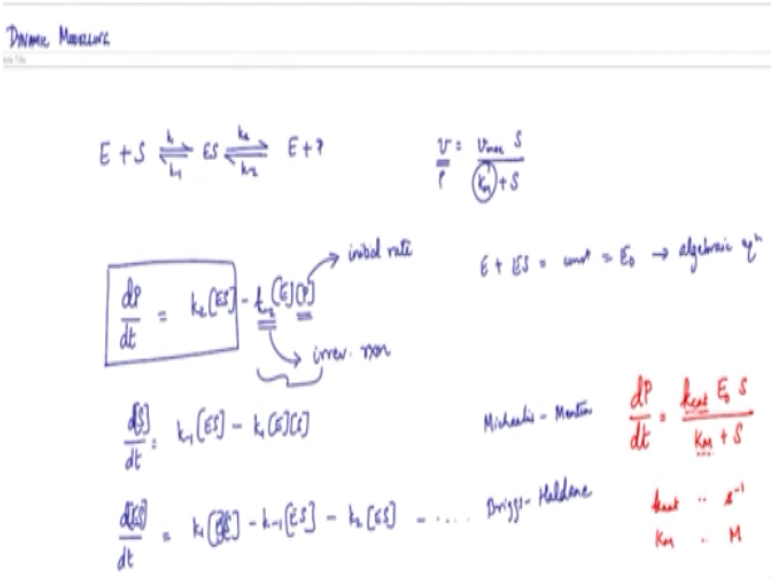

So this will be the wiring diagram. E+S, right from here the assumptions will start. You may say in most books that this may be written as a forward reaction, but that is not at all necessary. What do you mean by the rate of the enzyme catalyst reaction? You may all be familiar with this rate. V=v max *S/Km+S. What is this V, rate of what, dp/dt. So what is dp/dt? K2*ES-K-2*A*P. This is where the first important assumption for Michaelis-Menten comes.

People normally eliminate this term, why? So can be 0 in 3 scenarios. E=0 is a good point. We will not even have to worry about it. Either K-2 is 0 or P is 0. So P=0 means initial rate, or if you can have the product removed. K-2 would mean irreversible reaction, essentially, but that is a harsh assumption to stay with. So dp/dt is K2*ES. So we will just assume that it is K2*ES. So what ds/dt, K-1*ES-K1*ES, dES/dt will be a little more complex.

So it is going to be K-1*ES-K2*ES, actually minus this term as well, but we have already chosen to ignore that term and you have also another equation, E+ES=constant, in fact =E not, so this is 1 algebraic equation. You combine it with the rest, you can essentially compute that and you also have to usually apply 1 assumption. There are 2 popular assumptions, 1 is the classic Michaelis-Menten assumption, the other is the Briggs-Haldane assumption.

What is Briggs-Haldane assumption dES/dt is 0 quasi steady state. Michaelis-Menten assumption is basically ds/dt is 0. Based on this, you get different values of Km essentially, but the equation

remains as dp/dt is K cat*E not*S/Km+S. So Kcat is going to be involved in these terms. Kcat is a turn over number. So Vmax is actually Kcat*E not. So what are the parameters here. Kcat and Km. E not is like an initial condition more or less. It is the concentration of the enzyme.

But Kcat and Km are your parameters. Obviously units of Km concentration, substrate and Kcat will have its own units. Kcat will be units of inverse time and Km micromolar, nanomolar, some concentration units. This is classic Michaelis-Menten. You can obviously add layers to this, competitive inhibition, uncompetitive inhibition, irreversible inhibition, substrate inhibition, presence of other compounds and so on and so forth.

But the basic idea is there is a simple algorithm to derive the rate equations. This is not something that you will do on a daily basis, you will just look up a table. You do not want to work out the Michaelis-Menten carefully in every case, unless there is some special thing that is happening in your system. If you have multiple reactions or things like that, then you may have to worry about it. So what are the assumptions in Michaelis-Menten.

There are a bunch of fundamental assumptions and a bunch of assumptions that are needed for the Michaelis-Menten equation itself, like Briggs-Haldane and Michaelis-Menten assumptions. The fundamental assumptions are more important. It is an isothermal system, if neglected the effects of temperature. There is enough concentration of all these molecules, such that you will start worrying about concentrations rather than molecule numbers.

It is not in the stochastic domain. When you have very few numbers of molecules, you have to worry about the probability of collision between enzyme and substrate, whether that even happens. Now you basically start writing in terms of concentration and of course you assume free diffusion and you also assume that the cellular space where this happens is somewhat homogeneous. There are no spatial gradients of the components and so on.

There are so many implicit assumptions, when you just even wrote the laws of mass action. So remember, therefore it is not as I would have shown you here.

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Deriving Rate Equations Systematically

1. Draw a wiring diagram of all steps to consider. It contains all substrates and products (*S* and *P*), and *n* free or bound enzyme species (*E* and *ES*).
2. The RHS of ODEs for concentration changes sum up the rates of all steps leading to or away from a certain species. Rates follow mass action kinetics.
3. The sum of all enzyme-containing species is equal to the total enzyme concentration E_{total}. This constitutes **one equation**.
4. The assumption of quasi-steady state for $n - 1$ enzyme species together with (3) result in *n* **algebraic equations** for the concentrations of the *n* enzyme species.
5. The reaction rate is equal to the rate of product formation. Insert the respective concentrations of enzyme species resulting from (4).

It is not the law of mass action, but it is actually a model. So this is the systematic way to derive the equations. You first draw a wiring diagram and assume mass action kinetics and write out all the equations and some of all enzyme containing species are C total that is 1 algebraic equation and then assume quasi steady state of n-1 enzyme species. So this would mean E, ES, ESI, EI, if I is the inhibitor, whatever are these other species and the dp/dt is your final reaction rate, which you have to do some basic algebra to arrive at.

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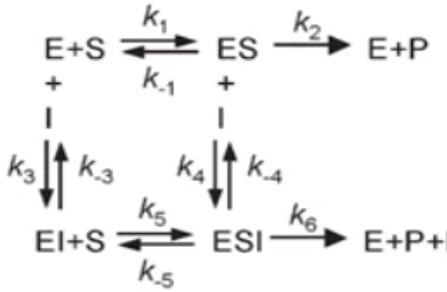
Inhibition in Michaelis–Menten Kinetics



standard Michaelis–Menten	reactions 1 and 2
competitive inhibition	reactions 1, 2, 3 *only*
uncompetitive inhibition	reactions 1, 2, 4 *only*
non-competitive inhibition	reactions 1, 2, 3, 4 and 5
partial inhibition	occurrence of reaction 6

This is the larger Michaelis-Menten system where you have all kinds of possible combinations. Standard Michaelis-Menten is just this and this, but if you have competitive inhibition, it is this, this, and EI+S with a reversible reaction. You can have uncompetitive inhibition or non-

competitive inhibition and partial inhibition. These are various types of inhibition that can happen. Each of these will give rise to different kinetic equations.

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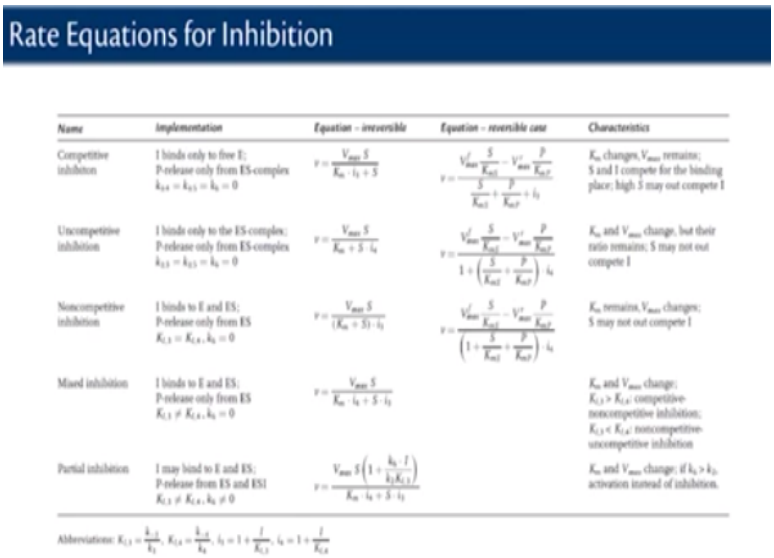

These have been derived in the book by clip. It is a very nice reference to look at. You will see that these are all the equations that you need to worry about. So the moment you see this kind of reaction happening in your system of interest, just plug in one of these equations.

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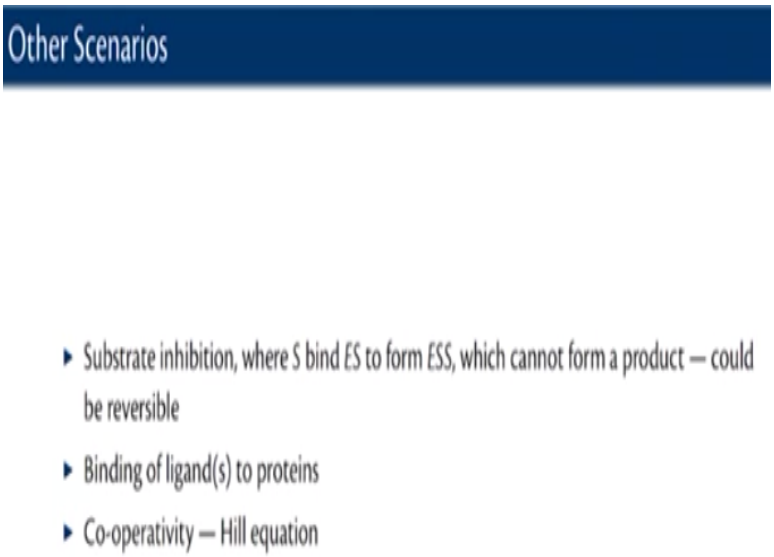

So you may also have substrate inhibition, where ES and S bind to form ESS, which cannot form a product and it is most likely irreversible. You can have other ligands going to the protein, you can have cooperativity. So how does the Michaelis-Menten equation plot look like.

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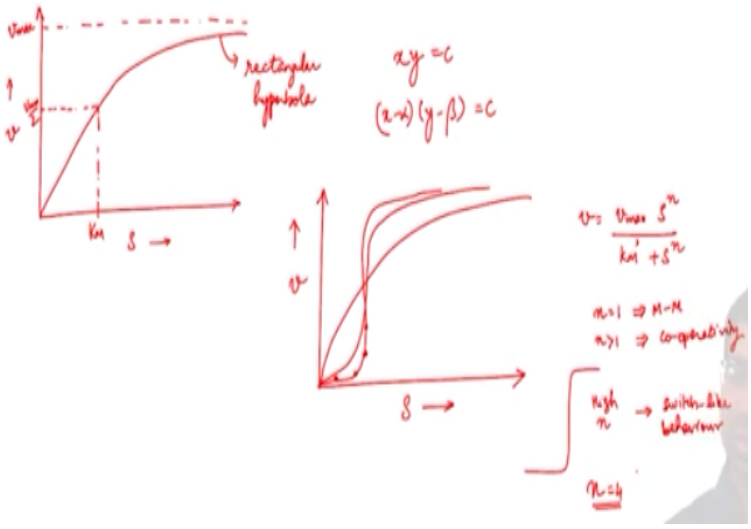

Initially it goes like this and goes to saturation, half of Vmax. What is the shape of this curve? What is a rectangular hyperbola, what is the canonical equation for a rectangular hyperbola, xy=C. You can see that you can write the Michaelis-Menten equation in that form, try it out. More interesting, very commonly in biology, you have the Hill equation, which can look like this, when there is no cooperativity.

So this comes from the V=Vmax*S/KmS except have a different Km when you have an exponent, n=1 classic Michaelis-Menten kinetics, n>1 cooperativity. So how does it look. It is difficult to draw this, something like this. As n increases it becomes more switch like. It will become step. For high n, switch like behavior. What is that you actually see on this curve. Just look at this curve.

When you have more substrate, the reaction really starts proceeding faster. So what happens is, 1 substrate typically binds to the enzyme, makes it nicer for the enzyme to bind more substrate and when a second substrate binds, then it makes it even better for the enzyme to bind a substrate, even have faster reaction and so on. So the presence of additional substrates on the enzyme enables it to bind more substrate till a point, so n=4 is commonly observed, a very classic example.

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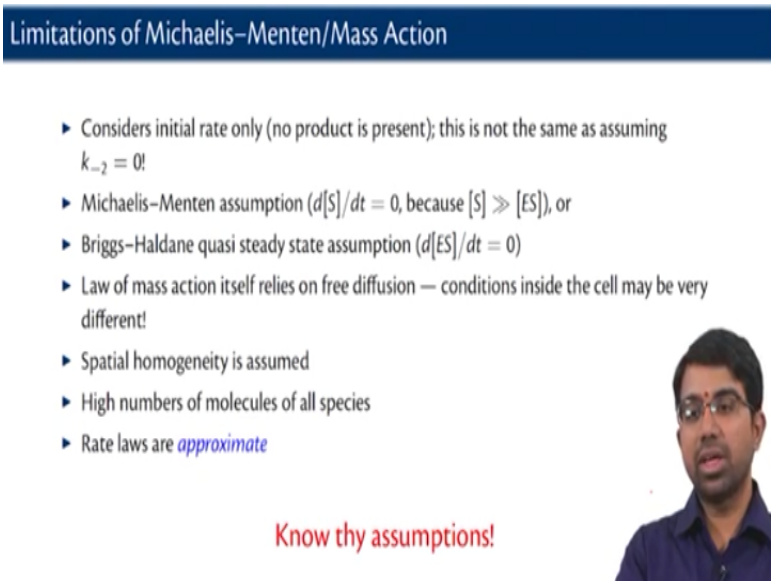

So it considers only initial rate, if you want to look at the limitations of Michaelis-Menten or applying mass action, it considers only initial rate and there is a Michaelis-Menten assumption. So remember that the initial rate is not the same as assuming K-2 is 0. K-2 is 0 would be in an irreversible ES to E+P reaction, as not mandatory or we have the Briggs-Haldane in quasi steady state assumption dES/dt is 0.

The law of mass action itself relies on free diffusion, whereas conditions inside the cell is actually a colloid, it is not just water. It feels more like a colloid and this spatial homogeneity is assumed, high numbers of all molecules, so that you do not have to worry about stochasticity and remember that rate loss are approximate. They are models finally. Know you assumptions, it is very important to not just start the modeling exercise with some assumptions, but they will fall by the way.

Once you are a few months into your modeling project and then you may end up with weird scenarios. Let us consider another classic example.

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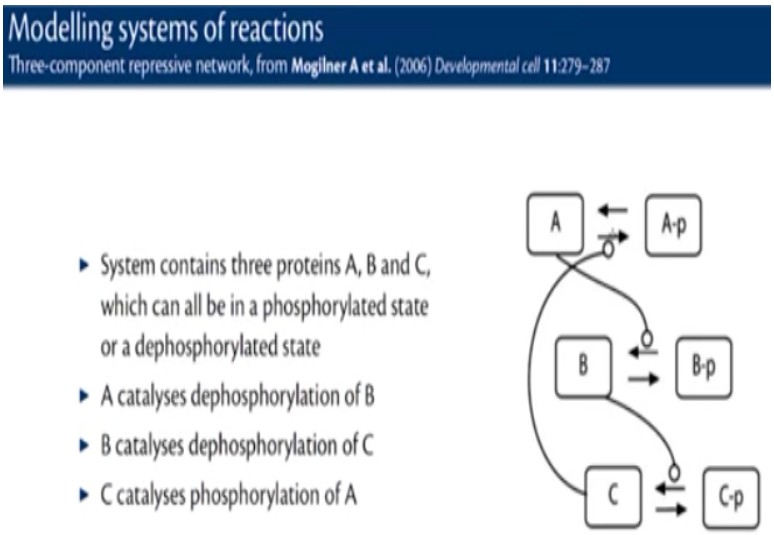

How do we model this system? We have a system that contains 3 proteins, A, B, and C where A catalyses the dephosphorylation of B, B catalyses the dephosphorylation of C and C catalyses the phosphorylation of A. It is not a repressilator as such. So all these are actually catalysis reaction, so it does not inhibit. Repressilator involves A inhibiting B, B inhibiting C, C inhibiting A. You will study that when you look at Boolean models.

Here basically you have 3 phosphatase kind of system, phosphorylase system. So A catalyses dephosphorylation of B, B catalyses dephosphorylation of C and C catalyses phosphorylation of A. So let us try to write out these equations.

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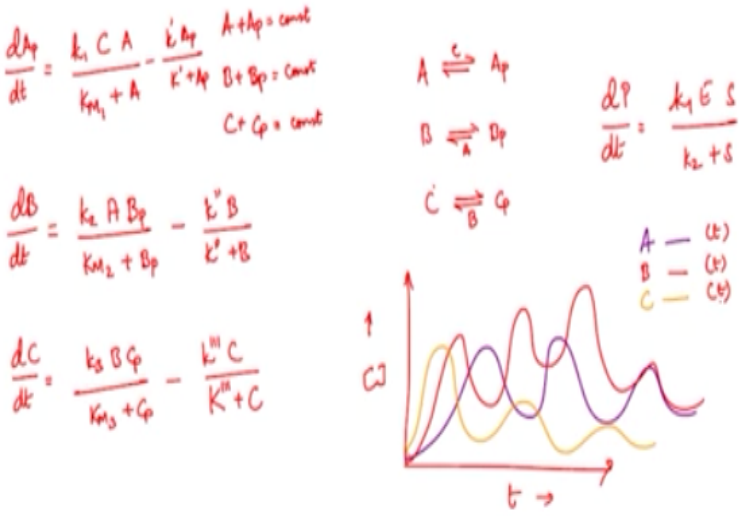

This is C, this is A, how do model this system? What do we know? We know that dp/dt is KES roughly of this form. This is what we know. If you want to assume Michaelis-Menten here, how would you write, dAp/dt. K2 is not the backward reaction. This is actually Km. let us not even write as K2, for simplicity I wrote it as K2. Let us call it Km1, dB/dt, remember we have consideration equations. What are consideration equations?

Which means dA/dt is –dAp/dt as simple as that. So what is dB/dt here? It is going to be K2A Bp/Km2+Bp and dC/dt will be K3B Cp/Km3+Cp. This is what we think they are, just look at the paper has suggested as the equations. So we only consider the forward reaction. There will also be a reverse reaction. You can think of it as a simple autocatalytic reaction, -K dash Ap/K dash+Ap, K-C/K1+A. So dAp/dt will just be the inverse of this.

Because A+Ap is A total, so you can eliminate 1 equation and basically get dA/dt is something of this sort. You do not have to use the term Ap any longer, you can just call it At-A, A total –A, A not-A. Does this sound reasonable? Does this sound similar to what we had written. We are missing the other equation, in both cases. Essentially what I wanted to emphasize is that these equations may look complex, but if you just stare at them, they are nothing but your basic Michaelis-Menten equations that we were able to write just by applying first principles.

No big deal, but when you start writing this for a large system of equations, it becomes incrementally complex and can get quite challenging. The next step is, you have now written down these equation, how do you solve them? What is it that we want in the end? You do not really want dA/dt or dB/dt, but you want A(t). How do solve these ODEs? You now have written out the ODEs, you finally need to solve these ODEs to find out what is A(t), B(t), and C(t).

You have to solve them numerically. It is very difficult to get analytical solution to these ODEs, so we will have to end up solving them numerically and you can basically set these up as initial value problems and solve it. In this video, we saw the classic example of Michaelis-Menten kinetics and you know all the modifications that can happen to Michaelis-Menten kinetics when there is an inhibitor and so on.

We also looked at another example system, wherein we had 3 enzymes that are being phosphorylated and so on and what are the potential kinetic equations for those kind of interactions and so on. The most important part is once you have these differential equations, you need to know how to solve them as we were just speaking about to get from dA/dt to A(t). So that is what, we will see in the next video.