Introduction to Dynamical Models in Biology
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Lecture 17
Modeling Molecular Processes - 2

Hello, welcome to module 5 of third week of our course. In this module we will discuss about writing ODE based model for simple enzymatic reaction processes.

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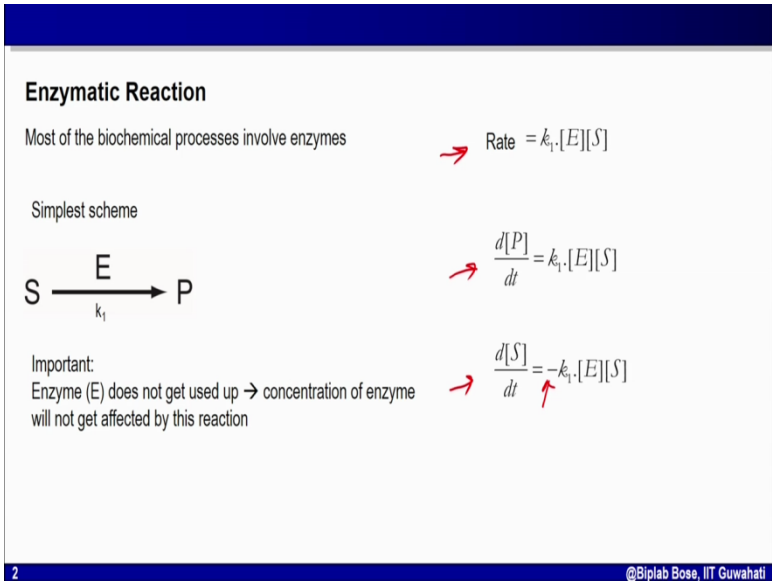

So let us start, enzymatic processes are ubiquitous, most of the bio-chemical reaction that happen inside the cell are actually enzymatic catalyst. So these are catalyst reaction so the enzyme is helping to happen the reaction at a favourable rate in normal temperature. So if I have to write a ODE for a simple enzymatic reaction, the scheme can be like this as shown in case in the slide and you have seen it earlier. S is becoming product, S is becoming product B and enzyme E is helping in this. And the rate constant is K1 in this process. Now remember this is a enzymatic reaction that means we have to remember that enzyme E is not getting used up in this reaction. Because initially it will form complex with substrate and then eventually it will be free again and the product will be formed.

So the concentration of enzyme does not get changed in the long run by this process that we have to keep in mind. That mean I have not to write ODE for if I have to write ODE for the enzyme I will not include this process in that ODE. Now if we keep thing in mind and do not look into any mechanistic aspect that how this particular enzymatic reaction is happening then I can simply write ODE which will be very simple as following a law of mass action which eventually the rate of the process is = K1*[E], concentration of E the enzyme concentration and concentration of the substitute S. Now remember using this rate, I can formulate the ODE's the rate of change of product concentration $\frac{d[P]}{dt} = K1 * [E][S]$.

And as the product is formed and as the processes are reversible the substrate gets used up that means $\frac{d[S]}{dt}$ change in rate of substrate will be $¿K1 * [S]$. This minus is here because this process is reducing the amount of substrate as the product is formed.

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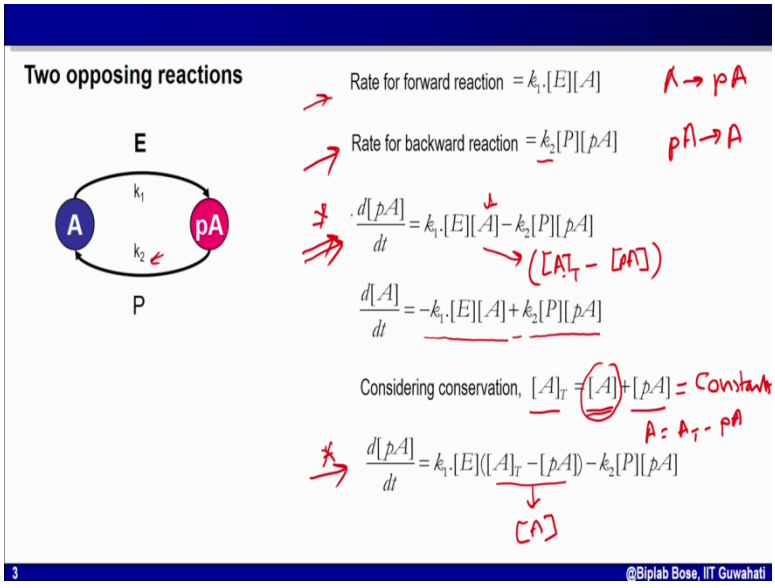

Now let us take this formulation for a reverse reaction and reversible enzymatic reactions are very common as we have discussed earlier in biology. See for example A may be enzyme... substrate which gets phosphorylated to Phospho A. So A maybe a protein and its stilosin residue may be getting phosphorylated by enzyme E to give rise to pA phosphorylated A. And the

rate constant associated with this process I have considered as K1. As there is Kinase to phosphorylate the protein , the kinase is E, there is also phosphatase to de-phosphorylated PA. So kinase E is creating pA and phosphatase P is removing the phosphate group and giving rise to A again so it is a reversible process. And I want to write ODE's for this.

So how I can write it, look into this rate of forward reaction that is A to pA is given by $K1*E*A$. K1 is the constant for forward reaction of the enzyme. E is the concentration enzyme, A is the concentration of the substrate, un-phosphorylated molecule. Similarly the backward reaction pA to A is catalysed by phosphatase, the enzyme is phosphatase here that is P, so the rate of backward reaction is equal to $K2*P$ that is the concentration of the phosphatase into phosphorylated A. So K2 here is the rate constant for the backward reaction.

So if I combine these two, forward and backward reaction then I get the ODE for both the variable that is for pA and A. So rate of change in concentration of phosphorylated A, $\frac{d[pA]}{dt}$ is equal to; first one is from the forward reaction because the forward reaction is forming pA. So $K1*[E]*[A]$ minus the backward reaction because backward reaction is removing pA and creating A, so the minus sign is there and the rest of the term is similar what I have got here for the backward reaction that is $K2*[P]*[pA]$.

For A the equation would be the same just with a minus sign because from A, pA is formed.

So $\frac{d[A]}{dt}=K1*[E]*[A]$, I have a minus sign here plus because the reverse reaction is forming A so $+K2*[P]*[pA]$. So what I have is reversible reaction while A is getting phosphorylated to pA by enzyme or kinase called E. And the pA de-phosphorylated by phosphatase P and I have two ODE's one for pA and another one for A. Now if I want to reduce these two numbers of ODE's into only 1 what I can do is configure the conversation of substrate.

If you remember many a times we use this conservation rule to reduce number of variable depending on the variable. So we can reduce number of ODE, so here I can assume that the total amount of the substrate that is the A remains constant and that is A_T and it is made up of free A

which is non- phosphorylated plus phosphorylated A and I can consider this whole sum is constant. So with time total amount of A is not changing some of the A is remaining as un-phosphorylated A and some it is becoming phosphorylated, so total A_T is remaining constant.

That means I can represent free A by $A_T - pA$. So I can represent free A as $A_T - pA$., so if I do that then I do not need a separate ODE for representing the rate of change in concentration of A. I can represent the system as a whole by the ODE for pA only. So if I do that what do I get, so in this initial reaction initial equation that we have written; let me make it a bit clear. So if I take this ODE, $\frac{d[pA]}{dt}$ I have A term here, I can replace this A term by concentration of total A minus concentration of phosphorylated A.

So I can replace this A, then I get this ODE, $\frac{d[pA]}{dt} = K1 * [E]$ the concentration of the enzyme into $A_T - pA$., which is actually nothing but free A $, -K2 * [P] * [pA]$. I am getting this ODE from this one. And as A is now represented in terms of pA and A_T, and A_T is constant so I don't require this $\frac{d[A]}{dt}$ ODE, so my whole system is represented by this ODE. So what I have done is I have written this two ODE's based on the forward and backward reaction and then I have used the rule of conservation of the substrate and reduce and combine them into 1 ODE. And this very frequently you have to do that to reduce the number of ODE in your problem.

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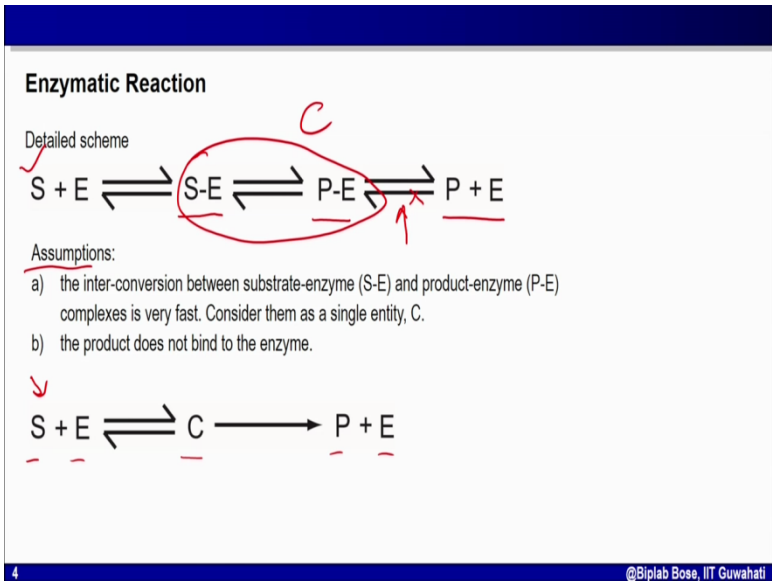

Till now what I have considered, I have not looked into mechanistic aspect of the enzymatic reaction. I have simply got inspired from the law of mass action and written down the ODE. Now in some cases actually the enzymatic reaction mechanism maybe critical and that may affect the dynamics of the whole process. So in that case you cannot use a simple ODE that we have discussed till now, things may get little complicated. So let us look into a bit of detailed mechanism, remember this mechanism that I show will be a generic one there can be a different variant of this also. And accordingly you may have to change your equations mathematical formula formulation as a whole.

So let us look at the detailed scheme as shown here in this slide. S is my substrate, S is reacting with E and giving rise to a substrate enzyme complex; that is what happened. You have enzyme that substrate comes and binds to the binding pocket the reaction pocket to give rise to S * E complex. Now at these binding pocket E helps S to become P so I get a product substrate complex that is PE and this PE separate now out to give rise to new product and free enzymes, so this one.

Now remember all this step we have considered at the beginning as reversible one because S and E is nothing but a binding process obviously it is reversible. Now SE gets combined into PE that is also considered usually as reversible. And now PE dislodge and break down, give rise to free E and free B and that is also reversibly reaction process. Now I can make certain assumptions here

which are rational assumptions and I can reduce this system further. What I can assume... I can assume that AC and PE converse, substrate enzyme complex and product enzyme complex conversion is very fast and in fact is very fast and is very difficult even in experiment to identify substrate enzyme complex and product enzyme complex separately.

So usually in chemistry lab also, people will consider both of them together as a single entity and will consider them as a C complex. So I will consider this whole thing as a single thing and C as a complex, I will not separately consider them. And the next assumption which is also very logical is that the product does not go back and bind to enzyme again that means I am not considering this step as reversible one but I am not considering this step as reversible one. I will consider this reverse path not possible only the forward thing, the product enzyme complex can breaks down into product and enzyme.

So the reverse would not happen, and that actually most of the times happen because there is a faster one. So if I make this two assumption then I can reduce this system into a simpler structure. So what is happening now, S is binding to E in a reversible fashion to form complex C and the complex C is the only unidirectional breaking down into the product plus E. So initially it started the details scheme and then now from this details scheme I have made some assumptions and I have written down a simplified scheme for the system. Now I will use this simplified system to write down the ODE's for the dynamics of this reaction system.

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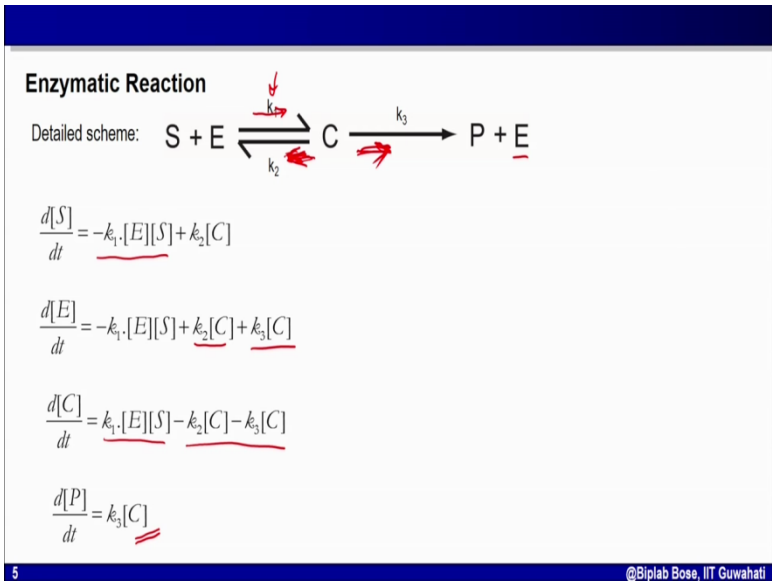

Let us start with that, so my scheme is S is binding to E to form C. It is reversible process so K1 and K2 are two rate constant. C unidirectional breaks into P and E and the rate constant is K3. So I can write the first ODE representing rate of change of S, concentration of S. $\frac{d[S]}{dt} = ¿$, remember S is getting consumed in formation of C and C breaks down into $S * E$ by this reverse mechanism, right this reverse path. So the first term in my ODE for S is $\frac{d[S]}{dt} = -K1 * [E] * [S]$, K1 is the rate constant for the forward reaction. E is the concentration of the enzyme. S is the concentration of the substrate + $K2 * [C]$ because $K2 * [C]$ is giving the formation of S by this reversible reaction.

Now let us write for the enzyme, $\frac{d[E]}{dt}$ the rate of change in concentration E equal to $K1 - K1 * [S]$ as same as the previous one in case of substrate S because enzyme is getting consumed in formation of the complex C, so I have a minus term here $-K1 * [S]$, next C is breaking down in both directions. In one direction here it is giving rise to S and E so I get back some E and on the other side S is breaking down into P and E and that also gives rise to some free E. So I have two terms, so $K2 * [C]$ is representing this reverse reaction from which C breaks and S and E is formed and $K3 * [C]$ is also giving rise; $K3 * [C]$ is representing the rate of this forward unidirectional reaction by which I also get E.

So $\frac{d[A]}{dt} = -K1*[E]*[S]$ that is the consumption plus $K2*[C] + K3*[C]$. Let us look into the ODE's for complex C; now complex C is formed by one path and broken down by two path. So obviously the formation is $K1*[E]*[S]$ that is the forward rate process by which C is formed. I have two processes by which it breaks down by one process it goes back to substrate enzyme and other process goes to product enzyme so I have minus $K2*[C]$ into sorry minus $K3*[C]$. So $\frac{d[C]}{dt}$ has three terms, one term the first one is for formation and the last two terms as they are minus sign because they are consumption of C.

Now let us look for the product, remember look into the skin and the product is formed only by the unidirectional process from C to P. So it is simple, $\frac{d[P]}{dt}$ rate of change of concentration of P is equal to $K3*C$ that is all. I have written down the ODE for all these 4 terms, all four dependent variable S, E, C and P. Now remember in this case, it is different for the enzyme. In the earlier simple case I have not considered any ODE for the enzyme because I have considered the enzyme concentration does not get changed with time. In this case E is forming a complex, some of the E is sequestered in the complex and I have detail mechanism, so I have considered E as a dependent variable and I have written down a ODE for E also; the enzyme also.

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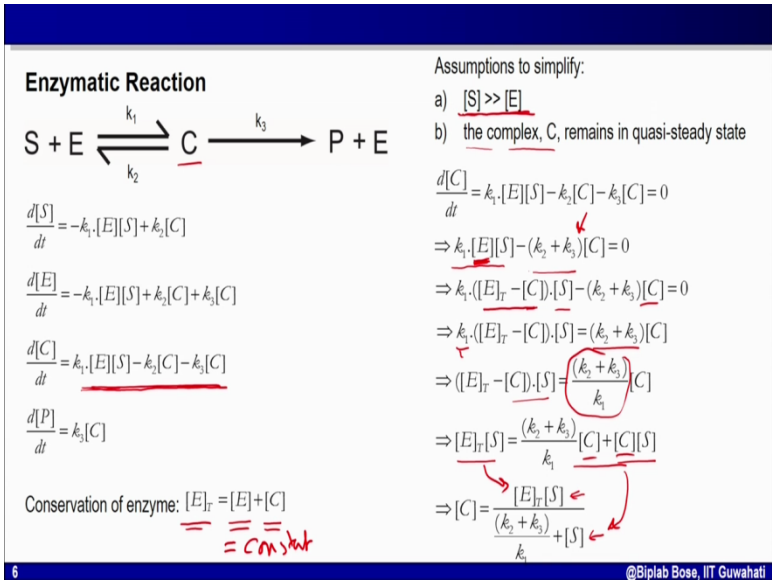

So now proceed further and let us make some further assumption to simplify this ODE and write down the ODE representing the proto formation of the product. So we can make some assumptions here, these assumptions you may have seen in your bio-chemistry text books. One assumption is, substrate concentration, the amount of substrate is much more than the concentration of the enzyme; that is the first assumption. That means you are concentrating the enzyme in the less concentration than the substrate and the second concentration... assumption is key 1 is that I am considering C, the complex formed between S and E.

E is at a quasi-state; that means with time concentration of C is not changing. It is quasi-stated because at the beginning of the process there will not be any steady state but as we assume that very fast it reached steady state and it is a quasi-state because you have multiple processes going on. C is formed; C is broken down so I have a steady state there. The formation and breaking rate are equal that is why I get a steady state for C. Now you can easily see that assumption 2 requires assumption A because if I have to continuously produce C and C is continuously broken down into P and E, I should have continuous supply of substrate so new C molecule can be formed.

So that is why you have to assume substrate concentration is much higher than enzyme concentration otherwise I will not get the steady state of C for a tangible time so if I have these two assumptions then I can assume that I can consider that C is at a steady state. What does that mean, if C has a steady state then this ODE, $\frac{d[C]}{dt}=0$ that is what I can write. Then $\frac{d[C]}{dt}=0$ that means $K1*[E]*[S]-K2*[C]-K3*[C]=0$ because that is how I have got ODE for $\frac{d[C]}{dt}$. Now I will rearrange terms so I can get C at one side and everything else on the other side because C is the complex form from where the product is formed, so I will try to separate C in one side from this algebraic relation and keep rest of the thing on the other side.

So let us try that, so what I can do is I have considered K2 and K3 both are associated with C so I have taken them together so I write the algebraic relation like this. $K1*[E]*[S]-K2+K3*[C]=0$ is very simple. Now I have assumed a conservation, obviously the total enzyme is conserved here as there is no consumption and de-gradation of

enzyme here so I can consider that the total enzyme E_T remain conserved with time and that is equal to free enzyme plus enzyme in the complex. Like if I consider one is to one stoichiometry then the concentration of the complex is equivalent to the concentration of the enzyme sequestered in that and that whole thing is equal to constant.

So with time $[E]_T$ is not changing, I can replace this E in my relation... algebraic relation using this $[E]_T - C$ that is what I will do, so I have replaced this E by $[E]_T - C$, concentration of C. So E is free enzyme and I am replacing it by $[E]_T$ by total enzyme minus complex so I get relation $K1*[E]_T - [C]*[S] - K2 + K3 = C$. So I again get another C here but I will take out that C and club with this C so what I get, $K2 + K3 * C$ on the other side then I re-write this whole thing like this taking the K1 on the other side here and so I get $\frac{(K2+K3)}{K1}$

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So the whole thing is constant, these are all combinations of 3 constants, then I can write multiply S inside this bracket so I get $[E]_T * [S] - [C] * [S]$ I take $[C] * [S]$ on the other side here on the right hand so I get $[E]_T * [S] = \frac{K2+K3}{K1} * [C] + [C] * [S]$. Let us separate C, so if I take C common from this, I get $\frac{K2+K3}{K1} + [S]$ that goes in the denominator and I have $[E]_T * S$ in the numerator. So this whole thing comes here and this one is here. So what I have got, I have separated out C and I have everything else on the right hand side. Right hand side has $[E]_T$ which is constant, the total amount of enzyme substrate which is varying with time, concentration of substrate is varying with time divided by $K2 + K3/K1$ as a whole is a constant because K3, K2 and K1 are rate constant plus concentration of the substitute.

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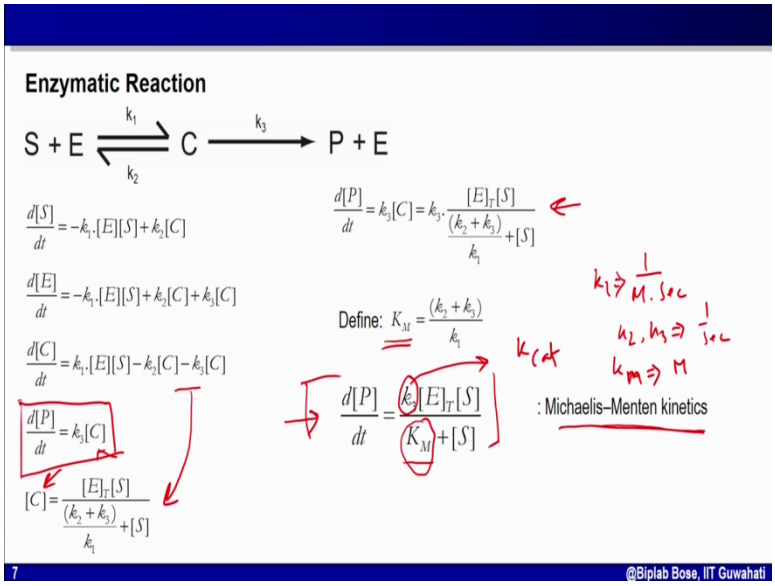

So let us clean a bit, what I have got this is my scheme and I have initially written down the ODE's from the $\frac{d[C]}{dt}$ I have got this relation, considering steady state for C that C ¿ $\frac{[E]_T * [S]}{\frac{K2+K3}{K1}+[S]}$. So now take this ODE, $\frac{d[P]}{dt}$ there is a rate of change in the concentration of the product that is what I am bothered over here because I want to know how with time the concentration of the product is changing. $\frac{d[P]}{dt} = K3 * [C]$, so I can replace this C by the C that I have considered here because I know C is in steady state and that is what my assumption is.

So I can replace C by this steady state value of C that is what my assumption is so I can replace C by this steady state of C so $\frac{d[P]}{dt} = \frac{K3[E]_T * [S]}{\frac{K2+K3}{K1}+[S]}$. Now $\frac{K2+K3}{K1}$ is a well known constant constant and that is called Michaelis Menten constant, it is called Km. And remember

this is very common in bio-chemistry book you will find many a time you find biology and bio-chemistry characterise enzyme, calculate this value Michaelis Menten for this enzymes and that is very helpful for our modelling because we can take those numerical values for those Michael's maintain constant and plug into our ODE.

So Km is defined as $\frac{K2+K3}{K1}$. Now I can re-write this ODE. $\frac{d[P]}{dt}=K3$ remain in the top... in the numerator. $K3*[S]$ concentration of the substrate, $[E]_T$ is the total concentration of the total enzyme divided by in the denominator plus Km+[S]. Km is the Michaelis Menten constant and remembers this K3 is all the time called K_{cat}. In some text book it is called K_{cat}, this whole ODE is essentially ODE for the product formation if I consider those two assumptions that substrate is much more than the enzyme and C is in a quasi-steady state. And this kinetic is called Michaelis Menten kinetics.

So if I have a biological system where the substrate is in large amount and I can safely if I can assume that the concentration of the complex formed between the substrate and the enzyme is remaining in a quasi-steady state for the period of my study. I can consider the enzymatic scheme is actually following Michaelis Menten kinetics and I can use this relation; this ODE that $\frac{d[P]}{dt}$ of a product formation equal to K3 which some book will call $\frac{K_{cat}*[E]_T*[S]}{Km+S}$. You can easily see K1 has a unit of 1 /molar and second, whereas K2 and K3 has unit 1 /second. So Km the Michaelis Menten unit you need to maintain is also molar.

And obviously you have in the denominator you have in the denominator substrate is in molar term and Km is also molar term so you can sum them because to some quantity their unit has to be same. Now K3 is second 1/ time, now if you look into this Michaelis Menten relationship which is very common in any bio-chemistry book. You can easily see that if my enzyme is following Michaelis Menten kinetics than I require two parameters; one is K3 that is the K_{cat} and you have to tell me Km. If you can tell me this parameter than depending upon how much enzyme and how much substrate I have I can easily understand the dynamics of product formation. So usually when you are dealing with Michaelis Menten kind of kinetics in a large

system with multiple enzyme you have to keep in mind that you have to note this K3 and Km values for each of this enzymes.

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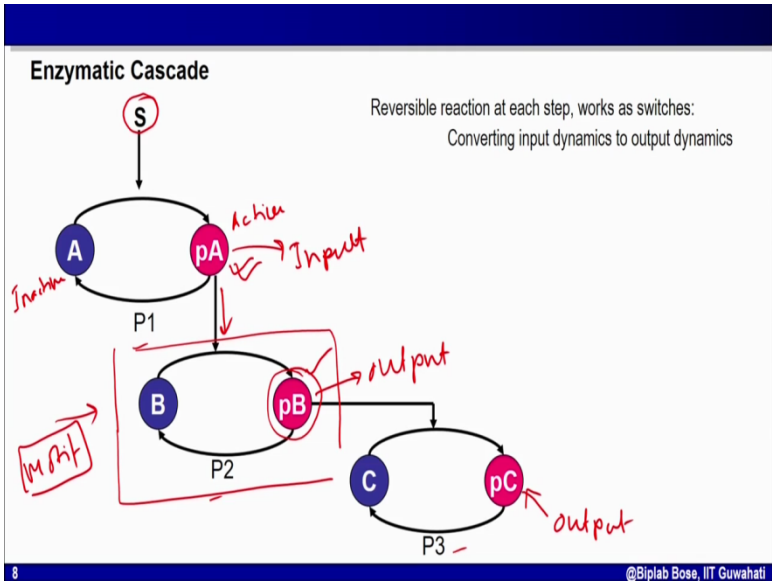

Now let us look in a scheme which is very common in biology that is enzymatic cascade. And in any biology text book you will find very frequently, we call them phosphorylate the way I have shown here. A signal comes which maybe a enzyme actually a kinase which activates A to phosphorylated and pA is active whereas your A is inactive enzyme. Once pA is active, pA is again a Kinase that activates B to pB phosphorylate to B. Phosphorylated B is again active as a Kinase and that phosphorylated C to pC. And each of this step I have phosphatase P1, P2 and P3.

Sometimes this phosphatase may be common, a single phosphatase maybe working on both pA, pB and pC. Whatever it is, it is a cascade you can see, first one phosphorylation is happening and then that converse the enzyme into active enzyme. Active enzyme which is a kinase now which will phosphorylate into another substance which is a inactive enzyme. On phosphorylation that will become active enzyme, active enzyme which is a kinase now will phosphorylate another sub-set which is a in active enzyme on phosphorylation that will become active and a chain of events happens that is a reversible action.

Now in this reversible processes each of this unit, each of this unit are actually I can assume as a switch. What type of switch, this arrow representing that the input is coming from this kinase and I can consider the concentration of this pB as output. So the concentration of pB at a particular

time is the output and this one is my input to the switch. So I have multiple input and output switch connected together one after another in a cascade so that this is my initial input in my system and the final output is this one. So if I have to model this type of system, how can I approach?

What I will do is I will focus into one single unit as this considers I can consider as a motif. A reversible reaction happening and is a switch made up of two reversible system by which one molecule is getting phosphorylated and that is again getting de-phosphorylated.

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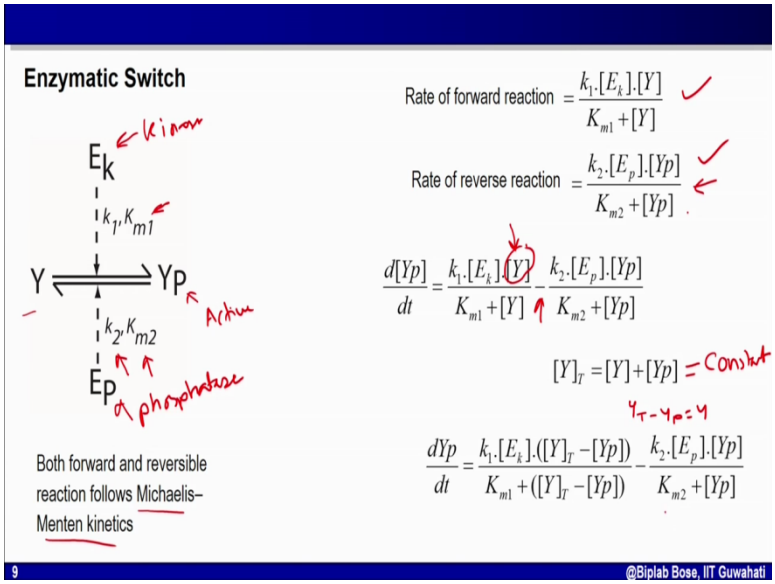

So let us look into this particular motif and try to understand the dynamics of that in terms of ODE. So I have written down this motif separately, here Y is the in active enzyme. It gets phosphorylated by this kinase Ek and it becomes Yp which is active, I can consider it as active enzyme or in some case it maybe inactive also. But we will consider it as a active enzyme. Ep is my phosphatise, so Ek the kinase is converting Y to Yp, phosphorylated Y and phosphates Ep is removing the phosphates group from Yp and giving rise to Y again.

And I assume this reaction forward and backward reaction follow Michaelis Menten kinetics so they are following Michaelis Menten kinetics. And as I said Michaelis Menten kinetics has two parameter one is the K_{cat} and other is the Michaelis Menten constant so for this forward reaction,

K_{cat} is suppose K1 and second one is suppose Michaelis Menten rate is Km1, and for the next one the reaction, the reverse reaction K2 is the capital K-cat and Km2 is my Michaelis Menten constant. So I can write down the rate of forward reaction will be as per Michaelis Menten kinetics, K1* Ek there is total concentration of kinase into Y that is substrate concentration by Michaelis Menten and concentration for that reaction plus substrate concentration.

Similarly for the reverse reaction, I have rate equal to $K2*Ep.Ep$ is the total concentration of the phosphates into Yp. Yp is the concentration of the sunset here that is phosphorylated to Y divided by the denominator Km that is Michaelis Menten constant plus the subset Yp. Now if I have to write down the $\frac{d[Yp]}{dt}$ the rate of change of Yp and I will club this two and I can get the equation $\frac{d[Yp]}{dt}=\frac{K1*[Ek]*[Y]}{Km1+[Y]}-\frac{K2*[Ep]*[Yp]}{[K2]}$, Km2 and Yp. I have taken this one and this one and added minus sign here because this is a reverse one and the other one is for the forward one.

Now, Yp is the amount of free one. I can reduce this into, I can covert this terms of amount of total energy total Y and phosphorylated Y considering a conservation. So I can consider total amount of the substance Y_T = free Y plus concentration of phosphorylated Y. And I can

consider this as constant. So with time Y_T is not changing. So I can replace this $\frac{[Y]}{¿}$. So

that's what I have done. So if I replace $\frac{Y}{¿}$. I get this relation, $\frac{d[Yp]}{dt} = ¿ K1 * [E_K] * ¿ \frac{2 * [¿¿P] * [Y_P]}{Km2 + [Y_P]}$.

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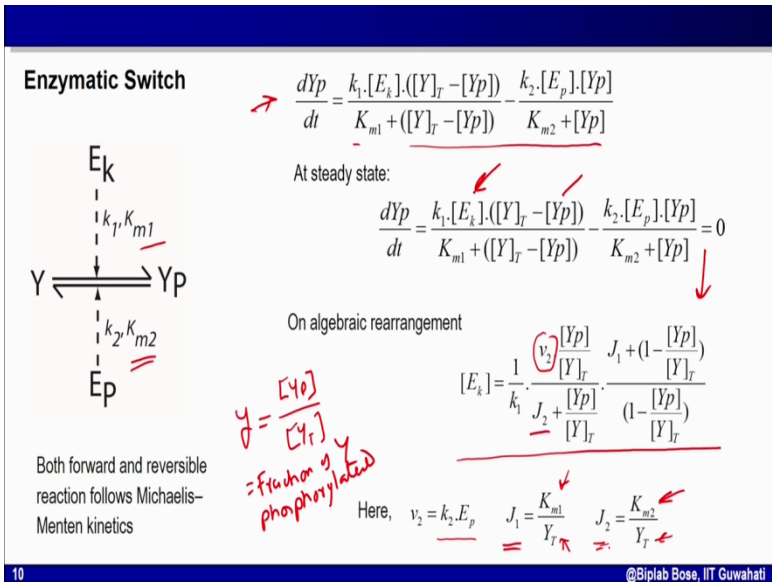

So let's clean it a bit. What I have? I have enzymatic switch which is a motif present in a enzymatic cascade. Y is becoming Yp by a kinase Ek and it is getting de-phosphorylated by Ep. Both are following my Michaelis menten Kinetics and I get a ODE like this, which represent rate of change of Yp with respect to time. Now, let us assume the system has reached a steady state I want to characterize the steady state behavior of this system. So that mean $\frac{d[Yp]}{dt}$ will be 0. So I can write $\frac{d[Yp]}{dt} = 0.$ That means this whole algebraic relation will be equal to 0.

And then I can separate out different terms on left hand side and right hand side. In this case what I will try, I'll try to separate out Ek that is the concentration of the kinase enzyme, total concentration of kinase enzyme and Yp on both side of my equal to sign. So I will keep Ek in one side and Yp on the other side. Remember Ek the concentration of kinase enzyme can be considered here just like a input because that Ek may have got activated by input signal. So that input signal can be represented by the concentration of the kinase enzyme.

So I want to keep that separate on the left hand side. And rest of the thing including Yp on the other side. So, what I can do. I can do lots of algebraic rearrangement. I have skipped them here to save time and eventually what you get is that Ek is equal to a relationship where I have

represented Yp as in terms of $\frac{Y}{[Yp]}$. So what I have done, I have represented not just Y, I have taken $\frac{Y}{[Yp]}$ so my relations become simple and it has a biological meaning also. So $\frac{Y}{[Yp]}$. So this is nothing but fraction of Y which is phosphorylated.

And in fact, biologists can measure this Y that is $\frac{Y}{[Yp]}$ the fraction of the total protein Y which is phosphorylated, by different assays for example western blot, by ELIZA I can actually measure not the total or real number of protein which is phosphorylated, but I can measure the fraction of the total protein which is phosphorylated. So that's why and as for the ease of algebra what we have done we are keeping Yp separate we have taken made it a ratio of $\frac{Y}{[Yp]}$. And that can be done easily, algebraically, from this relation. And I can get this whole relation this whole algebraic relation that we have seen here.

Now, this look bit complicated. And I have done some simplification also. For example, V2 is actually nothing but $K2*[Ep]$ obviously K2 is the rate constant, Ep is also a constant because of the concentration of the phosphatase enzyme. J1 is another parameter. J1 include two things one is Km1 that is the Michaelis Menten constant for the forward reaction the kinase

reaction and total amount of the substrate is Yt. Similarly J2 used here, which is represented here

by Km2 by Yt. So I have replaced Km2 / Y ¿ ¿ ¿ by the parameter J2.

So, Km2 is the Michaelis Menten constant for this reverse reaction, de-phosphorylation and Yt is the total amount of the substrate [Y ¿+[Yp] . This J1 and J2 will be crucial in our discussion so you have to keep in mind this is nothing but ratio of the Michaelis Menten constant and the total amount of the substrate that is Y. So what I have? I have a algebraic relation giving Ek on left

hand side $\frac{Y \text{ ¿ ¿ ¿ } [Yp]}{\text{¿}}$ that is the fraction of phosphorylated protein on the right hand side in terms of

J1, J2 and V.

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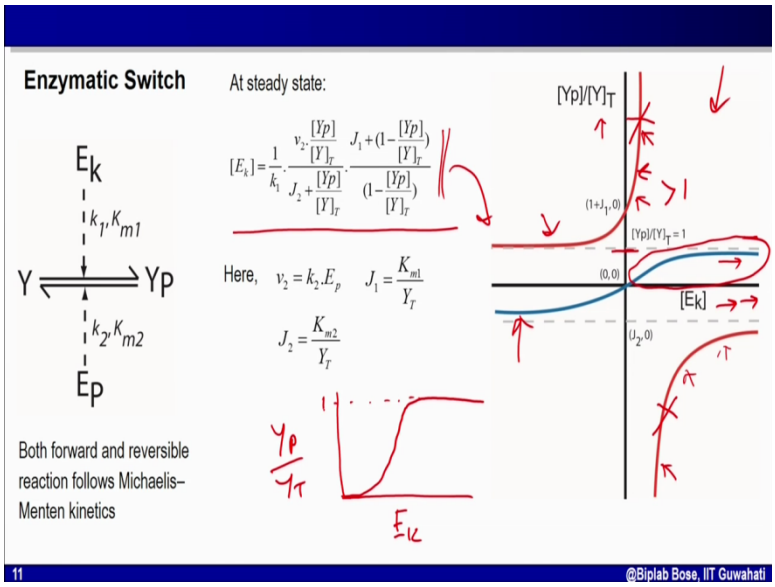

So if I try to plot this Ek, versus $\frac{[Yp]}{[Y]t}$ I get this type of plot. Pay attention to this plot. I have these two red lines. Here I have in a horizontal axis I have Ek and the vertical axis I have $\frac{[Yp]}{[Y]}$, the fraction of Y phosphorylated. I have two red lines. These red lines are all coming from this algebraic equation that we have derived just now. Now this particular red line Ek is positive but $\frac{[Yp]}{[Y]}$ is negative. That is not possible in our system. Mathematically that may be possible. But I cannot have a negative value for the fraction of a protein phosphorylated. So I will not bother about this one. Look into the other red line. This is also coming from my relationship. If you plot it using a plotter you will get that.

Now here what is happening, see, here in this part Ek is positive that is possible. $\frac{Y}{[Yp]}$ is also positive that is possible but look into it the values of Yt, $\frac{Y}{[Yp]}$ are all greater than 1 because this line, this dotted line is representing 1. Now $\frac{Y}{[Yp]}$ is fraction, fraction of phosphorylated protein. So, fraction of total protein that is phosphorylated so, that cannot be ever bigger than 1. You cannot have more than the protein be phosphorylated. So the fraction can never go above 1. So that mean I don't need to consider this red line. What I am left with? I am left with this blue line which is also coming from this relationship and if you look into this, positive domain, Ek is increasing in this direction, $\frac{[Yp]}{[Y]t}$ is also increasing but it saturate moves like a S and saturate at $\frac{Y}{T}$ 1.

And that is true that $\frac{Y}{[Yp]}$ should never be greater than 1. So this is the region of my interest. So in this region this blue line is working like a sigmoid curve. Initially increasing and then

saturating near 1. So what I get here from this algebraic relation is that if I remove all those not possible thing, then if I plot Ek in my horizontal axis and $\frac{[Yp]}{Y}$ in my vertical axis then the behavior will be a sigmoidal one, where it will saturate at a value much younger to 1.

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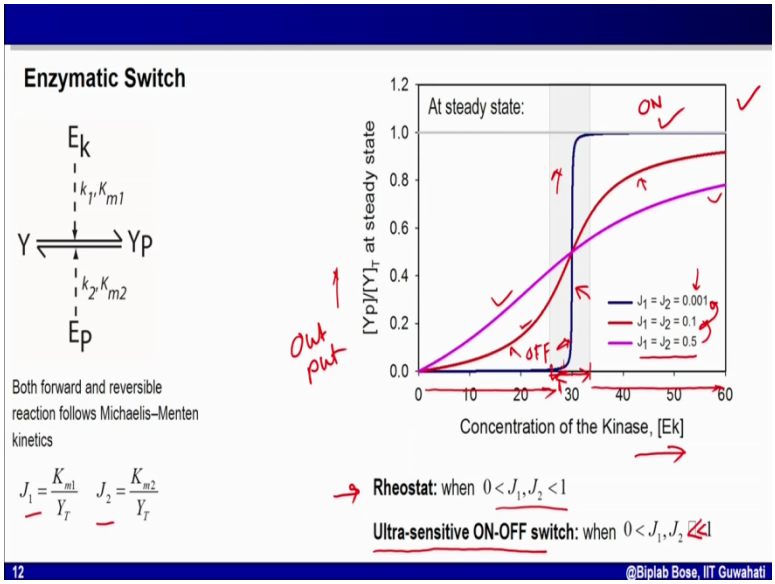

So let us look into different behavior of this one. What I have. See. If I have take this motif and if I derive that relationship between Ek and $\frac{[Yp]}{Y}$ that we have done, then I can make a plot like this that shown here. Here in the horizontal axis we have the concentration of the Ek which you

can consider as a input, $\frac{Y}{[Yp]}$ at steady state, you can consider this as a output in your vertical axis. So then you can draw different sigmoidal type curve based on values of J1 and J2. Let us look one by one. When J1 and J2 are equal to, both of them are equal to 0.5, right?

In that case I get this pink curve, slow very shallow sigmoid. If I make J1 and J2 slightly smaller from 0.5 I make to 0.1, then I get this red curve which is quite sigmoidal. Now make J1 J2 further small. Now, I have taken J1 and J2 both equal to 0.001 very small then this sigmoid become very sharp, this blue line. So what is happening? As I am making J1 and J2 very small with respect to 1 then, this sigmoidal curves are becoming very sharp and I am getting a sharp S type thing. So when I have very small J1 J2 for example equal to 0.001, I get this blue curve.

And what will happen? Up to this near 30 from 0 if you increase Ek there is no change of output, output remain close to 0. Then all of a sudden slight change in Ek will cause a sharp rise on in the output there is $\frac{Y}{[Yp]}$ and I'll reach the saturation. So this region shown in grey mark is the very sensitive region. We call ultra sensitive region. Here slight change in the value of Ek will sharply change the value of $\frac{Y}{[Yp]}$. And then, again in the rest of the region I'll remain at the higher value. So this lower value I can consider as a off and the higher value I can consider as a on. So when J1 and J2 are very small with respect to 1, I get a ultrasensitive ON-OFF switch.

Here you may not be seeing this one if there is a double less than 1. So J1 and J2 is very small with respect to 1. But when, in this one is like your ON-OFF switch the way you use in your house. ON and OFF two option, binary. But if you have J1 and J2 close to 1 but smaller than 1

then I get this sigmoidal thing, which is shallow that means you are increasing output slowly and input is also slowly increasing. There is no sharp jump in input output relation. So this is like a rheostat. So depending upon the value of J1 and J2, I can have this reversible reaction working as a rheostat or as a ON-OFF switch.

Before we close let us look into what is J1 and J2 once again. J1 and J2 is nothing but ratio of Michaelis Menten constant and total substrate. Michaelis Menten constant and total substrate. So when the total substrate concentration is very high with respect to Michaelis Menten constants then I will have J1 J2 very small with respect to 1, then I will have this ON-OFF switch. But when the concentration of the substrate is close to Michaelis Menten then I will get value of J1 J2 close to 1, then I will get this rheostat behavior. So depending on the substrate the same input can give rise to a sharp ON-OFF switch input-output relation or it may give a rheostat type slow shallow increase in change in dynamics in input-output.

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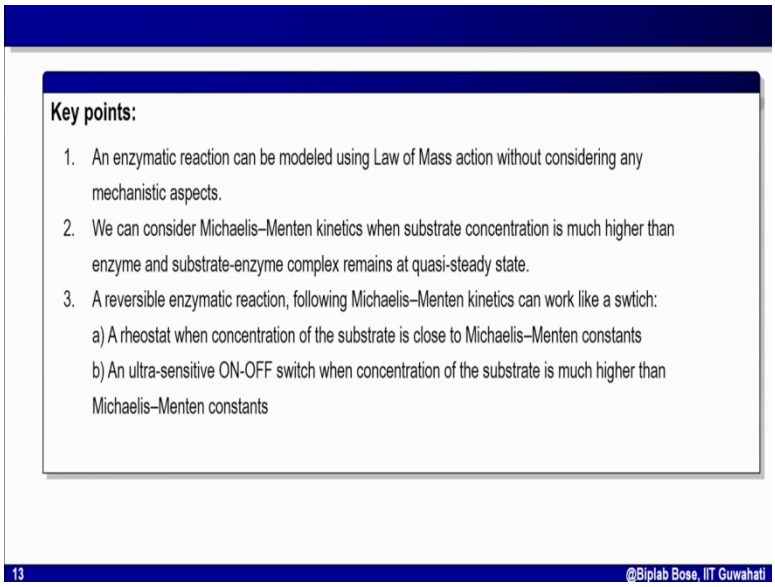

So if I jot down, if we don't look in need to look into mechanistic aspect or complications in the enzymatic process I can simply model law mass action, for a particular enzyme reaction. But in some cases I need to consider a complicated system and I can assume Michaelis Menten kinetics for those cases, where substrate concentration is much higher than the enzyme and the substrate-enzyme complex remains as a quasi steady state. If these two conditions are met I can consider Michaelis Menten kinetics otherwise I cannot. If I have a reversible reaction scheme which works as a switch in a large cascade and if both the processes the forward and reverse reaction follow Michaelis Menten kinetics then I can a switch which has two behavior.

When the substrate concentration is close to Michaelis Menten constant the switch will work like a rheostat, when the concentration of the substrate will be much higher than the Michaelis Menten constant the same reversible reaction switch will work as a ultra sensitive ON-OFF switch. That's all for this module. Thank you for watching.