Introduction to Dynamical Models in Biology
Dr. Biplab Bose
Associate Professor
Department of Biosciences & Bioengineering
Indian Institute of Technology, Guwahati
Lecture 19
Modeling A Signal Transduction Circuit

Hello, welcome to the first module of last week of this course. In this module, we will model a small signal to signal transaction module and analyse that and its behaviour will be analysed, so let us start with the simple signal conduction circuit that we will analyse today.

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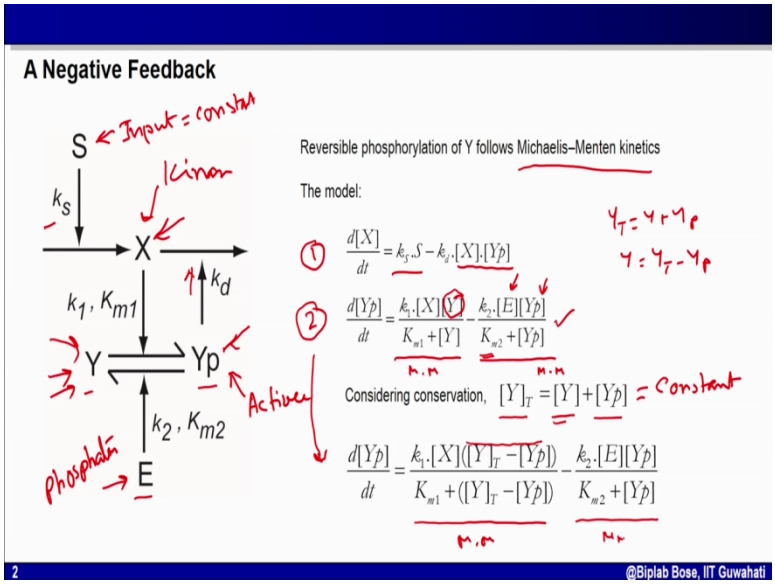

Here in this slide, I have shown the figure to represent that circuit, X is a Kinase, suppose. X is a Kinase and X is produced through a process which is controlled by S in input signal, and X controls phosphorylation, X is a Kinase so it's the enzyme, it controls phosphorylation of Y to YP. This phosphorylation is reversible, so YP get dephosphorylated into Y by a phosphatase E and YP is a phosphorylated protein Y and once it is phosphorylated, it becomes an active enzyme.

So now it is an active enzyme, and this active enzyme promotes degradation of X, that's what I have shown by a arrow here, so what we have, we are giving a input signal S, then induces production of X which is a Kinase and that X Kinase phosphorylate Y to YP, this stage of

phosphorylation is very common in signal conduction pathways and this stage of phosphorylation is a reversible process so if you have a Kinase, there will be a phosphatase to reversibly get it back from YP to Y and that phosphatase here shown as E. Now, usually in the phosphorylate system of signals transaction, usually the phosphorylation increases the activity of the molecule or it decreases.

In this case I have considered Y is inactive and when it is phosphorylated by X, it becomes YP and this YP is a active form and this active form promotes degradation of X, so what is happening here? S is promoting production of X, X is promoting formation of YP, and YP in turn is increasing (deg…) degradation of X, so that means I have a negative feedback here. X is producing YP, YP is degrading X, so that means I have a negative feedback, so the network or circuit shown here in this figure is a simple negative feedback circuit which can be triggered by a input signal S and we want to analyse how the input will affect the output for this particular circuit, so before we move into further, let us create mathematical model using ODE for this system.

How we will proceed (uh) before we proceed, let me assume that this reversible state, this Y to YP, I have two reaction, Y to YP controlled by X, and YP to Y, controlled by E, let us consider this step, these two steps are following Michaelis–Menten kinetics. If you remember in last week in a particular module, we had discussed where I have a reversible reaction system where both the reaction follows Michaelis–Menten kinetics, so in that module we have learned how to write the ODE for that type of simple reversible reaction.

We will use those concept here in this model, we have included some other molecules here to create a circuit, so the first thing is, I am considering this reversible reaction of Y to YP as Michaelis–Menten… as per Michaelis–Menten kinetics, so let us start writing the ODEs. I have three dependents variable X, YP and Y. I have three dependents variable X, Y and YP. S is input and this is constant. S is not changing with time, so as I have three dependents variable, I have to write three ODEs. The first one is for X, concentration, and I am writing ODE to represent rate of change of concentration of X.

So DXDT is equal to KS into S, KS is the rate constant as shown here, into S, so that is the term representing production of X minus KD into X into YP so this last term, KD into X into YP is

representing the degradation of X. Now the second ODE is for YP, that is DYPDT is equal to remember, I have considered this Y to YP trans change as a reversible reactions following Michaelis–Menten kinetics so the forward one from Y to YP will follow Michaelis–Menten and YP to Y will also follow Michaelis–Menten so the first one I have here is the Michaelis–Menten kinetics, so it follows Michaelis–Menten kinetics, that's why you have K1 rate constant into X.

X is the enzyme here, Y is the substrate, Y is becoming YP, so K1 into X into Y divided by Michaelis–Menten kinetics, KM1 for that reaction plus Y, the substrate concentration, so this first one is Michaelis–Menten kinetics equation for the forward reaction from Y to YP, the second one is also Michaelis–Menten. In this is the enzyme is E and the substrate is YP and the Michaelis–Menten constant is KM2, so these two Michaelis–Menten equations are formulations are stuck together to create the ODE for YP. Now I am left with ODE for Y.

Now if I consider that total amount of Y i.e. YT is equal to Y plus YP and if I consider that remain equal to constant, then I can replace Y in terms of YP, this type of reduction in number of variable, we have tried earlier, considering here a conservation of total amount of Y, a part of Y is free Y and another part is phosphorylated Y, so summation of these two is constant and equal to YT. So the second equation I can replace, the second equation I can rewrite in this form, DYPDT is equal to K1 into X, in place of Y in place of Y I am writing YT YP because YT is equal to Y plus YP that means Y is equal to YT minus YP, so that's what has been written here. This is the first Michaelis–Menten term. Second one remains same as here in the ODE2, so this is the second Michaelis–Menten term.

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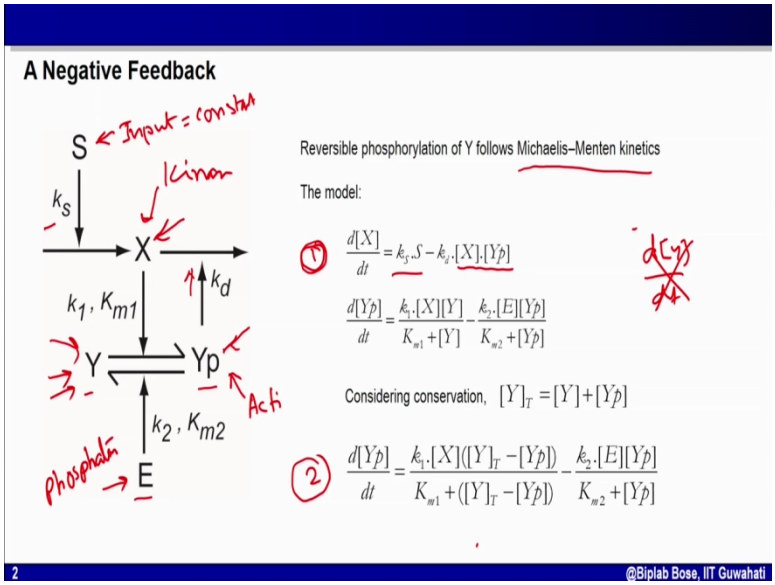

So if I clear my board a bit, what we have considered? We have considered conservation of Y in terms of Y and YP, so I am left with two ODEs. This is the first ODE1, that is called X and the second ODE for YP, I do not require a special ODE, separate ODE for DYPT because I do not require it, is because Y is dependent upon or express in terms of YP. So I have two ODEs, using these two ODE, I will try to analyse the behaviour of the system.

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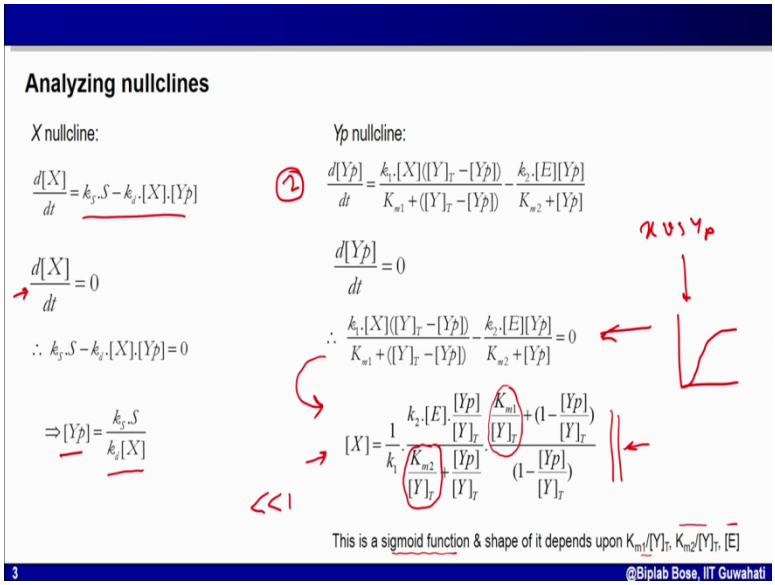

So let us first try the graphical method, we will try also numerical simulation, but first let us try the graphical method to understand how the system will behave. So if you remember to draw the graphical representation of the system, I have to plot phase plane plot and also draw the nullclines, so let us start analysing the nullclines first. So I will draw, I will try to understand the X nullcline first. So to get X nullcline, the deep ODE is DXDT is equal to KS into S minus KT into X into P YP, so for X nullcline, I have to consider DXDT equal to 0, so this is my consideration. If DXDT equal to 0, then this whole thing will become zero, so I get KS into X minus KD into X into YP equal to 0, so I will separate out X and YP on both side… on the sides of equal to sign, so that's what I do, I get YP equal to after rearrangement, YP equal to Ks into S divided by KD into X. It is essentially a hyperbolic equation.

Let us now look into YP nullcline. So to get the YP nullcline, start with the ODE, i.e. the second ODE representing the (Y…) YP, DYPDT. I will consider here, while DYPDT equal to 0 because I want to calculate the YP nullcline, so if I consider DYPDT equal to 0, then I put equal to 0 in the second ODE, so I get this formulation, K1 into X into YT minus YP divided by KM plus YT minus YP minus K2EYP divided by KM2 plus YP equal to 0. So again, I have to rearrange them to get YP in one side and X on the other side.

Now if you look into this relationship, in one module in the last week, where we have discussed this about reversible reaction following Michaelis–Menten kinetics, we have seen that to get the nullcline is not so easy to separate out the term, X and Y term and we have to play with trick to rearrange the term and what we can do, we can rearrange in this fashion. Xequal to I have a rearranged term here, where YP÷YT and other terms of KP, just like that. So this type of formulation we have done earlier and you can rearrange this whole thing algebraically and can get it.

Now, if you remember from that module, this X versus YP relation, X versus YP relation is actually sigmoidal. So this function of YP for X is actually a sigmoidal function and the shape of this sigmoid depends upon the values of KM1 by YT and KM2 by YT and E. If you remember, it's we have discussed earlier when KM1 by YT and KM2 by YT, they're much smaller than 1, then this sigmoid behaviour becomes very steep, very sharp. So, we will now draw these 2 nullclines in the phase plane X versus YP and try to see the behaviour.

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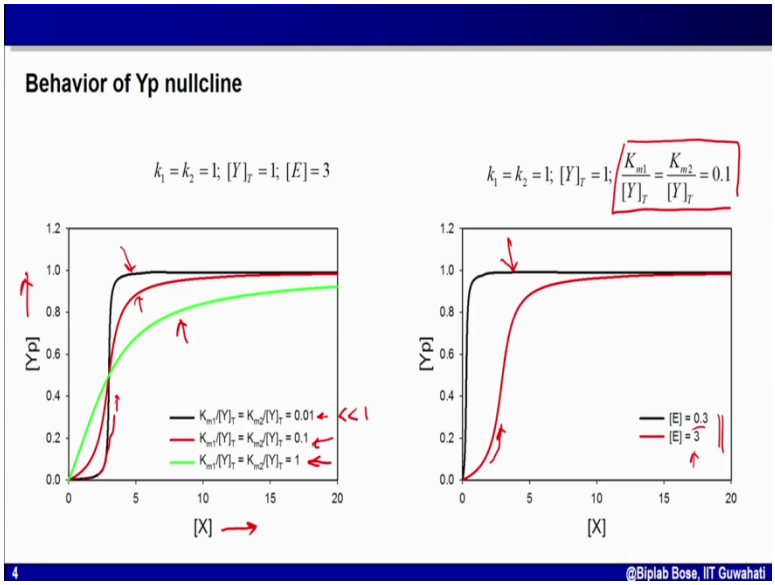

Let us start with the YP nullcline. As I said, YP nullcline will be a sigmoidal function from the YP nullcline that we have algebraically calculated. So I have X in the horizontal axis, YP in the vertical axis and I will have sigmoidal behaviour, sigmoidal function, for example when I take KM1 by YT, KM2 by YT equal to 0.01, this is much smaller than 1. Then we get a sharp sigmoid like this, this black line. When I reduce this value, KM1 by YT, i.e. the Michaelis–Menten constant divided by total amount of Y 2.1, we get still a sigmoidal curve, this red one but the sharpness, the steepness is bit narrowed down.

But when we consider, the Michaelis–Menten constant divided by total Y equal to 1 we get this green line which is hyperbolic. So this YP nullcline keeps on changing the shape from sigmoid to hyperbola, depending upon the ration of Michaelis–Menten constant for these two enzyme divided by the total amount of Y present. Another parameter will affect the shape of this nullcline, i.e. E, the total amount of phosphatase present. What I have drawn here, I have kept the ratio of Michaelis–Menten by the total amount of Y constant at 0.1 but what I have varied, I have varied the value of E here.

So when E is small, 0.3 I get this sharp curve, black one. Whereas, when E is bigger, 3, I get a smooth sigmoid behaviour and the curve shifts towards the right hand side. So you can see, the YP nullcline will have a sigmoidal to hyperbolic behaviour, depending upon the ratio of KMs

with the total amount of Y present in the system and also depending upon how much phosphatase you have in the system.

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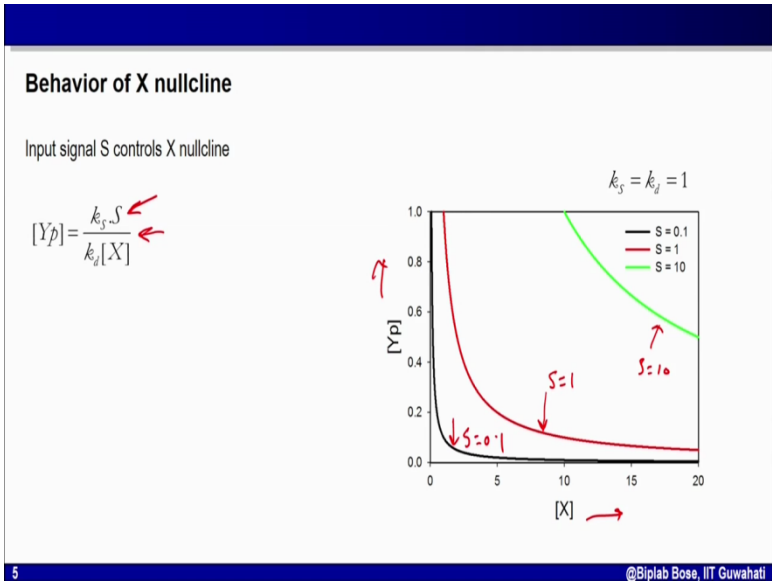

Now, let us look into the behaviour of X nullcline. The equation for X nullcline is this one, we have deduced that algebraically, you can see easily, it is a hyperbola and that hyperbola depend upon S, that input signal. Remember, in our system, we're considering S as a constant, it is not changing with time, but we can vary the value of S, so if I draw the nullclines in phase plane X versus YP, then as I change is, I can see the hyperbola keeps on shifting towards the right hand upper corner, so for this black one, S is equal to 0.1.

As I increase the signal, S from 0.1 to 1, I get this red curve, which is moving towards the upper right hand side, and when I increase this first for the ten times, to S equal to 10, I see it moving further right hand upper corner. So that means S, X nullcline is sensitive to S and remember Y YP nullcline was not sensitive to S, the input signal. Now we will jot down both these YP nullcline and X nullcline to identify the steady state. If you remember, intersection of two nullcline will give me the point of steady state.

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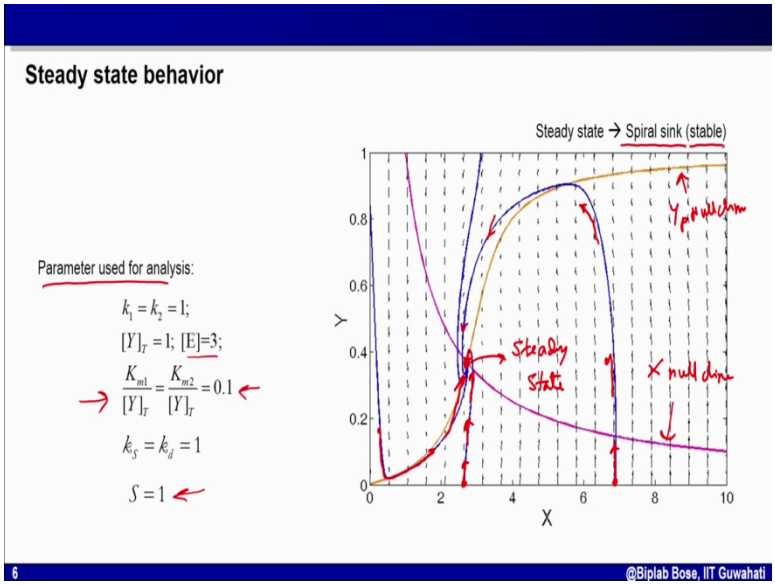

So let us look into that. I have drawn this phase plane plot using a MATLAB Tool and I have kept certain parameter values as constant, and those parameter values are given here. The ration of Michaelis–Menten constant to the total amount of Y is kept to value 0.1, and I have used the input signal S at equal to 1 and I have kept the phosphatase at 3. So this yellow line is my YP nullcline and this pink line is my X nullcline. These two nullclines are intersecting at this position, so this position is my steady state.

As you can see, the intersection can happen only at one position, so that means, in this system, I have only one steady state possible and this arrow here, which were not very clear in this picture are actually showing the phase portrait, so I can start from 1 point, I have shown one trajectories by this blue line, so if I start from here, the phase portrait tells me that the system will move along this trajectory and eventually collapse here. If I start from this point, at T equal to 0, then I will follow this trajectory and I will follow this line and eventually collapse at this steady state.

If I start somewhere here, then I will follow this trajectory and ultimately collapse at this steady state. So as you can see, wherever you're starting from the phase plane, you are eventually moving in a spiral path and collapsing at the steady state which is the intersection of YP and X nullclines, so that's why this steady state is a spiral, same steady state and obviously it is a stable one. So what I have tried to shown here in this phase portrait plot is that for this system, there will be only one steady state, and that steady state is a stable one, so it is the mono-stable system.

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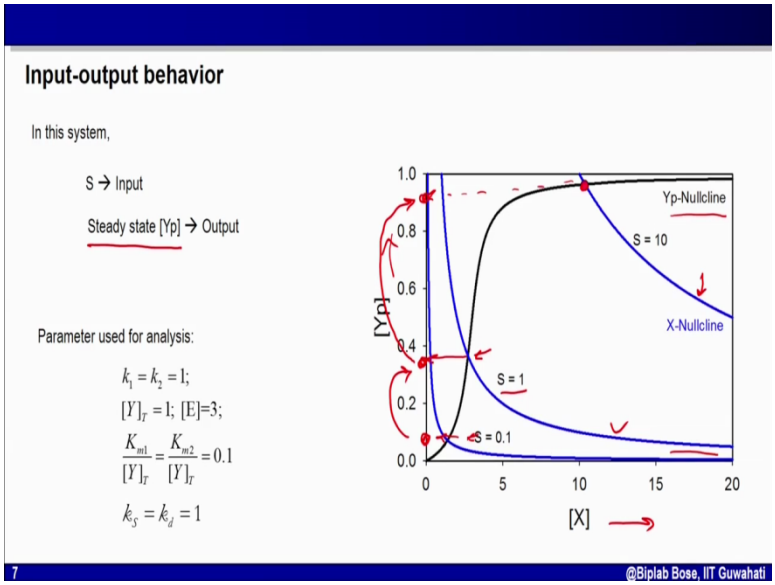

Let us further explore the behaviour of the steady state. Remember, the input to this circuit is S, the signal. What can be the output? I can consider the steady state value of YP as my output, so if this YP is, steady state value of YP is my output and S is my input signal, now I want to see how S as I change S, my output changes. So do that, I will again take the phase plane plot and try to understand. So what I had done here, I have drawn the phase plane plot with X in the horizontal axis, YP in the vertical axis, I have X nullcline here in the blue line and the YP nullcline is here in the black line and their point of intersection is shown here by this red dot.

Now this X nullcline that I have drawn is considering S equal to 0.1 so your input signal is 0.1 and at that time the YP value at steady state is this one, near 0.1. Now, if I change S 10 times, so S will become 0.1, so remember X nullcline is sensitive to S, YP nullcline is not sensitive to S so as I change S 10 times, I get a new nullcline, this one, for which S equal to 1 and the point of intersection is now this red dot, so this is where the steady state is and the steady state value for YP is now here, so initially the steady state value was here for S equal to .1, now the steady state value of YP is at this point. That means, my output has changed as I change the input S.

So now, in this S, my another tenfold change. So from 1, I will make it S equal to 10. So now the X nullcline has shifted here, the blue line and the intersection point is here by as shown by that red dot. Now if I draw a horizontal line, then this is my steady state value of YP, when S equal to 10 so that means, when I have changed S from 0.1 to 1 ten times, I have changed from here to

here but when I change from 1 to 10, another tenfold change in signal, I have a rapid change in YP with very large value. So this shows the input, output relation. Remember in the… as the phase plane plot is showing, every time I have only one stable steady state and the position of the steady state is changing as I changing the input signal.

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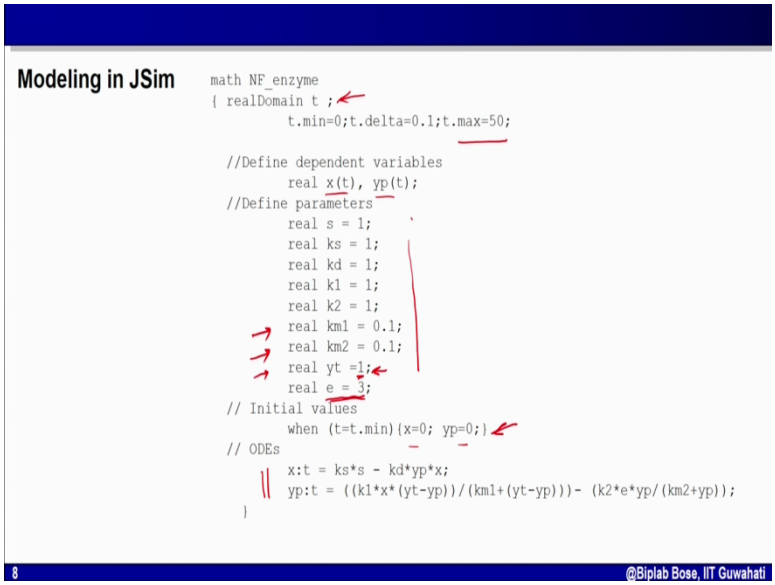

We'll explore this one further, but not using phase plane plot anymore, we'll do it using JSim. So I will advise you to write this code in JSim as shown here in the slide and then try to execute the way we're analysing the system here. So I have shown the code, as usual, this code have multiple segment, the first one is obviously degrading the time, how long you want to simulate, I want to simulate it for 50 time steps. I have two variables here, dependent variable here, YP and X and then I have all the parameters. Remember these parameters KM1, KM2 and YT will be critical, we can keep YT constant at 1, we will not change it much, rather we will change KM1 and KM2, so the ratio of KM1 to YT, KM2 and YT will change.

So we'll keep YT constant, we'll change the Michaelis–Menten constant in our simulation and also E, the amount of phosphatase will vary in our may vary in the simulation and see it. Now obviously we have to declare the initial position at T equal to 0, I have considered X equal to 0 and YP equal to 0 and the rest of these two are two ODEs that we have written earlier.

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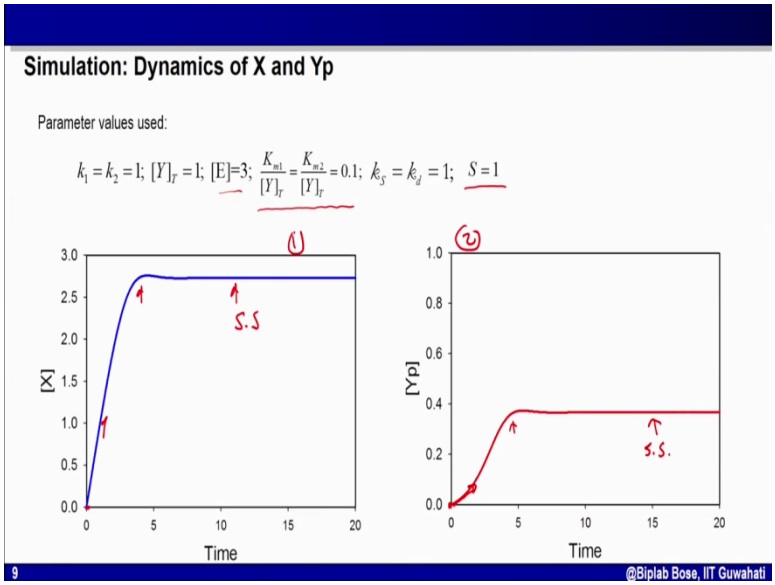

Now, if I simulate the system, I can get this type of result, so I have taken certain parameter value, the critical parameter, the ratio of Michaelis–Menten constant and total amount of Y, the substrate is kept at 0.1, so it is lesser than 1 so we're supposed to get a YP, which is the YP nullcline which is sigmoidal. I have kept E at 3 to the amount of enzyme as 3 and phosphatase as 3 and input signal I have taken S equal to 1. If I use this parameter value and simulate using JSim, I get these two type of dynamics for X and YP. As I am starting with 00, at time 0, X was 0, X increases.

I have a slight because if you remember, the phase plane plot has some spiral tendency, so the ampere and then eventually it reaches the steady state. Similarly, YP here start from 00, as I am starting at T equal to 0, YP I have considered equal to 0 and it moves up with a slight ampere and then eventually I reach a steady state. So here, I can easily see that for both XP YP and X, they're reaching steady state after some time, so that means this is a steady state, a stable one, that's why the system is moving there and it is expected also because the system is mono-stable.

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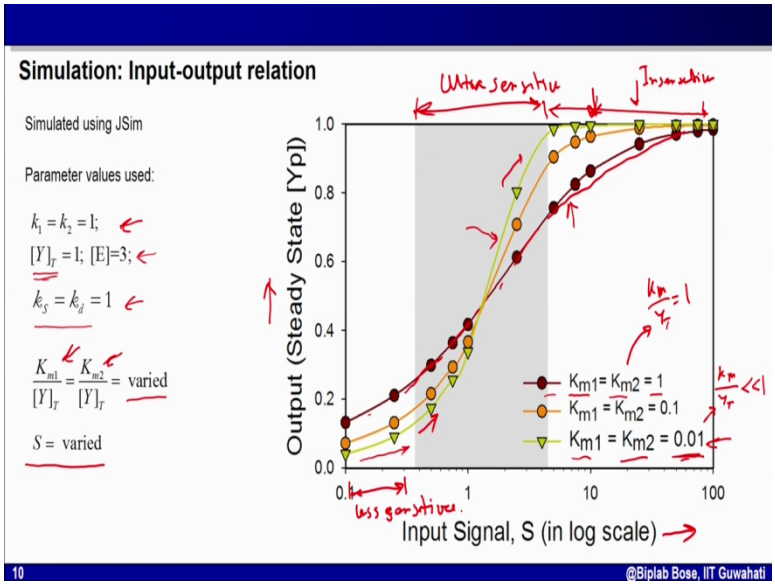

Now, let us look into the input, output relation using this JSim model. Previously I was trying to understand when S is changed, how YP steady state value, the output will change and I was trying to use the phase plane plot to plot, to understand. Here, I have done the JSim simulation to understand the similar thing. What I have done, I have kept parameter K1, K2 at one fixed, I have kept total amount of Y, T, Y as 1, fixed and I have kept the phosphatase amount fixed at 3 and the degradation and K's rate also kept fixed at 1, what I have varied is that, I have varied the ratio of Michaelis–Menten constant by YT.

Now if I keep YT constant at 1, then essentially I have varied this KM2 and KM1 and I have varied this S at I have taken different values of S from 0.1 to 10, 50, something like that. So as I vary S, my input signal I measure what is the steady state value of YP, the output using JSim simulation and then I have made this plot as shown here, so in this horizontal axis, I have plotted S, the input signal and I have plotted that in log scale because the figure looks better when you plot in log scale. In the vertical axis, I have plotted the output, i.e. the steady state of YP.

How do I get the steady state of YP? I have simulated using JSim for 50 time step, by 50 time step, the system which is steady state and I have taken the last time steps value of YP, i.e. my steady state value of YP and then I have plotted. Considered this greenish data, where KM1 and KM2 are very small with respect to 1. So that means in this case, the Michaelis–Menten constant

by YT is much smaller than 1 because YT is 1. So in that case, I have a sigmoidal behaviour. I have a sigmoidal behaviour with a saturation here.

Whereas, if you follow this brownish one, where KM1 and KM2 are taken as 1, that means in this case, the Michaelis–Menten constant by YTequal to 1 because YT is 1, so in that case the data is this brownish line. So as you can see here, for this particular case, where the ratio of Michaelis–Menten constant and the total Y is 1, as I change is the output, the steady state value of YP changes almost linear, it has slight sigmoidal behaviour. Whereas, when I change this ratio and make it much smaller than 1 at 0.01 behaviour, the input output behaviour becomes sigmoidal.

Now this change from almost linear to a sigmoidal input output relation has certain effect. For example, when my ratio of Michaelis–Menten constant to total Y is 1 or close to 1, I get this linear input output behaviour, this almost linear input output behaviour. That means, as I change S from lower value to higher value, my output also changes almost linear, so it is working like a rheostat, I am slowly changing the switch and slowly my output is also changing, but if my ratio of Michaelis–Menten constant to Y, total Y is smaller than 1, i.e. this green line, then I get this sharp sigmoidal behaviour and as you can see in this shaded region, in this region, if you vary S, the output, i.e. YP will change very drastically, sharp so this region is ultrasensitive.

Whereas, the higher value of S, YP is remaining almost constant at 1, so that means this region is insensitive. The similar insensitivity is at the lower end also, here also it is less sensitive. That means, if I have this sigmoidal input output relation, then in the middle region of signal, that is the input signal, I will have ultrasensitive behaviour but at the extreme end. At higher values of S and the lower values of S, the system will not be sensitive and it will not be able to differentiate between different signals.

Whereas if I have almost linear input output relation which is possible when Michaelis–Menten constant to the total of Y ratio is close to 1, then throughout the values of S, the input signal I will have defined values of steady state for YP, that means my output will have defined value, that means the system will be able to differentiate different S. So the same circuit, depending upon how much YT you have, how much substrate you have can behave like a ultrasensitive switch with ultrasensitivity in the middle region and no sensitivity at the end and as you change

total amount of Y, it can become a linear rheostat while it is sensitive to the input signal across all values of input signal.

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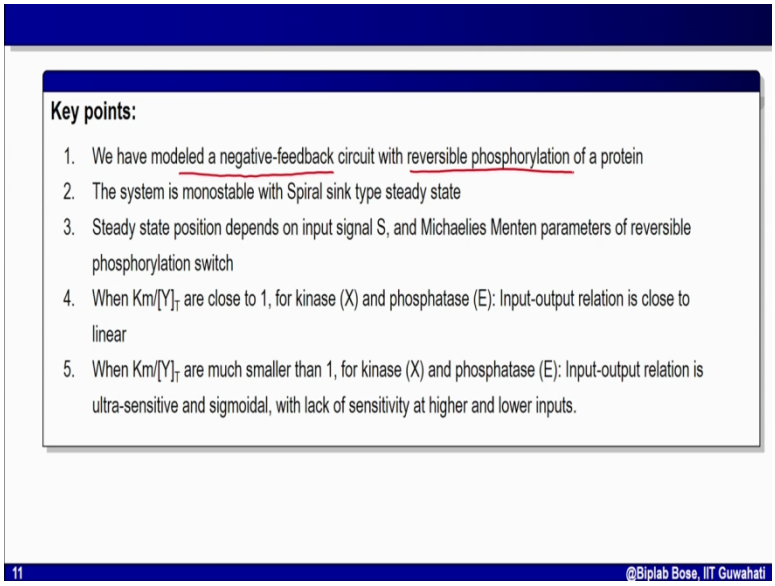

So let us jot down what we have learned in this module, we have module a negative feedback circuit with reversible phosphorylation of a protein. We have seen that this system is monostable with a spiral sink type study state. We have also seen that the steady state position depends upon input signal S, because its nullcline depend upon S and the Michaelis–Menten parameters of the reversible phosphorylation. We have considered the reversible phosphorylation of Y is following Michaelis–Menten kinetics and this Michaelis–Menten parameter are very crucial for the steady state position of the system.

When KM KM divided by YT, i.e. the ratio of Michaelis–Menten constant for both the enzymes and the total substrate is close to 1 for both, X and E that is the Kinase and phosphatase. The input output relation is almost linear throughout different values of input, but when these ratios for both, Kinase and phosphatase are smaller than 1. For example 0.1, 0.01, 0.001, then the input, output relation is ultrasensitive in the middle region, it is sigmoidal and it does not have sensitivity at higher and lower inputs. That's all for this module, thank you for watching.