Computational Systems Biology
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Lecture - 58
Flux Balance Analysis

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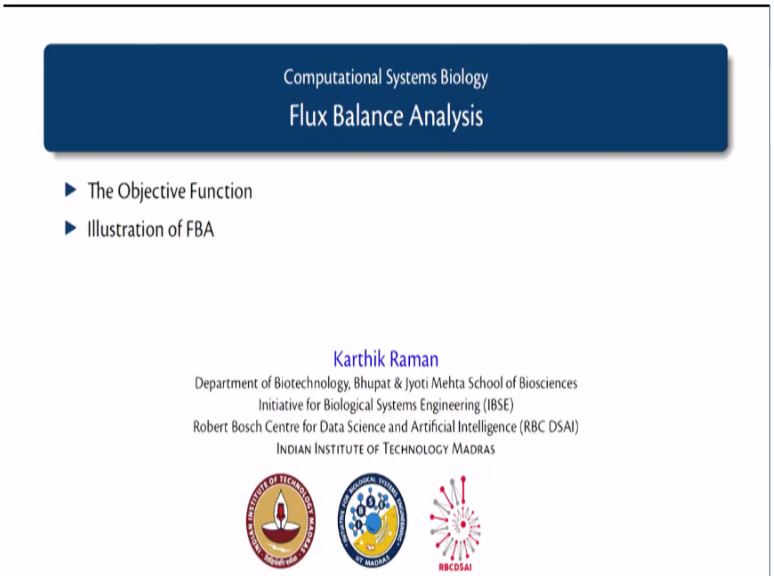

In today's video, we will continue with flux balance analysis. And I will start talking to you about the objective function which is a very important aspect of any FBA problem or any constraint based optimization problem in general. And we will look at a very simple illustration of flux balance analysis for a Toy system. So welcome back. Let us look at the most important aspect of FBA or what is potentially the most debated aspect of flux balance analysis.

How do you pick this objective function? In your school problem of chairs and tables objective functions are very clear, you wanted maximize the profit of the industry or factory or whatever, right. In this case, how do you what is the objective function that your pick.

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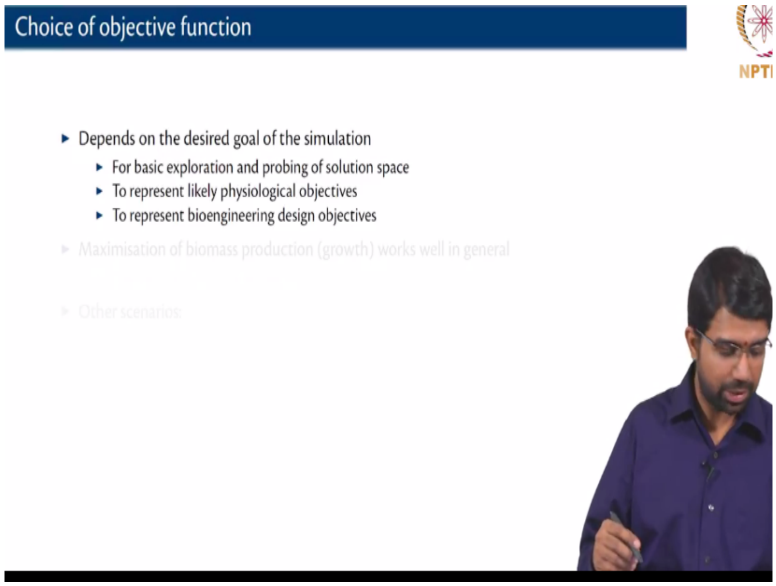

And this is where I want you to have a more fundamental understanding of FBA and let us see what that is. One obvious way to pick the objective function is based on the desired goal of the simulation maybe for basic explanation and probing of the solution space or to represent likely physiological objectives or to represent bioengineering design objective. Let us just look at the last two, that you can imagine what exploring the solution space is right you just try different objectives functions to see what kind of configurations are admissible in the cell.

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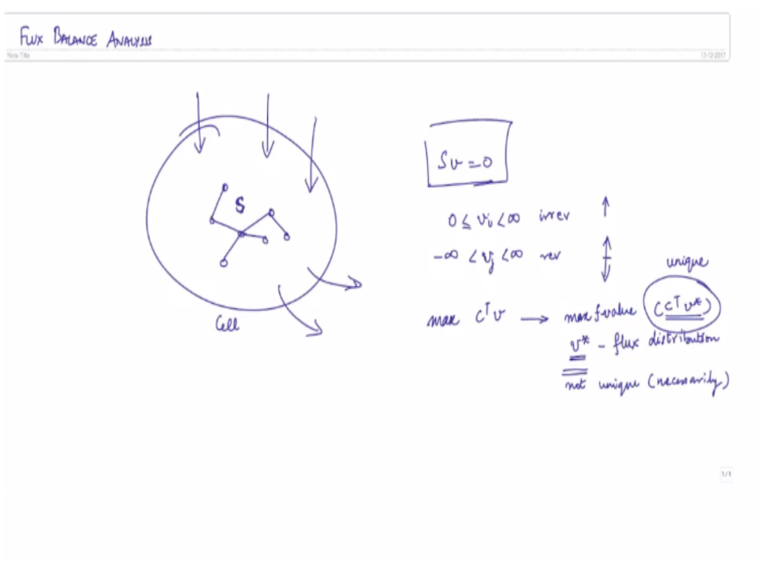

But let us remind ourselves what we have been doing. You have a cell which takes in several metabolites, gives out some metabolites and there is a complex metabolic network inside of it. This is metabolic network we essentially represent as S your stoichiometric matrix, and what you do it Sv=0 this is not-- you cannot violate this constraint, right. This is a fundamental constraint. Why? It arises from Stoichiometry.

You can only produce one mole of fructose from one mole of glucose, no other choice right. And this is thermodynamics, irreversible and reversible reactions and let us actually not a infinity but typically a some large value, right we know that this has to be only in this direction and this can be in either direction-reaction, right the values. **"Professor – student conversation starts"** (()) (02:52) Yes, you can have it, okay this variation can less than equal to infinity, right so it can be 0 of course. **"Professor – student conversation ends"**

So this is what we have so far. And we say we cannot solve the system unambiguously, right one unique solution. So to pick one solution we said let us now impose this objective function maximize C transverse v. What does this give you? It gives you two things when you solve; you will get the max value or what we call the max function value, this is basically C transverse into the v best and the v best which is basically your flux distribution.

What can you now tell me about the uniqueness of v* and C transverse v*? Have you study linear programming before; you may need to call in some logic of linear programming here. It is interesting to note that this is unique whereas this is not unique. This has major implications for everything we do with FBA and I think it is easy to understand the math of FBA but as always in this course what we want to understand is, you know the ramifications that any mathematical technique that we have has for modelling any given system.

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So let us look at a simple illustration of FBA. How does FBA actually work?

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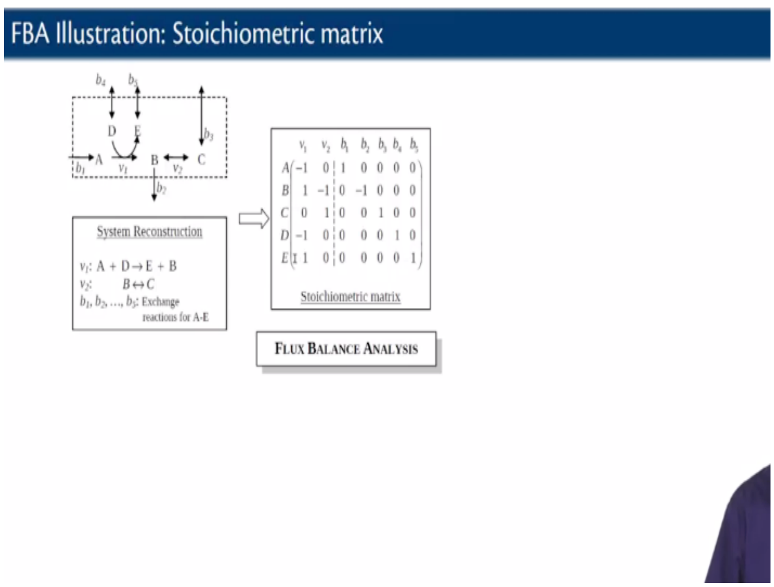

So you start with a system, so you reconstruct a system. How do you reconstruct the system? Go back a couple of modules pathway database, literature all the hard work to essentially assemble a long list of biochemical reactions that occur within equally or organism of your interest. Given this is your system, now how do you set up the-- what is the next step that you would do here for flux balance analysis?

So dx/dt=S*v. What is your stoichiometric matrix? What is going to be the size of your stoichiometric matrix? **"Professor – student conversation starts"** m cross n. What is m and what is r? m is 5. m is 5. This is one reaction, right this basically A+D giving E+B this is how you normally write in biochemistry, right ATP becoming ADP or something like that, so that makes it 7. **"Professor – student conversation ends"**

So you have to internal reactions v1 and v2 and you have a bunch of exchange reactions B2 to B5. Fair enough. So the stoichiometric matrix will therefore be 5 cross 7 and it will look like this. Fair enough. So now you can see the balance nicely v1 is b1 which really make sense, right —sorry, you will find that v1 yeah, –v1+ b1=0 balance for A. And B is involved in three reactions.

So we v1-v2-b2=0, whatever flow comes split across these two, right. This v2 in turn depend on b3 this v1 is turn depend on something else and so on. You will find all of this, right. But overall because v1 also is linked to b4, v1 is also linked to b5 and things like that. So one thing here it is a little there is a bit of a catch here, I cannot have reversible arrows here because if I say this is -5 I have to commit to one direction in the first place, right.

So here its reversible arrow is shown for the sake of highlighting that it is a reversible reaction but in essence when you start even writing a stoichiometric matrix you have to understand that b4 is in a particular direction v5 is in a particular direction, right. So what is b5 here? What is-- this is the reaction right, it is E on the right hand side alone which is basically to say it is something like **"Professor – student conversation starts"** Sir, why (()) (08:44). No, no so what I am saying is. **"Professor – student conversation ends"**

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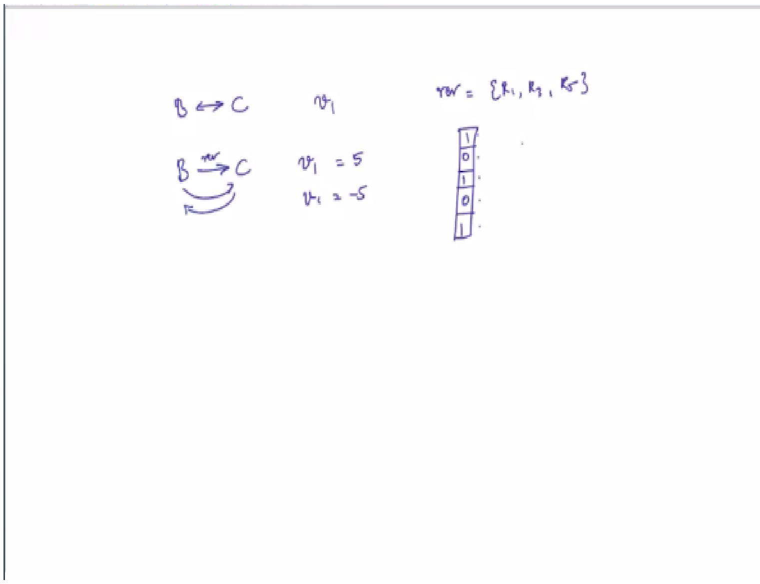

What was the reactions, let us take the same reaction? I have a reaction like this. If I say v1 what is it, is it the flux of the forward reaction or the backward reaction? So it makes sense if I say that this is a reversible reaction with v1 as the flux, right. So now if v1 is 5 it means B is going to C, if v1 is -5 it means C is going to B. **"Professor – student conversation starts"** ((')) (09:32) it does not tell you ((')) (09:37). Yeah so invariably in all these reconstructions you keep the reversibility information separate. **"Professor – student conversation ends"**

So you say reversibility r1, r3, r5 something like that, what are all the list of reversible reactions or typically you keep a Boolean vector it says 10101, right this means r1 is reversible, r2 is not, r3 is reversible, r4 is not, r5 is reversible, right. So when we do the lab sessions you will get familiar with this, okay.

Because to even fill up this column I need to know what the reaction is; with reversible I still need to know what is the forward or the backward reaction, right. So you can assume that all reactions are in the forward direction and if you get a negative flux it is in the reverse direction. But what do I even be in by forward direction, is this the forward or is this the backward, right. So I need to commit to one arrow direction. So assuming that we have this.

So here in fact you should-- this should have been a -1, right. E should be going out for example, otherwise you just have v1+b5=0, so they are going to be of opposite signs for sure, right.

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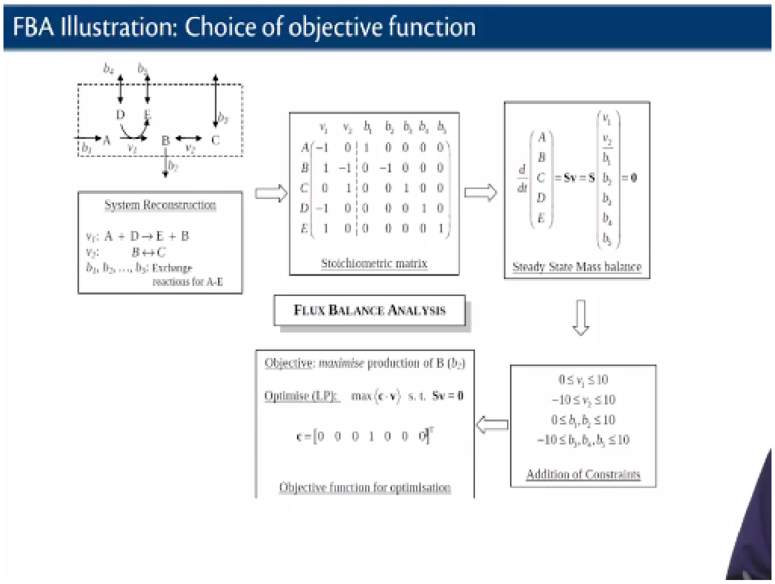

So now you set up this equation dx/dt=Sv and you say Sv=0. Now you have to solve the system of equations which basically reads –v1+b1=0, v1-v2-b2=0; v2+b3=0; -v1+b4=0 and so on. I am essentially multiplying this with this matrix multiplication. And note that this is just a partition. I am trying to show up, it might seem like a divided by something but it is a partition shows that there are some internal fluxes and there are some exchange fluxes in the same vector, okay.

So the next step you put in other constraints from your knowledge of biology or whatever you have put some toy constraints, I say 0<=v1<=10 -10<=v2<=10 so typically for any reversible reaction I would have the constraint in both directions. But for the Irreversible reaction it is only in one direction. What is the next step? I have assembled the constraints, yes I have to do the optimization.

So I say my product of interest is B. So I maximize the secretion of B outside the cell. How much B is coming out of this cell, right which means maximize b2 which means maximize 001, 0001 000* this vector, right. I am just putting it back into the Canonical form, right.

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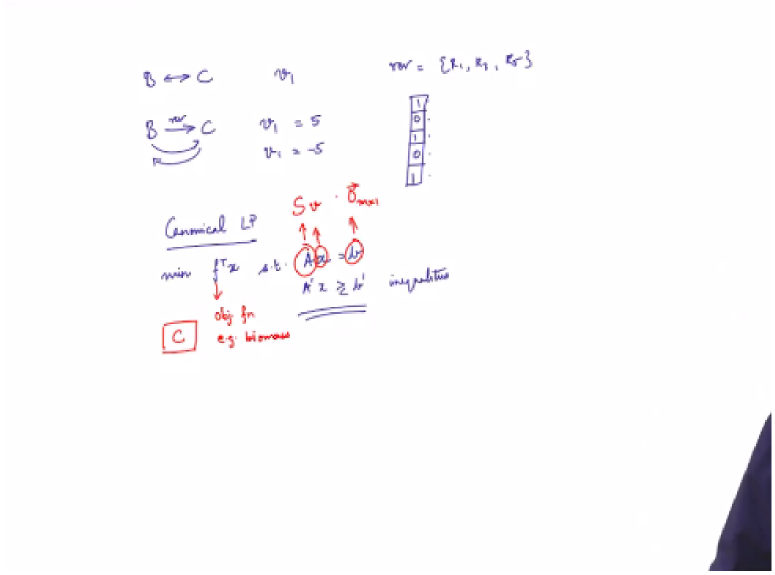

So what is the what is the Canonical LP? You know, in Canonical quadratic equation x squared+ bx + c=0, right. So the Canonical LP is minimize, f transpose x such that you can even have inequalities. This is an equality constraint; you can have inequality constraints as well. This will be your proper linear program.

Now if you map it back to our system. What is A? What is B? What is x? What is f? Your objective function like Biomass which is basically your C, this is the name we gave and it is useful to know this variable names as well because these are the same things that are used in the MATLAB toolbox for solving flux balance analysis problems. What is A, the equivalent of A? The stoichiometric matrix equivalent of B?

“Professor – student conversation starts” “0 0, 0 vector. What is the size of the 0 vector? (()) (15:16) m cross 1, very good. What is x? Is this clear? If you want me to repeat it I will repeat it, because this can be a little confusing, and it is important to fixate these ideas before we go any further, which is why I dropped the discussion on objective functions and jumped back to an example trivial toy FBA problem. **“Professor – student conversation ends”**

So basically start with a system, we extract the stoichiometric matrix out then we write this mass balance equation dx/dt=Sv, this is the mass balance equation. At steady state this becomes 0, right. And then we had other constraint based on a knowledge of biology, capacity whatever and then set up the optimization problem because this is going to close infinitely many solutions still, right.

Then we set up this optimization problem and we compute the best we-- like which will give you the max C transpose v. This is C.v right just a dot product between these two vectors such that Sv=0 and you see that my 0 is also bold meaning that is also a vector, right. This should give you solution.

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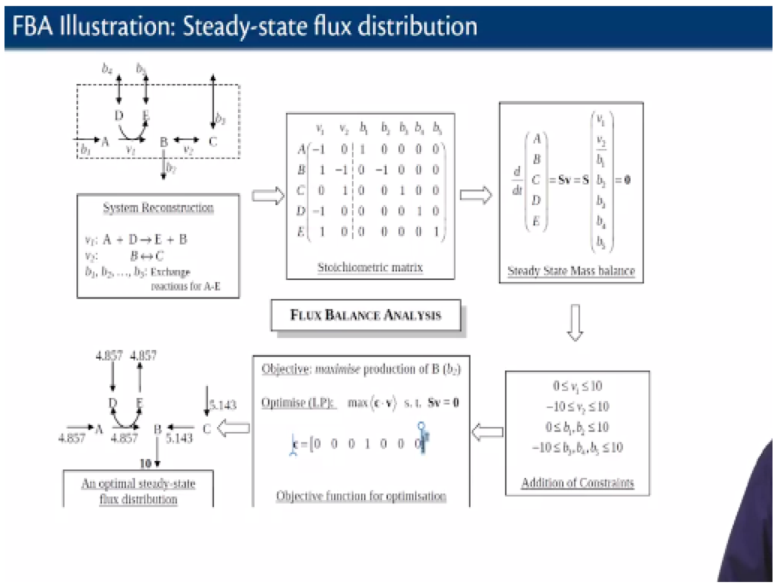

So one possible solution set is this; so you have 4.857 of A coming in 4.8 of A coming in; 5.143 of B coming in giving 10. But if you see this is just one arbitrary solution for a many equivalent solutions you might be able to come up with; you could just have 0000 10 10 10. **“Professor – student conversation starts”** What is C? This C? It is the objective function. I said maximize. **“Professor – student conversation ends”**

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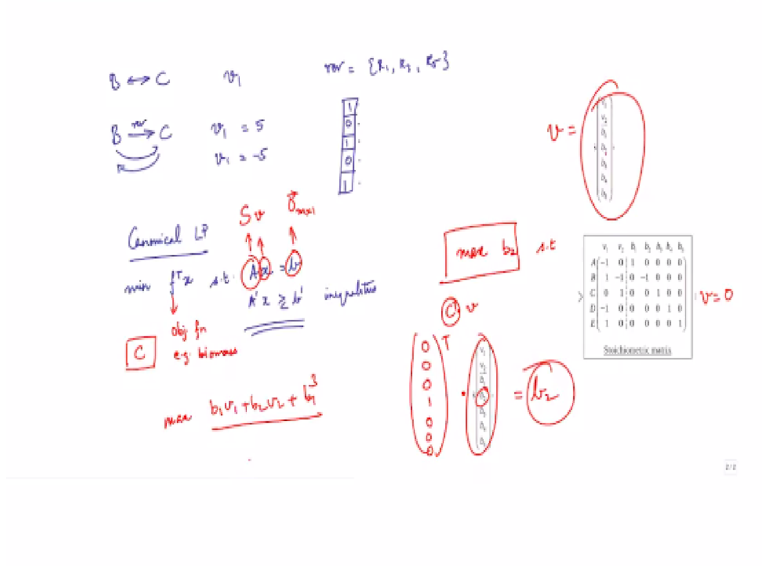

So I said max b2 such that S*v=0, right. And what is v? v is nothing but – this. So now this is your LP problem, right this is your LP objective function. So this has to be written as some C.v. So what is C vector that will multiply this to give you b2 it will be 0001000. So now C transpose this transpose into this vector, good. It just seems like a little concrete if you are not familiar with programming and linear algebra but essentially this is what it is, right.

I set this up as my objective function of my choice and I have to give my objective function as some linear combination of all these variables and that linear combination is 0 of all variables except b2. **"Professor – student conversation starts"** (()) (19:06) As some linear combination of V which is then it will remain linear programming. I can come up, you can say you are very well within your rights to say maximize b1 v1+b2 v2, this is quadratic; +b1 cube non-linear, right.

You can go in for any objective function constraints, right; your constraints are still the same old Sv=0 linear constraints. Of course this is therefore no longer linear programming. **"Professor – student conversation ends"** We will see examples of these, there are very interesting formulations that are non-linear programming based; the nonlinear optimization based which will give you interesting results.

So let us get back here. We see that they are all, there are different possible solutions that you can have, right. So clearly 0000 10-10-10 is one solution. A simply 5555 is another solution, right; of course this will be 10. And this is max out at 10 because that is the constant that you have yourself in posterior, you say 0<=b1b2 <=10. So now I want to draw your attention back to the fact that the solution is unique or the objective function value is unique whereas the possible flux distributions are many.

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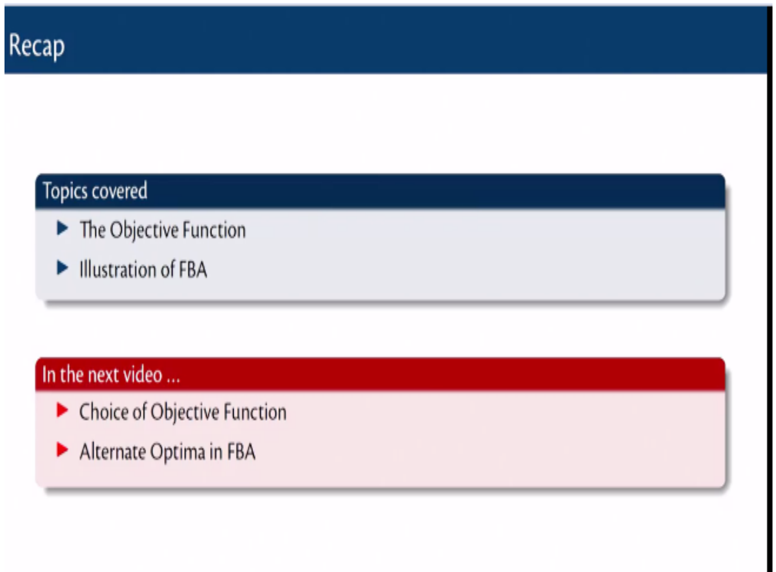

So I hope in this video you got a nice outline of FBA. We first looked at the objective function for flux balance analysis and then we also saw a simple toy illustration of flux balance analysis. In the next video, we will dew deeper and look at some very practical aspects namely what are the choice of objective function, how do you pick the objective function, we did have a bit of a discussion today. And more importantly we will discuss the fact that there are Alternate Optima in Flux Balance Analysis and what implications it has for modelling.