Computational Systems Biology
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Lecture – 65
Understanding FBA

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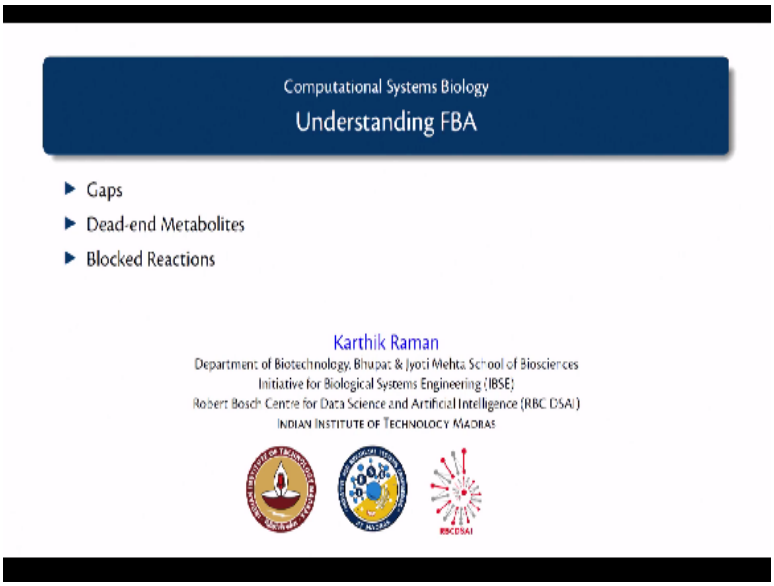

So in this class, we will delve deep into FBA and get a more practical understanding of what happens when you perform a flux balance analysis and we look at the concept of gaps, dead-end metabolites and what are known as blocked reactions.

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Welcome back. So let us spend some time understanding FBA little more. I think we have understood flux analysis through some lab sessions and some basic methods including FBA, MOMA, ROOM and so on but let us just revisit some of these concepts to fixate them better. There are a few points that deserve special mention.

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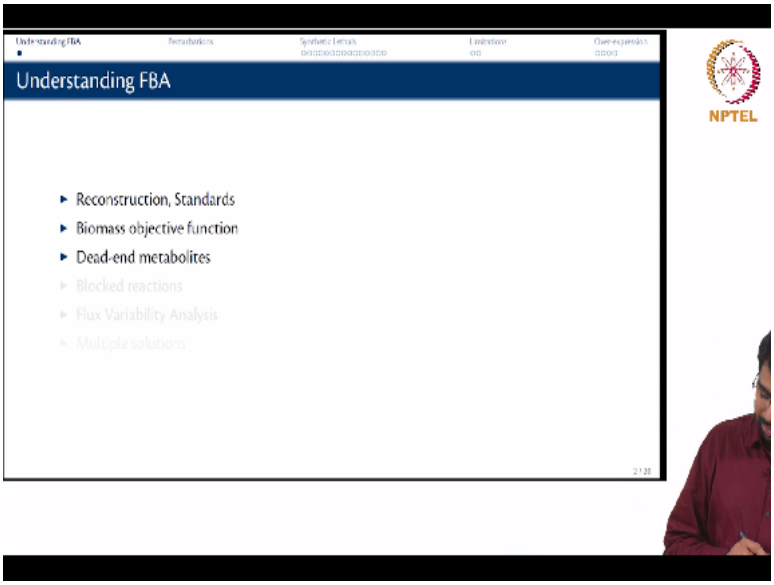

First thing is about reconstruction, right. We did use an E. coli core model in our earlier class and so on and there are other models as well.

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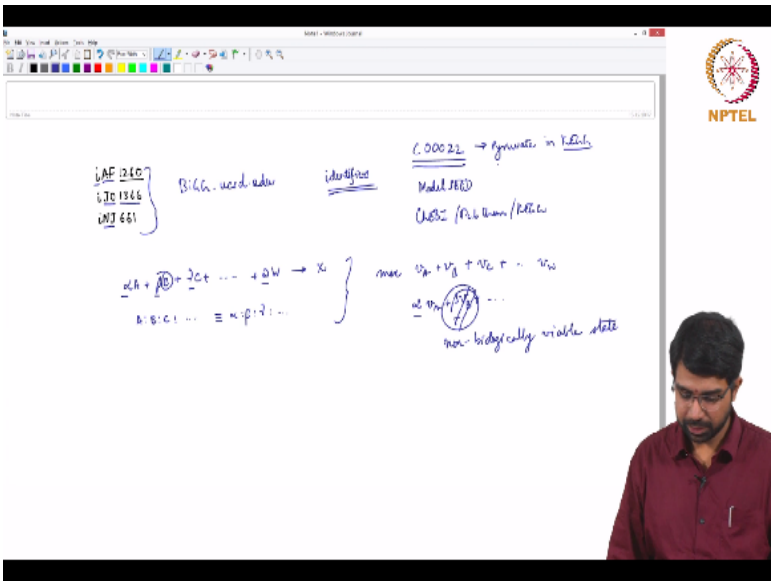

So typically a model is written like this or this is like a small nomenclature. This is the number of genes. This is the, these are initials of the first author of the paper that is involved. So this is Adam Feist, this is (()) (01:25) this is Neema Jamshidi and this just says that (()) (01:31) model. So this is the nomenclature that is normally used. So this means there are 1366 genes in this model.

It does not talk about reactions or metabolites or any of those, right and a very nice database that has most of these is called BIGG. So you can, I think, accesses from BIGG.ucsd.edu. So that is one important aspect which is, are these consistent like intraoperable and so on. How are the constraints mention? How are the metabolites referred to? One very important aspect is that you need to have sensible, identifiable identifiers for everything, right.

Suppose I say C00022, right. You just Google for this or you just, you know, look this up you will find that this stands for pyruvate in the KEGG Ligand and database, right. But this is important, right. So you need to have some intraoperable names, right. There is also another good database or reconstruction resource called model C which will use a bunch of nomenclature.

There is no problem with using multiple nomenclatures but the thing is they should all link back to some universal standard like ChEBI or PubChem or KEGG and so on. So the reconstructions

have to be interpretable in some sense, right. This is particularly important if you want to look at more complex models or community models where you want to integrate 2 organisms, make them talk to one another and things like that.

You cannot have GLC standing for glucoses in one and GLU standing for glucose in the other and so on. This sounds like a really minor issue but this becomes a big deal breaker when you are trying to merge models or study something across models or do anything on a large scale. So there are some other issues that are intimately related with reconstruction. Let us see what they are?

The first is the biomass objective function. So how do you decide the biomass objective function or how do you decide the objective functions in the first place. We did talk about a few ideas yesterday but there are, you know, several papers that have discussed how the optimality changes or how the predictions change based on the objective function and so on. So the chaise of objective function becomes supercritical when we are talking about FBA, right.

So FBA relies on that objective function to pick one flux distribution in the end. So this is quite important and usually this happens by sort of estimating the fractional composition of various things. You know what are the carbohydrates. What is the amount of glucose in the cell mass and so on, right. So we did see a longish reaction earlier in an earlier class where we saw that we have, you know, x moles of ATP+Y moles of erythrose for phosphate + something + something + something, giving something else.

So it is a fictitious reaction that is set up to basically capture growth and to recap, this could be your biomass equation. This means that you need to have ABC in this ratio, right. This is not the same as maximizing VA+VB+VC+VW or even alpha VA+beta VB+ (()) (05:44), right. This will attach some more importances to individual fluxes but you can still have one of these as 0 and still have the same function value but this sort of represents a non-biologically viable state, right. If I do not have like B, I cannot produce biomass actually.

"Professor - student conversation starts" Why cannot we multiply it and (()) (06:16) Why

cannot we multiply it and see the maximum. I did not understand. What do you mean multiply? (()) (06:20) that is a non-linear function, why would you want to… You can have a simple linear function. **"Professor - student conversation ends."**

In the next very interesting aspect of flux balance models is, there are dead-end metabolites. There are 2 kinds of dead-end metabolites. There are 2 things you need to understand.

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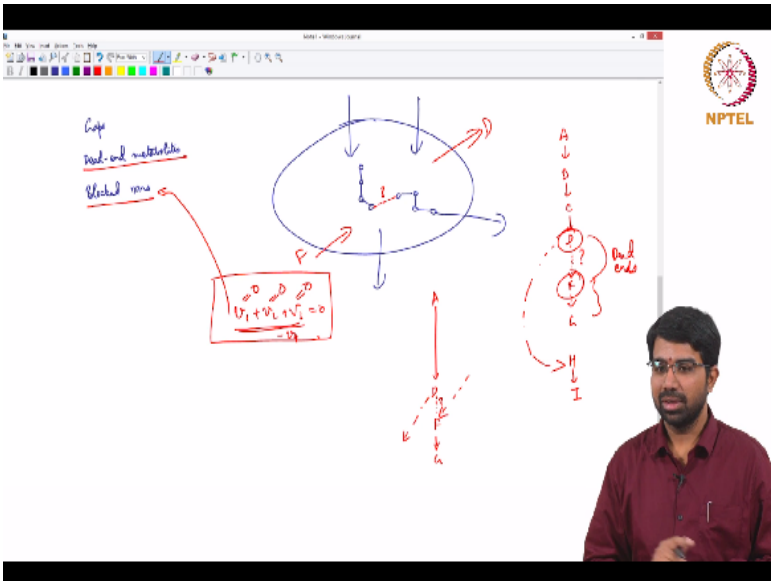

You need to worry about gaps and blocked reactions. Gaps, dead-end metabolites and blocked reactions. So gaps are easy to imagine. Let us say you have a cell and you have metabolic network of this sort. This could be a gap. You know that A goes to B, B goes to C, C goes to D and then you know that F goes to G but you do not know how this happens. This has major consequences.

The first thing is, do you put it in the model or you do not, right? Because I know that reaction is there, I'm inclined to put it into the model but it is not going to help by FBA cost. Why? Because D would be a, D and F would be what we call dead-ends. So these are metabolites that are only produced but not consumed or only consumed and not produced. Meaning how will they occur in a stoichiometric matrix.

They will have only 1 element in that, in that row. The row corresponding to that matrix. Well it

could have more than 1 element too but all of the same sign. What is the problem if all, all the coefficients are of the same sign? When you multiply that with the vector, you just going to basically say that V1+V2+V3=0 and if all of these potentially irreversible, meaning they can only be in the positive direction, then each of these are 0 independently which makes them a blocked reaction.

So blocked reactions are those that cannot carry flux in either direction under a given condition. You can also talk about, you know, unconditionally blocked reactions. They cannot carry reactions under, I mean flux under any condition. Can you think of, can you understand or appreciate the difference between the 2. Conditionally blocked and like totally blocked. So conditionally blocked reactions cannot carry, you know, the blocked reactions cannot carry a flux in any direction, right.

And there are conditionally blocked reactions that may not carry flux under some conditions but there are other reactions that will not carry a flux under any condition. What could be the difference between the 2? It could be environment specific. So when you turn off glucose, some glucose-based reactions may not carry flux. They will be blocked. In a fructose or in acetate environments, some glucose reactions will be blocked.

Whereas, you know, in a fructose and acetate environment, those reactions may come back alive. Whereas there will be some reactions, potentially like your gaps and so on, they are just going to be blocked. If I have, so if I then had some H giving I and may be even some, some other pathway from D to H, this F giving G is going to be forever blocked, right. Because I have no idea how to produce F, I have no idea how to use G as far as my network is concerned. Yes, so gap is when you, you do not know the intervening biochemistry. I do not know how D becomes F.

"Professor - student conversation starts" So D and F are kind of metabolites? Potentially. I mean if there was another way to produce F, so, see this is, there could be a problematic gap and a non-problematic gap so to speak, right. So if you have some A to D and I said F to G is my gap. As long as I have another set of reactions for D and another set of reactions producing F, they

will not be blocked but this might still be a gap. This may be an alternate pathway which has not yet been uncovered, right. **"Professor - student conversation ends."**

So these become practical issues when you start modelling. So the first thing you want to do with a given metabolic model is open it up and see how many reactions are blocked. Are there any dead-end metabolites? And the key reason why these, there are dead-end metabolites arises is that FBA does not admit any accumulation, right. All you have to do is just keep producing this and have D accumulate but no accumulation happen under steady-state conditions, right. So, therefore, it is basically a consequence of this identity, this equation, right.

If you have V1+V2+V3 is 0, you have this issue. They will all have to go to 0. How would you fix this? What is the simple way to fix this? You have an equation like this that is giving you trouble, a simple way to fix it would be to add -V4 meaning, what does it mean? This is mathematically what does it mean model-wise? Some exchange flux, right. You just now, you have this D giving you trouble, take D out. You have F giving you trouble, give some F. This should take care of it. So these are sources and sync reactions, sync and demand reactions.

"Professor - student conversation starts" (()) (13:16) you know that there is a path between D and F but we do not know how it occurs, is it like that? Yes. So if you had, if you had a scenario like this where D was anyway going through some other, to some other fate later on and F is already been produced, you would not even be worried about whether I can make D from it.

There could be several such reactions, right but in the case where it looks like this and you never going to know what is D and what is F, right. I am writing A B C D, so you think there is an E in between, right but there are some chemically related metabolites which, which seemed to not have a connection and you think there may be a connection. **"Professor - student conversation ends."**

This is very interesting. So there is a lot of recent research about underground metabolism and so on. This is underground metabolism. It exists but you are not seeing it yet. So you now understand the concept of a gap, a blocked reaction and dead-end metabolites and also how to

put a Band-Aid fix for dead-end metabolites.

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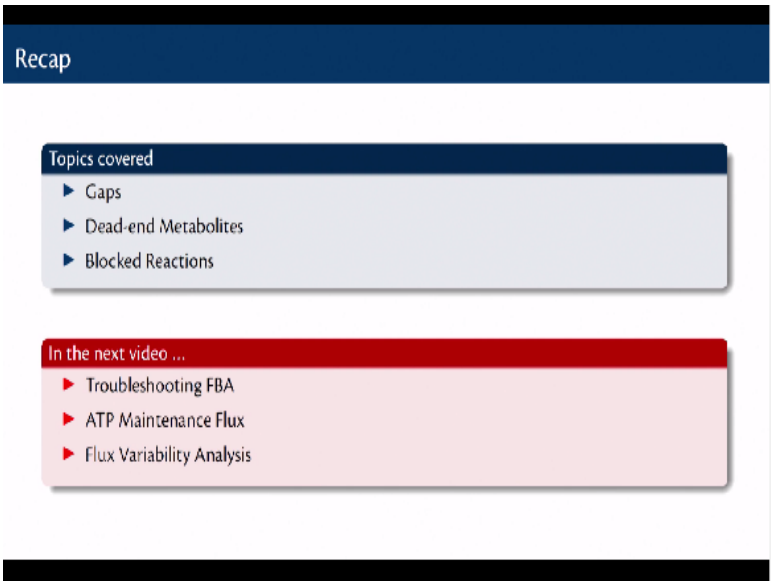

So I hope in this video, you got a good overview of what are the different types of gaps that can exist in metabolic networks, either the knowledge gaps or, you know, potential model mistakes and so on as well as the concept of dead-end metabolites and blocked reactions. In the next video, we will look at some more concepts related to troubleshooting FBA wherein we will also look at the ATP maintenance flux and this very interesting concept known as flux variability analysis.