Computational Systems Biology
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Lecture - 67
Perturbations to Metabolic Networks: Over-expression

In today's lecture, we will look at how we perturb metabolic networks, particularly how do we over-express genes and measure the effect of this over-expression and also how do we identify interesting over-expression targets for metabolic engineering using this method called FSEOF. It stands for flux scanning using enforced objective flux.

So we now look at over-expression. We did look at deletion. What would be the strategy for over-expressing the gene. Which genes would you over-express and typically you are going to be worried about deletion if you want to kill a cell like a pathogenic organism and so on and you are going to be worried about over-expression if you want to maximize the production of a metabolite. You want to push more flux through a particular pathway.

The other analogy that is often helpful while understanding these kind of systems is that you have a large system of pipes and there are a bunch of valves and so on and you have the final flux that comes out at the bottom. That is like your biomass flux. Now if you alter all of these, the steady state will vary. So, these are like perturbations that you can study. This is literally the same thing. Each of your reactions is like a pipe that is carrying a flux.

So to come back to over-expression, you would want to over-express genes that will improve the productivity in a particular pathway, where it turns out that there is no easy way to do this. First thing is how would you simulate over-expression. You have to manipulate the bounds. What happens if you increase the upper bound, it may not make a difference, but if you increase the lower bound, you are pushing it in the direction.

So, one way it is simulated would be you solve for your V Biostar and you will obtain a value for some V5, let us say that is the gene you want to over-express and now you fix V5 to twice that value and that would be over-expressed and now simulate what happens. **"Professor - student**

conversation starts" how do you choose that V5 like. How do you choose the V5 we will come to it? So, currently we are saying if I choose to over-express a gene it is like we still.

No h if you choose to over-express V5, you first get V Bio, but then the V5 that you get in that vector that has V Biostar you do not like there are technically many solutions, we would just take any solution that solve (()) (02:42) and h so you are saying you want to maximize V5 or something or is that what you are trying. You want to do a flux variability for V5, possibly I think that is doable too.

May be you can take max flux through V5 and double that and force that much flux through V5 and then see what is the impact on biomass. I think that is one way to look at it. **"Professor - student conversation ends."**

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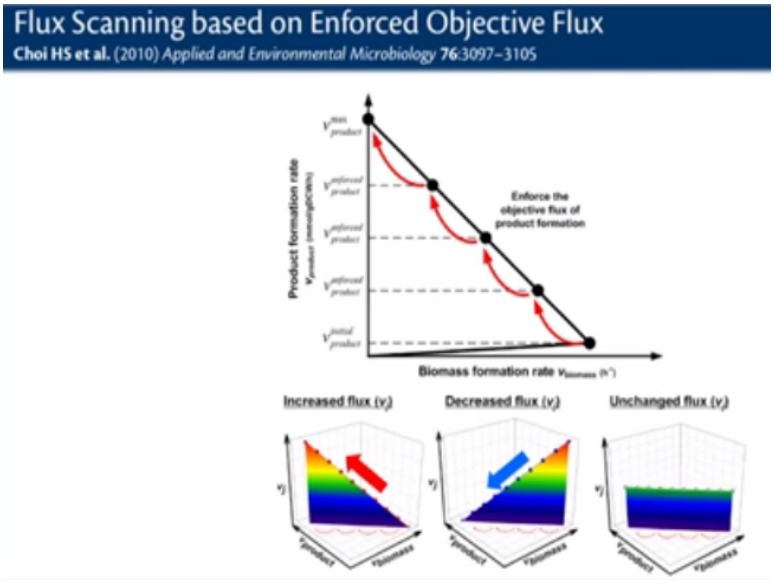

A very interesting approach was proposed by (()) (03:13) in 2010. They said let us study incremental increases in objective flux. Let us enforce the objective flux and scan what happens to various reaction fluxes. The idea is very nice; this is particularly useful if your metabolite is non-growth associated. You might know that there are some growth-associated metabolites and non-growth associated metabolites. Growth-associated metabolites will be higher in productivity when higher fluxes whenever the growth rate is higher.

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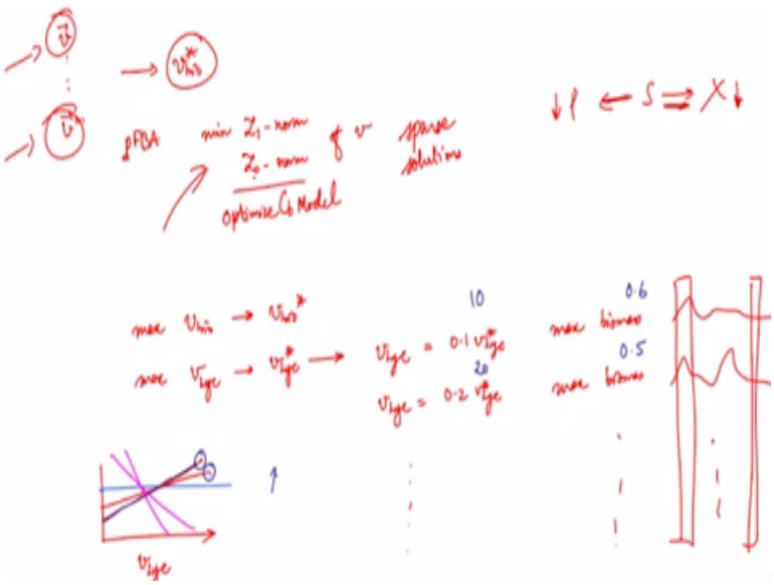

There are other secondary metabolites, which will basically be in opposite directions. The cells can either go in this direction or in this direction. If you go in this direction you will have lesser of biomass here. If you go in this direction, you will have lesser of product here. This is typical for several secondary metabolites. So, in these cases what they suggested was very nice. Let us always look at the cell trying to maximize its biomass.

That is what more cells are always trying to do. At least whenever you do that you seem to get very good predictions in terms of the growth rates. Now you max Vbio you are able to compute your Vbio star. Now you max V lycopene, a secondary metabolite of interest, you get V lycopene star. Now you set the Vlyc equals and maximize biomass. This will give you some flux distribution.

Now you say Vlyc = 0.2 and max biomass, you will get a little lesser biomass than the previous case. May be you get the situation of this sort. You do this multiple times, get multiple distributions. Is this simple, this is very clear. Now let us analyze, these reactions. Now, if you look at individual reactions, you will find that as you increase V lycopene, there are reactions that may go like this, the reactions that may go like this, there are reactions that may go like this and there could be reactions that go like this.

The authors came up with the hypothesis that these are the more interesting reactions. As you ask for more lycopene flux, these are the reactions that are carrying more flux and bringing you more lycopene in some sense. Could you over-express these reactions. The other way to look at this is you have snapshots let us say of a city where there is some traffic distribution. You find that whenever there is a lot of traffic flowing through some particular area, there was also a lot of traffic flowing through some other area, correlated.

If you widen that road or if you had more traffic flowing there, then you would end up getting more traffic here. Well in the road scenario, you want lesser traffic, whereas in the cellular scenario, you want more traffic, but the idea is kind of analog is. So, what are the roads if widened will admit more lycopene flux. What are the reactions if over-expressed will admit more lycopene flux? The idea is these are the reactions that kept increasing as you increase the lycopene flux.

Let us say here the biomass value was 0.6, here the biomass value may have been 0.5, here the lycopene value may have been 10 arbitrary units. Here it might have been 20. But, then you have certain reactions that are carrying more flux. So, these might be the reactions that are quite interesting and we have actually had very good success with this in two different systems, in both sunflower and lactococcus lactis.

Predictions made using this method, which is called FSEOF, flux scanning based on enforced objective flux. You basically enforce an objective flux and maximize only for biomass because that is what the cell is trying to do. You find that there are some reactions, which always increase in flux, some reactions which decrease in flux, some reactions that really do not matter.

"Professor - student conversation starts" you are setting lycopene something what objective flux are we enforcing, objective flux is biomass maximize. Objective, but we enforce the product. So, you enforce the product objective flux as well. You first do a maximize product and get the maximum value of product and then this is computed with probably 0 biomass. If you see biomass is actually 0 here. When you maximize the product?

It can be very low values of biomass like nearly 0. It can be 0 biomass. It is after all a simulation. h. This becomes the objective so you can very well have 0 biomass. It may be some small amounts of biomass if necessary, but you can very well have 0 biomass. But you cannot use this experimentally. We already discussed that right, so this just tells you what is the theoretical maximum flux that can be carried by the product forming reaction.

We did say that example, if you know that you are only at 60% of that value, you really need to invest in metabolic engineering of further product trying to do stain improvement, but if you are already at let us say 90-95% of this theoretical maximum, you do not need to worry about strain improvement, you have to look at some other avenue. **"Professor - student conversation ends"**

To repeat, you maximize the product, come up with a value and then you say I will do a very small amount of product, some 10% of the maximum value and then 20% of the maximum value, 40% of the maximum value, 60% of the maximum value of product and I will see how all the reaction fluxes vary, when I go from this state to this state to this state to this state to this state and you see that there are some reactions that monotonically increase, some reactions that keep decreasing and some reactions that remain unchanged.

These seem to be the interesting reactions. In fact, it would also be interesting to see what happens when you remove these reactions and so on. In fact, we have also tried building a bypass from these reactions to these reactions. These are the fluxes that are taking away our lycopene in some sense. Taking away something that is important for your product, so can you rear out them towards this that is an interesting thought. It is a project idea.

"Professor - student conversation starts" what kind of networks, like you have your model can you do network analysis or (()) (11:39). This is tricky, is tricky. We are trying some of those things, but typically the fluxes become very important. So, static network analysis does not lose a bit of gene when it comes to metabolic engineering. Of course it is very useful if you want to predict what kind of exchanges can happen between two organisms and so on. So, we are using those kinds of models for consocia and things like that.

Just to see which reactions correlate positively, if you have a model, just by looking at the network and if there are no analysis to say which. It seems possible, but it just seems hard as well. It just seems very reasonable right to be able to come up with that project idea. **"Professor - student conversation ends"**

There are flux coupling definitions. There are papers that talk about flux coupling in different reactions and so on. Like directionally coupled reactions, uncoupled reactions and so on. Basically when A carries a flux, B also carries a flux. When A does not carry a flux, B does not carry a flux. That is like proper directionally coupled and so on. Sometimes this can be non zero, this can be zero. This can be zero, this can also be nonzero and so on. They are uncorrelated.

There are different kinds of things, but these are being studied only through flux analysis. If you see some correlation that can be done with networks. What kind of networks do you use, substrate graphs are problem, you may want to use hypergraphs and things like that? This is interesting, you should go and talk to Aarthi about this, my PhD student. She has a very good algorithm that will take care of multiple metabolites in a network and it is based on using the bipartite representation and using some BFS on top of it.

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One way to evaluate these flux would be you can check what is the ratio of this V bio over-expressed/V bio wild type*V product or lycopene over-express/V product wild type. You could

rank your over-expression targets based on this. What is the net improvement in biomass and product? Let us say this was 0.3, this became 0.8 and this was 1.0, this became 0.4, this will tell you how good it is overall. It is one measure; you can come up with any other measures.

I think we will stop here. In this video I hope you got a good overview of how one goes about performing another kind of perturbation in addition to deletions, which is basically over-expression. So, how do you over-express a gene or how do you over-express a reaction in some sense in a model. We also looked at the technique called FSEOF, which tries to predict which genes need to be over-expressed to optimize the production of a given metabolite.

In the next video, we will look at another interesting type of perturbation namely synthetic lethals.