Computational Systems Biology
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Lecture – 82
13C-Metabolic Flux Analysis using Mass Spectrometry

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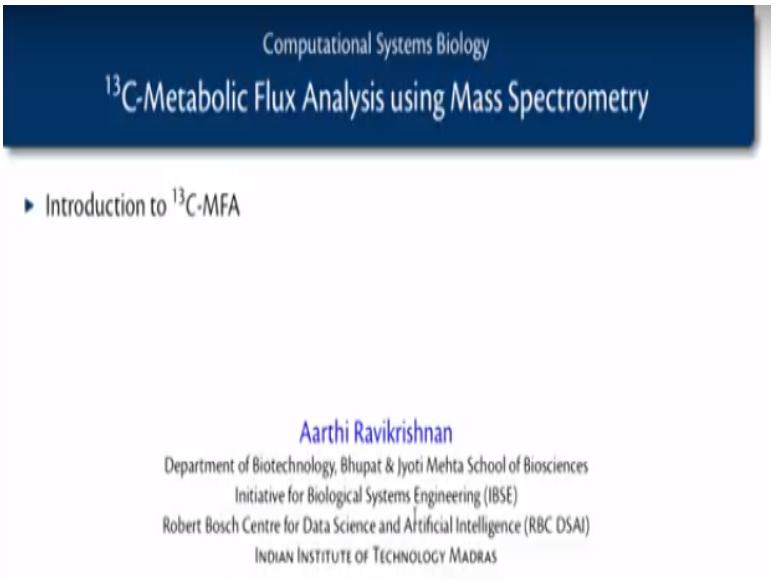

Hi, I am Aarthi Ravikrishnan a senior graduate student at the department of biotechnology IIT madras. I work on computational systems biology but specific focus on integrating computational and experimental framework to understand microbial communities. In this lecture and this lecture, I will be walking you through 13C metabolic flux analysis using ma spectrometry.

In this section you will be getting an idea of what exactly is 13C metabolic flux analysis and the basics to performing C13 MFA. Good morning so today well be looking at C13 metabolic flux analysis using mass spectrometry. So, this lecture would give you an idea of how to carry on metabolic flux analysis using labels substrate such as C13 label glucose and find out how the glucose is getting incorporated in different metabolites inside the cell.

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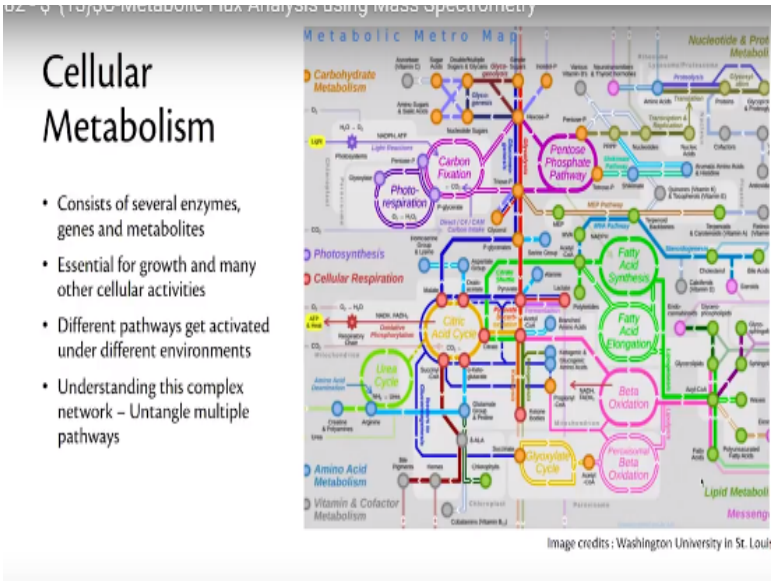

So, to start with us as we all know cellular metabolism is a complicated and a complex machinery which in while several enzymes, genes and metabolites and a lot of these enzymes take pathways in different reactions and these give rise to several metabolites which are essential for growth. And regulate other cellular activities and the cells have evolved different pathways under to basically metabolize different carbon sources under different environments.

And to understand these we need to entangle basically untangle multiple pathways and see how this carbon gets incorporated into the bio mass to see how the cell actually survives.

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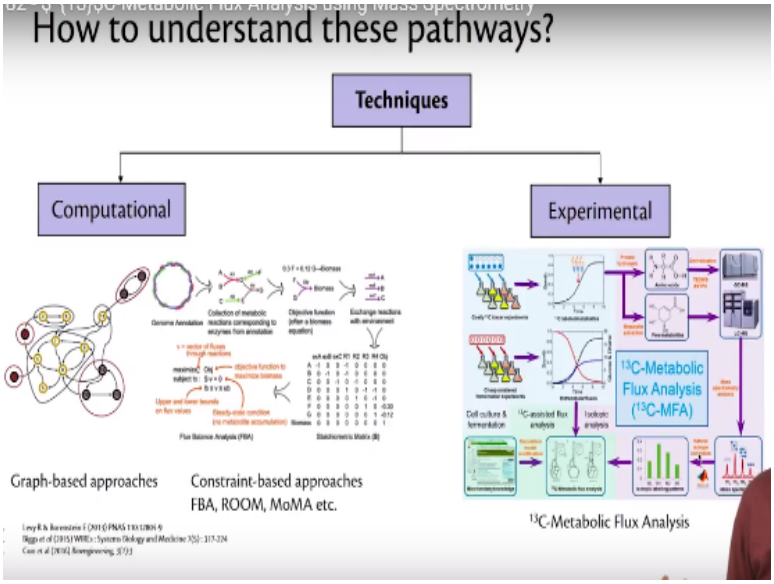

So, there are two different broadly classified techniques to understand these pathways. So, first

one is computational and the second one is experimental. So, in computational you might have already seen several techniques in the previous lectures where there are graphs based approaches where you basically take the metabolic network and try to find out different routes of conversion and also constrain based approaches where you have flux balance analysis room and MoMA.

Where you try to identify the fluxes through different reactions and see how it can be optimized for increasing the growth or a particular metabolite and so on and so forth. Whereas in experimental there are multiple techniques to understand these pathways how something like metabolic control analysis where you have a smaller pathway and you take only the few enzymes which are involved in that particular pathway and try to calculate their kinetic coefficients.

And also see 13 metabolic flux analysis where you trace how the substrate gets incorporated into the biomass and also take the substance and also to understand the pathways with in which the substrate gets incorporated. So, in this lecture will be can will be looking more about the C13 metabolic flux analysis and the methodologies involved and also how to calculate the fluxes from the experiment that we carried on.

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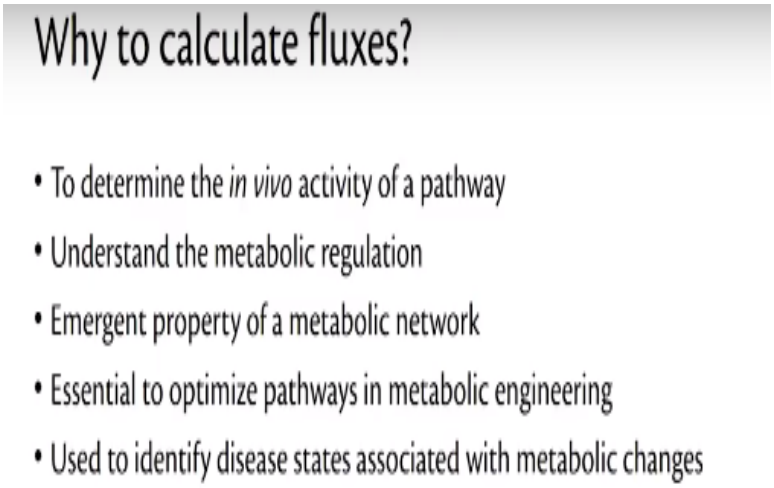

So, before we go into calculating fluxes we should first understand why you should be calculated fluxes. So, what is the importance of fluxes when you take a cellular metabolism so these fluxes are an emergent property of a metabolic network. And also they help you understand the

metabolic regulation that happens inside the cell say under different environmental conditions for instance you give glucose to the cell you give xylose to the cell.

You do not know how the cell acts to this particular carbon source. So, if you understand and calculate the fluxes you would be able to say that okay this is the pathway that being involved in in metabolizing this substrate and so on. So, this is basically to determine the in vivo activity of a pathway and to understand the metabolic regulation and as I say already said it is an emergent property of the metabolic network.

And also these fluxes are essential to optimize pathways in metabolic engineering. So, in metabolic engineering the goal of a particular process is to over produce a product say for example you want to overproduce lycopene you might want to understand what are the pathways which are involved in the production of lycopene. And there by how to overexpress the genes which are involved in that pathway to produce more of your product.

And for that you need to understand the fluxes so is to optimize the pathway and also in several diseases there are metabolic changes which happen something like diabetes mellitus where the metabolic changes. And you might want to understand how do they believe this in terms of the in terms of the metabolism and so again you might want to understand fluxes. So, again to identify the disease state.

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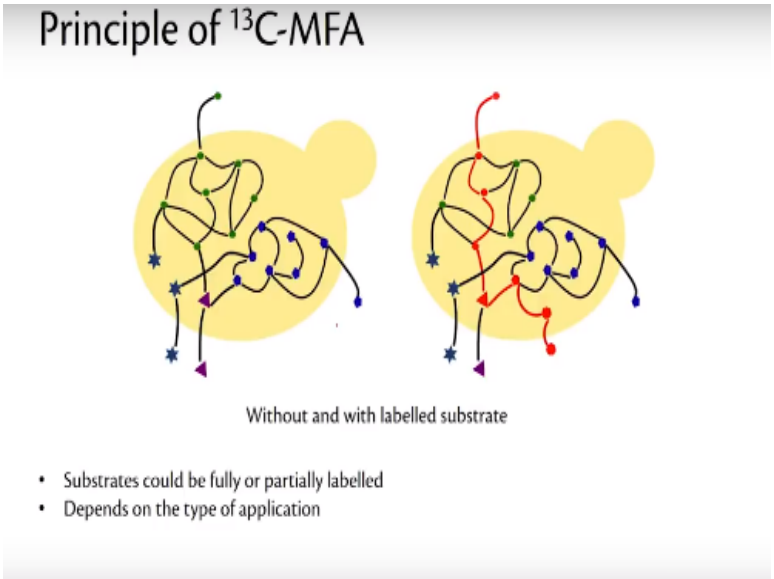

Having said that I would like to move to the principle of how we carry about this C13 metabolic flex analysis in experiments. So, consider two systems I mean consider two different systems where you have a pathway and the first one here and this is basically a depiction of a cell where multiple pathways that act in tandem and produce biomass. And you might want to trace the fate of this particular source that you give and see what what are the products.

Or what are the pathways which are activated based on this particular substrate. So, for that you give a label substrate that is C13 labeled substrate and see and trace where the C13 goes and check out the pathways with with these are involved. So, the choice of the substrate could be either I mean is determined by the application and it could either be fully or partially labeled and the details I will come to in the next slides.

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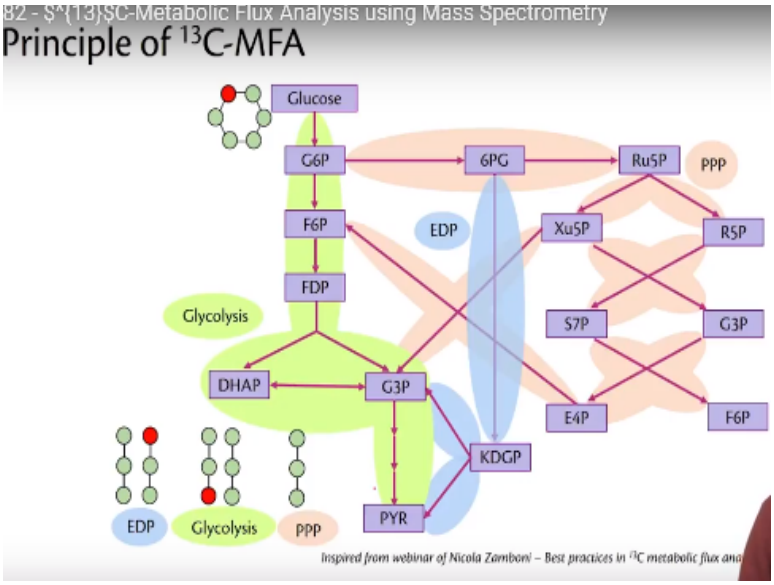

So, to understand in depth so you have glucose and say for example you have glucose which is labelled in the first position for instance and you have multiple pathways that happen inside the cell. So, for converting glucose to pyruvate which is the classic example you have three different pathways that is glycolysis EDP and pentodes phosphate pathway and this glucose can either form pyruvate from glycolysis or EDP or pentodes phosphate pathway.

And this depends on the environment in which the cell is so if you are able to observe labels like this where you have the label in the first first position you might want to infer that it has come from EDP and if you have a label in the other position you might want to infer that it comes from glycolysis. And if you have no labels at all it comes from pentodes phosphate pathway. So, this is just a general representation of the principle of C13 analysis.

So, with this you would be able to trace how the carbon flows from the source to the target and the target in this case is pyruvate.

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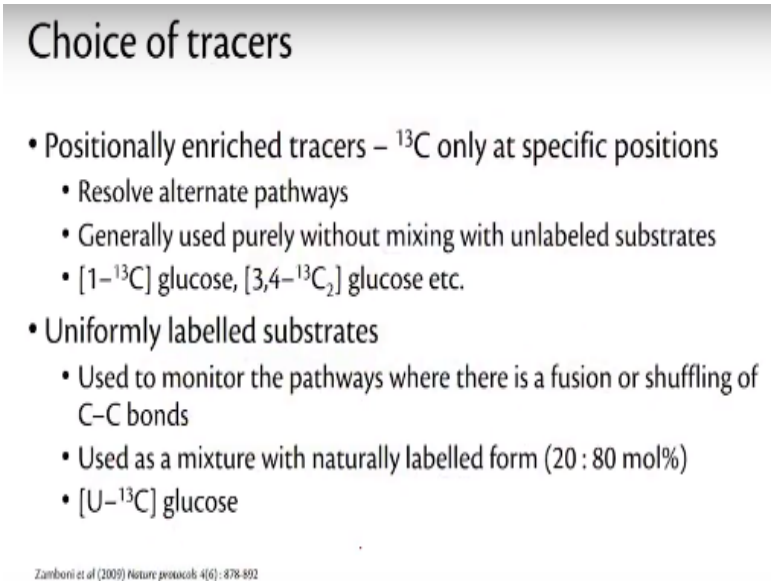

So, when I was talking about these particular pathways and how to identify them so here comes the choice of tracers. So, earlier I was telling you that if the pyruvate is coming from pentodes phosphate pathway it is not going to have a label. So, this can be inferred only if you use a positionally enriched tracer by this I mean you use a substrate which has labels only on a particular position of the carbon.

For instance, glucose which is labeled in the first position that is 1 C13 and glucose which is labeled in 3 4 this might be used in some other case. And these are generally used to resolve alternate pathways like what I was saying like pyruvate has three different pathways from glucose and if you want to resolve alternate pathways you basically use a positionally enriched tracer.

Whereas when you want to monitor the activity of different enzymes like say which basically form or shuffle bonds carbon carbon bonds you might want to use uniformly labelled substrates. And this uniformly label substrates are generally used in a mixture of 20:80 where 20% you have uniformly labelled substrate and 80% of non-uniformly labelled substrate. Simply because these tracer experiments are really expensive.

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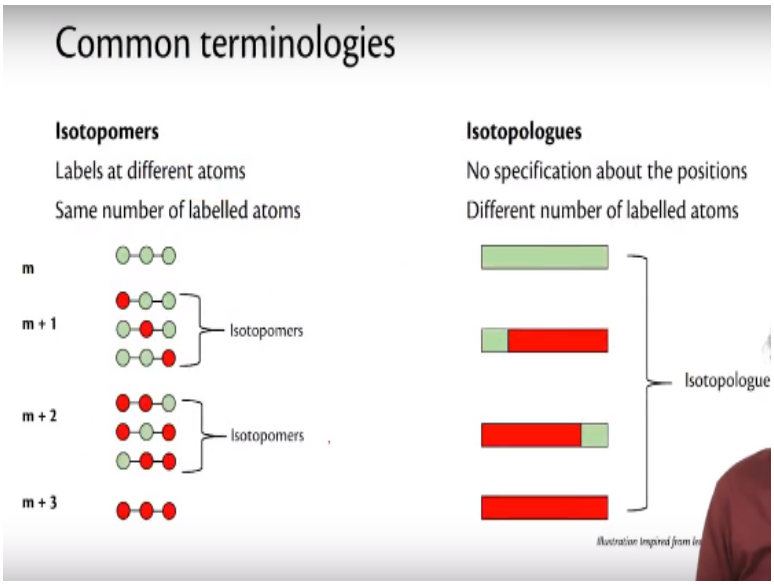

So, these are the common terminologies which would which one would be using when you are doing C13 metabolic flux analysis. The first one is isotopomers and the second one Isotopologues so Isotopomers are nothing but the molecules which have labels at different positions for instance if you have a 3 carbon substance and here in the first case you do not find any labels.

So, here the red color circles indicate that the other labels and green colored are the non-labels. So, this the first one is an Isotopomers whereas M+1 can mean that it has label in one of the positions. So, it could mean either in the first position second position or the third position and these collectively are called Isotopomers and similarly for M+2 or M+3 whereas Isotopologues are a collection of all these molecules which have labels have different positions.

And these joint combination of molecules are called Isotopologues and when you want to do a metabolic flux analysis generally isotopomers are calculated from the data that you get and this can be used to infer the relative abundance carbon abundance and also the positions where the labels occur which I will be talking to you in the next few slides.

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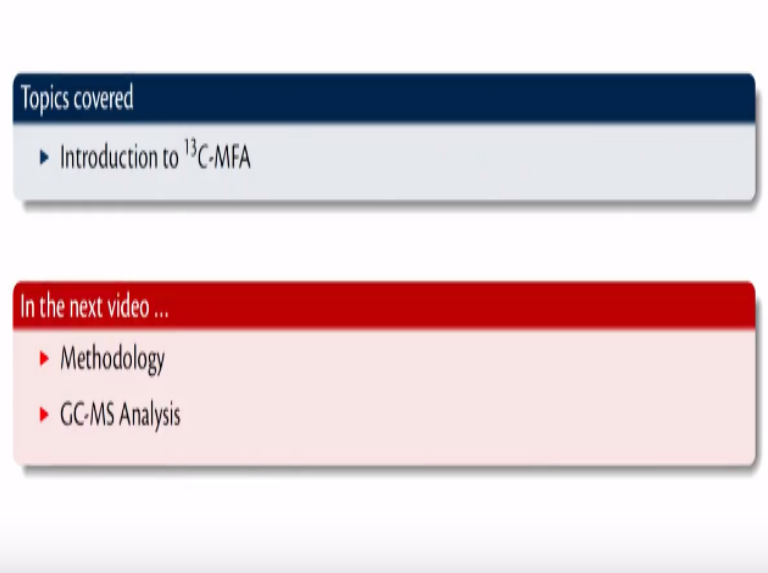

In this video we had a basic introduction to the 13C metabolic flex analysis and discuss the principles behind metabolic flux analysis using labelled substrates. In the next video we will be walking over the methodology to carry out experiments with label substrates and also deal with the GC-MS analysis of labelled amino acids to see how to carry out MFA.