Metabolic Engineering
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Lecture No-25
13 C MFA Formulations

Welcome to metabolic engineering course today will continue 13c metabolic flux analysis last class we discuss about 13C MFA. Today also we will continue with 13C MFA. In this class we will talk about the methodology the techniques. How do you formulate mathematically and how we can do the experiment.

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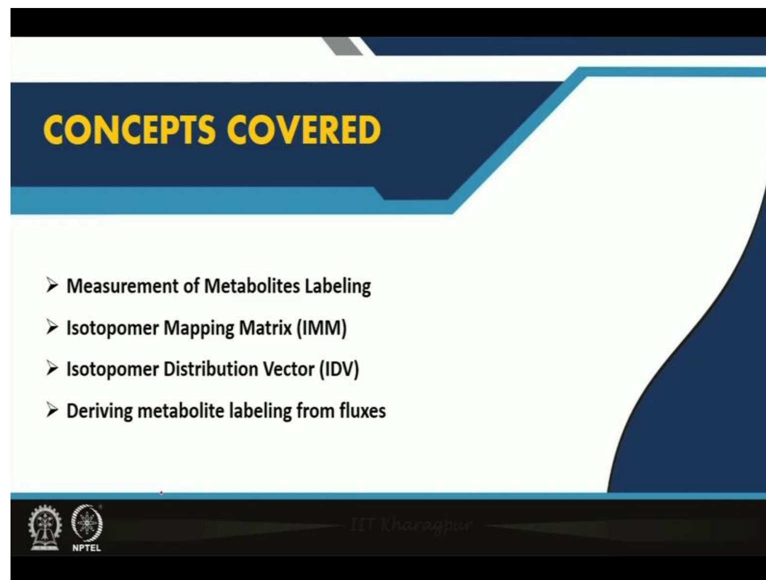

We will go more in detail. So mainly you are involved in measuring in the metabolite levelling that is one of the technique which is used in 13 c MFA metabolic labelling is a part of the procedure the protocol and then once you have the levelling then you will construct isotopomer mapping Matrix then isotopomer distribution vector. Using this 3 information you derive fluxes from metabolite labelling deriving metabolite labelling from fluxes or mathematical you derive this labelling and then compare with the GC mass labelling.

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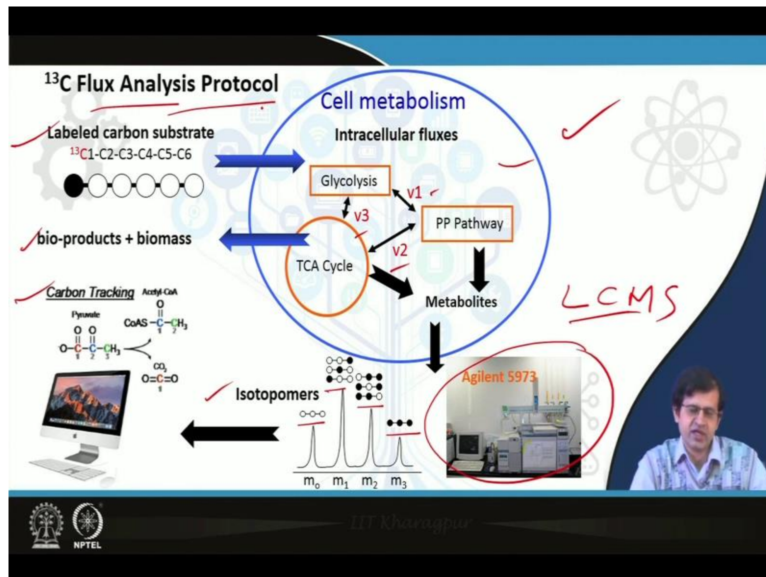

So this 13 metabolic flux analysis protocol is actually involved labelled carbon substrate, so here you can see glucose molecule but first carbon is 13 C labelled. So you can buy this type of glucose from Sigma Aldrich and Cambridge isotope they actually produced this kind of carbon labelling. First carbon level glucose are available, we can buy you can buy it from them. And once the cell consumes this level glucose and you will see that they will go through glycolysis and then it will go to TCA cycle in PPP pathway.

So these are the main centre metabolic pathway because 13 C deals with centre pathway. So cellular metabolism is almost like a black box. What is going inside you do not know so that is why is very important you characterize each and every reaction using 13 C MFA. So the input is the level carbon substrate and the output is biomass and bio product you can measure experimentally.

The extracellular flux is you can measure and biomass also you can measure as you do it for normal glucose culture. So everything will remain same so except you have a media where instead of normal glucose you have a level glucose and then you want to know what is happening inside. So, what is happening inside you want to know? And to know what is happening inside you have to use a GC Mass.

You can see, an Aglient GC mass shown over here. Using the GC mass you can measure the metabolite. So you can use also LC mass, LC mass is also available, LC mass is used for intracellular amino acid. So when you use this GC mass then you are measuring the protogenic amino acid this is the difference. So GC mass is generally used for protogenic amino acid and LC mass is used for intracellular amino acid.

So, these are 2 kinds amino acids this cell produces; protein also have amino acids but protein need to be cut into amino acids before it can be fed in the GC mass. Once you measure the metabolite and then what you will see that you will get a isotopomer profile m 0, m 1, m 2, m 3 and m 0 means that none of the carbons are level, m 1 means 1 carbon level and then m 2 means 2 carbon level and m 3 means 3 carbon level if we have a molecule or fragment which has 3 carbon then we have 4 peaks.

Using these, you will be able to predict class v3, v1, v2 using a software. The software actually does. The 13 C MFA softwares are available freely, some of them you have to buy. So there are 3 or 4 software which are available in the market you can buy them or you can buy academic licence to run the 13 C software you run on the computer that the isotopomer that you get from the GC mass and computationally also you can calculate the levelling.

So the isotopomer that you get from GC mass can also be obtained theoretically and then you match. So for every flux distribution you can have a levelling profile. So that theory that theory is very deep. So we will discuss today how we can derive the levelling from the flux profile. This is completely theoretical and then you match with the isotopomer value that you get from the GC mass. And this is the fitting procedure this 13 C MFA is basically you feed the experimental levelling profile with theoretical value.

And it is a fitting procedure that is why 13 C MFA does not require biomass maximization that is the benefit of 13 C MFA. That is why 13 C MFA is used in many types of problem in cancer cell, bio remediation not only in metabolic engineering.

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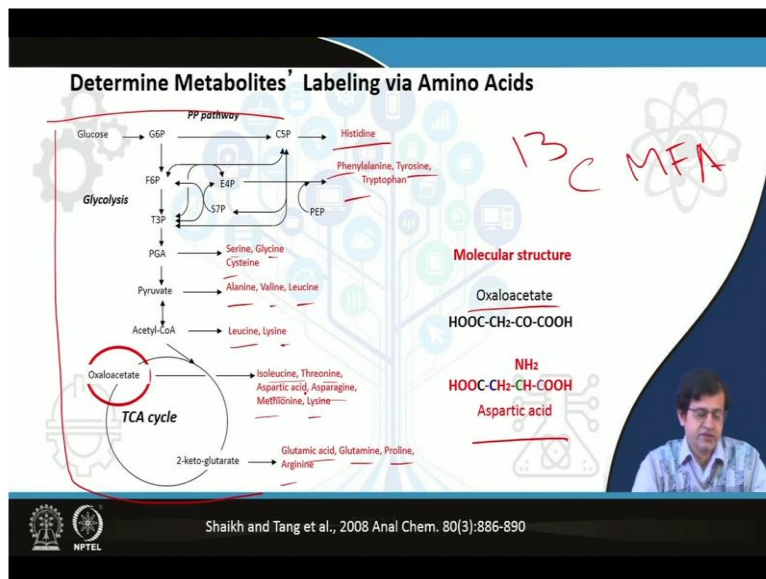

So, how do you determine metabolite labelling? You determine metabolite labelling via amino acid. As you know amino acids are the n metabolites and those amino acids can be measured using GC mass and this labelling profile can be used for flux analysis. What are those amino acid? We can see that the amino acid histidene then phenylalanine which comes from PPP pathway, pentose phosphate pathway we get this histidene and phenylalanine, tyrosine, tryptophan Serine and glysine you get; from glycolysis Cystiene, Alanine, Valine, Leucine you get; from Pyruvate, Acetyl Coenzyme A, Leucine, Lysine, Oxaloacetate you can get Isoleucine, Threonine, Aspartic acid, Asparagine, Methionine, Lysine and 2-keto-glutarate can get glutamic acid, glutamine Proline, Arginine. All 20 amino acid you can extract from the cell.

And since you are only considering the amino acid measurement that is why central metabolic pathway is enough. So if you consider the central metabolic pathway for 13 C MFA you can actually perform the experiment. Since we are not considered the biomass reaction that is why biomass component reaction may not be there in the network. So it is a reduced network, this 13 C MFA works on reduced network. So, it is a reduced network only Central metabolic pathway. Why we have only 20 amino acids present.

Using these 20 amino acids we would be able to do the 13 C MFA. We do not have to add all the biomass component, so it is easier. The network is small compare to FBA. So this is a molecular structure you can see, the molecular structure of aspartic acid oxaloacetate and they represent the

same number of carbon. Oxaloacetate acid produces aspartic acid; there is no LC association or dissociation of the metabolite.

So the labelling pattern is also same. So this way you can identify which compound you want to measure.

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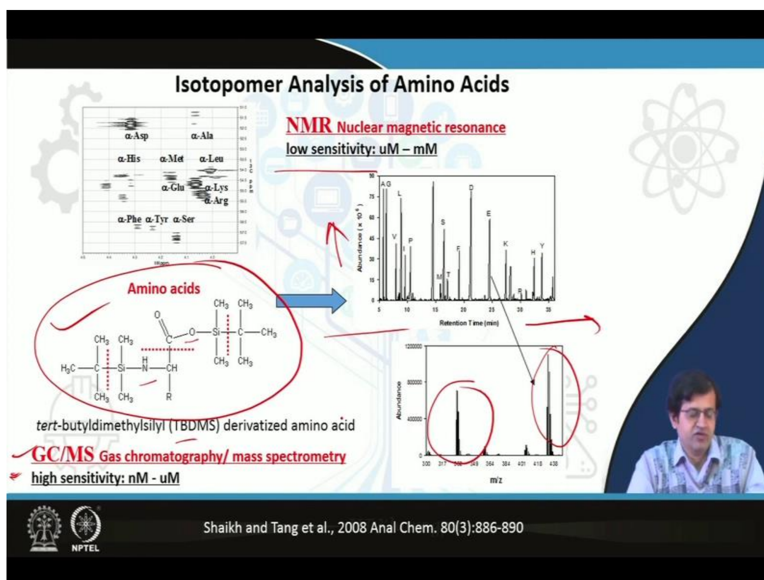

Apart from isotopomer analysis which involves another you can use GC mass and also you can use NMR. Using NMR also you can get the isotope distribution, but the resolution the sensitivity is low. So when you use NMR the sensitivity is low. And when you GC mass then the resolution is high-sensitivity is there in GC mass. Another thing what you do is when you use GC mass another issue is that GC mass is as you know the name suggests that gas chromatography-mass spectrometry.

So they actually convert mobile phase in to gas. So when you put the sample it under go through very high temperature and then your sample get gaseous phase that is why apolar molecule is better for GC mass. So we have to make the molecule apolar. So since the amino acids are mostly polar what do you do is basically you derivatise. when you derivatise you add methyl group. as many methyl groups so that molecule will become a polar.

So this is derivatization molecular which is shown over here. The derivatise molecule, so you can see that Carboxylic group is attached to the derivatise molecule. So, the derivatise molecules is attached and the name of the derivatise molecule is tret-butyldimethylsilyl you have silicone and lot of methyl groups are there which is attached to the carboxylic group and also at the amino group, so 2 molecules are attached.

So for every amino acid we have carboxylic group and amino group both are actually derivatised with TBD mass. So attached TBD mass for both end so that molecule become apolar and you can pass the sample in the GC mass once the measurement happens. This derivatisation is not required when we use LC Mass because LC mass is meant for Polar compound. So measurement of Polar compound is favourable in LC mass and that's why you do not derivatise when you use LC mass but when you are using GC mass definitely you have derivatise.

The derivatise technique is there where you can easily do it before passing the sample in the GC mass. So using gas chromatography and mass spectrometry you can measure this compound all 20 amino acids. But before measurement you have to derivatise so that it can vapourise as it goes in the gaseous phase. So that is why you need to derivatise. And once you pass through a mass spectrometry you will get abundance on the y-axis and on the x-axis is the retention time.

So every amino acid has a particular retention time which is best on the column we are using, the type of column you are using. So, you can see the retention time of serine is different from phenylalanine, prolene, isoleucine, valine, leucine, lysine, histidene, tyrosine, arginine so each one of them has different retention time so that the peaks are not overlapping. If you click on the peak you will get more resolved, then you will see that abundance get is plotted versus m by z.

So these are the levelling profile that you have to get from the mass spectrometer and that is why you get the levelling profile from this value you can store those value in a file and used in a software. So, the software if you feed those levelling profile along with the metabolic network then you would be able to calculate the fluxes.

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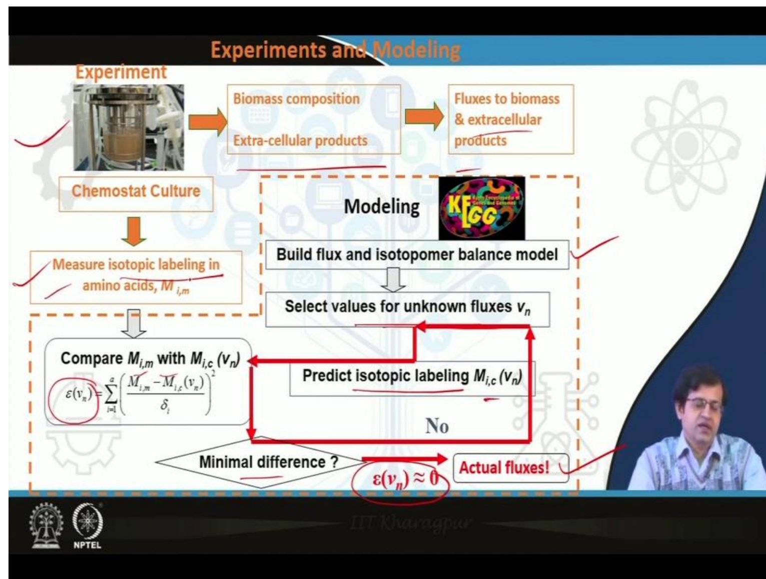

So the experiment and modelling it involves 13 C MFA requires extensive experiments. So 13 C levelling experiments are time-consuming. One or two days you have to do the experiment. One or two days you have to extract the metabolite and couple of days you have to spend in the GC mass. It is a long process and sometime it takes several weeks to conduct the 13 C MFA experiment, the experiment is laborious.

So ultimately you want to run a chemostat culture and then you measure the extracellular product. Not only Metabolite measurement extracellular product also you have to measure. You have to measure the Biomass, extracellular fluxes and through experiments will get those data. And then biomass measurement you have to do and then you to measure the isotope labelling of amino acid.

So, these are experimental part and in the modelling part you have to build flux and isotopomer balance models. So you have make a model Metabolic model. The isotopomer balance model that you have to make using KEGG database or any other database like metasaic, Kosaic, Brinda they also give metabolic reaction that we have to use for making the network. And once you have the network than what you do you select value, just choose randomly some flux values for all the reaction.

Suppose you have 50-60 reactions in the network, then you assume some value based on literature value, also you can use and using that value you predict the isotope labelling. So, I will discuss about that today, how we can calculate the isotope labelling from the flux profile and then you compared with the experimentally measured isotope labelling what I told you. You compare with the experimental value. So every flux profile has a mass distribution vector n that is this one. And experimentally also you get the Mass distribution vector and we have error function at the end.

So this error function is minimised if you repeatedly run the calculations such that the error function reduces to very, very close to zero and that is where you stop. When the difference is minimum that is where you stop the calculation and then you say that you have found the flux profile because every flux profile is given by the isotope labelling. So given an isotope levelling you say that when the difference is very, very less between experiment and simulation then you stop the calculation then you say that the flux which you are giving at that particular end is more close to experiment.

And that is most important for the experiment and you predict that this is much more close to the experiment and that is why, you are stopping the calculation. And that is the actual flux. When E is 0 in then you say that this is the actual flux that is very much close to experiment, because you are using the isotope labelling from the experiment. So that is we say that this is the actual flux. But again, it is a prediction only inferred only but since you are using the measured isotope you can say that is actual flux or experimental flux for a given network.

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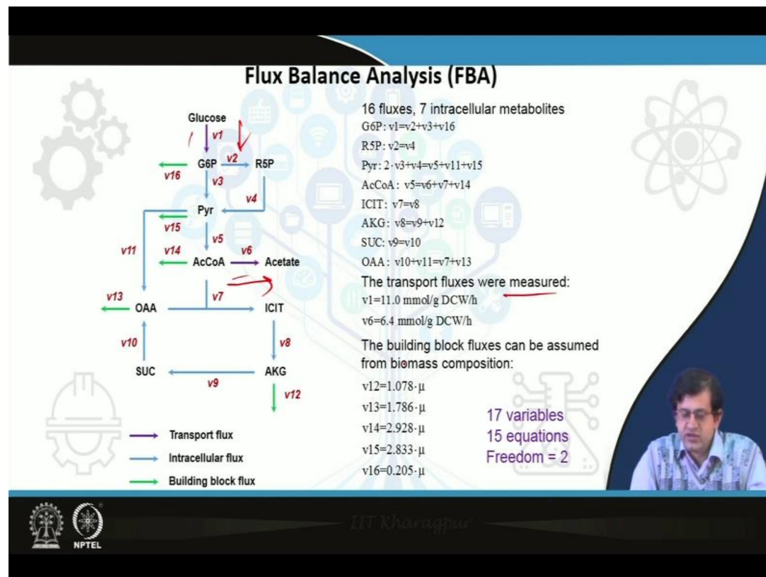

So I will discuss a simple example of FBA. In FBA also calculate the flux and this is a network where we can see that the transport flux is shown in Magenta. So, these are the transport flux 1, 2; there are 2 transport fluxes and v1 is basically glucose, it is taking up glucose and it is producing acetate. And the building blocks flux are basically the Biomass flux it goes to biomass basically G6P. Then Pyruvate acetyl coenzyme A, Oxaloacetate and Alpha ketoglutarate that goes to Biomass for building block, building the cell.

And then we have intercellular flux and intercellular fluxes are given in blue and building block fluxes are given in green. So, this is a small network where you can easily characterize how many reactions are there? There are 16 reaction, 7 intercellular fluxes and you can write the equation like v1 equal to v2 + v3 + v16 and then v3 is equal to v4 and these are all from the steady state condition you have those equation and the transport fluxes are measured.

So, transport fluxes you can measure because the extracellular fluxes that is how much glucose it is coming in and how much acetate it is produced. The extracellular fluxes can be measured so that you can measure from HPLC. I considering two time point and taking a time average you can get B1 and B6 and that is the uptake flux of glucose and secretion flux acetate that you can get. So, building block fluxes can be assumed from the Biomass composition.

So, for the building block fluxes if you know the growth rate and the composition of the biomass than you can get v 12, v 13, v 14, v 15, v 16 you can get it from the Biomass equation. So we have 17 variable and 15 equation and the degree of freedom is 2.

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Variables (fluxes)

Linear constraints

$$
\begin{bmatrix}
-1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -2 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & -1 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 1.078 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 1.786 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 2.928 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 2.833 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0.205
\end{bmatrix}
\cdot
\begin{bmatrix} v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \\ v14 \\ v15 \\ v16 \\ \mu \end{bmatrix}
=
\begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}
$$

$$S \cdot v = 0$$

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Now you can build a stoichiometric matrix based on the equation and the stoichiometric matrix you can see, and you have the reaction vector that is the flux v1 v2 v3 and then you would get this steady state condition, S dot v; and you get equation for v1 v2 v3 and you try to solve and get the fluxes.

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Flux Balance Analysis (FBA)

maximize μ

s.t. $S \cdot v = 0$

$0 < v < 20$ mmol/g DCW/h

$$obj = [0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 1]^T$$

$$lb = [11.0\ 0\ 0\ 0\ 0\ 6.4\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0\ 0]^T$$

$$ub = [11.0\ 20\ 20\ 20\ 20\ 6.4\ 20\ 20\ 20\ 20\ 20\ 20\ 20\ 20\ 20\ 20\ 20]^T$$

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So, in the FBA problem you maximize the Biomass. So you are maximising the biomass and you have S dot v is equal to zero and then we have a boundary condition like v greater than zero less than 20 and you have the objective function and the lower bound and upper bound you shown over here. So, using the FBA formula the FBA protocol you can calculate and there is no experimental data. You can see that there is no experimental data. The experimental data you do not have in FBA.

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^{13}C MFA Formulation

General Scheme

- Feed cells with stable isotopes
- Isotope-labeled atoms with distinct labeling distributions are incorporated into the structure of various metabolites
- GC/MS is used to quantify relative abundance of the isotope (e.g. ^{13}C) in the fragments of analyzed metabolites
  - Note: Different isomers of a metabolite are referred to as *isotopomers*

Adapted from Maranas, C. D. & Zomorrodi, A. R. *Optimization Methods in Metabolic Networks* (2016)

But in 13 C MFA I told you that you have the K isotopomer and this K isotopomer can be measured using GC mass. So for molecule of 3 carbon you have 4 peaks in m 0, m 1, m 2, m 3 in GC mass.

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Metabolic Flux Analysis

Flux Balance Analysis (FBA)

- *in silico* simulation
- Linear programming (LP)
- Genome-scale

maximize $\sum c_i \cdot v_i$

s.t. $S \cdot v = 0$

$lb < v < ub$

^{13}C-assisted Metabolic Flux Analysis

- *in vivo* search
- Nonlinear programming (NLP)
- Simplified model

minimize $(MDV_{exp} - MDV_{sim})^2$

s.t. $S \cdot v = 0$

$IDV = f(v, IMM, IDV)$

$MDV = IGM \cdot IDV$

$lb < v < ub$

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And the Mathematical formulations of these two methods are shown over here. In the left hand side is a insilico simulation and is a linear programming and it can be done a Genome-scale level. So that 13 C in vivo calculation because you measure the metabolite labelling in vivo and then it uses non linear programming and it is a simplified model, it is a reduced network.

And the equation that are used for FBA is basically maximize the Biomass and apply the steady state condition and then boundary condition. But in 13 C MFA you minimise the error function that is the experimental mass distribution vector minus the simulation mass distribution whole square that minimise that is the difference between these two experimental value. Now apply steady state condition as given in FBA.

And one more thing you calculate is the isotope distribution vector. Isotope distribution vector is a function of flux v, the isotope mapping matrix and isotope distribution vector. So you have a flux vector mapping Matrix and isotope distribution vector that goes for calculation of IDV and MDV you get mass distribution that you get from GC mass and for that you multiply isotope grouping Matrix with isotope distribution vector.

So you calculate here the isotope distribution vector and then you feed it here. So this way you can customise the MDV theoretically and then you have experimental MDV which you are supplying externally. And MDV from simulation you are basically calculating here theoretically

so you take a difference and you minimise. So you can see the steady state condition and the boundary condition as same as FBA.

But what is missing in 13 C MFA which is present in FBA is basically the maximization of the biomass the objective function. So you are not maximizing you are minimising the error function that is the major difference and it is a simplified model and uses nonlinear programming. So this is how these two problems are defined. So, it uses metabolic and isotopic steady state.

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So, the mathematical formulation of 13 C MFA involves isotopomer mapping matrices. In the previous slide I told you that you have IMM. How IMM is calculated. And what is isotopomer distribution vector. So get these 2 parameter you want to know what is IMM and what is IDB. So IDB is very simple is basically intensity of the peak, intensity of the labelling peak for all the isotopomer. So if you have a 2 carbon system then how many isotopomers are there that is 2 to the power 2 that is 4.

So, in 2 carbon system, 4 isotopomers are there. In one case all the two carbon are unlabeled. It is not filled so that is why it is unlabeled and in second case we have 1 carbon level, the second carbon level and then the first carbon level. So in this case this is first carbon and this is second carbon but both of them are one carbon level and the last one is basically both are level that is

why we have intensity I 00, I 01, I 10, I 11 and this is the isotope distribution vector for the metabolite of two carbon system.

So for 3 carbon system, what is IDV? It will be 2 to the power 3. So, 3 carbon, so the number of intensity you can get is 8; so we have I 000, I 001, I 010, I 011, I 100, I 101, I 110 and I 111. So this way you can actually have 8 components. And then for 2 carbon system we have 0 and 1. So either it is level or unlevel so 1 carbon system. So this isotope mapping matrix is basically you want to go from s1 to x.

So, s1 has 2 carbon and x metabolite has 3 carbons. So from 2 carbon system to 3 carbon system, how we can go? for that, you need a mapping matrix. So, in isotopomer mapping matrix so I have a substrate s1; and then I have a product x which has 3 carbon. So what do you do? You could draw a matrix and on the matrix, you can see that in the first one, both are unlevel. And the second case you have one carbon level and third case is also one carbon level and the fourth one is both are level.

So for unlevel, you can have 100 you can have a row 100 for this one because you are putting one on the first column that is why the first column is basically both are unlevel. And second row is also both are unlevel because you can have 2 possibilities. So these 2 unlevel can have a possibility but the third one is unlevel and the second isotopomer the third one can be level. So, there are 2 possibilities to fill the circle so that is why it can be levelled or unlevel.

So for every isotopomer since the carbon is increasing so we have 2 options. For one isotopomer you have 2 option. For this isotopomer I have 0110 0100 for this one. Twice you have to consider because one carbon you are adding one at a position 00 and another one. So that is why you can see that for this one I have 2 rows that is 1000 1000 for this one I have another two rows that is 0100 0100 since I am considering this one.

So, this becomes for the 2nd one and then for the third is one carbon level then also I include two rows that is 0010 0010 for this one. And you can see and you can fill either it is unlevelled or levelled for the last carbon. And for the final one you have both are levelled that can be 001 001

so that can be filled up in two ways like this 1 unlevel and this is level. So this way we can have matrix isotopomer mapping matrix.

That is when we going from 2 carbon system to 3 carbon system. Similarly I can go from one carbon system to 3 carbon systems. So one carbon system I can have 10, 01, 10, 01, 10, 01, 10, 01. So, ultimately you have to see that when you go from s2 to x, s2 is basically one carbon system is a one carbon. And this is a 3 carbon, 3 carbon means 2 to the power 8. So the number of columns will be 2 so since you are going from one carbon to 3 carbon so the number of isotopomer will be 2 for one carbon system, 2 into 8. You have to make sure that the dimension of the matrix is 2 into 8.

Here we have gone from 2 carbon system to 3 carbon that is why you have 4 into 8. Since we are going from one carbon system to 3 carbon that is why you have 8 into 2 and this is where we are going from 2 carbon system to 3 carbon system that is why you have 8 into 4, the dimension of the matrix and then you fill it up accordingly as I shown I have told you this technique how to fill it up.

Fill the elements. Once you decide the number of rows and number of column the dimension of matrix, then you can fill up the isotopomer mapping Matrix.

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A carbon exchange reaction involving the conversion of two reactants B (asymmetric) and C to a product A.

Adapted from Maranas, C. D. & Zomorrodi, A. R. *Optimization Methods in Metabolic Networks* (2016)

Now I want to calculate the isotopomer mapping matrix for an exchange reaction which involve conversion of 2 reactant and that is B and C to a product A. So have a reaction which has to reactant and one product. So it is a reaction which actually converts 2 metabolite into one metabolite and for that you can see you have k equal to 1 to 4 isotopomer k equal to 1 to 2 isotopomers and then it combined to form 2 to the power 8 isotopemers.

So these are the combinations you can see which I have already told you. And then the construction of the isotopomer mapping Matrix as I told previously that since you are going from B to A and C to A, B is basically 2 carbon system and A is basically 3 carbon system. So you are going from 2 carbon system to 3 carbon systems so the dimension will be 8 into 4 and then you fill it up accordingly. Since you starting with 2 carbon it can be 1000, 0100.. fill it up accordingly.

And then for 1 carbon to 3 carbon you have 8 rows 8 into 2. So you are going from 1 carbon to that form C to A you are going from one carbon to three carbon. So the mapping matrix, have dimension of 8 into 2.

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So this is the dissociation reaction where 3 carbon system which is actually dissociated into 2 carbon and 1 carbon and for that the isotopomer mapping Matrix since you are going from 3 carbon to 2 carbon so your mapping Matrix that is A to B will have 4 into 8. The dimension of

the matrix will be 4 into 8. And when you go from 3 carbon, that is from A to C 3 carbon to 1 carbon then it will become 2 into 8 dimension and then accordingly fill it up so that you have that many number of levelling.

So, this is you can practice and let me know how you can do it, if you have understood this one then obviously will be able to do this one.

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Now I will talk about the metabolite labelling from fluxes. How we can calculate the metabolite labelling from the fluxes. So given a flux profile you can calculate the metabolite labelling as I told you. So, this is a network I can show you that glucose is entering the cell and it is converted to glucose 6 phosphate and then glucose 6 phosphate is converted into fructose 6 phosphate. So we have bidirection reaction and then glucose 6 phosphate is also converted into the G6PDH glucose 6 phosphate dehydrogenase.

So I have 4 reaction and these 4 reaction you can write in terms of time derivative of glucose 6 phosphate, which is basically how much glucose 6 phosphate is form that is V PTS Plus how much glucose 6 phosphate is formed from fructose-6-phosphate is basically + PGIr reverse reaction and then minus since it is a used up that you have a minus sing, minus V glucose 6 phosphate dehydrogenase and PGI forward.

So this way we can write the time derivative of these concentration and this can be used to actually form the time derivative of the isotopomer distribution vector. So, isotopomer distribution vector for glucose 6 phosphate is basically dIDV by dt is nothing but V PTS multiplied by the IDV of glucose. So V PTS that is it flux coming through this site V PTS multiplied since it is a flux you have to multiply with the distribution vector.

The isotope distribution vector needs to be multiplied. Since we do not know the isotope distribution vector of G6P that is why you take, because G6P is same as glucose molecules that is why use the mapping Matrix for glucose to G6 phosphate multiplied by the IDV of glucose. Since the IDV of glucose is given because it is the media this cell is growing. So using IDV of glucose, you can calculate the IDV of G6P that is the formula you used.

That is V PTS multiplied by the isotopomer mapping matrix from glucose to glucose 6 phosphate multiplied by IDV of glucose. So, if I know idv of glucose and I can calculate the idv of glucose 6 phosphate, that is the mapping Matrix. For that you have to calculate the mapping matrix multiplied by the IDV of glucose. So this way you can get the IDV of G6P by using glucose IDV.

For that you have to multiply the isotope mapping matrix. Similarly for PGI reverse that is this reaction you can calculate the IDV of glucose 6 phosphate which is coming from fructose-6-phosphate. So IDV of fructose 6 phosphate is multiplied by mapping matrix of fructose 6 phosphate and glucose 6 phosphate. So how much isotopes are moving from this region to region that you can calculate by multiplying the mapping matrix of fructose 6 phosphate to glucose 6 phosphate multiplied by IDV of fructose 6 phosphate.

These 2 you are getting from glucose and Fructose that is why you have to multiply mapping matrix. The amount of isotopes you are getting from these to glucose and Fructose 6 phosphate that is why you are multiplying the mapping matrix but for this reaction you can see that Glucose 6 phosphate is actually used up. So that is why there is no mapping Matrix, the IDV of glucose 6 phosphate is you know, because that is variable you are using.

So that is why you have only the flux multiplied by IDV of glucose 6 phosphate. So, all the variable in this equation are in terms of IDV or glucose 6 phosphate. And you have fructose 6 phosphate. So now you can write the equation, since IDV under steady state condition. The time derivative of glucose 6 phosphate is 0. So if you rearrange the equation then IDV glucose 6 phosphate is nothing but the flux through that is V PTS from glucose and then multiplied by the istopomer mapping matrix.

Glucose to glucose 6 phosphate and multiplied by IDV of glucose this one, you know, and then we have another term that is the flux through PGI this direction. And then the isotopomer mapping matrix from Fructose 6 phosphate to glucose 6 phosphate multiplied by IDV fructose 6 phosphate. So if I want to know the isotopomer distribution vector for glucose 6 phosphate I should know the IDV of glucose and also IDV of fructose 6 phosphate.

Fluxes are already known because flux profile we are providing it is saying that deriving metabolite labelling from fluxes. So, fluxes are already know the flux value already we know. What do you do not know is basically the labelling. You want derive the labelling from the fluxes. So fluxes are given this one this one this one is given and IDV glucose is also given so you can easily calculate the IDV isotopomer distribution vector for glucose 6 phosphate.

And fructose 6 phosphate is also we can calculate by a similar equation. In this way, you can derive the metabolite labelling from the fluxes using this equation. So for each isotopomer you have to construct this type of equation to calculate the metabolite labelling and once you calculate the metabolite labelling then it can be used for comparing with the experimental data.

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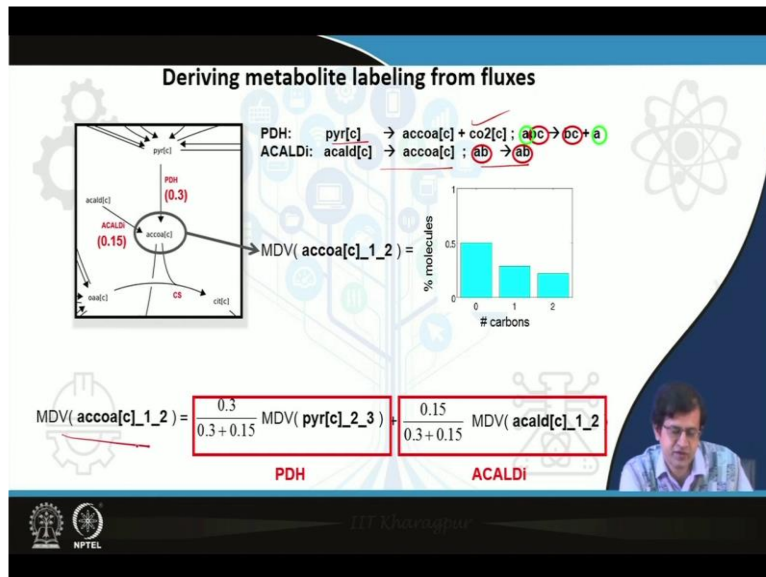

So, this is another derivation I have shown you for that you should know about the carbon atom transition. The carbon atom transition for pyruvate is shown over here. You can see that pyruvate has three carbon. So last two carbon goes into acetyl coenzyme A and the first carbon goes into carbon dioxide. So this is the carbon atom transition for pyruvate to acetyl coenzyme A you can get it from other database.

And acetaldehyde to acetyl coenzyme A there is no breakage or association or disassociation of reaction it is very simple and ab going to ab. Then you can see this is a reaction where pyruvate and acetaldehyde producing acetyl coenzyme A and we have the flux value 0.15 and 0.3. How do you know the labelling of acetyl coenzyme A? So if I know the labelling of pyruvate and acetaldehyde which is shown over here.

The MDV of these 2 are known then I can know the MDV of acetyl coenzyme A and the flux values are used to normalise. You see that the normalisation factor 0.3 divided by 0.3 + 0.15 that is a flux going through this reaction and to PDH. So PDH reaction I have a flux of 0.3. So 0.3 get normalised divided by 0.3 + 0.15 and the flux through acetaldehyde is 0.15. So, 0.15 divided by 0.3 + 0.15 is where it is normalised.

So, the MDV of acetyl coenzyme is nothing but the MDV of acetaldehyde and pyruvate and subsequently normalised by the fluxes going through this reaction. So, this way you can calculate

the MDV how we can derive the metabolite labelling fluxes I have shown you and it has to be evaluated for all the metabolites computationally not by hand and as for it is computationally is very expensive.

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So, the solution is a recursive algorithm where you choose a flux distribution and then predict the amino acid labelling and using the flux profile. So for V i is the flux of profile you are providing and then you compared with experimental data. So these are the equations which are used for calculating the labelling profile theoretically using simulation and then you compare those labelling pattern with the experimental data to find the best fitting.

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So, these are fluxes you can see which is shown in blue colour and then you have the flux variables and stoichiometric Matrix have a linear constraint and then you put a steady state condition is applied in 13 C MFA also but you do not use biomass equation the growth rate you do not, the flux through the biomass equation is not considered. So that is the difference in 13 C MFA and flux balance analyses. You do not consider the flux through biomass, because the biomass equation is not included in the reaction network.

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^{13}C-assisted Metabolic Flux Analysis (^{13}C-MFA)

minimize $(MDV_{exp}-MDV_{sim})^2$

s.t. $IDV = f(v, IMM, IDV)$

$MDV = IGM \cdot IDV$

$S \cdot v = 0$

$0 < v < 20$

$lb = [11.0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 6.4 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0 \;\; 0]^T$

$ub = [11.0 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 6.4 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 20 \;\; 20]^T$

So this 13 C assistant metabolic flux analyses you can see minimise the mass distribution vector that you obtain from experiment and simulation and take a difference and you may try to minimise. And the isotope distribution method you calculate from the flux profile isotopomer mapping matrix and IDV of the substrate. So IDV for all metabolise can be calculated theoretically and then you have an isotopomer grouping matrix that actually convert IDV into MDV.

It is very simple the isotopomer grouping matrix can be used to actually convert the IDV into MDV because MDV that you measure from experiments. So MDV for all the metabolise has to be converted so that it can be combined with the experimental data that is the amino acid MDV are available through experiment and then you apply the steady state condition and also the boundary condition that the upper bound and lower bound and try to minimise the error. And

when the error is very very small then and that is where do you stop the calculation and say that this is the exact flux experimentally obtained flux.

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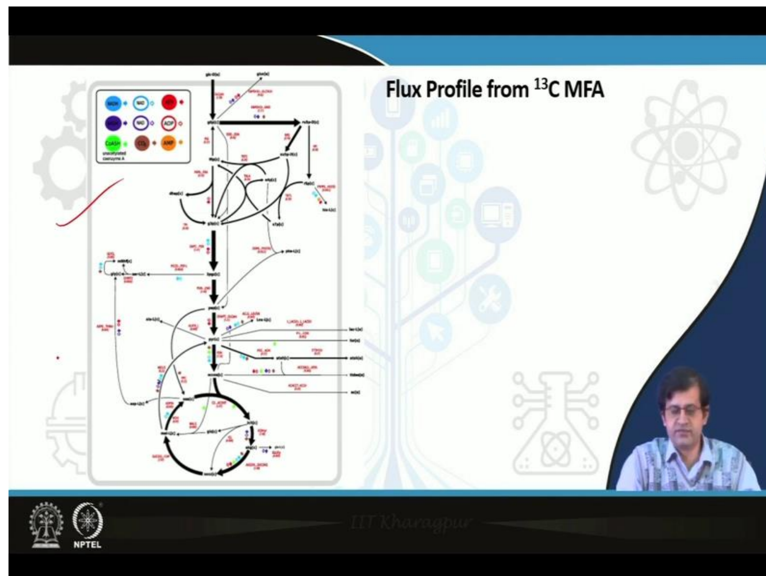

This is the flux profile. So, the flux profile you can see that after performing long experiment after doing modelling you get the flux profile like this which you can considered to be exact flux profile for that strain you are working on for metabolic engineering.

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CONCLUSION

1. The ^{13}C-MFA solution involves a recursive algorithm to solve for the fluxes
2. Incorporating MFA data into models gives a much higher resolution into the metabolic fluxes
3. No biomass maximization in ^{13}C-MFA
4. The main concept behind the formulation is steady state mass balances

So, in conclusion the 13 C MFA involves a recursive algorithm to solve the fluxes. So it is recursive algorithm iteratively solved so that the error function is very, very close to zero. Incorporation MFA data into model gives you higher resolution into metabolic fluxes. So the

flux profile and the flux value you get from 13C MFA are much more highly resolved. This range if you do flux variability analysis range value is very, very small because it is much more constraint using metabolite information data.

So, there is no biomass maximization in 13 C MFA the main concept defined the formulation of steady state mass balance that is S dot v is equal to 0. So, the approximation used is S dot v and there is no biomass maximization and the flux you get from the 13 C MFA have high resolution.

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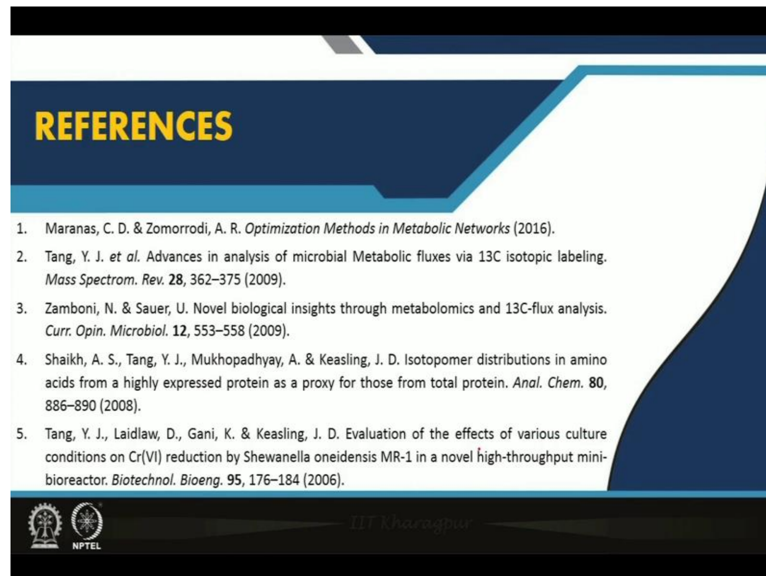

These are the references you can read further you can read about 13 C MFA using these references. So thank you for listening. I close here today. Hope you understand 13 C MFA mathematically. Thank you.