Metabolic Engineering
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Lecture - 23
Extreme Pathways and Elementary Modes

Welcome to metabolic engineering course, today we are going to learn a new topic that is extreme pathways and elementary modes. These are 2 important things in the metabolic network where you can get the flux distribution because of the elementary pathway, extreme pathway and the elementary mode present in the network.

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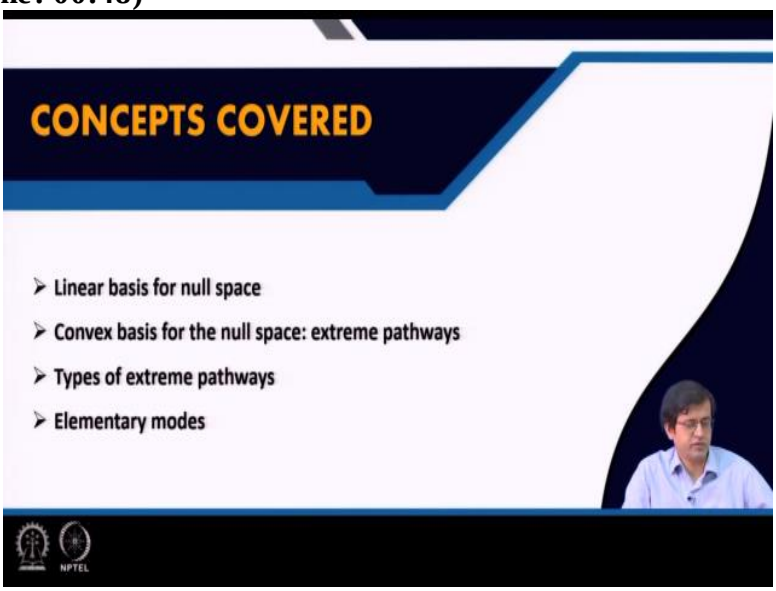

So to start with, we will cover some of the concept today like linear basis for null space, the null space we already know S dot v = 0. So we want to calculate the linear basis for the null space and then followed by convex basis for the null space, which is basically the extreme pathway and the type of extreme pathways and at the end we will learn about the elementary modes.

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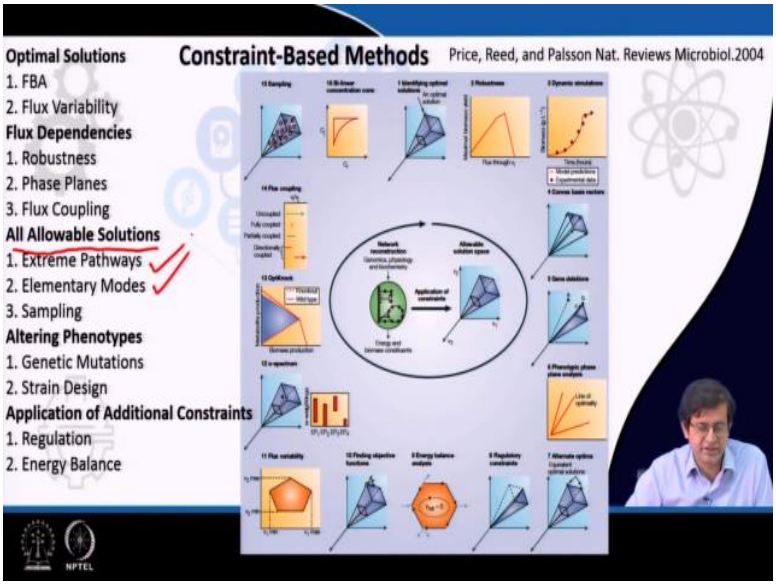

So this is the constant base method which are available, you can actually install MATLAB or Python version of anaconda can be installed and where you can run this constraint based method. So far we learned about FBA flux variability analysis. Also we learn about flux dependencies like robustness, phase planes, flux coupling. Today we are going to learn about the allowable solution among that we have the extreme pathways and elementary mode sampling we have already completed.

So today we will cover these 2 topics the extreme pathways and the elementary modes. To learn about the elementary modes and extreme pathways we start with the metabolic pathways.

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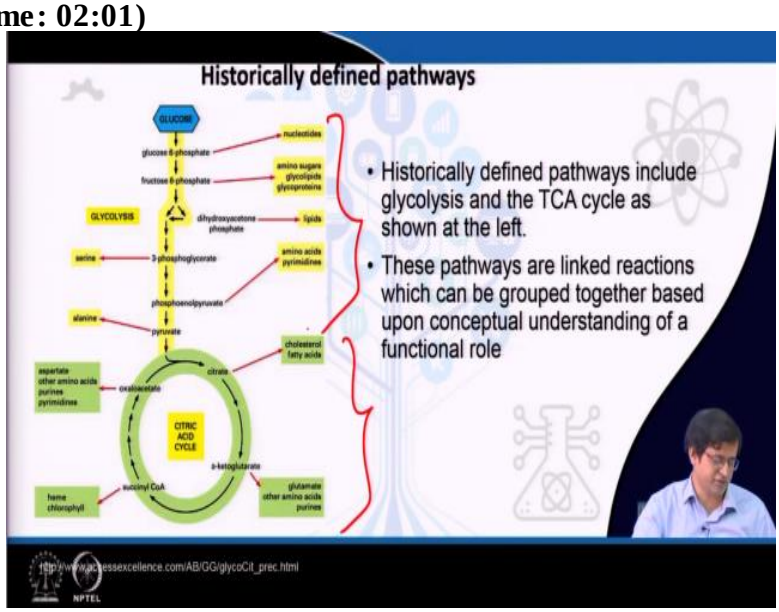

Historically, pathways include glycolysis, TCA cycle as shown in the left, you can see that in early in the history of biochemistry enzymes are isolated from cell, where it shown to carry

out specific chemical reaction. So, from biochemistry, you know the enzyme from the cell which actually carry out a specific biochemical reaction, it was then recognized that the product of one reaction was substrate of another.

So, here you can see that the glucose and then glucose 6 phosphate and that glucose 6 phosphate become converted into fructose 6 phosphate and so on. So, each of the product become the reactant of the other reaction. And it was then recognize that the product bound reaction was substrate of another and thus one could link different chemical transformation to form a series of reaction. So glycolysis is a series of reactions you can see to form a basic metabolic pathway.

So this is where glycolysis is the basic metabolic pathway, TCA cycle is again another metabolic pathway which you already know which are present in most of the organism and so on. Many pathways like TCA cycle, calvin cycle and then we have urea cycle. The definition and biochemical function of such pathway have been taught to generation of life scientists. In the classes previously in different courses, you have already learned about these biochemical pathways.

With the advent of whole genome sequencing and the development of network reconstruction method which I have already learned, we can now piece together in an entire network. So you can add these pathways into different metabolic pathway you can add to form a complete network for example, we have integrated the TCA cycle and the glycolysis together. So, that they can function as a whole. And these pathways are linked reaction which can be grouped together based upon conceptual understanding of a functional role. So, on the functional basis, you have to connect them this pathway together and try to bring about a function as a whole.

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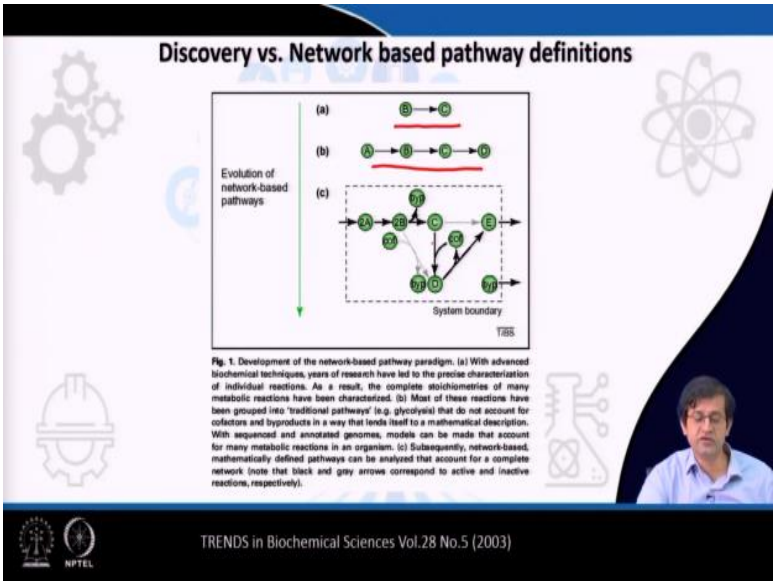

So, this is another example where you can see the network based pathway definition. In the first one you can see here only the reaction with the advanced biochemical techniques years of research have led to be precious characterization of the individual reactions. The individual reactions are characterized by the biochemistry in the lab for several years and as a result, the complete stoichiometry of many metabolic reactions can be characterized.

So that because we have biochemistry that is why; you would be able to make these stoichiometric matrix. But most of the reactions have been grouped into traditional pathway that is glycolysis, TCA cycle. So, in this section you can see that the individual reaction can be combined to form a pathway like glycolysis but they do not account for cofactors. So, there is no cofactor added or by product in a way that lends itself into a mathematical description with sequence.

And annotated genome you can account we can take into account many metabolic reaction in organism to make a model subsequently network based pathway can be analyzed that account for a complete network. So, you can see that some of the reactions which are active which are highlighted in a black arrow and the reaction whose arrow are given in grey arrows are basically inactive reaction.

So, in a network, we can see some of the flux flow for some of the reaction become active some of the reactions remain inactive depending on the regulation it has. So, the network was pathways where you can actually simulate and see which are the pathways and are the

functional state of the network. And the functional changes from one condition to the other condition that we have, we can actually verify using the flux calculation.

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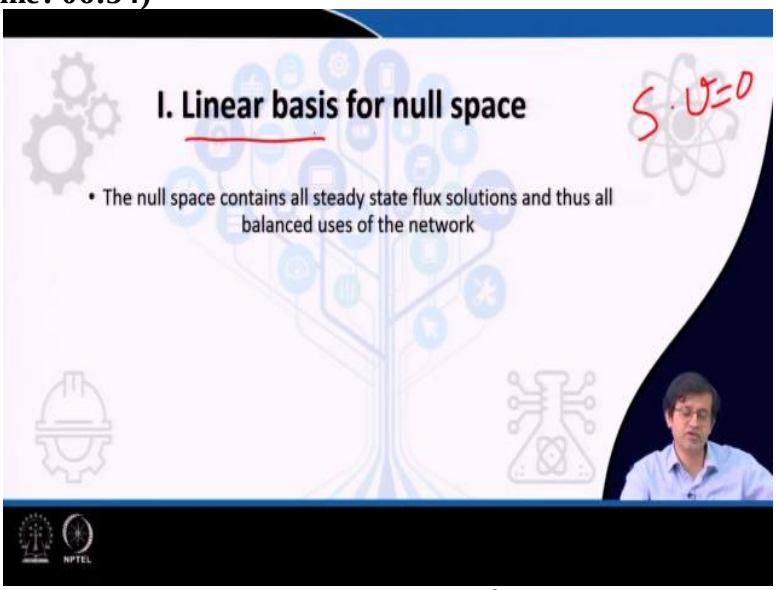

So, then in this class we will learn about linear bases of a null space, the linear bases of a null space, the null space contain all steady state solution, flux solution and thus all balanced to use of the network. So, this null space is basically all steady state solution that is S dot v = 0. In the last class was also I told that, we took a rectangular box and then within the box we have a plane which cuts the box and that is the intersection of the plane and the box is basically this steady state solution space.

And this steady state flux solution you can actually calculate by estimating the linear basis set. The linear basis set or the vector which are present which are actually spanning the steady state solution space. So, today we are going to see how we can calculate the linear basis set of a null space.

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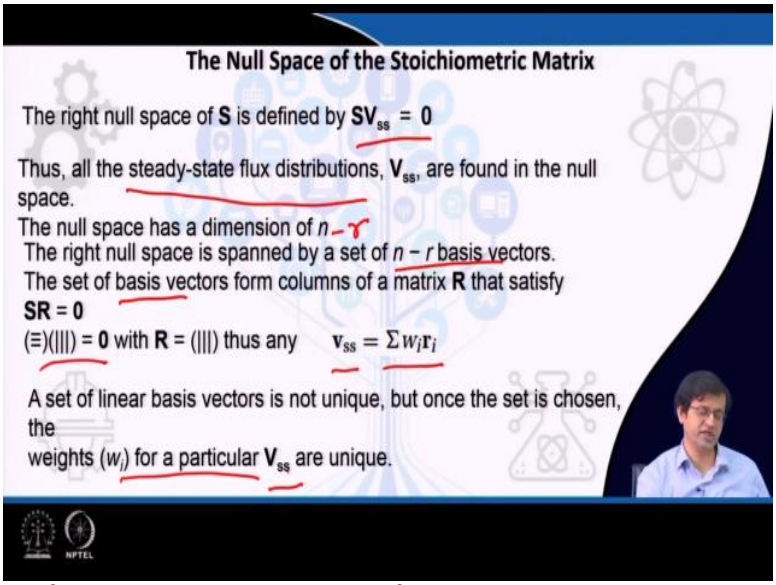

So, the null space of the stoichiometric matrix for any matrix you can calculate the null space yesterday I told you that the null space you can calculate for stoichiometric matrix as well. So, the null space of S is defined as S dot v = 0 or V ss is basically the steady state flux. So, when S dot v = 0 then the solution for this equation you will get the flux for only steady state thus all the steady state flux distribution are found in the null space.

So, whenever you calculate the null space there that is where we will get only the steady state flux distribution then the null space has a dimension of n, and the null space is spanned by n - r basis set. The set of basis vectors are represented of a matrix that satisfy SR = 0. So, the null space is actually have a spanned by n - r basis vector. So, R is the rank of the matrix and then the basis vector for that satisfy SR = 0.

So, if S is a stoichiometric matrix and R is the basis vector which is a column vector, and then if you multiply what you get is basically that is equal to 0 and then you solve this equation. So, you only solve this equation you get it gets steady state fluxes, which is actually a linear combination of r that is the basis vector. So, ultimately if you know the basis vector that is r, then you would be able to get these steady state fluxes. A set of linear basis vector is not unique.

But once the set is chosen, and when you chose the weights, that is W i then it become unique the steady state flux become unique. So, the null space has a dimension of n - r, r is missing. So, n is the number of reaction where n is number of reaction and r is the rank of the matrix.

So, given a matrix of m into n dimension, you can calculate the basis vector and basis vector is easy to calculate.

You can use MATLAB to actually calculate the basis vector of a matrix any matrix can be decomposed into a basis vector and the dimension and the null space has a dimension of n - r, where r is the rank of the matrix. And the basis set is actually if r i is the number of basis set where if you multiply it with a weight like non negative number W i then you get this steady state solutions. So, the weight for a particular v ss is actually unique. Once you choose the weight then your steady state fluxes are unique.

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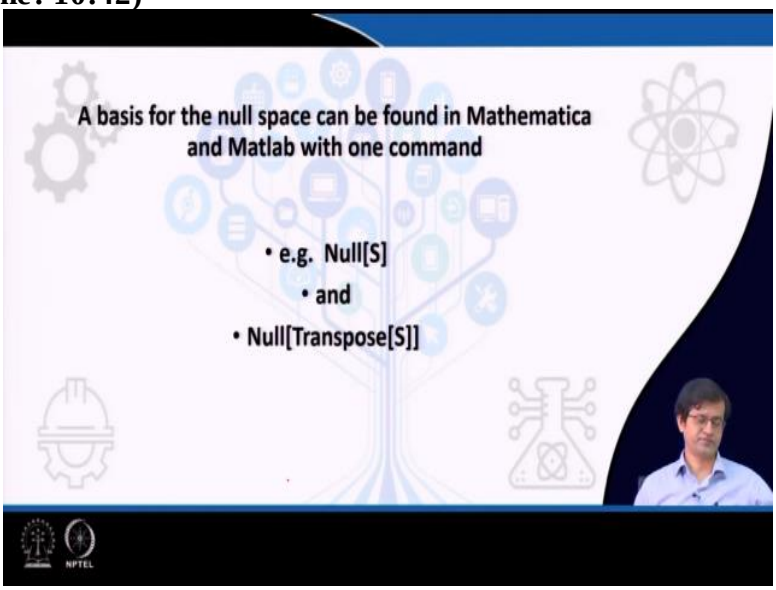

So, as I told the basis for the null space can be found in Mathematica and MATLAB with one command. So, in one command, you just have null, within bracket S that is the matrix we want to calculate the basis vector, immediately MATLAB will give the basis vector. And also you can use the command null for transpose of S. This way you can calculate the basis vector for any matrix and those basis vectors, the linear combination of your basis vector is basically the steady state fluxes.

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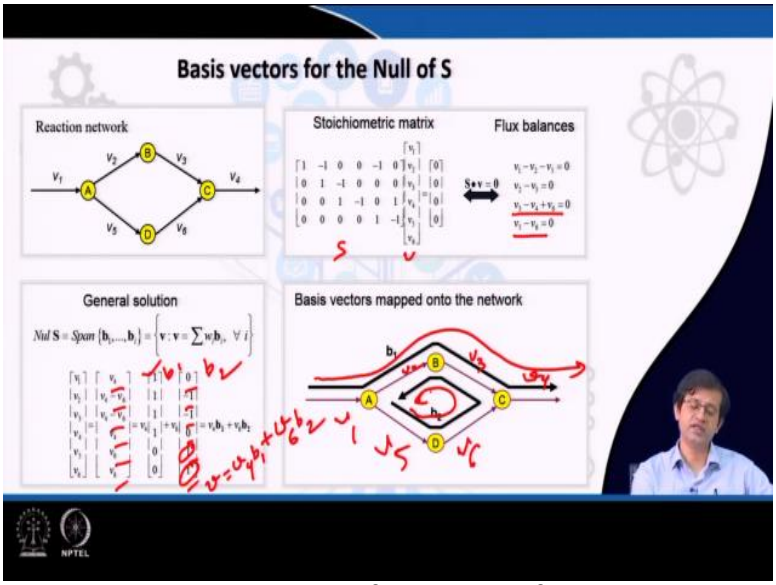

So, let us take an example. So basis vector for the null of S so how do you calculate for this small network reaction network where we have 6 reactions. So 6 reactions you can see v 1, v 2, v 3, v 4, v 5, v 6, and we have 4 metabolites. So, let us calculate the basis vector for this small network. How do you calculate? So, it is very straightforward like you do S dot v = 0. So you have the S matrix here and the flux v if you do S dot v, what do we get?

We will get 4 equations because there are 4 metabolites. So, we have 4 metabolites, that is why you have 4 equations. Now, you solve this equation and you get the flux out of it, very well, how do you do that? So, it is very, by handy you can do just you write the flux vector v 1, v 2, v 3, v 4, v 5, v 6 then you write v 1 in terms of v 4 v 6, because that is v 1 = v 1 the first, if you do the matrix multiplication, what you will get v 1 = v 1, v 2 = v 4 - v 6 that is nothing but this one v 2 = v 4 - v 6.

And then v 3 = v 4 - v 6, and then we have v 2 = v 4 - v 6, and then we have the v 4 = v 4, v 5 = v 6, v 6 = v 6. So, v 5 = v 6 is basically nothing but this one and v 2 = v 3 is basically so if I know v 2 = v 3, that is why we have v 4 - v 6 before that can also be further decomposed into in a vector. So v 4 is nothing but 1, 1, 1, 1, 0, 0.

Because we have 1, the coefficient of v 4 is 1 here, v4 is 1 here, v 4 is 1 here, 1 here, and this is 0, 0, got it? So, v 4 coefficients is only 1, 1, 1, 1 but here it is v 6 v 6, that is why you have a coefficient of 0, 0. And similarly, for v 6, we have coefficient over here is 0, here it is minus 1 here it is minus 1, here it is 0, and v 6. So, the coefficient over v 4 is 0 over here and the coefficient of v 6 is minus 1 here, here it is also minus 1 here v 4 is 0, v 6 is 1 and v 6 is 1.

So, you have that this linear set of equation can be written as v = v 4 multiplied by a constant non negative value b 1 + v 6 into b 2. So now we can see that the your this is vector v 4 and v 6, these 2 we have the basis vector. So that is basically b 1 so, b 1 is basically this one, and b 2 is this one. So b 1 and b 2 is basically your basis vector and v 4 and v 6 and can be any non-negative constant.

So, it is become a linear combination, your flux distribution v is basically nothing but the linear combination of the basis vector b 1 and b 2 multiplied by the constant value v 4 and v 6. So, if you draw these basis vectors on the network, we will see that these basis vectors the first basis vector b 1 that is 1, 1, 1, 1, 1 that is v 1, v 2, v 3, v 4 are actually carrying flux so, this which is nothing but this pathway.

So, this is v 1 and this is v 2 this is v 3 and this is v 4. So, your first equation is basically 1, 1, 1, 1; v 1, v 2, v 3, v 4. v 1, v 2, v 3, v 4 are actually carrying flux and the other b 2 is basically v 2, v 3 are actually v 5, v 6 are actually negative and it is going against the reaction. So, you are in the other basis vector which is v 5 and v 6, are positive so, these are positive these are positive.

So, v 2, v 5, v 6 are positive, so, it is going in this direction this way. So, these basis vectors basically we have 2 basis vector for this network and these basis vectors are actually encompassing the entire flux solution. The solution space is actually can expand by these 2 basis vectors and these basis vectors you can immediately calculate using a MATLAB given a matrix and just with one command you can calculate the basis vector.

Now, you can see the basis vector 2 is wrong, why it is wrong? Basis vector 1 is fine, because the reactions flux is along the direction of the basis vector, but if you look into basis vector b 2, it is going against the reaction, so it is going in the opposite direction. So, for reaction v 3 and v 2 basically, we have the reaction which is actually going against the reaction. So, the basis vector v 2 is kind of not satisfied.

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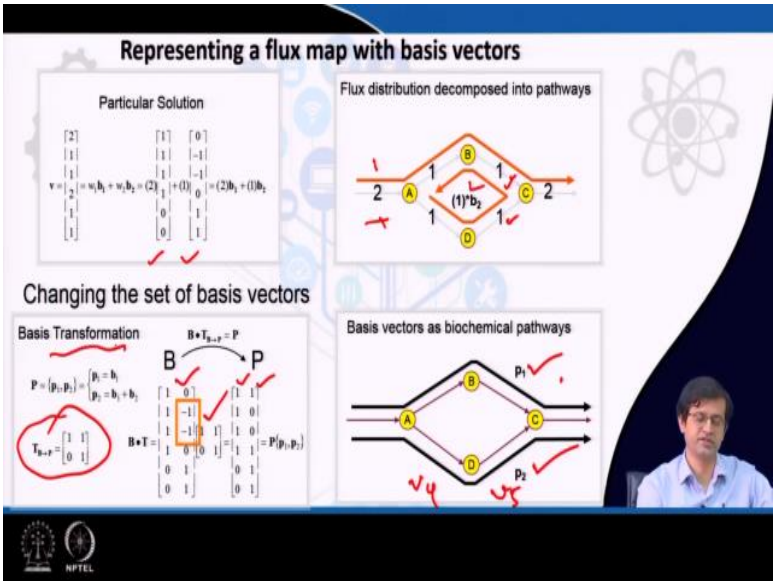

So, how do you correct that? So, this is what do we have the particular solution where you have the basis vector 1 basis vector 2 and we multiplied by a constant weight like 2 and 1 then it becomes a particular solution. Similarly, if you see the flux distribution can be decomposed into 2 pathways, first this is pathway 1 and this is pathway 2 and then you have a weightage.

So, if you put weightage 2 what do you have and the flux 2 which is entering from here which is distributed into 1, 1, 1 and then again it combined 1, 1 and become 2 so, it is fine. So, how do you change the basis set? Changing the basis it is also possible where you can multiply by a small 2 by 2 matrix. So, if you multiply it by a transformation matrix basis transformation matrix that is 1, 1, 0, 1 that is shown over here if you multiplied with the basis vector.

So, this is the original basis vector and this is the transformation basis vector. So, if you multiply these 2, you will get a new basis vector where the basis vector first basis vector remain the same only the second basis vector has changed where you can see that the negative sign, the basis vector p 2 is basically shown over here. So, if you plot it what you will see, this is basis this is the first basis vector p 1 and this is the p 2.

So, here you can see that, well the v 1 is carrying flux and then v 4 and v 5 carrying flux and v 4 and v 5 and then also and this is also carrying flux. So, this is the other basis with p 2. So, now, the basis vectors are correct because they are along the direction of the reaction. So,

none of the pathway is against the reactions. So, this is how you correct by using a basis transformation matrix, this one.

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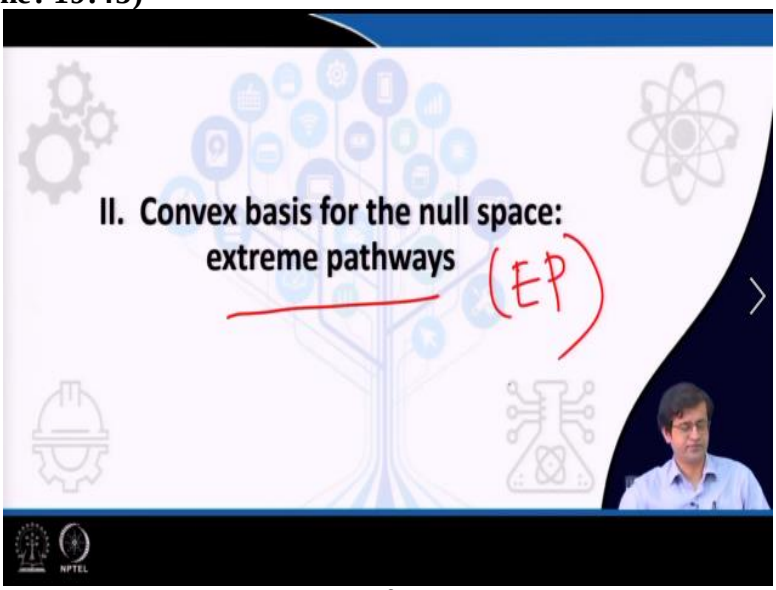

So, now we will talk about the convex basis for the null space that is extreme pathway. So now we will come to the extreme pathway. Some idea you have got about the basis vector. How do you can calculate the basis vector for a matrix and then how basis vector looks in terms of pathway and now what is extreme pathway based on null space? Once you have the null space you will be able to calculate the extreme pathways that is EP.

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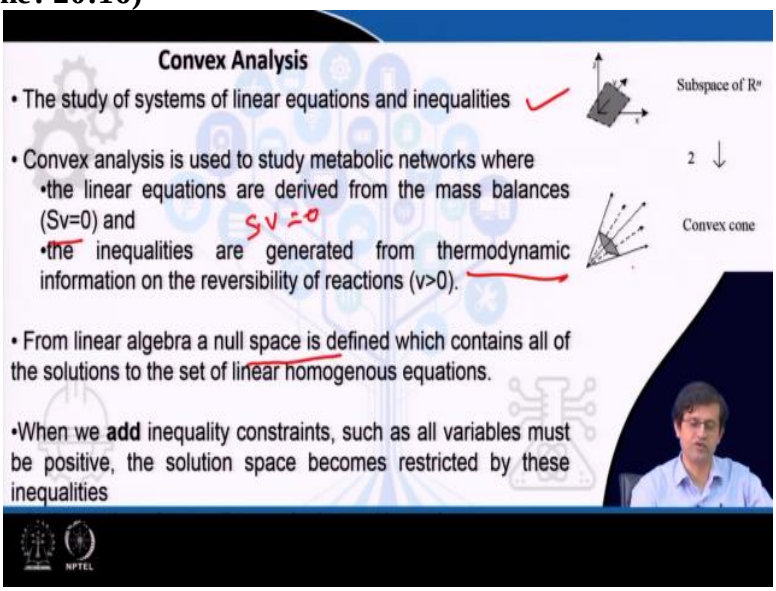

So, extreme pathways is very important for that you have to do convex analysis what is convex analysis that is study of a system of linear equation and inequality the convex analysis actually deal with linear equation and also inequality. The converse analysis is used to study metabolic network while the linear equations are derived from the mass balance involve

linear equation and that you get from the mass balance equation that is S dot v = 0. The inequality are generated from thermodynamic information or the reversibility of the reaction that is a boundary condition that is, v greater than 0.

From linear algebra a null spaces defined which contains all the solution space to the set of linear homogeneous equation. So, once you have defined null space, then you will see that all your solution actually lie in this linear homogeneous equation. When we add inequality constant such as all variables must be positive the solutions become restricted to positive quadrant, remain in the positive quadrant.

Because all the variables must be positive when you add inequalities and constantly make sure that all the variables are positive. So, the solution becomes restricted to positive quadrant. So, this is what we have the convex space when you apply the constraint that is the inequalities from thermodynamics and also apply the mass balance equations that is S dot v = 0 then you see becomes a convex cone and all your inequalities are positive that is why it remained in the positive quadrant.

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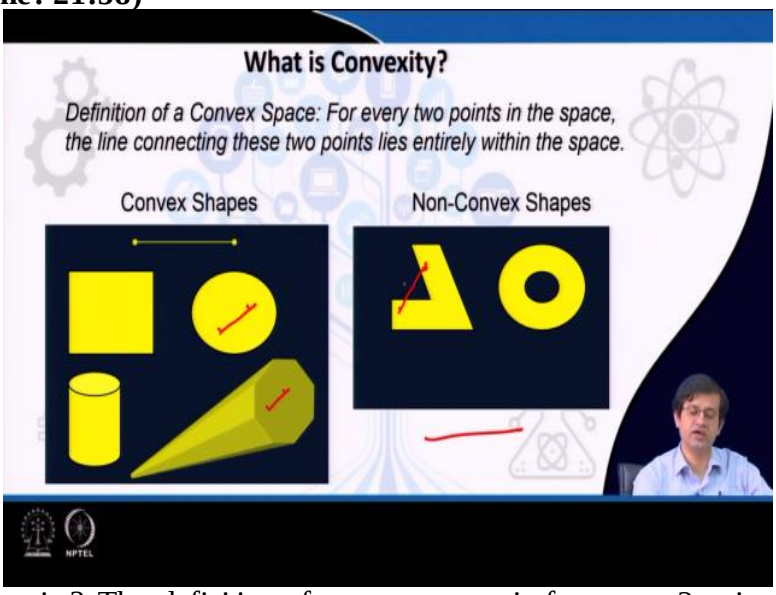

So, what is convexity? The definition of convex space is for every 2 point in this space the line connecting these 2 points should lie entirely within this space that it is solution space you are defining the convex space. So, any 2 point in the convex space if you connect it by a line and then if it lies within the space then it will entirely lie within the space for example, the convex shapes are given over here and these are non-convex space, shown over here, in the right hand side.

So, any 2 point if you draw in the convex space, you will see that if you connect this line, then it will lies within this sphere also in the cylinder also or in the rectangle as well you can see that any 2 point within this space if you connect it then it lies within that solution space. But in a non-convex space, if you draw a line over here and you try to connect it, it is going outside the convex space, so, it is actually a non-convex space not a convex one.

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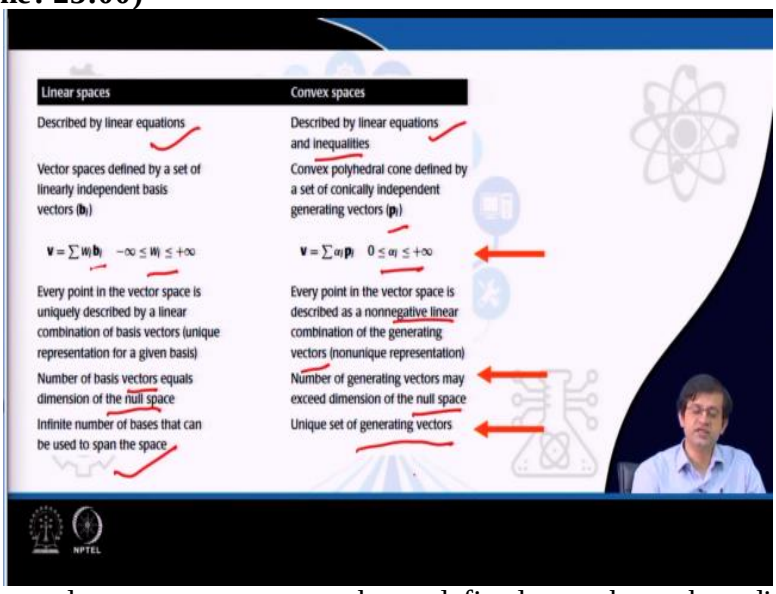

So, linear spaces and convex spaces, we have defined over here they distributed by both distribute by linear equation, both have linear equation but in convex space we have the inequalities that is the difference between the linear space. The vector space defined by a set of linearly independent basis vector b. The vector space in linear spaces are defined by the basis vector which I have already defined b i.

Whereas, in convex space polyhedral cone, it define a generating basis vector v which is basically an alpha p i linear combination of the basis vector, but your alpha is always positive see here that is the difference. So, in the linear space the w i goes from minus infinity to plus infinity, but whereas in convex where we have 0 to infinity that is the restrain the inequality you have a convex space where everything is positive.

So, every point in the vector space is uniquely described by a linear combination of the basis vector whereas, in convex space, the every point in the vector space is described by a non negative linear combination of the generating vector. So, this alpha is basically always

positive and the number of genetic vector may exceed the null space through these p i it can exceed the dimension of the null space and we have a unique set of generating vector.

So, the number of basis vector is actually equal to the dimension of the null space in case of linear space and there are in finite number of bases that can be used to span the space whereas, there are a unique set of generating vector to represent the convex space. So, this is a different way in the convex space and the linear space.

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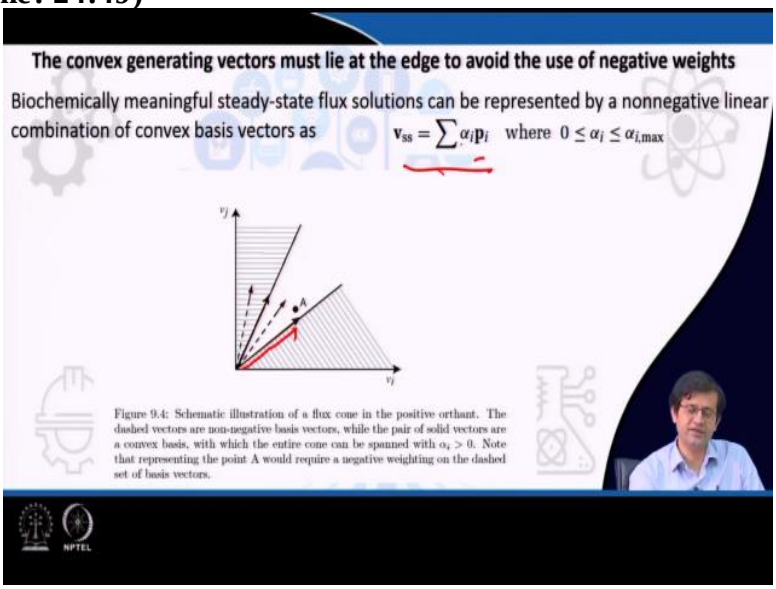

So, now the convex space generating vector must lie at the edge to avoid the use of negative weight. So, this is one of the outcome that you get from the convex space that the vector always lies at the edge. Lies at the edge of the solution space and biochemically, this steady state flux solution can be represented by non negative linear combination of the convex basis vector, which I have already told you.

So, the steady state fluxes are always can be represented by a non negative linear combination of the convex basis vector. So p i is the basis vector alpha i is a non negative parameter constant value that you multiply to get the steady state fluxes.

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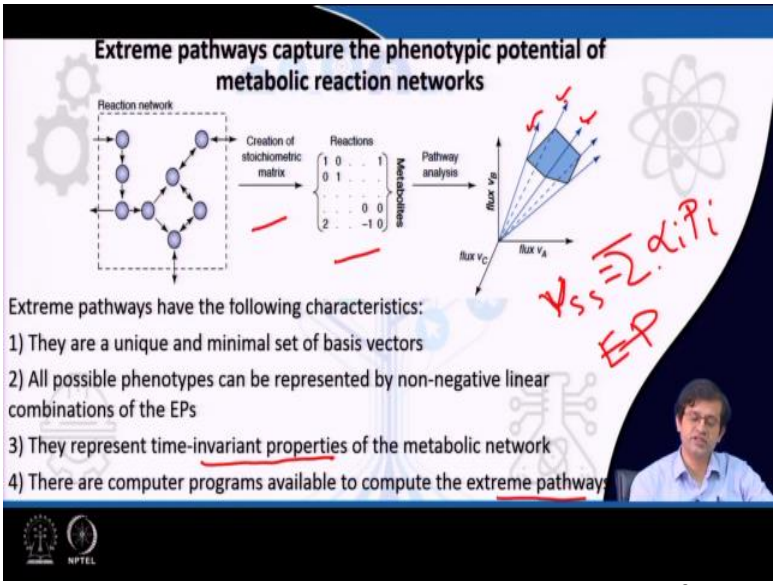

So, the extreme pathway you can capture the phenotypic potential of the metabolic network. How do you capture the phenotypic potential? We will start with the network and then you create this stoichiometric matrix and that is the reactions are column and metabolites are rows and then you construct the metabolic analysis that is in calculating the basis vector. So, once you calculate the basis vector this is one basis vector another vector which actually enclosing the entire solution space.

So, how many basis vector you have depend only based on that you can draw the solution space in this space. So, the extreme pathways have following characteristics, these are the extreme paths which are given by the basis vector. So, what are the properties for the extreme pathway they are unique and minimal set of basis vectors. So, they are unique and they have a minimum set of basis vector.

So, all possible phenotypes can be represented by non negative linear combination of the elementary pathway basically a linear combination, which I have already told. All possible phenotype V ss is basically sigma of alpha i p i. So, this is the steady state flux distribution, these are basically different phenotypes. So, these all phenotypes, you can calculate all possible phenotypes can be calculated from nonlinear combination of the elementary pathways.

So, you can change the alpha i, you get different phenotypes, that is what elementary pathway says and then they are represented by time variant properties of the metabolic network. So, the elementary pathway is basically not going to change, if we change the condition it is the

elementary pathways remain the same and that is why they are time invariant. And there are computer program available to calculate the extreme pathway. So, as I told as in MATLAB or Mathematica, you can easily calculate the elementary pathway.

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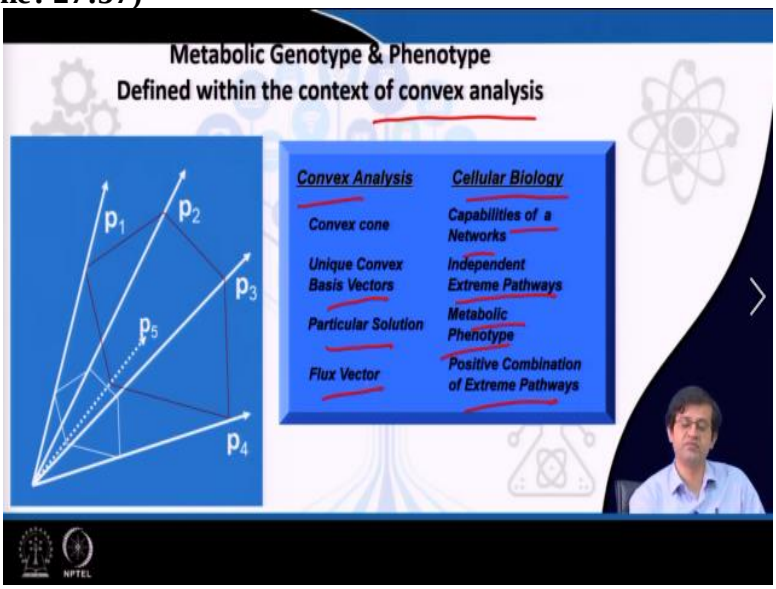

So, the metabolic genotype and phenotype which is defined within the context of convex analysis so, metabolic genotype and phenotype you can define by doing convex analysis, whereby if you do a convex analysis, you will be able to get the basis vector p 1, p 2, p 3, p 4, p 5 which actually encompasses the entire solution. So, the convex analysis can be related to cellular biology like convex cone, which is basically capability of the metabolic network in cellular biology.

And then unique convex that is the basis vector are nothing but the basis vector you get from the convex analysis is nothing but the extreme pathways which actually describe different phenotypic state of a cell and then we have a particular solution that is basically a metabolic phenotype. So, for whatever solution you get from the convex analysis is basically a metabolic phenotype and the flux vector is nothing but the positive combination of the extreme pathway. So, the combination of p 1, p 2, p 3, p 4, p 5 you get the flux vector that is different phenotype of the cell can be described by extreme pathways.

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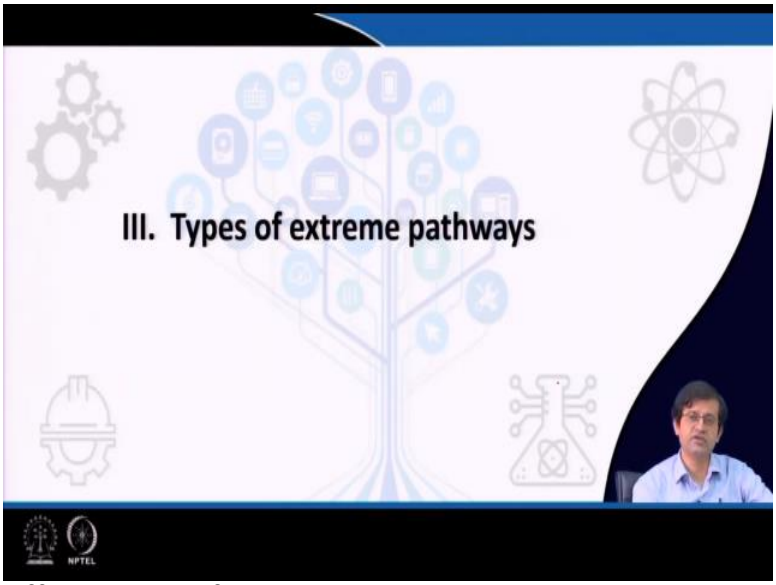

So, what are the different types of extreme pathways?

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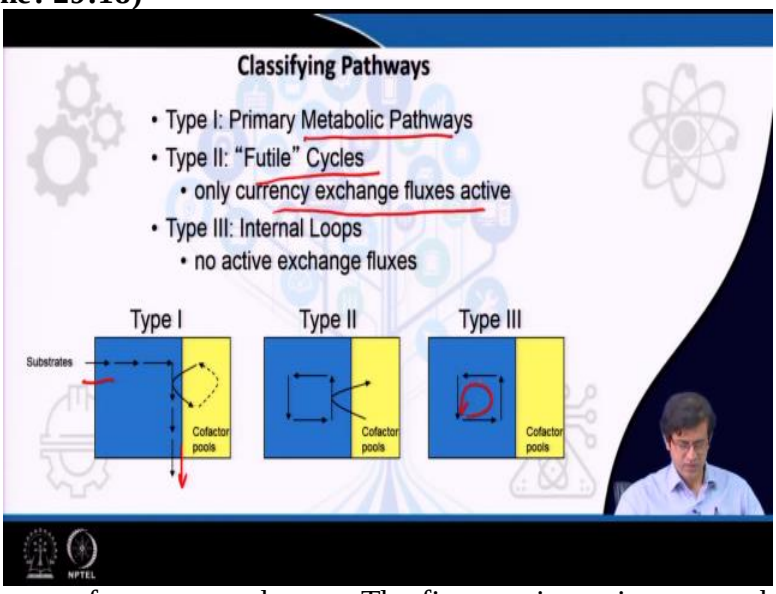

So, there are 3 types of extreme pathways. The first one is a primary metabolic pathway and then we have the type 2 futile cycle only currency exchange fluxes are active and then type 3 we have the internal loops. So, in the type 1 you can see that the metabolite the substrate is entering the cell and then some product is coming out. So, in primary type 1 metabolic pathway you can be see that the exchange reactions are involved. what is the difference between the main characteristic of type 1 pathway?

The type 1 extreme pathway actually involves the conversion of primary input into primary output and thus contains the exchange fluxes with the environment. So, these are the exchange fluxes, you can see that it is crossing the boundary and here also it is crossing the

boundary. So, in both the cases we see that I say extreme exchange reaction exchange fluxes and type 1 metabolic pathway is actually involving the exchange fluxes.

And the type 2 extreme pathway is actually internal there is an internal exchange of metabolites such as and that is currency metabolite. So, only the currency metabolites are exchanged in a futile cycle. So, this is a cycle which is present inside the cell and then the currency metabolites are exchanged in this loop, the type 2 extreme pathway involve the internal exchange of currency metabolite that is ADP energy, NADH.

And then we have type 3 pathway in type 3 extreme pathway, we have internal cycle. So, this is the internal cycle you can see over here and then important characteristic of type 3 pathways is that it does not carry any fluxes. There is 0 fluxes across all system boundaries. So, these are the extreme pathway, the type of extreme pathway.

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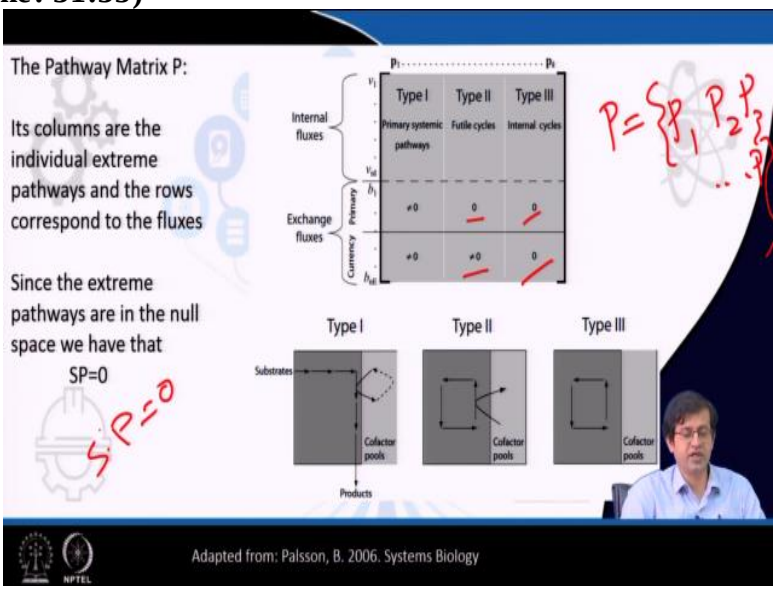

Then, we have the extreme pathways which are shown over here the type 1, type 2, type 3. In type 1 you can see the internal fluxes are involved and the exchange fluxes are actually not equal to 0 in case of type 1. Whereas, in type 2 the exchange fluxes are 0 for primary exchange fluxes but for the currency metabolite exchange fluxes is not 0. Whereas in type 3, which; is basically internal cycle in the both exchange fluxes are 0, either primary or currency.

And the pathway matrix P that is the matrix P is given by p 1, p 2, p 3, and so on. These are the extreme pathway that if you multiply it with S this stoichiometric matrix that is S dot P =

0 where P is the extreme pathways. So, these columns are the individual extreme pathways and the rows correspond to the fluxes.

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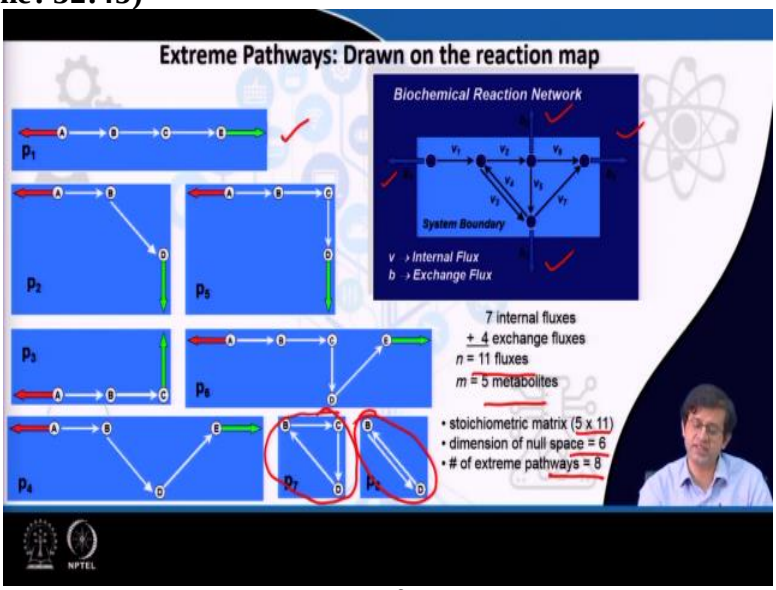

Now, we will calculate the extreme pathways for this metabolic network. So, these metabolic network what do you see is basically there are 7 internal fluxes v 1, v 2, v 3, v 4, v 5, v 6, v 7, these are the 7 internal fluxes. And then we have 4 exchange fluxes 1, 2, 3, 4 these are the 4 exchange fluxes. So, that is why how many metabolite we have in this network we have 5 metabolites.

So, we have 11 internal fluxes; total 11 internal fluxes, so, 7 + 4, 11, 7 internal fluxes and 4 exchange reaction that give rise to 11 fluxes and 5 metabolites. Now, you want to calculate this stoichiometric matrix. Stoichiometric matrix will have a dimension of 5 by 11 matrix and the dimension of the null space, either you can calculate in a pen and paper for null space also, you can use the computer like MATLAB to calculate the null space.

If you calculate the null space what you will get is basically the dimension of the null space will be 6 and the number of extreme pathway which is evaluated for this network is basically 8. How it is possible? So, we have p 1, p 2, p 3, p 4, p 5, p 6, p 7, p 8 these are the 8 extreme pathways. And out of that is 8 extreme pathways 6 are the basis vectors. So, the basis vector p 1, p 2, p 3, p 4, p 5, p 6 these are the basis vector which is basically p 1 is nothing but composed of reaction v 1, v 2, v 6 and p 2 is composed of v 1, v 4 and this exchange metabolite.

And then we have p 3 which is composed of metabolites A, B, C and p 6 is composed of metabolites A, B, C, D, E and p 4 composed of metabolite A, B, C, D, E. So, these are the extreme pathway, which are present, which you can calculate from the basis vector and those are plotted over here. And out of 7 basis vectors, 6 basis vectors are present in the extreme pathway, but the 2 pathway that is p 6, and p7, they are forming a futile loop so, this is a futile loop.

So, actually not carrying and not involve in any particular metabolite formation, but they are present in the network which is forming a loop inside the thermodynamically invisible loop inside the network. So, mainly, the metabolite formation and flux distribution are represented by the null space basis vector that was shown over from p 1 to p 6.

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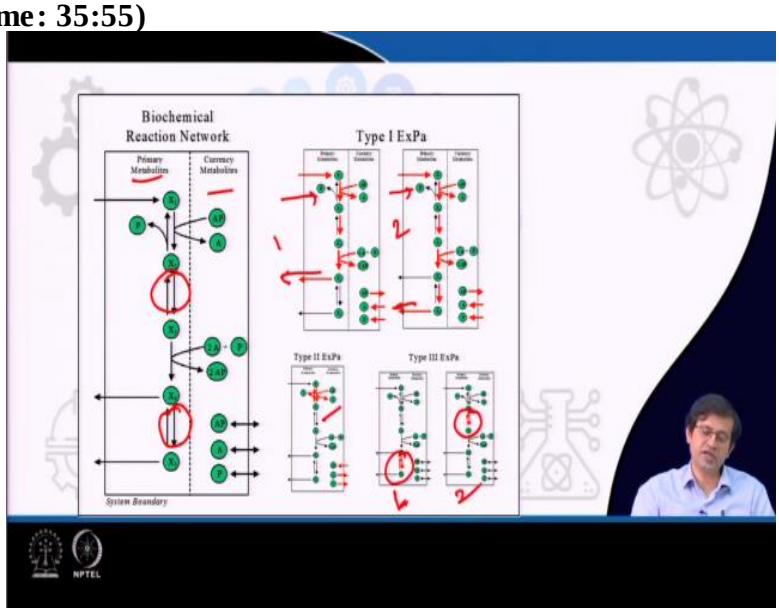

So, this is the extreme pathway analysis have been done in glycolysis where we have the primary metabolite and the currency metabolites. We have our primary metabolite and the currency metabolites and the type 1 pathways have been calculated for glycolysis. So, we have 2, type 1 pathway. So, what are those 2, type 1 pathway? This is the first pathway where substrate is entering and then subset is going out.

So, this is one of the type 1 pathway and the other one is a subset is going in and then product is going out. So, this is another, this is 1 this is 2; total type 1 pathway present is 2, 1 and 2. And they both are actually type 1 extreme pathways, then type 2 extreme pathways how many type 2 extreme pathways are present? there are only 1 type 2 extreme pathway. So, this

is the currency metabolite exchange you can see, so, that is why its a type 2 extreme pathway and type 3 extreme pathway there are 2 type 3 extreme pathway and this is 1 and this is 2.

So, these type 3 extreme patterns are basically the futile cycle over which is shown over here and is also shown over here. So, the futile cycles are basically this one and also this one. These 2 consists of type 3 extreme pathway. This way you can decompose any metabolic network or part of the network into different types of extreme pathway and type 1, type 2, type 3.

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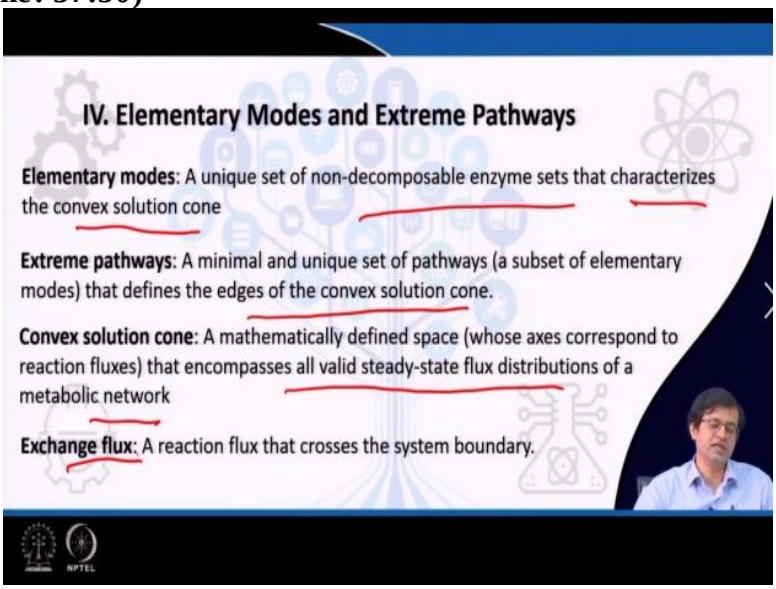

So, now we will discuss about the elementary mode and the extreme pathways. So, elementary modes are a unique set of non decomposable enzyme set that characterize the convex solution cone. The convex solution which you already described that you can actually decompose into enzyme set and that becomes your elementary mode. And extreme pathway which you already learned. The minimal and unique sets of pathway that; defines the edge of the convex solution cone that is a minimal and a unique set of pathway.

And the convex solution cone and mathematically you can define a space that encompasses all valid steady state flux distribution of a metabolic network. And inside the convex cone gives you the all feasible steady state flux distribution of the metabolic network and reaction flux that crosses the boundary is also known as exchange flux. So, these are the definition you should remember what is elementary mode, what is extreme pathway what is convex solution cone and the exchange fluxes?

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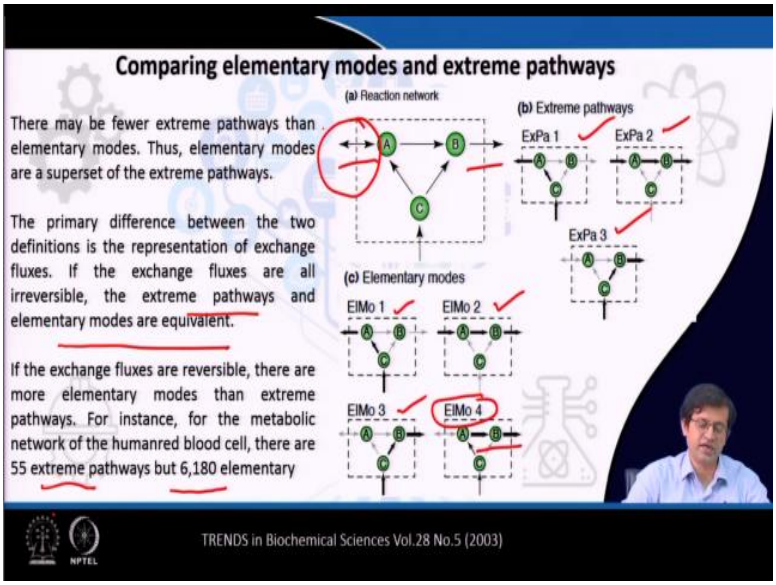

So, how do you compare the elementary modes with extreme pathway? So, I have a comparison between an extreme pathway and elementary modes you can see that this is a simple network which has 3 metabolites which consist of 3 reactions. The 3 reaction metabolic network is shown over here and these are the exchange reaction and these are the exchange reaction these are the exchange reaction.

Now, you actually convert this network into extreme pathways. So, how many extreme pathways are there? So, extreme pathways will depend on the number of basis vector, from this basis vector there are fewer extreme pathway. So, extreme pathway 1, extreme pathway 2, extreme pathway 3 so, for extreme pathway, you can see that the flux is going from here to here.

And then from extreme pathway 2 the flux is going from here to here. And then for extreme pathway 3 it is going from here. So, this way you can actually get the extreme pathway for a given network. And now, you want to actually calculate the elementary modes, the elementary modes also you can see the elementary modes for this network it has more than the extreme pathway.

So, there are fewer numbers of extreme pathways then elementary mode thus elementary modes are a superset of the extreme pathway. So, that if you consider extreme pathway as set A and elementary mode as set B then every set of A will be present in set B, but the reverse is not true. The primary difference between the 2 definitions is the representation of the exchange fluxes.

If the exchange fluxes are all irreversible then the extreme pathways and the elementary modes are equivalent, since we have 1 extreme pathway over here. So, that is why the number of elementary mode and the extreme, pathways are not equal. So, if they are equal, there are no reversible pathways, and then we would have the same number of extreme pathway and elementary mode. So, if the exchange fluxes are reversible, there are more elementary modes, than extreme pathways. So, the exchange fluxes are reversible.

So, here we have 1 reversible reaction that is why the number of extreme pathway and the elementary modes are different. For instance, for the metabolic network of human blood cells, there are 55 extreme pathways and number of elementary modes is 6000. So, you can understand that depending on the number of exchange reaction then it can multiply and you can have more number of elementary modes compared to the extreme pathway.

So they extreme pathway is a unique pathway. That is why the numbers are very less, but based on the exchange reaction you can see that these are the 3 elementary pathways which are already there in the elementary mode. So, this exchange pathway 1 is equivalent to elementary mode 1 and then we have a exchange pathway 2 is actually equivalent to elementary mode 2 exchange pathway 3 is equivalent to elementary mode 3 but since because you have the exchange reaction, that is why the elementary mode 4 is appearing.

So this is not present in the extreme pathway. So, the elementary modes 4, is not there in extreme pathway. So, this is the difference you are getting so, because of the exchange fluxes, because we have excess fluxes here that actually making it difference. So, these extreme pathway analyses have been performing a human red blood cell, where they actually got around 55 extreme pathways and also 6180 elementary modes.

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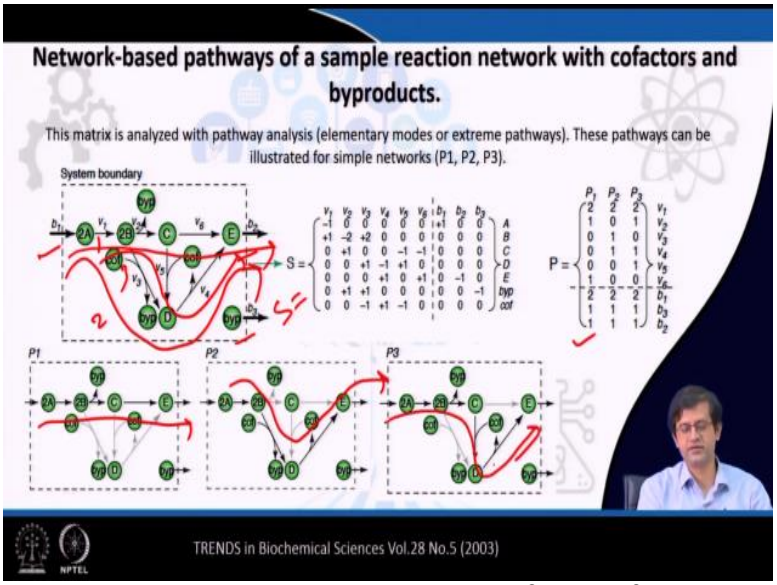

So the network based pathway analysis, have been performed for a reaction network with cofactor and byproducts. Here you can see metabolic network which is shown over here where you have the cofactors and we have byproduct as well. So the byproduct is shown over here and then these are the product and the substrate and we have the cofactor which is going in the reaction 3 and then we have byproduct forming here.

So if you construct the stoichiometric matrix for this network, you can easily make the stoichiometric matrix by considering the columns or the reaction v 1, v 2, v,3, v 4, v 5, v 6, and the exchange fluxes v 1, v 2, v 3 are toward the end of the stoichiometric matrix and the metabolites we have A, B, C, D, E, byb and cofactor and then once you have the S defined then you can actually calculate the basis vectors.

So, p 1, p 2, p 3 are the basis vector which are nothing but the your extreme pathways. So, the extreme pathways are given by p 1, p 2, p 3, so, p 1, p 2, p 3, if are the extreme pathways, then elementary modes also will also have p 1, p 2, p 3 because it is a superset. So, p 1 is shown over here, these are the 3 extreme pathways which are shown over here. This is the first extreme pathway and then we have the second extreme pathway like this and then we have the other extreme pathway going like this. This is 1 this is 2 and this is 3 clear? It is nothing but we have the p 1 going here and then p 2 going like this and then p 3 goes like this.

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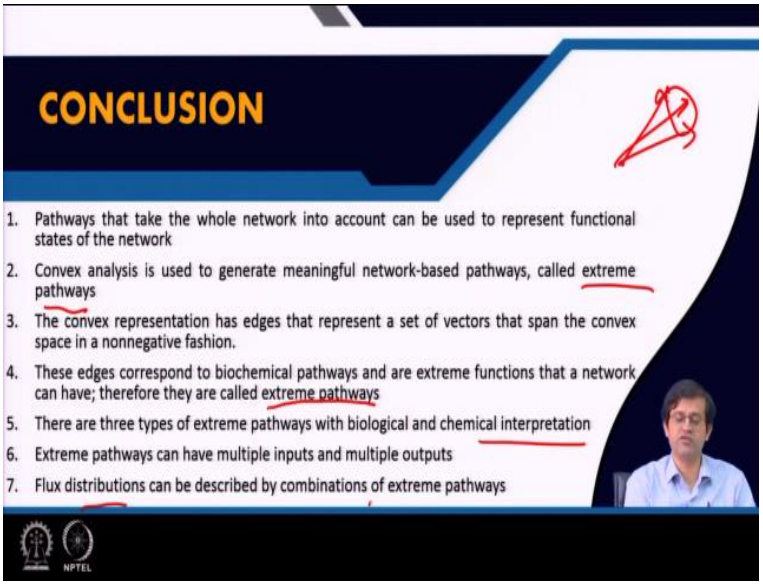

So, in conclusion, the pathway that takes the whole network into account can be used to present functional state of the network. So, using the pathway you can represent the functional state of the network like the phenotype of the cell. And the convex analysis is used to generate meaningful network this pathway called extreme pathway. So, from the conventional analysis you would be able to construct the extreme pathway, the convex representation has edge that represent a set of vector.

So, the convex representation as I told it form a, it has edge it has an edge and these edges are basically your extreme pathway. So, the convex representation has edge that represents a set of vectors that span the convex space in a non negative fashion. These edges correspond to biochemical pathways and are extreme function. And this pathway have an extreme function that a network can have therefore, they are called extreme pathways.

So, this is why they are called extreme pathway? because they can perform extreme function and that a network can have and therefore, we can call them as extreme pathway. There are 3 types of extreme pathway biological and chemical interpretation. And the extreme pathways can have multiple inputs and multiple outputs. The flux distribution can be described by combination of extreme pathway. The flux distribution of actually that is the phenotype of the cell can be described by combination of extreme pathway.

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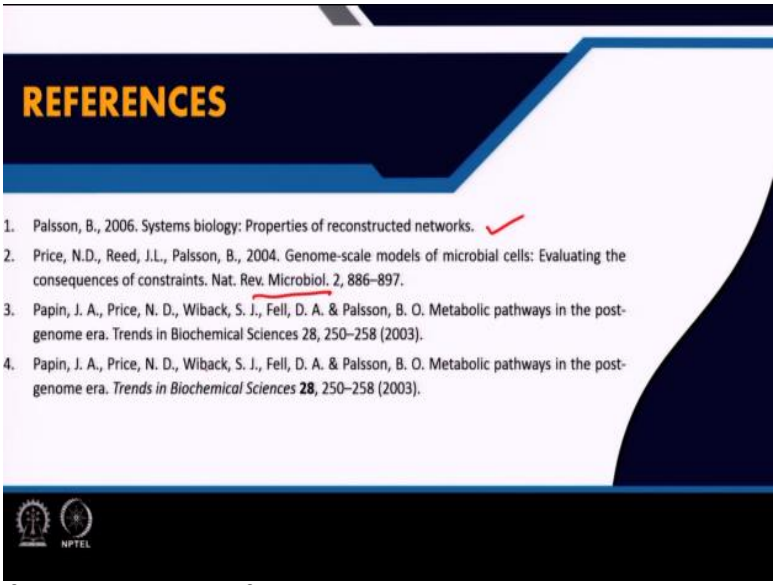

These are the references you can follow the book by Palsson in 2006 that is systems biology, properties of reconstructed network that you can read and Nat. review of microbiology also you can read and then trends in biochemical sciences paper published by Papin that also you can read for further study. So thank you, thank you for listening, we are closing here. Thank you.