An Introduction to Metabolic Control Analysis

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1 Introduction

The majority of genes within a cell encode for metabolic functions to create products. These functions can be thought of as pathways constituting of enzymes and metabolites. The metabolites are the intermediate substances used in making the product. Within a cell, amounts of different products from separate reactions come together to form the final product. The creation of a product can be thought of as a system of linear equations. Each equation in the system relies upon the moles (concentration) of each intermediate metabolite and reaction fluxes (amount applied per unit time.) Since the concentrations of enzymes help power the rate at which the creation of the product occurs, an enzyme basically controls each separate reaction in the system. Metabolic control analysis is the study of how these two variables are distributed among the different enzymes that control the system and most importantly how this distribution changes in response to perturbation. A perturbation can be described as a non-random disturbance such as a change in the concentration of a metabolite, a hormone secretion, or a cell proliferation (Cascante 244). Mathematical theories for analyzing reaction rate and metabolite concentration can be found in linear algebra. The ideas of several theorists over the 20th century, Reder, Cascante, Fell and Sauro, and their precursors, have come together to make one general method. Certain assumptions need be made for such methods:
1) All reactions in a metabolic system are interconnected.
2) A metabolic system can be studied at a steady state such that all concentrations of metabolites remain constant over time.
3) Metabolites in a system are distributed equally over the enzymes that act on them.
4) Rates of reaction are directly proportional to enzyme concentration.
5) Enzymes are parameters, not variables.
6) Amounts of certain substances do not affect a metabolic systems steady state.
(Fell 314)

2 Technique for Analyzing Metabolic Systems

In metabolic control analysis, a method has been developed for analyzing metabolic reactions to perturbations.

Reder developed an analyzing technique that relies simply on the stoichiometric relationships, describing how metabolites in the system of reactions combine to make products. Reder justifies relying only on this structural component on metabolic systems by saying it would be incoherent to work with detailed kinetic expressions before analyzing the structure of the model and its consequences (Reder 3). Defining environmental perturbations (temperature surrounding system, air flow, etc.) at the same time as analyzing the metabolite combinations would be overly intricate and result in disappointment. So the following is a way to represent chemical pathways as a system of equations, where the only system variables are metabolite concentrations, which are controlled by the rates of each reaction in the system, which are controlled by enzymes.

The rate at which a product of a system is created:

$$\frac{\Delta[X_i]}{\Delta t} = \Sigma_j S_{ij} v_j$$

Where S_{ij} is the number of moles metabolite X_i in reaction j and v_j is the rate of reaction j.

2.1 Stoichiometric Matrix

We now make a stoichiometric matrix, S, in which each reaction equation is listed vertically, as we are solving for rate of the reaction of each equation, rather than rates of the varying metabolites. The concentrations of substances, such as metabolites or ATP or NADP, with fixed concentrations are ignored. This is stated by assumption six. Let these substances be represented by $*$. Let M equal the number of varying metabolites (and therefore the number of concentrations) and let R equal the number of reactions (and therefore the number of enzymes and reaction rates).

Definition Stoichiometric Matrix
If S is a stoichiometric matrix, then S has M rows and R columns such that column j represents reaction j and the individual entires of S are such that:

$[S]_{ij} =$
$+\ \alpha$ if reaction j produces α molecules of M_i,
$-\alpha$ if reaction consumes α molecules of M_i,
0 otherwise

Example S1
A fake system of chemical equations: M=5 R=4
1. $* + M_5 \rightarrow M_1$
2. $M_1 \rightarrow M_2 + 2M_4$
3. $M_2 + 2M_4 \rightarrow M_3$
4. $M_3 \rightarrow M_5$

$$S = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \\ 0 & 2 & -2 & 0 \\ -1 & 0 & 0 & 1 \end{bmatrix}$$

And $\frac{\delta x}{\delta t} = S\mathbf{v}$

From here, we can analyze $\frac{\delta x}{\delta t} = S\mathbf{v}$ in a more complicated manner by introducing parameters to the rate of reactions. But first, here is an overview of what will happen without parameters to give you a basic idea of the process.

2.2 The Basics

If you assume no parameters on the reaction rates, then you can analyze the system in a simple matter. Assume a steady rate of reaction, then $\frac{\delta x}{\delta t} = 0$ and then

$$S\mathbf{v} = 0$$

Then we can simply say that the vector $\mathbf{v}$ may be found in the null space of S, $N(S)$, which dictates how reaction rate control is given to the individual enzymes of

the reactions in the system assuming that the rate of concentrations of metabolites does not change. Obviously, if the columns of S reduce to R pivot columns, the only solution for $\mathbf{v}$ is the trivial solution, a vector of zeros. This is problematic since that would mean the only way to achieve a steady rate of reaction would mean a halt of each reaction j.

A more likely situation in metabolism is that S does not reduce to R pivot columns. In this case conservation relationships hold.

Definition Conservation Sum
A conservation sum, $\mathbf{T}$, is the sum of the concentrations of metabolites which remain constant in a metabolic system regardless of parameter p.

What basis vectors should one choose for $N(S)$? Each vector in the null space is a solution to the rate of reactions vector $\mathbf{v}$. Meaning, if a vector, $\mathbf{v}$, in the $N(S)$ reads

$$\begin{bmatrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \end{bmatrix}$$

then 1 unit of flux, a measure of reaction rate control factor, is given to the enzyme which controls the first reaction, 2 to reaction two, and so on. Each choice of a vector $\mathbf{v}$ in the span of $N(S)$ may create a different product, for the metabolic system.

Example S2
Notice that in S from example S1, one conservation relationship can be found by adding rows 1, 2, 3, and 5 together. They sum to 0. From this we can say that $\frac{\delta x}{\delta t}(M_1 + M_2 + M_3 + M_5) = 0$. So the metabolites' concentrations remain constant such that $x_1 + x_2 + x_3 + x_5 = \mathbf{T}$.
Also,

$$N(S) =< \{ \begin{bmatrix} 1 \\ 1 \\ 1 \\ 1 \end{bmatrix} \} >$$

So each reaction in the metabolic system represented by S is controlled proportionally.

Any vector in the span of the vectors from $N(S)$ is theoretically and biologically feasible (Schilling 299). Therefore, in the past, a large aspect of MCA was designing computer programs to create and choose desirable basis vectors. Other methods for determining elegant vectors have been found in convex analysis (Schilling 300).

2.3 Adding a Parameter

By moving slightly away from the simple structural approach, one may incorporate parameters for the system. Doing so more or less just changes the look of the vectors $\mathbf{v}$ and $\mathbf{x}$. $\mathbf{x}$, the vector of concentrations of metabolites, is redefined as having dependent, $\mathbf{x}_d$, and independent, $\mathbf{x}_i$, aspects in correlation to the free and pivot columns of

S, and $\mathbf{v}$ becomes a vector representing steady state concentrations of metabolites, $\mathbf{x}_i$, $\mathbf{x}_d$, as well as of external parameters, p.

First define the concentration vector $\mathbf{x}$:
Let r equal the number of pivot columns in S, and therefore the dimension of the row space. Then, assuming S does in fact have non-pivot columns, $R \neq r$, rearrange S so that pivot columns come before non-pivot columns. Then, we can define a matrix L, named a link matrix, and a matrix S_r such that:

$$S = LS_r$$

Definition Link Matrix
A link matrix, L is the matrix with M rows and r columns, where the first r rows (and all r columns) make an $r \times r$ identity matrix. The last r rows of L are named L_0. This sub matrix, L_0, expresses the dependent reaction rates (of $\mathbf{v}$) in terms of the independent ones (Hofmeyr 2).

S_r is composed of the first r rows of S. L and L_0 will be important later. From here, the vector $\mathbf{x}$ should be written:

$\mathbf{x} = \begin{bmatrix} \mathbf{x}_i \\ \mathbf{x}_d \end{bmatrix}$ where $\mathbf{x}_i$ are the independent metabolic concentrations and the first r entries of $\mathbf{x}$, and $\mathbf{x}_d$ are the dependent ones and the last $m - r$ entries of $\mathbf{x}$. Then the solutions to

$$\frac{\delta}{\delta t}(\mathbf{x}_d - L_0\mathbf{x}_i) = 0$$

express concentrations of metabolites that remain constant in sum within the metabolic system regardless of perturbations such that $\mathbf{x}_d = L_0\mathbf{x}_i + \mathbf{T}$, where $\mathbf{T}$ is an $m - r$ dimensional vector of constant sums of concentrations (Hofmeyr 292). The main purpose of finding conservation sums is that the reaction rates depend on them.

Example S3
Use the same matrix S from example S1. S_r is composed of the first 3 rows of S, since S has three pivot columns, and they are already in front of the non-pivot columns.

$$S_r = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix}$$

L can simply be determined by finding the matrix that when multiplied by $S_r = S$

$$L = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 2 & 0 \\ -1 & -1 & -1 \end{bmatrix} \text{ And from this } L_0 = \begin{bmatrix} 0 & 2 & 0 \\ -1 & -1 & -1 \end{bmatrix}$$

Then we can find all conservation relationships.

$$\mathbf{x} = \begin{bmatrix} x_i \\ x_d \end{bmatrix} = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ - \\ x_4 \\ x_5 \end{bmatrix}$$

$\frac{\delta}{\delta t}(\begin{bmatrix} x_4 \\ x_5 \end{bmatrix} - \begin{bmatrix} 0 & 2 & 0 \\ -1 & -1 & -1 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix}) = 0$

$\frac{\delta}{\delta t}(x_4 - (2x_2)) = 0$

$\frac{\delta}{\delta t}(x_5 - (-x_1 - x_2 - x_3)) = 0$

$-2M_2 + M_4 = T$

$M_1 + M_2 + M_3 + M_5 = T$ (as seen previously in example S2)

Now define the vector $\mathbf{v}$:

If S does contain non-pivot columns in reduced row echelon form, then $\mathbf{v}$ becomes a function of $\mathbf{x}$ containing $\mathbf{x}_i$ and $\mathbf{x}_d$ and a perturbation, p (Reder 12). Note that if S does not have any non-pivot columns, there are no constant sums of metabolites ($\mathbf{T}$ is empty) and L is an identity matrix, and $\mathbf{v}$ is then a function of $\mathbf{x}$, with no distinction between independent and dependent aspects, and the parameter p.

A steady state vector $\mathbf{v}(\mathbf{x_i}, \mathbf{x_d}, p)$ is such that $S_r\mathbf{v}(\mathbf{x_i}, \mathbf{x_d}, p) = 0$.

So, the main question in MCA is how the determined steady-state rates (with their corresponding reactions) change in response to perturbations. A perturbation may affect the actual steady state by affecting $\mathbf{T}$ or p. The goal is now to determine the amount of flux that should be given to the individual reactions to adapt to a perturbation of the parameter.

This is done by finding the solutions for $\mathbf{v}$ in $S\mathbf{v}(\mathbf{x}_i, \mathbf{x}_d, p) = 0$. All solutions can be found in a null space. The null space represents all rates of reaction of a metabolic system, so the null space therefore defines what can and cannot be achieved by the reactions, and how efficient substrates are made into products (Schilling 298).

3 Perturbation Analysis

A change in the steady state, $\mathbf{v}(\mathbf{x}_i, \mathbf{x}_d, p)$, may be caused by a perturbation that causes a change in a parameter. The change in $\mathbf{v}$ may be modeled by

$$\mathbf{v}_2(\mathbf{x}_i, \mathbf{x}_d, p) = \mathbf{v}_1(\mathbf{x}_i, \mathbf{x}_d, p) + \frac{\delta \mathbf{V}}{\delta p}(p_2 - p_1)$$

(Hofmeyr 293)
Determining $\frac{\delta \mathbf{V}}{\delta p}$ in this equation involves the use of elasticity and control coefficients.

3.1 Elasticity and Control Coefficients

These two coefficients are related to reaction rate. Therefore, they define the response of a metabolic system to perturbations.

"A control coefficient is a relative measure of how much a perturbation affects a system variable" (Mendes Sec.1)

"An elasticity is a local property of an isolated enzyme that expresses how its rate varies with the concentration of any metabolite that affects it: this can be its substrate, product, or any other metabolite" (Cornish-Bowden Sec.2)

These coefficients are generally found through experiment (See Cascante 244). In 1991, Sauro and Fell worked on computer programming to determine the coefficients (Ainsworth). One of the major theorems in MCA relates control coefficients to elasticity coefficients. A definition for $\frac{\delta \mathbf{V}}{\delta p}$ of the above equation can be found in this theorems proof. Below is information compiled from Hofmeyr to the point of $\frac{\delta \mathbf{V}}{\delta p}$. Hofmeyr is a great reference for a complete proof relating a matrix of control coefficients to a matrix of elasticity coefficients of a metabolic system.
First, define an elasticity coefficient.

Definition Elasticity Coefficient
An elasticity coefficient is a partial derivative of a reaction rate function (in the vector $\mathbf{V}$) with respect to individual concentrations in $\mathbf{x}$, or to parameter p.

Definition Matrices of Elasticity Coefficients
Let E_p be a diagonal matrix of all elasticity coefficients with respect to parameters which effect each reaction in the metabolic system uniquely, and E_x be a matrix of elasticity coefficients with respect to $\mathbf{x}$ (concentration of metabolite).

Find $\frac{\delta \mathbf{V}}{\delta p}$:

Look at $S_r \mathbf{v}(\mathbf{x}_i, \mathbf{x}_d, p) = 0$

Since T is constant, differentiate with respect to p:

$$S_r[(\tfrac{\delta \mathbf{V}}{\delta x_i})_{x_d,T,p}(\tfrac{\delta X_i}{\delta p})_T + (\tfrac{\delta \mathbf{x}}{\delta x_d})_{x_i,T,p}(\tfrac{\delta x_i}{\delta p})_T + (\tfrac{\delta \mathbf{V}}{\delta p})_T] = 0$$

Now look at $x_d = L_0 x_i + \mathbf{T}$

Differentiate with respect to x_i:

$$(\tfrac{\delta x_d}{\delta x_i})_{T,p} = L_0$$

Combine these two differentiated equations and simplify:

$$S_r \begin{bmatrix} \frac{\delta \mathbf{v}}{\delta x_i} & | & \frac{\delta \mathbf{v}}{\delta x_d} \end{bmatrix} \begin{bmatrix} I_r \\ L_0 \end{bmatrix} (\tfrac{\delta x_i}{\delta p})_T + S_r(\tfrac{\delta \mathbf{v}}{\delta p})_T = 0$$

$$S_r(\tfrac{\delta \mathbf{v}}{\delta x})_{T,p} \begin{bmatrix} I_r \\ L_0 \end{bmatrix} (\tfrac{\delta x_i}{\delta p})_T + S_r(\tfrac{\delta \mathbf{v}}{\delta p})_T = 0$$

$$S_r E_x L(\tfrac{\delta \mathbf{x}_i}{\delta p})_T + S_r E_p = 0$$

Let $M = S_r E_x L$. M is invertible by assumption 2. *

$(\frac{\delta \mathbf{x}_i}{\delta p}) = -M^{-1} S_r E_p$

Since $(\frac{\delta x_d}{\delta x_i})_{T,p} = L_0$ we can say $(\frac{\delta x_d}{\delta p}) = L_0(\frac{\delta x_i}{\delta p}) = -L_0 M^{-1} S_r E_p$

Now differentiate the vector $\mathbf{v}(x_i, x_d, p)$ with respect to p:

$(\frac{\delta v(x_i,x_d,p)}{\delta p})_T = (\frac{\delta \mathbf{v}}{\delta x_i})_{x_d,T,p}(\frac{\delta x_i}{\delta p})_T + (\frac{\delta \mathbf{v}}{\delta x_d})_{x_i,T,p}(\frac{\delta x_d}{\delta x_i})_{T,p}(\frac{\delta x_i}{\delta p})_p + (\frac{\delta \mathbf{v}}{\delta p})_T$

Simplify

$(\frac{\delta v(x_i,x_d,p)}{\delta p})_T = E_x L(\frac{\delta x_i}{\delta p})_T + E_p$

$\frac{\delta V}{\delta p} = E_x(-LM^{-1}S_r)E_p + E_p$

We have now defined $\frac{\delta V}{\delta p}$.

*In the midst of this justification, the claim is made that the matrix $M = S_r E_x L$ is invertible. This is based from the assumption 2, that a metabolic system can be analyzed at a steady state. Hofmeyr states that if a steady state, V, exists, M is invertible. This is based off the fact that M is a Jacobian matrix (see Hofmeyr 298). A Jacobian matrix is like the derivative of a function, as it is the matrix of partial derivatives of a system of equations (with respect to a state of the metabolic system.)

In a full proof relating elasticity to control coefficients, the diagonalization of matrices containing elasticity coefficients as well as diagonalization of M should occur. E_p is already a diagonal matrix since it is in relation to parameters that affect single reactions in the system. Invertible diagonal matrices can be defined to diagonalize E_x and M (see Hofmeyr 295, 299)

4 Medical Applications

Once again, MCA is the study of how enzyme controlled reaction rates change in response to changes in parameter. Since genes or enzymes may control the parameters, MCA helps an understanding the effect of diseases, genes effect on controlling metabolism, and the use of enzymes in cancer treatment. MCA can also help in drug discovery (Cascante).

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