The Linkage between Flux Distributions and Elementary Modes Activity Patterns: An Interval Approach

F. Llaneras∗[a], J. Picó [a]

[a] *Dept. of Systems Engineering and Control, Technical University of Valencia, Valencia, Spain, e-mail: {frallaes@doctor.upv.es, jpico@ai2.upv.es}*

Keywords: Elementary modes, Extreme pathways, α-spectrum, Metabolic flux analysis.

1 Abstract

In this paper a new approach to determine the α-spectrum is presented. The approach is based on the use of an interval representation of fluxes, making it possible to compute α-spectrum from an uncertain or even partially unknown flux distribution. In addition, as a complement of metabolic flux analysis, a new method is proposed that allows the calculation of the ranges of possible values for each non-calculable flux. The presented methods are illustrated with the example of CHO cells.

2 Introduction

This work is focused on mathematical methods for translating a metabolic flux distribution into an elementary modes or extreme pathways activity pattern. These methods determine how much flux is being carried by each e. mode or e. pathway under some particular set of circumstances. Hence, the pooly informative flux distribution can be translated into a simpler and more meaningful representation. Unfortunately, this translation has not a unique solution but a range of solutions. Thus, two options are possible: choosing a particular solution (Poolman et al., 2004; Schwarz et al., 2005), or dealing with the whole solutions region. When choosing one solution, the validity of the obtained activity pattern depends on the validation of the underlying assumptions. Following the second option, the α-spectrum, the range of possible values for each e. mode or e. pathway activity, can be determined (Wiback et al. 2003).

Herein, a new approach that allows determining the α-spectrum when fluxes are represented with an interval is presented. This representation is useful when a) flux measurements are uncertain, and b) when some non-measured fluxes cannot be uniquely determined (Klamt et al., 2002). In addition, a method to flux calculation is presented as a complement of metabolic flux analysis (MFA). In many cases, when using MFA, the resulting system is undetermined and the complete flux distribution cannot be computed. In these cases, by using a similar procedure to the one used to determine the α-spectrum, it is possible to calculate the ranges of possible values for each non-calculable flux.

3 Theoretical

A biological network can be represented with a stoichiometric matrix N, where rows correspond to the m metabolites and columns to the n reactions. Including irreversible reactions as v_i, the mass balance of the network at steady state (Stephanopoulos et al., 1998) can be formulated as:

$$N \cdot v = 0 \qquad v_i \geq 0 \tag{1}$$

In general, as n is bigger than m, the system is undetermined. Nevertheless, the solution region can be spanned by convex combination with e. modes or e. pathways:

$$v_m = E \cdot \alpha \qquad \alpha_i \geq 0 \tag{2}$$

Where v_m is a flux distribution, E denotes the matrix formed with each e. mode or e. pathway as a column and α is a vector representing the non-negative activity for each e. mode or e. pathways. Despite differences between e. modes and e. pathways (Papin et al., 2004), the proposed methods can be applied in both cases, and therefore from this point only the term e. mode will be used.

∗ Corresponding author

3.1 TRANSLATING A FLUX DISTRIBUTION INTO A E. MODES ACTIVITY PATTERN

System (2) can be analyzed using the procedure proposed in (Klamt et al., 2002). The number of e. modes *e* is always bigger or equal than *n-m*, the number of linear independent vectors needed to span the solution region. Therefore the rank of *E* is equal to *n-m*. When *e=n-m* the system is exactly determined, and the unique solution can be calculated by using E^{-1}. But in general *e>n-m*, and the system is undetermined with *e-(n-m)* degrees of freedom. Then, the general solution of (2) can be considered:

$$\alpha_G = \alpha_p + K(E)\cdot\lambda \qquad \alpha_i \geq 0 \qquad (3)$$

Where α_p denotes a particular solution, *K(E)* the null space of *E* and λ an arbitrary vector representing the indeterminacy of equation. Thus, only such elements α_{Gi} of α_G whose corresponding row in *K* is a null row, are determined (its value can be taken from the non-negative least squares solution).

3.1.1 α-spectrum: The interval Approach

In (Wiback et al., 2003) the concept of α-spectrum is defined to work with the solution region. Basically, 2**e* linear programming problems are solved to compute the range of possible values for each e. mode activity. Here, a slight modification of the method makes it possible to compute the α-spectrum when the fluxes are represented as an interval:

$$\begin{aligned} &\forall \alpha_j, \min/\max\{\alpha_j\} \qquad j \in [1,e] \\ &subject: \quad E\cdot\alpha \leq v^+ \quad E\cdot\alpha \geq v^- \quad \alpha_i \geq 0 \end{aligned} \qquad (4)$$

where v^+ and v^- are vectors with extreme values for each flux. The interval representation implies reducing the restrictions of the problem, and therefore the solution ranges will be bigger. Nevertheless, if the interval representation is well justified, the obtained solution will be less precise, but more realistic.

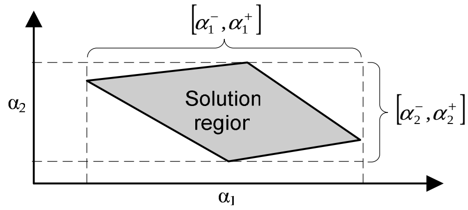

Figure 1. The α-spectrum.

This method makes it possible to compute the α-spectrum in two common situations: a) when the flux distribution is uncertain and b) when it is partially unknown. Additionally, it provides a straight method for dealing with inconsistency: Only if the flux region, defined with an interval, contains one consistent flux distribution, the linear programming problem has a solution. In figure 2, the different representations of fluxes based on certainty, consistency and completeness are summarized.

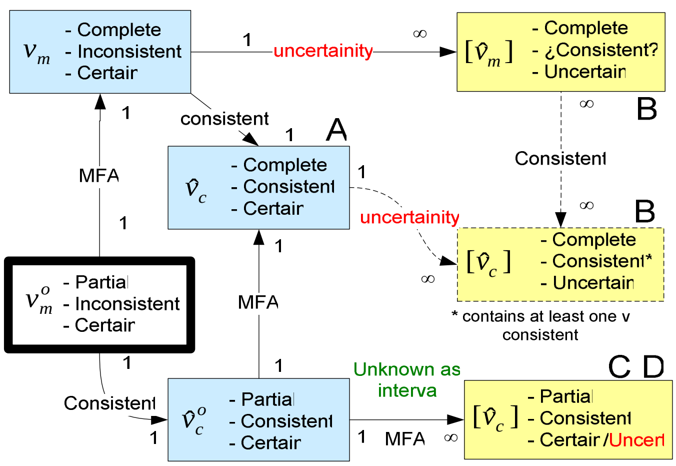

Figure 2. Fluxes as intervals.

3.2 METABOLIC FLUX ANALYSIS AND THE FLUX SPECTRUM

Although intracellular fluxes can be measured *in vivo* with tracer experiments (Sauer, 2004), there are several situations where these techniques are not suitable. In these cases, MFA can be used to calculate intracellular fluxes by using a set of measured fluxes and applying mass balances around metabolites (Stephanopoulos et al., 1998). Basically, making a partition between measured (subindex *m*) and unknown fluxes (subindex *u*), equation (1) can be transformed into:

$$N_u \cdot v_u = -N_u \cdot v_u \qquad (5)$$

Following (Klamt et al., 2002), the determinacy and the redundancy of (1) can be analyzed. If the system is determined, a unique solution can be computed; nevertheless, very often it is necessary to deal with underdetermined systems, where some fluxes cannot be uniquely computed (Klamt et al., 2002).

3.2.1 Flux-spectrum

To deal with these undetermined systems, a new approach is proposed that allows the calculation of the ranges of possible values for each non-calculable flux, resulting in a region that could be termed flux-spectrum. Again, these ranges can be obtained by solving a set of linear programming problems:

$$\begin{aligned} &\forall v_{uj}, \min/\max\{v_{uj}\} \qquad j \in [1, nu] \\ &subject: \quad N_u \cdot v_u = -N_m \cdot v_m \qquad v_i \geq 0 \end{aligned} \qquad (6)$$

Thus, when some fluxes cannot be calculated, the flux-spectrum provides a method to compute its ranges of values. Obviously, it is also possible to compute the flux-spectrum when the know fluxes are represented with an interval (as a previous step the extreme values of $-N_m \cdot v_m$ need to be calculated).

4 Results

In (Llaneras et al., 2006), the presented methods have been applied to the central metabolism of CHO cells (Provost and Bastin, 2004). Including a *6x18* matrix *P* linking extracellular fluxes with intracellular ones, the extended system has 16 metabolites (*me*) and 22 reactions (*ne*).

4.1 A-SPECTRUM AND. PARTIAL KNOWLEDGE

For example, when only v_1 *(G)*, v_{21} *(CO_2)* and v_{20} *(Q)* are measured, the system is undetermined: the rank of *Nu* (16) is less than the number of unknown (18). Therefore the complete flux distribution cannot be determined by using MFA. Nevertheless, even when the flux distribution is partially unknown, the α-spectrum can be computed by using the method presented in 3.1.1 (interval fluxes are given in table 9).

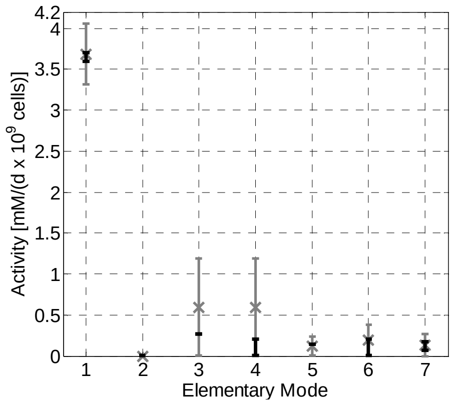

Figure 3. α-spectrum computed from the complete flux distribution (●) and from a incomplete one (x).

Table 1. Partially unknown flux distribution represented as a set of intervals (nM/(d x 10^9 cells)).

v_1 *(G)*	v_2- v_{19}	v_{20} *(Q)*	v_{21}*(CO_2)*	v_{22}
4.4305	[0,∞*]	1.186	2.5574	0

4.2 FLUX-SPECTRUM

As the system is undetermined, at least one flux cannot be uniquely determined. Moreover, there is not any calculable flux (matrix *K*, the kernel of *N*, has no null rows). Nevertheless, by using the concept of flux-spectrum it is possible to calculate the range of possible values for each non-calculable flux.

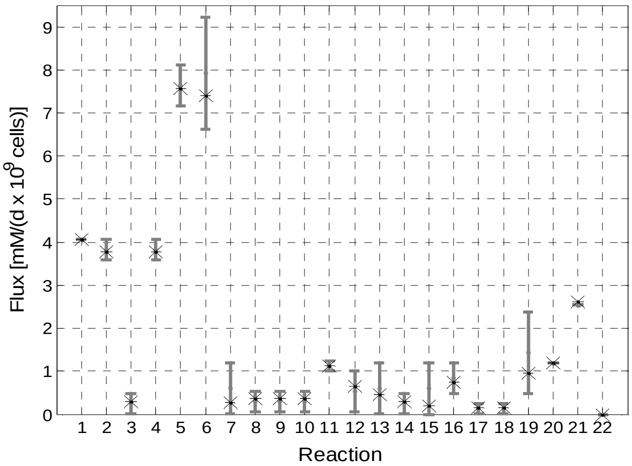

Figure 4. Exact flux distribution (x) and flux-spectrum computed from an partial flux distribution (●).

Moreover, if a unique and exact value is needed and depending on the size of the ranges, the use of each range middle point as an estimation is a sensible approach.

5 Conclusion

The translation of a metabolic flux distribution into an e. modes or e. pathways activity pattern has been investigated. A new approach to determine the α-spectrum was presented. Additionally, a method to calculate the ranges of possible values for non-calculable fluxes was proposed, as a complement to MFA.

Acknowledgements. This research has been partially supported by the Spanish Government (CICYT-FEDER DPI2005-01180). First author is recipient of a fellowship from the Spanish Ministry of Education and Science (FPU AP2005-1442).

References

Klamt S, Schuster S, Gilles ED. (2002) Calculability analysis in underdetermined metabolic networks illustrated by a model of the central metabolism in purple nonsulfur bacteria. Biotech. and Bioeng. 77(7):734-751.

Papin JA, Stelling J, Price ND, Klamt S, Schuster S, Palsson BO. (2004) Comparison of network-based pathway analysis methods. Trends in Biotechnol. 22(8):400-405.

Poolman MG, Venkatesh KV, Pidcock MK, Fell DA. (2004) A method for the determination of flux in e. modes, and its application to lactobacillus rhamnosus. Biotech. and Bioeng. 88(5):601-612.

Provost A and Bastin G. (2004). Dynamic metabolic modelling under the balanced growth condition. J. of Process Control 14(7):717-728.

Llaneras F, Picó J. (2006) The Linkage between flux distributions and elementary modes activity patterns: an interval approach. Internal Report. DISA, Technical University of Valencia.

Sauer U. (2004) High-throughput phenomics: Experimental methods for mapping fluxomes. Curr. Opinion in Biotechnol. 15(1):58-63.

Schwarz R, Musch P, von Kamp A, Engels B, Schirmer H, Schuster S, Dandekar T. (2005) YANA - a software tool for analyzing flux modes. BMC Bioinformatics 6(1):135.

Stephanopoulos GN, Aristidou AA, Nielsen J. (1998) Metabolic engineering : Principles and methodologies. San Diego: Academic Press.

Wiback SJ, Mahadevan R, Palsson BO. (2003) Reconstructing metabolic flux vectors from e. pathways: Defining the alpha-spectrum. J. of Theoretical Biol. 224(3):313-324.