
DEVELOPMENT OF A COMPUTATIONAL TOOL FOR ^{13}C - METABOLIC FLUX ANALYSIS

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ABSTRACT

^{13}C -Metabolic Flux Analysis (^{13}C MFA) has become a high-precision technique to estimate metabolic fluxes and get insights into metabolism. This method consists of experimental procedures, measurement techniques and data analysis calculations. In this context, metabolic engineering research groups demand accurate and suitable computational tools to perform the calculations. Here, we present a software implemented in MATLAB platform that performs ^{13}C MFA calculation, with metabolite and isotopomer balances, as well as a module to estimate the fluxes. At present, the program has been applied to many classical cases, and the results were comparable to those of well-established literature.

1. INTRODUCTION

System biology has emerged as a powerful tool for biotechnological process development, since it can give significant insights into how cells work and make predictions of genetic targets (Otero et al., 2009). The knowledge of genes and proteins and their interconnections is not enough to understand the metabolism of a microorganism, once the relationship of genome and phenotype is nonlinear and complex (Kitano, 2002). Metabolic Engineering uses system biology to design genetic modification to increase cell's yield of a product of interest. Thereby, metabolic engineering works in cycles of 3 stages: Genetic modifications are performed in a microorganism of interest through

genetic modification techniques; Analysis of bioprocess experiments is performed to collect data; and Models are used to evaluate the performance and define the next modifications. This process continues until the microorganism reaches a desirable yield and productivity (Stephanopoulos, 2002).

The modeling step in the metabolic engineering cycle needs a mathematical representation due to the considerable number of chemical reactions that are involved in a cell. Moreover, in many cases, an analysis of fluxomics is performed because the metabolism flux information is of great value to the study of pathways in a cell. Therefore, many types of models were created with this goal. There are models based on the stoichiometry of the reactions, carbon flux in a cell, mechanism of reaction, and genetic regulatory information. These models are ordered by complexity and the amount of information needed. The simpler one, Metabolic Flux Analysis (MFA), uses balances of metabolites and measures of extracellular fluxes to calculate intracellular fluxes in steady-state. However, this model is limited to metabolic networks without cycles and bidirectional fluxes (Stephanopoulos et al., 1998), which excludes important cases.

To overcome this limitation, experiments with labeled substrates, usually ^{13}C , have been proposed. In these experiments, the labeled substrates are fed into a system and the labeled carbon atoms are spread throughout a great number of metabolites according to carbon transitions in reactions. Then, the pattern of labeled carbon in intermediate metabolites that are formed from their precursors is analyzed with an analytical technique, usually RMN and mass spectroscopy. When carbon labeled information is available, it is possible to perform a more complex analysis, such as ^{13}C -Metabolic Flux Analysis (^{13}C MFA). This model, in addition to MFA, performs the balance of labeled carbon atoms or isotopomers, which are isomers with isotopic atoms at different positions. The labeled carbon atoms balance creates a balance for each carbon atom in each position of each metabolite in order to calculate the labeled carbon atom fractions. In the isotopomers balance, balances of all possible isotopomers are created to calculate each labeled isotopomer fraction. Isotopomers balance allows the complete representation of the system, and generates a more complex model. In the present work, we present software in MATLAB platform that performs ^{13}C MFA calculation with metabolite and isotopomer balances, as well as a module to estimate the fluxes.

2. METHODOLOGY

The problem of estimating metabolic fluxes can be formulated as a nonlinear constrained least squares problem, as follow:

$$\min \sum_{i \in I} \left(\frac{v_i^c - v_i^m}{\sigma_i} \right)^2 + \sum_{j \in J} \left(\frac{x_j^c - x_j^m}{\sigma_j} \right)^2$$

subject to the constraints : *Metabolite balances, Isotopomer balances and* $v \geq 0$ (1)

Where v is the vector of the metabolic fluxes (moles per time unit), and x is the isotopomer fractional labeling. The indexes c and m indicates the calculated and measured quantities respectively; σ corresponds to the standard deviation of the measures. The objective function is quadratic and the constraints are linear and non-linear, which gives rise to non-convexity. Therefore, it is necessary to use a numerical method to solve this estimation. This problem was implemented in MATLAB® v8.1 in a computer Intel Core i7, 3.40 GHz and 8 GB of ram. The function *sparse* was used to represent the matrices and the function *fmincon* was used to solve the estimation, by applying the Sequential Quadratic Programming method.

3. RESULTS

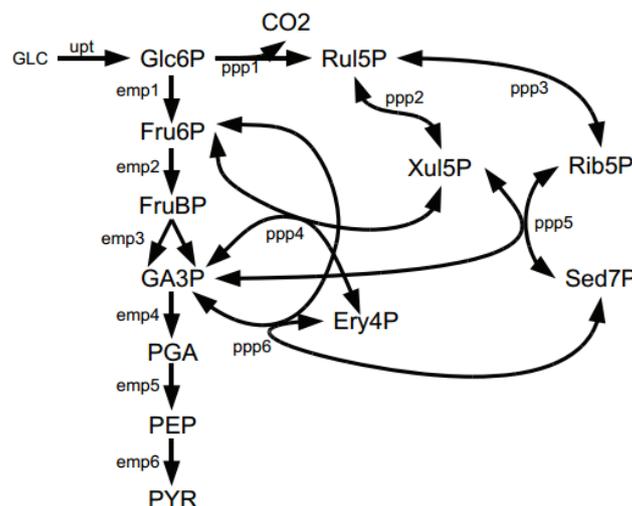


Figure 1. The Embden-Meyerhof and Pentose Phosphate metabolic pathways of *E.coli*.

In order to validate the tool created, we examined a network representing the Embden-Meyerhof and Pentose Phosphate metabolic pathways of *E.coli*, which is identical to those used by Srour et al. (2011). Also, the same substrate labeling and isotopic measurements were used. The estimated fluxes are presented in Table 1. Both works estimate similar fluxes for unidirectional reactions,

however the bidirectional fluxes present some disagreements, which was expected because of the poor identifiability of these fluxes.

Table 1. Comparison of estimates fluxes to E.coli network.

	upt	emp1	emp2	emp3	emp4	emp5	emp6	ppp1	ppp2
Srou et al. (2011)	1.02	0.51	0.85	0.85	1.87	1.87	1.87	0.51	4.42
This work	1.02	0.51	0.85	0.85	1.87	1.87	1.87	0.51	4.38
	ppp2r	ppp3	ppp3r	ppp4	ppp4r	ppp5	ppp5r	ppp6	ppp6r
FIA	4.08	4.47	4.30	4.25	4.08	0.42	0.25	0.42	0.25
This work	4.04	2.61	2.44	4.27	4.10	0.44	0.27	0.42	0.25

4. CONCLUSION

In this work a program that performs ^{13}C MFA calculation has been developed with metabolite and isotopomer balances to estimate fluxes. This tool can be useful to elucidate the metabolism of a range of different microorganisms and improve genetic modifications. The tool was compared with some results present in literature, and the results were satisfactory. At present, the tool is being applied to other case studies.

5. REFERENCES

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6. ACKNOWLEDGMENTS

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