Non-stationary Metabolic Control Analysis of *E. coli* Central Metabolism by a Mathematical Model Simulation

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Dedicated to the memory of Professor Dr. Valentin Koloini

A non-stationary metabolic control analysis (MCA) of *E. coli* central metabolism upon a response to a glucose impulse with an aim to discern the enzyme level regulation is applied. Results are obtained by a mathematical model simulation of the kinetic model derived from experimental data with a glucose impulse introduced after a 30-minute period of deprivation. The model includes 10 coupled metabolite balances, 24 highly nonlinear enzyme kinetic rate expressions and 132 kinetic parameters. Determined are the time courses of the flux control coefficients of the four fluxes (phosphotransferase system (PTS), nucleotide synthesis (NS), pyruvate and phosphoenolpyruvate to biomass) during the period of the first 15 seconds upon introduction of the impulse. Two methods, local and global, sensitivities of analysis are applied and compared. The local flux control coefficients are calculated by the finite difference formula for approximation of local derivatives applied one-by-one for each of the 24 enzyme levels (activities). The flux control coefficients based on global sensitivities are evaluated on the basis of partial variances corresponding to each enzyme under simultaneous variations of the all enzymes. Assumed are uniform probabilities of variations of the enzyme activities in the ranges (1 to 2) and (1 to 4) fold. The variances are calculated by the Fourier amplitude sensitivity test (FAST) numerical procedure. The two methods yielded similar overall features of the metabolic control, however identification of the key enzymes for particular fluxes are different. The results are an effort to broaden the scope of rational methodologies in application of genetic engineering.

Key words:
Non-stationary MCA, global sensitivity, *E. coli* central metabolism

Introduction

Mathematical theory of the metabolic control analysis (MCA) has been introduced by Kacser and Burns[1] and Heinrich and Rappaport.[2] It is based on systemic analysis of mathematical models of biochemical networks. Although mathematical models of individual enzyme activities are known, the behavior of networks requires a systemic approach due to complex interactions on the metabolic and cofactor levels. For example, there is no simple argument which would justify an assumption about how even complete knowledge of the rate equation for a particular "regulatory enzyme" would allow any quantitative prediction of the effect that change in its activity would have on the flux through the pathway in which it is embedded.[3]

The advancement of the experimental techniques for massive on-line throughput gene expression metabolomics and proteomics of numerous industrially important microorganisms leads fundamental research of industrial microbiology *in silico*.[4] Genomes and proteomes of numerous industrially important microorganisms are known and the corresponding stoichiometric matrix of metabolic reactions can be discerned, from which related pathways are determined. In spite of that, control of fluxes through the pathways on the cellular level is very complex and it is not uniquely defined by stoichiometric constraints and is very much open to experimental and theoretical (mathematical) research.

From the theoretical engineering point of view, possibilities for computer design of synthetic genome and proteins for development of new technologies are open, most importantly for bioenergetics based on synthetic microorganisms with integrated photosynthetic and fermentative metabolisms.[5] The main obstacles toward this far-reaching goal are not in the lack of experimental techniques for synthesis of genome, but rather in complex and unresolved biological and systems analysis of intricate metabolism control on a molecular level. Control of meta-

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bolic networks is species dependent and is a result of evolution encoded in a specie genome and epigenetic processes resulting in adaptation to external conditions. Results are highly structured and hierarchical systems of interrelated pathways responding to multi-objective life support goal functions. Experimental methods for massive *in vivo* sampling of gene expression profiles and proteome together with extensive computer modeling and simulation potentially lead to the development of complete *in silico* single cell life models.

The aim of this work is to apply two MCA methods, a deterministic local and single parameter analysis versus probabilistic and global sensitivity analysis, for evaluation of non-stationary flux control coefficients of *E. coli* central metabolism transients after glucose impulse.

Theory

At present, most of MCA analysis is based on steady state (homeostatic constraint) analysis and study of "one factor at a time" infinitesimal effects of perturbations of each individual enzyme and metabolite concentration on metabolic fluxes and individual reaction rates. The standard MCA analysis treats a linear pathway of enzyme reactions at steady state. Characteristic parameters and named control coefficients of the pathway are mathematically evaluated upon perturbations of each enzyme involved in the pathway. The coefficients are evaluated at assumed homeostatic conditions, i.e. at constant substrate concentrations entering the pathway and constant product concentrations leaving the pathway with constant concentrations of cofactors. Isolated enzyme rates are local properties and can be relatively easily analyzed. However, solutions to the steady state fluxes problem are intractable and control of the fluxes is distributed in a pathway (network) as a systemic property.

The control coefficients are determined as relative sensitivity quantities. The flux control coefficient is defined in the simplest (in a narrow) context by:[4–8]

$$C^{J}_{E_i} = \frac{E_i}{J} \cdot \left.\frac{\partial J}{\partial E_i}\right|_S = \left.\frac{\partial \ln J}{\partial \ln E_i}\right|_S \tag{1}$$

It is a relative measure of effect of i-th enzyme concentration, or activity, on the steady state flux J at constant concentrations of the rest of the enzymes imbedded in the pathway, the consumed substrate and products and at constant cofactor concentrations. The concentration control coefficients are defined similarly:

$$C_{i,j} = \frac{E_j}{x_i} \cdot \left.\frac{\partial x_i}{\partial E_j}\right|_S = \left.\frac{\partial \ln x_i}{\partial \ln E_j}\right|_S \tag{2}$$

From the experimental view point, application of a control coefficient of a metabolic reaction rate on metabolite concentration is the most important since it enables evaluation of flux control coefficients through the connectivity relations. The elasticities are defined by:

$$\varepsilon_{ij} = \frac{x_j}{v_i} \cdot \left.\frac{\partial v_i}{\partial x_j}\right|_S = \left.\frac{\partial \ln v_i}{\partial \ln x_j}\right|_S \tag{3}$$

In the case of chemical or mass action kinetics, the term reaction elasticity is identical to the reaction order. For a linear, i.e. un-branched pathway, Kacser and Burns[1] have derived the fundamental summation theorem for flux control coefficients, while Heinrich and Rappaport[2] have derived the equivalent summation theorem for concentration control coefficients. All of the summation and connectivity theorems can be express by the following matrix identity:

$$\begin{pmatrix} 1 & 1 & \cdots & 1 \\ \varepsilon_{1,1} & \varepsilon_{1,2} & \cdots & \varepsilon_{1,N} \\ \cdots & \cdots & \cdots & \cdots \\ \varepsilon_{N-1,1} & \varepsilon_{N-1,2} & \cdots & \varepsilon_{N-1,N} \end{pmatrix} \cdot$$

$$\cdot \begin{pmatrix} C^J_1 & -C_{1,1} & \cdots & -C_{1,N-1} \\ C^J_2 & -C_{2,1} & \cdots & -C_{2,N-1} \\ \cdots & \cdots & \cdots & \cdots \\ C^J_N & -C_{N,1} & \cdots & -C_{N,N-1} \end{pmatrix} = \tag{4}$$

$$= \begin{pmatrix} 1 & 0 & \cdots & \cdots \\ 0 & 1 & \cdots & 0 \\ \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & 0 & 1 \end{pmatrix} = \mathbf{I}$$

However, in general, control action is a systems dynamic property and can only be revealed from unsteady experiments, such as measuring flux and metabolite concentration responses due to an impulse perturbation of a substrate concentration fed to an investigated pathway. Under unsteady state conditions the standard steady state definitions of the control coefficients can be formally extended by definitions for the time varying flux control coefficient:

$$C^{J}_{E_i}(t) = \frac{E_i}{J(t)} \cdot \frac{\partial J(t)}{\partial E_i} =$$

$$= \frac{E_i}{J(t)} \cdot \lim_{\Delta E_i \to 0} \frac{J(t, E_i + \Delta E_i) - J(t, E_i)}{\Delta E_i} \tag{5}$$

Similarly holds for the concentration control coefficient and elasticities:

$$C_{i,j}(t) = \frac{E_j}{x_i(t)} \cdot \frac{\partial x_i(t)}{\partial E_j} = \tag{6}$$

$$= \frac{E_j}{x_i(t)} \cdot \lim_{\Delta E_j \to 0} \frac{x_i(t, E_i + \Delta E_i) - x_i(t, E_i)}{\Delta E_j}$$

$$\varepsilon_{i,j}(t) = \frac{x_j}{v_i(t)} \cdot \frac{\partial v_i(t)}{\partial x_j} = \tag{7}$$

$$= \frac{x_j}{v_i(t)} \cdot \lim_{\Delta x_j \to 0} \frac{v_i(t, x_j + \Delta x_j) - v_i(t, x_j)}{\Delta x_j}$$

The key issue in application of time varying control coefficients is local and/or global stability of metabolism steady state. In case of a locally stable steady state and relatively small perturbation transient trajectories along which they are evaluated, control coefficients will converge to the standard time independent values. When multiple steady states are present and perturbation is outside a limited range of local stability, transients may drive the time varying control coefficient to a new corresponding steady state. Only in the case of a single steady state convergence of the time varying coefficients to the values corresponding to steady state is assured.

Results of the metabolic control analysis are severely limited by unfulfilled steady state conditions and numerous errors involved in experimental and kinetic modeling. Problems arise due to measurement errors of on-line intracellular metabolites, inappropriate choice of kinetic rate expressions and large estimation errors in kinetic parameters.

Hence, we propose a probabilistic approach to the metabolic control analysis, which can account for uncertainness in kinetic modeling and time varying enzyme activities during transient experimental conditions.

Enzyme concentrations (activities) are assumed to be random variables within a finite range of values, minimum and maximum values, and uniform probability density functions:

$$E_i \in \rho_i(E_{i,\min}, E_{i,\max}) \qquad \rho_i \equiv U_i \tag{8}$$

Effects of variation of all the enzymes are evaluated by calculating the first and second moment of the joint probability distribution of a particular flux at a given time.

The first moment, the expected value of the flux J^i is the ensemble average value at a specific time t determined by:

$$E(J^i(t)) = \int_{E_{1,\min}}^{E_{1,\max}} \cdots \int_{E_{N,\min}}^{E_{N,\max}} J^i(t, E_1, \cdots E_N) \cdot \tag{9}$$

$$\cdot \rho_1(E_1) \cdots \rho_N(E_N) \cdot \mathrm{d}E_1 \cdots \mathrm{d}E_N$$

and the second moment is the corresponding variance

$$\sigma^2(J^i(t)) = E((J^i(t))^2) - E(J^i(t))^2 \tag{10}$$

where the squared term is calculated by

$$E(J_i^2(t)) = \int_{E_{1,\min}}^{E_{1,\max}} \cdots \int_{E_{N,\min}}^{E_{N,\max}} (J^i(t, E_1, \cdots E_N))^2 \cdot \tag{11}$$

$$\cdot \rho_1(E_1) \cdots \rho_N(E_N) \cdot \mathrm{d}E_1 \cdots \mathrm{d}E_N$$

General sensitivity corresponding to the probabilistic flux control coefficient $S_j^{J_i}$ of each individual enzyme E_j on the flux J^i is evaluated by the ratio of the conditional variance of a given enzyme and the total dispersion of the ensemble of enzymes

$$S_j^{J_i}(t) = \frac{\sigma^2(E(J^i(t)|\, E_j))}{\sigma^2(J^i(t))} \tag{12}$$

Numerical evaluation of the ensemble moments requires extensive unbiased sampling of the multidimensional enzyme space and unsteady flux evaluation at a set of discrete time.

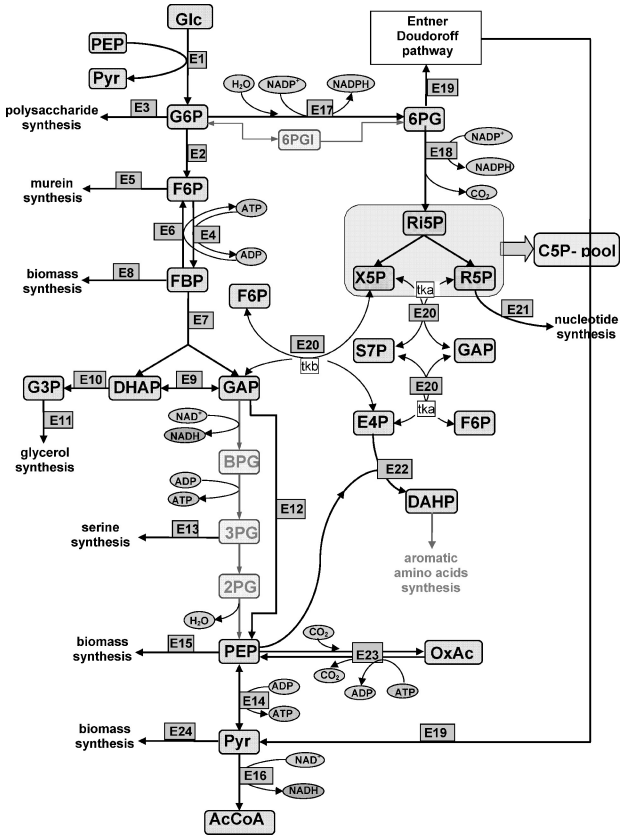
Experimental

Non-stationary flux control coefficients are calculated for *E. coli* central metabolism model based on data from on-line sampling. Intracellular metabolites are measured during 15 seconds after impulse of glucose introduced after 30 minutes of glucose deprivation, D. Degering e*t al.*[10] S. Čerić and Ž. Kurtanjek[11] have further improved the kinetic model and refitted the kinetic parameters. The network is depicted in Fig. 1.

The mathematical model is given as a set of ODE equations derived from the mass balances for the metabolites $\boldsymbol{x}$ as a product of the stoichiometric matrix **N** and the reaction rate vector **r** as function of vector of kinetic parameters **p**, and enzyme concentrations **E**:

$$\frac{\mathrm{d}}{\mathrm{d}t}\mathbf{x} = \mathbf{N} \cdot \mathbf{r}(\mathbf{E}, \mathbf{p}) \tag{13}$$

The dimensions of the model are: stoichiometric matrix **N**(10x24), reaction rates **r**(24x1), and parameters **p**(132x1), and enzyme concentrations **E**(24). The model equations, kinetic functions and



F i g . 1 – *Schematic presentation of the reactions included in the model of E. coli central metabolism with denoted fluxes and enzymes indexed according to Table 1*

parameters can be downloaded from the URL address[12] and the modeling details are given by A. Tušek and Ž. Kurtanjek.[13] The 24 enzymes and enzyme complexes included in the model are listed in Table 1.

The model equations are numerically solved during the interval of 15 seconds upon the impulse introduction. The model was a set of stiff ODE and was solved by applying a stable integration routine NDSolve available from Wolfram Research *Mathematica*.[14] Based on the simulation results the inlet flux $J^{PTS}(t)$ by PTS mechanism, and the following outlet fluxes from the central metabolism: pyruvate to biomass $J^{PYR \rightarrow X_{(t)}}$, phosphoenolpyruvate to biomass $J^{PEP \rightarrow X_{(t)}}$, and nucleotide synthesis $J^{Nucleotide\ synthesis}(t)$ are obtained fluxes as functions of time. For the given fluxes the time evolution of the control coefficients is determined by the finite difference approximation eqs. 5–7 with 5 % perturbation in each enzyme concentration.

The FAST method[15–16] is applied for probabilistic evaluation of the control coefficients in the concentration ranges 1:2 and 1:4. For each of the 24 enzymes, corresponding perturbations are associated with frequencies in the range from $\omega_1 = 29$ to $\omega_{24} = 75$ and a randomly selected phase φ_i. Each

T a b l e 1 – *Enumerated list of the enzymes and "pseudo-enzymes" for the lumped reactions*

E1	Enzymes in phosphotransferase system
E2	Phosphoglucose isomerase
E3	Polysaccharide synthesis
E4	Phosphofructokinase
E5	Murein synthesis
E6	Fructose 1,6-bisphosphate
E7	Aldolase
E8	Flux of FBP into biomass
E9	Triose phosphate isomerase
E10	Glycerol 3-phosphate dehydrogenase
E11	Glycerol synthesis
E12	Enzymes in the combined reactions GAP → PEP
E13	Serine synthesis
E14	Pyruvate kinase
E15	Reactions from PEP into biomass
E16	Pyruvate dehydrogenase
E17	Glucose 6-phosphate dehydrogenase
E18	6-phosphogluconate dehydrogenase
E19	Enzymes in Entner-Doudoroff pathway
E20	Combined transketolase and transaldolase reactions
E21	Nucleotide biosynthesis
E22	DAHP synthase
E23	Phosphoenolpyruvate carboxykinase
E24	Enyzmes in reactions from pyruvate to biomass

enzyme concentration is scaled to the range [0–1] and their perturbations are defined by the set of piecewise linear and periodic functions of a single "scan variable", $s \in [-1, 1]$:

$$E_i = \frac{1}{2} + \frac{1}{\pi} \cdot \arcsin\left(\sin\left(\pi \cdot \omega_i \cdot s + \varphi_i\right)\right) \quad (14)$$

The perturbations are mutually independent, cross-correlations are of order 10^{-8}, and are distributed by corresponding uniform probability density functions. The total enzyme concentration range is uniformly scanned from $s = -1$ to $s = 1$ in steps of $\Delta = 0.001$. Obtained flux values are linearly interpolated, and the interpolation functions $f(s)$ are expanded into Fourier series:

$$A_\omega = \frac{1}{2 \cdot \pi} \cdot \int_{-\pi}^{\pi} f(s) \cdot \cos(\omega \cdot s) \cdot \mathrm{d}s \quad (15)$$

$$B_\omega = \frac{1}{2 \cdot \pi} \cdot \int_{-\pi}^{\pi} f(s) \cdot \sin(\omega \cdot s) \cdot \mathrm{d}s \qquad (16)$$

The total dispersion of the flux data D_T is determined from the full spectrum by Fourier coefficients (maximum frequency $\omega_M = 1000$):

$$D_T = 2 \cdot \sum_{\omega=1}^{\omega_M} (A_\omega^2 + B_\omega^2) \qquad (17)$$

Contribution D_i of each enzyme perturbation E_i to the total data dispersion is determined from corresponding harmonics:

$$D_i = 2 \cdot \sum_{\omega=k\cdot\omega_i}^{\omega_M} (A_\omega^2 + B_\omega^2) \qquad (18)$$

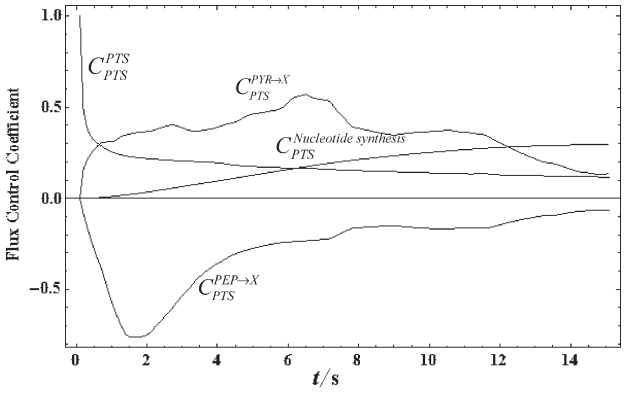
For each enzyme, the probabilistic flux control coefficients (sensitivities) are obtained as the ratios of partial dispersions and total dispersion:

$$C_{E_i}^J = \frac{D_i}{D_T} \qquad (19)$$

Results and discussion

Fig. 2 presents the time evolutions of the control coefficients for the inlet PTS flux and the following output fluxes: pyruvate to biomass flux, phosphoenolpyruvate to biomass, and the nucleotide synthesis flux on PTS "enzyme" activity. The results are obtained by the deterministic finite difference approximation, eq. (5), with 5 % perturbation of activity.

The coefficients are calculated continuously during the period of 15 seconds. Initially, the control coefficient of "PTS enzyme" on the rate of glucose assimilation is at its maximum value,

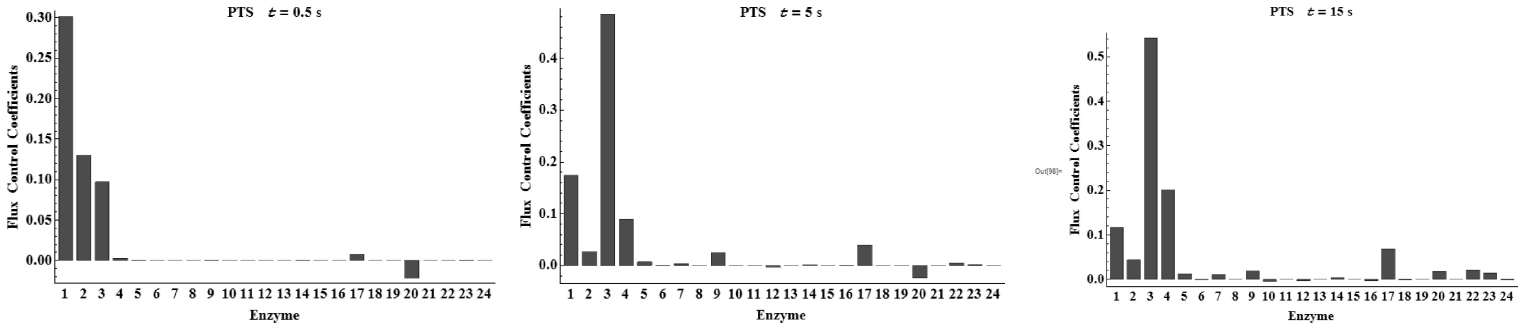


F i g . 2 – *Time evolutions of the control coefficients for PTS flux, pyruvate to biomass flux, phosphoenolpyruvate to biomas, and the nucleotide synthesis flux on PTS "enzyme" activity obtained by the deterministic finite difference approximation*

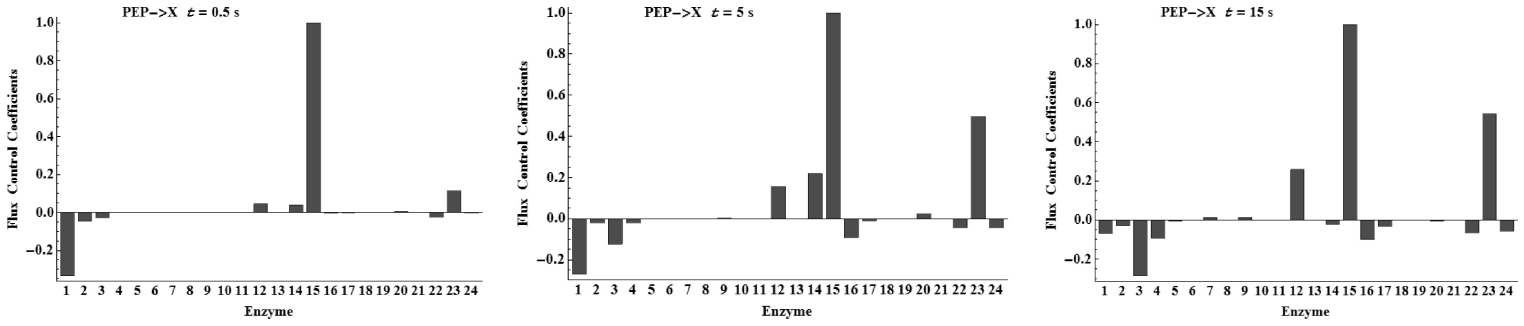
$C_{PTS}^{PTS}(t \le 0.5\ \mathrm{s}) \approx 1$, but afterwards sharply decreases and after 1 s reaches its steady value of 0.1. The control of PTS on the flux toward nucleotide biosynthesis has a time lag, and is initially negligible, $C_{PTS}^{Nucleotide\ synthesis}(t \le 1\ \mathrm{s}) \approx 0$, but after 1 s it proportionally increases with time and reaches 0.2 after 15 seconds. The increase of PTS control on the flux from pyruvate to biomass is initially zero, but without a noticeable time lag it "jumps" to a maximum value $C_{PTS}^{Pyr\to X}(t = 0.5\ \mathrm{s}) \approx 0.4$ and remains approximately at the same level through the whole period. The control of PTS on the flux from PEP to biomass has a negative effect. From initially zero value it reaches minimum value $C_{PTS}^{PEP\to X}(t \approx 1\ \mathrm{s}) \approx -0.75$ and afterwards it slowly returns to zero. The negative effect is due to the engagement of PEP in PTS process which diminishes the flux of PEP toward biomass synthesis.

The control effects of the enzyme assembly involved in the central metabolism are presented in Figs. 3a–d. The flux control coefficients are simultaneously evaluated for 24 enzymes, enumerated in Table 1, at the discrete times of 0.5 s, 5 s and 15 s. The results for PTS flux are depicted in Fig. 3a and show that the dominant role of PTS enzyme complex is only during the first few fractions of a second after the impulse. The control is immediately transferred to the enzymes "below" G6P, mostly by the enzymes involved in polysaccharide synthesis for fast replenishment of the exhausted pool during glucose starvation. The control coefficients for the flux from PEP to biomass are given in Fig. 3b. The dominance of the enzyme directly responsible for this reaction is pronounced during the whole period, $C_{E15}^{PEP\to X} \approx 1$. After 5 seconds there is a high control effect of phosphoenolpyruvate carboxykinase, $C_{E23}^{PEP\to X} \approx 0.6$. The negative effect is due to PTS enzymes, as observed in the time continuous result depicted in Fig. 2. The control profile for the nucleotide synthesis flux is presented in Fig. 3c. Throughout the period the flux is dominated by the enzyme responsible for the flux itself, $C_{E21}^{Nucleotide\ synthesis} \approx 1$. However, close to stabilization after 15 seconds, positive and negative control effects are observed for the rest of the enzymes. The results for flux from pyruvate to biomass, Fig. 3d, show a significant shift in control. Initially, it is controlled by the enzyme responsible for the reaction itself, $C_{E24}^{PYR\to X}(t \approx 0.5\ \mathrm{s}) \approx 0.9$, but after 5 seconds the control is transferred to the pyruvate dehydrogenase responsible for the flux toward TCA cycle, $C_{E16}^{PYR\to X}(t \ge 5\ \mathrm{s}) \approx -0.8$.

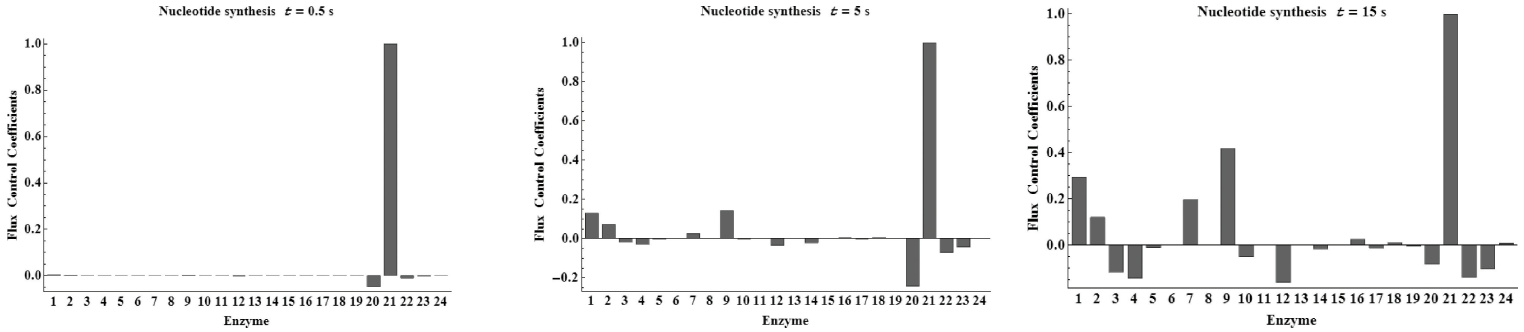
The probabilistic approach to metabolic control analysis is applied to account for the errors in-



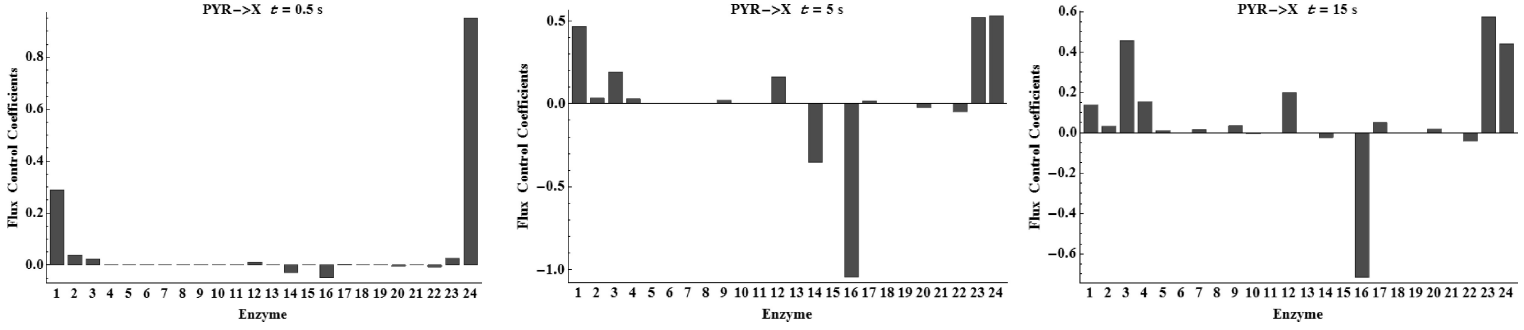
F i g . 3 a – *Standard control coefficients for PTS flux for the complete set of enzymes determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



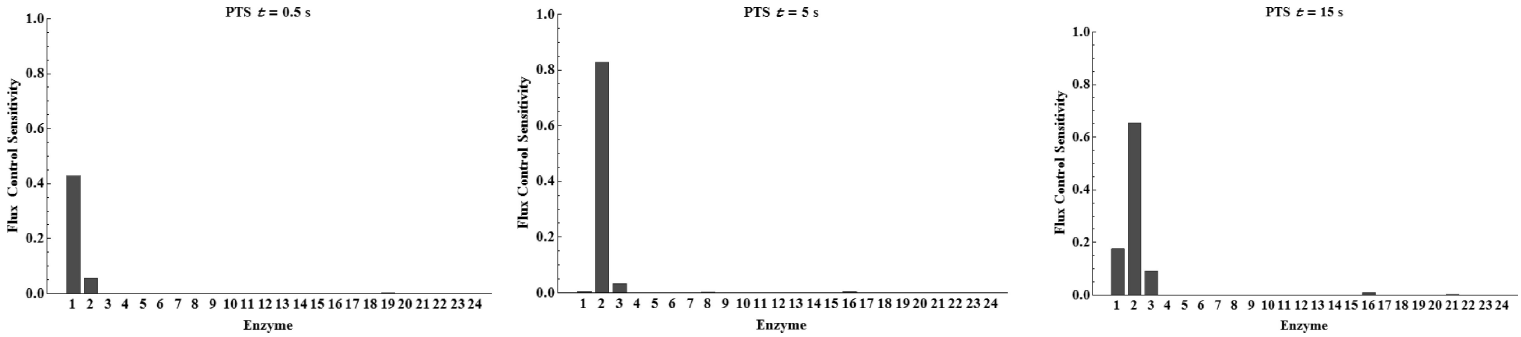
F i g . 3 b – *Standard control coefficients for the flux from phosphoenolpyruvate to biomass for the complete set of enzymes determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



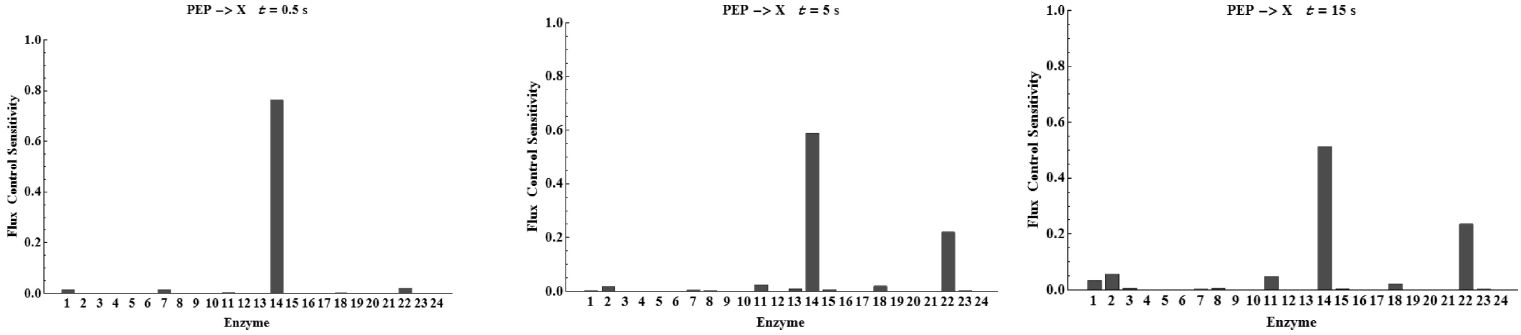
F i g . 3 c – *Standard control coefficients for the nucleotide biosynthesis flux for the complete set of enzymes determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



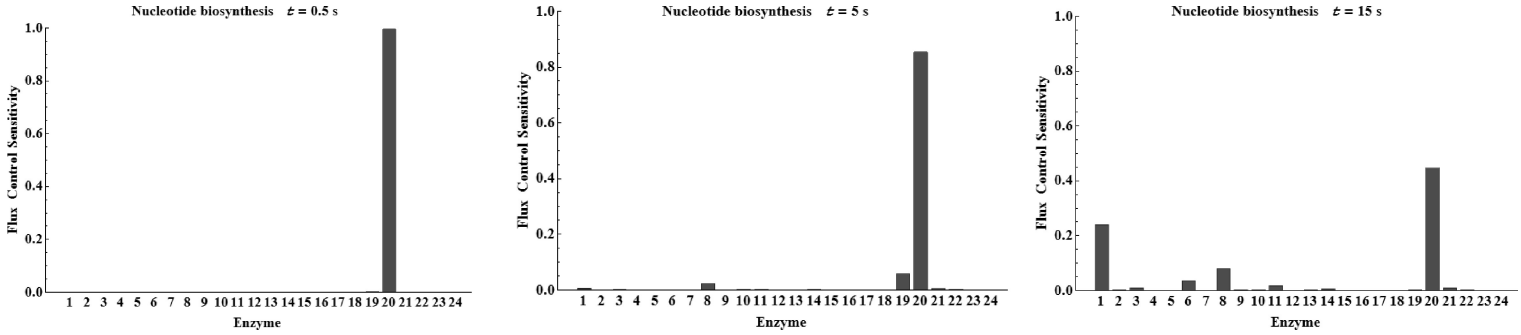
F i g . 3 d – *Standard control coefficients for the flux of pyruvate to biomass for the complete set of enzymes determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



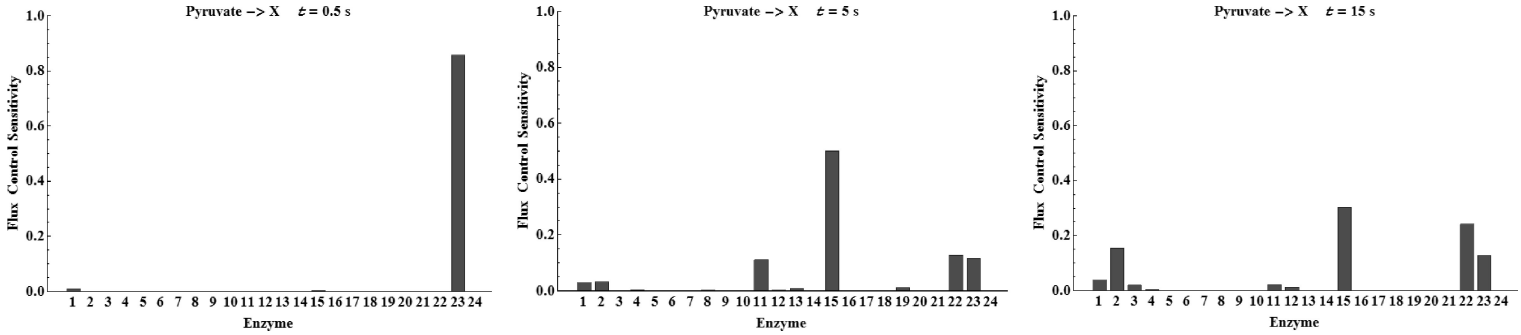
F i g . 4 a – *Probabilistic control coefficients for PTS flux for the complete set of enzymes in the range of 1:2-fold variation in their activity, determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



F i g . 4 b – *Probabilistic control coefficients for flux from phosphoenolpyruvate to biomass for the complete set of enzymes in the range of 1:2-fold variation in their activity, determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



F i g . 4 c – *Probabilistic control coefficients for the nucleotide biosynthesis flux for the complete set of enzymes in the range of 1:2-fold variation in their activity, determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*



F i g . 4 d – *Probabilistic control coefficients for the flux of pyruvate to biomass for the complete set of enzymes in the range of 1:2-fold variation in their activity, determined for the periods of t = 0.5 s, t = 5 s, and t = 15 s after the glucose impulse*

volved in the model parameter estimates and flux unsteadiness due to continuous perturbation imposed by the glucose impulse. Results are obtained by use of eqs. 14–19 and are shown in Figs. 4a–d. Since the method is based on the ratio between variances, the probabilistic control sensitivities are positive numbers, therefore the method cannot distinguish between positive and negative control effects. The calculations were obtained for a 2-fold and 4-fold increase in each enzyme concentration (activity) yielding almost identical results. Hence, only the values for the case of 2-fold increase are presented. The obtained control effects are here named as flux control sensitivities due to application of the FAST method for evaluation of systems global sensitivities. For the PTS flux and the probabilistic and the deterministic (standard) control effects show the same phenomena that the initial control of PTS enzyme complex lasts only for a fraction of the first second, but is soon transferred to the consequent reactions. The probabilistic result show the PTS flux is regulated by phosphoglucose isomerase, rather than the polysaccharide synthesis. For the flux from PEP to pyruvate, the control sensitivity indicates that pyruvate kinase is the key regulator, which is later partially supplemented by DAHP synthase. For nucleotide biosynthesis flux, the control sensitivity is maximum for the combined transketolase and transaldolase reactions, initial value is $C_{E20}^{Nucleotide\ synthesis}(t \leq 5\ \mathrm{s}) \approx 1$, and after impulse stabilization a part of the control sensitivity is with PTS, $C_{PTS}^{Nucleotide\ synthesis}(t \approx 15\ \mathrm{s}) \approx 0.3$. Results for the flux from pyruvate to biomass obtained by the probabilistic and standard control analysis imply different scenarios. The control sensitivity initially resides with phosphoenolpyruvate carboxykinase, but after 1 second is transferred to the enzymes which consume PEP for biomass synthesis, while the standard MCA analysis shows the control is with the pyruvate dehydrogenase.

Conclusions

In this work, the concept of probabilistic metabolic control analysis based on FAST algorithm is introduced. The motivation for the probabilistic approach is to account for a significant level of errors in estimates of kinetic parameter in complex metabolic networks, and to apply the method under unsteady conditions, such as for network control action in response to impulse perturbations. The method is here applied for control analysis of *E. coli* central metabolism perturbed by glucose impulse. The results are compared with numerical evaluation of non-stationary standard metabolic control analysis. Comparison of the two methods reveals essentially the same control features. Both methods show that enzymes of PTS flux dominate only for a fraction of a second after the impulse, immediately after which control is transferred to the branching points in glycolysis. Key enzymes for the "outlet" fluxes from pyruvate to biomass, phosphoenolpyruvate to biomass and the nucleotide synthesis flux have been identified. Shifts in the control during 15 seconds after the impulse have also been identified.

In conclusion, the standard MCA and the probabilistic control analysis are complementary, and may lead to a broader picture helpful in understanding the complexity of the problem.

Nomenclature

- $C_{E_i}^{J}$ – J-th flux control coefficient with respect to perturbation of E_i enzyme
- $C_{i,j}$ – concentration control coefficient of i-th metabolite perturbed by j-th enzyme
- D – data dispersion
- E – expected value
- E_i – i-th enzyme concentration (activity)
- $\mathbf{E}$ – vector of enzyme concentrations
- f – linear interpolation function
- $\mathbf{I}$ – identity matrix
- J – metabolic flux
- $\mathbf{N}$ – stoichiometric matrix
- $\mathbf{r}$ – reaction rates vector
- S – steady (cell homeostatic) state
- s – scan variable
- U_i – i-th uniform probability density distribution function
- t – time
- x_i – concentration of i-th metabolite
- $\boldsymbol{x}$ – vector of metabolite concentration
- Δ – finite difference
- φ_i – i-th random phase angle
- ω_i – frequency of i-th enzyme perturbation
- σ^2 – variance
- ρ_i – i-th probability density function
- v_i – i-th metabolic reaction rate
- $\varepsilon_{i,j}$ – elasticity of i-th reaction on j-th metabolite

Literature

1. *Kacser, H., Burns, J. A.,* Biochem. Soc. Trans. **23** (1973) 341.
2. *Heinrich, R., Rappaport, T. A.,* Acta Biol. Med. Ger. **31** (1974) 479.
3. *Cornish-Bowden, A.,* Fundamentals of Enzyme Kinetics, Portland Press, Oxford, 2004.

4. *Stephanopoulos, G., Aristidou, A., Nielsen, J.,* Metabolic Engineering – Principles and Methodologies, Academic Press, 1998.
5. *Stephanopoulos, G.,* Metab. Eng. **10** (2008) 293.
6. *Visser, D., Heijnen, J. J.,* Metab. Eng. **4** (2002) 115.
7. *Visser, D., Heijnen, J. J.,* Metab. Eng. **5** (2003) 164.
8. *Heijnen, J. J.,* Biotechnol. and Bioeng. **91** (5) (2005) 534.
9. *Ingalls, B. P., Sauro, H. M.,* arXiv: physics/0206075v1 [physics-bio-ph] 20 Jun. 2002.
10. *Degenring, D., Fromel, C., Dikata, G., Takors, R.,* J. Process Control **14** (2004) 729.
11. *Čerić, S., Kurtanjek, Ž.,* Chem. Biochem. Eng. Quart. **20** (3) (2006) 243.
12. http://www.pbf.hr/hr/zavodi/zavod_za_procesno_inzenjerstvo/ /laboratorij_za_mra/modeliranje_biotehnoloskih_procesa
13. *Tušek, A., Kurtanjek, Ž.,* Proceedings of MathMod 09 Vienna, Vienna, 11-12. Feb, 2009, p. 1178-1184.
14. Wolfram Research "Mathematica", v. 6.0 (2008).
15. *Cukier, R. I., Fortuin, C. M., Shuler, K. E., Petschek, A. G., Schaibly, J. H.,* J. Chem. Phys. **59** (1973) 3873.
16. *Saltelli, A., Ratto, M., Tarantola, S., Campolongo, F.,* Chem. Reviews **105** (7) (2005) 2811.