APPLICATION OF DYNAMIC MODELS OF GLYCOLYSIS DEVELOPING CONTROL SYSTEM

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Abstract. The paper deals with methodological items using computational methods for development and optimising of precision control of biological systems on molecular level. Systems biology approach to dynamics of cellular molecular processes using quantitative modelling allows describing these processes with differential equations. Thus automatic control theory methods optimising control activities accordingly to a set of efficiency criteria can be used. Possible simultaneous use of systems biology and automatic control theory methods are analysed depending on amount of available information and quality of model.

Glycolysis as a process of metabolism is used as a demonstration example. Glycolysis processes are analysed with biochemical network simulator COPASI 4.0 using SBML standard compatible models. Six glycolysis models with different scopes of different authors are analysed to find out common and different features as well as applied data. Models include following parameters: cellular compartments (1-3), number of species (15-34), reactions (14-29). Extraction of control systems development and optimisation related information from models is analysed. Methods of Metabolic Control Analysis (MCA) are analysed. Possibility of control development using dynamic models of molecular processes allows to optimise control activities afforcing experimental methods by mathematical ones. That is a promising direction in precision agriculture research due to savings of time and financial resources.

Key words: dynamic modelling, glycolysis, metabolism, SBML.

Introduction

Big part of activities in medicine, veterinary science, agriculture (precision agriculture in particular), food technology, ecology and biotechnology and other biological objects related branches can be defined as control of biological objects.

Control of biological object usually is carried out by changing parameters of environment of the process. Biological system reacts on those changes as a control system to compensate changes or reach new acceptable sate. Thus competition of two control systems occurs. One of those systems is biological control system and another one is artificial control system.

Biological control system (BCS) is in biological reproduction process developed control system that ensures internal processes within biological object and interaction processes with environment. Features of biological objects are metabolism and reproduction. BCS controlled biological objects are for example all living organisms (plants, animals, humans) as well as their subsystems (body temperature control, metabolism, processes within a cell).

Artificial control system (ACS) is a human designed control system. It can be executed by technical, chemical, biological or other means. By ACS in this paper is meant very wide range of control systems for example simple technical control system (climate control system in a building), complex technical control system (control system of an aircraft), control system for natural non biological objects (irrigation systems), human made control system of biological objects (fermentation process control), human designed control of biological object by another biological object (pest control by purposeful introduction of their biological enemies).

To assess and predict dynamic behaviour of BCS a dynamic model becomes necessary.

Systems Biology (SB) aims to understand and describe complexity and dynamics of biological systems (controlled by BCS) in holistic way confronting dynamic models (*in silico*) with dynamic experiments in the laboratory (*in vitro*). This approach partially is a result of unsuccessful trials to control biological objects. This relates to medicine, veterinary, industry, agriculture and other biology related branches. Dynamic models of particular processes are available on the Internet and can be used for development of control system.

Objective of the paper is to describe methodology of dynamic molecular process models application developing control algorithm for cellular processes.

Task of the paper is to demonstrate use of glycolysis dynamic models developing control system.

Materials and methods

Development process of control system consists on several steps: 1) mathematical description or model of the process of interest, 2) determination of control means and methods, 3) construction of the control system. In case of failure the cycle has to be repeated.

Models of the process of interest

Mathematical models of biological processes can be used from public data bases and simulations can be performed using free available modelling software. Dynamic models of cellular processes as well as necessary software usually are available in a common standard.

SBML – machine-readable model definition language based upon XML, the eXtensible Markup Language (Bray et al. 2000; Bosak and Bray 1999), which is a simple and portable text-based substrate that has gained widespread acceptance in computational biology.

SBML project is an effort to create a machine-readable format for representing computational models at the biochemical reaction level (Finney and Hucka 2003; Hucka et al. 2003). By supporting SBML as input and output formats, different software tools can operate on the identical representation of a model, removing chance for errors in translation and assuring a common starting point for analyses and simulations.

SBML standard software to operate SBML models is listed on the web (www.sbml.org). In this article software Complex Pathway Simulator (COPASI – www.copasi.org) (Hoops et al. 2006) will be used.

COPASI incorporates a model generator, different simulation techniques, optimization routines, methods from nonlinear dynamics and user-friendly visualization platforms enabling experimental biochemists simulate complex metabolic processes in cells without having to master complex mathematical and computer skills.

BioModels Database is an online resource for storing and serving quantitative models of biomedical and industrial interest. All the models in BioModels Database have been described in the peer-reviewed scientific literature.

The models stored in the curated branch of BioModels Database are compliant with MIRIAM (Le Novère et al. 2005), the standard of model curation and annotation. The models have been simulated by curators to check that when intentiated in simulations, they provide the same results that described in the publication. Model components are annotated, so the users can conveniently identify each model element and retrieve further information from other resources.

BioModels Database (http://www.ebi.ac.uk/biomodels) is developed in collaboration by the teams of Nicolas Le Novère (EMBL-EBI, United-Kingdom), Michael Hucka (SBML Team, Caltech, USA) in collaboration with Upinder Bhalla (DOQCS, National Center for Biological Sciences, India), Herbert Sauro (Keck Graduate Institute, USA), Hiroaki Kitano (Systems Biology Institute, Japan), Hans Westerhoff and Jacky Snoep (JWS Online, Stellenbosch (ZA) and Manchester (UK) Universities and Stellenbosh University, ZA), as part of the BioModels.net initiative.

JWS Online (http://jjj.biochem.sun.ac.za) aims to provide a service to the Systems Biology community by 1) giving access to a database of curated models of biological systems, and 2) allowing the users to run these models in a web browser via an easy to use interface, and 3) helping in reviewing of manuscripts containing kinetic models. In addition to this service role, JWS Online is an important component of an ambitious research initiative: The Silicon Cell. Whereas as a service JWS Online is a repository of published models, in the research activity models are not stored as published but are changed to reflect standardized notation of metabolites and enzymes, and a direct link between model and experimental data is provided.

SBML standard models contain several groups of parameters described below.

Compartments – fields of cells of distinct cell lineage, cell affinity, and genetic identity. In a developing organ, all cells within a compartment possess similar affinities, and so intermingle with each other. However, cells in neighbouring compartments have different cell affinity values and so never mix, thereby restricting the movement of cells to within compartments. Much cell proliferation may follow the appearance of compartments during development, hence this affinity-based subdivision has the effect of forcing cell lineages to stay within compartment boundaries.

Metabolites – species involved in metabolic reactions.

Moiety – a functional group, or part of a molecule. In organic chemistry, functional groups (or moieties) are specific groups of atoms within molecules that are responsible for the characteristic chemical reactions of those molecules. The same functional group will undergo the same or similar chemical reaction(s) regardless of the size of the molecule it is a part of.

Reactions – Different chemical reactions are used in combinations in chemical synthesis in order to get a desired product. In biochemistry, series of chemical reactions aided by enzymes form metabolic pathways, since straight synthesis of a product would be energetically impossible in conditions within a cell. Chemical reactions are also divided into organic reactions and inorganic reactions.

Determination of control means and methods

Possibility to simulate the process of interest allows optimisation of control means and methods. Criteria of efficiency can have several parameters where usually costs factor has one of the most important roles.

Methods of Metabolic Control Analysis (MCA) (Fell, 2005; Klipp et al., 2005) are used to optimise task setting of control system. Any metabolic network reaches steady state in constant environment. Several rules can be applied for a steady state and area near to that. MCA is a theory developed for processes in the steady state of biochemical system.

Control coefficients. When defining control coefficients, we refer to a stable steady state of the metabolic system characterized by steady-state concentrations $S = S(p)$ and steady-state fluxes $J = v(S(p),p)$. Any sufficiently small perturbation of an individual reaction rate by a parameter change, $v_k \rightarrow v_k + \Delta v_k$, drives the system to a new steady state in close proximity with $J \rightarrow J + \Delta J$ and $S \rightarrow S + \Delta S$. A measure for the change of fluxes and concentrations are the control coefficients.

The flux-control coefficient for the control of rate v_k over flux J_j is defined as

$$C_k^j = \frac{v_k}{J_j}\frac{\partial J_j}{\partial v_k},$$

while the concentration-control coefficient of concentration S_i with respect to v_k reads

$$C_k^i = \frac{v_k}{S_i}\frac{\partial S_i}{\partial v_k}.$$

The summation theorems make a statement about the total control over a certain steady-state flux of concentration. The flux-control coefficients fulfil

$$\sum_{k=1}^{r} C_{v_k}^{J_j} = 1,$$

where r is the number of reactions. The flux-control coefficients of a metabolic network for one steady-state flux sum up to 1. This means that all enzymatic reactions can share the control over this flux. For the concentration-control coefficients, we have

$$\sum_{k=1}^{r} C_{v_k}^{S_i} = 0.$$

The control coefficients of a metabolic network for one steady-state concentration are balanced. This means again that the enzymatic reactions can share the control over this concentration, but some exert a negative control while others exert a positive control.

Results and discussion

Search of dynamic models of glycolysis without focusing on particular organism resulted in 6 models summarised in the table. Number of models parameters indicate high variety in the scope of models. Appropriate model has to be chosen depending on the biological process or product of interest.

Table. **Comparison of glycolysis models by quantitative characteristics of models parameters**

Nr.	Source	Compartments	Metabolites	Moiety	Reactions
1	(Nielsen et al. 1998)	1	15	0	25
2	(Teusink et al. 2000)	2	21	1	17
3	(Bakker et al. 2001)	3	17	2	14
4	(Hynne et al. 2001)	2	25	2	24
5	(Hoefnagel 2002)	1	34	4	29
6	(Pritchard and Kell 2002)	1	25	2	19

	HXT	HK	PGI	PFK	ALD	TPI	GAPDH	PGK	PGM	ENO	PY
HXT	1.01425	0.21398	0.000450767	0.00138748	0.000474503	3.65247e-06	0.0218612	0.000497848	0.000207757	0.000928611	0.0
HK	1.01425	0.21398	0.000450767	0.00138748	0.000474503	3.65247e-06	0.0218612	0.000497848	0.000207757	0.000928611	0.0
PGI	1.26309	0.266478	0.000561361	0.0017279	0.000590919	4.54859e-06	0.0272247	0.000619992	0.00025873	0.00115644	0.0
PFK	1.26309	0.266478	0.000561361	0.0017279	0.000590919	4.54859e-06	0.0272247	0.000619992	0.00025873	0.00115644	0.0
ALD	1.26309	0.266478	0.000561361	0.0017279	0.000590919	4.54859e-06	0.0272247	0.000619992	0.00025873	0.00115644	0.0
TPI	1.45026	0.305967	0.000644547	0.00198395	0.000678486	2.76491e-05	0.164706	0.00375088	0.00156529	0.00699634	0
GAPDH	1.34068	0.282848	0.000595845	0.00183404	0.000627219	1.41247e-05	0.0842163	0.00191787	0.000800349	0.00357731	0.0
PGK	1.34068	0.282848	0.000595845	0.00183404	0.000627219	1.41247e-05	0.0842163	0.00191787	0.000800349	0.00357731	0.0
PGM	1.34068	0.282848	0.000595845	0.00183404	0.000627219	1.41247e-05	0.0842163	0.00191787	0.000800349	0.00357731	0.0
ENO	1.34068	0.282848	0.000595845	0.00183404	0.000627219	1.41247e-05	0.0842163	0.00191787	0.000800349	0.00357731	0.0
PYK	1.34068	0.282848	0.000595845	0.00183404	0.000627219	1.41247e-05	0.0842163	0.00191787	0.000800349	0.00357731	0.0
PDC	1.34068	0.282848	0.000595845	0.00183404	0.000627219	1.41247e-05	0.0842163	0.00191787	0.000800349	0.00357731	0.0
ADH	1.37968	0.291078	0.000613182	0.0018874	0.000645469	1.89389e-05	0.112868	0.00257037	0.00107264	0.00479438	0.0
ATPase	2.09576	0.442151	0.00093143	0.00286699	0.000980475	3.99555e-05	0.238016	0.00542037	0.00226198	0.0101104	0
AK	-inf	-inf	-inf	-inf	-inf	-inf	-inf	-inf	-inf	-inf	
G3PDH	0.809125	0.170704	0.000359604	0.00110688	0.000378539	-5.14783e-05	-0.306217	-0.00697352	-0.00291013	-0.0130074	-0
Glycogen Branch	0	0	0	0	0	0	0	0	0	0	
Trehalose Branch	0	0	0	0	0	0	0	0	0	0	
Succinate Branch	0.809125	0.170704	0.000359604	0.00110688	0.000378539	-5.14783e-05	-0.306217	-0.00697352	-0.00291013	-0.0130074	-0

Fig. 1. **Flux Control Coefficients**: COPASI screenshot of Metabolic Control Analysis of yeast glycolysis model (Pritchard and Kell, 2002)

In Figure 1, there can see connection between enzymes (columns) and fluxes (rows). With these coefficients we can determine how significant is connection between enzymes and fluxes. In first column there are coefficients between ~0.81 … ~2.1, that means HXT enzyme have very significant affect on almost all fluxes. In second column coefficients are between ~0.17 … ~0.44, which means HK enzyme have many times smaller significance, compared with HXT enzyme, but still have significant affect on almost all fluxes. In sixth (TPI) column coefficients are very small ~ –5.1e–05 … ~4.0e–05, which means TPI enzyme have insignificant affect on fluxes and this column we can discount and continue investigation of fluxes of glycolysis further with just significant enzymes. For example $C_{HK}^{PFK} \approx 0.27$, if we will increase/decrease concentration of enzyme v_{HK} per 1%, flux *PFK* (J_{PFK}) will increase/decrease per ~0.27%.

	HXT	HK	PGI	PFK	ALD	TPI	GAPDH	PGK	PGM	ENO	PYK
NAD	0.00424976	0.00089659	1.88875e-06	5.81366e-06	1.9882e-06	-3.73673e-06	-0.0222347	-0.000506354	-0.000211307	-0.000944478	-0.0
ADP	0.082251	0.0173528	3.65553e-05	0.000112519	3.84801e-05	1.56811e-06	0.00934127	0.00021273	8.87747e-05	0.000396795	5.8
G6P	2.97151	0.626912	-0.335187	-1.03172	-0.352836	2.17058e-06	-0.0817685	-0.00186213	-0.000777087	-0.00347334	-0.0
AcAld	0.809124	0.170704	0.000359604	0.00110688	0.000378539	-5.14782e-05	-0.306217	-0.00697352	-0.00291013	-0.0130074	-0.0
DHAP	1.565	0.330175	0.000695544	0.00214092	0.000732168	-0.000147766	-0.879079	-0.0200194	-0.00835432	-0.0373412	-0.0
GAP	1.56502	0.33018	0.000695553	0.00214095	0.000732178	2.60079e-05	-0.87926	-0.0200235	-0.00835604	-0.0373489	-0.0
P2G	2.28122	0.481279	0.00101386	0.0031207	0.00106724	2.45532e-05	0.146389	0.00333374	0.00139121	-1.43153	-
F6P	3.4095	0.719316	0.00151531	-1.38216	-0.472682	8.56825e-07	-0.121747	-0.00277256	-0.00115702	-0.00517153	-0.0
GLCi	-0.136152	-2.04513	-0.00430826	-0.013261	-0.00453511	-3.49089e-05	-0.20894	-0.00475823	-0.00198566	-0.0088753	-0.0
PYR	0.77341	0.16317	0.000343732	0.00105802	0.000361831	8.14825e-06	0.0485828	0.00110638	0.000461706	0.00206368	0.0
BPG	3.5181	0.742228	0.00156357	0.00481275	0.0016459	4.91431e-05	0.292865	-0.433026	-0.180706	-0.807702	-
PEP	1.87686	0.395969	0.000834145	0.00256754	0.000878067	2.25302e-05	0.134301	0.00305845	0.00127632	0.00570477	
F16bP	3.24552	0.684721	0.00144243	0.00443986	-1.48943	-5.46196e-05	-0.724704	-0.0165038	-0.00688722	-0.0307837	-0.0
P3G	2.24375	0.473374	0.000997205	0.00306945	0.00104971	2.40687e-05	0.143501	0.00326798	-0.264717	-1.1832	-
ATP	2.09576	0.442151	0.00093143	0.00286699	0.000980475	3.99555e-05	0.238016	0.00542037	0.00226198	0.0101104	0.0
NADH	-0.0742393	-0.0156626	-3.29946e-05	-0.000101559	-3.4732e-05	6.52772e-05	0.388419	0.00884553	0.00369134	0.0164991	0.0
AMP	-1.93125	-0.407445	-0.00085832	-0.00264195	-0.000903515	-3.68193e-05	-0.219333	-0.00499491	-0.00208443	-0.00931677	-0.0

Fig. 2. **Concentration Control Coefficients**: COPASI screenshot of Metabolic Control Analysis of yeast glycolysis model (Pritchard and Kell, 2002)

In Figure 2, there can see connection between enzymes (columns) and concentration (rows). With these coefficients, like it is with fluxes, we can determine how significant is connection between enzymes and concentration. Like it is with fluxes, there can select enzymes, which have significant affect on concentration, and work further just with them. For example $C_{ALD}^{F6P} \approx -0.47$, if we will

increase/decrease concentration of enzyme v_{ALD} per 1%, concentration *F6P* (J_{F6P}) will increase/decrease per ~–0.47%.

Conclusions

1. Systems Biology Markup Language (SBML) standard dynamic models of biological processes and available modelling and simulation software can be used to assess effect of particular enzyme on the whole network of metabolic reactions. That can be used developing a control system of biological processes in different branches.
2. Using option of Metabolic Control Analysis (MCA) within the software COPASI for steady state of metabolic networks effective control enzymes can be found to control fluxes (flux control coefficient) and concentrations of metabolites (concentration control coefficients).
3. Values of flux control coefficients indicate change of flux of reaction in percents within the metabolic after increase of value of particular enzyme for 1%. Values of concentration control coefficient indicate change of concentration of metabolite in percents within the metabolic after increase of value of particular enzyme for 1%.
4. Thus enzymes with high values of coefficients can be chosen as a good control mean while low values of coefficients mean its ineffectiveness in control of the network.
5. MCA methods allow prediction of side effects of control using particular enzymes.

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