APPLICATION OF THE MP THEORY TO SYSTEMS BIOLOGY

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Abstract: The main framework analysis for the most part of biological dynamics remains the theory of ordinary differential equations (ODEs). However, ODEs present some intrinsic limitations in the evaluation of the kinetic reaction rates. In contrast to ODEs, Metabolic P systems (MP systems), based on Păun's P systems, were introduced for modelling metabolic systems by means of suitable multiset rewriting grammars. In this work three applications of MP systems are presented, for discovering the internal regulation logic of three phenomena relevant in systems biology: *i)* the Goldbeter's mitotic oscillator; *ii)* the glucose/insulin dynamics in the Intravenous Glucose Tolerance Test; *iii)* the HER-2 oncogene-regulated transcriptome in human SUM-225 cells. Despite the differences between the considered phenomena, in all the cases a model was found that exhibits good approximation of the observed time series and highlights results which are new or that have been only theorized before.

1 INTRODUCTION

Systems biology (Ideker et al., 2001; Kitano, 2002) has been brought to the forefront of life-science research. Its goal is to understand biology at the system level by examining the whole structure and the dynamics of cellular and organismal function. However, the huge amount of experimental data which very often can be measured by means of high throughput technologies makes this job very difficult. For overcoming the problem, *mathematical modelling* is emerging as a suitable way for analysing data and developing new knowledge (Bailey, 1998). In particular, an important problem of systems biology is the mathematical definition of *dynamical systems* that explain observed dynamics of phenomena under investigation, by taking into account what is already known about each phenomenon.

The main framework analysis for the most part of biological dynamics remains the theory of ordinary differential equations (ODEs). However, ODEs present some intrinsic limitations in the evaluation of the kinetic reaction rates. In fact, very often, the evaluation of the kinetic reaction rates in differential models is problematic because it may require measurements hardly accessible in living organisms. Moreover, these measurements dramatically alter the context of the investigated processes. In contrast to ODEs, Metabolic P systems (MP systems) (Manca et al., 2005; Manca, 2010), based on Păun's P systems (Păun, 2002), were introduced for modelling *metabolic systems* by means of suitable multiset rewriting grammars (see (Decraene and Hinze, 2010; Hinze et al., 2007) for other discrete approaches compared to ODE systems).

A Metabolic P system is essentially a multiset grammar where multiset transformations are regulated by functions (MP grammar). Namely, a rule like $a+b \to c$ means that a number u of molecules of kind a and u of kind b are replaced by u molecules of type c. The value of u is the *flux* of the rule application. Assume to consider a system at some time steps $i = 0,1,2,\ldots,t$, and consider a substance x that is produced by rules r_1, r_3 and is consumed by rule r_2. If $u_1[i], u_2[i], u_3[i]$ are the fluxes of the rules r_1, r_2, r_3 respectively, in the passage from step i to step $i+1$, $i \in \mathbb{N}$, the set of natural numbers, then the variation of substance x is given by:

$$x[i+1] - x[i] = u_1[i] - u_2[i] + u_3[i].$$

In an MP system it is assumed that in any state the flux u_j of rule r_j is provided by a state function φ_j, called *regulator* of the rule. A state is essentially determined by the values of the system variables, that is, substances and parameters (quantities which are not transformed by the rules). However, usually only some variables enter as arguments of regulators, therefore if $u_j = \varphi_j(x,y,\ldots)$, the arguments $x,y,\ldots$ of φ_j will be called *tuners* of the regulator. Substances, reactions, and regulators specify the fol-

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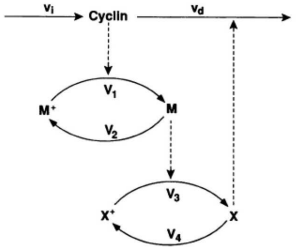
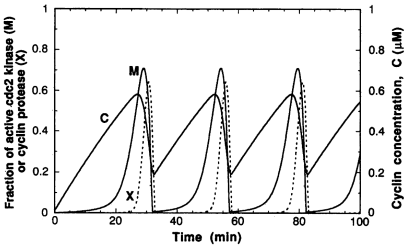
Table 1: Goldbeter's oscillator, which has a cycle of about 25 min (Goldbeter, 1991).



$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_d C$$
$$\frac{dM}{dt} = V_1 \frac{(1-M)}{K_1 + (1-M)} - V_2 \frac{M}{K_2 + M}$$
$$\frac{dX}{dt} = V_3 \frac{(1-X)}{K_3 + (1-X)} - V_4 \frac{X}{K_4 + X}$$
$$V_1 = \frac{C}{K_c + C} V_{M1}, V_3 = M V_{M3}$$



lowing discrete dynamics $(x[i]|i \in \mathbb{N})$ for any substance x, starting from the given value $x[0]$, called *Equational Metabolic Algorithm* (EMA):

$$x[i+1] = x[i] + \sum_{j=1}^{m} \alpha_j u_j[i] \qquad (1)$$

where m is the number of rules and α_j are integer stoichiometric coefficients determined by the reactions acting on substance x. Moreover, a *temporal interval* τ, a conventional *mole size* ν, and substances masses are considered, which specify the time and population (discrete) granularities respectively. In the following the MP dynamics we will present are computed in MATLAB by applying the EMA formula given in (1).

MP systems are equipped with a powerful regression algorithm, called *Log-Gain Stoichiometric Stepwise Regression* (LGSS), which derives MP models from the time series of observed dynamics and that can be applied independently from any knowledge about reaction rate kinetics (Manca and Marchetti, 2011). LGSS represents the most recent solution, in terms of MP systems, of the *dynamical inverse problem*, that is, of the identification of (discrete) mathematical models exhibiting an observed dynamics and satisfying all the constraints required by the specific knowledge about the modelled phenomenon. The LGSS algorithm combines and extends the log-gain principles developed in the MP system theory (Manca, 2008; Manca, 2009) with the classical method of Stepwise Regression (Hocking, 1976), which is a statistical regression technique based on Least Squares Approximation and statistical F-tests (Draper and Smith, 1981).

LGSS has been implemented by Luca Marchetti in 2010 as a set of MATLAB functions[1]. All the functions have been *ad hoc* implemented (including the stepwise regression function), and do not require additional MATLAB toolboxes. The code which needs harder computation (regression, simulation and tuning of regression parameters) has been implemented by taking advantage of the parallel processing facilities offered by the MATLAB Parallel Computing Toolbox (the software, however, runs also when this toolbox is not installed). When the Optimization Toolbox is installed in the system, LGSS supports also the usage of the *lsqlin* function which computes *constrained linear least squares problems*. This last feature is very important when we need to force complex constraints on the least squares estimation of the computed regressor coefficients.

The size of the systems of equations solved by LGSS depends on the number of substances and reactions of the MP system under examination and on its temporal interval τ (a smaller temporal interval require longer time series and so larger system of equations). However, the regression usually ends in few minutes (less than one minute in many cases, using a common laptop with a dual core CPU and 4 Gbyte of RAM memory), but it can increase to hours when the system is very big (i.e. a system with many hundreds of thousands of equations, and a regression dictionary of hundreds of regressors).

Even if computational tools are available for evaluating unknown parameters of ODE models (Maiwald and Timmer, 2008; Hoops et al., 2006), LGSS seems to point out a general methodology for solving dynamical inverse problems. In fact, LGSS not only discovers unknowns parameters, but suggests also the form of regulators as a combination of basic functions. This possibility could be very important in the case where the knowledge about the phenomenon under investigation is so poor that no clear idea is available about the kind of model underlying the observed behaviour.

In the following, three applications of MP systems will be presented for discovering, by means of LGSS, the internal regulation logic of three phenomena relevant in systems biology:

1. the Goldbeter's mitotic oscillator (Goldbeter, 1991);
2. the glucose/insulin dynamics in the Intravenous Glucose Tolerance Test (IVGTT);
3. the HER-2 oncogene-regulated transcriptome in human SUM-225 cells (working in progress with

[1] See http://www.mathworks.it/index.html for details on the MATLAB software.

the Karmanos Cancer Institute, Wayne State University, Detroit).

Despite the differences between the considered phenomena, in all the cases a model was found that exhibits good approximation of the observed time series and highlights results which are new or that have been only theorized before (Manca and Marchetti, 2010a; Manca et al., 2011; Marchetti and Manca, 2011).

2 MP GOLDBETER'S MITOTIC OSCILLATOR

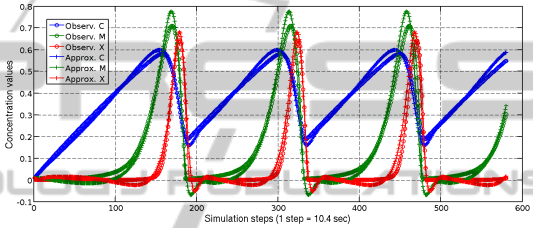
Rhythmic phenomena represent one of the most striking manifestations of dynamic behaviour in biological systems. Understanding the molecular and cellular mechanisms responsible for oscillations is crucial for unravelling the dynamics of life (Goldbeter, 2002).

The Goldbeter's mitotic oscillator represents the simplest form of mitotic trigger mechanism found in early amphibian embryos (Goldbeter, 1991). The fundamental mechanism of mitotic oscillations concerns the periodic change in the activation state of a protein produced by the *cdc2* gene in fission yeast or by homologous genes in other eukaryotes. The simplest form of this mechanism is found in early amphibian embryos. Here (see the picture in the left part of Table 1) cyclin (C) is synthesized at a constant rate and triggers the transformation of inactive (M^+) into active (M) *cdc2* protein, which leads to the formation of a complex known as M-phase promoting factor (*MPF*). MPF triggers mitosis, but at the same time M elicits the activation of a protease from state X^+ to X. The active protease then degrades cyclin resulting in the inactivation of *cdc*2. This brings the cell back to initial conditions and a new division cycle can take place. The ODE presented in Table 1 is the differential model of dynamics described in the right part of Table 1, where C, M, X are the concentrations of C, M, X respectively and $1-M, 1-X$ are the concentrations of M^+, X^+ respectively (the definitions of the parameters of the ODE model are not simple and are not relevant for our further discussion, however they can be found in (Goldbeter, 1991)).

In (Manca and Marchetti, 2010a) LGSS has been applied to Goldbeter's oscillator for showing that MP systems yield a robust method for biological modelling. In this manner, *were automatically generated 700 models of this oscillator*, which, for the most part, provide the same order of approximation of Goldbeter's model (see Table 2). Moreover, by considering the phenomenon at different values of τ, different models have been obtained and in many cases the analytical form of these models is simpler than Goldbeter's model. These models have been also categorised with respect to the analytical form of their regulators. In this way *a set of grammatical schemata was obtained* which express the regulation relationship acting on the systems in different intervals of the temporal interval τ.

Table 2: Example of MP mitotic oscillator ($\tau = 10.4$ sec). Constants and initial values as in (Manca and Marchetti, 2010a).

$r_1 : \emptyset \to C$	$\varphi_1 = v_i$
$r_2 : C \to \emptyset$	$\varphi_2 = k_1 + k_2\, M + k_3\, X - k_4\, CM$
$r_3 : M^+ \to M$	$\varphi_3 = k_5 + k_6\, CM$
$r_4 : M \to M^+$	$\varphi_4 = k_7\, M + k_8\, X$
$r_5 : X^+ \to X$	$\varphi_5 = k_9\, C + k_{10}\, M$
$r_6 : X \to X^+$	$\varphi_6 = k_{11} + k_{12}\, X + k_{13}\, C^2 + k_{14}\, CM$



3 GLUCOSE-INSULIN INTERACTIONS IN THE IVGTT

The Intra Venous Glucose Tolerance Test (IVGTT) is an experimental procedure used to study the glucose-insulin endocrine regulatory system. *Glucose* is the primary source of energy for body's cells. It is transported from the intestines or liver to body cells via the bloodstream, and is absorbed by the cells with the intervention of the hormone *insulin* produced by the pancreas. Normally, in mammals the blood glucose concentration is tightly regulated as a part of metabolic homeostasis (see Figure 1).

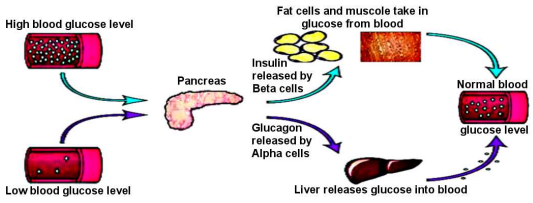


Figure 1: The glucose homeostasis.

If the plasma glucose concentration level is constantly out of the usual range, then we are in presence of blood glucose problems. In particular, when this level is constantly higher than the range upper bound, we are in presence of *Diabetes*: a dreadfully severe and pervasive illness which concerns a good number

of structures in the body. This motivates researches to study the glucose-insulin endocrine regulatory system.

The intravenous glucose tolerance test focuses on the metabolism of glucose in a period of 3 hours starting from the infusion of a bolus of glucose at time $t = 0$. It is based on the assumption that, in a healthy person, the glucose concentration decreases exponentially with time following the loading dose. In (Manca et al., 2011) Metabolic P systems theory has been applied for developing new physiologically based models of the glucose-insulin system which can be applied to the IVGTT. In that work, *ten data-sets obtained from literature were considered and, for each of them, an MP model which fits the data and explains the regulations of the dynamics was found* (see Figure 2).

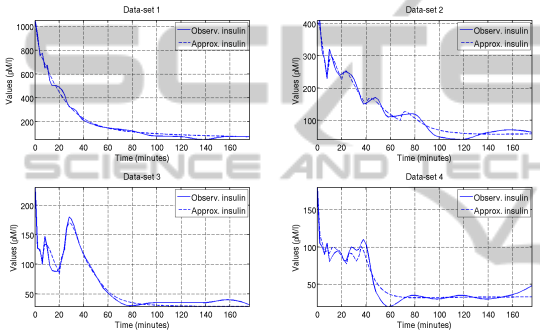


Figure 2: The calculated insulin dynamics related to four of the considered data-sets (Manca et al., 2011) ($\tau = 2$ min).

In the differential models proposed in literature, the delay of the insulin release is approached by adding artificial substances or by considering a delay integral kernel. Here, instead, the problem has been solved by assuming that the insulin production is regulated by the plasma glucose concentration level both at the current time and at some previous simulation steps (*glucose memories* as introduced in (Manca and Marchetti, 2010b)). This has permitted to point out, in a more natural and detailed way, the delays which act in the insulin production. Moreover, even if differences were found in the regulation governing the release of insulin, *it was possible to observe a common logic which before was only theorized during the development of the differential models* (see (Manca et al., 2011) for details). These preliminary results and analysis suggest that MP models seem to provide comprehensive tools for discovering *personalized glucose-insulin dynamics*.

4 MP ANALYSIS OF THE HER-2 ONCOGENE-REGULATED TRANSCRIPTOME IN HUMAN SUM-225 CELLS

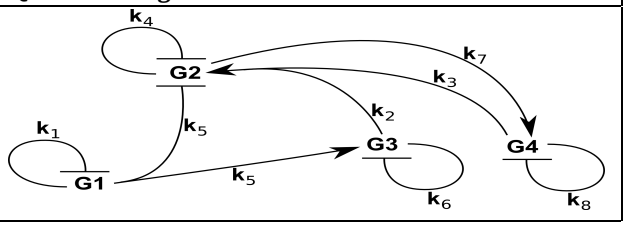
The identification of new gene networks are now an important part of systems biology. In addition to high-throughput experimental methods, mathematical and computational approaches are indispensable for the analysis of gene networks. Given the large number of components of most networks of biological interest, connected by positive and negative feedback loops, an intuitive comprehension of the dynamics of the system is often difficult, if not impossible to obtain. Mathematical modelling supported by computer tools can contribute to the analysis of a regulatory network by allowing the biologist to focus on a restricted number of plausible hypotheses. Many reviews of the modelling and simulation of gene networks have been published in recent years (e.g. (Cao et al., 2010; Bolouri and Davidson, 2002; Gilman and Arkin, 2002; Jong, 2002; Hasty et al., 2001; Smolen et al., 2000)), presenting the wide variety of formalisms that have been proposed in the literature, such as oriented graphs, Bayesian networks, Boolean networks, differential equations, stochastic master equations and stochastic P systems.

MP systems were initially introduced to model metabolic processes, but they can be successfully used in each context where we want to infer models of a system from a given set of time series. In (Marchetti and Manca, 2011) an application of the MP theory to gene expression analysis was developed. In this case, *a standard way for translating MP grammars involving gene expressions into corresponding quantitative gene networks was found* (see Table 3).

The number of the raw microarray time series which need to be processed for a generic experiment on human cells is usually of the order of tens of thousands. Generally, however, only a small part of them are related to the phenomenon under examination. For this reason, before to start with the modelling of the MP model, raw data need to be preprocessed following a methodology which comprises *normalization, filtering and clustering*. This methodology has been developed during a work in progress where *the MP theory has been successfully applied for defining the gene network underlying the regulations acting on the HER-2 oncogene-regulated transcriptome in human SUM-225 cells* in order to define new therapies for the breast cancer.

HER-2 is an epidermal growth factor receptor which have been implicated in radioresistance in

Table 3: An example of MP grammar related to a gene network (Marchetti and Manca, 2011).

MP grammar		Quantitative gene network
$r_1 : G1 \to \emptyset$	$\varphi_1 = k_1 \cdot G1$	
$r_2 : \emptyset \to G2$	$\varphi_2 = k_2 \cdot G3 + k_3 \cdot G4$	
$r_3 : G2 \to \emptyset$	$\varphi_3 = k_4 \cdot G2$	
$r_4 : G2 \to G3$	$\varphi_4 = k_5 \cdot G1$	
$r_5 : G3 \to \emptyset$	$\varphi_5 = k_6 \cdot G3$	
$r_6 : \emptyset \to G4$	$\varphi_6 = k_7 \cdot G2$	
$r_7 : G4 \to \emptyset$	$\varphi_7 = k_8 \cdot G4$	

breast cancer and other malignancies (see Figure 3). The analysis started by considering more than 24000 time series and finished by pointing out 1175 genes which seem to be HER-2 oncogene-regulated. These genes have been clustered following the ad hoc procedure defined in (Marchetti and Manca, 2011) and, finally, the MP model and the corresponding gene network have been provided, which seem to explain the regulation of the phenomenon.

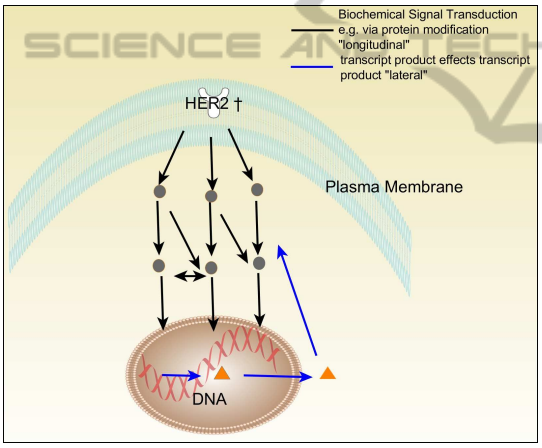


Figure 3: The action of the HER-2 growth factor on the cell transcriptome.

5 CONCLUSIONS

In contrast to ODEs, Metabolic P systems (MP systems), based on Păun's P systems, were introduced for modelling metabolic systems by means of suitable multiset rewriting grammars. In this work, three applications of MP systems for discovering the internal regulation logic of three phenomena relevant in systems biology have been presented.

The last two modelled phenomena are currently under development in order to extend the MP methodology in cases more complex (i.e. the insulin-glucose dynamics where C-peptide time series are taken into account) or other kind of gene expression analysis related to other pathological situations. In this perspective we intend to develop algorithmic and computational tools for making the MP modelling more adequate and useful in biomedical applications.

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