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The metabolic algorithm for P systems: Principles and applications

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ABSTRACT

Metabolic P systems, shortly MP systems, are a special class of P systems, introduced for expressing biological metabolism. An MP system evolution is given by a *metabolic algorithm*, a deterministic strategy, where the classical viewpoint on metabolic dynamics, in terms of ordinary differential equations, is replaced by suitable generalizations of chemical principles. The basic principles of MP systems are given and their main aspects are explained by means of examples of biological modeling. A new kind of regulation mechanism is outlined, which could be the basis for a more efficient construction of computational models from experimental data of specific metabolic processes.

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1. Introduction

P systems were introduced as a new computation model, inspired by **biology**. Membrane computing, based on P systems [39,40,50], was developed in the context of the formal language theory, as a new formal model of string rewriting, essentially based on *multisets* and *membranes*. This field has rapidly grown proving important results of computational universality for many classes of P systems.

The biological nature of this formalism, rooted in the membrane structure of living cells, suggested its use in the (discrete) mathematical modeling of several **biomolecular** phenomena acting at the cellular level, such as trans-membrane transport [36,38] and communication [3], consumption of energy [19,41] and even more specific biological processes [17,5,37,48,11]. However, these models had an essentially qualitative character. Metabolic P systems, shortly MP systems, were introduced in [32,30] for a better understanding of quantitative aspects of biological systems, meanwhile avoiding the use of complex systems of differential equations. Differently from the classical P systems, which are typically based on nondeterministic evolution strategies, MP systems have a discrete deterministic evolution strategy that links their **behaviour** to specific dynamical parameters.

Early attempts of symbolic descriptions of metabolic processes [28,29] considered some primitive notions of membrane systems, where the use of logical formulae driving metabolite concentrations resulted too general for expressing complex biological dynamics. P systems, based on membrane and multiset rewriting, provided the right environment for representing, in a discrete mathematical setting, biological dynamics. However, the main research perspective in P systems was essentially devoted to prove important computational properties for many classes of P systems, exploiting features of strong biological relevance. From a biological viewpoint, Turing's computation paradigm is not adequate for describing the evolution of living systems. The main point of discordance is the halting property which is the end of any terminating computation, giving a result. The main purpose of any living system is exactly opposite to termination. The most important strategy of any living system is to persist in a state where living processes can go on, by escaping from any cause stopping them, in fact, termination is death, and life is against it.

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A first step for a study of P systems, in a dynamical perspective, was introduced in [2], where the dynamical patterns of P systems were the main focus of investigation. In [46,47], a famous phenomenon of chemical oscillation, known as Belousov–Zabotinsky reaction, shortly BZ, was modeled, by multiset rewriting rules, and compared with the differential formulation due to Nicolis and Prigogine, also called Brusselator. These papers suggested us the idea of defining a general algorithmic strategy for computing the evolution of P systems for modeling biochemical processes.

A second step in developing P systems, according to a biochemical dynamical perspective, was achieved by the introduction of a molar perspective, borrowed from chemistry, for overcoming the limitations of standard rewriting mechanism of classical P systems. From a mathematical viewpoint it is very useful to apply the multiset rewriting rules in a *nondeterministic maximal parallel way*. This means that a maximal set of rules has to be chosen, among all the rules which can be applied, and these rules must be applied to a maximal multiset of all the objects to which the rules can be applied. However, this mechanism is too abstract when rules model biochemical reactions. For this reason, we introduced a molar perspective, and an abstract notion of “reaction strength” as a parameter able to regulate the cooperation/competition among the rules of P systems [30]. In fact, in a very first approximation, a cell is a membrane system, and its functioning is determined by all the types of molecules inside it, by the amount of molecules of these types, and by the cell compartments where they are located [1]. Therefore, it is of great importance to define a method for computing the evolution of a P system that is directly meaningful with respect to biochemical reactions. In this perspective, a transformation $AA \rightarrow BC$ is better read in chemical terms, as something which expresses the following prescription: “two moles of A produce one mole of B and a mole of C”. Here a *mole* is a conventional population unit like a *battalion*, or a *brigade*, which is not conceived in an absolute way, as it happens in the classical chemical setting (1 mole $\approx 6.02 \times 10^{23}$ molecules), but it is relative to the specific system. If we fix the number of objects of a mole, then the dimension of a multiset, in terms of moles, is a rational number.

MP systems [32–34,15,35] formalize these intuitions by considering P systems with a particular deterministic evolution procedure, called MP Algorithm, shortly MPA [33]. MP systems were proven to effectively model the dynamics of several biochemical processes: the Belousov–Zhabotinsky reaction (Brusselator) [7,9], the Lotka–Volterra dynamics [30,8,7,9,15], the SIR Susceptible–Infected–Recovered epidemic [7], the leukocyte selective recruitment in the immune response [17,7], the Protein Kinase C activation [9], circadian rhythms [14], mitotic cycles [33], and a *Pseudomonas* quorum sensing model [10]. Other phenomena under investigation concern Cdc25A degradation in tumor processes [18], an oscillatory circuit that includes Protein Kinases ERK2 and PK [27] and the intercellular communication which occurs in *Dictyostelium discoideum* [21]. The most part of these models are collected in [6]. In all these cases we compared the evolutions computed by our metabolic algorithm with the solutions of the differential models available in literature, and the comparison showed an almost full concordance. The reactivity of a rule can be also considered as a measure of the propensity or probability of applying it, in the line of Gillespie’s approach [20]. This perspective was investigated in [42,43], where some specific kinds of probabilistic approaches to biochemical kinetic were set in P systems frameworks.

In [16] a general relationship was discovered between ODE (ordinary differential equations) and MP models, and a general procedure was defined for deducing, from an ODE model, an MP system having the same dynamics. However, a natural question arises: “Why searching for metabolic models different from the classical ones based on ODE?” The answer is not only due to a theoretical interest. In fact, there are deep reasons making ODE models inadequate in many real situations. Very often, writing the differential equations regulating a metabolic dynamics is not difficult, because the stoichiometry of the biochemical reactions is well known, and what differential equations express is the *mass action* principle establishing the equilibrium between reactants and products quantities. But, in these equations kinetic constants occur which determine the exact rates of the metabolic variations. The determination of these constants depends on the chemo-physical details of the reactions, and moreover, even if carefully established in rigorous experimental settings, they may be completely different when many reactions are put together in real complex systems. This is the main limitation for a reliable scale-up of differential models in complex biological situations. Is it possible to overcome this limitation? The research initiated with MP systems intends to investigate discrete mathematical methods that consider dynamical parameters as “encoding” global behavioural patterns which are deducible from suitable macroscopic observation of real systems. This could be of great interest especially when kinetic constants are not available or difficult to be determined. In this paper we present the main principles of MP systems and will show an example of biological modeling by means of them. Finally, we outline some initial ideas on the identification and evaluation of a new kind of dynamical parameters which could replace the usual kinetic rates of biochemical reactions.

2. Metabolic P systems

MP systems are deterministic P systems where the transition to the next state (after some specified interval of time) is calculated according to a *mass partition strategy*, that is, the available matter of each substance is partitioned among all reactions which need to consume it. The policy of matter partition is regulated at each instant by real numbers, called *reactivities*, which represent the strength of any reaction.

A *discrete multiset* over an alphabet T is a function from T to the set \mathbb{N} of natural numbers. A *continuous multiset* over an alphabet T is a function from T to the set \mathbb{R} of real numbers. We use letter q (possibly with subscripts or exponents) for denoting a state of a metabolic system, that is a continuous multiset $q : T \rightarrow \mathbb{R}$. Moreover, we use capital letters for symbols of T and if $T = \{X_1, \dots, X_n\}$ and $q(X_1) = x_1, \dots, q(X_n) = x_n$, then we may identify q by the vector $\mathbf{x} = (x_1, \dots, x_n)$.

As it is customary in P systems, we will adopt the string notation for discrete multisets, that is, when a string denotes a multiset, the order of its symbols is not relevant. We write $X \in \alpha$ for saying that X is a symbol occurring in the string α (the symbol $+$ could be used for concatenation, in order to stress that in multisets concatenation is commutative; more details on P systems' notations can be found in [40]).

The set Q of states over an alphabet T are the continuous multisets over T . The passage from discrete to continuous states is motivated by the use of moles for determining the mass associated to each symbol of T .

The notion of MP system we consider here should be better identified by that of *zero level* MP system, because only one membrane is considered. The more general definition of MP systems with a given membrane structure is only matter of notational complication. Now we prefer to avoid it, for focusing our attention on the evolution strategy by means of our metabolic algorithm.

Definition 1 (*MP System*). An MP system is a construct

$$M = (T, R, F, \nu, \sigma, \tau, q_0, \Delta_q)$$

in which

- T is a finite set of symbols;
- R is a finite set of rules, i.e., pairs of discrete multisets over T (represented, as usual, in the arrow notation);
- F is the set of *reaction maps*, such that $F = \{f_r \mid r \in R\}$, where $f_r : Q \rightarrow \mathbb{R}$. Very often the *reactivity* $f_r(q)$ in the state $q \in Q$ depends only on the mass associated to some of the symbols of T , therefore, we write $f_r(x, y, \dots)$ to make the variables x, y, \dots which f_r depends on **explicit**;
- ν is a natural number which specifies the value of a (conventional) mole of M ;
- σ is a function which assigns to each $X \in T$, the mass $\sigma(X)$ of a mole of X , with respect to some measure unit;
- τ is the temporal interval between two consecutive states;
- q_0 is the initial state of M , an element of Q ;
- Δ_q is the temporal evolution of M , calculated by means of a *metabolic algorithm* which provides, for any state $q \in Q$, the function

$$\Delta_q : T \rightarrow \mathbb{R}$$

according to it, for the state q' following q in the temporal evolution of M , we have:

$$q'(X) = q(X) + \Delta_q(X)$$

for every $X \in T$.

The values of ν, σ, τ of the previous definition have no direct influence in the mathematical description of the dynamics of an MP system. Nevertheless, they are essential for the physical interpretation of the dynamics with respect to a specific mass/time measure scale according to which numerical values have to be read. The metabolic algorithm, for the class of MP systems considered in this paper, will be given in **Definition 5**.

In the case of ODE models the variation of substance quantities are considered in infinitesimal time intervals. Being these intervals infinitesimals, the competition among reactions can be disregarded. Therefore, all the mass present in the reactor is available to each reaction. This implicit assumption is the basis of the *mass action principle*, according to which, the infinitesimal variation of a substance produced by a reaction, is the product, by some reaction rate, of the whole reactant masses instantaneously available. In general, the dynamics can be expressed by a system of differential equations having the form (1) given below, where variables denote the quantities of the substances involved in the process. It is **worth** noting that in this form there is no explicit occurrence of the time variable. This means that quantities variations depend only on the state of the system, but not directly on the time instant. This kind of systems are called *autonomous* ODE and are the principal **methods** of the classical analyses of biological metabolism [49]. According to Picard's theorem if the functions f_1, f_2, \dots, f_n are of class C^1 (continuously differentiable functions), then the relative Cauchy's problem is solvable in a suitable interval: there exist n functions $x_1 = g_1(t), x_2 = g_2(t), \dots, x_n = g_n(t)$ that are solutions of the system and satisfy some given initial conditions [26].

$$\begin{aligned} \frac{dx_1}{dt} &= f_1(x_1, x_2, \dots, x_n) \\ \frac{dx_2}{dt} &= f_2(x_1, x_2, \dots, x_n) \\ &\dots \\ \frac{dx_n}{dt} &= f_n(x_1, x_2, \dots, x_n). \end{aligned} \tag{1}$$

If we pass from infinitesimal time intervals to macroscopic time intervals between observation steps, then we replace the "mass action" by a "mass partition" among reactions (rules). That is, in a given time the available mass of a substance A is divided among the reactions which need it, according to the percentage which they consume in the time interval of the observation step. The percentage of a reactant A taken by a rule r depends on the *weight* of r over A . This weight is due to the real number $f_r(q)$ which expresses the *reactivity* of the rule, in the state q . In fact, the function f_r , called *reaction map* of r , determines the reactivity of r for any state of the system. In conclusion, dynamical regulation is performed by the reaction

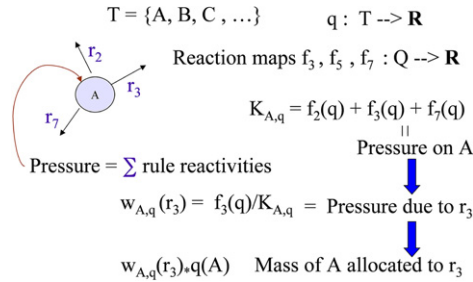


Fig. 1. Reaction maps, pressure, weights, and mass allocated to a rule.

maps of the rules. In a given step, the weight of a rule, over a reactant A , is obtained as the ratio between reactivity of the rule and the sum of the reactivities of all the rules that need the reactant A (this sum is also called the *global pressure* over A). The weight $w_{A,r}(q)$ of rule r over A in the state q , multiplied by the quantity of A and divided by $h(r)$, that is, $w_{A,r}(q)q(A)/h(r)$ is the mass $m_{A,r}(q)$ assigned, in the state q , to any occurrence of A in α_r (the same reactant can occur in many copies).

Fig. 1 illustrates, by an example, the mass partition principle adopted in Metabolic P systems. As a consequence of this principle two important aspects follow. In MP system rules act on object populations, rather than on single objects. Moreover, dynamics is deterministic at population level, but nothing can be said about the dynamical evolution of single objects. This situation resembles what happens in the macroscopic gas laws which specify deterministic relationships among pressure, volume and temperature measures, but do not describe the mechanical behaviour of single molecules.

The two following assumptions are directly related to the mass partition strategy adopted for MP systems evolution. An **inertial rule** is a rule $r_X : X \rightarrow X$ that does not transform a substance X . The following principle assumes an inertial rule for any substance. For this reason we do not mention explicitly inertial rules. As will be specified (see Definition 11), in the examples we will consider that all the inertial rules have the same constant value of reactivity.

Principle 2 (Inertia). In any MP system, for every $X \in T$, an inertial rule r_X for the substance X is present. The reactivity of r_X , in a given state, is the inertia of the substance X in that state, that is, its tendency to not be transformed into other substances.

An **input rule** is a rule that assumes the introduction of matter from outside. In the graphical representation that will be adopted, an input rule is indicated by a triangle with a vertex connected to the node of the reaction. An output rule is a rule which expels matter outside, its graphical representation is a triangle with an edge connected to the node of the reaction (Figs. 4 and 11).

The following assumption avoids rules without reactants and will simplify the use of mass partition principle.

Principle 3 (Creativity). Any input rule r of type $\lambda \rightarrow X$ is implicitly transformed into a rule $\lambda_r \rightarrow \lambda_r X$ where λ_r is a new symbol in T , called the input symbol of r . This means that a sort of input gate is assumed to feed the system from the outside, at a rate depending on the reactivity of the input rule.

In order to define our MP algorithm, which formalizes the intuition given at the beginning of Section 2, we use the following notation from [33], that will be adopted in the rest of the paper and it will always be related to a metabolic system $M = (T, R, F, \nu, \sigma, \tau, q_0, \Delta_q)$.

Definition 4 (MP Notation). • Each $r \in R$ is denoted by $r : \alpha_r \rightarrow \beta_r$; where α_r identifies the multiset of the reactants (substrates) of r , and β_r identifies the multiset of the products of r ;

- $h_r(X)$ is the number of occurrences of X in α_r ;
- $g_r(X)$ is the number of occurrences of X in β_r ;
- $d_r(X) = g_r(X) - h_r(X)$ is the *stoichiometric difference* of X in r ;
- $R_\alpha(X) = \{r \in R \mid X \in \alpha_r\}$;
- $R_\beta(X) = \{r \in R \mid X \in \beta_r\}$;
- $R(X) = R_\alpha(X) \cup R_\beta(X)$;
- $\Pi(\alpha_r) = \prod_{X \in \alpha_r} q(X)^{h_r(X)}$ ($\Pi(\alpha_r) = 1$ if $\alpha_r = \lambda$).

We assume that if $\alpha_r = \lambda$, then $\beta_r \in T$, and if $\beta_r = \lambda$ then $\alpha_r \in T$.

MP systems are based on some basic principles which generalize the well-known chemical principles [44] (other important principles, related to the energetic aspects of reactions are not relevant for the following discussion).

- **Lavoisier Principle.** The overall mass consumed by any non-input or non-output rule has to equate the overall mass of its products (mass conservation law).
- **Avogadro Principle.** Let $u_r(q)$ be the minimum among the moles of reactants allocated to r . We call such a number *the reaction unit* of rule r in the state q . Then, for any $X \in \alpha_r$, a number $h_r(X)u_r(q)$ moles of X are consumed by r and for any $Y \in \beta_r$, a number $g_r(Y)u_r(q)$ moles of Y are produced by r .

• **Dalton Principle.** For any $X \in T$, the global number of moles of X produced/consumed, in the passage from a state to the next state, is the algebraic sum of moles produced/consumed, according to Avogadro's principle, by all the rules where X occurs (additivity law).

The following definition provides the **metabolic algorithm** computing the single step transition of our MP systems.

Definition 5 (MPA). The function Δ_q computed by the metabolic algorithm of an MP system, in a state $q \in Q$, is given, for any $X \in T$, by (it is assumed that $K_{Y,q} \neq 0$):

$$\Delta_q(X) = \sum_{r \in R(X)} d_r(X) u_r(q)$$

where

$$u_r(q) = \min \{m_{Y,r}(q) \mid Y \in \alpha_r\}$$

and, for every $Y \in T$

$$K_Y(q) = \sum_{r \in R_\alpha(Y)} f_r(q), \quad w_{Y,r}(q) = \frac{f_r(q)}{K_Y(q)},$$

and

$$m_{Y,r}(q) = w_{Y,r}(q) \frac{q(Y)}{h_r(Y)}.$$

Now, from this definition, a very precise and intuitive meaning of reactivity follows. In fact, the reactivity of a rule r coincides with the number u of moles that r would take, for a reactant X , in the case of an "ideal" competition/cooperation situation on X , that is, if the other rules of the set $R_\alpha(X) - \{r\}$, competing for X , are globally demanding v moles of X , and the quantity of X in the reactor is exactly of $u + v$ moles. Of course, in the majority of cases, this "happy end" game does not happen, therefore the real number of moles taken from r is accordingly established. However, reactivity is always given by a number of moles for step, therefore it has a mole/time physical dimension (time is the interval τ between two consecutive steps).

Let us denote by $\text{Lim}_r(q)$ the set of reactants of r to which a mass equal to $u_r(q)$ is allocated, for each reactant occurrence in r . The following proposition is a simple consequence of Definition 5.

Proposition 6. For any $X \in T$ and for any $r \in R_\alpha(X)$, if $K_X(q) = q(X)$, then $f_r(q) = h_r(X) \cdot m_{X,r}(q)$, and if also $X \in \text{Lim}_r(q)$, then $f_r(q) = h_r(X) \cdot u_r(q)$.

Definition 7. Two MP systems M_1, M_2 are equivalent if they have the same dynamics, that is their evolutions provide the same sequence of states.

The following proposition follows from the previous definitions and from the equality $\sum_{r \in R_\alpha(X)} w_{X,r}(q) = 1$, which holds for any rule r and state q .

Proposition 8. Let M_1, M_2 be two MP systems such that the reaction maps of M_1 coincide with the weights of M_2 , then M_1 and M_2 are equivalent.

The extension of the metabolic algorithm to the case of MP systems with many membranes is obtained by replacing a variable X with the pair of variables (X, i) where i denotes an index of a membrane, and in a rule (X, i) means that X is inside the membrane i (by using the membrane boundary notation [3], this information is explicitly put in each rule).

In an MP system two parts are clearly distinguishable: the *signature* and the *quantities*. The first part (T, R, F) indicates the kinds of objects, the reaction and their regulation structure. The second part specifies the quantitative aspect which give meaning to the numbers which describe the evolution of systems. We represent the signature of metabolic P systems, in a way directly readable in terms of the metabolic algorithm, by means of *MP graphs* [33]. Similar graphical formalisms were developed in the context of complex reaction networks (SNA, *Stoichiometric Network Analysis*, and MCA, *Metabolic Control Analysis* [12,13,45]; see also [24,49]). Fig. 4 is an example of MP graph which translates in graphical terms all the information given in Table 2 (the biological meaning will be clarified in the next paragraph): circles are substances, full black circles are rules, squares are reaction maps, and triangles link to rules which feed the system from outside or expel substances.

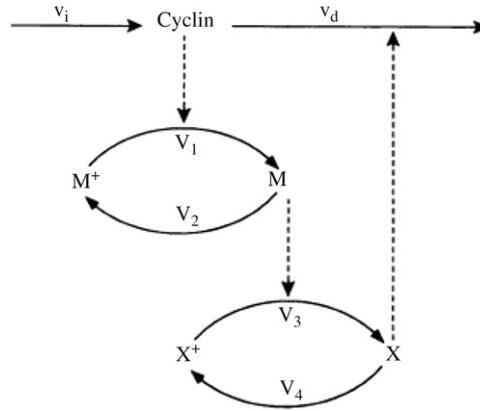


Fig. 2. The model provided by Goldbeter, from [21].

Table 1

Numerical values of Goldbeter's model

$K_1 = 0.005$	$K_2 = 0.005$	$K_3 = 0.005$	$K_4 = 0.005$
$V_{M1} = 3$	$V_2 = 1.5$	$V_{M3} = 1$	$V_4 = 0.5$
$v_i = 0.025$	$v_d = 0.25$	$K_c = 0.5$	$K_d = 0.02$
$k_d = 0.01$	$c(0) = 0.01$	$x(0) = 0.01$	$m(0) = 0.01$
$V_1 = \frac{V_{M1} * c}{(K_c + c)}$	$V_3 = m * V_{M3}$		

Table 2

An alternative formulation of the MP of Fig. 4

$r_1 : \lambda \rightarrow C$	$f_1 = v_i$
$r_2 : XC \rightarrow X$	$f_2 = v_d / k_d \pm \epsilon$
$r_3 : C \rightarrow \lambda$	$f_3 = K_d$
$r_4 : M^+C \rightarrow MC$	$f_4 = V_{M1} / (K_c + c)(K_1 + m^+)$
$r_5 : M \rightarrow M^+$	$f_5 = V_2 / (K_2 + m)$
$r_6 : X^+M \rightarrow XM$	$f_6 = V_{M3} / (K_3 + x^+)$
$r_7 : X \rightarrow X^+$	$f_7 = V_4 / (K_4 + x)$

Parameters are defined in [21], their numerical values are reported in Table 1.

3. A biological model based on MP systems

Now we consider an example of biological modeling which highlights the expressive power of MP formalisms and its relationship with classical differential models. Fig. 2 describes an important case study of mitotic oscillator, which is reported in [21]. Mitotic oscillations are mechanisms exploited by nature to regulate the onset of mitosis, that is, the process of cell division aimed at producing two identical daughter cells from a single parent cell. More precisely, mitotic oscillations concern the fluctuation in the activation state of a protein produced by *cdc2* gene in fission yeasts or by homologous genes in other eukaryotes. The model here considered focuses on the simplest form of this mechanism, as it is found in early amphibian embryos. Here (see Fig. 2) cyclin is synthesized at a constant rate and triggers the transformation of inactive (M^+) into active (M) *cdc2* kinase, by enhancing the rate of a phosphatase E_1 . Another kinase reverts this modification. On the other hand a kinase E_3 elicits the transformation from the inactive (X^+) to the active (X) form of a protease that degrades cyclin, and this activation is reverted by a phosphatase E_4 (E_1, E_2, E_3, E_4 are not indicated in the figure, $v_i, v_d, V_1, V_2, V_3, V_4$ denote rates of the processes, see Table 1). The activation of *cdc2* kinase provides the formation of a complex known as M-phase promoting factor (or MPF). The complex triggers mitosis and the degradation of cyclin leads to the inactivation of the *cdc2* kinase that brings the cell back to the initial conditions in which a new division cycle can take place. In yeasts and in somatic cells the cell cycle is subject to the control of many checkpoints, but the mechanism based on the activation-inactivation of *cdc2* kinase remains the same [1].

The following ODE is the differential model of dynamics described in Fig. 2, where c, m, x are the percentages of C, M, X respectively ($1 - m, 1 - x$ are the percentages of M^+, X^+ respectively):

$$\begin{aligned}
 \frac{dc}{dt} &= v_i - v_d x \frac{c}{K_d + c} - K_d c \\
 \frac{dm}{dt} &= V_1 \frac{(1-m)}{K_1 + (1-m)} - V_2 \frac{m}{K_2 + m} \\
 \frac{dx}{dt} &= V_3 \frac{(1-x)}{K_3 + (1-x)} - V_4 \frac{x}{K_4 + x}.
 \end{aligned} \tag{2}$$

Fig. 3 shows the solutions of these equations obtained by numerical integration for some value of parameters given in [21] and reported in Table 1.

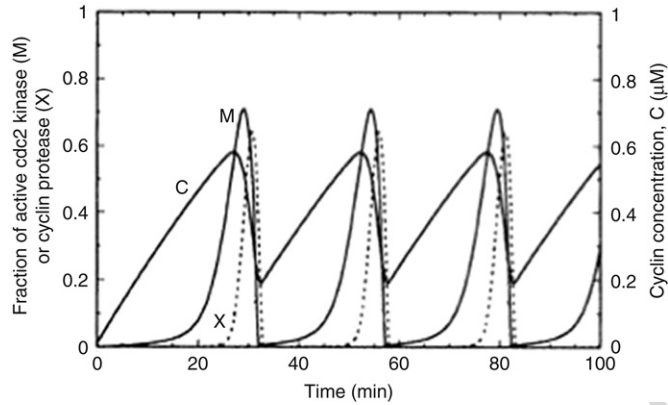


Fig. 3. A numerical solution of the set of differential equations (2) implementing the model provided by Goldbeter, from [21].

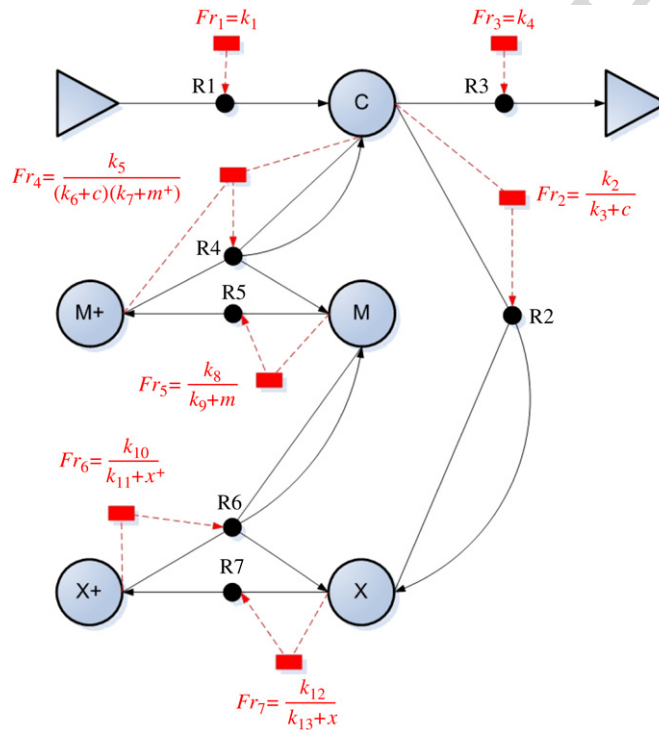


Fig. 4. A model of the mitotic oscillator of Fig. 2 represented by a MP graph, from [33]. The correspondences with Goldbeter's constants are: $k_1 = v_i$, $k_2 = v_d$, $k_3 = k_d$, $k_4 = K_d$, $k_5 = V_{M1}$, $k_6 = K_c$, $k_7 = K_1$, $k_8 = V_2$, $k_9 = K_2$, $k_{10} = V_{M3}$, $k_{11} = K_3$, $k_{12} = V_4$ and $k_{13} = K_4$.

A general relationship between MP graphs and ODE holds. In fact, MP Graphs transform naturally into ODE systems according to the mass action principle, on which differential models are based on: the amount of the products generated by a reaction is proportional to the product of quantities of substrates (considered with their multiplicity). This idea [16] is formalized by Definition 9, where the MP notation of Definition 4 is assumed, x is the real variable $q(X)$, and x' is the derivative of x with respect to time.

Definition 9 (MP-ODE Transformation). Let $G = (T, R, F)$ be an MP graph. For every $X \in T$, let $x = q(X)$ then the following is the ODE-transformed of G :

$$x' = \sum_{r \in R} d_r(X) f_r(q) \Pi(\alpha_r).$$

Fig. 4 shows an MP graph which provides the structure of an MP system. Bigger circles indicate the substances involved in the mitotic oscillator. Black, small circles denote the reactions transforming substances (the arrow verse going from reactants to products). Rectangles, connected to black circles by intermittent arrows, are labeled by the reaction maps which provide, for each state, the reactivity of the rule according to which the metabolic algorithm partitions substances among reactions. Triangles denote either reactions with reactants from outside, or reactions which expel their products outside. The MP

Table 3

A non-cooperative, slowly convergent MP model of mitotic oscillator

$r_1 : \lambda \rightarrow C$	$f_1 = v_i$
$r'_2 : X \rightarrow X$	$f_2 = v_d \cdot c / (k_d + c)$
$r''_2 : C \rightarrow \lambda$	$f_2 = v_d \cdot x / (k_d + c)$
$r_3 : C \rightarrow \lambda$	$f_3 = k_d$
$r'_4 : C \rightarrow MC$	$f_4 = V_{M1} \cdot m^+ / (K_c + c)(K_1 + m^+)$
$r''_4 : M^+ \rightarrow \lambda$	$f_4 = V_{M1} \cdot c / (K_c + c)(K_1 + m^+)$
$r_5 : M \rightarrow M^+$	$f_5 = V_2 / (K_2 + m)$
$r'_6 : X^+ \rightarrow XM$	$f_6 = V_{M3} \cdot m / (K_3 + x^+)$
$r''_6 : M \rightarrow \lambda$	$f_6 = V_{M3} \cdot x^+ / (K_3 + x^+)$
$r_7 : X \rightarrow X^+$	$f_7 = V_4 / (K_4 + x)$

Pairs of rules $\{r'_2, r''_2\}$, $\{r'_4, r''_4\}$, and $\{r'_6, r''_6\}$ correspond to the rules r_2, r_4, r_6 , respectively, given in Table 2.

graph of Fig. 4 is directly related to Goldbeter's model and is obtainable by means of the following procedure [16], which is completely motivated by the theorems we give at the end of this section. Consider an MP graph H where all the known biochemical reactions involving the mitotic phenomenon in amphibian embryos are indicated as rules, while reaction maps are unknown. Consider the ODE-Transform $ODE(H)$ of H , and equate the right members of (2) and $ODE(H)$. These equations determine the values of the reaction maps of H such that its ODE-Transform coincides with (2).

Table 2 is an alternative way to represent the MP graph in Fig. 4, where constants were put in the original format given in [21].

The following classes of MP systems play an important role in the relationship between ODE and MP systems.

Definition 10 (Non-cooperative MP System). A non-cooperative MP system is an MP system whose rules are non-cooperative, i.e., $\alpha_r \in T$ for every rule r of the system.

Definition 11 (Uniformly Inertial MP System). For some $\phi \in \mathbb{R}$, an MP system is ϕ -uniformly inertial if the reaction map of any inertial rule of the system has the same constant value ϕ in any possible state, called the (uniform) inertia of the system.

The following results are proved in [16].

Theorem 12. Given an ODE system E , there are MP graphs having E as their ODE-transforms.

Theorem 13. Given a non-cooperative, ϕ -uniformly inertial MP system M , if $ODE(M)$ satisfies the differential conditions of univocal solvability, then the evolution of M converges, as $\phi \rightarrow \infty$, to the solution of $ODE(M)$ having the initial conditions of M .

Theorem 14. For any MP system M , there exists a non-cooperative MP system M' having the same ODE transform of M .

Corollary 15. Approximate solutions of autonomous ODE which describe metabolic systems can be solved by computing the evolution of suitable MP systems.

Table 3 describes an MP graph which is obtained by a procedure relative to Theorem 14. It is easy to verify that the ODE-Transform of this graph (Definition 9) is equal to that of graph in Fig. 4, that is, to the Eq. (2). As it is asserted by Theorem 13, if we consider an uniform inertia acting in the system, then the MP evolution of this system approximates, for increasing inertia, the differential solution of Golbeter's model.

Fig. 5 shows the evolution of the MP system given in Table 2 for a small value of inertia, while Fig. 6 shows that the signal is completely lost when inertia is increased. This discrepancy between differential and P metabolic models is consistent with the previous theorems relating MP graphs and differential equations. In fact, the system of Table 2 does not fulfill the non-cooperativity requirement. But, if we apply the procedure providing a non-cooperative MP graph equivalent to that of Table 2, then we get many possible MP graphs. Two of them are given in Tables 3 and 4. Both these systems have the evolution given in Fig. 7. The similarity with Golbeter's solution 3 is evident and confirms the validity of the previous theorems, in a very significant biological model.

The only difference between the systems in Tables 3 and 4 is the convergence speed. In fact system of Table 4 converges more rapidly to the differential solution of (2), because, with a inertia value of 1.500, it provides a better result of the system of Table 3 with a inertia value of 7.500.

4. Log-gain regulation

In the definition of MPA it appears clear that what is essential in the evolution of an MP system is the knowledge of the reaction unit u_r of each rule r at each evolution step. A simple argument proves that reaction units determine completely the concentration variation of substances in the passage from a state to the next one. In fact, if we know the rules which produce and consume a given substance X , from the stoichiometry of these rules, given the value (number of moles) or their reaction units, we can deduce exactly the mole variation of X in the step. In other words, the knowledge of the number of

Table 4

A non-cooperative, rapidly convergent MP model of mitotic oscillator

$r_1 : \lambda \rightarrow C$	$f_1 = v_i$
$r_2' : C \rightarrow X$	$f_2 = v_d \cdot x / (k_d + c)$
$r_2'' : X \rightarrow \lambda$	$f_2 = v_d \cdot c / (k_d + c)$
$r_3 : C \rightarrow \lambda$	$f_3 = k_d$
$r_4' : C \rightarrow MC$	$f_4 = V_{M1} \cdot m^+ / (K_c + c)(K_1 + m^+)$
$r_4'' : M^+ \rightarrow \lambda$	$f_4 = V_{M1} \cdot c / (K_c + c)(K_1 + m^+)$
$r_5 : M \rightarrow M^+$	$f_5 = V_2 / (K_2 + m)$
$r_6' : X^+ \rightarrow XM$	$f_6 = V_{M3} \cdot m / (K_3 + x^+)$
$r_6'' : M \rightarrow \lambda$	$f_6 = V_{M3} \cdot x^+ / (K_3 + x^+)$
$r_7 : X \rightarrow X^+$	$f_7 = V_4 / (K_4 + x)$

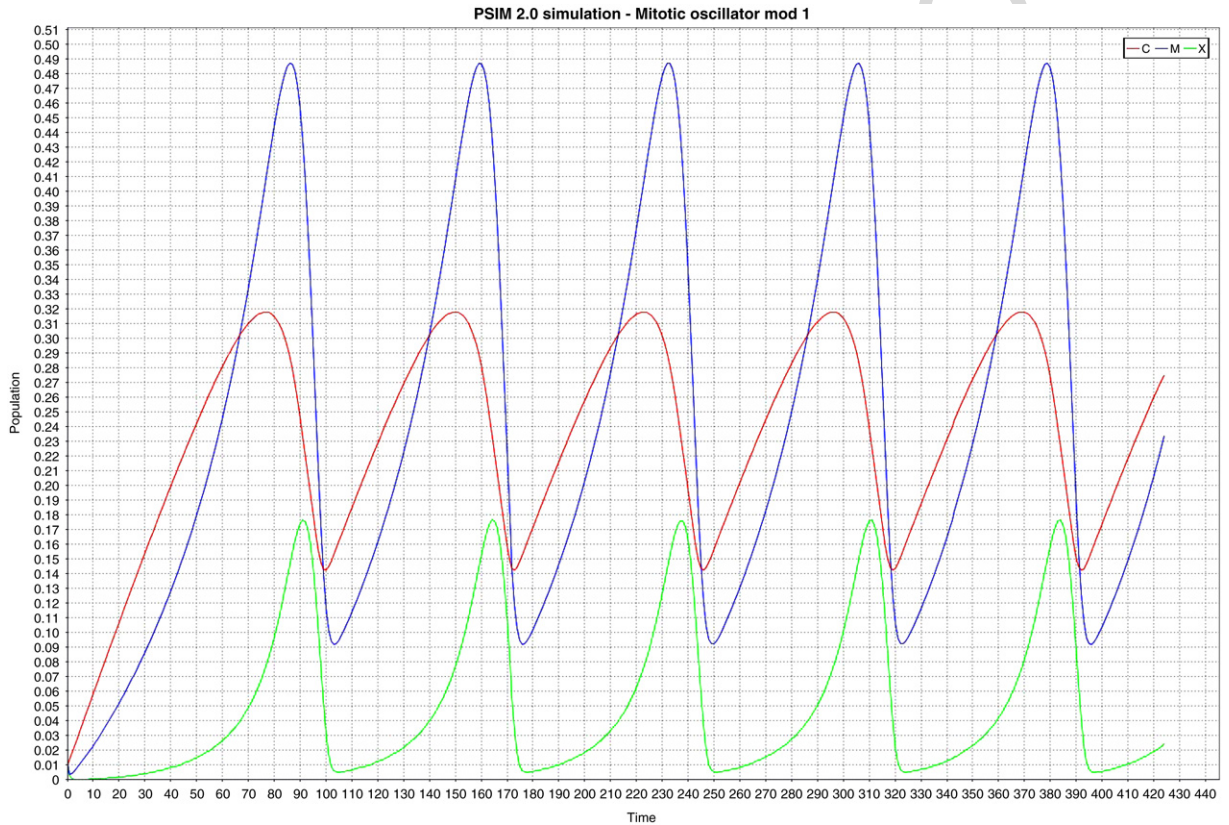


Fig. 5. The mitotic oscillator of Fig. 1 computed by means of a cooperative MP system with inertia 5.

objects transformed by any rule in the time unit is what we need for deriving the new concentrations of substances at the next step.

The reaction maps allow us to evaluate these values by computing some ratios and by choosing a suitable minimum, according to Avogadro's principle. However, we could follow a different strategy, by reversing the relationship between reaction units and reaction maps. As the reaction unit of a rule depends on the state of the system, it is reasonable to assume that really only a subset of all substance types influence it. The types such that a variation of their concentration determine a variation of reaction unit u_r of r are called *regulators* of rule r . We consider by default as regulators all the reactants of a rule. We denote the set of regulators of r by the string γ_r (the order of its symbols it is not significant). Another assumption seems to be perfectly natural: a proportion should exist among the relative variation of a regulator of a rule r and the relative variation of the reaction unit of r . The relative variation of a substance X is defined as the ratio $\Delta(x)/x$. In differential notation (with respect to time variable), this ratio is related to $\frac{dx}{dt}/x$, but from elementary calculus we know that it is the same as $\frac{d(\lg x)}{dt}$. This equation explains the term "log-gain" for expressing relative variations [49].

Now, it is very reasonable to claim that the effects of regulators are cumulative, therefore: *the passage from the value of the reaction unit of a rule r to its value in the next step can be computed as a linear combination of the relative variations of concentrations of the regulators of r* . This is the log-gain principle formally stated in the next Proposition 16. By applying this

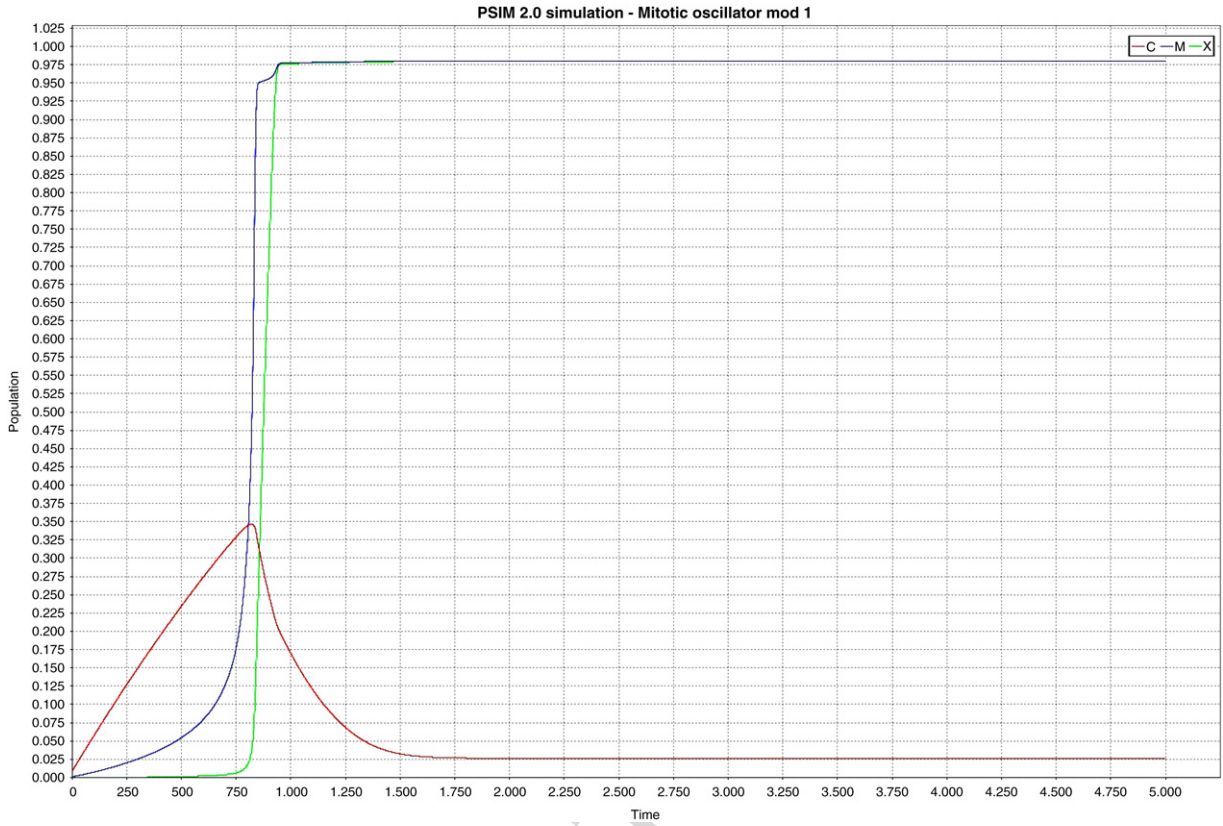


Fig. 6. The mitotic oscillator of Fig. 1 computed by means of a cooperative MP system with inertia 50.

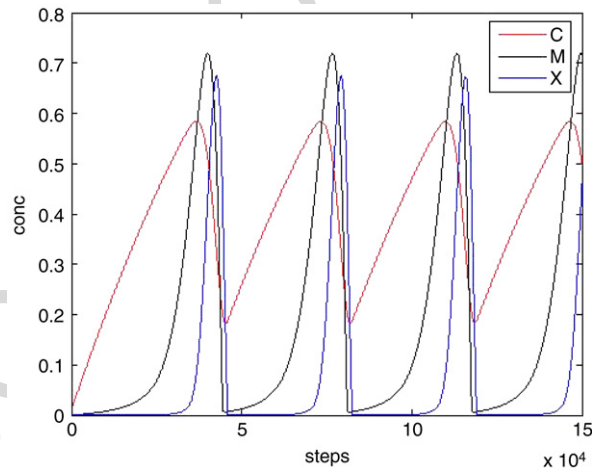


Fig. 7. The mitotic oscillator of Fig. 1 computed by means of non-cooperative MP systems having Eq. (2) as ODE-Transform.

principle, starting from some initial values of the reaction units we can derive their values at any observation time, therefore, these parameters determine the evolution of an MP system.

Log-gain principle extends a very important rule, well known in theoretical biology as the **allometric principle**. According to it a specific ratio holds between the relative variations of two related biological parameters (*e. g.* mass of an organism and its superficial area). As it is reported in [4], many empirical laws on metabolism are instances of *allometry* and also the abundance of power laws in biological systems is related to this principle. Therefore, it is not surprising that log-gain mechanism could suggest an analysis of regulation mechanism in metabolic MP systems.

Log-gain parameters determine the evolution strategy of MP systems. In fact, let us generalize the state of an MP system, having n substances and m rules, in such a way that it is defined by a real vector $(q, U(q))$ of $n + m$ values, where $q = (q(X) \mid X \in T)$ gives the quantity of each substance, and $U(q) = (u_r(q) \mid r \in R)$ gives the reaction unit of each rule. Let

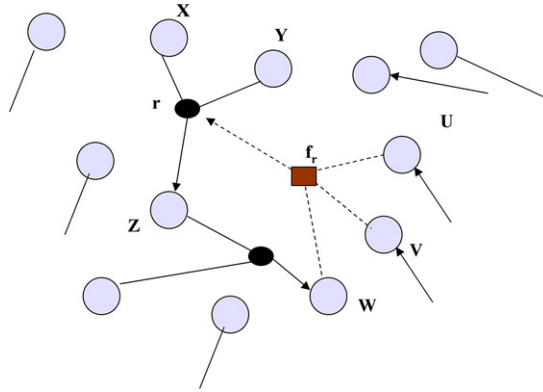


Fig. 8. Map reaction regulation.

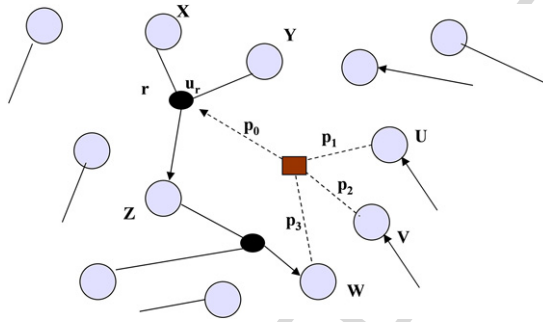


Fig. 9. Log-gain regulation.

$Lg(u_r(q)) = (u_r(q) - u_r(q'))/u_r(q)$ where $u_r(q)$ and $u_r(q')$ are the reaction units or rule r in the state q and in the next state q' following q . Let $Lg(q(X)) = (q(X) - q'(X))/q(X)$ be the log-gain of the substance X . As we already mentioned, according to a cumulative perspective of *effect additivity*, $Lg(u_r(q))$ could be expressed as a linear combination of the log-gains of the regulators of r . Of course, the adequacy of this assumption cannot be formally proved, however, as it will be shown, this hypothesis will suggest a possible method for deducing log-gain parameters which correspond to a given observed metabolic dynamics.

The relationship between reaction maps and log-gain parameters is illustrated by Figs. 8 and 9.

The following proposition formally states the log-gain principle.

Proposition 16 (*Log-Gain Principle*). Given an MP system $M = (T, R, F, v, \sigma, \tau, q_0, \Delta_q)$, let us assume to know the vector u_0 of initial reaction units, then the function Δ_q provided by the metabolic algorithm of M satisfies the following system of equations, with $X \in T$ and $r \in R$:

$$\Delta_q(X) = \sum_{r \in R(X)} d_r(X) u_r(q) \quad (3)$$

$$Lg(u_r(q)) = \sum_{Y \in \gamma_r} p_{(r,Y)} Lg(q(Y)) + p_r. \quad (4)$$

The equations of Proposition 16 can be expressed in terms of matrix product. In fact, let us represent a rule r as a (column) vector of dimension n , constituted by its stoichiometric coefficients: $r = (d_r(X) | X \in T)$. Then, consider Δ_q as the (column) vector given by the variations of substances between two consecutive steps q, q' :

$$\Delta_q = (q'(X) - q(X) | X \in T)$$

and the vector of the reaction units given by:

$$U(q) = (u_r(q) | r \in R)$$

then, if M_R is the matrix $(d_r(X) | r \in R, X \in T)$ of dimension $m \times n$, where T has cardinality n and R has cardinality m , we have:

$$\Delta_q = M_R * U(q).$$

Moreover, let

$$Lg(U(q)) = (Lg(u_r(q)) | r \in R)$$

Table 5
The MP signature of Sirius oscillator

r_1	$A \rightarrow AA$	$f_1 = k_1$
r_2	$A \rightarrow B$	$f_2 = k_2 \cdot c$
r_3	$B \rightarrow \lambda$	$f_3 = k_3$
r_4	$A \rightarrow C$	$f_4 = k_4 \cdot b$
r_5	$C \rightarrow \lambda$	$f_5 = k_5$

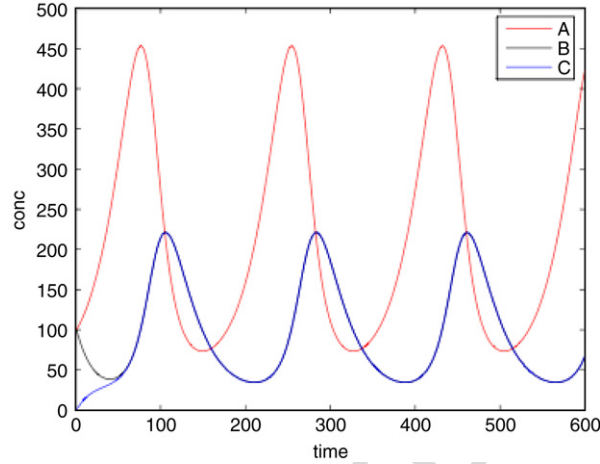


Fig. 10. Sirius oscillator dynamics.

and let the matrix P_R be constituted by the m (row) vectors of dimension n which provide the log-gain parameters of each rule with respect to the log-gain vector of substances, and the (column) vector P_0 by the log-gain vector ($p_r | r \in R$). With this notation, $Lg(U) = P_R * Lg(q) + P_0$ provides the m equations of the reaction unit variations. In conclusion:

$$\Delta_q = M_R * U \quad (5)$$

$$Lg(U) = P_R * Lg(q) + P_0. \quad (6)$$

Consider the metabolic system given in Table 5.

This system is interesting because it provides a simple metabolic oscillator which results very naturally in terms of metabolic algorithm. Let us call it *Sirius* (it is a beautiful star!). Its differential formulation, according to the correspondence stated in Definition 9, is the following.

$$\begin{aligned} \frac{da}{dt} &= k_1 a - k_2 c a - k_4 b a \\ \frac{db}{dt} &= k_2 a c - k_3 b \\ \frac{dc}{dt} &= k_4 a b - k_5 c. \end{aligned} \quad (7)$$

Setting the following values for the constants: $k_1 = 4$, $k_2 = 0.02$, $k_3 = 4$, $k_4 = 0.02$, $k_5 = 4$ and the the initial values of substance quantities as $a = 100$, $b = 100$, $c = 0$, with a uniform inertia of 100 it exhibits the dynamics given in Fig. 10 (when we fix τ a number of k steps correspond to the time $k\tau$).

Observe two consecutive steps n , $n + 1$ of its evolution. Let $a[n]$, $b[n]$, $c[n]$ and $a[n + 1]$, $b[n + 1]$, $c[n + 1]$ be the quantities (expressed in moles) of substances of this system at these steps. Substance a is produced by the rules r_1 and it is consumed by the rules r_2 , r_4 , and analogously for the other substances. Therefore, by applying the first formula of Proposition 16, we get the following system of equations.

$$a[n + 1] - a[n] = u_1[n] - u_2[n] - u_4[n]$$

$$b[n + 1] - b[n] = u_2[n] - u_3[n]$$

$$c[n + 1] - c[n] = u_4[n] - u_5[n].$$

Let the values $u_1[n]$, $u_2[n]$, $u_3[n]$, $u_4[n]$, $u_5[n]$ and $u_1[n + 1]$, $u_2[n + 1]$, $u_3[n + 1]$, $u_4[n + 1]$, $u_5[n + 1]$ be the reaction units at the steps n and $n + 1$, then by applying the second formula of Proposition 16 we get the following systems of equations, where $Lg(a[n]) = (a[n + 1] - a[n])/a[n]$, and analogously for $Lg(b[n])$, $Lg(c[n])$:

$$Lg(u_1[n]) = p_1 Lg(a[n]) + p_2$$

$$Lg(u_2[n]) = p_3 Lg(a[n]) + p_4 Lg(c[n]) + p_5$$

$$Lg(u_3[n]) = p_6 Lg(b[n]) + p_7$$

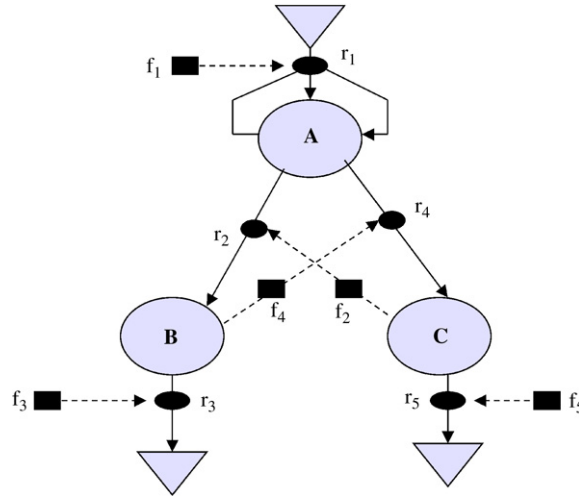


Fig. 11. Sirius oscillator as an MP graph. The top triangle indicates that duplication $A \rightarrow AA$ is supported by some external source.

$$Lg(u_4[n]) = p_8 Lg(a[n]) + p_9 Lg(b[n]) + p_{10}$$

$$Lg(u_5[n]) = p_{11} Lg(c[n]) + p_{12}.$$

In this case the matrices M_R and P_R , and the vector P_0 are the following.

$$\begin{pmatrix} 1 & -1 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix}, \begin{pmatrix} p_1 & 0 & 0 \\ p_3 & 0 & p_4 \\ 0 & p_6 & 0 \\ p_8 & p_9 & 0 \\ 0 & 0 & p_{11} \end{pmatrix}, \begin{pmatrix} p_2 \\ p_5 \\ p_7 \\ p_{10} \\ p_{12} \end{pmatrix}.$$

In general we set the following definition.

Definition 17 (Log-Gain Module). For any MP system, its log-gain module is the set of equations obtained by the system (4) of the Proposition 16 between two consecutive steps.

When we iterate a log-gain module, at the first step the number of variables is given by $\sum_{r \in R} |\gamma_r| + 3|R|$ ($|\gamma_r|$ is the length of γ_r and $|R|$ is the number of rules), while at the first step the number of equations is $|T| + |R|$. But, at each further step, $|R|$ variables are added, while $|T| + |R|$ equations are added too. This argument proves the next theorem.

Theorem 18 (Log-Gain Module Iteration). For any MP system, if we iterate the log-gain module for a sufficient number of steps, then we get a number of equations greater than the number of variables (reaction units and log-gain parameters).

In the case of Sirius oscillator, we have a log-gain module of 8 equations which initially has $5 + 5 + 12 = 22$ variables. At the second iteration of this module we get other 8 equations and other 5 variables. In this case, six steps of observation, that is, five iteration of the log-gain module provide a system of 48 equations and 47 variables. The solution of this system will provide the initial values of reaction units of this system.

A problem for future research will focus on general methods for deducing, with a good approximation, from a suitable sequence of observation steps, the reaction units of an MP fitting with the observed dynamics. When these reaction units are deduced, the reaction maps of this MP system will provide a complete, reliable model of the observed metabolism.

5. Conclusions

MP systems proved to be relevant in the analysis of dynamical systems expressing general metabolic processes. Moreover, their structure, where the reaction level and the regulation level are clearly interconnected but separated, correspond to a direct biological meaning and to interesting relationships with the differential models.

The search for MP systems where reaction maps can be deduced from experimental data is the main problem for a systematic applications of MP systems to complex dynamics. Some experiment in this direction are in progress and some positive results were obtained in this direction. In fact, we tested the log-gain method for deducing the dynamics of Sirius. We considered about one hundred steps of Sirius' evolution, then, by applying Theorem 18, we were able to evaluate the reaction units at the initial step of Sirius and, by means of suitable numerical experiments, we found some reaction maps giving an MP system with the same dynamics of Sirius. If we will prove that this method can be generalized in a systematic way, then MP models could disclose important fields of application. The future research in this field will focus on theoretical investigation, on suitable biological experiments, and on related computational tools for modeling biological phenomena by means of MP systems.

Uncited References

Q2 [22,23,25,31]

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