From P to MP Systems

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Summary. Metabolic P systems (MP systems) represent metabolic processes in a discrete mathematical framework based on P systems. MP systems are presented, with a special emphasis to their roots and to their relationship with P systems, which provided the right conceptual framework for their development. A synthetic algebraic formulation of MP system is given, and the log-gain theory of MP systems is outlined, by discussing the research perspectives and the methodological aspects of this approach.

1 Introduction

Metabolism is one of the basic phenomenon on which life is based. Any living organism has to maintain processes which introduce matter of some kind from the external environment, transform internal matter by changing its distribution in a number of biochemical species, and expel outside matter which is not useful or dangerous for the organism. Of course life cannot be reduced to this basic cycle of matter transformation, but no life can exist without such a kind of basic mechanism. To be more realistic, metabolism is not a unique process, but a network of strictly related processes, usually indicated as metabolic pathways. They differ for the involved substances, for the reactions and the enzymes performing them, for the shapes of the dynamical curves they determine (the amount of substances during time). The main question on the essence of life processes need to understand the origins of metabolic processes, their reliability, their integration and their relationship with other essential life functionalities which need metabolism as their basic energetic fuel.

A (finite) multiset is a collection of elements where the same kind of element may occur many times, therefore a chemical reaction is representable by a multiset rewriting rule. In a wide sense, metabolism is any kind of matter transformation which changes (bio)molecules of some types into molecules of other types (possibly allowing molecules come/go from/to the external environment).

A metabolic P system, shortly an MP system, is essentially a multiset grammar with maps *regulated* by functions. As it will results evident from the next section,

the letter P of MP systems comes from the theoretical framework of P systems introduced by Păun, in the context of membrane computing [39]. In fact, MP systems are a special class of P systems introduced for expressing metabolism in a discrete mathematical setting.

A peculiar aspect of MP systems is given by the Log-gain theory, specifically devised for them [28]. This theory, provides tools for solving the inverse dynamical problem for real metabolic processes. This means that, given a time series of the states of an observed metabolic system (at a specified time interval τ), then it is possible to deduce, by suitable algebraic manipulations, the functions regulating the rules which represent the metabolic transformations in terms of multiset rewriting. In this manner, an MP system can be defined which coincides, within a certain approximation, with the observed real system. This coincidence is, in many cases, an evidence of adequacy between the systemic logic of the observed real system and the mathematical structure of the deduced MP system.

Many phenomena were reconstructed in terms of MP systems (*e. g.*, Goldbeter's mitotic oscillator, Belousov-Zhabotinski reaction in the Nicolis and Prigogine's formulation, and Lotka-Volterra's Prey-Predator model [15, 30, 16]). In all these cases a complete concordance with the classical models was found. Moreover, some synthetic oscillators with interesting behaviors were easily discovered [27, 28, 33], and some MP models were directly deduced by using the Log-gain theory (a part of the photosynthetic NPQ phenomenon of NonPhotochemical Quencing, for which no standard reliable model is known) [36]. A specific software was developed for MP systems, starting from a prototypal version developed by Luca Bianco (Psim, MPsim, MetaPlab) [9, 11, 35, 31] which is downloadable from http://mplab.sci.univr.it, and http://www.cbmc.it).

In this paper we give a quick presentation of the theory of MP systems, with a special emphasis to its roots and to its relationship with P systems which provided the right conceptual framework for its development.

2 Historical backgrounds

The occasion for writing this paper, the decennial anniversary of Membrane Computing, suggested me to briefly reconstruct the initial ideas underlying the MP systems, aimed at developing a discrete theory of metabolic processes based on P systems. Along the line of this historical reconstruction it is possible to grasp in a deeper way the link between P systems and MP system, which rather than of a technical nature is based on the essential assimilation of P perspective in the context of symbolic analysis of metabolism.

My interests in this direction date around the late years 1990. The initial intuition of such a kind of research was the apparent similarity between processes of symbol transformation, typical of logic or formal language theory, with the processes of matter transformations typical of chemistry and biochemistry. If we represent atoms and molecules by suitable symbols, then any chemical reaction is directly translated by a rule of symbol manipulation.

Let me report an example which was a sort of initial formalization exercize. It describes a famous process known as Daniell's cell, a variant of Volta's pile. I presented this example during my invited talk in a meeting organized in 1997 by Gheorghe Păun in Mangalia (not so far from Curtea de Arges) [21].

Daniell' cell is constituted by two rods of two different metals, zinc and copper (Zn, Cu) which are partially immersed in two solutions where the respective salts in ionic state $ZnSO_4, CuSO_4$ are present (see Fig 1). The two salt solutions are separated, but a salt bridge allows ions to pass through the two compartments. In the zinc compartment, the Zn metal molecules prefer to pass from the metal state to the ion state Zn^{++}, therefore some electrons are in abundance on the zinc rod. If a conductor wire connects the two metal rods, these electrons, according to the greater electron affinity (electronegativity) of copper with respect to the zinc, flow from the zinc rod to the copper rod. After that, the copper ions in the copper solutions, after attracting these exceeding electrons, pass from the ion state to the metal state. At this point, a different electrical charge is present in the two solutions, because in the zinc compartment is present a quantity of SO_4^{--} ions which are not balanced by Zn^{++}, while in the copper solution, the opposite phenomenon happens, because a quantity of Cu^{++} is not balanced by the corresponding SO_4^{--} ions. In this situation, a passage happens of SO_4^{--} ions from the copper to the zinc compartment, in order to restore the electrical equilibrium. In conclusion, an electrical flow along the conductor wire between the rods is coupled with the ion flows through the salt bridge. This provides a cycle which persists, consuming the metal zinc, producing metal copper, and moving ions. In principle the cycle continues until zinc is available, and both kinds of ions are present in both compartments. The membrane perspective of this example is apparent. According to Păun's terminology, in this case a neuron-like membranes system represents the process, which is essentially based on transformation and passage of object symbols through membranes.

In my formalization the concept of membrane was explicit, but the symbol manipulation was based on a special kind of Post rules, which I was very familiar with, and which are a powerful formalism for symbol manipulation. But this is exactly the crucial point which made my formalization unsatisfactory in many aspects. Post rules are *too powerful*, and moreover, in this context strings are not the right data structure for expressing the chemical reactions.

Maybe Gheorghe Păun got some suggestions from my conference in Mangalia in August of 1997 (the paper [34]) including the Daniell's cell example was published in 1999). However, Gheorghe Păun (informally, George) sent me a preliminary version of his seminal paper on Membrane Computing [38] in the October of 1998. In his paper membrane were acutely conjugated with multiset rewriting, and from it I surely got the idea of using multisets in the representation of biochemical reactions.

This perspective emerged to me quite slowly, because I spent almost one year by searching the right form of a combinatorial mechanism for molecule manipulations, by essentially considering special forms of Post rules (with string variables

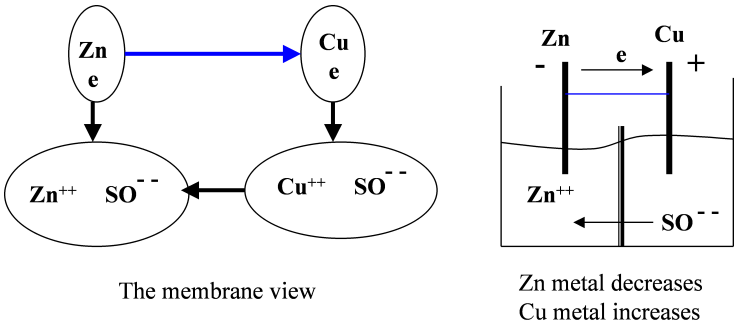

Fig. 1. A Daniell's cell (on the right) and its membrane representation (on the left).

and suitable constraints) [22]. In any case, in 2001, I realized what now seems to me almost obvious: that molecule populations and their transformations are the essence of metabolism and that multiset rewriting is the natural way to mathematically express this reality. However, an aspect of Paun's P systems was not the exact ingredient to use. The original way of applying rules in P systems was the nondeterministic maximal parallel approach. This perspective is mathematically clear and elegant, moreover allows the proof of computational universality for many variants of P systems. But it is not realistic to assume that biochemical reactions work in this way. For example, if a so efficient approach were applied to the ATP $\rightarrow$ ADP molecule transformation in our cells, then our bodies would almost instantaneously burned. Therefore, the next step, for a P system perspective to metabolism was the *molar perspective* and the *mass partition principle* which we will briefly recall in the next section. Another aspect deserves to be preliminarily remarked. Biological processes are subjected to noise, fluctuations, external influxes, but at large, they are essentially deterministic. This determinism is of statistical nature. In fact, the individual behavior is strongly variable, but populations obey to strict laws. This introduces a second level of considering multiset. A rule $W + 6C \rightarrow Z + 6O$ (we use multiset polynomial notation) has to be read not only as one molecule occurrence of W (water) and six of C (carbon) to be replaced by one of Z (sugar) and six of O (oxygen), but rather, as a replacement of populations of N and $6N$ objects. The size N is the *(molar) reaction unit*, depending, in general, on the state of the system. This perspective of multiset rewriting changed completely the discrete mathematical point of view about metabolism, providing the right conceptual framework for quantitative analysis of metabolic processes.

In 2004, I started to apply this idea during the supervision of Luca Bianco's Phd thesis [4] (in the meantime I moved from Pisa to Verona). Luca was asked to model some biological phenomena where differential models were available, by trying to find the same dynamics given by these known models, by using a P system perspective (a similar attempt, more devoted to aspects of biological localization, was afforded in [18]). Finding the rules was generally a simple task, but

the definition of the strategy for rewriting rules was very hard. Finally, we found a procedure, later called "Metabolic P Algorithm" (MPA), which was adequate for the example we considered, and which was based on a multiset representation of chemical transformations (I realized in [27] that they were an abstract formulation of Avogadro and Dalton principles in chemistry). The "official" appearance of MP system was in 2004 [30], but Initially, their focus was on a new rewriting strategy for P systems [5, 6, 7]. Later it was clear that this was only an aspect of the MP approach, because other radical changes were necessary, and MPA was a particular case of a regulation mechanism based on the notion of population mole. In fact, the name of MP systems was introduced in 2006, when this awareness emerged [23, 24]. In the membrane computing community, rewriting strategies different from maximal parallel rewriting were proposed, especially according to probabilistic approaches [40, 41], however neither of them adopted the molar perspective, which is peculiar to the development of the log-gain theory of MP systems. The interest in metabolism was a specific aspect of a more general interest in a dynamical, rather than computational, perspective in the study of P systems, addressed in [2], and more recently in [33]. The paper [42] was particularly influential in drawing my attention toward oscillatory phenomena.

3 The molar perspective in multiset rewriting

Let us give a first intuition of the molar perspective in the multiset representation of biochemical reactions. A reaction $2a + b \rightarrow c$ identifies a transformation such that, when it is applied to a population of objects where types a and b occur in more than 20000 and 10000 elements respectively, and when its flux regulation map specifies a reaction unit of, say 10000 elements, then, in the passage from two time instants at a given time distance τ, these 30000 elements are replaced by 10000 new objects of type c. For example, 20000 molecules of Hydrogen, plus 10000 molecules of Oxygen, are transformed into 10000 molecules of water. Time interval between consecutive instants depends on the macroscopic level is chosen for considering the dynamics of the system in question. The state, on which reaction units depend, is given by the value of some magnitudes, called parameters, which can influence the reactions (*e.g.*, temperature and pressure) and on the sizes of the different populations inside the system, in correspondence to the different kinds of objects.

A metabolic P system is a discrete representation of a metabolic system. It is essentially given by a set of *reactions*, each of them equipped with a corresponding *flux regulation map*. Such a map provides, for any state of the system, a *(flux) reaction unit* (*rules* and *reactions* are often used synonymously, and also *fluxes* and *reaction units* will be equivalently used).

The notion of MP system was explicitly defined, as a special class of P systems, during the Brainstorming Week on Membrane Computing, held in Sevilla in 2006 [23]. The initial formulations of MP systems were based on the usual string notation of P systems (sometimes using the additive notation). in Table

1 is given an example of this notation for Golbeter's model of mitotic oscillator, which we will consider later on. In this case, the rules are based on five substances $\{C, M, M_p, X, X_p\}$ (Cyclin, M-active kinase, M-inactive kinase, X-protease, X–inactive protease) .

However, the same multiset grammar can be easily expressed in algebraic notation. In fact any multiset over $\{C, M, M_p, X, X_p\}$ is easily denoted by a vector of $\mathbb{N}$ having as its first component the multiplicity of C, as second component the multiplicity of M, and so forth (in tis context, an implicit order is assumed over substances). In this manner a multiset rewriting rule $\alpha_r \to \beta_r$ becomes representable by a pair of vector (r^-, r^+) (left and right vector), where r^- is the vector expressing the multiset α_r, and r^+ is the vector expressing the multiset β_r. For example the rule $r_3 : C + M_p \to C + M$ is denoted by the pair of vectors

$$\begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix} \begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

$r_1 : \lambda \to C$
$r_2 : C \to \lambda$
$r_3 : C + M_p \to C + M$
$r_4 : C + X \to X$
$r_5 : M \to Mp$
$r_6 : X_p + M \to X + M$
$r_7 : X \to X_p$

Table 1. The rules of a mitotic oscillator.

The algebraic sum of the right component minus the left one provides the *stoichiometric balance* of the rule. It is important to distinguish in a rule its left part, its right part, and its stoichiometric balance. The left part (left vector) expresses the reactants necessary for activating the rule, the right part expresses the products replacing the reactants, while the stoichiometric balance expresses the effective variation performed by the application of the rule. Even if two rules have the same stoichiometric balance, they can be different in the amount of matter they need for their activation. For example the rule $2C + M_p \to 2C + M$ has the same stoichiometric balance of the rule $C + M_p \to C + M$, but the latter needs half of the quantity of c necessary for the activation of the former.

$$\begin{pmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 0 \end{pmatrix} - \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} 0 \\ 1 \\ -1 \\ 0 \\ 0 \end{pmatrix}$$

This algebraic representation of rules remarkably simplify the definition of MP system. The reader is advised to compare the next definition with the previous definitions of MP system [27, 28, 32]. However, it is not only matter of notation simplification. In fact, important properties of reactions need to be expressed by usual linear algebra concepts. For example, as it will be explained, the linear independence of some reactions is an essential requirement for discovering the fluxes responsible of a given dynamics.

Definition 1. *Let $\mathbb{R}^n$ be the vector (phase) space of n substance quantities (considered with a certain order). An MP system of type (n, m, k) is a deterministic discrete dynamical system, specified by a structure:*

$$(R, H, \Phi, X[0], \tau, \nu, \mu)$$

where:

- *R is a pair (R^-, R^+) of matrices $n \times m$ over $\mathbb{N}$, constituted by the m (column) vectors of $\mathbb{N}^n$ denoted by $r_1^-, \ldots, r_m^-$ and $r_1^+, \ldots, r_m^+$ respectively. A pairs (r_j^-, r_j^+) for $1 \leq j \leq m$ specifies a reaction of the system (left and right vectors). The $n \times m$ matrix $\mathbb{A} = R^+ - R^-$ over $\mathbb{Z}$ (the componentwise algebraic difference of the matrices R^+ and R^-) is the stoichiometric matrix associated to R;*
- *$H : \mathbb{N} \to \mathbb{R}^k$ is the function providing, at each step, the parameter vector;*
- *$\Phi : \mathbb{R}^n \times \mathbb{R}^k \to \mathbb{R}^m$ is the (vector) function $(\varphi_1, \ldots, \varphi_m)$ providing the flux vector corresponding to a state vector of $\mathbb{R}^n$ and to a parameter vector of $\mathbb{R}^k$;*
- *$X[0] \in \mathbb{R}^n$ is the initial state of the system;*
- *$\tau \in \mathbb{R}$ is the time interval between two consecutive steps;*
- *$\nu \in \mathbb{R}$ is the molar population (conventional) unit;*
- *$\mu :\in \mathbb{R}^n$ is the vector of the molar masses of substances.*

The dynamics of this system, that is, its state $X[i]$, at step $i \in \mathbb{N}, i > 0$, is given by the following recurrent vector equation, called $EMA[i]$ (Equational Metabolic Algorithm)

$$X[i] = \mathbb{A} \times \Phi(X[i-1], H[i-1]) + X[i-1] \tag{1}$$

for any step i, $\Phi(X[i], H[i])$ is abbreviated by $U[i]$, called the flux vector at step i.

□

The intuition behind the previous definition is that of a system defined by: *reactions* (among substances), *parameters*, *regulations*, *initial state*, and *scale factors* (time and population units, plus molecular masses). Reactions transform substances, while flux regulation maps regulate the amount of matter transformed by each reaction at each step, and parameters, which are not directly involved in reactions, together with the substance quantities, influence the flux regulation maps. Scale factors do not enter in the mathematical description of the dynamics, but they define its physical interpretation, according to an adequate time/mass scale of the phenomenon under investigation.

MP systems can be depicted by means of MP graphs [29, 19] with five kinds of nodes and four kinds of edges (see Fig. 3). Nodes are: substance nodes, reaction

nodes, regulation nodes, parameter nodes, and gate nodes denoting matter fluxes from/to the external environment (lambda rules). Edges are: transformation edges, regulation edges and dependency edges.

Table 2 specifies, an MP model, of type $(5, 7, 1)$, for a famous oscillator occurring in the mitosis of early amphibian embryos, established by Goldbeter in terms of differential equations [20]. In the order, are indicated: i) the constants (used for e better reading of formulae and including the temporal interval τ and the population unit ν, but leaving unspecified the molar weights), ii) the initial values of substance quantities, iii) the rules with the corresponding flux regulation maps, and iv) the parameters with their evolution functions ($i \in \mathbb{N}$ are the steps). This MP formulation is obtained by extending a procedure introduced in [17] and provides the same dynamics of the original differential model (see [27, 28] for Goldbeter's differential equations, for other MP models, and for discussions concerning their identification).

$K_1 = 0.005\ \nu$	$K_2 = 0.005\ \nu$	$K_3 = 0.005\ \nu$
$K_4 = 0.005\ \nu$	$V_{M1} = 3\ \nu$	$V_i = 0.025 \cdot 10^{-6}\ \nu$
$V_2 = 1.5\ \nu$	$V_4 = 0.5\ \nu$	$Q_d = 0.02 \cdot 10^{-6}\ \nu$
$V_d = 0.25$	$K_c = 0.5 \cdot 10^{-6}\ \nu$	$\tau = 0.001\ min$
$K_d = 0.01$	$S = 0.001$	$\nu = 6.02 \times 10^{23}$

$$C = 0.01 \cdot 10^{-6}\ \nu\ M = 0.01\ \nu\ M_p = 0.99\ \nu\ X = 0.01\ \nu\ X_p = 0.99\ \nu$$

$r_1 : \lambda \to C$	$\varphi_1 = S \cdot V_i$
$r_2 : C \to \lambda$	$\varphi_2 = S \cdot K_d \cdot C$
$r_3 : C + M_p \to C + M$	$\varphi_3 = (S \cdot V_1 \cdot M_p)/(K_1 + M_p)$
$r_4 : C + X \to X$	$\varphi_4 = (S \cdot V_d \cdot X \cdot C)/(Q_d + C)$
$r_5 : M \to Mp$	$\varphi_5 = (S \cdot V_2 \cdot M)/(K_2 + M)$
$r_6 : X_p + M \to X + M$	$\varphi_6 = (S \cdot M \cdot X_p)/(K_3 + X_p)$
$r_7 : X \to X_p$	$\varphi_7 = (S \cdot V4 \cdot X)/(K_4 + X)$

$$V_1[i] = (C[i] \cdot V_{M1})/(K_c + C[i])$$

Table 2. MP formulation of Goldbeter's mitotic oscillator.

4 The log-gain theory of MP Systems

The main question, at beginning of the log-gain theory for MP systems, is the following inverse dynamic problem. Given a time series $(X[i], H[i]) \in \mathbb{R}^{n+k}$ (for $i = 0, 1, 2, \ldots t$) of some consecutive states and parameters of a metabolic system (at a time interval τ), is it possible to deduce a corresponding time series of

vectors $U[i] \in \mathbb{R}^m$ (for $i = 0, 1, 2, \ldots t-1$) giving the reaction units at any step, which put in the equation (1) provide the time series of substance quantities (for $i = 1, 2, \ldots t$)? This is the discrete dynamical problem of reaction flux discovery. The deduction of time series $U[i]$ implies the knowledge, at the time granularity τ, of the systemic logic governing the matter transformations underlying the observed metabolic states. When vectors $U[i]$ are known, the discovery of maps Φ which provide $U[i]$, in correspondence to the vectors $(X[i], H[i])$, is a typical problem of approximation which can be solved with standard techniques of mathematical regression. Fig. 2 expresses graphically the two procedures, going in the opposite verses, of generation of a dynamics from a given MP system, and of providing an MP system fitting with an observed dynamics. The equation linear systems EMA provide the dynamics of an MP system, while the equation linear system OLGA, allow us to perform the opposite task. In the following, we will outline the log-gain theory, which determines the methods for construct the OLGA systems.

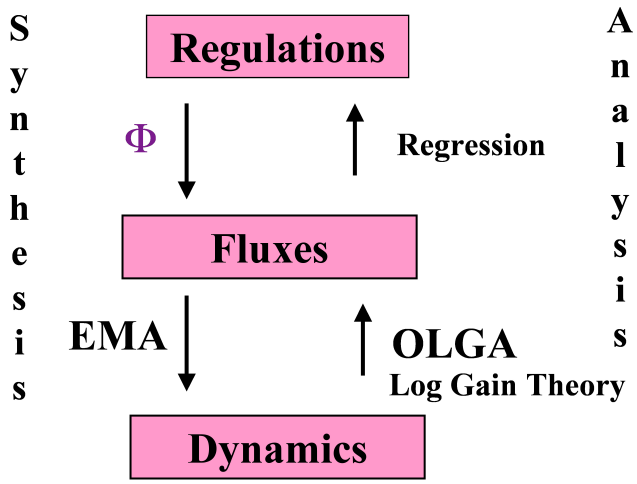

Fig. 2. Synthesis and analysis of dynamics by means of MP systems: direct and inverse dynamical problems.

An important remark is due in this context (which will be more extensively reconsidered, in the final section). The approach of flux discovery is essentially observational, macroscopic, and global, in a sense which is opposite to the perspective of differential models, which is infinitesimal, microscopic and local. In fact, we do not pretend to discover the real kinetic responsible, at a microscopic level, of the biochemical dynamics of each reaction, but we are determined to capture the global pattern of reaction ratios of an observed dynamics. In other words, leaving unknown the *real* local internal dynamics, we decide to consider the system at an abstraction level which is sufficient to reveal the logic of the behavior we

observe. This more abstract approach can be less informative, with respect to specific important details, but such a more generic information could be very useful in discriminating important aspects of the reality, and often, especially in the case of very complex systems, is the only way for grasping a kind of comprehension of the reality under investigation. From a mathematical point of view, the searched vectors $U[i]$ are the solutions of the equation system (1) (for $i = 0, 2, \ldots t-1$).

We call it EMA (Equational Metabolic Algorithm) when it is used for calculating the substance quantities, from the knowledge of flux regulation maps, while we call it ADA (Avogadro and Dalton Action), when we search so determine $U[i]$ from the knowledge of substance quantities (Avogadro refers to the integer stoichiometric coefficients, and Dalton to the summation of the effects of reactions). Unfortunately, often, ADA is not sufficient to provide the solutions because the number m of reactions is greater than the number n of substances. Therefore, we need to extend ADA by adding new equations.

The log-gain principle assist us in the search of further equations for identifying the fluxes. This principle derives from a general biological principle called *allometry*, according to which, in a living organism, the global variation of its typical magnitudes follow a sort of *harmonic rule* according to which their relative variations are proportional to the relative variations of the magnitudes related them. In differential terms the relative variation in time of a magnitude coincides with the variation of its logarithm, therefore we used the term "log-gain" for any law grounded on this assumption. In the specific context of our problem, we assume that the relative variations of a reaction flux is a linear combination of the relative variations of substance quantities and parameters affecting the reaction, and in a more restrict case, it is the sum of the relative variations of the reactants of the reaction. We refer to the papers [28] for a detailed account on the log-gain theory of MP systems. The principle was initially formulated starting from its general form. Then, in three subsequent transformations, it provided an equation system $COLG$ (Covering Offset Log-Gain), involving fluxes, with a number of equations equal to the number of reactions, but with additional unknown variables, called *offset log-gain*, equal to the number of substances. This means that the whole system constituted by ADA and $COLG$ has $2m+n$ variables. Moreover, if we consider the two systems, at the same observation step i, then it results a nonlinear system.

Here, an induction argument helps us to obtain a further reduction of variables, in order to get a *square equation linear system*. In fact, if we consider $ADA[i+1]$ and $COLG[i]$, assuming to know the fluxes at step i, we contemporarily reduce the variables to $n+m$ and remove the nonlinearity of the system.

Now we report the final form of a system of equations called $OLGA$ which solves our initial problem of flux discovery ($\times$ is the usual matrix product, while $+, \cdot, -, /$ are the componen-twise vector operations of sum, product, difference and division, respectively).

$$X[i+2] = \mathbb{A} \times U[i+1] + X[i+1] \tag{2}$$

$$(U[i+1] - U[i])/U[i] = B \times (W[i+1] - W[i])/W[i] + C \cdot P \tag{3}$$

where W is the (n+k) dimensional vector of substances and parameters, B is a boolean matrix choosing, for any reaction, its *tuners*, that is the magnitudes affecting its flux, and P is an m-dimensional vector of reals, expressing the reaction *offsets*, that is, the errors introduced in the log-gain approximations of fluxes, while C is a boolean m-dimensional vector, such that $\sum C = n$, that is, the sum of its components is equal to n.

We assume that the stoichiometric matrix $\mathbb{A}$ has maximum rank. This assumption is not restrictive because it implies that no substance variation is linear combination of the variations of other substance. If this were the case we can remove the substance variation which is combination of other variations, without loss of information, by obtaining a stoichiometric matrix of maximum rank.

We say that a rule is *linearly dependent* on other rules if its stoichiometric balance is a vector which is linearly dependent on the stoichiometric balance of other rules. A set of rules are linearly independent if no rule of this set is dependent on other rules of the set. We say that a subset R_0 of n rules is a *covering* of the set R of rules, if any substance is reactant o product of some rule in R_0.

The following theorems are a natural consequence of the algebraic formulation of rules and of the dynamics of MP system defined by EMA (we omit the proofs here).

Theorem 1. *Given a set of rules with stoichiometric matrix of maximum rank, then there exits a covering of linearly independent rules.*

Theorem 2. *Let R_0 subset of rules of R which are linearly independent. Let OLGA be a system with a covering vector C corresponding to R_0 ($C(i) = 1$ iff $r_i \in R_0$). Then, OLGA has one and only one solution.*

The previous theorems show that the problem of finding fluxes of a metabolic system is solvable under very general assumptions.

However, given the inductive nature of our method, in order to generate the time series of $U[i]$, we need the knowledge of $U[0]$. An algorithm for achieving this task was recently found [37], which was tested in many cases with a good success. This problem is essentially an optimum problem based on the notion of activation matrix. This matrix is the right component of the matrix R of rules. If we multiply it with the flux vector $U[i]$, then we get, for each component, the amount of a substance necessary, at step i, to activate all the rules which need that substance. Other constraints regard the positivity of fluxes and a sort of Lavoisier principle (the absolute variation of matter between two consecutive states has to equate the absolute difference between the sums of in-coming and out-coming fluxes).

The determination of the covering vector C is another important aspect in the construction of the OLGA system. Some investigations are in progress for the search of an *optimal* covering, or for showing that, under suitable conditions, the goodness of solutions can be independent on the choice of a specific covering. However, in the study of this aspect it seems useful to consider the Galois connection

arising between substances and reactions. Given a substance x, we denote by $R(x)$ the set of reactions where x occurs (as product or reactant), but symmetrically, given a reaction r, we can define $S(r)$ as the set of substances involved in the reaction r. If we extend R, S as functions from set of substances to set of reactions, and *viceversa*, we get a Galois connection, which is a very general and powerful algebraic concept. It seems possible that, rule covering, and other metabolic concepts, are related to properties which can be analyzed in this algebraic setting.

The following theorem shows a relevant aspect of the notion of covering. In fact, for the application of the log-gain principle, the flux log-gain of a rule should consider non only its reactants, but its tuners, that is, all magnitudes (substances and parameters) which influence the rule. Unfortunately, the knowledge of tuners of reactions is very often not available. The following theorem (we omit the proof) ensures that fluxes can be deduced even with this lack of knowledge. Therefore, the analysis about tuners, for determining fluxes, could be focused on the uncovered reactions.

Theorem 3. *Consider an OLGA system based on a linearly independent covering R_0. The fluxes which are solutions of this system do not depend on the tuners which are chosen for the rules of R_0 in the flux log-gains of these rules.*

In conclusion, tuners of rules of R_0 can be reduced only to the reactants of there rules, and the solutions of OLGA systems, one for each step, provide the time series $U[i]$ that solve the flux discovery problem, posed at the beginning of our discourse.

Results of equivalence of MP systems with other formalisms were developed [17, 13, 14]. However, the more relevant feature of MP system is the availability of the log-gain method here outlined, for the solution of the flux discovery problem.

5 Fluxes, reactivity, inertia, and differential models

The analysis process which provides an MP system from an observed dynamics is directly related to the notion of reaction fluxes. However, in the process of synthesizing dynamics is more natural to associate to every reaction a reactivity parameter determining a sort of score in the competition for getting the reactants necessary for the activation of the reaction. This competition concerns the part of matter available in a given state, therefore another parameter is necessary, for each substance, which provides the amount of substance that, in a given state, can be partitioned among all reactions competing for it, or equivalently, the amount of substance that is not transformed, called the *inertia* of the substance (at a given step). These systems were the first kind of MP systems formally defined [27], and correspond to the special class of *reactive* MP systems. In a reactive MP system of type (n, m, k), the inequality $k \geq n + m$ holds, because there is a parameter for each substance, providing its inertia and a parameter for each reaction, providing its reactivity. The evolutions of these parameters are specified by *inertial maps*

$(\psi_x | x \in X)$ and by *reaction maps* $(f_r | r \in R)$ respectively. In reactive MP systems, the flux regulation maps $\Phi = \{\varphi_r | r \in R\}$ are defined by the following equations for any $q \in \mathbb{R}^n$ (see [27, 28] for intuition and motivations of this class of MP systems)

$$\varphi_r(q) = \begin{cases} f_r(q) & \text{if } \alpha_r = \lambda; \\ min\{\frac{w_{r,y}(q) \cdot q(y)}{|\alpha_r|_x} \mid y \in \alpha_r\} & \text{otherwise.} \end{cases} \tag{4}$$

where

$$w_{r,x}(q) = \frac{f_r(q)}{\psi_x(q) + \sum_{r' \in R_\alpha(x)} f_{r'}(q)} \tag{5}$$

In reactive MP systems, being flux regulation maps φ_r $(r \in R)$ completely determined by the reaction maps and inertias, it is enough to specify only them (usually indicated f_r and ψ_r $(r \in R)$. In Fig. 3, an MP graph is given, which describes the simple metabolic oscillator *Sirius ternarius*, a variant of an oscillator widely studied in the context of MP systems [27, 28, 33]. The core of this oscillations is the reaction from $A \to B$, with a flux which linearly depends on the amount of B. In fact, when this quantity increases too much, then the reactant of $A \to B$ is greatly consumed, and consequently also the reaction flux diminishes. In such a way A, which is produced by $C \to A$ can increase and consequently also the reaction $A \to B$ returns again to work actively, so that the condition for a new cycle is restored.

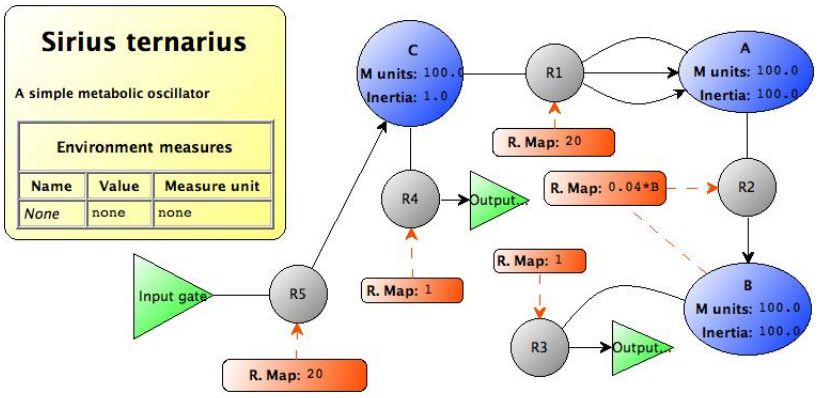

Fig. 3. The MP system Sirius ternarius. Big circles are substances, small circles are reactions, rectangles are reactivity parameters, and triangles indicate matter flows from/to the external environment. Fluxes are not indicated because determined by the reactivity parameters of reactions by means of Formula (4). The inertias of A, B, C are $100, 100$, and 1 respectively (all values are expressed in conventional moles of unspecified size).

In Fig. 4 is given the oscillatory dinamics of the MP system of Fig. 3, computed by Psim software.

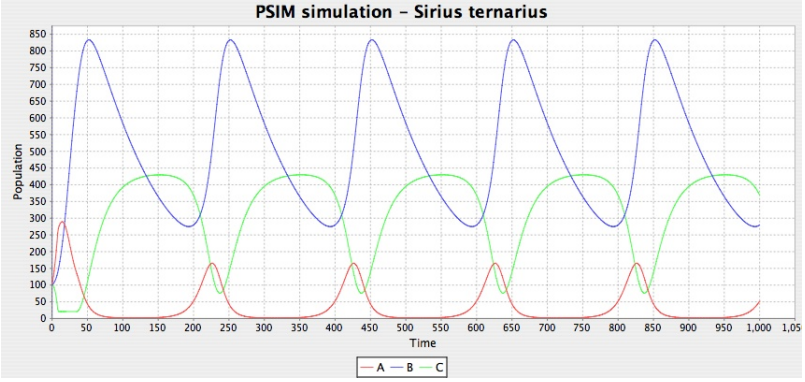

Fig. 4. Sirius ternarius' dynamics where EMA of Definition 1 is computed by Psim software (see: http://www.cbmc.it and http://mplab.sci.univr.it)

If we avoid the rule consuming C, the dynamics changes dramatically, even if we reduce sensibly the value of rule introducing C. This show that the analysis of metabolic processes is very complex and very often the behavior of a system is hardly deducible by the MP graph, without a direct inspection of its dynamics. The form of trajectories are related to the graph structure, but very often their shape is very robust for big changes of regulation maps and initial values, but very fragile with respect to some parameters. This kind of investigations applied to real metabolic oscillators are very important for establishing the key features responsible for maintain some dynamical regimes of interest.

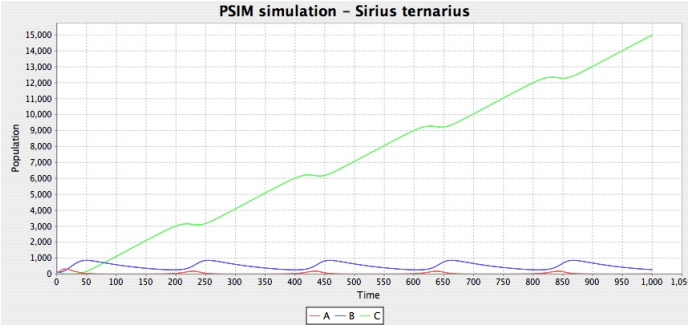

Fig. 5. Sirius ternarius' dynamics where the reaction $\lambda \rightarrow C$ is removed.

The following theorem (see [28] for a proof) states the dynamical equivalence between any MP system and a suitable reactive reactive MP system (starting by the same state they provide the same sequence of states).

Theorem 4. *For any MP system there exists a reactive MP system which is dynamically equivalent to it.*

A notion of *abstraction order* can be defined for MP systems, which result useful in the determination of models. A system M is more abstract of a system M' if the substance of M are a subset of those of M' and the dynamics of M coincide with the dynamics of M' on their common substances. In many cases a right abstraction level could be more informative of a too detailed system where it is difficult to grasp the main feature of the logic governing a dynamics. Some investigations are in progress about some basic mechanisms on which oscillatory phenomena are based, in particular, on the relationship between the MP graph and the corresponding oscillatory pattern, and on the numerical values and ranges ensuring some oscillatory forms. In some numerical experiments we found cases where few parameters have a crucial role in determining the dynamics, and some threshold values of them are discriminant for very specific behaviors.

Many special forms of reactions can be identified: *left-monic, right-monic, monic, assimilative, dispersive, cooperative, synthetic, dissociative, catalytic*, *replicative, monogenic* [33]. Monic refers to a rule involving only one substance (in the left, right, or both sides), assimilative to a rule producing without consuming substances, dispersive to a rule consuming without producing substances, cooperative to a rule with more than one reactant, synthetic to a rule with more than one reactant and only one product, dissociative to a rule with one reactant and more than one product, catalytic to a rule with a substance occurring contemporarily as reactant and as product, replicative to a rule where a substance occurs as product more times than as reactant, monogenic to a rule where any product and reactant occurs only once. These properties correspond to important biochemical aspects, and equivalence properties can be easily proved in the context of MP systems. The following theorem involves aspects peculiar to MP systems (we omit the proof).

Theorem 5. *For any MP system there exists a reactive MP system which is dynamically equivalent to it having only assimilative and dispersive rules.*

The notion of inertia is naturally related to the relationship between reactive MP models and differential models. In [17] equivalence results between these two kind of models were proven. In fact, it turns out that the inertia is inversely proportional to the discretization time of numerical integration methods. This equivalence holds by means of a limit process along a sequence of increasing values of inertia, which is supposed to be equal for all substances.

A general theorem can be easily proved stating an equivalence between the dynamics of a differential model, computed by the Euler method of numerical integration, and the dynamics computed by EMA for an MP model which is deduced by means of a straightforward "rule-driven translation" of the right members of

differential equations (the procedure used in Sect. 3 for the MP formulation of Goldbeter's mitotic oscillator). In this case, the MP time interval coincides with the discretization time of the numerical integration.

However, a deeper relationship can be established between differential and MP models. In fact, let us suppose, to have an ODE (Ordinary Differential Equation) model of a metabolic process. According to it, any derivative of substance quantity is the sum of some additive terms relative to the infinitesimal fluxes of the rules consuming and producing that substance. Assume to use a numerical integration method, and to solve the differential equations with a discretization time Δt. Now, if we consider a time interval τ and perform $\tau/\Delta t$ numerical integration steps (the natural number rounding this value), then we can deduce the fluxes of all the reactions involved in the system in the time interval τ. This means that we get exactly what the log-gain theory provides by solving the OLGA systems along a number of observation steps. In other words, we get the macroscopic fluxes from the ODE microscopic ones. From these fluxes, by approximation and correlation techniques we can derive the flux regulation maps of an MP system which provides the same dynamics along the steps separated at the time interval τ. It would be possible, that at this different temporal grain, some systemic effects emerge which could shed new light on the analysis of the modeled phenomenon.

6 Reconsidering membranes

MP system are described by focusing on the reactions, but disregarding the compartmetalization aspect of membrane computing. However, if we look at the MP graph we can see a neuron-like membrane structure given by the nodes along which the matter flows. This means that if we model substances as different membranes, and we fill them of a unique kind of substance (*e. g.* water) we are in a perfect membrane setting. This is a general aspect which it would be interesting to analyze in general terms. Objects and membrane are dual concepts which can be reciprocally reduced (an analogous situation arises in set theory). This duality is a special case of the space/matter duality formulated in the context of a discrete framework. In fact a physical object, having a spatial extension comprises a portion of space, the internal space occupied by it, that can be separated by an implicit membrane delimiting its internal region. Conversely, a membrane is an object with an internal region which can include other objects. Therefore, we may consider an object of type a as equivalent to an empty membrane $[\,]_a$. Analogously an object a inside the membrane of label j, $[a]_j$, is represented by as an object a_j with the index denoting the localization of a. In general, we may reverse the relationship of containment of membranes and objects, by expressing the localization of an object by putting its *membrane address* (for example, a string of membrane labels). Here we do not enter into further details. However, many aspects deserve a careful analysis. Namely, a sharp examination of the notion of object distinguishability could show some subtle implicit pitfalls. In multisets, this feature refers to object individuality, rather than to their enumerability (two undistinguishable balls are

different from only one of them). An important aspect of the relationship between objects and membrane concerns just the possibility of considering for them (or for some types of them) different processes of distinguishability.

According to the perspective of addressed objects, moving an object from a membrane to another one results to be a transformation acting on the index part of the object. In many modeling context this is the natural approach adopted for expressing localization changes. For example a protein p which can be localized in two places A, B is modeled by two species p_A and p_B and its displacement is assimilated to a transformation of matter. This discussion shows that the more appropriate way to model a reality depends on the specific aspects we are interested to model, but in principle "membranization" or "demembranization", or a mixing of the two strategies, are possible, and different viewpoints open different perspective of investigation.

In [1, 2] the boundary notation for membrane rules was introduced in order to cope with more general membrane rules. In fact, in Păun's original formulation, rules are inside membrane and everything is unknown to a rule, if it is outside the membrane where the rule is located. However, in many cases a transformation depends on the possibility of recognize configurations which can be defined only if the actors of the transformation have a visibility which is wider than interiors (windows could be necessary). The essential point of boundary representation is the idea of rules with a greater level of localization knowledge about the objects which they apply to. This idea can be further generalized, but the two perspectives could also be integrated for coping with different contexts of application.

Another natural generalization of P rules concerns the possibility of high-order multisets. This is not a mathematical generalization, but expresses a natural necessity for representing biochemical transformations. In fact, in many reactions two or three level multisets occur. Even in the simple case of water formation, the usual chemical notation is $2H_2+O_2 \rightarrow 2H_2O$. Here we have multiplicative numeric coefficients and numerical indexes, that we could express, by using parentheses, as $2(2H) + (2O) \rightarrow 2((2H)O)$. In this case, parentheses are not membrane parentheses, but express a two level multiset. In fact, the rule transforms a multiset of objects which are multisets too, that is, a second order (finite) multiset into in another one of the same kind.

In many phenomena the localization aspect is predominant, but in a way that membranes are not adequate. It is the case of *gradients* in morphogenesis. In this case, what is important, rather than containment relations, are the distances with respect to some coordination points, therefore indices memorizing these values are the natural way for handling this aspect.

In a discrete setting, *loci* could be represented by (localization) *binders* attached to the objects, which become relevant in relocation rules, while they are dummy when internal transformations are performed. Binders, for expressing loci, are useful for objects as far as for membranes where the importance of specific parameters for encoding physical feature was already investigated in membrane computing (*e. g.* polarization and thickness).

In conclusion, a very synthetic way for expressing the original P-system perspective could be: grammars of "parenthesized strings with commutative concatenation", or more simply, grammars of "parenthesized multisets". The passage from *boundaries* to *binders* and all the aspects mentioned above could enlarge the spectrum of modeling possibilities of P and MP systems toward the study of dynamics of high level discrete spatial complexity.

7 Open problems and methodological issues

Many lines of development emerged, in the context of MP systems. Some of them, as it was argued in the previous section, are related to the theory of P systems. Other research lines are specifically focused on the log-gain theory. The hot points in this direction are: i) the determination of the initial fluxes, ii) the determination of the more appropriate covering for the OLGA systems, iii) the determination of the tuners of reactions (initially for uncovered rules, and, after OLGA solutions, for all the rules), and iv) the determination of the flux regulation maps associated to the fluxes and to their tuners. Some investigations are in progress and some partial results are available. It is interesting that in the search of solutions a variety of methods naturally occurs, going from vector algebra and vector optimization to artificial neural networks [12, 37]. The next kinds of modeling applications which we intend to realize are phenomena related to gene regulation networks and to signal transduction mechanisms. From the computational side, many plugins are under development for extending the MetaPlab software, according to specific needs of the experiments which could orientate the theoretical and applicative research. Presently, a plug-in is available for computing MP dynamics by means of EMA, moreover a plug-in is also available for the flux discovery by means of OLGA, other visualizations and format translation plug-ins are available, and prototypal plug-ins for polynomial regression and artificial neural network correlation plug-ins were developed [11, 12, 31].

Other research lines of MP systems theory are more specifically related to the metabolism and to the population perspective of biological phenomena. Many aspects of metabolic dynamics can be expressed and abstractly studied on MP systems [33]. In particular, a general study of metabolic oscillators seems to be especially adapt to be investigated by using reactive MP systems. This class of systems are especially suited for synthesizing specific behaviors, in order to identify the specific structural features related to some dynamical properties. For example, a catalog of basic MP metabolic oscillators is under investigation, which is aimed to instantiate experiments of computational synthetic biology.

I want to conclude by stressing an important methodological aspect which is very often source of misunderstanding, because it remarkably differs from the usual modeling approaches in computational biology.

When we design an MP model by using the log-gain theory we start with time series of observations. The model we get at end of the process is a model of what

we observed. In the case of ODE models, from data kinetic rates of biochemical reactions are deduced. It is not the case for MP regulation maps. Although the term reaction is used, our reactions have to be more properly seen as transformations.

We adopt a perspective which could be described as the *Boltzmann's analogy*. According to Boltzmann's mechanical statistics, the macroscopic state of a thermodynamic system (a gas inside a volume at a given pressure and temperature) is given by the distribution function $f(z)$ providing the number of molecules in the ensemble z (a kind of energetic level). In our case, we claim that in a biochemical system, with a number of chemical species, its macroscopic state depends on the number of molecules which are present for each species. The passage from a state to another one is completely due to the change of molecule distribution per species.

We do non know and we do not pretend to describe what happens at the microscopic reaction level. We observe that some species are related by some reasonable transformatios and we assume that the variations are due to the action of these transformations. These transformations could be executed in many ways and maybe they involve other underlying very complex transformations, at different sublevels. However, this is outside the objective of the model. It tries to find the logic underlying the specified species and the chosen transformations. In other words, we explain what is observed in terms of the species and the transformations under investigation. If the choice of the species and of the transformations is not the right one, this means that the model was not adequate, but this is independent from the methodology, it is only a matter of the specific modeling design. In conclusion, MP modeling, according the log-gain analysis, is deliberately at a different, more abstract, level with respect to ODE models. This does not means that it is less adherent to the reality, but simply that it is focused on a different level of reality.

A model is either good or bad only if it helps us in predicting and explaining what we can observe. No other criterion can be discriminant, and it is ingenuous to adopt a mirror analogy with an absolute character. In fact, many mirrors could be available, and some could be more useful than others in certain contextes. Reality is different when it is considered at different levels of observation. When the level of phenomena under investigation is very different (too small or too big, or too complex) with respect to the observer level, the true scientific ability concerns the right theoretical and experimental choices about what has to be observed and about how the observation results have to be related. A priori is very hard to chose the "pertinent aspects" of a phenomenon and to disregard what is not relevant.

What is the reality adherence of the physical theories at quantum levels or at cosmological level? What is the reality of the probability wave in Shrödinger equation? We trust them because they work. No mirror principle can assist us for their evaluation. They are creations of the human invention. Modeling is an art, and it cannot follow easy prefixed procedures. This art is based on the right choice of what has to be observed, what relationships are relevant among the observed features, how translate them in a chosen conceptual universe, and how to interpret the findings which result from this translation.

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