Event-Driven Metamorphoses of P Systems

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Abstract. Complex reaction systems in molecular biology are often composed of partially independent subsystems associated with the activity of external or internal triggers. Occurring as random events or dedicated physical signals, triggers effect transitions from one subsystem to another which might result in substantial changes of detectable behavior. From a modeling point of view, those subsystems typically differ in their reaction rules or principle of operation. We propose a formulation of trigger-based switching between models from a class of P systems with progression in time employing discretized mass-action kinetics. Two examples inspired by biological phenomena illustrate the consecutive interplay of P systems towards structural plasticity in reaction rules: evolutionary construction of reaction networks and artificial chemistries with self-reproducible subunits.

1 Introduction

Structural dynamics in biological reaction networks, also known as *plasticity* [4], is a common property of complex processes in living systems and their evolution. Within the last years, its impetus for adaptation, variability, emergence, and advancement in biology became more and more obvious. Facets of life provide a plethora of examples for structural dynamics: Organisms undergo *metamorphosis* by the physical development of their form and metabolism. At a more specific level, synaptic plasticity within central nervous systems of animals [6] as well as photosynthesis of plants [3] are characterized by substantial structural changes of the underlying reaction networks over time, depending on external or even internal signals. In case of photosynthesis, light-dependent reactions differ from processes of the Calvin cycle. In nervous systems, presence of neurotransmitters for longer periods effects duplication or discreation of synapses. All these and many further biological phenomena can be divided into several stages of function. Typically, each stage corresponds to a subsystem of fixed components. In terms of modeling aspects, such a subsystem is defined by a dedicated set of species and unchanging reactions. Only the species concentrations vary in time or space according to identical rules.

Along with the development of systems biology, the integration of separately considered subsystems into more general frameworks comes increasingly into

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the focus of research to understand complex biological phenomena as a whole [1]. From this perspective, the question arises how to assemble multiple process models, each of which captures selected specific aspects of the overall system behavior.

Motivated by this question, we contribute to the specification of a framework able to incorporate correlated temporally "local" models whose dynamical behavior passes over from one to the other. In this context, time- and value-discrete approaches promise a high degree of flexibility in separate handling of atomic objects rather than analytical methods since singularities caused by transition between models can affect continuous gradients and amplify numerical deviations. We introduce a deterministic class Π_{PMA} of P systems with strict *prioritization* of reaction rules and a principle of operation based on discretized *mass-action* kinetics. Systems within this class enable an iterative progression in time. Representing temporally local models of chemical reaction systems, they are designed to interface to each other. An overlying state transition system manages the structural dynamics of P systems Π_{PMA} according to signals mathematically encoded by constraints (boolean expressions). Two case studies gain insight into the descriptional capabilities of this framework.

Related work addresses two aspects: discretization of chemical kinetics and structural network dynamics. On the one hand, metabolic or cell signalling P systems like [14] describe the dynamical behavior of a fixed reaction network based on concentration gradients, numerically studied in [8]. Results of [10] present a discretization of Hill kinetics mainly employed for gene regulatory networks. Artificial chemistries were explored in [7] along with issues of computability [13] and prioritization of reaction rules [20]. On the other hand, spatial structural dynamics in P systems was primarily considered as active membranes [16,17]. Dynamical reaction rules in probabilistic P systems were investigated in [18].

The paper is organized as follows: First we present a method for discretization of mass-action kinetics leading to P systems Π_{PMA} whose properties are discussed briefly. Section 3 introduces a transition framework for P systems of this class together with a description of the transition process. For demonstration, a chemical register machine with self-reproducible components for bit storage is formulated and simulated in Section 4. In Section 5, we discuss the transition framework for monitoring the evolutionary construction of reaction networks.

2 Deterministic P Systems for Chemistries Based on Mass-Action Kinetics

Multiset Prerequisites

Let A be an arbitrary set and $\mathbb{N}$ the set of natural numbers including zero. A multiset over A is a mapping $F : A \longrightarrow \mathbb{N} \cup \{\infty\}$. $F(a)$, also denoted as $[a]_F$, specifies the multiplicity of $a \in A$ in F. Multisets can be written as an elementwise enumeration of the form $\{(a_1, F(a_1)), (a_2, F(a_2)), \ldots\}$ since $\forall (a, b_1), (a, b_2) \in F : b_1 = b_2$. The support of F, $\mathrm{supp}(F) \subseteq A$, is defined by $\mathrm{supp}(F) = \{a \in A \mid F(a) > 0\}$. A multiset F over A is said to be empty iff

$\forall a \in A : F(a) = 0$. The cardinality $|F|$ of F over A is $|F| = \sum_{a \in A} F(a)$. Let F_1 and F_2 be multisets over A. F_1 is a subset of F_2, denoted as $F_1 \subseteq F_2$, iff $\forall a \in A : (F_1(a) \leq F_2(a))$. Multisets F_1 and F_2 are equal iff $F_1 \subseteq F_2 \wedge F_2 \subseteq F_1$. The intersection $F_1 \cap F_2 = \{(a, F(a)) \mid a \in A \wedge F(a) = \min(F_1(a), F_2(a))\}$, the multiset sum $F_1 \uplus F_2 = \{(a, F(a)) \mid a \in A \wedge F(a) = F_1(a) + F_2(a)\}$, and the multiset difference $F_1 \ominus F_2 = \{(a, F(a)) \mid a \in A \wedge F(a) = \max(F_1(a) - F_2(a), 0)\}$ form multiset operations. The term $\langle A \rangle = \{F : A \longrightarrow \mathbb{N} \cup \{\infty\}\}$ describes the set of all multisets over A.

Mass-Action Kinetics for Chemical Reactions

The dynamical behavior of chemical reaction networks is described by the species concentrations over the time course. According to biologically predefined motifs, a variety of models exists to formulate the reaction kinetics. Since most of them imply specific assumptions, we restrict ourselves to general mass-action kinetics [5]. Here, a continuous approach to express the dynamical behavior considers production and consumption rates v_p and v_c of each species S in order to change its concentration by $\frac{\mathrm{d}\,[S]}{\mathrm{d}\,t} = v_p([S]) - v_c([S])$. These rates result from the reactant concentrations, their stoichiometric factors $a_{i,j} \in \mathbb{N}$ (reactants), $b_{i,j} \in \mathbb{N}$ (products) and kinetic constants $\hat{k}_j \in \mathbb{R}_+$ assigned to each reaction quantifying its speed. For a reaction system with a total number of n species and h reactions

$$\begin{aligned}
a_{1,1}\mathrm{S}_1 + a_{2,1}\mathrm{S}_2 + \ldots + a_{n,1}\mathrm{S}_n &\xrightarrow{\hat{k}_1} b_{1,1}\mathrm{S}_1 + b_{2,1}\mathrm{S}_2 + \ldots + b_{n,1}\mathrm{S}_n \\
a_{1,2}\mathrm{S}_1 + a_{2,2}\mathrm{S}_2 + \ldots + a_{n,2}\mathrm{S}_n &\xrightarrow{\hat{k}_2} b_{1,2}\mathrm{S}_1 + b_{2,2}\mathrm{S}_2 + \ldots + b_{n,2}\mathrm{S}_n \\
&\ldots \\
a_{1,h}\mathrm{S}_1 + a_{2,h}\mathrm{S}_2 + \ldots + a_{n,h}\mathrm{S}_n &\xrightarrow{\hat{k}_h} b_{1,h}\mathrm{S}_1 + b_{2,h}\mathrm{S}_2 + \ldots + b_{n,h}\mathrm{S}_n,
\end{aligned}$$

the corresponding ordinary differential equations (ODEs) read [7]:

$$\frac{\mathrm{d}\,[S_i]}{\mathrm{d}\,t} = \sum_{\nu=1}^{h} \left(\hat{k}_\nu \cdot (b_{i,\nu} - a_{i,\nu}) \cdot \prod_{l=1}^{n} [S_l]^{a_{l,\nu}} \right) \quad \text{with} \quad i = 1, \ldots, n.$$

In order to obtain a concrete trajectory, all initial concentrations $[S_i](0) \in \mathbb{R}_+$, $i = 1, \ldots, n$ are allowed to be set according to the needs of the reaction system.

Discretization: Corresponding P Systems Π_{PMA}

The general form of a P system Π_{PMA} emulating the dynamical behavior of chemical reaction systems with strict *prioritization* of reaction rules based on discretized *mass-action* kinetics is a construct $\Pi_{\mathrm{PMA}} = (V, \Sigma, [_1\,]_1, L_0, R)$, where V denotes the system alphabet containing symbol objects (molecular species) and $\Sigma \subseteq V$ represents the terminal alphabet. Π_{PMA} does not incorporate inner membranes, so the only membrane is the skin membrane $[_1]_1$. The single membrane property results from the assumption of spatial globality in well-stirred reaction vessels. Within a single vessel, the finite multiset $L_0 \subset V \times (\mathbb{N} \cup \{\infty\})$ holds the initial configuration of the system.

We formulate reaction rules together with their kinetic constants by the system component R. The finite set $R = \{r_1, \ldots, r_h\}$ subsumes the reaction rules while each reaction rule $r_i \in \langle E_i \rangle \times \langle P_i \rangle \times \mathbb{R}_+$ is composed of a finite multiset of reactants (educts) $E_i \subset V \times \mathbb{N}$, products $P_i \subset V \times \mathbb{N}$, and the kinetic constant $k_i \in \mathbb{R}_+$. Multiplicities of elements in E_i and R_i correspond with according stoichiometric factors.

Since we strive for a deterministic P system, a strict prioritization among reaction rules is introduced in order to avoid conflicts that can appear if the amount of molecules in the vessel is too low to satisfy all matching reactions. In this case, running all matching reactions in parallel can lead to the unwanted effect that more reactant molecules are taken from the vessel than available violating conservation of mass. Prioritization provides one possible strategy to select applicable reaction rules in contrast to random decisions (introduction of stochasticity) or separate consideration of the combinatorial variety (nondeterministic tracing). For large amounts of molecules in the vessel, the strategy of conflict handling has no influence to the dynamical system behavior and can be neglected. We define the priority of a reaction rule by its index: $r_1 > r_2 > \ldots > r_h$.

For better readability, we subsequently write a reaction rule $r_i = \Big(\{(e_1, a_1), \ldots, (e_\mu, a_\mu)\}, \{(q_1, b_1), \ldots, (q_v, b_v)\}, k_i\Big)$ with $\mathrm{supp}(E_i) = \{e_1, \ldots, e_\mu\}$ and $\mathrm{supp}(P_i) = \{q_1, \ldots, q_v\}$ by using the chemical denotation $r_i : a_1\ e_1 + \ldots + a_\mu\ e_\mu \xrightarrow{k_i} b_1\ q_1 + \ldots + b_v\ q_v$.

Finally, the dynamical behavior of P systems of the form Π_{PMA} is specified by an iteration scheme updating the system configuration L_t at discrete points in time starting from the initial configuration L_0 whereas a second index $i = 1, \ldots, h$ reflects intermediate phases addressing the progress in employing reactions:

$$
\begin{aligned}
L_{t,0} &= L_t \\
L_{t,i} &= L_{t,i-1} \ominus \Bigg\{ \Bigg(a, \Bigg\lfloor k_i \cdot |E_i \cap \{(a,\infty)\}| \cdot \\
&\qquad \prod_{b \in \mathrm{supp}(E_i)} |L_{t,i-1} \cap \{(b,\infty)\}|^{|E_i \cap \{(b,\infty)\}|} \Bigg\rfloor \Bigg) \;\Bigg|\; \forall a \in \mathrm{supp}(E_i) \\
&\qquad \wedge \left(|L_{t,i-1} \cap \{(c,\infty)\}| \geq |E_i \cap \{(c,\infty)\}| \ \forall c \in \mathrm{supp}(E_i) \right) \Bigg\} \\
&\uplus \Bigg\{ \Bigg(a, \Bigg\lfloor k_i \cdot |P_i \cap \{(a,\infty)\}| \cdot \\
&\qquad \prod_{b \in \mathrm{supp}(E_i)} |L_{t,i-1} \cap \{(b,\infty)\}|^{|E_i \cap \{(b,\infty)\}|} \Bigg\rfloor \Bigg) \;\Bigg|\; \forall a \in \mathrm{supp}(P_i) \\
&\qquad \wedge \left(|L_{t,i-1} \cap \{(c,\infty)\}| \geq |E_i \cap \{(c,\infty)\}| \ \forall c \in \mathrm{supp}(E_i) \right) \Bigg\} \\
L_{t+1} &= L_{t,h}.
\end{aligned}
$$

The iteration scheme modifies the system configuration by successive application of reaction rules according to their priority in two stages. The first stage identifies the reactants of a reaction. For this purpose, the required amount of each reactant molecule $a \in \mathrm{supp}(E_i)$ is determined by the kinetic constant k_i, the stoichiometric factor of a (obtained by $|E_i \cap \{(a, \infty)\}|$), and the product of all discretized reactant concentrations. Therefore, the term $|L_{t,i-1} \cap \{(b, \infty)\}|$ describes the number of molecules b currently available in the vessel. Since a reaction is allowed to become employed if and only if it can be satisfied, the constraint $|L_{t,i-1} \cap \{(c, \infty)\}| \geq |E_i \cap \{(c, \infty)\}|\ \forall c \in \mathrm{supp}(E_i)$ checks this property. Along with removal of reactant molecules (multiset difference $\ominus$), corresponding product molecules are added ($\uplus$) to obtain the intermediate configuration $L_{t,i}$ after taking reactions $r_1, \ldots, r_i$ into consideration.

In order to adapt reaction rates for discretization, primary kinetic constants $\hat{k}_i$ defined in the continuous ODE approach have to be converted into counterparts for the discrete iteration scheme by using the transformation

$$k_i = \frac{\hat{k}_i}{\mathrm{V}^{|E_i|}} \cdot \Delta t.$$

Here, $\mathrm{V} \in \mathbb{R}_+ \setminus \{0\}$ expresses the volume of the reaction vessel while the exponent $|E_i|$ declares the sum of all stoichiometric factors of reactants occurring in reaction r_i. Constant $\Delta t \in \mathbb{R}_+ \setminus \{0\}$ specifies the discretization interval.

System Classification and Properties

Π_{PMA} belongs to deterministic P systems with symbol objects, strict prioritization of reaction rules, and progression in time according to mass-action kinetics that is time- and value-discretely approximated by a stepwise adaptation. Its principle of operation follows the idea of formulating one-vessel reaction systems together with their dynamical behavior.

Obviously, P systems Π_{PMA} can emulate finite automata M. To this end, each transition $(q, a) \mapsto q'$ is transformed into a reaction $q + a \xrightarrow{1} q' + a$. Taking all final states as terminal alphabet, $L(\Pi_{\mathrm{PMA}}) = \emptyset$ iff $L(M) = \emptyset$ holds.

From the perspective of computational completeness, P systems Π_{PMA} as defined before operate below Turing universality: Although system configurations might represent any natural number, we need to define an explicit control mechanism able to address dedicated items (configuration components) for arbitrary increment, decrement, and comparison to zero. Due to definition of mass-action kinetics, the number of molecules processed within one application of a reaction rule depends on the total amount of these molecules in the whole system. It seems that reaction rules should be *variable* during system evolution in order to enable enough flexibility. Allowing dynamical changes of kinetic constants or stoichiometric factors along with addition/deletion of reactions provides this flexibility, see Section 4.

3 Transitions between P Systems Π_{PMA}

In this section, we describe a framework enabling transitions between deterministic P systems Π_{PMA} on the fly. Initiated by an external trigger at a defined point in time, a transition manages three switching activities: Firstly, the running system stops its evolution. Secondly, the (only) resulting configuration of that system is mapped into the initial configuration of the subsequent system. This includes integration of possibly new species together with their initial number of copies put into the vessel as well as removal of vanished species iff specified. Reactions in R together with kinetic parameters are replaced. Thirdly, the subsequent system is set into operation.

For formulation of the transition framework, we utilize *state transition systems* [19] denoted as construct $\mathcal{A} = (Q, T, I, \Delta, F)$ with a set Q of states (not necessarily finite but enumerable), an alphabet T of input symbols, a set $I \subseteq Q$ of initial states, the transition relation $\Delta \subseteq Q \times T \times Q$, and a set $F \subseteq Q$ of final states. In general, state transition systems are known to be nondeterministic allowing multiple transitions. For our objective, we arrange the components as follows:

$$
\begin{aligned}
Q &= \{\Pi_{\mathrm{PMA}}^{(j)} \mid (j \in A) \wedge (A \subseteq \mathbb{N})\} \\
T &\subseteq \{(t = \tau) \mid (\tau \in B) \wedge (B \subseteq \mathbb{N})\} \cup \\
&\quad \{([a]\, \mathit{CMP}\, \kappa) \mid (\mathit{CMP} \in \{<, \leq, =, \neq, \geq, >\}) \wedge (\kappa \in \mathbb{N}) \wedge (a \in V^{(j)}) \wedge \\
&\qquad (\Pi_{\mathrm{PMA}}^{(j)} = (V^{(j)}, \Sigma^{(j)}, [\,]_1, L_0^{(j)}, R^{(j)}) \in Q) \wedge (j \in A)\}.
\end{aligned}
$$

While each state in Q is represented by a dedicated P system Π_{PMA}, the input alphabet T contains a number of constraints (triggering events) with regard to progress in operation time ($t = \tau$) or achievement of designated molecular amounts ($[a]\, \mathit{CMP}\, \kappa$). We assume that these constraints are related to the P system in Q currently in operation.

Each transition $\Pi_{\mathrm{PMA}}^{(j)} \overset{c}{\mapsto} \Pi_{\mathrm{PMA}}^{(m)} \in \Delta$ from $\Pi_{\mathrm{PMA}}^{(j)} = (V^{(j)}, \Sigma^{(j)}, [\,]_1, L_0^{(j)}, R^{(j)})$ to $\Pi_{\mathrm{PMA}}^{(m)} = (V^{(m)}, \Sigma^{(m)}, [\,]_1, L_0^{(m)}, R^{(m)})$ triggered by $c \in T$ allows addition of new species to system alphabets $V^{(j)}$ and $\Sigma^{(j)}$. Here, added species form sets $\mathit{AdditionalSpecies}V_{(j,m)}$ and $\mathit{AdditionalSpecies}\Sigma_{(j,m)}$ with $\mathit{AdditionalSpecies}V_{(j,m)} \cap V^{(j)} = \emptyset$ and $\mathit{AdditionalSpecies}\Sigma_{(j,m)} \cap \Sigma^{(j)} = \emptyset$. Furthermore, a species a is allowed to vanish if and only if $[a] = 0$. Corresponding sets $\mathit{VanishedSpecies}V_{(j,m)} \subset V^{(j)}$ and $\mathit{VanishedSpecies}\Sigma_{(j,m)} \subset \Sigma^{(j)}$ contain vanishing species. Within a transition $\Pi_{\mathrm{PMA}}^{(j)} \overset{c}{\mapsto} \Pi_{\mathrm{PMA}}^{(m)}$, new reactions might appear restricted to reactants and products available in $V^{(m)}$. New reactions $r_i \in \langle V^{(m)} \times \mathbb{N} \rangle \times \langle V^{(m)} \times \mathbb{N} \rangle \times \mathbb{R}_+$ with unique priority index i become accumulated by the multiset $\mathit{AdditionalReactions}_{(j,m)}$. Accordingly, we consider vanishing reactions present in multiset $\mathit{VanishedReactions}_{(j,m)}$. The scheme

$$
\begin{aligned}
V^{(m)} &= V^{(j)} \cup \mathit{AdditionalSpecies}V_{(j,m)} \setminus \mathit{VanishedSpecies}V_{(j,m)} \\
\Sigma^{(m)} &= \Sigma^{(j)} \cup \mathit{AdditionalSpecies}\Sigma_{(j,m)} \setminus \mathit{VanishedSpecies}\Sigma_{(j,m)}
\end{aligned}
$$

$$L_0^{(m)} = L_t^{(j)} \uplus \{(a,0) \mid a \in \mathit{AdditionalSpecies}V_{(j,m)}\}$$
$$R^{(m)} = R^{(j)} \uplus \mathit{AdditionalReactions}_{(j,m)} \ominus \mathit{VanishedReactions}_{(j,m)}$$

decomposes the P system transition into all single components. After performing the transition, the obtained system $\Pi_{\mathrm{PMA}}^{(m)}$ includes reactions $R^{(m)} = \{r_i \mid (i \in A) \wedge (A \subset \mathbb{N})\}$ where A is an arbitrary finite subset of natural numbers. In order to preserve the strict prioritization among reaction rules, pairwise distinctive indexes i are required in each set.

4 Chemical Register Machines with Self-reproducible Components

In the first example, we apply transitions between P systems to formulate a chemical register machine on binary numbers with self-reproducible components for bit storage units. Each time new storing capacity within a register is needed, a specific reaction subsystem for that purpose is added. A strict modularization of the reaction network forming bit storage units (chemical implementation of master-slave flip-flops) facilitates the system design towards achieving computational completeness. A chemical representation of binary numbers noticeably increases the reliability of operation from an engineering point of view.

Register Machines on Binary Numbers

A register machine on binary numbers is a tuple $M = (R, L, P, \#_0)$ consisting of the finite set of registers $R = \{\mathrm{R}_1, \ldots, \mathrm{R}_m\}$ each with binary representation of a natural number $\mathrm{R}_h \in \{0,1\}^*$, a finite set of jump labels (addresses) $L = \{\#_0, \ldots, \#_n\}$, a finite set P of instructions, and the jump label of the initial instruction $\#_0 \in L$. Available instructions are: $\#_i :$ INC R_h $\#_j$ (increment register R_h and jump to $\#_j$), $\#_i :$ DEC R_h $\#_j$ (non-negatively decrement register R_h and jump to $\#_j$), $\#_i :$ IFZ R_h $\#_j$ $\#_p$ (if $\mathrm{R}_h = 0$ then jump to $\#_j$ else jump to $\#_p$), and $\#_i$ HALT (terminate program and output register contents). We assume a pre-initialization of input and auxiliary registers at start with input data or zero. Furthermore, a deterministic principle of operation, expressed by unique usage of instruction labels: $\forall p, q \in P \mid (p = \#_i : v) \wedge (q = \#_j : w) \wedge ((i \neq j) \vee (v = w))$, is supposed.

Chemical Encoding of Binary Values

Each boolean variable $x \in \{0,1\}$ is represented by two correlated species X^T and X^F with complement concentrations $[X^T] \in \mathbb{R}_+$ and $[X^F] \in \mathbb{R}_+$ such that $[X^T] + [X^F] = c$ holds with $c = \mathit{const.}$ The boolean value of the variable x is determined whenever one of the following conditions is fulfilled: The inequality $[X^T] \ll [X^F]$ indicates "false" ($x = 0$) and $[X^F] \ll [X^T]$ "true" ($x = 1$). In case of none of these strong inequalities holds (e.g. $[X^T] = 0.6c$ and $[X^F] = 0.4c$), the system would consider the variable x to be in both states.

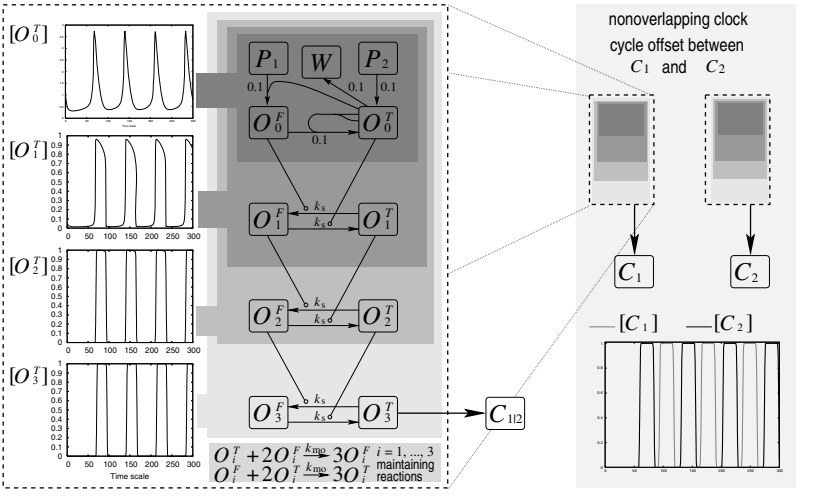

Fig. 1. Generation of chemical clock signals $[C_1]$, $[C_2]$ (right) by cascadization of toggle switches (left)

A Chemical Clock by Extending an Oscillating Reaction Network

A chemical implementation of a clock is necessary in order to synchronize the register machine instruction processing. Positive edges of clock signals can trigger micro-operations like register increment or jump to the next machine instruction. In our chemical machine model, an extended oscillating reaction network provides all clock signals. As preferred network template for permanent oscillation, we adopt the well-studied Belousov-Zhabotinsky reaction [2,21] depicted in the upper-left part of Figure 1 whose dynamical behavior results in periodic peak-shaped signals. By using a cascade of downstream switching and maintaining reactions, we extend that primary oscillator. In this way, a normalization with respect to signal shape and concentration course can be reached. Our idea employs both converse output signals O_i^T and O_i^F of the previous cascade stage as triggers for a subsequent chemical toggle switch. Thus, high and low concentration levels are more and more precisely separated over the time course, and the switching delay in between becomes shortened, see lower-left parts of Figure 1. After three cascade stages, the quality of the chemical clock signal turns out to be suitable for our purposes.

For technical reasons (two-phase register machine instruction processing), two offset clocks with designated output species C_1 and C_2 are employed. Owning the same network structure, they only differ in the time point when coming into operation caused by individual initializations (species producing clock signals C_1: $[O^F_{0,C_1}](0) = 2, [O^T_{0,C_1}](0) = 1$; corresponding species for clock signals C_2: $[O^F_{0,C_2}](0) = 0, [O^T_{0,C_2}](0) = 0$; species with identical initial concentrations: $[P_{1,C}](0) = 3, [P_{2,C}](0) = 1, [W_C](0) = 0, [O^F_{i,C}](0) = 1, [O^T_{i,C}](0) = 0), i \in \{1, 2, 3\}, C \in \{C_1, C_2\}$). C_1 and C_2 provide non-overlapping clock signals whose offset constitutes approximately one half of the clock cycle, see Figure 1 right.

Constructing Master-Slave Flip-Flops and Binary Registers

We introduce a reaction network that mimics a master-slave flip-flop (MSFF) based on the aforementioned chemical clocks and bit manipulating reaction

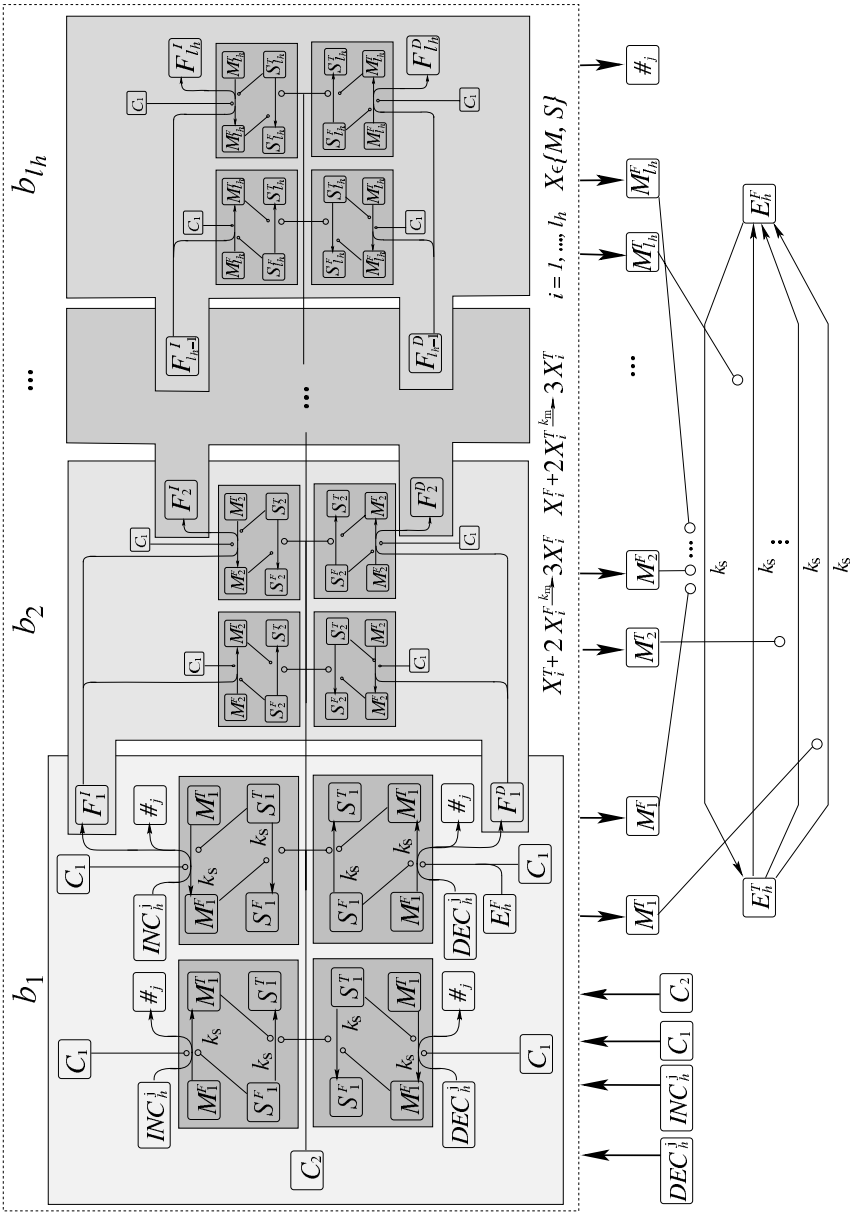

Fig. 2. Chemical reaction network of a register capable of processing a bitwise extendable binary number $\ldots b_{l_h} b_{l_h-1} \ldots b_2 b_1$ with $b_\alpha \in \{0, 1\}$ including interfaces for micro-operations increment, nonnegative decrement, and comparison to zero

motifs. Moreover, a chain of MSFFs forms a register R_h ($h \in \{1, \ldots, |R|\}$) with bitwise extendable initial length of one bit. In operation, it processes binary numbers $\ldots b_{l_h} b_{l_h-1} \ldots b_2 b_1$ with $b_\alpha \in \{0, 1\}$. Furthermore, each register is equipped with predefined triggers in order to carry out micro-operations "increment", "nonnegative decrement", and "comparison to zero", each of which is processed within one clock cycle.

Within a MSFF, bit setting is coupled to specific edges of the clock signal in order to prevent premature switches. In our MSFF implementation, bit setting consists of two phases (master and slave part). Within the master part, a bit can be preset using specific master species M^T and M^F co-triggered by positive edges of the clock signal C_1, while the subsequent slave part finalizes the setting by forwarding the preset bit from the master species to the correlated slave species S^T and S^F triggered by positive edges of the offset clock signal C_2. A subnetwork consisting of eight switching reactions (see darkest grey highlighted boxes within each MSFF in Figure 2) covers this task.

With regard to the functionality of a register machine, a sequence of interconnected MSFFs represents a register. Interconnections between neighbored MSFFs reflect the capability of incrementing and decrementing register contents. In case of increment, designated trigger molecules INC_h^j effect a successive bit flipping: Starting from the least significant bit b_1, "1" is consecutively converted into "0" until "0" appears first time which is finally converted into "1". Intermediate carry species F_α^I act as forwarding triggers between consecutive bits, see Figure 2. If the most significant bit b_{l_h} is reached increasing the concentration of carry species $F_{l_h}^I$, six new species $M_{l_h+1}^T$, $M_{l_h+1}^F$, $S_{l_h+1}^T$, $S_{l_h+1}^F$, $F_{l_h+1}^D$, and $F_{l_h+1}^I$ are added to the reaction system together with the corresponding set of reactions forming the subnetwork for managing bit b_{l_h+1} including update of $M_{l_h+1}^F$ and $M_{l_h+1}^T$ within reactions performing comparison to zero, see Figure 2.

Decrement is organized in a similar way using initial triggers DEC_h^j and intermediate molecules of carry species F_β^D. In order to achieve nonnegative processing, a species E_h^F indicating equality to zero, set by a satellite network, prevents decrement of binary strings $0 \ldots 0$. Figure 2 shows the reaction network structure of a register whose species F_α^I, F_β^D, M_γ^F, M_γ^T, S_γ^F, and S_γ^T are specific with respect to both register identifier h and bit position l_h within the register. Any comparison to zero is done by a satellite network which uses presence of any species M_κ^T with $\kappa = 1, \ldots, l_h$ as triggers in order to flip an equality indicator bit e (species E_h^T and E_h^F) onto "0", while all species M_κ^F with $\kappa = 1, \ldots, l_h$ are needed for flipping onto "1", respectively. The indicator e can be used for program control, see next section. As a further byproduct of each micro-operation on a register, molecules of the form $\#_j \in L$ encoding the jump label of the subsequent machine instruction are released.

Implementing a Chemical Program Control

A sequence of reactions directly derived from the given program P of the underlying register machine $M = (R, L, P, \#_0)$ carries out the program control as follows: For each jump label $\#_j \in L$ we introduce a dedicated *label species* $\#_j$

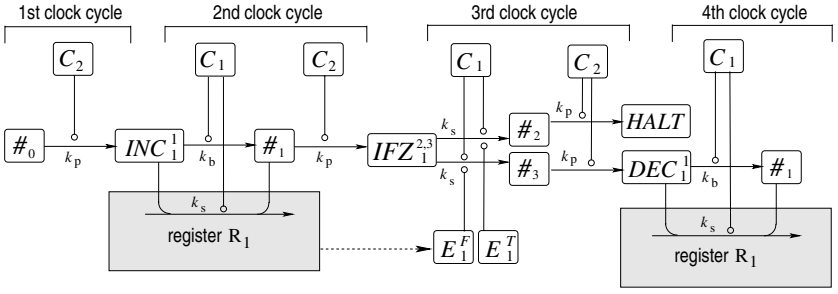

Fig. 3. Example: Chemical program control for $M = (\{\mathrm{R}_1\}, \{\#_0, \ldots, \#_3\}, P, \#_0)$ with $P = \{\#_0 : \mathrm{INC\ R}_1\ \#_1, \quad \#_1 : \mathrm{IFZ\ R}_1\ \#_2\ \#_3, \quad \#_2 : \mathrm{HALT}, \quad \#_3 : \mathrm{DEC\ R}_1\ \#_1\}$

with initial concentrations $[\#_0](0) = 1$ and $[\#_\kappa](0) = 0$ for $\kappa \in \{1, \ldots, |L| - 1\}$. Accordingly, a set of *instruction species* $I_\nu \in \{INC_h^j, DEC_h^j \mid \forall h \in \{1, \ldots, |R|\} \wedge \forall j \in \{0, \ldots, |L| - 1\}\} \cup \{IFZ_h^{j,q} \mid \forall h \in \{1, \ldots, |R|\} \wedge \forall j, q \in \{0, \ldots, |L| - 1\}\} \cup \{HALT\}$ is created with initial concentration $[I_\nu](0) = 0$. Furthermore, for each instruction in P a network motif consisting of a *program-control reaction* with kinetic constant $k_\mathrm{p} < k_\mathrm{s}$ and a consecutive *bypass reaction* with $k_\mathrm{b} \leq k_\mathrm{s}$ is defined. Following the two-phase structure of a register machine instruction, these reactions first consume its incipient label species, then produce the corresponding instruction species as an intermediate product and finally convert it into the label species of the subsequent instruction if available. In order to strictly sequentialize the execution of instructions according to the program P, clock species C_1 and C_2 with offset concentration course provided by both oscillators trigger program-control and bypass reactions alternating as catalysts.

The set of reactions for each type of register machine instruction is defined as in Table 1.

Instruction species of the form INC_h^j act as triggers for incrementing the contents of register R_h done within its reaction network part, see Figure 2. Here,

Table 1.

instruction	reactions
$\#_i : \mathrm{INC\ R}_h\ \#_j$	$\#_i + C_2 \xrightarrow{k_\mathrm{p}} INC_h^j + C_2$ $INC_h^j + C_1 \xrightarrow{k_\mathrm{b}} \#_j + C_1$
$\#_i : \mathrm{DEC\ R}_h\ \#_j$	$\#_i + C_2 \xrightarrow{k_\mathrm{p}} DEC_h^j + C_2$ $DEC_h^j + C_1 \xrightarrow{k_\mathrm{b}} \#_j + C_1$
$\#_i : \mathrm{IFZ\ R}_h\ \#_j\ \#_q$	$\#_i + C_2 \xrightarrow{k_\mathrm{p}} IFZ_h^{j,q} + C_2$ $IFZ_h^{j,q} + E_h^T + C_1 \xrightarrow{k_\mathrm{s}} \#_j + E_h^T + C_1$ $IFZ_h^{j,q} + E_h^F + C_1 \xrightarrow{k_\mathrm{s}} \#_q + E_h^F + C_1$
$\#_i : \mathrm{HALT}$	$\#_i + C_2 \xrightarrow{k_\mathrm{p}} HALT + C_2$

INC_h^j is converted into the byproduct $\#_j$ that provides the label species of the subsequent instruction. Accordingly, species DEC_h^j initiate a set of reactions decrementing register R_h non-negatively. Instruction species of the form $IFZ_h^{j,q}$ utilize a reaction network module attached to register R_h that releases two species E_h^T and E_h^F whose concentrations indicate whether or not $\mathrm{R}_h = 0$. Instruction species of the form INC_h^j, DEC_h^j, and $IFZ_h^{j,q}$ react into the corresponding label species $\#_j$ and $\#_q$. Since there is no reaction with instruction species $HALT$ as reactant, the program stops in this case. Figure 3 illustrates an example of a chemical program control that also gives an overview about the interplay of all predefined modules.

Although instruction species are consumed within register modules, this process could be too slow in a way that a significant concentration of an instruction species outlasts the clock cycle. This unwanted effect is eliminated by bypass reactions running in parallel to the designated register operation.

Case Study: Integer Addition

A chemistry processing $\mathrm{R}_2 := \mathrm{R}_2 + \mathrm{R}_1; \mathrm{R}_1 := 0$ including previous register initialization $(\mathrm{R}_1, \mathrm{R}_2) := (2, 1)$ on extendable bit word registers emulates a case study of the integer addition "$2 + 1$" whose dynamical behavior using $k_\mathrm{s} = 3$, $k_\mathrm{m} = 1$, $k_\mathrm{mo} = 3$, $k_\mathrm{b} = 0.5$, $k_\mathrm{p} = 1$ is shown in Figure 4 (upper part).

Starting with empty one-bit chemical registers $\mathrm{R}_1 = 0$ and $\mathrm{R}_2 = 0$, the primary P system $\Pi_{\mathrm{PMA}}^{(0)}$ is set into operation. Along with the second increment of R_1, concentration of the carry species $F_{1,1}^I$ becomes > 0 initiating the first P system transition into $\Pi_{\mathrm{PMA}}^{(1)}$, see Figure 4 (lower part). This system contains additional species and reactions (according to Figure 2) to enlarge register R_1 onto two bits. Four C_2 clock cycles later, carry species $F_{2,1}^I$ reaches a positive concentration transforming $\Pi_{\mathrm{PMA}}^{(1)}$ into $\Pi_{\mathrm{PMA}}^{(2)}$ by extending the chemical register R_2 from one into two bit storage capacity.

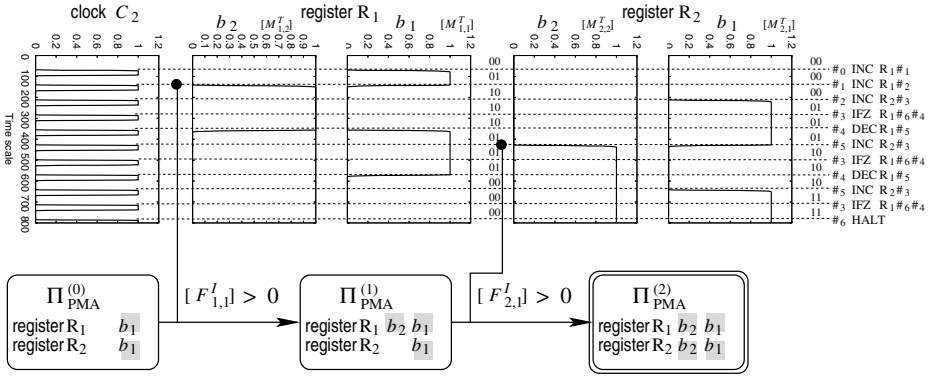

Fig. 4. Dynamical behavior of a chemical register machine acting as an adder (upper part) and transitions between corresponding P systems successive enlarging storage capacity (lower part)

All simulations of the dynamical register machine behavior were carried out using CellDesigner version 3.5.2, an open source software package for academic use [9]. The register machine (available from the authors upon request) was implemented in SBML (Systems Biology Markup Language) [11], a file format shown to be suitable for P systems representation [15].

5 Evolutionary Construction of Reaction Networks

Artificial evolution of reaction networks towards a desired dynamical behavior is a powerful tool to automatically devise complex systems capable of computational tasks. We have designed and implemented a software (SBMLevolver) [12] for evolutionary construction of single-compartmental biological models written in SBML. The SBMLevolver enables both, structural evolution (operators: adding/deleting species, adding/deleting reactions, connection/disconnection of a species to/from a reaction, species duplication) and network parameter fitting (adaptation of kinetic constants). Each reaction network generated within the process of artificial evolution forms a P system of the class Π_{PMA}. Evolutionary operators become activated randomly after a dedicated period for running a reaction network. When we understand evolutionary operators as (state) transitions between P systems, the arising phylogenetic graph (history of artificial evolution) is related to the corresponding state transition system. Because state transitions between P systems are not necessarily deterministic, the phylogenetic graph may have multiple branches. An example in Figure 5 shows P system transitions sketching an artificial evolution process towards a reaction network for addition of two numbers. In this procedure, selection can be incorporated by a network evaluation measure to be included as a component of Π_{PMA}.

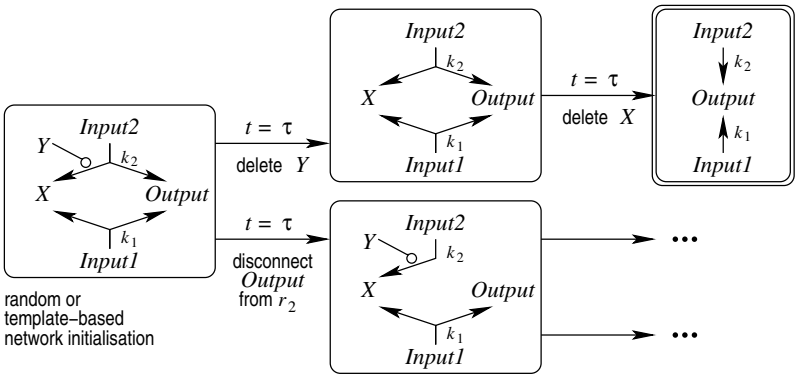

Fig. 5. Part of a state transition system sketching the trace of an artificial structural evolution towards a reaction network for addition of two numbers given as initial concentrations of species *Input1* and *Input2*. In the SBMLevolver, each network passes a separate supplementary parameter fitting (optimization) of kinetic constants (not shown).

6 Conclusions

The formalization of complex biological or chemical systems with structural dynamics within their reaction rules can contribute to explore the potential of their functionality as a whole. From the modeling point of view, coordination of temporally local subsystem descriptions in terms of well-defined interfaces might be a challenging task since it requires a homogeneous approach. The P systems framework inherently suits here because of its discrete manner and its ability to combine different levels of abstraction. We have shown a first idea for arranging previously separate subsystems into a common temporal framework. In our approach, transitions between subsystems have been initiated by constraints denoted as boolean expressions. Therefore, we allow for evaluation of internal signals (molecular amount) as well as external signals (time provided by a global clock). Beyond computational completeness, application scenarios are seen in systems and synthetic biology. Further work will be directed to comprise P systems of different classes and with compartmental structures into a common transition framework.

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