

Modelling Cellular Processes using Membrane Systems with Peripheral and Integral Proteins

Matteo Cavaliere, Sean Sedwards

Microsoft Research – University of Trento
Centre for Computational and Systems Biology, Trento, Italy
{matteo.cavaliere, sean.sedwards}@msr-unitn.unitn.it

Abstract. Membrane systems were introduced as models of computation inspired by the structure and functioning of biological cells. Recently, membrane systems have also been shown to be suitable to model cellular processes. We introduce a new model called *Membrane Systems with Peripheral and Integral Proteins*. The model has compartments enclosed by membranes, floating objects, objects associated to the internal and external surfaces of the membranes and also objects integral to the membranes. The floating objects can be processed within the compartments and can interact with the objects associated to the membranes. The model can be used to represent cellular processes that involve compartments, surface and integral membrane proteins, transport and processing of chemical substances. As examples we model a circadian clock and the G-protein cycle in yeast *saccharomyces cerevisiae* and present a quantitative analysis using an implemented simulator.

1 Introduction

Membrane systems are models of computation inspired by the structure and the function of biological cells. The model was introduced in 1998 by Gh. Păun and since then many results have been obtained, mostly concerning computational power. A short introductory guide to the field can be found in [12], while an updated bibliography is available via the web-page [18]. Recently (see, e.g., [10]), membrane systems have been successfully applied to systems biology and several models have been proposed for simulating biological processes (e.g., see the monograph dedicated to membrane computing applications [5]).

By the original definition, membrane systems are composed of an hierarchical nesting of membranes that enclose regions (*the cellular structure*), in which free-floating objects (*molecules*) exist. Each region can have associated rules, called *evolution rules*, for evolving the free-floating objects and modelling the biochemical reactions present in cell regions. Rules also exist for moving objects across membranes, called *symport* and *antiport* rules, modelling cellular transport. Recently, inspired by *brane calculus* [3], a model of a membrane system, having free-floating objects and objects attached to the membranes, was introduced in [2]. The attached objects model the proteins that are embedded in lipid bilayer cell membranes. In [2], however, objects are associated to an

indivisible membrane which has no concept of inner or outer surface while in [4] objects (peripheral proteins) are attached to either side of a membrane. In reality, many biological processes are driven and controlled by the presence of specific proteins on the appropriate side of and *integral* to the membrane: there is a constant interaction between floating chemicals and embedded proteins and between peripheral and integral proteins (see, e.g., [1]). Receptor-mediated processes, such as endocytosis (illustrated in Figure 1) and signalling, are crucial to cell function and by definition are critically dependent on the presence of peripheral and integral membrane proteins.

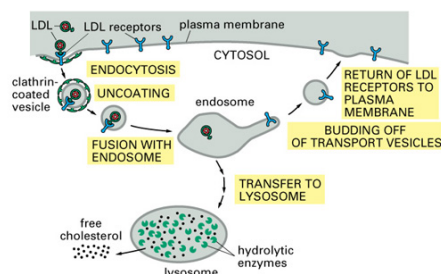


Fig. 1. Endocytosis of LDL (*Essential Cell Biology*, 2/e, ©2004 Garland Science)

One model of the cell is that of compartments and sub-compartments in constant communication, with molecules being passed from donor compartments to target compartments by interaction with membrane proteins. Once transported to the correct compartment, the substances are then *processed* by means of local biochemical reactions.

Motivated by these ideas we extend the model presented in [4], introducing a model having peripheral as well as integral proteins.

In each region of the system there are floating objects (the floating chemicals) and, in addition, objects can be associated to each side of a membrane or integral to the membrane (the peripheral and integral membrane proteins). Moreover, the system can perform the following operations: (i) the floating objects can be processed/changed inside the regions of the system (emulating biochemical rules) and (ii) the floating and attached objects can be processed/changed when they interact (modelling the interactions of the floating molecules with membrane proteins).

The proposed model can be used to represent cellular processes that involve floating molecules, surface and integral membrane proteins, transport of molecules across membranes and processing of molecules inside the compartments. As examples, we model a circadian clock and the G-protein cycle in *saccharomyces cerevisiae*, where the possibility to use, in an *explicit way*, compartments, membrane proteins and transport rules is very useful. A *quantitative analysis* of the models is also presented, performed using an implemented simu-

lator (presented in [4], downloadable at [19]). The simulator employs a stochastic algorithm and uses intuitive syntax based on chemical equations. The syntax is described in the appendix.

2 Formal Language Preliminaries

Membrane systems are based on *formal language theory* and *multiset rewriting*. We now briefly recall the basic theoretical notions used in this paper. For more details the reader can consult standard books, such as [8], [15], [6] and handbook [14].

Given the set A we denote by $|A|$ its cardinality and by \emptyset the empty set. We denote by \mathbb{N} and by \mathbb{R} the set of natural and real numbers, respectively.

As usual, an *alphabet* V is a finite set of symbols. By V^* we denote the set of all strings over V . By V^+ we denote the set of all strings over V excluding the empty string. The empty string is denoted by λ . The *length* of a string v is denoted by $|v|$. The concatenation of two strings $u, v \in V^*$ is written uv .

The number of occurrences of the symbol a in the string w is denoted by $|w|_a$.

A *multiset* is a set where each element may have a multiplicity. Formally, a multiset over a set V is a map $M : V \rightarrow \mathbb{N}$, where $M(a)$ denotes the multiplicity of the symbol $a \in V$ in the multiset M .

For multisets M and M' over V , we say that M is *included in* M' if $M(a) \leq M'(a)$ for all $a \in V$. Every multiset includes the *empty multiset*, defined as M where $M(a) = 0$ for all $a \in V$.

The *sum* of multisets M and M' over V is written as the multiset $(M + M')$, defined by $(M + M')(a) = M(a) + M'(a)$ for all $a \in V$. The *difference* between M and M' is written as $(M - M')$ and defined by $(M - M')(a) = \max\{0, M(a) - M'(a)\}$ for all $a \in V$. We also say that $(M + M')$ is obtained by *adding* M to M' (or viceversa) while $(M - M')$ is obtained by *removing* M' from M . For example, given the multisets $M = \{a, b, b, b\}$ and $M' = \{b, b\}$, we can say that M' is included in M , that $(M + M') = \{a, b, b, b, b, b\}$ and that $(M - M') = \{a, b\}$.

If the set V is finite, e.g. $V = \{a_1, \dots, a_n\}$, then the multiset M can be explicitly described as $\{(a_1, M(a_1)), (a_2, M(a_2)), \dots, (a_n, M(a_n))\}$. The *support* of a multiset M is defined as the set $\text{supp}(M) = \{a \in V \mid M(a) > 0\}$. A multiset is empty (hence finite) when its support is empty (also finite).

A compact notation can be used for finite multisets: if $M = \{(a_1, M(a_1)), (a_2, M(a_2)), \dots, (a_n, M(a_n))\}$ is a multiset of finite support, then the string $w = a_1^{M(a_1)} a_2^{M(a_2)} \dots a_n^{M(a_n)}$ (and all its permutations) precisely identify the symbols in M and their multiplicities. Hence, given a string $w \in V^*$, we can say that it identifies a finite multiset over V , written as $M(w)$, where $M(w) = \{a \in V \mid (a, |w|_a)\}$. For instance, the string bab represents the multiset $M(w) = \{(a, 1), (b, 2)\}$, that is the multiset $\{a, b, b\}$. The empty multiset is represented by the empty string λ .

3 Operations with Peripheral and Integral Proteins

Let V denote a finite alphabet of *objects* and Lab a finite set of labels.

As is usual in the membrane systems field, a membrane is represented by a pair of square brackets, $[]$. A *membrane structure* is an *hierarchical nesting* of membranes enclosed by a main membrane called the *root membrane*. To each membrane is associated a label that is written as a superscript of the membrane, e.g. $[]^1$. If a membrane has the label i we call it membrane i .

A membrane structure is essentially that of a tree, where the nodes are the membranes and the arcs represent the containment relation. In this paper we avoid a formal mapping in the interest of the intuitiveness of the description, however, being a tree, a membrane structure can be represented by a string of matching square brackets, e.g., $[[[]^2]^1 []^3]^0$.

To each membrane there are associated three multisets, u , v and x over V , denoted by $[]_{u|v|x}$. We say that the membrane is *marked* by u , v and x ; x is called the *external marking*, u the *internal marking* and v the *integral marking* of the membrane. In general, we refer to them as *markings* of the membrane.

The internal, external and integral markings of a membrane model the proteins attached to the internal surface, attached to the external surface and integral to the membrane, respectively.

In a membrane structure, the region between membrane i and any enclosed membranes is called region i . To each region is associated a multiset of objects w called the *free objects* of the region. The free objects are written between the brackets enclosing the regions, e.g., $[aa [bb]^1]^0$.

The free objects of a membrane model the floating chemicals within the regions of a cell.

We denote by $int(i)$, $ext(i)$ and $itgl(i)$ the internal, external and integral markings of membrane i , respectively. By $free(i)$ we denote the free objects of region i . For any membrane i , distinct from a root membrane, we denote by $out(i)$ the label of the membrane enclosing membrane i .

For example, the string

$$[ab [cc]_{a|}^2 [abb]_{bba|ab|c}^1]^0$$

represents a membrane structure, where to each membrane are associated markings and to each region are associated free objects. Membrane 1 is internally marked by bba (i.e., $int(1) = bba$), has integral marking ab (i.e., $itgl(1) = ab$) and is externally marked by c (i.e., $ext(1) = c$). To region 1 are associated the free objects abb (i.e., $free(1) = abb$). To region 0 are associated the free objects ab . Finally, $out(1) = out(2) = 0$. Membrane 0 is the root membrane. The string can also be depicted diagrammatically, as in Figure 2.

When a marking is omitted it is intended that the membrane is marked by the empty string λ , i.e., the empty multiset. For instance, in $[ab]_{u|v|}$ the external marking is missing, while in the case of $[ab]_{|v|x}$ the internal marking is missing.

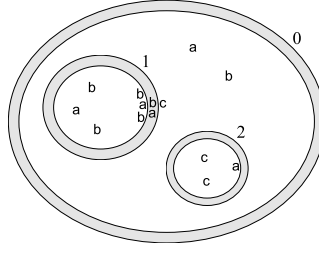


Fig. 2. Graphical representation of $[ab [cc]_a^2 \mid [abb]_{bba|ab|c}^1]^0$

3.1 Operations

We introduce rules that describe bidirectional interactions of floating objects with the membrane markings which we call *membrane rules*. These rules are motivated by the behaviour of cell membrane proteins (e.g., see [1]) and therefore permit a level of abstraction based on the behaviour of real molecules. We denote the rules as $attach_{in}$, $attach_{out}$, $de - attach_{in}$ and $de - attach_{out}$, defined:

$$\begin{aligned}
 attach_{in} &: [\alpha]_{u|v}^i \rightarrow [\alpha]_{u'|v'}^i, \quad \alpha \in V^+, u, v, u', v' \in V^*, i \in Lab \\
 attach_{out} &: [\alpha]_{v|x}^i \rightarrow [\alpha]_{v'|x'}^i, \quad \alpha \in V^+, v, x, v', x' \in V^*, i \in Lab \\
 de - attach_{in} &: [\alpha]_{u|v}^i \rightarrow [\alpha]_{u'|v'}^i, \quad \alpha, u', v', u, v \in V^*, |uv| > 0, i \in Lab \\
 de - attach_{out} &: [\alpha]_{v|x}^i \rightarrow [\alpha]_{v'|x'}^i, \quad \alpha, v', x', v, x \in V^*, |vx| > 0, i \in Lab
 \end{aligned}$$

The semantics of these rules is as follows.

The $attach_{in}$ rule is *applicable to membrane i* if $free(i)$ includes α , $int(i)$ includes u and $itgl(i)$ includes v . When the rule is applied to membrane i , α is removed from $free(i)$, u is removed from $int(i)$, v is removed from $itgl(i)$, u' is added to $int(i)$ and v' is added to $itgl(i)$. The objects not involved in the application of the rule are left unchanged in their original positions.

The $attach_{out}$ rule is applicable to membrane i if $free(out(i))$ includes α , $itgl(i)$ includes v , $ext(i)$ includes x . When the rule is applied to membrane i , α is removed from $free(out(i))$, v is removed from $itgl(i)$, x is removed from $ext(i)$, v' is added to $itgl(i)$ and x' is added to $ext(i)$. The objects not involved in the application of the rule are left unchanged in their original positions.

The $de - attach_{in}$ rule is applicable to membrane i if $int(i)$ includes u and $itgl(i)$ includes v . When the rule is applied to membrane i , u is removed from $int(i)$, v is removed from $itgl(i)$, u' is added to $int(i)$, v' is added to $itgl(i)$ and α is added to $free(i)$. The objects not involved in the application of the rule are left unchanged in their original positions.

The $de - attach_{out}$ rule is applicable to membrane i if $itgl(i)$ includes v and $ext(i)$ includes x . When the rule is applied to membrane i , v is removed from

$itgl(i)$, x is removed from $ext(i)$, v' is added to $itgl(i)$, x' is added to $ext(i)$ and α is added to $free(out(i))$. The objects not involved in the application of the rule are left unchanged in their original positions.

We denote by $\mathcal{R}_{V,Lab}^{att}$ the set of all possible *attach* and *de-attach* rules over the alphabet V and set of labels Lab . Instances of $attach_{in}$, $attach_{out}$, $de-attach_{in}$ and $de-attach_{out}$ rules are depicted in Figure 3.

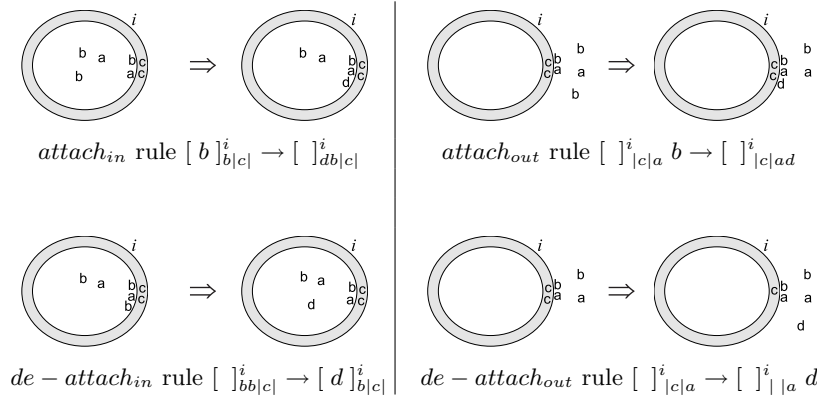


Fig. 3. Examples of $attach_{in}$, $attach_{out}$, $de-attach_{in}$ and $de-attach_{out}$ rules, showing how free and attached objects may be rewritten. E.g., in the $attach_{in}$ rule one of the two free instances of b is rewritten to d and added to the membrane's internal marking.

We next introduce *evolution rules* that rewrite the free objects contained in a region conditional on the markings of the enclosing membrane. These rules can be considered to model the biochemical reactions that take place within the cytoplasm of a cell. We define an evolution rule:

$$evol : [\alpha \rightarrow \beta]_{u|v|}^i$$

where $u, v, \beta \in V^*$, $\alpha \in V^+$, and $i \in Lab$.

The semantics of the rule is as follows. The rule is applicable to region i if $free(i)$ includes α , $int(i)$ includes u and $itgl(i)$ includes v . When the rule is applied to region i , α is removed from $free(i)$ and β is added to $free(i)$. The membrane markings and the objects not involved in the application of the rule are left unchanged in their original positions.

We denote by $\mathcal{R}_{V,Lab}^{ev}$ the set of all evolution rules over the alphabet V and set of labels Lab . An instance of an evolution rule is represented in Figure 4.

In general, when a rule has label i we say that a rule is associated to *membrane* i (in the case of *attach* and *de-attach* rules) or is associated to *region* i (in the case of *evol* rules). For instance, in Figure 3 the $attach_{in}$ is associated to membrane i .

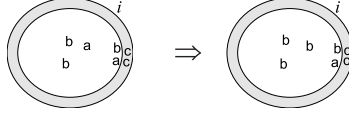


Fig. 4. *evol* rule $[a \rightarrow b]_{b|c|}^i$. Free objects can be rewritten inside the region and the rewriting can depend on the integral and internal markings of the enclosing membrane.

The objects of α , u and v for *attach_{in}*/*evol* rules, of α , v and x for *attach_{out}* rules, of u and v for *de-attach_{in}* rules and of v and x for *de-attach_{out}* rules are the *reactants* of the corresponding rules. E.g., in the attach rule $[b]_{a|c|} \rightarrow []_{d|c|}$, the reactants are a , b and c .

We note that a single application of an *evol* rule may be simulated by an application of an *attach_{in}* rule followed by an application of an *de-attach_{in}* rule. This may be biologically realistic in some cases, but not in all. Hence the need for evolution rules.

4 Membrane Systems with Peripheral and Integral Proteins

In this section we define membrane systems having membranes marked with peripheral proteins, integral proteins, free objects and using the operations introduced in Section 3.

Definition 1. A membrane system with peripheral and integral proteins and n membranes (in short, a P_{pi} system), is a construct

$$\mathcal{P} = (V_{\mathcal{P}}, \mu_{\mathcal{P}}, (u_0, v_0, x_0)_{\mathcal{P}}, \dots, (u_{n-1}, v_{n-1}, x_{n-1})_{\mathcal{P}}, w_{0,\mathcal{P}}, \dots, w_{n-1,\mathcal{P}}, R_{\mathcal{P}}, t_{in,\mathcal{P}}, t_{fin,\mathcal{P}}, rate_{\mathcal{P}})$$

- $V_{\mathcal{P}}$ is a finite, non-empty alphabet of objects.
- $\mu_{\mathcal{P}}$ is a membrane structure with $n \geq 1$ membranes injectively labelled by labels in $Lab_{\mathcal{P}} = \{0, 1, \dots, n-1\}$, where 0 is the label of the root membrane.
- $(u_0, v_0, x_0)_{\mathcal{P}} = (\lambda, \lambda, \lambda), (u_1, v_1, x_1)_{\mathcal{P}}, \dots, (u_{n-1}, v_{n-1}, x_{n-1})_{\mathcal{P}} \in V^* \times V^* \times V^*$ are called initial markings of the membranes.
- $w_{0,\mathcal{P}}, w_{1,\mathcal{P}}, \dots, w_{n-1,\mathcal{P}} \in V^*$ are called initial free objects of the regions.
- $R_{\mathcal{P}} \subseteq \mathcal{R}_{V, Lab_{\mathcal{P}} - \{0\}}^{att} \cup \mathcal{R}_{V, Lab_{\mathcal{P}}}^{ev}$ is a finite set of evolution rules, attach/de-attach rules.¹
- $t_{in,\mathcal{P}}, t_{fin,\mathcal{P}} \in \mathbb{R}$ are called the initial time and the final time, respectively.
- $rate_{\mathcal{P}} : R_{\mathcal{P}} \mapsto \mathbb{R}$ is the rate mapping. It associates to each rule a rate.

¹ The root membrane may contain objects and evolution rules but not attach or de-attach rules, since it has no enclosing region. It may therefore be viewed as an extended version of a membrane systems environment (as defined in [12]), with objects and evol rules. Alternatively, it can be seen as a membrane systems skin membrane, where the environment contains nothing and is not accessible.

Let Π be an arbitrary P_{pi} system. An *instantaneous description* I of Π consists of the membrane structure μ_Π with markings associated to the membranes and free objects associated to the regions. We denote by $\mathbb{I}(\Pi)$ the set of *all instantaneous descriptions* of Π . We shortly say *membrane (region) i of I* to denote the membrane (region, respectively) i present in I .

Let I be an arbitrary instantaneous description from $\mathbb{I}(\Pi)$ and r an arbitrary rule from R_Π . Suppose that r is associated to membrane $i \in Lab_\Pi$ if $r \in \mathcal{R}_{V, Lab_\Pi - \{0\}}^{att}$ (or to region $i \in Lab_\Pi$ if $r \in \mathcal{R}_{V, Lab_\Pi}^{ev}$).

Then, if r is applicable to membrane i (or to region i , accordingly) of I , we shortly say that r is *applicable to I* . We denote by $r(I) \in \mathbb{I}(\Pi)$ the instantaneous description of Π obtained when the rule r is applied to membrane i (or to region i , accordingly) of I (in short, we say r is *applied to I*).

The *initial instantaneous description* of Π , $I_{in, \Pi} \in \mathbb{I}(\Pi)$, consists of the membrane structure μ_Π with membrane i marked by $(u_i, v_i, x_i)_\Pi$ for all $i \in Lab_\Pi - \{0\}$ and free objects $w_{i, \Pi}$ associated to region i for all $i \in Lab_\Pi$.

A *configuration* of Π is a pair (I, t) where $I \in \mathbb{I}(\Pi)$ and $t \in \mathbb{R}$; t is called the *time* of the configuration. We denote by $\mathcal{C}(\Pi)$ the set of all configurations of Π . The *initial configuration* of Π is $C_{in, \Pi} = (I_{in, \Pi}, t_{in, \Pi})$.

Suppose that $R_\Pi = \{rule^1, rule^2, \dots, rule^m\}$ and let S be an arbitrary sequence of configurations $\langle C_0, C_1, \dots, C_j, C_{j+1}, \dots, C_h \rangle$, where $C_j = (I_j, t_j) \in \mathcal{C}(\Pi)$ for $0 \leq j \leq h$. Let $a_j = \sum_{i=1}^m p_j^i$, $0 \leq j \leq h$, where p_j^i is the product of $rate(rule^i)$ and the *mass action* combinatorial factor for $rule^i$ and I_j (see Appendix A).

The sequence S is an *evolution* of Π if

- for $j = 0$, $C_j = C_{in, \Pi}$
- for $0 \leq j \leq h - 1$, $a_j > 0$, $C_{j+1} = (r_j(I_j), t_j + dt_j)$ with r_j, dt_j as in [7]:
 - $r_j = rule^k$, $k \in \{1, \dots, m\}$ and k satisfies $\sum_{i=1}^{k-1} p_j^i < ran'_j \cdot a_j \leq \sum_{i=1}^k p_j^i$
 - $dt_j = (-1/a_j) \ln(ran''_j)$

where ran'_j, ran''_j are two random variables over the sample space $(0, 1]$, uniformly distributed.
- for $j = h$, $a_j = 0$ or $t_j \geq t_{fin, \Pi}$.

In other words, an *evolution* of Π is a sequence of configurations, starting from the initial configuration of Π , where, given the current configuration $C_j = (I_j, t_j)$, the next one $C_{j+1} = (I_{j+1}, t_{j+1})$, is obtained by applying the rule r_j to the current instantaneous description I_j and adding dt_j to the current time t_j . The rule r_j is applied as described in Section 3. Rule r_j and dt_j are obtained using the Gillespie algorithm [7] over the current instantaneous description I_j . The evolution halts when all rules have zero probability of being applied ($a_j = 0$) or when the current time is greater or equal to the specified final time.

5 Modelling and Simulation of Cellular Processes

Having established a theoretical basis, we now wish to demonstrate the quantitative behaviour of the presented model. To this end we have extended the simulator presented in [4] to produce evolutions of an arbitrary P_{pi} system. In Sections 5.2 and 5.3 we demonstrate the model and the simulator using two examples from the literature.

5.1 The Stochastic Algorithm

We use a discrete stochastic algorithm based on Gillespie’s which can more accurately represent the dynamical behaviour of small quantities of reactants, in comparison, say, to a deterministic approach based on *ordinary differential equations* [11]. Moreover, Gillespie has shown that the algorithm is fully equivalent to the chemical master equation.

The Gillespie algorithm is specifically designed to model the interaction of chemical species and imposes a restriction of a maximum of three reacting molecules. This is on the basis that the likelihood of more than three molecules colliding is vanishingly small. Hence the simulator is similarly restricted. Note that in the evolution of a P_{pi} system, the stochastic algorithm does not distinguish between floating objects and objects attached or integral to the membrane. That is, the algorithm is applied to the objects irrespective of where they are in the compartment on the assumption that the interaction between floating and attached molecules can be considered the same as between floating molecules. Our application of the Gillespie algorithm to membranes is further described in Appendix A.

5.2 Modelling a Noise-Resistant Circadian Clock

Many organisms use circadian clocks to synchronise their metabolisms to a daily rhythm, however the precise mechanisms of implementation vary from species to species. One common requirement is the need to maintain a measure of stability of timing in the face of perturbations of the system: *the clock must continue to tick and keep good time*. A general model which captures the essence of such stability, based on common elements of several real biological clocks, is presented in [16]. We choose this as an interesting, non-trivial example to model and simulate with a P_{pi} system using evolution rules alone. Moreover, we choose this example because it has been modelled in other formalisms, such as in stochastic Π calculus (see, e.g., [17], [13]).

The model is described diagrammatically in Figure 5. The system consists of two different genes (gA and gR) which produce two different proteins (pA and pR, respectively) via two different mRNA species (mA and mR, respectively). Protein pA up-regulates the transcription of its own gene and also the transcription of the gene that produces pR. The proteins are removed from the system by simple degradation to nothing (dashed lines) and by the formation of a complex AR. In this latter way the production of pR reduces the concentration of pA and

has the consequence of down-regulating pR's own production. Thus, in turn, pA is able to increase, increasing the production of pR and causing the cycle to repeat. Key elements of the stable dynamics are the rapid production of pA, by virtue of positive feedback, and the relative rate of growth of the complexation reaction.

A description of the P_{pi} system used to model the circadian clock is given in Figure 6, together with the corresponding simulator script for comparison. The alphabet, V_{clock} , is specified to contain all the reacting species. This corresponds to the **object** statement of the simulator script. The sixteen chemical reactions of Figure 5 are simply transcribed into corresponding rules mapped to reaction rates. In the simulator script they are grouped under one identifier, **clock**. The membrane structure, μ_{clock} , comprises just the *root* membrane. The root region initially contains one copy each of the two genes as free objects. These facts are reflected in the **system** statement of the simulator script, which also associates to the contents the set of rules **clock**.

The results of running the script are shown in Figure 5: the two proteins exhibit anti-phase periodicity of approximately 24 hours, as expected.

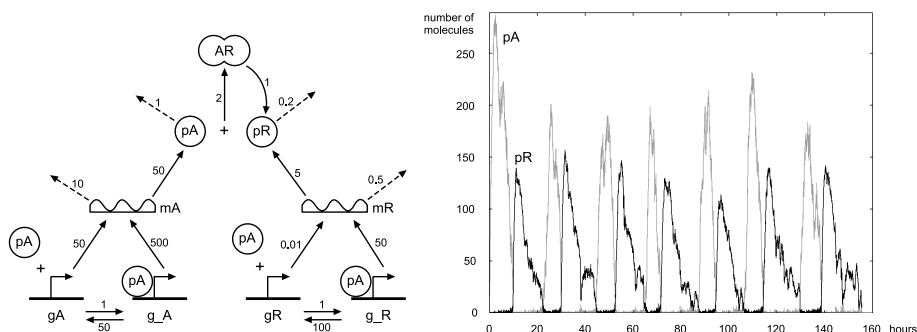


Fig. 5. Reaction scheme and simulation results of noise-resistant circadian clock of [16]

The simulator has the capability to add or subtract reactants from the simulation in runtime. We use this facility to discover the effect of switching off gR in the circadian clock by making the following addition to the **system** statement:

`-1 gR @50000, -1 g_R @50000`

These *instructions* request a subtraction from the system at time step 50000 of one gR and one g_R. Note that to switch off the gene it is necessary to remove both versions (i.e., with and without pA bound), since it is not possible to know in what state it will exist at a particular time step. Negative quantities are not allowed in the simulator, so only the existent specie will be deleted. In general, the number subtracted is the minimum of the existent quantity and the requested amount. The same syntax, without the negative sign, is used to add reactants.

The effect of switching off gR, shown in Figure 7, is to reduce the amount of pR to near zero and to thus allow pA to reach a maximum governed by its

P_{pi} system clock	Simulator script
$V_{clock} = \{gA, g_A, gR, g_R, mA, mR, pA, pR, RA\}$ $rate_{clock} =$ $\{$ $[gA \rightarrow gA \text{ } mA]_{ }^0 \mapsto 50$ $[pA \text{ } gA \rightarrow g_A]_{ }^0 \mapsto 1$ $[g_A \rightarrow g_A \text{ } mA]_{ }^0 \mapsto 500$ $[gR \rightarrow gR \text{ } mR]_{ }^0 \mapsto 0.01$ $[g_R \rightarrow g_R \text{ } mR]_{ }^0 \mapsto 50$ $[mA \rightarrow pA]_{ }^0 \mapsto 50$ $[mR \rightarrow pR]_{ }^0 \mapsto 5$ $[pA \text{ } pR \rightarrow AR]_{ }^0 \mapsto 2$ $[AR \rightarrow pR]_{ }^0 \mapsto 1$ $[pA \rightarrow \lambda]_{ }^0 \mapsto 1$ $[pR \rightarrow \lambda]_{ }^0 \mapsto 1$ $[mA \rightarrow \lambda]_{ }^0 \mapsto 10$ $[mR \rightarrow \lambda]_{ }^0 \mapsto 0.5$ $[g_R \rightarrow pA \text{ } gR]_{ }^0 \mapsto 100$ $[pA \text{ } gR \rightarrow g_R]_{ }^0 \mapsto 1$ $[g_A \rightarrow pA \text{ } gA]_{ }^0 \mapsto 50$ $\}$ $w_{0,clock} = gA \text{ } gR$ $\mu_{clock} = []_{ }^0$ $t_{in,clock} = 0$ $t_{fin,clock} = 155 \text{ hours}$	<pre> object gA,g_A,gR,g_R,mA,mR,pA,pR,RA rule clock { gA 50-> gA + mA pA+gA 1-> g_A g_A 500-> g_A + mA gR 0.01-> gR + mR g_R 50-> g_R + mR mA 50-> pA mR 5-> pR pA+pR 2-> AR AR 1-> pR pA 1-> OA pR 0.2-> OR mA 10-> OmA mR 0.5-> OmR g_R 100-> pA+gR pA+gR 1-> g_R g_A 50-> pA+gA } system 1 gA, 1 gR, clock evolve 0-150000 plot pA, pR </pre>

Fig. 6. P_{pi} system model of circadian clock of [16] with corresponding simulator script. Note the similarities between the definitions of V_{clock} and `object` and between the definitions of the elements of $rate_{clock}$ and of `rule clock`.

relative rates of production and decay. Note that a small amount of pR continues to exist long after its gene has been switched off. This is the result of a so-called *hidden* pathway from the AR complex, which decays at a much slower rate than pR (second graph of Figure 7). Although this model is a generalisation of biological circadian clocks and may not represent the behaviour of a specific example, the existence of an unexpected pathway exemplifies an important problem encountered when attempting to predict the behaviour of biological systems.

5.3 Modelling *Saccharomyces Cerevisiae* Mating Response

To demonstrate the ability of P_{pi} systems to represent compartments and membranes we model and simulate the G-protein mating response in yeast *saccharomyces cerevisiae*, based on experimental rates provided by [9]. The G-protein transduction pathway involves membrane proteins and the transport of substances between regions and is a mechanism by which organisms detect and respond to environmental signals. It is extensively studied and many pharmaceutical agents are aimed at components of the G-protein cycle in humans. The diagram in Figure 8 shows the relationships between the various reactants and regions modelled and simulated.

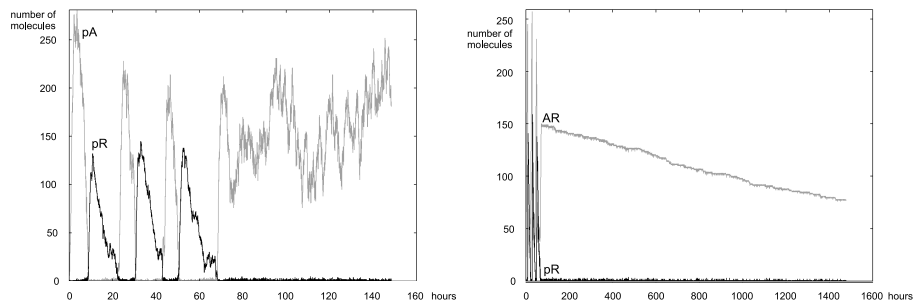


Fig. 7. Simulated effect of switching off **gA** in circadian clock of [16]

A description of the biological process is that the yeast cell receives a signal ligand (pL) which binds to a receptor pR, integral to the cell membrane. The receptor-ligand dimer then catalyses (dotted line in the diagram of Figure 8) the reaction that converts the inactive G-protein Gabg to the active GA. A competing sequence of reactions, which dominate in the absence of RL, converts GA to Gabg via Gd in combination with Gbg. The bound and unbound receptor (RL and pR, respectively) are degraded by transport into a vacuole via the cytoplasm. Figure 9 contains the P_{pi} system model and corresponding simulator script. Note that while additional quantities of the receptor pR are created in runtime, no species is *deleted* from the system; the dynamics are created by transport alone.

Figure 8 shows the results of the stochastic simulation plotted with experimental results from [16] equivalent to simulated GA. There is an apparent correspondence between the simulated and experimental data, in line with the deterministic simulation presented in the original paper. The stochastic noise evident in Figure 8 may explain why some measured points do not lie exactly on the deterministic curve, however further analysis of the original model is beyond the scope of this paper.

6 Perspectives

We have introduced a model of membrane systems (called a P_{pi} system) with objects integral to the membrane and objects attached to either side of the membrane. We have also introduced operations that can rewrite floating objects conditional on the existence of integral and attached objects and operations that facilitate the interaction of floating objects with integral and attached objects. With these we are able to model in detail many real biochemical processes occurring in the cytoplasm and in the cell membrane.

Evolutions of a P_{pi} system are obtained using an algorithm based on Gillespie [7] and in the second part of the paper we have presented a simulator which can produce evolutions of an arbitrary P_{pi} system, using syntax based on chemical equations. To demonstrate the utility of P_{pi} systems and of the simulator we

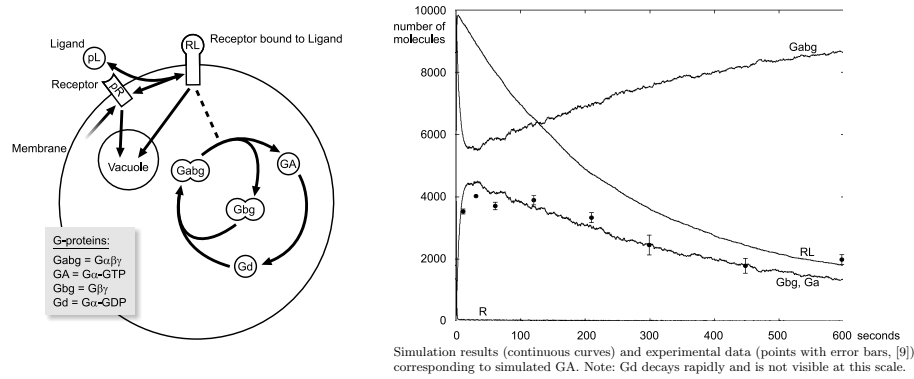


Fig. 8. Model and simulation results of *saccharomyces cerevisiae* mating response.

have modelled and simulated a circadian clock and the G-protein cycle mating response of *saccharomyces cerevisiae*. The latter makes extensive use of membrane operations.

Several different research directions are now proposed. The primary direction is the application of P_{pi} systems and of the simulator to real biological systems, with the aim of *prediction by in-silico experimentation*. Such application is likely to lead to the need for new bio-inspired features and these constitute another direction of research. The features will be implemented in the model and simulator as necessary, however it is already envisaged that operations of *fission* and *fusion* will be required to permit the modification of a membrane structure in runtime.

A further direction of research is the investigation of the theoretical properties of the model. Reachability of configurations and of markings have already been proved to be decidable for the more restricted model presented in [4] and these proofs should be extended accordingly for the model presented here. Other work in this area might include the modification of the way a P_{pi} system evolves, for example, to allow other semantics (such as that of *maximal parallel* [12]) or to use algorithms that more accurately model the behaviour of biological membranes. In this way we will be able to explore the limits of the model and perhaps discover a more useful level of abstraction.

References

1. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, 4th Ed., Garland Science, 2002, p. 593.
2. R. Brijder, M. Cavaliere, A. Riscos-Núñez, G. Rozenberg, D. Sburlan, Membrane Systems with Marked Membranes. *Electronic Notes in Theoretical Computer Science*. To appear.
3. L. Cardelli, Brane Calculi. Interactions of Biological Membranes. *Proceedings Computational Methods in System Biology 2004* (V. Danos, V. Schächter, eds.), Lecture Notes in Computer Science, 3082, Springer-Verlag, Berlin, 2005.

P_{pi} system $gprot$	Simulator script
$V_{gprot} = \{pL, pr, pR, RL, Gd, Gbg, Gabg, GA\}$ $rate_{gprot} =$ $\{$ $\quad []^1_{ pr } \rightarrow []^1_{ pR } pR \quad \mapsto 4.0$ $\quad []^1_{ pR } pL \rightarrow []^1_{ RL } \quad \mapsto 3.32e^{-18}$ $\quad []^1_{ RL } \rightarrow []^1_{ pR } pL \quad \mapsto 0.011$ $\quad []^1_{ RL } \rightarrow [RL]^1_{ } \quad \mapsto 4.1e^{-3}$ $\quad []^1_{ pR } \rightarrow [pR]^1_{ } \quad \mapsto 4.1e^{-4}$ $\quad [Gabg \rightarrow GA Gabg]^1_{ RL } \quad \mapsto 1.0e^{-5}$ $\quad [Gd Gbg \rightarrow Gabg]^1_{ } \quad \mapsto 1.0$ $\quad [GA \rightarrow Gd]^1_{ } \quad \mapsto 0.11$ $\quad []^2_{ } pR \rightarrow [pR]^2_{ } \quad \mapsto 4.1e^{-4}$ $\quad []^2_{ } RL \rightarrow [RL]^2_{ } \quad \mapsto 4.1e^{-3}$ $\quad \}$ $w_{2,gprot} = \lambda$ $(u_2, v_2, x_2)_{gprot} = (\lambda, \lambda, \lambda)$ $w_{1,gprot} = Gd^{3000} Gbg^{3000} Gabg^{7000}$ $(u_1, v_1, x_1)_{gprot} = (\lambda, pR^{10000} pr, \lambda)$ $w_{0,gprot} = pL^{6.022e17}$ $\mu_{gprot} = [[[]^2]^1]^0$ $t_{in,gprot} = 0$ $t_{fin,gprot} = 600 \text{ seconds}$	<pre> object pL,pr,pR,RL,Gd,Gbg,Gabg,GA rule g_cycle { pr 4-> pR,pr pR + pL 3.32e-18-> RL RL 0.011-> pR + pL RL 4.1e-3-> RL + pR 4.1e-4-> pR + Gabg + RL 1.0e-5-> GA, Gbg + RL Gd + Gbg 1-> Gabg GA 0.11-> Gd } rule vac_rule { + pR 4.1e-4-> pR + + RL 4.1e-3-> RL + } compartment vacuole [vac_rule] compartment cell [vacuole,3000 Gd,... ... 3000 Gbg,7000 Gabg,g_cycle : 10000 pR,pr] system cell, 6.022e17 pL evolve 0-600000 plot cell[Gd,Gbg,Gabg,GA: pR,RL] </pre>

Fig. 9. P_{pi} system model of G-protein cycle and corresponding simulator script.

4. M. Cavaliere, S. Sedwards, Membrane Systems with Peripheral Proteins: Transport and Evolution. *Electronic Notes in Theoretical Computer Science*. To appear.
5. G. Ciobanu, Gh. Păun, M.J. Pérez-Jiménez, eds., *Applications of Membrane Computing*. Springer-Verlag, Berlin, 2006.
6. J. Dassow, Gh. Păun, *Regulated Rewriting in Formal Language Theory*. Springer-Verlag, Berlin, 1989.
7. D. T. Gillespie, A General Method for Numerically Simulating the Stochastic Time Evolution of Coupled Chemical Reactions. *Journal of Computational Physics*, 22, 1976.
8. J.E. Hopcroft, J.D. Ullman, *Introduction to Automata Theory, Languages, and Computation*. Addison-Wesley, 1979.
9. T.-M. Yi, H. Kitano, M. I. Simon, A quantitative characterization of the yeast heterotrimeric G protein cycle. *Proceedings of the National Academy of Science*, 100, 19, 2003.
10. M. J. Pérez-Jiménez, F. J. Romero-Campero, Modelling EGFR signalling network using continuous membrane systems. *Proceedings of the Third Workshop on Computational Method in Systems Biology*, Edinburgh, 2005.
11. H. McAdams, A. Arkin, Stochastic mechanisms in gene expression. *Proceedings of the National Academy of Science*, 94, 1997.
12. Gh. Păun, G. Rozenberg, A Guide to Membrane Computing. *Theoretical Computer Science*, 287-1, 2002.
13. A. Regev, W. Silverman, N. Barkai, E. Shapiro, Computer Simulation of Biomolecular Processes using Stochastic Process Algebra. *Poster at 8th International Conference on Intelligent Systems for Molecular Biology, ISMB*, 2000.

14. G. Rozenberg, A. Salomaa, eds., *Handbook of Formal Languages*. Springer-Verlag, Berlin, 1997.
15. A. Salomaa, *Formal Languages*. Academic Press, New York, 1973.
16. M. G. Vilar, H. Y. Kueh, N. Barkai, S. Leibler, Mechanisms of noise-resistance in genetic oscillators. *Proceedings of the National Academy of Science*, 99, 9, 2002.
17. <http://www.wisdom.weizmann.ac.il/biospi/>
18. <http://psystems.disco.unimib.it>
19. <http://www.msr-unitn.unitn.it/downloads.php>

Appendices

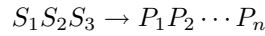
A The Gillespie Algorithm Applied To Membranes

The Gillespie algorithm is an exact stochastic simulation of a ‘spatially homogeneous mixture of molecular species which inter-react through a specified set of coupled chemical reaction channels’ [7]. It is unclear whether a biological cell contains a *spatially homogeneous mixture of molecular species* and less clear still whether integral and peripheral proteins can be described in this way, however for the purposes of the P_{pi} system model we choose to regard them as such. Hence we treat the objects attached to the membrane as homogeneously mixed with the floating objects, however objects of the same type (i.e. having the same name) but existing in different regions are considered to be of different types in the stochastic algorithm.

The mass action combinatorial factors of the Gillespie algorithm, defined by equations (14a...g) in [7], are calculated over the set of chemical reactions given in equations (2a...g) of [7], using standard stoichiometric syntax of the general form



S_1, S_2 and S_3 are the reactants and P_1, \dots, P_n are the products of the reaction. Since the order of the reactants and products is unimportant they may be represented as multisets $S_1 S_2 S_3$ and $P_1 P_2 \dots P_n$, respectively, over the set of objects V . Hence a chemical reaction may be expressed using the notation

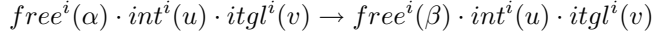


In the definition of the evolution of a P_{pi} system, the mass action combinatorial factor is calculated using equations (14a...g)[7] after transforming the membrane and evolution rules into chemical reactions and the objects of the current instantaneous description, using the following procedure.

Let $V_i = \{a_i | a \in V\}$, $V_{i,int} = \{a_{i,int} | a \in V\}$, $V_{i,itgl} = \{a_{i,itgl} | a \in V\}$ and $V_{i,out} = \{a_{i,out} | a \in V\}$. We then define morphisms $free^i : V \rightarrow V_i$, $int^i : V \rightarrow V_{i,int}$, $itgl^i : V \rightarrow V_{i,itgl}$ and $out^i : V \rightarrow V_{i,out}$ such that $free^i(a) = a_i$, $int^i(a) = a_{i,int}$, $itgl^i(a) = a_{i,itgl}$ and $out^i(a) = a_{i,out}$ for $a \in V$. Hence we map an evolution rule of the type

$$[\alpha \rightarrow \beta]_{u|v}^i$$

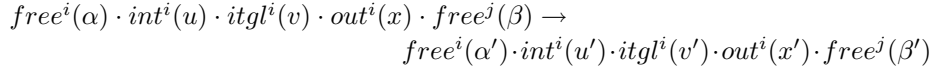
with $u, v, \alpha, \beta \in V^*$ and $i \in Lab$, to the chemical reaction



We map membrane rules, generally described by

$$[\alpha]_{u|v|x}^i \beta \rightarrow [\alpha']_{u'|v'|x'}^i \beta'$$

with $u, v, x, \alpha, \beta, u', v', x', \alpha', \beta' \in V^*$ and $i \in Lab$, to the chemical equation



where $j \in Lab$ is the marking of the membrane surrounding the region enclosing membrane i .

The objects of the current instantaneous description are similarly transformed, using the morphisms defined above, in order to correspond with the transformed membrane and evolution rules.

B The Simulator Syntax

The simulator syntax aims to be an intuitive interpretation of the P_{pi} system model. An example of a simple simulator script is shown below, together with its P_{pi} system counterpart.

Simulator script

// Lotka reactions

object X,Y1,Y2,Z

rule r1 X + Y1 0.0002-> 2Y1 + X

rule r2 Y1 + Y2 0.01-> 2Y2

rule r3 Y2 10-> Z

system 100000 X,1000 Y1,1000 Y2,r1,r2,r3

evolve 0-500000000

plot Y1,Y2

P_{pi} system *lotka*

$V_{lotka} = \{X, Y1, Y2, Z\}$

$rate_{lotka} = \{$

$[XY1 \rightarrow Y1Y1X]_{||}^0 \mapsto 0.0002$

$[Y1Y2 \rightarrow Y2Y2]_{||}^0 \mapsto 0.01$

$[Y2 \rightarrow Z]_{||}^0 \mapsto 10 \}$

$w_{0,lotka} = X^{100000}Y1^{1000}Y2^{1000}$

$\mu_{lotka} = []^0$

$t_{in,lotka} = 0$

With the exception of comments, which may appear anywhere in the script, the following subsections describe the statements in the order they must appear.

B.1 Comments

Comments begin with a double forward slash (//) and include all subsequent text on a single line. They may appear anywhere in the script.

B.2 Object definition

The reacting objects are defined in one or more statements beginning with the keyword **object** followed by a comma separated list of unique reactant names. The names are case-sensitive and must start with a letter but may include numbers and the underscore character (.). This corresponds to defining the alphabet, V , of the P_{pi} system.

B.3 Rules

The reaction rules are defined using rule definitions comprising the keyword **rule** followed by a unique name and the rewriting rule itself. These correspond to the attach / de-attach and evolution rules of the P_{pi} system model. Note, however, that simulator rules are user-defined types which may be instantiated in more than one region. The value preceding the implication symbol (\rightarrow) is the average reaction rate and corresponds to an element of the range of the mapping *rate* given in Definition 1. In the simulator it is also possible to define a reaction rate as the product of a constant and the rate of a previously defined rule using the name of the previous rule in the following way:

```
rule r2 Y1 + Y2 50 r1-> 2Y2
```

This has the meaning that rule **r2** has a rate 50 times that of **r1**. In addition, in the simulator it is possible to define a group of rules using a single identifier and braces. E.g.,

```
rule lotka {  
  X + Y1 0.0002-> 2Y1 + X  
  Y1 + Y2 0.01-> 2Y2  
  Y2 10-> Z }  
}
```

To include membrane operations the basic simulator rule syntax is also extended with the $||$ symbol, so

```
rule r4 X + |Y2| 0.1-> |X,Y2|
```

means that if one **X** exists within the compartment and one **Y2** exists integral to the membrane, then the **X** will be added to the integral marking of the membrane. The P_{pi} system equivalent is the following *attach_{in}* rule:

$$[X]_{|Y2|} \rightarrow []_{|XY2|}$$

To represent an *attach_{out}* rule in the simulator the following syntax is used:

```
rule r4 |Y2| + X 0.1-> |X,Y2|
```

Here the **X** appears to the right of the $||$ symbol, meaning that it must exist in the region surrounding the membrane for the rule to be applied. Hence the $+$ is non-commutative: the left side represents the internal part of the compartment and the right hand side is external. Similarly, the left side of the $||$ symbol represents the internal surface and the right hand side represents the external surface of the membrane. Objects integral to the membrane are listed between the vertical bars.

B.4 Compartments

Compartments may be defined using the keyword `compartment` followed by a unique name and a list of contents and rules, all enclosed by square brackets. For example,

```
compartment c1 [100 X, 100 Y1, r1, r2]
```

instantiates a compartment having the label `c1` containing 100 `X`, 100 `Y1` and rules `r1` and `r2`. In a P_{pi} system such a compartment would have a P_{pi} system initial instantaneous description

$$[X^{100}Y1^{100}]^1$$

Note that a P_{pi} system requires a numerical membrane label and that any rules associated to the region or membrane must be defined separately.

Compartments may contain other pre-defined compartments, so the following simulator statement

```
compartment c2 [100 Y2, c1]
```

corresponds to the P_{pi} system initial instantaneous description

$$[Y2^{100}[X^{100}Y1^{100}]^1]^2$$

Membrane markings in the simulator are added to compartment definitions using the symbol `||`, to the right of and separated from the floating contents by a colon. E.g.,

```
compartment c3 [100 X, c2 : 10 Y2||10 Y1]
```

has the meaning that the compartment `c3` contains compartment `c2`, 100 `X`, and the membrane surrounding `c3` has 10 `Y2` attached to its inner surface and 10 `Y1` attached to its outer surface. This corresponds to the P_{pi} system initial instantaneous description

$$[X^{100}[Y2^{100}[X^{100}Y1^{100}]^1]^2]_{Y2^{10}|Y1^{10}}^3$$

B.5 System statement

The system is instantiated using the keyword `system` followed by a list of constituents. This statement corresponds to the definition of $u_0 \dots u_n$, $v_0 \dots v_n$, $w_0 \dots w_n$, $x_0 \dots x_n$ and μ of the P_{pi} system.

It is also possible to add or subtract reactants from the simulation in runtime using the following syntax in the `system` statement:

```
-10 X @50000, 10 Y1 @50000
```

These *instructions* request a subtraction of ten `X` from the system and an addition of ten `Y1` to the system at time step 50000. Negative quantities are not allowed in the simulator, so if a subtraction requests a greater amount than exists, only the existing amount will be deleted.

B.6 Evolve statement

The simulator then requires a directive to specify the total number of evolution steps to perform and also the number of the evolution step at which to start recording data. This is achieved using the keyword `evolve` followed by the minimum and maximum evolution steps to record. Note that the minimum evolution step does not correspond to t_{in} of the P_{pi} system, since the simulation always starts from the 0^{th} step. By convention, the simulator sets the initial time of the simulation to 0, hence $t_{in} = 0$ for all simulations. Note too that in contrast to a P_{pi} system evolution, which halts when all rules have zero probability of being applied, the simulation may halt before this event if the user-defined maximum number of evolution steps is reached.

B.7 Plot statement

To specify which objects are to be observed during the evolution the `plot` keyword is used followed by a list of reactants. To plot the contents of a specific compartment the `plot` statement uses syntax similar to that used in the compartment definition. E.g.,

```
plot X, c3[X,Y1 : Y1|Y2|]
```

plots the number of free-floating `X` in the environment and the specified contents of compartment `c3` and its membrane.