Multi-level modelling via stochastic multi-level multiset rewriting

MULTI-LEVEL MODELLING VIA STOCHASTIC MULTI-LEVEL MULTISET REWRITING

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Abstract. We present a simple stochastic rule-based approach to multilevel modelling for computational systems biology. Populations are modelled using multilevel multisets; these contain both species and agents, with the latter possibly containing further such multisets. Rules are pairs of such multisets, but now allowing variables to occur (as well as species and agents), together with an associated stochastic rate.

We give two illustrative examples. The first is an extracellular model of virus infection, coupled with an intracellular model of viral reproduction; this model can demonstrate successive waves of infection. The second is a model of cell division in which a repressor protein is diluted in successive generations, when repression no longer occurs.

The multilevel multiset approach can also be seen in terms of stochastic term rewriting for the theory of a commutative monoid, equipped with extra constants (for the species) and unary operations (for the agents). We further discuss the relationship of this approach with two others: Krivine et al.'s stochastic bigraphs, restricted to Milner's place graphs, and Coppo et al.'s Stochastic Calculus of Wrapped Compartments. These various relationships provide evidence for the fundamental nature of the approach.

1. Introduction

We present a simple rule-based formalism for multilevel modelling of biological processes. We are thinking particularly of modelling both inter- and extra-cellular events, for example signalling and cell division. To that end we present a stochastic rule-based formalism of multilevel multisets. These are, essentially, nested multisets; more exactly they are finite multisets whose elements are either species names or pairs of an agent name, e.g., Cell, and a multilevel multiset, with this multiset nesting carried on only to finite depth. The agents serve to indicate a lower level and its kind. The rules use similar multisets, additionally allowing variables.

Multilevel modelling involves multiscale modelling and much work has been done on both. For reviews, see [MFK09, BBP09, GS09, CRT10, Nob02]; some particular systems are BioCharts [KLH10, HK10], Simmune [MXA06], and CompuCell3D [CAS07]. Most of these systems have specific modelling scenarios in mind. The computer science community has provided general purpose formalisms, often taking ideas from process calculus. The first of these is Regev et al.'s BioAmbients [RPS04], which has features of the stochastic pi-calculus [PR01] and constructs that enable the movement of agents into and out of

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other agents. Another is Cardelli's Brane Calculi [Car04], which has, essentially, our multilevel multisets in algebraic form together with other structure, including actions, thereby distinguishing it from rule-based approaches.

There are also rule-based formalisms, such as Mjolsness and Yosiphon's Dynamical Grammars [MY06], Milner's bigraphs [Mil09], adapted to biological ends in [KMT08], and the Stochastic Calculus of Wrapped Compartments of Coppo et al. [CD10a, CD10b], a descendent of the Stochastic Calculus of Looping Sequences [BCM08, BMM08]. Păun introduced dynamic compartments into membrane computing, which can also be considered a rule-based formalism: see, e.g., [Pau01, Pau08, Fri09], and see also [SMC08] for work on stochastic dynamic compartments. The present work is closely related, although, as so far formulated, the range of rules in membrane computing seems less varied. Bitonal membrane systems [Car08] are in the brane calculi family, but stripped of actions and instead equipped with rules; they can therefore be seen as particular rule-based systems, more or less of the kind considered here.

A standard formalism for reactions is multiset rewriting over a set of constants standing for various species. If each rule is given a rate, one obtains stochastic multiset rewriting, which is equivalent to stochastic Petri nets. All this is well-known, as are the uses of multiset rewriting and Petri nets for modelling biological systems. Here, as indicated above, we extend these ideas in just one way: we we add unary function symbols to the rewriting formalism: that single extension enables one to do multilevel modelling.

Here is an example stochastic multiset rewriting rule:

$$\text{Glucose}, \text{ATP} \xrightarrow{k_1} \text{Gluc-6-P}, \text{ADP}$$

It models a reaction from the glycolysis pathway with stochastic rate k_1. Suppose we further wish to indicate that the reaction only takes place inside cells. Then we introduce a unary function symbol Cell and write the same rule "one level down":

$$\text{Cell}(\text{Glucose}, \text{ATP}, x) \xrightarrow{k_1} \text{Cell}(\text{Gluc-6-P}, \text{ADP}, x)$$

The variable x is used to indicate that, other than the molecule of glucose and the molecule of ATP, the contents of the cell remain unchanged. We can also write rules that mix levels. For example, a rule of the form

$$\text{H}_2\text{O}, \text{Cell}(x) \xrightarrow{k_2} \text{Cell}(\text{H}_2\text{O}, x)$$

might be used for the passive transport of water into cells.

Multiset rewriting with species can be viewed algebraically as rewriting modulo an associative-commutative (AC) operation with a zero and with additional constants [The03]. Similarly our multilevel multiset rewriting can be seen as rewriting modulo an AC operation with a zero, with additional constants, and with additional unary function symbols. As stochasticity is also present, we are led to consider *stochastic* term rewriting. Curiously, although very natural, the idea of stochastic term rewriting seems novel; there is however, work on probabilistic term rewriting [BK02, BH03, AMS06].

We give a more detailed comparison of our formalism with Krivine et al.'s and Coppo et al.'s in Section 5 below; in particular it corresponds to "half" of Milner's bigraphs,

his place graphs. There is no doubt that the three formalisms are very closely related. We would argue that ours is particularly simple to understand, and easy and natural to use when modelling mutlilevel biological systems. Furthermore, by working within term rewriting, with its algebraic setting, one employs a very standard approach; this contrasts with other rule-based approaches which, while perfectly sound, are, perhaps, somewhat ad hoc. We expect the term rewriting approach to provide a sound basis for further extension; this point is discussed further in the concluding Section 6. Overall, the fact that all three approaches are very much the same encourages us to think that multilevel multisets provide a fundamental structure for multilevel rule-based modelling.

We begin, in Section 2, with an illustrative example of viral infection and reproduction. The model demonstrates a second wave of infection; it seems to be the first multilevel model to do so. Spicher et al [SMC08] have also given a stochastic viral model, a stochastic dynamical P systems model of the Semliki viral life cycle. We have implemented our rule-based formalism using a version of the standard Gillespie direct method [Gil77], and we give illustrative runs of our examples. We present our stochastic multilevel multiset rewriting formalism in Section 3. Populations are, as we have seen, modelled as multilevel multisets. Each rule has a given base rate, and the rate at which it applies in a given population is the product of the base rate and its activity, that is, the number of ways in which it can match the population.

We present another example in Section 4, again giving illustrative runs; this example was inspired by [RYA05], and features cell division. Next, we give the comparisons with the previous work of Krivine et al., and Coppo et al. in Section 5; we also discuss the algebraic formulation of stochastic multilevel multiset rewriting there. Finally, possibilities for future work are discussed in Section 6.

2. An Example: Viral Infection

We consider a simple model of viral infection, taken from [SYS02, HRY05]. There are two levels: intracellular and extracellular. A simple intracellular model of infected cells is given in [SYS02], and this is combined with an extracellular model of viral infection in [HRY05]. Intracellularly, infected cells can incorporate viral protein into their genome, and then produce viral structural protein and genomic viral nucleic acid, both of which may degrade. Extracellularly, viruses can invade uninfected cells, and infected cells can produce viruses, from viral structural protein and genomic viral nucleic acid, or die.

The intracellular part of the model involves the following species:

gen	genomic viral nucleic acid
str	viral structural protein
tem	template viral nucleic acid
V_1, V_2	viral enzymes

These take part in the following reactions, modelled in the standard way as stochastic multiset rewriting rules:

$$\begin{array}{lll} \mathrm{gen}, \mathrm{V}_1 & \xrightarrow{k_1} & \mathrm{tem}, \mathrm{V}_1 \\ \mathrm{tem}, \mathrm{V}_2 & \xrightarrow{k_2} & \mathrm{str}, \mathrm{tem}, \mathrm{V}_2 \\ \mathrm{tem} & \xrightarrow{k_3} & \mathrm{gen}, \mathrm{tem} \\ \mathrm{str} & \xrightarrow{k_4} & \\ \mathrm{gen} & \xrightarrow{k_{10}} & \end{array}$$

Turning to the extracellular part of the model, we introduce two new species, Virus and Cell, again modelled by constants, together with an "infected cell agent" ICell, modelled by a unary function symbol. (We may consider uninfected cells as species as the only internal activity we are modelling is that of infected cells which are rather modelled using the agent ICell.)

The rules are as follows:

$$\begin{array}{lll} \mathrm{Virus}, \mathrm{Cell} & \xrightarrow{k_6} & \mathrm{ICell}(\mathrm{gen}, 80\mathrm{V}_1, 40\mathrm{V}_2) \\ \mathrm{ICell}(\mathrm{gen}, \mathrm{str}, x) & \xrightarrow{k_5} & \mathrm{Virus}, \mathrm{ICell}(x) \\ \mathrm{Virus} & \xrightarrow{k_7} & \\ \mathrm{ICell}(x) & \xrightarrow{k_8} & \\ \mathrm{Cell} & \xrightarrow{k_9} & \end{array}$$

The first rule describes the infection of previously uninfected cells; note that there is no reinfection of infected cells in this model. If we wished to allow infected cells to be further infected, then, instead of working with a constant for uninfected cells and a unary function symbol for infected ones, we would simply work with a unary function symbol for cells — uninfected or not.

The second rule describes viral production; it can be read as saying that, given an infected cell whose population contains one molecule each of gen and str, and the rest of whose population is x, then a virus is produced and the population of the infected cell becomes x. The last three rules concern viral and cell death.

The intracellular reactions can only happen inside (multilevel multisets representing) infected cells, as it is only there that the relevant (sub)-populations will occur. Of course this can only be seen from the model as a whole. If we wished to make the matter explicit, then we could replace the intracellular part of the model by rules involving ICell. For example, the first rule would be replaced by the following rule:

$$\mathrm{ICell}(\mathrm{gen}, \mathrm{V}_1, x) \xrightarrow{k_1} \mathrm{ICell}(\mathrm{tem}, \mathrm{V}_1, x)$$

We illustrate the model by showing the results of a few example simulations of this system. Each run shows the number of cells and of the different molecules, plotted against

k_1	$3.125\ 10^{-4}$	k_6	5
k_2	25	k_7	0.08
k_3	1	k_8	0.005
k_4	1.99	k_9	0
k_5	$7.5\ 10^{-6}$	k_{10}	0.25

FIGURE 1. The stochastic rates used for the simulation

the time in days. Figure 1 gives the stochastic rates, in day^{-1}; they are taken from the original papers.

We first simulate the model with an initial population of one cell and one virus. The virus infects the cell and the mechanism to produce more virus is started inside the cell. The result of one such run is shown in Figure 2. In this simulation, we set k_9 to 0, in order to prevent the infected cell from dying before the end of the simulation.

As we simulate a stochastic system, different runs will give different results. For example, the delay before the first new virus is produced usually varies from 30 to 100 days. This simulation is similar to those obtained in [SYS02].

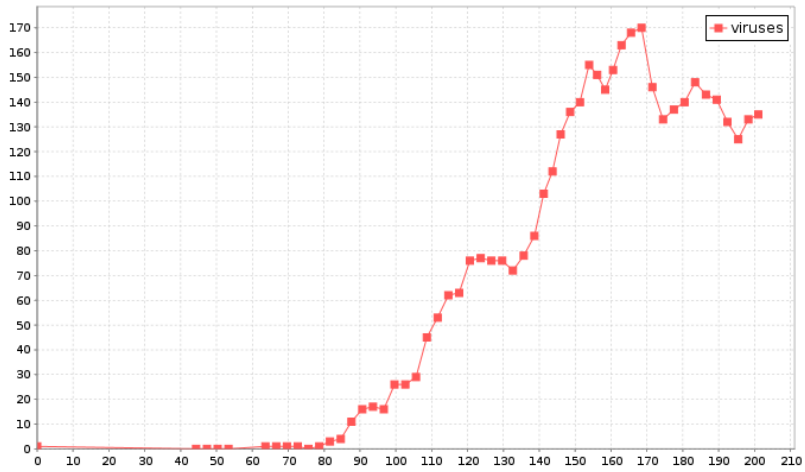
Next, we simulate an infection of many cells by many viruses. Figure 3 shows the result of the infection of 100 cells by 200 viruses. This simulation is similar to the problem studied in [HRY05]. However there, in order to be able to construct differential equations, the species in the different cells were averaged. Here, benefiting from multilevel multiset rewriting, we compute an exact stochastic simulation of every cell.

But the real benefit from this framework comes with the simulation of the infection of many cells with few viruses. In this situation, the averaging used in [HRY05] is no longer valid: 10% of cells being infected is different from each cell being 10% infected, which does not make much sense. In particular it is not clear how one would adapt the framework to simulate two or more rounds of viral infection. Figure 4 shows the result of the infection of 100 cells by 5 viruses. In particular, this simulation shows a second wave of infection starting after 125 days (this delay varies stochastically from simulation to simulation), when enough viral proteins have been made inside the cells infected during the first wave.

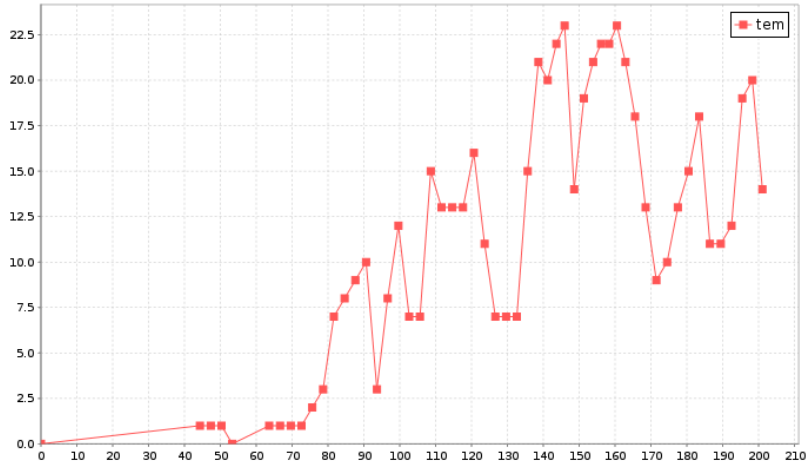
3. Stochastic Multilevel Multiset Rewriting

We explain successively: multilevel multiset terms, which we use to model population states; rules and their application, which we use to model system transformations such as reactions, transport and cell creation and division; and the activity of rules, which together with their application, determines a stochastic rate matrix. Our implementation samples a run from the corresponding stochastic process.

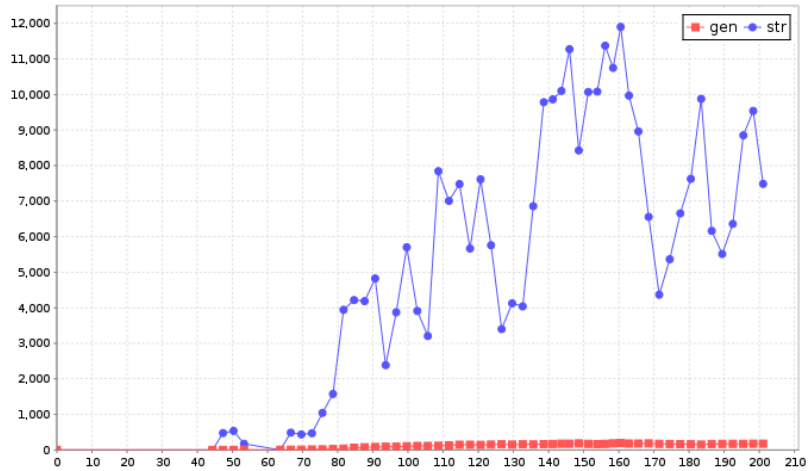
We need some notation and conventions for multisets. We may identify objects with their corresponding singleton multisets; we use possibly empty, lists $M_0, \ldots, M_{n-1}$ of multisets to denote their multiset sum; and we write nM (where $n \in \mathbb{N}$) for the n-fold multiset sum



(A) Virus

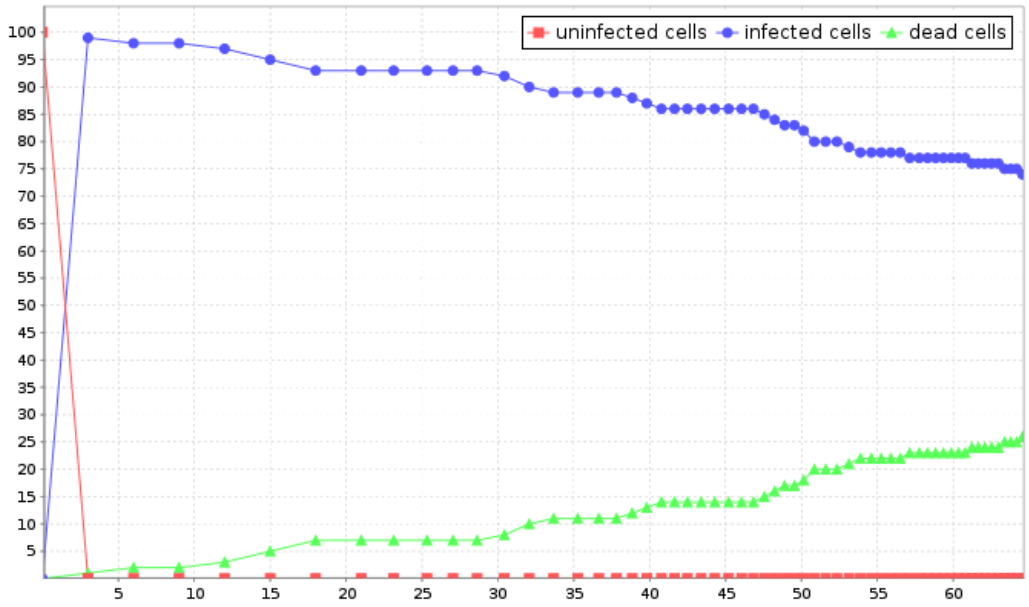


(B) tem

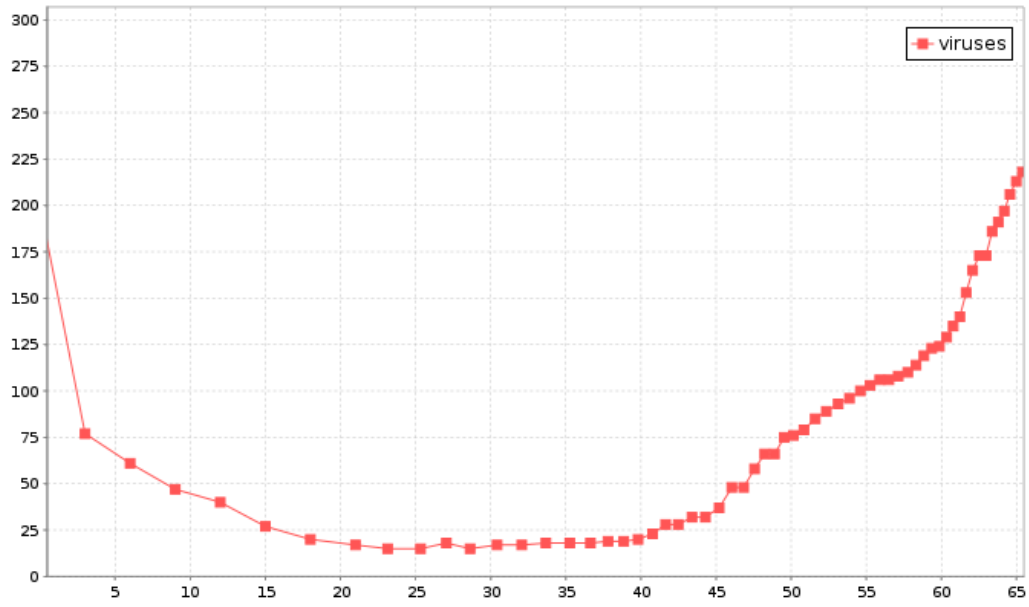


(C) str and a low level of gen

FIGURE 2. Simulation of the infection of one cell by one virus

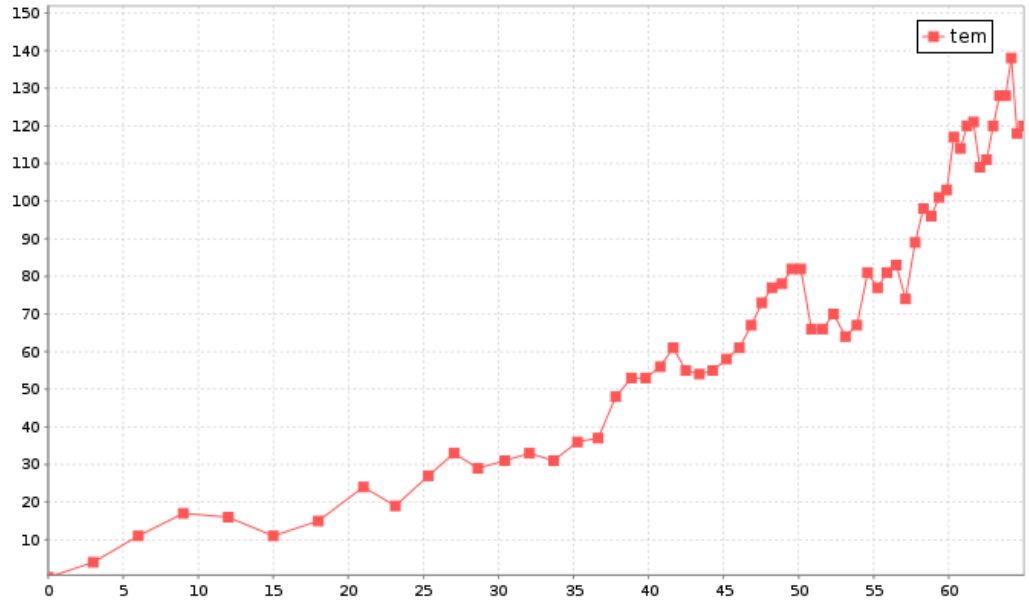


(A) uninfected, infected and dead cells

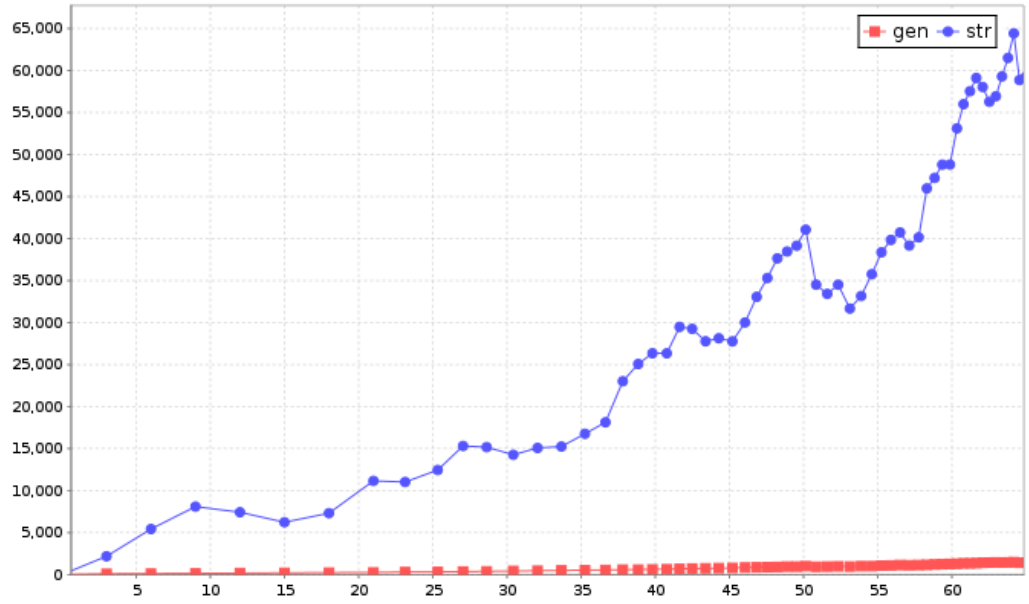


(B) Virus

FIGURE 3. Simulation of the infection of 100 cells by 200 viruses

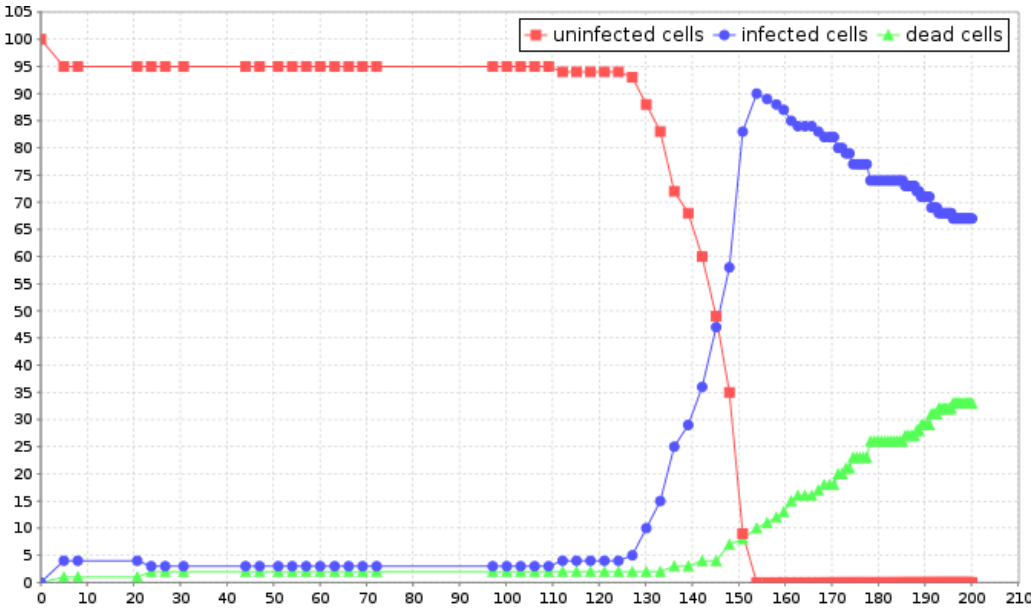


(C) tem

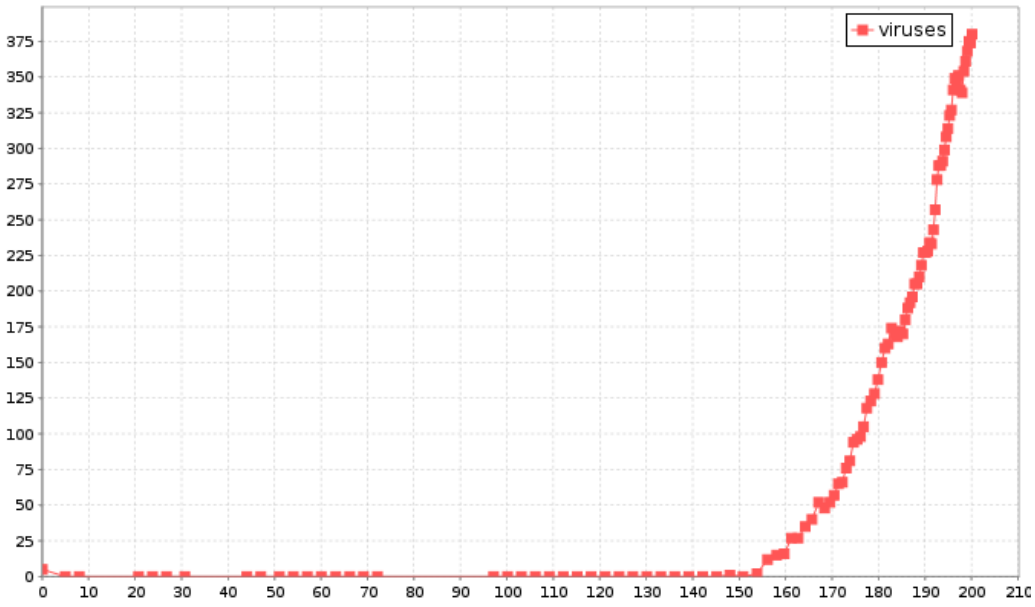


(D) str and a low level of gen

FIGURE 3. Simulation of the infection of 100 cells by 200 viruses – Continued

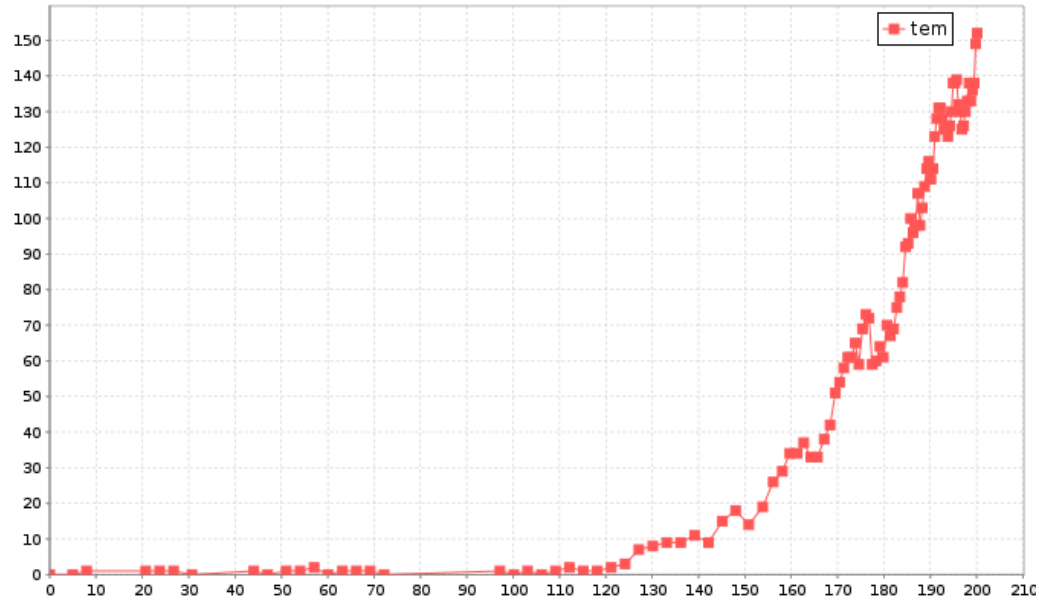


(A) uninfected, infected and dead cells

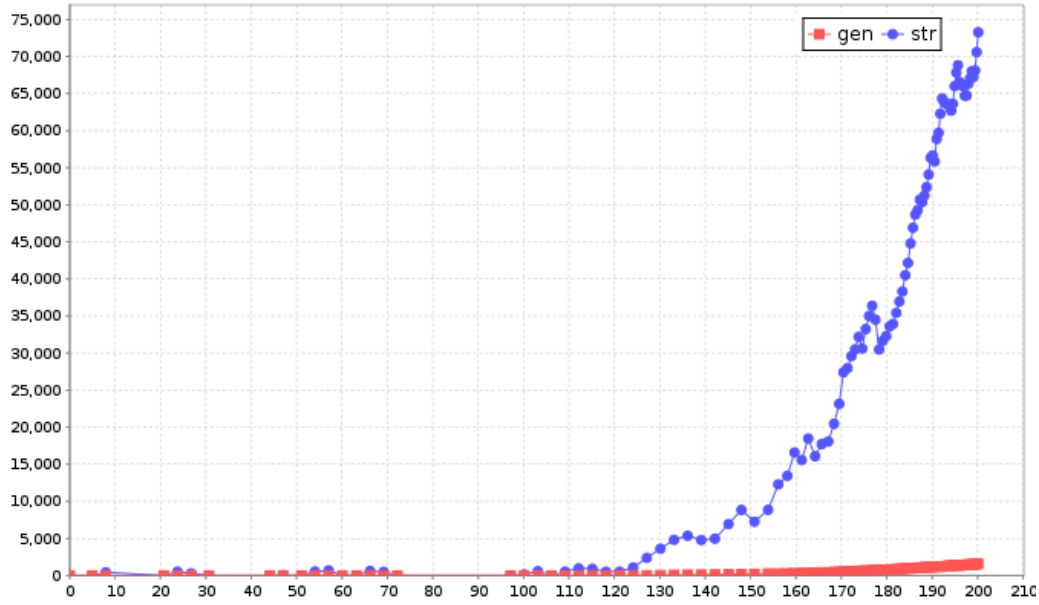


(B) Virus

FIGURE 4. Simulation of the infection of 100 cells by 5 viruses



(c) tem



(d) str and a low level of gen

FIGURE 4. Simulation of the infection of 100 cells by 5 viruses – Continued

of a multiset M with itself. We also use standard notation such as 0, $M + N$, or $\sum_{i=0}^{n-1} M_i$ for various sums of multisets.

Beginning with states, we assume given two disjoint sets, Spec of *species* and Agent of *agents* (a more neutral terminology, emphasizing the algebraic point of view, would be *constants* and *unary function symbols*). We then define *multilevel multisets* and *atomic multilevel multisets* as follows, where we further assume available a countably infinite set of variables (disjoint from Spec and Agent):

- Every finite multiset $a_0, \ldots, a_{n-1}$ ($n \geq 0$) of atomic multilevel multisets is a multilevel multiset.
- Every S in Spec is an atomic multilevel multiset.
- Every variable x is an atomic multilevel multiset.
- If t is a multilevel multiset and $A \in$ Agent then $A(t)$ is an atomic multilevel multiset.

It is convenient to refer to multilevel multisets (respectively atomic multilevel multisets) simply as *terms* (respectively *atomic terms*). A term, or atomic term, is *ground* if it contains no variables. Ground terms are used to model populations. For example:

$$\mathrm{Virus}, 20\mathrm{Cell}, \mathrm{ICell}(\mathrm{gen}, 80\mathrm{V}_1, 40\mathrm{V}_2), \mathrm{ICell}(\mathrm{gen}, \mathrm{tem}, \mathrm{str}, 80\mathrm{V}_1, 40\mathrm{V}_2)$$

models a population consisting of one virus, twenty uninfected cells, a cell which has (perhaps) just been infected and an infected cell which is ready to produce a virus.

A term of the form $A(t)$ is said to be an *agent atomic term (with agent A)*. We define the *height* of terms and atomic terms by setting:

$$|a_0, \ldots, a_{n-1}| = \max_{i=0}^{n-1} |a_i|$$

$$|S| = |x| = 0$$

and

$$|A(t)| = 1 + |t|$$

The *subterm* relation between terms is defined to be the least reflexive relation between them such that if t is a subterm of t' then it is a subterm of both $A(t')$ and $t' + t''$. The *wide subterm* relation between terms is defined to be the least reflexive relation between them such that if t is a wide subterm of t' then it is a wide subterm of $A(t')$. Both relations are easily seen to be partial orders. As an example S, x is a wide subterm of $A(S, x)$, but S is only a subterm of it.

Rules, ranged over by R, are pairs of terms l, r, together with an associated stochastic rate $k \in \mathbb{R}$, and are written:

$$l \xrightarrow{k} r$$

The height of such a rule is the maximum of the heights of l and r. For example the kind of rules used above for modelling reactions between species have height 0, with both sides ground.

It is interesting to consider the various forms such rules may take when used to model cellular behaviour. Here are some possible rules of height 1:

Transport:

$$M, A(N, x) \xrightarrow{k} M', A(N', x)$$

Creation:

$$M \xrightarrow{k} M', A(N', x)$$

Death:

$$M, A(N, x) \xrightarrow{k} M', N'$$

where M, N, etc are multisets of species. The names indicate their possible uses for modelling. One may ask if these provide sufficient possibilities for modelling extra- and intracellular behaviour, at least to a first approximation. In that respect the following, taken from [ASC08], and slightly rewritten, is germane:

> We have constructed the generic base of GemCell by appreciating the fact that any cell, in response to its surroundings, carries out only five types of behavior:
> - Export (secretion of molecules, electricity, etc.),
> - Import (receiving signals, metabolites, phagocytosis, etc.),
> - Death,
> - Movement (including shape change and adherence), and
> - Replication.

The transport rule accounts for export and import, and the death rule for death. Movement and replication raise important issues, and point to the need for, and possibilities of, a further development of our formalism. For movement we could try rules of the following form:

$$M, A(N, x) \xrightarrow{k} M', B(N', x)$$

where A and B are cells in two given different places, or of given different shapes. However this is an impoverished notion of place and shape. For adherence we might try:

$$M, A(N_1, x), B(N_2, y) \xrightarrow{k} M', C(N', x, y)$$

where C models A and B adhering to each other. Adherence is analogous to complex formation or binding, but at a higher level; as before, this is an impoverished notion.

As regards replication, we might consider the following kind of rule:

$$M, A(N, x, y) \xrightarrow{k} A(N', x), A(N'', y) \tag{1}$$

But there is a puzzle here, as the left-hand side is non-linear, and so there is a natural question as to how to interpret the stochastic rate: does each match of the left-hand side have the same rate, or is the rate somehow parcelled out among the possible matches? In the example below we follow [KMT08] and avoid the problem, preferring instead to explicitly model the division in two of the contents of the replicating cell.

There are several natural conditions one can place on rules, and, as alluded to in [KMT08], it is interesting to discuss which are natural when modelling biological systems, and which are not. The first such condition is:

No creation: $\mathrm{Var}(r) \subseteq \mathrm{Var}(l)$

with

This is standard in term rewriting; it is also required from a biological point-of-view, for where would the value of the "new" variable come from? For the others we first define some possible conditions on a term t.

Uniqueness: Any variable occurs at most once in t.
Unicity: t has no subterm containing two variable occurrences.
Generality: Every wide subterm of t contains a variable.

The last two conditions are equivalent to the condition that every wide subterm of t contains exactly one variable. The unicity condition means that not only cannot two distinct variables occur in the subterm, but also that any variable occurring in it can have multiplicity at most 1.

We say that a rule $l \xrightarrow{k} r$ satisfies the *no equality*, *no splitting*, or *no emptiness* condition according as, respectively, l satisfies the first, second or third of the above conditions. All three have a linearity flavour, and are all natural from a biological point of view. For the first, surely no step of a modelled biological process can depend on two subpopulations being identical? The third states that no step of a modelled biological process can depend on a population being exactly specified (for example, that there are *exactly* so many molecules rather than at *least* so many molecules). The "no splitting" requirement is less clearly unnatural biologically; the questions it presents in the case of replication were discussed above.

There are corresponding "dual" rule conditions. We say that a rule $l \xrightarrow{k} r$ satisfies the *no vanishing*, *no duplication*, *no merging*, or *no complete prescription* condition according as, respectively, $\mathrm{Var}(l) \subseteq \mathrm{Var}(r)$, or r satisfies the first, second or third of the above conditions on a term.

The "no duplication" condition, seems reasonable as a biological process that exactly duplicates a population seems unlikely. However, in contrast to the corresponding possible conditions on the left-hand side of a rule, there does not seem to be any strong reason from a biological point of view to impose any of the other conditions. For the rest, the first, cell death certainly does result in vanishing: it is at least natural not to be forced to model every detail of degradation. As regards the "no merging" condition, it is surely common for the contents of two agents to merge; equally one may wish to completely prescribe the initial modelled part of the population of a cell, as was done, for example, in the above example of infected cells.

Given this discussion, from now on we impose all of the first set of conditions on our rules but only the "no duplication" condition of the second set. The rules in our examples and discussions obey all these conditions except that they do not have a top level variable. However this is only for the sake of presentation, and every such rule written as $l \xrightarrow{k} r$ should be regarded as being in the form $l + x \xrightarrow{k} r + x$, where x is some canonically chosen variable not occurring in l or r.

We next turn to matching multiset terms against each other, more precisely to finding the multiset of matches and their multiplicities. This is needed in order to define the stochastic

process associated to a given finite set of rules. First a *substitution* σ is a finitely-based function from variables to terms, that is a function which acts as the identity on all but finitely many variables. Such a function σ can be denoted by $[t_0/x_0, \ldots, t_{n-1}/x_{n-1}]$ where the variables $x_0, \ldots, x_{n-1}$ are all distinct, $x_i\sigma = t_i$, for $i = 0, \ldots, n-1$ and σ acts as the identity on any other variable (it is common to use the postfix form of application for substitutions).

Substitutions are extended to act on all terms and atomic terms as follows:

$$\begin{array}{rcl}(a_0, \ldots, a_{n-1})\sigma & = & a_0\sigma, \ldots, a_{n-1}\sigma \\ S\sigma & = & S \\ A(t)\sigma & = & A(t\sigma)\end{array}$$

A *match* of a term l against another term t is a substitution σ such that $l\sigma = t$. For example, the substitution $\sigma = [(n-m)S/x]$ is a match of the term $l = mS + x$ against the term $t = nS$, assuming that $n \geq m$ (there is otherwise no match). This match can be thought of as occurring in several ways according to which of the m S's of l is matched against which of the n S's of t. So we say that the match has *multiplicity* the m-fold falling product of n, $n^{\underline{m}} =_{\text{def}} n(n-1)\ldots(n-(m-1))$. We also define a *symmetry* of l to be a permutation θ of the variables of l leaving it invariant, that is, such that $l\theta = l$, where we identify θ with the substitution that acts as the identity on all variables not in l.

We now define finite multisets $m(l;t)$ and $m(a;a')$ of substitutions of (atomic) terms against (atomic) ground terms; $m(l;t)$ is intended to be the multiset of matches of the term l against the term t, where a substitution has multiplicity its multiplicity as a match of l against t (and similarly for $m(a;a')$). The definition is only for terms l (respectively atomic terms al) satisfying the above three conditions, and ground terms t (respectively atomic ground terms at):

$$\begin{array}{rcl} m(a_0, \ldots, a_{m-1}, x; a'_0, \ldots, a'_{n-1}) & = & \sum_{f:[m]\hookrightarrow[n]} m(a_0, a'_{f(0)}) \circ \cdots \circ m(a_{m-1}, a_{f(m-1)}) \circ \\ & & \qquad [\sum_{j \notin f([m])} a_j/x] \end{array}$$

and

$$m(S;a') = \begin{cases} [\,] & (a' = S) \\ 0 & (\text{otherwise}) \end{cases}$$

$$m(A(l);a') = \begin{cases} m(l;t) & (a' = A(t)) \\ 0 & (\text{otherwise}) \end{cases}$$

where, by the composition of multisets of substitutions we mean the natural extension of the usual composition of substitutions to multisets. Note that if $m(l;t)(\sigma) \neq 0$ then σ acts as the identity on variables not in l.

One can show that σ is a match of l and t if, and only if, it has non-zero multiplicity in $m(l;t)$. This justifies its definition as far as as its elements are concerned. Below we give a reformulation in terms of counting tree embeddings.

We can separate $m(l;t)$ into a species and an agent part. Write l in the form $X + l' + x$, and t in the form $Y + t'$ where X and Y are finite multisets of species, and l' and t' are multisets of agent atomic terms. Define the *X-fold falling multiset sequential product of*

$Y$to be:

$$Y^{\underline{X}} =_{\text{def}} \prod_{S \in \text{Spec}} Y(S)^{\underline{X(S)}}$$

noting that this is essentially a finite product. Then we have:

$$m(l;t) = Y^{\underline{X}}(m(l' + x; t') + [(Y \dot{-} X)/x])$$

allowing ourselves to add substitutions pointwise. One might rather have expected to see the binomial of multisets

$$\binom{Y}{X} = \prod_{S \in \text{Spec}} \binom{Y(S)}{X(S)}$$

This would be the case if we rather counted the number of matches up to symmetry, by dividing $m(l;t)$ by the number of symmetries of l.

We now turn to defining the application of rules to terms. First we need to define *contexts*, which are terms $C[\,]$ with a (single) hole $[\,]$ in them. They are defined inductively, taking $[\,]$ to be a context, and $A(C[\,])$ and the multiset $C[\,], t$ to be contexts if $C[\,]$ is. Ground contexts are those containing no variables. Given a context $C[\,]$ one can obtain a term $C[u]$ (context $C[D[\,]]$) by filling-in the hole $[\,]$ with a term u (respectively, a context $D[\,]$); we omit the definition.

We can now define the transition relation $\longrightarrow_R$ between ground terms of application of rule of $R = l \xrightarrow{k} r$, by setting

$$t \longrightarrow_R t'$$

to hold when t has the form $C[u]$, for a context $C[\,]$ and there is a substitution σ which is a match of l against u, and is such that $t' = C[r\sigma]$; note that $C[\,]$ will necessarily be ground. A transition relation can then also be defined for a finite set $\mathcal{R}$ of rules by putting:

$$t \longrightarrow_{\mathcal{R}} t' \iff \exists R \in \mathcal{R}.\, t \longrightarrow_R t'$$

These are qualitative relations, by which we mean that no account is taken of the rates of the rules. To do so, we first need to define a narrower class of contexts, the *wide contexts* W$[\,]$. They are defined inductively, taking $[\,]$ to be a wide context, and the multiset $A(\text{W}[\,]), t$ to be a wide context if W$[\,]$ is. Note that every context can be written in the form $W[[\,], t]$. Using this, it is not hard to see that we get the same relation if we allow all contexts here, as l obeys the generality condition. Note too that a context C$[\,]$ is wide if, and only if, every term t is a wide subterm of C$[t]$.

Wide contexts are needed to avoid a possibility of double-counting when defining stochastic rates. For example, consider the rule

$$R = S, x \xrightarrow{k} S', x$$

We have $S, S' \to_R 2S'$, but that can be shown in two ways, using either of the contexts $[\,]$ or $[\,], S'$, and only the first of these is wide.

We need a count $\mathrm{occ}_t(\mathrm{W}[\,], u)$ of the number of ways in which a ground term t can have the form $\mathrm{W}[u]$, for a given wide context $W[\,]$ and term u:

$$\begin{aligned}\mathrm{occ}_t([\,], u) &= \begin{cases} 1 & (t = u) \\ 0 & (t \neq u) \end{cases} \\ \mathrm{occ}_{a_0,\ldots,a_{n-1}}((A(\mathrm{W}[\,]), l), u) &= \textstyle\sum_{i=0}^{n-1} \sum \{\mathrm{occ}_{t'}(\mathrm{W}[\,], u) \mid a_i = A(t'), \\ &\qquad\qquad a_0, \ldots, a_{i-1}, a_{i+1}, \ldots, a_{n-1} = l\}\end{aligned}$$

The corresponding quantitative notion for a rule $R = l \xrightarrow{k} r$ is a stochastic matrix Q_R. This is a function from pairs of ground terms to non-negative reals, where, for distinct ground terms t, t':

$$Q_R(t, t') = k \sum_{t = \mathrm{W}[u]} \mathrm{occ}_t(\mathrm{W}[\,], u) \sum_{\substack{u = l\sigma \\ t' = \mathrm{W}[r\sigma]}} m(l; u)(\sigma)$$

and, on the diagonal $Q_R(t, t)$ is, as usual, one minus the sum of the off-diagonal entries $Q_R(t, t')$ (one easily sees that almost all the off-diagonal entries are 0). Note the use of the representation of multisets as functions to $\mathbb{N}$ in this definition. Analogously to before, this can be extended to a stochastic matrix for a finite set $\mathcal{R}$ of rules by defining:

$$Q_{\mathcal{R}}(t, t') = \sum_{R \in \mathcal{R}} Q_R(t, t')$$

We next describe how to simulate the CTMC given by this stochastic rate matrix in terms of choosing and applying rules from $\mathcal{R}$. The *activity* of a rule $R =_{\mathrm{def}} l \xrightarrow{k} r$ on a term t is defined by:

$$\mathrm{Act}(R, t) = \sum_{t' \neq t} Q_R(t, t')$$

which is equal to

$$k \sum_{t = \mathrm{W}[u]} \mathrm{occ}_t(\mathrm{W}[\,], u) \sum_{u = l\sigma} m(l; u)(\sigma)$$

The simulation has a current time, initialised to 0, and a current state t, and proceeds by cycling through the following sequence, for as many times as are required:

- If $\lambda =_{\mathrm{def}} \sum_{R \in \mathcal{R}} \mathrm{Act}(R, t)$ is zero, stop the simulation.
- Chose τ from the exponential distribution $1 - e^{-\lambda\tau}$ and add it to the current time.
- Choose rule $R =_{\mathrm{def}} l \xrightarrow{k} r$ from $\mathcal{R}$ with probability $\lambda^{-1}\mathrm{Act}(R, t)$.
- Choose a wide context $\mathrm{W}[\,]$ and a u such that $t = \mathrm{W}[u]$ and a σ such that $l\sigma = u$ with probability
$$\frac{k\,\mathrm{occ}_t(\mathrm{W}[\,], u)(m(l; u)\sigma)}{\mathrm{Act}(R, t)}$$
- Update t to $\mathrm{W}[r\sigma]$.

Normally in a simulation one graphs the species populations against time. This is fine for species, of course, but what about agents? For example one might wish to graph the number of agents A containing 3 molecules of species S. We can achieve this by graphing the activity of suitable patterns given by terms l obeying the three conditions. For example, for a species S one graphs the term $S+x$ and for the agent example one graphs the pattern $A(3S+x)+y$. The activity of a pattern l in a ground term t is defined to be:

$$\mathrm{Act}(l,t) = \sum_{l\sigma=t} m(l;t)(\sigma)$$

Returning to the question of symmetries, the activity of a rule with left-hand side $mS+x$ in the population nS will be $n^{\underline{m}}$ rather than the more usual $\binom{n}{m}$. However it will not do simply to divide the activity by the number of symmetries of the left-hand side. Consider the rule:

$$A(x), A(y) \xrightarrow{k} B(x), C(y)$$

where we would not wish to divide by 2 as the right-hand side distinguishes x and y. Adapting a suggestion[1] of Russ Harmer made in the context of Danos and Laneve's κ [DL03], we may prefer to divide the activity of a rule by the number of symmetries of its left-hand side that extend to a symmetry of its right-hand-side. In a sense the matter is only one of convention since one can always absorb the division into the rate constant. In this paper, we adopt the simpler position of not doing any symmetry division, but do not argue it is the superior choice. In practice, the symmetry issue comes up for species, but, in our — very limited — experience, not for agents.

4. Another Example

In this section, we give a model of an experiment presented by Rosenfeld et al. in [RYA05]. We do not so much aim to model the experiment exactly as to illustrate how one can use multilevel multiset rewriting to model a biological process in which cell division plays an important rôle.

Rosenfeld et al. introduce a method to measure the gene regulation function of a given promoter. They create a high concentration of a repressor protein yfp in a single cell. This protein targets the chromosomally-integrated promoter Pro of the gene for a protein cfp. At cell division, each daughter cell receives approximately half the population of the repressor protein. So, after a few divisions, the concentration is low enough to allow the production of cfp to take place.

For our model we need to represent different cells with different content evolving independently. We begin by giving a set of rules for the reactions occurring inside cells. (For practical reasons, our model actually uses a modified version of these rules, as explained below.)

We first introduce two different species, yfp and cfp, for modelling the repressor protein and the gene product. We next introduce a species mRNA for the messenger RNA; this is used to give a (much-simplified) model of the transcription-translation of cfp,

[1] Personal communication.

Modelling the promoter is less straightforward. To approximate repression cooperativity, which has a measured value around 2 in the paper, we model the promoter as having two binding sites. Each of them can bind to a yfp. Transcription can only occur when both binding sites are empty. This could be modelled by using different species, one for each of the three possible promoter states: free, one binding site occupied, both binding sites occupied. This approach is very intuitive but would make it harder to model cell division.

Instead we use an agent Pro to model the promoter and two species FreeBS and OccBS to represent the state (respectively free or occupied) of each of the two binding sites. For example, a promoter with two free binding sites is modelled by Pro(2FreeBS), whereas a promoter with one binding site occupied is modelled by Pro(OccBS, FreeBS). (The model is symmetric in both binding sites; non-symmetric models are also possible.)

The following rules then model represent repression and transcription. (We do not model the nucleus and transport between it and the cytoplasm.)

$$\begin{array}{lcl} \mathrm{Pro}(\mathrm{FreeBS}, x), \mathrm{yfp} & \xrightarrow{r_{\mathrm{free-occ}}} & \mathrm{Pro}(\mathrm{OccBS}, x) \\ \mathrm{Pro}(\mathrm{OccBS}, x) & \xrightarrow{r_{\mathrm{occ-free}}} & \mathrm{Pro}(\mathrm{FreeBS}, x), \mathrm{yfp} \\ \mathrm{Pro}(\mathrm{2FreeBS}, x) & \xrightarrow{r_{\mathrm{transcr}}} & \mathrm{Pro}(\mathrm{2FreeBS}, x), \mathrm{mRNA} \end{array}$$

Note that whenever the last rule applies, x will be (matched to) 0.

Translation is modelled by the following rule:

$$\mathrm{mRNA} \xrightarrow{r_{\mathrm{transl}}} \mathrm{cfp}$$

Both mRNA and cfp can degrade, and so we have the following two rules:

$$\begin{array}{ll} \mathrm{mRNA} & \xrightarrow{r_{\mathrm{m-degrad}}} \\ \mathrm{cfp} & \xrightarrow{r_{\mathrm{c-degrad}}} \end{array}$$

This completes our representation of the rules for modeling the intracellular part of the process. However, there is a difficulty with modelling the extracellular part: the experiment produces an exponential number of cells, making faithful simulation impractical for our current implementation. There were 20 cell divisions in the experiment, and so we would have to simulate around 2^{20} cells.

To solve this problem, we model only one cell of each generation: the first simulated cell is given a marker, only one of the two descendants of a marked cell receives a marker, and the system simulates only cells that have been marked.

To this end, instead of a single agent Cell, we use a family of agents Cell_u, where $u \subseteq \{\mathrm{mark}, \mathrm{dup}\}$. The marker "mark" is used to determine the cells whose evolution is being modelled (the marker "dup" is used to control DNA replication).

To make sure we only simulate the marked cell, we adapt the above rules by embedding them in a marked agent: For example, the first three rules are replaced by the following six:

$$\begin{array}{lcll} \mathrm{Cell}_u(\mathrm{Pro}(\mathrm{FreeBS}, x), \mathrm{yfp}, y) & \xrightarrow{r_{\mathrm{free-occ}}} & \mathrm{Cell}_u(\mathrm{Pro}(\mathrm{OccBS}, x), y) & (\mathrm{mark} \in u) \\ \mathrm{Cell}_u(\mathrm{Pro}(\mathrm{OccBS}, x), y) & \xrightarrow{r_{\mathrm{occ-free}}} & \mathrm{Cell}_u(\mathrm{Pro}(\mathrm{FreeBS}, x), \mathrm{yfp}, y) & (\mathrm{mark} \in u) \\ \mathrm{Cell}_u(\mathrm{Pro}(\mathrm{2FreeBS}, x), y) & \xrightarrow{r_{\mathrm{transcr}}} & \mathrm{Cell}_u(\mathrm{Pro}(\mathrm{2FreeBS}, x), \mathrm{mRNA}, y) & (\mathrm{mark} \in u) \end{array}$$

(It would evidently be preferable to allow parametric agents directly in the formalism, thereby permitting parametric rules. For example, the above six rules would become three parametric ones. This point is discussed further in Section 6.)

Our remaining rules model cell division. The first rule models DNA replication. However we must ensure that this happens only once in the cell cycle. To that end we make use of the marker dup which acts as a token allowing DNA to be replicated, but consumed in the application of the rule.

$$\mathrm{Cell}_{\mathrm{dup,mark}}(\mathrm{Pro}(x), y) \xrightarrow{r_{\mathrm{replication}}} \mathrm{Cell}_{\mathrm{mark}}(\mathrm{Pro}(x), \mathrm{Pro}(\mathrm{2FreeBS}), y)$$

where, for example, we write $\mathrm{Cell}_{\mathrm{dup,mark}}$ instead of $\mathrm{Cell}_{\{\mathrm{dup,mark}\}}$ (and we employ similar notation below).

Next, we model cell division per se. Following the discussion in Section 3, we cannot use the analogous rule to (1), viz:

$$\mathrm{Cell}_{\mathrm{mark}}(\mathrm{Pro}(z), \mathrm{Pro}(z'), x, y) \xrightarrow{r_{\mathrm{division}}} \mathrm{Cell}_{\mathrm{mark,dup}}(\mathrm{Pro}(z), x), \mathrm{Cell}(\mathrm{Pro}(z'), y)$$

as it does not satisfy the uniqueness condition. We therefore use a set of rules with a similar effect. Much the same problem arose previously when applying bigraphs in a biological setting [KMT08], where a slightly different solution was adopted.

We use the expressivity of multilevel multiset rules to randomly partition the population of each species of a cell between its two children. To that end, we introduce a new unary function symbol CellPrec to model cell precursors, and add the rule

$$\mathrm{Cell}_{\mathrm{mark}}(\mathrm{Pro}(z), \mathrm{Pro}(z'), x) \xrightarrow{r_{\mathrm{division}}} \mathrm{Cell}(\mathrm{CellPrec}(\mathrm{Pro}(z)), \mathrm{CellPrec}(\mathrm{Pro}(z')), x),$$

The mark is removed to prevent any of the above rules from being applied while the cell species are being partitioned, i.e., during cell division.

The next three rules serve to partition the populations of the species in Cell (x in the preceding rule) between the two precursors CellPrec.

$$\begin{aligned}
\mathrm{Cell}(\mathrm{CellPrec}(y), \mathrm{mRNA}, x) &\xrightarrow{r_{\mathrm{partition}}} \mathrm{Cell}(\mathrm{CellPrec}(\mathrm{mRNA}, y), x) \\
\mathrm{Cell}(\mathrm{CellPrec}(y), \mathrm{yfp}, x) &\xrightarrow{r_{\mathrm{partition}}} \mathrm{Cell}(\mathrm{CellPrec}(\mathrm{yfp}, y), x) \\
\mathrm{Cell}(\mathrm{CellPrec}(y), \mathrm{cfp}, x) &\xrightarrow{r_{\mathrm{partition}}} \mathrm{Cell}(\mathrm{CellPrec}(\mathrm{cfp}, y), x)
\end{aligned}$$

We want cell division to happen instantaneously, so the rate $r_{\mathrm{partition}}$ must be very high with respect to all the other rates. It is important to understand that this high rate does not slow down the other parts of the simulation: these rules are only applicable in the presence of a CellPrec, which happens only when the other rules do not apply as the mark parameter is absent.

After the precursors are introduced, we need $|x|$ rule applications to partition all the species in x. After these simulation steps, we want the two precursors to become two new cells, and so might expect to use the rule:

$$\mathrm{Cell}(\mathrm{CellPrec}(x), \mathrm{CellPrec}(y)) \xrightarrow{r_{\mathrm{separation}}} \mathrm{Cell}_{\mathrm{dup,mark}}(x), \mathrm{Cell}(y)$$

$r_{\text{free-occ}}$	1.0	$r_{\text{m-degrad}}$	0.00556
$r_{\text{occ-free}}$	0.001	$r_{\text{replication}}$	$8.33\ 10^{-4}$
r_{transcr}	0.1	r_{division}	$4.17\ 10^{-4}$
r_{transl}	0.0167	$r_{\text{partition}}$	10^{12}
$r_{\text{c-degrad}}$	0	$r_{\text{separation}}$	10^{5}

FIGURE 5. The stochastic rates used for the simulation

However, such a rule is forbidden by the generality condition of Section 3: there is no variable in the left-hand side Cell. We instead use the following separation rule:

$$\text{Cell}(\text{CellPrec}(x), \text{CellPrec}(y), z) \xrightarrow{r_{\text{separation}}} \text{Cell}_{\text{dup,mark}}(x, z), \text{Cell}(y)$$

and make sure z is very unlikely to contain any species by choosing $r_{\text{separation}}$ very low with respect to $r_{\text{partition}}$. As the separation rule has constant stochastic activity, this ensures that it is very unlikely to be triggered if the partition rule can still be applied. The simulation is not slowed down, as there are at most $|x|$ steps of partition, after which separation is the only applicable rule. The cell division can therefore be simulated in $|x| + 1$ steps.

In practice, we want this process to happen instantly in simulated time, so we must fix both $r_{\text{separation}}$ and $r_{\text{partition}}$ very high with respect to the other rates of the system. In summary, we want:

$$r_{\text{partition}} >> r_{\text{separation}} >> r^*$$

where r^* is the maximum all the other rates of the system.

We ran a simulation of the above set of rules with an initial population of one cell containing 2500 repressor proteins:

$$\text{Cell}_{\text{mark,dup}}(2500\text{yfp})$$

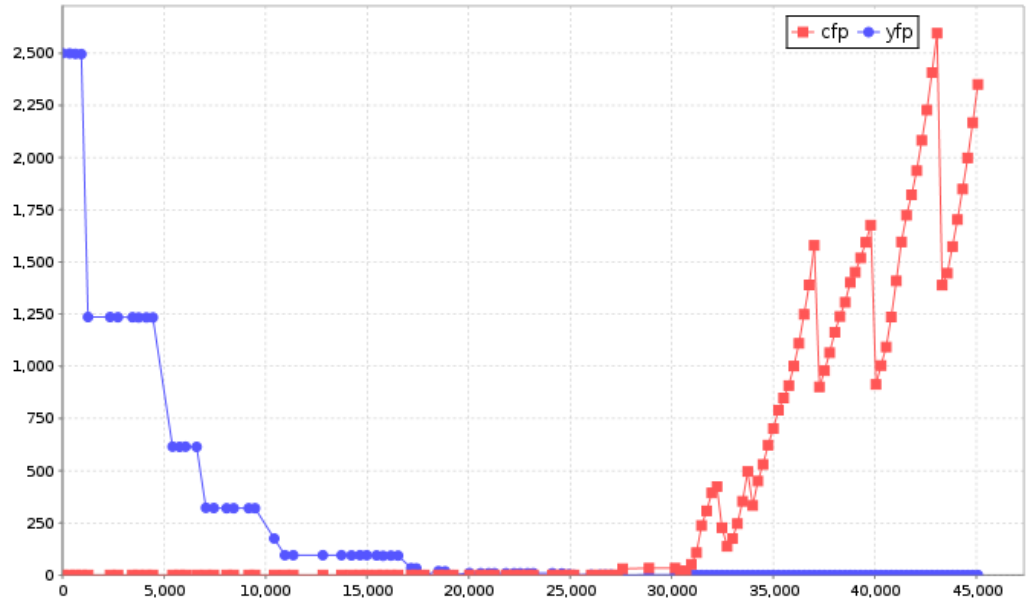
and with the rates, in s^{-1}, given in Figure 5. Figure 6 shows both the number of cells and the number of molecules of yfp and cfp in the run, plotted against time, measured in seconds

As expected, the evolution of the number of cells is linear. The run is similar to the one presented in the original article. With each division, the concentration of yfp decreases until the production of cfp becomes possible.

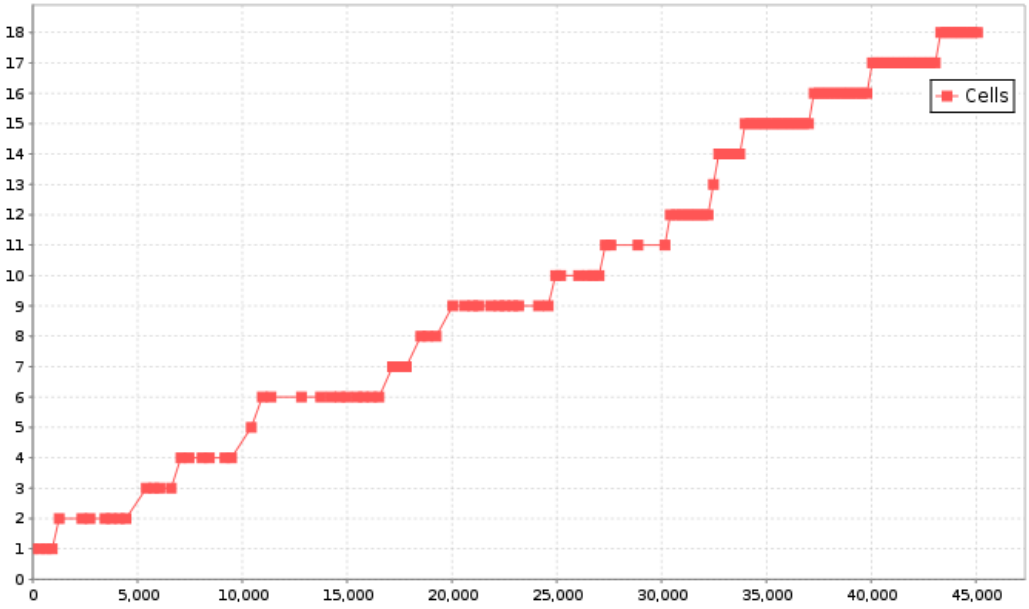
This second worked example gives some feeling for the strength and weaknesses of multi-level multiset rewriting. It allows a very direct expression of nested dynamic compartments, for both cell division and promoter representation, and an intuitive rule-based system expression. On the other hand, the very strict conditions on the rules make modelling cell division awkward to express: we return to this point in Section 6.

5. Other Formalisms

We begin by looking at a term rewriting formulation. There is an evident notion of stochastic term rewriting which seems, surprisingly, not to be in the literature. Suppose



(A) yfp and cfp



(B) cells

FIGURE 6. A run of the simulation

we have a a rule R of the form:

$$l \xrightarrow{k} r$$

where l and r are terms over a given signature, with $\mathrm{Var}(r) \subseteq \mathrm{Var}(l)$, and k is a nonnegative real. Then we can define a stochastic rate matrix on ground terms by setting:

$$Q_R(t, t') = k \,|\, \{C[\,] \mid \exists \sigma.\, t = C[l\sigma] \wedge t' = C[r\sigma]\} \,|$$

for distinct t and t'.

However to account for our multilevel multiset terms we need rather to work modulo a suitable equational theory. So, given the two disjoint sets, Spec of species and Agent of agents, consider the equational signature with constants the species and 0 (not a species), with unary operation symbols the agents, and with a binary operation symbol $+$. We work modulo the equational theory T that says $+$ is associative, commutative, and has a zero, 0. Our terms can then be seen as normal forms for the algebraic terms modulo T. More precisely, define a map N to the former from the latter by:

$$\begin{array}{rcl} \mathrm{N}(x) & = & x \\ \mathrm{N}(S) & = & S \\ \mathrm{N}(A(t)) & = & A(\mathrm{N}(t)) \\ \mathrm{N}(t+u) & = & \mathrm{N}(t), \mathrm{N}(u) \end{array}$$

Then N is onto and we have:

$$\vdash_{\mathrm{T}} t = u \quad \iff \quad \mathrm{N}(t) = \mathrm{N}(u)$$

Clearly a term and its normal form have the same variables. Say that an occurrence of a variable in a term is at *top level* if it is not within any unary operation symbol. Then $\mathrm{N}(t)$ obeys the above three conditions if, and only if, no variable occurs more than once in it, and there is exactly one top level variable occurrence, with the same being true of every term u such that $A(u)$ is a subterm of t, for some unary operation symbol A.

Given a rule $R = l \xrightarrow{k} r$ as above in the present signature, we can define a rewriting relation, modulo T, between ground terms in a standard way, by putting:

$$t \longrightarrow_R t' \iff \exists C[\,], \sigma.\ \vdash_{\mathrm{T}} t = C[l\sigma] \wedge \ \vdash_{\mathrm{T}} t' = C[r\sigma]$$

Then setting $\mathrm{N}(l \xrightarrow{k} r) = \mathrm{N}(l) \xrightarrow{k} \mathrm{N}(r)$, and assuming this rule obeys the above conditions on multilevel multiset rules, we have, for any ground terms t and t':

$$t \longrightarrow_R t' \iff \mathrm{N}(t) \longrightarrow_{\mathrm{N}(R)} \mathrm{N}(t')$$

However we do not know how to write the transition matrix between ground algebraic terms, modulo T, other than to use the normal form and the above definition of stochastic rates for multilevel multiset rules. We regard it as an interesting open problem to formulate a notion of stochastic rewriting for algebraic terms modulo equational theories. It may well be that one needs to restrict the class of equational theories considered; a possible such class is that of the *balanced theories*, those given by equations in which the same variables occur on each side, see [Man98].

The algebraic approach gives a possibility of generality for the development of useful term rewriting formalisms for computational systems biology. For example, one can argue that agents and compartments are different. Consider the term $A(B(x) + B(y) + z)$. Here one has two distinct B-agents with contents x and y within an agent A. However, if one instead takes B to be a compartment name, then the agent A should have only one compartment with a given name; it is therefore natural to make the identification $B(x) + B(y) = B(x + y)$, that B commutes with the AC operation. So an extension to our formalism of potential interest would be to add unary compartment operations that commute with the AC operation.

We next give a reformulation in terms of forests. We very largely follow in Milner's footsteps, and in Krivine et al.'s for the stochastic aspects: we essentially specialise to place graphs, making slight adaptations to allow for the presence of species. This last is a minor difference, as one can always simulate species by agents which never contain anything.

There are other small differences. For example we may impose slightly different conditions, and we do not bring in any categorical ideas, though they are certainly there in the background. Where appropriate we add comments on particular relationships with the bigraphical approach.

Fix a countably infinite set $\mathcal{V}$. An *(n-ary) concrete*(Spec, Agent)*-forest*, (n-ary) concrete forest, for short, is a structure

$$(V, \mathrm{p}, \lambda)$$

where

- $V \subseteq_{\mathrm{fin}} \mathcal{V}$ is a finite set of *nodes*,
- $\mathrm{p} : [n] \uplus V \to V \uplus [1]$ is the *parent* map, and
- $\lambda : v \to \mathrm{Spec} \cup \mathrm{Agent}$ is the *labelling map*

where $[m] =_{\mathrm{def}} \{0, \ldots, m-1\}$, for $m \geq 0$. The parent map is required to be acyclic, meaning that if $\mathrm{p}^i(v) = v$ then $i = 0$, and species can only label roots, which are nodes v such that $\mathrm{p}^{-1}(v) = \emptyset$.

If we drop species, these are the special case of Milner's concrete place graphs from n to 1 in which the controls have arity 0. It will prove convenient to confuse the set of nodes V with the entire structure (V, p, λ). We write $V : n$ to indicate that V is n-ary; we say that it is *ground* if $n = 0$; and we say it is an *atom* if $\mathrm{p}^{-1}(0)$ is a singleton. A homomorphism:

$$h : (V, \mathrm{p}, \lambda) \longrightarrow (V', \mathrm{p}', \lambda')$$

of n-ary concrete forests is a map from V to V' that respects structure in the evident sense that $h(\mathrm{p}(x)) = \mathrm{p}'(h(x))$, for any $x \in [n] \uplus V$, and $\lambda(v) = \lambda'(h(v))$, for any $v \in V$. We work with isomorphism equivalence classes $[(V, \mathrm{p}, \lambda)]$ of forests; following Milner, we call them *abstract forests*. This is helpful for definitions as we can always pick disjoint representatives of different equivalence classes. We say an abstract forest is n-ary (or ground) if any of its members is, and write $[V] : n$.

We next define the *composition* of n-ary forests with ground forests, beginning with concrete ones. Given pairwise disjoint n-ary (V, p, λ) and ground $(V_i, \mathrm{p}_i, \lambda_i)$, for $i = 0, \ldots, n-1$,

we define their composition

$$(V, \mathrm{p}, \lambda)((V_0, \mathrm{p}, \lambda), \ldots, (V_{n-1}, \mathrm{p}, \lambda))$$

to be the ground forest

$$(V \cup \bigcup_{i=0}^{n-1} V_i, \mathrm{p}', \lambda')$$

where:

$$\mathrm{p}'(v) = \begin{cases} v' & (v \in V_i, \mathrm{p}_i(v) = v' \in V_i) \\ \mathrm{p}(i) & (v \in V_i, \mathrm{p}_i(v) = i) \\ \mathrm{p}(v) & (v \in V) \end{cases}$$

and:

$$\lambda'(v) = \begin{cases} \lambda_i(v) & (v \in V_i) \\ \lambda(v) & (v \in V) \end{cases}$$

Note that if, for a given concrete V', n-ary V and ground V_i $(i = 0, \ldots, n-1)$ we have $V' = V(V_0, \ldots, V_{n-1})$ then the V_i are uniquely determined.

It is not hard to see that if $[V] = [V']$ and $[V_i] = [V_i']$, for $V, V' : 1$ and $V_i, V_i' : 0$ $(i = 0, n-1)$, then $V(V_0, \ldots, V_{n-1}) = V'(V_0', \ldots, V_{n-1}')$. So one can define the composition $[[(V, \mathrm{p}, \lambda)]([(V_0, \mathrm{p}, \lambda)], \ldots, [(V_{n-1}, \mathrm{p}, \lambda)])$ of an n-ary abstract forest $[(V, \mathrm{p}, \lambda)]$ with n ground ones $[(V_i, \mathrm{p}_i, \lambda_i)]$ $(i = 0, \ldots, n-1)$ to be

$$[(V, \mathrm{p}, \lambda)((V_0, \mathrm{p}, \lambda)], \ldots, [(V_{n-1}, \mathrm{p}, \lambda))]$$

with the understanding that disjoint members of the equivalence classes have been chosen.

A *stochastic rule* R is an n-ary equivalence class $[V_l]$ an m-ary equivalence class $[V_r]$ and a map $\eta : [m] \to [n]$ and a *rate* $k \in \mathbb{R}_0$, written as:

$$[V_l] \xrightarrow[\eta]{k} [V_r']$$

where we impose the further two conditions:

- restricted to $[n]$, the parent map of V_l is a bijection, and
- η is 1-1.

Regarding the first condition, in Krivine et al. [KMT08] the left-hand sides of rules are restricted to being *solid*, which here amounts to the condition that, restricted to $[n]$, the parent map of V_l is 1-1 and does not have 0 in its range. We do have 0 in its range; this causes an ambiguity in the application of rules of the kind discussed in Section 3; and the difficulty is again handled by the introduction of a suitable notion of wide context.

The requirement that, restricted to $[n]$, the parent map is onto, is, as will be seen, the correlate of the generality condition. Presumably we could as well have worked in the Krivine et al. style from the beginning, when an effectively equivalent generality condition would be imposed.

Regarding the second condition, we are rather following Milner [Mil09] than Krivine et al., as the latter's rules are linear in a certain sense, which here amounts to the requirement that η is a bijection. In the context of multilevel multiset rewriting this would correspond to adding the "no vanishing" condition, which we do not impose.

Qualitatively, we can assign the rule a transition relation between ground $[V]$, defined by:

$$[V] \longrightarrow_R [V'] \iff \begin{array}{l}\exists [C]:1, [V_0]:0, \ldots, [V_{n-1}]:0. \\ \quad [V] = [C]([V_l]([V_0], \ldots, [V_{n-1}])) \wedge \\ \quad [V'] = [C]([V_r]([V_{\eta(0)}], \ldots, [V_{\eta(m-1)}]))\end{array}$$

Quantitatively, as already remarked, we first need to restrict the "contexts" $[C] : 1$. We say that a concrete forest $C : 1$ is *wide* if 0 is the only child of its parent, and that an abstract forest $[C] : 1$ is wide if C is. Then we can assign the rule R a stochastic transition matrix Q_R where, off the diagonal:

$$\begin{array}{rcl} Q_R([V],[V']) & = & k \mid \{(C:1, V'':n, V_0:0, \ldots, V_{n-1}:0) \mid C \text{ is wide}, \\ & & \quad V = C(V''(V_0, \ldots, V_{n-1})) \ \wedge \ [V_l] = [V''], \\ & & \quad [V'] = [C]([V_r]([V_{\eta(0)}], \ldots, [V_{\eta(m-1)}]))\} \mid \end{array}$$

Here we count the number of factorisations of an element of $[V]$. Note that the V_i are determined, so do not enter into the count:only their existence is required. Unlike Krivine et al., we do not divide by the number of symmetries of the left-hand-side of the instance of the rule at hand: cf. the discussion of symmetry in Section 3.

We next give an equivalent definition that will help establish the relation with the multilevel multiset approach. For any concrete forest V define an equivalence relation on tuples $(C, V_0, \ldots, V_{n-1})$ such that $V = C(V_0, \ldots, V_{n-1})$, where $C : 1$ and $V_i : 0$ (for $i = 0, \ldots, n-1$), by:

$$(C, V_0, \ldots, V_{n-1}) \sim_V (C', V'_0, \ldots, V'_{n-1}) \iff [C] = [C'] \wedge \bigwedge_{i=0}^{n-1} [V_i] = [V'_i]$$

and write $[C, V_0, \ldots, V_{n-1}]_V$ for the corresponding equivalence class.

Proposition 5.1.

$$Q_R([V],[V']) = k \sum_{\substack{[C,V_{\mathrm{red}}]_V \\ C \text{ wide}}} |[C, V_{\mathrm{red}}]_V| \sum_{\substack{[\overline{V_l}, V_0, \ldots, V_{n-1}]_{V_{\mathrm{red}}} \\ [V'] = [C]([V_r]([V_{\eta(0)}], \ldots, [V_{\eta(m-1)}])) \\ [V_l] = [\overline{V_l}]}} |[C, V_0, \ldots, V_{n-1}]_{V_{\mathrm{red}}}|$$

As before, given a finite set of rules $\mathcal{R}$, we can then define a transition relation and stochastic matrix by putting:

$$[V] \longrightarrow_{\mathcal{R}} [V'] \iff \exists R \in \mathcal{R}. [V] \longrightarrow_R [V'] \qquad Q_{\mathcal{R}}([V],[V']) = \sum_{R \in \mathcal{R}} Q_R([V],[V'])$$

We next need some algebra on abstract forests. We begin with two constants: $\mathrm{merge}_1 : 1$ is the equivalence class of the unary forest with empty node set; and, for every $S \in \mathrm{Spec}$, $\underline{S}$ is the equivalence class of the nullary forest with a single node labelled by S. Next, for any agent A, we define a unary function $\underline{A}$ on abstract forests: for any forest $(V, \mathrm{p}, \lambda) : m$,

define $\underline{A}([V]) : m$ to be $[(V \uplus \{*\}, \mathrm{p}', \lambda')]$ where:

$$\mathrm{p}'(v) = \begin{cases} v' & (v \in V, \mathrm{p}(v) = v' \in V) \\ * & (v \in V, \mathrm{p}(v) = 0) \\ 0 & (v = *) \end{cases}$$

and

$$\lambda'(v) = \begin{cases} A & (v = *) \\ \lambda(v) & (v \in V) \end{cases}$$

Next for any $n \geq 0$ and bijection $\theta : \sum_{i=0}^{n-1} [m_i] \cong [\sum_{i=0}^{n-1} m_i]$ we define an n-ary *summation* operation by putting, for abstract forests $[(V_i, \mathrm{p}_i, \lambda_i)] : m_i$, where $i = 0, \ldots, n-1$:

$$\sum_{i=0,n-1}^{(\theta)} [V_i] = [(\bigcup_{i=0}^{n-1} V_i, \mathrm{p}, \lambda)]$$

where:

$$\mathrm{p}(v) = \begin{cases} \mathrm{p}_i(j) & (v = \theta(i,j), j \in [m_i]) \\ \mathrm{p}_i(v) & (v \in V_i) \end{cases}$$

and

$$\lambda(v) = \lambda_i(v) \quad (v \in V_i)$$

This operation is essentially commutative, by which we mean that for any permutation $\pi : [i] \cong [i]$ we have:

$$\sum_{i=0,n-1}^{(\theta)} [V_i] = \sum_{i=0,n-1}^{(\theta')} [V_{\pi i}]$$

where $\theta'(i,j) = \theta(\pi^{-1} i, j)$, for $0 \leq i \leq n-1$, $j \in m_{\pi i}$. We say that $[V'_{\pi(0)}], \ldots, [V'_{\pi(n-1)}], \theta'$ is a *reindexing* of $[V_0], \ldots, [V_{n-1}], \theta$.

Proposition 5.2. *Let $[V] : n$ be an abstract forest. Then, either it is an atom, when one of the following three mutually exclusive possibilities holds:*

1. $[V] = \mathrm{merge}_1$.
2. $[V] = \underline{S}$ *for a unique* $S \in \mathrm{Spec}$.
3. $[V] = \underline{A}([V'])$ *for a unique* $A \in \mathrm{Agent}$, *and abstract forest* $[V']$.

or else it is not an atom and:

$$[V] = \sum_{i=0,n-1}^{(\theta)} [V_i]$$

for a unique $n \geq 0$, and unique, up to reindexing, atoms $[V_0], \ldots, [V_{n-1}], \theta$.

We now turn to linking the multilevel multiset formalism to the forest one. First we need to translate terms to forests; we consider only terms in which no variable occurs more than once. Variables in one will correspond to numbers in the other. We assume a fixed ordering $z_0, \ldots, z_n, \ldots$ of all variables, and say that i is the index of z_i. For any term t write $\mathrm{Ind}(t)$ for the set of indices of its variables, and set $\mathrm{ar}(t) = \mid \mathrm{Ind}(t) \mid$. The translation assigns to every pair t, ρ of a term and a bijection $\rho : \mathrm{Ind}(t) \cong [\mathrm{ar}(t)]$ an abstract forest $\mathrm{F}_\rho(t) : \mathrm{ar}(t)$. For any $I \subseteq_{\mathrm{fin}} \mathbb{N}$ we fix a bijection $\rho_I : I \cong [|I|]$, and we write ρ_0 for $\rho_{[1]} (= 0 \mapsto 0)$.

For atomic terms we put:

$$\begin{array}{rcl} F_\rho(z_i) & = & \mathrm{merge}_1 \\ F_\rho(S) & = & \underline{S} \\ F_\rho(A(t)) & = & \underline{A}(F_\rho(t)) \end{array}$$

and for terms we put:

$$F_\rho(t_0,\ldots,t_{n-1}) \quad = \quad \textstyle\sum_{i=0,n-1}^{(\theta)} F_{\rho_{\mathrm{Ind}(t_i)}}(t_i)$$

where $\theta(i,j) = \rho(\rho^{-1}_{\mathrm{Ind}(t_i)}(j))$, for $0 \leq i \leq n-1, j \in \mathrm{Ind}(t_i)$. We see from the above remarks that this is well-defined. We will omit the (trivial) ρ when translating ground terms.

It follows from Proposition 5.2 that, for any $\rho : I \cong [n]$, the function $F_\rho(-)$ is a bijection between terms t with $I = \mathrm{Ind}(t)$ satisfying the uniqueness condition, and the $\mathrm{ar}(t)$-ary abstract forests. We note the further correspondences, where $F_\rho(t) = [(V, \mathrm{p}, \lambda)]$:

- t satisfies the unicity condition if, and only if, $\mathrm{p}{\restriction}[\mathrm{ar}(t)]$ is 1-1, and
- t satisfies the generality condition if, and only if, $\mathrm{p}{\restriction}[\mathrm{ar}(t)]$ is onto.

The translation maps substitution to composition in the following sense. Let t be a term with variables $z_{l_0},\ldots,z_{l_{n-1}}$, where $n = \mathrm{ar}(t)$, and let $t_0,\ldots,t_{n-1}$ be ground. Then:

$$F(t[t_0/z_{l_0},\ldots,t_0/z_{l_{n-1}}]) = F_\rho(F(t_0),\ldots,F(t_{n-1}))$$

Having mapped terms to terms, we can now map mutilevel multiset rules to forest rules. To any rule

$$R = l \xrightarrow{k} r$$

and bijections $\rho_l : \mathrm{Ind}(l) \cong [\mathrm{ar}(l)]$ and $\rho_r : \mathrm{Ind}(r) \cong [\mathrm{ar}(r)]$, we assign the rule

$$F_{\rho_l,\rho_r}(R) = F_{\rho_l}(l) \xrightarrow[\eta]{k} F_{\rho_r}(r)$$

where $\eta = \rho_l\rho_r^{-1}$. This map is a surjection from mutilevel multiset rules and pairs of such bijections to forest rules. According to the next proposition, the two rules are qualitatively equivalent:

Proposition 5.3. *For all ground terms t, t' we have:*

$$t \longrightarrow_R t' \iff F(t) \longrightarrow_{F_{\rho_l,\rho_r}(R)} F(t')$$

Proof. Let $\mathrm{Ind}(l) = \{i_1,\ldots,i_n\}$ and $\mathrm{Ind}(l) = \{j_1,\ldots,j_m\}$, where $n = \mathrm{ar}(l)$ and $m = \mathrm{ar}(r)$.

First suppose that $t \longrightarrow_R t'$. Then t has the form $C[u]$, and there is a substitution σ which is a match of l against u, and is such that $t' = C[r\sigma]$. Then:

$$t = C[l\sigma] = C[z_0][l[\sigma(i_0)/z_{i_0},\ldots,\sigma(i_{n-1})/z_{i_{n-1}}]/z_0]$$

and:

$$t' = C[r\sigma] = C[z_0][u[\sigma(j_0)/z_{j_0},\ldots,\sigma(j_{m-1})/z_{j_{m-1}}]/z_0]$$

It follows that:

$$F(t) = F_{\rho_0}(C[z_0])(F_{\rho_l}(l)(F(\sigma(\rho_l^{-1}(0))),\ldots,F(\sigma(\rho_l^{-1}(n-1))))))$$

and:

$$F(t') = F_{\rho_0}(C[z_0])(F_{\rho_r}(r)(F(\sigma(\rho_r^{-1}(0))), \ldots, F(\sigma(\rho_r^{-1}(m-1))))))$$

As we also have $F(\sigma(\rho_r^{-1}(j))) = F(\sigma(\rho_l^{-1}(\eta(j))))$, it follows that $F(t) \longrightarrow_{F_{\rho_l,\rho_r}(R)} F(t')$, as required.

Conversely, suppose that $F(t) \longrightarrow_{F_{\rho_l,\rho_r}(R)} F(t')$. Then we have:

$$F(t) = [V]([F_{\rho_l}(l)]([V_0], \ldots, [V_{n-1}]))$$

and

$$F(t') = [V]([F_{\rho_r}(r)]([V_{\eta(0)}], \ldots, [V_{\eta(m-1)}]))$$

for some $[V] : 1$ and $[V_0] : 0, \ldots, [V_{n-1}] : 0$. As F_{ρ_0} is a bijection between terms t with $\mathrm{Ind}(t) = \{0\}$ satisfying the uniqueness condition, and the unary abstract forests, there is a ground context $C[\,]$ such that $F_{\rho_0}(C[z_1]) = [V]$. Similarly there are ground terms t_i such that $F(t_i) = [V_i]$, for $i = 0, \ldots, n-1$. We then have:

$$[V]([F_{\rho_l}(l)]([V_0], \ldots, [V_{n-1}])) = F(C[l[t_0/z_{\rho_l^{-1}(0)}, \ldots, t_{n-1}/z_{\rho_l^{-1}(n-1)}]])$$

So, as F is injective, setting $\sigma = [t_0/z_{\rho_l^{-1}(0)}, \ldots, t_{n-1}/z_{\rho_l^{-1}(n-1)}]$ we see that $t = C[l\sigma]$. Similarly, we have:

$$t' = C[r[t_{\eta(0)}/z_{\rho_r^{-1}(0)}, \ldots, t_{\eta(n-1)}/z_{\rho_r^{-1}(m-1)}]] = C[r\sigma]$$

and the result follows. □

We now turn to showing that the two rules are are also quantitatively equivalent. First note that, for any context $C[\,]$, if $F_{\rho_0}(C[z_0]) = [C']$, then $C[\,]$ is wide if, and only if, C' is. Next we need two lemmas:

Lemma 5.4. *For any ground term t, wide context $\mathrm{W}[\,]$ and term u we have:*

$$\mathrm{occ}_t(\mathrm{W}[\,], u) = |\{(V_1 : 1, V_0 : 0) \mid V = V_1(V_0), [V_1] = F_{\rho_0}(\mathrm{W}[z_0]), [V_0] = F(u)\}|$$

where $V : 0$ is any concrete forest such that $F(t) = [V]$.

Lemma 5.5. *Let t be a ground term, and let l be a term with free variables $z_{i_0}, \ldots, z_{i_{n-1}}$, satisfying the above three conditions. Then for any ground terms $t_0, \ldots, t_{n-1}$ and any $\rho : \mathrm{Ind}(l) \cong [n]$ we have:*

$$m(l;t)([t_0/z_{i_0}, \ldots, t_{n-1}/z_{i_{n-1}}]) = |\{(V_l : n, V_0 : 0, \ldots, V_{n-1} : 0) \mid V = V_l(V_0, \ldots, V_{n-1}), [V_l] = F_\rho(l), \text{ and } [V_i] = F(t_i)(i = 0, \ldots, n-1)\}|$$

where $V : 0$ is any concrete forest such that $F(t) = [V]$.

Using Proposition 5.1, and Lemmas 5.4 and 5.5, we then obtain:

Proposition 5.6. *For all ground terms t, t' we have:*

$$Q_R(t, t') = Q_{F_{\rho_l,\rho_r}(R)}(F(t), F(t'))$$

We conclude this section by briefly considering the relation between our system and the Stochastic Calculus of Wrapped Compartments of Coppo et al. [CD10a]. This formalism introduces *wrapped compartments*, denoted using an infix operation $\rfloor$. The compartment notation allows one to represent both the content of a membrane, on the left of $\rfloor$, and the content of a compartment, on the right of $\rfloor$. The content of the membrane is a multiset of *atoms*, which correspond to our species. The content of the compartment is a multiset of atoms and nested compartments.

This formalism is extended in [CD10b] by allowing a a compartment to be labelled. For example, a cell containing an empty nucleus and having an atom a on its membrane would be denoted:

$$(a\rfloor(\rfloor)^{Nucleus})^{Cell}$$

We refer to this extended formalism as *SCWC*.

Both SCWC and our formalism allow one to express nested compartments containing species, and each can be encoded by the other. Regarding species as atoms and agents as compartment labels, a translation function C from our formalism into SCWC can be defined by:

$$C(M, A_0(t_0), \ldots, A_{n-1}(t_{n-1})) = (M, \rfloor(\rfloor \mathrm{C}(t_0))^{A_0}, \ldots, (\rfloor \mathrm{C}(t_{n-1}))^{A_{n-1}})^*$$

for any multiset of species $M = S_0, \ldots, S_{m-1}$, and where $* \notin$ Agent. It is notable that we have to make a choice for this translation: we put the species on the membrane, but we could put them inside the compartment, or even choose depending on the species. Because of this, the translation is not onto, though it is 1-1.

In the other direction, regarding atoms as species and compartment labels as agents, a translation function T from SCWC into our formalism can be defined by:

$$\begin{array}{rcl} \mathrm{T}(a) & = & a \\ \mathrm{T}((S_0, \ldots, S_{m-1}\rfloor t_0, \ldots, t_{n-1})^l) & = & l(M(\mathrm{T}(S_0, \ldots, S_{m-1})), \mathrm{T}(t_0), \ldots, \mathrm{T}(t_{n-1})) \end{array}$$

where M is an agent which is not a compartment label; it is thought of as a *membrane agent*. The translation T from SCWC to our formalism is again 1-1 but is again not onto, as there is no restriction on terms that only species can appear in the membrane agent M.

By using the algebraic approach we can get much closer to SCWC. Consider a two-sorted equational theory with: sorts m and c, for membrane and compartment; an AC operation with a zero for each sort; a unary operation over c for each compartment label; a set of constants of sort m, for the atoms which can be on a membrane; and a set of constants of sort c, for the atoms which can be in a compartment. If we allow overloading, in particular allowing the two sets of constants to be the same, then SCWC terms can be seen as normal forms for the terms of type c.

The restrictions imposed on rules in SCWC are similar to the ones we impose: for example, forms of uniqueness and unicity are imposed on the left-hand sides of rules, but the generality condition is only partially imposed. Based on the above translations, we conjecture that, once the differences in the conditions imposed have been reconciled, mutual simulation results, including stochastic rates, can be established.

Despite their (presumed) equivalence, the two formalisms have somewhat different orientations. SCWC has an elegant representation of membranes, whereas our formalism assigns no particular rôle or structure to them. Our formalism may therefore permit a more natural modelling of those parts of biological structures that do not involve membranes. As the translation from SCWC shows, we further lose little, if any, naturality as regards the expression of systems with membranes.

6. Discussion and Conclusion

There are several possibilities for future development. As regards related formalisms, it would be useful to have a notion of multilevel Petri net, to enable the graphical presentation of our multilevel multiset rule systems; indeed even such a notion for rules with terms of height ≤ 1 would be very helpful. As regards generality, it would be interesting to develop the algebraic approach discussed above: one should investigate both general theory and particular systems; the addition of compartments would be of particular immediate interest.

In another direction, it would be useful to have a type system. So far, for example, there is nothing that prevents cells being inside cells inside cells, etc., to arbitrary depths. One could imagine such a type system based on a forest, or dag, of types. Continuing the linguistic thought, very large lists of rules become difficult to understand and maintain, and often obscure underlying structure. This might be alleviated by a suitable module system. It would be interesting to design a language for multilevel systems along the lines of LBS [PP10], which is a modular language for the rule-based description of intracellular systems.

Facilities for parameterisation would also be useful. At the species level one could follow LBS and use parametrised species $S_{x_1,\ldots,x_n}$, where the x_i run over suitable parameter spaces describing, for example, modification states (phosphorylation, ubiquitination, etc). Similarly, one could make use of parametrised agents $A_{x_1,\ldots,x_n}$ where now the parameters might, for example, deal with cell fate, or volume, or location. Rules could make use of these parameters; for example, the stochastic rates could depend upon them and boolean conditions on them could determine their applicability.

Finally, it is most important to have facilities for complexes. A simple possibility would be to add another multiset operation to deal with complexes. A more sophisticated, not to mention more powerful, approach would be to be able to describe complexes as connected graphs, following the lead of κ [DL03]. Together with the multilevel multisets one would then have something very similar indeed to Milner's bigraphs, whose potential for biological application has, as mentioned above, already been noted in [KMT08].

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