PhD Dissertation

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A Natural Computation Approach To Biology

Modelling Cellular Processes and Populations of Cells With Stochastic Models of P Systems

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Abstract

According to the well-accepted paradigm, the underlying constituents of biology are discrete molecules, with some being in very small copy numbers. It is therefore most precise to model the interaction of biological substances as discrete events connecting discrete states. Using this abstraction it is then natural to treat molecular interactions as being part of a computation and to perform formal analysis on them using the techniques of computer science. In this way it is possible to extract useful information about biological systems in an automatic way.

Membranes and membrane proteins are fundamental to the operation of biological cells, hence this thesis presents three new computational models designed to represent biological systems, based on models of membrane computing: Membrane Systems with Peripheral Proteins *(MSPP),* Membrane Systems with Peripheral and Integral Proteins *(MSPIP) and* Colonies of Synchronizing Agents *(CSA). MSP(I)P is close to biologists' prevailing view of the cell and hence is highly compatible with existing biochemical models. CSA is an hierarchical paradigm designed to represent complex systems such as populations of cells and tissues. This work extends the corpus of knowledge about biology and the theory of computation by proving technical results related to these models.*

The MSPP, MSPIP and CSA models have associated software implementations which allow the simulation of the temporal evolution of biological models by means of multiset rewriting *under the control of a stochastic algorithm. One of these,* Cyto-Sim *(implementing the MSPP and MSPIP models), being most developed, is presented in detail with several examples.*

Stochastic simulation is inherently computationally intensive and hierarchical systems particularly so. This thesis presents a new state of the art stochastic simulation algorithm for hierarchical and agent-based systems (the Method of Partial Propensities) and uses this result to improve the state of the art of stochastic simulation algorithms for well stirred chemical systems (the *Method of Arbitrary Partial Propensities).*

The noise evident in stochastic simulations is a potentially useful characteristic, containing information about the system being simulated. To extract detailed measures of stochasticity and the behaviour of a system, a new technique using Fourier analysis is presented and illustrated. With this it is possible to create a space of phenotype to characterise models and the performance of simulation algorithms.

Keywords
[Natural Computing, Stochastic Simulation, Multiset Re-writing, Systems Biology, Biological Modelling, Agents, P Systems, Membranes, Fourier Analysis]

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Chapter 1

Introduction

1.1 The Context

The sequencing of the human genome is a well known and successful application of information technology to biology. Moreover, the information so derived is being successfully interrogated and manipulated using computational techniques. This apparent success and the continuing exponential growth in computational power (i.e., Moore's law) leads scientists to believe that computers may be successfully applied to other areas of biology [43]. It is expected that cells, tissues and even entire organisms may be accurately simulated by computational models, in order to accelerate biological research and so aid disease prevention and cure.

Large amounts of biological data are available from high throughput experimental techniques and the emerging field of Systems Biology thus requires predictive and informative models to make sense of it. Such models need to be intuitive and efficient in execution in order to minimise the apparent and real computational complexities. Much of the data gathered so far has been done in a piecemeal and informal way, being driven by the availability of technology. In order to gain inference from the data, it is necessary for models to be described in a structured way which may be formally analysed.

Natural computing is a field of computer science which derives inspiration for new computational paradigms from biology; Nature having evolved efficient ways to solve difficult problems. Turning this process around; having extracted the key elements of Nature's solution to form a formal framework, it is then conceivable to re-categorise natural models in a formal way and thus gain insight about how they work. This is one of the essential premises of this thesis.

1.2 The Problem

The reasonable expectation to use the power of computers to understand biology may be hampered by the inherent difference between having a *static* description of the biology (e.g., a genome or pathway) versus the *dynamic* system it describes. In computational terms, this is analogous to the difference between the *description* of a program versus the *behaviour* of the resulting process when the program is executed. It was hoped that knowing all the genes of an organism would allow its complete characterisation, however it seems that there are *epigenomic* and other effects which result in significant phenotypic differences. While it is not unreasonable to suppose that an organism's genes act to regulate its behaviour, it seems that precise prediction of future behaviour will depend critically on equally precise knowledge of its history. From this it becomes clear that analysis of the dynamics of biological models will be crucial and that simulation of such models in time will play an important role.

Molecular biology has been successfully modelled by the traditional tools of dynamical systems: differential equations. These accurately describe the interactions of molecular populations as flows when the number of similar discrete interaction events is sufficiently large for a continuous approximation to be valid. As the precision of biological measurements and experiments increases, however, it has become clear that some critical molecules are in very low copy number (single genes being an obvious example [28]) and that there are a great many *different* interactions. Under these conditions, differential equations can only describe an average behaviour, at best, and models using them tend to become unwieldy and inefficient. The paradigm of discrete molecules and discrete transitions, which in the thermodynamic limit makes differential equations plausible, more directly leads to the possibility to construct transition systems which may be analysed by the techniques of computer science formal methods.

1.3 The Solution

Starting from the premise of modelling biology using a computational paradigm, this work builds on one such that is inspired by biological cells and tissues: P Systems (a.k.a. Membrane Systems). The original purpose of the paradigm was to explore the computational power of computing devices having a cellular structure and using computational operations inspired by chemical reactions and the passage of molecules between cells. In order to more easily model the diversity of biology, however, it is necessary to extend the basic concept of a Membrane System to include specific biological features. Having done so, it is necessary to provide proofs of various properties of the extended models and to facilitate their simulation with software implementations. A further critical requirement is the analysis of the results of using such simulators.

This thesis presents two new classes of models of Membrane Systems, tailored to represent 'well-stirred' and hierarchical biochemical systems. The underlying formalism is intuitive to non-experts and has a wealth of existing technical results for reference as well as active ongoing research. Several results are presented here which extend the corpus and aid the understanding of biological *computations*. Since discrete and stochastic formalisms which model individual molecular interactions potentially give the most accurate dynamical representation of biological systems, the implementations of these models employ the concept of multiset rewriting controlled by a stochastic simulation algorithm. Two simulators and their associated languages have been created, one of which (Cyto-Sim) is presented in detail. To ameliorate the computational complexity of stochastic simulation two new state of the art simulation algorithms have been developed and are presented here. Finally, a new technique based on Fourier analysis is presented which extracts useful information from stochastic simulations.

1.4 Innovative Aspects

The specific innovative aspects relating to this thesis are listed below. The current author's original publications from which these innovations are drawn are listed in Section 1.6.

- **Membrane Systems with Peripheral Proteins**: A new model of membrane systems featuring objects attached to both inner and outer surfaces of a membrane. This model more accurately represents biological reality than previously considered models. Several technical results are presented which help to clarify the way biological processes work.

- **Membrane Systems with Peripheral and Integral Proteins**: An extension of the peripheral protein model to include integral proteins, further enhancing the model's biological plausibility by allowing known biological entities to be represented explicitly.

- **Cyto-Sim**: A software implementation of the two presented membrane systems models uses an efficient stochastic simulator, intuitive textual modelling language and provides various graphical and statistical outputs. Other features related to abstraction and compatibility with other formalisms include:

- ▷ Interconversion of native modelling language to various versions of SBML.
- ▷ Extension of native modelling language to represent models as Petri nets.
- ▷ Export of native models to ordinary differential equations (ODEs) in MATLAB m-file format.
- ▷ Extension of modelling language and simulator to accept arbitrary kinetic functions, as used by many existing biological models, thus facilitating an additional level of abstraction.

- **Colonies of Synchronizing Agents**: A new, ‘elegant’, agent-based paradigm that generalises and extends earlier models - a *multiset of multisets*. Provides a framework to model and analyse complex, hierarchical biological phenomena that can be extended to include space.
- **Method of Partial Propensities**: A state of the art stochastic simulation algorithm designed specifically for Colonies of Synchronizing Agents but with applicability to other complex hierarchical paradigms.
- **Method of Arbitrary Partial Propensities**: An improved stochastic simulation algorithm for chemical systems, based on the techniques developed for the Method of Partial Propensities but with more general applicability.
- **Fourier analysis of stochastic simulations**: A novel technique is presented which takes advantage of the additional information gained by stochastic simulation to create a *space of phenotype*.

1.5 Structure of the Thesis

- **Chapter 1** is this introduction.
- **Chapter 2** gives notational and formal language preliminaries necessary for the sequel and describes some basic conventions from the field of membrane computing.
- **Chapter 3** presents a model of Membrane Systems which includes a biologically realistic membrane proteins and explores its theoretical properties.
- **Chapter 4** extends the model in Chapter 3 to add further biologically-motivated features.
- **Chapter 5** describes the software implementation of the models presented in Chapters 3 and 4 and includes a number of examples which demonstrate its use.
- **Chapter 6** presents an hierarchical, agent-based paradigm called Colonies of Synchronizing Agents. Several technical results are proved and a computational tree logic over the model is defined.
- **Chapter 7** gives a brief background and state of the art relating to stochastic simulation algorithms.
- **Chapter 8** presents the Method of Partial Propensities; an algorithm which significantly improves the performance of Colonies of Synchronizing Agents and similar models with respect to current benchmark algorithms.
- **Chapter 9** extends and applies the work of Chapter 8 and presents an algorithm with general applicability to chemically reacting systems which improves on current benchmarks.

- **Chapter 10** presents a new technique for the analysis of stochastic simulations using Fourier decomposition.
- **Chapter 11** concludes and summarises the thesis.

1.6 Publications relating to the chapters presented in this thesis

Ch. 3: M. Cavaliere and S. Sedwards (2007) Membrane Systems with Peripheral Proteins: Transport and Evolution, Proceedings of MeCBIC06, *Electronic Notes in Theoretical Computer Science*, **171:2**, 37–53.

M. Cavaliere and Sedwards S. (2008) Decision Problems in membrane systems with peripheral proteins, transport and evolution, *Theoretical Computer Science*, **404**, 40–51.

Ch. 4: M. Cavaliere and S. Sedwards (2006) Modelling Cellular Processes Using Membrane Systems with Peripheral and Integral Proteins, Proceedings of the International Conference on Computational Methods in Systems Biology, CMSB06, *Lecture Notes in Bioinformatics*, **4210**, 108–126.

Ch. 5: S. Sedwards and T. Mazza (2008) Cyto-Sim: A formal language model and stochastic simulator of membrane-enclosed biochemical processes, *Bioinformatics*, **23:20**, 2800–2802.

Ch. 6: M. Cavaliere, R. Mardare and S. Sedwards (2007) Colonies of Synchronizing Agents: An Abstract Model of Intracellular and Intercellular Processes, *Proceedings of the International Workshop on Automata for Cellular and Molecular Computing, Budapest.*

M. Cavaliere, R. Mardare and S. Sedwards (2008) A multiset-based model of synchronizing agents: Computability and robustness, *Theoretical Computer Science*, **391:3**, 216–238.

R. Mardare, M. Cavaliere and S. Sedwards, A Logical Characterization of Robustness, Mutants and Species in Colonies of Agents, *International Journal of Foundations of Computer Science*, **19:5**, 1199–1221.

Chapter 2

Formal language preliminaries

This chapter briefly summarises the main ideas and notation from formal language theory used in the sequel. For more depth the reader can consult standard books, such as [46], [78], [26], and the respective chapters of the handbook [77].

$\mathbb{N}$ and $\mathbb{R}$ denote the set of natural and real numbers, respectively.

Given a set A, $|A|$ denotes its cardinality and 2^A its power set. The empty set is denoted by $\emptyset$.

As usual, an *alphabet* V is a finite and non-empty set of symbols. By V^* is denoted the set of all strings over V. By V^+ is denoted the set of all strings over V excluding the empty string, which is the string containing no symbols. The concatenation of two strings $u, v \in V^*$ is written uv. The empty string is denoted by λ.

The *length* of a string $w \in V^*$ is denoted by $|w|$, while the number of occurrences of $a \in V$ in w is denoted by $|w|_a$.

A grammar is a finite device generating in a well defined sense the syntactically correct strings of a language. Chomsky grammars are particular examples of *rewriting systems* where the action used to process strings is the replacement (rewriting) of a substring with another substring. A Chomsky grammar is a quadruple $G = (S, N, T, P)$, where N is a finite set of non-terminal symbols, T is a finite set of terminal symbols such that $T \cap N = \emptyset$, P is a finite set of production rules (productions) of the form $u \rightarrow v$, where $u = (N \cup T)^*N(N \cup T)^* \in V^*$ and $v = (N \cup T)^* \in V*$ and $S \in N$ is the start symbol (the axiom). The action of the rules is thus to rewrite the substring u with the substring v. The language of a grammar is the set of all terminal strings generated by applying its rules to the start symbol in all possible sequences . It is only possible to apply a rule to a string if the string contains a substring which matches the left side of the rule.

A *regular* language is produced by a regular grammar. A grammar is regular if each rule $u \rightarrow v \in P$ has $u \in N$ and $v \in T \cup TN \cup \{\lambda\}$.

A finite grammar is a regular grammar which produces a language with a finite number of strings: a finite language.

A *context free* language is produced by a context free grammar. A grammar is context free if each rule $u \rightarrow v \in P$ has $u \in N$.

A *context sensitive* language is produced by a context sensitive grammar. A grammar is context sensitive if each $u \rightarrow v \in P$ has $u = u_1Au_2, v = u_1xu_2$ for $u_1, u_2 \in (N \cup T)^*$, $A \in N$ and $x \in (N \cup T)^+$. The production $S \rightarrow \lambda$ is allowed so long as S does not appear in the right hand side of members of P.

A *recursively enumerable* language is produced by a recursively enumerable grammar. A recursively enumerable (arbitrary) grammar, is one which places no restrictions on $u \rightarrow v \in P$.

$L(G)$ denotes the language generated or produced by the grammar G.

FIN, REG, CF, CS, and RE denote the families of finite, regular, context-free, context-sensitive, and recursively enumerable languages, respectively.

The notation $Perm(x)$ indicates the set of all strings that can be obtained as a permutation of the string x.

For $x, y \in V^*$ their *shuffle* is defined by $x\xi y = \{x_1y_1 \cdots x_ny_n \mid x = x_1 \cdots x_n,\ y = y_1 \cdots y_n, x_i, y_i \in V^*, 1 \leq i \leq n, n \geq 1\}$. The operation can be extended in an intuitive way to languages: given two languages L_1 and L_2, their shuffle is $L_1\xi L_2 = \bigcup_{x_1 \in L_1, x_2 \in L_2} x_1\xi x_2$.

The following result is proved in [77] in a constructive way.

Theorem 2.0.1 *If $L_1, L_2 \in REG$, then $L_1\xi L_2 \in REG$.*

Given an alphabet $V = \{a_1, a_2, \ldots, a_n\}$, for all strings $x \in V^*$ it is possible to associate the *Parikh vector* $Ps_V(x) = (|x|_{a_1}, |x|_{a_2}, \ldots, |x|_{a_n})$. Given a language $L \subseteq V^*$, it is also possible to define the *Parikh image* of L as $Ps_V(L) = \{Ps_V(x) \mid x \in L\}$.

For a language $L \subseteq V^*$, the set *length*$(L) = \{|x| \mid |x \in L\}\}$ is called the *length set* of L, denoted by NL.

If FL is an arbitrary family of languages then NFL denotes the family of length sets of languages in FL (family of sets of natural numbers).

If FL is an arbitrary family of languages then $PsFL$ denotes the family of Parikh images of languages in FL (family of sets of vectors of natural numbers).

FL_A denotes the family of languages over the alphabet A, e.g., REG_A, the family of all regular languages over the alphabet A.

A *multiset* over a set V is a map $M : V \to \mathbb{N}$, where $M(a)$ denotes the multiplicity (i.e., number of occurrences) of the symbol $a \in V$ (where V can be infinite) in the multiset M. This fact can also be indicated by the forms $(a, M(a))$ or $a^{M(a)}$, for all $a \in V$. If the set V is finite, e.g. $V = \{a_1, \ldots, a_n\}$, then the multiset M can be explicitly described as $\{(a_1, M(a_1)), (a_2, M(a_2)), \ldots, (a_n, M(a_n))\}$. The *support* of a multiset M is the set *supp*$(M) = \{a \in V \mid M(a) > 0\}$. A multiset is empty (so finite) when its support is empty (also finite).

A compact notation can be used for finite multisets: if $M = \{(a_1, M(a_1)),$

$(a_2, M(a_2)), \ldots, (a_n, M(a_n))\}$ is a multiset of finite support, then the string $w = a_1^{M(a_1)} a_2^{M(a_2)} \ldots a_n^{M(a_n)}$ (and all its possible permutations) precisely identify the symbols in M and their multiplicities. Hence, given a string $w \in V^*$, it can be assumed that it identifies a finite multiset over V defined by $M(w) = \{(a, |w|_a) \mid a \in V\}$.

For multisets M and M' over V, M is said to be *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 $a \in V$. $(M + M')$ is also said to be obtained by *adding* M to M' (or vice versa) while $(M - M')$ is obtained by *removing* M' from M.

The *cardinality* of a multiset M is denoted by $card(M)$ and it indicates the number of objects in the multiset. It is defined in the following way. $card(M)$ is infinite if M has infinite support. If M has finite support then $card(M) = \sum_{a_i \in supp(M)} M(a_i)$ (i.e., all the occurrences of the elements in the support are counted).

$\mathbb{M}(V)$ denotes the set of all possible multisets over V and $\mathbb{M}_k(V)$ and $\mathbb{M}_{\leq k}(V)$, $k \in \mathbb{N}$, denote the set of all multisets over V having cardinality k and at most k, respectively. That is $\mathbb{M}_k(V) = \{M \mid M \in \mathbb{M}(V), card(M) = k\}$ and $\mathbb{M}_{\leq k}(V) = \{M \mid M \in \mathbb{M}(V), card(M) \leq k\}$.

Note that, since V could be infinite, $\mathbb{M}_k(V)$ and $\mathbb{M}_{\leq k}(V)$, for $k \in \mathbb{N}$ could also be infinite.

The empty multiset is represented by the empty string λ.

The notion of a *matrix grammar* is made use of.

A *matrix grammar with appearance checking (ac)* is a construct $G = (N, T, S, M, F)$, where N and T are disjoint alphabets of non-terminal and terminal symbols, $S \in N$ is the axiom, M is a finite set of matrices which are sequences of context-free rules of the form $(A_1 \rightarrow x_1, \ldots, A_n \rightarrow x_n)$, $n \geq 1$ (with $A_i \in N, x_i \in (N \cup T)^*$ in all cases), and F is a set of occurrences of rules in M.

For $w, z \in (N \cup T)^*$, $w \Longrightarrow z$ is written if there is a matrix $(A_1 \rightarrow x_1, \ldots, A_n \rightarrow x_n)$ in M and strings $w_i \in (N \cup T)^*, 1 \leq i \leq n+1$, such that $w = w_1, z = w_{n+1}$, and, for all $1 \leq i \leq n$, either
(i) $w_i = w_i' A_i w_i'', w_{i+1} = w_i' x_i w_i''$, for some $w_i', w_i'' \in (N \cup T)^*$
or
(ii) $w_i = w_{i+1}$, A_i does not appear in w_i, and the rule $A_i \rightarrow x_i$ appears in F.

The rules of a matrix are applied in order, possibly skipping the rules in F if they cannot be applied (one says that these rules are applied in *appearance checking* mode). The reflexive and transitive closure of $\Longrightarrow$ is denoted by $\Longrightarrow^*$. Thus the language generated by G is $L(G) = \{w \in T^* \mid S \Longrightarrow^* w\}$.

In other words, the language $L(G)$ is composed of all the strings of terminal symbols that can be obtained starting from S by applying iteratively the matrices in M.

The family of languages generated by matrix grammars with appearance checking is denoted by MAT_{ac}.

G is called a *matrix grammar without appearance checking* if and only if $F = \emptyset$. In this case the generated family of languages is denoted by MAT.

The following results are proved:

Theorem 2.0.2 ([26])

- *$CF \subset MAT \subset MAT_{ac} = RE$.*
- *Each language $L \in MAT$, $L \subseteq a^*$, $a \in V$, is regular (the proof of this statement is constructive).*

The following results are known (e.g., [26]) or they can be derived from the above assertions and from the definitions given earlier.

Theorem 2.0.3

- $PsMAT_{ac} = PsRE$.
- $NMAT_{ac} = NRE$.
- $PsREG \subset PsMAT \subset PsRE$.
- $PsCF = PsREG$.
- $NMAT = NREG = NCF$.

A matrix grammar is called *pure* if there is no distinction between terminals and non-terminals. The language generated by a pure matrix grammar is composed of all the sentential forms. The family of languages generated by pure matrix grammars without appearance checking is denoted by $pMAT$.

Theorem 2.0.4 ([26]) $pMAT \subset MAT$

Matrix grammars without appearance checking are equivalent to *partially blind counter machines* (introduced in [42]). That is, the family of Parikh images of languages generated by matrix grammars without a.c. is equal to the family of sets of vectors of natural numbers generated by partially blind register machines (a constructive proof of their equivalence can be found, for example, in [30]).

From this last assertion and using results in [42] the following corollaries are obtained which are of relevance to the sequel.

Corollary 2.0.4.a
Emptiness: *Given an arbitrary alphabet T, an arbitrary matrix grammar without a.c., G, with terminal alphabet T, it is decidable whether or not $Ps_T(L(G)) = \emptyset$.*

Union, intersection, complementation: *The sets of Parikh images of languages generated by matrix grammars without a.c. are closed under union and intersection but not under complementation.*

Containment, Equivalence: *Given an arbitrary alphabet, T, two arbitrary matrix grammars without a.c., G and G', with terminal alphabet T, it is undecidable whether or not $Ps_T(L(G)) \subseteq Ps_T(L(G'))$ or whether or not $Ps_T(L(G)) = Ps_T(L(G'))$.*

From Theorem 2.0.2 and using the fact that containment of regular languages is decidable [46] the following result is obtained.

Theorem 2.0.5 Containment, Equivalence
Given an arbitrary terminal alphabet T of cardinality one, two arbitrary matrix grammars without a.c. G and G' over T, it is decidable whether or not $NL(G') \subseteq NL(G)$ and whether or not $NL(G) = NL(G')$.

A context-free *programmed grammar* with appearance checking is a construct $G = (N, T, S, P)$, where N, T, S are the set of non-terminals, the set of terminals and the start symbol, respectively, and P is a finite set of rules of the form $(b : A \rightarrow x, E_b, F_b)$, where b is a label, $A \rightarrow x$ is a context-free rule over $N \cup T$, and E_b, F_b are two sets of labels of rules of G (E_b is called the *success field* and F_b the *failure field* of the rule). If the failure field is empty for any rule of P, then the grammar is without appearance checking. The set of labels of P are denoted and defined as $Lab(P) = \{b \mid (b : A \rightarrow x, E_b, F_b) \in P\}$.

The language $L(G)$ generated by G is defined as the set of all the words $w \in T^*$ such that there is a derivation

$$S = w_0 \Rightarrow_{b_1} w_1 \Rightarrow_{b_2} w_2 \Rightarrow_{b_3} \ldots \Rightarrow_{b_k} w_k = w,$$

$k \geq 1$, and, for $(b_i : A_i \rightarrow x_i, E_{b_i}, F_{b_i})$, $1 \leq i \leq k$, one of the following conditions hold: $w_{i-1} = w'_{i-1} A_i w''_{i-1}, w_i = w'_{i-1} x_i w''_{i-1}$ for some $w'_{i-1}, w''_{i-1} \in$

$(N \cup T)^*$ and $b_{i+1} \in E_{b_i}$ or A_i does not occur in w_{i-1}, $w_{i-1} = w_i$ and $b_{i+1} \in F_{b_i}$.

In other words, a rule $(b_i : A_i \to x_i, E_{b_i}, F_{b_i})$ is applied as follows: if A_i is present in the sentential form, the rule is used and the next rule to be applied is chosen from those having labels in E_{b_i}; otherwise the sentential form remains unchanged, the next rule is chosen from the rules labelled by elements of F_{b_i} and an attempt is made to apply it. Without loss of generality it is supposed that there is a unique initial production having the axiom S called the *initial production* of G.

PR denotes the family of languages generated by programmed grammars without appearance checking and PR_{ac} denotes the family of languages generated by programmed grammars with appearance checking.

The following theorem is proved, e.g., in [26].

Theorem 2.0.6 $MAT = PR \subset MAT_{ac} = PR_{ac} = RE.$

The literature is rich with parallel rewriting devices, where the rewriting of the current sentential form is performed in a parallel way, rather than sequentially (as in the previously described grammars). Of these, Lindenmayer systems (or *L systems* for short) are possibly the most well known parallel rewriting systems.

An ET0L system is a construct $G = (\Sigma, T, H, w)$, where Σ is the alphabet, $T \subseteq \Sigma$ is the terminal alphabet; $H = \{h_1, h_2, \cdots, h_k\}$ is a finite set of finite substitutions (tables) over Σ and $w \in \Sigma^*$ is the axiom; each $h_i \in H$, $1 \leq i \leq k$, can be represented by a list of context-free productions $A \to x$, such that $A \in \Sigma$ and $x \in \Sigma^*$ (moreover, for each symbol A of Σ and each table h_i, $1 \leq i \leq k$, there is a production in h_i with A as left hand side); G defines a derivation relation $\Rightarrow_{h_i}$ by $x \Rightarrow_{h_i} y$ iff $y \in h_i(x)$, for some $1 \leq i \leq k$ (h_i is used as substitution). Only $x \Rightarrow y$ is written if the table is of no interest.

The language generated by G is $L(G) = \{z \in T^* \mid w \Longrightarrow^* z\}$, where $\Longrightarrow^*$ is the the reflexive and transitive closure of $\Longrightarrow$. $ET0L$ denotes the family of languages generated by ET0L systems and note that it is known that $CF \subset ET0L \subset CS$ (see, e.g., [77]).

2.1 Membrane Systems

This thesis presents various models, results and applications associated to membrane computing [71, 72, 73]. The field is very large, hence reference is made to a context which has only direct relevance to the presented work. Several membrane computing paradigms are mentioned, together with some highlighted results, however a wider and more detailed treatment can be found in [72] and the books [71, 73].

Membrane Systems (i.e. P Systems) are models of computation inspired by the structure and function of biological cells; a so-called *natural* computational paradigm [50]. Since its introduction in 1998 by Gh. Păun, many extensions have been suggested and many results obtained; these latter mostly concerning computational power. A short introductory guide to the field can be found in [72], while an up-to-date bibliography is available via the web-page [81]. Recently, membrane systems have been successfully applied to systems biology and several models have been proposed for simulating biological processes (e.g., see [25], [67] and [62]).

The original definition describes membrane systems as being composed of an hierarchical nesting of membranes that enclose regions, modelling cellular structure, in which free-floating objects (i.e. 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. Some specific rules also exist for moving objects across membranes, for example, *symport* and *antiport* rules, modelling particular types of cellular transport. Some of these features are illustrated in Figure 2.1, which is a typical diagrammatic representation of a P System.

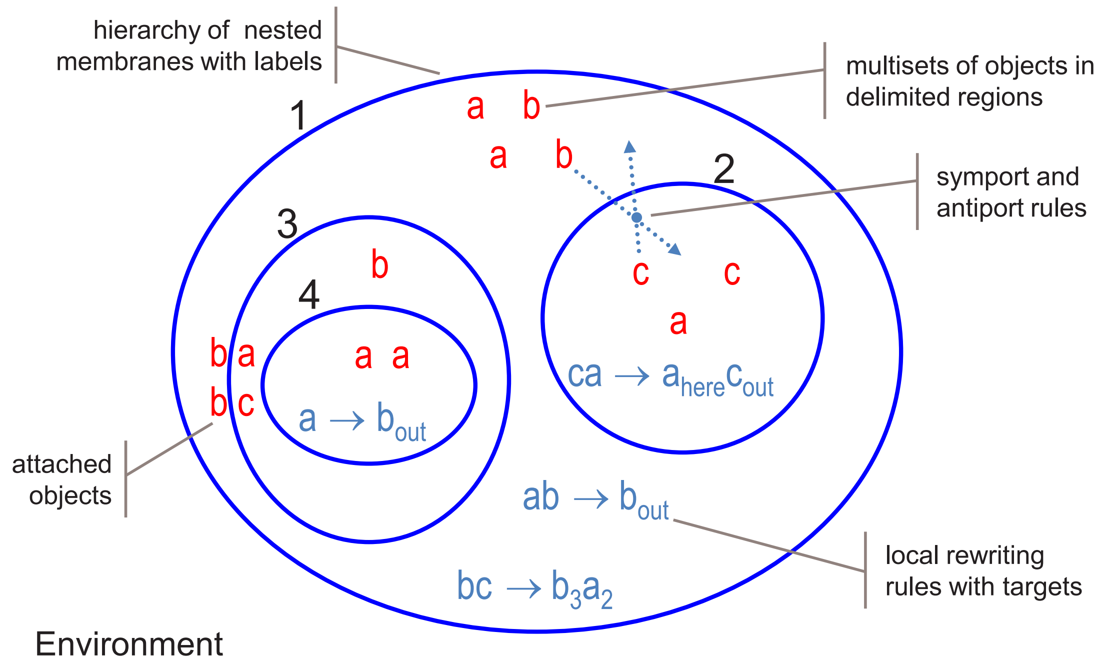
hierarchy of nested membranes with labels
multisets of objects in delimited regions
symport and antiport rules
attached objects
local rewriting rules with targets
1
2
3
4
a b
a b
b
c c
a
b a
b c
a a
$a \rightarrow b_{out}$
$ca \rightarrow a_{here}c_{out}$
$ab \rightarrow b_{out}$
$bc \rightarrow b_3a_2$
Environment

Figure 2.1: Diagrammatic representation of a P System (Membrane System) showing typical features.

In biology, all the compartments of a cell are in constant communication, with molecules being passed from a donor compartment to a target compartment, either via channels in the membrane or by means of membrane-enclosed transport *vesicles*. Once transported to the appropriate compartment, the molecules may then be processed by local biochemical reactions. Such behaviour is reminiscent of computer programs, where molecules represent the data being passed between functions, which thus correspond to the various compartments. Continuing the metaphor, the movement of vesicles (see, e.g., Figure 4.1) corresponds to the passing of classes or large data structures. This then is the inspiration of P Systems and other membrane-inspired natural computational paradigms.

While the original notion of a membrane system is apparently sufficient to emulate conventional computer programs, additional biological features have been added, both in order to investigate their relationship with computational power and to more accurately represent biology. In particular, the role of membranes, which was originally simply containment, has been extended to have associated objects which emulate the membrane proteins of biological membranes. One of the earliest models of this type can be found in [65], where a compartment exists within the phospholipid bilayer. Other variants are mentioned in later chapters, where they have more immediate relevance.

2.1.1 Membrane syntax

This section contains a summary of the syntax used to describe membrane systems in the remainder of the document.

A membrane is represented by a pair of square brackets, $[\ ]$. To each membrane may be associated a label that is written as a superscript to the right bracket denoting the membrane, e.g. $[\ ]^i$, and hence the membrane may then be referred to as membrane i. The structure of a membrane system (or *membrane structure*) is an hierarchical nesting of membranes enclosed by a root membrane, which in the literature is often referred to as the *skin* membrane. This structure is essentially that of a tree, where the nodes are the membranes and the arcs represent the containment relation. A formal mapping is avoided in the interest of intuitiveness, however, being a tree a membrane structure can be represented by a string of matched pairs of square brackets, e.g., $[\,[\,[\,]^2\,]^1\,[\,]^3\,]^0$.

Each membrane encloses a unique region and the contents of a region can consist of free objects as well as other membranes (hence it is possible to say that the region *contains* free objects as well as other membranes). Contained objects are written between the pair of brackets that define the containing membrane, either to the left, the right or between contained membranes. Objects may sometimes be individually represented as characters or strings of characters and sometimes as multisets denoted by single symbols or strings of symbols. For instance, in the system $[\ abb\ [\ aaaa\ ]^2\ ]^1$, the external membrane, labelled by 1, contains the free objects a, b, b and membrane 2. The same system could be represented in a more abstract way by $[\ \mathbf{ab}\ [\ \mathbf{c}\ ]^2\ ]^1$, where $\mathbf{a} = \{a\}$, $\mathbf{b} = \{b, b\}$ and $\mathbf{c} = \{a, a, a, a\}$ are multisets. For the remainder of this subsection the letters a, b and c denote individual objects. In subsequent sections and chapters the meanings of the symbols will be made clear as appropriate.

To each membrane there may be associated zero, one, two or three multisets (depending on the precise model) which correspond to the existence of membrane proteins attached to the membranes. In the case of zero, i.e. no membrane proteins, the syntax described above is sufficient. In the case of a model with one associated multiset, the multiset is written (in the various ways described above for the multiset contents of a membrane) as a subscript to the right bracket which denotes the membrane. E.g., the membrane written as $[\ abc\ ]^0_{bb}$ is membrane 0, contains the free objects a, b, c and has a single associated multiset containing objects b, b. The case of two associated multisets corresponds to proteins attached to the inner and outer surfaces of the membrane. In this case the inner surface multiset is written as a subscript on the left side of the right bracket which denotes the membrane, while the outer surface multiset is written as a subscript on the right side of the same bracket. E.g., the membrane written as $[\ aa\ {}_{bb}]^2_{cc}$ is membrane 2, contains free floating objects a, a, has b, b attached to its inner surface and c, c attached to its outer surface. Finally, in the case of three associated multisets, corresponding to inner, outer and *transmembrane* proteins[1], the three multisets are written as a subscript to

[1]The inner and outer surface proteins are later in this document referred to as *peripheral proteins* while transmembrane proteins are alternatively called *integral* proteins

the right bracket describing the associated membrane. To distinguish and identify the multisets they are written in the sequence inner, transmembrane, outer and separated by two | symbols. E.g., the membrane written as $[\ aabb\ ]^{v}_{bb|cc|dd}$ is membrane v containing free objects a, a, b, b, having inner surface multiset b, b, transmembrane multiset c, c and outer surface multiset d, d. When any of the associated multisets are empty they are simply omitted, however the two | symbols are always retained to avoid ambiguity. E.g., the membrane $[\ aa\ ]^{2}_{bb||cc}$ has essentially the same configuration as $[\ aa\ _{bb}]^{2}_{cc}$, however the former is using a three associated multiset membrane model while the latter a two associated multiset model.

Chapter 3

Membrane Systems with Peripheral Proteins

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The transport of substances and communication between compartments are fundamental biological processes mediated by the presence of complementary proteins attached to the surfaces of membranes, while within compartments substances are acted upon by local biochemical rules. Inspired by this knowledge, a model of Membrane Systems is created, having objects attached to the sides of the membranes and floating objects that can be moved between the regions of the system. Moreover, in each region there are evolution rules that *rewrite* the transported objects, mimicking chemical reactions.

This chapter investigates qualitative properties of the Membrane Systems with Peripheral Proteins (MSPP) model, such as configuration reachability in relation to the use of cooperative or non-cooperative evolution and transport rules and in the contexts of free-and maximal-parallel evolution.

3.1 Introduction and motivations

In the original definition, briefly discussed in Section 2.1, a Membrane System (a.k.a. a P System) is composed of an hierarchical nesting of membranes that enclose regions in which floating objects exist. Each region can have associated rules for evolving these objects (called evolution rules, modelling the biochemical reactions present in cell regions), and/or rules for moving objects across membranes (called symport/antiport rules, modelling some specific kinds of transport rules present in cells). Recently, inspired by *brane calculus*, [14], a model of membrane systems, having objects attached to the membranes, was introduced in [15]. Other models bridging brane calculus and membrane systems have been proposed in [55, 70]. A more general approach, considering both free floating objects and objects attached to the membranes has been proposed and investigated in [8]. The basic idea is that membrane operations are moderated by the objects (proteins) attached to the membranes. However, in these models objects are associated to an atomic membrane which has no concept of inner or outer surface. In reality, many biological processes are driven and controlled by the presence, on the appropriate side of a membrane, of specific proteins. For instance, receptor-mediated endocytosis, exocytosis and budding in eukaryotic cells are processes where the presence of proteins on the internal and external surfaces of a membrane is crucial (see e.g., [2]).

These processes are, for instance, used by eukaryotic cells to take up macromolecules and deliver them to digestive enzymes stored in lysosomes inside the cells. In general, all the compartments of a cell are in constant communication, with molecules being passed from a donor compartment to a target compartment by means of numerous membrane-enclosed transport packages, or *transport vesicles.* Once transported to the correct compartment the substances are then processed by means of local biochemical reactions (see e.g., [2]).

Motivated by this, a model called Membrane Systems with Peripheral Proteins is presented, combining some basic features found in biological cells: (*i*) *evolution* of objects (molecules) by means of multiset rewriting rules associated with specific regions of the systems (the rules model biochemical reactions); (*ii*) *transport* of objects across the regions of the system by means of rules associated with the membranes of the system and involving proteins attached to the membranes (on one or possibly both the two sides) and (*iii*) rules that take care of the *attachment/de-attachment* of objects to/from the sides of the membranes. Moreover, since it is desired to distinguish the functioning of different regions, a unique identifier (a label) is also associated to each membrane.

In this chapter a detailed qualitative investigation of the model is given using two alternative evolution strategies. The first is based on *free parallelism*: at each step of the evolution of the system an arbitrary number of rules may be applied. It is shown that, in this case, useful properties like configuration reachability can be decided, even in the presence of cooperative evolution and transport rules.

Maximal parallel evolution is also considered: if a rule can be applied then it *must* be applied, with alternative possible rules being chosen nondeterministically. This strategy models, for example, the behaviour in biology where a process takes place as soon as resources become available. In this case it is shown that configuration reachability becomes an undecidable property when the systems use non-cooperative evolution rules coupled with cooperative transport rules. However, several other cases where the problem remains decidable are also presented.

Note that the model presented in this chapter and the one in Chapter 4 have a static membrane structure. Some other P Systems models and paradigms based on Brane Calculi [14], for example, have a membrane structure which evolves under the control of objects bound to the membranes, using operations of the exo-, endo- and phagocytosis type. In further contrast to the models presented here, the membranes of Brane Calculi do not contain free objects; free objects being a standard feature of P systems models. Of more direct relevance to this thesis are P systems where objects fixed to membranes control the evolution of objects in the neighbouring regions. In a recently published book chapter [17], a distinction is drawn between 'Ruston models' [68, 70] and 'Trento models' [8, 18, 20]. The difference between these two approaches can be summarised by saying that the the Ruston models treat free objects and objects associated to membranes as disjoint sets (free objects being 'objects' and attached objects being 'proteins') while the Trento models allow free objects to attach and de-attach.

The formal language and notational preliminaries given in Chapter 2 are a prerequisite to understanding what follows.

3.2 Membrane Operations with Peripheral Proteins

This section presents the operations that govern the MSPP model and defines terminology used in the sequel. The membrane syntax and other conventions conforms to the description given in Section 2.1.1.

To each topological *side* of a membrane are associated multisets u and v (over a particular alphabet V) and this is denoted by $[\ _u]_v$. The membrane is said to be *marked* by u and v; v is called the *external marking* and u the *internal marking*; in general, they are referred to as *markings* of the membrane. The objects of the alphabet V are called *proteins* or, simply, *objects*. An object is called *free* if it is not attached to the sides of a membrane, so is not part of a marking.

As has been described in Section 2.1.1, each membrane is labelled and encloses a region whose contents may consist of free objects and/or other membranes.

Rules are considered that model the attachment of objects to the sides of the membranes. These rules extend the definition given in [8].

$$
\begin{aligned}
attach &: \ [\ a\ _{u}]^{i}_{v} \rightarrow [\ _{ua}]^{i}_{v}, \quad a[\ _{u}]^{i}_{v} \rightarrow [\ _{u}]^{i}_{va} \\
de-attach &: \ [\ \ _{ua}]^{i}_{v} \rightarrow [a\ _{u}]^{i}_{v}, \quad [\ _{u}]^{i}_{va} \rightarrow [\ _{u}]^{i}_{v}a
\end{aligned}
$$

with $a \in V$, $u, v \in V^*$ and $i \in Lab$.

The semantics of the attachment rules (*attach*) is as follows.

For the first case, the rule is *applicable* to the membrane i if the membrane is marked by multisets *containing* the multisets u and v on the appropriate sides, and region i contains an object a. In the second case, the rule is applicable to membrane i if it is marked by multisets containing the multisets u and v, as before, and is contained in a region that contains an object a. If the rule is applicable it is said that the objects defined by u, v and a can be *assigned* to the rule (so that it may be executed).

In both cases, if a rule is applicable and the objects given in u, v and a are assigned to the rule, then the rule can be executed and the object a is added to the appropriate marking in the way specified. The objects not involved in the application of a rule are left unchanged in their original positions.

The semantics of the detachment rule (*de-attach*) is similar, with the difference that the attached object a is detached from the specified marking and added to the contents of either the internal or external region.

Rules associated to the membranes are now considered that control the passage of objects across the membranes:

$$\begin{aligned} move_{in} &: \; a[\ _u]^i_v \rightarrow [\ a\ _u]^i_v \\ move_{out} &: \; [\ a\ _u]^i_v \rightarrow a[\ _u]^i_v \end{aligned}$$

with $a \in V$, $u, v \in V^*$ and $i \in Lab$.

The semantics of the rules is as follows.

In the first case, the rule is applicable to membrane i if it is marked by multisets containing the multisets u and v, on the appropriate sides, and the membrane is contained in a region containing an object a. The objects defined by u, v and a can thus be assigned to the rule.

If the rule is applicable and the objects a, u and v are assigned to the rule then the rule can be executed and, in this case, the object a is removed from the contents of the region surrounding membrane i and added to the contents of region i.

In the second case the semantics is similar, but here the object a is moved from region i to its surrounding region.

The rules of attach, de-attach, move$_{in}$, move$_{out}$ are generally called *membrane rules* (denoted collectively as mem_{rul}) over the alphabet V and the set of labels Lab.

Membrane rules for which $|uv| \geq 2$ are called *cooperative* membrane rules (in short, coo_{mem}). Membrane rules for which $|uv| = 1$ are called *non-cooperative* membrane rules (in short, $ncoo_{mem}$). Membrane rules for which $|uv| = 0$ are called *simple* membrane rules (in short, sim_{mem}).

Evolution rules are also presented that involve objects but not membranes. These can be considered to model the biochemical reactions that take place inside the compartments of the cell. They are evolution rules over the alphabet V and set of labels Lab and they follow the definition that can be found in evolution-communication P systems [16]. Defining

$$evol: \ [u \to v]^i$$

with $u \in V^+, v \in V^*$ and $i \in Lab$, an evolution rule is then called *cooperative* (in short, coo_e) if $|u| > 1$, otherwise the rule is called *non-cooperative* ($ncoo_e$).

The rule is applicable to region i if the region *contains* a multiset of free objects that *includes* the multiset u. The objects defined by u can thus be assigned to the rule.

If the rule is applicable and the objects defined by u are assigned to the rule, then the rule can be executed. In this case the objects specified by u are subtracted from the contents of region i while the objects specified by v are added to the contents of the region i.

3.3 Membrane Systems with Peripheral Proteins

Using the evolution and membrane rules defined in section 3.2, it is now possible to define membrane systems with peripheral proteins.

A *membrane system with peripheral proteins* (in short, a P_{pp} system) and n membranes, is a construct

$$\Pi = (V, \mu, (u_1, v_1), \ldots, (u_n, v_n), w_1, \ldots, w_n, R, R^m)$$

where:

- V is a finite, non-empty alphabet of objects (proteins).
- μ is a membrane structure with $n \geq 1$ membranes, injectively labelled by $1, 2, \ldots, n$.

- $(u_1, v_1), \dots, (u_n, v_n) \in V^* \times V^*$ are the markings associated, at the beginning of any evolution, to the membranes $1, 2, \dots, n$, respectively. They are called *initial markings* of Π; the first element of each pair specifies the internal marking, while the second one specifies the external marking.

- $w_1, \dots, w_n$ specify the multisets of free objects contained in regions $1, 2, \dots, n$, respectively, at the beginning of any evolution and they are called *initial contents* of the regions.

- R is a finite set of evolution rules over V and the set of labels $Lab = \{1, \dots, n\}$.

- R^m is finite set of membrane rules over the alphabet V and set of labels $Lab = \{1, \dots, n\}$.

3.4 Evolution of the System

A *configuration* of Π consists of a membrane structure, the markings of the membranes (internal and external) and the multisets of free objects present inside the regions. In what follows, configurations are denoted by writing the markings as subscripts (internal and external) of the parentheses which identify the membranes.

A standard labelling is supposed: 0 is the label of the *environment* that surrounds the entire system Π; 1 is the label of the *skin* membrane that separates Π from the *environment*.

The *initial configuration* consists of the membrane structure μ, the initial markings of the membranes and the initial contents of the regions; the environment is empty at the beginning of the evolution.

$\mathcal{C}(\Pi)$ denotes the set of all possible configurations of Π.

The existence of a clock is assumed which marks the timing of steps (single *transitions*) for the whole system.

A transition from a configuration $C \in \mathcal{C}(\Pi)$ to a new one is obtained by assigning the objects present in the configuration to the rules of the system and then executing the rules as described in Section 3.2.

Two possible ways of assigning the objects to the rules are defined: free-parallel and maximal-parallel.

- *Free-Parallel Evolution.*

  In each region and for each marking, *an arbitrary number* of applicable rules is executed (membrane and evolution rules have equal precedence). A single object (free or not) may only be assigned to a single rule.

  This implies that in one step, no rule, one rule or as many applicable rules *as desired* may be applied. That is, an arbitrary strategy of applying applicable rules can be chosen. This strategy is similar to the one introduced in ([71], Section 3.4).

  A single transition performed in a free-parallel way a is called a *free-parallel transition.*

- *Maximal-Parallel Evolution.*

  In each region and for each marking, applicable rules chosen in a non-deterministic way are assigned objects, also chosen in a non-deterministic way, such that after the assignment no further rule is applicable using the unassigned objects. As with free-parallel evolution, membrane and evolution rules have equal precedence and a single object (free or not) may only be assigned to a single rule.

  A single transition performed in a maximal-parallel way is called a *maximal-parallel transition.*

A sequence of free-parallel [maximal-parallel] transitions, starting from the initial configuration, is called a free-parallel [maximal-parallel, resp.] *evolution*. An evolution (free or maximal parallel) is said to be *halting* if it halts, that is, if it reaches a *halting configuration*, i.e., a configuration where no rule can be applied anywhere in the system.

A configuration of a P_{pp} system Π that can be reached by a free-parallel [maximal-parallel] evolution, starting from the initial configuration, is called free-parallel [maximal-parallel, resp.] *reachable*. A pair of multisets (u, v) is a free-parallel [maximal-parallel] *reachable marking* for Π if there exists a free-parallel [maximal-parallel, resp.] reachable configuration of Π which contains at least one membrane marked internally by u and externally by v.

$\mathcal{C}_R(\Pi, fp)$ [$\mathcal{C}_R(\Pi, mp)$] denotes the set of all free-parallel [maximal parallel, resp.] reachable configurations of Π and $\mathcal{M}_R(\Pi, fp)$ [$\mathcal{M}_R(\Pi, mp)$] denotes the set of all free-parallel [maximal-parallel, resp.] reachable markings of Π.

Moreover, $\mathcal{P}_{pp,m}(\alpha, \beta)$, $\alpha \in \{coo_e, ncoo_e\}$, $\beta \in \{coo_{mem}, ncoo_{mem}, sim_{mem}\}$ denotes the class of membrane systems with peripheral proteins, evolution rules of type α, membrane rules of type β, and m membranes (m is changed to $*$ if it is unbounded). α or β are omitted from the notation if the corresponding types of rules are not allowed. V_Π is also used to denote the alphabet V of the system Π.

3.5 Reachability with Free-Parallel Evolution

It is desirable to know whether or not a biological system can evolve to a particular specified configuration. Hence it would be useful to construct models having such qualitative properties to be decidable.

Using the presented model it is possible to prove that when the evolution is free-parallel it is decidable, for an arbitrary membrane system with peripheral proteins and an arbitrary configuration, whether or not such a configuration is reachable by the system. A proof can be given by showing that all the reachable configurations of a system Π can be produced by a pure matrix grammar without appearance checking. Moreover, it is shown that the reachability of an arbitrary marking can be decided.

Lemma 3.5.1 *It is decidable whether or not, for any P_{pp} system Π from $\mathcal{P}_{pp,1}(coo_e)$ and any configuration C of Π, $C \in \mathcal{C}_R(\Pi, fp)$.*

Proof Let $\Pi = (V, \mu = [\ ]^1, (u_1, v_1), w_1, R)$. First notice that since membrane rules are excluded, any configuration C of Π is effectively the contents of the unique region and therefore, being a multiset, can be represented by a string w_C, as described in Chapter 2 (every permutation of the string w_C represents the same contents, so the same configuration C). A pure matrix grammar without appearance checking G is constructed such that $L(G)$ contains all and only the strings representing the configurations in $\mathcal{C}_R(\Pi)$.

The grammar $G = (N, S, M)$ is defined in the following way. $N = V \cup V^{\#}$, with $V^{\#} = \{v^{\#} \mid v \in V\}$. Added to M is the matrix $(S \to w_1)$ and, for each rule $[x \to y]^1 \in R$, the matrix

$(x_1 \to x_1^{\#}, x_2 \to x_2^{\#}, \ldots, x_k \to x_k^{\#}, x_1^{\#} \to \lambda, x_2^{\#} \to \lambda, \ldots, x_k^{\#} \to y_1 y_2 \cdots y_q)$

where $x = x_1 x_2 \cdots x_k$ and $y = y_1 y_2 \cdots y_q$. Each application of a matrix simulates the application of an evolution rule inside the unique region of the system. The markings are not involved in the evolution of the system since membrane rules are not allowed. It can be seen immediately that, for each string w in $L(G)$ (i.e., all the sentential forms generated by G) there is an evolution of Π, starting from the initial configuration, that reaches the configuration represented by w. Moreover, it is easy to see that the reverse is also true since the evolution of Π is based on free parallelism:

for each reachable configuration C' of Π there exists a derivation of G that generates a string representing C'. In fact it can be seen that $L(G)$ contains all the strings representing configurations of Π reached by applying at each step a single evolution rule. In the case a configuration C' is reached by applying more than a unique evolution rule in a single step, a single step can be simulated in G by applying an appropriate sequence of matrices.

Therefore, to check whether or not an arbitrary configuration C of Π can be reached, it is only necessary to check whether any of the strings representing C is in $L(G)$. This can be done since there is only a finite number of strings representing C and the membership problem for pure matrix grammars without appearance checking is decidable (for the proof see [45]); therefore the Lemma follows. $\square$

Theorem 3.5.1 *It is decidable whether or not, for any P_{pp} system Π from $\mathcal{P}_{pp,*}(coo_e, coo_{mem})$ and any configuration C of Π, $C \in \mathcal{C}_R(\Pi, fp)$.*

Proof The main idea of the proof is that the problem can be reduced to check whether or not a configuration of a system from $\mathcal{P}_{pp,1}(coo_e)$ is reachable, and this is decidable (Lemma 3.5.1).

Suppose $\Pi = (V, \mu, (u_1, v_1), \ldots, (u_n, v_n), w_1, \ldots, w_n, R, R^m)$. By $cont(i)$ is denoted the label of the region surrounding membrane i (recall that 0 is the label of the environment and 1 is the label of the skin membrane).

$\overline{\Pi} = (\overline{V}, [\,]^1, (\lambda, \lambda), \overline{w_1}, \overline{R})$ from $\mathcal{P}_{pp,1}(coo_e)$ is constructed in the following way.

Defining $\overline{V} = \bigcup_{i \in \{1,\ldots,n\}} (V_i' \cup V_i'') \cup \bigcup_{i \in \{0,1,\ldots,n\}} V_i$ with $V_i = \{a_i \mid a \in V\}$, $V_i' = \{a_i' \mid a \in V\}$, $V_i'' = \{a_i'' \mid a \in V\}$.

Morphisms h_i, h_i', h_i'' are used, defined as follows.

- $h_i : V \to V_i$ defined by $h_i(a) = a_i, a \in V$, for $i \in \{0, 1, \ldots, n\}$
- $h_i' : V \to V_i'$ defined by $h_i'(a) = a_i', a \in V$, for $i \in \{1, \ldots, n\}$

- $h''_i : V \rightarrow V''_i$ defined by $h''_i(a) = a''_i, a \in V$, for $i \in \{1, \ldots, n\}$

$\overline{w_1}$ is defined as the string $h_1(w_1) \cdots h_n(w_n) h'_1(u_1) \cdots h'_n(u_n) h''_1(v_1) \cdots h''_n(v_n)$

For each rule $move_{in}$, $a[\ {}_u]^i_v \rightarrow [\ a\ {}_u]^i_v \in R^m$, $i \in \{1, \ldots, n\}$ the following rules are added to $\overline{R}$: $[\ a_k h'_i(u) h''_i(v) \rightarrow a_i h'_i(u) h''_i(v)]^1$, with $k = cont(i)$.

In the same way all the other rules present in $R \cup R^m$ can be *translated* in the evolution rules for $\overline{R}$.
Hence, given a configuration C of Π, one can construct the configuration $\overline{C}$ of $\overline{\Pi}$ having a unique region in the following way.

For each free object a contained in region i (the environment if $i = 0$) in C, $i \in \{0, 1, \ldots, n\}$ the object $h_i(a)$ is added to region 1 of $\overline{C}$. For each object a present in the internal marking of membrane i in C, $i \in \{1, \ldots, n\}$ is added the object $h'_i(a)$ to region 1 of $\overline{C}$ and finally for each object a present in the external marking of membrane i, $i \in \{1, \ldots, n\}$ is added the object $h''_i(a)$ to region 1 of $\overline{C}$.

Now it is possible to decide (Lemma 3.5.1) whether or not $\overline{C} \in \mathcal{C}_R(\overline{\Pi}, fp)$. From the way $\overline{\Pi}$ has been constructed it follows that:

- if $\overline{C} \in \mathcal{C}_R(\overline{\Pi}, fp)$ then $C \in \mathcal{C}_R(\Pi, fp)$.
- if $\overline{C} \notin \mathcal{C}_R(\overline{\Pi}, fp)$ then $C \notin \mathcal{C}_R(\Pi, fp)$.

and from this the Theorem follows.

□

Corollary 3.5.1.a *It is decidable whether or not, for any P system* Π *from* $\mathcal{P}_{pp,n}(mem_{rul}, coo), n \geq 1$ *and any pair of multisets* (u, v) *over* V_Π, $(u, v) \in \mathcal{M}_R(\Pi, fp)$.

Proof Given Π from $\mathcal{P}_{pp,n}(mem_{rul}, coo)$ and with alphabet of objects V, one can construct $\overline{\Pi} = (\overline{V}, \mu = [\ ]^1, (\lambda, \lambda), \overline{w_1}, \overline{R})$ from $\mathcal{P}_{pp,1}(coo)$ in the way described by Theorem 3.5.1.

Therefore, using $\overline{\Pi}$ one can construct the grammar G as described by Lemma 3.5.1 such that $L(G)$ contains all and only the strings representing the configurations in $\mathcal{C}_R(\overline{\Pi}, fp)$.

Now to check whether or not an arbitrary $(u, v) \in \mathcal{M}_R(\Pi, fp)$ one needs to check whether or not there exists an $i \in \{1, \ldots, n\}$ such that $(Perm(h'_i(u))\xi(\overline{V})^*) \cap L(G) \neq \emptyset$ *and* $(Perm(h''_i(v))\xi(\overline{V})^*) \cap L(G) \neq \emptyset$, where h'_i and h''_i are morphisms from V to V'_i and to V''_i, respectively, defined as in Theorem 3.5.1, and ξ denotes the shuffle operation.

The permutation and shuffle operation are used to construct all possible strings representing a configuration of $\overline{\Pi}$ containing the membrane i marked by multiset u internally and multiset v externally.

The languages $(Perm(h'_i(u))\xi(\overline{V})^*) \cap L(G)$ and $(Perm(h''_i(v))\xi(\overline{V})^*) \cap L(G)$ can be generated by matrix grammars without appearance checking (see, Theorem 2.0.1 and e.g., [26]) and the emptiness problem for this class of grammars is decidable (see, e.g., [26]). Therefore the Corollary follows.

□

The proof of the "reverse" Theorem is now sketched.

Theorem 3.5.2 *For any pure matrix grammar $G = (N, S, M)$ without a.c. there exists a P_{pp} system Π from $\mathcal{P}_{pp,*}(coo_e)$ such that, given an arbitrary string $w \in N^*$, $w \in L(G)$ if and only if $C_w \in \mathcal{C}_R(\Pi, fp)$ with C_w a configuration of Π obtained from w.*

Proof Let $G = (N, S, M)$ be a pure matrix grammar. Suppose, without loss of generality, that M has n matrices (indicated by $m_i, 1 \leq i \leq n$) and each matrix has p productions. So $m_{i,k}$, $1 \leq i \leq n, 1 \leq k \leq p$ indicates the production k of matrix i.

Π is then constructed in the following way.

$$\Pi = (V, [\,]^1, (\lambda, \lambda), w_1, R = R^{ev}, R^m = \emptyset)$$

with $V = N \cup \{(i,k) \mid 1 \leq i \leq n, 1 \leq k \leq p\}$. For each matrix $m_l : (1 : A_1 \to \alpha_1, 2 : A_2 \to \alpha_2, \ldots, p : A_p \to \alpha_p), 1 \leq l \leq n$ the evolution rules $[(l,1)A_1 \to \alpha_1(l,2)]^1$, $[(l,2)A_2 \to \alpha_2(l,3)]^1, \ldots, [(l,p)A_p \to \alpha_p(i,1)]^1$, $1 \leq i \leq n$ is added to R^{ev}.

From the construction it is clear that an arbitrary $w \in N^*$ is in $L(G)$ if and only if C_w is in $\mathcal{C}_R(\Pi, fp)$, where C_w is the configuration of Π represented by (any of) the permutations of string w. □

Corollary 3.5.2.b *It is decidable whether or not, for any P_{pp} system Π from $\mathcal{P}_{pp,*}(coo_e, coo_{mem})$ and any pair of multisets (u,v) over V_Π, $(u,v) \in \mathcal{M}_R(\Pi, fp)$.*

Proof Given Π from $\mathcal{P}_{pp,n}(coo_{mem}, coo_e)$ with alphabet of objects V, one can construct $\overline{\Pi} = (\overline{V}, \mu = [\ ]^1, (\lambda, \lambda), \overline{w_1}, \overline{R})$ from $\mathcal{P}_{pp,1}(coo_e)$ in the way described by Theorem 3.5.1.

Therefore, using $\overline{\Pi}$ it is possible to construct the grammar G as described by Lemma 3.5.1 such that $L(G)$ contains all and only the strings representing the configurations in $\mathcal{C}_R(\overline{\Pi}, fp)$.

Now, to check whether or not an arbitrary $(u,v) \in \mathcal{M}_R(\Pi, fp)$ one needs to check whether or not there exists an $i \in \{1, \ldots, n\}$ such that $(Perm(h'_i(u))\xi(\overline{V})^*) \cap L(G) \neq \emptyset$ *and* $(Perm(h''_i(v))\xi(\overline{V})^*) \cap L(G) \neq \emptyset$, where h'_i and h''_i are morphisms from V to V'_i and to V''_i, respectively, defined as in Theorem 3.5.1, and ξ denotes the shuffle operation.

The permutation and shuffle operations are used to construct all possible strings representing a configuration of $\overline{\Pi}$ containing the membrane i marked by multiset u internally and by multiset v externally.

The languages $(Perm(h'_i(u))\xi(\overline{V})^*) \cap L(G)$ and $(Perm(h''_i(v))\xi(\overline{V})^*) \cap L(G)$ can be generated by matrix grammars without appearance checking (see Theorem 2.0.1 and e.g., [26]) and the emptiness problem for this class of grammars is decidable (see, e.g., [26]). Therefore the Corollary follows.

□

3.6 Reachability with Maximal-Parallel Evolution

Using the presented model to describe a biological system which evolves in a maximal-parallel way, it is shown that the reachability of a specified configuration is decidable when the evolution rules used are non-cooperative and the membrane rules are simple or when the system uses only membrane rules (including cooperative membrane rules).

It is further shown that it is undecidable whether or not an arbitrary configuration can be reached by an arbitrary system working in the maximal-parallel way and using non-cooperative evolution rules coupled with cooperative membrane rules. The proof is based on the fact that, in this case, a P_{pp} system can simulate the derivations of a programmed grammar with appearance checking.

First, systems with only membrane rules are analysed.

Theorem 3.6.1 *It is decidable whether or not:*

- *For an arbitrary P_{pp} system Π from $\mathcal{P}_{pp,*}(coo_{mem})$ and an arbitrary configuration C of Π, $C \in \mathcal{C}_R(\Pi, mp)$.*
- *For an arbitrary P_{pp} system Π from $\mathcal{P}_{pp,*}(coo_{mem})$ and an arbitrary pair of multisets u, v over V_Π, $(u, v) \in \mathcal{M}_R(\Pi, mp)$.*

Proof Given a P_{pp} system from $\mathcal{P}_{pp,*}(coo_{mem})$ the number of possible reachable configurations for Π is finite because the system can only use membrane rules (which neither add nor remove objects). So the problem is decidable (by an exhaustive search). □

Systems having non-cooperative evolution and simple membrane rules are now investigated.

Lemma 3.6.1 *It is decidable whether or not, for an arbitrary P_{pp} system Π from $\mathcal{P}_{pp,1}(ncoo_e)$ and an arbitrary configuration C of Π, $C \in \mathcal{C}_R(\Pi, mp)$.*

Proof

Let $\Pi = (V, \mu = [\ ]^1, (u_1, v_1), w_1, R)$. As already mentioned in Lemma 3.5.1, any configuration C of Π is effectively the contents of the unique region and therefore, being a multiset, can be represented by a string w_C (every permutation of the string w_C represents the same contents, so the same configuration C). An $ET0L$ system $G = (\Sigma, \Sigma, h_1, w_1)$ (i.e., only one table and $\Sigma = T$) is constructed such that $L(G)$ contains all and only the strings representing the configurations in $\mathcal{C}_R(\Pi, mp)$.

The grammar $G = (\Sigma, \Sigma, h_1, w_1)$ is defined in the following way. $\Sigma = V$. Added to h_1 is the production $(S \to w_1)$ and, for each rule $[a \to \alpha]^1 \in R$, the production $a \to \alpha$.

The markings are not involved in the evolution of the system since membrane rules are not allowed. It is immediately clear that for each string w in $L(G)$ (i.e., all the sentential forms generated by G) there is an evolution of Π, starting from the initial configuration, that reaches the configuration represented by w. Moreover, it is easy to see that, for each reachable configuration C of Π, there exists a derivation of G that generates a string representing C (because Π works in maximal parallel way).

Therefore to check whether or not an arbitrary configuration C of Π can be reached, it is only necessary to check whether any of the strings representing C is in $L(G)$. This can be done since there is only a finite number of strings representing C and the membership problem for $ET0L$ systems is decidable (see, e.g., [26]); therefore the Lemma follows. $\square$

Theorem 3.6.2 *It is decidable whether or not, for an arbitrary P_{pp} system Π from $\mathcal{P}_{pp,*}(ncoo_e, sim_{mem})$ and an arbitrary configuration C of Π, $C \in \mathcal{C}_R(\Pi, mp)$.*

Proof

The idea of the proof closely follows the one given in Theorem 3.5.1 and is therefore only sketched here.

Suppose $\Pi = (V, \mu, (u_1, v_1), \ldots, (u_n, v_n), w_1, \ldots, w_n, R, R^m)$, then $\overline{\Pi} = (\overline{V}, [\,]^1, (\lambda, \lambda), \overline{w_1}, \overline{R})$ from $\mathcal{P}_{pp,1}(ncoo_e)$ is constructed using the morphisms h_i, h'_i, h''_i, as in Theorem 3.5.1. In this way it is easy to see that (using the same idea of Theorem 3.5.1), given an arbitrary configuration of Π, $C \in \mathcal{C}_R(\Pi, mp)$ if and only if $C \in \mathcal{C}_R(\overline{\Pi}, mp)$.

The Theorem follows using Lemma 3.6.1. □

Corollary 3.6.2.a *It is decidable whether or not, for any P_{pp} system Π from $\mathcal{P}_{pp,*}(ncoo_e, sim_{mem})$ and any pair of multisets (u, v) over V_Π, $(u, v) \in \mathcal{M}_R(\Pi, mp)$.*

Proof The idea of the proof follows closely the one given in Corollary 3.5.2.b so once again it is only sketched here.

Suppose $\Pi = (V, \mu, (u_1, v_1), \ldots, (u_n, v_n), w_1, \ldots, w_n, R, R^m)$, then $\overline{\Pi} = (\overline{V}, [\,]^1, (\lambda, \lambda), \overline{w_1}, \overline{R})$ from $\mathcal{P}_{pp,1}(ncoo_e)$ is constructed using the morphisms h_i, h'_i, h''_i, as in Theorem 3.5.1. Using $\overline{\Pi}$ one can construct an ET0L system G as described by Lemma 3.6.1 such that $L(G)$ contains all and only the strings representing the configurations in $\mathcal{C}_R(\overline{\Pi}, mp)$.

Now, to check whether or not an arbitrary pair of multisets over V_Π (u, v) is in $\mathcal{M}_R(\Pi, mp)$ one needs to check whether or not there exists an $i \in \{1, \ldots, n\}$ such that
$(Perm(h'_i(u))\xi(\overline{V})^*) \cap L(G) \neq \emptyset$ *and* $(Perm(h''_i(v))\xi(\overline{V})^*) \cap L(G) \neq \emptyset$ (ξ denotes the shuffle operation).

The permutation and shuffle operation are used to construct all possible strings representing a configuration of $\overline{\Pi}$ containing the membrane i marked by multiset u internally and by multiset v externally.

The languages $(Perm(h'_i(u))\xi(\overline{V})^*) \cap L(G)$ and $(Perm(h''_i(v))\xi(\overline{V})^*) \cap L(G)$ can be generated by an ET0L system (see Theorem 2.0.1 and e.g., [26]) and the emptiness problem for ET0L systems is decidable (see, e.g., [26]). Therefore the Corollary follows. $\square$

Systems having non-cooperative evolution rules and cooperative membrane rules are investigated now, showing that in this case the reachability of an arbitrary configuration becomes an undecidable problem.

Theorem 3.6.3 *It is undecidable whether or not, for an arbitrary P_{pp} system Π from $\mathcal{P}_{pp,*}(ncoo_e, coo_{mem})$ and an arbitrary configuration C of Π, $C \in \mathcal{C}_R(\Pi, mp)$.*

Proof Given a programmed grammar with appearance checking $G = (N, T, S, P)$, as defined in Chapter 2, suppose that $Lab(P) = \{0, 1, 2, \ldots, n\}$ and 0 is the label of the initial production of G. Defining $\overline{N} = \{\overline{x} \mid x \in N\}$ and $\overline{T} = \{\overline{a} \mid a \in T\}$, the morphism $h : N \cup T \to \overline{N} \cup \overline{T}$ is defined by $h(x) = \overline{x}$ for $x \in N \cup T$. A_i indicates the non-terminal on the left-hand side of the production with label i.

The P_{pp} system Π is defined as follows:

$$\Pi = (V, \mu, (u_1, v_1), (u_2, v_2), (u_3, v_3), w_1, w_2, w_3, R, R^m)$$

with

$$\begin{aligned}
V &= N \cup T \cup \overline{N} \cup \overline{T} \cup V' \cup V'' \text{ with} \\
V' &= \{l_i, l'_i, l''_i, \overline{l_i}, \overline{\overline{l_i}} \mid i \in Lab(P)\} \cup \{\#\} \cup \{Y', Y'', \ldots, Y^{vu}, d, l_{-1}\} \\
V'' &= \{h^s_i, \overline{h^s_i}, \overline{l^s_i}, \overline{\overline{l^s_i}}, \overline{\overline{\overline{l^s_i}}}, (l^s_i)' \mid i \in Lab(P)\} \\
&\cup \{X^s, (X^s)', (X^s)'', (X^s)''', (X^s)^{iv}, Y^s, (Y^s)', (Y^s)'', \ldots, (Y^s)^{vuu}\}
\end{aligned}$$

$$
\begin{aligned}
\mu &= [\,[\,]^2\,[\,]^3\,]^1 \\
u_1 &= v_1 = u_2 = v_2 = u_3 = v_3 = \lambda \\
w_2 &= \lambda, w_3 = \lambda, w_1 = l_{-1} S h_1^s \cdots h_n^s \\
R^m &= R' \cup R'' \text{ with} \\
R' &= \{l'_i[\,]^2 \to [\,]^2_{l'_i} \mid i \in Lab(P)\} && (3.1)\\
&\cup \{A[\,]^2_{l'_i} \to [A\,]^2_{l'_i} \mid i \in Lab(P), A \in N\} && (3.2)\\
&\cup \{[\overline{x}]^2 \to [\,]^2\overline{x} \mid x \in N \cup T\} && (3.3)\\
&\cup \{[\overline{l_i}]^2 \to [\,]^2\overline{l_i},\ [\,]^2_{l'_i} \to [\,]^2 l'_i,\ l''_i[\,]^3 \to [\,]^3_{l''_i} \mid i \in Lab(P)\} && (3.4)\\
&\cup \{\overline{\overline{l_i}}[\,]^3_{l''_i} \to [\overline{\overline{l_i}}\,]^3_{l''_i}, [\,\overline{\overline{l_i}}]^3_{l''_i} \to [\,{}_{\overline{\overline{l_i}}}]^3_{l''_i},\ \mid i \in Lab(P)\} && (3.5)\\
&\cup \{Y^{v\iota\iota}[\,{}_{\overline{\overline{l_i}}}]^3_{l''_i} \to [Y^{v\iota\iota}\,{}_{\overline{\overline{l_i}}}]^3_{l''_i},\ [\,{}_{\overline{\overline{l_i}}}]^3_{l''_i} \to [\,{}_{\overline{\overline{l_i}}}]^3 l''_i, \\
&\qquad\qquad [\,{}_{\overline{\overline{l_i}}}]^3 \to [\,\overline{\overline{l_i}}]^3 \mid i \in Lab(P)\} && (3.6)\\
R'' &= \{(l_i^s)'[\,]^2 \to [\,]^2_{(l_i^s)'},\ h_i^s[\,]^2_{(l_i^s)'} \to [\,h_i^s]^2_{(l_i^s)'}, \\
&\qquad\qquad [\,]^2_{(l_i^s)'} \to [\,]^2(l_i^s)' \mid i \in Lab(P)\} && (3.7)\\
&\cup \{[\,\overline{l_i^s}]^2 \to [\,]^2\overline{l_i^s},\ [\,\overline{h_i^s}]^2 \to [\,]^2\overline{h_i^s} \mid i \in Lab(P)\} && (3.8)\\
&\cup \{(l_i^s)'[\,]^3 \to [\,]^3_{(l_i^s)'},\ (X^s)^{iv}[\,]^3_{(l_i^s)'} \to [\,(X^s)^{iv}]^3_{(l_i^s)'} \mid i \in Lab(P)\} && (3.9)\\
&\cup \{A[\,]^3_{(l_i^s)'} \to [\,A]^3_{(l_i^s)'} \mid i \in Lab(P), A \in N\} && (3.10)\\
&\cup \{\overline{\overline{\overline{l_i^s}}}[\,]^3_{(l_i^s)'} \to [\,\overline{\overline{\overline{l_i^s}}}]^3_{(l_i^s)'},\ [\,\overline{\overline{\overline{l_i^s}}}]^3_{(l_i^s)'} \to [\,{}_{\overline{\overline{\overline{l_i^s}}}}]^3_{(l_i^s)'} \mid i \in Lab(P)\} && (3.11)\\
&\cup \{(Y^s)^{v\iota\iota\iota}[\,{}_{\overline{\overline{\overline{l_i^s}}}}]^3_{(l_i^s)'} \to [(Y^s)^{v\iota\iota\iota}\,{}_{\overline{\overline{\overline{l_i^s}}}}]^3_{(l_i^s)'} \mid i \in Lab(P)\} \\
&\qquad\qquad \cup \{l_{-1} \to l'_0 Y\} && (3.12)\\
&\cup \{[\,{}_{\overline{\overline{\overline{l_i^s}}}}]^3_{(l_i^s)'} \to [\,{}_{\overline{\overline{\overline{l_i^s}}}}]^3(l_i^s)',\ [\,{}_{\overline{\overline{\overline{l_i^s}}}}]^3 \to [\overline{\overline{\overline{l_i^s}}}]^3 \mid i \in Lab(P)\} && (3.13)\\
R &= (R^{ev})' \cup (R^{ev})'' \text{ with} \\
(R^{ev})' &= \{[l_j \to l'_i Y]^1 \mid i \in E(j), j \in Lab(P)\} \cup \{[l_{-1} \to l'_0 Y]^1\} && (3.14)\\
&\cup \{[Y \to Y']^1,\ [Y' \to Y'']^1, \ldots, [Y^{v\iota} \to Y^{v\iota\iota}]^1,\ [Y^{v\iota\iota} \to \#]^1\} && (3.15)\\
&\cup \{[\overline{x} \to x]^1 \mid x \in N \cup T\} && (3.16)\\
&\cup \{[\overline{l_i} \to \overline{\overline{l_i}}]^1 \mid i \in Lab(P)\} && (3.17)
\end{aligned}
$$

$$\begin{aligned}
&\cup \quad \{[l'_i \to l''_i]^1,\ [l''_i \to l_i]^1 \mid i \in Lab(P)\} && (3.18)\\
&\cup \quad \{[A \to h(\alpha)\overline{l_i}]^2 \mid (i : A \to \alpha, E(i), F(i)) \in P\} && (3.19)\\
&\cup \quad \{[Y^{v\iota\iota} \to \lambda]^3\} && (3.20)\\
&\cup \quad \{[\overline{\overline{l_i}} \to d]^3 \mid i \in Lab(P)\} && (3.21)\\
&\cup \quad \{[d \to \lambda]^3\} && (3.22)\\
(R^{ev})'' &= \quad \{[l_j \to (l^s_i)'Y^sX^s]^1 \mid i \in E(j), j \in Lab(P)\} && (3.23)\\
&\cup \quad \{[l^s_j \to l'_iY]^1 \mid i \in F(j), j \in Lab(P)\} && (3.24)\\
&\cup \quad \{[l^s_j \to (l^s_i)'Y^sX^s]^1 \mid i \in F(j), j \in Lab(P)\} && (3.25)\\
&\cup \quad \{[X^s \to (X^s)']^1,\ [(X^s)' \to (X^s)'']^1, [(X^s)'' \to (X^s)''']^1, && (3.26)\\
&\quad\quad [(X^s)''' \to (X^s)^{iv}]^1, [(X^s)^{iv} \to \#]^1\} && (3.27)\\
&\cup \quad \{[\overline{h^s_i} \to h^s_i]^1, [\overline{l^s_i} \to \overline{\overline{l^s_i}}]^1,\ [\overline{\overline{l^s_i}} \to \overline{\overline{\overline{l^s_i}}}]^1,\ [\overline{\overline{\overline{l^s_i}}} \to \#]^1, &&\\
&\qquad\qquad\qquad\qquad [(l^s_i)' \to l^s_i]^1 \mid i \in Lab(P)\} && (3.28)\\
&\cup \quad \{[(Y^s) \to (Y^s)']^1,\ [(Y^s)' \to (Y^s)'']^1,\ [(Y^s)'' \to (Y^s)''']^1, && (3.29)\\
&\quad\quad [(Y^s)''' \to (Y^s)^{iv}]^1, [(Y^s)^{iv} \to (Y^s)^v]^1,\ [(Y^s)^v \to (Y^s)^{v\iota}]^1, && (3.30)\\
&\quad\quad [(Y^s)^{v\iota} \to (Y^s)^{v\iota\iota}]^1,\ [(Y^s)^{v\iota\iota} \to (Y^s)^{v\iota\iota\iota}]^1, [(Y^s)^{v\iota\iota\iota} \to \#]^1\} && (3.31)\\
&\cup \quad \{[h^s_i \to \overline{h^s_i}\ \overline{l^s_i}]^2,\ [\overline{\overline{\overline{l^s_i}}} \to d]^3, \mid i \in Lab(P) && (3.32)\\
&\cup \quad \{[A \to \#]^3 \mid A \in N\} \cup \{[(Y^s)^{v\iota\iota\iota} \to \lambda]^3\}. && (3.33)
\end{aligned}$$

Note that where each numbered line contains a list of rules, the first in the list will be referred to in the text as *number*.a, the second as *number*.b etc.

The basic idea of the proof is that the system Π simulates the derivations of the grammar G, storing in region 2 a multiset of objects corresponding to the current sentential form of the grammar. In this way a reachability problem in G can be reduced to a reachability problem in Π and so, since programmed grammars with a.c. have been proved universal (in a constructive way, see Chapter 2) then the theorem holds.

The alphabet, evolution rules and transport rules have been divided into subsets. V', R' and $(R^{ev})'$ are used during the simulation of the application of production of G while V'', R'' and $(R^{ev})''$ are used for the simulation of the skipping of a production of G (the appearance checking case). The objects (present in region 1) $l_i, i \in Lab(P)$ are used to indicate the label (i) of the last simulated production, in case it was applied, and objects $l_i^s, i \in Lab(P)$ are used to indicate the label of the last simulated production (i) in case it was skipped.

The functioning of Π is now shown in detail.

Suppose that the last simulated production has label j and *it has been applied* (the case where the last simulated production has been skipped is similar).

Then, at some step $t-1$, the object l_j is present in region 1, together with the objects corresponding to the current sentential form of G and the objects $h_i^s, i \in Lab(P)$.

Region 2 and 3 as well as the markings are empty. The initial configuration is a particular case, where $l_j = l_{-1}$, the only applicable next rule is 3.14.b. However, in general, the next rule of Π to apply is chosen (in a non-deterministic way) from rules in groups 3.14.a and 3.23.

Two cases are distinguished.

- **Case 1**

A rule $[l_j \to l_i' Y]^1$ for some $i \in E(j)$ is applied at step t.

The application of such a rule means that Π has "guessed" that the next production of G that has to be simulated and that *can actually be applied* is the one with label i. The application of this rule produces two objects l_i' and Y.

(i) Suppose that, at step $t+1$, the object l_i' attaches to membrane 2 using the rule $l_i'[\ ]^2 \to [\ ]^2_{l_i'}$. In the same step the object Y is rewritten to

Y' (rule 3.15.a).

(ii) Suppose that at step $t + 2$ an object A present in region 1 (corresponding to the non-terminal A in N) is introduced to region 2 using one of the rules of group 3.2. In the same step Y' is rewritten to Y''.

At step $t + 3$ the object A is rewritten inside region 2 using one of the rules in group 3.19.

(iii) Suppose the rule used is $A \rightarrow h(\alpha)\overline{l_k}$ with $k = i$ (so $\overline{l_k} = \overline{l_i}$).

(iv) In the same step $t+3$, object l_i' is detached from membrane 2 using a rule from 3.4.b and Y'' is rewritten to Y'''.

At step $t + 4$ the objects of $h(\alpha)$ and $\overline{l_i}$ move from region 2 to region 1 (rules from group 3.3 and 3.4.a, resp.), while l_i' is rewritten to l_i'' (rule 3.18.a).

In the same step Y''' is rewritten to Y^{iv}.

In step $t+5$ the objects from $h(\alpha)$ are rewritten to α (the bar is removed) (rules 3.16); the multiset of objects in region 1 corresponding to the current sentential form of G is updated as the production i of G has been applied. Moreover, $\overline{l_i}$ is rewritten to $\overline{\overline{l_i}}$ (rules 3.17), l_i'' attaches to membrane 3 using the rule in 3.4.3 (it is the only rule that can use this object). In the same step, the object Y^{iv} is rewritten to Y^{v}.

In step $t + 6$ the object $\overline{\overline{l_i}}$ moves from region 1 to region 3 using the object l_i'' on membrane 3 and rule 3.5.a.

In the same step the object Y^{v} is rewritten to $Y^{v\iota}$.

In step $t + 7$, $Y^{v\iota}$ becomes $Y^{v\iota\iota}$ while $\overline{\overline{l_i}}$ is attached (internally) to membrane 3 using rule 3.5.a.

In step $t + 8$, the object $Y^{v\iota\iota}$ moves from region 1 to region 3 using the rule $Y^{v\iota\iota}[\ _{\overline{\overline{l_i}}}]^{3}_{l_i''} \rightarrow [Y^{v\iota\iota}\ _{\overline{\overline{l_i}}}]^{3}_{l_i''}$ from group 3.6.a.

In step $t+9$, $Y^{v\iota\iota}$ is deleted in region 3, while l_i'' detaches from membrane 3 using the rule 3.6.a.

It is possible for l_i'' to attach/de-attach to/from membrane 3 using, an arbitrary number of times, the rules from group 3.4.c and 3.6.b, respectively. At a certain step $t + 9 + p$, l_i'' is rewritten to l_i in region 1 (rule 3.18.b). This is necessary to start a new simulation of a production of G.

Moreover, at step $q \leq t + 9 + p + 1$, $\overline{\overline{l_i}}$ detaches from membrane 3 and goes into region 3 (rule 3.6.c) and is then rewritten to d at step $q + 2$ and then deleted at step $q + 3$ ($\overline{\overline{l_i}}$ cannot attach back to membrane since it would need l_i'' that is missing).

In this way, the production i of G with $i \in E(j)$ has been correctly simulated (in particular, applied) and at the step $t + 9 + p + 1$ a new rule among the rules in 3.14.a and 3.23. is applied and so the entire process can be iterated.

The assumptions made during the described evolution of Π are now discussed, showing that if they are not true then # is eventually produced in region 1 (notice that there are no rules to remove #).

For assumptions (i), (ii) & (iv):

When l_i' is produced (step t), Y is also produced and is ultimately rewritten to $Y^{v\iota\iota}$ at step $t + 7$.

If l_i' is not attached to membrane 2 at step $t + 1$ (and hence rewritten to l_i'') or it is detached from membrane 3 before an object A is transported from region 1 to region 2 (meaning it is detached from membrane 2 at step $t + 2$ or A is not present in region 1) then the rule $A \to h(\alpha)\overline{l_i}$ is not used in region 2 at step $t + 2$, and so $\overline{l_i}$ is not produced at step $t + 3$ and then it cannot be attached to membrane 3 at step $t + 7$. So, at step $t + 8$, the rule $Y^{v\iota\iota}[\ _{\overline{\overline{l_i}}}]^3_{l_i''} \to [Y^{v\iota\iota}\ _{\overline{\overline{l_i}}}]^3_{l_i''}$ cannot be used. Therefore, rule $Y^{v\iota\iota} \to \#$ is used and # is produced in region 1.

On the other hand, if l_i'' is not obtained (from l_i') in region 1 at step $t + 4$ then l_i'' cannot be attached to membrane 3 at step $t + 5$ (so $\overline{\overline{l_i}}$ cannot be attached to membrane 3 at step $t + 7$) and then Y cannot be moved inside region 3 at step $t+8$. Therefore $Y^{v\iota\iota} \rightarrow \#$ is used and $\#$ is produced in region 1.

Hence, to avoid creation of $\#$ in region 1, l'_i must attach to membrane 2 at step $t+1$, must detach from it at step $t+3$ and be rewritten to l''_i at step $t+4$.

Assumption (iii):

If the rule used is $A \rightarrow h(\alpha)\overline{l_k}$ with $k \neq i$ then at step $t+8$ the rule $Y^{v\iota\iota}[\ ]^{3}_{\overline{\overline{l_i}}\,l''_i} \rightarrow [Y^{v\iota\iota}\ ]^{3}_{\overline{\overline{l_i}}\,l''_i}$ cannot be used ($\overline{\overline{l_i}}$ is not attached to membrane 3) and so $Y^{v\iota\iota} \rightarrow \#$ is used in region 1.

Consider now the second case.

- **Case 2: appearance checking**

A rule $[l_j \rightarrow (l^s_i)'Y^sX^s]^1$ for some $i \in E(j)$ is applied at step t. The application of this rule means that Π has "guessed" that the next production of G that has to be simulated and that *should be skipped because it cannot be applied* is the one with label i. The application of this rule produces the objects $(l^s_i)'$, Y^s and X^s.

(i) At step $t+1$ the object $(l^s_i)'$ attaches to membrane 2 using a rule of group 3.7.a.

In the same step X^s is rewritten to $(X^s)'$ and Y^s is rewritten to $(Y^s)'$.

(ii) In step $t+2$, the object h^s_i moves from region 1 to region 2 using a rule of group 3.7.b. In the same step objects $(X^s)'$ and $(Y^s)'$ are rewritten to $(X^s)''$ and $(Y^s)''$ respectively.

In step $t+3$, object h^s_i, in region 2, is rewritten to $\overline{h^s_i}\ \overline{l^s_i}$ using rule 3.32.a. In the same step $(l^s_i)'$ detaches from membrane 2 using rule 3.7.c (it is the only rule that can involve the object). Also, the objects $(X^s)''$ and $(Y^s)''$ are rewritten to $(X^s)'''$ and $(Y^s)'''$ respectively.

In step $t+4$, objects $\overline{h^s_i}$ and $\overline{l^s_i}$ move from region 2 to region 1 using rules 3.8.a and 3.8.b.

(iii) In the same step object $(l_i^s)'$ attaches to membrane 3 using rule 3.9.a.

Moreover, objects $(X^s)'''$ and $(Y^s)'''$ are rewritten to $(X^s)^{iv}$ and $(Y^s)^{iv}$, respectively.

In step $t+5$, object $(X^s)^{iv}$ move from region 1 to region 3 using rule 3.9.b. In the same step $(Y^s)^{iv}$ is rewritten to $(Y^s)^{v}$. Moreover, in region 1, $\overline{h_i^s}$ is rewritten to h_i^s using rule 3.28.a and $\overline{l_i^s}$ is rewritten to $\overline{\overline{l_i^s}}$ using rule 3.28.b.

(iv) Suppose that, at step $t+6$, there is no object A_i in region 1. Then, in this step, $(Y^s)^{v}$ is rewritten to $(Y^s)^{v\iota}$ and $\overline{\overline{l_i^s}}$ is rewritten to $\overline{\overline{\overline{l_i^s}}}$ using rule 3.28.c.

In step $t+7$, $\overline{\overline{\overline{l_i^s}}}$ moves from region 1 to region 3 using rule 3.11.a while $(Y^s)^{v\iota}$ is rewritten to $(Y^s)^{v\iota\iota}$.

In step $t+8$, $\overline{\overline{\overline{l_i^s}}}$ attaches (internally) to membrane 3 using rule 3.11.b. Moreover, $(Y^s)^{v\iota\iota}$ is rewritten to $(Y^s)^{v\iota\iota\iota}$.

In step $t+9$, the object $(Y^s)^{v\iota\iota\iota}$ moves from region 1 to region 3 using rule 3.12.a.

In step $t+10$, the object $(Y^s)^{v\iota\iota\iota}$ is deleted inside region 3.

For an arbitrary number of steps the objects $(l_i^s)'$ and $\overline{\overline{\overline{l_i^s}}}$ can iterate their attachment/de-attachment to/from membrane 3 using rules 3.13.a, 3.9.a or 3.13.b and 3.11.b. However, to start a new simulation of a production of G the object $(l_i^s)'$ needs to be detached from membrane 3 (step $t+10+p$) and then rewritten (step $t+10+p+1$) to l_i^s using rule 3.28.e. So, at step $t+10+p+2$ the object l_i^s is obtained in region 1. The object indicates that the last simulated (and skipped) production of G is the one with label i.

Moreover, at step $q \leq t+10+p+1$ object $\overline{\overline{\overline{l_i^s}}}$ must de-attach from membrane 3 using 3.13.b (there are no other rules) and then rewritten to d (step $q+1$) inside region 3 using rule 3.32.b (there are no other rules available; l_i^s is not available any more on membrane 3). Finally d is deleted (step $q+2$ using rule 3.22.a).

In this way, the production i of G with $i \in E(j)$ has been correctly simulated (in particular, skipped) and at the step $t+10+p+2$ the process can then be iterated by choosing, in a non-deterministic way, one of the rules in 3.24.a or 3.25.a (then case 1 or case 2 can be applied again).

The assumptions made during the description of the process are now discussed, showing that if they are not true then # is produced in region 1.

Assumption (i): Suppose that at step $t+1$ the object $(l_i^s)'$ does not attach to membrane 2 (but chooses another possible rule). Then, in this case, object h_i^s cannot move from region 1 to region 2 at step $t+2$. Then it is not possible to fulfil both the conditions:

$(l_i^s)'$ attached to membrane 3 at step $t+4$ (to let $(X^s)^{iv}$ move from region 1 to region 3 at step $t+5$).

$(l_i^s)'$ and $\overline{\overline{\overline{l_i^s}}}$ attached both to membrane 3 at step $t+9$ to let $(Y^s)^{viii}$ move from region 1 to region 3.

So, the following result is obtained: at step $t+5$ the object $(X^s)^{iv}$ is rewritten to # using rule 3.27.b or at step $t+9$ the object $(Y^s)^{viii}$ is rewritten to # using rule 3.31.c.

Assumption (ii): In step $t+2$ the object h_i^s does not move from region 1 to region 2 using a rule of group 3.7.b. This can only happen if $(l_i^s)'$ detaches, at step $t+2$, from membrane 3 (using rule 3.7.c). But, in this case, the following condition cannot be fulfilled:

$(l_i^s)'$ and $\overline{\overline{\overline{l_i^s}}}$ both attached to membrane 3 at step $t+9$ (to let $(Y^s)^{viii}$ move from region 1 to region 3).

Therefore, at step $t+9$, the object $(Y^s)^{viii}$ is rewritten to # using rule 3.31.c.

Assumption (iii): At step $t+4$ the object $(l_i^s)'$ does not attach to membrane 3 using rule 3.9.a. In this case, at step $t+5$, the object $(X^s)^{iv}$ cannot be moved from region 1 to region 3 and, hence, it is rewritten to # using rule 3.27.b.

Assumption (iv): Suppose at step $t+6$ there is an object A_i in region 1. Then, in this step, using rule 3.10.a, A_i is moved inside region 3, where it is rewritten to # in the following step.

From the above description it follows that *all and only* the evolutions of Π that do not produce # in region 1 are the ones corresponding to correct simulations of derivations in G.

Moreover, as has been seen, when (one of) the rules that start a production simulation is applied (i.e., 3.14.a, 3.23, 3.24.a, 3.25.a), the objects $l_i'Y\ h_1 \cdots h_n$ or $(l_i^s)'Y^sX^sh_1 \cdots h_n$, for some $i \in Lab(P)$, and the objects corresponding to the current sentential form are the only ones present in region 1, while the object d is present in region 3 and region 2 and all the markings are empty.

Precisely:

There is a derivation in G producing the sentential form w if and only if there is an $i \in Lab(P)$ such that the two configurations of Π $[\ [\ ]^2 w' l_i' Y\ h_1 \cdots h_n\ [d\ ]^3\ ]^1$ *and* $[\ [\ ]^2 w' (l_i^s)' Y^s X^s\ h_1 \cdots h_n\ [d\ ]^3\ ]^1$ *with* $Ps_V(w) = Ps_V(w')$ *are in* $C_R(\Pi, mp)$.

Also, from the constructive universality (see [26]), it is easy to show that it is not decidable whether or not an arbitrary programmed grammar with a.c. has a derivation of a sentential form with an arbitrary Parikh vector.

From this the Theorem follows. $\square$

3.7 Conclusions and Open Problems

A model of membrane systems with objects attached to both sides of the membranes has been presented: a P_{pp} system. The model comes equipped with operations that can rewrite floating objects and move objects between regions depending on the attached objects. Qualitative properties of the P_{pp} system model have been investigated, such as configuration reachability in relation to the use of cooperative or non-cooperative evolution and transport rules and in the contexts of free- and maximal-parallel evolution. It has been proven that when the system works with free parallel evolution (i.e., allowing an arbitrary number of rules to be applied at each step) the reachability of a configuration or of a certain protein marking can be decided. It has also been shown that when the system works with maximal parallel evolution (all rules that can be applied must be applied) the reachability of configurations becomes an undecidable property for the case of non-cooperative evolution rules and cooperative membrane rules. The property remains decidable, however, for systems using non-cooperative evolution rules and simple membrane rules and for systems using only membrane rules. An interesting problem remains open: the decidability of reachability in the case of systems using non-cooperative evolution rules, non-cooperative membrane rules and maximal-parallel evolution.

While it is envisaged that other biologically-inspired operations may be introduced to the model, such as *fission* and *fusion* of regions, Chapter 4 extends the model by adding so-called *integral* proteins to the membrane. Another interesting direction of future research could be the analysis of the model in the presence of timed rules, following the idea of time-independent P systems [19].

In the mean time, the existing model can be used to simulate biological systems: having defined and investigated a qualitative model, it is possible to use it to examine quantitative properties using a simulator. Such a simulator has been created based on an extended version of the model presented in this chapter and is described in detail in Chapter 5 (the extended formal model is presented in Chapter 4). The simulator assumes discrete molecular interactions and uses the Gillespie algorithm [32] to stochastically choose at each step which single rule to apply and to calculate its stochastic time delay. The more general free parallel theoretical model is thus reduced to a specific sequential one. Examples of the simulator's use can be found in Chapter 4, Section 4.4.3, where the links between the formal model and its practical realisation are made evident. Other examples are presented in Section 5.7.

Chapter 4

Membrane Systems with Peripheral and Integral Proteins

The work presented in this chapter was originally published in
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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. The model of Membrane Systems with Peripheral Proteins (a P_{pp} system) presented in Chapter 3 is here extended to become a model called Membrane Systems with Peripheral and Integral Proteins (MSPIP, defined as a P_{pi} system below), initially introduced in [20]. 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

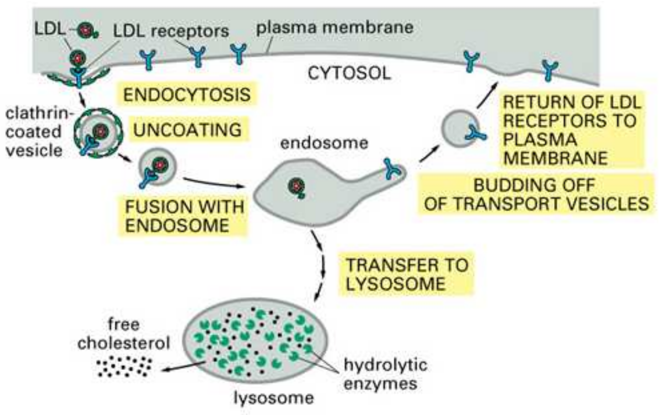


Figure 4.1: Endocytosis of LDL (*Essential Cell Biology*, 2/e, ©2004 Garland Science)

of chemical substances. As examples, a biologically inspired noise-resistant circadian oscillator and the G-protein cycle in Saccharomyces cerevisiae are modelled and a quantitative analysis using an implemented simulator are presented.

4.1 Introduction

Some basic notions of membrane systems have been given in Chapters 2 and 3, together with the specific context of those models featuring objects attached to membranes. In the model described in Chapter 3, 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., [2]). Receptor-mediated processes, such as endocytosis (illustrated in Figure 4.1) and signalling, are crucial to cell function and by definition are critically dependent on the presence of peripheral and integral membrane proteins.

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 the model presented in Chapter 3 is extended, 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, a biologically-inspired noise-resistant circadian oscillator and the G-protein cycle in *Saccharomyces cerevisiae* are modelled, 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 extended version of the simulator originally presented in [21] and described in Chapter 5. The simulator employs a stochastic algorithm and uses intuitive syntax based on chemical equations (described in Section 5.5).

In what follows it is assumed that the reader is already familiar with the contents of Chapters 2 and 3.

4.2 Operations with Peripheral and Integral Proteins

This section presents operations on the MSPIP model and defines terminology which will be used in the sequel. The membrane syntax and other conventions are those described in Section 2.1.1.

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

In contrast to the model presented in Chapter 3, to each membrane there are associated three multisets, u, v and x over V, denoted by $[\ ]_{u|v|x}$. This is reported by saying 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, they are referred to 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.

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

For example, the string

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

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 4.2.

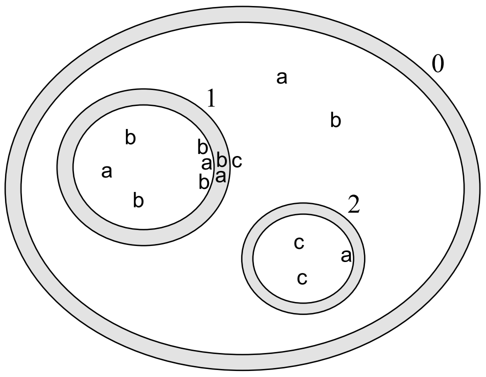


Figure 4.2: Graphical representation of membrane system $[\ ab\ [\ cc\ ]^2_{a|\ |}\ \ [\ abb\ ]^1_{bba|ab|c}\ ]^0$

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.

4.2.1 Operations

Rules are presented here that describe bidirectional interactions of floating objects with the membrane markings and are called *membrane rules*. These rules are motivated by the behaviour of cell membrane proteins (e.g., see [2]) and therefore permit a level of abstraction based on the behaviour of real molecules. The rules are denoted as $attach_{in}$, $attach_{out}$, $de - attach_{in}$ and $de - attach_{out}$, defined:

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

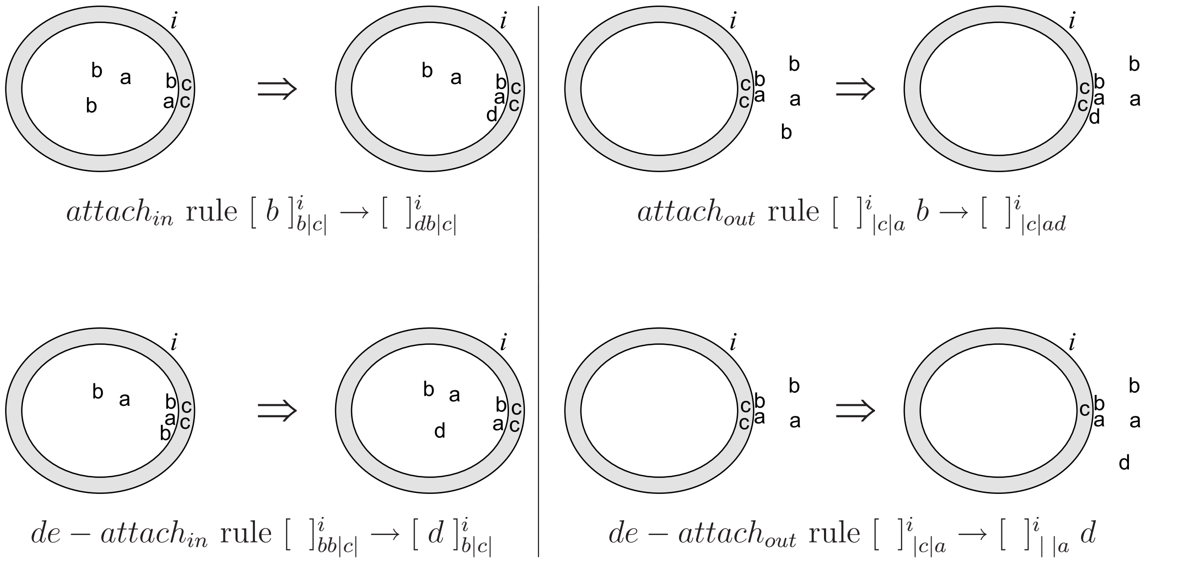
The semantics of these rules is as follows.

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



$attach_{in}$ rule $[\ b\ ]^i_{b|c|} \rightarrow [\ \ ]^i_{db|c|}$

$attach_{out}$ rule $[\ \ ]^i_{|c|a}\ b \rightarrow [\ \ ]^i_{|c|ad}$

$de-attach_{in}$ rule $[\ \ ]^i_{bb|c|} \rightarrow [\ d\ ]^i_{b|c|}$

$de-attach_{out}$ rule $[\ \ ]^i_{|c|a} \rightarrow [\ \ ]^i_{|\ |a}\ d$

Figure 4.3: 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.

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.

$\mathcal{R}^{att}_{V,Lab}$ denotes 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 4.3.

Next are presented *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. An evolution rule is defined thus:

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

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 $free(i)$.

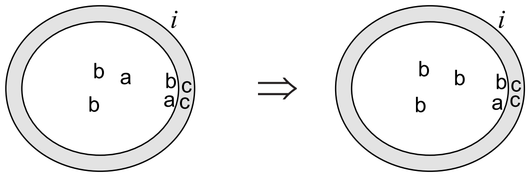


Figure 4.4: *evol* rule $[\ a \rightarrow b\ ]^{i}_{b|c|}$. Free objects can be rewritten inside the region and the rewriting can depend on the integral and internal markings of the enclosing membrane.

The membrane markings and the objects not involved in the application of the rule are left unchanged in their original positions.

$\mathcal{R}^{ev}_{V,Lab}$ denotes 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 4.4.

In general, when a rule has label i the 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 4.3 the $attach_{in}$ is associated to membrane i.

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.

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

4.3 Membrane Systems with Peripheral and Integral Proteins

In this section membrane systems are described having membranes marked with peripheral proteins, integral proteins, free objects and using the operations presented in Section 4.2.

Definition 4.3.1 *A* membrane system with peripheral and integral proteins *and n membranes (in short, a P_{pi} system), is a construct*

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

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

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. $\mathcal{I}(\Pi)$ denotes the set of *all instantaneous descriptions* of Π. As a shorthand, *membrane (region) i of I* denotes the membrane (region, respectively) i present in I.

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

Then, if r is applicable to membrane i (or to region i, accordingly) of I, in short, *r is applicable to I*. $r(I) \in \mathcal{I}(\Pi)$ denotes the instantaneous description of Π obtained when the rule r is applied to membrane i (or to region i, accordingly) of I (in short, *r is applied to I*).

The *initial instantaneous description* of Π, $I_{in,\Pi} \in \mathcal{I}(\Pi)$, 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 $I \in \mathcal{I}(\Pi)$ and $t \in \mathbb{R}$; t is called the *time* of the configuration. $\mathcal{C}(\Pi)$ denotes the set of all configurations of Π. The *initial configuration* of Π is $C_{in,\Pi} = (I_{in,\Pi}, t_{in,\Pi})$.

Suppose that $R_\Pi = \{rule^1, rule^2, \ldots, rule^m\}$ and let S be an arbitrary sequence of configurations $\langle C_0, C_1, \cdots, C_j, C_{j+1}, \cdots, C_h\rangle$, where $C_j = (I_j, t_j) \in \mathcal{C}(\Pi)$ for $0 \le j \le h$. Let $a_j = \sum_{i=1}^{m} p^i_j$, $0 \le j \le h$, where p^i_j is the product of $rate(rule^i)$ and the *mass action* combinatorial factor for $rule^i$ and I_j (see Section 4.5).

The sequence S is an *evolution* of Π *if*

- for $j = 0$, $C_j = C_{in,\Pi}$
- for $0 \le j \le h-1$, $a_j > 0$, $C_{j+1} = (r_j(I_j), t_j + dt_j)$ with r_j, dt_j as in [32]:

  $$r_j = rule^k,\ k \in \{1, \cdots, m\} \text{ and } k \text{ satisfies } \sum_{i=1}^{k-1} p^i_j < ran'_j \cdot a_j \le \sum_{i=1}^{k} p^i_j$$

  $$dt_j = (-1/a_j) ln(ran''_j)$$

  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 4.2. Rule* r_j *and* dt_j *are obtained using the Gillespie algorithm [32] 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.*

4.4 Modelling and Simulation of Cellular Processes

Having established a theoretical basis, it is now desirable to demonstrate the quantitative behaviour of the presented model. To this end the simulator first presented in [21] was extended to produce evolutions of an arbitrary P_{pi} system. In Sections 4.4.2 and 4.4.3 the model and the simulator are demonstrated using two examples from the literature. Chapter 5 contains a more detailed description of the simulator (Cyto-Sim) and features many examples.

4.4.1 The Stochastic Algorithm

A discrete and stochastic simulation algorithm is employed, based on Gillespie's, which can potentially represent the dynamical behaviour of small quantities of reactants more accurately, in comparison, say, to a deterministic approach based on *ordinary differential equations* [63]. Moreover, Gillespie has shown that the algorithm is fully consistent with 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. The application of the Gillespie algorithm to membranes is further described in Section 4.5.

4.4.2 Modelling a Noise-Resistant Circadian Oscillator

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 oscillators, is presented in [84]. This is chosen as an interesting, non-trivial example to model and simulate with a P_{pi} system using evolution rules alone. Moreover, this example has been chosen because it has also been modelled in other formalisms, such as in stochastic Π calculus (see, e.g., [86], [76]).

The model is described diagrammatically in Figure 4.5. 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 oscillator is given in Figure 4.6, 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 4.5 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 4.5: the two proteins exhibit anti-phase periodicity of approximately 24 hours, as expected.

The simulator has the capability to add or subtract reactants from the simulation in runtime. This facility is used to discover the effect of switching off `gR` in the circadian oscillator 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

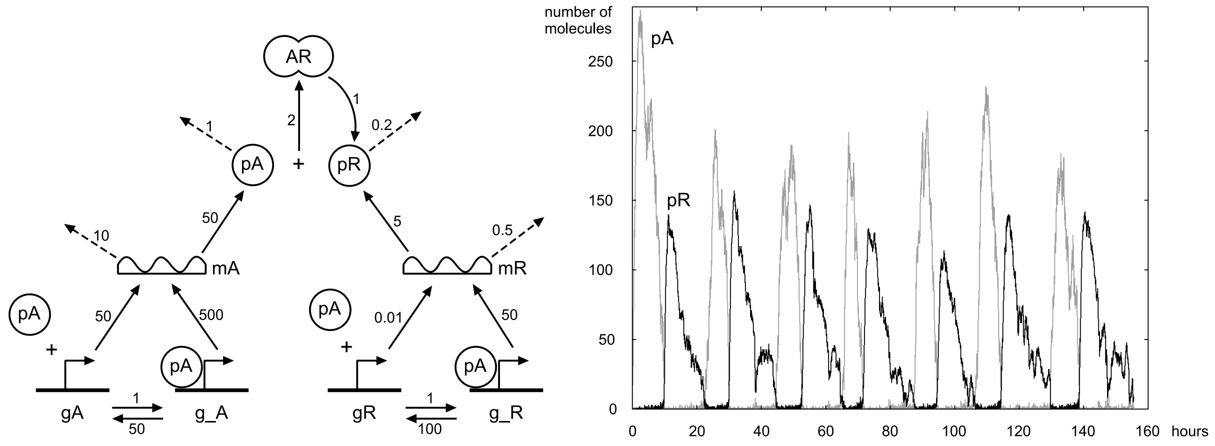


Figure 4.5: Reaction scheme and simulation results of noise-resistant circadian oscillator of [84]

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 4.7, is to reduce the amount of `pR` to near zero and to thus allow `pA` to reach a maximum governed by its 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 4.7). Although this model is a generalisation of biological circadian oscillators 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.

4.4.3 Modelling Saccharomyces Cerevisiae Mating Response

To demonstrate the ability of P_{pi} systems to abstract the notions compartments and membranes found in biological systems, the G-protein mating response in yeast *Saccharomyces cerevisiae* has been modelled and simulated, based on experimental rates provided by [92]. 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 4.8 shows the relationships between the various reactants and regions modelled and simulated.

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 4.8) 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 4.9 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 4.8 shows the results of a single stochastic simulation run plotted with experimental results from [84] 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 4.8 may explain why some measured points do not lie exactly on the deterministic curve. Testing this hypothesis would require multiple simulation runs in order to estimate a distribution at the times of each measured point, however such analysis is beyond the scope of this chapter.

4.5 Perspectives

A model of membrane systems (called a P_{pi} system) has been presented with objects integral to the membrane and objects attached to either side of the membrane. Operations have also been presented 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 it is possible 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 [32] and in the second part of the chapter a simulator has been presented 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, a circadian oscillator and the G-protein cycle mating response of Saccharomyces cerevisiae have been modelled. 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 Chapter 3 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* described in Chapter 3) or to use algorithms that more accurately model the behaviour of biological membranes. In this way it will be possible to explore the limits of the model and perhaps discover a more useful level of abstraction.

Appendix to Chapter 4
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' [32]. 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, this is the abstraction that has been chosen. Hence the objects attached to the membrane are treated as being 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 [32], are calculated over the set of chemical reactions given in equations (2a. . . g) of [32], using standard stoichiometric syntax of the general form

$$S_1 + S_2 + S_3 \rightarrow P_1 + P_2 + \ldots + P_n$$

S_1, S_2 and S_3 are the reactants and $P_1, \ldots, P_n$ are the products of the reaction. Since the order of the reactants and products is unimportant they may be represented as multisets $S_1S_2S_3$ and $P_1P_2 \cdots P_n$, respectively, over the set of objects V. Hence a chemical reaction may be expressed using the notation

$$S_1S_2S_3 \rightarrow P_1P_2 \cdots P_n$$

In the definition of the evolution of a P_{pi} system, the mass action combinatorial factor is calculated using equations (14a. . . g)[32] 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 $V_{i,out} = \{a_{i,out} | a \in V\}$. 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 map an evolution rule of the type

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

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

$$free^i(\alpha) \cdot int^i(u) \cdot itgl^i(v) \rightarrow free^i(\beta) \cdot int^i(u) \cdot itgl^i(v)$$

Map membrane rules, generally described by

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

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

$free^i(\alpha) \cdot int^i(u) \cdot itgl^i(v) \cdot out^i(x) \cdot free^j(\beta) \rightarrow$

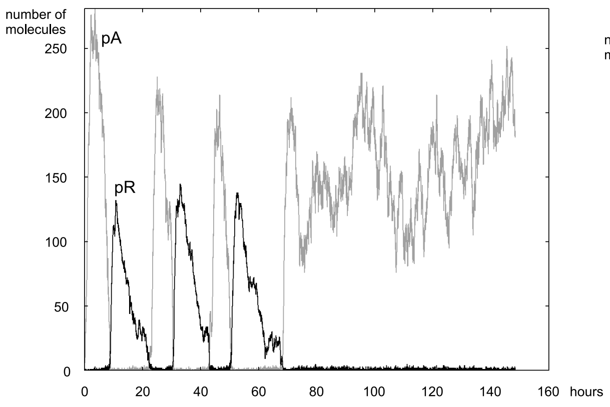
$$free^i(\alpha') \cdot int^i(u') \cdot itgl^i(v') \cdot out^i(x') \cdot free^j(\beta')$$

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.

P_{pi} system *clock*		Simulator script
$V_{clock} = \{gA, g\_A, gR, g\_R, mA, mR, pA, pR, RA\}$		`object gA,g_A,gR,g_R,mA,mR,pA,pR,RA`
$rate_{clock} =$		`rule clock`
$\{$		`{`
$[\, gA \rightarrow gA\; mA \,]^0_{\mid\,\mid}$	$\mapsto 50$	`gA 50-> gA + mA`
$[\, pA\; gA \rightarrow g\_A \,]^0_{\mid\,\mid}$	$\mapsto 1$	`pA+gA 1-> g_A`
$[\, g\_A \rightarrow g\_A\; mA \,]^0_{\mid\,\mid}$	$\mapsto 500$	`g_A 500-> g_A + mA`
$[\, gR \rightarrow gR\; mR \,]^0_{\mid\,\mid}$	$\mapsto 0.01$	`gR 0.01-> gR + mR`
$[\, g\_R \rightarrow g\_R\; mR \,]^0_{\mid\,\mid}$	$\mapsto 50$	`g_R 50-> g_R + mR`
$[\, mA \rightarrow pA \,]^0_{\mid\,\mid}$	$\mapsto 50$	`mA 50-> pA`
$[\, mR \rightarrow pR \,]^0_{\mid\,\mid}$	$\mapsto 5$	`mR 5-> pR`
$[\, pA\; pR \rightarrow AR \,]^0_{\mid\,\mid}$	$\mapsto 2$	`pA+pR 2-> AR`
$[\, AR \rightarrow pR \,]^0_{\mid\,\mid}$	$\mapsto 1$	`AR 1-> pR`
$[\, pA \rightarrow \lambda \,]^0_{\mid\,\mid}$	$\mapsto 1$	`pA 1-> 0A`
$[\, pR \rightarrow \lambda \,]^0_{\mid\,\mid}$	$\mapsto 1$	`pR 0.2-> 0R`
$[\, mA \rightarrow \lambda \,]^0_{\mid\,\mid}$	$\mapsto 10$	`mA 10-> 0mA`
$[\, mR \rightarrow \lambda \,]^0_{\mid\,\mid}$	$\mapsto 0.5$	`mR 0.5-> 0mR`
$[\, g\_R \rightarrow pA\; gR \,]^0_{\mid\,\mid}$	$\mapsto 100$	`g_R 100-> pA+gR`
$[\, pA\; gR \rightarrow g\_R \,]^0_{\mid\,\mid}$	$\mapsto 1$	`pA+gR 1-> g_R`
$[\, g\_A \rightarrow pA\; gA \,]^0_{\mid\,\mid}$	$\mapsto 50$	`g_A 50-> pA+gA`
$\}$		`}`
$w_{0,clock} = gA\; gR$		`system 1 gA, 1 gR, clock`
$\mu_{clock} = [\ \ ]^0$		
$t_{in,clock} = 0$		`evolve 0-150000`
$t_{fin,clock} = 155\ hours$		
		`plot pA, pR`

Figure 4.6: P_{pi} system model of circadian oscillator of [84] 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`.



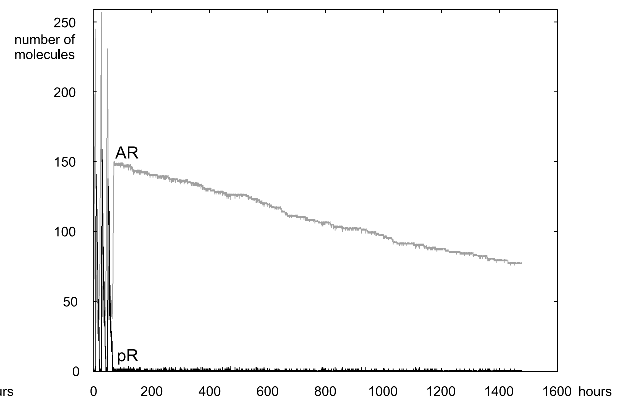
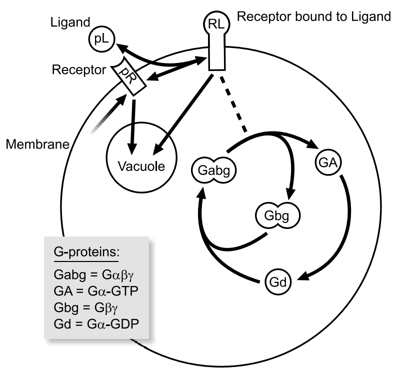
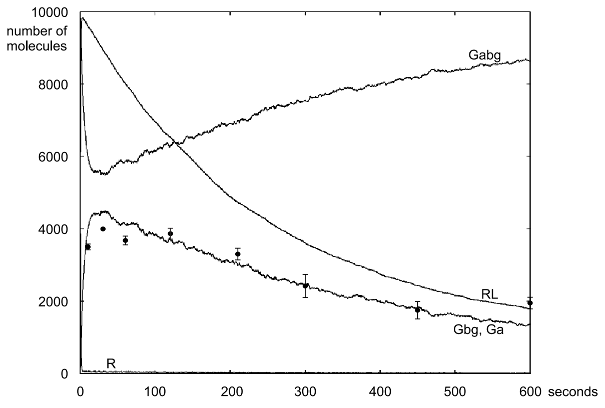


Figure 4.7: Simulated effect of switching off `gA` in circadian oscillator of [84]





Diagrammatic representation of model.

Simulation results (continuous curves) and experimental data (points with error bars, [92]) corresponding to simulated `GA`. Note: `Gd` decays rapidly and is not visible at this scale.

Figure 4.8: Model and simulation results of Saccharomyces cerevisiae mating response.

P_{pi} system *gprot*

$V_{gprot} = \{pL, pr, pR, RL, Gd, Gbg, Gabg, GA\}$

$rate_{gprot} =$

$\{$

$[\ ]^1_{\lvert pr\rvert} \to [\ ]^1_{\lvert pR\ pr\rvert}$	$\mapsto 4.0$
$[\ ]^1_{\lvert pR\rvert}\ pL \to [\ ]^1_{\lvert RL\rvert}$	$\mapsto 3.32e^{-18}$
$[\ ]^1_{\lvert RL\rvert} \to [\ ]^1_{\lvert pR\rvert}\ pL$	$\mapsto 0.011$
$[\ ]^1_{\lvert RL\rvert} \to [\ RL\ ]^1_{\lvert\ \rvert}$	$\mapsto 4.1e^{-3}$
$[\ ]^1_{\lvert pR\rvert} \to [\ pR\ ]^1_{\lvert\ \rvert}$	$\mapsto 4.1e^{-4}$
$[\ Gabg \to GA\ Gbg\ ]^1_{\lvert RL\rvert}$	$\mapsto 1.0e^{-5}$
$[\ Gd\ Gbg \to Gabg\ ]^1_{\lvert\ \rvert}$	$\mapsto 1.0$
$[\ GA \to Gd\ ]^1_{\lvert\ \rvert}$	$\mapsto 0.11$
$[\ ]^2_{\lvert\ \rvert}\ pR \to [\ pR\ ]^2_{\lvert\ \rvert}$	$\mapsto 4.1e^{-4}$
$[\ ]^2_{\lvert\ \rvert}\ RL \to [\ RL\ ]^2_{\lvert\ \rvert}$	$\mapsto 4.1e^{-3}$

$\}$

$w_{2,gprot} = \lambda$

$(u_2, v_2, x_2)_{gprot} = (\lambda, \lambda, \lambda)$

$w_{1,gprot} = Gd^{3000}\ Gbg^{3000}\ Gabg^{7000}$

$(u_1, v_1, x_1)_{gprot} = (\lambda, pR^{10000}pr, \lambda)$

$w_{0,gprot} = pL^{6.022e17}$

$\mu_{gprot} = [\ [\ [\ ]^2\ ]^1\ ]^0$

$t_{in,gprot} = 0$

$t_{fin,gprot} = 600\ seconds$

Simulator script

```
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|]
```

Figure 4.9: P_{pi} system model of G-protein cycle and corresponding simulator script.

Chapter 5

Cyto-Sim

Some of the work presented in this chapter was originally published in

S. Sedwards and T. Mazza (2008) Cyto-Sim: A formal language model and stochastic simulator of membrane-enclosed biochemical processes, *Bioinformatics*, **23:20**, 2800–2802.

Compartments and membranes are the basis of cell topology and more than 30% of the human genome codes for membrane proteins. It is possible to represent compartments and membrane proteins in a nominal way with many mathematical formalisms used in systems biology, however few explicitly model the topology of the membranes themselves.

Discrete stochastic simulation of molecular kinetics potentially offers the most accurate representation of cell dynamics. Since the details of every molecular interaction in a pathway are often not known, the relationship between chemical species in not necessarily best described by simple mass action chemistry. Moreover, modelling every individual molecular interaction in the cell is probably unnecessary and currently impractical.

Simulation is a form of *computer aided analysis*, relying on human interpretation to derive meaning. To improve efficiency and gain meaning in an automatic way, it is necessary to have a formalism based on a model which has *decidable* properties.

Cyto-Sim is a stochastic simulator of hierarchies of membrane-enclosed biochemical processes, where the membranes comprise an inner, outer and integral layer. The full underlying model is presented in Chapter 4, while the version of the model presented in Chapter 3 has been shown to have decidable properties in Chapter 3, allowing formal analysis in addition to simulation. The simulator provides arbitrary levels of abstraction based on chemical kinetics and ordinary differential equations; these latter providing a further dimension of analysability.

The paradigm is flexible and extensible, permitting adaptation to other types of simulation and analysis and integration within standard platforms. In addition to its compact native syntax, based on stoichiometric equations and reaction kinetics, Cyto-Sim currently supports models described as Petri nets, can import all versions of SBML and can export SBML and MATLAB® m-files.

5.1 Introduction

The function of membranes in cells is fundamental to their activity, separating them from other cells to permit differentiation of function and separating organelles within cells for similar purposes. Membrane proteins regulate the communication between the membrane-enclosed compartments and play a statistically important role in cell activity: more than 30% of the human genome codes for membrane proteins. Since membrane proteins control the entry of susbtances to cells, it is no surprise that in 2000 almost 50% of the drugs prescribed in the USA targetted one class of membrane proteins alone (GPCRs).

In computational terms, compartments correspond to *scope*, that is, regions where calculations can be performed in a local context. In computer programs, scope is the basis of functions, which are a means to perform the same calculation on different data without creating new code - computational differentiation. Biology has a finite repertoire of molecules to perform its *calculations* and uses compartments to increase computational power in the same way, i.e., by having a multiplicity of membrane-enclosed cells.

The aim is to create predictive or otherwise useful models of biological processes, with the current focus on inter- and intracellular pathways. Recognising the important roles of membrane proteins and biological scope, a model is required that allows the simultaneous representation of different (types of) cells which communicate via ligands and receptors. In addition to the obvious biological analogues, membranes can also be used to represent notional compartments in order to characterise, for example, diffusion or localised behaviour. Further, compartments can be used like assay plate wells, enabling several experiments to be run simultaneously and efficiently. These ideas and other have previously been considered, for example, in [22] and [25].

5.2 Approach

The formal basis for the simulator model has been presented in Chapter 4. The default level of abstraction in the implementation is thus chemistry: objects interact governed by stoichiometric rules at a rate defined by mass action kinetics. A variable level of abstraction is facilitated by the use of arbitrary kinetic laws: objects are consumed and produced at rates defined by arbitrary functions of reactants, thus modelling behaviour rather than mechanism. This allows complex systems to be reduced to single equations that nevertheless remain in the formal language and Markov framework for analysis. Such reduction may be a necessity if the structure of the system is unknown, or may be used to gain efficiency when the structure is unimportant.

The native simulator syntax language aims to be intuitive and uncluttered (see the description in Section 5.5). Objects, rules and compartments are defined, then (a subset of) these are composed to create the final system to simulate. In this way it is not necessary to explicitly define rules and objects for each compartment, as is necessary, for example, in SBML.

Petri nets are an intuitive graphical representation of logical flows, which have been successfully applied to biology (e.g., [66]). Hence, in addition to the native rule syntax, the simulator supports rule definitions in the form of Petri net incidence matrices. There is strong correspondence between stochastic Petri nets and stochastic chemical rules so, by default, the transitions adopt mass action kinetics, i.e., the rate of the transition is proportional to a constant multiplied by the numbers of tokens in the incoming places. Other Petri net dynamics are also possible by explicitly defining appropriate kinetic laws.

5.3 Methods

The software is written in J#, a Java-like language which is part of the .NET framework. This choice allows easy porting to both Java and C# and hence maximises cross-platform compatibility. There are currently two versions available on the internet [87]; an applet and a standalone version, both implemented in Java and having a graphical user interface (GUI). Other versions can be made available upon request.

The core simulation engine is an efficient implementation of a Markov chain Monte Carlo algorithm, based on that of Gillespie [33]. Performance is optimised for mass action chemical kinetics, however the simulator also supports chemical reactions with arbitrary kinetic laws based on functions of the reactants. This latter restriction guarantees the Markov property but permits the use of the many alternative kinetic laws used in systems biology. The addition of arbitrary kinetic laws is achieved in an efficient way, based on compiler technology and a virtual machine, and does not adversely affect the performance of the default kinetics.

5.4 Discussion

Much of the modelling in systems biology is done in the framework of deterministic differential equations, which are usually *solved* by numerical methods. Such solutions might more accurately be described as deterministic *simulations*. Recognising that molecular interactions are discrete stochastic events and that this stochasticity has a significant effect on the behaviour of models which have neutral or unstable manifolds [33], discrete stochastic simulations are now often used to give a more accurate representation of such behaviour. The principal qualitative difference between the two approaches is that, for a given set of simulation parameters, a deterministic simulation will have a unique, *average*, solution, whereas a discrete stochastic simulation is a single trajectory through the solution space. By using Monte Carlo techniques, the stochastic trajectory is guaranteed to be statistically consistent with the master equation describing the system, however this does not guarantee that a particular behaviour will be observed in any given simulation. Hence, a deterministic simulation gives a single average characterisation of the system but may not characterise all the behaviour, whereas a discrete stochastic simulation is only guaranteed to display all the behaviour in the limit of simulations. The choice between these two techniques is therefore dependent on the quality and robustness of the behaviour and the types of manifold present. Given that some robust behaviour may not be observed in a deterministic simulation, e.g., behaviour that relies on a stochastic divergence at a bifurcation point, performing many stochastic simulations is usually preferable. The efficiency of the Cyto-Sim algorithm, which can outperform the deterministic simulation of some models (particularly those with many reactions and low molecular concentrations, see Table 5.1) makes such statistical analysis feasible. In Cyto-Sim, models can also be exported as sets of differential equations in the form of a MATLAB® m-file. So, having performed initial stochastic simulations, it is thus possible to perform algebraic or numerical analysis or, at least, deterministic simulation, to gain further insight about the system.

Simulator	Yeast G prot. cycle	N-R oscillator
Cyto-Sim	**1.1s**	**0.41s**
CellDesigner[1]	1.5s	2.2s
MATLAB®[2] (deterministic)	617s	6.3s
Dizzy[3]	10^{13}s (Dizzy estd.)	19.3s

(Windows XP, JRE 1.6, Centrino M, 1.6GHz, 2MB L2 cache, 512 MB RAM)

[1]www.celldesigner.org [2]www.mathworks.com [3]www.systemsbiology.org

Table 5.1: Timings of Cyto-Sim and other popular simulators with models of yeast G protein coupled cycle [92] and a noise-resistant oscillator [84]. Simulated times were 600 seconds and 250 hours, respectively.

5.5 The Cyto-Sim language

The Cyto-Sim language syntax aims to be an intuitive interpretation of the P_{pi} system model presented in Chapter 4 and to provide sophisticated controls and ease of use to modellers. A simulator script conforms to the following grammar:

```
Simulator Script =  { Constant Declaration }
                    Object Declaration  { Object Declaration }
                    ( Rule Definition  { Rule Definition }
                    | Petri Net Definition )
                    { Compartment Definition }
                    System Statement
                    Evolve Statement
                    Plot Statement
```

An example of a simple simulator script is shown below, together with its P_{pi} system counterpart.

Simulator script	P_{pi} system *lotka*
`// Lotka reactions`	
`object X,Y1,Y2,Z`	$V_{lotka} = \{X, Y1, Y2, Z\}$
	$rate_{lotka} = \{$
`rule r1 X + Y1 0.0002-> 2Y1 + X`	$[\, XY1 \rightarrow Y1Y1X \,]^0_{\vert\,\vert} \mapsto 0.0002$
`rule r2 Y1 + Y2 0.01-> 2Y2`	$[\, Y1Y2 \rightarrow Y2Y2 \,]^0_{\vert\,\vert} \mapsto 0.01$
`rule r3 Y2 10-> Z`	$[\, Y2 \rightarrow Z \,]^0_{\vert\,\vert} \mapsto 10 \;\}$
`system 100000 X,1000 Y1,1000 Y2,r1,r2,r3`	$w_{0,lotka} = X^{100000}Y1^{1000}Y2^{1000}$
	$\mu_{lotka} = [\,]^0$
`evolve 0-1000000`	$t_{in,lotka} = 0$
`plot Y1,Y2`	

The syntax of the various sections of a simulator script are described below.

5.5.1 Comments

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

5.5.2 Constant Declaration

A constant declaration uses the keyword `constant` and contains a comma separated list of unique identifiers and assignments to define values which do not change. E.g.:

```
constant k1=0.0002,k2=0.01,k3=10
```

The constant declaration serves to perform both the declaration of an identifier as a constant and the assignment of its value. The right hand side of the assignment may be a mathematical expression containing literal values and previously defined constants. E.g.:

```
constant alpha=6.023e-17,x=0.1*alpha,y=0.02*alpha
```

Any number of consecutive constant declarations are permitted.

5.5.3 Object Declaration

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. E.g.:

```
object X,Y1,Y2,Z
```

The names are case-sensitive and must start with a letter but may include digits and the underscore character (_). This corresponds to defining the alphabet V of the P_{pi} system.

5.5.4 Rule Definition

The reaction rules are defined using rule definitions comprising the keyword `rule` followed by a unique name and the rewriting rule itself. E.g.:

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

These correspond to the attach / de-attach and evolution rules of the P_{pi} system model. Here `r1` is the name of the rule and `X` and `Y1` are the names of objects. Note, however, that simulator rules are user-defined types which may be instantiated in more than one region. The value preceding the implication symbol (`->`) is the average mass action reaction rate and corresponds to an element of the range of the mapping *rate* given in Definition 4.3.1. 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 simulator rule syntax is extended with `|` symbols to denote the membrane, in a deliberately similar way to that described in Section 2.1.1. In general, the membrane is represented by `||`; objects listed on the left hand side of the `||` represent the internal markings, objects listed on the right hand side represent the external markings and objects listed between the vertical bars are the integral markings of the membrane. E.g.:

```
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 following a `+`, meaning that it must exist in the region surrounding the membrane for the rule to be applied. Hence the `+` used in simulator membrane rules is non-commutative.

Arbitrary kinetics

Rules can be specified using kinetics other than mass action by writing a suitable parenthesis-enclosed function in place of the rate. E.g.:

```
rule enzyme E + S (Vmax*S/(S+Km))-> E + P
```

is an archetypal Michaelis-Menten reaction, where it is assumed that `Vmax` and `Km` are previously defined constants. The `S` used within the function refers to the object of the same name; during the simulation this variable takes the value of the current number of `S` objects in the local context of the rule. While one `E` and one `S` must exist for the rule to be applicable, the rate of the rule is given entirely by the function contained within the parenthesis. In general, this function need not necessarily refer to the objects on the left hand side of the rule. Cyto-Sim implements the following standard mathematical operations for use in arbitrary kinetic functions: `+`, `-`, `*`, `/`, `^` (exponentiation) and `(...)` (parentheses). Note that if an arbitrary kinetic function produces a negative rate, a rate of zero is used.

5.5.5 Petri Net Definition

An entire set of rules may be specified as a Petri net using the keyword `petri` and defining its incidence matrix. E.g.:

```
petri lotka_volterra
```

```
{
    X ,Y1,Y2,Z
t1  0 ,1 ,0 ,0  :(0.0002*X*Y1)
t2  0 ,-1,1 ,0  :(0.01*Y1*Y2)
t3  0 ,0 ,-1,1  :10
}
```

The keyword `petri` is thus used to define a block of rules having a single name (the name of the network). Within the block delimited by braces, the first line is a list of objects which define the data columns of the subsequent matrix. The first column of the matrix contains the names of the transitions which correspond to the rows. The rows are filled by comma separated values defining whether tokens are consumed (negative) or produced (positive) by the transition. Each row is terminated by a colon followed by a mass action rate, which may be replaced by a parenthesis-enclosed arbitrary kinetic function, as described in Section 5.5.4. In the case of enzymatic transitions, where an object is necessary but not consumed, the incidence matrix is ambiguous: the zero entry could also mean that the species is not involved. Under these circumstances the default mass action kinetics will be incorrectly calculated and it is necessary to write an explicit arbitrary kinetic function, as shown for transitions `t1` and `t2` in the above example.

5.5.6 Compartment Definition

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 (partial) 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 (partial) 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 (partial) initial instantaneous description

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

5.5.7 System Statement

The system is instantiated using the keyword `system` followed by a comma-separated list of constituents. E.g.:

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

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.

The system statement may be extended to multiple lines by enclosing the list of constituents between braces. E.g.:

```
system {
  100000 X,
  1000 Y1,
  1000 Y2,
  r1,r2,r3
}
```

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.

The numerical values in the `system` statement may be replaced by mathematical expressions containing constants and numeric literals. When these expressions evaluate to non-integer or negative values they are truncated or set to zero, respectively.

5.5.8 Evolve Statement

The simulator 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. E.g.,

```
evolve 0-1000000
```

To avoid storing and plotting unnecessarily many data points, the keyword `step` can be used. E.g.:

```
evolve 0-1000000 step 500
```

makes the simulator perform 1000000 evolution steps but records only 2000.

All the numerical values in the `evolve` statement can be replaced by mathematical expressions containing constants and numeric literals. When these expressions evaluate to non-integer or negative values they are truncated or set to zero, respectively.

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 that although t_{fin} of a P_{pi} system evolution *corresponds* to the maximum evolution step, the units are different and there is no explicit conversion.

5.5.9 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. The default behaviour of the `plot` statement is to plot its arguments against time, however the keyword `against` allows plots to be made relative to one of the objects, thus excluding time. E.g.:

```
plot X, c3[Y1] against c3[X]
```

plots free-floating `X` in the environment and `Y1` in compartment `c3` against free-floating `X` in compartment `c3`. Figure 5.1 demonstrates the use and effect of the `step` and `against` keywords: the graphical output is independent of time and the plotted points correspond to every 500th simulated point.

Cyto-Sim script

```
constant k1=10, k2=0.01
object X, Y
rule Lotka_Volterra {
X k1-> 2X
X + Y k2-> 2Y
Y k1-> *
}
system Lotka_Volterra, 1000 X, 1000 Y
evolve 0 - 500000 step 500
plot Y against X
```

Graphical output

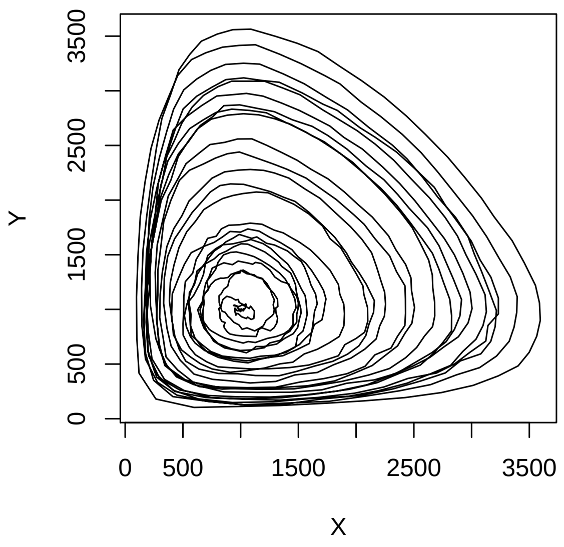


Figure 5.1: Phase plot of the Lotka-Volterra model, demonstrating the existence of a fixed point in the state space. The `step` size of 500 reduces the apparent stochasticity and thus improves the clarity of the diagram.

5.6 Extended syntax

An extended syntax has been implemented to investigate biological models defined as ordinary differential equations (ODE), differential algebraic equations (DAE), discontinuous differential equations (DDE) and delay differential equations. In particular, the extended syntax is motivated by the DDE models of the cell cycle in [82] and [24], which divide the mass of the cell when a species, *Cyclin B*, crosses a threshold with negative slope. Such discontinuities are cumbersome to implement with ordinary chemical reactions and have previously been deterministically simulated in WinPP / XPP [90] using 'global' constraints. Figure 5.21 shows the budding yeast cell cycle model of [82] in the extended syntax.

The extension thus allows reactions to be described as chemical equations (either elementary or with arbitrary kinetic functions) and in the format of differential equations. E.g., the differential equation

$$\frac{\mathrm{d}\,CycB_T}{\mathrm{d}t} = k_1 - (k_{2.1} + k_{2.2}\,Cdh1 + k_{2.3}\,Cdc20_A)CycB_T$$

could be entered as four elementary chemical reactions in the standard Cyto-Sim syntax:

```
* k1-> CycBT CycBT k2.1-> *
CycBT + Cdh1 k2.2-> Cdh1
CycBT + Cdc20A k2.3-> Cdc20A
```

or in a single line differential equation in the extended syntax:

```
CycBT' = k1 - (k2.1+k2.2*Cdh1+k2.3*Cdc20A)*CycBT
```

Algebraic equations such as

```
CycB = (1-2*CKIT/((BB=CycBT+CKIT+Kdiss)+sqrt(BB*BB-4*CycBT*CKIT)))*CycBT*M/beta
```

may also be included in the simulation script. These equations are evaluated when any of the values appearing on the right hand side of the equation change. If there is more than one equation that is affected, they are evaluated in the order they are written in the file. The above example also illustrates in-line evaluation (i.e., `BB=CycBT+CKIT+Kdiss`), which is performed using left-to-right priority.

To control the discontinuities of DDEs, a type of instantaneous reaction controlled by a predicate has been implemented. E.g., the following two reactions control when the cell divides in the cell cycle model used in Chapter 10:

```
div0 [CycB*M/beta<thresh0,M=M/2]-> div1
div1 [CycB*M/beta>thresh1]-> div0
```

As with the normal chemical paradigm, the reactants (i.e., `div0` and `div1`) must exist for the reaction to be enabled and when the reaction fires, the reactants are consumed and the products produced simultaneously. The difference in this case is that the reaction is also controlled by a comma-separated list of predicates or expressions interpreted as predicates (zero being false and values greater than zero being true) within square brackets. These are evaluated in left-to-right order with short-circuiting.

In the above example `div0` and `div1` are tokens which indicate the state of $CycB$. The initial condition is that `div0` exists and `div1` does not, hence only the first reaction can possibly fire. When $CycB * M/beta$ is lower than the threshold $thresh0$, the first predicate in the list returns true and the second one is evaluated. In this case the predicate is an expression which divides the mass by two and has a resulting value greater than zero, so the reaction is executed. Note that the short-circuiting prevents the mass division expression being evaluated more than once. With `div0` consumed, only the second reaction is now possible and it will only fire when $CycB * M/beta$ reaches threshold $thresh1$.

Other features of the extensions include the keyword `simulate`, which allow the possibility to run simulations with respect to time rather than steps.

5.7 Examples

The following subsections contain examples of Cyto-Sim scripts.

5.7.1 Lotka-Volterra Reactions

Possibly the simplest and most well known chemical oscillator is characterised by the Lotka autocatalytic reactions [59] (subsequently and independently modelled by Volterra). Figure 5.2 is a model which uses Cyto-Sim native chemical rules, which assume mass action and therefore require only a rate to fully specify the kinetics. Figure 5.4 is an equivalent model using Cyto-Sim Petri net incidence matrix syntax. Figure 5.3 is the corresponding Petri net diagram. Note that due to the limitations of incidence matrices in describing stoichiometry, it is necessary to explicitly describe the mass action kinetic laws for transitions `t1` and `t2`. These transitions have incoming and outgoing arcs incident on the same place, which result in a zero entry in the matrix. It is therefore necessary to explicitly state the kinetic dependence. Simulations of the two models are statistically indistinguishable and Figure 5.5 is a typical example. Note that this system of reactions produce neutrally stable oscillations and it has a fixed point at `Y1` = `Y2` = 0. Hence, if the simulation is run long enough, this condition will eventually be met and the simulator will halt because there is no auto-generation of either `Y1` or `Y2`.

5.7.2 Oregonator

Field and Noyes [29] produced a generalisation of the Belousov-Zhabotinskii reactions using five steps and three independent intermediate chemical species, which they called the Oregonator reactions. These demonstrate limit cycle (i.e. positively stable) oscillations using reactions that require no more than two reacting molecules (in contrast to the so-called Brusselator [69], which requires reactions involving three reactants). A model of the Oregonator reactions using Cyto-Sim native rules is shown in Figure 5.6. The Petri net version of the same model is given in Figure 5.8, with a corresponding Petri net diagram shown in figure 5.7. As with the previous Petri net model, the kinetics of some of the reactions must be explicitly defined. A simulation of this model is shown in Figure 5.9.

5.7.3 Noise-Resistant Oscillator

A sophisticated biochemically-inspired oscillator is the noise resistant oscillator of [84], represented in Figure 5.10 as a diagram adapted from the original article. This achieves temporal stability with small numbers of molecules by virtue of its topology. Figure 5.11 is the model described using Cyto-Sim's native chemical syntax. The assumption of mass action kinetics allows a compact description of each reaction, which require only the addition of a rate to specify the kinetic behaviour. Figure 5.13 is the same model given as a Petri net incidence matrix, shown diagrammatically in Figure 5.12. As with the previous Petri net models, the limitations of an incidence matrix require that the kinetic laws for some transitions be explicitly described.

Figure 5.14 is an example simulation of the noise resistant oscillator, demonstrating the expected periodicity. No explicit units are given in the model, however rates are assumed to be $hours^{-1}$ or $molecules^{-1}hours^{-1}$, as appropriate. Figure 5.15 shows the simulated effect of switching off the gene for protein `pR`. This is achieved in the simulator model by the inclusion of `-1 gR@50000,-1 g_R@50000` in the system statement:

```
system 1 gA,1 gR,circadian_clock,-1 gR@50000,-1 g_R@50000
```

This has the effect of deleting `gR` or `g_R` at evolution step 50000 (both must be deleted since it cannot be predicted which of these two states the gene

```
object X,Y1,Y2,Z
rule lotka_volterra
{
    X  + Y1 0.0002-> 2Y1 + X
    Y1 + Y2 0.01->   2Y2
    Y2      10->     Z
}
system 100000 X,1000 Y1,1000 Y2,lotka_volterra
evolve 0-500000 step 500
plot Y2,Y1
```

Figure 5.2: Lotka-Volterra reactions using Cyto-Sim native rule syntax.

will be in).

5.7.4 Oscillatory behaviour of NF-κB

The model is given in Figure 5.16 and a typical output plot is given in Figure 5.18. Very little stochastic noise is evident due to the relatively large numbers of molecules in the system.

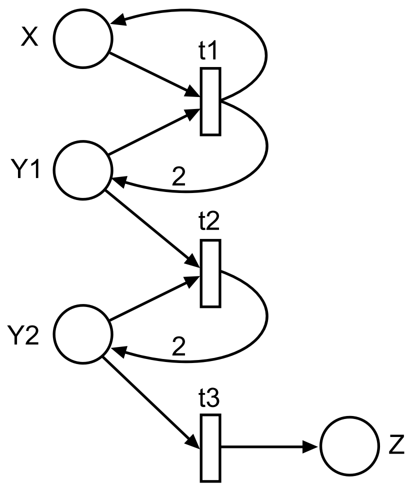


Figure 5.3: Petri net representation of Lotka-Volterra reactions (tokens omitted).

```
object X,Y1,Y2,Z
petri lotka_volterra
{
    X ,Y1,Y2,Z
t1  0 ,1 ,0 ,0  :(0.0002*X*Y1)
t2  0 ,-1,1 ,0  :(0.01*Y1*Y2)
t3  0 ,0 ,-1,1  :10
}
system 100000 X,1000 Y1,1000 Y2,lotka_volterra
evolve 0-500000 step 500
plot Y2,Y1
```

Figure 5.4: Lotka-Volterra reactions using a Petri net incidence matrix.

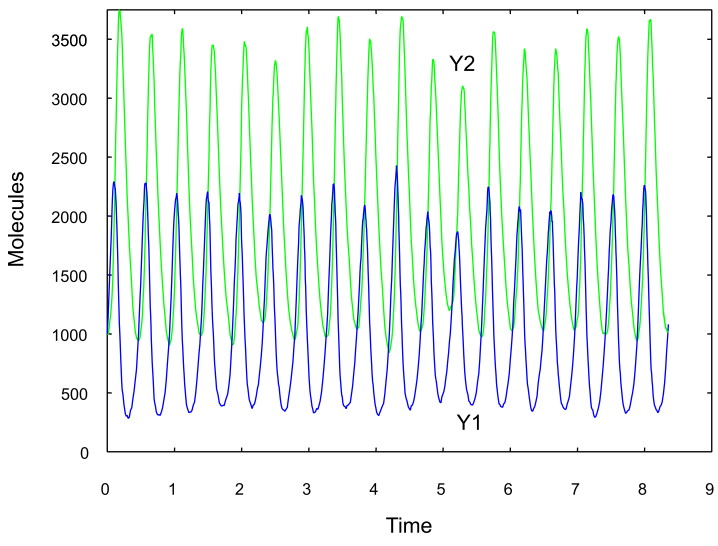


Figure 5.5: Typical simulation trace of Lotka-Volterra reactions.

```
object X1,X2,X3,Y1,Y2,Y3,Z1,Z2 rule oregonator {
X1 + Y2 0.004-> Y1 + X1
Y1 + Y2 0.1-> Z1
X2 + Y1 0.208-> 2Y1 + Y3 + X2
2Y1 0.016-> Z2
X3 + Y3 0.013-> Y2 + X3
}
system { 500 X1,500 Y1, 1000 X2,1000 Y2,2000 X3,2000 Y3,oregonator }
evolve 0-1000000 step 1000
plot Y1, Y2, Y3
```

Figure 5.6: Oregonator oscillator modelled using Cyto-Sim native rule syntax.

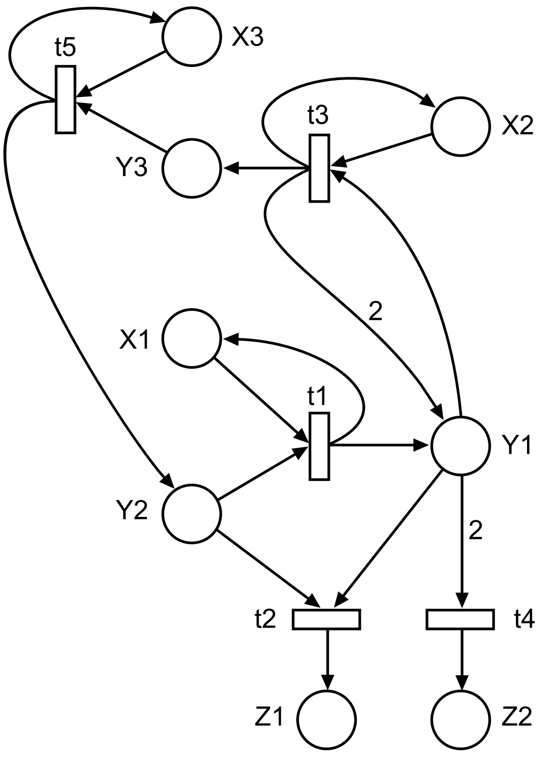


Figure 5.7: Petri net representation of Oregonator oscillator (tokens omitted).

```
object X1,X2,X3,Y1,Y2,Y3,Z1,Z2
petri oregonator
{
    X1,X2,X3,Y1,Y2,Y3,Z1,Z2
t1  0 ,0 ,0 ,1 ,-1,0 ,0 ,0  :(0.004*X1*Y2)
t2  0 ,0 ,0 ,-1,-1,0 ,1 ,0  :0.1
t3  0 ,0 ,0 ,1 ,0 ,1 ,0 ,0  :(0.208*X2*Y1)
t4  0 ,0 ,0 ,-2,0 ,0 ,0 ,1  :0.016
t5  0 ,0 ,0 ,0 ,1 ,-1,0 ,0  :(0.013*X3*Y3)
}
system { 500 X1,500 Y1,1000 X2,1000 Y2,2000 X3,2000 Y3,oregonator }
evolve 0-1000000 step 1000
plot Y1, Y2, Y3
```

Figure 5.8: Oregonator oscillator modelled using a Petri net incidence matrix.

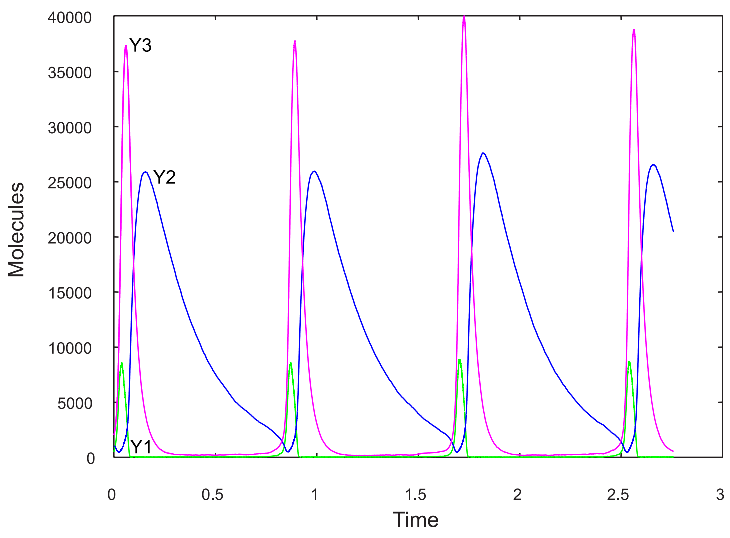


Figure 5.9: Typical simulation trace of Oregonator oscillator.

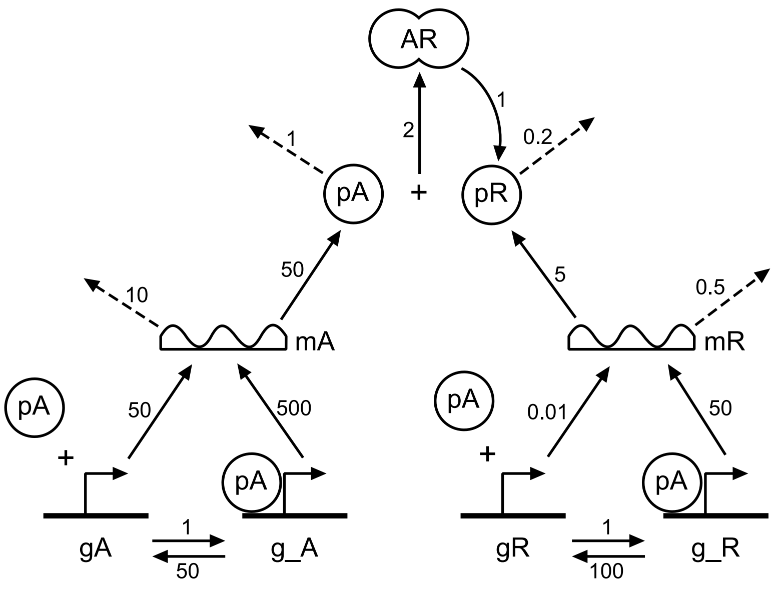


Figure 5.10: Reaction scheme of noise-resistant oscillator, adapted from [84].

```
object {
  IkBa,NF_kB,IkBa_NF_kB,IkBb,IkBb_NF_kB,IkBe,IkBe_NF_kB,IKK,NF_kBn,IkBan,
  IkBan_NF_kBn,IkBbn,IkBbn_NF_kBn,IkBen,IkBen_NF_kBn,IkBa_t,IkBb_t,IkBe_t,
  IKKIkBa,IKKIkBa_NF_kB,IKKIkBb,IKKIkBb_NF_kB,IKKIkBe,IKKIkBe_NF_kB
}
rule NFkB {                                           // Reaction no.
  IkBa + NF_kB 8.30E-7-> IkBa_NF_kB                         // 1.
  IkBa_NF_kB 0.5E-3-> NF_kB + IkBa                          // 2.
  IkBb + NF_kB 8.30E-7-> IkBb_NF_kB                         // 3.
  IkBb_NF_kB 0.5E-3-> NF_kB + IkBb                          // 4.
  IkBe + NF_kB 8.30E-7-> IkBe_NF_kB                         // 5.
  IkBe_NF_kB 0.5E-3-> NF_kB + IkBe                          // 6.
  IKKIkBa + NF_kB 8.30E-7-> IKKIkBa_NF_kB                   // 7.
  IKKIkBa_NF_kB 0.5E-3-> NF_kB + IKKIkBa                    // 8.
  IKKIkBa_NF_kB 2.04E-2-> IKK + NF_kB                       // 9.
  IKKIkBb + NF_kB 8.30E-7-> IKKIkBb_NF_kB                   // 10.
  IKKIkBb_NF_kB 0.5E-3-> NF_kB + IKKIkBb                    // 11.
  IKKIkBb_NF_kB 7.5E-3-> IKK + NF_kB                        // 12.
  IKKIkBe + NF_kB 8.30E-7-> IKKIkBe_NF_kB                   // 13.
  IKKIkBe_NF_kB 0.5E-3-> NF_kB + IKKIkBe                    // 14.
  IKKIkBe_NF_kB 1.1E-2-> IKK + NF_kB                        // 15.
  IkBa_NF_kB 2.25E-5-> NF_kB                                // 16.
```

```
object gA,g_A,gR,g_R,MA,MR,pA,pR,AR
rule circadian_clock
{
    gA 50-> MA + gA
    pA + gA 1-> g_A
    g_A 500-> MA + g_A
    gR 0.01-> MR + gR
    g_R 50-> MR + g_R
    MA 50-> pA
    MR 5-> pR
    pA + pR 2-> AR
    AR 1-> pR
    pA 1-> *
    pR 0.2-> *
    MA 10-> *
    MR 0.5-> *
    g_R 100-> pA + gR
    pA + gR 1-> g_R
    g_A 50-> pA + gA
}
system 1 gA, 1 gR, circadian_clock
evolve 0-150000 step 150
plot pA, pR
```

Figure 5.11: Noise-resistant oscillator [84] modelled using Cyto-Sim native reaction rules.

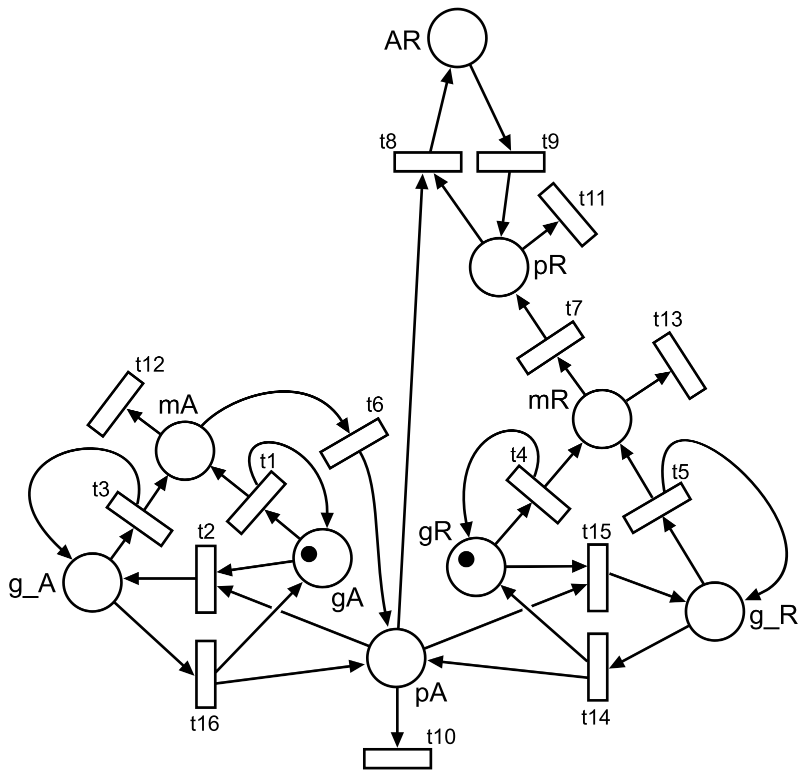


Figure 5.12: Petri net representation of the noise-resistant oscillator. Initial tokens shown.

```
object gA,g_A,gR,g_R,MA,MR,pA,pR,AR
petri circadian_clock
{
    gA,g_A,gR,g_R,MA,MR,pA,pR,AR
t1  0 ,0  ,0 ,0  ,1 ,0 ,0 ,0 ,0  :(50*gA)
t2  -1,1  ,0 ,0  ,0 ,0 ,-1,0 ,0  :1
t3  0 ,0  ,0 ,0  ,1 ,0 ,0 ,0 ,0  :(500*g_A)
t4  0 ,0  ,0 ,0  ,0 ,1 ,0 ,0 ,0  :(0.01*gR)
t5  0 ,0  ,0 ,0  ,0 ,1 ,0 ,0 ,0  :(50*g_R)
t6  0 ,0  ,0 ,0  ,-1,0 ,1 ,0 ,0  :50
t7  0 ,0  ,0 ,0  ,0 ,-1,0 ,1 ,0  :5
t8  0 ,0  ,0 ,0  ,0 ,0 ,-1,-1,1  :2
t9  0 ,0  ,0 ,0  ,0 ,0 ,0 ,1 ,-1 :1
t10 0 ,0  ,0 ,0  ,0 ,0 ,-1,0 ,0  :1
t11 0 ,0  ,0 ,0  ,0 ,0 ,0 ,-1,0  :0.2
t12 0 ,0  ,0 ,0  ,-1,0 ,0 ,0 ,0  :10
t13 0 ,0  ,0 ,0  ,0 ,-1,0 ,0 ,0  :0.5
t14 0 ,0  ,1 ,-1 ,0 ,0 ,1 ,0 ,0  :100
t15 0 ,0  ,-1,1  ,0 ,0 ,-1,0 ,0  :1
t16 1 ,-1 ,0 ,0  ,0 ,0 ,1 ,0 ,0  :50
}
system 1 gA, 1 gR, circadian_clock
evolve 0-150000 step 150
plot pA, pR
```

Figure 5.13: Noise-resistant oscillator modelled using a Petri net incidence matrix.

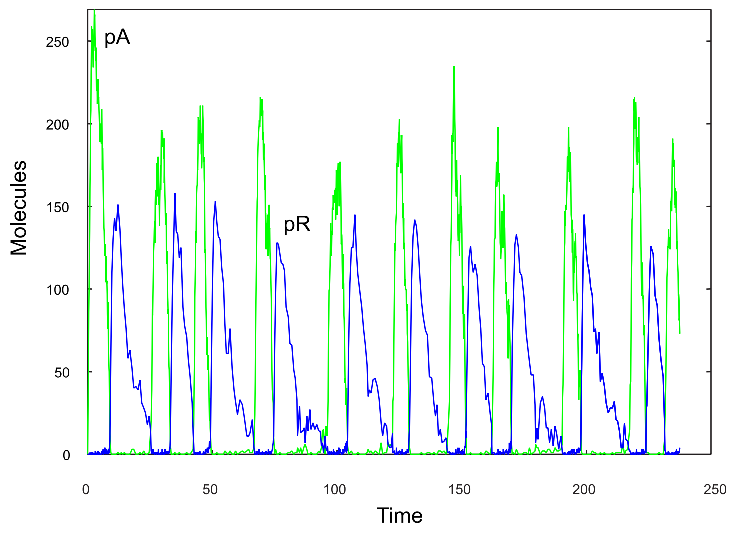


Figure 5.14: Typical single simulation trace of the noise-resistant oscillator.

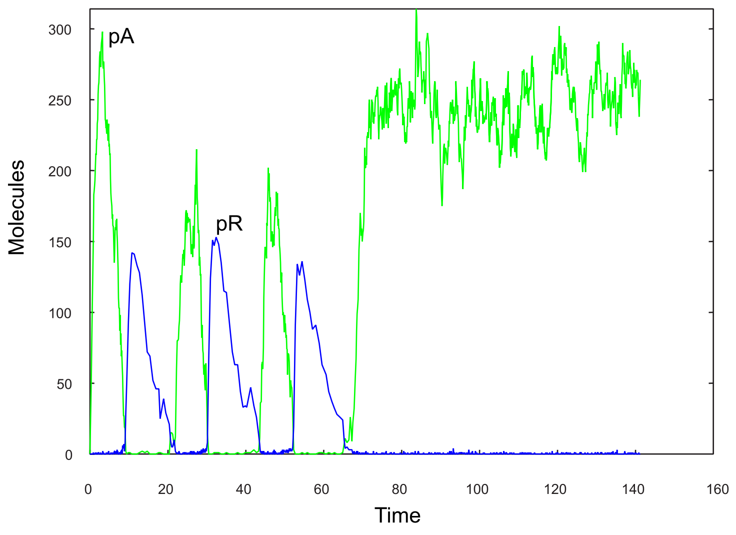


Figure 5.15: Typical single simulation trace of switching off the gene for pR in the noise-resistant oscillator.

Figure 5.16: (below) Model demonstrating oscillatory behaviour in NF-κB [47].

```
IkBb_NF_kB 2.25E-5-> NF_kB                         // 17.
IkBe_NF_kB 2.25E-5-> NF_kB                         // 18.
NF_kB 0.9E-1-> NF_kBn                              // 19.
NF_kBn 0.8E-4-> NF_kB                              // 20.
IkBan + NF_kBn 8.30E-7-> IkBan_NF_kBn              // 21.
IkBan_NF_kBn 0.5E-3-> NF_kBn + IkBan               // 22.
IkBbn + NF_kBn 8.30E-7-> IkBbn_NF_kBn              // 23.
IkBbn_NF_kBn 0.5E-3-> NF_kBn + IkBbn               // 24.
IkBen + NF_kBn 8.30E-7-> IkBen_NF_kBn              // 25.
IkBen_NF_kBn 0.5E-3-> NF_kBn + IkBen               // 26.
* 0.927-> IkBa_t                                   // 27.
NF_kBn + NF_kBn 2.74E-8*2-> IkBa_t + NF_kBn + NF_kBn // 28.
IkBa_t 2.8E-4-> *                                  // 29.
* 0.107-> IkBb_t                                   // 30.
IkBb_t 2.8E-4-> *                                  // 31.
* 0.0765-> IkBe_t                                  // 32.
IkBe_t 2.8E-4-> *                                  // 33.
IKK + IkBa 3.74E-08-> IKKIkBa                      // 34.
IKKIkBa 1.25E-3-> IKK + IkBa                       // 35.
IkBa_t 4.08E-3-> IkBa + IkBa_t                     // 36.
IkBa 1.13E-4-> *                                   // 37.
IkBa 3.0E-4-> IkBan                                // 38.
IkBan 2.0E-4-> IkBa                                // 39.
IKK + IkBb 9.96E-09-> IKKIkBb                      // 40.
IKKIkBb 1.75E-3-> IKK + IkBb                       // 41.
IkBb_t 4.08E-3-> IkBb + IkBb_t                     // 42.
IkBb 1.13E-4-> *                                   // 43.
IkBb 1.5E-4-> IkBbn                                // 44.
IkBbn 1.0E-4-> IkBb                                // 45.
IKK + IkBe 1.49E-08-> IKKIkBe                      // 46.
IKKIkBe 1.75E-3-> IKK + IkBe                       // 47.
IkBe_t 4.08E-3-> IkBe + IkBe_t                     // 48.
IkBe 1.13E-4-> *                                   // 49.
IkBe 1.5E-4-> IkBen                                // 50.
IkBen 1.0E-4-> IkBe                                // 51.
IKK + IkBa_NF_kB 3.07E-7-> IKKIkBa_NF_kB           // 52.
IKKIkBa_NF_kB 1.25E-3-> IKK + IkBa_NF_kB           // 53.
IkBan_NF_kBn 1.38E-2-> IkBa_NF_kB                  // 54.
```

```
  IKK + IkBb_NF_kB 7.97E-08-> IKKIkBb_NF_kB            // 55.
  IKKIkBb_NF_kB 1.75E-3-> IKK + IkBb_NF_kB             // 56.
  IkBbn_NF_kBn 5.2E-3-> IkBb_NF_kB                     // 57.
  IKK + IkBe_NF_kB 1.16E-7-> IKKIkBe_NF_kB             // 58.
  IKKIkBe_NF_kB 1.75E-3-> IKK + IkBe_NF_kB             // 59.
  IkBen_NF_kBn 5.2E-3-> IkBe_NF_kB                     // 60.
  IKK 1.2E-4-> *                                       // 61.
  IKKIkBa 4.07E-3-> IKK                                // 62.
  IKKIkBb 1.5E-3-> IKK                                 // 63.
  IKKIkBe 2.2E-3-> IKK                                 // 64.
}
system {
  NFkB,114218 IkBa,151 NF_kB,50184 IkBa_NF_kB,13028 IkBb,5001 IkBb_NF_kB,
  9295 IkBe,3568 IkBe_NF_kB,60221 IKK,130 NF_kBn,113576 IkBan,861 IkBan_NF_kBn,
  9939 IkBbn,189 IkBbn_NF_kBn,7092 IkBen,135 IkBen_NF_kBn,3314 IkBa_t,
  383 IkBb_t,273 IkBe_t
}
evolve 0-10000000 step 10000
plot {
  IkBa,NF_kB,IkBa_NF_kB,IkBb,IkBb_NF_kB,IkBe,IkBe_NF_kB,IKK,NF_kBn,IkBan,
  IkBan_NF_kBn,IkBbn,IkBbn_NF_kBn,IkBen,IkBen_NF_kBn,IkBa_t,IkBb_t,IkBe_t,
  IKKIkBa,IKKIkBa_NF_kB,IKKIkBb,IKKIkBb_NF_kB,IKKIkBe,IKKIkBe_NF_kB
}
```

5.7.5 Stable and unstable attractors

The Cyto-Sim script given in Figure 5.19 serves two purposes: to demonstrate the use of mathematical expressions to define parameters of the model and simulation and to illustrate a crucial difference between deterministic and stochastic models.

The model is essentially a one-dimensional system with three attractors, repeated three times in the script to simultaneously show the different attractors. Stable attractors at object numbers of 1000 and 7000 are separated by an unstable attractor at 4000. As a deliberately contrived model,

μ	Dependent reactions D_μ	$\lvert D_\mu \rvert$
1.	{1,2,3,5,7,10,13,16,19,34,37,38,52}	13
2.	{,1,2,3,5,7,10,13,16,19,34,37,38,52}	13
3.	{1,3,4,5,7,10,13,17,19,40,43,44,55}	13
4.	{1,3,4,5,7,10,13,17,19,40,43,44,55}	13
5.	{1,3,5,6,7,10,13,18,19,46,49,50,58}	13
6.	{1,3,5,6,7,10,13,18,19,46,49,50,58}	13
7.	{1,3,5,7,8,9,10,13,19,35,53,62}	12
8.	{1,3,5,7,8,9,10,13,19,35,53,62}	12
9.	{1,3,5,7,8,9,10,13,19,34,40,46,52,53,55,58,61}	17
10.	{1,3,5,7,10,11,12,13,19,41,56,63}	12
11.	{1,3,5,7,10,11,12,13,19,41,56,63}	12
12.	{1,3,5,7,10,11,12,13,19,34,40,46,52,55,56,58,61}	17
13.	{1,3,5,7,10,13,14,15,19,47,59,64}	12
14.	{1,3,5,7,10,13,14,15,19,47,59,64}	12
15.	{1,3,5,7,10,13,14,15,19,34,40,46,52,55,58,59,61}	17
16.	{1,2,3,5,7,10,13,16,19,52}	10
17.	{1,3,4,5,7,10,13,17,19,55}	10
18.	{1,3,5,6,7,10,13,18,19,58}	10
19.	{1,3,5,7,10,13,19,20,21,23,25,28}	12
20.	{1,3,5,7,10,13,19,20,21,23,25,28}	12
21.	{20,21,22,23,25,28,39,54}	8
22.	{20,21,22,23,25,28,39,54}	8
23.	{20,21,23,24,25,28,45,57}	8
24.	{20,21,23,24,25,28,45,57}	8
25.	{20,21,23,25,26,28,51,60}	8
26.	{20,21,23,25,26,28,51,60}	8
27.	{29,36}	2
28.	{29,36}	2
29.	{29,36}	2
30.	{31,42}	2
31.	{31,42}	2
32.	{33,48}	2
33.	{33,48}	2
34.	{1,7,34,35,37,38,40,46,52,55,58,61,62}	13
35.	{1,7,34,35,37,38,40,46,52,55,58,61,62}	13
36.	{1,34,37,38}	4
37.	{1,34,37,38}	4
38.	{1,21,34,37,38,39}	6
39.	{1,21,34,37,38,39}	6
40.	{3,10,34,40,41,43,44,46,52,55,58,61,63}	13
41.	{3,10,34,40,41,43,44,46,52,55,58,61,63}	13
42.	{3,40,43,44}	4
43.	{3,40,43,44}	4
44.	{3,23,40,43,44,45}	6
45.	{3,23,40,43,44,45}	6
46.	{5,13,34,40,46,47,49,50,52,55,58,61,64}	13
47.	{5,13,34,40,46,47,49,50,52,55,58,61,64}	13
48.	{5,46,49,50}	4
49.	{5,46,49,50}	4
50.	{5,25,46,49,50,51}	6
51.	{5,25,46,49,50,51}	6
52.	{2,8,9,16,34,40,46,52,53,55,58,61}	12
53.	{2,8,9,16,34,40,46,52,53,55,58,61}	12
54.	{2,16,22,52,54}	5
55.	{4,11,12,17,34,40,46,52,55,56,58,61}	12
56.	{4,11,12,17,34,40,46,52,55,56,58,61}	12
57.	{4,17,24,55,57}	5
58.	{6,14,15,18,34,40,46,52,55,58,59,61}	12
59.	{6,14,15,18,34,40,46,52,55,58,59,61}	12
60.	{6,18,26,58,60}	5
61.	{34,40,46,52,55,58,61}	7
62.	{7,34,35,40,46,52,55,58,61,62}	10
63.	{10,34,40,41,46,52,55,58,61,63}	10
64.	{13,34,40,46,47,52,55,58,61,64}	10

Figure 5.17: Reaction dependency of NF-κB model of Figure 5.16. D_μ is the set of reactions whose propensity is affected by reaction μ. Mean $|D_\mu| = 9.05$.

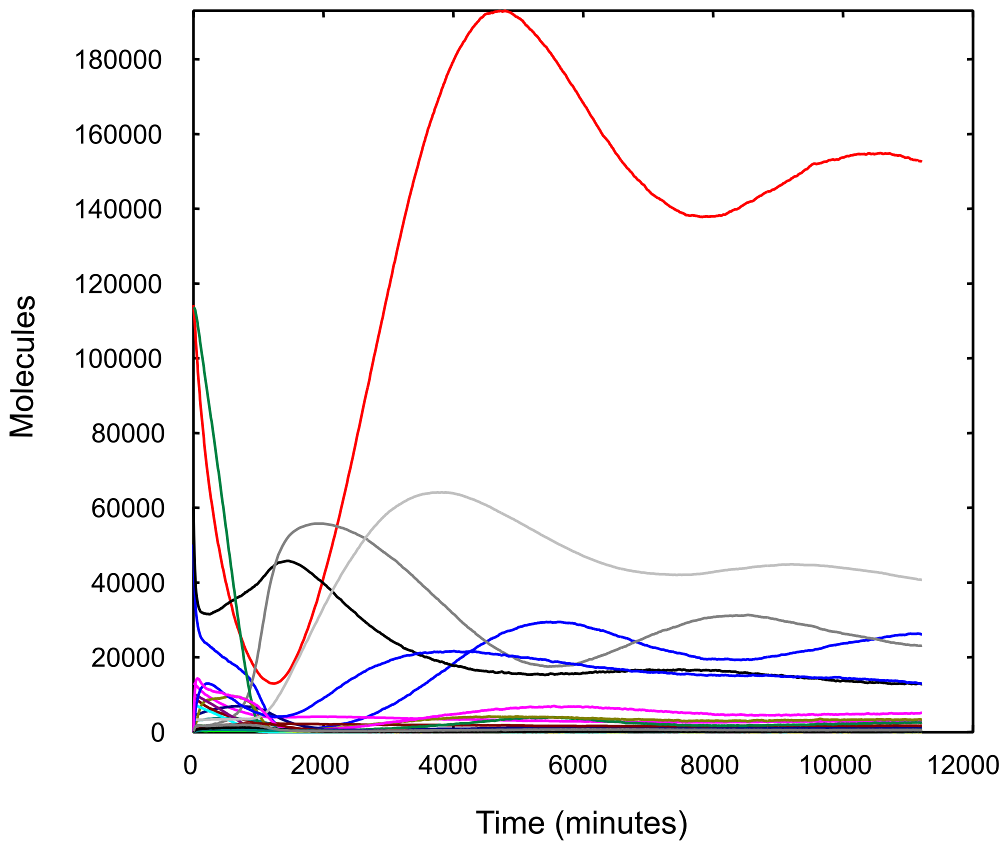


Figure 5.18: Typical simulation results for NF-κB model given in Figure 5.16.

these values can be set precisely using constants `x1`, `x2` and `x3`, respectively. With initial value `X` = 8000, `X` tends to the stable attractor at `X` = 7000. With initial value `Z` = 0, `Z` tends to the stable attractor at `Z` = 1000. With initial value `Y` = 4000, `Y` is placed directly on the unstable attractor and randomly tends to one of the stable attractors. This is illustrated in Figure 5.20. This behaviour is in contrast to that of a deterministic simulation of the same system using ordinary differential equations (ODEs), where `Y` will tend to remain on the unstable attractor. This is because in the ODE context the 'forces' on `Y` cancel to exactly zero and remain so.

```
constant alpha=1000000,x1=1000,x2=7*x1,x3=(x1+x2)/2
constant k=(x3*x3*(x3/3-x1)-x1*x1*(x1/3-x3))/(x3-x1)
constant a=x3*x3-k,c=x3*(x3*x3/3-k)
object X,Y,Z
rule Xattractor {
X a/alpha-> *
c/alpha-> X
X + X + X 2/alpha-> X + X
X + X 2*x3/alpha-> X + X + X
}
rule Yattractor {
Y a/alpha-> *
c/alpha-> Y
Y + Y + Y 2/alpha-> Y + Y
Y + Y 2*x3/alpha-> Y + Y + Y
}
rule Zattractor {
Z a/alpha-> *
c/alpha-> Z
Z + Z + Z 2/alpha> Z + Z
Z + Z 2*x3/alpha-> Z + Z + Z
}
system Xattractor, Yattractor, Zattractor, 8*x1 X, x3 Y, 0 Z
evolve 0-2000*x1 step x1/10
plot X,Y,Z
```

Figure 5.19: Cyto-Sim script to demonstrate stable and unstable attractors.

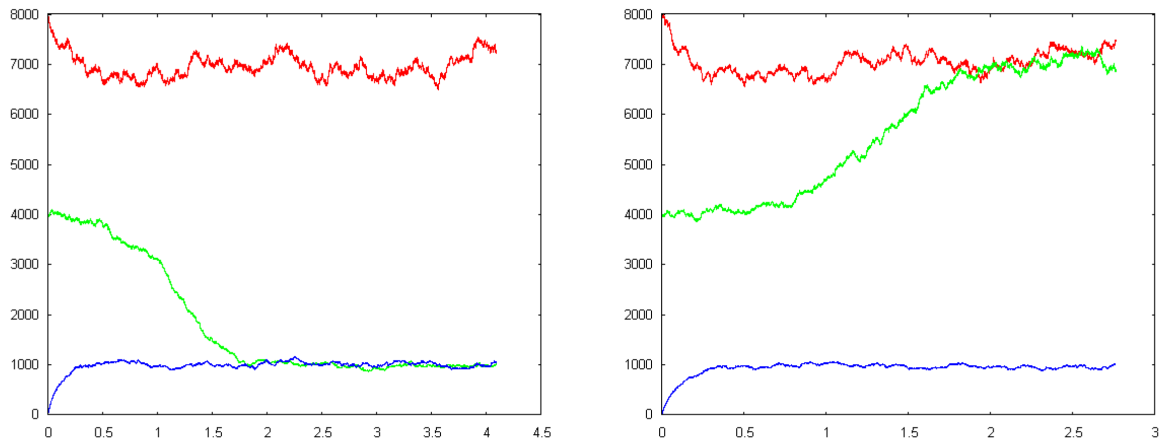


Figure 5.20: Two Cyto-Sim simulation traces showing how various initial conditions tend to stable attractors (`X`: upper trace in red, `Z`: lower trace in blue) and randomly away from an unstable attractor (`Y`: middle trace in green).

```
constant alpha=424,beta=1000,thresh0=0.1*alpha,thresh1=0.2*alpha
constant k1=0.04*alpha,k2.1=0.04,k2.2=1/alpha,k2.3=1/alpha
constant k3.1=alpha,k3.2=10,k4.1=2,k4=35,k5.1=0.005*alpha,k5.2=0.2*alpha,k6=0.1
constant J3=0.04*alpha,J4=0.04*alpha,J5=0.3*alpha,k7=1,k8=0.5,J7=0.001*alpha
constant J8=0.001*alpha,Mad1=alpha,k9=0.1/alpha,k10=0.02,k11=alpha,k12.1=0.2
constant k12.2=50/alpha,k12.3=100/alpha,Kdiss=0.001*alpha,k13.1=0*alpha,k13.2=1,k14=1
constant k15.1=1.5*alpha/beta,k15.2=0.05,k16.1=alpha,k16.2=3,J15=0.01*alpha
constant J16=0.01*alpha,mu=0.005,Mstar=10*beta
species CycBT=alpha,Cdh1,Cdc20T=alpha,Cdc20A,IE,CKIT,SK,TF,M=beta,BB,CycB,div0=1,div1
// Algebraic equation
CycB = (1-2*CKIT/((BB=CycBT+CKIT+Kdiss)+sqrt(BB*BB-4*CycBT*CKIT)))*CycBT*M/beta
div0 [CycB*M/beta<thresh0,M=M/2]-> div1  // Mass division control
div1 [CycB*M/beta>thresh1]-> div0        // using predicate reactions
// Creation & consumption reactions with summed rate functions
* k1-> CycBT
CycBT ((k2.1+k2.2*Cdh1+k2.3*Cdc20A)*CycBT)-> *
* ((k3.1+k3.2*Cdc20A)*(alpha-Cdh1)/(J3+alpha-Cdh1))-> Cdh1
Cdh1 ((k4.1*SK+k4*CycB)*Cdh1/(J4+Cdh1))-> *
* (k5.1+k5.2*CycB^4/(J5^4+CycB^4))-> Cdc20T
Cdc20T (k6*Cdc20T)-> *
* (k7*IE*(Cdc20T-Cdc20A)/(J7+Cdc20T-Cdc20A))-> Cdc20A
Cdc20A (k8*Mad1*Cdc20A/(J8+Cdc20A)+k6*Cdc20A)-> *
* (k9*(alpha-IE)*CycB)-> IE
IE (k10*IE)-> *
* k11-> CKIT
CKIT ((k12.1+k12.2*SK+k12.3*CycB)*CKIT)-> *
* (k13.1+k13.2*TF)-> SK
SK k14-> *
* ((k15.1*M+k15.2*SK)*(alpha-TF)/(J15+alpha-TF))-> TF
TF ((k16.1+k16.2*CycB)*TF/(J16+TF))-> *
* (mu*M*(1-M/Mstar))-> M
simulate 1000 step 0.5    // Simulation specification in terms of time
```

Figure 5.21: Budding yeast cell cycle model of [82] expressed in extended syntax (see Section 5.6). The terms of the original ODEs have been divided into stochastic creation and consumption reactions.

Chapter 6

From Single Cells to Tissues: Colonies of Synchronizing Agents

The work presented in this chapter was originally published in

M. Cavaliere, R. Mardare and S. Sedwards (2007) Colonies of Synchronizing Agents: An Abstract Model of Intracellular and Intercellular Processes, *Proceedings of the International Workshop on Automata for Cellular and Molecular Computing, Budapest.*

and

M. Cavaliere, R. Mardare and S. Sedwards (2008) A multiset-based model of synchronizing agents: Computability and robustness, *Theoretical Computer Science*, **391:3**, 216–238.

The models presented in Chapters 3 – 5 have considered a static and finite membrane structure. In order to now study systems whose structure may change or *emerge*, a modelling framework and computational paradigm is presented called Colonies of Synchronizing Agents (CSA), inspired by the intracellular and intercellular mechanisms in biological tissues.

The model is based on a multiset of agents in a common environment. Each agent has a local state stored in the form of a multiset of atomic objects, which is updated by global multiset rewriting rules either independently or synchronously with another agent.

The model is first defined and its computational power is then studied, considering trade-offs between internal rewriting (intracellular mechanisms) and synchronization between agents (intercellular mechanisms). The dynamic properties of CSAs are also investigated, including behavioural robustness (ability to generate a core behaviour despite agent loss or rule failure) and safety of synchronization (ability of an agent to synchronise with some other agent whenever needed).

6.1 Introduction and motivations

Inspired by *intracellular* and *intercellular* mechanisms in biological tissues, an abstract distributed model of computation called Colonies of Synchronizing Agents (in short CSA) is presented and investigated. The intention is to create a framework to model, analyse and simulate biological tissues in the context of formal language and multiset rewriting.

The model is based on a population of agents (e.g., corresponding to *cells* or *molecules*) in a common environment, able to modify their contents and to synchronise with other agents in the same environment. Each agent has a contents represented by a multiset of atomic objects (e.g., corresponding to *chemical compounds* or the characteristics of individual molecules) with some of the objects classified as terminals (e.g., corresponding to chemicals or properties visible to an external observer). The agents' contents may be modified by means of multiset rewriting rules (called *evolution rules*), which may mimic chemical or other types of *intracellular mechanisms*. Moreover, the agents can influence each other by synchronously changing their contents using pairwise *synchronization rules*. This models, in a deliberately abstract way, the various *intercellular mechanisms* present in biological tissues (e.g., signalling mechanisms that cells and biological systems use). *All rules are global*, so all agents obey the same rules: the only feature that may distinguish the agents is their contents.

Hence, a CSA is essentially a multiset of mutisets, acted upon by multiset rewriting rules.

In this chapter CSAs are considered as generative computing devices and various trade-offs between the power of the evolution rules and the power of the synchronizing rules are considered. CSAs are considered working in a maximally parallel way (all agents are updated synchronously), modelling the idea that if something can happen then it *must* happen. However, from both a biological and a mathematical point of view, it is also useful to investigate systems where the update of the agents is not obligatory (i.e., not synchronous). It is shown that the computational power of maximal parallel and asynchronous CSAs can range from that of finite sets of vectors to that of Turing machines, by varying the power of the evolution and synchronization rules. Moreover, an intermediate class of CSAs, equivalent to partially blind counter machines (hence, not universal [42]), is investigated.

Having investigated the computational power of CSAs, the *robustness* of colonies is studied by considering their ability to generate core behaviours despite the failure (i.e., removal) of agents or of rules. It is shown that for an arbitrary CSA, robustness cannot be decided but that it is possible to individuate classes of (non-trivial) CSAs where this property can be efficiently decided.

The final part of the chapter is concerned with dynamic properties of CSAs in regard to the applications of the rules.

For this reason, a decidable temporal logic is provided to specify and investigate *dynamic properties* of CSAs. For instance, it is shown that the proposed logic can be used to specify and then check whether or not in a CSA an agent has the ability to apply a synchronization whenever it needs: CSAs for which such a property is true are described *safe on synchronization* of rules. This models, in an abstract way, the ability of a cell to use an intercellular mechanism whenever it needs.

CSAs are computational devices that have features inspired by many different models. In particular, they have similarities (and significant differences) with other models inspired by cell-tissues and investigated in the area of membrane computing. Specifically, CSAs can be considered a generalization of P colonies [52], which is also based on interacting agents but has agents with limited contents (two objects) which can only change their contents using very restricted rewriting rules (following an earlier definition of an agent in formal language theory ([51]). In the case of CSAs, in order to be more general, the rewriting rules employed by an agent and the contents of an agent can be arbitrarily complex. Moreover, in P colonies objects can be introduced into the agent from an external environment (with unbounded copies of a given object) and the objects present in an agent may only be transferred to another agent by means of the common environment; no direct communication between agents is allowed (as is the case in CSAs).

CSAs also have similarities with population P systems [6], a class of tissue P systems [61], and in particular with EC tissue P systems [7], where evolution (rewriting) is combined with communication. Cells (i.e., agents) can change their contents by means of (non-cooperative) rewriting rules and hence different types of agents can have different sets of rules. It is also possible to move objects between the agents using 'bonds' and agents may also communicate with an environment that has unbounded resources. Computation is generally implemented in two different phases: local rewriting plus bond making rules, applied in an alternate manner. The main differences with these computing devices and the model described here are that CSAs do not have explicit bonds (edges) between agents (in a sense agents are linked by a complete graph), rewriting in CSAs is arbitrarily complex (i.e., it can be cooperative) for both evolution and synchronization rules, and CSA agents do not have explicit types: rules are global and only the agents' contents differentiate them. This latter characteristic makes CSAa similar to the model of self-assembly of graphs presented in [5], however in that case (*i*) a graph is constructed from an initial seed using multiset-based aggregation rules to enlarge the structure, (*ii*) there is no internal rewriting of the agent contents and (*iii*) there is no synchronization between the agents.

CSAs are also distinct from cellular automata [48], where cells exist on a regular grid, where each cell has a finite number of possible states and where cells interact with a defined neighbourhood. In the case of CSAs, as a result of the multiset-based contents and because of the general rewriting rules, the possible different internal states of a cell may be infinite. Although the initial definition does not include an explicit description of space, the proposed extensions include agents located at arbitrary positions and with the potential to interact with any other agent in the system.

6.2 Preliminaries

In this section, certain preliminary definitions and descriptions are given which have specific relevance to Colonies of Synchronizing Agents.

The following recalls some definitions and results from specific variants of membrane systems which are used in the proofs of this chapter. Readers not interested in the proofs can therefore skip this section.

Definition 6.2.1 ([71]) *A P system with symbol-objects and of degree $m \geq 1$ is defined as a construct*

$$\Pi = (V, T, \mu, w_1, \ldots, w_m, R_1, \ldots, R_m, i_0)$$

where

- *V is an alphabet and its elements are called objects; $T \subseteq V$ is a terminal alphabet;*

- *μ is a membrane structure consisting of m membranes arranged in an hierarchical tree structure; the membranes (and hence the regions that they delimit) are injectively labelled with $1, 2, \ldots, m$;*

- *w_i, $1 \leq i \leq m$, are strings that represent multisets over V associated to regions $1, 2, \ldots, m$ of μ;*

- *R_i, $1 \leq i \leq m$, are finite sets of evolution rules over V; R_i is associated to region i of μ; an evolution rule is of the form $u \rightarrow v$, where u is a string over V and v is a string over $\{a_{here}, a_{out} \mid a \in V\} \cup \{a_{in_j} \mid a \in V, 1 \leq j \leq m\}$.*

- *$i_0 \in \{0, 1, 2, \ldots, m\}$; if $i_0 \in \{1, \ldots, m\}$ then it is the label the membrane that encloses the output region; if $i_0 = 0$ then the output region is the environment.*

For any evolution rule $u \rightarrow v$ the length of u is called the *radius* of the rule and the symbols *here*, *out*, in_j, $1 \leq j \leq m$, are called target indications.

By virtue of the radius of the evolution rules it is possible to distinguish *cooperative* rules (if the radius is greater than one) and *non-cooperative* rules (otherwise).

The *initial configuration* of the system Π comprises the structure μ and the multisets represented by the strings $w_i, 1 \leq i \leq m$. In general, a *configuration* of the system is the m-tuple of multisets of objects present at any time in the m regions of the system.

An occurrence γ_r of the rule $r : u \rightarrow v \in R_i$, $i \in \{1, \cdots, m\}$ can be applied in region i by assigning to γ_r a multiset of objects u taken from the multiset of objects present in region i.

The application of an instance of the evolution rule $u \rightarrow v$ in a region i means to remove the multiset of objects u from the multiset of objects present in region i and to add the multiset v to the multisets of objects present in the adjacent regions, according to the target indications associated to each occurrence of the objects in v. In particular, if v contains an occurrence with target indication *here*, then the occurrence will be placed in the region i, where the rule has been applied. If v contains an occurrence with target indication *out*, then the occurrence will be moved to the region immediately outside the region i (this can be the environment if the region where the rule has been applied is the outermost or *skin* membrane). If v contains an occurrence with target indication in_j then the occurrence is moved from the region i and placed in region j (this can be done only if region j is directly contained by region i; otherwise the evolution rule $u \rightarrow v$ cannot be applied).

A *transition* between configurations is executed using the evolution rules in a *non-deterministic maximally parallel* manner at each step, in each region (it is supposed that a global clock exists, marking the instant of each step for the whole system). This means that occurrences of the objects are assigned to occurrences of the rules in such a way that, after the assignment is made, there are insufficient occurrences of the objects for further occurrences of any of the rules to be applied. This maximal assignment is performed simultaneously in every region of the system at each step. If an occurrence of an object can be assigned to more than one occurrence of the rules then the assignment is chosen in a non-deterministic way.

A sequence of transitions between configurations of a system is called a *evolution*; an evolution is a *successful computation* (or simply a *computation*) if and only if it starts from the initial configuration and *halts*, i.e., it reaches a halting configuration where no occurrence of any rule can be applied in any region.

The *output* of a computation is defined as the number of occurrences of objects from T present in the output region in the halting configuration of Π; the set of numbers computed (or generated) in this way by the system Π, considering any computation, is denoted by $N(\Pi)$.

It is possible to consider as the result of a computation the vector of numbers representing the multiplicities of the occurrences of objects from T present in the output region in the halting configuration. In this case $Ps_T(\Pi)$ denotes the set of vectors of numbers generated by Π, considering all the computations.

$NOP_m(\alpha, tar)$ and $PsOP_m(\alpha, tar)$ denote the family of sets of the form $N(\Pi)$ and $Ps(\Pi)$, respectively, generated by symbol-objects P systems of degree at most $m \geq 1$ (if the degree is not bounded the subscript m becomes $*$), using evolution rules of the type α.

α may be given as $\alpha = coo$, indicating that the systems considered use cooperative evolution rules, and $\alpha = ncoo$, indicating that the systems use only non-cooperative rules.

Moreover, the symbol tar indicates that the communication between the membranes (and hence the regions) is made using the target indication in_j in the way previously specified. If the degree of the system is 1 (only one membrane is present) then the only possible target indications that can be used are *here* and *out* and in such case the notation is $NOP_1(\alpha)$ and $PsOP_1(\alpha)$, respectively.

The following results are known (see, e.g., [71]).

Theorem 6.2.1

- $PsOP_*(ncoo, tar) = PsOP_1(ncoo) = PsCF$.
- $PsOP_*(coo, tar) = PsOP_m(coo, tar) = PsRE$ *for all* $m \geq 1$.

The definition and main results of evolution-communication P systems [16] are recalled here. This joins two basic models of membrane systems; that with evolution rules and symbol-objects and that with symport/antiport rules (see, e.g, [71]).

Definition 6.2.2 *An evolution-communication P system (in short, an EC P system) of degree $m \geq 1$, is defined as*

$$\Pi = (V, \mu, w_1, w_2, \ldots, w_m, R_1, \ldots, R_m, R'_1, \ldots, R'_m, i_0)$$

where:

- V, μ, i_0 *and* $w_i, 1 \leq i \leq m$ *as in Definition 6.2.1;*
- R_i*,* $1 \leq i \leq m$*, are finite sets of simple evolution rules over* V*;* R_i *is associated with the region* i *of* μ*; a simple evolution rule is of the form* $u \to v$*, where* $u \in V^+$ *and* $v \in V^*$*; hence, a simple evolution rule is an evolution rule as in P systems with symbol-objects, but with no target indications (in other words it uses an implicit* 'here' *for target indications);*
- R'_i*,* $1 \leq i \leq m$*, are finite sets of symport rules over* V *of the form* (x, in)*,* (y, out) *and of antiport rules* $(x, in; y, out)$ *with* $x, y \in V^+$*;* R'_i *is associated with membrane* i *of* μ*. For a symport rule* (x, in) *or* (x, out)*,* $|x|$ *is called the* weight *of the rule. For an antiport rule* $(x, in; y, out)$ *the weight is the* $max\{|x|, |y|\}$*.*

In an EC P system a configuration is represented by the membrane structure μ and by the m-tuple of multisets of objects present in the m regions of the system.

In particular, the *initial configuration* comprises the system of membranes μ and the multisets represented by the strings $w_i, 1 \leq i \leq m$.

Occurrences of evolution rules are applied as in P systems with symbol-objects.

An occurrence γ of a symport rule $(x, in) \in R'_i$ $((x, out) \in R'_i)$ can be applied to membrane i by assigning to γ the occurrences of the objects in x taken from the region surrounding region i (taken from region i, respectively).

The application of an instance of the *symport rule* (x, in) to membrane i consists of moving the occurrences of the objects in x from the region (or from the environment) surrounding the region i to region i . If an instance of the symport rule (x, out) is applied to membrane i, the occurrences of the objects in x are moved from region i to the region (or to the environment) that surrounds region i.

An occurrence γ of the antiport rule $(x, in; y, out) \in R'_i$ can be applied to membrane i by assigning to γ the occurrences of the objects in x taken from region i and the occurrences of the objects in y taken from the region surrounding region i.

If an instance of the *antiport rule* $(x, in; y, out)$ is applied to membrane i, the occurrences of the objects in x pass into the region i from the region surrounding it, while, at the same time, the occurrences of the objects in y move from the surrounding region to region i.

A *transition* between configurations is governed by the mixed application of occurrences of the evolution rules and of the symport/antiport rules. Instances of the rules from R_i are applied to occurrences of objects in region i while the application of the instances of rules from R'_i govern the communication of the occurrences of objects through membrane i. There is no distinction drawn between evolution rules and communication rules (*mixed approach*): they are applied in the *non-deterministic maximally parallel manner*, described above.

The system starts from the initial configuration and passes from one configuration to another by applying the above described transitions: this sequence of transitions is called an *evolution* of the system. The system halts when it reaches a halting configuration, i.e., a configuration where no occurrence of any rule (evolution rules or symport/antiport rules) can be applied in any region of Π.

In this case the evolution is called a successful *computation* of Π (or simply a computation of Π) and the number of occurrences of objects contained in the output region i_0 in the halting configuration is the result of the computation. The set of numbers computed (or generated) in this way by the system Π, considering any possible computation of Π, is denoted by $N(\Pi)$.

It is also possible to consider as a result of the computation the vector of numbers representing the multiplicities of the occurrences of the objects contained in the output region in the halting configuration. In this case $Ps(\Pi)$ denotes the set of vectors generated by Π, considering all computations.

The notation $NECP_m(i, j, \alpha)$, $\alpha \in \{ncoo, coo\}$, and $PsECP_m(i, j, \alpha)$, $\alpha \in \{ncoo, coo\}$, is used to denote the family of sets of numbers and the family of sets of vectors of numbers, respectively, generated by EC P systems with at most m membranes (as usual, $m = *$ if such a number is unbounded), using symport rules of weight at most i, antiport rules of weight at most j and simple evolution rules that can be cooperative (*coo*) or non-cooperative (*ncoo*).

The following results are known (see, e.g, [16, 3]).

Theorem 6.2.2 • *$NECP_1(1, 0, ncoo) = NCF$.*

- *$PsECP_1(1, 0, ncoo) = PsCF$.*
- *$NECP_2(1, 1, ncoo) = NRE$.*
- *$PsECP_2(1, 1, ncoo) = PsRE$.*

6.3 Colonies of Synchronizing Agents

In this section the notions of colonies and agents discussed in the Introduction are formally defined.

A *Colony of Synchronizing Agents* (a CSA) of degree $m \geq 1$ is a construct $\Pi = (A, T, C, R)$ with the components having the following meaning:

- A is a finite alphabet of symbols (its elements are called *objects*). $T \subseteq A$ is the alphabet of *terminal objects*.

- An *agent* over A is a multiset over the alphabet A (an agent can be represented by a string $w \in A^*$, since A is finite). C is the *initial configuration of* Π and it is a multiset over the set of all possible agents over A (with $card(C) = m$) .

  Using the notation presented in Chapter 2, $C \in \mathbb{M}_m(H)$ with $H = \mathbb{M}(A)$.

- R is a finite set of *rules* over A.

  There are *evolution rules* of type $u \to v$, with $u \in A^+$ and $v \in A^*$.

  An instance γ of an evolution rule $r : u \to v$ can be applied to an occurrence o_w of agent w by taking a multiset of objects u from o_w (hence, it is required that $u \subseteq w$) and *assigning* it to γ (i.e., assigning the occurrences of the objects in the taken multiset to γ).

  The application of an instance of rule r to the occurrence o_w of the agent w consists of removing from o_w the multiset u and then adding v to the resulting multiset.

  An evolution rule $u \to v$ is said to be *cooperative* (in short, coo_e) if $|u| > 1$, *non-cooperative* ($ncoo_e$) if $|u| = 1$ and *unary* (un_e) if $|v| \leq |u| = 1$.

There are *synchronization rules* of the type $\langle u, v\rangle \rightarrow \langle u', v'\rangle$ with $uv \in A^+$ and $u', v' \in A^*$.

An instance γ of a synchronization rule $r : \langle u, v\rangle \rightarrow \langle u', v'\rangle$ can be applied to the pair of occurrences o_w and $o_{w'}$ of, respectively, agents w and w' by: (i) taking from o_w a multiset of objects u and *assigning* it to γ; (ii) taking from $o_{w'}$ a multiset of objects v and *assigning* it to γ (hence, it is required that $u \subseteq w$ and $v \subseteq w'$).

The application of an instance of rule r to the occurrences o_w and $o_{w'}$ consists of: removing the multiset u from o_w and then adding u' to the resulting multiset; removing the multiset v from $o_{w'}$ and then adding v' to the resulting multiset.

Synchronization rules can be considered as matrices of two rules used simultaneously.

A synchronization rule $\langle u, v\rangle \rightarrow \langle u', v'\rangle$ is said to be cooperative (coo_s) if $|u| > 1$ or $|v| > 1$, non-cooperative ($ncoo_s$) if $|u| = 1$ and $|v| = 1$, *unary* (un_s) if $|u'| \leq |u| = 1$ and $|v'| \leq |v| = 1$.

A *configuration* of a CSA, Π, consists of the occurrences of the agents present in the system at a given time (the existence of a *global clock* which marks the passage of units of time is assumed).

$\mathcal{C}(\Pi)$ denotes the set of *all possible configurations* of Π. Therefore, using the notation presented in the Chapter 2, $\mathcal{C}(\Pi)$ is exactly $\mathbb{M}_m(H)$ with $H = \mathbb{M}(A)$.

A *transition* from an arbitrary configuration c of Π to the next lasts exactly one time unit and can be obtained in two different modes.

Maximally-parallel mode (in short *mp*): A *maximally-parallel transition* of Π (in short, an *mp-transition*) is obtained by applying the rules in the set R to the agents present in the configuration c in a *maximally parallel and non-deterministic* way. This means that for each occurrence o_w of an agent w and each pair of occurrences $o_{w'}$ and $o_{w''}$ of agents w' and w'' present in the configuration c, the occurrences of the objects present in o_w $(o_{w'}, o_{w''})$ are assigned to instances of the evolution (synchronization, resp.) rules, the occurrences of the agents, the occurrences of the objects and the instances of the rules chosen in a non-deterministic way but respecting the following condition. After the assignment of the occurrences of the objects to the instances of the rules is done there is no instance of any rule that can be applied by assigning the (still) unassigned occurrences of the objects.

A single occurrence of an object can only be assigned to a single instance of a rule.

Asynchronous mode (in short *asyn*): A single *asynchronous transition* of Π (in short, an *asyn-transition*) is obtained by applying the rules in the set R to the agents present in the configuration c in an *asynchronous* way.

This means that, for each occurrence o_w of an agent w and each pair of occurrences $o_{w'}$ and $o_{w''}$ of the agents w', w'', present in the configuration c, the occurrences of the objects of o_w $(o_{w'}, o_{w''})$ are *either* assigned to instances of the evolution (synchronization, resp.) rules *or* left unassigned. The occurrences of the agents, the occurrences of the objects and the instances of the rules are chosen in a non-deterministic way. A single occurrence of an object can only be assigned to a single instance of a rule.

In other words, in a single asynchronous transition, any number of instances of rules (zero, one or more) can be applied to the occurrences of the agents present in the configuration c.

A sequence (possibly infinite) $\langle C_0, C_1, \cdots, C_i, C_{i+1}, \cdots\rangle$ of configurations of Π, where C_{i+1} is obtained from C_i, $i \geq 0$, by a γ-transition is called a γ-*evolution* of Π, with $\gamma \in \{asyn, mp\}$. A configuration c of Π present in a γ-evolution of Π is said to be *reachable* using a γ-evolution of Π (or simply reachable if there is no confusion). Hence, it is frequently said that the evolution *reaches* the configuration c.

A γ-evolution of Π, with $\gamma \in \{asyn, mp\}$, is said to be *halting* if it halts, that is if it is finite and the last configuration of the sequence is a *halting configuration*, i.e., a configuration containing only occurrences of agents for which no rule from R is applicable.

A γ-evolution of Π that is halting and that starts with the initial configuration of Π is called a successful γ-computation or, because there is no confusion, it is simply called a γ-*computation* of Π, with $\gamma \in \{asyn, mp\}$.

The *result/output* of an mp- or asyn-computation is the set of vectors of natural numbers, one vector for each agent w present in the halting configuration (i.e., with a number of occurrences greater than zero) and with the vector describing the multiplicities of terminal objects present in w.

More formally, the result of an mp- or asyn-computation which stops in the configuration C_h is the set of vectors of natural numbers $\{Ps_T(w) \mid w \in supp(C_h)\}$.

Taking the union of all the results, for all possible mp- and asyn-computations, gives the set of vectors generated by Π, denoted by $Ps_T^{mp}(\Pi)$ and $Ps_T^{asyn}(\Pi)$, respectively.

It is also possible to consider only the total number of objects comprising the agent (the agent's *magnitude*), without considering the composition. In this case the result of an mp- or asyn-computation is the set of natural numbers, one number for each agent w present in the halting configuration and with the number being the length of w. More formally, in this case, the result of an mp- or asyn-computation that stops in the configuration C_h is then the set of numbers $\{|w| \mid w \in supp(C_h)\}$. In other words, in this case, there is no distinction between the objects composing the agents, in particular the terminals from T are ignored.

Again, taking the union of all the results, for all possible mp- and asyn-computations, gives the *set of numbers generated* by Π, denoted by $N^{mp}(\Pi)$

and $N^{asyn}(\Pi)$, respectively.

Note that in both cases, considering sets of vectors (or sets of numbers) one single computation delivers a finite family of vectors as output (or a finite set of numbers, resp.) because there could be several agents in the halting configuration. However, $Ps_T^{\gamma}(\Pi)$ ($N^{\gamma}(\Pi)$), $\gamma \in \{mp, asyn\}$, is obtained as the union of results of computations of Π, so as a union of sets of vectors (of sets of numbers, resp.).

Families of CSAs are now considered and then families of sets of vectors of numbers or of sets of numbers.

$CSA_m(\alpha, \beta)$, with $\alpha \in \{coo_e, ncoo_e, un_e\}$ and
$\beta \in \{coo_s, ncoo_s, un_s\}$, denotes the class of CSAs having evolution rules of type α, synchronization rules of type β and using at most m occurrences of agents in the initial configuration (m is changed to $*$ if it is unbounded). α or β are omitted if the corresponding rules are not allowed. In particular, notice that if β is omitted then there is no cooperation between the agents.

Hence, $PsCSA_m^{\gamma}(\alpha, \beta)$ (and $NCSA_m^{\gamma}(\alpha, \beta)$) with $\gamma \in \{mp, asyn\}$ denotes the family of sets of vectors (of sets of numbers, resp.) generated by CSAs from $CSA_m(\alpha, \beta)$ using γ-computations.

Example 6.3.1 *A CSA with degree* 3 *is defined by the following:*

$\Pi = (A, T, C, R)$ *with* $A = \{a, b, c\}$, $T = \{a\}$, $C = \{(abcba, 1), (abbcc, 1), (bab, 1)\}$ *and rules* $R = \{r_1 : abca \to ba,\ r_2 : \langle abc, cc\rangle \to \langle aa, cb\rangle\}$.

The application of an instance of the evolution rule r_1 *to the configuration* C *is shown diagrammatically in Figure 6.1. The application of an instance of the synchronization rule* r_2 *to the configuration* C *is shown in Figure 6.2.*

A more complex example is presented in Figure 6.3. Alternative maximally parallel and asynchronous (partial) evolutions of a CSA are shown,

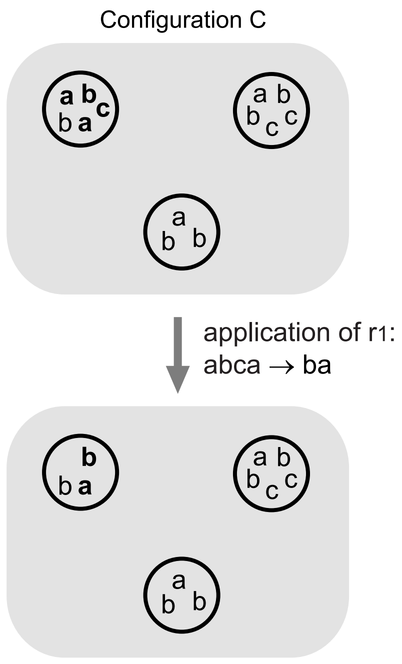


Figure 6.1: Application of an instance of the evolution rule r_1 to configuration C from Example 6.3.1.

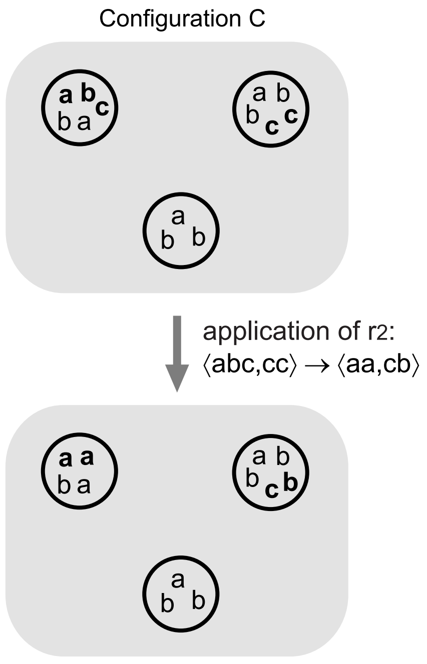


Figure 6.2: Application of an instance of the synchronization rule r_2 to configuration C from Example 6.3.1.

starting from the configuration $\{(ac, 2), (a, 1)\}$ *with rules* $\{ac \rightarrow aa, a \rightarrow b, \langle aa, aa\rangle \rightarrow \langle ab, ab\rangle, \langle ab, d\rangle \rightarrow \langle bb, d\rangle, b \rightarrow d\}$.

The following considers the equality of families of sets of vectors modulo the null vector, i.e., whether two families differ only by the null vector then they are considered to be equal. A_Π denotes the alphabet of the CSA Π, T_Π denotes the terminal alphabet of Π and C_Π denotes the initial configuration of Π.

Moreover, because there is no confusion, it is possible to avoid using "occurrences of ...", writing the entities involved (objects, rules or agents) directly.

For instance, the expression "an object c is used ..." actually means "one occurrence of object c is used ..." and similarly, the expression "the rule r is applied ..." means "one instance of rule r is used ...".

6.4 Computational Power of CSAs

The following Theorem is obtained from the definitions of CSAs and invoking the Turing-Church thesis:

Theorem 6.4.1

$$PsCSA_m^\gamma(\alpha) \subseteq PsCSA_m^\gamma(\alpha, \beta) \subseteq PsRE.$$

with $\alpha \in \{coo_e, ncoo_e, un_e\}$, $\beta \in \{coo_s, ncoo_s, un_s\}, \gamma \in \{mp, asyn\}$ *and* $m \geq 1$.

As soon as there are cooperative evolution rules and maximal-parallelism there is, as expected, maximal computational power.

Theorem 6.4.2

$$PsCSA_2^{mp}(coo_e) = PsCSA_2^{mp}(coo_s) = PsRE.$$

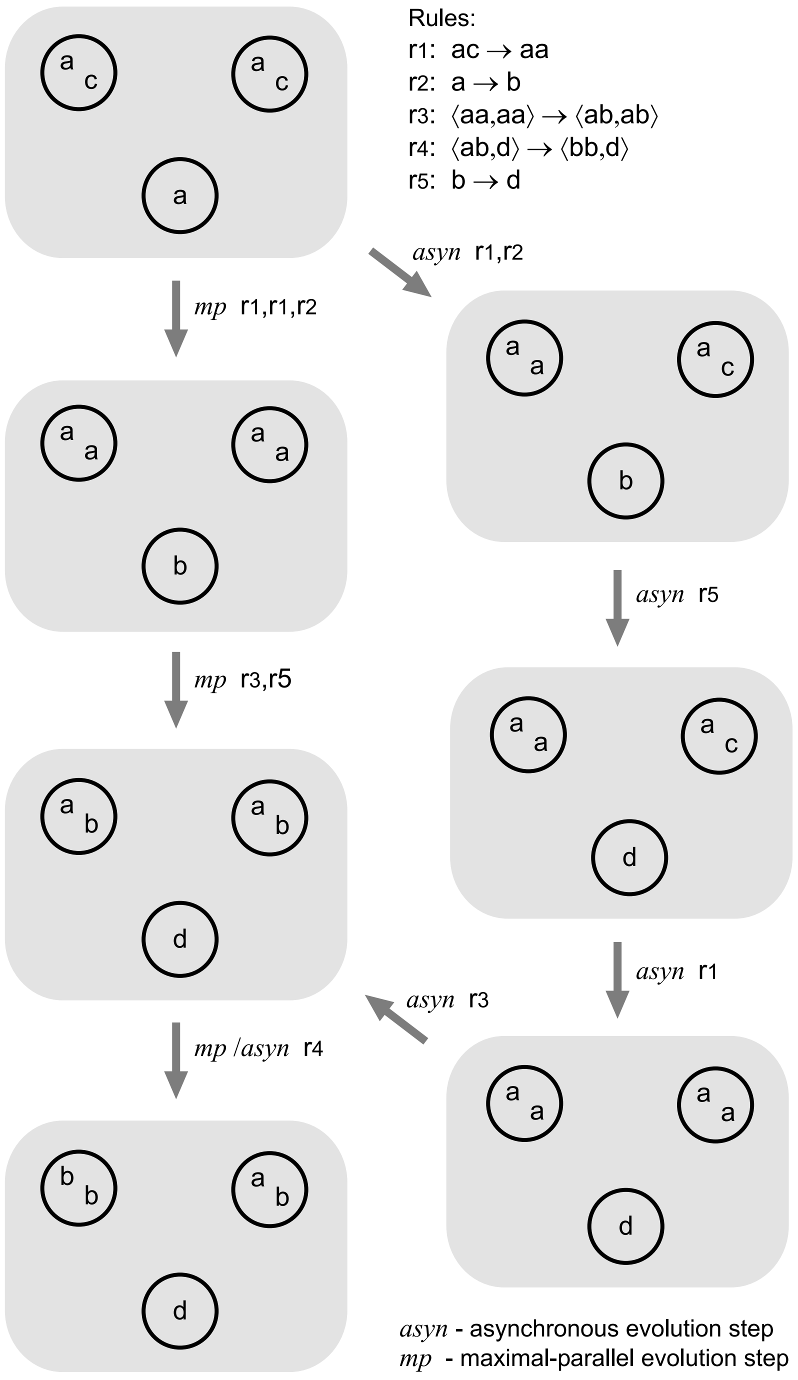


Figure 6.3: Alternative maximally-parallel and asynchronous evolutions of a CSA.

Proof The proofs of the two equalities are straightforward, hence only a short sketch is given here. For each P system Π with symbol-objects, one membrane, cooperative evolution rules, working in maximally-parallel mode and producing as output the set of vectors of natural numbers S, there exists a CSA, Π', from $CSA_1(coo_e)$ such that $Ps_T^{mp}(\Pi') = S$ for an adequate terminal alphabet T. Just take Π' having in the initial configuration one single agent corresponding to the initial configuration of Π and with cooperative evolution rules as those defined in Π (with no loss of generality we suppose that Π uses only rules with the target indication 'here': any evolution rule with target indication 'out' that sends objects to the environment, where they are effectively lost, can be replaced by appropriate rules that delete the objects).

Also there exists a CSA Π'' from $CSA_2(coo_s)$ (i.e, using only synchronization rules) such that $Ps_T^{mp}(\Pi'') = S$, for an adequate terminal alphabet T. Again, Π'' has in the initial configuration one agent corresponding to the initial configuration of Π, while the other agent is necessary for applying synchronization rules, since a synchronization requires two different agents in order to be executed. The cooperative evolution rules of Π can easily be implemented by using cooperative synchronization rules in Π''.

The equalities follow from the fact that P systems with symbol-objects, cooperative evolution rules, one membrane and working in the maximally-parallel mode are known to be computational complete (Theorem 6.2.1). $\square$

Removing maximal parallelism decreases the computational power of the considered colonies.

Theorem 6.4.3

$$PsCSA_*^{asyn}(coo_e, coo_s) = PsCSA_*^{asyn}(coo_e) = PsMAT.$$

Proof First it is proved that for an arbitrary CSA, $\Pi = (A, T, C, R)$ from $CSA_*(coo_e, coo_s)$, there exists a matrix grammar without appearance checking, G, with terminal alphabet T, such that $Ps_T^{asyn}(\Pi) = Ps_T(L(G))$.

It is supposed that $card(C) = m$. In particular, it is supposed that C consists of m agents $w_1, w_2, \cdots, w_m$ with $w_i \in A^*$ for $i \in \{1, \cdots, m\}$.

The sets $A_i = \{a_i \mid a \in A\}$ for $i \in \{1, 2, \ldots, m\}$ are constructed.

The morphisms $h_i : A \to A_i$ for $i \in \{1, 2, \ldots, m\}$ defined as $h_i(a) = a_i$, $a \in A$ are constructed. The inverse morphisms are denoted by h_i^{-1} for $i \in \{1, 2, \ldots, m\}$. Hence, $h_i^{-1}(a_i) = a$, $a \in A$.

The pure matrix grammar without appearance checking, $G = (N, N, S, M)$, is then constructed in the following way.

Defining $N = \{S\} \cup A_1 \cup A_2 \cup \cdots \cup A_m$ with $S \notin A_1 \cup A_2 \cdots \cup A_m$, the matrices of M are constructed in the following manner (grouping them according to their use).

Group I
Add to M is the the matrix $(S \to h_1(w_1)h_2(w_2)\cdots h_m(w_m))$.

Group II
For each evolution rule $u \to v$ in R, with $u = u_1u_2\cdots u_k$, $u_i \in A$ for $i \in \{1, 2, \ldots, k\}$ are added the following matrices: $\{(h_j(u_1) \to \lambda, h_j(u_2) \to \lambda, \ldots, h_j(u_{k-1}) \to \lambda, h_j(u_k) \to h_j(v)) \mid j \in \{1, 2, \ldots, m\}\}$.

Group III
For each synchronization rule $\langle u, v\rangle \to \langle u', v'\rangle$ with $u = u_1u_2\cdots u_k$, $u_r \in A$ for $r \in \{1, 2, \ldots, k\}$ and $v = v_1v_2\cdots v_p$, $v_r \in A$ for $r \in \{1, 2, \ldots, p\}$ are added the matrices: $\{(h_i(u_1) \to \lambda, h_i(u_2) \to \lambda, \ldots, h_i(u_{(k-1)}) \to \lambda, h_i(u_k) \to h_i(u'), h_j(v_1) \to \lambda, h_j(v_2) \to \lambda, \ldots, h_j(v_{p-1}) \to \lambda, h_j(v_p) \to h_j(v')) \mid i, j \in \{1, 2, \ldots, m\}, i \neq j\}$.

The basic idea of the simulation is that the matrix in group I is used to start a derivation of G by creating the string $h_1(w_1)h_2(w_2)\cdots h_m(w_m)$

corresponding to the initial configuration of Π (distinguishing the objects of the different agents by using different indexes). The matrices of group *II* are used to simulate the evolution rules present in the set R, while the matrices of group *III* are used to simulate the synchronization rules present in R.

The language $L(G)$ is the set of all the (strings representing) the configurations of Π reachable by asynchronous evolutions of Π starting with the initial configuration C.

Precisely, if there is an asynchronous evolution e of Π, starting from the initial configuration C and reaching the configuration $\{w'_1, w'_2, \cdots, w'_m\}$, then in $L(G)$ there is the string $h_1(w'_1)h_2(w'_2)\cdots h_m(w'_m)$.

In particular, a transition of the evolution e obtained by applying an evolution (synchronization) rule of R to one (to a pair, resp.) of agents is simulated in G by applying the corresponding matrix from group *II* (or from group *III*, resp.). However, since Π works in an asynchronous way, it is necessary to take care of the transitions of e that are obtained by using more than one rule. Precisely, a transition of the evolution e that is obtained by applying *several* rules from R to the agents is simulated in G by applying, *sequentially*, the corresponding matrices from groups *II* and *III*.

The reverse is also true : if there is a string w in $L(G)$ then it must be (by the way G functions) of type $h_1(w'_1)h_2(w'_2)\cdots h_m(w'_m)$ with $w'_1, w'_2, \cdots, w'_m \in A^*$. And by the way G has been constructed, if there is a derivation d in G that produces the string $h_1(w'_1)h_2(w'_2)\cdots h(w'_m)$, then there is an asynchronous evolution of Π starting from the initial configuration C and reaching the configuration $\{w'_1, w'_2, \cdots, w'_m\}$. In fact, Π works in the asynchronous mode and, in particular, can have evolutions comprising sequential transitions. That is, only one rule is applied at each application of a rule that simulates the application of a matrix in the derivation d.

From the language $L(G)$ is selected, by an appropriate regular intersection, the language L' of all the strings corresponding to halting configurations reached by asynchronous computations of Π. This can clearly be done by intersecting the language $L(G)$ with a regular set R_h of strings over N representing the halting configurations of Π (i.e., the set R_h represents the strings over N where no matrix can be applied and it is clearly a regular set).

$L' = L(G) \cap R_h$ is obtained. The language L' can still be generated by a matrix grammar without appearance checking since matrix grammars without a.c. are closed under regular intersection ([26]).

The morphisms $d_i : N \longrightarrow N \cup \{\lambda\}$ for each $i \in \{1, \cdots, m\}$ are then constructed, defined in the following manner.

$$d_i(a_i) = a_i, a \in T.$$
$$d_i(a_i) = \lambda, a \in (A - T).$$
$$d_i(a_j) = \lambda, a \in A, j \neq i.$$

For each $i \in \{1, \cdots, m\}$, the language $d_i(L')$ selects from each string in L' the substring corresponding to the agent with objects indexed by i.

Moreover, from each agent the objects not in T are deleted.

The language $L'' = \bigcup_{1 \leq i \leq m}(h_i^{-1}(d_i(L')))$ is now constructed.

L'' is the language that collects all the agents present in the halting configurations, considering all the computations of Π.

Note that the language L'' is a language over T and can also be obtained using a matrix grammar without appearance checking (with terminal alphabet T) because matrix grammars without appearance checking are closed under arbitrary morphisms and under union ([26]).

For the above construction it follows that $Ps_T(L'') = Ps_T^{asyn}(\Pi)$.

Then $PsCSA_*^{asyn}(coo_e, coo_s) \subseteq PsMAT$.

On the other hand, a CSA with only one agent in the initial configuration and using only cooperative evolution rules can simulate a matrix grammar $G = (N, T, S, M)$ without appearance checking. To make matters simpler and without loss of generality it is supposed that M has p matrices (labelled by $1, \cdots, p$) each one with k productions (labelled by $1, \cdots, k$). It is always possible to add "dummy" matrices. It is also supposed, again with no loss of generality, that the only production that rewrites S is the first production of matrix 1.

The set $L_M = \{(m_i, m_j) \mid 1 \leq i \leq p, 1 \leq j \leq k\}$ is constructed.

A CSA is then constructed, $\Pi = (A = N \cup T \cup L_M \cup \{x\}, T, C, R)$, with $C = \{S(m_1, m_1)\}$ and $x \notin N \cup T \cup L_M$.

The set of rules R is obtained in the following way. For each matrix $i : (a_1 \to u_1, a_2 \to u_2, \ldots, a_k \to u_k)$ in M and with $i \in \{1, \cdots, p\}$, $a_1, a_2, \ldots, a_k \in N$, and $u_1, u_2, \cdots, u_k \in (N \cup T)^*$, the following cooperative evolution rules are added to R: $\{(m_i, m_1)a_1 \to u_1(m_i, m_2)x, (m_i, m_2)a_2 \to u_2(m_i, m_3), \cdots, (m_i, m_{k-1})a_{k-1} \to (m_i, m_k)u_{k-1} \mid 1 \leq i \leq m\} \cup \{x(m_i, m_k)a_k \to u_k(m_j, m_1) \mid 1 \leq i \leq p, 1 \leq j \leq p\} \cup \{x \to x\} \cup \{a \to a \mid a \in N\}$.

It is straightforward to see that any successful derivation in G producing the string w can be simulated in Π by starting from the initial configuration C and applying the corresponding evolution rules in R until a halting configuration $\{(m_j, m_1)w\}$, for some $1 \leq j \leq p$, is reached.

Moreover, for any asynchronous computation c in Π halting in a configuration $\{(m_j, m_1)w\}$, for some $1 \leq j \leq p$, there is a derivation in G producing w.

In fact, due to the way R is defined, all computations of Π are obtained by having iterative applications of "blocks" of rules.

Each block of rules is a sequence of applications of rules, $(m_i, m_1)a_1 \to u_1(m_i, m_2)x, (m_i, m_2)a_2 \to u_2(m_i, m_3), \cdots, (m_i, m_{k-1})a_{k-1} \to (m_i, m_k)u_{k-1}$, $x(m_i, m_k)a_k \to u_k(m_j, m_1)$ for some $i \in \{1, \cdots, p\}$ and some $j \in \{1, \cdots, p\}$.

Once a block has been started (i.e., $(m_i, m_1)a_1 \rightarrow u_1(m_i, m_2)x$ is applied) it must also be completed (i.e., $x(m_i, m_k)a_k \rightarrow u_k(m_j, m_1)$ applied): in a computation, a block cannot be interrupted because this would lead to the object x being present in the configuration of the system, which would then make the evolution non-halting (because of the rule $x \rightarrow x$ in R).

It is easy to see that each element of this block of rules can be simulated in G by applying the corresponding matrix.

Moreover, there are no computations in Π halting in a configuration $\{(m_j, m_1)w\}$ for some $1 \leq j \leq p$ with w having objects from N (non terminals of G). This because of the rules $a \rightarrow a$, $a \in N$ present in R.

Hence, from the above description, $Ps_T^{asyn}(\Pi) = Ps_T(L(G))$.

Thus, $PsCSA_*^{asyn}(coo_e, coo_s) \supseteq PsMAT$ and the Theorem follows.

□

Using a similar construction to that in the proof of Theorem 6.4.3 and from the last statement of Theorem 2.0.2 the following Corollary is obtained.

Corollary 6.4.3.a *For an arbitrary CSA,* Π, *there exists a regular grammar* G *with one-letter terminal alphabet such that* $N^{asyn}(\Pi) = NL(G)$.

Proof The proof is obtained by a slight modification of the first part of Theorem 6.4.3.

Given the CSA, $\Pi = (A, T, C, R)$, a matrix grammar without a.c., $G = (N, N, S, M)$, is constructed as given in the first part of Theorem 6.4.3. Then, again following Theorem 6.4.3, the language L' is constructed to contain all the strings corresponding to halting configurations reached by asynchronous computations of Π. For instance, if $\{w'_1, w'_2, \cdots, w'_m\}$ is a halting configuration reached by Π, then in L' there is the string $\{h_1(w_1), h_2(w_2), \cdots, h_m(w_m)\}$, where $h_1, h_2, \ldots, h_m$ are morphisms defined as in the proof of Theorem 6.4.3. As explained in the proof of Theorem 6.4.3 L' can be generated by a matrix grammar without a.c.

The morphisms $d_i : N \longrightarrow \{z\} \cup \{\lambda\}$, for each $i \in \{1, \cdots, m\}$ are then constructed, defined in the following manner (z is a new symbol not in N).

$$d_i(a_i) = z, a \in A.$$
$$d_i(a_j) = \lambda, a \in A, j \neq i.$$

Then, for each $i \in \{1, \cdots, m\}$, the language $d_i(L')$ selects from each string in L' the substring corresponding to the agent with objects indexed by i and replaces all the objects of the agent by the symbol z.

The language $L'' = \bigcup_{1 \leq i \leq m} d_i(L')$ is now constructed.

L'' is the language that collects all the agents present in the halting configurations, considering all the computations of Π.

From the construction it is clear that $N^{asyn}(\Pi) = NL''$.

Moreover, L'' can also be generated by a matrix grammar without a.c., using terminal alphabet $\{z\}$. Matrix grammars without a.c. are closed under union and arbitrary morphism, see, e.g., [26].

The result then follows from the fact that a language generated by a matrix grammar without a.c. over a one letter alphabet is regular (Theorem 2.0.2). □

Using Theorem 6.4.2 and Theorem 6.4.3 the following corollary is obtained:

Corollary 6.4.3.b

$$\begin{aligned} PsCSA_*^{asyn}(coo_e, coo_s) \subset PsCSA_1^{mp}(coo_e, coo_s) \\ = PsCSA_1^{mp}(coo_e) \\ = PsCSA_1^{mp}(coo_s) \end{aligned}$$

When using unary rules the computational power is equivalent to that of finite sets of vectors of natural numbers, even for CSAs working in the maximally parallel mode.

Theorem 6.4.4 $PsCSA_*^{asyn}(un_e, un_s) = PsCSA_*^{mp}(un_e, un_s)$
$= PsFIN.$

Proof In CSAs using only unary rules the sizes of the agents present in the initial configuration cannot be increased, so, because of the finite number of possible combinations, these systems can only generate finite sets of vectors of numbers as output. On the other hand, any finite set, S, of vectors of numbers can be obtained as output of a CSA, Π, by having in the initial configuration of Π, for each vector v in S, one agent w with Parikh vector v (with respect to an adequate terminal alphabet). $\square$

However, by combining unary synchronization rules and non-cooperative evolution rules, computational completeness is obtained for CSAs working in the maximally parallel way with two agents in the initial configuration. The proof of this result is by simulation of EC P systems.

Theorem 6.4.5 $PsCSA_2^{mp}(ncoo_e, un_s) = PsRE.$

Proof Programmed grammars with appearance checking are grammars known to be computationally complete, as discussed in Chapter 2 and shown in Theorem 2.0.6. In [3] it has been shown that for any programmed grammar with appearance checking, G, with terminal alphabet T, there exists an EC P system Π with two membranes, non-cooperative evolution rules, symport/antiport rules of weight at most one such that $Ps_T(L(G)) = Ps(\Pi)$. This proves that $PsECP_2(1, 1, ncoo) = PsRE$.

It is now shown that any evolution-communication P systems with two membranes, non- cooperative evolution rules and antiport rules of weight one can be simulated by using a CSA system with two agents, non-cooperative evolution rules, unary synchronization rules and working in the maximally parallel way (the two agents represent the two regions enclosed by the two membranes in the EC P system).

For an arbitrary programmed grammar with a.c., G, with terminal alphabet T, an EC P system $\Pi = (V, [\ [\ ]_2\ ]_1, w_1, w_2, R_1, R_2, R'_1, R'_2, i_0)$, with $T \subseteq V$, is constructed using the construction proposed in [3] such that $Ps_T(L(G)) = Ps(\Pi)$. Π is constructed in such a way that its output at the end of a computation consists of objects corresponding to the terminals T collected in the environment. These objects are immediately sent into the environment once they are obtained in region 1 and remain there unchanged until the end of the computation (the symport rules associated to membrane 1 are used only to send to the environment these objects and no other antiport or symport rules are associated to membrane 1).

Defining $V_1 = \{a_1 \mid a \in V\}$ and $V_2 = \{a_2 \mid a \in V\}$, two morphisms are defined that map the objects of V into indexed objects (the index denotes the region of Π where the object is present).

Precisely, $h_1 : V \to V_1$ is defined as $h_1(a) = a_1$ for each $a \in V$ and $h_2 : V \to V_2$ is defined as $h_2(a) = a_2$ for each $a \in V$.

The CSA $\Pi' = (A, T', C, R)$ is now constructed as follows.

Setting $A = \{h_1(a), h_2(a) \mid a \in V\}$ and $C = \{h_1(w_1), h_2(w_2)\}$, the terminal alphabet T' is defined as $\{h_1(a) \mid a \in T\}$.

The rules in R are constructed in the following manner.

For each rule $a \to v$ in R_i, $i \in \{1, 2\}$, add to R the rule $h_i(a) \to h_i(v)$.

For each symport rule (a, in) present in R'_2, add to R the synchronization rule $\langle h_1(a), \lambda\rangle \to \langle \lambda, h_2(a)\rangle$.

For each symport rule (a, out) present in R'_2, add to R the synchronization rule $\langle h_2(a), \lambda\rangle \to \langle \lambda, h_1(a)\rangle$.

For each antiport rule $(a, in; b, out)$ present in R_2', add to R the synchronization rule $\langle h_1(a), h_2(b)\rangle \to \langle h_1(b), h_2(a)\rangle$.

All (and only) the computations of Π are simulated by computations of Π'.

The idea is that the two agents in Π' represent the contents of the regions and of the environment of Π: the agent with objects indexed by 1 represents the contents of region 1 and the objects in the environment, while the agent with objects indexed by 2 represents the contents of region 2.

Evolution rules and symport/antiport rules in Π are simulated by the corresponding constructed evolution and synchronization rules, respectively, present in R.

The use of indexed objects for the agents guarantees that the two agents are maintained separate, such that no incorrect interaction (i.e., synchronization) can take place and every configuration of Π', reached during any computation, will always have two agents; one with all objects indexed by 1 and one with all objects indexed by 2. That is, there are no computations in Π' that reach a configuration having agents with objects with different indexes.

From the way Π' is constructed, it can easily be seen that for each computation in Π, producing in the environment a multiset of objects w, for $w \in T^*$ (i.e., the output of the computation of Π is the vector $v = Ps_T(w)$), there exists a computation for Π' having, in the halting configuration, the agents $h_1(ww'), h_2(w'')$ with $w'' \in V^*$, $w \in T^*$, $w' \in (V - T)^*$. That is, the output of the computation is the set composed of the vectors $v = Ps_{T'}(h_1(ww')) = Ps_{T'}(h_1(w))$ and $Ps_{T'}(h_2(w'')) = \overline{0}$. The empty vector is also present since in $h(w'')$ there are no objects from T.

On the other hand, for each computation in Π', with the agents $h_1(ww')$ and $h_2(w'')$ in the halting configuration, with $w' \in V^*$, $w \in T^*$ and $w'' \in V^*$

(i.e., the output is the set composed of the vectors $v = Ps_{T'}(h_1(ww')) = Ps_{T'}(h_1(w))$ and $Ps_{T'}(h_2(w'')) = \overline{0}$), there exists a computation in Π producing the multiset of objects w in the environment in the halting configuration (i.e., having as output the vector $v = Ps_T(w)$).

Because in the equality of sets of vectors the null vector is not considered, the Theorem follows. $\square$

Note that the role of synchronization rules, even if only unary, is crucial: when this type of rule is not used, the computational power of CSAs is only regular (in terms of Parikh images).

Theorem 6.4.6 $PsCSA_*^{mp}(ncoo_e) = PsCF$.

Proof For an arbitrary CSA, $\Pi = (A, T, C, R)$, with m agents $w_1, w_2, \ldots, w_m$ (no bound on m) there exists a P system, Π', with symbol-objects and non-cooperative evolution rules working in the maximally parallel way, such that $Ps_T(\Pi') = Ps_T^{mp}(\Pi)$. The P system, $\Pi' = (A \cup \{S\}, T, [\,]_1, S, R_1)$, needs only one region labelled 1. Added to R_1 are the following rules: $\{S \to w_1, S \to w_2, \ldots, S \to w_m\}$ and all the rules present in R. Clearly, for each vector v in $Ps_T^{mp}(\Pi)$ there is a computation in Π' that halts with a multiset of objects w in region 1, such that $Ps_T(w) = v$. Equally, for each vector v obtained as the output of a computation in Π' there exists a computation in Π halting in a configuration containing the agent w with $Ps_T(w) = v$.

Vice versa, for a P system, $\Pi' = (V, T, [\,]_1, w_1, R_1)$, with symbol-objects, non-cooperative evolution rules and working in the maximally-parallel way, it is possible to construct an equivalent CSA, $\Pi = (V, T, C, R)$, with $C = \{w_1\}$ and $R = R_1$. It can be seen directly that $Ps_T^{mp}(\Pi) = Ps_T(\Pi')$. Using Theorem 6.2.1, the result follows.

$\square$

6.5 Robustness of CSAs: A Formal Study

In this Section is presented a study of the robustness of CSAs against perturbations of some of the features of the system.

For this purpose a notion of robustness of CSAs is used which is similar to that employed in [53] in the framework of grammar systems.

It is desired to investigate situations where either some of the agents or some of the rules of the colony do not function. What are the consequences to the behaviour of the colony?

Of particular interest will be systems that are robust, e.g., where the behaviour does not change critically if one or more agents cease to exist in the system.

Let $\Pi = (A, T, C, R)$ be an arbitrary CSA.

Π' is said to be an an *agent-restriction* of Π if $\Pi' = (A, T, C', R)$ with $C' \subseteq C$. Π' is a CSA where some of the agents originally present in Π no longer work, i.e., the CSA behaves as though they were absent from the system.

A *rule-restriction* of Π is also considered, obtained by removing some or possibly all of the rules. Then, $\Pi' = (A, T, C, R')$ is a *rule-restriction* of Π if $R' \subseteq R$. In this case some of the rules do not work, i.e., the CSA behaves as if they were absent from the system.

A CSA, Π, is said to be *robust* when a core behaviour, i.e., the minimally accepted behaviour, is preserved when considering proper restrictions of it. A measure of the robustness of Π is the *difference* between the initial system and the *minimum* restriction preserving the core behaviour, where *difference* and *minimum* are to be defined.

By a *core behaviour* of Π is meant a subset of the set of vectors of natural numbers generated by Π.

These subsets are defined by making an intersection with a set of vectors from $PsREG$ that defines the regular property of the core behaviour of interested. Note that the core behaviour may be infinite.

Questions about robustness can then be formalised in the following manner.

Consider an arbitrary CSA, Π, an arbitrary agent- or rule- restriction Π' of Π, and an arbitrary set S from $PsREG$. Is it possible to decide whether or not $Ps(\Pi) \cap S \subseteq Ps(\Pi')$ (i.e., whether Π is robust against the restriction Π', in the sense that it will continue to generate, at least, the core behaviour)?

Example 6.5.1 *What follows is a small example that clarifies the presented notion of robustness in the case of agent-restriction and asynchronous computations. The other cases (rule-restriction, maximally- parallel computations) are conceptually similar.*

Consider a CSA $\Pi = (A, T, C, R)$ *with* $A = \{a, b, c, d, e, f\}$, $T = \{e, f\}$, $C = \{(ab, 1), (bc, 1), (bd, 1), (a, 1)\}$. *The rules in* R *are* $\{\langle ab, bc\rangle \rightarrow \langle eff, eff\rangle, \langle ab, bd\rangle \rightarrow \langle eff, eff\rangle\}$.

There are two possible asynchronous computations of Π, *which are represented diagrammatically in Figure 6.4.*

Collecting the results (vectors representing the multiplicities of the terminal objects in the agents in the halting configurations) it is possible to see that $Ps_T^{asyn}(\Pi) = \{(1, 2), \overline{0}\} \cup \{(1, 2), \overline{0}\} = \{(1, 2), \overline{0}\}$, *where* $\overline{0}$ *denotes* $(0, 0)$.

In fact, there are two halting configurations (for the two computations), two agents eff, *whose associated Parikh vector (with respect to* T*) is* $(1, 2)$ *and the other agents,* bd, bc *and* a, *whose associated Parikh vectors, with respect to* T, *are the null vector (the agents do not contain any terminal object).*

Now, suppose a core behaviour is fixed to be the set of vectors $\{(1, 2)\}$ *(it can be clearly obtained by an intersection of* $Ps_T^{asyn}(\Pi)$ *with* $\{(1, 2)\}$,

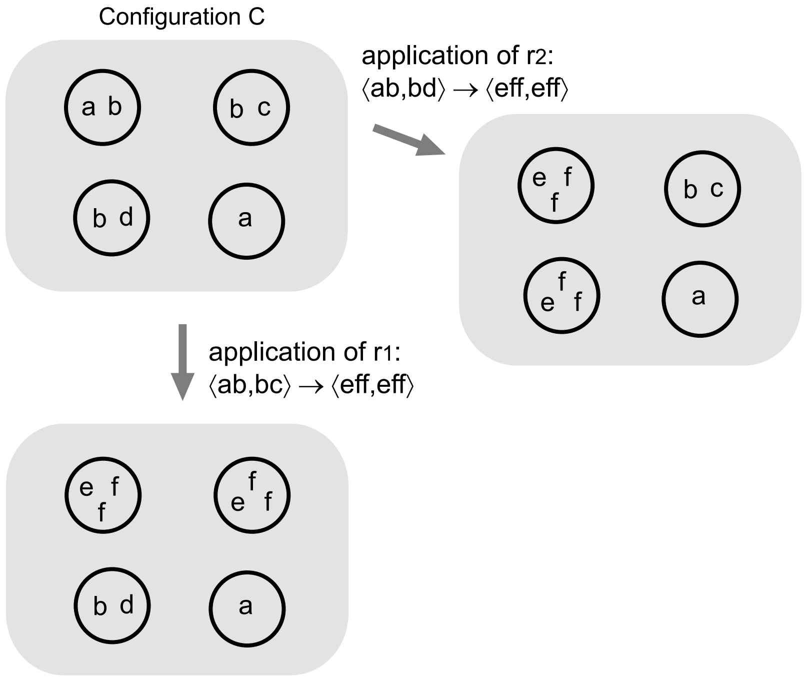


Figure 6.4: The two possible asynchronous computations of Π of Example 6.5.1

which is in $PsREG$).

The system Π is robust when the agent bc is deleted from its initial configuration. In fact, considering $\Pi' = (A, T, C', R)$, with $C' = \{(ab, 1), (bd, 1), (a, 1)\}$, then $Ps_T^{asyn}(\Pi') = \{(1, 2), \overline{0}\}$, which still contains the defined core behaviour. The only possible computation of Π' is represented in Figure 6.5.

On the other hand, the system Π is not robust when the agent ab is deleted from its initial configuration. Considering $\Pi'' = (A, T, C'', R)$, with $C'' = \{(bd, 1), (a, 1)\}$, then $Ps_T^{asyn}(\Pi'') = \{\overline{0}\}$, which does not contain the core behaviour. The system Π'' is represented in Figure 6.6. The only possible computation of Π'' is the one that halts in the initial configuration C''.

The case of rule-restrictions with asynchronous evolution is now analysed, demonstrating a negative result.

In what follows it is supposed that an arbitrary set S from $NREG$ (from

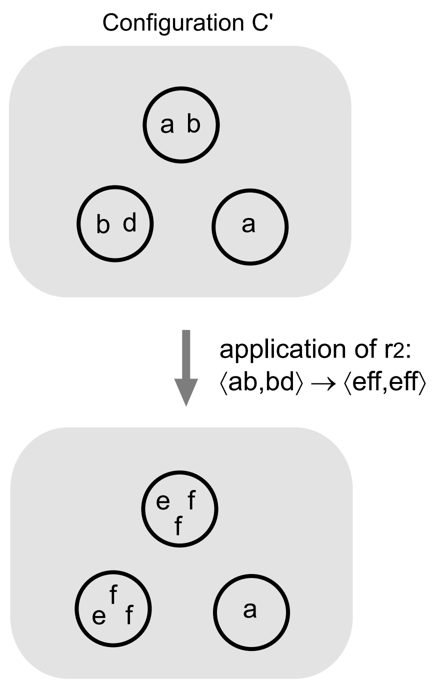


Figure 6.5: Robust behaviour of Π' of Example 6.5.1 when agent bc is removed from C.

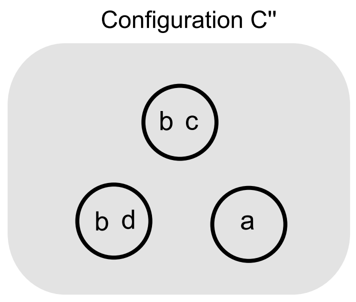


Figure 6.6: No robustness displayed by Π'' of Example 6.5.1 when agent ab is removed from C.

$PsREG$) is given by having the corresponding grammar G from REG such that $NL(G) = S$ ($Ps(L(G)) = S$, resp.).

Theorem 6.5.1 *It is undecidable whether or not for an arbitrary CSA,* Π*, with terminal alphabet* T*, arbitrary rule restriction* Π' *of* Π *and arbitrary set* S *from* $PsREG_T$*,* $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$*.*

Proof Starting with two arbitrary matrix grammars without a.c., $G = (N, T, S, M)$ and $G' = (N', T, S, M')$ with $N \cap N' = \{S\}$, it is undecidable whether or not $Ps_T(L(G)) \subseteq Ps_T(L(G'))$ (see Corollary 2.0.4.a).

To make matters simpler and without loss of generality it is supposed that M has p matrices (labelled by $1, 2, \cdots, p$) of k productions (labelled by $1, 2, \cdots, k$) and M' has m' matrices (labelled by $1, 2, \cdots, m'$) of k' productions (labelled by $1, 2, \cdots, k'$). Again with no loss of generality it is also supposed that the only production in M (and in M') that rewrite the axiom S is the production 1 of matrix 1.

The sets $L_M = \{(m_i, m_j) \mid 1 \leq i \leq p, 1 \leq j \leq k\}$ and $L_M = \{(m'_i, m'_j) \mid 1 \leq i \leq m', 1 \leq j \leq k'\}$ are constructed.

As in the second part of Theorem 6.4.3, a CSA is constructed, Π, equivalent to G in the following way.

$\Pi = (A = N \cup N' \cup T \cup L_M \cup L'_M \cup \{x\}, T, C, R)$ with $C = \{S(m_1, m_1)(m'_1, m'_1)\}$ and with the set of rules R obtained in the following way.

For each matrix $i : (a_1 \to u_1, a_2 \to u_2, \ldots, a_k \to u_k)$ in M with $a_1, a_2, \ldots, a_k \in N$ and $u_1, u_2, \cdots, u_k \in (N \cup T)^*$, with $i \in \{1, \cdots, p\}$, the following cooperative evolution rules are added to R: $\{(m_i, m_1)a_1 \to u_1(m_i, m_2)x, (m_i, m_2)a_2 \to u_2(m_i, m_3), \cdots, (m_i, m_{k-1})a_{k-1} \to (m_i, m_k)u_{k-1}\}$ $\cup \{x(m_i, m_k)a_k \to u_k(m_j, m_1) \mid 1 \leq j \leq p\} \cup \{a \to a \mid a \in N\} \cup \{x \to x\}$.

Using the same arguments as in the proof of Theorem 6.4.3 it is possible to see that $Ps_T^{asyn}(\Pi) = Ps_T(L(G))$.

In a similar way a CSA is constructed, $\Pi' = (A, T, C, R')$, which is the same as Π except that has rules R', constructed as follows.

For each matrix $i : (a_1 \rightarrow u_1, a_2 \rightarrow u_2, \cdots, a_{k'} \rightarrow u_{k'})$ in M' with $a_1, a_2, \cdots, a_k \in N'$ and $u_1, u_2, \cdots, u_k \in (N' \cup T)^*$, with $i \in \{1, \cdots, m'\}$, the following cooperative evolution rules are added to R:
$\{(m'_i, m'_1)a_1 \rightarrow u_1(m'_i, m'_2)x, (m'_i, m'_2)a_2 \rightarrow u_2(m'_i, m'_3), \cdots, (m'_i, m'_{k'-1})a_{k'-1} \rightarrow (m'_i, m'_{k'})u_{k'-1}\} \cup \{x(m'_i, m'_{k'})a_{k'} \rightarrow u_{k'}(m'_j, m'_1) \mid 1 \leq j \leq m'\} \cup \{a \rightarrow a \mid a \in N'\} \cup \{x \rightarrow x\}$.

Again, using the same arguments as in the proof of Theorem 6.4.3, it is clear that $Ps_T^{asyn}(\Pi') = Ps_T(L(G'))$.

The CSA $\Pi'' = (A, T, C, R' \cup R)$ is then constructed. It can be seen that $Ps_T^{asyn}(\Pi'') = Ps_T^{asyn}(\Pi) \cup Ps_T^{asyn}(\Pi') = Ps_T(L(G)) \cup Ps_T(L(G'))$. In fact, applying rules from R one gets $Ps_T^{asyn}(\Pi)$, while applying rules from R' one gets $Ps_T^{asyn}(\Pi')$. The application of the rules cannot be "mixed" since $N \cap N' = \{S\}$.

Now suppose an algorithm exists to decide whether or not, for arbitrary CSA, Π, arbitrary rule restriction Π' of Π and arbitrary set S from $PsREG_T$, $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$.

This algorithm could be applied to decide whether or not $Ps_T^{asyn}(\Pi'') \cap Ps_T(T^*) \subseteq Ps_T^{asyn}(\Pi')$. Notice that Π' is a rule restriction of Π''.

If the answer is true then $Ps_T^{asyn}(\Pi) \subseteq Ps_T^{asyn}(\Pi')$, otherwise (answer false) $Ps_T^{asyn}(\Pi) \not\subseteq Ps_T^{asyn}(\Pi')$.

So it is also possible to decide whether or not $Ps_T(L(G)) \subseteq Ps_T(L(G'))$, which is not possible. Hence, by contradiction, the Theorem follows. $\square$

Note, however, that the result is different when the considered core behaviour is finite.

Theorem 6.5.2 *It is decidable whether or not, for an arbitrary CSA, Π, with terminal alphabet T, arbitrary rule restriction Π' of Π and arbitrary* *finite set S from $PsREG_T$, $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$.*

Proof To check whether or not $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$ it is only necessary to construct $S' = Ps_T^{asyn}(\Pi) \cap S$ and then to check whether or not each vector in S' is in $Ps_T^{asyn}(\Pi')$. This can be done because:

- S is finite.
- For any arbitrary CSA, Π, with terminal alphabet T, it is possible to construct a matrix grammar without a.c., G, with terminal alphabet T, such that $Ps_T^{asyn}(\Pi) = Ps_T(L(G))$ (Theorem 6.4.3) and the membership problem for matrix grammars without a.c. is decidable, as shown in Corollary 2.0.4.a in Chapter 2.
- Given a vector v, there is only a finite set of strings (over T) whose Parikh vector with respect to T is exactly v.

□

Suppose that only the *size* of the agents is of interest and not their internal structure. This means that the set of numbers $N(\Pi)$ is collected for a colony Π. In this case the robustness problem can be rephrased in the following manner.

Consider an arbitrary CSA, Π, with an arbitrary agent- / rule-restriction Π' of Π and an arbitrary set S from $NREG$.

Is it possible to decide whether or not $N(\Pi) \cap S \subseteq N(\Pi')$ (i.e., whether Π is robust against the restriction Π')? Note that in this case the core behaviour is defined by specific sizes of the agents.

In this case the following positive results are obtained, even when considering infinite core behaviour.

Theorem 6.5.3 *It is decidable whether or not, for an arbitrary CSA, Π, arbitrary rule restriction Π' of Π and arbitrary set S from $NREG$, $N^{asyn}(\Pi) \cap S \subseteq N^{asyn}(\Pi')$.*

Proof

It is known from Corollary 6.4.3.a that for an arbitrary CSA Π' it is possible to construct a regular grammar G' with a one-letter terminal alphabet such that $N^{asyn}(\Pi') = NL(G')$.

Moreover, it is also possible to construct a regular grammar G over a one-letter alphabet such that $N(L(G)) = N^{asyn}(\Pi') \cap S$.

The result then follows from the fact that, given two arbitrary regular grammars G_1 and G_2 it is decidable whether or not $L(G_1) \subseteq L(G_2)$ (see, e.g., [46]). In particular, this is true when the terminal alphabet of the two regular grammars is of cardinality one and the decidability result can be easily extended to the length sets of the languages. $\square$

The same positive result holds when, considering vectors of numbers, the CSAs work in maximally-parallel mode but use only non-cooperative evolution rules.

Theorem 6.5.4 *It is decidable whether or not, for an arbitrary CSA,* Π *from* $CSA_*(ncoo_e)$*, with terminal alphabet* T*, arbitrary rule restriction* Π' *of* Π *and arbitrary set* S *from* $PsREG_T, Ps_T^{mp}(\Pi) \cap S \subseteq Ps_T^{mp}(\Pi')$.

Proof For any CSA Π from $CSA_*(ncoo_e)$ with terminal alphabet T it is possible to construct a regular grammar G with terminal alphabet T' such that $Ps_T^{mp}(\Pi) = Ps_{T'}(L(G))$ (because of Theorem 6.4.6 and Theorem 2.0.3). Then it is also possible to construct a regular grammar G' over T' such that $Ps_T^{mp}(\Pi) \cap S = Ps_{T'}(L(G'))$. The result then follows from the fact that containment is decidable for regular languages (see, e.g., [46]) and this result can easily be extended to the Parikh images of regular languages because there is only a finite number of strings over an alphabet T' having a given Parikh vector with respect to T'. $\square$

Note, however, that even if robustness against rule absence is in many cases undecidable when the core behaviour is infinite, it is still possible to decide whether a rule (evolution or synchronization) is used or not by a CSA. So, if a rule is not used it can be removed and the system will be robust against the deletion.

Theorem 6.5.5 *It is decidable whether or not, for an arbitrary CSA* $\Pi = (A, C, T, R)$ *and an arbitrary rule* r *from* R*, there exists at least one asynchronous computation for* Π *containing at least one configuration obtained by applying at least one instance of rule* r*.*

Proof Given an arbitrary CSA, $\Pi = (A, C, T, R)$, and an arbitrary rule r from R it is possible to construct, by modifying the construction given in the first part of the proof of Theorem 6.4.3, a matrix grammar without a.c., G, with terminal alphabet T, such that $Ps_T(L(G))$ is not the empty set if and only if there exists at least one asynchronous computation for Π having at least one transition where r is applied. This can be done, for instance, by modifying the matrix grammar G given in the proof of Theorem 6.4.3 as follows. A matrix is added that is applied at the beginning of each derivation of G and that introduces a non-terminal, X, which is removed only when the matrix that simulates the rule r is used. In this case $L(G)$ (also its Parikh image) is not the empty set if and only if there is a derivation in G where the matrix that simulates rule r is used. The Theorem follows from the fact that it is possible to decide whether or not $Ps_T(L(G))$ is the empty set (Corollary 2.0.4.a). □

The case of agent-restrictions are now considered and analysed. In this case the problems remain undecidable when the core behaviour is infinite.

Theorem 6.5.6 *It is undecidable whether or not, for an arbitrary CSA,* Π*, with terminal alphabet* T*, arbitrary agent restriction* Π' *of* Π *and arbitrary set* S *from* $PsREG_T$*,* $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$*.*

Proof Starting with two arbitrary matrix grammars without a.c., $G = (N, T, S, M)$ and $G' = (N', T, S, M')$ with $N \cap N' = \{S\} \cup T$, it is undecidable whether or not $Ps_T(L(G)) \subseteq Ps_T(L(G'))$ (see Corollary 2.0.4.a).

To make matters simpler and without loss of generality it is supposed that M has p matrices (labelled by $1 \cdots, p$) of k productions (labelled by $1, \cdots, k$) and M' has m' matrices (labelled by $1, \cdots, m'$) of k' productions (labelled by $1, \cdots, k'$). Again with no loss of generality it is supposed that in M and M' only the production 1 of matrix 1 can rewrite S.

The sets $L_M = \{(m_i, m_j) \mid 1 \leq i \leq p, 1 \leq j \leq k\}$ and $L'_M = \{(m'_i, m'_j) \mid 1 \leq i \leq m', 1 \leq j \leq k'\}$ are constructed.

As in the second part of the proof of Theorem 6.4.3 a CSA Π is constructed equivalent to G in the following way.

$\Pi = (A = N \cup N' \cup T \cup L_M \cup L'_M \cup \{x\}, T, C, R)$ with $C = \{S(m_1, m_1)\}$ and with the set of rules R obtained in the following way.

For each matrix $i : (a_1 \to u_1, a_2 \to u_2, \ldots, a_k \to u_k)$ in M with $a_1, a_2, \ldots, a_k \in N$ and $u_1, u_2, \cdots, u_k \in (N \cup T)^*$ with $i \in \{1, \cdots, p\}$, the following cooperative evolution rules are added to R:
$\{(m_i, m_1)a_1 \to u_1(m_i, m_2)x, (m_i, m_2)a_2 \to u_2(m_i, m_3), \cdots, (m_i, m_{k-1})a_{k-1} \to (m_i, m_k)u_{k-1}\} \cup \{x(m_i, m_k)a_k \to u_k(m_j, m_1) \mid 1 \leq j \leq p\}$.

For each matrix $i : (a_1 \to u_1, a_2 \to u_2, \ldots, a_{k'} \to u_{k'})$ in M' with $a_1, a_2, \ldots, a_k \in N'$ and $u_1, u_2, \cdots, u_k \in (N' \cup T)^*$ with $i \in \{1, \cdots, m'\}$, the following cooperative evolution rules are added to R:
$\{(m'_i, m'_1)a_1 \to u_1(m'_i, m'_2)x, (m'_i, m'_2)a_2 \to u_2(m'_i, m'_3), \cdots, (m'_i, m'_{k'-1})a_{k'-1} \to (m'_i, m'_{k'})u_{k'-1}\} \cup \{x(m'_i, m'_{k'})a_{k'} \to u_{k'}(m'_j, m'_1) \mid 1 \leq j \leq m'\}$.

Also added to R is the set of rules $\{a \to a \mid a \in N\} \cup \{x \to x\}$.

Using the same arguments as in the proof of Theorem 6.4.3 it is clear that $Ps_T^{asyn}(\Pi) = Ps_T(L(G))$.

In a similar way a CSA, $\Pi' = (A, T, C', R)$, is constructed with the only difference being the initial configuration $C' = \{S(m'_1, m'_1)\}$.

In this case, $Ps_T^{asyn}(\Pi') = Ps_T(L(G'))$.

Then the CSA $\Pi'' = (A, T, C{+}C', R)$ is constructed and thus $Ps_T^{asyn}(\Pi'') = Ps_T^{asyn}(\Pi) \cup Ps_T^{asyn}(\Pi') = Ps_T(L(G)) \cup Ps_T(L(G'))$. Suppose that $Ps_T^{asyn}(\Pi)$ and $Ps_T^{asyn}(\Pi')$ are not the empty set.

Now suppose that there is an algorithm to decide, for an arbitrary CSA, Π, arbitrary agent restriction Π' of Π and arbitrary set S from $PsREG_T$, whether or not $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$.

This algorithm may be used to decide whether or not $Ps_T^{asyn}(\Pi'') \cap Ps_T(T^*) \subseteq Ps_T^{asyn}(\Pi')$.

In fact, Π' is an agent restriction of Π''.

If the proposition is true then $Ps_T^{asyn}(\Pi) \subseteq Ps_T^{asyn}(\Pi')$, while if the proposition is false, $Ps_T^{asyn}(\Pi) \nsubseteq Ps_T^{asyn}(\Pi')$ (the case when $Ps_T^{asyn}(\Pi)$ or/and $Ps_T^{asyn}(\Pi')$ is the empty set is trivial, emptiness for Parikh images of languages generated by matrix grammars without a.c. is decidable, Corollary 2.0.4.a).

So it is possible to decide whether or not $Ps_T(L(G)) \subseteq Ps_T(L(G'))$ and this is not possible (Corollary 2.0.4.a). From this, by contradiction, the Theorem follows. $\square$

Using the same ideas as in the proof of Theorem 6.5.2 the following result is obtained.

Theorem 6.5.7 *It is decidable whether or not, for an arbitrary CSA,* Π, *with terminal alphabet* T, *arbitrary agent restriction* Π' *of* Π *and arbitrary finite set* S *from* $PsREG_T$, $Ps_T^{asyn}(\Pi) \cap S \subseteq Ps_T^{asyn}(\Pi')$.

Using the same ideas as in the proof of Theorem 6.5.3 the following result is obtained.

Theorem 6.5.8 *It is decidable whether or not, for an arbitrary CSA,* Π, *arbitrary agent restriction* Π' *of* Π *and arbitrary set* S *from* $NREG$, $N(\Pi) \cap S \subseteq N(\Pi')$.

Obviously, for CSAs that are computationally complete (in a constructive way), every non-trivial property is undecidable (Rice's Theorem, see, e.g., [46]). This is already shown to be true for CSAs with non-cooperative evolution rules, unary synchronization rules and working in the maximally parallel mode.

Therefore, from Theorem 6.4.5, the following result is obtained.

Theorem 6.5.9 *It is undecidable whether or not, for an arbitrary CSA,* Π *from* $CSA_*(coo_e, un_s)$ *with terminal alphabet* T*, arbitrary agent or rule restriction* Π' *of* Π *and arbitrary set* S *from* $PsREG_T$*,* $Ps_T^{mp}(\Pi) \cap S \subseteq Ps_T^{mp}(\Pi')$*.*

Note that, invoking Rice's Theorem once again, the same negative results are obtained even when considering finite core behaviour and length sets.

Theorem 6.5.10 *It is undecidable whether or not for an arbitrary CSA,* Π *from* $CSA_*(coo_e, un_s)$*, with terminal alphabet* T*, arbitrary agent or rule restriction* Π' *of* Π *and arbitrary finite set* S *from* $PsREG_T$*,* $Ps_T^{mp}(\Pi) \cap S \subseteq Ps_T^{mp}(\Pi')$*.*

Theorem 6.5.11 *It is undecidable whether or not, for an arbitrary CSA,* Π *from* $CSA_*(coo_e, un_s)$*, arbitrary agent or rule restriction* Π' *of* Π *and arbitrary set* S *from* $NREG$*,* $N^{mp}(\Pi) \cap S \subseteq N^{mp}(\Pi')$*.*

6.6 A Computational Tree Logic for CSAs

In this section the investigation of the dynamic properties of CSAs continues and a *computational tree logic (CTL temporal logic)* is presented to formally specify, verify and model-check properties of CSAs. An introduction to the basic notions and results of temporal logics can be found in [4, 83].

Temporal logics are the most used logics in model-checking analysis: efficient algorithms and tools having already been developed for them, e.g. NuSMV [88]. They are devised with operators for expressing and quantifying on possible evolutions or configurations of systems. For instance, for an arbitrary system it is possible to specify properties such as *'for any possible evolution, ϕ is fulfilled'*, *'there exists an evolution such that ϕ is not true'*, *'in the next state ϕ will be satisfied'*, *'eventually ϕ will be satisfied'* and *'ϕ happens until ψ is satisfied'*, with ϕ and ψ properties of the system. What follows is a demonstration of how to use these operators to formally specify and verify complex properties of CSAs, such as *'the agent will always eventually reach a certain configuration'*, or *'rule r is not applicable until rule r' is used'*, etc.

Definition 6.6.1 (Preconditions) *Let A be an arbitrary alphabet and R an arbitrary set of rules over A. The mapping $prec : R \rightarrow 2^{\mathbb{M}(A)}$ is defined by*

- *if $r \in R$ is the evolution rule $u \rightarrow v$ then $prec(r) = \{u\}$;*
- *if $r \in R$ is a synchronization rule $\langle u, v\rangle \rightarrow \langle u', v'\rangle$ then $prec(r) = \{u\} \cup \{v\}$.*

$prec(R)$ is thus defined as $prec(R) = \bigcup_{r\in R} prec(r)$.

The definition of γ-evolutions for a given CSA is now extended by presenting the notion of *γ-complete evolution* defined for arbitrary classes of CSAs.

In what follows, let $\mathcal{C} = CSA_m^{A,T,R}$ be a class of all the CSAs having alphabet A, terminal alphabet T, set of rules R over A, degree m, with A, T, R and m arbitrarily chosen.

Definition 6.6.2 (γ-complete evolutions) *A sequence of CSAs $\langle \Pi_0, \Pi_1, \Pi_2, \ldots, \Pi_i, \ldots\rangle$ with $\Pi_i = (A, T, C_i, R) \in \mathcal{C}$, $i \geq 0$, is called* γ-complete evolution *in $\mathcal{C}$ starting in Π_0 if $\langle C_0, C_1, C_2, \ldots C_i, \ldots\rangle$, $i \geq 0$, is a halting or an infinite γ-evolution of Π_0, with $\gamma \in \{asyn, mp\}$.*

$E^{\gamma}_{\mathcal{C}}(\Pi_0)$ denotes the set of all γ-complete evolutions in $\mathcal{C}$ starting at Π_0.

Let $e = \langle \Pi_0, \Pi_1, \ldots, \Pi_i, \Pi_{i+1} \ldots\rangle$ be an arbitrary γ-complete evolution in $\mathcal{C}$ starting in Π_0. $\langle \Pi_i, \Pi_{i+1}, \ldots\rangle$, $i \geq 0$, is called an i-suffix evolution[1] *of e and is denoted by e_i.*

Definition 6.6.3 (Syntax of $\mathcal{L}_{\mathcal{C}}$) *The set $AP(\mathcal{C})$ is defined by:*

- *$\top \in AP(\mathcal{C})$.*
- *$prec(R) \subseteq AP(\mathcal{C})$.*
- *if $w_1, w_2, \ldots, w_i \in prec(R) \cup \{\top\}$, $i \leq m$, then $w_1 \oplus \ldots \oplus w_i \in AP(\mathcal{C})$.*

The elements of $AP(\mathcal{C})$ are called atomic formulas *of the logic $\mathcal{L}_{\mathcal{C}}$.*
The configuration formulas *of $\mathcal{L}_{\mathcal{C}}$ and the* evolution formulas *of $\mathcal{L}_{\mathcal{C}}$ are defined in the following way.*

- *any atomic formula of $\mathcal{L}_{\mathcal{C}}$ is a configuration formula of $\mathcal{L}_{\mathcal{C}}$.*
- *if ϕ, ψ are configuration formulas of $\mathcal{L}_{\mathcal{C}}$ then $\neg\phi$ and $\phi \wedge \psi$ are configuration formulas of $\mathcal{L}_{\mathcal{C}}$.*
- *if ϕ is an evolution formula of $\mathcal{L}_{\mathcal{C}}$ then $E\phi$ is a configuration formula of $\mathcal{L}_{\mathcal{C}}$.*
- *if ϕ, ψ are configuration formulas of $\mathcal{L}_{\mathcal{C}}$ then $X\phi$ and $\phi U \psi$ are evolution formulas of $\mathcal{L}_{\mathcal{C}}$.*

The configuration formulas and evolution formulas of $\mathcal{L}_{\mathcal{C}}$ form the language of $\mathcal{L}_{\mathcal{C}}$.

[1]Observe that for an arbitrary γ-complete evolution e in $\mathcal{C}$, for each $i \geq 0$, e_i is also a γ-complete evolution in $\mathcal{C}$.

The meanings of $\top, \neg, \wedge$ are those from classical logic. In addition, there are the temporal operators: $E\phi$ that expresses an existential quantification on evolutions, $X\phi$ which means "at the next configuration ϕ is satisfied" and $\phi U \psi$ which means "ϕ is satisfied until ψ is satisfied". In what follows, the properties that can be expressed by using these operators are checked for some models called temporal structures.

Definition 6.6.4 (Temporal structures) *The structure* $\mathcal{T}_{\mathcal{C}}^{\gamma} = (\mathcal{S}, \mathfrak{R})$, $\gamma \in \{asyn, mp\}$, *is defined as follows:*

- $\mathcal{S} \subseteq \mathcal{C}$, *such that if* $\Pi_0 \in \mathcal{S}$ *then* $\{\Pi_1, \Pi_2, \ldots \mid \langle \Pi_0, \Pi_1, \Pi_2, \ldots \rangle \in E_{\mathcal{C}}^{\gamma}(\Pi_0)\} \subseteq \mathcal{S}$.
- $\mathfrak{R} \subseteq \mathcal{S} \times \mathcal{S}$, *such that* $(\Pi_1, \Pi_2) \in \mathfrak{R}$ *iff there exists* $\langle \Pi_1, \Pi_2, \ldots \rangle \in E_{\mathcal{C}}^{\gamma}(\Pi_1)$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}$ *is called a temporal structure in* $\mathcal{C}$.

Definition 6.6.5 (CSA-Semantics) *Let* $\mathcal{T}_{\mathcal{C}}^{\gamma} = (\mathcal{S}, \mathfrak{R})$ *be a temporal structure in* $\mathcal{C}$. *For an arbitrary* $\Pi \in \mathcal{S}$, *an arbitrary* $e \in E_{\mathcal{C}}^{\gamma}(\Pi)$ *and an arbitrary formula* ϕ *from the language of* $\mathcal{L}_{\mathcal{C}}$, *the satisfiability relations* $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \phi$ *and* $\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models \phi$ *are defined co-inductively by:*

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \top$ *always.*

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models w$ *for* $w \in prec(R)$ *iff* $C_\Pi = \{(w', 1)\}$ *and* $w \subseteq w'$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models w_1 \oplus w_2 \oplus \ldots \oplus w_i$ *for* $w_j \in prec(R) \cup \{\top\}, 1 \leq j \leq i$ *iff* $C_\Pi = C_1 + C_2 + \ldots + C_i$ *s.t. for any* $w_j \neq \top$, $1 \leq j \leq i$, $C_j = \{(w_j + u_j, 1)\}$ *for some* $u_j \in \mathbb{M}(A)$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \phi \wedge \psi$ *iff* $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \phi$ *and* $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \psi$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \neg\phi$ *iff* $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \not\models \phi$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models E\phi$ *iff there exists* $e \in E_{\mathcal{C}}^{\gamma}(\Pi)$ *such that* $\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models \phi$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models \phi U \psi$ *iff there exists* $i \geq 0$ *such that* $\mathcal{T}_{\mathcal{C}}^{\gamma}, e_i \models \psi$ *and for all* $j \leq i$ $\mathcal{T}_{\mathcal{C}}^{\gamma}, e_j \models \phi$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models X\phi$ *iff* $\mathcal{T}_{\mathcal{C}}^{\gamma}, e_1 \models \phi$.

Definition 6.6.6 (Validity and satisfiability) *A configuration formula ϕ (evolution formula ϕ) from $\mathcal{L}_{\mathcal{C}}$ is* valid *iff for every temporal structure $\mathcal{T}_{\mathcal{C}}^{\gamma} = (\mathcal{S}, \mathfrak{R})$ in $\mathcal{C}$ and any $\Pi \in \mathcal{S}$ (any $e \in E_{\mathcal{C}}^{\gamma}(\Pi)$, resp.) the following holds $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \phi$ ($\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models \phi$, resp.). A configuration formula ϕ (evolution formula ϕ) is satisfiable iff there exists a temporal structure $\mathcal{T}_{\mathcal{C}}^{\gamma} = (\mathcal{S}, \mathfrak{R})$ and a $\Pi \in \mathcal{S}$ (an $e \in E_{\mathcal{C}}^{\gamma}(\Pi)$, resp.) such that $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models \phi$ ($\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models \phi$, resp.).*

Definition 6.6.7 (Derived formulas) *The following derived formulas are defined for $\mathcal{L}_{\mathcal{C}}$.*

$A\phi = \neg E \neg \phi$.

$F\phi = \top U \phi$.

$G\phi = \neg F \neg \phi$.

The semantics of the derived formulas are the following.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi \models A\phi$ iff for any $e \in E_{\mathcal{C}}^{\gamma}(\Pi)$ the following holds $\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models \phi$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models F\phi$ iff there exists $i \geq 0$ such that $\mathcal{T}_{\mathcal{C}}^{\gamma}, e_i \models \phi$.

$\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models G\phi$ iff for any $i \geq 0$ the following holds $\mathcal{T}_{\mathcal{C}}^{\gamma}, e_i \models \phi$.

$A\phi$ is a universal quantification on evolutions. $F\phi$ means "eventually ϕ is satisfied" (i.e., $F\phi$ is satisfied by an evolution that contains at least one configuration that has the property ϕ). $G\phi$ means "globally ϕ is satisfied" (i.e., $G\phi$ is satisfied by an evolution that contains only configurations satisfying ϕ).

Theorem 6.6.1 (Decidability) *The satisfiability, validity and model-checking problems for $\mathcal{L}_{\mathcal{C}}$ against the CSA-semantics are decidable.*

Proof The result derives from the fact that CTL logic is decidable (see, e.g., [83, 4]) and from the fact that $AP(\mathcal{C})$, the set of atomic formulas, is a finite set. $\square$

To show the potential of the presented logic a small example is given of properties that can be specified. The question is posed whether or not during any evolution the agents can always synchronise when they are *ready* to do so.

In other words, given an arbitrary CSA, Π, and an arbitrary rule $r : \langle u, v\rangle \rightarrow \langle u', v'\rangle$, it is desired to check whether or not it is true that, whenever during an evolution of Π, a configuration with an agent w_1, where $u \subseteq w_1$, is reached, then in the same configuration there is also an agent w_2 with $v \subseteq w_2$ (so rule r can actually be applied). If this is true, Π is said to be *safe on synchronization* of rule r.

This property can be expressed in the proposed temporal logic by the following formula.

$$AG((u \oplus \top) \rightarrow (u \oplus v \oplus \top)).$$

Taking a CSA, Π_0, from $\mathcal{C}$. If the presented CSA-semantics are considered then:

$\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi_0 \models AG((u \oplus \top) \rightarrow (u \oplus v \oplus \top))$

iff for any $e \in E_{\mathcal{C}}^{\gamma}(\Pi_0)$ the following holds $\mathcal{T}_{\mathcal{C}}^{\gamma}, e \models G((u \oplus \top) \rightarrow (u \oplus v \oplus \top))$

iff for any $e = \langle \Pi_0, \Pi_1, \ldots, \Pi_i, \ldots\rangle \in E_{\mathcal{C}}^{\gamma}(\Pi_0)$ and any $i \geq 0$ the following holds $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi_i \models (u \oplus \top) \rightarrow (u \oplus v \oplus \top)$.
This means that if any configuration present in a γ-evolution of Π_0 satisfies $u \oplus \top$ then it will also satisfy $u \oplus v \oplus \top$.

In fact, it is known that $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi_i \models u \oplus \top$ iff $C_{\Pi_i} = C_1 + C_2$, $C_1, C_2 \in \mathbb{M}(\mathbb{M}(A))$ and $C_1 = \{(u + u', 1)\}$, i.e., the configuration of Π_i contains an agent w that contains u.

Similarly, $\mathcal{T}_{\mathcal{C}}^{\gamma}, \Pi_i \models u \oplus v \oplus \top$ iff $C_{\Pi_i} = C_1' + C_2' + C_3'$, $C_1', C_2', C_3' \in \mathbb{M}(\mathbb{M}(A))$ and $C_1' = \{(u + u'', 1)\}$, $C_2' = \{(v + v', 1)\}$, i.e., the configuration of Π_i contains two agents w_1 and w_2 such that $u \subseteq w_1$ and $v \subseteq w_2$, which precisely indicates that Π_0 is safe on synchronization of rule $r : \langle u, v\rangle \rightarrow \langle u', v'\rangle$.

6.7 Prospects

In this chapter a basic model of Colonies of Synchronizing Agents has been presented, however several enhancements to the theoretical model are already in prospect. Primary among these is the addition of *space* to the colony. Precisely, each agent would then have a triple of co-ordinates corresponding to its position in Euclidean space and the rules would be similarly endowed with the ability to modify an agent's position. A further extension of this idea is to give each agent an *orientation*, i.e. a rotation relative to the spatial axes, which may also be modified by the application of rules.

The idea is to make the application of a rule dependent on either an absolute position (thus directly simulating a chemical gradient) or on the relative distance between agents in the case of synchronization. Moreover, in the case of the application of a synchronization rule, the ensuing translation and rotation of the two agents may be defined *relative to each other*. In this way it will be possible to simulate reaction-diffusion effects, movement and local environments.

Some additional biologically-inspired primitives are also planned, such as agent *division* (one agent becomes two) and agent *death* (deletion from the colony) (as, for instance, done in [6]). These primitives can simulate, for example, the effects of mitosis, apoptosis and morphogenesis. In combination with the existing primitives, it will be possible (and is planned) to model, for example, many aspects of the complex multi-scale behaviour of the immune system.

With the addition of the features just mentioned, it will also be interesting to extend the investigation and proofs given above to identify further classes of CSAs demonstrating robustness and having decidable properties. Moreover, it is planned to investigate classes of CSAs where model-checking based on the presented temporal logic can be efficiently implemented, e.g., having an associated temporal structure that can be algorithmically constructed in efficient time and space. It would also be desirable to extend the investigation to design efficient algorithms that implement distributed computations, as used, for instance, in the area of amorphous computing [1].

It is clear that this flexible and elegant paradigm already has many applications related to biology and other complex systems. One such, not detailed in this document, is *A Logical Characterization of Robustness, Mutants and Species in Colonies of Agents* [60], which defines formal ways to characterise fundamental biological concepts using the basic CSA model. The enhancements described above can only increase the possibilities.

Chapter 7

Stochastic Simulation Algorithms

Chapters 3 and 4 present theoretical models which has been implemented in the form of a stochastic simulator peresented in Chapter 5. While it was noted in Chapter 5 that stochastic simulation is inherently computationally intensive, the algorithm was not described nor any mathematical treatment given. These details are included below and in Chapter 9, where an improved algorithm is introduced. It turns out that the model of synchronising agents presented in Chapter 6 poses additional computational challenges for stochastic simulation and these are described in detail in Chapter 8, where a state of the art algorithm is introduced to ameliorate them.

This chapter thus provides the context for Chapters 8 and 9. In particular it describes key stochastic simulation algorithms and their relationship with the basic Markovian assumption and with deterministic simulation.

7.1 Stochastic Simulation

Much of the current use of stochastic simulation in Systems Biology has been facilitated or inspired by the stochastic simulation algorithm(s) created by Gillespie in the 70s [32, 33]. Using Monte-Carlo techniques, these allow the relatively efficient *exact*[1] stochastic simulation in time of *well-stirred* chemically reacting systems by means of only considering the interactions which change the state of the system. In 1998 Gibson and Bruck [31] made a significant improvement by devising modifications which take advantage of the nature of biochemical systems and use an efficient data structure to effectively improve the efficiency of Gillespie's algorithms from $\mathcal{O}(M)$ to $\mathcal{O}(\log M)$ (where M is the number of different reaction types in the system). This asymptotic performance has not yet been bettered for simple chemical systems and hence the Gibson-Bruck algorithm has become the benchmark for all those wishing to improve the state of the art.

The drawback of exact algorithms is that they necessarily simulate every molecular interaction and hence their utility is dependent on the number of molecules in the system. In biological cells, which have volumes of the order of nanolitres, the number of molecules is relatively small, however simulations are still potentially intractable. Even when the *numbers* of molecules are tractable, some phenomena require many reaction events to be observable. When this is compounded with the need to perform multiple simulation runs to gain statistical confidence, the need for efficiency is clear.

This being the case, approximate stochastic algorithms have been developed, such as that of *tau-leaping* [37] (see Section 7.3.3), which allows several reaction steps to be executed simultaneously with minimal error when instantaneous conditions during the simulation permit. This approximate technique has been further refined to remove instability due to stiffness and increase the leap size. Work continues to accommodate stiffness and improve efficiency using similar steady state assumptions to those used in Michaelis-Menten kinetics [11, 74].

When all molecular species are in large copy number, the evolution of

[1]Exact in the sense that the distribution of simulations is consistent with the master equation in the limit of simulations.

the system may be well approximated by a relatively efficient deterministic simulation of a system of ordinary differential equations (ODE). In biological cells however, one or more species is often in low copy number at some point during the simulation, resulting in significant stochasticity. This will not be represented in the deterministic simulation, motivating the need for a stochastic approach. The choice of a discrete and stochastic modelling framework to represent a biological model, such as those presented in Chapters 3–6, thus offers the potential of increased accuracy at a price of increased computational complexity.

The most accurate paradigm might conceivably use molecular dynamics and track the position and velocity of every molecule in the system, but this would be extremely computationally intense and does not seem necessary. A more tractable approach is to work at the levels of populations of molecules and to make the assumption that the system is *well stirred*. This latter assumption removes the concept of individual molecules and is a reasonable approximation when the number of inelastic collisions between molecules (i.e., the reactions) is much lower than the elastic collisions (the unreactive collisions) which serve to stir the system.

In what follows no results are proved, however the reader is assumed to be familiar with the terminology and notation of probability and Markov processes. A good reference is [34].

7.2 Markov processes

This section briefly describes how the chemical master equation arises from basic Markovian assumptions, drawing some specific ideas from [38].

The well accepted paradigm of chemically reacting systems is that molecules are discrete objects that react with one another via well-defined discrete reactions (reaction *channels*) in a random way [64, 34]. Moreover, this random process (denoted here $X(t)$) is usually considered to be a Markov process, which can thus be specified by the probability density function P as follows:

$$P(x_2, t_2|x_1, t_1)dx_2 \equiv \qquad (7.1)$$
$$\text{Probability}\{X(t_2) \in [x_2, x_2 + dx_2),\ \text{given } X(t_1) = x_1, \forall t_1 \leq t_2\}$$

In words, this says that P is a function such that the system's existence in state x_2 at time t_2 is only dependent on it being in state x_1 at time t_1. By imposing on Equation 7.2 the standard requirements of a probability density function (e.g., $P(x_2, t_2|x_1, t_1) \geq 0$ and $\int_{-\infty}^{\infty} P(x_2, t_2|x_1, t_1)dx_2 = 1$ etc.) it is possible to derive the Chapman-Kolmogorov equation in the following form:

$$P(x, t + dt|x_0, t_0) = \int_{-\infty}^{\infty} P(x, t + dt|x - \xi, t)P(x - \xi, t|x_0, t_0)d\xi \qquad (7.2)$$

This essentially says that the probability of arriving at state x at time t is the sum of the probabilities of all the possible paths from an initial state x_0 at t_0. In the case of a real valued jump Markov processes, $P(x, t+dt|x-\xi, t)$ is chosen to ensure that for any positive function $W(\xi; x, t)$,

$$W(\xi; x, t)d\xi\, dt \equiv \text{Probability}\{\text{given } X(t) = x,\ \text{the process will jump}$$
$$\text{to some state in the interval } [x + \xi, x + \xi + d\xi) \text{ during } [t, t + dt)\} \qquad (7.3)$$

Substituting W into Equation 7.2 results in the forward master equation:

$$\frac{\partial}{\partial t}P(x, t|x_0, t_0) =$$
$$\int_{-\infty}^{\infty} [W(\xi|x - \xi, t)P(x - \xi, t|x_0, t_0) - W(\xi|x, t)P(x, t|x_0, t_0)]d\xi$$
$$(t_0 \leq t) \qquad (7.4)$$

A heuristic interpretation of the master equation is that $W(\xi|x - \xi, t)$ is a flow rate from state $x - \xi$ to state x and $W(\xi|x, t)$ is a flow rate from state

x to state $x+\xi$. Equation 7.4 can be re-formulated for integer valued jump Markov processes and, in particular, for chemically reacting systems:

$$\frac{\partial}{\partial t}P(\mathbf{x},t|\mathbf{x}_0,t_0) = \sum_{\mu=1}^{M}[a_\mu(\mathbf{x}-\nu_\mu)P(\mathbf{x}-\nu_\mu,t|\mathbf{x}_0,t_0) - a_\mu(\mathbf{x})P(\mathbf{x},t|\mathbf{x}_0,t_0)] \tag{7.5}$$

Equation 7.5 is then the chemical master equation (CME), where $\mathbf{x}$ is a vector of chemical species under the influence of M chemical reactions, $P(\mathbf{x},t|\mathbf{x}_0,t_0)$ is the probability of $\mathbf{x}$ at time t given initial conditions $\mathbf{x}_0$ at time t_0, $a_\mu(\cdot)$ takes the place of $W(\cdot)$ and is an instantaneous reaction *propensity* function and ν_μ is a constant per-reaction vector of species change.

The CME thus describes the flow of probability between discrete states in a chemically reacting system.

7.3 Hierarchy of simulation methods

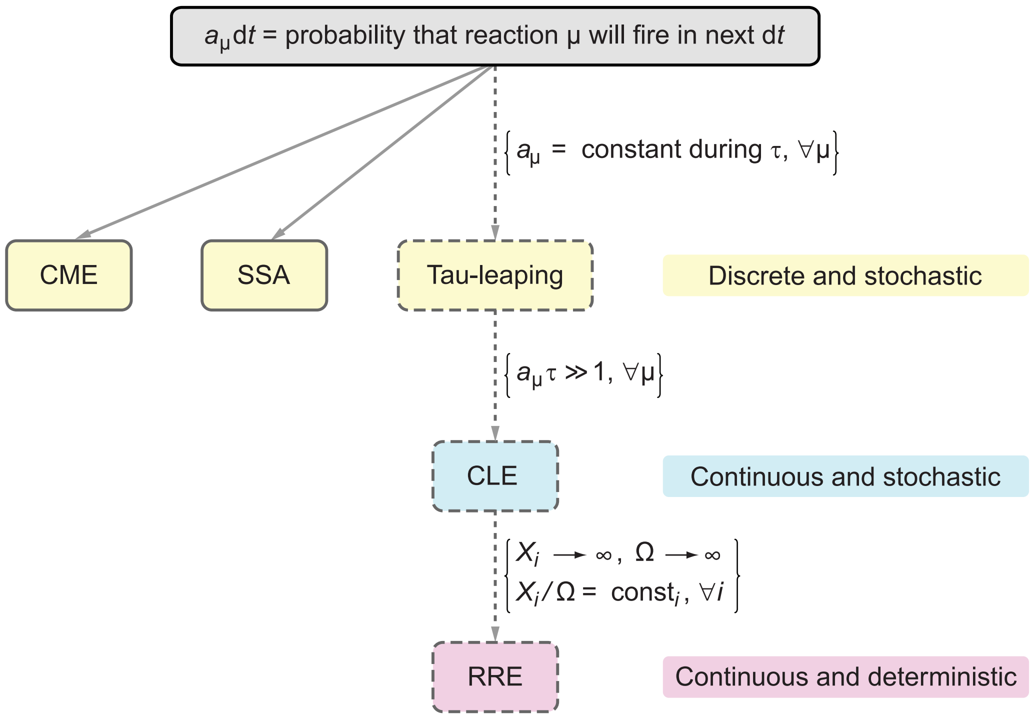
This section places the various simulation approaches in context, drawing some ideas from [39]. Figure 7.1 summarizes the relationship between the various commonly used simulation techniques used to represent well-stirred chemically reacting systems.

The axiomatic Markovian premise is that a reaction's propensity, a_μ, is the only parameter controlling the probability that reaction μ will be executed in the next infinitesimal time dt. A direct consequence of this is the CME (Equation 7.5) and the Stochastic Simulation Algorithm (SSA) [32, 35]. When all a_μ do not change significantly in time τ, the time between reaction events, it is possible to employ a strategy of *tau-leaping* [37] (Section 7.3.3) to simulate the system: a good approximation to the SSA is thus achieved by executing several reaction steps at once, the number chosen according to a Poisson process. When $a_\mu\tau \gg 1$, it is possible to use

Gaussian noise as an approximation of Poisson noise and hence simulate the Chemical Langevin Equation [36] (CLE, Section 7.3.3). In the thermodynamic limit, the noise term of the CLE becomes vanishingly small and the CLE is well approximated by the traditional deterministic Reaction Rate Equation (RRE, Section 7.3.3).

7.3.1 Exact methods

Gillespie created an efficient way to simulate a trajectory of a chemically reacting system which is exactly consistent with the underlying principles of the CME [32, 35]. The algorithm therefore simulates a jump Markov process and is based on the assumption that two events take place at the



$a_\mu dt$ = probability that reaction μ will fire in next dt
$\{a_\mu$ = constant during τ, ∀μ$\}$
CME
SSA
Tau-leaping
Discrete and stochastic
$\{a_\mu \tau \gg 1, \forall\mu\}$
CLE
Continuous and stochastic
$X_i \to \infty, \Omega \to \infty$
$X_i/\Omega = \text{const}_i, \forall i$
RRE
Continuous and deterministic

Figure 7.1: Diagram showing the relation of various simulation methods and representations of chemical systems arising from the from the premise that the next reaction to fire depends only on the current state of the system. Solid arrows indicate exact inference, dotted arrows indicate approximation. Adapted from [39].

same time with zero probability. Specifically, Gillespie defines the *reaction probability density function* as

$$P(\tau, \mu) \equiv$$

$$\text{Probability}\{\text{at time } t \text{ the next reaction will occur in the differential time interval } (t+\tau, t+\tau+d\tau) \text{ and it will be reaction } \mu\} \tag{7.6}$$

To simulate the interaction of populations of chemical species, it samples the random variable so defined, chooses an appropriate sequence of individual reactions to execute and calculates the time intervals between them.

Gillespie proposes two mathematically equivalent methods to achieve this: the 'direct method' (DM) and the 'first reaction method' (FRM). The FRM is conceptually simple: at each step a random putative reaction time is calculated for each reaction and the one with the shortest time is chosen and executed. The DM transforms the process into two separate calculations: the choice of reaction and the evaluation of the time. Gibson and Bruck later achieved a significant reduction in complexity to the Gillespie algorithms, however the underlying principles remain the same.

To maintain coherence with earlier work, where possible the nomenclature from [32] and its derivatives is used. When reference is made to algorithmic complexity, the computational operations given in Table 7.1 are considered to be $\mathcal{O}(1)$.

The Direct Method

Gillespie considers a well-stirred chemically reacting system at thermal equilibrium at some constant temperature, confined in constant volume Ω and containing M reaction *channels* (chemical reactions described by stoichiometric equations) indexed by $1 \leq \mu \leq M$, with corresponding rate constants c_μ. E.g.,

$$\textit{reaction}\,1: \; X_1 + X_2 \xrightarrow{c_1} X_3 \qquad \textit{reaction}\,2: \; X_3 \xrightarrow{c_2} X_4 \tag{7.7}$$

Operation	**Relative time**
`= + - * ++ -- == < > <= >=`	
Integer division	1
`if(...) for(...) while(...)`	0 + (...)
Function call	2 + no. of args.
Double precision division	4
Double precision division by 0.0	
Natural logarithm	20
Double precision random number	60

Table 7.1: Computational operations considered to be $\mathcal{O}(1)$ with their approximate relative time cost. Values are based on timings of Java and C$^{\#}$ instructions on an Intel Pentium machine running Windows.

where $X_1, \ldots, X_4$ are different chemical species.

When a reaction is executed, the numbers of species defined as reactants (the left side) of the reaction are subtracted from the population and the numbers of species defined as products (the right side) of the reaction are added to the population.

Gillespie defines reaction μ's *propensity*, denoted a_μ, to be the product of its rate constant, c_μ, and a combinatorial factor, h_μ, equal to the instantaneous number of ways the reaction may take place given the current numbers of reactants in the system. For example, if species X_1, X_2 and X_3 have instantaneous populations equal to S_1, S_2 and S_3 molecules, respectively, then the propensities of reactions 1 and 2 from Equation 7.7 are $c_1S_1S_2$ and c_2S_3, respectively. The propensity is then a valid measure of how likely a reaction is to be executed next because the well stirred assumption implies that all molecules have simultaneous access to all others[2].

The *total propensity*, denoted a, is the sum of all the individual reaction propensities and is an instantaneous measure of how likely *any* of the reactions is to be executed next. When this value is zero, no reactions are

[2]A rigorous derivations of c_μ and h_μ can be found in [35].

possible and the system can not change. The total propensity is defined by the following equation:

$$a \equiv \sum_{\mu=1}^{M} a_\mu = \sum_{\mu=1}^{M} h_\mu c_\mu \tag{7.8}$$

From the foregoing, it can be appreciated that the time until the next reaction is related to the total propensity. Using a Monte Carlo technique, the algorithm finds the time τ to the next reaction by selecting a value r_1 from a uniform random variable in the interval $(0, 1]$ and evaluating the following equation:

$$\tau = -\ln(r_1)/a \tag{7.9}$$

To select which reaction to execute, a sample is drawn, denoted r_2, from a uniform random variable in the interval $(0, a)$. The algorithm decides which reaction to execute next by finding the minimum value of ν which satisfies the following equation:

$$r_2 \leq \sum_{\mu=1}^{\nu} a_\mu \tag{7.10}$$

This is shown diagrammatically in Figure 7.2. At any step in the simulation, the probability of choosing a particular reaction next is given by its propensity divided by the total propensity.

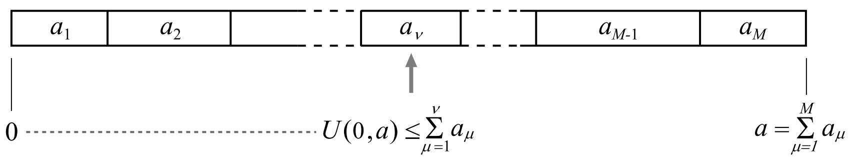

a_1
a_2
a_ν
$a_{M\text{-}1}$
a_M
0
$U(0,a) \leq \sum_{\mu=1}^{\nu} a_\mu$
$a = \sum_{\mu=1}^{M} a_\mu$

Figure 7.2: Selection of reaction ν using the Direct Method. a_μ is the reaction propensity for reaction μ, a is the total propensity and $U(0, a)$ is a sample of the uniform random variable in the interval $(0, a)$.

The First Reaction Method

At each step, the FRM calculates putative time increments for all possible reactions in the system and executes the one with the shortest. The increment in simulated time for reaction μ is denoted τ_μ and calculated using a similar Monte Carlo method to that used in Equation 7.9:

$$\tau_\mu = -\ln(ran[0,1))/(c_\mu h_\mu) \tag{7.11}$$

where $ran[0,1)$ is a number drawn from a uniform random variable in the range $[0,1)$ and $c_\mu h_\mu$ is the propensity of reaction μ.

7.3.2 The Next Reaction Method

The Next Reaction Method (NRM) [31] builds on the FRM to achieve a significant improvement in efficiency for typical biochemically reacting systems. An improvement in complexity from $\mathcal{O}(M)$ to $\mathcal{O}(\log M)$ of an individual reaction step is gained, where M is the number of reactions in the system. The key modifications are as follows:

- Reaction dependency graph: After the execution of each reaction, Gillespie's FRM re-calculates all the propensities in the system and then re-generates the random putative times of the reactions. This has complexity $\mathcal{O}(M)$. In biological systems, however, it is often the case that reactions have limited dependency and only a fraction of all the propensities change at any step. By initially constructing a reaction dependency graph, during the simulation the algorithm need only update those propensities which it knows could have changed. Reaction dependency in typical biological pathways is typically much less than M (e.g., see Figures 7.3 and 5.17) and hence the authors of the NRM assume the reaction dependency graph to be 'sparse', i.e. dependency asymptotically unimportant.[3] Under this assumption the propensity update step drops out of the overall complexity of the algorithm.

- Absolute time: The FRM and DM generate simulated time increments which are relative to the current time of the simulation. By considering reaction times relative to t_0, thus *absolute time*, the NRM is able to re-use the putative times of reactions whose propensities have been changed by the firing of the selected reaction. The formula

$$\tau_{\mu,new} = t + (\tau_{\mu,old} - t)a_{\mu,old}/a_{\mu,new} \tag{7.12}$$

  where τ_μ and a_μ are the absolute putative time and the propensity of reaction μ, respectively, and t is the current simulated time, is thus used to update the putative times of the dependent reactions.[4] In this way the NRM need only generate one random number per simulation step - an additional saving over the DM.

- Indexed priority queue: The FRM requires $\mathcal{O}(M)$ operations to find the reaction with the shortest time in an unordered linear data structure. By arranging the reaction times in a partially ordered binary tree (called an *indexed priority queue* in [31]), such that the root holds the reaction with the shortest time and reactions further from the root always have longer times than those closer (reactions at the same height are not ordered), it is possible to select the fastest reaction in $\mathcal{O}(1)$ operations. When the root reaction is executed, its propensity and those of an arbitrarily located set of dependent reactions in the tree changes. Since it is not necessary to maintain a horizontal ordering, the authors provide an algorithm which applies pairwise exchanges

[3]It seems likely from empirical evidence that the dependency is something like $\mathcal{O}(\log M)$ or $\mathcal{O}(M^{1/k})$, where $k > 1$.

[4]While $a_{\mu,new} = 0$, $t_{\mu,new}$ is set to ∞. Immediately thereafter τ_μ is calculated afresh.

of reactions at adjacent heights in the tree to maintain the tree's invariant property. This has complexity proportional to the number of reactions that change (the reaction dependency) multiplied by the height of the tree. Since it has already been assumed that the average reaction dependency of the system is sparse, the overall complexity of selecting a reaction becomes $\mathcal{O}(\log M)$.

Combining the effects of the itemised modifications gives an overall asymptotic complexity for the NRM of $\mathcal{O}(\log M)$.

A particular drawback of the NRM is that even if there is a well defined average order of reaction speeds that is constant throughout the course of the simulation, the algorithm may still spend much time re-ordering the tree. Following the execution of a reaction, the new times calculated for the dependent reactions, being samples of random variables, alter their positions in random ways. The significance of this effect depends on the stochasticity of the reactions and how inherently well ordered the reactions are.

7.3.3 Approximate methods

This section contains a brief overview of approximate simulation methods which link the exact methods to the commonly used reaction rate equation of chemical kinetics. In what follows $X_i(t)$ is the number of molecules of species i at time t and $\mathbf{X}(t)$ is the vector of such quantities at time t, given that the system was at $\mathbf{X}(t_0) = \mathbf{x}_0$ at some initial time t_0. $\mathbf{x}$ is an instantaneous system state vector and $a_j(\mathbf{x})$ is the propensity of the j^{th} reaction, given that $\mathbf{X}(t) = \mathbf{x}$. This nomenclature is consistent with a recent review of stochastic simulation [39], which may be consulted for more detail.

μ	Reaction	Species affected	Dependent reactions D_μ	$\lvert D_\mu\rvert$
1.	gA → MA + gA	{MA}	{6,12}	2
2.	pA + gA → g_A	{pA,gA,g_A}	{1,2,3,8,10,15,16}	7
3.	g_A → MA + g_A	{MA}	{6,12}	2
4.	gR → MR + gR	{MR}	{7,13}	2
5.	g_R → MR + g_R	{MR}	{7,13}	2
6.	MA → pA	{MA,pA}	{2,6,8,10,12,15}	6
7.	MR → pR	{MR,pR}	{7,8,11,13}	4
8.	pA + pR → AR	{pA,pR,AR}	{2,8,9,10,11,15}	6
9.	AR → pR	{AR,pR}	{8,9,11}	3
10.	pA → *	{pA}	{2,8,10,15}	4
11.	pR → *	{pR}	{8,11}	2
12.	MA → *	{MA}	{6,12}	2
13.	MR → *	{MR}	{7,13}	2
14.	g_R → pA + gR	{g_R,pA,gR}	{2,4,5,8,10,14,15}	7
15.	pA + gR → g_R	{pA,gR,g_R}	{2,4,5,8,10,14,15}	7
16.	g_A → pA + gA	{g_A,pA,gA}	{1,2,3,8,10,15,16}	7
			Mean $\lvert D_\mu\rvert$ =	4.06

Figure 7.3: Reaction dependencies of the noise-resistant oscillator model of Figure 5.11, whose average dependency is 4.06. If S is the set of species and R is the set of reactions $r_1 \ldots r_\mu \ldots$, $D_\mu = \{r : R \mid |product_j(r_\mu)| \neq |reactant_j(r_\mu)| \wedge product_j(r_\mu) \cup reactant_j(r_\mu) \in reactants(r), 1 \leq j \leq |S|\}$. That is, for a chosen reaction r_μ, D_μ is the set of reactions whose propensities are affected by its execution.

Tau-leaping

The term 'tau-leaping', coined in [37], refers to the process in an approximate stochastic simulation algorithm of simulating several individual reaction events by a single aggregated event, thus *leaping* over several steps. Under the condition that the $a_j(\mathbf{x})$ remain approximately constant during the time interval $[t, t + \tau]$, the following equation (the *tau-leaping formula*) is a good approximation of the evolution of the system:

$$\mathbf{X}(t+\tau) \doteq \mathbf{x} + \sum_{j=1}^{M} \mathcal{P}_j(a_j(\mathbf{x})\tau)\nu_j \tag{7.13}$$

where $\mathcal{P}(m)$ is the Poisson random variable with mean m and τ is the length of the leap. When the conditions of applicability are met, a tau-leaping simulation algorithm thus generates samples of the appropriate Poisson random variable and uses the value to make multiple simultaneous applications of the corresponding reaction.

Selecting values of τ which maximise leap size to gain efficiency, yet avoid inaccuracy, instability and negative populations, is non-trivial and the subject of ongoing research [41, 12, 9]. Stiff systems, which have both fast and slow time-scales and where the fastest modes are stable, require τ to be small on the fastest time-scale to retain accuracy. Since stiff systems are common, several strategies have been devised, such as the implicit tau-leaping method [75] and the Slow Scale Stochastic Simulation Algorithm (ssSSA) [11]. See Section 7.3.3.

The Chemical Langevin Equation

Knowing that $\mathcal{N}(m, \sigma^2) = m + \sigma\mathcal{N}(0, 1)$, where $\mathcal{N}(m, \sigma^2)$ is the normal random variable with mean m and variance σ^2, and that $\mathcal{P}(m) \approx \mathcal{N}(m, m)$

for $m \gg 1$, Equation 7.13 can be re-written

$$\mathbf{X}(t+\tau) \doteq \mathbf{x} + \sum_{j=1}^{M} \nu_j a_j(\mathbf{x})\tau + \sum_{j=1}^{M} \nu_j \sqrt{a_j(\mathbf{x})\tau}\, \mathcal{N}_j(0,1)\sqrt{\tau} \tag{7.14}$$

Equation 7.14 is then a good approximation to the evolution of the system when both $a_j(\mathbf{x})$ remain sufficiently constant and the copy number of molecules is much greater than 1. Equation 7.14 can be written in continuous form

$$\frac{d\mathbf{X}(t)}{dt} \doteq \sum_{j=1}^{M} \nu_j a_j(\mathbf{X}(t)) + \sum_{j=1}^{M} \nu_j \sqrt{a_j(\mathbf{X}(t))}\, \Gamma_j(t) \tag{7.15}$$

where $\Gamma_j(t)$ are statistically independent white noise processes. Equation 7.14 is the standard form Chemical Langevin Equation (CLE) while Equation 7.15, an alternative form of the CLE, has the canonical form of a stochastic differential equation, containing a deterministic *drift* term and a stochastic *diffusion* term.

The Reaction Rate Equation

In the *thermodynamic limit*, that is, the case when the system volume Ω and species populations $\mathbf{X}$ tend to infinity such that their concentrations $\mathbf{X}/\Omega$ remain constant, the system is well approximated by Equation 7.15. Under these conditions the propensity functions $a_j(\mathbf{x})$ grow in proportion to the size of the system (i.e., the magnitudes of the species populations) and the drift and diffusion terms of the CLE are thus affected unequally. The drift term increases in proportion to the system size while the diffusion term grows as the square root of the system, hence as the system approaches the thermodynamic limit, the stochastic term disappears and what remains is the familiar deterministic reaction rate equation (RRE):

$$\frac{d\mathbf{X}(t)}{dt} = \sum_{j=1}^{M} \nu_j a_j(\mathbf{X}(t)) \tag{7.16}$$

Quasi Steady State Stochastic Simulation Algorithms

Inspired by the well-known Michaelis-Menten approximation for enzyme kinetics, several approaches to stochastically simulating stiff chemical systems are based on a *quasi steady-state* approximation [74]. See also, e.g., [44], [10] and [11]. The following subsection describes the slow scale Stochastic Simulation Algorithm (ssSSA) [11] to exemplify these approaches.

The slow scale Stochastic Simulation Algorithm

The ssSSA first makes a partition of the reactions into fast and slow sets. The fast set contains those reactions whose propensity functions tend to have the largest values and the slow set contains the rest. The species are also partitioned into into fast and slow subsets. Any species which is affected by a fast reaction is classified as a fast species, the rest are classified as slow. It is then possible to partition the overall process into fast and slow processes $X^f(t)$ and $X^s(t)$, respectively, where $X^s(t)$ involves only slow species and slow reactions but $X^f(t)$ involves fast reactions and both fast and slow species.

The virtual fast process $\hat{X}^f(t)$ is defined as the fast species evolving as a result of the fast reactions with the slow reactions turned off.

For the ssSSA to be applicable, the system must satisfy two stochastic stiffness conditions: $\hat{X}^f(t)$ must approach a well defined time-independent random variable $\hat{X}^f(\infty)$ as $t \to \infty$ and $\hat{X}^f(t) \to \hat{X}^f(\infty)$ must be achieved in a time which is small compared with the time to the next slow reaction. The slow scale approximation [11] then asserts that it is acceptable to ignore the fast reactions and simulate the system one slow reaction at a time, provided that the propensity function of each slow reaction is replaced by its average with respect to the asymptotic virtual fast process $\hat{X}^f(\infty)$.

The ssSSA thus simulates the slow reactions using the average propensity functions and ignores the fast reactions. The fast species can be obtained by Monte Carlo sampling.

Note that the multiscale stochastic simulation algorithm [10] differs from the ssSSA in the way the averages are computed and the way in which the fast species populations are generated, but is otherwise similar.

7.3.4 Numerical precision

It is important to note here that the random numbers used in stochastic simulations as described above should have sufficient precision to adequately represent the granularity of the range of propensities and times that occur during a simulation. A simple illustration of how a problem might arise for an algorithm based on the Direct Method is the case of simulated system having three reaction channels but the random number used to select them having only one bit of precision: a single sample would not be able to distinguish the reactions. A more likely problem is that the *ratios* of instantaneous propensities or times can not be *accurately* represented by the random number. For example, suppose a simulation algorithm has a uniform random number generator with eight bits of precision (so 256 different equally likely values) and a model to be simulated contains two reactions which have respective propensities of 1 and 400 at some point during the simulation: the reactions can not be accurately selected by the algorithm because the random number generator does not have sufficient precision to represent the ratio 1:400. In general, floating point numbers are represented in a computer with a fixed window of precision plus an exponent which allows the window to slide over a much wider range of values. The problems of precision may therefore be more subtle.

For algorithms based on the first reaction method an analogous situation is that two or more reactions have exactly the same time of execution or that a reaction ends up having zero or infinite time. For the case of the next reaction method, which uses absolute time, there is an additional burden of numerical precision. Reaction events continue to occur separated by the same time-scales found in the other exact algorithms (since they all produce equivalent traces), yet the values of time used and stored by the NRM are of the order of the total length of the simulation. To distinguish new reaction events, the numbers are thus required to maintain precision at extreme orders of magnitude, with the eventual result that lower order bits get lost.

Concerning the Direct Method, if the lowest significant figure of the largest propensity represents a greater value than the highest significant figure of another propensity, then the reaction corresponding to the smaller propensity can not be selected. Moreover, algorithms which update the total propensity using the change in propensity at each step, rather than performing a complete summation, run the risk of additional error due to a related limitation. The conditions that cause the error to arise are that during a simulation there are a number of reactions which have individual propensities whose lowest significant figure has a lower value than the value of the lowest *possible* significant figure of the total propensity at its current value. It is then conceivable that the change in propensity caused by the application of two or more such reactions is different to the change that would be caused by applying the sum of their changes.

The consequences are that the stored total propensity is different to the *real* total propensity and that the error may grow, dependent on the nature of the model, the simulation conditions and other factors such as the machine's precision and rounding policy. For the case that the stored total propensity is less than the real total propensity, the effect will be most strongly felt by the reactions that occur at the end of the summation: they will not be fully covered by the random sample r_2 and will be selected less frequently. In the worst case this could be not at all: if the reactions are ordered to increase the performance of selection, then those with the lowest propensities will lie at the end of the summation and their propensities might be less than the accumulated error. Reactions with low propensities might also fall at the end of the summation by chance, so this problem is not limited to algorithms which order the reactions in such a way.

Chapter 8

The Method of Partial Propensities

Agent based paradigms are emerging as a promising computational formalism to represent the complexity of biological systems and one such, Colonies of Synchronising Agents, has been presented in Chapter 6. It turns out, however, that to simulate the CSA model involves a high level of computational complexity. This is in part due to the fact that agents, not being atomic, must be represented individually and simulated discretely.

The Gillespie algorithm [32] and its derivatives are widely used in Computational Biology as relatively efficient means to simulate the discrete interaction of chemical species. This efficiency relies on a description of chemistry which treats populations of molecules as a ‘well stirred’ mixture of identical hard spheres. This, in effect, removes the concepts of space and molecular individuality from the formulation and exploits the resulting symmetries. By contrast, agent based paradigms such as CSA reintroduce individuality and thus lose the advantageous symmetries. The computational complexity rises in a combinatorial way.

In developing a software realisation of the CSA model, a stochastic simulation algorithm has been devised which significantly ameliorates the computational complexity in comparison to standard approaches. It is observed that while some of the symmetries of a well stirred system have been removed, others have been introduced and it is these that are exploited.

Though the present motivation is to model complex biological systems as they might be in-vivo, the algorithm can also be applied to efficiently simulate experiments where there is structured repetition of chemical reactions. Moreover, the technique may be beneficial in other stochastic agent-based or hierarchical systems where there is combinatorial interaction.

8.1 Introduction

There is growing interest in Computational Systems Biology to perform discrete and stochastic simulations of chemically reacting systems; the intuition being that this technique provides additional insight about what are essentially stochastic biological processes. More precisely, the rationale is that stochastic simulation using discrete quantities of molecules, rather than continuous concentrations, can reveal behaviour that is hidden by the averaging effect of deterministic simulation of ordinary differential equations. In addition, the discrete approach lends itself to analysis by the tools of computing science formal methods, such as model checking. The penalty of this technique is the computational complexity of simulating every individual interaction between populations, although the current algorithms and hardware keep the task more or less tractable, if not trivial.

As systems biologists' ambitions grow, however, and bigger systems with more complex phenomena are modelled, there will be an inevitable increase in the computational complexity associated with simulation. In particular, agent-based modelling frameworks offer the possibility to model the complex interaction of populations of objects which may themselves have complex internal behaviour. By using such a formalism it is possible to reduce the complexity of the *description* of a complex system by hierarchical composition: define behaviour at a microscopic level, call that an agent and then define the interaction of populations of agents at a macroscopic level. Each member of the population is then potentially a unique individual with a unique internal state which must be tracked. While the description may be simple, the complexity of the system manifests itself as a combinatorial explosion of states in the unfolding simulation.

In this context, the hierarchical computational paradigm and modelling framework Colonies of Synchronizing Agents (CSA) has been presented in Chapter 6. The key points to note about CSA for the present context are that agents may contain arbitrary, independent chemically reacting systems and may interact with each other in a chemistry-like way according to their type (a dynamic property), optionally controlled by their internal chemistry (internal reactions). A set of synchronisation reactions (agent reactions) is specified to define how agents react with one another and these depend on the internal state of the agents. While the representation of the agents is necessarily discrete, the representation of their internal chemistry is also represented in a discrete way in the CSA model to facilitate homogeneous simulation and logical analysis (see Chapter 6).

The quantities of agents and chemical species are therefore discrete and the evolution of the system is stochastic. Contrary to intuition perhaps, using a discrete approach to represent agents is potentially *more* efficient than simulating the same system with ordinary differential equations (ODEs). The reason behind this is that ODEs are efficient to represent large populations of small numbers of species, since small and large populations have equivalent complexity when represented by a single real number, but each species represents a differential equation to be solved. In the CSA model, each agent corresponds to a unique species in a population of magnitude one, which would therefore be computationally cumbersome in an ODE framework.

Currently, the most popular and efficient approach to discrete and stochastic simulation is the implementation of some derivative of the Gillespie algorithm [32] (e.g. [13, 11, 31, 37]), which was designed to simulate trajectories of chemically reacting systems which are exactly consistent with the chemical master equation. In what follows, a basic understanding of the Gillespie algorithm is assumed and may be gained from Section 7.3.1. More detailed treatment can be found in the original papers [32, 33, 35], the reviews [39, 40] and the book [34]. Section 8.2 first recaps some basic terminology then considers the computational complexity of the direct method (DM) and the additional complexity entailed in its application to the CSA model. Techniques are then described which significantly reduce this complexity and it is shown show how these improve on benchmark algorithms. In doing this, it is also shown how the techniques have general applicability in contexts where there is combinatorial-like interaction between populations of objects.

In what follows the reader is assumed to be familiar with the basic concepts and terminology of exact stochastic simulation methods as outlined in Section 7.3.1.

8.2 The Direct Method

The Gillespie algorithm is an efficient way to *exactly* simulate a trajectory of a chemically reacting system, which are often intractable to analysis and more easily investigated by simulation [33]. Exact in this context means that the trajectories so generated are fully consistent with the premises that underlie the chemical master equation (CME). In what follows, the focus is on the direct method (DM) since it is most readily adapted for the current purpose and most easily understood. It is noted here, however, that the techniques described in Section 8.3 are not limited to the DM.

8.2.1 Computational complexity of the DM

The average number of steps of an *exact* discrete and stochastic simulation of a chemically reacting system is critically dependent on the nature of the model, the initial numbers of molecules and the length of time being simulated, but it is *independent* of the algorithm used because, by definition of the term exact, the simulations are samples of the sme distribution. Since the number of steps cannot therefore be reduced without skipping reaction events and thus losing exactness, improving the efficiency of an exact stochastic simulation algorithm requires the reduction of the cost of an individual iterative step.

The asymptotic computational complexity of the DM is now considered in terms of input being the number of reactions, M, using the terminology defined in Section 7.3.1.

The direct method can be summarised thus:

1. initialise species and set $time = 0$.
2. iterate for sufficient steps or until all propensities are zero:
   - a calculate M propensities
   - b sum M propensities to give a, the total propensity
   - c generate random number r_1
   - d calculate τ using Equation 7.9 and add to *time*
   - e generate random number r_2
   - f sum reaction propensities in order until value just greater than r_2
   - g update species according to the reaction reached in step 2.f

Steps 2.a and 2.b each require a number of operations proportional to M and hence are $\mathcal{O}(M)$. Steps 2.c, 2.d and 2.e involve calls to costly mathematical functions which generate random numbers and logarithms, however the cost does not increase with input size and is therefore $\mathcal{O}(1)$ (see Table 7.1). Step 2.f will usually take fewer than M operations, but scales as $\mathcal{O}(M)$. The number of species affected by a reaction is often a small number only weakly related to M, so step 2.g is often assumed to be $\mathcal{O}(1)$. Summing these, the overall asymptotic complexity is $\mathcal{O}(M)$

8.2.2 Optimizations of the DM

The following optimisations are features of the Optimized Direct Method (ODM) of [13], not to be confused with the algorithm of the same name featured in the appendix of [31].

The DM relies on a selection made from the cumulative sum of propensities, where each value in the accumulation is dependent on all summations up to that point. If one propensity changes, then all the cumulative propensities that follow it must be re-calculated. In general, the application of a reaction affects the propensities of the other reactions unpredictably, although it is unlikely to affect *all* the other reactions. Hence, it is not always necessary to recalculate all the propensities, but it does seem necessary to re-calculate all the cumulative propensities. The following optimizations address these issues:

1. By creating a reaction dependency graph [31] it is necessary to update only the propensities of those reactions which have changed. Step 2.a then reduces to $\mathcal{O}(1)$, under the assumption that reaction dependency is only weakly related to M.

2. By storing the intermediate sums of step 2.b in an array it is possible to reduce step 2.f to an $\mathcal{O}(\log M)$ search. Since the values are monotonic, the desired reaction can be found by iteratively dividing the array

according to whether the desired value is above or below a mid point. However, this optimization is superseded by the following:

3. By storing the total propensity, a, and updating it at each step by the sum of the *differences* of changed propensities, it is possible to avoid summing M propensities in either step 2.b or step 2.f. It is only necessary to sum until the value defined by r_2 is reached, which is $\leq M$ operations. If it is possible to arrange the reactions in descending order of frequency, the worst case average summation will be $M/2$ operations (when all the reactions are equally likely), with a best case being significantly better (see Figures 9.1 and 9.2). Like Optimization 1., this optimization relies on the assumption that reaction dependency is $\mathcal{O}(1)$.

4. Thus, by initially calculating and storing all the propensities, it is possible to avoid re-calculating unchanged propensities during the iteration.

8.3 The Method of Partial Propensities

The Method of Partial Propensities (MPP) has been specifically designed to ameliorate the computational complexity of simulation the CSA model. The terminology given in Section 7.3.1 and used above is thus extended to accommodate the various extended concepts it contains.

A colony consists of N agents, each containing a maximum of S different chemical species, M_I *internal reactions* and M_A *agent reactions*, where $M \equiv M_I + M_A$. The reactions are *global*, i.e., they apply to all agents, however reactions may be disabled with respect to certain agents by altering the agents' contents. Internal reactions can be considered chemistry of the type described in Section 8.2, however agent reactions are an abstraction of this concept and need further explanation.

An agent reaction is applicable to a pair of agents and contains two sets (strictly *multisets*, see Chapter 2) of reactants which must exist in their corresponding agents for the reaction to be executed. In contrast to internal reactions and the chemical reactions defined in [32], which have a maximum of three reactants, there is no theoretical limit to the number of elements of either of these two sets. An agent reaction also contains two sets of products which correspond with the sets of reactants. When an agent reaction is executed, the species in the sets of reactants are removed from their corresponding agents and species defined in the corresponding sets of products are added accordingly. An agent reaction therefore has the general form

$$\{V_1, \ldots, V_k\} + \{W_1, \ldots, W_l\} \xrightarrow{c_\mu} \{X_1, \ldots, X_m\} + \{Y_1, \ldots, Y_n\} \tag{8.1}$$

where c_μ is the constant reaction rate, $\{V_1, \ldots, V_k\}$ and $\{W_1, \ldots, W_l\}$ are sets of necessary reactants in the first and second agents, respectively, $\{X_1, \ldots, X_m\}$ and $\{Y_1, \ldots, Y_n\}$ are the respective sets of products, and $k \ldots n$ are arbitrary integers.

The state of the colony is evolved by applying a sequence of reactions which are chosen randomly according to their average rate and an exponential distribution. In order to maintain this Markovian property, so that the system may be simulated in the manner of the Gillespie algorithm, the propensities of agent reactions, like those of internal reactions, are functions of the current state of the system only. The propensity of an internal reaction is calculated with respect to the populations of species contained within a specific agent. The propensity of an agent reaction refers to a specific pair of agents and is calculated as the number of ways that the reaction's first set of reactants may be selected from the first agent, multiplied by the number of ways its second set of reactants may be selected from the second agent, multiplied by a rate constant.

8.3.1 Computational complexity of CSAs using the DM

In this section the cost of simulating the CSA model is considered with respect to the DM, in order to gain an understanding of the increased complexity. The following description therefore takes the same form as that of Section 8.2.1, however in this case the number of agents is also considered as part of the input when evaluating the complexity. This is because the number of reactions alone does not determine the total number of propensities used by the algorithm.

$a_{\mu i}$ denotes the propensity of internal reaction μ in agent i. $a_{\pi ij}$ denotes the propensity of agent reaction π applied to agents i and j. In general, $a_{\pi ij} \neq a_{\pi ji}$, hence propensities for all ordered pairs of agents are calculated except the case when $i = j$, which is defined to be impossible and therefore $a_{\pi ii} \equiv 0$.

The total propensity for internal reactions, a_I, is defined by:

$$a_I \equiv \sum_{i=1}^{N} \sum_{\mu=1}^{M_I} a_{\mu i} \tag{8.2}$$

The total propensity of agent reactions, a_A, is defined by:

$$a_A \equiv \sum_{i=1}^{N} \sum_{\substack{j=1 \\ j \neq i}}^{N} \sum_{\pi=1}^{M_A} a_{\pi ij} \tag{8.3}$$

Hence, the total propensity of all the possible reactions at a given instant is defined to be:

$$a \equiv a_I + a_A \tag{8.4}$$

The total number of propensities is thus:

$$(N^2 - N)M_A + NM_I \tag{8.5}$$

The time until execution of the next reaction, τ, will be calculated, as in the case of the standard DM, by sampling the uniform random variable in the range $(0, 1]$ and evaluating Equation 7.9 using a defined in Equation 8.4.

A reaction is selected, as before, by first drawing a sample, r_2, from the uniform random variable in the interval $(0, a)$. In general, propensities are summed in order and the reaction to execute is that reached when the value of the summation is just greater than or equal to r_2.

Hence, by arranging the propensities such that propensities $1 \ldots M_I$ correspond to the set of internal reactions and propensities $(M_I + 1) \ldots M$ correspond to the set of agent reactions, it is possible to say that for $r_2 \leq a_I$ the reaction will be an internal reaction and an agent reaction otherwise. For the case that $r_2 \leq a_I$, the DM will choose internal reaction ν in agent α, where α and ν satisfy

$$r_2 \leq \sum_{i=1}^{\alpha} \sum_{\mu=1}^{\nu} a_{\mu i} \tag{8.6}$$

such that α is minimum and ν is minimum given α. For the case that $r_2 > a_I$, the reaction will be agent reaction δ between agents β and γ, where β, γ and δ satisfy

$$r_2 \leq a_I + \sum_{i=1}^{\beta} \sum_{\substack{j=1 \\ j \neq i}}^{\gamma} \sum_{\pi=1}^{\delta} a_{\pi i j} \tag{8.7}$$

such that β is minimum, γ is minimum given β and δ is minimum given β and γ. Note that, in general, to find α and ν or β, γ and δ to satisfy Equations 8.6 and 8.7 requires the summation of $\mathcal{O}(NM_I)$ or $\mathcal{O}(N^2 M_A)$ propensities, respectively.

The DM applied to CSA can thus be summarised as:

1. initialise agents and internal species and set *time* $= 0$.

```
2. iterate for sufficient steps or until all propensities are zero:
    a calculate all $NM_I + N^2M_A$ propensities
    b sum all $NM_I$ and $N^2M_A$ propensities to give $a_I$, $a_A$ and thus $a$
    c generate random number $r_1$ using $a$
    d calculate $\tau$ using Equation 7.9 and add to *time*
    e generate random number $r_2$ using $a$
    f if $r_2 \leq a_I$:
        (i) sum propensities of internal reactions in order until just greater than $r_2$
    g else if $r_2 > a_I$:
        (i) sum propensities of agent reactions in order until just greater than $r_2 - a_I$
    h execute the reaction reached in the previous step
```

Steps 2.c, 2.d, 2.e and 2.h are $\mathcal{O}(1)$, as with the DM. Step 2.(f)(i) has complexity $\mathcal{O}(NM_I)$ and Step 2.(g)(i) has complexity $\mathcal{O}(N^2M_A)$. Steps 2.a and 2.b have complexity $\mathcal{O}(NM_I) + \mathcal{O}(N^2M_A)$. Hence the overall complexity is $\mathcal{O}(NM_I) + \mathcal{O}(N^2M_A)$.

While it might be expected that the internal chemistry of N independent agents will require N times as much computation as a single system, the N^2 term may be seen as something of a surprise. The fact that each agent potentially constitutes a *unique agent specie* means that agent reactions effectively define a class of reactions containing all the possible pairs of agents. In systems where N, M_I and M_A are all large, this result constitutes a significant increase in complexity.

8.3.2 Details of the Method of Partial Propensities

The method of partial propensities (MPP) partitions the selection of one reaction from a choice of $(N^2-N)M_A+NM_I$ alternatives to a combination of three or four simpler choices. These choices correspond to (i) whether the reaction is internal or external; (ii) which agent is affected; (iii) which other agent is affected (if an agent reaction); (iv) which agent reaction is to be applied (if this is so) or (v) which internal reaction is to be applied. The total propensity is constructed from summations which can be divided into *partial propensities* which correspond to these choices. The individual propensities form the contents of a two dimensional matrix and a three dimensional matrix, corresponding to a_I and a_A, respectively. The partial propensities are sums of elements in the rows and columns of these matrices and are characterised as follows.

The choice of whether a reaction is internal or an agent reaction can be made from partial propensities a_I and a_A, as described in Section 8.3.1. Once this choice has been made, the choice of the first agent of an agent reaction (or the only agent if an internal reaction has been chosen) is made using N partial propensities $A_1, \ldots, A_N$, where

$$A_i \equiv \sum_{\substack{j=1 \\ j\neq i}}^{N} \sum_{\pi=1}^{M_A} a_{\pi ij} + \sum_{\mu=1}^{M_I} a_{\mu i} \tag{8.8}$$

The first agent, α, is chosen to be the minimum value which satisfies:

$$r_2 \leq \sum_{i=1}^{\alpha} A_i \tag{8.9}$$

If the reaction is an agent reaction, the choice of the second agent is made using N partial propensities $B, \ldots, B_N$, where

$$B_j \equiv \sum_{\substack{i=1 \\ i\neq j}}^{N} \sum_{\pi=1}^{M_A} a_{\pi ij} \tag{8.10}$$

The second agent, β, is chosen to be the minimum value which satisfies:

$$r_2 \leq \sum_{i=1}^{\beta} B_i \tag{8.11}$$

The choice of the agent reaction is made using M_A partial propensities $C_1, \ldots, C_{M_A}$, where

$$C_\pi \equiv \sum_{i=1}^{N} \sum_{\substack{j=1 \\ j\neq i}}^{N} a_{\pi ij} \tag{8.12}$$

Agent reaction γ is chosen to be the minimum value which satisfies:

$$r_2 \leq \sum_{i=1}^{\gamma} C_i \tag{8.13}$$

If the reaction was chosen to be internal, the choice of which one is made using M_I partial propensities $D_1, \ldots, D_{M_I}$, where

$$D_\mu \equiv \sum_{i=1}^{N} a_{\mu i} \tag{8.14}$$

Internal reaction ν is chosen to be the minimum value which satisfies:

$$r_2 \leq \sum_{i=1}^{\nu} D_i \tag{8.15}$$

There are therefore $3N + M_I + M_A$ partial propensities generated by Equations 8.8, 8.10, 8.12 and 8.14, plus a_I and a, which must all be updated after the execution of a reaction. This value is $O(N)$ times fewer than the total number of propensities.

The execution of an internal reaction affects a maximum of M_I other propensities (i.e., only those relating to reactions in the same agent), while the execution of an agent reaction affects the propensities of a maximum of $2(M_A N - 1 + M_I)$ agent reactions (i.e., the reactions of all the other agents in the colony reacting with the two involved in the current reaction, plus the internal reactions of the two changed agents.). If the set of internal reactions is based on biochemical pathways, the number of internal reactions affected by the execution of an internal reaction is likely to be much less than M_I, even when an agent contains multiple pathways (see Figures 5.17 and 7.3), however the cost of updating propensities, which is $\mathcal{O}(NM_A) + \mathcal{O}(M_I)$, still contains an $\mathcal{O}(M_I)$ term as a result of the effect of the agent reactions. This is nevertheless $\mathcal{O}(N)$ times less than the total number of propensities given in Equation 8.5.

Hence, the MPP makes an initial calculation of all the propensities and partial propensities, but thereafter updates only the propensities and partial propensities that change. By then selecting the next reaction to execute using the partial propensities, the overall gain in efficiency relative to the direct method applied to the CSA model is therefore $\mathcal{O}(N)$.

The method of partial propensities can be summarised as follows:

1. initialise agents and internal species and set *time* $= 0$.
2. ascertain and store internal reaction dependency and internal reaction dependency on agent reactions
3. calculate and store all $(N^2 - N)M_A + M_I$ propensities
4. sum propensities to generate and store $3N + M_I + M_A$ partial propensities plus a_I and a
5. iterate for sufficient steps or until all propensities are zero:
    a generate random number r_1 using a
    b calculate τ using Equation 7.9 and add to *time*
    c generate random number r_2 using a
    d sum partial propensities A_i to find α, the minimum value to satisfy Equation 8.9

e if $r_2 \leq a_I$:

(i) sum partial propensities D_i to find ν that is the minimum value that satisfies Equation 8.15

(ii) execute reaction ν in agent α

(iii) re-calculate propensities of internal reactions of agent α according to internal reaction dependency.

(iv) re-calculate the $M_A(N-1)$ agent reaction propensities involving agent α

f else if $r_2 > a_I$:

(i) sum partial propensities B_i to find β, the minimum value to satisfy Equation 8.11

(ii) sum partial propensities C_i to find γ, the minimum value to satisfy Equation 8.13

(iii) execute agent reaction γ between agents α and β

(iv) re-calculate internal reaction propensities for agents α and β according to internal reaction dependency on agent reaction γ.

(v) re-calculate the $2M_A(N-1)$ agent reaction propensities involving agents α and β

8.4 Results

While the theoretical basis for the MPP is clear, it is felt necessary to have a practical demonstration of its increased efficiency relative to benchmark algorithms. In particular, it is desirable to see evidence of the algorithms' comparative scaling with increased model size. This is a somewhat artificial requirement in terms of biological modelling, hence it is necessary to construct a set of biologically plausible artificial models which present an increasing load.

The unit of load is chosen to be the noise-resistant oscillator (NRO) of Figure 5.11, which although not a real biological model is nevertheless biologically inspired. Moreover, it has the characteristic of being 'multi-scale' in a plausibly biological way (see Figure 9.2) and has a suitable number of reactions. The more biological model of NF-κB oscillation (Figure 5.16) was also considered, however its larger size makes it unsuitable as the unit of model generation: the problem almost immediately becomes intractable to all algorithms and no clear trend is established.

In order to allow the widest possible valid comparison of algorithms and models, the NRO is used in a way that simulates the increased computational load of a CSA model using standard chemical reactions. Precisely, the NRO reactions are considered to be a set of 16 agent reactions which have an average dependency of approximately four (see Figure 7.3). For the simplest case, a benchmark model is created which contains two sets of identical NRO reactions. This then has double the dependency of the single model, since each reaction has one set of dependent reactions from the first instance and an identical set from the second. Such a model replicates the situation of a colony with two agents.

By thus creating models with increasing numbers of copies of the NRO model it is possible to simulate the algorithmic load presented by colonies with increasing numbers of agents: the dependency grows proportional to the number of copies but the number of different types of reaction does not grow.[1]

By adopting this strategy it is then possible to create a set of benchmark models which may be simulated on a variety of platforms for comparison.

[1]Note that by using a single set of species and dividing the original reaction rates by the number of 'agents', the observed behaviour of the species populations will be identical to a single instance of the model.

The results of a preliminary investigation are shown in Figure 8.1, where the ‘impossible’ case of one agent is included for reference (this made possible by virtue of the way the benchmarks are generated). In order to include the MPP it was necessary to create a version of the algorithm which takes standard chemical systems as input but can take advantage of the structure of the models. Algorithmic scaling of systems containing up to ten agents seems to conform to theory: the DM scales as $\mathcal{O}(N^2)$, the MPP scales as $\mathcal{O}(N)$ and the NRM scales as $\mathcal{O}(N \log N)$. The ODM initially scales at a similar rate to the MPP and NRM but eventually reverts to $\mathcal{O}(N^2)$. This is because the ODM is initially able to take advantage of the inherently logarithmic distribution of reactions but combining parallel instances of logarithmically distributed systems, as here, does not maintain a logarithmic distribution. See Figure 9.4.

In addition to the asymptotic scaling, the constant ratios between the algorithms are also evident in Figure 8.1. The relatively light implementational weight of the MPP and ODM make them approximately three times faster than the NRM at the left hand side of the graph. The DM, included for reference, is slow due to not having the benefit of the optimisations of the ODM and MPP. The small additional implementational complexity of the MPP relative to the ODM also seems evident on the left hand side of the figure: the MPP’s technique does not have an advantage for the case of one agent. Perhaps surprisingly, above ten agents the effects of low level memory caching (i.e., *not* disk caching and therefore not under the control of the operating system) affect the measured performance and the theoretical trends are lost. Instruction counting confirms that the theoretical trends continue, however it is not possible to overcome the limitations of the hardware. The memory effects eventually dominate the performance and since the memory usage of all the algorithms is $\mathcal{O}(N^2)$, this is the asymptotic scaling. Importantly, however, although the algorithms scale at the same rate they are separated by the constant ratio that exists when the memory effects become significant. Above ten agents the MPP maintains a factor of four performance lead over the NRM and the ODM is also marginally faster.

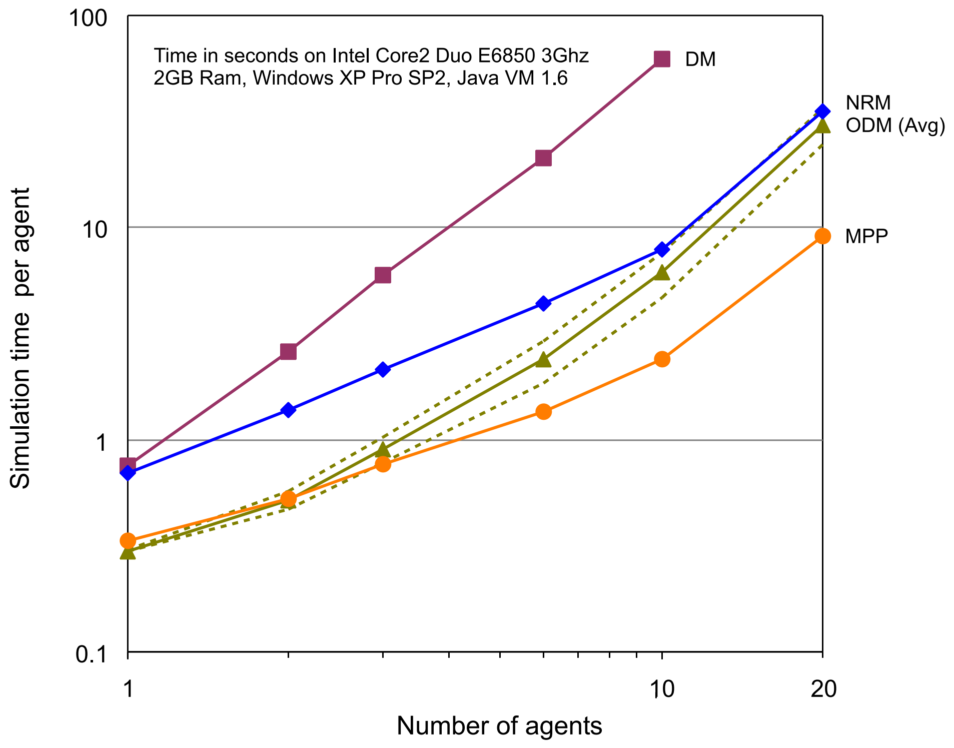


Figure 8.1: Algorithm scaling with increasing numbers of agents, where agents have 16 synchronisation reactions based on the noise-resistant oscillator of Figure 5.11. The dashed lines of the ODM trace are the maximum and minimum (without and with sorting, respectively) used to calculate the average trace (solid line). The algorithm performance below ten agents broadly agrees with theory. Above ten agents the effects of memory caching become visible in the traces of the MPP and NRM which thus diverge from theoretical predictions.

8.5 Discussion

The method of partial propensities makes an apparent $\mathcal{O}(N)$ saving over the direct method naïvely applied to a model of Colonies of Synchronizing Agents with N agents. While this is significant, it might have been conjectured that the Next Reaction Method (NRM) [31], which apparently offers $\mathcal{O}(\log(\textit{number of propensities}))$ complexity, will be trivially more efficient. The fallacy of this is that the NRM has complexity more accurately described as $\mathcal{O}(d\log(\textit{number of propensities}))$, where d is the average dependency of reactions. In biochemical systems d is usually only weakly related to M, perhaps $\mathcal{O}(\log M)$ or $\mathcal{O}(M^{1/k})$ for some $k > 1$). In contrast, the dependency of CSA agent reactions is $\mathcal{O}(NM_A)$, so the NRM applied to these has complexity $O(NM_A\log(NM_A))$, compared to $\mathcal{O}(NM_A)$ of the MPP. It seems that for agent reactions, at least, the method of partial propensities will outperform the benchmark NRM.

It has been observed by others [10, 13] that biochemical systems often have a ‘multiscale’ distribution and this is corroborated by the examples in this chapter (see Figures 9.2 and 9.1). If agent reactions (modelling, e.g., cell to cell communication) also have this distribution, then the method of partial propensities will likely be the optimal choice even when $\log_2 N < M_A$. Moreover, the MPP can take advantage of such multi-scale distributions by judicious ordering of the reactions. Note, however, that this has a lesser effect than in the ODM, for example, because the MPP is already optimised not to need such ordering.

It might be supposed that internal reactions, corresponding to intracellular pathways, do not have the same kind of combinatorial complexity of agent reactions and therefore can not benefit from the techniques of the MPP. Chapter 9 presents an algorithm which draws inspiration from the MPP (the method of *arbitrary* partial propensities - MAPP) which is able to consistently outperform the NRM when applied to test case models constructed in a similar way to the benchmark models described in Section 8.4. For the sake of presenting the main result relating to agent reactions, the selection of an internal reaction has so far been assumed to be via an optimised version of the direct method. The internal reactions may constitute significant complexity in themselves however, so it is conceivable that once the MPP has decided that the next reaction to execute is internal, its selection will be achieved using a more efficient algorithm such as the MAPP.

The method of partial propensities can also be applied to simulations where there is a significant amount of either deliberate or accidental combinatorial or repetitious reactions. For example, the technique has been previously used in a stochastic model of aggregation [57] and has application in modelling microarray experiments. The general conditions of applicability are that the system is large and consists of reactions which form a well defined n-dimensional non-sparse array of propensities. The requirement of being large is so that the gains can compensate the additional overhead. The requirement of the array not being sparse is so that the number of propensities is large with respect to the number of partial propensities such that a potential gain exists. In general, the number of reactions in a given order (i.e., a class of reactions having the same number of species used to calculate the propensity) must approximate S^n, where S is the number of species and n is the order of the reaction. It is observed here that under certain circumstances it might be advantageous to add a 'dummy species' to the set of species so that reactions of a lower order can be included in a higher order for greater overall efficiency. For example, $X_1 \rightarrow X_2$ might be re-written as $X_0 + X_1 \rightarrow X_2$, where X_0 would have a constant value of 1.

8.6 Conclusion

One of the goals of systems biology is to model and simulate large systems comprising smaller systems. When the smaller systems are themselves complex there is a potential of high combinatorial complexity. In solving such a problem specific to the implementation of Colonies of Synchronizing Agents, a simple method has been devised that has utility and wider application: the method of partial propensities. By partitioning the simulation algorithm along the lines which generate the complexity, it is possible to significantly ameliorate it. The methods presented in this chapter may also be relevant to other hierarchical stochastic formalisms, such as those based on process algebra or state charts, where complexity derives from combinatorial effects.

In prospect is the incorporation into the MPP of an efficient way to select internal reactions. One possible candidate is a related algorithm resented in Chapter 9: the method of arbitrary partial propensities.

Chapter 9

The Method of Arbitrary Partial Propensities

Chapter 5 presented a stochastic simulator implementing the models described in Chapters 3 and 4: Cyto-Sim. In Chapter 8 an algorithm was introduced which significantly ameliorates the computational cost of the stochastic simulation of Colonies of Synchronizing Agents, with particular regard to the cost of agent reactions. This chapter addresses the computational cost of stochastic simulations of standard chemically reacting systems and therefore has relevance to both Cyto-Sim and the internal reactions of the CSA model.

The Next Reaction Method (NRM) [31] is widely considered to be the fastest alternative to the benchmark Direct Method (DM) [32], due to apparent asymptotically superior performance, however it has been shown in [13] that an *Optimized* Direct Method (ODM) can improve on the NRM under certain circumstances[1]. Indeed, Chapter 8 has shown that the NRM is not necessarily the best choice under all circumstances, especially when reaction dependency is high, as in the case of CSA agent reactions. Investigations presented in this chapter confirm this view under more normal

[1]This work refers to the algorithm presented in [13] as *the* optimized direct method but notes that this term was coined earlier in [31] to refer to an algorithm which introduced some of the same optimizations.

conditions of chemically reacting systems but do not entirely corroborate the findings in [13].

Optimizations are applied to the NRM and DM and a re-assessment of their relative performances is made; theoretically and using new benchmark examples. The optimizations aim to remove implementational differences and reveal the core behaviour of the algorithms, thus allowing the possibility to account for the anomalies mentioned above. By doing this it is then possible to introduce the Method of Arbitrary Partial Propensities (MAPP) and show that it can outperform both the NRM and ODM in practical applications.

9.1 Introduction and motivation

Motivation has been given throughout this thesis for taking a discrete approach to modelling biochemical systems. Moreover, in Chapters 5 and 7 further motivation is given for taking a stochastic approach to simulation; the current most popular varieties of stochastic simulation algorithms having been outlined in Chapters 7 and 8.

The Next Reaction Method (NRM) [31] is based on the slower Gillespie method (the FRM), but its optimizations make it widely considered to be the most efficient formulation of an exact stochastic simulation algorithm. In addition to some useful lesser optimizations, the NRM stores the putative reaction times in a vertically ordered binary tree (an 'indexed priority queue'), thus allowing the selection of the next reaction (i.e. the root of the tree) in constant time. The computational complexity of the NRM is thus dominated by the maintenance of the ordered tree, which turns out to be proportional to the logarithm of the number of reactions.

A detailed analysis of the relative complexities of the NRM and DM was performed in [13], resulting in the proposal of an Optimized Direct Method

(ODM) claiming superiority over the NRM for certain large models. This claim is in part based on the idea that large biological models are likely to be 'multiscale', i.e., having a wide range of reaction speeds, and that the reactions can thus be ordered to minimize the average time it takes to find the reaction to execute. Some of the results in [13] are not borne out by the current work, however analysis presented here is able to provide plausible explanations for some of the apparent anomalies.

As a philosophical aside, it is noted that when the creation of a new algorithm results in a significant increase in performance, minor optimizations and small conceptual errors may at first be overlooked. In order to compare the relative merits of algorithms in an objective way, however, it is necessary to give each one considered the benefit of any optimizations which allow its core performance to be isolated. Specifically, in this chapter it is desired to compare the core concepts of the DM and NRM; in essence comparing the cost of searching unordered (or pre-ordered) data versus that of dynamically ordering the data, respectively.

A number of optimizations are therefore applied equally (where appropriate) to the DM and NRM. Where possible, code is shared between the implementations in order to remove implementational differences and isolate the comparison of their fundamental applicability. With this clarity of conceptual difference, it is then possible to introduce a new algorithm (the Method of Arbitrary Partial Propensities) and show that it improves on the benchmarks for a wide range of practical instances.

9.2 Computational cost of exact stochastic simulation algorithms

This section makes reference to the concepts, notation and equations of Section 7.3.1. In particular, it makes frequent reference to computational complexity in terms of the total number of reaction channels in the system, M.

9.2.1 The First Reaction Method

For the general case, with no a priori knowledge of the model, the complexity of an FRM step consists of the time to calculate all the putative times using Equation 7.11 ($\mathcal{O}(M)$), the time needed to select the fastest reaction ($\mathcal{O}(M)$) and the time needed to execute the chosen reaction ($\mathcal{O}(1)$). These operations have an overall asymptotic time complexity of $\mathcal{O}(M)$.

9.2.2 The Direct Method

The asymptotic complexity of the DM is shown in Section 8.2.1 to be $\mathcal{O}(M)$, which is the same as the FRM. Implementations of the DM are often faster than the FRM, however, because the DM requires only two samples of the uniform random variable in contrast to M samples for the FRM. As can be seen in Table 7.1, sampling random variables is computationally costly. Moreover, as was mentioned in Section 8.2.2, by storing the partial sums of propensities in an array, it is possible to find the reaction to execute in $\mathcal{O}(\log M)$ operations because the values are monotonic.

9.2.3 The Next Reaction Method

The NRM [31] refines the FRM. Recall that in the FRM the total propensity is not calculated; only the individual putative times of the reactions. This avoids the $\mathcal{O}(M)$ summation of the DM but incurs the $\mathcal{O}(M)$ cost of re-calculating new putative times after a reaction has been executed. The NRM initially constructs a static reaction dependency graph in order to avoid re-calculating times during the simulation which are guaranteed not to have changed. This reduces the complexity of this operation to

$\mathcal{O}(D)$, where D is the average dependency of the reaction scheme and is often much less than M in biological models. The FRM incurs a penalty relative to the DM in that it must select the fastest of a set of putative times which are unordered: $\mathcal{O}(M)$ versus the $\mathcal{O}(\log M)$ selection of the DM. To overcome this, the NRM therefore uses a vertically ordered binary tree (an *indexed priority queue*) to store the times, maintaining the invariant property that all nodes lower in the tree have longer times. In this way, choosing the shortest time is simply a matter of looking at the root node and is therefore $\mathcal{O}(1)$. The complexity of the NRM is thus shifted to maintaining the invariant property. Since the ordering is only vertical (i.e., nodes at the same depth are in arbitrary horizontal order), the cost of maintaining the tree is proportional to its height, which is $\lceil \log_2 M \rceil$. This is because although the executed reaction will always be at the root, its dependent reactions will in general be arbitrarily distributed in the tree. If it assumed that there are an average of D dependent reactions in a system, the complexity of ordering the tree after each step is $\mathcal{O}(D \log M)$.

Calculating putative times is computationally expensive, requiring both a random number and a logarithm (see Table 7.1 for relative costs). The NRM refines the FRM further by transforming the putative times from relative to absolute (i.e., relative to t_0, the initial time), thus allowing the dependent but unused putative reaction times to be re-used following a simple transformation (Equation 7.12). In this way, only a single random number and logarithm need be calculated at each step, which is $\mathcal{O}(1)$. While this does not change the order of the complexity, it represents a considerable saving over the FRM and also a moderate saving over the DM (trading a random number for a simpler arithmetical transformation).

The overall asymptotic complexity of the NRM is thus $\mathcal{O}(D\, log\, M)$.

9.2.4 The Optimized Direct Method

The computational cost of the ODM has been described in Section 8.2.2, however for the current treatment it is necessary to include the concepts of the average reaction dependency of the model, D, and the average search depth of the simulation, S.

The ODM uses a NRM-style reaction dependency graph to reduce the cost of re-calculating propensities in the DM from $\mathcal{O}(M)$ to $\mathcal{O}(D)$. The ODM avoids an $\mathcal{O}(M)$ summation and maintains a correct value of the total propensity by adding to it the difference between the new and old propensities as they are updated. This is therefore also $\mathcal{O}(D)$. Hence, by maintaining the total propensity independent of the summation, it is possible to reduce the complexity of summation to $\mathcal{O}(S)$, where S is the average summation depth of the simulation and is dependent on the ordering of the reactions and their relative frequency of execution. Since execution of the chosen reaction is $\mathcal{O}(1)$, as before, the overall complexity of the ODM is

$$\mathcal{O}(S) + \mathcal{O}(D) \tag{9.1}$$

, where $M \geq S > D$ for large models and S can be expected to be less than M because it is an average. For 'multiscale' models, i.e., those that have reactions which vary widely in frequency of execution, the authors of the ODM show that an optimum ordering of reactions can be found to reduce S, apparently allowing the ODM to equal and better the performance of the NRM in certain cases.

In essence, the ODM takes the view that many models are inherently multi-scale and can be statically ordered to achieve the performance that the NRM achieves by costly dynamic re-ordering. Examples of where this assertion is apparently true are the noise-resistant oscillator (Figure 5.11) and the model of NF-κB oscillation (Figure 5.16). Typical reaction frequencies for simulations of these models are plotted in Figures 9.1 and 9.2.

Comments on the ODM

Discerning in advance which models are multi-scale and the consequent optimal order of reactions is non-trivial, hence the authors advocate 'one or a few pre-simulations'. While this strategy seems to preclude the notion of experimenting with many different models, it should be remembered that a single stochastic simulation is but one possible trace through the solution space and results only attain validity as part of a *distribution*. Hence, it is not unreasonable to expect to make more than one simulation of a system.[2]

The authors' claim in [13] that 'in a large system, the reactions will undoubtedly be multiscale' is somewhat rash. Moreover, a model being multiscale *on average* does not specify how stable an ordering is over the course of the simulation. This variance should not unduly affect the ODM, since its performance is only related to the average, however it can have a significantly deleterious consequence on the NRM. A change of instantaneous order will result in the NRM engaging in costly re-ordering of the tree.

It has been found by experimentation by the present author that the large linear models constructed to demonstrate the superiority of the ODM over the NRM (see Figure 1 in [13]) achieve this result through somewhat artificial means. The initial conditions are such that only the first reaction in the 'chain' is initially enabled and the other reactions are only enabled slowly as the simulation progresses. The chosen maximum simulation time

[2]As an alternative, adopting an adaptive strategy is suggested which maintains a frequency count of reaction execution and re-orders them during the course of the simulation, say every $1000\, log_{10} M$ reaction steps.

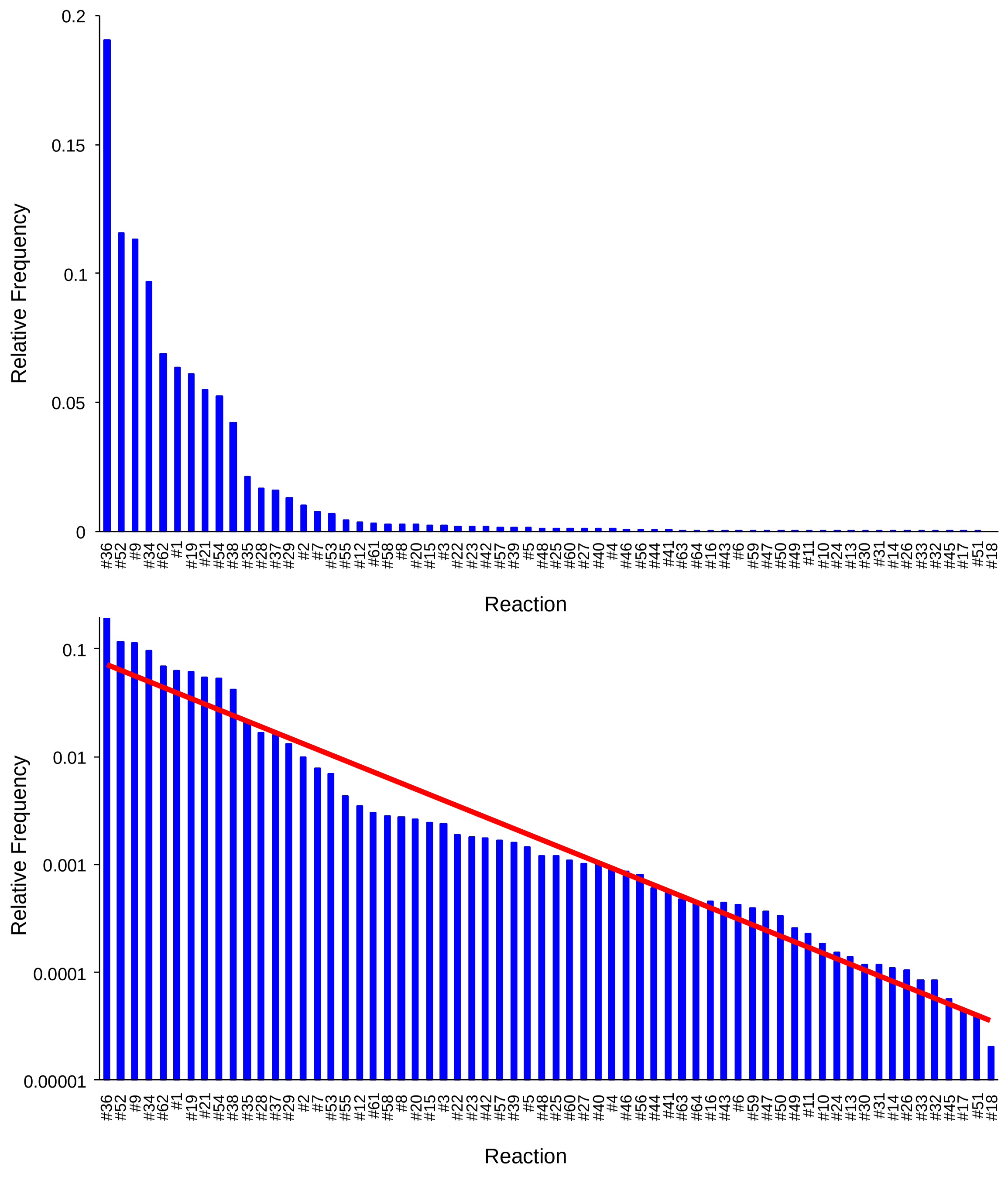


Figure 9.1: Relative reaction frequency in the simulation of a stochastic version of the model of NF-κB oscillation of Figure 5.16. The upper graph has a linear frequency scale and shows the wide range of reaction frequencies. The lower graph re-plots the data on a logarithmic scale and shows that the multi-scaledness is approximately logarithmic.

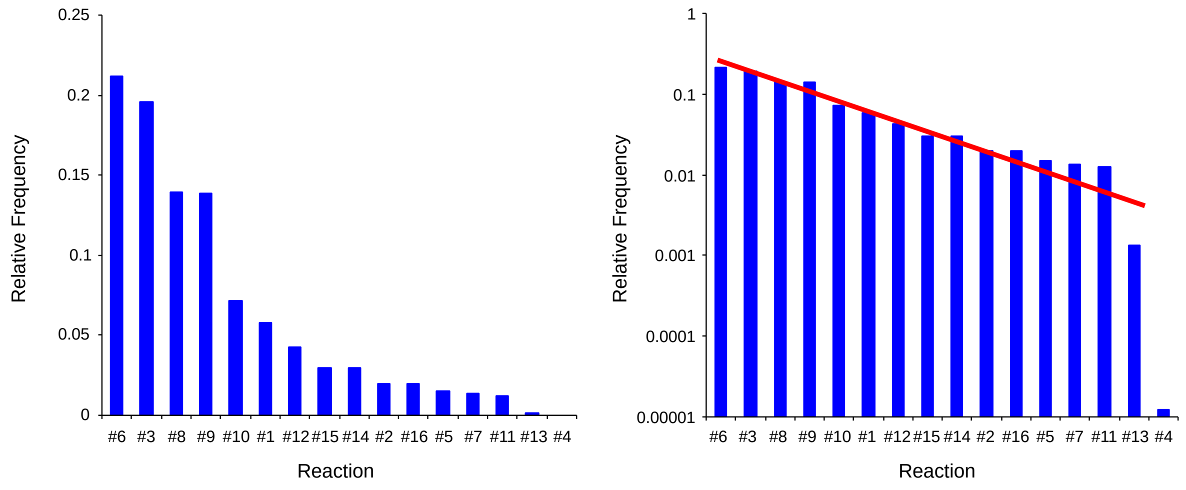


Figure 9.2: Relative reaction frequency in the simulation of the noise-resistant oscillator of Figure 5.11. The left graph has a linear frequency scale while the right graph re-plots the data on a logarithmic scale to show the approximately logarithmic distribution. For such an optimal ordering of reactions, the average search depth of the ODM corresponds to the third category, i.e., where the sum of relative frequency reaches 0.5.

in [13] results in many reactions (i.e., those at the end of the chain) not actually being used at all, hence the ODM does not have to venture too far into the list of reactions and can thus match the performance of the NRM. Since the reactions are ordered and the last reactions in the chain are unused, lengthening the chain by adding more unused reactions has minimal or no effect on the cost of searching for the reaction to execute. Note that this beneficial effect is inherent in the NRM because its re-ordering of the tree at every step automatically places unused reactions far from the root.

The reasons behind the increase in computational time of the ODM and NRM as the model size increases, as shown in Figure 1 of [13], are therefore unclear, since the additional reactions are not active and do not contribute. Given that the two algorithms apparently scale at the same rate, it is suspected that the cause of this phenomenon is some unforeseen common $\mathcal{O}(M)$ task, such as storing the system state. Evidence for this supposition is that in Figure 1 of [13], the DM applied to the same linear models reveals that the ODM is slightly more than twice as fast across the range of values plotted; an improvement which is consistent with the known reduction of the reaction selection from $\mathcal{O}(M)$ to $\mathcal{O}(1)$ given some other $\mathcal{O}(M)$ cost. A possible amelioration is suggested in Section 9.3.1.

9.3 Optimizations

In this section more detailed analysis of complexity is performed and it is shown that the NRM outperforms the DM with all but trivial models. It is further shown that, contrary to the conclusions of [13], the NRM also outperforms the ODM on non-trivial models. Finally, the Method of Arbitrary Partial Propensities (MAPP) is presented and shown to outperform the NRM on a wide range of biologically plausible instances.

In algorithmic research the goal is usually to reduce the *order* of complexity of an algorithm and constants are often ignored due to being asymptotically unimportant. Depending on the application however, such constants can be highly significant in practice: a division by two of running time is usually considered worth having. In a practical context, pursuing a low asymptotic complexity at the expense of a greater complexity in the useful range of an algorithm is clearly absurd. Hence, in Table 7.1 the relative time cost of a range of computational operations is defined in order to better understand the performance of the algorithms.

9.3.1 Optimization 1

The number of reactions in a system is often related in an approximately linear way to the number of reactants: a reactant usually has one or more reactions in which it is used ‘constructively’, i.e., linked in some way to another reactant, plus perhaps one or more reactions in which it is degraded. In large systems, however, a reactant is unlikely to react with the majority of the other reactants. Hence, the number of reactants can be considered $\mathcal{O}(M)$. While the Gillespie algorithm limits the total number of reactants of any reaction to a maximum of two or three, on the basis that the collision of more than two molecules at any instant is extremely unlikely [32, 33, 35], it imposes no theoretical bound on the number or nature of products. Hence, a general purpose implementation must allow for reactions with an arbitrary number and combination of products. A naïve implementation might then have an array of product species for each reaction, where the size of the array is the total number of species: $\mathcal{O}(M)$ by the previous assumption. This array contains many zeros, but in order to execute a reaction and update the species, it would be necessary to perform a mostly redundant iteration through the product array. This effect can be repeated when the simulation time-course is updated or output: by writing out every species instead of only those which have changed, the algorithm incurs an additional $\mathcal{O}(M)$ cost.

The proposed optimization is therefore to have reactions that use a linked list of products which will usually be much shorter than the full list of species (typically less than four). The complexity of the execution of a reaction can then be considered $\mathcal{O}(1)$, since this number is not generally linked to the size of the model. In a similar way, the simulation traces must be recorded as a series of changes (or a series of reactions) rather than a series of states.

It is noted there that this problem is common to all algorithms and may be unavoidable if the data need to be represented as a series of states for graph plotting or other post-processing. Not all the simulated points are necessarily plotted, however, hence when the traces are sampled at fixed time intervals during the simulation, for example, the cost of generating states from changes in state becomes $\mathcal{O}(1)$ relative to M.

9.3.2 Optimization 2

The major computational complexity of the NRM is contained in maintaining the invariant property of the vertically ordered tree. When a value of a node in the tree is altered by the execution of a reaction, the NRM uses an update algorithm which applies pairwise swaps of nodes until the vertical ordering is restored. In [31] the authors describe a SWAP algorithm which 'swaps the tree nodes ... and updates the index structure'. Two arbitrary adjacent nodes in the tree have a total of ten internal and external unidirectional links which must be modified when they are swapped. This is because each node must know its parent and its children (three links) and its parent and children must also know it (three more links). This gives six unidirectional links per node to be swapped, with two links being common, hence ten in total. Swapping the nodes will therefore involve ten variable swaps, requiring thirty individual variable assignments. It is noted, however, that the tree structure itself is constant and it is only the *contents* of the nodes that need to be swapped.

The proposed optimization is therefore to have a static tree containing pointers to reaction structures which hold all the information about the reaction (reactants, products, rate, putative time etc.). When a reaction is executed by the NRM, the putative times of its dependent reactions are updated and their positions in the tree are also updated accordingly. It is therefore necessary for a reaction to keep a record of its position in the tree, hence an optimized swap algorithm involves swapping a total of two variables (a link from the tree node to the reaction and one from the reaction to the tree node), which require a total of six individual variable assignments. This optimization thus constitutes a local saving of a factor of five.

9.3.3 Optimization 3

A further optimization is to re-order the tree without using pair-wise swapping ('bubbling'), which is not an efficient way of moving a reaction to a new position (recall the inefficiency of bubble sort). Assuming an updated reaction has a shorter time than its parent, the optimized technique is to shift down by one node all the progenitors of the current reaction which have longer times and to move the current reaction to the position of its progenitor having the shortest time greater than it. This forms a kind of rotational shift where each movement is an assignment of two variables. The case of the current reaction having a longer time than one of its children works in an analogous way: a rotational shift in the opposite direction.

A tree that requires s swaps to be re-ordered, using $6s$ variable assignments, can thus be re-ordered by $s/2 + 1$ shifts using only $2s + 2$ variable assignments. An improvement in all cases is therefore guaranteed, with a local factor of improvement of between 1.5 and 3.

9.4 The Method of Arbitrary Partial Propensities

The Method of Arbitrary Partial Propensities (MAPP) is designed to overcome the drawbacks of the NRM and ODM and give good performance for a range of models on the first run, without pre-ordering (although adaptive ordering is beneficial). It is a descendent of the DM, in that it sums propensities and selects a reaction using a uniformly distributed random number, however by employing a unique data structure and some of the optimizations described above it is able to outperform previous algorithms.

The NRM aims to achieve asymptotic $\mathcal{O}(log\, M)$ performance by dynamically arranging the reactions in an ordered tree, but suffers a penalty with models that are not large due to the overhead that this creates. The ODM seeks to overcome this limitation by statically ordering the reactions to minimise the search time, but this is only successful for models which have an overall multiscale property. In fact, both the NRM and ODM rely on the multiscale property: the advantage of the NRM is limited in the case that all the reactions have similar speeds because the reactions at the bottom of the tree must then be brought to the top more frequently.

9.4.1 Details of the MAPP

The MAPP uses a static tree-like data structure of height two, where the leaf nodes of the tree each contain an array of reaction propensities. The single parent node (the parent *layer*) also contains an array, each element of which is associated with a different leaf (or child) node and contains the sum of the propensities in the corresponding child. The parent array has length $\left\lceil \sqrt{M} \right\rceil$ and each of its $\left\lceil \sqrt{M} \right\rceil$ children contain arrays of length $\left\lceil M / \left\lceil \sqrt{M} \right\rceil \right\rceil$, such that the total number of array elements in the child layer is $\geq M$. Simply put, the child layer contains all the individual reaction propensities and the parent layer contains their partial sums - the *partial propensities*.[3] These sums are therefore *arbitrary* partial propensities, since unlike the case of the method of partial propensities presented in Chapter 8, they do not correspond to any feature of the model.

The sum of the elements of either layer is the total propensity. When the square root of the number of reactions is not an integer, the total number of array elements in the child layer will be greater than the number of reactions. In this case the unused array elements contain zero, although the value is arbitrary since the algorithm will not consider them. Figure 9.3 illustrates the two layer data structure for $M = 11$.

The MAPP algorithm is initialized by calculating all the propensities

[3]This is reminiscent of the Optimized Direct Method proposed in the Appendix of [31], which uses a binary tree to store propensities. The crucial difference is that the MAPP uses a *constant* height data structure, such that the cost of updating the propensities is $\mathcal{O}(1)$.

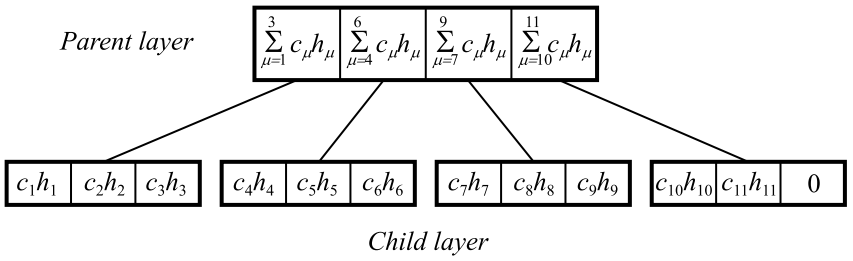

Parent layer
$\sum_{\mu=1}^{3} c_\mu h_\mu$
$\sum_{\mu=4}^{6} c_\mu h_\mu$
$\sum_{\mu=7}^{9} c_\mu h_\mu$
$\sum_{\mu=10}^{11} c_\mu h_\mu$
c_1h_1 c_2h_2 c_3h_3
c_4h_4 c_5h_5 c_6h_6
c_7h_7 c_8h_8 c_9h_9
$c_{10}h_{10}$ $c_{11}h_{11}$ 0
Child layer

Figure 9.3: An example of the two layer data structure used by the MAPP for $M = 11$. Each child node contains an array of reaction propensities. The parent node contains an array of partial sums of propensities which correspond to the children.

and summing them according to the description above and as illustrated in Figure 9.3. The total propensity is also calculated and, as with the NRM and ODM, a static reaction dependency graph is constructed to avoid updating propensities unnecessarily during the simulation. After the execution of a reaction during the simulation, individual propensities in the child layer and corresponding partial propensities in the parent layer are updated according to the dependency graph.

Selecting a reaction to execute is a two stage process. As with the DM, a value is sampled from a uniform random number in the range between zero and the total propensity. This has complexity $\mathcal{O}(1)$. The partial propensities are then summed in sequence to find the interval, and therefore the child, which contains the reaction to execute. Since there are $\mathcal{O}(\sqrt{M})$ children, the average length of the search (assuming random ordering) will be ca.$\sqrt{M}/2$, which is $\mathcal{O}(\sqrt{M})$. Having found the child, the same random sample also specifies the position within the child's subinterval which corresponds to the reaction to be executed.[4] The child's propensities are summed, in a similar way to that described above, until the reaction is found. There are $\mathcal{O}(\sqrt{M})$ propensities per child, so the average length of the search (again assuming arbitrary ordering) will be ca.$\sqrt{M}/2$, which is

[4]The random number generator is assumed to have sufficient precision for this to be valid, although this is not an assumption peculiar to the MAPP. See Section 7.3.4.

$\mathcal{O}(\sqrt{M})$. Hence, selecting a reaction has overall complexity $\mathcal{O}(\sqrt{M})$.

For every propensity updated, a partial propensity and the total propensity must also be updated. Assuming that the model has average dependency D, updating the propensities and the partial propensities requires on average $3D$ operations, which has complexity $\mathcal{O}(D)$. Calculating the incremental time, using Equation 7.9, is $\mathcal{O}(1)$, as before. Hence, the overall asymptotic time complexity of the MAPP is

$$\mathcal{O}(D) + \mathcal{O}(\sqrt{M}) \tag{9.2}$$

.

9.4.2 Comparison of the MAPP, NRM and ODM

To recap, the three algorithms have the following asymptotic complexities. NRM:
$\mathcal{O}(D \log M)$; ODM: $\mathcal{O}(S) + \mathcal{O}(D)$ and MAPP: $\mathcal{O}(D) + \mathcal{O}(\sqrt{M})$. M is the number of reactions in the model, D is the average dependency of the reactions in the model, S is the average search depth and, in general for a large model, $D < S < M$. Note that S is strictly less than M in the case of a model where the average number of reactions used during the simulation is > 1. For an ordered model, $S \leq M/2$.

For unordered models or models which do not benefit from ordering, $\mathcal{O}(S) = \mathcal{O}(M)$, hence the MAPP will be faster than the ODM. The relative performance of these two algorithms with multiscale models will depend on the ratio of S to $\sqrt{M}$, i.e., exactly how multiscale it is. By comparison of Equations 9.1 and 9.2, the model must satisfy the condition $S < \sqrt{M}$ for the ODM to outperform the MAPP. Note that although the MAPP is designed to achieve good performance without ordering, its performance will also benefit from ordering the reactions. The NRM re-orders the reactions at each step during the course of the simulation, so does not benefit from pre-ordering.

For an ordered multiscale model where $S \leq \sqrt{M}$ but $S \not\ll \sqrt{M}$, the asymptotic complexity of the MAPP is $\mathcal{O}(D) + \mathcal{O}(\sqrt{M})$ because the selection of the child node will be $\mathcal{O}(1)$ (the first child is almost always chosen since, by definition, the average search depth is less than the partial propensity of the first child), but the selection of the reaction within the child will not necessarily be speeded up. If $S \ll \sqrt{M}$, the complexity reduces to $\mathcal{O}(D) + \mathcal{O}(S/\sqrt{M}))$ because now the choice of the reaction within the child is influenced by the ordering. The MAPP will in fact outperform the ODM on all models except those very rare cases which satisfy $D \approx S \lll \sqrt{M}$, i.e., those which are *extremely* multiscale and are heavily dominated by very few reactions. In such cases the increased overhead of the MAPP relative to the ODM becomes significant.

In comparing the performances of NRM and MAPP applied to real model instances, the asymptotic complexities given above are not very informative, even when the models are large. Since it has been observed that average reaction dependency, D, in biological models is generally much less than M, it might be supposed that the NRM will outperform the MAPP because $\log M$ will tend to be less than $\sqrt{M}$. In real biological models D scales weakly with M, perhaps somewhere between $\mathcal{O}(\log M)$ and $\mathcal{O}(\sqrt{M})$, and the logarithmic term of the NRM is *multiplied* by D. The relative performances thus depend on the model being simulated, its stochasticity and the constants which have so far been omitted from the asymptotic complexities. With high dependency, such as in the case of the Colonies of Synchronising Agents model with agent reactions, the NRM has been shown in Chapter 8 to be less efficient than the ODM. With low dependency and the typical dependency found in single instances of the noise resistant oscillator and NF-κB models, experimental results show that the MAPP is generally faster, but the differences are smaller (see

Figures 9.5, 9.6 and 9.7).

9.4.3 Generalization of the MAPP

So far a data structure has been considered for the MAPP with only two layers (Figure 9.3), since this is already efficient, but the concept of the MAPP can be generalised to include an arbitrary number of layers. In general, increasing the number of layers has the effect of reducing the order of complexity of the algorithm because for n layers, each node contains $\sqrt[n]{M}$ reactions. The cost of reaction selection is thus reduced and since n is a constant it can be increased arbitrarily without appearing in the asymptotic complexity. The effect of increasing n is felt in the cost of updating the partial propensities: a partial propensity on each layer must be updated for every reaction propensity that changes. n therefore manifests itself as a multiplier of the average reaction dependency, D.

The minimum number of layers is one, which effectively reduces the MAPP to the ODM: update cost is $\mathcal{O}(D)$ and selection by summation is $\mathcal{O}(S)$, where $S \leq M$ for unordered reactions and $S \leq M/2$ when they are ordered.

The maximum number of layers is $\lceil log_2(M+1)\rceil$, which produces the binary tree structure suggested in the Appendix of [31] (the original optimized direct method). Here the cost of updating is proportional to $D \lceil \log_2 (M+1) \rceil$ and the cost of selection is proportional to $\lceil \log_2 (M+1) \rceil$ (no summation is performed, just a choice between the two alternatives). It can be seen that the bulk of the cost has shifted from selection to update. The apparent $\mathcal{O}(D \log M)$ performance might seem ideal, however in this case the algorithm does not benefit from ordering the reactions and can not take advantage of multiscale models, as can the other algorithms considered here. Moreover, the hidden constants and increased memory requirement (there are as many partial propensities as propensities) make this configuration less than optimal for practical purposes.

An approximate optimum number of layers is proposed to be

$$k(M) = \log_2(M)/\log_2\log_2(M) \tag{9.3}$$

such that each node in the tree contains an array of $\log_2 M$ partial propensities. In this way the summation on each layer of the tree is $\mathcal{O}(\log M)$. The cost of updating the tree is then $\mathcal{O}(kD)$ and the cost of selection is $\mathcal{O}(k \log M)$. This is reminiscent of the complexity of the NRM ($\mathcal{O}(D) + \mathcal{O}(D \log_2 M)$), noting that k is also a function of M but one which grows very slowly. In practice, for $M = 16$, $k = 2$; for $M = 1000$, $k \approx 3$; for $M = 2^{16}$, $k = 4$. Hence, for models having $D \approx 4$, for example, the MAPP might be expected to outperform the NRM on models up to 2^{16} reactions by choosing the number of layers according to Equation 9.3.

9.5 Results

In order to verify the theoretical treatment of the algorithms it is necessary to construct benchmark model instances which demonstrate their asymptotic scaling and relative performance. Since the motivation of the present work is the simulation of large biological systems, it is desirable that the benchmarks be biological in nature. Thus, the models of the noise-resistant oscillator (NRO) of Figure 5.11 and the oscillatory model of NF-κB (Figure 5.16) were used as the basis to generate models with increasing complexity. Although both oscillatory in nature, the models have distinct characteristics: the NRO has few reactions, average dependency around four, continuous oscillations, few molecules and is very stochastic; the NF-κB model is much larger, has average dependency around nine, damped oscillations, large numbers of molecules and is not very stochastic.

Benchmark models were therefore constructed using multiple *independent* instances of the NRO and NF-κB pathways. That is, multiple instances of either the NRO or NF-κB models were included in a single model file, each instance having species with an appended index and a unique set of corresponding reactions.

The average dependency within a network of reactions has a significant effect on the performance of stochastic algorithms used to simulate them, as was shown in Chapter 8 in relation to the CSA model. It is not clear how average reaction dependency scales with biological network size so this was treated as an external parameter. Since the constituent instances of the benchmark models are independent, the overall average reaction dependency in the multiple models is the same as the individual case.

Having thus created a set of models with average dependency of (approximately) four and nine, respectively for NRO and NF-κB, a further entirely artificial set was created with average dependency equal to one. This was achieved by creating models containing multiple parallel instances of reactions of the form

$$S_i \to S_i' \tag{9.4}$$

where for all $i = 1 \ldots M$, $S_i \neq S_i'$, the initial number of molecules of S_i is 1000 and the simulation was run for $1000M$ steps.

The results of preliminary investigations using the benchmark instances described above are shown in Figures 9.5, 9.6 and 9.7. Figure 9.5 serves to broadly categorize the models in accordance with theory: the DM is consistently $\mathcal{O}(M)$; the ODM is initially good but inevitably becomes $\mathcal{O}(M)$; the MAPP and NRM both have significantly better performance than both the DM and ODM, with the NRM slightly ahead for the largest models. With the implemented optimizations and in the unrealistic (trivial) case of average reaction dependency equal to one, the NRM needs to perform only one swap to re-order its tree data structure. Above 3000 reactions the performance of the NRM and MAPP appear to diverge from what is expected from theory. More evident in Figures 9.6 and 9.7, this is the result low level memory caching limitations.

For the biological models, below 10 NF-κB systems (below 40 systems in the case of the NRO), the algorithms seem to scale in accordance with theory. The DM is the reference with $\mathcal{O}(M)$ scaling. The ODM, NRM and MAPP are similarly efficient for small instance size, but the ODM progressively drifts towards $\mathcal{O}(M)$ as larger instance sizes have reaction distributions which are less logarithmic. In fact, the average search depth of the ODM is proportional to the number of parallel instances of the models in the way shown in Figure 9.4. The NRM and MAPP scale similarly and at similar levels in the case of the NF-κB models. Although the NRM and MAPP also scale similarly in the case of the NRO models, the increased stochasticity gives the MAPP an advantage: the NRM has to constantly re-order relatively deeper parts of the reaction tree in comparison to the almost deterministic NF-κB system.

Above 10 (40 for NRO) systems, the performance of the NRM and MAPP algorithm increasingly reflect the effects of low level memory caching (i.e., *not* disk caching). Whereas the times of the NRM might be expected to flatten relative to the MAPP and eventually drop below it (i.e., $\mathcal{O}(\log M)$ versus $\mathcal{O}(\sqrt{M})$), both rise with increasing memory use. Importantly, when these effects start to become significant, the inherently lower memory requirements of the MAPP give it a lead which it maintains thereafter.

A final point to observe is the performance of an ODE solver (lsoda in Scilab 4.0) relative to the stochastic simulation algorithms. In the case of the NRO models, all the stochastic simulation algorithms, including the DM, outperform the deterministic simulation. This is because the NRO models have low copy numbers of molecules and the stochastic simulation algorithms have relatively little to do. Moreover, the waveforms contain a lot of high frequencies (in part resulting from the flat bottoms) so the ODE solver has to use small time steps. By comparison, the NF-κB mod-

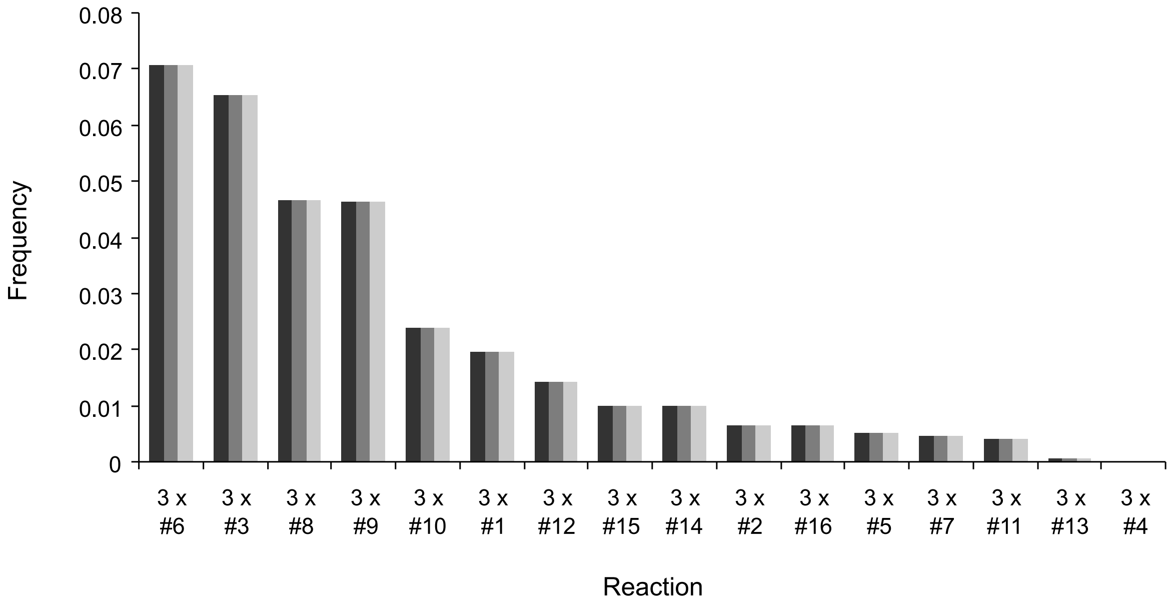


Figure 9.4: The reaction frequency distribution of three instances of the noise resistant oscillator of Figure 5.11. The shape of the distribution remains apparently similar to that of a single instance of the model (Figure 9.2), however each category now corresponds to three reactions. With this optimal ordering the average search depth of the ODM once again corresponds to the third category in the figure, however the 0.5 total relative frequency now falls in the eighth reaction. In general, the average search depth will be proportional to the number of instances.

els have high copy numbers of molecules and smooth waveforms. While the stochastic simulation algorithms have to simulate all the molecular interactions, the ODE solver can take advantage of the smoothness and use long time steps. In the case of the NF-κB models, the ODE solver therefore consistently outperforms the stochastic simulation algorithms in the range of model sizes considered. The trend of the ODE curve seems to be tending towards $\mathcal{O}(M)$, in line with the DM and ODM, presumably as a result of memory requirements. It seems plausible from this trend that larger models will favour the performance of the MAPP and NRM.

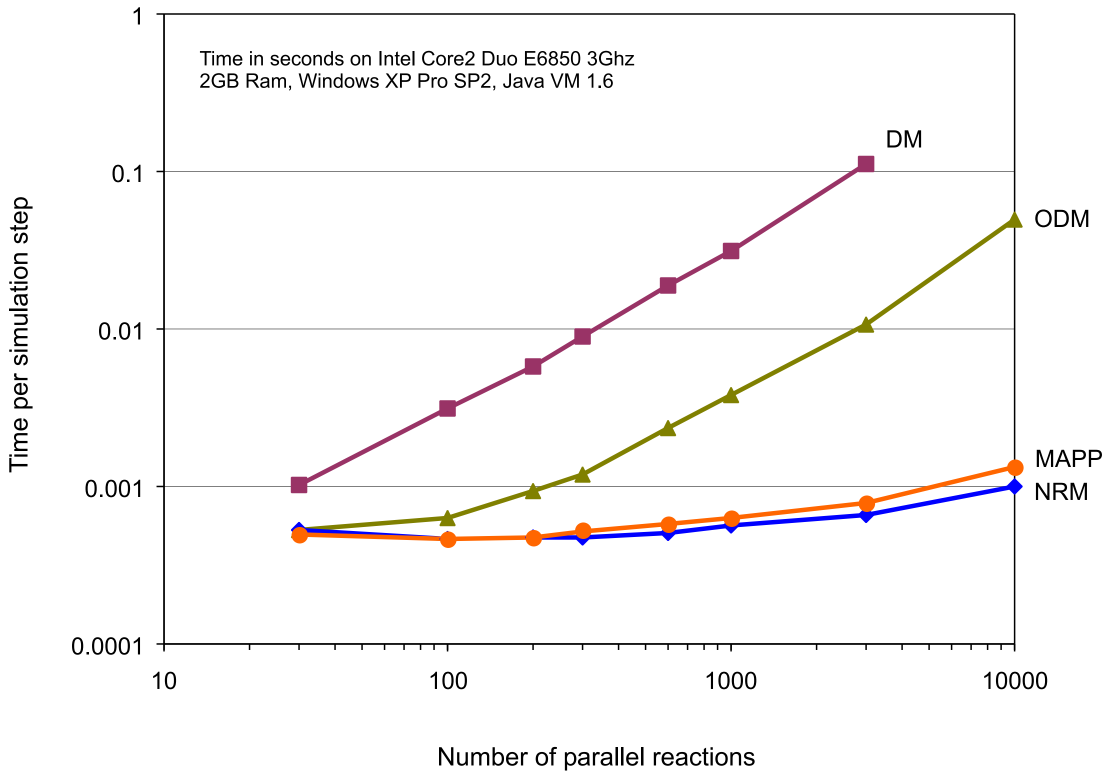


Figure 9.5: Algorithm scaling with increasing numbers of parallel reactions having average dependency 1.

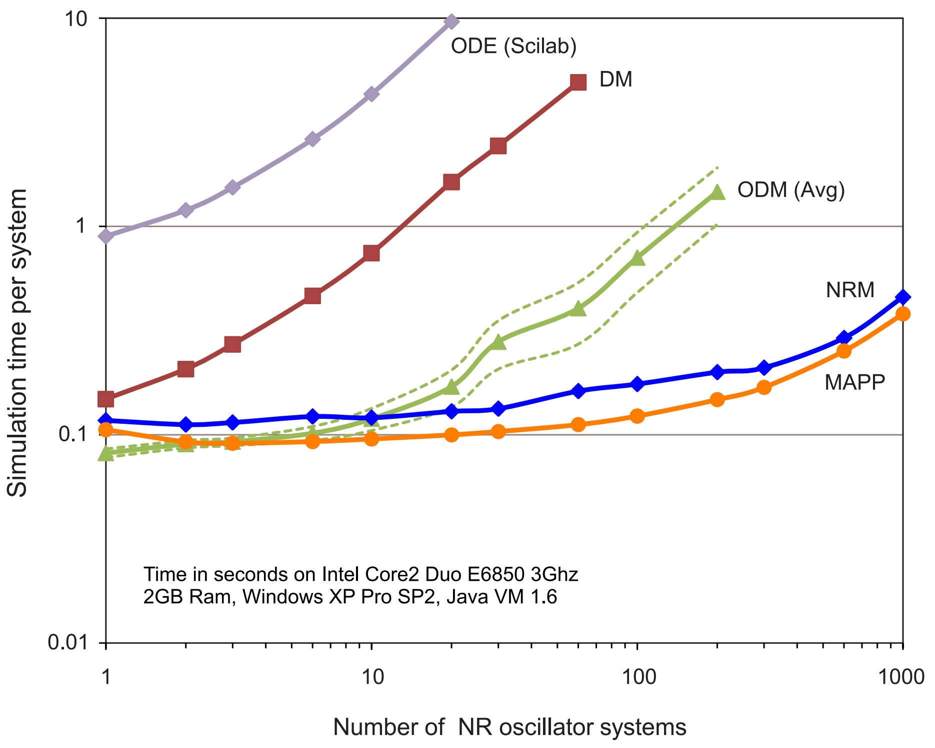


Figure 9.6: Algorithm scaling with increasing numbers of reactions, based on multiple parallel instances of the noise resistant oscillator of Figure 5.11. The dashed lines of the ODM trace are the maximum and minimum values (from unsorted and sorted reactions, respectively) used to calculate the average trace (solid line). The MAPP outperforms the NRM below 300 noise-resistant oscillator systems due to its inherently low computational overhead. Above 300 systems, corresponding to 4800 reactions, low level memory caching counteracts the theoretical $\mathcal{O}(D \log M)$ performance of the NRM and the relatively light computational footprint of the MAPP allows it to maintain supremacy. Note that the deterministic simulation (using lsoda via Scilab 4.0) is slower than any of the stochastic simulations. This is because the stochastic model has low copy numbers of molecules (hence requiring less computation) and the deterministic traces have sharp changes which require small time steps (hence requiring more computation).

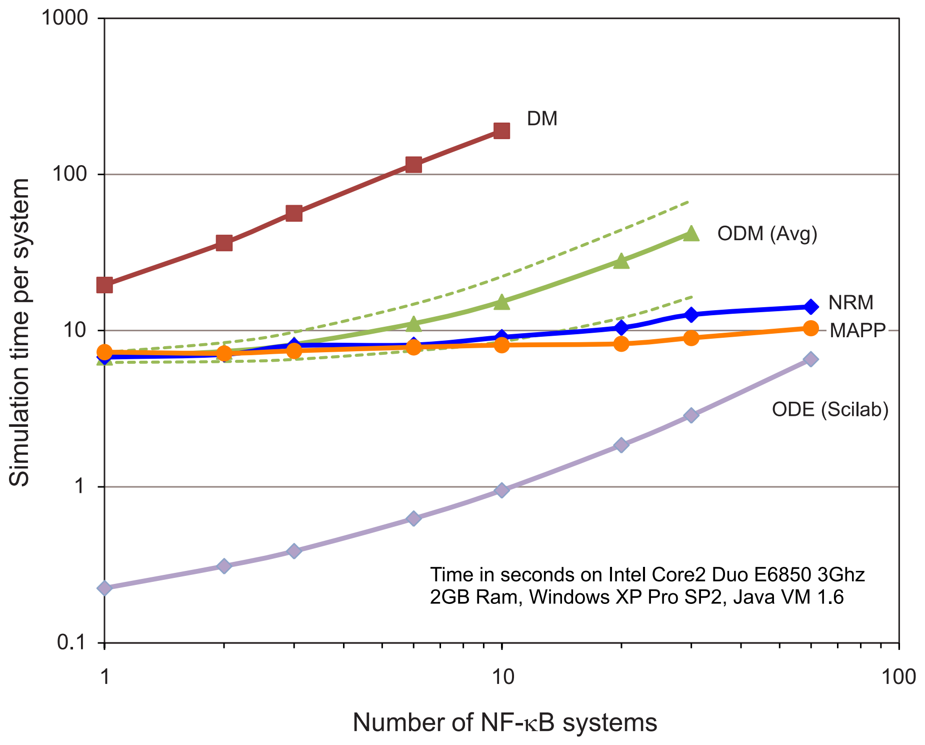


Figure 9.7: Algorithm scaling with increasing numbers of reactions, based on multiple parallel instances of the NF-κB oscillatory model of Figure 5.16. The dashed lines of the ODM trace are the maxima and minima (corresponding to unsorted and unsorted reactions, respectively) used to calculate the average (solid trace). As in Figure 9.6, memory caching affects the reported performance of the algorithms when they are used with very large numbers of reactions. The MAPP nevertheless manages to maintain its performance edge due to its inherently low computational overhead. Note that the model has large copy number of molecules and the trajectories are relatively smooth: the ODE solver is therefore able to outperform all the stochastic algorithms.

9.6 Conclusion

By taking advantage of the observation that reaction dependency in chemically reacting systems tends to be low, the Next Reaction Method [31] (NRM) is able to improve on the performance of both the Direct Method (DM) and First Reaction Method (FRM) of [32, 33]. These latter apparently make no assumptions about the system and feature few optimizations. The NRM uses a tree data structure and a dynamic technique to order the reactions to thus allows an efficient selection of the next reaction to be executed. For very large systems, the cost of dynamically ordering the active reactions is offset by not having to search through all the inactive ones.

The authors of [13] made a further observation that some systems are inherently multiscale, containing reactions which are used significantly more frequently than others during the course of a simulation. By applying an optimal static ordering to these systems it is therefore possible to take advantage of this property using the simple linear storage structure of the DM and thus create an Optimised Direct Method (ODM). For certain systems, the sub-optimal performance of linear storage is more than offset by the elimination of the costly dynamic ordering of the NRM.

By minimising implementational differences and applying additional optimizations, it has been possible in this chapter to lay bare the core performance of the competing algorithms mentioned above. In doing so, it has also been possible to devise an algorithm which apparently more closely suits the models of current interest: the Method of Arbitrary Partial Propensities (MAPP). This has been achieved by a balanced strategy which acknowledges the existence of multiscale systems and the benefits of tree structures for data retrieval: the algorithm includes both, without completely relying on either. This is clearly a practical approach.

There is scope for improvement, but the law of diminishing returns prevails. Re-ordering of the reactions between runs of multiple simulation runs has already been implemented and is computationally cheap, however it has been found that the gains are minimal. A further gain may be achieved by adaptive re-ordering during simulation runs: not at every step like the NRM but on a timescale that reflects a slower trend of reaction dependency. It is noted, however, that in the worst case there may not be such a trend.

Chapter 10

Fourier analysis of stochastic simulations

Some the work presented in this chapter was originally published in the poster

S. Sedwards and A. Csikasz-Nagy (2008) Characterization of mutants by Fourier analysis of stochastic simulations of yeast cell cycle, 9th International Conference of Systems Biology, Göteborg, Sweden.

The choice of a discrete paradigm to represent chemically reacting systems, such as those presented in Chapters 3 to 6, has the potential to investigate biology in the most precise way. For example, using the techniques of model checking (e.g., [23]) it is possible to define exact logical properties (or exact probabilities in the case of probabilistic model checking [56, 89]) of a given system. In the case of paradigms which have the chemical master equation (CME) at their root, it is also conceivable to solve the CME to give a description to arbitrary precision of the distribution of a species at a given time point. It is often the case, however, that non-trivial biological systems have an intractably large state space, rendering model checking impractical. Solving the master equation also has drawbacks, especially in the case of systems which exhibit oscillatory behaviour: the oscillations are not necessarily evident in the distribution (e.g., see Figure 10.2).

Motivated by the above, an analysis technique based on Fourier transformation is presented. By transforming the time series produced by stochastic simulation into the frequency domain, it is possible to characterise the behaviour of both oscillatory and non-oscillatory systems. Moreover, this technique reveals more information about the system than is possible to (easily) extract from deterministic simulations.

In what follows it is assumed that the reader is familiar with the notions of stochastic simulation presented in Chapter 7. It is also assumed that the reader has a basic familiarity with analysis and statistics. While no deep theoretical treatment is given, some of the presented methods involve advanced concepts not likely to be found in introductory textbooks. [54] is a good reference, but otherwise it is recommended that the reader perform an Internet search on specific topics of interest.

10.1 Average behaviour

While a single stochastic simulation run often has an apparent characteristic behaviour, such behaviour may have little statistical significance relative to the total space of possible trajectories. In order to draw general conclusions about the model it is thus necessary to characterise some kind of average trajectory, as might be realised by the deterministic simulation of ordinary differential equations. A simplistic approach to averaging time series of multiple stochastic simulation runs does not produce an average trajectory, however: the instantaneous phase shift between simulations is a random variable, hence oscillatory behaviour of summed time series will tend to disappear with increasing numbers of cycles. An example of this effect is shown in Figure 10.1. While there may still be evident oscillation, this is not in general the case and information will be lost to the averaging process.

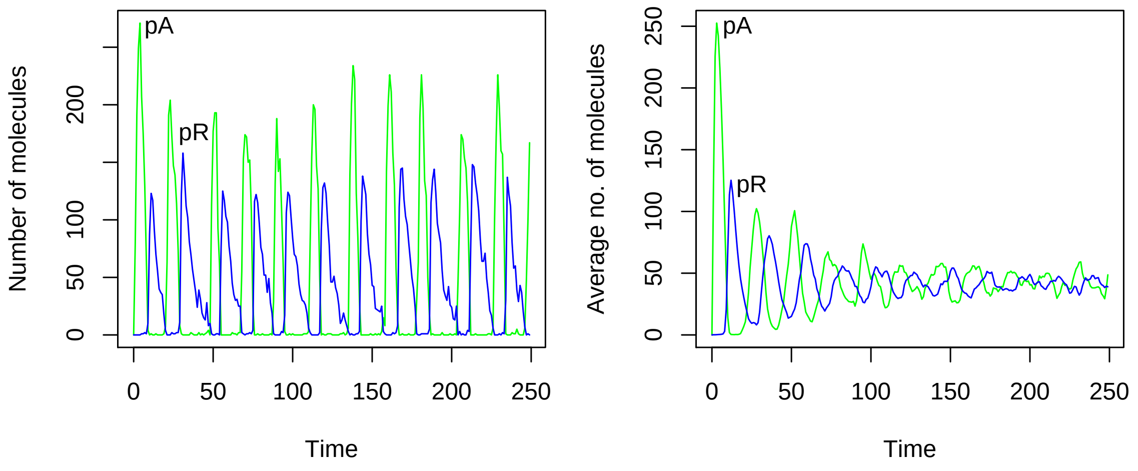


Figure 10.1: The left graph shows typical plots of proteins pA and pR of the noise-resistant oscillator [84] (see Figures 5.10 and 5.11). The right graph shows average plots of pA and pR, based on 100 simulation runs. The lack of phase stability in the stochastic time series causes the amplitude of oscillations to diminish in the average plots.

While the average gained in this way appears to conceal information about the time evolution of the system, it gathers information about the distribution of trajectories at given time points. This distribution approximates a solution to the chemical master equation that describes the system. Examples of such distributions are shown in Figure 10.2 for two species and two time points of the noise resistant oscillator model of [84] as shown in Figures 5.10 and 5.11. The significant observation here is that these distributions do not reveal the nature of the observed oscillatory behaviour of a typical trajectory.

10.2 The Fourier transform

The forward Fourier transform is a linear transformation from the time domain to the frequency domain:

$$X(f) = \int_{-\infty}^{+\infty} x(t)e^{-j2\pi ft}dt \tag{10.1}$$

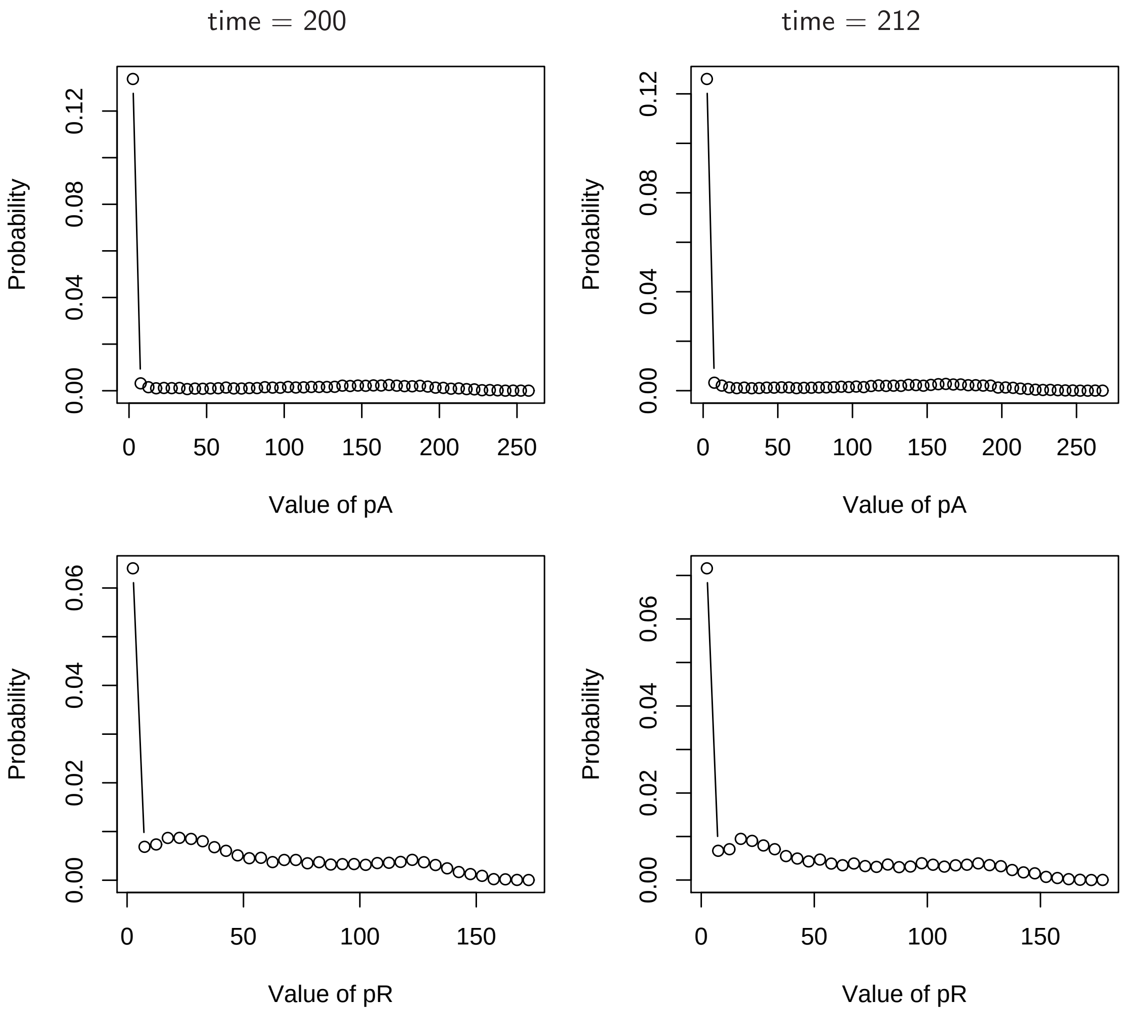


Figure 10.2: Sampled distributions of proteins pA and pR of the noise-resistant oscillator [84] (see Figures 5.10 and 5.11) evaluated at time = 200 and time = 212. Each plot is based on samples taken from 10000 simulation runs. Despite being separated by half the period of oscillation, there is little to distinguish the distributions at the two time points since the mode of oscillation is equally robust at both. Moreover, the existence of oscillation is not revealed by such distributions.

By evaluating this integral for a function of time (i.e., $x(t)$), it is possible to find a function of frequency, $X(f)$, which is a frequency spectrum that *exactly* describes $x(t)$. Since the transformation is linear, no information is gained or lost, however the *representation* of the information may be more intuitive or compact in the frequency domain. In particular, systems having oscillatory behaviour are often more conveniently described in the frequency domain. An example is shown graphically in Figure 10.3, where it is assumed that the time course extends to infinity in the positive and negative time directions. By comparison, the two spectral lines (plus their corresponding phase components, here equal to zero) are a complete *finite* description of the infinite time series. Of course, the frequency axis also continues to positive (and negative) infinity, but it is only necessary to represent a finite segment of it to completely describe the infinite time course.

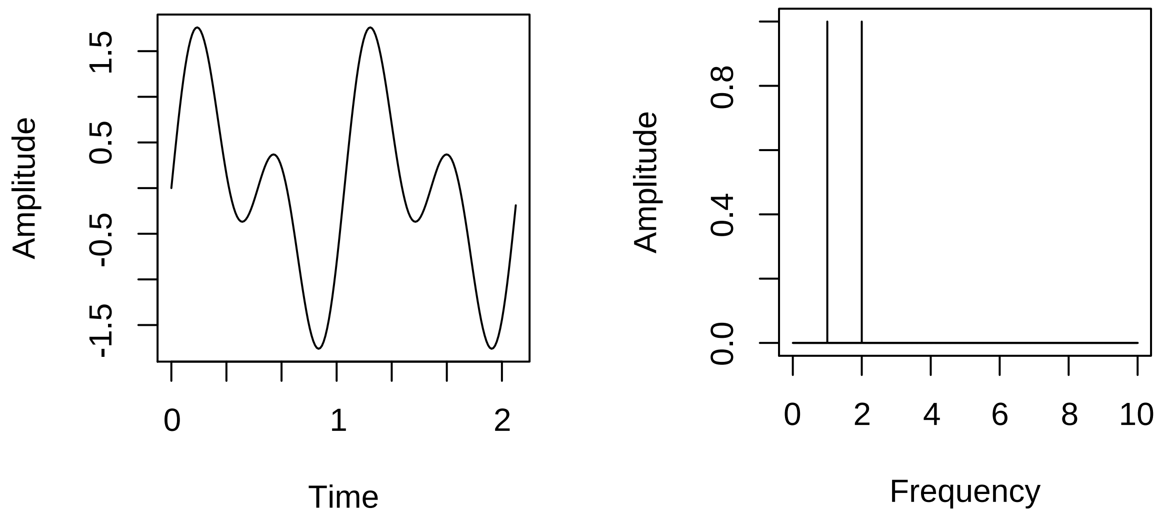


Figure 10.3: Time and frequency domain representations of $x(t) = sin(2\pi t) + sin(4\pi t)$.

In the case of the function of time given in Figure 10.3 it is possible apply the Fourier transfom (Equation 10.1) and derive an analytical solution:

$$\int_{-\infty}^{+\infty} (sin(2\pi t) + sin(4\pi t))e^{-j2\pi ft}dt = \delta(f-1) + \delta(f-2) \tag{10.2}$$

where $\delta(\cdot)$ is the Kronecker delta function ($\delta(d) = \{1 \text{ for } d = 0, 0 \text{ otherwise}\}$. See, e.g., [54]). As expected, the result is a function which gives two spectral peaks and contains no imaginary part because there is no phase difference between the components.

For this contrived example, while there is an evident simplification of the description of the behaviour when it is represented graphically, it can be argued that the time and frequency domain representations are equivalently compact and intuitive. In the case of biological systems described by non-linear differential equations (or a stochastic alternative), no such explicit functional description of the time course usually exists. What is often available are the (discrete) time courses of simulations and possibly also the corresponding experimental time courses.

The nature of experimental data is thus discrete, requiring that the discrete Fourier transform (DFT, Equation 10.3) is used to transform them. The DFT is also a linear transformation from the time domain to the frequency domain for which an inverse transformation is defined. A common application of the DFT is the removal of noise. With sufficient data it is possible to distinguish the noise from the principal signal by Fourier decomposition of the time series using the DFT, as illustrated in Figure 10.4. By deleting the unwanted part of the frequency spectrum and applying the reverse transformation it is then possible to recover (a better approximation to) the principal signal.

The time courses resulting from the stochastic simulation of biological models have arbitrary shape and arbitrary amounts of noise. Some of this noise may be considered an inherent part of the model, i.e., correlated noise, whereas other noise may give no insight about the model. In Section 10.4 a new technique using Fourier analysis is introduced as a means to usefully characterise the behaviour observed in stochastic simulation traces.

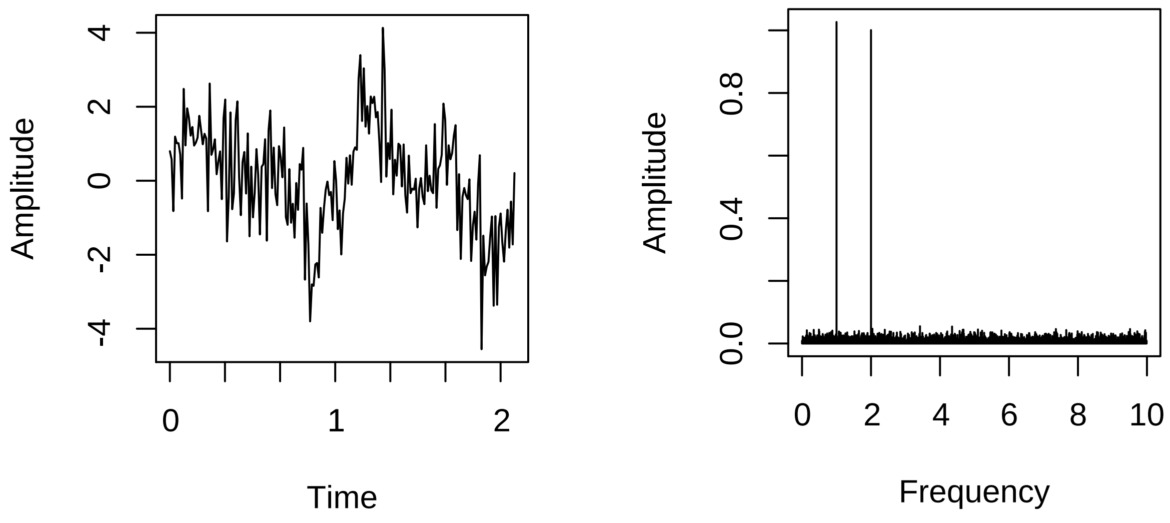


Figure 10.4: Time and frequency domain representations of $x(t)$ with added uncorrelated Gaussian noise.

10.3 Computational cost

Simulating individual molecular interactions has an obvious computational cost; its amelioration being the subject of Chapters 8 and 9. Despite this, the technique presented here can be significantly more efficacious than techniques which require the enumeration of the entire state space, especially in discerning dynamical properties relating to oscillation. The technique characterizes typical behaviour, as exemplified by an average of simulation runs. By definition and in general, simulation runs tend to occupy the most interesting part of the state space, hence the technique essentially avoids generating the uninteresting, often sparse regions.

In order for the obtained distributions to appear smooth and for the statistical measures to converge, it is usually necessary to perform a large number of simulation runs. However, the memory overhead of these runs is low because (i) the number of points required to create the distribution is mostly independent of the number of simulated points (it is more related to the required precision of the results) and (ii) the distribution can be stored cumulatively. As a stochastic process, it is conceivable that successive simulation runs visit the same states, thus perhaps being inefficient in comparison to enumeration. In practice, the total number of points generated by all simulation runs is far fewer than the entire state space. Formalisations of the relationships between state space, number of simulations and the convergence of distributions and measures remain open, however an illustration of convergence is shown in Figure 10.5.

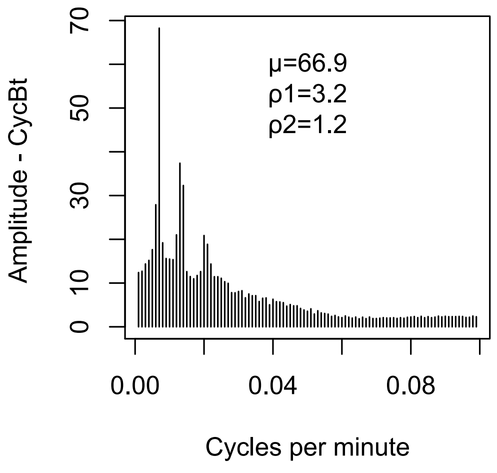
Having performed the simulation runs it is necessary to transform the data according to the DFT given in Equation 10.3. This is achieved by applying a so-called fast Fourier transform (FFT) algorithm, which generally have asymptotic complexity $\Theta(N \log N)$, where N is the number of sampled points. There is not a single most efficient algorithm for arbitrary N, however it is possible to achieve the given asymptotic complexity by choosing the algorithm most appropriate for a given value (see [27] for a review). Since N is generally less than the number of points in a simulation run, the discrete Fourier transformation does not add significantly to the overall computational burden.

10.4 Statistical measures over DFT spectra

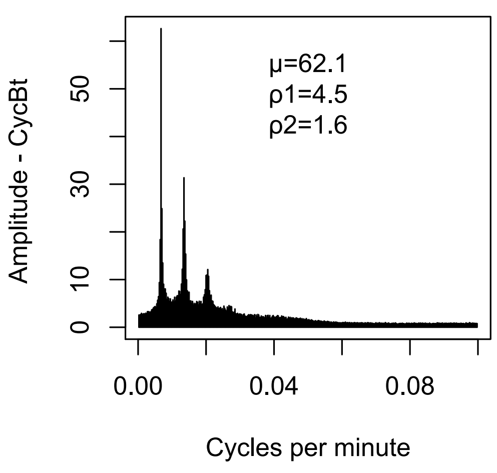
Multiple simulation runs are made having identical initial conditions and length (in simulated time) and the resulting time series are converted to frequency spectra using a discrete Fourier transformation:

$$f_\omega = \sum_{n=0}^{N-1} x_n e^{-\frac{2i\omega n}{N}} \tag{10.3}$$

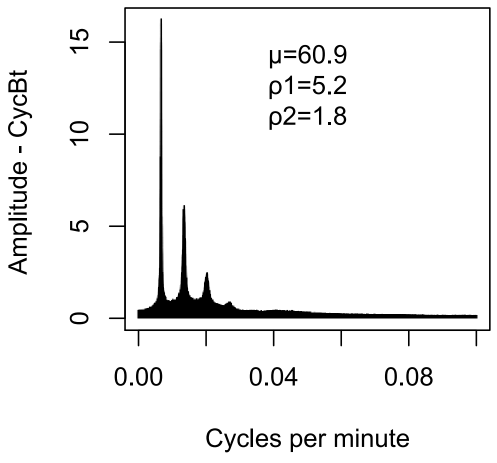
where f_ω is the ω^{th} frequency component and x_n is the n^{th} time sample of a given molecular species. Stochastic simulations based on a variant of the Gillespie algorithm produce time series having irregular time spacing between points, hence to apply Equation 10.3 it is necessary to sample the



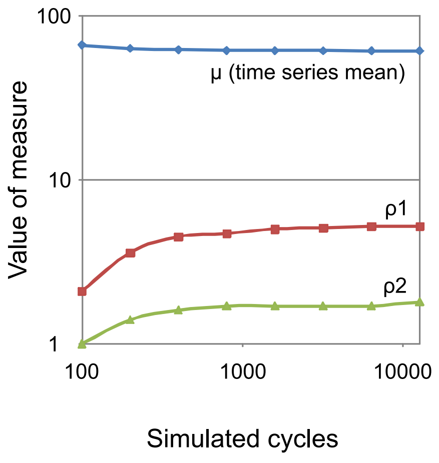
DFT of 100 cycles.



DFT of 400 cycles.



DFT of 12800 cycles.



Convergence with increasing cycles.

Figure 10.5: Convergence of statistical measures relating to stochastic simulations of the budding yeast cell cycle model of Figure 5.21. The three spectra demonstrate increasing smoothness and illustrate the convergence of μ, $\rho 1$ and $\rho 2$ with increasing number of simulated cycles.

stochastic time series at regular time intervals. The method adopted is to choose $x_n = x_t \mid \max(t \leq n\ \delta t)$, where x_t is the simulation point having value x at time t and δt is the chosen sampling time step. That is, the value of species at the time less than or equal to the required sample time.

The result of the DFT is N complex numbers per simulation run, containing amplitude and phase information for each of the N frequencies. Since these frequencies correspond between runs (by definition), the data can be combined to give a distribution (runs will be different from each other by virtue of the stochasticity). Note, however, that it is not sufficient to simply add the complex numbers. Since the DFT is a linear transformation, adding the transformed data is equivalent to performing a transform on the sum of the time series. The result would suffer the same limitations described in Section 10.1 and shown in Figure 10.1.

The solution is not to add the raw complex data but to add the moduli of the spectral data.

The spectra created in this way form distributions which characterize the observed behaviour in a compact form. Figure 10.6 shows typical results, comparing the spectra of deterministic and stochastic simulations of the noise resistant oscillator of [84] (the model is shown in Figures 5.10 and 5.11). Note in particular how high frequency components of the deterministic spectrum are not evident in the stochastic spectrum, being *lost in the noise*. This behaviour is typical and is an interesting result in itself. In Section 10.5 Fourier decomposition of stochastic simulations are used to classify the viability of budding yeast mutants [91]. Typical distributions of $CycB_T$, relative cell mass and SK ($Cdc20_A$ in the case of Mutant 2) for wild type and three mutant yeast strains are shown in Figures 10.11, 10.12, 10.13 and 10.14. The corresponding DFT spectra from deterministic simulations are also shown for comparison with wild type and mutant 3. The key point to observe is that there is qualitatively little to discern the

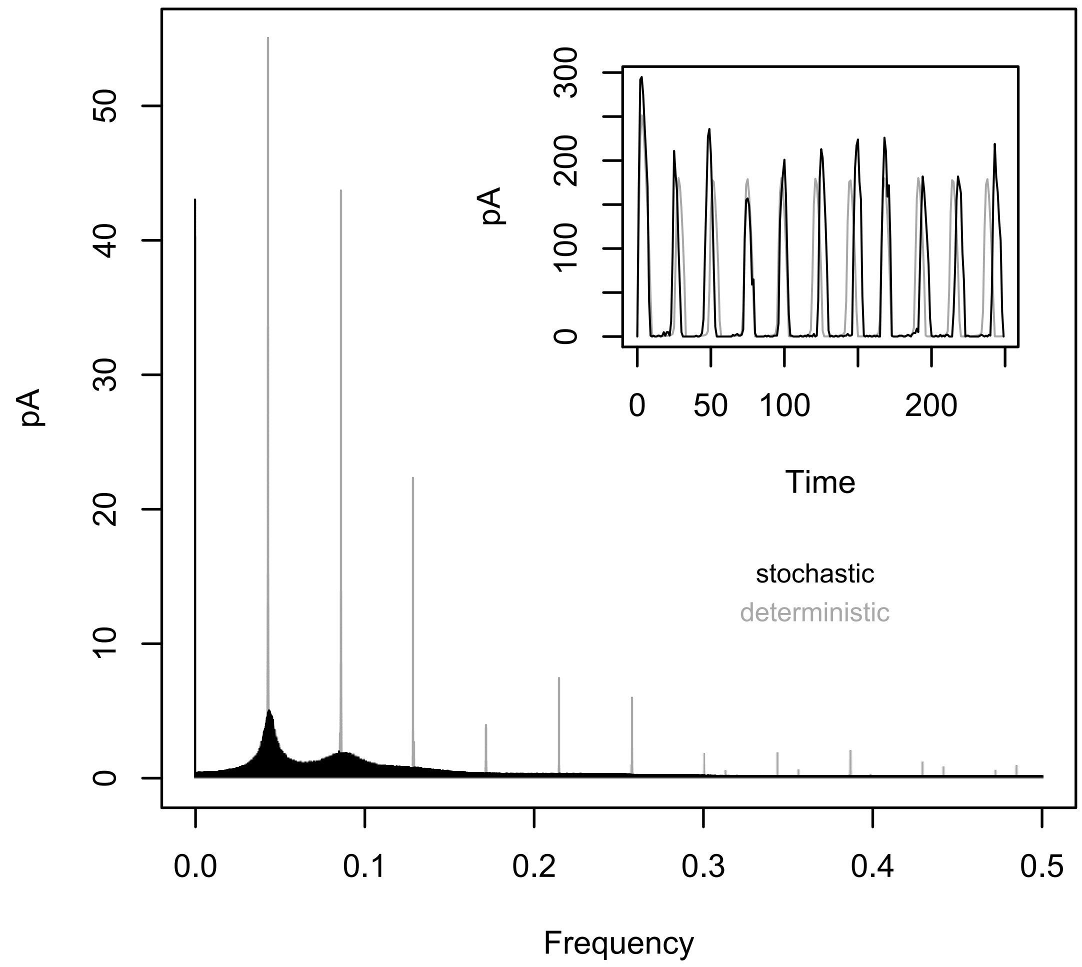


Figure 10.6: Comparison of DFT spectra of stochastic and deterministic simulations of protein A (`pA`) of the noise resistant oscillator of [84]. Note that (i) high frequency components of the deterministic spectrum are not evident in the stochastic spectrum and (ii) the 'mass' of the deterministic spectral peaks is distributed in the noise of the stochastic spectrum. A comparison of typical time series is inset.

deterministic spectra of wild type and mutant 3, yet mutant 3 is known to be the least viable [80, 49, 85]. The difference is clearly shown in the stochastic spectra, demonstrating value of the stochastic simulation and this technique.

10.4.1 Statistical measures

Once spectra have been created, it is possible to define statistical measures which characterise them. Three such measures are introduced here and defined as follows:

$$\rho 1 = \log_2(\max(f_{1..N-1})/\langle f_{1..N-1}\rangle) \tag{10.4}$$

$$\rho 2 = \sigma(f_{1..N-1})/\langle f_{1..N-1}\rangle \tag{10.5}$$

$$\rho 3 = \sup|F^1_{0..N-1} - F^2_{0..N-1}| \tag{10.6}$$

where $\langle\cdot\rangle$ denotes the expected value of its argument, $\sigma(\cdot)$ denotes the standard deviation of its argument and $\sup|\cdot|$ is the supremum of the absolute values of its argument. F^1 and F^2 are the cumulative distributions of the empirical distributions given as f_1 and f_2. Note that

- $\rho 1$ and $\rho 2$ are independent measures of robustness of behaviour: values decrease to 0 with increasing stochasticity.
  - ▷ $\rho 1$ is the maximum of the distribution divided by the mean (excluding μ, the zero frequency component, which is the mean of the time series).
  - ▷ $\rho 2$ is the coefficient of variance of the distribution (excluding μ).
- $\rho 3$ is a relative measure, measuring the difference between two distributions.
  - ▷ $\rho 3$ is the Kolmogorov-Smirnov statistic, with values between 0 and 1, where 0 indicates no difference.

- ▷ $\rho 3$ can be seen to define a *space of phenotype* which can be applied to compare models, mutants or stochastic algorithms.

Figure 10.7 demonstrates the use the technique in comparing models of NF-κB oscillation.

10.5 Fourier analysis of budding yeast mutants

The cell cycle of budding yeast has been thoroughly modelled and simulated using differential equations (e.g., [82]) but much less so using stochastic techniques. The budding yeast cell cycle models are particularly sophisticated, with one of the latest being able to accurately represent the behaviour of more than 100 mutant strains [24]. Using a simplified model adapted from [82], shown diagrammatically in Figure 10.8 and as a simulator script in Figure 5.21, the proof of concept of a new technique to classify the viability of mutants using Fourier analysis applied to stochastic simulations is presented.

Typical deterministic traces are shown in Figure 10.10, which includes the change in relative mass for reference. Typical stochastic traces for the same model are shown in Figure 10.10. The stochastic model was derived from the differential equation model by expanding the differential equations into corresponding creation and consumption reactions, in accordance with the reaction scheme. While the goal is to create a set of elementary reactions from the ODEs, some of the reactions in this model use Michaelis-Menten kinetics and Hill functions and do not contain sufficient information to make an exact conversion. Moreover, the model uses algebraic equations to balance mass and model fast equilibria. These functions and equations were used without modification, but advisedly.

The three statistical measures define in Section 10.4.1 are used to categorize the viability of wild type and mutant varieties of budding yeast.

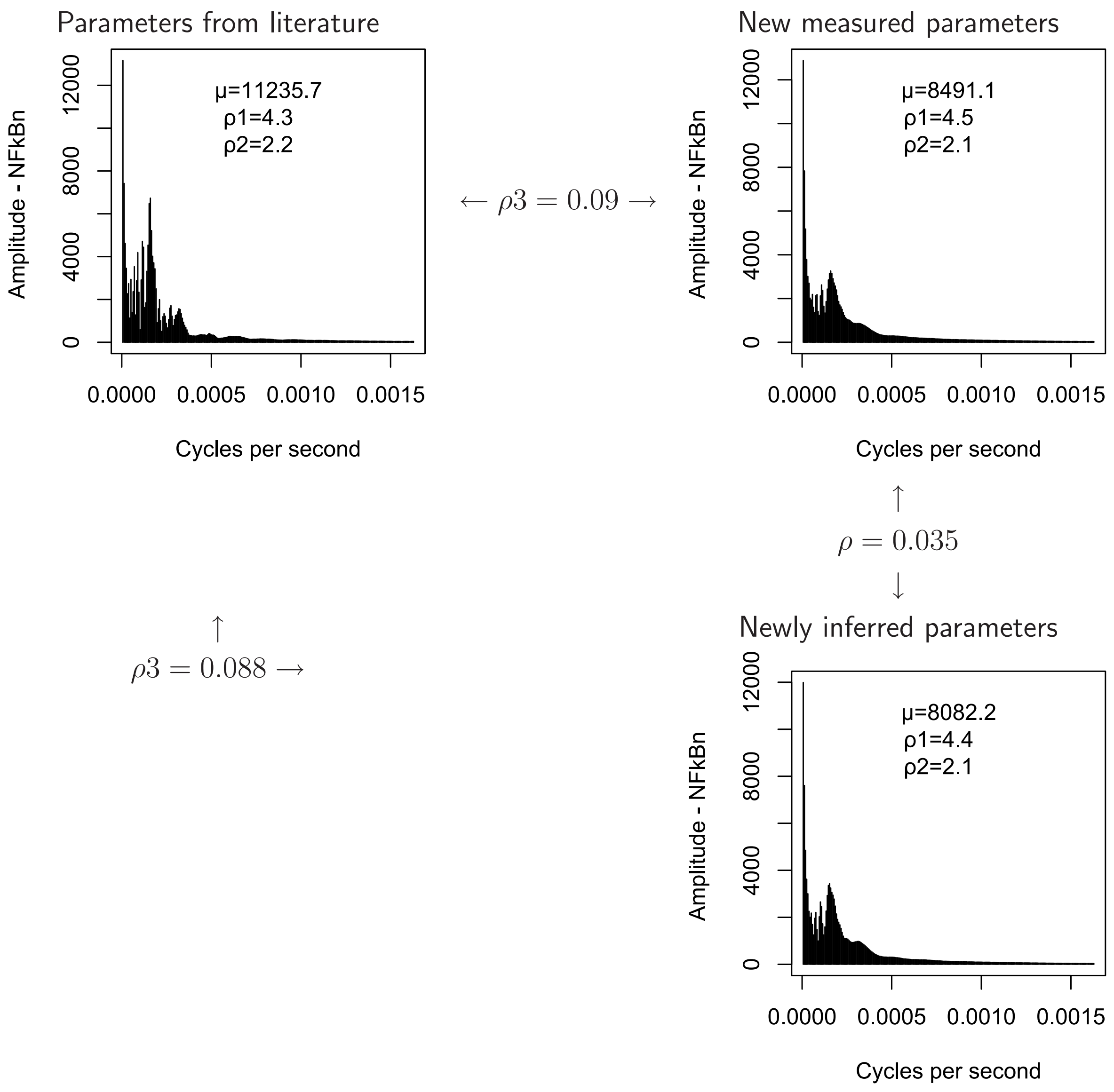


Figure 10.7: DFT spectra and associated statistical measures of NF-κBn oscillation using the model given in Figure 5.16 and three parameter sets. The spectrum on the left features parameters taken from the literature, while that in the top right uses parameters obtained from new measurements. The bottom right spectrum uses parameters inferred from the raw measured data using a new algorithm [58]. The values of $\rho3$ show that the two parameter sets obtained from the new measurements are closer to each other than either of them are to the parameters obtained from the literature.

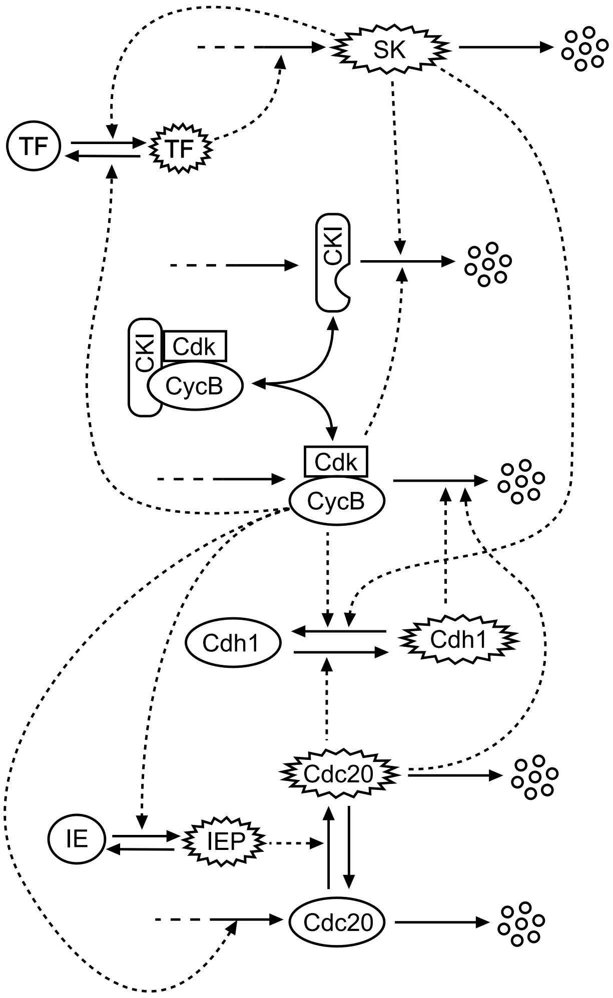


Figure 10.8: The simplified generic model of budding yeast cell cycle. Dashed lines indicate enzymatic action. Active forms of species are contained in stellated ovals.

Overall viability of three mutant strains in comparison to wild type is shown in Figure 10.15. The theoretical results agree well with the experimental results relating to the same mutants (e.g., [79, 80]). Note that $\rho 1$ and $\rho 2$ increase with decreasing viability, while $\rho 3$ decreases.

10.6 Prospects

A technique based on Fourier decomposition has been presented which is able to extract useful information contained in stochastic simulation time

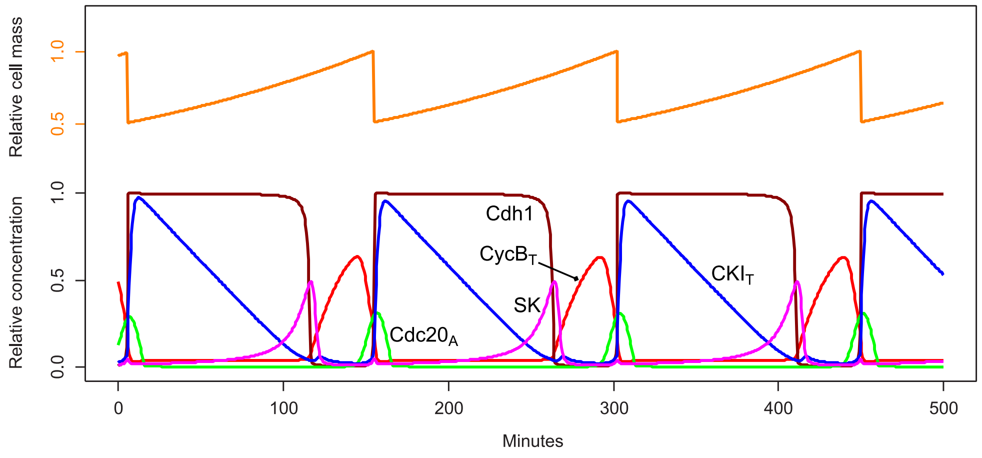


Figure 10.9: Typical deterministic time series of concentrations of species plus relative cell mass of simplified cell cycle model.

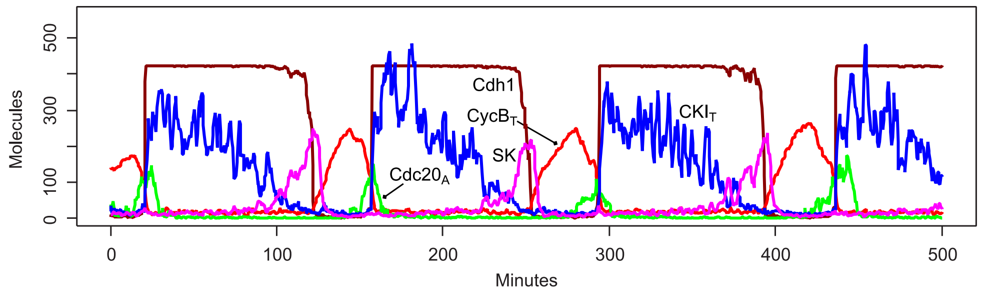


Figure 10.10: Typical stochastic simulation traces of species of the simplified cell cycle model given in Figure 5.21.

series. This information is not easy to obtain by other means and is not available by analysis of deterministic time series.

Three statistical measures have been defined to characterize the distributions generated by the technique and these have been used to accurately classify the viability of wild type and mutant varieties of budding yeast. One of these measures ($\rho 3$) can be used to define a space of behaviour and can thus be used to compare species, varieties within a species, different models of the same species or different simulation algorithms applied to the same model.

With the low memory overhead and relatively low computational cost

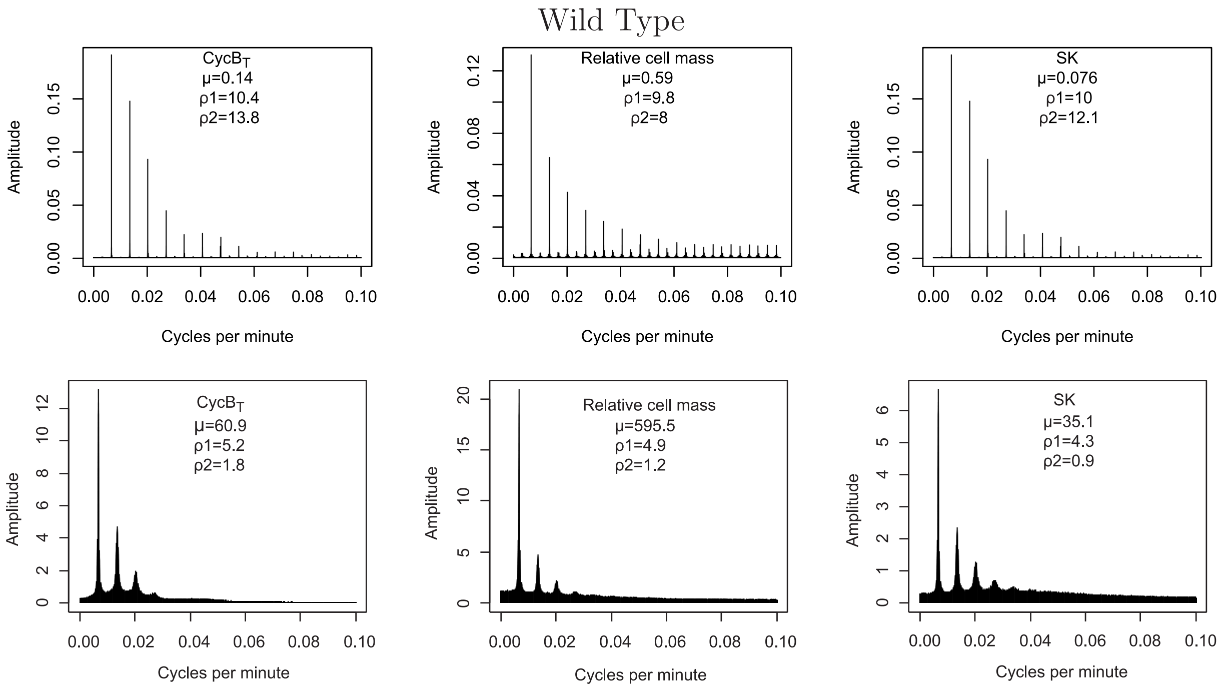


Figure 10.11: Typical DFT distributions of $CycB_T$, relative cell mass and SK for wild type yeast. The top plots are obtained from deterministic simulation, the bottom from stochastic.

of stochastic simulation, the technique has wide application within and beyond systems biology. Further application of the technique to the budding yeast cell cycle will involve the model of [24] and many more mutants. The technique has recently been applied to re-investigate the coupling of a model of the mammalian cell cycle with a circadian oscillator, following initial work in [93], where it has proved revealing and provided inspiration for further investigation. In the same vein, the technique is also being used to investigate a model linking three oscillatory systems, namely those of NF-κB, p53 and the mammalian cell cycle.

Several enhancements to the technique are envisaged, in particular the better characterisation of non-oscillatory transient waveforms, while many existing results may already be re-examined in the light of the existing technique. For example, measuring the merits of various approximate sim-

Mutant 1: CKI deleted (*sic*1Δ)

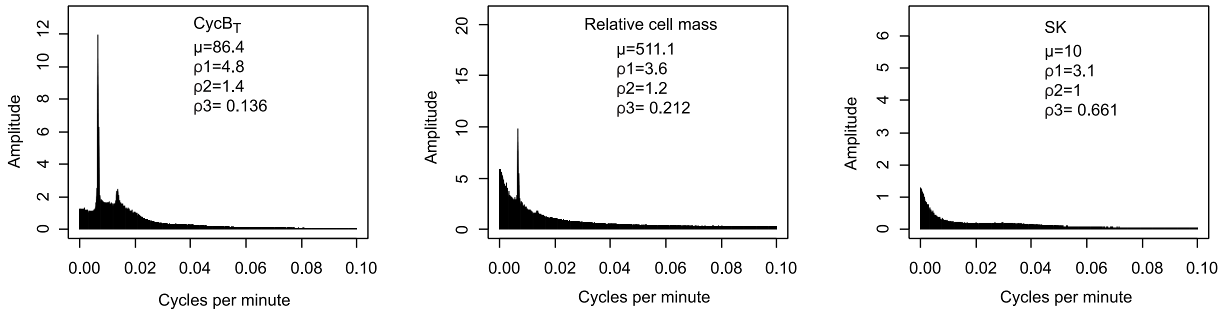


Figure 10.12: Typical DFT distributions of $CycB_T$, relative cell mass and SK for *sic*1Δ mutant.

Mutant 2: CKI & SK deleted ($cln1\Delta, cln2\Delta, cln3\Delta, sic1\Delta$)

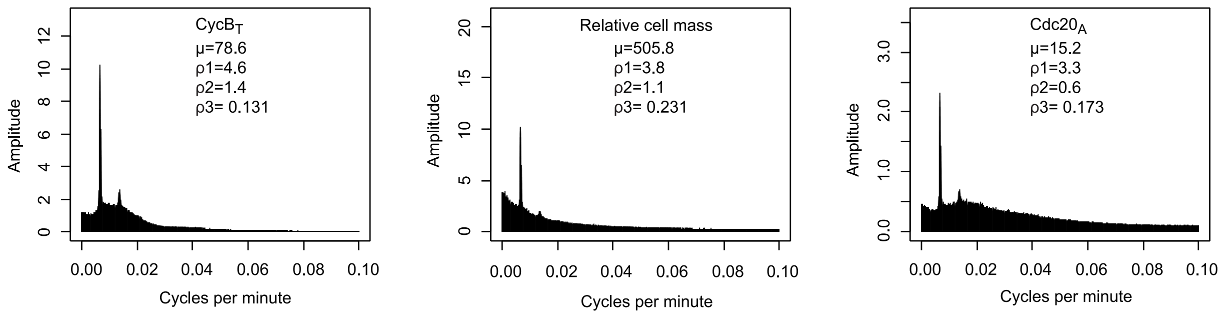


Figure 10.13: Typical DFT distributions of $CycB_T$, relative cell mass and $Cdc20_A$ for $cln1\Delta, cln2\Delta, cln3\Delta, sic1\Delta$ mutant.

ulation algorithms to verify and ascertain the quality of their approximation.

Mutant 3: Cdh1 deleted ($cdh1\Delta$)

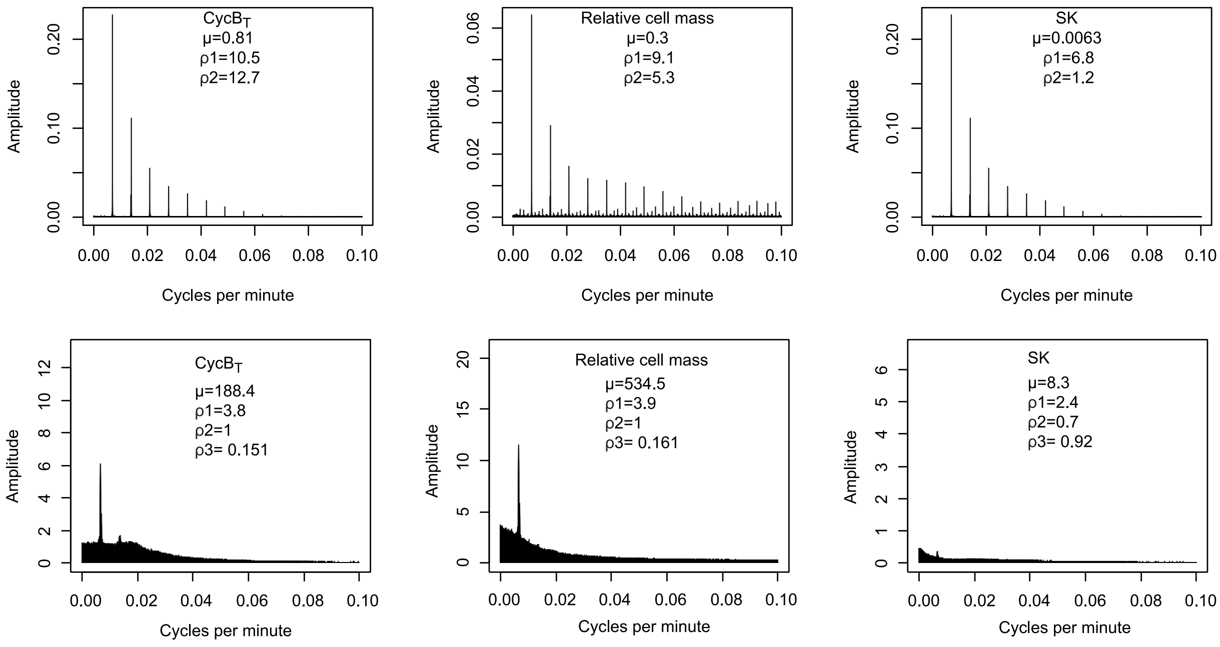


Figure 10.14: Typical DFT distributions of $CycB_T$, relative cell mass and SK for $cdh1\Delta$ mutant. The top plots are obtained from deterministic simulation, the bottom from stochastic.

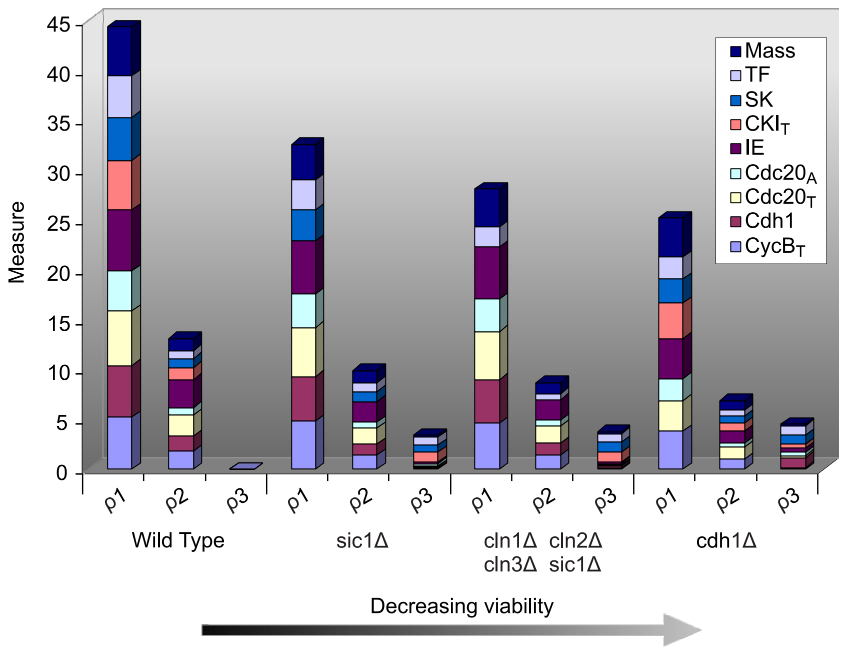


Figure 10.15: Total measures of viability, using all modelled species, for wild type and three mutant yeast strains.

Chapter 11

Conclusions

The widespread availability of high computational power and the sequencing of the human and other genomes has seen an explosion in computational and systems biology research. It is believed that such power can be applied to the vast repositories of biological data in order to advance biological and medical science. The data has been gathered over many generations of scientists and much of the knowledge is unstructured. In order to gain inference from the data using computers it will therefore be necessary to re-interpret it in the context of formal models, such as that of Membrane Systems.

This thesis has presented two new classes of models of Membrane Systems, tailored to represent simple and hierarchical biochemical systems. The underlying formalisms are intuitive to non-experts and have a wealth of existing technical results for reference, as well as active ongoing research. Several results have been presented which extend the corpus and thus aid the understanding of biology. The implementations of these models employ the concept of multiset rewriting controlled by a stochastic simulation algorithm. Two simulators and their associated languages have been created, one of which, Cyto-Sim, has been presented in detail with many examples. To ameliorate the computational complexity of stochastic simulation, two new state of the art simulation algorithms have been presented here. Finally, a new technique based on Fourier analysis was presented which extracts useful information from stochastic simulations.

Membrane Systems with Peripheral Proteins

In Chapter 3 a model of membrane systems with objects attached to both sides of the membranes was presented: a P_{pp} system. The model is equipped with operations that can rewrite floating objects and move objects between regions depending on the attached objects. Qualitative properties of the P_{pp} system model were investigated, such as configuration reachability in relation to the use of cooperative or non-cooperative evolution and transport rules and in the contexts of free- and maximal-parallel evolution. It was proved that when the system works with free parallel evolution, the reachability of a configuration or of a certain protein marking can be decided. It was also shown that when the system works with maximal parallel evolution the reachability of configurations becomes an undecidable property for the case of non-cooperative evolution rules and cooperative membrane rules. The property remains decidable, however, for systems using non-cooperative evolution rules and simple membrane rules and for systems using only membrane rules. An interesting problem remains open: the decidability of reachability in the case of systems using non-cooperative evolution rules, non-cooperative membrane rules and maximal-parallel evolution.

Membrane Systems with Peripheral and Integral Proteins

In Chapter 4 a model of membrane systems was presented with objects integral to the membrane and objects attached to either side of the membrane: a P_{pi} system. Operations were presented that can rewrite floating objects conditional on the existence of integral and attached objects and other operations were presented that facilitate the interaction of floating objects with integral and attached objects. With these it was shown that it is possible to model in detail many real biochemical processes occurring in the cytoplasm and in the cell membrane.

Evolutions of the system are obtained using an enhancement of the Gillespie algorithm and in the second part of the chapter a noise-resistant circadian oscillator and the G-protein cycle mating response of Saccharomyces cerevisiae were modelled using Cyto-Sim, the simulator presented in Chapter 5.

Cyto-Sim

The implementation of the P_{pp} system and P_{pi} system models, Cyto-Sim, was described in detail in Chapter 5, including its syntax and many examples. Cyto-Sim is a tool which has been developed to provide efficient stochastic simulations of biological processes in compartments, using an intuitive representation of the underlying formal model. The explicit modelling of compartments and membranes facilitates the construction of models by composing instances of the variously defined parts, making them compact and transparent. The simulation engine is based on Markov Chain Monte Carlo techniques and can be used at arbitrary and mixed levels of abstraction; micro- and macroscopic biological processes can thus be simulated using arbitrary kinetic laws best suited to each defined interaction. It was noted in Chapter 5 that the use of hierarchical compartments has wide application in biological modelling and in other fields, while the way in which membranes are modelled in Cyto-Sim is specifically aimed at molecular cell biology. For maximum utility, Cyto-Sim also accepts models defined as Petri net matrices and SBML and exports to SBML and Matlab.

Colonies of Synchronizing Agents

A new paradigm designed to model complex, hierarchical systems called Colonies of Synchronizing Agents (CSA) was presented and explored in Chapter 6. Inspired by the intracellular and intercellular mechanisms in biological tissues, the model is based on a multiset of agents, where each agent has a local state stored in the form of a multiset of atomic objects. Hence a CSA can be seen as a multiset of multisets updated by a set of global multiset rewriting rules either independently or synchronously with another agent.

The model was first defined then its computational power was studied, considering the trade-off between internal rewriting and agent synchronization. The dynamic properties of CSAs was also investigated, including behavioural robustness with respect to the loss of an agent or rule and safety of synchronization.

Several prospective enhancements to the theoretical model were proposed and primary among these is the addition of space to the colony: agents have two associated triples corresponding to their position and orientation in space and new rules have the ability to modify these dependent on distance. Other biologically-inspired primitives considered include agent division (mitosis) and agent death (apoptosis).

CSAs have been independently described as an ‘elegant model’ and it is clear that it has many applications related to biology and other complex systems. One such, not detailed in this document, is described in *A Logical Characterization of Robustness, Mutants and Species in Colonies of Agents* [60], which defines formal ways to characterise fundamental biological concepts using the basic CSA model.

The Method of Partial Propensities

In solving a problem specific to the implementation of Colonies of Synchronizing Agents, a simple method was devised that has utility and wider application: the Method of Partial Propensities. By partitioning the simulation algorithm along the lines which generate the complexity, it is possible to significantly ameliorate it and improve on the performance of benchmark algorithms. The methods presented in Chapter 8 may also be relevant to other hierarchical stochastic formalisms, such as those based on process algebra, where complexity derives from combinatorial effects.

The Method of Arbitrary Partial Propensities

By applying some of the ideas of Chapter 8 to more general chemically reacting systems, it was possible to introduce the Method of Arbitrary Partial Propensities in Chapter 9; an algorithm which improves on the performance of the current benchmark, the Next Reaction Method (Section 7.3.2). In order to give the comparison the maximum validity, Chapter 9 also introduced a number of optimisations for the NRM which aimed to minimise implementational differences between the algorithms.

Fourier analysis

In Chapter 10 a novel and promising analysis technique based on Fourier decomposition was presented. The technique is able to extract useful information from the time series created by stochastic simulation. The information so obtained is not easy to obtain by other means and is not available by analysis of deterministic time series.

Three statistical measures were defined to characterise the distributions generated by the technique and these were used to characterise the viability of varieties of budding yeast. One of these measures can be used to define a space of behaviour and has broad application: it can be used to compare species, varieties within a species, different models of the same species or simulation algorithms applied to a single model.

11.1 Prospects and open problems

While the P_{pp} system model has already been extended to include integral proteins, becoming a P_{pp} system, other biologically-inspired operations may be introduced, such as fission and fusion of regions, all still dependent on the objects attached to the membranes. On the other hand, the CSA paradigm might be a more appropriate framework for these, since fission, i.e. agent division, already exists in the CSA implementation. It would be relatively simple to implement a fusion operation, with the interesting prospect of then exploring fission and fusion with space.

A more fruitful line of research with the P_{pp} system and P_{pi} system models is probably their application to the wealth of biological problems available from the literature which have so far only been explored in the context of a continuous paradigm via ordinary differential equations. For example, using Cyto-Sim, which can often import existing models unaltered, it would be possible to gain new insight by applying the techniques of Fourier analysis presented in Chapter 10.

With the addition of the features already in prospect for the CSA model, it will be interesting to extend the investigation and proofs to identify further classes of CSAs demonstrating robustness and having decidable properties. Moreover, it would be interesting to link such theoretical classes with observed robustness in real biological colonies, such as embryos, tissues, tumours and the immune system. In this way it might be possible to gain useful insight about important aspects of biology.

The method of partial propensities (MPP) offers a considerable improvement over standard stochastic simulation algorithms applied to the CSA model. This improvement mostly concerns the amelioration of the effects of agent reactions, however the internal reactions of the same model may constitute a difficult computational task in themselves. The method of arbitrary partial propensities (MAPP) has been shown to be effective for these types of reactions and so may profitably be incorporated in the MPP. The MAPP, being highly optimised already, seems to offer limited scope for further optimisation. Plenty of scope exists, however, for its application to biological systems and in particular to large biological systems.

With the low overhead and relatively low computational cost of stochastic simulation, Fourier analysis of stochastic simulations seems to have wide application within and beyond systems biology. Further application to the budding yeast cell cycle and several enhancements to the technique are in prospect (e.g., better characterisation of non-oscillatory, transient waveforms), while it is envisaged that many existing results and techniques may be re-examined in the light of the new technique. For example, measuring the merits of various approximate simulation algorithms.

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