Bio-PEPA: a framework for the modelling and analysis of biological systems

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Abstract

In this work we present Bio-PEPA, a process algebra for the modelling and the analysis of biochemical networks. It is a modification of PEPA, originally defined for the performance analysis of computer systems, in order to handle some features of biological models, such as stoichiometry and the use of general kinetic laws. The domain of application is the one of biochemical networks. Bio-PEPA may be seen as an intermediate, formal, compositional representation of biological systems, on which different kinds of analysis can be carried out. Bio-PEPA is enriched with some notions of equivalence. Specifically, the isomorphism and strong bisimulation for PEPA have been considered. Finally, we show the translation of three biological models into the new language and we report some analysis results.

Key words: Process Algebras, Biochemical Networks, Modelling, Analysis

1 Introduction

In recent years there has been increasing interest in the application of process algebras in the modelling and analysis of biological systems [37,18,20,36,13,33,9]. Process algebras have some interesting properties that make them particularly useful in describing biological systems. First of all, they offer *compositionality*, i.e. the possibility of defining the whole system starting from the definition of its subcomponents. Secondly, process algebras give a formal representation of the system avoiding ambiguity. Thirdly, biological systems can be abstracted by concurrent systems described by process algebras: species may be seen as processes that can

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interact with each other and reactions may be modelled using actions. Finally, different kinds of analysis can be performed on a process algebra model. These analyses provide conceptual tools which are complementary to established techniques: it is possible to detect and correct potential inaccuracies, to validate the model and to predict its possible behaviours.

The process algebra PEPA, originally defined for the performance analysis of computer systems, has been recently applied in the context of signalling pathways [9,10]. Two different approaches have been proposed: one based on reagents (the so-called *reagent-centric view*) and another based on pathways (*pathway-centric view*). In both cases the species concentrations are discretized into levels, each level abstracting an interval of concentration values. In the reagent-centric view the PEPA sequential components represent the different concentration levels of the species. In this approach the abstraction is "*processes as species*" and not "*processes as molecules*", as in other process algebras such as the π-calculus and Beta-binders [37,36]. In the pathway-centric approach we have a more abstract view: the processes represent sub-pathways. Here multiple copies of components represent levels of concentration. The two views have been shown to be equivalent [10].

Even though PEPA has proved useful in studying signalling pathways, it does not allow us to represent all the features of biological networks. The main difficulties are the definition of *stoichiometric coefficients* (i.e. the coefficients used to show the quantitative relationships of the reactants and products in a biochemical reaction) and the representation of *kinetic laws*. Indeed, stoichiometry is not represented explicitly and the reactions are assumed to be elementary (with constant rate). The problem of extending to the domain of kinetic laws beyond basic mass-action (hereafter called *general kinetic laws*) is particularly relevant, as these kinds of reactions are frequently found in the literature as abstractions of complex situations whose details are unknown. Reducing all reactions to the elementary steps is complex and often impractical. This problem impacts also on other process algebras. Indeed, generally they rely on Gillespie's stochastic simulation for analysis which considers only elementary reactions. Some recent works have extended the approach of Gillespie to deal with complex reactions [1,12] but these extensions are yet to be reflected in the work using process algebras. Previous work concerning the use of general kinetic laws in process algebras and formal methods was presented in [6,14]. These are discussed in Section 3.1.

In this paper we present Bio-PEPA, a language for the modelling and the analysis of biochemical networks. A preliminary version of the language has been proposed in [15]. Here we describe the final version of the language, we introduce new definitions and more details about our approach. Furthermore, we enrich Bio-PEPA with some notions of *equivalence*. In particular, we extend the definition of isomorphism and *strong bisimulation* proposed for PEPA in [29] to Bio-PEPA.

Bio-PEPA is based on the reagent-centric view in PEPA, modified in order to rep-

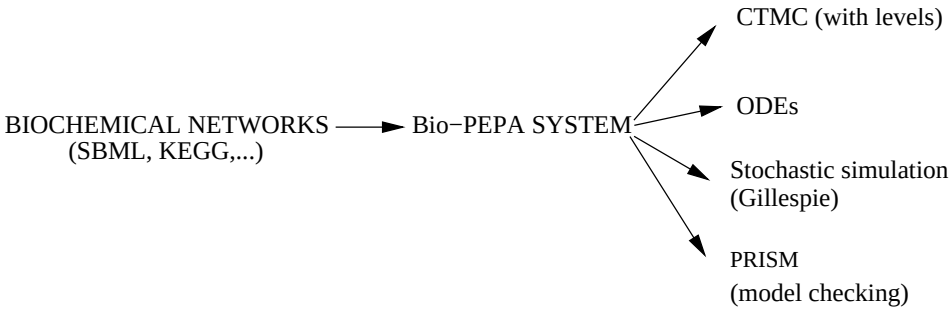


Fig. 1. Schema of the Bio-PEPA framework

resent explicitly some features of biochemical models, such as stoichiometry and the role of the different species in a given reaction. A major feature of Bio-PEPA is the introduction of functional rates to express general kinetic laws. Each action type represents a reaction in the model and is associated with a functional rate.

The idea underlying our work is represented schematically in the diagram in Fig. 1. The context of application is biochemical networks. Broadly speaking, biochemical networks consist of some chemical species, which interact with each other through chemical reactions. The reaction dynamics are described in terms of some kinetic laws. The biochemical networks can be obtained from databases such as *KEGG* [31,30] and *BioModels Database* [5,35]. From the biological model, we develop the Bio-PEPA specification of the system. This is an *intermediate, formal, compositional* representation of the biological model. At this point we can apply different kinds of analysis, including stochastic simulation [25], analysis based on ordinary differential equations, numerical solution of continuous time Markov chains (CTMC) and stochastic model checking using PRISM [38,28]. It is worth noting that each of these analyses can help in understanding the system. The choice of one or more methods depends on the context of application [42]. There exist some relations between the different kinds of analysis. It is well-known that the results of stochastic simulations tend to the ODEs solution when the number of elements is relatively high. Similarly, it is shown in [24] that the numerical solution of the CTMC with levels (derived from the PEPA pathway-centric view) tends to the solution of the ODEs when the number of levels increases.

The paper is structured as follows. In the next section a description of biochemical networks is reported. Section 3 describes PEPA and reports the application of PEPA to the modelling of some signalling pathways. Furthermore, some related works concerning the application of process algebras in systems biology are discussed. After that, in Section 4, we define Bio-PEPA. In Section 6 we enrich the calculus with strong bisimulation. In Section 7 we discuss the main kinds of analysis that can be used from a Bio-PEPA model. The translation of three biological models into Bio-PEPA and their subsequent analysis is described in Section 8. Finally, Section 9 reports some final observations and future investigations.

2 Biochemical networks

In this work we focus on biochemical networks, such as those collected in the Biomodels Database [35] and KEGG [31]. A widely-used classification of such networks distinguishes *gene regulatory networks*, *signalling pathways* and *metabolic pathways*. Broadly speaking, the first class concerns genes and transcription/ translation reactions, signalling pathways involve signals and stimuli that cause the activation/inhibition of other reactions and, finally, the last class considers reaction cascades that describe metabolic processes involving enzymes and cofactors. This classification is not exhaustive, but majority of models collected in databases belong to one of these classes.

A biochemical system $\mathcal{M}$ is composed of:

(1) a set of *compartments* **C**. These represent the locations of the various species;
(2) *a set of chemical species* **S**. These species may be genes, proteins, etc. For each species an initial concentration is given;
(3) *a set of (irreversible) reactions* **R**. The general form of an irreversible reaction j is given by:

$$\kappa_{1j}A_1 + \kappa_{2j}A_2 + ... + \kappa_{n_jj}A_{n_j} \xrightarrow{E_1,E_2,...I_1,I_2,...;f_j} \kappa'_{1j}B_1 + \kappa'_{2j}B_2 + ... + \kappa'_{m_jj}B_{m_j} \quad (1)$$

where A_h, $h = 1, ..., n_j$, are the reactants, B_l, $l = 1, ..., m_j$, are the products, E_v are the enzymes and I_u, the inhibitors. Enzymes and inhibitors are represented differently from the reactants and products. Their role is to enhance or inhibit the reaction, respectively. We call species that are involved in a reaction without changing their concentration (i.e. enzymes/activators and inhibitors) *modifiers*. The parameters κ_{hj} and κ'_{lj} are the stoichiometry coefficients. These express the degree to which species participate in a reaction. The dynamics associated with the reaction is described by a kinetic law f_j, depending on some parameters and on the concentrations of some species. Reversible reactions can be seen as a pair of forward and inverse reactions.

The best known kinetic law is *mass-action*: the rate of the reaction is proportional to the product of the reactants' concentrations. In published models it is common to find *general kinetic laws*, which describe approximations of sequences of reactions. They are useful when it is difficult to derive certain information from the experiments, e.g. the reaction rates of elementary steps, or when there are different timescales for the reactions. Generally these laws are valid under some conditions, such as the *quasi-steady-state assumption (QSSA)*. This describes the situation where one or more reaction steps may be considered faster than the others and so the intermediate elements can be considered to be constant. There is a long list of kinetic laws; for details see [40].

3 PEPA and biological systems

PEPA was originally defined for the performance modelling of systems with concurrent behaviour [29]. Systems are represented as the composition of components or agents which undertake actions. In PEPA each action is assumed to have a duration, which is represented by a random variable with a negative exponential distribution. PEPA has a small set of combinators that allows the system description to be built up as the concurrent interaction of simple sequential components. We informally introduce the syntax of the language below. For more details see [29].

Prefix The basic term is the *prefix combinator* $(\alpha, r).S$. It denotes a component which has action of type α and an exponentially distributed duration with parameter r (mean duration $1/r$), and it subsequently behaves as S.

Choice The component $S + R$ represents a system which may behave either as S or as R. The activities of both S and R are enabled. The first activity to complete distinguishes one of them and the other is discarded.

Constant Constants are components whose meaning is given by a defining equation $C \stackrel{def}{=} S$. They allow us to assign names to patterns of behaviour associated with components.

Hiding In $S/\mathcal{H}$ the set $\mathcal{H}$ identifies those activities which can be considered internal or private to the component S.

Cooperation The term $P \underset{\mathcal{L}}{\bowtie} Q$ denotes cooperation between P and Q over the cooperation set $\mathcal{L}$, that determines those activities on which the cooperands are forced to synchronise. PEPA supports *multiway synchronisation* between components: the result of synchronising on an activity α is thus another α, available for further synchronisation. For action types not in $\mathcal{L}$, the components proceed independently and concurrently with their enabled activities. In the context of performance evaluation the rate for the synchronised activities is the minimum of the rates of the synchronising activities.

PEPA has a structured operational semantics which generates a labelled transition system and from this a continuous time Markov chain (CTMC) is derived.

Recently, PEPA has been applied to the modelling and analysis of signalling pathways. A first study concerns the influence of the Raf Kinase Inhibitor Protein (RKIP) on the Extracellular signal Regulated Kinase (ERK) [9], whereas in [10] the PEPA system for Schoeberl's model [21] involving the MAP kinase and EFG receptors is reported. In [9] two different modelling styles have been proposed, one based on the *reagent-centric view* and the other on the *pathway-centric view*. The former focuses on the variation in the concentrations of the reagents: the concentrations are discretized in levels, each level representing an interval of concentration values. The level l can assume values between 0 and N_{max} (maximum level). The granularity of the representation can vary; the coarsest possibility is $N_{max} = 1$, corresponding to the case of *low* and *high* levels. The pathway-centric style provides

a more abstract view of the system and focuses on the subpathways. The two representations were shown to be equivalent [9]. In addition to the standard analysis offered by process algebras, in [8] a mapping from reagent-centric PEPA models to a system of ordinary differential equations (ODEs), has been proposed.

From these works PEPA has been shown to be appropriate for the modelling of biological systems: it offers a high level of abstraction for the model and focuses on compositionality and on the interactions. Furthermore, by using PEPA as a modelling language it is possible to apply different kinds of analysis, not only stochastic simulation, but also differential equations and the study of properties by means of model checking.

However, not all the features of biochemical networks can be expressed using the present version of PEPA: the various kinetic laws are not considered and stoichiometry is added by hand in the conversion of PEPA into ODEs. With a few exceptions (e.g. [6]) and a few cases (dimerization), these features cannot be represented in other process algebras either.

3.1 Related work

Other process algebras have been considered in the context of biological systems. Initial work focused upon the *π-calculus* and its biochemical stochastic extension [37]. Several case studies have been considered, e.g. [18,33] and some simulation tools have been implemented [41,3]. The translation of biochemical models into this language is based on the abstraction "*processes as single molecules*": molecules are represented by processes and the biological interactions are abstracted by communications between processes.

Beta-binders [36,39] is an extension of the π-calculus inspired by biological phenomena. This calculus is based on the concept of *bio-process*, a box with some sites (*beta-binders*) to express the interaction capabilities of the element, in which π-like processes (*pi-processes*) are encapsulated. Beta-binders enrich the standard π-calculus with some constructs that allow us to represent biological features, such as the join between two bio-processes, the split of one bio-process into two, the change of the bio-process interface by hiding, unhiding and exposing a site.

In both π-calculus and Beta-binders it is not possible to represent all the features that are present in the biochemical networks proposed in this paper. The kinetic law is assumed to be mass-action (constant rates) and reactions can have at most two reactants. Therefore it is not possible to represent stoichiometry with the exception of dimerization. In order to represent multiple-reactant multiple-product reactions the two process algebras have been enriched with transactions [16,17]. Finally, in both cases the analysis of the model is based on stochastic simulation using Gillespie's algorithm [25].

Another language used for the modelling of biological systems is the κ-calculus [19], based on the description of protein interactions. Processes describe proteins and their compounds, a set of processes models solutions and protein behaviour is given by a set of rewriting rules, driven by suitable side-conditions. The two main rules concern activation and complexation.

Previous works concerning the use of general kinetic laws and stoichiometry in process algebras and formal methods have been proposed in [6,14]. The authors of [6] present a stochastic extension of *Concurrent Constraint Programming* (CCP) and show how to apply it in the case of biological systems. Here each species is represented by a variable and the reactions are expressed by constraints on these variables. The domain of application is extended to any kind of reactions and the rate can be expressed by a generic function. The analysis is limited to stochastic simulation using Gillespie's algorithm. *BIOCHAM* [14] is a programming environment for modeling biochemical systems, making simulations and querying the model in temporal logic. In its current version BIOCHAM is based on a rule-based language for modeling biochemical systems, in which species are expressed by objects and reactions by reaction rules. The rates are expressed by using some functions, whose definition is similar to the one proposed in our work. This language permits to evaluate temporal logic queries by using the *NuSMV* model checker [34]. Differently from PRISM, only qualitative queries can be formulated.

4 Bio-PEPA

The aim of this work is to define a new process algebra in order to model some of the features of biochemical networks that are not possible to represent in PEPA. We will show that the new language is able to represent all the reactions in a straightforward way and it deals with stoichiometry and general kinetic laws. It extends the *reagent-centric view* previously used in PEPA models of biochemical pathways.

We adopt a high level of abstraction similar to the one proposed in formalisms such as *SBML* [4] [1] . Furthermore we have made the following assumptions:

(1) compartments are *static*, i.e. compartments are not actively involved in the reactions —they are simply containers. The transport of a species from one compartment to another is modelled by introducing two distinct components for representing the species. The translocation is abstracted by a transformation of one species into another. Compartments are added to the definition of the Bio-PEPA system as in the analysis it can be necessary to have the size of the compartments where the species are.

[1] This is a widely used XML-based format for representing models of biochemical reaction networks. Many SBML models are collected in the *BioModels Database* [5,35].

(2) reactions are *irreversible reactions*.

4.1 Discrete concentrations and granularity

Following the reagent-centric view, models are based not on individual molecules, but on discrete levels of concentration within a species: each component represents a species and it is parametric in terms of concentration levels. Some advantages of this view are:

- it allows us to deal with uncertainty/incomplete information in the exact number of elements (semi-quantitative data);
- the focus is on the concentration levels not on the number of elements: this leads to a reduction of the state space as there are less states for each component.

This view was presented in [11]. The authors focused on the case of reactions with mass-action kinetics and stoichiometry equal to one for all the reactants and products. The granularity of the system has been expressed in terms of the number of levels, representing concentration intervals. Furthermore they considered the same step size h and the same maximum level N for all the species.

In the following we adapt this approach to general kinetic laws, stoichiometry greater than one and different numbers of levels for the species. The granularity of the system is defined in terms of the step size h of the concentration intervals instead of the number of levels. We define the same step size h for all the species. This is motivated by the fact that, following the *law of conservation of mass*, there must be a "balance" between the concentrations consumed (reactants) and the ones created (products). In the case the stoichiometry is greater than one we need to consider concentration quantities proportional to stoichiometric coefficients. Given a species i, we can assume that it has a maximum finite concentration M_i. The number of levels for the species i is given by $N_i + 1$ where $N_i = \lceil \frac{M_i}{h} \rceil$ (the integer value greater than or equal to $\frac{M_i}{h}$). Each species can assume the discrete concentration levels from 0 (concentration null) to N_i (maximum concentration).

If l_i is the concentration level for the species i, the concentration is taken to be $x_i = l_i \times h$.

Some observations about this approach are due. First of all, we assume that there is a maximum concentration for the species i. This is to ensure a finite state space in the corresponding CTMC, making numerical solution feasible. However, we can have a species without a limiting value. In these cases we can consider a maximum level for the values greater than a certain (high) value. It is worth noting that this assumption affects only the CTMC and not the other kinds of analysis. A second point concerns the assumption that all the species have the same step size. There can be some exceptions to this assumption. First of all, since modifiers remain constant

during reaction, we may define a different step size for each species which is only a modifier. Secondly, we can consider a different step size for all species that are involved only in creation/degradation reactions.

4.2 The syntax

The syntax is designed in order to collect the biological information we need:

$$S ::= (\alpha, \kappa)\ \mathsf{op}\ S \mid S + S \mid C \qquad P ::= P \underset{\mathcal{L}}{\bowtie} P \mid S(l)$$

where $\mathsf{op} = \downarrow \mid \uparrow \mid \oplus \mid \ominus \mid \odot$.

The component S is called *sequential component* (or *species component*) and represents the species. The component P, called a *model component*, describes the system and the interactions among components. C is a Bio-PEPA constant, defined as in PEPA. We suppose a countable set of model components $\mathcal{C}$ [2] and a countable set of action types $\mathcal{A}$. The parameter $l \in \mathbb{N}$ represents the discrete level of concentration. The prefix term in PEPA is replaced by a new one, $(\alpha, \kappa)\ \mathsf{op}\ S$, containing information about the role of the species in the reaction associated with the action type α:

- (α, κ) is the prefix, where $\alpha \in \mathcal{A}$ is the *action type* and κ is the *stoichiometry coefficient* of the species in that reaction;
- the *prefix combinator* "op" represents the role of the element in the reaction. Specifically, $\downarrow$ indicates a *reactant*, $\uparrow$ a *product*, $\oplus$ an *activator*, $\ominus$ an *inhibitor* and $\odot$ a generic *modifier*.

The choice operator and cooperation are unchanged. In contrast to PEPA the hiding operator is omitted, as it is not necessary for our purposes.

In order to fully describe a biochemical network in Bio-PEPA we need to define structures that collect information about the compartments, the maximum concentrations, number of levels for all the species, the constant parameters and the functional rates. In the following the function *name* returns the names of the elements of a given Bio-PEPA component.

First of all we define the set of compartments.

Definition 1 *Each compartment is described by "V: v unit", where V is the compartment name, "v" is a positive real number expressing the compartment size and the (optional) "unit" denotes the unit associated with the compartment size. The set of compartments is denoted $\mathcal{V}$.*

[2] This is different from C, the costant in the definition of sequential components.

In this version of Bio-PEPA compartments are static and they cannot change their structure/size. The list of compartment is composed of at least one compartment. In the case we have no information about compartments we need to add a default compartment whose size is 1 and the unit depends on the model.

For each species represented in the system we need to define the number of possible levels, the step, the initial concentration, the maximum concentration, the enclosing compartment name and the compartment for the species concetration.

Definition 2 *For each species we define the element* $C : H, N, M_0, M, V, unit$*, where:*

- C *is the species component name,*
- $H \in \mathbb{N}$ *is the step size,*
- $N \in \mathbb{N}$ *is the maximum level,*
- $M_0 \in \mathbb{R}^+ \cup \{\_\}$ *is the initial concentration,*
- $M \in \mathbb{R}^+ \cup \{\_\}$ *is the maximum concentration,*
- $V \in name(\mathcal{V}) \cup \{\_\}$ *is the name of the enclosing compartment,*
- *unit is the unit for the species concentrations.*

The set of all the elements $C : H, N, M_0, M, V, unit$ *is denoted* $\mathcal{N}$.

In the definition the symbol "_" denotes the empty string. The last four components are optional. Specifically, the initial concentration is added in the case we want to compare our model results with the results in the literature. The maximum concentration is used in the definition of the number of levels, but generally it can be derived from the step size and the maximum number of levels. Concerning compartments, if there is only one compartment for all the species in the model we can omit it in the definition of $\mathcal{N}$.

In order to collect the information about the dynamics of the system, we associate a functional rate f_{α_j} with each action α_j. This function represents the kinetic law of the associated reaction. For the definition of functional rates we consider mathematical expressions with simple operations and operators involving constant parameters and components. All the kinetic laws proposed in the book by Segel [40] can be defined in this way. In addition, for convenience, we include some predefined functions to express the most commonly used kinetic laws.

Definition 3 *The functional rates are expressed by the following grammar:*

$$
\begin{aligned}
f\_rate &::= f_\alpha(\bar{k}, \bar{C}) = sk \mid f_\alpha(\bar{k}) = sk2 \\
sk &::= int \mid float \mid name \mid sk + sk \mid sk \times sk \mid sk/sk \mid sk - sk \mid sk^{sk} \mid \\
&\quad exp(x) \mid log(sk) \mid sin(sk) \mid cos(sk) \\
sk2 &::= fMA(sk) \mid fMM(sk, sk) \mid fH(sk, sk, int)
\end{aligned}
$$

The set of functional rates is denoted $\mathcal{F}_R$.

The mathematical expressions are defined by some mathematical operators (*sk*) and the predefined functions (*sk2*). The general expression for the functional rate contains the names of the parameters and the names of the species components involved in the associated reaction. The predefined kinetic laws considered here are mass-action (fMA), Michaelis-Menten (fMM) and Hill kinetics (fH). They depend only on some parameters; the components/species are derived from the context [3] . The functional rates are defined externally to the components and are evaluated when the system is derived. They are used to derive the transition rates of the system. In the functional rates some parameter constants can be used. These must be defined in the model by means of the set of parameter definitions $\mathcal{K}$.

Definition 4 *Each parameter is defined by "k_{name} = value unit", where "$k_{name} \notin C$" is the parameter name, "value" denotes a positive real number and the (optional) "unit" denotes the unit associated with the parameter. The set of the parameters is denoted $\mathcal{K}$.*

Finally, we have the following definition for the set of sequential components:

Definition 5 *The set Comp of sequential components is defined as*

$$Comp ::= \{C \stackrel{\text{def}}{=} S, \text{ where } S \text{ is a sequential component } \}$$

We can define the Bio-PEPA system in the following way:

Definition 6 *A Bio-PEPA system $\mathcal{P}$ is a 6-uple $\langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}_R, Comp, P \rangle$, where:*

- *$\mathcal{V}$ is the set of compartments;*
- *$\mathcal{N}$ is the set of quantities describing each species;*
- *$\mathcal{K}$ is the set of parameter definitions;*
- *$\mathcal{F}_R$ is the set of functional rate definitions;*
- *Comp is the set of definitions of sequential components;*
- *P is the model component describing the system.*

Now we consider when a Bio-PEPA system is *well-defined*. In a well-defined Bio-PEPA system each element has to satisfy some conditions.

[3] In the case of mass-action, the function $fMA(r)$ is $r \times \prod_{i=1}^{n_j} (C_i)^{\kappa_i}$, where C_i $i = 1, ..., n_j$ are the n_j distinct reactants involved in the reaction and κ_i is the associated stoichiometric coefficients. The information about the reactants are derived from the Bio-PEPA specifications of the system. In the case of Michaelis-Menten, the function $fMM(v_M, K_M)$ is $v_M \times E \times S/(K_M + S)$, where E is the enzyme and S the substrate. Also in this case E and S are derived from the Bio-PEPA specifications. In the case of Hill kinetics, the function $fH(v, K, n)$ is $v \times C^n/(K + C^n)$, where C is the element involved in the reaction.

Definition 7 *A Bio-PEPA system $\mathcal{P}$ is well-defined if and only if all its elements are well-defined.*

The definition of well-definedeness for each element is reported below. First of all we define the set of action types enabled in a species or model component.

Definition 8 *The set of current action types enabled in the model component P, denoted $\mathcal{A}(P)$, is defined as:*

$$\mathcal{A}((\alpha, \kappa) \ op \ S) = \{\alpha\}$$

$$\mathcal{A}(S_1 + S_2) = \mathcal{A}(S_1) \cup \mathcal{A}(S_2)$$

$$\mathcal{A}(C) = \mathcal{A}(S) \text{ where } C \stackrel{\text{def}}{=} S$$

$$\mathcal{A}(S\,(l)) = \mathcal{A}(S)$$

$$\mathcal{A}(P_1 \underset{\mathcal{L}}{\bowtie} P_2) = \mathcal{A}(P_1)\backslash\mathcal{L} \cup \mathcal{A}(P_2)\backslash\mathcal{L} \cup (\mathcal{A}(P_1) \cap \mathcal{A}(P_2) \cap \mathcal{L})$$

If $\mathcal{P}$ is a Bio-PEPA system with model component P, the set of current action types enabled in $\mathcal{P}$ is $\mathcal{A}(\mathcal{P}) = \mathcal{A}(P)$.

Definition 9 *The list $\mathcal{N} = \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}_R, Comp, P \rangle$ is well-defined if and only if:*

- *$name(\mathcal{N}) = name(Comp)$;*
- *for each species specification "$C_i : H_i, N_i, M_{i0}, M_i, V_i, unit$", we have:*
  - *C_i is defined in $name(Comp)$;*
  - *$H_i > 0$ and $H_i \in \mathbb{R}^+$;*
  - *$N_i \in \mathbb{N}$ with $N_i \geq 1$;*
  - *either $M_{i0}, M_i \in \mathbb{R}^+$ with $0 \leq M_{i0} \leq M_i$ or empty;*
  - *$V_i \in \mathcal{V}$ or empty.*

Definition 10 *A functional rate f_α in $\mathcal{F}_R$ is well-defined if and only if:*

- *$\alpha \in \mathcal{A}(P)$;*
- *if $f_\alpha = f_\alpha(\bar{k}, \bar{C})$, $\bar{C}$ are defined in Comp and $\bar{k}$ in $\mathcal{K}$;*
- *if $f_\alpha = f_\alpha(\bar{k})$ then $\bar{k}$ are defined in $\mathcal{K}$.*

The list of functional rates $\mathcal{F}_R$ is well-defined if all the functional rates are well-defined.

Definition 11 *A species component $C \in Comp$ is well-defined if and only if:*

- *$\forall \alpha_h, \alpha_k \in \mathcal{A}(C)$ with $h \neq k$, $\alpha_h \neq \alpha_k$;*
- *each subterm of C is of the form "(α, κ) op C".*

The list of component definitions Comp is well-defined if all the components C are well-defined.

Definition 12 *The model component P is well-defined if and only if:*

- $\forall C_{i(h)}(l_{i(h)0}) \in components(P)$, $C_{i(h)}$ *is defined in Comp;*
- $\forall C_{i(h)}(l_{i(h)0}) \in components(P)$, $0 \leq (l_{i(h)0}) \leq N_{i(h)}$;
- *For each cooperation set* $\mathcal{L}_j$ *in P,* $\mathcal{L}_j \subseteq \mathcal{A}(P)$.

In the definition above the function $components(P)$ returns the set of the sequential components used in the model component P.

In the following we consider only well-defined Bio-PEPA systems. We indicate with $\tilde{\mathcal{P}}$ the set of well-defined Bio-PEPA systems.

4.3 The semantics

The semantics of Bio-PEPA is defined in terms of an operational semantics. We define two relations over the processes. The former, called the *capability relation*, supports the derivation of quantitative information and it is auxiliary to the latter which is called the *stochastic relation*. The stochastic relation gives us the rates associated with each action. The rates are obtained by evaluating the functional rate associated with the action, divided by the step size of the species involved, and by using the quantitative information derived from the capability relation.

The capability relation is $\rightarrow_c \subseteq C \times \Theta \times C$, where the label $\theta \in \Theta$ contains the quantitative information needed for the evaluation of the functional rate. We define the labels θ as:

$$\theta := (\alpha, w)$$

where w is defined as $w ::= [S : op(l, \kappa)] \mid w :: w$, with $S \in C$, l the level and κ the stoichiometry coefficient of the components. The order of the components is not important. The relation $\rightarrow_c$ is defined as the minimum relation satisfying the rules reported in Table 1.

The first three axioms describe the behaviour of the three different prefix terms. In the case of a reactant, the level decreases, in the case of a product the level increases whereas in the case of a modifier the level remains the same. Concerning the reactants and the products, the number of levels that changes depends on the stoichiometric coefficient κ. This expresses the degree to which a species (reactant or product) participates in a reaction. Therefore some side conditions concerning the present concentration level must be added to the rules. Specifically, for the reactants the level has to be greater than or equal to κ, whereas for the products the level has to be less than or equal to $(N-\kappa)$, where N is the maximum level. The modifiers can have any possible value between 0 and N. In all three cases the label records the level and the stoichiometry of the associated component. The rules `choice1` and `choice2` have the usual meaning. The rule `constant` is used to define the behaviour of the constant term, defined by one or more prefix terms in summation.

`prefixReac`	$((\alpha,\kappa)\downarrow S)(l) \xrightarrow{(\alpha,[S:\downarrow(l,\kappa)])}_c S(l-\kappa) \quad \kappa \leq l \leq N$
`prefixProd`	$((\alpha,\kappa)\uparrow S)(l) \xrightarrow{(\alpha,[S:\uparrow(l,\kappa)])}_c S(l+\kappa) \quad 0 \leq l \leq (N-\kappa)$
`prefixMod`	$((\alpha,\kappa)\, op\, S)(l) \xrightarrow{(\alpha,[S:op(l,\kappa)])}_c S(l)$ with $op = \odot, \oplus, \ominus$ and $0 \leq l \leq N$
`choice1`	$\dfrac{S_1(l) \xrightarrow{(\alpha,w)}_c S_1'(l')}{(S_1+S_2)(l) \xrightarrow{(\alpha,w)}_c S_1'(l')}$
`choice2`	$\dfrac{S_2(l) \xrightarrow{(\alpha,w)}_c S_2'(l')}{(S_1+S_2)(l) \xrightarrow{(\alpha,w)}_c S_2'(l')}$
`constant`	$\dfrac{S(l) \xrightarrow{(\alpha,S':[op(l,\kappa)])}_c S'(l')}{C(l) \xrightarrow{(\alpha,C:[op(l,\kappa)])}_c S'(l')}$ with $C \stackrel{def}{=} S$
`coop1`	$\dfrac{P_1 \xrightarrow{(\alpha,w)}_c P_1'}{P_1 \underset{\mathcal{L}}{\bowtie} P_2 \xrightarrow{(\alpha,w)}_c P_1' \underset{\mathcal{L}}{\bowtie} P_2}$ with $\alpha \notin \mathcal{L}$
`coop2`	$\dfrac{P_2 \xrightarrow{(\alpha,w)}_c P_2'}{P_1 \underset{\mathcal{L}}{\bowtie} P_2 \xrightarrow{(\alpha,w)}_c P_1 \underset{\mathcal{L}}{\bowtie} P_2'}$ with $\alpha \notin \mathcal{L}$
`coop3`	$\dfrac{P_1 \xrightarrow{(\alpha,w_1)}_c P_1' \quad P_2 \xrightarrow{(\alpha,w_2)}_c P_2'}{P_1 \underset{\mathcal{L}}{\bowtie} P_2 \xrightarrow{(\alpha,w_1::w_2)}_c P_1' \underset{\mathcal{L}}{\bowtie} P_2'}$ with $\alpha \in \mathcal{L}$

Table 1
Axioms and rules for Bio-PEPA.

The label contains the information about the level and the stoichiometric coefficient related to the action α. The last three rules report the case of cooperation. The rules `coop1` and `coop2` concern the case when the action enabled does not belong to the cooperation set. In this case the label in the conclusion contains only the information about the component that fires the action. The rule `coop3` describes the case in which the two components synchronize and the label reports the information from both the components.

In order to associate the rates with the transitions we introduce the stochastic relation $\rightarrow_s \subseteq \tilde{\mathcal{P}} \times \Gamma \times \tilde{\mathcal{P}}$, where the label $\gamma \in \Gamma$ is defined as $\gamma := (\alpha, r_\alpha)$, with $r_\alpha \in \mathbb{R}^+$. In this definition r_α represents the parameter of a negative exponential distribution. The dynamic behaviour of processes is determined by a *race condition*: all activities enabled attempt to proceed but only the fastest succeeds.

The relation $\rightarrow_s$ is defined as the minimal relation satisfying the rule

$$\texttt{Final} \quad \frac{P \xrightarrow{(\alpha_j, w)}_c P'}{\langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P \rangle \xrightarrow{(\alpha_j, r_\alpha[w, \mathcal{N}, \mathcal{K}])}_s \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P' \rangle}$$

The second element in the label of the conclusion represents the rate associated with the transition. The rate is calculated from the functional rate f_α in the following way:

$$r_\alpha[w, \mathcal{N}, \mathcal{K}] = \frac{f_\alpha[w, \mathcal{N}, \mathcal{K}]}{h}$$

where h is the step size for the species involved in the reaction and the notation $f_\alpha[w, \mathcal{N}, \mathcal{K}]$ means that the function f_α is evaluated over w, $\mathcal{N}$ and $\mathcal{K}$. In detail, for each component C_i we derive the concentration as $l_i \times h$. Then we replace each free occurrence of C_i with $(l_i \times h)^{\kappa_{ij}}$, where κ_{ij} is the stoichiometric coefficient of the species i with respect to the reaction R_j. Some observations about the derivation of the rate are reported in Paragraph 4.3.1.

A *Stochastic Labelled Transition System* can be defined for a Bio-PEPA system.

Definition 13 *The Stochastic Labelled Transition System (*SLTS*) for a Bio-PEPA system is* $(\tilde{\mathcal{P}}, \Gamma, \rightarrow_s)$*, where* $\rightarrow_s$ *is the minimal relation satisfying the rule* `Final`*.*

The states of *SLTS* are defined in terms of the concentration levels of the system components and the transitions from one state to another represent reactions that cause changes in the concentration levels of some components.

Note that using the relation $\rightarrow_c$ it is possible to define another labelled transition system (*LTS*) as $(C, \Theta, \rightarrow_c)$. Given a Bio-PEPA system $\mathcal{P}$ with model component P, the associated transition systems *SLTS* (for $\mathcal{P}$) and *LTS* (for P) have the same states and transitions, but have different transition labels.

The derivation of the CTMC associated with a Bio-PEPA system is reported in Section 7.

4.3.1 Derivation of rates

In the *SLTS* the states represent *levels of concentration* and the transitions cause a change in these levels for one or more species. The number of levels depends on the stoichiometric coefficients of the species involved.

In [11] it was shown how to derive the transition rates in some specific cases. In the following we extend this approach to Bio-PEPA. The derivation is valid even when species have different numbers of levels and maximum concentrations.

Let us consider a reaction j described by a *kinetic law* f_j and with all stoichiometric coefficients equal to one. Following [11], we can define the transition rate as $(\Delta t)^{-1}$, where Δt is the time to have a variation in the concentration of one step for both the reactants and the products of the reaction. Let y be a variable describing one product of the reaction. We can consider the rate equation for that species with respect to the given reaction. This is $dy/dt = f_j(\bar{x}(t))$, where $\bar{x}$ is the set (or a subset) of the reactants/modifiers of the reaction. We can apply the *Taylor expansion* up to the second term and we obtain

$$y_{n+1} \approx y_n + f(\bar{x}_n) \times (t_{n+1} - t_n)$$

Now we can fix $y_{n+1} - y_n = h$ and then derived the time interval $(t_{n+1} - t_n) = \Delta t$ as $\Delta t \approx h/f(\bar{x}_n)$. From this we obtain the transition rate as $f(\bar{x}_n)/h$.

When the reaction has stoichiometric coefficients different from one, we can consider an approach similar to the one above. Let y be a product of the reaction. The approximation gives:

$$y_{n+1} \approx y_n + r \times \kappa \times \prod_{i=1}^{n_r} x_{i,n}^{\kappa_i} \times (t_{n+1} - t_n)$$

where r is the reaction constant rate, κ is stoichiometric coefficient of the product y, x_i $i = 1, ..., n_r$ are the reactants of the reaction, κ_i $i = 1, ..., n_r$ are the associated stoichiometric coefficients, n_r is the number of distinct reactants.

Now we can fix $y_{n+1} - y_n = \kappa \times h$ and then derive the respective $(t_{n+1} - t_n) = \Delta t$ as $\Delta t \approx h/(r \times \prod_{i=1}^{n_r} x_{i,n}^{\kappa_i})$. From this expression we can derive the rate as usual.

Some observations follow. First of all, this approach is based on an *approximation*; this depends on the time/concentration steps. Secondly, we assume that the species can vary by one step size h (fixed) in an interval time (if all the reactants and products are not at 0 or at the maximum level). In particular, reactants are assumed to decrease until 0 is obtained and products increase until a given value. This implies that the kinetic law has to satisfy some properties. Specifically, it must be *monotonic* (non-decrescent in terms of the reactant concentration). Mass-action, Hill-kinetics and Michaelis-Menten are all monotonic, as are many other kinetic laws.

4.4 From biochemical networks to Bio-PEPA

The translation *tr_BM_BP* of a biochemical network $\mathcal{M}$ into a Bio-PEPA system $\mathcal{P} = \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}_R, Comp, P \rangle$ is based on the following abstraction:

(1) Each compartment is defined in the set $\mathcal{V}$ in terms of a name and an associated volume. In this version of Bio-PEPA compartments are not involved actively in the reactions and therefore are not represented by processes.
(2) Each species i in the network is described by a species component $C_i \in Comp$. The constant component C_i is defined by the "sum" of *elementary components* (i.e.prefix terms) describing the interaction capabilities of the species. We suppose that there is at most one elementary component in each species component with an action of type α. A single definition can express the behaviour of the species at any level.
(3) Each reaction j is associated with an action type α_j and its dynamics is described by a specific function $f_{\alpha_j} \in \mathcal{F}_R$. The constant parameters used in the function can be defined in $\mathcal{K}$.
(4) The model P is defined as the cooperation of the different components C_i.

4.5 Some examples

In the following we report some simple examples in order to show how some biochemical situations can be specified in Bio-PEPA.

4.5.1 Example 1: Mass-action kinetics

Consider the reaction $2X + Y \xrightarrow{-;f_M} 3Z$, described by the mass-action kinetic law $f_M = r \times X^2 \times Y$. The three species can be specified by the syntax:

$$X \stackrel{def}{=} (\alpha, 2)\downarrow X \quad Y \stackrel{def}{=} (\alpha, 1)\downarrow Y \quad Z \stackrel{def}{=} (\alpha, 3)\uparrow Z$$

The system is described by $(X(l_{X0}) \underset{\{\alpha\}}{\bowtie} Y(l_{Y0})) \underset{\{\alpha\}}{\bowtie} Z(l_{Z0})$, where l_{X0}, l_{Y0} and l_{Z0} denote the initial level of the three components. The functional rate is $f_\alpha = fMA(r)$. The rate associated with a transition is given by:

$$r_\alpha = \frac{r \times (l_X \times h)^2 \times (l_Y \times h)}{h}$$

where l_X, l_Y are the concentration levels for the species X and Y in a given state and h is the step size of all the species. The reaction can happen only if we have at least 3 levels (0, 1, 2) for X and 4 levels (0, 1, 2, 3) for Z.

4.5.2 Example 2: Michaelis-Menten kinetics

One of the most commonly used kinetic laws is Michaelis-Menten. It describes a basic enzymatic reaction from the substrate S to the product P and is written as $S \xrightarrow{E;f_E} P$, where E is the enzyme involved in the reaction. This reaction is an

approximation of a sequence of two reactions, under the quasi-steady state assumption (QSSA). The whole sequence of reactions is described by the kinetic law $f_E = \frac{v_M \times E \times S}{(K_M + S)}$. For more details about the derivation of this kinetic law and the meaning of parameters see [40].

The three species can be specified in Bio-PEPA by the following components:

$$S \stackrel{def}{=} (\alpha, 1)\downarrow S \quad P \stackrel{def}{=} (\alpha, 1)\uparrow P \quad E \stackrel{def}{=} (\alpha, 1) \oplus E$$

The system is described by $(S(l_{S0}) \underset{\{\alpha\}}{\bowtie} E(l_{E0})) \underset{\{\alpha\}}{\bowtie} P(l_{P0})$ and the functional rate is $f_\alpha = fMM(v_M, K_M)$.

The transition rate is given by:

$$r_\alpha = \frac{v_M \times (l_S \times h) \times (l_E \times h)}{(K_M + l_S \times h)} \times \frac{1}{h}$$

where l_S, l_E are the concentration levels for the species S and E in a given state and h is the step size of all the species. The reaction can happen only if we have at least 2 levels (0, 1) for all the species involved.

4.5.3 Example 3: competitive inhibition

Competitive inhibition is a form of enzyme inhibition where binding of the inhibitor to the enzyme prevents binding of the substrate and vice versa. In classical competitive inhibition, the inhibitor binds to the same active site as the normal enzyme substrate, without undergoing a reaction. The substrate molecule cannot enter the active site while the inhibitor is there, and the inhibitor cannot enter the site when the substrate is there. This reaction is described as:

$$\begin{array}{l} S + E + I \longleftrightarrow SE \longrightarrow P + E \\ \updownarrow \\ EI \end{array}$$

where S is the substrate, E the enzyme, I the inhibitor and P the product. Under QSSA the intermediate species SE and EI are constant and we can approximate the reactions above by a sinlge reaction $S \xrightarrow{E,I:f_I} P$, with rate $f_I = \frac{v_c \times S \times E}{S + K_M(1 + \frac{I}{K_I})}$, where v_c is the the turnover number (catalytic constant), K_M is the Michaelis-Menten constant and K_I is the inhibition constant.

The specification in Bio-PEPA is:

$$S \stackrel{def}{=} (\alpha, 1)\downarrow S \quad P \stackrel{def}{=} (\alpha, 1)\uparrow P \quad E \stackrel{def}{=} (\alpha, 1) \oplus E \quad I \stackrel{def}{=} (\alpha, 1) \ominus I$$

The system is described by $((S(l_{S0}) \underset{\{\alpha\}}{\bowtie} E(l_{E0})) \underset{\{\alpha\}}{\bowtie} I(l_{I0})) \underset{\{\alpha\}}{\bowtie} P(l_{P0})$ with functional rate

$$f_\alpha = f_{CI}((v_c, K_M, K_I), S, E, I) = \frac{v_c \times S \times E}{S + K_M(1 + \frac{I}{K_I})}.$$

The transition rate is given by:

$$r_\alpha = \frac{v_c \times (l_S \times h) \times (l_E \times h)}{(l_S \times h + K_M(1 + \frac{l_I \times h}{K_I}))} \times \frac{1}{h}$$

where l_S, l_E, l_I are the concentration levels for the species S, E, I in a given state and h is the step size of all the species. The reaction can happen only if we have at least 2 levels (0, 1) for all the species involved.

4.5.4 Example 4: degradation and synthesis of a species

Two particular reactions are those which describe the degradation and the creation of a species. In order to model these reactions we need to add two auxiliary species components to represent respectively the *residue* (*Res*) of the reaction and the *creation factor* (*CF*), i.e. genes or DNA.

Let us consider the degradation reaction $A \rightarrow \emptyset$. We describe this reaction in Bio-PEPA by introducing the component *Res* as the residue/product of the reaction. The two species A and *Res* are defined as:

$$A \stackrel{def}{=} (\alpha, 1)\downarrow A \qquad Res \stackrel{def}{=} (\alpha, 1) \odot Res$$

The component *Res* is described by one or more sub-terms each of which describes a different degradation reaction.

In contrast the synthesis of a species $\emptyset \rightarrow A$ is described by a new component *CF*. The two species A and *CF* are described by:

$$A \stackrel{def}{=} (\alpha, 1)\uparrow A \qquad CF \stackrel{def}{=} (\alpha, 1) \odot CF$$

In the definitions of the components *Res* and *CF* we use the symbol $\odot$ to indicate that they do not change with the reaction.

5 Auxiliary definitions

In this section we report some auxiliary definitions. Firstly we consider the derivative of a component, the derivative set and the derivative graph. We refer to the

relation $\rightarrow_s$. The case of $\rightarrow_c$ is analogous, the only differences are in the label and in the fact that the former relation refers to Bio-PEPA systems and the latter refers to model components.

Definition 14 *If $\mathcal{P} \xrightarrow{(\alpha,r)}_s \mathcal{P}'$ then $\mathcal{P}'$ is a one-step $\rightarrow_s$ system derivative of $\mathcal{P}$.*
If $\mathcal{P} \xrightarrow{(\alpha_1,r_1)}_s \mathcal{P}_1 \xrightarrow{(\alpha_2,r_2)}_s .... \xrightarrow{(\alpha_n,r_n)}_s \mathcal{P}'$ then $\mathcal{P}'$ is a system derivative of $\mathcal{P}$.

We can indicate the sequence $\xrightarrow{\gamma_1}_s \xrightarrow{\gamma_2}_s .... \xrightarrow{\gamma_n}_s$ with $\xrightarrow{\mu}_s$, where μ denotes the sequence $\gamma_1\gamma_2, ...\gamma_n$ (possibly empty).

Definition 15 *A system α-derivative of $\mathcal{P}$ is a system $\mathcal{P}'$ such that $\mathcal{P} \xrightarrow{(\alpha,r)}_s \mathcal{P}'$. For each $\alpha \in \mathcal{A}$ we have at most one system α-derivative of a system $\mathcal{P}$.*

Definition 16 *The system derivative set ds($\mathcal{P}$) is the smallest set such that:*

- $\mathcal{P} \in ds(\mathcal{P})$;
- *if $\mathcal{P}' \in ds(\mathcal{P})$ and there exists $\alpha \in \mathcal{A}(\mathcal{P}')$ such that $\mathcal{P}' \xrightarrow{(\alpha,r)}_s \mathcal{P}''$ then $\mathcal{P}'' \in ds(\mathcal{P})$.*

Definition 17 *The system derivative graph $\mathcal{D}(\mathcal{P})$ is the labelled directed multi-graph whose set of nodes is ds($\mathcal{P}$) and whose multi-set of arcs are elements in $ds(\mathcal{P}) \times ds(\mathcal{P}) \times \Gamma$.*

It is worth noting that in the case of well-defined Bio-PEPA components the multiplicity of $\langle \mathcal{P}_i, \mathcal{P}_j, \gamma \rangle$ is always one.

The definitions above refer to Bio-PEPA systems. The only element of the system $\mathcal{P} = \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P \rangle$ that evolves is the model component P. The other elements collect information about the compartments, the species, the rates and report the definition of the species components. They remain unchanged in the evolution of the system. In some cases it can be useful (and simpler) to focus on the model component instead of considering the whole system and use the other elements for the derivation of the rates. We define a function $\pi_P(\mathcal{P}) = P$, that, given a Bio-PEPA system returns the model component. Then we define a *(component) derivative* of P by considering the model component P' of the system derivative of $\mathcal{P}$. Similarly, we define a *(component) α-derivative* of P, *(component) derivative set ds(P)* and the *(component) derivative graph $\mathcal{D}(P)$* starting from the definitions for the associated system $\mathcal{P}$.

In the derivation of the CTMC (see Section 7.1) we need to identify the actions describing the interactions from one state to another.

Definition 18 *Let $\mathcal{P}$ be a Bio-PEPA system and let $P = \pi_P(\mathcal{P})$. Let P_u, P_v be two derivatives of a model component P with P_v a one-step derivative of P_u. The set of action types associated with the transitions from the process P_u to the process P_v is denoted $\mathcal{A}(P_u|P_v)$.*

The next definition concerns the *complete action type set* of a system $\mathcal{P}$ and of a component P.

Definition 19 *The complete action type set of a system $\mathcal{P}$ is defined as:*

$$\bar{\mathcal{A}} = \cup_{\mathcal{P}_i \in ds(\mathcal{P})} \mathcal{A}(\mathcal{P}_i)$$

The complete action type set of a component P is defined similarly.

Other useful definitions are the ones concerning the exit rate and transition rates. In the following we report the definition for the model components, but a similar definition can be used for Bio-PEPA systems.

Definition 20 *Let us consider a Bio-PEPA system $\mathcal{P} = \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P \rangle$ and let $P_1, P_2 \in ds(P)$. The exit rate of a process P_1 is defined as:*

$$rate(P_1) = \sum_{\{\alpha | \exists \mathcal{P}_2 . \mathcal{P}_1 \xrightarrow{(\alpha, r_\alpha[w,\mathcal{N},\mathcal{K}])}_s \mathcal{P}_2,\ P_1 = \pi_P(\mathcal{P}_1)\}} r_\alpha[w, \mathcal{N}, \mathcal{K}]$$

Similarly, the transition rate is defined as:

$$rate(P_1 \mid P_2) = \sum_{\{\alpha | \mathcal{P}_1 \xrightarrow{(\alpha, r_\alpha[w,\mathcal{N},\mathcal{K}])}_s \mathcal{P}_2,\ P_1 = \pi_P(\mathcal{P}_1),\ P_2 = \pi_P(\mathcal{P}_2)\}} r_\alpha[w, \mathcal{N}, \mathcal{K}]$$

Given the transition labels it can be useful to define some functions to extract information from them. For the label θ in the capability relation, the function $action(\theta) = \alpha$ extracts the former element of the pair (i.e. the action type) and $list(\theta) = w$ returns the second element (i.e. the vector of quantitative information). Furthermore, the functions $reacts(\theta)$, $prods(\theta)$ $mods(\theta)$, $enzs(\theta)$, $inhibs(\theta)$, $totMods(\theta)$ return the sets of component names that are indicated as reactants, products, generic modifiers, enzymes, inhibitors and any of the last 3 possibilities from the vector w, respectively. The functions $\#reacts$, $\#prods$,... return the number of elements involved as reactants, products and so on. For the label γ in the stochastic relation, the function $action(\gamma) = \alpha$ extracts the first element of the pair (i.e. the action type) and the function $rate(\gamma) = r \in \mathbb{R}$ returns the second element (i.e. the rate).

6 Equivalences

It is sometimes useful to consider *equivalences* between models in order to determine whether the systems represented are in some sense the "same". In this section we present some notions of equivalence for Bio-PEPA. Some characteristics of the language impact on the definitions of equivalence and we start by highlighting

those. Firstly, there is no hiding operator or τ actions. Therefore, in Bio-PEPA we do not have weaker forms of equivalence based on abstracting τ actions. Secondly, in well-defined systems we have at most one action of a given type in each sequential term and each component describes the behaviour of a single species. So we cannot have processes of the form "$S + S$" or terms such as "$A = a.C$" (where A and C differ). Thirdly, if we have two transitions between the processes P and P', they involve different action types and they represent similar reactions that differ only in the kind/number of modifiers. Finally, we have defined two relations within the semantics. In the former relation the labels contain the information about the action type and about the elements involved. This is used as an auxiliary relation for the derivation of the second one, in which the labels contain the information about the action type and the rate (similarly to PEPA activity). Thus we have a a notion of equivalence for each relation.

In the case of Bio-PEPA we need to define equivalences both for systems and model components. It is worth noting that the only element that changes in the transitions of a Bio-PEPA system is the model component. All the other elements remain unchanged. We define equivalences for the Bio-PEPA systems in terms of equivalences for the model components. Specifically, we say that two Bio-PEPA systems $\mathcal{P}_1$ and $\mathcal{P}_2$ are equivalent if their respective model components are equivalent.

In the following we use the same symbol to denote equivalences for both the system and the corresponding model component. In this section we present definitions of isomorphism and strong bisimulation which are similar to the relations defined for PEPA in [29]. Furthermore we show some relationships between the defined equivalences.

6.1 Isomorphism

Isomorphism is a strong notion of equivalence based on the derivation graph of the components (systems). Broadly speaking, two components (systems) are isomorphic if they generate derivation graphs with the same structure and capable of carrying out exactly the same activities.

We have the following definition of isomorphism based on the capability relation:

Definition 21 *Let $\mathcal{P}_1$, $\mathcal{P}_2$ be two Bio-PEPA systems whose model components are P and Q, respectively. A function $\mathcal{F} : ds(P) \to ds(Q)$ is a component isomorphism between P and Q, with respect to $\to_c$, if $\mathcal{F}$ is an injective function and for any component $P' \in ds(P)$, $\mathcal{A}(P') = \mathcal{A}(\mathcal{F}(P'))$, with $r_\alpha[P', \mathcal{N}, \mathcal{K}] = r'_\alpha[\mathcal{F}(P'), \mathcal{N}', \mathcal{K}']$ for each $\alpha \in \mathcal{A}(P)$, and for all $\alpha \in \mathcal{A}$ the set of α-derivatives of $\mathcal{F}(P')$ is the same as the set of $\mathcal{F}-$images of the α-derivatives of P', with respect to $\to_c$.*

The definition of isomorphism based on the capability relation is very strong since

the labels in the derivative graph contain a lot of information. Formally, we can define isomorphic components in the following way:

Definition 22 *Let $\mathcal{P}_1$, $\mathcal{P}_2$ be two Bio-PEPA systems whose model components are P and Q. P and Q are isomorphic with respect to $\rightarrow_c$ (denoted $P =_c Q$), if there exists a component isomorphism $\mathcal{F}$ between them such that $\mathcal{D}(\mathcal{F}(P)) = \mathcal{D}(Q)$, where $\mathcal{D}$ denotes the derivative graph.*

We can now define when two Bio-PEPA systems are isomorphic.

Definition 23 *Let $\mathcal{P}_1$, $\mathcal{P}_2$ be two Bio-PEPA systems whose model components are P and Q. $\mathcal{P}_1$ and $\mathcal{P}_2$ are isomorphic with respect to $\rightarrow_c$ (denoted $\mathcal{P}_1 =_c \mathcal{P}_2$), if $P =_c Q$.*

For the stochastic relation we have the following three definitions.

Definition 24 *A function $\mathcal{F} : ds(\mathcal{P}_1) \rightarrow ds(\mathcal{P}_2)$ is a system isomorphism between $\mathcal{P}_1$ and $\mathcal{P}_2$, with respect to $\rightarrow_s$, if $\mathcal{F}$ is an injective function and for any system $\mathcal{P}'_1$, $\mathcal{A}(\mathcal{P}'_1) = \mathcal{A}(\mathcal{F}(\mathcal{P}'_1))$, and for all $\alpha \in \mathcal{A}$, the set of system α-derivatives of $\mathcal{F}(\mathcal{P}'_1)$ is the same as the set of $\mathcal{F}-$images of the system α-derivatives of $\mathcal{P}'_1$, with respect to $\rightarrow_s$.*

Definition 25 *Let $\mathcal{P}_1$, $\mathcal{P}_2$ be two Bio-PEPA systems whose model components are P and Q. P and Q are isomorphic with respect to $\rightarrow_s$ (denoted $P =_s Q$), if there exists a system isomorphism $\mathcal{F}$ between $\mathcal{P}_1$ and $\mathcal{P}_2$ such that $\mathcal{D}(\mathcal{F}(\mathcal{P}_1)) = \mathcal{D}(\mathcal{P}_1)$.*

Definition 26 *Let $\mathcal{P}_1$, $\mathcal{P}_2$ be two Bio-PEPA systems whose model components are P and Q. $\mathcal{P}_1$ and $\mathcal{P}_2$ are isomorphic with respect to $\rightarrow_s$ (denoted $\mathcal{P}_1 =_s \mathcal{P}_2$), if $P =_s Q$.*

The next proposition reports some properties of the two notions of isomorphism.

Proposition 1 *The following properties hold.*

(1) Both $=_c$ and $=_s$ are equivalence relations.
(2) Both $=_c$ and $=_s$ are congruences.
(3) Isomorphic components ($=_c$ or $=_s$) generate identical Markov processes.
(4) $=_c \subset =_s$.

The proof of the first three points is analogous to the case of isomorphism for PEPA in [29]. The last point follows from the fact that in the former isomorphism we take into account the information in the vector w on the label of the capability relation, in addition to the rate and the action type. Thus isomorphism $=_c$ is more strict.

6.1.1 Equational laws

In the following the symbol "=" denotes either $=_c$ or $=_s$. The proof follows the definition of isomorphism and the semantic rules.

Choice The laws for choice are:

1) $(P + Q) \underset{\mathcal{L}}{\bowtie} S = (Q + P) \underset{\mathcal{L}}{\bowtie} S$

2) $(P + (Q + R)) \underset{\mathcal{L}}{\bowtie} S = ((P + Q) + R) \underset{\mathcal{L}}{\bowtie} S$

Cooperation The laws for cooperation are:

(1) $P \underset{\mathcal{L}}{\bowtie} Q = Q \underset{\mathcal{L}}{\bowtie} P$

(2) $P \underset{\mathcal{L}}{\bowtie} (Q \underset{\mathcal{L}}{\bowtie} R) = (P \underset{L}{\bowtie} Q) \underset{\mathcal{L}}{\bowtie} R$

(3) $P \underset{\mathcal{K}}{\bowtie} Q = P \underset{\mathcal{L}}{\bowtie} Q \quad$ if $\mathcal{K} \cap (\bar{\mathcal{A}}(P) \cup \bar{\mathcal{A}}(Q)) = \mathcal{L}$

(4) $(P \underset{\mathcal{L}}{\bowtie} Q) \underset{K}{\bowtie} R = \begin{cases} P \underset{\mathcal{L}}{\bowtie} (Q \underset{\mathcal{K}}{\bowtie} R) \text{ if } \bar{\mathcal{A}}(R) \cap (\mathcal{L} \backslash \mathcal{K}) = \emptyset \wedge \bar{\mathcal{A}}(P) \cap (\mathcal{K} \backslash \mathcal{L}) = \emptyset \\ Q \underset{\mathcal{L}}{\bowtie} (P \underset{\mathcal{K}}{\bowtie} R) \text{ if } \bar{\mathcal{A}}(R) \cap (\mathcal{L} \backslash \mathcal{K}) = \emptyset \wedge \bar{\mathcal{A}}(Q) \cap (\mathcal{K} \backslash \mathcal{L}) = \emptyset \end{cases}$

Constant The law for constant is: If $A \stackrel{def}{=} P$ then $A = P$

In the case of Bio-PEPA systems we have the following law, that follows directly from the definition.

Bio-PEPA systems The law for Bio-PEPA systems is:

Let $\mathcal{P}_1$ and $\mathcal{P}_2$ be two Bio-PEPA systems, with $P = \pi_P(\mathcal{P}_1)$ and $Q = \pi_P(\mathcal{P}_2)$.

If $P = Q$ then $\mathcal{P}_1 = \mathcal{P}_2$.

6.2 Strong bisimulation

The definition of bisimulation is based on the *labelled transition system*. Strong bisimulation captures the idea that bisimilar components (systems) are able to perform the same actions with same rates resulting in derivatives that are themselves bisimilar. This makes the components (systems) indistinguishable to an external observer. We give two definitions according to the two relations.

In the case of the capability relation the label contains a lot of information. We can define different relations according to the information we want to consider. In the following we report two possible relations.

Definition 27 *A binary relation $\mathcal{R} \subseteq C \times C$ is a strong capability bisimulation if $(P, Q) \in \mathcal{R}$ implies for all $\alpha \in \mathcal{A}$:*

- *if* $P \xrightarrow{\theta_1}_c P'$ *then, for some* Q' *and* θ_2, $Q \xrightarrow{\theta_2}_c Q'$ *with* $(P', Q') \in \mathcal{R}$ *and*

(1) $action(\theta_1) = action(\theta_2) = \alpha$;

(2) $\#reacts(list(\theta_1)) = \#reacts(list(\theta_2))$, $\#prods(list(\theta_1)) = \#prods(list(\theta_2))$, $\#enzs(list(\theta_1)) = \#enzs(list(\theta_2))$,$\#inhibs(list(\theta_1)) = \#inhibs(list(\theta_2))$;

- *the symmetric definition with* Q *replacing* P.

Definition 28 *Let* $\mathcal{P}_1$, $\mathcal{P}_2$ *be two Bio-PEPA systems whose model components are* P *and* Q, *respectively.* P *and* Q *are strong capability bisimilar, written* $P \sim_c Q$, *if* $(P, Q) \in \mathcal{R}$ *for some strong capability bisimulation* $\mathcal{R}$ *and* $r_\alpha[P, \mathcal{N}, \mathcal{K}] = r'_\alpha[Q, \mathcal{N}', \mathcal{K}']$ *for all* $\alpha \in \mathcal{A}$.

A condition concerning the transition rate is added. In the case of Bio-PEPA systems we have the following definition.

Definition 29 *Let* $\mathcal{P}_1$, $\mathcal{P}_2$ *be two Bio-PEPA systems whose model components are* P *and* Q, *respectively.* $\mathcal{P}_1$, $\mathcal{P}_2$ *are strong capability bisimilar, written* $\mathcal{P}_1 \sim_c \mathcal{P}_2$, *if* $P \sim_c Q$.

We can relax the second point omitting it entirely. In this way we obtain a weaker form of strong capability bisimulation. We denote this $P \sim_c^2 Q$ in the case of model components and $\mathcal{P}_1 \sim_c^2 \mathcal{P}_2$ in the case of systems.

The definition of *strong stochastic bisimulation* is reported below.

Definition 30 *A binary relation* $\mathcal{R} \subseteq \tilde{\mathcal{P}} \times \tilde{\mathcal{P}}$ *is a strong stochastic bisimulation, if* $(\mathcal{P}_1, \mathcal{P}_2) \in \mathcal{R}$ *implies for all* $\alpha \in \mathcal{A}$:

- *if* $\mathcal{P}_1 \xrightarrow{\gamma_1}_s \mathcal{P}'_1$ *then, for some* $\mathcal{P}'_2$ *and* γ_2, $\mathcal{P}_2 \xrightarrow{\gamma_2}_s \mathcal{P}'_2$ *with* $(\mathcal{P}'_1, \mathcal{P}'_2) \in \mathcal{R}$ *and*

(1) $action(\gamma_1) = action(\gamma_2) = \alpha$

(2) $rate(\gamma_1) = rate(\gamma_2)$

- *the symmetric definition with* $\mathcal{P}_2$ *replacing* $\mathcal{P}_1$.

Definition 31 *Let* $\mathcal{P}_1$, $\mathcal{P}_2$ *be two Bio-PEPA systems whose model components are* P *and* Q, *respectively.* P *and* Q *are strong stochastic bisimilar, written* $P \sim_s Q$, *if* $(\mathcal{P}_1, \mathcal{P}_2) \in \mathcal{R}$ *for some strong stochastic bisimulation* $\mathcal{R}$.

Definition 32 *Let* $\mathcal{P}_1$, $\mathcal{P}_2$ *be two Bio-PEPA systems whose model components are* P *and* Q, *respectively.* $\mathcal{P}_1$, $\mathcal{P}_2$ *are strong stochastic bisimilar, written* $\mathcal{P}_1 \sim_s \mathcal{P}_2$, *if* $P \sim_s Q$.

Some facts about the strong bisimulation relations are reported in the following proposition.

Proposition 2 *The following facts hold:*

(1) the bisimulations $\sim_c$, $\sim_c^2$ *and* $\sim_s$ *are all equivalences and congruences;*

(2) $\sim_c \subset \sim_c^2$;
(3) $\sim_s = \sim_c^2$;
(4) $=_c \subset \sim_c$ *and* $=_s \subset \sim_s$

The last point reports that two components that are isomorphic are also strong bisimilar. The proof is identical to the case for PEPA. From this some equational laws are defined for the bisimulation relation too.

6.2.1 Example

Consider the following systems representing two biological systems. The former system $\mathcal{P}_1$ represents a system described by an enzymatic reaction with kinetic law $\frac{v_1 \times E \times S}{K_1 + S}$, where S is the substrate and E the enzyme. We have that the set $\mathcal{N}$ is defined as "$S : h, N_S;\ P : h, N_P; E : 1, 1;$ " for some step size h and maximum levels N_S and N_P. The component and the model components are defined as:

$$S \stackrel{def}{=} (\alpha, 1)\downarrow S \quad E \stackrel{def}{=} (\alpha, 1) \oplus E \quad P \stackrel{def}{=} (\alpha, 1)\uparrow P$$

The model component P_1 is $(S(l_{S0}) \underset{\{\alpha\}}{\bowtie} E(1)) \underset{\{\alpha\}}{\bowtie} P(l_{P0})$. The functional rate is $f_\alpha = fMM(v_1, K_1)$.

The second system $\mathcal{P}_2$ describes an enzymatic reaction where the enzyme is left implicit (it is constant). The rate is given by $\frac{v_1 \times S'}{K_1 + S'}$, where S' is the substrate.

We have that the set $\mathcal{N}$ is defined as "$S' : h, N_{S'};\ P' : h, N_{P'};$ ".

The components are defined as $S' \stackrel{def}{=} (\alpha, 1)\downarrow S'$ and $P' \stackrel{def}{=} (\alpha, 1)\uparrow P'$ and the model component $P2$ is $S'(l_{S0}) \underset{\{\alpha\}}{\bowtie} P'(l_{P0})$. In this case $f_\alpha = fMM'((v_1, K_1), S') = \frac{v_1 \times S'}{K_1 + S'}$ and the component S' and P' have the same number of levels/maximum concentration of S and P.

We have that $P_1 \sim_s P_2$, but $P_1 \nsim_c P_2$, because the number of enzymes is different. The same relations are valid if the systems rather than the model components are considered.

7 Analysis

A Bio-PEPA system is an *intermediate, formal, compositional* representation of the biological model. Based on this representation we can perform different kinds of analysis. In this section we discuss briefly how to use a Bio-PEPA system to derive

a *CTMC with levels*, a set of *Ordinary Differential Equations* (*ODEs*), a *Gillespie simulation* and a *PRISM* model.

7.1 From Bio-PEPA to CTMC

As for the reagent-centric view of PEPA, the CTMC associated with the system refers to the concentration levels of the species components. Specifically, the states of the CTMC are defined in terms of concentration levels and the transitions from one state to the other describe some variations in these levels. Hereafter we call the CTMC derived from a Bio-PEPA system (or from a PEPA reagent-centric view system) *CTMC with levels*.

Theorem 1 *For any finite Bio-PEPA system $\mathcal{P} = \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}_R, Comp, P \rangle$, if we define the stochastic process X(t) such that $X(t) = P_i$ indicates that the system behaves as the component P_i at time t, then X(t) is a Markov Process.*

The proof is not reproduced here but it is analogous the one presented for PEPA [29]. Instead of the PEPA activity we consider the label γ and the rate is obtained by evaluating the functional rate in the system. We consider finite models to ensure that a solution for the CTMC is feasible. This is equivalent to supposing that each species in the model has a maximum level of concentration.

Theorem 2 *Given $(\tilde{\mathcal{P}}, \Gamma, \rightarrow_s)$, let $\mathcal{P}$ be a Bio-PEPA system, with model component P. Let $n_c = |ds(P)|$, where $ds(P)$ is the derivative set of P. Then the infinitesimal generator matrix of the CTMC for $\mathcal{P}$ is a square matrix Q $(n_c \times n_c)$ whose elements $q_{u,v}$ are defined as*

$$q_{u,v} = \sum_{\alpha_j \in \mathcal{A}(P_u|P_v)} r_{\alpha_j}[w_u, \mathcal{N}, \mathcal{K}] \quad \text{if } u \neq v \qquad q_{u,u} = -\sum_{u \neq v} q_{u,v} \quad \text{otherwise.}$$

where P_u, P_v are two derivatives of P.

It is worth noting that the states of CTMC are defined in terms of the derivatives of the model component. These derivatives are uniquely identified by the levels of species components in the system, so we can give the following definition of CTMC states:

Definition 33 *The CTMC states derived from a Bio-PEPA system can be defined as vectors of levels $\sigma = (l_1, l_2, ..., l_n)$, where l_i, for $i = 1, 2, ..., n$, is the level of the species i and n is the total number of species. We can avoid consideration of the two levels for Res and CF as they are always constant.*

This leads to the following proposition.

Proposition 3 *Let $\mathcal{P}$ be a Bio-PEPA system with model component P. Let P_u and*

P_v be two derivatives of P such that the latter is one-step derivative of the former. If there exist two action types α_1 and α_2 that belong to $\mathcal{A}(P_u|P_v)$ then:

(1) $\alpha_1 \neq \alpha_2$*;*
(2) the two action types refer to two transitions/biological reactions that differ only in the modifiers.

If two transitions are possible between a pair of states, the actions involved are different and they represent reactions that differ only in the modifiers and/or the number of enzymes used. The former point follows from the definition of well-defined Bio-PEPA system. The second point follows because the only possibility to have two transitions between two given states is that the associated reactions have the same reactants and products. We can see this by observing that the states depend on the levels and the reactions cause some changes in these levels. The only elements involved that do not change during a reaction are the modifiers.

7.2 From Bio-PEPA to ODEs

The translation into ODEs is similar to the method proposed for PEPA (reagent-centric view) [8]. It is based on the syntactic presentation of the model and on the derivation of the stoichiometry matrix $D = \{d_{ij}\}$ from the definition of the components. The entries of the matrix are the stoichiometric coefficients of the reactions and are obtained in the following way: for each component C_i consider the prefix subterms C_{ij} representing the contribution of the species i to the reaction j. If the term represents a reactant we write the corresponding stoichiometry κ_{ij} as $-\kappa_{ij}$ in the entry d_{ij}. For a product we write $+\kappa_{ij}$ in the entry d_{ij}. All other cases are null.

The derivation of ODEs from the Bio-PEPA system $\mathcal{P}$, hereafer called t_{ODE}, is based on the following steps:

(1) definition of the stoichiometry $(n \times m)$ matrix D, where n is the number of species and m is the number of molecules;
(2) definition of the *kinetic law vector* $(m \times 1)$ v_{KL} containing the kinetic laws of each reaction;
(3) association of the variable x_i with each component C_i and definition of the vector $(n \times 1)$ $\bar{x}$.

The ODE system is then obtained as:

$$\frac{d\bar{x}}{dt} = D \times v_{KL}$$

with initial concentrations $x_{i0} = l_{i0} \times h$, for $i = 1, ..., n$.

The following property holds:

Property 1 *For a biochemical network $\mathcal{M}$ and a Bio-PEPA system $\mathcal{P}$= tr_BM_BP($\mathcal{M}$), we have that $t_{ODE}(\mathcal{P}) = t_{BODE}(\mathcal{M})$, where t_{ODE} and t_{BODE} are the translation functions from Bio-PEPA and the biological system into ODEs, respectively.*

The ODE system derived from a Bio-PEPA system $\mathcal{P}$ is "equal" to the one obtained directly from the biological network itself. This means that in the translation into Bio-PEPA no information for the derivation of ODEs is lost. This result follows from the fact that in both cases we derive the stoichiometric matrix and, for construction, they are the same in both cases. However the Bio-PEPA model can collect generally more information than the respective ODEs. We have this further result:

Property 2 *Given two biochemical networks $\mathcal{M}_1$ and $\mathcal{M}_2$ we define the corresponding Bio-PEPA models $P_1 = tr\_BM\_BP(\mathcal{M}_1)$ and $\mathcal{P}_2 = tr\_BM\_BP(\mathcal{M}_2)$. Let $t_{ODE}(\mathcal{P}_1)$ and $t_{ODE}(\mathcal{P}_2)$ be the two ODE systems obtained from $\mathcal{P}_1$ and $\mathcal{P}_2$ respectively. If $t_{ODE}(\mathcal{P}_1) = t_{ODE}(\mathcal{P}_2)$ it does not imply that $\mathcal{P}_1$ is "equivalent" to $\mathcal{P}_2$.*

The above result can be easily seen with appropriate counterexamples. For example consider the Bio-PEPA models corresponding to the following sets of reactions: $\{A \xrightarrow{r} B + C; A \xrightarrow{r} B; A \xrightarrow{r} C + D\}$ and $\{A \xrightarrow{2r} B + C; A \xrightarrow{r} D\}$. The two Bio-PEPA models are different, but the ODE systems that we derived from them coincide.

7.3 From Bio-PEPA to stochastic simulation

Gillespie's stochastic simulation algorithm [25] is a widely-used method for the simulation of biochemical reactions. It deals with homogenous, well-stirred systems in thermal equilibrium and constant volume, composed of n different species that interact through m reactions. Broadly speaking, the goal is to describe the evolution the system $\mathbf{X}(t)$, described in terms of the number of molecules of each species, starting from an initial state. Every reaction is characterized by a stochastic rate constant c_j, termed the *basal rate* (derived from the constant rate r by means of some simple relations proposed in [25,42]). Using this it is possible to calculate the *actual rate* $a_j(\mathbf{X}(t))$ of the reaction, that is the probability of the reaction R_j occurring in time $(t, t + \Delta t)$ given that the system is in a specific state.

The algorithm is based essentially on the following two steps:

- calculation of the next reaction that occurs in the system;
- calculation of the time when the next reaction occurs.

We derive the information above from two conditional density functions: $p(j \mid \mathbf{X}(t)) = a_j(\mathbf{X}(t))/a_0$, that is, the probability that the next reaction is R_j and $p(\tau|\mathbf{X}(t)) = a_0 e^{a_0 \mathbf{X}(t)\tau}$, the probability that the next reaction occurs in $[t+\tau, t+\tau+d\tau]$,

where $a_0 = \sum_{v=1}^{m} a_v(\mathbf{X}(t))$.

The translation of a Bio-PEPA model to a Gillespie's simulation is similar to the approach proposed for ODEs. The main drawbacks are the definition of the rates and the correctness of the approach in the case of general kinetic laws. Indeed Gillespie's stochastic simulation algorithm supposes elementary reactions and constant rates (mass-action kinetics). If the model contains only this kind of reactions the translation is straightforward. If there are non-elementary reactions and general kinetic laws, it is a widely-used approach to consider them translated directly into a stochastic context. This is not always valid and some counterexamples have been demonstrated [7]. The authors of [7] showed that when Gillespie's algorithm is applied to Hill kinetics in the context of the transcription initiation of autoregulated genes, the magnitude of fluctuations is overestimated. The application of Gillespie's algorithm in the case of general kinetics laws is discussed by several authors [1,12]. Rao and Arkin [1] show that this approach is valid in the case of some specific kinetic laws, such as Michaelis-Menten and inhibition. However, it is important to remember that these laws are approximations, based on some assumptions that specific conditions (such as "$S \gg E$" in the case of Michaelis-Menten) hold. The approach followed here is as in [32]: we apply Gillespie's algorithm, but particular attention must be paid to the interpretation of the simulation results and to their validity.

The definition of a Gillespie model is based on:

- definition of the state vector $\bar{X}$. It is composed of n elements X_i, representing the number of molecules for each species i.
- Definition of the initial condition $\bar{X}_0$. The values are given by:

$$X_{i0} = l_{i0} \times h \times N_A \times v_i \text{ molecules}$$

  where N_A is the Avogadro's number that indicates the number of molecules in a mole of a substance and v_i is the volume size of the containing compartment V_i.
- Definition of the actual rate for each reaction. We have two cases:

(1) reactions whose dynamics is described by mass-action law and with constant rate r_j. The actual rate for the reaction is:

$$a_j(\bar{X}_j) = c_j \times f_h(\bar{X}_j)$$

where c_j is the stochastic rate constant, f_h is a function that gives the number of distinct combinations of reactant molecules and $\bar{X}_j$ are the species involved in the reaction j. The stochastic rate constant is defined in [42] as:

$$c_j = \frac{r_j}{(N_A \times v)^{n_{tot}-1}} \times \prod_{u=1}^{n_j} \kappa_{uj}!$$

where n_j is the number of distinct reactants in the reaction j, r_j is the rate of the reaction and $n_{tot} = \sum_{u=1}^{n_j} \kappa_{uj}$ is the total number of reactants [4].

Finally, the number of possible combinations of reactants is defined as

$$f_h(\bar{X}_j) = \prod_{u=1}^{n_j} \binom{X_{p(u,j)}}{\kappa_{uj}} \sim \frac{\prod_{u=1}^{n_j} (X_{p(u,j)})^{\kappa_{uj}}}{\prod_{u=1}^{n_j} \kappa_{uj}!}$$

(2) reactions with general kinetic laws $f_{\alpha_j}(\bar{k}, \bar{C})$. The actual rate is:

$$a_j(\bar{X}_j) = f_{\alpha_j}(\bar{k}, \bar{X}_j)$$

7.4 From Bio-PEPA to PRISM

PRISM [38] is a probabilistic model checker, a tool for the formal modelling and analysis of systems which exhibit random or probabilistic behaviour. PRISM has been used to analyse systems from a wide range of application domains. Models are described using the PRISM language, a simple state-based language and it is possible to specify quantitative properties of the system using a temporal logic, called *CSL* [2] (Continuous Stochatic Logic). For our purposes the underlying mathematical model of a PRISM model is a CTMC and the PRISM models we generate from Bio-PEPA correspond to the CTMCs with levels. However we present the translation separately as the models are specified in the PRISM language.

The PRISM language is composed of *modules* and *variables*. A model is composed of a number of modules which can interact with each other. A module contains a number of local variables. The values of these variables at any given time constitute the state of the module. The global state of the whole model is determined by the local state of all modules. The behaviour of each module is described by a set of commands. Each update describes a transition which the module can make if the guard is true. A transition is specified by giving the new values of the variables in the module, possibly as a function of other variables. Each update is also assigned a probability (or in some cases a rate) which will be assigned to the corresponding transition. It is straightforward to translate a Bio-PEPA system into a PRISM model. We have the following correspondences:

- The model is defined as **stochastic** (this term is used in PRISM for CTMC).

[4] We assume that all the species that are involved in the reaction as reactants are inside the same compartment with volume v.

- Each element in the set of parameters $\mathcal{K}$ is defined as a *global constant*.
- The maximum levels, the concentration steps and the volume sizes are defined as *global constants*.
- Each species component is represented by a *PRISM module*. The species component concentration is represented by a *local variable* and it can (generally) assume values between 0 and N_i. For each sub-term (i.e. reaction where the species is involved) we have a definition of a *command*. The name of the command is related to the action α (and then to the associated reaction). The guards and the change in levels are defined according to whether the element is a reactant, a product or a modifier of the reactions.
- The functional rates are defined inside an auxiliary module.
- In PRISM the rate associated with an action is the *product* of the rates of the commands in the different modules that cooperate. For each reaction, we give the value "1" to the rate of each command involved in the reaction, with the exception of the command in the module containing the functional rates. In this case the rate is the functional rate f, expressing the kinetic law. The rate associated with a reaction is given by $1 \times 1 \times ... \times f = f$, as desired.

8 Examples

This section reports the translation of three biological models into Bio-PEPA and some analysis results. The first example is taken from [26] and describes a minimal model for the cascade of post-translational modifications that modulate the activity of cdc2 kinase during the cell cycle. The second model is taken from [7] and concerns a simple genetic network with a negative feedback loop. The last example is the repressilator [22], a synthetic genetic network with an oscillating behaviour.

In the present work the stochastic and deterministic simulations are obtained exporting the Bio-PEPA system by means of the maps described in Section 7. An automatic translation is under implementation.

8.1 The Goldbeter's model

In the following we show the translation of the Goldbeter's model presented in [26] into Bio-PEPA and we discuss the kinds of analysis that are possible from it. Broadly speaking, the model describes the activity of the *protein cyclin* in the cell cycle. The cyclin promotes the activation of a *cdk* (*cdc2*) which in turn activates a *cyclin protease*. This protease promotes cyclin degradation, and therefore a negative feedback loop is obtained.

id	name	react.	prod.	mod.	kinetic laws
R1	creation of cyclin	-	C	-	v_i
R2	degradation of cyclin	C	-	-	$k_d \times C$
R3	activation of cdc2 kinase	M'	M	-	$\frac{C \times V_1}{(K_c + C)} \frac{M'}{(K_1 + M')}$
R4	deactivation of cdc2 kinase	M	M'	-	$\frac{M \times V_2}{(K_2 + M)}$
R5	activation of cyclin protease	X'	X	M	$\frac{X' \times M \times V_3}{(K_3 + X')}$
R6	deactivation of cyclin protease	X	X'	-	$\frac{X \times V_4}{K_4 + X}$
R7	degradation of cyclin triggered by protease	C	-	X	$\frac{C \times V_d \times X}{C + K_d}$

Table 2
Goldbeter model. The list of reactions.

8.1.1 The biological model

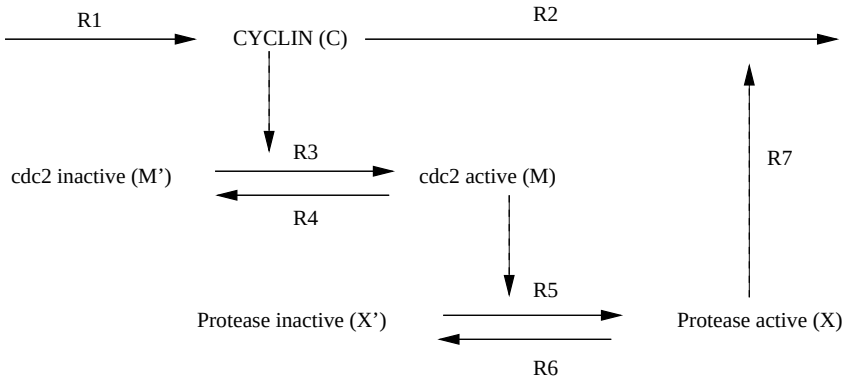


Fig. 2. Goldbeter's model.

A schema of the model is shown in Fig. 2. There are three distinct species involved:

- *cyclin*, the protein protagonist of the cycle, represented by variable C;
- *cdc2 kinase*, in both active (i.e. dephosphorylated) and inactive form (i.e. phosphorylated). The variables used to represent them are M and M', respectively;
- *cyclin protease*, in both active (i.e. phosphorylated) and inactive form (i.e. dephosphorylated). The variables are X and X'.

A detailed list of reactions is reported in Table 2. The first two reactions are the creation of cyclin and its degradation. The reactions *R3-R6* are enzymatic reactions describing the activation/deactivation of the biological species cdc2 and protease. These reactions are activated through phosphorylation/dephosphorylation. The last reaction is the degradation of the cyclin triggered by the protease.

Concerning the kinetic laws, the first two reactions have mass-action kinetics, whereas all the others have Michaelis-Menten kinetics. We have some kinetic laws in which the enzyme is explicit (reactions 3, 5), others in which is not explicit (reactions 4,

6) as it is constant and abstracted within the Michaelis-Menten parameter V_i.

8.1.2 The Bio-PEPA system

The translation of the Goldbeter's model into Bio-PEPA is achieved in the following steps.

- *Definition of the list $\mathcal{V}$*. In the model compartments are not considered. Here we add the default compartment:

$$V: \ 1.10^{-14} \text{ litre}$$

- *Definition of the set $\mathcal{N}$*. This is defined as:

$$C : h, N_C, 0.01, 0.6, V, \_; \quad M' : h, N_{M'}, 0.99, 1, V, \_; \quad M : h, N_M, 0.01, 0.7, V, \_;$$
$$X' : h, N_{X'}, 0.99, 1, V, \_; \quad X : h, N_X, 0.01, 0.65, V, \_;$$
$$Res : 1, 1, \_, \_, \_, \_; \quad CF : 1, 1, \_, \_, \_, \_;$$

The components Res and CF are added to represent degradation reactions and the synthesis of the cyclin, respectively. The information about the initial and the maximum concentrations are derived from the paper. We can fix the step size to 0.05. In this case the maximum levels are: $N_C = 12$, $N_M = 14$, $N_X = 13$, $N_{M'} = N_{X'} = 20$. If we wanted to consider the finer granularity $h = 0.01$ (corresponding to the initial concentration of some of the species) we would have $N_C = 60$, $N_M = 70$, $N_X = 65$, $N_{M'} = N_{X'} = 100$.

- *Definition of functional rates ($\mathcal{F}_R$) and parameters ($\mathcal{K}$)*. The functional rates are:

$$f_{\alpha_1} = fMA(v_i); \qquad f_{\alpha_2} = fMA(k_d); \qquad f_{\alpha_4} = fMM(V_2, K_2);$$
$$f_{\alpha_5} = fMM(V_3, K_3); \quad f_{\alpha_6} = fMM(V_4, K_4); \quad f_{\alpha_7} = fMM(V_d, K_d);$$
$$f_{\alpha_3} = fMM'((V_1, K_c, K_1), M', C) = \frac{V_1 \times C}{K_c + C} \frac{M'}{K1 + M'};$$

The parameters are those reported in the original paper and we have:

$$v_i = 0.025\ \mu M.min^{-1}; \quad k_d = 0.01\ min^{-1}; \quad V_1 = 12\ \mu M.min^{-1}; \quad K_1 = 0.02\ \mu M;$$
$$V_2 = 1.5\ \mu M.min^{-1}; \quad K_2 = 0.02\ \mu M; \quad V_3 = 12\ min^{-1}; \quad K_3 = 0.02\ \mu M;$$
$$V_d = 0.0625\ \mu M.min^{-1}; \quad V_4 = 2\ \mu M.min^{-1}; \quad K_4 = 0.02\ \mu M; \quad K_d = 0.02\ \mu M\ ;$$
$$K_c = 0.5\ \mu M$$

- *Definition of species components (Comp) and of the model component (P).*

$$
\begin{aligned}
C &\stackrel{def}{=} (\alpha_1, 1)\uparrow C + (\alpha_2, 1)\downarrow C + (\alpha_7, 1)\downarrow C + (\alpha_3, 1) \oplus C;\\
M' &\stackrel{def}{=} (\alpha_4, 1)\uparrow M' + (\alpha_3, 1)\downarrow M';\\
M &\stackrel{def}{=} (\alpha_3, 1)\uparrow M + (\alpha_4, 1)\downarrow M + (\alpha_5, 1) \oplus M;\\
X' &\stackrel{def}{=} (\alpha_6, 1)\uparrow X' + (\alpha_5, 1)\downarrow X';\\
X &\stackrel{def}{=} (\alpha_5, 1)\uparrow X + (\alpha_6, 1)\downarrow X + (\alpha_7, 1) \oplus X;\\
Res &\stackrel{def}{=} (\alpha_2, 1) \odot Res; \quad CF = (\alpha_1, 1) \odot CF;
\end{aligned}
$$

$$
C(l_{0C}) \underset{\{\alpha_3\}}{\bowtie} M(l_{0M}) \underset{\{\alpha_3,\alpha_4\}}{\bowtie} M'(l_{0M'}) \underset{\{\alpha_5,\alpha_7\}}{\bowtie} X(l_{0X}) \underset{\{\alpha_5,\alpha_6\}}{\bowtie} X'(l_{0X'}) \underset{\{\alpha_2\}}{\bowtie} Res(0) \underset{\{\alpha_1\}}{\bowtie} CF(1)
$$

The levels represent the initial values of the system and are set to $l_{0C} = l_{0M} = l_{0X} = 0$ and $l_{0M'} = l_{0X'} = 20$.

8.1.3 Analysis

In the following we report some observations about the analysis of the Bio-PEPA system.

8.1.3.1 SLTS and CTMC By considering the step size $h = 0.05$ and the number of levels given in the Bio-PEPA system we obtain a CTMC with 52 states and 185 transitions. The states are described by the vector:

$$(C(l_C), M'(l_{M'}), M(l_M), X'(l_{X'}), X(l_X))$$

where the different components can assume different values according to the possible number of levels for each species. This CTMC is not reported.

In the following we present a simpler CTMC for our model, obtained assuming $h = 1$ and considering only two levels for each species. The vector $\mathcal{N}$ is modified accordingly. We show how to define the states and the transition rates of this CTMC starting from the Bio-PEPA system and the associated transition system. The initial situation is with C, M and X absent (0) and the other elements present (1). The initial state is $(C(0), M'(1), M(0), X'(1), X(0))$. Figure 3 reports the stochastic transition system in this simplified case.

The numbers indicate the different transitions. Each transition is characterized by a label γ_i containing the information about the action type and the rate. We have:

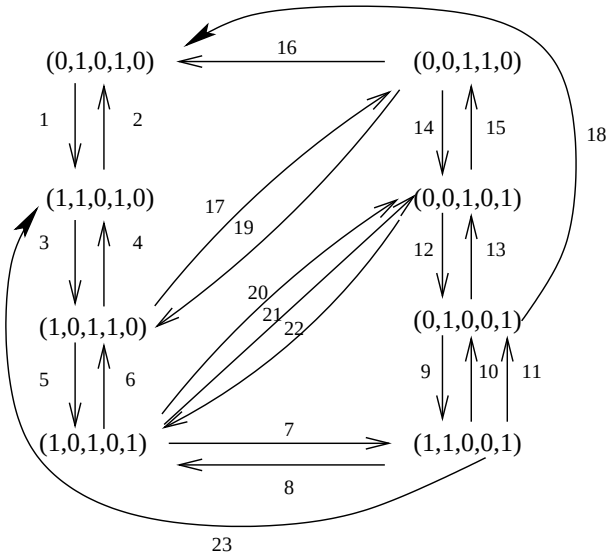


Fig. 3. The transition system for the Goldbeter's model in the case of two levels.

$$\gamma_1 = (\alpha_1, r_1) \quad \gamma_2 = (\alpha_2, r_2) \quad \gamma_3 = (\alpha_3, r_3) \quad \gamma_4 = (\alpha_4, r_4)$$
$$\gamma_5 = (\alpha_5, r_5) \quad \gamma_6 = (\alpha_6, r_6) \quad \gamma_7 = (\alpha_4, r_7) \quad \gamma_8 = (\alpha_3, r_8)$$
$$\gamma_9 = (\alpha_1, r_9) \quad \gamma_{10} = (\alpha_2, r_{10}) \quad \gamma_{11} = (\alpha_7, r_{11}) \quad \gamma_{12} = (\alpha_4, r_{12})$$
$$\gamma_{13} = (\alpha_3, r_{13}) \quad \gamma_{14} = (\alpha_5, r_{14}) \quad \gamma_{15} = (\alpha_6, r_{15}) \quad \gamma_{16} = (\alpha_4, r_{16})$$
$$\gamma_{17} = (\alpha_2, r_{17}) \quad \gamma_{18} = (\alpha_6, r_{18}) \quad \gamma_{19} = (\alpha_1, r_{19}) \quad \gamma_{20} = (\alpha_2, r_{20})$$
$$\gamma_{21} = (\alpha_7, r_{21}) \quad \gamma_{22} = (\alpha_1, r_{22}) \quad \gamma_{23} = (\alpha_6, r_{23})$$

where

$$r_1 = r_9 = r_{19} = r_{22} = v_i = 0.025, \quad r_2 = r_{10} = r_{20} = r_{17} = k_d \times M_C = 0.0001,$$
$$r_3 = r_{13} = \frac{V_1 * M_C}{K_c + M_C} \frac{M_{M'}}{(K_1 + M_{M'})} = 0.23, \quad r_4 = r_7 = r_{12} = r_{16} = \frac{V_2 \times M_M}{(K_2 + M_M)} = 2.66,$$
$$r_5 = r_{14} = \frac{V_3 \times M_M \times M_{X'}}{(K_3 + M_{X'})} = 0.117, \quad r_6 = r_{15} = r_{23} = r_{18} = \frac{V_4 \times M_X}{(K_4 + M_X)} = 2.66,$$
$$r_{11} = r_{21} = \frac{V_d \times M_C \times M_X}{(K_d + M_C)} = 0.00086$$

The states and transitions of the CTMC correspond to those of the SLTS with the exception of multiple transitions between the same two states. In this case in the CTMC we have only two transitions whose rate is the sum of the rates of two single transitions in the SLTS. In the graph above these cases correspond to the degradation of cyclin, that can happen both with and without the protease. In the CTMC the rate associated with the transition between the states $(C(1), M'(0), M(1), X'(0), X(1))$ and $(C(0), M'(0), M(1), X'(0), X(1))$ and between $(C(1), M'(1), M(0), X'(0), X(1))$ and $(C(0), M'(1), M(0), X'(0), X(1))$ is given by the sum of the rates of the two degradation reactions $k_d \times M_C + \frac{v_d \times M_C \times M_X}{(K_d + M_C)} = 0.00093\ \mu M.min^{-1}$. The rates associated with the other transitions are the ones contained in the labels γ_i above.

8.1.3.2 ODEs The stoichiometry matrix D associated with the Bio-PEPA system above is

	R1	R2	R3	R4	R5	R6	R7	
C	+1	-1	0	0	0	0	-1	x_C
M'	0	0	-1	+1	0	0	0	$x_{M'}$
M	0	0	+1	-1	0	0	0	x_M
X'	0	0	0	0	-1	+1	0	$x_{X'}$
X	0	0	0	0	+1	-1	0	x_X

The vector that contains the kinetic laws is:

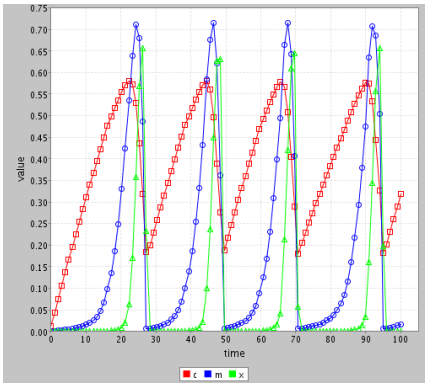
$$v_{KL}^T = \left(v_i \times 1, k_d \times x_C, \frac{V_1 \times x_C}{K_c + x_C} \frac{x_{M'}}{(K_1 + x_{M'})}, \frac{V_2 \times x_M}{(K_2 + x_M)}, \frac{V_3 \times x_M \times x_{X'}}{(K_3 + x_{X'})}, \frac{V_4 \times x_X}{(K_4 + x_X)}, \frac{v_d \times x_C \times x_X}{(K_d + x_C)} \right)$$

where "T" indicates the transpose of a vector (or a matrix). The system of ODEs is obtained as $\frac{d\bar{x}}{dt} = D \times v_{KL}$, with $\bar{x}^T := (x_C, x_{M'}, x_M, x_{X'}, x_X)$, the vector of the species variables:

$$\begin{aligned}
\frac{dx_C}{dt} &= v_i \times 1 - k_d \times x_C - \frac{v_d \times x_C \times x_X}{(K_d + x_C)}; \\
\frac{dx_{M'}}{dt} &= -\frac{V_1 \times x_C}{K_c + x_C} \times \frac{x_{M'}}{(K_1 + x_{M'})} + \frac{V_2 \times x_M}{(K_2 + x_M)}; \\
\frac{dx_M}{dt} &= \frac{V_1 \times x_C}{K_c + x_C} \times \frac{x_{M'}}{(K_1 + x_{M'})} - \frac{V_2 \times x_M}{(K_2 + x_M)}; \\
\frac{dx_{X'}}{dt} &= -\frac{V_3 \times x_M \times x_{X'}}{(K_3 + x_{X'})} + \frac{V_4 \times x_X}{(K_4 + x_X)}; \\
\frac{dx_X}{dt} &= \frac{V_3 \times x_M \times x_{X'}}{(K_3 + x_{X'})} - \frac{V_4 \times x_X}{(K_4 + x_X)};
\end{aligned}$$

The initial conditions are the ones reported in the set $\mathcal{N}$. It is worth noting that the system is equivalent, after some arithmetic manipulations, to the ODE model presented in [26]. The analysis of the model using ODEs is reported in Figure 11. The graphs coincide with results in the original paper.

8.1.3.3 PRISM The full translation of the model into PRISM is reported in the Appendix A. The number of levels, the maximum concentrations and the parame-



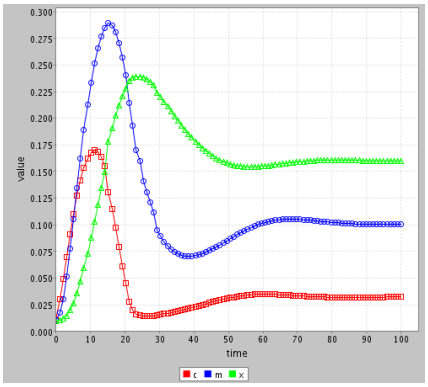


Fig. 4. ODE simulation results. In both the figures we consider the same parameters with the exception of Michaelis-Menten constants. For K_i $i = 1, 2, 3, 4$ we have that $K_i = 0.02\ \mu M$ for the graph on the left and $K_i = 40\ \mu M$ for the graph on the right. The initial values are the ones reported in the original Goldbeter's paper: $0.01\ \mu M$ for C, X and M. The simulation time is 100 minutes. In the figure on the left we have sustained oscillations whereas in the figure on the right we have no oscillations.

ters used in the kinetic laws are expressed using global constants. For each species a module is constructed. The module representing the cyclin is:

module cyclin

$cyclin : [0..Nc]$ **init** 0;

$[creationC]\ cyclin < Nc \rightarrow (cyclin' = cyclin + 1);$

$[degradationC]\ cyclin > 0 \rightarrow (cyclin' = cyclin - 1);$

$[activationM]\ cyclin > 0 \rightarrow (cyclin' = cyclin);$

$[degradationCX]\ cyclin > 0 \rightarrow (cyclin' = cyclin - 1);$

endmodule

The variable cyclin is *local* and represents the species "cyclin". The possible values are $[0..Nc]$ (where Nc is the maximum level for cyclin) and the initial value is set to 0. Cyclin is involved in four different reactions represented by four commands. The name in the square brackets denotes the reaction. The *guards* are defined according to whether cyclin is a reactant, product or modifier of the reaction (this can be derived from the Bio-PEPA specification of the model). The rate associated with each command is "1" with the exception of the command in the module describing the functional rates. The functional rates are defined in a specific module.

8.1.4 Extension of the model with a control mechanism based on inhibition

The authors of [23] proposed an extension of Goldbeter's model in order to represent a control mechanism for the cell division cycle (CDC). Their approach is based on the introduction of a protein that binds to and inhibits one of the proteins

involved in the CDC. This influences the initiation and the conclusion of cell division and modulates the frequency of oscillations. Their approach is based on the basic biochemical network of the CDC oscillations and not on the details of the model so that it may work for other models of this kind. One possible extension for Goldbeter's model is reported in Figure 5.

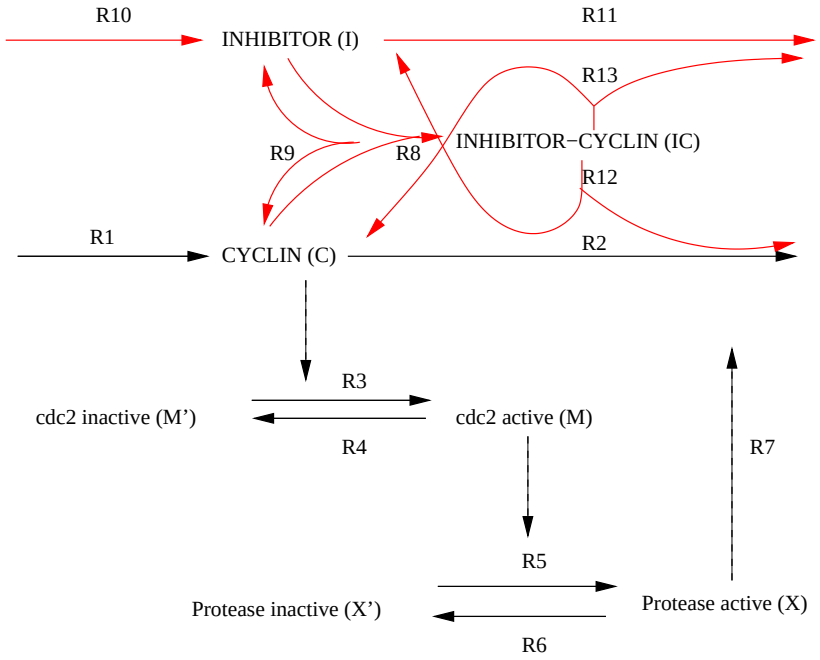


Fig. 5. Extension of the Goldbeter's model. An inhibitor is added.

Generally speaking, given a general CDC model with l proteins U_1, U_2, ...,U_l, Gardner *et al.* show that the ODE model is modified in the following way (see [23] for details):

$$
\begin{aligned}
\frac{dU_1}{dt} &= f_1(U_1, U_2, \cdots, U_l) - a_1 \times U_1 \times Y + (a_2 + \theta \times d_1);\\
\frac{dU_2}{dt} &= f_2(U_1, U_2, \cdots, U_l);\\
&\vdots\\
\frac{dU_l}{dt} &= f_l(U_1, U_2, \cdots, U_l);\\
\frac{dY}{dt} &= v_s - d_1 \times Y - a_1 \times U_1 \times Y + (a_2 + \theta \times k_d) \times Z;\\
\frac{dZ}{dt} &= a_1 \times U_1 \times Y - (a_2 + \theta \times d_1 + \theta \times k_d) \times Z;
\end{aligned}
$$

where:

- $f_i(U_1, U_2, ...)$ with $i = 1, 2, ..., l$ are the functions of the standard model;

- U_1 is the concentration of the target protein of the inhibitor, Y is the inhibitor and Z denotes the concentration of the inhibition-target complex. U_2,...,U_l are the other proteins involved in the cycle;
- a_1 and a_2 are the constant rates for the binding and for the release;
- v_s and d_1 are the rate for the inhibitor synthesis and degradation;
- $\theta < 1$ is the fraction of the degradation rates for the complex Z.

In the following we show how to modify the Bio-PEPA system in order to capture the new reactions and species. Bio-PEPA offers a *compositional approach*: it is possible to compose the whole system by defining the simple subcomponents that compose it. As observed in Section 1, compositionality is one of the main properties of process algebras, that makes them particularly useful in the case of complex models. In our example, the new reactions and species are indeed added in a straightforward way, with minor modifications of the system specification. Broadly speaking, we need to define components for the new species, some new terms to describe the new reactions and new functional rates. Finally, the new components are added to the system component.

Here we consider $l = 3$, $U_1 = C$, $U_2 = M$ and $U_3 = X$. However we can obtain modulation of CDC frequency by using an inhibitor of any of the proteins.

We need to extend the Bio-PEPA model in the following way:

$$
\begin{array}{lcl}
C & \stackrel{def}{=} & \cdots + (\alpha_8, 1)\downarrow C + (\alpha_9, 1)\uparrow C + (\alpha_{12}, 1)\uparrow C \\
\vdots & & \vdots \\
Res & \stackrel{def}{=} & \cdots + (\alpha_{11}, 1) \odot Res \quad CF \stackrel{def}{=} \cdots + (\alpha_{10}, 1) \odot CF \\
I & \stackrel{def}{=} & (\alpha_8, 1)\downarrow I + (\alpha_9, 1)\uparrow I + (\alpha_{10}, 1)\uparrow I + (\alpha_{11}, 1)\downarrow I + (\alpha_{13}, 1)\uparrow I \\
IC & \stackrel{def}{=} & (\alpha_8, 1)\uparrow IC + (\alpha_9, 1)\downarrow IC + (\alpha_{12}, 1)\downarrow IC + (\alpha_{13}, 1)\downarrow IC
\end{array}
$$

where I stands for the inhibitor and IC for the inhibitor-cyclin complex in Figure 5. The new functional rates, all described by mass-action kinetics, are reported below.

$$
\begin{array}{lll}
f_{\alpha_8} = v_s; & f_{\alpha_9} = fMA(d_1); & f_{\alpha_{10}} = fMA(a_1); \\
f_{\alpha_{11}} = fMA(a_2); & f_{\alpha_{12}} = fMA(\theta \times d_1); & f_{\alpha_{13}} = fMA(\theta \times k_d)
\end{array}
$$

The list of parameters is extended in order to consider the new values.

Finally the Bio-PEPA model is:

$$C(l_{0C}) \underset{\{\alpha_3\}}{\bowtie} M(l_{0M}) \underset{\{\alpha_3,\alpha_4\}}{\bowtie} M'(l_{0M'}) \underset{\{\alpha_5,\alpha_7\}}{\bowtie} X(l_{0X}) \underset{\{\alpha_5,\alpha_6\}}{\bowtie} X'(l_{0X'})$$
$$\underset{\{\alpha_2\}}{\bowtie} Res(0) \underset{\{\alpha_1\}}{\bowtie} CF(1) \underset{\{\alpha_8,\alpha_9,\alpha_{10},\alpha_{11}\}}{\bowtie} I(l_{0I}) \underset{\{\alpha_8,\alpha_9,\alpha_{12},\alpha_{13}\}}{\bowtie} IC(l_{0IC})$$

The results of the ODE simulations corresponding to the new model are reported in Fig. 6.

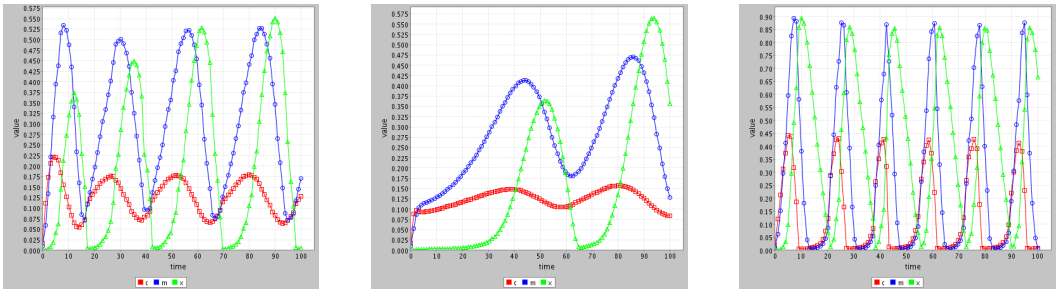


Fig. 6. ODE simulation results for the extended model. The parameters of Goldbeter's model are as before. For the new parameters, in all the graphs $d_1 = 0.05$, $\theta = 0.1$ and $K_{diss} = \frac{a_1}{a_2} = 1$. In the model on the left $a_1 = a_2 = 0.3$ and $v_s = 0.6$, in the graph in the middle $a_1 = a_2 = 0.7$ and $v_s = 1.4$ and in the graph on the right $a_1 = a_2 = 0.05$ and $v_s = 0.1$. The initial values of C, X, M and I are $0.01\mu M$. Simulation time is 100 minutes.

8.2 A simple genetic network

Information processing in biological cells is often implemented by a *genetic network*. The state of such a network is represented by the concentrations and locations of the different species of molecules. The interactions between these molecules occur in a random fashion. In order to prevent large fluctuations in the number of molecules of some species, the genetic network itself can contain negative feedback mechanisms that suppress these fluctuations.

In order to show how to model genetic networks in Bio-PEPA, we consider a model from [7]. The model describes a general genetic network with negative feedback through dimers, such as the one representing the control circuit for the λ repressor protein CI of λ-phage in *E.Coli*. Here we focus on the second model presented in the paper, which uses an inhibition reaction to describe the negative feedback loop (3 species and 5 reactions, one of which is reversible).

In the present work the stochastic and deterministic simulations are obtained exporting the Bio-PEPA system by means of the maps described in the previous section.

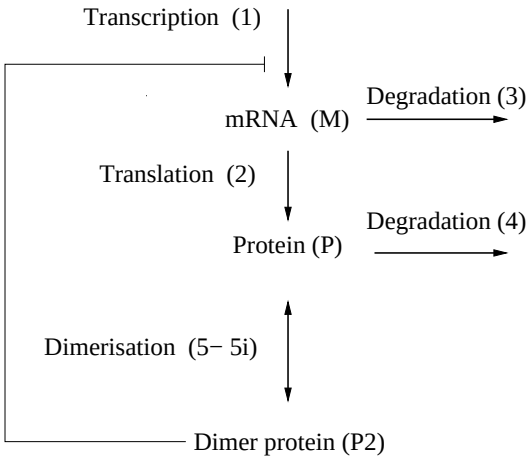


Fig. 7. Genetic network model

8.2.1 The biological model

A schema of the model is reported in Figure 7. The model is composed of three biological entities that interact with each other through five reactions (of which one is reversible). The biological entities are the mRNA molecule (M), the protein in monomer form (P) and the protein in dimeric form ($P2$). The first reaction (1) is the transcription of the mRNA (M) from the genes/DNA (not considered explicitly). The protein P in the dimer form ($P2$), which is the final result of the network, has an inhibitory effect on this process. The second reaction (2) is the translation of the protein P from M. Another two reactions represent the degradation of M (3) and the degradation of P (4). Finally there is the dimerization of P and its inverse process (5,5i). All the reactions are described by mass-action kinetics with the exception of the first reaction, which has an inhibition/Michaelis-Menten kinetics.

8.2.2 The Bio-PEPA system

The translation of the model in Bio-PEPA is based on the following steps:

- *Definition of compartments.* The only compartment is defined as $v_{cell}: \ 1\ (nM)^{-1}$.
- *Definition of the set* $\mathcal{N}$.

$$C : M : 1, 1, 1, 1, v_{cell}, nM; \quad P : 30, 2, 0, 60, v_{cell}, nM;$$
$$P2 : 30, 6, 0, 180, v_{cell}, nM; \quad Res : 1, 1, \_, \_, \_, \_; \quad CF : 1, 1, \_, \_, \_, \_;$$

From the original paper the maximum concentrations are $M_M = 1\ nM$, $M_P = 60\ nM$ and $M_{P2} = 180\ nM$. We can consider $h_M = 1\ nM$ and $h_P = h_{P2} = 30\ nM$. Indeed the stoichiometry of P in the dimerization reaction is 2 and we need to consider at least two levels for P. The maximum levels for the three species are: $N_M = 1$, $N_P = 2$ and $N_{P2} = 6$.

- *Definition of the set of functional rates $\mathcal{F}_R$.*

$$f_{\alpha_1} = fI((v, K_M), [P2, CF]) = \frac{v \times CF}{K_M + P2};$$
$$f_{\alpha_2} = fMA(k2); \qquad f_{\alpha_3} = fMA(k3); \qquad f_{\alpha_4} = fMA(k4);$$
$$f_{\alpha_5} = fMA(k5); \qquad f_{\alpha_{5i}} = fMA(k5i)$$

  where the suffix of the action type α refers to the number of the reaction as reported in Fig. 7.
- *Definition of the set of parameters.* The parameter values are

$$K_M = 356\ nM; \quad v = 2.19\ s^{-1}; \quad k_2 = 0.043\ s^{-1}; \quad k_3 = 0.0039\ s^{-1};$$
$$k_4 = 0.0007\ s^{-1}; \quad k_5 = 0.025\ s^{-1} nM^{-1}; \quad k_{5i} = 0.5\ s^{-1}$$

- *Definition of the set of species components and of the model component.*

$$M \stackrel{def}{=} (\alpha_2, 1) \oplus M + (\alpha_3, 1)\downarrow M + (\alpha_1, 1)\uparrow M;$$
$$P \stackrel{def}{=} (\alpha_4, 1)\downarrow P + (\alpha_5, 2)\downarrow P + (\alpha_{5i}, 2)\uparrow P) + (\alpha_2, 0)\uparrow P;$$
$$P2 \stackrel{def}{=} (\alpha_1, 1) \ominus P2 + (\alpha_{5i}, 1)\downarrow P2 + (\alpha_5, 1)\uparrow P2;$$
$$Res \stackrel{def}{=} (\alpha_3, 1) \odot Res + (\alpha_4, 1) \odot Res;$$
$$CF \stackrel{def}{=} (\alpha_1, 1) \odot CF;$$
$$(((CF(1) \underset{\{\alpha_1\}}{\bowtie} M(0)) \underset{\{\alpha_2\}}{\bowtie} P(0)) \underset{\{\alpha_5, \alpha_{5i}\}}{\bowtie} P2(0)) \underset{\{\alpha_3, \alpha_4\}}{\bowtie} Res(0)$$

8.2.3 Analysis

The model is amenable to a number of different analyses as we report in the following paragraphs.

8.2.3.1 SLTS and CTMC From the Bio-PEPA model we can derive the SLTS and the CTMC. The transition system consists of 42 states and 108 transitions, in the case we consider the information about species listed above.

The states are described by the levels of the single components. Specifically, we can define a state using a vector $(CF(l_1), M(l_2), P(l_3), P2(l_4), Res(l_5))$, where l_i, for $i = 1, ..., 5$, represents the level of each component. The parameter l_i can assume the values 0 and 1 in the case of M, the values 0, 1, 2 for P and values between 0 and 6 for $P2$. Res and CF can assume only one value (0 and 1, respectively). The labels γ_t of the stochastic transition system contain the action type α_j and the rate r_{α_j}, calculated by applying the associated function f_{α_j} to the quantitative information collected in the labels of the capability relation and dividing this by the step size of the reactants/products involved in the reaction. These rates are the ones associated with the CTMC transitions.

8.2.3.2 ODEs and Gillespie A second kind of analysis concerns differential equations. The stoichiometry matrix D associated with the system is

	R1	R2	R3	R4	R5	R5i	
CF	0	0	0	0	0	0	x_{CF}
M	+1	0	-1	0	0	0	x_1
P	0	+1	0	-1	-2	+2	x_2
P2	0	0	0	0	+1	-1	x_3
Res	0	0	0	0	0	0	x_{Res}

The kinetic-law vector is $v_{KL}^T = (\frac{v \times x_{CF}}{K+x_3}; k_2 \times x_1; k_3 \times x_1; k_4 \times x_2; k_5 \times x_2^2; k_{5i} \times x_3)$. The system of ODEs is obtained as $\frac{d\bar{x}}{dt} = D \times v_{KL}$:

$$\frac{dx_1}{dt} = \frac{v \times 1}{K + x_3} - k_3 \times x_1$$
$$\frac{dx_2}{dt} = k_2 \times x_1 - k_4 \times x_2 - 2 \times k_5 \times x_2^2 + 2 \times k_{5i} \times x_3$$
$$\frac{dx_2}{dt} = k_5 \times x_2^2 - k_{5i} \times x_3$$

The derivation of the Gillespie's simulation model is straightforward and not reported here.

The simulation results are depicted in Figure 12. We consider both deterministic and stochastic simulation. The two simulation graphs show the same behaviour (with the exception of some noise in the Gillespie's simulation), as expected.

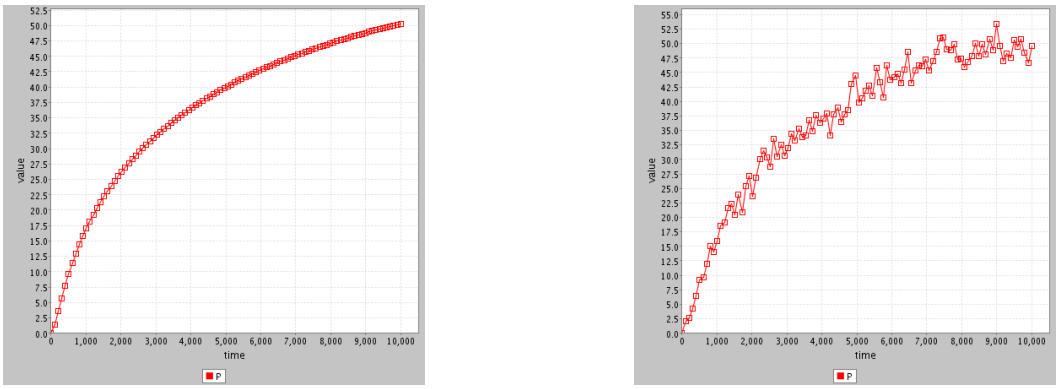


Fig. 8. ODE and Gillespie simulation results. In the case of Gillespie we consider 10 runs. In both cases the rates are as in the original paper.

8.2.3.3 PRISM The full translation of the model into PRISM is reported in Appendix B. Each species is represented by a PRISM module and the reactions in which it is involved are captured by commands. In the following we report the definition of the modules representing the protein in the monomer and dimer form respectively.

module p

$p : [0..Np]$ **init** 0;

$[a2]\ p < Np \rightarrow (p' = p + 1);$

$[a4]\ p > 0 \rightarrow (p' = p - 1);$

$[a5]\ p > 0 \rightarrow (p' = p - 2);$

$[a5i]\ p < Np \rightarrow (p' = p + 2);$

endmodule

module pd

$pd : [0..Npd]$ **init** 0;

$[a5i]\ pd > 0 \rightarrow (pd' = pd - 1);$

$[a5]\ pd < Npd \rightarrow (pd' = pd + 1);$

endmodule

The variables p and pd are *local* with respect to each of the two modules and represent the species "protein in monomer form" and "protein in dimer form", respectively. The possible values are $[0..Np]$ for p and $[0..Npd]$ for pd, while the initial values are 0. The monomer P is involved in four different reactions while the dimer form $P2$ in just two. We have an additional module with the functional rates.

Properties of the system can be expressed formally in *CSL* and analysed against the constructed model. Two examples of possible queries are considered below. A first query considers the probability that the monomer is at level i at time T. The property is expressed by the form "P =?[...]", that returns a numerical value representing the probability of the proposition inside the square brackets. In our case the query is P =?[$true\ \ U[T,T]\ \ p = i$], where U is *the bounded until operator* and $[T,T]$ indicates a single time instant. A property of the form "*prop1* U[*time*] *prop2*" is true for a path if *time* defines an interval of real values and the path is such that *prop2* becomes true at a time instant which falls within the interval and *prop1* is true in all time instants up to that point. The second query concerns the proportion of the protein in monomer form (P) relative to the total quantity of the protein (i.e. $P+P2$). In order to define this property, we need a reward structure. State rewards can be specified using multiple reward items, each of the form "*guard:reward*;", where guard is a predicate and reward is an expression. States of the model which satisfy the predicate in the guard are assigned the corresponding reward. Specifically, in our case we define the reward:

rewards

$true : \frac{p}{(p+pd)}$;

endrewards

This reward assigns the value $\frac{p}{(p+pd)}$ to each state of the system. We can ask for the frequency of P by using the query $R =?[I = T]$. This is an *instantaneous reward property*, i.e. it refers to the reward of a model at a particular instant in time T. The property "$I = T$" associates with a path the reward in the state of that path when exactly T time units have elapsed. The letter "R" indicates that the property refers to a reward structure. The results of the two queries are reported in Fig. 9.

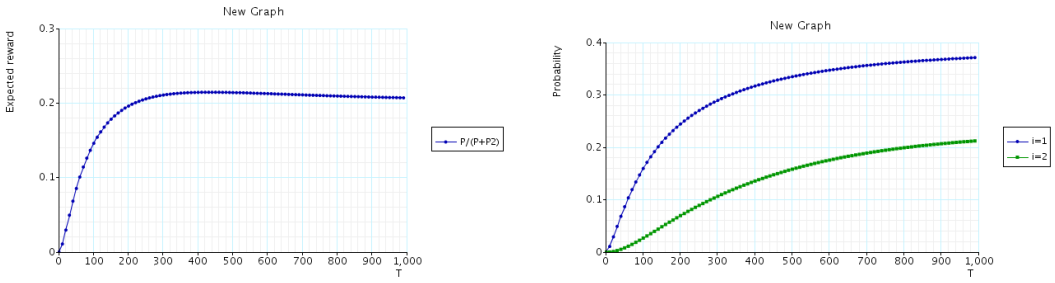


Fig. 9. PRISM query results. In the figure on the left reports the graph of the proportion of monomer P over the total protein with respect to time. On the right it is depicted the probability that the monomer protein is at level 1 and 2, with respect to time.

8.3 The repressilator

The *repressilator* is a synthetic genetic regulatory network with oscillating behaviour reported in [22]. The repressilator consists of three genes connected in a *feedback loop*, such that the transcription of a gene is inhibited by one of the other proteins. In the following we present the translation of the original model into Bio-PEPA and we report some analysis results.

8.3.1 The biological model

A schema of network is reported in Figure 10.

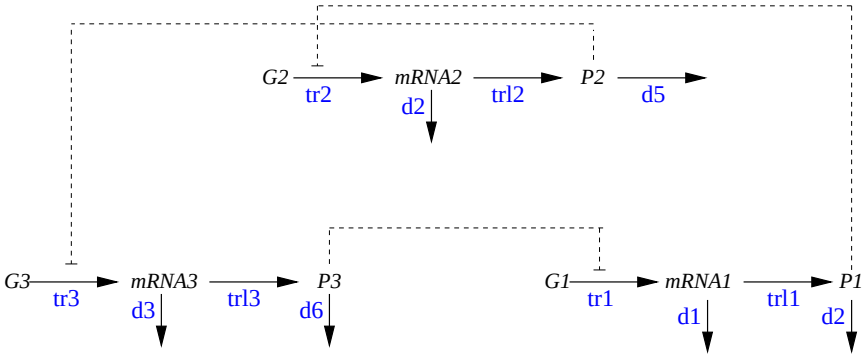


Fig. 10. Repressilator model.

The species involved are:

- three kinds of genes, hereafter denoted *G1, G2, G3*. These represent the genes *lacI*, *tetR* and *cI*, respectively;
- the mRNAs transcribed from the three genes, hereafter denoted *mRNA1, mRNA2, mRNA3,* respectively;
- the proteins corresponding to the three genes, denoted *P1, P2, P3*, respectively. These represents the protein associated with the previous genes and are *LacI, TetR, CI*.

The reactions are:

- the *transcription* of the three mRNAs with inhibition by one of the proteins. These reactions are indicated as $tr1$, $tr2$, $tr3$. The genes are constant and are kept implicit;
- the *translation* of mRNAs into the proteins, indicated as $trl1$, $trl2$, $trl3$;
- *degradation* of both mRNAs and proteins, indicated as d_i with $i = 1, ..., 6$.

The transcription reactions are described by Hill kinetics, while the other reactions have mass-action kinetic laws.

8.3.2 The Bio-PEPA system

The definition of the Bio-PEPA corresponding to the repressilator model is reported below. The parameters and the initial concentrations are one of the possibilities defined in the paper [22].

- *Definition of compartments.* There are no compartments defined explicitly in the model. We consider the default compartment:

$$v_{Cell} : 1;$$

- *Definition of the set* $\mathcal{N}$ It is defined as:

$$mRNA1 : 1, 1, 0, \_, v_{Cell}, \_; \quad mRNA2 : 1, 1, 0, \_, v_{Cell}, \_; \quad mRNA3 : 1, 1, 0, \_, v_{Cell}, \_;$$

$$P1 : 1, 1, 5, \_, v_{Cell}, \_; \quad P2 : 1, 1, 0, \_, v_{Cell}, \_; \quad P3 : 1, 1, 15, \_, v_{Cell}, \_;$$

$$Res : 1, 1, \_, \_, v_{Cell}, \_; \; CF : 1, 1, \_, \_, v_{Cell}, \_;$$

  It is worth noting that in the original model the genes are not represented explicitly. In Bio-PEPA we introduce CF to define the transcription. For all the species we consider two levels (*high* and *low*) and step $h = 1$. The initial values (third components) are the ones reported in the paper.
- *Definition of the set* $\mathcal{F}_R$ *and of the set of parameters*. The set of functional rates is:

$$
\begin{aligned}
f_{tr1} &= fI((\alpha, \alpha 0), [P3], 2) = \tfrac{\alpha}{1+P3^2} + \alpha 0; \\
f_{tr2} &= fI((\alpha, \alpha 0), [P1], 2) = \tfrac{\alpha}{1+P1^2} + \alpha 0; \\
f_{tr3} &= fI((\alpha, \alpha 0), [P2], 2) = \tfrac{\alpha}{1+P2^2} + \alpha 0; \\
f_{trl1} &= fMA(\beta) \\
f_{trl2} &= fMA(\beta) \\
f_{trl3} &= fMA(\beta) \\
f_{di} &= fMA(1), \quad i = 1, 2, 3, 4, 5, 6;
\end{aligned}
$$

All the three repressors have same behaviour except for their DNA-binding specificities. We assume that all the degradation reactions have rate 1.

The other parameters are:

$$\alpha = 250; \quad \alpha 0 = 0; \quad \beta = 5$$

These parameters have the following meaning:

· $\alpha 0$ is the number of protein copies per cell produced from a given promoter type during growth in the presence of saturating amounts of the repressor. In the case of the absence of the repressor this number is $\alpha 0 + \alpha$;

· β is the ratio of the protein decay rate to the mRNA decay rate.

- *Definition of the species components*. The species components are:

$$
\begin{aligned}
mRNA1 &\stackrel{def}{=} (d1, 1)\downarrow mRNA1 + (tr1, 1)\uparrow mRNA1 + (trl1, 1) \oplus mRNA1; \\
mRNA2 &\stackrel{def}{=} (d2, 1)\downarrow mRNA2 + (tr2, 1)\uparrow mRNA2 + (trl2, 1) \oplus mRNA2; \\
mRNA3 &\stackrel{def}{=} (d3, 1)\downarrow mRNA3 + (tr3, 1)\uparrow mRNA3 + (trl3, 1) \oplus mRNA3; \\
P1 &\stackrel{def}{=} (d4, 1)\downarrow P1 + (trl1, 1)\uparrow P1 + (tr3, 1) \ominus P1; \\
P2 &\stackrel{def}{=} (d5, 1)\downarrow P2 + (trl2, 1)\uparrow P2 + (tr1, 1) \ominus P2; \\
P3 &\stackrel{def}{=} (d6, 1)\downarrow P3 + (trl3, 1)\uparrow P3 + (tr2, 1) \ominus P3; \\
CF &\stackrel{def}{=} (tr1, 1) \odot CF + (tr1, 1) \odot CF + (tr1, 1) \odot CF; \\
Res &\stackrel{def}{=} (d1, 1) \odot Res + (d2, 1) \odot Res + (d3, 1) \odot Res \\
&\quad +(d4, 1) \odot Res + (d5, 1) \odot Res + (d6, 1) \odot Res;
\end{aligned}
$$

- *Definition of the model component*. The model is defined as:

$$
\begin{aligned}
((((((M1(l_{M10}) <> M2(l_{M20})) \bowtie M3(l_{M30})) \underset{\{trl1,tr3\}}{\bowtie} P1(l_{P10})) \underset{\{trl2,tr1\}}{\bowtie} P2(l_{P20})) \underset{\{trl3,tr2\}}{\bowtie} \\
P3(l_{P30})) \underset{\{tr1,tr2,tr3\}}{\bowtie} \underset{\{tr1,tr2,tr3\}}{\bowtie} CF(1)) \underset{\{d1,d2,d3,d4,d5,d6\}}{\bowtie} Res(0)
\end{aligned}
$$

The initial levels are defined according to the initial values of the model.

8.3.3 Analysis

We consider both ODEs and stochastic simulation using Gillespie's algorithm. The analysis results are reported in Figures 11 and 12. In Figure 11 we have used the parameters reported in the paper. On the left, the ODE simulation results are reported. An oscillating behaviour is shown by all the three proteins. The figure on the right shows a run by using Gillespie's algorithm. We have an oscillating behaviour also in this case, but the trajectories are different with respect to the deterministic simulation. The difference between determistic and stochastic simulations is probably due to the use of Hill kinetics with Gillespie. Indeed, as discussed in Section 7.3 the use of this kinetic law with Gillespie can lead to different results from the expected ones. Note that varying the values of α and β for the different elements we obtain different amplitudes for the oscillations. In the case of Figure 12, the three proteins reach a steady state, both with ODE and stochastic simulation.

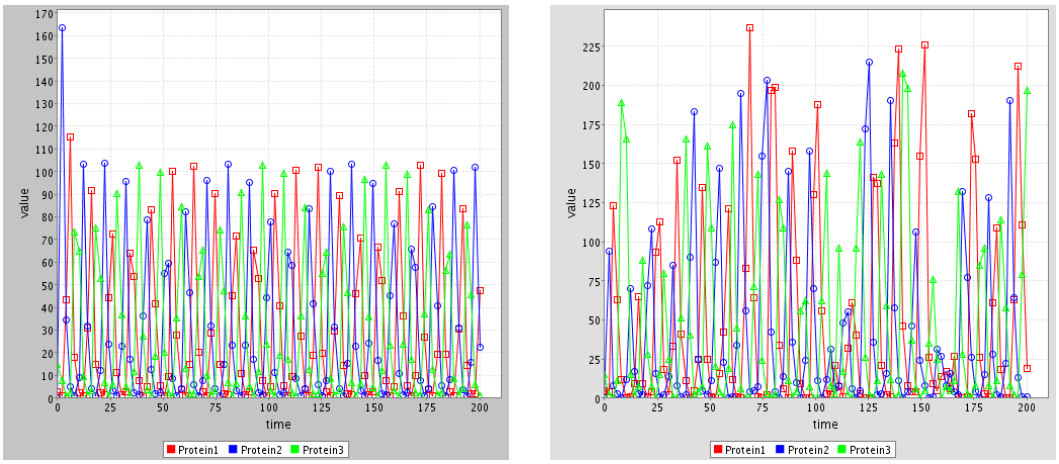


Fig. 11. Analysis of the model: ODE (on the left) and Gillespie (on the right). The parameters are the ones reported in the text. In the case of Gillespie's simulation 1 run is considered.

9 Conclusions

In this paper we have presented Bio-PEPA, a modification of the process algebra PEPA for the modelling and the analysis of biochemical networks. Bio-PEPA allows us to represent explicitly some features of biochemical networks, such as stoichiometry and general kinetic laws. Thus not only elementary reactions with constant rates, but also complex reactions described by general kinetic laws can be considered. Indeed each reaction in the model is associated with an action type and with a functional rate. The potential to consider various kinds of kinetic laws permits us to model a vast number of biochemical networks. Indeed complex reactions are frequently found in models as abstractions of sequences of elementary steps and reducing to elementary reactions is often impossible and undesirable.

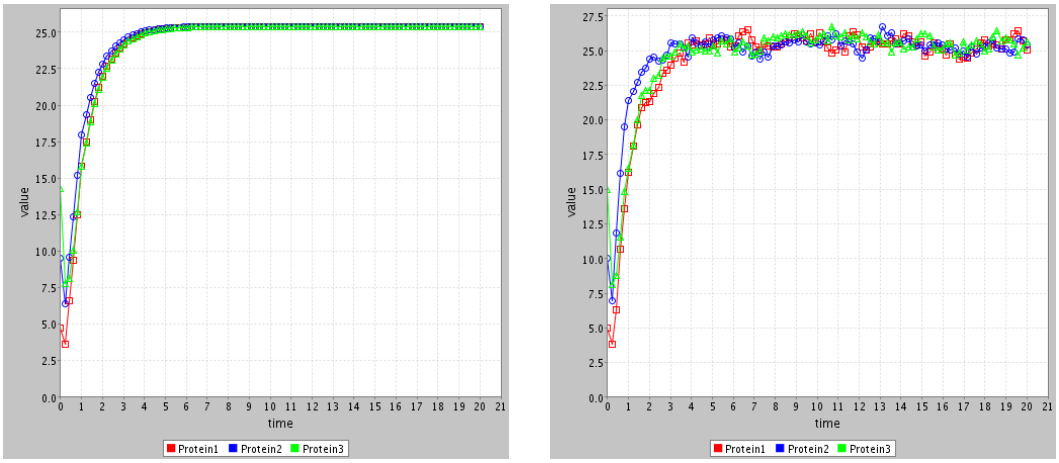


Fig. 12. Analysis of the model: ODE (on the left) and Gillespie (on the right). The parameters are different from the ones reported in the text. The parameter $\alpha 0$ is 25 and the initial values are $P1 = 5$, $P2 = 10$ and $P3 = 15$. In Gillespie, 100 runs are considered.

Bio-PEPA is in enriched with some notions of equivalence. We have presented definitions of isomorphism and strong bisimulation which are similar to the relations defined for PEPA in [29]. These equivalences are quite strict. A further investigation concerns the definition of other forms of equivalence, more appropriate for studying biological systems.

A principal feature of Bio-PEPA is the possibility of mapping the system to different kinds of analysis. In this work we have shown how to derive a CTMC from a Bio-PEPA model and we have discussed the derivation of ODEs, stochastic simulation and PRISM models. Indeed Bio-PEPA has been defined as an intermediate language for the formal representation of the model. We have extended the definition of CTMC with levels, defined in [11], to the case of general kinetic laws and to different levels for the species. The main benefit of this approach is a reduction in the state space. Some assumptions are made. First of all all the species must have a finite maximum concentration. This is to ensure a finite state space in the corresponding CTMC, making numerical solution feasible. However, we can have a species without a limiting value. In these cases we can consider a maximum level for the values greater than a certain (high) value. A second point concerns the assumption that all the species have the same step size. This may be a problem when the species can have maximum concentrations belonging to different concentration scales. In this case some species can have only few levels whereas other can have many. Furthermore, some species (for instance genes) are present in the system only in few copies and in this case the representation in terms of continuous concentration is wrong. In order to handle this situation, Bio-PEPA could be enriched with discrete variables. The possibility to consider different step sizes and discrete variables is a topic for future work.

The different kinds of analysis proposed for Bio-PEPA are strongly related. An area for further work will concern a deeper study of these relationships, in partic-

ular for the CTMC with levels and Gillespie models. An outstanding problem is the application of Gillespie's stochastic simulation with general kinetic laws. The approach proposed in this work is to use Gillespie simulation also in this context, but to be careful about the interpretation of the results. An important topic for the future will be deeper investigation of the relation between Gillespie simulation and general kinetic laws. Another aspect that needs further study is the determination of the number of concentration levels for each species. The definition of these numbers is critical for the definition of the CTMC and must be done carefully. Indeed for some analyses (ODEs and Gillespie simulation) two levels are enough to capture the behaviour of some systems, but for numerical analysis of the CTMC and PRISM model checking a finer granularity for levels is necessary to capture the full behaviour of the system.

Another aspect that we will consider in further work is the possibility of incorporating *SBML-like events*. SBML events describe the time and the form of explicit instantaneous state changes in the model. For example, an event may describe that one species quantity is halved when another species quantity exceeds a given threshold value. An *Event* object defines when the event can occur, the variables that are affected by the event, and how the variables are affected. The idea is to add the definition of the events to the system and to investigate some possible analyses that can be carried out.

Finally, a tool for the analysis of biochemical networks using Bio-PEPA is under implementation and a translation from SBML into Bio-PEPA is planned.

A Appendix A: PRISM specification of the Goldbeter's model

```
//Kind of model
stochastic

//Volume
const double cell = 1;

// Levels
const int Nc = 1;
const int Nm = 1;
const int Nx = 1;
const int Nxi = 1;
const int Nmi = 1;

//Steps
const double Hc = 0.01;
const double Hm = 0.01;
const double Hx = 0.01;
```

```
const double Hxi = 0.01;
const double Hmi = 0.01;

//Parameters
const double vi = 0.05;
const double vd = 0.025;
const double kd = 0.01;
const double Kc = 0.5;
const double V1 = 3;
const double V3 = 1;
const double Kd = 0.2;
const double V2 = 1.5;
const double V4 = 0.5;
const double K1 = 0.005;
const double K2 = 0.005;
const double K3 = 0.005;
const double K4 = 0.005;


//Modules

//module Cyclin
module cyclin
cyclin : [0..Nc] init 0;
[creationC] cyclin<Nc --> 1: (cyclin' = cyclin+1);
[degradationC] cyclin>0 --> 1: (cyclin' = cyclin-1);
[activationM] cyclin>0 --> 1: (cyclin' = cyclin);
[degradationCX] cyclin>0 --> 1:  (cyclin' = cyclin-1);
endmodule

//module kinase inactive
module kinasei
kinasei : [0..Nmi] init  1;
[activationM] kinasei>0 --> 1: (kinasei' = kinasei-1);
[deactivationM] kinasei<Nmi --> 1: (kinasei'= kinasei+1);
endmodule

//module kinase active
module kinase
kinase : [0..Nm] init 0;
[activationM] kinase<Nm --> 1: (kinase'= kinase+1);
[deactivationM] kinase>0 --> 1: (kinase' = kinase-1);
[activationX] kinase>0 --> 1: (kinase' = kinase);
endmodule

//module protease inactive
module proteasei
proteasei : [0..Nxi] init 1;
```

```
[activationX] proteasei>0 --> 1: (proteasei'= proteasei-1);
[deactivationX] proteasei<Nxi --> 1: (proteasei'= proteasei+1);
endmodule

//module protease active
module protease
protease : [0..Nx] init 0;
[activationX] protease<Nx  --> 1: (protease' = protease+1);
[deactivationX] protease>0 --> 1: (protease' = protease-1);
[degradationCX] protease>0 --> 1: (protease' = protease);
endmodule

module Functional_rates
dummy: bool init true;
[creationC] cyclin<Nc --> vi/Hc: (dummy'=dummy);
[degradationC] cyclin<Nc --> (kd*cyclin*Hc)/Hc: (dummy'=dummy);
[activationM] cyclin>0 & kinasei>0 -->
   ((cyclin*Hc*V1 )/(Kc + cyclin*Hc))*((kinasei*Hmi)/(K1+kinasei*Hmi))
   *(1/Hmi): (dummy'=dummy);
[activationX] kinase>0 & proteasei>0 -->
   (kinase*Hm*proteasei*Hxi*V3/(K3+proteasei*Hxi))*(1/Hxi):
   (dummy'=dummy);
[deactivationM] kinase>0 --> ((kinase*Hm*V2)/(K2 +kinase*Hm))*(1/Hm):
   (dummy'=dummy);
[deactivationX] protease>0 --> (protease*Hx*V4/(K4 + protease*Hx))
   *(1/Hx): (dummy'=dummy);
[degradationCX] cyclin>0 & protease>0 -->
   ((cyclin*Hc*vd *protease*Hx)/(cyclin*Hc + Kd))*(1/Hc): (dummy'=dummy);
endmodule
```

B AppendixB: PRISM specification of the genetic network model

```
//Kind of model
stochastic

//Volume
const double cell = 1*10^{-6};

//Levels
const int NCF = 1;
const int Nm = 1;
const int Np = 2;
const int Npd = 6;

//Steps
```

```
const double HCF = 1;
const double Hm = 1.0;
const double Hp = 30.0;
const double Hpd = 30.0;

//Parameters
const double v = 2.19;
const double KM = 356;
const double k3 = 0.0039;
const double k2 = 0.043;
const double k4 = 0.0007;
const double k5 = 0.025;
const double k5i = 0.5;

//Modules
module CF
CF: [0..NCF] init NCF;
[a1] CF>0 --> 1: (CF'=CF);
endmodule

module p
p: [0..Np] init 0;
[a2] p<Np --> 1: (p'=p+1);
[a4] p>0 --> 1: (p'=p-1);
[a5] p>0 --> 1: (p'=p-2);
[a5i] p<Np --> 1: (p'=p+2);
endmodule

module pd
pd: [0..Npd] init 0;
[a5i] pd>0 --> 1: (pd'=pd-1);
[a5] pd<Npd --> 1: (pd'=pd+1);
endmodule

module m
m: [0..Nm] init 0;
[a1] m<Nm --> 1: (m'=m+1);
[a2] m>0 --> 1: (m'=m);
[a3] m>0 --> 1: (m'=m-1);
endmodule

module Functional_rates
dummy: bool init true;
[a1] dummy = true --> (v/(KM  + pd*Hpd))*(1/Hm): (dummy' = dummy);
[a2] dummy = true --> (k2*m*Hm)/Hm: (dummy' = dummy);
[a3] dummy = true --> (k3*m*Hm)/Hm: (dummy' = dummy);
[a4] dummy = true --> (k4*p*Hp)/Hp: (dummy' = dummy);
```

```
[a5] dummy = true --> (k5*(p*Hp)^2)/Hp: (dummy' = dummy);
[a5i] dummy = true --> (k5i*pd*Hpd)/Hpd: (dummy' = dummy);
endmodule
```

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