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# Some Investigations Concerning the CTMC and the ODE Model Derived From Bio-PEPA

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## Abstract

Bio-PEPA is a recently defined language for the modelling and analysis of biochemical networks. It supports an abstract style of modelling, in which *discrete levels of concentration* within a species are considered instead of individual molecules. A finer granularity for the system corresponds to a smaller concentration step size and therefore to a greater number of concentration levels. This style of model is amenable to a variety of different analysis techniques, including numerical analysis based on a CTMC with states reflecting the levels of concentration.

In this paper we present a formal definition of the CTMC with levels derived from a Bio-PEPA system. Furthermore we investigate the relationship between this CTMC and the system of ordinary differential equations (ODEs) derived from the same model. Using Kurtz's theorem, we show that the set of ODEs derived from the Bio-PEPA model is able to capture the limiting behaviour of the CTMC obtained from the same system. Finally, we define an empirical methodology to find the granularity of the Bio-PEPA system for which the ODE and the CTMC with levels are in a good agreement. The proposed definition is based on a notion of distance between the two models. We demonstrate our approach on a model of the Repressilator, a simple biochemical network with oscillating behaviour.

**Keywords:** Systems Biology, process algebras, analysis, ordinary differential equations, Markov chains

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## 1 Introduction

In the recent years there have been various applications of process algebras for the study of biochemical networks [18,17,3,8,1]. An attractive feature of process algebras is the simple abstraction they offer for representing biological entities. In the  $\pi$ -calculus and related calculi [18,17] each biochemical molecule is abstracted by a process and reactions are represented by means of communications between

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processes. In the recently defined Bio-PEPA formalism [5,6] a different view has been proposed: each component abstracts the behaviour of a species. In particular, species concentrations are discretized into levels and the components capture concentration levels within a species. The *granularity* of the system is expressed in terms of the concentration step size  $h$ : when  $h$  decreases, the number of levels increases. This modelling style, based on discrete concentration levels, gives rise to an underlying continuous time Markov chain (CTMC) which we will call the “CTMC with levels” to distinguish it from the CTMC which underlies a stochastic simulation based on individual molecules.

Since Bio-PEPA is an *intermediate, formal, compositional* representation of the biological model it supports different kinds of analysis, including stochastic simulation [11], analysis based on ordinary differential equations (ODEs), numerical solution of the CTMC with levels and stochastic model checking using PRISM [16]. It is worth noting that each of these analyses can aid understanding different aspects of the behaviour of the system. Furthermore, when two analyses overlap in scope, the results obtained can be used for verification.

This paper makes the following contributions:

- a formal definition of the CTMC with levels;
- an investigation of the relationship between CTMC with levels and ODEs obtained from the same Bio-PEPA system;
- a proposal for a methodology to find the granularity  $h$  for which these two underlying models are in good agreement.

Whilst our work is presented in the context of Bio-PEPA it has potential application to a much wider class of models.

The CTMC with levels was introduced in [2] together with the PEPA reagent-centric view and was subsequently also used in [4]. One advantage of this approach is that it is based on discrete levels of concentration instead of exact numbers of molecules, allowing us to deal with incomplete information about molecular concentrations, as given in real experimental settings. Furthermore, in comparison to the CTMC underlying a stochastic simulation, it leads to a reduction of the state space, leading to models which may be amenable to numerical solution and approaches such as stochastic model checking. The authors of [2] focused on the case of reactions with mass-action kinetics and stoichiometry equal to one for all the reagents. Here we extend this approach to the general case and we investigate some properties of these Markov chains. Note that this approach is an approximation and does represent some loss of information compared to both the stochastic simulation (in which all molecules are represented individually) and ODE model (in which concentrations vary continuously rather than in discrete jumps).

The second aspect of our work concerns an investigation of the relationship between the CTMC with levels and the set of ODEs obtained from the same Bio-PEPA system. Confidence in the compatibility between the two models is important since we can use them to perform different kinds of analysis. For instance we can check some properties of the system using model checking before simulating the

model using ODEs. The validity of the results depends on the agreement between the two approaches. The relationship between the ODEs and the CTMC derived from a process algebra model has been previously investigated in [10], but in that case the authors focused on the pathway centric-view in PEPA. Here we adapt their approach to the reagent-centric style modelling supported by Bio-PEPA. Using Kurtz’s theorem [13] we show that the set of ODEs derived from Bio-PEPA is able to capture the limiting behaviour of the CTMC with levels representing the discretised system. This involves showing that the CTMC belongs to the family of *density dependent CTMCs*, i.e. the rates of the CTMC may depend on a scaled representation of states, in our case the step size of the species concentrations.

The last challenge is to determine a value for the step size  $h$  which gives good agreement between the two models. In other words, for a fixed error  $\epsilon$ , we want to find a value  $h$  for which the two models differ by less than  $\epsilon$ . This leads us to consider how to express the difference between models and propose an approach based on the definition of a distance function between the models.

The rest of the paper is structured as follows. Section 2 gives a brief introduction to Bio-PEPA, a description of the definition of discrete levels and transition rates in the language. In Section 3 the CTMC with levels is defined. The mappings from Bio-PEPA to ODEs and CTMCs with levels are described in Section 4 and the relationship between these models is discussed in Section 5. In Section 6 the repressilator model, a genetic network with oscillating behaviour, is considered to illustrate and test our approach. Finally, in Section 7, some conclusions and directions for future work are reported.

## 2 Bio-PEPA

In this section we present a short description of Bio-PEPA [5,6] and then we discuss the definition of discrete levels of concentration and how to derive the transition rates from the reaction kinetic laws. Some auxiliary definitions for Bio-PEPA and the operational semantics are reported in the Appendix A.

The context of application is biochemical networks. A biochemical network is composed of  $n$  species that interact through  $m$  reactions in  $o$  compartments. The dynamics of reaction  $j$  is described by a kinetic law  $f_j$ . The stoichiometric coefficients of the reactions are assumed to be integer and bounded. We make the following assumptions:

- only irreversible reactions are considered: reversible reactions can be seen as the union of a pair of forward and inverse reactions;
- the reactants of the reaction can only decrease their concentration whereas the products can only increase it. Enzymes and inhibitors do not change;
- the same species in different situations (e.g. phosphorylated, free, bound...) are regarded as different species and represented by distinct Bio-PEPA components;
- compartments are static and do not play an active role in reactions. Throughout this paper we assume that all reactions take place within a single compartment.

The syntax of Bio-PEPA is defined as:

$$S ::= (\alpha, \kappa) \text{ op } S \mid S + S \mid C \quad P ::= P \boxtimes_{\mathcal{L}} P \mid S(l) \quad \text{where } \text{op} = \downarrow \mid \uparrow \mid \oplus \mid \ominus \mid \odot .$$

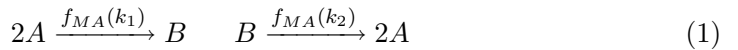
The component  $S$  is a *sequential component* (or *species component*) and represents the species; the component  $P$ , a *model component*, describes a system as the interactions between components. The parameter  $l \in \mathbb{N}$  represents the discrete level of concentration. In the prefix term  $(\alpha, \kappa) \text{ op } S$ ,  $\kappa$  is the *stoichiometry coefficient* of the species and 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 operator “+” expresses the choice between possible actions and the constant  $C$  is defined by an equation  $C \stackrel{\text{def}}{=} S$ . Finally, the process  $P \boxtimes_{\mathcal{L}} Q$  denotes the synchronisation between components: the set  $\mathcal{L}$  determines those activities on which the operands are forced to synchronise. We can define a Bio-PEPA system as follows:

**Definition 2.1** A Bio-PEPA system  $\mathcal{P}$  is a 6-tuple  $\langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}_R, \text{Comp}, P \rangle$ , where:  $\mathcal{V}$  is the set of compartments,  $\mathcal{N}$  is the set of quantities describing each species,  $\mathcal{K}$  is the set of parameters,  $\mathcal{F}_R$  is the set of functional rates,  $\text{Comp}$  is the set of definitions of sequential components,  $P$  is the model component describing the system.

Each element in the set  $\mathcal{N}$  associates the step size, initial concentration, number of levels and compartment with a species. We denote the set of well-defined Bio-PEPA systems  $\tilde{\mathcal{P}}$  (see [6] for more details). The behaviour of the system is defined in terms of an operational semantics (see Appendix A). The derivation of the rate is reported in Section 2.3.

### 2.1 Dimerization example

Let us consider the system composed of the following two reactions, representing the dimerization of a protein and its inverse process:



The dynamics are mass-action kinetics ( $f_{MA}(k_1) = k_1 \cdot A^2$  and  $f_{MA}(k_2) = k_2 \cdot B$ , respectively). We assume that initially  $A = 10 \text{ mol/l}$  and  $B = 0 \text{ mol/l}$  and  $k_1 = k_2 = 1.0$ . This simple network will be used as a running example throughout the remainder.

In Bio-PEPA, we define for each species the step size ( $h$ ), the number of levels ( $N$ ), the initial concentration ( $M_0$ ) and the compartment containing the species ( $cell$ ):

$$A : h = 5, N = 2, M_0 = 10, cell; \quad B : h = 5, N = 1, M_0 = 0, cell;$$

The stoichiometry of  $A$  in the reactions is two and so we need at least two levels for  $A$ . This is the coarsest granularity possible. We denote the two reactions  $\alpha_1$  and  $\alpha_2$  respectively, and define the functional rates:  $f_{\alpha_1} = f_{MA}(k_1)$  and  $f_{\alpha_2} = f_{MA}(k_2)$ .

At this point we can define the set of species components:

$$A \stackrel{\text{def}}{=} (\alpha_1, 2)\downarrow A + (\alpha_2, 2)\uparrow A \quad B \stackrel{\text{def}}{=} (\alpha_1, 1)\uparrow B + (\alpha_2, 1)\downarrow B;$$

The model component is:  $A(2) \boxtimes_{\{\alpha_1, \alpha_2\}} B(0)$ .

## 2.2 Discrete levels of concentration

Each species is characterised by a number of concentration levels, with step size (granularity)  $h$ . Specifically, we assume that all the species in the same compartment have the same step size<sup>5</sup>. This follows from the *law of conservation of mass*: there must be a “balance” between the number of molecules consumed (reactants) and the ones created (products). Note that a finer granularity of a Bio-PEPA system corresponds to a smaller step size.

We assign to each species different concentration levels, from 0 to a maximum number  $N$ . This ensures that the CTMC has a finite state space — a condition which is necessary to make numerical analysis feasible. The maximum level  $N_i$  for each species  $i$  is defined according to prior knowledge and experimental evidence. Given a maximum concentration  $M_i$  for the species  $i$ , the maximum level is given by  $\lceil M_i/h \rceil$ . If the maximum values are not available we can consider stochastic simulation to obtain an estimate for the upper bound of the concentration [7].

If  $l_i$  is the current level for species  $i$ , the concentration is taken to be  $x_i = l_i \cdot h$ . The initial concentration and the initial level of  $i$  are  $x_{i,0}$  and  $l_{i,0}$ , respectively.

## 2.3 Derivation of rates

In the following we show how to derive the transition rates when discrete concentrations are used. The transition rate is defined by  $(\Delta t)^{-1}$ , where  $\Delta t$  is the time taken to vary the concentration of reactants/products a number of levels in the CTMC.

**Stoichiometry equal to one:** Let  $f_j$  be the kinetic law and let  $y$  be one product of the reaction  $j$ . The rate equation for that species with respect to the given reaction is  $dy/dt = f_j(\bar{x})$ , where  $\bar{x}$  is the set (or a subset) of the reactants/modifiers of the reaction. Applying the Taylor expansion (up to two terms) we obtain:

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

We define  $y_{n+1} - y_n = 1 \cdot h$  and then derive the respective time interval  $(t_{n+1} - t_n) = \Delta t$  as  $\Delta t = \frac{h}{f_j(\bar{x}_n)}$ . From this we obtain the transition rate  $\frac{f_j(\bar{x}_n)}{h}$ . Note that if stoichiometry one we have a variation of one level between the states.

**Stoichiometry possibly different from one:** We assume the kinetic law is mass-action in this case. Let  $y$  be a product of the reaction and let  $\kappa$  be its stoichiometric

<sup>5</sup> For modifiers the step size can be chosen arbitrarily since their concentration is unchanged by a reaction.

coefficient with respect to that reaction. Applying the expansion again we obtain:

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

where  $r$  is the rate constant,  $x_i$ , with  $i = 1, \dots, n_r$  are the reactants of the reaction,  $\kappa_i$  are the associated stoichiometric coefficients and  $n_r$  is the number of distinct reactants in the reaction.

Now we can fix  $y_{n+1} - y_n = \kappa \cdot h$ . and derive the rate as  $\frac{r \cdot \prod_{i=1}^{n_r} x_{i,n}^{\kappa_i}}{h}$ .

To summarise, the rate associated with a transition (representing a reaction  $j$ ) from one state  $u$  to another state  $v$  can be calculated as:  $r_j = \frac{f_j[u]}{h}$ , where  $h$  is the step size of the reactants and  $f_j[u]$  is the evaluation of the functional rate in the state  $u$ . When the stoichiometric coefficient of a reagent is  $\kappa$  then the reagent varies by  $\kappa$  levels as a result of the transition.

The kinetic law function  $f_j$  for reaction  $j$  must satisfy some mild conditions:

- it is continuously differentiable;
- it is strictly positive.

The first condition is useful to prove some results about the CTMC, whereas the second one follows because we assume that the reactants decrease their concentration and products increase it. All the most well-known kinetic laws satisfy these properties.

### 3 CTMC with levels

The term *CTMC with levels* indicates a CTMC whose states capture levels of concentration of the species and the transitions from one state to another reflect changes of these levels.

**Definition 3.1** A *state* of a CTMC with levels is defined as a vector of levels  $\sigma = (l_1, l_2, \dots, l_n)$ , where  $l_i$ , for  $i = 1, 2, \dots, n$ , is the level of the species  $i$ . The *transitions* of a CTMC with levels represent biochemical reactions. Each transition causes a change in the number of levels of one or more species, as determined by the stoichiometry. The transition rates are as defined in Section 2.3.

For the analysis, it is necessary that the CTMCs are finite. Starting from a finite number of levels, is possible to obtain an infinite CTMC only if there are some reactions of the kind “ $\rightarrow A$ ” or “ $C \rightarrow C + A$ ”. We call these *creation reactions*. We term a biochemical network without creation reactions a *bounded chemical network*. We then have the following result.

**Proposition 3.2** Let  $X_h$  be a CTMC corresponding to a bounded biochemical network with granularity  $h$ . Let  $\sigma_0 = (l_{1,0}, l_{2,0}, \dots, l_{n,0})$  be the initial state. If the values  $l_{i,0}$  ( $i = 1, \dots, n$ ) are finite then  $X_h$  is finite and the maximum value of the level depends on  $\sigma_0$  and the stoichiometric coefficients of the reactions.

In particular, if all stoichiometric coefficients are equal to one and there are no reactions with more than one product, in each state  $\sigma$  each component  $l_i$  satisfies:  $l_i \leq (\sum_{j=1}^n l_{j,0})$ .

If we allow creation reactions, it could happen that some species do not have a maximum concentration. In this case it is necessary to assume a maximum level also for the species that grows infinitely in order to guarantee a finite CTMC. Note that this is an approximation and we have to pay attention to the results obtained from the analysis (e.g. model checking). However, in many situations, unbounded network may have a pragmatic (average) bound value because of the quantitative relations between the molecules and the reactions composing the network.

Finally, the complexity of the CTMC is expressed in terms of the number of states. This number depends on the amount of levels of the species. An upper bound for the number of states is given by  $\prod_{i=1}^n (N_i + 1)$ , where  $N_i$  is the maximum level of the species  $i$  and  $n$  the number of species.

## 4 From Bio-PEPA to CTMC with levels and to ODEs

In this section we outline how the CTMC with levels and the system of ODEs underlying a Bio-PEPA model are derived.

### 4.1 From Bio-PEPA to CTMC with levels ( $\pi_{CTMC}$ )

Let  $\pi_{CTMC}$  be the function that derives a CTMC with levels from a Bio-PEPA system. We do not define this function formally here, but states are derived from a labelled transition system, via the operational semantics, and transition rates are as described in Section 2.3. From any Bio-PEPA system we can apply the semantic rules to generate the labelled transition system where each node is a *derivative* of the system. We have the following result:

**Theorem 4.1** *For any finite Bio-PEPA system  $\mathcal{P} = \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}_R, \text{Comp}, P \rangle$ , if we define the stochastic process  $X_h(t)$  such that  $X_h(t) = P_i$  indicates that the system behaves as derivative  $P_i$  at time  $t$ , then  $X_h(t)$  is a CTMC.*

The proof is analogous to that presented for PEPA [12]. The rate associated with each activity is obtained by evaluating the functional rate in the system.

The CTMC is characterised by an infinitesimal generator matrix  $\mathbf{Q}$  whose off-diagonal entries are the transition rates, and whose diagonal entries are the negative row sums.

#### 4.1.1 Dimerization example (continued)

Consider the dimerization example again; we derive the CTMC with levels for two values of  $h$ :  $h = 5$  and  $h = 1$ , as illustrated in Figures 1 and 2. When  $h = 5$  there are two states,  $(2, 0)$  and  $(0, 1)$ , and two transitions with rates:

$$q_{1,2} = k_1 \cdot A^2/h = 1 \cdot (10)^2/5 = 20, \quad q_{2,1} = k_2 \cdot B/h = 1 \cdot 5/5 = 1.$$

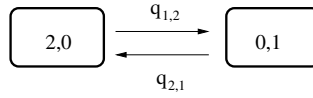


Fig. 1. CTMC with levels for the dimerization example ( $h = 5$ ).

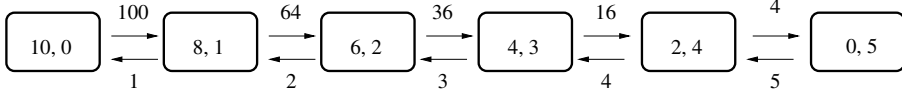


Fig. 2. CTMC with levels for the dimerization example ( $h = 1$ ).

When  $h$  is smaller ( $h = 1$ ) then there is a finer granularity. There are six states and ten transitions (see Figure 2). As a further example (not illustrated), when  $h = 0.1$  there are 51 states and 100 transitions.

#### 4.2 From Bio-PEPA to ODEs

Let  $\pi_{ODE}$  be the definition of the set of ODEs from a Bio-PEPA model. A crucial part is the derivation of the stoichiometry matrix  $D = \{d_{ij}\}$ . The entries of the matrix are obtained as follows: for each sequential 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  $\kappa_{ij}$  as  $-\kappa_{ij}$  in the entry  $d_{ij}$ . In the case of a product we write  $+\kappa_{ij}$ . All other cases are null.

$\pi_{ODE}$  entails three 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* ( $m \times 1$ )  $\mathbf{v}_{\mathbf{KL}}$  containing the kinetic laws of each reaction; 3) definition of the vector ( $n \times 1$ )  $\mathbf{x}$ , with  $\mathbf{x}^T = (x_1, x_2, \dots, x_n)$ .

The ODE system thus obtained has the form:

$$\frac{d\mathbf{x}}{dt} = D \times \mathbf{v}_{\mathbf{KL}}$$

where the vector of initial concentrations is  $\mathbf{x}_0$ , with  $x_{i,0} = l_{i,0} \cdot h$ ,  $i = 1, \dots, n$ .

#### 4.3 Dimerization example (continued)

We define the vector  $\mathbf{x}^T = (x_A, x_B)$  and the kinetic vector  $\mathbf{v}_{\mathbf{KL}}^T = (k_1 \cdot x_A^2, k_2 \cdot x_B)$ . The stoichiometry matrix  $D$  associated with the system is

$$\begin{pmatrix} -2 & +2 \\ +1 & -1 \end{pmatrix}$$

The system of ODEs obtained by  $\pi_{ODE}$  is:

$$\begin{aligned} \frac{dx_A}{dt} &= -2 \cdot k_1 \cdot x_A + 2 \cdot k_2 \cdot x_B \\ \frac{dx_B}{dt} &= +k_1 \cdot x_A - k_2 \cdot x_B \end{aligned}$$



with initial conditions  $(x_{A,0}, x_{B,0})^T = (10, 0)$ .

## 5 Comparison of CTMC with levels and ODEs

In this section we consider how to compare the two models derived from a Bio-PEPA system  $\mathcal{P}$  and how to define the granularity  $h$  so that the difference between the two models is acceptable.

First we apply Kurtz's Theorem [13,15] to our case. This theorem tells us that, under some conditions, the limit of a sequence of density dependent CTMCs (the CTMCs with levels), as  $h$  approaches 0, is a set of ODEs.

Second we consider the difference between the two models. We define a distance measure and discuss the factors to consider when choosing  $h$  in order to ensure that the distance between the two models is less than an acceptable error  $\epsilon$ .

In the following we introduce the notation used, then we show that the CTMC with levels derived from a Bio-PEPA system satisfies the conditions of Kurtz's Theorem. Finally, we observe that the set of ODEs extracted from the Bio-PEPA system coincides with those in the theorem.

### 5.1 Application of Kurtz's Theorem

Kurtz's Theorem applies to a sequence of *density dependent Markov chains*. In the original theorem the dependency is expressed in terms of the volume  $V$ , but we express the dependency in terms of the granularity  $h$ . Note that when  $h$  decreases, the number of levels in the system increases. The formal definition of the Theorem with its conditions is reported in the Appendix B.

Let  $X_h$  be the CTMC describing the model with granularity  $h$ . Given a state of the CTMC  $\sigma$ , we denote by  $\mathbf{h}\sigma$  the vector  $(h \cdot l_1, h \cdot l_2, \dots, h \cdot l_n)$ , where  $h$  is the step size and  $l_i$  is the level of the species  $i$ . Let  $D$  be the stoichiometry matrix obtained from the Bio-PEPA system and  $D^j$  the  $j$ th column of  $D$ . This vector represents the stoichiometric coefficients for all the species in a given reaction  $j$ . In the following the kinetic law associated with the reaction  $j$  is denoted by  $f_j(\mathbf{h}\sigma, D^j)$ , where the dependency from both the state and the stoichiometric coefficients is expressed explicitly. This notation is different from the one generally used in Bio-PEPA. Here we focus on Bio-PEPA systems representing bounded biochemical networks, also pragmatically.

In order to apply Kurtz's Theorem, we first show that the CTMC  $X_h$  is density dependent and that all the conditions of the theorem are satisfied.

**$X_h$  is density dependent** From the definition of CTMC with levels, the entry  $q_{u,v}$  of the infinitesimal generator matrix is (according to the notation above):

$$q_{u,v} = \sum_{\mathcal{A}(\sigma_u|\sigma_v)} f_j(\mathbf{h}\sigma_u, D^j) \cdot h^{-1} \quad \text{if } u \neq v \quad q_{u,u} = -\sum_{u \neq v} q_{u,v} \quad \text{otherwise.}$$

where  $\mathcal{A}(\sigma_u|\sigma_v) = \{\alpha \mid \sigma_u \xrightarrow{\alpha} \sigma_v\}$  and  $f_j(\mathbf{h}\sigma_u, D^j)$  is the evaluation of the

functional rate in state  $\sigma_u$ . This definition of transition rates clearly satisfies the definition of density dependent reported in the Appendix B.

**Conditions of Kurtz's theorem** Let  $\mathbf{x}_0$  be the initial concentration vector for the ODEs. The initial level vector is  $\mathbf{l}_0 = \lceil \mathbf{x}_0/h \rceil$ . Thus  $\lim_{h \rightarrow 0} h \cdot \mathbf{l}_0 = \mathbf{x}_0$ .

Consider the system of ODEs  $dX(t)/dt = F(\mathbf{x})$  where  $F(\mathbf{x}) = \sum D^j f_j(\mathbf{x}, D^j)$  with initial condition  $X(0) = \mathbf{x}_0$ . By hypothesis, the trajectory of  $X(t)$  is bounded, so we can assume it is bounded by some open set  $E$ . Since each kinetic law is continuously differentiable (the first condition for the kinetic laws in Section 2.3), it follows that  $f$  is Lipschitz. This the first condition of Kurtz's Theorem. The second and third conditions of Kurtz's Theorem state that for each transition the rate of change is bounded and that there is a bound for the whole state space so that the impact of each transition is bounded. By the assumptions made for the kinetic laws and because stoichiometric coefficients are assumed to be integer and bounded, it is clear that both these conditions are also satisfied. In particular, for the third condition, we can observe that  $f(\mathbf{x}, D^j) = 0$  for all  $|D^j| > C$  with  $C = \sum_{i,j} d_{ij}$ .

**ODE systems** Consider the ODE system  $\pi_{ODE}(\mathcal{P})$ , for a given Bio-PEPA system  $\mathcal{P}$ . We can observe that  $F(\mathbf{x}) = D \times \mathbf{v}_{\mathbf{KL}}$ , as the kinetic law vector  $\mathbf{v}_{\mathbf{KL}}$  contains all the functions  $f_j$  for all the reactions. The ODE system  $\pi_{ODE}(\mathcal{P})$  coincides with the one in Kurtz's Theorem, with initial condition  $x_{i,0} = l_{i,0} \cdot h$ , for  $i = 1, 2, \dots, n$ .

## 5.2 Distances between the two models

The result in Section 5 confirms that, in the limit, the agreement between the ODEs and the CTMC with levels derived from a Bio-PEPA system is complete. However it does not tell us about the relationship between the two models for a given finite  $h$ . In [4] the authors showed experimentally that in some pathways the two models are indistinguishable for just few levels, for example when  $h = 1$  and  $h = 7$ , but these results are not generalised. Here we investigate the relationship between the step size  $h$  of the CTMC and the agreement with ODEs. In [14,15] Kurtz reported some estimates for the probability of convergence between the two models. However the estimation is complex and offers a poor guide for choosing  $h$ . In the following we propose a measure of distance between the two models. There are various possible ways to define this measure. One possibility is to define it in terms of the difference between the ODE simulation trajectory and the expected value (numerical solution) of the CTMC, for all the species in the biological network, with respect to a time interval. This gives the following definition of distance:

$$f_{dist} = \sum_{t=1}^{T_{sim}} \sum_{i=1}^n \left( X_i^h(t) \cdot h - x_i(t) \right)^2$$

where  $x_i$  is the ODE trajectory for the species  $i$ ,  $X_i^h(t)$  is the numerical solution of the CTMC for the species  $i$  at time  $t$ ,  $n$  is the number of species in the network,  $T_{sim}$  is the simulation time and  $t$  indicates a simulation time point.

We propose the following empirical approach to find the value of  $h$  for which we have good agreement between the models.

- Let us consider a well-defined Bio-PEPA system  $\mathcal{P}$ , the CTMC  $X_h = \pi_{CTMC}(\mathcal{P})$  and the ODEs solution  $X$  of the model  $\pi_{ODE}(\mathcal{P})$ .
- Let  $T_{sim}$  be the simulation time (this depends on the model) and  $\epsilon > 0$  the acceptable discrepancy between the two models.
- Starting from an initial granularity we calculate the distance measure for the simulation time. If the measure is greater than  $\epsilon$  then change  $h$  and try again.

Clearly the choice of  $\epsilon$  is crucial. Furthermore, the numerical solution of the CTMC may be unfeasible for even moderately sized models. In this case, instead of considering the expected value from the numerical solution of the CTMC, we can define the distance in terms of the average (mean) of some value over repeated CTMC simulation runs. This leads to the following distance function:

$$f_{dist,avg} = \sum_{t=1}^{T_{sim}} \sum_{i=1}^n \left( \bar{X}_i^h(t) \cdot h - x_i(t) \right)^2$$

where  $\bar{X}_i^h(t)$  is the average level for species  $i$  in the CTMC at time  $t$  over  $N_{run}$  runs and the other variables are as before. In this approach the main challenge is the definition of the number of simulation runs needed for a good approximation of the expected value. Increasing the number of runs we obtain a better approximation of the expected value for the CTMC, however the calculations become more expensive. Generally we obtain indistinguishable curves for a relative small number of runs.

In both the definitions, the distance between the two models generally decreases with the step size  $h$ . However, note that for very small  $h$  the number of states becomes large and even the simulation of the CTMC may become prohibitively expensive. Thus there is a trade-off between accuracy (in terms of both number of runs and step size) and tractability. The resolution of this trade-off is left to the modeller.

### 5.3 Dimerization example (continued)

In Figure 3 we report some analysis results for the dimerization example. The ODE simulation is reported at the top. The other two graphs show the time evolution of the expected value of the CTMC with levels for  $h = 5$ ,  $h = 1$ ,  $h = 0.1$  and  $h = 0.01$ .

By comparing the ODE trajectory and the numerical solutions, we can observe that for a large step sizes ( $h = 5$ ) there is a discrepancy between the two curves, both for  $A$  and  $B$ . When we decrease the step size  $h$ , the discrepancy between the two curves becomes smaller and for  $h = 0.1$  (corresponding to 100 levels) the expected value of the CTMC (almost) coincides with the ODE. This is as predicted by Kurtz's Theorem. If we consider the average of some simulation runs instead of the expected value, we obtain similar results for 100 runs. However, for  $h = 5$  and  $h = 1$  there is a large variability between the different simulation runs.

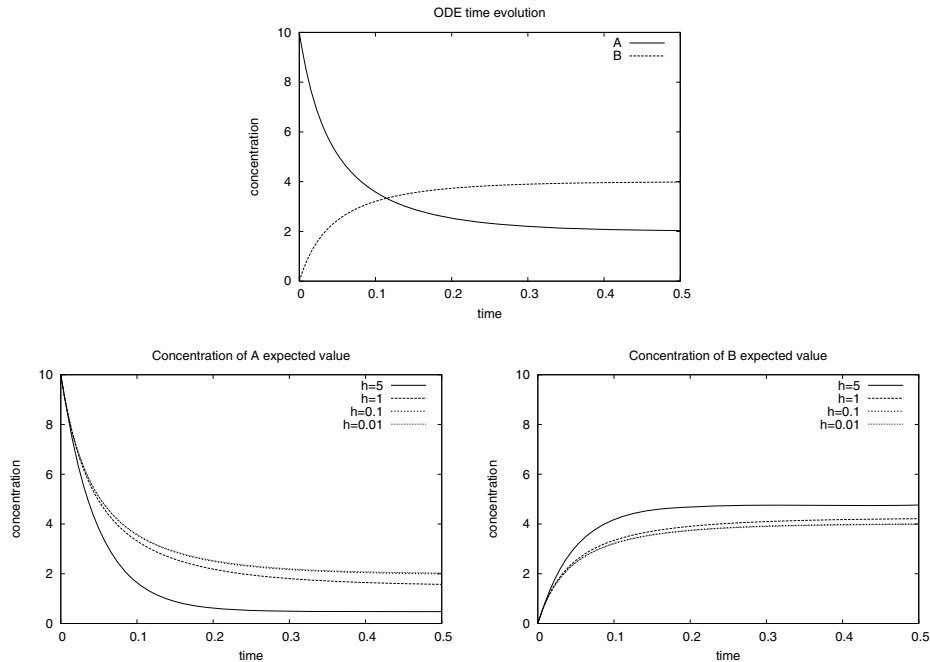


Fig. 3. Dimerization example. On the top: ODE simulation. The other two graphs represent the numerical solution for the CTMC for  $A$  (on the left) and  $B$  (on the right) for  $h = 5$ ,  $h = 1$ ,  $h = 0.1$  and  $h = 0.01$ .

In the table below we report the distance  $f_{dist}$  between the ODE and CTMC for different values of  $h$ . If we fix the admissible distance between the models as  $\epsilon = 1.05$ , then we have  $h = 0.1$ .

distance	$h = 5$	$h = 1$	$h = 0.1$	$h = 0.01$
$f_{dist}$	53.69	3.7	1.02	1.00

## 6 The repressilator

The *repressilator* is a synthetic genetic regulatory network with oscillating behaviour [9]. It consists of three genes (denoted  $G1$ ,  $G2$ ,  $G3$ ) connected in a *feedback loop*, such that the transcription of a gene is inhibited by one of the other proteins (denoted  $P1$ ,  $P2$ ,  $P3$ ). A schema of the network is reported in Figure 4.

The reactions are: the *transcription* of the three mRNAs with inhibition by one of the proteins (reactions  $tr1$ ,  $tr2$ ,  $tr3$ ), the *translation* of mRNAs into the proteins ( $trl1$ ,  $trl2$ ,  $trl3$ ), *degradation* of both mRNAs and proteins ( $d_i$  with  $i = 1, \dots, 6$ ).

Note that this network contains some creation reactions (transcription and translation reactions) therefore it is an unbounded network according to our definition. However, it is pragmatically bounded, This is due to the regulatory effect of degradation and inhibition.

This network is described in Bio-PEPA as follows:

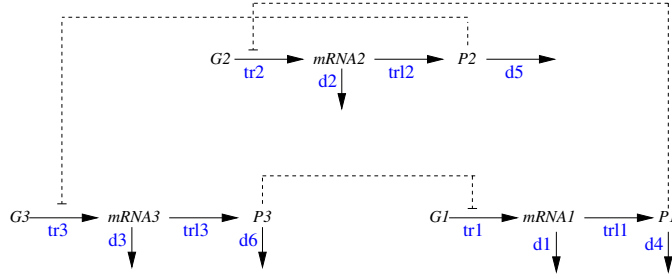


Fig. 4. Repressilator model.

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

while the model is defined as:

$$\begin{aligned}
 &(((mRNA1(0) \boxtimes_{\emptyset} mRNA2(2)) \boxtimes_{\emptyset} mRNA3(0)) \boxtimes_{\{trl1, tr3\}} \\
 &\quad P1(1)) \boxtimes_{\{trl2, tr1\}} P2(0)) \boxtimes_{\{trl3, tr2\}} P3(3)
 \end{aligned}$$

The parameters and the initial concentrations are defined as in [9] i.e. the initial levels are defined according to the initial values of the model. There are no compartments defined explicitly in the model. So we consider the default compartment  $v_{Cell} : 1$ . The step size, the number of levels, the initial concentration and location of species are declared as:

$$\begin{aligned}
 mRNA1 : h = 5, N = 2, M_0 = 0, -, v_{Cell}; & \quad mRNA2 : h = 5, N = 2, M_0 = 0, -, v_{Cell}; \\
 mRNA3 : h = 5, N = 2, M_0 = 0, -, v_{Cell}; & \quad P1 : h = 5, N = 50, M_0 = 5, -, v_{Cell}; \\
 P2 : h = 5, N = 50, M_0 = 0, -, v_{Cell}; & \quad P3 : h = 5, N = 50, M_0 = 15, -, v_{Cell};
 \end{aligned}$$

For all the species we consider the step size  $h = 5$ . The numbers of levels are derived in terms of the concentration in the biological model. The set of functional rates is:

$$\begin{aligned}
 f_{tr1} &= \frac{\alpha}{1 + P3^2} + \alpha_0; & f_{tr2} &= \frac{\alpha}{1 + P1^2} + \alpha_0; & f_{tr3} &= \frac{\alpha}{1 + P2^2} + \alpha_0; \\
 f_{trl1} &= fMA(\beta); & f_{trl2} &= fMA(\beta); & f_{trl3} &= fMA(\beta); \\
 f_{di} &= fMA(1) \quad i = 1, 2, 3, 4, 5, 6.
 \end{aligned}$$

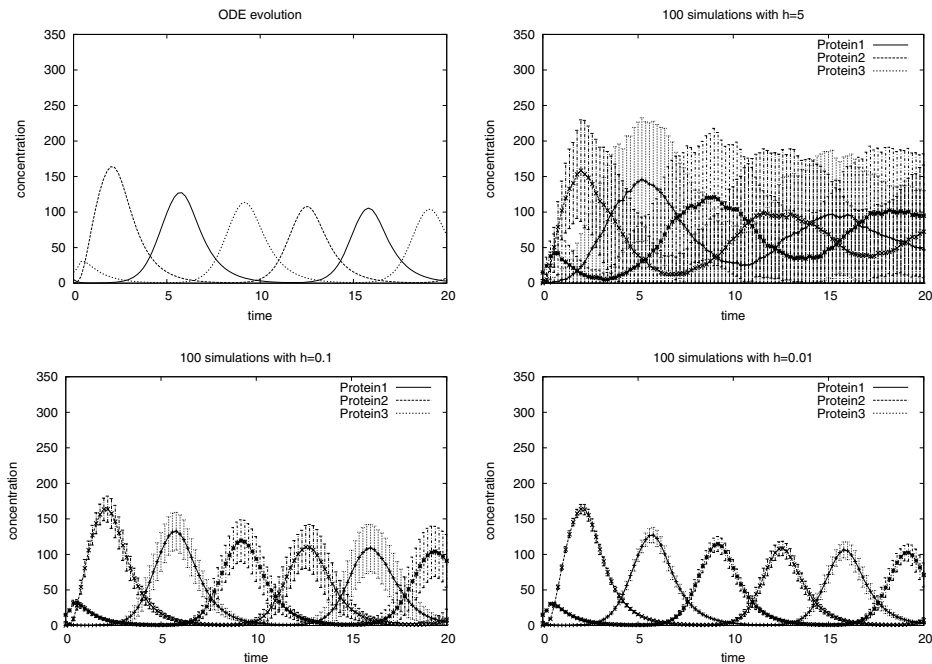


Fig. 5. Some analysis results for the Repressilator.

$fMA(r)$  denotes mass-action with rate constant  $r$ . All 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:  $\alpha = 250$ ,  $\alpha_0 = 0$ , and  $\beta = 5$ .

From the Bio-PEPA system we can derive the CTMC and the ODE model as usual (reported in the Appendix C). For each temporal point we show the mean and the standard deviation of the 100 runs. In Figure 5 we report some analysis results. The ODE simulation is reported at the top, left. The other graphs show the time evolution of the average of 100 simulation runs for the CTMCs with  $h = 5$ ,  $h = 0.1$  and  $h = 0.01$ . For the Repressilator the numerical calculation of the expected value of the CTMC is too expensive.

For relatively large  $h$ , there is a great variability among the different simulation runs and the mean value is very different from the ODE results. For smaller  $h$  this variability decreases and the mean value approaches the ODE trajectory.

In the Table below we report the distances between ODE and CTMC for different values of  $h$ . We consider the definition of distance in terms of the mean value. Note that these distances are not normalised and the sum is over all the six species of the system. By observing these values we can see that the distance between the two models decreases with smaller step sizes.

distance	h = 5	h = 1	h = 0.1	h = 0.01
$f_{dist,avg}$	969105	209817	21796	540

## 7 Discussion and conclusions

There are three main contributions of this work. Firstly, we gave a formal definition of the CTMC derived from a Bio-PEPA system. We called it the *CTMC with levels*, as its states are characterised in terms of the concentration levels for each of the species of the system. Secondly, we investigated the relationship, at the limit, between the ODE model and the CTMC obtained from the same Bio-PEPA system using Kurtz's Theorem. Thirdly, we proposed a distance measure between the CTMC and ODE models and this has been used for finding a “good” granularity for the system. We tested our approach against a simple example describing a dimerization reaction and the Repressilator network.

Based on our results, in the case of a low number of levels (i.e. coarse granularity), the behaviour shown by the expected value of the CTMC might or might not agree from the ODE time evolution. We use a smaller  $h$  in order to decrease the variability of the CTMC model, and as predicted by Kurtz's Theorem, obtain a global behaviour that is closer to that given by the deterministic approach. This can allow more flexibility to the modeller. For instance, in the presence of experimental observations that suggest a certain degree of uncertainty, we can choose the model that better agrees with those observations.

We proposed a distance measure between the CTMC and ODE models and this has been used for finding a “good” granularity for the system. The definition of distance is based on the numerical solution of CTMC. As observed in the paper, the derivation of the numerical solution is often impractical. In order to overcome this drawback we proposed an alternative definition of distance based on the average of a number of simulation runs. The selection of the appropriate number of runs remains an open problem. A deeper investigation of this point and the study of other definitions of distance between models is planned.

Finally, other future investigations concern the validation of the system against experimental data and existing knowledge.

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## A Auxiliary definitions for Bio-PEPA

In the following we report some auxiliary definitions. For more details see [5,6].

**Definition A.1** The set of current action types enabled in the model component  $P$ , denoted  $\mathcal{A}(P)$ , is defined as:

$$\begin{aligned}
 \mathcal{A}((\alpha, \kappa) \text{ op } S) &= \{\alpha\} & \mathcal{A}(S_1 + S_2) &= \mathcal{A}(S_1) \cup \mathcal{A}(S_2) \\
 \mathcal{A}(S(l)) &= \mathcal{A}(S) & \mathcal{A}(C) &= \mathcal{A}(S) \text{ where } C \stackrel{\text{def}}{=} S \\
 \mathcal{A}(P_1 \boxtimes_c P_2) &= \mathcal{A}(P_1) \setminus \mathcal{L} \cup \mathcal{A}(P_2) \setminus \mathcal{L} \cup (\mathcal{A}(P_1) \cap \mathcal{A}(P_2) \cap \mathcal{L})
 \end{aligned}$$

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

The behaviour of the system is defined in terms of an operational semantics. We define two relations over the processes. The former, called the *capability relation* (indicated with  $\rightarrow_c$ ), supports the derivation of quantitative information and it is auxiliary to the latter which is called the *stochastic relation* (indicated with  $\rightarrow_s$ ). The stochastic relation gives us the rates associated with each action.

The formal definition of these relations in terms of structured operational rules is presented in Table A.1.

The following definitions concern 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.



<b>prefixReac</b>	$((\alpha, \kappa) \downarrow S)(l) \xrightarrow{(\alpha, [S: \downarrow(l, \kappa)])}_c S(l - \kappa) \quad \kappa \leq l \leq N$	
<b>prefixProd</b>	$((\alpha, \kappa) \uparrow S)(l) \xrightarrow{(\alpha, [S: \uparrow(l, \kappa)])}_c S(l + \kappa) \quad 0 \leq l \leq (N - \kappa)$	
<b>prefixMod</b>	$((\alpha, \kappa) op S)(l) \xrightarrow{(\alpha, [S: op(l, \kappa)])}_c S(l) \quad \text{with } op = \odot, \oplus, \ominus \text{ and}$ $0 < l \leq N \text{ if } op = \oplus, \quad 0 \leq l \leq N \text{ otherwise}$	
<b>choice1</b>	$\begin{array}{c} S_1(l) \xrightarrow{(\alpha, w)}_c S'_1(l') \\ (S_1 + S_2)(l) \xrightarrow{(\alpha, w)}_c S'_1(l') \end{array}$	<b>choice2</b> $\begin{array}{c} S_2(l) \xrightarrow{(\alpha, w)}_c S'_2(l') \\ (S_1 + S_2)(l) \xrightarrow{(\alpha, w)}_c S'_2(l') \end{array}$
<b>constant</b>	$\begin{array}{c} S(l) \xrightarrow{(\alpha, S: [op(l, \kappa)])}_c S'(l') \\ C(l) \xrightarrow{(\alpha, C: [op(l, \kappa)])}_c S'(l') \end{array} \quad \text{with } C \stackrel{def}{=} S$	
<b>coop1</b>	$\begin{array}{c} P_1 \xrightarrow{(\alpha, w)}_c P'_1 \\ P_1 \boxtimes_{\mathcal{L}} P_2 \xrightarrow{(\alpha, w)}_c P'_1 \boxtimes_{\mathcal{L}} P_2 \end{array} \quad \text{with } \alpha \notin \mathcal{L}$	
<b>coop2</b>	$\begin{array}{c} P_2 \xrightarrow{(\alpha, w)}_c P'_2 \\ P_1 \boxtimes_{\mathcal{L}} P_2 \xrightarrow{(\alpha, w)}_c P_1 \boxtimes_{\mathcal{L}} P'_2 \end{array} \quad \text{with } \alpha \notin \mathcal{L}$	
<b>coop3</b>	$\begin{array}{c} P_1 \xrightarrow{(\alpha, w_1)}_c P'_1 \quad P_2 \xrightarrow{(\alpha, w_2)}_c P'_2 \\ P_1 \boxtimes_{\mathcal{L}} P_2 \xrightarrow{(\alpha, w_1 :: w_2)}_c P'_1 \boxtimes_{\mathcal{L}} P'_2 \end{array} \quad \text{with } \alpha \in \mathcal{L}$	
<b>Final</b>	$\frac{P \xrightarrow{(\alpha_j, w)}_c P'}{\langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P \rangle \xrightarrow{(\alpha_j, r_\alpha)}_s \langle \mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P' \rangle}$	

Table A.1  
Axioms and rules for Bio-PEPA.

**Definition A.2** If  $\mathcal{P} \xrightarrow{(\alpha, r)}_s \mathcal{P}'$  then  $\mathcal{P}'$  is a one-step  $\rightarrow_s$  system derivative of  $\mathcal{P}$ .  
If  $\mathcal{P} \xrightarrow{(\alpha_1, r_1)}_s \mathcal{P}_1 \xrightarrow{(\alpha_2, r_2)}_s \dots \xrightarrow{(\alpha_n, r_n)}_s \mathcal{P}'$  then  $\mathcal{P}'$  is a system derivative of  $\mathcal{P}$ .

**Definition A.3** 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 A.4** 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  $ds(\mathcal{P}) \times ds(\mathcal{P}) \times \Gamma$ , with  $\Gamma$  the set of labels for  $\rightarrow_s$ .

In the following definition we identify the actions describing the transitions from one state to another.

**Definition A.5** Let  $\mathcal{P}$  be a Bio-PEPA system and let  $P$  be the associated model component. 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  $\mathcal{A}(P_u|P_v)$ .

## B Kurtz's theorem

In the following we report the main theorem described in [13]. First of all we give the definition of *Density Dependent Markov Chain*, as here we limit our attention to this kind of CTMCs.

**Definition B.1** A family of CTMCs  $X_V$ , for some parameter  $V$ , is called *density dependent* if and only if there exists a continuous function  $f(x, s)$ ,  $x \in \mathbb{R}^n$ ,  $s \in \mathbb{Z}^n$ , such that the entries of the infinitesimal generators are given by:

$$q_{k,k'} = f(\sigma V^{-1}, s) \cdot V \quad s \neq 0$$

with  $\sigma$  the state vector and  $s$  a transition vector containing the modifications to element of each state (i.e. the number of copies to add or subtract) when the transition is taken.

**Theorem B.2** Let  $X_V$  be a family of density dependent CTMCs with the infinitesimal generator matrix as in the definition above. Assume  $X(t)$  is the solution of the ODE system  $dX/dt = F(X)$ , where  $F(X) = \sum_s s f(x, s)$  and let  $X(0) = x_0$ . If there exists an open set  $E \subset \mathbb{R}^n$  such that  $X(t) \in E$  and

- (i)  $\exists M, \forall x, y \in E \quad |F(x) - F(y)| < M |x - y|$ ;
- (ii)  $\sup \sum_s |s| f(x, s) < \infty$ ;
- (iii)  $\lim_{d \rightarrow \infty} \sup_{x \in E} \sum_{|s| > d} |s| f(x, s) = 0$

then

$$\lim_{V \rightarrow \infty} V^{-1} X_V(0) = x_0 \implies \forall \delta > 0, \forall t > 0 \quad \lim_{V \rightarrow \infty} \mathbb{P}(\sup_{z < t} |V^{-1} X_V(z) - X(z)| > \delta) = 0$$

The theorem states that, under the given conditions, the system of ODEs can be defined as the limit of a sequence of density dependent CTMCs. As the theorem phrased above the states represent numbers of individuals and are normalised with respect to a parameter  $V$  (in this case, the volume). Therefore  $V^{-1} X_V(z)$  represents the scaled Markov process with concentrations. In our case the scaling factor is in terms of  $h$  instead of  $V$ .

## C ODEs and CTMC for the Bio-PEPA Repressilator

From the Bio-PEPA system in Section 6 we can derive both the CTMC and the ODE system corresponding to the Repressilator. In the former case we obtain a CTMC with  $2^3 \cdot 50^3$  states (not reported), where 50 is the number of levels considered for the proteins and 2 is the number of levels for the mRNAs.

Concerning the derivation of the ODE system, the first step is the derivation of

the stoichiometry matrix D. For this model we have:

$$\left( \begin{array}{cccccccccccc|c} tr1 & tr2 & tr3 & trl1 & trl2 & trl3 & d1 & d2 & d3 & d4 & d5 & d6 & \\ \hline +1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & x_{m1} \\ 0 & +1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & x_{m2} \\ 0 & 0 & +1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & x_{m3} \\ 0 & 0 & 0 & +1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & x_{p1} \\ 0 & 0 & 0 & 0 & +1 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & x_{p2} \\ 0 & 0 & 0 & 0 & 0 & +1 & 0 & 0 & 0 & 0 & 0 & -1 & x_{p3} \end{array} \right)$$

Each row describes the stoichiometry for a given species in each reaction. The last column reports the name of the variables associated with the species in the network ( $m_i$  stands for  $mRNA_i$  and  $p_i$  for  $P_i$ ,  $i = 1, 2, 3$ ). The kinetic vector  $\mathbf{v}_{\mathbf{KL}}$  is:

$$\left( \begin{array}{c} \frac{\alpha}{1+x_{p3}^2} + \alpha_0, \frac{\alpha}{1+x_{p1}^2} + \alpha - 0, \frac{\alpha}{1+x_{p2}^2} + \alpha_0, \beta \cdot x_{m1}, \beta \cdot x_{m2}, \beta \cdot x_{m3}, \\ 1 \cdot x_{m1}, 1 \cdot x_{m2}, 1 \cdot x_{m3}, 1 \cdot x_{p1}, 1 \cdot x_{p2}, 1 \cdot x_{p3} \end{array} \right)^T$$

The ODE system is:

$$\begin{aligned} \frac{dx_{m1}}{dt} &= + \frac{\alpha}{1+x_{p3}^2} + \alpha_0 - 1 \cdot x_{m1} & \frac{dx_{m2}}{dt} &= + \frac{\alpha}{1+x_{p1}^2} + \alpha_0 - 1 \cdot x_{m2} \\ \frac{dx_{m3}}{dt} &= + \frac{\alpha}{1+x_{p2}^2} + \alpha_0 - 1 \cdot x_{m3} & \frac{dx_{p1}}{dt} &= +\beta \cdot x_{m1} - 1 \cdot x_{p1} \\ \frac{dx_{p2}}{dt} &= +\beta \cdot x_{m2} - 1 \cdot x_{p2} & \frac{dx_{p3}}{dt} &= +\beta \cdot x_{m3} - 1 \cdot x_{p3} \end{aligned}$$

with the initial conditions  $(x_{m1}, x_{m2}, x_{m3}, x_{p1}, x_{p2}, x_{p3})^T = (0, 0, 0, 5, 0, 15)$ .