Formalizing a Notion of Concentration Robustness for Biochemical Networks

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Abstract. The main goal of systems biology is to understand the dynamical properties of biological systems by investigating the interactions among the components of a biological system. In this work, we focus on the robustness property, a behaviour observed in several biological systems that allows them to preserve their functions despite external and internal perturbations. We first propose a new formal definition of robustness using the formalism of continuous Petri nets. In particular, we focus on robustness against perturbations to the initial concentrations of species. Then, we demonstrate the validity of our definition by applying it to the models of three different robust biochemical networks.

Keywords: Robustness · Biochemical networks · Petri nets

1 Introduction

From the discovery of DNA structure, in 1953, there has been an increasing interest in the morphological and functional organization of living cells. A cell is a complex system. It consists of a huge number of components that interact with each other through chemical reaction networks. The cell's global behaviour, both internal and with the environment, emerges from such an interaction.

Chemical reaction networks, also called *pathways* are often based on long series of chemical reactions, also known as *signalling cascades*, activated by an initial stimulus (a chemical in the environment or entering the cell), that is perceived by a *transductor* (e.g. a receptor protein in the cell surface). The transductor causes the cascade of reactions to start, leading to the amplification and the filtering of the stimulus (or input signal), in order to suitably regulate and reconfigure cell activities as a response. Signalling pathways play a crucial role for the cell functioning. Many severe diseases, such as cancer and diabetes, are caused by the malfunctioning or the corruption of a crucial signalling pathway.

In this context, the main challenge is to explore how the components of the cells interact with each other as a *system* in order to predict how perturbations can influence the cell functioning. This is the aim of *systems biology* [1,21].

In this perspective, we focus on the definition of the *robustness* property, a fundamental feature of complex evolving systems, for which the functionality

M. Mazzara et al. (Eds.): STAF 2018 Workshops, LNCS 11176, pp. 81–97, 2018.
https://doi.org/10.1007/978-3-030-04771-9_8

of the system remains essentially intact despite the presence of internal and external perturbations.

In nature, there are different mechanisms ensuring robustness, such as system control, redundancy, modularity and structural stability [22]. *System control* is based on negative and positive feedback which, together, amplify the pathway input signals filtering out noise (other chemicals that may interfere). In this context, the most popular example is the chemotaxis of *E. Coli* [1] because it shows an evident robust adaptation to environmental changes. *Redundancy* plays a key role in robustness: pathways often have different ways to produce the same molecules, allowing them to tolerate problems such as the absence of a specific reactant. *Modularity* ensures that, if there is a damage in one of the parts of the system, this does not affect also the other parts. In this way, it is possible to avoid a total collapse, due to a local error. *Structural stability* is the quality according to which a system is able to adapt to changes even in presence of different external perturbations. Some examples of this can be found in gene regulatory circuits, that are stable for a broad range of stimuli and genetic polymorphisms [21].

The robustness of a pathway can be tested by performing wet-lab (in vitro) experiments, or through mathematical or computational (in silico) approaches on a pathway model. Model-based approaches are usually based either on mathematical analysis methods, or on numerical and simulation methods. Unfortunately, the applicability of these approaches is often hampered by the complexity of the models to be analyzed (usually expressed as ODEs or Markov chains).

To avoid analyzing complex models, Shinar and Feinberg in [34,35] proposed a *sufficient condition* that, in some particular cases, allows robustness to be derived directly from a syntactical property of the pathway, without the need of studying or simulating its dynamics. The sufficient condition states that a mass action system can be considered robust if it admits a positive steady state, the underlying reaction network has a *deficiency* (that is a measure of *linear independence* among its reactions) equal to one and there are distinct non-terminal complexes that differ only in a single species (see [16] for the details).

This approach has the great advantage to prove robustness without executing the system. Indeed, verifying robustness would require, in general, to consider all possible initial states of the system. In particular, regarding the signalling pathways, it would be necessary to test the system behaviour by examining all the possible combinations of initial concentrations of chemical species and, in practice, this would require a huge number of simulations. On the other hand, the sufficient condition proposed in [34,35] is not general: its syntactic constraint makes it applicable only to a particular class of pathways.

A further step towards the formal study of robustness was made in [8], where the concept of *adaptability* of a system is introducted. This consists in the capacity of the system to adapt its behaviour to different initial concentrations of some chemical species with, possibly, different degrees of robustness.

Both robustness and adaptability can be formally studied by applying the methodology proposed by Rizk et al. in [32,33]. Such a methodology is based

on the definition of robustness given by Kitano in [22] as *the ability of a system to maintain specific functionalities against perturbations*. The robustness of a system is measured as the *distance* of the system behaviour under perturbations from its reference behaviour expressed as a temporal logic formula. The distance is computed by using a notion of *violation degree* measuring how much the temporal logic formula should be changed in order to match traces of perturbed behaviours obtained, for instance, through simulations.

The approach proposed by Rizk et al. is very general, both in the description of the reference behaviour and as regards perturbations. In this paper, instead, we focus on *concentration robustness*, namely on the influence of the initial concentrations of species on what will be the steady state of the system. What we propose is a notion of α-robustness, based on continuous Petri nets [17] and interval markings, which extends the notion of *absolute concentration robustness* considered in [34,35] with the notion of *adaptability* proposed in [8].

Our definition of robustness is simpler and much less general than the one considered by Rizk et al. However, it is conceived with the aim of enabling further studies on sufficient conditions that could allow robustness to be assesses by avoiding (or significantly reducing) the number of simulations to be perfomed. This could be obtained, for instance, by adapting conditions already considered in the context of monotonicity analysis [3].

We validate our definition by modelling and simulating three different systems, two related to the Escherichia coli organism (the *EnvZ/OmpR* and bacterial chemotaxis) and the last one dealing with enzyme activity at saturation. By simulations, we verify the robustness of the system and, by varying the initial parameters, we test the degree of the robustness.

We proceed by first introducing the continuous Petri nets formalism in Sect. 2.1, which is the base of our new formal definition of robustness presented in Sect. 2.2. In Sect. 3 we validate our definition using the three biochemical examples. Finally, Sect. 4 contains some conclusions and future work.

2 Formal Definition of the Robustness Property

Many formalisms have been used to describe biological systems at different abstraction levels, as for example Petri nets [19,31], P systems [28,29], reaction systems [13], BioPepa [11] and Hybrid Automata [2,20,24,27]. These notations allow systems to be modeled unambiguously and enable the application of formal analysis techniques such as model checking [10,23], abstract interpretation [12,15,18] and, in general, logic and symbolic reasoning approaches [4–7,14].

In this work, we formalize the robustness property, using the formalism of continuous Petri nets [17]. Petri nets have many applications in different areas, since they are able to model static and dynamic behavioural aspects. They are a valid tool to study concurrent and parallel programs, communication protocols, business processes as well as biological systems.

2.1 Continuous Petri Nets

A *continuous Petri net* N can be defined as a quintuple $\langle P, T, F, W, m_0 \rangle$ where:

- P is the set of continuous *places*, conceptually one for each considered kind of system resource;
- T is the set of continuous *transitions* that consume and produce resources;
- $F \subseteq (P \times T) \bigcup (T \times P) \rightarrow \mathbb{R}_{\geq 0}$ represents the set of arcs in terms of a function giving the weight of the arc as result: a weight equal to 0 means that the arc is not present;
- $W : F \rightarrow \mathbb{R}_{\geq 0}$ is a function, which associates each transition with a *rate*;
- m_0 is the *initial marking*, that is the initial distribution of *tokens* (representing resource instances) among places. A marking is defined formally as $m : P \rightarrow \mathbb{R}_{\geq 0}$.

Tokens are movable objects, assigned to places, that are consumed by transitions in the input places and produced in the output places. Graphically, a Petri net is drawn as a graph with nodes representing places and transitions. Circles are used for places and rectangles for transitions. Tokens are drawn as black dots inside places. Graph edges represent arcs and are labeled with their weights. For simplicity, the labels of arcs with weight 1 is omitted. To faithfully model biochemical networks, the marking of a place is not an integer (the number of tokens) but a positive real number (called *token value* representing the concentration of a chemical species. Each transition is associated with a kinetic constant, that determines the rate of (continuous) flow of tokens from the input to the output places of the transition.

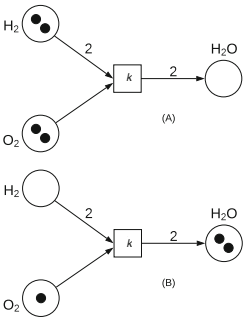

Fig. 1. Example of Petri net. In this case, it shown how represent the chemical reaction: $2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$. (A) and (B) represent two different markings for the same Petri net. The marking in (B) is obtained from the one in (A) as the result of firing transition with the rate k.

Figure 1 shows a simple example of continuous Petri net modeling the chemical reaction $2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$. In sub-figure (A), each place, H and O, has

two tokens: the transition is enabled since it requires two tokens from H_2 and only one from O_2. Sub-figure (B) shows the situation after the transition has been fired: the tokens are moved (in a continuous way) to the output places. Note that in (B) the transition is no longer enabled.

The dynamics of a Continuous Petri net can be expressed in terms of ODEs (in agreement with the standard mass action kinetics of chemical reactions). Each place corresponds to a continuous variable whose value corresponds the place's marking. The dynamics of the variable is expressed by a differential equation consisting of a summation of terms corresponding to the transitions connected to the place. Each term has a positive sign if the transition is connected to the place by an outgoing arc. The sign is negative otherwise. Moreover, the term is the product of the weight of the arc with the values of the variables corresponding to all the places providing resources to the transition (i.e., having and outgoing arc connecting them to the transition). Those variables have as exponent the weight of the arc connecting them to the transition.

For example, considering the continuous Petri net in Fig. 1. The ODEs describing the dynamics of the net are as follows:

$$\frac{dH_2}{d_t} = -2kH_2^2O_2 \qquad \frac{dO_2}{d_t} = -kH_2^2O_2 \qquad \frac{dH_2O}{d_t} = +2kH_2^2O_2$$

An alternative (stochastic) dynamics can be given by using the terms of the ODEs computed for each transition as rates of a Continuous Time Markov Chain (CTMC). Both ODEs and CTMCs offer standard analytic ways to compute the steady state of the system.

Hereinafter, we refer to continuous Petri nets simply as Petri nets and we assume their dynamics to be expressed in terms of ODEs.

2.2 Formal Definition of Robustness

Given a biochemical network, the idea is to verify whether by varying the initial concentrations of some *input* species, the *output* of the network (the concentration of a species of interest) remains either constant or bounded within a given interval. We will assume the initial concentration of the input species to vary within given intervals, and the initial concentrations of all the other molecules to be fixed. Under these assumptions, we define the property of robustness of the system and we formalize it by using Petri nets.

We introduce some auxiliaries definitions. First, we extend the concept of marking. Recall that in Sect. 2.1 we defined the initial marking as an assignment of a fixed value to each place p. Now, we generalize the idea of initial marking by considering a marking as an assignment of a *interval of values* to each place p of the Petri net.

We first define the domain of intervals.

Definition 1 (Intervals). *We define the interval domain as*

$$\mathcal{I} = \{[n, m] \mid n, m \in \mathbb{R}_{\geq 0} \cup \{+\infty\} \text{ and } n \leq m\}.$$

An interval $[n, m] \in \mathcal{I}$ *is* trivial *iff* $n = m$. *Moreover, we say that* $x \in [n, m]$ *iff* $n \leq x \leq m$.

We now define interval markings.

Definition 2 (Interval marking). *Given a set of places* P, *an* interval marking *is a function* $m_{[\,]} : P \to \mathcal{I}$. *We call* $M_{[\,]}$ *the domain of all interval markings.*

An interval marking in which at least one interval is non-trivial represents an infinite set of markings, one for each possible combination of values of the non-trivial intervals. Therefore, given an interval marking, we relate it with the markings as in the original Petri nets formalism in the following way:

$$\text{Given } m \in M \text{ and } m_{[\,]} \in M_{[\,]},\ m \in m_{[\,]} \text{ iff } \forall p \in P, m(p) \in m_{[\,]}(p).$$

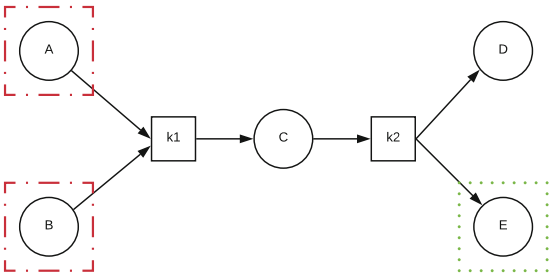

Fig. 2. Example of Petri nets, in which A and B are marked as input of the system (red dot-line) and E is marked as output (green dots). (Color figure online)

In a Petri net we assume that there exists *at least one* input place and *exactly one* output place representing input and output species of the modeled biochemical network, respectively. See Fig. 2 for an example. Under this assumption, we can give our formal definition of robustness.

Definition 3. (α-Robustness). *A Petri net* N *with output place* O *is* α*-robust with respect to a given interval marking* $m_{[\,]}$ *iff* $\exists k \in \mathbb{R}$ *such that* $\forall m \in m_{[\,]}$, *the marking* m' *corresponding to the steady state reachable from* m, *is such that*

$$m'(O) \in [k - \frac{\alpha}{2}, k + \frac{\alpha}{2}] \ .$$

Note that the definition of α-robustness does not explicitly mention the input places of the net. Actually, input places will be those having a non-trivial initial in $m_{[\,]}$. In other words, input places are those whose initial marking is not fixed.

Given the previous definition, it can be observed that:

– the wider are the intervals of the initial interval marking, the more robust is the network, because it means that the system gives similar outputs regardless the initial inputs;

– the smaller is the value of α, the more robust is the network.

Here, we have given a general definition that can be modified in different ways. For example, rather than considering the marking at the steady states, it could be possible to consider the marking reached at a given time T, or when the system terminates its execution (no transition is enabled).

It is worth noting that our definition is general enough to capture several notions of robustness available in literature. For example, by considering the initial intervals $[1, \infty]$ for the initial concentration of the input species and $\alpha = 0$ we obtain a formal definition for the robustness notions considered in [8,34].

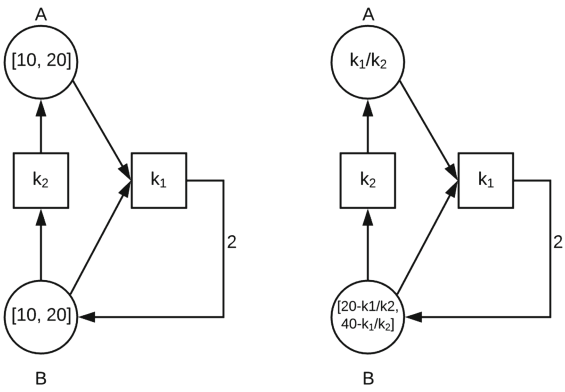

Fig. 3. Example of robust biochemical network, considering the species A as output of the system.

A simple example of robust biochemical network is given by the following two reactions:

$$\mathrm{A} + \mathrm{B} \xrightarrow{k_1} 2\,\mathrm{B} \qquad \mathrm{B} \xrightarrow{k_2} \mathrm{A}$$

The Petri net representation of the network is shown in Fig. 3 (on the left with the initial marking, on the right with the steady state marking). In this case, the steady state is such that

$$A = \frac{k_2}{k_1} \qquad B = \theta - \frac{k_2}{k_1}$$

where θ is the sum of initial concentrations of A and B. If A is the output of the system, then its concentration in the steady state does not depend from the initial quantity of the (input) chemical species A and B (0-robustness with $k = \frac{k_2}{k_1}$). If we consider $[10, 20]$ as the initial interval for both A and B, we obtain that θ will be in $[20, 40]$. So, for B as the output we obtain:

$$B \in [20 - \frac{k_2}{k_1}, 40 - \frac{k_2}{k_1}]$$

Thus, for output B we have α-robustness with $\alpha = 20$, suggesting that B is not independent from the initial concentrations of A and B.

Moreover, in Fig. 4 we can see a network that is never robust neither considering A as output, nor B. Their chemical reactions are: A $\xrightarrow{k_1}$ B, B $\xrightarrow{k_2}$ A. In this case, the concentrations of A and B at the steady state are both always influenced by the input values. The reason of this behaviour is related to the fact that in this case the chemical species are transformed, but not consumed.

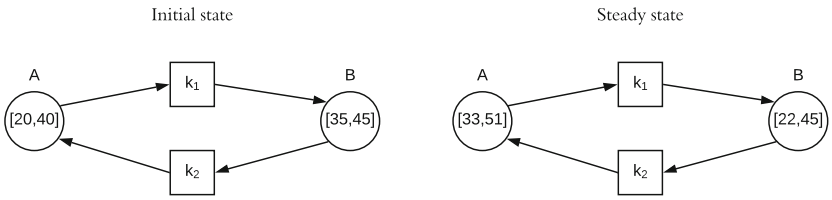

Fig. 4. Example of non robust network. In this case we chose $k_1 = 2$ and $k_2 = 3$.

3 Validating the Definition of Robustness

To validate our definition of robustness, we consider three examples of biological networks: the two component *EnvZ/OmpR* osmoregulatory signalling system and the bacterial chemotaxis, which are related to E. coli, and a model of the behaviour of the enzyme kinetics at saturation. The first example shows absolute concentration robustness, corresponding to 0-robustness in our setting. The other two examples show a concentration robustness that it is not absolute (α-robustness with α greater than 0).

3.1 EnvZ/OmpR Osmoregulatory Signalling System in E. Coli

The *EnvZ/OmpR* system regulates the expression of two porins, *OmpF* and *OmpC*, which are proteins having many roles in the cell, as for example nutrients transportation, elimination of toxins and many others [9,35].

The regulatory system consists of two components. The first one is the *histine kinase EnvZ*, a particular kind of protein having the role of adding and removing a phosphate to an aspartame acid usually on the other component of the signalling pathway, the *response regulator OmpR*, which mediates a response of the cell to changes in its environment. The role of *EnvZ* is bifunctional because it phosphorylates and dephosphorylates *OmpR*: the model predicts that the steady state level of $OmpR_P$ (the phosporylated form of *OmpR*) is insensitive to variations in the concentration of *EnvZ* and *OmpR*.

Table 1. The initial concentrations, the rates and the chemical reactions of *EnvZ/OmpR* system. The concentration of X and Y, marked by the symbol $\diamond$, can vary to prove the robustness in Y_P.

Initial concentrations	Rates	Chemical reactions
$X = 25\ \diamond$	$k_1, k_2, k_3, k_4 = 0.5$	$XD \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X$
$Y = 150\ \diamond$	$k_5, k_{11} = 0.1$	$XT \underset{k_4}{\overset{k_3}{\rightleftharpoons}} X$
$XT = 0$	$k_6, k_9 = 0.02$	$XT \xrightarrow{k_5} X_P$
$X_P = 0$	$k_7, k_8, k_{10} = 0.5$	$X_P + Y \underset{k_7}{\overset{k_6}{\rightleftharpoons}} X_PY$
$X_PY = 0$		$X_PY \xrightarrow{k_8} X + Y_P$
$Y_P = 10$		$XD + Y_P \xrightarrow{k_9} XDY_P$
$XDY_P = 0$		$XDY_P \xrightarrow{k_{10}} XD + Y_P$
$XD = 50$		$XDY_P \xrightarrow{k_{11}} XD + Y$

Modeling and Simulation of the EnvZ/OmpR System in E.coli. The main components of this chemical network are *EnvZ* and *OmpR*, denoted in Table 1 respectively as X and Y. *EnvZ* phosporylates *OmpR* (Y_P) and itself (X_P), by binding and breaking down *ATP*. In this sequence of chemical reactions, in fact, *ATP* and *ADP* act as cofactors (denoted as T and D).

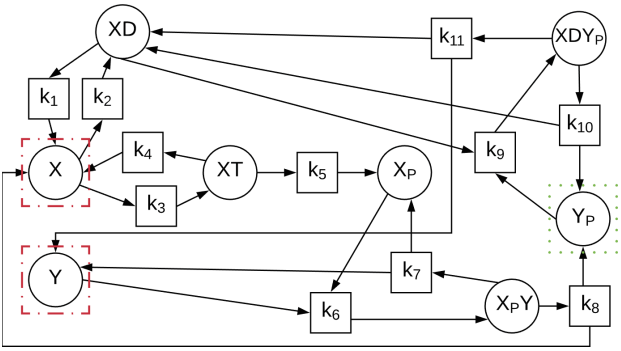

Fig. 5. The Petri nets model for the reaction network of the *EnvZ/OmpR* system. The input of the network are X and Y (red dot-lines), the output is Y_P (green dots). (Color figure online)

In order to check whether the system satisfies our definition of robustness we build the Petri nets model shown in Fig. 5, where X and Y are the input and Y_P is the output. To study the equilibrium configuration, we compute the steady state by setting the time-derivatives to zero and solving the obtained equations.

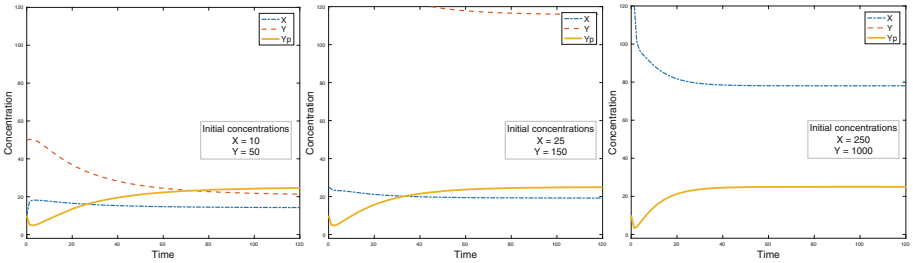

Fig. 6. Graphical results of the simulation of the *EnvZ/OmpR* system. We vary the concentrations of X and Y to show robustness in Y_P. Note that in the third case the curve of Y is out of the graph.

At the steady state, the concentration of Y_P does not depend from the input chemical species, thus, the system satisfies 0-robustness (absolute concentration robustness) for the widest intervals ($[1, \infty]$) of initial concentrations.

To illustrate the robustness of this system we show some simulation results obtained by using Dizzy [30]. Simulation results are in Fig. 6, where it is shown that the concentration of Y_P is constant even varying the initial concentrations of the input species X and Y.

Moreover, note that in this case, we can also apply the theorem in [34]: the *deficiency* of the network is 1 and the sufficient conditions required by the theorem to assure absolute concentration robustness in Y_P can be verified.

3.2 Bacterial Chemotaxis

In nature, one of the most important examples of robustness is in bacterial chemotaxis. It is the process through which bacteria sense and move along concentration gradients of specific chemicals like sugars or amino acids (as serine and aspartame) [1]. Despite their physical limitations, bacteria can detect concentration gradients to guide their motion, which consists in runs in which they alternate keeping a constant direction with *tumbles* in which they randomly change direction. The bacterium continuously compares the current attractant concentration with its concentration in the past. If it detects an increment, it reduces the tumbling frequency. After a while, if the concentration of the attractant remains constant, the bacterium increases the tumbling frequency back to the original level. This phenomenon is an example of *exact adaptation*, because the concentration of attractant does not influence the bacterium response to the ambient change (it is the gradient that matters).

Modeling and Simulation of Chemotaxis of E. coli. A detailed description of bacterial chemotaxis can be found in [1,8]. The E. coli senses the concentration of an attractant L through receptors on its external membrane. Each receptor is bound to a protein kinase, constituting a group denoted X. Rapidly, this group

Table 2. The initial concentrations, the rates and the chemical reactions of chemotaxis phenomenon of the E. coli. The concentration of the attractant L, marked by the symbol $\diamond$, can vary to prove the robustness in $CheY_P$.

Initial concentration	Rates	Chemical reaction
$X = 10$	$k_1, k_{13} = 1.15$	$X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X^*$
$X^* = 10$	$k_2, k_{12} = 0.25$	$X^* + CheY \xrightarrow{k_3} CheYp + X$
$L = 0 \diamond$	$k_3 = 0.1$	$CheYp + Z \xrightarrow{k_4} CheY + Z$
$CheY = 10$	$k_4 = 10$	$CheYp \xrightarrow{k_5} CheY$
$Z = 1$	$k_5 = 0.002$	$L + X^* \xrightarrow{k_6} L + XY$
$CheYp = 1$	$k_6, k_7, k_{11} = 1$	$CheBp \xrightarrow{k_7} CheB$
$XL = 0$	$k_8 = 80$	$XY \xrightarrow{k_8} XL$
$X^*m = 1$	$k_9 = 0.01$	$CheR + XL \xrightarrow{k_9} X^*m + CheR$
$CheR = 1000$	$k_{10} = 0.2$	$X^*m + CheB \xrightarrow{k_{10}} Bp + X^*m$
$XY = 0$	$k_{14} = 0.18$	$X^*m + CheBp \xrightarrow{k_{11}} X^* + Bp$
$CheB = 2$		$X^*m \underset{k_{13}}{\overset{k_{12}}{\rightleftharpoons}} Xm$
$CheBp = 0$		$X^*m + CheY \xrightarrow{k_{14}} CheYp + Xm$
$Xm = 0$		

passes from inactive state X to an active state $X*$, and starts modifying the state of a regulator protein, $CheY$, by adding a phosphate group to it (which becomes $CheY_P$). The complex $CheY_P$ is the main responsible of tumbles: in fact, the higher is its concentration, the higher is the tumbling frequency.

During this process, the binding of X with attractants reduces its probability to reach the active state that, consequently, reduces also the probability to attach a phospate group to $CheY$. As a consequence, the tumbling frequency is lowered.

Since the attractant (L) reduces the activity of X, there is the *methylation* mechanism to switch on again the chemical group. An enzyme $CheR$ adds at constant rate a methyl group to the XL complex, which becomes X_m and restarts behaving as X. The methyl group is removed by the enzyme $CheB$, which is influenced by X that, adding a phosphoryl group to $CheB$, makes it more active, constituting a *negative feedback loop*: the higher is the activity of X, the higher is that of $CheB$, which, in turn, reduces the activity of X. Exact adaptation is achieved because of the feedback circuit: the increased methylation of X precisely balances the reduction in activity caused by the attractant.

In Table 2 we summarize the chemical reactions of the chemotaxis network, together with rates and initial concentrations. The Petri net of the reaction network is in Fig. 7. By computing the steady state for $CheY_P$ we find that it

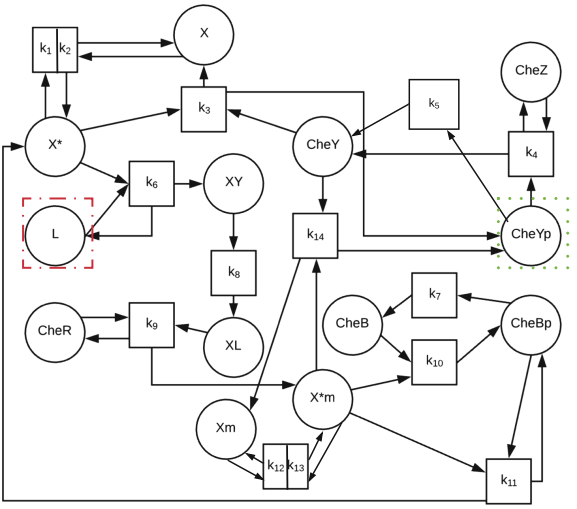

Fig. 7. The Petri nets model for the reaction network of the bacterial chemotaxis network. The input of the network are L (red dot-line), the output is the concentration of $CheYp$ (green dots). (Color figure online)

does not depend on L. Thus, according to our definition, this system is 0-robust on $CheY_P$ with respect to the variation of input L.

We simulated the chemical reactions network using again Dizzy [30]. Some simulation results are shown in Fig. 8. The first sub-figure shows that the bacterium sense the initial concentration of L and reacts by reducing the concentration of $CheY_P$ (and hence the frequency of tumbles). Since the concentration of L does not change over time, the concentration of $CheY_P$ is brought back to its original value. The second sub-figure shows that, as an effect of 0-robustness with respect to the input L, the dynamics of $CheY_P$ is the same even with a different concentration of L. The third sub-figure, instead, shows that by varying the concentration of the enzyme $CheR$, the concentration of $CheY_P$ does not

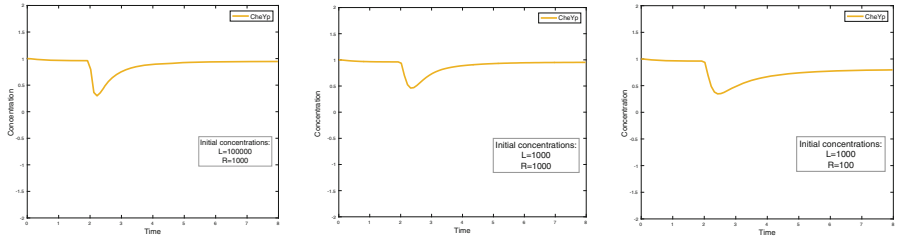

Fig. 8. Graphical results of the simulation of the bacterial chemotaxis. To show how robustness is preserved, we change the concentration of the attractant L, to study how this influences $CheY_P$.

return exactly to the initial value, hence the system is α-robust with $\alpha = 0.3$ when the species considered as input is $CheR$.

Note that the *deficiency* of this network is 3, hence the sufficient condition of [34] cannot be applied.

3.3 Enzyme Activity at Saturation

The well-known Lotka-Volterra reactions [25,26] can be interpreted as abstract chemical reactions and, in fact, they have been proposed to investigate the oscillatory dynamics of autocatalytic enzymes. Similarly, the logistic equation [36] is a model of population growth that is commonly used also in the context of biochemical reaction kinetics. It describes the growth of a population by taking the amount of available environmental resources into account (the *carrying capacity* of the environment) and it is used also to model enzyme dynamics at saturation. In this section we consider an abstract model of enzyme activity inspired by the Lotka-Volterra reactions and the logistic equation (Table 3).

Modeling and Simulation of Enzyme Activity at Saturation. We consider an abstract chemical reaction network in which an enzyme R produces a molecule X. To ensure mass conservation, we add to this idealized example the species Z, which has the role to preserve the concentration of R (i.e. R is never consumed nor produced, but transformed into Z and back).

Table 3. The initial concentrations, the rates and the chemical reactions of enzyme activity at saturation model. The concentration of P, marked by the symbol $\diamond$, can vary to prove the robustness in X.

Initial concentrations	Rates	Chemical reactions
$\mathrm{R} = 1000$	$k_1 = 100$	$\mathrm{R} + \mathrm{X} \xrightarrow{k_1} \mathrm{X} + \mathrm{X} + \mathrm{Z}$
$\mathrm{X} = 30$	$k_2 = 10$	$\mathrm{X} \xrightarrow{k_2} \mathrm{W}$
$\mathrm{Z} = 0$	$k_3 = 0.5$	$\mathrm{Z} \xrightarrow{k_3} \mathrm{R}$
$\mathrm{P} = 1 \diamond$	$k_4 = 0.01$	$\mathrm{X} + \mathrm{P} \xrightarrow{k_4} \mathrm{P} + \mathrm{P}$
$\mathrm{C} = 0$	$k_5 = 0.5$	$\mathrm{P} \xrightarrow{k_5} \mathrm{C}$
$\mathrm{W} = 10$		

As in Lotka-Volterra, the production of X is autocatalytic (the more X are present, the higher is the production rate), but the concentration of enzymes R is limited. Hence, the enzyme activity can easily reach saturation. This reaction system is of the kind typically modeled by the logistic equation. It is expected to reach a dynamic equilibrium in which the concentration of X does not depend on its initial concentration, but only on the concentration of R. We add to

this system a molecular species P acting as a "predator" for X (again, as in Lotka-Volterra). Species X can be consumed and transformed into P, by another autocatalytic reaction. In this model it can be interesting to investigate how the initial concentration of P influences the steady state concentration of X.

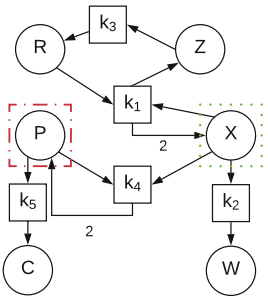

Fig. 9. The Petri nets model for enzyme activity at saturation system. The input of the network is P (red dot line), the output is X(green dots). (Color figure online)

The Petri nets model of the reactions network is shown in Fig. 9, with P as the input and X as the output species. At the steady state, the concentration of X is always constant and its value only loosely depends on the initial concentration of P. We chose $[1, 20000]$ as initial interval marking for P and we found, by the means of simulations, that the concentration reached by X is in the range $[50, 47]$, (see Fig. 10). Therefore, the system is α-robust with $\alpha = 3$ with respect to input P and the considered initial interval marking.

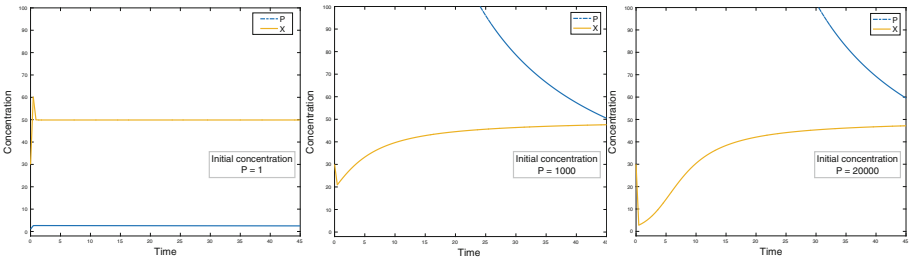

Fig. 10. Graphical results of the enzyme activity at saturation model. We change the concentration of the P to test robustness in X.

4 Conclusions

We proposed the notion of α-robustness with extends the notion of absolute concentration robustness considered in [34,35] with the notion of adaptability [8] in

a way that could capture a large class of pathways exhibiting robust behaviours. We illustrated α-robustness with three examples of robust pathways.

As future work, we plan to formulate and study α-robustness by applying the more general methodology proposed by Rizk et al. in [32,33]. Moreover, we will investigate new ways to verify our α-robustness property. For example, we would like to find sufficient conditions under which the property could be verified efficiently, without computing the steady state of the system and without performing simulations in an exhaustive way. This could be obtained, for instance, by adapting conditions already considered in the context of monotonicity analysis of chemical reaction networks [3].

Acknowledgements. This work has been supported by the project "Metodologie informatiche avanzate per l'analisi di dati biomedici" funded by the University of Pisa (PRA_2017_44).

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