Formalizing robustness of 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 system components. 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. 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 study of living cells, to understand their morphological and functional organization. A cell, in fact, is a very complex system. It consists of a huge number of components that interact with each other essentially through chemical reaction networks. The cell's global behaviour, both internal and with the environment, emerges from such an interaction.

Chemical reaction networks, called *pathways* in the context of cells, govern the basic activities of cells and how the cell reacts to external stimuli. They 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 allow 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 particular context, the main challenge is to explore how the components of the cells interact with each other as a *system*, reproducing the observed behaviours, and how the concentration of particular species can influence the whole complex structure. To achieve this goal, one of the possible solutions is

to follow the systems biology approach that allows us to design predictive and multiscale models. Systems biology, in fact, has two main purposes: to analyze the interactions between single parts of each cell and to examine the working principles in its entirety, exploring their robust and complex structure.

In this perspective, we focus on the definition of the *robustness* property, a fundamental feature of complex evolving systems, for which the functionality of the system remain 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 [13]. *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 components. In this way, it is possible to avoid a total collapse, due to a local error. Cells are clearly organized into functional units separated by membranes (the nucleus, the Golgi apparatus, endosomes, etc.). *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 [12].

It emerges that robustness can be seen as an internal quality or as an architectural characteristic of the system that enables complex systems to evolve after a specific environmental perturbation. 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 all of these approaches is often hampered by the complexity of the models to be analyzed (often expressed in terms of ODEs or Markov chains).

To avoid analyzing complex models, Shinar and Feinberg in [22,23] 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 [7] 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 [22,23] is not general: its syntactic constraint make them applicable to a particular class of robust pathways.

A further step towards the formal study of robustness was made in [4], where the authors introduced the concept of *adaptability* of a system. This consists in the capacity of the system to adapt itself to initial concentrations of chemical species. From this work emerges the possibility to have different degrees in robustness representing how the system reacts to internal and external changes.

The first problem in formally studying robustness is that in the literature there is no formal characterization of this property, able to generalize the different aspects of robustness and adaptability of a biochemical network. For this reason, we propose a formal definition of robustness based on the continuous Petri nets formalism, able to capture different aspects of this property. In particular, we focus on the role of the initial concentrations of the species involved in the chemical reactions to study how they influence the system functionality.

In many biochemical networks the initial set of chemical species can have impact on the internal features of the system. An example is the chemotaxis of the E. coli, in which the enzymes initial concentration has effect on the bacterium perception of the external environment.

With our definition, we are able to describe and study which perturbation influences more the functionality of the system and how. We validate our definition by modelling and simulating three different systems, two related to the Escherichia coli organism (the *EnvZ/OmpR* and the isocitrate dehydrogenase (*IDH*) regulatory systems) and the last one dealing with enzyme activity at saturation. By simulations, we verify the robustness of the system and, changing the initial parameters, we test the degree of the robustness.

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

2 Formal definition of the robustness property

Many formalisms have been used in Computer Science to describe biological systems at different abstraction levels, as for example Petri nets [10,21], P Systems [18,19] and Hybrid Automata [2,11,14].

In this work, we formalize the robustness property, using the formalism of continuous Petri nets. 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 [17], communication protocols, decision models and neural networks.

2.1 Continuous Petri nets formalism definition

Formally, we can define a continuous Petri net N[8] as a quintuple:

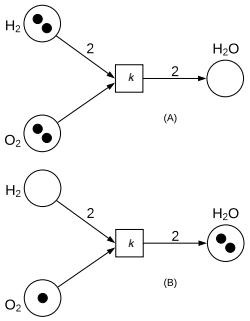

Fig. 1: Example of use of Petri net. In this case, it shown how represent the chemical reaction: $2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$. (A) and (B) represent two different markings for the same Petri net. The marking in (B) is obtained from the one in (A) as the result of firing transition with the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

$$N =< P, T, F, W, m_0 >$$

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 $m : P \rightarrow \mathbb{R}_{\geq 0}$.

The 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 network, the marking of a place is not an integer, but a positive real number, called token value, representing the concentration of chemical species. To each transition is associated a chemical rate, which represents a continuous flow.

Figure 1 shows a simple example of continuous Petri net modeling the chemical reaction $2\,H_2 + O_2 \xrightarrow{k} 2\,H_2O$ taken from [17]. 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 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. The term has a positive sign if the the place is connected to the transition 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 Figure 1. The ODEs describing the dynamics of the Petri 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 analytical 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, like a signalling pathway, our idea is to verify whether, even by varying the initial concentrations of some chemical species, the *output* of the chemical reactions remains either constant or bounded within a given interval of values. We will assume the initial concentration of the input molecules of the pathway to vary within given intervals, and the initial concentrations of all the other molecules (that are neither input, nor output) 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 section 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*

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

Moreover we say that $x \in [n, m]$ *iff* $n \leq x \leq m$.

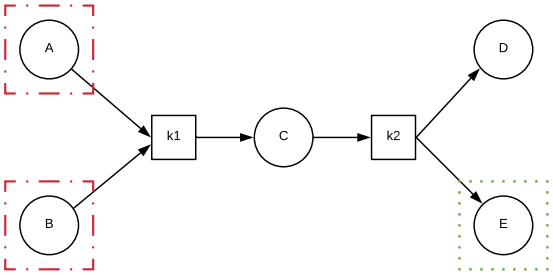

Fig. 2: Example of Petri nets, in which A and B are marked as input of the system (red dot-line) and E is marked as output (green dots).

We now define interval markings.

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

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

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

In a Petri net PN we assume that there exists at least one place p that we consider as the *input* of the network. In addition, we assume that there exists also a (unique) place p that we consider the *output* of the net. See Figure 2 for an example. Within this framework, we can give our formal definition of robustness.

Definition 3 (α-Robustness). *A Petri net PN with output place O is defined as* 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*

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

Given the previous definition, it can be observed that:

- wider are the intervals of the initial interval marking, more robust is the network, because it means that the system gives the same output regardless the initial inputs;
- smaller is the value of α, more robust is the system.

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).

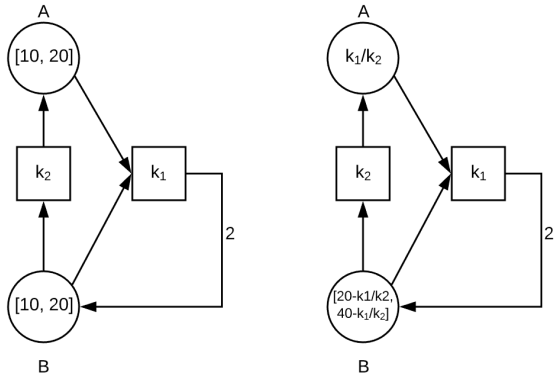

Fig. 3: Example of robust biochemical network, considering the species A as output of the system.

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 [4,22].

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 Figure 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:

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

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 Figure 4 we can see a network that is never robust neither considering A as output, nor B. Their chemical reactions are: $\mathrm{A} \xrightarrow{k_1} \mathrm{B}$, $\mathrm{B} \xrightarrow{k_2}$

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.

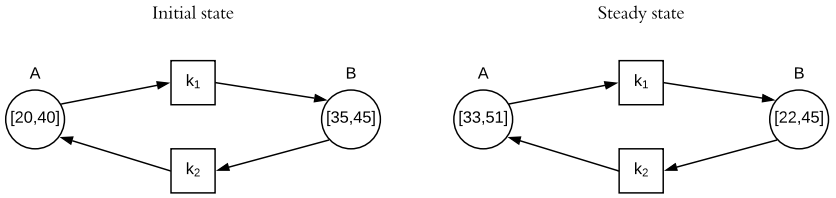

Fig. 4: Example of non robust network. In this case we chose $k_1 = 2$ and $k_2 = 3$.

3 Validating the definition of robustness

To validate our definition of robustness, we consider three examples of biological networks. The first two, the two component *EnvZ/OmpR* osmoregulatory signalling system and the isocitrate dehydrogenase regulatory system of E. coli, show an absolute concentration robustness (the α parameter of Definition 3 will be equal to 0). The third example models an enzyme kinetics at saturation behaviour, inspired from the Lotka-Volterra reactions [9,24], which shows a concentration robustness that it is not absolute (in this case the α parameter of Definition 3 will be greater than 0).

3.1 EnvZ/OmpR osmoregulatory signalling system

In bacteria and in particular in E. coli, the *EnvZ/OmpR* system has the function to regulate 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 [5].

The regulatory system consists of two components. The first one is the *histine kinase EnvZ*, a particular kind of protein having the role to transmit information, 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 when *EnvZ* is much less abundant than *OmpR*, or when the concentration of this species is sufficiently high, 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
$\mathrm{X} = 25\ \diamond$	$k_1, k_2, k_3, k_4 = 0.5$	$\mathrm{XD} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{X}$
$\mathrm{Y} = 150\ \diamond$	$k_5, k_{11} = 0.1$	$\mathrm{XT} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \mathrm{X}$
$\mathrm{XT} = 0$	$k_6, k_9 = 0.02$	$\mathrm{XT} \xrightarrow{k_5} \mathrm{X_P}$
$\mathrm{X}_P = 0$	$k_7, k_8, k_{10} = 0.5$	$\mathrm{X_P} + \mathrm{Y} \underset{k_7}{\overset{k_6}{\rightleftharpoons}} \mathrm{X_PY}$
$\mathrm{X}_P\mathrm{Y} = 0$		$\mathrm{X_PY} \xrightarrow{k_8} \mathrm{X} + \mathrm{Y_P}$
$\mathrm{Y}_P = 10$		$\mathrm{XD} + \mathrm{Y_P} \xrightarrow{k_9} \mathrm{XDY_P}$
$\mathrm{XDY}_P = 0$		$\mathrm{XDY_P} \xrightarrow{k_{10}} \mathrm{XD} + \mathrm{Y_P}$
$\mathrm{XD} = 50$		$\mathrm{XDY_P} \xrightarrow{k_{11}} \mathrm{XD} + \mathrm{Y}$

Modeling and simulation of the EnvZ/OmpR system in E.coli. The main components of this chemical network are *EnvZ* and *OmpR* [5,23], 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 cofactor (denoted as T and D).

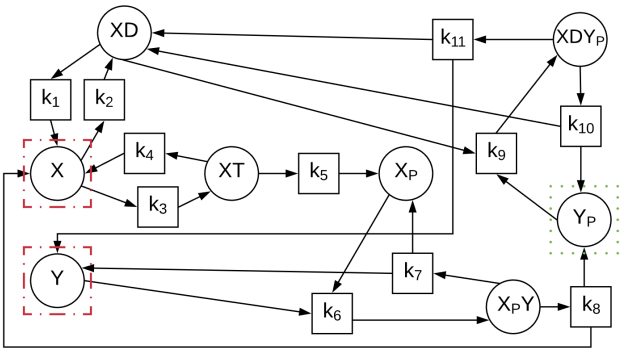

Fig. 5: The Petri nets model for the reaction network of the *EnvZ/OmpR* system. The input of the network are X and Y(red dot line), the output is the concentration of Y_P(green dots).

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

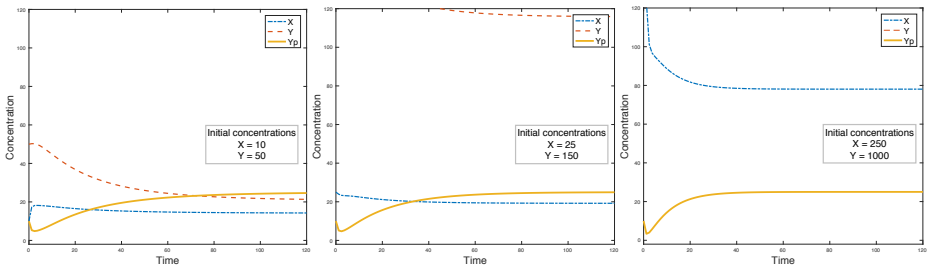

Fig. 6: Graphical results of the simulation of the *EnvZ/OmpR* system. We vary the concentrations of X and Y to show robustness in Y_P. Note that in the third case the curve of Y is out of the graph.

equations. 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 [20]: a simulator of chemical reactions. Simulation results are in Figure 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 [22]: the *deficiency* [7] of the network is 1 and the sufficient conditions required by the theorem to assure robustness in Y_P can be verified (see [3] for details).

3.2 The isocitrate dehydrogenase regulatory system

In the literature, metabolic and regulatory pathways that contain multifunctional proteins, as the *Envz/OmpR* system described above, have frequently been observed to exhibit robustness, thanks to their ability to perform their tasks even in presence of internal and external perturbations. Among several examples, there is the isocitrate dehydrogenase regulatory system (*IDHKP-IDH)* of E. coli [6].

This system controls the partioning of carbon flux and it is useful when the bacterium of E. coli grows on substances, like for example an acetate, which contains only a small quantity of carbon. Without this regulation system, in fact, the organism would not have enough carbon available for biosynthesis of cell constituents [23].

Modeling and simulation of the IDHKP-IDH system. The isocitrate dehydrogenase regulatory system works regulating the phosphorylation level of the TCA cycle enzyme isocitrate dehydrogenase (*IDH*), denoted as I in Table 2. In its active form, the protein I has the role of regulating how much carbon will flow through the system, while it is inactive in its phosporylated form I_P. The enzyme E is bifunctional: it phosporylates and dephosporylates I.

Table 2: The initial concentrations, the rates and the chemical reactions of *IDHKP-IDH* system. The concentration of E and I_P, marked by the symbol $\diamond$, can vary to prove the robustness in I.

Initial concentrations	Rates	Chemical reactions
$\mathrm{E} = 0.001\ \diamond$	$k_1, k_4 = 0.02$	$\mathrm{E} + \mathrm{I_P} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{EI_P}$
I=100	$k_2, k_3, k_5, k_{11} = 0.5$	$\mathrm{EI_P} \xrightarrow{k_3} \mathrm{E} + \mathrm{I}$
$\mathrm{I}_P = 10000\ \diamond$	$k_6 = 0.1$	$\mathrm{EI_P} + \mathrm{I} \underset{k_5}{\overset{k_4}{\rightleftharpoons}} \mathrm{EI_PI}$
$\mathrm{EI}_P = 10$		$\mathrm{EI_PI} \xrightarrow{k_6} \mathrm{EI_P} + \mathrm{I_P}$

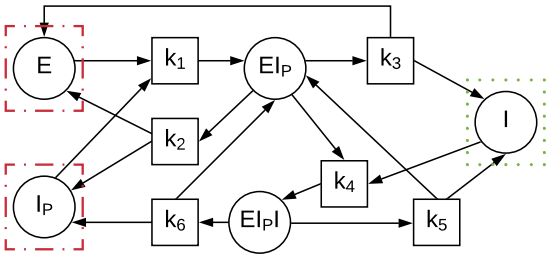

Fig. 7: The Petri nets model for the reaction network of the *IDHKP/IDH* system. The input of the network are E and I_P(red dot line), the output is the concentration of I(green dots).

We build the Petri net model of the chemical networks and we identify I_P and E as the input of the model and I as the output, as shown in Figure 7. By studying the equilibrium configuration, we find that at the steady state the concentration of I is completely independent from the concentration of I_P and E. Thus, the system shows robustness in this species: choosing a wide range of possible initial concentrations for the input, we obtain a constant value for I, as it is possible to notice from the simulation results shown in Figure 8. Therefore, we verified 0-robustness for the widest interval range of initial concentrations of the input species.

As in the case of the EnvZ/OmpR system, we have that the theorem in [22] could be applied to prove absolute concentration robustness of this system.

3.3 Enzyme activity at saturation

The well-known Lotka-Volterra reactions [15,16] 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 [25] 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*

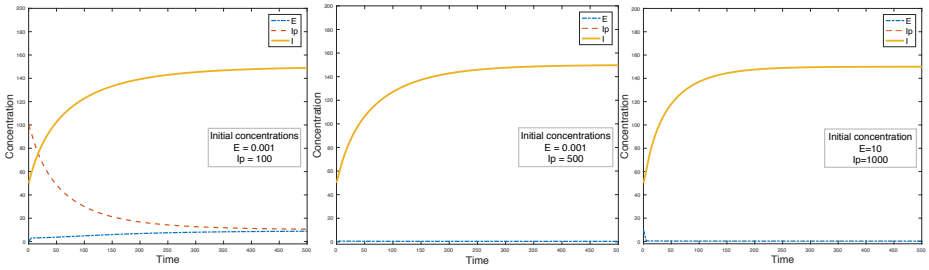

Fig. 8: Graphical results of the simulation of the *IDHKP-IDH* system. We change the concentration of E and I_P to test robustness in I.

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

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.

Modeling and simulation of enzyme activity at saturation model. We consider an abstract chemical reaction network in which an enzyme R produces a molecule X. To guarantee the mass conservation, we add to this idealized example the species Z, which has the role to preserve the concentration of R.

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 an additional molecular species P acting as a "predator" for X (as in Volterra's equations). What happens is that X can be consumed and transformed into P, and the reaction performing this action is autocatalytic (i.e., stimulated by P itself). In

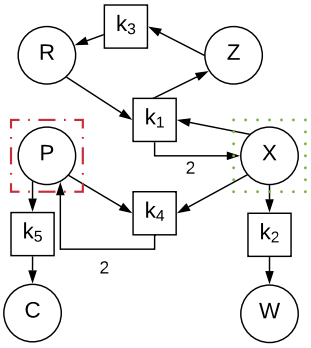

Fig. 9: The Petri nets model for enzyme activity at saturation system. The input of the network is P (red dot line), the output is X(green dots).

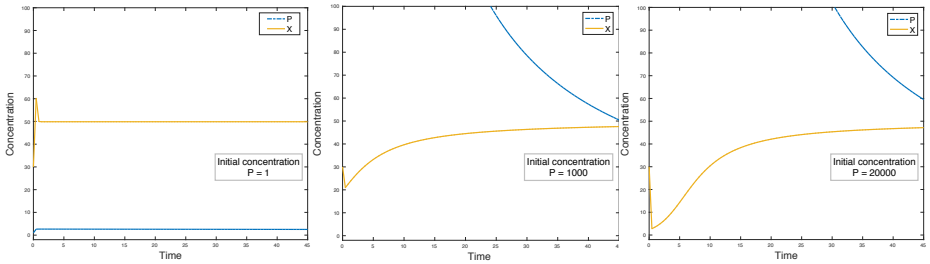

Fig. 10: Graphical results of the enzyme activity at saturation model. We change the concentration of the P to test robustness in X.

this model it can be interesting to investigate how the initial concentration of P influences the steady state concentration of X.

Building the Petri nets model of the reactions network, as shown in Figure 9, we identify P as the input and X as the output of the network. At the steady state, it emerges that the concentration of X is always constant and its constant value only loosely depends on the concentration of P. Indeed, even varying the concentration of the molecular species P in a wide interval, the concentration of X at the steady state assumes a value in a very small interval. It is worth noting that this kind of robustness of the system was not captured by the previous definitions presented in the literature. Instead, our definition is able to express not only absolute robustness but also weaker levels of robustness by tuning the α parameter and the amplitude of the intervals of the input species.

In this case, to apply Definition 3, we choose an interval marking for $P = [1, 20000]$ and we find, by the means of simulations, that the concentration of X is in the range $[50, 47]$, as shown in Figure 10. Therefore, the system is α-robust with $\alpha = 3$.

4 Conclusions

In this paper we presented the notion of α-robustness. This new notion is very general and flexible and allows us to formalize different informal notions of robustness that are present in the literature. As a first step we verify the relevance of our definition by considering different behaviours present in nature. To this aim, we presented here two examples of absolute concentration robustness and one system that shows a robust behaviour even if it cannot be classified as robust before the introduction of our more general definition. To illustrate α-robustness, we computed the steady states and performed some simulations of the three systems by varying the initial concentrations of the input species.

As future work, we intend to 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.

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