Towards an Efficient Verification Method for Monotonicity Properties of Chemical Reaction Networks

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Abstract: One of the main goals of systems biology is to understand the behaviour of (bio)chemical reaction networks, which can be very complex and difficult to analyze. Often, dynamical properties of reaction networks are studied by performing simulations based on the Ordinary Differential Equations (ODEs) models of the reactions' kinetics. For some kinds of dynamical properties (e.g. robustness) simulations have to be repeated many times by varying the initial concentration of some components of interest. In this work, we propose sufficient conditions that guarantee the existence of monotonicity relationships between the variation of the initial concentration of an "input" biochemical species and the concentration (at all times) of an "output" species involved in the same reaction network. Our sufficient conditions allow monotonicity properties to be verified efficiently by exploring a dependency graph constructed on the set of species of the reaction network. Once established, monotonicity allows us to drastically restrict the number of simulations required to prove dynamical properties of the chemical reaction network.

1 INTRODUCTION

The dynamics of biological systems can be very difficult to analyze, because they often consist of a huge number of components that interact with each other as a *system*. This is true in particular for systems considered at the molecular level, in which components interact essentially through chemical reactions.

From the computational viewpoint, the dynamics of (bio)chemical reaction networks is often studied by performing simulations. The two most common approaches to the simulation of reaction networks are the deterministic and the stochastic ones (Barnes and Chu, 2010). The deterministic approach is usually based on a description of the behaviour of the system in terms of Ordinary Differential Equations (ODEs). The stochastic approach usually requires the application of Gillespie's simulation algorithm (Gillespie, 1977), or of one of its more recent (and efficient) variants (Cao et al., 2006; Salis and Kaznessis, 2005).

In the deterministic approach the time evolution of the model is studied as a continuous process. Concentrations are continuous values and reactions change their values in a continuous way. In the stochastic approach, concentrations are discretized (these approaches actually deal with number of species, rather than concentrations) and reactions occur one by one

in discrete time steps. In both the deterministic and the stochastic cases, simulations can become computationally very expensive. For this reason, other less accurate analysis approaches are often considered, which are based for instance on a qualitative description of the system behaviour, or on abstractions. Examples of analysis approaches of this kind are those based on Boolean networks (Shmulevich et al., 2002; Schlitt and Brazma, 2007; Barbuti et al., 2018), on interaction graphs (Jeong et al., 2001; Stelzl et al., 2005; Brohee and Van Helden, 2006), on abstract interpretation (Fages and Soliman, 2008; Gori and Levi, 2010; Bodei et al., 2015; Fages et al., 2017; Carvalho et al., 2018) and, in general, on logic and symbolic approaches (Eker et al., 2001; Antoniotti et al., 2003; Barbuti et al., 2016a; Barbuti et al., 2015; Barbuti et al., 2016b).

In this paper, we focus on how to make more efficient the analysis of the dynamics of a biological systems in a deterministic setting. One of the problems in this case is that often the behaviour has to be studied by varying the initial concentrations of some species. This happens, for example, when the initial concentrations of some species is not precisely known, or when a complex or general biological property (e.g. robustness (Kitano, 2004; Fages and Soliman, 2018)) is investigated. In principle, this requires performing

many simulations, or to analytically solve the non-linear differential equations by considering many initial conditions.

In this context it would be very useful to assess monotonicity properties between species that could allow us to significantly reduce the set of initial conditions that have to be considered. This is the aim of this work: to define sufficient conditions for chemical reactions networks that guarantee the existence of monotonic relationships between the chemical species involved in the network. More in detail, given two species, that we call *input* and *output* species of the network, we say that they are in a monotonicity relation if the concentration of output species at any time either increases or decreases due to an increase in the initial concentration of the input. This result would allow us to reduce substantially the number of simulations required to explore the system. Indeed, if two species are in a monotonicity relationship and we are interested in studying the dynamics of the output when varying the input, we can avoid to simulate the chemical reaction network for all possible values of the initial concentrations of the input species.

It is worth noting that the idea of finding sufficient conditions that guarantee some biological properties useful to analyze the behaviour of chemical reaction networks is not completely new. In (Angeli et al., 2006), the authors show a graphical method to study a global notion of monotonicity for certain classes of chemical reaction networks. The sufficient conditions they propose says that a chemical reaction network is globally monotone if a particular form of graph associated to the chemical reaction network under study contains only certain kinds of loop (see (Angeli et al., 2006) for details). However, global monotonicity is a very strong property, since it is based on a unique ordering on the whole set of species of the reaction network. Unfortunately, such a strong property does not hold on most realistic chemical reaction network. For this reason we introduce new definitions of monotonicity concerning the relation between a given input and output species. We then propose sufficient conditions for verifying our monotonicity relations.

Another example of sufficient conditions proposed to infer dynamical properties of chemical reaction networks are related with the study of absolute concentration robustness (Shinar and Feinberg, 2010; Shinar and Feinberg, 2011). This property holds for a species of a reaction systems if its concentration at the steady state is independent from perturbations in the initial concentration of some other species. In (Shinar and Feinberg, 2010; Shinar and Feinberg, 2011) the authors propose a sufficient condition on the structure

of the reaction network that allows absolute concentration robustness to be assessed without performing simulations. In this case, both the considered property (absolute concentration robustness) and the sufficient condition are very strong, and can be applied to a limited class of networks. For this reason, more general notions of robustness have been proposed (Rizk et al., 2009), but for which verification requires considerable efforts. In (Nasti et al., 2018) we proposed a notion of concentration robustness based on concentration intervals, which is more general than absolute concentration robustness and for which an efficient verification method could be designed by exploiting the monotonic properties we are considering in this paper.

We proceed by introducing some basic definition in Section 2, which are assumed in the rest of the paper. In Sections 3 and 4 we give our new definitions of monotonicity and propose sufficient conditions to assess them. In Section 5, we apply our methodology on some simple systems and to study the case of the ERK signaling pathway. Finally, in Section 6 we draw our conclusions and discuss future work.

2 BACKGROUND

A chemical reaction is a transformation that involves one or more chemical species, in a specific situation of volume and temperature.

The chemical species that are transformed are called *reactants*; while those that are the result of the transformation are called *products*. We can represent a chemical reaction as an equation, showing all the species involved in the process.

A simple example of chemical reaction is the following elementary reaction:

$$a\text{A} + b\text{B} \xrightleftharpoons[k_{-1}]{k_1} c\text{C} + d\text{D} \qquad (1)$$
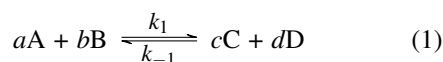
In this case, A, B, C, D are the species involved in the process: A and B are the reactants, C and D are the products. The parameters a, b, c, d are called *stoichiometric coefficients* and represent the number of reactants and products participating in the reaction. They are always integer, because elementary reactions involve the whole participants. The arrow is used to indicate the direction in which a chemical reaction takes place. When we have only one arrow, it means that the reaction is *irreversible*, that is it is not possible to have the opposite process. To describe the dynamical behaviour of the chemical reaction network, we can use the *law of mass action*, which states that: the rate of a reaction is proportional to the product of the reactants.

Applying the *law of mass action* to the system, we obtain, for each chemical species, a differential equation describing the production and the consumption of the considered species. Considering the generic chemical equation 1, we obtain:

$$\frac{d[A]}{dt} = \overbrace{-ak_1[A]^a[B]^b}^{\text{direct reaction term}} \overbrace{+ak_{-1}[C]^c[D]^d}^{\text{inverse reaction term}}$$

$$\frac{d[B]}{dt} = -bk_1[A]^a[B]^b + bk_{-1}[C]^c[D]^d$$

$$\frac{d[C]}{dt} = +ck_1[A]^a[B]^b - ck_{-1}[C]^c[D]^d$$

$$\frac{d[D]}{dt} = +dk_1[A]^a[B]^b - dk_{-1}[C]^c[D]^d.$$

where, in each equation, we isolated the term describing the direct reaction from the one describing the inverse reaction. With these two terms, we implicitly considered, for each element, the processes of consumption and production.

3 DEFINITION OF MONOTONICITY

We start with a formal definition of chemical reaction.

Definition 1 (Reaction). *Given a set of species S, a reaction is a tuple (u,v,k) denoted $u \xrightarrow{k} v$, where $u, v \in S^*$ (multisets over S) and $k \in \mathcal{R}^+$.*

Consider a set of reactions R, over a set of species $S = S_1, \ldots S_n$. Let us indicate with S_I the input and with S_O the output species. Moreover, let $\frac{dS_1}{dt} = f_{S_1}(S), \ldots, \frac{dS_n}{dt} = f_{S_n}(S)$ be the ODEs obtained from R according to the mass action kinetics. With $F_{S_i}(t, S_1^0, \ldots, S_n^0)$ we indicate the solution of the ODEs for the species S_i considering $S_1^0, \ldots, S_n^0$ as the initial values of the species $S_1, \ldots, S_n$ in R.

The following two definitions describe the concept of monotonicity that we are interested in. Our properties of monotonicity describe whether the output species react in a monotone way to the increase of the input concentration.

Definition 2 (Positive Monotonicity). *Given a set of reactions R, species S_O is* positively monotonic *with respect to S_I in R if and only if, for every time $t \in \mathcal{R}^{\geq 0}$:*

$$S_I^0 < \overline{S_I^0} \implies F_{S_O}(t, S_1^0, \ldots, S_I^0, \ldots S_n^0) \leq F_{S_O}(t, S_1^0, \ldots, \overline{S_I^0}, \ldots S_n^0)$$

Definition 3 (Negative Monotonicity). *Given a set of reactions R, species S_O is* negatively monotonic *with respect to S_I in R if and only if, for every time $t \in \mathcal{R}^{\geq 0}$:*

$$S_I^0 < \overline{S_I^0} \implies F_{S_O}(t, S_1^0, \ldots, S_I^0, \ldots S_n^0) \geq F_{S_O}(t, S_1^0, \ldots, \overline{S_I^0}, \ldots S_n^0)$$

Example 1. Consider a network R consisting of the following chemical reactions:

$$A + B \xrightarrow{k_1} C \qquad (R_1)$$

$$D + B \xrightarrow{k_2} E \qquad (R_2)$$

The differential equations describing the behaviour of R are the following

$$\begin{cases} \frac{d[A]}{dt} = -k_1[A][B] \\ \frac{d[B]}{dt} = -k_1[A][B] - k_2[B][D] \\ \frac{d[C]}{dt} = +k_1[A][B] \\ \frac{d[D]}{dt} = -k_2[B][D] \\ \frac{d[E]}{dt} = +k_2[B][D] \end{cases}$$

Consider A as the input species and C as the output species. Assume now that the initial concentrations of species B, D, C and E are fixed, but that the initial concentration of species A can vary from 10 to 1000. In principle, in order to study the dynamics of the concentration of C we would need to perform many simulations, one for each possible (continuous) value of the initial concentration of A. However, if species C is *positively monotonic* w.r.t. A, then just two simulations are necessary: one with $A = 10$ and one with $A = 1000$. The dynamics of C in all the other (intermediate) cases is included in the results we obtained from these two simulations. A similar simplification could be done by assessing the *negatively monotonicity* of E w.r.t. A. In addition, if the species to vary were two, say A and B with the latter varying from 20 to 200, and if species C was also *positively monotonic* w.r.t. B, then just two simulations would be necessary to study the behaviour of C: one with $A = 10$ and $B = 20$ and another with $A = 1000$ and $B = 200$. Finally, if we could prove that species E is *positively monotonic* w.r.t. B then, to study its behaviour we would need at most four different simulations, $A = 10$ and $B = 20$, $A = 10$ and $B = 200$, $A = 100$ and $B = 20$, and $A = 100$ and $B = 200$.

It is worth noting that interesting weaker notions of monotonicity could be defined. For example, for some reaction networks it could be interesting to study steady state monotonicity, defined (in its positive formulation) as follows.

Definition 4. *S_O in R is* steady state positively monotonic *with respect to S_I if and only if $S_I^0 < \overline{S_I^0}$ implies*

$$\lim_{t \to \infty} F_{S_O}(t, S_1^0, \ldots, S_I^0, \ldots S_n^0) \leq \lim_{t \to \infty} F_{S_O}(t, S_1^0, \ldots, \overline{S_I^0}, \ldots S_n^0)$$
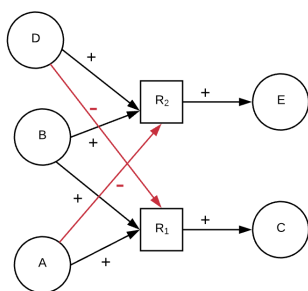
Figure 1: Dependency graph of the reaction network in Example 1. The red edges have sign − and express competition among reactions, the remaining ones have sign +.

4 EFFICIENT ASSESSMENT OF MONOTONICITY

We now define a dependency graph on species that we will use to define our sufficient conditions. Intuitively, the graph highlights the positive or negative influence that each species has on each reaction.

In the following, we denote the cardinality of a set S with $\sharp S$, and the number of instances of s in a multiset u with $\sharp_s u = \sharp\{a \mid a = s \text{ and } a \in u\}$.

Definition 5 (Dependency graph). *Given a finite set of reactions R over a set of species S, the dependency graph G of R is the tuple $\langle S, R, E_+, E_- \rangle$, where $E_+ \subseteq (S \times R) \cup (R \times S)$ and $E_- \subseteq (S \times R)$, are such that given $s \in S$, $u \xrightarrow{k} v \in R$:*

- $(s, u \xrightarrow{k} v) \in E_+ \iff s \in u$
- $(u \xrightarrow{k} v, s) \in E_+ \iff s \in v \wedge \sharp_s v > \sharp_s u$
- $(s, u \xrightarrow{k} v) \in E_- \iff s \notin u \wedge \exists u' \xrightarrow{k'} v' \in R \, (u \cap u' \neq \emptyset \wedge s \in u')$

Consider again the reaction network R of Example 1. The corresponding dependency graph is shown in Figure 1. The nodes representing species are depicted as circles, while nodes representing reaction as squares. To build the dependency graph, we draw an edge labelled with the sign + between each reactant and its reaction, and between each reaction and each of its product. Moreover, if a species is a reactant of two or more reactions (like B, in the example) we build an edge with the sign − between all the species that are not in common and each competing reaction. Considering the example, we build an edge with sign − between A and reaction R_2 since reaction R_2 competes with reaction R_1 for species B. Analogously, we draw an edge with sign − between D and the competing reaction R_1.

We are interested in characterizing the paths over the previously defined dependency graph.

Definition 6 (Path). *Given a dependency graph $\langle S, R, E_+, E_- \rangle$, a path is a finite sequence $S_o R_1 S_1 R_2 ... R_n S_n$ where $S_o, ..., S_n \in S$, $R_1, ..., R_n \in R$ and $\forall i \in [1, n] \, (S_{i-n}, R_i) \in E_+ \cup E_-$ and $(R_i, S_i) \in E_+$. A path is positive iff $\sharp\{(S_{i-1}, R_i) \in E_- | i \in (1, n)\}$ is even. A path is negative otherwise.*

Going back to the dependency graph depicted in Figure in 1, we can observe that there is a positive path from species A to C, while there is a negative path from species A to E. Analogously, there is a negative path from D to C and a positive path from D to E. Finally, there are positive paths from B to D and E.

We are now ready to present our conjectures.

Conjecture 1 (Positive Paths). *Given a set of reactions R over a set of species S. Let $G = \langle S, R, E_+, E_- \rangle$ be the dependency graph of R. Let $S_I, S_O \in S$. If paths from S_I to S_O in G are all positive, then S_O is positively monotonic with respect to S_I.*

Conjecture 2 (Negative Paths). *Given a set of reactions R over a set of species S. Let $G = \langle S, R, E_+, E_- \rangle$ be the dependency graph of R. Let $S_I, S_O \in S$. If paths from S_I to S_O in G are all negative, then S_O is negatively monotonic with respect to S_I.*

As a consequence of our conjectures, in the reaction network of Example 1, we can affirm that C is positively monotonic with respect to A and B, while it is negatively monotonic with respect to D. Analogously, we can conclude that E is positively monotonic with respect to D and B, while it is negatively monotonic with respect to A.

5 APPLICATION EXAMPLES

We show the application of our methodology to two small examples of reaction networks and to the case study of the ERK signaling pathway.

5.1 Example of Monotonic Behaviours

Consider again the reaction network of Example 1 and let A be the input species. By applying our conjectures we can derive (by observing paths in the dependency graph in Figure 1) that C is positively monotonic w.r.t. A, and E is negatively monotonic w.r.t. A. This behaviour is confirmed by the simulation results shown in Figures 2 and 3: by increasing the initial concentration of A, the concentration of C is (at all times) increased, while that of E is (at all times) decreased. In the simulations, initial concentrations of B, C, D and E are $B_0 = 20$, $C_0 = 0$, $D_0 = 10$, and $E_0 = 0$.
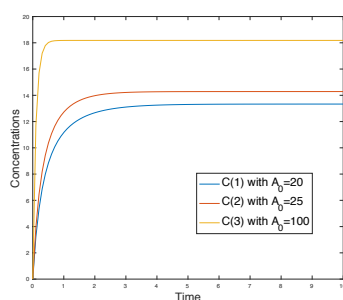
Figure 2: Simulation results of Example 1. Changes in the dynamics of species *C* by varying the initial concentration of species *A*.
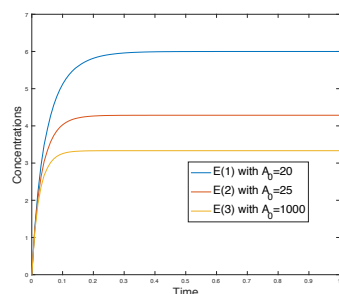

Figure 3: Simulation results of Example 1. Changes in the dynamics of species *E* by varying the initial concentration of species *A*.

For this particular example, monotonicities between species that can be deduced from our conjectures are stated in the following proposition.

Proposition 1. *In the reaction network of Example 1, species C and D are positively monotonic w.r.t. A, and species B and E are negatively monotonic w.r.t. A.*

An empirical assessment of the validity of the previous proposition can be obtained by using the small tool we developed and we made available at http://www.di.unipi.it/msvbio/software/MonotoneExample.html. The tool executes 1000 simulations of the reaction network of Example 1 by using the Euler method. Each simulation actually computes two sets of variables: A, B, C, D, E and A', B', C', D', E'. The dynamics of both sets of variables is governed by the same ODEs, but the initial value of variable A' is greater than the initial value of variable A (the initial value of each other primed variable is the same as that of the corresponding unprimed variable). In this way the tool can compare step by step the dynamics of the reaction network with the dynamics of the same network with a (positive) perturbation in the initial concentration of the input species A.

In each of the 1000 simulations, the initial values of the variables, the perturbation of the input species and the values of the kinetic constants are randomly chosen. To assess the validity of Proposition 1, at each step of each simulation the tool checks whether the

following inequalities hold: $A'_i \geq A_i$, $B'_i \leq B_i$, $C'_i \geq C_i$, $D'_i \geq D_i$ and $E'_i \leq E_i$. If one of such inequalities does not hold, an error message is displayed.

We ran the tool 10 times, corresponding to 10000 simulations overall. The error message never appeared.

5.2 Example of Non-monotonic Behaviour

Example 2. Consider the following example of chemical reaction network

$$
\begin{aligned}
A + B &\xrightarrow{k_1} C \\
A + E &\xrightarrow{k_2} N \\
E &\xrightarrow{k_3} C
\end{aligned}
$$
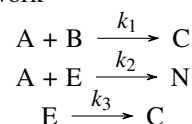
for which, applying the law of mass action, we obtain the following system of ODEs:

$$
\begin{cases}
\frac{d[A]}{dt} = -k_1[A][B] - k_2[A][E] \\
\frac{d[B]}{dt} = -k_1[A][B] \\
\frac{d[C]}{dt} = +k_1[A][B] + k_3[E] \\
\frac{d[E]}{dt} = -k_2[A][E] - k_3[E] \\
\frac{d[N]}{dt} = +k_2[A][E]
\end{cases}
$$

The dependency graph is shown in Figure 4. Considering A as the input species and the C as the output of the chemical reaction network, we observe two different paths, one positive and one negative, relating species A and C. Hence, the sufficient conditions of our conjectures do not apply and we cannot conclude anything on the monotonicity of the selected species.

However, observing the differential equations, we can notice that the behaviour of the chemical species A is ambivalent with respect to the chemical species C. Indeed, A produces C in R_1, but - at the same time - consumes the chemical species E (another reactant producing C) in the reaction R_2. This intuition is confirmed by the result of simulations where we augmented the concentration of A and plot the concentration of C (see Figure 5). The simulations shows that in this case C is not monotonic with respect to the variation of the initial concentration of A. Initial concentrations considered in the simulations are $B_0 = 10$, $C_0 = 0$, $E_0 = 20$ and $N_0 = 10$.

5.3 Application to the ERK Signaling Pathway

We now apply our method to a more complex example. We consider the mathematical modeling of the influence of RKIP on the ERK signaling pathway presented in (Kwang-Hyun et al., 2003), a series of chemical transformations which contributes to
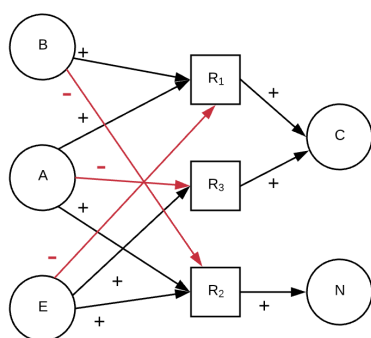
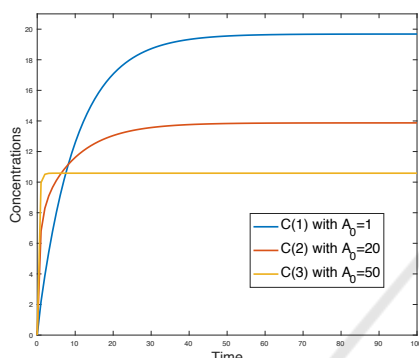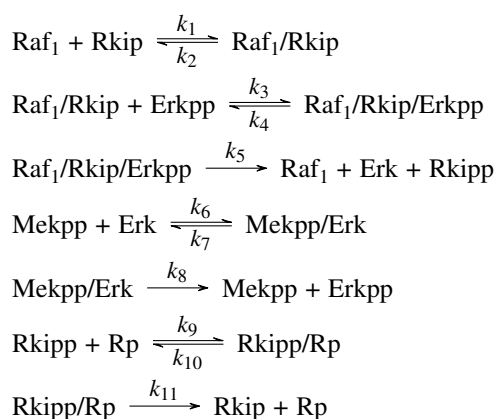Figure 4: Dependency graph of the reaction network in Example 2.

Figure 5: Simulation results of Example 2. Changes in the dynamics of species *C* by varying the initial concentration of species *A*.

the control of a large number of cellular processes. The activated kinase $Raf1$ phosphorylates the kinase MAPK/ERK (MEK), which phosphorylates and activates the Extracellular Signal Regulated kinase (ERK).

Example 3. This chemical reaction network consists of 11 chemical species and 11 reactions:

$$\text{Raf}_1 + \text{Rkip} \underset{k_2}{\overset{k_1}{\rightleftarrows}} \text{Raf}_1/\text{Rkip}$$

$$\text{Raf}_1/\text{Rkip} + \text{Erkpp} \underset{k_4}{\overset{k_3}{\rightleftarrows}} \text{Raf}_1/\text{Rkip}/\text{Erkpp}$$

$$\text{Raf}_1/\text{Rkip}/\text{Erkpp} \overset{k_5}{\longrightarrow} \text{Raf}_1 + \text{Erk} + \text{Rkipp}$$

$$\text{Mekpp} + \text{Erk} \underset{k_7}{\overset{k_6}{\rightleftarrows}} \text{Mekpp}/\text{Erk}$$

$$\text{Mekpp}/\text{Erk} \overset{k_8}{\longrightarrow} \text{Mekpp} + \text{Erkpp}$$

$$\text{Rkipp} + \text{Rp} \underset{k_{10}}{\overset{k_9}{\rightleftarrows}} \text{Rkipp}/\text{Rp}$$

$$\text{Rkipp}/\text{Rp} \overset{k_{11}}{\longrightarrow} \text{Rkip} + \text{Rp}$$

To assess monotonicity relationships between the chemical species, we build the dependency graph of the chemical reaction network, shown in Figure 6. In this case, we notice that only reactions R_2 and R_4 have

common reactants, that is the species $Raf1/Rkip$. Hence, in the dependency graph, we build only a negative edge between the species $Erkpp$ and the reaction R_2.

Once the dependency graph is constructed, we inspect all possible paths among the species in order to verify the conditions of our conjectures. Our conjectures allow us to draw conclusions on the monotonicity of many pairs of species, that we validated by performing simulations.

As an example, consider the species $Mekpp$ and Erk, respectively as input and output of the chemical reaction network. The paths from $Mekpp$ to Erk in the dependency graph are all positive. Hence, according to our conjecture Erk is positively monotonic with respect to $Mekpp$. This is confirmed by the result of simulations, shown in Figure 7, where we increase the initial concentration of $Mekpp$ to study the concentration variation of Erk. Consider now the species $Raf1/Rkip/Erkpp$ and $Raf1/Rkip$ as input and output, respectively, of the chemical reaction network. In this case we find two paths (one positive and one negative) which link the two species. Hence, $Raf1/Rkip$ could be not monotonic with respect to $Raf1/Rkip/Erkpp$. Indeed, the fact that $Raf1/Rkip$ does not react in a monotonic way to the increase of the initial concentration of $Raf1/Rkip/Erkpp$ is verified by the result of simulations (see Figure 8). Parameters and initial concentrations are as in (Kwang-Hyun et al., 2003).
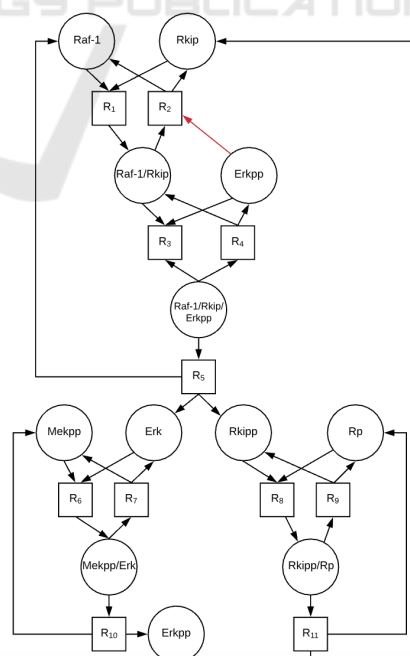

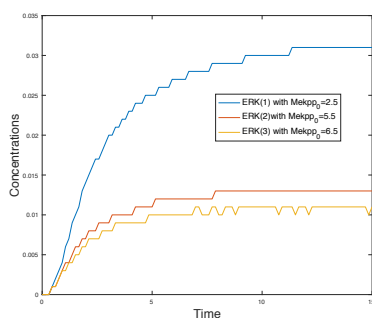Figure 6: Dependency graph of the ERK signaling pathway.

Figure 7: Simulation results of Example 3. In this case, we increase the initial concentration of $Mekpp$ and compare the concentrations of the output Erk. The results show that Erk is monotonic with respect to the variation of the input.
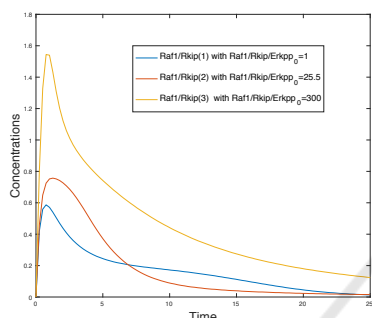

Figure 8: Simulation results of Example 3. In this case, we increase the initial concentration of $Raf1/Rkip/Erkpp$ and compare the concentrations of the output $Raf1/Rkip$. The results show that $Raf1/Rkip$ is not monotonic with respect to the variation of the input.

6 CONCLUSION AND FUTURE WORK

In this paper we have studied dynamical properties of chemical reaction networks. We have given a new notion of monotonicity, for which two species, considered as input and output of the network, are monotonic if the variation of the initial concentration of the input implies a monotonic variation in the concentration of the output. We have proposed new sufficient conditions based on a dependency graph that guarantee the monotonicity property to hold. Monotonicity assessment can drastically reduce the number of simulations necessary to study the dynamical behaviour of a chemical reaction network. We have shown the application of our sufficient conditions to two small models and to the quite complex model of the ERK signaling pathway (Kwang-Hyun et al., 2003).

As regards ongoing and future work, we are working on the proof of our conjectures and we plan to validate the approach by applying it extensively to a benchmark collection of models of chemical reaction networks as we did, for example, in (Pardini et al.,

2014) to validate an algorithm for the modularization of biochemical pathways.

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