Universität Stuttgart

SimTech
Cluster of Excellence

S. Waldherr · F. Allgöwer

Robust stability and instability of biochemical networks with parametric uncertainty

Stuttgart, May 2011

Institute for Systems Theory and Automatic Control, University of Stuttgart,
Pfaffenwaldring 9, 70569 Stuttgart/ Germany
{waldherr,allgower}ist.uni-stuttgart.de
www.ist.uni-stuttgart.de

Abstract Parameter perturbations in dynamical models of biochemical networks affect the qualitative dynamical behaviour observed in the model. Since this qualitative behaviour is in many cases the key model output used to explain biological function, the robustness analysis of the model's behaviour with respect to parametric uncertainty is a crucial step in systems biology research. In this paper, we develop a new method for robustness analysis of the dynamical behaviour. As a first step, we provide a characterization of non-robust perturbations as a system of polynomial equalities and inequalities. In the second step, we apply the Positivstellensatz and Handelman representation of polynomials to check for the non-existence of solutions to this system, which can be relaxed to solving a linear program. Thereby, a solution to the linear program yields a robustness certificate for the considered dynamical behaviour. With these robustness certificates, we propose an algorithm to compute a lower robustness bound corresponding to a level of parametric uncertainty up to which no local bifurcations can occur. The applicability of the proposed method to biochemical network models is illustrated by analysing the robustness of oscillations in a model of the NF-κB signalling pathway. The results may be used to define a level of confidence in the observed model behaviour under parametric uncertainty, making them valuable for evaluating dynamical models of biological networks.

Keywords analysis of systems with uncertainties · biochemical network dynamics · bifurcation analysis · polynomial programming

Preprint Series **Issue No. 2011-12**
Stuttgart Research Centre for Simulation Technology (SRC SimTech)
SimTech – Cluster of Excellence
Pfaffenwaldring 7a
70569 Stuttgart
publications@simtech.uni-stuttgart.de
www.simtech.uni-stuttgart.de

Robust stability and instability of biochemical networks with parametric uncertainty ⋆

Steffen Waldherr and Frank Allgöwer

Institute for Systems Theory and Automatic Control, University of Stuttgart, Pfaffenwaldring 9, Stuttgart, Germany
email: (waldherr,allgower)@ist.uni-stuttgart.de

Abstract

Parameter perturbations in dynamical models of biochemical networks affect the qualitative dynamical behaviour observed in the model. Since this qualitative behaviour is in many cases the key model output used to explain biological function, the robustness analysis of the model's behaviour with respect to parametric uncertainty is a crucial step in systems biology research. In this paper, we develop a new method for robustness analysis of the dynamical behaviour. As a first step, we provide a characterization of non-robust perturbations as a system of polynomial equalities and inequalities. In the second step, we apply the Positivstellensatz and Handelman representation of polynomials to check for the non-existence of solutions to this system, which can be relaxed to solving a linear program. Thereby, a solution to the linear program yields a robustness certificate for the considered dynamical behaviour. With these robustness certificates, we propose an algorithm to compute a lower robustness bound corresponding to a level of parametric uncertainty up to which no local bifurcations can occur. The applicability of the proposed method to biochemical network models is illustrated by analysing the robustness of oscillations in a model of the NF-κB signalling pathway. The results may be used to define a level of confidence in the observed model behaviour under parametric uncertainty, making them valuable for evaluating dynamical models of biological networks.

Key words: analysis of systems with uncertainties, biochemical network dynamics, bifurcation analysis, polynomial programming

1 Introduction

A significant portion of systems biology research is based on dynamical models of biochemical networks on the cellular level. These models are mostly given in the form of parametrised ordinary differential equations. For many such networks, the qualitative dynamical behaviour, such as sustained oscillations or bistability, is the key model output used to describe the biological function of the network (see [1] for an overview of important examples). In particular, the dynamical behaviour around equilibrium points is frequently the most distinct aspect of the global dynamical properties for these networks. A problem with parametrised models on the cellular level is that parameter values are usually highly uncertain, or may vary significantly subject to the environment or cell type being considered. The fact that the qualitative dynamical behaviour depends on the parameter values thus directly gives rise to the question of robustness: how much uncertainty or perturbation in the network's parameters can be tolerated without affecting the qualitative dynamical behaviour of the system?

Under parametric uncertainty, changes in the qualitative dynamical behaviour around equilibrium points are always related to the occurence of local bifurcations of equilibrium points. Bifurcation analysis is therefore frequently applied to biochemical reaction networks [3,9,5]. In particular, local bifurcations typically correspond to emergence or loss of complex dynamical behaviour such as sustained oscillations or bistability. In this framework, the robustness analysis problem is to quantify the deviations from nominal parameter values that the system may tolerate without any local bifurcations occuring. Such a robustness concept has been utilized in several previous studies. Based on the work in [14], we define a robustness measure as the maximal parameter variation around a nominal parameter value p_0 which does not affect the qualitative dynamical behaviour of the system. Bifurcation analysis with numerical continuation can in fact be applied for robustness analysis if only one or two parameters are assumed uncertain [15,14]. Yet, major difficulties are that the bifurcation surface can usually

⋆ This paper was not presented at any IFAC meeting. Corresponding author S. Waldherr. Email `waldherr@ist.uni-stuttgart.de`. Tel. +49-711-685-67657. Fax +49-711-685-67735.

not be computed explicitly in a high-dimensional parameter space, and that continuation methods may miss parts of the bifurcation surface, even if only one or two parameters are uncertain. To deal with multiparametric uncertainty, it was suggested to use the structured singular value as analysis tool [14,10,19]. However, a significant problem with the approaches based on the structured singular value is that the uncertainty in the location of the steady state due to parameter variations usually cannot be taken into account directly.

Although robustness analysis is a classical topic in control engineering, it remains surprisingly challenging to apply established methods to the analysis of biological networks. This shortcoming of classical control methods has several reasons. First, most methods have been developed for linear systems, whereas biological networks are almost always non-linear. Second, if methods are applicable to non-linear systems, it is typically assumed that the relevant steady state is at the origin and not affected by perturbations. In addition, the typical question in control engineering is the robustness of stability, whereas in biochemical networks also the robustness of complex dynamical behaviour, such as bistability or sustained oscillations, is highly relevant. In fact, biological function based on complex dynamical behaviour like sustained oscillations or bistability is typically directly related to instability of an equilibrium point [18,5].

The approach developed in this article overcomes the outlined problems of classical robustness analysis methods and is directly applicable to typical models for biochemical reaction networks. The proposed robustness analysis method is an application of the feedback loop breaking approach [21], which we introduced previously in order to characterise parameter values leading to a Jacobian with eigenvalues on the imaginary axis, i.e. eigenvalues at zero or conjugate imaginary. Based on the feedback loop breaking, we propose conditions for robustness of stability or instability as well as a computational algorithm to compute a lower bound on the corresponding dynamical robustness radius. Thereby, the occurence of local bifurcations is determined by the necessary condition that the system's Jacobian, evaluated at a steady state, has an eigenvalue on the imaginary axis. Importantly, the variation in the location of the steady state that occurs upon variations in the parameters is explicitly accounted for by the proposed method.

The paper is structured as follows. In Section 2, we introduce the considered model class and give the robustness definition to be used in this paper. In Section 3, we derive a mathematical characterisation of non-robust perturbations, from which an efficient computational robustness algorithm is constructed. An application of this algorithm to the analysis of oscillations in a specific signalling pathway model is presented in Section 4.

2 Model class and robustness definition

2.1 Models of biochemical networks

As a first step, let us define the class of models that is considered in this paper. Biochemical reaction networks are composed of two main elements: Biochemical species, each of which represents an ensemble of chemically identical molecules in a specific compartment of the cell, and chemical reactions, which are processes transforming one group of species into another one.

The structure of a biochemical reaction network is characterised completely by the list of involved species, denoted as $X_1, X_2 \ldots, X_n$, and the list of reactions, denoted as

$$\sum_{i=1}^{n} S_{ij}^{(s)} X_i \to \sum_{i=1}^{n} S_{ij}^{(p)} X_i, \quad j = 1, \ldots, r, \tag{1}$$

where r is the number of reactions in the network, and the factors $S_{ij}^{(s)} \in \mathbb{N}_0$ and $S_{ij}^{(p)} \in \mathbb{N}_0$ are the stoichiometric coefficients of the substrate and product species, respectively [8].

The structural information of the reaction network is usually subsumed in the stoichiometric matrix, given by

$$S = \left(S_{ij}^{(p)} - S_{ij}^{(s)}\right)_{i=1,\ldots,n,\ j=1,\ldots,r} \in \mathbb{R}^{n\times r}. \tag{2}$$

The state vector of the system consists of the concentrations of the involved chemical species and is denoted by

$$x = ([X_i])_{i=1,\ldots,n} \in \mathbb{R}^n,$$

where $[X_i]$ represents the concentration of species X_i. The kinetic information for the reaction network is given by reaction rate functions, which depend on the state $x \in \mathbb{R}^n$ and the kinetic parameters $p \in \mathcal{P}_0$. Thereby, the set

$$\mathcal{P}_0 \subset \mathbb{R}^q \tag{3}$$

is the set of admissible parameter values. The reaction rates are given by the vector

$$v(x,p) = (v_j(x,p))_{j=1,\ldots,r} \in \mathbb{R}^r,$$

where $v_j(x,p)$ is the rate of the j–th reaction in (1). Usually, reaction rate laws are polynomial or rational expressions in both kinetic parameters and state variables, arising for example from the law of mass action or the Michaelis-Menten mechanism [1].

Independently of the chosen reaction rate mechanisms, a model for the dynamics of the reaction network is obtained by mass balancing. The dynamics are described

by an ordinary differential equation given as

$$\dot{x} = Sv(x,p). \tag{4}$$

A crucial assumption in the paper is that the Jacobian $S\frac{\partial v}{\partial x}(x,p)$ of (4) does not have eigenvalues on the imaginary axis at equilibria of (4) for nominal parameters. However, conservation relations, which are commonly present in biochemical networks, structurally lead to eigenvalues at zero [7]. To remove these eigenvalues, the model (4) can always be reduced to an equivalent system of differential equations where these zero eigenvalues are not present [7].

2.2 Robustness definition

The proposed concept for robustness of dynamical properties is formalised in the following definitions. We assume throughout that all steady states are hyperbolic for nominal parameters p_0, i.e. none of the nominal steady states yields eigenvalues of the system's Jacobian on the imaginary axis. Furthermore, we assume that the state of the system (4) is restricted to a known compact set $\mathcal{X}_0 \subset \mathbb{R}^n$. For most biochemical networks, such bounds can be derived either from conservation relations, or by exploiting positive invariance of a sufficiently large compact set in the state space.

As discussed in the introduction, the notion of robustness considered in this paper corresponds to the non-occurence of local bifurcations of steady states under parameter variations. With this notion, one can define the set of robust parameters $\mathcal{P}^*(p_0)$ as the largest connected set within the set of admissible parameters $\mathcal{P}_0 \subset \mathbb{R}^q$ which contains the nominal parameter values p_0 and for which there does not exist a $\bar{p} \in \mathcal{P}^*(p_0)$ and a corresponding steady state $\bar{x} \in \mathcal{X}_0$, with $Sv(\bar{x},\bar{p}) = 0$, such that the Jacobian $S\frac{\partial v}{\partial x}(\bar{x},\bar{p})$ has eigenvalues on the imaginary axis, i.e. at zero or conjugate imaginary. With knowledge of $\mathcal{P}^*(p_0)$, one could decide whether the system is robust against a given parametric perturbation or not. In most cases, it will however not be possible to compute the robust parameter set $\mathcal{P}^*$ explicitly, as it would require to compute the bifurcation surfaces of the system which delimit this set. Also, in many cases the exact global shape of $\mathcal{P}^*$ is not even relevant for robustness analysis. For robustness issues, it is more informative how large a compact region of regular shape (like a hyperrectangle or -ellipsoid) around the nominal parameters can be, while still being contained in $\mathcal{P}^*$.

To develop a precise definition from this perspective, let us consider the hyperrectangle

$$\mathcal{P}_r(\psi,p_0) = \Big\{p \in \mathcal{P}_0 \mid \frac{1}{\psi} \leq \frac{p_j}{p_{0,j}} \leq \psi,\ j = 1,\dots,q\Big\}. \tag{5}$$

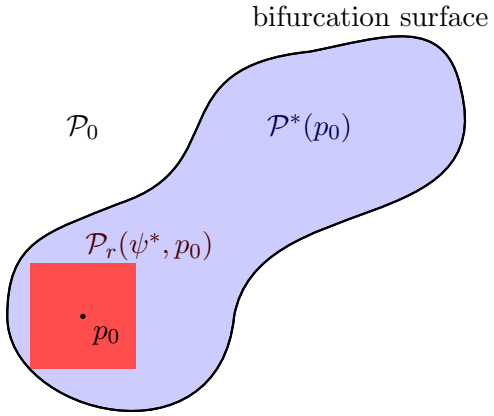

Fig. 1. Illustration of the considered robustness regions. The irregular shaped region is the set of robust parameters, which is delimited by bifurcation surfaces, while the rectangle centered at p_0 is $\mathcal{P}_r(\psi^*,p_0)$. Perturbing parameters by a factor of ψ^* or more from p_0 may be non-robust by leaving the rectangle through the lower left corner.

of all parameter values within a factor variation of at most ψ from p_0. The dynamical robustness radius $\psi^*(p_0)$ will be defined by the supremum of the logarithmic radius ψ of all such hyperrectangles in which no parameter values yield steady states where the Jacobian has eigenvalues on the imaginary axis. Thus, if the dynamical robustness radius is finite, it is equal to the minimal factor by which parameter values have to be varied from p_0 in order to leave the robust parameter set and to induce a local bifurcation of steady states. Note that we have used a hyperrectangle for $\mathcal{P}_r(\psi,p_0)$ mainly for convenience and ease of interpretation, in principle any compact polytope could have been used for the analysis developed in this paper. The precise robustness definition then is as follows.

Definition 1 *The* dynamical robustness radius $\psi^* \in (1,\infty]$ *is defined as*

$$\begin{aligned}\psi^*(p_0) = \sup\Big\{\psi \in \mathbb{R} \mid \forall p \in \mathcal{P}_r(\psi,p_0) \forall x \in \mathcal{X}_0 :\\ Sv(x,p) = 0 \Rightarrow \sigma\big(S\frac{\partial v}{\partial x}(x,p)\big) \cap j\mathbb{R} = \emptyset\Big\},\end{aligned} \tag{6}$$

where $\sigma(A)$ is the spectrum of the matrix A.

The robustness definition is illustrated in Figure 1. In the following section, the goal is to compute a lower bound $\widehat{\psi^*} \leq \psi^*$ on the dynamical robustness radius. By Definition 1, knowledge of a lower bound allows to guarantee that the system does not undergo local bifurcations of steady states for parameter variations up to a factor of $\widehat{\psi^*}$. Upper bounds on ψ^* can be found by bifurcation analysis, for example via continuation methods [12]. Using the feedback loop breaking approach also discussed

below, an efficient bifurcation search can be done in biochemical networks with a high-dimensional parameter space [21].

3 Robustness analysis method

3.1 The feedback loop breaking approach

For the analysis of dynamical behaviour of biological feedback systems, we have previously introduced the *feedback loop breaking* approach [21], where properties of the original system are characterised by studying an appropriately constructed input–output system. The original system is thereby interpreted as the closed loop description of the artificially constructed input–output system.

To simplify the notation, let us rewrite the right hand side of the system (4) as $Sv(x,p) = F(x,p)$. Thus, the system to be considered is given by

$$\dot{x} = F(x,p), \tag{7}$$

with $x \in \mathcal{X}_0 \subset \mathbb{R}^n$, $p \in \mathcal{P}_0 \subset \mathbb{R}^q$ and $F : \mathbb{R}^n \times \mathbb{R}^q \to \mathbb{R}^n$ a smooth vector field. To deal with steady states for uncertain parameter values, we introduce the notation of a state–parameter pair $\chi = (x,p) \in \mathbb{R}^n \times \mathbb{R}^q$. We call χ a *steady state–parameter pair* if the corresponding x and p satisfy the equation

$$F(x,p) = 0. \tag{8}$$

Also define the set of steady state–parameter pairs

$$\mathcal{M} = \{\chi = (x,p) \in \mathcal{X}_0 \times \mathcal{P}_0 \mid F(\chi) = 0\}. \tag{9}$$

Definition 2 ([21]) *A* feedback loop breaking *for the system (7) is a tuple (f,h), where $f : \mathbb{R}^n \times \mathbb{R} \times \mathbb{R}^q \to \mathbb{R}^n$ is a smooth vector field and $h : \mathbb{R}^n \to \mathbb{R}$ is a smooth function, such that*

$$F(x,p) = f(x,h(x),p). \tag{10}$$

The corresponding open loop system is then given by

$$\begin{aligned} \dot{x} &= f(x,u,p) \\ y &= h(x), \end{aligned} \tag{11}$$

and the closed loop system (7) is recovered by setting $u = y$. Importantly, there is a direct relation between steady states in the closed and the open loop system: for a steady state–parameter pair (x_0,p) of the closed loop system (7), setting the input $u = h(x_0)$ in the open loop system (11) leads to (x_0,p) being a steady state–parameter pair of the open loop system (11).

3.2 Characterisation of critical points via the loop transfer function

The feedback loop breaking introduced in the previous section is a useful tool to characterise steady state–parameter pairs for which the system's Jacobian has eigenvalues on the imaginary axis, and where thus changes in the qualitative dynamical behaviour should be expected. In the following, let us denote the Jacobian of the closed loop system (7) by

$$A(\chi) = \frac{\partial F}{\partial x}(\chi). \tag{12}$$

Definition 3 *The steady state–parameter pair χ_c is called a* critical point*, if the Jacobian $A(\chi_c)$ has an eigenvalue on the imaginary axis, i.e. a zero eigenvalue or a pair of conjugate imaginary eigenvalues.*

In the neighbourhood of the steady state–parameter pair $\chi = (x_0,p) \in \mathcal{M}$, the open loop system (11) has a linear approximation given by the state space representation $(A_o(\chi), B_o(\chi), C_o(\chi))$, with $A_o(\chi) = \frac{\partial f}{\partial x}(x_0, h(x_0), p)$, $B_o(\chi) = \frac{\partial f}{\partial u}(x_0, h(x_0), p)$, and $C_o(\chi) = \frac{\partial h}{\partial x}(x_0)$. The linearised open loop system can also be described by its transfer function, which is defined as

$$G(\chi,s) = C_o(\chi)\left(sI_n - A_o(\chi)\right)^{-1} B_o(\chi) \tag{13}$$

with the complex variable $s \in \mathbb{C}$. The following lemma, adopted from [21], is a tool to characterise eigenvalues of the closed loop Jacobian $A(\chi)$ by properties of the open loop system (11), specifically the transfer function G.

Lemma 4 ([21]) *Assume that $s_0 \in \mathbb{C}$ is not an eigenvalue of $A_o(\chi)$. Then s_0 is an eigenvalue of $A(\chi)$, if and only if*

$$G(\chi, s_0) = 1. \tag{14}$$

In the following, Lemma 4 is used with s_0 on the imaginary axis to characterise critical points χ_c with the condition (14). To this end, the transfer function G is represented as a complex rational function with real coefficients

$$G(\chi,s) = \frac{Q(\chi,s)}{R(\chi,s)}, \tag{15}$$

where $Q(\chi,s)$, $R(\chi,s)$ are multi-variate polynomials in χ and s. The following result then characterises critical steady state–parameter pairs.

Theorem 5 *Assume that the open loop Jacobian $A_o(\chi)$ does not have an eigenvalue on the imaginary axis for any $\chi \in \mathcal{M}$. Then the following two conditions are equivalent.*

(i) There exists a critical point $\chi_c \in \mathcal{M}$.

(ii) The system of equations

$$Q(\chi, j\omega) = R(\chi, j\omega) \tag{16a}$$
$$F(\chi) = 0 \tag{16b}$$

in the variables $\chi \in \mathbb{R}^{n+q}$ and $\omega \in \mathbb{R}$ has a solution with $\chi \in \mathcal{X}_0 \times \mathcal{P}_0$.

PROOF. By assumption, there does not exist $\omega \in \mathbb{R}$ and $\chi \in \mathcal{M}$ such that $j\omega$ is an eigenvalue of $A_o(\chi)$.

(i) $\Rightarrow$ (ii): Let $j\omega_c$ be an imaginary eigenvalue of $A(\chi_c)$. By Lemma 4, χ_c and ω_c solve (16).

(ii) $\Rightarrow$ (i): Let χ and ω be a solution of (16). In particular, by (9), we have $\chi \in \mathcal{M}$. By Lemma 4, χ is a critical point, with $j\omega$ being an eigenvalue of $A(\chi)$.

The assumption that $A_o(\chi)$ does not have an eigenvalue on the imaginary axis represents the idea of feedback loop breaking, that the feedback loop which is responsible for the considered change in the dynamical behaviour is removed in the open loop system. From a control engineering perspective, this assumption assures that no pole–zero cancellations of eigenvalues on the imaginary axis occur in the transfer function $G(\chi, s)$. The assumption on $A_o(\chi)$ can often be satisfied by structural properties. For instance, if the open loop system does not have a feedback circuit, the eigenvalues can directly be read from the Jacobian. In this case, it is typically easy to check whether zero or conjugate imaginary eigenvalues are possible for $p \in \mathcal{P}_0$ and $x \in \mathcal{X}_0$.

3.3 Robustness certificates from the Positivstellensatz

In this section, we develop an approach to test whether the dynamical behaviour of the system (4) is robust with respect to uncertain parameters inside a given region $\mathcal{P} \subset \mathbb{R}^q$. From Theorem 5, this is equivalent to checking infeasibility of (16). Observe that, for reaction rates $v(x, p)$ which are rational in state variables and parameters, (16) is a system of polynomial equations. Thus, in order to obtain a robustness certificate, we have to assert that the system of polynomial equations (16) does not have a solution χ with $\chi \in \mathcal{X}_0 \times \mathcal{P}$. A useful result from real algebraic geometry in this context is the Positivstellensatz [2], which provides sufficient and necessary conditions for the non-existence of solutions to a system of polynomial equalities and inequalities.

First, we need to introduce some notation of algebraic geometry. Let $\mathbb{R}[\xi]$ be the ring of polynomials in the vector variable $\xi \in \mathbb{R}^m$ over the field of real numbers. The ideal generated by a set of polynomials $\mathcal{Y} = \{Y_1, \ldots, Y_N\} \subset \mathbb{R}[\xi]$ is defined as

$$\mathcal{I}(Y_1, \ldots, Y_N) = \Big\{ \sum_{i=1}^{N} T_i Y_i \mid T_i \in \mathbb{R}[\xi] \Big\}. \tag{17}$$

The cone generated by $\mathcal{Y}$ is denoted by $\mathcal{C}(Y_1, \ldots, Y_N)$ and defined by the properties

(i) $Y_i \in \mathcal{C}(Y_1, \ldots, Y_N)$, $i = 1, \ldots, N$,
(ii) $T \in \mathbb{R}[\xi] \Rightarrow T^2 \in \mathcal{C}(Y_1, \ldots, Y_N)$,
(iii) $Y \in \mathcal{C}(Y_1, \ldots, Y_N)$, $\bar{Y} \in \mathcal{C}(Y_1, \ldots, Y_N) \Rightarrow Y + \bar{Y} \in \mathcal{C}(Y_1, \ldots, Y_N)$, $Y\bar{Y} \in \mathcal{C}(Y_1, \ldots, Y_N)$.

Theorem 6 (Positivstellensatz, [2]) *Consider a system of polynomial (in-)equalities given by*

$$\begin{aligned} Y_i(\xi) &= 0, \quad i = 1, \ldots, N \\ Z_j(\xi) &\geq 0, \quad j = 1, \ldots, M, \end{aligned} \tag{18}$$

with $\xi \in \mathbb{R}^m$. System (18) does not have a solution in $\mathbb{R}^m$, if and only if there exist $Y \in \mathcal{I}(Y_1, \ldots, Y_N)$ and $Z \in \mathcal{C}(Z_1, \ldots, Z_M)$ such that

$$Y + Z + 1 = 0. \tag{19}$$

In the recent literature, the Positivstellensatz has been combined with sum of squares relaxations to obtain computationally efficient proofs for the infeasibility of inequality systems of the form (18), based on semidefinite programming [17]. However, the sum of squares relaxation typically leads to very large semidefinite programs, which may pose computational problems even for the efficient solvers which are available. To avoid this computational issue, we follow an alternative approach where the problem reduces to the solution of a linear program, for which solvers are more efficient than for semidefinite programs. The basic tool in the proposed approach is the Handelman representation theorem [6]. This theorem makes use of so-called Handelman monomials H_d. These are constructed from the inequality constraints Z as

$$H_d(\xi) = \prod_{j=1}^{M} Z_j(\xi)^{d_j}, \tag{20}$$

where $d \in \mathbb{N}_0^M$ is the vectorial degree of the Handelman monomial H_d. The Handelman representation theorem is given in the following statement.

Theorem 7 ([6]) *Let $\mathcal{K} \subset \mathbb{R}^m$ be a compact polytope defined by the equations*

$$Z_j(\xi) \geq 0, \quad j = 1, \ldots, M, \tag{21}$$

with $\xi \in \mathbb{R}^m$ and $Z_j : \mathbb{R}^m \to \mathbb{R}$ affine functions. The polynomial $Y : \mathbb{R}^m \to \mathbb{R}$ is non-negative on $\mathcal{K}$, if and

only if Y can be represented as

$$Y = \sum_{d \in \mathbb{N}_0^M} c_{H,d} H_d, \tag{22}$$

with non-negative coefficients $c_{H,d}$.

Example 8 *Consider the polynomial $Y(x) = -2x^2 + 7x - 5$ in the variable $x \in \mathbb{R}$. The polynomial is non-negative on the domain $1 \leq x \leq 2$. This domain is represented by the constraints $Z_1(x) = x - 1 \geq 0$ and $Z_2(x) = 2 - x \geq 0$. Using an ansatz with Handelman monomials up to degree 2,* (22) *can be solved for the coefficients $c_{H,d}$ by equating coefficients. In this way, we find a representation of Y with the two Handelman monomials $H_{(1,0)}(x) = x - 1$ and $H_{(1,1)}(x) = (x-1)(2-x)$ with coefficients $c_{H,(1,0)} = 1$ and $c_{H,(1,1)} = 2$ as $Y(x) = -2x^2 + 7x - 5 = 1 \cdot (x-1) + 2 \cdot (x-1)(2-x)$.*

The original result by [6] gives a necessary and sufficient condition for positivity of a single polynomial Y on a compact polytope. A serious restriction compared to the Positivstellensatz is that the result concerns positivity of a single polynomial only. However, in the present problem, it is necessary to guarantee non-existence of solutions for a set of polynomial equations within a polytope. The following result combines the Positivstellensatz with the Handelman representation theorem to achieve a statement suitable for this purpose.

Theorem 9 *Let $\mathcal{K} \subset \mathbb{R}^m$ be a compact polytope defined as in Theorem 7. Then the following two conditions are equivalent.*

(i) The system of equations

$$Y_i(\xi) = 0, \quad i = 1, \ldots, N \tag{23}$$

with $\xi \in \mathbb{R}^m$ and polynomials $Y_i \in \mathbb{R}[\xi]$ does not have a solution in $\mathcal{K}$.

(ii) There exist polynomials $T_i \in \mathbb{R}[\xi]$, $i = 1, \ldots, N$ and non-negative coefficients $c_{H,d}$ such that the polynomial

$$Y = \sum_{i=1}^{N} T_i Y_i - 1 \tag{24}$$

can be represented as

$$Y = \sum_{d \in \mathbb{N}_0^M} c_{H,d} H_d. \tag{25}$$

PROOF. *(i) $\Rightarrow$ (ii).* By the Positivstellensatz, there exist polynomials $\tilde{Y} \in \mathcal{I}(Y_1, \ldots, Y_N)$ and $Z \in \mathcal{C}(Z_1, \ldots, Z_M)$ such that $\tilde{Y} + Z + 1 = 0$. By $Z \geq 0$ on $\mathcal{K}$, we have that $-\tilde{Y} - 1 \geq 0$ on $\mathcal{K}$. Since $\tilde{Y} \in \mathcal{I}(Y_1, \ldots, Y_N)$, it can be represented as $\tilde{Y} = -\sum_{i=1}^N T_i Y_i$ with $T_i \in \mathbb{R}[\xi]$. Thus, there exist T_i, $i = 1, \ldots, N$ such that the polynomial $Y = -\tilde{Y} - 1$ as defined in (24) is non-negative on $\mathcal{K}$. The result (25) then follows from Theorem 7.

(ii) $\Rightarrow$ (i). Note that the Handelman monomials satisfy $H_d \in \mathcal{C}(Z_1, \ldots, Z_M)$. Let $\tilde{Y} = \sum_{i=1}^N (-T_i) Y_i \in \mathcal{I}(Y_1, \ldots, Y_N)$ and $\tilde{Z} = \sum_{d \in \mathbb{N}_0^M} c_{H,d} H_d \in \mathcal{C}(Z_1, \ldots, Z_M)$. From (24) and (25), we have $\tilde{Y} + \tilde{Z} + 1 = -\sum_{i=1}^N T_i Y_i + \sum_{d \in \mathbb{N}_0^M} c_{H,d} H_d + 1 = 0$. Thus, by the Positivstellensatz, (23) does not have a solution on $\mathcal{K}$.

Example 10 *Consider the system of equations in $\mathbb{R}^2$*

$$\begin{aligned} 7 + 3x_1 - 4x_2 &= 0 \\ x_2 - x_1 - 1 &= 0. \end{aligned} \tag{26}$$

Using Theorem 9, we want to establish that (26) *does not have a solution in the set $\mathcal{K} = \{x \in \mathbb{R}^2 \mid 0 \leq x_i \leq 2, i = 1, 2\}$. Similar as in Example 8, we can use a (in this case affine) ansatz for the multipliers T_i, and, with Handelman monomials up to degree 2, equate coefficients in* (24) *and* (25) *in order to determine the multipliers and the Handelman coefficients. For the given example* (26)*, we find the multipliers $T_1 = 1$ and $T_2 = x_2$, yielding the polynomial*

$$Y = 7 + 3x_1 - 4x_2 + x_2(x_2 - x_1 - 1) - 1,$$

according to (24)*, with the Handelman representation*

$$Y = x_1 + x_1(2 - x_2) + (2 - x_2) + (2 - x_2)^2. \tag{27}$$

In this case, all Handelman coefficients are equal to one. The Handelman representation shows that (26) *does not have a solution in $\mathcal{K}$.*

A relaxed result for which only the conclusion *(ii) $\Rightarrow$ (i)* in Theorem 9 is valid can be obtained by restricting the degree of the multipliers T_i. With this relaxation, a finite parametrisation of the problem is achieved, where the coefficients of the polynomials T_i are free parameters and the coefficients $c_{H,d}$ in (25) are non-negatively constraint parameters. The procedure to compute an infeasibility certificate, i.e. specific multipliers T_i such that condition *(ii)* is satisfied, then reduces to the solution of a linear program and is outlined as follows.

(i) Construct an ansatz for the multiplier polynomials T_i, $i = 1, \ldots, N$, according to

$$T_i(\xi) = \sum_{d \in \mathcal{D}_i} c_{T,d}^{(i)} \prod_{i=1}^{m} \xi_i^{d_i}, \tag{28}$$

where $\mathcal{D}_i \subset \mathbb{N}_0^m$ contains all vectorial degrees to be used in the multiplier T_i and $c_{T,d}^{(i)} \in \mathbb{R}$, $i = 1, \ldots, N$, $d \in \mathcal{D}_i$, are free parameters to be chosen later. With the multipliers T_i, construct the polynomial Y according to (24).

(ii) Construct all Handelman monomials $H_d(\xi)$ of the form (20), for all $d \in \mathbb{N}_0^M$ such that the vectorial degrees of the H_d do not exceed the vectorial degree of Y.

(iii) Make an ansatz for the Handelman polynomial

$$Y_H = \sum_{d \leq d_{max}} c_{H,d} H_d, \tag{29}$$

where $d_{max} \in \mathbb{N}_0^M$ is the maximal vectorial degree of Y_H, and the $c_{H,d} \in \mathbb{R}$, $d \leq d_{max}$, are non-negatively constrained parameters to be determined in the next step.

(iv) Check whether the linear program

$$\begin{aligned} \text{find } & c_{T,d}^{(i)} \qquad i = 1, \ldots, N,\ d \in \mathcal{D}_i \\ & c_{H,d} \qquad d \leq d_{max} \\ \text{s.t. } & \text{coeff}_\xi(Y) = \text{coeff}_\xi(Y_H) \\ & c_{H,d} \geq 0 \end{aligned} \tag{30}$$

is feasible or not, where $\text{coeff}_\xi(Y)$ denotes the coefficient vector of the polynomial Y with respect to monomials in ξ.

The above procedure may be used to compute robustness certificates for the system (7), in the sense that one can certify the non-existence of solutions for (16) and thus non-existence of critical points. To this end, choose $\mathcal{K}$ in Theorem 9 as $\mathcal{K} = \mathcal{X}_0 \times \mathcal{P} \times [0, \omega_{max}]$, where $\omega_{max} > 0$ is an upper bound on the imaginary eigenvalues to be considered. Moreover, in Theorem 9, set $\xi = (\chi, \omega) \in \mathbb{R}^{n+q+1}$, and take the equality constraints Y_i, $i = 1, \ldots, N$ from (16). The resulting robustness certificate is given in the following result.

Corollary 11 *If the linear program* (30)*, constructed for the equations* (16) *with affine constraints* $\xi = (\chi, \omega) \in \mathcal{X}_0 \times \mathcal{P} \times [0, \omega_{max}]$*, has a feasible solution, then there does not exist a critical point* $\chi_c \in \mathcal{M} \cap \mathcal{X}_0 \times \mathcal{P}$ *where the Jacobian* $A(\chi_c)$ *has an eigenvalue* $j\omega$ *with* $|\omega| \leq \omega_{max}$*.*

Corollary 11 provides a Positivstellensatz robustness certificate for the system (7) under the parametric uncertainty $p \in \mathcal{P}$, in the sense that any feasible solution to the corresponding linear program proves that no local bifurcations of equilibria can occur for any $p \in \mathcal{P}$, at least considering eigenvalues with imaginary parts up to ω_{max}.

3.4 Robustness analysis algorithm

Next, we discuss the application of the robustness certificate provided in the previous section to the computation of a lower bound $\widehat{\psi}^*$ on the robustness radius ψ^*. The proposed algorithm is a bisection on the logarithmic radius ψ of the parameter uncertainty region $\mathcal{P}_r(\psi, p_0)$, using the robustness certificate according to Corollary 11 in each step to decide whether the estimate of the robustness radius should be increased or decreased. The robustness analysis algorithm is implemented according to the following steps.

(i) *Initialisation.* Set the initial estimate for the lower bound on the robustness radius: $\psi_{est} = 2$. Set initial bounds for $\widehat{\psi}^*$: $\psi_{lo} = 1$, and ψ_{hi} to some sufficiently large number. Define a termination tolerance *tol*.

(ii) *Bisection step.* Try to obtain a robustness certificate according to Corollary 11 for $\mathcal{P} = \mathcal{P}_r(\psi_{est}, p_0)$.
   (a) If successful: $\psi_{lo} := \psi_{est}$.
   (b) Otherwise: $\psi_{hi} := \psi_{est}$.
   Bisect for next step: $\psi_{est} := \frac{1}{2}(\psi_{hi} + \psi_{lo})$.

(iii) *Termination criterion.* If $(\psi_{hi} - \psi_{lo}) \leq tol$, proceed to step (iv). Otherwise return to step (ii).

(iv) *Output.* Return the lower bound on the robustness radius

$$\widehat{\psi}^* := \psi_{lo}. \tag{31}$$

The lower bound $\widehat{\psi}^*$ on the robustness radius ψ^* obtained in the proposed algorithm certifies that the set $\mathcal{X}_0 \times P_r(\widehat{\psi}^*, p_0)$ does not contain critical points of the system (7). It thereby provides a certified level of uncertainty in the parameter values, up to which local bifurcations of equilibria cannot occur in the system (7).

The computational cost of the method is basically caused by two factors: first the construction of the Handelman monomials $H_d(\xi)$ and the computation of the coefficients of the Handelman polynomial $Y_H(\xi)$ with respect to ξ, which is done once in the algorithm, and second the solution of the resulting linear program in each step of the bisection algorithm. For increasing network complexity, the computational cost grows quickly due to the fast increase of required Handelman polynomials with the number of affine constraints M and the polynomial degree of the equality constraints (23). To deal with medium to large scale biochemical networks, it will become necessary to improve the computational efficiency, which is possible via several approaches. For example, the polynomial degree of (23) is expected to grow mainly in the frequency variable ω, while for the other variables, i.e. state variables and uncertain parameters, relatively low degrees are expected even for large scale networks. Thus, simplifications based on structural features have the potential to significantly reduce the number of polynomial coefficients which need to be considered, as was previously suggested for sum-of-squares

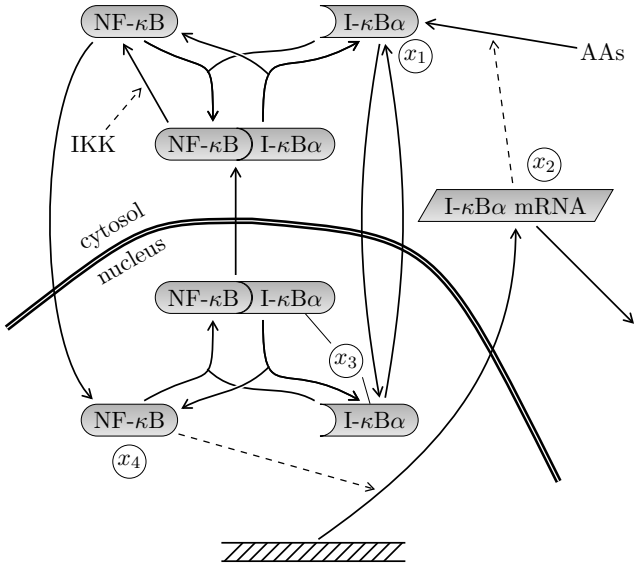

Fig. 2. Reaction scheme for the NF-κB pathway model according to [11].

problems [16]. Furthermore, the construction of the Handelman monomials and corresponding polynomial coefficients could easily be parallelized, thus improving computational efficiency on modern computing architectures. Despite these possibilities, the combinatorial increase in computational cost may prohibit the application to large scale networks. Yet, it is feasible to apply the proposed algorithm to biologically relevant models of small to medium scale networks, as the following example illustrates.

4 Oscillations in a NF-κB pathway model

Recently, the TNF induced NF-κB signalling pathway has attracted much attention in systems biology. This is due to the fact that NF-κB is a central transcription factor involved in the inflammatory response of mammalian cells and directly interacts with the apoptotic pathway by upregulation of anti-apoptotic proteins [13]. Therefore, the NF-κB pathway is highly relevant for understanding cancer or autoimmune diseases. We consider an ODE model suggested in [11], which reproduces experimentally observed oscillations. The robustness analysis method developed in the previous section will be applied to this model in order to investigate the robustness of oscillations with respect to parameter variations.

The original model consists of seven chemical species and a twelve reactions. A scheme of the model is depicted in Figure 2. From a conservation relation for the amount of NF-κB and quasi-stationarity assumptions on the complex formation between NF-κB and its inhibitor I-κB, a reduced order model with four state variables is derived [11]. The four state variables correspond to species concentrations according to the following list: x_1 – free cytosolic I-κBα, x_2 – I-κBα mRNA, x_3 – total nuclear I-κBα, x_4 – free nuclear NF-κB. The reaction parameters are given in Table 1. As a short-hand notation, the additional dependent parameters

$$K_I = \frac{k_b + \alpha}{k_f}$$
$$K_N = \frac{k_b + k_{NI,out}}{k_f}$$

are introduced. The model is then given by the equations

$$\begin{aligned}
\dot{x}_1 &= k_{tl}x_2 - \frac{\alpha(N_{tot} - x_4)x_1}{K_I + x_1} - k_{I,in}x_1 + \frac{k_{I,out}K_N x_3}{K_N + x_4} \\
\dot{x}_2 &= k_t x_4^2 - \gamma_m x_2 \\
\dot{x}_3 &= k_{I,in}x_1 - \frac{k_{I,out}K_N x_3}{K_N + x_4} - \frac{k_{NI,out}x_3 x_4}{K_N + x_4} \\
\dot{x}_4 &= \frac{k_{N,in}K_I(N_{tot} - x_4)}{K_I + x_1} - \frac{k_{NI,out}x_3 x_4}{K_N + x_4}.
\end{aligned} \tag{32}$$

Table 1
Nominal parameter values for the NF-κB model (32) [11].

$k_{N,in} = 5.4\,\mathrm{min}^{-1}$	$k_{I,in} = 0.018\,\mathrm{min}^{-1}$
$k_{I,out} = 0.012\,\mathrm{min}^{-1}$	$k_{NI,out} = 0.83\,\mathrm{min}^{-1}$
$k_f = 30\,(\mu\mathrm{M\,min})^{-1}$	$k_b = 0.03\,\mathrm{min}^{-1}$
$k_t = 1.03\,(\mu\mathrm{M\,min})^{-1}$	$k_{tl} = 0.24\,\mathrm{min}^{-1}$
$\alpha = 0.525\,\mathrm{min}^{-1}$	$\gamma_m = 0.017\,\mathrm{min}^{-1}$
$N_{tot} = 1\,\mu\mathrm{M}$	

The robustness analysis method developed in the previous section is applied [1] to the reduced order NF-κB pathway model (32), with parameter values as given in Table 1. For nominal parameter values, there is an unstable equilibrium point and a stable limit cycle, giving rise to periodic oscillations.

For the purpose of this example, let us assume that the translation of the I-κBα gene and the activity of IKK are uncertain, i.e. $p = (k_t, \alpha)$. In the cell, these two processes are highly suspectible to further influences that have not been included in the model, and therefore the robustness of the pathway with respect to uncertainties therein is an important question. The question to be addressed is how much the two parameters k_t and α may be varied while maintaining instability of the equilibrium point with two right half plane eigenvalues. The algorithm computes a lower bound on the robustness

[1] The implementation of the presented example is available for download as a Matlab script from the website `http://www.ist.uni-stuttgart.de/research/sysbio/sw-fa-automatica-robustness` .

radius for instability of the equilibrium point by bisection on the parametric uncertainty factor ψ. In each bisection step, the algorithm tries to obtain an infeasibility certificate for the critical point condition (16), with $\mathcal{P}(\psi, k_t, \alpha) = [\frac{1}{\psi}k_t, \psi k_t] \times [\frac{1}{\psi}\alpha, \psi\alpha] \subset \mathbb{R}^2$, where k_t and α are taken from the nominal parameter values in Table 1. As loop breaking point, the influence of nuclear NF-κB on the transcription of the I-κBα gene is used, i.e. $h(x) = x_4$ and the x_4^2 term in $\dot{x}_2$ is substituted by u^2. This influence is part of the negative feedback circuit responsible for oscillations in the NF-κB pathway model, and therefore is a reasonable choice for the loop breaking point.

In the computation of the robustness radius for the NF-κB pathway model, it is crucial to get good bounds on the set $\mathcal{X}_0$ of possible steady states to be considered for any given parameter uncertainty. In this study, suitable bounds are obtained by the steady state uncertainty analysis previously described in [22]. Another critical factor is the number of variables which need to be considered in the polynomial equations (16), as the computational effort grows significantly with the number of variables. For the NF-κB pathway model, a good way to reduce the number of variables is to solve partially for the equilibrium point of (32). In steady state, it holds that

$$x_2 = k_t \frac{x_4^2}{\gamma_m}$$
$$x_3 = \frac{k_{I,in} x_1 (K_N + x_4)}{k_{I,out} K_N + k_{NI,out} x_4}.$$

Exploiting this relation, the critical point conditions (16) involve only the five variables x_1, x_4, k_t, α, and the frequency ω. From (16), we obtain two steady state equations for x_1 and x_4, and two equations for the real and imaginary part of (16a), thus $N = 4$.

The maximum degree of the critical point conditions (16) for the NF-κB pathway model is four, resulting from the degree four with respect to the frequency variable ω in the characteristic polynomial of the fourth order system (32). In the analysis, the degree sets $\mathcal{D}_i$ for the multipliers T_i are chosen such that all terms $T_i Y_i$ in the construction of Y have degree five with respect to any individual variable. Considering (24), it is reasonable to choose the ansatz for the multipliers such that all terms $T_i Y_i$ are of the same degree. In this example, a degree of five, i.e. one larger than the maximum degree of the considered equality constraints Y_i, was the lowest degree for which a non-trivial robustness region could be found. In the resulting ansatz for the multipliers T_i, a total of 632 unknown coefficients $c_{T,d}^{(i)}$ has to be used. With a lower and upper bound on each individual variable, we have $M = 10$ inequality constraints. Constructing the Handelman polynomial Y_H according to (29) up to the required degree results in 187787 Handelman monomials of the form (20). Expanding the Handelman polynomial $Y_{H,d}$ in monomials based on the original five variables gives 2282 terms,

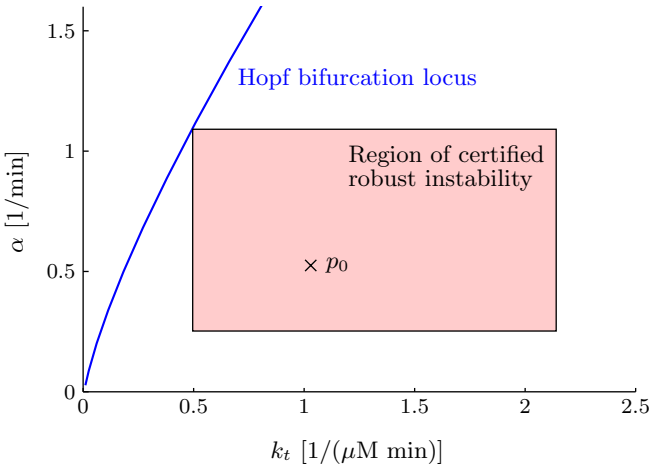

Fig. 3. Region of guaranteed robustness and Hopf bifurcation locus for the NF-κB pathway model (32) in the k_t–α plane. The Hopf bifurcation locus is computed with the bifurcation analysis software `auto` [4], while the region with certified non-existence of bifurcations is computed by the algorithm developed in this paper.

with coefficients depending affinely on the 187787 unknown parameters c_H in the Handelman representation. On a standard desktop computer (Intel(R) Core(TM)2 Duo CPU E4500 2.20GHz, 2GB RAM), constructing these coefficients takes about 2 hours. In each iteration of the bisection algorithm, a linear program with 632 free parameters, 187787 non-negatively constrained parameters and 2282 equality constraints from the comparison of coefficients has to be solved. To solve the linear program, we use the Matlab toolbox SeDuMi [20], which deals well with the sparsity of the equality constraints and the large number of non-negatively constrained parameters. One call to the linear program solver requires about 15 minutes of computation time on a standard desktop computer (as above) for this example.

The lower bound on the dynamical robustness radius obtained for the NF-κB pathway model is $\widehat{\psi}^* = 2.078 \leq \psi^*$, up to a tolerance of $tol = 0.01$ used as termination criterion for the bisection. To find an upper bound, we compute a Hopf bifurcation locus by numerical continuation methods [12]. In this way, a Hopf bifurcation is discovered at $(k_t, \alpha)^* = (0.495, 1.094)$, corresponding to an upper bound of $2.084 \geq \psi^*$. Notice that the lower bound computed with our method is exact within the chosen tolerance. The results are also depicted in Figure 3. In conclusion, the NF-κB pathway as modelled by [11] can tolerate an uncertainty in the considered parameters of more than a factor 2 without experiencing a loss of sustained oscillations. The pathway is therefore expected to maintain the biological function related to the oscillations for a considerable amount of uncertainty in the uncertain processes.

5 Conclusions

For control engineers, checking robustness of instability is an uncommon problem. Yet, in biochemical networks, this problem is of high relevance in the analysis of complex dynamical behaviour such as sustained oscillations or bistability. To deal with this type of problems, we propose a feedback loop breaking approach to obtain conditions for non-existence of local bifurcations under a parametric uncertainty. The conditions are checked computationally by constructing a Handelman representation for a Positivstellensatz robustness certificate. This construction is efficiently accomplished with linear programming. Although theoretically such a certificate does always exist, if the equations are infeasible, in practice the method is conservative due to limitations on the polynomial degree. Yet, the approach is quite efficient, and is, to the best of our knowledge, presently the only method to check robust instability of non-linear systems with respect to a generic parametric uncertainty.

We have also illustrated the application of the proposed method by studying robustness of oscillations in a model of the NF-κB signalling pathway. This example in particular shows that the proposed method is suitable for the robustness analysis of dynamical behaviour in small to medium size biochemical reaction networks.

References

[1] B. D. Aguda and A. Friedman. *Models of Cellular Regulation.* Oxford University Press, 2008.

[2] J. Bochnak, M. Coste, and M.-F. Roy. *Real Algebraic Geometry.* Springer, Berlin, 1998.

[3] M. T. Borisuk and J. J. Tyson. Bifurcation analysis of a model of mitotic control in frog eggs. *J. Theor. Biol.*, 195(1):69–85, Nov 1998.

[4] E. J. Doedel, R. C. Paffenroth, A. R. Champneys, T. F. Fairgrieve, Y. A. Kuznetsov, B. E. Oldeman, B. Sandstede, and X. Wang. *AUTO 2000: continuation and bifurcation software for ordinary differential equations.* Concordia University, Montreal, Canada, 2006.

[5] T. Eissing, S. Waldherr, F. Allgöwer, P. Scheurich, and E. Bullinger. Steady state and (bi-)stability evaluation of simple protease signalling networks. *BioSystems*, 90:591–601, 2007.

[6] D. Handelman. Representing polynomials by positive linear functions on compact convex polyhedra. *Pac. J. Math.*, 132:35–62, 1988.

[7] R. Heinrich and S. Schuster. *The Regulation of Cellular Systems.* Chapman & Hall, New York, 1996.

[8] D. J. Higham. Modeling and simulating chemical reactions. *SIAM Rev.*, 50(2):347–368, Jan 2008.

[9] J. Keener and J. Sneyd. *Mathematical Physiology.* Springer, New York, 2004.

[10] J. Kim, D. G. Bates, I. Postlethwaite, L. Ma, and P. A. Iglesias. Robustness analysis of biochemical network models. *IEE Proc. Syst. Biol.*, 153(3):96–104, May 2006.

[11] S. Krishna, M. H. Jensen, and K. Sneppen. Minimal model of spiky oscillations in NF-kappaB signaling. *Proc. Natl. Acad. Sci.*, 103(29):10840–45, Jul 2006.

[12] Y. A. Kuznetsov. *Elements of Applied Bifurcation Theory.* Springer-Verlag, New York, 1995.

[13] Q. Li and I. M. Verma. NF-kappaB regulation in the immune system. *Nat. Rev. Immunol.*, 2(10):725–734, Oct 2002.

[14] L. Ma and P. A. Iglesias. Quantifying robustness of biochemical network models. *BMC Bioinform.*, 3:38, 2002.

[15] M. Morohashi, A. E. Winn, M. T. Borisuk, H. Bolouri, J. Doyle, and H. Kitano. Robustness as a measure of plausibility in models of biochemical networks. *J. Theor. Biol.*, 216(1):19–30, May 2002.

[16] P. A. Parrilo. Exploiting structure in sum of squares programs. In *Proc. of the 42th IEEE Conf. on Dec. and Control, Maui, Hawai*, pages 4664–4669, 2003.

[17] P. A. Parrilo. Semidefinite programming relaxations for semialgebraic problems. *Math. Program.*, 96(2):293–320, May 2003.

[18] H. Schmidt and E. W. Jacobsen. Linear systems approach to analysis of complex dynamic behaviours in biochemical networks. *IEE Proc. Syst. Biol.*, 1:149–158, 2004.

[19] J. E. Shoemaker and F. J. Doyle III. Identifying fragilities in biochemical networks: Robust performance analysis of Fas signaling-induced apoptosis. *Biophys. J.*, 95:2610–23, Jun 2008.

[20] J. F. Sturm. Using SeDuMi 1.02, a Matlab toolbox for optimization over symmetric cones. *Optim. Meth. Softw.*, 11(1):625–653, 1999.

[21] S. Waldherr and F. Allgöwer. Searching bifurcations in high-dimensional parameter space via a feedback loop breaking approach. *Int. J. Syst. Sci.*, 40:769–782, 2009.

[22] S. Waldherr, R. Findeisen, and F. Allgöwer. Global sensitivity analysis of biochemical reaction networks via semidefinite programming. In *Proc. of the 17th IFAC World Congress, Seoul, Korea*, pages 9701–06, 2008.