

Robustness Analysis, Prediction and Estimation for Uncertain Biochemical Networks

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Abstract: Mathematical models of biochemical reaction networks are important tools in systems biology and systems medicine to understand the reasons for diseases like cancer, and to make predictions for the development of effective treatments. In synthetic biology, for instance, models are used for the design of circuits to reliably perform specialized tasks. For analysis and predictions, plausible and reliable models are required, i.e., models must reflect the properties of interest of the considered biochemical networks. One remarkable property of biochemical networks is *robust functioning* over a wide range of perturbations and environmental conditions. Plausible mathematical models of such robust networks should also be robust. However, capturing, describing, and analyzing robustness in biochemical reaction networks is challenging. First, including uncertainty in the structures, parameters, and perturbations into the model is not straightforward due to different types of uncertainties encountered. Second, robustness as well as system and thus model properties are often itself inherently uncertain, such as qualitative (i.e., nonquantitative) descriptions. Finally, analyzing nonlinear models subject to different uncertainties and with respect to quantitative and qualitative properties is still in its infancy. In the first part of this perspective article, network functions and behaviors of interest are formally defined. Furthermore, different classes of uncertainties and perturbations in the data and model are consistently described. In the second part, we review frequently used approaches and present our own recent developments for robustness analysis, estimation, and model-based prediction. We illustrate their capabilities to deal with the different types of uncertainties and robustness requirements.

Keywords: Biochemical reaction network; complex dynamical system; estimation; robustness; systems and control theory.

1. INTRODUCTION

Biochemical reaction networks form the structural basis of most cellular processes such as in metabolism, signal transduction, and gene expression. In these networks, many species dynamically interact and are transformed by biochemical reactions to perform and maintain biological functions. Intertwined and possibly redundant feedback and feedforward mechanisms give rise to complex dynamical behaviors and their lack or improper functioning can result in malfunctioning or diseases. To minimize these risks, the biological networks must perform their tasks reliably under various changes of the cellular environment and conditions (Bullinger et al., 2007; Kim et al., 2006; Kitano, 2004; Ma and Iglesias, 2002). This property is generally called *robustness* and refers to the *persistence of*

a behavior or the *insensitivity of function characteristics* in the presence of perturbations (Trané and Jacobsen, 2008).

Typical examples of *biological behaviors* that are robust to environmental changes are oscillations or multistability, e.g., in the cell cycle or in apoptosis, respectively, or adaptation in chemotaxis and phototaxis (Streif et al., 2010; Alon et al., 1999). The readers are referred to (Aguda and Friedman, 2008) for other examples and mechanisms of cellular regulation. *Robust functioning* is of particular interest in synthetic biology or metabolic engineering. One core task in synthetic biology is the design of motifs or building blocks that perform a function robustly when connected into larger networks and under various perturbations of the cellular environment (Purnick and Weiss, 2009). Function characteristics of interest include certain types of dynamic input-output behavior such as the time derivation of inputs and adaptation to persistent stimuli, logical combinations of different inputs, or oscillatory behavior (Sontag, 2005).

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Besides the *robustness of qualitative behaviors* or function characteristics, *quantitative predictions* of system responses have become increasingly important especially in therapy design, and quantitative or synthetic biology (Yordanov and Belta, 2011). Often mathematical models are developed and employed to analyze and quantitatively predict, estimate, and control the response of the considered systems with respect to applied inputs or environmental changes (Kitano, 2002). The main challenges hereby are not only to consider the various external perturbations of these systems, but also to take into account the various uncertainties that arise both in the analysis and in the models (Sontag, 2005).

This article provides a perspective on the modeling of biochemical reaction networks with a focus on describing, capturing, and analyzing robustness, taking qualitative as well as quantitative aspects into account. More precisely, robustness analysis requires a formal specification and definition of the analyzed behavior or function characteristics, and of the uncertainties with respect to which robustness is to be analyzed. Robustness can then be analyzed and quantified by determining the allowable uncertainties for which the desired system behaviors or function characteristics are still observed. Because descriptions of biological functions are inherently uncertain, robustness analysis is inevitable linked to uncertainty analysis. To this end, the following two key challenges are outlined:

- (1) specification of the different types of uncertainties encountered in the description of system behaviors, functions, and in the data and models;
- (2) methods for analyzing and quantifying robustness under consideration of uncertainties using prediction and estimation.

While the first challenge is mainly of a conceptual nature, the second is still a widely open field of research once it comes to *efficient* methods for analysis and predictions.

1. Challenge:

Specification of Uncertainties and System Behaviors

Mathematical models of robust biological systems should exhibit appropriate levels of robustness when analyzed (Kim et al., 2010, 2006; Ma and Iglesias, 2002). For robustness analysis and prediction of system responses to perturbations and uncertainties, two crucial ingredients are required: first, a clear *description of external perturbations* under which the biological system (represented by the model) should function robustly; second, a clear *formulation of the behavior or function characteristics* that is about to be analyzed for its robustness.

However, considering only external perturbations is far too limited for the analysis of biological models because already the models are the largest source of uncertainty. This is simply due to the fact that experimental data are sparse, limited, and incomplete and measurement techniques are mostly indirect and have very low accuracy and resolution (Rumschinski et al., 2010a; van Riel and Sontag, 2006; Sontag, 2005). This results in large uncertainties of the absolute quantities of the measured physical or chemical entities or species. In addition, due to low sampling times and missing normalization standards for

absolute quantification, the data are usually not *quantitative and time-resolved*. Often, the data available for model construction are supplemented by *qualitative information* such as conditional or temporal statements or *if-then* observations (Samaga and Klamt, 2013; Rumschinski et al., 2012; De Jong, 2002).

For the analysis of robustness and for prediction and analysis, different uncertainties must be modeled and considered: first, *external perturbations*; second, *uncertainties in the formulation of the investigated behaviors or function characteristics* due to the qualitative character and the *uncertain measurement data*; third, *structural uncertainties* such as due to incomplete knowledge of the reaction kinetics or intermediate reaction steps; fourth, *parameter uncertainties* such as unknown reaction rate constants. Methods must be chosen for robustness analysis, robust prediction, and estimation that can account for the encountered and largely different types of uncertainties. Currently no suitable tools exist that can capture all described uncertainties.

2. Challenge:

Robustness Analysis, Estimation and Prediction Methods

Methods for the analysis of robustness and robust prediction for biochemical reaction networks should allow nonlinearities to be taken into account. The methods should be able to handle different types of uncertainties and to make robust statements on network performance, qualitative behavior, and the influence of uncertainties to be made (see Fig. 1). In particular, we are interested in making dynamical predictions of system outputs under uncertainties and perturbations (left to right in Fig. 1). To quantify robustness of function characteristics, parameter estimation can be used (right to left in Fig. 1) where the volume of the consistent and robust parameter set could serve as a measure of robustness (Chaves et al., 2009).

Robustness analysis, robust estimation, and prediction are classical topics in control engineering, see e.g. (Zhou et al., 1995). Most existing methods are limited to linear systems, whereas realistic biochemical networks are nonlinear. Methods that can handle nonlinear systems are often limited or assume that the steady-state is not affected by uncertainties or perturbations. In contrast to most technical systems where robustness of stability is the main objective, the robustness of instability is important in biological systems, because instability is related to biological behaviors such as oscillations or multistability (Waldherr and Allgöwer, 2011; Angeli et al., 2004). The direct application of classical systems and control methods is therefore limited. In addition, the different types of uncertainties encountered in biological and medicinal research differ compared to technical systems. For example, in human-made technical systems, sensors can often be placed as wished or uncertainties can often be avoided by a suitable design.

Outline of this Paper

In the last decade, several approaches have been developed that can handle or overcome some of the mentioned challenges. This paper considers methods for the analysis and

estimation of uncertain dynamical quantitative models described by ordinary differential or difference equations (Sec. 2). Note that we do not consider or review qualitative or structural modeling frameworks or methods allowing for qualitative predictions using these models. For reviews of those methods see, e.g., (Samaga and Klamt, 2013; Wilhelm et al., 2004; Barabási and Oltvai, 2004; De Jong, 2002; Stelling et al., 2002). We furthermore restrict the perspective toward methods that allow a closed form or algebraic analysis. In particular, we do not cover methods that employ Monte Carlo simulation or related analysis methods. Descriptions of stochastic analysis techniques can be found, for example, in (Schwarick et al., 2010).

Sec. 3 provides possible ways to describe and capture the uncertainties in the models (mainly probabilistic and set-based descriptions). Because the presented methods have individual and different advantages and limitations and can not all deal with all uncertainty types, it is important to classify these different types. We discuss the selection of suitable methods and increases the awareness of the statements that can actually be made for the robustness analysis and model verification.

Sec. 4 reviews *classical approaches* and extensions thereof. These methods are often restricted to local or structural analysis, but can still give valuable insight into the system. However, these methods are not well-suited for quantitative predictions and analysis in the case of large uncertainties. Sec. 5 reviews set-based approaches that can efficiently deal with set-based uncertainty descriptions. Well-known and computationally efficient approaches such as interval analysis fall into this class. Interval analysis methods can produce results that are too conservative, and less restrictive methods are outlined in Sec. 5.

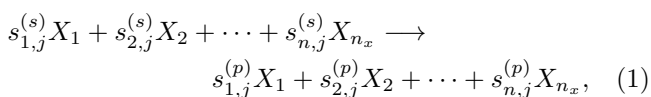
While set-based approaches allow robust and guaranteed statements, the results can also be conservative. This conservatism can be reduced by employing approaches that provide statements in terms of probabilities and probability distributions with the premises that definite and guaranteed statements are only possible asymptotically. Several existing probabilistic methods are reviewed in Sec. 6.

Sec. 7 discusses various problems that have not yet been solved in the current literature. This section also provides an outlook for future research.

2. BIOCHEMICAL NETWORK MODELS

This paper considers a wide class of biological systems, including metabolic, signal transduction, and gene regulation networks. Most of these processes can be formally modeled as biochemical reaction networks.

Basically, biochemical networks have two main elements, namely, species and reactions. The biochemical species X_1, X_2, \dots, X_{n_x} represent ensembles of chemically identical molecules in a specific cell compartment. These species are interconverted by chemical reactions of the general form



where $j = 1, 2, \dots, n_v$ is the reaction index, and the factors $s_{i,j}^{(s)}, s_{i,j}^{(p)} \in \mathbb{N}_0$ are the stoichiometric coefficients of the substrate and product species, respectively.

The structural information of the reaction network is usually collected in the stoichiometric $S \in \mathbb{R}^{n_x \times n_v}$ matrix with entries given by

$$S_{i,j} := s_{i,j}^{(p)} - s_{i,j}^{(s)}, \quad i = 1, \dots, n_x, \quad j = 1, \dots, n_v. \quad (2)$$

For simplicity of presentation, the system's state vector $x(t) \in \mathbb{R}^{n_x}$ comprises the species' concentrations³ $[X_i]$ and is denoted by

$$x := ([X_1], [X_2], \dots, [X_{n_x}])^\top \in \mathbb{R}^{n_x}. \quad (3)$$

The kinetics of the reactions are given by rate functions, which depend on the state x , time-invariant parameters $p \in \mathbb{R}^{n_p}$, and time-varying signals $w(t) \in \mathbb{R}^{n_w}$. In the context of biochemical networks, $w(t)$ can represent external inputs or stimuli, changes of the cellular environment, or control inputs such as cooling temperature or added nutrients or substrates in fed-batch processes.

The transformation of the species are described by reaction rates that are given by the vector

$$v(x, p, w) := (v_1(x, p, w), \dots, v_{n_v}(x, p, w))^\top \in \mathbb{R}^{n_v}. \quad (4)$$

Usually, reaction rates are polynomial or rational expressions arising from the law of mass action or the Michaelis-Menten mechanism (Aguda and Friedman, 2008). Some of the analysis frameworks are restricted to certain types of equations such as polynomial equations. In principle and under rather mild assumptions, it is possible to convert other nonlinearity expressions including quasi-polynomial kinetics or non-algebraic functions into polynomial form using state immersion (see references in (Hancock and Papachristodoulou, 2013; Motee et al., 2012; Ohtsuka and Streif, 2009)) or by approximation techniques such as Taylor series (see references in (Kishida and Braatz, 2012)).

Outputs are often used to capture the measured variables. However, outputs are also used to quantitatively capture the uncertain/robust behavior, or to qualitatively characterize robustness. Consider outputs of the form $y(t) \in \mathbb{R}^{n_y}$ that are nonlinear functions of the states, parameters, and inputs:

$$y = h(x, p, w) \in \mathbb{R}^{n_y}. \quad (5)$$

In summary, the biochemical reaction network is concisely written as:

$$\dot{x} = Sv(x, p, w) = f(x, p, w), \quad (6a)$$

$$y = h(x, p, w), \quad (6b)$$

where $f(\cdot)$ is introduced to simplify notation and is nonlinear in general. The rest of this section considers some mathematical preliminaries that are needed before moving on to the inclusion of uncertainties in the models.

A common feature in biochemical networks are conservation relations x_T among the state variables x of the form $x_{T,j} = \sum_{i=1}^{n_x} l_{j,i} x_i, j = 1, \dots, n_c$ with non-negative coefficients $l_{j,i}$ (Heinrich and Schuster, 1996). Such relations reduce the degrees of freedom and usually the system of differential equations is treated in its reduced form with $n_x - n_c$ state variables.

³ Concentrations are always greater than or equal to zero.

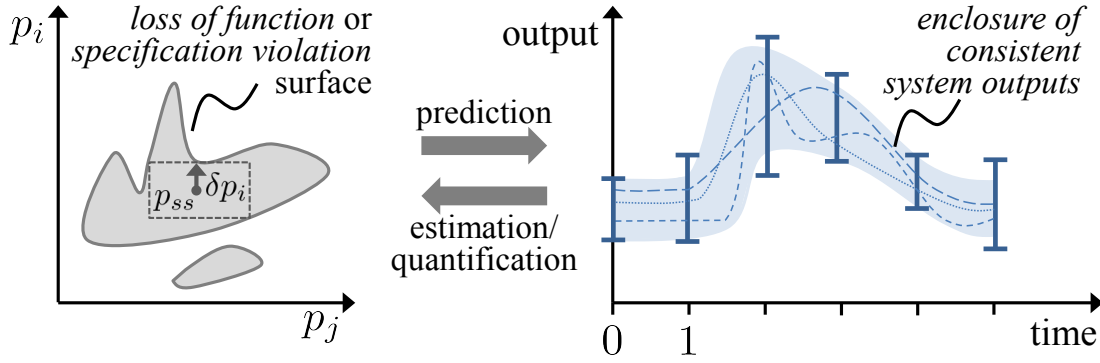


Fig. 1. Robustness analysis and quantification is closely related to prediction of uncertainty propagation and estimation. Prediction can be used to analyze the influence of uncertainties and perturbations on the model outputs/response. Estimation can be used to determine, e.g., parameter uncertainties (gray shaded areas in the figure on the left) such that certain qualitative or quantitative specifications of system behaviors or properties are satisfied (light blue shaded areas on the right). Robustness of system behavior can be either quantified with respect to some nominal parameters p_{ss} and the distance δp_i from the surface where the specifications are no longer satisfied, or the volume of the consistent parameter set or some approximation of it such as the dashed box. Note that, in this figure, only the set-based description is shown for easier presentation. The ideas are transferable to probabilistic uncertainties and predictions. In this case, constraint violation is formulated in terms of probabilities.

The discrete-time version of the continuous-time system (6) is employed in later sections. To account for implicit and explicit integration schemes, we use the representation

$$0 = F(x(k+1), x(k), p, w(k)), \quad (7a)$$

$$0 = H(y(k), x(k), p, w(k)), \quad (7b)$$

where $k \in \mathbb{N}$ is the time index with associated time points t_k . The functions $F(\cdot)$ and $H(\cdot)$ represent the discrete-time versions of $f(\cdot)$ and $h(\cdot)$, respectively.

For several subsequent analyses, the linearization of the nonlinear dynamical system (6) at a steady-state will be used. To shorten the notation, the steady-state will be written as

$$\xi_{ss} := (x_{ss}, p_{ss}, w_{ss}), \quad (8)$$

where the parameter values $p = p_{ss}$ and constant perturbations $w = w_{ss}$. The steady-states are given by solving

$$f(\xi_{ss}) = 0. \quad (9)$$

The linearization of (6) around (8) is then given by

$$\delta \dot{x} = A \delta x + B_w \delta w + B_p \delta p, \quad (10a)$$

$$\delta y = C \delta x + D_w \delta w + D_p \delta p, \quad (10b)$$

where the matrices $A := \left. \frac{\partial f}{\partial x} \right|_{\xi_{ss}}$, $B_w := \left. \frac{\partial f}{\partial w} \right|_{\xi_{ss}}$, $B_p := \left. \frac{\partial f}{\partial p} \right|_{\xi_{ss}}$, $C := \left. \frac{\partial h}{\partial x} \right|_{\xi_{ss}}$, $D_w := \left. \frac{\partial h}{\partial w} \right|_{\xi_{ss}}$, $D_p := \left. \frac{\partial h}{\partial p} \right|_{\xi_{ss}}$ are evaluated at the steady-state ξ_{ss} . This formulation also considers perturbations of the nominal parameter values p_{ss} by δp , through the matrices B_p and D_p .

3. UNCERTAINTY DESCRIPTIONS AND ROBUSTNESS SPECIFICATIONS

Robustness is typically analyzed and quantified by determining the allowable uncertainties for which a desired system behavior or function characteristic can be guaranteed (see Fig. 1). Thus robustness analysis is inevitably linked to uncertainty characterization. In addition, uncertainty

can enter the analysis from many sources such as the quantitative or even qualitative description of the robustness property or function characteristic. Other sources include limited structural knowledge and data uncertainties. Note that we use the term *data uncertainty* in a rather broad sense. By this term, we refer not only to the sparse and uncertain measurement data, but also to qualitative observations and information from expert knowledge. *Model uncertainties* are simply considered here as a consequence of data uncertainty, as explained in the next subsection.

To be able to make correct statements and predictions despite these uncertainties, it is important to choose suitable methods that can capture and handle uncertainties. Suitable methods are presented in the Sections 4–6. In this section, the required formulation of the different uncertainties (see Fig. 2) and analysis questions are presented. Furthermore, we illustrate the uncertainties with some observed biological behaviors and state commonly considered analysis questions (see Fig. 3).

3.1 Model Uncertainties: Hypotheses and Structural Uncertainties

It is often unclear whether relevant species or reactions and interaction between species have been missed in the construction of a model for a biochemical reaction network. The structure of the models is uncertain in the sense that either the stoichiometric coefficients in S are not precisely known, some reactions $v(\cdot)$ might be absent or present, or the mathematical expression of the reaction rates (i.e., involved species and reaction kinetics) are unknown.

The uncertain structure often leads to different model hypotheses that need to be compared against the data. Models with structures that are inconsistent with the data can then be ruled out or may indicate that further circles of iterative modeling and tests of consistency are required.

Methods to test model consistency while taking uncertainties into account are presented in Sec. 5. Obviously, such tests require the comparison of model outputs with data.

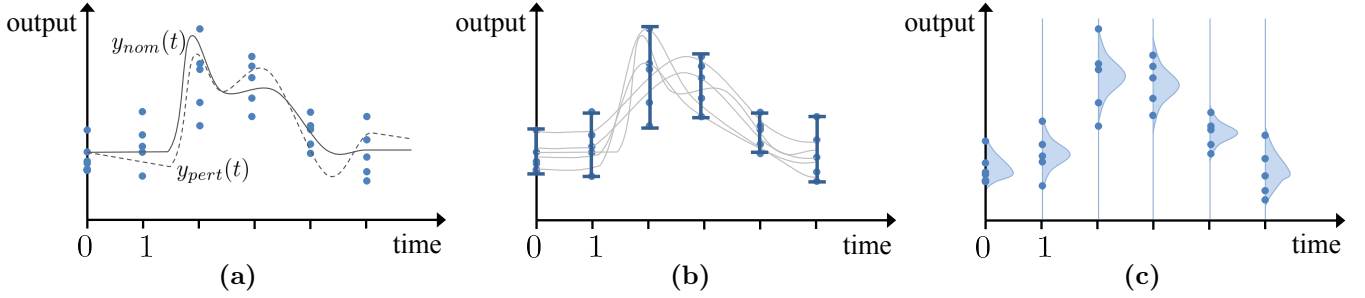


Fig. 2. Different quantitative data uncertainties at time points $0, 1, \dots$ and system outputs/response. (a) Pointwise measurements at the time points. The curves show the effect of a parameter perturbation on the system output $y_{nom}(t)$ resulting in $y_{pert}(t)$. (b) Set-based or worst-case uncertainty description, which in this case was derived from (a) by the minimum and maximum measurement values. Different trajectories (gray solid curves) are consistent with the measurements. (c) If the number of samples is sufficiently large and under certain conditions, the probability density of the underlying uncertainty distribution may be assumed or derived.

For comparison purposes, it is important to formulate the uncertain data such that a comparison can be made based on unbiased tests. These criteria can be formulated mathematically as *constraints*, see details below.

3.2 Set-Based Uncertainties

Due to limited measurement precision, low sampling frequency, and low number of experimental replicates (see Fig. 2a), it is not always possible to make any conclusion with respect to the actual probabilistic uncertainty distribution. Therefore, error bars, as depicted in Fig. 2b, derived from standard deviations or worst-case approximations, can be helpful. Such data correspond to uncertainty intervals or more generally, uncertainty sets. This set-based uncertainty description is also encountered when parameter values cannot be specified exactly. Indeed, parameters are usually highly uncertain and possible ranges can span several orders of magnitude.

Interval Uncertainties. Often uncertainties in the variables are described by an upper and lower bound (see Fig. 2). Such bounds on uncertain parameters p or uncertain initial conditions x_0 are represented by

$$\begin{aligned} \underline{p}_i &\leq p_i \leq \bar{p}_i, & i = 1, \dots, n_p, \\ \underline{x}_{0,i} &\leq x_{0,i} \leq \bar{x}_{0,i}, & i = 1, \dots, n_x. \end{aligned} \quad (11)$$

Interval uncertainties are a special class of set-based uncertainties.

To describe such uncertainties more generally, assume that the variables such as parameters or measurements $y(t_k)$ at time points t_k take values from a set defined by possibly nonlinear inequalities $g_i(\xi) \geq 0$, i.e.,

$$\xi \in \{\xi : g_i(\xi) \geq 0, i = 1, 2, \dots, n_m\} \subseteq \mathbb{R}^{n_\xi}, \quad (12)$$

where $\xi := (x, p, w)$.⁴ This description also allows for the next type of data.

Parameter or Data Relations. This special class of set-based uncertainties is encountered when there are known relations between measurements of different outputs or of the same output at different time points. A common example is when an observed peak of a biological output is known to be twice as high after a stimulus compared

⁴ Equalities can be handled by introducing $g_i(\xi) \geq 0$ and $-g_i(\xi) \geq 0$.

to its value before the stimulus. In general, this results in relations between (unknown or uncertain) variables and can be represented as a manifold, which can be implicitly expressed by (12).

Methods to deal with set-based uncertainty descriptions are presented in Sec. 5.

3.3 Probabilistic Uncertainties

The set-based uncertainty description only describes the *possibility* for a parameter value, but does not make any statements about the *probability* that a particular parameter value is taken. For certain cases, a set-based description is reasonable and less assumptive especially if only few replicates are available to make meaningful statements on probabilistic measurement uncertainties or parameter distributions. However, due to rapidly improving measurement and high-throughput techniques, statistics and thus probabilistic descriptions become more and more available.

In case meaningful statistics or distributions can be derived, as in Fig. 2c, these uncertainties are referred to as *probabilistic uncertainties*, which often can be characterized as in

$$\xi_i \sim \text{Prob}\left(E(\xi_i), E(\xi_i^2), \dots\right), \quad (13)$$

where $\text{Prob}(\cdot)$ is a specified probability distribution with specified expected values and moments $E(\cdot)$, which are related to such properties as mean and variance (ξ_i is an element of the vector ξ).

Chance constraints are another way of handling probabilistic uncertainties (Ben-Tal et al., 2009; Prékopa, 1994), which can be formalized by

$$\text{Prob}\left(g_i(\xi) \geq 0\right) \geq \nu_{g_i}, \quad (14)$$

where ν_{g_i} is the *confidence level* associated with a constraint $g_i(\cdot)$.

Methods that can handle probabilistic uncertainties are presented in Sec. 6.

3.4 Qualitative Information on System Behavior

Very often the system behavior of interest is not described by quantitative measures or values. Instead, so-called *qual-*

*itative information*⁵ is available on how the system behaves (see Fig. 3). To analyze robustness of these kind of system behaviors requires a different definition, which is given in this subsection. A thorough formulation and description can be found in (Rumschinski et al., 2012). In many cases, the qualitative data directly follows from the lack of data and that absolute and quantitative measurement techniques are either not available or too expensive.

Steady-state Location. Due to lack of affordable measurement techniques for fast sampling and time-consuming experiments, only measurements of the steady-state before and after a perturbation or stimulus might be available (see Fig. 3a). This can result in a particular qualitative pattern of (some) outputs. Because quantification standards might not be available, the data are normalized, typically with respect to the maximum. Different levels are then defined such as *high* or *low* (see Fig. 3a) to verbalize the qualitative system behavior as, for instance, gene activation patterns (De Jong, 2002).

This information can be written similarly as (12):

$$\xi_{ss,j} \in \{\xi : f(\xi) = 0, g_{ss,j}(\xi) \geq 0\}, j = 1, \dots, n_{ss}. \quad (15)$$

The first constraints $f(\cdot)$ are the steady-state conditions, cf. (8), $g_{ss,j}(\cdot) \geq 0$ is the location information, and n_{ss} is the number of steady-states. Alternatively, probabilistic uncertainties as in (13) could be used.

Stability, Instability, and Oscillation. In addition to the steady-state location, the dynamical properties of the biochemical reaction network are of interest. Typically, biological functions are related to certain qualitative dynamical behaviors such as multistability, i.e., the existence of several stable steady-states, limit cycle oscillations, or non-periodic oscillations (see Fig. 3b). Examples are the cell cycle, apoptosis, or circadian rhythm (Aguda and Friedman, 2008; Sontag, 2005). From a dynamical systems point of view, bistability, multistability, and sustained oscillations are related to stability and instability of an equilibrium point.

A steady-state ξ_{ss} is locally stable if all eigenvalues of the Jacobian A (see also (10)) have negative real parts. Here stability is meant in the sense of Hurwitz or asymptotical stability, which can be checked by the characteristic equation:

$$q(s, \xi_{ss}) = \det(sI - A(\xi_{ss})) = \sum_{i=0}^{n_x} c_i(\xi_{ss}) s^i = 0. \quad (16)$$

The question then is whether the family of polynomials $q(s, \xi_{ss})$ with coefficients $c_i(\xi_{ss})$ is (robustly) stable for all uncertainties $\xi_{ss} \in \mathcal{X}_{ss}$, where $\xi_{ss} \in \mathcal{X}_{ss}$ is a subset of \mathbb{R}^{n_x} of interest. Answering this question is equivalent to assessing whether $q(s, \xi_{ss}) \neq 0$ for all $s \in \mathbb{C}$ with $\text{Real}(s) \geq 0$.

Other ways besides (16) exist to check Hurwitz stability, e.g., see (Horn and Johnson, 1991). For nonlinear systems, various conditions exist that can be used for the analysis of stability of nonlinear systems, e.g., see standard textbooks such as (Khalil, 2002).

⁵ Mixed classes of *qualitative* and *quantitative* data are also often encountered.

Adaptation and Inverse Response. Besides stability- and instability-related behaviors, the qualitative dynamical responses to perturbations of external conditions or stimuli are of interest (Fig. 3c). Most prominent examples where robustness has been observed in vivo and in silico are excitation and adaptation in bacterial chemotaxis (Alon et al., 1999; Barkai and Leibler, 1997) and archaeal phototaxis (Streif et al., 2010). In this context, adaptation denotes the property that an observed output initially changes in response to a stimulus, but then returns to the value before the stimulus, even though the stimulus persists. Adaptation is important to keep cells fit in changing environments by maintaining homeostasis under perturbations, or by expanding the dynamic range of sensory receptors. From a systems theoretic point of view, the conditions for exact (or perfect) adaptation have been extensively investigated (see (Waldherr et al., 2012; Sontag, 2003; Yi et al., 2000) and references within): if a system adapts to a class of input signals, then it necessarily contains a subsystem that is capable of generating signals of this class, which is known under the term *internal model principle*.

For constant inputs, adaptation is obviously related to the steady-state gain of the system. The relation between the output deviation from steady-state and the stimulus deviation from steady-state in the Laplace domain is characterized by the transfer function $G(s) = C(sI - A)^{-1}B_w + D_w$ (assuming $p = 0$ and $D_w = 0$) as computed from the matrices in (6), with the complex variable $s \in \mathbb{C}$. A system with scalar input $w(t)$ and output $y(t)$ has perfect local adaptation at ξ_{ss} if and only if $G(s) = 0$ for $s = 0$. This condition is equivalent to

$$\det \begin{pmatrix} A & B_w \\ C & 0 \end{pmatrix} = 0 \quad (17)$$

and A has nontrivial eigenvalues.

In a similar manner, *inverse response behavior* (see Fig. 3d) could be analyzed at the linearization and is related to unstable zero dynamics, i.e., zeros of the transfer function $G(s)$ in the right half plane. However, to the best of our knowledge, inverse response behavior has so far only been reported once (Hartmann and Oesterhelt, 1977). From a systems theoretic perspective, inverse response behavior is not unlikely, though. This raises the question of whether measurement data showing such inverse response behavior have simply been trashed by experimentalists, because an inverse behavior was counterintuitive and unexpected.

Robustness or invariance of concentrations of particular species despite the variations of the concentrations of other species is another important class that has been considered, e.g., in (Steuer et al., 2011) and (Shinar and Feinberg, 2010). Robustness or invariance of concentration can be more general and can also refer to invariance of the considered concentrations on short or long time scales. In contrast, adaptive system behavior is usually associated with an initial excitation, i.e., large changes of the concentrations of one or several species.

Conditional and Temporal Observations. Very often, only a limited amount of quantitative and temporally resolved data is available for model construction and estimation. Instead, only qualitative statements such as *if a stimulus is given, then the concentration increases*

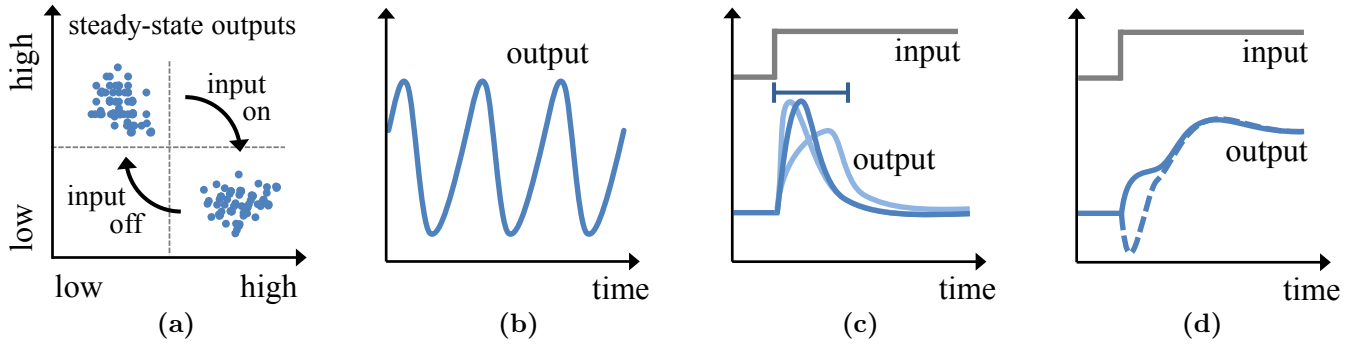


Fig. 3. Qualitative biological system behaviors. (a) Steady-state pattern in the presence or absence of inputs, i.e., stimuli. (b) Stable oscillations like a cell cycle or circadian rhythm. (c and d) Qualitatively different transient system outputs. In both cases, a persistent stimulus is applied as an input. In (c), the output transiently increases and then decreases again to converge versus the prestimulus level even though the stimulus persists, which is called *adaptation* in biology. Due to a low sampling frequency, the time when the maximum occurs may not be known, which then corresponds to temporal uncertainties as shown by the horizontal bar. In (d), the output increases to an elevated steady-state level without (solid line) and with (dashed line) initial inverse response behavior.

transiently, before returning to its prestimulus level even though the stimulus persists. Such information is often also provided by experts and experimentalists from biological insight or knowledge. Such constraints can be formally captured using conditional statements such as

$$\begin{aligned} &\text{IF } (g_A(\xi) \geq 0 \text{ AND } g_B(\xi) \geq 0) \text{ OR} \\ &\quad (g_C(\xi) \geq 0 \text{ AND } \dots) \text{ OR } \dots \\ &\text{THEN } (g_X(\xi) \geq 0 \text{ AND } \dots). \end{aligned} \quad (18)$$

Generally, the conditions can be constraints involving different variables or the same variable at different time points, which allows the formulation of *temporal uncertainties*, i.e., where the time point when an event happens is not exactly known. This is illustrated in Fig. 3c where the time point when the transient response peaks is temporally uncertain.

As shown in (Rumschinski et al., 2012), such a formulation allows the capture of many qualitative observations, biological knowledge, and data using Boolean logic (Rizk et al., 2011; Karaman et al., 2008; Bemporad and Morari, 1999). Note that robustness of such qualitative behaviors, such as having oscillations or not, is most often of interest.

3.5 Problem Statements for Robustness Analysis

Robustness analysis can be informally stated as *quantification of the perturbations that a system can tolerate before losing a specific function* (Kim et al., 2006; Kitano, 2004; Stelling et al., 2004; Ma and Iglesias, 2002; Morohashi et al., 2002). More formally, the remainder of the paper considers the question of robustness analysis as (see also Fig. 1):

- (1) *Quantification of robustness by estimation* of parameter sets or distributions that are consistent with uncertain data (sections 3.2–3.3) and qualitative specifications of system behavior (Sec. 3.4).
- (2) *Prediction of uncertainty propagation* and how uncertainties affect robustness and output specifications.

The volume of the consistent parameter set is an immediate measure of robustness. As illustrated and discussed in (Chaves et al., 2009), however, a large volume of the robust parameter set does not imply large robustness because

the set might be very thin with a small perturbation into one direction of the parameter space leading to a loss of function, while the system can still be robust for perturbations in other directions (see also Fig. 1). Thus, the geometry and topology of the robust set contains very important information on robustness (Chaves et al., 2009).

Sections 4–6 present different methods to tackle these questions for quantitative dynamical models of biochemical reaction networks. Monte Carlo sampling and related algorithms that do not provide conclusive results are not discussed due to their high computational cost compared to alternative methods. Our main focus is on set-based methods that provide guarantees, and methods that allow probabilistic statements with reasonable computational cost.

4. ANALYSIS OF LOCAL PERTURBATIONS AND NETWORK STRUCTURES

This section reviews methods from systems and control theory that are well-suited to analyze the influence and propagation of uncertainties in biochemical reaction networks. In particular, *local perturbations* of variables such as the parameters are considered, which then lead to perturbations of the *nominal system behavior*. The term *local* refers to being either small or infinitesimal perturbations that can lead to a different output trajectory $y_{pert}(t)$ as illustrated in Fig. 2.

The presented methods allow a characterization of steady-state solutions and a quantification of robustness by the distance between nominal parameters and the parameters for which constraints on the qualitative or quantitative behavior are violated (see Fig. 1). While these methods are conceptually simple and well-known, their applicability for the analysis of the usually large uncertainties is limited, because they focus on nominal points that are often assumed to be invariant to the perturbations, or because the methods rely on linearizations that requires small perturbations.

In addition, methods are reviewed that allow statements about the qualitative behavior to be drawn from the nominal network structure or its perturbation. These methods

are, at least to a large extent, independent of parameters. Due to this fact, the methods are well-suited for qualitative analyses, but are limited for quantitative predictions.

4.1 Sensitivity Analysis

One robustness analysis question is how the nominal dynamical system behavior or the steady-state changes in response to perturbations of the parameters, which then can be used to quantify robustness (see Fig. 2a). The analysis of the influence of the parameters is denoted as *parametric sensitivity analysis*, which has been used for many purposes such as the identification of targets for the design of drugs and therapies, or the identification of limiting steps in a metabolic network to achieve a maximum yield of a product. For reviews of applications and the general methods, see, e.g., (Zi, 2011; Streif et al., 2009; Ingalls, 2008; Saltelli et al., 2000). Sensitivity analysis provides a good starting point to identify the parameters and corresponding key factors that have strong impact on the output. This analysis can provide valuable insights about how robust the biological responses are with respect to parameter changes. In general, sensitivity analysis methods can be classified as *local* and *global* as detailed further below. The sensitivity with respect to probabilistic uncertainties is addressed in Sec. 6.

Local Sensitivity Analysis. Local sensitivity analysis concentrates on a nominal point in the parameter space, such as a nominal operating condition or steady-state ξ_{ss} . To approximate the perturbed output trajectory $y_{nom}(t)$ (see Fig. 1) in the case of a small perturbation δp_j of parameter p_j , the first-order sensitivity system can be used that is obtained from the linearization (10) of (6) in which the input perturbation δw is set to zero:

$$\frac{d}{dt} \frac{\partial x}{\partial p_j} = A(\xi_{nom}(t)) \frac{\partial x}{\partial p_j} + B_{p_j}(\xi_{nom}(t)), \quad (19a)$$

$$\frac{\partial y}{\partial p_j} = C(\xi_{nom}(t)) \frac{\partial x}{\partial p_j} + D_{p_j}(\xi_{nom}(t)). \quad (19b)$$

The sensitivity equations (19) describe a linear time-varying system of ordinary differential equations in which the matrices $A(\xi_{nom}(t))$, $B_{p_j}(\xi_{nom}(t))$, $C(\xi_{nom}(t))$ and $D_{p_j}(\xi_{nom}(t))$ are evaluated along the nominal trajectory $\xi_{nom}(t)$.

The sensitivity $\frac{\partial x}{\partial p_j}$ of the steady-state ξ_{ss} can be obtained from the steady-state solution of (19) if A is Hurwitz:

$$\frac{\partial x}{\partial p_j} = -A^{-1}B_{p_j} \quad \text{and} \quad \frac{\partial y}{\partial p_j} = -CA^{-1}B_{p_j} + D_{p_j},$$

where the matrices are evaluated at ξ_{ss} . The latter equation can then be used to determine the shift of the steady-state output for finite perturbations δp_j :

$$\delta y = \left(-CA^{-1}B_{p_j} + D_{p_j}\right)\delta p_j.$$

Often, scaled or normalized sensitivities $\frac{p_{ss,j}}{x_{ss}} \frac{\partial x}{\partial p_j}$ are more meaningful to measure the changes of the steady-state (Saltelli et al., 2000).

Besides these classical approaches, several extensions have been presented, such as for oscillating systems (Zi, 2011; Wilkins et al., 2009; Taylor et al., 2008).

Local sensitivity analysis, also known as *metabolic control analysis*, provides a first-order approximation of the effect of parameter perturbations. However, the local parametric sensitivity analysis results should be used with care for the prediction of large perturbations. For this reason, higher-order sensitivities (e.g., (Streif et al., 2007; Hwang, 1983; Cascante et al., 1991)) and global sensitivity methods have been considered. The latter are described next.

Global Sensitivity Analysis. Global sensitivity analysis aims to predict model behavior either for larger parameter values, or for local sensitivities averaged over a domain in parameter values. Often statistical methods are used to guide the sampling of values from within the specified domains in the parameter space. An introduction to global sensitivity analysis can be found in (Marino et al., 2008).

In (Streif et al., 2009, 2006), a global sensitivity analysis method was presented based on an input-output control engineering view. The idea employs a combination of observability and controllability Gramians (see also (Singh and Hahn, 2005)), the so-called *cross Gramian*, and an empirical extension for nonlinear systems. Even though this approach allows larger parameter perturbations to be considered, the statements are still made with respect to a nominal operating point.

4.2 Bifurcation Analysis

Bifurcation analysis based on numerical continuation has often be used to measure robustness in cases when only few parameters are assumed uncertain and varied (Kim et al., 2006; Ma and Iglesias, 2002; Morohashi et al., 2002). As discussed in (Waldherr and Allgöwer, 2011), a major limitation is that bifurcation surfaces (see Fig. 1) can usually not be computed explicitly in a high-dimensional parameter space. In addition, continuation methods may miss parts of the bifurcation surface, even if only one or two parameters are uncertain. To deal with multi-parametric uncertainty, it was suggested to use the structured singular value as an analysis tool (Shoemaker and Doyle, 2008; Kim et al., 2006; Ma and Iglesias, 2002). 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. For more detailed discussion see (Waldherr, 2009).

Mönnigmann and Marquardt (2002) use normal vectors on manifolds of critical points to measure the distance between these manifolds and equilibrium solutions. This approach allows the characterization of an equilibrium solution by their parametric distance to manifolds at which the behavior of the system changes qualitatively, i.e., bifurcation points, or points at which state variable constraints or output constraints are violated. Statements are only made with respect to a nominal operating point.

4.3 Kinetic Perturbations

A kinetic perturbation is a modification of the network's reaction rate vector from $v(\cdot)$ to $\tilde{v}(\cdot)$. The key restriction is that the steady-state reaction rates should remain unchanged, i.e., $v(\xi_{ss}) = \tilde{v}(\xi_{ss})$. Thus, kinetic perturbations

change the slope of the reaction rates at a steady-state, but leave the nominal steady-state and the stoichiometric matrix unchanged. Defining a suitable perturbation matrix $\Delta \in \mathbb{R}^{n_r \times n_x}$, the change in the slope of the reaction rate at steady-state ξ_{ss} is given by

$$\delta v := \frac{\partial \tilde{v}}{\partial x}(\xi_{ss}) - \frac{\partial v}{\partial x}(\xi_{ss}) = \text{diag}(\tilde{v}(\xi_{ss})) \Delta \text{diag}(\xi_{ss})^{-1}.$$

Considering the linear approximation (10) of the network at steady-state, the Jacobian is

$$\tilde{A}(\delta v) = S \frac{\partial v}{\partial x}(\xi_{ss}) + \text{diag}(\tilde{v}(\xi_{ss})) \Delta \text{diag}(\xi_{ss})^{-1}.$$

Using the notion of kinetic perturbations, Waldherr et al. (2009) studied the robustness problem of finding a perturbation δv such that $\tilde{A}(\delta v)$ has eigenvalues on the imaginary axis, i.e., where the qualitative behavior of the system changes.

In a similar approach, Waldherr et al. (2012) investigated the adaptation problem for a network with scalar input and output. Then the adaptation problem is to find Δ such that (17) is satisfied and $\tilde{A}(\delta v)$ is Hurwitz. Both the robustness and adaptation problem are solved by robust control techniques (Zhou et al., 1995).

4.4 Qualitative Behavior and its Dependence on Network Structure and Feedback Loops

In principle, using parametric sensitivity (see previous subsections) as a measure of robustness is based on the assumption that the underlying model structure is exactly known and that all relevant perturbations can be represented by changes in the model parameters. However, model structures are in general uncertain due to incomplete knowledge of the reaction kinetics, neglected intermediate reaction steps, and unmodeled transport phenomena such as diffusion and delays (Jacobsen and Cedersund, 2005).

A large number of methods exist to analyze the robustness of the qualitative system behavior with respect to perturbation or addition or removal of interactions between the species in the network. Below is a review of a selection of methods for structural network analysis.

Structural Robustness. In (Jacobsen and Cedersund, 2008; Trané and Jacobsen, 2008; Jacobsen and Cedersund, 2005), linear systems analysis, transfer functions, and structured singular values are used to analyze perturbations that affect the model structure. In (Jacobsen and Nenchev, 2011), structural uncertainty is particularly considered as unmodeled dynamics and transfer function analysis is used to compute robustness with respect to structural changes in reaction networks. In addition, it was shown that robustness analysis can be used to validate/invalidate a hypothesized model structure and to detect structural fragilities.

Monotone Systems. For certain classes of biochemical reaction networks, model structures (or reaction structures) can be related to dynamical system properties such as multi-stability. In (Craciun et al., 2011), for monotonic reaction rates, analysis of the associated graphical models is used to characterize structures of biochemical reaction

networks in terms of dynamical properties including multi-stability and convergence to an equilibrium point. Monotonicity of reaction rates means that, for the reaction network dynamics (6), the nonlinear function $v(x, p)$ corresponding to a reaction rate vector satisfies the relations

$$\frac{\partial v_i(x, p)}{\partial x_j} = \begin{cases} \geq 0 & \text{if } s_{i,j}^{(s)} > 0, & \forall i = 1, \dots, n_r, \\ = 0 & \text{if } s_{i,j}^{(s)} = 0, & \forall j = 1, \dots, n_x, \end{cases}$$

for all p .⁶ These results follow from extensive studies of monotone systems (Enciso and Sontag, 2005; Angeli et al., 2004; Angeli and Sontag, 2004).

A finding in (Kim et al., 2012; Venkatesh et al., 2004) is that existence of positive and negative feedback loops in a reaction network plays a key role in robustifying a dynamical reaction network system against both parametric and structural perturbations.

5. SET-BASED UNCERTAINTIES AND ANALYSIS

The methods presented in Sec. 4 allow the analysis of perturbations of single or few parameters, and do not easily allow the consideration of more general set-based uncertainties. In the analysis of biochemical networks, however, it is important to consider simultaneous perturbations and uncertainties in all parameters, and to derive rigorous enclosures of all solutions for iterative modeling or classification of motifs. This section presents methods that can deal with set-based uncertainties and provide bounds on all solutions (see Fig. 2b), as well as how to employ set-based approaches for robustness analysis.

5.1 Interval Analysis

Interval analysis was introduced by Moore (1966) as an approach to bound rounding and truncation errors in mathematical computations. Due to its general simplicity and computational efficiency, as well as many sophisticated improvements, interval analysis has gained much attention. Several reviews have been published (Moore et al., 2009; Hijazi et al., 2007; Jaulin et al., 2001), with many discussing applications to robust prediction, estimation and control.

In interval analysis, interval uncertainties (11) are considered. These uncertainties result in different possible output trajectories. Guaranteed bounds $\underline{y}_i \leq y_i \leq \bar{y}_i, i = 1, \dots, n_y$, on the outputs can be computed using interval functions and interval arithmetic. Interval arithmetic is a logical extension of standard arithmetic. Operations like addition and subtraction are simply defined by operations on the lower and upper bounds.

Though a simple idea, interval analysis is a very powerful technique with numerous applications in mathematics, computer science, and engineering (see reviews cited above). However, the computation of the tightest possible interval solution set that completely contains all solutions is difficult, primarily due to dependence or correlations among uncertain variables. This difficulty is discussed in more detail in the next section.

⁶ Since monotonicity of reaction rates are required for all p in the set, this property might be called *robust* monotonicity.

Stability Analysis Using Interval Matrices. Stability and instability are related to many biological functions as highlighted in Sec. 3.4. Consider a system (6) with box-shaped parameter uncertainties $\mathcal{P} := [\underline{p}_1, \overline{p}_1] \times \dots \times [\underline{p}_{n_p}, \overline{p}_{n_p}]$ as defined in (11). A standard approach for robustness analysis of nonlinear systems in the presence of such parametric uncertainties is to examine the linearization (10) and its associated characteristic equation. In mass action networks, the parameter uncertainties appear affinely in the entries of the Jacobian A , denoted by $A(p)$, and consequently multi-affinely in the coefficients $c_i(p)$ of the each term of the characteristic equation (16).

A common approach is to relax the polynomial dependencies and assume each $c_i(p)$ as an independent uncertainty interval. Lower and upper bounds for $c_i(p)$ can then be computed using interval arithmetic and the polynomial becomes consequentially an interval polynomial. In 1978, Kharitonov proposed a theorem on robust Hurwitz stability of interval polynomials that reduces the stability analysis to the stability of four deterministic polynomials, where each polynomial corresponds to a certain combination of extreme values $\underline{c}_i(p)$ and $\overline{c}_i(p)$ of each coefficient (Kharitonov, 1978).

Interval polynomials and the well-known Kharitonov theorem is thus an approach for the stability analysis of biochemical reaction networks with interval uncertainties. Albeit this approach is elegant, a drawback is that it neglects the parameter correlation in each coefficient $c_i(p)$. In general, algorithms that are based on positivity of the Hurwitz determinant associated with (16) suffer from the fact that the order of the polynomials $c_i(p)$ grow polynomially in the number of states, n_x , and in the order of the entries in $A(p)$. This approach can lead to high-order polynomials $c_i(p)$, and neglecting the parameter correlations by an overapproximation using interval analysis is a rough approximation. Because of this, Kharitonov's theorem can introduce conservativeness when it comes to the stability problem of the original nonlinear dynamical system.

It is therefore important to apply other appropriate methods that take care of the dependence of the parameters to remove conservativeness due to correlation of the uncertain parameters whenever possible (for specific numerical examples, see (Goh et al., 2012) and citations therein). Root locus is a general method but too computationally expensive when the number of parameters is large. Generalized Kharitonov (Barmish, 1989) methods are more generally useful but still only apply to limited parameter dependencies. Numerous other approaches for robust Hurwitz stability that handle more general polynomial parameter dependencies have been presented in the systems and control communities, see e.g., (Zettler and Garloff, 1998) and references within. The next section presents other set-based approaches that are useful not only for stability analysis, but also for addressing other questions stated in Sec. 3. These methods reduce this conservatism by improved relaxations.

5.2 Linear and Semidefinite Relaxations

The general idea behind the methods presented next is to construct a *feasibility problem* (or sometimes called a

constraint satisfaction problem) and to derive the entire set of solutions, i.e., not only a single solution. This feasibility problem is a set of nonlinear equations and inequalities, concisely written as:

$$\begin{aligned} & \text{find } \xi \\ & \text{subject to } g_i(\xi) \geq 0, \quad i = 1, \dots, n_g \end{aligned} \quad (20)$$

where the vector ξ contains all time-variant and time-invariant variables that appear in the problem. The constraints $g_i(\xi)$ are used to represent the nonlinear dynamics (6) after a suitable time discretization on a finite-time horizon t_0, t_1, \dots, t_{n_t} , as well as all set-based uncertainties and relation of variables and other information on the outputs in the form of (12).

Due to nonlinearities and hence nonconvexities, the solution set of (20) cannot be derived directly. Therefore, relaxations into linear and semidefinite feasibility problems are applied. In plain words, the basic idea of relaxations are to replace nonlinearities with simpler expressions. For example, a linear relaxation introduces variables that are linear in the relaxation (or lifting) variables. The resulting relaxed problems can be solved efficiently, and due to this relaxation procedure, each solution of the original nonlinear feasibility problem is also a solution of the relaxed feasibility problem. The converse is, however, not true.

Interval analysis (see Sec. 5.1) follows the same idea and relaxes a nonlinear expression by overapproximating it by an interval. As discussed, interval-based relaxations can be quite conservative. The following approach produces, in our experience, tighter bounds (Streif et al., 2012).

With the assumption of real-valued⁷, bounded, and non-negative⁸ variables ξ , as well as polynomial or rational expressions⁹ $g_i(\xi) \geq 0$. Under the outlined assumptions, the constraints $g_i(\cdot)$ in (20) can be reformulated in terms of a matrix X composed of monomials needed to represent the inequalities. This new representation allows to capture the present nonconvexities in form of a rank-one condition on X . By relaxing this rank condition, a convex semidefinite program (SDP) is obtained, i.e., $X \succeq 0$ as a relaxation of $\text{rank}(X) = 1$. To deal with larger biochemical reaction networks with more constraints and variables, the SDP can be relaxed to a linear program (LP) by replacing $X \succeq 0$ by the weaker constraints $X \geq 0$ and by assuming symmetry of X . More details can be found elsewhere (Streif et al., 2012; Rumschinski et al., 2012; Streif et al., 2009).

The relaxed SDP or LP contains information about the model dynamics (6) and the set-based uncertainties (12). In general, this approach is very flexible and allows different robust analysis questions to be tackled as done further below. Formulation of feasibility problems with associated constraints on data and models with set-based uncertainties, the involved relaxation steps, and the respective solution of the problems by outer approximations

⁷ Sec. 5.3 shows that mixed real and integer valued variables can be handled if qualitative (e.g., conditional and temporal) constraints are needed.

⁸ Due to the boundedness of the variables, the non-negativity of the variables poses no limitation and can be obtained by suitable translation.

⁹ Assuming polynomial expressions poses few limitations, because different solutions exist to convert the rational and transcendental into polynomial form, see Sec. 2.

can be performed in ADMIT, which is a freely available toolbox (Streif et al., 2012).

Structural Uncertainties and Model Invalidation.

To prove that a model hypothesis, reformulated as a feasibility problem (20), is inconsistent with some qualitative behavior or with output constraints despite set-based uncertainties, it is necessary to determine if a solution for the feasibility problem (20) exists. An efficient approach (Rumschinski et al., 2012) in this case is to consider the Lagrangian dual formulation of the semidefinite or linear relaxation. The weak duality theorem and the relaxation process guarantee that, if the objective of the dual program is unbounded, then (20) does not admit a solution, hence is inconsistent. This approach enables entire families of models to be ruled out and thus deals efficiently with structural uncertainties.

Uncertainty Propagation and Parameter Estimation.

The feasibility approach also enables the derivation of outer approximations of consistent parameter sets or state variable sets. It can therefore be used to address the robust estimation of states and parameters. Outer approximations of uncertain variables can be obtained if the feasibility problem is replaced by an optimization problem in which the single variables are minimized or maximized. A tighter lower bound of variable ξ_j can be obtained by the formulation

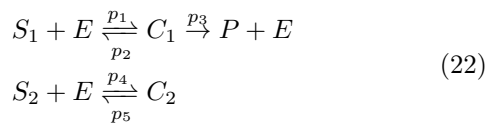
$$\begin{aligned} \min \xi_j \\ \text{subject to } g_i(\xi) \geq 0, i = 1, \dots, n_g. \end{aligned} \quad (21)$$

followed by application of the same relaxations as above. In this way, box-shaped outer approximations can be easily determined on state variables of parameters.

Outer approximations provide an intuitive measure of robustness—the larger the volume of the outer approximation, the more robust the system is. As discussed in Sec. 3.5, however, volume-based robustness measures should be not over-interpreted because the shape of the robust set contain important robustness information. A better description of the robust set can be obtained by the following approach or by so-called *inner approximations* as presented in Sec. 5.3.

Besides such box-shaped outer approximations, the solution sets of (20) can be approximated by partitioning the initial uncertainties into regions for which it is then checked whether they contain a solution or not. With suitable recursive algorithms, this approach allows the derivation of an outer approximation of consistent parameter values as in Fig. 4 and as shown in the following example (Streif et al., 2013b).

Consider the enzyme-catalyzed reaction



Here, enzyme E and a substrate S_1 reversibly form a complex C_1 that is converted into product P . Furthermore, the enzyme is bound by a second substrate S_2 forming the inhibitory complex C_2 . The parameters p_1, p_2, \dots, p_5 denote the uncertain reaction rate constants. The reaction

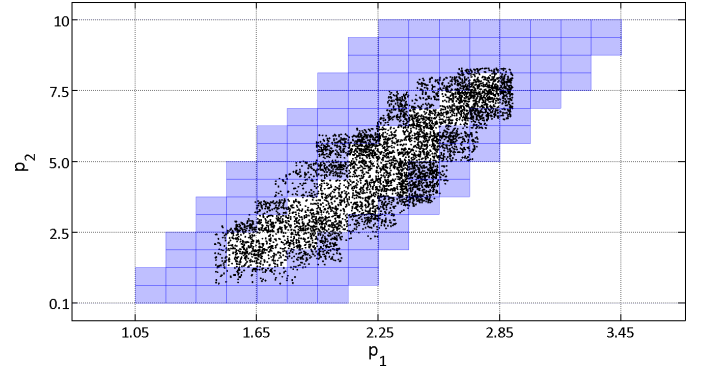


Fig. 4. Outer (blue boxes) and inner (white boxes) approximation of parameter sets. Valid samples are given by the black dots. Image was taken with permission from (Streif et al., 2013b).

mechanism (22) is modeled in discrete time (fixed step size t_Δ) by

$$\begin{aligned} x_1(k+1) &= x_1(k) + t_\Delta(p_1 x_4(k) x_5(k) - (p_2 + p_3) x_1(k)) \\ x_2(k+1) &= x_2(k) + t_\Delta(p_4 x_5(k) x_6(k) - p_5 x_2(k)) \\ x_3(k+1) &= x_3(k) + t_\Delta(p_3 x_1(k)). \end{aligned}$$

with the conservation relations: $x_{T,4} = x_4(k) + x_1(k) + x_3(k)$, $x_{T,5} = x_5(k) + x_1(k) + x_2(k)$, $x_{T,6} = x_6(k) + x_2(k)$. The variables $x_1(k)$, $x_2(k)$, $x_3(k)$, $x_4(k)$, $x_5(k)$, and $x_6(k)$ represent the concentrations of C_1 , C_2 , P , S_1 , E , and S_2 , respectively.

Now consider the capability of the set-based method to provide guaranteed predictions despite set-based uncertainties. To this end, introduce artificial measurements of the product P from the simulation of the system with nominal initial condition $[x_1(0), x_2(0), x_3(0)]^\top = [0.10, 0.10, 0.05]^\top$ for a step size of $t_\Delta = 0.1$ hours, and all parameter values set to 2. To simulate data uncertainties, an absolute error of 5% was added. Initial parameter uncertainties were assumed as $0.1 \leq p_j \leq 10$. Fig. 4 shows an outer approximation of the parameter sets consistent with the uncertain output data. The outer approximation was obtained by invalidating different regions in parameter space.

Stability and Instability. Biological networks should perform their functions robustly despite uncertainties. To check robustness of stability- and instability-related behaviors, (16) and its associated Hurwitz determinant could be used to test asymptotic stability, similar as for the interval analysis approach. However, this approach suffers from similar conservatism problems as the interval polynomial approach.

In an approach that also employs linear programming uses a feedback loop-breaking approach to obtain conditions for non-existence of local bifurcations under a parametric uncertainty (Waldherr and Allgöwer, 2011). The conditions are checked computationally by applying the Positivstellensatz, which is relaxed into a linear program similar as above. A solution to the linear program yields a robustness certificate for the considered dynamical behavior and lower robustness bound corresponding to a level of parametric uncertainty up to which no local bifurcations can occur.

5.3 Mixed-Integer Linear Relaxations

Semidefinite and linear relaxations enables the determination of *outer approximations* of parameters and state variables consistent with formulated *quantitative robustness constraints*. The methods presented in this subsection use mixed-integer programming¹⁰ and allow the determination of *inner approximations* and to analyze *qualitative information* (discussed in Sec. 3.4).

Inner Approximations. Robustness can be quantified by volume of the outer approximation of the consistent sets. However, this measure can be misleading for two reasons. First of all, the outer approximation is conservative and usually contains inconsistent parameterizations. Second, even if the outer approximation is tight, the consistent parameter set might not be simply connected such as discussed in (Chaves et al., 2009) (see also Sec. 3.5). It is therefore important to find *inner approximating sets* for which it is guaranteed that all parameter combinations from this set lead to consistent solutions. This determination will then help to elucidate the geometry and topology of the consistent parameter set.

Streif et al. (2013b) proposed a method to determine inner approximations. The two basic ideas are, first, to reformulate the constraints $g_i(\xi) \geq 0$ in (12) by associating a binary variable b_i with each constraint such that each binary variable is constrained to be true if and only if the associated inequality is satisfied. This approach leads to an equivalent mixed-integer nonlinear programming problem. An inner approximation is then obtained by adding a logical combination using *OR*-statements and by checking if the feasibility problem has a solution or not. Sets of parameters or initial conditions for which the feasibility problem provides no solution then gives an inner approximation. The relaxation of the mixed-integer nonlinear feasibility problem into a mixed-integer linear feasibility problem allows the inner approximations to be determined efficiently. Inner approximations for the example (22) are shown in Fig. 4 and the union of the boxes gives a robustness measure.

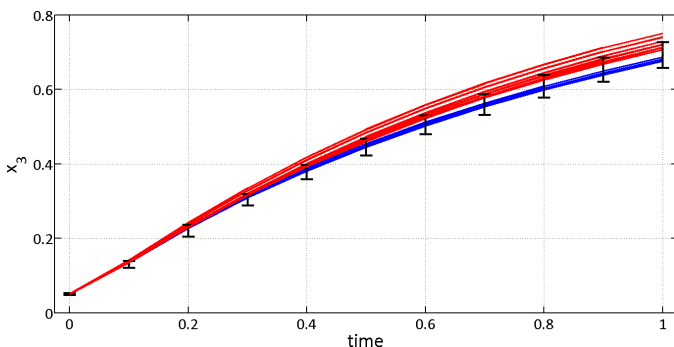


Fig. 5. Inner approximation of parameter sets that lead to guaranteed satisfaction of constraints. Blue trajectories result from parameter samples taken from the inner approximation. Red trajectories are inconsistent samples from the outer approximation. Figure taken from (Streif et al., 2013b).

¹⁰This approach also allows for discrete state, input, or parameter variables.

Including Qualitative Information in Robustness Analysis.

Rumschinski et al. (2012) considered qualitative, temporal, and conditional statements of the general form given in Eq. (18). In general, *AND* combinations of constraints (e.g., $g_A(\xi) \geq 0$ *AND* $g_B(\xi) \geq 0$) do not pose any problem because they can simply be added to the feasibility problem. However, *OR*-combinations (e.g., $g_A(\xi) \geq 0$ *OR* $g_B(\xi) \geq 0$) have to be treated specially. Similar to the inner approximation idea, one approach is to introduce binary variables (e.g., b_A) and imposing constraints on them such that they are *true* (i.e., equal to 1) if a condition (e.g., $g_A(\xi) \geq 0$) is satisfied. By suitably combining the binary variables in linear constraints, the statement *A OR B* (associated with g_A and g_B) can be enforced to be true, which then allows the estimation of consistent parameters.

This approach is computationally demanding, but very versatile. By suitable formulation of qualitative information and statements, using Boolean algebra and reformulation in terms of the mixed-integer program, and allows many different types of uncertainties to be considered, such as temporal uncertainties (i.e., either *A* is true at time t_1 *OR* at time t_2 *OR* ...), or conditional statements (*A OR B* is true if *C* is true). This approach has been used for the robust analysis of adaptation networks (Rumschinski et al., 2012).

5.4 Methods for Continuous-Time Dynamical Systems

A possibility to address continuous-time systems within the presented framework is by discrete-time approximations, e.g., obtained by numerical integration. Due to the discretization error, the consistent parameter sets of continuous-time and discrete-time model do not necessarily overlap and, thus, wrong conclusions on model robustness are possible (Rumschinski et al., 2010b). One assumption that still allows the direct application of the presented methods is to assume that the time derivatives of the state variables are available as measurements (Fey and Bullinger, 2010), but this is not often the case.

Interval analysis methods that can deal with continuous-time dynamics rely on higher-order Taylor approximations and are usually termed *verified integration* (see e.g., (Lin and Stadtherr, 2007; Nedialkov et al., 1999; Berz and Makino, 1998)). The tightness of the overapproximations produced by these methods heavily depend on the underlying integration algorithms. Especially for large times, it can easily happen that the overapproximation explodes. Verified integration can easily be used for prediction of uncertainty propagation, but considering e.g., stability or output constraints can be difficult.

Other methods employing semidefinite programming and the related *Sum-of-Squares* approach exist, such as *barrier certificates* (Anderson and Papachristodoulou, 2009; Prajna, 2006; Prajna and Rantzer, 2007) and *occupation measures* (Streif et al., 2013a). These approaches allow the continuous-time dynamics to be considered directly without numerical integration and allow the certification of parameter regions as inconsistent with data and model. Occupation measures have been used recently (Streif et al., 2013a) to derive both polynomial inner- and outer-approximations of the consistent parameter sets for continuous-time nonlinear systems.

An advantage for approaches based on barrier certificate and occupation measure is that these approaches directly incorporate the right-hand side of the differential equations between the measurement points without using any approximation by time discretization. However, both approaches are computationally demanding for realistic systems and the number of decision variables required for this construction increases polynomially with the number of variables and relaxation order (see discussion and references in (Streif et al., 2013a)).

5.5 Robustness Analysis via Skewed Structured Singular Values

For rational systems, the system's parameter set and output set can be related by the skewed structured singular value along with the scaled main loop theorem (Ferrerres, 1999; Zhou et al., 1995). This theorem states that the maximum norm of the linear fractional transformation (LFT) over an uncertainty set is the corresponding skewed structured singular value. The advantage of using the skewed structured singular value is that it can handle very general parameter dependencies, which usually reduces the conservativeness.

As stated in Sec. 2, many biochemical reaction networks can be written in terms of polynomial or rational functions. Such systems together with parametric uncertainties, noise, and disturbances can be expressed in the form of an LFT assuming that the function is well-defined at the nominal system (Zhou et al., 1995). The resulting LFT consists of two terms: a term for the nominal system and a term for the uncertain/varying portion of the system.

To find a parameter set that is consistent with the given specification of the system output/response, two steps are required after expressing the system as an LFT. The first step is to decide the *shape* of the box of the allowable uncertain set (e.g., the ratio of δp_1 and δp_2 in Fig. 1). The shape can be chosen based on the relative expected variations in the parameters, the magnitude of the nominal parameters, or other methods (Kishida and Braatz, 2012). This step is necessary because the skewed structured singular value gives only one value, and cannot compute multiple values at once. In the second step, the skewed structured singular value is used to stretch or shrink the chosen box equally in all directions to find a maximum volume uncertain set that guarantees that satisfaction of the specification on the system output/response (Kishida and Braatz, 2012).

To see how the parametric uncertainties propagate in the output, consider a discrete-time system with a scalar output (7), which corresponds to continuous-time system (6). In order to derive lower and upper bounds on the output variables $y(k)$, an LFT is constructed with a constant matrix and a perturbation matrix Δ that contains information on the interval uncertainties, respectively.¹¹ For systems with parametric uncertainties, Δ is a block-diagonal matrix where each block is a scalar times the identity matrix.

¹¹ For systems with ellipsoidal uncertainties, the skewed spherical structured singular value should be used instead of the skewed structured singular value (Kishida and Braatz, 2013).

By using the main loop theorem, the bounds on uncertainty propagation can be determined from

$$\overline{y(k)} = -\nu\Delta(N_l) + M, \quad (23a)$$

$$\underline{y(k)} = \nu\Delta(N_u) - M, \quad (23b)$$

where N_l and N_u are constant matrices and $M > 0$ is any large enough scalar value. Variations that balance the conservativeness and computational complexities of the algorithm can be found in (Kishida et al., 2011).

In applications, it is desired to employ an LFT of minimal order, whose construction is equivalent to a multi-dimensional realization and non-trivial. For this purpose, order reduction algorithms such as (Marcos et al., 2007; D'Andrea and Khatiri, 1997; Russell and Braatz, 1998) are available. Once the system is written in LFT form, the computational difficulty and robustness is the same as for computing lower/upper bounds on structured singular values, which can be computed in polynomial time. For further discussions, see e.g., (Kishida et al., 2011).

6. PROBABILISTIC UNCERTAINTIES AND ANALYSIS

As more measurements can be made for biochemical experiments as a result of technological advancements, parameters are more often reported in probabilistic form, which increases the importance of methods that analyze the influence and propagation of probabilistic uncertainties (see Fig. 2c). Two classes of methods for the propagation and analysis of probabilistic uncertainties are summarized in this section. The first class of methods uses an approximation of Liouville's Equation and its solution using the Fokker-Planck equation. The second class of methods employs Polynomial Chaos Theory. The latter expands system responses to probabilistic uncertainties in appropriate polynomial basis functions, which are determined by the uncertainty distributions. Approximating system responses by these polynomial chaos expansions (PCEs) has the advantage of decreasing computation time compared to Monte Carlo simulation that requires the solution of the system equations for each sample.

6.1 Approximate Analysis using the Fokker-Planck Equation

Analysis of the effects of randomness (i.e., probabilistic uncertainty) of the initial concentrations and kinetic reaction parameter variations can be studied by solving a partial differential equation (PDE) known as the Fokker-Planck or Liouville equation for a given probability distribution of initial concentration profiles and kinetic parameters. A difficulty of this problem is the hardness of computing an exact solution for such PDEs, and there have been made many efforts to either avoid solution of the PDE or compute an approximate solution instead. Such methods for stochastic stability analysis and sensitivity analysis are described next.

Stochastic Stability Analysis. El Samad and Khammash (2004) analyzed the stochastic stability of two gene regulatory networks that has been built and analyzed in the biological literatures. They specialized some standard theorems on in the literature (Lasota and Mackey, 1994) that

enable the analysis of stochastic stability using Lyapunov functions, which is much simpler than direct analysis of the Fokker-Planck equation for the probability distribution. Kim et al. (2012) characterized the dynamics of a motif consisting of interlinked fast and slow positive feedback loops, which regulate polarization of budding yeast, calcium signaling, Xenopus oocyte maturation, and other processes (Brandman et al., 2005). Interest in this motif as a component in synthetic genetic networks is that it provides a dual-time switch that can be rapidly and reliably induced while being relatively insensitive to noise in the stimulus (Brandman et al., 2005). Kim et al. (2012) discussed how the expressions derived from this approach can be used to *design* robust biological gene switch circuits that perform programmed desired behaviors in the presence of intrinsic and extrinsic perturbations.

Sensitivity Analysis. Horenko et al. (2005) applied the method called the Trapezoid Rule for Adaptive Integration of Liouville dynamics (TRAIL) to analyze the propagation of randomness (distribution) in nonlinear dynamical systems. This technique consists of two steps: (a) *prediction*, in which linear ordinary differential equations (ODEs) corresponding to reaction dynamics and the associated Fokker-Planck equations are used, and (b) *correction*, in which nonlinear effects are treated to refine the accuracy.

In (Elf and Ehrenberg, 2003), the reaction dynamics (called macroscopic dynamics) was linearized with respect to a steady-state, and the covariance of a solution of the corresponding Fokker-Planck equation was computed in terms of a solution of Lyapunov equations. It was shown that the method can be used to efficiently estimate sensitivity criteria such as the Fano factor and analyze the effects of elimination of fast variables (i.e., removing fast subsystems, which corresponds to unmodeled dynamics).

6.2 Polynomial Chaos Theory

This section considers a stochastic spectral method of uncertainty propagation and quantification called *polynomial chaos* including its generalization. The approach belongs to the class of analysis of stochastic system responses and uses PCEs as a functional approximation of the mathematical model. The PCE approach is suitable for studying probabilistic uncertainty quantification and propagation.

Recent introductory tutorials of the use of PCE with emphasis on the application to systems and control problems (Kim et al., 2013; Nagy and Braatz, 2010) and a comprehensive overview on the use of PCE methods for sensitivity analysis (Sudret, 2008) are available. This section focuses on tutorial introduction to the ideas of PCE methods with some simple examples of biochemical reaction network systems.

Propagation of Probabilistic Uncertainty. The method of PCE to analyze uncertainty in a stochastic dynamical system was first introduced by Wiener (1938) for turbulence modeling for uncertainties that are Gaussian random variables, which was later extended to other random variables (Xiu and Karniadakis, 2002). The underlying idea is to use a spectral decomposition for which solutions for stochastic differential equations in an infinite-dimensional probability space are projected onto a finite-dimensional

subspace spanned by a set of certain polynomial basis functions. Selection of the types of basis functions depends on the types of random variables whose stochasticity is to be propagated, and the methods of projections determine ways of computing the associated coefficients of basis functions that minimize an approximation error.

To illustrate the use of PCE methods for uncertainty propagation, consider the biochemical reaction network model in (Ma and Iglesias, 2002) underlying cAMP oscillations observed in chemotactic *Dictyostelium discoideum* cells:

$$\dot{x} = \begin{bmatrix} k_1 x_7 - k_2 x_1 x_2 \\ k_3 x_5 - k_4 x_2 \\ k_5 x_7 - k_6 x_2 x_3 \\ k_7 - k_8 x_3 x_4 \\ k_9 x_1 - k_{10} x_4 x_5 \\ k_{11} x_1 - k_{12} x_6 \\ k_{13} x_6 - k_{14} x_7 \end{bmatrix} \quad (24)$$

where $p = [k_1, k_2, \dots, k_{14}]^\top$ are the parameters, and $x = [x_1, \dots, x_7]^\top$ represents the concentrations of seven proteins: [ACA], [PKA], [ERK2], [REG A], [Internal cAMP], [External cAMP], and [CAR1], respectively. Suppose that the only source of uncertainty is in kinetic parameters¹² described by a normal distribution with mean 0.8 and variance 0.1:

$$k_6 \sim \text{norm}(0.8, 0.1).$$

Here the state solution $x_i(t; k_6)$ is approximated by a PCE of the form $\hat{x}_i(t; \theta) = \sum_{j=1}^{m_i} \xi_{ij}(t) \phi_j(\theta)$, where $\theta \sim \text{norm}(0, 1)$, i.e., $k_6 = 0.1\theta + 0.8$, and ϕ_j 's refer to the Hermite polynomial basis functions. A least-squares fit was used to determine $\xi_{ij}(t)$, which minimizes $[x_i(t; k_6) - \hat{x}_i(t; \theta)]^2$ for m_i distinct θ 's that are roots of ϕ_{m_i+1} . Fig. 6 shows the average and variance of [ACA] computed from an 8th-order PCE ($m_i = 9$) and from Monte Carlo simulation (10^4 samples). Compared to Monte Carlo simulation, which takes 342.7 seconds, PCE significantly decreases the computational time to 0.644 seconds. As shown by Fig. 6, PCE agrees with Monte Carlo for short-time behavior; however, as time progresses, disagreement in the variance increases. This result suggests that PCE should be applied to oscillatory systems with caution, which has motivated the development of modified PCE methods that are more accurate for such systems (Le Meitour et al., 2010).

The PCE methods require the determination of the associated coefficients $\xi_{ij}(t)$. In addition to the least-squares fit, there are several methods available for the determination of the coefficients of a PCE. For details of methods of coefficient determination, the readers are referred to the research monographs (Le Maitre et al., 2010; Xiu, 2010).

In addition to quantifying uncertainty propagation in dynamical systems, PCE methods are used to facilitate solving Bayesian inference problems (Ma and Zabaras, 2009; Marzouk et al., 2007). Direct Monte Carlo simulations are replaced by PCEs to efficiently approximate the likelihood functions associated with the Bayesian rule of computing posterior distributions. Computational efficiency and approximation accuracy of using the PCE methods for solv-

¹²PCE methods can be also used for uncertainty propagation and quantification of uncertain initial conditions and/or inputs in straightforward ways, see (Kim, 2013; Kim and Braatz, 2012a,b) for technical details.

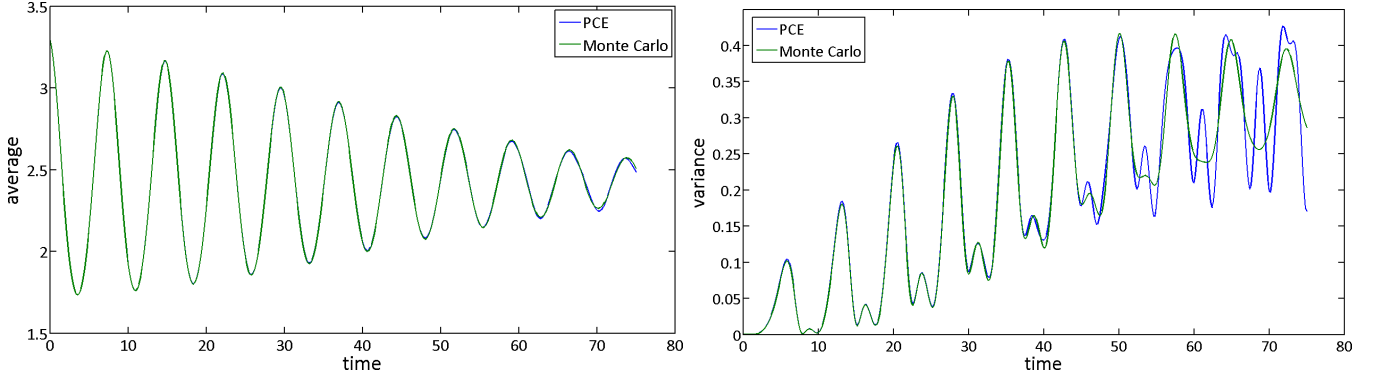


Fig. 6. Average and variance of the state x_1 computed from an 8th-order PCE and from Monte Carlo simulation for 10^4 samples (computation time: 0.644 seconds for PCE and 342.7 seconds for Monte Carlo simulation).

ing Bayesian inference problems have been demonstrated with genetic circuit models (Marzouk and Xiu, 2009) and a diffusion process (Marzouk et al., 2007).

Steady-State Probability Distribution. In the presence of randomness of kinetic parameters, the steady-state of a biochemical reaction network has a probability distribution in which the probability density function of the state or the output converges as time goes to infinity. For analysis of the steady-state of the output y_{ss} , a goal is to compute or estimate such a converging probability distribution. One approach to estimate the steady-state distribution is to use a sampling-based method such as Monte Carlo simulations that can be computationally inefficient, especially for analysis of a large-scale biochemical reaction network. A spectral method based on PCE can be used as a computationally efficient alternative to analyze the probability distribution of the steady-state.

To illustrate the use of PCE methods for analysis of a steady-state, consider the mathematical model for the dual-positive feedback loops motif (Kim et al., 2012; Bornholdt, 2005; Brandman et al., 2005),

$$\begin{aligned} \frac{dA}{dt} &= \tau_a \left(k_{\min} - A + \frac{O^n}{O^n + ec_{50}}(1 - A)S \right), \\ \frac{dB}{dt} &= \tau_b \left(k_{\min} - B + \frac{O^n}{O^n + ec_{50}}(1 - B)S \right), \\ \frac{dO}{dt} &= k_{\text{on}}^{\text{out}}(A + B)(1 - O) - k_{\text{off}}^{\text{out}}O + k_{\min}^{\text{out}}, \end{aligned} \quad (25)$$

which has been normalized and nondimensionalized. The parameter vector is $p = [ec_{50}, k_{\min}, k_{\min}^{\text{out}}, k_{\text{on}}^{\text{out}}, k_{\text{off}}^{\text{out}}, \tau_a, \tau_b]^T$, and the state vector is $x = [A, B, O]^T$. The state variables O , A , and B refer to concentrations of the corresponding genes. O is activated by A and B , and a nonlinear Hill function $h(O) := \frac{O^n}{O^n + ec_{50}}$ characterizes the relationship between the concentration of O and the rate of production of A and B . The Hill coefficient is n and the concentration for half-maximum response for the feedback is ec_{50} . The mutual activations O shared with A and B form the dual positive feedback loops. A and B are also activated by an external stimulus S .

Fig. 7 shows estimated probability distributions of the steady-state output that are computed from a Monte Carlo simulation and the second degree

Hermite polynomial chaos expansion. The parameter vector $p = [ec_{50}, k_{\min}, k_{\min}^{\text{out}}, k_{\text{on}}^{\text{out}}, k_{\text{off}}^{\text{out}}, \tau_a, \tau_b]^T$ is assumed to have a Gaussian distribution with mean $[0.35, 0.01, 0.001, 2, 0.3, 0.5, 0.008]$ and covariance $\text{diag}\{0.03, 0.1, 0.06, 0.15, 0.13, 0.2, 0.01\}$, and the Hill coefficient is $n = 3$. The resultant histograms in Fig. 7 are obtained with the same set of samples of the random vector p for both Monte Carlo simulation and the PCE method.

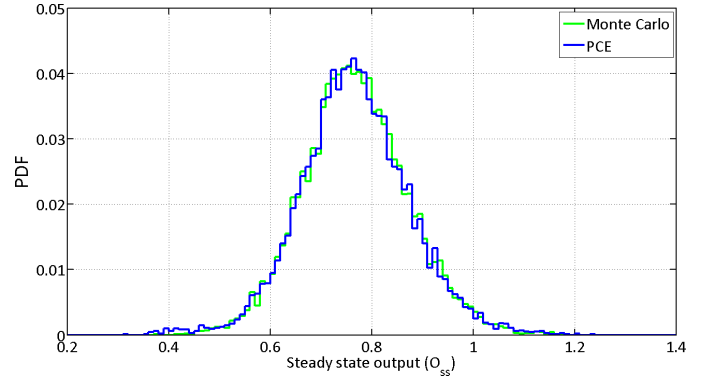


Fig. 7. A comparison of the steady-state probability density functions y_{ss} obtained from Monte Carlo simulation (with 10^4 samples) and a Hermite PCE (with degree 2), where the applied step stimulus is unity, i.e., $S = 1$.

Stochastic Stability. Sec. 6.1 discusses methods to rigorously analyze stochastic stability without approximation by employing Lyapunov theory proven to be rigorous using the Fokker-Planck equation for the probability distribution of the states. Alternative approaches for analysis of stochastic stability have been proposed based on the PCE approximation of the probability distribution. For example, (Fisher and Bhattacharya, 2008; Fisher, 2008) presented stability tests for linear and polynomial stochastic systems that contain random variables. The tests were based on PCEs for which the intrusive Galerkin projection method was used to obtain deterministic dynamical system equations for the associated coefficients, and the convergence of the coefficients was used to define notions of stochastic stability, for which Lyapunov methods were applied. As another example, Hover (2006) applied PCEs to estimate short-term statistics and stability of a solution trajectory in the presence of random parameters and initial conditions with known probability distributions.

However, it was unfortunate that the stability analysis using PCE methods presented in (Fisher and Bhattacharya, 2008; Fisher, 2008) is very limited. Most importantly, the notion of stability was very restrictive, requiring the probability distribution of a state solution to converge to a Dirac delta function that peaks at the origin in some sense of probabilistic convergence. However, the main purpose of stability analysis of a stochastic dynamical system is to determine *whether there exists* a stationary probability distribution to which distribution of a state solution converges (El Samad and Khammash, 2004; Lasota and Mackey, 1994). This issue can be corrected by employing the following steps:

- (1) compute/check the existence of equilibrium points of an extended state space model corresponding to an ODE for the coefficients of a PCE,
- (2) rewrite the ODE in terms of the deviations from the equilibrium points, and
- (3) test stability of the equilibrium points by using Lyapunov analysis.

Note that checking the existence of an equilibrium point in Step 1 should be considered as *approximate*¹³ existence of a stationary probability distribution in the sense of convergence in distribution, since it implies that there exists a random vector with well-defined probability distribution such that its moments have a convergent stationary point for the associated stochastic differential equation.

7. DISCUSSION AND CONCLUSIONS

This work presented an overview of various approaches to model and analyze robustness in biochemical reaction networks. Usually, *robustness analysis* aims to quantify the perturbations for which a network loses or gains some qualitative behavior.

When constructing or working with mathematical models of biochemical networks, there are not only a large amount of uncertainties, but also significantly different classes of uncertainties such as set-based, qualitative, or probabilistic uncertainties. These uncertainties typically result from limited or expensive measurement techniques, precision, and sampling frequency. Moreover, the descriptions of the network functions or characteristics analyzed for robustness are also inherently uncertain. Robustness analysis is hence inevitably linked to uncertainty analysis.

Due to the different types of encountered uncertainties, the analysis method must be chosen carefully. Moreover, it is important to examine the influence of the uncertainties on the model response and consequently to assess the validity of the analysis conclusions. We feel that the different approaches have not been reviewed systematically so far, especially within the context of biochemical reaction networks. Therefore, this paper, first, defined the different uncertainty classes and, secondly, formally stated the different robustness analysis questions of interest. Finally, recent developments for the uncertainty and robustness analysis were presented.

Besides a quantification of robustness, we expect that in the future quantitative predictions of responses to pertur-

¹³Notice that PCE methods provide an approximation, not an exact solution for the associated stochastic differential equation.

bations and uncertainty propagation will become increasingly important, for instance, in therapy design and synthetic biology. This paper presented methods that are— from our perspective—potentially suited for robustness analysis, prediction, and estimation. Due to lack of space and for consistency, the focus was on a particular class of methods and models that we feel can be used to deal with the mentioned challenges. The presentation was weighted towards set-based deterministic methods and stochastic methods based on the Fokker-Planck equation or polynomial chaos theory, with minimal discussion of Monte Carlo sampling methods or local analyses.

This perspectives paper also touched on advances being made in robust estimation and prediction and the design of biochemical reaction networks, also known as *synthetic biology*. Substantial research is still needed in the development of practically implementable algorithms, especially for larger networks. A natural approach to the development of scalable methods is to exploit the interconnection (or reaction) structure and to decompose the whole system into many several subsystems and analyze these in isolation, see e.g. (Del Vecchio et al., 2008).

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