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A robustness measure for the stationary behavior of qualitative gene regulation networks

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Abstract: In this paper the stationary behavior of uncertain and possibly multistable gene regulation networks is considered. We first introduce a modeling framework which is able to represent the qualitative knowledge which is typically available for these systems. Then we turn to the problem of model discrimination: Given several alternative model structures that can all reproduce the experimental observations, is it possible to decide which structure may be the most appropriate description of the real system. To this end, a robustness measure for qualitative multistable gene regulation networks is introduced and also a method for the computation of this measure is presented. The benefit of the developed method is twofold: On the one hand it allows to compare the robustness properties of different model structures, on the other hand also the most fragile interconnections of a network can be detected. Finally, an example network is analyzed with this method.

Keywords: gene regulation networks, robustness, convex optimization

1. INTRODUCTION

Developing models of gene regulation networks is in general a difficult task as the knowledge about these systems is usually very vague. Especially exact concentrations and reaction kinetics can mostly not be determined. However, from numerous experiments and extensive literature research it is often possible to develop hypotheses about the interaction structure of a regulation network. These hypotheses can then be implemented and tested in a qualitative model, as for example a Boolean description. However, it will most likely be possible to construct several alternative models which are all able to reproduce the main characteristics of the biological system, such as for example the steady state behavior, but differ in the interaction structure. In (Wittmann et al., 2009) for example several alternative model structures could be developed that were all able to reproduce a certain gene expression pattern around the midbrain-/hindbrain boundary.

In this work we focus on the steady state behavior of gene regulation networks and explicitly consider networks which are able to show multistability as this is a common phenomenon in biology. Assuming that we have given several alternative model structures which can all in principle reproduce the required steady states, we address the question of model discrimination: How to decide which of the given model structures may be a more appropriate description of the real system.

To approach this problem we adopt the definition from Kitano (2004) that robustness is a system's ability to maintain its function even in the presence of perturbations, and we make use of the common assumption that biological systems have evolved such that they became very robust against common perturbations. In this context, a network which can tolerate the largest perturbations until it loses its function will be regarded as the most robust and thus biologically most plausible network. For the purpose of this paper we consider multistability, i.e., the network's ability to reproduce several steady states at specified locations, as the function to be generated in a maximally robust way.

Many authors have studied robustness properties of regulation networks, modeled in different frameworks. Considering Boolean models, Chaves et al. (2005) have analyzed a Boolean model for the segment polarity genes with respect to its ability to generate a certain expression pattern under synchronous and asynchronous updates. For regulation networks described by ordinary differential equations, Eißing et al. (2005) have analyzed the robustness of an apoptosis model with respect to intrinsic and parametric perturbations. For the same class of models, Jacobsen and Cedersund (2008) have analyzed dynamic perturbations of the interactions using methods from robust control theory. Kinetic Perturbations have been introduced in (Waldherr et al., 2009). There also have been approaches focusing on the relation between network topology and robustness. Prill et al. (2005) have for example analyzed small network motifs and their stability properties and correlated them with their relative abundance in large regulation networks. Also Klemm and Bornholdt (2005) discuss the influence of small motifs on the performance of the whole network. Similar to a Boolean framework, our approach will only use structural information about the system and make no specific assumptions about the exact reaction kinetics. However, we build upon a framework based on ordinary differential equations which allows a more elaborate treatment of robustness aspects.

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The paper is structured as follows: In Section 2 we first introduce a nonparametric modeling framework which is appropriate to incorporate the incomplete knowledge which is typically available for gene regulation networks. It is also explained how steady state measurements can be represented in this framework and several important definitions are given there. In Section 3, conditions for forward-invariance are stated which are then used to develop the robustness measure. In Section 4, a method for the efficient computation of this robustness measure is presented. Finally, the application of the method is illustrated with an example in Section 5.

2. PRELIMINARIES

2.1 Qualitative modeling framework

In gene regulation networks, genes can code for proteins which may in turn influence the production of other proteins. These proteins are called transcription factors and can either activate or inhibit the production of other proteins by binding to specific binding sites of the DNA strand. As described in (Chaves et al., 2008; Breindl and Allgöwer, 2009), these systems can be modeled by differential equations of the form

$$\dot{x}_i = -k_i \cdot x_i + f_i(x), \quad i = 1, \dots, n, \tag{1}$$

with $x = [x_1, \dots, x_n]^T \in \mathbb{R}^n$. A variable x_i represents the concentration of the i-th protein in the network. For simplicity it is assumed that the degradation rates $k_i > 0$ are constant. The production rate of a protein x_i can be influenced by other proteins in the system. Therefore, the functions f_i are combinations of activation and inhibition functions which are defined next (Chaves et al., 2008).

Definition 1. Let $N \in \mathbb{R}_+$. An *activation (inhibition) function* is a function $\nu : [0, \infty) \to [0, N)$ ($\mu : [0, \infty) \to (0, N]$) with:

i) ν (μ) is continuously differentiable,
ii) $\nu(0) = 0$ and $\nu(x) \to N$ as $x \to \infty$ ($\mu(0) = N$ and $\mu(x) \to 0$ as $x \to \infty$),
iii) $\nu(x)$ ($\mu(x)$) is monotonously increasing (decreasing).

We denote the set of all activation functions $\mathcal{N}$ and the set of all inhibition functions $\mathcal{M}$. We furthermore use the symbol φ to denote either an activation function $\nu \in \mathcal{N}$ or an inhibition function $\mu \in \mathcal{M}$. $\mathcal{S}_\varphi$ then either denotes $\mathcal{N}$ if φ is an activation function, or $\mathcal{M}$ if φ is an inhibition function.

In order to achieve a compact notation for the production terms $f_i(x)$ we use the symbol "$\circ$" for sums "$+$" as well as for multiplications "$\cdot$". Then, the general form of a production term $f_i(x)$ can be written as $f_i(x) = \varphi_{i,1}(x_{j_1}) \circ \ldots \circ \varphi_{i,q_i}(x_{jq_i})$, with indices $j_k \in \{1, \dots, n\}$, $k \in \{1, \dots, q_i\}$ and $q_i \in \{1, \dots n\}$. This means that for a function $\varphi_{i,k}(x_{j_k})$, the index i denotes the protein which is regulated, i.e., x_i, the index k enumerates the transcription factors of x_i, and the index j_k specifies the transcription factor. Furthermore, the index q_i denotes the number of transcription factors of x_i. As it is assumed that each protein can only either activate, inhibit, or have no influence on the production of another protein, a state variable x_j can be the argument of at most one function $\varphi_{i,k}$, $k \in \{1, \dots, q_i\}$, for each $i = \{1, \dots, n\}$. Without knowledge of the reaction kinetics, the values k_i and the exact shapes of the monotonous functions cannot be specified which leads to an uncertain model.

2.2 Measurements

Considering the location of steady states in the state space two facts are important. Firstly, the typical variability between individual cells makes it impossible to specify exact values for the steady state protein concentrations, but rather intervals for these concentrations should be considered. Secondly, also measurements usually show large uncertainties. For these reasons we assume that a stable steady state can be represented by a hyperrectangular forward-invariant set $\mathcal{F} = \mathcal{I}_{x_1} \times \ldots \times \mathcal{I}_{x_n}$ in the state space, with intervals $\mathcal{I}_{x_i} = [\underline{x}_i, \overline{x}_i]$.

Definition 2. A set $\mathcal{P} \subseteq \mathbb{R}^n$ is forward-invariant for the system (1) if, for each initial condition $x(0) = x_0 \in \mathcal{P}$, the corresponding solution $x(t; x_0)$ remains in $\mathcal{P}$ for all positive times, i.e., $\forall t > 0 : x(t; x_0) \in \mathcal{P}$.

Additionally, we assume that the maximal concentrations $x_i^{\max}$ of each protein are biologically well characterized and we suppose that only high and low protein concentrations are distinguished such that each interval $\mathcal{I}_{x_i}$ has either the form $\mathcal{I}_{x_i} = [0, x_i^{\text{low}}]$ or $\mathcal{I}_{x_i} = [x_i^{\text{high}}, x_i^{\max}]$, with $0 < x_i^{\text{low}} < x_i^{\text{high}} < x_i^{\max}$.

2.3 Definitions

For deriving conditions on the monotonous functions $\varphi_{i,k}$ such that a set $\mathcal{F}$ is forward-invariant, we will use the values and variables specified in Figure 1. Also several additional definitions are needed. We begin with the definition of a tube for a monotonous function.

Definition 3. The 3-tuple of pairs of positive real numbers $T_\mathcal{N} = \big((x^{\text{low}}, \gamma^{\text{low}}), (x^{\text{high}}, \gamma^{\text{high}}), (x^{\max}, \gamma^{\max})\big)$ such that $\gamma^{\text{low}} \leq \gamma^{\text{high}} \leq \gamma^{\max}$ and $x^{\text{low}} \leq x^{\text{high}} \leq x^{\max}$ is called tube for activation functions.

Equivalently, the 3-tuple of pairs of positive real numbers $T_\mathcal{M} = \big((x^{\text{low}}, \gamma^{\text{high}}), (x^{\text{high}}, \gamma^{\text{low}}), (x^{\max}, \gamma^{\min})\big)$ such that $\gamma^{\min} \leq \gamma^{\text{low}} \leq \gamma^{\text{high}}$ and $x^{\text{low}} \leq x^{\text{high}} \leq x^{\max}$ is called tube for inhibition functions.

Definition 4. An activation function $\nu \in \mathcal{N}$ ($\mu \in \mathcal{M}$) is said to satisfy a tube $T_\mathcal{N}$ ($T_\mathcal{M}$), denoted as $\nu \vDash T_\mathcal{N}$ ($\mu \vdash T_\mathcal{M}$), if the following inequalities hold.

$$\forall x \leq x^{\text{low}} : \ \nu(x) \leq \gamma^{\text{low}} \ (\mu(x) \geq \gamma^{\text{high}}) \tag{2}$$

$$\forall x \geq x^{\text{high}} : \ \nu(x) \geq \gamma^{\text{high}} \ (\mu(x) \leq \gamma^{\text{low}}) \tag{3}$$

$$\forall x \leq x^{\max} : \ \nu(x) \leq \gamma^{\max} \ (\mu(x) \geq \gamma^{\min}) \tag{4}$$

If these inequalities are not satisfied we write $\nu \nvDash T_\mathcal{N}$, or $\mu \nvdash T_\mathcal{M}$, respectively. Furthermore, we use T as abbreviation for both, $T_\mathcal{N}$ and $T_\mathcal{M}$. A tube assigned to a monotonous function $\varphi_{i,k}$ will be indexed $T^{i,k}$.

Next, a measure for the perturbation of a monotonous function is defined.

Definition 5. Given a monotonous function φ and a perturbed function $\varphi^p \in \mathcal{S}_\varphi$. Then,

$$\mathcal{P}(\varphi, \varphi^p) = \int_0^\infty |\varphi(x) - \varphi^p(x)| \, \mathrm{d}x \tag{5}$$

is a measure for the perturbation of φ.

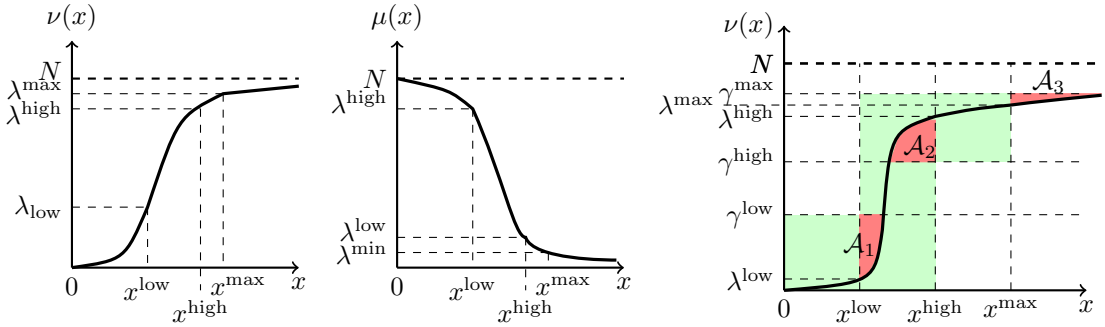


Fig. 1. Left two figures: Activation and inhibition functions. Right figure: Illustration of a tube and the areas $\mathcal{A}_1, \mathcal{A}_2$ and $\mathcal{A}_3$ for an activation function.

With this the purpose of this paper can now be stated more precisely. Suppose a set of m forward-invariant sets $\mathcal{F}_z$, $z \in \{1, \dots, m\}$, is given and every interval $\mathcal{I}_{x_{i,z}}$ is either a low or a high interval, i.e., $\mathcal{I}_{x_{i,z}} = [0, x_{i,z}^{\text{low}}]$, or $\mathcal{I}_{x_{i,z}} = [x_{i,z}^{\text{high}}, x_{i,z}^{\max}]$. Furthermore, an interaction structure of the regulation network is given in the form of Equation (1), but with unspecified monotonous functions. The goal is now to evaluate the capability of the given model structure to generate the observed steady states in the sense of Kitano's robustness definition. The function which shall be maintained and also the perturbations have already been defined in this section such that the next step is the development of a measure which quantifies this capability. This measure will be referred to as robustness measure and is developed in the following section. A further goal is then the development of a method that allows to compute this robustness measure and thus to also compare different network structures.

3. CONDITIONS FOR FORWARD-INVARIANCE

We first state sufficient and necessary conditions for the forward-invariance of a set $\mathcal{F}$ (Blanchini, 1999).

Theorem 1. [Nagumo] Consider the system $\dot{x} = g(x)$, $x \in \mathbb{R}^n$. Let $\mathcal{F} \subseteq \mathbb{R}^n$ be a closed and convex set. Then $\mathcal{F}$ is forward-invariant if and only if

$$\forall x \in \mathcal{F}: \quad g(x) \in \mathcal{K}_{\mathcal{F}}(x), \tag{6}$$

where $\mathcal{K}_{\mathcal{F}}(x)$ is the tangent cone to $\mathcal{F}$ in x.

In words, this means that at each point x at the boundary of $\mathcal{F}$ the vector field has to be directed inwards or tangent to $\mathcal{F}$. For further explanations, see for example (Blanchini, 1999).

For the class of systems considered here and for a hyperrectangular set $\mathcal{F}$, the following proposition is equivalent to Theorem 1.

Proposition 2. A hyperrectangular set $\mathcal{F} = \mathcal{I}_{x_1} \times \ldots \times \mathcal{I}_{x_n}$, $\mathcal{I}_{x_j} = [\underline{x}_j, \overline{x}_j]$, is forward-invariant for system (1) if and only if

$$\forall i \in \{1, \dots n\}: \; -k_i \cdot \underline{x}_i + \underline{\lambda}_{i,1} \circ \ldots \circ \underline{\lambda}_{i,q_i} \geq 0 \tag{7}$$

where $\underline{\lambda}_{i,k} = \min_{x_j \in \mathcal{I}_{x_j}} \varphi_{i,k}(x_j)$, and

$$\forall i \in \{1, \dots n\}: \; -k_i \cdot \overline{x}_i + \overline{\lambda}_{i,1} \circ \ldots \circ \overline{\lambda}_{i,q_i} \leq 0 \tag{8}$$

where $\overline{\lambda}_{i,k} = \max_{x_j \in \mathcal{I}_{x_j}} \varphi_{i,k}(x_j)$.

The proof directly follows from the fact that all activation and inhibition functions in the network are monotonous. Therefore we can conclude that, if all monotonous functions $\varphi_{i,k}$ of system (1) satisfy Equations (7) and (8) for every set $\mathcal{F}_z$, $z \in \{1, \dots, m\}$, these sets are indeed forward-invariant for the system. As in our case we have no nominal system, Proposition 2 cannot be applied directly. However, if only high and low intervals are considered it can be reformulated in terms of tubes.

Proposition 3. Given a set $\mathcal{F} = \mathcal{I}_{x_1} \times \ldots \times \mathcal{I}_{x_n}$ such that $\mathcal{I}_{x_i} = [0, x_i^{\text{low}}]$ or $\mathcal{I}_{x_i} = [x_i^{\text{high}}, x_i^{\max}]$. Furthermore, given tubes $T^{i,k}$ which satisfy the conditions

$$\forall i \in \{1, \dots n\}: \; -k_i \cdot \underline{x}_i + \underline{\gamma}_{i,1} \circ \ldots \circ \underline{\gamma}_{i,q_i} \geq 0 \tag{9}$$

with either $\underline{x}_i = 0$ or $\underline{x}_i = x_i^{\text{high}}$, and

$$\underline{\gamma}_{i,k} = \begin{cases} 0 & \text{if } 0 \in \mathcal{I}_{x_{i,k}} \wedge \varphi_{i,k} \in \mathcal{N} \\ \min\{\gamma : (x, \gamma) \in T^{i,k} \wedge x \in \mathcal{I}_{x_{i,k}}\} & \text{otherwise} \end{cases}$$

and

$$\forall i \in \{1, \dots n\}: \; -k_i \cdot \overline{x}_i + \overline{\gamma}_{i,1} \circ \ldots \circ \overline{\gamma}_{i,q_i} \leq 0 \tag{10}$$

where either $\overline{x}_i = x_i^{\text{low}}$ or $\overline{x}_i = x_i^{\max}$, and $\overline{\gamma}_{i,k} = \max\{\gamma : (x, \gamma) \in T^{i,k} \wedge x \in \mathcal{I}_{x_{i,k}}\}$.

If $\forall i, k: \; \varphi_{i,k} \vDash T^{i,k}$, then the set $\mathcal{F}$ is forward-invariant for the system (1).

Proof. Note that $\varphi_{i,k} \vDash T^{i,k}$ means that each $\underline{\gamma}_{i,k}$ is a lower bound on $\underline{\lambda}_{i,k}$ and each $\overline{\gamma}_{i,k}$ is an upper bound on $\overline{\lambda}_{i,k}$. Therefore, if Equations (9) and (10) hold for a tube $T^{i,k}$ and a set $\mathcal{F}$, then Equations (7) and (8) hold for $\varphi_{i,k} \vDash T^{i,k}$ and the set $\mathcal{F}_z$. □

However, the other direction is not necessarily true. Therefore Equations (9) and (10) are conservative.

Two more definitions are needed to state the robustness measure.

Definition 6. Given a tube T and a monotonous function $\varphi \vDash T$. Then,

$$\mathcal{R}^{\min}(\varphi, T) = \min_{\varphi^p \in S_\varphi \wedge \varphi^p \not\vDash T} \mathcal{P}(\varphi, \varphi^p) \tag{11}$$

is the minimal perturbation of φ with respect to T, i.e., the smallest perturbation of φ according to Equation (5) such that φ^p is no longer contained in the tube T.

Definition 7. The maximal robustness value for a given tube T is defined by

$$\mathcal{R}^{\max}(T) = \max_{\varphi \vDash T} \mathcal{R}^{\min}(\varphi, T) \tag{12}$$

The solution of Equation (12) can be computed analytically as will be shown in Section 4. With this, we are now ready to give the definition of the robustness measure.

Definition 8. Given a system (1) with unspecified activation and inhibition functions. The robustness measure $\mathcal{G}$ for the system is given by

$$\begin{aligned}\mathcal{G} = &\max_{T^{i,k}} \min_{i,k} \mathcal{R}^{\max}(T^{i,k}) \\ \text{s.t.: } &\forall T^{i,k} \text{ and } \forall \mathcal{F}_z : \text{Equations (9) and (10) hold.}\end{aligned} \tag{13}$$

The measure $\mathcal{G}$ can be interpreted as a guarantee. It involves the computation of an optimal system, i.e., functions $\tilde{\varphi}_{i,k}$ such that $\mathcal{G}$ is maximized. Then, for this optimal system it can be guaranteed that all sets $\mathcal{F}_z$ are still forward-invariant if no function $\tilde{\varphi}_{i,k}$ is perturbed by more than $\mathcal{G}$, i.e., $\forall i,k : \ \mathcal{P}(\tilde{\varphi}_{i,k}, \tilde{\varphi}^p_{i,k}) \leq \mathcal{G}$. Note also that the optimal system is designed such that the smallest value $\mathcal{R}^{\max}(T^{i,k})$ among all tubes is maximized. This is reasonable as the tube with the smallest value $\mathcal{R}^{\max}(T^{i,k})$ represents the most fragile interconnection of the network and thus determines the robustness of the whole network. Maximizing this value therefore increases the robustness of the complete network.

As Proposition 3 is only a sufficient but not a necessary condition for the forward-invariance of the sets $\mathcal{F}_z$, $\mathcal{G}$ is a lower bound on the maximally achievable robustness value.

4. COMPUTATION OF THE ROBUSTNESS MEASURE

First, the analytical solution for Equation (12) is given.

Proposition 4. Given a tube T. The maximal value $\mathcal{R}^{\max}(T)$ is given by

$$\mathcal{R}(T_{\mathcal{N}}) = \frac{\gamma_{\text{low}} \cdot (\gamma_{\max} - \gamma_{\text{high}})}{\gamma_{\text{low}} + (\gamma_{\max} - \gamma_{\text{high}})} \cdot (x_{\text{high}} - x_{\text{low}}) \tag{14}$$

if T is a tube for activations functions and it is

$$\mathcal{R}(T_{\mathcal{M}}) = \frac{(N - \gamma_{\text{high}}) \cdot (\gamma_{\text{low}} - \gamma_{\min})}{(N - \gamma_{\text{high}}) + (\gamma_{\text{low}} - \gamma_{\min})} \cdot (x_{\text{high}} - x_{\text{low}}) \tag{15}$$

if T is a tube for inhibition functions.

The proof is only given for a tube for activation functions as the proof for a tube for inhibition functions works with equivalent arguments.

Proof. Given a tube $T_{\mathcal{N}}$, an activation function $\varphi \vDash T_{\mathcal{N}}$ and a perturbed activation function $\nu^p \nvDash T_{\mathcal{N}}$. Then ν^p violates at least one of the Inequalities (2) - (4) and we can give the following estimates on $P(\nu, \nu^p)$. For an illustration see Figure 1.

Assume Inequality (2) is violated and let $\hat{x}$ be the smallest value such that $\nu(\hat{x}) = \gamma^{\text{low}}$. Then it holds that $\mathcal{P}(\nu, \nu^p) \geq \int_{x^{\text{low}}}^{\hat{x}} (\gamma^{\text{low}} - \nu(x)) \, \mathrm{d}x = \mathcal{A}_1(\nu)$.

Now assume Inequality (3) is violated. Let $\hat{x}$ be the smallest value such that $\nu(\hat{x}) = \gamma^{\text{high}}$. Then $\mathcal{P}(\nu, \nu^p) \geq \int_{\hat{x}}^{x^{\text{high}}} (\nu(x) - \gamma^{\text{high}}) \, \mathrm{d}x = \mathcal{A}_2(\nu)$.

Finally assume that Inequality (4) is violated. Then, with $\hat{x}$ being the largest value such that $\nu(\hat{x}) = \gamma^{\max}$ it holds that $\mathcal{P}(\nu, \nu^p) \geq \int_{x^{\max}}^{\hat{x}} (\gamma^{\max} - \nu(x)) \, \mathrm{d}x = \mathcal{A}_3(\nu)$.

Thus, for every $\nu^p \in \mathcal{N}$ such that $\nu^p \nvDash T$ it holds that $\mathcal{R}(\nu, \nu^p) \geq \mathcal{A}(\nu) = \min\{\mathcal{A}_1(\nu), \mathcal{A}_2(\nu), \mathcal{A}_3(\nu)\}$ and thus $\mathcal{R}^{\min}(\nu, T) = \mathcal{A}(\nu)$. In order to compute $\mathcal{R}^{\max}(T)$, we first derive the function $\nu^\star$ which maximizes $\mathcal{A}$.

To do so, first note that for every given $\nu \vDash T_{\mathcal{N}}$ it is possible to find another $\bar{\nu} \vDash T_{\mathcal{N}}$ which is identical with ν in $[0, \tilde{x}]$, with $\nu(\tilde{x}) = \gamma^{\max} - \epsilon$, ϵ arbitrarily small, such that $\mathcal{A}_3(\bar{\nu}) = \max\{\mathcal{A}_1(\bar{\nu}), \mathcal{A}_2(\bar{\nu}), \mathcal{A}_3(\bar{\nu})\}$. One possibility to achieve this is keeping $\bar{\nu}$ constant at $\gamma^{\max} - \epsilon$ in the interval $[\tilde{x}, \hat{x}]$ and choose $\hat{x}$ large enough. But this means that for computing the function $\nu^\star$ which maximizes $\mathcal{A}$ it is sufficient to compute the function $\nu^\star$ which maximizes $\min\{\mathcal{A}_1, \mathcal{A}_2\}$, and satisfies the modified tube $\bar{T}_{\mathcal{N}} = \{(x^{\text{low}}, \gamma^{\text{low}}), (x^{\text{high}}, \gamma^{\text{high}}), (x^{\max}, \bar{\gamma}^{\max})\}$, with $\bar{\gamma}^{\max} = \gamma^{\max} - \epsilon$.

Now, to compute this $\nu^\star$, first define a step function

$$h_{x_s}(x) = \begin{cases} 0 & x \leq x_s \\ \bar{\gamma}_{\max} & x > x_s \end{cases}. \tag{16}$$

Next, for the given function $\bar{\nu}$, let $\bar{x}_s$ be the smallest value such that $\bar{\nu}(\bar{x}_s) = \frac{(\gamma_{\text{high}} + \gamma_{\text{low}})}{2}$. Then, $h_{\bar{x}_s} \vDash \bar{T}_{\mathcal{N}}$ and with the above definitions of $\mathcal{A}_1$ and $\mathcal{A}_2$ it follows that $\min\{\mathcal{A}_1(\bar{\nu}), \mathcal{A}_2(\bar{\nu})\} \leq \min\{\mathcal{A}_1(h_{\bar{x}_s}), \mathcal{A}_2(h_{\bar{x}_s})\}$. This means we can always find a step function with a larger value $\mathcal{A}$ than the function $\bar{\nu}$. Next, among all possible step functions $h_{x_s} \vDash \bar{T}_{\mathcal{N}}$, the step function $h_{x_s^\star}$ which maximizes $\min\{\mathcal{A}_1, \mathcal{A}_2\}$ has to satisfy $\mathcal{A}_1 = \mathcal{A}_2$, i.e., $(\bar{\gamma}^{\max} - \gamma^{\text{high}}) \cdot (x^{\text{high}} - x^{s^\star}) = \gamma^{\text{low}} \cdot (x_s^\star - x^{\text{low}})$, from which it follows that $x_s^\star = \frac{\gamma^{\text{low}} \cdot x^{\text{low}} + (\bar{\gamma}^{\max} - \gamma^{\text{high}}) \cdot x^{\text{high}}}{\gamma_{\text{low}} + (\bar{\gamma}^{\max} - \gamma^{\text{high}})}$. With this and as ϵ can be arbitrarily small we get

$$\mathcal{R}^{\max}(T_{\mathcal{N}}) = \frac{\gamma^{\text{low}} \cdot (\gamma^{\max} - \gamma^{\text{high}})}{\gamma^{\text{low}} + (\gamma^{\max} - \gamma^{\text{high}})} \cdot (x^{\text{high}} - x^{\text{low}}). \tag{17}$$

□

Note that step functions are no admissible activation function as they are not continuously differentiable. Therefore, Equation (17) is an upper bound on $\mathcal{R}^{\max}(T)$. But as step functions can be approximated arbitrarily closely in the l_1-Norm by differentiable activation functions it is the least upper bound.

With this result and the new variables

$$\begin{aligned} c^h_{i,k} &= \begin{cases} \gamma^{\max}_{i,k} - \gamma^{\text{high}}_{i,k} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ N_{i,k} - \gamma^{\text{high}}_{i,k} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \\ c^l_{i,k} &= \begin{cases} \gamma^{\text{low}}_{i,k} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ \gamma^{\text{low}}_{i,k} - \gamma^{\min}_{i,k} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \end{aligned} \tag{18}$$

Equation (13) can now be rewritten as

$$\begin{aligned}\mathcal{G} = &\max_{T^{i,k}} \min_{i,k} \left\{ \frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} (x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k}) \right\} \\ \text{s.t.: } &\forall T^{i,k} \text{ and } \forall \mathcal{F}_z : \text{Equations (9) and (10) hold.}\end{aligned} \tag{19}$$

Again an equivalent formulation is the following one. For similar examples see for example (Boyd and Vandenberghe, 2004).

$$\begin{aligned}\mathcal{G} = &\min \frac{1}{t} \\ \text{s.t.: } &\forall i,k : \ t \leq \left\{ \frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} (x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k}) \right\} \\ &\forall T^{i,k} \text{ and } \forall \mathcal{F}_z : \text{Equations (9) and (10) hold}\end{aligned} \tag{20}$$

While Equation (20) gives a lower bound on the perturbation which will not lead to a loss of the desired forward-

invariant sets, further constraints need to be considered for the overall optimization problem. Firstly, as we assumed maximum concentrations $x_i^{\max}$ for each protein, we can consider $\mathcal{X} = [0, x_i^{\max}] \times \ldots \times [0, x_n^{\max}]$ as another forward-invariant set. Using again Proposition 2 gives the following conditions

$$-k_i \cdot x_i^{\max} + \hat{\gamma}_{i,1} \circ \ldots \circ \hat{\gamma}_{i,q_i} \leq 0 \tag{21}$$

where $\hat{\gamma}_{i,k} = N$ if $\varphi_{i,k}$ is an inhibition function and $\hat{\gamma}_{i,k} = \gamma_{i,k}^{\max}$ if it is an activation function.

Also the monotonicity constraints from the definition of the tube have to be included. For every tube $T_{\mathcal{N}}^{i,k}$ the constraint

$$0 \leq \gamma_{i,k}^{\text{low}} \leq \gamma_{i,k}^{\text{high}} \leq \gamma_{i,k}^{\max} \tag{22}$$

and for every tube $T_{\mathcal{M}}^{i,k}$ the constraint

$$0 \leq \gamma_{i,k}^{\min} \leq \gamma_{i,k}^{\text{low}} \leq \gamma_{i,k}^{\text{high}} \leq N \tag{23}$$

has to be included.

Finally, we require all optimization variables to be positive.

4.1 Formulation as convex optimization problem

We aim to formulate the optimization problem (20) and the additional constraints as convex optimization problem and first recall its definition.

Definition 9. A convex optimization problem has the standard form

$$\begin{aligned} \min \quad & f_0(x) \\ \text{s.t.} \quad & f_i(x) \leq 0, \quad i = 1, \ldots, m \\ & h_i = 0, \quad i = 1, \ldots, p \end{aligned} \tag{24}$$

where the objective function $f_0 : \mathbb{R}^n \to \mathbb{R}$ and the inequality constraints $f_i : \mathbb{R}^n \to \mathbb{R}$, $i = 1, \ldots, m$, are convex functions, and the equality constraints $h_i : \mathbb{R}^n \to \mathbb{R}$ are affine in x.

Unfortunately, it is not always possible to transfer the presented problem into an equivalent convex formulation. However, in the cases where each "$\circ$" either only represents a sum or a multiplication, the problem is already convex or can be easily transformed into an equivalent convex form. These two cases are studied next. In the general case it is still possible to compute upper and lower bounds on the optimal value, but this will not be treated here.

Proposition 5. If the system (1) contains only additive combinations of monotonous functions, the optimization problem (20) together with the additional constraints (21) - (23) is convex.

Proof. It has to be checked that all requirements from Definition 9 are fulfilled. Clearly, the objective $\mathcal{G} = f_0 = \frac{1}{t}$ is convex in t. Also the constraints $f_{i,k}^c = t - \left(\frac{c_{i,k}^h \cdot c_{i,k}^l}{c_{i,k}^h + c_{i,k}^l} (x_{i,k}^{\text{high}} - x_{i,k}^{\text{low}}) \right) \leq 0$ are convex. To see this, the second-order condition (Boyd and Vandenberghe, 2004) can be applied. The eigenvalues of the Hessian of $f_{i,k}^c$ are $\{0, 0, \frac{2((c_{i,k}^h)^2 + (c_{i,k}^l)^2)}{((c_{i,k}^h)^2 + (c_{i,k}^l)^2)^3}\}$. Therefore, the Hessian is positive semi-definite on the domain $(c_{i,k}^h, c_{i,k}^l, t) \in \mathbb{R}_+^3$ and $f_{i,k}^c$ is convex in this domain. Next, all inequality constraints from (9), (10), (21), (22) and (23) are affine in the optimization variables and thus convex. Finally, the equality constraints from (18) are affine in the variables as required. □

Next, the special case is considered where all "$\circ$" signs stand for a multiplication. Then by changing the constraints from Equation (18) into

$$\begin{aligned} c_{i,k}^h &\leq \begin{cases} \gamma_{i,k}^{\max} - \gamma_{ij}^{\text{high}} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ N_{i,k} - \gamma_{i,k}^{\text{high}} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \\ c_{i,k}^l &\leq \begin{cases} \gamma_{i,k}^{\text{low}} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ \gamma_{i,k}^{\text{low}} - \gamma_{i,k}^{\min} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \end{aligned} \tag{25}$$

yields an equivalent convex optimization problem as stated next.

Proposition 6. Assume System (1) has only multiplicative combinations. The relaxed optimization problem with Equations (25) instead of Equations (18) is convex and has the same optimal value $\mathcal{G}$ as the original problem.

To prove this, two definitions from (Boyd and Vandenberghe, 2004) are needed.

Definition 10. A function $f : \mathbb{R}^n \to \mathbb{R}$ with domain $\mathbf{dom}\ f = \mathbb{R}_+^n$ of the form $f(x) = c x_1^{a_1} x_2^{a_2} \ldots x_n^{a_n}$, $c > 0$ and $a_i \in \mathbb{R}$ is a monomial. A finite sum of monomials $F(x) = \sum_{k=1}^K f_k(x)$, $K \in \mathbb{N}_+$, is called posynomial.

Definition 11. An optimization problem

$$\begin{aligned} \min \quad & f_0(x) \\ \text{s.t.} \quad & f_i(x) \leq 1, \quad i = 1, \ldots, m \\ & h_i = 1, \quad i = 1, \ldots, p \end{aligned} \tag{26}$$

with domain $\mathbf{dom} = \mathbb{R}_+^n$, where f_0 and f_i, $i = 0, \ldots, m$, are posynomials, and h_i, $i = 1, \ldots, p$, are monomials is called a geometric program.

A geometric program can be transformed into a convex problem of the form of Equation (24) by the variable transformation $y_i = \log x_i$. With this Proposition 6 can now be proved.

Proof. *Convexity:* We will show that the optimization problem is given as a geometric program. First, the objective $\mathcal{G} = f_0 = \frac{1}{t}$ is a posynomial. Also, the constraints $f_{i,k}^c = t - \frac{c_{i,k}^h \cdot c_{i,k}^l}{c_{i,k}^h + c_{i,k}^l} (x_{i,k}^{\text{high}} - x_{i,k}^{\text{low}}) \leq 0$ can be reformulated as posynomial $f_{i,k}^c = t \cdot a^{-1} \cdot (c_{i,k}^l)^{-1} + t \cdot a^{-1} \cdot (c_{i,k}^h)^{-1} \leq 1$, with constant $a = (x_{i,k}^{\text{high}} - x_{i,k}^{\text{low}})$. In an equivalent way, the inequality constraints from Equations (9), (10), (21), (22) and (23) can be rewritten as posynomials. Also for the modified constraints from Equation (25) this is possible. Therefore the optimization problem is given as a geometric problem.

Equivalence: First note that the feasible set of the original problem is contained in the feasible set of the relaxed problem. Denote $p_{\text{mod}}^\star$ the optimal value of the relaxed problem. Then it holds that $p_{\text{mod}}^\star \geq p^\star$. It now has to be shown that the optimal value for the modified problem is obtained when all relaxed inequalities (25) are satisfied with equality, i.e., $p_{\text{mod}}^\star = p^\star$.

Denote ξ the vector of variables, which takes the value $\xi^\star$ at the optimal point. Recall that the optimal value

Table 1. Forward-invariant sets

Protein	Steady State 1	Steady State 2
x_1	0 - 0.2	0.8 - 1
x_2	0.8 - 1	0 - 0.2
x_3	0.8 - 1	0 - 0.2

$p^\star$ equals the smallest value $\mathcal{R}_{i,k}^{\max}$ of all tubes $T^{i,k}$ in the system. Then, all tubes $T^{i,k}$ in the system can be partitioned into two sets. The first set V contains all tubes for which it holds that $\mathcal{R}_{i,k}^{\max} = p^\star$ and the second set W contains all remaining tubes. For these tubes we have that $\mathcal{R}_{i,k}^{\max} > p_{\text{mod}}^\star$.

Now assume that at $\xi = \xi^\star$ there is a relaxed constraint (25) which does not hold with equality. Then, without influencing any other constraint, equality of this constraint can be achieved by increasing the respective value $c_{i,k}^h$ or $c_{i,k}^l$. Then, as $\mathcal{R}_{i,k}^{\max}$ is monotonically increasing in $c_{i,k}^h$ and $c_{i,k}^l$, this value will also increase. As $p_{\text{mod}}^\star$ is optimal, there has to be at least one tube $T^{i,k}$ in the set V for which it holds that all constraints (25) hold with equality. Then modifying all other constraints which do not hold with equality in the way described above yields an optimal solution $p^\star$ for the original problem with $p^\star = p_{\text{mod}}^\star$. □

5. EXAMPLE

The method and its application shall now be illustrated with an example. Consider the two alternative networks in Table 2 involving 3 species. The second model is a submodel of the first one. We want to compare these two models with respect to their ability to reproduce the desired forward-invariant sets from Table 1. The maximal concentration of each $x_i^{\max}$ as well as all parameters k_i are set to 1.

The question is now, which model structure is better suited to fulfill this task under perturbations of the monotonous functions. Solving the presented optimization problem for each model with Yalmip (Löfberg, 2004) gives the guarantee $\mathcal{G}$ and also a tube for each monotonous function in the system. From these tubes the optimal step functions as shown in the proof of Proposition 4 can then be reconstructed. The results are summarized in Table 3. In this case, for the weaker connected model a larger guarantee can be given with our method. Considering Table 3, in model 1, $\nu_{3,1}(x_2)$ is the most robust interaction while the areas $\mathcal{A}$ are equal for all other functions and can not be increased further, making them the most fragile links. In the second model, all functions have an equal area $\mathcal{A} = \mathcal{G}_2$, which makes them all equally robust or fragile.

It is also interesting to investigate how these results depend on the choice of the steady state concentrations in Table 1. A case study showed that, if all intervals were chosen of equal size, always the weaker connected model has a higher robustness value. However, this situation can change for different interval sizes.

Table 2. Alternative Models

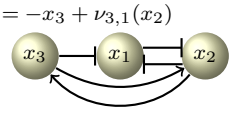
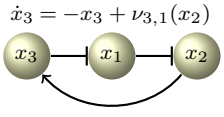
Model 1	Model 2
$\dot{x}_1 = -x_1 + \mu_{1,1}(x_2) \cdot \mu_{1,2}(x_3)$	$\dot{x}_1 = -x_1 + \mu_{1,1}(x_3)$
$\dot{x}_2 = -x_2 + \mu_{2,1}(x_1) \cdot \nu_{2,2}(x_3)$	$\dot{x}_2 = -x_2 + \mu_{2,1}(x_1)$
$\dot{x}_3 = -x_3 + \nu_{3,1}(x_2)$	$\dot{x}_3 = -x_3 + \nu_{3,1}(x_2)$
	

Table 3. Tubes resulting from optimization

Model 1, $\mathcal{G}_1 = 0.05$				Model 2, $\mathcal{G}_2 = 0.06$			
	γ^{low}	γ^{high}	$\gamma^{\max}, N$		γ^{low}	γ^{high}	$\gamma^{\max}, N$
$\mu_{1,1}$	0.447	0.894	1	-	-	-	-
$\mu_{1,2}$	0.447	0.894	1	$\mu_{1,1}$	0.2	0.8	1
$\mu_{2,1}$	0.447	0.894	1	$\mu_{2,1}$	0.2	0.8	1
$\nu_{2,2}$	0.447	0.894	1	-	-	-	-
$\nu_{3,1}$	0.2	0.8	1	$\nu_{3,1}$	0.2	0.8	1

6. CONCLUSION

In this paper we have defined a robustness measure that characterizes the ability of a given network structure to produce forward-invariant sets representing the steady states of the system. Also a method for its computation has been developed and it was demonstrated that the resulting optimization problem is convex in special cases. The purpose of this method is twofold. On the one hand it allows to compare different network structures with respect to their ability to generate a desired multistable behavior. On the other hand the most fragile interactions of a network can be detected.

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