INTERNATIONAL JOURNAL OF ROBUST AND NONLINEAR CONTROL
Int. J. Robust. Nonlinear Control 2011; **21**:1742–1758
Published online 28 July 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/rnc.1786

Steady-state robustness of qualitative gene regulation networks

C. Breindl[1,*,†], S. Waldherr[1], D. M. Wittmann[2,3], F. J. Theis[2,3] and F. Allgöwer[1]

[1]*Institute for Systems Theory and Automatic Control, University Stuttgart, 70550 Stuttgart, Germany*
[2]*Institute for Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Centre for Environmental Health, 85764 Munich-Neuherberg, Germany*
[3]*Zentrum Mathematik, Technische Universität München, 85747 Garching, Germany*

SUMMARY

In this paper, we define a robustness measure for gene regulation networks, which allows to quantify how well a given model structure can reproduce a desired steady-state pattern in the absence of detailed knowledge about the kinetic mechanisms and parameters. To develop this measure, a modeling framework is introduced, which is able to represent the qualitative knowledge typically available for gene regulation networks. With this framework, the robustness measure as well as tools for its efficient computation are developed. The benefit of our method is twofold: On the one hand, it allows to compare the robustness properties of different model structures and thus may help modelers to decide which model is biologically more plausible. On the other hand, the most fragile interconnections within a network can be detected. To demonstrate its use, the new method is applied to various models of a gene regulation network, which is responsible for the maintenance of the mid-hindbrain boundary. We find that for this example system, weaker connected networks are more robust.

Received 1 September 2010; Revised 3 May 2011; Accepted 20 June 2011

KEY WORDS: gene regulation networks; qualitative modeling; multistability; robustness; convex optimization; mid-hindbrain boundary

1. INTRODUCTION

Gene regulation networks are at the heart of almost all cellular processes. The information stored on the DNA segments is transcribed into complementary messenger RNA, which is then translated into proteins. Some of these gene products, called transcription factors, can also act back on the transcription of other genes in an activating or inhibiting way by binding to specific sites on their DNA strands. Closing the loop of transcription and translation in this way enables a cell to respond to changing intracellular and extracellular conditions in an appropriate way. Well-studied systems that involve such a feedback on the transcriptional level are, for example, the *lactose utilization network* of *Escherichia coli* [1], which regulates the expression of the *lac operon* depending on the presence of glucose and lactose, or the *apoptosis signaling network* [2, 3], which functions as a switch between death or survival of a cell depending on the presence of certain proteins. In addition, in fate determination, gene regulation networks play a crucial role. In this context, it is commonly assumed that different cell types correspond to different stable expression patterns of the underlying gene regulation network. Examples for that are models of hematopoietic or mesenchymal stem cells [4,5] and for the formation and maintenance of the mid-hindbrain boundary (MHB) in mammals [6].

*Correspondence to: C. Breindl, Institute for Systems Theory and Automatic Control, University Stuttgart, 70550 Stuttgart, Germany.

†E-mail: breindl@ist.uni-stuttgart.de

Unfortunately, despite their great importance for the correct functioning of cells, the knowledge about these networks is in general very limited. This applies for the kinetic mechanisms and parameters as well as for the interaction structure or even the involved molecular species. Although knockout or over-expression experiments may allow for conclusions, if a certain protein is influencing the production of another protein in a positive or negative way, the exact kinetic parameters can usually not be determined. Furthermore, common measurement techniques such as DNA chips and western blots usually show large uncertainties. For western blots, for example, measurement errors in the range of 15% are not unusual [7]. Moreover, many experiments yield merely relative data as absolute values are hard to obtain.

As the first goal of this paper, we therefore want to present a modeling framework that is able to deal with the measurement uncertainties and the uncertainties about the system itself. This framework has already been used in [8–10] and shall be recalled here.

Considering the asymptotic behavior of gene regulation networks, multistability is a very common phenomenon. All example systems mentioned earlier indeed show this behavior, and in this work, we want to explicitly focus on multistable networks. A natural requirement for a model of a multistable network is that the model shows a multistable behavior too. A further goal of this paper is therefore the development of tools to assure that the model exhibits the desired multistable behavior, that is, the desired number and locations of the steady states.

However, when a model for a specific gene regulation network shall be developed from only qualitative information, several plausible models might be found that can all reproduce the experimental observations. These models may, for example, differ in the ranges of the parameters or even in the roles of certain species (A inhibits B in the first model and activates B in the second model,...). The previous study of a gene regulation network maintaining a certain expression pattern at the MHB [6] is an example for such a situation.

The third and main goal of this paper is therefore the development of a computationally attractive method to assess different model structures with respect to their biological plausibility. As an underlaying idea of our approach, we make use of the concept of robustness, which is defined according to Kitano [11] as a system's ability to maintain its function in the presence of perturbations. Assuming that biological systems have evolved such that they have become more robust against common perturbations, we consider the most robust system, that is, the system that can tolerate the largest perturbations until it loses its function, as biologically most plausible. In the context of this paper, the function that shall be realized by the network is, as mentioned earlier, a specified multistable behavior. We consider this a reasonable choice because of the importance of multistability in regulatory networks as explained earlier. A further motivation for this choice are the studies [12] and [13]. It was shown there that the polarity segmentation regulatory network in Drosophila indeed robustly maintains its desired multistability properties against large ranges of parameter variations. Of course, depending on the concrete networks to be studied, also other network functions can be reasonable. This is however not in the scope of this paper.

Several authors have studied multistable regulation networks before. Angeli and Sontag [14, 15] found sufficient conditions for multistability in the context of monotone systems. Results about necessary conditions on the graph structure of gene regulation networks are, for example, summarized in [16]. Robustness properties of regulation networks, modeled in different frameworks, were also studied before in the literature. Considering Boolean models, the authors of [17] and [18] proposed different methods for robustness analysis. For networks described by ordinary differential equations, the authors of [19] have analyzed the robustness properties of a bistable apoptosis model with respect to intrinsic and parametric perturbations. For the same class of models, dynamic perturbations of the interactions using methods from robust control theory have been analyzed in [20]. Kinetic perturbations have been introduced in [21]. There also have been approaches focusing on the relation between network topology and robustness. The authors of [22] have analyzed small network motifs and their stability properties, and correlated them with their relative abundance in large regulation networks. In [23], the influence of small motifs on the performance of the whole network is discussed. A different approach is taken in [24] and [25] where the attractor landscape of gene regulation networks modeled in a Boolean framework is studied. The authors found that networks, which show good robustness properties but are still able to respond to stimuli, operate close to the

DOI: 10.1002/rnc

so-called 'critical regime'. There are also approaches that focus on the robustness of the network's graph structure against perturbations while not considering specific dynamic properties. In [26] for example, the effects of concentrated versus distributed perturbations of the interconnections on a graph property, which was called 'network efficiency', were studied.

Similar to a Boolean framework, our approach only uses structural information about the system and makes no specific assumptions about the exact reaction kinetics. Only activating and inhibiting influences of transcription factors on their target genes are distinguished. This is in accordance with our main goal to evaluate the robustness of the interaction structure itself and not of a given set of nominal parameters. As mentioned before, we mean with robustness the ability to generate a desired multistable behavior even in the presence of perturbations. As mutations in the promoter regions, environmental influences such as temperature or chemicals, and inherent stochastic fluctuations can have strong effects on gene regulation, we consider variations of the activating and inhibiting regulatory interactions as relevant perturbations.

With these assumptions, we define a robustness measure for the network that takes into account the interaction structure and the desired number and positions of the steady states. Its computation can be formulated as an optimization problem. In some respects, our approach also resembles flux-balance and energy-balance analysis of metabolic networks [27,28]. Also there, the structure, that is, the stoichiometry, is known, and only qualitative constraints on the reaction kinetics are imposed. Another similarity is that in both approaches, an objective function is formulated. In the case of flux-balance/energy-balance analysis, this objective function usually aims to maximize energy production, growth rate, or other biologically important factors, and shall further restrict the space of possible steady-state flux distributions. In our case, it aims to maximize the network's robustness. Both methods can furthermore be used to study the influence of changes in the network structure on the respective objective. However, the two approaches are not directly related because of the inherent differences between signaling and metabolic networks.

The paper is structured as follows: In Section 2, we introduce a nonparametric modeling framework based on ordinary differential equations. It is shown that this framework can deal with the uncertainties described earlier. Our approach of considering the desired steady states as forward-invariant sets in the state space is also explained in this section. Furthermore, several definitions needed later in the paper are given there. In Section 3, we show how the requirement for the invariance of certain regions in the state space can be translated into conditions on the interactions between the individual species. In Section 4, the robustness measure will be defined precisely. We also show that computing this measure can be formulated as a convex optimization problem for special classes of systems. In Section 5, we introduce the gene regulation network that is responsible for the maintenance of the mid-hindbrain boundary, and present several plausible model structures that can reproduce the desired expression pattern around this boundary. These models are analyzed with the new method in order to rate them with respect to their robustness properties. The paper ends with a conclusion in Section 6.

2. MODELING FRAMEWORK AND PRELIMINARIES

2.1. Qualitative modeling framework

As there is usually no detailed information about the reaction kinetics, we aim to base our modeling framework upon as few assumptions as possible. However, we will not use a Boolean framework but prefer a model description based on ordinary differential equations. Among other advantages, this also facilitates the robustness analysis, which is the main goal of this paper.

As discussed in Section 1, transcription factors can activate or inhibit the production of other proteins. The first assumption is that now, this positive or negative influence can be modeled by monotonously increasing or decreasing activation or inhibition functions whose definitions are given next [8,9].

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

DOI: 10.1002/rnc

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

Definition 2 (Inhibition function)
Let $N \in \mathbb{R}_+$. An *inhibition function* is a function $\mu : [0, \infty) \to (0, N]$ with the following:

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

We furthermore denote the set of all activation functions $\mathcal{N}$ and the set of all inhibition functions $\mathcal{M}$. The symbol φ is used to denote either an activation function $\nu \in \mathcal{N}$ or an inhibition function $\mu \in \mathcal{M}$. Finally, $\mathcal{S}_\varphi$ represents the class of a function φ, that is, $\mathcal{S}_\varphi = \mathcal{N}$ if φ is an activation function and $\mathcal{S}_\varphi = \mathcal{M}$ if φ is an inhibition function. Note that commonly used reaction kinetics such as Michaelis–Menten or Hill type kinetics satisfy the aforementioned definitions of activation and inhibition functions and are thus covered by this modeling framework.

With the second modeling assumption that all proteins are degraded at a rate proportional to their concentrations, the model equations take the form

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

with $k_i \in \mathbb{R}_+$, $x = [x_1, \ldots, x_n]^T \in \mathbb{R}^n$, and x_i representing the concentration of the ith protein in the network. As proteins can have several transcription factors, the terms f_i in Equation (1) can take more complex forms according to our third modeling assumption: The effective influence of several transcription factors can be represented by arbitrary sums and products of individual activation and inhibition functions. 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 '$\cdot$'. With these assumptions and conventions, the general form of a production term $f_i(x)$ can be written as

$$f_i(x) = \varphi_{i,1}(x_{j_1}) \circ \cdots \circ \varphi_{i,q_i}(x_{j q_i}) \tag{2}$$

with indices $j_k \in \{1, \ldots, n\}$, $k = 1, \ldots, q_i$ and $q_i \in \mathbb{N}_+$. To illustrate this notation, consider a function $\varphi_{i,k}(x_{j_k})$. In this, the index i denotes the protein, which is regulated, the index k enumerates the transcription factors of the regulated protein, and the index j_k specifies the transcription factor. Finally, the index q_i denotes the number of transcription factors of x_i. Note that self-regulation is explicitly allowed. Furthermore, it is even possible that a transcription factor, say x_j, has an activating as well as an inhibiting influence on x_i. By allowing such constellations also, cases can be modeled where, for example, x_j is an activator of x_i at low concentrations of x_j but turns into an inhibitor of x_i at high concentrations. Using activation and inhibitions functions as defined earlier, this case could be modeled as the product $\mu(x_j) \cdot \nu(x_j)$.

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 where only the interaction structure is specified.

2.2. Measurements

As outlined in Section 1, we focus on steady-state measurements. In this context, we however suggest to treat a steady state of a gene regulation network not as a single point in the state space but to consider a forward-invariant region. The following two arguments shall motivate this approach. Firstly, because of differences between individual cells, the determination of an exact value for the steady state is not possible, but rather, intervals for these concentrations should be considered. Secondly, in addition, the fact that measurements usually show large uncertainties favors the consideration of intervals.

Furthermore, again, because of the large measurement uncertainties, only high and low concentrations shall be distinguished, and we assume that, at least approximately, values x_i^{low}, x_i^{high}, and

DOI: 10.1002/rnc

$x_i^{\max}$ with $0 \leqslant x_i^{\text{low}} \leqslant x_i^{\text{high}} \leqslant x_i^{\max}$ are known such that a low or high concentration of x_i lies in the interval $\mathcal{I}_{x_i} = [0, x_i^{\text{low}}]$ or $\mathcal{I}_{x_i} = [x_i^{\text{high}}, x_i^{\max}]$, respectively.

With this, a steady state is represented as a hyper-rectangular set $\mathcal{F} = \mathcal{I}_{x_1} \times \cdots \times \mathcal{I}_{x_n}$, where each interval $\mathcal{I}_{x_i}$ is either a low interval, that is, $\mathcal{I}_{x_i} = [0, x_i^{\text{low}}]$, or a high interval, that is, $\mathcal{I}_{x_i} = [x_i^{\text{high}}, x_i^{\max}]$. Furthermore, we require this set $\mathcal{F}$ to be forward invariant, that is, $\forall x_0 \in \mathcal{F}, \forall t > 0 : x(t; x_0) \in \mathcal{F}$, with $x(t, x_0)$ denoting the solution of system (1) for initial condition x_0.

This concept shall be illustrated with an example. Consider the mutual inhibition network

$$\begin{aligned} \dot{x}_1 &= -k_1 x_1 + \mu_{1,1}(x_2) \\ \dot{x}_2 &= -k_2 x_2 + \mu_{2,1}(x_1). \end{aligned} \tag{3}$$

The phase diagram of this system is shown in Figure 1 for a specific choice of parameters k_1 and k_2 and inhibition functions $\mu_{1,1}$ and $\mu_{2,1}$. This specific system has two stable steady states, which are depicted as circles. For our approach, we are not interested in their precise positions but only in the forward-invariant sets $\mathcal{F}_1$ and $\mathcal{F}_2$ that contain the corresponding steady states.

2.3. *Definitions*

In Section 3, we will derive conditions on the activation and inhibition functions such that a number of sets $\mathcal{F}_z$, $z = 1, \ldots, m$, is forward invariant. To arrive at these conditions, some definitions are needed. First, the concept of tubes for activation and inhibition functions is introduced.

Definition 3
The 3-tuple of pairs of positive real numbers $T_{\mathcal{N}} = \left((x^{\text{low}}, \gamma^{\text{low}}), (x^{\text{high}}, \gamma^{\text{high}}), (x^{\max}, \gamma^{\max})\right)$ such that $\gamma^{\text{low}} \leqslant \gamma^{\text{high}} \leqslant \gamma^{\max}$ and $x^{\text{low}} \leqslant x^{\text{high}} \leqslant x^{\max}$ is called tube for activation functions. Furthermore, an activation function $\nu \in \mathcal{N}$ is said to satisfy a tube $T_{\mathcal{N}}$, denoted as $\nu \vDash T_{\mathcal{N}}$, if the following inequalities hold.

$$\forall x \leqslant x^{\text{low}} : \ \nu(x) \leqslant \gamma^{\text{low}} \tag{4}$$

$$\forall x \geqslant x^{\text{high}} : \ \nu(x) \geqslant \gamma^{\text{high}} \tag{5}$$

$$\forall x \leqslant x^{\max} : \ \nu(x) \leqslant \gamma^{\max} \tag{6}$$

An equivalent definition can be given for inhibition functions.

Definition 4
The 3-tuple of pairs of positive real numbers $T_{\mathcal{M}} = \left((x^{\text{low}}, \gamma^{\text{high}}), (x^{\text{high}}, \gamma^{\text{low}}), (x^{\max}, \gamma^{\min})\right)$, such that $\gamma^{\min} \leqslant \gamma^{\text{low}} \leqslant \gamma^{\text{high}}$ and $x^{\text{low}} \leqslant x^{\text{high}} \leqslant x^{\max}$, is called tube for inhibition functions.

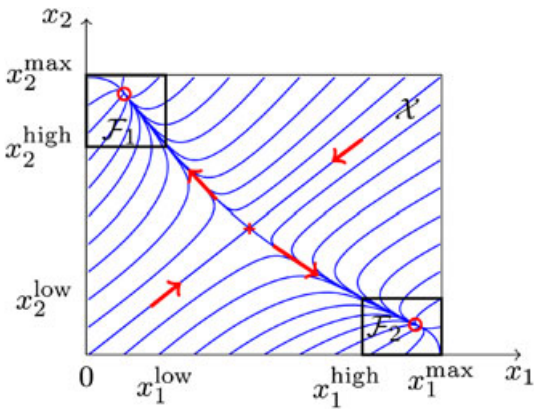

Figure 1. Phase portrait and forward-invariant sets $\mathcal{F}_1 = [0, x_1^{\text{low}}] \times [x_2^{\text{high}}, x_2^{\max}]$ and $\mathcal{F}_2 = [x_1^{\text{high}}, x_1^{\max}] \times [0, x_2^{\text{low}}]$ for the mutual inhibition network.

DOI: 10.1002/rnc

Furthermore, an inhibition function $\mu \in \mathcal{M}$ is said to satisfy a tube $T_{\mathcal{M}}$, denoted as $\mu \vDash T_{\mathcal{M}}$, if the following inequalities hold.

$$\forall x \leqslant x^{\text{low}} : \ \mu(x) \geqslant \gamma^{\text{high}} \tag{7}$$

$$\forall x \geqslant x^{\text{high}} : \ \mu(x) \leqslant \gamma^{\text{low}} \tag{8}$$

$$\forall x \leqslant x^{\text{max}} : \ \mu(x) \geqslant \gamma^{\text{min}} \tag{9}$$

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 $T^{i,k}$. An exemplary tube for an activation function to illustrate the aforementioned definitions and the meaning of the λ, γ, and x values are depicted in Figure 2.

For the definition of the robustness measure in Section 4, it will be necessary to define a measure for the perturbation of a monotonous function. For this paper, the l_1 norm is used. More precisely, given a monotonous function φ and a perturbed function $\varphi^p \in \mathcal{S}_\varphi$,

$$\|\varphi - \varphi^p\|_1 = \int_0^\infty |\varphi(x) - \varphi^p(x)| \, \mathrm{d}x \tag{10}$$

is a measure for the perturbation of φ.

3. CONDITIONS FOR FORWARD INVARIANCE

As explained in Section 1, the function that should be realized by the system is the forward invariance of several sets $\mathcal{F}_z$, $z = 1, \ldots, m$. In this section, we will now derive conditions on the monotonous functions such that these sets are indeed forward invariant. As basis for the computations, a theorem from [29] can be used, which is recalled next.

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

$$\forall x \in \mathcal{F} : \quad f(x) \in \mathcal{K}_{\mathcal{F}}(x), \tag{11}$$

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

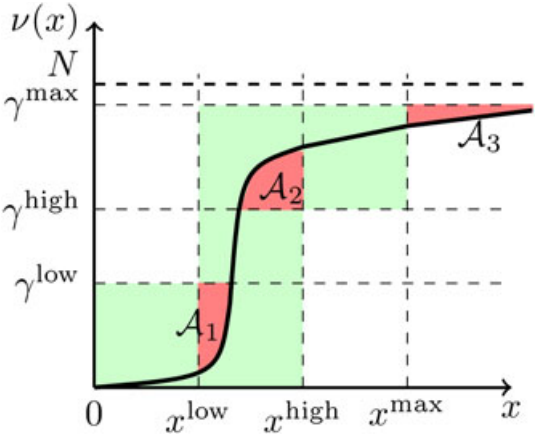

Figure 2. Illustration of a tube for an activation function and the areas $\mathcal{A}_1$, $\mathcal{A}_2$, and $\mathcal{A}_3$ as used in the proof of Proposition 3.

DOI: 10.1002/rnc

This means that at each point x at the boundary of $\mathcal{F}$, the vector field has to be directed inwards or tangent to $\mathcal{F}$. Further details and explanations can be found in [29]. As the sets $\mathcal{F}_z$ considered in this paper are hyper-rectangular and thus convex, Theorem 1 can be applied directly.

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

$$\forall i \in \{1, \ldots, n\} : -k_i \cdot \underline{x}_i + \underline{\lambda}_{i,1} \circ \cdots \circ \underline{\lambda}_{i,q_i} \geqslant 0 \tag{12}$$

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

$$\forall i \in \{1, \ldots, n\} : -k_i \cdot \overline{x}_i + \overline{\lambda}_{i,1} \circ \cdots \circ \overline{\lambda}_{i,q_i} \leqslant 0 \tag{13}$$

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

Proof
Consider the hypersurface of the set $\mathcal{F}$ where $x_i = \underline{x}_i$. If Equation (12) holds, then the vector field is directed inwards $\mathcal{F}$ everywhere on this hypersurface. Equivalently, the vector field is directed inwards $\mathcal{F}$ on the hypersurface $x_i = \overline{x}_i$ if Equation (13) holds. □

From this proposition, we can conclude that the sets $\mathcal{F}_z$, $z = 1, \ldots, m$, are forward invariant if and only if all monotonous functions $\varphi_{i,k}$ satisfy Proposition 1 for every set $\mathcal{F}_z$.

As in our case, we have no nominal system given, the values $\underline{\lambda}_{i,k}$ and $\overline{\lambda}_{i,k}$ from Proposition 1 are unknown, and the proposition can therefore not be applied directly. However, restricting the setup to the case where each interval $\mathcal{I}_{x_i}$ is either a low or high interval as explained earlier, Proposition 1 can be restated in terms of tubes. This reformulation will have the consequence that, if each monotonous function lies in its associated tube, the forward invariance of the sets $\mathcal{F}_z$ can be guaranteed.

Given a set $\mathcal{F} = \mathcal{I}_{x_1} \times \cdots \times \mathcal{I}_{x_n}$ where each interval is either $\mathcal{I}_{x_i} = [0, x_i^{\text{low}}]$ or $\mathcal{I}_{x_i} = [x_i^{\text{high}}, x_i^{\max}]$, define for a monotonous function $\varphi_{i,k}(x_j)$ the values

$$\underline{\gamma}_{i,k} = \begin{cases} 0 & \text{if } 0 \in \mathcal{I}_{x_j} \wedge \varphi_{i,k} \in \mathcal{N} \\ \min\{\gamma : (x, \gamma) \in T^{i,k} \wedge\ x \in \mathcal{I}_{x_i}\} & \text{otherwise} \end{cases} \tag{14}$$

and

$$\overline{\gamma}_{i,k} = \max\{\gamma : (x, \gamma) \in T^{i,k} \wedge x \in \mathcal{I}_{x_i}\}. \tag{15}$$

That is, $\underline{\gamma}_{i,k}$ is the smallest γ value of a tube $T^{i,k}$ in the interval $\mathcal{I}_{x_j}$. Equivalently, $\overline{\gamma}_{i,k}$ is the largest γ value of a tube $T^{i,k}$ in this interval. With this, we can state the following proposition.

Proposition 2
Given a set $\mathcal{F} = \mathcal{I}_{x_1} \times \cdots \times \mathcal{I}_{x_n}$ 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 $T^{i,k}$ that satisfy the conditions

$$\forall i \in \{1, \ldots, n\} : -k_i \cdot \underline{x}_i + \underline{\gamma}_{i,1} \circ \cdots \circ \underline{\gamma}_{i,q_i} \geqslant 0 \tag{16}$$

where $\underline{x}_i = \min_{x_i \in \mathcal{I}_{x_i}} x_i$, and $\underline{\gamma}_{i,k}$ as defined in Equation (14), and

$$\forall i \in \{1, \ldots, n\} : -k_i \cdot \overline{x}_i + \overline{\gamma}_{i,1} \circ \cdots \circ \overline{\gamma}_{i,q_i} \leqslant 0 \tag{17}$$

where $\overline{x}_i = \max_{x_i \in \mathcal{I}_{x_i}} x_i$, and $\overline{\gamma}_{i,k}$ as defined in Equation (15).

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

DOI: 10.1002/rnc

Proof
Note that $\varphi_{i,k} \vDash T^{i,k}$ means that $\underline{\gamma}_{i,k}$ is a lower bound on $\underline{\lambda}_{i,k}$ and $\overline{\gamma}_{i,k}$ is an upper bound on $\overline{\lambda}_{i,k}$. Therefore, if Equations (16) and (17) hold for a tube $T^{i,k}$ and a set $\mathcal{F}_z$, then Equations (12) and (13) hold for $\varphi_{i,k} \vDash T^{i,k}$ and the set $\mathcal{F}_z$. □

However, the other direction is not necessarily true. That is, given tubes $T^{i,k}$, which satisfy inequalities (16) and (17), it is possible to construct activation and inhibition functions $\varphi_{i,k}$ such that at least for some of these functions, it holds that $\varphi_{i,k} \nvDash T^{i,k}$, but the sets $\mathcal{F}_z$ are still forward invariant. Therefore, Proposition 2 only states a sufficient but not necessary condition for the forward invariance of the sets $\mathcal{F}_z$.

4. THE ROBUSTNESS MEASURE

4.1. Definition and interpretation of the robustness measure

In this section, we finally introduce the robustness measures for uncertain gene regulations networks with respect to a specified multistable behavior. As a preparation step, let

$$\mathcal{R}^{\min}(\varphi, T) = \inf_{\varphi^p \in S_\varphi \wedge \varphi^p \nvDash T} \|\varphi - \varphi^p\|_1 \tag{18}$$

be the minimal perturbation of a given function $\varphi \vDash T$ with respect to the given tube T, that is, the smallest perturbation of φ according to Equation (10) such that $\varphi^p \nvDash T$. Also, define

$$\mathcal{R}^{\max}(T) = \sup_{\varphi \vDash T} \mathcal{R}^{\min}(\varphi, T). \tag{19}$$

If this supremum is achieved by a function $\tilde{\varphi}$, this function can be viewed as best centered with respect to the tube T. The solution of Equation (19) can even be computed analytically as will be shown in Section 4.2. With this, the robustness measure can now be defined.

Definition 5
Given a system (1) with unspecified activation and inhibition functions. The robustness measure $\mathcal{R}$ for the system is defined as

$$\begin{aligned} \mathcal{R} = &\max_{T^{i,k}} \min_{i,k} \mathcal{R}^{\max}(T^{i,k}) \\ &\text{s.t.: } \forall T^{i,k} \text{and } \forall \mathcal{F}_z : \text{Equations (16) and (17) hold.} \end{aligned} \tag{20}$$

The measure $\mathcal{R}$ can be interpreted as a guarantee. It involves the computation of an optimal system, that is, of optimal tubes $\tilde{T}^{i,k}$ and monotonous functions $\tilde{\varphi}_{i,k}$ such that $\mathcal{R}$ 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 $\mathcal{R}$, that is, $\forall i, k : \|\tilde{\varphi}_{i,k} - \tilde{\varphi}^p_{i,k}\|_1 \leqslant \mathcal{R}$. The optimal system is also 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.

As Proposition 2 is only a sufficient but not a necessary condition for the forward invariance of the sets $\mathcal{F}_z, z = 1, \ldots, m$, $\mathcal{R}$ is a lower bound on the maximally achievable robustness value.

4.2. Computation of the robustness measure

In this section, a computationally attractive method to compute $\mathcal{R}$ is presented. Therefore, first, an analytical solution of Equation (19) is given.

DOI: 10.1002/rnc

Proposition 3
Let $T_{\mathcal{N}}$ be a tube for an activation function. Then, the maximal value $\mathcal{R}^{\max}(T_{\mathcal{N}})$ is given by

$$\mathcal{R}^{\max}(T_{\mathcal{N}}) = \frac{\gamma^{\text{low}} \cdot (\gamma^{\max} - \gamma^{\text{high}})}{\gamma^{\text{low}} + (\gamma^{\max} - \gamma^{\text{high}})} \cdot (x^{\text{high}} - x^{\text{low}}). \tag{21}$$

Equivalently, let $T_{\mathcal{M}}$ be a tube for an inhibition function. Then, $\mathcal{R}^{\max}(T_{\mathcal{M}})$ is given by

$$\mathcal{R}^{\max}(T_{\mathcal{M}}) = \frac{(N - \gamma^{\text{high}}) \cdot (\gamma^{\text{low}} - \gamma^{\min})}{(N - \gamma^{\text{high}}) + (\gamma^{\text{low}} - \gamma^{\min})} \cdot (x^{\text{high}} - x^{\text{low}}). \tag{22}$$

The proof for an activation function is given in Appendix A. The proof for inhibition functions works with equivalent arguments.

With this result and the new variables

$$\begin{aligned} c^h_{i,k} &= \begin{cases} \gamma^{\max}_{i,k} - \gamma^{\text{high}}_{i,k} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ N_{i,k} - \gamma^{\text{high}}_{i,k} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \\ c^l_{i,k} &= \begin{cases} \gamma^{\text{low}}_{i,k} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ \gamma^{\text{low}}_{i,k} - \gamma^{\min}_{i,k} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \end{aligned} \tag{23}$$

Equation (20) can now be rewritten as

$$\begin{aligned} \mathcal{R} = \max_{T^{i,k}} \min_{i,k} &\left\{ \frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} (x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k}) \right\} \\ \text{s.t.: } \forall T^{i,k} \text{ and } \forall \mathcal{F}_z &: \text{ Equations (16) and (17) hold.} \end{aligned} \tag{24}$$

Again, an equivalent formulation is

$$\begin{aligned} \mathcal{R} = &\min_{T^{i,k}} \frac{1}{t} \\ \text{s.t.: } \forall i,k : \; t \leqslant &\left\{ \frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} (x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k}) \right\} \\ \forall T^{i,k} \text{ and } \forall \mathcal{F}_z &: \text{Equations (16) and (17) hold.} \end{aligned} \tag{25}$$

The optimization problem of Equation (25) still involves minimization over all admissible tubes. This can be made explicit by adding constraints on the γ values of the individual tubes. These additional constraints are derived in the remainder of this section.

Firstly, the tubes have to satisfy the maximum concentration constraints $x^{\max}_i$ for each protein. Therefore, the set $\mathcal{X} = [0, x^{\max}_i] \times \cdots \times [0, x^{\max}_n]$ can be considered as an additional forward-invariant set and, applying Proposition 1, yields the conditions

$$-k_i \cdot x^{\max}_i + \hat{\gamma}_{i,1} \circ \cdots \circ \hat{\gamma}_{i,q_i} \leqslant 0 \quad i = 1, \ldots, n, \tag{26}$$

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

Secondly, also, the monotonicity constraints from the definition of the tubes have to be considered. Therefore, for every tube $T^{i,k}_{\mathcal{N}}$, the constraint

$$0 \leqslant \gamma^{\text{low}}_{i,k} \leqslant \gamma^{\text{high}}_{i,k} \leqslant \gamma^{\max}_{i,k} \leqslant N \tag{27}$$

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

$$0 \leqslant \gamma^{\min}_{i,k} \leqslant \gamma^{\text{low}}_{i,k} \leqslant \gamma^{\text{high}}_{i,k} \leqslant N \tag{28}$$

have to be included.

DOI: 10.1002/rnc

Finally, we require all optimization variables to be positive. The resulting optimization problem can then be written as

$$\begin{aligned} \mathcal{R} = & \min_{\gamma} \frac{1}{t} \\ & \text{s.t.: } \forall i,k: \; t \leqslant \left\{ \frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} (x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k}) \right\} \\ & \quad \forall \mathcal{F}_z : \text{Equations(16) and (17) hold} \\ & \quad \text{Equations (26), (27), and (28) hold} \\ & \quad \gamma \geqslant 0. \end{aligned} \tag{29}$$

4.3. Formulation as convex optimization problem

We aim to formulate the optimization problem of Equation (29) as convex problem and first recall its definition.

Definition 6
A convex optimization problem has the standard form

$$\begin{aligned} \min \quad & f_0(x) \\ \text{s.t.} \quad & f_i(x) \leqslant 0, \quad i = 1, \ldots, m \\ & h_i = 0, \quad i = 1, \ldots, p \end{aligned} \tag{30}$$

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 problems into an equivalent convex formulation. However, we could identify two cases where these optimization problems are already given in a convex form or can be easily transferred into such a form. These two cases are studied in this section.

4.3.1. Additive combinations. In the first case, each '$\circ$' symbol represents an addition. Then, the following result can be stated.

Proposition 4
If the system (1) contains only additive combinations of monotonous functions, the optimization problem of Equations (25) together with the additional constraints (26)–(28) is convex.

Proof
It has to be checked that all requirements from Definition 6 are fulfilled. Clearly, the objective $\mathcal{G} = f_0 = \frac{1}{t}$ is convex in t. Furthermore, the constraints $t - \left(\frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} (x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k}) \right) \leqslant 0$ are convex. To see this, the second-order condition [30] can be applied. The eigenvalues of the Hessian of this constraint are computed as $\left\{ 0, 0, \frac{2((c^h_{i,k})^2 + (c^l_{i,k})^2)}{((c^h_{i,k})^2 + (c^l_{i,k})^2)^3} \right\}$. Therefore, the Hessian is positive semi-definite on the domain $(c^h_{i,k}, c^l_{i,k}, t) \in \mathbb{R}^3_+$, and the constraint is thus convex on this domain. Next, the inequality constraints (16), (17), (26), (27), and (28) are affine in the optimization variables and thus convex. Finally, the equality constraints from Equation (23) are affine in the variables as required. □

DOI: 10.1002/rnc

4.3.2. Multiplicative combinations. In the second case, all '$\circ$' symbols stand for a multiplication. If the constraints from Equation (23) are changed into

$$\begin{aligned} c_{i,k}^{h} &\leqslant \begin{cases} \gamma_{i,k}^{\max} - \gamma_{ij}^{\text{high}} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ N_{i,k} - \gamma_{i,k}^{\text{high}} & \text{if } \varphi_{i,k} \text{ is an inhibition function} \end{cases} \\ c_{i,k}^{l} &\leqslant \begin{cases} \gamma_{i,k}^{\text{low}} & \text{if } \varphi_{i,k} \text{ is an activation function} \\ \gamma_{i,k}^{\text{low}} - \gamma_{i,k}^{\min} & \text{if } \varphi_{i,k} \text{ is an inhibition function,} \end{cases} \end{aligned} \tag{31}$$

the following result can be given.

Proposition 5
Assume system (1) has only multiplicative combinations. Then, the optimization problem to compute $\mathcal{R}$ with the relaxed constraints from Equation (31) instead of Equation (23) is convex and yields the same optimal value.

In order to prove this proposition, two definitions are needed [30].

Definition 7
A function $f : \mathbb{R}^n \to \mathbb{R}$ with domain $\mathbf{dom}\ f = \mathbb{R}_+^n$ of the form $f(x) = c x_1^{a_1} x_2^{a_2} \dots x_n^{a_n}$, $c > 0$ 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 8
An optimization problem

$$\begin{aligned} \min \quad & f_0(x) \\ \text{s.t.} \quad & f_i(x) \leqslant 1, \quad i = 1, \dots, m \\ & h_i = 1, \quad i = 1, \dots, p \end{aligned} \tag{32}$$

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

A geometric program can be transformed into a convex problem of the form of Equation (30) by the variable transformation $y_i = \log x_i$ [30].

With this, Proposition 5 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. Additionally, the constraints $t - \frac{c_{i,k}^{h} \cdot c_{i,k}^{l}}{c_{i,k}^{h} + c_{i,k}^{l}} (x_{i,k}^{\text{high}} - x_{i,k}^{\text{low}}) \leqslant 0$ can be reformulated as posynomial $t \cdot a^{-1} \cdot (c_{i,k}^{l})^{-1} + t \cdot a^{-1} \cdot (c_{i,k}^{h})^{-1} \leqslant 1$, with constant $a = (x_{i,k}^{\text{high}} - x_{i,k}^{\text{low}})$. In an equivalent way, the inequality constraints from Equations (16), (17), (26), (27), and (28) can be rewritten as posynomials. Moreover, for the modified constraints from Equation (31), 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 $p_{\text{mod}}^{\star} \geqslant p^{\star}$. It now has to be shown that the optimal value for the modified problem is obtained when all relaxed inequalities (31) are satisfied with equality, that is, $p_{\text{mod}}^{\star} = p^{\star}$. Denote ξ the vector containing all optimization variables. It takes the value $\xi^{\star}$ at the optimal point $p^{\star}$. Recall that the optimal value $p^{\star}$ 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 (31) that does not hold with

DOI: 10.1002/rnc

equality. Then, without influencing any other constraint, equality of this constraint can be achieved by increasing the respective value $c^h_{i,k}$ or $c^l_{i,k}$. Then, as $\mathcal{R}^{\max}_{i,k}$ is monotonically increasing in $c^h_{i,k}$ and $c^l_{i,k}$, this value will also increase. As $p^\star_{\text{mod}}$ is optimal, there has to be at least one tube $T^{i,k}$ in the set V for which it holds that all constraints (31) hold with equality. Then, modifying all other constraints that do not hold with equality in the way described above yields an optimal solution $p^\star$ for the original problem with $p^\star = p^\star_{\text{mod}}$. □

5. EXAMPLE

In this section, we apply the methodology developed in the previous sections to several models of a gene regulatory network, which is responsible for the maintenance of the MHB.

This boundary is formed during vertebrate development, when the central nervous system arises from a precursor tissue called neural plate. Shortly after gastrulation, this neural plate is patterned along the anterior–posterior axis into four regions, which continue to develop into forebrain, midbrain, hindbrain, and spinal cord. This patterning is determined by a well-defined and locally restricted expression of genes and by the action of short-range and long-range signaling centers. The development of mid-hindbrain and hindbrain, which we are interested in, is controlled by the activity of the isthmic organizer (IsO) located at the boundary between the prospective mid-hindbrain and hindbrain, the so-called MHB.

The IsO is characterized by the localized expression of several transcription and secreted factors. In this contribution, we focus on the following four IsO genes: *Otx2*, *Gbx2*, *Fgf8*, and *Wnt1*. Various *in situ* hybridization experiments revealed the spatio-temporal expression patterns of these genes around the MHB. Figure 3 shows a schematic snapshot of these expression patterns at embryonic day 10.5 (E10.5). It has been shown that the patterns at that time continue to be stably maintained. Moreover, various loss-of-function and gain-of-function experiments demonstrated that by E10.5 the aforementioned genes have become interdependent and form the core module of a regulatory network that guarantees the sharpening and subsequent maintenance of their specific expression patterns [31]. For a biological review, see for example [31,32]. In [6] and [33], several model structures that are able to reproduce the observed expression pattern around the MHB in a multi-compartment model have been developed. Nine such model structures are depicted in Figure 4.

As all nine model structures from Figure 4 are in principle able to reproduce the desired pattern, we now focus on how these models differ and which model structure might be best suited. To approach this question, the networks are first translated into the modeling framework presented in Section 2. Then, a robustness analysis according to Section 4 is performed for each of the models.

5.1. Modeling

In order to analyze the models, the spatial expression pattern, which is stably maintained after E10.5, is discretized into six regions, and for each of these regions, the expression strength of the four genes

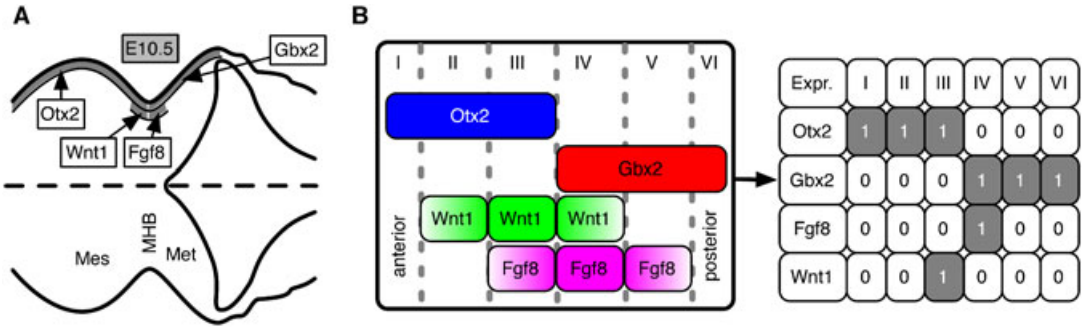

Figure 3. (A) Schematic snapshot of the expression of the four genes *Otx2*, *Gbx2*, *Fgf8*, and *Wnt1* at the mid-hindbrain boundary (MHB) at day E10.5. (B) Spatial discretization of the expression pattern at the MHB into six segments. The left figure shows the presence of the proteins in each segment. The right figure shows the expression level on the four proteins.

DOI: 10.1002/rnc

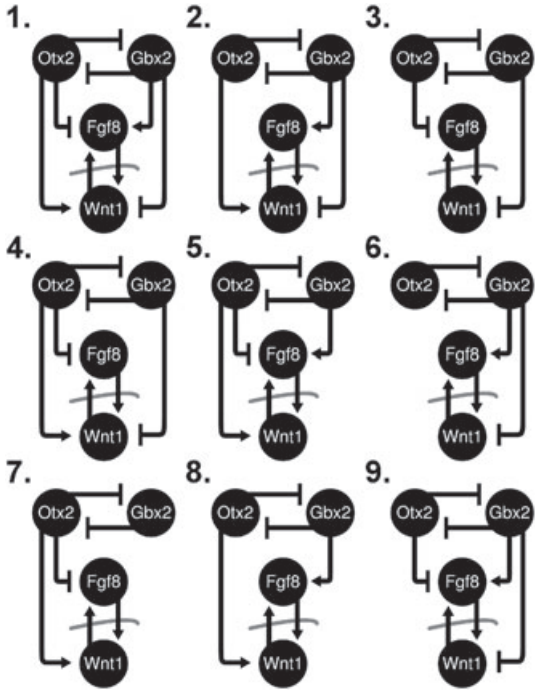

Figure 4. Nine different gene regulation networks for the maintenance of the mid-hindbrain boundary.

Otx2, *Gbx2*, *Fgf8*, and *Wnt1* is characterized as highly expressed or weakly expressed, which is also depicted in Figure 3. Equivalently, the presence of the corresponding proteins Otx2, Gbx2, Fgf8, and Wnt1 is characterized by present or not present. As Fgf8 and Wnt1 are secreted, Wnt1 and Fgf8 are not only present in the regions where *Fgf8* and *Wnt1* are highly expressed but also diffused in neighboring sections.

For our approach, each of these six regions is considered as stably maintained operation mode of the system, and thus, six forward-invariant sets in the state space can be assigned to the model. In the real network, cells can communicate via the secreted factors Wnt1 and Fgf8. For our analysis, however, we break this communication between the cells and replace the influence of Wnt1 and Fgf8 by external inputs $\tilde{\text{Fgf8}}$ and $\tilde{\text{Wnt1}}$. For each of regions I to VI, these inputs are then set to a high or low concentration as observed in the respective region (Figure 3).

The resulting forward-invariant sets corresponding to the six regions are listed in Table I. As we have no information about the maximum concentrations and degradation rates, we assumed them to be similar for all species and normalized them to 1, that is, $\forall i : x_i^{\max} = 1, k_i = 1$. Finally, we define a low concentration of a protein x_i to lie in an interval $\mathcal{I}_{x_i} = [0, 0.2]$ and a high concentration to lie in an interval $\mathcal{I}_{x_i} = [0.8, 1]$.

With these modeling assumptions, each of the nine networks can be modeled with activation and inhibition functions. The equations for network 1 from Figure 4 for example read as

Table I. The six forward-invariant sets corresponding to the six regions I to VI.

Steady state	Otx2	Gbx2	Fgf8	Wnt1	$\tilde{\text{Fgf8}}$	$\tilde{\text{Wnt1}}$
I	High	Low	Low	Low	Low	Low
II	High	Low	Low	Low	Low	High
III	High	Low	Low	High	High	High
IV	Low	High	High	Low	High	High
V	Low	High	Low	Low	High	Low
VI	Low	High	Low	Low	Low	Low

DOI: 10.1002/rnc

$$\begin{aligned}
\dot{\text{Otx2}} &= -\text{Otx2} + \mu_{1,1}(\text{Gbx2}) \\
\dot{\text{Gbx2}} &= -\text{Gbx2} + \mu_{2,1}(\text{Otx2}) \\
\dot{\text{Fgf8}} &= -\text{Fgf8} + \mu_{3,1}(\text{Otx2}) \cdot \nu_{3,2}(\text{Gbx2}) \cdot \nu_{3,3}(\tilde{\text{Wnt1}}) \\
\dot{\text{Wnt1}} &= -\text{Wnt1} + \mu_{4,1}(\text{Gbx2}) \cdot \nu_{4,2}(\text{Otx2}) \cdot \nu_{4,3}(\tilde{\text{Fgf8}}).
\end{aligned} \tag{33}$$

5.2. Analysis and discussion of the results

For each of the nine networks, the respective geometric optimization problems from Equation (29) were generated and solved using YALMIP [34]. The obtained robustness values for the nine networks are listed in Table II.

For the given specification and according to Table II, there are only two classes of systems: those with the higher $\mathcal{R}$ value of 0.0423 (networks 3,6,7 and 8) and those with a lower $\mathcal{R}$ value of 0.0357 (all other networks). This means that for the optimal realizations of networks 3,6,7, and 8, a larger perturbation of each individual activation and inhibition function, measured according to Equation (10), can be guaranteed to not violate the invariance of the specified six regions. According to our definition, these four models are therefore considered to be more robust.

A closer look at the model structures reveals an important difference between the two classes. All models with the lower $\mathcal{R}$ value have at least one node with three incoming links (nodes Fgf8 or Wnt1), whereas for all models in the class with the higher $\mathcal{R}$ value, the maximal number on incoming links is two.

That this number is indeed the determining factor can be seen by further inspecting the results of the optimization problems for the computation of the $\mathcal{R}$ values. Recall that for every tube $T^{i,k}$, there is one constraint of the form

$$t \leq \left\{ \frac{c^h_{i,k} \cdot c^l_{i,k}}{c^h_{i,k} + c^l_{i,k}} \left(x^{\text{high}}_{i,k} - x^{\text{low}}_{i,k} \right) \right\}. \tag{34}$$

When optimizing the $\mathcal{R}$ value for networks with a maximal indegree of 3, the constraints of Equation (34) were active for all incoming links of nodes with indegree 3. Equivalently, for all networks with maximal indegree of 2, the constraints (34) were active for incoming links of nodes with indegree 2. The constraints (34) for incoming links of nodes of indegree 1 (Otx2 and Gbx2) were never active.

In summary, computing the $\mathcal{R}$ values for the nine networks structures suggests the conclusion that a higher indegree requires finer tuning of the activation and inhibition functions. On the other side, a larger total perturbation might still be possible for stronger connected networks. If one measures a total perturbation as the sum of the perturbations of individual links, network 1 can for example tolerate at least a total perturbation of $8 \cdot 0.0357 = 0.2856$. For the four networks with the higher $\mathcal{R}$ value, however, only an admissible perturbation of $6 \cdot 0.0423 = 0.2538$ can be guaranteed. As we have not maximized over the sum of individual perturbations as this would lead to a non-convex optimization problem, this observation should only be considered as a possible advantage of stronger connected networks but not as a general statement.

Table II. Robustness values of the nine network structures.

System	$\mathcal{R}$ value
1	0.0357
2	0.0357
3	0.0423
4	0.0357
5	0.0357
6	0.0423
7	0.0423
8	0.0423
9	0.0357

DOI: 10.1002/rnc

We therefore conjecture that stronger connected networks may tolerate larger total perturbations at the price that individual links have to be tuned finer. When formulated differently, for sparse network topologies, it is easier to find activation and inhibition functions such that a given steady-state pattern can be achieved. This conjecture is also in agreement with the observations made in [35] although the setup there is slightly different from ours. In this paper, the so-called 'gross average cost of perturbation' was introduced as robustness measure that explicitly takes network complexity, that is, the number of links in network, into account. The result of this study was that sparser networks outperform densely connected networks and will thus be preferred during evolution. This conjecture is furthermore in agreement with the observation that, for example, well-studied gene regulation networks from *Escherichia coli*, *Arabidopsis*, or *Drosophila* show a relatively small connectivity of only 1.5–2 regulating factors per gene in average [35].

6. CONCLUSIONS AND OUTLOOK

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. Furthermore, a method for its computation has been developed, and it was demonstrated that the resulting optimization problem is convex in special cases. This method can be applied to answer two types of questions: 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. To demonstrate its use, the method was applied to several hypothetical model structures for a gene regulation network, which is responsible for the stable maintenance of a well-defined spatio-temporal expression pattern. We found that the maximal number of transcription factors of a target gene limits the achievable robustness in the studied networks.

Concerning the reasoning behind our approach, we have argued that postulating a robust multistable behavior is biologically meaningful for a large class of gene regulation networks, especially for those involved in differentiation and pattern formation processes. However, depending on the precise function of the network, also, other aspects should be considered. For differentiation networks for example, a well-regulated transition between steady states upon certain stimuli has to be guaranteed in a robust way. Our future research will therefore address different network functions in order to obtain other biologically meaningful and more differentiated definitions of robust behavior.

APPENDIX A: PROOF OF PROPOSITION 3

Let $T_{\mathcal{N}}$ be a tube for an activation function. Then, the maximal value $\mathcal{R}^{\max}(T_{\mathcal{N}})$ is given by

$$\mathcal{R}^{\max}(T_{\mathcal{N}}) = \frac{\gamma_{\text{low}} \cdot (\gamma_{\max} - \gamma_{\text{high}})}{\gamma_{\text{low}} + (\gamma_{\max} - \gamma_{\text{high}})} \cdot (x_{\text{high}} - x_{\text{low}}). \tag{35}$$

Equivalently, let $T_{\mathcal{M}}$ be a tube for an inhibition function. Then, $\mathcal{R}^{\max}(T_{\mathcal{M}})$ is given by

$$\mathcal{R}^{\max}(T_{\mathcal{M}}) = \frac{(N - \gamma_{\text{high}}) \cdot (\gamma_{\text{low}} - \gamma_{\min})}{(N - \gamma_{\text{high}}) + (\gamma_{\text{low}} - \gamma_{\min})} \cdot (x_{\text{high}} - x_{\text{low}}). \tag{36}$$

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 Inequalities (4)–(6), and we can give the following estimates on $\|\nu - \nu^p\|_1$.

Assume that Inequality (4) is violated, and let $\hat{x}$ be the smallest value such that $\nu(\hat{x}) = \gamma^{\text{low}}$. Then, it holds that $\|\nu - \nu^p\|_1 \geqslant \int_{x^{\text{low}}}^{\hat{x}} (\gamma^{\text{low}} - \nu(x))\, \mathrm{d}x = \mathcal{A}_1(\nu)$.

Now assume Inequality (5) is violated. Let $\hat{x}$ be the smallest value such that $\nu(\hat{x}) = \gamma^{\text{high}}$. Then $\|\nu - \nu^p\|_1 \geqslant \int_{\hat{x}}^{x^{\text{high}}} (\nu(x) - \gamma^{\text{high}})\, \mathrm{d}x = \mathcal{A}_2(\nu)$.

DOI: 10.1002/rnc

Finally, assume that Inequality (6) is violated. Then, with $\hat{x}$ being the largest value such that $\nu(\hat{x}) = \gamma^{\max}$, it holds that $\|\nu - \nu^p\|_1 \geqslant \int_{x^{\max}}^{\hat{x}} (\gamma^{\max} - \nu(x))\, \mathrm{d}x = \mathcal{A}_3(\nu)$.

For an illustration of these areas, see also Figure 2.

Thus, for every $\nu^p \in \mathcal{N}$ such that $\nu^p \not\models T$, it holds that $\|\nu - \nu^p\|_1 \geqslant \mathcal{A}(\nu) = \min\{\mathcal{A}_1(\nu), \mathcal{A}_2(\nu), \mathcal{A}_3(\nu)\}$, 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 $\nu \models T_{\mathcal{N}}$, it is possible to find another $\bar{\nu} \models T_{\mathcal{N}}$ that 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

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

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, $h_{\bar{x}_s} \models \bar{T}_{\mathcal{N}}$, and with the aforementioned definitions of $\mathcal{A}_1$ and $\mathcal{A}_2$, it follows that $\min\{\mathcal{A}_1(\bar{\nu}), \mathcal{A}_2(\bar{\nu})\} \leqslant \min\{\mathcal{A}_1(h_{\bar{x}_s}), \mathcal{A}_2(h_{\bar{x}_s})\}$. This means that we can always find a step function with a larger value $\mathcal{A}$ than the function $\bar{\nu}$. Next, among all possible step functions $h_{x_s} \models \bar{T}_{\mathcal{N}}$, the step function $h_{x_s^\star}$, which maximizes $\min\{\mathcal{A}_1, \mathcal{A}_2\}$, has to satisfy $\mathcal{A}_1 = \mathcal{A}_2$, that is, $(\bar{\gamma}^{\max} - \gamma^{\text{high}}) \cdot (x^{\text{high}} - x^{s^\star}) = \gamma^{\text{low}} \cdot (x_s^\star - x^{\text{low}})$, 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

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

□

Note that step functions are not admissible activation functions as they are not continuously differentiable. Therefore, Equation (A) 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.

ACKNOWLEDGEMENT

The authors gratefully acknowledge partial funding through the Helmholtz Society, project 'Control of Regulatory Networks with focus on non-coding RNA' (CoReNe); F. A., C. B., and S. W. thank the German Research Foundation (DFG) for partial funding within the Cluster of Excellence in Simulation Technology (EXC 310/1) at the University of Stuttgart.

REFERENCES

1. Ozbudak E, Thattai M, Lim H, Shraiman B, van Oudenaarden A. Multistability in the lactose utilization network of escherichia coli. *Nature* 2004; **427**:737–740. DOI: 10.1038/nature02298.
2. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death and Differentiation* 2003; **10**:45–65. DOI: http://dx.doi.org/10.1038/sj.cdd.4401189.
3. Eissing T, Conzelmannm H, Gilles ED, Allgöwer F, Bullinger E, Scheurich P. Bistability analyses of a caspase activation model for receptor-induced apoptosis. *The Journal of Biological chemistry* 2004; **279**:36892–36897. DOI: 10.1074/jbc.M404893200.
4. Roeder I, Glauche I. Towards an understanding of lineage specification in hematopoietic stem cells: a mathematical model for the interaction of transcription factors GATA-1 and PU.1. *Journal of Theoretical Biology* 2006; **241**(4):852–865. DOI: 10.1016/j.jtbi.2006.01.021.
5. Lemon G, Waters SL, Rose FR, King JR. Mathematical modelling of human mesenchymal stem cell proliferation and differentiation inside artificial porous scaffolds. *Journal of Theoretical Biology* 2007; **249**:543–553. DOI: 10.1016/j.jtbi.2007.08.015.

6. Wittmann DM, Blöchl F, Trümbach D, Wurst W, Prakash N, Theis FJ. Spatial analysis of expression patterns predicts genetic interactions at the mid-hindbrain boundary. *PLoS Computational Biology* 2009; **5**(11):e1000569. DOI: 10.1371/journal.pcbi.1000569.
7. Szallasi Z. Biological data acquisition or system level modeling an exercise in the art of compromise. In *System Modeling in Cellular Biology*. MIT Press: Cambridge, Massachusetts, USA, 2006; 201–220.
8. Chaves M, Eissing T, Allgöwer F. Bistable biological systems: A characterization through local compact input-to-state stability. *IEEE Transactions on Automatic Control, Special Issue on Systems Biology* 2008; **53**:87–100. DOI: 10.1109/TAC.2007.911328.
9. Breindl C, Allgöwer F. Verification of multistability in gene regulation networks: a combinatorial approach. *Proceedings of the 48th IEEE Conference on Decision and Control (CDC)*, 2009; 5637–5642, DOI: 10.1109/CDC.2009.5400809.
10. Breindl C, Waldherr S, Allgöwer F. A robustness measure for the stationary behavior of qualitative gene regulation networks. *Proceedings of the 11th Symposium on Computer Applications in Biotechnology (CAB)*, 2010; 36–41.
11. Kitano H. Biological robustness. *Nature Reviews Genetics* 2004; **5**:826–837. DOI: 10.1038/nrg1471.
12. von Dassow G, Meir E, Munro EM, Odell GM. The segment polarity network is a robust developmental module. *Nature* 2000; **406**(6792):188–192. DOI: 10.1038/35018085.
13. Ingolia NT. Topology and robustness in the drosophila segment polarity network. *PLoS Biology* 2004; **2**(6):e123. DOI: 10.1371/journal.pbio.0020123.
14. Angeli D, Sontag ED. Monotone control systems. *IEEE Transactions on Automatic Control* 2003; **48**:1684–1698. DOI: 10.1109/TAC.2003.817920.
15. Angeli D, Sontag ED. Multi-stability in monotone input/ouptut systems. *Systems & Control Letters* 2004; **51**:185–202. DOI: 10.1109/TAC.2007.894507.
16. Thomas R, Thieffry D, Kaufman M. Dynamical behaviour of biological regulatory networks–I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bulletin of Mathematical Biology* 1995; **57**(2):247–276. DOI: 10.1016/0092-8240(94)00036-C.
17. Chaves M, Albert R, Sontag ED. Robustness and fragility of boolean models for genetic regulatory networks. *Journal of Theoretical Biology* August 2005; **235**(3):431–449. DOI: 10.1016/j.jtbi.2005.01.023.
18. Klamt S, Saez-Rodriguez J, Lindquist JA, Simeoni L, Gilles ED. A methodology for the structural and functional analysis of signaling and regulatory networks. *BMC Bioinformatics* 2006; **7**(56). DOI: 10.1186/1471-2105-7-56.
19. Eißing T, Allgöwer F, Bullinger E. Robustness properties of apoptosis models with respect to parameter variations and intrinsic noise. *IET Systems Biology* 2005; **152**(4):221–228. DOI: 10.1049/ip-syb:20050046.
20. Jacobsen EW, Cedersund G. Structural robustness of biochemical network models-with application to the oscillatory metabolism of activated neutrophils. *IET Systems Biology* 2008; **2**(1):39–47. DOI: 10.1049/iet-syb:20070008.
21. Waldherr S, Allgöwer F, Jacobsen EW. Kinetic perturbations as robustness analysis tool for biochemical reaction networks. *Proceedings of the 48th IEEE Conference on Decision and Control (CDC)*, 2009; 4572–4577, DOI: 10.1109/CDC.2009.5400939.
22. Prill RJ, Iglesias PA, Levchenko A. Dynamic properties of network motifs contribute to biological network organization. *PLoS Biology* 2005; **3**(11):e343. DOI: 10.1371/journal.pbio.0030343.
23. Klemm K, Bornholdt S. Topology of biological networks and reliability of information processing. *PNAS* 2005; **102**(51):18414–18419. DOI: 10.1073/pnas.0509132102.
24. Aldana M, Balleza E, Kauffman S, Resendiz O. Robustness and evolvability in genetic regulatory networks. *Journal of Theoretical Biology* 2007; **245**(3):433–448. DOI: 10.1016/j.jtbi.2006.10.027.
25. Balleza E, Alvarez-Buylla ER, Chaos A, Kauffman S, Shmulevich I, Aldana M. Critical dynamics in genetic regulatory networks: examples from four kingdoms. *PLoS One* 2008; **3**(6):e2456. DOI: 10.1371/journal.pone.0002456.
26. Agoston V, Csermely P, Pongor S. Multiple weak hits confuse complex systems: a transcriptional regulatory network as an example. *Physical Review E* 2005; **71**:051909. DOI: 10.1103/PhysRevE.71.051909.
27. Edwards JS, Ibarra RU, Palsson BO. In silico predictions of escherichia coli metabolic capabilities are consistent with experimental data. *Nature Biotechnology* 2001; **19**:125–130. DOI: 10.1038/84379.
28. Beard DA, Liang S, Qian H. Energy balance for analysis of complex metabolic networks. *Biophysical Journal* 2002; **83**(1):79–86. DOI: 10.1016/S0006-3495(02)75150-3.
29. Blanchini F. Set invariance in control. *Automatica* 1999; **35**:1747–1767. DOI: 10.1016/S0005-1098(99)00113-2.
30. Boyd S, Vandenberghe L. *Convex Optimization*. Cambridge University Press: Cambridge, UK, 2004.
31. Wurst W, Bally-Cuif L. Neural plate patterning: upstream and downstream of the isthmic organizer. *Nature Reviews Neuroscience* 2001; **2**(2):99–108. DOI: 10.1038/35053516.
32. Prakash N, Wurst W. Specification of midbrain territory. *Cell and Tissue Research* 2004; **318**:5–14. DOI: 10.1007/s00441-004-0955-x.
33. Krumsiek J, Pölsterl S, Wittmann DM, Theis FJ. Odefy - From discrete to continuous models. *BMC Bioinformatics* 2010; **11**:233. DOI: 10.1186/1471-2105-11-233.
34. Löfberg J. YALMIP: a toolbox for modeling and optimization in MATLAB. *Proceedings of the CACSD Conference*, Taipei, Taiwan, 2004. http://control.ee.ethz.ch/~joloef/yalmip.php.
35. Leclerc RD. Survival of the sparsest: robust gene networks are parsimonious. *Molecular Systems Biology* 2008; **4**:213. DOI: 10.1038/msb.2008.52.