Proceedings of the 9th International Symposium on
Dynamics and Control of Process Systems (DYCOPS 2010),
Leuven, Belgium, July 5-7, 2010
Mayuresh Kothare, Moses Tade, Alain Vande Wouwer, Ilse Smets (Eds.)

Robustification as a Tool in Modeling Biochemical Reaction Networks

Vladislav Nenchev * **Elling W. Jacobsen** *

* *Automatic Control Lab, KTH, S-100 44 Stockholm, Sweden (email: jacobsen@kth.se)*

Abstract: Biological functions have evolved to become robust against a multitude of perturbations such as gene mutations, intracellular noise and changes in the physical and chemical environment. This robustness should be reflected in models of the underlying biochemical networks, and robustness analysis is frequently employed in validating models of intracellular biochemical reaction networks. However, at present there are no tools or guidelines available to support postulation of model modifications that can serve to improve the robustness. Herein we propose a method based on computing the sensitivity of the robustness with respect to generic dynamic perturbations applied to the individual network edges. To quantify robustness we compute the smallest simultaneous change in the activity of the network nodes that induces a bifurcation in the network, resulting in a qualitative change in the network behavior. The focus is on biological functions related to bistable switches and sustained oscillations, and the proposed methodology is demonstrated through application to metabolic oscillations in white blood cells and bistable switching in MAPK signal transduction.

Keywords: Systems biology, gene regulatory networks, modeling, dynamic behavior, biochemical networks, robustness, bifurcations

1. INTRODUCTION

While dynamic modeling is a key to understand biological functions at the cellular level, experimental data collected under non-steady-state conditions are relatively scarce. To (in)validate models one must therefore usually rely also on other sources of information. One key aspect of normal biological functions is that, through evolution, they have developed robustness against potentially harmful internal and external perturbations that occur with some probability. Based on this fact, robustness analysis is routinely used in validating dynamic models of biochemical networks, e.g., (6; 4). A model which is found to be relatively unrobust to biologically probable perturbations is considered invalidated, and the key problem is then how to identify biologically relevant model modifications that will improve the robustness.

Robustness analysis has previously been employed to postulate hypotheses concerning network structures (3) and strength of interactions between network components within a given topology (11). Wagner (3) considers simple gene regulatory networks providing sustained oscillations and performs a search over all possible interconnections to find the most robust topology. The robustness is quantified in terms of parametric sensitivity. In Chen et al (11), several model candidates for a bistable switch in the network underlying apoptosis are compared in terms of parametric robustness and based on this the most plausible mechanisms are identified. In the case of biological functions related to sustained oscillations, a common postulation is that adding a delay in the primary feedback loop will increase the robustness of the oscillations, e.g., (8). However, in the case of more complex networks, a delay may also have the adverse effect, i.e., reducing the robustness or even removing the oscillatory behavior altogether (5).

In this work we propose a systematic method for postulating model modifications that will increase the robustness of the predicted behavior. To quantify robustness we employ the structural robustness analysis method proposed in (5) and (7). Essentially, the method is based on perturbing the activity of all network components and computing the smallest overall perturbation that induces a bifurcation, corresponding to a qualitative change in the network behavior. This is a convex and computationally inexpensive method. In order to identify network modifications that will have the most significant impact on the overall robustness, we apply generic dynamic perturbations to the individual network edges and compute the maximum change in robustness for perturbations within a norm-bounded set. An advantage of applying generic dynamic perturbations, as compared e.g., to parametric perturbations, is that the impact of unmodelled phenomena can be taken into account. This implies that also the impact of topological modifications, e.g., unmodelled nodes and edges, can be determined.

The focus here is on functions related to sustained oscillations, i.e., limit cycle behavior, and bistable switches. Such functions are commonplace in cell biology, and include for instance cell cycle control, cell differentiation, apoptosis and circadian rhythms to mention a few.

We start the paper by introducing a simple network, consisting of three components forming a single feedback loop, in order to motivate and conceptually explain the proposed method. We then briefly review the robustness

analysis employed and present the method for computing the sensitivity of the robustness with respect to dynamic perturbations applied to the network edges. The method is then applied to the introductory example to confirm that it systematically identifies plausible modifications to improve the robustness. As we show, the method supports the intuitive insight that a time delay in the feedback loop is an effective means of increasing robustness of the oscillations. However, the method also identifies an alternative modification corresponding to closing an additional positive feedback loop, i.e. a change in network topology, which has a similar impact on the robustness. We then consider a model of the oscillatory metabolism in white blood cells. This model corresponds to a complex and highly interconnected network, and has previously been found to be highly unrobust. Robustification of this model is a challenging task (5) and served as the original motivation for deriving the method presented here. We show how the proposed method can be used to significantly robustify this model using plausible model modifications. Finally, we consider robustification of the bistable switch in a model of MAPK signal transduction.

2. INTRODUCTORY EXAMPLE

In order to illustrate the basic idea behind the method proposed in this paper, we start by considering a simple network involving 3 components, corresponding to the much studied Goodwin model (2). See Figure 1. The model is given by the ODEs

$$\dot{X} = v_0 \frac{K_0^n}{K_0^n + Z^n} - v_1 \frac{X}{K_1 + X} \tag{1}$$

$$\dot{Y} = K_4 X - v_2 \frac{Y}{K_2 + Y} \tag{2}$$

$$\dot{Z} = K_5 Y - v_3 \frac{Z}{K_3 + Z} \tag{3}$$

For the parameter values $v_0 = 0.7, v_1 = v_2 = v_3 = 0.35, K_0 = K_1 = K_2 = K_3 = 1, K_4 = K_5 = 0.7, n = 4.5$, the model displays a limit cycle as shown in Figure 1. This simple network structure has been used to model sustained oscillations in a variety of biological functions, most notably circadian timekeeping. A weakness of the model is that it requires an unrealistically large cooperativity with a Hill coefficient $n > 4$ to produce sustained oscillations. Furthermore, even with the large value considered here, $n = 4.5$, the model displays a relatively poor robustness; as shown below, a change in the activity (concentrations) of the three components by only 2% will remove the oscillatory behavior. A common postulation to improve the robustness of the model, and possibly also reproduce oscillations for more realistic values of n, is to add a time delay to the loop, e.g., (8). A delay of θ time units can for instance be implemented by adding a relative perturbation δ_{32} to the edge connecting component 2 and component 3, see Figure 1, and letting

$$1 + \delta_{32} = e^{-\theta s}$$

Indeed, such a modification will increase the robustness of the oscillations as we will show below. Furthermore, a delay can be justified biologically by the fact that there are intracellular transport phenomena that impose a delay in the interactions between e.g., proteins and genes. Thus, in

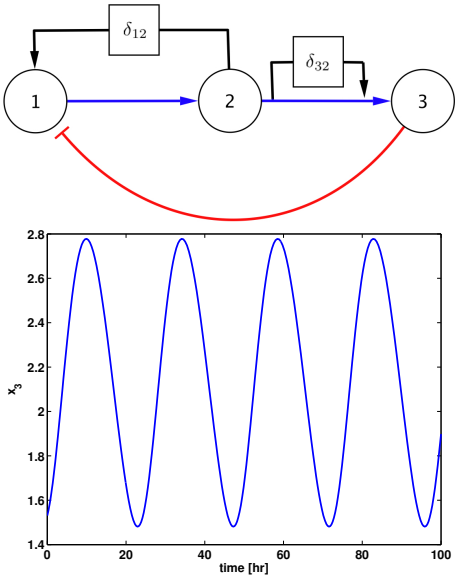

Fig. 1. Three component network in Introductory example. The components form a negative feedback loop which produces sustained oscillations.

this case intuitive reasoning alone can be used to postulate model modifications that will improve the robustness of the model and hence, by assumption, increase the confidence in the model. However, most biochemical network models are significantly more complex than the simple model considered here and in such cases it is not likely that intuitive reasoning alone can be used to determine model modifications that will improve the robustness. Hence, there is a need for a systematic tool that can identify modifications that will have a significant impact on the overall robustness. This is considered below. We first present a scalar quantitative measure of robustness.

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3. QUANTIFYING ROBUSTNESS

The robustness considered here concerns the persistence of a qualitative behavior in the presence of perturbations of the network properties. Since changes in the qualitative behavior of a dynamical system are directly related to the existence of bifurcation points, we consider determining perturbations that induce a bifurcation in the network. Furthermore, since most behaviors of interest in the context of biological functions can be related to steady-state bifurcations, the consideration can be limited to static and Hopf bifurcations of steady-states. In particular, in the case of limit cycle behaviors and bistable switches, which are the focus of this paper, one can quantify robustness by determining the smallest perturbation, within a given class, that induces a Hopf or saddle-node bifurcation, respectively, at the underlying unstable steady-state (7).

The existence of a bifurcation at a given steady-state is determined by the linear part of the nonlinear model at the steady-state. The nonlinear part is required to ensure that a transversality condition is fulfilled, and to determine if a Hopf bifurcation is sub- or supercritical. However, for robustness analysis we are only concerned with the existence of a bifurcation point as such and hence can limit

ourselves to analyze the linear part of the model. Assume that the nonlinear model is on the form

$$\dot{y} = f(y, p), \quad y \in \mathbb{R}^n, p \in \mathbb{R}^m$$

where y are the state variables, corresponding e.g., to biochemical component activities, and p is a vector of model parameters. Assume also that the nominal parameter vector is p^* and that the corresponding steady-state of interest is y^*, i.e., $f(y^*, p^*) = 0$. For the cases of stable limit cycle behavior and bistable switches, we consider the underlying unstable steady-state provided such a state exists. The linearized dynamics around the steady-state are then given by

$$\dot{x} = Ax(t) \; ; \quad A = \left(\frac{\partial f}{\partial y}\right)_{y^*, p^*}$$

where $x = y - y^*$. The robustness analysis aims at determining the smallest perturbation that imposes eigenvalues on the imaginary axis for the perturbed system. We consider relative perturbations of the activity of the individual components according to

$$\dot{x} = Ax(t) + (A - \tilde{A})x_\Delta(t) \tag{4}$$

$$x_\Delta(t) = \Delta_I x(t) \tag{5}$$

where $\tilde{A}$ is a diagonal matrix with the diagonal elements of A and Δ_I is a diagonal matrix with diagonal elements δ_i. This implies that the impact of component i on all other components is perturbed by a relative perturbation $(1+\delta_i)$. The perturbation can be made dynamic by letting Δ_I be frequency dependent. Denote

$$L(j\omega) = (j\omega - A)^{-1}(A - \tilde{A})$$

Then the perturbed system will have eigenvalues on the imaginary axis, corresponding to a bifurcation point in the corresponding nonlinear system, if

$$\det\left(I - \Delta_I(j\omega)L(j\omega)\right) = 0$$

for some frequency $\omega \geq 0$. If $\omega = 0$ the bifurcation is static, while $\omega > 0$ implies a Hopf bifurcation. The robustness radius R, corresponding to the smallest Δ_I inducing eigenvalues at the imaginary axis, is then defined as

$$R(\omega) = \inf\{\|\Delta_I\| \mid \Delta_I \in \mathbf{\Delta} \subset \mathbb{C}^{n\times n}, \\ \det\left(I - \Delta_I L(j\omega)\right) = 0\}, \quad \omega \geq 0$$

As a scalar robustness measure we employ the minimum robustness radius over all frequencies, i.e.,

$$\mathbf{R} = \min_{\omega} R(\omega)$$

Further motivation for employing the above measure as a robustness measure for biochemical networks can be found in (5; 7).

4. IMPACT OF PERTURBING NETWORK EDGES ON OVERALL ROBUSTNESS

Given a scalar quantitative measure of the robustness of a network model, we can systematically search for model modifications with the largest impact on the robustness of a given network function. The approach adopted here is to consider the role of individual network edges, i.e., the direct interactions between any two network nodes (components), by perturbing these using generic dynamic perturbations. Since the robustness, as defined above, can be restricted to consideration of the linearized model we also restrict the edge perturbations to be linear dynamic systems.

We first consider the case in which existing network interactions are perturbed. For the linearized model, the direct effect of component j on component i in the unperturbed model is

$$X_i(s) = \underbrace{\frac{A_{ij}}{s - A_{ii}}}_{G_{ij}(s)} X_j(s)$$

A dynamic perturbation will in general affect both the amplification and the phase lag of $G_{ij}(j\omega)$. To allow for both effects we consider the perturbation

$$G^p_{ij}(s) = G_{ij}(s)(1 + k)e^{-Ts}$$

where k is the relative change in the amplification while e^{-Ts} corresponds to introducing a time-delay of T time units. Rewriting the perturbed system in the form of a relative perturbation

$$G^p_{ij}(s) = G_{ij}(s)\left(1 + \delta_{ij}(s)\right)$$

yields $\delta_{ij}(s) = (1+k)e^{-Ts} - 1$. To determine the maximum robustification obtained by perturbing the edge from x_j to x_i, we solve

$$d\mathbf{R}^{ij} = \sup\{\mathbf{R} \mid |\delta_{ij}(\omega_0)| < \epsilon\} \tag{6}$$

where ω_0 is the frequency corresponding to the minimum robustness radius R. That is, we determine the maximum robustness radius $\mathbf{R}$ over all δ_{ij} with a maximum perturbation size given by ϵ. Since the impact of the perturbation on the robustness radius is a nonlinear function of ϵ, it is useful to perform the computation for different values of ϵ. Note that determination of $d\mathbf{R}^{ij}$ is computationally inexpensive since the perturbation is parametrized by two parameters k and T only, and these are strictly correlated through the constraint $|\delta_{ij}(\omega_0)| < \epsilon$. Thus, one can for instance compute the robustness radius over a grid of values for one of the parameters in order to determine the maximum value of $\mathbf{R}$.

To illustrate the method we consider the oscillatory three component network in the introductory example. See also Figure 1. The robustness radius of the unperturbed model is $\mathbf{R} = 0.022$ implying that a change of only 2% in the activity of the three components will remove the oscillatory behavior. Thus, the model can be said to be relatively unrobust. Computing the robustification for perturbations of the three individual edges using (6) with $\epsilon = 0.01$ we find that $d\mathbf{R}^{ij} = 0.0256$ for all edges, and that the optimal perturbation has $k = 0.004$ and $T = 0.032$. Thus, a 1% perturbation of in the strength of an interaction yields a 17% increase in the robustness radius. A pure time-delay perturbation, with size $\epsilon = 0.01$, yields a 15% increase in the robustness radius. Thus, the maximum robustification is obtained with a combined perturbation of amplification and delay, and as expected the effect is independent of where in the loop the perturbation is added. By increasing the size of the perturbation to $\epsilon = 0.05$ and $\epsilon = 0.1$, respectively, we obtain $d\mathbf{R}^{ij} = 0.039$ and $d\mathbf{R}^{ij} = 0.0543$, respectively. For the latter case, a pure delay is close to optimal. Thus, a 10% relative change in the strength of one of the interactions serves to increase the robustness radius by a factor of 2.5. In order to increase the robustness radius to $d\mathbf{R}^{ij} = 0.1$ we find that we need

to increase ϵ to 0.25, corresponding to introducing a time-delay $T = 0.8\ hr$ in one of the interactions. The impact of this perturbation on the robustness radius is shown in Figure 2, together with the impact of the perturbation on the bifurcation diagram with the Hill coefficient n as the bifurcation parameter. As can be seen, the robustifying perturbation computed at $n = 4.5$ implies that sustained oscillations now exist for a Hill coefficient as low as $n = 2$.

Above we limited ourselves to consider modifying the strength and lag of interactions already included in the model. However, the actual topology of intracellular biochemical networks is usually also uncertain. In particular, it is not always clear which components that affect the kinetics of a given reaction. Furthermore, essentially all models leave out a large number of components that are assumed to have a small impact on the considered biological function. The latter implies that there even are an uncertain number of nodes in a typical network model. The method above, based on adding dynamic perturbation may in principle be used to analyze the impact of adding new edges as well as new nodes on the network robustness. We here limit ourselves to consider adding new edges between existing nodes, i.e., connections for which the nominal Jacobian has $A_{ij} = 0$. The perturbation introduced in this case is given by

$$X_i(s) = \frac{\delta_{ij}(s)}{s - A_{ii}} X_j(s)$$

with $\delta_{ij}(s) = ke^{-Ts}$. For each new edge, we then search for the maximum robustification subject to the constraint $|\delta_{ij}(\omega_0)| = \|\delta_{ij}\|_\infty = |k| < \epsilon$.

For the network in the Introductory example and Figure 1, we find that the addition of an edge from component 2 to component 1 has the largest impact on the robustness. For $\epsilon = 0.01$ we get $d\mathbf{R}^{12} = 0.035$ and with $\epsilon = 0.05$ we get $d\mathbf{R}^{12} = 0.1$. In both cases, the maximum robustifying δ_{12} is found to be a pure positive amplification with $k = \epsilon$ and $T = 0$. This corresponds to closing a positive feedback loop between components 1 and 2, and as can be seen from Figure 2, this has a significant impact on the robustness of the oscillations. This result is interesting in view of the fact that it has been found that the combination of positive and negative feedback loops is a recurring motif in many intracellular oscillators. The positive feedback loop is illustrated in Figure 1 and the effect of the loop on the robustness radius is shown in Figure 2.

The robustifying network modifications identified with the proposed method serve as hypotheses for biological features that have not been included in the original model. Indeed, a key task in modeling intracellular networks is to postulate hypotheses that can be tested against existing knowledge and new experiments. The method proposed above is a systematic way of identifying possible network properties that can be judged against existing data and knowledge, and eventually be used to design new experiments for testing plausible hypotheses.

5. APPLICATION TO THE OSCILLATORY METABOLISM OF ACTIVATED NEUTROPHILS

Neutrophils are white blood cells that activate when sensing the presence of an invader, e.g., in the form of a bacteria or a virus. Upon activation, the cell encapsulates the invader and then destroys it using highly reactive chemicals that are produced in an oscillating metabolic process. An unconfirmed hypothesis is that the oscillations enable the cell to produce high levels of toxics during short transients that are sufficient to kill the invader, while not being harmful to the cell itself. Olsen et al (10) propose a mechanistic lumped model for the oscillatory metabolism. However, *in vivo* the oscillations have been observed to be spatially extended and the authors therefore also attempted to extend the model by dividing it into compartments with mutual exchange driven by diffusion. However, even for very high diffusion rates the authors are not able not reproduce any oscillations. In Jacobsen and Cedersund (2008) it is shown that the model predictions indeed are highly fragile to the introduction of small delays in the involved reactions. We here consider employing the method proposed above to identify plausible model modifications that will improve the robustness and eventually enable a spatial extension.

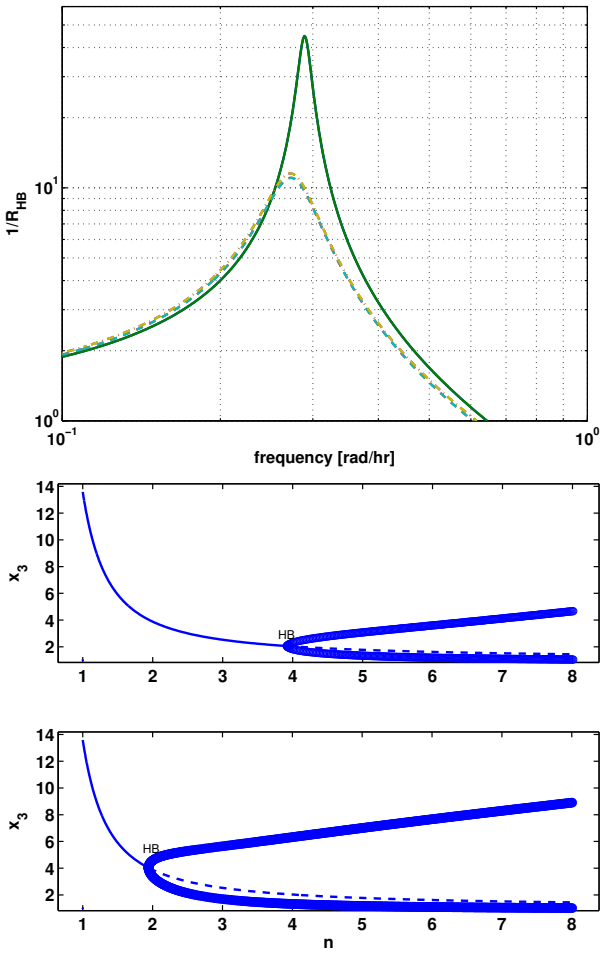

Fig. 2. Three component network. Upper: Reciprocal of robustness radius for nominal (solid), perturbation δ_{32} with $\epsilon = 0.25$ (dashed) and δ_{21} with $\epsilon = 0.1$ (dashdot). Lower: bifurcation diagram for nominal network and after adding perturbation δ_{32} of size $\epsilon = 0.25$.

The metabolic network, illustrated in Figure 3, involves 14 components and a total of 86 direct interactions corresponding to network edges. The nominal model has a

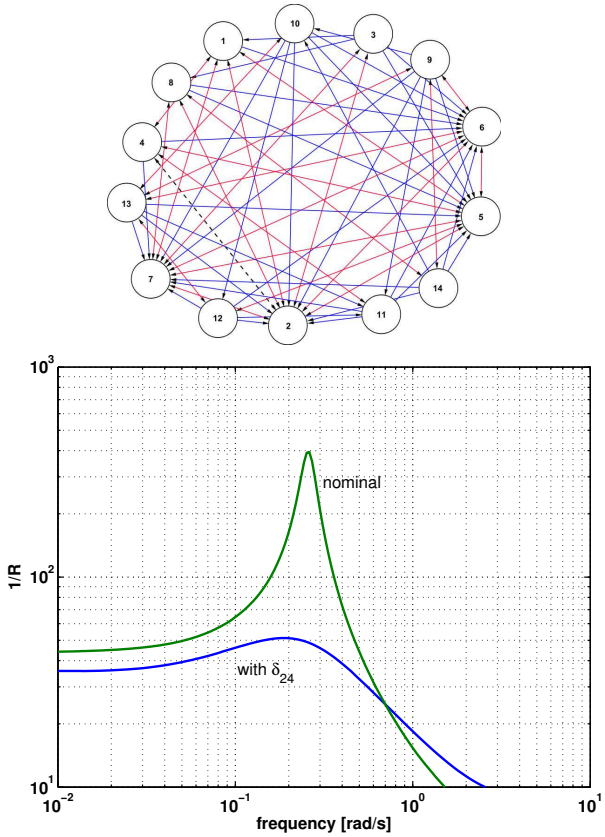

Fig. 3. Neutrophil metabolic network. The dashed edge corresponds to the edge with the largest impact on the robustness of the oscillations. Lower figure: Reciprocal of robustness radius for nominal model (solid) and after relative perturbation δ_{24} with $\epsilon = 0.1$.

robustness radius $\mathbf{R} = 0.0025$ implying that very small changes in the network properties will make the sustained oscillations disappear. This probably also explains the difficulties in extending the model to include spatial phenomena.

Computing the maximum robustification for perturbations of existing individual edges we find that the most effective modification is a perturbation of the effect of metabolite 4 (H_2O_2) on metabolite 2 (coI). For $\epsilon = 0.1$, corresponding to a 10% change in the strength of interaction, we find an almost tenfold increase in the robustness radius to $d\mathbf{R}^{24} = 0.02$. The corresponding perturbation corresponds to an increase in the amplification combined with a small time delay. The change in the amplification can be implemented by modifying the corresponding reaction kinetics as proposed in (9).

Considering the addition of new network edges, the most significant effects are obtained by introducing direct interactions between components residing in different cell compartments which is biochemically irrelevant. However, introducing a small direct effect of $NADPH$ on O_2^- in the main cell compartment has a significant robustifying effect and is also an interaction that is relevant to study closer experimentally.

The effect of the above considered network modifications on the frequency dependent robustness radius of the metabolic oscillations are shown in Figure 3.

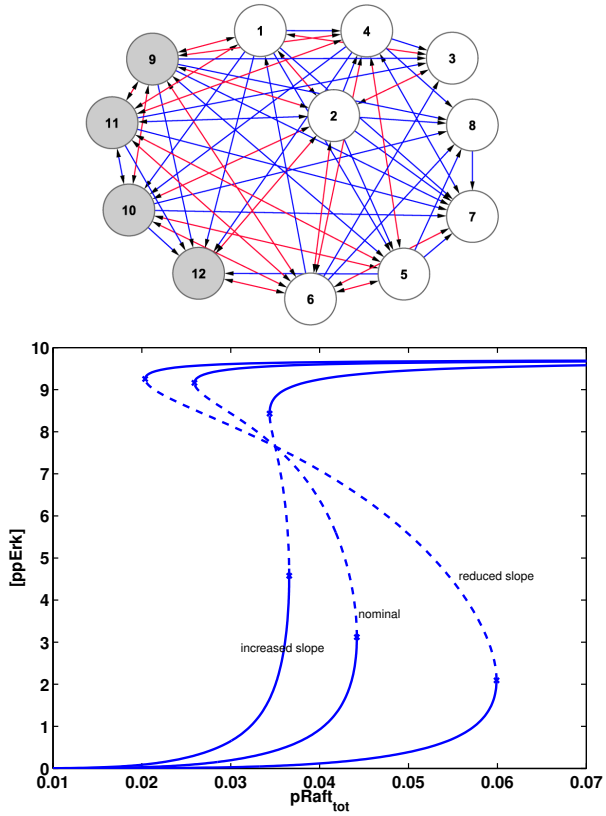

Fig. 4. MAPK signal transduction network in (15). The lower figure shows the bifurcation diagram in terms of response in $ppErk$ as a function of the stimuli $pRad_{tot}$. Also shown are the bifurcation diagrams resulting when decreasing and increasing the kinetic slope V_{64}, respectively, by 50%.

In summary, we find that small and potentially highly relevant modifications of the network interactions in the metabolic network of white blood cells can significantly increase the robustness of the oscillations in activated cells.

6. APPLICATION TO BISTABLE SWITCH IN MAPK SIGNALING

The mitogen-activated protein kinase (MAPK) pathway, one of the most important signal transduction cascades in eukaryotic cells, is involved in the control of crucial cell functions such as cell division, programmed cell death and differentiation. Experimentally it has been shown that the cascade can exhibit an ultrasensitive (12), bistable (13), (14)) or oscillatory response (15) to stimuli. Legewie et al. (15) propose a network model, involving 12 components and a total of 84 direct interactions, which displays a bistable response. The network and the bifurcation diagram with the stimuli $pRaf_{tot}$ as the bifurcation parameter is shown in Figure 4. The robustness radius for the unstable steady-state at $pRaf_{tot} = 0.035$ is $\mathbf{R} = R(0) = 0.00072$, which reflects a poor robustness of the bistable behavior.

Since bistability is related to purely static bifurcations, robustifying modifications will also be static. As discussed in Waldherr et al. (9), implementing static perturbations of individual edges, i.e., Jacobian elements, in a biochemically meaningful way can be a challenging task. As they

propose, one can instead consider decomposing the Jacobian into a stoichiometric part S and a kinetic part V according to

$$A = S\frac{\partial v}{\partial x}\bigg|_{x^*} = SV(x^*) \tag{7}$$

and perturb elements of the kinetic slopes V. Here $v(x)$ are the expressions for the reaction kinetics. Note, that a perturbation in some element of V usually will result in perturbation of several edges in the network.

We employ the method for robustification proposed above, but apply the (static) perturbations to the kinetic slopes V instead of directly to the Jacobian A. Considering first perturbing non-zero kinetic slopes, we find that the most robustifying modification involves decreasing the slope V_{64}, i.e., the dependency of reaction rate v_6 on component x_4. With $\epsilon = 0.5$, corresponding to halving the slope V_{64}, the robustness radius is doubled to $\mathbf{R} = 0.0015$. Although this may seem a small increase in robustness, it has a significant impact on the width of the bistable region as can be seen in Figure 4. Considering adding new kinetic dependencies, we find that introducing a small kinetic slope $V_{36} = -0.001$ increases $\mathbf{R}$ to 0.0017 while making $V_{36} = -0.01$ increases the robustness radius by a factor 13 to $\mathbf{R} = 0.01$. Introducing a non-zero slope V_{36} corresponds to making the degradation of $pRafMek$ depend on $pERK$ which is not implausible since these two components interact in the network.

7. CONCLUSIONS

The fact that normal biological functions have developed significant robustness through evolution implies that also models of the underlying biochemical networks should be robust to biologically probable perturbations. Indeed, some form of robustness analysis is used more or less routinely as part of the validation of biochemical network models. However, systematic tools to aid in identifying model modifications that will serve to increase the robustness are lacking.

In this paper we proposed a method for systematic robustification of biochemical network models based on adding generic dynamic perturbations to existing network edges, or by introducing new nodes or edges. The robustness was quantified by computing the smallest change in the activity of the network components that would induce a bifurcation in the model, and hence a qualitative change in the predicted behavior. The method was first demonstrated by application to a simple 3 component feedback loop, used to model e.g., circadian oscillations, for which it was shown that the method automatically identified the intuitively plausible addition of a delay in the loop as the modification of the existing network structure with the largest impact on the robustness. However, the method also found that modifying the network topology corresponding to closing a positive feedback loop between two components had a similar effect on the robustness. This is an interesting result as intertwined negative and positive feedback loops appears to be a recurring motif in intracellular oscillators. Finally, we identified relatively small modifications of the interactions in the complex metabolic network of neutrophils that served to increase the robustness by more than an order of magnitude. It was also shown how small changes in a single kinetic slope could significantly increase the robustness of the bistable switch in MAPK signaling. It was stressed that the identified model modifications should serve as hypotheses that need to be evaluated against existing biological data and knowledge as well as against dedicated experiments.

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