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Kinetic perturbations as robustness analysis tool for biochemical reaction networks

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Abstract— **Models of biochemical reaction networks can be decomposed into a stoichiometric part and a kinetic part. The stoichiometric part describes the structural mass flows while the kinetic part describes how the flow rates vary with substrate concentrations and regulatory interactions. Herein a method for analyzing the robustness of biochemical networks with respect to perturbations of the kinetic part is proposed. In particular, we consider a class of perturbations that modify the local kinetic slopes while leaving the reaction flow rates in steady state unchanged. A method for computing the associated robustness radii for perturbations of single or multiple kinetic slopes is devised. The corresponding non-robust perturbations can be implemented in the original nonlinear model through specific parameter variations described by the perturbation class. The proposed method is illustrated through application to the Huang–Ferrell model of MAPK signaling cascades. In particular, we compute the smallest kinetic perturbations that translate the nominal utltrasensitive response into a bistable and oscillatory response, respectively. The results are highly relevant since MAPK cascades are conserved pathways known to produce bistability as well as sustained oscillations depending on the context in which they operate.**

I. INTRODUCTION

Robustness of biochemical reaction networks has become an important issue in systems biology. Typically, robustness of qualitative properties is of interest, with the most frequently studied property being the qualitative dynamical behaviour of a biochemical network. As perturbations, generic variations in biochemical parameters are often considered. Such perturbations suggest the use of analysis tools based on bifurcation analysis, which may however be challenging in a high-dimensional parameter space [1], [2], [3]. To a certain extent, measures based on the structured singular value have been proposed for the robustness analysis of biochemical reaction networks [4], [1]. Another approach is the consideration of structural uncertainties, which may e.g. arise from unmodelled dynamics [5], [6]. For such perturbations, the use of classical robust control tools is very efficient, but it is sometimes not so clear how to interpret the resulting non-robust perturbations.

In this paper, we aim to develop a notion which is an intermediate between parametric and structural uncertainties. The basic idea is to consider static variations in the reaction rate expressions that leave the steady state reaction rates unaffected, but lead to changes in the reaction rate slopes. Such perturbations are defined in this paper as *kinetic perturbations*. A systematic approach to analyse the robustness of the qualitative dynamical behaviour with respect to kinetic perturbations is proposed. It turns out that the problem can be transformed nicely to a classical setup studied extensively in robust control theory, and admits explicit solutions in relevant simple cases.

The paper is structured as follows. In Section II, we develop the theory of kinetic perturbations and show that it applies naturally to common biochemical modelling frameworks. The use of kinetic perturbations for robustness analysis is discussed in Section III. Section IV contains the application of the proposed robustness analysis method to the Huang–Ferrell model of the MAPK signalling cascade.

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II. THEORY OF KINETIC PERTURBATIONS

A. Definition of a kinetic perturbation

Consider a biochemical reaction network described by the ordinary differential equation

$$\dot{x} = Sv(x), \tag{1}$$

where $x \in \mathbb{R}^n$ is the concentration vector, $v(x) \in \mathbb{R}^m$ the reaction flux vector, and $S \in \mathbb{R}^{n\times m}$ the stoichiometric matrix.

We are interested in analysing the dynamical behaviour of the network around a given steady state x_0, i.e. $Sv(x_0) = 0$. The corresponding steady state reaction fluxes are denoted as $v_0 = v(x_0)$. For ease of notation, we will frequently write $V = \frac{\partial v}{\partial x}$. Locally around x_0, the dynamical behaviour is characterised by the Jacobian of the system (1) evaluated at x_0, which we denote by

$$A = SV(x_0). \tag{2}$$

From a biochemical perspective, the stoichiometric matrix contains the structural mass flow interactions, while the reaction flux vector $v(x)$ describes the dependence of reaction rates on the substrate concentrations, and also contains the structural regulatory interactions.

Perturbations to the system that leave the mass flow structure unaffected can therefore be characterised by a change of the reaction flux vector, yielding the perturbed system

$$\dot{x} = S\tilde{v}(x), \tag{3}$$

where $\tilde{v}(x)$ is the perturbed reaction flux vector.

As can be seen from (2), the Jacobian depends on the stoichiometry as well as the local kinetic slopes, i.e., the rate derivatives with respect to reactants at the steady state.

A perturbation of some kinetic parameter will in general have two separate effects on the local kinetic slopes. First, the perturbation will modify the slope of the corresponding reaction. Second, the perturbation will in general also affect the reaction rates and thereby the steady state of the complete network. This secondary effect implies that the local slopes of all reaction rates will change as a result of a single parameter perturbation, something that complicates the robustness analysis and, more importantly, obscures structural insight. The fact that steady state changes affect most interactions in the network simultaneously is a major obstacle for the efficient application of bifurcation analysis techniques to the problem of parametric robustness. Here, we introduce *kinetic perturbations*, which affect the steady state Jacobian $V(x_0)$ of the reaction fluxes, but leave the stationary fluxes v_0, and as a consequence also the steady state concentrations x_0, unchanged.

Definition 1: The system (3) is said to be subject to a *kinetic perturbation*, if

$$\tilde{v}(x_0) = v_0. \tag{4}$$

From condition (4), we obtain directly $S\tilde{v}(x_0) = Sv_0 = 0$, and conclude that x_0 is also a steady state of the perturbed system.

Perturbations are often related to parameter variations in a parameter dependent reaction flux vector $v(x,p)$, where $p \in \mathbb{R}^q$ are reaction parameters. The nominal reaction flux vector is then given by $v(x) = v(x,p)$, and the perturbed reaction rate vector is

$$\tilde{v}(x) = v(x,\tilde{p}), \tag{5}$$

with perturbed parameters $\tilde{p}$.

Kinetic perturbations of parametrised reaction rate expressions can also be related to structural perturbations by introducing implicit parameters. With the term "implicit parameters", we denote any parameters that do not appear in the nominal model (1), but are introduced as free parameters only during the model analysis. Reasons for this might be that these parameters are supposed to be fixed at trivial constant values through choice of the model class (as in mass action networks), or that they are related to interactions within the system that have been neglected in formulating the nominal model. Still, perturbations that affect implicit parameters may be relevant to the system and thus should be considered in robustness analysis.

While the steady state reaction rates are not affected by a kinetic perturbation, the reaction rate Jacobian at the steady state will in general change. Similar to the nominal case, we denote $\tilde{V} = \frac{\partial \tilde{v}}{\partial x}$. The change in the reaction rate Jacobian V at the steady state is denoted by

$$\bar{\Delta} = \tilde{V}(x_0) - V(x_0). \tag{6}$$

The perturbed Jacobian of the system at the steady state x_0 will be denoted by $\tilde{A} \in \mathbb{R}^{n\times n}$ and is given by

$$\tilde{A} = S\tilde{V}(x_0) = A + S\bar{\Delta}. \tag{7}$$

We conclude that as far as the dynamical behaviour in the neighbourhood of the steady state x_0 is concerned, any kinetic perturbation is completely characterised by the perturbation matrix $\bar{\Delta}$.

For kinetic perturbations introduced by parameter variations, it is important that for any given perturbation matrix $\bar{\Delta}$, the corresponding parameter change is determined uniquely. In the following section, we show that the parameter change corresponding to a given $\bar{\Delta}$ can be computed analytically for the common modelling framework of generalised mass action networks. Similar results can be derived for enzyme reaction networks with Michaelis-Menten kinetics, and metabolic networks where enzymes are subject to allosteric regulation.

B. Kinetic perturbations in GMA networks

In a GMA network [7], reaction rates are given by the expression

$$v_i(x) = k_i \prod_{j=1}^{n} x_j^{\alpha_{ij}}, \quad i = 1,\dots,m, \tag{8}$$

where $k_i > 0$ is the nominal reaction rate constant, and $\alpha_{ij} \in \mathbb{R}$ is the nominal kinetic order of the j-th species. Notice that as a generalisation from classical mass action networks, non-integer kinetic orders α_{ij} are allowed in GMA networks. Such kinetic orders are supported by simulation studies of reaction systems under diffusion constraints [8], or may represent the aggregation of mechanistic detail in a single reaction step [7]. For a GMA network with reaction rates as given in (8), a parameter vector p is introduced that contains the rate constants k_i as well as the kinetic orders α_{ij}, $i = 1,\dots,m$, $j = 1,\dots,n$.

If the nominal model is a classical mass action network, the α_{ij} can be considered as implicit parameters, fixed to the values given by the stoichiometry of the reaction. Also, a value of $\alpha_{ij} = 0$ means that the j-th species does not affect the i-th reaction. Changing this value away from 0 then corresponds to a structural perturbation of the nominal model.

In the following, we derive explicit expressions for parameter variations in a GMA network subject to a kinetic perturbation. From (4), a kinetic perturbation is characterised by the condition

$$\tilde{k}_i \prod_{j=1}^{n} x_{0,j}^{\tilde{\alpha}_{ij}} = k_i \prod_{j=1}^{n} x_{0,j}^{\alpha_{ij}}$$

or equivalently

$$\tilde{k}_i = k_i \prod_{j=1}^{n} x_{0,j}^{\alpha_{ij}-\tilde{\alpha}_{ij}}.$$

Considering the elements of the reaction rate Jacobian V, we obtain

$$V_{ij}(x) = \alpha_{ij} x_j^{-1} v_i(x)$$

and a kinetic perturbation where the reaction rate Jacobian is changed additively by $\bar{\Delta}$ is given by

$$\tilde{\alpha}_{ij} x_{0,j}^{-1} v_{0,i} = \alpha_{ij} x_{0,j}^{-1} v_{0,i} + \bar{\Delta}_{ij},$$

or equivalently

$$\tilde{\alpha}_{ij} - \alpha_{ij} = \frac{x_{0,j}}{v_{0,i}} \bar{\Delta}_{ij}.$$

In what follows, let

$$\Delta = (\operatorname{diag} v_0)^{-1} \bar{\Delta} \operatorname{diag} x_0, \tag{9}$$

where $\Delta \in \mathbb{R}^{m \times n}$ is a suitably scaled perturbation matrix. Notice that this scaling is only possible if both x_0 and v_0 have only non-zero components. If this is not the case, the relation of the perturbation $\bar{\Delta}$ to the parameter perturbation has to be computed differently.

In terms of Δ, kinetic perturbations by parameter variations in a GMA network are characterised by

$$\tilde{\alpha}_{ij} = \alpha_{ij} + \Delta_{ij} \tag{10}$$

and

$$\tilde{k}_i = k_i \prod_{j=1}^{n} x_{0,j}^{-\Delta_{ij}}. \tag{11}$$

In conclusion, we see that kinetic perturbations of a GMA network change the kinetic order of species in reactions, while at the same time adjusting the reaction rate constants to keep steady state reaction rates and steady state concentration values unperturbed. This result offers an illustrative biochemical interpretation of kinetic perturbations. Biochemically, a positive Δ_{ij} corresponds to an increase in the cooperativity of reaction i with respect to species j, while a negative Δ_{ij} corresponds to an increase in the saturation.

III. ROBUSTNESS ANALYSIS

A. Problem statement

The problem of local robustness analysis is to evaluate the effects of perturbations on the dynamical behaviour of the system (1) in a neighbourhood of the steady state x_0. Since steady state concentration values and reaction fluxes are often well characterised for biochemical networks, we are specifically interested in perturbations which do not affect these values, i.e. kinetic perturbations.

Using kinetic perturbations with a scaled uncertainty matrix Δ, the perturbed Jacobian becomes

$$\tilde{A}(\Delta) = A + S \operatorname{diag}(v_0) \Delta (\operatorname{diag} x_0)^{-1}. \tag{12}$$

Notice that this representation of the perturbed Jacobian does not rely on the results of Section II-B and is independent of the considered network class.

In general, the goal of robustness analysis is the computation of the smallest perturbation that leads to a qualitative change in the dynamical behaviour of the system. To do this analysis with kinetic perturbations, one first needs to introduce a measure for the perturbation strength. This is commonly done by considering a suitable operator norm $\|\Delta\|$. Next, define the robustness radius of the system (1) as the smallest perturbation for which the perturbed Jacobian $\tilde{A}(\Delta)$ has an eigenvalue on the imaginary axis:

$$R = \inf\{\|\Delta\| \mid \sigma(\tilde{A}(\Delta)) \cap \mathbf{j}\mathbb{R} \neq \emptyset\}, \tag{13}$$

where $\sigma(A)$ denotes the spectrum of a square matrix A. In local robustness analysis, two goals are ususally pursued. The first goal is to compute the robustness radius R, or at least lower and upper bounds. As a second goal, we want to compute a minimum-size non-robust perturbation Δ^* such that $\|\Delta^*\| = R$ and $\tilde{A}(\Delta^*)$ has an eigenvalue on the imaginary axis. For GMA networks, a corresponding parameter vector $\tilde{p}^*$ can be obtained through the expressions derived in Section II-B.

Due to the proposed reformulation, the robustness problem with respect to kinetic perturbations is equivalent to a real μ–problem. From classical robust control theory, the μ–value is defined as [9]

$$\mu_{\mathbf{\Delta}}(G(\mathbf{j}\omega)) = \big(\inf\{\|\Delta\| \mid \Delta \in \mathbf{\Delta} \subset \mathbb{R}^{m \times n}, \det(I_m - \Delta G(\mathbf{j}\omega)) = 0\}\big)^{-1}.$$

For the perturbed Jacobian in (12), the transfer function $G : \mathbb{C} \to \mathbb{C}^{n \times m}$ that needs to be considered is given by

$$G(\mathbf{j}\omega) = (\operatorname{diag} x_0)^{-1} (\mathbf{j}\omega I_n - A)^{-1} S \operatorname{diag} v_0. \tag{14}$$

Computation of the robustness radius from the μ–value is then a standard problem in robust control theory [9], with the solution

$$R = \Big(\sup_{\omega} \mu_{\mathbf{\Delta}}(G(\mathbf{j}\omega))\Big)^{-1}. \tag{15}$$

Computation of the μ–value is a difficult problem in general. For general matrix perturbations, usually only lower and upper bounds can be obtained. Fortunately, in the analysis of biochemical reaction networks, already scalar and vector perturbations provide useful results. These cases are discussed in the following two sections.

B. Scalar perturbations

The scalar case is encountered if we assume that all elements of Δ apart from one are zero. For a biochemical reaction network, this corresponds to the case where only the influence of a single species on a single reaction rate is subject to a perturbation. In the analysis of biochemical networks, this approach will be useful for the detection of single fragile interactions. In terms of the robust control approach outlined in Section III-A, such a perturbation translates into a scalar uncertainty problem, for which the robustness radius and a non-robust perturbation are easily computed.

Assume that the derivative V_{ij} of reaction i with respect to the species j is subject to perturbations. Then we have

$$\Delta = e_i^m \Delta_{ij} e_j^{n\mathrm{T}}, \tag{16}$$

with the uncertainty $\Delta_{ij} \in \mathbb{R}$, where $e_i^m \in \mathbb{R}^m$ ($e_j^n \in \mathbb{R}^n$) is the unit vector in the i–th (j–th) coordinate direction. The perturbed Jacobian is then given by

$$\tilde{A}(\Delta) = A + S \operatorname{diag}(v_0) e_i^m \Delta_{ij} e_j^{n\mathrm{T}} (\operatorname{diag} x_0)^{-1}. \tag{17}$$

Denote $B = S \operatorname{diag}(v_0) e_i^m$ and $C = e_j^{n\mathrm{T}} (\operatorname{diag} x_0)^{-1}$. Define the transfer function

$$G(\mathbf{j}\omega) = C(\mathbf{j}\omega I_n - A)^{-1} B. \tag{18}$$

For the robustness radius we obtain [9]

$$R = \left(\sup_{\omega} \mu_{\mathbf{\Delta}}(G(\mathbf{j}\omega)) \right)^{-1} = \left(\sup_{\omega \in \mathcal{R}_G} |G(\mathbf{j}\omega)| \right)^{-1}, \quad (19)$$

where

$$\mathcal{R}_G = \{\omega \in \mathbb{R} : \mathrm{Im}(G(\mathbf{j}\omega)) = -\omega C(\omega^2 I + A^2)^{-1} B = 0\}$$

is the realness locus of $G(\mathbf{j}\omega)$.

The remaining task is now to construct a minimum–norm non-robust perturbation Δ_{ij}^*, for which the Jacobian $\tilde{A}(\Delta)$ has an eigenvalue on the imaginary axis. To this end, let $\omega^* = \arg\max_{\omega \in \mathcal{R}_G} |G(\mathbf{j}\omega)|$. A minimum–norm non-robust scalar perturbation is then given by

$$\Delta_{ij}^* = \left(G(\mathbf{j}\omega^*) \right)^{-1}. \quad (20)$$

C. Vector perturbations

We can distinguish two perturbation cases which both lead to a vector uncertainty. In the first case, all elements of the perturbation matrix Δ apart from one column are equal to zero. Biochemically, this corresponds to a simultaneous perturbation of the influence of one species on several reactions. In the second case, all elements of Δ apart from one row are equal to zero, which corresponds to a perturbation of the influence of several species on one reaction. Both cases are of interest for biochemical networks, because medical drug development currently focuses on tackling a single target to achieve a medical effect [10]. Such targets may either be a single molecular species or a single reaction, corresponding to the two vector perturbation cases.

Let us first consider the case where the influence of several species on a single reaction is subject to perturbations. Denote the index of the perturbed reaction as i and the indices of the affected species as $j_1, \ldots, j_{n_j}$. Define e_i^m, e_j^n as in Section III-B and let

$$E_x = (e_{j_1}^n, \ldots, e_{j_{n_j}}^n).$$

The perturbation matrix can then be written as

$$\Delta = e_i^m \Delta_{i\bullet} E_x^{\mathrm{T}},$$

with the vector uncertainty $\Delta_{i\bullet} \in \mathbb{R}^{1 \times n_j}$. Then the perturbed Jacobian is given by

$$\tilde{A}(\Delta) = A + S \,\mathrm{diag}(v_0) e_i^m \Delta_{i\bullet} E_x^{\mathrm{T}} (\mathrm{diag}\, x_0)^{-1}. \quad (21)$$

1) Computation of the robustness radius: Define the transfer function

$$G(\mathbf{j}\omega) = E_x^{\mathrm{T}} (\mathrm{diag}\, x_0)^{-1} (\mathbf{j}\omega I_n - A)^{-1} S \,\mathrm{diag}(v_0) e_i^m. \quad (22)$$

The transfer function G is split into its real and imaginary part via

$$G(\mathbf{j}\omega) = X(\omega) + \mathbf{j} Y(\omega),$$

where $X(\omega), Y(\omega) \in \mathbb{R}^{n_j}$.

The robustness radius is computed as [9, Th. 5.3.16]

$$R = \left(\sup_{\omega} \mathrm{dist}_p(X(\omega), \mathbb{R}Y(\omega)) \right)^{-1}, \quad (23)$$

where

$$\mathrm{dist}_p(X, \mathbb{R}Y) = \min_{\alpha \in \mathbb{R}} \|X - \alpha Y\|_p$$

is the distance of X to the linear subspace with basis Y, measured in the p–norm. If $Y = 0$, then $\mathrm{dist}_p(X, \mathbb{R}Y) = \|X\|_p$. For the 2–norm, we have the explicit formula

$$\mathrm{dist}_2(X, \mathbb{R}Y) = \left(\|X\|_2^2 - \frac{\langle X, Y \rangle^2}{\|Y\|_2^2} \right)^{\frac{1}{2}}.$$

For the 1– and ∞–norms, the distance is obtained by solving a linear program:

$$\mathrm{dist}_1(X, \mathbb{R}Y) = \min_{\alpha \in \mathbb{R}, t \in \mathbb{R}_+^{n_j}} \mathbf{1}^{\mathrm{T}} t \quad \text{s.t. } -t \le X - \alpha Y \le t$$

$$\mathrm{dist}_\infty(X, \mathbb{R}Y) = \min_{\alpha \in \mathbb{R}, t \in \mathbb{R}_+} t \quad \text{s.t. } -t\mathbf{1} \le X - \alpha Y \le t\mathbf{1},$$

where $\mathbf{1} \in \mathbb{R}^{n_j}$ is a vector with all elements equal to 1, and the inequalities are to be taken element-wise.

Note that in the computation of dist_p, we have to use the dual of the norm which we use to measure the uncertainty $\Delta_{i\bullet}$. So to compute the robustness radius for $\Delta_{i\bullet}$ measured in the 1–norm, dist_∞ should be used, and vice versa. The 2–norm is self-dual.

2) Construction of non-robust perturbations: At a given frequency $\omega^* \in \mathbb{R}$, a non-robust perturbation is characterised by the condition

$$\det(I - \Delta_{i\bullet} G(\mathbf{j}\omega^*)) = 0, \quad (24)$$

which results in an eigenvalue at $\mathbf{j}\omega^*$ for the perturbed system.

In the vector case, the product $\Delta_{i\bullet} G(\mathbf{j}\omega^*)$ is scalar. Thus condition (24) is equivalent to $1 = \Delta_{i\bullet} G(\mathbf{j}\omega^*)$ or

$$\begin{aligned} \Delta_{i\bullet} X(\omega^*) &= 1 \\ \Delta_{i\bullet} Y(\omega^*) &= 0. \end{aligned} \quad (25)$$

Now if $R < \infty$, choose ω^* such that $\mu_{\mathbf{\Delta}}(\omega^*) = R^{-1} > 0$. By construction, $X(\omega^*) \neq 0$ and $X(\omega^*) \neq \alpha Y(\omega^*)$ for any $\alpha \in \mathbb{R}$. Thus (25) is guaranteed to have a solution, which however will not be unique in general. We are typically interested in obtaining a solution of minimum norm. Depending on the norm that is used, different non-robust perturbations are obtained. An efficient computation of non-robust perturbations is possible with the 1-, 2-, and ∞-norms. To keep the exposition concise, we give the results for the 1-norm only. Using this norm is the biologically most plausible choice, if perturbations on the different interactions are assumed to act independently from each other.

For ease of notation, we write (25) as $M \Delta_{i\bullet}^{\mathrm{T}} = b$ in the following. Let K be a matrix whose columns span the kernel of M and $\kappa = \dim \mathrm{kern}\, M$. Then all solutions of $M \Delta_{i\bullet}^{\mathrm{T}} = b$ are parametrised by $\Delta_{i\bullet}^{\mathrm{T}} = \Delta_{i\bullet,0}^{\mathrm{T}} + K\lambda$, with $\lambda \in \mathbb{R}^\kappa$, where $\Delta_{i\bullet,0}$ is any solution of $M \Delta_{i\bullet}^{\mathrm{T}} = b$. For a solution $\Delta_{i\bullet}^*$ of minimum 1-norm, the parameter λ is taken from the solution of the linear program

$$\min_{t \in \mathbb{R}_+^{n_j},\ \lambda \in \mathbb{R}^\kappa} \mathbf{1}^{\mathrm{T}} t$$
$$\text{s.t. } t \le \Delta_{i\bullet,0}^{\mathrm{T}} + K\lambda \le t.$$

In the case where he influence of a single species on several reactions is subject to a perturbation, a column vector uncertainty can be used to describe Δ. This is the dual to the previous case, where we had the row vector uncertainty $\Delta_{i\bullet}$. Up to this duality, the analysis of this case is equivalent to the analysis of the row vector case.

The results of Sections III-B and III-C thus provide efficient ways to compute a robustness measure for dynamical properties with respect to kinetic perturbations and the associated non-robust perturbations. For the class of GMA networks, these perturbations can be related to actual parameter variations by the results of Section II-B.

IV. APPLICATION TO THE MAPK CASCADE

The Mitogen-Activated Protein Kinase (MAPK) cascade is involved in a large number of eukaryotic signal transduction pathways, controlling functions such as cell division, differentiation and apoptosis and playing a key role in development of cancer. It is also one of the most studied and modeled intracellular signaling pathways [11]. One of the first models [12] predicted an ultrasensitive response from stimuli to activated MAPK. However, experimental evidence reveals that the cascade in certain contexts displays a bistable response [13] while in others the response to stimuli may be sustained oscillations [14]. Subsequent models have embedded the signaling cascade in positive [13] and negative feedback loops [15] from response to stimuli, to reproduce bistability and sustained oscillations, respectively. Markevich et al [16] show, by including more detailed kinetic models, that bistability can be predicted also in the absence of external feedback. Based on extensive parameter searches, Qiao et al. [2] show that even the original Huang-Ferrell model can display bistability and sustained oscillations in some parameter regions. Here we employ the robustness analysis proposed above to determine if perturbations of existing interactions, or addition of new interactions, in the nominal Huang and Ferrell model can translate the ultrasensitive response into bistable or oscillatory responses.

The Huang-Ferrell model involves 22 biochemical components, but due to 7 moiety conservations only 15 independent states. The components interact through a total of 30 reactions, described using mass action kinetics and imposing a total of 61 direct binary interactions in the network. A schematic of the network and the nominal input-output response is shown in Figure 1. The nominal stimulus considered here corresponds to $E1_{tot} = 3 \cdot 10^{-6}\,\mu$M.

For this example, we consider scalar perturbations only, and compute the robustness radii R and corresponding non-robust perturbations Δ^* using (19) and (24), respectively. Note that, under certain non-degeneracy conditions, a non-robust perturbation imposes a bifurcation in the corresponding nonlinear network. In particular, if the non-robust perturbation is at frequency $\omega^* = 0$ the network will undergo a saddle-node bifurcation while for $\omega^* \neq 0$ the system will undergo a Hopf bifurcation. Saddle-node bifurcations underly bistability, while Hopf bifurcations result in sustained oscillations. Table I shows some of the smallest non-robust

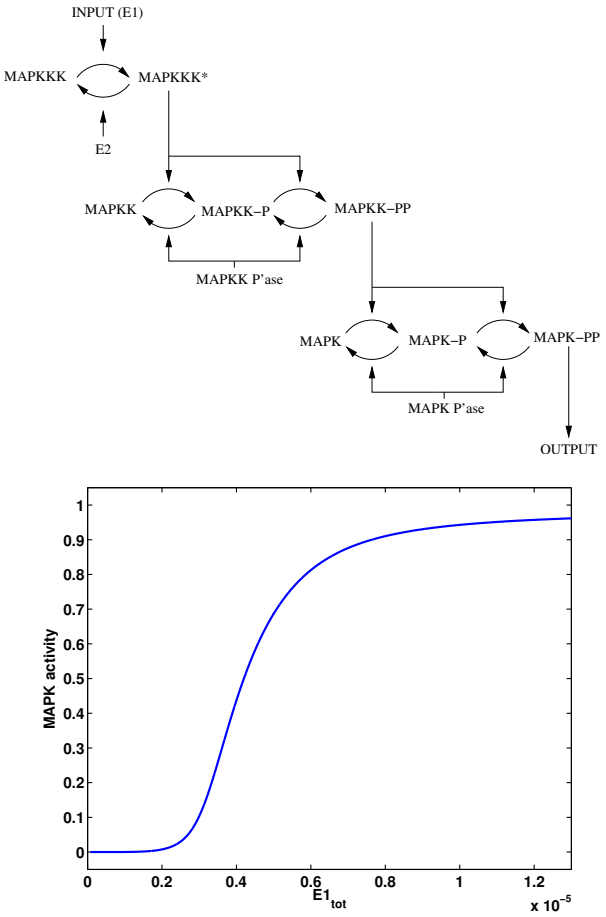

Fig. 1. MAPK cascade. Schematic of phosphorylation cascade and steady-state input-output response curve.

perturbations required to induce saddle-node (SN) and Hopf (HB) bifurcations for perturbation of existing ($\alpha_{ij} \neq 0$) as well as non-existing binary interactions ($\alpha_{ij} = 0$).

As can be seen from Table I, it is possible to induce bistability as well sustained oscillations by perturbing a single existing interaction, or by introducing one new interaction. The smallest non-robust perturbation corresponds to adding a feedback from the cascade output, activated MAPK, to the first reaction in the cascade (reaction 4 in Table I) and corresponds to the positive feedback loop proposed in [13]. As can be seen from the table, a similar effect can also be obtained by a shorter feedback path from activated MAPK to the second or third level of the cascade (reactions 13 and 25, respectively). However, it is also interesting to note that all the observed qualitative behaviors in MAPK signaling cascades can be replicated by the Huang-Ferrell model simply by tuning the kinetics of a single reaction. In particular, modifying the kinetics of the decomposition of KPase-MAPKPP complex can induce a saddle-node bifurcation as well as a Hopf bifurcation. The input-output response curve after perturbing this reaction with $\Delta_{30,15} = -0.91$ is shown in Figure 2. As can be seen, the perturbation results in two saddle-node and two Hopf bifurcation points, imply-

TABLE I

NON-ROBUST PERTURBATIONS FOR SCALAR KINETIC PERTURBATIONS OF THE MAPK CASCADE.

Reaction	Component	α_{ij}	Δ^*_{ij}	ω^*
30	KPase-MAPKPP	1	-0.91	0 (SN)
18	KKPase-MAPKKPP	1	-0.98	0 (SN)
1	MAPKPP	0	0.150	0 (SN)
4	MAPKPP	0	-0.149	0 (SN)
4	KPase	0	0.52	0 (SN)
13	MAPKPP	0	0.37	0 (SN)
25	MAPKPP	0	0.90	0 (SN)
30	KPase-MAPKPP	1	-0.79	0.24 (HB)
30	MAPKPP	0	-0.94	0.25 (HB)

The reaction numbers are: 30 – decomposition of KPase-MAPKPP, 18 – decomposition of KKPase-MAPKKPP, 1 – first step in phosphorylation of MAPKKK, 4 – first step in dephosphorylation of MAPKKKP, 13 – first step in phosphorylation of MAPKKP, 25 – first step in phosphorylation of MAPKP,

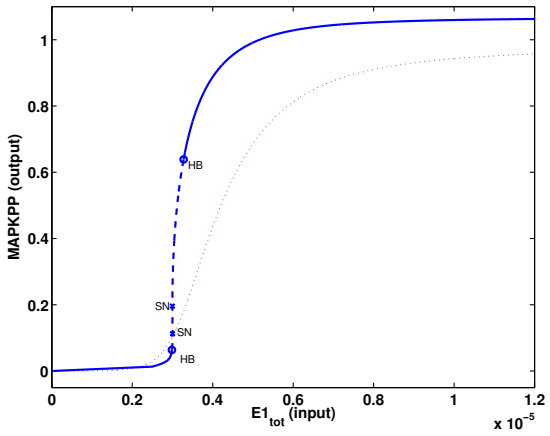

Fig. 2. MAPK cascade. Input-output response after a scalar non-robust perturbation $\Delta_{30,15} = -0.91$ of the reaction kinetics for decomposition of KPase-MAPKPP complex. SN – saddle-node, HB – Hopf bifurcation.

ing that the perturbed systems displays a narrow range of multistationarity as well as sustained oscillations. Increasing the size of the perturbation will increase this region.

In conclusion, the analysis of the MAPK cascade does not reveal any severe fragilities for the perturbation of existing interactions, but shows that the qualitative predictions of the Huang-Ferrell model can be changed by adding relatively weak additional interactions to the network.

V. CONCLUSIONS

The paper introduces kinetic perturbations as new uncertainty class for biochemical reaction networks. It is shown that these perturbations are directly related to variations in the reaction order for general mass action networks. The advantage of kinetic perturbations is that the robustness analysis problem can be solved using the well developed theory of linear robust control, and even admits exact solutions for the robustness radius in the scalar and vector uncertainty cases. The motivation for using kinetic perturbations is that steady state concentration values and reaction fluxes are often well characterized in biochemical networks, whereas the exact reaction kinetics are much less known. In this respect, an important application of robustness analysis with kinetic perturbations will be model validation. It should also be pointed out that our approach does not require an explicit model of the network, apart from the problem of relating kinetic perturbations to parameter variations. It is in fact sufficient to know the steady state concentrations, reaction fluxes, and Jacobian elements for the robustness analysis, which can be inferred more easily from experiments than explicit rate expressions [17].

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REFERENCES

[1] J. Kim, D. G. Bates, I. Postlethwaite, L. Ma, and P. A. Iglesias, "Robustness analysis of biochemical network models," *IEE Proc. Syst. Biol.*, vol. 153, pp. 96–104, May 2006.

[2] L. Qiao, R. B. Nachbar, I. G. Kevrekidis, and S. Y. Shvartsman, "Bistability and oscillations in the Huang–Ferrell model of MAPK signaling," *PLoS Comp. Biol.*, vol. 3, p. e184, 9 2007.

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

[4] L. Ma and P. A. Iglesias, "Quantifying robustness of biochemical network models," *BMC Bioinform.*, vol. 3, p. 38, 2002.

[5] E. W. Jacobsen and G. Cedersund, "Structural robustness of biochemical network models—with application to the oscillatory metabolism of activated neutrophils," *IET Syst. Biol.*, vol. 2, pp. 39–47, Jan 2008.

[6] C. Trané and E. W. Jacobsen, "Network structure and robustness of intracellular oscillators," in *Proc. of the 17th IFAC World Congress, Seoul, Korea*, 2008, pp. 10 989–94.

[7] J. Vera, E. Balsa-Canto, P. Wellstead, J. R. Banga, and O. Wolkenhauer, "Power-law models of signal transduction pathways," *Cell. Signal.*, vol. 19, pp. 1531–41, Jul 2007.

[8] R. Kopelman, "Fractal reaction kinetics." *Science*, vol. 241, pp. 1620–26, Sep 1988.

[9] D. Hinrichsen and A. J. Pritchard, *Mathematical Systems Theory I*. Berlin: Springer-Verlag, 2005.

[10] E. C. Butcher, E. L. Berg, and E. J. Kunkel, "Systems biology in drug discovery," *Nat. Biotechnol.*, vol. 22, pp. 1253–59, Oct 2004.

[11] J. J. Hornberg, B. Binder, F. J. Bruggeman, B. Schoeberl, R. Heinrich, and H. V. Westerhoff, "Control of MAPK signalling: from complexity to what really matters." *Oncogene*, vol. 24, pp. 5533–5542, Aug 2005.

[12] C. Y. Huang and J. E. Ferrell, "Ultrasensitivity in the mitogen-activated protein kinase cascade," *Proc. Natl. Acad. Sci.*, vol. 93, pp. 10 078–83, Sep 1996.

[13] J. E. Ferrell and E. M. Machleder, "The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes." *Science*, vol. 280, pp. 895–898, May 1998.

[14] Z. Hilioti, W. Sabbagh, S. Paliwal, *et al.*, "Oscillatory phosphorylation of yeast Fus3 MAP kinase controls periodic gene expression and morphogenesis." *Curr. Biol.*, vol. 18, pp. 1700–06, Nov 2008.

[15] B. N. Kholodenko, "Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades," *Eur. J. Biochem.*, vol. 267, pp. 1583–88, Mar 2000.

[16] N. I. Markevich, J. B. Hoek, and B. N. Kholodenko, "Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades," *J. Cell Biol.*, vol. 164, pp. 353–359, Feb 2004.

[17] B. N. Kholodenko, A. Kiyatkin, F. J. Bruggeman, E. Sontag, H. V. Westerhoff, and J. B. Hoek, "Untangling the wires: A strategy to trace functional interactions in signaling and gene networks," *Proc. Natl. Acad. Sci.*, vol. 99, pp. 12 841–46, 2002.