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Implication of dynamics in signal transduction and targeted disruption analyses of signaling networks

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Abstract

Modeling and analysis of the dynamics of signaling transduction networks can be powerful tools to understand and predict how cells will respond to native signals and artificial perturbations. This is of special interest for analyzing disease processes associated with signal transduction malfunctioning and to contribute to the development of efficient drug treatment strategies. In this work we examine the advantages of a kinetics-based framework as compared with purely topological approaches to identify input sets and disruption strategies that preserve desired cellular functions while blocking undesired disease states in signaling networks. These differences are highlighted through two examples where the mechanistic-based approach captures information that the topological-based analysis is unable to reveal.

Keywords: Signaling networks; Mechanistic model

1. Introduction and background

Regulation of the physiological functions of a living system depends in part on its ability to process information coming from its environment. The mechanism by which cells achieve this task is known as signal transduction. Broadly speaking, signal transduction requires first sensing of an environmental perturbation and then its transduction into a suitable signal for the regulatory machinery in charge of the coordination of gene expression and protein levels. Generally, sensing is carried out by proteins at the membrane of the cell (receptors) that bind with high specificity biochemical signals (ligands) such as hormones, neurotransmitters, growth factors and cytokines. Ligand–receptors complexes then trigger or suppress cascades of various proteins which can undergo and/or catalyze biochemical transformations (e.g., phosphorylation, complex formation) that ultimately result in the activation or deactivation of transcription factors. The collection of proteins and other biomolecules that participate in the transduction of a signal form a signaling pathway. Signaling pathways are not necessarily linear series of reactions but multiple branches and feedback loops are common motifs. Moreover, signaling pathways do not operate independently but interact with others through proteins that participate in multiple pathways. These interacting pathways constitute an intricate web, commonly referred to as signaling network, comprising a large number of components. In humans the signaling network is comprised by the products of more than two thousand genes that encode mainly for receptors, kinases and phosphatases (Papin, Hunter, Palsson, & Subramaniam, 2005). This vast collection of proteins has to process, generally in parallel, multiple signals coming from a complex microenvironment.

The complexity of signaling process and the availability of an enormous amount of experimental data have motivated the development of mathematical models to systematically integrate and analyze information and generate meaningful hypotheses that can be tested experimentally. Clearly, due to the large number of components and their intricate interactions, mapping the complete hierarchy of receptor signaling networks and their activation sequences is a significant challenge. A comprehensive review of modeling frameworks is beyond the scope of this paper. Here, we comment on the main features of the stoichiometric and kinetic models. The reader may refer to (Eungdamrong & Iyengar, 2004; Orton et al., 2005; Vayttaden, Ajay, & Bhalla, 2004) for a broad classification of models typically used for modeling signaling networks.

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The simplest signaling network models available are graphical abstractions representing various species as nodes and biochemical transformations as interconnections between them. Due to the large amount of stoichiometric information regarding protein interactions, these models are able to span large networks (i.e., hundreds to thousands of biotransformations). This information is available in a number of databases developed to systematically organize protein interaction data such as TRANSPATH (Krull et al., 2003; Schacherer et al., 2001), Molecular Pages database (Li et al., 2002), database of interacting proteins (Xenarios et al., 2002), and PANTHER (Mi et al., 2005). Graphical models can be converted into a mathematical representation analogous to the stoichiometric models of metabolic networks. These models are amenable to network analyses such as correlation and topological analysis (Dasika, Burgard, & Maranas, 2006; Maslov & Sneppen, 2002; Papin & Palsson, 2004). However, such models do not handle explicitly the level of the network components, but instead inputs and outputs are considered to be either active or inactive whereas the presence of inhibitors/activators implies complete disruption or activation of the corresponding transformation, respectively.

Knowledge of network components and their interactions is a very important step toward understanding the signaling cascades. However, to understand many critical signaling events that control cell responses such as growth, survival and differentiation, it is imperative to analyze their dynamic behavior (Haugh & Lauffenburger, 1998; Hornberg et al., 2005; Ni & Savageau, 1996). Ideally, detailed models for signaling networks should explain their behavior in both space (e.g., across the entire cell volume) and time. In recent years, considerable amount of research has been directed to achieve this goal. Notable contributions include kinetic modeling of signaling networks (Schoeberl, Eichler-Jonsson, Gilles, & Muller, 2002), spatially distributed analysis (Takahashi, Arjunan, & Tomita, 2005), and stochastic modeling of signaling networks (Tian & Burrage, 2006). In contrast to stoichiometric models, current mechanistic models include only a few pathways. The use of kinetic information enables a more detailed description of inputs/outputs and the effect of activators/inhibitors on process dynamics. This is important to track in cases where responses such as high-detect, low-detect or band-detect behavior change from low to high intensity and vice-versa depending upon the strength of the input (Basu, Gerchman, Collins, Arnold, & Weiss, 2005).

Proper functioning of every component of a signaling network is important for the correct response to an environmental signal. Mutations in one of several of its proteins may cause serious disregulations in a signaling pathway, contributing to the development of severe illness such as cancer (Adnane, Trail, Taylor, & Wilhelm, 2006; Janssens, Janicot, & Perera, 2006). This has motivated considerable research towards understanding signal transduction in normal and impaired pathways in order to design strategies to prevent, correct and/or compensate for malfunctions. Contributions in this direction make use of computational models to address relevant questions in the design of therapeutic strategies and target identification. Topological models have been used to elucidate the structural properties of signaling networks within the framework of extreme pathways analysis (Papin & Palsson, 2004) based on the algorithm of Schilling, Letscher, and Palsson (2000). Dasika et al. (2006) proposed an optimization-based framework to efficiently explore the topological space of signaling networks seeking for solutions for two meaningful problems in drug development studies. The first one is the identification of all sets of inputs that can trigger an output of interest (coined Min-Input problem). The second one is the identification of targeted disruptions within the network to "shape" its response, to eliminate undesired outputs while preserving the desired ones (coined Min-Interference problem). Mechanistic models have also been used to identify key component of signaling networks through an extension of metabolic control analysis to account for transient behavior (Hornberg et al., 2005) and time depending sensitivity analysis (Hu & Yuan, 2006). In other works, mechanistic models were used to predict the effect of perturbations of intuition-guided selected components of biological networks (Araujo, Petricoin, & Liotta, 2005; Christopher et al., 2004).

In this work, using kinetic descriptions we extent the framework introduced in Dasika et al. (2006) to address the relevant problems previously described (Min-Input and Min-Interference). We illustrate these frameworks using a prototype model of overlapping MAPK cascades and a simplified MAPK signaling network where a unique targeted disruption is identified due to the bistability of the network. The rest of the paper is organized as follows. We begin with the description of necessary mathematical preliminaries to formulate the generic kinetic description of signaling networks. Subsequently, we mathematically formulate the Min-Input and Min-Interference problems. Finally, the advantages of incorporating network dynamics into the optimization problems are illustrated through numerical examples using the models just mentioned.

2. Mathematical modeling

The dynamics of a signaling network is described by a set of coupled, generally nonlinear, ordinary differential equations (ODEs) derived from component balances. These ODEs for a network comprised of $N = \{1, \ldots, n\}$ chemical transformations and $M = \{1, \ldots, m\}$ chemical species are written as:

$$\frac{dC_i}{dt} = \sum_{j=1}^{n} S_{ij} r_j(\mathbf{C}, \mathbf{p}), \quad \forall i \in M \tag{1}$$

where C_i denotes the concentration of species i, S_{ij} is the stoichiometric coefficient of chemical entity i in the transformation j, and r_j is the corresponding flux of transformation j. The flux of each transformation is a nonlinear function of the concentration of the participating species including reactants, products, activators and inhibitors, and the kinetic parameters ($\mathbf{p}$). Typical examples of r_j ($\mathbf{C}$, $\mathbf{p}$) are Michaelis–Menten, Hill equation, Generalized mass action (GMA) kinetics, or S-system descriptions (Savageau, 1979).

3. Mathematical formulations

3.1. Min-Input problem

The initial objective is to identify sets of inputs that can activate an output of interest. The kinetic description of a signaling network confers flexibility to represent the state of its components. For instance, an output can be considered activated if the concentration level of a given chemical species is higher than a biological meaningful threshold value either at steady state or at some point in time during its trajectory. Considering this output description and the kinetic description outlined in the previous section the Min-Input problem can be mathematically recast as follows:

$$\min \sum_{i \in M_{\text{in}}} Y_i \tag{2}$$

s.t.

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = \sum_{j=1}^{n} S_{ij} r_j, \quad C_i(0) = C_{0,i}, \ \forall i \in M_{\text{bal}} \tag{3}$$

$$C_i \in \mathcal{W}^*, \quad \forall i \in M_{\text{out}}, \tag{4}$$

$$0 \le C_i(t) \le C_i^u Y_i, \quad \forall t \in [0, T], \forall i \in M_{\text{in}}, \tag{5}$$

$$Y_i \in \{0, 1\}, \quad \forall i \in M_{\text{in}} \tag{6}$$

The objective function represents the total number of inputs required to activate a desired set of outputs. The optimization variables in this formulation are Y_i and $C_i(t)$, $\forall i \in M_{\text{in}}$, where $M_{\text{in}} \subset \mathrm{M}$ is the set of inputs. $C_i(t)$ is the concentration profile of input i, which is allowed to be active (i.e., $C_i(t) > 0$) only if $Y_i = 1$ (constraint 5). Constraint 3 represents the dynamics of the signaling network, where $M_{\text{bal}} \subset M$ is the set of species for which a mass balance is available. $M_{\text{out}} \subset M$ is the set of outputs of interest, T is the final time, $C_{0,i}$ is the initial concentration of specie i, and C_i^{u} is the upper limit on the input concentrations. $\mathcal{W}^*$ is the set of active outputs. For example, if one is interested in the steady-state level of C_i, the set is defined as $\mathcal{W}^* = \{C_i(T) | C_i(T) > C_i^*\}$ and constraint 4 assumes the form $C_i(T) \ge C_i^*$. When the maximum level of C_i at any point in time is important, constraint 4 is replaced by $\max_{0 \le t \le T} C_i(t) \ge C_i^*$.

3.2. Min-Interference problem

The objective of the problem is to identify the set of possible manipulations within the network to obtain a desired temporal response from the network. For example, identify a set of disruptions within a network such that a given set of undesired outputs can be eliminated while preserving the rest of the desired outputs. In Dasika et al. (2006) the problem was formulated as a bilevel optimization problem. The inner level problem identifies the worst-case scenario response of the network by maximizing the flow to the undesirable output. Subsequently, the outer problem guarantees that the solution to the inner problem is equal to zero by systematically disrupting a minimal number of transformations. We reformulate this problem by accounting for the dynamical behavior of the network into the optimization problem. The aim of the current study is to demonstrate the qualitative and quantitative effect of accounting of network dynamics on the output of the Min-Interference problem. Given the dynamics of the network and the maximum number of allowable disruptions, the optimization problem successively computes strategies such that an undesirable output is blocked by disrupting minimal number of transformations. Mathematically, the problem is formulated as follows:

$$\min_{Y_j, w_j} \sum_{j=1}^{n} Y_j \tag{7}$$

s.t.

$$\frac{\mathrm{d}C_i}{\mathrm{d}t} = \sum_{j=1}^{n} S_{ij} R_j, \quad C_i(0) = C_{0,i}, \ \forall i \in M_{\text{bal}} \tag{8}$$

$$R_j = w_j r_j(\mathbf{C}, \mathbf{p}), \tag{9}$$

$$1 + (w_j^{\text{l}} - 1) Y_j \le w_j \le 1 + (w_j^{\text{u}} - 1) Y_j \tag{10}$$

$$C_i \in \mathcal{W}^*, \quad \forall i \in M_{\text{out}}^{\text{des}}, \tag{11}$$

$$C_i \in \mathcal{W}, \quad \forall i \in M_{\text{out}}^{\text{undes}}, \tag{12}$$

$$\sum_{j=1}^{n} Y_j \le K \tag{13}$$

where w_j represents the efficacy of the disruptions, $w_j = 0$ indicating complete disruption of the corresponding transformation and $w_j = 1$ indicating no disruption. w_j^{l} and w_j^{u} represent the lower and upper bounds for the available disruptions and $w_j^{\text{l}} = 0$ and $w_j^{\text{u}} = 1$ for the nominal case. The objective function minimizes the total number of targeted disruptions (identified by the corresponding binary number $Y_i = 1$). $M_{\text{out}}^{\text{des}} \subset M_{\text{out}}$ is the set of desirable outputs and $M_{\text{out}}^{\text{undes}} \subset M_{\text{out}}$ is the set of undesired outputs where M_{out} is the set of all outputs of the network. Constraint 8 represents the dynamics of the signaling network. The disruption efficiency w_j affects the dynamics such that the rate of the corresponding transformation is reduced by the corresponding factor (constraint 9). Constraints 11 and 12 define whether the desired output is "present" (i.e., belongs to set $\mathcal{W}^*$) and the undesired output is "absent" (i.e., belongs to set $\mathcal{W}$). Incorporation of kinetic models in the formulation allows flexibility in the definition of sets $\mathcal{W}^*$ and $\mathcal{W}$. For instance, we define the output as preserved if the corresponding output concentration is greater than a lower threshold; this is mathematically captured by enforcing a constraint such as $C_i \ge C_{\text{threshold}}$ can be enforced. Another way to ensure that the output is persistently greater than a given threshold, a constraint of the form $f \int_0^{t_f} ||C_i|| \ge \Delta$ can be enforced. Finally, constraint 13 ensures that the total number of disruptions are less than or equal to the maximum number of allowable disruptions.

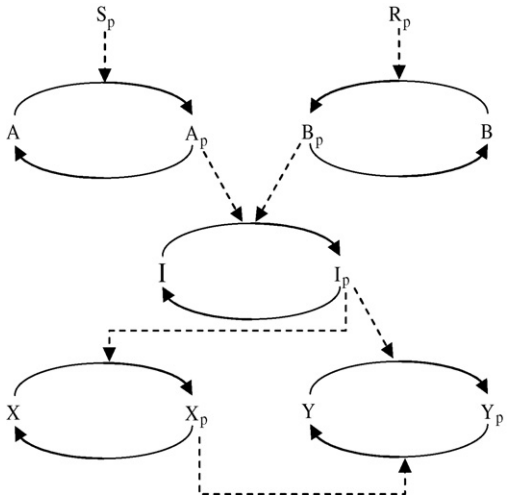

Fig. 1. Schematic diagram of overlapping MAPK cascade. Dashed arrows indicate the enzymes that catalyze the biochemical transformations, denoted by continuous arrows.

It should be noted that there may exist multiple solutions of the Min-Input and Min-Interference problems (i.e., multiple optima). A given output may be blocked through multiple disruption strategies or a desired output can be triggered by several sets of inputs. Such alternate optima can be successively identified by iteratively solving the optimization problem and excluding the previously obtained solutions in the next iterations using integer cut constraints (Floudas, 1995).

4. Numerical examples

4.1. Overlapping MAPK cascades

Incorporation of kinetic descriptions of signaling networks in the formulation of the Min-Input problem allows to obtain results that are different from the ones obtained with stoichiometric models. This is demonstrated through an example using a prototype model of overlapping MAPK cascades proposed by Somsen, Siderius, Bauer, Snoep, and Westerhoff (2002) to explain a possible mechanism for selective output activation (Fig. 1). The model is comprised of the mass balances of five chemical entities that participate in twelve reactions and it has two inputs (S_P and R_P) that can activate two outputs (X_P and Y_P). The model is represented by the following set of coupled differential equations:

$$\begin{aligned}
\dot{A}_P &= k_{AS} S_P \frac{A}{A + K_{AS}} - V_A \frac{A_P}{A_P + K_A} \\
\dot{B}_P &= k_{BR} R_P \frac{B}{B + K_{BR}} - V_B \frac{B_P}{B_P + K_B} \\
\dot{I}_P &= k_{IA} A_P \frac{I}{I + K_{IA}} + k_{IB} B_P \frac{I}{I + K_{IB}} - V_I \frac{I_P}{I_P + K_I} \\
\dot{X}_P &= k_{XI} I_P \frac{X}{X + K_{XI}} - V_X \frac{X_P}{X_P + K_X} \\
\dot{Y}_P &= k_{YI} I_P \frac{Y}{Y + K_{YI}} - k_{YX} X_P \frac{Y_P}{Y_P + K_{YX}} - V_Y \frac{Y_P}{Y_P + K_Y}
\end{aligned} \tag{14}$$

Table 1
Input–output relationships for the overlapping MAPK cascades model

Inputs		Outputs					
		Stoichiometry Steady state		Kinetic Steady state		Kinetic Dynamic	
S_P	R_P	X_P	Y_P	X_P	Y_P	X_P	Y_P
●	○	●	○	●	○		
		○	●	○	●	○	●
		●	●			●	●
○	●	●	○				
		○	●	○	●	○	●
		●	●				
●	●	●	○	●	○		
		○	●	○	●	○	●
		●	●			●	●

The corresponding input or output is activated (●) or deactivated (○).

For this small problem all possible input combinations can be analyzed exhaustively. We first consider only the steady-state value of the outputs, implying that constraint 4 assumes the form $C_i(T) \geq C_i^*$. The outputs that can be activated by each input combination are shown in the second and third columns of Table 1. Notably, the stoichiometry-only approach predicts that any output can be triggered by any input combination, however, using kinetics even at steady state reveals that output X_P cannot be elicited by the input R_P and that no input combination can activate simultaneously both outputs. Next, we consider dynamic effects and explore whether at any time the maximum level of X_P and Y_P are above a given threshold. In this case constraint 4 takes the form $\max_{0 \leq t \leq T} C_i(T) \geq C_i^*$. The results for this case are presented in the forth column of Table 1. It can be seen that R_P can only activate Y_P, whereas X_P cannot be triggered without activating Y_P by any input combination. Overall, the results from this small example demonstrate that kinetic information reveals that a number of stoichiometrically valid input–output combinations are not feasible. In addition, the answer to the question of what input can trigger what output becomes dependent upon the dynamic or static nature of the response and stimuli.

4.2. Simplified MAPK cascade

We consider a three-tier MAPK cascade based on the Mos/MEK1/p42 MAPK cascade present in *Xenopus* oocytes (Angeli, Ferrell, & Sontag, 2004). The network is schematically shown in Fig. 2. Active Mos (x) activates the phosphorylation of unphosphorylated MEK (y_1) to monophosphorylated MEK (y_2) and also activates phosphorylation of y_2 to biphosphorylated state (y_3). In turn, y_3 activates phosphorylation of p42 MAPK (z_1) at two residues resulting in the formation of active p42 MAPK (z_3). Ultimately, z_3 promotes the Mos synthesis, thus forming a positive feedback loop. The above cascade has been extensively studied experimentally and some of the relevant kinetic parameters have been measured (Sohaskey & Ferrell, 1999). The dynamics of the system can be modeled using a set

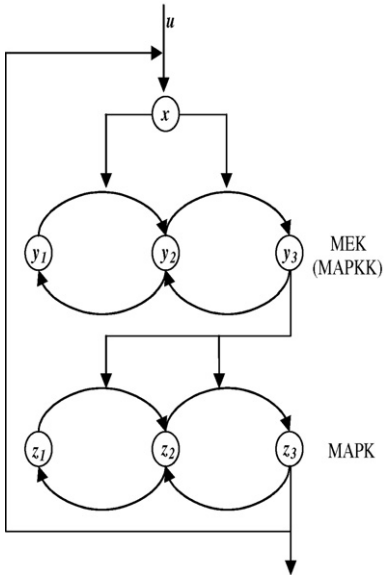

Fig. 2. Schematic diagram of Mos-MEK-p42 MAPK cascade.

of coupled differential equations:

$$
\begin{aligned}
\dot{x} &= -\frac{V_2 x}{K_2 + x} + v V_0 z_3 x + V_1 \\
\dot{y}_1 &= \frac{V_6 y_2}{K_6 + y_2} - \frac{V_3 x y_1}{K_3 + y_1} \\
\dot{y}_2 &= \frac{V_3 x y_1}{K_3 + y_1} + \frac{V_5 y_3}{K_5 + y_3} - \frac{V_4 x y_3}{K_4 + y_3} - \frac{V_6 y_2}{K_6 + y_2} \\
\dot{y}_3 &= \frac{V_4 x y_3}{K_4 + y_3} - \frac{V_5 y_3}{K_5 + y_3} \\
\dot{z}_1 &= \frac{V_1 0 z_2}{K_1 0 + z_2} - \frac{V_7 y_3 z_1}{K_7 + z_1} \\
\dot{z}_2 &= \frac{V_7 y_3 z_1}{K_7 + z_1} + \frac{V_9 z_3}{K_9 + z_3} - \frac{V_8 y_3 z_2}{K_8 + z_2} - \frac{V_1 0 z_2}{K_1 0 + z_2} \\
\dot{z}_3 &= \frac{V_8 y_3 z_2}{K_8 + z_2} - \frac{V_9 z_3}{K_9 + z_3}
\end{aligned}
\tag{15}
$$

where v denotes the strength of the feedback. The relevant parameter values are tabulated in Table 2. For the nominal value of feedback strength, $v = 2$, the p42 MAPK activation exhibits bistability. This implies that depending upon the initial condition, MAPK concentration evolves to either a positive stable steady state (termed as the "on"-state) or to a stable "off"-state. Fig. 3 shows the system evolution from a number of initial conditions where the profiles converging to the on-state are drawn in solid lines and profiles converging to the off-state are drawn in dotted lines.

Using the modified Min-Interference framework we exhaustively compute network disruption strategies that lead to suppression of the output MAPK (z_3).

Also, we seek solutions that are obtainable only through inclusion of network dynamics into the optimization framework and which are otherwise unattainable through stoichiometry alone. Fig. 4 shows one such disruption for the MAPK cascade, where blocking the feedback path in which MAPK promotes Mos synthesis ultimately leads to the inhibition of MAPK. The

Table 2
Kinetic parameters used in the MAPK cascade

Parameter		Value
y_{tot}	Total MEK concentration	1200 nM
z_{tot}	Total p42 MAPK concentration	300 nM
V_0	$\ldots \xrightarrow{\text{MAPK-PP}}$ Mos	0.0015 s^{-1} nM^{-1}
V_1	$\ldots \rightarrow$ Mos	0.000002 s^{-1}
V_2	Mos $\rightarrow \ldots$	1.2 nM s^{-1}
K_2	Mos $\rightarrow \ldots$	200 nM
V_3	MEK $\xrightarrow{\text{Mos-P}}$ MEK-P	0.064 s^{-1}
K_3	MEK $\xrightarrow{\text{Mos-P}}$ MEK-P	1200 nM
V_4	MEK-P $\xrightarrow{\text{Mos-P}}$ MEK-PP	0.064 s^{-1}
K_4	MEK-P $\xrightarrow{\text{Mos-P}}$ MEK-PP	1200 nM
V_5	MEK-PP $\rightarrow$ MEK-P	5 nM s^{-1}
K_5	MEK-PP $\rightarrow$ MEK-P	1200 nM
V_6	MEK-P $\rightarrow$ MEK	5 nM s^{-1}
K_6	MEK-P $\rightarrow$ MEK	1200 nM
V_7	MAPK $\xrightarrow{\text{MEK-PP}}$ MAPK-P	0.06 s^{-1}
K_7	MAPK $\xrightarrow{\text{MEK-PP}}$ MAPK-P	300 nM
V_8	MAPK-P $\xrightarrow{\text{MEK-PP}}$ MAPK-PP	0.06 s^{-1}
K_8	MAPK-P $\xrightarrow{\text{MEK-PP}}$ MAPK-PP	300 nM
V_9	MAPK-PP $\rightarrow$ MAPK-P	5 nM s^{-1}
K_9	MAPK-PP $\rightarrow$ MAPK-P	300 nM
V_{10}	MAPK-P $\rightarrow$ MAPK	5 nM s^{-1}
K_{10}	MAPK-P $\rightarrow$ MAPK	300 nM

above solution, that is non-intuitive based on the topography of the network, is a manifestation of the dynamics of the network. To understand this, we plot a set of trajectories from different initial conditions by setting the feedback strength to zero (i.e., $v = 0$, which amounts to disruption of the feedback to the network). It is observed that disruption of feedback in this case eliminates the bistability property of the network and renders the off-state to be the only stable steady state.

It should be noted that both the Min-Input the Min-Interference problems are mixed integer dynamic optimization (MIDO) problems due to the presence of ODE constraints arising from the dynamic representation of signaling networks. Moreover, the ODEs are nonlinear in nature due to the nonlinear dependence of reaction rates on the concentrations of

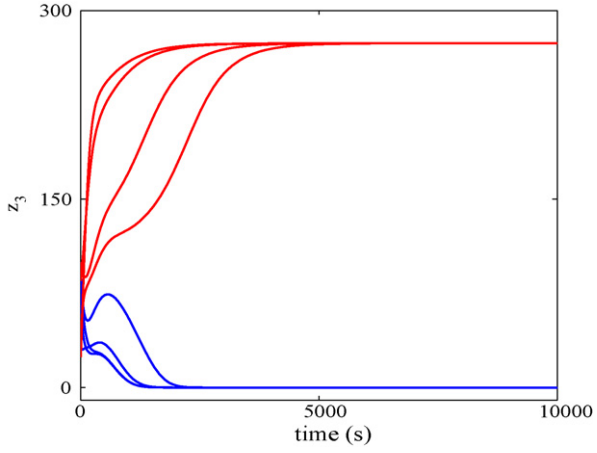

Fig. 3. Trajectories of z_3 for a number of distinct initial conditions.

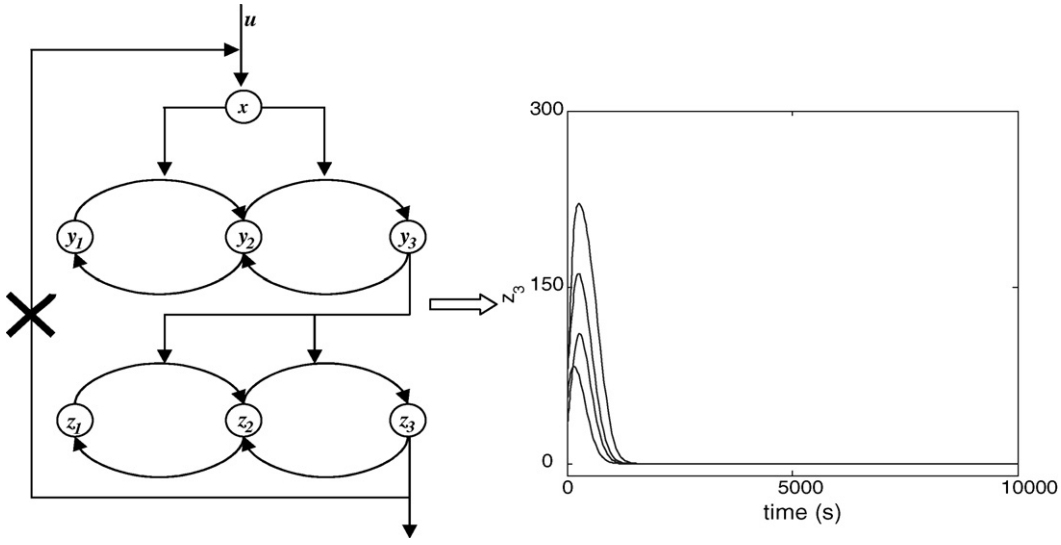

Fig. 4. Schematic diagram of Mos-MEK-p42 MAPK cascade with the inhibition of feedback and corresponding trajectories of z_3.

participating species, activators and inhibitors. Consequently, the solution of the resulting optimization problem is challenging. In the current work, the ODE constraints are first discretized in time using finite differences to obtain a set of nonlinear algebraic constraints. This reformulated the original MIDO problem to a mixed integer nonlinear program (MINLP), which is subsequently solved using standard solver DICOPT accessed through GAMS. An alternative approach is to integrate the ODE constraints separately, keeping track of possible constraint violations, a technique known as control vector parameterization (Vassiliadis, Sargent, & Pantelides, 1994).

5. Summary

This work outlines the development of a general framework to address two important problems regarding the design of signaling networks. The first is the identification of input sets that are capable of eliciting a given output. The second is the identification of disruption strategies within cell signaling networks to manipulate their responses to external stimuli. At the core of the solution strategies lies an optimization problem that systematically identifies input sets or the disruptions required to achieve the above objectives. The dynamics of signaling networks have been incorporated to (a) allow flexible and more detailed representations for inputs, outputs, inhibitors and activators involved and (b) to capture emergent properties of networks originated due to their dynamics. Finally, the effectiveness of the proposed approach is demonstrated using a prototype model of overlapping MAPK cascades and a three-tier MAPK cascade. Even though the examples considered correspond to relatively small networks, the scheme can be readily extended to larger and more comprehensive networks that have greater descriptive and predictive capabilities. Examples include detailed models of MAPK activated by EGF receptor activation (Hornberg et al., 2005; Schacherer et al., 2001). Controlling the output of the above signaling network has practical significance since ERK profile has implications on ultimate cell fate.

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