# Dealing with Discontinuities in the Qualitative Simulation of Genetic Regulatory Networks

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**Abstract.** Methods developed for the qualitative simulation of dynamical systems have turned out to be powerful tools for studying genetic regulatory networks. We present a generalization of a simulation method based on piecewise-linear differential equation models that is able to deal with discontinuities. The method is sound and has been implemented in a computer tool called GNA.

## 1 Introduction

Methods developed for the qualitative simulation of dynamical systems have turned out to be powerful tools for studying the networks of regulatory interactions between genes, proteins, and small molecules which underlie the functioning of living organisms. The interest in qualitative methods for analyzing these *genetic regulatory networks* derives from a general absence of quantitative information on kinetic parameters and molecular concentrations. As a consequence, traditional methods for numerical simulation are difficult to apply (see [1] for a review).

The qualitative simulation method described in [3] is able to handle large and complex networks of regulatory interactions. The method is based on a class of piecewise-linear (PL) differential equations that has been well-studied in mathematical biology [7, 11, 16]. The PL models provide a coarse-grained description of genetic regulatory networks, well-adapted to state-of-the-art measurement techniques in genomics. Moreover, they have mathematical properties that favour qualitative analysis of the steady-state and transient behavior of regulatory systems. The qualitative simulation method has been implemented in a publicly-available computer tool, called Genetic Network Analyzer (GNA). The program has been used to analyze several genetic regulatory networks of biological interest, including the network controlling the initiation of sporulation in *B. subtilis* [3].

The PL models contain step functions describing the regulatory interactions in a network. This introduces discontinuities in the right-hand side of the differential equations, which may give rise to non-trivial mathematical problems [11]. In the above-mentioned simulation method, these problems were treated by redefining the discontinuous, piecewise-linear models as a limit case of continuous, non-linear models [3, 11]. The resulting simulation method has turned out to be unsatisfactory, because it is not sound, in the sense that it is not guaranteed to predict all possible qualitative behaviors of the system [10]. Soundness is critical for many applications, such as model validation and model discrimination.

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Recent work on the generalization of the PL differential equations to differential inclusions allows the discontinuities to be dealt with in a mathematically proper and practically useful manner [8]. The generalization is based on an approach developed by Filippov [5], which has been widely used in control theory. The major contribution of this paper is to show how the method for the qualitative simulation of genetic regulatory networks can be generalized in the line of Filippov's work. This generalization results in a simulation algorithm with the desired soundness property. Beyond their application in the context of genetic regulatory networks, the ideas underlying the generalization may be a useful complement to other methods for the qualitative analysis of dynamical systems based on the approximation of complex nonlinear functions by piecewise-linear functions (*e.g.*, [14]).

After a brief review of PL models of regulatory networks (sec. 2), we will discuss the Filippov generalization in sec. 3. The qualitative simulation method based on this approach is described in sec. 4, followed by a discussion in the context of related work in sec. 5.

## 2 PL models of genetic regulatory networks

The dynamics of genetic regulatory networks can be modeled by a class of piecewise-linear differential equations of the following general form [7, 11, 16]:

$$\dot{\boldsymbol{x}} = \boldsymbol{f}(\boldsymbol{x}) - \boldsymbol{g}(\boldsymbol{x})\,\boldsymbol{x},\ \boldsymbol{x} \geq \boldsymbol{0}, \tag{1}$$

where $\boldsymbol{x} = (x_1, \ldots, x_n)'$ is a vector of cellular protein concentrations, and $\boldsymbol{f} = (f_1, \ldots, f_n)'$, $\boldsymbol{g} = \mathrm{diag}(g_1, \ldots, g_n)$. The rate of change of each concentration $x_i$, $1 \leq i \leq n$, is defined as the difference of the rate of synthesis $f_i(\boldsymbol{x})$ and the rate of degradation $g_i(\boldsymbol{x})\,x_i$ of the protein. The function $f_i : \mathbb{R}^n_{\geq 0} \to \mathbb{R}_{\geq 0}$ consists of a sum of step function expressions, each weighted by a rate parameter, which expresses the logic of gene regulation [11, 16]. The function $g_i : \mathbb{R}^n_{\geq 0} \to \mathbb{R}_{> 0}$ is defined analogously.

Figure 1 gives an example of a simple genetic regulatory network. Genes *a* and *b*, transcribed from separate promoters, encode proteins A and B, each of which controls the expression of both genes. More specifically, proteins A and B repress gene *a* as well as gene *b* at different concentrations. Repression of the genes is achieved by binding of the proteins to regulatory sites overlapping with the promoters.

The network in figure 1 can be described by means of the following pair of state equations:

$$\dot{x}_a = \kappa_a\, s^-(x_a, \theta_a^2)\, s^-(x_b, \theta_b^1) - \gamma_a\, x_a \tag{2}$$

$$\dot{x}_b = \kappa_b\, s^-(x_a, \theta_a^1)\, s^-(x_b, \theta_b^2) - \gamma_b\, x_b. \tag{3}$$

Gene *a* is expressed at a rate $\kappa_a > 0$, if the concentration of protein A is below its threshold $\theta_a^2$ and the concentration of protein B below

its threshold $\theta_b^1$, that is, if $s^-(x_a, \theta_a^2)\, s^-(x_b, \theta_b^1) = 1$. Recall that $s^-(x, \theta)$ is a step function evaluating to 1, if $x < \theta$, and to 0, if $x > \theta$. Protein A is spontaneously degraded at a rate proportional to its own concentration ($\gamma_a > 0$ is a rate constant). The state equation of gene *b* is interpreted analogously.

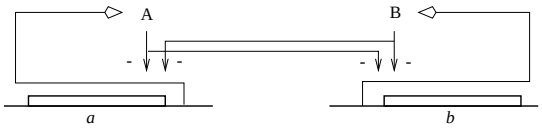


**Figure 1.** Example of a genetic regulatory network of two genes (*a* and *b*), each coding for a regulatory protein (A and B).

The dynamical properties of PL models of the form (1) can be analyzed in the $n$-dimensional phase space box $\Omega = \Omega_1 \times \ldots \times \Omega_n$, where $\Omega_i = \{x_i \in \mathbb{R}_{\geq 0} \mid 0 \leq x_i \leq max_i\}$, $1 \leq i \leq n$, and $max_i$ is a parameter denoting a maximum concentration for the protein.

In general, a protein encoded by a gene is involved in different interactions at different threshold concentrations, which after ordering are denoted by $\theta_i^1, \ldots, \theta_i^{p_i}$. The $n-1$-dimensional hyperplanes $x_i = \theta_i^{k_i}$, $1 \leq k_i \leq p_i$, divide $\Omega$ into regions that are called *domains*. More precisely, a domain $D \subseteq \Omega$ is defined by $D = D_1 \times \ldots \times D_n$, where every $D_i$, $1 \leq i \leq n$, is defined by one of the equations below:

$$\begin{aligned} D_i &= \{x_i \mid 0 \leq x_i < \theta_i^1\}, \\ D_i &= \{x_i \mid x_i = \theta_i^1\}, \\ D_i &= \{x_i \mid \theta_i^1 < x_i < \theta_i^2\}, \\ &\ldots \\ D_i &= \{x_i \mid \theta_i^{p_i} < x_i \leq max_i\}. \end{aligned} \tag{4}$$

If for a domain $D$, there are some $i, j$, $1 \leq i \leq n$, $1 \leq j \leq p_i$, such that $D_i = \{x_i \mid x_i = \theta_i^j\}$, then $D$ is called a *switching* domain. The corresponding variables $x_i$ are called *switching variables*. The *order* of a switching domain is a number between 1 and $n$, equal to the number of switching variables. A domain that is not a switching domain is called a *regulatory* domain. $\Delta$ denotes the set of domains in $\Omega$.

In figure 2(a) the two-dimensional phase space box $\Omega$ for the example network is shown. As proteins A and B each have two thresholds, the phase space box is partitioned into 9 regulatory and 16 switching domains. For example, $D^1 = \{(x_a, x_b) \in \mathbb{R}^2 \mid 0 \leq x_a < \theta_a^1,\ 0 \leq x_b < \theta_b^1\}$ is a regulatory domain, whereas $D^4 = \{(x_a, x_b) \in \mathbb{R}^2 \mid 0 \leq x_a < \theta_a^1,\ x_b = \theta_b^2\}$ is a (first-order) switching domain.

When evaluating the step function expressions in (1) in a regulatory domain, $f_i$ and $g_i$ reduce to sums of rate constants. More precisely, in a regulatory domain $D$, $f_i$ reduces to some $\mu_i^D \in M_i \equiv \{f_i(\boldsymbol{x}) \mid \mathbf{0} \leq \boldsymbol{x} \leq \boldsymbol{max}\}$, and $g_i$ to some $\nu_i^D \in N_i \equiv \{g_i(\boldsymbol{x}) \mid \mathbf{0} \leq \boldsymbol{x} \leq \boldsymbol{max}\}$. Inside $D$, the state equations thus simplify to linear and orthogonal differential equations

$$\dot{\boldsymbol{x}} = \boldsymbol{\mu}^D - \boldsymbol{\nu}^D\, \boldsymbol{x}, \tag{5}$$

where $\boldsymbol{\mu}^D = (\mu_1^D, \ldots, \mu_n^D)'$ and $\boldsymbol{\nu}^D = \mathrm{diag}(\nu_1^D, \ldots, \nu_n^D)$. Since the step functions are not defined at the thresholds, the state equations are not defined in the switching domains.

For every regulatory domain $D \in \Delta$, we define the function $\phi_i(D) = \mu_i^D / \nu_i^D$. Analysis of (5) shows that all solution trajectories in $D$ monotonically tend towards a *target equilibrium*, a stable equilibrium given by $\boldsymbol{x} = \boldsymbol{\phi}(D)$, with $\boldsymbol{\phi} = (\phi_1, \ldots, \phi_n)'$ [4, 16].

In the example, we have $M_a = \{0, \kappa_a\}$, $N_a = \{\gamma_a\}$ for protein A, and $M_b = \{0, \kappa_b\}$, $N_b = \{\gamma_b\}$ for protein B. In regulatory domain $D^1$ in figure 2(a), the trajectories tend towards the target equilibrium $\boldsymbol{\phi}(D^1) = (\kappa_a/\gamma_a, \kappa_b/\gamma_b)$. Different regulatory domains generally have different target equilibria. For instance, in regulatory domain $D^3$, the target equilibrium is given by $(0, \kappa_b/\gamma_b)$.

The global solution of (1) could be obtained by piecing together the local solutions in regulatory domains, in such a way as to guarantee continuity of the global solution across the threshold hyperplanes [4, 16]. This works fine as long as trajectories arriving at a threshold hyperplane can be continued in another regulatory domain, *e.g.*, trajectories arriving at the switching domain $D^2$ from the regulatory domain $D^1$ (figure 2(a)). However, when the trajectories on both sides of a threshold hyperplane evolve towards this plane, as in the case of trajectories arriving from $D^3$ and $D^5$ at $D^4$, mathematical perplexities arise. There is no indication on how the local solutions in $D^3$ and $D^5$ can be continued.

## 3 Analysis of discontinuities in PL models

The troubles at the threshold hyperplanes are caused by discontinuities in the right-hand side of (1), due to the use of step functions. In order to deal with these discontinuities, we will use a method originally proposed by Filippov [5]. This method, recently applied by Gouzé and Sari [8] to PL systems of the form (1), consists of extending a system of differential equations with discontinuous right-hand sides into a system of differential inclusions.

Let $D$ be a switching domain of order $k$. Let $C$ be the hyperplane of dimension $n-k$ containing $D$. The *boundary* of $D$ in $C$ is the set $B(D)$ of all points $\boldsymbol{x} \in C$, such that each ball $B_C(\boldsymbol{x}, \varepsilon)$ in $C$ of center $\boldsymbol{x}$ and radius $\varepsilon > 0$ intersects both $D$ and $C \setminus D$ [9]. In the case that $D$ is a regulatory domain, $C$ equals $\Omega$.

Now, for every $D \in \Delta$ we define the sets

$$\begin{aligned} A(D) &= \{D' \in \Delta \mid D' \subseteq B(D)\} \\ R(D) &= \{D' \in \Delta \mid D' \text{ regulatory domain}, D \subseteq B(D')\} \end{aligned}$$

$A(D)$ contains the domains in the boundary of $D$, whereas $R(D)$ contains the regulatory domains that have $D$ in their boundary.

In the case of the regulatory domain $D^1$ in figure 2(a), we find $A(D^1) = \{D^2, D^6, D^7\}$, while $A(D^2) = \{D^7\}$. Furthermore, $R(D^1) = \{\}$ and $R(D^2) = \{D^1, D^3\}$.

The basic idea of the Filippov approach is to extend the differential equations (1) into differential inclusions

$$\dot{\boldsymbol{x}} \in \boldsymbol{H}(\boldsymbol{x}), \tag{6}$$

where $\boldsymbol{H} : \Omega \to S(\Omega)$ is a set-valued function.[2]

For $\boldsymbol{x} \in D$, and $D$ a regulatory domain, we define $\boldsymbol{H}(\boldsymbol{x})$ as

$$\boldsymbol{H}(\boldsymbol{x}) = \{\boldsymbol{\mu}^D - \boldsymbol{\nu}^D\, \boldsymbol{x}\}. \tag{7}$$

Notice that, since the set $\boldsymbol{H}(\boldsymbol{x})$ contains a single element, the extension of the PL system agrees with the original system in the regulatory domains. If $D$ is a switching domain, $\boldsymbol{H}(\boldsymbol{x})$ is defined by

$$\boldsymbol{H}(\boldsymbol{x}) = \overline{co}\,(\{\boldsymbol{\mu}^{D'} - \boldsymbol{\nu}^{D'}\, \boldsymbol{x} \mid D' \in R(D)\}). \tag{8}$$

The smallest closed convex set $\overline{co}\,(E)$ of a set $E$ is the intersection of all closed convex sets containing $E$ [5]. In the case of switching domains, $\boldsymbol{H}(\boldsymbol{x})$ will not generally be single-valued.

Let $\boldsymbol{\theta} = (\ldots, \theta_i^{k_i}, \ldots)'$, $\boldsymbol{\kappa} = (\ldots, \kappa_{il}, \ldots)'$, and $\boldsymbol{\gamma} = (\gamma_1, \ldots, \gamma_n)'$ be numerical parameter values. Furthermore, let

[2] $S(E)$ represents the powerset of a set $E$.

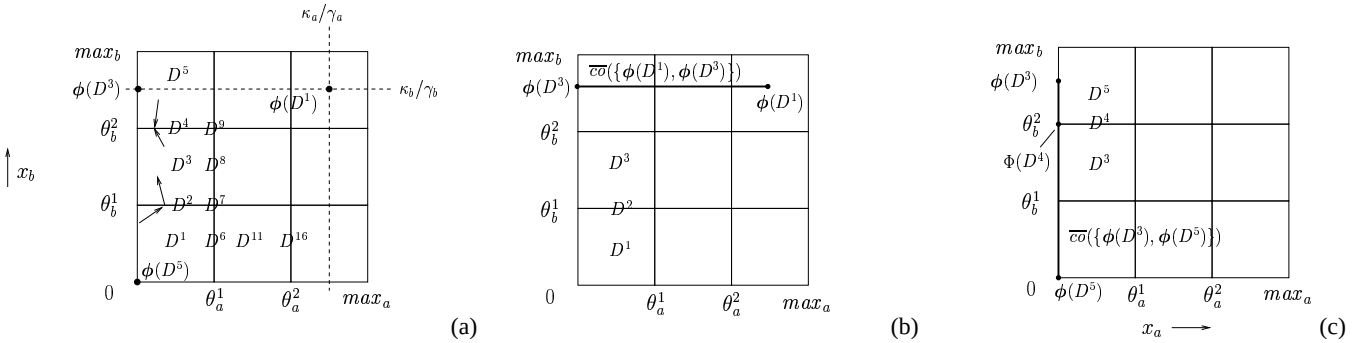


**Figure 2.** (a) Phase space box for the example network in figure 1. $\boldsymbol{\phi}(D^1)$, $\boldsymbol{\phi}(D^3)$, $\boldsymbol{\phi}(D^5)$ denote the target equilibria of the regulatory domains $D^1$, $D^3$, $D^5$, and are assumed to lie in the upperright, upperleft, and lowerleft regulatory domains, respectively. In addition, the figure shows the discontinuities at the switching domains $D^2$ and $D^4$. (b)-(c) Determination of the target equilibrium sets $\Phi(D^2)$ and $\Phi(D^4)$.

$\boldsymbol{x}(0) = \boldsymbol{x}_0 \in \Omega$ represent the initial conditions. An absolutely continuous function $\boldsymbol{x}(t) = \boldsymbol{\xi}(t, 0, \boldsymbol{x}_0, \boldsymbol{\theta}, \boldsymbol{\kappa}, \boldsymbol{\gamma})$ is a solution of (6) in the sense of Filippov on $[0, \tau[$, $\tau > 0$, if $\boldsymbol{x}(0) = \boldsymbol{x}_0$ and for almost all $t \in [0, \tau[$ it holds that $\dot{\boldsymbol{x}}(t) \in \boldsymbol{H}(\boldsymbol{x}(t))$ [5]. The qualification 'for almost all $t \in [0, \tau[$' means that the set of time-points for which the condition does not hold is of measure 0. For all initial values $\boldsymbol{x}_0 \in \Omega$ there exists a solution of (6) on $[0, \tau[$. However, this solution is not guaranteed to be unique.

For every domain $D$, a so-called *target equilibrium set* $\Phi(D)$ can be defined. If $D$ is a regulatory domain, then

$$\Phi(D) = \{\boldsymbol{\phi}(D)\}. \tag{9}$$

If $D$ is a switching domain, the definition is a little bit more complicated. Let $D$ be a switching domain of order $k$, contained in the $n-k$-dimensional hyperplane $C$. Then

$$\Phi(D) = C \cap \{\overline{co}\,(\{\boldsymbol{\phi}(D') \mid D' \in R(D)\}). \tag{10}$$

That is, $\Phi(D)$ is the smallest closed convex set of the target equilibria of regulatory domains $D'$ having $D$ in their boundary, intersected with the hyperplane containing $D$.

A solution either instantaneously crosses a switching domain or remains in it for some time $\tau > 0$, sliding along the threshold hyperplane containing the domain. Gouzé and Sari [8] have shown that the latter *sliding mode* solutions exist in a switching domain $D$, iff $\Phi(D) \neq \{\}$. The sliding mode solutions monotonically tends towards a target equilibrium in $\Phi(D)$ [8]. Because $\Phi(D)$ does not generally include a single point, the behavior of the system is not uniquely determined by the differential inclusion (6).

Consider the examples in figure 2(b)-(c). The target equilibrium set $\Phi(D^2)$ of the switching domain $D^2$ is defined, following (10), by the intersection of $\overline{co}(\{\boldsymbol{\phi}(D^1), \boldsymbol{\phi}(D^3)\})$ and the threshold hyperplane $x_b = \theta_b^1$. The smallest closed convex set consists of the linear segment connecting the points $(\kappa_a/\gamma_a, \kappa_b/\gamma_b)$ and $(0, \kappa_b/\gamma_b)$. $\Phi(D^2)$ and the threshold plane $x_b = \theta_b^1$ do not intersect in the figure, so $\Phi(D^2) = \{\}$ and all solutions instantaneously cross $D^2$.

This is different in the case of $D^4$. Here, the target equilibrium set $\Phi(D^4)$ is given by the intersection of $\overline{co}(\{\boldsymbol{\phi}(D^3), \boldsymbol{\phi}(D^5)\})$, the linear segment connecting the points $(0, \kappa_b/\gamma_b)$ and $(0, 0)$, and the threshold hyperplane $x_b = \theta_b^2$. Consequently, $\Phi(D^4)$ equals $\{(0, \theta_b^2)\}$, and there exists a (unique) sliding mode solution in $D^4$, tending towards $(0, \theta_b^2)$. Because the target equilibrium lies inside $D^4$, it is also a steady state of the system. Closer analysis reveals that the equilibrium $(0, \theta_b^2)$ is stable. Notice the intuitive validity of the Filippov approach: solutions arriving at $D^4$ from $D^3$ or $D^5$ slide along the threshold plane towards the equilibrium.

# 4 Method for qualitative simulation

## 4.1 Qualitative constraints on parameters

Most of the time, precise numerical values for the threshold and rate parameters in (1) will not be available. Instead, we will specify qualitative constraints on the parameter values, as explained in [3]. These constraints, having the form of algebraic inequalities, can usually be inferred from biological data.

The first constraint is obtained by ordering the $p_i$ threshold concentrations of gene $i$, yielding the *threshold inequalities*. In the case of protein A, there are two threshold concentrations: $\theta_a^1$ and $\theta_a^2$. Assuming the first to be lower than the second, we obtain the threshold inequalities $0 < \theta_a^1 < \theta_a^2 < max_a$. The ordering of the thresholds of protein B give rise to $0 < \theta_b^1 < \theta_b^2 < max_b$.

Second, the possible target equilibrium levels $\mu_i^D/\nu_i^D$ of $x_i$ in different regulatory domains $D \in \Delta$ can be ordered with respect to the threshold concentrations. The resulting *equilibrium inequalities* for $x_a$ in the example are $\theta_a^2 < \kappa_a/\gamma_a < max_a$. In the absence of protein B, while protein A has not yet reached its highest level, gene *a* is expressed at a rate $\kappa_a$. The corresponding target equilibrium value $\kappa_a/\gamma_a$ of $x_a$ must be above the second threshold $\theta_a^2$, otherwise the concentration of the protein would not be able to reach or maintain a level at which the observed negative autoregulation of gene *a* occurs. In a similar way, we set $\theta_b^2 < \kappa_b/\gamma_b < max_b$ for $x_b$.

A *quantitative* PL model of a genetic regulatory network consists of state equations (1) and numerical parameter values $\boldsymbol{\theta}, \boldsymbol{\kappa}, \boldsymbol{\gamma}$. In a *qualitative* PL model, on the other hand, the state equations are supplemented by threshold and equilibrium inequalities. Every quantitative PL model can be uniquely abstracted into a qualitative PL model.

## 4.2 Qualitative states and behaviors

An intuitive qualitative description of the state of a regulatory system consists of the domain in which the system resides, supplemented by the position with respect to this domain of the set of target equilibria to which the state of the system tends. A qualitative behavior is then given by the sequence of qualitative states traversed by the system.

We first define a function $v : \Delta \times \Omega \to \{-1, 0, 1\}^n$ that maps a domain $D$ and a point $\boldsymbol{e}$ to a sign vector $\boldsymbol{r}$ describing the relative position of $D$ and $\boldsymbol{e}$. If $x_i$ is a non-switching variable, then

$$r_i = \begin{cases} 1 & , \text{ if } e_i \geq \sup D_i, \\ 0 & , \text{ if } \inf D_i < e_i < \sup D_i, \\ -1 & , \text{ if } e_i \leq \inf D_i. \end{cases}$$

On the other hand, if $x_i$ is a switching variable, then $D_i$ contains a single threshold value $\theta_i^j$, and

$$r_i = \begin{cases} 1 & , \text{ if } e_i > \theta_i^j, \\ 0 & , \text{ if } e_i = \theta_i^j, \\ -1 & , \text{ if } e_i < \theta_i^j. \end{cases}$$

Generalizing the definition, we obtain the set function $V : \Delta \times S(\Omega) \rightarrow S(\{-1, 0, 1\}^n)$ that maps a domain $D$ and a set $E$ to a set of sign vectors: $V(D, E) = \{v(D, \boldsymbol{e}) \mid \boldsymbol{e} \in E\}$.

Let $\boldsymbol{x}(t) = \boldsymbol{\xi}(t, 0, \boldsymbol{x}_0, \boldsymbol{\theta}, \boldsymbol{\kappa}, \boldsymbol{\gamma})$ be the solution of a quantitative PL model describing a regulatory network on the time-interval $[0, \tau[$. Now suppose that for some $t$, $0 \leq t < \tau$, we have $\boldsymbol{x}(t) \in D$, $D \in \Delta$. The point $\boldsymbol{x}(t)$ corresponds to a *qualitative state* of the system defined by

$$QS(\boldsymbol{x}, t) = \langle D, V(D, \Phi(D))\rangle.$$

The solution $\boldsymbol{x}(t)$ on $[0, \tau[$ passes through a sequence of domains $D^0, \ldots, D^m$. The corresponding sequence of qualitative states is called the *qualitative behavior* of the system on the time-interval. More specifically, a qualitative behavior of the system is defined by

$$QB(\boldsymbol{x}, 0, \tau) = (\langle D^0, V(D^0, \Phi(D^0))\rangle, \ldots, \langle D^m, V(D^m, \Phi(D^m))\rangle)$$

Consider the solution trajectory in figure 3(a), which for given parameter values moves from an initial state in $D^1$ towards a stable equilibrium in $D^4$. Following the above definitions, the solution can be abstracted into the qualitative behavior $QB = (QS^1, QS^2, QS^3, QS^4)$, where $QS^1 = \langle D^1, \{(1,1)\}\rangle$, $QS^2 = \langle D^2, \{\}\rangle$, $QS^3 = \langle D^3, \{(0,1)\}\rangle$, and $QS^4 = \langle D^4, \{(0,0)\}\rangle$. For regulatory domain $D^1$, we have $\Phi(D^1) = \{(\kappa_a/\gamma_a, \kappa_b/\gamma_b)\}$. For the parameter values in figure 3, we find $\kappa_a/\gamma_a > \theta_a^2 > \theta_a^1$ and $\kappa_b/\gamma_b > \theta_b^2 > \theta_b^1$. As a consequence, $V(D^1, \Phi(D^1)) = \{(1,1)\}$, and hence $QS^1 = \langle D^1, \{(1,1)\}\rangle$. In the case of the switching domain $D^2$, the smallest closed convex set of the target equilibria in $D^1$ and $D^3$ consists of the linear segment connecting $(\kappa_a/\gamma_a, \kappa_b/\gamma_b)$ and $(0, \kappa_b/\gamma_b)$. For the parameter values in figure 3, this segment does not intersect with $x_b = \theta_b^1$, so that $V(D^2, \Phi(D^2)) = \{\}$ and $QS^2 = \langle D^2, \{\}\rangle$. The other qualitative states are derived analogously.

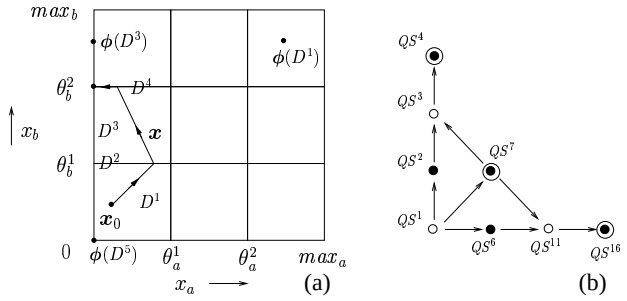


**Figure 3.** (a) Solution trajectory of the PL model (2)-(3), obtained for given numerical values for the parameters and initial conditions. (b) Transition graph resulting from a simulation of the example system starting in the domain $D^1$. Qualitative states associated with regulatory domains and switching domains are indicated by unfilled and filled dots, respectively. Qualitative states associated with domains containing an equilibrium point are circled [2].

The example shows how a numerical solution of the system can be abstracted into a qualitative behavior consisting of a sequence of qualitative states. More generally, every solution $\boldsymbol{x}$ of a quantitative PL model on a time-interval $[0, \tau[$ can be uniquely abstracted into a qualitative behavior $QB(\boldsymbol{x}, 0, \tau)$.

## 4.3 Simulation algorithm

Given a qualitative PL model and qualitative initial conditions consisting of a regulatory domain $D^0$, one can ask what are the possible qualitative behaviors of the system. Determining these qualitative behaviors is the aim of *qualitative simulation*. Phrased in a different way, denoting by $X$ the set of solutions $\boldsymbol{x}(t)$ on some time-interval $[0, \tau[$ of all quantitative PL models corresponding to the qualitative model, such that $\boldsymbol{x}(0) = \boldsymbol{x}_0 \in D^0$, the aim of qualitative simulation is to find the set of qualitative behaviors that abstract from some $\boldsymbol{x} \in X$.

The simulation algorithm generates a set of qualitative behaviors by recursively determining transitions between qualitative states, starting from the qualitative state associated with the initial domain $D^0$ [2]. In order to achieve this, two issues need to be addressed. First of all, how can we determine the qualitative state associated with a domain from the constraints on the parameters? Second, how can we find the possible transitions from this qualitative state?

In order to determine the qualitative state associated with a domain $D$, we need to derive $V(D, \Phi(D))$ from the threshold and equilibrium inequalities. This is achieved by computing $V(D, \Psi(D))$, where $\Psi(D) \subseteq \Omega$ is a hyperrectangular, closed convex set that is sure to include $\Phi(D)$, but that may be an overapproximation of the latter. The details of the procedure, which consists of a rather straightforward comparison of the upper and lowerbounds of $D$ and either the target equilibrium $\boldsymbol{\phi}(D)$ or the target equilibria $\boldsymbol{\phi}(D')$, $D' \in R(D)$, depending on whether $D$ is a regulatory or switching domain, are given in [2].

The possible transitions are defined by two *transition rules*. The relative position of the domains $D$ and $D'$ is given by $V(D, D')$. As can be easily verified, $V(D, D')$ always consists of a single sign vector, that is, $V(D, D') = \{\boldsymbol{w}\}$. The domains $D$ and $D'$ are associated with qualitative states $QS$ and $QS'$, calculated to be $\langle D, V(D, \Psi(D))\rangle$ and $\langle D', V(D', \Psi(D'))\rangle$, respectively.

**Rule 1** Let $D' \in A(D)$. There is a transition from $QS$ to $QS'$, if (1) $V(D, \Psi(D)) \neq \{\}$, and (2) there is some $\boldsymbol{v} \in V(D, \Psi(D))$, such that $v_i\, w_i = 1$, if $x_i$ is a switching variable in $D'$, but not in $D$.

**Rule 2** Let $D \in A(D')$. There is a transition from $QS$ to $QS'$, if (1) $V(D', \Psi(D')) \neq \{\}$, and (2) there is some $\boldsymbol{v}' \in V(D', \Psi(D'))$, such that $v_i'\, w_i \neq -1$, if $x_i$ is a switching variable in $D$, but not in $D'$.

Intuitively, the first transition rule says that, in order to enter a switching domain $D'$ in the boundary of $D$, some trajectories must tend towards $D'$ (condition (2)). If $D$ is a switching domain, then there must exist sliding mode trajectories in $D$ (condition (1)). The second transition rule says that, in order to enter a domain $D'$ from a switching domain $D$ in the boundary of $D'$, the trajectories in $D'$ must not tend towards $D$ (condition (2)). If $D'$ is a switching domain, then there must exist sliding mode trajectories in $D'$ (condition (1)).

Given an initial domain $D^0$, describing the initial protein concentrations $\boldsymbol{x}_0$, the simulation algorithm computes the initial qualitative state $QS^0$, and then determines all possible transitions from $QS^0$ to successor qualitative states by means of the rules above. The generation of successor states is repeated in a recursive manner. This results in a directed graph of qualitative states and transitions, the *state transition graph*, which contains all qualitative states reachable from the initial qualitative state. The simulation algorithm has been implemented in a new version of the program GNA.

Figure 3(b) shows the transition graph for a qualitative simulation of the example system, starting in the regulatory domain $D^1$. Con-

sider the possible transitions from the qualitative state $QS^3$ associated with regulatory domain $D^3$ to qualitative states associated with the boundary domains $A(D^3) = \{D^2, D^4, D^7, D^8, D^9\}$. We have to verify whether the conditions (1) and (2) of rule 1 are verified. $V(D^3, \Psi(D^3))$ is calculated to be $\{(0,1)\}$, by means of the procedure in [2], while $V(D^3, D^4)$ equals $\{(0,1)\}$. With $x_b$ a switching variable in $D^4$, but not in $D^3$, we find that (1) and (2) are satisfied. Consequently, there exists a transition from $QS^3$ to $QS^4$. Transitions from $QS^3$ to the other candidate successor states are ruled out, because they violate condition (2).

### 4.4 Soundness

Given a qualitative PL model and an initial regulatory domain $D^0$, what can be said about the correctness of the behaviors produced by qualitative simulation? We demand that for every $\boldsymbol{x} \in X$, the transition graph contains a qualitative behavior $QB$, such that $QB = QB(\boldsymbol{x}, 0, \tau)$ (*soundness*).

**Theorem 1** The qualitative simulation algorithm is sound.

**Proof sketch** Let $\boldsymbol{x}$ be a solution in $X$. On $[0, \tau[$, $\boldsymbol{x}(t)$ traverses a sequence of domains $D^0, \ldots, D^m$, where $\boldsymbol{x}_0 \in D^0$. Like in the proof of the soundness of QSIM [10], it can be shown by induction that the qualitative behavior $QB(\boldsymbol{x}, 0, \tau)$ is generated by the algorithm. The proof in [2] rests on two properties of the simulation algorithm. First of all, the set $\Psi(D)$ used in sec. 4.3 is sure to include $\Phi(D)$. Second, the transition rules cover all solutions leaving a domain $D$ and entering a domain $D'$, $D' \in A(D)$ or $D \in A(D')$. □

## 5 Discussion

We have presented a method for the qualitative simulation of genetic regulatory networks described by a class of piecewise-linear (PL) differential equations. The method is an extension of [3], which allows one to deal with discontinuities in the right-hand side of the differential equations, occasioned by the use of step functions. The soundness of the qualitative simulation method guarantees that no solution of a quantitative PL model consistent with the qualitative PL model is omitted.

The qualitative simulation method is supported by the computer tool GNA, which has been used to analyze several genetic regulatory networks of biological interest, such as the network underlying the initiation of sporulation in *B. subtilis* described in [3]. A qualitative simulation of this system under conditions conducive to sporulation, using a model consisting of 9 state variables, 2 input variables, and 58 parameter inequalities, gives rise to a state transition graph with 465 states. Only 82 states remain after elimination of the states corresponding to switching domains without sliding mode solutions, which are of limited interest from a biological point of view.

Several ways to deal with the step function discontinuities in (1) have been proposed in the literature, such as restricting the analysis to an easy-to-handle subclass of PL models [4] or relaxing the discontinuous PL models to continuous nonlinear models [11, 13]. The approach presented here has the advantage of putting no restrictions on the class of genetic regulatory networks that can be handled, while explicitly defining the behavior of the system in the threshold planes by means of simple-to-analyze PL models. On a formal level, the generalized logical method of Thomas and colleagues [17] is related to the method presented here. For a subclass of the PL models (1), the logical method can identify equilibrium points in the threshold hyperplanes, but a general way to deal with the discontinuities is currently missing.

PL models of the form (1) can be interpreted as representing a class of *hybrid systems* [6, 15], consisting of modes in which the system evolves in a continuous way and discrete transitions between the modes controlled by a switching logic. In order to deal with discontinuities entailed by mode transitions, hybrid-system simulation methods based on Filippov solutions have been developed [12]. These methods are suitable for (semi-)quantitative, but not for qualitative PL models.

The ideas underlying the qualitative simulation method discussed in this paper seem more widely applicable in qualitative reasoning, especially when nonlinear functions are approximated by piecewise-linear functions (*e.g.*, [14]). This may give rise to discontinuities on the boundaries separating the regions where the system behaves linearly. Current methods for the qualitative analysis of piecewise-linear systems, including the general simulation method QSIM [10], are not able to deal with the problems occasioned by sliding modes, shown in figure 2(a). Generalization of the Filippov approach to other qualitative reasoning methods raises a host of interesting research problems.

**Acknowledgements** The authors would like to thank Grégory Batt and Ivayla Vatcheva.

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