Qualitative Analysis of the Dynamics of Genetic Regulatory Networks using Piecewise-Linear Models

Grégory Batt,[1,2] Richard Casey,[3] Hidde de Jong,[1]
Johannes Geiselmann,[4] Jean-Luc Gouzé,[3] Michel Page,[1,5]
Delphine Ropers,[1] Tewfik Sari,[6] Dominique Schneider[4]

[1]Institut National de Recherche en Informatique et en Automatique (INRIA),
Unité de recherche Rhône-Alpes,
655 avenue de l'Europe, Montbonnot, 38334 Saint Ismier Cedex, France.
Email: {Gregory.Batt, Hidde.de-Jong, Michel.Page, Delphine.Ropers}@inrialpes.fr
[2]Université Joseph Fourier, Grenoble, France.
[3]Institut National de Recherche en Informatique et en Automatique (INRIA),
Unité de recherche Sophia Antipolis,
2004 route des Lucioles, BP 93, 06902 Sophia Antipolis, France.
Email: {Richard.Casey, Jean-Luc.Gouze}@sophia.inria.fr
[4]Laboratoire Adaptation et Pathogénie des Microorganismes (CNRS UMR 5163),
Université Joseph Fourier, Bâtiment Jean Roget, Faculté de Médecine-Pharmacie,
Domaine de la Merci, 38700 La Tronche, France.
Email: {Hans.Geiselmann, Dominique.Schneider}@ujf-grenoble.fr
[5]École Supérieure des Affaires, Université Pierre Mendès France, Grenoble, France.
[6]Laboratoire de Mathématiques, Université de Haute-Alsace
4 rue des Frères Lumière, 68093 Mulhouse, France.
Email: T.Sari@uha.fr

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1 Introduction

The functioning and development of living organisms is controlled by large and complex networks of genes, proteins, small molecules, and their interactions, so-called *genetic regulatory networks*. The concerted efforts of genetics, molecular biology, biochemistry, and physiology have led to the accumulation of enormous amounts of data on the molecular components of genetic regulatory networks and their interactions, reported in the literature or stored in databases. Notwithstanding the advances in the mapping of the network structure, surprisingly little is understood about how the behavior of the systems controlled by these networks emerges from the interactions between the molecular components. This has incited an increasingly large group of researchers to turn from the structure to the behavior of genetic regulatory networks, against the background of a broader movement variously referred to as functional genomics, systems biology, or integrative biology [12, 18, 49, 54].

In the last decade the toolbox of the molecular biologist has been extended with high-throughput experimental techniques for transcriptomics, proteomics, and metabolomics allowing the structure and behavior of genetic regulatory networks to be studied on a genome-wide scale [36, 58, 69, 71, 80, 100]. In addition, techniques providing an in-depth characterization of individual components and interactions of the network have been developed, such as techniques for monitoring gene expression in a single cell and quantifying molecular interactions [56, 68, 72, 81]. However, it is clear that, in addition to powerful experimental tools, the study of the behavior of genetic regulatory networks also requires the support of mathematical and computational tools. Since most networks of biological interest consist of a large number of molecular components involved in complex feedback loops, predicting the behavior of the system by intuition alone quickly becomes unfeasible or fraught by error. The use of mathematical models in combination with computer tools allows for the precise and unambiguous description of a network and the systematic and efficient prediction of its behavior.

A variety of methods for the *modeling, analysis, and simulation of genetic regulatory networks* have been proposed in the past forty years [21, 40, 62, 84]. However, most of the work seems to have focused on two approaches, based on the use of *differential equations* and *stochastic master equations*, respectively. The application of these classical approaches rests on well-established theoretical frameworks for the modeling of the kinetics of biochemical reaction systems, from either a deterministic or stochastic point of view [19, 34, 42, 96]. In addition, a variety of mathematical methods and computer tools is available for transforming the model equations into experimentally-testable predictions. Many excellent examples exist to demonstrate the capability of the classical approaches to help gaining insight into the functioning of genetic regulatory networks of biological importance, such as the networks underlying the response of *Escherichia coli* to bacteriophage λ infection [3, 63], the regulation of the cell cycle in yeast and higher eukaryotes [94], and the control of circadian rhythms in *Drosophila* [55].

The classical approaches allow precise numerical predictions of deterministic or stochastic dynamic properties of genetic regulatory networks to be made. However, for most networks of biological interest the application of differential equations and stochastic master equations is far from straightforward. First, the biochemical reaction mechanisms underlying the interactions are usually not or incompletely known, which complicates the formulation of the models. Second, quantitative data on kinetic parameters and molecular concentrations is generally absent, even for extensively-studied systems, which makes standard numerical methods difficult to apply. In practice, the modeler disposes of much weaker information on the network components and their interactions. Instead of details on the mechanisms through which a protein regulates a gene, she typically only knows whether the protein is an activator or an inhibitor. And even if it had been shown, for example, that the protein binds to one or several sites upstream of the coding region of the gene, numerical values of dissociation constants and other parameters are rarely available. At best, it is possible to infer that the regulatory protein strongly or weakly binds to the DNA, with a greater affinity for one site than for another.

Although the classical approaches require precise, quantitative information on biochemical reaction mechanisms and parameter values, one may pose the question whether this information is really essential for understanding the functioning of genetic regulatory networks. In fact, it is reasonable to assume that many important dynamic properties of living systems, such as the response of a bacterial cell to an environmental perturbation or the differentiation of an insect cell in the course of development, do not depend on precise numerical values or

a specific regulatory mechanism. While different individuals of the same species often display the same response to a perturbation or follow the same developmental trajectory, the values of the parameters describing the interactions are likely to differ from one individual to another, due to genetic polymorphism and physiological fluctuations. Similarly, as a consequence of evolutionary diversification, different species may implement the same regulatory function by way of different biochemical reaction mechanisms [74]. In other words, *qualitative dynamic properties*, that is, dynamic properties that are invariant for a range of parameter values and reaction mechanisms, may be more important than quantitative dynamic properties. The qualitative properties express the intimate connection between the behavior of the system and the structure of the network of molecular interactions, independently from the quantitative details of the latter.

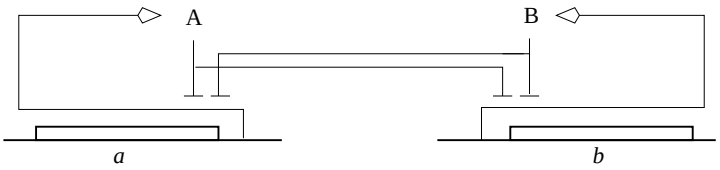
For all of the above reasons, there is a growing interest in qualitative approaches for the modeling, analysis, and simulation of genetic regulatory networks and other networks of biological interactions. These approaches have in common that they are capable of inferring qualitative properties of the system dynamics from currently-available incomplete and non-quantitative data. However, the multiplication of formalisms being proposed betrays the diverse origins of the qualitative approaches: Boolean networks [47, 92], Petri nets [50, 76], directed graphs [20, 51, 82], process algebras [77], qualitative differential equations [41], hybrid automata [33],... A comprehensive overview of the qualitative approaches is still missing, although there recently appeared reviews motivating their use and comparing some of the existing formalisms [25, 32].

The aim of this chapter is to give an overview of one particular method for the qualitative modeling and simulation of genetic regulatory networks [26], based on a class of *piecewise-linear (PL) differential equations* proposed by Glass and Kauffman some thirty years ago [35]. While abstracting from the precise biochemical reaction mechanisms involved, the PL models capture essential aspects of gene regulation. Moreover, their simple mathematical form permits a qualitative analysis of the behavior of a genetic regulatory network to be carried out. More precisely, the method presented in this chapter describes the qualitative dynamics of a PL system by means of a so-called state transition graph consisting of qualitative states and transitions between qualitative states. The qualitative states correspond to regions in the phase space where the system behaves in a qualitatively-homogeneous way, whereas the transitions correspond to solution trajectories that connect adjacent regions. We have shown that the state transition graph is invariant for sets of parameter values defined by inequality constraints that can be easily inferred from the experimental literature. Moreover, the qualitative states and transitions between qualitative states can be computed from these constraints by means of simple, symbolic rules. The qualitative modeling and simulation method has been implemented in the computer tool Genetic Network Analyzer (GNA) [24], and applied to the analysis of a number of bacterial regulatory networks [23, 78, 95].

In the next two sections, we introduce the PL models of genetic regulatory networks and review some basic mathematical results concerning their dynamics. The results underlie the discrete abstraction used in Section 4 to describe the qualitative dynamics of a network by means of a state transition graph. The application of the qualitative modeling and simulation method to the analysis of the complex regulatory network controlling the nutritional stress response of the bacterium *Escherichia coli* is the subject of Section 5. The chapter ends with a discussion of the method in the context of related work and gives a tentative response to the question of how increasingly-large networks could be handled by the method.

2 PL models of genetic regulatory networks

Figure 1(a) shows an example of a simple genetic regulatory network. The genes a and b, transcribed from separate promoters, encode the proteins A and B, each of which controls the expression of both genes.[1] Proteins A and B repress genes a and b at different concentrations. Repression of the genes is achieved by binding of the proteins to regulatory sites overlapping with the promoters. Simple as it is, this *mutual-inhibition network* is a basic component of more complex, real networks and allows the analysis of some characteristic aspects of cellular differentiation [70, 92].



(a)

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

(b)

Figure 1: (a) Example of a genetic regulatory network of two genes (a and b) coding for a regulatory protein (A and B). For the notation, see Figure 8. (b) State equations for the network in (a).

The dynamics of genetic regulatory networks can be modeled by a class of piecewise-linear (PL) differential equations of the following general form [35, 67, 92]:

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

where $\boldsymbol{x} = (x_1, \ldots, x_n)' \in \Omega$ is a vector of cellular protein concentrations, $\boldsymbol{f} = (f_1, \ldots, f_n)'$, $\boldsymbol{g} = \mathrm{diag}(g_1, \ldots, g_n)$, and $\Omega \subset \mathbb{R}^n_{\geq 0}$ is a bounded n-dimensional phase space box. The rate of change of each protein concentration x_i, $i \in [1, \ldots, n]$, is thus 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 PL models can be easily extended to take into account *input variables* $\boldsymbol{u} = (u_1, \ldots, u_m)'$, representing the concentration of proteins and small molecules whose synthesis and degradation are regulated outside the system [26].

The function $f_i : \Omega \to \mathbb{R}_{\geq 0}$ expresses how the rate of synthesis of the protein encoded by gene i depends on the concentrations $\boldsymbol{x}$ of the proteins in the cell. More specifically, the function f_i is defined as

$$f_i(\boldsymbol{x}) = \sum_{l \in L_i} \kappa_i^l \, b_i^l(\boldsymbol{x}), \tag{2}$$

where $\kappa_i^l > 0$ is a rate parameter, $b_i^l : \Omega \to \{0, 1\}$ a piecewise-continuous *regulation function*, and L_i a possibly empty set of indices of regulation functions. The function g_i expresses the

[1] As a notational convention, names of genes are printed in italic and names of proteins start with a capital.

regulation of protein degradation. It is defined analogously to f_i, except that we demand that g_i is strictly positive. In addition, in order to formally distinguish degradation rate parameters from synthesis rate parameters, we will denote the former by γ instead of κ. Notice that with the above definitions, $\boldsymbol{h}$ is a *piecewise-linear (PL)* vector-valued function.

A regulation function b_i^l describes the conditions under which the protein encoded by gene i is synthesized (degraded) at a rate κ_i^l ($\gamma_i^l\, x_i$). It is defined in terms of step functions and is the arithmetic equivalent of a Boolean function expressing the logic of gene regulation [35, 67, 92]. More precisely, the conditions for synthesis or degradation are expressed using the step functions s^+, s^-:

$$s^+(x_j,\theta_j) = \begin{cases} 1, \text{ if } x_j > \theta_j, \\ 0, \text{ if } x_j < \theta_j, \end{cases} \quad \text{and } s^-(x_j,\theta_j) = 1 - s^+(x_j,\theta_j), \tag{3}$$

where x_j is an element of the state vector $\boldsymbol{x}$ and θ_j a constant denoting a threshold concentration.

In Figure 1(b) the state equations for the example network are shown. Gene a is expressed at a rate κ_a, if the concentration of protein A is below its threshold θ_a^2 and the concentration of protein B below its threshold θ_b^1, that is, if $s^-(x_a,\theta_a^2)\, s^-(x_b,\theta_b^1)$ evaluates to 1. Analogously, gene b is expressed at a rate κ_b, if the concentration of protein A is below the threshold θ_a^1 and the concentration of protein B below the threshold θ_b^2. The degradation of the proteins is not regulated in this case and therefore proportional to the concentration of the proteins (with degradation parameters γ_a or γ_b).

The use of step functions $s^{\pm}(x_j,\theta_j)$ in (1) gives rise to complications, because the step functions are discontinuous at $x_j = \theta_j$, and therefore $\boldsymbol{h}$ might be discontinuous on the union of the threshold hyperplanes

$$\Theta = \bigcup_{i\in[1,\ldots,n], l_i\in[1,\ldots,p_i]} \{\boldsymbol{x}\in\Omega \mid x_i = \theta_i^{l_i}\}, \tag{4}$$

where p_i denotes the number of threshold concentrations of the protein encoded by gene i. In order to deal with this problem, we can follow an approach widely used in control theory, originally proposed by Filippov [31]. It consists in extending the differential *equation* $\dot{\boldsymbol{x}} = \boldsymbol{h}(\boldsymbol{x})$, $\boldsymbol{x}\in\Omega\setminus\Theta$, to the differential *inclusion*

$$\dot{\boldsymbol{x}} \in K(\boldsymbol{x}), \text{ with } K(\boldsymbol{x}) = \overline{co}(\{\lim_{\boldsymbol{y}\to\boldsymbol{x},\, \boldsymbol{y}\notin\Theta} \boldsymbol{h}(\boldsymbol{y})\}),\ \boldsymbol{x}\in\Omega, \tag{5}$$

where $\overline{co}(P)$ denotes the smallest closed convex set containing the set P and $\{\lim_{\boldsymbol{y}\to\boldsymbol{x},\, \boldsymbol{y}\notin\Theta} \boldsymbol{h}(\boldsymbol{y})\}$, the set of all limit values of $\boldsymbol{h}(\boldsymbol{y})$, for $\boldsymbol{y}\notin\Theta$ and $\boldsymbol{y}\to\boldsymbol{x}$. This approach has been applied in the context of genetic regulatory network modeling by Gouzé and Sari [37].

In practice, $K(\boldsymbol{x})$ may be difficult to compute because the smallest closed convex set can be a complex polyhedron in Ω. We therefore employ an alternative extension of the differential equation:

$$\dot{\boldsymbol{x}} \in H(\boldsymbol{x}), \text{ with } H(\boldsymbol{x}) = \overline{rect}(\{\lim_{\boldsymbol{y}\to\boldsymbol{x},\, \boldsymbol{y}\notin\Theta} \boldsymbol{h}(\boldsymbol{y})\}),\ \boldsymbol{x}\in\Omega, \tag{6}$$

where $\overline{rect}(P)$ denotes the smallest closed *hyperrectangular* set containing the set P [26]. The advantage of using $\overline{rect}$ is that we can rewrite $H(\boldsymbol{x})$ as a system of differential inclusions $\dot{x}_i \in H_i(\boldsymbol{x})$, $i\in[1,\ldots,n]$. Notice that $H(\boldsymbol{x})$ is an overapproximation of $K(\boldsymbol{x})$, for all $\boldsymbol{x}\in\Omega$.

Formally, we define the *PL system* Σ as the triple (Ω,Θ,H), that is, the set-valued function H given by (6), defined on the n-dimensional phase space Ω, with Θ the union of the threshold

hyperplanes. A *solution* of the PL system Σ on a time interval I is a solution of the differential inclusion (6) on I, that is, an absolutely-continuous vector-valued function $\boldsymbol{\xi}(t)$ such that $\dot{\boldsymbol{\xi}}(t) \in H(\boldsymbol{\xi}(t))$ holds almost everywhere on I [31].

For all $\boldsymbol{x}_0 \in \Omega$ and $\tau \in \mathbb{R}_{>0}$, $\Xi_\Sigma^\omega(\boldsymbol{x}_0, \tau)$ will denote the set of solutions $\boldsymbol{\xi}(t)$ of the PL system Σ, for the initial condition $\boldsymbol{\xi}(0) = \boldsymbol{x}_0$ and $t \in [0, \tau]$. The existence of at least one solution $\boldsymbol{\xi}$ on some time interval $[0, \tau]$, $\tau > 0$, with initial condition $\boldsymbol{\xi}(0) = \boldsymbol{x}_0$ is guaranteed for all $\boldsymbol{x}_0$ in Ω [31]. However, there is, in general, not a unique solution. $\Xi_\Sigma^\omega = \bigcup_{\boldsymbol{x}_0 \in \Omega, \tau > 0, \tau \text{ finite}} \Xi_\Sigma^\omega(\boldsymbol{x}_0, \tau)$ denotes the set of all solutions of Σ on a finite time interval $[0, \tau]$. We restrict our analysis to the set Ξ_Σ of solutions in Ξ_Σ^ω that reach and leave a threshold hyperplane finitely-many times.

3 Mathematical analysis of PL systems

The dynamic properties of Σ can be analyzed in the n-dimensional phase space box $\Omega = \Omega_1 \times \ldots \times \Omega_n$, where $\Omega_i = \{x_i \in \mathbb{R} \mid 0 \leq x_i \leq max_i\}$, $i \in [1, \ldots, n]$, and max_i denotes a maximum concentration of the protein encoded by gene i. The $(n-1)$-dimensional threshold hyperplanes $\{\boldsymbol{x} \in \Omega \mid x_i = \theta_i^{l_i}\}$, $l_i \in [1, \ldots, p_i]$, $i \in [1, \ldots, n]$, partition Ω into (hyper)rectangular regions that are called *domains*. Within each such region, the concentration of a protein equals a threshold or is bounded by thresholds. More precisely, a domain $D \subseteq \Omega$ is defined by $D = D_1 \times \ldots \times D_n$, with every D_i, $i \in [1, \ldots, n]$, given by one of the following equations:

$$
\begin{aligned}
D_i &= \{x_i \mid 0 \leq x_i < \theta_i^1\},\\
D_i &= \{x_i \mid x_i = \theta_i^1\},\\
D_i &= \{x_i \mid \theta_i^1 < x_i < \theta_i^2\},\\
D_i &= \{x_i \mid x_i = \theta_i^2\},\\
&\ldots\\
D_i &= \{x_i \mid x_i = \theta_i^{p_i}\},\\
D_i &= \{x_i \mid \theta_i^{p_i} < x_i \leq max_i\}.
\end{aligned}
\tag{7}
$$

$\mathcal{D}$ denotes the set of all domains in Ω. Figure 2(a) shows the partitioning into domains of the two-dimensional phase space of the example network. We distinguish between domains like D^2 and D^7, which are located on (intersections of) threshold hyperplanes, and domains like D^1 and D^3, which are not. The former domains are called *singular* domains and the latter *regular* domains. We denote by $\mathcal{D}_s$ and $\mathcal{D}_r$ the sets of singular and regular domains, respectively.

As a preliminary step for the analysis of the dynamic properties of (1) in regular and singular domains, we define some simple topological concepts. For every domain, $D \subseteq \Omega$, of dimension $k \in [0, \ldots, n]$, we define the *supporting hyperplane* of D, $supp(D) \subseteq \Omega$, as the k-dimensional hyperplane containing D. The *boundary* of D in $supp(D)$ is denoted by ∂D. Suppose that D is a singular domain, *i.e.* $D \in \mathcal{D}_s$. Then $R(D)$ is defined as the set of regular domains D' having D in their boundary, *i.e.* $R(D) = \{D' \in \mathcal{D}_r \mid D \subseteq \partial D'\}$.

Using the definition of the differential inclusion (6), it can be shown that in a regular domain D, $H(\boldsymbol{x})$ reduces to the singleton set $\{\boldsymbol{\mu}^D - \boldsymbol{\nu}^D \boldsymbol{x}\}$, for all $\boldsymbol{x} \in D$, where $\boldsymbol{\mu}^D$ is a vector of (sums of) synthesis rate constants and $\boldsymbol{\nu}^D$ a diagonal matrix of (sums of) degradation rate constants. This yields the classical result that all solutions $\boldsymbol{\xi}$ in D monotonically converge towards the *focal set*

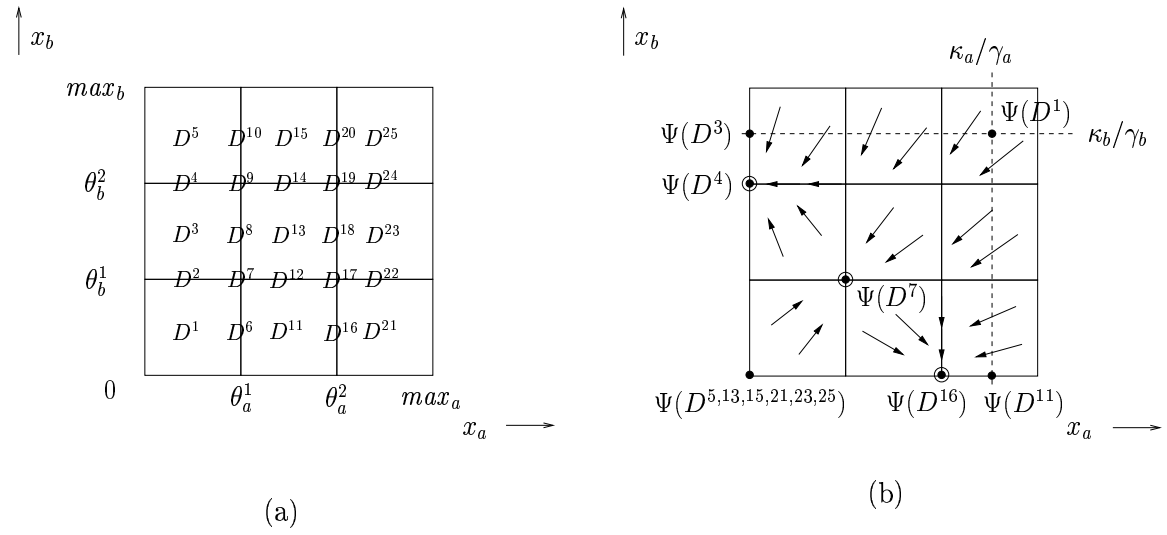


Figure 2: (a) Domain partition of the phase space corresponding to the model of Figure 1(b). (b) Vector field and focal sets for the (persistent) domains in (a). The circled focal sets are equilibrium points of the system. The following parameter values have been assumed: $\theta_a^1 = 4$, $\theta_a^2 = 8$, $\theta_b^1 = 4$, $\theta_b^2 = 8$, $\kappa_a = 20$, $\kappa_b = 20$, $\gamma_a = 2$, and $\gamma_b = 2$.

$$\Psi(D) = \{\boldsymbol{\psi}(D)\}, \tag{8}$$

where $\boldsymbol{\psi}(D)$ is called the *focal point*, defined as $\boldsymbol{\psi}(D) = (\boldsymbol{\nu}^D)^{-1}\boldsymbol{\mu}^D$ [35, 73, 85]. We make the generic assumption that the focal sets $\Psi(D)$, for all $D \in \mathcal{D}_r$, are not located in the threshold hyperplanes Θ. If $\boldsymbol{\psi}(D) \in D$, then for $t \to \infty$ all solutions in D approach the focal set, which is then an asymptotically stable equilibrium point of the system. If $\boldsymbol{\psi}(D) \notin D$, all solutions will leave D at some point.

Figure 2(b) shows the focal sets of the regular domains in the phase space of the example system. For instance, in D^1 the PL model in Figure 1(b) reduces to $\dot{x}_a = \kappa_a - \gamma_a x_a$ and $\dot{x}_b = \kappa_b - \gamma_b x_b$, so that the focal set $\Psi(D^1)$ equals $\{(\kappa_a/\gamma_a, \kappa_b/\gamma_b)\}$. Because $\Psi(D^1)$ lies outside D^1, the trajectories in D^1 will leave the domain at some point. Different regular domains generally have different focal sets. For instance, the focal set of D^3 is given by $\Psi(D^3) = \{(0, \kappa_b/\gamma_b)\}$.

In a singular domain, the right-hand side of the differential inclusion (6) reduces to $H(\boldsymbol{x}) = \overline{rect}(\{\boldsymbol{\mu}^{D'} - \boldsymbol{\nu}^{D'}\boldsymbol{x} \mid D' \in R(D)\})$, for all $\boldsymbol{x} \in D$ [26, 37]. The focal set associated with the domain now becomes

$$\Psi(D) = supp(D) \cap \overline{rect}(\{\boldsymbol{\psi}(D') \mid D' \in R(D)\}), \tag{9}$$

which is generally not a single point in higher-dimensional domains [26, 37]. Two different cases can be distinguished. If $\Psi(D)$ is empty, then every solution passes through D instantaneously and D is called an *instantaneous* domain. If not, then some (but not necessarily all) solutions arriving at D will remain in D for some time, sliding along the supporting hyperplane of D. The domain is then called *persistent*. If $\Psi(D)$ is a single point, then all solutions in D monotonically converge towards this point. In the case that $\Psi(D)$ is not a single point, a

weaker monotonicity property holds [26, 37].

If $\Psi(D) \cap D = \emptyset$ all solutions will leave D at some point. On the other hand, if $\Psi(D) \cap D \neq \emptyset$, there exist solutions in D that asymptotically approach or reach the focal set $\Psi(D)$ as $t \to \infty$. Using (6), it can be shown that for every $\boldsymbol{\psi} \in \Psi(D) \cap D$ it holds that $\mathbf{0} \in H(\boldsymbol{\psi})$, and hence that there exists a solution $\boldsymbol{\xi}(t)$, such that $\boldsymbol{\xi}(t) = \boldsymbol{\psi}$ for all $t \geq 0$. Consequently, $\Psi(D) \cap D$ is a set of equilibrium points of the system, called *equilibrium set*. The occurrence of equilibrium sets, as well as the non-uniqueness of solutions of differential inclusions (Section 2), require extended definitions of stability [13]. For simple cases, in particular when the equilibrium set consists of a single point, these definitions reproduce the intuitive notion of stability suggested by the direction of the vector field around an equilibrium point.

The singular domain D^2 in the example is instantaneous ($\Psi(D^2) = \emptyset$), because the intersection of $\overline{rect}(\{\boldsymbol{\psi}(D^1), \boldsymbol{\psi}(D^3)\})$ and $supp(D^2)$ is empty (Figure 2(b)). On the other hand, D^4 is a persistent domain in which solutions slide along the threshold plane and converge towards the focal set $\Psi(D^4) = supp(D^4) \cap \overline{rect}(\{\boldsymbol{\psi}(D^3), \boldsymbol{\psi}(D^5)\}) = \{(0, \kappa_b/\gamma_b)\}$. Since this focal set is a single point included in D^4, it is an equilibrium point of the system. The equilibrium point is asymptotically stable, according to the definitions in [13], because it is reached or asymptotically approached by all solutions in the neighborhood of $\Psi(D^4)$, as a consequence of the direction of the vector field in the regular domains D^3 and D^5. The figure also shows the focal sets of the persistent singular domains, D^7 and D^{16}. The focal set of D^7 coincides with the domain itself, which is a point in Ω. $\Psi(D^7)$ is an unstable equilibrium point, because solutions starting in a neighborhood of $\Psi(D^4)$ tend to escape, as suggested by the vector field in D^1, D^3, D^{11}, and D^{13}. Like $\Psi(D^4)$, $\Psi(D^{16})$ is an asymptotically stable equilibrium point.

In summary, the use of differential inclusions makes it possible to extend the definition of the PL models (1) to the whole of the phase space in a systematic and mathematically proper way. In particular, the above results show that the local dynamics of PL systems Σ are relatively straightforward: in every domain of the phase space, the system converges monotonically – in the usual sense for regular domains, in a weaker sense for singular domains – towards a focal set. This does not imply that the global dynamics of the PL systems are equally simple. On the contrary, because the focal set usually changes when going from one domain to another, the global dynamics of the PL systems may be quite complex [29, 57, 66]. However, the fact that in every domain the system behaves in a qualitatively-homogeneous way suggests a discrete abstraction of the dynamics that will turn out to be quite useful for studying qualitative properties of the behavior of genetic regulatory networks.

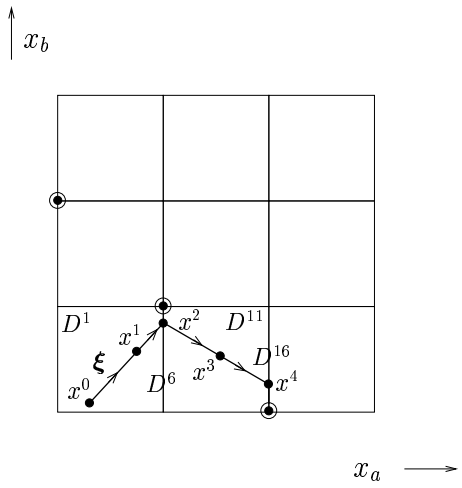
4 Qualitative simulation using PL models

As a preparatory step to the definition of a discrete or *qualitative abstraction* of the dynamics of PL systems, we first introduce a continuous transition system having the same reachability properties as the original PL system Σ. Consider $\boldsymbol{x} \in D$ and $\boldsymbol{x'} \in D'$, where $D, D' \in \mathcal{D}$ are domains. If there exists a solution $\boldsymbol{\xi}$ of Σ passing through $\boldsymbol{x}$ at time τ and reaching $\boldsymbol{x'}$ at time τ', without leaving $D \cup D'$ in the time interval $[\tau, \tau']$, then the absolute continuity of $\boldsymbol{\xi}$ implies that D and D' are either equal or contiguous. More precisely, one of the three following cases holds: $D = D'$, $D \in \partial D'$, or $D' \in \partial D$. We consequently distinguish three types of continuous transition that correspond to these three cases: *internal*, denoted by $\boldsymbol{x} \xrightarrow{int} \boldsymbol{x'}$, *dimension increasing*, denoted by $\boldsymbol{x} \xrightarrow{dim^+} \boldsymbol{x'}$, and *dimension decreasing*, denoted by $\boldsymbol{x} \xrightarrow{dim^-} \boldsymbol{x'}$. The latter two terms refer to the increase or decrease in dimension when going from D to

D'. The *continuous PL transition system* of a PL system $\Sigma = (\Omega, \Theta, H)$ is then given by Σ-TS $= (\Omega, L, \rightarrow)$, where $L = \{int, dim^+, dim^-\}$ is the set of labels denoting the types of transition, and $\rightarrow \subseteq \Omega \times L \times \Omega$ is the transition relation describing the continuous evolution of the system. More precisely, $\boldsymbol{x} \stackrel{l}{\rightarrow} \boldsymbol{x'}$ if and only if there exist $\boldsymbol{\xi} \in \Xi_\Sigma$ and τ, τ', such that $0 \leq \tau < \tau'$, $\boldsymbol{\xi}(\tau) = \boldsymbol{x}$, $\boldsymbol{\xi}(\tau') = \boldsymbol{x'}$, and

1. if $l = int$, then for all $t \in [\tau, \tau']$ it holds that $\boldsymbol{\xi}(t) \in D$ and $D = D'$,
2. if $l = dim^+$, then for all $t \in (\tau, \tau']$ it holds that $\boldsymbol{\xi}(t) \in D'$ and $D' \neq D$,
3. if $l = dim^-$, then for all $t \in [\tau, \tau')$ it holds that $\boldsymbol{\xi}(t) \in D$ and $D \neq D'$.

We define any sequence of points $(\boldsymbol{x}^0, \ldots, \boldsymbol{x}^m)$ in Ω, $m \geq 0$, as a *path* of Σ-TS if and only if one can reach $\boldsymbol{x}^m$ from $\boldsymbol{x}^0$ by a sequence of transitions, that is, if and only if for all $i \in [0, \ldots, m\text{–}1]$, there exists some $l \in L$ such that $\boldsymbol{x}^i \stackrel{l}{\rightarrow} \boldsymbol{x}^{i+1}$. It is not difficult to show that a PL system Σ and its corresponding continuous PL transition system Σ-TS have equivalent reachability properties, in the sense that for all $\boldsymbol{x}, \boldsymbol{x'} \in \Omega$, there exists a path in Σ-TS leading from $\boldsymbol{x}$ to $\boldsymbol{x'}$ if and only if there exists a solution $\boldsymbol{\xi}$ of Σ on a time interval $[\tau, \tau']$ that passes through $\boldsymbol{x}$ at τ and $\boldsymbol{x'}$ at τ'.



(a)

Σ-TS $= (\Omega, L, \rightarrow)$, where

- $\Omega = \{\boldsymbol{x}^0, \boldsymbol{x}^1, \boldsymbol{x}^2, \boldsymbol{x}^3, \boldsymbol{x}^4, \ldots\}$,
- $L = \{int, dim^+, dim^-\}$,
- $\rightarrow = \{\boldsymbol{x}^0 \stackrel{int}{\longrightarrow} \boldsymbol{x}^1,\ \boldsymbol{x}^0 \stackrel{dim^-}{\longrightarrow} \boldsymbol{x}^2,\ \boldsymbol{x}^1 \stackrel{dim^-}{\longrightarrow} \boldsymbol{x}^2,$ $\boldsymbol{x}^2 \stackrel{dim^+}{\longrightarrow} \boldsymbol{x}^3,\ \boldsymbol{x}^3 \stackrel{dim^-}{\longrightarrow} \boldsymbol{x}^4, \ldots\}$.

(b)

Figure 3: Trajectory of a solution $\boldsymbol{\xi}$ of the PL system Σ starting in D^1, reaching D^{16}, and passing through the points $\boldsymbol{x}^0, \ldots, \boldsymbol{x}^4$. (b) Partial description of the continuous PL transition system Σ-TS, illustrating its relation with the PL system Σ.

As an illustration, consider a solution $\boldsymbol{\xi}$ of the PL system Σ for our two-gene example, as shown in Figure 3(a). $\boldsymbol{\xi}$ passes through the points $\boldsymbol{x}^0, \ldots, \boldsymbol{x}^4$, at time instants $\tau^0, \ldots \tau^4$, respectively. Using the definition of the continuous transition system, and the fact that $\boldsymbol{x}^0$ and $\boldsymbol{x}^1$ belong to the same domain D^1, we infer the existence of an *int* transition from $\boldsymbol{x}^0$ to $\boldsymbol{x}^1$ in Σ-TS. Again, following the definition of Σ-TS, since $\boldsymbol{x}^1 \in D^1$, $\boldsymbol{x}^2 \in D^6$, and $\boldsymbol{\xi}(t) \in D^1$ for all $t \in [\tau^1, \tau^2)$, there exists a transition of type dim^- from $\boldsymbol{x}^1$ to $\boldsymbol{x}^2$. Similarly, there exists a dim^- transition from $\boldsymbol{x}^0$ to $\boldsymbol{x}^2$. Also, there exists a dim^+ transition from $\boldsymbol{x}^2$ to $\boldsymbol{x}^3$, since $\boldsymbol{x}^2 \in D^6$, $\boldsymbol{x}^3 \in D^{11}$, and $\boldsymbol{\xi}(t) \in D^{11}$ for all $t \in (\tau^2, \tau^3]$. Note that the transition labels dim^+ and dim^- are consistent with the dimension of the domains, which equals 2 for D^1 and D^{11}, and 1 for D^6. The sequence of points $(\boldsymbol{x}^0, \boldsymbol{x}^1, \boldsymbol{x}^2, \boldsymbol{x}^3, \boldsymbol{x}^4)$ is a path of Σ-TS.

Using the partition $\mathcal{D}$ of the phase space Ω, introduced in Section 3, a discrete or qualitative abstraction of the continuous transition system can be formulated. The abstraction is based on the equivalence relation $\sim_\Omega$, stating that two points are equivalent when they belong to the same domain, *i.e.* $\boldsymbol{x} \sim_\Omega \boldsymbol{x'}$, if and only if $\boldsymbol{x} \in D$ and $\boldsymbol{x'} \in D$. The discrete description of the PL system Σ, called the *qualitative PL transition system*, is now defined as the quotient transition system of Σ-TS with the equivalence relation $\sim_\Omega$ [2, 16]. More precisely, given that $\Sigma\text{-TS} = (\Omega, L, \rightarrow)$, we have $\Sigma\text{-QTS} = (\Omega/_{\sim_\Omega}, L, \rightarrow_{\sim_\Omega})$, where $\Omega/_{\sim_\Omega} = \mathcal{D}$ and $\rightarrow_{\sim_\Omega} \subseteq \mathcal{D} \times L \times \mathcal{D}$, such that $D \stackrel{l}{\rightarrow}_{\sim_\Omega} D'$ if and only if there exist $\boldsymbol{\xi} \in \Xi_\Sigma$ and $\tau, \tau',\ 0 \leq \tau < \tau'$, such that $\boldsymbol{\xi}(\tau) \in D$, $\boldsymbol{\xi}(\tau') \in D'$, and

1. if $l = int$, then for all $t \in [\tau, \tau']$ it holds that $\boldsymbol{\xi}(t) \in D$ and $D = D'$,
2. if $l = dim^+$, then for all $t \in (\tau, \tau']$ it holds that $\boldsymbol{\xi}(t) \in D'$ and $D' \neq D$,
3. if $l = dim^-$, then for all $t \in [\tau, \tau')$ it holds that $\boldsymbol{\xi}(t) \in D$ and $D \neq D'$.

In words, there is a transition from a domain D to another domain D' if and only if there exists a solution of Σ which on a time interval I reaches D' from D, without leaving $D \cup D'$. The main difference with Σ-TS is that Σ-QTS is a *finite* rather than an *infinite* transition system, due to the fact that the state space of Σ-QTS equals the finite set $\mathcal{D}$. This is a key property, since it makes it possible to actually compute and represent Σ-QTS, as well as to conveniently analyze its dynamics.

The domains in a qualitative PL transition system are also called *qualitative states*. As for Σ-TS, we define any sequence of domains $(D^0, \ldots, D^m)$, $m \geq 0$, as a *path* of Σ-QTS if and only if for all $i \in [0, \ldots, m\text{–}1]$, there exists $l \in L$ such that $D^i \stackrel{l}{\rightarrow}_{\sim_\Omega} D^{i+1}$. A path in Σ-QTS corresponds to a possible qualitative evolution of the protein concentrations over time. If a domain D contains an equilibrium set, that is, if $\Psi(D) \cap D \neq \emptyset$, the domain is called an *qualitative equilibrium state* of the transition system. The qualitative states and transitions between qualitative states of Σ-QTS are often represented in the form of a *state transition graph*, defined as $G = (\mathcal{D}, \rightarrow_{\sim_\Omega})$.

Consider again the situation illustrated in Figure 3. Following the above definition of a qualitative transition system, the existence of the transitions from $\boldsymbol{x^0}$ to $\boldsymbol{x^1}$, from $\boldsymbol{x^1}$ to $\boldsymbol{x^2}$, and from $\boldsymbol{x^2}$ to $\boldsymbol{x^3}$ in Σ-TS, leads to an *int* transition from D^1 to D^1, a dim^- transition from D^1 to D^6, and a dim^+ transition from D^6 to D^{11}, respectively. The qualitative transition system and corresponding state transition graph for the two-gene example are represented in Figure 4. The temporal evolution of the protein concentrations along the path $(D^1, D^6, D^{11}, D^{16})$ of Σ-QTS is shown in Figure 5.

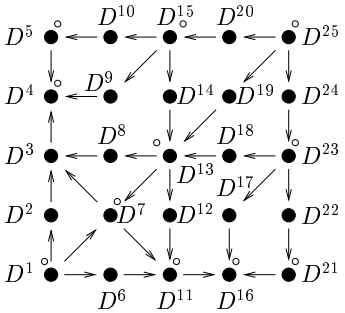
Using standard results from hybrid systems theory [2, 16], it directly follows from the definition of the qualitative PL transition system Σ-QTS as a quotient transition system of the continuous PL transition system Σ-TS that the former is a *conservative approximation* of the latter. That is, if there exists a path $(\boldsymbol{x}^0, \ldots, \boldsymbol{x}^m)$ in Σ-TS, then there also exists a path $(D^0, \ldots, D^m)$ in Σ-QTS, such that $\boldsymbol{x}^i \in D^i$ for all $i \in [0, \ldots, m]$. Note that the converse does not necessarily hold.[2] Using the reachability equivalence between the continuous PL transition system Σ-TS and the PL system Σ, it follows that Σ-QTS is a conservative approximation

[2] In the hybrid systems terminology, Σ-QTS is a *simulation*, but not a *bisimulation* of Σ-TS. Another way to phrase this, using qualitative reasoning terminology, is that any algorithm for constructing Σ-QTS is *sound*, but *incomplete* [26].

Σ-QTS $= (\mathcal{D}, L, \rightarrow_{\sim_\Omega})$, where

- $\mathcal{D} = \{D^1, D^2, \ldots, D^{25}\}$,
- $L = \{int, dim^+, dim^-\}$,
- $\rightarrow_{\sim_\Omega} = \{D^1 \xrightarrow{int} \sim_\Omega D^1,\ D^1 \xrightarrow{dim^-} \sim_\Omega D^2,$ $D^1 \xrightarrow{dim^-} \sim_\Omega D^6, \ldots, D^{25} \xrightarrow{dim^-} \sim_\Omega D^{24}\}$.

(a)



(b)

$$0 < \theta_a^1 < \theta_a^2 < max_a,\ \theta_a^2 < \kappa_a/\gamma_a < max_a,$$
$$0 < \theta_b^1 < \theta_b^2 < max_b,\ \theta_b^2 < \kappa_b/\gamma_b < max_b.$$

(c)

Figure 4: (a) Qualitative transition system Σ-QTS and (b) state transition graph $G = (\mathcal{D}, \rightarrow_{\sim_\Omega})$ corresponding to the two-gene example of Figure 1. Self-transitions are represented by small open circles. (c) Parameter inequality constraints defining a region in the parameter space in which the graph in (b) is invariant.

of Σ. This means that we can safely use the former to study the qualitative dynamics of the latter.

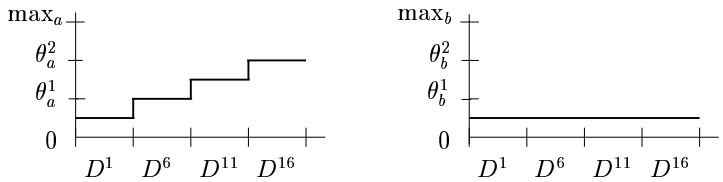


Figure 5: Temporal evolution of the protein concentrations along the path $(D^1, D^6, D^{11}, D^{16})$ of the qualitative transition system Σ-QTS in Figure 4.

The continuous dynamics of a PL system depends on the values of the model parameters, that is, the synthesis and degradation rate parameters, and the threshold concentrations. Nevertheless, two PL systems differing by their parameter values often yield the same qualitative PL transition system, and thus satisfy the same qualitative dynamic properties. We introduce a second equivalence relation $\sim_\Gamma \subseteq \Gamma \times \Gamma$, defined on the parameter space Γ of the PL system Σ, such that two parameter vectors $\boldsymbol{p}$ and $\boldsymbol{p'}$ are equivalent, if their corresponding qualitative PL transition systems, or state transition graphs, are isomorphic. $\Gamma/_{\sim_\Gamma}$ denotes the quotient parameter space, given the equivalence relation $\sim_\Gamma$. We have shown that a certain class of parameter inequality constraints define regions $P \subseteq \Gamma$, such that for every $\boldsymbol{p}, \boldsymbol{p'} \in P$, it holds that $\boldsymbol{p} \sim_\Gamma \boldsymbol{p'}$. More precisely, there exists some $Q \in \Gamma/_{\sim_\Gamma}$, such that $P \subseteq Q$ [26]. As a consequence, for all parameter values satisfying the inequality constraints, the system has the same qualitative dynamic properties.

The regions are defined by two types of inequality constraints that can usually be inferred from the available experimental data. The first type are the so-called *threshold inequalities*,

which are obtained by ordering the threshold concentrations $\theta_i^{l_i}$ of each protein. The second type are the *focal inequalities*, which are obtained by ordering the coefficients of the focal points, $\psi_i(D)$, $D \in \mathcal{D}_r$, with respect to the threshold concentrations of the protein. Figure 4(c) shows the inequality constraints for our example network. The constraints state that protein A inhibits the expression of gene b at a lower concentration than that required for the expression of its own gene ($\theta_a^1 < \theta_a^2$). Moreover, when gene a is active, the concentration of protein A tends towards a level above which autoinhibition occurs ($\kappa_a/\gamma_a > \theta_a^2$). The inequality constraints for protein B are interpreted analogously. The state transition graph in Figure 4(b) is invariant for all parameter values satisfying the inequality constraints in Figure 4(c).

The inequality constraints also play an important role in the actual computation of the qualitative PL transition system [26]. This computation is greatly simplified by the fact that the domains D and the focal sets $\Psi(D)$ are hyperrectangular sets, which allows the sets to be expressed as product, *i.e.* $D = D_1 \times \ldots \times D_n$ and $\Psi(D) = \Psi_1(D) \times \ldots \times \Psi_n(D)$. As a consequence, the computation can be carried out for each dimension separately, using the inequality constraints. For instance, in order to determine the transitions from domain D^1, it is sufficient to know the ordering of κ_a/γ_a and θ_a^1 in the x_a-dimension, as well as the ordering of κ_b/γ_b and θ_b^1 in the x_b-dimension. Given the inequality constraints in Figure 4(c), and the monotonic convergence of the solutions in D^1 towards $\Psi(D^1)$, it can be inferred that there are solutions starting in D^1 and arriving at D^2, D^6, or D^7. Simple symbolic rules for performing these computations are given in [26].

The symbolic rules for computing a qualitative PL transition system from a PL model and inequality constraints have been implemented in Java, giving rise to the computer tool *Genetic Network Analyzer (GNA)* [24] (Figure 6).[3] In order to facilitate its use, the program is equiped with a graphical user interface, called *VisualGNA*. In practice, since the number of domains in the phase space grows exponentially with the number of genes in the network, it is not possible to compute the complete qualitative PL transition system or state transition graph. GNA is therefore mostly used to perform what is called a *qualitative simulation*, that is, the determination of the subgraph of the state transition graph that is reachable from a specified set of initial domains. GNA has been used for the analysis of several genetic regulatory networks, such as the initiation of sporulation in the soil bacterium *Bacillus subtilis* [23] and quorum sensing in the pathogenic bacterium *Pseudomonas aeruginosa* [95].

5 Qualitative simulation of nutritional stress response in *E. coli*

In their natural environment, bacteria like *Escherichia coli* rarely encounter conditions allowing continuous, balanced growth. While nutrients are available, *E. coli* cells grow quickly, leading to an exponential increase of their biomass, a state called *exponential phase*. However, upon depletion of an essential nutrient, the bacteria are no longer able to maintain fast growth rates, and the population consequently enters a non- or slow-growth state, called *stationary phase* (Figure 7(a)). During the transition from exponential to stationary phase, each individual *E. coli* bacterium undergoes numerous physiological changes, concerning among other things the morphology and the metabolism of the cell, as well as gene expression (Figure 7(b)) [44]. These changes enable the cell to survive prolonged periods of starvation and resistant to multiple stresses. This *nutritional stress response* can be reversed and growth resumed, as soon as nutrients become available again.

[3] GNA is available for non-profit academic research purposes at `http://www-helix.inrialpes.fr/gna`.

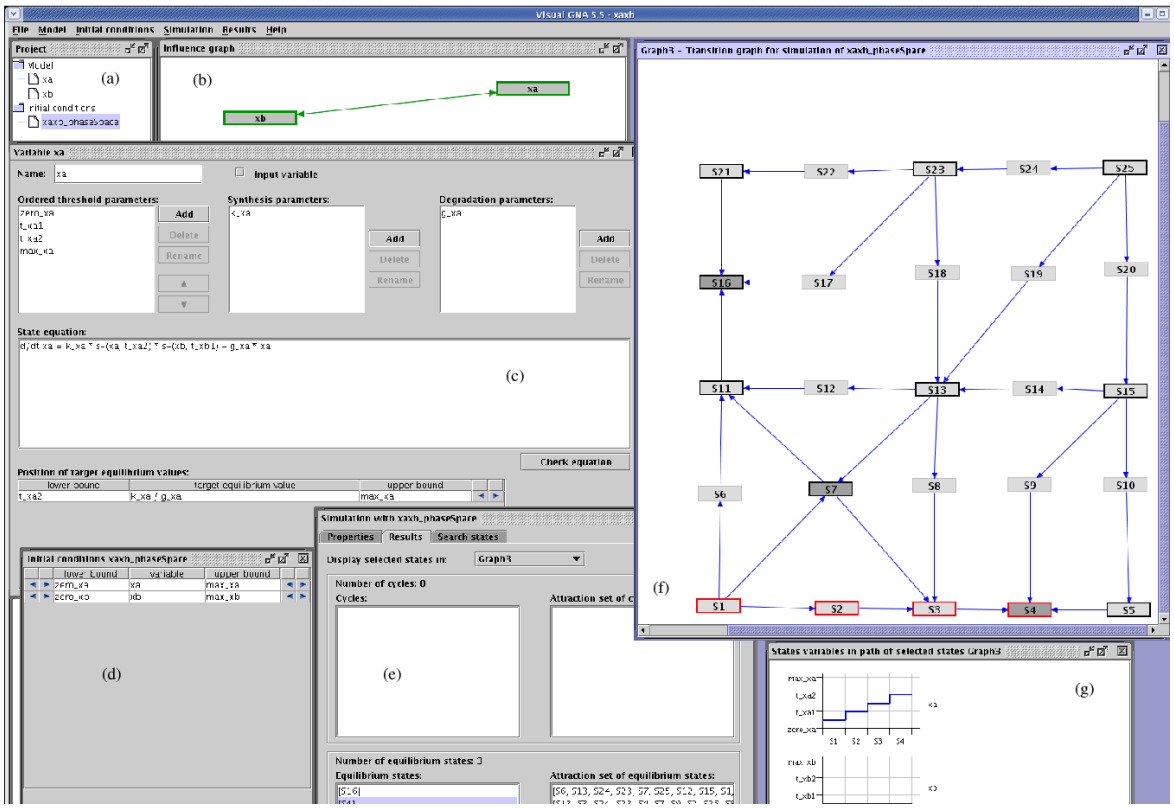


Figure 6: Screenshot of the graphical user interface of GNA, for the simulation of the two-gene network in Figure 1: (a) Tree of concentration variables and initial conditions, (b) interaction graph, (c) specification of differential equation and inequality constraints for a variable, (d) specification of initial domains, (e) summary of simulation results, (f) state transition graph produced by simulation, and (g) qualitative temporal evolution of the concentration variables.

On the molecular level, the transition from exponential phase to stationary phase is controlled by a complex genetic regulatory network integrating various environmental signals [43, 61, 98]. The molecular basis of the adaptation of the growth of *E. coli* to nutritional stress conditions has been the focus of extensive studies for decades [43]. However, notwithstanding the enormous amount of information accumulated on the genes, proteins, and other molecules known to be involved in the stress adaptation process, there is currently no global understanding of how the response of the cell emerges from the network of molecular interactions. Moreover, with some exceptions [11, 38, 97], numerical values for the parameters characterizing the interactions and the molecular concentrations are absent, which makes it difficult to apply traditional methods for the dynamic modeling of genetic regulatory networks.

The above circumstances have motivated the qualitative analysis of the nutritional stress response network in *E. coli* by means of the method presented in the previous sections [78]. The objective of the study was to simulate the response of an *E. coli* bacterium to the absence or presence of nutrients in the growth medium. To this end, an initial, simple model of the nutritional stress response network has been built on the basis of literature data. It includes six genes that are believed to play a key role in the nutritional stress response (Figure 8).

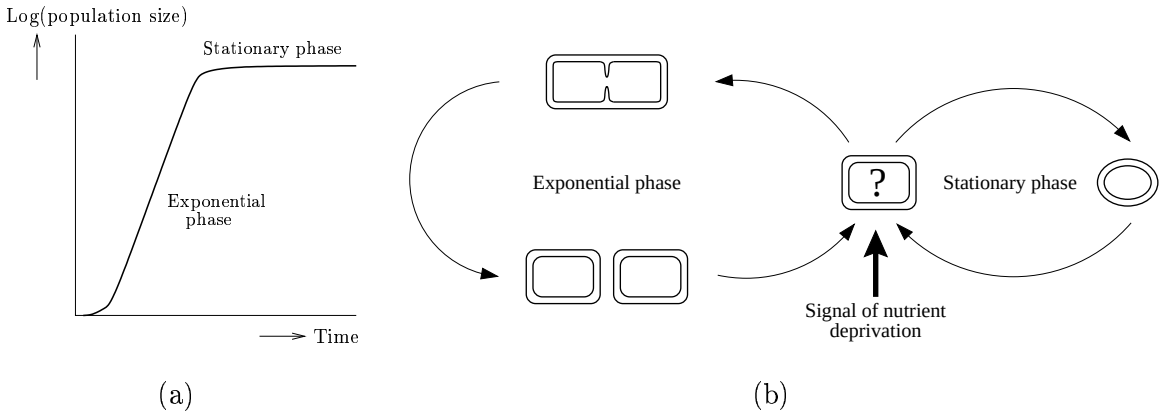


Figure 7: (a) Growth states of a bacterial population: exponential and stationary phase. (b) Nutrient-stress response of bacteria during the transition from exponential to stationary phase.

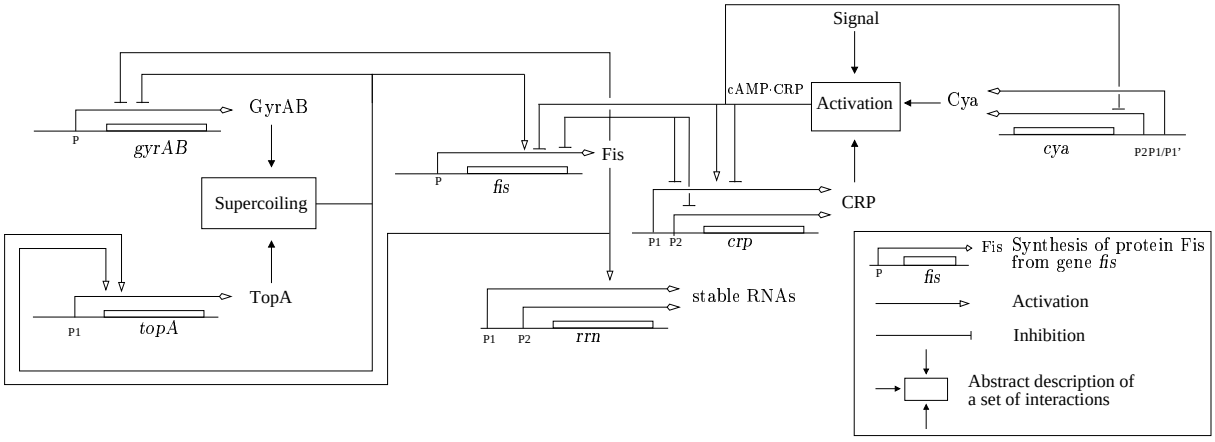


Figure 8: Network of key genes, proteins, and regulatory interactions involved in the nutritional stress network in *E. coli*. The notation follows, in a somewhat simplified form, the graphical conventions proposed by Kohn [52]. The contents of the boxes labeled 'Activation' and 'Supercoiling' are detailed in [78].

More specifically, the network includes genes encoding proteins whose activity depends on the transduction of the nutritional stress signal (the global regulator *crp* and the adenylate cyclase *cya*), genes involved in the metabolism (the global regulator *fis*), cellular growth (the *rrn* genes coding for stable RNAs), and DNA supercoiling, an important modulator of gene expression (the topoisomerase *topA* and the gyrase *gyrAB*).

The graphical representation of the network has been translated into a PL model supplemented with parameter inequality constraints. The resulting model consists of seven variables, one concentration variable for the product of each of the six genes and one input variable representing the presence or absence of a nutritional stress signal [78]. The 38 parameters are constrained by 54 parameters inequalities, the choice of which is largely determined by experimental data. As an illustration, the piecewise-linear differential equation and the inequality constraints for the state variable x_{topA} are given here.

$$\dot{x}_{topA} = \kappa^1_{topA} + \kappa^2_{topA}\, s^+(x_{gyrAB}, \theta^3_{gyrAB})\, s^-(x_{topA}, \theta^1_{topA})\, s^+(x_{fis}, \theta^4_{fis}) - \gamma_{topA}\, x_{topA},$$
$$0 < \theta^1_{topA} < \theta^2_{topA} < \theta^3_{topA} < max_{topA},$$
$$0 < \kappa^1_{topA}/\gamma_{topA} < \theta^1_{topA},$$
$$\theta^3_{topA} < (\kappa^1_{topA} + \kappa^2_{topA})/\gamma_{topA} < max_{topA}.$$

The equation and inequalities state that the basal expression of *topA* is low ($\kappa^1_{topA}/\gamma_{topA} < \theta^1_{topA}$), whereas in the presence of a high concentration of Fis ($s^+(x_{fis}, \theta^4_{fis}) = 1$) and of a high level of DNA supercoiling ($s^+(x_{gyrAB}, \theta^3_{gyrAB})\ s^-(x_{topA}, \theta^1_{topA}) = 1$), the concentration of TopA increases, converging towards a high value ($(\kappa^1_{topA} + \kappa^2_{topA})/\gamma_{topA} > \theta^3_{topA}$).

The computer tool GNA has been used to simulate the response of an *E. coli* cell to nutrient depletion [78]. Starting from initial conditions representing exponential growth, the system is perturbed with a nutritional stress signal that makes the adenylate cyclase active. Simulation of the network takes less than one second to complete on a PC (2.4 GHz, 512 Mb) and gives rise to a transition graph of 128 qualitative states. Many of these states are associated with singular domains that the system traverses instantaneously. Since the biological relevance of the latter states is limited, they can be eliminated. This leads to a reduced transition graph of 39 qualitative states.

In all qualitative behaviors in the state transition graph, the system starts from a qualitative state corresponding to exponential-phase conditions, to reach a qualitative equilibrium state corresponding to the physiological conditions found in stationary phase [78]. A sequence of qualitative states typical for this behavior is shown in Figure 9. The first event after receiving the nutritional stress signal is the decrease of the Fis concentration, followed by the decrease of the stable RNA concentration. The next event concerns the increase of the level of CRP. In parallel, the concentration of GyrAB increases, whereas the concentration of TopA remains constant.

The level of the stable RNAs is a reliable indicator of cellular growth. Since the level of RNAs is low in the qualitative equilibrium state reached by the system after a nutritional stress signal, we conclude that this state is representative for a stationary-phase cell. The process driving the cell's growth arrest can be explained by relating the qualitative behavior to the nutritional stress response network in Figure 8. During exponential phase, the adenylate cyclase is present, but inactive. When the nutritional stress signal is switched on, it activates the protein and thus enables it to produce a metabolite, cAMP. The small molecule binds to

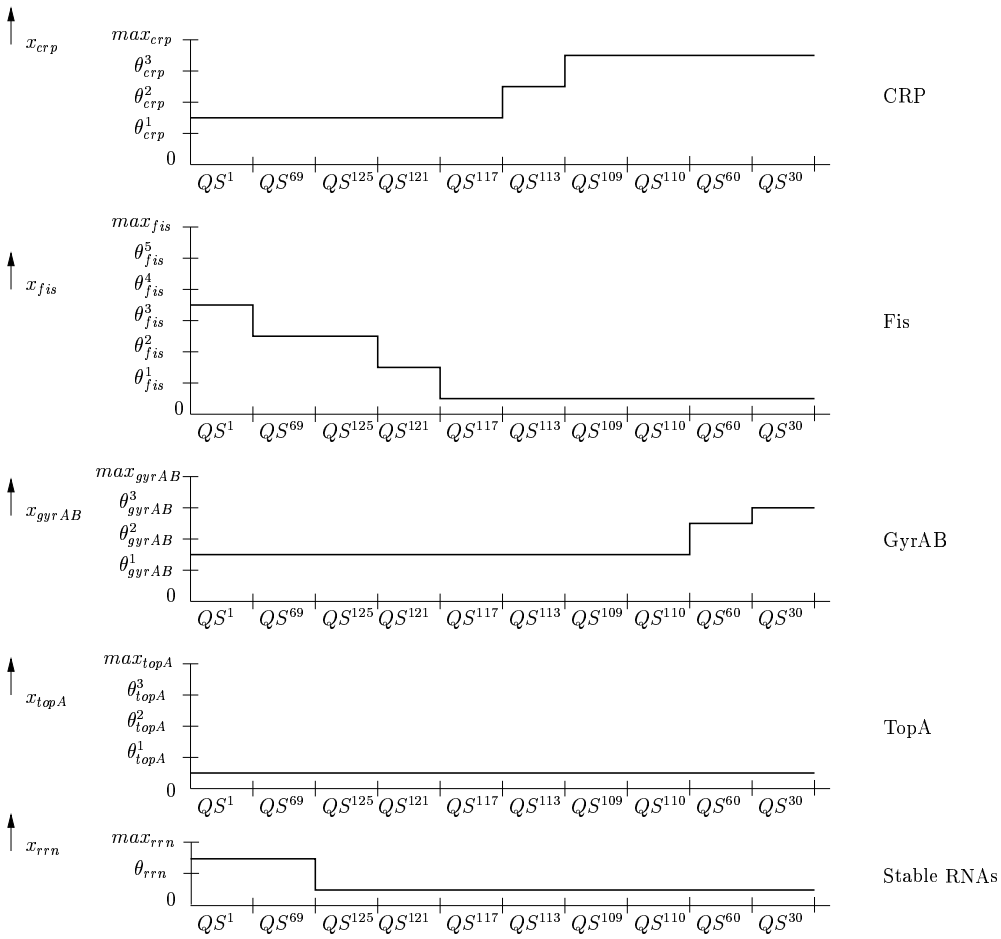


Figure 9: Temporal evolution of selected protein concentrations in a typical qualitative behavior in the state transition graph generated from the *E. coli* nutritional stress response model. The behavior represents the molecular events accompanying the transition from exponential to stationary phase.

CRP, which is not yet abundant, thus giving rise to a low concentration of the cAMP·CRP complex. The level of cAMP·CRP is nevertheless high enough to start repressing the expression of *fis*. This stimulates further accumulation of CRP, and thus further repression of Fis, through the derepression of the Fis-controlled promoters of *crp*. The decrease of the Fis concentration causes the downregulation of the expression of the *rrn* genes. As a consequence, the level of the stable RNAs decreases and the cell enters stationary phase.

We conclude from our model of the nutritional stress response network that a positive feedback mechanism, the mutual inhibition of *fis* and *crp*, plays a key role in the transition from exponential phase to stationary phase. This kind of control mechanism is known to play a key role in developmental processes [92]. In the presence of a nutritional stress signal, it causes a switch from a state with a high Fis concentration and a low CRP concentration to a state with a low Fis concentration and a high CRP concentration. The positive feedback circuit thus enables the cell to abandon the exponential phase in the absence of nutrients and enter stationary phase, while also making it possible for the cell to reenter exponential phase in case of nutrient recovery.

The proteins GyrAB and TopA control the DNA supercoiling level, which constrains the topological structure of DNA and thus influences gene expression [28]. Depending on the relative concentration of the two enzymes, DNA supercoiling can reach different levels in the cell. In the presence of high amounts of GyrAB, many negative supercoils are introduced into the DNA, whereas these are relaxed when the TopA concentration is high. The predicted qualitative evolution of the concentrations of the proteins GyrAB and TopA in Figure 9 implies that the DNA supercoiling level increases at the onset of stationary phase. However, this is not what has been observed experimentally. On the contrary, the DNA supercoiling level has been shown to decrease when *E. coli* cells enter stationary phase [5]. The inconsistency between the predicted and observed level of supercoiling suggests that our picture of the nutritional stress response network is incomplete, in the sense that interactions between the global regulators in Figure 8, or additional regulators not shown in the figure, are missing. Carrying out experiments and extending the model may help to further investigate these possibilities.

In addition to the increased understanding of the transition from exponential to stationary phase, the model has also yielded predictions on the occurrence of damped oscillations in some of the protein concentrations after a nutrient upshift [78]. These predictions, as well as other model predictions on the expression of key genes in the network, have never been observed experimentally. This has motivated us to start experiments aiming at the validation of the model predictions, in particular experiments for measuring the temporal evolution of the expression of key genes in the network by means of reporter genes. Comparison of the observed and predicted expression profiles might lead to refinements of the model, thus initiating a new cycle of prediction and experimental verification.

6 Discussion

In order to understand how the functioning and development of living organisms are controlled by the networks of interactions between genes, proteins, and small molecules in and between cells we need mathematical methods and computer tools. In particular, we have insisted on the demand for qualitative approaches for the modeling, analysis, and simulation of genetic regulatory networks, that is, approaches capable of inferring properties of the dynamics of genetic regulatory networks that are invariant for a range of parameter values and

reaction mechanisms. The interest of these qualitative approaches derives from the fact that, for most networks of biological interest, we do not dispose of precise numerical values for the parameters and detailed information on the reaction mechanisms [45]. Moreover, it is reasonable to assume that many dynamic properties of living organisms are robust to at least some changes in parameter values and variations of reaction mechanisms [6, 30, 87]. This does not mean that qualitative approaches always impose themselves: there are biological questions for which quantitative precision is required, and there do exist systems for which detailed, quantitative information is available. Quantitative and qualitative approaches should be seen as complementary rather than mutually exclusive.

In this chapter, we have reviewed our work on a method for the qualitative modeling, analysis, and simulation of genetic regulatory networks that is well-adapted to currently-available experimental data and many interesting biological questions [26]. The method is based on a class of piecewise-linear differential equation models, originally introduced by Glass and Kauffman [35], that capture essential aspects of gene regulation and have favorable mathematical properties. We have shown that the qualitative dynamics of PL systems can be represented by a state transition graph, consisting of qualitative states and transitions between qualitative states. The major result underlying our method is that this graph is invariant for large sets of parameter values, defined by inequality constraints that can be easily inferred from the experimental literature. Moreover, the state transition graph can be computed from the inequality constraints by means of simple, symbolic rules. In order to support its application to large and complex genetic regulatory networks, the method has been implemented in the computer tool Genetic Network Analyzer (GNA).

The use of PL models is justified by the intuition that, to a first approximation, genes can be considered logical switches that transform continuous inputs – *i.e.*, the concentration of regulatory proteins – into discrete outputs – *i.e.*, the activation state of the genes [88, 99]. Instead of developing this intuition for models with continuous time and concentration variables, one could also decide to employ discrete models. The major example of this approach is the *Boolean network* formalism developed by Kauffman, Thomas, and others [46, 47, 86, 91, 92]. The application of Boolean networks rests on the assumption that a gene is either active or inactive, and that genes change their activation state synchronously. For the purpose of modeling actual genetic regulatory networks, these assumptions are usually too strong. In response to this problem, generalized formalisms with multivalued activation states and asynchronic transitions have been proposed and successfully applied to the analysis of complex developmental regulatory networks [65, 79]. The advantage of Boolean networks and their generalizations is that they provide a convenient way to express the logic of gene expression regulation. However, they have difficulty in treating dynamic properties of genetic regulatory networks taking place at the threshold of activation or inactivation of a gene. The analysis of the dynamics of PL models in Section 3 shows that these phenomena are critical, since equilibrium points of the system may be located at the thresholds.

The method presented in this chapter preserves the ability of continuous models to characterize the dynamics of the system at the thresholds, while at the same time employing discrete abstractions to facilitate the analysis of qualitative dynamic properties. The use of discrete abstractions to study qualitative properties of dynamic systems has been proposed before, in the context of the qualitative reasoning about physical systems [22, 53, 93] and the analysis of hybrid systems [2, 16, 59]. The methods have been applied to a variety of prokaryotic and eukaryotic model systems [1, 33, 41], some applications using the same PL models as in this chapter. Our method borrows ideas and terminology from both qualitative reasoning and the

analysis of hybrid systems, but differs from the latter work in an important respect. Instead of analyzing a broad class of models using general-purpose algorithms, we have developed a method that is tailored to a class of PL models particularly appropriate for genetic regulatory networks. In addition, the method employs algorithms that have been adapted to the favorable mathematical properties of the PL models, thus promoting the upscalability of the analysis to large and complex networks.

In the end, there is only one criterion for evaluating the usefulness of a method for the analysis of the dynamics of genetic regulatory networks: which biologically-interesting results have been obtained through its application? Thus far, our method has been applied to a number of bacterial regulatory networks, such as the initiation of sporulation in *Bacillus subtilis* [23], quorum sensing in *Pseudomonas aeruginosa* [95], and the nutritional stress response in *Escherichia coli* [78]. The description of the latter application in Section 5 has shown that the qualitative simulation method can be used to obtain predictions of the behavior of networks that are currently not yet well understood by biologists. While some of the predictions help clarifying the role of particular regulatory mechanisms (the mutual inhibition of *fis* and *crp*), others contradict measurements reported in the literature (the increase of the DNA supercoiling level upon the entry into stationary phase) or concern phenomena that have not yet been experimentally investigated (the occurrence of damped oscillations after a nutrient upshift). The latter two predictions are particularly interesting from a biological point of view, because they generate new questions and suggest further experiments.

The currently-available data suggest that the network of global regulators of transcription involved in the nutritional stress response is probably an order of magnitude larger than the network presented in Figure 8, while the complete genetic regulatory network of *E. coli* is even several orders of magnitude larger [4, 15, 43, 60, 61, 64, 82, 89]. This raises the question whether the method presented in this chapter, and qualitative approaches more generally, are upscalable to larger networks than those treated thus far. The relevance of this question is confirmed by our experience that the state transition graphs for networks with more than fifteen genes usually consist of several hundreds or even thousands of states, which make them too large to be analyzed by visual inspection alone. Several directions can be chosen to address the problems posed by the size of actual genetic regulatory networks. A promising alternative to the visual inspection of state transition graphs would be to couple the qualitative simulator with model-checking tools for the automatic verification of dynamic properties expressed in temporal logic [17, 83]. We have started to explore this direction and other groups have proposed similar ideas [7, 8, 9, 14]. Another way to deal with large networks would be to avoid the generation of state transition graphs altogether and focus on equilibrium sets and other phase-space attractors, which are usually of special significance for understanding the dynamics of a system [13, 27].

In parallel to the development of computer tools for analyzing and interpreting qualitative simulation results, the upscalability challenge also requires a reflection on the utility of building ever larger and ever-more complex models of cellular systems [75]. For many biological questions, it is not obvious that the prediction of the temporal evolution of the concentration of all proteins in an *E. coli* cell will be the surest road to gaining a better comprehension of the stress response of the bacterium. In many cases, it seems more appropriate and informative to distinguish subnetworks of the network of interest, describe the dynamics of these subnetworks individually – using more abstract models than those presented here –, and couple the abstract models in order to analyze the interactions between subnetworks [10, 48]. This modular approach can be justified by what we know or suspect about the structure of biological

regulatory networks [39, 90]. For instance, the interactions between the ten to twenty global regulators of transcription of *E. coli* seem to be the backbone of the genetic regulatory network of this organism, hierarchically connecting and coordinating the functioning of modules for specific cellular functions.

Qualitative approaches towards the modeling, analysis, and simulation of genetic regulatory networks are promising extensions of the modeler's toolbox, as testified by the results obtained by means of the method presented in this chapter and other, similar methods. Their widespread adoption necessarily passes through the experimental verification of the predictions of qualitative properties of the network dynamics.

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