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► **To cite this version:**

Grégory Batt, Hidde de Jong, Johannes Geiselman, Michel Page, Delphine Ropers, et al.. Symbolic Reachability Analysis of Genetic Regulatory Networks using Qualitative Abstraction. [Research Report] RR-5362, INRIA. 2004, pp.37. inria-00070641

**HAL Id: inria-00070641**

**<https://inria.hal.science/inria-00070641>**

Submitted on 19 May 2006

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# *Symbolic Reachability Analysis of Genetic Regulatory Networks using Qualitative Abstraction*

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**N° 5362**

October 2004

Thème BIO



*Rapport  
de recherche*



# Symbolic Reachability Analysis of Genetic Regulatory Networks using Qualitative Abstraction

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Thème BIO — Systèmes biologiques  
Projet HELIX

Rapport de recherche n° 5362 — October 2004 — 37 pages

**Abstract:** The switch-like character of the dynamics of genetic regulatory networks has attracted much attention from mathematical biologists and researchers on hybrid systems alike. While powerful techniques for the analysis, verification, and control of hybrid systems have been developed, the specificities of the biological application domain pose a number of challenges. In particular, while most networks of biological interest are large and complex, quantitative information on the kinetic parameters and molecular concentrations are usually absent. We introduce a method for the analysis of reachability properties of genetic regulatory networks that is based on a class of discontinuous piecewise-affine (PA) differential equations well-adapted to the above constraints. More specifically, we introduce a partition of the phase space by hyperrectangular regions in each of which the derivatives of the concentration variables have a unique sign pattern. This partition forms the basis for the definition of a discrete abstraction transforming the continuous transition system associated with a PADE model into a discrete or qualitative transition system. The discrete transition system is a simulation of the continuous transition system, thus providing a conservative approximation of the qualitative dynamics of the network. Moreover, the discrete transition system can be easily computed in a symbolic manner from inequality constraints on the parameters. The method has been implemented in a new prototype version of the computer tool Genetic Network Analyzer (GNA), which has been applied to the analysis of a regulatory system whose functioning is not well-understood by biologists, the nutritional stress response in the bacterium *Escherichia coli*

**Key-words:** hybrid systems, piecewise-affine differential equations, differential equations with discontinuous righthand sides, qualitative analysis, discrete abstraction, formal verification, genetic regulatory networks, Genetic Network Analyzer, nutritional stress response, *Escherichia coli*

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# Analyse symbolique d'atteignabilité de réseaux de régulation génique par abstraction qualitative

**Résumé :** Le caractère discret/continu de la dynamique des réseaux de régulation génique a attiré l'attention à la fois des biomathématiciens et des chercheurs en systèmes hybrides. Bien que des techniques efficaces pour l'analyse, la vérification et le contrôle des systèmes hybrides existent, les particularités de ce domaine d'application posent un certain nombre de problèmes. Ainsi, alors que la plupart des réseaux de régulation génique ayant un intérêt biologique sont grands et complexes, il n'existe en général pas d'information quantitative sur les valeurs des paramètres cinétiques et des concentrations moléculaires. Nous proposons une méthode pour l'analyse des propriétés d'atteignabilité des réseaux de régulation génique fondée sur une classe d'équations différentielles affines par morceaux (EDAPM) particulièrement bien adaptées aux contraintes précitées. Plus précisément, nous introduisons une partition de l'espace de phase en régions hyperrectangulaires dans lesquelles les dérivées des variables de concentration ont même signes. Cette partition sert de base à l'introduction d'une abstraction discrète transformant le système de transition continu associé au modèle EDAPM en un système de transition discret et qualitatif. Ce système de transition discret est une simulation du système de transition continu, représentant ainsi une approximation conservatrice de la dynamique du réseau. De plus, le système de transition discret peut être facilement calculé de manière symbolique en utilisant des contraintes d'inégalités sur les paramètres. La méthode a été implémentée dans une nouvelle version prototype de l'outil informatique Genetic Network Analyzer et a été appliquée à l'analyse d'un système de régulation génique dont le fonctionnement est encore mal compris par les biologistes, à savoir, la réponse au stress nutritionnel chez la bactérie *Escherichia coli*.

**Mots-clés :** systèmes hybrides, équations différentielles affines par morceaux, équations différentielles à second membre discontinu, analyse qualitative, abstraction discrète, vérification formelle, réseaux de régulation génique, Genetic Network Analyzer, réponse au stress nutritionnel, *Escherichia coli*

# 1 Introduction

*Hybrid systems* are dynamical systems which exhibit both discrete and continuous behaviors. Typical examples of hybrid systems are systems with a discontinuous dynamics, like bouncing balls or electrical circuits with diodes. The hybrid character of a system may also arise from the interactions of a continuous process with a discrete controller, such as a thermostat controlling the temperature of a room. Finally, complex purely-continuous systems are sometimes preferably represented by hybrid models, using discontinuities as simplifications of rapid and highly nonlinear phenomena. The hybrid paradigm has been fruitfully applied to problems in automotive engine control [8, 9], chemical processing plants [24], automated highway systems [36, 46], manufacturing [50, 56], and air traffic management [60].

The analysis of *genetic regulatory networks* has recently emerged as a promising application field for the hybrid-systems community (*e.g.*, [2, 7, 11, 26, 37, 45]). Genetic regulatory networks, composed of the regulatory interactions between genes, proteins, and small molecules, underly the functioning and development of all living organisms. The dynamics of these networks is hybrid in nature, in the sense that the continuous evolution of the concentration of proteins and other molecules is punctuated by discrete changes in the activity of genes coding for the proteins. More precisely, the cellular concentration of the proteins is the result of two antagonist phenomena: protein synthesis and protein degradation. The synthesis of a protein depends on the expression (*i.e.*, activity) of its gene, which can be regulated by the concentration of other proteins. The degradation of a protein can also be controlled through regulatory interactions with other proteins. While powerful techniques for the analysis, verification, and control of hybrid systems have been developed (see [47, 6] for reviews), the specificities of the biological application domain pose a number of challenges [18]. First, except for a few paradigmatic examples, quantitative information on kinetic parameters and molecular concentrations is usually not available. Second, most networks of biological interest involve a large number of genes and interlocked feedback loops. So their dynamics is complex and an intuitive understanding of their functioning is difficult to obtain. These constraints motivate the development of a method for the qualitative analysis of models of large and complex genetic regulatory networks.

In order to achieve this, genetic regulatory networks are represented by a class of hybrid models originally proposed by Glass and Kauffman [29] in the early seventies. In these models, the continuous phenomena of protein synthesis and degradation are described by means of affine differential equations, while the discontinuous nature of their interaction is captured by means of step functions. The resulting *piecewise-affine differential equations* (PADE) models have mathematical properties that favor qualitative analysis of the steady-state and transient behavior of the networks (*e.g.*, [23, 27, 30, 31, 48, 51, 54]). On a formal level, the PADE models are related to a class of asynchronous logical models proposed by Thomas and colleagues [57]. PADE models and their logical relatives have been used for the study of a number of prokaryotic and eukaryotic regulatory networks (*e.g.*, [19, 26, 49, 53, 58]).

In this report, the analysis of the PADE models of genetic regulatory networks is recast in a hybrid-systems framework. The phase space of the system is partitioned by hyperrectangular regions with qualitatively-identical dynamics, that is, the sign pattern of the time derivative of the state variables is identical in each of these regions. We exploit this partition to associate to each PADE model a continuous transition system having equivalent reachability properties and then, applying a *discrete* or *qualitative abstraction* [4, 5, 14, 15, 16, 33, 59], a discrete transition system providing a compact and qualitative description of the dynamics of the continuous system. Formally, the discrete transition system is a simulation, that is, a conservative approximation of the continuous transition system. We show that the discrete transition system can be easily computed in a symbolic manner from inequality constraints on the parameters. This enables the design of tailored algorithms that scale up to large and complex genetic regulatory networks.

The hybrid, switch-like character of the dynamics of genetic regulatory networks has attracted much attention from researchers on hybrid systems (*e.g.* [2, 7, 11, 26, 37, 45]). Our work differs from this work in three respects. First, we base our approach on piecewise-affine models, whereas some groups have employed piecewise-multi-affine or piecewise-nonlinear models [2, 7, 11, 45], and others have used stochastic instead of deterministic models [37]. While PADE models provide a more approximate description of the regulatory interactions in a network, they are well-adapted to the currently available experimental data and they permit an analysis of high-dimensional systems using inequality constraints on parameters instead of numerical values. Second, while the PADE models have also been used by Ghosh and colleagues [26], these authors do not take into account the dynamics of the system on the planes of discontinuity, where equilibrium points and other phenomena of interest may occur [31]. We use an approach inspired by the treatment of discontinuities originally proposed by Filippov [25] and recently applied to PADE models of genetic regulatory networks by Gouzé and Sari [31]. Third, we introduce tailored algorithms for the computation of the discrete transition system that do not require

the use of complex decision procedures such as the quantifier elimination technique used in [26]. This allows us to fully exploit the favorable mathematical properties of the PADE models and thus promote the upscalability of the method.

In comparison with our previous work [21, 22], the method presented here is based on a finer-grained partition of the phase space which guarantees a unique sign pattern of the derivatives in each of the hyperrectangular regions. This obviously leads to a refined discrete abstraction, and hence to a more precise description of the reachability properties of the system. However, the refinement of the abstraction also ensures a better match between the model predictions and the currently available experimental data, given that the latter usually take the form of changes in the sign pattern of the derivatives of the variables.

The method presented here has been implemented in a new prototype version of the tool *Genetic Network Analyzer (GNA)* [20]. Its applicability to the analysis of complex genetic regulatory networks, whose functioning is not yet well understood by biologists, is illustrated by means of the example of the nutritional stress response in the bacterium *Escherichia coli*. We show how a qualitative analysis of a PADE model of this network results in a discrete transition system of 73 biologically relevant states, whose reachability properties can be validated by means of experimental data using standard model-checking tools. This has led to new insights into how the adaptation of cell growth to nutritional stress emerges from the molecular interactions. Moreover, it has given rise to predictions of the behavior of the system after a nutrient upshift, which are currently being tested in our laboratory [52].

The paper is organized as follows. In Section 2 we introduce PADE models of genetic regulatory networks and we explain how to deal with the discontinuities in the righthand side of the differential equations. The dynamical properties of the PADE models are analyzed in Section 3, which leads us to introduce a partition of the phase space by hyperrectangular regions in which the sign pattern of the derivatives is identical. This will serve as a basis for the discrete or qualitative abstraction presented in Section 4. We reformulate the PADE models as a continuous transition system with equivalent reachability properties, and define a discrete transition system through the use of abstraction. In Section 5, we show how the discrete transition system can be easily computed in a symbolic manner from inequality constraints on parameters. The applicability of the method is illustrated in Section 6 by the qualitative analysis of the reachability properties of the genetic regulatory network underlying the nutritional stress response in *Escherichia coli*. The final section of the paper discusses the results in the context of related work and gives some indications for further work.

## 2 PADE models of genetic regulatory networks

### 2.1 PA systems with step functions

The dynamics of genetic regulatory networks can be modeled by a class of piecewise-affine differential equations (PADE) of the following general form [29, 57]:

$$\dot{\mathbf{x}} = \mathbf{h}(\mathbf{x}) = \mathbf{f}(\mathbf{x}) - \mathbf{g}(\mathbf{x}), \quad (1)$$

where  $\mathbf{x} = (x_1, \dots, x_n)' \in \Omega$  is a vector of cellular protein concentrations,  $\mathbf{f} = (f_1, \dots, f_n)'$ ,  $\mathbf{g} = \text{diag}(g_1, \dots, g_n)$ , and  $\Omega \subset \mathbb{R}_{\geq 0}^n$  is a bounded  $n$ -dimensional phase space box. The rate of change of each protein concentration  $x_i$ ,  $1 \leq i \leq n$ , is thus defined as the difference of the rate of synthesis  $f_i(\mathbf{x})$  and the rate of degradation  $g_i(\mathbf{x}) x_i$  of the protein.

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

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

where  $\kappa_i^l > 0$  is a rate parameter,  $b_i^l : \Omega \rightarrow \{0, 1\}$  a piecewise-continuous *regulation function*, and  $L_i$  a possibly empty set of indices of regulation functions. The function  $g_i$  expresses the 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  $\gamma$  instead of  $\kappa$ . Notice that with the above definitions,  $\mathbf{h}$  is a *piecewise-affine (PA)* function.

A regulation function  $b_i^l$  describes the conditions under which the protein encoded by gene  $i$  is synthesized (degraded) at a rate  $\kappa_i^l$  ( $\gamma_i^l x_i$ ). It is defined in terms of step functions and is the arithmetic equivalent of a

Boolean function expressing the logic of gene regulation [29, 57]. 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  $\mathbf{x}$  and  $\theta_j$  a constant denoting a threshold concentration.  $\boldsymbol{\kappa} = (\dots, \kappa_i^{k_i}, \dots)$ ,  $\boldsymbol{\gamma} = (\dots, \gamma_i^{g_i}, \dots)$  and  $\boldsymbol{\theta} = (\dots, \theta_i^{t_i}, \dots)$  will denote the parameter values.

Figure 1 gives an example of a simple genetic regulatory network consisting of two genes,  $a$  and  $b$ . When a gene ( $a$  or  $b$ ) is expressed, the corresponding protein (A or B) is synthesized. Proteins A and B regulate the expression of genes  $a$  and  $b$ . More specifically, protein B inhibits the expression of gene  $a$ , above a certain threshold concentration, while protein A inhibits the expression of gene  $b$  above one threshold concentration, and of its own gene, above a second, higher threshold concentration. The degradation of the proteins is not regulated.

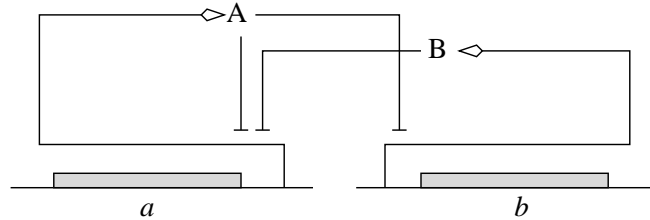


Figure 1: Example of a genetic regulatory network of two genes ( $a$  and  $b$ ), each coding for a regulatory protein (A and B). The notation follows, in a somewhat simplified form, the graphical conventions proposed by Kohn [41].

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

$$\dot{x}_a = \kappa_a s^-(x_a, \theta_a^2) s^-(x_b, \theta_b) - \gamma_a x_a \tag{4}$$

$$\dot{x}_b = \kappa_b s^-(x_a, \theta_a^1) - \gamma_b x_b, \tag{5}$$

where  $\theta_a^1$  and  $\theta_a^2$  denote the threshold concentrations for protein A, with  $\theta_a^1 < \theta_a^2$ , and  $\theta_b$ , the threshold concentration for protein B. We will temporarily assume the following parameter values:  $\kappa_a = 22$ ,  $\kappa_b = 10$ ,  $\gamma_a = \gamma_b = 2$ ,  $\theta_a^1 = 4$ ,  $\theta_a^2 = 8$ ,  $\theta_b = 4$ ,  $max_a = 12$  and  $max_b = 8$ , where  $max_a$  and  $max_b$  denote the maximum concentration of each protein.

Equation (5) describes the rate of change of the concentration of protein B. It states that protein B is produced (at a rate  $\kappa_b$ ), if and only if  $s^-(x_a, \theta_a^1) = 1$ , that is, if and only if  $x_a$ , the concentration of protein A, is below threshold  $\theta_a^1$ . Protein B is degraded at a rate proportional to its own concentration ( $\gamma_b > 0$  is a rate constant). Equation (4) states that protein A is produced (at a rate  $\kappa_a$ ), if and only if the concentration of protein A is below  $\theta_a^2$  ( $s^-(x_a, \theta_a^2) = 1$ ) and  $x_b$ , the concentration of protein B, is below  $\theta_b$  ( $s^-(x_b, \theta_b) = 1$ ). Protein A is also degraded at a rate proportional to its own concentration.

## 2.2 Solutions of PA systems

We have presented above a class of differential equations used to model genetic regulatory networks. The differential equation (1) incorporates step functions to describe the influence of cellular protein concentrations on gene expression. Our goal is to analyze the dynamical properties of the system in the phase space  $\Omega$ . Complications arise from the use of step functions. The step function  $s^\pm(x_j, \theta_j)$  is discontinuous at  $x_j = \theta_j$ . As a consequence, the righthand side of (1),  $\mathbf{h}$ , might be discontinuous on  $\Theta$ , the union of the threshold hyperplanes, given by  $\Theta = \bigcup_{i \in [1..n], t_i \in [1..p_i]} \{ \mathbf{x} \in \Omega \mid x_i = \theta_i^{t_i} \}$ , where the protein encoded by gene  $i$  is assumed to have  $p_i$  threshold concentrations. Since  $\mathbf{h}$  is not fully continuous on  $\Omega$ , classical integration techniques do not apply.

In order to deal with this problem, we will follow an approach widely-used in control theory, originally proposed by Filippov [25]. It consists in extending the differential equation

$$\dot{\mathbf{x}} = \mathbf{h}(\mathbf{x}), \quad \mathbf{x} \in \Omega \setminus \Theta, \tag{6}$$

where  $\mathbf{h}$  is piecewise-continuous in  $\Omega$ , with points of discontinuity in  $\Theta$ , to the differential inclusion

$$\dot{\mathbf{x}} \in K(\mathbf{x}), \quad \text{with } K(\mathbf{x}) = \overline{\text{co}}(\{ \lim_{\mathbf{y} \rightarrow \mathbf{x}, \mathbf{y} \notin \Theta} \mathbf{h}(\mathbf{y}) \}), \quad \mathbf{x} \in \Omega, \tag{7}$$



where  $\overline{co}(P)$  denotes the smallest closed convex set containing the set  $P$ , and  $\{\lim_{\mathbf{y} \rightarrow \mathbf{x}, \mathbf{y} \notin \Theta} \mathbf{h}(\mathbf{y})\}$ , the set of all limit values of  $\mathbf{h}(\mathbf{y})$ , for  $\mathbf{y} \notin \Theta$  and  $\mathbf{y} \rightarrow \mathbf{x}$ . This approach has been applied in the context of genetic regulatory network modeling by Gouzé and Sari [31].

In practice,  $K(\mathbf{x})$  may be difficult to compute because the smallest closed convex set can be a complex polyhedron in  $\Omega$ . We therefore employ an alternative extension of the differential equation (6),

$$\dot{\mathbf{x}} \in H(\mathbf{x}), \text{ with } H(\mathbf{x}) = \overline{rect}(\{\lim_{\mathbf{y} \rightarrow \mathbf{x}, \mathbf{y} \notin \Theta} \mathbf{h}(\mathbf{y})\}), \mathbf{x} \in \Omega, \quad (8)$$

where  $\overline{rect}(P)$  denotes the smallest closed *hyperrectangular* set containing the set  $P$ . By using  $\overline{rect}$ , we can rewrite  $H(\mathbf{x})$  as a product of sets, *i.e.*  $H(\mathbf{x}) = H_1(\mathbf{x}) \times \dots \times H_n(\mathbf{x})$ . Thus, the differential inclusion (8) can be rewritten as a system of differential inclusions  $\dot{x}_i \in H_i(\mathbf{x})$ ,  $i \in [1..n]$ . Notice also that  $H(\mathbf{x})$  is an overapproximation of  $K(\mathbf{x})$ , for all  $\mathbf{x} \in \Omega$ .

Formally, we define a PA system  $\Sigma$  as a triple  $(\Omega, \Theta, H)$ . The solutions of a PA system are given by the solutions of the differential inclusion (8).

**Definition 1 (Solution of differential inclusion).** A solution of the differential inclusion (8) on a time interval  $I$  is an absolutely-continuous vector-valued function  $\xi(t)$ , such that

$$\dot{\xi}(t) \in H(\xi(t)), \text{ almost everywhere on } I, \quad (9)$$

where ‘almost everywhere on  $I$ ’ means everywhere on  $I$  except on a set of measure zero.

This definition of a solution allows us to distinguish few cases. If at time  $t$  a solution  $\xi$  is in a region where  $\mathbf{h}$  is continuous (*i.e.*,  $\xi(t) \in \Omega \setminus \Theta$ ), then  $H(\xi(t)) = \{\mathbf{h}(\xi(t))\}$ , and  $\xi$  is differentiable and satisfies  $\dot{\xi}(t) = \mathbf{h}(\xi(t))$ . If for some  $i \in [1..n]$ ,  $\xi_i$  reaches or leaves a threshold value  $\theta_i^{l_i}$  at time  $t$ , the solution  $\xi$  might not be differentiable, and consequently  $\dot{\xi}(t) \in H(\xi(t))$  might not hold. Finally, if at time  $t$  a solution  $\xi$  slides along a surface of discontinuity,  $\xi$  is differentiable and  $\dot{\xi}(t) \in H(\xi(t))$  holds.

For all  $\mathbf{x}_0 \in \Omega$  and  $\tau \in \mathbb{R}_{>0} \cup \{\infty\}$ ,  $\Xi_\Sigma(\mathbf{x}_0, \tau)$  will denote the set of solutions  $\xi$  of the PA system  $\Sigma$  for the initial condition  $\xi(0) = \mathbf{x}_0$  on  $[0, \tau]$ , if  $\tau$  is finite, or on  $[0, \infty)$ , otherwise.<sup>1</sup> Since the righthand side of (8) is upper semi-continuous, the existence of at least one solution  $\xi$  on some time interval  $[0, \tau]$ ,  $\tau > 0$ , with initial condition  $\xi(0) = \mathbf{x}_0$  is guaranteed, for all  $\mathbf{x}_0 \in \Omega$  [25]. However, there is in general not a unique solution. The set  $\Xi_\Sigma^\circ = \bigcup_{\mathbf{x}_0 \in \Omega, \tau > 0} \Xi(\mathbf{x}_0, \tau)$  is the set of all solutions, on a finite or infinite time interval, of the PA system  $\Sigma$ . We restrict our analysis to the set  $\Xi_\Sigma$  of the solutions in  $\Xi_\Sigma^\circ$  that reach and leave finitely-many times a threshold hyperplane.

### 3 Analysis of the dynamics of PA systems

In this section, we analyze the solutions of a PA system  $\Sigma = (\Omega, \Theta, H)$  and we propose a characterization of the solutions in the phase space that will be used to perform a symbolic and qualitative reachability analysis in the latter section. In order to achieve this, we partition the phase space into regions having qualitatively-identical dynamics. In fact, we successively introduce two partitions: an initial, coarse-grained partition (Section 3.1), that is a preliminary to a second, more refined partition (Section 3.3).

#### 3.1 Partitioning the phase space into mode domains

The dynamical properties of the solutions of  $\Sigma$  can be analyzed in the  $n$ -dimensional phase space box  $\Omega = \Omega_1 \times \dots \times \Omega_n$ , where  $\Omega_i = \{x_i \in \mathbb{R} \mid 0 \leq x_i \leq \max_i\}$  and  $\max_i$  denotes a maximum concentration of the protein encoded by gene  $i$ ,  $1 \leq i \leq n$ .

The  $(n - 1)$ -dimensional threshold hyperplanes  $\{\mathbf{x} \in \Omega \mid x_i = \theta_i^{l_i}\}$ ,  $1 \leq l_i \leq p_i$ ,  $1 \leq i \leq n$ , partition  $\Omega$  into (hyper)rectangular regions. Since the regulation of gene expression is identical everywhere in such a region (Section 3.2), it corresponds to a regulation *mode*. Consequently, the regions are called *mode domains*.<sup>2</sup>

**Definition 2 (Mode domains).**  $\mathcal{M}$  is the set of hyperrectangular regions  $M = M_1 \times \dots \times M_n \subseteq \Omega$ , such that for all  $i \in [1..n]$ ,  $M_i$  is given by one of the following equations:

<sup>1</sup>In the sequel, we say, by abuse of terminology, that  $\xi$  is a solution of  $\Sigma$  on  $[0, \tau]$ ,  $\tau \in \mathbb{R}_{>0} \cup \{\infty\}$ .

<sup>2</sup>Mode domains were called *domains* in our previous publications. [19, 21, 22]

$$\begin{aligned}
 M_i &= \{x_i \in \Omega_i \mid 0 \leq x_i < \theta_i^1\}, \\
 M_i &= \{x_i \in \Omega_i \mid x_i = \theta_i^1\}, \\
 &\dots \\
 M_i &= \{x_i \in \Omega_i \mid x_i = \theta_i^{p_i}\}, \\
 M_i &= \{x_i \in \Omega_i \mid \theta_i^{p_i} < x_i \leq \max_i\}.
 \end{aligned}$$

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

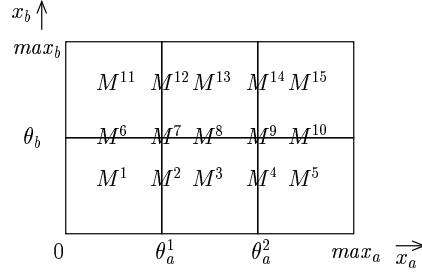


Figure 2: Partition by mode domains of the phase space corresponding to the regulatory network in Figure 1.

We introduce some simple topological concepts. For every hyperrectangular region  $R \subseteq \Omega$  of dimension  $k$ ,  $0 \leq k \leq n$ , we define the supporting hyperplane of  $R$ ,  $\text{supp}(R) \subseteq \Omega$ , as the  $k$ -dimensional hyperplane containing  $R$ . The boundary of  $R$  in  $\text{supp}(R)$ ,  $\partial R$ , is given by the set of points  $\mathbf{x} \in \text{supp}(R)$ , such that each ball  $B(\mathbf{x}, \epsilon)$  with center  $\mathbf{x}$  and radius  $\epsilon > 0$  intersects both  $R$  and  $\text{supp}(R) \setminus R$ . Then, we define the set  $R(M)$  of regular mode domains  $M'$  having the singular mode domain  $M$  in their boundary as:

$$R(M) = \{M' \in \mathcal{M}_r \mid M \subseteq \partial M'\}, \quad M \in \mathcal{M}_s.$$

For example, in the phase space corresponding to our two-gene example shown in Figure 2, it holds that  $\text{supp}(M^1) = \Omega$ ,  $\text{supp}(M^2) = \{\mathbf{x} \in \Omega \mid x_a = \theta_a^1\}$ , and  $\text{supp}(M^7) = \{\mathbf{x} \in \Omega \mid x_a = \theta_a^1 \text{ and } x_b = \theta_b\} = M^7$ , with the dimensions of  $M^1$ ,  $M^2$ , and  $M^7$  being equal to 2, 1, and 0, respectively. The boundary of  $M^2$  in  $\text{supp}(M^2)$  corresponds to the single point in  $M^7$ , while the boundary of  $M^1$  in  $\text{supp}(M^1)$  corresponds to the union of the domains  $M^2$ ,  $M^6$  and  $M^7$ . So,  $M^1$  is included in  $R(M^2)$ ,  $R(M^6)$  and  $R(M^7)$ . More precisely,  $R(M^2) = \{M^1, M^3\}$ ,  $R(M^6) = \{M^1, M^{11}\}$  and  $R(M^7) = \{M^1, M^3, M^{11}, M^{13}\}$ .

### 3.2 Characterization of solutions in mode domains

#### 3.2.1 Analysis in regular mode domains

It follows from the definition of the partition of  $\Omega$  that the functions  $b_i^l$  in equation (2) reduce to *constants* in a regular mode domain  $M$ , so that the functions  $f_i$  and  $g_i$  reduce to sums of constants. More specifically, for every regular mode domain  $M$ , we have for all  $\mathbf{x} \in M$ ,  $\mathbf{f}(\mathbf{x}) = \boldsymbol{\mu}^M$  and  $\mathbf{g}(\mathbf{x}) = \boldsymbol{\nu}^M$ , with  $\boldsymbol{\mu}^M = (\mu_1^M, \dots, \mu_n^M)' \geq \mathbf{0}$  and  $\boldsymbol{\nu}^M = \text{diag}(\nu_1^M, \dots, \nu_n^M) > \mathbf{0}$ .

Our goal is to analyze the differential inclusion (8) in a regular mode domain  $M$ , where  $\dot{\mathbf{x}} \in H(\mathbf{x})$ , with  $H(\mathbf{x}) = \overline{\text{rect}(\{\lim_{\mathbf{y} \rightarrow \mathbf{x}, \mathbf{y} \notin \Theta} \mathbf{h}(\mathbf{y})\})}$ ,  $\mathbf{x} \in M$ ,  $M \in \mathcal{M}_r$ . Since the function  $\mathbf{h}$  is continuous in  $M$ , the set of all limit values of  $\mathbf{h}$  at  $\mathbf{x}$ ,  $\{\lim_{\mathbf{y} \rightarrow \mathbf{x}, \mathbf{y} \notin \Theta} \mathbf{h}(\mathbf{y})\}$ , is a singleton set and equals  $\{\mathbf{h}(\mathbf{x})\}$ . So,  $H(\mathbf{x}) = \{\mathbf{h}(\mathbf{x})\} = \{\boldsymbol{\mu}^M - \boldsymbol{\nu}^M \mathbf{x}\}$  and (8) reduces in  $M$  to the differential inclusion:

$$\dot{\mathbf{x}} \in H(\mathbf{x}), \text{ with } H(\mathbf{x}) = \{\boldsymbol{\mu}^M - \boldsymbol{\nu}^M \mathbf{x}\}, \mathbf{x} \in M \tag{10}$$

Note that (10) can be rewritten as the system of *uncoupled* differential inclusions  $\dot{x}_i \in H_i(\mathbf{x}) = \{\mu_i^M - \nu_i^M x_i\}$ ,  $i \in [1..n]$ . To each regular mode domain is associated a *focal set* [28, 51, 54].<sup>3</sup>

<sup>3</sup>In our earlier work, focal sets were called target equilibrium sets [19, 21, 22].

**Definition 3 (Focal set: regular case).** The focal set  $\Psi(M)$  associated with the regular mode domain  $M$  is  $\{\psi(M)\}$ , where  $\psi(M) = (\nu^M)^{-1}\mu^M$ .

It is not difficult to show that all solutions  $\xi$  in  $M$  monotonically converge towards the point,  $\psi(M)$ . Moreover, if  $\psi(M) \in M$ , then all solutions in  $M$  asymptotically approach  $\psi(M)$ , which is then an equilibrium point of the system. On the contrary, if  $\psi(M) \notin M$ , then all solutions will leave  $M$  at some point [28, 51, 54].

For the sake of simplicity, we make the generic assumption that that the focal sets of regular mode domains are *not* located in  $\Theta$ , the union of threshold planes, where discontinuities occur.

**Assumption 1.** For all  $M \in \mathcal{M}_r$ ,  $i \in [1..n]$ , and  $l_i \in [1..p_i]$ , it holds that  $\mu_i^M/\nu_i^M \neq \theta_i^{l_i}$ .

Additionally, for all  $M \in \mathcal{M}_r$ , and  $i \in [1..n]$ , we require that  $\mu_i^M/\nu_i^M < \max_i$ , which guarantees that every solution starting in  $\Omega$  will remain in it.

Consider the PA system representing the genetic regulatory network in Figure 1. Every point  $x = (x_a, x_b)'$  in  $M^{11}$  satisfies  $0 \leq x_a < \theta_a^1$  and  $\theta_b < x_b \leq \max_b$  (Figure 3(a)), by definition of the partition  $\mathcal{M}$  (Definition 2). Then, equation (5) reduces to  $\dot{x}_b = \kappa_b - \gamma_b x_b$ , since for every  $x \in M^{11}$ ,  $s^-(x_a, \theta_a^1) = 1$ . Similarly, for every  $x \in M^{11}$ ,  $s^-(x_a, \theta_a^2) s^-(x_b, \theta_b) = 0$ , so that equation (4) reduces to  $\dot{x}_a = -\gamma_a x_a$ . Consequently, the constants associated with  $M^{11}$ ,  $\mu^{M^{11}}$  and  $\nu^{M^{11}}$  equal  $(0, \kappa_b)'$  and  $\text{diag}(\gamma_a, \gamma_b)$  respectively, while the focal set  $\Psi(M^{11})$  equals  $\{\psi(M^{11})\}$ , where  $\psi(M^{11}) = (0, \kappa_b/\gamma_b)'$ . With the parameter values of Section 2.1,  $\theta_b < \kappa_b/\gamma_b \leq \max_b$ ,  $\Psi(M^{11}) \in M^{11}$ , and all solutions in  $M^{11}$  monotonically converge towards the equilibrium point  $\psi(M^{11})$ , as illustrated in Figure 3(b). The focal sets associated with all regular mode domains in the example are shown in Figure 4.

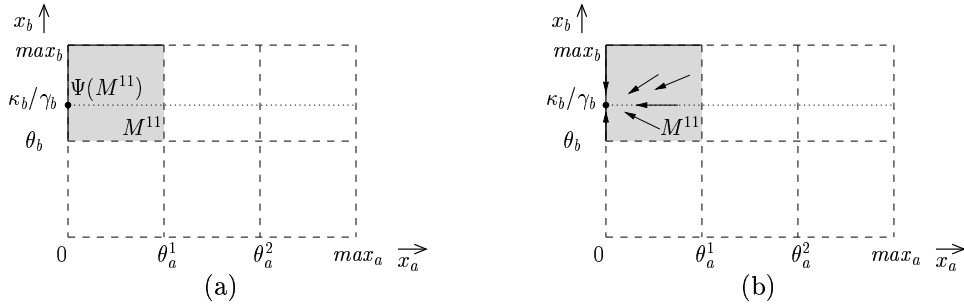


Figure 3: Analysis of the example PA system in the regular mode domain  $M^{11}$ : (a) focal set and (b) direction of the flow.

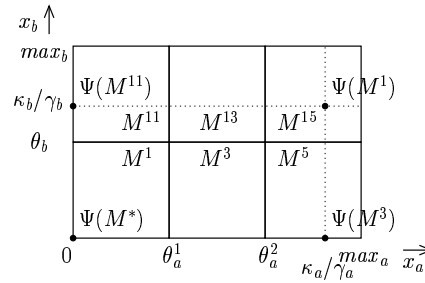


Figure 4: Focal sets associated with all regular mode domains ( $M^*$  denotes  $M^5$ ,  $M^{13}$ , and  $M^{15}$ ).

### 3.2.2 Analysis in singular mode domains

Now our goal is to analyze the differential inclusion (8) in a singular mode domain  $M$ :  $\dot{x} \in H(x)$ , with  $H(x) = \overline{\text{rect}}(\{\lim_{y \rightarrow x, y \notin \Theta} h(y)\})$ ,  $x \in M$ ,  $M \in \mathcal{M}_s$ . Since  $h$  may be discontinuous at the point  $x$ , the set of all limit values of  $h$  at  $x$ ,  $\{\lim_{y \rightarrow x, y \notin \Theta} h(y)\}$  is not necessarily a singleton set.  $y \rightarrow x$  and  $y \notin \Theta$  implies that  $y$  is in a *regular* mode domain  $M'$  having  $x$  in its boundary, that is  $y \in M' \in R(M)$ . Since  $h$  is continuous in every regular mode domain  $M'$ ,  $M' \in R(M)$ ,  $\lim_{y \rightarrow x, y \in M'} h(y) = \mu^{M'} - \nu^{M'} x$ . Consequently, the differential inclusion (8) reduces to

$$\dot{x} \in H(x), \text{ with } H(x) = \overline{\text{rect}}(\{\mu^{M'} - \nu^{M'} x \mid M' \in R(M)\}), x \in M. \quad (11)$$

Like in the case of regular mode domains, the differential inclusion (11) can be rewritten as the system of *uncoupled* differential inclusions  $\dot{x}_i \in H_i(\mathbf{x}) = \overline{\text{rect}}(\{\mu_i^{M'} - \nu_i^{M'} x_i \mid M' \in R(M)\})$ ,  $i \in [1..n]$ .

Similarly to what was proposed for regular mode domains, we introduce a *focal set* associated with a singular mode domain [22, 31].

**Definition 4 (Focal set: singular case).** The focal set associated with the singular mode domain  $M$  is  $\Psi(M) = \text{supp}(M) \cap \overline{\text{rect}}(\{\psi(M') \mid M' \in R(M)\})$ .

Two different cases can be distinguished, depending on whether  $\Psi(M)$  is empty or not. Note that if  $\Psi(M)$  is not empty, we have  $\Psi(M) = \Psi_1(M) \times \dots \times \Psi_n(M)$ , since it has a (hyper)rectangular shape, possibly reduced to a single point. If  $\Psi(M)$  is empty, then every solution passes through  $M$  instantaneously [22].<sup>4</sup>  $M$  is then called an *instantaneous* mode domain. If not, then some (but not necessarily all) solutions arriving at  $M$  will remain in  $M$  for more than a single instant of time, sliding along one or several threshold planes [22]. We denote by  $\mathcal{M}_{pers}$  the set of (regular and singular) non-instantaneous, also called *persistent*, mode domains in  $\Omega$ :  $\mathcal{M}_{pers} = \{M \in \mathcal{M} \mid \Psi(M) \neq \emptyset\}$ .

In [22], it was also proven that all solutions in  $M$ ,  $M \in \mathcal{M}_{pers}$ , converge towards the focal set,  $\Psi(M)$ . As for the regular case, the convergence towards  $\Psi(M)$  of the solutions in  $M$  is monotonic, but in a weak sense (see Lemma 1 in Appendix A). Additionally, if  $\Psi(M) \cap M$  is not empty, then some solutions in  $M$  reach or asymptotically approach  $\Psi(M) \cap M$ , which is a set of equilibrium points of the system. In the special case where  $\Psi(M) \subseteq M$ , all solutions in  $M$  reach or asymptotically approach  $\Psi(M)$ , which is a set of equilibrium points. If, on the contrary,  $\Psi(M) \cap M = \emptyset$ , then all solutions will leave  $M$  at some point. Note that the preceding properties hold for *solutions in  $M$* , where  $M$  is a persistent mode domain and by ‘solutions in  $M$ ’, we mean ‘solutions lying within  $M$  longer than a single time instant’.

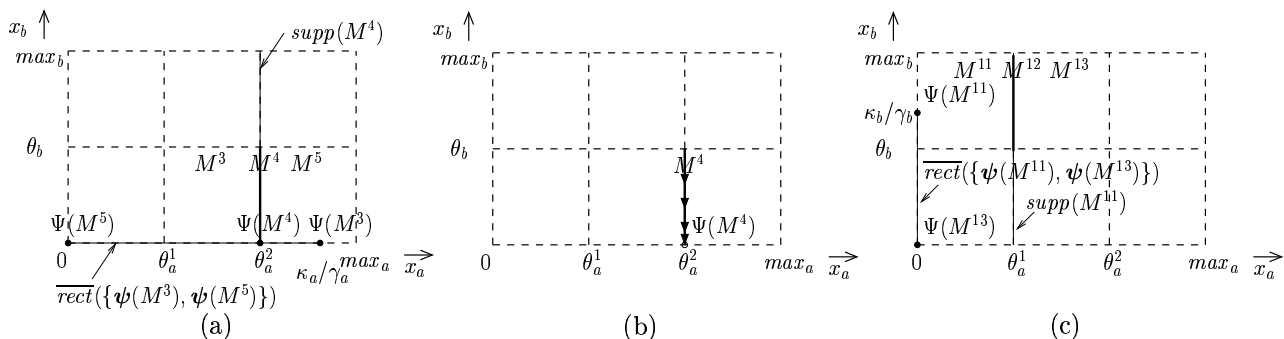


Figure 5: Analysis of the example PA system in the singular mode domains  $M^4$  and  $M^{12}$ : (a) determination of the focal set of  $M^4$ , and (b) direction of the flow in  $M^4$ , and (c) determination of the focal set of  $M^{12}$ .

Consider again our two-gene example. For the singular mode domain  $M^4$ , represented in Figure 5(a), we can determine the focal set, using Definition 4. First, the supporting hyperplane of  $M^4$ ,  $\text{supp}(M^4)$ , equals  $\{\mathbf{x} \in \Omega \mid x_a = \theta_a^2\}$ . Second, we have  $R(M^4) = \{M^3, M^5\}$  and  $\overline{\text{rect}}(\{\psi(M') \mid M' \in R(M^4)\}) = \{\mathbf{x} \in \Omega \mid 0 \leq x_a \leq \kappa_a/\gamma_a, x_b = 0\}$ , since  $\psi(M^3) = (\kappa_a/\gamma_a, 0)'$  and  $\psi(M^5) = (0, 0)'$ . Consequently,  $\Psi(M^4)$ , defined as  $\text{supp}(M^4) \cap \overline{\text{rect}}(\{\psi(M') \mid M' \in R(M^4)\})$ , equals the singleton set  $\{(\theta_a^2, 0)'\}$ .<sup>5</sup>  $\Psi(M^4) \neq \emptyset$ , hence  $M^4$  is a persistent mode domain, and the solutions in  $M^4$ , sliding along the  $\theta_a^2$ -hyperplane, converge towards  $\Psi(M^4)$  (Figure 5(b)). Moreover, since  $\Psi(M^4) \subseteq M^4$ , all solutions in  $M^4$  reach  $\Psi(M^4)$ , for  $t \rightarrow \infty$ , which is an equilibrium point.

A similar analysis can be carried out for the singular mode domain  $M^{12}$  (Figure 5(c)). We have  $\text{supp}(M^{12}) = \{\mathbf{x} \in \Omega \mid x_a = \theta_a^1\}$ . Since  $R(M^{12}) = \{M^{11}, M^{13}\}$ ,  $\psi(M^{11}) = (0, \kappa_b/\gamma_b)'$  and  $\psi(M^{13}) = (0, 0)'$ , we have  $\overline{\text{rect}}(\{\psi(M') \mid M' \in R(M^{12})\}) = \{\mathbf{x} \in \Omega \mid x_a = 0, 0 \leq x_b \leq \kappa_b/\gamma_b\}$ . Consequently,  $\Psi(M^{12})$ , defined as  $\text{supp}(M^{12}) \cap \overline{\text{rect}}(\{\psi(M') \mid M' \in R(M^{12})\})$ , is empty and  $M^{12}$  is an instantaneous mode domain: all solutions arriving at  $M^{12}$  instantaneously traverse it.

In the two preceding sections, we have defined the focal set associated with a mode domain and we have given some important properties that can be used to sketch the local phase portrait in any mode domain, as illustrated for both types of mode domains in Figures 3 and 5. The local phase portraits can be pieced together

<sup>4</sup>These results are based on work of [31], which applies to Filippov solutions, that is, to solutions of (7), instead of solutions of (8).

<sup>5</sup>Note that, although we do not encounter this situation in our two-gene example, a focal set can be a non-singleton set.

to obtain the global phase portrait for the whole phase space. This is illustrated in Figure 6 for our two-gene example. The currently-distributed version of the computer tool Genetic Network Analyzer (GNA 5.5) uses this partition to sketch the dynamics of the system in the phase space.

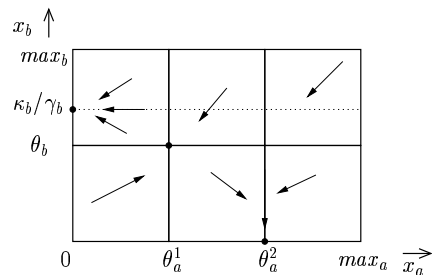


Figure 6: Global phase space portrait corresponding to the two-gene example of Figure 1. The flow is sketched in every persistent mode domain. Dots represent equilibrium points.

Remind that our goal is to use the results of the analysis to test the validity of a genetic regulatory network model. The data produced by state-of-the-art experimental techniques, such as quantitative RT-PCR, reporter genes, and DNA microarrays, usually provide information on changes in the sign of the derivatives of the concentration variables. Consequently, in order to compare simulated with experimental data, we have to be able to determine the signs of the derivatives of the solutions in each domain of the phase space. Unfortunately, all solutions in a mode domain do not necessarily have the same derivative signs (consider, for example, mode domain  $M^{11}$ , in the  $x_b$ -dimension, in Figure 3(b)). So, it is difficult to give an appropriate characterization of the derivative signs of the solutions in a *mode* domain. In the next section, we will show that it is possible to find a more fine-grained partition of the phase space, such that the dynamics is qualitatively-identical in every region. This is achieved by repartitioning the mode domains into so-called *flow domains*.

### 3.3 Partitioning mode domains into flow domains

In this section, we will show how to partition mode domains into flow domains. We will consider separately the two types of mode domains we have defined so far: regular and singular mode domains.

#### 3.3.1 Partitioning regular mode domains into flow domains

Let  $M$  be a regular mode domain. Then  $M$  is partitioned by the  $(n - 1)$ -dimensional hyperplanes  $\{\mathbf{x} \in \Omega \mid x_i = \psi_i(M)\}$ ,  $i \in [1..n]$ , into (hyper)rectangular regions, called *flow domains*. More precisely, the regular mode domain  $M$  is split in the  $x_i$ -dimension, if and only if the hyperplane  $\{\mathbf{x} \in \Omega \mid x_i = \psi_i(M)\}$  intersects  $M$ , that is, if and only if  $\psi_i(M) \in M_i$ .

**Definition 5 (Flow domains: regular case).** The set of flow domains  $\mathcal{D}^M$  associated with a regular mode domain  $M$  is the set of non-empty hyperrectangular regions  $D = D_1 \times \dots \times D_n \subseteq M$ , such that for all  $i \in [1..n]$ :

- if  $\psi_i(M) \notin M_i$ , then  $D_i = M_i$ ,
- otherwise,  $D_i$  is given by one of the following equations:

$$\begin{aligned} D_i &= \{x_i \in M_i \mid x_i < \psi_i(M)\}, \\ D_i &= \{x_i \in M_i \mid x_i = \psi_i(M)\}, \\ D_i &= \{x_i \in M_i \mid \psi_i(M) < x_i\}. \end{aligned}$$

The partitioning of the two regular mode domains  $M^1$  and  $M^{11}$  is illustrated in Figure 7. Definition 5 states that  $M^1$  is neither split in the  $x_a$ -dimension, since  $\psi_a(M^1) \notin M_a^1$ , nor in  $x_b$ -dimension, since  $\psi_b(M^1) \notin M_b^1$ , as illustrated in Figure 7(a). Consequently, a single flow domain,  $D^{1.1}$ , with  $D^{1.1} = M^1$ , makes up the new partitioning of  $M^1$ . The partitioning of  $M^{11}$ , illustrated in Figure 7(b), is more interesting. Since  $\psi_b(M^{11}) \in M_b^{11}$ ,  $M_b^{11}$  is partitioned into three subsets:  $\{x_b \in \Omega_b \mid \theta_b < x_b < \psi_b(M^{11})\}$ ,  $\{x_b \in \Omega_b \mid x_b = \psi_b(M^{11})\}$  and  $\{x_b \in \Omega_b \mid \psi_b(M^{11}) < x_b \leq \max x_b\}$ . Analogously,  $M_a^{11}$  is partitioned into two subsets:  $\{x_a \in \Omega_a \mid x_a = \psi_a(M^{11})\}$  and  $\{x_a \in \Omega_a \mid \psi_a(M^{11}) < x_a < \theta_a^1\}$ , bearing in mind that the subset  $\{x_a \in \Omega_a \mid 0 \leq x_a < \psi_a(M^{11})\}$  is empty, since  $\psi_a(M^{11}) = 0$ . Consequently,  $M^{11}$  is partitioned into  $2 \times 3 = 6$  flow domains, denoted  $D^{11.1}, \dots, D^{11.6}$ , as shown in Figure 7(c).

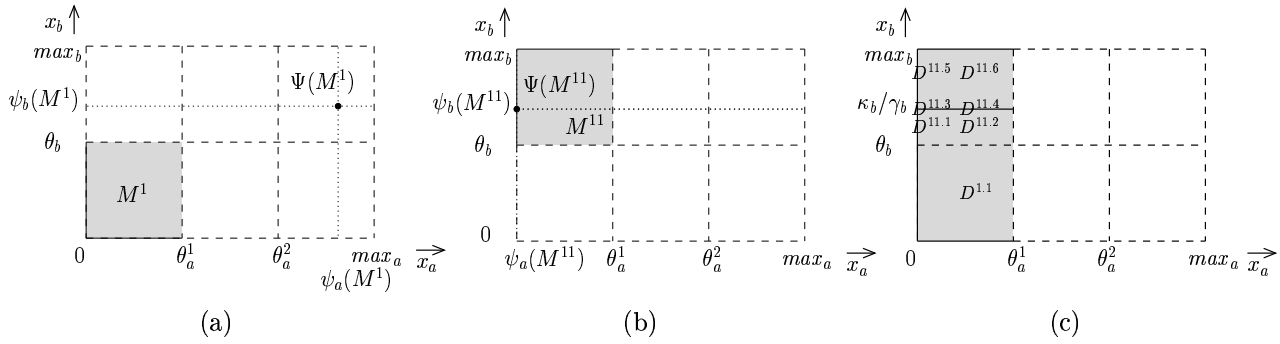


Figure 7: Partition of the regular mode domains  $M^1$  and  $M^{11}$ . (a) The partition of  $M^1$  is trivial, because  $\psi_a(M^1) \notin M_a^1$  and  $\psi_b(M^1) \notin M_b^1$ . (b)  $M^{11}$  is partitioned in the  $x_a$ - and  $x_b$ -dimension, because  $\psi_a(M^{11}) \in M_a^{11}$  and  $\psi_b(M^{11}) \in M_b^{11}$ . (c) The partitioning of  $M^1$  and  $M^{11}$  yields the flow domains  $D^{1..1}$  and  $D^{11.1}, \dots, D^{11.6}$ , respectively.

### 3.3.2 Partitioning singular mode domains into flow domains

Let  $M$  be a singular mode domain. Then,  $M$  is partitioned into flow domains by the  $(n-1)$ -dimensional hyperplanes  $\{\mathbf{x} \in \Omega \mid x_i = \psi_i(M')\}$ ,  $M' \in R(M)$ ,  $i \in [1..n]$ . We consequently define the set of focal concentrations partitioning  $M$  in the  $x_i$ -dimension  $\Lambda_i(M)$  as  $\Lambda_i(M) = \{\psi_i(M') \in M_i \mid M' \in R(M)\}$ ,  $i \in [1..n]$ . We denote by  $\lambda_i^1, \dots, \lambda_i^{q_i}$  the ordered elements of  $\Lambda_i(M)$ , such that  $\lambda_i^1 < \dots < \lambda_i^{q_i}$ . Thus,  $M$  is split in the  $x_i$ -dimension, if and only if  $M_i$  is not a singleton set and  $\Lambda_i(M) \neq \emptyset$ , that is at least one of the hyperplanes  $\{\mathbf{x} \in \Omega \mid x_i = \psi_i(M')\}$ ,  $M' \in R(M)$ , intersects  $M_i$ .

**Definition 6 (Flow domains: singular case).** The set of flow domains  $\mathcal{D}^M$  associated with a singular mode domain  $M$  is the set of non-empty hyperrectangular regions  $D = D_1 \times \dots \times D_n \subseteq M$ , such that for all  $i \in [1..n]$ :

- if  $M_i$  is a singleton set or  $\Lambda_i(M) = \emptyset$ , then  $D_i = M_i$ ,
- otherwise,  $\Lambda_i(M) = \{\lambda_i^1, \dots, \lambda_i^{q_i}\}$  and  $D_i$  is given by one of the following equations:

$$\begin{aligned} D_i &= \{\mathbf{x}_i \in M_i \mid x_i < \lambda_i^1\}, \\ D_i &= \{\mathbf{x}_i \in M_i \mid x_i = \lambda_i^1\}, \\ D_i &= \{\mathbf{x}_i \in M_i \mid \lambda_i^1 < x_i < \lambda_i^2\}, \\ &\dots \\ D_i &= \{\mathbf{x}_i \in M_i \mid x_i = \lambda_i^{q_i}\}, \\ D_i &= \{\mathbf{x}_i \in M_i \mid \lambda_i^{q_i} < x_i\}. \end{aligned}$$

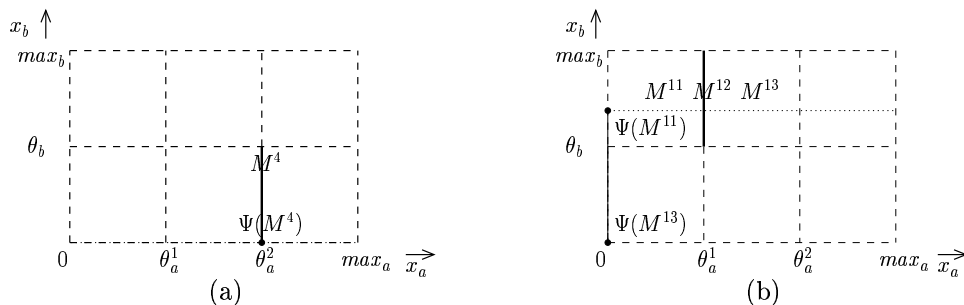


Figure 8: Partition of the singular mode domains  $M^4$  and  $M^{12}$ . (a)  $M^4$  is not split in the  $x_a$ -dimension, since  $M_a^4$  is a singleton set, whereas it is split in the  $x_b$ -dimension, since  $M_b^4$  is not a singleton set and  $\Lambda_b(M^4) = \{0\} \neq \emptyset$ . (b)  $M^{12}$  is not split in the  $x_a$ -dimension, since  $M_a^{12}$  is a singleton set, whereas it is split in the  $x_b$ -dimension, since  $\Lambda_b(M^{12}) = \{\kappa_b/\gamma_b\} \neq \emptyset$ .

The partitioning of the singular mode domain  $M^4$  is illustrated in Figure 8(a). Definition 6 states that  $M^4$  is not split in the dimension  $x_a$ , since  $M_a^4 = \{\theta_a^2\}$  is a singleton set. Recall that  $R(M^4) = \{M^3, M^5\}$ ,

$\psi(M^3) = (\kappa_a/\gamma_a, 0)'$  and  $\psi(M^5) = (0, 0)'$ . Consequently,  $\psi_b(M^3) = \psi_b(M^5) = 0 \in M_b^4$  such that  $\Lambda_b(M^4) = \{0\}$ , and the application of Definition 6 causes  $M_b^4$  to be split into two subsets:  $\{0\}$  and  $(0, \theta_b)$ . That is,  $M^4$  is partitioned into the flow domains  $D^{4.1} = \{\theta_a^2\} \times \{0\}$  and  $D^{4.2} = \{\theta_a^2\} \times (0, \theta_b)$  (Figure 9). The partitioning of the singular mode domain  $M^{12}$  is illustrated in Figure 8(b). Since  $M_a^{12}$  is a singleton set,  $M^{12}$  is not split in the  $x_a$ -dimension. Recall that  $R(M^{12}) = \{M^{11}, M^{13}\}$ ,  $\psi(M^{11}) = (0, \kappa_b/\gamma_b)'$ , and  $\psi(M^{13}) = (0, 0)'$ . Consequently,  $\psi_b(M^{11}) = \kappa_b/\gamma_b \in M_b^{12}$  and  $\psi_b(M^{13}) = 0 \notin M_b^{12}$ , so that  $\Lambda_b(M^{12}) = \{\kappa_b/\gamma_b\}$ . According to Definition 6,  $M_b^{12}$  is split into three subsets:  $(\theta_b, \kappa_b/\gamma_b)$ ,  $\{\kappa_b/\gamma_b\}$ , and  $(\kappa_b/\gamma_b, \max_b]$ .  $M^{12}$  is thus partitioned into three flow domains, denoted by  $D^{12.1}, \dots, D^{12.3}$  (Figure 9).

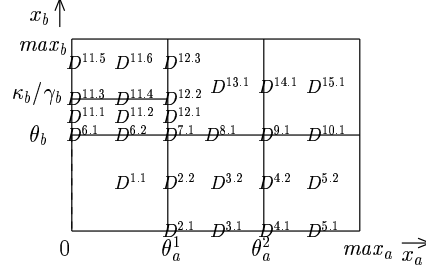


Figure 9: Partition by flow domains of the phase space corresponding to the regulatory network in Figure 1.

The set of flow domains,  $\mathcal{D}$ , is the union of all flow domains obtained by partitioning the mode domains:

$$\mathcal{D} = \bigcup_{M \in \mathcal{M}} \mathcal{D}^M.$$

$\mathcal{D}$  is a partition of the phase space  $\Omega$ . By Definition 6, every flow domain is included in a single mode domain. We introduce the function  $mode: \mathcal{D} \rightarrow \mathcal{M}$  to denote this surjective mapping:  $mode(D) = M$ , if and only if  $D \subseteq M$ . The concepts of instantaneous and persistent domain are extended to flow domains. We have  $\mathcal{D}_{pers} = \{D \mid mode(D) \in \mathcal{M}_{pers}\}$ . In the same way, we introduce the function  $flow: \Omega \rightarrow \mathcal{D}$  to denote the surjective mapping that associates a point in the phase space to a flow domain:  $flow(\mathbf{x}) = D$ , if and only if  $\mathbf{x} \in D$ .

The partition of  $\Omega$  into 27 flow domains is represented in Figure 9.  $\mathcal{D}$  is the set of the flow domains  $D^{1.1}, \dots, D^{15.1}$ . For example, it holds that  $mode(D^{1.1}) = M^1$ ,  $mode(D^{2.1}) = M^2$ , and  $mode(D^{2.2}) = M^2$ . Examples of persistent flow domains are  $D^{1.1}, D^{11.1}, \dots, D^{11.6}$ , while  $D^{12.1}, D^{12.2}, D^{12.3}$  illustrate instantaneous flow domains.

### 3.4 Characterization of solutions in flow domains

In the previous section, we introduced a partition of the phase space  $\Omega$ , the union  $\mathcal{D}$  of all flow domains in  $\Omega$ , while claiming that this yields a qualitatively-identical dynamics in every flow domain. The goal of this section is to formalize and prove this notion, which will be critical for the use of qualitative abstraction in the next section.

Consider a point  $\mathbf{x}$  in a flow domain  $D \in \mathcal{D}$  and the set of solutions  $\xi \in \Xi_\Sigma$  in  $D$  passing through  $\mathbf{x}$ . We denote by  $S(\mathbf{x}) \in 2^{\{-1,0,1\}^n}$  the derivative sign pattern at  $\mathbf{x}$ , that is the set of derivative sign vectors of these solutions at  $\mathbf{x}$ .

**Definition 7 (Derivative sign).** The set of derivative sign vectors of the solutions  $\xi \in \Xi_\Sigma$  in  $D$  passing through  $\mathbf{x}$  is  $S(\mathbf{x})$ :  $S(\mathbf{x}) = \{\mathbf{sign}(\dot{\xi}(t_x)) \mid \xi \in \Xi_\Sigma \text{ in } D, \xi(t_x) = \mathbf{x}, \text{ and } \dot{\xi}(t_x) \in H(\xi(t_x))\}$

$S(\mathbf{x})$  describes, in a qualitative way, the dynamics of the system at the point  $\mathbf{x}$ . Note that this definition does not take into account the solutions of  $\Sigma$  that traverse  $D$  instantaneously, or that do not satisfy (9) at  $\mathbf{x}$ . In particular, if  $\mathbf{x}$  is in an instantaneous flow domain  $D$ , then  $S(\mathbf{x})$  equals the empty set, since no solution remains in  $D$  longer than a single time instant. Like in the case of  $H(\mathbf{x})$ , we can rewrite  $S(\mathbf{x})$  as a product of sets if  $S(\mathbf{x})$  is not empty, i.e.,  $S(\mathbf{x}) = S_1(\mathbf{x}) \times \dots \times S_n(\mathbf{x})$ .  $S_i(\mathbf{x}) \in 2^{\{-1,0,1\}}$  describes the flow at point  $\mathbf{x}$  in the  $x_i$ -dimension,  $i \in [1..n]$ .

In order to illustrate the definition, consider the flow domain  $D^{11.4}$ , shown in Figure 7(c). All solutions in  $D^{11.4}$  are (strictly) decreasing in the  $x_a$ -dimension and steady in the  $x_b$ -dimension, since they are monotonically converging towards the focal set  $\Psi(mode(D^{11.4})) = \Psi(M^{11})$ , located in  $D^{11.3}$  (Figure 3). Consequently, for

every  $\mathbf{x} \in D^{11.4}$ ,  $S(\mathbf{x})$  equals  $\{(-1, 0)'\}$ . In the flow domain  $D^{4.2}$ , shown in Figure 8(a), all solutions are steady in the  $x_a$ -dimension and decreasing in the  $x_b$ -dimension (Figure 5). Consequently, for every  $\mathbf{x} \in D^{4.2}$ ,  $S(\mathbf{x}) = \{(0, -1)'\}$ . Finally, since  $D^{12.1}$  is an instantaneous flow domain, every solution instantaneously traverses  $D^{12.1}$ , so that for every  $\mathbf{x} \in D^{12.1}$ ,  $S(\mathbf{x})$  equals  $\emptyset$ .

Note also that since we use differential inclusions, the solutions of  $\Sigma$  in a persistent flow domain  $D$  that pass through  $\mathbf{x}$  may have different derivatives, and consequently,  $S(\mathbf{x})$  may be a non-singleton set. For example, if for some  $\mathbf{x}$  in a flow domain  $D$ ,  $S_i(\mathbf{x}) = \{-1, 0, 1\}$ , then there exist at least three solutions in  $D$  that are, in the  $x_i$ -dimension, increasing, decreasing, and steady, respectively, when passing through  $\mathbf{x}$ . This rather unusual situation is a direct consequence of the use of differential inclusions instead of differential equations and does not arise in our two-gene example. In fact, one can prove that this can only happen if  $D_i \subset \Psi_i(\text{mode}(D))$ .

The following theorem, which is proven in Appendix A, states that all points in a flow domain  $D$  have the same derivative sign pattern. This explains the name ‘flow domain’ for  $D$ , as the sign description of the flow is identical everywhere in a flow domain. Phrased differently, Theorem 1 states that  $\mathcal{D}$  is a partition of  $\Omega$ , dividing the phase space into regions having qualitatively-identical dynamics.

**Theorem 1 (Qualitatively-identical dynamics in flow domains).**

For all  $D \in \mathcal{D}$  and for all  $\mathbf{x}, \mathbf{x}' \in D$ ,  $S(\mathbf{x}) = S(\mathbf{x}')$ .

This theorem serves as a hinge between the mathematical and computational analyses of models of genetic regulatory networks, since it provides a justification for the partition of the phase space introduced in this section as well as for the use of discrete abstractions, in the next section.

## 4 Qualitative abstraction of the dynamics of PA systems

In this section, we define a discrete abstraction [4, 5, 14, 15, 16, 33, 59] of the original PA system that yields an intuitive qualitative description of the dynamics of the system in the phase space. We therefore call this abstraction a *qualitative abstraction*.

In order to have a clear relation between the original and the abstract system, we will first introduce a *continuous transition system* having the same reachability properties as the original PA system. The abstract system is then defined as the *discrete quotient* of the continuous transition system, using a *proposition preserving* equivalence relation [5]. In such a way, interesting properties of the continuous transition system, and consequently of the original PA system, are preserved in the abstract transition system.

### 4.1 PA transition systems

Given a PA system  $\Sigma$ , we define a corresponding continuous transition system,  $\Sigma$ -TS, such that  $\Sigma$  and  $\Sigma$ -TS have equivalent reachability properties. Consider  $\mathbf{x} \in D$  and  $\mathbf{x}' \in D'$ , with  $D, D' \in \mathcal{D}$  being two flow domains. If there exists a solution  $\xi$  of  $\Sigma$  passing through  $\mathbf{x}$  at time  $\tau \in \mathbb{R}_{\geq 0}$  and reaching  $\mathbf{x}'$  at time  $\tau' \in \mathbb{R}_{> 0} \cup \{\infty\}$ , without leaving  $D \cup D'$  in the time interval  $[\tau, \tau']$ , then the absolute continuity of  $\xi$  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 correspondingly distinguish three types of continuous transition: *internal*, denoted by  $\mathbf{x} \xrightarrow{int} \mathbf{x}'$ , *dimension increasing*, denoted by  $\mathbf{x} \xrightarrow{dim^+} \mathbf{x}'$ , and *dimension decreasing*, denoted by  $\mathbf{x} \xrightarrow{dim^-} \mathbf{x}'$ . The labels  $dim^+$  and  $dim^-$  refer to the change in dimension of the domain when going from  $D$  to  $D'$ . This leads us to the following definition:

**Definition 8 (PA transition system).** The transition system corresponding to the PA system  $\Sigma = (\Omega, \Theta, H)$  is  $\Sigma$ -TS =  $(\Omega, L, \Pi, \rightarrow, |=)$  where:

- $\Omega$  is the state space.
- $L = \{int, dim^+, dim^-\}$  is a set of labels denoting the three different types of transition.
- $\Pi = \{Dsign = S \mid S \in 2^{\{-1, 0, 1\}^n}\}$  is a set of propositions providing a qualitative description of the state of the system.
- $\rightarrow$  is the transition relation describing the continuous evolution of the system, defined by  $\rightarrow \subseteq \Omega \times L \times \Omega$ , such that  $\mathbf{x} \xrightarrow{l} \mathbf{x}'$  iff there exists  $\xi \in \Xi_\Sigma$  and  $\tau, \tau'$ , such that  $0 \leq \tau < \tau'$ ,  $\xi(\tau) = \mathbf{x}$ ,  $\xi(\tau') = \mathbf{x}'$ , and
  - if  $l = int$ , then  $flow(\mathbf{x}) = flow(\mathbf{x}')$  and for all  $t \in [\tau, \tau']$ , it holds that  $\xi(t) \in flow(\mathbf{x})$ ,



- if  $l = \dim^+$ , then  $\text{flow}(\mathbf{x}) \neq \text{flow}(\mathbf{x}')$  and for all  $t \in (\tau, \tau']$ , it holds that  $\boldsymbol{\xi}(t) \in \text{flow}(\mathbf{x}')$ ,
  - if  $l = \dim^-$ , then  $\text{flow}(\mathbf{x}) \neq \text{flow}(\mathbf{x}')$  and for all  $t \in [\tau, \tau')$ , it holds that  $\boldsymbol{\xi}(t) \in \text{flow}(\mathbf{x})$ .
- $\models$  is the satisfaction relation of the propositions in  $\Pi$ , defined by  $\models \subseteq \Omega \times \Pi$ , such that  $\mathbf{x} \models \text{Design} = S$  iff  $S = S(\mathbf{x})$ .

The satisfaction relation  $\models$  associates to each point  $\mathbf{x}$  in the phase space the qualitative description of the dynamics of the system at  $\mathbf{x}$  introduced in the previous section.

We define any sequence of points in  $\Omega$ ,  $(\mathbf{x}^0, \dots, \mathbf{x}^m)$ ,  $m \geq 0$ , as a *run* of  $\Sigma$ -TS if and only if for all  $i \in [0..m-1]$ , there exists some  $l \in L$  such that  $\mathbf{x}^i \xrightarrow{l} \mathbf{x}^{i+1}$ . The following theorem, which is proven in Appendix A, states that a PA system  $\Sigma$  and its corresponding PA transition system  $\Sigma$ -TS have equivalent reachability properties.

**Theorem 2 (Reachability equivalence).** For all  $\mathbf{x}, \mathbf{x}' \in \Omega$ , there exists some solution  $\boldsymbol{\xi}$  of  $\Sigma$  and  $\tau, \tau'$  such that  $0 \leq \tau \leq \tau'$ ,  $\boldsymbol{\xi}(\tau) = \mathbf{x}$ , and  $\boldsymbol{\xi}(\tau') = \mathbf{x}'$  if and only if there exists a run  $(\mathbf{x}^0, \dots, \mathbf{x}^m)$  of  $\Sigma$ -TS such that  $\mathbf{x}^0 = \mathbf{x}$  and  $\mathbf{x}^m = \mathbf{x}'$ .

As an illustration, consider solution  $\boldsymbol{\xi}$  of the PA system  $\Sigma$  corresponding to our two-gene example, as shown in Figure 10.  $\boldsymbol{\xi}$  passes through the points  $\mathbf{x}^0$ ,  $\mathbf{x}^1$ ,  $\mathbf{x}^2$ , and  $\mathbf{x}^3$  at time instants 0,  $\tau^1$ ,  $\tau^2$ , and  $\tau^3$ , respectively. Let  $\Sigma$ -TS =  $(\Omega, L, \Pi, \rightarrow, \models)$  be the PA transition system corresponding to  $\Sigma$ . Using Definition 8, and the fact that  $\mathbf{x}^0$  and  $\mathbf{x}^1$  belong to the same flow domain  $D^{1.1}$ , we directly infer the existence of an *int* transition from  $\mathbf{x}^0$  to  $\mathbf{x}^1$ . Again, by a direct application of Definition 8, since  $\text{flow}(\mathbf{x}^1) = D^{1.1}$ ,  $\text{flow}(\mathbf{x}^2) = D^{2.2}$  and  $\boldsymbol{\xi}(t) \in D^{1.1}$ , for all  $t \in [\tau^1, \tau^2)$ , there exists a transition of type  $\dim^-$  from  $\mathbf{x}^1$  to  $\mathbf{x}^2$ . Similarly, there exists a  $\dim^-$  transition from  $\mathbf{x}^0$  to  $\mathbf{x}^2$ . Also, there exists a  $\dim^+$  transition from  $\mathbf{x}^2$  to  $\mathbf{x}^3$ , since  $\text{flow}(\mathbf{x}^2) = D^{2.2}$ ,  $\text{flow}(\mathbf{x}^3) = D^{3.2}$  and  $\boldsymbol{\xi}(t) \in D^{3.2}$ , for all  $t \in (\tau^2, \tau^3]$ . Note that the transition labels,  $\dim^+$  or  $\dim^-$ , are consistent with the dimension of the domains:  $\dim(D^{1.1}) = \dim(D^{3.2}) = 2$  and  $\dim(D^{2.2}) = 1$ . The sequence of points  $(\mathbf{x}^0, \mathbf{x}^1, \mathbf{x}^2, \mathbf{x}^3)$  is a run of  $\Sigma$ -TS. Finally, from the monotonic convergence towards  $\Psi(\text{mode}(D^{1.1}))$  of the solutions of  $\Sigma$  in  $D^{1.1}$ , one can infer that all solutions in  $D^{1.1}$  are (strictly) increasing in the  $x_a$ - and  $x_b$ -dimension. In particular, since  $\text{flow}(\mathbf{x}^1) = D^{1.1}$ , we have  $S(\mathbf{x}^1) = \{(1, 1)'\}$ . So, by Definition 8, it holds that  $\mathbf{x}^1 \models \text{Design} = \{(1, 1)'\}$ , which describes the flow at  $\mathbf{x}^1$  in a qualitative manner.

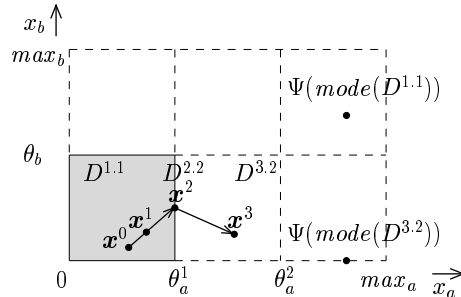


Figure 10: Trajectory starting in  $D^{1.1}$ , reaching  $D^{3.2}$ , and passing through the points  $\mathbf{x}^0$ ,  $\mathbf{x}^1$ ,  $\mathbf{x}^2$ , and  $\mathbf{x}^3$  at time instants 0,  $\tau^1$ ,  $\tau^2$ , and  $\tau^3$ , respectively.

## 4.2 Qualitative PA transition systems

In this section, we define and characterize the qualitative abstraction of a PA transition system. The qualitative abstraction is defined by means of an equivalence relation,  $\sim_\Omega$ , on the points of  $\Omega$ .

Formally, we define  $\sim_\Omega \subseteq \Omega \times \Omega$  as the equivalence relation induced by the partition  $\mathcal{D}$  of the phase space:  $\mathbf{x} \sim_\Omega \mathbf{x}'$  iff  $\text{flow}(\mathbf{x}) = \text{flow}(\mathbf{x}')$ . Consider two equivalent points,  $\mathbf{x}$  and  $\mathbf{x}'$ , that is, two points in a same flow domain. Then, applying Theorem 1, it holds that  $S(\mathbf{x}) = S(\mathbf{x}')$ . So, given that the propositions  $\pi \in \Pi$  are of type ‘ $\text{Design} = S$ ’ and hold true if and only if  $S = S(\mathbf{x})$ , it follows that  $\mathbf{x} \models \pi$  is equivalent to  $\mathbf{x}' \models \pi$ . Phrased differently, two equivalent points satisfy the same properties. Consequently,  $\sim_\Omega$  is a *proposition preserving* equivalence relation [5].<sup>6</sup>

We now define the qualitative abstraction of a PA transition system,  $\Sigma$ -TS as the *quotient transition system* of  $\Sigma$ -TS, given the proposition preserving equivalence relation  $\sim_\Omega$  [5]. It is called a *qualitative PA transition system*, and denoted by  $\Sigma$ -QTS.

<sup>6</sup>Equivalently, one can say that  $\mathcal{D}$  is a *consistent partition*, in the sense of [14].

**Definition 9 (Qualitative PA transition system).** The qualitative PA transition system corresponding to the PA transition system  $\Sigma\text{-TS} = (\Omega, L, \Pi, \rightarrow, \models)$  is  $\Sigma\text{-QTS} = (\Omega/\sim_\Omega, L, \Pi, \rightarrow_{\sim_\Omega}, \models_{\sim_\Omega})$ .

Proposition 1 immediately follows from the above definition.

**Proposition 1 (Qualitative PA transition system).** Let  $\Sigma\text{-QTS} = (\Omega/\sim_\Omega, L, \Pi, \rightarrow_{\sim_\Omega}, \models_{\sim_\Omega})$  be the qualitative PA transition system corresponding to the PA transition system  $\Sigma\text{-TS} = (\Omega, L, \Pi, \rightarrow, \models)$ . Then,

- $\Omega/\sim_\Omega = \mathcal{D}$ .
- $\rightarrow_{\sim_\Omega} \subseteq \mathcal{D} \times L \times \mathcal{D}$ , such that  $D \xrightarrow{l}_{\sim_\Omega} D'$  iff there exists  $\xi \in \Xi_\Sigma$  and  $\tau, \tau'$  such that  $0 \leq \tau < \tau'$ ,  $\xi(\tau) \in D$ ,  $\xi(\tau') \in D'$ , and
  - if  $l = \text{int}$ , then  $D = D'$  and for all  $t \in [\tau, \tau']$ , it holds that  $\xi(t) \in D$ ,
  - if  $l = \text{dim}^+$ , then  $D \neq D'$  and for all  $t \in (\tau, \tau']$ , it holds that  $\xi(t) \in D'$ ,
  - if  $l = \text{dim}^-$ , then  $D \neq D'$  and for all  $t \in [\tau, \tau')$ , it holds that  $\xi(t) \in D$ .
- $\models_{\sim_\Omega} \subseteq \mathcal{D} \times \Pi$ , such that  $D \models_{\sim_\Omega} \text{Design} = S$  iff for all  $x \in D$ ,  $S(x) = S$ .

Contrary to  $\Sigma\text{-TS}$ ,  $\Sigma\text{-QTS}$  is a *finite* transition system, as the state space of  $\Sigma\text{-QTS}$  equals the finite set  $\mathcal{D}$ . This is a key property, since it makes it possible to actually compute and represent  $\Sigma\text{-QTS}$ , as well as to analyze it using formal verification techniques [12, 17].

In a similar way to the satisfaction relation  $\models$ , which associates to each point  $x \in \Omega$  a qualitative description of the dynamics, the satisfaction relation  $\models_{\sim_\Omega}$  associates to each flow domain  $D \in \mathcal{D}$  a qualitative description of the dynamics. Consequently, the states of  $\Sigma\text{-QTS}$  have been called *qualitative states* in [19, 21, 22]. Qualitative states correspond to flow domains, but for the sake of clarity, we will only use the latter term.

The transitions labeled by  $\text{dim}^+$  or  $\text{dim}^-$  connect two different flow domains, since in Proposition 1 we require that  $D \neq D'$ . This corresponds to a continuous evolution of the system along which a switch from one flow domain to another occurs. On the contrary, the transitions labeled by  $\text{int}$  are self-transitions, and correspond to a continuous evolution during which the system remains in the same flow domain.

As for  $\Sigma\text{-TS}$ , we define any sequence of flow domains  $(D^0, \dots, D^m)$ ,  $m \geq 0$  as a *run* of  $\Sigma\text{-QTS}$ , if and only if for all  $i \in [0..m-1]$  there exists some  $l \in L$  such that  $D^i \xrightarrow{l}_{\sim_\Omega} D^{i+1}$ . The satisfaction relation  $\models_{\sim_\Omega}$  associates to every run a qualitative description of the evolution of the derivatives over time. A run of a qualitative PA transition system has also been called a *qualitative behavior* in [19, 21, 22].

As usual,  $\Sigma\text{-QTS}$  can be represented by the directed graph  $G = (\mathcal{D}, \rightarrow_{\sim_\Omega})$ , called the *state transition graph*. In the sequel, for the sake of clarity, the labels on the transitions between flow domains will be omitted. Paths in  $G$  are runs.

Consider again the situation illustrated in Figure 10. Applying Proposition 1, it holds that the qualitative description of  $D^{1.1}$  is given by  $D^{1.1} \models_{\sim_\Omega} \text{Design} = \{1, 1\}$ . Based on Proposition 1, we conclude that there is an  $\text{int}$  transition on  $D^{1.1}$ , a  $\text{dim}^-$  transition from  $D^{1.1}$  to  $D^{2.2}$ , and a  $\text{dim}^+$  transition from  $D^{2.2}$  to  $D^{3.2}$ . The state transition graph  $G = (\mathcal{D}, \rightarrow_{\sim_\Omega})$  corresponding to the two-gene example is shown in Figure 11(a).  $(D^{1.1}, D^{2.2}, D^{3.2}, D^{4.2}, D^{4.1})$  and  $(D^{1.1}, D^{6.2}, D^{11.2}, D^{11.3})$  are two examples of runs, which are described in more detail in Figure 11(b).

It directly follows from the definitions of quotient transition system and simulation of transition systems [5, 14] that  $\Sigma\text{-QTS}$  is a *simulation* of  $\Sigma\text{-TS}$ . The converse is not true in general, so that  $\Sigma\text{-QTS}$  and  $\Sigma\text{-TS}$  are not bisimilar.

**Proposition 2.**  $\Sigma\text{-QTS}$  is a simulation of  $\Sigma\text{-TS}$ .

As a consequence of Proposition 2, if there exists a run  $(x^0, \dots, x^m)$  of  $\Sigma\text{-TS}$ , then there also exists a run  $(D^0, \dots, D^m)$  of  $\Sigma\text{-QTS}$  such that  $x^i \in D^i$ , for all  $i \in [0..m]$ . In other words,  $\Sigma\text{-QTS}$  is a *conservative approximation* of  $\Sigma\text{-TS}$ .

Consider a solution  $\xi$  of the PA system  $\Sigma$ , defined on some (finite or infinite) time interval  $I$ , which passes through the time-ordered sequence of flow domains  $(D^0, \dots, D^m)$  on the time interval  $I$ . Then, by Theorem 2 and Proposition 2,  $(D^0, \dots, D^m)$  is a run of  $\Sigma\text{-QTS}$ . On the other hand, it may happen that no solution of  $\Sigma$  passes through a sequence of flow domains corresponding to a run in  $\Sigma\text{-QTS}$ .

Consider again the solution  $\xi$  whose trajectory on the time interval  $I = [0, \tau^3]$  is shown in Figure 10. It passes through  $D^{1.1}$ ,  $D^{2.2}$ , and  $D^{3.2}$  during the time interval  $I$ . One can immediately check that  $(D^{1.1}, D^{2.2}, D^{3.2})$  is a run of  $\Sigma\text{-QTS}$  or, equivalently, a path in  $G$  (Figure 11). Note that the run  $(D^{1.1}, D^{2.2}, D^{3.2})$  of  $\Sigma\text{-QTS}$  is a

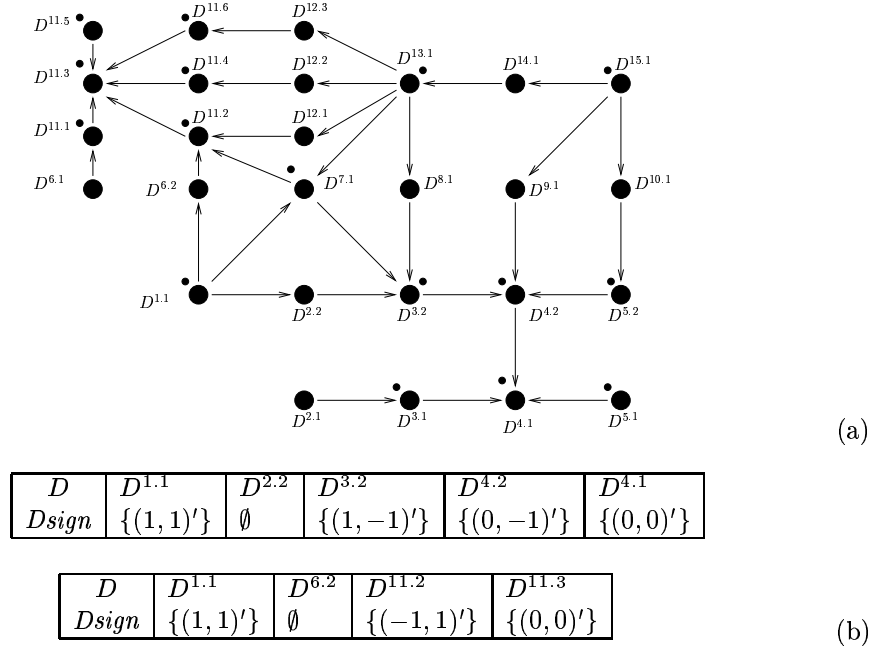


Figure 11: (a) State transition graph  $G = (\mathcal{D}, \rightarrow_{\sim_{\Omega}})$  corresponding to the two-gene example of Figure 1. Self-transitions are represented by a dot. (b) Two runs,  $(D^{1.1}, D^{2.2}, D^{3.2}, D^{4.2}, D^{4.1})$  and  $(D^{1.1}, D^{6.2}, D^{11.2}, D^{11.3})$ , and their  $Dsign$  properties.

qualitative representation of not only the solution depicted in Figure 10, but of all solutions starting in  $D^{1.1}$ , passing through  $D^{2.2}$ , and reaching  $D^{3.2}$ . A single run of  $\Sigma$ -QTS may represent infinitely-many runs of  $\Sigma$ -TS (solutions of  $\Sigma$ ).

### 4.3 Qualitative PADE models

A PA system  $\Sigma$  has been defined as a triple  $(\Omega, \Theta, H)$ . As seen in Section 2.1,  $\Theta$  and  $H$ , and consequently  $\Sigma$ , depend on the parameter vectors  $\kappa$ ,  $\gamma$ , and  $\theta$ . Let  $m$  be the number of parameters in  $\Sigma$  and define  $\Gamma$  as the  $m$ -dimensional parameter space of  $\Sigma$ , that is,  $\Gamma \subseteq \mathbb{R}_{>0}^m$ . We denote by  $\mathbf{p} \in \Gamma$  a vector of numerical values for the parameters in  $\Sigma$ . A pair  $(\Sigma, \mathbf{p})$  formally defines a *quantitative PADE model*, also called quantitative model. Given a quantitative PADE model  $(\Sigma, \mathbf{p})$ , we denote by  $\Sigma\text{-QTS}_{\mathbf{p}}$  the corresponding qualitative PA transition system.

The quantitative model defining our two-gene example consists of the PA system (12) and the parameter vector (13), defined as follows (see Section 2.1 for the numerical values).

$$\begin{cases} \dot{x}_a = \kappa_a s^-(x_a, \theta_a^2) s^-(x_b, \theta_b) - \gamma_a x_a, \\ \dot{x}_b = \kappa_b s^-(x_a, \theta_a^1) - \gamma_b x_b. \end{cases} \quad (12)$$

$$(\kappa_a, \kappa_b, \gamma_a, \gamma_b, \theta_a^1, \theta_a^2, \theta_b)' = (20, 10, 2, 2, 4, 8, 4)'. \quad (13)$$

Intuitively, two different quantitative models can have an equivalent corresponding qualitative PA transition system. In order to make this intuition more precise, we introduce the notion of *isomorphic* qualitative PA transition systems. Two qualitative PA transition systems,  $\Sigma\text{-QTS}_{\mathbf{p}}$  and  $\Sigma\text{-QTS}_{\mathbf{p}'}$ , are *isomorphic*, denoted by  $\Sigma\text{-QTS}_{\mathbf{p}} \cong \Sigma\text{-QTS}_{\mathbf{p}'}$ , if and only if there exists a bijection between their flow domains preserving the relations  $\rightarrow_{\sim_{\Omega}}$  and  $\models_{\sim_{\Omega}}$ . As a consequence, two corresponding flow domains have the same connectivity and state properties. Two isomorphic qualitative PA transition systems have isomorphic state transition graphs. For all practical purposes, isomorphic qualitative PA transition systems can be considered the same.

Given a PA system  $\Sigma$ , let  $\sim_{\Gamma} \subseteq \Gamma \times \Gamma$  be the equivalence relation induced by the isomorphism on qualitative PA transition systems:  $\mathbf{p} \sim_{\Gamma} \mathbf{p}'$ , iff  $\Sigma\text{-QTS}_{\mathbf{p}} \cong \Sigma\text{-QTS}_{\mathbf{p}'}$ . Given the equivalence relation  $\sim_{\Gamma}$ , we denote by  $\Gamma/\sim_{\Gamma}$  the quotient parameter space. That is,  $\Gamma/\sim_{\Gamma}$  is a partition of the parameter space consisting of regions where all parameters yield the same qualitative PA transition system.

In the sequel, we will define regions in the parameter space by means of parameter inequality constraints. Denote by  $\Theta_i$  and  $\Lambda_i$  the sets of threshold and focal concentrations of gene  $i$ :  $\Theta_i = \{\theta_i^1, \dots, \theta_i^{p_i}\}$  and  $\Lambda_i = \{\psi_i(M) \mid M \in \mathcal{M}_r\}$ .  $\Theta_i$  and  $\Lambda_i$  can be easily inferred from the parameter vectors  $\kappa$ ,  $\gamma$ , and  $\theta$ , since  $\Theta_i$  is a set of threshold concentrations, given in  $\theta$ , and  $\Lambda_i$ , a set of ratios between (sums of) synthesis rate constants in  $\kappa$ , and (sums of) degradation rate constants in  $\gamma$ . We now define the *parameter inequality constraints* of  $\Sigma$  as follows.

**Definition 10 (Parameter inequality constraints).** The parameter inequality constraints of  $\Sigma$  are a set of total strict orders on  $\Theta_i \cup \Lambda_i$ ,  $i \in [1..n]$ .

The set of parameter vectors  $\mathbf{p}$  whose elements satisfy the parameter inequality constraints of  $\Sigma$  corresponds to a region  $P$  in the parameter space. We consequently interpret  $\mathbf{p} \in P$  either as ‘the elements of the parameter vector  $\mathbf{p}$  satisfy the parameter inequality constraints of  $\Sigma$ ’ or ‘the point  $\mathbf{p}$  is included in the region  $P$ ’.

The following theorem, which is proven in Appendix A, states that the region defined by the parameter inequality constraints of  $\Sigma$  is included in a unique equivalence class of  $\Gamma/\sim_\Gamma$ . In other words, all quantitative models  $(\Sigma, \mathbf{p})$ ,  $\mathbf{p} \in P$ , yield the same qualitative PA transition system.

**Theorem 3 (Model equivalence).** Let  $P$  be a region of the parameter space  $\Gamma$  defined by the parameter inequality constraints of  $\Sigma$ . Then, there exists some  $Q \in \Gamma/\sim_\Gamma$  such that  $P \subseteq Q$ .

Theorem 3 underlies the definition of qualitative models. A pair  $(\Sigma, P)$  is called a *qualitative PADE model*, also called qualitative model. A qualitative model  $(\Sigma, P)$  is a qualitative abstraction of the set of quantitative models  $(\Sigma, \mathbf{p})$  satisfying the parameter inequality constraints of  $\Sigma$ .

Whereas exact numerical values for the parameters are usually not available, the weaker information required for the formulation of parameter inequality constraints can often be obtained from the experimental literature. The following parameter inequality constraints corresponds to our two-gene example, where  $\Theta_a = \{\theta_a^1, \theta_a^2\}$ ,  $\Lambda_a = \{0, \kappa_a/\gamma_a\}$ ,  $\Theta_b = \{\theta_b\}$ ,  $\Lambda_b = \{0, \kappa_b/\gamma_b\}$ :

$$\begin{cases} 0 < \theta_a^1 < \theta_a^2 < \kappa_a/\gamma_a < \max_a, \\ 0 < \theta_b < \kappa_b/\gamma_b < \max_b. \end{cases} \quad (14)$$

One can check that the parameter vector  $\mathbf{p}$  given in (13) satisfies (14). Consequently, the state transition graph in Figure 11(a) represents the qualitative transition system corresponding not only to the numerical PA model  $(\Sigma, \mathbf{p})$ , given by (12)-(13), but also to all numerical PA models  $(\Sigma, \mathbf{p}')$  whose parameters  $\mathbf{p}'$  satisfy the parameter inequalities (14).

To each qualitative model  $(\Sigma, P)$ , we associate a corresponding qualitative PA transition system, denoted by  $\Sigma\text{-QTS}_P$ . More precisely,  $\Sigma\text{-QTS}_P = \Sigma\text{-QTS}_{\mathbf{p}}$ , for every  $\mathbf{p} \in P$ , that is,  $\Sigma\text{-QTS}_P$  qualitatively describes the dynamics of a whole class of quantitative models. This transition system can be used to test the validity of the models, given experimental observations of the system, and to answer other questions of biological interest (Section 6).

Thus far, we have only defined the qualitative PA transition system  $\Sigma\text{-QTS}_P$  of a qualitative model  $(\Sigma, P)$ , without giving any hints on how to actually compute it. We will see in the next section how this can be achieved in a simple way, using the parameter inequality constraints of  $\Sigma$ .

## 5 Symbolic computation of the qualitative PA transition system

In this section, we consider a qualitative model  $(\Sigma, P)$ , where  $\Sigma = (\Omega, \Theta, H)$ . Our goal is to compute its corresponding qualitative PA transition system,  $\Sigma\text{-QTS}_P = (\mathcal{D}, L, \Pi, \rightarrow_{\sim_\Omega}, \models_{\sim_\Omega})$ , as defined in the previous section.  $L$  and  $\Pi$  are constant:  $L = \{int, dim^+, dim^-\}$  and  $\Pi = \{Dsign = S \mid S \in 2^{\{-1,0,1\}^n}\}$ . So, this leaves the set of states as well as the transition and satisfaction relations of  $\Sigma\text{-QTS}_P$  to be computed.

The above computations rely on two essential features. First, all sets we consider —  $H(\mathbf{x})$ ,  $S(\mathbf{x})$ , mode domains, flow domains, supporting hyperplanes, smallest closed rectangular sets, and focal sets — are *hyper-rectangular* sets. Consequently, they can be decomposed as the product of their  $x_i$ -components. This makes it possible to carry out the computations separately in each dimension. Secondly, all sets that represent regions in the phase space are delimited by threshold or focal concentrations, that is, by elements in  $\Theta_i \cup \Lambda_i$ ,  $i \in [1..n]$ . As a consequence, in every dimension  $i$ , the evaluation of the relative position, the intersection, and the inclusion of the  $x_i$ -components of these sets simply amounts to comparing the relative position of the threshold and focal concentrations. Since these concentrations are elements in  $\Theta_i \cup \Lambda_i$ ,  $i \in [1..n]$ , their order is given by the

parameter inequality constraints of  $\Sigma$ . This means that the computations reduce in practice to simple checks of ordering relations, which can be carried out symbolically. In other words, the determination of the qualitative PA transition system  $\Sigma\text{-QTS}_P$  does not require numerical techniques.

In Section 5.1, we will see how to compute the set of states of  $\Sigma\text{-QTS}_P$ . This amounts to determining the partition  $\mathcal{D}$ , that is, the set of all flow domains in  $\Omega$ . The computation of the satisfaction relation  $\models_{\sim_\Omega}$  is the topic of Section 5.2. We indicate how to test whether a flow domain  $D$  satisfies a property of the type ' $D\text{sign} = S$ '. In Section 5.3, finally, we will see how to compute the transition relation  $\rightarrow_{\sim_\Omega}$ . This amounts to determining whether, given two flow domains, there is a transition from one domain to the other.

## 5.1 Symbolic computation of the set of flow domains

Our goal is to compute the set of states of  $\Sigma\text{-QTS}_P$ , that is, to determine the set of all flow domains in  $\Omega$ . From the parameter inequality constraints of  $\Sigma$ , one can infer the relative order of the thresholds in  $\Theta_i = \{\theta_i^1, \dots, \theta_i^{p_i}\}$ ,  $i \in [1..n]$ . Consequently, one can use Definition 2 and determine the partition  $\mathcal{M}$  of  $\Omega$  by mode domains.  $\mathcal{D}$  is then obtained by repartitioning every mode domain.

If  $M \in \mathcal{M}$  is a regular mode domain, the evaluation of  $\mathbf{f}$  and  $\mathbf{g}$  in  $M$  gives  $\boldsymbol{\mu}^M$  and  $\boldsymbol{\nu}^M$ . Using Definition 3, the focal set of  $M$  equals  $\{\boldsymbol{\psi}(M)\}$ , where  $\psi_i(M) = \mu_i^M / \nu_i^M$ ,  $i \in [1..n]$ . Then, for every dimension  $i$ , one can decide whether  $\psi_i(M) \in M_i$  or not, by means of the total order on  $\Theta_i \cup \Lambda_i$ , given by the parameter inequality constraints of  $\Sigma$ . More precisely,  $M_i$  equals either  $\{\lambda_i\}$  or  $(\lambda_i, \lambda'_i)$ , for some  $\lambda_i, \lambda'_i \in \Theta_i$ .<sup>7</sup> Therefore, testing whether  $\psi_i(M) \in M_i$ , amounts to testing whether  $\psi_i(M) = \lambda_i$ , in the first case, or whether  $\lambda_i < \psi_i(M)$  and  $\psi_i(M) < \lambda'_i$ , in the second case. Given the modeling assumptions made in Section 3.2.1, the first case never holds. The second case can be easily tested, since  $\lambda_i, \lambda'_i$  and  $\psi_i(M)$  are elements of  $\Theta_i \cup \Lambda_i$ , so that their order can be inferred from the parameter inequality constraints of  $\Sigma$ . By applying Definition 5, we can then partition  $M$  into flow domains.

If  $M \in \mathcal{M}$  is not a regular mode domain, then it is a singular mode domain. Given the partition  $\mathcal{M}$ , one can determine  $R(M)$ , the set of regular mode domains having  $M$  in their boundary. For every regular mode domain  $M'$  in  $R(M)$ , one can evaluate  $\boldsymbol{\mu}^{M'}$ ,  $\boldsymbol{\nu}^{M'}$ , and  $\boldsymbol{\psi}(M')$  as described above. Again, one can use the parameter inequality constraints of  $\Sigma$  to decide, for every dimension  $i$  and for every  $M'$  in  $R(M)$ , whether  $\psi_i(M') \in M_i$  or not. Then, denoting by  $\Lambda_i(M)$  the elements of  $\{\psi_i(M') \mid M' \in R(M)\}$ , included in  $M_i$ , one can infer a total order on  $\Lambda_i(M)$  from the parameter inequality constraints of  $\Sigma$ . By applying Definition 6,  $M$  can then be partitioned into flow domains.

The set of all flow domains in the phase space corresponds to the partition  $\mathcal{D}$ , or equivalently, to the set of states of  $\Sigma\text{-QTS}_P$ . In the next section, we show how to compute the satisfaction relation  $\models_{\sim_\Omega}$ , that is, how to determine for every  $D \in \mathcal{D}$  and  $S \in 2^{\{-1,0,1\}^n}$ , whether  $D \models_{\sim_\Omega} D\text{sign} = S$  or not.

## 5.2 Symbolic computation of flow domain properties

From Proposition 1, it follows that  $D \models_{\sim_\Omega} D\text{sign} = S$  is equivalent to  $S = S(\mathbf{x})$ , for all  $\mathbf{x} \in D$ . So, computing the relation  $\models_{\sim_\Omega}$  amounts to computing  $S(\mathbf{x})$ , which represents the signs of the derivatives of the solutions passing through the points  $\mathbf{x}$  in  $D$ . First, we state computation rules for the satisfaction relation. Second, we show how they can be applied by means of the parameter inequality constraints of  $\Sigma$ .

Consider a point  $\mathbf{x}$  in a persistent flow domain  $D \in \mathcal{D}_{pers}$  and a solution  $\boldsymbol{\xi}$  in  $D$  passing through  $\mathbf{x}$  at time  $t_x$ , satisfying  $\dot{\boldsymbol{\xi}}(t_x) \in H(\boldsymbol{\xi}(t_x))$ . We consider each dimension  $i$  separately,  $i \in [1..n]$ . If  $D_i$  is a singleton set, that is,  $D_i = \{\lambda_i\}$  for some  $\lambda_i \in \Theta_i \cup \Lambda_i$ , then  $\boldsymbol{\xi}$  is sliding along the plane  $\{\mathbf{x} \in \Omega \mid x_i = \lambda_i\}$  and necessarily satisfies  $\xi_i(t) = \lambda_i$  when in  $D$ , so that  $\xi_i(t_x) = 0$ . Consequently,  $S_i(\mathbf{x}) = \{0\}$ , by Definition 7. If  $D_i$  is not a singleton set, then the sign of the derivative of  $\xi_i$  at  $t_x$  corresponds to the relative position of  $x_i$  with respect to  $\Psi_i(\text{mode}(D))$ , that is, to the sign of  $\psi_i - x_i$ , for  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$ , since, as shown in Sections 3.2.1 and 3.2.2,  $\xi_i$  is converging monotonically towards  $\Psi_i(\text{mode}(D))$ . For example, if  $\Psi(\text{mode}(D))$  is located above  $D$  in some dimension  $i$ , that is,  $\psi_i - x_i > 0$  for all  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$  and  $\mathbf{x} \in D$ , then all solutions in  $D$  are necessarily increasing in the  $x_i$ -dimension:  $S_i(\mathbf{x}) = \{1\}$ , for all  $\mathbf{x} \in D$ . These intuitions are formalized in the following proposition, which is proven in Appendix A.

**Proposition 3 (Computation of  $D\text{sign}$ ).** Let  $D \in \mathcal{D}$  be a persistent flow domain and  $S \in 2^{\{-1,0,1\}^n}$  such that  $D \models_{\sim_\Omega} D\text{sign} = S$ . Then,  $S = S_1 \times \dots \times S_n$  and for all  $i \in [1..n]$ ,

<sup>7</sup>The  $x_i$ -component of a mode (flow) domain,  $M_i$  ( $D_i$ ), can also be a semi-open interval, in the case where  $M_i$  ( $D_i$ ) is delimited by 0 or  $\max x_i$ . For the sake of clarity, these special cases will not be exposed here. However, the reader can check that all subsequent computational rules remain valid in these cases. Moreover, it is easy to adapt the proofs to take these special cases into account.

- if  $D_i$  is a singleton set, then  $S_i = \{0\}$ ,
- if  $D_i$  is not a singleton set, then
  - $-1 \in S_i$  iff there exists  $\mathbf{x} \in D$  and  $\psi \in \Psi(\text{mode}(D))$  such that  $\psi_i - x_i < 0$ ,
  - $0 \in S_i$  iff there exists  $\mathbf{x} \in D$  and  $\psi \in \Psi(\text{mode}(D))$  such that  $\psi_i - x_i = 0$ ,
  - $1 \in S_i$  iff there exists  $\mathbf{x} \in D$  and  $\psi \in \Psi(\text{mode}(D))$  such that  $\psi_i - x_i > 0$ .

If  $D$  is an instantaneous flow domain, no solution remains in  $D$  longer than a single time instant, so that, by Definition 7,  $S(\mathbf{x}) = \emptyset$ , for all  $\mathbf{x} \in D$ . It immediately follows that  $D \models_{\sim\Omega} \text{Dsign} = S$  if and only if  $S = \emptyset$ .

**Proposition 4 (Computation of  $\text{Dsign}$ ).** Let  $D \in \mathcal{D}$  be an instantaneous flow domain and  $S \in 2^{\{-1,0,1\}^n}$  such that  $D \models_{\sim\Omega} \text{Dsign} = S$ . Then,  $S = \emptyset$ .

We will show how to apply these computation rules by means of the parameter inequality constraints of  $\Sigma$ . In order to apply Propositions 3 or 4 to a flow domain  $D$ , one first has to compute  $\Psi(\text{mode}(D))$ . In the sequel, we set  $\text{mode}(D) = M$ . We have seen in the previous section how to compute the focal set of a regular mode domain. So, here, we only consider the case where  $M$  is a singular mode domain. Recall that  $\Psi(M)$  is defined as the intersection of  $\text{supp}(M)$  and  $\overline{\text{rect}}(\{\psi(M') \mid M' \in R(M)\})$  (Definition 4). Since we consider each dimension  $i$  separately,  $i \in [1..n]$ , we have to compute the intersection of  $\text{supp}_i(M)$  and  $\overline{\text{rect}}_i(\{\psi(M') \mid M' \in R(M)\})$ . It can be directly seen that the latter equals the segment  $[\min_{M' \in R(M)} \psi_i(M'), \max_{M' \in R(M)} \psi_i(M')]$ . Since the relative order of the elements in  $\{\psi_i(M') \mid M' \in R(M)\}$  is given by the parameter inequality constraints of  $\Sigma$ ,  $\overline{\text{rect}}_i(\{\psi(M') \mid M' \in R(M)\})$  can be symbolically determined. If  $M_i$  is not a singleton set, then  $\text{supp}_i(M) = \Omega_i$ , and the intersection of  $\text{supp}_i(M)$  and  $\overline{\text{rect}}_i(\{\psi(M') \mid M' \in R(M)\})$  simply equals the latter set. If  $M_i$  is a singleton set, then there exists a threshold  $\theta_i \in \Theta_i$  such that  $M_i = \{\theta_i\}$ . As a consequence,  $\text{supp}_i(M) = \{\theta_i\}$ , and the intersection of  $\text{supp}_i(M)$  and  $\overline{\text{rect}}_i(\{\psi(M') \mid M' \in R(M)\})$  is either reduced to  $\{\theta_i\}$  or empty, according to whether these two sets intersect or not, respectively. This can be simply decided by means of the total order specified by the parameter inequality constraints of  $\Sigma$ . It follows that the focal set  $\Psi(M)$  of all (regular and singular) mode domains in  $\mathcal{M}$  can be computed.

How can the relation  $\models_{\sim\Omega}$  be computed from the focal sets? Consider a flow domain  $D \in \mathcal{D}$ , and its corresponding mode domain  $\text{mode}(D) \in \mathcal{M}$ . If  $\Psi(\text{mode}(D))$  is empty, then  $D$  is an instantaneous flow domain, and the application of Proposition 4 directly yields  $D \models_{\sim\Omega} \text{Dsign} = \emptyset$ . If not, then  $D$  is a persistent flow domain, and one can apply Proposition 3. It holds that  $D \models_{\sim\Omega} \text{Dsign} = S$ , with  $S = S_1 \times \dots \times S_n$ , and  $S_i$  to be determined for every  $i \in [1..n]$ . If  $D_i$  is a singleton set, then  $S_i = \{0\}$ . If  $D_i$  is not a singleton set, then  $D_i$  equals  $(\lambda_i, \lambda'_i)$ , for some  $\lambda_i, \lambda'_i \in \Theta_i \cup \Lambda_i$ . It holds that  $1 \in S_i$  if and only if there exists  $M' \in R(\text{mode}(D))$  such that  $\psi_i(M')$  is strictly greater than  $\lambda_i$ . Whether  $-1 \in S_i$  can be tested analogously. Finally, it is not difficult to show that  $0 \in S_i$  if and only if  $-1 \in S_i$  and  $1 \in S_i$ . Again,  $S_i$  can be symbolically computed, since the total order on  $\{\lambda_i, \lambda'_i\} \cup \{\psi_i(M') \mid M' \in R(\text{mode}(D))\}$  is given by the parameter inequality constraints of  $\Sigma$ .

In conclusion, using Propositions 3 or 4, one can compute whether a flow domain  $D \in \mathcal{D}$  satisfies a property of type ‘ $\text{Dsign} = S$ ’. Together, these propositions provide criteria that can be tested symbolically by means of the parameter inequality constraints, and yield a qualitative description of the flow in every flow domain.

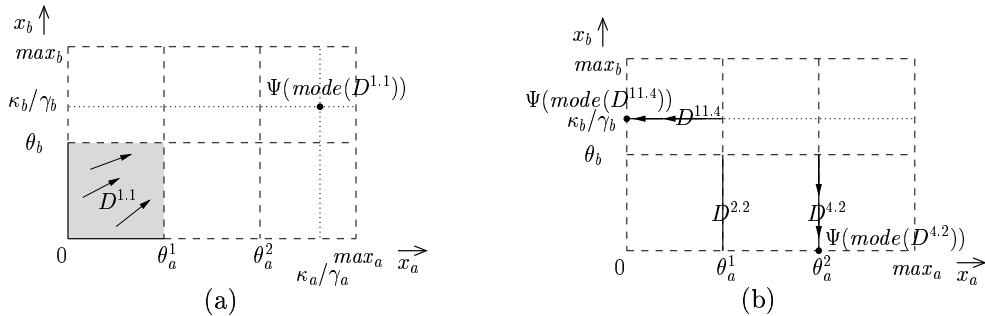


Figure 12: Derivative signs of flow domains: (a)  $\text{Dsign}(D^{1.1}) = \{(1, 1)'\}$ , (b)  $\text{Dsign}(D^{11.4}) = \{(-1, 0)'\}$ ,  $\text{Dsign}(D^{4.2}) = \{(0, -1)'\}$  and  $\text{Dsign}(D^{2.2}) = \emptyset$ .

The application of Propositions 3 and 4 is illustrated in Figure 12 for our two-gene example. First, consider the flow domain  $D^{1.1}$ , represented in Figure 12(a).  $\Psi(\text{mode}(D^{1.1}))$  equals  $\{(\kappa_a/\gamma_a, \kappa_b/\gamma_b)'\}$  and is not empty. So  $D^{1.1}$  is a persistent flow domain, and by Proposition 3 it holds that  $D^{1.1} \models_{\sim\Omega} \text{Dsign} = S$ , with  $S =$

$S_a \times S_b$ , and  $S_a$  and  $S_b$  determined as follows. Neither  $D_a^{1.1}$  nor  $D_b^{1.1}$  is a singleton set. From the parameter inequality constraints (14) given in Section 4.3, it follows that  $\theta_a^1 < \kappa_a/\gamma_a$ , so, given that  $D_a^{1.1} = [0, \theta_a^1)$  and  $\Psi_a(\text{mode}(D^{1.1})) = \{\kappa_a/\gamma_a\}$ , it holds that  $\psi_a - x_a > 0$ , for all  $x \in D^{1.1}$  and  $\psi \in \Psi(\text{mode}(D^{1.1}))$ . The same observation applies in the  $x_b$ -dimension. Consequently, we infer from Proposition 3 that  $S_a = S_b = \{1\}$ , so that  $D^{1.1} \models_{\sim_\Omega} \text{Dsign} = \{(1, 1)'\}$ . Intuitively speaking, Proposition 3 states that, given the parameter inequality constraints of  $\Sigma$  given in Section 4.3, the focal set corresponding to  $D^{1.1}$  is located ‘above’  $D^{1.1}$  in both dimensions, so that the (monotonic) solutions in  $D^{1.1}$  are increasing in both dimensions.

As a second example, consider the flow domain  $D^{4.2}$ , represented in Figure 12(b).  $M^4$  equals  $\text{mode}(D^{4.2})$  and is a singular mode domain, so we first have to compute the focal set corresponding to  $D^{4.2}$ ,  $\Psi(M^4)$ . This can be done using Definition 4. The supporting hyperplane of  $M^4$  is  $\{x \in \Omega \mid x_a = \theta_a^2\}$ . The regular mode domains having  $M^4$  in their boundary are  $M^3$  and  $M^5$ , which are shown, together with their focal sets, in Figure 4. The smallest closed rectangular set containing the two points  $\psi(M^3) = (\kappa_a/\gamma_a, 0)'$  and  $\psi(M^5) = (0, 0)'$  equals  $[0, \kappa_a/\gamma_a] \times \{0\}$ . It follows from the parameter inequality constraints (14) that  $0 < \theta_a^2 < \kappa_a/\gamma_a$ , so that  $\text{supp}(M^4)$  and  $\overline{\text{rect}}(\{\psi(M^3), \psi(M^5)\})$  intersect and their intersection equals  $\{\theta_a^2\} \times \{0\}$ , which is the focal set corresponding to  $D^{4.2}$ ,  $\Psi(M^4)$ . In order to determine the signs of the derivatives of the solutions in  $D^{4.2}$ , we apply Proposition 3. First,  $\Psi(M^4)$  is not empty, so  $D^{4.2}$  is a persistent flow domain. By Proposition 3, it holds that  $D^{4.2} \models_{\sim_\Omega} \text{Dsign} = S$ , with  $S = S_a \times S_b$ , and  $S_a$  and  $S_b$  determined as follows.  $D_a^{4.2}$  is a singleton set, so  $S_a = \{0\}$ . Applying the same reasoning as for  $D^{1.1}$ , with  $D_b^{4.2} = (0, \theta_b)$  and  $\Psi_b(M^4) = \{0\}$ , yields  $S_b = \{-1\}$ . So, it holds that  $D^{4.2} \models_{\sim_\Omega} \text{Dsign} = \{(0, -1)'\}$ . This is of course consistent with the fact that the solutions in  $D^{4.2}$  monotonically converge towards  $\Psi(M^4)$ , sliding along the hyperplane  $\{x \in \Omega \mid x_a = \theta_a^2\}$ , and are therefore strictly decreasing in the  $x_b$ -dimension (Figure 12(b)). In much the same way, one can check that Proposition 3 yields  $D^{11.4} \models_{\sim_\Omega} \text{Dsign} = \{(-1, 0)'\}$ .

Finally, consider the flow domain  $D^{2.2}$ , shown in Figure 12(b). Since  $\text{mode}(D^{2.2})$  is a singular mode domain, we first have to determine  $\Psi(\text{mode}(D^{2.2}))$ . In a similar way as above, it can be inferred that  $\Psi(\text{mode}(D^{2.2}))$  equals the empty set, using  $R(\text{mode}(D^{2.2})) = \{M^1, M^3\}$ ,  $\Psi_a(M^1) = \Psi_a(M^3) = \{\kappa_a/\gamma_a\}$ , and  $D_a^{2.2} = \{\theta_a^1\}$ . The application of Proposition 4 directly yields  $D^{2.2} \models_{\sim_\Omega} \text{Dsign} = \emptyset$ . This result is consistent with the fact that no solution remains in  $D^{2.2}$  longer than a single time instant.

In this section, we have shown how to symbolically compute, given a qualitative model  $(\Sigma, P)$ , the satisfaction relation  $\models_{\sim_\Omega}$ , representing the properties of flow domains  $D \in \mathcal{D}$ . In the next section, we present rules that can be used to compute the set of all transitions between flow domains,  $\rightarrow_{\sim_\Omega}$ .

### 5.3 Symbolic computation of flow domain transitions

In Section 4.2, we have seen that there is a transition between two flow domains  $D$  and  $D'$  if and only if there exists a solution  $\xi$  defined on some time interval  $[\tau, \tau']$ , which passes through a point  $x \in D$  at  $\tau$  and through a point  $x' \in D'$  at  $\tau'$ , without leaving  $D \cup D'$  in the time interval  $[\tau, \tau']$ . Additionally, as noticed earlier, the absolute-continuity of the solution  $\xi$  implies that  $D = D'$ ,  $D' \subseteq \partial D$ , or  $D \subseteq \partial D'$ , which corresponds to a transition of type *int*, *dim*<sup>-</sup>, or *dim*<sup>+</sup> in Proposition 1, respectively. In the sequel, for each of these three cases, we state transition rules and show how they can be computed by means of the parameter inequality constraints of  $\Sigma$ . These rules are proven in Appendix A.

The transitions of type *int* are easily characterized. In fact, an internal transition  $D \xrightarrow{\text{int}}_{\sim_\Omega} D'$  is necessarily a self-transition:  $D = D'$ . Moreover, Proposition 1 states that the existence of an *int* transition on a flow domain  $D$  is equivalent to the existence of a solution remaining in  $D$  on some  $[\tau, \tau']$ , with  $0 \leq \tau < \tau'$ , that is, a solution remaining in  $D$  longer than a single time instant. This means that all and only persistent flow domains have an *int* transition.

**Proposition 5 (Computation of transitions: *int*).** Let  $D$  and  $D'$  be two flow domains.  $D \xrightarrow{\text{int}}_{\sim_\Omega} D'$  iff

1.  $D = D'$ , and
2.  $D \in \mathcal{D}_{\text{pers}}$

We have seen in the two previous sections how to determine, by means of the parameter inequality constraints, the focal set  $\Psi(\text{mode}(D))$  associated with a flow domain  $D \in \mathcal{D}$ . By testing the emptiness of  $\Psi(\text{mode}(D))$ , we decide whether  $D$  is persistent or not, and thus decide by means of Proposition 5 whether there is an *int* transition on  $D$ .

In our two-gene example, the focal sets associated with the flow domains  $D^{1.1}$  and  $D^{4.2}$  are not empty. So,  $D^{1.1}$  and  $D^{4.2}$  are persistent, and there is an *int* transition on  $D^{1.1}$  and  $D^{4.2}$ . On the contrary,  $\Psi(\text{mode}(D^{2.2}))$  is empty, so  $D^{2.2}$  is instantaneous and does not have an *int* transition.

Now, consider the  $dim^+$  transition type:  $D \xrightarrow{dim^+}_{\sim_\Omega} D'$ . By Proposition 1, there exists a  $dim^+$  transition from  $D$  to  $D'$  if and only if there exists a solution  $\xi$  defined on some interval  $[\tau, \tau']$ , passing through  $x$  in  $D$  at  $\tau$  and through  $x'$  in  $D'$  at  $\tau'$ , while remaining in  $D'$  in the time interval  $(\tau, \tau']$ . As already mentioned, this implies that  $D \subseteq \partial D'$ . Moreover, since  $\xi$  is monotonic on  $(\tau, \tau']$ , at least in a weak sense, the sign of the derivative of  $\xi_i$  on  $(\tau, \tau']$  is determined by the sign of  $x'_i - x_i$ ,  $i \in [1..n]$ . Additionally, because while being in  $D'$ ,  $\xi$  converges towards  $\Psi(mode(D'))$ , the sign of the derivative of  $\xi_i$  on  $(\tau, \tau']$  is also determined by the relative position of  $x'$  with respect to  $\Psi(mode(D'))$ , that is, the sign of  $\psi'_i - x'_i$ , for  $\psi' \in \Psi(mode(D'))$ . These intuitions are formalized in the following proposition.

**Proposition 6 (Computation of transitions:  $dim^+$ ).** Let  $D$  and  $D'$  be two flow domains.  $D \xrightarrow{dim^+}_{\sim_\Omega} D'$  iff

1.  $D \subseteq \partial D'$ , and
2. there exist  $x \in D$ ,  $x' \in D'$ , and  $\psi' \in \Psi(mode(D'))$ , such that for all  $i \in [1..n]$  for which  $D_i$ , but not  $D'_i$ , is a singleton set, it holds that

$$(\psi'_i - x'_i)(x'_i - x_i) > 0.$$

Again, we have to show how, by means of the parameter inequality constraints of  $\Sigma$  and Proposition 6, we can decide whether there is a  $dim^+$  transition between two flow domains  $D$  and  $D'$ . We have seen in Section 5.1 how to determine the partition  $\mathcal{D}$ . So, given two flow domains  $D$  and  $D'$ , we can decide whether  $D \subseteq \partial D'$  or not. Suppose that  $D \subseteq \partial D'$  and let  $i \in [1..n]$  be such that  $D_i$ , but not  $D'_i$ , is a singleton set. There exist some  $\lambda_i, \lambda'_i \in \Theta_i \cup \Lambda_i$ , such that  $D_i = \{\lambda_i\}$  and  $D'_i$  equals either  $(\lambda_i, \lambda'_i)$  or  $(\lambda'_i, \lambda_i)$ , depending on whether  $D'$  is located above or below  $D$  in the  $x_i$ -dimension, respectively. Suppose that  $D'$  is located above  $D$  in the  $x_i$ -dimension, the other case being symmetrical. For a transition to occur, there must exist  $x_i \in D_i$ ,  $x'_i \in D'_i$ , and  $\psi'_i \in \Psi_i(mode(D'))$ , such that  $(\psi'_i - x'_i)(x'_i - x_i) > 0$ . This is equivalent to the existence of  $M' \in R(mode(D'))$  such that  $\psi_i(M') > \lambda_i$ . Since, the order on the elements in  $\{\lambda_i\} \cup \{\psi_i(M') \mid M' \in R(mode(D'))\}$  can be deduced from the parameter inequality constraints of  $\Sigma$ , we can decide symbolically whether this holds or not. Using a similar reasoning for every dimension, we can decide whether there is a  $dim^+$  transition between  $D$  and  $D'$ , or not.

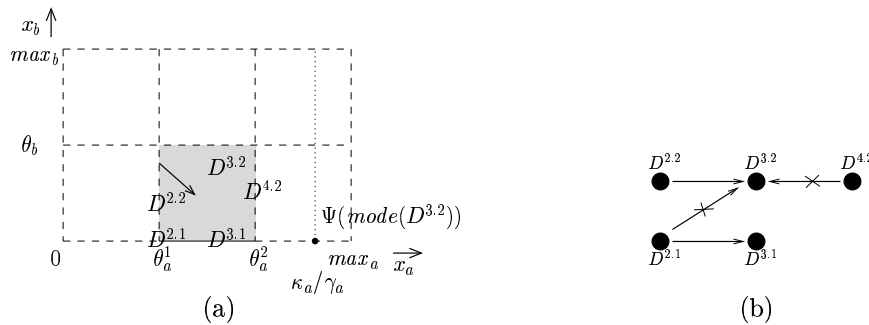


Figure 13: Computation of  $dim^+$  transitions between flow domains. (a) Representation of the phase space, with a trajectory passing from  $D^{2.2}$  to  $D^{3.2}$ . (b) Representation of some possible and impossible  $dim^+$  transitions in the state transition graph  $G$ .

As an illustration, consider some of the possible transitions between flow domains, in our two-gene example. Let  $\xi$  be the solution represented in Figure 13 which satisfies  $\xi(\tau) \in D^{2.2}$  and  $\xi(t) \in D^{3.2}$ , for  $t \in (\tau, \tau']$ . By means of Proposition 6 and the parameter inequality constraints (14), one test whether there exists a  $dim^+$  transition from  $D^{2.2}$  to  $D^{3.2}$ . First, it holds that  $D^{2.2} \in \partial D^{3.2}$ . Second, since  $D_a^{2.2} = \{\theta_a^1\}$  and  $D_a^{3.2} = (\theta_a^1, \theta_a^2)$ , it holds that  $x'_a - x_a > 0$ , for all  $x \in D^{2.2}$  and  $x' \in D^{3.2}$ . Moreover, since  $\Psi_a(mode(D^{3.2}))$  equals  $\{\kappa_a/\gamma_a\}$ , and the parameter inequality  $\theta_a^2 < \kappa_a/\gamma_a$  holds by (14), we have  $\psi'_a - x'_a > 0$ , for all  $x' \in D^{3.2}$  and  $\psi' \in \Psi(mode(D^{3.2}))$ . Since the only dimension  $i$  for which  $D_i^{2.2}$ , but not  $D_i^{3.2}$ , is a singleton set, is the dimension  $a$ , we can deduce from Proposition 6 that there is a  $dim^+$  transition from  $D^{2.2}$  to  $D^{3.2}$ , as illustrated in Figure 13. Using Proposition 6, one can also rule out a transition from  $D^{4.2}$  to  $D^{3.2}$ , since  $\psi'_a - x'_a > 0$ , whereas  $x'_a - x_a < 0$ , for all  $x \in D^{4.2}$ ,  $x' \in D^{3.2}$  and  $\psi' \in \Psi(mode(D^{3.2}))$ , given that  $D_a^{4.2} = \{\theta_a^2\}$ ,  $D_a^{3.2} = (\theta_a^1, \theta_a^2)$  and  $\Psi_a(mode(D^{3.2})) = \{\kappa_a/\gamma_a\}$ , with  $\theta_a^2 < \kappa_a/\gamma_a$  (see (14)). In a similar way, we can infer that there is a  $dim^+$  transition from  $D^{2.1}$  to  $D^{3.1}$ , but not to  $D^{3.2}$ , which is of course consistent with the flow in  $D^{3.2}$ .



Now, consider the third and last possible transition type:  $D \xrightarrow{\dim^-} \sim_{\Omega} D'$ . By Proposition 1, there exists a  $\dim^-$  transition from  $D$  to  $D'$  if and only if there exists a solution  $\xi$  defined on some interval  $[\tau, \tau']$ , passing through  $x$  in  $D$  at  $\tau$  and through  $x'$  in  $D'$  at  $\tau'$ , while remaining in  $D'$  in the time interval  $[\tau, \tau']$ . Remark that, as said in Section 4.1,  $\tau \in \mathbb{R}_{\geq 0}$  and  $\tau' \in \mathbb{R}_{> 0} \cup \{\infty\}$ . As a consequence, one can distinguish transitions that happen in finite time ( $\tau' \in \mathbb{R}_{> 0}$ ) from transitions that happen in infinite time ( $\tau' = \infty$ ). It is possible to prove that there exists a transition in finite time from  $D$  to  $D'$  if and only if  $D' \subseteq \partial D$  and there exist  $x \in D$ ,  $x' \in D'$ , and  $\psi \in \Psi(\text{mode}(D))$ , such that  $(\psi_i - x'_i)(x'_i - x_i) > 0$ , for all  $i \in [1..n]$  for which  $D'_i$ , but not  $D_i$ , is a singleton set, similarly to Proposition 6. If  $\tau' = \infty$ , then a solution  $\xi$  satisfying the conditions for a  $\dim^-$  transition from  $D$  to  $D'$  remains in  $D$  on  $[0, \infty)$ , and reaches  $D'$  only as time goes to infinity. However,  $\xi$  converges towards  $\Psi(\text{mode}(D))$ , while remaining in  $D$ . So, if  $\tau' = \infty$ , then  $\xi$  must reach  $\Psi(\text{mode}(D))$ , and  $D' \cap \Psi(\text{mode}(D))$  is not empty. One can prove that these conditions are also sufficient. The following proposition summarizes these considerations.

**Proposition 7 (Computation of transitions:  $\dim^-$ ).** Let  $D$  and  $D'$  be two flow domains.  $D \xrightarrow{\dim^-} \sim_{\Omega} D'$  iff

1.  $D' \subseteq \partial D$ , and
2. there exist  $x \in D$ ,  $x' \in D'$ , and  $\psi \in \Psi(\text{mode}(D))$ , such that
  - (a) for all  $i \in [1..n]$  for which  $D'_i$ , but not  $D_i$ , is a singleton set, it holds that

$$(\psi_i - x'_i)(x'_i - x_i) > 0,$$

or (b) it holds that

$$x' = \psi.$$

Again, we have to show how, by means of the parameter inequality constraints of  $\Sigma$  and Proposition 7, we can decide whether there is a  $\dim^-$  transition between two flow domains  $D$  and  $D'$ . It is possible to determine, as for Proposition 6, whether  $D' \subseteq \partial D$ , and whether there exist  $x \in D$ ,  $x' \in D'$ , and  $\psi \in \Psi(\text{mode}(D))$  satisfying  $(\psi_i - x'_i)(x'_i - x_i) > 0$ , for all  $i \in [1..n]$  for which  $D'_i$ , but not  $D_i$ , is a singleton set. In order to test whether there exist  $x \in D$  and  $\psi \in \Psi(\text{mode}(D))$  such that  $\psi = x'$ , we have to determine whether the intersection of  $D'$  and  $\Psi(\text{mode}(D))$  is empty, that is whether, for some  $i \in [1..n]$ ,  $D'_i$  and  $\Psi_i(\text{mode}(D))$  do not intersect. Again, this can be decided by means of the total order on the threshold and focal concentrations delimiting  $D'_i$  and  $\Psi_i(\text{mode}(D))$ , specified by the parameter inequality constraints of  $\Sigma$ .

We illustrate the application of Proposition 7 by considering some of the possible transitions between flow domains in our two-gene example. The phase space, together with some interesting trajectories, is represented in Figure 14. Let  $\xi^1$  be the solution satisfying  $\xi^1(t) \in D^{1.1}$ , for  $t \in [\tau, \tau']$  and  $\xi^1(\tau') \in D^{2.2}$ . By means of Proposition 7 and the parameter inequality constraints (14), we test whether there exists a  $\dim^-$  transition from  $D^{1.1}$  to  $D^{2.2}$ . First, it holds that  $D^{2.2} \in \partial D^{1.1}$ . Second, given that  $D_a^{1.1} = [0, \theta_a^1]$ ,  $D_a^{2.2} = \{\theta_a^1\}$ , and  $\Psi_a(\text{mode}(D^{1.1})) = \{\kappa_a/\gamma_a\}$ , with  $\theta_a^1 < \kappa_a/\gamma_a$ , it holds that  $x'_a - x_a > 0$  and  $\psi_a - x'_a > 0$ , for every  $x \in D^{1.1}$ ,  $x' \in D^{2.2}$ , and  $\psi \in \Psi(\text{mode}(D^{1.1}))$ . Since the only dimension  $i$  for which  $D_i^{2.2}$ , but not  $D_i^{1.1}$ , is a singleton set, is the dimension  $a$ , the condition 2(a) of Proposition 7 holds, and we can conclude that there exists a  $\dim^-$  transition from  $D^{1.1}$  to  $D^{2.2}$ . Now, consider  $D^{1.1}$  and  $D^{2.1}$ . It holds that  $D^{2.1} \in \partial D^{1.1}$ . However, it holds that  $D_b^{2.1}$  is a singleton set but not  $D_b^{1.1}$ , and that  $\psi_b - x'_b > 0$ , whereas  $x'_b - x_b \leq 0$ , for every  $x \in D^{1.1}$ ,  $x' \in D^{2.1}$ , and  $\psi \in \Psi(\text{mode}(D^{1.1}))$ , so that condition 2(a) does not hold. Moreover,  $D^{2.1}$  and  $\Psi(\text{mode}(D^{1.1}))$  do not intersect, so that condition 2(b) does not hold either. Consequently, by Proposition 7, one can rule out a transition from  $D^{1.1}$  to  $D^{2.1}$ . Intuitively, such a transition would be in contradiction with the direction of the flow in the  $x_b$ -dimension in  $D^{1.1}$ .

Consider the solution  $\xi^{11}$  represented in Figure 14 and satisfying  $\xi^{11}(t) \in D^{11.6}$ , for  $t \in [\tau, \infty)$  and,  $\lim_{t \rightarrow \infty} \xi^{11}(t) \in D^{11.3}$ . First,  $D^{11.3} \in \partial D^{11.6}$  holds. Second,  $D^{11.3} \cap \Psi(\text{mode}(D^{11.6})) \neq \emptyset$ , since in this case we have  $D^{11.3} = \Psi(\text{mode}(D^{11.6}))$ . So, the condition 2(b) of Proposition 7 holds, and we can conclude that there exists a  $\dim^-$  transition from  $D^{11.6}$  to  $D^{11.3}$ . On the other hand, we can rule out a transition from  $D^{11.6}$  to  $D^{11.5}$ . Since  $\psi_a - x'_a = 0$ , whereas  $x'_a - x_a < 0$ , for every  $x \in D^{11.6}$ ,  $x' \in D^{11.5}$ , and  $\psi \in \Psi(\text{mode}(D^{11.6}))$ , given that  $D_a^{11.6} = (0, \theta_a^1)$ ,  $D_a^{11.5} = \{0\}$ , and  $\Psi_a(D^{11.6}) = \{0\}$ , the condition 2(a) of Proposition 7 is not satisfied. Additionally, since  $D^{11.5} \cap \Psi(\text{mode}(D^{11.6})) = \emptyset$ , the condition 2(b) of Proposition 7 is not satisfied either. Consequently, there is no transition from  $D^{11.6}$  to  $D^{11.5}$ . Intuitively, such a transition would be in contradiction with the asymptotic convergence of the solutions towards  $\Psi(\text{mode}(D^{11.6}))$  in  $D^{11.6}$ .

Using Propositions 5, 6, and 7, it is possible to compute all and only possible transitions between flow domains. The qualitative PA transition system  $\Sigma\text{-QTS}_P$  of a qualitative model  $(\Sigma, P)$ , and its corresponding

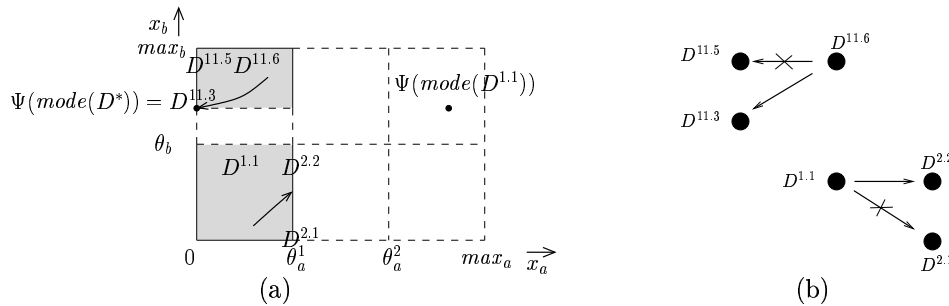


Figure 14: Computation of  $\dim^-$  transitions between flow domains. (a) Representation of the phase space, with two trajectories, passing from  $D^{1.1}$  to  $D^{2.2}$  and from  $D^{11.6}$  to  $D^{11.3}$ .  $D^*$  denotes  $D^{11.3}$ ,  $D^{11.5}$  and  $D^{11.6}$ . (b) Representation of some possible and impossible  $\dim^-$  transitions in the state transition graph  $G$ .

state transition graph  $G = (\mathcal{D}, \rightarrow_{\sim_{\Omega}})$ , can be derived by applying the transition rules to any pair of flow domains in  $\mathcal{D}$ . Again, note that these computations can be done symbolically, since they reduce to the determination of the relative order of elements in  $\Theta_i \cup \Lambda_i$ ,  $i \in [1..n]$ , representing threshold and focal concentrations. This order is given by the parameter inequality constraints of  $\Sigma$ , which specify a total order on  $\Theta_i \cup \Lambda_i$ ,  $i \in [1..n]$ .

## 5.4 Implementation

In summary, given a qualitative model  $(\Sigma, P)$ , where  $\Sigma$  is a PA system and  $P$  a region of the parameter space defined by the parameter inequality constraints of  $\Sigma$ , we have seen in the above sections how to compute symbolically its qualitative abstraction, that is, the qualitative PA transition system  $\Sigma\text{-QTS}_P$ . As shown in Section 4,  $\Sigma\text{-QTS}_P$  represents, in an abstract and qualitative way, the dynamics in the phase space of an entire class of genetic regulatory networks models. The computation of  $\Sigma\text{-QTS}_P$  has been implemented in a new prototype version of the computer tool *Genetic Network Analyzer (GNA)* [20]. In order to facilitate the analysis of  $\Sigma\text{-QTS}_P$ , the state transition graph generated by GNA can be exported to standard model-checking tools like NuSMV and CADP [10].

In practice, since the number of flow domains in the phase space grows exponentially with the number of genes in the network, it is not always possible to compute the complete qualitative PA transition system or state transition graph. However, knowledge of the part of the state transition graph reachable from a (set of) initial flow domain(s) is often sufficient to answer most of the questions of biological interest. In GNA it is possible to either compute the complete qualitative PA transition system or carry out a *reachability analysis* from a specified set of initial domains. Moreover, GNA identifies the equilibrium states, that is, the flow domains corresponding to equilibrium points, by testing whether  $D \models_{\sim_{\Omega}} \mathbf{0} \in \text{Dsign}$ . For each of the equilibrium states, the *attractor set* is computed, that is, the set of states in the graph  $G$  from which the equilibrium state is reachable.

The state transition (sub)graphs, corresponding to the reachability analysis starting from  $D^{1.1}$  and  $D^{15.1}$ , are shown in Figure 15(a) and (b), respectively. Figure 16 displays a screen capture of GNA, with the state transition graph of Figure 15(b).

## 6 Application: qualitative analysis of nutritional stress response in *Escherichia coli*

In case of nutritional stress, an *Escherichia coli* population abandons exponential growth and enters a non-growth state called *stationary phase*. This growth-phase transition is accompanied by numerous physiological changes in the bacteria, concerning among other things the morphology and the metabolism of the cell, as well as gene expression [38]. On the molecular level, the transition from exponential phase to stationary phase is controlled by a complex genetic regulatory network integrating various environmental signals. The molecular basis of the adaptation of the growth of *Escherichia coli* to nutritional stress conditions has been the focus of extensive studies for decades [32, 42]. 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 [61], numerical values for the parameters characterizing the

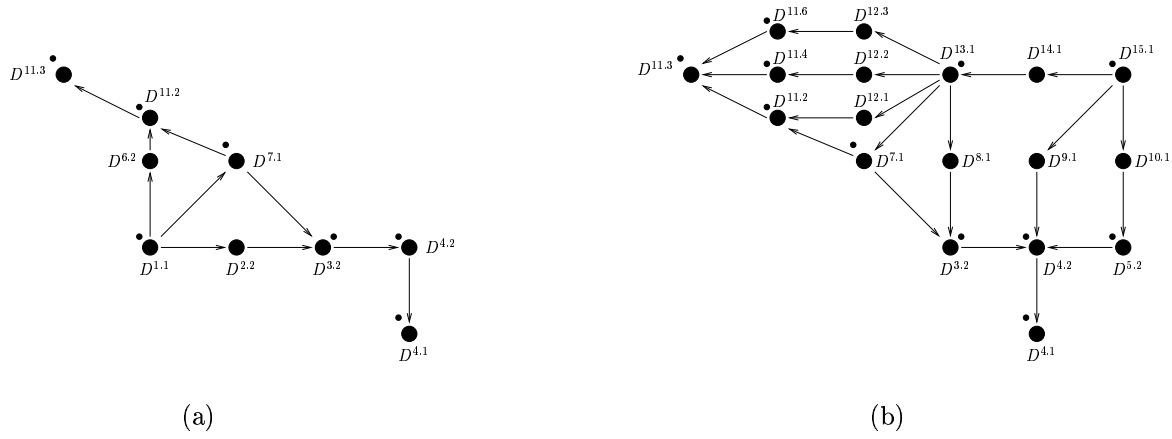


Figure 15: State transition graphs resulting from the reachability analysis with initial flow domain (a)  $D^{1.1}$  and (b)  $D^{15.1}$ .

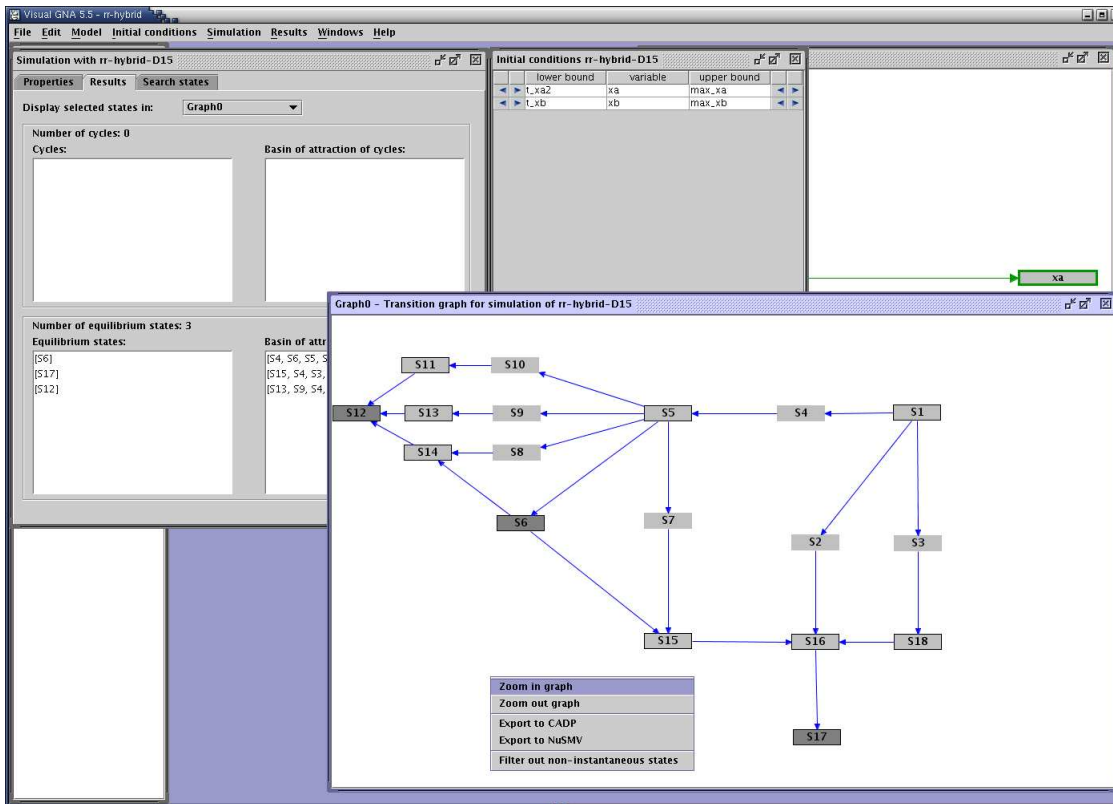


Figure 16: Screen capture of GNA for the model of the genetic regulatory network in Figure 1, showing the result of the reachability analysis corresponding to Figure 15(b).

interactions and the molecular concentrations are absent, which makes it difficult to apply traditional methods for the dynamical modeling of genetic regulatory networks.

The above circumstances have motivated the qualitative analysis of the nutritional stress response network in *Escherichia coli* by means of the method presented in this report. On the basis of literature data, we have decided to focus, as a first step, on a network of six genes that are believed to play a key role in the nutritional stress response (Figure 17). The network includes genes involved in the transduction of the nutritional stress signal, metabolism, DNA topology, and cellular growth. Based on this information, a PADE model of seven variables has been constructed, one protein concentration variable for each of the six genes and one input variable representing the presence or absence of a nutritional stress [52]. The PADE model has been supplemented by 46 inequality constraints on the parameters, inferred from information available in the experimental literature.

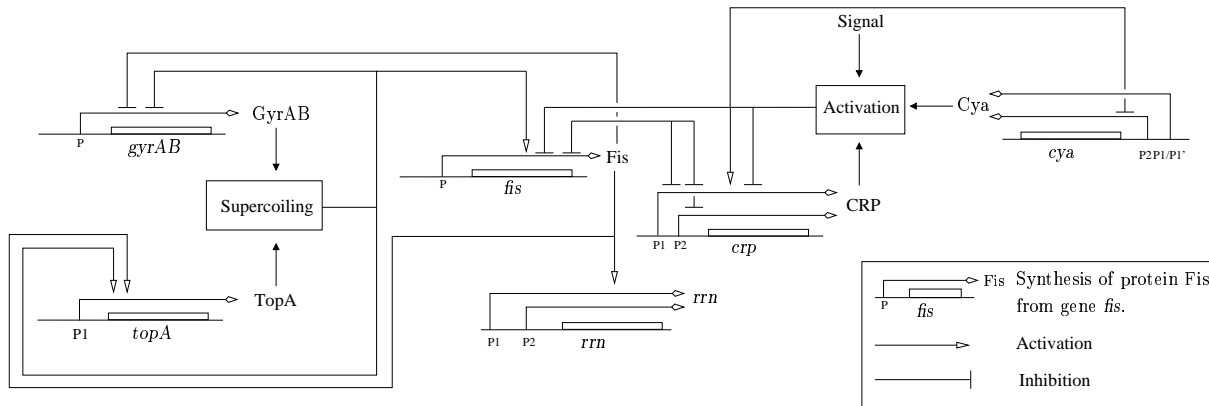


Figure 17: Network of key genes, proteins, and regulatory interactions involved in the nutritional stress network in *Escherichia coli*. The contents of the boxes labeled ‘activation’ and ‘supercoiling’ are detailed in [52].

Using the computer tool GNA, we have performed a reachability analysis on the qualitative PA transition system associated with the PADE model. In particular, we have studied the response of the cells to nutrient depletion occurring during the transition from exponential phase to stationary phase. This has given rise to a state transition graph of 331 states, computed in a few seconds. By eliminating the instantaneous states, which are of limited biological interest, a graph of 73 states remains. The graph indicates which states are reachable from an initial state in which a fast-growing bacterium receives a nutritional stress signal.

The coupling of GNA with model-checking tools [10] has allowed the verification of a number of observed dynamical properties of the *Escherichia coli* nutritional stress response. For instance, we have verified the temporal-logic formula

$$E(\dot{x}_{fis} < 0 \text{ U } AG \dot{x}_{fis} = 0),$$

which expresses the observed decrease of the concentration of the protein Fis to a new equilibrium level during the transition from exponential phase to stationary phase [1]. The formula holds for the state transition graph, as does a similar formula concerning Crp, whose concentration has been observed to increase to a new equilibrium level [40]:

$$E(\dot{x}_{crp} \geq 0 \text{ U } AG \dot{x}_{crp} = 0).$$

The transition from exponential to stationary phase is characterized by the growth arrest of the individual bacteria [38]. This can be formally expressed as the attainment of a state in which the stable RNAs encoded by the *rrn* genes, a reliable indicator of cellular growth, are present in low concentrations:

$$EFAG(x_{rrn} < \theta_{rrn}).$$

This formula is also verified by the state transition graph, for  $x_{rrn} < \theta_{rrn}$  holds in the single equilibrium state reachable from all states in the graph.

In summary, our analysis of the PADE model of the nutritional stress response has emphasized the role of the activation of the transcription factor Crp by cAMP (see [52] for details). The resulting complex inhibits the expression of the gene *fis*, which entails a decrease in the expression of the *rrn* genes, that is, a slowing-down of cellular growth. In addition to this increased understanding of the transition from exponential to stationary phase, the model has yielded predictions on the occurrence of oscillations in some of the protein concentrations after a nutrient upshift, predictions that are currently being tested in our laboratory.

We are currently working on extended models of the nutritional stress response network. The increase in the number of variables naturally leads to the generation of larger state transition graphs by our method. In order to investigate the upscalability of the method more systematically, we have applied it to a PADE model with nine state and two input variables, describing the initiation of sporulation in the bacterium *Bacillus subtilis* [19]. More specifically, we have analyzed the response of the cell to nutrient deprivation in the case of the wild-type and a dozen of mutant strains. On average, the state transition graphs generated by our method consist of 180 persistent states, with a minimum of 9 and a maximum of 923 states. The analysis of graphs of this size does not pose any problems for current model-checking tools.

## 7 Discussion

In this report, we have presented a method for the reachability analysis of genetic regulatory networks described by a class of piecewise-affine differential equation models originally proposed by Glass and Kauffman [29]. While the PADE models abstract from precise molecular mechanisms, they capture essential aspects of the network dynamics, in particular the hybrid character of the regulation of gene expression and proteolysis. Moreover, the simple mathematical form of the equations allows a qualitative analysis of the dynamics of higher-order systems to be carried out. This enables our method to deal with two major challenges confronting the analysis of genetic regulatory networks: the general absence of quantitative information on kinetic parameters and molecular concentrations as well as the size and complexity of most networks of biological interest.

In order to achieve this, the analysis of the PADE models of genetic regulatory networks has been recast in a hybrid-systems framework. More precisely, we have introduced a partition of the phase space by hyperrectangular regions in which the sign pattern of the derivatives of the concentration variables is identical, as suggested by the analysis of the dynamical properties of the PADE models. Based on this, we have shown how a PADE model can be reformulated as a so-called continuous PA transition system with equivalent reachability properties. This has prepared the application of a discrete abstraction [4, 5, 14, 15, 16, 33, 59] in a next step, which has resulted in a qualitative PA transition system providing a finite description of the dynamics of the continuous system. More specifically, the qualitative PA transition system has been shown to be a simulation of the continuous PA transition system, thus providing a conservative approximation of the reachability properties of the original system. Furthermore, inequality constraints on the parameters have been introduced, which define a region in the parameter space where the network dynamics are described by the same qualitative PA transition system. Whereas exact numerical values for the parameters are usually not available, the weaker information required for the formulation of inequality constraints can often be obtained from the experimental literature. Moreover, the inequality constraints can be exploited for the symbolic computation of the corresponding qualitative PA transition system, using simple, tailored algorithms that favor the upscaling of the method to large and complex networks.

We have implemented our approach in a new prototype version of the computer tool *Genetic Network Analyzer (GNA)* [20]. The implementation has been applied to a biological system whose functioning is not yet well-understood by biologists, the nutritional stress response in the bacterium *Escherichia coli*. The analysis of the reachability properties of qualitative PA transition system corresponding to the network model has given rise to moderately-sized state transition graphs. The coupling of GNA with model-checking tools [10] has allowed the verification of a number of observed dynamical properties of the *Escherichia coli* nutritional stress response on these graphs. This has led to new insights into how the adaptation of cell growth to nutritional state emerges from the molecular interactions. Moreover, it has given rise to predictions of the behavior of the system after a nutrient upshift, which are currently being tested in our laboratory.

The hybrid, switch-like character of the dynamics of genetic regulatory networks has attracted much attention from researchers on hybrid systems (*e.g.* [2, 7, 11, 26, 37, 45]). Our work differs from this work in three respects. First, we base our approach on piecewise-affine models, whereas other groups have employed more general types of models. For example, multi-affine differential equation models, where the flow has only product-type nonlinearities, are used in [2, 7, 11]. In [2], the quorum sensing in *Vibrio fischeri* has been modeled in Charon [3], a language for modular specification of interacting hybrid systems. The same model illustrates the applicability of the method presented in [7], which consists in reducing the dimensionality of a reachability problem by means of projection. In [11], Belta and colleagues develop a tailored method to exploit the specificities of this class of models, assuming that the flow is continuous. This method is illustrated on the analysis of the stringent response in *Mycobacterium tuberculosis* and *Escherichia coli*. More recently, stochastic hybrid models have been used to model the subtilin production in *Bacillus subtilis* [37]. Stochastic models and their deterministic equivalents yielded results similar in the average sense. While PADE models provide a more approximate description of the regulatory interactions in a network, they are well-adapted to the currently available experimental data and they permit an analysis of high-dimensional systems using inequality constraints on parameters instead of numerical values.

Second, while the PADE models have also been used in other work, notably for the analysis of Delta-Notch signalling in *Xenopus laevis* by Ghosh and colleagues [26, 39], these approaches do not take into account the dynamics of the system on the planes of discontinuity. However, it is well-known that equilibrium points and other phenomena of functional relevance, such as sliding modes, may occur on the threshold hyperplanes separating the regulatory domains [31, 55, 48, 51, 13]. In order to deal with these problems, we use an approach inspired by the treatment of discontinuities originally proposed by Filippov [25] and recently applied to PADE

models of genetic regulatory networks by Gouzé and Sari [31]. This approach has the advantage of being at the same time mathematically proper and practically useful.

Third, we introduce tailored symbolic algorithms for the computation of the qualitative PA transition system, instead of numerical simulation or general-purpose symbolic approaches. With the exception of the analytical characterization of steady-states, the results presented in [2, 7, 11, 37] solely rely on numerical simulations. As pointed out above, the lack of quantitative information on the structure and dynamics of genetic regulatory networks hampers the practical application of the above-mentioned numerical approaches. In [26, 45], symbolic approaches are proposed as an attractive alternative. These two approaches rely on predicate abstraction. However, the computation of the discrete, abstract system is performed by the general-purpose tool (Hybrid)SAL [59], which requires the use of complex decision procedures for quantifier elimination [35], in combination with theorem-proving strategies [26]. In comparison, the computation of the qualitative PA transition system in this report uses simple computational rules that exploit the favorable mathematical properties of PADE models, thereby trading genericity for upscalability.

From a more general perspective, our approach can be seen as an application of the notion of discrete abstraction, which has been introduced to study the dynamics of systems with an infinite number of states [4, 5, 14, 15, 33, 59]. Much work has focused on the identification of classes of continuous and discrete dynamical systems for which bisimulation relations with finite transition systems are guaranteed to exist [5, 33, 34, 44]. The results of this paper can be seen as showing that the weaker simulation relation may also be of considerable practical interest, especially for classes of systems for which the existence of a finite bisimulation cannot be guaranteed. Discrete abstraction criteria similar to the one used in this paper, based on the sign of the (higher) derivatives of continuous variables, have also been proposed by other authors in the fields of hybrids systems [4, 59] and qualitative reasoning [43]. Our work differs from these approaches in that it deals with the dynamics of the system on surfaces of discontinuity and uses tailored algorithms for symbolically computing the discrete or qualitative abstraction.

The method presented in this report extends our previous work [22, 21] in that it introduces a refined partition of the phase space, using flow domains instead of mode domains (Section 3). This partition underlies a discrete abstraction preserving stronger properties of the qualitative dynamics of the system, *i.e.* the sign pattern of the derivative of the variables. The qualitative PA transition system resulting from the abstraction is well-adapted to currently-available experimental data, which usually consist of information on changes in the sign pattern of the derivatives of the variables. The increased precision of the predictions obtained by means of the abstraction presented in this report comes at a price, though. The refined partition leads to an increase in the number of states of the qualitative PA transition system, which may threaten the upscalability of the method. However, as explained in Section 5.4, in practice we only determine which states are reachable from a given initial state when analyzing large systems. Under such conditions, we have found that the increase of the size of the state transition graphs generated from our models is limited, *e.g.* only a factor two on average for the analyses carried out on the sporulation model (Section 6). This observation can be explained by the fact that the increased precision of the analysis also reduces the part of the phase space that is reachable from the domain corresponding to the initial state, thus reducing the number of reachable states.

The possibility to use efficient, tailored algorithms for the computation of the qualitative PA transition system rests, to a large extent, on the approximation of the set  $K(\mathbf{x})$  in (7) by the set  $H(\mathbf{x})$  in (8). Because the latter set is hyperrectangular, the computation of domains, transitions, and sign patterns can be carried out separately in every dimension, using the ordering of parameter values fixed by inequality constraints. Because  $H(\mathbf{x})$  is an overapproximation of  $K(\mathbf{x})$ , the state transition graph may contain sequences of states that would not occur in the graph obtained by using  $K(\mathbf{x})$ . As a consequence, a PADE model may fail to be rejected by an observed time-series of measurements of the concentration variables. However, due to the fact that the approximation of  $H(\mathbf{x})$  by  $K(\mathbf{x})$  is conservative, a PADE model will never be falsely rejected. An obvious direction for further research would be to see whether finer approximations of  $H(\mathbf{x})$  can be found that still allow tailored symbolic algorithms to be used that do not compromise the upscalability of the method to large and complex genetic regulatory networks.

## Acknowledgments

The authors would like to thank Jean-Luc Gouzé and Tewfik Sari for discussions on the mathematical analysis of PADE systems. The authors acknowledge the financial support of the Programme Bioinformatique Inter-EPST and the ACI IMPBio initiative of the French Ministry for Research.

## A Proofs

### A.1 Qualitatively-identical dynamics in flow domains

**Lemma 1.** [13, 22] For every  $M \in \mathcal{M}_{pers}$  and  $\xi \in \Xi_\Sigma$  in  $M$ , it holds that

1. if  $M$  is a regular mode domain, then  $\xi$  monotonically converges towards  $\Psi(M)$ ,
2. if  $M$  is a singular mode domain, then  $\xi_i$  monotonically converges towards  $\Psi_i(M)$  for every  $i \in [1..n]$  for which  $\xi_i(0) \notin \Psi_i(M)$ .

*Proof.* Let  $M$  be a persistent mode domain and  $\xi \in \Xi_\Sigma$  a solution in  $M$ . If  $M$  is a regular mode domain, then  $\Psi(M) = \{\psi(M)\}$  and  $\xi$  is given by:  $\xi(t) = \psi(M) - (\psi(M) - x_0) e^{-\nu^M t}$ , for some  $x_0 \in M$ . It is clear that  $\xi$  monotonically converges towards  $\psi(M)$  while remaining in  $M$ . If  $M$  is not a regular mode domain, then it is a singular mode domain, and  $\Psi(M)$  might be set-valued. Consider  $i \in [1..n]$  for which  $\xi_i(0) \notin \Psi_i(M)$ . Because  $M$  is persistent,  $\Psi(M) \neq \emptyset$  and  $\Psi_i(M) = [\min_{M' \in R(M)} \psi_i(M'), \max_{M' \in R(M)} \psi_i(M')]$  (Definition 4). Assume that  $\xi_i(t) < \min \Psi_i(M)$  holds for  $t \in [0, \tau]$ , with  $\tau \in \mathbb{R}_{>0} \cup \{\infty\}$ . It holds that  $\xi_i(t) < \psi_i(M') = \mu_i^{M'} / \nu_i^{M'}$ , for all  $M' \in R(M)$ . By (11),  $\min H_i(\xi(t)) = \min_{M' \in R(M)} \{\mu_i^{M'} - \nu_i^{M'} \xi_i(t)\}$ . So  $\min H_i(\xi(t)) > 0$  and, since  $\dot{\xi}_i(t) \in H_i(\xi(t))$  holds almost everywhere on  $[0, \tau]$ ,  $\dot{\xi}_i(t) > 0$  holds almost everywhere on  $[0, \tau]$ , and  $\xi_i$  is strictly increasing on  $[0, \tau]$ . Consequently,  $\xi_i$  monotonically converges towards  $\Psi_i(M)$  while being in  $M$ . The case  $\xi_i(t) > \max \Psi_i(M)$  is proven in a similar way.  $\square$

**Lemma 2.** For every  $M \in \mathcal{M}_{pers}$ ,  $x \in M$ , and  $\psi \in \Psi(M)$ , there exists some  $\xi \in \Xi_\Sigma$  in  $M$  such that  $\xi(t_x) = x$  for some  $t_x \geq 0$ , and for every  $i \in [1..n]$ ,  $\xi_i$  monotonically converges towards  $\psi_i$ .

*Proof.* Let  $M$  be a persistent mode domain,  $x$  a point in  $M$ , and  $\psi$  a point in  $\Psi(M)$ . If  $M$  is a regular mode domain, then it follows from Definition 4 that  $\Psi(M) = \{\psi\}$ . As a consequence, there exists a unique solution  $\xi \in \Xi_\Sigma$  such that  $\xi(t_x) = x$  for a given  $t_x \geq 0$ :  $\xi(t) = \psi - (\psi - x) e^{-\nu^M (t - t_x)}$ . It is clear that for every  $i \in [1..n]$ ,  $\xi_i$  monotonically converges towards  $\psi_i$  while  $\xi$  remains in  $M$  (see also case 1 in Lemma 1).

If  $M$  is not a regular mode domain, then it is a singular mode domain. Let  $\xi$  be an absolutely-continuous function defined as follows. If  $x_i = \psi_i$ ,  $1 \leq i \leq n$ , then  $\xi_i(t) = x_i$ , for all  $t \geq 0$ . Alternatively, suppose that  $x_i < \psi_i$  (the case for  $x_i > \psi_i$  goes similarly). From Definition 4 and the fact that  $M$  is persistent, as in Lemma 1, it follows that  $x_i < \psi_i(M') = \mu_i^{M'} / \nu_i^{M'}$ , for some  $M' \in R(M)$ . Now, set  $\xi_i(t) = \psi_i(M') - (\psi_i(M') - x_i) e^{-\nu_i^{M'} (t - t_x)}$ , if  $\xi_i(t) < \psi_i$ , and  $\xi_i(t) = \psi_i$ , otherwise.  $\xi = (\xi_1, \dots, \xi_n)'$  satisfies  $\xi(t_x) = x$ , remains in  $M$  longer than a single time instant, and  $\dot{\xi}(t) \in H(\xi(t))$  holds almost everywhere while  $\xi$  remains in  $M$ . As a consequence,  $\xi$  is a solution in  $M$  according to Definition 1. Moreover, while  $\xi$  remains in  $M$ , and for every  $i \in [1..n]$ ,  $\xi_i$  monotonically converges towards  $\psi_i$ .  $\square$

**Theorem 1 (Qualitatively-identical dynamics in flow domains).**

For all  $D \in \mathcal{D}$  and for all  $x, x' \in D$ ,  $S(x) = S(x')$ .

*Proof.* Let  $D$  be a flow domain, and  $x$  and  $x'$  be two points in  $D$ . If  $D$  is an instantaneous flow domain, no solution remains in  $D$  longer than a single time instant. Consequently, the set of the solutions  $\xi \in \Xi_\Sigma$  in  $D$  that are differentiable when passing through  $x$  is empty, and by Definition 7,  $S(x) = \emptyset$ . By the same reasoning,  $S(x') = \emptyset$ . If  $D$  is not an instantaneous flow domain, then  $D$  is persistent. Let  $\xi \in \Xi_\Sigma$  and  $t_x \geq 0$  be such that  $\xi$  is a solution in  $D$ ,  $\xi(t_x) = x$ , and  $\dot{\xi}(t_x) \in H(\xi(t_x))$ . First, consider the case that  $D_i$  is a singleton set,  $1 \leq i \leq n$ , which means that  $D$  is located in a threshold or focal hyperplane. It holds that  $\dot{\xi}_i(t_x) = 0$ , since  $\xi_i$  is differentiable at  $t_x$  and remains constant in  $D_i$ , and therefore,  $S_i(x) = \{0\}$ . By the same argument, we find  $S_i(x') = \{0\}$ . Second assume that  $D_i$  is not a singleton set, and that  $\dot{\xi}_i(t_x) > 0$  (the cases for  $\dot{\xi}_i(t_x) = 0$  and  $\dot{\xi}_i(t_x) < 0$  go similarly). It is easy to see that  $\dot{\xi}(t_x) \in H(\xi(t_x))$  and  $\dot{\xi}_i(t_x) > 0$  imply the existence of some  $\psi \in \Psi(\text{mode}(D))$  such that  $\psi_i - \xi_i(t_x) > 0$  (see (10) and Definition 3 for the regular case, and (11) and Definition 4 for the singular case). More precisely, there exists some  $\psi \in \Psi(\text{mode}(D)) \cap \text{supp}(D)$  satisfying the above conditions, given that  $\Psi(\text{mode}(D))$  is an hyperrectangular set and that  $\Psi(\text{mode}(D)) \cap \text{supp}(D) \neq \emptyset$  (due to the persistence of  $D$  and Lemma 1). From the definition of the partition of  $\Omega$  given in Section 3.3, it follows that there exists some  $\psi' \in \Psi(\text{mode}(D)) \cap \text{supp}(D)$ , possibly equal to  $\psi$ , such that  $\psi_i - x_i$  and  $\psi'_i - x'_i$  have the same sign, that is, such that  $\psi'_i - x'_i > 0$ . Then, using Lemma 2, it holds that there exists  $\xi' \in \Xi_\Sigma$  in  $\text{mode}(D)$  such that  $\xi'(t'_x) = x'$  for some  $t'_x \geq 0$ , and  $\xi'_i$  monotonically converges towards  $\psi'_i$ . Since,  $\psi' \in \text{supp}(D)$ ,  $\xi'$  remains in  $D$  while converging towards  $\psi'$ . Moreover, it holds that  $\dot{\xi}'_i(t'_x) > 0$ . By Definition 7, the above

implies that if  $1 \in S_i(\mathbf{x})$ , then  $1 \in S_i(\mathbf{x}')$ , and therefore that  $S_i(\mathbf{x}) \subseteq S_i(\mathbf{x}')$ . Since this holds for every  $i \in [1..n]$ , and the roles of  $\mathbf{x}$  and  $\mathbf{x}'$  are symmetric, we conclude that  $S(\mathbf{x}) = S(\mathbf{x}')$ .  $\square$

## A.2 Qualitative abstraction of PA systems

**Theorem 2 (Reachability equivalence).** For all  $\mathbf{x}, \mathbf{x}' \in \Omega$ , there exists a solution  $\xi$  of  $\Sigma$  and  $\tau, \tau'$ , such that  $0 \leq \tau \leq \tau'$ ,  $\xi(\tau) = \mathbf{x}$ , and  $\xi(\tau') = \mathbf{x}'$  if and only if there exists a run  $(\mathbf{x}^0, \dots, \mathbf{x}^m)$  of  $\Sigma$ -TS such that  $\mathbf{x}^0 = \mathbf{x}$  and  $\mathbf{x}^m = \mathbf{x}'$ .

*Proof.*

(Only if) Consider two points  $\mathbf{x}, \mathbf{x}' \in \Omega$ , a solution  $\xi \in \Xi_\Sigma$  and  $\tau, \tau'$  such that  $0 \leq \tau \leq \tau'$ ,  $\xi(\tau) = \mathbf{x}$ , and  $\xi(\tau') = \mathbf{x}'$ . If  $\tau = \tau'$ , then  $(\xi(\tau))$  is a trivial run satisfying the conditions of Theorem 2. Otherwise,  $\tau < \tau'$  and we denote by  $D^0, \dots, D^m$ , the time-ordered sequence of flow domains traversed by  $\xi$  on the time interval  $[\tau, \tau']$ .  $D^0, \dots, D^m$  is a finite sequence, since, by definition, any solution of  $\Sigma$  reaches and leaves finitely-many times a threshold hyperplane during a time interval. If  $m = 0$ , then by Definition 8 there exists an *int* transition from  $\xi(\tau)$  to  $\xi(\tau')$ , and consequently,  $(\xi(\tau), \xi(\tau'))$  is a run satisfying the conditions of Theorem 2. Otherwise,  $m > 0$ , and we denote by  $\sigma^0, \dots, \sigma^{m-1}$ , the switching times, formally defined as  $\sigma^i = \sup\{t \in [\tau, \tau'] \mid \xi(t) \in D^i\}$ . Finally, we introduce a sequence of time instants  $\tau^0, \dots, \tau^m$  such that  $\xi(\tau^i) \in D^i$ ,  $i \in [0..m]$ . More precisely, we define  $\tau^0$  as  $\tau$ ,  $\tau^i$  as  $(\sigma^{i-1} + \sigma^i)/2$ , for all  $i \in [1..m-1]$ , and  $\tau^m$  as  $\tau'$ . Then, we will prove by induction on  $i$  that  $(\xi(\tau^0), \dots, \xi(\tau^i))$  is a run, for every  $i \in [0..m]$ . It is clear that the induction hypothesis holds for  $i = 0$ . Suppose that  $(\xi(\tau^0), \dots, \xi(\tau^i))$  is a run, for some  $i \in [0..m-1]$ . Then, we distinguish three cases, according to whether  $\xi$  instantaneously traverses  $D^i$ ,  $D^{i+1}$ , or neither of these. Note that an absolutely-continuous function cannot instantaneously traverse two consecutive flow domains. In the first case, it holds that  $\tau^i = \sigma^i < \tau^{i+1}$ . So  $\xi$  leaves  $D^i$  at  $\tau^i$  and lies in  $D^{i+1}$  on  $(\tau^i, \tau^{i+1}]$ . So, by Definition 8, there exists a *dim*<sup>+</sup> transition from  $\xi(\tau^i)$  to  $\xi(\tau^{i+1})$ . In the second case, it holds that  $\tau^i < \sigma^{i+1} = \tau^{i+1}$ . It is easy to see that this corresponds to a *dim*<sup>-</sup> transition from  $\xi(\tau^i)$  to  $\xi(\tau^{i+1})$ . In the last case, it holds that  $\tau^i < \sigma^i < \tau^{i+1}$ . This situation corresponds to an *int* (*dim*<sup>-</sup>) transition from  $\xi(\tau^i)$  to  $\xi(\sigma^i)$ , followed by a *dim*<sup>+</sup> (*int*) transition from  $\xi(\sigma^i)$  to  $\xi(\tau^{i+1})$ , if  $\xi(\sigma^i) \in D^i$  ( $\xi(\sigma^i) \in D^{i+1}$ ). In all of the above cases, we conclude that  $(\xi(\tau^0), \dots, \xi(\tau^{i+1}))$  is a run of  $\Sigma$ -TS.

(If) Consider a run  $(\mathbf{x}^0, \dots, \mathbf{x}^m)$  of  $\Sigma$ -TS, with  $\mathbf{x}^0 = \mathbf{x}$  and  $\mathbf{x}^m = \mathbf{x}'$ . If  $m = 0$ , then  $\mathbf{x} = \mathbf{x}'$  and any solution  $\xi \in \Xi_\Sigma$ , satisfying  $\xi(0) = \mathbf{x}$  satisfies the conditions of Theorem 2, with  $\tau = \tau' = 0$ . In the sequel, we suppose  $m > 0$ . Then, by Definition 8, there exists a sequence of solutions,  $(\xi^i)_{i \in [0..m-1]}$ , and of time instants,  $(\tau^i)_{i \in [0..m]}$ , such that  $\xi^i$  is defined on the time interval  $[\tau^i, \tau^{i+1}]$ , with  $\tau^i < \tau^{i+1}$ , and satisfies  $\xi^i(\tau^i) = \mathbf{x}^i$  and  $\xi^i(\tau^{i+1}) = \mathbf{x}^{i+1}$ ,  $i \in [0..m-1]$ . We will prove that the concatenation of those solutions is a solution that satisfies the conditions of Theorem 2.

Define the piecewise-constant map  $I : [\tau^0, \tau^m] \rightarrow [0..m-1]$ , such that  $I(t) = \{i \mid t \in [\tau^i, \tau^{i+1})\}$ , for all  $t \in [\tau^0, \tau^m)$ , and  $I(\tau^m) = m-1$ . Then, we define the concatenation  $\xi$  of the solutions  $\xi^i$  as  $\xi(t) = \xi^{I(t)}(t)$  on  $[\tau^0, \tau^m]$ . First, note that  $\xi(\tau^0) = \xi^0(\tau^0) = \mathbf{x}$  and that  $\xi(\tau^m) = \xi^{m-1}(\tau^m) = \mathbf{x}'$ . Then, the absolute-continuity of every solution  $\xi^i$  on  $[\tau^i, \tau^{i+1}]$ , together with the continuity of  $\xi$  at  $\tau^i$ ,  $i \in [1..m-1]$ , implies the absolute-continuity of  $\xi$  on  $[\tau^0, \tau^m]$ . Additionally,  $\xi^i$  satisfies (9) almost everywhere on  $[\tau^i, \tau^{i+1}]$ ,  $i \in [0..m-1]$ , so  $\xi$  satisfies (9) almost everywhere on  $[\tau^0, \tau^m]$ . Finally, since, by Definition 8, the solution  $\xi^i$  switches from a domain to another at most once on the time interval  $[\tau^i, \tau^{i+1}]$ ,  $\xi$  traverses at most  $m$  flow domains on the time interval  $[\tau^0, \tau^m]$ . So  $\xi$  is a solution of  $\Sigma$  that satisfies the conditions of Theorem 2.  $\square$

**Proposition 1 (Qualitative PA transition system).** Let  $\Sigma$ -QTS =  $(\Omega/\sim_\Omega, L, \Pi, \rightarrow_{\sim_\Omega}, \models_{\sim_\Omega})$  be the qualitative PA transition system corresponding to the PA transition system  $\Sigma$ -TS =  $(\Omega, L, \Pi, \rightarrow, \models)$ . Then,

1.  $\Omega/\sim_\Omega = \mathcal{D}$ .
2.  $\rightarrow_{\sim_\Omega} \subseteq \mathcal{D} \times L \times \mathcal{D}$ , such that  $D \xrightarrow{l}_{\sim_\Omega} D'$  iff there exists  $\xi \in \Xi_\Sigma$  and  $\tau, \tau'$  such that  $0 \leq \tau < \tau'$ ,  $\xi(\tau) \in D$ ,  $\xi(\tau') \in D'$ , and
  - if  $l = \textit{int}$ , then  $D = D'$  and for all  $t \in [\tau, \tau']$ , it holds that  $\xi(t) \in D$ ,
  - if  $l = \textit{dim}^+$ , then  $D \neq D'$  and for all  $t \in (\tau, \tau']$ , it holds that  $\xi(t) \in D'$ ,
  - if  $l = \textit{dim}^-$ , then  $D \neq D'$  and for all  $t \in [\tau, \tau')$ , it holds that  $\xi(t) \in D$ .
3.  $\models_{\sim_\Omega} \subseteq \mathcal{D} \times \Pi$ , such that  $D \models_{\sim_\Omega} D\textit{sign} = S$  iff for all  $\mathbf{x} \in D$ ,  $S(\mathbf{x}) = S$ .



*Proof.* We first restate the definition of the quotient transition system  $\Sigma$ -QTS of  $\Sigma$ -TS given the equivalence relation  $\sim_\Omega$ , and then prove the proposition. By definition of a quotient transition system,  $\Sigma$ -QTS equals  $(\Omega/\sim_\Omega, L, \Pi, \rightarrow_{\sim_\Omega}, \models_{\sim_\Omega})$ , where

- $\Omega/\sim_\Omega$  is the quotient phase space,
- $\rightarrow_{\sim_\Omega} : \Omega/\sim_\Omega \times L \times \Omega/\sim_\Omega$  such that  $D \xrightarrow{l} D'$  iff there exists  $\mathbf{x} \in D$ , and  $\mathbf{x}' \in D'$  such that  $\mathbf{x} \xrightarrow{l} \mathbf{x}'$ , and
- $\models_{\sim_\Omega} : \Omega/\sim_\Omega \times \Pi$  such that  $D \models_{\sim_\Omega} \pi$  iff there exists  $\mathbf{x} \in D$  such that  $\mathbf{x} \models \pi$ .

First,  $\Omega/\sim_\Omega$  equals  $\mathcal{D}$  since  $\sim_\Omega$  is defined as the equivalence relation on  $\Omega$  induced by  $\mathcal{D}$ , so the first part of the proposition holds. The second part of the proposition holds by definition of  $\mathbf{x} \xrightarrow{l} \mathbf{x}'$  in  $\Sigma$ -TS (Definition 8). Finally, by definition,  $D \models_{\sim_\Omega} \pi$  if and only if there exists  $\mathbf{x} \in D$  such that  $\mathbf{x} \models \pi$ . More specifically, given that  $\pi$  is of type ' $Dsign = S$ ',  $D \models_{\sim_\Omega} \pi$  if and only if there exists  $\mathbf{x} \in D$  such that  $S(\mathbf{x}) = S$  (Definition 8). Theorem 1 then immediately yields the last part of the proposition.  $\square$

**Theorem 3 (Model equivalence).** Let  $P$  be the region of the parameter space  $\Gamma$  defined by the parameter inequality constraints of  $\Sigma$ . Then there exists some  $Q \in \Gamma/\sim_\Gamma$  such that  $P \subseteq Q$ .

*Proof.* We have to prove that two quantitative models  $(\Sigma, \mathbf{p})$  and  $(\Sigma, \mathbf{p}')$ , whose parameters  $\mathbf{p}$  and  $\mathbf{p}'$  satisfy the same parameter inequality constraints, are equivalent, that is, yield isomorphic qualitative PA transition systems. It is not difficult to check that, if  $\mathbf{p}$  and  $\mathbf{p}'$  satisfy the same parameter inequality constraints,  $\mathbf{p}$  and  $\mathbf{p}'$  satisfy exactly the same conditions for the computation of the partition  $\mathcal{D}$  (Definitions 2, 5, and 6), of the relation  $\models_{\sim_\Omega}$  (Propositions 3 and 4), and of the relation  $\rightarrow_{\sim_\Omega}$  (Propositions 5, 6, and 7). As a consequence,  $\Sigma$ -QTS $_{\mathbf{p}}$  and  $\Sigma$ -QTS $_{\mathbf{p}'}$  have the same set of state, the same transition relation and the same satisfaction relation. So,  $\Sigma$ -QTS $_{\mathbf{p}}$  and  $\Sigma$ -QTS $_{\mathbf{p}'}$  are isomorphic qualitative PA transition systems.  $\square$

### A.3 Symbolic computation of qualitative PA transition system

**Proposition 3 (Computation of  $Dsign$ ).** Let  $D \in \mathcal{D}$  be a persistent flow domain and  $S \in 2^{\{-1,0,1\}^n}$  such that  $D \models_{\sim_\Omega} Dsign = S$ . Then,  $S = S_1 \times \dots \times S_n$  and for all  $i \in [1..n]$ ,

- if  $D_i$  is a singleton set, then  $S_i = \{0\}$ ,
- if  $D_i$  is not a singleton set, then
  - $-1 \in S_i$  iff there exists  $\mathbf{x} \in D$  and  $\psi \in \Psi(mode(D))$  such that  $\psi_i - x_i < 0$ ,
  - $0 \in S_i$  iff there exists  $\mathbf{x} \in D$  and  $\psi \in \Psi(mode(D))$  such that  $\psi_i - x_i = 0$ ,
  - $1 \in S_i$  iff there exists  $\mathbf{x} \in D$  and  $\psi \in \Psi(mode(D))$  such that  $\psi_i - x_i > 0$ .

*Proof.* Let  $D \in \mathcal{D}$  be a persistent flow domain. Recall that, by definition of a qualitative PA transition system,  $D \models_{\sim_\Omega} Dsign = S$  is equivalent to  $S = S(\mathbf{x})$ , for some  $\mathbf{x} \in D$ . Consider  $i \in [1..n]$  such that  $D_i$  is a singleton set. Then, as shown in the proof of Theorem 1, it holds for every  $\mathbf{x} \in D$  that  $S_i(\mathbf{x}) = \{0\}$ . Now, consider  $i \in [1..n]$  such that  $D_i$  is not a singleton set. We will consider the case where  $-1 \in S_i$ , bearing in mind that the cases  $1 \in S_i$  and  $0 \in S_i$  can be proven in a similar manner.

- (If) Let  $\mathbf{x} \in D$ ,  $\psi \in \Psi(mode(D))$ , and assume that  $\psi_i - x_i < 0$ . More precisely, assume that  $\psi \in \Psi(mode(D)) \cap supp(D)$ , the existence of such a  $\psi$  being guaranteed by the persistence of  $D$  and Lemma 1. It follows from Lemma 2 that there exists  $\xi \in \Xi_\Sigma$  in  $mode(D)$  passing through  $\mathbf{x}$  at  $t_x$  such that  $\xi_i$  monotonically converges towards  $\psi_i$ , and consequently, such that  $\dot{\xi}_i(t_x) < 0$ . Since  $\psi \in supp(D)$ ,  $\xi$  is a solution remaining in  $D$  while converging towards  $\psi$ . This implies that  $-1 \in S_i(\mathbf{x})$ , for  $\mathbf{x} \in D$  (Definition 7).
- (Only if) By contraposition, we suppose that for every  $\mathbf{x} \in D$  and  $\psi \in \Psi(mode(D))$  it holds that  $\psi_i - x_i \geq 0$ . Given that  $D_i$  is not a singleton set, and taking into account the definition of the partition of  $\Omega$  (Definitions 5 and 6), it even holds that  $\psi_i - x_i > 0$ . We can therefore apply Lemma 1 which implies, by the monotonicity of the convergence of the solutions in  $D$  towards  $\Psi(mode(D))$ , that all solutions are steady or increasing in the  $x_i$ -dimension. So,  $-1 \notin S_i(\mathbf{x})$  for every  $\mathbf{x} \in D$ , which proves necessity.  $\square$

**Proposition 4 (Computation of  $D_{sign}$ ).** Let  $D \in \mathcal{D}$  be an instantaneous flow domain and  $S \in 2^{\{-1,0,1\}^n}$  such that  $D \models_{\sim_\Omega} D_{sign} = S$ . Then,  $S = \emptyset$ .

*Proof.* Let  $D \in \mathcal{D}$  be an instantaneous flow domain and  $\mathbf{x}$  a point in  $D$ . As shown in the proof of Theorem 1, the set of the solutions  $\xi \in \Xi_\Sigma$  in  $D$  that are differentiable when passing through  $\mathbf{x}$  is empty, so that by Definition 7,  $S(\mathbf{x}) = \emptyset$ . We conclude that  $S = \emptyset$  by means of Proposition 1.  $\square$

**Proposition 5 (Computation of transitions:  $int$ ).** Let  $D$  and  $D'$  be two flow domains.  $D \xrightarrow{int}_{\sim_\Omega} D'$  iff

1.  $D = D'$ , and
2.  $D \in \mathcal{D}_{pers}$

*Proof.*

(Only if) From Proposition 1, if there exists an  $int$  transition from  $D$  to  $D'$ , then  $D = D'$ , and at least one solution remains in  $D$  longer than a single time instant. So,  $D$  is a persistent flow domain.

(If) By definition of a persistent flow domain  $D$ , there exists at least one solution  $\xi$  that remains in  $D$  more than a single time instant. Then, this solution satisfies the conditions of Proposition 1 for an  $int$  transition, with  $D = D'$ .  $\square$

**Proposition 6 (Computation of transitions:  $dim^+$ ).** Let  $D$  and  $D'$  be two flow domains.  $D \xrightarrow{dim^+}_{\sim_\Omega} D'$  iff

1.  $D \subseteq \partial D'$ , and
2. there exist  $\mathbf{x} \in D$ ,  $\mathbf{x}' \in D'$ , and  $\psi' \in \Psi(mode(D'))$ , such that for all  $i \in [1..n]$  for which  $D_i$ , but not  $D'_i$ , is a singleton set, it holds that

$$(\psi'_i - x'_i)(x'_i - x_i) > 0.$$

*Proof.* <sup>†</sup>

(Only if) By Proposition 1, a  $dim^+$  transition from  $D$  to  $D'$  implies the existence of a solution  $\xi$  on a time interval  $[\tau, \tau']$ , which starts in  $D$  and reaches  $D'$ . The absolute-continuity of the solution imposes that either  $D = D'$ ,  $D' \subseteq \partial D$ , or  $D \subseteq \partial D'$ . Since  $\xi$  additionally satisfies  $\xi(\tau) \in D$  and  $\xi(t) \in D'$ , for all  $t \in (\tau, \tau']$ , there exists at least one dimension  $i$  such that  $D_i$  is a closed interval, and  $D'_i$  is an open interval, and consequently,  $D \subseteq \partial D'$ . So condition 1 must hold.

Suppose that condition 2 does not hold. So, there exists  $i \in [1..n]$  with  $D_i$ , but not  $D'_i$ , singleton set such that  $(\psi'_i - x'_i)(x'_i - x_i) \leq 0$  for every  $\mathbf{x} \in D$ ,  $\mathbf{x}' \in D'$ , and  $\psi' \in \Psi(mode(D'))$ . If  $x'_i < x_i$ , then this implies that  $\psi'_i \geq x'_i$ . Given that  $D'_i$  is not a singleton set, and taking into account the definition of the partition of  $\Omega$  (Definitions 5 and 6), the strict inequality must hold. Similarly, if  $x'_i > x_i$ , then  $\psi'_i < x'_i$ . In both cases, it holds that  $D'_i \cap \Psi_i(mode(D')) = \emptyset$ , and we can therefore apply Lemma 1, which implies for all solutions  $\xi \in \Xi_\Sigma$  in  $D'$  that  $\xi_i$  monotonically converges towards  $\Psi_i(mode(D'))$ . As a consequence, no solution can enter  $D'$  from  $D$ . Hence, there cannot be a transition from  $D$  to  $D'$ . So condition 2 must hold.

(If) We want to construct a solution  $\xi$  satisfying the conditions of Proposition 1. We will define for all  $i$ ,  $\xi_i$ , starting in  $D_i$  and entering  $D'_i$ , and prove that  $\xi = (\xi_1, \dots, \xi_n)'$  satisfies the transition conditions of Proposition 1.

If  $D'_i$  is a singleton set, then  $D_i = D'_i = \{x_i \in \Omega_i \mid x_i = \lambda_i\}$ , for some  $\lambda_i \in \Lambda_i \cup \Theta_i$ , since  $D \subseteq \partial D'$ . Then, we define  $\xi_i(t) = \lambda_i$ , for all  $t \geq 0$ . If  $D'_i$  and  $D_i$  are both non singleton sets, then  $D_i \subseteq D'_i$  and, since  $D'_i$  is an open interval,  $\xi_i$  will remain in  $D'_i$  longer than a single time instant, for every solution  $\xi$  starting in  $D$ . The last possible case is when  $D_i$ , but not  $D'_i$ , is a singleton set. Assume again that  $D_i = \{x_i \in \Omega_i \mid x_i = \lambda_i\}$ . If  $x'_i < \lambda_i$ , then  $\psi'_i < x'_i$  must hold for some  $\psi' \in \Psi(mode(D'))$ . This implies that for some  $M \in \mathcal{M}_r$ ,  $\psi_i(M) < \lambda_i$ , with  $M = mode(D')$ , if  $mode(D')$  is a regular mode domain, or  $M \in R(mode(D'))$ , otherwise. Similarly, if  $x'_i > \lambda_i$ , there exists  $M \in \mathcal{M}_r$ , such that  $\psi_i(M) > \lambda_i$ . Then, we define  $\xi_i(t) = \psi_i(M) + (\lambda_i - \psi_i(M))e^{-\nu_i^M t}$ . Finally, we define  $\xi$  as  $(\xi_1, \dots, \xi_n)'$  on  $[0, \tau]$ . We can choose a sufficiently small  $\tau$  such that  $\xi$  starts in  $D$  and remains in  $D'$  on  $(0, \tau]$ . It can be easily verified that  $\xi$  is a solution of  $\Xi_\Sigma$  satisfying the transition conditions of Proposition 1.  $\square$

**Proposition 7 (Computation of transitions:  $dim^-$ ).** Let  $D$  and  $D'$  be two flow domains.  $D \xrightarrow{dim^-}_{\sim_\Omega} D'$  iff

1.  $D' \subseteq \partial D$ , and

<sup>†</sup>The proof is similar to the ones given in [13, 22].

2. there exist  $\mathbf{x} \in D$ ,  $\mathbf{x}' \in D'$ , and  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$ , such that

(a) for all  $i \in [1..n]$  for which  $D'_i$ , but not  $D_i$ , is a singleton set, it holds that

$$(\psi_i - x'_i)(x'_i - x_i) > 0,$$

or (b) it holds that

$$\mathbf{x}' = \boldsymbol{\psi}.$$

*Proof.* †

(Only if) By Proposition 1, a  $\text{dim}^-$  transition from  $D$  to  $D'$  implies the existence of an absolutely-continuous solution  $\boldsymbol{\xi}$  on a time interval  $[\tau, \tau']$  satisfying  $\boldsymbol{\xi}(t) \in D$  for all  $t \in [\tau, \tau')$ , and  $\boldsymbol{\xi}(\tau') \in D'$ . Then, applying the same reasoning as in the previous proof, we find that  $D' \subseteq \partial D$ . So condition 1 must hold.

Suppose that condition 2 does not hold. That is, we assume that (a) there exists  $i \in [1..n]$  with  $D'_i$ , but not  $D_i$ , singleton set but not  $D_i$  such that  $(\psi_i - x'_i)(x'_i - x_i) \leq 0$  for every  $\mathbf{x} \in D$ ,  $\mathbf{x}' \in D'$ , and  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$ , and that (b)  $D' \cap \Psi(\text{mode}(D)) = \emptyset$ . Assume that  $D'_i = \{x'_i \in \Omega_i \mid x'_i = \lambda_i\}$ , with  $\lambda_i \in \Lambda_i \cup \Theta_i$ . If  $x'_i < x_i$ , then (a) implies that  $\psi_i \geq x'_i$ . Similarly, if  $x'_i > x_i$ , then  $\psi_i \leq x'_i$ . If for all  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$  the above inequalities are strict, then we can prove, by means of Lemma 1, that no solution can reach  $D'$  from  $D$ , as for Proposition 6. Otherwise, one can prove that the solutions  $\boldsymbol{\xi}$  reaching the hyperplane  $\{\mathbf{x}' \in \Omega \mid x'_i = \lambda_i\}$  do so only asymptotically. Since  $\boldsymbol{\xi}$  in  $D$  converges towards  $\Psi(\text{mode}(D))$ , if such a solution  $\boldsymbol{\xi}$  reaches  $D' \subset \{\mathbf{x}' \in \Omega \mid x'_i = \lambda_i\}$ , this would imply that  $D' \cap \Psi(\text{mode}(D)) \neq \emptyset$ , which is in contradiction with (b). So condition 2 must hold.

(If) Assume that either conditions 1 and 2(a), or 1 and 2(b) hold. In both cases,  $\Psi(\text{mode}(D)) \neq \emptyset$ , so that  $\text{mode}(D)$ , and consequently  $D$ , are persistent. First, suppose that conditions 1 and 2(a) hold. Let  $i$  be such that  $D'_i$ , but not  $D_i$ , is a singleton set. Then,  $D'_i = \{x'_i \in \Omega_i \mid x'_i = \lambda_i\}$ , for some  $\lambda_i \in \Lambda_i \cup \Theta_i$ . It follows from condition 2(a) that there exist  $\mathbf{x} \in D$  and  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$ , such that  $(\psi_i - x'_i)(x'_i - x_i) > 0$ . More precisely, we assume that  $\boldsymbol{\psi} \in \Psi(\text{mode}(D)) \cap \text{supp}(D)$ , the existence of such a  $\boldsymbol{\psi}$  being guaranteed by the persistence of  $D$  and Lemma 1. Suppose that  $x'_i < x_i$ . Given the definition of domains, this inequality holds for every  $\mathbf{x} \in D$ . Moreover, it holds that  $\psi_i < x'_i$ . So, for every  $\mathbf{x} \in D$  and for some  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$ , it holds that  $\psi_i < x'_i < x_i$ . Symmetrically, if there exists  $\mathbf{x} \in D$  such that  $x'_i > x_i$ , then for every  $\mathbf{x} \in D$  and for some  $\boldsymbol{\psi} \in \Psi(\text{mode}(D))$ , it holds that  $\psi_i > x'_i > x_i$ . From Lemma 2, and taking into account that  $\boldsymbol{\psi} \in \Psi(\text{mode}(D)) \cap \text{supp}(D)$ , there exists, for every  $\mathbf{x} \in D$ , a solution  $\boldsymbol{\xi}$  in  $D$  passing through  $\mathbf{x}$  and converging monotonically towards  $\boldsymbol{\psi}$ . As a consequence, these solutions will reach in finite time one or more hyperplanes  $\{\mathbf{x}' \in \Omega \mid x'_i = \lambda_i\}$ ,  $i \in I$ , with  $I$  the set of indices  $i$  such that  $D'_i$ , but not  $D_i$ , is a singleton set. One can choose  $\mathbf{x} \in D$  such that the corresponding solution  $\boldsymbol{\xi}$  in  $D$  passes through  $D' \subset \cap_{i \in I} \{\mathbf{x}' \in \Omega \mid x'_i = \lambda_i\}$ . It can easily be verified that such a solution  $\boldsymbol{\xi}$  satisfies the transition conditions of Proposition 1.

Second, suppose that conditions 1 and 2(b) hold. Consider  $\boldsymbol{\psi} \in \Psi(\text{mode}(D)) \cap D'$  and a solution  $\boldsymbol{\xi}$  in  $\text{mode}(D)$ , given by Lemma 2, that starts in  $D$  and monotonically converges towards  $\boldsymbol{\psi}$ . Since  $D' \subseteq \partial D$ , it holds that  $\boldsymbol{\psi} \in D' \subseteq \text{supp}(D)$ , and consequently, that  $\boldsymbol{\xi}$  is a solution in  $D$ .  $\boldsymbol{\xi}$  asymptotically reaches  $\boldsymbol{\psi}$ , and hence  $D'$ . So,  $\boldsymbol{\xi}$  satisfies the transition conditions of Proposition 1. □

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<http://www.inria.fr>  
ISSN 0249-6399