A class of Switched Piecewise Quadratic Systems for coupling gene expression with growth rate in bacteria ⋆

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Abstract: In this paper we propose a novel qualitative formalism to model gene expression dynamics dependent on dilution due to growth rate of the cell. We extend the piecewise linear (PL) systems by keeping the use of step functions to model the interactions between the elements and adding a growth rate expression to model the dilution effect. Focusing on the global gene expression machinery in bacteria, we model the growth rate as the minimum of two limiting factors: RNA polymerase (RNAP) and ribosomes. The resulting system is a switched system with two piecewise quadratic (PQ) modes. We study the stability of such switched piecewise quadratic (SPQ) system starting from the stability analysis of the (PQ) modes. We also present and analyze by means of phase-planes a bidimensional SPQ model involving RNAP and ribosomes concentrations, which brings out the important differences with respect to PL systems. Finally, we qualitatively show that our growth rate expression acts well in different biological conditions.

1. INTRODUCTION

One of the aims of systems biology is to link molecular-level mechanisms (e.g. gene expression) to cell-level behavior (e.g. growth rate) (Kitano [2002]). In the last years much work has focused on the impact of molecular and gene networks on cellular physiology, but less is known about how cellular physiology can influence the machinery of transcription and translation (Klumpp et al. [2010]).

In bacteria, the rate of cell proliferation, i.e. the growth rate, is known to be intimately coupled with gene expression (Scott et al. [2010]). In fact, bacterial gene expression depends not only on transcription factors-promoter interactions, but also on bacterial growth, because important components of the gene expression machinery (CGEMs), such as RNA polymerase (RNAP) and ribosomes, are all growth rate dependent (Bremer et al. [1996]). Studying these global effects is crucial for a better understanding of the gene expression on its whole and for the design of synthetic gene circuits (Carta et al. [2012]). In this paper we focus on two of the major CGEMs of E. coli, i.e. ribosomes and RNAP, which account for transcription and translation, respectively, and use them to develop a model for bacterial growth rate.

This growth rate expression leads to a *switched piecewise quadratic* (SPQ) formalism–derived from *piecewise linear* (PL) systems–to model gene expression dependent on dilution due to bacterial growth.

2. PIECEWISE LINEAR SYSTEMS OVERVIEW

Mathematical modeling and computational techniques are fundamental to the understanding of these genetic regulatory networks (De Jong [2002]). The principal modeling challenges come from incomplete knowledge of the networks, and the dearth of quantitative data for identifying kinetic parameters required for detailed mathematical models. Qualitative methods overcome both of these difficulties and are thus well-suited to the modeling and simulation of genetic networks (Ropers et al. [2006]).

A class of widespread and well studied qualitative models are piecewise linear (PL) systems, originally introduced by Glass and Kauffman [1973]. PL models of regulatory genetic networks are built with discontinuous (step) functions. The use of step functions has been motivated by the experimental observation that the activity of certain genes changes in a drastic manner at a threshold concentration of a regulatory protein (Yagil and Yagil [1971]). The PL model has the general form

$$\dot{x}_i = f_i(x) - d_i x_i, \quad 1 \leq i \leq n, \tag{1}$$

where $x = (x_1, ..., x_n)^T \in \mathbb{R}^n_{\geq 0}$ is a vector of protein concentrations. The non-negative quantities $f_i(x)$ and $d_i x_i$ represent synthesis and degradation rates for each protein x_i respectively. The function $f_i : \mathbb{R}^n_{\geq 0} \to \mathbb{R}_{\geq 0}$ represents the expression rate of the gene i depending on the whole state x. However, $f_i(x)$ can be detailed as:

$$f_i(x) = \sum_{l=1}^{L_i} k_{il} b_{il}(x)$$

where $k_{il} > 0$ is a rate parameter and $b_{il}(x)$ is a combination of step functions s^+, s^- defined as:

$$s^+(x_i, \theta_i^j) = \begin{cases} 1 & \text{if } x_i > \theta_i^j \\ 0 & \text{if } x_i < \theta_i^j \end{cases}; \quad s^-(x_i, \theta_i^j) = 1 - s^+(x_i, \theta_i^j).$$

More details on dynamical analysis and applications of PL models can be found in Casey et al. [2006] and references therein.

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3. THE GROWTH RATE MODEL

Focusing on the gene expression machinery in bacteria, we assume that growth rate is intimately related to the capacity of cells to produce *bulk proteins*, which represent cell building and maintenance proteins essential for bacterial growth. Bulk proteins, as any other protein, are produced in a two-step process (gene expression) in which RNAP and ribosomes play a pivotal role (George and Danny [2002]). The first step, i.e. transcription, is catalyzed by RNAP which allows the synthesis of mRNA from DNA. During the second step, i.e. translation, the mRNA is translated into proteins by ribosomes. Taking this into account, the cell's growth rate–considered as a sort of production rate of bulk proteins–is thus limited by two potential limiting factors: RNAP and ribosomes. Thus, let x_p, $x_r \in \mathbb{R}_{\geq 0}$ be the concentrations of RNAP and ribosomes, respectively we modeled the bacterial growth rate $\mu : \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$ as:

$$\mu(t) = \min(\mu_p\, x_p(t), \mu_r\, x_r(t)) \tag{2}$$

where μ_p and μ_r are proportion factors depending on the carbon source used. Moreover, we note that expressions of the type (2) are widely used in ecology to model the specific growth rate of species, determined by the resource that is most limiting according to Liebig's "law of the minimum" (Huisman and Weissing [1999]), and recently, a similar expression to (2) has also been applied in a model of ribosomal regulation in E. coli (Shachrai et al. [2010]).

4. SWITCHED PIECEWISE QUADRATIC (SPQ) SYSTEM

Since our purpose is dealing with gene expression dependent on bacterial growth, we take into account the fact that cells remove proteins by two processes: degradation and dilution due to cell growth (Eden et al. [2011]). Thus, the PL formalism (1) can be extended setting $d_i = \mu + \gamma_i$ in (1), where μ of the form (2), is the bacterial growth rate accounting for proteins' dilution and γ_i is a degradation constant. Therefore, letting $p = n-1$ and $r = n$ in (2), the PL system with dilution effect has the general form:

$$\dot{x}_i = f_i(x) - [\min(\mu_{n-1}\, x_{n-1}, \mu_n\, x_n) + \gamma_i]\, x_i, \quad 1 \leq i \leq n\,. \tag{3}$$

We note that system (3), according to the evaluation of the function min in μ, can be split into two subsystems (or modes):

$$\begin{aligned} &\text{I: } \dot{x}_i = f_i(x) - [\mu_{n-1}\, x_{n-1} + \gamma_i]\, x_i \ (1 \leq i \leq n), \text{ if } x_{n-1} < \frac{\mu_n x_n}{\mu_{n-1}} \\ &\text{II: } \dot{x}_i = f_i(x) - [\mu_n\, x_n + \gamma_i]\, x_i \ (1 \leq i \leq n), \text{ if } x_n < \frac{\mu_{n-1} x_{n-1}}{\mu_n} \end{aligned} \tag{4}$$

which share the same structure and properties. Thus, system (3) belongs to the class of *switched systems* (Liberzon and Morse [1999]) in which the growth rate μ acts as a rule that orchestrates the switching between the two subsystems in (4). Thus, we named system (3) *switched piecewise quadratic* (SPQ). Moreover, each piecewise quadratic (PQ) system (or mode) in (4) can be written in matrix form as

$$\dot{x} = f(x) - d(x_q)x, \tag{5}$$

where $f = (f_1, ..., f_n)$, $d(x_q) = diag(\mu_q\, x_q + \gamma_1, ..., \mu_q\, x_q + \gamma_n)$, *diag* is the diagonal matrix corresponding to the vector and $q = n-1$ or $q = n$ depending on whether we refer to mode I or mode II in (4), respectively. To study the dynamics of the SPQ system (3) we need first to characterize the dynamics of its PQ modes (5), and then investigate the properties arising from the switching condition. To this end, in the next section we present a dynamical study of the PQ subsystem.

5. THE PQ SUBSYSTEM: DYNAMICAL STUDY

For simplicity, we provide a dynamical study only for mode II in (4), that is when $x_q = x_n$ in (5), but equivalent results can be derived for mode I.The dynamics of the PQ subsystem can be studied in the n-dimensional state-space $\Omega = \Omega_1 \times \Omega_2 \times ... \times \Omega_n$, where $\Omega_i = \{x_i \in \mathbb{R}_{\geq 0} | 0 \leq x_i \leq max_i\}$ for some maximum concentration value max_i. A protein encoded by a gene will be involved in different interactions at different concentration thresholds, so for each variable x_i, we assume there are p_i ordered thresholds $\theta_i^1, ..., \theta_i^{p_i}$ (we also define $\theta_i^0 = 0$ and $\theta_i^{p_i+1} = max_i$). The $(n-1)$-dimensional hyper-planes defined by these thresholds partition Ω into hyper-rectangular regions we call *domains*. Specifically, a domain $D \subset \Omega$ is defined to be a set $D = D_1 \times ... \times D_n$, where D_i is one of the following:

$$\begin{aligned} D_i &= \left\{ x_i \in \Omega_i | \theta_i^j < x_i < \theta_i^{j+1} \right\} \quad \text{for } j \in \{0, ..., p_i\} \\ D_i &= \left\{ x_i \in \Omega_i | x_i = \theta_i^j \right\} \quad \text{for } j \in \{0, ..., p_i\}. \end{aligned}$$

Let $\mathscr{D}$ be the set of domains in Ω. A domain $D \in \mathscr{D}$ is called a *regulatory domain* if none of the variables x_i has a threshold value in D.In contrast, a domain $D \in \mathscr{D}$ is called a *threshold domain* of order $k \leq n$ if exactly k variables have threshold values in D (in Mestl et al. [1995] threshold domains are called switching domains, but we avoid this definition to prevent misunderstandings with switched system). The corresponding variables x_i are called *threshold variables* in D. The two sets of domains are respectively denoted by $\mathscr{D}_r$ and$\mathscr{D}_t$.

5.1 Solutions and Stability in Regular Domains

For any regulatory domain D, the function $f(x)$ is constant for all $x \in D$, and it follows that the PQ system (5) (for $x_q = x_n$) can be written as

$$\dot{x} = f^D - d(x_n)x, \tag{6}$$

where f^D is constant in D. We note that (6) is a cascaded system, since the differential equation governing $x_n(t)$ depends only on $x_n(t)$ while $\dot{x}_i(t)$ depends only on $x_i(t)$ and $x_n(t)$, but not on $x_j(t)$ for $j > i$. Thus, for any $x(t_0) \in D$ the unique solution of (6) can be found explicitly by solving first the n-component of (6)— $\dot{x}_n = f_n^D - (\mu_n\, x_n + \gamma_n)x_n$—which is an autonomous differential equation, and then solving for the remaining components. Hence, it can be shown that $x_i(t)$ $(i = 1, ..., n-1)$ is given by: $x_i(t) = \left(b(t_0)x_i(t_0) + f_i^D \int_{t_0}^t b(s)ds \right) / b(t)$, where $b(t) = exp\left(\int_{t_0}^t (\mu_n\, x_n(\tau) + \gamma_n) d\tau \right)$. Moreover, regarding the stability of system (6) we can state the following two theorems.

Theorem 1. Assuming that $D = \mathbb{R}^n_{\geq 0}$, then point $\Phi(D) = (\bar{x}_1, ..., \bar{x}_n)^T$ defined as

$$\begin{aligned} \bar{x}_i &= \eta(\bar{x}_n, f_i^D, \mu_n, \gamma_i) = \frac{f_i^D}{\mu_n \bar{x}_n + \gamma_i}, \quad i = 1, ..., n-1 \\ \bar{x}_n &= \varphi(f_n^D, \mu_n, \gamma_n) = \frac{-\gamma_n + \sqrt{\gamma_n^2 + 4\mu_n f_n^D}}{2\mu_n}, \end{aligned} \tag{7}$$

is a globally asymptotically stable equilibrium of system (6)

Proof. Note that $\bar{x}_n$ is the only positive solution of $\dot{x}_n = 0$ and thus it is easy to check that $\bar{x}_n$ is a globally asymptotically stable equilibrium for $\dot{x}_n = f_n^D - (\mu_n x_n + \gamma_n)x_n$ in $\mathbb{R}_{\geq 0}$. Moreover, $\bar{x}_i$ is a globally asymptotically stable equilibrium for $\dot{x}_i = f_n^D - (\mu_n \bar{x}_n + \gamma_n)x_i$ (which is of the form of PL systems).

Finally, the trajectories are bounded. Hence, the proof follows by Theorem 1 in Viel et al. [1995].

Next we consider $D \in \mathscr{D}_r$. It follows from Theorem 1 that $\Phi(D)$ is locally asymptotically stable only if $\Phi(D) \in D$. Otherwise solutions will leave D.

Theorem 2. Let $D \in \mathscr{D}_r$. If $\Phi(D) \in D$, then $\Phi(D)$ is a locally asymptotically stable point of system (6).

We note that Theorem 2 states a novel behavior of the PQ systems with respect to PL systems, that is the convergence towards the equilibrium point is not assured from every point within the domain containing the equilibrium as, conversely, it is for PL systems.

***Definition** 1.* Given a regulatory domain $D \in \mathscr{D}_r$, the point $\Phi(D) = (\bar{x}_1, ..., \bar{x}_n)^T \in \Omega$ (defined by (7)) is called the *focal point* for the flow in D.

The focal points are equilibrium points of the PQ system (5) provided that they belong to their respective regular domain, i.e. $\Phi(D) \in D$. If this is the case, the focal points are referred to as *regular equilibria*. Different regulatory domains will usually have different focal points. In general, all solutions in a regulatory domain D flow towards the focal point $\Phi(D)$ until they either reach it or leave the domain D. At threshold domains ($x_i = \theta_i^{p_i}$ for some i) the step functions and vector fields are undefined. We need to precise our definition of solutions.

5.2 Solutions and Stability in Threshold Domains

To provide the existence and the possibility for solutions to be continued on all domains, we have to define the right-hand side of system (5) at the points of discontinuity of the function f. To this end, we use a construction originally proposed by Filippov [1988] and then applied to PL systems (Casey et al. [2006]). The method consists of extending the system (5) to a differential inclusion,

$$\dot{x} \in H(x), \tag{8}$$

where H is a set valued function defined as: $H(x) = \{f^D - d(x_n)x\}, \quad \forall x \in D$, if $D \in \mathscr{D}_r$. If $D \in \mathscr{D}_t$, we define H as

$$H(x) = \overline{co}(\{f^{D'} - d(x_n)x \mid D' \in R(D)\}), \quad \forall x \in D, \tag{9}$$

where $R(D) = \{D' \in \mathscr{D}_r \mid D \subseteq \partial D'\}$ is the set of all regulatory domains with D in their boundary, and $\overline{co}(X)$ is the closed convex hull of X. For threshold domains, $H(x)$ is typically multi-valued so solutions of the differential inclusion are defined as follows.

***Definition** 2.* A solution of (8) on $[0,T]$ in the sense of Filippov is an absolutely continuous function (w.r.t. t) $\xi_t(x_0)$ such that $\xi_0(x_0) = x_0$ and $\dot{\xi}_t \in H(\xi_t)$, for almost all $t \in [0,T]$.

Now, we shall show how to construct solutions at discontinuity points of f. Consider the case where x belongs to a threshold domain S separating two n-regular domains D_1 and D_2. Hence,

$$H(x) = \overline{co}(\{f^{D_1} - d(x_n)x, f^{D_2} - d(x_n)x\})$$

represents the segment joining the endpoints of the vectors $g_1 = f^{D_1} - d(x_n)x$ and $g_2 = f^{D_2} - d(x_n)x$. Trajectories can cross S if the vector fields g_1 and g_2 point in a similar direction, slide along S if g_1 and g_2 point in opposite directions towards S and be repelled from S if g_1 and g_2 point in opposite directions away from S. The last two cases are known as *stable* and *unstable sliding motion* in the literature (Filippov [1988]). Moreover, the velocity of the sliding motion (stable or unstable) on S is given by

$$\dot{x} = f^S - d(x_n)x. \tag{10}$$

Theorem 3. Assume that S is in the hyper-plane $C_i^j = \{x \in \mathbb{R}^n_{\geq 0} \ : \ x_i = \theta_i^j\}$ for some i, and a sliding motion (stable or unstable) occurs on S. The vector f^S in (10) is given by

$$f^S = \alpha f^{D_1} + (1-\alpha) f^{D_2},$$
$$\alpha = \begin{cases} \dfrac{f_i^{D_2} - (\gamma_i + \mu_n x_n)\theta_i^j}{f_i^{D_2} - f_i^{D_1}}, \text{ if } i \in \{1, ..., n-1\} \\ \dfrac{f_i^{D_2} - (\gamma_i + \mu_n \theta_i^j)\theta_i^j}{f_i^{D_2} - f_i^{D_1}}, \text{ if } i = n. \end{cases} \tag{11}$$

Proof. The segment joining the endpoints of the vectors $f^{D_1} - d(x_n)x$ and $f^{D_2} - d(x_n)x$ is expressed by

$$\alpha f^{D_1} + (1-\alpha) f^{D_2} - d(x_n)x, \quad 0 \leq \alpha \leq 1.$$

Since the trajectories during sliding motion are on the hyper-plane $x_i = \theta_i^j$, the parameter α in (11) is selected such that $\dot{x}_i = 0$.Thus, α can found from the conditions

$$\alpha f_i^{D_1} + (1-\alpha) f_i^{D_2} - (\gamma_i + \mu_n x_n)\theta_i^j = 0, \text{ if } i \in \{1, ..., n-1\}$$
$$\alpha f_i^{D_1} + (1-\alpha) f_i^{D_2} - (\gamma_i + \mu_n \theta_i^j)\theta_i^j = 0, \text{ if } i = n.$$

We notice that in the case $i = n$ the value of α is constant $\forall x \in S$ and thus a sliding motion occurs along the entire threshold domain S. By contrast, in the case $i \in \{1, ..., n-1\}$ the value of α depends on x_n, that means that a sliding motion occurs on S as long as the vector fields point in opposite direction towards (or away) S or, equivalently, as long as $0 < \alpha(x_n) < 1 \ \forall x_n \in S$. Specifically, it could happen that solutions slide for a while along S and then leave it as soon as the condition $0 < \alpha(x_n) < 1$ does not hold any more. It is useful to define a concept analogous to the focal points defined for regulatory domains, extended to deal with threshold domains.

***Definition** 3.* We recall that $supp(D)$ is the $(n-k)$-dimensional hyperplane supporting D. Let D be a threshold domain of order k, then its focal set is defined as $\Phi(D) = supp(D) \cap \{x : \ 0 \in H(x)\}$, where $H(x)$ is defined as in (9).

Hence, $\Phi(D)$ for $D \in \mathscr{D}_t$ is the set containing all the equilibrium points of the differential inclusion (8), which lie on $supp(D)$. $\Phi(D)$ can be a singleton, but more generally is a closed convex bounded set and hence is referred to as a focal set. To rule out some difficult cases when proving results on stability, we make a technical assumption on the focal sets for our system.

***Assumption** 1.* $\forall D \in \mathscr{D}, \Phi(D) \cap supp(D') = \{\}, \forall D' \in \partial(D)$.

It essentially says that for every regular or threshold domain D, the focal set $\Phi(D)$ does not intersect the supporting hyperplane of any domain D' in the boundary of D. It is possible that solutions of (5) reach equilibria that lie in threshold domains and such equilibria are called *singular equilibria*. In general, a singular equilibrium $\bar{x}$ of system (5) is a point that satisfies the condition $0 \in H(\bar{x})$ and that belongs to some threshold plane. Determining in the most general case whether a singular equilibrium is stable or unstable requires a detailed analysis that for the sake of space is not mentioned in this paper. However, in the following theorem we present a procedure to assess the stability of singular equilibria that can occur on x_n-hyperplane.

Theorem 4. Assume that a sliding motion occurs on a threshold domain S, in the hyper-plane $C_n^j = \{x \in \mathbb{R}^n_{\geq 0} \ : \ x_n = \theta_n^j\}$,

separating two n-domains D_1 and D_2. Let $\bar{x} = (\bar{x}_1, ..., \bar{x}_n)$ be the singular equilibrium point of the sliding motion. If $\bar{x} \in S$ and if the sliding motion is stable (resp. unstable), then $\bar{x}$ is locally stable (resp. unstable).

Proof. Assuming the presence of a such stable sliding motion in S and $\bar{x} \in S$, this implies that there exists a neighborhood of $\bar{x}$ where the n-component of trajectories are approaching $\bar{x}_n = \theta_n^j$. Notably, the velocity of motion of the other i-components ($i = 1, ..., n-1$) is given by

$$\dot{x}_i = \alpha f_i^{D_1} + (1-\alpha) f_i^{D_2} - (\mu_n \theta_n^j + \gamma_i) x_i \tag{12}$$

with α equal to the second value in (11). Hence, the stability of $\bar{x}$ follows by the fact that (12) is of the PL form. If the $\bar{x} \in S$, but the sliding motion is unstable, the instability of $\bar{x}$ follows from the instability of the sliding motion.

6. STABILITY ANALYSIS OF THE SPQ SYSTEM

Typically, Multiple Lyapunov Functions are used to prove Lyapunov stability for switched systems (Liberzon and Morse [1999], Branicky [1994]). However, many results using this approach are not directly applicable to systems with sliding motions and/or cases when the domains do not have a common focal point. Moreover, the structure of the SPQ system (3) is particular and the problem we consider quite specific, which allows us to take a different approach. More specifically, we can define two regions χ_I and $\chi_{II} \subset \mathbb{R}^n$, in which system (3) is active following the I-mode and the II-mode, respectively:

$$\begin{aligned} \chi_I &= \{[x_1, ..., x_n]^T \in \mathbb{R}^n : \mu_{n-1} x_{n-1} - \mu_n x_n < 0\} \\ \chi_{II} &= \{[x_1, ..., x_n]^T \in \mathbb{R}^n : \mu_{n-1} x_{n-1} - \mu_n x_n > 0\} . \end{aligned} \tag{13}$$

In addition, a switching surface between the I and II modes, i.e. a boundary between χ_I and χ_{II}, is given by:

$$S_{I,II} = \{[x_1, ..., x_n]^T \in \mathbb{R}^n : \mu_{n-1} x_{n-1} - \mu_n x_n = 0\} . \tag{14}$$

We will now state two definitions and an hypothesis useful to enunciate a theorem for the stability of system (3).

Definition 4. Let Ψ_m (resp. Λ_m) ($m = I, II.$) be the set containing all the locally stable (resp. unstable) points of the m-mode.

We recall that the procedures to determine Ψ_m and Λ_m, that is the stable and unstable points of the two modes, have been presented in Section 5.

Hypothesis 5. Assume that:

$$\Psi_m \cap S_{I,II} = \{\} \ (\forall m = I, II), \quad \Lambda_m \cap S_{I,II} = \{\} \ (\forall m = I, II).$$

Hypothesis 5 states that equilibria of the I and II modes do not lay on switching surface $S_{I,II}$ (very particular cases).

Theorem 6. Assume that hypothesis 5 holds, then the set of locally stable points of (3), i.e. Ψ, and set of unstable points of (3), i.e. Λ, are given by:

$$\Psi = (\Psi_I \cap \chi_I) \cup (\Psi_{II} \cap \chi_{II}) , \ \Lambda = (\Lambda_I \cap \chi_I) \cup (\Lambda_{II} \cap \chi_{II}) . \tag{15}$$

Proof. The proof follows by observing that a stable (resp. unstable) point of the m-mode, is also a stable (resp. unstable) point of the switched system (3) only if it is within the space region in which the m-mode is active, i.e. χ_m.

7. CASE STUDY: THE RNAP-RIBOSOMES SYSTEM

Here, we investigate a system to express bacterial growth rate in terms of the machinery of the cell, to improve on a previous model (Carta et al. [2012]). It is known that both RNAP and ribosomes affect growth rate, but a dynamical model describing this double dependence is not available. In fact, in most cases, growth rate is assumed to be constant (Marr [1991]) or dependent only on RNAP (Tan et al. [2009]) or on ribosomes (Shachrai et al. [2010]). We propose a bidimensional SPQ model describing the concentrations' dynamics of RNAP and ribosomes. Let x_p, x_r be the concentration of RNAP and ribosomes respectively, the SPQ model is given by:

$$\begin{aligned} \dot{x}_r &= k_r^1 s^+(x_p, \theta_p^1) + k_r^2 s^+(x_p, \theta_p^2) - (\min(\mu_p x_p, \mu_r x_r) + \gamma_r) x_r \\ \dot{x}_p &= k_p^0 s^+(x_p, \theta_p^1) s^+(x_r, \theta_r^1) + k_p^1 s^+(x_p, \theta_p^2) s^+(x_r, \theta_r^2) \\ &\quad - (\min(\mu_p x_p, \mu_r x_r) + \gamma_p) x_p \end{aligned} \tag{16}$$

We have considered that the synthesis of ribosomes is limited by the production rate of stable-RNAs and do not model the expression of ribosomal proteins (Marr [1991]). Stable RNA genes are essentially regulated by RNAP at the level of two promoters, i.e. P1 and P2, of the *rnn* operons. We assumed that a lower concentration of RNAP, i.e. θ_p^1, activates the housekeeping promoter P2 while a higher RNAP concentration, i.e. θ_p^2, is needed to stimulate the promoter P1, whose activity increases with growth rate. Regarding RNAP, it has been shown (Bremer et al. [2003]) that $\beta\beta'$ subunits limit RNAP production. Hence we assume that RNAP amount reflects that of $\beta\beta'$ subunits and we omit other subunits production and assembling. Notably, a lower concentration of RNAP (accounting for transcription), i.e. θ_p^1, and a lower concentration of ribosomes (according for translation), i.e. θ_r^1, are required for the basal synthesis (k_p^0) of RNAP whereas, for the main synthesis of RNAP ($k_p^0 + k_p^1$), higher concentrations of RNAP (θ_p^2) and ribosomes (θ_r^2) are needed. From the considerations above, it follows that: $0 \le \theta_r^1 \le \theta_r^2 \le max_r$ and $0 \le \theta_p^1 \le \theta_p^2 \le max_p$. Therefore, the state space of each of two modes of system (16) can be partitioned into nine *regular domains*:

$$\begin{aligned} D_1 &= \left\{x \in \mathbb{R}^2_{\ge 0} : 0 \le x_r < \theta_r^1,\ 0 \le x_p < \theta_p^1\right\} \\ D_2 &= \left\{x \in \mathbb{R}^2_{\ge 0} : \theta_r^1 < x_r < \theta_r^2,\ 0 \le x_p < \theta_p^1\right\} \\ D_3 &= \left\{x \in \mathbb{R}^2_{\ge 0} : \theta_r^2 < x_r \le max_r,\ 0 \le x_p < \theta_p^1\right\} \\ D_4 &= \left\{x \in \mathbb{R}^2_{\ge 0} : 0 \le x_r < \theta_r^1,\ \theta_p^1 < x_p < \theta_p^2\right\} \\ D_5 &= \left\{x \in \mathbb{R}^2_{\ge 0} : \theta_r^1 < x_r < \theta_r^2,\ \theta_p^1 < x_p < \theta_p^2\right\} \\ D_6 &= \left\{x \in \mathbb{R}^2_{\ge 0} : \theta_r^2 < x_r \le max_r, \theta_p^1 < x_p < \theta_p^2\right\} \\ D_7 &= \left\{x \in \mathbb{R}^2_{\ge 0} : 0 \le x_r < \theta_r^1,\ \theta_p^2 < x_p \le max_p\right\} \\ D_8 &= \left\{x \in \mathbb{R}^2_{\ge 0} : \theta_r^1 < x_r < \theta_r^2,\ \theta_p^2 < x_p \le max_p\right\} \\ D_9 &= \left\{x \in \mathbb{R}^2_{\ge 0} : \theta_r^2 < x_r \le max_r, \theta_p^2 < x_p \le max_p\right\} . \end{aligned}$$

The *threshold domains* are not listed here, but they are as defined in Section 5. Let mode I be active when $\min(\mu_p x_p, \mu_r x_r) = \mu_p x_p$ and mode II be active when $\min(\mu_p x_p, \mu_r x_r) = \mu_r x_r$ in (16). Hence, according to (7) we can calculate the focal points of modes I and II for each regular domains D_j ($j = 1, ..., 9$) as shown in Table 7. Then, regarding the I-mode, we set $\theta_p^1 < \bar{x}_{p,1}^I = \varphi(k_p^0, \mu_p, \gamma_p) < \theta_p^2$ and $\bar{x}_{r,1}^I = \eta(\bar{x}_{p,1}^I, k_r^1, \mu_r, \gamma_r) > \theta_r^1$ to allow RNAP to reach its basal concentration at which it can stimulate its own expression and the expression of ribosomes. Moreover, we also set $\bar{x}_{p,2}^I = \varphi(k_p^0 + k_p^1, \mu_p, \gamma_p) > \theta_p^2$ and $\bar{x}_{r,2}^I = \eta(\bar{x}_{p,2}^I, k_r^1 + k_r^2, \mu_r, \gamma_r) > \theta_r^1$ to allow RNAP to reach the level at which it can activate its main expression and the P1 promoter of ribosomes. With similar arguments, we can

D_j	I-mode	II-mode
D_1	$\bar{x}_p = \varphi(0,\mu_p,\gamma_p)$	$\bar{x}_r = \varphi(0,\mu_r,\gamma_r)$
D_2	$\bar{x}_r = \eta(\bar{x}_p,0,\mu_r,\gamma_r)$	$\bar{x}_p = \eta(\bar{x}_r,0,\mu_p,\gamma_p)$
D_3		
D_4	$\bar{x}_p = \varphi(0,\mu_p,\gamma_p)$ $\bar{x}_r = \eta(\bar{x}_p,k_r^1,\mu_p,\gamma_p)$	$\bar{x}_r = \varphi(k_r^1,\mu_r,\gamma_r)$ $\bar{x}_p = \eta(\bar{x}_r,0,\mu_p,\gamma_p)$
D_5	$\bar{x}_p = \varphi(k_p^0,\mu_p,\gamma_p)$	$\bar{x}_r = \varphi(k_r^1,\mu_r,\gamma_r)$
D_6	$\bar{x}_r = \eta(\bar{x}_p,k_r^1,\mu_r,\gamma_r)$	$\bar{x}_p = \eta(\bar{x}_r,k_p^0,\mu_p,\gamma_p)$
D_7	$\bar{x}_p = \varphi(0,\mu_p,\gamma_p)$ $\bar{x}_r = \eta(\bar{x}_p,k_r^1+k_r^2,\mu_r,\gamma_r)$	$\bar{x}_r = \varphi(k_r^1+k_r^2,\mu_r,\gamma_r)$ $\bar{x}_p = \eta(\bar{x}_r,0,\mu_p,\gamma_p)$
D_8	$\bar{x}_p = \varphi(k_p^0,\mu_p,\gamma_p)$ $\bar{x}_r = \eta(\bar{x}_p,k_r^1+k_r^2,\mu_r,\gamma_r)$	$\bar{x}_r = \varphi(k_r^1+k_r^2,\mu_r,\gamma_r)$ $\bar{x}_p = \eta(\bar{x}_r,k_p^0,\mu_p,\gamma_p)$
D_9	$\bar{x}_p = \varphi(k_p^0+k_p^1,\mu_p,\gamma_p)$ $\bar{x}_r = \eta(\bar{x}_p,k_r^1+k_r^2,\mu_r,\gamma_r)$	$\bar{x}_r = \varphi(k_r^1+k_r^2,\mu_r,\gamma_r)$ $\bar{x}_p = \eta(\bar{x}_r,k_p^0+k_p^1,\mu_p,\gamma_p)$

Table 1. Focal points of modes I and II for each regular domains D_j ($j = 1,\ldots,9$) of model (16).

set for the II-mode: $\bar{x}_{r,1}^{II} = \varphi(k_r^1,\mu_r,\gamma_r) > \theta_r^1$, $\theta_p^1 < \bar{x}_{p,1}^{II} = \eta(\bar{x}_{r,1}^{II},k_p^0,\mu_p,\gamma_p) < \theta_p^2$, $\bar{x}_{r,2}^{II} = \varphi(k_r^1+k_r^2,\mu_r,\gamma_r) > \theta_r^2$, $\bar{x}_{p,2}^{II} = \eta(\bar{x}_{r,2}^{II},k_p^0+k_p^1,\mu_p,\gamma_p) > \theta_p^2$. Considering these inequalities and applying Theorem 2, it turns out that both the I-mode and the II-mode have three locally stable points. Notably, $\Psi_I = \{(0,0), (\bar{x}_{r,1}^I,\bar{x}_{p,1}^I), (\bar{x}_{r,2}^I,\bar{x}_{p,2}^I)\}$ is the set containing the stable points of the I-mode while $\Psi_{II} = \{(0,0), (\bar{x}_{r,1}^{II},\bar{x}_{p,1}^{II}), (\bar{x}_{r,2}^{II},\bar{x}_{p,2}^{II})\}$ is the set containing the stable points of the II-mode.

In order to find out which of the stable points of the two modes are also stable points of the SPQ system (16) we need to consider their position with respect the switching surface $x_p = \frac{\mu_r}{\mu_p} x_r$ (see Theorem 6), except for the origin which will surely be a locally stable point of the SPQ system because it is a locally stable point of both modes. Notably, we can have the case when the set of locally stable points of the SPQ system coincides with the set of locally stable points of the I-mode, i.e. $\Psi = \Psi_I$ (Figure 1(a)) or the case when $\Psi = \Psi_{II}$ (not shown) or the case when Ψ shares one focal point with the I-mode and one with the II-mode (Figure 1(b)). Summarizing, we can have the case when a mode is only transiently active, in the sense that all stable equilibria lay in the active region of the other mode (Figure 1(a)) or the case when both modes contribute to the equilibria of the SPQ system (Figure 1(b)). Other possible scenarios are omitted for the sake of space. Moreover, we note that unstable sliding modes can occur on the threshold domains $x_p = \theta_p^1$ and $x_p = \theta_p^2$.

To conclude our study, we qualitatively analyze the temporal growth rate expression of the SPQ system (16), expressed in (2), for the scenario depicted in Figure 1(b) in two relevant cases, and we compare it with the growth rate resulting from the two PQ modes taken separately. Notably, in the first case we considered a point within the D_7 domain as initial condition, which corresponds to very high RNAP and very low ribosomes while, in the second case, we considered a point within the D_6 domain as initial condition, which corresponds to low RNAP and very high ribosomes. For the first case, depicted in Figure 2(a), we note that the growth rate of the I-mode (in blue), being simply proportional to RNAP, is decreasing in the first part of the picture, but this is not biologically realistic because the very high amount of RNAP at $t = 0$ should boot up the gene expression machinery leading to a gradual increasing of the growth rate, as it is effectively shown by the SPQ growth rate (in green), which in this case it is equal to the growth rate of the II-mode. A similar argument applies in Figure 2(b). Thus,

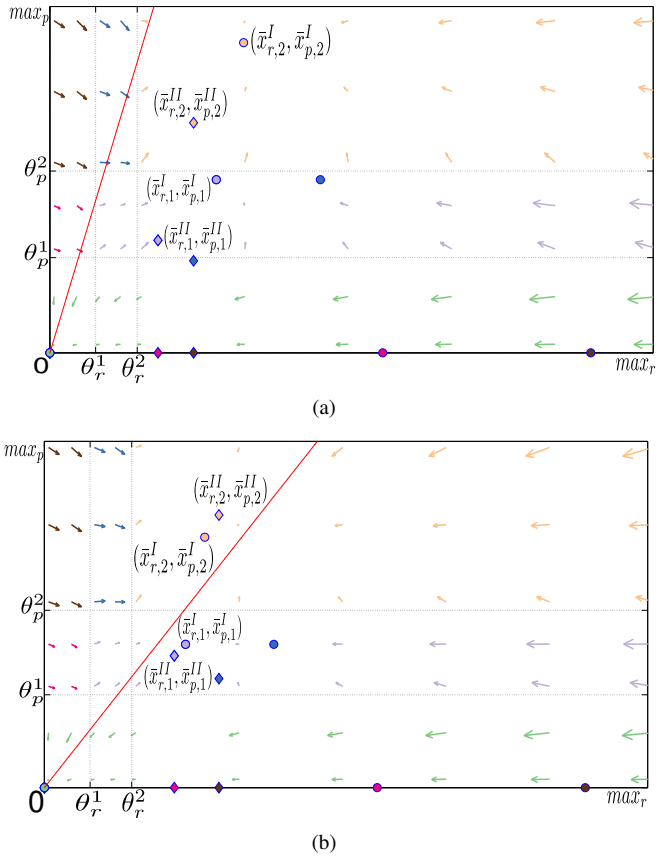


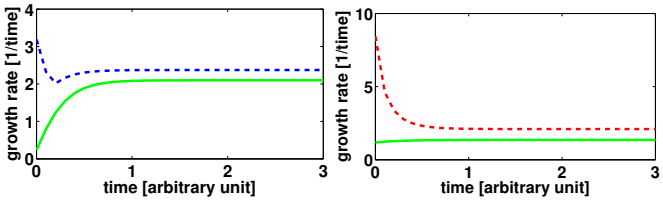
Fig. 1. Phase planes of the SPQ system (16) for two different scenarios. For each regular domain are drawn the focal points of the I-mode (circles) and the II-mode (diamonds). The color of the focal points is the same of the arrows of the domain whence they are originated. The red line represents the switching surface. The stable points of the two modes are marked with their coordinates. (a): the stable points of the SPQ system are $(0,0)$, $(\bar{x}_{r,1}^I,\bar{x}_{p,1}^I)$, $(\bar{x}_{r,2}^I,\bar{x}_{p,2}^I)$. (b): the stable points of the SPQ system are $(0,0)$, $(\bar{x}_{r,1}^I,\bar{x}_{p,1}^I)$, $(\bar{x}_{r,2}^{II},\bar{x}_{p,2}^{II})$.

Figure 2 shows that a switching model is better suited to model the bacterial growth rate than either of the two PQ modes taken separately.

Moreover, we note that the growth rate (Figure 2, in green) reaches a constant value after a while, because the SPQ system has achieved a locally stable equilibrium, notably $(\bar{x}_{r,2}^{II},\bar{x}_{p,2}^{II})$ for Figure 2(a), and $(\bar{x}_{r,1}^I,\bar{x}_{p,1}^I)$ for Figure 2(b). This constant growth rate is biological meaningful because it corresponds to the slope of the exponential phase of a bacterial growth curve (Monod [1949]).

8. CONCLUSION

In this paper we proposed a new mathematical formalism to model bacterial gene expression dependent on dilution due to growth rate. This novel modeling approach can be considered as an extension of the piecewise linear (PL) systems, which have been modified by introduction of an expression for the growth rate to model the dilution effect. This leads to a switched system with two piecewise quadratic (PQ) modes. We have first focused on the characterization of equilibria of the PQ subsys-



(a) (b)

Fig. 2. Growth rate curves of the SPQ system ($\min(\mu_p\ x_p(t), \mu_r\ x_r(t))$, in solid green), the PQ I-mode ($\mu_p\ x_p(t)$, in dashed blue) and the PQ II-mode ($\mu_r\ x_r(t)$, in dashed red) for the scenario in Figure 1(b), in two different cases: (a) initial condition within D_7; (b) initial condition within D_6.

tems, both for equilibria within regular domains and equilibria that lie on surfaces of discontinuity (threshold domains) due to the use of step functions (as in PL models). Then, we took into account the switching behavior of the SPQ system to formulate a criterium assessing the stability of its equilibria.

Notably, focusing on the global gene expression machinery, we identified two possible limiting factors of the growth rate: the RNAP, which accounts for transcription, and the ribosomes, which are responsible for translation. Hence, we modeled the growth rate as the minimum between RNAP and ribosomes. As a case study we proposed a bidimensional SPQ model whose variables are RNAP and ribosomes concentrations. The stability of such a system has been studied by means of phase-planes in three possible scenarios. Moreover, we performed a qualitative analysis of the temporal expression of the SPQ growth rate and showed that it is well suited to model biologically pertinent bacterial growth rate curves.

To conclude, we believe that the SPQ formalism can be a promising approach for qualitative modeling gene expression dynamics dependent on dilution and a valid starting point to address fine tuned growth rate control problems in synthetic biology (Carta et al. [2012]).

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