

Repressilators and Promotilators: Loop Dynamics in Synthetic Gene Networks

H. El Samad, D. Del Vecchio, and M. Khammash

Abstract—Oscillators and switches are ubiquitous in gene regulatory networks. Understanding the dynamical properties of these systems is a necessity not only to reverse engineer their structure, but also to forward engineer novel structures in the context of the nascent field of “Synthetic Biology”. In this work, we analyze two classes of cyclic gene networks. The first class corresponds to the “Repressilator”, which is topologically equivalent to a class of negative gain networks. The second class corresponds to the “promotilator” which is topologically equivalent to a class of positive gain networks. We demonstrate that the repressilator can exhibit oscillatory behavior under conditions that we derive. We also demonstrate that the promotilator and its equivalent circuits belong to the class of strongly monotone systems, and as such, the behavior they can exhibit is convergence to stable equilibria.

I. INTRODUCTION

The successful implementation of synthetic genetic circuits with designed functionalities is a promising approach that can expedite biological discovery. Building gene regulatory networks that mimic naturally occurring systems generates the possibility of probing in a controlled environment the interactions of the various components and assessing the capability of these components to reproduce observed phenotypic behaviors. Furthermore, programming novel functionalities into gene networks can prove valuable for a variety of applications, such as targeted drug administration. Similar to the engineering of man-made control systems, the success of such an endeavor critically hinges on the capability of carrying successful designs and predicting the full range of dynamical behavior expected from these designs. A particularly challenging task in synthetic biology has been the successful implementation of systems that can exhibit sustained oscillations. Oscillations in the concentration of some key proteins in the cell are often present to implement clocks that synchronize behavior in response to various growth and adaptation demands (such as day-night cycles). One of the first attempts to generate oscillatory behavior in synthetic gene networks was carried for the so called “Repressilator” [1]. The Repressilator is a bacterial

synthetic network consisting of three genes, *Tet*, *CI* and *lac*. The *LacI* protein represses the promoter of the *Tet* gene, the *TetR* protein represses the promoter of the *ci* gene, and the *CI* protein represses the promoter of the *lac* gene, therefore closing the loop. The repressilator, constructed using some numerical analysis of an ODE model describing the system, produces self sustained oscillations in the concentration of its three proteins. The repressilator is the first synthetic oscillator to be built in the biological substrate and is currently being used to understand the synchronization behavior of coupled biological oscillators [2]. It is therefore necessary to carry a detailed dynamical study of the system, explore the array of dynamical behaviors it can exhibit and specify the parameter regions where the different behaviors are present. This can be accomplished by transforming the repressilator into a standard form of loop oscillators then exploit various results concerning these oscillators to systematically for analysis [4]. We also study the symmetric counterpart of the repressilator, that we call the “promotilator”. Instead of repressing each other, the promotilator gene products enhance the production of each other, therefore generating a cooperative monotone system. We use known results in monotone systems to demonstrate that the promotilator and its equivalent loop circuits are practically switches.

II. THE CLASS OF SYSTEMS CONSIDERED

In this paper, we consider the class of cyclic gene models. We specialize in three genes forming the network, but the analysis can be readily generalized to any number of genes. The equations we consider are of the form

$$\begin{aligned} \dot{r}_1 &= g_1(p_3) - \alpha_1 r_1 \\ \dot{p}_1 &= a_1 r_1 - \gamma_1 p_1 \\ \dot{r}_i &= g_i(p_{i-1}) - \alpha_i r_i \\ \dot{p}_i &= a_i r_i - \gamma_i p_i \end{aligned} \quad (1)$$

for $i = 2, 3$ and where $r_i, p_i \in \mathbb{R}_+$, $\forall i, 1, 2, 3$ are the concentrations the of *ith mRNA* and its corresponding protein, respectively. g_i are nonlinear functions that are either monotonically decreasing, i.e. $g'_i < 0$, or monotonically increasing, i.e. $g'_i > 0$. When g_i are monotonically decreasing functions, we assume throughout that $g_i(0)$ assume constant values and that $g_i(\infty) = 0$. When g_i are monotonically increasing functions, we assume that $g_i(\infty)$ are bounded and that $g_i(0) = 0$. These conditions on g_i are certainly true for the Hill type functions that are commonly used to model the dynamics of feedback in the context of gene regulatory

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networks. When the nonlinear functions are monotonically decreasing, we will perform a change of variable that transforms the system in (1) into a normalized form that has the benefit of making the analysis more transparent. Define $\bar{r}_1 = \frac{r_1}{g_1(0)}, \bar{p}_1 = \frac{p_1}{g_1(0)a_1}, \bar{r}_2 = \frac{r_2}{g_2(0)}, \bar{p}_2 = \frac{p_2}{g_2(0)a_2}, \bar{r}_3 = \frac{r_3}{g_3(0)}, \bar{p}_3 = \frac{p_3}{g_3(0)a_3}$. The resulting system, with some abuse of notation (we remove the bars from the new variables) is

$$\begin{aligned} \dot{r}_1 &= f_1(p_3) - \alpha_1 r_1 \\ \dot{p}_1 &= r_1 - \gamma_1 p_1 \\ \dot{r}_i &= f_i(p_{i-1}) - \alpha_i r_i \\ \dot{p}_i &= r_i - \gamma_i p_i \end{aligned} \quad (2)$$

for $i = 2, 3$ and where $f_1(p_3) = \frac{g_1(g_3(0)a_3 p_3)}{g_1(0)}, f_2(p_1) = \frac{g_2(g_1(0)a_1 p_1)}{g_2(0)}, f_3(p_2) = \frac{g_3(g_2(0)a_2 p_2)}{g_3(0)}$. Therefore, $f_1(0) = f_2(0) = f_3(0) = 1$. When the functions are monotonically increasing, we perform the same change of coordinate as in the monotonically decreasing case, but replace $g_1(0), g_2(0)$, and $g_3(0)$ by $g_1(\infty), g_2(\infty)$, and $g_3(\infty)$ respectively. In either case, the normalized functions f_i will be bounded by 1.

III. THE PROMOTILATOR AND THE REPRESSILATOR

Two dynamically distinct sets of systems arise when the variables in the system compete with each other, i.e. $f'_i < 0$ for all i (repressilator) or cooperate with each other, i.e. $f'_i > 0$ for all i (promotilator). Before we get into the details of this distinction, we introduce the concept of the loop gain.

Definition 1: The loop gain LG of the circuits in Figure 1 and described by equations (2) is given by

$$LG := \text{sign}(f'_1(p_3)f'_2(p_1)f'_3(p_2)).$$

Claim 2: The systems with positive loop gain are equivalent to the promotilator and the systems with negative loop gain are equivalent to the repressilator.

Proof: let $\tilde{r}_i = \frac{1}{\alpha_i} - r_i, \tilde{p}_i = \frac{1}{\alpha_i \gamma_i} - r_i, \tilde{r}_j = r_j, \tilde{p}_j = p_j, \tilde{r}_k = r_k, \tilde{p}_k = p_k$. When $i = 1, j = 2, k = 3$ we get the transformations corresponding to the row relations of Figure 1. When $i = 2, j = 1, k = 3$, we get the transformations corresponding to the column relations, while when $i = 3, j = 1, k = 2$, we get the transformations corresponding to the diagonal relations. ■

Let $\mathcal{B}(\eta, \theta, \vartheta)$, $\eta, \theta, \vartheta \geq 1$ be defined by $\mathcal{B}(\eta, \theta, \vartheta) = [0, \frac{\eta}{\alpha_1}] \times [0, \frac{\eta}{\alpha_1 \gamma_1}] \times [0, \frac{\theta}{\alpha_2}] \times [0, \frac{\theta}{\alpha_2 \gamma_2}] \times [0, \frac{\vartheta}{\alpha_3}] \times [0, \frac{\vartheta}{\alpha_3 \gamma_3}]$

Lemma 3: Let $X_0 = X(0) = \{r_i(0), p_i(0)\}$, $i = 1, 2, 3$ be any vector of initial conditions taking values in $\mathcal{B}(\eta, \theta, \vartheta)$. Then the trajectory of (2) starting from $X(0)$ (denoted here by $X(t, X_0)$) for the repressilator and promotilator, belongs to $\mathcal{B}(\eta, \theta, \vartheta)$ for $t \geq 0$, i.e. $\mathcal{B}(\eta, \theta, \vartheta)$ is a positively invariant set for (2). Furthermore, $\mathcal{B}(1, 1, 1)$ contains an attractor for (2) which is compact and connected.

Proof: We prove the claim for the repressilator. The case of the promotilator proceeds in a similar fashion. For the proof of the invariance of $\mathcal{B}(\eta, \theta, \vartheta)$, we only need to check the direction of the vector field on the boundaries of $\mathcal{B}(\eta, \theta, \vartheta)$. When $r_1(0) = 0, p_1(0) = 0$, $\dot{r}_1 = f_1(p_3) > 0, \dot{p}_1 = 0$, similarly, when $r_2(0) = 0, p_2(0) = 0$ or $r_3(0) =$

$0, p_3(0) = 0$, then $\dot{p}_2 = f_2(p_1) > 0$ and $\dot{r}_3 = f_3(p_2) > 0$, respectively. Therefore, the vector field is pointing inward in the set, and the boundaries at zero are reflecting, in the sense that none of the trajectories can escape to negative values. When $r_1(0) = \frac{\eta}{\alpha_1}, p_1(0) = \frac{\eta}{\alpha_1 \gamma_1}$, then $\dot{r}_1 = f_1(p_3) - \eta < 0$ since $f_1(p_3) < 1$ and $\eta \geq 1$. Also, $\dot{p}_1 = \frac{\eta}{\alpha_1} - \frac{\eta \gamma_1}{\alpha_1 \gamma_1} = 0$. Similar relations can be easily verified for the other variables. Hence, the vector field is again pointing inward in the set $\mathcal{B}(\eta, \theta, \vartheta)$ and cannot escape to larger values. The proof of the second part relies on the observation that $\mathcal{B}(1, 1, 1)$ attracts all trajectories $X(t, X_0)$, $X_0 \in \mathbb{R}_+^6$. This could be easily checked by the use of differential inequalities. For example, we know that if $\dot{r}_1(t) \leq 1 - \alpha_1 r_1(t)$, then for $t \geq 0$, $r_1(t) \leq R_1(t)$ where $R_1(t) = \frac{1}{\alpha_1} + (R_1(0) - \frac{1}{\alpha_1})e^{-\alpha_1 t}$ [5]. The same argument can be repeated for the other variables. Therefore, $\mathcal{B}(1, 1, 1)$ is globally attracting, and all solutions eventually enter it and stay there as it is also invariant. At this point, we can use a known theorem [theorem 8.1, chap. 1, [6]], which states that if a trajectory is bounded, then its limit set is nonempty, compact, and connected, therefore completing our proof. ■

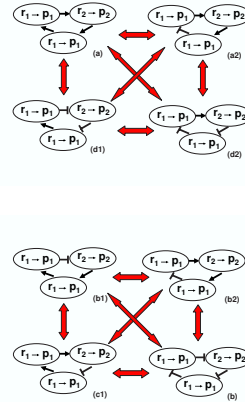


Fig. 1. The promotilator (top) and repressilator (bottom) and their equivalent circuit topologies.

We now invoke a theorem by Mallet-Paret and Smith [3] in order to limit the omega-limit set of any trajectory of (2) to either a single equilibrium, a single nonconstant periodic solution, or a structure consisting of a set of equilibria connected together through homoclinic and heteroclinic orbits. In fact, the main result in [3] implies that the omega-limit set of any bounded orbit of (2) can be embedded in \mathbb{R}^2 and therefore must be of the type encountered in two dimensional systems. Therefore, bifurcations with null spaces of dimensions of at most two are the only type of bifurcations possible. In a general sense, chaos can be ruled out from the dynamics of this class of systems. We therefore proceed to determining the relations that dictate the system's equilibria. The steady-state solution of (2) is given by

$$f_1(p_3) = \alpha_1 \gamma_1 p_1; f_2(p_1) = \alpha_2 \gamma_2 p_2; f_3(p_2) = \alpha_3 \gamma_3 p_3; \quad (3)$$

let $g : [0, \infty) \rightarrow [0, \infty)$ be a map given by the following composition of the f_i

$$g(u) = \left(\frac{1}{\alpha_3 \gamma_3} f_3\right) \circ \left(\frac{1}{\alpha_2 \gamma_2} f_2\right) \circ \left(\frac{1}{\alpha_1 \gamma_1} f_1\right)(u). \quad (4)$$

The fixed points of (2) are the intersection of this map with the diagonal, i.e. u^* such that $g(u^*) = u^*$. If u^* is one such equilibrium point, it will translate to $X^* = \{r_i^*, p_i^*\}, i = 1, 2, 3$ in terms of the state variables

$$X^* = \left(\frac{f_1(u^*)}{\alpha_1}, \frac{f_1(u^*)}{\alpha_1 \gamma_1}, \frac{1}{\alpha_2} f_2\left(\frac{f_1(u^*)}{\alpha_1 \gamma_1}\right), \frac{1}{\alpha_2 \gamma_2} f_2\left(\frac{f_1(u^*)}{\alpha_1 \gamma_1}\right), \gamma_3 u^*, u^*\right).$$

The Jacobian D of the system (2) is given as

$$D = \begin{bmatrix} -\alpha_1 & 0 & 0 & 0 & 0 & f_1'(p_3^*) \\ 1 & -\gamma_1 & 0 & 0 & 0 & 0 \\ 0 & f_2'(p_1^*) & -\alpha_2 & 0 & 0 & 0 \\ 0 & 0 & 1 & -\gamma_2 & 0 & 0 \\ 0 & 0 & 0 & f_2'(p_2^*) & -\alpha_3 & 0 \\ 0 & 0 & 0 & 0 & 1 & -\gamma_3 \end{bmatrix} \quad (5)$$

and the characteristic polynomial is given by

$$P(\lambda) = \prod_{i=1}^3 (\lambda + \alpha_i)(\lambda + \gamma_i) - f_1'(p_3^*) f_2'(p_1^*) f_2'(p_2^*).$$

We need to specialize our study to the particular steady state and stability properties of the two classes of systems represented by the repressilator and the promotilator.

IV. THE REPRESSILATOR

Consider the system corresponding to (2), with f_1, f_2, f_3 being monotonically decreasing, i.e. the repressilator case. The repressilator has one equilibrium point in \mathbb{R}_+^6 . We have already established that the equilibria of (2) are the intersection of g in (4) with the diagonal. Notice that $g(0) > 0$ and $g'(u) < 0, \forall u > 0$ since it is the composition of an odd number of monotonically decreasing functions. Therefore, g is a monotonically decreasing function itself, with a positive value at 0, and hence intersects the diagonal only in one point u^* . We now state a theorem by Hastings and co-workers in [4] which relates the instability of this equilibrium point to the existence of limit cycles.

Theorem 4: ([4]) Consider a system of differential equations of the form

$$\begin{aligned} \dot{x}_1 &= f_1(x_n, x_1) \\ \dot{x}_j &= f_j(x_{j-1}, x_j), \quad 2 \leq j \leq n \end{aligned} \quad (6)$$

Let P be the interior of a closed set \bar{P} in \mathbb{R}_+^n . Assume the following to hold in P

$$\begin{aligned} \frac{\partial f_i}{\partial x_i} &< 0, \quad \frac{\partial f_i}{\partial x_{i-1}} > 0 \quad 2 \leq i \leq n \\ \frac{\partial f_1}{\partial x_n} &< 0 \\ f_i(0, 0) &\geq 0, \quad f_i(x_n, 0) > 0 \quad \forall x_n \geq 0 \\ \frac{\partial f_1}{\partial x_1} &\text{ is bounded above in } \bar{P} \end{aligned}$$

and the system has a unique steady state $x^* = (x_1^*, x_2^*, \dots, x_n^*) \in P$ such that $f_1(x_n, x_1) < 0$ if $x_n > x_n^*$ and $x_1 > x_1^*$, while $f_1(x_n, x_1) > 0$ if $x_n < x_n^*$ and $x_1 < x_1^*$. Under these conditions, let $D = f_x(x^*)$ be the Jacobian matrix of f at x^* such that D has no repeated eigenvalues. Then, if D has any eigenvalues with positive real parts, (6) has a nonconstant periodic solution in P .

The proof of this theorem is elaborate and relies heavily on the Brouwer fixed point theorem. The details can be found in [4]. Theorem 5 follows.

Theorem 5: Let D be the Jacobian matrix of (2) evaluated at X^* . Suppose D has no repeated eigenvalues. If D has any eigenvalues with positive real parts, then (b) has a nonconstant periodic solution in $\mathcal{B}(1, 1, 1)$.

Proof: First notice that the system (b2) in Figure (1) exactly satisfies the conditions on the vector field given in Theorem 4. Also recall that we know from Fact 2 that the repressilator can be transformed into system (b2) through a change of variables. Furthermore, Lemma 3 guarantees the remaining conditions of the theorem, and hence the conclusion follows. ■

Before considering more specific analysis of the repressilator, we investigate the dynamical properties of the promotilator.

V. THE PROMOTILATOR

Our treatment of the promotilator and its equivalent circuits will be in the context of monotone systems. Monotone systems theory has been previously put to use for the study of biological systems in [13]. We provide a short list of the definitions and basic concepts that will be used in this sequel.

A. Monotone Systems and Monotonicity of Promotilator

Definition 6: An ordered Euclidean space is a Euclidean space \mathbb{R}^n , together with an order \succeq induced by a positivity cone K . A positivity cone $K \subseteq \mathbb{R}^n$ is a nonempty, closed, convex, pointed (in the sense that $K \cap (-K) = \{0\}$) and solid cone, such that $x_1 \succeq x_2$ means that $x_1 - x_2 \in K$.

Definition 7: Given a cone K , in addition to the ordering notion above, two notions of strict ordering are possible. By saying $x_1 \succ x_2$, one means that $x_1 \succeq x_2$ and $x_1 \neq x_2$. By saying $x_1 \gg x_2$ one means that $x_1 - x_2 \in \text{int}(K)$.

For example, we are mainly interested in the ‘‘NorthEast’’ orthant where $x_1 \succeq x_2$ means that each coordinate of x_1 is larger or equal than the corresponding coordinate of x_2 , $x_1 \gg x_2$ means that each coordinate of x_1 is strictly larger than the corresponding coordinate of x_2 , and $x_1 \succ x_2$ mean that some coordinate is strictly larger. We now define the notion of a monotone system. Let $\dot{x} = h(x)$ be a finite dimensional continuous time system specified by a state-space $X \subseteq \mathbb{R}_+^n$, satisfying the usual differentiability and Lipschitz conditions that insure the existence and uniqueness of solutions. Denote by $x(t, \xi)$ the solution of $\dot{x} = h(x)$ with initial condition $x(0) = \xi$.

Definition 8: Monotone systems The system $\dot{x} = h(x)$ is said to be monotone with respect to the order induced from K if $\xi_1 \succeq \xi_2 \Rightarrow x(t, \xi_1) \succeq x(t, \xi_2), \forall t \geq 0$ and strongly monotone if it is monotone and $\xi_1 \succ \xi_2 \Rightarrow x(t, \xi_1) \gg x(t, \xi_2), \forall t \geq 0$.

Definition 9: In an n -dimensional systems, the incidence graph [7] is a graph with n -nodes (vertices), where the nodes are labelled for example by the name of the variables, say $x_i, i = 1, \dots, n$. A labelled edge (an arrow with + or - sign attached to it) is drawn whenever a variable x_j affects directly the rate of change of variable $x_i, j \neq i$ ($\partial h_i(x)/\partial x_j \neq 0$). The edge is directed from x_j to x_i , and bears a positive sign if the effect of x_j on x_i is positive ($\partial h_i(x)/\partial x_j > 0$) and a negative sign if $\partial h_i(x)/\partial x_j < 0$. A path between two vertexes is a series of edges that connects the vertexes. A cycle is a closed graph. A simple cycle is a cycle consisting of distinct edges and vertexes. The sign of a path or cycle is the product of the signs of the edges comprising it.

Graphical test for monotonicity (Theorem 1, [7]) A system $\dot{x} = h(x)$ induces an order preserving flow with respect to an orthant if and only if there are no negative simple cycles in its incidence graph. If in addition the Jacobian matrix $[\partial h_i/\partial x_j]$ corresponding to the system is irreducible $\forall x \in X$, then the system is strongly monotone.

Claim 10: The promotilator is an irreducible (strongly) monotone system.

Proof: The incidence graph of the promotilator corresponds exactly to the diagram given in Figure 1(a), with the arrows and bars replaced by positive and negative signs. In this incidence graph, there is only one positive cycle. Therefore, the promotilator is a monotone system with respect to the order induced by $K = \mathbb{R}_+^n$. It is then easy to check that the Jacobian matrix in (5) is irreducible. ■

B. The equilibria of the Promotilator and their stability

The equilibria of the promotilator are given by the intersection of a monotonically increasing function g in (4) with the diagonal. So, in principle, this intersection could occur in 0, 1, 2, or 3 points. However, if we impose the physically motivated condition that f_1, f_2 , and f_3 have bounded derivatives, then we can guarantee that there is at least one equilibrium. Furthermore, if we impose the nondegeneracy condition that $g'(u) \neq 1$ when $g(u) = u$, we further guarantee that the number of steady states is odd. To study the stability of these equilibria, we state a useful theorem due to Berman and Plemmons [8].

Theorem 11: Let $A = \{a_{ij}\}$ be an $n \times n$ matrix having nonnegative off-diagonal elements. Then the following two conditions are equivalent

- 1) $\max \operatorname{Re}(\lambda) < 0$, λ is an eigenvalue running over the spectrum of A .

2)

$$(-1)^k \det \begin{pmatrix} a_{11} & a_{12} & \cdot & \cdot & a_{1k} \\ a_{21} & a_{22} & a_{23} & \cdot & a_{2k} \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot \\ a_{k1} & \cdot & \cdot & \cdot & |a_{k,k-1}|a_{kk} \end{pmatrix} > 0.$$

The sup-diagonal elements of the jacobian of the promotilator are zero, and the sub-diagonal are either 1 or $f'(i)$, which are obviously nonnegative since the functions are monotonically increasing. Therefore, investigating the stability of an equilibrium would simply boil down to checking that the jacobian evaluated at the equilibrium satisfies condition (2) in the theorem above. This boils down to checking that the principal minors of the matrix alternate in sign, which is certainly true, except for the largest minor (the determinant), whose positivity needs to be enforced. For the jacobian in (5), this condition would correspond to having

$$\frac{f'_1(p_3^*)}{\alpha_1 \gamma_1} \frac{f'_2(p_1^*)}{\alpha_2 \gamma_2} \frac{f'_3(p_2^*)}{\alpha_3 \gamma_3} < 1.$$

This is simply the condition that $g'(p_3^*) < 1$ which is obviously only satisfied at the odd points. Therefore, odd numbered equilibria are asymptotically stable, while even numbered equilibria are unstable. We can now invoke a theorem to rule out the existence of periodic orbits for the promotilator [10].

Theorem 12: Let $x(t)$ be the flow of an irreducible monotone system with the property that all forward orbits have compact closures. If the set of steady states of the system is discrete, then the set of points ξ for which $x(t, \xi)$ does not converge to a steady state has Lebesgue measure zero. The application of this theorem to the promotilator is immediate in light of the previous section, in addition to Lemma 3. The results for the promotilator have been established for more general strongly monotone irreducible systems in [9].

VI. OSCILLATIONS IN REPRESSILATOR

Using the machinery detailed above, a two-gene circuit can be shown to exhibit oscillatory behavior for certain parameter ranges. We derive conditions on these parameters, and demonstrate that they fall into regions that not likely to be encountered in realistic biological elements. We illustrate how this can be remedied by adding a third gene to the system, therefore obtaining the repressilator.

A. A two-element circuit

The equations describing the circuit are given by

$$\begin{aligned} \dot{r}_1 &= -\delta_1 r_1 + f_1(p_2) \\ \dot{p}_1 &= r_1 - \delta_2 p_1 \\ \dot{r}_2 &= -\delta_1 r_2 + f_2(p_1) \\ \dot{p}_2 &= r_2 - \delta_2 p_2, \end{aligned} \tag{7}$$

where $f_1(p_2) = \frac{\alpha_2^2}{1+p_2^n}$ and $f_2(p_1) = \frac{\alpha_1^2 p_1^n}{1+p_1^n}$ are commonly referred to as Hill functions, with n being the Hill coefficient. The protein concentrations have been normalized with respect to the affinity (equal for p_1 and p_2) and the time has been rescaled assuming equal translation constants.

Proposition 13: The linearization of the system in equations (7) does not have positive eigenvalues if $n \leq 2$.

Proof: The characteristic polynomial is given by

$$p(\lambda) = f_1'(p_2)f_2'(p_1) - (\delta_1 + \lambda)^2(\delta_2 + \lambda)^2,$$

where it is important to notice that $f_1'(p_2)f_2'(p_1) < 0$. Thus the eigenvalues of the system are given by

$$\lambda_{1,2,3,4} = \frac{1}{2} \left(-\delta_1 - \delta_2 \pm \sqrt{(\delta_1 - \delta_2)^2 \pm 4i \sqrt{|f_1'(p_2)| |f_2'(p_1)|}} \right).$$

Thus, the necessary condition for oscillations is that at least one eigenvalue has positive real part, that is

$$-\delta_1 - \delta_2 + \frac{\sqrt{2}}{2} \left(\sqrt{\sqrt{(\delta_1 - \delta_2)^4 + 16|f_1'(p_2)| |f_2'(p_1)|} + (\delta_1 - \delta_2)^2} \right) > 0,$$

which can be reduced after some manipulations to $16|f_1'(p_2)| |f_2'(p_1)| > 4(\delta_1 + \delta_2)^4 - 4(\delta_1^2 - \delta_2^2)^2$, that is

$$16\alpha_1^2 \alpha_2^2 \frac{n^2 p_1^{n-1} p_2^{n-1}}{(1+p_1^n)^2 (1+p_2^n)^2} > 4(\delta_1 + \delta_2)^4 - 4(\delta_1^2 - \delta_2^2)^2.$$

From the equilibrium relations $f_1(p_2) = \delta_1 \delta_2 p_1$ and $f_2(p_1) = \delta_1 \delta_2 p_2$ we deduce that

$$\alpha_1^2 \alpha_2^2 = \frac{(\delta_1 \delta_2)^2 p_1 p_2 (1+p_1^n)(1+p_2^n)}{p_1^n}.$$

If $n \geq 1$, then $\frac{p_2^n p_1}{(1+p_1^n)(1+p_2^n)} < 1$. Therefore, a necessary condition is $16n^2(\delta_1 \delta_2)^2 > 4(\delta_1 + \delta_2)^4 - 4(\delta_1^2 - \delta_2^2)^2$ that in turn implies $n > 2$, considering in the right hand side that $(a^2 + b^2) > 2ab$. ■

In the next section, we show that adding an element to the loop, the value of n needed for oscillations decreases.

B. Three-element circuit: Fully Symmetric case

For the analysis of a three element circuit, we consider the system of the form

$$\begin{aligned} \dot{r}_1 &= -\delta r_1 + f_1(p_3) \\ \dot{p}_1 &= r_1 - \delta p_1 \\ \dot{r}_i &= -\delta r_i + f_i(p_{i-1}) \\ \dot{p}_i &= r_i - \delta p_i, \end{aligned} \quad (8)$$

for $i = 1, 2$ and where for the symmetric case we have $f_1(p) = f_2(p) = f_3(p) = \frac{\alpha^2}{1+p^n}$.

Proposition 14: The symmetric repressilator has a periodic solution for the ratio α/δ satisfying the relation

$$\alpha^2/\delta^2 > \sqrt[n]{\frac{4/3}{n-3/4} \left(1 + \frac{4/3}{n-4/3}\right)}.$$

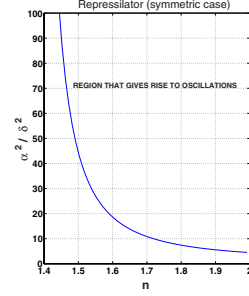


Fig. 2. Space of parameters that give rise to oscillations for the repressilator.

Proof: First notice that in this case, the equilibrium is given by $p_1 = p_2 = p_3 := u$, where u is given by the relation $u = \frac{\alpha^2/\delta^2}{1+u^n}$. The Jacobian of the system has a positive eigenvalue if and only if $-2\delta + \sqrt{3}|f'(u)| > 0$. But $|f'(u)| = \alpha^2 \frac{nu^{n-1}}{(1+u^n)^2}$. Therefore, the positivity condition of the eigenvalue becomes the condition that

$$\frac{u^n}{1+u^n} > \frac{4}{3n}. \quad (9)$$

Notice that $\frac{u^n}{1+u^n}$ is monotonically increasing function bounded by 1 from above. It is also an increasing function of the ratio α/δ . Therefore, (9) is satisfied if and only if $\frac{4}{3n} \doteq \varepsilon < 1$ and $\frac{\alpha}{\delta} > \frac{\alpha_0}{\delta_0}$ where u_0 is generated using $\frac{\alpha_0}{\delta_0}$ such that $\frac{u_0^n}{1+u_0^n} = \varepsilon$. This last equation implies that $u_0 = \sqrt[n]{\frac{\varepsilon}{1-\varepsilon}}$.

Therefore, $\frac{\alpha_0^2}{\delta_0^2} = \sqrt[n]{\frac{\varepsilon}{1-\varepsilon}} \left(1 + \frac{\varepsilon}{1-\varepsilon}\right)$ yielding the result. ■

This relationship is plotted in Figure 2. Notice that when n decreases, the existence of an unstable equilibrium necessitates α/δ to increase. Physically, this corresponds to a large number of protein molecules. Although a sufficient condition for the existence of oscillations has been established to be for a value of cooperativity $n > 4/3$, a necessary condition for these oscillations was established by Banks and Mahaffy [11] to be $n > 1$. Indeed, for $n = 1$ they have established (for general values of the parameters) that the unique equilibrium point is globally asymptotically stable.

C. The Non-Symmetric Case

The parameter space that gives rise to oscillations for the non-symmetric repressilator can also be computed. For the case where the genes are not identical, we consider a nonsymmetric case where, for example,

$$f_1(p) = \frac{\alpha_3^2}{1+p^n}, f_2(p) = \frac{\alpha^2}{1+p^n}, f_3(p) = \frac{\alpha^2}{1+p^n}. \quad (10)$$

The condition for oscillations becomes

$$(0.86)^2 n^3 \sqrt{\frac{p_3^n}{(1+p_3^n)(1+p_2^n)(1+p_1^n)}} > 1. \quad (11)$$

We rewrite p_1 and p_3 as functions of p_2 by using two of the equilibrium relations $p_1 = \sqrt[n]{\frac{p_2}{\alpha^2/\delta^2 - p_2}}$, $p_3 = \frac{\alpha^2/\delta^2 p_2^n}{1+p_2^n}$.

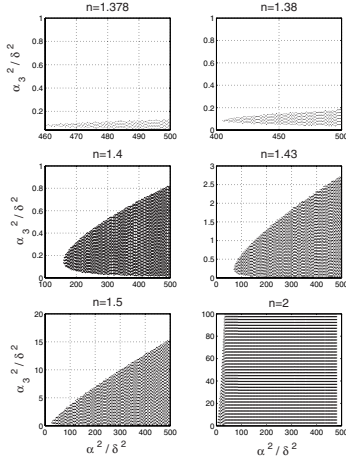


Fig. 3. Space of parameters that give rise to oscillations for the repressilator (non-symmetric case).

Using these expressions in (11), we can find all possible values of p_2 that satisfy (11) for a fixed pair $(\alpha^2/\delta^2, n)$. These values of p_2 correspond to the possible values of α_3^2/δ^2 by means of the third equilibrium condition $\alpha_3^2/\delta^2 = p_1(1 + p_3^2)$. For each pair $(\alpha^2/\delta^2, n)$, we finally obtain all possible values of α_3^2/δ^2 that satisfy the equilibrium conditions and inequality (11). These values are reported in Figure 3. Our results, summarized in Figures 3 and 2, give a clear insight into the design criteria that generate oscillations. In addition, these plots indicate the robustness margins of these oscillations with respect to parameter variations. In naturally occurring oscillators, such as the circadian rhythm, perhaps a more plausible type of robustness that needs to be investigated is related to the robustness of the period with respect to parameter variations. This investigation is in general quite difficult. However, for simple loop oscillators, the computation of the period as a function of the circuit parameters can in principle be carried out using harmonic balancing techniques as pointed out in [12]. Approximate computations along these lines, by using only one harmonic of the oscillations spectrum indicated that the period T is such that $T \propto \frac{1}{\delta}$, and varied only mildly with α . We report such results for exact numerical period computations in Figure 4. Notice that increasing values for δ decrease the sensitivity of the period to the variation of δ itself. However, increasing δ necessitates the increase of the cooperativity n , therefore indicating a possible tradeoff that must be taken into account in the design process. Also note that the value of α has no considerable effect on the period. Instead, it strongly affects the oscillation amplitude.

VII. CONCLUSIONS

The analysis carried out in this paper is aimed to demonstrate that careful dynamical analysis must accompany the implementation of genetic circuits designed to exhibit robustly some desired behavior. We have focused on the dynamical properties of two classes of gene regulatory

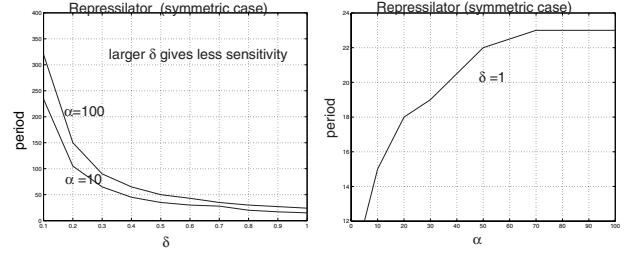


Fig. 4. Repressilator-symmetric case: Period as a function of δ and α .

networks. Using standard results from dynamical systems theory, we gave conditions for the repressilator type of circuits to exhibit oscillations, then used the conditions to find the parameter space for oscillations. For the promoter circuits, we showed that oscillations cannot be possible as such a class of systems belongs to a monotone class of dynamical systems for which it is known that oscillations cannot occur. In addition to phase oscillators, such as the repressilator, we plan perform a similar kind of analysis for the relaxation type of oscillators. We expect that such a dynamical analysis will be useful to investigate the mechanisms at the basis of phase and period adaptation in naturally occurring oscillators.

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REFERENCES

- [1] M. Elowitz and S. Leibler A synthetic oscillatory network of transcriptional regulators *Nature* 403 (6767), pp. 335-338, 2000.
- [2] J. Garcia Ojalvo, M. B. Elowitz, and S. H. Strogatz, Modeling a synthetic multicellular clock: Repressilators coupled by quorum sensing, *PNAS*, Vol. 101, pp. 10955-10960, 2004.
- [3] J. Mallet-Paret and H.L. Smith, The Poincare-Bendixon Theorem for Monotone Cyclic Feedback Systems, *J. Dyn. Diff. Equ.*, Vol. 2, No. 4, pp. 367-421, 1990.
- [4] S. Hastings, J. Tyson, D. Webster Existence of Periodic Solutions for Negative Feedback Cellular Systems, *Journal of Differential Equations*, Vol. 25, pp. 39-64, 1977.
- [5] W.A. Coppel, *Stability and Asymptotic Behavior of Differential Equations* D.C. Heath and Co., 1965.
- [6] J.K. Hale, *Ordinary Differential Equations* Wiley-Interscience, 1980.
- [7] H. Kunze and D. Siegel A graph theoretical Approach To Monotonicity with Respect to Initial Conditions, in *Comparison Methods and Stability Theory*, eds. X. Liu and D. Siegel, Dekker, New York, 1994.
- [8] A. Berman and R.J. Plemmons, *Nonnegative Matrices in the Mathematical Sciences*, Academic Press, New York, 1979.
- [9] H.L. Smith, *Monotone Dynamical System: An introduction to the Theory of Competitive and Cooperative Systems*, American Mathematical Society, Providence, Rhode Island, 1995.
- [10] M. Hirsh, Stability and Convergence in Strongly Monotone Dynamical Systems, *J. Reine Angew.*, Vol. 383, pp. 1-53, 1988.
- [11] H.T. Banks and J.M. Mahaffy, Stability of Cyclic Gene Models for Systems Involving Repression, *J. Theor. Biol.*, Vol. 74, pp. 323-334, 1978.
- [12] P. Rapp, Analysis of Biochemical Phase Shift Oscillators by Harmonic Balancing Technique, *J. of Mathematical Biology*, Vol. 3, pp. 203-224, 1976.
- [13] D. Angeli and E. Sontag, An Analysis Of a Circadian Model Using the Small-Gain Approach To Monotone Systems, *Proc. IEEE CDC*, pp. 575-578, 2004.