Condition for Sustained Oscillations in Repressilator Based on a Hybrid Modeling of Gene Regulatory Networks

Honglu Sun\textsuperscript{1,}\textsuperscript{a}, Jean-Paul Comet\textsuperscript{2,}\textsuperscript{b}, Maxime Folschette\textsuperscript{3,}\textsuperscript{c} and Morgan Magnin\textsuperscript{1,}\textsuperscript{d}

\textsuperscript{1}Nantes Université, École Centrale Nantes, CNRS, LS2N, UMR 6004, F-44000 Nantes, France
\textsuperscript{2}University Côte d’Azur, I3S Laboratory, UMR CNRS 7271, CS 40121, 06903 Sophia Antipolis Cedex, France
\textsuperscript{3}Univ. Lille, CNRS, Centrale Lille, UMR 9189 CRISIAL, F-59000 Lille, France

Keywords: Hybrid Modeling, Repressilator, Sustained Oscillation, Gene Regulatory Network, Synthetic Biology.

Abstract: In this work, we study the existence of sustained oscillations in the “canonical” repressilator, a basic synthetic circuit of 3 genes leading to sustained oscillations. Previous works mostly used differential equations to study the repressilator. In our work, a pre-existing hybrid modeling framework of gene regulatory networks called HGRN is used to model this system. Compared to differential equations, dynamical properties of HGRNs are easier to prove theoretically due to its lower dynamical complexity. The objective of this work is to find conditions for the existence of sustained oscillations described by separable constraints on parameters. With such separable constraints, each parameter is constrained individually by an interval, which can provide useful information for the design of synthetic circuits. Our two major contributions are the following: firstly, we develop, by using the Poincaré map, a sufficient and necessary condition for the existence of sustained oscillations; then, based on this condition, we give a method using the range enclosure property of Bernstein coefficients to compute compatible separable constraints. By applying this method, we successfully obtain sets of conditions for the existence of sustained oscillations described as separable constraints.

1 INTRODUCTION

With the developments in the field of synthetic biology in the recent years, the construction of synthetic gene regulatory networks in living cells which satisfy certain properties becomes possible. Stable and controllable synthetic circuits could have potential medical applications. Mathematical modeling is one of the ways to guide the design of these synthetic circuits. The modeling of many different synthetic circuits have been studied in the literature (Chaves and Jong, 2021; Firippi and Chaves, 2020; Chaves and Gouzé, 2011; Bușe et al., 2010).

In this work, we focus on one class of synthetic circuit: the repressilators, which are gene regulatory networks consisting of at least one feedback loop, in which the expression of each gene inhibits the expression of the next gene in the loop leading to an oscillatory behavior. Among different repressilators, in this work, we focus on the canonical repressilator with three components (see Figure 1). Our objective is to study the conditions for the existence of sustained oscillations in this network. Long term perspective application of sustained oscillations in a repressilator could be the treatment of diseases related to circadian rhythms, for instance, by allowing drug delivery at a particular pace.

Figure 1: The influence graph of the canonical repressilator.

The existence of sustained oscillations in the repressilator has been studied both mathematically (Bușe et al., 2009; Bușe et al., 2010) and biologically (Potvin-Trottier et al., 2016; Elowitz and Leibler, 2000). In particular, the first biological implementation of the repressilator uses three natural repressor
proteins, the TetR, LacI and CI repressors (Elowitz and Leibler, 2000).

In this work, we analyze mathematically the existence of sustained oscillations based on a pre-existing formalism, which has not yet been used for modeling the repressilator. In fact, how to biologically implement a repressilator with sustained oscillations is still an open question, particularly in eukaryotic cells, therefore exploring new models to search for conditions for sustained oscillations is of high interest.

Previous works about mathematical analysis of oscillations in the repressilator are mainly based on differential equations. Many models of the repressilator with three components are developed from a differential equation model using 6 variables (Elowitz and Leibler, 2000), with 3 variables for repressor-protein concentrations and 3 variables for corresponding mRNA concentrations. This 6-variable model can also be reduced to a 3-variable model with only repressor-protein variables under certain assumptions (Buße et al., 2010). These models and their variations are extensively studied in the literature (Dukare et al., 2019; Dilão, 2014; Kuznetsov and Afratimovich, 2012; Buße et al., 2009; Müller et al., 2006; El Samad et al., 2005). One major limit about differential equations is that some dynamical properties are hard to analyze. Finally, let us mention some pre-existing tools that analyze dynamical properties on models such as GNA (De Jong et al., 2003) and RoVerGeNe (Batt et al., 2007), which are based mostly on qualitative properties.

In this work, a class of hybrid model called hybrid gene regulatory network (HGRN) (Behaegel et al., 2016; Cornillon et al., 2016) is used to model the repressilator. The HGRN is an extension of Thomas’ discrete modeling framework (Thomas, 1973; Thomas, 1991). In HGRNs, the state space is separated into several discrete states, as for discrete models, and in each discrete state, the temporal derivative of the system is described by a constant vector making the system evolve continuously over time, as for differential equations. The most important property of HGRNs is that the “sliding mode” is allowed, which means that when a trajectory reaches a black wall (a boundary of the discrete state which cannot be crossed by trajectories) it is forced to move along the black wall.

There are two major reasons why we choose HGRNs to study synthetic circuits. Firstly, the dynamical complexity of HGRNs is lower than differential equations, making some dynamical properties of HGRNs easier to prove theoretically, such as the stability of limit cycles (Sun et al., 2022). Secondly, from the parameters of a HGRN, we can easily derive the time required for the expression value of one gene to move from one threshold to another under certain regulation, and this information can be useful for the biological design of synthetic circuits.

The objective of this work is to find separable constraints on the parameters of a HGRN of the repressilator to ensure the existence of sustained oscillations. “Separable constraints on the parameters” means that the constraints are separable in a conjunction of constraints which cover a unique parameter each: each parameter is included individually in an interval. Thus we are looking for constraints which can be evaluated variable by variable. When all these constraints are satisfied, the global system shows the desired behavior. The reason why we choose constraints of separable form is that they can be easily interpreted and used. If such separable constraints can be found and if the individual intervals are not degenerated, the measure of the solution space is not null leading to a null chance to be able to implement it in biological cells. These separable constraints represent a bounding box in the parameter space.

This work has the following contributions:

- It is the first study of sustained oscillations in the canonical repressilator based on HGRN.
- Similarly to the work (Sun et al., 2022), the Poincaré map is also used to analyze the stability of a certain cycle in HGRN. But contrary to (Sun et al., 2022) where the values of parameters are known, in this work, the Poincaré map is analyzed symbolically. By doing so, a sufficient and necessary condition for the existence of sustained oscillations in a HGRN of the canonical repressilator is proposed for the first time.
- An intermediate result implies some new control strategies for sustained oscillations in this HGRN of the canonical repressilator: controlling certain parameters such that their absolute values are sufficiently small compared to others.
- The range enclosure property of Bernstein coefficients, which can be used to over-approximate the image of a polynomial function on a bounding box, is adapted for the first time in this work to find bounding boxes in which all models satisfy certain conditions. Based on this method, some bounding boxes which only contain models with sustained oscillations are obtained.

The paper is organized as follows. In Section 2, the HGRN framework is defined and a HGRN of the canonical repressilator is introduced. In Section 3, we discuss different qualitative properties of this HGRN. In Section 4, the Poincaré map is used to compute a sufficient and necessary condition for the existence of
sustained oscillations. In Section 5, a method based on the range enclosure property of Bernstein coefficients is proposed to find separable constraints on parameters under which this HGRN has sustained oscillations. Finally, in Section 6, we make a conclusion and discuss our future works.

2 MODELING REPRESSILATOR WITH HGRN

This section first defines a hybrid gene regulatory network (HGRN). Then a HGRN of the repressilator is introduced.

2.1 Hybrid Gene Regulatory Network (HGRN)

Consider a gene regulatory network with \( N \) genes, the \( p \)th gene has \( n_i + 1 \) discrete levels which are represented by integers: \{0, 1, 2, ..., \( n_i \}\). A discrete state \( s \) is obtained by attributing a valuation for each gene among its discrete levels. We denote \( d_s \) the integer vector which describes the discrete levels of all genes in \( s \) in order; in the following, for simplicity, we also call \( d_s \) a discrete state. The set of all discrete states is \( E_d = \{d_s \in \mathbb{N}^n \mid \forall i \in \{1, 2, ..., N\}, d_i \in \{0, 1, ..., n_i\}\} \), where \( d_i^s \) is the \( i \)th component of \( d_s \). Based on the notion of discrete state, HGRNs are defined as follows:

Definition 1 (Hybrid gene regulatory network (HGRN)). A hybrid gene regulatory network (HGRN) is noted \( \mathcal{H} = (E_d, c) \) where \( E_d \) is the set of all discrete states and \( c \) is a function from \( E_d \) to \( \mathbb{R}^N \). For each \( d_s \in E_d \), \( c(s) \), also noted \( c_d \), is called the celerity of discrete state \( d_s \) and describes the temporal derivataive of the system in \( d_s \).

In HGRNs, a hybrid state is used to fully describe the state of the system: it contains the discrete state in which the system currently is, and a fractional part that represents the (normalized) position of each variable inside this discrete state.

Definition 2 (Hybrid state of a HGRN). A hybrid state of a HGRN is a couple \( h = (\pi, d_s) \) containing a fractional part \( \pi \), which is a real vector in \([0, 1]^N\), and a discrete state \( d_s \) in \( E_d \). \( E_h \) is the set of all hybrid states.

Unless there is an ambiguity, a hybrid state will be called simply a state. Based on this notion of state, a trajectory and a boundary are defined as follow.

Definition 3 (Hybrid trajectory). A (hybrid) trajectory \( \tau \) is a function from a time interval \([0, t_0]\) to \( \mathcal{H} = E_h \cup E_d \), where \( t_0 \in \mathbb{R}^+ \cup \{\infty\} \). \( E_d \) is the set of all states, and \( E_h \) is the set of all sequences of states \( E_h = \{(h_0, h_1, ..., h_m) \in (E_h) m+1 \mid m \in \mathbb{N} \cup \{\infty\}\} \).

A trajectory represents a simulation of the system over time. Consider a trajectory \( \tau \) on \([0, t_0]\). For any \( t \in [0, t_0] \), if \( \tau(t) = (h_0, h_1, ..., h_m) \in E_h \), this means that there is a sequence of instant transitions at \( t \), which begins from \( h_0 \), reaches \( h_1 \) at first, then reaches \( h_2 \), ..., and finally reaches \( h_m \); otherwise, if \( \tau(t) \in E_d \), then the trajectory in \( t \) is made of a regular point. See below for an illustration.

Definition 4 (Boundary). A boundary in a discrete state \( d_s \) is a set of states defined by \( e(i, \pi_0, d_s) = \{(\pi, d_s) \in E_h \mid \pi = \pi_0 \} \), where \( i \in \{1, 2, ..., N\} \). The boundary \( e(i, \pi_0, d_s) \) is inside the discrete state \( d_s \). In the rest of this paper, we simply use \( e \) to represent a boundary.

A toy example of HGRN, not based on any real-world biological system, is shown in Figure 2. This example is related to a negative feedback loop with two genes: A (first dimension) and B (second dimension), where A activates B and B inhibits A. Each gene has two discrete levels, so there are four discrete states in this system. Black arrows represent the celerities of each discrete state and red arrows represent a possible trajectory of this system, which happens, in this particular case, to be a closed trajectory.

The state \( h_M = ((\pi_M^1, 1), (1, 1)) \) of point \( M \) belongs to the upper boundary \( e_1 \) in the second dimension of the discrete state \( 11 \), which is a shorthand notation of \((1, 1)\). Since there is no other discrete state on the other side of \( e_1 \), the trajectory from \( h_M \) cannot cross \( e_1 \) and has to slide along \( e_1 \). Boundaries like \( e_1 \), which can be reached by trajectories but cannot be crossed, are defined as attractive boundaries. If there was another discrete state on the other side of \( e_1 \), in which the celerity is negative in the second dimension (towards the boundary), then the trajectory from \( h_M \) could still not cross it, and in this case \( e_1 \) would also be an attractive boundary.

The state \( h_P = ((\pi_P^1, 0), (0, 1)) \) of point \( P \) belongs to the lower boundary \( e_2 \) in the second dimension of the discrete state \( 01 \). The trajectory from \( h_P \) will reach instantly \( h_0 = ((\pi_0^1, 0), (0, 0)) \), which belongs to the upper boundary \( e_3 \) in the second dimension of discrete state \( 00 \), because the celerities on both sides allow this (instant) discrete transition. \( e_2 \) is defined as an output boundary of \( 01 \) and \( e_3 \) is defined as an input boundary of \( 00 \).

The trajectory in Figure 2 only reaches one new boundary at a time, however generally a trajectory can reach several new boundaries at the same time.
2.2 HGRN of the Repressilator

Here we only focus on the influence graph of the canonical repressilator, see Figure 1. We assume that each gene has one threshold when it influences another gene. Although, a priori, one gene can have multiple thresholds for one another gene, in this work we only consider the simplest case. Based on this assumption, since each gene only influences one other gene in this influence graph, it has only two discrete levels separated by only one threshold.

The parameters (celerities) of this HGRN of the repressilator are shown symbolically in Table 1. Each parameter in Table 1 is strictly positive and is denoted by $C_{yzji}$, which represents the absolute value of the celerity of variable $y$ when the discrete level of variable $z$ is $i$ and the discrete level of variable $y$ is $j$. Consider the influence of A on B; when the discrete level of A is 1, meaning that the expression of A is above the threshold to inhibit B, then the temporal derivative of B is always negative, no matter the discrete level of B (0 or 1) which corresponds to the negative values $-C_{ab10b}$ and $-C_{ab1b1}$. On the other hand, when the expression of A is below the threshold to inhibit B, the temporal derivative of B is always positive, corresponding to parameters $C_{ab00b}$ and $C_{ab0b1}$. From the parameters in this table, we can also see that the number of different parameters (12) is smaller than the multiplication of the number of dimensions by the number of discrete states (24), because some discrete states have celerities in common (same regulation on some variables).

In addition to the threshold which separates the discrete levels 0 and 1, each gene also has a maximal value and a minimal value. For example, when A inhibits B (see Figure 1), B will continue to decrease until it reaches the minimal value (most of the time this minimal value is 0) which is related to the lower boundary in the second dimension (the dimension of gene B) of discrete state 10*, where * can be 0 or 1. Similarly, when A does not inhibit B, B will continue to increase until it reaches the upper boundary in the second dimension of 01*.

### Table 1: Parameters of the HGRN of the repressilator.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>$C_A$</th>
<th>$C_B$</th>
<th>$C_C$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>$C_{ac00a}$</td>
<td>$C_{ac00b}$</td>
<td>$C_{ac00c}$</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>$-C_{ac10a}$</td>
<td>$C_{ac10b}$</td>
<td>$C_{ac10c}$</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>$C_{ac01a}$</td>
<td>$C_{ac01b}$</td>
<td>$-C_{ac11c}$</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
<td>$-C_{ac11a}$</td>
<td>$C_{ac11b}$</td>
<td>$C_{ac11c}$</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>$C_{ac01a}$</td>
<td>$-C_{ac11b}$</td>
<td>$C_{ac11c}$</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>$-C_{ac11a}$</td>
<td>$-C_{ac11b}$</td>
<td>$C_{ac11c}$</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>$C_{ac01a}$</td>
<td>$-C_{ac11b}$</td>
<td>$-C_{ac11c}$</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>$-C_{ac11a}$</td>
<td>$-C_{ac11b}$</td>
<td>$-C_{ac11c}$</td>
</tr>
</tbody>
</table>

![Figure 2: Example of a HGRN of negative feedback loop with 2 genes: gene A and gene B, where A activates B and B inhibits A. Abscissa represents the first gene (gene A) and ordinate represents the second gene (gene B).](image)

When a trajectory reaches several output boundaries at the same time, it can cross any of them but can only cross one boundary at a time, which causes non-deterministic behaviors.

### Figure 3

Figure 3 gives two simulations with two different choices of parameters. The simulation on the left represents a sustained oscillation while the simulation on the right represents a damped oscillation. In the simulation on the left, gene C continues to increase from $t = 0$ until it reaches the maximal value, which also means that the trajectory reaches an attractive boundary, then the trajectory will slide along this boundary (called the sliding mode) so that the value of gene C stays unchanged for some time. The sliding mode is an important property of HGRNs.

3 QUALITATIVE BEHAVIORS IN THIS HGRN OF THE REPRESSILATOR

In this section, we discuss different qualitative properties of this HGRN. To analyze dynamical properties of a HGRN, we need to firstly analyze the transition graph of discrete states, which is determined by the signs of celerities. The signs of celerities of this HGRN of the repressilator can be found in Table 1, based on which the transition graph of discrete states can be constructed using the classical discrete asyec-
Figure 3: Simulations of the HGRN of the repressilator with two different choices of parameters (Abscissa represents time and ordinate represents the sum of the fractional part and the discrete state of each gene). Parameters of the model on the left: \( C_{\text{abs000}} = 1, C_{\text{abs25}} = 1.9, C_{\text{abs16}} = 1.3, C_{\text{abs14}} = 0.4, C_{\text{abs000}} = 3.8, C_{\text{abs16}} = 2.5, C_{\text{abs16}} = 2.7, C_{\text{abs16}} = 3.3, C_{\text{abs000}} = 1.5, C_{\text{abs16}} = 0.8, C_{\text{abs16}} = 1.9, C_{\text{abs16}} = 1.5. \) Parameters of the model on the right: \( C_{\text{abs000}} = 1.5, C_{\text{abs001}} = 0.7, C_{\text{abs000}} = 0.6, C_{\text{abs16}} = 1.6, C_{\text{abs000}} = 2.1, C_{\text{abs000}} = 0.4, C_{\text{abs000}} = 0.3, C_{\text{abs16}} = 3.3, C_{\text{abs16}} = 1.25, C_{\text{abs16}} = 0.25, C_{\text{abs16}} = 0.23, C_{\text{abs16}} = 1.23. \)

Figure 4: Transition graph of discrete states of the HGRN of the repressilator.

From the transition graph, we can see that there is a unique cycle of discrete states, which is 001 \( \rightarrow \) 010 \( \rightarrow \) 110 \( \rightarrow \) 100 \( \rightarrow \) 101 \( \rightarrow \) 001. This cycle is a global attractor of discrete states (the unique terminal strongly connected component), which means that any trajectory in this HGRN will finally enter this cycle.

Following the same sequence of discrete states, there is a special hybrid trajectory:

\[
\begin{align*}
(1,1,0),(0,0,1) & \rightarrow (1,0,0),(0,1,1) \\
(1,0,1),(0,1,0) & \rightarrow (0,1,0),(1,0,0) \\
(0,1,1),(1,0,0) & \rightarrow (0,1,0),(1,0,1) \\
(1,1,0),(0,0,1)
\end{align*}
\]

It is illustrated in Figure 5 by green arrows. This trajectory contains 6 different states (the last and the first one are identical). From each state in this trajectory, there is an instant transition (transition which crosses boundaries between the discrete states and takes no time), which reaches the next state. This trajectory represents also a closed trajectory, meaning that beginning from each of these 6 states, the trajectory will return to the initial state. When representing trajectories, we often use an embedding of hybrid states in \( R^N \): a state \((\pi,d_s)\) is represented in \( R^N \) by summing its discrete and fractional parts: \( \pi + d_s \). We say that the hybrid state \((\pi,d_s)\) and the point \( \pi + d_s \in R^N \) are related. When doing so, the six previous hybrid states are embedded in the same point \( H_0 \) in \( R^2 \): \( H_0 = (1.0,1.0,1.0) \).

is called a characteristic state of this HGRN. The characteristic state is formally defined as follows:

**Definition 5** (Characteristic state). A characteristic state of a HGRN is a state \( H \) in the continuous space such that: for any hybrid state \( h_0 \) related to \( H \), all trajectories from \( h_0 \) will never reach a hybrid state which is not related to \( H \), and there exist oscillations in any small neighborhood of \( H \).

In this paper, we say that a trajectory of a HGRN is an oscillation if it is related to an oscillation in the continuous space. Likewise, the nature of an oscillation of a HGRN (damped or sustained) and the relation between an oscillation and a characteristic state (converging to the characteristic state, moving away from it, etc.) are determined by the related oscillation in the continuous space. A neighborhood of a characteristic state \( H \) is defined as a set \( N(H) = \{(\pi,d_s) \in E_h | \|\pi + d_s - H\| < r\} \) where \( r \in R \) is the radius of the neighborhood.

In the continuous space, a characteristic state is a fixed point, because from any hybrid state \( h \) related to a characteristic state, all trajectories can only reach hybrid states related to this characteristic state.

We can easily prove that \( H_0 \) is a characteristic state: apart from the 6 hybrid states on this closed trajectory, there are two other hybrid states which are related to \( H_0 \): \((1,1,1),(0,0,0)\) and \((0,0,0),(1,1,1)\) From each of these two other states, all trajectories reach directly the closed trajectory, and once they do, they can never leave it. Finally, we can find oscillations which follow the unique cycle of discrete states in any neighborhood of \( H_0 \). In this HGRN, there is only one characteristic state. This can be proved by verifying all “corners” of discrete states.

All trajectories in this HGRN will oscillate in this unique cycle of discrete states, except some special trajectories from discrete state 000 or 111 which can reach directly a hybrid state related to the characteris-
Figure 5: Illustration of the closed trajectory with only instant transitions (green arrows), two special trajectories which can reach directly the characteristic state (black arrows), and a trajectory without sliding mode (red arrows) in this HGRN of the canonical repressilator.

The Poincaré map was initially proposed to study limit cycles of nonlinear dynamical systems and has also been used later to study limit cycles of hybrid systems (Belgacem et al., 2020; Firrippi and Chaves, 2020; Znegui et al., 2020; Flieller et al., 2006; Edwards and Glass, 2005; Girard, 2003; Hiskens, 2001; Edwards, 2000; Mestl et al., 1996). The Poincaré map is the intersection of a periodic orbit with a lower dimension subspace which is called the Poincaré section. The properties of the periodic orbit can be derived from the Poincaré map. In the case of the repressilator studied in this paper, the blue boundary in Figure 5 could be chosen as a Poincaré section.

4 ANALYSIS OF THE POINCARÉ MAP

In this section, a method based on the Poincaré map is proposed to compute a sufficient and necessary condition for the existence of sustained oscillations.

4.1 Poincaré Map in HGRNs

The Poincaré map was initially proposed to study limit cycles of nonlinear dynamical systems and has also been used later to study limit cycles of hybrid systems (Belgacem et al., 2020; Firrippi and Chaves, 2020; Znegui et al., 2020; Flieller et al., 2006; Edwards and Glass, 2005; Girard, 2003; Hiskens, 2001; Edwards, 2000; Mestl et al., 1996). The Poincaré map is the intersection of a periodic orbit with a lower dimension subspace which is called the Poincaré section. The properties of the periodic orbit can be derived from the Poincaré map. In the case of the repressilator studied in this paper, the blue boundary in Figure 5 could be chosen as a Poincaré section.
A method based on the Poincaré map was proposed in (Sun et al., 2022) to analyze the stability of limit cycles in HGRNs. To illustrate this method, consider another example in 2 dimensions, with an additional discrete level in the first dimension, as shown in Figure 7. In this example, we assume that all parameters (celerities) are known, that is, their real values can directly be used. The upper boundary in the second dimension of discrete state 00 is chosen as Poincaré section. We can see that there is a closed trajectory which intersects this Poincaré section at state $P = (\pi_P, (0, 0))$, where $\pi_P \in [0, 1]^2$ is the fractional part of state $P$. We note that in this particular case, $\pi_P = (x, 1)$ where $x \in [0, 1]$, thus there is only one varying dimension to study in this Poincaré section. According to the properties of HGRNs, we can find on the Poincaré section a neighborhood $\mathcal{N}$ around $P$, such that trajectory from any state $M = (\pi_M, (0, 0))$ from $\mathcal{N}$ will return to the Poincaré section at state $M' = (\pi_{M'}, (0, 0))$ and the relation between $\pi_M$ and $\pi_{M'}$ can be described by an affine matrix application:

$$\pi_{M'} = G(\pi_M) = A\pi_M + b \tag{1}$$

where $A$ is a matrix and $b$ is a vector. We note that the form of Equation (1) is general and applies to any case, including those of dimension higher than 2. At this point, the stability of the closed trajectory depends on the eigenvalues of $A$. For more details, see (Sun et al., 2022).

In this work, we adapt this method based on the Poincaré map to analyze the stability of the characteristic state, which is a special case of closed trajectory, in order to find conditions for sustained oscillations. As opposed to (Sun et al., 2022) where parameters are assumed to be known, here we compute and analyze symbolically the Poincaré map.

### 4.2 Symbolic Poincaré Map

In order to analyze the stability of the characteristic state, we only need to consider trajectories without sliding mode around this state. Indeed, the characteristic state is stable if we can find a small neighborhood around the characteristic state such that all oscillations which begin from this neighborhood converge to the characteristic state. Such a trajectory exists because otherwise, the celerities would prevent the characteristic state from existing. Without loss of generality, we choose the lower boundary in the second dimension of discrete state 011 as Poincaré section; see the blue boundary in Figure 5. Now, we consider any trajectory $\tau$ which begins from a state $s_1 = ((x_1, 0, z_1), (0, 1, 1))$ on the Poincaré section and returns to the Poincaré section for the first time at $s_7 = ((x_7, 0, z_7), (0, 1, 1))$ without sliding mode; such a trajectory is illustrated in red in Figure 5. Thus, the Poincaré map is an affine application describing the relation between $(x_1, 0, z_1)$ and $(x_7, 0, z_7)$. Since $s_1$ is on an input boundary of the discrete state 011, from $s_1$, $\tau$ will first (continuously) cross the discrete state 011 and reach a state $((x_2, y_2, 0), (0, 1, 1))$ on the lower boundary in the third dimension of 011 which is the output boundary of this discrete state towards 010. We name this output boundary $e_1$. Then, it crosses instantaneously $e_1$ and reaches an input boundary of 010 in state $((x_2, y_2, 1), (0, 1, 0))$. The duration of crossing in discrete state 011 is:

$$t_1 = \frac{0 - z_1}{-C_{t1}c_{1e1}} \tag{2}$$

It should be noted that, to ensure that this trajectory $\tau$ has no sliding mode in 011, we also need to ensure that the lower boundary in the first dimension and the upper boundary in the second dimension of 011 are not reached before $e_1$, which gives us two additional inequalities:

$$t_1 < \frac{0 - x_1}{-C_{ac1a0}} \tag{3}$$

$$t_1 < \frac{0 - x_1}{-C_{ac1a0}} \tag{3}$$

where $C_{t1}$ is the celerity of the trajectory and $c_{1e1}$ is the celerity of the input boundary.
\[ t_1 < \frac{1 - 0}{C_{\text{bud}01}} \]  \hspace{1cm} (4)

In fact, these inequalities can always be satisfied if \( \tau \) is sufficiently close to the characteristic state, which is the case we consider here. Therefore, in the rest of this section, we do not consider these additional constraints.

Based on the duration \( t_1 \) and the fact that there is no sliding mode, we can get the relation between \((x_1, 0, z_1)\) and \((x_2, y_2, 1)\):

\[ x_2 = x_1 - C_{\text{x}1\text{a}0} \times t_1 \]  \hspace{1cm} (5)

\[ y_2 = 0 + C_{\text{b}0\text{a}1} \times t_1 \]  \hspace{1cm} (6)

Following the same process, we can get the duration of \( \tau \) in each discrete state and the relations between states from one input boundary to another input boundary:

\[ t_2 = \frac{1 - x_2}{C_{\text{x}2\text{a}0}} \]  \hspace{1cm} (7)

\[ t_3 = \frac{0 - y_3}{C_{\text{b}0\text{a}1}} \]  \hspace{1cm} (8)

\[ t_4 = \frac{1 - z_4}{C_{\text{c}1\text{b}0}} \]  \hspace{1cm} (9)

\[ t_5 = \frac{0 - x_5}{C_{\text{x}1\text{a}1}} \]  \hspace{1cm} (10)

\[ t_6 = \frac{1 - y_6}{C_{\text{b}1\text{a}0}} \]  \hspace{1cm} (11)

\[ t_7 = \frac{1 - z_7}{C_{\text{c}1\text{b}1}} \]  \hspace{1cm} (12)

where \( t_2, t_3, t_4, t_5, t_6 \) are the durations of \( \tau \) in discrete states 010, 110, 100, 101, 001 respectively, and \((0, y_3, z_3), (x_4, 1, z_4), (x_5, y_5, 0), (1, y_6, z_6), (x_7, 0, z_7)\) are the fractional parts of the states when \( \tau \) first reaches 110, 100, 101, 001, 011 respectively.

Based on the above equations, the Poincaré map can be calculated to describe the relation between \((x_1, 0, z_1)\) and \((x_7, 0, z_7)\) as follows. One dimension is missing in the matrix below; indeed, this dimension is useless in the computation of the stability, for more details see (Sun et al., 2022).

\[
\begin{pmatrix}
  x_7 \\
  z_7
\end{pmatrix} = \begin{pmatrix}
  b_1 & c_1 \\
  b_2 & c_2
\end{pmatrix} \begin{pmatrix}
  x_1 \\
  z_1
\end{pmatrix} + \begin{pmatrix}
  a_1 \\
  a_2
\end{pmatrix}
\]  \hspace{1cm} (14)

In the above equation, \( a_1, a_2, b_1, b_2, c_1, c_2 \) are non-linear combinations of the celerity values. Their expressions are given in Supporting Material Section 1. We can easily derive that \( b_1 \) and \( c_2 \) are strictly positive, while \( b_2 \) and \( c_1 \) are strictly negative.

### 4.3 Analysis of Eigenvales

The stability of the characteristic state depends on the two eigenvalues of \( \begin{pmatrix}
  b_1 & c_1 \\
  b_2 & c_2
\end{pmatrix} \), which are:

\[ \lambda_1 = \frac{b_1 + c_2 + \sqrt{(b_1 - c_2)^2 + 4c_1b_2}}{2} \]  \hspace{1cm} (15)

\[ \lambda_2 = \frac{b_1 + c_2 - \sqrt{(b_1 - c_2)^2 + 4c_1b_2}}{2} \]  \hspace{1cm} (16)

**Property 1.** These two eigenvalues are real and strictly positive.

The proof of Property 1 is given in Supplementary Material Section 3.

Suppose that two eigenvectors which are related to \( \lambda_1 \) and \( \lambda_2 \) respectively are \( v_1 = (v_1^1, v_1^2) \) and \( v_2 = (v_2^1, v_2^2) \). We have the following property on \( v_1 \) and \( v_2 \).

**Property 2.** \( v_1^1 \times v_2^2 < 0 \) and \( v_2^1 \times v_2^1 > 0 \).

The proof of Property 2 is given in Supplementary Material Section 4.

Based on these properties, we develop the following theorem to verify the stability of the characteristic state.

**Theorem 1.** The characteristic state is unstable if and only if \( \lambda_1 \geq 1 \).

The proof of Theorem 1 is given in Supplementary Material Section 5.

Based on Hypothesis 1 and Theorem 1, the condition \( \lambda_1 \geq 1 \) is a sufficient and necessary condition for the existence of sustained oscillations in this HGRN of canonical repressilator.

Since our final objective is to provide practical information for the construction of synthetic networks, conditions like \( \lambda_1 \geq 1 \) might not be a good result, because the set of models under this constraint is not easy to figure. In the next section, a method to compute separable constraints based on the condition \( \lambda_1 \geq 1 \) is presented.

### 5 COMPUTATION OF SUFFICIENT SEPARABLE CONSTRAINTS ON PARAMETERS

In this section, we propose a method to compute separable constraints on parameters based on the condition \( \lambda_1 \geq 1 \) which is developed in the previous section. In this paper, separable constraints mean constraints with separable form: each parameter is constrained by an interval, for example \( C_{\text{a}0\text{a}0} \in [C_{\text{a}0\text{a}0}, C_{\text{a}0\text{a}0}] \). In other words, these separable constraints represent a \( n \)-dimensional bounding box in the space of parameters (celerities). What we want to do is to find such a \( n \)-dimensional box so that any model in this bounding box satisfies the condition \( \lambda_1 \geq 1 \), which means that any model in this box has sustained oscillations.

Firstly, we present a simplification of the condition \( \lambda_1 \geq 1 \). Secondly, we introduce a method to verify if all models in a given bounding box satisfy this.
simplified condition. At last, using the method in the second part, we propose a search algorithm to find some separable constraints.

5.1 Condition Simplification

The condition:

\[ \lambda_1 = \frac{b_1 + c_2 + \sqrt{(b_1 - c_2)^2 + 4c_1b_2}}{2} \geq 1 \]  \hspace{1cm} (17)

can be reformulated as:

\[ b_1 + c_2 - 2 \geq -\sqrt{(b_1 - c_2)^2 + 4c_1b_2} \]  \hspace{1cm} (18)

which is equivalent to:

\begin{align*}
(b_1 + c_2 - 2 \geq 0) & \lor \\
((b_1 + c_2 - 2 < 0) & \land \\
((b_1 + c_2 - 2)^2 \leq (b_1 - c_2)^2 + 4c_1b_2))
\end{align*}

or:

\begin{align*}
(b_1 + c_2 - 2 \geq 0) & \lor \\
(b_1c_2 - c_1b_2 - b_1 - c_2 + 1 \leq 0)
\end{align*}

This last condition is equivalent to \((P_1 \geq 0) \lor (P_2 \geq 0)\) where \(P_1\) and \(P_2\) are polynomials on parameters. The expressions of \(P_1\) and \(P_2\) are given in Supplementary Material Section 2.

Condition \((P_1 \geq 0) \lor (P_2 \geq 0)\) seems preferable to \(\lambda_1 \geq 1\) because it only contains polynomials. In fact, one can easily prove that solutions for \((P_1 \geq 0) \lor (P_2 \geq 0)\) exist. For example, by only considering \(Cac0a0\) and \(Cac0a1\), which are two parameters describing the derivative of gene \(A\) when gene \(C\) does not inhibit gene \(A\), \(P_1\) and \(P_2\) can be expressed by:

\begin{align*}
P_1 &= p_{11} \times Cac0a0 + p_{12} \times Cac0a1 + p_{13} \times Cac0a0 \times Cac0a1 + p_{14} \\
P_2 &= p_{21} \times Cac0a0 + p_{22} \times Cac0a1 + p_{23} \times Cac0a0 \times Cac0a1 + p_{24}
\end{align*}

where \(p_{ij}\) are expressions of parameters which do not include \(Cac0a0\) and \(Cac0a1\). We can see that if \(Cac0a0\) and \(Cac0a1\) converge to 0 while other parameters remain unchanged, \(P_1\) and \(P_2\) converge to \(p_{14}\) and \(p_{24}\) respectively, which are both positive. This indicates that the solutions of \((P_1 \geq 0) \lor (P_2 \geq 0)\) exist and also implies a new control strategy for the existence of sustained oscillations: controlling the derivatives of gene \(A\) when \(A\) is not inhibited by \(C\) such that these derivatives are sufficiently small, while keeping other parameters unchanged.

5.2 Satisfiability Under Separable Constraints

In this subsection, we adapt the range enclosure property of Bernstein coefficients to verify if all models in a given bounding box satisfy the condition \((P_1 \geq 0) \lor (P_2 \geq 0)\). The Bernstein coefficients have been used in the literature to, for example, compute images for polynomial dynamical system (Dang and Salinas, 2009; Dang and Testylier, 2012), or compute affine lower bound functions for polynomials (Garloff and Smith, 2003), etc.

Before introducing the Bernstein coefficients, we firstly introduce the notion of multi-index. A multi-index is a vector of non-negative integers. Given two multi-index \(i = (i_1, i_2, \ldots, i_n)\) and \(j = (j_1, j_2, \ldots, j_n)\), we write \(i \leq j\) if \(\forall k \in \{1, 2, \ldots, n\}, i_k \leq j_k\). We also write \(\binom{i}{j}\) for \(\binom{\frac{n}{i_1, i_2, \ldots, i_n}}{\frac{n}{j_1}}\) which is the multiplication of all binomial coefficient \(\binom{N}{n}\), \(k \in \{1, 2, \ldots, n\}\).

Using the multi-index, a polynomial \(f : \mathbb{R}^n \rightarrow \mathbb{R}\) can be represented as follows:

\[ f(x) = \sum_{i \in \mathbb{I}} a_i x^i \]  \hspace{1cm} (23)

where \(a_i \in \mathbb{R}\), \(i\) and \(d\) are multi-indices, \(\mathbb{I}\) is set of all multi-indices \(i\) such that \(i \leq d\), and \(x^i = x_1^{i_1} x_2^{i_2} \cdots x_n^{i_n}\) which is the product of all \(x_j^i\), where \(x_j\) is the \(j\)th variable of polynomial \(f\).

\(f\) can also be expressed by Bernstein expansion as follows:

\[ f(x) = \sum_{i \in \mathbb{I}_d} b_i \mathcal{B}_{di}(x) \]  \hspace{1cm} (24)

where

\[ \mathcal{B}_{di}(x) = \beta_{d_{i_1}, i_1}(x_1) \cdots \beta_{d_{i_n}, i_n}(x_n) \]  \hspace{1cm} (25)

\[ \beta_{d_{i_k}, i_k}(x_k) = \binom{d_k}{i_k} x_k^{i_k} (1 - x_k)^{d_k - i_k} \]  \hspace{1cm} (26)

\[ b_i = \sum_{j \leq d} \binom{i}{j} a_j \]  \hspace{1cm} (27)

where \(d\) and \(i\) are multi-indices and \(k \in \{1, 2, \ldots, n\}\). The values \(b_i\), for \(i \in \mathbb{I}_d\) are called Bernstein coefficients.

One fundamental property of Bernstein coefficients for our approach is the range enclosure property, which can be derived from the convex hull property. The convex hull of a set \(S\), noted \(\text{Conv}(S)\), is the smallest convex set that contains \(S\).
Lemma 1 (Convex hull property). \[ \text{Conv}\{ (x, f(x)) \mid x \in B \} \subseteq \text{Conv}\{ (i/d, b_i) \mid i \in I_d \}. \]

Lemma 2 (Range enclosure property). \( \min \{ b_i \mid i \in I_d \} \leq f(x) \leq \max \{ b_i \mid i \in I_d \}, \forall x \in B, \)

where \( B = [0, 1]^n \) is the unit box.

The range enclosure property over-approximates the range of image of \( f \) on \( B \) and can be used to verify if all models in a given bounding box satisfy the condition \( (P_1 \geq 0) \lor (P_2 \geq 0) \). To do so, we need to firstly make a change of variables of polynomials \( P_1 \) and \( P_2 \), such that all variables are included in [0, 1]. For example, the variable \( C_{ac00} \in [C_{ac00}, C_{ac00}] \) is replaced by \( C_{ac00} = (C_{ac00} - C_{ac00}) \times X_{ac00} + C_{ac00} \), where \( X_{ac00} \in [0, 1] \). By doing so, we get two new polynomials \( P'_1 \) and \( P'_2 \).

So now, to verify if \( (P_1 \geq 0) \lor (P_2 \geq 0) \) is always true in a given bounding box, we only need to verify if \( (P'_1 \geq 0) \lor (P'_2 \geq 0) \) is always true in the unit box. To do so, we compute the Bernstein coefficients \( \{b_{1,j}\} \) and \( \{b_{2,j}\} \) (where \( i \in I_d \)) of \( P'_1 \) and \( P'_2 \) respectively. A sufficient condition for the condition \( "(P'_1 \geq 0) \lor (P'_2 \geq 0) \) is always true in the unit box” (condition1) is \( "\forall i \in I_d, b_{1,j} \geq 0 \lor \forall i \in I_d, b_{2,j} \geq 0" \) (condition2), according to the range enclosure property. In fact, since the minimum value of the image of \( P'_1 \) on the unit box is always larger or equal to the minimum value of \( \{b_{1,j}\} \), \( \{b_{2,j}\} \) are not negative \( \forall i \in I_d, b_{1,j} \geq 0 \) indicates that \( P'_1 \) are not negative on the unit box, and the same holds for \( P'_2 \). Since there is a finite number of Bernstein coefficients, condition2 can be verified. Therefore, in this work, condition2 is used to verify if all models in a given bounding box always have sustained oscillations.

5.3 Search of Separable Constraints

Based on the method introduced in the previous subsection, we propose a depth first algorithm to find some bounding boxes which satisfy condition2. This algorithm is illustrated in Figure 8. Initially, each parameter is included in an interval. In this implementation, without loss of generality, we assume that each parameter is included initially in [0, 1]. Then, we verify if condition2 is satisfied for this bounding box using the method proposed in the previous subsection. If it is satisfied, then it is a bounding box such that all models in it have sustained oscillations. If condition2 is not satisfied, then there might be some models in this bounding box which do not have sustained oscillations, in this case the bounding box is split into two smaller bounding boxes (by splitting the largest interval into two) which have the same volume and the process is repeated on each of these two new bounding boxes. Each path in this algorithm will stop, either when a bounding box which satisfies condition2 is found or when the length of the largest interval is smaller than a certain threshold. In fact, similar ideas are widely used to find solution sets under non-linear constraints (Ziat et al., 2019; Pelleau et al., 2013).

Figure 8: Illustration of the algorithm to search for some bounding boxes.

Since the HGRN of the canonical repressilator has 12 parameters (see Table 1), and if we assume that the number of possible smallest intervals for each parameter are the same, noted \( m \), then there are at most \( m^{12} \) smallest bounding boxes to check. Verifying all these boxes can be time consuming. In our implementation, we assume that the intervals of these three genes are identical, which means that we search for bounding boxes such that \( C_{aiaj} = C_{biaj} = C_{cbicj} \) and \( C_{aciaj} = C_{bajc} = C_{cbicj} \) for any \( i, j \in \{0, 1\} \), where, for example, \( C_{ac00}, C_{ac00} \) is the interval of the parameter \( C_{ac00} \), so that we only need to consider 4 independent intervals when searching for bounding boxes. This assumption is only applied here to decrease the number of possible bounding boxes. Similar assumption about the symmetry between these three genes was also made in works based on differential equations, see for example (Busse et al., 2010). We also assume that the minimal length of interval is greater or equal to 0.5. A value smaller than 0.5 could naturally be chosen, but this might exponentially increase the number of possible bounding boxes, which could also exponentially increase the execution time. With these assumptions, we obtain 5 bounding boxes which satisfy the condition2. In the results below, \( (y, x) \) represents \((c, a), (a, b)\) and \((b, c)\), where \( y \) inhibits \( x \), for example \( C_{cy00} \) presents \( C_{ac00}, C_{bc00} \) and \( C_{cb00} \).

- **Bounding box 1**: \( C_{cy00} \in [0, 0.5], C_{cy01} \in [0, 0.5], C_{cy10} \in [0.5, 1], C_{cy11} \in [0, 1] \)
- **Bounding box 2**: \( C_{cy00} \in [0, 0.5], C_{cy01} \in [0.5, 1], C_{cy10} \in [0, 0.5], C_{cy11} \in [0, 0.5] \)
- **Bounding box 3**: \( C_{cy00} \in [0, 0.5], C_{cy01} \in [0.5, 1], C_{cy10} \in [0, 0.5], C_{cy11} \in [0, 0.5] \)
In this work, a HGRN of the canonical repressilator is constructed. By computing and analyzing analytically a Poincaré map and based on a hypothesis, a sufficient and necessary condition for the existence of sustained oscillations is developed. Then the range enclosure property of Bernstein coefficients is adapted to find some bounding boxes in parameters space that satisfy this sufficient and necessary condition. These bounding boxes (intervals of parameters) can provide useful information for the design of synthetic circuits. Moreover, an intermediate result implies some new control strategies for sustained oscillations: controlling the absolute values of the derivatives of one gene under certain regulation such that these values are sufficiently small, while keeping other parameters of the system unchanged.

Naive assumptions about the bounding boxes are made in this work. For example, we assume that the influence between these three genes are symmetrical. In other words, some groups of parameters (such as \( C_{xy00}, C_{xy0100} \) and \( C_{xy000} \)) are constrained by the same intervals. These assumptions could be replaced by more realistic ones with access to more biological knowledge. For instance, knowing the intervals of possible values for some parameters would allow to speed up the enumeration of the bounding boxes.

Only the canonical repressilator with three components is considered in this work. This method could be extended for more complex influence graphs that exist in the literature (Page, 2019; Perez-Carrasco et al., 2018; Goh et al., 2008). However, for more complex influence graphs, conditions for the existence of sustained oscillations can be harder to develop, as there could be several cycles of discrete states around one characteristic state: in such a case, one would have to consider the disjunction of the conditions associated with each cycle. It is also possible to extend this work to find condition for other dynamical properties expressed with temporal logics.

In this work, constraints are obtained based on given dynamical properties. In future works, the converse approach might be considered, that is, given a bounding box of parameters, predict possible behaviors of the system.

6 CONCLUSIONS

This work is partly supported by China Scholarship Council.

ACKNOWLEDGEMENTS

We would like to thank Gilles Bernot and Thao Dang for their fruitful discussions.

ADDITIONAL INFORMATION

Supplementary Material is available at https://hal.archives-ouvertes.fr/hal-03890505/document. The code of this work is available at https://github.com/Honglu42/HGRN_repressilator.

REFERENCES


Dang, T. and Salinas, D. (2009). Image computation for polynomial dynamical systems using the bernstein ex-


