Invariance kernel of Biological Regulatory Networks

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Abstract: The analysis of Biological Regulatory Network (BRN) leads to compute the set of the possible behaviours of the biological components. These behaviours are seen as trajectories and we are specifically interested in cyclic trajectories since they stand for stability. The set of cycles is given by the so-called invariance kernel of a BRN. This paper presents a method for deriving symbolic formulae for the length, volume and diameter of a cylindrical invariance kernel. These formulae are expressed in terms of delay parameters expressions and give the existence of an invariance kernel and a hint of the number of cyclic trajectories.

Keywords: regulatory networks; gene networks; hybrid modelling; delays; HyTech; PolyLib; cycles; invariance kernel.

Reference to this paper should be made as follows: Ahmad, J. and Roux, O. (2010) ‘Invariance kernel of Biological Regulatory Networks’, Int. J. Data Mining and Bioinformatics, Vol.

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1 Introduction

Biological Regulatory Networks (BRN) represent interaction among biological entities. For example, genetic regulatory networks are graphs where vertices
represent genes or regulatory products e.g., RNA, proteins and edges represent interactions between them. These interactions are further directed (regulators are distinct from targets) and signed (+ for activation and − for inhibition).

The semantics of the BRN lies in the dynamics of the BRN. There are different modelling approaches for the dynamics of BRN. The most realistic one is the differential equation model or non-linear model; but the drawback of this approach is that it incorporates too many parameters in the model and which are a priori unknown. The other two well adopted approaches are the discrete modelling formalism of René Thomas (Thomas and D’Ari, 1990) and the piecewise linear differential equation (Glass and Kauffman, 1973). The discrete modelling highlights the effects of thresholds to discretise the concentrations and the dynamics in these modelling depend on discrete parameters called the attractors are targets.

We adopt the discrete approach of René Thomas to model BRN and uses the semantic described in Bernot et al. (2003) to derive the qualitative model of BRN. Further, from the qualitative or discrete model of BRN we derive the hybrid model by introducing time delays.

Cycles in the BRN show interesting phenomena while analysing the different observable pathways. The biologists observe these paths for different behaviours of the biological system underlying a regulatory network. For example, the mucoid and non-mucoid states in the mucous production system of *Pseudomonas aeruginosa* can be distinguished in the discrete model of its two genes network (see Section 3). The cyclic behaviour of this system is the one where *Pseudomonas aeruginosa* does not produce mucus but can always lead to other state where mucus can be produced. We have shown in Ahmad et al. (2007, 2008) the cyclic and non-cyclic behaviour of BRN as a constrained region in their hybrid model that incorporates time delays.

Viability theory is an area of mathematics which is concerned with the viable behaviour of controlled dynamics (Aubin, 1991). A system execution is considered viable if the system trajectory remains within a prescribed region, the viability domain. The viability kernel is the largest viability domain. Roughly speaking the viability domain is a set of states such that there exist at least one execution (trajectory) from every point that remains viable in this set. The invariant set is a set of points such that each point must keep rotating within the set forever. The invariance kernel is the largest of such sets.

The goal of this paper is to compute the length, volume and diameter of the cylindrical invariance kernel of BRN using PolyLib (Wilde, 1993) library. These properties have some biological significance. For instance, the diameter of the invariance kernel, which is a function of the delay parameters, can be modified (e.g., in order to obtain that the diameter be equal to zero) by ascribing different values to delay parameters. Increasing the diameter of the invariance kernel will increase the stability of the cycles and vice versa. The length of the invariance kernel is also important as this tells about the time length of the cycles. We will go further in the discussion about these issues in Section 4. For the operations on polyhedra we use PolyLib library. This library can operate on both parameterised and non-parameterised polyhedra.

Other related works that deals with time delays are Siebert and Bockmayr (2006) and Batt et al. (2007). The approach of Siebert and Bockmayr (2006) is similar
to ours as they enrich the formalism of René Thomas by timing information and use timed automata to replace the discrete modelling by hybrid modelling while we replace it by using Linear Hybrid Automata (LHA). LHA is a class of hybrid automata where the continuous variables can be increased, decreased and reset; however unlike timed automata, this class in general is undecidable. The approach of Batt et al. (2007) is different from ours as they adopt the formalism of Maler and Pnueli (1995) that gives a formal treatment of asynchronous network of Boolean gates with uncertain (bi-bounded) delays and a systematic method for translating such networks into timed automata.

This paper is organised as follows. In Section 2, we introduce the discrete modelling of BRN. Section 3 illustrate the modelling formalism through two examples and Section 4 focuses on the impact of cycle analysis in living systems. In Section 5, we introduce the hybrid modelling formalism of BRN. The notion invariance kernel is presented in Section 6. The method to compute the length of an invariance kernel is also presented in Section 6. In Section 7, we explain the polyhedral modelling of an invariance kernel and present a method to compute the volume and diameter of an invariance kernel. Finally, in Section 8, we apply our method on biological examples and then we conclude this paper in Section 9.

2 Discrete modelling of BRN

In this section, we introduce the discrete modelling formalism of René Thomas (Thomas, 1991) for BRN. We use this formalism to derive the qualitative state graph of a BRN. We recall only the main definitions related to the discrete modelling framework and the readers may refer to Bernot et al. (2003) for more detail information.

In a directed graph \( G = (V, A) \), we denote \( G^{-}(v) \) and \( G^{+}(v) \) the set of predecessors and successors of a node \( v \in V \) respectively.

**Definition 1** (Biological Regulatory Network): A Biological Regulatory Network, or BRN for short, is a graph \( G = (V, A) \) where \( V \) is the set of nodes representing the biological entities and \( A \) is the set of edges representing the interaction between entities. Each edge \( u \rightarrow v \) is labelled by a pair \( (t_{uv}, \alpha_{uv}) \), where \( t_{uv} \) is a positive integer and \( \alpha_{uv} \in \{+,-\} \) is the sign of interaction (\( + \) for an activation and \( - \) for an inhibition). Each node \( u \) has a limit \( l_{u} \), which is equal to the out-degree of \( u \), such that for \( \forall v \in G^{+}(u) \) each \( t_{uv} \in \{1, \ldots, m_{u}\} \) where \( m_{u} \leq l_{u} \). Each entity \( u \) holds its abstract concentration in the set \( C_{u} = \{0, \ldots, m_{u}\} \).

For the behaviour of the BRN we need to know first its possible number of states and then the transitions among them.

**Definition 2** (States): Let \( G = (V, A) \) be a BRN. A state of a BRN is a tuple \( s \in S \), where

\[
S = \prod_{v \in V} C_{v}.
\]
Formally, we represent a qualitative state like a vector \((x_v)_{v \in V}\), where \(x_v\) is the level of concentration of the product \(v\).

The number of activators of a variable at a given level is formally represented by its set of resources.

**Definition 3 (Resources):** Let \(G = (V, A)\) be a BRN. The set of resources \(R_{x_v}\) of a variable \(v \in V\) at a level \(x\) is defined as

\[
R_{x_v} = \{ u \in G^- (v) \mid (x_u \geq t_{uv} \text{ and } \alpha_{uv} = +) \text{ or } (x_u < t_{uv} \text{ and } \alpha_{uv} = -) \}.
\]

In the above definition, it can be noticed that the absence of inhibitors are treated as activators.

The dynamics of BRN depend on the logical parameters which are also called targets. The set of logical parameters of a BRN are defined as

\[
K(G) = \{ K_v, R_{x_v} \in \{0, \ldots, m_v\} \mid x_v \in C_v \forall v \in V\}.
\]

At a level \(x\) of \(v\), \(K_v, R_{x_v}\) gives the level towards which the variable \(v\) tends to evolve. We consider three cases, (1) if \(x_v < K_v, R_{x_v}\) then \(x_v\) can increase by one unit, (2) if \(x_v > K_v, R_{x_v}\) then \(x_v\) can decrease by one unit and (3) if \(x_v = K_v, R_{x_v}\) then \(x_v\) cannot evolve.

The state graph of a BRN represents the set of the states that a BRN can adopt with transitions among them. It can be deduced from the previous definitions.

In the following, we define the evolution operator \(\mapsto\) (Bernot et al., 2003) for the asynchronous state graph of BRN. Let \(x\) and \(k \in \mathbb{Z}_{\geq 0}\), then:

\[
x \mapsto k = \begin{cases} 
x + 1 & \text{if } x < k \\
x - 1 & \text{if } x > k \\
x & \text{if } x = k
\end{cases}
\]

**Definition 4 (State graph):** Let \(G = (V, A)\) be a BRN and \(s_v\) represent the level of a gene \(v\) in a state \(s \in S\). The state graph of a BRN is a directed graph \(G = (S, T)\) with a transition relation \(T \subseteq S \times S\) such that \(s \rightarrow s' \in T\) iff:

- there exists a unique \(v \in V\) such that \(s_v \neq s'_v\) and \(s'_v = s_v \mapsto K_v, R_{x_v}\)
- and \(s'_u = s_u \forall u \in V \setminus \{v\}\).

From the definition of the state graph it is clear that a state differs from a successor state in one component only. If the state \(s\) in a state graph has \(n\) components to be evolved then there will be \(n\) successor states of \(s\).

### 3 Examples of BRN

In this section, we present the discrete modelling of two real biological examples. The first example is the mucous production system of *Pseudomonas aeruginosa* while the second example is the circadian clock in mammals.
3.1 Example of Pseudomonas aeruginosa

*Pseudomonas aeruginosa* is an opportunistic pathogen, which is often encountered in chronic lung diseases such as cystic fibrosis. The mucus can cause the respiratory deficiency in the patients of cystic fibrosis. The regulatory network which controls the mucus production is given in Figure 1.

The main regulator for the mucus production, AlgU, supervises an operon which is made of four genes among which one codes for a protein that is an inhibitor of AlgU. Moreover AlgU favours its own synthesis. The mucus production regulatory network can then be simplified into the regulatory graph of Figure 1, where node $x$ represents AlgU, and node $y$ its inhibitor.

**Figure 1** The BRN of the mucus production in *Pseudomonas aeruginosa*

![Figure 1](image)

Figure 2 shows the state graph of the *P. aeruginosa* according to the parameters $K_{x,\{\}} = 0$, $K_{x,\{y\}} = 2$, $K_{x,\{x,y\}} = 2$, $K_{x,\{x\}} = 2$, $K_{y,\{\}} = 0$ and $K_{y,\{x\}} = 1$.

**Figure 2** The state graph for the mucus production of *P. aeruginosa*

![Figure 2](image)

The abstract cycle $(0,0) \rightarrow (1,0) \rightarrow (1,1) \rightarrow (0,1)$ shows the non-mucoid state while the bifurcation towards abstraction in the state $(2,1)$ reveals the mucoid production of *P. aeruginosa*.

3.2 Example of circadian rhythm

Chronobiology is concerned with the formal study of biological temporal rhythms such as daily, tidal, weekly, seasonal and annual rhythms. A circadian rhythm produces an autonomous oscillation and periodic or aperiodic changes of an external light stimulus affect the circadian oscillation. The key regulatory elements involved in the circadian system of mammals are *Clock, Bmal1, Per, Cry* and *Rev–Erbα*. The core circadian regulatory network of mammals consists of coupled negative and positive feedback loops (Sriram et al., 2007). Figure 3 presents the regulatory network of circadian rhythm.

**Figure 4** The state graph according to the parameters $K_{b,\{\}} = 0$, $K_{p,\{\}} = 0$, $K_{r,\{\}} = 0$, $K_{b,\{p\}} = 1$, $K_{b,\{r\}} = 1$, $K_{b,\{p,r\}} = 1$, $K_{p,\{b\}} = 1$, $K_{p,\{p\}} = 1$, $K_{p,\{r\}} = 1$. **Figure 3** The BRN of the circadian production in *P. aeruginosa*
$K_{p,b} = 1$, $K_{r,p} = 1$, $K_{r,b} = 1$, and $K_{r,p,b} = 1$. The variables $b$, $p$ and $r$ represent the heterodimer BMAL1–LOCK, PER–CRY complex and REV–ERB $\alpha$ protein concentrations respectively. For more details about these variables, we refer to Sriram et al. (2007).

Figure 3 The BRN of the circadian rhythm

Figure 4 The state graph of the circadian rhythm

4 Biological significance of cycles in BRN

The abstract cycle of the discrete model e.g., Figure 2, represents the discretisation of the continuous time trajectories. These cyclic-idealised trajectories are abstract representations of behaviours which are either

- the evolution towards an asymptotic limit cycle (see Figure 5-right)
- or the spiral concentrating towards an asymptotic point (see Figure 5-left)
- or nested cycles (see Figure 6-right)
- or a perfect rough cycle (see Figure 6-right).

As the René Thomas’ discrete model is too coarse to predict these time trajectories, hence time is required to be added in order to investigate in an accurate fashion the evolutions of the time trajectories. Ahmad et al. (2007) tackle this problem with a new hybrid modelling formalism. This formalism provides greater advantages for example, to distinguish different behaviours like, reachable states, cyclic trajectories, stable states, basins of attraction for cycles and stable states.
Mostly, biological experiments are performed to observe the stable cycles and steady states as the transitory states are difficult to observe. We focus in this paper on the volume computation of the cyclic trajectories, the so-called invariance kernel. Indeed, the volume and the diameter of the invariance kernel can be exploited to investigate the sensibility of the invariance kernel to time delays. Intuitively, the convergence towards the equilibrium point is highlighted by the valuation of the delay parameters of the model. We conjecture that the larger the diameter of the invariance kernel is, the slower the convergence towards the equilibrium point will be and vice versa. Also, we are interested in the fact that if the volume of the invariance kernel is null for certain values of the parameters, then, it means that this invariance kernel is empty.

5 Hybrid modelling of BRN

In this section, we recall the hybrid modelling framework from Ahmad et al. (2007) that was proposed to go from a pure discrete modelling (Thomas, 1991) to a hybrid modelling taking into account delays in the expression space.

5.1 Delays and clocks

During the activation of a gene $v$, its expression moves from the abstract level $n$ to $n+1$ after a time delay $d^+_v$. Similarly, during the inhibition of a gene $v$, its
expression changes from level \( n + 1 \) to \( n \) after a time delay \( d_v \). A sufficient amount of change in corresponding protein concentration occurs after the activation or inhibition time delay. Figure 7 highlights the activation and inhibition time delays in the evolution of a gene’s expression.

**Figure 7** Activation and inhibition delays in the actual evolution of a gene’s expression

[Figure 7 image]

Figure 8 represents the discrete approximation of the actual evolution of a gene’s expression.

**Figure 8** The discrete model

[Figure 8 image]

The piecewise linear approximation of the actual evolution of a gene’s expression is shown in Figure 9.

**Figure 9** The piecewise linear model

[Figure 9 image]
Let $V$ be the set of regulatory variables in a BRN. Each $v \in V$ is associated a clock $h_v$. The clocks measure the time from one abstract expression level $n$ to expression level $n+1$ and vice versa. The total time that a clock measure from one expression level to another level is considered as the time delay between these two expression levels. Figure 9 shows how clocks and delay can be associated with a gene’s expressions. Initially, the clock is set to zero and when it reaches the associated delay time $d^+_v$ or $d^-_v$, then a discrete transition to the next level occurs. The advantage of this approach is that we get a hybrid model which has both continuous and discrete transitions and which is consistent with the discrete model.

5.2 Temporal zones

Each discrete location of the discrete model presented in Section 2 is transformed in a temporal zone by embodying it with continuous variables clocks and delay parameters. Each clock $h_v$ evolves with a particular speed such that $\frac{dh_v}{dt} = c$, where $c \in \{0, 1, -1\}$. These rates determine the tendency of the genes’ expressions evolution in the current as well as in the next successor states (Ahmad et al., 2007).

The hybrid modelling presented in the previous section possesses continuous and discrete transitions. These transitions represent the dynamics of expression levels. A continuous transition stands for a time elapsing in a zone until the border of the zone. A discrete transition stands for instantaneous change of zone and leads to the appropriate clock reset. A trajectory is any sequence of points related by such transitions. A cycle is a trajectory that starts at a point $p$ and later on arrives at the same point $p$. In the next section, we are going to broadly distinguish the cyclic and non-cyclic trajectories in a model by introducing the concept of invariance kernels.

6 Invariance kernel

In this section, we briefly recall the notion of invariance kernel (Aubin, 1991; Asarin et al., 2002) and then define the length of an invariance kernel and present a method for computing such a length.

In a hybrid model of a BRN, we denote $\varphi(t)$ with $t \in \mathbb{R}_{\geq 0}$ and $S$ the sequence of points of a trajectory and the set of all points in its state space.

**Definition 5** (Invariance Set and Invariance Kernel): A trajectory $\varphi(t)$ is viable in $S$ if $\varphi(t) \in S$ for all $t \geq 0$. A subset $K$ of $S$ is said to be invariant if for any point $p \in K$ there exists at least one trajectory starting in $p$, and every trajectory starting in $p$ is viable in $K$. Given a set $S$, its largest invariant subset is called the invariance kernel of $S$.

**Example 1:** In Figure 10, the invariance kernel is the set of all points of the regions labelled by letters $A, B, C$ and $D$.

In Aubin (1991) and Asarin et al. (2002), the authors also define the notions of the viability domain and viability kernel which in our hybrid modelling can be confused with the notions of invariance set and invariance kernel because the trajectories are deterministic.
In the rest of this paper, we will focus on invariance kernels and their featuring measures volume, length and diameter in order to know about the existence and stability of invariance kernels.

6.1 Length and diameter of an invariance kernel

In this section, first, we present a method to compute the length of an invariance kernel and then we present the definitions of the volume and diameter of an invariance kernel.

6.1.1 Length

The cylindrical\(^1\) invariance kernel consists of plain\(^2\) and nested cycles (Ahmad et al., 2007). We define the length of an invariance kernel as the total time of a plain cycle. To find the length of cycle we use a variable \(t\) to accumulate time in the hybrid model. We update \(t\) with each discrete transition as \(t = t + t_e\) where \(t_e\) is the total time to be elapsed in a zone.

The algorithm presented in Ahmad et al. (2007) finds the set of constraints which characterises an invariance kernel. To compute the length of the invariance kernel, we suppose any constrained region of the invariance kernel as the initial region \(I_{reg}\) and then use Algorithm 1 to compute the length of the invariance kernel. Algorithm 1 proceeds as follows. At the beginning, the variable \(t\) is initialised to zero. The algorithm contains one loop that iterates until the fixed point \(I_{new} = post(I_{new}) \cup I_{new}\). With each iteration the algorithm accumulates the time elapsing in the temporal zones. Finally, the length of the invariance kernel is equal to the value of the variable \(t\) which is a symbolic formula in terms of delay parameters.

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**Algorithm 1** Finds the time length of the invariance kernel

1: \(I_{new} := I_{reg}\)
2: \(t := 0\)
3: repeat
4: \(I_{old} := I_{new}\)
5: \(I_{new} := post(I_{new}) \cup I_{new}\)
6: \(t := t + t_e\)
7: until \((I_{new} = I_{old})\)
The length of the invariance kernel gives the time duration of one plain cycle. However, the invariance kernel may consist of many cycles thus we further need to compute the volume and diameter of an invariance kernel.

We conjecture that the problem of computing an invariance kernel is undecidable. Therefore, in case there is no result from the algorithm (Ahmad et al., 2007), then, we use the same algorithm by just replacing the relational equality operator ‘=’ by the inclusion operator ‘⊆’. The algorithm then finds the convergence domain instead of the invariance kernel.

**Definition 6** (Convergence Domain): A subset \( K \) of \( S \) is called a convergence domain if each trajectory starting at a state \( p \in K \) converges asymptotically.

### 6.1.2 Volume and diameter of an invariance kernel

The phase portrait of an invariance kernel is the union of all the constrained regions (Ahmad et al., 2007). Each constrained region is represented by constraints of clocks and delay parameters. It is of great interest to find the sub-domains for parameter values (constraints on parameters only), such that the invariance kernel exists if the values of parameters verify the domain constraints. These are the so-called validity domains used in the polyhedral calculus which we are presenting in Section 7 (see Definition 11). We compute the Ehrhart polynomials (Loechner, 1999) of associated validity domains for estimating the volume of an invariance kernel. Ehrhart polynomials are symbolic formulae which represent the volume of parameterised polyhedron (see Section 7).

Finally, we define the diameter of an invariance kernel.

**Definition 7** (Diameter): The diameter of an invariance kernel is defined as its volume divided by its length.

In the next section, we present a method for computing the volume and diameter of an invariance kernel.

### 7 Polyhedral modelling of an invariance kernel

In this section, first, we present some definitions related to polyhedra and then we present a method to compute the

#### 7.1 Definitions

The following are the relevant definitions for the polyhedra operations. The readers may refer to Loechner and Wilde (1997) for the detail description of polyhedra.

**Definition 8** (Hyperplane and Half-space): An affine subspace of co-dimension 1 is called hyperplane. For example, for the \( n \)-dimensional space the subspace of \( n-1 \) dimension represents a hyperplane. A hyperplane can be represented by one affine equation. A \( n-1 \) dimensional hyperplane divides a \( n \)-dimensional space into two half-spaces, therefore a half-space can be represented by one affine inequality.
Definition 9 (Polyhedron): A polyhedron is a finite intersection of half-spaces. Thus, it can be represented by a finite set of equalities and inequalities

\[ D = \{ x \in \mathbb{Q}^d | Ax = b, Cx \leq d \}, \tag{1} \]

where \( A \) is a \( j \times n \) matrix and \( b \) a \( j \)-vector, \( C \) is \( k \times n \) matrix and \( d \) is a \( k \)-vector, and where \( n \) is the dimension of the space containing the polyhedron, \( k \) the number of inequalities, and \( j \) the number of equalities. A polyhedron of dimension \( n \) is called a \( n \)-polyhedron.

Definition 10 (Parameterised polyhedron): A parameterised polyhedron is defined as a set of constraints (equalities and inequalities), in which the constant part depends linearly on a parameter vector

\[ D(p) = \{ x \in \mathbb{Q}^d | Ax = Bp + a, Cx \geq Dp + b \}, p \in \mathbb{Q}^m \tag{2} \]

where \( A \) is a \( k \times n \) integer matrix, \( B \) a \( k \times m \) integer matrix, \( a \) is an integer \( k \)-vector, \( C \) is a \( k' \times n \) integer matrix, \( D \) a \( k' \times m \) integer matrix and \( b \) is an integer \( k' \)-vector.

\( D(p) \) can also be thought as a family of polyhedra where each valid assignment of values to the vector \( p \) gives one member of the set.

Definition 11 (Validity domain): Let \( D(p) \) be a parameterised polyhedron. The set \( C_v \) of parametric constraints is called the validity domain of \( D(p) \) such that \( D(p) \) exists only when the values of the parameters verify all the constraints of \( C_v \).

Now, we present the valuation function.

Definition 12 (Valuation): A parameter valuation is a function \( \nu : p \rightarrow \mathbb{R} \) assigning a real value to each parameter in a parameter vector \( p \). If \( c \) is a constraint then, \( c(\nu) \) denotes a constraint obtained by replacing \( p \) in \( c \) with \( \nu(p) \). If \( c \) is a parametric constraint then \( c(\nu) \) evaluates to either true or false. A validity domain \( C_v \) evaluates to true if the valuation satisfies each constraint in the set, otherwise false. Likewise, \( P(\nu) \) evaluates to a value where \( P \) is a polynomial in terms of parameters.

An invariance kernel of a BRN is the union of parameterised polyhedra.

Definition 13 (Member polyhedron): A parameterised polyhedron that represents a region of an invariance kernel in a temporal zone is called a member polyhedron of an invariance kernel.

Equation (2) can be used to model each member polyhedron of an invariance kernel. Now, we want to further investigate each member polyhedron of an invariance kernel for the validity domains and their associated volumes which will be expressed as Ehrhart polynomials.
7.2 Using PolyLib for parameterised polyhedra

In order to compute the volume of an invariance kernel, we use the PolyLib library (Wilde, 1993; Loechner, 1999) for the operations on polyhedra. The library can handle both parameterised and non-parameterised polyhedra.

7.2.1 Computing the volume and the diameter of an invariance kernel

As already said that the parameterised polyhedra depend on the parameter vector \( p \). Of course, the volume of the parameterised polyhedra also depends on the parameter vector \( p \). The peculiarity of PolyLib library is that it offers library functions to compute the validity domains of the parameters of a parameterised polyhedron along with their associated Ehrhart polynomials which are symbolic formulae for estimating the volumes.

The following steps can be followed to compute the validity domains and their volumes for an invariance kernel.

- For each member polyhedron of an invariance kernel we write an input file for PolyLib programme (Loechner, 1999). The input file contains the two matrices for the coefficients of the constraints (equalities or inequalities) in homogeneous form of the member polyhedron. The first matrix contains the coefficient of the constraints consisting of both clock variables and parameters. The second matrix represents the coefficient of the constraints consisting of the parameters only.

- A PolyLib programme (Loechner, 1999) that first reads the two matrices of a member polyhedron and then finds the validity domains and their associated Ehrhart polynomials. The programme can also evaluate both the validity domain which is a set of parametric constraints and the Ehrhart polynomial for a given valuation of parameters.

- The above steps are repeated for all the member polyhedra of an invariance kernel.

The PolyLib programme uses the following functions. The function PolyhedronEnumerate returns a list of validity domains and their corresponding Ehrhart polynomials. The function compute_poly evaluates Ehrhart polynomial according to the given valuation parameters. For the detailed description of these functions we refer to Loechner and Wilde (1997).

To compute the diameter of an invariance kernel we divide the sum of volumes of the member polyhedra of an invariance kernel by its length (cf. Definition 7).

8 Results on the examples

In this section, we apply our method on two biological examples which were introduced in Section 3. First we show the volume and diameter computations for Pseudomonas aeruginosa and then for the circadian clock.
8.1 Example of Pseudomonas aeruginosa

In this section, we show the results about the volume and diameter of the invariance kernel for the example of Pseudomonas aeruginosa (see Section 3). We show that by applying the presented method, since the trajectories outside the invariance kernel diverge towards the zone (2,1) and which stands for the mucus production (see Figure 12), the stability of non-mucoid state of Pseudomonas aeruginosa depends on its diameter of the invariance kernel. Furthermore, the volume (and consequently the diameter too) is highly related to the parameters values.

For the sake of simplicity, we only deal with fewer delay parameters, assuming that all $d_{ij}$ are equal, whatever the actual value of $j$ is, and similarly for all $d_{ij}$, whatever the actual value of $i$ is. The major consequence is that, from now on, zones are adjacent (see Figure 11).

![Figure 11](image)

8.2 Phase portrait and length of the invariance kernel

The hybrid model of Pseudomonas aeruginosa (see Section 5) is analysed with HyTech (Henzinger et al., 1997) by using the algorithm (Ahmad et al., 2007) for computing the phase portrait of the invariance kernel. Table 1 shows the different members polyhedra of the invariance kernel of Pseudomonas aeruginosa. Each member polyhedron corresponds to a region in a temporal zone of the BRN. The member polyhedra $A$, $B$, $C$ and $D$ correspond to the zones (0,0), (1,0), (1,1) and (0,1) respectively. The length of the invariance kernel is computed along execution of Algorithm 1 and the result is: $L = d_{y0}^+ + |d_{y1}^-|$. 

...
8.3 Volume and diameter

According to the the method presented in Section 7.2.1, Table 2 presents different volumes and diameters according to the given valuations of the parameters for
the invariance kernels as described in Table 1. In Table 2, \( \nu(p) \), \( \mathcal{C}_\nu(\nu) \), \( VA(\nu) \), \( VB(\nu) \), \( VC(\nu) \), and \( VD(\nu) \) represent the valuation of parameter vector \( p \), the validity domain, the volumes of the member polyhedra A, B, C and D respectively. The table shows that if a given valuation satisfies a validity domain then the invariance kernel exists and its volume will be equal to the sum of its members' volumes. The zero diameter or zero volume means that there exist no invariance kernel. The larger the diameter of the invariance kernel is, the greater number of cycles it will contain and the slower will be the convergence towards an equilibrium point. The invariance kernels of diameters 3, 1 and 0 are shown in Figures 11–13 respectively.

**Table 2** Volumes and diameters of the invariance kernels according to different valuations of the parameters. The measures for length, volume and diameter are given in integer points.

<table>
<thead>
<tr>
<th>( \nu_1(p) )</th>
<th>( d_{x_0}^+ = 4, d_{x_0}^- = -3, d_{x_1}^+ = 3, d_{x_1}^- = -2, d_{x_2}^+ = 2, d_{x_2}^- = -4, )</th>
<th>( d_{y_0}^+ = 5, d_{y_0}^- = -3, d_{y_1}^+ = 2, d_{y_1}^- = -5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mathcal{C}_\nu(\nu_1) ) for ( i \in {1, 3} )</td>
<td>True</td>
<td>False</td>
</tr>
<tr>
<td>Members</td>
<td>Member-A</td>
<td>Member-B</td>
</tr>
<tr>
<td>Volumes</td>
<td>( VA(\nu_1) = 12 )</td>
<td>( VB(\nu_1) = 9 )</td>
</tr>
<tr>
<td>Diameter</td>
<td>diameter = volume/length = 42/12 = 3</td>
<td></td>
</tr>
<tr>
<td>( \nu_2(p) )</td>
<td>( d_{x_0}^+ = 4, d_{x_0}^- = -4, d_{x_1}^+ = 3, d_{x_1}^- = -2, d_{x_2}^+ = 2, d_{x_2}^- = -4, )</td>
<td>( d_{y_0}^+ = 7, d_{y_0}^- = -3, d_{y_1}^+ = 2, d_{y_1}^- = -5 )</td>
</tr>
<tr>
<td>( \mathcal{C}_\nu(\nu_2) ) for ( i \in {1, 3} )</td>
<td>True</td>
<td>False</td>
</tr>
<tr>
<td>Members</td>
<td>Member-A</td>
<td>Member-B</td>
</tr>
<tr>
<td>Volumes</td>
<td>( VA(\nu_2) = 5 )</td>
<td>( VB(\nu_2) = 4 )</td>
</tr>
<tr>
<td>Diameter</td>
<td>diameter = volume/length = 16/14 = 1</td>
<td></td>
</tr>
<tr>
<td>( \nu_3(p) )</td>
<td>( d_{x_0}^+ = 4, d_{x_0}^- = -4, d_{x_1}^+ = 3, d_{x_1}^- = -2, d_{x_2}^+ = 2, d_{x_2}^- = -4, )</td>
<td>( d_{y_0}^+ = 8, d_{y_0}^- = -2, d_{y_1}^+ = 2, d_{y_1}^- = -5 )</td>
</tr>
<tr>
<td>( \mathcal{C}_\nu(\nu_3) ) for ( i \in {1, 2, 3, 4, 5} )</td>
<td>False</td>
<td></td>
</tr>
<tr>
<td>Member</td>
<td>Member-A</td>
<td>Member-B</td>
</tr>
<tr>
<td>Volumes</td>
<td>( VA(\nu_3) = 0 )</td>
<td>( VB(\nu_3) = 0 )</td>
</tr>
<tr>
<td>Diameter</td>
<td>diameter = volume/length = 0</td>
<td></td>
</tr>
</tbody>
</table>

The advantage of our approach is that it is possible to verify the existence of the invariance kernel according to the delay constraints. Our analysis shows that the invariance kernel has zero volume for the constraints: \( d_{x_0}^+ < d_{y_0}^+ \land d_{x_1}^+ < d_{y_0}^+ \land d_{x_0}^+ > |d_{y_1}^-| \land d_{x_1}^+ < |d_{y_1}^-| \).
8.4 Example of circadian rhythm

Here, we show only the length of a circadian cycle. The valuations of parameters to obtain the invariance kernels of different diameters can be done in the same way as for the example of *Pseudomonas aeruginosa*. The results show that the length of the circadian cycle is the expression $L = d_{p0}^+ + |d_{p1}^-|$ with $d_{p0}^+ + |d_{p1}^-| = d_{r0}^+ + |d_{r1}^-|$. The length is therefore the sum of the positive and negative regulation delays of the variables $p (PER–CRY)$ which is also equal to the sum of the positive and negative regulation delays of the variables $b$ and $r$. This also confirms the length of the limit cycle in Sriram et al. (2007, p.191).

9 Conclusion

We have shown in this paper a method to compute the length, volume and diameter of an invariance kernel. The invariance kernel represents an important biological feature of the behaviour of a BRN. The featuring measures of an invariance kernel i.e., length, volume and diameter give the importance and existence of this invariance kernel. The larger the diameter size is, the slower will be the convergence towards equilibrium point and vice versa (cf. Section 4). From the results, it is clear that by varying the length and diameter, the stability of the invariance kernel can be increased or decreased. The results also show that it is possible that for certain valuations of the parameters the invariance kernel will not exist. In general, we are now able to show, when a set of paths in the hybrid model of BRN is empty. Our current work consists in applying the principles described here to more complex regulation processes, like the network of carbon starvation response in the bacterium Escherichia coli. The discrete model of this network contains 810 qualitative states and some relevant results are obtained, specifically about the lengths of some cycles.
References


Notes

1The trajectories evolve in a parallel fashion because of the unique rate of each clock in a zone.

2A plain cycle of an invariance kernel has the minimum period.