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Modeling Biological Regulatory Networks with Singular States

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Abstract : In the field of biological regulation, models extracted from experimental works are usually complex networks comprising intertwined feedback circuits. R. Thomas and coworkers introduced a qualitative description of such regulatory networks, then they used the concept of circuit-characteristic states to identify all steady states and functional circuits. These characteristic states play an essential role for the dynamics of the system, but they are not represented in the state graph. In this paper we present an extension of this formalization in which all singular states including characteristic ones are represented. Consequently, the state graph contains all steady states. We easily demonstrate in our qualitative modeling the previously demonstrated theorems giving the conditions for steadiness of characteristic states. We also prove that this new modeling is coherent with all the dynamics of the Thomas modeling since all paths of the Thomas dynamics are represented in the new state graph, which in addition shows the influence of singular states in the dynamics.

Keywords : biological networks, feedback circuits, singular states, steady states.

1 Introduction

It is now becoming clear for a lot of researchers that to elucidate the fundamental principles that govern how genomic information translates into organismal complexity, one has to overcome the current habit of ad hoc explanations and instead embrace novel and formal concepts that will involve computer modeling[9]. These new approaches form the *systems biology*[30] which tends to deal with functioning of modular circuits, including their robustness, design and manipulation[12, 10, 8]. Computational systems biology addresses questions fundamental to our understanding of life. For this, we need to establish methods and techniques that enable us to understand biological systems as systems, which means to understand : the structure of the system, such as gene/metabolic/signal transduction networks and physical structures, the dynamics of such systems, methods to control systems, and methods to design and modify systems to generate desired properties[11].

Most modeling approaches deal with simulation of a recreated living system with computer in which are put as much as possible details. Traditionally, biochemical systems are modeled using kinetics and differential equations, using the mathematical language of dynamic systems[27], in a quantitative simulator.

Biological regulatory systems often are complex networks comprising several intertwined feedback circuits. The behavior of such systems is extremely anti-intuitive and cannot be solved without adequate formalization. They can be accurately described by non-linear ordinary differential equations which, however, cannot be solved analytically. The discrete approach developed by R. Thomas for describing biological regulatory networks extracts the essential qualitative features of the dynamics of such systems. But this description does not consider explicitly all the steady states.

In this paper we provide an extension of R. Thomas modeling which considers also *singular states* leading to represent all the steady states. Then we study how the introduction of singular states gives a new light on the properties of characteristic states of feedback circuits. In section 2 we present the continuous dynamics of biological regulatory networks based on ordinary differential equations which constitute the common grounds of our and R. Thomas' qualitative approaches. Then we introduce our discretization map, different from the R. Thomas one, which links qualitative descriptions to the continuous one. Section 3 treats of the discretization of the continuous dynamics leading to the definition of the state graphs. We also define the resources and the qualitative parameters which allows us to define the qualitative dynamics independently of the continuous system. In section 4 we define the characteristic states of feedback circuits in a qualitative and formal manner. They are singular and play a major role since they make a circuit functional when one of them is steady. We show that the conditions of steadiness of characteristic states, proved by R. Thomas, are similar in our modeling. After having given some comparisons between both modelings, we prove that the R. Thomas' state graph is in a certain sense included in our qualitative state graph. Finally in section 5 perspectives are presented.

2 Qualitative values and qualitative regulatory networks

Interactions between biological entities, often macromolecules or genes, are classically represented by labelled graphs, where vertices abstracts biological entities and edges their interactions. If the interaction is an inhibition (resp. activation), the label is $-$ (resp. $+$). This static representation is formally defined as following.

Definition 1 (Regulatory network) *A regulatory graph is a labelled directed graph $G = (V, E)$ where :*
- *each vertex v of V, called* variable*, represents a biological entity,*
- *each edge $(v_1 \to v_2)$ of E is labelled with a sign of the interaction $\alpha_{uv} \in \{+, -\}$.*

In the sequel we denote, for each vertex $v \in V$, $G^-(v)$ (resp. $G^+(v)$) the set of predecessors (resp. successors) of v, and $\#E$ denotes the cardinal of the set E.
We now present the continuous dynamics of such systems based on ordinary differential equations. This kind of approaches has been fruitfully applied to different systems [29, 21, 3, 28] and will be the grounds of the qualitative approach first introduced by Thomas. To each variable v is associated a continuous variable $x_v \in \mathbb{R}^+$ which represents its concentration. At a given time, each variable x_v, $v \in V$ has a unique concentration and the vector x composed of all variables defines the state of the regulatory network : $x = (x_v)_{v \in V}$. The evolution of the system is thus given by the following system of ordinary differential equation :

$$\frac{\mathrm{d}x_v}{\mathrm{d}t} = \mathcal{S}_v(x) - \lambda_v x_v, \qquad \forall v \in V \tag{1}$$

where $\lambda_v > 0$ and $\mathcal{S}_v(x)$ represent respectively the degradation coefficient and the synthesis rate of the variable v. The synthesis rate $\mathcal{S}_v : \mathbb{R}_+^{\#V} \to \mathbb{R}_+$ is often defined by :

$$\mathcal{S}_v(x) = \sum_{u \in G^-(v)} \mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) \tag{2}$$

where the function $\mathcal{I}^{\alpha_{uv}} : \mathbb{R}_+^2 \to \mathbb{R}_+$ describes the influence of a regulator u on the synthesis rate of v. α_{uv} and θ_{uv} are respectively the sign and the threshold of the interaction $u \to v$. Indeed for the majority of the biological interactions, under a certain threshold θ_{uv} of the concentration of u, the interaction $u \to v$ has a quasi null effect on v, and a saturated effect over it. More precisely the function $\mathcal{I}^{\alpha_{uv}}$ is near 0 on one side of the threshold and near the saturation effect $k_{uv} > 0$ on the other, it can be represented by a sigmoid as a Hill function (see figure 1). In such a case, the threshold is the inflexion point of the Hill function.

With a such non linear description of interactions the system 1 has no analytical solution. The solution can be numerically approximated but the precision may be misleading[25] because the parameters are most often unknown. Thomas and Snoussi [17] proposed to estimate the sigmoid function $\mathcal{I}^{\alpha_{uv}}$ by the step function $\widetilde{\mathcal{I}}^{\alpha_{uv}}$ (figure 1) defined by :

$$\widetilde{\mathcal{I}}^+(x_u, \theta_{uv}) = \begin{cases} 0 & \text{if } x_u < \theta_{uv} \\ k_{uv} & \text{if } x_u > \theta_{uv} \end{cases} \qquad \widetilde{\mathcal{I}}^-(x_u, \theta_{uv}) = \begin{cases} k_{uv} & \text{if } x_u < \theta_{uv} \\ 0 & \text{if } x_u > \theta_{uv} \end{cases}$$

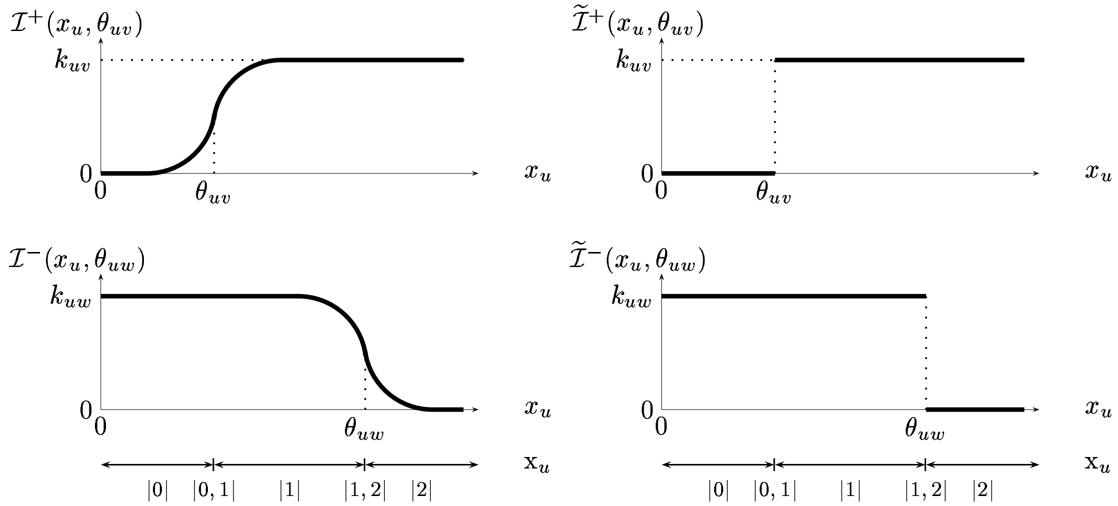


FIG. 1 – Approximation of sigmoids by step functions and discretization

In such a case the system 1 has an analytic solution on each interval where the synthesis rates are constant functions. But with this approximation, $\widetilde{\mathcal{I}}^{\alpha_{uv}}$ is undefined when $x_u = \theta_{uv}$. A state in which there is at least one variable on a threshold is thus called a *singular* state. To define the system 1 for the singular states Snoussi and Thomas represent the *uncertain* influence of u on v when $x_u = \theta_{uv}$ by an open interval : $\widetilde{\mathcal{I}}^{\alpha_{uv}}(\theta_{uv}, \theta_{uv}) =]0, k_{uv}[$. This interval represents the set of possible influences of u on v strictly included between the case where u acts on v ($x_u > \theta_{uv}$) and the case where it does not ($x_u < \theta_{uv}$). Then the system has to be seen as a system of differential inclusions[5] :

$$\tfrac{dx_v}{dt} \in \mathcal{S}_v(x) - \lambda_v x_v, \qquad \forall v \in V, \qquad \text{with} \qquad \mathcal{S}_v(x) = \textstyle\sum_{u \in G^-(v)} \widetilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) \tag{3}$$

We now introduce our qualitative approach based on a notion of discretization of this continuous description. Before defining the discretization map which gives the qualitative concentration of a continuous one, let us introduce some definitions. In our qualitative approach we modelize the evolution of variables even if the regulators are on thresholds. We then introduce the *qualitative values* which can represent the concentration of variables even if it is on a threshold.

Definition 2 (Qualitative Values)

- *A qualitative value, noted* $|a, b|$ *is a couple of integers* ($|a, b| \in \mathbb{N}^2$) *where* $a \leq b$. *The set of qualitative values is noted* Q.
- *The relations* $=, <, >, \subseteq$ *are defined for 2 qualitative values* $|a, b|$ *and* $|c, d|$:
  - $|a, b| = |c, d|$ *if* $(a = c)$ *and* $(b = d)$.
  - $|a, b| < |c, d|$ *if* $(b < c)$ *or* $(b = c$ *and* $(a < b$ *or* $c < d))$
  - $|a, b| > |c, d|$ *if* $|c, d| < |a, b|$
  - $|a, b| \subseteq |c, d|$ *if* $\begin{cases} |a, b| = |c, d| \text{ or} \\ (a = b) \text{ and } (c < a) \text{ and } (b < d) \text{ or} \\ (a < b) \text{ and } (c \leq a) \text{ and } (b \leq d). \end{cases}$

The intuition of the qualitative values is the following. On one hand, if $a < b$ then $|a, b|$ represents the open interval $]a, b[$. On the other hand, if $a = b$, the qualitative value is similar to the close interval $[a, b]$ which contains only the value a. Then two open intervals are comparable if they are not overlapping : $]a, b[<]c, d[$ if $b < c$ and $]a, b[>]c, d[$ if $a > d$. The relation $\subseteq$ is simply the inclusion relation on intervals. The previous definition leads to two kinds of qualitative values : a qualitative value $|a, b|$ is said *regular* if $a = b$, it is said *singular* otherwise. The notation $|a|$ denotes the regular qualitative value $|a, a|$.

Let us now introduce for each variable u the set of out-thresholds defined by $\Theta_u = \{\theta_{uv} : v \in G^+(u)\}$. The thresholds of Θ_u are ranked from the smallest to the largest : $\Theta_u = \{\theta_u^1, \theta_u^2, ..., \theta_u^{b_u}\}$ where θ_u^i is the i-th smallest value of Θ_u and b_u is the cardinal of Θ_u. We have $\theta_u^1 < \theta_u^2 < ... < \theta_u^{b_u}$.

Definition 3 (Discretization map) *The discretization map* $d_u : \mathbb{R}_+ \to \mathrm{Q}$ *which associates a qualitative value to each concentration of variable* u, *is defined as follows :*

$$\mathrm{x}_u = d_u(x_u) = \begin{cases} |q| & \text{if } \theta_u^q < x_u < \theta_u^{q+1} \\ |q-1, q| & \text{if } x_u = \theta_u^q \end{cases} \quad \text{where} \quad \theta_u^0 = -\infty \quad \text{and} \quad \theta_u^{b_u+1} = +\infty$$

Property 1 *The function* $d_v : \mathbb{R}_+ \to \mathrm{Q}$ *is an incresing function.*

The proof is just the application of the definition 2.

To understand why d_u gives the qualitative behavior of u, let us consider a regulatory network in which u acts positively on v and negatively on w. Let us suppose that $\theta_{uv} < \theta_{uw}$ (figure 1). $\mathrm{x}_u = |0|$ means that u does not act neither on v nor on w, $\mathrm{x}_u = |0, 1|$ means that u does not act on w and acts uncertainly on v, $\mathrm{x}_u = |1|$ means that u acts only on v, $\mathrm{x}_u = |1, 2|$ means that u acts on v and acts uncertainly on w and finally $\mathrm{x}_u = |2|$ means that u acts on both.

More generally let us suppose that the number of out-thresholds of u (cardinal of Θ_u) is b_u. The qualitative value x_u can have $2b_u + 1$ different values : $b_u + 1$ regular qualitative values $\mathrm{x}_u = |q|, q \in \{0, ..., b_u\}$ which indicate that u effectively acts on all targets t for which we have $\theta_{ut} \leq \theta_u^q$, and b_u singular qualitative values $\mathrm{x}_u = |q, q+1|, q \in \{0, ..., b_u - 1\}$, which indicate that u effectively acts on the same targets t and that for all targets t such as $\theta_{ut} = \theta_u^{q+1}$, the regulation is uncertain. In other words when a variable has a concentration on a threshold (has a singular qualitative value) its influence on the associated target is uncertain.

Definition 4 (Qualitative regulatory network) *A qualitative regulatory network, denoted by* QR *is a regulatory network* $G = (V, E)$ *in which*

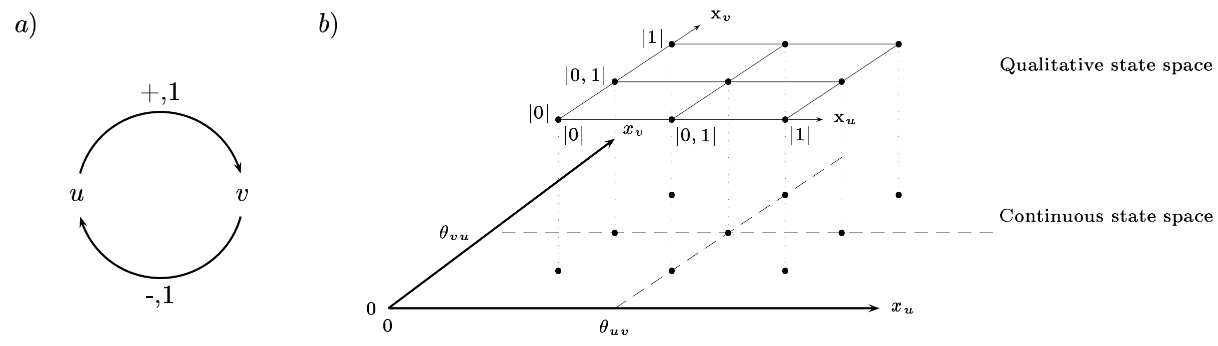


FIG. 2 – *Qualitative regulatory network (a) and its qualitative state space (b)*

- *to each vertex u, is associated a bound* $b_u \leq \#G^+(u)$*,*
- *each interaction* $u \to v \in E$ *is labelled by a couple* $(\alpha_{uv}, q_{uv}) \in \{-,+\} \times \{1,...,b_u\}$*, such that if* $q_{uv} > 1$ *then* $\exists w \in G^+(u)$ *such that* $q_{uw} = q_{uv} - 1$.

q_{uv} *is called the* threshold rank *and the qualitative threshold* s_{uv} *of the interaction* $u \to v$ *is defined by* $\mathrm{s}_{uv} = |q_{uv} - 1, q_{uv}|$.

Note that for each vertex u there exists a surjective function f_u from $G^+(u)$ to $\{1, 2, ..., b_u\}$ such that $f_u(v) = q_{uv}$. In a practical point of view we associate to each variable a bound b_u. Then with a surjective function from the set of all edges going out from u to the set $\{1, 2, ..., b_u\}$, we associate to each edge from u the threshold rank. The qualitative threshold on an edge is directly deduced from its rank. This construction of qualitative regulatory networks is then independent of the differential equations. Contrary to the continuous approach, for a given regulatory network $G = (V, E)$, there is a finite number of qualitative regulatory networks.

Definition 5 (Qualitative state and regular/singular states) *Let* QR *be a qualitative regulatory network built on* $G = (V, E)$.

- *The set of possible values of* x_u *is noted* Q_u : $\mathrm{Q}_u = \{|0|, |0,1|, |1|..., |q-1|, |q-1,q|, |q|, ..., |b_u|\}$.
- *The qualitative state* x *of the network is the vector composed by qualitative concentrations* x_v *associated to each vertex* $v \in V$: $\mathrm{x} = (\mathrm{x}_v)_{v \in V}$. *The qualitative state* x *belongs to the finite space of qualitative states* $\mathrm{Q}_{\mathrm{QR}} = \prod_{v \in V} \mathrm{Q}_v$.
- *A state is* singular *if one of its coordinates is singular, otherwise it is* regular.

Figure 2 gives an example of a qualitative regulatory network. Its qualitative state space is composed of 9 states, 4 regular states and 5 singular ones. Since the network contains only two variables, the 4 regular states correspond to the 4 open domains in the continuous space, and the 5 others to 4 segments and one point.

In the modeling of R. Thomas, the qualitative concentration of a variable is an integer given by the following discretization function $d_v^{RT} : \mathbb{R}_+ \to \mathbb{N} : d_v^{RT}(x_v) = q$ iff $\theta_v^q < x_v < \theta_v^{q+1}$ with $\theta_v^0 = -\infty$ and $\theta_v^{b_v+1} = +\infty$. Thus the qualitative concentrations of the Thomas model are integers $\{0, ..., q, ..., b_v\}$ corresponding to the regular values of our model $\{|0|, ..., |q|, ..., |b_v|\}$. In other words if $x_v \notin \Theta_v$ then $d_v(x_v) = |d_v^{RT}(x_v)|$. The singular states are not represented in the Thomas approach. Yet since they can be stable, they can play an important role for the dynamics of the networks (see following section).

3 The dynamics of regulatory networks

The discretization map will be able to extract the essential qualitative features of the continuous dynamics. We first present briefly the analytic solution when the interactions are approximated by step functions. In such a case the different thresholds define domains in which the synthesis rates are constant. Let us introduce two functions $\mathcal{D}_v : \mathrm{Q}_v \to \mathcal{P}(\mathbb{R}_+)$ and $\mathcal{D} : \mathrm{Q}_{\mathrm{QR}} \to \mathcal{P}(\mathbb{R}_+)^{\#V}$ defined by :

$$\mathcal{D}_v(\mathrm{x}_v) = \{x_v \in \mathbb{R}_+ : d_v(x_v) = \mathrm{x}_v\} \qquad \text{and} \qquad \mathcal{D}(\mathrm{x}) = (\mathcal{D}_v(\mathrm{x}_v))_{\mathrm{x}_v \in \mathrm{x}}$$

where $\mathcal{P}(\mathcal{E})$ will denote the set of parts of the set $\mathcal{E}$. These functions give respectively the set of continuous concentrations and the set of continuous states for which the discretization corresponds to the qualitative value x_u and to the qualitative state x. $\mathcal{D}_v(\mathrm{x}_v)$ and $\mathcal{D}(\mathrm{x})$ are called the *domains* of x_v and x.

If x is a qualitative regular state, $\forall x_u \in \mathcal{D}_u(\mathrm{x}_u)$ and $\forall v \in G^+(u)$, $\widetilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) \in \{0, k_{uv}\}$ because $x_u \notin \Theta_u$. $u \in V$. Thus we can deduce that $\forall x \in \mathcal{D}(\mathrm{x})$ and $\forall v \in V$, the synthesis rate $\mathcal{S}_v(x)$ is constant. Since the degradation $\lambda_v x_v$ of v is linear, the differential equation system 1 has one solution on each domain $\mathcal{D}(\mathrm{x})$ corresponding to each regular state. If the initial state is $x^0 \in \mathcal{D}(\mathrm{x})$, the solution is :

$$x_v(t) = \mathcal{X}_v(x^0) - (\mathcal{X}_v(x^0) - x_v^0)e^{-\lambda_v t}, \qquad \forall v \in V$$

where $\mathcal{X}_v(x) = \frac{\mathcal{S}_v(x)}{\lambda_v}$. Thus all continuous states of the domain $\mathcal{D}(\mathrm{x})$ tend to the same constant state $\mathcal{X}(x^0) = (\mathcal{X}_v(x^0))_{v \in V}$ that is called the *attractor* of the domain $\mathcal{D}(\mathrm{x})$. If $\mathcal{X}(x^0) \in \mathcal{D}(\mathrm{x})$, all states of $\mathcal{D}(\mathrm{x})$ will never go out of the domain $\mathcal{D}(\mathrm{x})$ and they will reach (in $+\infty$) the continuous steady state $\mathcal{X}(x^0)$. Otherwise if $\mathcal{X}(x^0) \notin \mathcal{D}(\mathrm{x})$, then a state x of $\mathcal{D}(\mathrm{x})$ will evolve towards $\mathcal{X}_v(x^0)$ up to go out of the domain $\mathcal{D}(\mathrm{x})$. Outside the domain, the solution of the system is not the same and the attractor is modified. In such a case the state $\mathcal{X}_v(x^0)$ can be never be reached. We define naturally the qualitative attractor of a variable x_v according to the regular qualitative state x as the discretization of the attractor $\mathcal{X}_v(x)$:

$$\mathrm{X}_v(\mathrm{x}) = d_v(\mathcal{X}_v(x)), \quad \forall x \in \mathcal{D}(\mathrm{x}). \tag{4}$$

If x is a qualitative singular state, there is at least one variable u such that for all continuous states x in $x \in \mathcal{D}(\mathrm{x})$ we have $x_u \in \Theta_u$. There exists v such that $x_u = \theta_{uv}$. Then $\widetilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) =]0, k_{uv}[$ so $\mathcal{X}_v(x)$ is an interval. Thus for generalizing the qualitative attractor of equation 4 to a singular qualitative state we define the discretization of an interval as follows : $d_v(]a, b[) = |d_v(a), d_v(b)|$. If a and b are not elements of Θ_v, $|d_v(a), d_v(b)|$ is equal to $||q_1|, |q_2||$ with $q_1 \leq q_2$ because $a \leq b$ and d_v is an increasing function. Identifying qualitative values $|q_1|$ and $|q_2|$ to integers q_1 and q_2 (section 2), we pose $||q_1|, |q_2|| = |q_1, q_2|$. Thus the attractor of a uncertainly regulated variable is defined.

The attractor of a qualitative variable x_v does not give the value at the next step, but only the tendence. If $\mathrm{x}_v < \mathrm{X}_v(\mathrm{x})$ the variable tends to increase, if $\mathrm{x}_v > \mathrm{X}_v(\mathrm{x})$ the variable tends to decrease, and if $\mathrm{x}_v \subseteq \mathrm{X}_v(\mathrm{x})$ the variable is steady. Then one considers that a state x is steady if all variables are steady. With this definition of steadiness we prove the following theorem.

Theorem 1 *A qualitative state* x *is steady iff there is a continuous steady state in* $\mathcal{D}(\mathrm{x})$.

Proof : Let QR be a qualitative regulatory network built on $G = (V, E)$, let x be a qualitative state and let x be a continuous one. Using definition of the steadiness given in [19] x is steady if :

$$x_v = \mathcal{X}_v(x) \text{ if } x_v \notin \Theta_v \qquad \text{and} \qquad x_v \in \mathcal{X}_v(x) \text{ if } x_v \in \Theta_v, \qquad \forall v \in V$$

If $x \in \mathcal{D}(\mathrm{x})$ is steady then x is steady because for all $v \in V$:

1. If $x_v \notin \Theta_v$, we have $x_v = \mathcal{X}_v(x)$ so $d_v(x_v) = d_v(\mathcal{X}_v(\mathrm{x}))$ which is equivalent to $\mathrm{x}_v = \mathrm{X}_v(\mathrm{x})$.
2. If $x_v \in \Theta_v$, $\mathrm{x}_v = d_v(x_v)$ is a singular qualitative value and we have $x_v \in \mathcal{X}_v(x) =]a, b[$. Since the function d_v is increasing, $d_v(a) \leq d_v(x) \leq d_v(b)$. Then $d_v(x_v) \subseteq |d_v(a), d_v(b)|$ (see definition 2) that is $d_v(x_v) \subseteq d_v(]a, b[)$ which is equivalent to $\mathrm{x}_v \subseteq \mathrm{X}_v(\mathrm{x})$.

If x is steady there is one continuous steady state in $\mathcal{D}(\mathrm{x})$ because for all v in V :

1. if x_v has a regular value $\mathrm{x}_v = |q|$ then $\mathrm{x}_v = \mathrm{X}_v(\mathrm{x}) \iff d_v(x_v) = d_v(\mathcal{X}_v(x))$ for all $x_v \in \mathcal{D}_v(\mathrm{x}_v)$ and $x \in \mathcal{D}(\mathrm{x})$. As $x_v \notin \Theta_v$ and $\mathcal{X}_v(x) \in \mathcal{D}_v(\mathrm{x}_v)$, if $x_v = \mathcal{X}_v(x)$, x_v is steady.
2. if x_v has a singular value $\mathrm{x}_v = |q-1, q|$ then

$$\begin{aligned}
\mathrm{x}_v \subseteq \mathrm{X}_v(\mathrm{x}) &\iff |q-1, q| \subseteq |a, b| \text{ with } \mathrm{X}_v(\mathrm{x}) = |a, b| \\
&\iff |a| < |q-1, q| < |b| \\
&\iff d_v(\alpha) < d_v(x_v) < d_v(\beta) \text{ with } x_v = \theta_v^q \text{ and } \forall (\alpha, \beta) \in \mathcal{D}_v(|a|) \times \mathcal{D}_v(|b|)
\end{aligned}$$

So $\alpha < x_v < \beta \iff x_v \in]\alpha, \beta[\iff x_v \in \mathcal{X}_v(x)$ with $x \in \mathcal{D}(\mathrm{x})$. Thus x_v is steady.

□

Since the function $\mathrm{X}_v : \mathrm{Q}_{\mathrm{QR}} \to \mathrm{Q}$ gives the attractor of a qualitative variable x_v according to the qualitative state x, it would be more suitable to express X_v as a function of the qualitative state x independently of the continuous state $x \in \mathcal{D}(\mathrm{x})$. In this perspective let us define the regular/singular resources and the qualitative parameters.

Definition 6 (Regular/singular resources) *Let* QR *be a qualitative regulatory network built on* $G = (V, E)$ *and* $v \in V$.

- *The set of regular resources* $\mathrm{R}_v(\mathrm{x})$ *of* v *according to the state* x *is the set of predecessors of* v *which acts positively on* v *(effective activators or non effective inhibitors) :*

$$\mathrm{R}_v(\mathrm{x}) = \{u \in G^-(v) : (\mathrm{x}_u > \mathrm{s}_{uv} \text{ and } \alpha_{uv} = +) \text{ or } (\mathrm{x}_u < \mathrm{s}_{uv} \text{ and } \alpha_{uv} = -)\}$$

- *The set of singular resources* $\mathrm{S}_v(\mathrm{x})$ *of* v *according to the state* x *is the set of predecessors of* v *which acts uncertainty on* v *:*

$$\mathrm{S}_v(\mathrm{x}) = \{u \in G^-(v) : \mathrm{x}_u = \mathrm{s}_{uv}\}$$

Note that $u \in \mathrm{R}_v(\mathrm{x})$ if and only if $\widetilde{\mathcal{I}}^{\alpha_{uv}}(x_u, \theta_{uv}) = k_{uv}$ and $u \in \mathrm{S}_v(\mathrm{x})$ if and only if $\widetilde{\mathcal{I}}^{\alpha_{uv}}(x_v, \theta_{uv}) =]0, k_{uv}[$.

Definition 7 (Qualitative parameters) *Let* QR *be a qualitative regulatory network built on* $G = (V, E)$ *and* $v \in V$. *The qualitative parameters* $\mathrm{K} = \{\mathrm{K}_{v,\omega}\}$ *is a family of integers indexed by couples* (v, ω) *such that :*

- v *belongs to* V *and* ω *is a subset of* $G^-(v)$
- $\mathrm{K}_{v,\omega} = 0$ *if* $\omega = \emptyset$ *and* $\mathrm{K}_{v,\omega} \in \{0, ..., b_v\}$ *otherwise.*
- $\omega \subseteq \omega' \Longrightarrow \mathrm{K}_{v,\omega} \leq \mathrm{K}_{v,\omega'}$

The definition 7 allows us to set down $|\mathrm{K}_{v,\omega}| = d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v})$, because

1. if $\omega = \emptyset$ then $d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) = d_v(0) = |0|$ else $d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \in \{|0|, |1|, ..., |b_v|\}$ under the hypothesis that $(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \notin \Theta_v$.
2. if $\omega \subseteq \omega'$ then $(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \leq (\sum_{u\in\omega'} \frac{k_{uv}}{\lambda_v})$, and since d_v is an increasing function, $d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \leq d_v(\sum_{u\in\omega'} \frac{k_{uv}}{\lambda_v})$.

Then it is possible to define the function $\mathrm{X}_v : \mathrm{Q}_{\mathrm{QR}} \to \mathrm{Q}$ as a function of regular/singular resources and of the qualitative parameters $\{\mathrm{K}_{v,\omega}\}_{\omega\subseteq G^-(v)}$.

Theorem 2 *Setting* $|\mathrm{K}_{v,\omega}| = d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v})$ *the function* X_v *is given by* $\mathrm{X}_v(\mathrm{x}) = \left|\mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})}, \mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})}\right|$

Proof : Using equations 2 and 4, we have $\mathcal{X}_v(x) = \frac{\sum_{u\in G^-(v)} \widetilde{\mathcal{I}}^{\alpha_{uv}}(x_u,\theta_{uv})}{\lambda_v}$. The contribution of all predecessors which are not resources (regular or singular), is null. So we can write : $\mathcal{X}_v(x) = (\sum_{u\in\mathrm{R}_v(\mathrm{x})} k_{uv}/\lambda_v) + \sum_{u\in\mathrm{S}_v(\mathrm{x})}]0, k_{uv}[/\lambda_v$ with the convention $\sum_{u\in\emptyset} a = 0$. If $\mathrm{S}_v(\mathrm{x})$ is empty, then $\mathcal{X}_v(x) = \sum_{u\in\mathrm{R}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}$. Thus $\mathrm{X}_v(\mathrm{x}) = d_v(\sum_{u\in\mathrm{R}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}) = |\mathrm{K}_{v,\omega}| = |\mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})}, \mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})}|$.

On the other hand, if $\mathrm{S}_v(\mathrm{x})$ is not empty, $\mathcal{X}_v(x) = (\sum_{u\in\mathrm{R}_v(\mathrm{x})} k_{uv}/\lambda_v) + \left]0, \sum_{u\in\mathrm{S}_v(\mathrm{x})} k_{uv}/\lambda_v\right[$. This can be written as $\mathcal{X}_v(x) = \left]\sum_{u\in\mathrm{R}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}, \sum_{u\in\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}\right[$. Thus

$$\begin{aligned}
\mathrm{X}_v(\mathrm{x}) &= d_v\left(\left]\sum_{u\in\mathrm{R}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}, \sum_{u\in\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}\right[\right) \\
\mathrm{X}_v(\mathrm{x}) &= \left|d_v\left(\sum_{u\in\mathrm{R}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}\right), d_v\left(\sum_{u\in\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}\right)\right| \\
\mathrm{X}_v(\mathrm{x}) &= \left||\mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})}|, |\mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})}|\right| \\
\mathrm{X}_v(\mathrm{x}) &= \left|\mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})}, \mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})}\right|
\end{aligned}$$

□

With this theorem the qualitative regulatory network is sufficient to define the attractor of each state of the networks. We deduce from these attractors the tendency of each variable that allows us to define the dynamics of the network expressed with the following state graph.

Definition 8 (Asynchronous state graph associated to a qualitative model) *Let* QR *be a qualitative regulatory network built on* $G = (V, E)$. *The asynchronous state graph (or state graph for short)* SG *associated to a model of* QR, *is a directed graph* $\mathrm{SG} = (\mathrm{S}, \mathrm{T})$ *where* S *is the set of qualitative states of* QR, *and* T *is the set of transitions between qualitative states such that :*

1. $\mathrm{x}^a \to \mathrm{x}^a \in \mathrm{T}$ *if* x^a *is steady.*
2. $\mathrm{x}^a \to \mathrm{x}^b \in \mathrm{T}$ *if* $\exists\, v \in V$ *such that* $\begin{cases} \mathrm{x}^b_v = \Delta^+_v(\mathrm{x}^a_v) \text{ if } \mathrm{x}^a_v < \mathrm{X}_v(\mathrm{x}^a) \\ \mathrm{x}^b_v = \Delta^-_v(\mathrm{x}^a_v) \text{ if } \mathrm{x}^a_v > \mathrm{X}_v(\mathrm{x}^a) \end{cases}$ *and* $\mathrm{x}^b_u = \mathrm{x}^a_u\ \forall\, u \in V \setminus \{v\}$

where Δ_v^+ and Δ_v^- are the evolution operators defined as following :

$$\Delta_v^+(\mathrm{x}_v) = \begin{cases} |q, q+1| \ \textit{if}\ \mathrm{x}_v = |q| \\ |q| \ \textit{if}\ \mathrm{x}_v = |q-1, q| \end{cases} \quad \textit{and} \quad \Delta_v^-(\mathrm{x}_v) = \begin{cases} |q-1, q| \ \textit{if}\ \mathrm{x}_v = |q| \\ |q| \ \textit{if}\ \mathrm{x}_v = |q, q+1| \end{cases}$$

To explain this definition we have to notice that the attractor defines the state towards which the system tends to evolve. We consider that two variables cannot evolve simultaneously, that is why the state graph is said asynchronous. When several variables tend to evolve at a given state, additional information is needed to select which one first changes. In fact it is the knowledge of time delays associated to each variation of variables, which defines which one effectively evolve first[25]. As we have no information about time delays, all possible variations are considered since all of them could occur first. As a consequence a state for which n variables tend to evolve, has n successors.

Practically to built a state graph associated to a qualitative regulatory network built on $G = (V, E)$, we have to instantiate the qualitative parameters $\mathrm{K} = \{\mathrm{K}_{v,\omega} : v \in V, \omega \subseteq G^-(v)\}$. The data composed of a qualitative regulatory network and an instantiation of its parameters is called a *model*. Note that there is a finite number of models associated to a qualitative regulatory network, since the number of possible instantiations of parameters is itself finite. Thus, the qualitative approach allows with a finite number of models to study the qualitative features of the infinity of continuous dynamics associated to a regulatory network.

In the Thomas' approach, the logical parameters is defined in the same way. Thus the models associated to a qualitative regulatory network are the same in both approaches (in particular there is the same number of models), but the state graphs deducing from these models are different. Indeed, the attractor of a variable x_v of R. Thomas can be written with our notation by $\mathrm{X}_v^{RT}(\mathrm{x}) = \mathrm{K}_{v,\mathrm{R}_v(\mathrm{x}')}$ where x is a state of R. Thomas (a vector of integers) and where x' is the qualitative regular state identifiable to x ($\mathrm{x}'_v = |\mathrm{x}_v|$ for all $v \in V$). Thus the attractors of the states of Thomas' approach are the attractors of our regular states : $\mathrm{X}_v(\mathrm{x}') = |\mathrm{X}_v^{RT}(\mathrm{x})|$. Then the R. Thomas' state graph contains only transitions between regular states such that $\mathrm{x}^a \to \mathrm{x}^a$ is a transition if x^a is steady and $\mathrm{x}^a \to \mathrm{x}^b$ is a transition if :

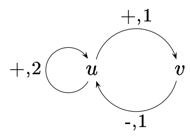
$$\exists\, v \in V \text{ such that } \begin{cases} \mathrm{x}_v^b = \mathrm{x}_v^a + 1 \text{ if } \mathrm{x}_v^a < \mathrm{X}_v^{RT}(\mathrm{x}^a) \\ \mathrm{x}_v^b = \mathrm{x}_v^a - 1 \text{ if } \mathrm{x}_v^a > \mathrm{X}_v^{RT}(\mathrm{x}^a) \end{cases} \text{ and } \quad \mathrm{x}_u^b = \mathrm{x}_u^a \ \forall\, u \in V \setminus \{v\}$$

Let us consider the model corresponding to the qualitative regulatory network of figure 3, it corresponds to the modeling of the mucus production of *Pseudomonas aeriginosa*[14]. The variable u acts positively on v and on itself, and v acts negatively on u. For each possible qualitative state, one can deduce the symbolic attractor expressed as the vector of the qualitative values $\mathrm{X}_v(\mathrm{x}) = \left|\mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})}, \mathrm{K}_{v,\mathrm{R}_v(\mathrm{x})\cup\mathrm{S}_v(\mathrm{x})}\right|, v \in V$. Then the attractor is explicitly computed for the following instanciation of qualitative parameters : $\mathrm{K}_{u,\emptyset} = 0$, $\mathrm{K}_{u,v} = 2$, $\mathrm{K}_{u,u} = 2$, $\mathrm{K}_{u,uv} = 2$, $\mathrm{K}_{v,\emptyset} = 0$ and $\mathrm{K}_{v,u} = 1$. Finally the definition 8 allows us to construct the asynchronous state graph. It is compared to the R. Thomas' state graph obtained with the same parameters. One can notice that our state graph contains 2 more steady states than the R. Thomas' state graph which are thus steady qualitative singular states.

4 Functionality of feedback circuits

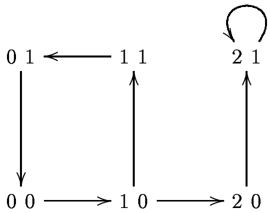
Most often, there is a huge number of models associated to a regulatory network. Indeed, for each $v \in V$, the number of possible instantiations of all parameters $K_{v,\omega}$ is exponential according to the number of parameters $K_{v,\omega}$. Moreover the number of parameters $K_{v,\omega}$ associated to v is also an exponential function of the number of its predecessors. Then, the major issue of the modeling activity is to select the suitable set of parameters which give a qualitative behavior coherent with the experimental knowledge on the system. Three different kinds of dynamic properties are often used to aid the selection of suitable models : steady states, multistationarity and homeostasis. The selection of models which presents a given set of steady states remains a simple application of definition of steady states (see section 3). On the other hand the two other dynamic properties are not directly expressed in term of parameters, and their detection in a given model is not trivial. Hopefully the feedback circuits theory [26] allows us to link dynamic properties (multistationarity and homeostasis) to parameters and to select appropriate models.

In a feedback circuit, each variable has an influence on its target but also an indirect effect on all following elements including itself. A feedback circuit is said *positive* (resp. *negative*) if each variable has a positive (resp. negative) influence on itself. The sign of the circuit is determined by the number of negative interactions : the circuit is negative if the number of negative interactions is odd, otherwise it is positive. It has been shown that it is possible to associate to a feedback circuit a typical dynamic behavior : in a **negative circuit**, a



states x		symbolic attractors		attractors		tendencies	
x_u	x_v	$X_u(x)$	$X_v(x)$	$X_u(x)$	$X_v(x)$	u	v
$\|0\|$	$\|0\|$	$\|K_{u,v}\|$	$\|K_{v,\emptyset}\|$	$\|2\|$	$\|0\|$	$\nearrow$	$\leadsto$
$\|0\|$	$\|0,1\|$	$\|K_{u,\emptyset}, K_{u,v}\|$	$\|K_{v,\emptyset}\|$	$\|0,2\|$	$\|0\|$	$\nearrow$	$\searrow$
$\|0\|$	$\|1\|$	$\|K_{u,\emptyset}\|$	$\|K_{v,\emptyset}\|$	$\|0\|$	$\|0\|$	$\leadsto$	$\searrow$
$\|0,1\|$	$\|0\|$	$\|K_{u,v}\|$	$\|K_{v,\emptyset}, K_{v,u}\|$	$\|2\|$	$\|0,1\|$	$\nearrow$	$\nearrow$
$\|0,1\|$	$\|0,1\|$	$\|K_{u,\emptyset}, K_{u,v}\|$	$\|K_{v,\emptyset}, K_{v,u}\|$	$\|0,2\|$	$\|0,1\|$	$\leadsto$	$\leadsto$
$\|0,1\|$	$\|1\|$	$\|K_{u,\emptyset}\|$	$\|K_{v,\emptyset}, K_{v,u}\|$	$\|0\|$	$\|0,1\|$	$\searrow$	$\searrow$
$\|1\|$	$\|0\|$	$\|K_{u,v}\|$	$\|K_{v,u}\|$	$\|2\|$	$\|1\|$	$\nearrow$	$\nearrow$
$\|1\|$	$\|0,1\|$	$\|K_{u,\emptyset}, K_{u,v}\|$	$\|K_{v,u}\|$	$\|0,2\|$	$\|1\|$	$\leadsto$	$\nearrow$
$\|1\|$	$\|1\|$	$\|K_{u,\emptyset}\|$	$\|K_{v,u}\|$	$\|0\|$	$\|1\|$	$\searrow$	$\leadsto$
$\|1,2\|$	$\|0\|$	$\|K_{u,v}, K_{u,uv}\|$	$\|K_{v,u}\|$	$\|2\|$	$\|1\|$	$\nearrow$	$\nearrow$
$\|1,2\|$	$\|0,1\|$	$\|K_{u,\emptyset}, K_{u,uv}\|$	$\|K_{v,u}\|$	$\|0,2\|$	$\|1\|$	$\leadsto$	$\nearrow$
$\|1,2\|$	$\|1\|$	$\|K_{u,\emptyset}, K_{u,u}\|$	$\|K_{v,u}\|$	$\|0,2\|$	$\|1\|$	$\leadsto$	$\leadsto$
$\|2\|$	$\|0\|$	$\|K_{u,uv}\|$	$\|K_{v,u}\|$	$\|2\|$	$\|1\|$	$\leadsto$	$\nearrow$
$\|2\|$	$\|0,1\|$	$\|K_{u,u}, K_{u,uv}\|$	$\|K_{v,u}\|$	$\|2\|$	$\|1\|$	$\leadsto$	$\nearrow$
$\|2\|$	$\|1\|$	$\|K_{u,u}\|$	$\|K_{v,u}\|$	$\|2\|$	$\|1\|$	$\leadsto$	$\leadsto$

R. Thomas' state graphe



Asynchronous state graph

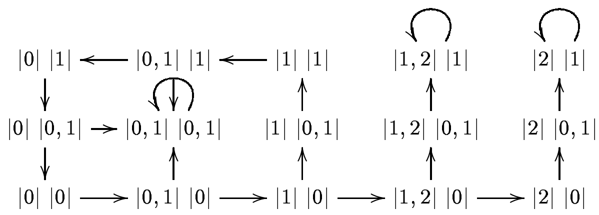


FIG. 3 – An example of regulatory state graph (see text).

high concentration of a variable tends to make decrease itself (and reversely). Thus the circuit makes the concentration of each variable to tend to (or oscillate around) an equilibrium concentration. It then generates homeostasis. In a **positive circuit**, a high (resp. low) concentration of a variable tends to make it increase (resp. decrease). Thus each variable can stay at a low or high concentration and the positive circuit generates multistationnarity. A feedback circuit, which presents a typical dynamic behavior is said 'functional'. Several other properties on the behabior of feedback circuits have been proved : at least one positive regulatory circuit is necessary to generate multistationarity whereas at least one negative circuit is necessary to generate a stable oscillatory behavior[26, 23, 15, 6, 18, 20]. Thus the positive feedback circuit can generate differentiation[22]. One can then demonstrate that m functional disjointed positive feedback circuits generate 3^m steady states of which 2^m are regular.

In the sequel we first introduce the notion of characteristic states of a feedback circuit which play a fundamental role : the steadiness of one of them leads to the functionality of the circuit. We then give the conditions for the steadiness of a singular state : it has to be characteristic and some constraints on parameters have to be verified. The proof of these conditions by Snoussi and Thomas is quite technical in their modeling because they do not explicitly take into account the singular states in the state graph. This leads us to compare the two states graphs for a model which verifies the condition of functionality of some circuits and to give a theorem which shows how the dynamics of Thomas are present in our modeling.

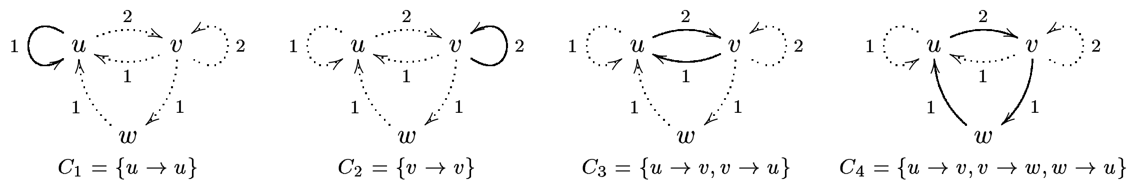
4.1 Characteristic states

A circuit can be described by the finite set of edges which compose it. A characteristic state of a circuit[19] is defined as a state in which u is a singular resource of v iff $(u \to v)$ is an edge of the circuit. This notion of characteristic state can be extended to the union of circuits. Two cases are to be considered : a disjointed union of circuits is a union in which all couple of circuits have no vertex in common, otherwise the union is said jointed.

Definition 9 (Characteristic state of an union of circuits) *Let* QR *be a qualitative regulatory network built on* $G = (V, E)$ *and let* C *an union of circuits. The state* x *is characteristic of the union of circuits if we have : u is a singular resource of v iff $u \to v$ is an edge of the union of circuits, that is* $C = \bigcup_{v \in V} \{u \to v, u \in S_v(x)\}$.

Note that a characteristic state is singular and that when an union of circuits does not contain all variables of the network, several characteristic states are associated to the union. Some examples of circuits with their characteristic states are given in Figure 4. E. H. Snoussi and R. Thomas proved for their formalism that

a)



b)

C_1

x_u	x_v	x_w
$\lvert 0,1\rvert$	$\lvert 0\rvert$	$\lvert 0\rvert$
$\lvert 0,1\rvert$	$\lvert 0\rvert$	$\lvert 1\rvert$
$\lvert 0,1\rvert$	$\lvert 1\rvert$	$\lvert 0\rvert$
$\lvert 0,1\rvert$	$\lvert 1\rvert$	$\lvert 1\rvert$
$\lvert 0,1\rvert$	$\lvert 2\rvert$	$\lvert 0\rvert$
$\lvert 0,1\rvert$	$\lvert 2\rvert$	$\lvert 1\rvert$

C_2

x_u	x_v	x_w
$\lvert 0\rvert$	$\lvert 1,2\rvert$	$\lvert 0\rvert$
$\lvert 0\rvert$	$\lvert 1,2\rvert$	$\lvert 1\rvert$
$\lvert 1\rvert$	$\lvert 1,2\rvert$	$\lvert 0\rvert$
$\lvert 1\rvert$	$\lvert 1,2\rvert$	$\lvert 1\rvert$

$C_1 \cup C_2$

x_u	x_v	x_w
$\lvert 0,1\rvert$	$\lvert 1,2\rvert$	$\lvert 0\rvert$
$\lvert 0,1\rvert$	$\lvert 1,2\rvert$	$\lvert 1\rvert$

$C_3 \cup C_4$

x_u	x_v	x_w
$\lvert 1,2\rvert$	$\lvert 0,1\rvert$	$\lvert 0,1\rvert$

FIG. 4 – Sets of circuits (a) and all possible characteristics states (b) of a regulatory network. The interactions are not labelled by any sign because they do not play a role in the notion of characteristic states. Note that there is no characteristic state for C_3 or for C_4 separately since if v is a singular resource of u, then it is also a singular resource of w (both interactions have the same threshold).

a singular state can be steady only if it characterizes a feedback circuit. This property is preserved in our qualitative modeling.

Property 2 *Among singular states, only characteristic states can be steady.*

Proof : Let QR be a qualitative regulatory network built on $G = (V, E)$ and let x a non characteristic singular state. Then there is an edge $v \to w$ such that v is a singular resource of w and such that all resources of v are regular. Then x_v is a singular value and it can be deduced that the attractor of x_v is a regular qualitative value $X_v(x) = |K_{v,R_v(x)}|$. Since a singular value cannot be contained in a regular qualitative value, $x_v \not\subseteq X_v(x)$, and x cannot be steady. □

4.2 Constraints for functionality of feedback circuits

To select suitable models, one has to translate functionality of feedback circuits in terms of constraints on parameters. It has been proved with the formalism of Thomas that the functionality of feedback circuits is directly linked to the stationnarity of characteristic states.

Theorem 3 *[19] All circuits of a disjointed union are functional if one characteristic state of the union is steady.*

Intuitively a characteristic state of a negative circuit acts as an attractor since for all variables implicated in the circuit, if the concentration is above (resp. below) the out-threshold in the circuit, the negative effect on itself tends to make it decrease (resp. increase). On the other hand, a characteristic state of a positive circuit is unstable, because for each variable of the circuit the slightest departure from the threshold is sufficient to make topple down the variable under or over its threshold.

With the previous theorem 3, the constraints for functionality are equivalent to the constraints for steadiness of characteristic states. In our modeling these constraints are expressed trivially : let x a characteristic state of an union C of circuits. It is steady iff for each variable v, $x_v \subseteq X_v(x)$.

- For each variable u not implicated in C, $S_u(x) = \emptyset$ and $x_u \subseteq X_u(x) \iff x_u = X_u(x) \iff x_u = |K_{u,R_u(x)}|$

- For each variable u implicated in C, we have $\mathrm{S}_u(\mathrm{x}) \neq \emptyset$ and $\mathrm{x}_u = \mathrm{s}_{uv} = |q_{uv}-1, q_{uv}|$ where $u \to v \in C$, then

$$\begin{aligned} \mathrm{x}_u \subseteq \mathrm{X}_u(\mathrm{x}) &\iff |q_{uv}-1, q_{uv}| \subseteq |\mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})}, \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})\cup \mathrm{S}_u(\mathrm{x})}| \\ &\iff \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})} \leq q_{uv}-1 \text{ and } q_{uv} \leq \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})\cup \mathrm{S}_u(\mathrm{x})} \\ &\iff \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})} < q_{uv} \leq \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})\cup \mathrm{S}_u(\mathrm{x})} \end{aligned}$$

Snoussi and Thomas deduced also constraints on parameters from functionality of circuits which are expressed in the following theorem.

Theorem 4 *[19] For a given model, there exists a steady characteristic state associated to a circuit C if there exist two regular qualitative states (in the Thomas model) x^+ and x^- such that :*
- *for each variable u not implicated in C, $\mathrm{x}^-_u = \mathrm{x}^+_u$ and $\mathrm{X}^{RT}_u(\mathrm{x}^\epsilon) = \mathrm{x}^\epsilon_u$ with $\epsilon \in \{+,-\}$*
- *for each variable u implicated in C whose the successor in C is v,*

$$\left\{ \begin{array}{l} \text{if } \alpha_{uv} = + \text{ then } \left\{ \begin{array}{l} \mathrm{x}^+_u = q_{uv} \\ \mathrm{x}^-_u = q_{uv}-1 \end{array}\right. \\ \text{if } \alpha_{uv} = - \text{ then } \left\{ \begin{array}{l} \mathrm{x}^+_u = q_{uv}-1 \\ \mathrm{x}^-_u = q_{uv} \end{array}\right. \end{array} \right. \quad \text{and} \quad \mathrm{X}^{RT}_u(\mathrm{x}^-) < q_{uv} \leq \mathrm{X}^{RT}_u(\mathrm{x}^+)$$

where q_{uv} is the integer labelling the interaction $u \to v$. The states x^+ and x^- are called the adjacent regular states of the characteristic state of the functional circuit which give respectively the minimal and maximal attractors.

The notion of resources permits us to develop the constraints of the previous theorem in terms of parameters. We assimilate both x^+ and x^- of the modeling of Thomas to the corresponding qualitative regular states in our modeling. The steady characteristic state x which has x^+ and x^- for adjacent states, is the only characteristic state of C which verifies $\mathrm{x}_u = \mathrm{x}^-_u = \mathrm{x}^+_u$ for all u not implicated in the circuit. By definition, the resources of each variable of the circuit at the state x^- are not in the circuit. Since $\mathrm{x}_u = \mathrm{x}^-_u$ for all u not implicated in the circuit, we have for all u, $\mathrm{R}_u(\mathrm{x}^-) = \mathrm{R}_u(\mathrm{x})$. In contrast, by definition of the state x^+ each variable implicated in the circuit is a resource of its successor in the circuit. So we have for all u, $\mathrm{R}_u(\mathrm{x}^+) = \mathrm{R}_u(\mathrm{x}) \cup \mathrm{S}_u(\mathrm{x})$ (with $\mathrm{S}_u(\mathrm{x}) \neq \emptyset$ if u is implicated in the circuit and $\mathrm{S}_u(\mathrm{x}) = \emptyset$ otherwise). Thus :
- for each variable u not implicated in C, we have $\mathrm{S}_u(\mathrm{x}) = \emptyset$ and

$$\mathrm{x}^\epsilon_u = \mathrm{X}^{RT}_u(\mathrm{x}^\epsilon) \iff \mathrm{x}^\epsilon_u = |\mathrm{K}_{u,\mathrm{R}_u(\mathrm{x}^\epsilon)}| \iff \mathrm{x}_u = |\mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})}| \qquad \text{with } \epsilon \in \{+,-\}$$

- for each variable u implicated in C whose the successor in C is v, we have $\mathrm{S}_u(\mathrm{x}) \neq \emptyset$ and

$$\mathrm{X}^{RT}_u(\mathrm{x}^-) < q_{uv} \leq \mathrm{X}^{RT}_u(\mathrm{x}^+) \iff \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x}^-)} < q_{uv} \leq \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x}^+)} \iff \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})} < q_{uv} \leq \mathrm{K}_{u,\mathrm{R}_u(\mathrm{x})\cup \mathrm{S}_u(\mathrm{x})}$$

Thus the constraints elaborated for the Thomas model from which the proof is technical is equivalent to the constraints expressed in our modeling.

4.3 Examples and comparison with R. Thomas modeling

In the previous subsections, we highlight that characteristic states play a central role in the circuit functionality and we show that the conditions for functionality are the same in the R. Thomas modeling and in our one. Now we compare the dynamics of models for which some circuits are functional in both modelings. Let us start with a first example which contains a unique vertex which acts on itself positively or negatively :

We deduce from these two loops the following symbolic attractors :

L_- : x_v	$\mathrm{X}_v(\mathrm{x})$		L_+ : x_v	$\mathrm{X}_v(\mathrm{x})$
$\lvert 0 \rvert$	$\lvert \mathrm{K}_{v,v} \rvert$		$\lvert 0 \rvert$	$\lvert \mathrm{K}_{v,\emptyset} \rvert$
$\lvert 0,1 \rvert$	$\lvert \mathrm{K}_{v,\emptyset}, K_{v,v} \rvert$	← characteristic state →	$\lvert 0,1 \rvert$	$\lvert \mathrm{K}_{v,\emptyset}, K_{v,v} \rvert$
$\lvert 1 \rvert$	$\lvert \mathrm{K}_{v,\emptyset} \rvert$		$\lvert 1 \rvert$	$\lvert \mathrm{K}_{v,v} \rvert$

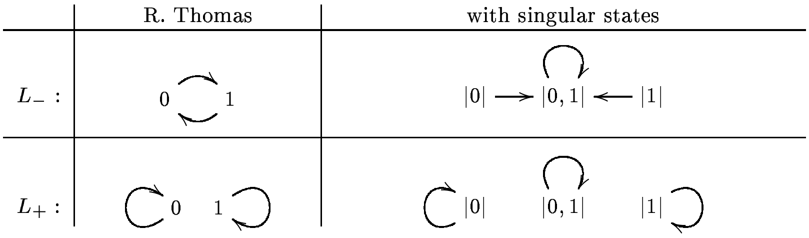
The two loops are functional, if the associated characteristic state $|0,1|$ is steady. In terms of parameters we have :

$$|0,1| \subseteq \mathrm{X}_v(|0,1|) \iff |0,1| \subseteq |\mathrm{K}_{v,\emptyset}, \mathrm{K}_{v,v}| \iff \mathrm{K}_{v,\emptyset} \leq 0 \text{ and } \mathrm{K}_{v,v} \geq 1$$

Thus, $K_{v,\emptyset} = 0$ and $K_{v,v} = 1$ is the only one possible instantiation of parameters for which the loops are functional. The attractors and the tendencies of each state for both models are :

L_- : x_v	$X_v(x)$	Tendencies		L_+ : x_v	$X_v(x)$	Tendencies
$\|0\|$	$\|1\|$	$\nearrow$		$\|0\|$	$\|0\|$	$\leadsto$
$\|0,1\|$	$\|0,1\|$	$\leadsto$	$\leftarrow$ steady characteristic state $\rightarrow$	$\|0,1\|$	$\|0,1\|$	$\leadsto$
$\|1\|$	$\|0\|$	$\searrow$		$\|1\|$	$\|1\|$	$\leadsto$

One then deduces the four state graphs :



In the 4 state graphs, homeostasis or multistationarity induced by the loop functionality is present. The greatest difference of representation between both modelings concerns the negative circuit :

1. The paths of the Thomas' state graph do not correspond to paths between regular states in our state graph. When a characteristic state of a negative loop $v \rightarrow v$ is steady, then the Thomas' state graph is not "contained" in the state graph with singular states (see the property 3 for details).
2. The state graph reflects an softened oscillation towards the characteristic state in our modeling and an infinite oscillation for Thomas modeling.

In the Thomas modeling it is not possible to represent the softening generated by the functionality of negative circuits. That can infer a confusion about the interpretation of the circuit functionality. Let us consider now the qualitative regulatory network of figure 3 containing 2 variables with a negative circuit of length 2 and a positive loop. The following table gives for each characteristic states the constraints for steadiness.

	characteristic states		Symbolic attractors		Contraints for the functionnality	
	x_u	x_v	$X_u(x)$	$X_v(x)$		
Circuit -	$\|0,1\|$	$\|0,1\|$	$\|K_{u,\emptyset}, K_{u,v}\|$	$\|K_{v,\emptyset}, K_{v,u}\|$	$K_{u,v} \geq 1$	$K_{v,u} \geq 1$
Circuit +	$\|1,2\|$	$\|0\|$	$\|K_{u,v}, K_{u,uv}\|$	$\|K_{v,u}\|$	$K_{u,v} \leq 1, K_{u,uv} \geq 2$	$K_{v,u} = 0$
Circuit +	$\|1,2\|$	$\|1\|$	$\|K_{u,\emptyset}, K_{u,u}\|$	$\|K_{v,u}\|$	$K_{u,u} \geq 2$	$K_{v,u} = 1$

The necessary conditions for functionality of both circuits are compatible in the case of steadiness of the second characteristic state of the positive circuits. For example the following instantiation of qualitative parameters $K_{u,\emptyset} = 0$, $K_{u,v} = 2$, $K_{u,u} = 2$, $K_{u,uv} = 2$, $K_{v,\emptyset} = 0$ and $K_{v,u} = 1$ makes functional both circuits and the associated state graphs are depicted in figure 3. Then a multistationarity is predicted (functional positive loop). But in the Thomas' state graph there is only one steady state (the two others are singular) and from each state it is possible to go to it. Then the state graph does not illustrate the predicted multistationarity.

In both state graphs, the paths between regular states are coherent. Indeed, each transition $x^1 \rightarrow x^2$ of the Thomas' state graph corresponds to a path $x^a \rightarrow x^s \rightarrow x^b$ where x^a and x^b are identifiable to x^1 and x^2 and where x^s is a singular state. Note that the network does not contain negative loop (circuit of length 1) then for all models deduced from this network the R. Thomas' state graph is "contained" in our one. This results from the following property.

Property 3 *Let QR be a qualitative regulatory network built on $G = (V, E)$. Let $x^1 \rightarrow x^2$ (with x^1 distinct from x^2) be a transition of a state graph of R. Thomas deducing from a given possible instantiation of parameters and let x_u be the only one variable which evolves during the transition $x^1 \rightarrow x^2$ ($x^1_u \neq x^2_u$). Let us define*

- *x^a and x^b, the qualitative regular states identifiable to x^1 and x^2 ($x^a_v = |x^1_v|$ and $x^b_v = |x^2_v|$ for all v in V)*
- *x^s the qualitative singular state between x^a and x^b ($x^s_v = x^a_v = x^b_v$ for all $v \neq u$ and $x^s_u = |x^1_u, x^2_u|$ if $x^1_u < x^2_u$ and $x^s_u = |x^2_u, x^1_u|$ otherwise).*

Then the qualitative state graph contains the path $x^a \rightarrow x^s \rightarrow x^b$ if x^s_u is not steady (x^s_u is steady imposes that x^s is a characteristic state of the negative loops $u \rightarrow u$).

Proof : Let us set down $x^a_u = |q|$. Since $x^1 \rightarrow x^2$ is a transition of the state graph of R. Thomas and since $x^1 \neq x^2$, $x^1_u \neq X^{RT}_u(x^1) \iff x^a_u \neq |K_{u,R_u(x^a)}| \iff |q| \neq |K_{u,R_u(x^a)}|$.

- If $|q| < |K_{u,R_u(x^a)}|$ then $x^s_u = |q, q+1|$, $x^b_u = |q+1|$, so $x^a \rightarrow x^s$ is a transition of our state graph, and $|q+1| \leq |K_{u,R_u(x^a)}|$.

- If x^s is not a characteristic state then the variable u does not regulate itself or the threshold of the auto-regulation is not equal to $x^s_u = |q, q+1|$. So $S_u(x^s) = \emptyset$ and $X_u(x^s) = |K_{u,R_u(x^s)}| = |K_{u,R_u(x^a)}|$. So $x^s_u = |q, q+1| < |K_{u,R_u(x^a)}|$ and $x^s \to x^b$ is a transition of our state graph.
- If x^s is a characteristic state ($u \to u \in E$ and $s_{uu} = |q, q+1|$) then $S_u(x^s) = \{u\}$.
  - If $\alpha_{uu} = +$ then $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s)\cup\{u\}}| = |K_{u,R_u(x^a)}, K_{u,R_u(x^a)\cup\{u\}}|$. So $x^s_u = |q, q+1| < |K_{u,R_u(x^a)}, K_{u,R_u(x^a)\cup\{u\}}|$ and $x^s \to x^b$ is a transition of our state graph.
  - If $\alpha_{uu} = -$ then $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s)\cup\{u\}}| = |K_{u,R_u(x^a)\setminus\{u\}}, K_{u,R_u(x^a)}|$. So, if x^s_u is not steady we have $x^s_u = |q, q+1| \not\subseteq |K_{u,R_u(x^a)\setminus\{u\},R_u(x^a)}| \implies q < K_{u,R_u(x^a)\setminus\{u\}} \iff q+1 \leq K_{u,R_u(x^a)\setminus\{u\}}$. So $|q, q+1| < |K_{u,R_u(x^a)\setminus\{u\}}, K_{u,R_u(x^a)}|$ and $x^s \to x^b$ is a transition of our state graph.

- If $|q| > |K_{u,R_u(x^a)}|$ then $x^s_u = |q-1, q|$, $x^b_u = |q-1|$, so $x^a \to x^s$ is a transition of our state graph, and $|q-1| \geq |K_{u,R_u(x^a)}|$.
  - If x^s is not a characteristic state then the demonstration is identical.
  - If x^s is a characteristic state ($u \to u \in E$ and $s_{uu} = |q-1, q|$) then $S_u(x^s) = u$.
    - If $\alpha_{uu} = +$ then $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s)\cup\{u\}}| = |K_{u,R_u(x^a)\setminus\{u\}}, K_{u,R_u(x^a)}|$. So $x^s_u = |q-1, q| > |K_{u,R_u(x^a)\setminus\{u\}}, K_{u,R_u(x^a)}|$ and $x^s \to x^b$ is a transition of our state graph.
    - If $\alpha_{uu} = -$ then $X_u(x^s) = |K_{u,R_u(x^s)}, K_{u,R_u(x^s)\cup\{u\}}| = |K_{u,R_u(x^a)}, K_{u,R_u(x^a)\cup\{u\}}|$. So, if x^s_u is not steady we have $x^s_u = |q-1, q| \not\subseteq |K_{u,R_u(x^a)}, K_{u,R_u(x^a)\cup\{u\}}| \implies q > K_{u,R_u(x^a)\cup\{u\}} \iff q-1 \geq K_{u,R_u(x^a)\cup\{u\}}$. So $|q-1, q| > |K_{u,R_u(x^a)}, K_{u,R_u(x^a)\cup\{u\}}|$ and $x^s \to x^b$ is a transition of our state graph.

□

This approach has been successfully applied to the mucus production in *Pseudomonas aeruginosa*. This bacteria is commonly present in the environment and secretes mucus only in lungs affected by cystic fibrosis. As it increases the respiratory deficiency of the patient, it is the major cause of mortality. The regulatory network of the mucus production has been widely studied[13, 7] and can be sketched by the system presented in the figure 3. The instantiation of parameters of the figure makes both circuits functional. The multistationarity due to the functionality of the positive loop is clearly represented in the qualitative state graph. It is then possible to associate particular behaviors concerning the mucus production to some steady states.

5 Conclusion and perspectives

In this paper we present a new qualitative modeling based on the R. Thomas works which allows us to represent the singular states in the dynamics. In both cases the models are built as a dicretization of of piecewise-linear differential equations system but our modeling, taking into account the singular states, permits us to represent all the steady states of the continuous dynamics. Moreover, the introduction of singular states leads to some other remarks : the increase in the number of states does not imply an increase in the number of models associated to a networks, the state graph reflects the softening of the negative functional circuits and it does not contradict the dynamics of R. Thomas. Finally, the theorems of the functionality of feedback circuits in the modeling of R. Thomas have been extended to our modeling : the introduction of singular states and of singular/regular resources make the demonstration more straightforward.

The R. Thomas modeling supposes that all interactions of a regulator on its targets have different thresholds. This constraint has been relaxed. Thus it leads to define the characteristic states of jointed union of circuits, of which the functionality is still to be defined.

Now that the all steady states are present in the state graph, we will take advantage of the corpus of formal methods to confront the models to biological knowledge. Indeed we want to select models which are coherent not only with the static conditions (functionality of feedback circuits) but also with some known dynamic properties extracted from biological experiments or hypothesis. We have already implemented a software, `SMBioNet`[2, 16] (Selection of Models of Biological Networks), which allows one to select models of given regulatory networks according to their temporal properties. The software takes as input qualitative regulatory network (with a graphical interface), some temporal properties expressed as a CTL formulae and a set of functional loops. Then it generates all the R. Thomas models and gives as output those which satisfy the specified temporal properties (using the NuSMV model checker[4]). A short-term perspective is to introduce in `SMBioNet` the new modeling with singular states.

More generally the formal methods can be applied in the field of biological regulatory networks in order to explicit some behaviors or to model some other biological knowledge. Let us mention for example that the introduction of transitions in the regulatory graph could help to specify how the different regulators cooperate for inducing or repressing their common target [1]. One can also separate inhibitors from regulators to increase the readability of the approach, or take into account time delays[24] between the beginning of the activation order and the synthesis of the product and conversely for the turn-off delays. These constitute ongoing or future works of our genopole® and G^3 research groups.

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