Graph-based Modeling of Biological Regulatory Networks : Introduction of 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 the dynamics of such regulatory networks, called the generalized logical analysis, and used the concept of circuit-characteristic states to identify all steady states and functional circuits. These characteristic states play an essential role on the dynamics of the system, but they are not represented in the state graph. In this paper we present an extension of this formalism in which all singular states including characteristic ones are represented. Consequently, the state graph contains all steady states. Model checking is then able to verify temporal properties concerning singular states. Finally, we prove that this new modeling is coherent with R. Thomas' modeling since all paths of R. Thomas' dynamics are represented in the new state graph, which in addition shows the influence of singular states on the dynamics.

1 Introduction

Biological regulatory systems are often 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 [1,2,3,4] which, however, cannot be solved analytically and use kinetic parameters which are most often unknown. The generalized logical analysis developed by R. Thomas and coworkers [5,6,7,8] to describe biological regulatory networks extracts the essential qualitative features of the dynamics of such systems by logical parameters [9,5] which can take a finite number of values. But some states, the *singular states*, are not explicitly represented in the state graph obtained with this formalism whereas they can be steady. Even if the steady singular states can be detected with the concept of circuit-characteristic states [10,11], it is not possible to use model checking for verifying temporal properties concerning singular states. This paper provides, in section 2, our extension of R. Thomas' modeling. This new formalism considers the singular states and consequently represents all the steady states of a classic continuous description of regulatory networks. This continuous description is also the ground of the work of R. Thomas. Section 3 presents this description and shows, by the introduction of a discretisation map, why our qualitative modeling extracts similarly its essential qualitative features. Then we study in section 4 how the introduction of singular states gives a new light on the properties of characteristic states of feedback circuits. Finally conclusions and perspectives are presented.

2 Qualitative dynamics of regulatory networks

In our qualitative approach, the entities of a biological regulatory network, often macromolecules or genes, have discrete expression levels defined as *qualitative values*.

Definition 1 (Qualitative Values). *A* qualitative value*, denoted by $|a, b|$, is a couple of integers ($|a, b| \in \mathbb{N}^2$) where $a \leq b$. The relations $=,<,>,\subseteq$ are defined for two qualitative values $|a, b|$ and $|c, d|$ by:*

- $|a, b| = |c, d|$ *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* $(|a, b| = |c, d|)$ *or* $(a = b$ *and* $c < a$ *and* $b < d)$ *or* $(a < b$ *and* $c \leq a$ *and* $b \leq d)$.

Intuitively, if $a < b$ then $|a, b|$ is said *singular* and represents the open interval $]a, b[$. Otherwise, if $a = b$ then $|a, b|$ is said *regular* and represents the closed interval $[a, b]$ which only contains the integer a. Then two qualitative values are comparable if the corresponding intervals are not overlapping and the relation $\subseteq$ is simply the inclusion relation between these intervals. To shorten the notation of the qualitative values we denote by $|a|$ the regular qualitative value $|a, a|$.

Interactions between biological entities are classically represented by directed graphs, where vertices abstracts biological entities and edges their interactions. In the sequel we denote by $\#S$ the cardinal of a set S and by $G^-(v)$ (resp. $G^+(v)$) the set of predecessors (resp. successors) of a vertex v in a graph G.

Definition 2 (QRN). *A* qualitative regulatory network *(QRN for short) is a labelled directed graph $N = (V, E)$ where:*

- *each vertex $v \in V$, called* variable*, represents a biological entity. The set Q_v of all* possible qualitative expression levels *of v is defined as $Q_v = \{|0|, |0, 1|, |1|, ..., |q-1|, |q-1, q|, |q|, ..., |\#N^+(v)|\}$.*
- *each edge $u \to v \in E$, called* interaction*, is labelled by a couple (α_{uv}, q_{uv}) where α_{uv} is the* sign *of the interaction ($\alpha_{uv} = +$ (resp. $\alpha_{uv} = -$) if $u \to v$ is an activation (resp. inhibition)) and where q_{uv} is an integer in $\{1, 2, ..., \#N^+(u)\}$ such that $q_{uv} \neq q_{uw}$ for all $w \in N^+(u)$ distinct from v. The* threshold *t_{uv} of the interaction is defined as $t_{uv} = |q_{uv} - 1, q_{uv}|$.*

At a given time, the data made of the expression level of each variable is called the state of the network.

Definition 3 (States of a QRN). *Let $N = (V, E)$ be a QRN. A* state x *of N is a vector $\mathrm{x} = (\mathrm{x}_v)_{v \in V}$ such that $\mathrm{x}_v \in Q_v$ for all $v \in V$. A state is said* singular *if one of its component is singular and* regular *otherwise.*

As a majority of biological interactions behave in a cooperative way and have a sigmoid nature, they are in a QRN labelled by thresholds and they model switch-like reactions: at a given state x, an interaction $u \to v$ is said effective when $\mathrm{x}_u > t_{uv}$, not effective when $\mathrm{x}_u < t_{uv}$ and uncertain when $\mathrm{x}_u = t_{uv}$. Thus $\mathrm{x}_u = |q|$ means that u is an effective regulator for q of its successors and $\mathrm{x}_u = |q, q+1|$ means that u is an effective regulator for the same q successors and it is an uncertain regulator for the successor v such that $t_{uv} = \mathrm{x}_u$ (v exists inevitably).

Definition 4 (Resources). *Let $N = (V, E)$ be a QRN, v be a variable of N and x be a state of N. The sets of* regular resources $R_v(\mathrm{x})$ *and* singular resources $S_v(\mathrm{x})$ *of v at the state x are given by:*

- $R_v(\mathrm{x}) = \{u \in N^-(v) \mid (\mathrm{x}_u > t_{uv} \textit{ and } \alpha_{uv} = +) \textit{ or } (\mathrm{x}_u < t_{uv} \textit{ and } \alpha_{uv} = -)\}$
- $S_v(\mathrm{x}) = \{u \in N^-(v) \mid \mathrm{x}_u = t_{uv}\}$

A regular resource of v is a variable which acts positively on v, that is to say an effective activator or a non effective inhibitor of v. A singular resource is just an uncertain regulator.

Definition 5 (Qualitative model). *A* qualitative model *M of a QRN $N = (V, E)$ is a couple $M = (N, K)$ where $K = \{K_{v,\omega} \mid v \in V \text{ and } \omega \subseteq N^-(v)\}$ is a set of integers, called* qualitative parameters, *such that :*

- *if $\omega = \emptyset$ then $K_{v,\omega} = 0$ and $K_{v,\omega} \in \{0, 1, ..., \#N^+(v)\}$ otherwise.*
- *if $\omega \subseteq \omega'$ then $K_{v,\omega} \leq K_{v,\omega'}$.*

At a given state x, the expression level of a variable v evolves toward a qualitative value according to its regular and singular resources. This qualitative value, called attractor and noted $A_v(\mathrm{x})$, is defined with two parameters indexed by the regular and singular resources of v:

Definition 6 (Attractors). *Let $M = (N, K)$ be a qualitative model and x be a state of N. The* attractor *$A_v(\mathrm{x})$ of $v \in V$ at the state x is :*

$$A_v(\mathrm{x}) = \left| K_{v,R_v(\mathrm{x})}, K_{v,R_v(\mathrm{x}) \cup S_v(\mathrm{x})} \right|$$

At a given state x, if v does not have singular resources ($S_v(\mathrm{x}) = \emptyset$) then it evolves toward the qualitative value $|K_{v,R_v(\mathrm{x})}|$. Otherwise ($S_v(\mathrm{x}) \neq \emptyset$) v has singular resources and evolves toward an expression level greater than $|K_{v,R_v(\mathrm{x})}|$, i.e. the case where all the singular resources are not regarded as regular resources, and less than $|K_{v,R_v(\mathrm{x}) \cup S_v(\mathrm{x})}|$, i.e. the case where all the singular resources are regarded as regular resources: $|K_{v,R_v(\mathrm{x})}| < |K_{v,R_v(\mathrm{x})}, K_{v,R_v(\mathrm{x}) \cup S_v(\mathrm{x})}| < |K_{v,R_v(\mathrm{x}) \cup S_v(\mathrm{x})}|$ if $|K_{v,R_v(\mathrm{x})}| < |K_{v,R_v(\mathrm{x}) \cup S_v(\mathrm{x})}|$. Naturally, if $\mathrm{x}_v < A_v(\mathrm{x})$ then v tends to increase, if $\mathrm{x}_v > A_v(\mathrm{x})$ then v tends to decrease, and otherwise ($\mathrm{x}_v \subseteq A_v(\mathrm{x})$) v is steady.

A steady state is thus a state where all the variables are steady, that is, a state x such that for all variable $v \in V$:

$$|K_{v,R_v(\mathrm{x})}| = \mathrm{x}_v = |K_{v,R_v(\mathrm{x})\cup S_v(\mathrm{x})}| \quad \text{or} \quad |K_{v,R_v(\mathrm{x})}| < \mathrm{x}_v < |K_{v,R_v(\mathrm{x})\cup S_v(\mathrm{x})}| \quad (1)$$

In section 4, we show how these static constraints (in which the dynamics of the system does not matter) can be used for the detection of homeostasis and multistationnarity. It can be proved that these constraints are equivalent to those given by E. H. Snoussi and R. Thomas in [10].

To sum up, we deduce from a model the tendencies of variables at each state, which is sufficient to define its dynamics with the following state graph.

Definition 7 (State graph). *The* state graph *of a qualitative model $M = (N, K)$, is a directed graph where the set of vertices is the set of states of N, and where* $\mathrm{x} \to \mathrm{y}$ *is an edge, called* transition*, if there is a variable v verifying :*

$$\textit{for all } u \neq v,\ \mathrm{y}_u = \mathrm{x}_u \quad \textit{and} \quad \begin{cases} \mathrm{y}_v = \Delta^+(\mathrm{x}_v) \ \textit{if } \mathrm{x}_v < A_v(\mathrm{x}) \\ \mathrm{y}_v = \Delta^-(\mathrm{x}_v) \ \textit{if } \mathrm{x}_v > A_v(\mathrm{x}) \end{cases}$$

with Δ^+ and Δ^- the evolution operators *defined by:*

$$\Delta^+(\alpha) = \begin{cases} |q, q+1| \ \textit{if } \alpha = |q| \\ |q| \ \textit{if } \alpha = |q-1, q| \end{cases} \quad \textit{and} \quad \Delta^-(\alpha) = \begin{cases} |q-1, q| \ \textit{if } \alpha = |q| \\ |q| \ \textit{if } \alpha = |q, q+1| \end{cases}$$

In this definition two variables cannot evolve simultaneously towards their respective attractors, the state graph is thus asynchronous and can be deduced from a synchronous one as in [6]. Indeed, when several variables tend to evolve at a given state, additional information (time delays associated to each transition [8]) is needed to select which one first changes. As this information is most often unknown, all possible transitions are considered. Thus the system is non deterministic and can translate the stochastic character of biological interactions. Consequently, a state for which n variables tend to evolve has n successors. In particular, if $n = 0$ then the state is steady and does not have any successor.

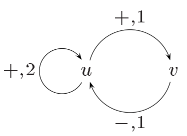
A qualitative model in R. Thomas' approach can be defined in the same way but the state graph deduced from it just gives transitions between regular states. Indeed, in R.Thomas' state graph of a model $M = (N, K)$ the vertices are all the regular states of N and $x \to y$ is a transition if there is a variable v verifying:

$$\text{for all } u \neq v,\ \mathrm{y}_u = \mathrm{x}_u \quad \text{and} \quad \begin{cases} \mathrm{y}_v = |q+1| \text{ if } \mathrm{x}_v < A_v(\mathrm{x}) \\ \mathrm{y}_v = |q-1| \text{ if } \mathrm{x}_v > A_v(\mathrm{x}) \end{cases} \quad \text{with } |q| = \mathrm{x}_v$$

As we can see in figure 1, for a given model, R. Thomas' state graph is present in our state graph: our state graph can be viewed as a refinement of R. Thomas' one formally expressed in theorem 3.

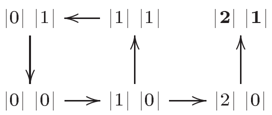
We now illustrate our formalism with the QRN $N = (V, E)$ whose representation is given in figure 1. It represents a small genetic network controlling the mucus production of *Pseudomonas aeruginosa* [12,13,14]. We have $V = \{u, v\}$ and

QRN



states x		symbolic attractors		attractors		tendencies	
x_u	x_v	$A_u(\mathrm{x})$	$A_v(\mathrm{x})$	$A_u(\mathrm{x})$	$A_v(\mathrm{x})$	u	v
$\lvert 0\rvert$	$\lvert 0\rvert$	$\lvert K_{u,\{v\}}\rvert$	$\lvert K_{v,\emptyset}\rvert$	$\lvert 2\rvert$	$\lvert 0\rvert$	$\nearrow$	$\rightsquigarrow$
$\lvert 0\rvert$	$\lvert 0,1\rvert$	$\lvert K_{u,\emptyset}, K_{u,\{v\}}\rvert$	$\lvert K_{v,\emptyset}\rvert$	$\lvert 0,2\rvert$	$\lvert 0\rvert$	$\nearrow$	$\searrow$
$\lvert 0\rvert$	$\lvert 1\rvert$	$\lvert K_{u,\emptyset}\rvert$	$\lvert K_{v,\emptyset}\rvert$	$\lvert 0\rvert$	$\lvert 0\rvert$	$\rightsquigarrow$	$\searrow$
$\lvert 0,1\rvert$	$\lvert 0\rvert$	$\lvert K_{u,\{v\}}\rvert$	$\lvert K_{v,\emptyset}, K_{v,\{u\}}\rvert$	$\lvert 2\rvert$	$\lvert 0,1\rvert$	$\nearrow$	$\nearrow$
$\lvert 0,1\rvert$	$\lvert 0,1\rvert$	$\lvert K_{u,\emptyset}, K_{u,\{v\}}\rvert$	$\lvert K_{v,\emptyset}, K_{v,\{u\}}\rvert$	$\lvert 0,2\rvert$	$\lvert 0,1\rvert$	$\rightsquigarrow$	$\rightsquigarrow$
$\lvert 0,1\rvert$	$\lvert 1\rvert$	$\lvert K_{u,\emptyset}\rvert$	$\lvert K_{v,\emptyset}, K_{v,\{u\}}\rvert$	$\lvert 0\rvert$	$\lvert 0,1\rvert$	$\searrow$	$\searrow$
$\lvert 1\rvert$	$\lvert 0\rvert$	$\lvert K_{u,\{v\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 2\rvert$	$\lvert 1\rvert$	$\nearrow$	$\nearrow$
$\lvert 1\rvert$	$\lvert 0,1\rvert$	$\lvert K_{u,\emptyset}, K_{u,\{v\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 0,2\rvert$	$\lvert 1\rvert$	$\rightsquigarrow$	$\nearrow$
$\lvert 1\rvert$	$\lvert 1\rvert$	$\lvert K_{u,\emptyset}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 0\rvert$	$\lvert 1\rvert$	$\searrow$	$\rightsquigarrow$
$\lvert 1,2\rvert$	$\lvert 0\rvert$	$\lvert K_{u,\{v\}}, K_{u,\{u,v\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 2\rvert$	$\lvert 1\rvert$	$\nearrow$	$\nearrow$
$\lvert 1,2\rvert$	$\lvert 0,1\rvert$	$\lvert K_{u,\emptyset}, K_{u,\{u,v\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 0,2\rvert$	$\lvert 1\rvert$	$\rightsquigarrow$	$\nearrow$
$\lvert 1,2\rvert$	$\lvert 1\rvert$	$\lvert K_{u,\emptyset}, K_{u,\{u\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 0,2\rvert$	$\lvert 1\rvert$	$\rightsquigarrow$	$\rightsquigarrow$
$\lvert 2\rvert$	$\lvert 0\rvert$	$\lvert K_{u,\{u,v\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 2\rvert$	$\lvert 1\rvert$	$\rightsquigarrow$	$\nearrow$
$\lvert 2\rvert$	$\lvert 0,1\rvert$	$\lvert K_{u,\{u\}}, K_{u,\{u,v\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 2\rvert$	$\lvert 1\rvert$	$\rightsquigarrow$	$\nearrow$
$\lvert 2\rvert$	$\lvert 1\rvert$	$\lvert K_{u,\{u\}}\rvert$	$\lvert K_{v,\{u\}}\rvert$	$\lvert 2\rvert$	$\lvert 1\rvert$	$\rightsquigarrow$	$\rightsquigarrow$

R. Thomas' state graph



state graph

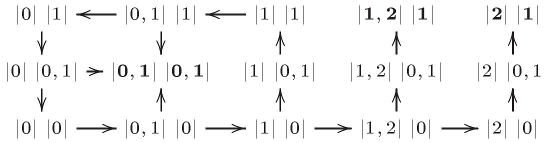


Fig. 1. The table gives, all the states of the *QRN*, the attractors of variables at each state, the values of the attractors deduced from the qualitative model where $K_{u,\emptyset} = 0$, $K_{u,\{v\}} = 2$, $K_{u,\{u\}} = 2$, $K_{u,\{u,v\}} = 2$, $K_{v,\emptyset} = 0$ and $K_{v,\{u\}} = 1$, and finally the corresponding tendencies ($\nearrow$ if $x_u < A_u(\mathrm{x})$, $\searrow$ if $x_u > A_u(\mathrm{x})$ and $\rightsquigarrow$ if $\mathrm{x}_u \subseteq A_u(\mathrm{x})$). These tendencies allow us to construct our state graph and R. Thomas' one.

$E = \{u \to u, u \to v, v \to u\}$. Variable u activates v and itself ($\alpha_{uu} = \alpha_{uv} = +$) when its expression level respectively reaches the thresholds $t_{uv} = |0,1|$ and $t_{uu} = |1,2|$. In return, variable v inhibits u ($\alpha_{vu} = -$) when its expression level reaches the threshold $t_{vu} = |0,1|$. Consequently, the possible expression levels of u are $Q_u = \{|0|, t_{uv}, |1|, t_{uu}, |2|\}$ and those of v are $Q_v = \{|0|, t_{vu}, |1|\}$. Thus 15 states are associated to the network, 6 are regular and 9 are singular (see the table of figure 1). The qualitative parameters corresponding to N are $K_{u,\emptyset}, K_{u,\{u\}}, K_{u,\{v\}}, K_{u,\{u,v\}} \in \{0,1,2\}$ and $K_{v,\emptyset}, K_{v,\{u\}} \in \{0,1\}$. The attractors expressed with the qualitative parameters are given in the table of figure 1. For a given model, the values of attractors allows us to deduce the tendencies of each variable at each state and to build, for both formalisms, the corresponding state graphs. For example, at the state $([1|, |0|)$ both u and v have an expression level less than their attractors (respectively equal to $|2|$ and $|1|$) and thus both variables tend to increase. Consequently $([1|, |0|)$ has two successors:

- in our state graph we have $(|1|, |0|) \to (|1,2|, |0|)$ for the increase of u and $(|1|, |0|) \to (|1|, |0,1|)$ for the increase of v.

– in R. Thomas' state graph we have $(|1|,|0|) \to (|2|,|0|)$ for the increase of u and $(|1|,|0|) \to (|1|,|1|)$ for the increase of v.

One can notice that our state graph contains two more steady states than R. Thomas' one (they are both singular states).

3 Discretization map

R. Thomas' approach has been built as a discretization of the continuous approach presented in this section. Our formalism can also be viewed as a discretization of this description, which gives the dynamics of the regulatory networks defined as follow.

Definition 8 (*RN*). *A* regulatory network *(RN for short) is a labelled directed graph $\mathcal{N} = (V, E)$ where :*

- *each vertex v of V, called* variable*, represents a biological entity,*
- *each edge $u \to v$ of E, called* interaction*, is labelled by a couple $(\alpha_{uv}, \theta_{uv})$ where α_{uv} is the* sign *of the interaction and where $\theta_{uv} \in \mathbb{R}_+$ is its* threshold*.*

To each variable v of $\mathcal{N} = (V, E)$ is associated a continuous variable $x_v \in \mathbb{R}_+$ which represents its expression level. At a given time, each variable x_v has a unique expression level and the vector $x = (x_v)_{v \in V}$ defines the state of the *RN*. The continuous dynamics of $\mathcal{N}$ can be given by the following system of piecewise-linear differential equations [9]:

$$\frac{\mathrm{d}x_v}{\mathrm{d}t} = \mathcal{S}_v(x) - \lambda_v x_v \quad \forall v \in V \quad \text{with} \quad \mathcal{S}_v(x) = \sum_{u \in \mathcal{N}^-(v)} \mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) \tag{2}$$

where $\lambda_v > 0$ is the degradation coefficient of v, $\mathcal{S}_v(x)$ is its synthesis rate and $\mathcal{I}^{\alpha_{uv}}$ is a step function (figure 2) describing the effect of u on the synthesis rate of v:

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

With such a definition, $\mathcal{I}^{\alpha_{uv}}$ is undefined for $x_u = \theta_{uv}$. A state in which there is at least one variable on a threshold is thus called a *singular* state. To define the system (2) for the singular states E. H. Snoussi and R. Thomas proposed in [10] to represent the *uncertain* influence of u on v when $x_u = \theta_{uv}$ by an open interval: $\mathcal{I}^{\alpha_{uv}}(\theta_{uv}, \theta_{uv}) =]0, k_{uv}[$. This interval represents the set of possible effects 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 [15]:

$$\frac{\mathrm{d}x_v}{\mathrm{d}t} \in \mathcal{S}_v(x) - \lambda_v x_v \quad \forall v \in V \quad \text{with} \quad \mathcal{S}_v(x) = \sum_{u \in \mathcal{N}^-(v)} \mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) \tag{3}$$

Definition 9 (Model). *A* model $\mathcal{M}$ *is a tuple* $\mathcal{M} = (\mathcal{N}, k, \lambda)$ *where* $\mathcal{N} = (V, E)$ *is a RN,* $k = \{k_{uv}\}_{u \to v \in E}$ *is the set of parameters associated to each interaction, and* $\lambda = \{\lambda_v\}_{v \in V}$ *is the set of degradation rates associated to each variable.*

Definition 10 (Discretization map). *Let* $\mathcal{N}$ *be a RN and* u *a variable. The* discretization map $d_u : 2^{\mathbb{R}_+} \setminus \emptyset \to \mathbb{N}^2$ *is defined for all non empty open intervals* $I =]\alpha, \beta[$ *and for all singletons* $I = [\alpha, \beta]$ *with* $\alpha = \beta$ *by :*

$$d_u(I) = \Big|\ \#\{\theta \in \Theta_u \mid \theta < \alpha\}\ ,\ \#\{\theta \in \Theta_u \mid \theta \leq \beta\}\ \Big|$$

where $\Theta_u = \{\theta_{uv} \mid v \in \mathcal{N}^+(u)\}$ *is the* set of out-thresholds *of* u*.*

Let us highlight some properties of d_u which are useful in the sequel. The order relations $<$, $>$ and $\subseteq$ on non empty open intervals and singletons are defined similarly to the corresponding order relations on qualitative values. Thus, d_u is an increasing function: if $A < B$ then $d_u(A) \leq d_u(B)$. Then if $d_u([\alpha, \alpha]) = |a|$ and $d_u([\beta, \beta]) = |b|$ with $\alpha \leq \beta$ we have $d_u(]\alpha, \beta[) = |a, b|$. Finally $d_u(A) \subseteq d_u(B)$ iff $A \subseteq B$, in particular $d_u([\alpha, \alpha]) \subseteq d_u(B)$ iff $\alpha \in B$. In the remainder, for a singleton $[\alpha, \alpha]$, $d_u(\alpha)$ denotes $d_u([\alpha, \alpha])$ by abuse of notation.

Definition 11 (Qualitative form). *The* qualitative form of *a RN* $\mathcal{N} = (V, E)$ *is the QRN* $N = (V, E)$ *such that each interaction* $u \to v$ *has the sign of the corresponding interaction in* $\mathcal{N}$ *and is such that* $t_{uv} = d_u(\theta_{uv})$*.*

According to the previous definition, a *RN* has a qualitative form iff it has no variable which acts on two successors with the same threshold, but it is a marginal case since thresholds are real values a priori different.

Let $\mathcal{N}$ be a *RN* and N its qualitative form. The discretization $d_u(x_u)$ of a continuous expression level x_u is a qualitative expression level of u in N:

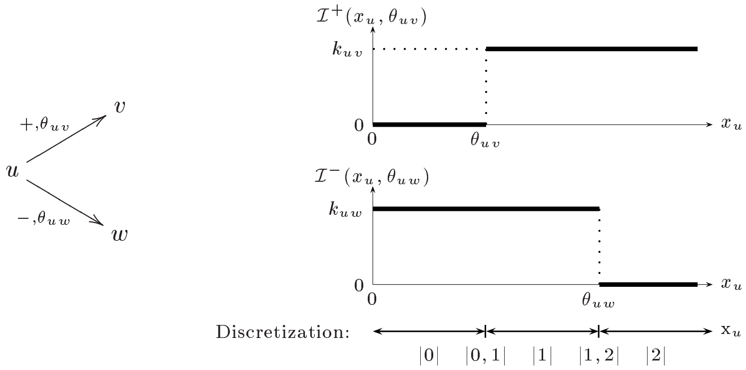


Fig. 2. Step functions associated to the interactions $u \to v$ and $u \to w$ with $\theta_{uv} < \theta_{uw}$ and discretization of the expression levels of u.

$d_u(x_u) \in Q_u$ (figure 2). Thus each continuous state of $\mathcal{N}$ corresponds to one qualitative state of N but a qualitative state of N can correspond to an infinity of continuous states of $\mathcal{N}$. To link the states of N with those of $\mathcal{N}$ we define $\mathcal{D}_v : Q_v \to 2^{\mathbb{R}_+}$ and $\mathcal{D} : \prod_{v\in V} Q_v \to \prod_{v\in V} 2^{\mathbb{R}_+}$ by:

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

$\mathcal{D}_v(\mathrm{x}_v)$ and $\mathcal{D}(\mathrm{x})$ are respectively called the *domains* of x_v and x. Let $\mathcal{M} = (\mathcal{N}, k, \lambda)$ be a model. The differential equation system (3) has one analytic solution on each domain $\mathcal{D}(\mathrm{x})$ where x is regular. For the initial state of the system $x^0 \in \mathcal{D}(\mathrm{x})$, the solution is:

$$x_v(t) = \mathcal{A}_v(x^0) - (\mathcal{A}_v(x^0) - x_v^0)e^{-\lambda_v t} \quad \forall v \in V \quad \text{with} \quad \mathcal{A}_v(x) = \frac{\mathcal{S}_v(x)}{\lambda_v}$$

Thus all continuous states of the domain $\mathcal{D}(\mathrm{x})$ tend to the same constant state $\mathcal{A}(x^0) = (\mathcal{A}_v(x^0))_{v\in V}$ called the *attractor* of the domain $\mathcal{D}(\mathrm{x})$. If $\mathcal{A}(x^0) \in \mathcal{D}(\mathrm{x})$, 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 $\mathcal{A}(x^0)$. Otherwise, if $\mathcal{A}(x^0) \notin \mathcal{D}(\mathrm{x})$, then a state x of $\mathcal{D}(\mathrm{x})$ will evolve towards $\mathcal{A}_v(x^0)$ until it goes 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 $\mathcal{A}_v(x^0)$ can never be reached. To sum up, at the state x if $x_v < \mathcal{A}_v(x)$ (resp. $x_v > \mathcal{A}_v(x)$) then v tends to increase (resp. decrease) and if $x_v = \mathcal{A}_v(x)$ then v is steady.

If $x \in \mathcal{D}(\mathrm{x})$ with x a singular state then x is also singular and there is at least one variable u such that $x_u = \theta_{uv}$ with $v \in \mathcal{N}^+(u)$. Thus $\mathcal{A}_v(x)$ is an open interval and the tendencies of v are defined in the same way except that v is considered steady if $x_v \in \mathcal{A}_v(x)$.

Definition 12 (Qualitative form of a model). *The* qualitative form of a model *$\mathcal{M} = (\mathcal{N}, k, \lambda)$ is the qualitative model $M = (N, K)$ such that N is the qualitative form of $\mathcal{N}$ and such that for all parameters $K_{v,\omega}$ of K we have $|K_{v,\omega}| = d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v})$.*

Notice that $\mathcal{M}$ has a qualitative form iff $(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \notin \Theta_v$ for all v and ω which is a reasonable hypothesis. By setting down $|K_{v,\omega}| = d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v})$ we respect the constraints given in the definition of qualitative parameters. Indeed, if $\omega = \emptyset$ then $K_{v,\omega} = 0$ and $d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) = d_v(0) = |0|$. Otherwise, $K_{v,\omega} \in \{0, 1, ..., \#N^+(v)\}$ and since $(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \notin \Theta_v$ we have $d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \in \{|0|, |1|, ..., |\#N^+(v)|\}$. Then, if $\omega \subseteq \omega'$ we have $\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v} \le \sum_{u\in\omega'} \frac{k_{uv}}{\lambda_v}$ and since d_v is an increasing map we have $d_v(\sum_{u\in\omega} \frac{k_{uv}}{\lambda_v}) \le d_v(\sum_{u\in\omega'} \frac{k_{uv}}{\lambda_v})$, that is $K_{v,\omega} \le K_{v,\omega'}$.

Theorem 1. *Let $\mathcal{M}$ be a model and M its qualitative form. For all variable u, for all qualitative state* x *of M and for all continuous state $x \in \mathcal{D}(\mathrm{x})$ of $\mathcal{M}$, we have $A_u(\mathrm{x}) = d_u(\mathcal{A}_u(x))$.*

Proof. According to the definition of d_u, we have $\mathrm{x}_u > t_{uv}$ iff $x_u > \theta_{uv}$, $\mathrm{x}_u < t_{uv}$ iff $x_u < \theta_{uv}$ and $\mathrm{x}_u = t_{uv}$ iff $x_u = \theta_{uv}$. Thus $u \in R_v(\mathrm{x})$ iff $\mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) =$

k_{uv} and $u \in S_v(\mathrm{x})$ iff $\mathcal{I}^{\alpha_{uv}}(x_u, \theta_{uv}) =]0, k_{uv}[$. Thus, if $S_v(\mathrm{x}) = \emptyset$ we have $A_v(\mathrm{x}) = |K_{v,R_v(\mathrm{x})}|$ and $\mathcal{A}_v(x) = \sum_{u \in R_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}$. Since $\sum_{u \in R_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v} \notin \Theta_v$ we have $A_u(\mathrm{x}) = d_u(\mathcal{A}_u(x))$. If $S_v(\mathrm{x}) \neq \emptyset$ we have $A_v(\mathrm{x}) = |K_{v,R_v(\mathrm{x})}, K_{v,R_v(\mathrm{x}) \cup S_v(\mathrm{x})}|$ and $\mathcal{A}_v(x) = \sum_{u \in R_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v} + \sum_{u \in S_v(\mathrm{x})} \frac{]0, k_{uv}[}{\lambda_v}$. Thus $\mathcal{A}_v(x)$ is equal to the open interval $]\alpha, \beta[=] \sum_{u \in R_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}, \sum_{u \in R_v(\mathrm{x}) \cup S_v(\mathrm{x})} \frac{k_{uv}}{\lambda_v}[$. Since α and β are not in Θ_v we have $A_u(\mathrm{x}) = d_u(\mathcal{A}_u(x))$.

Consequently we have, for all $x \in \mathcal{D}(\mathrm{x})$ and for each variable v, $\mathrm{x}_v < A_v(\mathrm{x})$ iff $x_v < \mathcal{A}_v(x)$ and $\mathrm{x}_v > A_v(\mathrm{x})$ iff $x_v > \mathcal{A}_v(x)$. Thus if v is not steady, it tends to evolve in the same way at the state x and at the state $x \in \mathcal{D}(\mathrm{x})$. Moreover, $\mathrm{x}_v \subseteq A_v(\mathrm{x})$ iff there exists $x \in \mathcal{D}(\mathrm{x})$ such that $x_v = \mathcal{A}_v(x)$ or $x_v \in \mathcal{A}_v(x)$. So if v is steady at the state x then v can be steady in $\mathcal{D}(\mathrm{x})$. Thus, a qualitative state x is steady iff there is a continuous steady state in $\mathcal{D}(\mathrm{x})$. We can sketch these properties by saying that the dynamics of M extracts the essential qualitative features of the dynamics of $\mathcal{M}$, and in particular both kinds of dynamics have the same number of steady states.

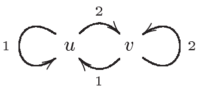
In practice, the values of the kinetic parameters k and λ are most often unknown. If we want to carry out a continuous modeling of a biological system, an infinity of models has to be considered corresponding to all the possible values of real parameters. The qualitative parameters K which define the dynamics of a *QRN* (definition 5) are also most often unknown but they can take a finite number of qualitative values. Then we can use the following fruitful exhaustive strategy to model a system: *to generate all the models with the aim to select those which give a dynamics coherent with the experimental knowledge of the system.* We have developed a software, called `SMBioNet` [16], which automatically carries out this generation and selection of models using three approaches : feedback circuit functionality, temporal logic and model checking. It has been used successfully to model the mucoidy and the cytotoxicity of *Pseudomonas aeruginosa* [13,14].

4 Circuit characteristic states

The most important generalized logical analysis concepts are certainly those of positive and negative circuits, which respectively generate multistationarity and cycle in the state graph, when the corresponding circuit is functional [17,18,19,20,21]. These concepts are especially important when modeling biological systems where differentiation and homeostasis need to be represented [13,14]. A circuit is said positif (resp. negatif) when it contains an even (resp. odd) number of inhibitions. It is said functionnal when there is a steady circuit characteristic state (which is singular by definition) associated to it [10]. Consequently, the steady singular states play an essential role on the dynamics of a system. A characteristic state of a circuit is a singular state in which the set of uncertain interactions is equal to the set of edges of the circuit. This notion of characteristic state can be extended to the union of disjoint circuits.

Definition 13 (Characteristic state). *Let N be a QRN and C be a circuit of N. A state* x *is a* characteristic state *of C if $C = \bigcup_{v \in V}\{u \to v \mid u \in S_v(\mathrm{x})\}$.*

Some examples of circuits with their characteristic states are given in Figure 3. E. H. Snoussi and R. Thomas proved for their formalism that a singular state can



$C_1 = \{u \to u\}$		$C_2 = \{v \to v\}$		$C_3 = \{u \to v, v \to u\}$		$C_1 \cup C_2$	
x_u	x_v	x_u	x_v	x_u	x_v	x_u	x_v
$\lvert 0,1 \rvert$	$\lvert 0 \rvert$	$\lvert 0 \rvert$	$\lvert 1,2 \rvert$	$\lvert 1,2 \rvert$	$\lvert 0,1 \rvert$	$\lvert 0,1 \rvert$	$\lvert 1,2 \rvert$
$\lvert 0,1 \rvert$	$\lvert 1 \rvert$	$\lvert 1 \rvert$	$\lvert 1,2 \rvert$				
$\lvert 0,1 \rvert$	$\lvert 2 \rvert$	$\lvert 2 \rvert$	$\lvert 1,2 \rvert$				

Fig. 3. Characteristic states of all the circuits and unions of disjoint circuits in a QRN (C_1 and C_2 have three characteristic states and the others only one). The interactions of are not labelled by any sign since they do not play a role in the definition of characteristic states.

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

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

Proof. Let $N = (V, E)$ be a QRN 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 the attractor $A_v(\mathrm{x})$ is a regular qualitative value: $A_v(\mathrm{x}) = |K_{v,R_v(\mathrm{x})}|$ since $S_v(\mathrm{x}) = \emptyset$. A singular qualitative value cannot be contained in a regular one, thus $\mathrm{x}_v \nsubseteq A_v(\mathrm{x})$ and x cannot be steady.

We now compare the dynamics of models for which some circuits are functional in both modelings with the aim to hightlight how the presence of singular states makes more explicit the functionality. Let us start with the example of figure 4. In both formalisms, homeostasis induced by the stationarity of the negative circuit characteristic state is represented. But the steady characteristic state towards which tends the softened oscillation, representing the homeostasis, in the continuous description is represented in our state graph while the homeostasis is reflected as an infinite oscillation in R. Thomas' one. Thus, our state graph extracts more precisely the qualitative features of the continuous formalism. Notice that the paths of R. Thomas' state graph do not correspond to paths between regular states in our state graph. The presence a of steady characteristic state of a negative loop is the only case where R. Thomas' state graph is not "included" in our state graph (see theorem 3 for formal explanation). In a general way, the softening generated by the functionality of negative circuits is not represented in R. Thomas' modeling. That can lead to a confusion about the interpretation of the circuit functionality. Let us consider the QRN of figure 1 containing a negative and a positive circuit. The model presented in the same figure makes

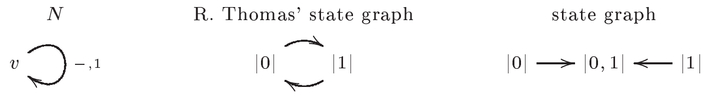


Fig. 4. State graphs, in the two approaches, deduced from the model $M = (N, \{K_{v,\emptyset} = 0, K_{v,\{v\}} = 1\})$ which makes functional the negative circuit N.

functional both circuits, there is two steady singular states (equation 1) which are characteristic of both circuits, and thus multistationarity and homeostasis are predicted. For the homeostasis let us remark that our state graph describes a dynamics in which infinite and softened oscillations are possible. For the multistationnarity, in R. Thomas' state graph there is only one steady state and from each state it is possible to reach it. The state graph does not really illustrate the multistationarity. In our state graph, the presence of all the steady states (two singular steady states and the previous regular one) makes more explicit the multistationarity. In both state graphs, the paths between regular states are coherent. Indeed, each transition $\mathrm{x} \to \mathrm{z}$ of R. Thomas' state graph corresponds, in our state graph, to a path $\mathrm{x} \to \mathrm{y} \to \mathrm{z}$ where y is the singular state adjacent to the regular states x and z. Note that, according to the following theorem, for all models deduced from this network, R. Thomas' state graph is "included" in our one since the network does not contain a negative loop.

Theorem 3. *Let* $\mathrm{x} \to \mathrm{z}$ *be a transition of R. Thomas' state graph deduced from a qualitative model, and let* v *be the only variable which evolves during the transition* $\mathrm{x} \to \mathrm{z}$ $(\mathrm{x}_v \neq \mathrm{z}_v)$. *Let* y *be the singular state adjacent to* x *and* z *defined by :* $\mathrm{y}_u = \mathrm{x}_u = \mathrm{z}_u$ *for all* $u \neq v$ *and, setting down* $\mathrm{x}_v = |q|$, $\mathrm{y}_v = |q, q+1|$ *if* $\mathrm{z}_v = |q+1|$ *and* $\mathrm{y}_v = |q-1, q|$ *if* $\mathrm{z}_v = |q-1|$. *Then our state graph contains the path* $\mathrm{x} \to \mathrm{y} \to \mathrm{z}$ *if* $\mathrm{y}_v \not\subseteq A_v(\mathrm{y})$ *(*$\mathrm{y}_v \subseteq A_v(\mathrm{y})$ *imposes that* y *is a characteristic state of the negative circuit* $v \to v$*).*

Proof. As x is a regular state, we have $A_v(\mathrm{x}) = |K_{v,R_v(\mathrm{x})}|$. Let us suppose that $\mathrm{x}_v = |q| < |K_{u,R_u(\mathrm{x})}|$. We have $\mathrm{y}_v = |q, q+1|$ and so $\mathrm{x} \to \mathrm{y}$ is a transition of our state graph. Moreover, $\mathrm{z}_v = |q+1|$ since $\mathrm{x} \to \mathrm{z}$ is a transition of R. Thomas' state graph.

As y_v is the only component of y which is singular, y is not a characteristic state if v does not regulate itself or if it regulates itself with a threshold not equal to y_v. In this case, $S_v(\mathrm{y}) = \emptyset$ and $A_v(\mathrm{y}) = |K_{v,R_v(\mathrm{y})}| = |K_{v,R_v(\mathrm{x})}|$. Thus $\mathrm{y} \to \mathrm{z}$ is a transition of our state graph since $|q| < |K_{u,R_u(\mathrm{x})}|$ implies that $|q, q+1| < |K_{u,R_u(\mathrm{x})}|$.

If y is a characteristic state (v regulates itself and $t_{vv} = \mathrm{y}_v$) then $S_v(\mathrm{y}) = \{v\}$. Thus, if $\alpha_{vv} = +$ then $A_v(\mathrm{y}) = |K_{v,R_v(\mathrm{y})}, K_{v,R_v(\mathrm{y})\cup\{v\}}| = |K_{v,R_v(\mathrm{x})}, K_{v,R_v(\mathrm{x})\cup\{v\}}|$. So $\mathrm{y}_v = |q, q+1| < |K_{v,R_v(\mathrm{x})}, K_{v,R_v(\mathrm{x})\cup\{v\}}|$ and $\mathrm{y} \to \mathrm{z}$ is a transition of our state graph (if $\alpha_{vv} = +$ then v cannot be steady at the state y). Otherwise, if $\alpha_{vv} = -$ then $A_v(\mathrm{y}) = |K_{v,R_v(\mathrm{y})}, K_{v,R_v(\mathrm{y})\cup\{v\}}| = |K_{v,R_v(\mathrm{x})\setminus\{v\}}, K_{v,R_v(\mathrm{x})}|$. So, if v is not steady at the state y we have $\mathrm{y}_v = |q, q+1| \not\subseteq |K_{v,R_v(\mathrm{x})\setminus\{v\}}, K_{v,R_v(\mathrm{x})}|$

which implies that $q < K_{v,R_v(\mathrm{x})\backslash\{v\}}$ equivalent to $q+1 \leq K_{v,R_v(\mathrm{x})\backslash\{v\}}$. Thus $|q, q+1| < |K_{v,R_v(\mathrm{x})\backslash\{v\}}, K_{v,R_v(\mathrm{x})}|$ and $\mathrm{y} \rightarrow \mathrm{z}$ is a transition of our state graph.

In the other case, if $\mathrm{x}_v = |q| > |K_{u,R_u(\mathrm{x})}|$, the proof is similar.

5 Conclusions and perspectives

In this paper we present a new qualitative modeling based on the generalized logical analysis of R. Thomas which allows us to represent the singular states in the dynamics. Both modeling are built as a dicretization of a 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. In spite of an exponential increase in the number of states, there is not an increase in the number of models associated to a network. Moreover, the state graph reflects the softening of the negative functional circuits and it is a refinement of the dynamics of R. Thomas.

The representation of all steady states is essential to confront with precision the models to biological knowledge. The concepts of circuit functionality allow us to select models which present homeostasis and/or multistationarity with only static constraints, that is inequality constraints for the steadiness of singular states.

To still go further such static conditions must be reinforced by properties on the dynamics. To achieve the selection of the acceptable models (with temporal properties coherent with all available biological knowledge) we will take advantage of the corpus of formal methods. We have already implemented a user-friendly software, `SMBioNet` [13,14,16] (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 a QRN (with a graphical interface) and some temporal properties expressed as CTL formulae and a set of functional circuits. It generates all models associated to the network which makes functional the specified circuits and gives as output those whose corresponding R. Thomas' state graph satisfy the specified temporal properties (using the NuSMV model checker [22]). Then, the selected models can be used to make and formally test hypotheses or to run simulations. The input of `SMBioNet` not consists to a complex file with several reaction-rules or parameters assignment as in several other tools using a qualitative approach to model biological regulatory networks [23,24,25,26,27]. Indeed, R. Thomas' formalism catches the qualitative structure of a system in a simple graphical object (a QRN) easily extractable from present biological data. Nevertheless, it is difficult to represent with this approach a physical change of state of biological entities after an interaction or the formation/breakage of complexes. However, the effectiveness of the generalized logical analysis has been demonstrated in the study of a number of genetic regulatory systems [28,29,5,30,31,32,13,14].

Naturally, a short term perspective is to introduce in this software our new formalism. The generation of the models making some circuits functional will remains the same but the increase in the number of states will makes more difficult to check a CTL formula. However, this formula will be able to express temporal properties concerning regular and singular states. To struggle against this increase of states, we can already propose to automatically remove from the state graph some singular states, for example those whose the set of successors is reduced to a single regular state (the states $(|1|, |0, 1|)$, $(|1, 2|, |0, 1|)$ and $(|2|, |0, 1|)$ can be removed from the state graph of figure 1).

More generally the formal methods can be applied in the field of biological regulatory networks in order to explicit some behaviors or to take into account not yet modelled 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 [33]. One can also separate inhibitors from regulators [34] to increase the readability of the approach, or take into account time delays [8] 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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