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Arnaud Poret[1,*], Claudio Monteiro Sousa[2], Jean-Pierre Boissel[3]

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[1] arnaud.poret@gmail.com
UMR CNRS 5558 Biometry and Evolutionary Biology Laboratory
Villeurbanne
France
https://lbbe.univ-lyon1.fr/

[2] claudio.ms@universite-lyon.fr
UMR CNRS 5558 Biometry and Evolutionary Biology Laboratory
Villeurbanne
France
https://lbbe.univ-lyon1.fr/

[3] jean-pierre.boissel@novadiscovery.com
Novadiscovery
Lyon
France
http://www.novadiscovery.com

[*] Corresponding author.

Abstract

Quantitative modeling in systems biology can be difficult due to the scarcity of quantitative details about biological phenomenons, especially at the subcellular scale. An alternative to escape this difficulty is qualitative modeling since it requires few to no quantitative information. Among the qualitative modeling approaches, the Boolean network formalism is one of the most popular. However, Boolean models allow variables to be valued at only true or false, which can appear too simplistic when modeling biological processes. Consequently, this work proposes a modeling approach derived from Boolean networks where fuzzy operators are used and where edges are tuned. Fuzzy operators allow variables to be continuous and then to be more finely valued than with discrete modeling approaches, such as Boolean networks, while remaining qualitative. Moreover, to consider that in a given biological network some interactions are slower and/or weaker relative to other ones, edge states are computed in order to modulate in speed and strength the signal they convey. The proposed formalism is illustrated through its implementation on a tiny sample of the epidermal growth factor receptor signaling pathway. The obtained simulations show that continuous results are produced, thus allowing finer analysis, and that modulating the signal conveyed by the edges allows their tuning according to knowledge about the modeled interactions, thus incorporating more knowledge. The proposed modeling approach is expected to bring enhancements in the ability of qualitative models to simulate the dynamics of biological networks while not requiring quantitative information.

Contents

1 Introduction

Quantitative modeling in systems biology allows scientists to produce formal models of biological systems and then to implement them on computers [1, 2]. With such computational models, scientists can perform *in silico* experiments which have the advantage of being less costly in time and resources than the traditional wet lab experiments. However, the stumbling block of *in silico* approaches is that they are built from the available knowledge: not all is known about everything. Nevertheless, an impressive and ever increasing amount of biological knowledge is already available in the scientific literature, databases and knowledge bases such as KEGG [3] and Reactome [4]. In addition to the difficulty of integrating an increasing body of knowledge comes the inherent complexity of biological systems themselves [5–10]: this is where computational tools can help owing to their integrative power [11–18]. This interplay between wet lab and computational biology is synergistic rather than competitive [19]. Since wet lab experiments produce factual results, they can be considered as trustworthy sources of knowledge. Once these factual pieces of knowledge are obtained, computational tools can help to integrate them and infer new ones. This computationally obtained knowledge can be subsequently used to direct further wet lab experiments, thus mutually potentiating the whole.

One of the main difficulties encountered when quantitatively modeling biological systems with, for example, systems of differential equations [20] is that the required quantitative parameter values are often not easy to obtain due to experimental limitations, particularly at the subcellular scale. One solution to overcome this barrier is qualitative modeling since it requires few to no quantitative information while producing informative predictions [21]. Several qualitative modeling approaches already exist and are mostly based on logic [22–25] such as Boolean networks [26, 27] which are based on Boolean logic [28]. However, this is at the cost of being qualitative: no quantification is performed. This does not mean that qualitative modeling is a downgrade of the quantitative one. This means that scientists have different approaches at their disposal, each with its advantages and disadvantages, depending on the pursued goals and available resources. If accurate numerical results are expected, quantitative modeling is required. However, if tendencies and global properties are the main concerns, qualitative modeling is entirely fitting and proved itself through several works [29–54].

The present work proposes a logic-based modeling aimed at enhancing the Boolean network formalism. The basic principles remain the same as in Boolean networks: given a biological network [55–57], entities are modeled by variables and their interactions by functions allowing their value to be updated at each iteration of the simulation. However, Boolean operators are replaced by the operators of fuzzy logic [58, 59], allowing variables to be valued at any real number between 0 and 1, that is to consider all the possible truth degrees between the absolutely true and the definitively false. Results obtainable with fuzzy operators, while remaining qualitative, can be finer than those obtainable with Boolean operators. In some cases, the ON/OFF nature of Boolean logic is a relevant choice, as for example with gene regulatory networks where gene expression level can be approximated by Boolean states [60–63]. However, in some other cases where things are not necessarily binary, such as in signaling pathways where enzymes can be more or less active, using fuzzy operators can

be an interesting choice.

In addition to using fuzzy operators, some additional features are introduced in order to capture more behavioral aspects of biological networks. These additional features concern the edges of the network, which are seen as conveyors of signals corresponding to influences exerted by entities of the network onto other ones. This signal, together with its modulation, are taken into account so that edges can be tuned. To do so, edge states are computed and the signal they convey can be slowed or weakened. This results in a qualitative modeling approach intended to bring a fine qualitative quantification of biological network behaviors.

Talking about a qualitative quantification can appear somewhat contradictory but is common in thinking processes, which are at the basis of any scientific reasoning. Simple examples of such qualitative quantification could be to state that an enzyme is more active than another one, or to state that an enzyme is moderately active: quantification is expressed by perceptions and tendencies. Indeed, qualitative quantification is expressed by words rather than measurements, hence its qualitative nature, and is characteristic of fuzzy logic [64, 65].

It should be noted that fuzzy logic-based modeling is a promising approach successfully developed in several works [66–74]. However, this work is not fuzzy logic-based: there are no fuzzy sets, no fuzzy membership functions, no degrees of membership and no fuzzy inference systems. Only the operators are taken from fuzzy logic to replace the Boolean ones, the goal being to enhance the Boolean network formalism by extending it to a continuous formalism and by adding edge tuning.

2 Methods

This section introduces some basic principles, namely biological and Boolean networks, introduces fuzzy operators and then describes how the proposed enhancement of Boolean networks is built. An example to illustrate it, together with its implementation, are also described.

2.1 Basic principles

2.1.1 Biological networks

A biological network is a way to conceptualize a set of interacting biological entities where entities are represented by nodes and interactions by edges. It is based on graph theory [75–80], thus bringing formal tools to encode information about biological systems, particularly their topology. Moreover, being graphs, biological networks offer a convenient visualization [81] of the complex interconnections lying in biological systems. As said Napoleon Bonaparte:

> "A good sketch is better than a long speech."

Several types of biological networks can be encountered, depending on the scale, the involved entities and their interconnections. For example, at the ecological scale, food webs are biological networks where nodes represent species and edges represent trophic relations [82–84]. At the subcellular scale there is, for example, gene regulatory networks where nodes represent gene products and edges

represent gene expression modulations. Whatever the scale or entities, the principle remains the same: given a biological system, nodes represent entities and edges represent interactions between them.

Mathematically, a network can be seen as a digraph $G = (V, E)$ where $V = \{v_1, \ldots, v_n\}$ is the set of cardinality n containing exactly all the nodes v_i of the network and where $E = \{(v_{i,1}, v_{j,1}), \ldots, (v_{i,m}, v_{j,m})\} \subseteq V^2$ is the set of cardinality m containing exactly all the edges (v_i, v_j) of the network. In practice, nodes represent entities and edges represent binary relations $R \subseteq V^2$ involving them: $v_i \ R \ v_j$.

2.1.2 Boolean networks

Boolean networks, pioneered in biology by Kauffman [85], Ostrander [86], Thomas [87] and Glass [88], are one of the existing qualitative modeling approaches. While being conceptually simple, Boolean networks are able to predict and reproduce features of biological systems and then to bring relevant insights [89–93]. This makes them an attractive and efficient approach, especially when the complexity of biological systems renders quantitative approaches unfeasible due to the amount of quantitative details they require.

As their name indicates, Boolean networks are based on Boolean logic and, like biological networks, are also based on graph theory: nodes represent Boolean variables and edges represent interdependencies between them. Boolean networks can be classified according to their updating scheme as synchronous or asynchronous: if all the variables are updated simultaneously at each iteration of the simulation then the network is synchronous, otherwise it is asynchronous. While there is only one synchronous updating scheme, different asynchronous updating schemes exist:

- the random order asynchronous updating scheme where, at each iteration, an updating order for the variables is randomly selected
- the general asynchronous updating scheme where, at each iteration, a randomly selected variable is updated
- the deterministic asynchronous updating scheme where a divisor is assigned to each variable and then, at each iteration, a variable is updated if and only if the iteration is a multiple of its divisor

With the exception of deterministic asynchronous Boolean networks, only synchronous Boolean networks are deterministic since, at each iteration, the variables have only one possible successor. This makes synchronous Boolean networks easier to compute than asynchronous ones [94].

Mathematically, a Boolean network is a network where nodes are Boolean variables x_i and where edges (x_i, x_j) represent the binary *is input of* relation: x_i *is input of* x_j. Each x_i has $b_i \in [\![0, n]\!]$ inputs $x_{i,1}, \ldots, x_{i,b_i}$. The variables which are not inputs of x_i have no direct influence on it. If $b_i = 0$ then x_i is a parameter and does not depend on other variables. At each iteration $k \in [\![k_0, k_{end}]\!]$ of the simulation, the value $x_i(k) \in \{0, 1\}$ of each x_i is updated to the value $x_i(k+1)$ using a Boolean function f_i and the values $x_{i,1}(k), \ldots, x_{i,b_i}(k)$ of its inputs, as in the following pseudocode:

1 **for** $k \in [\![k_0, k_{end} - 1]\!]$ **do**

2 $x_1(k+1) = f_1(x_{1,1}(k), \ldots, x_{1,b_1}(k))$
3 ...
4 $x_n(k+1) = f_n(x_{n,1}(k), \ldots, x_{n,b_n}(k))$
5 **end for**

which can be written in a more concise form:

1 **for** $k \in [\![k_0, k_{end} - 1]\!]$ **do**
2 $\boldsymbol{x}(k+1) = \boldsymbol{f}(\boldsymbol{x}(k))$
3 **end for**

where $\boldsymbol{f} = (f_1, \ldots, f_n)$ is the Boolean transition function and $\boldsymbol{x} = (x_1, \ldots, x_n)$ is the state vector. In the particular case where $k = k_0$, $\boldsymbol{x}(k_0) = \boldsymbol{x}_0$ is the initial state. If the values of all the x_i are updated simultaneously at each k, as in the above pseudocode, then the network is synchronous, otherwise it is asynchronous. With synchronous Boolean networks, $\boldsymbol{x}(k)$ has a unique possible successor $\boldsymbol{x}(k+1)$: synchronous Boolean networks are deterministic and then easier to compute than asynchronous ones.

2.1.3 Fuzzy operators

The main difference between Boolean and fuzzy logic is that the former is discrete, that is valued in $[\![0;1]\!] \subset \mathbb{N}$, whereas the latter is continuous, that is valued in $[0;1] \subset \mathbb{R}$. Fuzzy logic can be seen as a generalization of Boolean logic, implying that the fuzzy counterparts of the Boolean operators have to behave like them on $[\![0;1]\!]$ but have to be defined on $[0;1]$. The generalization of the Boolean AND operator is the *t-norm*, the generalization of the Boolean OR operator is the *s-norm* and the generalization of the Boolean NOT operator is the *complement*:

$$\begin{aligned} \textit{t-norm}\colon [0;1]^2 &\to [0;1]\colon (x,y) \mapsto \textit{t-norm}(x,y) \\ \textit{s-norm}\colon [0;1]^2 &\to [0;1]\colon (x,y) \mapsto \textit{s-norm}(x,y) \\ \textit{complement}\colon [0;1] &\to [0;1]\colon x \mapsto \textit{complement}(x) \end{aligned}$$

where $x, y \in [0;1]$. There exist different mathematical formulations of the *t-norm*, *s-norm* and *complement*, all fulfilling the rules of Boolean algebra [95] but defined on $[0;1]$. For convenience, both the Boolean and fuzzy operators can be named AND, OR and NOT, the context specifying which of them is referred to.

Due to the ability of fuzzy operators to be continuous, variables can take their value in $[0;1]$. Therefore, they can be equal to 1 (true), 0 (false) or all the other real numbers of $[0;1]$ (more or less true): all the truth degrees between true and false are considered. This can be more realistic in a world where things are not necessarily binary. For example, a Boolean model of a signaling pathway allows enzymes to be ON or OFF and nothing between. However, one can expect that an enzyme is allowed to be in an intermediate activity level, an expectation not implementable with Boolean models but which is with continuous ones. Whatever the truth degrees represent, using fuzzy operators enables to consider all the intermediate levels of what is modeled without leaving the qualitative modeling formalism.

2.2 The proposed logic-based modeling

First of all, it should be mentioned that a distinction is made between quantitative and qualitative parameters, this distinction residing in what parameters translate. A quantitative parameter translates a quantification obtained by experimental measurements whereas a qualitative parameter translates a perception by means of truth degrees. For example, regarding the velocity of a biochemical reaction, "slow" could be expressed by the truth degree 0.2 whereas "fast" by 0.8: this is the truth degree of the statement "This biochemical reaction is fast.". Unlike an experimental quantification which is *de facto* objective, a perception is subjective, so the same applies to its associated truth degree. Incorporating qualitative parameters should not yield the scarcity of parameter values encountered in quantitative modeling since qualitative information is relatively easy to obtain.

To build the proposed logic-based modeling from Boolean networks, the Boolean operators AND, OR and NOT have to be replaced by the fuzzy operators *t-norm*, *s-norm* and *complement*. Furthermore, the initial states $x_i(k_0)$ of the x_i have to belong to $[0;1]$. As a consequence, the value of the x_i belongs to $[0;1]$: $x_i(k) \in [0;1]$, the f_i become functions from $[0;1]^n$ to $[0;1]$:

$$f_i \colon [0;1]^n \to [0;1] \colon \boldsymbol{x} \mapsto f_i(\boldsymbol{x})$$

the value of $\boldsymbol{x}$ and $\boldsymbol{x}_0$ belongs to $[0;1]^n$: $\boldsymbol{x}(k), \boldsymbol{x}_0 \in [0;1]^n$ and $\boldsymbol{f}$ becomes a function from $[0;1]^n$ onto itself:

$$\boldsymbol{f} \colon [0;1]^n \to [0;1]^n \colon \boldsymbol{x} \mapsto \boldsymbol{f}(\boldsymbol{x})$$

Finally, some additional features are added in order to capture more behavioral aspects of biological networks. These features concern the edges of the network and are presented separately for the sake of clarity before being integrated all together.

2.2.1 Edge computation

As with node states, edge states are computed. For convenience, edges can be notated e_{ij} instead of (x_i, x_j). An edge e_{ij} is seen as a channel conveying the signal sent by its source x_i to its target x_j which uses it to compute its state thanks to f_j. Practically, e_{ij} conveys the value $x_i(k)$ of x_i to x_j and then f_j uses it to compute $x_j(k+1)$. This is implicitly done in Boolean networks where $x_j(k+1) = f_j(\dots, x_i(k), \dots)$ but, in this work, this is made explicit in order to modulate the signal conveyed by the edges. Consequently, the f_j no longer directly accept the $x_i(k)$ as arguments but accept the $e_{ij}(k)$. Since e_{ij} conveys $x_i(k)$, its value $e_{ij}(k)$ should be $x_i(k)$, but this is where additional features are added. Indeed, a function f_{ij}^{edge} is attributed to each e_{ij}:

$$e_{ij}(k+1) = f_{ij}^{edge}(x_i(k), e_{ij}(k))$$

It should be noted that, in addition to the value $x_i(k)$ of the source x_i, f_{ij}^{edge} also takes as argument the value $e_{ij}(k)$ of e_{ij} itself. This is required for the

additional feature *edge reactivity* described below. As mentioned above, the f_j have now to accept the $e_{ij}(k)$ instead of the $x_i(k)$. For convenience, the f_j are renamed f_j^{node}:

$$x_j(k+1) = f_j^{node}(\boldsymbol{e}(k))$$

where $\boldsymbol{e} = (\dots, e_{ij}, \dots)$ is the counterpart of $\boldsymbol{x} = (\dots, x_i, \dots)$, namely the state vector of the edges, its value at the iteration k being $\boldsymbol{e}(k) = (\dots, e_{ij}(k), \dots)$. Consequently, $\boldsymbol{f}$ becomes $\boldsymbol{f}^{node} = (\dots, f_i^{node}, \dots)$:

$$\boldsymbol{x}(k+1) = \boldsymbol{f}^{node}(\boldsymbol{e}(k))$$

and its counterpart the transition function of the edges $\boldsymbol{f}^{edge} = (\dots, f_{ij}^{edge}, \dots)$ is introduced:

$$\boldsymbol{e}(k+1) = \boldsymbol{f}^{edge}(\boldsymbol{x}(k), \boldsymbol{e}(k))$$

On the basis of the updating scheme of synchronous Boolean networks, the computation becomes:

for $k \in [\![k_0, k_{end} - 1]\!]$ **do**
    ...
    $e_{ij}(k+1) = f_{ij}^{edge}(x_i(k), e_{ij}(k))$
    ...
    $x_i(k+1) = f_i^{node}(\dots, e_{ij}(k), \dots)$
    ...
end for

which can be written in a more concise form:

for $k \in [\![k_0, k_{end} - 1]\!]$ **do**
    $\boldsymbol{e}(k+1) = \boldsymbol{f}^{edge}(\boldsymbol{x}(k), \boldsymbol{e}(k))$
    $\boldsymbol{x}(k+1) = \boldsymbol{f}^{node}(\boldsymbol{e}(k))$
end for

2.2.2 Edge reactivity

The additional feature *edge reactivity* is implemented by a qualitative parameter $p_{ij} \in [0; 1]$ attributed to each e_{ij}. p_{ij} is the portion of the signal conveyed by e_{ij} which is updated at each k, namely the portion of the value $e_{ij}(k)$ which is updated to $x_i(k)$:

$$e_{ij}(k+1) = (1 - p_{ij}) \cdot e_{ij}(k) + p_{ij} \cdot x_i(k)$$

The higher p_{ij} is, the higher is the portion of $e_{ij}(k)$ which is updated: a highly reactive edge has a p_{ij} close to 1 whereas a faintly reactive edge has a p_{ij} close to 0. Biologically, *edge reactivity* can take into account that some biological interactions can be slower, or of higher inertia, than other ones. For example, an edge modeling a gene expression activation of a gene product by a transcription factor should have a lower p_{ij} than an edge modeling an activating phosphorylation of an enzyme by another one. Indeed, gene expression is a complex mechanism involving several steps and then takes more time to be accomplished and terminated than a simple phosphorylation.

2.2.3 Edge weakening

The additional feature *edge weakening* is implemented by a qualitative parameter $q_{ij} \in [0; 1]$ attributed to each e_{ij}. q_{ij} is a weakening coefficient applied at each k to the signal conveyed by e_{ij}, that is to $x_i(k)$:

$$e_{ij}(k+1) = q_{ij} \cdot x_i(k)$$

The higher q_{ij} is, the lower is the weakening of the signal $x_i(k)$ conveyed by e_{ij}: a strong edge has a q_{ij} close to 1 whereas a weak edge has a q_{ij} close to 0. Biologically, *edge weakening* can take into account that some biological interactions can be weaker than other ones. For example, given a receptor, an edge modeling its activation by a partial agonist should have a lower q_{ij} than an edge modeling its activation by a full agonist.

2.2.4 Combining the all

Edge reactivity and *edge weakening* are described separately for the sake of clarity but are both computed at each iteration:

$$e_{ij}(k+1) = (1 - p_{ij}) \cdot e_{ij}(k) + p_{ij} \cdot q_{ij} \cdot x_i(k)$$

hence the mathematical formulation of the f_{ij}^{edge}:

$$f_{ij}^{edge}(x_i, e_{ij}) = (1 - p_{ij}) \cdot e_{ij} + p_{ij} \cdot q_{ij} \cdot x_i$$

2.3 Implementation

In this work, k is not the time, it only represents the iterations performed during a run. Although quantifying time through k is possible, here the goal is to visualize sequences of events linked by causal connections without time quantification. To do so, $k_0 = 1$ and $k_{end} = 50$: 49 iterations are performed during a run. Furthermore, the initial state $e_{ij}(k_0)$ of each e_{ij} is assumed to be equal to the initial state $x_i(k_0)$ of its source x_i: $e_{ij}(k_0) = x_i(k_0)$. To illustrate the proposed logic-based modeling, it is implemented on an example with GNU Octave[1]. The code is available on GitHub[2] at `https://github.com/arnaudporet/kali-sim`.

2.3.1 Example

The used example is a tiny sample of the epidermal growth factor receptor signaling pathway [96] adapted from [24]. It is chosen for its simplicity so that it can be mentally computed in order to easily judge the produced results. A digital electronic representation is shown in *Figure 1* page 10. Below are the corresponding Boolean functions where AND, NOT and OR stand for the Boolean operators:

[1] `http://www.gnu.org/software/octave/`
[2] `https://github.com/`

$$
\begin{aligned}
EGF(k+1) &= \text{input set manually} \\
HRG(k+1) &= \text{input set manually} \\
EGFR(k+1) &= OR(EGF(k), HRG(k)) \\
PI3K(k+1) &= AND(EGFR(k), NOT(ERK(k))) \\
AKT(k+1) &= PI3K(k) \\
Raf(k+1) &= OR(EGFR(k), AKT(k)) \\
ERK(k+1) &= Raf(k)
\end{aligned}
$$

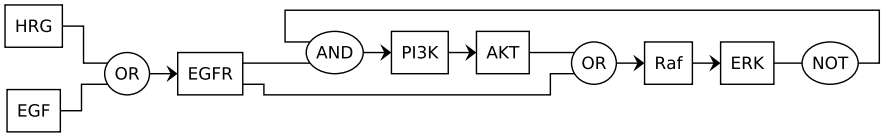

Figure 1: Digital electronic representation of the example. Nodes are rectangles whereas logical gates are ellipses. This digraph should be read from left to right. For example, the node $PI3K$ is an input of the node AKT and the node ERK, due to a feedback loop, is an input of the node $PI3K$. Logical gates are not nodes and, as such, edges only pass through them. For example, the edge $(ERK, PI3K)$ passes through a NOT and AND gate whereas the edge (Raf, ERK) does not pass through any logical gate.

By applying the above-described methodology, below are the obtained f_{ij}^{edge} and f_i^{node} where AND, NOT and OR stand for the fuzzy operators:

$$
\begin{aligned}
(EGF, EGFR)(k+1) &= (1 - p_{EGF,EGFR}) \cdot (EGF, EGFR)(k) \\
&\quad + p_{EGF,EGFR} \cdot q_{EGF,EGFR} \cdot EGF(k) \\
(HRG, EGFR)(k+1) &= (1 - p_{HRG,EGFR}) \cdot (HRG, EGFR)(k) \\
&\quad + p_{HRG,EGFR} \cdot q_{HRG,EGFR} \cdot HRG(k) \\
(EGFR, PI3K)(k+1) &= (1 - p_{EGFR,PI3K}) \cdot (EGFR, PI3K)(k) \\
&\quad + p_{EGFR,PI3K} \cdot q_{EGFR,PI3K} \cdot EGFR(k) \\
(ERK, PI3K)(k+1) &= (1 - p_{ERK,PI3K}) \cdot (ERK, PI3K)(k) \\
&\quad + p_{ERK,PI3K} \cdot q_{ERK,PI3K} \cdot ERK(k) \\
(PI3K, AKT)(k+1) &= (1 - p_{PI3K,AKT}) \cdot (PI3K, AKT)(k) \\
&\quad + p_{PI3K,AKT} \cdot q_{PI3K,AKT} \cdot PI3K(k) \\
(EGFR, Raf)(k+1) &= (1 - p_{EGFR,Raf}) \cdot (EGFR, Raf)(k) \\
&\quad + p_{EGFR,Raf} \cdot q_{EGFR,Raf} \cdot EGFR(k) \\
(AKT, Raf)(k+1) &= (1 - p_{AKT,Raf}) \cdot (AKT, Raf)(k) \\
&\quad + p_{AKT,Raf} \cdot q_{AKT,Raf} \cdot AKT(k) \\
(Raf, ERK)(k+1) &= (1 - p_{Raf,ERK}) \cdot (Raf, ERK)(k) \\
&\quad + p_{Raf,ERK} \cdot q_{Raf,ERK} \cdot Raf(k)
\end{aligned}
$$

$$
\begin{aligned}
EGF(k+1) &= \text{input set manually} \\
HRG(k+1) &= \text{input set manually} \\
EGFR(k+1) &= OR((EGF, EGFR)(k), (HRG, EGFR)(k)) \\
PI3K(k+1) &= AND((EGFR, PI3K)(k), NOT((ERK, PI3K)(k))) \\
AKT(k+1) &= (PI3K, AKT)(k) \\
Raf(k+1) &= OR((EGFR, Raf)(k), (AKT, Raf)(k)) \\
ERK(k+1) &= (Raf, ERK)(k)
\end{aligned}
$$

It should be noted that f_{EGF}^{node} and f_{HRG}^{node} do not accept any $e_{ij}(k)$ as argument. This is because they are associated to the two inputs EGF and HRG of the network and are consequently set manually.

2.3.2 Fuzzy operators

As mentioned above, there exist different mathematical formulations of the fuzzy operators, all fulfilling the rules of Boolean algebra but defined on $[0; 1]$. In this work, the algebraic formulation is used:

$$
\begin{aligned}
AND(x, y) &= x \cdot y \\
OR(x, y) &= x + y - x \cdot y \\
NOT(x) &= 1 - x
\end{aligned}
$$

which is one of the most simple and convenient.

2.3.3 Additional features

Since $p_{ij} \in [0; 1]$, its value can be set to any real number of $[0; 1]$. However, p_{ij} is a qualitative parameter and rather than requiring to precisely valuate it as in quantitative models, its value is randomly picked in specified intervals of $[0; 1]$ from a uniform distribution. By the way, this random selection introduces a little of a rudimentary stochasticity, although introducing randomness is not the purpose of this work. To do so, $[0; 1]$ is split into intervals of truth degrees reflecting various edge reactivities:

instantaneous	$p_{ij} = 1$
faster	$p_{ij} \in [0.75; 1]$
fast	$p_{ij} \in [0.5; 0.75]$
slow	$p_{ij} \in [0.25; 0.5]$
slower	$p_{ij} \in [0; 0.25]$
down	$p_{ij} = 0$

plus the entire interval $[0; 1]$ in case of an undetermined *edge reactivity*. For example, $p_{ij} = fast$ means that the value of p_{ij} is randomly picked in $[0.5; 0.75]$ from a uniform distribution. This random selection occurs before each run and, once selected, the value of p_{ij} remains the same during the run. To better approach the behavior of the modeled biological network, replicates are made: r runs are performed and the results are superposed. In this work, $r = 10$. $q_{ij}, x_i(k_0) \in [0; 1]$ are subjected to the same replication with the following splits of $[0; 1]$:

strong	$q_{ij} = 1$
weak	$q_{ij} \in [0.75; 1]$
weaker	$q_{ij} \in [0.5; 0.75]$
faint	$q_{ij} \in [0.25; 0.5]$
fainter	$q_{ij} \in [0; 0.25]$
down	$q_{ij} = 0$

and

full	$x_i(k_0) = 1$
much more	$x_i(k_0) \in [0.75; 1]$
much	$x_i(k_0) \in [0.5; 0.75]$
few	$x_i(k_0) \in [0.25; 0.5]$
fewer	$x_i(k_0) \in [0; 0.25]$
none	$x_i(k_0) = 0$

plus the entire interval $[0; 1]$ in case of an undetermined *edge weakening* or initial state.

3 Results

In this section, results obtained with the example through five simulations are presented. Although the obtained curves are continuous due to the use of fuzzy operators, they are not quantitative. As qualitative results, rather than looking for numerical values, one can say, for example, that $PI3K$ is totally inhibited or that ERK is partly activated, two simple examples of qualitative quantification expressed by words and perceptions.

3.1 Simulation 1

EGF and HRG are the two inputs of the example and, since both can activate $EGFR$, one is sufficient to initiate the signaling cascade. It is assumed that, at the resting state, both the inputs are down: $\forall k,\ EGF(k) = HRG(k) = none$. However, at $k_{EGF} = k_{end}/10$, EGF is activated: $\forall k > k_{EGF},\ EGF(k) = full$. Therefore, f^{node}_{EGF} and f^{node}_{HRG} become:

$$EGF(k+1) = \begin{cases} full & \text{if } k \geq k_{EGF} \\ none & \text{if } k < k_{EGF} \end{cases}$$
$$HRG(k+1) = none$$

The network being assumed to be at the resting state, $\boldsymbol{x}_0 = (\dots, none, \dots)$. The p_{ij} are set to $fast$ and the q_{ij} to *strong*. The corresponding results are shown in *Figure 2* page 13. As expected, before EGF activation, the network is at rest: the signaling cascade is not active. However, once EGF activated, the signaling cascade activates. This ultimately activates ERK, hence the subsequent inactivation of $PI3K$ despite sustained $EGFR$ activity. Since AKT is activated by $PI3K$, it also deactivates.

3.2 Simulation 2

In addition to the inputs described in simulation 1, a perturbation is introduced. It consists in disabling the inhibitory effect of ERK on $PI3K$, that is in disabling the edge $(ERK, PI3K)$. It points out an advantage of computing the edge states: disturbing a node disturbs all its effects while selectively disturbing the edges prevents this. To implement this perturbation, the parameter values are as in simulation 1, except $q_{ERK,PI3K}$ which is set to $weaker$. With $q_{ERK,PI3K} = weaker$, the signal conveyed by the edge $(ERK, PI3K)$ is weakened throughout this simulation. The corresponding results are shown in *Figure 3* page 14. As expected, weakening the edge $(ERK, PI3K)$ results in a weakened inhibition of $PI3K$ by ERK: ERK does not totally inhibit $PI3K$.

3.3 Simulation 3

A perturbation is again applied to the edge $(ERK, PI3K)$. However, in this simulation the perturbation concerns its reactivity, namely $p_{ERK,PI3K}$, which is set to $slower$. The other parameter values are as in simulation 1. With $p_{ERK,PI3K} = slower$, the signal conveyed by the edge $(ERK, PI3K)$ is slowed throughout this simulation. The corresponding results are shown in *Figure 4* page 14. As expected, slowing the edge $(ERK, PI3K)$ results in a slowed inhibition of $PI3K$ by ERK: although ERK totally inhibits $PI3K$, it does it slower than in simulation 1 where $p_{ERK,PI3K} = fast$.

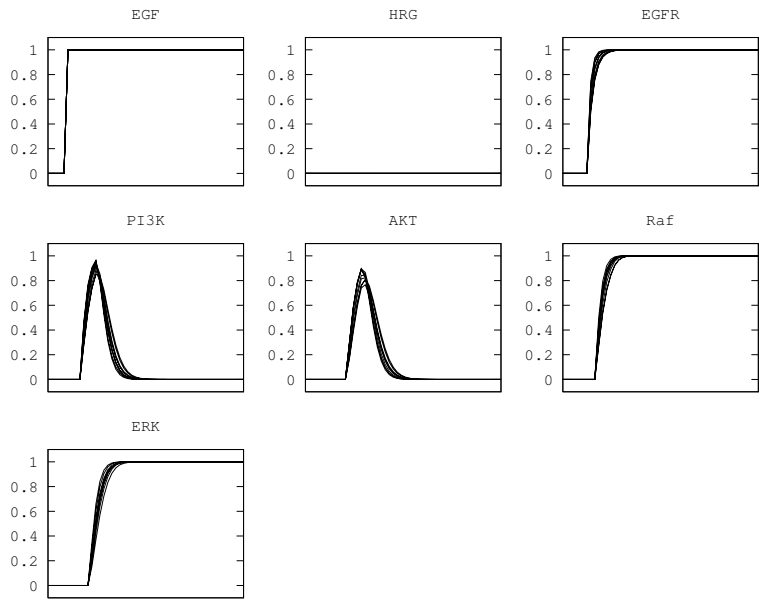

Figure 2: Activation of the signaling cascade by EGF and subsequent inhibition of $PI3K$ by ERK.

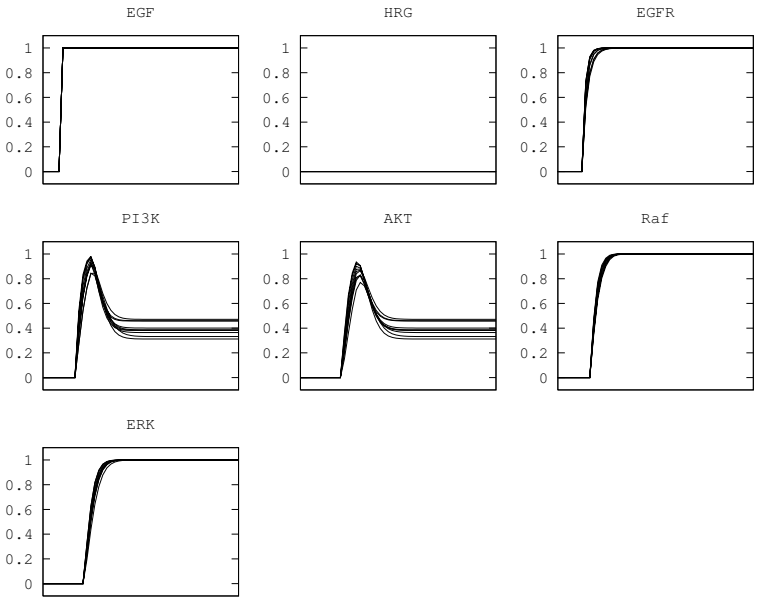

Figure 3: Weakening the inhibitory effect of ERK on $PI3K$.

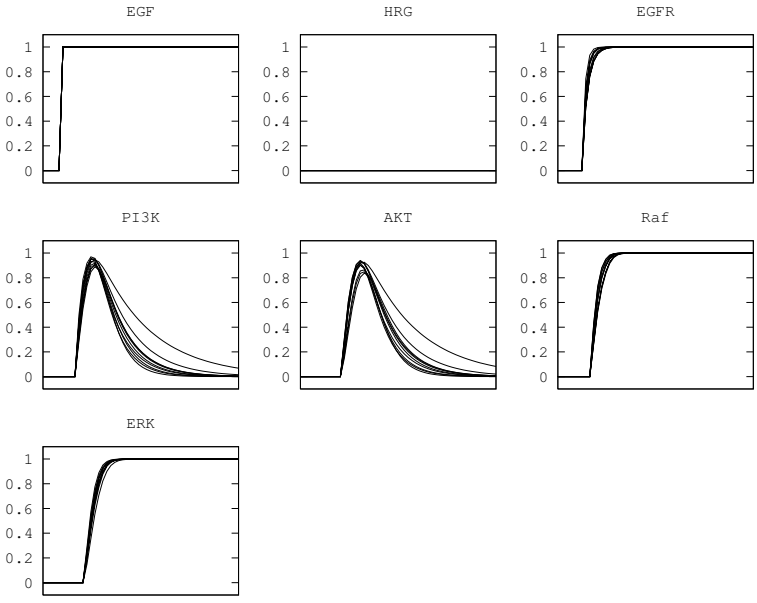

Figure 4: Slowing the inhibitory effect of ERK on $PI3K$.

3.4 Simulation 4

In this simulation, no perturbations are applied and the parameter values are as in simulation 1. However, rather than totally activating EGF, it is set to few. Therefore, f_{EGF}^{node} and f_{HRG}^{node} become:

$$EGF(k+1) = \begin{cases} few & \text{if } k \geq k_{EGF} \\ none & \text{if } k < k_{EGF} \end{cases}$$
$$HRG(k+1) = none$$

The corresponding results are shown in *Figure 5* page 15. As expected, the activation of EGF is not total and the same applies to the entire signaling cascade. For example, $PI3K$ does not totally activate since $EGFR$ does not. Furthermore, $PI3K$ is not totally inhibited by ERK since ERK itself does not totally activate.

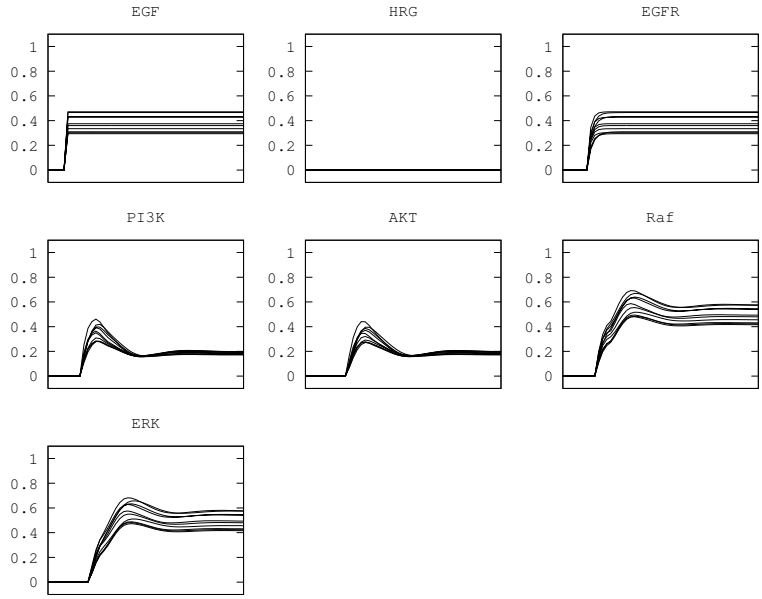

Figure 5: Consequences on the signaling cascade of a partial activation of EGF.

3.5 Simulation 5

In this simulation, both EGF and HRG are set to few. Therefore, f_{EGF}^{node} and f_{HRG}^{node} become:

$$EGF(k+1) = \begin{cases} few & \text{if } k \geq k_{EGF} \\ none & \text{if } k < k_{EGF} \end{cases}$$

$$HRG(k+1) = \begin{cases} few & \text{if } k \geq k_{HRG} \\ none & \text{if } k < k_{HRG} \end{cases}$$

with $k_{HRG} = k_{EGF}$, the other parameter values being as in simulation 1. The corresponding results are shown in *Figure 6* page 16. It points out that the effect of EGF and HRG on $EGFR$ is cumulative due to an OR gate. Indeed, although both EGF and HRG are set to few, cumulating their effect on $EGFR$ makes the signaling cascade more active than in simulation 4 where only EGF is set to few.

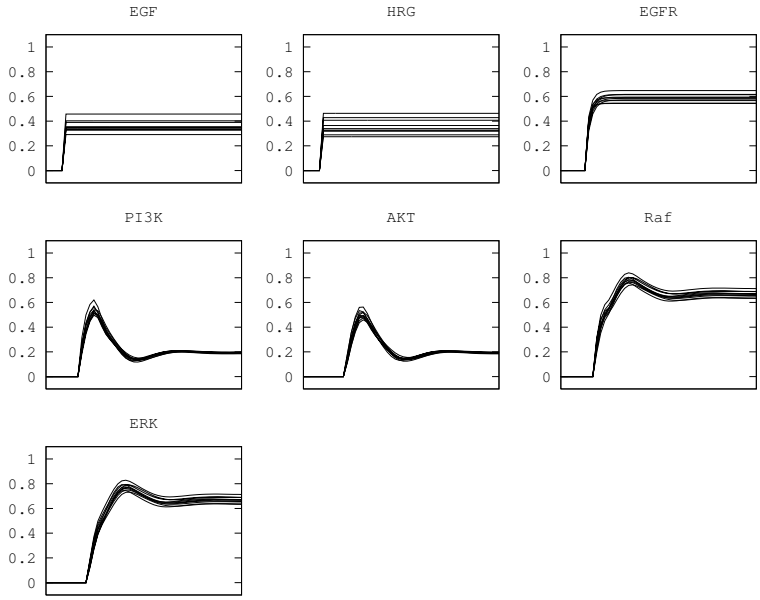

Figure 6: Cumulative effect of partly activated EGF and HRG on the signaling cascade.

4 Conclusion

Owing to the use of fuzzy operators, the simulations performed with the example show that the proposed logic-based modeling is able to produce continuous results while remaining qualitative. This allows qualitative variables to be more finely valued than with discrete approaches, such as Boolean networks, by taking into account all the possible levels of what is modeled. Moreover, thanks to the additional features *edge reactivity* and *edge weakening* attributed to each edge of the network, it is possible to tune in speed and strength the interactions taking

place in the modeled biological network according to knowledge about them. This is expected to take into account that some interactions can be weaker or slower relative to other ones and therefore to be more realistic in their qualitative modeling.

These enhancements should enable to incorporate more knowledge, notably about biological processes, and to obtain more accurate results. In exchange, they require the parameters controlling how the signal flows in the edges to be valued. These parameters are intended to be qualitative, that is parameters whose the valuation is knowledge-based, by opposition to quantitative parameters whose the valuation is data-based. In other words, qualitative parameters translate qualitative information, an information which should be easier to obtain than the quantitative one. Indeed, quantitative models require their parameters to be valued by data obtained through experimental measurements. However, due to experimental limitations, such measurements can be challenging. Qualitative information is easier to obtain but at the cost of being qualitative, as its name indicates. This is the well-known trade-off between what is wished and what is obtainable.

A little of stochasticity on the two additional features *edge reactivity* and *edge weakening* is also realized through the random selection of their value in specified intervals followed by replication and superposition of the produced results. This stochasticity, although very rudimentary, constitutes a line of improvement which should yield more realism since events taking place in biological systems are themselves subjected to stochasticity [97–101]. Another improvement could be to apply information theory [102] on the signal conveyed by the edges, as previously introduced for cell signaling [103–106]. This improvement should enable to better model how the information flows in a biological network and particularly, starting from its sender, how the information is altered by noise before reaching its receiver. Such alterations of the information could have significant consequences on the functionalities of a biological network, such as an inappropriate response to an input. Altogether, starting from Boolean networks and still founded on their basic principles, this work is expected to bring a fine qualitative quantification of the behavior of biological networks.

It should be noted that a qualitative quantification remains qualitative and should not be confused with a true quantification which involves experimental measurements, values and units [107]. The qualitative quantification proposed in this work has the goal of bringing enhancements in the ability of qualitative models to simulate the behavior of biological networks. One of the main goals, and advantages, of qualitative modeling remains to propose an alternative to, but not a replacement of, quantitative approaches when the frequently encountered scarcity in quantitative information makes the work unreasonably or unnecessarily difficult.

It is also possible to use qualitative and quantitative approaches in combination. For example, qualitative modeling can be used to explore global properties and then quantitative modeling can be used to focus on particular aspects. Knowing the difficulty of quantitative modeling in systems biology, this two-steps approach could make modeling more efficient by highlighting where to deploy quantitative approaches. Qualitative and quantitative approaches can also be merged into hybrid models [108–111] which attempt to exploit the advantages of these two approaches in one. Hybrid models, or semi-quantitative models, can be good compromises between the convenience of qualitative mod-

eling and the accuracy of quantitative modeling.

Finally, continuous dynamical systems are mostly modeled by differential equations for which advanced solvers are available, such as LSODE (the Livermore Solver for Ordinary Differential Equations) [112]. This work introduces continuous dynamical systems made of logical equations, for which advanced solvers do not seem to exist. However, mathematically speaking, it is likely that these continuous logical equations are differential equations thought and built in a different way. Consequently, it would be possible to mathematically express them as differential equations and then to use available computational tools aimed at analyzing continuous dynamical systems.

References

[1] Raymond Cheong and Andre Levchenko. Wires in the soup: quantitative models of cell signaling. *Trends in cell biology*, 18(3):112–118, 2008.

[2] Alex Mogilner, Roy Wollman, and Wallace F Marshall. Quantitative modeling in cell biology: what is it good for? *Developmental cell*, 11(3):279–287, 2006.

[3] Mao Tanabe and Minoru Kanehisa. Using the kegg database resource. *Current Protocols in Bioinformatics*, pages 1–12, 2012.

[4] David Croft, Antonio Fabregat Mundo, Robin Haw, Marija Milacic, Joel Weiser, Guanming Wu, Michael Caudy, Phani Garapati, Marc Gillespie, Maulik R Kamdar, et al. The reactome pathway knowledgebase. *Nucleic acids research*, 42(D1):D472–D477, 2014.

[5] Fulvio Mazzocchi. Complexity and the reductionism–holism debate in systems biology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 4(5):413–427, 2012.

[6] Christof Koch. Modular biological complexity. *Science*, 337(6094):531–532, 2012.

[7] Robert Longtin. An integrated approach: systems biology seeks order in complexity. *Journal of the National Cancer Institute*, 97(7):476–478, 2005.

[8] Danail Bonchev. On the complexity of directed biological networks. *SAR and QSAR in Environmental Research*, 14(3):199–214, 2003.

[9] Zoltan N Oltvai and Albert-Laszlo Barabasi. Life's complexity pyramid. *Science*, 298(5594):763–764, 2002.

[10] Gezhi Weng, Upinder S Bhalla, and Ravi Iyengar. Complexity in biological signaling systems. *Science*, 284(5411):92–96, 1999.

[11] Md Altaf-Ul-Amin, Farit Mochamad Afendi, Samuel Kuria Kiboi, and Shigehiko Kanaya. Systems biology in the context of big data and networks. *BioMed research international*, 2014, 2014.

[12] Joseph O Dada and Pedro Mendes. Multi-scale modelling and simulation in systems biology. *Integrative Biology*, 3(2):86–96, 2011.

[13] Samik Ghosh, Yukiko Matsuoka, Yoshiyuki Asai, Kun-Yi Hsin, and Hiroaki Kitano. Software for systems biology: from tools to integrated platforms. *Nature Reviews Genetics*, 12(12):821–832, 2011.

[14] Saket Navlakha and Ziv Bar-Joseph. Algorithms in nature: the convergence of systems biology and computational thinking. *Molecular Systems Biology*, 7(1), 2011.

[15] Eric A Sobie, Young-Seon Lee, Sherry L Jenkins, and Ravi Iyengar. Systems biology–biomedical modeling. *Science signaling*, 4(190):tr2, 2011.

[16] Casey S Greene and Olga G Troyanskaya. Integrative systems biology for data-driven knowledge discovery. *Seminars in nephrology*, 30(5):443–454, 2010.

[17] Jean-Pierre Boissel, Michel Cucherat, Patrice Nony, Francois Gueyffier, and Francois-Henri Boissel. Modeling and medical product r& d. In *International pharmaceutical product registration*, pages 762–773. Informa, 2009.

[18] Jean-Pierre Boissel, Benjamin Ribba, Emmanuel Grenier, Guillemette Chapuisat, and Marie-Aimee Dronne. Modelling methodology in physiopathology. *Progress in biophysics and molecular biology*, 97(1):28–39, 2008.

[19] Barbara Di Ventura, Caroline Lemerle, Konstantinos Michalodimitrakis, and Luis Serrano. From in vivo to in silico biology and back. *Nature*, 443(7111):527–533, 2006.

[20] M Ilea, M Turnea, M Rotariu, et al. Ordinary differential equations with applications in molecular biology. *Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi*, 116(1):347–352, 2011.

[21] Michelle L Wynn, Nikita Consul, Sofia D Merajver, and Santiago Schnell. Logic-based models in systems biology: a predictive and parameter-free network analysis method. *Integrative Biology*, 4(11):1323–1337, 2012.

[22] K Ballerstein, UU Haus, JA Lindquist, T Beyer, B Schraven, and R Weismantel. Discrete, qualitative models of interaction networks. *Frontiers in bioscience (Scholar edition)*, 5:149–166, 2013.

[23] Claudine Chaouiya and Elisabeth Remy. Logical modelling of regulatory networks, methods and applications. *Bulletin of mathematical biology*, 75(6):891–895, 2013.

[24] Melody K Morris, Julio Saez-Rodriguez, Peter K Sorger, and Douglas A Lauffenburger. Logic-based models for the analysis of cell signaling networks. *Biochemistry*, 49(15):3216–3224, 2010.

[25] Steven Watterson, Stephen Marshall, and Peter Ghazal. Logic models of pathway biology. *Drug discovery today*, 13(9):447–456, 2008.

[26] Assieh Saadatpour and Reka Albert. Boolean modeling of biological regulatory networks: a methodology tutorial. *Methods*, 2012.

[27] Istvan Albert, Juilee Thakar, Song Li, Ranran Zhang, and Reka Albert. Boolean network simulations for life scientists. *Source code for biology and medicine*, 3(1):1–8, 2008.

[28] George Boole. The mathematical analysis of logic, being an essay towards a calculus of deductive logic, 1847.

[29] Silvia Von der Heyde, Christian Bender, Frauke Henjes, Johanna Sonntag, Ulrike Korf, and Tim Beissbarth. Boolean erbb network reconstructions and perturbation simulations reveal individual drug response in different breast cancer cell lines. *BMC systems biology*, 8(1):75, 2014.

[30] Herman F Fumia and Marcelo L Martins. Boolean network model for cancer pathways: predicting carcinogenesis and targeted therapy outcomes. *PLOS ONE*, 8(7):e69008, 2013.

[31] Luca Grieco, Laurence Calzone, Isabelle Bernard-Pierrot, Francois Radvanyi, Brigitte Kahn-Perles, and Denis Thieffry. Integrative modelling of the influence of mapk network on cancer cell fate decision. *PLoS computational biology*, 9(10):e1003286, 2013.

[32] Oscar Andres Moreno-Ramos, Maria Claudia Lattig, and Andres Fernando Gonzalez Barrios. Modeling of the hypothalamic-pituitary-adrenal axis-mediated interaction between the serotonin regulation pathway and the stress response using a boolean approximation: a novel study of depression. *Theor. Biol. Med. Model*, 10:59, 2013.

[33] Hedi Peterson, Raed Abu Dawud, Abhishek Garg, Ying Wang, Jaak Vilo, Ioannis Xenarios, and James Adjaye. Qualitative modeling identifies il-11 as a novel regulator in maintaining self-renewal in human pluripotent stem cells. *Frontiers in physiology*, 4:303, 2013.

[34] Tomas Tokar, Zdenko Turcan, and Jozef Ulicny. Boolean network-based model of the bcl-2 family mediated momp regulation. *Theor Biol Med Model*, 10:40, 2013.

[35] Ozgur E Akman, Steven Watterson, Andrew Parton, Nigel Binns, Andrew J Millar, and Peter Ghazal. Digital clocks: simple boolean models can quantitatively describe circadian systems. *Journal of The Royal Society Interface*, page rsif20120080, 2012.

[36] Alfredo Rodriguez, David Sosa, Leda Torres, Bertha Molina, Sara Frias, and Luis Mendoza. A boolean network model of the fa/brca pathway. *Bioinformatics*, 28(6):858–866, 2012.

[37] Amit Singh, Juliana M Nascimento, Silke Kowar, Hauke Busch, and Melanie Boerries. Boolean approach to signalling pathway modelling in hgf-induced keratinocyte migration. *Bioinformatics*, 28(18):i495–i501, 2012.

[38] G Bhardwaj, CP Wells, R Albert, DB van Rossum, and RL Patterson. Exploring phospholipase c-coupled ca2+ signalling networks using boolean modelling. *IET systems biology*, 5(3):174–184, 2011.

[39] Chu C Kang, Yung J Chuang, Kai C Tung, Chun C Chao, Chuan Y Tang, Shih C Peng, and David SH Wong. A genetic algorithm-based boolean delay model of intracellular signal transduction in inflammation. *BMC bioinformatics*, 12(Suppl 1):S17, 2011.

[40] Amy L Bauer, Trachette L Jackson, Yi Jiang, and Thimo Rohlf. Receptor cross-talk in angiogenesis: mapping environmental cues to cell phenotype using a stochastic, boolean signaling network model. *Journal of theoretical biology*, 264(3):838–846, 2010.

[41] Aurelien Naldi, Jorge Carneiro, Claudine Chaouiya, and Denis Thieffry. Diversity and plasticity of th cell types predicted from regulatory network modelling. *PLoS computational biology*, 6(9):e1000912, 2010.

[42] Juilee Thakar and Reka Albert. Boolean models of within-host immune interactions. *Current opinion in microbiology*, 13(3):377–381, 2010.

[43] Hao Ge and Min Qian. Boolean network approach to negative feedback loops of the p53 pathways: synchronized dynamics and stochastic limit cycles. *Journal of Computational Biology*, 16(1):119–132, 2009.

[44] Zhongxing Mai and Haiyan Liu. Boolean network-based analysis of the apoptosis network: irreversible apoptosis and stable surviving. *Journal of Theoretical Biology*, 259(4):760–769, 2009.

[45] Ozgur Sahin, Holger Frohlich, Christian Lobke, Ulrike Korf, Sara Burmester, Meher Majety, Jens Mattern, Ingo Schupp, Claudine Chaouiya, Denis Thieffry, et al. Modeling erbb receptor-regulated g1/s transition to find novel targets for de novo trastuzumab resistance. *BMC Systems Biology*, 3(1):1, 2009.

[46] Regina Samaga, Julio Saez-Rodriguez, Leonidas G Alexopoulos, Peter K Sorger, and Steffen Klamt. The logic of egfr/erbb signaling: theoretical properties and analysis of high-throughput data. *PLoS computational biology*, 5(8):e1000438, 2009.

[47] Rebekka Schlatter, Kathrin Schmich, Ima Avalos Vizcarra, Peter Scheurich, Thomas Sauter, Christoph Borner, Michael Ederer, Irmgard Merfort, and Oliver Sawodny. On/off and beyond-a boolean model of apoptosis. *PLoS computational biology*, 5(12):e1000595, 2009.

[48] Maria I Davidich and Stefan Bornholdt. Boolean network model predicts cell cycle sequence of fission yeast. *PLOS ONE*, 3(2):e1672, 2008.

[49] Gwenael Kervizic and Laurent Corcos. Dynamical modeling of the cholesterol regulatory pathway with boolean networks. *BMC systems biology*, 2(1):99, 2008.

[50] Ranran Zhang, Mithun Vinod Shah, Jun Yang, Susan B Nyland, Xin Liu, Jong K Yun, Reka Albert, and Thomas P Loughran. Network model of survival signaling in large granular lymphocyte leukemia. *Proceedings of the National Academy of Sciences*, 105(42):16308–16313, 2008.

[51] Simone Gupta, Siddharth S Bisht, Ritushree Kukreti, Sanjeev Jain, and Samir K Brahmachari. Boolean network analysis of a neurotransmitter signaling pathway. *Journal of theoretical biology*, 244(3):463–469, 2007.

[52] Adrien Faure, Aurelien Naldi, Claudine Chaouiya, and Denis Thieffry. Dynamical analysis of a generic boolean model for the control of the mammalian cell cycle. *Bioinformatics*, 22(14):e124–e131, 2006.

[53] Luis Mendoza. A network model for the control of the differentiation process in th cells. *Biosystems*, 84(2):101–114, 2006.

[54] Sui Huang and Donald E Ingber. Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. *Experimental Cell Research*, 261(1):91–103, 2000.

[55] Xiaowei Zhu, Mark Gerstein, and Michael Snyder. Getting connected: analysis and principles of biological networks. *Genes & Development*, 21(9):1010–1024, 2007.

[56] Albert-Laszlo Barabasi and Zoltan N Oltvai. Network biology: understanding the cell's functional organization. *Nature Reviews Genetics*, 5(2):101–113, 2004.

[57] Eric Alm and Adam P Arkin. Biological networks. *Current Opinion in Structural Biology*, 13(2):193–202, 2003.

[58] Ilia Nikolaevich Bronshtein, Konstantin A Semendyayev, Gerhard Musiol, and Heiner Muehlig. Fuzzy logic. In *Handbook of mathematics*, chapter 5, pages 360–378. Springer, 2007.

[59] Lotfi Asker Zadeh. Fuzzy logic. *Computer*, 21(4):83–93, 1988.

[60] Frank Emmert-Streib, Matthias Dehmer, and Benjamin Haibe-Kains. Gene regulatory networks and their applications: understanding biological and medical problems in terms of networks. *Frontiers in cell and developmental biology*, 2, 2014.

[61] Yufei Xiao. A tutorial on analysis and simulation of boolean gene regulatory network models. *Current Genomics*, 10(7):511, 2009.

[62] Guy Karlebach and Ron Shamir. Modelling and analysis of gene regulatory networks. *Nature Reviews Molecular Cell Biology*, 9(10):770–780, 2008.

[63] John C Rockett and David J Dix. Gene expression networks. *eLS*, 2006.

[64] Lotfi A Zadeh. From computing with numbers to computing with words. *Annals of the New York Academy of Sciences*, 929(1):221–252, 2001.

[65] Lotfi A Zadeh. Fuzzy logic = computing with words. *Fuzzy Systems, IEEE Transactions on*, 4(2):103–111, 1996.

[66] Eugene PEM Cleophas and Ton J Cleophas. Fuzzy modeling, a novel approach to studying pharmacodynamics. *American journal of therapeutics*, 21(1):20–25, 2014.

[67] Melody K Morris, Julio Saez-Rodriguez, David C Clarke, Peter K Sorger, and Douglas A Lauffenburger. Training signaling pathway maps to biochemical data with constrained fuzzy logic: quantitative analysis of liver cell responses to inflammatory stimuli. *PLoS computational biology*, 7(3):e1001099, 2011.

[68] Yonghui Sun, Gang Feng, and Jinde Cao. A new approach to dynamic fuzzy modeling of genetic regulatory networks. *NanoBioscience, IEEE Transactions on*, 9(4):263–272, 2010.

[69] Bree B Aldridge, Julio Saez-Rodriguez, Jeremy L Muhlich, Peter K Sorger, and Douglas A Lauffenburger. Fuzzy logic analysis of kinase pathway crosstalk in tnf/egf/insulin-induced signaling. *PLoS computational biology*, 5(4):e1000340, 2009.

[70] Zuyi Huang and Juergen Hahn. Fuzzy modeling of signal transduction networks. *Chemical Engineering Science*, 64(9):2044–2056, 2009.

[71] Ezequiel Franco-Lara and Dirk Weuster-Botz. Application of fuzzy-logic models for metabolic control analysis. *Journal of theoretical biology*, 245(3):391–399, 2007.

[72] Pan Du, Jian Gong, Eve Syrkin Wurtele, and Julie A Dickerson. Modeling gene expression networks using fuzzy logic. *Systems, Man, and Cybernetics, Part B: Cybernetics, IEEE Transactions on*, 35(6):1351–1359, 2005.

[73] Bogju Lee, John Yen, Linyu Yang, and James C Liao. Incorporating qualitative knowledge in enzyme kinetic models using fuzzy logic. *Biotechnology and bioengineering*, 62(6):722–729, 1999.

[74] Liang Wang and Reza Langari. Complex systems modeling via fuzzy logic. *IEEE transactions on systems, man, and cybernetics. Part B, Cybernetics: a publication of the IEEE Systems, Man, and Cybernetics Society*, 26(1):100–106, 1996.

[75] Avi Ma'ayan. Insights into the organization of biochemical regulatory networks using graph theory analyses. *Journal of Biological Chemistry*, 284(9):5451–5455, 2009.

[76] Avi Ma'ayan. Network integration and graph analysis in mammalian molecular systems biology. *Systems Biology, IET*, 2(5):206–221, 2008.

[77] Ilia Nikolaevich Bronshtein, Konstantin A Semendyayev, Gerhard Musiol, and Heiner Muehlig. Algorithms of graph theory. In *Handbook of Mathematics*, chapter 5, pages 348–359. Springer, 2007.

[78] Wolfgang Huber, Vincent J Carey, Li Long, Seth Falcon, and Robert Gentleman. Graphs in molecular biology. *BMC Bioinformatics*, 8(Suppl 6):S8, 2007.

[79] Oliver Mason and Mark Verwoerd. Graph theory and networks in biology. *Systems Biology, IET*, 1(2):89–119, 2007.

[80] Tero Aittokallio and Benno Schwikowski. Graph-based methods for analysing networks in cell biology. *Briefings in bioinformatics*, 7(3):243–255, 2006.

[81] Jill H Larkin and Herbert A Simon. Why a diagram is (sometimes) worth ten thousand words. *Cognitive science*, 11(1):65–100, 1987.

[82] Ross M Thompson, Ulrich Brose, Jennifer A Dunne, Robert O Hall, Sally Hladyz, Roger L Kitching, Neo D Martinez, Heidi Rantala, Tamara N Romanuk, Daniel B Stouffer, et al. Food webs: reconciling the structure and function of biodiversity. *Trends in ecology & evolution*, 27(12):689–697, 2012.

[83] Thomas C Ings, Jose M Montoya, Jordi Bascompte, Nico Bluthgen, Lee Brown, Carsten F Dormann, Francois Edwards, David Figueroa, Ute Jacob, J Iwan Jones, et al. Ecological networks–beyond food webs. *Journal of Animal Ecology*, 78(1):253–269, 2009.

[84] Oswald J Schmitz and Andrew P Beckerman. Food webs. *eLS*, 2007.

[85] Stuart A Kauffman. Metabolic stability and epigenesis in randomly constructed genetic nets. *Journal of theoretical biology*, 22(3):437–467, 1969.

[86] Lee E Ostrander and Joe E Brand. Functional boolean models for systems with continuous variables. *Journal of theoretical biology*, 38(3):665–675, 1973.

[87] Rene Thomas. Boolean formalization of genetic control circuits. *Journal of theoretical biology*, 42(3):563–585, 1973.

[88] Leon Glass. Classification of biological networks by their qualitative dynamics. *Journal of Theoretical Biology*, 54(1):85–107, 1975.

[89] Reka Albert and Juilee Thakar. Boolean modeling: a logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 6(5):353–369, 2014.

[90] Rui-Sheng Wang, Assieh Saadatpour, and Reka Albert. Boolean modeling in systems biology: an overview of methodology and applications. *Physical biology*, 9(5):055001, 2012.

[91] Stefan Bornholdt. Boolean network models of cellular regulation: prospects and limitations. *Journal of the Royal Society Interface*, 5(Suppl 1):S85–S94, 2008.

[92] Sui Huang. Genomics, complexity and drug discovery: insights from boolean network models of cellular regulation. *Pharmacogenomics*, 2(3):203–222, 2001.

[93] J Leclerco and Jacques Emile Dumont. Boolean analysis of cell regulation networks. *Journal of theoretical biology*, 104(4):507–534, 1983.

[94] Abhishek Garg, Alessandro Di Cara, Ioannis Xenarios, Luis Mendoza, and Giovanni De Micheli. Synchronous versus asynchronous modeling of gene regulatory networks. *Bioinformatics*, 24(17):1917–1925, 2008.

[95] Ilia Nikolaevich Bronshtein, Konstantin A Semendyayev, Gerhard Musiol, and Heiner Muehlig. Boolean algebras and switch algebra. In *Handbook of mathematics*, chapter 5, pages 342–347. Springer, 2007.

[96] Kanae Oda, Yukiko Matsuoka, Akira Funahashi, and Hiroaki Kitano. A comprehensive pathway map of epidermal growth factor receptor signaling. *Molecular systems biology*, 1(1), 2005.

[97] Tamas Szekely and Kevin Burrage. Stochastic simulation in systems biology. *Computational and structural biotechnology journal*, 12(20):14–25, 2014.

[98] Marcello Buiatti and Giuseppe Longo. Randomness and multilevel interactions in biology. *Theory in Biosciences*, 132(3):139–158, 2013.

[99] Mukhtar Ullah and Olaf Wolkenhauer. Stochastic approaches in systems biology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 2(4):385–397, 2010.

[100] Vahid Shahrezaei and Peter S Swain. The stochastic nature of biochemical networks. *Current opinion in biotechnology*, 19(4):369–374, 2008.

[101] Alexei Kurakin. Stochastic cell. *IUBMB Life*, 57(2):59–63, 2005.

[102] CE Shannon. A mathematical theory of communication. *The Bell System Technical Journal*, 27(3):379–423, 1948.

[103] Siobhan S Mc Mahon, Aaron Sim, Sarah Filippi, Robert Johnson, Juliane Liepe, Dominic Smith, and Michael PH Stumpf. Information theory and signal transduction systems: from molecular information processing to network inference. *Seminars in cell & developmental biology*, 35:98–108, 2014.

[104] Matthew D Brennan, Raymond Cheong, and Andre Levchenko. How information theory handles cell signaling and uncertainty. *Science*, 338(6105), 2012.

[105] Alex Rhee, Raymond Cheong, and Andre Levchenko. The application of information theory to biochemical signaling systems. *Physical biology*, 9(4):045011, 2012.

[106] Christian Waltermann and Edda Klipp. Information theory based approaches to cellular signaling. *Biochimica et Biophysica Acta*, 1810(10):924–932, 2011.

[107] Walter Benenson and Horst Stocker. Measurements and measurement errors. In *Handbook of physics*, chapter 30, pages 1097–1113. Springer, 2002.

[108] Faiz M Khan, Ulf Schmitz, Svetoslav Nikolov, David Engelmann, Brigitte M Putzer, Olaf Wolkenhauer, and Julio Vera. Hybrid modeling of the crosstalk between signaling and transcriptional networks using ordinary differential equations and multi-valued logic. *Biochimica et Biophysica Acta*, 1844(1):289–298, 2014.

[109] Natalie Berestovsky, Wanding Zhou, Deepak Nagrath, and Luay Nakhleh. Modeling integrated cellular machinery using hybrid petri-boolean networks. *PLoS computational biology*, 9(11):e1003306, 2013.

[110] Regina Samaga and Steffen Klamt. Modeling approaches for qualitative and semi-quantitative analysis of cellular signaling networks. *Cell Commun Signal*, 11:43, 2013.

[111] Leon Glass and Stuart A Kauffman. The logical analysis of continuous, non-linear biochemical control networks. *Journal of theoretical Biology*, 39(1):103–129, 1973.

[112] Alan C Hindmarsh. Odepack, a systematized collection of ode solvers. *IMACS transactions on scientific computation*, 1:55–64, 1983.