Operational interactions in genetic networks: application to an apoptosis signalling pathway

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Abstract— **Discrete dynamical systems, and in particular non-deterministic Boolean automata, offer a convenient framework to analyse complex regulatory networks motivated by biological systems. In this paper, a method is proposed to analyse the dynamics of Boolean networks under the realistic context-sensitive asynchronous strategy. The main goal is to identify the operational interactions responsible for a given dynamical behaviour of the network. The application to a model of programmed cell death (apoptosis) uncovers two families of operational interactions, used by the cell to guide its decision between the survival or death pathways.**

I. Introduction

Boolean networks (and more generally discrete dynamical systems) have been playing an increasingly important role in the qualitative study of gene networks [1], [2], [6], [13]. The components of a genetic network are typically messenger RNAs and proteins, which are represented simply as "expressed" ($X = 1$) or "not expressed" ($X = 0$). The evolution of each variable X along a discrete time is given by a logical rule which reflects the influence of all other variables on X (typically, a combination of activation/repression effects). The state space of a Boolean model is finite, and a transition graph characterizes all the *qualitative* dynamical trajectories of the network, based simply on the structure of interactions and an updating strategy. Such a qualitative description does not depend on specific kinetic parameters and so provides a measure of the robustness of the network with respect to fluctuations in the environment [1]. These general properties and "easier" handling of the state space counterbalance the loss of detailed information on time evolution and (more realistic) continuous concentration changes. This is therefore an appropriate framework to study complex networks and gain intuition on the mechanisms responsible for a particular qualitative dynamical behaviour. For instance, a logical model of the mammalian cell cycle developed in [6] was later used to study possible strategies to control the cell cycle, so that the cell spends less time in "undesirable states" [5]. In another study [13], a logical model of T cell receptor signalling was used to find which interactions prevent a given target biological function. These applications are useful from the point of view of control and therapeutical interventions.

In this context, we propose a rigorous method to analyse Boolean networks, combining the information contained on the asynchronous transition graph of the system (state space dynamics) with the structure of the network (diagram of interactions). This method may be viewed as a *model reduction* technique, where a smaller network is identified to be responsible for the dynamics within a given region of the state space. The first part of the method involves the simplification and hierarchical organization of the asynchronous transition graph (based on the well-known strongly connected components decomposition). A new "reduced" transition graph is then constructed which describes the transitions between the strongly connected components (Section III). The second part involves the identification of the operational network (active interactions) within a given region of the state space (Section IV). Finally, in Section V, probabilities of transition are associated to each link in the transition graph (thus generating a Markov chain), enabling to consider updating strategies in a more quantitative manner. The probability matrix may reflect different biological scenarios, such as different relative degradation rates, or other experimental situations that make one reaction more probable than another. Relevant quantities, such as the expected times for convergence to a given attractor, can then be computed.

The methods proposed above are illustrated by an application to an apoptosis (or programmed cell death) network [2], [14]. The dynamics of the network in response to death-receptor stimulation is studied, and two core groups of variables and pathways are identified. They correspond to two mechanisms responsible for the decision between programmed cell death or cell survival.

II. Asynchronous Boolean models of gene regulatory networks

A. Structure of a Boolean network

Discrete networks have often been proposed to represent the dynamics of gene regulatory networks [10], [15]. The mathematical basis of any discrete model consists of a finite set of discrete variables that interact with one another through discrete *activation functions*. Usually, these interactions are comprised in a (finite) directed graph, called *interaction graph*. This graph, together with the family of activation functions, define the structure of a discrete system.

In this paper, we will consider *Boolean* networks, where variables can take only two qualitative values: "0" represents a basal level (inhibition -or weak activation- of the transcription of a gene, absence of a protein) and "1" represents a high level (activation of the transcription of a gene, presence of a protein). As most of discrete models are based on the same mathematical objects (with slightly different definitions), the

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two following definitions only set the notations that will be used in the rest of this paper (for a detailed explanation, one can refer to the extensive literature on nondeterministic automata).

Definition 1 *The* interaction graph *of a n-dimensional Boolean network is denoted by $\mathcal{G} = (\mathcal{V}, \mathcal{E})$, where $\mathcal{V} = \{v_1, \ldots, v_n\}$ is the set of nodes (each node being associated with a biological species) and $\mathcal{E} \subset \mathcal{V} \times \mathcal{V}$ is the set of directed edges (representing the interactions between these species). The edge (v_j, v_i) exists if node v_j influences node v_i (e.g. v_j activates or inhibits v_i).*

Definition 2 *The* structure *of a n-dimensional Boolean network is defined by an interaction graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$ together with a collection $\mathcal{F} = \{f_i,\, i = 1, \ldots, n\}$ of Boolean functions. For each $i \in \{1, \ldots, n\}$, f_i designates the activation function of node v_i.*

Let x_i be the Boolean variable associated with node v_i. The updated value of x_i, denoted by x_i' is given by:

$$x_i' = f_i\left(x_{i_1}, \ldots, x_{i_{k_i}}\right),$$

where $\left\{v_{i_1}, \ldots, v_{i_{k_i}}\right\}$ is the set of nodes that *influence* v_i.

Remark 1 *In the study of discrete models of biochemical networks, the arrows of the interaction graph are usually signed, indicating whether the arrow represents an* activation *or an* inhibition*. It is to be noted that, for a general network given by Def. 2, it is not always possible to associate a sign to each arrow in an unequivocal manner. Nevertheless, for networks constructed from the description of a particular biological system, most of the edges can actually be signed unequivocally.*

In order to illustrate these definitions, we describe in the following a 12-dimensional Boolean network, modelling an apoptosis signalling pathway. This example will be analysed throughout the whole paper.

B. Working example: an apoptosis signalling pathway

Apoptosis, or programmed cell death, is a physiological process which allows an organism to remove damaged or unwanted cells, thus maintaining normal cellular homeostasis. Diseases such as cancer result from malfunctioning apoptotic pathways. The apoptosis signalling pathway to be considered in this paper is based on the model presented in [2], which is, in fact, a discrete version of a continuous model of apoptosis first developed in [14]. For details on the modelling step, the reader is referred to [2], [4], [9], [12], [14] and references therein. Fig. 1 shows the interaction graph of the system. The activation functions can be found in Table I (the notations used are classical in Boolean algebra: a bar over a variable represents the logical negation, and the symbols $\vee$ and $\wedge$ represent respectively the logical disjunction and conjunction).

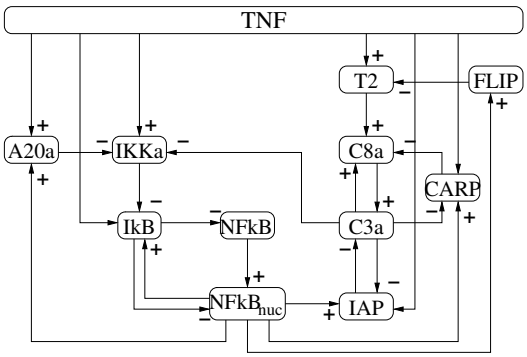

Fig. 1. Interaction graph of the simplified model of regulation of apoptosis via the NFκB pathway. As noted in Remark 1, some edges could not be signed unequivocally (influence of TNF on IκB, IAP and CARP).

TABLE I

BOOLEAN RULES FOR THE APOPTOSIS NETWORK DEPICTED IN FIG. 1.

Node	Boolean rule
TNF	TNF (input of the whole system)
T2	TNF $\wedge$ $\overline{\text{FLIP}}$
IKKa	TNF $\wedge$ $\overline{\text{A20a}}$ $\wedge$ $\overline{\text{C3a}}$
NFκB	$\overline{\text{I}\kappa\text{B}}$
NFκB$_{\text{nuc}}$	NFκB $\wedge$ $\overline{\text{I}\kappa\text{B}}$
IκB	[TNF $\wedge$ (NFκB$_{\text{nuc}}$ $\wedge$ $\overline{\text{IKKa}}$)] $\vee$ [$\overline{\text{TNF}}$ $\wedge$ (NFκB$_{\text{nuc}}$ $\vee$ $\overline{\text{IKKa}}$)]
A20a	TNF $\wedge$ NFκB$_{\text{nuc}}$
IAP	[TNF $\wedge$ (NFκB$_{\text{nuc}}$ $\wedge$ $\overline{\text{C3a}}$)] $\vee$ [$\overline{\text{TNF}}$ $\wedge$ (NFκB$_{\text{nuc}}$ $\vee$ $\overline{\text{C3a}}$)]
FLIP	NFκB$_{\text{nuc}}$
C3a	$\overline{\text{IAP}}$ $\wedge$ C8a
C8a	$\overline{\text{CARP}}$ $\wedge$ (C3a $\vee$ T2)
CARP	[TNF $\wedge$ (NFκB$_{\text{nuc}}$ $\wedge$ $\overline{\text{C3a}}$)] $\vee$ [$\overline{\text{TNF}}$ $\wedge$ (NFκB$_{\text{nuc}}$ $\vee$ $\overline{\text{C3a}}$)]

Note that TNF stimulation triggers two opposite effects: activation and inhibition (through NFκB) of caspases C3a, C8a. An abundance of active caspases typically leads to cell death, while a high concentration of IAP and a low level of C3a, C8a typically characterizes a living cell. The dynamics of the network will ultimately lead to a decision between cell death or cell survival.

C. Synchronous vs asynchronous dynamics

Consider a n-dimensional network $\mathcal{N} = (\mathcal{V}, \mathcal{E}, \mathcal{F})$ (see Def. 2). The state space of $\mathcal{N}$ is the set $\Omega = \{0,1\}^n$ whose cardinality is 2^n. As the state space is finite, one can represent the discrete dynamical behaviour of the network with a finite directed graph, called *transition graph*. In order to define it properly, one needs to assign an operating mode, or updating strategy. There exist two main operating modes studied in the literature. The first one is the *synchronous* strategy, where all variables are simultaneously updated at each discrete instant [10]. The dynamics implied by the synchronous strategy presents some nice mathematical properties (mainly, the transition graph is deterministic) that allow one to simulate high-dimensional networks, randomly generated, in order to find statistically relevant types of dynamical behaviour. However, if one wants to model a given biological system in a more realistic manner, the synchronous updating strategy may be quite a strong assumption, poorly

related to the reality. This is why other approaches have been proposed, by developing *asynchronous* strategies, where discrete variables are updated in a heterogeneous (*context-sensitive*) way over time. Known as "nondeterministic automata" in computer science, asynchronous networks as models of biological regulatory networks are often called Thomas' networks [15].

For any state $X \in \Omega$, we introduce the following notations:

- $F(X) \in \Omega$ designates the synchronous successor of X, ie: for $i = 1 \dots n$, $F_i(X) = x'_i$.
- For each $i \in \{1, \dots, n\}$, $\widetilde{X}^i$ designates the Boolean vector: $(x_1, \dots, x_{i-1}, \overline{x_i}, x_{i+1}, \dots, x_n) \in \Omega$.
- $U(X) = \{v_i \in \mathcal{V} \mid x_i \neq x'_i\} \subset \mathcal{V}$ denote the (possibly empty) set of nodes that can be updated in the state X.

Throughout this paper, simultaneous updates of several nodes is forbidden (which is reasonable from the biological point of view). Furthermore, every possible update is taken into account (i.e. if at state X the node v_i is liable to change, then that update *must* be present in the transition graph). These two conditions lead to the following:

Definition 3 *The* asynchronous transition graph *of a network $\mathcal{N} = (\mathcal{V}, \mathcal{E}, \mathcal{F})$ is the directed graph $G = (V, E)$ where the set of nodes V is the state space $\Omega = \{0, 1\}^n$ and the set of directed edges E is given by:*

$$E = \left\{ \left(X \to \widetilde{X}^i \right) \mid X \in \Omega, v_i \in U(X) \right\}.$$

Contrary to the synchronous case, the asynchronous transition graph is non-deterministic (one state may have several successors). One consequence is for instance that the notions of attractor, or basin of attraction, are not straightforward (they will be defined later). This non-determinism is a fundamental property as the asynchronous transition graph comprises all possible trajectories in a finite structure, which allows to find general dynamical properties, valid whatever the updating strategy.

Obviously, although finite, the size of this graph grows exponentially with the dimension of the system (in the boolean case, its size is exactly 2^n). This limits the use of general graph algorithms (see next section) to relatively low dimensional systems (on the order of $n =$ 10-20), with respect to the synchronous case, where the dimension of the system under study can be higher.

III. Hierarchical organization of the asynchronous transition graph

In this section, a general methodology to analyse the asynchronous transition graph of a Boolean network is presented. This methodology is based on different algorithms that are classical in the field of graph theory (mainly the strongly connected components decomposition and the topological sort). One can refer to [3] for a detailed analysis of these algorithms. The method provides answers to many biological issues tackled by Boolean models, such as the existence and characterization of attractors, reachability or controllability of a given state, etc. More generally, our main goal is to find dynamical properties that are *independent* of the choice of a particular updating strategy, and therefore *robust* with the structure of the network. Some of the following results are related to results of [6], [7].

A. SCC decomposition and hierarchical organization

The notion of hierarchical organization of a directed graph (or *digraph*) relies on the well known strongly connected components (SCC) decomposition algorithm. Let $G = (V, E)$ be a digraph. A strongly connected component of G is a maximal set of vertices $C \subseteq V$ such that for every pair of vertices $u, v \in C$, u and v are reachable from each other (see [3] for a precise definition). The SCC *decomposition* of G consists in computing its SCCs: $C_1, \dots, C_p$ and then in computing the digraph $G^{scc} = (V^{scc}, E^{scc})$ defined as follows:

- $V^{scc} = \{C_1, \dots C_p\}$,
- given $1 \leq i, j \leq p$, the directed edge (C_i, C_j) belongs to E^{scc} if and only if there are $u \in C_i$ and $v \in C_j$ such that $(u, v) \in E$.

It can be easily proved (see [3]) that the digraph G^{scc} contains no directed cycles. It is called a *dag* (for directed acyclic graph). This is a key property of G^{scc}, because every dag can be *topologically sorted* (see [3], section 22.4). A topological sort of a dag can be viewed as a classification of its vertices in several hierarchical levels $H_1, H_2, \dots$ such that the vertices of the first level H_1 are vertices with no predecessors, and the predecessors of vertices of level H_i, $i > 0$, are contained in inferior levels H_j with $j < i$.

The main interest of this hierarchical organization, applied to the asynchronous transition graph of a Boolean network, is that, whatever path we choose in the graph (i.e. whatever updating order we choose for the variables), once it leaves a hierarchical level H_i, the system cannot return to this level. So, any trajectory will travel "down" the hierarchical levels: $H_{i_1} \to H_{i_2} \to \dots$ (with $i_1 < i_2 < \dots$).

Definition 4 *Let $\mathcal{N}$ be a Boolean network, a SCC $c^* \in V^{scc}$ that has no successor in G^{scc} is called an (asynchronous) attractor of $\mathcal{N}$.*

In graph theory, such SCCs are often called *terminal* SCCs. In other words, the asynchronous attractors of a Boolean network are the strongly connected components of the transition graph that cannot be escaped by the system, whatever the updating strategy. It should be noted that it is still possible to construct specific asynchronous updating strategies such that the system gets "stuck" in a non terminal SCC. Nevertheless, such intermediate SCCs will not be considered as attractors, as we seek general dynamical properties that are valid *for all* the choices of updating rules. Also note that in a probabilistic approach (see last part), given any probability distribution for the transitions (provided that any asynchronous successor of a state has a non-zero probability), the transition graph can be seen as an *absorbing* Markov chain, and its attractors are actually its absorbing classes.

Definition 5 *Let c be a SCC of the asynchronous transition graph. $\mathcal{A}(c)$ (resp. $\mathcal{R}(c)$) designates the attraction set (resp. the reachability set) of c, that is, the set of all SCCs that can lead to c (resp., the set of all SCCs that can be reached from c). If c is an attractor of the network, the set $\mathcal{A}(c)$ is its basin of attraction.*

As G^{scc} has no cycles, the sets $\mathcal{A}(c)$ and $\mathcal{R}(c)$ can be easily computed by straightforward recursive procedures.

B. Application to the apoptosis network

The results presented here were obtained with codes implemented in `Matlab`. Following the `matlab_bgl`[1] library specifications, graphs were represented with sparse matrices, allowing a quite efficient implementation.

Recall that the system under study is of dimension $n = 12$. The state space is $\Omega = \{0,1\}^{12}$, and the size of the asynchronous transition graph G is $2^{12} = 4096$. The number of strongly connected components is $p = 1472$, therefore the size of G^{scc} is only 40% of the size of G. The hierarchical organization of this graph has only 38 hierarchical levels and 3 attractors. As TNF is an input, its value remains constant, whatever the path in the graph. Therefore G (and also G^{scc}) has two regions that are completely separated (i.e., there exist no directed edges between them). They will be denoted $\mathcal{T}^0$ (where TNF $= 0$) and $\mathcal{T}^1$ (where TNF $= 1$).

The attractors of the system (corresponding to three terminal SCCs) will be denoted a_1, a_2 and a_3. Attractors a_1 and a_2 both contain only one state (they are therefore equilibrium points) whereas a_3 contains 56 states. Table II indicates the boolean values taken by the variables within each attractor (symbol * indicates variables that oscillate within the attractor). As can be seen in Table II, a_1 and a_2 belong to $\mathcal{T}^0$

TABLE II
BOOLEAN PATTERNS OF THE THREE ATTRACTORS.

	TNF	T2	IKKa	NFκB	NFκB$_{nuc}$	IκB
a_1	0	0	0	0	0	1
a_2	0	0	0	0	0	1
a_3	1	*	0	*	*	*
	A20a	IAP	FLIP	C3a	C8a	CARP
a_1	0	1	0	0	0	1
a_2	0	0	0	1	1	0
a_3	*	0	*	1	1	0

(TNF$=0$), the first one corresponding to the survival of the cell (caspases C3a and C8a are absent) and the second one corresponding to the triggering of apoptosis (with activation of the caspases). Attractor a_3 belongs to $\mathcal{T}^1$ (TNF$=1$), within this attractor the caspases are activated while NFκB, IκB and other factors oscillate. At first, the presence of caspases might seem to indicate that apoptosis will be the final outcome. Of course, this "phenotypic" interpretation is only valid provided the input TNF is effectively sustained for a sufficiently long time (long enough for the caspases to initiate the effective steps of apoptosis, not represented in our model). However, for "pulse"-like stimulation, with removal of TNF after a certain time interval, trajectories starting from a_3 (and then switching to the corresponding state with TNF=0) may still converge to the survival state (see Section V). Therefore, considering the model as a *decision* process, we can conclude that attractor a_3 is not really "apoptotic", in the sense that survival outcome is still possible upon TNF removal.

[1] see http://www.stanford.edu/~dgleich/programs/matlab_bgl/

Remark 2 *In attractor a_3, six variables oscillate, which accounts for $2^6 = 64$ states. So the concise description of a_3 in Table II is not enough to completely describe the attractor (which contains only 56 states). Actually, if we consider the projection of a_3 onto the 3-dimensional subspace composed of NFκB, NFκB_{nuc}, and IκB (the other variables A20a, FLIP, and T2 being only outputs, as can be seen in Fig. 2, top), we obtain a SCC lying in $\{0,1\}^3$ composed of seven states. This explains why a_3 contains only $7 \times 2^3 = 56$ states.*

IV. IDENTIFICATION OF OPERATIONAL INTERACTIONS

The set of SCCs can be seen as a new state space, reduced from 2^n to p states. Thus, the graph G^{scc} describes the (asynchronous) dynamics of a new, reduced, system. As a second step of our *model reduction* technique, we propose in this section to identify a subset of rules that govern the dynamics in a given region of the new state space.

This method makes use of the algorithm REVEAL, developed in [11]. The primary goal of this algorithm is to identify, given a set of discrete transitions $\{X \to X' : X, X' \in \Omega\}$, the set of Boolean networks (Def. 2) that are consistent with these data. Initially, this algorithm was proposed as an inference algorithm, identifying Boolean networks from (qualitative) *experimental* data, such as time series issued from DNA microarrays. With respect to that goal, its main limitation is that these time series are supposed to obey the *synchronous* update of the variables, which is, as already said, quite a strong assumption from a biological point of view. Nevertheless, we show in the following that adapting this algorithm to the asynchronous framework provides interesting results, that improve the understanding of the underlying biological system. For details about REVEAL (correctness, complexity analysis etc.), the reader is referred to [11], [16].

A. Algorithmic search for operational interactions

Let $\mathcal{N} = (\mathcal{G}, \mathcal{F})$ be a n-dimensional Boolean network, and let G, G^{scc} denote respectively its asynchronous transition graph and its SCC decomposition. The principle of the method relies on the following observations.

1) Suppose the system is in an initial state X^0. This state belongs to a unique SCC, denoted by c.
2) Compute the reachability set $\mathcal{R}(c)$ (Def. 5). This set contains all the states that are reachable by the system from c, whatever the updating strategy.
3) Reconstruct the partial synchronous successor function on $\mathcal{R}(c)$.
4) Applying REVEAL to this partial synchronous successor function identifies the Boolean rules (and the

corresponding interaction graph) that are *operational* in the system, starting from c.

A detailed description of this algorithmic procedure can be found in [17]. It is to be noted that the structure identified by REVEAL is not *a priori* unique (see [17] for more details).

B. Application to the apoptosis network

The previous procedure has been applied to the asymptotic behaviours in the two separated regions $\mathcal{T}^0$ and $\mathcal{T}^1$. As seen previously, region $\mathcal{T}^1$ contains a unique oscillatory attractor a_3 (see Table II). The operational graph of this attractor is depicted in Fig. 2 (top). In the region $\mathcal{T}^0$, we applied the method to a SCC c^* situated just before the fork between the two equilibria. The corresponding operational graph is depicted in Fig. 2 (bottom). The first graph represents the interactions that stay ultimately active as long as TNF stimulation is sustained. The second one represents the interactions that are eventually responsible for the choice between apoptotic and anti-apoptotic pathways upon TNF removal. The interesting fact about these two graphs is that the first one contains only two feedback loops (both negative), and the second one contains only one positive loop. They actually illustrate a very general result in systems biology (see for instance [8]) that links multistationarity with positive feedback loops and the presence of oscillations with negative feedback loops. Indeed, what our method shows on this example is that it is algorithmically possible, within the asynchronous boolean framework, to isolate in a complex interaction graph (Fig. 1), comprising multiple feedback loops, which loops are eventually responsible for two global dynamical behaviours: oscillations and multistationarity.

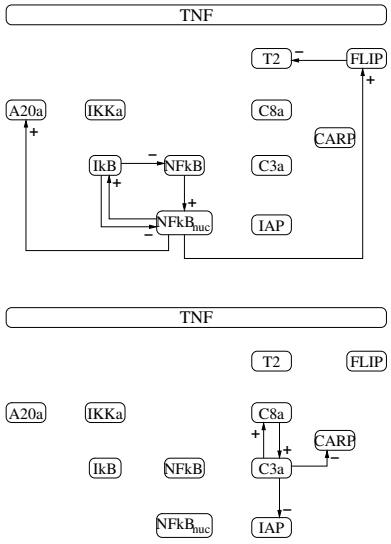

Fig. 2. Top: operational graph within attractor a_3 (oscillatory behaviour, TNF= 1). Bottom: operational graph prior the choice between equilibria a_1 and a_2 (survival of the cell/apoptosis). The isolated variables have a fixed value, that can be found in Table II.

V. Probabilistic analysis of asynchronous dynamics

The previous sections dealed with a systematic *qualitative* study of the trajectories of an asynchronous Boolean model. The transition graph G provides all possible successors without indicating which is more probable at a given time. A more *quantitative* model may be obtained by associating a transition probability with each edge of G. In other words, one can construct a discrete time Markov chain from the dynamics on G.

The graph G can be characterized by its adjacency matrix, $A(G) = (a_{i,j})_{1\leq i,j\leq 2^n}$, where $a_{ij} = 1$ if state j is a successor of state i, and $a_{ij} = 0$ otherwise. If a given state i has only one successor j, then any trajectory going through i moves to j with probability $p_{ij} = 1$. If successor i has $N(i) \geq 2$ successors, then different probabilities, $p_{ij} < 1$, may be assigned to each transition, with $\sum_{j=1}^{N(i)} p_{ij} = 1$.

A transition from i to j occurs due to a change in one of the variables so, to assign the different transition probabilities, biological knowledge can be used to decide which variables change more frequently than others (based, for instance, on relative turn-over rates). Following the notion of *priority classes* [6], the idea is to divide the variables into several groups and assign a weight to each of these groups: higher weights denote a more probable transition. A similar idea was used in [1], where two classes were considered, one for proteins and another for mRNAs. More generally, consider ρ classes $\mathcal{C}_1$, ..., $\mathcal{C}_\rho$ and their respective weights, $W_1 > W_2 > \cdots > W_\rho$, and associate with each transition (i, j) the value $w_{ij} = W_r$, where r is the index of the class containing the variable that has been updated between i and j. Then define the transition matrix, $\mathcal{P}(G) = (p_{i,j})_{1\leq i,j\leq 2^n}$, as follows:

$$\forall 1 \leq i, j \leq 2^n \ , \ p_{ij} = \frac{w_{ij}a_{ij}}{\sum_{k=1}^{2^n} w_{ik}}. \qquad (1)$$

To compute the transition probabilities between two SCCs c and c', in the reduced graph G^{scc}, it then suffices to apply the following formula:

$$p^{scc}_{c,c'} = \frac{1}{|S(c)|} \sum_{i\in S(c)} \sum_{j\in S(c')} p_{ij}. \qquad (2)$$

(where $S(c)$ denotes the set of states that are contained in the SCC c).

For the apoptosis network, four priority classes were chosen (Table III) based on the parameters reported in [4], [14] and references therein. The first class of proteins ($w_1 = 7$) corresponds to variables with higher degradation rates. The matrix $\mathcal{P}^{scc}$ was then constructed following (2).

We used this matrix to analyse the response of the cell to a shutdown of TNF stimulation. Technically, we considered any state $X \in \mathcal{T}^1$ (i.e. where TNF is present), and for all such states, we computed the corresponding state Y in $\mathcal{T}^0$ (where TNF is absent). Denoting by c the SCC that contains Y, we can then compute the probability to reach attractor a_1 (survival) and attractor a_2 (apoptosis) from c. We then

TABLE III

PRIORITY CLASSES AND RESPECTIVE WEIGHTS.

Class	Weights	Variables
$\mathcal{C}_1$	$w_1 = 7$	NFκB, NFκB$_{nuc}$, IκB, CARP
$\mathcal{C}_2$	$w_2 = 5$	T_2, IKKa
$\mathcal{C}_3$	$w_3 = 3$	C3a, C8a
$\mathcal{C}_4$	$w_4 = 1$	A20a, IAP, FLIP

averaged these probabilities with respect to the hierarchical levels of initial states X. The result is given in Fig. 3. This figure shows that cell survival is always more probable

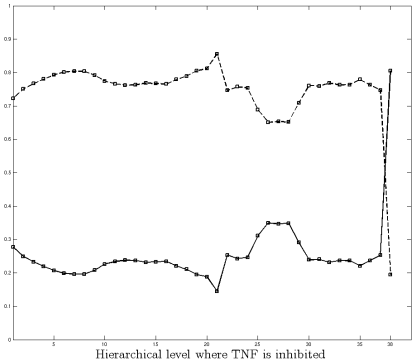

Fig. 3. System's response to TNF switch off at level H_i (x-axis). The two curves represent the average probability of "cell survival" (dashed) or "cell death" (solid), as the system starts from a state in hierarchical level H_i.

(around 85%) than apoptosis, except when TNF is switched off once the trajectory has reached the last hierarchical level of $\mathcal{T}^1$. This suggests that, to promote apoptosis, TNF should be sustained long enough for the system to reach attractor a_3. This observation is also in agreement with experimental results, which indicate that longer exposure to TNF leads to higher cell death rates (see [4] and references therein).

VI. CONCLUSION AND FUTURE WORK

The qualitative dynamics of genetic networks were studied by analysing the asynchronous transition graph of the Boolean model associated with the network's diagram of interactions. The notion of a family of operational interactions was introduced, defined with respect to a given (self-contained) region of the transition graph, as a set of interactions which generate the transition dynamics in that particular region of the graph.

A method for identifying operational interactions was developed, based on the decomposition of the asynchronous transition graph (or a sub-graph) into its strongly connected components, followed by the reconstruction of the Boolean rules that represent the graph of transitions among the SCCs. The identification algorithm known as REVEAL was adapted and used to determine a family of Boolean rules that describe the dynamics represented by a (sub-)graph of transitions.

As illustrated with the apoptosis example, identifying operational interactions allows to uncover which mechanisms are mainly responsible for a given asymptotic behaviour of the system, for instance, the existence of oscillatory dynamics or (multi-)stability.

Altogether, these are useful tools to test hypotheses and generate predictions concerning the structure of interconnections and the importance of each variable to the overall dynamics. Future work will focus on the control of the system, towards either the apoptotic or the survival states. Possible approaches include finding and implementing a suitable matrix $\mathcal{P}_{bio}^{scc}$, or re-wiring the diagram of interactions in an appropriate way.

VII. ACKNOWLEDGMENTS

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