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Predicting the asymptotic dynamics of large biological networks by interconnections of Boolean modules

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Abstract— **The dynamics of Boolean networks can be represented by asynchronous transition graphs, whose attractors describe the system's asymptotic behavior. This paper shows that the attractors of the feedback interconnection of two Boolean modules can be fully identified in terms of cross-products of the attractors of each module. Based on this observation, a model reduction technique is proposed, aiming at analysing the asymptotic behavior of a high-dimensional network through the computation of the dynamics of two isolated smaller subnetworks. The method is applied to a large network which models cell-fate decision: all the attractors of the full network are exactly calculated by representing the network as an interconnection of two 3-input/3-output modules.**

I. INTRODUCTION

Models of biological regulatory networks frequently involve a large number of variables and interactions, and this introduces mathematical problems related to the analysis of high dimensional dynamical systems. Searching for model reduction techniques is therefore a central point in mathematical modeling [1]. The development of reduction techniques appropriate to describe biological systems remains a very challenging task, due to the constraints on positivity or interpretation of the reduced variables. The method proposed in this paper concerns the special class of asynchronous Boolean networks (ABN). Boolean models are specially useful to represent large biological networks for which available information is essentially *qualitative* rather than *quantitative*, in the sense that interactions among a group of biochemical species (genes, enzymes, transcription factors, etc.) are clearly identified and experimentally confirmed, but little is known about rate constants and kinetic parameters. In these cases, Boolean models are able to rapidly predict gene expression patterns under various experimental conditions as well as mimick, in a rather straightforward manner "mutant" behaviors (for instance by forcing one or several variables to a given value, thus modeling genetic deletions and over-expressions, or specific drug effects). Furthermore, Boolean models generally provide an efficient and practical framework to implement complex influence diagrams, with dozens of interacting variables, intertwined feedback loops and multiple crosstalks between several antagonistic pathways. Some successful examples include Drosophila's segment polarity network [2], a cell-fate decision network [3], or apoptosis [4].

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Due to the nature of its state space (the variables take only the values 0 or 1), a Boolean model is quite easy to implement and simulate and one can, in principle, exactly compute all its possible trajectories and "steady states" or attractors. However, as the dimension n of the model increases, the full characterization of the state space rapidly becomes computationally expensive, since the number of calculations increases with 2^n. In such cases, one solution can be to focus the analysis on a particular (and wisely chosen) set of initial conditions, which leads to a partial characterization of the state space and attractors (see for instance [2]). This has the disadvantage that possibly interesting global dynamical behaviors and attractors may remain unidentified. Another reduction technique is described in [5] and consists in projecting the transition graph onto a subset of the variables. When a variable is "hidden", its logical rule is injected into the rules of the variables it regulates (self-regulated variables are thus irreducible), therefore guaranteeing no loss in terms of interactions. From a dynamical point of view, this process assumes that the variable is rapid with respect to the others, as its update is immediately transmitted to its targets.

In this paper we propose a method to analyse large Boolean networks as the interconnection of two input/ouput modules, A and B, of smaller dimensions. The idea of decomposing a system into two smaller modules to deduce some properties of the composed system is a classical idea in automatic control. It has been used in several contexts, notably to characterize the equilibria of the interconnection of two monotone ODE systems, under appropriate conditions on the input/output characteristics [6], [7]. For discrete systems the fact that the state space of an interconnection can be easily computed as the set of all cross-products of the states of the two systems, has been used to predict several facts on the composed system, such as reachability regions [8].

In this work, we will consider the interconnection of Boolean networks, with Boolean inputs and outputs. For each fixed input, the dynamics of the network is characterized by an asynchronous transition graph (see Section II). To study the attractors of the interconnection of systems A and B, a new *asymptotic graph* is introduced, where the nodes are the cross products of (same-output subsets of the) attractors of the networks A and B, and the edges are those induced by the asynchronous dynamics (Section III). The main result states that the attractors of the interconnected system can be recovered from the attractors of this asymptotic graph (Section IV), a result which is valid for general multiple input-multiple output systems (Section V). Finally, an application

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to the high-dimensional cell-fate decision model developed in [3] shows that this method can be a very powerful tool for model reduction and analysis.

II. DYNAMICS OF BOOLEAN MODULES

A Boolean multiple input-multiple output (MIMO) system A is characterized by its state space $\Omega^A : \{0,1\}^{n_A}$ on n_A variables $a = (a_1, \ldots, a_{n_A})$, its input $u \in \mathcal{U}^A = \{0,1\}^{p_A}$, output function $h_A : \Omega^A \to \mathcal{H}^A$, with $\mathcal{H}^A = \{0,1\}^{q_A}$, and a logical vector function $f^A(a;u) : \Omega^A \times \mathcal{U}^A \to \Omega^A$.

To characterize the dynamics of system A one needs to specify the updating strategy, by defining a rule for calculating the state at the next instant ($a[t^+] \equiv a^+$) as a function of the current state ($a[t]$). There are different possible strategies, among them the synchronous case (where all coordinates a_i are simultaneously updated), and the more general asynchronous case (where a single coordinate is updated at each iteration). In both cases, the dynamics of a Boolean system can be fully characterized in terms of a directed graph, called *transition graph*. Asynchronous updating is more complex as the transition graph is not deterministic, nevertheless it is more general and also much more realistic for biological applications, as different events may happen at very different time scales. Throughout this paper, we will consider asynchronous Boolean networks (ABN). The description and analysis of an ABN without inputs or outputs can be found in [9] (see also [10] and [11] for general references on graphs and positive matrices). For MIMO ABNs, the relevant objects can be defined as follows.

Definition 1: The *asynchronous transition graph*, $G^{A,u} = (\Omega^A, \mathcal{E}^{A,u})$, of system A under fixed input u is a directed graph among the elements (or nodes) of Ω^A, where the edges are given by $\mathcal{E}^{A,u}$. An edge "$a \to \tilde{a}$" is in $\mathcal{E}^{A,u}$ iff:

$$\exists j,\ \tilde{a}_j = f_j^A(a;u) = 1 - a_j \text{ and } \tilde{a}_i = a_i\ \forall i \neq j.$$

If $f^A(a;u) \equiv a$, then there are no outgoing arrows from this node and, in this case, an edge $a \to a$ is added. Such states are called *single state attractors*.

Let $\mathcal{S}(\Omega^A)$ represent the set of subsets of Ω^A.

Definition 2: The set of asynchronous *successors*, $\sigma_{A,u} : \Omega^A \to \mathcal{S}(\Omega^A)$, of an element of Ω^A in $G^{A,u}$ is given by:

$$\sigma_{A,u}(a) = \{\tilde{a} \in \Omega^A :\ a \to \tilde{a} \text{ is in } \mathcal{E}^{A,u}\}, \tag{1}$$

(therefore, if $f^A(a;u) = a$, $\sigma_{A,u}(a)$ is the singleton $\{a\}$). By abuse of notation, define also the set of successors of a set $S \subset \Omega^A$: $\sigma_{A,u}(S) = \cup_{a \in S}\ \sigma_{A,u}(a)$.

A pathway in $G^{A,u}$ connecting two of its vertices a_0 to a_f will be represented in the form:

$$a_0 \overset{G^{A,u}}{\rightsquigarrow} a_f.$$

Definition 3: The *basin of attraction* and the *reachable set* of a set $S \subset \Omega^A$, in $G^{A,u}$, are given by:

$$\mathcal{B}(S;G^{A,u}) = \{a_0 \in \Omega^A :\ \exists\ a_0 \overset{G^{A,u}}{\rightsquigarrow} a_f,\ \text{ for some } a_f \in S\},$$
$$\mathcal{R}(S;G^{A,u}) = \{a_f \in \Omega^A :\ \exists\ a_0 \overset{G^{A,u}}{\rightsquigarrow} a_f,\ \text{ for some } a_0 \in S\}.$$

Definition 4: A *strongly connected component (SCC)* of $G^{A,u}$ is a maximal subset $C \subset \Omega^A$, that contains a pathway joining any pair of its elements:

$$\forall a, \tilde{a} \in C,\ \ \exists\ \ a \overset{G^{A,u}}{\rightsquigarrow} \tilde{a}.$$

Define also the set $\mathcal{C}^{A,u}$ of all strongly connected components of $G^{A,u}$.

Definition 5: An *attractor* T is a *terminal strongly connected component*, that is, $\cup_{a \in T}\sigma_{A,u}(a) = T$. The output set of an attractor will be denoted $h_A(T) = \{h_A(a), a \in T\} \subseteq \mathcal{H}^A$. Each attractor can be decomposed into (at most) 2^{q_A} disjoint subsets, according to their output, which will be called *semi-attractors* :

$$T = \cup_{\alpha \in \mathcal{H}^A} T_\alpha,\ \text{where } T_\alpha = \{a \in T :\ h_A(a) = \alpha\ \}.$$

In the following, we will only consider the nonempty semi-attractors (it is immediate to see that at least one of the semi-attractors will be nonempty). To deal with systems that have more than one attractor we will adopt the following notation:

$$A_u^i = \cup_{\alpha \in \mathcal{H}^A} A_{u,\alpha}^i, \quad i = 1, \ldots, r_{A,u}, \tag{2}$$

where A_u^i is the i-th attractor of the graph $G^{A,u}$. The set of all semi-attractors of the graphs $G^{A,u}$, for all $u \in \mathcal{U}^A$, is denoted $\mathcal{T}^A$. To avoid confusions between SCCs, sets of SCCs and their corresponding states, introduce the projection function $\pi : \mathcal{S}(\mathcal{C}^{A,u}) \to \mathcal{S}(\Omega^A)$:

$$\pi(R) = \{a \in \Omega^A :\ a \in C \text{ for some } C \in R\}.$$

For any two semi-attractors, define a function $\Delta : \mathcal{T}^A \times \mathcal{T}^A \to \{0,1\}$ which indicates whether they belong to the same parent attractor:

$$\Delta(A_{u,\alpha}^i, A_{u_1,\alpha_1}^{i_1}) = \begin{cases} 1, & u = u_1, \alpha \neq \alpha_1,\ \text{and } \exists A_u^{\tilde{\imath}} \in \text{ such that} \\ & \pi(A_{u,\alpha}^i) \cup \pi(A_{u_1,\alpha_1}^{i_1}) \subset \pi(A_u^{\tilde{\imath}}) \\ 0, & \text{otherwise.} \end{cases} \tag{3}$$

III. THE ASYMPTOTIC GRAPH

Consider two Boolean MIMO systems A and B, characterized by the objects defined above, with respective output functions $\bar{h}_A$ and $\bar{h}_B$. The interconnection of A and B can be described by two feedback functions that transform the outputs of one system into the inputs of the other (see Fig. 1):

$$\kappa_{AB} : \mathcal{H}^A \to \mathcal{U}^B, \qquad \kappa_{BA} : \mathcal{H}^B \to \mathcal{U}^A.$$

To simplify the statements, without loss of generality, we can consider the composition of $\bar{h}_*$ and κ_* to be a new output function with consistent dimensions:

$$h_A : \Omega^A \to \mathcal{U}^B,\ \ h_A(a) = \kappa_{AB}(\bar{h}_A(a)),$$
$$h_B : \Omega^B \to \mathcal{U}^A,\ \ h_B(b) = \kappa_{BA}(\bar{h}_B(b)).$$

Therefore, we have: $\mathcal{H}^A \equiv \mathcal{U}^B$ and $\mathcal{H}^B \equiv \mathcal{U}^A$. Under this transformation, the *interconnection of A and B* is the Boolean system Σ, with no inputs or outputs, with state space

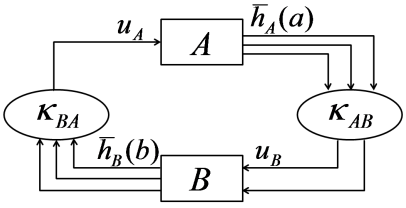

Fig. 1. The interconnection of two multiple input-multiple output systems with $(p_A, q_A) = (1, 3)$ and $(p_B, q_B) = (2, 3)$.

$\Omega = \Omega^A \times \Omega^B$, and Boolean rule $f^\Sigma : \Omega \to \Omega$ constructed in the following way:

$$f^\Sigma(a, b) = \left(f^A(a; h_B(b)), f^B(b; h_A(a))\right). \tag{4}$$

The *successors* $\sigma(a, b)$ of an element of Ω with the asynchronous updating strategy are of the form

$$\sigma(a, b) := \Big\{(a, \tilde{b}), (\tilde{a}, b) \in \Omega : \tilde{a} \in \sigma_{A,h_B(b)}(a) \text{ and } \tilde{b} \in \sigma_{B,h_A(a)}(b)\Big\}. \tag{5}$$

The notation $\sigma(S)$ will also be used to designate the successors of a set $S \in \Omega$ (see Def. 2). Let G denote the asynchronous transition graph of Σ. To motivate the concepts discussed in this section, suppose that systems A and B are two single input- single output systems that have single state attractors of the form A^1_{01} and B^1_{10}. Then the Boolean rules for the interconnection give:

$$\begin{aligned} f^\Sigma(A^1_{01}, B^1_{10}) &= \left(f^A(A^1_{01}; 0), f^B(B^1_{10}; 1)\right) \\ &= \left(A^1_{01},\ B^1_{10}\right), \end{aligned} \tag{6}$$

where the last equality follows by definition of the attractors. Therefore, the cross-product $A^1_{01} \times B^1_{10}$ is itself an attractor of the interconnected system. So, one may ask the following questions: are all cross-products $A^i_{u\alpha} \times B^k_{v\beta}$, also attractors of the interconnected system? Are all attractors of Σ of this form? The answer to the first question is, obviously, no. The answer to the second question is also negative but, in this paper, we will show that all the attractors of Σ can nevertheless be identified in terms of products $A^i_{u\alpha} \times B^k_{v\beta}$. Consider thus the set of *cross-products of semi-attractors*:

$$\mathcal{V}^{\text{as}} = \{V^{ik}_{u\alpha;v\beta} := A^i_{u\alpha} \times B^k_{v\beta} :\ A^i_{u\alpha} \in \mathcal{T}^A, B^k_{v\beta} \in \mathcal{T}^B\}.$$

Note that the asynchronous updating strategy for systems A and B induces a similar strategy for Σ, since the successors of a state are defined according to (5). Now, observe that for any fixed input u, any state $a \in \Omega^A$ must belong to at least one basin of attraction, that is, there exist indexes $i_\ell = i_\ell(a)$, $\ell = 1, \ldots, 2^{p_A}$ such that

$$a \in \mathcal{B}(A^{i_\ell}_{u_\ell \alpha_a}; G^{A,u^\ell}),\ \forall\ \ell = 1, \ldots, 2^{p_A} \tag{7}$$

where $\alpha_a = h_A(a)$ and u^ℓ is the binary representation of ℓ on p_A digits ($1 = 0 \cdots 0$, $2 = 0 \cdots 01$, etc.). Using (5), we can construct a pathway where coordinates b remain fixed and a follows a path in $G^{A,\beta}$ until it reaches an attractor with $u = h_B(b) = \beta$:

$$A^i_{u\alpha} \times B^k_{v\beta} \ni (a_1, b) \overset{G^{A,\beta}}{\rightsquigarrow} (a_f, b) \in A^{i_f}_{\beta\alpha_f} \times B^k_{v\beta} \tag{8}$$

with $h_A(a_f) = \alpha_f$. Based on these observations, one can define a set of edges $\mathcal{E}^{\text{as}}$ between nodes of $\mathcal{V}^{\text{as}}$, and thus generate a transition graph, G^{as}, as follows.

Definition 6: Let $u, u_1, \beta, \beta_1 \in \mathcal{U}^A$ and $v, v_1, \alpha, \alpha_1 \in \mathcal{U}^B$. The *asymptotic graph* associated with the interconnected system Σ is $G^{\text{as}} = (\mathcal{V}^{\text{as}}, \mathcal{E}^{\text{as}})$, where the edges are given by:
(i) $V^{ik}_{u\alpha;v\beta} \to V^{ik_1}_{u\alpha;v_1\beta_1}$ iff
either $\alpha = v_1 = v$ and $\Delta(B^k_{v\beta}, B^{k_1}_{v\beta_1}) = 1$,
or $\alpha = v_1 \neq v$ and $\mathcal{R}(B^k_{v\beta}; G^{B,v_1}) \cap \mathcal{B}(B^{k_1}_{v_1\beta_1}; G^{B,v_1}) \neq \emptyset$;

(ii) $V^{ik}_{u\alpha;v\beta} \to V^{i_1k}_{u_1\alpha_1;v\beta}$ iff
either $\beta = u_1 = u$ and $\Delta(A^i_{u\alpha}, A^{i_1}_{u\alpha_1}) = 1$,
or $\beta = u_1 \neq u$ and $\mathcal{R}(A^i_{u\alpha}; G^{A,u_1}) \cap \mathcal{B}(A^{i_1}_{u_1\alpha_1}; G^{A,u_1}) \neq \emptyset$.

To illustrate this construction, see Examples 1-3 below. Since the number of attractors for each graph is typically much smaller than the total number of states, the graph G^{as} will be much faster to construct and analyze than the full G. Note that the transitions in the asymptotic graph follow an asynchronous strategy in $\mathcal{T}^A \times \mathcal{T}^B$, since no edges are allowed to connect elements $V^{ik}_{u\alpha;v\beta} \to V^{i_1k_1}_{u_1\alpha_1;v_1\beta_1}$, with $u \neq u_1$, $\alpha \neq \alpha_1$, $v \neq v_1$, and $\beta \neq \beta_1$.

Remark 1: A timescale hypothesis. An edge of G^{as} of type (ii) in Def. 6 corresponds to a trajectory in G of the form (8), $\mathcal{P}_b = \{(a_j, b), j = 1, \ldots, f\}$ for some fixed $b \in B^k_{v\beta}$ and a_1, a_f in distinct semi-attractors. One may say that pathway $\mathcal{P}_b$ evolves according to the Σ^A dynamics, with no update of the interconnecting function $v = h_A(\cdot)$. Similar trajectories, $\mathcal{P}_a$, correspond to type (i) edges. Pathways in G^{as} correspond, therefore, to concatenations of type $\mathcal{P}_b$ and $\mathcal{P}_a$ pathways in G, along which the interconnecting functions are updated at a "low" frequency (w.r.t. the dynamics of each system). In fact, v (resp., u) can be updated only at the endpoint of a type $\mathcal{P}_b$ (resp., $\mathcal{P}_a$) sequence.

IV. ATTRACTORS OF AN INTERCONNECTION

The main result states that the set of attractors of G^{as} generates all the attractors of the full system G.

Theorem 1: If Q is an attractor of G, then there exists at least one corresponding attractor in G^{as}, $Q^{\text{as}} = Q^{\text{as}}(Q)$. Moreover, if $Q_1 \neq Q_2$ are two distinct attractors of G, then $Q^{\text{as}}(Q_1) \neq Q^{\text{as}}(Q_2)$.

In broad terms, Theorem 1 says that any attractor of G generates an attractor in G^{as}, but the converse is not necessarily true and G^{as} may have more attractors than G. Moreover, the individual states of $Q^{\text{as}}(Q)$ are contained in Q (*ie.* $\pi(Q^{\text{as}}(Q)) \subset \pi(Q)$). The proof is given in Section V.

To decide which of the attractors of G^{as} actually correspond to attractors of G, one way is to compute the reachable set of each of them in G. However, using (6), it is immediate to see that those Q^{as} formed by a cross-product of two single state (semi-)attractors are also attractors of G:

Lemma 1: Suppose that R is a single state attractor of G^{as} i.e., $\pi(R)$ contains a single element of Ω. Then $\pi(R)$ is also an attractor of G.

We next give two simple examples to illustrate Def. 6 and Theorem 1.

Example 1: Consider the interconnection between two positive feedforward cascades, A, B both of the form:

$$\Sigma^A:\ f^A(a;u)=\begin{pmatrix}u\\ a_1\end{pmatrix},\ h_A(a)=a_2,$$

It is easy to check that each system has only one attractor for each fixed input:

$$A^1_{00}=\{00\},\ A^1_{11}=\{11\},\ B^1_{00}=\{00\},\ B^1_{11}=\{11\}.$$

The interconnection Σ obtains by setting $u=h_B(b)=b_2$ and $v=h_A(a)=a_2$, and the asymptotic graph is, therefore,

$$\begin{array}{ccc} A^1_{00}\times B^1_{00} & \leftarrow & A^1_{00}\times B^1_{11}\\ \uparrow & & \downarrow\\ A^1_{11}\times B^1_{00} & \rightarrow & A^1_{11}\times B^1_{11}\end{array}.$$

By Theorem 1 and Lemma 1, Σ has exactly two attractors: $\{0000\}$ and $\{1111\}$. Indeed, it is easy to see that Σ is a positive feedback loop, hence a bistable system, with exactly these two attractors.

Example 2: Consider next the interconnection between a positive and a negative cascade, with Σ^A as in Example 1 and:

$$\Sigma^B:\ f^B(b;v)=\begin{pmatrix}v\\ \neg b_1\end{pmatrix},\ h_B(b)=b_2,$$

where $\neg b_1=1-b_1$ denotes negation. Again there is only one attractor for each fixed input, as follows:

$$A^1_{00}=\{00\},\ A^1_{11}=\{11\},\ B^1_{01}=\{01\},\ B^1_{10}=\{10\}.$$

As in the previous example, the interconnection Σ obtains by setting $u=h_B(b)=b_2$ and $v=h_A(a)=a_2$, and the asymptotic graph becomes:

$$\begin{array}{ccc} A^1_{00}\times B^1_{01} & \leftarrow & A^1_{00}\times B^1_{10}\\ \downarrow & & \uparrow\\ A^1_{11}\times B^1_{01} & \rightarrow & A^1_{11}\times B^1_{10}\end{array}.$$

By Theorem 1, there is only one attractor for Σ, and it contains the elements: $\{0001,1101,1110,0010\}$. Indeed, it is easy to check that Σ is a negative feedback loop, with a cyclic attractor: $T^\Sigma=\{0001,1001,1101,1111,1110,0110,0010,0000\}$.

The third example shows that there is not a one-to-one correspondence between G and G^{as}: an interconnected system where G^{as} has three attractors but G only two.

Example 3: Consider two 3-dimensional systems, characterized by their asynchronous transition graphs, shown in Fig. 2. Assume that the output for each system is the last coordinate, $h_A(x)=h_B(x)=x_3$. Each system has four semi-attractors, so the asymptotic graph has 16 states, while the graph G of the interconnected system has $8\times 8=64$ states, which is a very significant reduction. The asymptotic graph G^{as} corresponding to the interconnection of the two systems is given in Fig. 3. Theorem 1 says that G has at most three attractors, characterized by: $Q^{\text{as}}_1=A^1_{01}\times B^1_{10}=\{001000\}$, $Q^{\text{as}}_2=A^2_{01}\times B^1_{10}=\{111000\}$, and $Q^{\text{as}}_3=\{A^1_{10}\times B^1_{00},\ldots,A^2_{11}\times B^2_{10}\}$. The two single state attractors are immediately attractors of the interconnected system, by Lemma 1. However, we can also see that Q^{as}_3 is not a true attractor of G. Indeed, consider the following set of transitions

$$A^1_{10}\times B^3_{11}\overset{G^{B,0}}{\rightsquigarrow}A^1_{10}\times 000\overset{G^{A,0}}{\rightsquigarrow}001\times 000=Q^{\text{as}}_1$$

which shows there is a pathway connecting Q^{as}_3 to Q^{as}_1. This pathway is "hidden" in G^{as}, as it is not allowed in Def. 6: the first part of this trajectory stops at 000 in $G^{B,0}$ instead of at $110=B^1_{00}$.

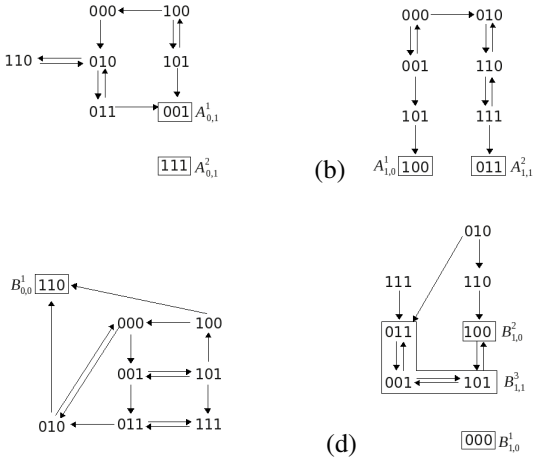

Fig. 2. The transitions graphs for Example 3: (a) $G^{A,0}$, (b) $G^{A,1}$, (c) $G^{B,0}$, (d) $G^{B,1}$.

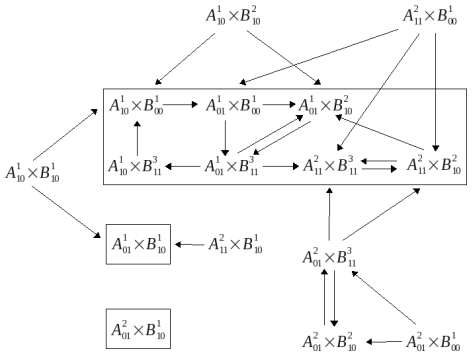

Fig. 3. The asymptotic transition graph G^{as} for Example 3.

V. PROOF OF THEOREM 1

To prove Theorem 1, it is useful to introduce a function that assigns elements of $\mathcal{V}^{\text{as}}$ to subsets of Ω.

Definition 7: The *asymptotic reducing function* $\psi:\mathcal{S}(\Omega^A\times\Omega^B)\to\mathcal{S}(\mathcal{V}^{\text{as}})$ is defined as:

$$\psi(Q)\ =\ \{A^i_{u\alpha}\times B^k_{v\beta}\in\mathcal{V}^{\text{as}}:\ \pi(A^i_{u\alpha})\times\pi(B^k_{v\beta})\subset Q\}.$$

Recall that, without loss of generality, we are assuming $p_A = q_B$ and $p_B = q_A$, which implies $\mathcal{H}^B = \mathcal{U}^A$, $\mathcal{H}^A = \mathcal{U}^B$.

Lemma 2: If Q is an attractor of G, then $\psi(Q) \neq \emptyset$.

Proof: Suppose Q is an attractor of G, and let $(a, b) \in Q$, with $a \in \Omega^A$ and $b \in \Omega^B$. Set $u := h_B(b) = \beta$ and let $a \in \mathcal{B}(A^i_{\beta\alpha}; G^{A,\beta})$, for some i and some $\alpha \in \mathcal{U}^B$. Since Q is a terminal SCC, it contains all the paths starting from any of its elements, in particular Q must contain the set $\pi(A^i_{\beta\alpha}) \times \{b\}$, for all $\alpha \in \mathcal{U}^B$, because A^i_β is connected. Since $A^i_{\beta\alpha}$ is nonemtpy, then the set $\{\tilde{a}\} \times \pi(B^k_{\alpha\delta})$ belongs to Q for all $\tilde{a} \in A^i_{\beta\alpha}$ and $b \in \mathcal{B}(B^k_{\alpha\delta}; G^{B,\alpha})$, for some $\delta \in \mathcal{U}^A$ (since $B^k_{\alpha\delta}$ is nonemtpy for some $\delta \in \mathcal{U}^A$, by (7)). Therefore, one can conclude that $A^i_{\beta\alpha} \times B^k_{\alpha\delta} \in \psi(Q)$. ∎

Lemma 3: Let Q be an attractor of G and let $A^i_{u\alpha} \times B^k_{v\beta} \in \psi(Q)$. Then all its successors in G^{as} are also in $\psi(Q)$.

Proof: Assume that $A^{i_1}_{u_1\alpha_1} \times B^k_{v\beta}$ is a successor. Then, by Definition 6, either

1. $u_1 = \beta$ and $\mathcal{R}(A^i_{u\alpha}; G^{A,u_1}) \cap \mathcal{B}(A^{i_1}_{u_1\alpha_1}; G^{A,u_1}) \neq \emptyset$
2. or $u = u_1 = \beta$ and $\alpha_1 \neq \alpha$.

In the first case, since Q is an attractor, for all $(a_0, b_0) \in \pi(A^i_{u\alpha}) \times \pi(B^k_{v\beta}) \subset Q$ then also $\mathcal{R}(a_0; G^{A,\beta}) \times \{b_0\} \subset Q$. Because $h_B(B^k_{v\beta}) \equiv \beta$ we can conclude $\mathcal{R}(A^i_{u\alpha}; G^{A,\beta}) \times \pi(B^k_{v\beta}) \subset Q$. In particular, $\pi(A^{i_1}_{u_1\alpha_1}) \subset \mathcal{R}(A^i_{u\alpha}; G^{A,\beta})$. Therefore, $A^{i_1}_{u_1\alpha_1} \times B^k_{v\beta} \in \psi(Q)$.

In the second case, one has that $A^{i_1}_{u_1\alpha_1} = A^{i_1}_{\beta,\alpha_1}$ and $A^i_{\beta,\alpha}$ are two semi-attractors belonging to the same attractor A^j_β of the graph $G^{A,\beta}$. By connectedness of A^j_β, $\pi(A^{i_1}_{\beta,\alpha_1}) \times \{b_0\} \subset Q$, for all $b_0 \in \pi(B^k_{v\beta})$. Hence $A^{i_1}_{u_1\alpha_1} \times B^k_{v\beta}$ is indeed in $\psi(Q)$. ∎

Theorem 1 follows immediately as a Corollary:

Corollary 1: If Q is an attractor of G, then $\psi(Q)$ contains an attractor of G^{as}. If Q_1, Q_2 are two distinct attractors of G, then $\psi(Q_1) \cap \psi(Q_2) = \emptyset$ and so $\psi(Q_i)$ $(i = 1, 2)$ contain distinct attractors of G^{as}.

Proof: By Lemma 3 the set $\psi(Q)$ contains all of its successors. Recall that all states in a digraph eventually converge to an attractor. If $\psi(Q)$ contains no attractor of G^{as} then it does not contain all the successors of its states, which is a contradiction.

To prove the second statement, first note that $Q_1 \cap Q_2 = \emptyset$ since they are two distinct attractors of G. Hence, by construction of ψ, also $\psi(Q_1) \cap \psi(Q_2) = \emptyset$. Therefore, since each $\psi(Q_i)$ contains an attractor of G^{as} (by the first statement), they have to be distinct attractors. ∎

VI. APPLICATION: CELL-FATE DECISION

To further illustrate the reduction method, it is applied to the Boolean model of cell-fate decision developed in [3]. This model represents the interplays between three major cellular pathways: apoptotic cell death (intrinsic or mitochondria-dependent pathway), non-apoptotic cell death (RIP1-dependent necrosis in this case) and survival (through activation of the pro-survival transcription factor NFκB). It includes the engagement of two different death receptors: TNF and Fas. For a more thorough biological description of the network, the reader is referred to [3] and references therein.

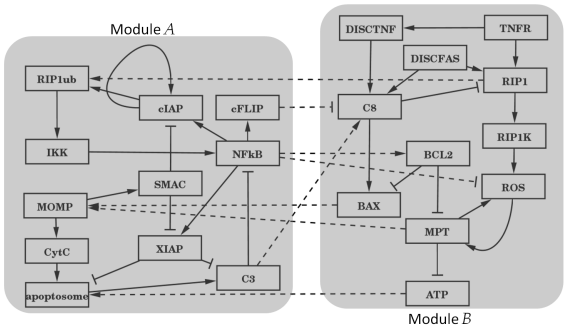

Fig. 4. The cell-fate decision network developed in [3]. Only internal variables are depicted (external inputs FASL, TNF and FADD are considered as constants and are not represented). The full system (22 variables) is expressed as the interconnection of two modules A and B of eleven variables, each with 3 inputs and 3 outputs (dashed arrows correspond to modules' interconnections).

Remark 2: The original system of [3] has 25 variables, three of which do not have specific logical rules as they correspond to external inputs of the system: TNF (Tumor Necrosis Factor), FASL (Fas ligand) and FADD (Fas-associated protein with Death Domain). These external inputs are represented in italic in Table I. In this study, we will consider them as constants and focus our analysis on the 22-dimensional graph proposed in Fig. 4. The $2^3 = 8$ different combinations of the triple (TNF,FASL,FADD) will then be analysed separately.

The first step of the method consists in partitioning the 22 variables into two 11-dimensional, interconnected MIMO modules A and B. The chosen modules with their inputs, outputs and logical rules are defined in Table I (the rules are taken from [3, Suppl.Mat.]). Mathematically, the interconnection of A and B is defined by:

$$\begin{cases} u_1 := h_{B,1}(b) = \text{RIP1}, \\ u_2 := h_{B,2}(b) = \text{ATP}, \\ u_3 := h_{B,3}(b) = \text{MPT} \vee \text{BAX}, \end{cases} \qquad \begin{cases} v_1 := h_{A,1}(a) = \text{cFLIP}, \\ v_2 := h_{A,2}(a) = \text{NF}\kappa\text{B}, \\ v_3 := h_{A,3}(a) = \text{C3}. \end{cases}$$

The next step consists in computing transition graphs $G^{A,u}$ and $G^{B,v}$ (for $u, v \in \{0,1\}^3$) and their sets of attractors and semi-attractors $A^i_{u,\alpha}$ and $B^k_{v,\beta}$. Each one of these graphs contains only $2^{11} = 2048$ states, which makes these computations (involving SCC decomposition and terminal SCC detection) much more rapid than for the transition graph of the whole system (which contains $2^{22} > 4$ million states). Once the semi-attractors are found, the last step consists in the construction of the asymptotic graph. As an example, consider the case where TNF,FASL,FADD=1,0,1. The asymptotic graph has only 120 nodes. This graph has three attractors, denoted a_1, a_2 and a_3, each one containing a single state (see Table II). By virtue of Theorem 1 and Lemma 1, these states are attractors of the interconnected system. They can be associated to the three expected phenotypes: a_1 to non-apoptotic cell death (necrosis), a_2 to apoptosis and a_3 to survival through NFκB activation (see [3]

TABLE I

DEFINITION OF MODULES.

Module A		
Inputs: $u = (u_1, u_2, u_3)$		
Outputs: $h_{A,1}$ = cFLIP, $h_{A,2}$ = NFκB, $h_{A,3}$ = C3		
Logical rules:		
RIP1ub'	=	cIAP $\wedge$ u_1
cIAP'	=	(NFκB $\vee$ cIAP) $\wedge$ $\neg$SMAC
cFLIP'	=	NFκB
IKK'	=	RIP1ub
NFκB'	=	IKK $\wedge$ $\neg$C3
SMAC'	=	MOMP
MOMP'	=	u_3
CytC'	=	MOMP
XIAP'	=	NFκB $\wedge$ $\neg$SMAC
apoptosome'	=	CytC $\wedge$ $\neg$XIAP $\wedge$ u_2
C3'	=	apoptosome $\wedge$ $\neg$XIAP

Module B		
Inputs: $v = (v_1, v_2, v_3)$		
Outputs: $h_{B,1}$ = RIP1, $h_{B,2}$ = ATP, $h_{B,3}$ = MPT$\vee$BAX		
Logical rules:		
DISC-TNF'	=	*FADD* $\wedge$ TNFR
TNFR'	=	*TNF*
DISC-FAS'	=	*FADD* $\wedge$ *FASL*
RIP1'	=	$\neg$C8 $\wedge$ (TNFR $\vee$ DISC-FAS)
C8'	=	(DISC-TNF $\vee$ DISC-FAS $\vee$ v_3) $\wedge$ $\neg v_1$
BCL2'	=	v_2
RIP1K'	=	RIP1
ROS'	=	$\neg v_2$ $\wedge$ (RIP1K $\vee$ MPT)
BAX'	=	C8 $\wedge$ $\neg$BCL2
MPT'	=	ROS $\wedge$ $\neg$BCL2
ATP'	=	$\neg$MPT

for details). In total, when considering all combinations of constants TNF, FASL and FADD, 27 attractors are retrieved, all containing single state attractors. Due to lack of space, the 27 retrieved steady states are not reproduced here, but they exactly correspond to the ones shown in [3, Fig. 2].

Interconnection of Boolean modules is therefore an efficient and practical method to simplify the analysis of a large Boolean network, by reducing it to the analysis of two half-sized isolated subnetworks, as shown in this example. The fact that the 27 uncovered semi-attractor cross-products correspond exactly to the attractors of the whole system is a direct consequence of Theorem 1 and Lemma 1, and is based on the structure of the asymptotic graph. In [3] the same conclusion is obtained through a different argument based on the reduction method developed in [5]. In contrast to [5], our interconnection method does not involve the projection onto a subset of variables, with the advantage that every variable of the original system is preserved. A further development of the method will be to extend the asymptotic graph to include (biological) transition probabilities (see for instance [3], [9]),

TABLE II

STEADY STATES WHEN TNF=1, FASL=0 AND FADD=1.

	RIP1ub	cIAP	cFLIP	IKK	NFκB	SMAC	MOMP	CytC
a_1	0	0	0	0	0	1	1	1
a_2	0	0	0	0	0	1	1	1
a_3	1	1	1	1	1	0	0	0
	XIAP	apop.	C3	D-TNF	TNFR	D-FAS	RIP1	C8
a_1	0	0	0	1	1	0	0	1
a_2	0	1	1	1	1	0	0	1
a_3	1	0	0	1	1	0	1	0
	Bcl2	RIP1K	ROS	BAX	MPT	ATP	phenotype	
a_1	0	0	1	1	1	0	*necrosis*	
a_2	0	0	0	1	0	1	*apoptosis*	
a_3	1	1	0	0	0	1	*survival*	

in order to compute the probability to reach each attractor from a given initial condition. This work is currently in progress.

VII. CONCLUSIONS AND FUTURE WORK

The main result in this paper is the identification of all the possible attractors of a Boolean interconnected system in terms of the attractors of the two isolated subsystems. This result has important applications to the analysis of large Boolean networks which appear frequently in the biological sciences. For any Boolean network, the size of the transition graph grows exponentially with the dimension n of the network, thus rapidly limiting the use of algorithms such as SCC decomposition or hierarchical organization. Therefore, by decomposing a large system into two smaller MIMO modules, it may become possible to analytically compute the asymptotic behavior of the system without computing the whole 2^n-dimensional transition graph. Several questions have arised from our analysis, in particular the problem of deciding when one of the predicted attractors is spurious (Lemma 1 provides a first answer, sufficient in the given example, but possible extensions are currently being investigated by the authors). Another question concerns the possibility of decomposing a network into more than two modules and, more generally, the necessity of developing algorithms for decomposing a network into modules with minimal numbers of inputs and outputs. Future work is to explore the trade-off between model decomposition/reduction and the computational load in the construction of the corresponding asymptotic graph.

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