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Categorizing Attractor-Effective Canalyzing Functions in Boolean Networks ⋆

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Abstract: Canalyzing Boolean functions have shown their popularity in various biological networks and established themselves to be biologically meaningful at the system level, marking their importance in the analysis of stability and robustness of such complex systems. Based on a matrix representation of Boolean networks due to the recently developed tool called semi-tensor product, we categorize canalyzing functions in terms of their capabilities of affecting the number of attractors in the Boolean network, which is one key index for the stability and robustness of Boolean networks. We show that there exist only three categories of attractor-effective canalyzing functions for any network size larger than 1, while the number of all the interested canalyzing functions is proportional to the square of the network size. We also give the explicit expression of the mean number of attractors with any length for Boolean networks with a single canalyzing function. Compared with Boolean networks without any canalyzing functions, we are able to show quantitatively how canalyzing functions can affect the mean number and length of attractors in Boolean networks for the first time.

Keywords: Boolean networks, Canalyzing functions, Attractors

1. INTRODUCTION

Boolean networks are simple yet meaningful models for biological networks. One can investigate universal properties of biological systems such as robustness, evolutionary preference, etc. based on such models(Zhao and Krishnan, 2016; Akman et al., 2012; Handorf and Klipp, 2012; Zhao and Krishnan, 2014). Within this Boolean network modeling framework, canalyzing functions have shown their popularity in various Boolean network models of biological networks. A Boolean function is said to be canalyzing on one of its variables if a specific logical value of this variable (either TRUE or FALSE) can determine the output of the whole Boolean function (either TRUE or FALSE) whatever values other variables choose. Canalyzing functions are believed to be biologically significant at the systems level, and are closely related to the system and control concepts in the general sense (Kauffman et al., 2004, 2003; Paul et al., 2006; Murrugarra and Laubenbacher, 2011). For example, genetic networks with canalyzing functions are shown to be always stable (Kauffman et al., 2004, 2003). Boolean networks with a generalized version of canalyzing functions "exhibit more robust dynamics than random networks, with few attractors and short limit cycles" (Murrugarra and Laubenbacher, 2011). Therefore, to understand the properties of canalyzing functions would be one key aspect of understanding general biological systems from the perspectives of systems biology and synthetic biology.

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However, on the one hand, all these existing results on canalyzing functions have been obtained essentially based on simulations, as there had been no efficient analytical tools for such logical networks. On the other hand, it is realized that the number of all the canalyzing functions is fast increasing with the network size (see Remark 17) (Just et al., 2004), thus making it impossible for the simulation-based approach to exhaust all the possible canalyzing functions even for networks with only dozens of nodes. Therefore, we have sufficient reasons to doubt about all existing results since they have been obtained by examining only a very limited part of all the possibilities, but a thorough understanding of canalyzing functions has to rely on more systematic analysis.

An analytical tool for Boolean networks is recently proposed based on a new product defined for matrices called "semi-tensor product"(Cheng and Qi, 2010a,b; Cheng et al., 2011, 2012). This new product allows us to write Boolean networks as linear discrete systems, and logical systems can then be solved algebraically. Within this framework, we investigate canalyzing functions in terms of their capabilities of affecting the number of attractors of the Boolean network they belong to. Surprisingly we find that there exist only three different categories of attractor-effective canalyzing functions regardless of the network size. Furthermore, for the first time we are able to show quantitatively the way how canalyzing functions affect the number and length of attractors.

The remainder of the paper is organized as follows. In Section 2, we introduce the matrix representation of Boolean networks based on semi-tensor product for completeness, and discuss canalyzing functions within this framework. The problem under study is also formally formulated. The main results are presented in Section 3, including both the number of different categories of canalyzing functions and how these canalyzing functions affect the number and length of attractors. Section 4 concludes the paper.

Notations: The following notations are used throughout the paper.

(1) $\mathcal{M}_{n\times m}$: the set of $n\times m$ real matrices;
(2) δ_n^k: the kth column of the identity matrix with dimension n, I_n;
(3) $\mathrm{Col}(A)$ ($\mathrm{Row}(A)$): the set of columns (rows) of matrix A; $\mathrm{Col}_i(A)$ ($\mathrm{Row}_i(A)$) is the ith column (row) of A and $\mathrm{Col}_P(A)$ ($\mathrm{Row}_P(A)$) is the set of all columns (rows) with their indexes belonging to the set P;
(4) $\Delta_n := \{\delta_n^k | 1\leq k\leq n\}$ and for simplicity $\Delta := \Delta_2$;
(5) $A\in\mathcal{M}_{n\times m}$ is called a logical matrix if $\mathrm{Col}(A)\subset\Delta_n$. The set of $n\times m$ logical matrices is denoted by $\mathcal{L}_{n\times m}$. A logical matrix $[\delta_n^{i_1}\ \delta_n^{i_2}\ \ldots\ \delta_n^{i_m}]$ is briefly denoted by $\delta_n[i_1\ i_2\ \ldots\ i_m]$.

2. PRELIMINARIES

2.1 Matrix representation of Boolean networks

Definition 1. (Semi-tensor product, (Cheng et al., 2011)). Let $A\in\mathcal{M}_{r_1\times c_1}$ and $B\in\mathcal{M}_{r_2\times c_2}$. The semi-tensor product of A and B, denoted by $A\ltimes B$, is defined as follows,

$$A\ltimes B := (A\otimes I_{d/c_1})(B\otimes I_{d/r_2}) \tag{1}$$

where $d := \mathrm{lcm}(c_1, r_2)$ is the least common multiple of c_1 and r_2 and $\otimes$ represents the Kronecker product.

Throughout the paper the product is assumed to be semi-tensor product. It is readily to check that semi-tensor product is a generalization of normal product of matrices. Therefore in what follows we might omit the symbol $\ltimes$ wherever no confusion can be caused.

If we map the logical values as follows: TRUE $\sim\delta_2^1$ and FALSE $\sim\delta_2^2$, a Boolean function $f(x_1,x_2,\ldots,x_n)$ is then a mapping from Δ^n to Δ. We have the following fundamental result based on semi-tensor product.

Theorem 2. ((Cheng and Qi, 2010a)). Let $f(x_1,x_2,\ldots,x_n)$ be a Boolean function. There exists a unique $M_f\in\mathcal{L}_{2\times 2^n}$, called the structure matrix of f, such that

$$f(x_1,x_2,\ldots,x_n) = M_f\ltimes_{i=1}^n x_i \tag{2}$$

Consider a Boolean network with n nodes, as follows,

$$\begin{cases} x_1(t+1) = f_1(x_1(t),\ldots,x_n(t)) \\ \vdots \\ x_n(t+1) = f_n(x_1(t),\ldots,x_n(t)) \end{cases} \tag{3}$$

According to Theorem 2, the Boolean network in (3) can be equivalently represented in its component-wise matrix representation, as follows,

$$\begin{cases} x_1(t+1) = L_1x(t) \\ \vdots \\ x_n(t+1) = L_nx(t) \end{cases} \tag{4}$$

where $x(t) := \ltimes_{i=1}^n x_i(t)$. The above component-wise matrix representation can further be rewritten in a compact form, as follows,

$$x(t+1) = Lx(t) \tag{5}$$

with the structure matrix for the Boolean network being

$$L = L_1 * L_2 * \ldots * L_n \tag{6}$$

where $*$ is the Khatri-Rao product. That is,

$$\mathrm{Col}_i(L) = \ltimes_{j=1}^n \mathrm{Col}_i(L_j), i = 1,\ldots,2^n \tag{7}$$

Remark 3. We consider Boolean functions in the functionally equivalent sense. That is, two Boolean functions are regarded to be the same if and only if they are functionally equivalent. According to this principle, the two Boolean functions, $f(x_1,x_2) = x_1$ and $g(x_1,x_2) = (x_1\wedge x_2)\vee(x_1\wedge\neg x_2)$ where $\wedge$, $\vee$ and $\neg$ represent conjunction, disjunction and negation, respectively, are the same despite their different expressions, as the same input can guarantee the same output for the two functions. In this sense, the mapping of Boolean functions from the logical representation to the matrix representation is bijective and thus we are free to use the matrix representation in all cases.

2.2 Canalyzing functions

Definition 4. ((Kauffman et al., 2003)). A Boolean function $f(x_1,x_2,\ldots,x_n)$ is said to be canalyzing on x_i if there exist $u,y\in\Delta$ such that

$$y\equiv f(x_1,x_2,\ldots,x_{i-1},u,x_{i+1},\ldots,x_n),\ \forall x_j\in\Delta, j\neq i \tag{8}$$

where u and y are referred to as the canalyzing and canalyzed values, respectively.

If the condition in Definition 4 is held, we say that function f is $\mathrm{CF}(x_i^u, f^y)$ for brevity, where we use 1 and 0 to represent δ_2^1 and δ_2^2 for u, y, respectively. For example, it is readily to check that the following Boolean function is $\mathrm{CF}(x_2^0, f^0)$ since $f(x_1,\delta_2^2,x_3)\equiv\delta_2^2, \forall x_1,x_3\in\Delta$.

$$f(x_1,x_2,x_3) = (x_2\wedge\neg x_1)\vee(x_3\wedge x_2)$$

Regardless of the canalyzing variable, canalyzing functions can be categorized into four types according to the four combinations of the canalyzing and canalyzed values, namely, (1, 1)-type, (1, 0)-type, (0, 1)-type, (0, 0)-type. We borrow the following proposition from (Li and Cheng, 2010) which offers a criterion of determining the canalyzing type for a given Boolean function. This proposition also implies that canalyzing functions can be fully described using the matrix representation of logics based on semi-tensor product.

Proposition 5. ((Li and Cheng, 2010)). A Boolean function $f(x) = M_fx$ is canalyzing on x_i with (1, 1)-type (res. (1, 0)-type, (0, 1)-type, (0, 0)-type) if and only if

$$M_f(S_i^n)^T = \left(\,2^{n-1}\delta_2^1\ *\,\right)$$

$$\left(res.\left(\,2^{n-1}\delta_2^2\ *\,\right),\left(\,*\ 2^{n-1}\delta_2^1\,\right),\left(\,*\ 2^{n-1}\delta_2^2\,\right)\right) \tag{9}$$

where $S_i^n\in\mathcal{L}_{2\times 2^n}$ is constructed by 2^i blocks with equal size of $2\times 2^{n-i}$ and the odd and even blocks being $\delta_2[1,1,\ldots,1]$ and $\delta_2[2,2,\ldots,2]$, respectively.

Remark 6. Suppose f is $\mathrm{CF}(x_i^u, f^y)$. $\neg u$ is normally not the canalyzing value for x_i at the same time, since otherwise function f will depend only on x_i and is thus trivial. It thus follows that a canalyzing variable can determine half of all the possible outputs of a non-trivial Boolean function. This shows the importance of the canalyzing variables and consequently canalyzing functions.

Remark 7. Throughout the paper we assume that a canalyzing function can only be canalyzing on exactly one variable. This assumption is due to the following fact: when multiple canalyzing variables exist in a single function, no individual variable can determine the output of the function by itself which is conflicting with the concept of canalization (Remark 6). Suppose, for example, function f is canalyzing on both x_1 and x_2. It immediately follows that the canalyzed values for x_1 and x_2 have to be the same and therefore neither x_1 nor x_2 is "independently" canalyzed.

2.3 Canalyzing functions in a Boolean network

Although the concept of canalization is applying to a single Boolean function, the evaluation of it has to be done at the Boolean network level. For Boolean network models, it is very useful to study the general properties in the mean sense, that is, the ensemble-based approach. This approach works as follows. Suppose we are interested in the effects of certain characteristic in Boolean networks (canalizing functions in the present study). We can construct all the possible Boolean networks that satisfy this characteristic (the ensemble) and then study the mean properties of this ensemble. Then, it is fair to claim that the concerned characteristic contributes to the observed properties in the general sense.

For brevity we denote by $\mathrm{CF}(x_i^u, f_j^y)$ if function f_j in a Boolean network is $\mathrm{CF}(x_i^u, f^y)$. All such canalyzing functions in Boolean networks with size n are denoted by the following set

$$\mathcal{C}_{\mathrm{CF}}^n := \{\mathrm{CF}(x_i^u, f_j^y), 1 \le i, j \le n, u, y \in \Delta\} \tag{10}$$

Denote by $\mathrm{BN}(n : x_i^u, f_j^y)$ a Boolean network with size n and a canalyzing function $\mathrm{CF}(x_i^u, f_j^y)$ while other functions in it are arbitrarily constructed. For this specific canalyzing function, $\mathrm{CF}(x_i^u, f_j^y)$, the ensemble of interest is

$$\mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n := \{\mathrm{BN}(n : x_i^u, f_j^y)\} \tag{11}$$

The functionality of $\mathrm{CF}(x_i^u, f_j^y)$ can be inferred from the mean properties of the ensemble $\mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n$.

In particular, the ensemble of Boolean networks without any restrictions is referred to as "absolute random Boolean network" (ARBN), that is,

$$\mathcal{B}^n := \{\mathrm{BN}(n)\} \tag{12}$$

where $\mathrm{BN}(n)$ represents any Boolean network with size n.

In the present study we are particularly interested in how canalyzing functions can affect the number of attractors, for which the following definition is useful.

Definition 8. (Attractor-effective equivalence). Two canalyzing functions in a Boolean network of size n, $\mathrm{CF}(x_{i_1}^{u_1}, f_{j_1}^{y_1})$ and $\mathrm{CF}(x_{i_2}^{u_2}, f_{j_2}^{y_2})$, are said to be attractor-effective equivalent if the two ensembles $\mathcal{B}_{\mathrm{CF}(x_{i_1}^{u_1}, f_{j_1}^{y_1})}^n$ and $\mathcal{B}_{\mathrm{CF}(x_{i_2}^{u_2}, f_{j_2}^{y_2})}^n$ have exactly the same mean number of attractors of any length.

Attractor-effective equivalence defines an equivalence relation over $\mathcal{C}_{\mathrm{CF}}^n$. It then divides $\mathcal{C}_{\mathrm{CF}}^n$ into several equivalence classes. Canalyzing functions in the same attractor-effective equivalence class affect the mean number of attractors in the same way.

Denote the set of the attractor-effective equivalence classes by $\mathbb{E}_{\mathrm{CF}}^n$. The problem of interest in the present study is mainly regarding $\mathbb{E}_{\mathrm{CF}}^n$. Specifically,

Problem: For any n, find out

(1) The structure of $\mathbb{E}_{\mathrm{CF}}^n$: the number of attractor-effective equivalence classes and how these equivalence classes are constructed;
(2) The mean number of attractors for all the attractor-effective equivalence classes: how different attractor-effective equivalent classes affect the number of attractors in different ways.

2.4 Properties of the ensemble of interest

The following proposition discovers the properties of the ensemble of $\mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n$ which are useful preparations for the main results to be presented in the next section. For brevity hereafter we call i_j the "state" of the column j for a Boolean network with structure matrix L and $\mathrm{Col}_j(L) = \delta_{2^n}^{i_j}$.

Proposition 9. Consider $\mathrm{BN}(n : x_i^u, f_j^y) \in \mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n$ with its structure matrix being L. The following inclusion relationships are held for different types of canalyzing functions.

$$(1,1)\text{-type} \rightarrow \mathcal{V}(P_i^n) \subseteq P_j^n \tag{13a}$$
$$(1,0)\text{-type} \rightarrow \mathcal{V}(P_i^n) \subseteq \bar{P}_j^n \tag{13b}$$
$$(0,1)\text{-type} \rightarrow \mathcal{V}(\bar{P}_i^n) \subseteq P_j^n \tag{13c}$$
$$(0,0)\text{-type} \rightarrow \mathcal{V}(\bar{P}_i^n) \subseteq \bar{P}_j^n \tag{13d}$$

where $\mathcal{V}(P)$ is the set of the possible states of $\mathrm{Col}_P(L)$ and

$$\begin{aligned} P_i^n :=& \{k \mid \mathrm{Col}_k(S_i^n) = \delta_2^1\} \\ \bar{P}_i^n :=& \{k \mid \mathrm{Col}_k(S_i^n) = \delta_2^2\} \end{aligned} \tag{14}$$

Proof. We prove the case of $(1,1)$-type. Other cases follow similarly.

Consider the component-wise matrix representation in (4). A canalyzing function, $\mathrm{CF}(x_i^1, f_j^1)$ means that the values in $\mathrm{Col}_{P_i^n}(L_j)$ must be δ_2^1. Then, from the transformation from the component-wise matrix representation in (4) to the compact matrix representation in (5), it is readily to check that the states of those columns in L must belong to P_j^n. This completes the proof.

Example 10. Consider the following Boolean network where $*$ means the values at these positions can be arbitrary.

$$\begin{cases} x_1(t+1) = \delta_2[* * * * * * * *]x(t) \\ x_2(t+1) = \delta_2[2\ 2\ 2\ 2\ * * * *]x(t) \\ x_3(t+1) = \delta_2[* * * * * * * *]x(t). \end{cases}$$

It is not difficult to verify that this Boolean network is $\mathrm{BN}(3 : x_1^1, f_2^0)$. Furthermore, according to Proposition 9 it is immediately seen that columns belonging to $P_1^3 = \{1, 2, 3, 4\}$ in its compact structure matrix L can only choose states from $\bar{P}_2^3 = \{3, 4, 7, 8\}$.

3. CATEGORIZING ATTRACTOR-EFFECTIVE CANALYZING FUNCTIONS

Before proceeding with the main results in this section, we first discuss how the mean number of attractors of an ensemble of Boolean networks can be calculated in general.

Consider the compact matrix representation of Boolean networks in (5). For any $\mathrm{Col}_j(L) = \delta_{2^n}^{i_j}$, we write it as a 2-tuple with both the column index and the state, i.e., (j, i_j). L can thus be rewritten as

$$L := \{(j, i_j)\} \tag{15}$$

Definition 11. A chain in a Boolean network is a sequence of different states with the following form using the 2-tuple representation

$$(i_1, i_2) \to (i_2, i_3) \to \ldots \to (i_{k-1}, i_k) \tag{16}$$

where $i_{j_1} \neq i_{j_2}, 1 \leq j_1, j_2 \leq k-1$.

Simple calculations show that a column (i_1, i_2) in L has the capability of mapping $\delta_{2^n}^{i_1}$ to $\delta_{2^n}^{i_2}$. The above chain can thus transform $\delta_{2^n}^{i_1}$ to $\delta_{2^n}^{i_k}$ through $k-1$ steps.

The following proposition is straightforward yet important.

Proposition 12. A group of states can form an attractor if and only if they can form a chain as in (16) with $i_1 = i_k$. The length of the attractor is k.

Example 13. Consider Example 10 again. L can now be written as

$$L = [(1,3)(2,7)(3,7)(4,8)(5,1)(6,5)(7,5)(8,6)]$$

It is readily seen that $(1,3) \to (3,7)$ is a chain by Definition 11 and $(1,3) \to (3,7) \to (7,5) \to (5,1)$ is an attractor with length 4 by Proposition 12.

Theorem 14. (Mean number of attractors). Given an ensemble of Boolean networks. Suppose for any j and i_j, the probability of the state of $\mathrm{Col}_j(L)$ being i_j is known independently as p_{ji_j} (we refer to it as the "transition probability" hereafter). The mean number of attractors of any lengths for this ensemble, N_k, can be obtained as follows,

$$N_k = \sum_{\substack{i_1=1,\ldots,2^n \\ i_2 \neq i_1 \\ \cdots \\ i_k \neq i_1,\ldots,i_{k-1}}} p_{i_1 i_2} p_{i_2 i_3} \ldots p_{i_{k-1} i_k} p_{i_k i_1}, \forall k \geq 1 \tag{17}$$

Proof. The theorem is straightforward by Proposition 12.

Corollary 15. (Mean number of attractors of ARBN). The mean number of attractors of any length for the ensemble $\mathcal{B}^n$ is as follows,

$$N_k^{n,0} = \frac{2^n!}{2^{kn}(2^n-k)!}, \forall k \geq 1 \tag{18}$$

Proof. Notice first that for ARBN, $p_{ij} \equiv \frac{1}{2^n}, \forall i, j$. Therefore the product $p_{i_1 i_2} p_{i_2 i_3} \ldots p_{i_{k-1} i_k} p_{i_k i_1}$ always contribute a constant $\frac{1}{2^{kn}}$. The number of all these possible products equals to all the possible choices of the following procedures: 1) choose k states out of 2^n possible states; and 2) permutate these k states. It is readily to calculate that the latter gives $C_k^{2^n} k!$ possible choices where C_i^j is the combination of selecting i out of j items.

The corollary is thus correct by Theorem 14.

3.1 The attractor-effective equivalence classes

Theorem 16. $|\mathbb{E}_{\mathrm{CF}}^n| = 3, \forall n \geq 2$ and is constructed as follows

$$\mathbb{E}_{\mathrm{CF}}^n = \{\mathbb{E}_{\mathrm{CF}}^{n,1}, \mathbb{E}_{\mathrm{CF}}^{n,2}, \mathbb{E}_{\mathrm{CF}}^{n,3}\} \tag{19}$$

where $|\cdot|$ denotes the cardinality of a set and

$$\mathbb{E}_{\mathrm{CF}}^{n,1} := \{\mathrm{CF}(x_i^u, f_j^y) | i = j, (u, y) = (1,1) \text{ or } (0,0)\} \tag{20a}$$
$$\mathbb{E}_{\mathrm{CF}}^{n,2} := \{\mathrm{CF}(x_i^u, f_j^y) | i = j, (u, y) = (1,0) \text{ or } (0,1)\} \tag{20b}$$
$$\mathbb{E}_{\mathrm{CF}}^{n,3} := \{\mathrm{CF}(x_i^u, f_j^y) | i \neq j\} \tag{20c}$$

In particular,

$$\mathbb{E}_{\mathrm{CF}}^1 = \{\mathbb{E}_{\mathrm{CF}}^{1,1}, \mathbb{E}_{\mathrm{CF}}^{1,2}\} \tag{21}$$

Proof. We prove the theorem in the following three steps.

(1) Let $x' := x_i \ltimes x_1 \ltimes x_2 \ldots \ltimes x_{i-1} \ltimes x_{i+1} \ldots \ltimes x_n$ and rewrite the compact matrix representation in (5) to be dependent on x'. Such a process obviously does not affect the attractors of the Boolean network as attractors are intrinsic properties which do not rely on specific representations. This implies that, in terms of the capability of affecting the number of attractors, any canalyzing function can be equivalent to a canalyzing function that is canalyzing on its first variable. Therefore, we may consider only canalyzing functions with the form $\mathrm{CF}(x_1^u, f_j^y)$. In addition, it is easy to check that in this simplification process $\mathrm{CF}(x_1^u, f_1^y)$ represents and only represents those canalyzing functions with $i = j$.

(2) Consider $\mathrm{CF}(x_1^u, f_1^y)$, i.e., $\mathrm{CF}(x_i^u, f_j^y)$ with $i = j$. According to Proposition 9, we have the following inclusion relationships with different canalyzing types,

$$(1,1)\text{-type} \to \mathcal{V}(P_1^n) \subseteq P_1^n \tag{22a}$$
$$(1,0)\text{-type} \to \mathcal{V}(P_1^n) \subseteq \bar{P}_1^n \tag{22b}$$
$$(0,1)\text{-type} \to \mathcal{V}(\bar{P}_1^n) \subseteq P_1^n \tag{22c}$$
$$(0,0)\text{-type} \to \mathcal{V}(\bar{P}_1^n) \subseteq \bar{P}_1^n \tag{22d}$$

(a) $(1,1)$-type and $(0,0)$-type canalyzing functions, that is, $\mathbb{E}_{\mathrm{CF}}^{n,1}$. The transition probability of these two types of canalyzing functions are given as follows.

$$p_{ij}^{(x_1^1, f_1^1)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i, j \in P_1^n \\ 0, & i \in P_1^n, j \in \bar{P}_1^n \\ \dfrac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{23a}$$

$$p_{ij}^{(x_1^0, f_1^0)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i, j \in \bar{P}_1^n \\ 0, & i \in \bar{P}_1^n, j \in P_1^n \\ \dfrac{1}{2^n}, & i \in P_1^n \end{cases} \tag{23b}$$

It is not difficult to see that these two types of transition probabilities can give the same N_k in (17) as the exchange of P_1^n and $\bar{P}_1^n$ does not change the value of N_k. That is, all canalyzing functions in $\mathbb{E}_{\mathrm{CF}}^{n,1}$ are attractor-effective equivalent.

(b) $(1,0)$-type and $(0,1)$-type canalyzing functions, that is, $\mathbb{E}_{\mathrm{CF}}^{n,2}$. The transition probability of these two types of canalyzing functions are given as follows.

$$p_{ij}^{(x_1^1,f_1^0)} = \begin{cases} 0, & i,j \in P_1^n \\ \dfrac{1}{2^{n-1}}, & i \in P_1^n, j \in \bar{P}_1^n \\ \dfrac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{24a}$$

$$p_{ij}^{(x_1^0,f_1^1)} = \begin{cases} 0, & i,j \in \bar{P}_1^n \\ \dfrac{1}{2^{n-1}}, & i \in \bar{P}_1^n, j \in P_1^n \\ \dfrac{1}{2^n}, & i \in P_1^n \end{cases} \tag{24b}$$

Due to the same reason as above we can confirm that all canalyzing functions in $\mathbb{E}_{\mathrm{CF}}^{n,2}$ are attractor-effective equivalent but are different from $\mathbb{E}_{\mathrm{CF}}^{n,1}$.

(3) Consider $\mathrm{CF}(x_1^u, f_{j\neq 1}^y)$, that is, $\mathbb{E}_{\mathrm{CF}}^{n,3}$. The inclusion relationships are as follows,

$$(1,1)\text{-type} \rightarrow \mathcal{V}(P_1^n) \subseteq P_j^n \tag{25a}$$
$$(1,0)\text{-type} \rightarrow \mathcal{V}(P_1^n) \subseteq \bar{P}_j^n \tag{25b}$$
$$(0,1)\text{-type} \rightarrow \mathcal{V}(\bar{P}_1^n) \subseteq P_j^n \tag{25c}$$
$$(0,0)\text{-type} \rightarrow \mathcal{V}(\bar{P}_1^n) \subseteq \bar{P}_j^n \tag{25d}$$

The transition probability for $\mathrm{CF}(x_1^1, f_{j\neq 1}^1)$ can be written as follows.

$$p_{ij}^{(x_1^1,f_{j\neq 1}^1)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i \in P_1^n, j \in P_j^n \cap P_1^n \\ \dfrac{1}{2^{n-1}}, & i \in P_1^n, j \in P_j^n \backslash P_1^n \\ \dfrac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{26}$$

Notice that for any $j \neq 1$, $|(P_j^n)^{\pm} \cap (P_1^n)^{\pm}| = |(P_j^n)^{\pm} \backslash (P_1^n)^{\pm}| = \frac{1}{2^{n-2}}$ where $(P_j^n)^{\pm}$ can be either P_j^n or $\bar{P}_j^n, j = 1, \ldots, n$. It is then not difficult to verify that all $\mathrm{CF}(x_1^u, f_{j\neq 1}^y)$ give the same N_k in (17) (more details can be referred in the proof of Theorem 18 to be presented later), meaning that all canalyzing functions in $\mathbb{E}_{\mathrm{CF}}^{n,3}$ are attractor-effective equivalent.

Furthermore, (21) is true since for $n = 1$ we do not have canalyzing function of the form $\mathrm{CF}(x_1^u, f_{j\neq 1}^y)$ and thus $\mathbb{E}_{\mathrm{CF}}^{1,3}$ does not exist.

Remark 17. The numbers of all canalyzing functions in the presence of possibly multiple canalyzing variables has shown to be increasing exponentially with the network size (Just et al., 2004). The set of canalyzing functions we consider in this paper, $\mathcal{C}_{\mathrm{CF}}^n$, is believed to be the most meaningful subset. It is readily to calculate that $|\mathcal{C}_{\mathrm{CF}}^n| = 4n^2$. Theorem 16 means that there exist only three different attractor-effective canalyzing functions (for $n \geq 2$) out of the $4n^2$ possible candidates. This simplifies significantly all analysis regarding the number of attractors affected by canalyzing functions.

3.2 Mean number of attractors for different attractor effective equivalence classes

Theorem 18. The mean number of attractors of the three attractor-effective equivalence classes in $\mathbb{E}_{\mathrm{CF}}^n$ are as follows.

(1) $\mathbb{E}_{\mathrm{CF}}^{n,1}$.

$$N_k^{n,1} = (1 + \frac{1}{2^k})N_k^{n-1,0} \tag{27}$$

(2) $\mathbb{E}_{\mathrm{CF}}^{n,2}$.

$$N_k^{n,2} = \sum_{i=0}^{\lfloor \frac{k}{2} \rfloor} k(k-i-1)!i!C_i^{k-i}C_i^{2^{n-1}}C_{k-i}^{2^{n-1}}2^{i-nk} \tag{28}$$

(3) $\mathbb{E}_{\mathrm{CF}}^{n,3}$.

$$N_k^{n,3} = \sum_{i=0}^{\lfloor \frac{k}{2} \rfloor}\sum_{j=i}^{k-i}\sum_{l=0}^{k-i-j} p_{i,j,l,k} C_i^{2^{n-2}}C_j^{2^{n-2}}C_l^{2^{n-2}} \times C_{k-i-j-l}^{2^{n-2}} 2^{k+j+l-nk} \tag{29}$$

where $p_{i,j,l,k}$ is defined as follows

$$\begin{cases} k(j-1)!G_j^{k-i-j-l}C_i^j i!G_j^l, & j > 0 \\ k! & i = j = 0, l = k, 0 \\ 0 & \text{otherwise.} \end{cases} \tag{30}$$

and G_i^j is the number of choices when allocating j items into i groups.

Proof. We give the proof for $\mathbb{E}_{\mathrm{CF}}^{n,1}$ and $\mathbb{E}_{\mathrm{CF}}^{n,3}$. $\mathbb{E}_{\mathrm{CF}}^{n,2}$ can readily follow from $\mathbb{E}_{\mathrm{CF}}^{n,3}$.

(1) $\mathbb{E}_{\mathrm{CF}}^{n,1}$. Consider $\mathcal{B}_{\mathrm{CF}(x_1^1,f_1^1)}^n$. The transition probability in (23a) implies that no attractors can include states from both P_1^n and $\bar{P}_1^n$ and therefore the attractors contributed by $i,j \in P_1^n$ and $i \in \bar{P}_1^n$ can be calculated independently. By Theorem 14, it is seen that the contribution of the former is exactly equivalent to an ARBN with size $n-1$ while the latter with an additional factor $\frac{1}{2^k}$. (27) readily follows.

(2) $\mathbb{E}_{\mathrm{CF}}^{n,3}$. Consider $\mathcal{B}_{\mathrm{CF}(x_1^1,f_2^1)}^n$. From (26) it is known

$$p_{ij}^{(x_1^1,f_2^1)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i \in P_1^n, j \in P_2^n \cap P_1^n \\ \dfrac{1}{2^{n-1}}, & i \in P_1^n, j \in P_2^n \backslash P_1^n \\ \dfrac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{31}$$

Define

$$A = P_1^n \cap P_2^n, B = P_1^n \backslash A,$$
$$C = P_2^n \backslash A, D = \Omega \backslash (A \cup B \cup C)$$

where $\Omega := \{1, 2, \ldots, 2^n\}$. The transition probabilities in (31) can be reformed as

$$p_{ij}^{(x_1^1,f_2^1)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i \in A, j \in B \\ \dfrac{1}{2^{n-1}}, & i \in B, j \in C \\ \dfrac{1}{2^n}, & i \in C, j \in D \\ \dfrac{1}{2^n}, & i \in D, j \in A \end{cases} \tag{32}$$

Consider $N_k^{n,3}$. Choose, in order, the following different number of states from the four sets,

$$i \rightarrow B, j \rightarrow C, l \rightarrow D, k-i-j-l \rightarrow A$$

In total we have $C_i^{2^{n-2}}C_j^{2^{n-2}}C_l^{2^{n-2}}C_{k-i-j-l}^{2^{n-2}}$ different choices. By (32), it is also known that each product in (17) is the same as $\frac{1}{2^{nk-k-j-l}}$.

Table 1. Number of attractors for various ensembles with network size 3.

Ensemble \Number \Length	1	2	3	4	5	6	7	8
$\mathcal{B}^n$	1	0.875	0.6563	0.4102	0.2051	0.0769	0.0192	0.0024
$\mathbb{E}_{\text{CF}}^{n,1}$	1.5000	0.9375	0.4219	0.0996	0	0	0	0
$\mathbb{E}_{\text{CF}}^{n,2}$	0.5000	1.1875	0.6094	0.4746	0.2051	0.0747	0.0154	0.0011
$\mathbb{E}_{\text{CF}}^{n,3}$	1	0.8125	0.6094	0.3496	0.1465	0.0439	0.0077	0.0005

Now consider all the possible permutations of these k states, the total number of which is denoted by $p_{i,j,l,k}$. These permutations can be valid in only the following two cases.

(a) $j > 0$. In this case we first allocate j states from C into the k possible positions, resulting in $k(j-1)!$ possible choices. Note that all the states in A can be put either before or behind the states in C, therefore we can allocate the $k-i-j-l$ states from A to j groups, i.e., $G_j^{k-i-j-l}$. Although now we have $k-i-l$ available positions, the states from B are not able to be put arbitrarily, i.e., they can not be behind A (the states from A can never be transformed to states in B directly), and therefore the available positions are only j. Notice that states from B can not be transformed to states in itself, meaning that we can only permutate them but not allocate them into groups. This will contribute a factor of $C_i^j i!$. At last, l states from D can only be grouped into j groups (they can not be behind A nor B), which is G_j^l.

(b) $i = j = 0, k = k, 0$. In this case there are no states from B nor C and the states can only be from either A or D. It is immediately clear that all the possible choices are $k!$.

The above analysis gives (30).

This completes the proof.

Remark 19. From Theorem 18 we are able to calculate the mean number of attractors for different canalyzing functions directly, which had been impossible before. More properties of canalyzing functions can also be possibly examined. For example, the comparison of the mean number of attractors of Boolean networks with size 3 between without and with the three types of different attractor-effective canalyzing functions is shown in Table 1. It is observed that in general Boolean networks with canalyzing functions tend to have less attractors with shorter lengths, which confirms previous simulation-based findings (Murrugarra and Laubenbacher, 2011).

4. CONCLUSIONS

Canalyzing functions have been shown to be biologically significant. For the first time we describe canalyzing functions algebraically using a novel matrix representation of logics based on semi-tensor product. Within this new framework, we categorize canalyzing functions in terms of their capabilities of affecting the number of attractors in the Boolean network. Surprisedly we find that there exist only three different attractor-effective canalyzing functions for all sizes of Boolean networks larger than 1, despite the fast increasing number of all possible canalyzing functions. The calculation of the mean number of attractors for Boolean networks with canalyzing functions confirms previous simulation-based findings. It is believed that further analysis within this analytical framework will result in meaningful findings which are impossible to obtain using simulations.

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Categorizing Attractor-Effective Canalyzing Functions in Boolean Networks ⋆

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Abstract: Canalyzing Boolean functions have shown their popularity in various biological networks and established themselves to be biologically meaningful at the system level, marking their importance in the analysis of stability and robustness of such complex systems. Based on a matrix representation of Boolean networks due to the recently developed tool called semi-tensor product, we categorize canalyzing functions in terms of their capabilities of affecting the number of attractors in the Boolean network, which is one key index for the stability and robustness of Boolean networks. We show that there exist only three categories of attractor-effective canalyzing functions for any network size larger than 1, while the number of all the interested canalyzing functions is proportional to the square of the network size. We also give the explicit expression of the mean number of attractors with any length for Boolean networks with a single canalyzing function. Compared with Boolean networks without any canalyzing functions, we are able to show quantitatively how canalyzing functions can affect the mean number and length of attractors in Boolean networks for the first time.

Keywords: Boolean networks, Canalyzing functions, Attractors

1. INTRODUCTION

Boolean networks are simple yet meaningful models for biological networks. One can investigate universal properties of biological systems such as robustness, evolutionary preference, etc. based on such models(Zhao and Krishnan, 2016; Akman et al., 2012; Handorf and Klipp, 2012; Zhao and Krishnan, 2014). Within this Boolean network modeling framework, canalyzing functions have shown their popularity in various Boolean network models of biological networks. A Boolean function is said to be canalyzing on one of its variables if a specific logical value of this variable (either TRUE or FALSE) can determine the output of the whole Boolean function (either TRUE or FALSE) whatever values other variables choose. Canalyzing functions are believed to be biologically significant at the systems level, and are closely related to the system and control concepts in the general sense (Kauffman et al., 2004, 2003; Paul et al., 2006; Murrugarra and Laubenbacher, 2011). For example, genetic networks with canalyzing functions are shown to be always stable (Kauffman et al., 2004, 2003). Boolean networks with a generalized version of canalyzing functions "exhibit more robust dynamics than random networks, with few attractors and short limit cycles" (Murrugarra and Laubenbacher, 2011). Therefore, to understand the properties of canalyzing functions would be one key aspect of understanding general biological systems from the perspectives of systems biology and synthetic biology.

However, on the one hand, all these existing results on canalyzing functions have been obtained essentially based on simulations, as there had been no efficient analytical tools for such logical networks. On the other hand, it is realized that the number of all the canalyzing functions is fast increasing with the network size (see Remark 17) (Just et al., 2004), thus making it impossible for the simulation-based approach to exhaust all the possible canalyzing functions even for networks with only dozens of nodes. Therefore, we have sufficient reasons to doubt about all existing results since they have been obtained by examining only a very limited part of all the possibilities, but a thorough understanding of canalyzing functions has to rely on more systematic analysis.

An analytical tool for Boolean networks is recently proposed based on a new product defined for matrices called "semi-tensor product"(Cheng and Qi, 2010a,b; Cheng et al., 2011, 2012). This new product allows us to write Boolean networks as linear discrete systems, and logical systems can then be solved algebraically. Within this framework, we investigate canalyzing functions in terms of their capabilities of affecting the number of attractors of the Boolean network they belong to. Surprisingly we find that there exist only three different categories of attractor-effective canalyzing functions regardless of the network size. Furthermore, for the first time we are able to show quantitatively the way how canalyzing functions affect the number and length of attractors.

The remainder of the paper is organized as follows. In Section 2, we introduce the matrix representation of Boolean networks based on semi-tensor product for completeness, and discuss canalyzing functions within this framework. The problem under study is also formally formulated. The

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main results are presented in Section 3, including both the number of different categories of canalyzing functions and how these canalyzing functions affect the number and length of attractors. Section 4 concludes the paper.

Notations: The following notations are used throughout the paper.

(1) $\mathcal{M}_{n\times m}$: the set of $n \times m$ real matrices;
(2) δ_n^k: the kth column of the identity matrix with dimension n, I_n;
(3) $\mathrm{Col}(A)$ ($\mathrm{Row}(A)$): the set of columns (rows) of matrix A; $\mathrm{Col}_i(A)$ ($\mathrm{Row}_i(A)$) is the ith column (row) of A and $\mathrm{Col}_P(A)$ ($\mathrm{Row}_P(A)$) is the set of all columns (rows) with their indexes belonging to the set P;
(4) $\Delta_n := \{\delta_n^k | 1 \le k \le n\}$ and for simplicity $\Delta := \Delta_2$;
(5) $A \in \mathcal{M}_{n\times m}$ is called a logical matrix if $\mathrm{Col}(A) \subset \Delta_n$. The set of $n\times m$ logical matrices is denoted by $\mathcal{L}_{n\times m}$. A logical matrix $[\delta_n^{i_1}\ \delta_n^{i_2}\ \ldots\ \delta_n^{i_m}]$ is briefly denoted by $\delta_n[i_1\ i_2\ \ldots\ i_m]$.

2. PRELIMINARIES

2.1 Matrix representation of Boolean networks

Definition 1. (Semi-tensor product, (Cheng et al., 2011)). Let $A \in \mathcal{M}_{r_1\times c_1}$ and $B \in \mathcal{M}_{r_2\times c_2}$. The semi-tensor product of A and B, denoted by $A\ltimes B$, is defined as follows,

$$A \ltimes B := (A \otimes I_{d/c_1})(B \otimes I_{d/r_2}) \tag{1}$$

where $d := \mathrm{lcm}(c_1, r_2)$ is the least common multiple of c_1 and r_2 and $\otimes$ represents the Kronecker product.

Throughout the paper the product is assumed to be semi-tensor product. It is readily to check that semi-tensor product is a generalization of normal product of matrices. Therefore in what follows we might omit the symbol $\ltimes$ wherever no confusion can be caused.

If we map the logical values as follows: TRUE $\sim \delta_2^1$ and FALSE $\sim \delta_2^2$, a Boolean function $f(x_1, x_2, \ldots, x_n)$ is then a mapping from Δ^n to Δ. We have the following fundamental result based on semi-tensor product.

Theorem 2. ((Cheng and Qi, 2010a)). Let $f(x_1, x_2, \ldots, x_n)$ be a Boolean function. There exists a unique $M_f \in \mathcal{L}_{2\times 2^n}$, called the structure matrix of f, such that

$$f(x_1, x_2, \ldots, x_n) = M_f \ltimes_{i=1}^n x_i \tag{2}$$

Consider a Boolean network with n nodes, as follows,

$$\begin{cases} x_1(t+1) = f_1(x_1(t), \ldots, x_n(t)) \\ \vdots \\ x_n(t+1) = f_n(x_1(t), \ldots, x_n(t)) \end{cases} \tag{3}$$

According to Theorem 2, the Boolean network in (3) can be equivalently represented in its component-wise matrix representation, as follows,

$$\begin{cases} x_1(t+1) = L_1 x(t) \\ \vdots \\ x_n(t+1) = L_n x(t) \end{cases} \tag{4}$$

where $x(t) := \ltimes_{i=1}^n x_i(t)$. The above component-wise matrix representation can further be rewritten in a compact form, as follows,

$$x(t+1) = Lx(t) \tag{5}$$

with the structure matrix for the Boolean network being

$$L = L_1 * L_2 * \ldots * L_n \tag{6}$$

where $*$ is the Khatri-Rao product. That is,

$$\mathrm{Col}_i(L) = \ltimes_{j=1}^n \mathrm{Col}_i(L_j), i = 1, \ldots, 2^n \tag{7}$$

Remark 3. We consider Boolean functions in the functionally equivalent sense. That is, two Boolean functions are regarded to be the same if and only if they are functionally equivalent. According to this principle, the two Boolean functions, $f(x_1, x_2) = x_1$ and $g(x_1, x_2) = (x_1 \wedge x_2) \vee (x_1 \wedge \neg x_2)$ where $\wedge$, $\vee$ and $\neg$ represent conjunction, disjunction and negation, respectively, are the same despite their different expressions, as the same input can guarantee the same output for the two functions. In this sense, the mapping of Boolean functions from the logical representation to the matrix representation is bijective and thus we are free to use the matrix representation in all cases.

2.2 Canalyzing functions

Definition 4. ((Kauffman et al., 2003)). A Boolean function $f(x_1, x_2, \ldots, x_n)$ is said to be canalyzing on x_i if there exist $u, y \in \Delta$ such that

$$y \equiv f(x_1, x_2, \ldots, x_{i-1}, u, x_{i+1}, \ldots, x_n),\ \forall x_j \in \Delta, j \neq i \tag{8}$$

where u and y are referred to as the canalyzing and canalyzed values, respectively.

If the condition in Definition 4 is held, we say that function f is $\mathrm{CF}(x_i^u, f^y)$ for brevity, where we use 1 and 0 to represent δ_2^1 and δ_2^2 for u, y, respectively. For example, it is readily to check that the following Boolean function is $\mathrm{CF}(x_2^0, f^0)$ since $f(x_1, \delta_2^2, x_3) \equiv \delta_2^2, \forall x_1, x_3 \in \Delta$.

$$f(x_1, x_2, x_3) = (x_2 \wedge \neg x_1) \vee (x_3 \wedge x_2)$$

Regardless of the canalyzing variable, canalyzing functions can be categorized into four types according to the four combinations of the canalyzing and canalyzed values, namely, $(1,1)$-type, $(1,0)$-type, $(0,1)$-type, $(0,0)$-type. We borrow the following proposition from (Li and Cheng, 2010) which offers a criterion of determining the canalyzing type for a given Boolean function. This proposition also implies that canalyzing functions can be fully described using the matrix representation of logics based on semi-tensor product.

Proposition 5. ((Li and Cheng, 2010)). A Boolean function $f(x) = M_f x$ is canalyzing on x_i with $(1,1)$-type (res. $(1,0)$-type, $(0,1)$-type, $(0,0)$-type) if and only if

$$M_f (S_i^n)^T = \begin{pmatrix} 2^{n-1}\delta_2^1 & * \end{pmatrix}$$

$$(res. \begin{pmatrix} 2^{n-1}\delta_2^2 & * \end{pmatrix}, \begin{pmatrix} * & 2^{n-1}\delta_2^1 \end{pmatrix}, \begin{pmatrix} * & 2^{n-1}\delta_2^2 \end{pmatrix}) \tag{9}$$

where $S_i^n \in \mathcal{L}_{2\times 2^n}$ is constructed by 2^i blocks with equal size of $2 \times 2^{n-i}$ and the odd and even blocks being $\delta_2[1, 1, \ldots, 1]$ and $\delta_2[2, 2, \ldots, 2]$, respectively.

Remark 6. Suppose f is $\mathrm{CF}(x_i^u, f^y)$. $\neg u$ is normally not the canalyzing value for x_i at the same time, since otherwise function f will depend only on x_i and is thus trivial. It thus follows that a canalyzing variable can determine half of all the possible outputs of a non-trivial Boolean function. This shows the importance of the canalyzing variables and consequently canalyzing functions.

Remark 7. Throughout the paper we assume that a canalyzing function can only be canalyzing on exactly one variable. This assumption is due to the following fact: when multiple canalyzing variables exist in a single function, no individual variable can determine the output of the function by itself which is conflicting with the concept of canalization (Remark 6). Suppose, for example, function f is canalyzing on both x_1 and x_2. It immediately follows that the canalyzed values for x_1 and x_2 have to be the same and therefore neither x_1 nor x_2 is "independently" canalyzed.

2.3 Canalyzing functions in a Boolean network

Although the concept of canalization is applying to a single Boolean function, the evaluation of it has to be done at the Boolean network level. For Boolean network models, it is very useful to study the general properties in the mean sense, that is, the ensemble-based approach. This approach works as follows. Suppose we are interested in the effects of certain characteristic in Boolean networks (canalizing functions in the present study). We can construct all the possible Boolean networks that satisfy this characteristic (the ensemble) and then study the mean properties of this ensemble. Then, it is fair to claim that the concerned characteristic contributes to the observed properties in the general sense.

For brevity we denote by $\mathrm{CF}(x_i^u, f_j^y)$ if function f_j in a Boolean network is $\mathrm{CF}(x_i^u, f^y)$. All such canalyzing functions in Boolean networks with size n are denoted by the following set

$$\mathcal{C}_{\mathrm{CF}}^n := \{\mathrm{CF}(x_i^u, f_j^y), 1 \le i, j \le n, u, y \in \Delta\} \tag{10}$$

Denote by $\mathrm{BN}(n : x_i^u, f_j^y)$ a Boolean network with size n and a canalyzing function $\mathrm{CF}(x_i^u, f_j^y)$ while other functions in it are arbitrarily constructed. For this specific canalyzing function, $\mathrm{CF}(x_i^u, f_j^y)$, the ensemble of interest is

$$\mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n := \{\mathrm{BN}(n : x_i^u, f_j^y)\} \tag{11}$$

The functionality of $\mathrm{CF}(x_i^u, f_j^y)$ can be inferred from the mean properties of the ensemble $\mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n$.

In particular, the ensemble of Boolean networks without any restrictions is referred to as "absolute random Boolean network" (ARBN), that is,

$$\mathcal{B}^n := \{\mathrm{BN}(n)\} \tag{12}$$

where $\mathrm{BN}(n)$ represents any Boolean network with size n.

In the present study we are particularly interested in how canalyzing functions can affect the number of attractors, for which the following definition is useful.

Definition 8. (Attractor-effective equivalence). Two canalyzing functions in a Boolean network of size n, $\mathrm{CF}(x_{i_1}^{u_1}, f_{j_1}^{y_1})$ and $\mathrm{CF}(x_{i_2}^{u_2}, f_{j_2}^{y_2})$, are said to be attractor-effective equivalent if the two ensembles $\mathcal{B}_{\mathrm{CF}(x_{i_1}^{u_1}, f_{j_1}^{y_1})}^n$ and $\mathcal{B}_{\mathrm{CF}(x_{i_2}^{u_2}, f_{j_2}^{y_2})}^n$ have exactly the same mean number of attractors of any length.

Attractor-effective equivalence defines an equivalence relation over $\mathcal{C}_{\mathrm{CF}}^n$. It then divides $\mathcal{C}_{\mathrm{CF}}^n$ into several equivalence classes. Canalyzing functions in the same attractor-effective equivalence class affect the mean number of attractors in the same way.

Denote the set of the attractor-effective equivalence classes by $\mathbb{E}_{\mathrm{CF}}^n$. The problem of interest in the present study is mainly regarding $\mathbb{E}_{\mathrm{CF}}^n$. Specifically,

Problem: For any n, find out

(1) The structure of $\mathbb{E}_{\mathrm{CF}}^n$: the number of attractor-effective equivalence classes and how these equivalence classes are constructed;
(2) The mean number of attractors for all the attractor-effective equivalence classes: how different attractor-effective equivalent classes affect the number of attractors in different ways.

2.4 Properties of the ensemble of interest

The following proposition discovers the properties of the ensemble of $\mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n$ which are useful preparations for the main results to be presented in the next section. For brevity hereafter we call i_j the "state" of the column j for a Boolean network with structure matrix L and $\mathrm{Col}_j(L) = \delta_{2^n}^{i_j}$.

Proposition 9. Consider $\mathrm{BN}(n : x_i^u, f_j^y) \in \mathcal{B}_{\mathrm{CF}(x_i^u, f_j^y)}^n$ with its structure matrix being L. The following inclusion relationships are held for different types of canalyzing functions.

$$(1,1)\text{-type} \rightarrow \mathcal{V}(P_i^n) \subseteq P_j^n \tag{13a}$$
$$(1,0)\text{-type} \rightarrow \mathcal{V}(P_i^n) \subseteq \bar{P}_j^n \tag{13b}$$
$$(0,1)\text{-type} \rightarrow \mathcal{V}(\bar{P}_i^n) \subseteq P_j^n \tag{13c}$$
$$(0,0)\text{-type} \rightarrow \mathcal{V}(\bar{P}_i^n) \subseteq \bar{P}_j^n \tag{13d}$$

where $\mathcal{V}(P)$ is the set of the possible states of $\mathrm{Col}_P(L)$ and

$$\begin{aligned} P_i^n &:= \{k \mid \mathrm{Col}_k(S_i^n) = \delta_2^1\} \\ \bar{P}_i^n &:= \{k \mid \mathrm{Col}_k(S_i^n) = \delta_2^2\} \end{aligned} \tag{14}$$

Proof. We prove the case of $(1,1)$-type. Other cases follow similarly.

Consider the component-wise matrix representation in (4). A canalyzing function, $\mathrm{CF}(x_i^1, f_j^1)$ means that the values in $\mathrm{Col}_{P_i^n}(L_j)$ must be δ_2^1. Then, from the transformation from the component-wise matrix representation in (4) to the compact matrix representation in (5), it is readily to check that the states of those columns in L must belong to P_j^n. This completes the proof.

Example 10. Consider the following Boolean network where $*$ means the values at these positions can be arbitrary.

$$\begin{cases} x_1(t+1) = \delta_2[* \ * \ * \ * \ * \ * \ * \ *]x(t) \\ x_2(t+1) = \delta_2[2 \ 2 \ 2 \ 2 \ * \ * \ * \ *]x(t) \\ x_3(t+1) = \delta_2[* \ * \ * \ * \ * \ * \ * \ *]x(t). \end{cases}$$

It is not difficult to verify that this Boolean network is $\mathrm{BN}(3 : x_1^1, f_2^0)$. Furthermore, according to Proposition 9 it is immediately seen that columns belonging to $P_1^3 = \{1, 2, 3, 4\}$ in its compact structure matrix L can only choose states from $\bar{P}_2^3 = \{3, 4, 7, 8\}$.

3. CATEGORIZING ATTRACTOR-EFFECTIVE CANALYZING FUNCTIONS

Before proceeding with the main results in this section, we first discuss how the mean number of attractors of an ensemble of Boolean networks can be calculated in general.

Consider the compact matrix representation of Boolean networks in (5). For any $\mathrm{Col}_j(L) = \delta_{2^n}^{i_j}$, we write it as a 2-tuple with both the column index and the state, i.e., (j, i_j). L can thus be rewritten as

$$L := \{(j, i_j)\} \tag{15}$$

Definition 11. A chain in a Boolean network is a sequence of different states with the following form using the 2-tuple representation

$$(i_1, i_2) \to (i_2, i_3) \to \ldots \to (i_{k-1}, i_k) \tag{16}$$

where $i_{j_1} \neq i_{j_2}, 1 \leq j_1, j_2 \leq k-1$.

Simple calculations show that a column (i_1, i_2) in L has the capability of mapping $\delta_{2^n}^{i_1}$ to $\delta_{2^n}^{i_2}$. The above chain can thus transform $\delta_{2^n}^{i_1}$ to $\delta_{2^n}^{i_k}$ through $k-1$ steps.

The following proposition is straightforward yet important.

Proposition 12. A group of states can form an attractor if and only if they can form a chain as in (16) with $i_1 = i_k$. The length of the attractor is k.

Example 13. Consider Example 10 again. L can now be written as

$$L = [(1,3)(2,7)(3,7)(4,8)(5,1)(6,5)(7,5)(8,6)]$$

It is readily seen that $(1,3) \to (3,7)$ is a chain by Definition 11 and $(1,3) \to (3,7) \to (7,5) \to (5,1)$ is an attractor with length 4 by Proposition 12.

Theorem 14. (Mean number of attractors). Given an ensemble of Boolean networks. Suppose for any j and i_j, the probability of the state of $\mathrm{Col}_j(L)$ being i_j is known independently as p_{ji_j} (we refer to it as the "transition probability" hereafter). The mean number of attractors of any lengths for this ensemble, N_k, can be obtained as follows,

$$N_k = \sum_{\substack{i_1=1,\ldots,2^n \\ i_2 \neq i_1 \\ \cdots \\ i_k \neq i_1,\ldots,i_{k-1}}} p_{i_1 i_2} p_{i_2 i_3} \ldots p_{i_{k-1} i_k} p_{i_k i_1}, \forall k \geq 1 \tag{17}$$

Proof. The theorem is straightforward by Proposition 12.

Corollary 15. (Mean number of attractors of ARBN). The mean number of attractors of any length for the ensemble $\mathcal{B}^n$ is as follows,

$$N_k^{n,0} = \frac{2^n!}{2^{kn}(2^n-k)!}, \forall k \geq 1 \tag{18}$$

Proof. Notice first that for ARBN, $p_{ij} \equiv \frac{1}{2^n}, \forall i, j$. Therefore the product $p_{i_1 i_2} p_{i_2 i_3} \ldots p_{i_{k-1} i_k} p_{i_k i_1}$ always contribute a constant $\frac{1}{2^{kn}}$. The number of all these possible products equals to all the possible choices of the following procedures: 1) choose k states out of 2^n possible states; and 2) permutate these k states. It is readily to calculate that the latter gives $C_k^{2^n} k!$ possible choices where C_i^j is the combination of selecting i out of j items.

The corollary is thus correct by Theorem 14.

3.1 The attractor-effective equivalence classes

Theorem 16. $|\mathbb{E}_{\mathrm{CF}}^n| = 3, \forall n \geq 2$ and is constructed as follows

$$\mathbb{E}_{\mathrm{CF}}^n = \{\mathbb{E}_{\mathrm{CF}}^{n,1}, \mathbb{E}_{\mathrm{CF}}^{n,2}, \mathbb{E}_{\mathrm{CF}}^{n,3}\} \tag{19}$$

where $|\cdot|$ denotes the cardinality of a set and

$$\mathbb{E}_{\mathrm{CF}}^{n,1} := \{\mathrm{CF}(x_i^u, f_j^y) | i = j, (u, y) = (1,1) \text{ or } (0,0)\} \tag{20a}$$

$$\mathbb{E}_{\mathrm{CF}}^{n,2} := \{\mathrm{CF}(x_i^u, f_j^y) | i = j, (u, y) = (1,0) \text{ or } (0,1)\} \tag{20b}$$

$$\mathbb{E}_{\mathrm{CF}}^{n,3} := \{\mathrm{CF}(x_i^u, f_j^y) | i \neq j\} \tag{20c}$$

In particular,

$$\mathbb{E}_{\mathrm{CF}}^1 = \{\mathbb{E}_{\mathrm{CF}}^{1,1}, \mathbb{E}_{\mathrm{CF}}^{1,2}\} \tag{21}$$

Proof. We prove the theorem in the following three steps.

(1) Let $x' := x_i \ltimes x_1 \ltimes x_2 \ldots \ltimes x_{i-1} \ltimes x_{i+1} \ldots \ltimes x_n$ and rewrite the compact matrix representation in (5) to be dependent on x'. Such a process obviously does not affect the attractors of the Boolean network as attractors are intrinsic properties which do not rely on specific representations. This implies that, in terms of the capability of affecting the number of attractors, any canalyzing function can be equivalent to a canalyzing function that is canalyzing on its first variable. Therefore, we may consider only canalyzing functions with the form $\mathrm{CF}(x_1^u, f_j^y)$. In addition, it is easy to check that in this simplification process $\mathrm{CF}(x_1^u, f_1^y)$ represents and only represents those canalyzing functions with $i = j$.

(2) Consider $\mathrm{CF}(x_1^u, f_1^y)$, i.e., $\mathrm{CF}(x_i^u, f_j^y)$ with $i = j$. According to Proposition 9, we have the following inclusion relationships with different canalyzing types,

$$(1,1)\text{-type} \to \mathcal{V}(P_1^n) \subseteq P_1^n \tag{22a}$$

$$(1,0)\text{-type} \to \mathcal{V}(P_1^n) \subseteq \bar{P}_1^n \tag{22b}$$

$$(0,1)\text{-type} \to \mathcal{V}(\bar{P}_1^n) \subseteq P_1^n \tag{22c}$$

$$(0,0)\text{-type} \to \mathcal{V}(\bar{P}_1^n) \subseteq \bar{P}_1^n \tag{22d}$$

(a) $(1,1)$-type and $(0,0)$-type canalyzing functions, that is, $\mathbb{E}_{\mathrm{CF}}^{n,1}$. The transition probability of these two types of canalyzing functions are given as follows.

$$p_{ij}^{(x_1^1, f_1^1)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i, j \in P_1^n \\ 0, & i \in P_1^n, j \in \bar{P}_1^n \\ \dfrac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{23a}$$

$$p_{ij}^{(x_1^0, f_1^0)} = \begin{cases} \dfrac{1}{2^{n-1}}, & i, j \in \bar{P}_1^n \\ 0, & i \in \bar{P}_1^n, j \in P_1^n \\ \dfrac{1}{2^n}, & i \in P_1^n \end{cases} \tag{23b}$$

It is not difficult to see that these two types of transition probabilities can give the same N_k in (17) as the exchange of P_1^n and $\bar{P}_1^n$ does not change the value of N_k. That is, all canalyzing functions in $\mathbb{E}_{\mathrm{CF}}^{n,1}$ are attractor-effective equivalent.

(b) $(1,0)$-type and $(0,1)$-type canalyzing functions, that is, $\mathbb{E}_{\mathrm{CF}}^{n,2}$. The transition probability of these two types of canalyzing functions are given as follows.

$$p_{ij}^{(x_1^1, f_1^0)} = \begin{cases} 0, & i, j \in P_1^n \\ \frac{1}{2^{n-1}}, & i \in P_1^n, j \in \bar{P}_1^n \\ \frac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{24a}$$

$$p_{ij}^{(x_1^0, f_1^1)} = \begin{cases} 0, & i, j \in \bar{P}_1^n \\ \frac{1}{2^{n-1}}, & i \in \bar{P}_1^n, j \in P_1^n \\ \frac{1}{2^n}, & i \in P_1^n \end{cases} \tag{24b}$$

Due to the same reason as above we can confirm that all canalyzing functions in $\mathbb{E}_{\mathrm{CF}}^{n,2}$ are attractor-effective equivalent but are different from $\mathbb{E}_{\mathrm{CF}}^{n,1}$.

(3) Consider $\mathrm{CF}(x_1^u, f_{j\neq 1}^y)$, that is, $\mathbb{E}_{\mathrm{CF}}^{n,3}$. The inclusion relationships are as follows,

$$(1,1)\text{-type} \rightarrow \mathcal{V}(P_1^n) \subseteq P_j^n \tag{25a}$$
$$(1,0)\text{-type} \rightarrow \mathcal{V}(P_1^n) \subseteq \bar{P}_j^n \tag{25b}$$
$$(0,1)\text{-type} \rightarrow \mathcal{V}(\bar{P}_1^n) \subseteq P_j^n \tag{25c}$$
$$(0,0)\text{-type} \rightarrow \mathcal{V}(\bar{P}_1^n) \subseteq \bar{P}_j^n \tag{25d}$$

The transition probability for $\mathrm{CF}(x_1^1, f_{j\neq 1}^1)$ can be written as follows.

$$p_{ij}^{(x_1^1, f_{j\neq 1}^1)} = \begin{cases} \frac{1}{2^{n-1}}, & i \in P_1^n, j \in P_j^n \cap P_1^n \\ \frac{1}{2^{n-1}}, & i \in P_1^n, j \in P_j^n \backslash P_1^n \\ \frac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{26}$$

Notice that for any $j \neq 1$, $|(P_j^n)^{\pm} \cap (P_1^n)^{\pm}| = |(P_j^n)^{\pm} \backslash (P_1^n)^{\pm}| = \frac{1}{2^{n-2}}$ where $(P_j^n)^{\pm}$ can be either P_j^n or $\bar{P}_j^n, j = 1, \ldots, n$. It is then not difficult to verify that all $\mathrm{CF}(x_1^u, f_{j\neq 1}^y)$ give the same N_k in (17) (more details can be referred in the proof of Theorem 18 to be presented later), meaning that all canalyzing functions in $\mathbb{E}_{\mathrm{CF}}^{n,3}$ are attractor-effective equivalent.

Furthermore, (21) is true since for $n = 1$ we do not have canalyzing function of the form $\mathrm{CF}(x_1^u, f_{j\neq 1}^y)$ and thus $\mathbb{E}_{\mathrm{CF}}^{1,3}$ does not exist.

Remark 17. The numbers of all canalyzing functions in the presence of possibly multiple canalyzing variables has shown to be increasing exponentially with the network size (Just et al., 2004). The set of canalyzing functions we consider in this paper, $\mathcal{C}_{\mathrm{CF}}^n$, is believed to be the most meaningful subset. It is readily to calculate that $|\mathcal{C}_{\mathrm{CF}}^n| = 4n^2$. Theorem 16 means that there exist only three different attractor-effective canalyzing functions (for $n \geq 2$) out of the $4n^2$ possible candidates. This simplifies significantly all analysis regarding the number of attractors affected by canalyzing functions.

3.2 Mean number of attractors for different attractor effective equivalence classes

Theorem 18. The mean number of attractors of the three attractor-effective equivalence classes in $\mathbb{E}_{\mathrm{CF}}^n$ are as follows.

(1) $\mathbb{E}_{\mathrm{CF}}^{n,1}$.

$$N_k^{n,1} = (1 + \frac{1}{2^k}) N_k^{n-1,0} \tag{27}$$

(2) $\mathbb{E}_{\mathrm{CF}}^{n,2}$.

$$N_k^{n,2} = \sum_{i=0}^{\lfloor \frac{k}{2} \rfloor} k(k-i-1)!i!C_i^{k-i} C_i^{2^{n-1}} C_{k-i}^{2^{n-1}} 2^{i-nk} \tag{28}$$

(3) $\mathbb{E}_{\mathrm{CF}}^{n,3}$.

$$N_k^{n,3} = \sum_{i=0}^{\lfloor \frac{k}{2} \rfloor} \sum_{j=i}^{k-i} \sum_{l=0}^{k-i-j} p_{i,j,l,k} C_i^{2^{n-2}} C_j^{2^{n-2}} C_l^{2^{n-2}} \times C_{k-i-j-l}^{2^{n-2}} 2^{k+j+l-nk} \tag{29}$$

where $p_{i,j,l,k}$ is defined as follows

$$\begin{cases} k(j-1)!G_j^{k-i-j-l} C_i^j i! G_j^l, & j > 0 \\ k! & i = j = 0, l = k, 0 \\ 0 & \text{otherwise.} \end{cases} \tag{30}$$

and G_i^j is the number of choices when allocating j items into i groups.

Proof. We give the proof for $\mathbb{E}_{\mathrm{CF}}^{n,1}$ and $\mathbb{E}_{\mathrm{CF}}^{n,3}$. $\mathbb{E}_{\mathrm{CF}}^{n,2}$ can readily follow from $\mathbb{E}_{\mathrm{CF}}^{n,3}$.

(1) $\mathbb{E}_{\mathrm{CF}}^{n,1}$. Consider $\mathcal{B}_{\mathrm{CF}(x_1^1, f_1^1)}^n$. The transition probability in (23a) implies that no attractors can include states from both P_1^n and $\bar{P}_1^n$ and therefore the attractors contributed by $i, j \in P_1^n$ and $i \in \bar{P}_1^n$ can be calculated independently. By Theorem 14, it is seen that the contribution of the former is exactly equivalent to an ARBN with size $n-1$ while the latter with an additional factor $\frac{1}{2^k}$. (27) readily follows.

(2) $\mathbb{E}_{\mathrm{CF}}^{n,3}$. Consider $\mathcal{B}_{\mathrm{CF}(x_1^1, f_2^1)}^n$. From (26) it is known

$$p_{ij}^{(x_1^1, f_2^1)} = \begin{cases} \frac{1}{2^{n-1}}, & i \in P_1^n, j \in P_2^n \cap P_1^n \\ \frac{1}{2^{n-1}}, & i \in P_1^n, j \in P_2^n \backslash P_1^n \\ \frac{1}{2^n}, & i \in \bar{P}_1^n \end{cases} \tag{31}$$

Define

$$A = P_1^n \cap P_2^n, B = P_1^n \backslash A,$$
$$C = P_2^n \backslash A, D = \Omega \backslash (A \cup B \cup C)$$

where $\Omega := \{1, 2, \ldots, 2^n\}$. The transition probabilities in (31) can be reformed as

$$p_{ij}^{(x_1^1, f_2^1)} = \begin{cases} \frac{1}{2^{n-1}}, & i \in A, j \in B \\ \frac{1}{2^{n-1}}, & i \in B, j \in C \\ \frac{1}{2^n}, & i \in C, j \in D \\ \frac{1}{2^n}, & i \in D, j \in A \end{cases} \tag{32}$$

Consider $N_k^{n,3}$. Choose, in order, the following different number of states from the four sets,

$$i \rightarrow B, j \rightarrow C, l \rightarrow D, k-i-j-l \rightarrow A$$

In total we have $C_i^{2^{n-2}} C_j^{2^{n-2}} C_l^{2^{n-2}} C_{k-i-j-l}^{2^{n-2}}$ different choices. By (32), it is also known that each product in (17) is the same as $\frac{1}{2^{nk-k-j-l}}$.

Table 1. Number of attractors for various ensembles with network size 3.

Ensemble \Number \Length	1	2	3	4	5	6	7	8
$\mathcal{B}^n$	1	0.875	0.6563	0.4102	0.2051	0.0769	0.0192	0.0024
$\mathbb{E}_{\text{CF}}^{n,1}$	1.5000	0.9375	0.4219	0.0996	0	0	0	0
$\mathbb{E}_{\text{CF}}^{n,2}$	0.5000	1.1875	0.6094	0.4746	0.2051	0.0747	0.0154	0.0011
$\mathbb{E}_{\text{CF}}^{n,3}$	1	0.8125	0.6094	0.3496	0.1465	0.0439	0.0077	0.0005

Now consider all the possible permutations of these k states, the total number of which is denoted by $p_{i,j,l,k}$. These permutations can be valid in only the following two cases.

(a) $j > 0$. In this case we first allocate j states from C into the k possible positions, resulting in $k(j-1)!$ possible choices. Note that all the states in A can be put either before or behind the states in C, therefore we can allocate the $k-i-j-l$ states from A to j groups, i.e., $G_j^{k-i-j-l}$. Although now we have $k-i-l$ available positions, the states from B are not able to be put arbitrarily, i.e., they can not be behind A (the states from A can never be transformed to states in B directly), and therefore the available positions are only j. Notice that states from B can not be transformed to states in itself, meaning that we can only permutate them but not allocate them into groups. This will contribute a factor of $C_i^j i!$. At last, l states from D can only be grouped into j groups (they can not be behind A nor B), which is G_j^l.

(b) $i = j = 0, k = k, 0$. In this case there are no states from B nor C and the states can only be from either A or D. It is immediately clear that all the possible choices are $k!$.

The above analysis gives (30).

This completes the proof.

Remark 19. From Theorem 18 we are able to calculate the mean number of attractors for different canalyzing functions directly, which had been impossible before. More properties of canalyzing functions can also be possibly examined. For example, the comparison of the mean number of attractors of Boolean networks with size 3 between without and with the three types of different attractor-effective canalyzing functions is shown in Table 1. It is observed that in general Boolean networks with canalyzing functions tend to have less attractors with shorter lengths, which confirms previous simulation-based findings (Murrugarra and Laubenbacher, 2011).

4. CONCLUSIONS

Canalyzing functions have been shown to be biologically significant. For the first time we describe canalyzing functions algebraically using a novel matrix representation of logics based on semi-tensor product. Within this new framework, we categorize canalyzing functions in terms of their capabilities of affecting the number of attractors in the Boolean network. Surprisedly we find that there exist only three different attractor-effective canalyzing functions for all sizes of Boolean networks larger than 1, despite the fast increasing number of all possible canalyzing functions. The calculation of the mean number of attractors for Boolean networks with canalyzing functions confirms previous simulation-based findings. It is believed that further analysis within this analytical framework will result in meaningful findings which are impossible to obtain using simulations.

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