Physica D 353-354 (2017) 39–47

Contents lists available at ScienceDirect

Physica D

journal homepage: www.elsevier.com/locate/physd

The influence of canalization on the robustness of Boolean networks

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HIGHLIGHTS

- The Boolean algebra concept of sensitivity is generalized to c-sensitivity.
- Activities and c-sensitivities of various Boolean canalizing functions are computed.
- Derrida values of canalizing Boolean networks are weighted sums of c-sensitivities.

ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form 3 May 2017
Accepted 8 May 2017
Available online 17 May 2017
Communicated by: K. Josic

Keywords:
k-canalizing function
Derrida value
Boolean network
Nested canalizing function
Stability

ABSTRACT

Time- and state-discrete dynamical systems are frequently used to model molecular networks. This paper provides a collection of mathematical and computational tools for the study of robustness in Boolean network models. The focus is on networks governed by k-canalizing functions, a recently introduced class of Boolean functions that contains the well-studied class of nested canalizing functions. The variable activities and sensitivity of a function quantify the impact of input changes on the function output. This paper generalizes the latter concept to c-sensitivity and provides formulas for the activities and c-sensitivity of general k-canalizing functions as well as canalizing functions with more precisely defined structure. A popular measure for the robustness of a network, the Derrida value, can be expressed as a weighted sum of the c-sensitivities of the governing canalizing functions, and can also be calculated for a stochastic extension of Boolean networks. These findings provide a computationally efficient way to obtain Derrida values of Boolean networks, deterministic or stochastic, that does not involve simulation.

1. Introduction

The robustness of dynamic networks has long been an important topic of investigation in a wide range of contexts, using various definitions of the concept [1,2]. Due to the important role of stochasticity in the dynamic behavior of biological networks, in particular gene regulatory networks, the concept of robustness has been studied extensively in this context [3]. Since the introduction of Boolean and logical network models to the study of the properties of gene regulatory networks [4,5], time- and state-discrete dynamical systems have become an increasingly popular representation of molecular networks [6–8]. For the most part, these consist of Boolean networks and various generalizations thereof. Questions regarding the robustness of molecular networks, modeled in the time- and state-discrete dynamical systems framework, frequently involve the relationship between structural features of the network and its resulting dynamics. One commonly used measure of the robustness of such a network is the so-called Derrida value of the network, a measure of how perturbations propagate through the network [9]. This measure can then be related to network structure, such as the type of logical rules used to represent the regulatory mechanisms for individual network nodes; see, e.g., [10].

A frequently investigated concept related to robustness is that of *canalization* in developmental biology, introduced by Waddington in the 1940s [11]. It was intended to account for the absence of a known mechanism that enables the genetic regulatory protocols driving embryonic development to accurately produce a specific phenotype, even in light of substantial variation in the developing organism's environment. The underlying idea is that phenotypes can be thought of as valleys in a landscape, and canalization is a "force" that channels the developmental trajectory accurately into a particular valley, protecting it from perturbations. Kauffman introduced a version of this concept to Boolean network modeling

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http://dx.doi.org/10.1016/j.physd.2017.05.002
0167-2789/

of gene regulatory networks by studying canalizing functions [12], as well as the special subclass of so-called nested canalizing functions [13]. Several authors recently extended this work by considering canalization as a property of Boolean functions [14,15]. Generalizing findings from [16], they showed that every Boolean function has a unique algebraic form, characterized by three invariants: canalizing depth, number of layers, and the non-canalizing core-polynomial. The canalizing depth of a function describes its degree of canalization and a k-canalizing function is defined to have a canalizing depth of at least k. This extension neatly stratifies the set of all Boolean functions on n variables by their canalizing depth, including so-called nested canalizing functions (n-canalizing), canalizing functions (1-canalizing) and non-canalizing functions (0-canalizing but not 1-canalizing).

A *canalizing* function possesses at least one input variable such that, if this variable takes on a certain "canalizing" value, then the output value is already determined, regardless of the values of the remaining input variables. If this variable takes on another value, and there is a second variable with this same property, the function is 2-canalizing. If k variables follow this pattern, the function is *k-canalizing*, and if all variables follow this pattern, the function is *nested canalizing* (NCF). By definition, any $(k+1)$-canalizing function is also k-canalizing, and the Boolean function $f(x_1, \ldots, x_n) = x_1 \wedge \cdots \wedge x_n$ is an example of an NCF.

The relationship between network stability, frequently measured using Derrida values [9], and the proportion of canalizing functions and their degree of canalization has received much attention in recent years. Boolean networks governed by canalizing functions are more stable than those constructed using random functions [13,17–19]. In general, network stability and the degree of canalization are positively correlated, with networks governed by NCFs exhibiting the most stable dynamics [20–23]. Derrida himself already described the positive link between network stability and function bias (also called internal homogeneity) [24]. Canalizing functions exhibit a high degree of bias, as do functions which are part of some special Post classes [19]. On average, these latter functions need to be present in a network at an even lower proportion than 1-canalizing functions in order to give rise to stable dynamics [19]. It is however unknown how their ability to confer robustness compares to k-canalizing functions. These findings clearly motivate the study of k-canalizing functions in the context of understanding the regulatory logic of gene networks.

In this paper, we introduce a closed formula for the efficient computation of the Derrida values of a network governed by k-canalizing functions. In detail, the paper is organized as follows. In Section 2, we formally introduce the concept of canalization, see also, e.g., [13,15,16]. The activity of a variable in a function quantifies the influence of that variable on the whole function, while the average sensitivity of a function measures how sensitive a function is to a random input change and can be expressed as a weighted sum of the activities. Both quantities have been extensively studied by the Boolean modeling community, see, e.g., [14,25–27]. In Section 3, we derive formulas for the expected activities of k-canalizing functions for various degrees of knowledge of their structure. We also introduce the c-sensitivity of a function as a natural generalization of the sensitivity and compute the average c-sensitivity of a canalizing function from the activities of its variables. In Section 4, we use the normalized average c-sensitivities to derive a formula for the Derrida values of a Boolean network that is governed by k-canalizing functions. This greatly simplifies the application of this tool for robustness analyses of networks, which otherwise requires extensive simulations (difficult or infeasible for large networks). We explore the relationship between the Derrida value, the bias, the canalizing depth, the size of each layer and the non-canalizing core-polynomial of a Boolean function. In Section 5, we extend the formula for the Derrida values to the case of stochastic networks, which are an important modeling paradigm for gene regulatory networks. Section 6 concludes the paper with some remarks and possible avenues of future work.

2. The concept of canalization

In this section we review some well-known concepts and definitions, mainly from [15], to introduce the concept of *canalization*. Throughout, let $\oplus$ denote addition modulo 2 when used in a polynomial, and the "exclusive or" (XOR) function when used in a Boolean logical expression.

Definition 2.1. A Boolean function $f(x_1, \ldots, x_n)$ is essential in the variable x_i if there exists some $\mathbf{x} \in \{0,1\}^n$ such that

$$f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_i),$$

where e_i is the ith unit vector.

Definition 2.2. A Boolean function $f(x_1, \ldots, x_n)$ is canalizing if there exists a variable x_i, a Boolean function $g(x_1, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n)$ and $a, b \in \{0,1\}$ such that

$$f(x_1, \ldots, x_n) = \begin{cases} b & \text{if } x_i = a, \\ g \not\equiv b & \text{if } x_i \neq a, \end{cases}$$

in which case x_i is called a canalizing variable, the input a is the canalizing input, and the output value b when $x_i = a$ is the corresponding canalized output.

Definition 2.3. A Boolean function $f(x_1, \ldots, x_n)$ is k-canalizing, where $1 \leq k \leq n$, with respect to the permutation $\sigma \in \mathcal{S}_n$, inputs $a_1, \ldots, a_k$ and outputs $b_1, \ldots, b_k$, if

$$f(x_1, \ldots, x_n) = \begin{cases} b_1 & x_{\sigma(1)} = a_1, \\ b_2 & x_{\sigma(1)} \neq a_1, x_{\sigma(2)} = a_2, \\ b_3 & x_{\sigma(1)} \neq a_1, x_{\sigma(2)} \neq a_2, x_{\sigma(3)} = a_3, \\ \vdots & \vdots \\ b_k & x_{\sigma(1)} \neq a_1, \ldots, x_{\sigma(k-1)} \neq a_{k-1}, x_{\sigma(k)} = a_k, \\ g \not\equiv b_k & x_{\sigma(1)} \neq a_1, \ldots, x_{\sigma(k-1)} \neq a_{k-1}, x_{\sigma(k)} \neq a_k, \end{cases} \tag{2.1}$$

where $g = g(x_{\sigma(k+1)}, \ldots, x_{\sigma(n)})$ is a Boolean function on $n-k$ variables. When g is not canalizing, the integer k is the canalizing depth of f (as in [14]), and if, in addition, g is not constant, it is called the core function of f, denoted by f_C. If g is constant, the variables $x_{\sigma(k+1)}, \ldots, x_{\sigma(n)}$ are not essential and f does not have a core function. We further define all Boolean functions to be 0-canalizing.

While the representation of a k-canalizing function as in Eq. (2.1) is generally not unique (certain canalizing variables may be reordered), its canalizing depth as well as its core function (if it exists) are independent of the representation [15].

Example 2.4. The Boolean function $f(w, x, y, z) = w \wedge \bar{x} \wedge (y \oplus z)$ has canalizing depth 2 and core function $f_C = y \oplus z$.

Remark 2.5. If we consider the set of all Boolean functions on n variables, then

(a) The n-canalizing functions are precisely the well-studied nested canalizing functions.
(b) The 1-canalizing functions are precisely the canalizing functions.
(c) Every Boolean function is 0-canalizing.
(d) Every non-canalizing function has canalizing depth 0.
(e) As in [15], constant functions are - contrary to Kauffman's original definition [28] - not canalizing. This implies that (i) g is uniquely determined and (ii) since $g \not\equiv b_k$, a k-canalizing function is essential in each of its canalizing variables.

3. Activities and normalized average sensitivities

Throughout, we use the following definition to compare the distance between two vectors.

Definition 3.1. The distance between two vectors $\mathbf{x}, \mathbf{y} \in \{0,1\}^n$ is given by their Hamming distance (the standard ℓ^1 metric),

$$d(\mathbf{x},\mathbf{y}) = \left|\{j \mid x_j \neq y_j\}\right| \in \{0,\dots,n\}.$$

Two vectors $\mathbf{x}, \mathbf{y} \in \{0,1\}^n$ with $d(\mathbf{x},\mathbf{y}) = c$ are *c-Hamming neighbors*.

Some variables of a Boolean function have greater influence over the output of the function than others. The activity of variable x_i in the function $f(x_1,\dots,x_n)$ is defined as

$$\alpha_i^f = \frac{1}{2^n} \sum_{\mathbf{x}\in\{0,1\}^n} \chi[f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_i)],$$

where χ is an indicator function. A change in a highly active variable frequently affects the function f, while a non-essential variable has activity 0 and never alters f.

Another important quantity, directly related to the activity of a variable and introduced in [29], measures how sensitive the output of a function is to input changes. The sensitivity of a function f at a vector $\mathbf{x}$ is defined as the number of 1-Hamming neighbors of $\mathbf{x}$ with a different function value than $f(\mathbf{x})$. That is,

$$S^f(\mathbf{x}) = \sum_{i=1}^{n} \chi[f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_i)].$$

The average sensitivity S^f is the expected value of $S^f(\mathbf{x})$. Assuming a uniform distribution of $\mathbf{x}$,

$$S^f = \mathbb{E}[S^f(\mathbf{x})] = \frac{1}{2^n} \sum_{\mathbf{x}\in\{0,1\}^n} \sum_{i=1}^{n} \chi[f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_i)] = \sum_{i=1}^{n} \alpha_i^f.$$

Next, we will generalize the concept of sensitivity to c-sensitivity and calculate the normalized average c-sensitivity for different classes of canalizing functions, which we will use in the next section for the calculation of stability properties of Boolean networks.

Definition 3.2. The c-sensitivity of $f(x_1,\dots,x_n)$ at $\mathbf{x}$ is defined as the number of c-Hamming neighbors of $\mathbf{x}$ on which the function value is different from its value on $\mathbf{x}$. That is,

$$S_c^f(\mathbf{x}) = \sum_{\substack{I\subseteq\{1,\dots,n\}\\ |I|=c}} \chi[f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_I)],$$

where e_I is a vector with 1 at all indices in I and 0 everywhere else. Note that, by definition, $S_0^f(\mathbf{x}) = 0$. The average c-sensitivity of f is

$$S_c^f = \mathbb{E}[S_c^f(\mathbf{x})] = \frac{1}{2^n} \sum_{\mathbf{x}\in\{0,1\}^n} \sum_{\substack{I\subseteq\{1,\dots,n\}\\ |I|=c}} \chi[f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_I)].$$

S_c^f must lie in the interval $[0, \binom{n}{c}]$. Let us therefore define the normalized average c-sensitivity of f as

$$s_c^f = \frac{S_c^f}{\binom{n}{c}} \in [0,1].$$

This definition generalizes the concept of sensitivity in a natural way ($S_1^f = S^f$) and allows the impact of a simultaneous change in more than one input of a function to be studied. We will now derive the expected activities of a k-canalizing function, where the expectation is taken uniformly over all k-canalizing functions.

Theorem 3.3. *Let f be a k-canalizing function of n variables. By relabeling the variables if necessary, assume that f is k-canalizing in the variable order $x_1,\dots,x_k$. The expected activity of x_j in f is*

$$\mathbb{E}[\alpha_j^f] = \begin{cases} \dfrac{1}{2^j} & \text{if } j < k \\ \dfrac{1}{2^{k-1}} \dfrac{2^{2^{n-k}-1}}{2^{2^{n-k}}-1} & \text{if } j = k \\ \dfrac{1}{2^k} \dfrac{2^{2^{n-k}-1}}{2^{2^{n-k}}-1} & \text{if } j > k. \end{cases}$$

Proof. See Appendix. □

The average c-sensitivity of any k-canalizing function is a weighted sum of the activities of its variables.

Theorem 3.4. *By relabeling the variables if needed, assume that $f(x_1,\dots,x_n)$ is a k-canalizing function in the variable order $x_1,\dots,x_k$. The average c-sensitivity of f is*

$$S_c^f = \sum_{j=1}^{n-c+1} \binom{n-j}{c-1} \alpha_j^f.$$

Proof. See Appendix. □

Corollary 3.5. *The expected activities of the variables $(x_{\sigma(1)},\dots,x_{\sigma(n)})$ in an NCF f are*

$$\mathbb{E}[\alpha^f] = \left(\frac{1}{2}, \frac{1}{4}, \dots, \frac{1}{2^{n-2}}, \frac{1}{2^{n-1}}, \frac{1}{2^{n-1}}\right),$$

and the expected normalized average c-sensitivity can be expressed in terms of a hypergeometric function:

$$\mathbb{E}[s_c^f] = \frac{c}{2n} \cdot {}_2F_1\left[1, c-n; 1-n; \frac{1}{2}\right].$$

In particular, for $c = 1$, $\mathbb{E}[s_1^f] = \frac{1}{n}$.

Theorem 4.6 of [15] shows that any Boolean function can be written in a unique standard monomial form, in which the variables are grouped into different layers based on their dominance (see also [16,30] for earlier work on this topic for NCFs). Any canalizing variable is part of the first layer. All variables that become canalizing once the variables from the first layer are excluded, are part of the second layer, etc. The number of layers is denoted by r. The number of variables in the ith layer is the *size of the ith layer*, denoted by k_i, and the number of all variables that become eventually canalizing is the *canalizing depth* $k = \sum k_i$. All remaining variables that never become canalizing are part of the *core polynomial*, which is simply an affine transformation of the core function (see [15] for details).

Definition 3.6. The *layer structure* of a Boolean function $f(x_1,\dots,x_n)$ with canalizing depth k is defined as the vector $(k_1,\dots,k_r)$, where r is the number of layers and k_i is the size of the ith layer, $i = 1,\dots,r$.

Theorem 3.3 yields the expected activities of the variables in a function for which only the minimal canalizing depth is known. If instead the exact canalizing depth and layer structure are known, we can quantify the dynamic properties of a function much more accurately. In [16], the authors computed the activities and average sensitivity of an NCF with known layer structure. We will now determine the activities of any Boolean function, for which the canalizing depth, the layer structure, and the Hamming weight of its core function are known. In this case, we do not require an expected value since all such functions share the same activities.

Theorem 3.7. *Let $f(x_1,\dots,x_n)$ be a Boolean function with canalizing depth k, layer structure $(k_1,\dots,k_r)$, and $v \in \{1,\dots,2^{n-k}\}$ entries*

$\neq b_k$ in the truth table of its non-canalizing core g. By relabeling the variables if necessary, assume that f is k-canalizing in the variable order $x_1, \dots, x_k$. The activity of x_j in f is

$$\alpha_j^f = \begin{cases} \varphi_{L(x_j)} + \dfrac{1}{2^{k-1}}\psi_{L(x_j)} & \text{if } j \le k, \\ \dfrac{v(2^{n-k}-v)}{2^{n-1}(2^{n-k}-1)} & \text{if } j > k, \end{cases}$$

where $L(x_j) \in \{1, \dots, r\}$ denotes the layer of variable x_j and

$$\varphi_{r+1} = \varphi_r = 0, \qquad \varphi_i = \varphi_{i+2} + \sum_{s=0}^{k_{i+1}-1} \left(\frac{1}{2}\right)^{s+k_1+\cdots+k_i}$$

for $i = r-1, \dots, 1$,

$$\psi_r = \frac{v}{2^{n-k}}, \qquad \psi_i = 1 - \psi_{i+1} \text{ for } i = r-1, \dots, 1.$$

Proof. See Appendix. □

4. Derrida values of networks governed by k-canalizing functions

Gene regulatory networks need to be robust to perturbations, which, consequently, is a property of Boolean network models that is important to determine. The so-called Derrida plot is a common technique to evaluate the robustness of a Boolean network [9]. It describes how a perturbation of a given size propagates through the network on average over time. If a small perturbation vanishes over time, the network is considered to be in the ordered regime, in which case it typically has many steady states and short limit cycles. If the perturbation amplifies over time, the network is in the chaotic regime, which typically possesses long limit cycles. Lastly, if the perturbation remains of similar size, then the network is situated in the narrow region between these regimes, often called the critical threshold [25,28]. Many biological systems seem to operate at this "edge of chaos"; they must be robust enough to withstand perturbations caused by environmental changes but also flexible enough to allow adaptation [10,31,32].

In this section we formally define the concept of Derrida value in the framework of Boolean networks, using an annealed approximation. Although this approximation corresponds to a system in which interactions are randomly rewired at each time step, it has been shown that its use does not significantly change the Derrida plot of random Boolean networks [33]. With few exceptions [23], the calculation of Derrida values has thus far required extensive Monte Carlo simulations [13,17]. Using the concept of c-sensitivities from Section 3, we derive a closed formula for the Derrida values of synchronously updated Boolean networks. Especially for networks with many inputs, this offers a substantial improvement, since the time required to approximate the Derrida plot through simulations increases exponentially in the number of inputs.

Definition 4.1. A *synchronous Boolean network* of N nodes is defined as

$$F = (f_1, \dots, f_N) : \{0,1\}^N \to \{0,1\}^N,$$

where each $f_i : \{0,1\}^N \to \{0,1\}$ is called an *update function* of F. All nodes are updated at the same time so the state of the network at the next time step only depends on the state at the current time step. That is, for any state $\mathbf{x}(t) \in \{0,1\}^N$,

$$\mathbf{x}(t+1) = F(\mathbf{x}(t)) = \left(f_1(\mathbf{x}(t)), \dots, f_N(\mathbf{x}(t))\right).$$

Further, let $I(f_i)$ be the set of all indices of essential variables of f_i, and we set $n_i := |I(f_i)|$, $i = 1, \dots, N$.

Definition 4.2. Let $F = (f_1, \dots, f_N)$ be a synchronous Boolean network. For a given perturbation size m, the Derrida value of F is defined as the average size of the perturbation after one update,

$$D(F, m) = \mathbb{E}\Big[d\big(F(\mathbf{x}), F(\mathbf{y})\big) \;\Big|\; d(\mathbf{x}, \mathbf{y}) = m\Big], \tag{4.1}$$

where the expected value is taken uniformly over all m-Hamming neighbors.

Theorem 4.3. *The Derrida value of a synchronous Boolean network $F = (f_1, \dots, f_N)$ can be expressed as a weighted sum of the normalized average c-sensitivities of its update functions,*

$$\begin{aligned} D(F, m) &= \sum_{i=1}^{N} \mathbb{P}\left(f_i(\mathbf{x}) \neq f_i(\mathbf{y}) \;\middle|\; d(\mathbf{x}, \mathbf{y}) = m\right) \\ &= \sum_{i=1}^{N} \sum_{c=0}^{m} H_{N,m,n_i}(c) s_c^{f_i}, \end{aligned} \tag{4.2}$$

where

$$H_{N,m,n_i}(c) = \frac{\binom{m}{c}\binom{N-m}{n_i-c}}{\binom{N}{n_i}} = \frac{\binom{n_i}{c}\binom{N-n_i}{m-c}}{\binom{N}{m}}$$

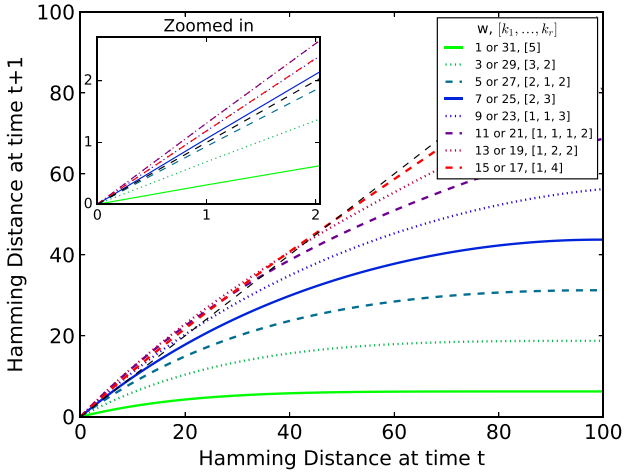
denotes the hypergeometric probability mass function.

Proof. Let $F = (f_1, \dots, f_N)$ be a synchronous Boolean network, and let $\mathbf{x}, \mathbf{y} \in \{0,1\}^N$ be m-Hamming neighbors. The update of each component in a synchronous Boolean network is by definition independent from the update of other components. The Derrida value is therefore simply the sum of the probabilities that $f_i(\mathbf{x})$ and $f_i(\mathbf{y})$ differ, $i = 1, \dots, N$. These probabilities, in turn, equal the normalized average c-sensitivities of the update functions.

To finish the proof of the second equality, let $J(f_i) = I(f_i) \cap \{j \mid x_j \neq y_j\}$ be the restriction of $I(f_i)$ to those indices where $\mathbf{x}$ and $\mathbf{y}$ differ, $i = 1, \dots, N$. $J(f_i)$ is the intersection of two sets of sizes n_i and m so that its magnitude $|J(f_i)|$ follows a hypergeometric distribution. Conditioning with respect to $|J(f_i)|$ leads to the second equality. Note that since $H_{N,m,n_i}(c) = 0$ if $c \notin \{\max(m + n_i - N, 0), \dots, \min(m, n_i)\}$, we can expand the inner sum from 0 to m. □

We will now use this theorem together with the results from Section 3 to calculate average Derrida values for different Boolean networks.

The Hamming weight w of a Boolean function is defined as the number of 1s in its truth table, and the bias is the probability that a randomly chosen entry in the truth table is 1 minus the probability that it is 0. The absolute bias is the absolute value of the bias. A Boolean function with equally many 0s and 1s has bias 0 and is called balanced. Constant functions are the most biased with absolute bias 1, and it is easy to see that there is a 1-1 correspondence between the absolute bias and the layer structure of an NCF (see also [16]). Fig. 1 depicts the Derrida values for networks of $N = 100$ nodes, which are governed by NCFs with $n = 5$ inputs and varying layer structures (specified by the Hamming weight w of the NCF or, correspondingly, the layer structure). The calculation of all the 800 plotted values took less than a second on a regular desktop computer. In networks governed by highly unbalanced NCFs with Hamming weights 1, 3, 29 and 31, small perturbations vanish on average over time; these networks operate in the stable regime. Networks of NCFs with Hamming weights 5, 7, 25 and 27 operate close to the critical threshold, while networks of NCFs with Hamming weights between 11 and 23 operate in the chaotic regime. Surprisingly, networks of NCFs with Hamming weights 11, 13, 19, and 21 are more chaotic than those governed by almost balanced NCFs with Hamming weights 15 and 17. One possible explanation for this observation may be the number of layers. NCFs with Hamming weight 15 or 17 consist of two layers, while NCFs with Hamming weight 13 and 19 (11 and 21) have three (four)



w	|bias|	r	[k_1,...,k_r]	D(F,1)	D(F,2)	D(F,3)
1 or 31	0.9375	1	[5]	0.3125	0.6124	0.9000
3 or 29	0.8125	2	[3, 2]	0.6875	1.3548	2.0024
5 or 27	0.6875	3	[2, 1, 2]	0.9375	1.8523	2.7448
7 or 25	0.5625	2	[2, 3]	1.0625	2.1048	3.1273
9 or 23	0.4375	3	[1, 1, 3]	1.1875	2.3548	3.5023
11 or 21	0.3125	4	[1, 1, 1, 2]	1.3125	2.6023	3.8698
13 or 19	0.1875	3	[1, 2, 2]	1.3125	2.6048	3.8774
15 or 17	0.0625	2	[1, 4]	1.1875	2.3624	3.5250
expected value when r = 1				0.3125	0.6124	0.9000
expected value when r = 2				0.9792	1.9407	2.8849
expected value when r = 3				1.1458	2.2706	3.3748
expected value when r = 4				1.3125	2.6023	3.8698
expected value when r and [k_1,...,k_r] are unknown				1	1.9811	2.9436

Fig. 1. Derrida plot for Boolean networks of $N = 100$ nodes governed by NCFs with $n = 5$ inputs and varying layer structure. A black dashed line shows the line $y = x$ that separates the ordered from the chaotic region. The table shows the Derrida values for perturbations of up to size 3 (first 8 rows), as well as average values for cases where only the number of layers but not the exact layer structure is known (rows 9–12) and average values when both are unknown (last row).

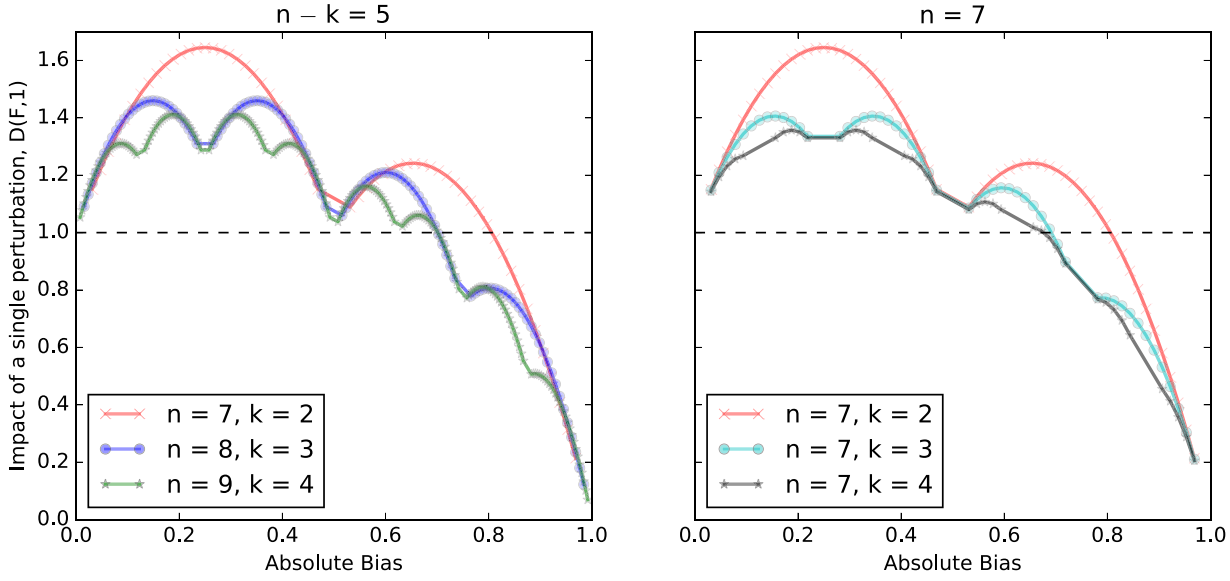


Fig. 2. For all different functions of n variables with canalizing depth k, the impact of a single perturbation ($D(F, 1)$) is plotted against the absolute bias of the function. In the left plot, the number of non-canalizing variables is constant ($n - k = 5$), while in the right plot, the total number of variables is constant ($n = 7$). For visualization purposes, the scatter points are connected by a line.

different layers. The number of layers is positively correlated with the Derrida value for small perturbations (see rows 9-12 in Fig. 1B and Table 1). Similarly, the number of variables in the first layer, k_1, and the absolute bias are negatively correlated. Interestingly, the correlation of the Derrida value for small perturbations with k_1 and with the absolute bias remains high for NCFs with many inputs, whereas the correlation with the number of layers decreases with increasing number of inputs (Table 1).

Table 2 shows the impact of a single perturbation on networks governed by canalizing functions with $n = 7$ inputs and canalizing depth $k = 4$ for varying numbers of layers (r), layer structures $(k_1, \ldots, k_r)$ and numbers of entries $\neq b_k$ in the truth table of the core function (v). As for NCFs, the Derrida value increases as the number of layers increases and decreases with increasing number of most dominant variables, k_1. Each combination of layer structure and v yields a canalizing function with a different absolute bias and Fig. 2 exhibits the connection between Derrida value and absolute bias. Again, almost balanced functions give rise to more robust networks than functions with intermediate absolute bias, while networks governed by highly biased functions operate in the stable regime. Moreover, networks with a higher proportion of canalizing variables are more robust. The gain of additional dynamical stability decreases however quickly when adding canalizing variables. Similar behavior is reported for networks governed by partially nested canalizing functions where the non-canalizing inputs follow a complementary threshold function [22].

Table 1
For a Boolean network governed by NCFs with n inputs, this table shows the Spearman correlations between the Derrida value of a single perturbation ($D(F, 1)$) and the size of the first layer (first column), the absolute bias (second column), and the number of layers (last column).

	k_1	|bias|	r
n = 5	-0.944	-0.892	0.747
n = 7	-0.920	-0.869	0.636
n = 10	-0.912	-0.862	0.528
n = 15	-0.911	-0.862	0.420
n = 20	-0.911	-0.862	0.357

Table 2
Impact of a single perturbation ($D(F, 1)$) on networks governed by canalizing functions with $n = 7$ inputs and canalizing depth $k = 4$ for varying layer structures $(k_1, \ldots, k_r)$ and numbers of entries $\neq b_k$ in the truth table of the core function (v). Average values for cases where only the number of layers but not the exact layer structure is known (rows 9–12), average values when both are unknown (last row), as well as average values when v is unknown (last column) are also shown. The top row shows the number (#) of different functions that exist for a given v.

r	$(k_1,\ldots,k_r)$	v = 2 (# = 4)	v = 3 (# = 32)	v = 4 (# = 64)	v = 5 (# = 32)	v = 6 (# = 4)	expected value when v is unknown
1	(4)	0.2054	0.2879	0.3571	0.4129	0.4554	0.3524
2	(3,1)	0.7679	0.7567	0.7321	0.6942	0.6429	0.7274
2	(2,2)	1.0804	1.1004	1.1071	1.1004	1.0804	1.1024
3	(2,1,1)	0.8929	0.9442	0.9821	1.0067	1.0179	0.9774
3	(1,1,2)	1.1429	1.1942	1.2321	1.2567	1.2679	1.2274
4	(1,1,1,1)	1.3304	1.3504	1.3571	1.3504	1.3304	1.3524
2	(1,3)	1.1429	1.1942	1.2321	1.2567	1.2679	1.2274
3	(1,2,1)	1.3304	1.3504	1.3571	1.3504	1.3304	1.3524
expected value when r = 1		0.2054	0.2879	0.3571	0.4129	0.4554	0.3524
expected value when r = 2		0.9970	1.0171	1.0238	1.0171	0.9970	1.0191
expected value when r = 3		1.1220	1.1629	1.1905	1.2046	1.2054	1.1857
expected value when r = 4		1.3304	1.3504	1.3571	1.3504	1.3304	1.3524
expected value when r and $(k_1,\ldots,k_r)$ are unknown		0.9866	1.0223	1.0446	1.0536	1.0491	**1.0399**

Theorems 3.3 and 3.7 are used in Table 3 to investigate the difference between k-canalizing functions (i.e., functions with canalizing depth $\geq k$) and functions with exact canalizing depth k. As expected, k-canalizing functions give rise to slightly more stable networks. This difference in robustness is however only of noticeable size for functions with few non-canalizing variables; if $k \ll n$, the vast majority of k-canalizing functions have also exact canalizing depth k [15]. To our knowledge, the number of non-canalizing functions with a given Hamming weight is unknown. For $n - k > 4$, we therefore approximated the distribution by generating 10^7 random non-canalizing functions. For $n - k \leq 4$, we used exhaustive enumeration.

In all these analyses, we did not specify the size of the network since we focused on the impact of a single perturbation, for which it does not matter. When considering larger perturbations, the network size has a theoretical impact on the Derrida value. By comparing the computed values for different network sizes, we found that the empirical impact was, however, negligible as long as the proportion of perturbed nodes remained small.

5. Derrida values of stochastic discrete dynamical systems

Gene regulatory networks are stochastic in nature. A recently introduced generalization of Boolean networks, called Stochastic Discrete Dynamical Systems (SDDS), captures this inherent stochasticity by assigning gene-specific activation probabilities $p_i^{\uparrow}$ and degradation probabilities $p_i^{\downarrow}$, which describe how likely a specific state change happens at a given update step [34]. This framework allows modeling of different time scales as well as stochastic variability, while preserving the simplicity of a synchronous Boolean network. The Derrida value concept (Eq. (4.1)) can be directly applied to this type of system, and we derive a formula for its computation.

Definition 5.1 (*[34]*). An SDDS in the variables $x_1, \ldots, x_N$ is defined as a collection of N triples

$$F = \left(f_i, p_i^{\uparrow}, p_i^{\downarrow}\right)_{i=1}^{N},$$

where $f_i : \{0,1\}^N \to \{0,1\}$ is the update function, $p_i^{\uparrow}$ the activation probability, and $p_i^{\downarrow}$ the degradation probability for x_i, $i = 1, \ldots, N$.

For all $i \in \{1, \ldots, N\}$ such that $f_i(\mathbf{x}) \neq x_i$,

$$\mathbb{P}(x_i \to x_i) = \begin{cases} 1 - p_i^{\uparrow} & \text{if } x_i < f_i(x), \\ 1 - p_i^{\downarrow} & \text{if } x_i > f_i(x). \end{cases}$$

$$\mathbb{P}(x_i \to f_i(\mathbf{x})) = \begin{cases} p_i^{\uparrow} & \text{if } x_i < f_i(x), \\ p_i^{\downarrow} & \text{if } x_i > f_i(x). \end{cases}$$

Furthermore, as in Section 4, let $I(f_i)$ be the set of all indices of essential variables of f_i, and $n_i := |I(f_i)|$.

Theorem 5.2. *Let $F = \left(f_i, p_i^{\uparrow}, p_i^{\downarrow}\right)_{i=1}^{N}$ be an SDDS. For a perturbation size m, the Derrida value of F is*

$$D(F, m) = \sum_{i=1}^{N} \sum_{c=0}^{m} \begin{cases} \frac{m}{N} H_{N-1,m-1,n_i-1}(c)\left[\gamma_1 s_{c+1}^{f_i} + \gamma_2\left(1 - s_{c+1}^{f_i}\right)\right] \\ \quad + \frac{N-m}{N} H_{N-1,m,n_i-1}(c)\left[\gamma_3 s_c^{f_i} + \gamma_4\left(1 - s_c^{f_i}\right)\right] \\ \quad \text{if } i \in I(f_i), \\ \frac{m}{N} H_{N-1,m-1,n_i}(c)\left[\gamma_1 s_c^{f_i} + \gamma_2\left(1 - s_c^{f_i}\right)\right] \\ \quad + \frac{N-m}{N} H_{N-1,m,n_i}(c)\left[\gamma_3 s_c^{f_i} + \gamma_4\left(1 - s_c^{f_i}\right)\right] \\ \quad \text{if } i \notin I(f_i), \end{cases}$$

where

$$\gamma_1 = 1 - \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right) + p_i^{\uparrow} p_i^{\downarrow}, \qquad \gamma_2 = 1 - \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right),$$

$$\gamma_3 = \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right), \qquad \gamma_4 = \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right) - \frac{1}{2}\left(p_i^{\uparrow} p_i^{\uparrow} + p_i^{\downarrow} p_i^{\downarrow}\right),$$

and H denotes the hypergeometric probability mass function as in Eq. (4.2).

Proof. See Appendix. □

6. Discussion

A characteristic ability of organisms is their capability to operate in highly variable environments, striking a fine balance between the ability to adapt to changing conditions and the robustness to function predictably. This extends to the molecular networks that drive everything from embryonic development to metabolism. Understanding the mechanisms that confer this ability is one of the central challenges in studying biology and its fundamental principles. As in physics, mathematical modeling and

Table 3
Impact of a single perturbation ($D(F, 1)$) on networks governed by k-canalizing functions (gray shaded rows) or functions with exact canalizing depth k (white rows) for varying numbers of inputs (n) and varying canalizing depths (k). Approximated values are marked with *.

		k = n-7	k = n-6	k = n-5	k = n-4	k = n-3	k = n-2	k = n-1	k = n
n = 5	canalizing depth ≥ k	d.n.e.	d.n.e.	2.5	1.50002	1.12745	1.01667	1	1
	canalizing depth = k	d.n.e.	d.n.e.	2.50003*	1.51087	1.15966	1.04167	d.n.e.	1
n = 6	canalizing depth ≥ k	d.n.e.	3	1.75	1.25001	1.06373	1.00833	1	1
	canalizing depth = k	d.n.e.	3.00042*	1.75018*	1.25543	1.07983	1.02083	d.n.e.	1
n = 7	canalizing depth ≥ k	3.5	2	1.375	1.12501	1.03186	1.00417	1	1
	canalizing depth = k	3.50013*	2.00011*	1.37504*	1.12772	1.03992	1.01042	d.n.e.	1

analysis is an enabling technology for the drive to connect structural properties of dynamic biological networks to their dynamics. The study of robustness, as instantiated in the concept of canalization, is no exception, and many such studies have been published, see, e.g., [10,17,33,35].

Here, we have focused on one computational instantiation of robustness, that of canalization in the context of Boolean networks. This includes, as a special case, nested canalization. We have provided a collection of practical and theoretical tools for the analysis of systems governed by k-canalizing functions. In order to study the impact of a simultaneous change in more than one input of a function, we have, at the function level, generalized the concept of sensitivity. At the network level, this allowed us to provide easy-to-use closed form formulas for the Derrida values, a commonly used metric for the stability of networks. We explored the relationship between Derrida value, canalizing depth, absolute bias, number of layers, and size of the first layer of a function. In addition, we derived formulas for the Derrida values of a stochastic discrete dynamical system, a modeling framework that can cope with the inherent stochasticity of gene regulatory networks.

The presented formulas significantly simplify the study of robustness via Derrida values. We only started to disentangle the influence of the different parameters of a canalizing function on the robustness of the Boolean network it appears in, and much work remains to be done. Knowledge of the exact number of non-canalizing functions with a certain number of variables and a certain Hamming weight would be helpful in this effort.

Acknowledgments

First and third authors were supported by NSF Grant CMMI-0908201 and US DoD Grant W911NF-14-1-0486.

Appendix

Theorem 3.3

Proof. Let $f(x_1, \dots, x_n)$ be a k-canalizing function with canalizing order $x_1, \dots, x_k$, inputs a_i and outputs b_i, $1 \le i \le k$. We will use an argument similar to one in [14] to find the expected activities of f. By definition, the activity of x_j in f is the probability that a change in x_j changes the output of f.

Case 1, $j \le k$, i.e., x_j is a canalizing variable: A change in x_j can only affect the output of f if none of the variables $x_1, \dots, x_{j-1}$ receive their canalizing input. Thus,

$$\begin{aligned}\alpha_j^f &= \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_j)\big)\\ &= \mathbb{P}(x_1 \neq a_1, \dots, x_{j-1} \neq a_{j-1})\\ &\quad \times \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_j) \mid x_1 \neq a_1, \dots, x_{j-1} \neq a_{j-1}\big).\end{aligned}$$

Since each canalizing variable receives its canalizing input with probability $\frac{1}{2}$,

$$\mathbb{P}(x_1 \neq a_1, \dots, x_{j-1} \neq a_{j-1}) = \frac{1}{2^{j-1}}.$$

The subfunction $f(1 - a_1, \dots, 1 - a_{j-1}, x_j, \dots, x_n)$ is canalizing in x_j and can therefore be written as $(x_j + a_j)\tilde{g}(x_{j+1}, \dots, x_n) + b_j$ for some Boolean polynomial $\tilde{g} \not\equiv 0$, as in Lemma 4.1 from [15]. Hence,

$$\begin{aligned}&\mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_j) \mid x_1 \neq a_1, \dots, x_{j-1} \neq a_{j-1}\big)\\ &\quad = \mathbb{P}\big(\tilde{g}(x_{j+1}, \dots, x_n) = 1\big).\end{aligned}$$

Case 1a, $j < k$: Since a k-canalizing function must be essential in all of its canalizing variables (Remark 2.5e), $\tilde{g} \not\equiv 1$ must hold. Both constant functions are thus excluded from the set of possible choices for $\tilde{g}$ so that

$$\mathbb{P}\big(\tilde{g}(x_{j+1}, \dots, x_n) = 1\big) = \frac{1}{2}.$$

Case 1b, $j = k$: $\tilde{g} \equiv 1$ does not cause a contradiction. In this case, there are $2^{2^{n-k}}$ choices of Boolean functions for $\tilde{g}$, half of which satisfy $\tilde{g}(x_{k+1}, \dots, x_n) = 1$. Only 0 is excluded from the set of choices for $\tilde{g}$ so that

$$\mathbb{P}\big(\tilde{g}(x_{k+1}, \dots, x_n) = 1\big) = \frac{\frac{1}{2}2^{2^{n-k}}}{2^{2^{n-k}} - 1} = \frac{2^{2^{n-k}-1}}{2^{2^{n-k}} - 1}. \tag{A.1}$$

Case 2, $j > k$, i.e., x_j is not one of the first k canalizing variables: A change in x_j can only affect the output of f if none of the k canalizing variables receive their canalizing input. Thus,

$$\begin{aligned}\alpha_j^f &= \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_j)\big)\\ &= \mathbb{P}(x_1 \neq a_1, \dots, x_k \neq a_k)\\ &\quad \times \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_j) \mid x_1 \neq a_1, \dots, x_k \neq a_k\big)\\ &= \frac{1}{2^k}\mathbb{P}\big(g(x_{k+1}, \dots, x_n) \neq g(y_{k+1}, \dots, y_n)\big),\end{aligned}$$

where $g \not\equiv b_k$ from Definition 2.3, and $\mathbf{y} = \mathbf{x} \oplus e_j$. Similar arguments as for Eq. (A.1) yield

$$\begin{aligned}&\mathbb{P}\big(g(x_{k+1}, \dots, x_n) \neq g(y_{k+1}, \dots, y_n)\big)\\ &\quad = \frac{\frac{1}{2}2^{2^{n-k}}}{2^{2^{n-k}} - 1} = \frac{2^{2^{n-k}-1}}{2^{2^{n-k}} - 1}. \qquad \square\end{aligned} \tag{A.2}$$

Theorem 3.4

Proof. Let f be a k-canalizing function in the variable order $x_1, \dots, x_k$. For $\mathbf{x} \in \{0,1\}^n$, let $\mathbf{y} = \mathbf{x} \oplus e_l$ be a randomly chosen c-Hamming neighbor. In a k-canalizing Boolean function, the probability that $f(\mathbf{x})$ and $f(\mathbf{y})$ differ only depends on the index of the first variable where $\mathbf{x}$ and $\mathbf{y}$ differ. This index is $\min(I)$. Thus,

$$\begin{aligned}\frac{1}{2^n}\sum_{\mathbf{x}\in\{0,1\}^n} \chi[f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_l)] &= \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_l)\big)\\ &= \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x} \oplus e_{\min(I)})\big)\\ &= \alpha^f_{\min(I)}.\end{aligned}$$

There are $\binom{n}{c}$ c-subsets in the set $\{1, \dots, n\}$, of which $\binom{n-j}{c-1}$ contain j as its smallest element for each $j = 1, \dots, n - c + 1$.

Therefore,

$$S_c^f = \sum_{\substack{I\subseteq\{1,\dots,n\}\\|I|=c}} \frac{1}{2^n} \sum_{\mathbf{x}\in\{0,1\}^n} \chi[f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_I)]$$
$$= \sum_{\substack{I\subseteq\{1,\dots,n\}\\|I|=c}} \alpha^f_{\min(I)} = \sum_{j=1}^{n-c+1} \binom{n-j}{c-1} \alpha_j^f. \qquad \square$$

Theorem 3.7

Proof. (i) As in the proof of Theorem 3.3, the activity of any canalizing variable x_j ($j \leq k$) is

$$\alpha_j^f = \mathbb{P}(x_1 \neq a_1, \dots, x_{j-1} \neq a_{j-1})$$
$$\times\ \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_j) \mid x_1 \neq a_1, \dots, x_{j-1} \neq a_{j-1}\big)$$
$$= \frac{1}{2^{j-1}} \mathbb{P}\big(f(1-a_1, \dots, 1-a_j, x_{j+1}, \dots, x_n) \neq b_j\big).$$

Due to the canalizing nature of f, the probability can be further written as

$$\mathbb{P}\big(f(1-a_1, \dots, 1-a_j, x_{j+1}, \dots, x_n) \neq b_j\big)$$
$$= \sum_{i=j+1}^{k} \Big[\mathbb{P}\big(x_{j+1} \neq a_{j+1}, \dots, x_{i-1} \neq a_{i-1}, x_i = a_i\big)$$
$$\cdot\, \mathbb{P}\big(f(1-a_1, \dots, 1-a_{i-1}, a_i, x_{i+1}, \dots, x_n)$$
$$\neq b_j \mid x_{j+1} \neq a_{j+1}, \dots, x_{i-1} \neq a_{i-1}, x_i = a_i\big)\Big]$$
$$+\ \mathbb{P}\big(x_{j+1} \neq a_{j+1}, \dots, x_k \neq a_k\big)$$
$$\times\ \mathbb{P}\big(f(1-a_1, \dots, 1-a_k, x_{k+1}, \dots, x_n)$$
$$\neq b_j \mid x_{j+1} \neq a_{j+1}, \dots, x_k \neq a_k\big)$$
$$= \sum_{i=j+1}^{k} \frac{1}{2^{i-j}} \chi(b_i \neq b_j) + \frac{1}{2^{k-j}} \mathbb{P}\big(g(x_{k+1}, \dots, x_n) \neq b_j\big).$$

Let $L := L(x_j)$ and $L(x_i)$ be the layers of the variables x_j and x_i, resp., $i \leq k$. The canalized output is equal for all variables of the same layer and alternates among layers. Therefore, $b_i \neq b_j$ if and only if $L(x_i) - L(x_j)$ is odd, and the first sum can be rewritten as a sum over all k_{L+2t-1} variables of every second layer $L + 2t - 1 > L$ ($t \geq 1$),

$$\sum_{i=j+1}^{k} \frac{1}{2^{i-j}} \chi(b_i \neq b_j) = \sum_{t=1}^{\lceil (r-L)/2 \rceil} \sum_{s=1}^{k_{L+2t-1}} \left(\frac{1}{2}\right)^{s-j+k_1+\dots+k_{L+2t-2}}.$$

Also, since g contains v entries $\neq b_k$ in its truth table,

$$\psi_l := \mathbb{P}\left(g(x_{k+1}, \dots, x_n) \neq b_j\right)$$
$$= \begin{cases} \mathbb{P}\left(g(x_{k+1}, \dots, x_n) \neq b_k\right), & \text{if } r-l \text{ is even;} \\ \mathbb{P}\left(g(x_{k+1}, \dots, x_n) = b_k\right), & \text{if } r-l \text{ is odd;} \end{cases}$$
$$= \begin{cases} \dfrac{v}{2^{n-k}}, & \text{if } r-l \text{ is even;} \\ 1 - \dfrac{v}{2^{n-k}}, & \text{if } r-l \text{ is odd.} \end{cases}$$

Altogether,

$$\alpha_j^f = \frac{1}{2^{j-1}} \left[\sum_{t=1}^{\lceil (r-L)/2 \rceil} \sum_{s=1}^{k_{L+2t-1}} \left(\frac{1}{2}\right)^{s-j+k_1+\dots+k_{L+2t-2}} + \frac{1}{2^{k-j}} \psi_l \right]$$
$$= \underbrace{\sum_{t=1}^{\lceil (r-L)/2 \rceil} \sum_{s=0}^{k_{L+2t-1}-1} \left(\frac{1}{2}\right)^{s+k_1+\dots+k_{L+2t-2}}}_{:=\varphi_l} + \frac{1}{2^{k-1}} \psi_l.$$

By induction, φ_l and ψ_l can be calculated recursively with

$$\varphi_{r+1} = \varphi_r = 0, \qquad \varphi_i = \varphi_{i+2} + \sum_{s=0}^{k_{i+1}-1} \left(\frac{1}{2}\right)^{s+k_1+\dots+k_i}$$
for $i = r-1, \dots, 1$,
$$\psi_r = \frac{v}{2^{n-k}}, \qquad \psi_i = 1 - \psi_{i+1} \text{ for } i = r-1, \dots, 1.$$

(ii) On the other hand, the activity of any non-canalizing variable x_j ($j > k$) is simply

$$\alpha_j^f = \mathbb{P}(x_1 \neq a_1, \dots, x_k \neq a_k)$$
$$\times\ \mathbb{P}\big(f(\mathbf{x}) \neq f(\mathbf{x}\oplus e_j) \mid x_1 \neq a_1, \dots, x_k \neq a_k\big)$$
$$= \frac{1}{2^k} \mathbb{P}\big(g(x_{k+1}, \dots, x_n) \neq g(x_{k+1}, \dots, x_{j-1}, 1-x_j, x_{j+1}, \dots, x_n)\big)$$
$$= \frac{1}{2^k} \frac{v(2^{n-k} - v)}{\binom{2^{n-k}}{2}}$$
$$= \frac{v(2^{n-k} - v)}{2^{n-1}(2^{n-k} - 1)}. \qquad \square$$

Theorem 5.2

Proof. Let $\mathbf{x}, \mathbf{y} \in \{0,1\}^N$ be randomly chosen m-Hamming neighbors. As in the proof of Theorem 4.3, let $J(f_i) = I(f_i) \cap \{j \mid x_j \neq y_j\}$ be the restriction of $I(f_i)$ to those indices where $\mathbf{x}$ and $\mathbf{y}$ differ, $i = 1, \dots, N$. For each node $i \in \{1, \dots, N\}$, define three events

$$A_i = \{x_i \neq y_i\},$$
$$B_i = \left\{f_i(\mathbf{x}) \neq f_i(\mathbf{y}) \text{ before applying } p_i^{\uparrow}, p_i^{\downarrow}\right\},$$
$$C_i = \left\{f_i(\mathbf{x}) \neq f_i(\mathbf{y}) \text{ after applying } p_i^{\uparrow}, p_i^{\downarrow}\right\}.$$

- Since $\mathbf{x}$ and $\mathbf{y}$ differ in m of N bits, $\mathbb{P}(A_i) = \frac{m}{N}$.
- $\mathbb{P}(B_i|c) := \mathbb{P}\big(B_i \big| |J(f_i)| = c\big)$ is simply the normalized average c-sensitivity of f_i, i.e., $\mathbb{P}(B_i|c) = s_c^{f_i}$.
- Lastly, the probability that $\mathbf{x}$ and $\mathbf{y}$ differ after applying the propensity probabilities needs to be calculated. This probability depends on both A_i and B_i. If $x_i \neq y_i$ and $f_i(\mathbf{x}) \neq f_i(\mathbf{y})$ before applying $p_i^{\uparrow}, p_i^{\downarrow}$, we can assume that $x_i = 0, y_i = 1$. Then, either $f_i(\mathbf{x}) = 0, f_i(\mathbf{y}) = 1$ or $f_i(\mathbf{x}) = 1, f_i(\mathbf{y}) = 0$, both with probability $\frac{1}{2}$. In the first case, there is no change in values so that the propensity probabilities $p_i^{\uparrow}, p_i^{\downarrow}$ play no role and $f_i(\mathbf{x}) \neq f_i(\mathbf{y})$ after applying $p_i^{\uparrow}, p_i^{\downarrow}$ with probability 1. In the second case, $f_i(\mathbf{x})$ and $f_i(\mathbf{y})$ only differ after applying $p_i^{\uparrow}, p_i^{\downarrow}$, if either both updates happen (probability $p_i^{\uparrow} p_i^{\downarrow}$) or neither update happens (probability $(1-p_i^{\uparrow})(1-p_i^{\downarrow})$). That means,

$$\gamma_1 := \mathbb{P}(C_i|A_i, B_i) = \frac{1}{2} \cdot 1 + \frac{1}{2}\left(p_i^{\uparrow} p_i^{\downarrow} + \left(1-p_i^{\uparrow}\right)\left(1-p_i^{\downarrow}\right)\right)$$
$$= 1 - \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right) + p_i^{\uparrow} p_i^{\downarrow}.$$

Similarly, we can derive

$$\gamma_2 := \mathbb{P}(C_i|A_i, \neg B_i) = 1 - \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right),$$
$$\gamma_3 := \mathbb{P}(C_i|\neg A_i, B_i) = \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right),$$
$$\gamma_4 := \mathbb{P}(C_i|\neg A_i, \neg B_i) = \frac{1}{2}\left(p_i^{\uparrow} + p_i^{\downarrow}\right) - \frac{1}{2}\left(p_i^{\uparrow} p_i^{\uparrow} + p_i^{\downarrow} p_i^{\downarrow}\right).$$

Since the exact update functions of F are known, we need to distinguish two cases in the calculation of $\mathbb{P}(C_i)$.

Case 1, $i \in I(f_i)$: If node i is self-regulatory, its update depends only on $n_i - 1$ other inputs. Moreover, if A_i is true, $\mathbf{x}$ and $\mathbf{y}$ differ in at least one of the essential variables of f_i, i.e., $|J(f_i)| \geq 1$. Due to the

same arguments as in Theorem 4.3, $|J(f_i)|$ follows a hypergeometric distribution, and we have

$$\mathbb{P}(|J(f_i)| = c+1 \mid A_i) = H_{N-1,m-1,n_i-1}(c),$$
$$\mathbb{P}(|J(f_i)| = c \mid \neg A_i) = H_{N-1,m,n_i-1}(c).$$

For these reasons,

$$\begin{aligned}\mathbb{P}(C_i) = &\sum_{c=0}^{m-1} \mathbb{P}(|J(f_i)| = c+1 \mid A_i)\Big(\mathbb{P}(C_i|A_i, B_i)\mathbb{P}(B_i|c+1)\mathbb{P}(A_i)\\ &+ \mathbb{P}(C_i|A_i, \neg B_i)\mathbb{P}(\neg B_i|c+1)\mathbb{P}(A_i)\Big)\\ &+ \sum_{c=0}^{m} \mathbb{P}(|J(f_i)| = c \mid \neg A_i)\Big(\mathbb{P}(C_i|\neg A_i, B_i)\mathbb{P}(B_i|c)\mathbb{P}(\neg A_i)\\ &+ \mathbb{P}(C_i|\neg A_i, \neg B_i)\mathbb{P}(\neg B_i|c)\mathbb{P}(\neg A_i)\Big)\\ = &\sum_{c=0}^{m-1} \frac{m}{N} H_{N-1,m-1,n_i-1}(c)\left[\gamma_1 s_{c+1}^{f_i} + \gamma_2\left(1 - s_{c+1}^{f_i}\right)\right]\\ &+ \sum_{c=0}^{m} \frac{N-m}{N} H_{N-1,m,n_i-1}(c)\left[\gamma_3 s_c^{f_i} + \gamma_4\left(1 - s_c^{f_i}\right)\right].\end{aligned}$$

Case 2, $i \notin I(f_i)$: If node i does not regulate its own update, the n_i inputs of f_i are among the remaining $N-1$ nodes. Similar to Case 1, we have

$$\mathbb{P}(|J(f_i)| = c \mid A_i) = H_{N-1,m-1,n_i}(c),$$
$$\mathbb{P}(|J(f_i)| = c \mid \neg A_i) = H_{N-1,m,n_i}(c),$$

and

$$\begin{aligned}\mathbb{P}(C_i) = &\sum_{c=0}^{m} \mathbb{P}(|J(f_i)| = c \mid A_i)\Big(\mathbb{P}(C_i|A_i, B_i)\mathbb{P}(B_i|c)\mathbb{P}(A_i)\\ &+ \mathbb{P}(C_i|A_i, \neg B_i)\mathbb{P}(\neg B_i|c)\mathbb{P}(A_i)\Big)\\ &+ \sum_{c=0}^{m} \mathbb{P}(|J(f_i)| = c \mid \neg A_i)\Big(\mathbb{P}(C_i|\neg A_i, B_i)\mathbb{P}(B_i|c)\mathbb{P}(\neg A_i)\\ &+ \mathbb{P}(C_i|\neg A_i, \neg B_i)\mathbb{P}(\neg B_i|c)\mathbb{P}(\neg A_i)\Big)\\ = &\sum_{c=0}^{m} \frac{m}{N} H_{N-1,m-1,n_i}(c)\left[\gamma_1 s_c^{f_i} + \gamma_2\left(1 - s_c^{f_i}\right)\right]\\ &+ \sum_{c=0}^{m} \frac{N-m}{N} H_{N-1,m,n_i}(c)\left[\gamma_3 s_c^{f_i} + \gamma_4\left(1 - s_c^{f_i}\right)\right].\end{aligned}$$

Since $H_{N,m,n}(c) = 0$ if $c \notin \{\max(m+n-N, 0), \ldots, \min(m, n)\}$, we can use the same summation limits for all cases. Thus,

$$\begin{aligned}D(F, m) &= \sum_{i=1}^{N} \mathbb{P}(C_i)\\ &= \sum_{i=1}^{N}\sum_{c=0}^{m}\begin{cases}\frac{m}{N} H_{N-1,m-1,n_i-1}(c)\left[\gamma_1 s_{c+1}^{f_i} + \gamma_2\left(1 - s_{c+1}^{f_i}\right)\right]\\ \quad + \frac{N-m}{N} H_{N-1,m,n_i-1}(c)\left[\gamma_3 s_c^{f_i} + \gamma_4\left(1 - s_c^{f_i}\right)\right]\\ \quad \text{if } i \in |I(f_i)|\\ \frac{m}{N} H_{N-1,m-1,n_i}(c)\left[\gamma_1 s_c^{f_i} + \gamma_2\left(1 - s_c^{f_i}\right)\right]\\ \quad + \frac{N-m}{N} H_{N-1,m,n_i}(c)\left[\gamma_3 s_c^{f_i} + \gamma_4\left(1 - s_c^{f_i}\right)\right]\\ \quad \text{if } i \notin |I(f_i)|. \quad \square\end{cases}\end{aligned}$$

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