The number and probability of canalizing functions

Winfried Just

corresponding author; just@math.ohiou.edu, phone: (740)-593-1260, fax: (740)-593-9805, Department of Mathematics, Ohio University, Athens, Ohio 45701, U.S.A.

Ilya Shmulevich

is@ieee.org, Cancer Genomics Laboratory, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, U.S.A.

John Konvalina

johnkon@unomaha.edu, Department of Mathematics University of Nebraska at Omaha Omaha, NE 68182-0243, U.S.A.

Abstract

Canalizing functions have important applications in physics and biology. For example, they represent a mechanism capable of stabilizing chaotic behavior in Boolean network models of discrete dynamical systems. When comparing the class of canalizing functions to other classes of functions with respect to their evolutionary plausibility as emergent control rules in genetic regulatory systems, it is informative to know the number of canalizing functions with a given number of input variables. This is also important in the context of using the class of canalizing functions as a constraint during the inference of genetic networks from gene expression data. To this end, we derive an exact formula for the number of canalizing Boolean functions of \( n \) variables. We also derive a formula for the probability that a random Boolean function is canalizing for any given bias \( p \) of taking the value 1. In addition, we consider the number and probability of Boolean functions that are canalizing for exactly \( k \) variables. Finally, we provide an algorithm for randomly generating canalizing functions with a given bias \( p \) and any number of variables, which is needed for Monte Carlo simulations of Boolean networks.

Key words: canalizing function, forcing function, Boolean network

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1 Introduction

A Boolean function (on \( n \) variables) is a function \( f : \{0,1\}^n \rightarrow \{0,1\} \). A canalizing function (also called a forcing function) is a type of Boolean function in which at least one of the input variables is able to determine the function output regardless of the values of the other variables. For example, the function \( f(x_1, x_2, x_3) = x_1 + x_2 x_3 \), where the addition symbol stands for disjunction and the multiplication for conjunction, is a canalizing function, since setting \( x_1 \) to 1 guarantees that the function value is 1 regardless of the value of \( x_2 \) or \( x_3 \). On the other hand, the function \( f(x_1, x_2) = x_1 \oplus x_2 \), where \( \oplus \) is addition modulo 2, is not a canalizing function, since the values of both variables always need to be known in order to determine the function output.

Canalizing functions have been implicated in a number of phenomena related to discrete dynamical systems as well as nonlinear filters. Concerning the latter, they have been used to study the convergence behavior of an important class of nonlinear digital filters called stack filters [1,2,3]. For example, stack filters defined by canalizing functions are known to possess a convergence property whereby a filter is guaranteed to converge to a so-called root signal or fixed point of the filter after a finite number of passes [2]. In [4], some learning schemes were proposed to find minimal filters defined by canalizing functions.

Canalizing functions also play an important role in the study of phase transitions in random Boolean networks [5,6,7,8,9]. Boolean networks have been one of the most intensively studied models of discrete dynamical systems and have been used to gain insight into the behavior of large genetic networks [5], evolutionary principles [10,11], and the development of chaos [12,13]. Although structurally simple, these systems are capable of displaying a remarkably rich variety of complex behavior. Canalizing functions represent one of the few known mechanisms capable of preventing chaotic behavior in Boolean networks [5]. By increasing the percentage of canalizing functions in a Boolean network, one can move closer toward the ordered regime and, depending on the connectivity and the distribution of the number of canalizing variables, cross the phase transition boundary [14]. In fact, there is overwhelming evidence that canalizing functions are abundantly utilized in higher vertebrate gene regulatory systems [5]. A recent large-scale study of the literature on transcriptional regulation in eukaryotes demonstrated an overwhelming bias towards canalizing rules [15]. Canalization is also a natural mechanism for designing robustness against noise [16].

Knowledge of the number of possible canalizing functions with a given number of input variables is important for determining the degree to which these functions are evolutionarily plausible as regulatory rules in genetic networks. There are two related issues here. First, a class of functions that is overly
limited in size is unlikely to emerge via the mechanism of random selection. Thus, when comparing different classes of functions vis-à-vis their likelihood of giving rise to regulatory control rules, it is informative to know their respective sizes [9]. Second, when gene regulatory rules are inferred from real gene expression measurements [17], it is often beneficial to constrain the inferential algorithms to a certain class of functions that can be produced. It may seem that imposing a constraint (e.g., restricting all functions to be canalizing) can only result in a degradation of the performance of the algorithm, thus yielding a larger estimation or prediction error relative to an algorithm with no imposed constraints. But it turns out that doing so can often improve the tractability and precision of the inference. This can be particularly noticeable when an inference is made from small sample sizes. In order to quantify the reduction in ‘design cost’ owing to the constraint, it is again informative to consider the size of the class of functions used as a constraint. Thus, it is an important goal to establish the number of canalizing functions of a given number of input variables.

Of course, one approach is to generate all Boolean functions with $n$ variables and check whether each one is canalizing. However, despite efficient methods to test the canalizing property [18], this approach becomes prohibitive for large values of $n$ and the exact number has only been known for $n \leq 5$ [9]. It has also been known that the number of canalizing functions with $n$ variables is upper bounded by $4n \cdot 2^{2^n-1}$ [19].

In this paper, we derive an exact formula for the number of canalizing functions with $n$ variables. In addition, we also derive a formula for the probability that a random Boolean function whose truth table is a Bernoulli($p$) random vector is canalizing. The latter is important because the ‘bias’ $p$ of Boolean functions also plays a crucial role in the order-disorder transition in Boolean networks and it is known that canalizing functions are likely to be biased, meaning that they are expected to have a large number of ones or zeros in their truth tables [8,9]. Since a canalizing function can have one or more canalizing variables, we also consider the number and probability of Boolean functions that are canalizing for exactly $k$ variables. This is also a relevant issue because it is known that tuning the number of canalizing inputs in a random Boolean network can dramatically affect its dynamical behavior. Moreover, according to the formulas derived in our paper, real genetic regulatory rules appear to be highly skewed towards large numbers of canalizing inputs [15] relative to what should be expected by chance in a canalizing function.
2 The probability of canalizing functions

Throughout this paper, let \( n \geq 1 \) be a fixed positive integer. For each positive integer \( k \), the set \( \{0, 1, \ldots, k - 1\} \) will be denoted by \([k]\). The cardinality of a set \( A \) will be denoted by \(|A|\). Let \( 0 \leq p \leq 1 \). We will consider the following probability measure on the space of all Boolean functions:

\[
Pr_p(f) = p^{|f^{-1}(1)|}(1 - p)^{|f^{-1}(0)|}.
\]

We call \( Pr_p(f) \) the probability of \( f \) for bias \( p \).

Recall that a Boolean function \( f \) is canalizing if there exist \( i \in n \) (called a canalizing variable) and \( s, v \in \{0, 1\} \) such that:

\[
\forall x \in \{0, 1\}^n (x_i = s \Rightarrow f(x_i) = v). \tag{1}
\]

If \( v = 1 \), then we will say that \( f \) is positively canalizing; if \( v = 0 \), then we will say that \( f \) is negatively canalizing.

Let \( C \) be the set of all canalizing Boolean functions; let \( PC \) be the set of all positively canalizing Boolean functions, let \( NC \) be the set of all negatively canalizing Boolean functions, and let \( BC \) be the set of Boolean functions that are both positively and negatively canalizing.

Our goal in this section is to calculate \( Pr_p(C) \). It is clear that

\[
Pr_p(C) = Pr_p(PC) + Pr_p(NC) - Pr_p(BC). \tag{2}
\]

Let us first dispose of the easy part and calculate \( Pr_p(BC) \). Note that it cannot be the case that a Boolean function \( f \) is positively canalizing for a canalizing variable \( x_i \) and negatively canalizing for a different canalizing variable \( x_j \neq x_i \).

Thus for every \( f \in BC \) there exists a unique canalizing variable \( x_i(f) \), and we either have

\[
\forall x \in \{0, 1\}^n (x_i = 0 \Rightarrow f(x_i) = 0) \& (x_i = 1 \Rightarrow f(x_i) = 1),
\]
or we have

\[
\forall x \in \{0, 1\}^n (x_i = 0 \Rightarrow f(x_i) = 1) \& (x_i = 1 \Rightarrow f(x_i) = 0).
\]

Thus \(|BC| = 2n\); and for every function \( f \in BC \) we have \( Pr_p(f) = p^{2n-1}(1 - \)
It follows that

$$Pr_p(BC) = 2np^{2n-1}(1-p)^{2n-1}. \quad (3)$$

In our calculations of $Pr_p(PC)$ and $Pr_p(NC)$ it will be convenient to work only with nonconstant canalizing functions. Let $PC^- = PC \setminus \{1\}$ and $NC^- = NC \setminus \{0\}$, where 1, 0 are the Boolean functions that take the value 1 respectively 0 everywhere. In this terminology, equation (2) is equivalent to:

$$Pr_p(C) = Pr_p(PC^-) + Pr_p(NC^-) + p^{2n} + (1-p)^{2n} - 2np^{2n-1}(1-p)^{2n-1}. \quad (4)$$

Now we need to be a little more specific about the number of variables for which a function is canalizing.

**Definition 1** Let $f : \{0,1\}^n \to \{0,1\}$ and let $I$ be a nonempty subset of $[n]$. We say that $f$ is positively canalizing on $I$ if there exists a function $\sigma : I \to \{0,1\}$ called a signature of $f$ on $I$ such that

$$\forall x \in \{0,1\}^n \ ((\exists i \in I x_i \neq \sigma(i)) \Rightarrow f(x_i) = 1). \quad (5)$$

The notion of being negatively canalizing on $I$ is defined analogously. The set of all nonconstant Boolean functions that are positively canalizing on a given index set $I$ will be denoted by $PC_I^-$; the set of all nonconstant Boolean functions that are negatively canalizing on a given index set $I$ will be denoted by $NC_I^-$. 

**Fact 1** Let $f \in PC_I^-$ or $f \in NC_I^-$. Then there exists exactly one signature for $f$ on $I$.

**Proof.** Without loss of generality suppose $f \in PC_I^-$, and assume towards a contradiction that $\sigma, \tau : I \to \{0,1\}$ are two different signatures for $f$. Let $i \in I$ be such that $\sigma(i) \neq \tau(i)$. Then for every $x \in \{0,1\}^n$ we have $x_i \neq \sigma(i)$ or $x_i = \tau(i)$, and it follows from equation (5) that $f(x) = 1$. Thus $f = 1$, which contradicts the assumption that $f \in PC_I^-$. ■

If $f \in PC_I^-$ or $f \in NC_I^-$, then we let $\sigma_{I,f}$ denote the unique signature of $f$ on $I$.

**Lemma 1** Let $I$ be a nonempty subset of $[n]$. Then $PC_I^- = \bigcap_{i \in I} PC_{\{i\}}^-$ and $NC_I^- = \bigcap_{i \in I} NC_{\{i\}}^-$. 

**Proof.** Suppose $f \in PC_I^-$ and $i \in I$. It is easy to see that the restriction of $\sigma_{I,f}$ to $\{i\}$ is a signature for $f$ on $\{i\}$, and thus $f \in PC_{\{i\}}^-$. Now suppose
\( f \in \text{PC}_{\{i\}}^- \) for all \( i \in I \). Let \( \sigma = \bigcup \{ \sigma_{\{i\}, f} : i \in I \} \). Then \( \sigma \) is a signature for \( f \) on \( I \), and it follows that \( f \in \text{PC}_I^- \).

The proof of the second equation is analogous. \( \blacksquare \)

It follows from the definition of canalizing functions that

\[
\text{PC}^- = \bigcup_{i<n} \text{PC}_{\{i\}}^-, \quad \text{NC}^- = \bigcup_{i<n} \text{NC}_{\{i\}}^- \tag{6}
\]

Unfortunately, the sets \( \text{PC}_{\{i\}}^- \) are not pairwise disjoint. So we have to use the Inclusion-Exclusion Principle to calculate the probability of the union of these sets. This gives:

\[
\Pr_p(\text{PC}^-) = \sum_{0 \leq i < n} \Pr_p(\text{PC}_{\{i\}}^-) - \sum_{0 \leq i_1 < i_2 < n} \Pr_p(\text{PC}_{\{i_1\}}^- \cap \text{PC}_{\{i_2\}}^-) + \ldots \\
+ (-1)^{k+1} \sum_{0 \leq i_1 < i_2 < \ldots < i_k < n} \Pr_p(\text{PC}_{\{i_1\}}^- \cap \text{PC}_{\{i_2\}}^- \cap \ldots \cap \text{PC}_{\{i_k\}}^-) + \ldots \tag{7}
\]

By Lemma 1, equation (7) can be written as:

\[
\Pr_p(\text{PC}^-) = \sum_{k=1}^{n} (-1)^{k+1} \sum_{0 \leq i_1 < i_2 < \ldots < i_k < n} \Pr_p(\text{PC}_{\{i_1, i_2, \ldots, i_k\}}^-). \tag{8}
\]

Since for \( |I| = |J| \) we obviously have \( \Pr_p(\text{PC}_I^-) = \Pr_p(\text{PC}_J^-) \), we can rewrite equation (8) as follows:

\[
\Pr_p(\text{PC}^-) = \sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} \Pr_p(\text{PC}_{\{k\}}^-). \tag{9}
\]

The analogous reasoning shows that

\[
\Pr_p(\text{NC}^-) = \sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} \Pr_p(\text{NC}_{\{k\}}^-). \tag{10}
\]

Now it remains to compute \( \Pr_p(\text{PC}_{\{k\}}^-) \) and \( \Pr_p(\text{NC}_{\{k\}}^-) \).

**Lemma 2** Let \( 1 \leq k < n \). Then

\[
\Pr_p(\text{PC}_{\{k\}}^-) = 2^k (p^{2^n-2^{n-k}} - p^n). \tag{11}
\]
\[ Pr_p(\text{NC}_{[k]}) = 2^k((1 - p)^{2^n-2^n-k} - (1 - p)^{2^n}). \]  
(12)

\textbf{Proof.} We prove equation (11); the proof of equation (12) is analogous. By Fact 1 we have

\[ Pr_p(\text{PC}_{[k]}) = \sum_{\sigma \in \{0,1\}^n} Pr_p(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma). \]  
(13)

It is clear that for any \(\sigma, \tau : [k] \rightarrow \{0,1\}\) we have \(Pr_p(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma) = Pr_p(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \tau)\). Pick an arbitrary \(\sigma^* : [k] \rightarrow \{0,1\}\).

Equation (13) now implies:

\[ Pr_p(\text{PC}_{[k]}) = 2^k Pr_p(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma^*). \]  
(14)

Let us calculate \(Pr_p(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma^*)\). If \(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma^*\), then \(f(x) = 1\) whenever the restriction of \(x\) to the first \(k\) variables is not equal to \(\sigma^*\). So there are \(2^{n-k}\) arguments \(x\) of \(f\) on which \(f\) can take arbitrary values (except taking value 1 everywhere), and \(2^n - 2^{n-k}\) arguments \(x\) where \(f\) has to take value 1. In other words,

\[ Pr_p((f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma^*) \land f = 1) = p^{2^n-2^{n-k}}. \]  
(15)

Since \(Pr_p(1) = p^{2^n}\), equation (15) implies

\[ Pr_p(f \in \text{PC}_{[k]} \land \sigma_{[k],f} = \sigma^*) = p^{2^n-2^{n-k}} - p^{2^n}. \]  
(16)

This in turn implies equation (11). \(\blacksquare\)

Now let us put all our formulas together. We get:

\[ Pr_p(C) = p^{2^n} + (1 - p)^{2^n} - 2np^{2^{n-1}}(1 - p)^{2^{n-1}} + \]
\[ \sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} 2^k(p^{2^n-2^{n-k}} + (1 - p)^{2^n-2^{n-k}} - p^{2^n} - (1 - p)^{2^n}). \]  
(17)
Note that

\[
\sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} 2^{k} (-p^{2n} - (1-p)^{2n}) =
\]

\[
(p^{2n} + (1-p)^{2n}) \sum_{k=1}^{n} \binom{n}{k} (-2)^{k} =
\]

\[
(p^{2n} + (1-p)^{2n})((1-2)^{n} - 1) = (p^{2n} + (1-p)^{2n})((-1)^{n} - 1).
\]

Thus equation (17) simplifies to:

\[
Pr_{p}(C) = (-1)^{n}(p^{2n} + (1-p)^{2n}) - 2np^{2n-1}(1-p)^{2n-1} +
\]

\[
\sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} 2^{k}(p^{2n-2^{n-k}} + (1-p)^{2n-2^{n-k}}).
\]

\[\text{(19)}\]

3 The number of canalizing functions

Equation (19) allows us to derive a formula for the number of canalizing functions as follows. Set \( p = 0.5 \). Then all functions have equal probability, and we simply can compute:

\[
|C| = Pr_{0.5}(C)2^{2n} =
\]

\[
2^{2n} 2((-1)^{n} - n)2^{-2^{n}} + \sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} 2^{k} 2^{-2^{n}+2^{n-k}} =
\]

\[
2((-1)^{n} - n) + \sum_{k=1}^{n} (-1)^{k+1} \binom{n}{k} 2^{k+1} 2^{n-k}.
\]

\[\text{(20)}\]

The values of \(|C|\) for \( n = 1, \ldots, 10 \) are shown in Table 1.

It is interesting to note that for large \( n \) the value of \(|C|\) given by equation (20) asymptotically approaches the upper bound of \( 4n \cdot 2^{2n-1} \) given in [19]. To see this, let \( S_{k} = \binom{n}{k} 2^{k+1} 2^{n-k} \) for \( 1 \leq k \leq n \). Then

\[
|C| = 2((-1)^{n} - n) + \sum_{k=1}^{n} (-1)^{k+1} S_{k}.
\]

\[\text{(21)}\]

For sufficiently large \( n \), the first term becomes negligible, and we can concentrate on the asymptotic behavior of \( S = \sum_{k=1}^{n} (-1)^{k+1} S_{k} \). Moreover, it is not hard to see that \( S_{k} > S_{k+1} \) for all \( k < n \). Thus the partial sums of \( S \) with an odd number of terms form an upper bound for \( S \), while the partial sums with
an even number of terms form a lower bound. In particular, for the first and second partial sums we have the inequalities:

\[ S_1 - S_2 \leq S \leq S_1. \]  \hspace{1cm} (22)

Dividing by \( S_1 \) we obtain

\[ 1 - \frac{S_2}{S_1} \leq \frac{S}{S_1} \leq 1. \]  \hspace{1cm} (23)

As \( n \) approaches infinity, \( \frac{S_2}{S_1} \) approaches zero, and therefore, \( S \) is asymptotic to \( S_1 \). Now it suffices to note that \( S_1 = \binom{n}{1}2^{1+1}2^{n-1} = 4n \cdot 2^{2n-1} \) is exactly the upper bound given in [19].

4 Functions that are canalizing for \( k \) variables

By definition, a Boolean function is canalizing if and only if it is canalizing for at least one variable. How can we compute the number and probability of Boolean functions that are canalizing for exactly \( k \) variables? To solve this problem, we need a generalization of the Inclusion-Exclusion Principle. The following lemma appears as Corollary 5B.4 in [20].

**Lemma 3** Let \( f_0, \ldots, f_{n-1} \) be real-valued functions with a common domain, and let \( u \) be the function that is identically 1 on the common domain of the \( f_i \)'s. Let \( I \subseteq [n] \), and let \( I^c = [n]\setminus I \). Then

\[ \prod_{i \in I} f_i \prod_{i \in I^c} (u - f_i) = \sum_{R \supseteq I} (-1)^{|R|-|I|} \prod_{i \in R} f_i. \]  \hspace{1cm} (24)

Now suppose that \( E_0, \ldots, E_{n-1} \) are events in a fixed probability space \( \Omega \), and that \( f_i \) is the characteristic function of \( E_i \) on \( \Omega \) for \( i = 0, \ldots, n - 1 \). Then we have for \( I \subseteq [n] \):

\[ Pr(\prod_{i \in I} f_i = 1) = Pr(\bigcap_{i \in I} E_i), \]

\[ Pr(\prod_{i \in I} f_i \prod_{i \in I^c} (u - f_i) = 1) = Pr(\bigcap_{i \in I} E_i \cap \bigcap_{i \in I^c} E_i^c), \]

and equation (24) translates into:

\[ Pr(\bigcap_{i \in I} E_i \cap \bigcap_{i \in I^c} E_i^c) = \sum_{R \supseteq I} (-1)^{|R|-|I|} Pr(\bigcap_{i \in R} E_i). \]  \hspace{1cm} (25)
Now let $E_0, \ldots, E_{n-1}$ and $I$ be as above. Following Definition 5B.5 of [20] we define:

$$IN(I) = \{ \omega \in \Omega : \omega \in E_i \Leftrightarrow i \in I \},$$

and for $k \leq n$:

$$IN(k) = \bigcup_{|I|=k} IN(I).$$

The following lemma is a straightforward generalization of Corollary 5B.6 of [20]:

**Lemma 4** In the terminology introduced above we have:

$$Pr(IN(I)) = \sum_{R \supseteq I} (-1)^{|R|-|I|} Pr(\bigcap_{i \in R} E_i). \quad (26)$$

$$Pr((IN(k)) = \sum_{r=k}^{n} \binom{r}{k} (-1)^{r-k} \sum_{R \subseteq [n], |R|=r} Pr(\bigcap_{i \in R} E_i). \quad (27)$$

Let us apply equation (27) to the situation where $\Omega$ is the space of all Boolean functions of $n$ variables with probability function $Pr_p$ defined above. For $1 \leq k \leq n$, let $Pr_p(PCE_k)$ denote the probability that a randomly chosen Boolean function with bias $p$ is positively (but not negatively) canalizing on exactly $k$ variables, and let $Pr_p(NCE_k)$ denote the probability that a randomly chosen Boolean function with bias $p$ is negatively (but not positively) canalizing on exactly $k$ variables. We will compute $Pr_p(PCE_k)$. For $i < n$, let $E_i = PC_{\{i\}}$. Note that for this choice of $E_i$ and $k < n$, $IN(k)$ is the set of functions that are positively canalizing for exactly $k$ variables; and the set of functions that are positively canalizing for $n$ variables is $IN(n) \cup \{1\}$. In view of Lemma 1, equation (27) now boils down to the following:

$$Pr_p((IN(k)) = \sum_{r=k}^{n} \binom{r}{k} (-1)^{r-k} \sum_{R \subseteq [n], |R|=r} Pr_p(PC^{-}_R). \quad (28)$$

Since the probability of $PC^{-}_R$ depends only on $|R|$, we get

$$Pr_p((IN(k)) = \sum_{r=k}^{n} \binom{r}{k} (-1)^{r-k} \binom{n}{r} Pr_p(PC^{-}_{[r]}). \quad (29)$$
It follows from Lemma 2 that for $k > 1$ we have:

$$Pr_p(IN(k)) = \sum_{r=k}^{n} \binom{n}{r} (-1)^{r-k} \binom{n}{r} Pr_p(PC^+_r) = \sum_{r=k}^{n} \binom{n}{r} (-1)^{r-k} \binom{n}{r} 2^r (p^{2^n-2^n-r} - p^{2^n}).$$  \hspace{1cm} (30)

Thus it follows that for $1 < k < n$ we have:

$$Pr_p(PCE_k) = \sum_{r=k}^{n} \binom{n}{r} (-1)^{r-k} \binom{n}{r} 2^r (p^{2^n-2^n-r} - p^{2^n}).$$  \hspace{1cm} (31)

A similar argument shows that for $1 < k < n$ we have:

$$Pr_p(NCE_k) = \sum_{r=k}^{n} \binom{n}{r} (-1)^{r-k} \binom{n}{r} 2^r ((1 - p)^{2^n-2^n-r} - (1 - p)^{2^n}).$$  \hspace{1cm} (32)

For $1 < k = n$ we need to add the two constant functions:

$$Pr_p(PCE_n) = 2^n (p^{2^n-1} - p^{2^n}) + p^{2^n}. \hspace{1cm} (33)$$

$$Pr_p(NCE_n) = 2^n ((1 - p)^{2^n-1} - p^{2^n}) + (1 - p)^{2^n}. \hspace{1cm} (34)$$

For $1 = k < n$ we need to subtract the probability that the function is canalizing both ways. This gives:

$$Pr_p(PCE_1) = n(Pr_p(PC^+_[1]) - 2p^{2^n-1} (1 - p)^{2^n-1}) +$$

$$\sum_{r=2}^{n} r(-1)^{r-1} \binom{n}{r} (Pr_p(PC^+_r)) + 2n(p^{2^n-2^n-1} - p^{2^n} - p^{2^{n-1}} (1 - p)^{2^{n-1}}) +$$

$$\sum_{r=2}^{n} r(-1)^{r-1} \binom{n}{r} 2^r (p^{2^n-2^n-r} - p^{2^n}) =$$

$$2n(p^{2^n-1} - p^{2^n} - p^{2^{n-1}} (1 - p)^{2^{n-1}}) +$$

$$\sum_{r=2}^{n} r(-1)^{r-1} \binom{n}{r} 2^r (p^{2^n-2^n-r} - p^{2^n}). \hspace{1cm} (35)$$

For 1 = k < n we need to subtract the probability that the function is canalizing both ways. This gives:
\[ Pr_p(NCE_1) = n(Pr_p(NC_{[1]}) - 2p^{2n-1}(1-p)^{2n-1}) + \]
\[ \sum_{r=2}^{n} r(-1)^{r-1} \binom{n}{r} (Pr_p(NC_{[r]})) = \]
\[ 2n((1-p)^{2n-2^{n-1}} - (1-p)^{2n} - p^{2n-1}(1-p)^{2n-1}) + \]
\[ \sum_{r=2}^{n} r(-1)^{r-1} \binom{n}{r} 2^r((1-p)^{2n-2^{n-r}} - (1-p)^{2n}) = \]
\[ 2n((1-p)^{2n-1} - (1-p)^{2n} - p^{2n-1}(1-p)^{2n-1}) + \]
\[ \sum_{r=2}^{n} r(-1)^{r-1} \binom{n}{r} 2^r((1-p)^{2n-2^{n-r}} - (1-p)^{2n}). \]

Let \( c(k) \) denote the number of functions that are canalizing for exactly \( k \) variables. We have:

\[ c(k) = (Pr_{0.5}(PCE_k) + Pr_{0.5}(NCE_k))2^{2n} \quad \text{if } 1 < k < n, \quad (37) \]

\[ c(k) = 2 + (Pr_{0.5}(PCE_k) + Pr_{0.5}(NCE_k))2^{2n} \quad \text{if } 1 < k = n, \quad (38) \]

\[ c(k) = (Pr_{0.5}(PCE_1) + Pr_{0.5}(NCE_1) + Pr_{0.5}(BC))2^{2n} \quad \text{if } 1 = k < n, \quad (39) \]

\[ c(k) = 2 + (Pr_{0.5}(PCE_1) + Pr_{0.5}(NCE_1) + Pr_{0.5}(BC))2^{2n} \quad \text{if } 1 = k = n. \quad (40) \]

This implies the following formulas for \( c(k) \):

For \( 1 < k < n \):

\[ c(k) = \sum_{r=k}^{n} \binom{r}{k} (-1)^{r-k} \binom{n}{r} 2^{r+1}(2^{2^{n-r}} - 1). \quad (41) \]

For \( 1 < k = n \):

\[ c(k) = 2 + 2^{n+1}. \quad (42) \]
For $1 = k < n$:

$$c(1) = 2n(2^{1+2^{n-1}} - 3) + \sum_{r=2}^{n} \binom{r}{1}(-1)^{r-1}\binom{n}{r}2^{r+1}(2^{2^{n-r}} - 1).$$  \hspace{1cm} (43)

For $1 = k = n$:

$$c(1) = 2 + 2 \cdot 1(2^{1+2^{1-1}} - 3) + 0 = 4.$$ \hspace{1cm} (44)

5 Randomly generating canalizing functions

In simulating the behavior of random Boolean networks, it is important to be able to randomly generate canalizing functions with a given bias $p$ [9]. Our results in Section 4 allow us to do so by means of the following algorithm:

Recall that for $1 \leq k \leq n$, $Pr_p(PE_k)$ denotes the probability that a randomly chosen Boolean function with bias $p$ is positively (but not negatively) canalizing on exactly $k$ variables, and that $Pr_p(NCE_k)$ denotes the probability that a randomly chosen Boolean function with bias $p$ is negatively (but not positively) canalizing on exactly $k$ variables. Here is the algorithm.

**Algorithm** *CanalizingFunctionGenerator*(p)

1. Let $q = 0$ with probability $Pr_p(BC)$; for $1 \leq k \leq n$, let $q = k$ with probability $Pr_p(PE_k)+Pr_p(NCE_k)$.  
   \hspace{1cm} Pr_p(C)

2. If $q \geq 1$ then let $r = 1$ with probability $Pr_p(PE_k)$ and let $r = 0$ with probability $Pr_p(NCE_k)$.  

3. If $q == 0$ then
   - Randomly pick an input variable $x_i$.  
   - Randomly pick one of the two functions that are canalizing both ways on input $x_i$.  
   - **return** the function $f$ that was just picked.

4. Else if $r == 1$ then
   - Randomly pick a subset $S$ of $[n]$ of size $q$.  
   - Randomly pick a function $s : S \rightarrow \{0, 1\}$.  
   - For each input vector $x$ that contains some $x_i$ with $x_i = s(i)$ let
\[ f(x) = 1. \]

\textbf{repeat}

For each of the remaining input vectors \( x \) let independently and randomly \( f(x) = 1 \) with probability \( p \) and let \( f(x) = 0 \) with probability \( 1 - p \).

\textbf{until} the resulting function \( f \) is in \( PCE_q \).

\textbf{return} \( f \).

\textbf{else} // \( r == 0 \)

Randomly pick a subset \( S \) of \( [n] \) of size \( q \).

Randomly pick a function \( s : S \to \{0, 1\} \).

For each input vector \( x \) that contains some \( x_i \) with \( x_i = s(i) \) let \( f(x) = 0. \)

\textbf{repeat}

For each of the remaining input vectors \( x \) let independently and randomly \( f(x) = 1 \) with probability \( p \) and let \( f(x) = 0 \) with probability \( 1 - p \).

\textbf{until} the resulting function \( f \) is in \( NCE_q \).

\textbf{return} \( f \).

Note that the \textit{repeat} \ldots \textit{until} loops in this algorithm are necessary since when parts of the vectors \( x \) are assigned randomly, the resulting function might, by chance, become canalizing for more than \( q \) canalizing variables. Should this occur, we would need to throw the function away and generate another one.

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| $n$ | $|C|$ |
|-----|------|
| 1   | 4    |
| 2   | 14   |
| 3   | 120  |
| 4   | 3514 |
| 5   | 1292276 |
| 6   | 103071426294 |
| 7   | 516508833342349371376 |
| 8   | 10889035741470030826695916769153787968498 |
| 9   | $4.168515213 \times 10^{78}$ |
| 10  | $5.363123172 \times 10^{155}$ |

Table 1
The number of canalizing functions with $n$ input variables.