Nested canalizing functions minimize sensitivity and simultaneously promote criticality

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We prove that nested canalizing functions are the minimum-sensitivity Boolean functions for any given activity ratio and we characterize the sensitivity boundary which has a nontrivial fractal structure. We further observe, on an extensive database of regulatory functions curated from the literature, that this bound severely constrains the robustness of biological networks. Our findings suggest that the accumulation near the “edge of chaos” in these systems is a natural consequence of a drive towards maximum stability while maintaining plasticity in transcriptional activity.

Keywords: nested canalizing function, gene regulatory network, criticality, Boolean network, sensitivity, robustness

Canalizing functions are a class of Boolean functions for which a specific value of a (typically small) subset of the input variables dictates or “canalizes” the output to be 0 or 1. Waddington [1] and Schmalhausen [2] recognized the significance of the concept of canalization in biological development and evolution early on, suggesting it as a mechanism that promotes coordinated response to environmental/genetic perturbations and supresses genetic variation. Biological signatures and implications of canalization is still an active research area [3,4]. Canalizing functions entered the radar of the biophysics community due to seminal works by Kauffman et al. [5,6] who observed that they are particularly suited to describe the transcriptional states of genes subject to multiple regulatory inputs, once a binary representation of gene expression is granted. If the regulatory inputs to a gene are hierarchically organized in their capacity for dictating the gene’s expression state, we get a highly specific subclass of Boolean functions known as “nested canalizing functions” (NCFs). In addition to their relevance to biochemical networks [12], NCFs are known in the realm of computer science, under the alternate identity of “unate cascade functions”, due to their optimal properties in the context of binary decision processes [13].

We here prove that NCFs realize the minimum possible sensitivity (or maximum robustness) across all Boolean functions with a given dimension and activity, with precise definitions for “sensitivity” and “activity” given below. Despite numerous studies attesting to the improved robustness of Boolean network dynamics under the canalization rule [11,14,18], this central mathematical fact appears to have been overlooked so far. Moreover, the proven bound turns out to serve as a decisive limit on the stability of gene regulation models curated from past studies on numerous organisms.

Below, we provide some background and the relevant mathematical framework, then outline the proof. Next, we investigate its relevance to biological systems by quantifying - for more than 2000 regulatory functions in the Cell Collective database [19] - the distance from the obtained sensitivity minimum. We show that the proven bound acts as a strong constraint, with 90% of the functions realizing the minimum and the rest deviating by ≈20% from it on average. We finally reconcile our findings with the fact that these systems simultaneously reside near the order-chaos boundary [6].

Motivated by the switch-like behavior of transcriptional activity, representing the continuum of gene expression levels by two (on/off) states is a widely adopted simplification which captures many essential features of the complex gene regulation dynamics in living cells [20,21]. In this framework, one models the (discrete) time evolution of gene expression by Boolean networks, where the vertices represent genes, directed edges encode regulatory interactions, and the state of a vertex is updated at each time step by a vertex-specific Boolean function of its neighbors’ states. Abundance of canalization in these “gene regulation networks” is well established [7,9]. This can be rationalized in physical terms through the mechanisms of interaction between transcription factors and the DNA [14], or in biological terms by the evolutionary advantage it lends the organism through stabilization of the regulatory dynamics against random fluctuations [12]. In fact, a Boolean network utilizing random vertex update functions with k inputs on average and a mean probability p of outputting “1” is typically unstable for

\[ k^{-1} < 2p(1-p), \]

yielding a stability threshold of \( k = 2 \) for \( p = 1/2 \) [22,23], while a network utilizing canalizing rules is not [10,14].

A NCF \( f(\cdot) \) with \( n \) inputs \( \{s_i\} \) is a Boolean function which is canalizing in all of its inputs. It is uniquely defined in terms of a set of canalizing (input) values \( \{\sigma_i\} \) and canalized (output) values \( \{r_i\} \) where \( s_i, \sigma_i, r_i \in \{0,1\} \), \( i = 1,..,n \). An algorithmic definition for a NCF \( f(\cdot) \) is \( f(\{s_i\}) = F(\{s_i\}, 1) \) with \( F(\cdot) \) recursively defined as

\[
F(\{s_i\}, m) = \begin{cases} r_m, & \text{if } s_m = \sigma_m \\ r_{m+1}, & \text{if } m = n + 1 \\ F(\{s_i\}, m + 1), & \text{otherwise.} \end{cases}
\]

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Eq.\(\text{(2)}\) implies a full hierarchy among the inputs, here chosen as \(s_1 \succ s_2 \succ \cdots \succ s_n\) without loss of generality. The first condition above (with the notation \(\bar{r} = 1 - r\)) ensures that all inputs of \(f(\cdot)\) are relevant. In other words, \(\{s_i\}_{i \neq j}\) being the “context” of the input \(s_j\), there exists a context for all \(j\) such that \(s_j \rightarrow \bar{s}_j\) changes the output.

Note that the Hamming weight (the number of “1”s) of the truth table, henceforth referred to as the activity, for a NCF is given by \(h = (r_1 r_2 \cdots r_{n-1} 1)\) in base two and is always odd. Even values of \(h\) can be incorporated into the definition by relaxing the second condition in Eq.\(\text{(2)}\). Such a generalized NCF may have one or more irrelevant inputs, in which case it can be reduced either to a “proper” NCF of the relevant subset of its inputs or to a constant function. We introduce this generalization for not only the completeness of the following discussion, but also the fact that the Cell Collective database contains a significant number of such reducable functions\([7]\). Below, we use the term NCF in both the strict sense of Eq.\(\text{(2)}\) and the generalized sense, except when stated otherwise.

The stability—in the Lyapunov sense—of the discrete-time dynamics for a Boolean network is quantified by its “sensitivity”\([24, 25]\). The sensitivity \(\xi\) of a Boolean function to its \(j\)th input is defined as the fraction of contexts for which \(s_j \rightarrow \bar{s}_j\) flips the output. The overall sensitivity of the function \(f(\{s_i\})\) is then

\[
\xi[f] = \sum_{i=1}^{n} \xi_i = 2^{-n} \sum_{i=1}^{n} \sum_{s_j} f(\ldots, s_i, \ldots) \oplus f(\ldots, \bar{s}_i, \ldots)
\]

(3)

It is clear that, \(\xi[f]\) for a NCF is independent of the choice of canalizing inputs \(\{s_i\}\). Furthermore, the activity ratio, \(p = h/2^n\), uniquely determines the sensitivity (see below). It has been shown that the tight upper bound on \(\xi\) for a NCF is \(4/3\)\([26]\), while \(\langle\xi\rangle = n/2\) for a random Boolean function with \(n\) inputs\([27]\).

The following geometric interpretation of \(\xi[f]\) is helpful: a Boolean function with \(n\) inputs is a 2-coloring of vertices on the \(n\)-dimensional hypercube graph, \(C_n\). It follows from Eq.\(\text{(2)}\) that, a NCF has its \(C_{n-1}\) hyperface corresponding to \(s_1 = \bar{s}_1\) uniformly colored, while the opposite hyperface \((s_1 = s_1)\) conforms to the similar condition with \(n \rightarrow n - 1\) on the remaining variables, as depicted in Fig.\(\text{1}\). The choice of color for the uniform hyperface at step \(i < n\) is encoded by \(r_i\), say, \(r_i = 0 \rightarrow \text{black}\) and \(r_i = 1 \rightarrow \text{white}\). In this picture, the sensitivity in Eq.\(\text{(3)}\) becomes \(\xi[f] = b[f]/2^{n-1}\), where \(b[f]\) is the number of “boundary” edges with different terminal colors (shown in red in Fig.\(\text{1}\)). Therefore, minimizing the sensitivity subject to fixed \((n, h)\) is equivalent to finding the ground-state energy of the Ising model on \(C_n\) subject to fixed magnetization.

A lower bound on \(\xi[f]\) for a given activity \(h\) is provided by spectral graph theory: considering \(C_n\) as a graph and using a well-known result\([28]\) on the so-called “isoperimetric ratio” of a subgraph of size \(h\) yields \(\xi[f] \geq 2\lambda_2 p_f (1 - p_f)\) (see Fig.\(\text{2}\)). Here, \(p_f\) is the activity ratio of the function \(f\) and \(\lambda_2 = 2\) is the smallest nonzero eigenvalue (a.k.a., spectral graph or algebraic connectivity) of the graph Laplacian for \(C_n\). The similarity between this bound and Eq.\(\text{(1)}\) is not coincidental, since the role of \(\lambda_2\) on the stability of network dynamics is well known and has multiple applications (see, e.g., \([29, 30]\) and references therein).

Below, we outline a proof by induction for the fact that the sensitivity minimum is realized by NCFs and refer the reader to the Appendix for further details. To this end, let \(B_{n, h}\) be the set of all Boolean functions with dimension \(n\) and activity \(h\), and let \(\beta(n, h)\) be the number of boundary edges of a NCF in \(B_{n, h}\). Our objective is to prove that

\[
\beta(n, h) = \min_{f \in B_{n, h}} b[f], \forall n, h.
\]

(4)

Seed the induction with \(n = 2\): two representative NCFs with \(h = 1, 3\) are \(s_1 \land s_2\) and \(s_1 \lor s_2\), respectively, while the NCFs with \(h = 2\) are \(s_1, s_2\), and their negations. They all satisfy

\[
\beta(2, h) = 2 = \min_{f \in B_{2, h}} b[f], \text{ for } h = 1, 2, 3.
\]

(5)

\(h = 0, 4\) correspond to constant functions (which, too, are NCFs in the generalized sense) and trivially realize the minimum with \(b(2, 0) = b(2, 4) = 0\). Now let’s assume

\[
\beta(n, h) = \min_{f \in B_{n, h}} b[f], \forall n, h.
\]
that \( \min_{f \in \mathcal{B}_{n,h}} b[f] = \beta(d, h) \) is true for all \( d \in \{2, \ldots, n-1\} \) and for all \( h \in \{0, \ldots, 2^d\} \). It suffices to show that,

\[
\beta(n,h) \leq \beta(n-1,h_1) + \beta(n-1,h-h_1) + |h-2h_1| \tag{6}
\]

for all allowed \( h \) and \( h_1 \), that is, \( 0 \leq h \leq 2^n \) and \( \max(0, h - 2^{n-1}) \leq h_1 \leq \min(h, 2^{n-1}) \). In order to make sense of Inequality (6), consider the hypercube-coloring picture and imagine the following search algorithm for \( \min_{f \in \mathcal{B}_{n,h}} b[f] \): we distribute \( h \) white corners of \( C_n \) to two opposite \( C_{n-1} \) hyperfaces by \( h_1 \) and \( h-h_1 \). The number of boundary edges connecting the two hyperfaces is at least \( |h-2h_1| \). The remaining boundary edges lie within the hyperfaces and, upon minimization, add up to \( \beta(n-1,h_1) + \beta(n-1,h-h_1) \) by the induction hypothesis. Then, the inequality (6) states that no 2-coloring of \( C_n \) in \( \mathcal{B}_{n,h} \) yields boundary edges less than that of a NCF, which is the statement of Eq. (4).

Note that, it is sufficient to consider \( h \leq 2^{n-1} (r_1 = 0) \) since \( f \rightarrow \bar{f} \) preserves both the sensitivity and the NCF designation, and as a corollary yields

\[
\beta(n,h) = \beta(n,2^n-h). \tag{7}
\]

Furthermore,

\[
\beta(n,h) = \beta(n-1,h) + h \tag{8}
\]

which observes that the number of boundary edges connecting hyperfaces \( s_1 = 0 \) and \( s_1 = 1 \) is that of minority-color vertices (all of which reside on \( s_1 = \bar{s}_1 \), see Fig. 1).

**Case I.** \( h \leq 2^{n-2} \): By the induction hypothesis,

\[
\beta(n-1,h) \leq \beta(n-2,h_1)+\beta(n-2,h-h_1)+|h-2h_1| \tag{9}
\]

is true for all allowed \( h, h_1 \). Substituting Eq. (8) in the form \( \beta(n-1,h) = \beta(n,h) - h \) above, first arguments of \( \beta(.) \) can be promoted by one to reach the saught relation in Eq. (6).

**Case II.** \( 2^{n-2} < h \leq 2^{n-1} \): The argument used in Case I still holds for the moderate values \( h-2^{n-2} \leq h_1 \leq 2^{n-2} \). For the remaining values of \( h_1 \) on the left/right of the interval above, we make use of Eq. (8) and the relation \( \beta(n,h) = \beta(n,2^n-h) \) (by \( \xi[f] = \xi[\bar{f}] \) symmetry) to obtain (see Appendix):

\[
\beta(n-1,h - 2^{n-2}) = \beta(n,h) + h - 3 \times 2^{n-2}. \tag{10}
\]

For the “left” region with \( h_1 \in \{0, \ldots, h - 2^{n-2}\} \), we use the induction hypothesis in the form

\[
\beta(n-1,h - 2^{n-2}) \leq \beta(n-2,h_1) + \beta(n-2,h - 2^{n-2} - h_1) + |h - 2^{n-2} - 2h_1| \tag{11}
\]

and substitute Eq. (10) to obtain

\[
\beta(n,h) \leq \beta(n-1,h_1) + \beta(n-1,h-h_1) + 2^{n-2} - 2h_1 + |h - 2^{n-2} - 2h_1|. \tag{12}
\]

The desired inequality (6) follows from \( (2^{n-2} - 2h_1) + |h - 2^{n-2} - 2h_1| < |h - 2h_1| \) (see Appendix).

For \( h_1 \in \{2^{n-2}, \ldots, h\} \) values on the “right”, the inequality (11) can be utilized again after substituting \( h_1 \to (h_1 + 2^{n-2}) \), yielding

\[
\beta(n,h) \leq \beta(n-1,h_1) + \beta(n-1,h-h_1) + 2h_1 - 2h + 2^{n-2} + |h + 2^{n-2} - 2h_1|. \tag{13}
\]

The proof is completed by observing that \( (2h_1 - 2h + 2^{n-2}) + |h + 2^{n-2} - 2h_1| < |h - 2h_1| \) (see Appendix).

It is interesting to consider the sensitivity as a function of the activity ratio \( p \). The support of this function can be extended onto the real interval \([0,1]\) as

\[
\xi^{\ast}(p) = \lim_{n \to \infty} \beta(n, [p \times 2^n])/2^{n-1}. \tag{14}
\]

The existence of \( \xi^{\ast}(p) \) is granted by the fact that \( \beta(n,h) = 2\beta(n-1,h/2) \) for \( h \) even. In other words,
\( \xi(p) \) is the closure of the set of points \((p, \xi) = (2^{-n}h, 2^{1-n} \beta(n, h))\) for all \( n \) and \( h \). Fig. 2 shows the nontrivial self-similar structure of \( \xi(p) \) (also see Ref. 31), a consequence of the recursion relation

\[
\frac{\xi'(p)}{2} = \xi\left(\frac{p}{2}\right) - p
\]  

which follows from Eq.(5), Eq.(15) and the symmetry condition \( \xi(p) = \xi^*(1-p) \) from Eq.(7) fully determine \( \xi(p) \), subject to the boundary condition \( \xi^*(1) = 0 \).

Having proven that NCFs realize the lower bound of the sensitivity in \( B_{n,h} \), we next ask whether this bound is consequential to biology at all. To this end, we downloaded all regulatory functions of the 78 biochemical networks in the Cell Collective database 19 which contains models curated from previously published work for a wide selection of cellular processes from multiple organisms. Out of 3460 regulatory functions, we discarded 1310 which take a single variable as input (they convey no valuable information for our study) and calculated the activity ratio and sensitivity values for the rest, using Eq.(3).

Superimposing the scatter plot of the compiled values on top of the calculated theoretical minimum in Fig.(2) unveils the relevance of the constraint imposed by the proven bound. The region occupied by the ensemble of randomized functions obtained by shuffling the truth table of each distinct function in the database is also shown as an overhanging shaded region in the figure. The precipitation of the biological networks onto the minimal curve is a clear manifestation of the drive towards maximum stability.

For a quantitative assessment of the degree of sensitivity minimization in the dataset, we calculate the “normalized excess sensitivity” of each regulatory function measured relative to the corresponding value of \( \xi(p) \) as \( \delta[\xi] = \langle \xi(f) - \xi^*(p_f) \rangle / \xi^*(p_f) \). The dominating feature of the distribution of \( \delta \) (shown in Fig.(3)) is the peak at \( \delta = 0 \) (Fig.(3a)) which reveals the fact that all but 215 functions out of 2150 lie on the sensitivity minimum (i.e., are NCFs, consistent with an earlier analysis on a much smaller set 9). A comparison with an unbiased reference histogram derived from the random ensemble shows that the remaining 10% (non-NCFs) are also significantly closer to the minimum.

It is interesting to consider our findings in conjunction with a recent analysis on the same dataset by Daniels et al. 6, who observe an impressive accumulation around the order-chaos boundary \( \xi = 1 \) (also reproduced here in Fig.(2)). The observation serves as a confirmation of the well-known “edge-of-chaos” hypothesis by Kauffman, that is, most biological systems are tuned to the vicinity of the critical point 12,20, striking a balance between robustness to transient environmental changes and adaptability to persistent shifts. Mechanisms leading to criticality in living cells are still unclear 32. Our results underline the somewhat counterintuitive fact that, although the gene regulatory networks “live at the edge of chaos”, they barely stray away from the minimum boundary of the sensitivity. Upon inspection, the uneven preference for certain activity ratios (Fig.(2), top panel), stemming from over-representation of functions with few inputs, is partially responsible for the peak at \( \xi = 1 \). Yet, it is evident that the shape of \( \xi^*(p) \) favors the vicinity of the critical point, even in absence of such bias. In fact, 50% and 85% of NCFs selected randomly from a uniform distribution on \( p \) deviate, respectively, by less than 25% and 35% from the critical boundary (Fig.(3b)). Therefore, the organization of gene regulation near the critical point may, after all, emerge as a generic feature of selection for minimum sensitivity.

Finally, it is worth noting that some caution is required while interpreting the above from the perspective of network dynamics. Although the network sensitivity can be expressed as \( \langle \xi_\alpha \rangle \) (averaged over the network nodes, \( \alpha \)) in an annealed approximation, existence of correlations between the inputs of different nodes generally necessitates a more refined treatment 11,33,34. It would be interesting to investigate the limits of sensitivity at the network scale, in conjunction with the derived bound at the node level.

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[1] C. H. Waddington, Nature 150, 563 (1942).
[2] I. I. Schmalhausen, (1949).
[3] S. C. Stearns, Proceedings of the National Academy of Sciences 99, 10229 (2002).
[4] M. Marques-Pita and L. M. Rocha, PloS one 8, e55946 (2013).
[5] A. J. Gates and L. M. Rocha, Scientific reports 6, 1
[6] B. C. Daniels, H. Kim, D. Moore, S. Zhou, H. B. Smith, B. Karas, S. A. Kauffman, and S. I. Walker, Physical review letters 121, 138102 (2018).

[7] A. J. Gates, R. B. Correia, X. Wang, and L. M. Rocha, Proceedings of the National Academy of Sciences 118 (2021).

[8] S. E. Harris, B. K. Sawhill, A. Wuenesch, and S. Kauffman, Complexity 7, 23 (2002).

[9] S. Kauffman, C. Peterson, B. Samuelsson, and C. Troein, Proceedings of the National Academy of Sciences 100, 14796 (2003).

[10] S. Kauffman, C. Peterson, B. Samuelsson, and C. Troein, Proceedings of the National Academy of Sciences 101, 17102 (2004).

[11] I. Shmulevich, S. A. Kauffman, and M. Aldana, Proceedings of the National Academy of Sciences 102, 13439 (2005).

[12] S. A. Kauffman et al., The origins of order: Self-organization and selection in evolution (Oxford University Press, USA, 1993).

[13] A. S. Jarrah, B. Raposa, and R. Laubenbacher, Physica D: Nonlinear Phenomena 233, 167 (2007).

[14] T. P. Peixoto, The European Physical Journal B 78, 187 (2010).

[15] Y. Li and J. O. Adeyeye, Theoretical Computer Science 791, 116 (2019).

[16] K. Jansen and M. T. Matache, The European Physical Journal B 86, 1 (2013).

[17] F. Karlsson and M. Hörnquist, Physica A: Statistical Mechanics and its Applications 384, 747 (2007).

[18] I. Shmulevich and S. A. Kauffman, Physical review letters 93, 048701 (2004).

[19] “The cell collective database,” http://cellcollective.org.

[20] S. Kauffman, Nature 224, 177 (1969).

[21] L. Glass and S. A. Kauffman, Journal of theoretical Biology 39, 103 (1973).

[22] B. Derrida and Y. Pomeau, EPL (Europhysics Letters) 1, 45 (1986).

[23] B. Derrida and D. Stauffer, EPL (Europhysics Letters) 2, 739 (1986).

[24] S. Cook, C. Dwork, and R. Reischuk, SIAM Journal on Computing 15, 87 (1986).

[25] B. Luque and R. V. Solé, Physica A: Statistical Mechanics and its Applications 284, 33 (2000).

[26] Y. Li, J. O. Adeyeye, D. Murrugarra, B. Aguilar, and R. Laubenbacher, Theoretical Computer Science 481, 24 (2013).

[27] I. Shmulevich and E. Dougherty, Probabilistic Boolean Networks: The Modeling and Control of Gene Regulatory Networks Other Titles in Applied Mathematics (Society for Industrial and Applied Mathematics, 2010).

[28] N. Alon and V. D. Milman, Journal of Combinatorial Theory, Series B 38, 73 (1985).

[29] J. A. Almendral and A. Díaz-Guilera, New Journal of Physics 9, 187 (2007).

[30] Y. Kim and M. Mesbahi, in Proceedings of the 2005, American Control Conference, 2005. (IEEE, 2005) pp. 99–103.

[31] C. Kadelka, J. Kuipers, and R. Laubenbacher, Physica D: Nonlinear Phenomena 353, 39 (2017).

[32] B. Vidiella, A. Guillamon, J. Sardanyés, V. Maull, J. Pla, N. Conde, and R. Solé, Nature Communications 12, 1 (2021).

[33] T. Rohlf and S. Bornholdt, Physica A: Statistical Mechanics and its Applications 310, 245 (2002).

[34] A. A. Moreira and L. A. N. Amaral, Physical review letters 94, 218702 (2005).