Boolean networks with robust and reliable trajectories

Christoph Schmal, Tiago P. Peixoto and Barbara Drossel
Institut für Festkörperphysik, TU Darmstadt, Hochschulstrasse 6, 64289
Darmstadt, Germany
E-mail: schmal@physik.uni-bielefeld.de, tiago@fkp.tu-darmstadt.de,
drossel@fkp.tu-darmstadt.de

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Abstract. We construct and investigate Boolean networks that follow a given
reliable trajectory in state space, which is insensitive to fluctuations in the
updating schedule, and which is also robust against noise. Robustness is quantified
as the probability that the dynamics return to the reliable trajectory after a
perturbation of the state of a single node. In order to achieve high robustness, we
navigate through the space of possible update functions by using an evolutionary
algorithm. We constrain the networks to having the minimum number of
connections required to obtain the reliable trajectory. Surprisingly, we find that
robustness always reaches values close to 100 percent during the evolutionary
optimization process. The set of update functions can be evolved such that it
differs only slightly from that of networks that were not optimized with respect to
robustness. The state space of the optimized networks is dominated by the basin
of attraction of the reliable trajectory.
1. Introduction

Boolean networks (BNs) are used as simplified models of gene regulation, where the expression levels of genes are described by Boolean variables, and their mutual regulation by Boolean functions. This simplification permits in particular the analysis of larger networks, the full dynamics of which would include many nonlinear equations and many parameters [1].

The simplest class of BNs are Random Boolean Networks (RBNs) [2, 3], i.e., BNs with connections and update functions assigned at random to each node. These networks undergo a phase transition from a frozen phase to a "chaotic" phase at a critical value $K = 2$ of the number of inputs per node. It has been argued [2] that real networks may share properties with RBNs that lie at the boundary between two phases, since these “critical” networks are capable of responding to perturbations, but without an exponentially fast divergence of trajectories in state space.

However, critical RBNs are not robust against noise [4], due to their large number of dynamical attractors. In contrast, BNs that are modeled on the basis of real biological data, such as the yeast cell cycle regulation network [5], go faithfully through the correct sequence of states even in the presence of noise. This is due to the structure of the state space: most states of the network lead after a few update steps to the dynamical attractor that corresponds to the cell cycle.

In this paper, we will construct and investigate BNs that are robust against two types of noise. The first type of noise is applied to the update schedule, and it delays or advances the update time of a given node [6, 7, 8, 9]. The second type of noise is applied to the update rule, and it flips the state of a node to the opposite of the value imposed by the update function [10, 11, 12, 13, 14, 15, 4, 16]. Both types of noise are present in real systems, since genes lack a global update clock and are therefore not updated at fixed time intervals, and since expression levels are subject to stochastic fluctuation [17]. However, these two types of noise are quite different, and require different strategies to attain robustness: With respect to the first type of noise, it is possible for the dynamics of BNs to be entirely reliable, simply by requiring that consecutive states of an attractor differ by the state of at most one node [9]. In order to make BNs robust against the second type of noise, it is necessary to introduce redundancy [18], or to build networks with a state space dominated by the basin of attraction of one attractor [19]. These methods lead to a good level of robustness, but can never entirely remove the effects of noise.

In order to obtain networks that are robust against both types of noise, we will first construct minimal networks that have a reliable dynamical trajectory, which is insensitive to the sequence in which the nodes are updated [9]. Then, by using an evolutionary algorithm, we will optimize the set of update functions of all nodes such that the dynamics return to this attractor with a large probability when the state of a node is perturbed. We investigate the extent of robustness attainable for these networks, and characterize the distribution of their update functions and their state space properties.

This paper is structured as follows: In section 2 we provide a definition of the model, and a description of the minimal reliable BNs defined in [9] as well as of the evolutionary algorithm used for the optimization process. In section 3 we analyze the robustness, the set of update functions, and the state space of the networks obtained by the optimization process. Section 4 summarizes and discusses our main findings.
2. Construction of reliable and robust BNs

Our goal is to obtain and investigate BNs that are robust with respect to the update schedule and with respect to perturbation of the state of a node. To this purpose, we first construct reliable networks (i.e., networks that have an attractor that is robust with respect to the update sequence), which we will then optimize with respect to robustness against perturbations.

2.1. Reliable BNs

A Boolean network is specified by its topology and dynamical update rules. The topology is specified by the number $N$ of nodes, and by the connections between these nodes. Each node obtains an index $i \in \{0,1,\ldots,N-1\}$ and can be either in the state $\sigma_i = 0$ or $\sigma_i = 1$. Its time evolution is given by the iterative map

$$\sigma_i(t+1) = f_i(\overline{\sigma}_{j(i)}(t))u_i(t) + \sigma_i(t)[1 - u_i(t)]$$  \hspace{1cm} (1)

where $f_i : \{0,1\}^{k_i} \rightarrow \{0,1\}$ is the update function of node $i$, which depends exclusively on the states of its $k_i$ input nodes $\overline{\sigma}_{j(i)}$. $\overline{u}(t)$ represents the update schedule, and has the components $u_i(t) = 1$ if node $i$ is updated at time $t$, and $u_i(t) = 0$ if it is not updated at time $t$.

We construct networks with entirely reliable trajectories in the same way as in [9]. Reliable trajectories have the property that two consecutive states (under any update schedule) $\overline{\sigma}(t)$ and $\overline{\sigma}(t+1)$ can differ by the value of at most one node, i.e. the Hamming distance between these states is one. Entirely reliable attractors can therefore be represented as closed walks over the $N$-dimensional Hamming hypercube, as shown in Fig. 1. The length of the attractor can be written as $L = \sum_i l_i$, where $l_i$ denotes the number of times node $i$ changes its state during the full period. Given a reliable trajectory of length $L$ it is possible to construct a minimal network that realizes it, by finding for each node a minimal set of inputs and a corresponding Boolean function which is compatible with the trajectory (see [9] for details). Since there are possibly many such networks, we sample randomly from the ensemble of all possibilities. From all possible functions that realize the same trajectory, given a specific choice of inputs, we choose those which are more homogeneous, i.e., that have the smallest number of outputs that deviate from the majority bit in their truth table.

We generate the reliable trajectories at random, given the average number of flips per node $\ell$. The number of flips of node $i$ is $l_i = 2 + 2\ell_i$, where $\ell_i$ is a random variable.

![Figure 1: Example of a reliable trajectory of length $L = 6$ on a system of size $N = 3$.](image-url)
sampled from a Poisson distribution with average $l/2 - 1$. The average total length of the trajectory is given simply by $Nl$. Fig. 2 shows an example of a random trajectory and one of its minimal networks.

![Image of a random trajectory and one corresponding minimal network.](image-url)

Figure 2: Example of a random reliable trajectory and one corresponding minimal network.

### 2.2. Optimizing the networks for dynamical robustness

We define robustness as the probability that the dynamics return to the reliable trajectory after a perturbation of a single node. Such a perturbation moves the system to one of the $N$ neighboring states on the Hamming hypercube representing the state space. More precisely, considering the set

$$
\mathcal{H}_1(\sigma_a) = \{ \sigma \in \{0, 1\}^N : H(\sigma, \sigma_a) = 1 \}
$$

of all states with Hamming-distance 1 from a given state $\sigma_a$ of our reliable attractor, we define the local fitness $f_a$ of this state as the fraction of these $N$ neighbors that return to the reliable attractor. The total fitness of the network is given by the average $f = \frac{1}{L} \sum_{a=1}^{L} f_a$. In order to avoid stochasticity in the measurement of $f$, we always use a parallel update schedule, where all nodes are updated at the same time.

The fact that two successive states on the reliable trajectory differ only by the value of one node means that there is a lower bound on the fitness value of $f_{\text{min}} = 2/N$, since two of the $N$ possible perturbations generate a state that is on the reliable attractor.

Given this definition of the fitness of the network, we apply an evolutionary algorithm in order to maximize it, modifying the update functions but retaining the network topology and the reliable trajectory. When exploring the search space $\mathcal{S}$ of possible update functions, we can only change the truth table entries of the output values that do not interfere with the given reliable trajectory. Let us assume that node $i$ has $k_i$ input nodes. If its function has $\kappa_i$ truth table entries that are fixed by the reliable trajectory and $\kappa_i$ entries that are not, then there are $2^{\kappa_i}$ different possible output combinations for these entries. For $N$ nodes, we have $|\mathcal{S}| = \prod_{i=1}^{N} 2^{\kappa_i} = 2^{\sum_{i=1}^{N} \kappa_i}$ for the size of the search space. The typical number of entries not fixed by the reliable trajectory scales as $\kappa \sim 2^{l} - \langle \kappa \rangle \sim 2^{l - l}$. Hence the size of the search space scales as $|\mathcal{S}| \sim 2^{N(2^{l} - l)}$ and therefore grows exponentially with $N$ and superexponentially with $l$. Finding a global optimum by searching through all update functions is possible.
only for very small networks. Instead, we use an evolutionary algorithm, specified as follows:

(i) A node \( i \in \{1, 2, \ldots, N\} \) is chosen at random.
(ii) An output in the truth table of this node is chosen at random. If it does not belong to a configuration of the input nodes that occurs during the course of the reliable trajectory, we change its value.
(iii) When this mutation increases the fitness (positive mutation) or has no effect (neutral mutation) we accept the modification, otherwise (negative mutations) we reject it.
(iv) The adaptive walk obtained by iterating steps 1 to 3 stops when the maximum possible fitness value (evaluated below) is reached, or after a certain number of attempted mutations, which was set to \( 5 \times 10^3 \) for \( N = 10 \), to \( 10 \times 10^3 \) for \( N = 20 \), and to \( 30 \times 10^3 \) for simulations that use the approximate fitness \( f^* \) (see below).

3. Results

3.1. Robustness of reliable networks before evolution

Fig. 3 shows the initial fitness \( f \) of minimal reliable networks for several combinations of \( N \) and \( l \), averaged for \( 6 \times 10^3 \) (for \( N < 40 \)) or \( 2 \times 10^3 \) (for \( N > 40 \)) independent network realizations.

![Figure 3](image)

Figure 3: Average fitness \( \langle f \rangle \), for several parameter combinations.

A large proportion of networks with small \( N \) and \( l \) have \( f = 1 \). As was observed in [9], for these networks the reliable trajectory often has a basin of attraction which dominates the entire state space, which explains why \( f \) is close to 1. When \( N \) and \( l \) increase, this changes, and the basin of the reliable trajectory no longer dominates the state space, resulting in smaller values of \( \langle f \rangle \). The only trivial exception is when \( l \) is so large that the reliable trajectory occupies a large portion of the state space (i.e. \( NL \sim 2^N \)). This explains the positive slope of the curve for \( N = 5 \). In the more interesting case \( N \gg 1 \) and \( NL \ll 2^N \), the fitness is far from the maximum value, and the optimization procedure can considerably increase the fitness.
3.2. Fitness of the optimized networks

3.2.1. Upper bound on the fitness  In contrast to our initial expectation, even a full search of the space of update functions does not always lead to a fitness value of 1. The reason for this is that the search space is constrained by the reliable trajectory, which cannot change during the evolutionary algorithm. This means that the truth table entries that cannot be modified by the evolutionary algorithm (since they are necessary to regulate the given trajectory) may also regulate other portions of the state space. This portion, therefore, cannot be modified by the optimization. If some of these states are reached after a perturbation, and they do not inherently lead back to the reliable trajectory, then the value of \( f = 1 \) can never be reached. If \( \phi \) is the number of perturbations which lead to one of these “locked” states, the maximum fitness will then be \( f_{\text{max}} = 1 - \frac{\phi}{N_L} \). Fig. 4 shows the state space of such a network with a maximal fitness smaller than \( f = 1 \). For five possible perturbations of the reliable attractor, this network will unavoidably be trapped in a spurious attractor of size two, and thus \( f_{\text{max}} = 1 - \frac{5}{N_L} = 67/72 \approx 0.93 \). We evaluated \( f_{\text{max}} \) for ensembles of

![Figure 4: State space of the example network in Fig 2 before and after evolution. The states are color-coded as follows. Blue: reliable attractor. Green: states to which the network is brought by a perturbation. Yellow: the attractor that cannot be modified by the optimization procedure and is reached by a perturbation. Red: remaining states.](image-url)
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Figure 5: Upper limit on fitness, averaged over networks of different sizes $N$ and trajectory lengths $l$ (top). Average deviation from $f_{\text{max}}$ after the evolutionary process for those networks that did not reach $f_{\text{max}}$ (middle). Average deviation from $f_{\text{max}}$ after the evolutionary process for all networks; the dashed curves being obtained by evolving the functions based on the approximate fitness $f^*$ (bottom).
3.2.2. Approximate fitness

The computer time required for the fitness evaluation at each evolutionary step depends on the number \( L \approx Nl \) of states in the reliable trajectory, and on the number \( N \) of possible perturbations per state, which leads to a complexity of \( \mathcal{O}(N^2l) \). Thus, the optimization process becomes computationally too expensive for larger \( N \) and \( l \) as we have to determine the fitness after each mutation. In order to reduce computer time for larger \( Nl \), we used an approximate fitness function \( f^* \), which uses only a random subset of \( \xi \) different perturbations, which remains fixed during the optimization. Thus, if \( k \) of these \( \xi \) perturbed states return to the reliable trajectory the approximate fitness is \( f^* := k/\xi \). Such an approximation introduces a probability of accepting a negative mutation or rejecting a positive or neutral one. In order to minimize this effect, we re-sample the \( \xi \) perturbations after the maximum \( f^*_{\text{max}} \) has been reached (which can be computed analogously to \( f_{\text{max}} \) above).

We have investigated the performance of this approximation, as can be seen in Fig. 6, which compares the approximate and real fitness during two evolutionary processes applied to the same network, using \( f^* \) as the selection criterion, with sampling sizes of \( \xi = 20 \) and and \( \xi = 40 \). One can see that the real fitness increases in both cases, and that it fluctuates around \( f^* \), but does not deviate strongly from it. The amplitude of the fluctuation gets smaller for larger \( \xi \).

![Figure 6: Evolution via approximate fitness of a network with \( N = 20 \) and \( l = 4.3 \), and with sample sizes of \( \xi = 20 \) and \( \xi = 40 \). Vertical lines mark the instances when new sets of perturbed nodes were chosen.](image)

3.2.3. Fitness results

We optimized networks for \( N = 10, 15 \) and \( l = 2, 2.5, 3, 4, 6 \) as well as for \( N = 20 \) and \( l = 2, 2.5, 3 \) using the fitness function \( f \). Networks with \( N > 20 \) and \( N = 20 \) with \( l = 4, 6 \) were optimized via the approximate fitness function \( f^* \). The number of networks evolved ranged from \( 10^4 \) for \( N = 10 \) to 800 networks for larger values of \( N \) and \( l \).

The results are shown in Fig. 5. A significant fraction of networks did not reach \( f_{\text{max}} \), which can be potentially due to three reasons:

(a) The evolution got stuck in a local fitness maximum.
(b) The global fitness maximum of the network is smaller than \( f_{\text{max}} \).
(c) The algorithm stopped before the optimization reached \( f_{\text{max}} \).

For \( N = 10 \), the fraction of networks that did not reach \( f_{\text{max}} \) decreases monotonically with increasing \( l \) which indicates that the probability of reaching \( f_{\text{max}} \) increases with
the growth of the search space. We tried to optimize these networks further with a simulated annealing algorithm \[20\] by introducing a probability \( p = e^{-|\Delta f|/T} \) of accepting a negative mutation in order to leave a local maximum. As this never resulted in better values of fitness, and since all networks suffered their last positive mutation after approximately 10\% of total running time of the algorithm, we concluded that reason (b) is more probable than either (a) or (c). The fraction of networks that did not reach \( f_{\text{max}} \) increases with \( l \) for \( N = 15 \) and \( N = 20 \). These networks often suffered their last positive mutation almost at the end of the optimization run, and thus one could increase the fitness if we would evolve them further, but it would take a much longer time for it to increase significantly. However, despite the fact that many networks did not reach the values of \( f_{\text{max}} \), the deviation from \( f_{\text{max}} \) for the final values of fitness are very small, as can be seen in Fig. 5. This deviation is worsened if the approximate fitness is used, as seen in the bottom graph, which can be improved only if the number of samples \( \xi \) is increased, as the change from \( \xi = 40 \) to 80 shows. The total number of mutations needed to evolve the networks can be as large as a few thousands, for larger \( N \) and \( l \), and is therefore much larger than in the work of Szejka and Drossel [19]. This is due the fact that the optimization done here is much more restricted, as we only search through the space of possible update functions whereas in [19] both the topology and dynamics were allowed to change, and there was no particular trajectory imposed on the system.

### 3.3. Update functions

We evaluated the frequency of the possible update functions that occur in the optimized networks. Let us first discuss the functions with \( k = 2 \). Before and after optimization for robustness, the distribution is almost entirely dominated by the eight canalizing functions that have three bits of one type and one bit of the other type in the truth table. The reasons for the dominance of these functions were explained in [9]. For functions with larger \( k \), we evaluated the homogeneity \( d \), which is equal to the number of entries in the truth table that have the minority bit. Fig. 7 shows the distribution of \( d \) before and after the optimization process. Before the optimization process, functions with smaller values of \( d \) dominate. After the process, the number of functions with higher values of \( d \) is significantly larger. This means that the distribution of functions has become more random, as there exist many more functions with larger \( d \), their number being \( 2 \left( \begin{array}{c} 2^k \\ d \end{array} \right) \) for \( d < 2^{k-1} \), and \( \left( \begin{array}{c} 2^k \\ d \end{array} \right) \) for \( d = 2^{k-1} \).

In order to investigate whether the differences in homogeneity are a fundamental property of the optimized networks or an artifact of neutral mutations, we tried to decrease the values of \( d \) while retaining the value of fitness. To achieve this, we let the evolutionary algorithm continue from the final configuration, with the modification that a mutation is only accepted if it simultaneously does not lower the fitness and increases the homogeneity of the randomly chosen update function. This was done for the evolved networks with \( N = 10 \) and \( N = 15 \). The distribution of \( d \) after homogenization is shown in the right column of Fig. 7. It can be clearly seen that the shift to less homogeneous functions can be reversed to a large degree, except for \( l = 2 \). This means that the increase of the values of \( d \) during the evolutionary process is mainly due to neutral mutations. The fact that it is possible to homogenize the update functions after reaching the global optimum \( f_{\text{max}} \) gives an insight into the fitness landscape: The global optimum is a plateau, instead of isolated peaks, on which the networks can move via neutral mutations, similar to what was found in [19].
Lastly, we investigated the influence of the optimization and homogenization processes on the state space of the minimal networks. We evaluated the entire state space for optimized networks of size $N = 10, 15$, and we sampled the state space for $N = 20$, under parallel update. In particular, we enlisted the attractors and the sizes of their basins of attraction (i.e., the number of states leading to the attractor). As expected, the optimization process increases the average basin size of the reliable trajectory $\omega_r$, as can be seen in Fig. 5. Fig. 6 shows the state space of a typical network with $N = 10$ nodes and $l = 6.8$, before and after evolution, and after homogenization. Before evolution, the state space is divided into six basins of attraction, five of which belong to fixed points, and one being the basin of the reliable attractor. The network has an unevolved fitness of $f \approx 0.64$. The short transients of $T \approx 1.3$ steps on average indicate that the system resembles an RBN in the frozen phase. After the evolutionary process, the basin of the reliable trajectory occupies the entire state space on average, leading a fitness of $f = 1$. The dynamics are less frozen, with the average transient time to the attractor having increased to $T \approx 10.1$. After homogenization, the transient time has decreased to $T \approx 2.9$.

### 3.4. State space

![Graphs showing distribution of functions with different values of $d$, for different $l$ and $N = 10$ before and after evolutionary optimization, after evolution, and after homogenization.](image)

Figure 7: Distribution of functions with different values of $d$, for different $l$ and $N = 10$ before the evolutionary optimization (left), after evolution (middle), and after homogenization (right).
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Figure 8: Average basin size $\langle \omega_r \rangle$ of the reliable attractors for different values of $N$ and $l$. The dotted line shows the averages after the evolutionary process. The dashed lines represents networks evolved via the approximate fitness.

Figure 9: Influence of the evolution and homogenization on the state space of a network with $N = 10$ and $L = 68$.

4. Conclusion

We have shown that there exists a large ensemble of minimal Boolean networks that show reliable and robust dynamics. The networks are minimal in the respect that the number of connections of a node is not larger than necessary for obtaining a desired reliable trajectory. A reliable trajectory is an attractor of the dynamics of the network that does not change when the update schedule is changed or randomized. This means that under parallel update, at each time step only one node changes its state. The reliable trajectories were chosen at random, given a fixed average number of flips per node. High robustness was achieved by using an evolutionary algorithm that modifies the update functions and that accepts only those changes that do not decrease robustness. For all investigated parameter sets, we obtained networks with a robustness close to 100 percent. The set of update functions associated with the final
robustness value is not unique, but can be varied over a broad range of homogeneity
domination. The state space of the resulting
etworks is dominated by the basin of attraction of the reliable trajectory.

Dynamical reliability and robustness to noise are important features of biological
etworks, such as gene regulation networks. While the networks constructed by our
procedure are random in many respects and still far from the very specific networks
found in biological systems, our study shows that there exist many solutions to the
task of constructing such networks.

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