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On the Long-run Sensitivity of Probabilistic Boolean Networks

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Abstract

Boolean networks and, more generally, probabilistic Boolean networks, as one class of gene regulatory networks, model biological processes with the network dynamics determined by the logic-rule regulatory functions in conjunction with probabilistic parameters involved in network transitions. While there has been significant research on applying different control policies to alter network dynamics as future gene therapeutic intervention, we have seen less work on understanding the sensitivity of network dynamics with respect to perturbations to networks, including regulatory rules and the involved parameters, which is particularly critical for the design of intervention strategies. This paper studies this less investigated issue of network sensitivity in the long run. As the underlying model of probabilistic Boolean networks is a finite Markov chain, we define the network sensitivity based on the steady-state distributions of probabilistic Boolean networks and call it *long-run sensitivity*. The steady-state distribution reflects the long-run behavior of the network and it can give insight into the dynamics or momentum existing in a system. The change of steady-state distribution caused by possible perturbations is the key measure for intervention. This newly defined long-run sensitivity can provide insight on both network inference and intervention. We show the results for probabilistic Boolean networks generated from random Boolean networks and the results from two real biological networks illustrate preliminary applications of sensitivity in intervention for practical problems.

Index Terms

Genetic regulatory networks; Boolean networks; probabilistic Boolean networks; Markov chains; sensitivity; steady-state distribution; intervention; metastasis

I. Introduction

Gene regulatory networks facilitate understanding of biological processes and provide a mathematical framework for future gene therapeutic intervention strategies through the study of network dynamics. For a function-based network like a probabilistic Boolean network

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(PBN) [25], which is a probabilistic extension of the classical Boolean network (BN) [11], the transition probability matrix of the corresponding Markov chain is determined by the logic-rule regulatory functions defining the network and several probabilistic parameters involved in network transitions. In this paper, we study the sensitivity of network dynamics with respect to possible perturbations, including changes made to regulatory rules and probabilistic network parameters.

Previous sensitivity research has focused on ensemble dynamical behavior for networks possessing different properties, such as connectivity, bias, and function classes. Different sensitivity measures have been proposed for this purpose, including the Derrida curve [6] based on the Hamming distances between trajectories starting from different initial states, the activity and sensitivity measure [11], [12], [24], [27] or critical complexity [3] with Boolean partial derivatives of regulatory Boolean functions, and another Boolean-derivative-based order parameter, Lyapunov exponents [16], etc. Basically, these measures provide ways to classify random Boolean networks into ordered, chaotic and critical (the transition phase from ordered to chaotic) regimes. They show that the network behavior is dependent on connectivity, bias and the function class of random Boolean networks.

The underlying probabilistic model for Boolean networks with random perturbation (BNps) and probabilistic Boolean networks (PBN) is a Markov chain. The steady-state distribution provides insight into long-run dynamics [13]. Intervention strategies aim to beneficially alter network dynamics. One type of intervention involves a one-time state perturbation [12], [18], [22], [26], [27] to flip gene-expression states to modulate the dynamics. The effect is transient, in the sense that it simply resets the initial state of the underlying Markov chain and does not alter the network structure or the steady-state distribution. Intervention via function perturbation [21], [26], [29] has a more fundamental impact by altering the underlying rule-based structure. This can alter the steady-state distribution and thus has the potential for guiding the development of drug-based gene therapies to act on gene products and thereby alter the extant cell behavior. Although not directly relevant to the present paper, we should add that a significant amount of research has been done on the development of long-run intervention strategies based on the theory of control [7], [19], [20].

Whereas classical sensitivity measures based on temporary gene perturbation measure transient effects, the literature lacks research on network sensitivity relevant to steady-state distributions. If we are concerned with network changes that alter the steady-state distribution, then we need to consider sensitivity relative to such changes. In this paper we provide a new definition of network sensitivity based on the steady-state distribution of the underlying Markov chain model. This new *long-run sensitivity* is defined in terms of the difference between the steady-state distributions before and after perturbation. Its particular form will depend upon the change being considered – that is, change to the network structure or to other network parameters. This new sensitivity measure facilitates analysis of network robustness and controllability. Therefore, it can be used to provide guidelines for network inference and control problems. In addition, the computation of the new sensitivity measure can be derived from Markov chain theory in a straightforward way. To efficiently compute the long-run sensitivity with respect to structural perturbation, the change of regulatory functions, we implement an analytic solution adapted from perturbation theory in Markov chains [9], [21], [23] to derive the exact quantitative change of steady-state distributions.

With this newly defined long-run sensitivity, we investigate the behavior of Boolean and probabilistic Boolean networks with different network properties, including connectivity and bias. The results demonstrate that the new definition is more suitable for the analysis of long-run network behavior in comparison to the previous sensitivity measures in the ensemble analysis of random Boolean networks [3], [6], [12], [16], [24], [27].

The rest of the paper is organized as follows: Section II briefly reviews the background material for BNs and PBNs. In Section III, we define the network long-run sensitivity based on the concept of steady-state distribution from Markov chain theory, including the sensitivity with respect to the parameters involved in PBNs and the sensitivity with respect to the regulatory rules of the underlying Boolean networks. For probabilistic parameters, it is natural to use the partial derivatives of the steady-state distribution since they are continuous. Since changes to regulatory rules are discrete, we define the long-run sensitivity directly as the change of the steady-state distribution, which provides a suitable measure for either network inference or intervention. We apply a computationally efficient analytic method to compute the steady-state distribution change owing to regulatory rule perturbations. Section IV provides simulation results and discusses sensitivity behavior for networks with different network properties. We discuss the potential applications of the new sensitivity measures in network inference and intervention in Section V. Section VI gives two preliminary practical examples demonstrating the potential use of long-run sensitivity analysis in structural intervention of probabilistic Boolean networks. We conclude the paper and describe future research directions in Section VII.

II. Background

We focus on the analysis of binary BNs and PBNs in this paper but these results directly extend to more general BNs and PBNs since the underlying models are always finite Markov chains. Following the standard definitions of the Boolean network model, including BNps and PBNs [11], [25], BNps and PBNs are described by truth tables determined by Boolean regulatory rules and the related parameters, including various probabilities. In a binary Boolean network of n genes, each gene $x_i \in \{0, 1\}$ at time $t+1$ is determined by the values of a set V_i of predictor genes at t via a Boolean function $f_i: \{0, 1\}^{K_i} \mapsto \{0, 1\}$, where $K_i = |V_i|$ denotes the number of predictor genes in V_i and is called the input degree of x_i in the network. Given a truth table, the network evolves as a trajectory of gene expression states $\mathbf{X}_t \in \{0, 1\}^n$. From the initial state, a BN will eventually reach a set of states, called an *attractor cycle*, through which it will cycle endlessly. Each state corresponds to a unique attractor cycle and the set of states leading to a specific attractor cycle is known as its *basin of attraction* (BOA).

For BNps, perturbation is introduced with a positive probability p by which the current state of each gene in the network can be randomly flipped. Introduction of perturbation makes the corresponding Markov chain of a BNp irreducible. Hence, the network possesses a steady-state distribution π describing its long-run behavior. A BNp inherits the attractor structure from the original Boolean network without perturbation. With sufficiently small p , π will reflect the attractor structure within the original network. For developing therapeutic intervention, we are especially interested in the proportion of time the network occupies an attractor in its steady state. To study network dynamics, the steady-state distribution is a natural descriptor for BNps.

A binary probabilistic Boolean network (PBN) is composed of a family $\{B_1, B_2, \dots, B_m\}$ of BNps together with probabilities governing the selection of a BNp at time t . The m constituent BNps are characterized by m network functions, $\mathbf{F} = \{\mathbf{f}_1, \mathbf{f}_2, \dots, \mathbf{f}_m\}$, where $\mathbf{f}_j$ is the multi-output function for the j th constituent network B_j . At any time point there is a positive probability q of switching from the current governing constituent BNp to another. If a switch is called for at time point t , then one function from among $\mathbf{f}_1, \dots, \mathbf{f}_m$ is randomly selected according to the probability distribution $\mathbf{c} = \{c_1, \dots, c_m\}$. There are two types of PBNs with different interpretations regarding q . If $q < 1$, the interpretation is that there are latent variables governing the model network to behave stochastically. In this case the PBN is said to be *context-sensitive* and q is usually assumed to be small. If $q = 1$, as in the original formulation of PBNs [25], the interpretation is that the uncertainty in the BNp arises from uncertainty in model inference. In this case the PBN is said to be *instantaneously random*. A PBN inherits the

attractor cycles of its constituent BNps and its steady-state distribution π again reflects the long-run behavior.

The dynamics of BNps and PBNs can be analyzed via their associated homogeneous irreducible finite Markov chains. We can derive the transition probability matrix P from the truth tables and the involved probabilistic parameters, and from there derive the steady-state distribution π . We will define sensitivity via the steady-state distribution change caused by the change to P via perturbation to the network structure determined by regulatory functions or the probabilistic parameters. For BNps and instantaneously random PBNs we write the transition probability from $\mathbf{y}$ to $\mathbf{x}$ at arbitrary time t as $P_{\mathbf{y}}(\mathbf{x}) = P[\mathbf{X}_{t+1} = \mathbf{x} | \mathbf{X}_t = \mathbf{y}]$. For a context-sensitive PBN, we need to take into account the constituent BN. The following theorem provides explicit expressions for the transition probabilities, the derivation being given in the Appendix.

Theorem 1

The transition probabilities for a BNp, an instantaneously random PBN and a context-random PBN are given, respectively, by

$$P_{\mathbf{y}}(\mathbf{x}) = \mathbf{1}_{[\mathbf{f}(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]}p^{\eta(\mathbf{x},\mathbf{y})}(1-p)^{n-\eta(\mathbf{x},\mathbf{y})}, \quad (1)$$

where $\eta(\mathbf{x}, \mathbf{y})$ is the Hamming distance between $\mathbf{x}$ and $\mathbf{y}$, and $\mathbf{1}_{[\mathbf{f}(\mathbf{y})=\mathbf{x}]}$ is the indicator function that takes value 1 if $\mathbf{f}(\mathbf{y}) = \mathbf{x}$ according to the truth table and is equal to 0 otherwise;

$$P_{\mathbf{y}}(\mathbf{x}) = \sum_{j=1}^m c_j \mathbf{1}_{[\mathbf{f}_j(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]}p^{\eta(\mathbf{x},\mathbf{y})}(1-p)^{n-\eta(\mathbf{x},\mathbf{y})}, \quad (2)$$

and

$$P_{s,\mathbf{y}}(r,\mathbf{x}) = \mathbf{1}_{[r=s]}(1-q)\{\mathbf{1}_{[\mathbf{f}_s(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]}p^{\eta(\mathbf{x},\mathbf{y})}(1-p)^{n-\eta(\mathbf{x},\mathbf{y})}\} + \mathbf{1}_{[r \neq s]}q \frac{c_r}{\sum_{j \neq s} c_j} \{\mathbf{1}_{[\mathbf{f}_r(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]}p^{\eta(\mathbf{x},\mathbf{y})}(1-p)^{n-\eta(\mathbf{x},\mathbf{y})}\}, \quad (3)$$

where r, s denote the r th and s th BNp, which are the BNps at time $t+1$ and t .

For a given transition matrix P , we have

$$\pi^T = \pi^T P, \quad (4)$$

where π is the corresponding steady-state distribution for P and T denotes transpose. In general, both P and π for PBNs are functions of the network regulatory functions $\mathbf{F}$ and the involved probabilistic parameters $p, q, \mathbf{c}$. Hence, we rewrite equation (4) as

$$\pi^T = (\mathbf{F}, p, q, \mathbf{c}) = \pi^T(\mathbf{F}, p, q, \mathbf{c})P(\mathbf{F}, p, q, \mathbf{c}). \quad (5)$$

The perturbations we can apply to network functions and probabilistic parameters have different properties. For the parameters involved, both P and π are continuous functions of p , q , $\mathbf{c}$. Hence, we can define long-run sensitivity as the partial derivative of the steady-state distribution with respect to any one of them. Since the change to regulatory rules of networks is discrete (binary in our examples), we will have a different long-run sensitivity defined by the change of steady-state distribution as the steady-state distribution reflects the long-run behavior of the network.

III. Long-run Sensitivity

A. Long-run Sensitivity with respect to Probabilistic Parameters

We first consider the long-run sensitivity of BNPs and PBNs with respect to the parameters p , q , and $\mathbf{c}$. For these continuous parameters, the long-run sensitivity is defined via the partial derivatives of the steady-state distribution π . To obtain these derivatives, using (5), we take derivatives on both sides with respect to some parameter ξ :

$$\frac{\partial \pi^T(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi} = \frac{\partial \pi^T(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi} P(\mathbf{F}, p, q, \mathbf{c}) + \pi^T(\mathbf{F}, p, q, \mathbf{c}) \frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi},$$

where ξ can be any of p , q , c_1 , ..., c_m . After re-arranging the above equation, we have

$$\frac{\partial \pi^T(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi} [I - P(\mathbf{F}, p, q, \mathbf{c})] = \pi^T(\mathbf{F}, p, q, \mathbf{c}) \frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi}, \quad (6)$$

The generalized inverse of $[I - P(\mathbf{F}, p, q, \mathbf{c})]$ (for example, the fundamental matrix $Z(\mathbf{F}, p, q, \mathbf{c})$) is easy to compute when we compute $\pi(\mathbf{F}, p, q, \mathbf{c})$ [9], [14], [23]. Hence, the extra computational cost of computing $\frac{\partial \pi^T(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi}$ is just the cost of computing the multiplication of the vector $\pi(\mathbf{F}, p, q, \mathbf{c})$ and matrix $\frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi} Z(\mathbf{F}, p, q, \mathbf{c})$, and, in the case of BNPs and PBNs, $\frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi}$ is easy to compute.

We now define long-run sensitivity with respect to the probabilistic parameters.

Definition 1—The **long-run sensitivity (LRS)** with respect to any probabilistic parameter ξ in $\{p, q, c_1, \dots, c_m\}$ for a given PBN defined by $P(\mathbf{F}, p, q, \mathbf{c})$ is given by

$$\omega_p[\xi] = \left\| \frac{\partial \pi^T(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi} \right\|_l = \left\| \pi^T(\mathbf{F}, p, q, \mathbf{c}) \frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi} Z(\mathbf{F}, p, q, \mathbf{c}) \right\|_l, \quad (7)$$

where $\|\cdot\|_l$ denotes the l -norm and we use the l_1 -norm in our simulations.

For a given network, $\pi(\mathbf{F}, p, q, \mathbf{c})$ and $Z(\mathbf{F}, p, q, \mathbf{c})$ can be computed. To get $\omega_p[\xi]$, we need to derive $\frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi}$ based on the transition probabilities given by Theorem 1 in Section II. We now do this for BNPs, instantaneously random PBNs, and context-sensitive PBNs.

For BNPs, the only probabilistic parameter in $P_{\mathbf{y}}(\mathbf{x})$ in (1) is the perturbation probability p . By taking the derivative with respect to p on both sides of (1), we have:

$$\frac{\partial P_{\mathbf{y}}(\mathbf{x})}{\partial p} = -n \mathbf{1}_{[\mathbf{f}(\mathbf{y})=\mathbf{x}]}(1-p)^{n-1} + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} \{ \eta(\mathbf{x}, \mathbf{y}) p^{\eta(\mathbf{x}, \mathbf{y})-1} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} - [n - \eta(\mathbf{x}, \mathbf{y})] p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})-1} \}. \quad (8)$$

For instantaneously random PBNs, we need to compute $\frac{\partial P_{\mathbf{y}}(\mathbf{x})}{\partial p}$ and $\frac{\partial P_{\mathbf{y}}(\mathbf{x})}{\partial c_j}$ using (2):

$$\frac{\partial P_{\mathbf{y}}(\mathbf{x})}{\partial p} = -n \sum_{j=1}^m c_j \mathbf{1}_{[\mathbf{f}_j(\mathbf{y})=\mathbf{x}]}(1-p)^{n-1} + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} \{ \eta(\mathbf{x}, \mathbf{y}) p^{\eta(\mathbf{x}, \mathbf{y})-1} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} - [n - \eta(\mathbf{x}, \mathbf{y})] p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})-1} \} \quad (9)$$

and

$$\frac{\partial P_{\mathbf{y}}(\mathbf{x})}{\partial c_j} = \mathbf{1}_{[\mathbf{f}_j(\mathbf{y})=\mathbf{x}]}(1-p)^n. \quad (10)$$

We have $p, q, c_1, \dots, c_m$ in (3) for the transition probability in context-sensitive PBNs:

$$\begin{aligned} \frac{\partial P_{s,y}(r,\mathbf{x})}{\partial p} &= -\mathbf{1}_{[r=s]}(1-q) \{ n \mathbf{1}_{[\mathbf{f}_s(\mathbf{y})=\mathbf{x}]}(1-p)^{n-1} - \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} \{ \eta(\mathbf{x}, \mathbf{y}) p^{\eta(\mathbf{x}, \mathbf{y})-1} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} \\ &\quad - [n - \eta(\mathbf{x}, \mathbf{y})] p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})-1} \} \} \\ &\quad - \mathbf{1}_{[r \neq s]} q \frac{c_r}{\sum_{j \neq s} c_j} \{ n \mathbf{1}_{[\mathbf{f}_r(\mathbf{y})=\mathbf{x}]}(1-p)^{n-1} - \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} \{ \eta(\mathbf{x}, \mathbf{y}) p^{\eta(\mathbf{x}, \mathbf{y})-1} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} \\ &\quad - [n - \eta(\mathbf{x}, \mathbf{y})] p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})-1} \} \}; \end{aligned} \quad (11)$$

$$\begin{aligned} \frac{\partial P_{s,y}(r,\mathbf{x})}{\partial q} &= -\mathbf{1}_{[r=s]} \{ \mathbf{1}_{[\mathbf{f}_s(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} \} \\ &\quad + \mathbf{1}_{[r \neq s]} \frac{c_r}{\sum_{j \neq s} c_j} \{ \mathbf{1}_{[\mathbf{f}_r(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} \}; \end{aligned} \quad (12)$$

and

$$\frac{\partial P_{s,y}(r,\mathbf{x})}{\partial c_j} = \begin{cases} \frac{\mathbf{1}_{[r \neq s]} q}{\sum_{k \neq s} c_k} \{ \mathbf{1}_{[\mathbf{f}_r(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} \} & \text{if } j=r, \\ 0 & \text{if } j=s, \\ -\frac{\mathbf{1}_{[r \neq s]} q}{(\sum_{k \neq s} c_k)^2} \{ \mathbf{1}_{[\mathbf{f}_r(\mathbf{y})=\mathbf{x}]}(1-p)^n + \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})} \} & \text{otherwise.} \end{cases} \quad (13)$$

By computing all the matrix entries, we can obtain all possible matrices $\frac{\partial P(\mathbf{F}, p, q, \mathbf{c})}{\partial \xi}$ and then the long-run sensitivity.

B. Long-run Sensitivity with respect to Regulatory Functions

Sensitivity analysis with respect to perturbations to regulatory functions is more complicated. Function perturbations are not continuous and therefore computation of the partial derivative of the transition matrix is not straightforward. Since our focus is on the long-run impact caused by function perturbations, we define the long-run sensitivity by the difference of the steady-state distributions due to perturbations to the regulatory Boolean functions.

We start with the perturbation theory for finite Markov chains. The results for general Markov chains have appeared in the literature [2], [9], [10], [14], [23]. We have adapted these results for both BNPs and PBNs in [21]. With function perturbations, the original transition matrix P is changed to a new transition matrix $\tilde{P} = P + E$, where E represents the perturbation. Our task is to find the difference between the perturbed steady-state distribution $\tilde{\pi}$ and the original steady-state distribution π based on $\tilde{P}$ and P . For BNPs and PBNs, the underlying Markov chains are always irreducible and we have

$$\tilde{\pi}^T - \pi^T = \tilde{\pi}^T \tilde{P} - \pi^T P = \tilde{\pi}^T (P + E) - \pi^T P,$$

Hence,

$$(\tilde{\pi} - \pi)^T (I - P) = \tilde{\pi}^T E. \quad (14)$$

Based on this relationship, one natural choice for network sensitivity with respect to regulatory functions is to compute the l -norm, $\|\tilde{\pi} - \pi\|_l$, of the steady-state distribution difference. However, (14) requires another round of computing the steady-state distribution $\tilde{\pi}$ besides π , which is time-consuming. One possible approach is to apply the results in [2], [23] and write

$$(\tilde{\pi} - \pi)^T = \pi^T E Z + O(E^2).$$

Through this, we can compute norm-type bounds of $\|\tilde{\pi} - \pi\|_l$ and define the network sensitivity approximately by them. Since our concern is with PBNs and perturbations to regulatory functions in Boolean networks have special forms, we aim to derive an explicit expression of $\tilde{\pi} - \pi$ for the given P and $\tilde{P}$.

If $\mathbf{F}$ and $\tilde{\mathbf{F}}$ denote the vectors of regulatory functions for a PBN before and after perturbation, respectively, we define the sensitivity with respect to regulatory functions as follows:

Definition 2—Let $\mathbf{F}$ denote the vector of regulatory functions for a PBN defined by $P(\mathbf{F}, p, q, \mathbf{c})$ and $\tilde{\mathbf{F}}$ denote the vector of regulatory functions for the new PBN defined by $P(\tilde{\mathbf{F}}, p, q, \mathbf{c})$ following function perturbation. The **long-run sensitivity** with respect to the perturbation mapping $\varphi : \mathbf{F} \rightarrow \tilde{\mathbf{F}}$ is defined by

$$\omega[\varphi] = \|\tilde{\pi}^T(\tilde{\mathbf{F}}, p, q, \mathbf{c}) - \pi^T(\mathbf{F}, p, q, \mathbf{c})\|_l. \quad (15)$$

For a class of perturbation mappings $\Phi = \{\varphi_\alpha : \alpha \in A\}$, where A is a probability space, the long-run sensitivity for the class, or **average long-run sensitivity**, is defined as the expectation $\omega[\Phi] = \mathbb{E}_\alpha [\omega[\varphi_\alpha]]$.

The preceding definition is general. In order to introduce an efficient way to compute the average long-run sensitivity for some special function perturbations, we first reiterate the following theorem for general Markov chains which has appeared in [9], [21], [23].

Theorem 2—For a rank-one perturbation $E = ab^T$, where a and b are two arbitrary vectors,

$$\tilde{\pi}^T = \frac{(\pi^T a)\beta^T + (1 - \beta^T a)\pi^T}{(\pi^T a)(\beta^T e) + 1 - \beta^T a} \quad (16)$$

where t and u are any vectors such that $\pi^T t \neq 0$ and $u^T e \neq 0$, and $\beta^T = b^T [I - P + tu^T]^{-1}$.

If we let $t = e$ and $u = \pi$ in (16), we can derive

$$\tilde{\pi} = \pi + \frac{\pi^T a}{1 - \beta^T a} \beta, \quad (17)$$

where $\beta^T = b^T Z$. Hence, the steady-state distribution of the rank-one perturbation is expressed in terms of π and Z , the steady-state distribution and fundamental matrix of the original network. Thus, for rank-one perturbations to regulatory functions, we have an explicit way to compute the exact long-run sensitivity defined in (15).

We will now consider some special cases and derive the exact analytic long-run sensitivity.

1) 1-predictor function perturbations: Let us begin by recalling the sensitivity definition for perturbations defined in [24], [27]. For a Boolean function f in a random Boolean network, the average sensitivity s^f is the expected number of Hamming neighbors of gene states $\mathbf{x}$ on which the function value is different:

$$s^f = \mathbb{E}_{\mathbf{x}}[s^f(\mathbf{x})] = \sum_{i=1}^n \mathbb{E}_{\mathbf{x}} \left[\left| \frac{\partial f(\mathbf{x})}{\partial x_i} \right| \right],$$

where $\mathbb{E}_{\mathbf{x}}[\cdot]$ takes the expectation with respect to the distribution of $\mathbf{x}$; $\frac{\partial f(\mathbf{x})}{\partial x_i}$ is the partial derivative of Boolean function f to x_i defined by $\frac{\partial f(\mathbf{x})}{\partial x_i} = \mathbf{1}[f(\mathbf{x} \oplus e_i) \neq f(\mathbf{x})]$, which is also a Boolean function. Letting e_i denote the elementary vector with a 1 in the i th position and 0s elsewhere, $\mathbf{x} \oplus e_i$ defines a Hamming neighbor with the i th bit different from $\mathbf{x}$. With some intuitive reasoning, we can get [24], [27] that $s^f = 2np_b(1 - p_b)$, where we assume that the truth table of f with 2^n output values follows an independent and identically distributed *Bernoulli* (p_b) distribution, where p_b is known as the bias for the Boolean function f . Clearly, s^f measures the Hamming distance before and after we flip x_i 's expression state, which provides a measure of different network trajectories. This measure can be used to check whether a Boolean network is chaotic or ordered: With $s^f > 1$, the network is chaotic; the network is ordered if $s^f < 1$.

Since our interest is with steady-state distribution change when we perturb a regulatory function, we take a different route to define sensitivity. For a PBN with multi-output function $\mathbf{F}$, consider the multi-output Boolean function $\mathbf{f}_j$ for the j th constituent BNp. Instead of flipping gene state x_i , we alter the truth table directly by $\mathbf{f}_j(\mathbf{x} \oplus e_i)$ for a particular predictor state $\mathbf{x}$ (for BNps there is only one network function $\mathbf{f}$). This leads to the following definition:

Definition 3—The **long-run sensitivity** with respect to a 1-predictor function perturbation $\mathbf{f}_j(\mathbf{x} \oplus e_i)$ for a given state $\mathbf{x}$ for a PBN defined by $P(\mathbf{F}, p, q, \mathbf{c})$ is given by

$$\omega_b[\mathbf{f}_j, \mathbf{x}, e_i] = \|\pi^T(\tilde{\mathbf{F}}_j[\mathbf{x}], p, q, \mathbf{c}) - \pi^T(\mathbf{F}, p, q, \mathbf{c})\|_l \quad (18)$$

where $\tilde{\mathbf{F}}_j[\mathbf{x}]$ differs from $\mathbf{F}$ only in $\mathbf{f}_j$ for the state $\mathbf{x}$ by replacing $\mathbf{f}_j(\mathbf{x})$ by $\mathbf{f}_j(\mathbf{x} \oplus e_i)$ and $\|\cdot\|_l$ denotes the l -norm (we use the l_1 norm in our simulations). Letting Π denote the class of all such perturbations, the **average long-run sensitivity** is defined by

$$\omega_b[\Pi] = \frac{1}{n} \sum_{j=1}^m c_j \sum_{i=1}^n \mathbb{E}_{\mathbf{x}}[\omega_b(\mathbf{f}_j, \mathbf{x}, e_i)] \quad (19)$$

A 1-predictor function perturbation on $\mathbf{f}_j$ simply replaces the output state for the predictor state $\mathbf{x}$ with the output state for the predictor $\mathbf{x} \oplus e_i$ in the truth table. If we ignore the perturbation part in (1) and (2), then each row in the transition matrix corresponds to a single input state $\mathbf{i}$ and the row consists of all 0s except for a 1 in the column of the output state $\mathbf{f}(\mathbf{i})$ in the BNp case, c_j 's ($1 \leq j \leq m$) in several columns for instantaneously random PBNs, or more complicated non-zero values for context-sensitive PBNs. We note here that we use the bold font to denote the gene states in BNs while we write them in the regular font to represent the relative positions in matrices when we use them as the subscripts for matrix entries. The 1-predictor perturbation $\tilde{\mathbf{f}}_j(\mathbf{x}) = \mathbf{f}_j(\mathbf{x} \oplus e_i)$ from $\mathbf{f}_j$ for input state $\mathbf{x}$ means that $\mathbf{v} = \mathbf{f}_j(\mathbf{x} \oplus e_i) = \tilde{\mathbf{f}}_j(\mathbf{x}) \neq \mathbf{f}_j(\mathbf{x}) = \mathbf{u}$ and $\tilde{\mathbf{f}}_j(\mathbf{i}) = \mathbf{f}_j(\mathbf{i})$ for all $\mathbf{i} \neq \mathbf{x}$. This perturbation changes the transition matrix P by a special rank-one perturbation. Based on this observation, we can apply the analytic solution in Theorem 2 to derive the following theorem (proof in the appendix):

Theorem 3—For 1-predictor function perturbations, the long-run sensitivity can be computed with a closed form for BNps, instantaneously random PBNs, context-sensitive PBNs as follows:

1. For BNps and instantaneously random PBNs,

$$\omega_b[\mathbf{f}_j, \mathbf{x}, e_i] = \left\| \frac{\varepsilon \pi_x(z_v - z_u)}{1 - \varepsilon(z_{vx} - z_{ux})} \right\|_l, \quad (20)$$

where z_u and z_v are the u th and v th row, respectively, in the fundamental matrix Z of the original network; and π_x is the stationary mass for the state $\mathbf{x}$ in the original network. For BNps, $\varepsilon = (1 - p)^n$; while for instantaneously random PBNs, $\varepsilon = (1 - p)^n c_j$.

2. For context-sensitive PBNs,

$$\omega_b[\mathbf{f}_j, \mathbf{x}, e_i] = \left\| \frac{(1 - p)^n \pi^T a(z_{(j-1)2^n+v} - z_{(j-1)2^n+u})}{1 - (1 - p)^n (z_{(j-1)2^n+v} - z_{(j-1)2^n+u})^T a} \right\|_l, \quad (21)$$

where $z_{(j-1)2^n+u}$ and $z_{(j-1)2^n+v}$ are again the two rows of the fundamental matrix Z corresponding to states $(j, \mathbf{u})$ and $(j, \mathbf{v})$, respectively, in the PBN; and a is a $m2^n$ -dimensional vector with

$$a[y] = \begin{cases} \frac{q^c_j}{\sum_{k \neq j} q^c_k} & \text{if } y = (r-1)2^n + x, \text{ and } r = 1, \dots, j-1, j+1, \dots, m \\ (1-q) & \text{if } y = (r-1)2^n + x, \text{ and } r = j \\ 0 & \text{otherwise.} \end{cases}$$

Since we now have an analytic solution for the long-run sensitivity, we can compute the average long-run sensitivity efficiently. Note that we have defined the average long-run sensitivity in this case as averaging over all constituent networks, target genes, and input states, but one could, if desired, average with respect to any of these.

2) 1-bit function perturbations: Again, our interest is with steady-state distribution change when we perturb the regulatory function. A simple type of function perturbation is just to change one bit in the truth table for the multi-output function $\mathbf{F}$. For example, we can simply flip the output for the Boolean function f_j^i that decides the gene state x_i in the j th constituent BNp at a particular state $\mathbf{x}$. Thus, we alter the function by $\mathbf{f}_j(\mathbf{x}) \oplus e_i$ (for BNps, there is only one network function $\mathbf{f}$). This leads to the following definition:

Definition 4—The **long-run sensitivity** with respect to a 1-bit function perturbation $\mathbf{f}_j(\mathbf{x}) \oplus e_i$ for a given state $\mathbf{x}$ for a PBN defined by $P(\mathbf{F}, p, q, \mathbf{c})$ is given by

$$\omega_f[f_j^i, \mathbf{x}] = \|\pi^T(\tilde{\mathbf{F}}_j^i[\mathbf{x}], p, q, \mathbf{c}) - \pi^T(\mathbf{F}, p, q, \mathbf{c})\|_l \quad (22)$$

where $\tilde{\mathbf{F}}_j^i[\mathbf{x}]$ differs from $\mathbf{F}$ only in f_j^i for the state $\mathbf{x}$ and $\|\cdot\|_l$ denotes the l -norm (we use the l_1 norm in our simulations). Letting Π again denote the class of all such perturbations, the **average long-run sensitivity** is defined by

$$\omega_f[\Pi] = \frac{1}{n} \sum_{j=1}^m c_j \sum_{i=1}^n \mathbb{E}_{\mathbf{x}}[\omega_f[f_j^i, \mathbf{x}]]$$

A 1-bit function perturbation on $\mathbf{f}_j$ flips one target gene at the response side for the predictor state $\mathbf{x}$ in the truth table. Similar as the derivation for the previous definition of the long-run sensitivity for 1-predictor function perturbations, the 1-bit perturbation $\tilde{\mathbf{f}}_j(\mathbf{x}) = \mathbf{f}_j(\mathbf{x}) \oplus e_i$ from $\mathbf{f}_j$ for input state $\mathbf{x}$ means that $\mathbf{v} = \tilde{\mathbf{f}}_j(\mathbf{x}) \neq \mathbf{f}_j(\mathbf{x}) = \mathbf{u}$ and $\tilde{\mathbf{f}}_j(\mathbf{i}) = \mathbf{f}_j(\mathbf{i})$ for all $\mathbf{i} \neq \mathbf{x}$. Here, $\mathbf{u}$ and $\mathbf{v}$ differ only for 1 bit. Hence, this type of function perturbation changes the transition matrix for either BNps, instantaneously random PBNs or context-sensitive PBNs by either a single row perturbation or a rank-one perturbation in the exactly same form as in the previous definition. Therefore, we can use the same equations (20) and (21) to compute the long-run sensitivity for 1-bit function perturbations.

To further emphasize our objective, this new long-run sensitivity definition takes the steady-state distribution as the important measure reflecting the long-run behavior of a PBN. Hence, it can give insight on the controllability of PBNs with different network properties. It is also straightforward to extend this definition for the long-run sensitivity for multiple-bit function perturbations and other more general perturbations.

3) Function perturbations considering one gene regulation: There exist biochemistry techniques to suppress or express certain genes in living cells. With this type of intervention in mind, we want to study network sensitivity considering either permanent blocking or expressing a gene in the network, this being an ideal case as the available techniques do not work perfectly. We consider perturbing $\mathbf{F}$ by setting all the output values of f_j^i equal to either 0 or 1.

Definition 5—The **long-run sensitivity** with respect to 1-gene function perturbation in $\mathbf{F}$ for a given PBN defined by $P(\mathbf{F}, p, q, \mathbf{c})$ can be described by

$$\omega_g[f_j^i] = \max \left\{ \|\pi^T(\tilde{\mathbf{F}}_j^{i,0}, p, q, \mathbf{c}) - \pi^T(\mathbf{F}, p, q, \mathbf{c})\|_l, \|\pi^T(\tilde{\mathbf{F}}_j^{i,1}, p, q, \mathbf{c}) - \pi^T(\mathbf{F}, p, q, \mathbf{c})\|_l \right\}, \quad (23)$$

where $\tilde{\mathbf{F}}_j^{i,0}$ differs from $\mathbf{F}$ by suppressing gene x_i with $f_j^i(\mathbf{x})=0$ for any $\mathbf{x}$ in the j th constituent network and $\tilde{\mathbf{F}}_j^{i,1}$ differs from $\mathbf{F}$ by expressing gene x_i with $f_j^i(\mathbf{x})=1$ for any $\mathbf{x}$ in the j th constituent network and $\|\cdot\|_l$ denotes the l -norm (we use the l_1 norm in our simulations). Letting Γ denote the class of all such perturbations, the **average long-run sensitivity** is defined by

$$\omega_g[\Gamma] = \frac{1}{n} \sum_{j=1}^m c_j \sum_{i=1}^n \omega_g[f_j^i].$$

A 1-gene function perturbation changes the transition matrix P in multiple rows for BNPs, instantaneously random PBNs, and context-sensitive PBNs. Theorem 2 cannot be directly applied to obtain the analytic solution; however, we can extend Theorem 2 by an iterative procedure to compute the steady-state distribution difference, as shown in [9], [21]. To be succinct, let P^w , Z^w and π^w be the transition matrix, fundamental matrix and steady-state distribution, respectively, after w single-row (or more general rank-one) perturbations. Let $P^{w+1} = P^w + e_{w+1} b_{w+1}^T$ be the transition matrix with the next single-row perturbation. We can compute π^w and Z^w using the results in [9], [21] to update the new steady-state distribution and fundamental matrix iteratively.

The computation of the steady-state distribution difference can still be accomplished analytically as a sequential procedure; however, for complicated perturbations, the computation for the iterative algorithm is in the form of vector-matrix multiplication and the complexity of the procedure increases linearly with the increasing number of perturbations.

We next study the properties of the various long-run sensitivity definitions for networks possessing different properties.

IV. Long-run Sensitivity with Randomly Generated PBNS

In this section, we examine the different definitions of long-run sensitivity. As with ensemble analysis in [12], [24], [27], we study long-run sensitivity based on a large number of randomly generated networks with similar network properties. The two most important parameters for generating random Boolean networks are the bias (p_b) and connectivity (K). Again, p_b is the mean of the *Bernoulli* distribution to generate the truth table of one Boolean function in a Boolean network and K is the maximum input degree of the Boolean functions in the network.

All simulation results in this subsection are based on 1,000 randomly generated Boolean networks or PBNs with different p_b 's and K 's. We compute the l_1 -norm in our experiments.

A. Sensitivity with respect to rule perturbations

We first consider long-run sensitivity (LRS) with respect to rule perturbations, beginning with simulations of Boolean networks possessing 6 nodes (representing 6 genes).

We start with the average LRS, $\omega_b[\Pi]$, in Definition 3 and compare it to the average sensitivity, s^f , in [24], which reflects transient BN dynamics. We focus on BNPs to illustrate the impact of different parameters. In Fig. 1(a) and (b), we plot the change of average LRS with respect to p_b and K , respectively. In general, long-run sensitivity is a complex function of regulatory functions $\mathbf{F}$, perturbation probability p , and the other parameters. We plot average LRS for different p values. With small p values ($p = 0.0005$), BNPs inherit the original attractor structures of their corresponding Boolean networks. As shown in [24], [27], $s^f = 2Kp_b(1 - p_b)$. Simulated results in Fig. 2 with the same sets of networks shows the networks are more chaotic with increasing connectivity K or when $p_b \rightarrow 0.5$. Note that s^f does not change with p since the original BNs do not depend on p .

To gain a better appreciation of long-run sensitivity, we plot the average number of attractors ($|A|$) with respect to p_b and K in Fig. 3 and the average number of changed attractors ($|DA|$) by 1-predictor function perturbation in Fig. 4. Since these numbers do not change with p , we only plot the results for one set of 1,000 random BNs. From these figures, it is clear that there are more attractors with increasing K or when $p_b \rightarrow 0.5$. At the same time, the average number of changed attractors also increases with increasing K or with $p_b \rightarrow 0.5$. Hence, with small p values, the steady-state distribution difference comes mainly from the mass shift for these attractors. With increasing K or $p_b \rightarrow 0.5$, the number of changed attractors increases. Thus, the steady-state distribution difference increases and $\omega_b[\Pi]$ increases. However, when p gets large, $\omega_b[\Pi]$ shows different trends. With increasing perturbation probability p , the state can flip with larger probability, so that the attractor states can jump out of attractor cycle more easily. Thus, there will be more steady-state mass in transient states and the total steady-state distribution difference can come from the difference of steady-state mass in those transient states. In addition, the change caused by changed attractors by 1-predictor function perturbation is relatively small (almost less than 1) as shown in Fig. 4. Hence, with larger K or p_b close to 0.5, there are less transient states, as shown in Fig. 3 indirectly, and the resulting steady-state distribution difference is more complicated, as shown in Fig. 1. With increasing K or $p_b \rightarrow 0.5$, the average LRS, in fact, decreases when p is large. If we think of different $\omega_b[\Pi]$ plots in Fig. 1 as cross-sections of the function surface $\omega_b[\Pi](p_b, K, p)$, it is clear that this function is a complex nonlinear function. In addition, when $p = 0.5$, indicating that the state flips very often, we can see that a 1-predictor function perturbation does not significantly perturb the steady-state distributions (the maximum value here being 0.0005) as we can consider all the transient states. This phenomenon indicates PBNs are hard to control (perturb) when the perturbation probability p is large.

For 1-bit function perturbations, we plot the change of average long-run sensitivity, $\omega_f[\Pi]$, in Definition 4 with respect to p_b and K in Fig. 5(a) and (b), respectively. We see a different trend in Fig. 5(a) compared to the average LRS for 1-predictor function perturbation in Fig. 1(a). The reason is that 1-bit function perturbation changes the bias p_b of the regulating functions $\mathbf{F}$ in BNs and the amount of bias change directly determines the change to steady-state distributions as shown in the previous simulations. To illustrate the bias change caused by 1-bit function perturbations, we plot the absolute difference between the original bias p_b and $\tilde{p}_b$ after 1-bit function perturbations ($\Delta p_b = |\tilde{p}_b - p_b|$) in Fig. 6 for 1,000 random BNs. Figure 6(a) shows that the bias change is becoming smaller when $p_b \rightarrow 0.5$ as 1-bit function perturbations have small impact considering the ratio change between 1's and 0's in truth tables

when p_b is 0.5 while they can have relative large impact when there are either less 1's or 0's. While with different K 's, the bias changes caused by 1-bit function perturbations are similar and relatively small as shown in Fig. 6(b). Thus, the change of average LRS for 1-bit function perturbations with respect to the connectivity K (Fig. 5(b)) is similar as the LRS change shown in Fig. 1(b) for 1-predictor function perturbations. Also similarly as previous simulations, the long-run sensitivity decreases with increasing perturbation probability p .

We consider long-run sensitivity with respect to 1-gene regulation, $\omega_g[\Gamma]$, in Definition 5. The change of $\omega_g[\Gamma]$ with respect to p_b and K is illustrated in Fig. 7. Because we are either suppressing or forcing the expression of one gene, the number of bits changed in the truth table (Boolean functions) depends on p_b . With $p_b \rightarrow 0.5$, the number of changed bits is minimum and thus the steady-state distribution difference, or $\omega_g[\Gamma]$, is minimum. With increasing K , as in the case of $\omega_b[\Pi]$, $\omega_g[\Gamma]$ increases when p is small because the steady-state mass shift mainly comes from the change of attractors by function perturbation. When p is large, because the number of transient states in BNs decreases with K and the steady-state distribution difference mainly comes from transient states, $\omega_g[\Gamma]$ decreases with increasing K . Again, when $p = 0.5$, all states become transient and it is hard to control these networks.

B. Sensitivity with respect to probabilistic parameters

Continuing with 6-gene BNs, we analyze the LRS with respect to probabilistic parameters (Definition 1). Figures 8(a) and (b) show the change of $\|\frac{\partial \pi}{\partial p}\|_{l_1}$ with respect to p_b and K , respectively. Once again, with small p values, the LRS with respect to p increases with increasing K or $p_b \rightarrow 0.5$. This is because there are more attractors with large K or p_b close to 0.5, as shown in Fig. 3. Perturbation to p results in changes in the steady-state distributions mainly from the steady-state mass change of attractors in BNs. With p increasing, the steady-state distribution difference is dominated by the mass shift for transient states in BNs. Another important feature in the figure is that the values of $\|\frac{\partial \pi}{\partial p}\|_{l_1}$ are relatively large but they decrease with increasing p . We now replace the partial derivatives by the finite difference values to show how the steady-state distribution changes by perturbing p . Using the standard differential approximation

$$\|\Delta \pi\|_{l_1} \approx \Delta p \left\| \frac{\partial \pi}{\partial p} \right\|_{l_1} \quad (24)$$

Assuming that we perturb p by $\Delta p = 0.1p$, we find that $\|\Delta \pi\|_{l_1}$ is very small (with the maximum about 0.03 in this set of simulations). Hence, inaccuracy in the estimation of p during network inference does not significantly affect the steady-state distribution. In other words, the long-run network dynamics are very robust with respect to p .

Figures 9(a) and (b) show the change of $\|\frac{\partial \pi}{\partial c_j}\|_{l_1}$ with respect to p_b and K , respectively. In this set of experiments, we use instantaneously random PBNs for simulations, where there are 5 constituent BNs in each PBN with randomly assigned selection probabilities c_j with $\sum_j c_j = 1$. Therefore, the expectation of c_j is 0.2. Considering the absolute values of $\|\frac{\partial \pi}{\partial c_j}\|_{l_1}$ in the figure, the long-run behavior of PBNs is again robust to c_j estimates. For instantaneously random PBNs, each state in the network can have different properties in different constituent BNs. It can be transient in one BN while belonging to an attractor cycle in another. Hence, on account of instantaneously random perturbations, much of the steady-state mass does not lie within the attractor cycles. Therefore, based on the previous explanations, with increasing K or p_b close to 0.5, the steady-state mass at transient states decreases and the LRS decreases. We show the

similar trend in Fig. 9 even with small p value ($p = 0.0005$). To further emphasize, we can clearly see that LRS is a complex nonlinear function of p_b, K, p , and c .

Finally, we plot $\|\frac{\partial \pi}{\partial q}\|_{l_1}$ in Fig. 10 and Fig. 11. In these simulations, we use context-sensitive PBNs with 2 constituent BNs. In Fig. 10, we fix $p = 0.0005$. The figures illustrate that when we have small $q = 0.0005$, indicating that switches between constituent BNs are rare, the LRS is large and with increasing q values, $\|\frac{\partial \pi}{\partial q}\|_{l_1}$ decreases. When q is very small, a small increase in q results in a multiplicative factor on q and this same factor is applied to networks switches, which has a large impact on π , relative to a small Δq . This factor is much smaller relative to Δq for large q . We also see that the LRS increases as a function of decreasing K and $p_b \rightarrow 0.5$, notably for small q . We observe some similar trends in Fig. 11 when we fix $q = 0.001$ and have different perturbation probability p values. To begin with, the LRS decreases with increasing p values. In both Fig. 10(a) and Fig. 11(a), we see that the LRS increases when $p_b \rightarrow 0.5$. When p_b is close to 0.5, there are more possible attractors and switching to different constituent networks will lead to steady-state distribution change from these attractors. The LRS is large with small K in both Fig. 10(b) and Fig. 11(b) when q is small. The change to steady-state distributions caused by perturbing q can be considered having similar effects by changing K since q controls the probability of switching between constituent BNs. Hence, the change to the steady-state distribution caused by perturbation to q is relatively small with large K . If we compute the approximate $\|\Delta \pi\|_{l_1}$ caused by perturbed q with $\Delta q = 0.1q$, the maximum value is about 0.001 in Fig. 11 and 0.04 in Fig. 10. Therefore, the long-run behavior of PBNs is again robust to q .

C. Simulations for larger networks

We now plot the change of different LRS measures for randomly generated Boolean networks with 8 nodes. Since, with $p = 0.5$, all the LRS measures are very small, we do not consider this situation for 8-gene networks. We plot these LRS measures in the exact same settings as with 6-gene networks. Figures 12, 13 14, 15, 16, 17 and 18 show similar trends as in the figures for 6-gene networks.

Next, for networks with 10 nodes, we only plot different LRS measures with one setting, $p = 0.0005$, and plot them in Figs. 19, 20, 21, 22, and 23. To further illustrate the similar trends of LRS change, we plot $\|\frac{\partial \pi}{\partial q}\|_{l_1}$ for 6-, 8-, and 10-gene networks together in Fig. 24, where $p = 0.001$ and $q = 0.001$. We see that the values of $\|\frac{\partial \pi}{\partial q}\|_{l_1}$ are very similar.

V. Sensitivity and Robustness of Control

Since the l-norm distance between the steady-state distributions constitutes one measure of network closeness, and therefore a measure to quantify inference accuracy [5], the LRS can *ipso facto* be used in the analysis of inference robustness with respect to various network parameters, and we have considered this point in the preceding section. Instead of writing (24) in its approximation form, let us rewrite it in terms of a bound on the difference between the two steady-state distributions,

$$\|\pi_{p'} - \pi_p\|_{l_1} \leq \left\| \frac{\partial \pi}{\partial p}(p) \right\|_{l_1} |p' - p| + \varepsilon(p' - p) \quad (25)$$

where $\pi_{p'}$ and π_p are the steady-state distributions with perturbation parameters p' and p , respectively, and $\frac{\varepsilon(p' - p)}{p' - p} \rightarrow 0$ as $p' - p \rightarrow 0$. Suppose now that $\pi_{p'}$ and π_p correspond to the estimated PBN and actual PBN, respectively. If we are interested in finding the 1-bit optimal

structural intervention [21] then in practice this intervention is determined on the estimated PBN.

Referring to (25), let $\pi_{p'}[f_j^i, \mathbf{x}]$ and $\pi_p[f_j^i, \mathbf{x}]$ denote the steady-state distributions resulting from the optimal structural intervention derived from the estimated PBN on $\pi_{p'}$ and π_p , respectively, meaning that the intervention is derived via the estimated PBN but also applied to the original PBN. Analogously, let $\pi_{p'}[f_s^r, \mathbf{y}]$ and $\pi_p[f_s^r, \mathbf{y}]$ denote the steady-state distributions resulting from optimal structural intervention derived from the original PBN applied on $\pi_{p'}$ and π_p , respectively. We would like to know that $\|\pi_{p'}[f_j^i, \mathbf{x}] - \pi_p[f_s^r, \mathbf{y}]\|_{l_1}$ is small, because this would mean that applying the intervention on the estimated PBN yields a steady-state distribution close to the optimal one we would obtain if we actually knew the true PBN. Application of the triangle inequality yields

$$\begin{aligned} \|\pi_{p'}[f_j^i, \mathbf{x}] - \pi_p[f_s^r, \mathbf{y}]\|_{l_1} &\leq \|\pi_{p'}[f_j^i, \mathbf{x}] - \pi_p[f_j^i, \mathbf{x}]\|_{l_1} + \|\pi_p[f_j^i, \mathbf{x}] - \pi_p[f_s^r, \mathbf{y}]\|_{l_1} \\ &\leq \left\| \frac{\partial \pi_p[f_j^i, \mathbf{x}]}{\partial p} \right\|_{l_1} (p') \|p' - p\| + \varepsilon (p' - p) + \omega_b[f_j^i, \mathbf{x}](p) + \omega_b[f_s^r, \mathbf{y}](p) \end{aligned}$$

Hence, the norm of interest is bounded in terms of long-run sensitivities for the probability parameter and 1-bit perturbations, along with the fast-converging term $\varepsilon (p' - p)$. The sensitivities can be computed as discussed previously and standard methods of calculus can be used to obtain $\varepsilon (p' - p)$. Other probabilistic parameters can be treated similarly.

VI. Sensitivity and Robustness in Biological Applications

In this section, we use biologically derived networks to illustrate the relationship between long-run sensitivity (in particular, with respect to the perturbation probability) and the robustness of optimal intervention strategies, the expectation being that greater long-run sensitivity means less robust intervention strategies.

A. Mammalian Cell Cycle Network

We first study a probabilistic Boolean network (PBN) model of the mammalian cell cycle recently proposed in [8]. For a normal mammalian organism, cell division should coordinate with overall growth. This process is tightly controlled via extra-cellular signals. These signals indicate whether a cell should divide or remain in a resting state. The positive signals, or growth factors, instigate the activation of Cyclin D (CycD), which is one of the key genes in the mammalian cell cycle. The other two important genes are retinoblastoma (Rb) and p27. Rb is a tumor-suppressor gene. This gene is expressed in the absence of the cyclins, which inhibit Rb by phosphorylation. Gene p27 is also active in the absence of the cyclins. Whenever p27 is present, it blocks the action of CycE or CycA and Rb can also be expressed, even in the presence of CycE or CycA. Hence, it stops the cell cycle.

The preceding explanation represents the wild-type cell cycle model. In this model, when p27 is active, the cell cycle can be stopped in cancerous situations. Hence, we can define the logical states in which all of CycD, Rb, p27 are down-regulated as undesirable states since the cell can cycle forever even in the absence of any growth factor. The goal of intervention is to minimize the stationary mass for the undesirable states.

We construct the instantaneously random PBN of the cell cycle based on the Boolean functions given in Table I, reproduced from [8]. This PBN consists of 10 genes: CycD, Rb, p27, E2F, CycE, CycA, Cdc20, Cdh1, UbcH10, and CycB. The regulatory graph is shown in Fig. 25. The above order of genes is used in the binary representation of the logical states, with CycD as

the most significant bit and CycB as the least significant bit. The order of genes in the logical states does not affect our analysis or intervention. We assume that the extra-cellular signal to the cell cycle model is a latent variable. The growth factor is not part of the cell and its value is determined by the surrounding cells. The expression of CycD changes independently of the cell's content and reflects the state of the growth factor. Depending on the expression status of CycD, we obtain two constituent Boolean networks. The first constituent Boolean network is determined based on the Boolean functions in Table I when the value of CycD is equal to 0. Similarly, the second constituent Boolean network is determined by setting the value of CycD to 1. To completely define the PBN, we set the perturbation probability $p = 0.01$, and the probability of selecting each constituent Boolean network $c_j = 0.5, j = 1, 2$.

We first compute the proposed long-run sensitivities with respect to the changes to all the elements (including the underlying regulatory functions and involved probabilistic parameters) in this instantaneously random PBN model. In Table II, we give the values of the long-run sensitivities with respect to the perturbation probability p , BN selection probability c_1, c_2 , and the long-run sensitivities for 1-predictor function perturbations, 1-bit function perturbations, and 1-gene function perturbations. Comparing with the previous plots for random networks, this real biological network appears to be less sensitive to all these perturbations.

We then apply the proposed structural intervention in [21] to avoid the logical states with simultaneously down-regulated CycD, Rb and p27. In other words, we aim to steer the network dynamics away from these undesirable states, where $x_1 = 0, x_2 = 0$, and $x_3 = 0$. Here, we search for the optimal 1-bit function perturbation to hinder the cell growth and minimize $\sum_{x_1=0, x_2=0, x_3=0} \pi(\mathbf{x})$. We check all possible controllable functions to find the one perturbation that minimizes this objective function. We list the objective function values with the optimal 1-bit function perturbations respectively for each f_i in the two constituent Boolean networks in Table III (As CycD is determined by extra-cellular signals, we can not perturb it and therefore the table does not have CycD included.) From the table, we see that the perturbations to the function regulating Rb significantly reduce the steady-state mass of the undesirable states. Flipping the output expression in the truth table for the second Boolean network leads to the minimum objective function value. Hence, in practice, we can choose to intervene the function regulating Rb to shift the stationary mass away from undesirable states. If we further check Table III, the function regulation E2F is another possible choice.

To test the robustness of this optimal intervention strategy with respect to the perturbation probability p , we solve the same optimization problem with different perturbation probabilities: $p = 0.001, 0.01, 0.02, 0.03, 0.04, 0.05$. For $p \leq 0.04$, we obtain the same optimal intervention strategy; however, for $p = 0.05$ we obtain a different strategy.

Next, we follow one of the proposed mutations in [8], in which p27 is mutated and it is always off. In this cancerous scenario, p27 can never be activated. This mutation introduces a situation where both CycD and Rb might be inactive. As a result, in this mutated phenotype, the cell cycles in the absence of any growth factor. In other words, we consider the logical states in which both Rb and CycD are down-regulated as undesirable states, when p27 is mutated.

We use the PBN that postulates the cell cycles with mutated phenotype in our experiments. Table IV summarizes the mutated Boolean functions derived from the functions given in [8]. Using the same parameters (with $p = 0.01$) in the previous wild-type PBN, We construct the instantaneously random PBN of the cell cycle based on the Boolean functions in Table IV with mutated p27. This PBN consists of 9 genes. The illustration of the relationship between these genes in the PBN is shown in Fig. 26.

We again compute the proposed long-run sensitivities, which are illustrated in Table V, with respect to the changes to both the underlying regulatory functions and probabilistic parameters

in the new PBN. From these values, it is obvious that the mutated PBN is more stable (and therefore more resistant to intervention) compared to the original PBN. In particular, the mutated PBN is less sensitive to the perturbation probability p .

We apply structural intervention to avoid the logical states with simultaneously down-regulated CycD and Rb in this cancerous scenario. We now aim to steer the network dynamics away from the undesirable states, where $x_1 = 0$ and $x_2 = 0$. Here, we search for the optimal 1-bit function perturbation to minimize $\sum_{x_1=0, x_2=0} \pi(\mathbf{x})$. We also list the objective function values with the optimal 1-bit function perturbations, respectively, for each f_i in the two constituent Boolean networks in Table VI. Again, the perturbation to the function regulating Rb in the second context BN significantly reduces the steady-state mass of the undesirable states and we can intervene with the function regulating Rb to shift the stationary mass away from undesirable states. The function regulating E2F is again the second optimal target for applying intervention. Note that the ranking of the values in Table III and Table VI remains the same.

Regarding robustness of this optimal intervention strategy with respect to the perturbation probability p , as in the previous wild-type network we solve the same optimization problem with different perturbation probabilities: $p = 0.001, 0.01, 0.02, 0.03, 0.04, 0.05$. In all cases, we obtain the same optimal strategy. This differs from the wild-type case, where a different optimal strategy is obtained for $p = 0.05$. Comparing the long-run sensitivity $\|\frac{\partial \pi}{\partial p}\|_{l_1}$ in the two PBNs, the value (19.6017) in the mutated network is smaller than the value (25.9195) in the wild-type network. The greater sensitivity in the wild-type network is consistent with its being less robust with regard to optimal intervention across different perturbation probabilities.

B. Melanoma-related Network

In this section, we consider a network derived from gene expression data collected in a study of metastatic melanoma [1], [28]. In this study, it has been shown that experimentally increasing the levels of the WNT5A protein secreted by a melanoma cell line via genetic engineering methods directly alters the metastatic competence of that cell as measured by the standard *in vitro* assays for metastasis. Furthermore, blocking the WNT5A protein from activating its receptor by the use of an antibody that binds the WNT5A protein could substantially reduce WNT5A's ability to induce a metastatic phenotype. This suggests that a reasonable control strategy would be to reduce the WNT5A gene's action in affecting biological regulation, since the available data suggests that disruption of this influence could reduce the chance of a melanoma metastasizing, a desirable outcome. It is precisely because we have a criterion of desirability, specifically, the down-regulation of WNT5A, that networks involving WNT5A have been used in a number of control studies [4], [18], [21], [29].

A previous study in [15] chose a set of 587 well-measured genes that show sufficient changes in expression values over 31 samples of melanoma patients. The authors quantified the multivariate relationships among them. The set of genes was filtered and reduced to the set of 10 genes on the basis of their close predictive relationships, and either their known or likely roles in the WNT5A driven induction of metastasis in melanoma cells. They obtained the wiring diagram as illustrated in Fig. 27 and constructed a ten-gene regulatory network that contains the genes WNT5A, pirin, S100P, RET1, MMP3, PHOC, MART1, HADHB, synuclein, and STC2, which we label as $x_1, x_2, \dots, x_{10}$, respectively. In [18], due to exponentially increasing complexity of dynamic programming with the number of genes, the authors approximated a ten-gene PBN with a seven-gene PBN. They proposed to exert external controls to steer the network dynamics towards desirable states, where $x_1 = 0$ (WNT5A not expressed). Here we apply function perturbations [21] to the ten-gene network to permanently change the network structure and thereafter the long-run behavior of the network. We note that we have selected a Boolean network whose states with up-regulated WNT5A possess larger aggregated

stationary mass to arrive at a network resembling a cancerous situation. With that, control of the network resembles a therapeutic situation in which the goal of the control is to reduce the likelihood of reaching undesirable states so that the risk of metastasis decreases.

We compute the proposed long-run sensitivities for the BNp with $p = 0.01$ with respect to the perturbation probability, 1-predictor function perturbations, 1-bit function perturbations, and 1- gene function perturbations. We note that the contents in Table VII are different from the values in the mammalian cell cycle network since here we use a BNp to model the melanoma-related network. With a single Boolean network, we can closely study its BOA structure for the network intervention.

To reduce the risk of metastasis, we apply intervention to the network. While the intervention strategy to minimize the stationary mass for the undesirable states in the previous section appears obvious, it has the effect of significantly altering the attractor structure. The intervention could introduce new attractors. If we lack knowledge of phenotypes corresponding to these new attractors, we may not want such a large mass associated with little known phenotypes. Since we have clear understanding of the BOA structure of the selected Boolean network, we can use an intervention strategy that avoids this problem. The original network has four attractors possessing the following steady-state probabilities: $\pi(0010001110) = 0.2571$, $\pi(0100110001) = 0.2382$, $\pi(0101110011) = 0.1394$, and $\pi(1011101110) = 0.1276$ with the perturbation probability $p = 0.01$. Since we want to down-regulate WNT5A, the attractor 1011101110 is undesirable and we want to reduce its steady-state probability. In addition, if we do not want to introduce other attractors that might lead to unexpected cellular behavior, then we can maximize the objective function $\tilde{\pi}(0010001110) + \tilde{\pi}(0100110001) + \tilde{\pi}(0101110011)$. The optimal function perturbation in this case is to perturb the function f_4 regulating RET1. The perturbed steady-state probabilities are $\tilde{\pi}(0010001110) = 0.3872$, $\tilde{\pi}(0100110001) = 0.3155$, $\tilde{\pi}(0101110011) = 0.0500$, and $\tilde{\pi}(1011101110) = 7.0147 \times 10^{-4}$. The steady-state mass for the undesirable states is equal to 0.0705. Figure 28(2-b) illustrates that the steady-state distribution shifts from undesirable to desirable states after this 1-bit function perturbation. Clearly, the strategy does not introduce any new attractor. When we check the total stationary mass $\sum_{x_1=1} \tilde{\pi}(\mathbf{x})$ for the undesirable states with $x_1 = 1$ in Table VIII, we can see that the stationary mass is the third smallest one. While the smallest stationary mass 0.0591 is achieved by perturbing the function regulating S100P, it will introduce a new attractor 0101111011, which takes stationary mass $\pi(0101111011) = 0.1289$.

As in the case of the mammalian cell cycle, to examine the robustness of the optimal intervention strategy with respect to the perturbation probability we solve the same optimization problem with different perturbation probabilities: $p = 0.001, 0.01, 0.02, 0.03, 0.04, 0.05$. The same optimal intervention strategy is achieved for $p < 0.03$ and a different strategy is obtained for $p \geq 0.03$. This decreased stability with respect to robustness of intervention is consistent with the fact that the long-run sensitivity with respect to p is greater (41.3624) than that of the mutated cell cycle PBN.

VII. Conclusion

In this paper, we define several measures of long-run sensitivity that focus on the change of steady-state distributions caused by different perturbations, including changes to regulatory functions and changes to probabilistic parameters in PBNs. We have presented ways to compute these measures, provided simulation results, and have discussed a role for long-run sensitivity in network inference and control. The experiments on two real biological networks illustrate the relationship between the new sensitivity measures and the robustness of network intervention. In our future research, we hope to analytically characterize this relationship. We emphasize that our ultimate goal of studying networks is to apply intervention to living

organisms. We and our colleagues at the Translational Genomics Research Institute are currently developing the experimental capability to alter and follow the gene-state trajectories of cancer cells, hoping that in the not too distant future we will be able to implement mathematically derived intervention strategies in living cells.

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Appendix A

Proof of Theorem 1

With perturbation, the dynamics of a BNp can be analyzed as a homogeneous irreducible finite Markov chain $\mathbf{X}_t$ with the state space $\{0, 1\}^n$. The state transitions of a BNp over time are governed by transition rules $\mathbf{f}$ and perturbation. Implicitly, we assume that there is an i.i.d. random perturbation vector $\gamma \in \{0, 1\}^n$, where the i th gene flips if the i th component of γ is equal to 1. Here, $p = P\{\gamma_i = 1\} = E[\gamma_i]$ is the same for all the genes. If $\mathbf{X}_t \in \{0, 1\}^n$ is the state of the network at time t , then the next state $\mathbf{X}_{t+1}$ is either $\mathbf{f}(\mathbf{X}_t)$ with probability of $(1-p)^n$ or $\mathbf{X}_t \oplus \gamma$ with probability $1 - (1-p)^n$ (at least one perturbation), where $\oplus$ is component-wise addition modulo 2. For each pair of states $\mathbf{x}$ and $\mathbf{y}$, We write the transition probability from $\mathbf{y}$ to $\mathbf{x}$ at arbitrary time t as $P_{\mathbf{y}}(\mathbf{x}) = P[\mathbf{X}_{t+1} = \mathbf{x} | \mathbf{X}_t = \mathbf{y}]$. This probability is a weighted sum of two transition probabilities:

$$P_{\mathbf{y}}(\mathbf{x}) = \mathbf{1}_{[\mathbf{f}(\mathbf{y})=\mathbf{x}]}(1-p)^n + p_{\mathbf{y}}(\mathbf{x})[1 - (1-p)^n], \quad (26)$$

where $\mathbf{1}_{[\mathbf{f}(\mathbf{y})=\mathbf{x}]}$ is the indicator function that takes value 1 if $\mathbf{f}(\mathbf{y}) = \mathbf{x}$ according to the truth table and is equal to 0 otherwise, and $p_{\mathbf{y}}(\mathbf{x})$ is the probability that the perturbation moves from $\mathbf{y}$ to $\mathbf{x}$. With $\eta(\mathbf{x}, \mathbf{y})$ as the Hamming distance between $\mathbf{x}$ and $\mathbf{y}$, we can write:

$$p_{\mathbf{y}}(\mathbf{x}) = \mathbf{1}_{[\mathbf{x} \neq \mathbf{y}]} \frac{p^{\eta(\mathbf{x}, \mathbf{y})} (1-p)^{n-\eta(\mathbf{x}, \mathbf{y})}}{1 - (1-p)^n}.$$

Plugging this into (26), we obtain (1). Based on this, we can derive the transition matrix P of the underlying Markov chain by computing all of its matrix entries.

For instantaneously random PBNs, we assume that we select one function from among $\mathbf{f}_1, \dots, \mathbf{f}_m$, representing one of constituent BNps, with probabilities $\mathbf{c} = \{c_1, \dots, c_m\}$ at any time point t . Therefore, the transition probability for the state pair $\mathbf{x}$ and $\mathbf{y}$ can be written according to (2).

The transition probability for context-sensitive PBNs is more complicated because $P_{\mathbf{y}}(\mathbf{x})$ depends on the function $\mathbf{f}_j$ selected at time t . The state space of the underlying Markov chain for context-sensitive PBNs is in fact the joint space of the gene expression states and the constituent BNps. Thus, the transition probability of the underlying homogeneous Markov chain $(B_t, \mathbf{X}_t)$ is given by (3), where r, s denote the r th and s th BNp, which are the BNps at time $t+1$ and t .

Appendix B

Proof of Theorem 3

We start with the proof for the equation (20). We have reasoned that 1-predictor function perturbation on $\mathbf{f}_j$ changes the output state from $\mathbf{u}$ to $\mathbf{v}$ at the predictor state $\mathbf{x}$. In general, $\mathbf{v} = \mathbf{f}_j(\mathbf{x} \oplus \mathbf{e}_i) = \mathbf{f}_j(\mathbf{x}) \neq \mathbf{f}_j(\mathbf{x}) = \mathbf{u}$; while $\mathbf{f}_j(\mathbf{i}) = \mathbf{f}_j(\mathbf{i})$ for all $\mathbf{i} \neq \mathbf{x}$. Hence, there is now (with $\mathbf{f}_j$) a 1 (for BNps) or c_j (for instantaneously random PBNs) in the column of the output state $\mathbf{v}$ and a 0 in the column of state $\mathbf{u}$, whereas originally (with $\mathbf{f}_j$) there was a 0 in the column of the output state $\mathbf{v}$ and a 1 (for BNps) or c_j (for instantaneously random PBNs) in the column of state $\mathbf{u}$, and all other entries in the matrix are unchanged. Consequently, there are changes to the transition matrix P only at p_{xu} and p_{xv} . Thus, $\tilde{p}_{xu} = p_{xu} - \varepsilon$ and $\tilde{p}_{xv} = p_{xv} + \varepsilon$, where ε is decided by the change to the truth table and the perturbation probability p : $\varepsilon = (1-p)^n$ for BNps and $\varepsilon = (1-p)^n c_j$ for instantaneously random PBNs. Hence, we write the perturbation matrix as

$$E = e_x \xi^T,$$

where

$$\xi^T = [0, \dots, 0, \underbrace{-\varepsilon}_u, 0, \dots, 0, \underbrace{\varepsilon}_v, 0, \dots, 0].$$

Insofar as averaging in (19) is concerned, using (17) in Theorem 2 with $a = e_x$ and $b = \xi$ gives the equation (20):

$$\omega_b[\mathbf{f}_j, \mathbf{x}, e_i] = \left\| \frac{\varepsilon \pi_x(z_v - z_u)}{1 - \varepsilon(z_{vx} - z_{ux})} \right\|_l,$$

where z_u and z_v are the u th and v th row, respectively, in the fundamental matrix Z .

For a context-sensitive PBN, each state of the Markov chain is of the form $(r, \mathbf{i})$, where r denotes the r th constituent BNp and $\mathbf{i}$ the gene state vector. Since there are m constituent networks and n genes, the size of the transition matrix is $m2^n \times m2^n$. A 1-predictor perturbation for $\mathbf{f}_j$ causes changes to multiple rows (m rows to be exact). But only two columns are changed, both being columns of the matrix corresponding to the j th constituent BN as the output changes from $(j, \mathbf{u})$ to $(j, \mathbf{v})$. Hence, we can proceed similarly using (17) but now the perturbation matrix is

$$E = a \xi^T,$$

where a and ξ are $m2^n$ -dimensional vectors,

$$\xi^T = [0, \dots, 0, \underbrace{-(1-p)^n}_{(j-1)2^n+u}, 0, \dots, 0, \underbrace{(1-p)^n}_{(j-1)2^n+v}, 0, \dots, 0],$$

and a is defined by

$$a[(r-1)2^n+x] = \frac{q^c_j}{\sum_{k \neq j} c_k}$$

for $r = 1, \dots, m$, and $r \neq j$,

$$a[(r-1)2^n+x] = (1-q)$$

for $r = j$, and $a[y] = 0$ elsewhere. Again, we can compute the average sensitivity efficiently with the same analytic form using Theorem 2 and (17) to get the equation (21):

$$\omega_b[\mathbf{f}_j, \mathbf{x}, e_i] = \left\| \frac{(1-p)^n \pi^T a(z_{(j-1)2^n+v} - z_{(j-1)2^n+u})}{1 - (1-p)^n (z_{(j-1)2^n+v} - z_{(j-1)2^n+u})^T a} \right\|_l,$$

where $z_{(j-1)2^n+u}$ and $z_{(j-1)2^n+v}$ are again the two rows of the fundamental matrix Z corresponding to states $(j, \mathbf{u})$ and $(j, \mathbf{v})$, respectively, in the PBN.

Fig. 1.

The change of average long-run sensitivity ($\omega_b[\Pi]$) with respect to different network properties for 6-gene Boolean networks: (a) Bias (p_b); (b) Connectivity (K).

Fig. 2.

The change of average sensitivity (s^f) defined in [24] with respect to different network properties for 6-gene Boolean networks: (a) Bias (p_b); (b) Connectivity (K). Notice that the simulated average sensitivity values are roughly equal to $2Kp_b(1-p_b)$.

Fig. 3. The average number of attractors ($|A|$) for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 4. The average number of changed attractors ($|DA|$) by 1-predictor function perturbation for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 5. The change of average long-run sensitivity ($\omega_f[II]$) with respect to 1-bit function perturbation for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 6.

The change of bias (p_b) with respect to 1-bit function perturbation for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 7.

The change of long-run sensitivity ($\omega_g[\Pi]$) with respect to 1-gene regulation for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 8.

The change of long-run sensitivity with respect to the perturbation probability $p(\|\frac{\partial \pi}{\partial p}\|_{l_1})$ for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 9.

The change of long-run sensitivity with respect to the selection probability $c_j(\|\frac{\partial \pi}{\partial c_j}\|_{l_1})$ for 6-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 10.

The change of long-run sensitivity with respect to the switching probability $q(\|\frac{\partial \pi}{\partial q}\|_{l_1})$ for 6-gene Boolean networks with different network properties with fixed $p = 0.0005$: (a) Bias (p_b); (b) Connectivity (K).

Fig. 11.

The change of long-run sensitivity with respect to the switching probability q ($|\frac{\partial \pi}{\partial q}|_{l_1}$) for 6-gene Boolean networks with different network properties with fixed $q = 0.001$: (a) Bias (p_b); (b) Connectivity (K).

Fig. 12.

The change of average long-run sensitivity ($\omega_b[\Pi]$) with respect to different network properties for 8-gene Boolean networks: (a) Bias (p_b); (b) Connectivity (K).

Fig. 13.

The change of average long-run sensitivity ($\omega_f[\Pi]$) with respect to different network properties for 8-gene Boolean networks: (a) Bias (p_b); (b) Connectivity (K).

Fig. 14.

The change of average long-run sensitivity ($\omega_g[\Gamma]$) with respect to 1-gene regulation for 8-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 15.

The change of long-run sensitivity with respect to the perturbation probability $p(\|\frac{\partial \pi}{\partial p}\|_{l_i})$ for 8-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 16.

The change of long-run sensitivity with respect to the selection probability $c_j(\|\frac{\partial \pi}{\partial c_j}\|_{l_1})$ for 8-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 17.

The change of long-run sensitivity with respect to the switching probability $q(\| \frac{\partial \pi}{\partial q} \|_{l_1})$ for 8-gene Boolean networks with different network properties with fixed $p = 0.0005$: (a) Bias (p_b); (b) Connectivity (K).

Fig. 18.

The change of long-run sensitivity with respect to the switching probability $q(|\frac{\partial \pi}{\partial q}|_{l_1})$ for 8-gene Boolean networks with different network properties with fixed $q = 0.001$: (a) Bias (p_b); (b) Connectivity (K).

Fig. 19.

The change of average long-run sensitivity ($\omega_b[\Pi]$) with respect to different network properties for 10-gene Boolean networks: (a) Bias (p_b); (b) Connectivity (K).

Fig. 20.

The change of average long-run sensitivity ($\omega_f[\Pi]$) with respect to different network properties for 10-gene Boolean networks: (a) Bias (p_b); (b) Connectivity (K).

Fig. 21.

The change of average long-run sensitivity ($\omega_g[\Gamma]$) with respect to 1-gene regulation for 10-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 22.

The change of long-run sensitivity with respect to the perturbation probability $p(\left\| \frac{\partial \pi}{\partial p} \right\|_{l_1})$ for 10-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 23.

The change of long-run sensitivity with respect to the selection probability c_j ($|\frac{\partial \pi}{\partial c_j}|_{I_1}$) for 10-gene Boolean networks with different network properties: (a) Bias (p_b); (b) Connectivity (K).

Fig. 24.

The change of long-run sensitivity with respect to the switching probability q ($\| \frac{\partial \pi}{\partial q} \|_{l_1}$) for Boolean networks with different network properties with fixed $p = 0.001$, $q = 0.001$: (a) Bias (p_b); (b) Connectivity (K).

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Fig. 26.
 Logical regulatory graph for the mutated mammalian cell cycle network (modified from Fig. 1 in [8]). Blunt arrows stand for inhibitory effects; normal arrows for activations.

Fig. 27.
Multivariate relationship between the genes in the WNT5A network (modified from Fig. 2 in [15])

Fig. 28.

Steady-state distribution shifts for BNps with (1) $p = 0.001$ (left), (2) $p = 0.01$ (middle), and (3) $p = 0.02$ (right): a. Original steady-state distributions (top); b. After optimal perturbation to f_4 (bottom).

TABLE I

Definitions of Boolean Functions for the wild-type mammalian cell cycle PBN with 10 genes (reproduced from Table 1 in [8]).

Order	Gene	Regulating function
x_1	<i>CycD</i>	extra-cellular signals
x_2	<i>Rb</i>	$(\overline{CycD} \sqcap \overline{CycE} \sqcap \overline{CycA} \sqcap \overline{CycB}) \sqcap (p27 \sqcap \overline{CycD} \sqcap \overline{CycB})$
x_3	<i>p27</i>	$(\overline{CycD} \sqcap \overline{CycE} \sqcap \overline{CycA} \sqcap \overline{CycB}) \sqcap (p27 \sqcap (\overline{CycE} \sqcap \overline{CycA}) \sqcap \overline{CycD} \sqcap \overline{CycB})$
x_4	<i>E2F</i>	$(Rb \sqcap \overline{CycA} \sqcap \overline{CycB}) \sqcap (p27 \sqcap Rb \sqcap \overline{CycB})$
x_5	<i>CycE</i>	$(E2F \sqcap Rb)$
x_6	<i>CycA</i>	$(E2F \sqcap Rb \sqcap \overline{Cdc20} \sqcap (\overline{Cdh1} \sqcap \overline{UbcH10})) \sqcap (CycA \sqcap Rb \sqcap \overline{Cdc20} \sqcap (\overline{Cdh1} \sqcap \overline{UbcH10}))$
x_7	<i>Cdc20</i>	<i>CycB</i>
x_8	<i>Cdh1</i>	$(\overline{CycA} \sqcap \overline{CycB}) \sqcap Cdc20 \sqcap (p27 \sqcap \overline{CycB})$
x_9	<i>UbcH10</i>	$\overline{Cdh1} \sqcap (Cdh1 \sqcap UbcH10 \sqcap (Cdc20 \sqcap CycA \sqcap CycB))$
x_{10}	<i>CycB</i>	$(\overline{Cdc20} \sqcap \overline{Cdh1})$

Long-run sensitivities with respect to the changes to both the underlying regulatory functions and probabilistic parameters in the 10-gene cell cycle PBN.

TABLE II

$\ \partial\pi/\partial p\ _{l_1}$	$\ \partial\pi/\partial c_1\ _{l_1}$	$\ \partial\pi/\partial c_2\ _{l_1}$	ω_b	ω_f	ω_g
25.9195	1.7125	1.6379	0.1895	0.3672	1.2963

TABLE III
 $\Sigma_{x_1=0, x_2=0, x_3=0} \tilde{\pi}(\mathbf{x})$ with the optimal 1-bit function perturbations for all f_i s in the 10-gene cell cycle PBN.

Gene	Rb	p27	E2F	CycE	CycA	Cdc20	Cdh1	UbeH10	CycB
BN_1	0.1099	0.0996	0.1441	0.1436	0.1477	0.1191	0.1484	0.1467	0.1600
BN_2	0.0346	0.1329	0.0380	0.1228	0.1423	0.1723	0.1356	0.1447	0.1614

TABLE IV

Definitions of Boolean functions for the mutated 9-gene PBN

Order	Gene	Regulating function
x_1	<i>CycD</i>	extra-cellular signals
x_2	<i>Rb</i>	$(\overline{CycD} \sqcap \overline{CycE} \sqcap \overline{CycA} \sqcap \overline{CycB})$
x_3	<i>E2F</i>	$(Rb \sqcap \overline{CycA} \sqcap \overline{CycB})$
x_4	<i>CycE</i>	$(E2F \sqcap Rb)$
x_5	<i>CycA</i>	$(E2F \sqcap Rb \sqcap \overline{Cdc20} \sqcap (\overline{Cdh1} \sqcap \overline{UbcH10})) \sqcap (CycA \sqcap Rb \sqcap \overline{Cdc20} \sqcap (\overline{Cdh1} \sqcap \overline{UbcH10}))$
x_6	<i>Cdc20</i>	<i>CycB</i>
x_7	<i>Cdh1</i>	$(\overline{CycA} \sqcap \overline{CycB}) \sqcap Cdc20$
x_8	<i>UbcH10</i>	$\overline{Cdh1} \sqcap (Cdh1 \sqcap UbcH10 \sqcap (Cdc20 \sqcap CycA \sqcap CycB))$
x_9	<i>CycB</i>	$(\overline{Cdc20} \sqcap \overline{Cdh1})$

Long-run sensitivities with respect to the changes to both the underlying regulatory functions and probabilistic parameters in the mutated 9-gene PBN.

TABLE V

$\ \partial \pi / \partial p\ _{l_1}$	$\ \partial \pi / \partial c_1\ _{l_1}$	$\ \partial \pi / \partial c_2\ _{l_1}$	ω_b	ω_f	ω_g
19.6017	1.5724	1.5541	0.0794	0.1061	1.1758

TABLE VI
 $\Sigma_{x_1=0, x_2=0} \tilde{\pi}(\mathbf{x})$ with the optimal 1-bit function perturbations for all f_i s in the mutated 9-gene PBN.

Gene	Rb	E2F	CycE	CycA	Cdc20	Cdh1	UbcH10	CycB
BN_1	0.1350	0.2064	0.1882	0.2028	0.1703	0.2134	0.2160	0.2190
BN_2	0.0317	0.0505	0.1842	0.2042	0.2330	0.2083	0.2064	0.2221

TABLE VII

Long-run sensitivities with respect to the changes to both the underlying regulatory functions and probabilistic parameters in the 10-gene melanoma-related BNp.

$\ \partial\pi/\partial p\ _{l_1}$	ω_b	ω_f	ω_g
41.3624	0.2374	0.1748	1.1911

TABLE VIII
 $\sum_{x_l=1} \tilde{\pi}(\mathbf{x})$ with the optimal 1-bit function perturbations for all f_i s in the 10-gene melanoma-related BNp.

WNT5A	pirin	S100P	RET1	MMP3	PHOC	MART1	HADHB	synuclein	STC2
0.1829	0.0804	0.0591	0.0705	0.1942	0.2138	0.0675	0.2086	0.1969	0.2077