International Journal of Computational Intelligence in Control, 7(1), 2015

A QUANTITATIVE ROBUSTNESS MEASURE FOR GENE REGULATORY NETWORKS

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ABSTRACT: Biologists have known that gene regulatory networks (GRNs) are robust against mutations and the interactions between genes maybe the major mechanism that contributes to compensating the phenotypic effects of mutations. However, biologists do not know how to measure the robustness quantitatively. Therefore, it is needed to develop a quantitative robustness measure for GRNs. In this study, the dynamics of a GRN is described by a set of nonlinear coupled differential equations in power-law formalism. Based on this mathematical representation, a quantitative robustness measure for GRNs is proposed. Using the proposed robustness measure, one can quantitatively compute how the steady state of a GRN is affected by small changes of the interactions between genes due to mutations or diseases. Moreover, the proposed robustness measure could be used to quantitatively compare the robustness of different GRN topologies, which has very important applications in studying the evolution of the robustness of GRNs. In addition, the proposed robustness measure is useful for designing a robust GRN, which has very important applications in synthetic biology.

Keywords: gene regulatory network, robustness, synthetic biology, differential equations in power-law formalism

INTRODUCTION

Robustness is the invariance of phenotypes in the face of perturbation [1, 2]. It is a ubiquitous property of biological systems and has been found to be present in living organisms at all levels of organizational complexity ranging from the signal molecular level such as RNA secondary structure [3] and protein structure [4] to the level of genome-scale networks such as gene regulatory networks (GRNs) [5, 6, 7], biochemical networks [8, 9] and metabolic networks [10, 11]. To better understand robustness is of paramount importance for understanding organismal evolution [12, 13].

Several GRNs have been represented by mathematical models and shown to be robust to parameter variations by computer simulations. von Dassow *et al.* [5] modeled the Drosophila segment polarity network using nonlinear differential equations and showed that this network is resistant to variations in the kinetic constants that govern its behavior. Eldar *et al.* [6] modeled the Drosophila dorsal patterning network using reaction-diffusion equations and showed that the bone morphogenic protein (BMP) activation gradient is robust to changes in gene dosage. Li *et al.* [7] modeled the yeast cell-cycle network using Boolean network and showed that the cell-cycle network is robustly designed to against small perturbations.

Although the above studies all successfully demonstrated in computer simulations that the specific GRN is robust to parameter variations, these authors did not propose any quantitative robustness measure for GRNs. Therefore, they can not quantitatively measure how robust a GRN is and can not quantitatively compare the robustness of different GRN topologies. Having a quantitative robustness measure for GRNs is very important especially for the studying the evolution the robustness [14, 15] and for designing robust gene circuits in synthetic biology [16, 17]. The aim of this study is to propose a quantitative robustness measure for GRNs.

In this paper, a GRN is represented as a dynamical system. Its dynamics is described by a set of nonlinear coupled differential equations in power-law formalism [18]. This model is very general and is capable of capturing virtually any complicated phenomenon of a dynamical system, including complex oscillations and even chaos [18]. Moreover, the regular structure of this model makes theoretically analyses of the nonlinear dynamical system possible and relatively simple [18]. For example, using this model one can analytically compute the steady state of a GRN, which is a formidable task when using other kinds of mathematical models such as Boolean networks [7], dynamic Bayesian networks [19], neural networks [20] or other general classes of nonlinear differential equations like Michael-Menten kinetics [21] or Hill functions [21]. In addition, using this model all the regulatory interactions between genes in the GRN can be explicitly represented by the parameters $k_{ij}'s$, where k_{ij} denotes the regulatory effect of gene product j on the synthesis of gene product i, and the small changes of the interactions between genes due to mutations or diseases can be represented by $\Delta k_{ij}'s$ (see Methods for details).

Given this mathematical representation, the robustness of the steady state of a GRN in response to small changes of the interactions between genes due to mutations or diseases can be studied. In this paper, a quantitative robustness measure R for GRNs is proposed. Using the proposed robustness measure, one can analytically compute how the steady state of a GRN is affected by small changes of the interactions between genes due to mutations or diseases. Moreover, the robustness of different GRNs can be quantitatively compared using the proposed robustness measure. For example, the first gene regulatory network (GRN1) is said to be more robust against mutations or diseases than the second gene regulatory network (GRN2) if $R_{GRN1} > R_{GRN2}$. In addition, the proposed robustness measure R is useful for designing a robust GRN, which has very important applications in synthetic biology [16, 17]. Given a desired robustness value R_d, if the original GRN does not have an enough robustness value, i.e. $R_{GRNo} < R_d$, some $\Delta k_{ij}'s$ can be introduced to change the regulatory interactions between genes in the original GRN so that the modified GRN has a robustness value $R_{GRNm} > R_d$. A GRN of four genes will be used as an example to demonstrate how the robust GRN design procedure could be done.

METHODS

Mathematical Notations

For convenience, some mathematical notations are given here. The length of a vector $\vec{b} = [b_1, \cdots, b_n]^T$ is defined as $\left\|\vec{b}\right\|_2 = \sqrt{b_1^2 + \cdots + b_n^2}$. The size of a matrix A is defined as $\|A\|_2 = \max_{\vec{b} \neq \vec{0}} \left\|A\vec{b}\right\|_2 \Big/ \left\|\vec{b}\right\|_2$. It could be shown $\|A\|_2 = \max_i \sigma_i(A)$, where $\sigma_i(A)$ is the i-th singular value of matrix A [22].

A Dynamic System Model of GRNs

A GRN of n genes is represented by a dynamical system, with state vector $\vec{X}(t) = \left(X_1(t), \cdots, X_n(t)\right)^T$ containing the concentration of each gene product at time t. The dynamics of $\vec{X}(t)$ is modelled by a set of nonlinear coupled differential equations in power-law formalism [18]

$$dX_i(t)/dt = \alpha_i \prod_{j=1}^{n} X_j^{k_{ij}}(t) - \beta_i X_i(t) \, i = 1, \cdots, n \quad (1)$$

where α_i (or β_i) represents the rate constant of the synthesis (or degradation) of gene product i, k_{ij} represents the regulatory effect of gene product j on the synthesis of gene product i. The magnitude of k_{ij} quantifies the regulatory capability and the sign of k_{ij} reflects the regulatory mode. Gene product j is said to stimulate (or inhibit) the synthesis of gene product i if $k_{ij} > 0$ (or $k_{ij} < 0$) and have no regulatory effect if $k_{ij} = 0$. The biological meaning of Eq. (1) is that the change in the concentration of gene product i over time, i.e. $dX_i(t)/dt$, results from the difference of its synthesis rate $\alpha_i \prod_{j=1}^{n} X_j^{k_{ij}}(t)$ and degradation

rate $\beta_i X_i(t)$. The synthesis rate depends on those gene products that have regulatory effects (stimulation or inhibition) on the synthesis of gene product i, and the degradation rate is a first-order process that only depends on the concentration of gene product i itself.

The regulatory interactions within a GRN can be represented by the following regulatory matrix $K = \begin{bmatrix} k_{11} & k_{12} & \cdots & k_{1n} \\ k_{21} & k_{22} & \cdots & k_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ k_{n1} & k_{n2} & \cdots & k_{nn} \end{bmatrix}$. All the regulators of gene i can be known by looking at the nonzero terms in the i-th row $[k_{i1} \;\; k_{i2} \;\; \cdots k_{in}]$ of the regulatory matrix. All the target genes that are regulated by gene product j can be known by looking at the nonzero terms in the j-th column $[k_{1j} \;\; k_{2j} \;\; \cdots \;\; k_{nj}]^T$ of the regulatory matrix.

Steady State Analysis

One of the advantages of modelling GRNs using differential equations in power-law formulation compared to other mathematical models (e.g. Boolean networks [7], dynamic Bayesian networks [19], neural networks [20] or other general classes of nonlinear differential equations like Michael-Menten kinetics [21] or Hill functions [21]) is that the steady state analysis of GRNs can be performed easily and analytically [18]. The steady state $\vec{X}_S = (X_{1S}, \cdots, X_{nS})^T$ of the GRN can be computed by equalizing the synthesis rate and the degradation rate in Eq. (1). That is, by letting $dX_i(t) / dt = 0$, we have

$$\alpha_i \prod_{j=1}^{n} X_{jS}^{k_{ij}} = \beta_i X_{iS} \qquad i = 1, \cdots, n \tag{2}$$

Assume $\alpha_i > 0$, $\beta_i > 0$, $i = 1, 2, \cdots, n$. Then by taking the logarithm on both sides of Eq. (2) and after some rearrangements, we get

$$(k_{ii} - 1) \ln X_{iS} + \sum_{j=1, j \neq i}^{n} k_{ij} \ln X_{jS} = \ln \beta_i - \ln \alpha_i \;\; i = 1, \cdots, n$$

Denoting $y_j = \ln X_{jS}$, $b_i = \ln \beta_i - \ln \alpha_i$, the steady state $\vec{X}_S$ of the GRN satisfies the following system of linear algebraic equations

$$\begin{aligned} (k_{11} - 1) y_1 + k_{12} y_2 + \cdots + k_{1n} y_n &= b_1 \\ k_{21} y_1 + (k_{22} - 1) y_2 + \cdots + k_{2n} y_n &= b_2 \\ &\vdots \\ k_{n1} y_1 + k_{n2} y_2 + \cdots + (k_{nn} - 1) y_n &= b_n \end{aligned} \tag{3}$$

Denoting

$$\vec{y} = \begin{pmatrix} y_1 \\ \vdots \\ y_n \end{pmatrix}, \vec{b} = \begin{pmatrix} b_1 \\ \vdots \\ b_n \end{pmatrix}, A = \begin{bmatrix} k_{11} - 1 & k_{12} & \cdots & k_{1n} \\ k_{21} & k_{22} - 1 & \cdots & k_{2n} \\ \vdots & \vdots & \cdots & \vdots \\ k_{n1} & k_{n2} & \cdots & k_{nn} - 1 \end{bmatrix},$$

Eq. (3) becomes the following matrix equation

$$A\vec{y} = \vec{b} \tag{4}$$

We call the matrix A the system matrix of the GRN. The system matrix contains all the information of $k_{ij}'s$ (all the regulatory relationships between genes in the GRN). If A is invertible, then the steady state in the logarithm domain can be solved uniquely as $\vec{y} = A^{-1}\vec{b}$. Hence, the steady state of the GRN can be computed uniquely as $\vec{X}_S = \exp\left(A^{-1}\vec{b}\right)$.

RESULTS

A Quantitative Robustness Measure for GRNs

Biologists have known that GRNs are robust against mutations and the interactions between genes maybe the major mechanism that contributes to compensating the phenotypic effects of mutations [23]. However, robustness is only a qualitative concept for most biologists, it is informative to have a quantitative robustness measure for GRNs.

For a robust GRN, its steady state should not have a dramatic change caused by small perturbations of the regulatory interactions between genes due to mutations or diseases [2]. Using the dynamic system model of GRNs, how the steady state of a GRN is affected by small

changes of the regulatory interactions between genes can be quantitatively calculated. Assume that the original GRN becomes the perturbed GRN due to mutations or diseases and denote the system matrix of the perturbed GRN as $A+\Delta A$ and the steady state of the perturbed GRN as $\vec{y}+\Delta\vec{y}$, then $\vec{y}+\Delta\vec{y}$ satisfies the following matrix equation which is similar to Eq. (4)

$$(A+\Delta A)(\vec{y}+\Delta\vec{y})=\vec{b} \tag{5}$$

where $A, \vec{y}, \vec{b}$ are defined in Eq. (4) and

$$\Delta A=\begin{bmatrix} \Delta k_{11} & \Delta k_{12} & \cdots & \Delta k_{1n} \\ \Delta k_{21} & \Delta k_{22} & \cdots & \Delta k_{2n} \\ \vdots & \vdots & \cdots & \vdots \\ \Delta k_{n1} & \Delta k_{n2} & \cdots & \Delta k_{nn} \end{bmatrix}$$

denotes any possible perturbations of the regulatory effects $k_{ij}'s$ due to mutations or diseases, and $\Delta\vec{y}$ denotes the difference between the steady state $\vec{y}+\Delta\vec{y}$ of the perturbed GRN and the steady state $\vec{y}$ of the original GRN.

A quantitative robustness measure R for a GRN is proposed as a function of the system matrix A of the GRN as follows:

$$R \triangleq \frac{1}{\|A\|_2 \cdot \|A^{-1}\|_2} \tag{6}$$

Then the following theorem can be proved.

Theorem 1

The relative change of the steady state $\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2}$ of the GRN caused by the relative change of the system matrix $\frac{\|\Delta A\|_2}{\|A\|_2}$ of the GRN due to mutations or diseases satisfies the following inequality:

$$\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2} \le \frac{1/R}{1-\|A^{-1}\Delta A\|_2} \cdot \frac{\|\Delta A\|_2}{\|A\|_2} \tag{7}$$

When $\|A^{-1}\Delta A\|_2 \ll 1$, this inequality becomes

$$\frac{\|\Delta\vec{y}\|_2/\|\vec{y}\|_2}{\|\Delta A\|_2/\|A\|_2} \le \frac{1}{R} \tag{8}$$

As seen in Eq. (8), the upper bound of the ratio $\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2} \Big/ \frac{\|\Delta A\|_2}{\|A\|_2}$ is $\frac{1}{R}$, which is the inverse of the robustness measure for a GRN. That is, $\frac{1}{R}$ represents the maximal possible relative change of the steady state of a GRN caused by the relative change of the system matrix of a GRN. Therefore, the proposed robustness measure R can be used to quantitatively calculate the largest possible change of the steady state of a GRN affected by small changes of the regulatory effects $k_{ij}'s$ due to mutations or diseases. Moreover, the robustness of different GRNs can be quantitatively compared using the proposed robustness measure R. The first GRN (GRN1) is said to be more robust against mutations or diseases than the second GRN (GRN2) if $R_{GRN1} > R_{GRN2}$. In addition, the proposed robustness measure R is useful for designing a robust GRN, which has very important applications in synthetic biology. Given a desired robustness value R_d, if the original GRN does not have an enough robustness value, i.e. $R_{GRNo} < R_d$, then some $\Delta k_{ij}'s$ can be introduced to change the regulatory interactions between genes in the original GRN so that the modified GRN has a robustness value $R_{GRNm} > R_d$.

Design Examples

Fig. 1a shows a GRN of four genes, where gene product 1 activates gene 3, gene product 2 activates gene 3 and gene 4, gene product 3 activates gene 2 and gene product 4 activates gene 1 and gene 4 but represses gene 3. Assume the dynamics of this GRN can be written as follows

$$dX_1(t)/dt = 5X_4^{0.98}(t) - 10X_1(t)$$
$$dX_2(t)/dt = 10X_3^{0.38}(t) - 5X_2(t)$$
$$dX_3(t)/dt = 1.25X_1^{0.51}(t)X_2^{0.72}(t)X_4^{-0.49}(t) - 2X_3(t)$$
$$dX_4(t)/dt = 5X_2^{1.24}(t)X_4(t) - 8X_4(t)$$

then its system matrix equals to $A = \begin{bmatrix} -1 & 0 & 0 & 0.98 \\ 0 & -1 & 0.38 & 0 \\ 0.51 & 0.72 & -1 & -0.49 \\ 0 & 1.24 & 0 & 0 \end{bmatrix}$ and robustness value equals to 0.0011 calculated by Eq. (6). Since the robustness value of this GRN is very small, it may not meet the desired robustness value, say 0.09. Therefore, some $\Delta k_{ij}'s$ should be introduced to change the system matrix of this GRN so that the modified GRN has a robustness value > 0.09. Three possible modifications of the system matrix are shown as follows. If we

(i) change the value of k_{14} from 0.98 to -1.1 (see Fig. 1b), the robustness value increases to 0.0924;

(ii) change the value of k_{34} from -0.49 to 1.25 (see Fig. 1c), the robustness value increases to 0.1112;

(iii) introduce a new interaction $k_{24} = -1.49$ so that gene product 4 can repress gene 2 (see Fig. 1d), the robustness value increases to 0.2838.

In order to prove the effectiveness of the proposed robustness measure, it is needed to show that the larger the robustness value of a GRN, the smaller the relative change of its steady state in response to the change of its system matrix A caused by small perturbations of the regulatory interactions between genes due to mutations or diseases. Fig. 1a to 1d can be regarded as four different GRNs with robustness value equals to 0.0011, 0.0924, 0.1112, and 0.2838, respectively. The goal is to show that the GRN with a larger robustness value has a smaller $\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2}$ value. That is, GRN in Fig. 1d should have the smallest $\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2}$ among these four GRNs since its robustness value is the largest among these four GRNs. In order to test this hypothesis, the following computer simulation was conducted. The perturbations $\Delta A = \begin{bmatrix} \Delta k_{11} & \Delta k_{12} & \cdots & \Delta k_{1n} \\ \Delta k_{21} & \Delta k_{22} & \cdots & \Delta k_{2n} \\ \vdots & \vdots & \cdots & \vdots \\ \Delta k_{n1} & \Delta k_{n2} & \cdots & \Delta k_{nn} \end{bmatrix}$ were introduced into the above four GRNs, where $\Delta k_{ij}'s$ are Gaussian random noises with μ = 0, σ = 0.01. Then the relative changes of the steady states $\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2}$ of these four GRNs were calculated. The same procedure was done for 1000 times. As shown in Table 1, the GRN with a larger robustness value does have a smaller $\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2}$ value, which demonstrates the usefulness of the proposed robustness measure to calculate the robustness of a GRN.

Compare with other Existing Robustness Measures

Two robustness measures are widely used. The simplest one is to do computer simulations for large numbers of different $\Delta k_{ij}'s$, observing the effects of the steady state changes. By doing a lot of computer simulations, biologists can get a rough idea of how robust a GRN is. However, this is not an anlytical solution and as the number of $\Delta k_{ij}'s$ increases, a brute force sweep through the parameter space becomes impossible. The second robustness measure is derived from the local sensitivity analysis [18]. Local means that only one k_{ij} is allowed to have a small perturbation while the other $k_{ij}'s$ must remain fixed. This has limited relevance in the typical situation where many $k_{ij}'s$ could be perturbed at the same time. Moreover, since this kind of local sensitivity analysis takes no account of interactions between $\Delta k_{ij}'s$, it may give a misleading result. For example, if a small perturbation in k_{ij} has no visible effect on the steady state of a GRN, but it makes the steady state much more sensitive to a small perturbation in k_{gh}, local sensitivity analysis will incorrectly say that the GRN is robust to Δk_{ij} [24].

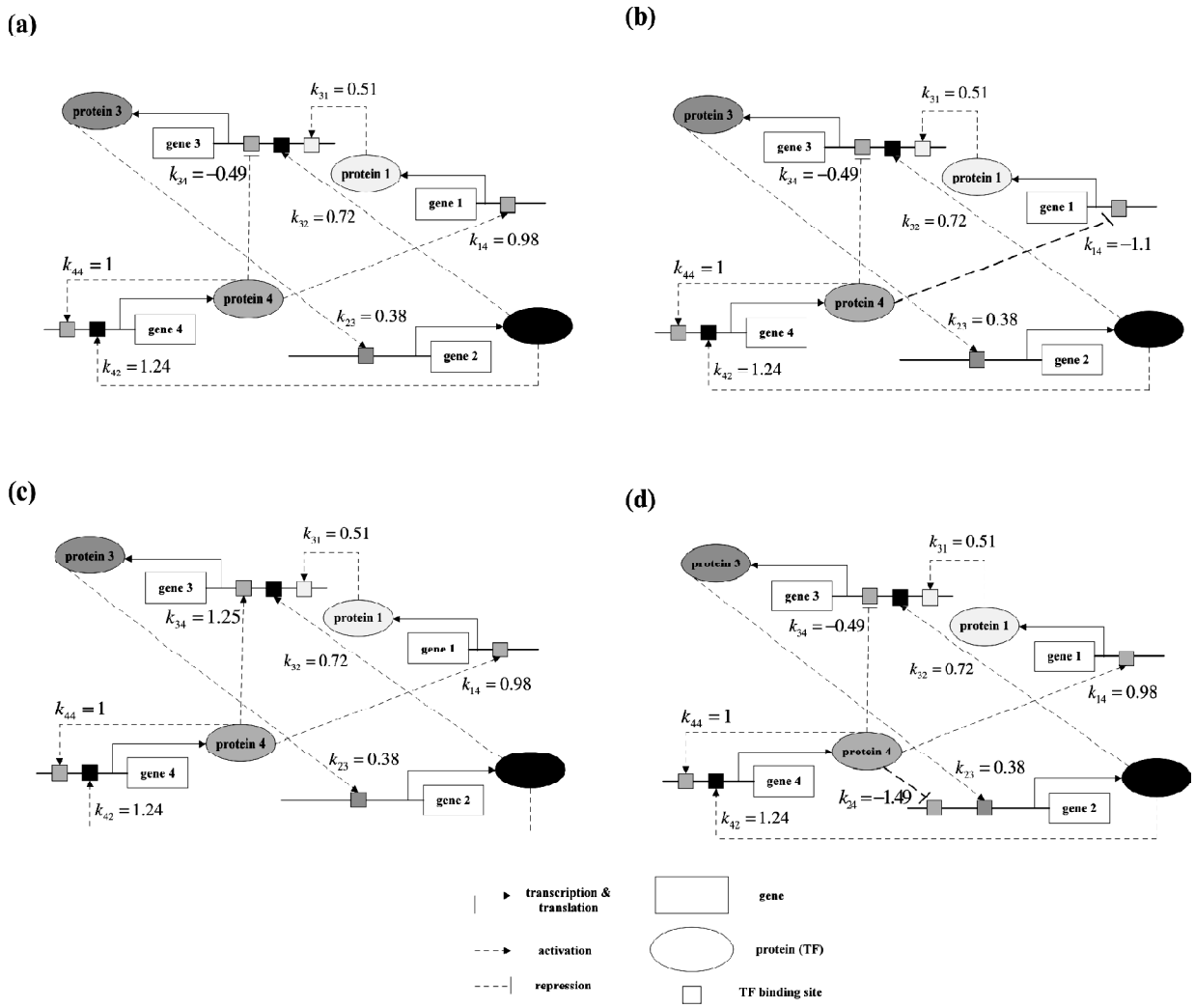

Figure 1: Four different GRNs of four genes. (a) The original GRN. (b) A GRN modified from the original GRN by changing the value of k_{14} from 0.98 to -1.1. (c) A GRN modified from the original GRN by changing the value of k_{34} from -0.49 to 1.25. (d) A GRN modified from the original GRN by adding a new interaction k_{24} with the value -1.49

Table 1
The Mean and Standard Error of the Relative Change of the Steady State in Response to ΔA (in 1000 Simulations) for four Different GRNs in Fig. 1a to Fig. 1d

	Robustness value	*Mean±S.E.*
GRN in Fig. 1a	0.0011	2.9263 ± 0.9458
GRN in Fig. 1b	0.0924	0.0477±0.011
GRN in Fig. 1c	0.1112	0.0362±0.008
GRN in Fig. 1d	0.2838 (the largest among the four GRNs)	0.0205±0.003 (the smallest among the four GRNs)

The quantitative robustness measure proposed in this study could remedy the weakness of the above two widely used robustness measure. The proposed robustness measure directly considers the effect of $\Delta A = \begin{bmatrix} \Delta k_{11} & \Delta k_{12} & \cdots & \Delta k_{1n} \\ \Delta k_{21} & \Delta k_{22} & \cdots & \Delta k_{2n} \\ \vdots & \vdots & \cdots & \vdots \\ \Delta k_{n1} & \Delta k_{n2} & \cdots & \Delta k_{nn} \end{bmatrix}$, the perturbation of the system matrix A. That is, the interactions between all combinations of $\Delta k_{ij}'s$ could be analyzed. Moreover, the proposed robustness measure, which is defined as

$R \triangleq \dfrac{1}{\|A\|_2 \cdot \|A^{-1}\|_2}$, is an analytical formula. Therefore, the robustness of different GRNs could be compared without performing any computer simulation. In addition, one can still compute the robustness value of a GRN even when the number of $\Delta k_{ij}'s$ is too large to do computer simulations.

CONCLUSIONS

In this paper, a GRN is represented as a dynamical system. Its dynamics is described by a set of nonlinear coupled differential equations in power-law formalism. The regular structure of this model makes theoretically analyses of the steady state of a GRN relative simple, which is a formidable task when using other kinds of mathematical models. Moreover, all the regulatory interactions between genes in the GRN can be explicitly represented by the system matrix A and the small changes of the interactions between genes due to mutations or diseases can be represented by ΔA. Based on the system matrix, a quantitative robustness measure $R \triangleq \dfrac{1}{\|A\|_2 \cdot \|A^{-1}\|_2}$ is proposed. Using computer simulations, the effectiveness of the proposed robustness measure was validated by showing that the larger the robustness value of a GRN, the smaller the relative change of its steady state in response to ΔA caused by small perturbations of the regulatory interactions between genes due to mutations or diseases. The proposed robustness measure is an analytical formula and can be computed without performing any computer simulation. Most importantly, the proposed robustness measure has two important applications. First, it could be used to quantitatively compare the robustness of different GRN topologies, which has very important applications in studying the evolution of the robustness of GRNs. Second, the proposed robustness measure is useful for designing a robust GRN, which has very important applications in synthetic biology.

APPENDIX

Proof of Theorem 1

After some reorganization, Eq. (5) can be rewritten as follows

$$A\Delta\vec{y} = -\Delta A\left(\vec{y} + \Delta\vec{y}\right) \tag{9}$$

Assume that A is invertible, Eq. (9) can be written as

$$\Delta\vec{y} = -A^{-1}\Delta A\left(\vec{y} + \Delta\vec{y}\right) \tag{10}$$

Compute the 2-norm of the both sides of Eq. (10), we have

$$\|\Delta\vec{y}\|_2 = \left\|A^{-1}\Delta A\left(\vec{y} + \Delta\vec{y}\right)\right\|_2 \tag{11}$$

Using the property that $\left\|A^{-1}\Delta A\left(\vec{y} + \Delta\vec{y}\right)\right\|_2 \le \left\|A^{-1}\Delta A\right\|_2 \cdot \left\|\left(\vec{y} + \Delta\vec{y}\right)\right\|_2$, we have

$$\|\Delta\vec{y}\|_2 \le \left\|A^{-1}\Delta A\right\|_2 \cdot \left\|\left(\vec{y} + \Delta\vec{y}\right)\right\|_2 \tag{12}$$

Using the property that $\left\|\left(\vec{y} + \Delta\vec{y}\right)\right\|_2 \le \|\vec{y}\|_2 + \|\Delta\vec{y}\|_2$, we have

$$\|\Delta\vec{y}\|_2 \le \left\|A^{-1}\Delta A\right\|_2 \cdot \left(\|\vec{y}\|_2 + \|\Delta\vec{y}\|_2\right) \tag{13}$$

After some reorganization, Eq. (13) can be rewritten as follows

$$\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2} \le \frac{\left\|A^{-1}\Delta A\right\|_2}{\left(1 - \left\|A^{-1}\Delta A\right\|_2\right)} \tag{14}$$

Using the property that $\left\|A^{-1}\Delta A\right\|_2 \le \left\|A^{-1}\right\|_2 \cdot \|\Delta A\|_2$, we have

$$\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2} \le \frac{\left\|A^{-1}\right\|_2 \cdot \|\Delta A\|_2}{\left(1 - \left\|A^{-1}\Delta A\right\|_2\right)} \tag{15}$$

Using the definition that $1/R = \|A\|_2 \cdot \left\|A^{-1}\right\|_2$ in Eq. (15), we have

$$\frac{\|\Delta\vec{y}\|_2}{\|\vec{y}\|_2} \le \frac{1/R}{\left(1 - \left\|A^{-1}\Delta A\right\|_2\right)} \cdot \frac{\|\Delta A\|_2}{\|A\|_2} \tag{16}$$

Remark: It is shown by Chen *et al.* [25] that when $\left\|A^{-1}\Delta A\right\|_2 \ge 1$, the steady state of the perturbed GRN may cease to exist. That is, the perturbed GRN may undergo a dramatic change and is qualitatively different from the original GRN. Such changes in qualitative properties of a GRN are called bifurcations (a term used in nonlinear system analysis [26]). In this situation, the biological function of the original GRN will not be maintained in the perturbed GRN. The perturbed GRN is therefore regarded as a lethal mutant. For this reason, the robustness analysis of a

GRN in this study only focuses on the perturbations ΔA (due to mutations or diseases) that satisfy the criterion $\left\|A^{-1}\Delta A\right\|_2 < 1$.

Acknowledgements

This study was supported by the Taiwan National Science Council NSC-099-2628-B-006-015-MY3.

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