On the Robust Circuit Design Schemes of Biochemical Networks: Steady-State Approach

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***Abstract*—Based on the steady-state analyses of the synergism and saturation system (S-system) model, a robust control method is proposed for biochemical networks via feedback and feedforward biochemical circuits. Two robust biochemical circuit design schemes are developed. One scheme is to improve the system's structural stability so as to tolerate larger kinetic parameter variations, whereas the other is to compensate for the kinetic parameter variations to eliminate their effects. In addition, a multi-objective biochemical circuit design scheme is introduced for both the robust design against kinetic parameter variations and a desired sensitivity design to eliminate the effect of external disturbance simultaneously.**

The proposed robust circuit design schemes will provide a systematic method with potential applications in synthetic circuit design for biotechnological purpose and drug design purpose. Recent advances in both metabolic and genetic engineering have made the robust biochemical circuit control approach feasible through the design and implementation of synthetic biological networks amenable to mathematical modeling and quantitative analysis. Finally, several examples including the robust circuit design of the tricarboxylic acid cycle are used in silico to illustrate the design procedure and to confirm the performance of the proposed design method.

***Index Terms*—Biochemical network, multi-objective design, robust circuit design, S-system, sensitivity, synthetic biological network.**

I. Introduction

A BIOCHEMICAL network is robust if the steady states of its metabolite concentrations (phenotype) are preserved despite changes in its kinetic parameter values. Robustness is defined as a measure of tolerance of kinetic parameter variations with the existence of the steady states of the biochemical network preserved. In addition, sensitivity analyses are conventionally employed to assess the robustness of biochemical networks [1]. The sensitivity of the steady-state concentration of a metabolite with respect to kinetic parameter changes and external disturbance is considered as the inverse of robustness of a biochemical network [2].

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Robustness plays an important role in the fail-safe mechanism of biochemical networks. A robust biochemical network should be able to cope with environmental changes, not be sensitive to kinetic parameter variations, and have a slow rate of degradation of the system function. Therefore, for a biochemical network that lacks robustness to tolerate kinetic parameter variations and environmental changes, it is desirable to have an efficient control design to improve its robustness. Since there does not exist a systematic design method for this purpose, it is highly desirable to develop such a robust circuit design method.

In recent years, robustness of biochemical systems has attracted much attention [3]–[11]. As pointed out in [12], robustness emerges as a central issue of systems biology and understanding its property may have an impact on the future of medicine. Robustness in metabolism, cell cycle and intercellular signaling pathways has been widely investigated [10], [11]. These biochemical networks must operate reliably under various environmental conditions that can cause change in the internal "parameters" of the network.

Recently, a new robustness measure of biochemical networks has been introduced on the basis of the S-system model to estimate the upper bound of the tolerated kinetic parameter variations with the preservation of the steady state [13]. It is found that the robustness is related to the system matrix of biochemical networks at steady state, i.e., the structural stability of biochemical networks. In general, a healthy biochemical network must be robust enough to tolerate parametric and environmental perturbations. However, the robustness of a biochemical network can be weakened by the effect of mutation or disease. To develop a method for biotechnological application or to design an engineered circuit for synthetic biochemical networks, improvement in the robustness of biochemical networks is necessary. This robust circuit design of a biochemical network is an important topic in systems and synthetic biology [9], [14]–[17]. Because biochemical networks are complex and highly nonlinear, it is difficult to develop an efficient robust circuit design method for biochemical networks. Therefore, it is more appealing to have a systematic biocircuit control design for biochemical systems to improve robustness to tolerate larger parameter variation and external disturbance.

In this study, based on robust stability and sensitivity analysis, we develop a robustness design method for biochemical systems to tolerate the kinetic parameter perturbations within some prescribed ranges. We use the S-system representation, a well-studied approach in modeling biochemical systems [2]. It is a type of power-law formalism that uses nonlinear differential equations in which the component processes are characterized by power-law functions. The structure of the S-system is rich enough to capture many relevant biological dynamics and the S-system allows standardizing analytical and computational

methods [2], [18], especially for steady-state evaluation, control analysis, robustness and sensitivity analysis. Taking logarithm on the state variables makes the steady state of a S-system equivalent to an algebraic linear system [2], facilitating the robustness analysis and robust circuit design of a biochemical network.

Traditionally, metabolic engineering has been approached with experimental biotechnological methods, but it is becoming popular to precede the experimental phase by a mathematical modeling step that allows objective prescreening of possible improvement strategies [19]. Based on biochemical models and biochemical systems theory, biochemical circuit designs are developed for biotechnological or engineered control purpose by regulatory features like feedback inhibition and other modulations of enzyme-catalyzed steps in biochemical networks. At present, metabolic engineering is on the verge of being able to design pathways de novo. The recent progress in genetic engineering has made the implementation of synthetic networks feasible. Several biocircuits have been experimentally constructed [20]–[23]. It can be presumed that synthetic biochemical networks will be used for significant enzymatic steps to which the microorganism or cell line had no access previously. Similarly, it will be possible to modulate the existing or introduced biochemical reaction steps through internal or external signals [19].

In this study, a method for robust circuit design is developed by the feedback control via catalytic circuit linkages in biochemical networks through the way of metabolic pathway engineering. Elementary biochemical circuits can be linked catalytically in biochemical networks without their individual properties changing appreciably, because the molecules in a circuit acting catalytically on another circuit are not consumed in the process of interaction [18]. Therefore, the catalytic linkage circuit approach by synthetic biocircuit technologies through metabolic and genetic engineering is suitable for the implementation of robust circuit design of biochemical networks.

Based on robust analysis at steady state, we design some catalytic linkage circuits and specify their kinetic parameter values to achieve a desired robustness design of a biochemical network at the steady state. Two robust biochemical circuit design schemes are developed. One design scheme is to improve the structural stability of a biochemical network to tolerate larger kinetic parameter variations. The other design scheme is to compensate for the kinetic parameter variations to eliminate their effect on the biochemical network. Finally, by considering the desired sensitivity (for example, below a prescribed sensitivity value) to environmental perturbations into the robust circuit design, the multi-objective biochemical circuit design can also be achieved simultaneously. The multi-objective biochemical circuit design scheme via feedback or feedforward catalytic circuit design can be implemented by transfection and transformation biotechnologies through internal or external signals in the near future [19]. In this study, several examples of robust circuit design are given in silico to illustrate the design procedure and to confirm the performance of the proposed design methods.

II. Model of Biochemical Networks

In general, a biochemical network is a collection of enzymatic reactions that serve to process cellular and intercellular metabolites. In biochemistry, one often measures the rates of reactions or fluxes, and the rates correspond directly to changes in concentrations of substrates, enzymes, factors or products. When we express such changes in concentrations, we can express the relationship in terms of differential equations. The following S-system representation has been an efficient model for describing a dynamic network for the last three decades [2], [24]

$$\begin{aligned}
\dot{X}_1 &= \alpha_1 \prod_{j=1}^{n+m} X_j^{g_{1j}} - \beta_1 \prod_{j=1}^{n+m} X_j^{h_{1j}} \\
&\vdots \\
\dot{X}_i &= \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}} = V_i - V_{-i}, \quad i=1,\ldots,n, \\
&\vdots \\
\dot{X}_n &= \alpha_n \prod_{j=1}^{n+m} X_j^{g_{nj}} - \beta_n \prod_{j=1}^{n+m} X_j^{h_{nj}}
\end{aligned} \tag{1}$$

where $X_1,\ldots,X_{n+m}$ are metabolites, such as substrates, enzymes, factors or products of the biochemical network, in which $X_1,\ldots,X_n$ denote n dependent variables and $X_{n+1},\ldots,X_{n+m}$ denote the independent variables. In a biochemical network, intermediate metabolites and products are dependent variables, whereas substrates and enzymes are independent variables. The rate of change in X_i, $\dot{X}_i$, is equal to the difference between two terms, one for production or accumulation and the other for degradation or clearance. Each term is the product of the rate constant, α_i or β_i, which is positive, and all dependent and independent variables that affect directly the production or degradation, respectively. Each variable X_j is raised to the power of a kinetic parameter g_{ij} or h_{ij}, which represents an activating effect of X_j on X_i when its value is positive and an inhibitive effect when its value is negative. The symbols V_i and V_{-i} represent aggregate flux into and out of the X_i pool.

Remark 1: g_{ij} and h_{ij}, which are defined, respectively, as $(\partial V_i/\partial X_j)(X_j/V_i)$ and $(\partial V_{-i}/\partial X_j)(X_j/V_{-i})$, and are called elasticities, quantify the sensitivity of a reaction rate to the change of a concentration of a metabolite, effector or enzyme [25], [26]. Elasticity represents the total effect of activity, selectivity, substrate specificity, stability, and solubility [27].

The nonlinear system in (1) describes the dynamic evolution among dependent variables and is called an S-system, where S represents synergism and saturation of the investigated biochemical system. Synergism and saturation are two fundamental properties of biochemical and biological systems. S-system equations are types of power-law formalism that use nonlinear differential equations in which the component processes are characterized by power law functions [18]. How to construct the S-system representation of a biochemical network and how to estimate its kinetic parameters from experimental data can be found in [2] and [24] and references therein. Recently, the nonlinear parameter estimation problem of S-systems has been efficiently solved by the evolution optimization methods [28], [29].

In general, it is difficult to study the robustness or the sensitivity of such a nonlinear system given in (1). Fortunately, many important characteristics of a S-system at or close to the steady state can be analyzed using simple algebraic methods. Since most biochemical systems in nature operate close to the steady

state at which inputs and outputs are almost balanced, we shall focus on the robustness of biochemical systems at the steady state (i.e., $V_i = V_{-i}$) [2], [13].

Remark 2: While the S-System formalism is powerful, it should be noticed that not all systems can be modeled by S-system adequately. A recent example is the study done by Voit *et al.* [30] on regulation of glycolysis in *Lactococcus lactis*, where only a GMA model is possible.

III. Robust Analysis of a Biochemical Network at Steady State

Consider the steady state of the biochemical network in (1), i.e., inputs and outputs are balanced [2]

$$\alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} = \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}}, \quad i = 1, 2, \ldots, n. \tag{2}$$

Taking the logarithm on both sides of (2) and making some rearrangements, we obtain

$$\sum_{j=1}^{n}(g_{ij} - h_{ij}) \ln X_j = \ln \beta_i - \ln \alpha_i - \sum_{j=n+1}^{n+m} (g_{ij} - h_{ij}) \ln X_j, \quad i = 1, 2, \ldots, n. \tag{3}$$

Introduce new variables and coefficients as

$$y_j = \ln X_j, \quad a_{ij} = g_{ij} - h_{ij}, \quad b_i = \ln\left(\frac{\beta_i}{\alpha_i}\right). \tag{4}$$

The steady state of the biochemical system is obtained as follows: [2]

$$A_D Y_D = b - A_I Y_I \tag{5}$$

where

$$Y_D = \begin{bmatrix} y_1 \\ \vdots \\ y_n \end{bmatrix}, \; b = \begin{bmatrix} b_1 \\ \vdots \\ b_n \end{bmatrix}, \; Y_I = \begin{bmatrix} y_{n+1} \\ \vdots \\ y_{n+m} \end{bmatrix}$$

$$A_D = \begin{bmatrix} a_{11} & \cdots & a_{1n} \\ \vdots & \ddots & \vdots \\ a_{n1} & \cdots & a_{nn} \end{bmatrix}, \; A_I = \begin{bmatrix} a_{1,n+1} & \cdots & a_{1,n+m} \\ \vdots & \ddots & \vdots \\ a_{n,n+1} & \cdots & a_{n,n+m} \end{bmatrix}.$$

In (5), A_D denotes the system matrix of the interactions between dependent variables Y_D, and A_I indicates the interactions between the dependent variables Y_D and independent variables Y_I.

In the nominal parameter case, we assume that the inverse of A_D exists, so that Y_D can be solved uniquely, i.e., the biochemical network will result in only one steady state (phenotype). Therefore, the steady state (phenotype) of the biochemical system is given by

$$Y_D = A_D^{-1}(b - A_I Y_I). \tag{6}$$

Suppose that the parameter variations owing to mutation or disease can alter the kinetic properties of the steady state of a biochemical network as follows [13]:

$$(A_D + \Delta A_D)(Y_D + \Delta Y_D) = (b + \Delta b) - (A_I + \Delta A_I)(Y_I + \Delta Y_I) \tag{7}$$

where the parameter perturbations of the biochemical network are defined by the equations shown at bottom of the page, where ΔA_D denotes the parameter perturbations owing to the kinetic parameter variations (Δg_{ij} and Δh_{ij}) of dependent variables, Δb denotes the parameter perturbations owing to the rate constant variations and ΔA_I denotes the parameter perturbations owing to the kinetic parameter variations between dependent and independent variables. The parameter perturbation ΔA_D can influence the existence of the steady state of the biochemical network.

By singular value decomposition [31], we get

$$A_D = U^T \Sigma V = \sum_{i=1}^{n} \sigma_i u_i^T v_i$$

where σ_i denotes the ith singular value and u_i, $v_i \in R^{1\times n}$ denotes the corresponding left and right singular vectors, respectively. Therefore, if a parameter variation is specified as follows

$$\Delta A_D = -\sigma_i u_i^T v_i, \quad i = 1, 2, \ldots, n. \tag{8}$$

Then

$$A_D + \Delta A_D = U^T \begin{bmatrix} \sigma_1 & & & & & & \\ & \ddots & & & & 0 & \\ & & \sigma_{i-1} & & & & \\ & & & 0 & & & \\ & & & & \sigma_{i+1} & & \\ & & & & & \ddots & \\ & 0 & & & & & \sigma_n \end{bmatrix} V.$$

Obviously, the inverse $(A_D + \Delta A_D)^{-1}$ or $(I + A_D^{-1}\Delta A_D)^{-1}$ does not exist under the parameter perturbation in (8).

Remark 3: The parameter perturbations in the direction of singular vectors like (8) are the points of fragility (Achilles'

$$\Delta A_D = \begin{bmatrix} \Delta a_{11} & \cdots & \Delta a_{1n} \\ \vdots & \Delta a_{ij} & \vdots \\ \Delta a_{n1} & \cdots & \Delta a_{nn} \end{bmatrix} = \begin{bmatrix} \Delta g_{11} - \Delta h_{11} & \cdots & \Delta g_{1n} - \Delta h_{1n} \\ \vdots & \Delta g_{ij} - \Delta h_{ij} & \vdots \\ \Delta g_{n1} - \Delta h_{n1} & \cdots & \Delta g_{nn} - \Delta h_{nn} \end{bmatrix}$$

$$\Delta b = \begin{bmatrix} \Delta b_1 \\ \vdots \\ \Delta b_n \end{bmatrix}, \quad \Delta A_I = \begin{bmatrix} \Delta g_{1,n+1} - \Delta h_{1,n+1} & \cdots & \Delta g_{1,n+m} - \Delta h_{1,n+m} \\ \vdots & \Delta g_{i,n+j} - \Delta h_{i,n+j} & \vdots \\ \Delta g_{n,n+1} - \Delta h_{n,n+1} & \cdots & \Delta g_{n,n+m} - \Delta h_{n,n+m} \end{bmatrix}$$

heels). Hence, the robustness should prevent this kind of parameter variations to guarantee the existence of the steady state of biochemical networks. When unexpected perturbations like (8) are encountered, it will lead to catastrophic failure of a biochemical network. In this situation, robust circuit design is necessary as a fail-safe mechanism of biochemical networks. For example, the trehalose pathway in yeast consists of only a few metabolites, which form a substrate cycle, and is governed by a surprisingly complex control system that comprises several inhibiting or activating signaling mechanisms [32]. Though a small system, it has a lot of robustness features, whose biological function is stress handling. However, even though the system is highly robust, the existence of the steady states still can not be preserved under the perturbation in (8) (details about the fragility of the trehalose pathway can be seen in Supplementary Material S1[1]). Obviously, the parameter perturbations in (8) are the Achilles' heels of biochemical networks.

For the illustration of robustness analysis and circuit design, a simple example is given in the following. Consider the cascaded network in Fig. 1(a). Cascaded mechanisms are found in diverse areas of biochemistry and physiology, including hormonal control, gene regulation, immunology, blood clotting and visual excitation [2], [24]. The S-system model is given as

$$\begin{aligned}
\dot{X}_1 &= 10X_2^{-0.1}X_3^{-0.05}X_4 - 5X_1^{0.5}, \;\; X_1(0) = 0.2 \\
\dot{X}_2 &= 2X_1^{0.5} - 1.44X_2^{0.5}, \;\; X_2(0) = 0.5 \\
\dot{X}_3 &= 3X_2^{0.5} - 7.2X_3^{0.5}, \;\; X_3(0) = 0.1, \;\; X_4 = 0.75 \qquad (9)
\end{aligned}$$

$$A_D = \begin{bmatrix} -0.5 & -0.1 & -0.05 \\ 0.5 & -0.5 & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix}, \;\; A_I = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}. \qquad (10)$$

The time responses of the cascaded network are shown in Fig. 1(b). Suppose the kinetic parameters A_D suffer from parameter perturbations as

$$\Delta A_D = \begin{bmatrix} 0.04675 & 0.11756 & 0.1655 \\ 0.04667 & 0.2826 & 0 \\ 0 & 0.06494 & 0.0914 \end{bmatrix}. \qquad (11)$$

System (9) will then be perturbed as

$$\begin{aligned}
\dot{X}_1 &= 10X_2^{0.01756}X_3^{0.1155}X_4 - 5X_1^{0.45325}, \;\; X_1(0) = 0.2 \\
\dot{X}_2 &= 2X_1^{0.54667} - 1.44X_2^{0.2174}, \;\; X_2(0) = 0.5 \\
\dot{X}_3 &= 3X_2^{0.56494} - 7.2X_3^{0.4086}, \;\; X_3(0) = 0.1, \;\; X_4 = 0.75. \qquad (12)
\end{aligned}$$

In this situation, the robustness is violated and the steady state ceases to exist [Fig. 1(c)]. Hence, a robust circuit design is necessary to improve robustness to tolerate this parameter perturbation. It will be discussed in the following sections.

IV. Robust Circuit Design Schemes of a Biochemical Network

The robustness may be violated by larger parameter perturbation ΔA_D due to DNA mutation, environmental changes or disease. That is to say, the system structure matrix of the biochemical network, A_D, is not robust enough to tolerate the kinetic parameter perturbation ΔA_D. For biotechnological or engineered control design purpose, we wish to improve the robustness to tolerate larger ΔA_D. Therefore, the biochemical circuit design for the robust control of such a biochemical network is an important topic in systems biology and synthetic biological networks [15], [16], [33], [34].

Exploring further, we find two mechanisms for a robust circuit design to tolerate large ΔA_D in a biochemical network. One is to strengthen the structural stability of the nominal system to tolerate large ΔA_D by making $A_D A_D^T$ larger; the other is to diminish ΔA_D so that the robustness can be preserved.

Suppose a robust circuit design is developed for (1) by the state feedback method via biochemical circuits in a more general biochemical network form:

$$\dot{X}_i = \alpha_i \prod_{j=1}^{n+m} X_j^{g_{ij}} \prod_{k=1}^{n} X_k^{f_{ik}} - \beta_i \prod_{j=1}^{n+m} X_j^{h_{ij}} \prod_{k=1}^{n} X_k^{l_{ik}}, \quad i = 1, \ldots, n \qquad (13)$$

where $X_k^{f_{ik}}$ denotes a new biochemical control circuit with X_k regulating the production of X_i by the kinetic parameter f_{ik} and $X_k^{l_{ik}}$ denotes a new biochemical control circuit with X_k regulating the degradation of X_i by the kinetic parameter l_{ik}. The choice of regulating objects, X_k and X_i, and the specification of the kinetic parameters, f_{ik} and l_{ik}, are to be designed according to the feasibility of biochemical circuit linkage to achieve the desired robustness to tolerate ΔA_D within the prescribed range of kinetic parameter perturbations in a biochemical network. Since f_{ik} and l_{ik} are the elasticities of the corresponding enzymes in the designed control circuits, the implementation of control circuits are heavily dependent on the specification of elasticities of these enzymes.

A. Systematic Robust Circuit Design: Steady-State Approach

If a biochemical network cannot tolerate parameter perturbations, robust control via a biochemical circuit design is desirable to remedy for. Based on the robustness analysis, we develop two biochemical circuit design schemes for the robust control of biochemical networks as follows. Consider the robust control system of biochemical network in (13). By a similar procedure from (2) to (7), at steady state, we obtain

$$\begin{aligned}
&(A_D + F + \Delta A_D)(Y_D + \Delta Y_D) \\
&\qquad = (b + \Delta b) - (A_I + \Delta A_I)(Y_I + \Delta Y_I) \qquad (14)
\end{aligned}$$

where the control parameter matrix F is defined as

$$F \equiv \begin{bmatrix} f_{11} - l_{11} & \cdots & f_{1n} - l_{1n} \\ \vdots & f_{ij} - l_{ij} & \vdots \\ f_{n1} - l_{n1} & \cdots & f_{nn} - l_{nn} \end{bmatrix} \qquad (15)$$

where f_{ij} and l_{ij} are the kinetic parameters of the biochemical control circuit to be specified in (13).

Suppose we can find some F such that the inverse of $(A_D + F)$ matrix exists. Then (14) is equivalent to

$$\begin{aligned}
&(A_D + F)\left(I + (A_D + F)^{-1}\Delta A_D\right)(Y_D + \Delta Y_D) \\
&\qquad = (b + \Delta b) - (A_I + \Delta A_I)(Y_I + \Delta Y_I). \qquad (16)
\end{aligned}$$

[1]Supplementary Material can be found at http://www.ee.nthu.edu.tw/bschen/robust_circuit_design/

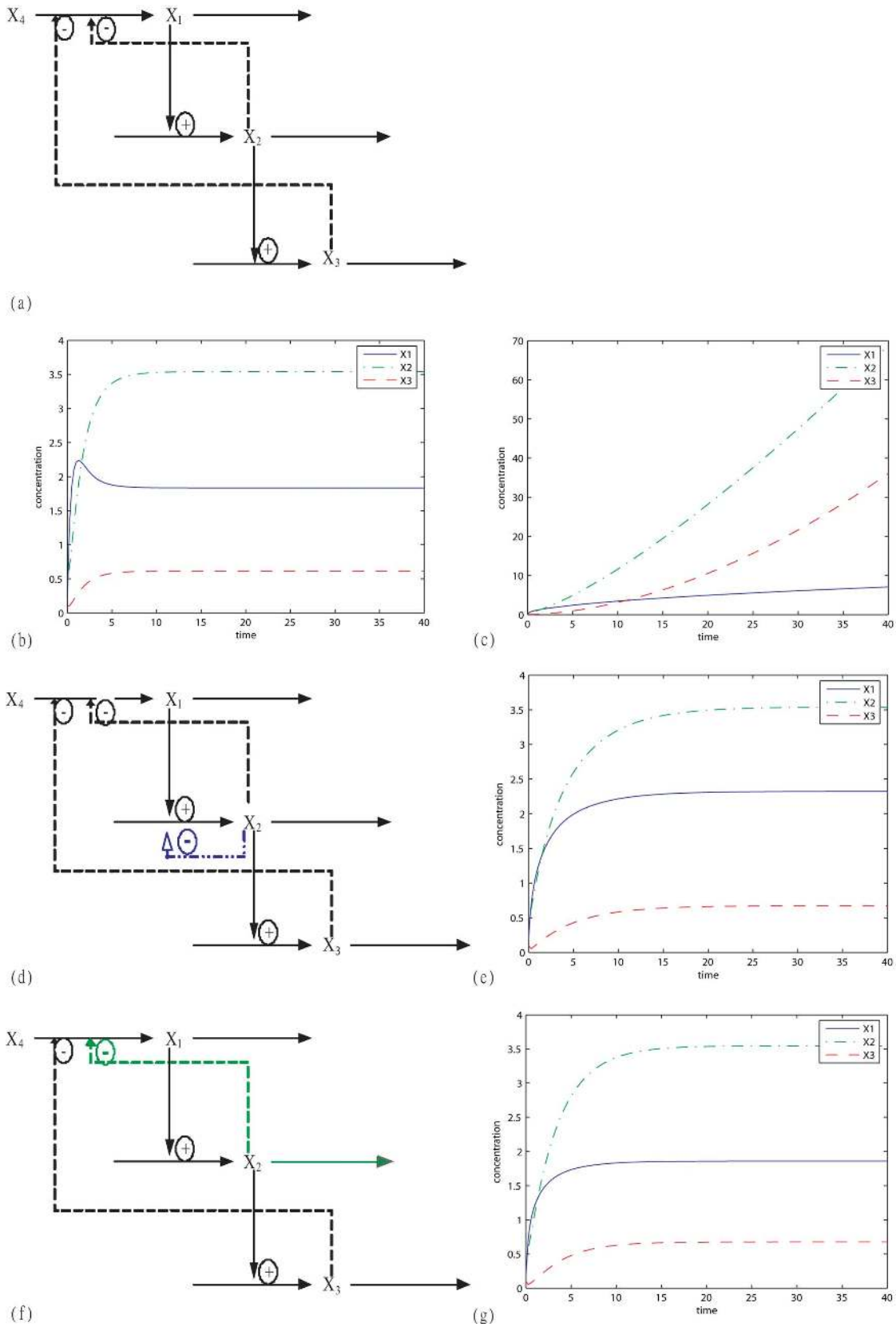

Fig. 1. (a) Cascaded biochemical network. (b) Time responses of (a) in the nominal parameter case. (c) Time responses of (a) under parameter perturbations in (11). (d) Designed cascaded biochemical network with $f_{22} = -0.407$ (the dash-dot line from X_2 to its production) by the multi-objective design in Example 3. (e) Time responses of the designed biochemical network in (d) under parameter perturbations in (11). (f) Designed cascaded biochemical network with $f_{12} = -0.08$ (the dashed line from X_2 to the production of X_1) and $l_{22} = 0.31$ (the solid line of the degradation of X_2) by the multi-objective design in Example 4. (g) Time responses of the designed biochemical networks in (f) under parameter perturbations in (11).

By the fact that if the matrix norm $\|X\|_2 < 1$, then the inverse $(I+X)^{-1}$ exists [31], [35], the robust design scheme I for the controlled biochemical system in (14) is given by

$$\left\|(A_D+F)^{-1}\Delta A_D\right\|_2 < 1$$

or

$$\Delta A_D \Delta A_D^T < (A_D+F)(A_D+F)^T \tag{17}$$

where $\|X\|_2 = \sigma_{\max}(A) = \max_i \sqrt{\lambda_i(X^TX)}$ and $\lambda_i(M)$ denotes the ith eigenvalue of M, i.e., if the inequality in (17) holds, then the inversibility of $I+(A_D+F)^{-1}\Delta A_D$ could be guaranteed [31], [35]. In this case, our design purpose is to specify F such that the robustness of structural stability of the biochemical network is improved so that the robust design scheme I in (17) can be guaranteed to tolerate larger parameter perturbations ΔA_D. Then the phenotype, i.e., the steady state of the controlled biochemical network in (16), is given by

$$\begin{aligned} Y_D+\Delta Y_D = &\left(I+(A_D+F)^{-1}\Delta A_D\right)^{-1}(A_D+F)^{-1} \\ &\times\left[(b+\Delta b)-(A_I+\Delta A_I)(Y_I+\Delta Y_I)\right]. \end{aligned} \tag{18}$$

Since the robust design scheme I in (17) is only a sufficient condition for the robustness of the perturbed system in (16), we can get another robust design rule of the system in (14) as follows. If ΔA_D is known beforehand, (14) can be expressed as

$$\begin{aligned} &A_D\left(I+A_D^{-1}(F+\Delta A_D)\right)(Y_D+\Delta Y_D) \\ &\quad = (b+\Delta b)-(A_I+\Delta A_I)(Y_I+\Delta Y_I). \end{aligned} \tag{19}$$

Similarly, robust design scheme II of the controlled biochemical system in (14) is obtained by

$$\left\|A_D^{-1}(F+\Delta A_D)\right\|_2 < 1$$

or

$$(F+\Delta A_D)(F+\Delta A_D)^T < A_D A_D^T. \tag{20}$$

In this situation, our design purpose is to specify F to cancel or compensate for ΔA_D such that the parameter perturbation effect on the biochemical network is diminished, i.e., $(F+\Delta A_D)(F+\Delta A_D)^T$ should be sufficiently small so that the robust design scheme II in (20) could be guaranteed to tolerate the parameter perturbations ΔA_D. Then the steady state of the controlled biochemical network in (19) is given by

$$\begin{aligned} Y_D+\Delta Y_D = &\left(I+A_D^{-1}(F+\Delta A_D)\right)^{-1}A_D^{-1}\left[(b+\Delta b)\right. \\ &\left.-(A_I+\Delta A_I)(Y_I+\Delta Y_I)\right]. \end{aligned} \tag{21}$$

From the above analysis based on two robustness criteria in (17) and (20), there are two robust circuit control design schemes for biochemical networks. Robust design scheme I is to specify F to enhance, i.e., to make $(A_D+F)(A_D+F)^T$ larger, the robustness of the structural stability of the biochemical network to tolerate larger parameter perturbations, ΔA_D, in (17). An adequate negative feedback is of this kind of design [3], [14]. Robust design scheme II is to specify F to efficiently cancel or compensate for parameter perturbations, i.e., to make $(\Delta A_D+F)(\Delta A_D+F)^T$ smaller, so that the robustness condition in (20) is more easily guaranteed. The self-regulation and redundancy in real biochemical systems are of this kind of design [13], [20], [36].

Consider the cascade biochemical network example again. Suppose a biochemical control circuit can be designed [see Fig. 1(d)] such that X_2 can self-regulate its production to achieve the desired robustness to tolerate the parameter perturbations ΔA_D in (11). Then, the second equation in (9) can be modified as

$$\dot{X}_2 = 2X_1^{0.5}X_2^{f_{22}} - 1.44X_2^{0.5}. \tag{22}$$

In order to tolerate the parameter perturbations in (11), f_{22} in (22) should be specified to satisfy the robust design scheme I in (17), i.e., we need to design a new cascaded network with a more robust property by adding a new biochemical control circuit to tolerate ΔA_D. On the other hand, if we can adjust the enzyme activities via metabolite pathway engineering to change the kinetic parameters, an alternative design is to enhance an existing pathway by modulating its kinetic parameter value to tolerate ΔA_D. For instance, suppose a catalytic control circuit can be designed such that X_2 can regulate the production of X_1, i.e., f_{12}, and X_2 can self-regulate its degradation, i.e., l_{22} (see Fig. 1(f)), to satisfy the robust design scheme to tolerate ΔA_D. Then, the differential equations of the cascaded network in (9) should be modified as

$$\begin{aligned} \dot{X}_1 &= 10X_2^{-0.1+f_{12}}X_3^{-0.05}X_4 - 5X_1^{0.5},\ \ X_1(0)=0.2 \\ \dot{X}_2 &= 2X_1^{0.5} - 1.44X_2^{0.5+l_{22}},\ \ X_2(0)=0.5 \\ \dot{X}_3 &= 3X_2^{0.5} - 7.2X_3^{0.5},\ \ X_3(0)=0.1,\ \ X_4=0.75. \end{aligned} \tag{23}$$

In the former design case, the biochemical circuit design work is reduced to how to specify the range of f_{22} in (22) so that the robust design scheme I or II is satisfied. In the latter design case, both f_{12} and l_{22} in (23) are to be specified to meet the robust design schemes simultaneously.

Remark 4: If ΔA_D is unknown but within some range, for example, $\|\Delta A_D\|_2 \le a$ for some prescribed value a to be tolerated, then the robust design scheme I in (17) should be changed to [31], [35]

$$a^2 I < (A_D+F)(A_D+F)^T. \tag{24}$$

Example 1: Consider the nominal cascaded biochemical network in (9) which cannot tolerate the parameter perturbations in (11). Suppose the catalytic control circuit in (22) [see Fig. 1(d)] is to be designed, i.e., the new designed circuit is a X_2 self-regulation on its production. The kinetic parameter f_{22} should be specified such that the robust design scheme I in (17) is satisfied as follows:

$$\begin{aligned} &\begin{bmatrix} 0.043396 & 0.035404 & 0.022761 \\ 0.035404 & 0.082041 & 0.018352 \\ 0.022761 & 0.018352 & 0.012571 \end{bmatrix} \\ &\quad < \begin{bmatrix} -0.5 & -0.1 & -0.05 \\ 0.5 & -0.5+f_{22} & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix} \\ &\qquad \times \begin{bmatrix} -0.5 & 0.5 & 0 \\ -0.1 & -0.5+f_{22} & 0.5 \\ -0.05 & 0 & -0.5 \end{bmatrix}. \end{aligned} \tag{25}$$

A simple procedure how to find f_{22} in (25) is given as follows. At first, candidate interval of f_{22} is determined subject to biochemical limitation. Then, by exhaustive algorithm, we search

all the values in the candidate interval to find the feasible interval which satisfies the inequality (25). If no solution can be found in this candidate interval, we could extend the candidate interval and repeat the above procedure.

In this example, the candidate interval is chosen within [−1,1]. With the help of Matlab, the range of f_{22} is found to be within [−1,−0.081] to tolerate ΔA_D in (11).

Example 2: Similarly, consider the cascaded network in (9) with parameter perturbations ΔA_D in (11). Suppose that the control circuit design in (23) [see Fig. 1(f)] is employed to achieve the desired robustness, i.e., to specify f_{12} and l_{22} in (23) to achieve the robust design scheme I in (17) as follows:

$$\begin{bmatrix} 0.043396 & 0.035404 & 0.022761 \\ 0.035404 & 0.082041 & 0.018352 \\ 0.022761 & 0.018352 & 0.012571 \end{bmatrix} < \begin{bmatrix} -0.5 & -0.1+f_{12} & -0.05 \\ 0.5 & -0.5-l_{22} & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix} \times \begin{bmatrix} -0.5 & 0.5 & 0 \\ -0.1+f_{12} & -0.5-l_{22} & 0.5 \\ -0.05 & 0 & -0.5 \end{bmatrix}. \quad (26)$$

With the help of Matlab, the range of f_{12} and l_{22} are found to be within [−1,0] and [0,1], respectively, to tolerate ΔA_D in (11).

B. Implementation of Biochemical Circuit Design

In the former design case in (22), one needs to implement a novel regulatory pathway. In the latter design case in (23), one needs to change the enzymes' kinetic properties (elasticity). Recent progress in genetic (recombinant DNA technology) and metabolic engineering have made these implementations feasible. At present, rational design [37], [38] and directed evolution [39]–[41] are two efficient methods to change the elasticity, and dynamic controller design [21], [42] is a useful technique to develop a novel regulatory pathway.

Rational design is an implementation method to change the elasticity on the basis of the enzyme structure, which mimics the evolution of enzymes in nature, through the acquisition of new catalytic or binding properties by an existing protein scaffold [37]. Directed evolution, which is also termed molecular breeding, uses sequence recombination (e.g., block shuffling [43]) and functional selection to breed quickly the desired enzyme. The design method also mimics the natural evolution but doesn't need structural information in comparison with rational design. Directed evolution approaches rely on mutagenesis and recombination to create an efficient selection or screening strategy to achieve a desired enzyme elasticity through a library of variants [27], [39]. There are some successful design examples by using directed evolution [39]–[41] and by using combination of directed evolution approaches and rational design methods to change enzyme's elasticity [37], [38].

In order to change the kinetic parameter f_{12} in (23), we could use rational design and directed evolution to modify the elasticity of the corresponding catalytic enzyme. The implementation of l_{22} by changing the elasticity of the corresponding catalytic enzyme is obtained in a similar manner.

A dynamic controller scheme [21], [42] is to create an artificial feedback loop by using an intracellular signal to control the expression of desired genes which are responsible for the key

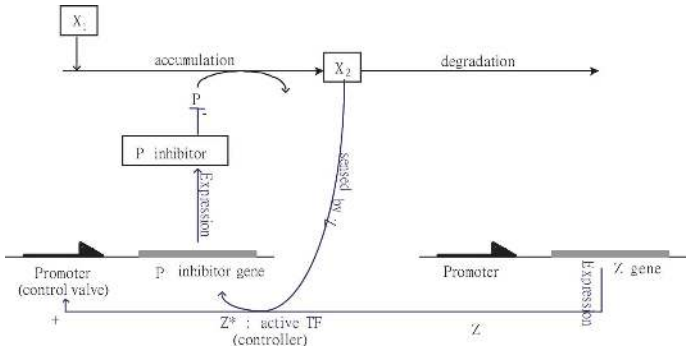

Fig. 2. Flowchart of the control circuit implementation based on the dynamic controller design [42] in Fig. 1(d). X_2 is required to activate TF Z, and active TF Z^*, should bind to the promoter of the P inhibitor gene so that its protein P can inhibit the accumulation of X_2 with negative elasticity f_{22}.

enzymes of the designed pathway. To implement such a biochemical control loop, f_{22} in (22), X_2 is required to activate a transcription factor (TF or controller), Z, at first (Fig. 2). The TF should bind to the promoter and then control the expression of corresponding gene (P gene) of the catalytic enzyme whose elasticity is responsible for the designed circuit, f_{22}. Farmer and Liao [42] have designed a dynamic controller by sensing glucose to control the rate-limiting enzymes to improve the lycopene production.

In the design case of (22), we need to construct a self-regulatory biochemical circuit to regulate the catalytic enzyme P in the accumulation of X_2. First, a controller (Z) should be found to sense X_2 to bind to the promoter of the P gene (by constructing a binding site) that produces the enzyme P. Then, the expression product of the P enzyme is thus proportional to X_2. In the succeeding step, we may need to modify the elasticity of the P enzyme by rational design and directed evolution methods to achieve a desired performance. Then, implementation of f_{22} is achieved. Of course, we can insert the P gene sequence to an existing transcriptional regulation rather than constructing a binding site on the promoter region [42]. Several synthetic biocircuits and engineered metabolic pathways have successfully been implemented by above methods [18], [33], [36], [37], [42]. In this study, the robust circuit design of biochemical network could be implemented by these developed methods.

After a discussion on biocircuit implementation methods, a multipurpose circuit control design of a biochemical network under parameter perturbations will be considered in the following section.

V. Multipurpose Circuit Control Design of a Biochemical Network

The above robustness design focuses on the tolerance of kinetic parameter perturbations ΔA_D. The effects of the rate constant variations Δb and the environmental changes ΔY_I on the output variations ΔY_D should also be considered in the robust circuit design of biochemical networks in order to guarantee the robustness against both the intrinsic parameter variations and the extrinsic environmental perturbations. The sensitivity from the rate constant variations Δb to output variations ΔY_D in the designed biochemical network of (14) is given by

$$\frac{\Delta Y_D}{\Delta b} = (A_D + F)^{-1}. \quad (27)$$

The sensitivity from the environmental variations ΔY_I to output variations ΔY_D in the designed biochemical network is given by

$$\frac{\Delta Y_D}{\Delta Y_I} = -(A_D + F)^{-1} A_I. \tag{28}$$

It is more appealing to design a robust biochemical network with desired sensitivities to the variations of rate constants and environmental signals, i.e.,

$$\left\| \frac{\Delta Y_D}{\Delta b} \right\|_2 < s_1, \quad \left\| \frac{\Delta Y_D}{\Delta Y_I} \right\|_2 < s_2 \tag{29}$$

where the upper bounds, s_1 and s_2, are prescribed by the biochemical circuit designer beforehand.

From (27)–(29) and the definition of the l_2-induced matrix norm, we obtain the equivalent sensitivity criteria for (29) as

$$\begin{aligned} I - s_1^2 (A_D + F)(A_D + F)^T &< 0 \\ A_I A_I^T - s_2^2 (A_D + F)(A_D + F)^T &< 0. \end{aligned} \tag{30}$$

Therefore, if the robust circuit design in a biochemical network is intended to achieve both the robust design scheme in (17) or (20) to tolerate kinetic parameter perturbations ΔA_D and the sensitivity condition in (29) to eliminate the effect of rate constant variations Δb and environmental perturbations ΔY_I on ΔY_D below the prescribed values s_1 and s_2, simultaneously, we should specify F to satisfy the inequalities in (17) [or (20)] and (30) simultaneously. In general, s_1 and s_2 can be specified as the sensitivities in the nominal system case, i.e., $s_1 = \|A_D^{-1}\|_2$ and $s_2 = \|A_D^{-1} A_I\|_2$, in order not to change the sensitivities of the designed biochemical network too much from the nominal system case, because the sensitivities of a nominal (healthy) biochemical network are the desired sensitivities in the real world.

Example 3: In Example 1, suppose that we want to design a robust biochemical circuit to tolerate kinetic parameter variations ΔA_D in (11) and to achieve desired sensitivities $\|(\Delta Y_D/\Delta b)\|_2 < \|(\Delta Y_D/\Delta b)\|_{2,\text{nominal}} \equiv s_1 = \|A_D^{-1}\|_2 = 3.42$ and $\|(\Delta Y_D/\Delta Y_I)\|_2 < \|(\Delta Y_D/\Delta Y_I)\|_{2,\text{nominal}} \equiv s_2 = \|A_D^{-1} A_I\|_2 = 2.66$. Then we should specify f_{22} to satisfy the robust design scheme I in (25) and the following two inequalities simultaneously:

$$\begin{aligned} &\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} - s_1^2 \begin{bmatrix} -0.5 & -0.1 & -0.05 \\ 0.5 & -0.5 + f_{22} & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix} \\ &\quad \times \begin{bmatrix} -0.5 & 0.5 & 0 \\ -0.1 & -0.5 + f_{22} & 0.5 \\ -0.05 & 0 & -0.5 \end{bmatrix} < 0 \\ &\begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} - s_2^2 \begin{bmatrix} -0.5 & -0.1 & -0.05 \\ 0.5 & -0.5 + f_{22} & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix} \\ &\quad \times \begin{bmatrix} -0.5 & 0.5 & 0 \\ -0.1 & -0.5 + f_{22} & 0.5 \\ -0.05 & 0 & -0.5 \end{bmatrix} < 0. \end{aligned} \tag{31}$$

The two inequalities in (31) are based on the desired sensitivity criteria in (30). With the help of Matlab, the range of f_{22} are found to be within [−1,−0.081] to tolerate ΔA_D in (11) by robust design scheme I in (25) and satisfy the desired sensitivity criteria in (31) simultaneously. We choose $f_{22} = -0.407$ as a design example, which is a negative self-regulation and has been found to efficiently eliminate the effect of parameter variations by negative compensation. About 10% of yeast genes encoding regulators are negative self-regulation so that the mechanism seems to be important to maintain robustness in yeast [44]. The biochemical network is shown in Fig. 1(d) and the time responses are shown in Fig. 1(e).

Example 4: Similarly, in the design case we described in Example 2, suppose that we want to specify f_{12} and l_{22} to satisfy the robust design scheme I in (26) and the following two desired sensitivity constraints simultaneously:

$$\begin{aligned} &\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} - s_1^2 \begin{bmatrix} -0.5 & -0.1 + f_{12} & -0.05 \\ 0.5 & -0.5 - l_{22} & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix} \\ &\quad \times \begin{bmatrix} -0.5 & 0.5 & 0 \\ -0.1 + f_{12} & -0.5 - l_{22} & 0.5 \\ -0.05 & 0 & -0.5 \end{bmatrix} < 0 \\ &\begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} - s_2^2 \begin{bmatrix} -0.5 & -0.1 + f_{12} & -0.05 \\ 0.5 & -0.5 - l_{22} & 0 \\ 0 & 0.5 & -0.5 \end{bmatrix} \\ &\quad \times \begin{bmatrix} -0.5 & 0.5 & 0 \\ -0.1 + f_{12} & -0.5 - l_{22} & 0.5 \\ -0.05 & 0 & -0.5 \end{bmatrix} < 0 \end{aligned} \tag{32}$$

where s_1 and s_2 are the same as example 4. With the help of Matlab, the ranges of f_{12} and l_{22} are found to be within [−1,0] and [0,1], respectively, to tolerate ΔA_D in (11) by the robust design scheme I in (25) and satisfy the desired sensitivity criteria in (32) simultaneously. We choose $f_{12} = -0.08$ and $l_{22} = 0.31$ as a design example. The biochemical network is shown in Fig. 1(f) and the time responses are shown in Fig. 1(g).

The design method we proposed above will offer a systematic biochemical circuit design method for the multipurpose robust control of a biochemical network under kinetic parameter variations and environmental perturbations. There is a high potential for drug design for therapeutic purpose in the future.

VI. Computational Simulation

Consider the Tricarboxylic Acid (TCA) cycle biochemical network in *Dictyostelium discoideum* [2]. The TCA cycle, a cyclic reaction, can produce ATP very efficiently, and serves as the core of the metabolic network in most living cells. The condensation of acetyl-CoA and oxaloacetic acid (OAA) results in the products, citric acid and acetyl-CoA. In succeeding reactions, the products cooperate with the electronic delivering mechanism and oxidative-phosphorylation (ADP→ATP) at the cell membrane of prokaryotes or at the intima of eukaryotic mitochondria to oxidize an oxaloacetic acid molecule to equivalent water, CO_2 and 12 ATP molecules simultaneously.

In this case, the TCA cycle mode, shown in Fig. 3(a), is simplified reasonably to involve the following 13 dependent metabolites, 35 independent metabolites and 26 enzyme-catalyzed process [2], [45], [46].

X_1 Oxaloacetate 1 (OAA 1);
X_2 Oxaloacetate 2 (OAA 2)
X_3 Acetyl-CoA (ACO);
X_4 Isocitrate (ISOC);
X_5 Pyruvate (PYR);

X_6 Glutamate (GLU);
X_7 Aspartate (ASP);
X_8 Alanine (ALA);
X_9 Citrate 1 (CIT 1);
X_{10} α-Ketoglutarate (KG1);
X_{11} Succinate (SUC);
X_{12} Fumarate (FUM);
X_{13} Malate (MAL 1);
X_{14} Glutamate dehydrogenase;
X_{15} α-Ketoglutarate dehydrogenase complex;
X_{16} Succinate dehydrogenase;
X_{17} Fumarase;
X_{18} Malate dehydrogenase;
X_{19} Malic enzyme;
X_{20} Ala $\rightarrow$ Pyr;
X_{21} Pyruvate dehydrogenase complex;
X_{22} Oaa 2 $\rightarrow$ Asp;
X_{23} Asp $\rightarrow$ Oaa 2;
X_{24} Citrate synthetase;
X_{25} Aconitase;
X_{26} Isocitrate dehydrogenase;
X_{27} Glu $\rightarrow$ Suc;
X_{28} Aspartate transaminase;
X_{29} Alanine transaminase;
X_{30} Oaa1 $\rightarrow$ Oaa 2;
X_{31} Asp $\rightarrow$ Oaa 1;
X_{32} Suc $\rightarrow$ Glu;
X_{33} Oaa1 $\rightarrow$ Asp;
X_{34} Protein $\rightarrow$ Asp;
X_{35} Protein $\rightarrow$ AcCoA;
X_{36} Protein $\rightarrow$ Suc;
X_{37} Protein $\rightarrow$ Fum;
X_{38} Protein $\rightarrow$ Ala;
X_{39} Protein $\rightarrow$ Glu;
X_{40} Asp $\rightarrow$ Protein;
X_{41} Acetyl-CoA $\rightarrow$ Protein;
X_{42} Suc $\rightarrow$ Protein;
X_{43} Fum $\rightarrow$ Protein;
X_{44} Ala $\rightarrow$ Protein;
X_{45} Glu $\rightarrow$ Protein;
X_{46} NAD;
X_{47} CoA;
X_{48} NADH;

The S-system model of the TCA cycle network in *Dictyostelium discoideum* is written as follows [2]:

$$
\begin{aligned}
\dot{X}_1 =& 0.8282X_1^{-0.038}X_6^{-0.0204}X_7^{0.106}X_{10}^{0.114}X_{13}^{0.7}X_{18}^{0.807} \\
&\times X_{28}^{0.108}X_{31}^{0.0848}X_{46}^{0.599}X_{48}^{-0.181} \\
&- 1.3423X_1X_{30}^{0.915}X_{33}^{0.0847} \\
\dot{X}_2 =& 1.3401X_1^{0.915}X_7^{0.0848}X_{23}^{0.0848}X_{30}^{0.915} \\
&- 17.166X_2^{0.706}X_3^{0.0716}X_{22}^{0.0848}X_{24}^{0.915}X_{47}^{-0.0341} \\
\dot{X}_3 =& 0.3231X_3^{-0.405}X_5^{0.156}X_{21}^{0.427}X_{35}^{0.573}X_{46}^{0.422} \\
&\times X_{47}^{0.405}X_{48}^{-0.418} - 9.6952X_2^{0.376}X_3^{0.489} \\
&\times X_{24}^{0.554}X_{41}^{0.446}X_{47}^{-0.00206} \\
\dot{X}_4 =& X_9X_{25} - 0.152X_4^{0.958}X_{26}X_{46}^{0.0348}X_{48}^{-0.862} \\
\dot{X}_5 =& 1.875X_7^{0.0274}X_8^{0.465}X_{13}^{0.336}X_{19}^{0.535}X_{20}^{0.465} \\
&- 0.01923X_3^{-0.717}X_5^{0.413}X_6^{0.306}X_8^{-0.29} \\
&\times X_{10}^{-0.0883}X_{21}^{0.756}X_{29}^{0.244}X_{46}^{0.748}X_{47}^{0.718}X_{48}^{-0.741} \\
\dot{X}_6 =& 2.459X_1^{-0.00921}X_6^{-0.0154}X_7^{0.0162}X_{10}^{0.086} \\
&\times X_{11}^{0.276}X_{28}^{0.813}X_{32}^{0.276}X_{39}^{0.6413} \\
&- 1.1528X_5^{0.0963}X_6^{1.01}X_8^{-0.204}X_{10}^{-0.062} \\
&\times X_{14}^{0.0518}X_{27}^{0.277}X_{29}^{0.171}X_{45}^{0.5}X_{46}^{0.0222}X_{48}^{-0.0191} \\
\dot{X}_7 =& 2.1167X_1^{0.129}X_2^{0.129}X_{22}^{0.129}X_{33}^{0.129}X_{34}^{0.741} \\
&- 3.4893X_1^{-0.0187}X_6^{-0.0311}X_7^{0.868}X_{10}^{0.174} \\
&\times X_{23}^{0.129}X_{28}^{0.165}X_{31}^{0.129}X_{40}^{0.577} \\
\dot{X}_8 =& 0.5724X_5^{0.111}X_6^{0.247}X_8^{0.234}X_{10}^{0.0713}X_{29}^{0.197}X_{38}^{0.803} \\
&- 1.9369X_8X_{20}^{0.375}X_{44}^{0.625} \\
\dot{X}_9 =& 16.242X_2^{0.679}X_3^{0.0782}X_{24}X_{47}^{-0.0372} - X_9X_{25} \\
\dot{X}_{10} =& 0.156X_4^{0.724}X_5^{0.106}X_6^{0.259}X_8^{-0.223}X_{10}^{-0.0679} \\
&\times X_{14}^{0.0568}X_{26}^{0.756}X_{29}^{0.188}X_{46}^{0.0506}X_{48}^{-0.672} \\
&- 0.8063X_1^{-0.0101}X_6^{-0.0168}X_7^{0.0177}X_{10}^{0.99} \\
&\times X_{11}^{-0.879}X_{15}^{0.911}X_{28}^{0.0891}X_{46}^{0.882}X_{47}^{0.879}X_{48}^{-0.881} \\
\dot{X}_{11} =& 2.0031X_6^{0.166}X_{10}^{0.491}X_{11}^{-0.481}X_{15}^{0.499}X_{27}^{0.166} \\
&\times X_{36}^{0.335}X_{46}^{0.483}X_{47}^{0.481}X_{48}^{-0.483} \\
&- 2.4373X_{11}^{0.495}X_{12}^{-0.00542}X_{16}^{0.574}X_{32}^{0.166}X_{42}^{0.261} \\
\dot{X}_{12} =& 1.271X_{11}^{0.106}X_{12}^{-0.00836}X_{16}^{0.885}X_{37}^{0.115} \\
&- 9.1694X_{12}^{1.89}X_{13}^{-1.24}X_{17}^{0.911}X_{43}^{0.0893} \\
\dot{X}_{13} =& 8.289X_{12}^{1.98}X_{13}^{-1.36}X_{17} - 0.9387X_1^{-0.0197}X_7^{0.0196}X_{13}^{0.775} \\
&\times X_{18}^{0.618}X_{19}^{0.382}X_{46}^{0.458}X_{48}^{-0.139}
\end{aligned} \tag{33}
$$

where

$$
\begin{aligned}
&X_1(0) = 0.003, X_2(0) = 0.003, X_3(0) = 0.065, \\
&X_4(0) = 0.01, X_5(0) = 0.32, X_6(0) = 6.63, \\
&X_7(0) = 2.035, X_8(0) = 5.313, X_9(0) = 0.0275, \\
&X_{10}(0) = 0.01, X_{11}(0) = 0.9, X_{12}(0) = 0.04, \\
&X_{13}(0) = 0.24, X_{14} = 0.977, X_{15} = 7610, X_{16} = 3.15, \\
&X_{17} = 25.7, X_{18} = 77.8, X_{19} = 3.08, X_{20} = 0.196, \\
&X_{21} = 258, X_{22} = 74, X_{23} = 0.1, X_{24} = 8.24, \\
&X_{25} = 80, X_{26} = 271, X_{27} = 0.133, X_{28} = 9.95, \\
&X_{29} = 2.67, X_{30} = 800, X_{31} = 0.1, X_{32} = 1, \\
&X_{33} = 74, X_{34} = 1.06, X_{35} = 2.07, X_{36} = 1.62, \\
&X_{37} = 0.36, X_{38} = 2.03, X_{39} = 1.86, X_{40} = 0.446, \\
&X_{41} = 27.2, X_{42} = 1.57, X_{43} = 7, X_{44} = 0.326, \\
&X_{45} = 0.24, X_{46} = 0.072, X_{47} = 0.1, X_{48} = 0.18.
\end{aligned}
$$

The time responses of the TCA cycle network in (33) are shown in Fig. 3(b). A_D and A_I are given in Supplementary Material S2.[1] From A_D and the robustness condition, we can calculate the upper bound of the perturbation tolerance, $A_D A_D^T$. Suppose the biochemical network suffers the following perturbation, ΔA_D, which violates the upper bound of robustness condition, so that the steady states of the TCA cycle network ceases to exist and the time responses are shown in Fig. 3(c). [See (34), shown at the bottom of the next page.]

Suppose we want to design a robust biochemical circuit to tolerate the perturbation ΔA_D and to achieve the desired sensitivities, s_1 and s_2 in (30). The robust control problem can be reduced to specifying F to satisfy the following multipurpose control design derived from (17) and (30):

$$\begin{aligned} &\Delta A_D \Delta A_D^T < (A_D + F)(A_D + F)^T \\ &I - s_1^2 (A_D + F)(A_D + F)^T < 0 \\ &A_I A_I^T - s_2^2 (A_D + F)(A_D + F)^T < 0 \end{aligned} \tag{35}$$

where the nominal sensitivities $s_1 = \|A_D^{-1}\|_2 = 8.3685$, $s_2 = \|A_D^{-1} A_I\|_2 = 7.5464$.

Suppose that we design one biochemical control pathway with kinetic parameter $f_{1,2}$ to satisfy the multi-objective design criteria in (35), the range of $f_{1,2}$ is found within $[-0.8, -0.1]$. If $f_{1,2}$ is chosen as "-0.2," for example [blue line in Fig. 3(a)], the time responses of the designed TCA cycle network are shown in Fig. 3(d), which matches the desired properties of the proposed design method. That is, the robust controlled biochemical network not only can tolerate ΔA_D (to preserve its phenotype under the parameter perturbations) but also can reserve the sensitivity in the nominal case. The control parameter matrix F is given in the Supplementary Material S2.[1]

If the dynamic controller design is employed to implement the biochemical control circuit, $f_{1,2}$, we need to find an enzyme that can catalyze the reaction, $X_2 \longrightarrow X_1$. We also need to find a TF, Z, so that Oxaloacetate2, X_2, could bind to the promoter of the enzyme's inhibitor gene because $f_{1,2}$ is negative. Therefore, the concentration of X_2 could regulate X_1 through the kinetic parameter $f_{1,2}$. Then, we need to modulate the elasticity of the enzyme inhibitor's gene sequence to the specified performance by rational design or directed evolution. Thus, the biochemical control circuit, $f_{1,2}$, can be achieved.

VII. Discussion

Mutation and disease are unavoidable and can permanently alter the kinetic properties of a biochemical network. The less the sensitivity to the rate constant variations and the environmental signal perturbations, the more appealing for biochemical networks in the evolutionary process. In this study, for biotechnological or engineered control design purpose, a robust biochemical circuit control design method based on the S-system is proposed for biochemical networks to achieve a robustness condition to tolerate kinetic parameter variations.

$$\Delta A_D = \begin{bmatrix} 0.0209 & 0 & 0 & 0 & 0 & 0.0267 & 0.000292 \\ 0.0219 & 0.0412 & 0 & 0 & 0 & 0 & 0.000305 \\ 0 & -0.0278 & 0 & 0 & 0.058 & 0 & 0 \\ 0 & 0 & 0.0157 & 0.0157 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.0611 & -0.0198 & -0.0002 \\ 0.0087 & 0 & 0 & 0 & -0.0344 & 0.0111 & 0.0001 \\ 0.00039 & 0.00726 & 0 & 0 & 0 & 0.00492 & 0.000054 \\ 0 & 0 & 0 & 0 & 0.0413 & -0.0133 & 0 \\ 0 & 0.0216 & 0 & 0 & 0 & 0 & 0 \\ 0.0119 & 0 & 0.0179 & 0.0179 & -0.0472 & 0.0153 & 0.00017 \\ 0 & 0 & 0 & 0 & 0 & 0.0207 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0.0124 & 0 & 0 & 0 & 0 & 0 & 0.00017 \end{bmatrix}$$

$$\begin{bmatrix} 0 & 0 & 0.0565 & 0 & 0 & 0.0142 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0.0135 & 0 & 0 & 0 & 0 \\ 0.00571 & 0 & -0.0418 & 0 & 0 & -0.0105 \\ -0.0032 & 0 & 0.0235 & 0.0157 & 0 & 0 \\ 0 & 0 & 0.0104 & 0 & 0 & 0 \\ 0.0039 & 0 & -0.0282 & 0 & 0 & 0 \\ 0 & 0.0149 & 0 & 0 & 0 & 0 \\ -0.0044 & 0 & 0.0323 & 0.0215 & 0 & 0 \\ 0 & 0 & 0.0438 & 0.0293 & 0.0103 & 0 \\ 0 & 0 & 0 & 0.0239 & 0.00843 & 0.009 \\ 0 & 0 & 0 & 0 & 0.0079 & 0.0084 \end{bmatrix} \tag{34}$$

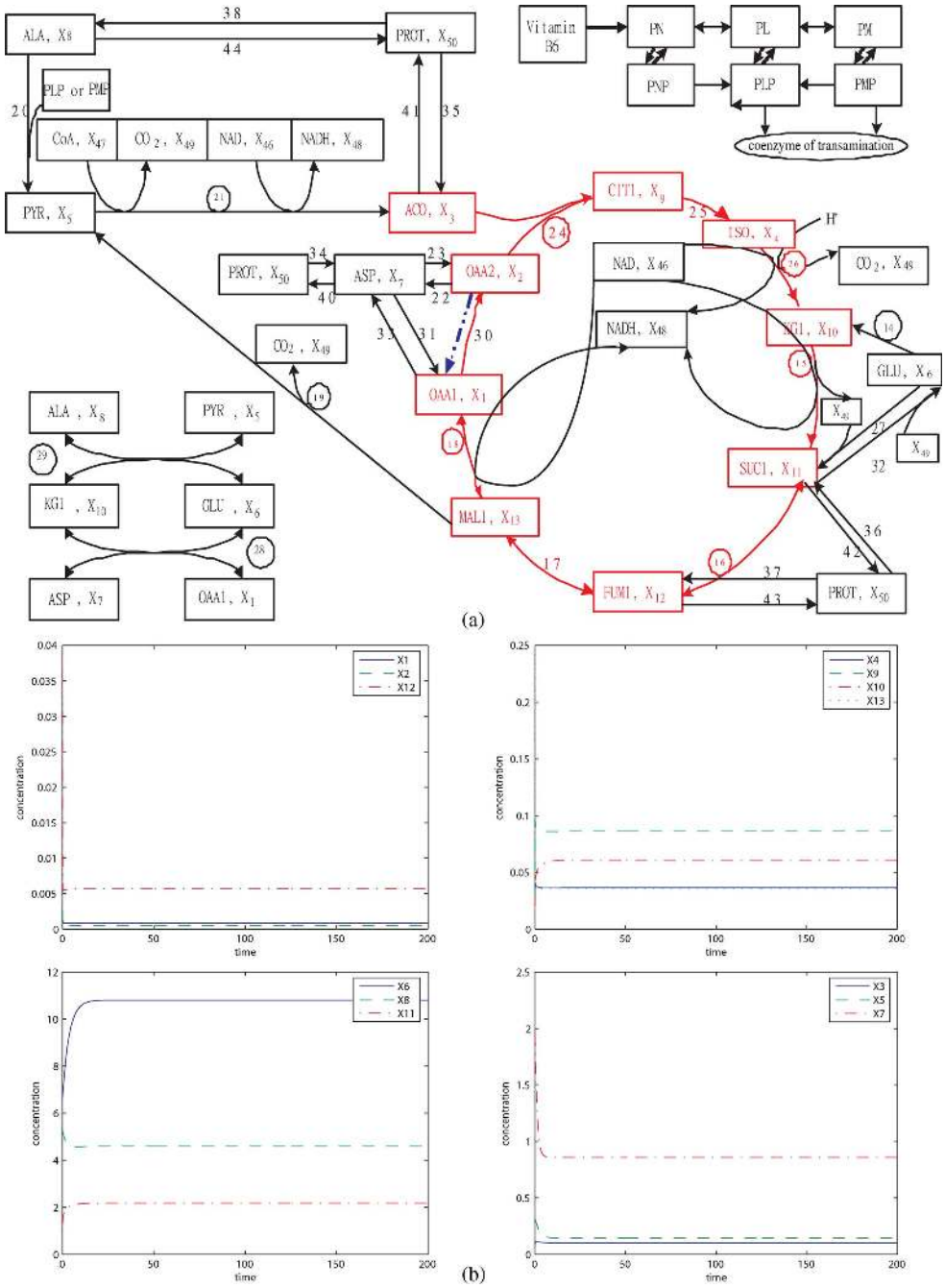

Fig. 3. (a) TCA cycle network redrawn from KEGG database and [2], [47]. (b) Time responses of (a) in the nominal parameter case.

According to the arguments in [9] and [11], system controls, modularity, alternative mechanisms (redundancy and diversity) and decoupling serve as basic mechanisms to provide robustness to the system. Enhancement of robustness against perturbation can be made through the combination of these mechanisms, but the system control is the primary mechanism for coping with environmental perturbations. Therefore, evolution of organisms can be viewed, at least in one aspect, as evolution of control systems. Modularity, alternative mechanism, and decoupling, in part, support the robust maintenance of control loops, but are also controlled by control loop either explicitly or implicitly. The perspective on biological robustness would provide effective guiding principles for understanding many biological phenomena, and for therapy design [9] and [10]. In this study, we have developed three robust schemes. For the first design scheme $\Delta A_D \Delta A_D^T < (A_D + F)(A_D + F)^T$ in (17), the control feedback F is to improve the system's structural stability to tolerate parameter perturbation ΔA_D. Negative feedback scheme in biochemical networks is of this design case. For the second design scheme $(F+\Delta A_D)(F+\Delta A_D)^T < A_D A_D^T$ in (20), our design purpose is to specify F to cancel or compensate ΔA_D such that the parameter perturbation effect could be attenuated to meet the robust design scheme. The specification of F based on the mechanisms of modularity, redundancy and decoupling could achieve the effective compensation of parameter perturbations. Furthermore, the multiple purpose design

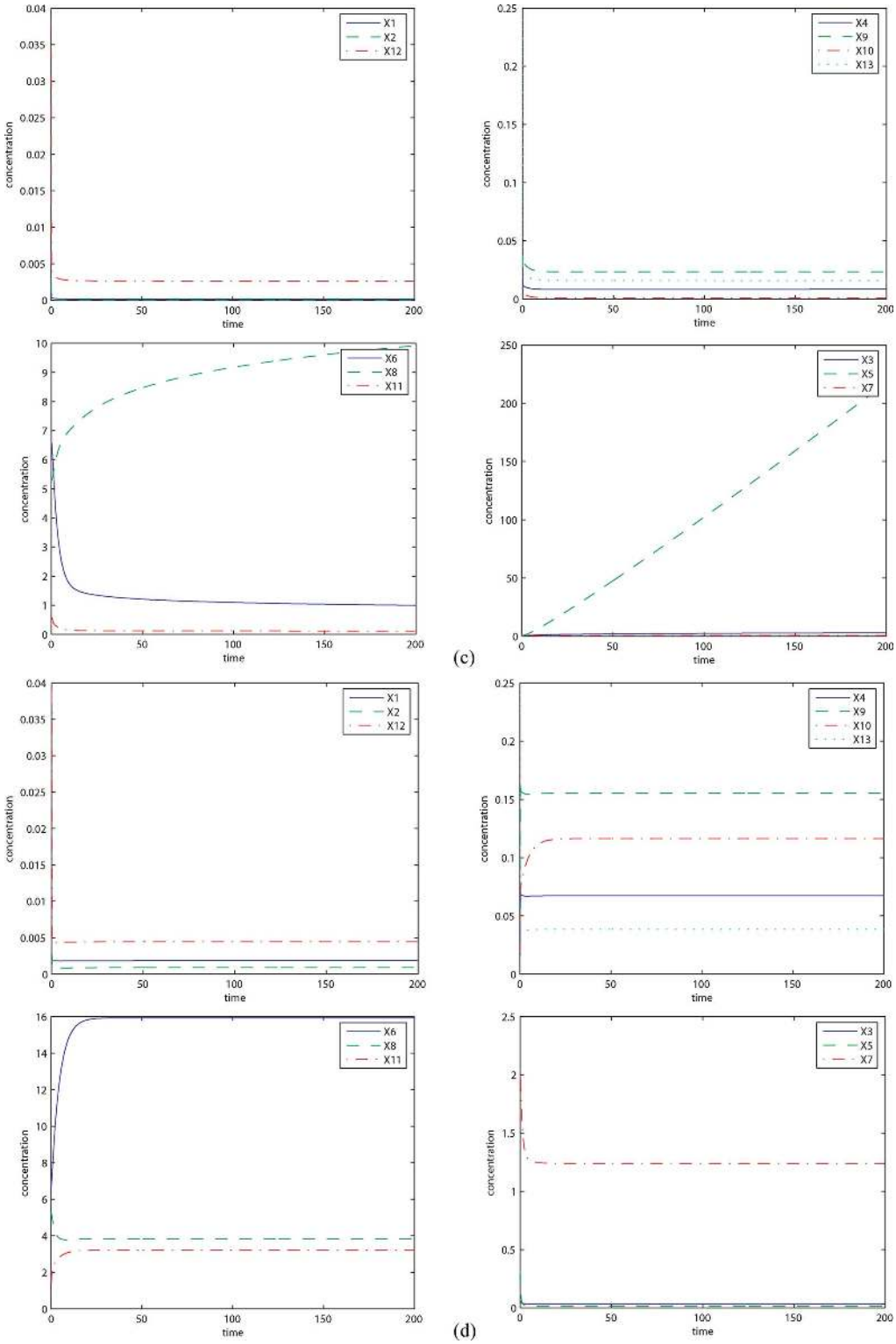

Fig. 3. *(Continued)* (c) Time responses of the TCA cycle network under the parameter perturbations ΔA_D in (34). (d) Time responses of the designed TCA cycle network with $f_{12} = -0.2$ (the dash-dot line from X_2 to X_1) under parameter perturbations in (34).

in (35) with combination of different schemes could effectively achieve robust design to attenuate the parameter variation and external disturbance.

In order to maintain the robustness of functionality against the parameter variations and disturbance, the feedback circuit with parameter compensation F should be imposed. In other words, robustness allows changes in the structure and components of the system owing to perturbations, but specific functions are maintained. This also matches Kitano's argument, i.e., the evolution of organisms can be viewed, at least in one aspect, as evolution of control systems.

The multi-objective circuit control design for biochemical networks is transformed to three corresponding inequalities in (35), which can be solved easily using Matlab to search for the

adequate circuit control parameters. Therefore, the proposed biochemical circuit design method provides efficient design strategies from the system perspective to modify or design biochemical networks with desired robust and sensitive properties based on control design principles and simulations, instead of trial-and-error experimental search. In other words, through the multi-objective design criteria in (35), we propose a framework of multi-objective circuit control design for biochemical networks to tolerate intrinsic kinetic parameter variations and to attenuate the effect of environmental variations. If there are several sets of design parameters that can meet the multi-objective robust control design inequalities in (35), then a set of parameters with proper time response or easy implementation can be specified for the robust biochemical control design. Further, several metabolic engineering and synthetic biocircuit methods are also discussed to implement the proposed robust circuit control designs of biochemical networks.

VIII. Conclusion

We have proposed a systematic circuit design method to achieve a robust biochemical network with a prescribed tolerance to the parameter perturbations and a desired sensitivity to the external disturbances. Two robust circuit design schemes are developed. One scheme is to improve the structural stability of a biochemical network via adequate feedback synthetic circuits and the other is to compensate parameter variations, which mimics the self-regulation and redundancy in biochemical networks. Further, a multi-objective biochemical network is also introduced for both the robust control design against intrinsic parameter variation and a desired sensitivity against external disturbances, which is with a more potential application for biotechnological purpose and drug design purpose. Recent advances in both metabolic and genetic engineering have made the proposed robust biochemical circuit control design feasible from the theoretical and experimental viewpoints. Finally, several computational simulation examples of robust circuit design are also given to illustrate the design procedure and to confirm the performance of the proposed robust design method. Based on the mathematical model, the proposed systematic circuit design schemes will provide a powerful tool for robust synthetic biology design of biochemical networks in the near future.

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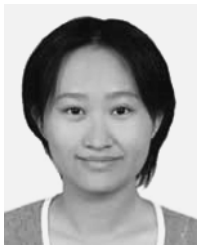

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