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Optimal Design of Robust Synthetic Biological Oscillators

Chia-Hua Chuang and Chun-Liang Lin*

Department of Electrical Engineering, National Chung Hsing University, Taichung 402, Taiwan, Republic of China

Abstract

This paper applies a real structural genetic algorithm (RSGA) to simultaneously identify network structure and estimate system parameters for a robust biological oscillator with specific oscillation frequency. At present, a repressilator with oscillation phenomenon has been successfully built in *Escherichia coli (E. coli)* and its function is to harmonize cell-cell communication. However, stochastic molecular perturbations may affect the oscillatory behaviors and the network structure is unknown beforehand. We use a stochastic S-system model to capture the dynamic behavior and the network topology for the biological oscillator under the perturbational environments, transform a robust synthetic design problem to a robust multi-objective optimization problem, and introduce the RSGA to solve this problem based on the design specification. The optimal parameters and the simplest structure are simultaneously searched such that the cost function related to tracking error of sinusoidal signal and the number of reaction pathways is minimized. Numerical experimental results *in silico* show that the proposed method is effective to synthesize robust biological oscillators implementing the particular oscillatory functions when the networks are influenced by intrinsic and extrinsic stochastic molecular perturbations.

Keywords: Synthetic biology; Biological oscillator; Modeling; Genetic algorithm; Robust optimization

Introduction

Synthetic biology and systems biology are new interdisciplinary researches, which combine the knowledge of various fields to understand the behavior of biological systems from the system perspective and allow one to construct an artificial biological circuit embedding into host cells to perform the new tasks or modify the behavior of organisms [1-3]. Similar to the standard electronic components such as resistors, inductors, capacitors, and transistors in electronics, the biological system also includes the basic components of DNA, RNA, protein, and metabolites at the bottom of the hierarchy of synthetic biology Recently, many biological circuits have been successfully built to achieve the basic functions, such as toggle switches [4,5], oscillators [5-10], pulse generator, genetic counter, logic evaluator, sensor, filter, and cell-cell communicator. Based on a bottom-up approach, the more complicated large-scale biological systems can be constructed, such as tunable filters, analog-to-digital and digital to analog converters, and adaptive learning networks, and applied in medicine, biotechnology, bioremediation, and bioenergy [11].

Oscillation is an important natural phenomenon and widely occurs in physical, biological, chemical, and social systems. In biological systems, the oscillation phenomena have been found at various levels of biological organization, ranging from neuronal rhythms to biochemical oscillations, and circadian clocks. For different cell types, it depends on various rhythmic frequencies to control cell physiology and harmonize cell-cell communication [12,13]. Presently, a simplest oscillator composes a single gene repressing itself [7]. An extension of the simplest oscillator is known as the repressilator which consists of one and more genes repressing other genes resulting limit cycles in the concentration responses. The topology of repressilator is also observed in other fields. In electronic circuits, a cycle of an odd number of NOT gates is called a ring oscillator, and cyclic networks of neurons are known as neural ring network in neuroscience [14,15]. The oscillation signal can also be generated by the repressive and activating links in mammalian cells [10]. These oscillators are potentially applicable in the control of dosage of drugs, such as melatonin can be released at night to help sleeping.

To understand the characteristics of these oscillatory processes from system-level, the biological models ranging from Boolean network, deterministic model, and stochastic model are developed to capture dynamic responses of the complex systems, and further analyze or design the synthetic biological networks [16-19]. A nonlinear S-system model is widely used to describe the dynamic behavior and the network topology. In the known network topology, many parameter estimation methods with multiple-objective optimization have been proposed to reconstruct S-system models from time data [20-22]. To characterize the delayed genetic regulatory networks with the S-system models has further been developed [23,24]. However, the information of the network structure is lack beforehand, there are many computational algorithms proposed to identify network topology and estimate system parameters [25-30]. To combine a threshold value to genetic algorithm (GA), the genetic networks with a combined structure and parameter optimization can be constructed [25-27,30].

However, a robust synthetic network is difficultly built to achieve the desired biological behaviors while considering the environment of stochastic molecular perturbations [31-34]. The oscillatory behaviors are more difficult than the steady state behaviors for designing a synthetic network because the equilibrium point of the former is dynamic, and the latter has only one fixed equilibrium point [33]. This can be regarded as a multi-objective optimization problem accompanied by the requirement of robust optimization.

This paper introduces a real structural genetic algorithm (RSGA) [35-37] to simultaneously estimate system parameters and identify

***Corresponding author:** Chun-Liang Lin, Department of Electrical Engineering, National Chung Hsing University, Taichung 402, Taiwan, Tel: +886-4-2285-1549; Fax: +886-4-2285-1410; E-mail: chunlin@dragon.nchu.edu.tw

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network structure for synthesizing a robust sustained biological oscillator described by the stochastic S-system model under the perturbational environments. The RSGA incorporates the major advantages of real genetic algorithm (RGA) with structured genetic algorithm (SGA) to deal with the combined parameter and structure optimization problem. The RGA, which emphasizes on coding of the chromosomes with floating point, is more efficient than the conventional GAs to handle the parameter optimization problem, while the SGA was developed to deal with the multi-objective optimization problem, i.e. the problem of simultaneous structure and parameter optimization. In the adopted RSGA, the function of structured genetic mapping is provided to regulate the structure of biological oscillators by using control genes to control the activities of parameter genes in the chromosome structure.

To synthesize the robust biological oscillator with the desired oscillation by the RSGA, a sinusoidal wave with the specific amplitude, frequency and phase is used to describe the desired reference oscillation and the standard deviation of intrinsic and extrinsic noises is chosen to mimic the stochastic molecular perturbations in biological systems. According to the objectives of optimal parameters and simplest structure, we establish two criteria relating our design objectives. All system parameters including rate constants and kinetic orders in S-system model are regarded the parameter genes in RSGA, the control genes are used to control parameter genes to switch the link of reaction pathways. The obtained solution which has the simplest structure and the optimal parameters can achieve the design of the desired robust synthetic oscillator based on the design specifications. Numerical experimental results *in silico* are illustrated to confirm the proposed method is effective to synthesize the robust biological oscillator when the network is influenced by the intrinsic and extrinsic stochastic molecular perturbations, and further show that the RSGA-based design converges faster and yields better performance in comparison to RGA and SGA approaches because of the strategies corresponding to parameters and structure optimization are consistent.

RSGA-Based Robust Biological Oscillator

Stochastic dynamic model for biological systems

The simplest structure of synthetic oscillators composes a single gene repressing itself. In the mouse, the Hes7 protein inhibits itself Hes7 promoter [7]. The extension of the simplest oscillator is called as repressilator which consists of one and more genes repressing the other genes in the limit cycle. Recently, the biologists have successfully built a repressilator which consists of three in-chain repressor genes. The first repressor protein, *lac I* from *E. coli,* inhibits the transcription of the second repressor gene, *tet R* from the tetracycline-resistance transposon *Tn*10, whose protein product in turn inhibits the expression of the third gene, *cI* from the λ phage. Finally, *cI* inhibits *lac I* expression, completing the negative feedback cycle [6]. The topological types of the above oscillators only focus on the repressive links. The oscillatory signal can also be generated by the repressive and activating links in mammalian cells [10]. However, there is lacking of a systematic approach to identify the network topology and determine the system parameters for synthesizing a robust biological oscillator with desired amplitude, frequency, and phase in stochastic environments. To tackle the problem, consider here the sinusoidal wave describing for the desired oscillatory phenomenon as follows

$$x_{di}(t) = A_i \sin(\omega_i t + \varphi_i) + x_{d0,i},\ i = 1,\ 2,\ldots,\ n \tag{1}$$

where $x_{di}(t), \forall i$ are the reference oscillatory signals with the desired amplitudes A_i, frequencies ω_i, phases ϕ_i, and $x_{d\,0,i}$ is the base level to ensure nonnegative protein concentration.

To understand the nonlinear characteristics of biological systems from the system-level, we adopt the S-system model to describe the architecture of synthetic oscillatory networks constructed by many molecules, and active and inactive pathways. It is typical power-law formalism and is well enough to capture the nonlinear dynamic characteristics and the connection information of reaction pathways. The S-system model can be described as follows [16].

$$\dot{x}_i = V_i^+ - V_i^- = \alpha_i \prod_{i=1}^{n} x_j^{g_{ij}} - \beta_i \prod_{i=1}^{n} x_j^{h_{ij}},\ i = 1,\ 2,\ldots,\ n \tag{2}$$

where x_i is a state which denotes the concentration of protein, V_i^+ and V_i^- are respectively input flux and output flux, $\alpha_i \geq 0$ and $\beta_i \geq 0$ are rate constants which denote, respectively, production and degradation effects; g_{ij} and h_{ij} are kinetic orders. For the kinetic orders g_{ij}, the j-th state actives the state i when the values of kinetic orders are positive and the state j inhibits the state i when the values of kinetic orders are negative. The kinetic orders h_{ij} have the opposite effects to g_{ij}. Zero values of kinetic orders represent the state j won't affect the state i, that is, the reaction pathway from state j to state i doesn't exist. The model contains 2 n rate constants and $2n^2$ kinetic orders. The total number of system parameters is 2n (n +1). Figure 1shows the topology of a generalized S-system model.

For example, the S-system model for *Dictyostelium* network can be illustrated as follows [19]

$$\dot{x}_1 = 2x_7 - 0.9x_1x_2,$$
$$\dot{x}_2 = 2.5x_5 - 1.5x_2,$$
$$\dot{x}_3 = 0.6x_7 - 0.8x_2x_3,$$
$$\dot{x}_4 = 1 - 1.3x_3x_4,$$
$$\dot{x}_5 = 0.3x_1 - 0.8x_4x_5,$$
$$\dot{x}_6 = 0.7x_1 - 4.9x_6,$$
$$\dot{x}_7 = 23x_6 - 4.5x_7$$

where the variables $x_1, x_2, x_3, x_4, x_5, x_6$ and x_7 respectively denote the concentrations of ACA, PKA, ERK2, REGA, internal cAMP, external cAMP, and CAR1. The cAMP oscillation in *Dictyostelium* is a robust spontaneous phenomenon by the interplay of activating and inhibiting pathways.

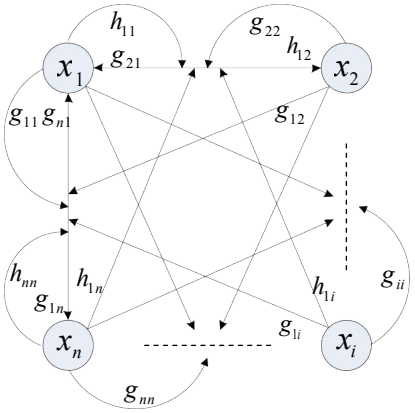


Figure 1: Topology of a generalized S-system model

The capability of oscillations depends on the network topology and system parameters for the design of synthetic biologically oscillatory systems. Most importantly, the synthetic oscillator must be robust to the internal parameter uncertainties such as thermal fluctuation and external environment disturbances in the host cell because these fluctuations may lead sustained oscillations to be damped oscillations, stable steady states or chaos. At present, these synthetic oscillators still can't perform reliably for a long time and need further tuning before application, hence, design a robust synthetic oscillator to withstand the influences of intrinsic and extrinsic stochastic molecular perturbations is necessary [33]. The robust oscillatory networks based on the S-system model when the networks are influenced by intrinsic fluctuations and extrinsic disturbances can be described as

$$\dot{x} = f(x) + \sum_{k=1}^{L} g_k(x) n_k + w \tag{3}$$

where $x = [\, x_1\ x_2 \cdots x_i \cdots x_n\,] \in R^n$ denotes a concentration vector which denotes the concentration of proteins; $f(\cdot)$ and $g_k(\cdot)$ denote, respectively, the vectors of interaction of synthetic oscillatory networks which display to the right term of (2) and coupling vectors of intrinsic noises. The intrinsic noises $n_k, \forall k$ are state-dependent with zero mean and standard deviation σ_k, and w is the extrinsic noise.

The purpose here is to synthesize a robust biological oscillator by introducing a systematic design method. We apply two models to describe the reference oscillatory signals desired for the biologists, and the dynamic response of realistic biological systems mentioned above. The robust synthetic design problem can be transformed to a robust optimization tracking problem accompained by parameter and structure estimation formulation. To achieve the desired outcome with low cost and less structure complexity, we consider two criteria related by minimizing the concentration error between the desired reference oscillation and synthetic concentration, and the number of reaction pathways in the following objective function such that the synthetic oscillators have satisfactory performance and simpler structure respectively:

$$J_p = E\left[\sum_{i=1}^{n}\int_0^{t_f}\left(\frac{x_i(t) - x_{di}(t)}{x_{di,\max}}\right)^2 dt\right] \tag{4}$$

and

$$J_s = \frac{N_g + N_h}{2n^2} \tag{5}$$

where J_p is the normalized performance index, J_s reflecting the normalized index of structure complexity complexity, E denotes the expectation, t_f is the final time for collecting data, $x_{di,\ max}$ is the i-th maximal reference concentration, N_g and N_h are respectively the number of nonzero g_{ij} and h_{ij} is *large*, the structure will be much complexity, otherwise.

The design specifications of robust synthetic oscillator are given as follows.

1) Given a desired oscillation signal with the desired amplitudes, frequencies and phases as described by (1).

2) Given the standard deviation of intrinsic noises and extrinsic noises according to the situation in vivo of synthetic oscillators in the host cells.

3) Given the feasible ranges of design parameters according to the feasible design condition.

$$\begin{aligned} &\alpha_i \in \left[\alpha_{i,\min} \quad \alpha_{i,\max}\right],\ \beta_i \in \left[\beta_{i,\min} \quad \beta_{i,\max}\right], \\ &g_{ij} \in \left[g_{ij,\min} \quad g_{ij,\max}\right],\ h_{ij} \in \left[h_{ij,\min} \quad h_{ij,\max}\right] \end{aligned} \tag{6}$$

where $\alpha_{i,min}$ ($\beta_{i,min}$) and $\alpha_{i,\max}$ ($\beta_{i,max}$) are, respectively, lower and upper bounds for rate constants α_i (β_i) and $g_{ij,\ min}$ ($h_{ij,min}$) and $g_{ij,max}$ ($h_{ij,max}$) are lower and upper bounds for rate constants g_{ij} (h_{ij}), respectively.

4) Define the objective function which consists of the corresponding costs of two parts related to oscillation performance and network complexity as follows

$$J_{tot} = \min_{\alpha_i, \beta_i, N_g, N_h} \rho J_s(N_g, N_h) + (1-\rho) J_p(\alpha_i, \beta_i) \tag{7}$$

where the weighting factor $\rho \in [0,1]$ representing the desired emphasis on the corresponding terms. If $\rho > 0.5$, the performance of oscillation accuracy is treated heavily than the structure complexity of the oscillators and vice versa.

The above formulation is a simple multi-objective optimization problem. The design should be able to predict the parameters of S-system model within the feasible range (6) and identify the network topology to achieve the design of the robust nonlinear synthetic oscillators in the stochastic environments such that the number of reaction pathways and the synthetic error for the desired oscillation with the amplitude, frequency, and phases are minimized.

Real structural genetic algorithm

In general, it is not easy to obtain a solution to solve the multi-objective optimal tracking design problem (7) for the nonlinear stochastic synthetic biological networks [3] to satisfy the design specifications of robust synthetic oscillator.

The RSGA is a stochastic optimization algorithm [35-37] which is a variant from the traditional GA which simultaneously considering optimization of structure and parameters mimics the mechanisms of natural selection and evolution genetics. It's different from other traditional optimization methods which get the possibility of local optimal solution. Because it simultaneously evaluates many points in the parameter space, it is suitable to deal with the stochastic optimal tracking problem for designing the biological oscillators to obtain the global optimal solutions. Moreover, this algorithm combines the advantages of RGA and SGA, thus it may have the properties of highly computation speed and precision. One may refer to [36] for an introductory reference of this algorithm. The synthetic design procedure of robust biological oscillatory networks via RSGA is reformed and applied to deal with the current problem. Figure 2 illustrates the design process solving for the current problem. The RSGA is used to search for the optimal parameters and simplest structure such that the cost function (7) related to the concentration errors between the reference signal (1) and the synthetic signals (3) and the number of reaction pathways is minimized.

Initialization: We start by initializing a population of possible solutions, that is, randomly generate a population of candidate chromosomes. In RSGA, the chromosome structure consists of control genes and parameter genes. The control genes are used to regulate the parameter genes. For the current problem, all rate constants and all kinetic orders in the S-system model are arranged as the parameter gene's strings. The number of control gene's strings is equal to the

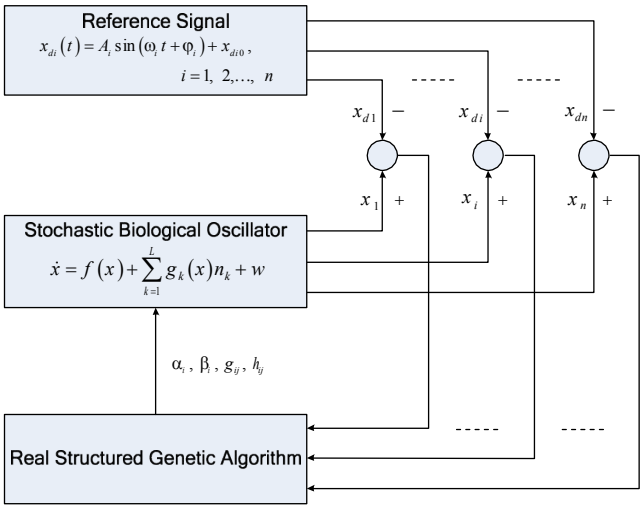


Figure 2: Block diagram for designing the robust synthetic biological oscillators via RSGA.

number of kinetic orders, which is used to determine the network structure and the type of reaction pathways. The computational complexity isproportional to the number of system states. There are 2n(n+1) parameter genes including all rate constants and all kinetic orders and $2n^2$ control genes for the system with n states The overall length of the chromosome structure contains genes. Both of the control genes and parameter genes are real numbers within feasible ranges in (6). The fundamental chromosome structure is shown in Figure 3 and the mathematical model is defined as follows

$$X = (C, P) = ([c_m], [p_l]),\ m = 2n^2,\ l = 2n(n+1) \tag{8}$$

where X represents an ordered set consisting of the control genes' strings $C = [c_m]$ and the parameter genes' string $P = [p_l] \in g_{ij}, h_{ij}, \alpha_i, \beta_i$ in which the kinetic orders are placed in the front of rate constants, and then the control genes control the corresponding kinetic orders.

Structured genetic mapping: The strategy of structured genetic mapping is used to regulate the topology of biological networks, and further identify the important reaction pathways for synthesizing the biological oscillators. The mathematical model of structured genetic mapping from C to P is defined as

$$\tilde{X} = \langle C, \tilde{P} \rangle = \langle [c_m], [c_m] \otimes [p_l] \rangle = \langle [c_m], [\tilde{p}_l] \rangle \tag{9}$$

with

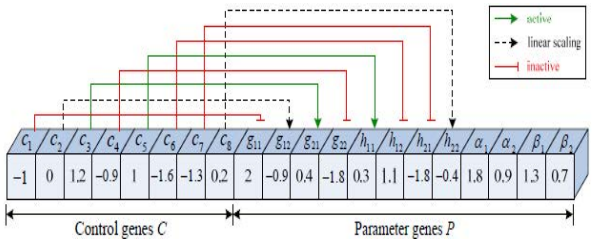
$$\tilde{p}_l = \begin{cases} p_l,\ B_{\max} \le c_m \text{ or } l > m \\ p_l \varepsilon,\ B_{\min} < c_m < B_{\max},\ \varepsilon = \dfrac{c_m - B_{\min}}{B_{\max} - B_{\min}} \\ 0,\ c_m \le B_{\min} \end{cases} \tag{10}$$

where $\tilde{X}$ is new chromosome after structured genetic mapping, $\otimes$ acts as the genetic switch to determine the status of each element in the parameter gene's string, and denotes the effective gain for c_m within the boundary of $B_{\min}$ and $B_{\max}$ The activation function of the control genes is determined by $B_{\min}$ and $B_{\max}$, which corresponds to the boundaries for OFF (inactive) and ON (active) respectively. The control genes regulate the parameter genes by linearly scaling their original values when c_m with the boundary of $B_{\min}$ and $B_{\max}$. If the value of the control gene exceeds $B_{\max}$ the corresponding parameter gene is maintained; the parameter gene is deactivated if otherwise, that is, this parameter gene of kinetic order is set to be 0, and thus the corresponding reaction pathway doesn't exist. A boundary sizing technique is applied to regulate the values of $B_{\min}$ and $B_{\max}$ in each generation.

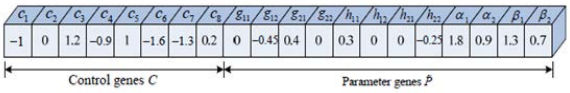
Example: The S-system model for two-state biological network can be described by

$$\dot{x}_1 = \alpha_1 x_1^{g_{11}} x_2^{g_{12}} - \beta_1 x_1^{h_{11}} x_2^{h_{12}},$$
$$\dot{x}_2 = \alpha_2 x_1^{g_{21}} x_2^{g_{22}} - \beta_2 x_1^{h_{21}} x_2^{h_{22}} \tag{11}$$

From the above model, there are 4 rate constants and 8 kinetic orders. Suppose that a randomly generated chromosome with B_{max} = 0.8 and B_{min} = -0.8 is given as follows



The new chromosome after using the structured genetic mapping becomes



and its corresponding synthetic S-system model is characterized as

$$\dot{x}_1 = 1.8 x_2^{-0.45} - 1.3 x_1^{0.3},$$
$$\dot{x}_2 = 0.9 x_1^{0.4} - 0.7 x_2^{-0.25} \tag{12}$$

The new model possesses only 4 rate constants and 4 kinetic orders, i.e. the network has reduced 4 reaction pathways. One can further obtain a simpler structure which may consist of the repressive and activating reaction pathways.

Fitness: To employ the RSGA to solve the searching problem, the solution have to be related the fitness function F which is inversely proportional J_{tot}. The large fitness meant the synthetic network has a smaller tracking error and the less reaction pathways and vice versa.

Genetic operators: Similar to the standard GAs, the RSGA involves fundamental genetic operations including reproduction, crossover, and mutation. Reproduction operates on the principle of survival of the fittest in the process of natural selection. A new population in the next generation is created from the current populations according to the reproduction probability which is defined as $F_i / \sum_{i=1}^{M} F_i$ where F_i is the fitness value of the i-th member and M is the population size. A higher probability tends to be assigned to chromosomes associated with higher fitness value among the population. Crossover operator provides a mechanism to mix the information of two chosen chromosome. As in the usual GAs, the number of individuals joining

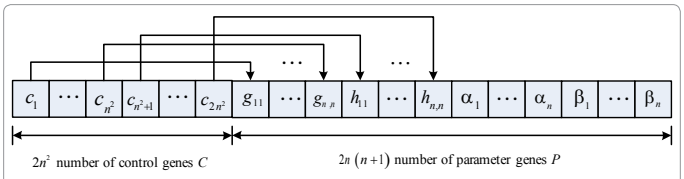


Figure 3: Chromosome structure of RSGA for synthesizing the biological oscillators based on the S-system model.

the operation is determined by the crossover probability. The crossover of randomly selected pairs of individuals is a combination of an extrapolation/interpolation method with a crossover process. It starts by extrapolation and switches to interpolation when the parameters of the offspring exceed the permissible range. Interpolation avoids parameters from going over the admissible range during a boundary value search. Mutation operator applies for the randomly chosen individuals. The number of individuals to be varied is determined by the mutation probability. The mutation operator adopted is the non-uniform mutation method. The dynamic mutation and crossover probability adjustment method based on the idea of the Butterworth filter is utilized. Applying this scheme to the crossover and mutation probabilities, one can ensure that the emphasis of the algorithm is placed on the structure first, and then on the parameters.

The design steps of RSGA are summarized as follows.

Step 1: Specify the design specifications.

Step 2: Generate randomly a population of chromosome.

Step 3: Apply the structure genetic mapping to switch the parameter genes.

Step 4: Calculate the fitness value for each candidate chromosomes.

Step 5: Determine the crossover and mutation probabilities by using the dynamic probability adjustment

method.

Step 6: Perform the genetic operators such as reproduction, crossover, and mutation to create offspring.

Step 7: If the stop condition is achieved, then the optimal solution is obtained. Otherwise, go to Step 3.

The flow chart of RSGA for designing robust synthetic biological oscillators is shown as in Figure 4.

Remark 1: The elitist strategy can be incorporated to enhance the convergence performance. This strategy copies the best chromosomes from the old population to the next population to prevent losing the best solutions.

Remark 2: Both control and parameter genes apply the same crossover operation for generating new individuals. This unified mechanism avoids the need to utilize two kinds of crossover operators with different attributes as required in the traditional SGA. The unified mechanism results in better computation efficiency and consistency of chromosome crossover.

Results

To demonstrate effectiveness of the proposed RSGA-based robust synthetic biological oscillators, the following examples are given to illustrate the design procedure and confirm the performance related to the simplest network structure and the optimal model parameters under the stochastic molecular noises.

Example 1: Two-component biological oscillator

Consider the synthetic biological oscillator with two-components in (11) while the network isn't corrupted by any molecular noises, and suppose the prescribed reference model as follows.

$$x_{d1}(t) = 0.3\sin(t) + 1, \qquad x_{d2}(t) = 0.3\cos(t) + 0.5 \tag{13}$$

where $[\, x_{d1}(0)\ x_{d2}(0)] = [1 \quad 0.8]$, α_i , $\beta_i \in (0, 2]$ and g_{ij} , $h_{ij} \in [-2, 2]$. For the two-component synthetic network, the total number of reaction pathways is 8 in the S-system model. To solve the parameter and structure optimization problems, we apply the proposed RSGA method to predict the optimal system parameters and identify the simplest network structure. For the chromosome structure, there are 12 parameter genes including 8 kinetic orders and 4 rate constants, and 8 control genes to control the activities of 8 kinetic orders and regulate the structure complexity of the biological oscillator. The initial conditions for RSGA are selected as follows: generation size 5000, population size 1000, crossover rates 0.8 and 0.7 corresponding to the control and parameter genes, respectively, and mutation rates 0.3 and 0.2 corresponding to the control and parameter genes, respectively. The weighting factor ρ is set to be 0.5 for a balanced weight on the oscillation accuracy and network complexity. The dynamic concentration response of the designed RSGA-based synthetic biological oscillator is shown in Figure 5a-5c respectively display the best fitness value approximates to 8 and the number of reaction pathways converges to 2. Clearly, the RSGA focuses on minimizing the model structure in the earlier generations and optimizing the parameters in the latter generations.

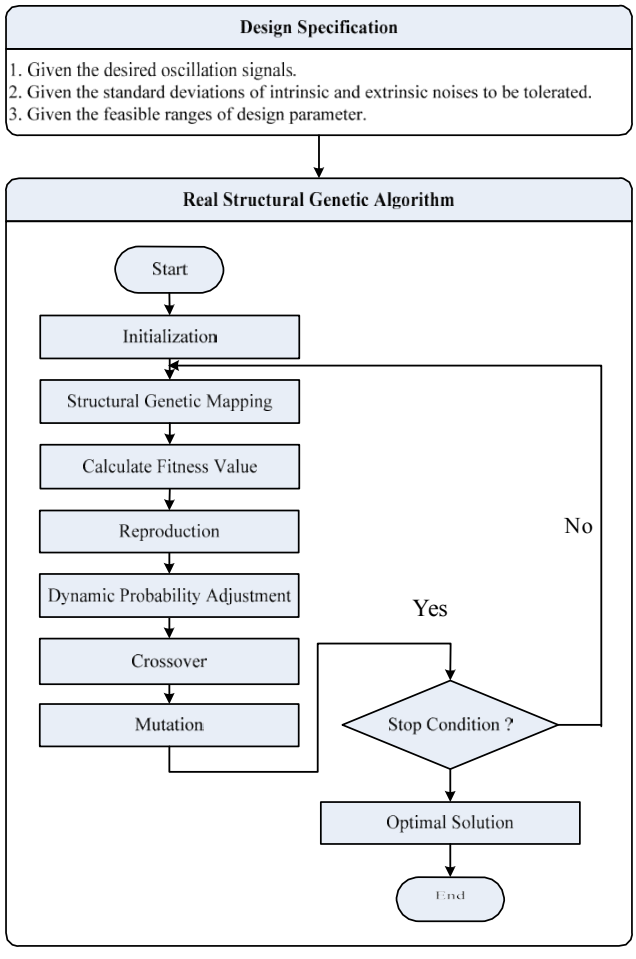


Figure 4: Flow chart of RSGA for designing the robust synthetic biological oscillators.

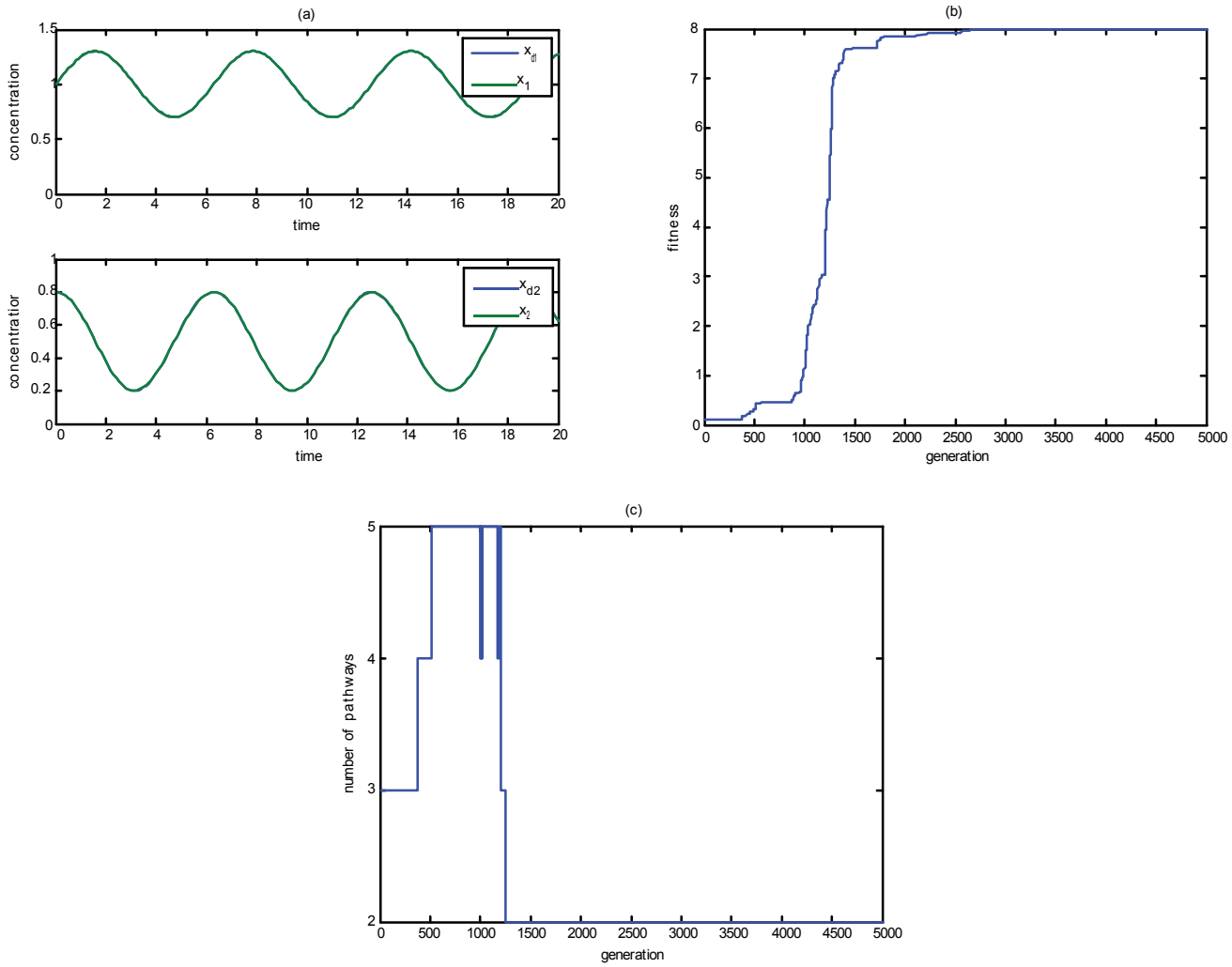


Figure 5: Dynamic simulations for the biological oscillator. (a) Concentration response of the reference and synthetic states; (b) Convergence of the fitness value, and (c) Convergence of the number of reaction pathways.

For the synthetic oscillator, there are 2 reaction pathways, state 1 activates the output flux of state 2, and the state 2 activates the input flux of state 1. The network topology is shown in Figure 6. The optimal parameters searching by RSGA, SGA, and RGA methods are compared and summarized in Table 1. The number of pathways for RGA is fixed at 8 corresponding to the model. The network topology of SGA is the same as that of RSGA. The comparison of concentration error for RSGA, SGA, and RGA-based design methods in terms of the mean error defined by $e_i = E\left(\int \left|x_i - x_{d\,i}\right| dt\right)$ with the final time t_f is given in Table 2.

To compare parameter sensitivity, the sensitivities of rate constants and kinetic orders are defined by [16]

$$S\left(x_i,\alpha_j\right)=\frac{\partial \ln x_i}{\partial \ln \alpha_j},\ S\left(x_i,\beta_j\right)=\frac{\partial \ln x_i}{\partial \ln \beta_j} \tag{14}$$

and

$$S\left(x_i,g_{ij}\right)=\frac{\partial \ln x_i}{\partial \ln g_{ij}},\ S\left(x_i,h_{ij}\right)=\frac{\partial \ln x_i}{\partial \ln h_{ij}} \tag{15}$$

where $S\left(x_i,\alpha_j\right)$ and $S\left(x_i,\beta_j\right)$ are the sensitivities of rate constants, $S\left(x_i,g_{ij}\right)$ and $S\left(x_i,h_{ij}\right)$ are the sensitivities of kinetic orders. The comparison of parameter sensitivity of the designed biological oscillator using RSGA, SGA, and RGA is given in Table 3. The sensitivities reflect the approximate percentage change in the state variables caused by 1% changes in rate constants or kinetic orders. The sensitivities of all system parameters for RSGA are smaller than that of SGA. As shown, the performance of oscillation accuracy and network structure resulted from applying the RSGA is better than others.

Example 2: Robust biological oscillator

Because the stochastic fluctuations in the host cell may affect the oscillatory properties of synthetic biological oscillator, the robust synthetic oscillator must be constructed to tolerate these stochastic disturbances.

Suppose that the two-component synthetic network is affected by the intrinsic parameter fluctuations and external environmental disturbances; the stochastic nonlinear model based on the S-system model is given by

$$\begin{aligned}\dot{x}_1(t)&=\left(\alpha_1+n_1\right)x_1^{g_{11}}x_2^{g_{12}}-\left(\beta_1+n_1\right)x_1^{h_{11}}x_2^{h_{12}}+w_1,\\ \dot{x}_2(t)&=\left(\alpha_2+n_2\right)x_1^{g_{21}}x_2^{g_{22}}-\left(\beta_2+n_2\right)x_1^{h_{21}}x_2^{h_{22}}+w_2\end{aligned} \tag{16}$$

	α_1	g_{11}	g_{12}	β_1	h_{11}	h_{12}
RSGA	0.9999	0	1.0003	0.4998	0	0
SGA	1.0029	0	0.9734	0.5096	0	0
RGA	0.6113	0.3273	1.4547	0.1286	0.3497	-0.6960
	α_2	g_{21}	g_{22}	β_2	h_{21}	h_{22}
RSGA	0.9214	0	0	0.9191	1.0889	0
SGA	1.2921	0	0	1.2980	0.7713	0
RGA	0.3792	-1.1530	0.1463	0.3874	1.6410	0.1386

Table 1: Parameter comparison of biological oscillator for RSGA, SGA, and RGA methods

state	RSGA	SGA	RGA
1	0.00098	0.0014	0.0057
2	0.0012	0.0023	0.0031

Table 2: Comparison of concentration error of biological oscillator for RSGA, SGA and RGA methods

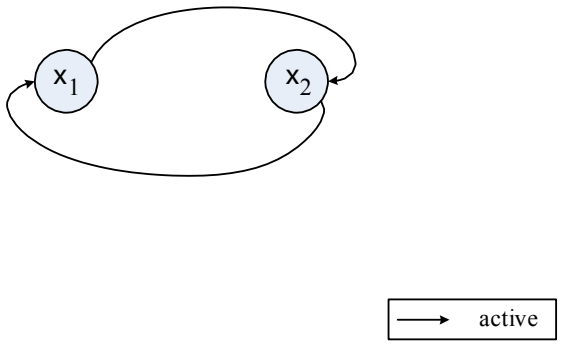


Figure 6: Network topology for the simplified two-component synthetic oscillator.

where w_k are and n_k , k=1,2 Gaussian noises with zero mean and standard deviation 0.01. The reference signals remain the same as that of [13]. The initial conditions of RSGA are the same to Example 1. The dynamic simulation for concentration response with 50 rounds is shown in Figure 7(a) and convergences of the fitness and the number of reaction pathways are respectively displayed in Figure 7(b) and 7(c). When the networks are influenced by the intrinsic and extrinsic stochastic molecular noises, the periodic oscillatory phenomenon is still sustained in Figure 7(a). There are spikes in Figure 7(b) due to noise corruption affecting the convergence of fitness value. The detail optimal parameters searched by adopting the RSGA, SGA, and RGA methods are summarized in Table 4. The number of pathways for RSGA is 2 which is less than the number of pathways for RGA. Define the standard deviation of concentration error as $SD_i = \sqrt{\left(\sum_{j=1}^{N}\left(e_{ij}-\bar{e}_i\right)^2\right)/N}$ with the mean $\bar{e}_i = \left(\sum_{j=1}^{N} e_{ij}\right)/N$ where N is the number of simulation rounds. Comparison of mean and standard deviation of concentration error for the robust biological oscillators with 50 simulation rounds are summarized in Table 5 and shown in Figure 8. The results of comparison of parameter sensitivity for the robust biological oscillator are displayed in Table 6. It is seen that the mean of concentration error by the design of RSGA is smaller than other GA-based approaches. From Tables 4-6, we can find that performance and structure complexity for the proposed method are superior to other approaches because the RSGA searches for the optimal solution in the specific parameter and structure spaces.

It is seen from case studies presented above that the synthetic oscillator design by using the proposed RSGA shows robust oscillatory characteristics under intrinsic parameter fluctuations and extrinsic environment disturbances. Random fluctuations in genetic networks are practically inevitable as chemical reactions are known to be probabilistic and many genes, RNAs and proteins are presented in low numbers per cell. It was depicted in [38] that such noise significantly affects all life processes. Thus, a synthetic network is difficult to build to present sustainable oscillation. If the noise effect could be incorporated into the network model with precise noise statistics, the present approach could be used as a way to synthesize a robust oscillator with the optimal performance with cheaper structure.

Discussions

The goal of synthetic biology is to construct artificial biological circuits performing the desired biological behaviors in the face of intracellular fluctuations and environmental disturbances. Because these stochastic perturbations can significantly affect oscillatory properties of the synthetic biological system, design of a robust synthetic oscillator is highly desirable by using a systematic method. For the nonlinear characteristics of a complicated biological system, one has to deal with the problem from the system perspective. By constructing the biological model and applying engineering methodology, one can be more efficient to design complex biological circuits. The S-system model is widely used to describe the dynamic behaviors of that kind of systems. The architecture of S-system model clearly reveals topological information and is well enough to capture the nonlinear property of synthetic biological oscillators, although there are always many system parameters to be determined under a specific structure.

To select the applicable promoter-RBS components from a promoter-RBS library, a class of robust genetic circuits has been constructed [39,40]. Green fluorescent protein (GFP) has been used in real-world experiments to exhibit genetic expression. The intensity of fluorescence of GFP can be measurable by using a flow cytometer. Following this idea, one can acquire input and output concentrations for different combination of promoter and RBS from measurement and identify system parameters in terms of our proposed model by system identification. With the information of these parameters, one is capable of using the RSGA to search a suitable combination of the corresponding promoters and RBS meeting the design specifications from the reconstructed libraries to realize the biological oscillator.

Conclusions

This paper has proposed an efficient design method based on RSGA to construct a robust synthetic biological oscillator with the optimal performance and simplest structure under stochastic molecular fluctuations. A robust synthetic design problem is converted to a robust multi-objective optimal tracking problem and a stochastic

		α_1	g_{11}	g_{12}	β_1	h_{11}	h_{12}
RSGA	x_1	0.00000	*	0.00000	0.00000	*	*
	x_2	-0.99970	*	0.69324	0.99970	*	*
SGA	x_1	0.00000	*	0.00000	0.00000	*	*
	x_2	-1.02733	*	0.69553	1.02733	*	*
RGA	x_1	-0.00128	0.00000	0.00135	0.00128	0.00000	0.00065
	x_2	-0.46498	0.00147	0.49034	0.46498	-0.00157	0.23460
		α_2	g_{21}	g_{22}	β_2	h_{21}	h_{22}
RSGA	x_1	0.91836	*	*	-0.91836	-0.00230	*
	x_2	0.00000	*	*	0.00000	0.00000	*
SGA	x_1	1.29651	*	*	-1.29651	0.00591	*
	x_2	0.00000	*	*	0.00000	0.00000	*
RGA	x_1	0.35792	0.00398	-0.03796	-0.35792	0.00567	0.03596
	x_2	0.00373	0.00004	-0.00040	-0.00373	0.00006	0.00037

* denotes no value because the corresponding kinetic order is zero.

Table 3: Comparison of parameter sensitivity of the biological oscillator design using RSGA, SGA and RGA

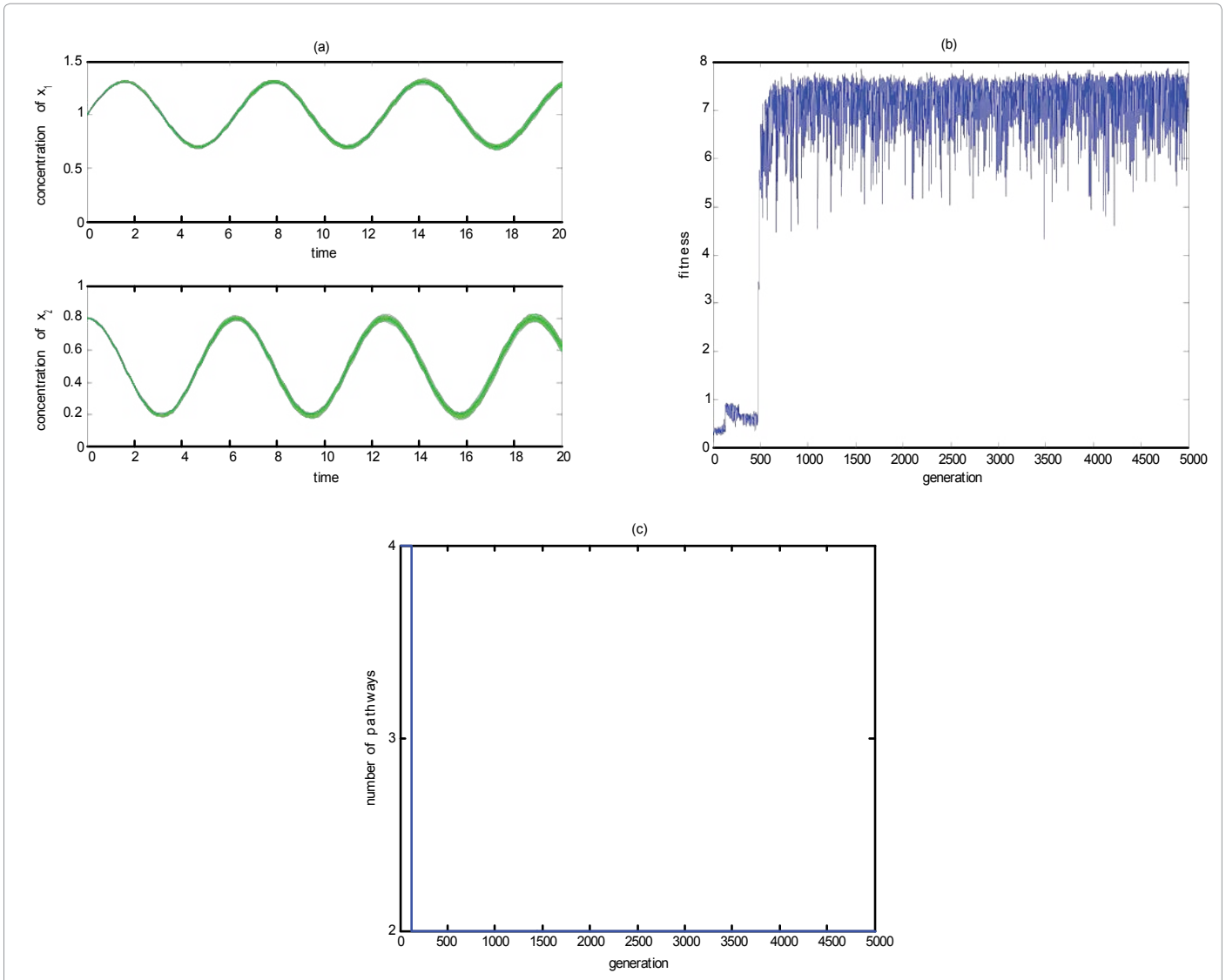


Figure 7: Dynamic simulations for the robust biological oscillator. (a) Concentration response of the reference and synthetic states with 50 rounds; (b) Convergence of the fitness value, and (c) Convergence of the number of reaction pathways.

	α_1	g_{11}	g_{12}	β_1	h_{11}	h_{12}
RSGA	1.0433	0	0.8635	0.5651	0	0
SGA	1.7745	0	0.3345	1.3749	0	0
RGA	1.3544	0.2000	0.6262	0.9094	0.1901	0.0745
	α_2	g_{21}	g_{22}	β_2	h_{21}	h_{22}
RSGA	1.0793	0	0	1.0791	0.9273	0
SGA	0.8528	0	0	0.8547	1.1637	0
RGA	0.6728	-0.5841	0.1078	0.6874	1.0109	0.1133

Table 4: Parameter comparison of robust biological oscillator for RSGA, SGA and RGA methods

	RSGA		SGA		RGA	
state	1	2	1	2	1	2
ei	0.0086	0.0083	0.0178	0.0125	0.0089	0.0086
SDi	0.0025	0.0028	0.0019	0.0035	0.0027	0.0026

Table 5: Comparison of mean and standard deviation of concentration error of the robust biological oscillator with 50 simulation rounds using RSGA, SGA and RGA

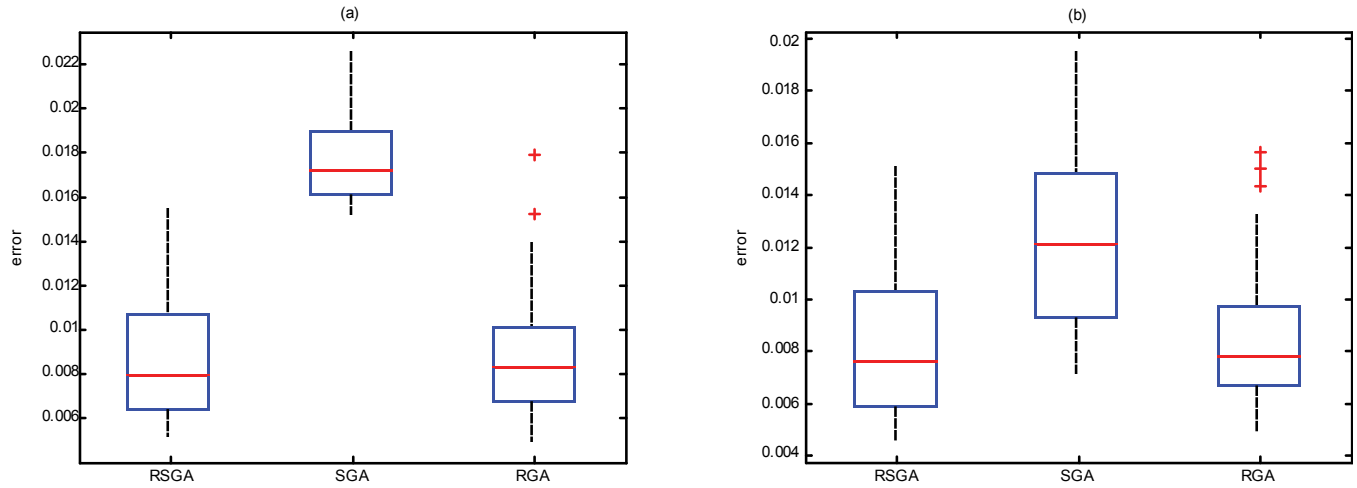


Figure 8: Boxplot of concentration error for the robust biological oscillators with 50 rounds using RSGA, SGA and RGA methods. (a) Concentration error of x1; Concentration error of x

		α_1	g_{11}	g_{12}	β_1	h_{11}	$h12$
RSGA	x_1	0.00000	*	0.00000	0.00000	*	*
	x_2	-1.15808	*	0.71007	1.15808	*	*
SGA	x_1	0.00000	*	0.00000	0.00000	*	*
	x_2	-2.98954	*	0.76274	2.98954	*	*
RGA	x_1	0.00625	-0.00001	-0.00283	-0.00625	0.00001	0.00034
	x_2	-1.81269	0.00398	0.81933	1.81269	-0.00378	-0.09748
		α_2	g_{21}	g_{22}	β_2	h_{21}	h_{22}
RSGA	x_1	1.07840	*	*	-1.07840	-0.00020	*
	x_2	0.00000	*	*	0.00000	0.00000	*
SGA	x_1	0.85933	*	*	-0.85933	0.00191	*
	x_2	0.00000	*	*	0.00000	0.00000	*
RGA	x_1	0.62700	0.00402	-0.04879	-0.62700	0.00695	0.05128
	x_2	-0.01125	-0.00007	0.00088	0.01125	-0.00012	-0.00092

* denotes no value because the corresponding kinetic order is zero.

Table 6: Comparison of parameter sensitivity of the robust biological oscillator design using RSGA, SGA and RGA

S-system model is used to describe the oscillation behaviors of biological systems. The proposed RSGA-based design method mimics the mechanism of natural selection to simultaneously search for the system parameters and network topology to achieve the desired robust oscillation with simple network complexity when the network topology is unknown beforehand and is possibly influenced by stochastic noises. Numerical demonstrative examples for two-component synthetic oscillator are illustrated *in silico* to confirm that the proposed method is superior than RGA and SGA in the design of biological oscillators with simultaneous optimization of the structure and parameter and can increase the robust oscillatory characteristics.

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