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Robust model matching design methodology for a stochastic synthetic gene network

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ABSTRACT

Synthetic biology has shown its potential and promising applications in the last decade. However, many synthetic gene networks cannot work properly and maintain their desired behaviors due to intrinsic parameter variations and extrinsic disturbances. In this study, the intrinsic parameter uncertainties and external disturbances are modeled in a non-linear stochastic gene network to mimic the real environment in the host cell. Then a non-linear stochastic robust matching design methodology is introduced to withstand the intrinsic parameter fluctuations and to attenuate the extrinsic disturbances in order to achieve a desired reference matching purpose. To avoid solving the Hamilton–Jacobi inequality (HJI) in the non-linear stochastic robust matching design, global linearization technique is used to simplify the design procedure by solving a set of linear matrix inequalities (LMIs). As a result, the proposed matching design methodology of the robust synthetic gene network can be efficiently designed with the help of LMI toolbox in Matlab. Finally, two *in silico* design examples of the robust synthetic gene network are given to illustrate the design procedure and to confirm the robust model matching performance to achieve the desired behavior in spite of stochastic parameter fluctuations and environmental disturbances in the host cell.

1. Introduction

Synthetic biology is essentially an engineering discipline to design and to construct simplified biological systems that provide a desired behavior [1–6]. The ability to design and construct biological systems can also offer scientists a useful way to test their understanding of the complex functional networks of genes and biomolecules that mediate life processes [7]. Therefore, synthetic biology is foreseen to have important applications in biotechnology and medicine. Other practical applications such as biosensing, the production of biofuels and novel biomaterials are also thought to be accomplished by synthetic biology [8]. At present, the construction of biological networks of inter-regulating genes, so-called gene networks, has demonstrated the feasibility of synthetic biology [9]. A bottom–up approach, which couples simple, well-characterized modules into more complex networks with behaviors that can be predicted from that of the individual components, has also shown to be achievable in synthetic gene network construction [10]. However, the development of gene networks is still difficult, and most newly created networks are non-functioning and need tuning [11]. One important reason for this difficulty is that a synthetic gene network suffers from intrinsic parameter variations due to thermal fluctuations, transcription factor (TF) bindings, gene expression noises, mutations, and extrinsic disturbances caused by changing extracellular environments [2,11,12]. These uncertainties and disturbances come from current biotechnological limitations and also from the fluctuations of intra- and extra-cellular environments in the host cell [11,13]. Furthermore, current approaches of gene network construction typically use a small set of components taken from nature systems, which are then assembled and tested *in vivo*, often without guidance from a priori mathematical modeling for design and assembly [12,14]. In this case, the networks rarely behave as intended the first time. They usually need to be resolved over months of iterative retrofitting, often by fine-tuning imperfect parts by mutation, identifying alternative parts or adding extra features to counterbalance the problem [12,14]. Because of these limitations that hamper the design of synthetic gene networks, the stability robustness against parameter fluctuation and the filtering ability to attenuate the environmental noises, which have been widely discussed from the systems biology perspective [13,15–21], are taken into consideration for synthetic gene network design.

Recently, Batt et al. proposed an approach to analyze a class of uncertain piecewise-multiaffine differential equation model of a synthetic gene network [11]. This modeling framework is adapted to the experimental data and allows the development for solving robustness analysis and tuning problems under parameter variations. This approach can find parameter sets for which the system

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presents a particular behavior. However, the external disturbances are not included in their design procedure. Kuepfer et al. developed an approach based on semi-finite programming for partitioning the parameter space of polynomial differential equation models into the so-called feasible and infeasible regions [22]. In this approach, 'feasible' simply refers to the existence of a steady state of the system. Chen et al. developed a circuit design scheme to implement some gene circuits into an existing gene network in an organism to improve the stability robustness to tolerate parameter variations [23,24]. However, this gene circuit design is different from the synthetic gene network design. The conventional gene circuit design implements some gene circuits to an existing gene network to improve its function, but the synthetic gene network designs a new artificial gene network, which has to be inserted into a host cell (for example, *E. coli*) to perform its function. In the host cell, the synthetic gene network will suffer from intrinsic parameter fluctuations and external disturbances, which will destroy the function of the synthetic gene network if it is not robustly designed. More recently, a robust synthetic biology design with molecular noises was developed to achieve a desired steady state [25]. A robust synthetic biology design was also proposed to achieve a desired steady state despite parameter variations and external disturbances via engineering design specifications [26]. These design schemes are of the robust stabilization scheme to a desired steady state (or an equilibrium point). There are, however, many other behaviors of interest in synthetic gene network designs to which the stabilization scheme behavior cannot be applied. In fact, other behaviors of interest such as oscillations or transient behaviors are more complex than the steady state behaviors. How to engineer a synthetic gene network with desired oscillations or transient behaviors is a model matching (tracking) design problem. In this tracking design case, the desired behavior should be generated by a reference dynamic model $\dot{x}_r = f_r(x_r) + h_r(x_r)u_r$ and then the parameters of the synthetic gene network should be designed so that the time responses of the synthetic gene network can match the desired behavior x_r of reference model despite the parameter fluctuations and external disturbances in the host cell. More effort is needed for the robust model matching design than the conventional robust stabilization design of synthetic gene network with a desired steady state. Based on a fuzzy approximation method, a suboptimal H_2 tracking design method is proposed for synthetic gene network [27].

In this study, the intrinsic parameter variations and external disturbances are modeled into the non-linear stochastic equation of synthetic gene networks to mimic the real environments in the host cell [13]. The desired behavior is simulated by the input/output response of a reference model that has been widely used to generate a desired output response for model reference control design [28]. In this situation, the design problem of a synthetic gene network becomes how to specify the kinetic parameters and degradation rates within allowable ranges for the synthetic gene network to robustly track the desired response of the reference model in spite of intrinsic parameter variations and external disturbances in the host cell (see Fig. 1). A stochastic H_∞ robust model matching design methodology is introduced for the synthetic gene network to tolerate intrinsic parameter perturbations and to attenuate the extrinsic disturbances on the matching error to the reference model response to achieve robust tracking of the desired behavior (see Fig. 1).

In general, it is still very difficult to solve the so-called non-linear stochastic model matching design problems. It is necessary to solve a non-linear Hamilton–Jacobi inequality (HJI) for the robust matching design of non-linear stochastic synthetic gene network, and there are currently no good analytic or numerical methods to solve the HJI [29,30]. In order to avoid solving the HJI for the synthetic gene network design, the global linearization technique [31] is employed to simplify the model matching design procedure of the robust synthetic gene regulatory network by solving a set of linear matrix inequalities (LMIs) instead [13,32]. In this case, the robust model matching problem for synthetic gene networks can be efficiently solved with the help of LMI toolbox in Matlab [33]. Finally, two *in silico* design examples of synthetic gene network are given to illustrate the design procedure and to confirm the robust model matching performance to achieve a desired input/output behavior under stochastic parameter fluctuations and environmental disturbances in the host cell.

2. Design specifications and problem description

Before the introduction of a general design methodology of the robust model matching synthetic gene network, an example is provided for the convenience of illustrating the design specifications and problem formulation. We consider a cascade of transcriptional inhibition built in *E. coli* by Hooshangi et al. [34]. The synthetic gene network is represented in Fig. 2. It is made of four genes: *tetR*, *lacI*, *cI* and *eyfp* that codes respectively for three repressor proteins, TetR, LacI, CI, and the fluorescent protein EYFP (enhanced yellow fluorescent protein). The system can be controlled by the addition or removal of a chemical anhydrotetracycline (aTc), which can inhibit protein TetR and is considered as the control input $u(t)$.

We denote the concentrations of these proteins as

$$\begin{bmatrix} x_1 & x_2 & x_3 & x_4 \end{bmatrix}^T = \begin{bmatrix} x_{tetR} & x_{lacI} & x_{cI} & x_{eyfp} \end{bmatrix}^T, \tag{1}$$

where x_{tetR}, x_{lacI}, x_{cI}, x_{eyfp} denote the concentrations of proteins TetR, LacI, CI, EYFP, respectively and u denotes the constant input of aTc. Then the dynamic model of synthetic transcriptional cascade in Fig. 2 can be represented by the following differential equations [11]

$$\begin{aligned} \dot{x}_1 &= k_{10} - r_1 x_1, \\ \dot{x}_2 &= k_{20} + k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - r_2 x_2, \\ \dot{x}_3 &= k_{30} + k_3 d_3(x_2) - r_3 x_3, \\ \dot{x}_4 &= k_{40} + k_4 d_4(x_3) - r_4 x_4, \end{aligned} \tag{2}$$

where the constants k_{10}, k_{20}, k_{30}, k_{40} are the basal levels of the corresponding production rates of proteins due to the regulations other than upstream regulatory genes, which are not easy to measure in the host cell and can be considered as system uncertainties in our design procedure; k_2, k_3, k_4 are the kinetic parameters which represent regulation abilities of the gene network. The magnitudes of k_i, $i = 2,\ldots,4$ are proportional to the lengths or affinities of the transcription factor binding sites inserted in the promoter region of target genes by using a high-efficiency phage-based homologous recombination system, called recombineering [35,36]. The parameters r_1, r_2, r_3, r_4 are the degradation rates of the corresponding proteins in the host cell. These parameters (k_i and r_i) can be designed by the synthetic biological designers; $d_2(x_1)$, $d_3(x_2)$ and $d_4(x_3)$ are Hill (or sigmoid) functions (see Fig. 3) of inhibition regulation on the gene expression by the corresponding proteins TetR, LacI, CI and $a(u)$ for activation regulation by chemical aTc.

In the synthetic gene network (2), suppose k_i and r_i suffer from parameter perturbations, due to gene expression disturbances in TF bindings, transcription and translation processes, alternative splicing, mutation, etc. [37]. Based on our previous work [13], the intrinsic fluctuations can be represented as the following additive noises

$$\begin{aligned} & & r_1 &\to r_1 + \Delta r_1 n_1, \\ k_2 &\to k_2 + \Delta k_2 n_2, & r_2 &\to r_2 + \Delta r_2 n_2, \\ k_3 &\to k_3 + \Delta k_3 n_3, & r_3 &\to r_3 + \Delta r_3 n_3, \\ k_4 &\to k_4 + \Delta k_4 n_4, & r_4 &\to r_4 + \Delta r_4 n_4, \end{aligned} \tag{3}$$

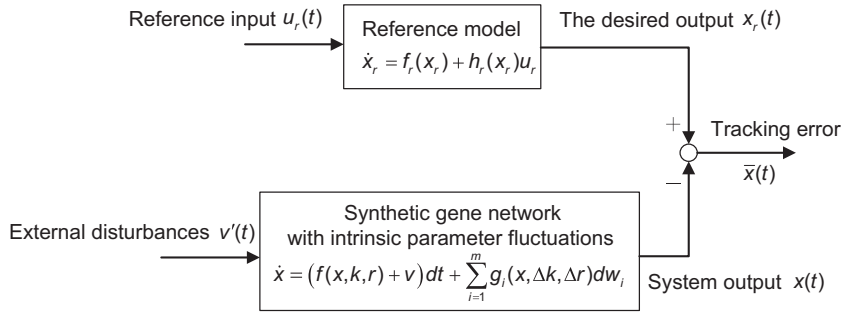

Fig. 1. System block description of robust model matching design for a synthetic gene network. The desired behavior is simulated by the input/output response of a reference model. The synthetic gene network is designed to match the desired output response of the reference model to achieve the desired behavior x_r in spite of external disturbances and intrinsic parameter fluctuations. Here, $u_r(t)$ is the reference input, $x_r(t)$ is the desired state vector to be matched, and $x(t)$ is the state vector of the synthetic gene network. $v'(t)$ are the stochastic external disturbances and $v = v' + k_0$ denote the total external disturbances and uncertain basal values.

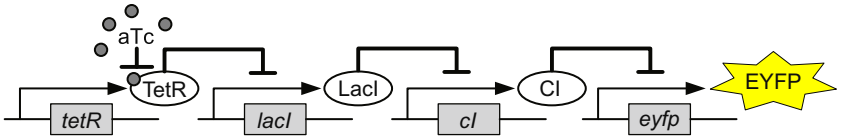

Fig. 2. The 4-gene synthetic transcriptional cascade. The protein TetR represses gene *lacI*. The protein LacI represses gene *cI*, and the protein CI represses gene *eyfp*. aTc controls the repression of TetR and is considered as system input.

where $\Delta k_i n_i$ and $\Delta r_i n_i$ denotes the additive parameter noises, in which Δk_i and Δr_i denote the amplitudes of the kinetic parameter variations; n_i is a white noise with zero mean and unit variance, i.e., Δk_i and Δr_i denote the amplitudes of the corresponding parametric variations and n_i absorbs the stochastic property of intrinsic parametric variations. n_1, n_2, n_3 and n_4 are independent white noises to indicate that there are four independent stochastic sources of random parameter fluctuations over time, whose covariances are given as

$$\begin{aligned} &\mathrm{Cov}(\Delta k_i n_i(t), \Delta k_i n_i(\tau)) = \Delta k_i^2 \delta_{t,\tau}, \\ &\mathrm{Cov}(\Delta r_i n_i(t), \Delta r_i n_i(\tau)) = \Delta r_i^2 \delta_{t,\tau}, \\ &\mathrm{Cov}(\Delta k_i n_i(t), \Delta r_i n_i(\tau)) = \Delta k_i \Delta r_i \delta_{t,\tau} \end{aligned} \tag{4}$$

in which $\delta_{t,\tau}$ denotes the delta function, i.e., $\delta_{t,\tau} = 1$ if $t = \tau$ and $\delta_{t,\tau} = 0$ if $t \neq \tau$, i.e., Δk_i and Δr_i denote the corresponding standard deviations of stochastic kinetic parameter fluctuations $\Delta k_i n_i$ and $\Delta r_i n_i$ respectively. Further, the synthetic gene network (2) is also affected by the environmental disturbances from the cellular context and interactions with other networks in the host cell. In this situation, the perturbative synthetic gene network is represented as follows [13]

$$\begin{aligned} \dot{x}_1 &= k_{10} - r_1 x_1 - \Delta r_1 n_1 x_1 + v'_1, \\ \dot{x}_2 &= k_{20} + k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - r_2 x_2 \\ &\quad + (\Delta k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - \Delta r_2 x_2) n_2 + v'_2, \\ \dot{x}_3 &= k_{30} + k_3 d_3(x_2) - r_3 x_3 + (\Delta k_3 d_3(x_2) - \Delta r_3 x_3) n_3 + v'_3, \\ \dot{x}_4 &= k_{40} + k_4 d_4(x_3) - r_4 x_4 + (\Delta k_4 d_4(x_3) - \Delta r_4 x_4) n_4 + v'_4, \end{aligned} \tag{5}$$

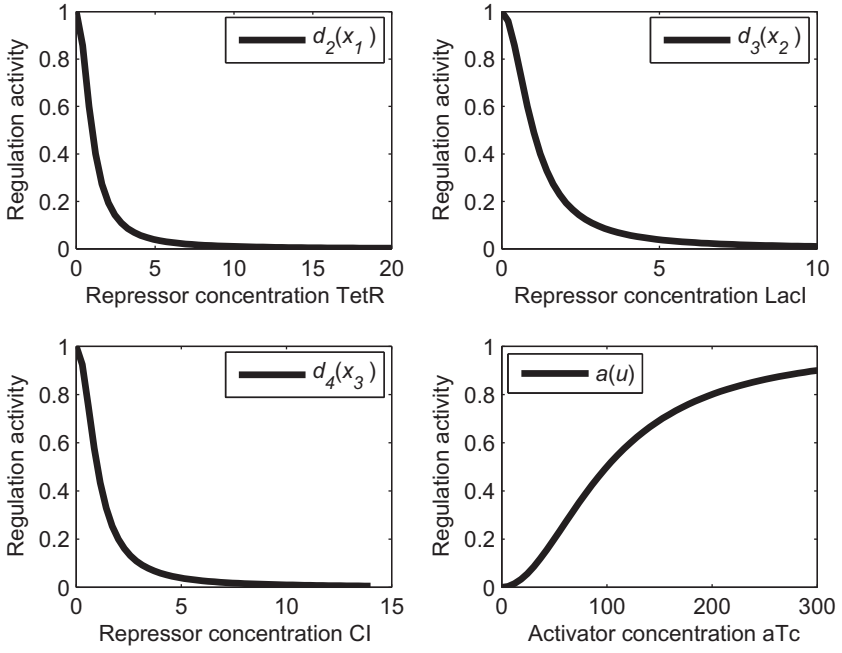

Fig. 3. Hill functions (sigmoid function) as regulatory functions of the gene expression. The Hill functions indicate the relation between the regulatory protein and its regulatory activity. TetR, LacI and CI are repressors, whereas aTc acts as an activator.

where v'_1, v'_2, v'_3 and v'_4 denote the corresponding stochastic external disturbances, whose statistics are assumed to be unavailable in general, i.e., a real synthetic gene network within the host cell must suffer from intrinsic stochastic parameter fluctuations and environmental disturbances. If a synthetic gene network wants to function properly to achieve its design goal, these parameter fluctuations and external disturbances should be overcome and the elimination of their effects should be considered in the design procedure of synthetic gene network.

For the convenience of analysis and design, the stochastic synthetic gene regulatory network of (5) in the host cell can be represented by the following equivalent Ito stochastic differential equations [29,30]

$$\begin{aligned} dx_1 &= (-r_1x_1 + v_1)dt + (-\Delta r_1x_1)dw_1,\\ dx_2 &= (k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - r_2x_2 + v_2)dt\\ &\quad + (\Delta k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - \Delta r_2x_2)dw_2\\ dx_3 &= (k_3d_3(x_2) - r_3x_3 + v_3)dt + (\Delta k_3d_3(x_2) - \Delta r_3x_3)dw_3,\\ dx_4 &= (k_4d_4(x_3) - r_4x_4 + v_4)dt + (\Delta k_4d_4(x_3) - \Delta r_4x_4)dw_4, \end{aligned} \tag{6}$$

where $v_i = v'_i + k_{i0}$ denote the total external disturbances and uncertain basal value of the ith gene because the basal levels k_{i0} are always unavailable and maybe fluctuant. $w_i(t)$ is a standard Wiener process or Brownian motion with $dw_i(t) = n_i(t)dt$. The above stochastic gene network could be represented by

$$\begin{aligned} \begin{bmatrix} dx_1\\ dx_2\\ dx_3\\ dx_4 \end{bmatrix} &= \left(\begin{bmatrix} -r_1x_1\\ k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - r_2x_2\\ k_3d_3(x_2) - r_3x_3\\ k_4d_4(x_3) - r_4x_4 \end{bmatrix} + \begin{bmatrix} v_1\\ v_2\\ v_3\\ v_4 \end{bmatrix} \right) dt\\ &\quad + \begin{bmatrix} -\Delta r_1x_1\\ 0\\ 0\\ 0 \end{bmatrix} dw_1 + \begin{bmatrix} 0\\ \Delta k_2(d_2(x_1) + a(u) - d_2(x_1)a(u)) - \Delta r_2x_2\\ 0\\ 0 \end{bmatrix} dw_2\\ &\quad + \begin{bmatrix} 0\\ 0\\ \Delta k_3d_3(x_2) - \Delta r_3x_3\\ 0 \end{bmatrix} dw_3 + \begin{bmatrix} 0\\ 0\\ 0\\ \Delta k_4d_4(x_3) - \Delta r_4x_4 \end{bmatrix} dw_4. \end{aligned}$$

If a synthetic gene regulatory network consists of n genes, a more general perturbative synthetic network can be represented by the following stochastic differential equation

$$dx = (f(x,k,r) + h(x)v)dt + \sum_{i=1}^{m} g_i(x,\Delta k_i,\Delta r_i)dw_i, \quad x(0) = x_0, \tag{7}$$

where $x = [x_1,\ldots,x_n]^T$ denotes a vector of protein concentrations of n genes; $v = [v_1,\ldots,v_n]^T$ denotes the total external disturbances of the synthetic gene network; $k = [k_1,\ldots,k_n]$ and $r = [r_1,\ldots,r_n]$ denote the kinetic parameters and degradation rates to be specified by the designer; $h(x)$ denotes the non-linear coupling between the external disturbances and the synthetic gene network. The term $\sum_{i=1}^{m} g_i(x,\Delta k_i,\Delta r_i)dw_i$ denotes the stochastic parameter fluctuations due to m random sources. Obviously, a synthetic gene network is affected by a large number of disturbances and parameter fluctuations in the host cell. If their effects on the synthetic gene network cannot be efficiently attenuated by the robustness of synthetic gene network, the synthetic gene network cannot work properly and its desired behaviors will decay quickly [11]. After the stochastic parameter variations to be tolerated and environmental disturbances to be attenuated are both modeled into the dynamic equation of synthetic gene network in (7) to mimic the realistic behavior *in vivo*, some design specifications for robust synthetic gene network to remedy these uncertainties to achieve reference model matching in the host cell are described as follows:

2.1. Design specifications for robust reference model matching

(i) The kinetic parameters and the degradation rates should be designed from the following feasible ranges, respectively

$$k \in [k_\ell, k_u], \quad r \in [r_\ell, r_u]. \tag{8}$$

These feasible ranges are defined by the designer according to the engineering ability of biotechnologies and the characteristics of the genes in the network.

(ii) The intrinsic parameter fluctuations $\sum_{i=1}^{m} g_i(x,\Delta k_i,\Delta r_i)dw_i$ should be tolerated by the synthetic gene network, i.e., the designed gene network could tolerate the stochastic parameter fluctuations with standard deviations Δk_i and Δr_i prescribed by designers according to the statistics of realistic parameter variations in the host cell.

(iii) The following prescribed non-linear reference model should be input/output matched

$$\dot{x}_r = f_r(x_r) + h_r(x_r)u_r, x_r(0) = x_{r0}, \tag{9}$$

where $f_r(x_r)$ and $h_r(x_r)$ are specified beforehand such that $x_r(t)$ could represent the desired behavior of the designed stochastic synthetic gene network in (7). $u_r(t)$ denotes the reference input to generate the desired reference behavior $x_r(t)$ by (9). This is the non-linear reference model to be tracked by synthetic gene network in the robust model matching design. If a linear reference model will be tracked, then (9) is modified as

$$\dot{x}_r = A_rx_r + B_ru_r, x_r(0) = x_{r0}, \tag{10}$$

where A_r and B_r are specified beforehand so that the desired reference behavior of synthetic gene network could be generated by the linear reference model in (10). In general, the reference input signal $u_r(t)$ is related to the steady state of $x_r(t)$ and is always given or changed by users. Therefore, $u_r(t)$ may be unavailable at the design stage and can be considered as a disturbance.

(iv) The following prescribed disturbance attenuation should be achieved by filtering uncertain external disturbances [30]

$$\begin{aligned} &\frac{E\int_0^\infty (x-x_r)^TQ(x-x_r)dt}{E\int_0^\infty (v^Tv + u_r^Tu_r)dt} \leqslant \rho^2 \quad \text{or}\\ &E\int_0^\infty (x-x_r)^TQ(x-x_r)dt \leqslant \rho^2 E\int_0^\infty (v^Tv + u_r^Tu_r)dt \end{aligned} \tag{11}$$

for all possible bounded external disturbances $v(t)$, where ρ is a prescribed attenuation level, i.e., the effect of external disturbances $v(t)$ and u_r on the matching error $x - x_r$ should be less than ρ from the average energy perspective. Q is a symmetric weighting matrix on the matching errors. If $Q = I$, then all state variables are required to match the desired state variables. If $Q = diag(0,\ldots,0,1)$, then only the output variable x_n is required to match the last variable x_{rn}. If the external disturbances are of deterministic molecular signals rather than of stochastic noises, then $E\int_0^\infty (v^Tv + u_r^Tu_r)dt$ in (11) is replaced by $\int_0^\infty (v^Tv + u_r^Tu_r)dt$, i.e., the expectation E can be neglected.

Remark.

(1) The physical meaning of (11) is that the effect of external disturbances on matching errors should be attenuated to the level ρ^2 from the energy point of view. For example, if $\rho = 0.1$, then the effect of v and u_r on matching error $x - x_r$ should be attenuated to the level of $\rho^2 = 0.01$ from the energy perspective.

(2) If the effect of the uncertain initial condition $x_0 - x_{r0}$ is also considered in the disturbance attenuation problem, Eq. (11) should be modified as follows [30]

$$E\int_0^\infty (x-x_r)^T Q(x-x_r)dt \leqslant EV(x_0-x_{r0}) + \rho^2 E \times \int_0^\infty (v^T v + u_r^T u_r)dt \qquad (12)$$

for some positive function $V(x) > 0$

(3) For the convenience of analysis and the simplicity of design, the origin of non-linear stochastic gene network should be shifted to the desired steady state of the reference model in (9).

Based on the above four specifications given by users, our goal is to design a synthetic gene network by selecting kinetic parameters $k \in [k_\ell, k_u]$ and degradation rates $r \in [r_\ell, r_u]$ to satisfy the engineering specifications (8)–(11) so that the synthetic gene network can achieve the reference model response under the intrinsic parameter fluctuations and external disturbances which appear in the host cell. In other words, the design specifications (i)–(iv) can guarantee that the synthetic gene network has enough robust stability and filtering ability to tolerate the parameter fluctuations and to attenuate the external disturbances to track the desired reference signals.

3. Robust model matching design methodology of synthetic gene network

From the analyses in the above section, the robust model matching design of the synthetic gene network is to select the kinetic parameters k and degradation rates r to engineer a stochastic gene network in (7) to achieve the design specifications (i)–(iv). In general, it is not easy to design the robust model matching gene network directly. Before further discussion of the robust matching design methodology of synthetic gene network, the attenuation level of disturbance on the matching error $x - x_r$ in (12) can be derived as

$$E\int_0^\infty \bar{x}^T \overline{Q}\bar{x}dt \leqslant EV(\bar{x}_0) + \rho^2 E\int_0^\infty \bar{v}^T\bar{v}dt, \qquad (13)$$

where the augmented state vector $\bar{x}$, the generalized disturbance $\bar{v}$ and $\overline{Q}$ are denoted, respectively, by

$$\bar{x} = \begin{bmatrix} x_r \\ x \end{bmatrix}, \quad \overline{Q} = \begin{bmatrix} -I \\ I \end{bmatrix} Q[-I \quad I] = \begin{bmatrix} Q & -Q \\ -Q & Q \end{bmatrix}, \quad \bar{v} = \begin{bmatrix} u_r \\ v \end{bmatrix}.$$

Since u_r can be arbitrarily assigned by users and can not be predicted by the designer, for the convenience of design, it can be considered as a disturbance in the design procedure. Let us denote

$$F(\bar{x},k,r) = \begin{bmatrix} f_r(x_r) \\ f(x,k,r) \end{bmatrix}, \quad \bar{g}_i(\bar{x},\Delta k_i,\Delta r_i)\begin{bmatrix} 0 \\ g_i(x,\Delta k_i,\Delta r_i) \end{bmatrix},$$
$$H(\bar{x}) = \begin{bmatrix} h_r(x_r) & 0 \\ 0 & h(x) \end{bmatrix}. \qquad (14)$$

Then the non-linear augmented system of (7) and (9) is given by

$$d\bar{x} = (F(\bar{x},k,r) + H(\bar{x})\bar{v})dt + \sum_{i=1}^m \bar{g}_i(\bar{x},\Delta k_i,\Delta r_i)dw_i, \quad \bar{x}(0) = \bar{x}_0. \qquad (15)$$

Based on the above analysis, the matching design of robust synthetic gene network becomes how to specify the kinetic parameters $k \in [k_\ell, k_u]$ and degradation rates $r \in [r_\ell, r_u]$ in the augmented system (15) such that the disturbance attenuation in (13) is achieved for a prescribed disturbance attenuation level ρ. Then we get the following modeling matching design method for robust synthetic gene network.

Proposition 1. *If we can specify kinetic parameters $k \in [k_\ell, k_u]$ and degradation rates $r \in [r_\ell, r_u]$ such that the following HJI has a positive solution $V(\bar{x}) > 0$*

$$\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T F(\bar{x},k,r) + \bar{x}^T\overline{Q}\bar{x} + \frac{1}{4\rho^2}\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T H(\bar{x})H(\bar{x})^T\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right) + \frac{1}{2}\sum_{i=1}^m g_i^T(\bar{x},\Delta k_i,\Delta r)\frac{\partial^2 V(\bar{x})}{\partial \bar{x}^2}g_i(\bar{x},\Delta k_i,\Delta r_i) \leqslant 0 \qquad (16)$$

then the stochastic synthetic gene network in (7) can achieve the robust model matching design specifications (i)–(iv) from (8) to (11).

Proof. see Appendix A. □

In general, it is not easy to solve HJI in (16) for $V(\bar{x}) > 0$ analytically or numerically at present. In such a situation, the global linearization technique is employed to globally linearize the augmented system in (15) to simplify the model matching design problem of robust synthetic gene network. By the global linearization theory [31], if

$$\begin{bmatrix} \frac{\partial F(\bar{x},k,r)}{\partial \bar{x}} \\ \frac{\partial H(\bar{x})}{\partial \bar{x}} \\ \frac{\partial g_i(\bar{x},\Delta k_i,\Delta r_i)}{\partial \bar{x}} \end{bmatrix} \in \mathrm{Co}\left\{\begin{bmatrix} \overline{A}_1(k,r) \\ \overline{B}_1 \\ \overline{C}_{i1} \end{bmatrix}, \begin{bmatrix} \overline{A}_2(k,r) \\ \overline{B}_2 \\ \overline{C}_{i2} \end{bmatrix}, \ldots, \begin{bmatrix} \overline{A}_M(k,r) \\ \overline{B}_M \\ \overline{C}_{iM} \end{bmatrix}\right\}, \quad \forall \bar{x} \qquad (17)$$

where Co denotes the convex hull of a polytope with M vertices defined in (17), then the state trajectories of the non-linear augmented system of (7) and (9) in (15) will belong to the convex combination of the state trajectories of the following M linearized augmented systems at the M vertices of the convex hull

$$d\bar{x} = (\overline{A}_j(k,r)\bar{x} + \overline{B}_j\bar{v})dt + \sum_{i=1}^m \overline{C}_{ij}\bar{x}dw_i, \quad j = 1,\ldots,M, \qquad (18)$$

where $\overline{A}_j(k,r)$, $\overline{B}_j$ and $\overline{C}_{ij}$ are obtained from M vertices in (17).

The physical meaning of (18) is that if all the linearizations of non-linear augmented system in (15) at all possible points $\bar{x}(t)$ belong to the convex hull Co which consists of linear systems at the M vertices in (17), then the behavior of non-linear augmented system in (15) can be represented by the convex combinations of M linear systems in (18) [31]. Then the augmented system's matrices of the globally linearized system of (15) at the M vertices are given as follows

$$\overline{A}_j(k,r) = \begin{pmatrix} A_{rj} & 0 \\ 0 & A_j(k,r) \end{pmatrix}, \quad \overline{B}_j = \begin{pmatrix} B_{rj} & 0 \\ 0 & B_j \end{pmatrix}, \quad \overline{C}_{ij} = \begin{pmatrix} 0 & 0 \\ 0 & C_{ij} \end{pmatrix}.$$

Remark. In the linear reference model case in (10), the above system matrices of global linearization are modified as

$$\overline{A}_j(k,r) = \begin{pmatrix} A_r & 0 \\ 0 & A_j(k,r) \end{pmatrix}, \quad \overline{B}_j = \begin{pmatrix} B_r & 0 \\ 0 & B_j \end{pmatrix}.$$

Based on the global linearization theory [31], the augmented non-linear stochastic system in (15) can be represented by the following interpolation system

$$d\bar{x} = \sum_{j=1}^M \alpha_j(\bar{x})\left[(\overline{A}_j(k,r)\bar{x} + \overline{B}_j\bar{v})dt + \sum_{i=1}^m \overline{C}_{ij}\bar{x}dw_i\right], \qquad (19)$$

where the interpolation function $\alpha_j(\bar{x})$ must satisfy the constraints $0 \leqslant \alpha_j(\bar{x}) \leqslant 1$ and $\sum_{j=1}^{M}\alpha_j(\bar{x}) = 1$. Unlike the conventional linearization method to approximate the non-linear system by the linearized system at the origin, the global linearization method interpolates several linearized systems at the vertices of convex hull Co in (17) to represent the non-linear system.

Proposition 2. *If we can specify the kinetic parameters $k \in [k_\ell, k_u]$ and degradation rates $r \in [r_\ell, r_u]$ such that the following inequalities has a common solution $P = P^T > 0$*

$$P\overline{A}_j(k,r) + \overline{A}_j^T(k,r)P^T + \overline{Q} + \frac{1}{\rho^2}P\overline{B}_j\overline{B}_j^T P^T + \sum_{i=1}^{m}\overline{C}_{ij}^T P\overline{C}_{ij} \leqslant 0, \quad j = 1,....,M \tag{20}$$

then the robust model matching design of stochastic gene network satisfies the design specifications (i)–(iv)

Proof. see Appendix B. □

For convenience, we replace the difficult HJI in (16) by a set of easier algebraic inequalities in (20) to simplify the design procedure of robust model matching problem of non-linear synthetic gene networks. By Schur complement [31], the inequalities in (20) are equivalent to the following LMIs

$$\begin{pmatrix} P\overline{A}_j(k,r) + \overline{A}_j^T(k,r)P^T + \overline{Q} + \sum_{i=1}^{m}\overline{C}_{ij}^T P\overline{C}_{ij} & P\overline{B}_j \\ \overline{B}_j^T P^T & -\rho^2 I \end{pmatrix} \leqslant 0, \quad j = 1,\ldots,M \tag{21}$$

Then the model matching problem for synthetic gene network design becomes how to select the kinetic parameters $k \in [k_\ell, k_u]$ and degradation rates $r \in [r_\ell, r_u]$ such that the LMIs in (21) have a positive definite solution $P > 0$ to satisfy the design specifications (i)–(iv). Because the LMIs in (21) can be easily solved by the LMI toolbox in Matlab, the proposed method has good potential for the desired model reference matching design of robust synthetic gene network in the future.

Remark.

(1) If we want to design a robust synthetic gene network, which can tolerate intrinsic parameter fluctuation and attenuate external disturbance to optimally achieve reference model matching, then we need to solve the following constrained optimization problem

$$\min_{\substack{k\in[k_\ell,k_u]\\ r\in[r_\ell,r_u]}} \rho^2, \quad \text{subject to LMIs in (21)}, \quad P > 0. \tag{22}$$

This constrained optimization can be obtained by decreasing ρ until there exists no solution $P > 0$ for all $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$.

(2) In the oscillation design case, the eigenvalues of A_{rj} in reference model are always on the $j\omega$-axis, i.e., one half of eigenvalues of $\overline{A}_j$ in (21) are on the $j\omega$-axis (i.e., with zero real parts). In this situation, it is not easy to specify $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$ to satisfy the LMIs in (21). In order to overcome this design difficulty, an eigenvalue-shifted technique is proposed as an expedient scheme to deal with the oscillation tracking design problem. Let us adjust the system variables in (18) by $\bar{x}_s(t) = e^{-\lambda t}\bar{x}(t)$ and $\bar{v}_s(t) = e^{-\lambda t}\bar{v}(t)$ for some positive value λ, then $\bar{x}_s(t)$ can be obtained by the following [31].

$$d\bar{x}_s = \sum_{j=1}^{M}\alpha_j(\bar{x}_s)\left[\left((\overline{A}_j(k,r) - \lambda I)\bar{x}_s + B_j\bar{v}_s\right)dt + \sum_{i=1}^{m}\overline{C}_{ij}\bar{x}_s dw_i\right]. \tag{23}$$

For the eigenvalue-shifted system in (23), suppose we want to specify $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$ to achieve the following attenuation level of disturbances

$$E\int_0^\infty \bar{x}_s^T\overline{Q}\bar{x}_s dt \leqslant EV(\bar{x}_0) + \rho^2 E\int_0^\infty \bar{v}_s^T\bar{v}_s dt, \tag{24}$$

which is the same as (13) except $\bar{x}(t)$ and $\bar{v}(t)$ are replaced by $\bar{x}_s(t)$ and $\bar{v}_s(t)$, respectively, i.e., we use the matching performance in (24) to replace the matching performance in (13).

In this transformation case, the robust matching design problem is relaxed to how to specify k and r in (23) to achieve the disturbance attenuation performance in (24). By Proposition 2 and (21), we need to specify $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$ to solve $P > 0$ for the following LMIs.

$$\begin{pmatrix} P\left(\overline{A}_j(k,r) - \lambda I\right) + \left(\overline{A}_j^T(k,r) - \lambda I\right)P^T + \overline{Q} + \sum_{i=1}^{m}\overline{C}_{ij}^T P\overline{C}_{ij} & P\overline{B}_j \\ \overline{B}_j^T P^T & -\rho^2 I \end{pmatrix} \leqslant 0, \ j = 1,\ldots,M. \tag{25}$$

In the above expedient method, due to the more negative eigenvalues of $\overline{A}_j^{(k,r)} - \lambda I$, we have more feasible way to solve the robust reference matching problem for periodic reference signals. However, in order to avoid some distortion due to signal transformation, λ should be selected as small as possible.

Based on the analyses above, a design procedure for robust model matching synthetic gene network is proposed as follows:

3.1. Design procedure

(a) Formulate a stochastic dynamic equation to mimic a perturbative gene network as (7) in the host cell.
(b) Give design specifications (i)–(iv) to the synthetic gene networks to robustly match a desired input/output response model $\dot{x}_r = f_r(x_r) + h_r(x_r)u_r$ in spite of intrinsic parameter fluctuations, external disturbances and any input signal u_r.
(c) Augment the stochastic dynamic equation and the desired reference model as (15).
(d) Perform the global linearization as (17).
(e) Solve the LMIs in (21) for design parameters k and r from the feasible intervals $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$, respectively.

4. Design examples in silico

4.1. Case 1: Synthetic transcriptional cascade design

Based on the above analyses of model matching design methodology of robust synthetic gene network, two *in silico* design examples are given to illustrate the proposed design procedure and to confirm the robust matching performance of the proposed method. The first case is the synthetic transcriptional cascade design. The synthetic gene network in Fig. 2 is made of four genes: *tetR*, *lacI*, *cI* and *eyfp* that code respectively for three repressor proteins, TetR, LacI and CI, and the fluorescent protein EYFP. The additional chemical aTc is considered as the system input to be used to control the behavior of the system output of EYFP. The regulatory dynamic equation of the synthetic gene network is given in (2). In the dynamic Eq. (2), the basal levels k_{10}, k_{20}, k_{30} and k_{40} are given as constants 3, 1, 0.9, and 2.5, respectively. In addition, the inhibition functions $d_2(x_1)$, $d_3(x_2)$, $d_4(x_3)$ are all Hill functions as $d_i(x) =$

$\frac{\beta_d}{1+(x/K_i)^n}$, where β_d is maximal expression level of promoter. K_i is the repression coefficient. The Hill coefficient n governs the steepness of the input function. For the activator $a(u)$, Hill function can be described in the form $a(u)=\frac{\beta_a u^n}{K_a^n+u^n}$. β_a is also the maximal expression level of promoter. K_a is the activation coefficient. n determines the steepness of the input function [38]. These Hill functions are shown in Fig. 3. The kinetic parameters k_2, k_3, k_4 and degradation rates r_1, r_2, r_3, r_4 also suffer from parameter perturbations in the host cell, and we want to design the three kinetic parameters and four degradation rates from the biological allowable range to satisfy the following four design specifications to achieve robust model matching design.

(i) The kinetic parameters and degradation rates are to be designed in the following allowable ranges.

$$r_1 \in [0.1, 3],$$
$$k_2 \in [1, 40], \quad r_2 \in [0.1, 5],$$
$$k_3 \in [30, 600], \quad r_3 \in [0.1, 1.5],$$
$$k_4 \in [300, 1200], \quad r_4 \in [0.1, 1].$$

(ii) The standard deviations of parameter fluctuations to be tolerated in the host cell are assumed as follows

$$\Delta r_1 = 0.05,$$
$$\Delta k_2 = 5, \quad \Delta r_2 = 0.05,$$
$$\Delta k_3 = 5, \quad \Delta r_3 = 0.05,$$
$$\Delta k_4 = 5, \quad \Delta r_4 = 0.05.$$

(iii) The reference model of the synthetic gene network is given as follows

$$\dot{x}_r = A_r x_r + B_r u_r = \begin{pmatrix} -1 & 0 & 0 & 0 \\ 3 & -2 & 0 & 0 \\ 0 & 2 & -1.5 & 0 \\ 0 & 0 & 1.5 & -1 \end{pmatrix} x_r + \begin{pmatrix} 3 & 0 & 0 & 0 \\ 0 & 5 & 0 & 0 \\ 0 & 0 & 7 & 0 \\ 0 & 0 & 0 & 10 \end{pmatrix} u_r, \tag{26}$$

where x_r is the reference state to be matched, and u_r is the reference input.

(iv) The disturbance attenuation level is described as $\rho = 0.05(\rho^2 = 0.0025)$.

In order to apply the global linearization technique to simplifying the design procedure, the desired steady state x_r of the reference model in (26) to be matched by the synthetic gene network in (2) is shifted to the origin, and the global linearization is employed to obtain A_j, B_j and C_{ij} with 6 vertices ($M = 6$) (see Appendix C). The non-linear augmented system in (15) can be approximated via the six linearized augmented systems in (19) through the following suitable interpolation functions [39]

$$\alpha_j(\bar{x}) = \frac{1}{\|\bar{x}_j - \bar{x}(t)\|_2^2} \Big/ \sum_{j=1}^{M} \frac{1}{\|\bar{x}_j - \bar{x}(t)\|_2^2}, \quad j = 1, \ldots, M, \tag{27}$$

where $\bar{x}_j$ are the polytope operating points.

The next step is to solve LMIs in (21) by tuning the kinetic parameters k_i and degradation rates r_i iteratively within the allowable ranges to find a common positive definite matrix solution P. The weighting matrix is selected as $Q = \mathrm{diag}([0.0005 \quad 0.0005 \quad 0.00050.0005])$. Following the design procedure above and using LMI Toolbox in Matlab, we find that a $P > 0$ will be solved when the kinetic parameters k_i are selected from the following feasible parameter sets (the green cubic region in Fig. 4).

$$k_2 \in [12, 19], \quad k_3 \in [174, 466], \quad k_4 \in [510, 977]$$

and degradation rates r_i within the following ranges

$$r_1 \in [1.0106, 1.3366], \quad r_2 \in [1.538, 2.4585],$$
$$r_3 \in [0.3311, 0.77817], \quad r_4 \in [0.1633, 0.2422]. \tag{28}$$

In order to verify the disturbance filtering ability and model matching performance, a set of kinetic parameters are chosen from the feasible parameter sets which lie in the green cubic region in Fig. 4 and the degradation rates are selected from the design-specified ranges in (28) as follows

$$[k_2 \quad k_3 \quad k_4] = [18 \quad 309 \quad 713],$$
$$[r_1 \quad r_2 \quad r_3 \quad r_4] = [1.0691 \quad 2.0071 \quad 0.39826 \quad 0.18203].$$

Thirty simulation results of the synthetic gene network are shown in Fig. 5 with random initial condition of normal distribution under intrinsic parameter fluctuations mentioned above and external disturbances $v'(t) = [n_1(t) \quad n_2(t) \quad n_3(t) \quad n_4(t)]^T$ where $n_i(t), i = 1, \ldots, 4$ denote the standard white noises with zero mean and unit variance. The desired model reference states x_r are also shown as red dashed curves in Fig. 5. The desired transient and steady states can be properly matched under the external disturbances and intrinsic parameter perturbations and the filtering ability of the synthetic gene network can be estimated by Monte Carlo simulation as follows

$$\frac{E\int_0^{40} \bar{x}^T \bar{Q} \bar{x} dt - EV(\bar{x}_0)}{E\int_0^{40} \bar{v}^T \bar{v} dt} \approx (0.0188)^2 < (0.05)^2.$$

Obviously, the effect of disturbances is attenuated more significantly than the prescribed attenuation level of $\rho = 0.05(\rho^2 = 0.0025)$. The conservative is mainly due to the inherent conservativeness in solving LMIs in (21).

If we select a set of kinetic parameter k_i from the infeasible parameter sets (the pink region in Fig. 4) and degradation rate r_i out of the design-specified ranges in (28), for instance, the kinetic parameter $[k_2 \quad k_3 \quad k_4] = [2 \quad 159 \quad 498]$ and degradation rate $[r_1 \quad r_2 \quad r_3 \quad r_4] = [1.8843 \quad 1.4872 \quad 0.10967 \quad 0.52228]$. The simulation results under intrinsic parameter fluctuations and random external disturbances with random initial conditions of normal distribution with unit standard deviation are shown in Fig. 6. It can be seen that the model matching cannot be achieved

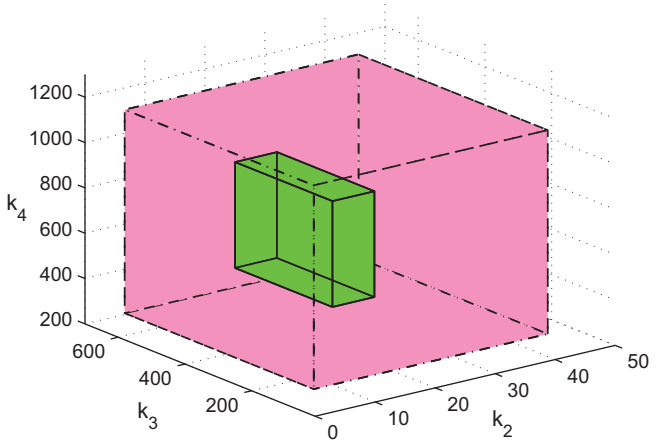

Fig. 4. The parameter space of kinetic parameters (k_2,k_3 and k_4). The biological feasible ranges for kinetic parameters are located in the green cubic region. The infeasible parameters are spread widely outside the green region i.e., the pink region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

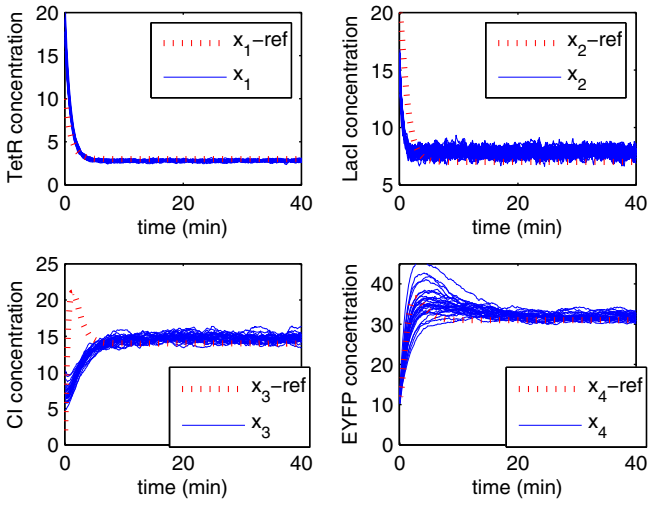

Fig. 5. Monte Carlo simulation results of the synthetic transcriptional cascade with feasible parameters for 30 rounds. The 4-genes synthetic transcriptional cascade matches the reference dynamic model (red dashed curve) under external disturbances and parameter fluctuations with random initial conditions. The feasible kinetic parameters are $k_i = [18\ 309\ 713]$ (which lie in the green cubic region in Fig. 4) and the degradation rates are in the design-specified ranges with $r_i = [1.0691\ 2.0071\ 0.39826\ 0.18203]$.

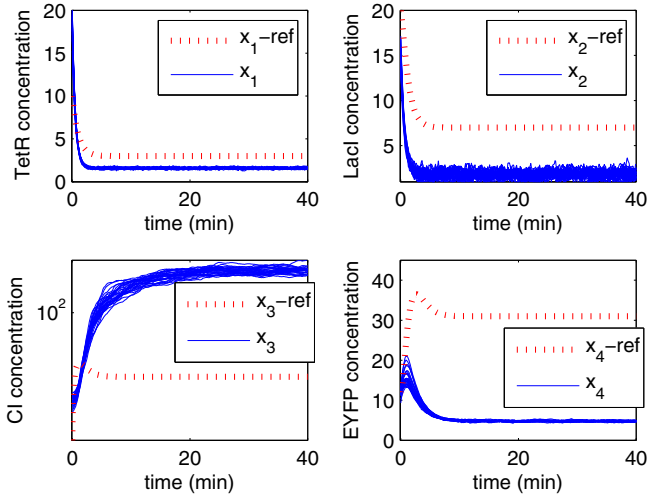

Fig. 6. Monte Carlo simulation results of the synthetic transcriptional cascade with infeasible parameters for 30 rounds. We select a set of kinetic parameter k_i from the infeasible parameter sets and the degradation rate r_i out of the design-specified ranges, i.e., the pink region in Fig. 4. The expression of the synthetic transcriptional cascade cannot match the desired reference model (red dashed curve) under external disturbances and parameter fluctuations with random initial conditions. The infeasible kinetic parameters are $k_i = [2\ 159\ 498]$ and the degradation rates are $r_i = [1.8843\ 1.4872\ 0.10967\ 0.52228]$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

because the robust matching conditions in (i)–(iv) are disobeyed, and the filtering ability to attenuate disturbances is estimated by Monte Carlo simulation as follows

$$\frac{E\int_0^{40}\bar{x}^T\bar{Q}\bar{x}dt - EV(\bar{x}_0)}{E\int_0^{40}\bar{v}^T\bar{v}dt} \approx (2.1811)^2 > (0.05)^2.$$

Apparently, the design specification of filtering ability is violated. Thus, the proposed model matching design method for the robust synthetic gene network can be validated by the simulation example.

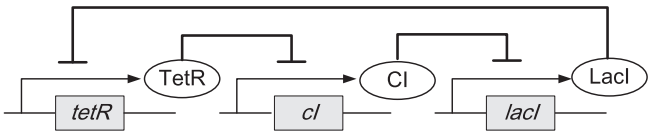

Fig. 7. The synthetic coupled repressilator. The repressor protein TetR inhibits the transcription of the repressor gene *cI*, whose protein product in turn inhibits the expression of the repressor gene *lacI*. Finally, the repressor protein LacI inhibits *tetR* expression.

4.2. Case 2: Synthetic coupled repressilator design

The second example is the synthetic coupled repressilator [40,41] shown in Fig. 7. The repressor protein TetR inhibits the transcription of the repressor gene *cI*, whose protein product in turn inhibits the expression of the repressor gene *lacI*. Finally, the repressor protein LacI inhibits *tetR* expression. The negative feedback loop leads to temporal oscillations in the concentration of each of its components which can be seen from a simple model of transcriptional regulation. The dynamic equations for the synthetic coupled repressilator under stochastic external disturbances are given as

$$\begin{aligned}\frac{dx_1}{dt} &= \frac{k_1\alpha}{\mu + x_3^m} - r_1x_1 + v_1',\\ \frac{dx_2}{dt} &= \frac{k_2\alpha}{\mu + x_1^m} - r_2x_2 + v_2',\\ \frac{dx_3}{dt} &= \frac{k_3\alpha}{\mu + x_2^m} - r_3x_3 + v_3',\end{aligned} \tag{29}$$

where $[x_1\ \ x_2\ \ x_3]^T = [x_{TetR}\ \ x_{CI}\ \ x_{LacI}]^T$ denote the protein concentrations of TetR, CI and LacI, respectively. k_1, k_2, k_3 are the kinetic parameters which represent regulation abilities of the gene network. r_1, r_2, r_3 are the degradation rates of the corresponding proteins in the host cell. The parameters are set as $\alpha = 1.8$, $\mu = 1.3$, and the Hill coefficient $m = 4$. v_1', v_2', v_3' denote the corresponding stochastic external disturbances.

We want to design the three kinetic parameters and three degradation rates from the biologically allowable range to satisfy the following four design specifications to achieve robust oscillation matching design.

(i) The kinetic parameters and degradation rates are to be designed in the following allowable ranges.

$$\begin{aligned}k_1 &\in [0.1\ \ 2], \quad r_1 \in [0.01\ \ 1],\\ k_2 &\in [0.1\ \ 2], \quad r_2 \in [0.01\ \ 1],\\ k_3 &\in [0.1\ \ 2], \quad r_3 \in [0.01\ \ 1].\end{aligned}$$

(ii) The standard deviations of parameter fluctuations to be tolerated in the host cell are assumed as follows

$$\begin{aligned}\Delta k_1 &= 0.1, \quad \Delta r_1 = 0.01,\\ \Delta k_2 &= 0.1, \quad \Delta r_2 = 0.01,\\ \Delta k_3 &= 0.1, \quad \Delta r_3 = 0.01.\end{aligned}$$

(iii) The non-linear reference model of the synthetic gene network is given as follows

$$\begin{aligned}\dot{x}_{r1} &= \frac{1}{1 + x_{r3}^7} - 0.5x_{r1} + 0.2u_r,\\ \dot{x}_{r2} &= \frac{1}{1 + x_{r1}^7} - 0.5x_{r2} + 0.2u_r,\\ \dot{x}_{r3} &= \frac{1}{1 + x_{r2}^7} - 0.5x_{r3} + 0.2u_r,\end{aligned} \tag{30}$$

where u_r is the reference input. The desired oscillations to be matched by the synthetic network are shown with black dashed curves in Fig. 8.

(iv) The disturbance attenuation level is prescribed as $\rho = 0.8$ ($\rho^2 = 0.64$).

After applying the global linearization technique, we can obtain A_j, A_{rj}, and C_{ij} with 5 vertices (M=5), which are shown in Appendix D. In order to deal with the oscillation tracking design problem, we consider the eigenvalue-shift method with $\bar{x}_s(t) = e^{-\lambda t}\bar{x}(t)$ and $\bar{v}_s(t) = e^{-\lambda t}\bar{v}(t)$ as an expedient scheme for a positive value $\lambda = 0.616$. The next step is to solve LMIs in (25) by tuning the kinetic parameters k_i and degradation rates r_i iteratively within the allowable ranges to find a common positive definite matrix solution P. The weighting matrix is selected as $Q = \mathrm{diag}([0.01 \quad 0.01 \quad 0.01 \quad 0.01])$. Finally, we find that a $P > 0$ will be solved when the kinetic parameters k_i and degradation rates r_i are selected from the following feasible parameter ranges for this robust oscillation tracking design problem.

$$\begin{aligned} &k_1 \in [0.8, 1], \quad k_2 \in [0.8, 1], \quad k_3 \in [0.8, 1], \\ &r_1 \in [0.45, 0.51], \quad r_2 \in [0.45, 0.51], \quad r_2 \in [0.45, 0.51]. \end{aligned} \tag{31}$$

In order to confirm the design result, we choose a set of the feasible parameters for kinetic parameters k_i and degradation rates r_i from the feasible ranges in (31) for the synthetic coupled repressilator in (29) as follows

$$\begin{aligned} &[k_1 \quad k_2 \quad k_3] = [0.9133 \quad 0.91330.9133], \\ &[r_1 \quad r_2 \quad r_3] = [0.46637 \quad 0.46637 \quad 0.46637]. \end{aligned} \tag{32}$$

Thirty simulation results for the synthetic coupled repressilator by (32) are shown in Fig. 8 with random initial condition of normal distribution under intrinsic parameter fluctuations and external disturbances $v(t) = [0.3n_1(t) \quad 0.3n_2(t) \quad 0.3n_3(t)]^T$ where $n_i(t)$, i = 1,...,3 denote the standard white noises with zero mean and unit variance. The desired oscillation can be properly matched by the synthetic coupled repressilator in (32) under the intrinsic parameter perturbations and external disturbances. If we select a set of kinetic parameter k_i and degradation rates r_i out of the design-specified ranges in (31), for example, $[k_1 \quad k_2 \quad k_3] = [1.5 \quad 1.5 \quad 1.5]$ and $[r_1 \quad r_2 \quad r_3] = [0.4 \quad 0.4 \quad 0.4]$. The simulation results of the synthetic coupled repressilator under intrinsic parameter fluctuations and external disturbances with random initial conditions are shown in Fig. 9. It can be seen that the model matching cannot be achieved because the robust matching conditions in (i)–(iv) are disobeyed.

5. Discussion

Due to intrinsic perturbations and extrinsic disturbances in the host cell, the synthetic gene networks engineered so far in bacteria to behave in a particular way seem decay rapidly after a short period of activity [37,42]. Therefore, the development of a robust design scheme is an important topic for synthetic gene network to work properly and robustly in spite of intrinsic and external noises. In general, noise originates from many sources, including environmental disturbances, fluctuations in gene expression, cell cycle variations, differences in the concentrations of metabolites and continuous mutational evolution. The synthetic gene network can reduce significant intrinsic genetic noise [34], even at the level of a single gene [43,44], by the circuit topology. In previous studies [45–48], robust gene circuit designs have been proposed to attenuate the parameter variations or noises. In this study, the stochastic parameter fluctuations to be tolerated and the environmental disturbances to be attenuated below a prescribed level are simultaneously considered. By doing this, we attempt to reduce the effect of noise by constructing the synthetic gene network with desired behaviors. In this situation, noise is assumed to have a negative influence on cellular behaviors. The noise should be avoided or attenuated by choosing adequate kinetic parameters and degradation rates based on the feasible ranges. Murphy et al. attempted to tune and control gene expression noise in synthetic gene net-

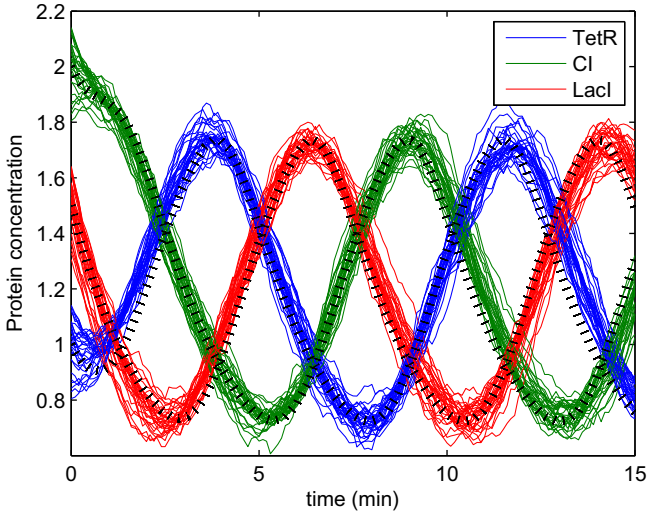

Fig. 8. Monte Carlo simulation results of the synthetic coupled repressilator with feasible parameters for 30 rounds. The 3-genes synthetic coupled repressilator can match the non-linear reference model (black dashed curves) under external disturbances and parameter fluctuations with random initial conditions. In these Monte Carlo simulations, the feasible kinetic parameters k_i = [0.9133 0.9133 0.9133] and the degradation rates r_i = [0.46637 0.46637 0.46637] are selected for the synthetic coupled repressilator from the design-specified ranges in (31). These simulations confirm that the proposed design scheme can achieve robust oscillation matching for the synthetic coupled repressilator. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

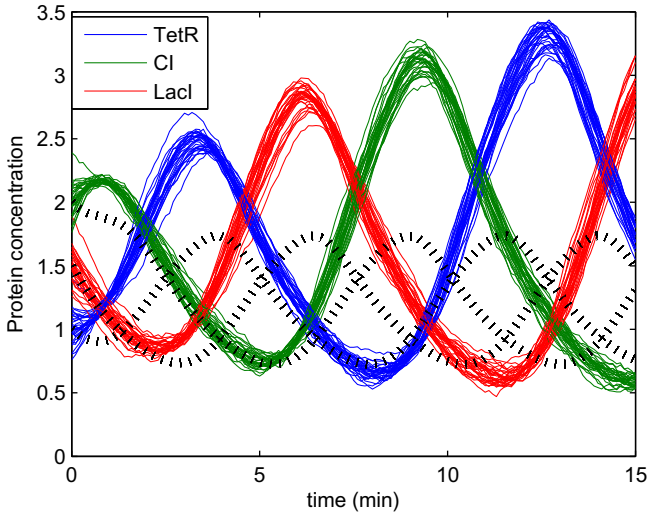

Fig. 9. Monte Carlo simulation results of the synthetic coupled repressilator with infeasible parameters for 30 rounds. We select a set of kinetic parameters k_i from the infeasible parameter sets and the degradation rates r_i out of the design-specified ranges. The gene expressions of the repressilator cannot match the desired nonlinear reference model (black dashed curve) under external disturbances and parameter fluctuations with random initial conditions. The infeasible kinetic parameters k_i = [1.5 1.5 1.5] and degradation rates r_i = [0.4 0.4 0.4] are selected in this simulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

work so that it can be suppressed in some cases and harnessed in others [49], but our purpose is to design the kinetic parameters and degradation rates to satisfy four design specifications that guarantee the desired behaviors of the engineered synthetic gene network in the host cell.

Based on the design specifications, a simple design procedure is developed in this study. From the *in silico* design examples, the four design specifications can be achieved for the robust synthetic gene network according to the proposed robust model matching design scheme. Specifically, we can specify robust kinetic parameters k_i and degradation rates γ_i within the feasible parameter ranges to achieve the desired transient and steady states of the synthetic gene network. The kinetic parameters and degradation rates are approximately dependent on the promoters and protein decay rates, respectively. In synthetic biology, the promoter activities of different promoter architectures are provided as a combinatorial promoter libraries [50]. The promoter activities are determined largely by the position of single operator. In bacteria, operators are classified as being in the core, proximal or distal regions of the promoter, and the repressor can repress expression from all three subregions [51,52]. In eukaryotes, the chromatin structure also strongly influences expression level [53,54]. Although we still engineered the synthetic gene network in the bacteria, a more clearly understanding of the relationship between the promoter activities and the promoter structure will help us engineer a complex synthetic gene network in eukaryotes. As for the biological implementation, we could refer to standard biological parts in biological device datasheets to construct the genetic circuits with the fine-tuned kinetic parameters k_i and degradation rates γ_i. In this way, synthetic biologists can efficiently design the gene circuit through registries of biological parts and standard datasheets.

Quantitative descriptions of devices in the form of standardized, comprehensive datasheets are widely used in many engineering disciplines. A datasheet is intended to allow an engineer to quickly determine whether the behavior of a device will meet the requirements of a system in which a device might be used [55]. Such a determination is based on a set of standard characteristics of device behavior, which are the product of engineering theory and experience. In the datasheets of engineering, the characteristics typically reported are common across a wide range of device types, such as sensors, logic elements and actuators. Recently, biological datasheets have been set as standards for characterization, manufacture and sharing of information about modular biological devices for a more efficient, predictable and design-driven genetic engineering science [55,56]. Because datasheets of biological parts or devices are an embodiment of engineering standard for synthetic biology [55], a good device standard should define sufficient information about biological parts or devices to allow the design of synthetic gene networks with the optimal parameters. Datasheets usually contain a formal set of input–output transfer functions, dynamic behaviors, compatibility, requirements and other details about a particular part or device [55,56]. Since parameters k_i are combinations of transcription and translation, they could be measured from the input–output transfer functions and dynamic behaviors of biological parts or devices in biological device datasheets. When the biological parts and devices in datasheets become more complete in future, we can rapidly select from a vast list the parts that will meet our design parametersk_i. Therefore we can ensure that devices selected from datasheets can fit the feasible parameters, and systems synthesized from them can satisfy the requirements of design specifications for robust synthetic gene networks.

In the present integrated circuit (IC) industry, due to high complexity and difficulty, some system design companies, like Intel, respond for system design of VLSI systems, and some implementation companies, like TSMC (Taiwan Semiconductor Manufacturing Company), are responsible for manufacturing VLSI systems. In future, there may exist some system design companies for system design of synthetic gene networks, and other implementation companies will respond for manufacturing complex synthetic gene networks. If this is the case, the development of synthetic design tools will become an important work for system design of robust synthetic gene networks.

Since the design examples *in silico* meets the four design specifications, the synthetic gene network can overcome different kinds of internal parameter fluctuations and external disturbances. The design specification (ii) includes the prescribed standard deviations of stochastic parameter variations to be tolerated by the synthetic gene network in the host cell, and in the design specification (iv), we introduce a disturbance filtering method to guarantee that the effect of disturbances on the matching error is attenuated by a prescribed level ρ. However, in reality, the amplitudes of external disturbances are uncertain, and so it is not easy to estimate their statistics outside the host cell. But from the energy perspective, the effect of these disturbances on matching error can be prescribed below a desired level ρ by the proposed design method to ensure the matching performance without the knowledge of the disturbance statistics. In addition, specification (iii) provides a desired system behavior of synthetic gene networks. Unlike the synthetic gene network design methods in [25,26], which can only asymptotically achieve some desired steady states, the proposed design method can make synthetic gene network track the transient and steady behaviors prescribed beforehand by a desired reference model. Based on the H_∞ matching performance and global linearization technique, we can also validate the robustness of the proposed design method under the internal parameter fluctuations and external disturbances. Therefore, the proposed matching design method might be more useful than the conventional methods for practical synthetic biological design and will have a great impact on the synthetic biology in the near future.

6. Conclusions

In this study, we have shown a stochastic model for dealing with the dynamic properties of synthetic gene networks under parameter uncertainties and external disturbances in the host cell. In order for the synthetic gene networks to achieve a desired response matching for a reference model under parameter fluctuations and environmental disturbances, four design specifications are proposed in the design procedure of robust synthetic gene network. Finally, a systematic method for robust model matching design of synthetic gene networks is introduced to satisfy these design specifications. To simplify the design procedure, a global linearization technique is employed to avoid solving the complex non-linear stochastic filtering and tracking problems directly, and to represent the non-linear gene network by interpolating a set of simple linearized gene networks. Therefore the design procedures of a robust model matching design of synthetic gene network can be simplified by solving a set of LMIs via the help of LMI toolbox in Matlab. Two design examples *in silico* can confirm the robust model matching performance of a desired reference response.

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Appendix A

Let us choose a Lyapunov function $V(\bar{x}) > 0$ with $V(0) = 0$, then we get

$$E\int_0^\infty \bar{x}^T\overline{Q}\bar{x}dt = E\left\{V(\bar{x}(0)) - V(\bar{x}(\infty)) + \int_0^\infty \left[\bar{x}^T\overline{Q}\bar{x} + \frac{dV(\bar{x})}{dt}\right]dt\right\}. \tag{A.1}$$

By the Ito's formula [57] and $E\frac{d}{dt}w_i = En_i = 0$, we get

$$E\frac{d}{dt}V(\bar{x}) = E\left\{\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T (F(\bar{x},k,r) + H(\bar{x})\bar{v}) + \frac{1}{2}\sum_{i=1}^m \bar{g}^T(\bar{x},\Delta k_i,\Delta r_i)\frac{\partial^2 V(\bar{x})}{\partial \bar{x}^2}\bar{g}(\bar{x},\Delta k_i,\Delta r_i)\right\}. \tag{A.2}$$

Substituting (A.2) into (A.1), we have

$$E\int_0^\infty \bar{x}^T\overline{Q}\bar{x}dt = E\left\{V(\bar{x}(0)) - V(\bar{x}(\infty)) + \int_0^\infty \left[\bar{x}^T\overline{Q}\bar{x} + \left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T F(\bar{x},k,r) + \left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T H(\bar{x})\bar{v} + \frac{1}{2}\sum_{i=1}^m \bar{g}^T(\bar{x},\Delta k_i,\Delta r_i)\frac{\partial^2 V(\bar{x})}{\partial \bar{x}^2}\bar{g}(\bar{x},\Delta k_i,\Delta r_i)\right]dt\right\}. \tag{A.3}$$

By the inequality (16), we have

$$\begin{aligned} E\int_0^\infty \bar{x}^T\overline{Q}\bar{x}dt &\leqslant E\left\{V(\bar{x}(0)) - V(\bar{x}(\infty)) + \int_0^\infty \left[\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T H(\bar{x})\bar{v} - \frac{1}{4\rho^2}\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T H(\bar{x})H^T(\bar{x})\frac{\partial V(\bar{x})}{\partial \bar{x}} - \rho^2\bar{v}^T\bar{v} + \rho^2\bar{v}^T\bar{v}\right]dt\right\} \\ &< E\left\{V(\bar{x}(0)) + \int_0^\infty \left[-\left(\frac{1}{2\rho}H^T(\bar{x})\frac{\partial V(\bar{x})}{\partial \bar{x}} - \rho\bar{v}\right)^T \times \left(\frac{1}{2\rho}H^T(\bar{x})\frac{\partial V(\bar{x})}{\partial \bar{x}} - \rho\bar{v}\right) + \rho^2\bar{v}^T\bar{v}\right]dt\right\} \\ &\leqslant E\left\{V(\bar{x}_0) + \rho^2\int_0^\infty \bar{v}^T\bar{v}dt\right\}. \end{aligned} \tag{A.4}$$

This is the inequality of robust model matching in (13). Then the attenuation level ρ in (13) is achieved. If we specify $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$ such that the inequality in (16) is satisfied, then the specifications (i)–(iv) are satisfied for non-linear stochastic synthetic network in (15).

Appendix B

Now we will derive the sufficient condition to ensure that the linearized augmented systems in (19) can attenuate the external disturbance below a prescribed attenuation level ρ in (13). By choosing a Lyapunov function as $V(\bar{x}) = \bar{x}^TP\bar{x}$ for some $P = P^T > 0$, we have

$$E\int_0^\infty \bar{x}^T\overline{Q}\bar{x}dt = E\left\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(\infty)P\bar{x}(\infty) + \int_0^\infty (\bar{x}^T\overline{Q}\bar{x} + \frac{d}{dt}\bar{x}^TP\bar{x})dt\right\}. \tag{B.1}$$

By Ito formula [57] and $E\frac{d}{dt}w_i = 0$, we have

$$\begin{aligned} E\int_0^\infty \bar{x}^T\overline{Q}\bar{x}dt &= E\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(\infty)P\bar{x}(\infty) \\ &\quad + \int_0^\infty \left[\bar{x}^T\overline{Q}\bar{x} + \sum_{j=1}^M \alpha_j(x)\left(\left[\overline{A}_j(k,r)\bar{x} + \overline{B}_j\bar{v}\right]^TP\bar{x} + \bar{x}^TP\left[\overline{A}_j(k,r)\bar{x} + \overline{B}_j\bar{v}\right] + \sum_{i=1}^m \left(\bar{x}^T\overline{C}_{ij}^TP\overline{C}_{ij}\bar{x}\right)\right)\right]dt\right\} \\ &< E\left\{\bar{x}(0)^TP\bar{x}(0) + \int_0^\infty \left(\sum_{j=1}^M \alpha_j(x)\left(\bar{x}^T\left[P\overline{A}_j(k,r) + \overline{A}_j^T(k,r)P + \overline{Q} + \sum_{i=1}^m \left(\overline{C}_{ij}^TP\overline{C}_{ij}\right)\right]\bar{x} + \bar{v}^T\overline{B}_j^TP\bar{x} + \bar{x}^TP\overline{B}_j\bar{v}\right)\right)dt\right\}. \end{aligned} \tag{B.2}$$

By the inequality in (20), we have

$$\begin{aligned} E\int_0^\infty \bar{x}^T\overline{Q}\bar{x}dt &< E\left\{\bar{x}(0)^TP\bar{x}(0) + \int_0^\infty \left[-\frac{1}{\rho^2}\bar{x}^TP\overline{B}_j\overline{B}_j^TP^T\bar{x} + \bar{v}^T\overline{B}_j^TP\bar{x} + \bar{x}^TP\overline{B}_j\bar{v} - \rho^2\bar{v}^T\bar{v} + \rho^2\bar{v}^T\bar{v}\right]dt\right\} \\ &\leqslant E\left\{\bar{x}(0)^TP\bar{x}(0) + \int_0^\infty \left[-\left(\frac{1}{\rho}\overline{B}_j^TP\bar{x} - \rho\bar{v}\right)^T\left(\frac{1}{\rho}\overline{B}_j^TP\bar{x} - \rho\bar{v}\right) + \rho^2\bar{v}^T\bar{v}\right]dt\right\} \leqslant E\left\{\bar{x}(0)^TP\bar{x}(0) + \int_0^\infty \rho^2\bar{v}^T\bar{v}dt\right\}. \end{aligned} \tag{B.3}$$

This is the robust disturbance attenuation with a prescribed level ρ in (13). When $\bar{x}(0) = 0$, it is reduced to the robust disturbance attenuation in (11). Therefore, if we select $k \in [k_\ell, k_u]$ and $r \in [r_\ell, r_u]$ such that the inequalities in (20) are satisfied, then the specifications (i)–(iv) are satisfied for non-linear stochastic synthetic gene network in (7).

Appendix C

The global linearization technique can be employed to transform the non-linear stochastic gene network of (2) into an equivalent interpolation of a set of globally linearized gene networks. In this design example, the global linearizations are bound by a polytope consisting of 6 vertices, shown as follows

$$dx = (A_j(k,r)x + B_jv)dt + \sum_{i=1}^4 C_{ij}xdw_i, \quad j = 1,\ldots,6, \tag{C.1}$$

where

$$A_1 = \begin{bmatrix} -1.0691 & 0 & 0 & 0 \\ -0.030482 & -2.0071 & 0 & 0 \\ 0 & -0.47223 & -0.39826 & 0 \\ 0 & 0 & -0.18915 & -0.18203 \end{bmatrix},$$

$$A_2 = \begin{bmatrix} -1.0691 & 0 & 0 & 0 \\ -0.079495 & -2.0071 & 0 & 0 \\ 0 & -2.9315 & -0.39826 & 0 \\ 0 & 0 & -0.32622 & -0.18203 \end{bmatrix},$$

$$A_3 = \begin{bmatrix} -1.0691 & 0 & 0 & 0 \\ -0.20458 & -2.0071 & 0 & 0 \\ 0 & -16.931 & -0.39826 & 0 \\ 0 & 0 & -0.55293 & -0.18203 \end{bmatrix},$$

$$A_4 = \begin{bmatrix} -1.0691 & 0 & 0 & 0 \\ -3.4286 & -2.0071 & 0 & 0 \\ 0 & -160.14 & -0.39826 & 0 \\ 0 & 0 & -2.3385 & -0.18203 \end{bmatrix},$$

$$A_5 = \begin{bmatrix} -1.0691 & 0 & 0 & 0 \\ -7.7445 & -2.0071 & 0 & 0 \\ 0 & 16.964 & -0.39826 & 0 \\ 0 & 0 & -50.28 & -0.18203 \end{bmatrix},$$

$$A_6 = \begin{bmatrix} -1.0691 & 0 & 0 & 0 \\ -0.000211 & -2.0071 & 0 & 0 \\ 0 & 1.2959 & -0.39826 & 0 \\ 0 & 0 & -78.005 & -0.18203 \end{bmatrix}.$$

$$B_j = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}, \quad j = 1, \ldots, 6.$$

$$C_{11} = \begin{bmatrix} -0.05 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, \quad C_{12} = \begin{bmatrix} -0.05 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{13} = \begin{bmatrix} -0.05 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{14} = \begin{bmatrix} -0.05 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}, \quad C_{15} = \begin{bmatrix} -0.05 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{16} = \begin{bmatrix} -0.05 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{21} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ -0.0084672 & -0.05 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{22} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ -0.022082 & -0.05 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{23} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ -0.056827 & -0.05 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{24} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ -0.9524 & -0.05 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{25} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ -2.1513 & -0.05 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{26} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ -5.8681e-5 & -0.05 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{31} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & -0.0076413 & -0.05 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{32} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & -0.0050056 & -0.05 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{33} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & -0.0032505 & -0.05 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{34} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & -0.00039857 & -0.05 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{35} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 4.2854e-5 & -0.05 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{36} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 7.4721e-6 & -0.05 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix},$$

$$C_{41} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & -0.0013264 & -0.05 \end{bmatrix},$$

$$C_{42} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & -0.0022876 & -0.05 \end{bmatrix},$$

$$C_{43} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & -0.0038775 & -0.05 \end{bmatrix},$$

$$C_{44} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & -0.016399 & -0.05 \end{bmatrix},$$

$$C_{45} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & -0.35259 & -0.05 \end{bmatrix},$$

$$C_{46} = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & -0.54702 & -0.05 \end{bmatrix}.$$

Appendix D

The global linearization technique can be employed to transform the non-linear synthetic coupled repressilator (29) into an equivalent interpolation of a set of globally linearized repressilator networks. Also the non-linear reference model in (30) can be transformed into an equivalent interpolation of a set of globally linearized reference models. In this design example, the global linearizations are bound by a polytope consisting of 5 vertices, shown as follows

$$d\bar{x} = (\overline{A}_j(k,r)\bar{x} + \overline{B}_j\bar{v})dt + \sum_{i=1}^{3}\overline{C}_{ij}\bar{x}dw_i, \quad j = 1,\ldots,5, \tag{D.1}$$

where $\overline{A}_j(k,r) = \begin{pmatrix} A_{rj} & 0 \\ 0 & A_j(k,r) \end{pmatrix}$, $\overline{B}_j = \begin{pmatrix} B_{rj} & 0 \\ 0 & B_j \end{pmatrix}$, $\overline{C}_{ij} = \begin{pmatrix} 0 & 0 \\ 0 & C_{ij} \end{pmatrix}$,

$$A_1 = \begin{bmatrix} -0.46637 & 0 & -0.013703 \\ 0.41972 & -0.46637 & 0 \\ 0 & -0.08117 & -0.46637 \end{bmatrix},$$

$$A_2 = \begin{bmatrix} -0.46637 & 0 & -0.035303 \\ 0.052859 & -0.46637 & 0 \\ 0 & -1.0817 & -0.46637 \end{bmatrix},$$

$$A_3 = \begin{bmatrix} -0.46637 & 0 & 0.29891 \\ -1.8016e-5 & -0.46637 & 0 \\ 0 & -0.20433 & -0.46637 \end{bmatrix},$$

$$A_4 = \begin{bmatrix} -0.46637 & 0 & 0.41972 \\ -0.08014 & -0.46637 & 0 \\ 0 & -0.013653 & -0.46637 \end{bmatrix},$$

$$A_5 = \begin{bmatrix} -0.46637 & 0 & 0.099206 \\ -0.49461 & -0.46637 & 0 \\ 0 & 0.058579 & -0.46637 \end{bmatrix},$$

$$A_{r1} = \begin{bmatrix} -0.5 & 0 & -1.4452 \\ -1.0311 & -0.5 & 0 \\ 0 & -0.11258 & -0.5 \end{bmatrix},$$

$$A_{r2} = \begin{bmatrix} -0.5 & 0 & -1.7195 \\ -1.786 & -0.5 & 0 \\ 0 & -0.082919 & -0.5 \end{bmatrix},$$

$$A_{r3} = \begin{bmatrix} -0.5 & 0 & -1.2062 \\ -0.97277 & -0.5 & 0 \\ 0 & -0.1657 & -0.5 \end{bmatrix},$$

$$A_{r4} = \begin{bmatrix} -0.5 & 0 & -0.84548 \\ -0.31308 & -0.5 & 0 \\ 0 & -0.52263 & -0.5 \end{bmatrix},$$

$$A_{r5} = \begin{bmatrix} -0.5 & 0 & -1.6181 \\ -0.098182 & -0.5 & 0 \\ 0 & -1.5431 & -0.5 \end{bmatrix}.$$

$$B_j = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}, \quad B_{rj} = \begin{bmatrix} 0.2 & 0 & 0 \\ 0 & 0.2 & 0 \\ 0 & 0 & 0.2 \end{bmatrix}, \quad j = 1,\ldots,5.$$

$$C_{11} = \begin{bmatrix} -0.01 & 0 & -0.0015003 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{12} = \begin{bmatrix} -0.01 & 0 & -0.0038654 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{13} = \begin{bmatrix} -0.01 & 0 & 0.032728 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{14} = \begin{bmatrix} -0.01 & 0 & 0.045956 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{15} = \begin{bmatrix} -0.01 & 0 & 0.010862 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{21} = \begin{bmatrix} 0 & 0 & 0 \\ 0.045956 & -0.01 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{22} = \begin{bmatrix} 0 & 0 & 0 \\ 0.0057876 & -0.01 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{23} = \begin{bmatrix} 0 & 0 & 0 \\ -1.9726e-6 & -0.01 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{24} = \begin{bmatrix} 0 & 0 & 0 \\ -0.0087747 & -0.01 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{25} = \begin{bmatrix} 0 & 0 & 0 \\ -0.054156 & -0.01 & 0 \\ 0 & 0 & 0 \end{bmatrix},$$

$$C_{31} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -0.0088876 & -0.01 \end{bmatrix},$$

$$C_{32} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -0.11844 & -0.01 \end{bmatrix},$$

$$C_{33} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -0.022373 & -0.01 \end{bmatrix},$$

$$C_{34} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & -0.0014949 & -0.01 \end{bmatrix},$$

$$C_{35} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0.006414 & -0.01 \end{bmatrix}.$$

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