Robust Optimal Reference-Tracking Design Method for Stochastic Synthetic Biology Systems: T–S Fuzzy Approach

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***Abstract*—At present, the development in the nascent field of synthetic gene networks is still difficult. Most newly created gene networks are nonfunctioning due to intrinsic parameter fluctuations, uncertain interactions with unknown molecules and external disturbances of intra and extracellular environments on the host cell. How to design a completely new gene network, that is to track some desired behaviors under these intrinsic and extrinsic disturbances on the host cell, is the most important topic in synthetic biology. In this study, the intrinsic parameter fluctuations, uncertain interactions with unknown molecules and environmental disturbances, are modeled into the nonlinear stochastic systems of synthetic gene networks *in vivo*. Four design specifications are introduced to guarantee the stochastic synthetic gene network, which can achieve robust optimal tracking of a desired reference model in spite of these intrinsic and extrinsic disturbances on the host cell. However, the robust optimal reference-tracking design problem of nonlinear synthetic gene networks is still hard to solve. In order to simplify the design procedure of the robust optimal nonlinear stochastic-tracking design for synthetic gene networks, the Takagi–Sugeno (T–S) fuzzy method is introduced to solve the nonlinear stochastic minimum-error-tracking design problem. Hence, the robust optimal reference-tracking design problem under four design specifications can be solved by the linear matrix inequality (LMI)-constrained optimization method using convex optimization techniques. Further, a simple design procedure is developed for synthetic gene networks to meet the four design specifications to achieve robust optimal reference tracking. Finally, an eigenvalue-shifted design method is also proposed as an expedient scheme to improve the stochastic optimal-tracking design method of synthetic gene oscillators.**

***Index Terms*—Eigenvalue-shifted design method, linear matrix inequality (LMI), stochastic optimal reference-tracking design, synthetic gene network, Takagi–Sugeno (T–S) fuzzy model.**

I. Introduction

THE MAIN goal of the nascent field of synthetic biology is to engineer an artificial gene network and then insert it into the host cell to perform new tasks. One useful analogy to conceptualize both the goal and methods of synthetic biology is the computer engineering hierarchy. At the bottom of the hierarchy of synthetic biology are DNA, RNA, proteins, and metabolites (including lipids and carbohydrates, amino acids, and nucleotides), which are analogous to the physical layer of transistors, capacitors, and resistors in computer engineering [1]. The next layer, i.e., the device layer, comprises biochemical reactions that regulate the flow of information and manipulate physical processes, which is equivalent to engineered logic gates that perform computations in a computer. At the module layer, synthetic biologists use a diverse library of biological devices to assemble complex pathways that function like the integrated circuits (ICs).

However, building biological systems entails a unique set of design problems and solutions. Biological devices and modules are not independent objects, and they are not built in the absence of a biological context. Biological devices and modules of synthetic biology typically function within cellular environments. When synthetic biologists engineer the devices or modules, they do so using the resources and machinery of the host cell, but in the process, they also modify the cells themselves. A major concern in this process is our present inability to fully predict the functions of even the simple devices in the engineered cells and construct the systems that perform complex tasks with precision and reliability [1]. The lack of predictive power stems from several resources of uncertainty, some of which signify the incompleteness of the available information about inherent cellular characteristics. The effects of gene expression noises, uncertain initial conditions, mutations, cell death, undefined and changing extracellular environments, and interactions with cellular contexts currently hinder us from engineering biological systems with the confidence that we can engineer computers to do specific tasks.

Recently, Kuepfer *et al*. have developed an approach for synthetic biology based on semidefinite programming to partition the parameter spaces of polynomial differential equation models into the so-called feasible and infeasible regions [2]. In this approach, the feasible region simply refers to the existence of a steady state of the synthetic system. Batt *et al*. propose an approach for the analysis of a class of uncertain piecewise-multiaffine differential equation models of synthetic biological systems to adapt to the experimental data [3]. These models allow the development of efficient algorithms to solve robustness analysis and parameter tuning problems. Even though the robustness to tolerate the parameter variations of synthetic gene networks has been analyzed in the earlier literature, the parameter fluctuations of synthetic gene networks are inherently

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stochastic in the nanoscale systems and should be modeled by nonlinear stochastic dynamic systems. Further, the uncertain interactions with unknown molecules and environmental disturbances of synthetic gene networks have not been considered in their design procedure to describe the undefined and changing extracellular environments as well as the interactions with the cellular context. Recently, some gene circuit designs have been proposed to embed gene circuits into an existing gene network to improve its robust stability [4], [5]. However, the synthetic gene network is a different topic. We need to design a completely new gene network and then insert it into a host cell to perform new tasks in spite of parameter fluctuations, uncertain interactions with unknown molecules in the cellular context, and environmental disturbances on the host cell. More recently, a robust synthetic gene network despite environmental disturbances is designed to achieve a desired steady state via dynamic game theory from the worst-case disturbance point of view [6]. A robust synthetic gene network is also designed via H_∞ stabilization method to achieve a desired steady state [7]. The design purpose of all the aforementioned robust stabilization methods is to design synthetic gene networks with a desired steady state. However, some synthetic gene networks with a desired behavior like oscillation or a transient behavior cannot be designed by these kinds of robust stabilization methods. Therefore, how to design a synthetic gene network to achieve a desired behavior like oscillation or transient behavior despite parameter fluctuations, uncertain interactions with unknown molecules in the cellular context, and environmental disturbances belongs to a robust-tracking design problem. More effort is needed than the conventional robust stabilization methods. In the robust-tracking design case, a reference model must be chosen at first to generate a desired behavior, and then, the robust-tracking design method must be developed for synthetic gene networks to track the reference model to achieve the desired behavior.

In this study, a synthetic gene network is designed so that it can robustly track the desired behavior of a reference model, despite the intrinsic parameter fluctuations, uncertain interactions with the cellular context, and environmental disturbances on the host cell. In this situation, intrinsic parameter fluctuations of synthetic gene networks are modeled as the state-dependent stochastic noises, uncertain interactions with unknown molecules in the cellular context are modeled as uncertain nonlinear state-dependent couplings with unknown molecules, and environmental disturbances are also modeled as the uncertain external signals, i.e., a synthetic gene network in the host cell is described by a nonlinear stochastic system with state-dependent noises, uncertain interactions with unknown molecules, and external disturbances. Based on the theories of stochastic stability and nonlinear filtering [8], [9], the robust optimal reference-tracking design of synthetic gene networks is discussed from the perspective of the nonlinear stochastic-tracking system. Further, based on four prescribed design specifications, i.e., the allowable ranges of kinetic parameters and decay rates, the bound of uncertain interactions with unknown molecules, the tolerable variances of parameter fluctuations, and the desired reference model and the optimal reference-tracking ability, a design procedure is developed for robust synthetic gene networks. The procedure that matches the prescribed four design specifications will achieve the robust optimal reference-tracking purpose, despite the intrinsic parameter fluctuations, uncertain interactions with unknown molecules, external disturbances, and uncertain initial conditions. The optimal-tracking design of synthetic gene networks is different from the conventional optimal-tracking design of control systems. The first one is to design a completely new gene network with embedded feedback and feedforward gene circuits so that the gene network can track reference behaviors by itself; the second one is to design a controller for a controlled system through outside feedback and feedforward loops so that the state variables of controlled system can track reference signals. In other words, the synthetic gene network can track the desired behavior through intrinsic feedback and feedforward circuits, while the controlled system tracks the desired behaviors through outside feedback and feedforward control loops.

Because of the uncertainties of exogenous uptakes and interactions with the cellular context, i.e., the environmental disturbances, uncertain interactions with unknown molecules, and uncertain initial conditions, their effects on the desired behavior tracking of synthetic gene networks should be considered in the design procedure. From the robust optimal reference-tracking point of view, the uncertain effects can be efficiently attenuated. Therefore, for the purpose of robust-tracking design of synthetic gene networks, the effects of all possible uncertainties on the desired behavior tracking should be minimized, i.e., the proposed robust optimal reference-tracking design of synthetic gene networks is solved from the minimum mean-square-tracking error perspective. In general, it is not easy to directly solve the optimal-tracking design problem for nonlinear stochastic synthetic gene networks. In this study, a suboptimal-tracking design method is developed for nonlinear stochastic gene networks. Since a synthetic gene network is highly nonlinear, it is still difficult to solve the suboptimal reference-tracking design problem. This is because it needs to solve a highly nonlinear second-order Hamilton–Jacobi inequality (HJI), which cannot be solved analytically or numerically at present. In this study, a Takagi–Sugeno (T–S) fuzzy system is employed to efficiently approximate the nonlinear stochastic system of a synthetic gene network to simplify the design procedure of optimal-tracking design problem of robust synthetic gene networks.

Recently, T–S fuzzy systems have been employed to approximate nonlinear dynamic systems to efficiently solve the nonlinear control problem [10]–[12]. A fuzzy model is proposed to interpolate several linearized synthetic gene networks to approximate a nonlinear synthetic gene network via smooth fuzzy membership functions. Then, with the help of the T–S fuzzy approximation method, a fuzzy dynamic-tracking design scheme is developed so that the optimal desired behavior-tracking design problem of synthetic gene networks could be easily solved by the linear dynamic-tracking method, which can be easily solved by a constrained optimization scheme via the linear matrix inequality (LMI) technique [13] with the help of Robust Control Toolbox in MATLAB [14]. The design procedure is also developed to meet the four design specifications so that the robust optimal reference-tracking design can be achieved

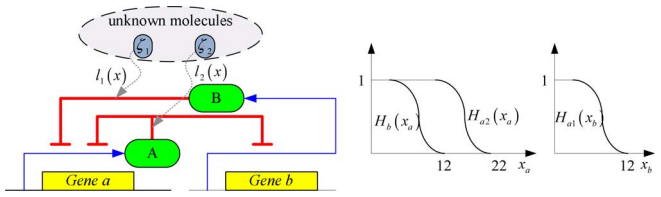

Fig. 1. Cross-inhibition network and its regulation functions in (1). Suppose there exist uncertain interactions with unknown molecules (i.e., ζ_1 and ζ_2) that intertwine with the synthetic gene network.

to track its desired behaviors *in vivo*, in spite of the intrinsic parameter perturbations, uncertain interactions with unknown molecules, and environmental disturbances on the host cell. In order to avoid the design difficulty to solve LMIs in tracking a desired periodic or oscillatory reference model with eigenvalues all on image axis (i.e., $j\omega$-axis), an eigenvalue-shifted technique is also proposed as an expedient method to provide design feasibility to overcome the LMI-constrained problem in the periodicity or oscillation-tracking design of synthetic gene networks. Obviously, the proposed robust optimal-tracking design method has much potential application to the nascent field of synthetic gene networks. Finally, two *in silico* examples are given to illustrate the design procedure and to confirm the efficiency under uncertain initial conditions, uncertain interactions with unknown molecules, intrinsic parameter fluctuations, and external disturbances.

II. Stochastic Synthetic Gene Network Model With Intrinsic Parameter Fluctuations, Uncertain Interactions With Unknown Molecules, and External Disturbances

The parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances on the host cell make synthetic gene networks difficult to track their desired behaviors. To overcome this design problem, the parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances should be considered in the dynamic model of synthetic gene networks to mimic their real-dynamic behaviors in the host cell. Besides, the tracking performance should be considered in the design procedure. For the convenience of illustration, a simple example is provided to outline the robust optimal reference-tracking design of synthetic gene networks under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances on the host cell. Consider the cross-inhibition network shown in Fig. 1 [3]. The network is synthesized by two genes a and b, which code for two repressor proteins A and B. More specifically, protein B represses the expression of gene a, whereas protein A represses the expression of gene b and, at a higher concentration, the expression of its own gene. This system can be modeled by differential equations as follows [3]:

$$\begin{aligned}\dot{x}_a &= \kappa_a H_{a1}(x_b) H_{a2}(x_a) - \gamma_a x_a \\ \dot{x}_b &= \kappa_b H_b(x_a) - \gamma_b x_b. \end{aligned} \tag{1}$$

The state variables $x_a(t)$ and $x_b(t)$ denote the concentrations of proteins A and B. $x(t) = [x_a(t), x_b(t)]^T$ is the vector of the state variables. κ_i and γ_i are the production and degradation rate parameters, respectively, and $H_i(\cdot)$ is the regulation function, which captures the regulatory effect of an effector protein on gene expression and is a smooth sigmoidal function (e.g., Hill function, see Fig. 1) [15], [16]. The product of the regulation function can be used to capture complex genetic regulations. For example, the product of the regulation function in (1) captures the hypothesis, which, in order to have a maximal expression of a gene, must show how the concentration changes. Some parameters are inherently uncertain in this nanoscale biochemical system [16]. We assume that the production and/or the degradation rate parameters (i.e., κ_i and γ_i) of synthetic gene networks are stochastically uncertain due to gene expression noises in transcriptional and translational processes, thermal fluctuations, DNA mutation, and evolution [16], as follows:

$$\begin{aligned}&\kappa_a \to \kappa_a + \Delta\kappa_a n_a, \kappa_b \to \kappa_b + \Delta\kappa_b n_b \\ &\gamma_a \to \gamma_a + \Delta\gamma_a n_a, \gamma_b \to \gamma_b + \Delta\gamma_b n_b \end{aligned} \tag{2}$$

where $\Delta\kappa_i$ and $\Delta\gamma_i$, $i = a, b$ denote the amplitudes of stochastic parameter variations, and $n_i(t)$, $i = a, b$ are white noises with zero means and unit variances. Thus, $\Delta\kappa_i$ and $\Delta\gamma_i$ denote the deterministic part of parameter variations, and $n_i(t)$ denotes the stochastic property of intrinsic parameter fluctuations. $n_a(t)$ and $n_b(t)$ are two independent stochastic sources of random parameter fluctuations. The covariance of $\Delta\kappa_a n_a(t)$ and $\Delta\gamma_a n_a(t)$ are given as $\mathrm{Cov}(\Delta\kappa_a n_a(t), \Delta\kappa_a n_a(\tau)) = \Delta\kappa_a^2 \delta_{t,\tau}$ and $\mathrm{Cov}(\Delta\gamma_a n_a(t), \Delta\gamma_a n_a(\tau)) = \Delta\gamma_a^2 \delta_{t,\tau}$, respectively, where $\delta_{t,\tau}$ denotes the delta function, i.e., $\delta_{t,\tau} = 1$ if $t = \tau$ and $\delta_{t,\tau} = 0$ if $t \neq \tau$. Therefore, $\Delta\kappa_a$ and $\Delta\gamma_a$ denote the standard deviations of the stochastic parameter fluctuations $\Delta\kappa_a n_a(t)$ and $\Delta\gamma_a n_a(t)$, respectively.

Suppose the synthetic gene network suffers from the external disturbances $v_a(t)$ and $v_b(t)$ on the host cell due to the changing extracellular environment, uncertain interactions $l_1(x)$ and $l_2(x)$ with unknown molecules $\zeta_1(t)$ and $\zeta_2(t)$, respectively, in the cellular context, etc. In general, the external disturbances and unknown molecules are also stochastic. Then, the stochastic synthetic gene network in the host cell can be described as follows:

$$\begin{aligned}\begin{bmatrix}\dot{x}_a \\ \dot{x}_b\end{bmatrix} &= \begin{bmatrix}(\kappa_a + \Delta\kappa_a n_a) H_{a1}(x_b) H_{a2}(x_a) - (\gamma_a + \Delta\gamma_a n_a) x_a \\ (\kappa_b + \Delta\kappa_b n_b) H_b(x_a) - (\gamma_b + \Delta\gamma_b n_b) x_b\end{bmatrix} \\ &\quad + \begin{bmatrix}l_1(x)\zeta_1 \\ l_2(x)\zeta_2\end{bmatrix} + \begin{bmatrix}v_a \\ v_b\end{bmatrix} \\ &= \begin{bmatrix}\kappa_a H_{a1}(x_b) H_{a2}(x_a) - \gamma_a x_a \\ \kappa_b H_b(x_a) - \gamma_b x_b\end{bmatrix} \\ &\quad + \begin{bmatrix}l_1(x) & 0 \\ 0 & l_2(x)\end{bmatrix}\begin{bmatrix}\zeta_1 \\ \zeta_2\end{bmatrix} + \begin{bmatrix}\Delta\kappa_a & -\Delta\gamma_a \\ 0 & 0\end{bmatrix} \\ &\quad \times \begin{bmatrix}H_{a1}(x_b) H_{a2}(x_a) \\ x_a\end{bmatrix} n_a \\ &\quad + \begin{bmatrix}0 & 0 \\ \Delta\kappa_b & -\Delta\gamma_b\end{bmatrix}\begin{bmatrix}H_b(x_a) \\ x_b\end{bmatrix} n_b + \begin{bmatrix}v_a \\ v_b\end{bmatrix}. \end{aligned} \tag{3}$$

For the convenience of analysis and design, the stochastic synthetic gene network in (3) can be represented by the following Ito stochastic differential equation:

$$\begin{aligned}\begin{bmatrix} dx_a \\ dx_b \end{bmatrix} &= \left\{ \begin{bmatrix} \kappa_a H_{a1}(x_b) H_{a2}(x_a) - \gamma_a x_a \\ \kappa_b H_b(x_a) - \gamma_b x_b \end{bmatrix} \right. \\ &\quad \left. + \begin{bmatrix} l_1(x) & 0 \\ 0 & l_2(x) \end{bmatrix} \begin{bmatrix} \zeta_1 \\ \zeta_2 \end{bmatrix} + \begin{bmatrix} v_a \\ v_b \end{bmatrix} \right\} dt \\ &\quad + \begin{bmatrix} \Delta\kappa_a & -\Delta\gamma_a \\ 0 & 0 \end{bmatrix} \begin{bmatrix} H_{a1}(x_b) H_{a2}(x_a) \\ x_a \end{bmatrix} dW_a \\ &\quad + \begin{bmatrix} 0 & 0 \\ \Delta\kappa_b & -\Delta\gamma_b \end{bmatrix} \begin{bmatrix} H_b(x_a) \\ x_b \end{bmatrix} dW_b, \begin{bmatrix} x_a(0) \\ x_b(0) \end{bmatrix} \\ &= \begin{bmatrix} x_{a0} \\ x_{b0} \end{bmatrix} \end{aligned} \tag{4}$$

where $W_i,\ i = a,\ b$ is a standard Wiener process or a Brownian motion with $dW_i = n_i(t)\,dt$ [9]. $[x_a(0),\ x_b(0)]^T$ denotes the initial values of the synthetic gene network and is always uncertain. The stochastic nonlinear differential equation in (4) is given to illustrate the synthetic gene network in a biological milieu, which suffers from intrinsic parameter fluctuations, uncertain interactions with cellular context, and extrinsic disturbances on the host cell. Instead of (1), the robust optimal reference-tracking design should consider the stochastic nonlinear system in (4) as a synthetic gene network model to mimic the dynamic behaviors in a real-host cell.

In this simple synthetic gene network design case, we want to design two kinetic parameters κ_a and κ_b and two decay rates γ_a and γ_b within the following allowable ranges:

$$\kappa_a \in [\underline{\kappa}_a, \bar{\kappa}_a], \kappa_b \in [\underline{\kappa}_b, \bar{\kappa}_b], \gamma_a \in \left[\underline{\gamma}_a, \bar{\gamma}_a\right], \gamma_b \in \left[\underline{\gamma}_b, \bar{\gamma}_b\right] \tag{5}$$

such that the following desired reference model could be robustly tracked by the stochastic synthetic gene network in (4):

$$\begin{bmatrix} \dot{x}_{r1}(t) \\ \dot{x}_{r2}(t) \end{bmatrix} = A_r \begin{bmatrix} x_{r1}(t) \\ x_{r2}(t) \end{bmatrix} + \begin{bmatrix} r_1(t) \\ r_2(t) \end{bmatrix} \tag{6}$$

where A_r and $[r_1(t),\ r_2(t)]^T$ are specified by a designer such that the vector $[x_{r1}(t),\ x_{r2}(t)]^T$ in (6) has the desired behaviors to be tracked by $[x_a(t),\ x_b(t)]^T$ in (4), despite the intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances on the host cell.

Now, consider a more general synthetic gene network design case. We extend the two-genes system in (4) to an n-genes system as follows:

$$\begin{aligned} dx &= \left(f(x, \kappa,\ \gamma) + l(x)\zeta + v\right) dt + \sum_{j=1}^{m} M_j g_j(x) dW_j \\ x(0) &= x_0 \end{aligned} \tag{7}$$

where the state vector $x = [x_1, \ldots, x_n]^T$ denotes the concentrations of proteins in the synthetic gene network, and $f(x,\ \kappa,\ \gamma)$ denotes the nonlinear regulation consisting of sigmoidal regulation functions between these genes. $l(x)$ denotes the uncertain interactions with unknown molecules $\zeta(t) = [\zeta_1(t) \ldots \zeta_l(t)]^T$. Suppose the uncertain interactions $l(x)$ with unknown molecules can be bounded as follows:

$$\|l(x)\| \le c \tag{8}$$

for some positive constant c.

The kinetic parameters $\kappa = [\kappa_1, \ldots, \kappa_n]$ and decay rates $\gamma = [\gamma_1, \ldots, \gamma_n]$ are both to be designed so that the state vector $x(t)$ in (7) can track the desired behavior $x_r(t)$. The last term in (7) denotes the stochastic parameter fluctuations due to m random sources, and $v(t) = [v_1(t),\ \ldots,\ v_n(t)]^T$ denotes the vector of external disturbances. The elements of the perturbative matrix M_j denote the corresponding perturbation amplitudes or the standard deviations of the corresponding stochastic parameter fluctuations due to m random noise sources. In the synthetic gene network design $M_j, j = 1,\ \ldots,\ m$ given by the designer, denote the prescribed amplitudes (or standard deviations) of parameter fluctuations to be tolerated by synthetic gene networks according to a real-statistical estimation on the host cell.

Remark 1: 1) If some components of κ and γ are contained in $g_j(x)$, then $g_j(x)$ can be replaced by $g_j(x,\ \kappa,\ \gamma)$. 2) If the equilibrium point of $dx = f(x, \kappa, \gamma)\,dt$ is not at the origin, it should be shifted to the origin for the convenience of design. 3) Unlike the conventional-tracking control design for a plant by control inputs from the outside feedback loops, the proposed optimal synthetic design method is to select adequate parameters κ and γ to influence the feedback abilities and kinetic properties of imbedded biochemical circuits so that the synthetic gene network can robustly track some desired behaviors by itself in the host cell. Therefore, there are some differences between the proposed robust optimal reference-tracking design and the conventional-tracking control design. The proposed robust synthetic gene network lies in the design of embedded gene circuits to track the desired behaviors via intrinsic gene circuits by itself, but the conventional-tracking control system is through the outside control signals based on feedback and/or feedforward loops to track the desired behaviors for controlled plants [17]–[19].

III. Robust Synthetic Gene Network Design via Design Specifications

In general, the stochastic system in (7) is an extension of the stochastic system of a synthetic gene network in (4) from two to n genes. Based on the earlier analysis, the design specifications of the robust optimal reference-tracking design problem for a synthetic gene network are described in the following.

i) Formulate the nonlinear stochastic system in (7) with the prescribed standard deviations of parameter fluctuations M_j and the bounded uncertain interactions $l(x)$ in (8) with unknown molecules $\zeta(t)$ to be tolerated by the synthetic gene network.

ii) Provide the allowable ranges of kinetic parameters and decay rates to be designed

$$\kappa \in [\underline{\kappa},\ \bar{\kappa}],\ \gamma \in \left[\underline{\gamma},\ \bar{\gamma}\right] \tag{9}$$

where $\underline{\kappa}$ and $\bar{\kappa}$ and $\underline{\gamma}$ and $\bar{\gamma}$ denote the lower and upper bounds of the feasible kinetic parameter vector κ and the decay rate vector γ, respectively. The allowable ranges

of design parameters are dependent on the corresponding biotechnology and the kinds of the host cell.

iii) Provide a reference model that can generate the desired behavior to be tracked as follows:

$$\dot{x}_r(t) = A_r x_r(t) + r,\ x_r(0) = 0 \tag{10}$$

where A_r and r are specified beforehand to generate a desired behavior $x_r(t)$ to be tracked by $x(t)$. Since r can be an arbitrary value specified by the designer, it can be also considered as an uncertain signal to the synthetic gene network.

iv) Specify κ and γ to achieve the following robust optimal-tracking design:

$$\min_{\substack{\kappa\in[\underline{\kappa},\ \bar{\kappa}]\\ \gamma\in[\underline{\gamma},\ \bar{\gamma}]}} E\int_0^{t_f} \left(x(t) - x_r(t)\right)^T Q\left(x(t) - x_r(t)\right) dt \tag{11}$$

i.e., the tracking error $x(t) - x_r(t)$ should be minimized to efficiently eliminate the effects of parameter fluctuations, uncertain interactions with unknown molecules, and environmental disturbances from the average energy perspective. Q is a symmetric weighting matrix. For example, if we only want to track the output $x_n(t)$, then Q can be chosen as $Q = \mathrm{diag}\left([0\ 0\cdots 0\ 1]\right)$.

Remark 2:

1) The reference matrix A_r in (10) is determined by the transient time of the reference model. The input r is determined by the steady state $x_{rs} = -A_r^{-1} r$, where x_{rs} is the desired steady state given by the user of the synthetic gene network [18]. Moreover, if we want to track desired oscillations with frequencies at $\omega_1,\ \omega_2,\ \ldots,\ \omega_l$, then we can let $A = \mathrm{diag}\left[j\omega_1,\ -j\omega_1,\ j\omega_2,\ -j\omega_2,\ \ldots,\ j\omega_l,\ -j\omega_l\right]$ for the reference model in (10).
2) The stochastic optimal-tracking design in (11) is an optimal-tracking design method with efficient attenuation of parameter fluctuations, uncertain interactions with unknown molecules in the cellular context, and external disturbances on the host cell in which the synthetic gene network will be inserted [20].
3) In general, the H_∞ tracking design is formulated as a robust-tracking problem to attenuate the $v(t)$ on the tracking error $x(t) - x_r(t)$ below a prescribed level as follows [5], [6]:

$$\frac{E\int_0^{t_f} \left(x(t) - x_r(t)\right)^T Q\left(x(t) - x_r(t)\right)dt}{E\int_0^{t_f} v^T(t)\, v(t)dt} \le \rho^2. \tag{12}$$

The H_∞ robust-tracking design is based on the fact that statistical information of $v(t)$ and $x(0)$ is unavailable or unknown. Therefore, the upper bound of the ratio [or the effect of $v(t)$ on $x(t) - x_r(t)$] is considered for all possible $v(t)$. In general, the result of the robust H_∞ tracking design is more conservative because we do not need the statistical information of $x(0)$ and $v(t)$. In the study, we assume the statistical knowledge of $x(0)$ and $v(t)$ are available from this experiment or the other statistical measurement. Therefore, the stochastic optimal-tracking design is employed to minimize the mean-square-tracking error due to all stochastic uncertainties. In this situation, the proposed robust-tracking design is with a less-conservative result.

From the aforementioned analysis, our design purpose is to specify kinetic parameters κ and decay rates γ such that the design specifications i)–iv) for synthetic gene networks can be achieved simultaneously. If the design specifications i)–iv) are guaranteed, then the purpose of the robust optimal reference-tracking design for synthetic gene networks can be achieved within some feasible parameter ranges in spite of parameter fluctuations, uncertain interactions with unknown molecules, and environmental disturbances on the host cell. In general, it is hard to solve the robust optimal-tracking design problem in (11) subject to design specifications i)–iv). The robust optimal-tracking design problem should be transformed to an equivalent robust optimal-regulation design problem via an augmented system to simplify the design procedure.

Let us combine the reference model in (10) with the stochastic gene network in (7) as an augmented system

$$\begin{bmatrix} dx_r \\ dx \end{bmatrix} = \left(\begin{bmatrix} A_r x_r \\ f(x,\ \kappa, \gamma) \end{bmatrix} + \begin{bmatrix} I & 0 & 0 \\ 0 & I & l(x) \end{bmatrix} \begin{bmatrix} r \\ v \\ \zeta \end{bmatrix} \right) dt + \sum_{j=1}^{m} \begin{bmatrix} 0 \\ M_j g_j(x) \end{bmatrix} dW_j \tag{13}$$

or equivalently

$$d\bar{x} = \left(\bar{f}(\bar{x}, \kappa, \gamma) + \bar{l}\left(\bar{x}\right)\bar{v}\right) dt + \sum_{j=1}^{m} \bar{M}_j \bar{g}_j\left(\bar{x}\right) dW_j \tag{14}$$

where $\bar{x} = \begin{bmatrix} x_r \\ x \end{bmatrix}$, $\bar{v} = \begin{bmatrix} r \\ v \\ \zeta \end{bmatrix}$, $\bar{f}(\bar{x},\ \kappa, \gamma) = \begin{bmatrix} A_r x_r \\ f(x,\ \kappa, \gamma) \end{bmatrix}$, $\bar{M}_j = \begin{bmatrix} 0 \\ M_j \end{bmatrix}$, $\bar{l}\left(\bar{x}\right) = \begin{bmatrix} I & 0 & 0 \\ 0 & I & l(x) \end{bmatrix}$, $\bar{g}_j\left(\bar{x}\right) = g_j(x)$.

Let us denote

$$J(\kappa, \gamma) = E\int_0^{t_f} \left(x - x_r\right)^T Q\left(x - x_r\right) dt.$$

Then, the tracking error in (11) can be represented as the following regulation error of the augmented system in (14):

$$J(\kappa, \gamma) = E\int_0^{t_f} \bar{x}^T \bar{Q} \bar{x} dt \tag{15}$$

where $\bar{Q} = \begin{bmatrix} -I \\ I \end{bmatrix} Q \begin{bmatrix} -I & I \end{bmatrix}$.

After the augmented system in (13) is obtained, the robust optimal-tracking design is discussed in the following. How to design kinetic parameters κ and decay rates γ of the stochastic synthetic gene network in (7) to optimally track the reference model in (10) is equivalent to how to design κ and γ to solve the following optimal regulation design problem for the augmented system in (14):

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} J(\kappa,\ \gamma) = \min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} E\int_0^{t_f} \left(x - x_r\right)^T Q\left(x - x_r\right) dt$$

$$= \min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} E\int_0^{t_f} \bar{x}^T\bar{Q}\bar{x}dt. \tag{16}$$

Based on the augmented system in (14), the robust optimal-tracking design problem is transformed to an equivalent robust regulation design problem of the augmented system in (14) and (16). However, it is still very complicated to solve the nonlinear stochastic optimal regulation design problem in (14) and (16). A suboptimal regulation design method is introduced to simplify the design problem, i.e., to minimize the upper bound of $J(\kappa,\gamma)$ in (16) instead of minimizing $J(\kappa,\gamma)$ directly.

In this case, let us choose a Lyapunov (energy) function $V(\bar{x})>0$ for the augmented stochastic system in (14). Based on the Lyapunov function, we obtain the following result.

Proposition 1: The suboptimal-tracking design for synthetic gene networks is to solve the following constrained optimization problem:

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} EV(\bar{x}(0)) \tag{17}$$

subject to

$$\begin{aligned} &V(\bar{x})>0\\ &\bar{x}^T\bar{Q}\bar{x}+\left(\frac{\partial V(\bar{x})}{\partial\bar{x}}\right)^T\bar{f}(\bar{x},\kappa,\gamma)\\ &\quad+\frac{1}{4}\left(\frac{\partial V(\bar{x})}{\partial\bar{x}}\right)^T S\left(\frac{\partial V(\bar{x})}{\partial\bar{x}}\right)\\ &\quad+\frac{1}{2}\sum_{j=1}^{m}\bar{g}_j^T(\bar{x})\bar{M}_j^T\frac{\partial^2V(\bar{x})}{\partial\bar{x}^2}\bar{M}_j\bar{g}_j(\bar{x})<0 \end{aligned} \tag{18}$$

where $S=\begin{bmatrix} I & 0\\ 0 & \left(c^2+1\right)I\end{bmatrix}$ with c defined in (8).

Proof: See Appendix A.

$EV(\bar{x}(0))$ in (17) is the upper bound of the tracking error in (15) if the HJI in (18) holds, and $V(\bar{x})$ is a positive solution of HJI in (18), i.e., the suboptimal-tracking design problem is to specify kinetic parameters $\kappa\in[\underline{\kappa},\bar{\kappa}]$ and decay rates $\gamma\in[\underline{\gamma},\bar{\gamma}]$ of the gene circuit of the synthetic gene network to minimize the upper bound of the tracking error under the HJI constraint in (18). In general, there is no good method to solve HJI in (18) for the nonlinear stochastic optimal-tracking design problem. Therefore, it is very difficult to solve the constrained optimization problem in (17) to get the design kinetic parameters κ and decay rates γ to achieve the robust optimal reference-tracking performance under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances on the host cell. In the following section, the fuzzy stochastic scheme will be employed to simplify the design procedure of robust optimal-tracking design problem for the nonlinear stochastic gene network. ■

Remark 3:

1) When the H_∞ tracking design (12) is employed for robust-tracking design for a prescribed attenuated level ρ, we need to solve a $V(x)>0$ for the following HJI:

$$\begin{aligned} &\left(\frac{\partial V(\bar{x})}{\partial\bar{x}}\right)^T\bar{f}(\bar{x},\kappa,\gamma)+\frac{1}{2}\sum_{j=1}^{m}\bar{g}_j^T(\bar{x})\bar{M}_j^T\frac{\partial^2V(\bar{x})}{\partial\bar{x}^2}\bar{M}_j\bar{g}_j(\bar{x})\\ &+\frac{1}{4\rho^2}\left(\frac{\partial V(\bar{x})}{\partial\bar{x}^2}\right)^T S\left(\frac{\partial V(\bar{x})}{\partial\bar{x}^2}\right)+\bar{x}^T\bar{Q}\bar{x}<0 \end{aligned} \tag{19}$$

and the optimal H_∞ tracking design solves the following constrained optimization:

$$\min_{\kappa,\gamma}\rho \tag{20}$$

subject to $V(\bar{x})>0$, HJI in (19).

2) According to [6], we want to design a gene circuit $\dot{x}=f(x,\kappa,\gamma)+v$ under disturbance to achieve minimax regulation

$$\min_{\kappa,\gamma}\max_{\tilde{x}(0),v}\frac{E\int_0^{t_f}(x-x_d)^TQ(x-x_d)dt}{\int_0^{t_f}(v^Tv)dt+\tilde{x}(0)\tilde{x}(0)}\le g^2 \tag{21}$$

in which x_d is a constant steady state and the worse-case effect of $v(t)$ and $x(0)$ on the regulation $x-x_d$ is to be minimized. g^2 is the upper bound of this effect. When the worse case disturbance $v^*=(1/2g^2)(\partial V(\tilde{x})/\partial\tilde{x})$ is considered in the minimax (game theory) design, the robust synthetic design problem becomes to how to solve the following constrained optimization problem [6, Prop. 1]:

$$\min_{\kappa,\gamma} g^2$$

subject to

$$\begin{cases}\frac{\partial V(\tilde{x})}{\partial\tilde{x}}f(\tilde{x}+x_d,\kappa,\gamma)+\tilde{x}^TQ\tilde{x}+\frac{1}{4g^2}\left(\frac{\partial V(\tilde{x})}{\partial\tilde{x}}\right)^T\left(\frac{\partial V(\tilde{x})}{\partial\tilde{x}}\right)<0\\ E[V(\tilde{x}(0))]\le g^2E\left[\tilde{x}^T(0)\tilde{x}(0)\right]\end{cases} \tag{22}$$

where $\tilde{x}=x-x_d$.

Since the worse-case disturbance v^* is used, the design is more conservative. Further the synthetic gene network is designed to achieve the desired constant-tracking steady state x_d, it cannot track the changeable or periodic target.

In this study, the proposed method can design a robust synthetic gene network with parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances in (7) such that it can achieve the following stochastic optimal tracking:

$$\min_{\kappa,\gamma}E\int_0^{t_f}(x-x_r)^TQ(x-x_r)dt$$

where $\sum_{i=1}^{m}M_ig_idW_i$ is due to parameter fluctuations, $l(x)\zeta$ is due to interactions with unknown molecules, and x_r is any reference signal generated by the reference model $\dot{x}_r=A_rx_r+r$. By the suboptimal design method, the stochastic optimal tracking becomes how to solve the constrained optimization (17) and (18) (see Proposition 1). Obviously, they are two different constrained optimization design problems. The main differences with [6] are pointed out as follows.

1) The study in [6] considered a constant steady-state regulation design problem under disturbance; the proposed

method is a model reference-tracking design problem under parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances.

2) The design performance indices are different. The method in [6] considers the minimization of worst-case effect of external disturbances; the proposed method provides four design specifications to achieve the robust stochastic optimal mean-square-tracking design.
3) Since the Ito stochastic model is considered for intrinsic parameter fluctuations, the second-order HJI constraint in our method is different from the first-order HJI constraint in [6].

IV. Robust Optimal Reference-Tracking Design of Synthetic Gene Network: Fuzzy Dynamic Approach

In general, the robust optimal reference-tracking design of synthetic gene networks under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances needs to solve an HJI-constrained optimization problem in (18). At present, there is no analytic or numerical solution for HJI in (18). Recently, the T–S fuzzy model [9], [10], [17] had been widely applied to approximate the nonlinear system via interpolating several linearized systems at different operation points. Hence, the robust optimal reference-tracking design of nonlinear stochastic synthetic gene networks can be transformed to a tracking design problem of fuzzy-interpolation linear systems.

Suppose the augmented system in (14) can be represented by the T–S fuzzy model [21]. The T–S fuzzy model is a piecewise interpolation of several linearized models through the membership functions. The fuzzy model is described by several *if–then* rules and will be employed to deal with the robust model-tracking design problem of nonlinear stochastic gene networks under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances. The *i*th rule of the fuzzy model for the nonlinear stochastic system in (14) can be expressed as the following form [8], [9], [18]–[32]:

Rule i: If x_1 is F_{i1}, x_2 is F_{i2}, ..., and x_n is F_{in}, then

$$d\bar{x} = (\bar{A}_i(\kappa,\gamma)\bar{x} + \bar{l}(\bar{x})\bar{v})dt + \sum_{j=1}^{m} \bar{M}_j \bar{B}_{ij}\bar{x}dW_j,\ i = 1,\ \ldots,\ L \tag{23}$$

where $\bar{A}_i(\kappa,\gamma) = \begin{bmatrix} A_r & 0 \\ 0 & A_i(\kappa,\gamma) \end{bmatrix}$, $\bar{l}(x) = \begin{bmatrix} I & 0 & 0 \\ 0 & I & l(x) \end{bmatrix}$, $\bar{M}_j = \begin{bmatrix} 0 \\ M_j \end{bmatrix}$, $\bar{B}_{ij} = [\,0 \quad B_{ij}\,]$ and F_{ij} is the fuzzy set, $A_i(\kappa,\gamma)$ denotes a matrix with the components of kinetic parameters κ and decay rates γ in its elements, L is the number of *if–then* rules, n is the number of premise variables, and $x_1, ..., x_n$ are the premise variables.

Remark 4: 1) If $\bar{g}_j(\bar{x})$ in (14) is replaced by $\bar{g}_j(\bar{x},\ \kappa,\ \gamma)$, then $\bar{B}_{ij}$ in (23) should be replaced by $\bar{B}_{ij}(\kappa,\gamma)$ to contain some components of κ and γ in its elements. 2) The fuzzy approximation errors are also included into uncertain disturbances $\bar{v}$. Therefore, the effects of fuzzy approximation errors on the tracking errors could be attenuated by the minimum-error-tracking design of synthetic gene networks.

The fuzzy system can be inferred as follows [10], [11], [21]–[37]:

$$d\bar{x} = \frac{\sum_{i=1}^{L} \mu_i(x)\left(\left[\bar{A}_i(\kappa,\gamma)\bar{x} + \bar{l}(\bar{x})\bar{v}\right]dt + \sum_{j=1}^{m} \bar{M}_j\bar{B}_{ij}\bar{x}dW_j\right)}{\sum_{i=1}^{L}\mu_i(x)} = \sum_{i=1}^{L} h_i(x)\left([\bar{A}_i(\kappa,\gamma)\bar{x} + \bar{l}(\bar{x})\bar{v}]dt + \sum_{j=1}^{m}\bar{M}_j\bar{B}_{ij}\bar{x}dW_j\right) \tag{24}$$

where $\mu_i(x) = \prod_{j=1}^{n} F_{ij}(x_j(t))$, $h_i(x) = \mu_i(x) / \sum_{i=1}^{L}\mu_i(x)$, $x = [x_1, ..., x_n]^T$, and $F_{ij}(x_j)$ is the grade of the membership function of x_j in F_{ij}.

We assume [22]

$$\mu_i(x) \geq 0 \text{ and } \sum_{i=1}^{L}\mu_i(x) > 0\,\forall\, t \tag{25}$$

Therefore, we get the fuzzy bases as $h_i(x) \geq 0$ and $\sum_{i=1}^{L} h_i(x) = 1\ \forall\, t$.

The T–S fuzzy model in (24) is to interpolate L linear stochastic systems to approximate the nonlinear stochastic system in (14) via the fuzzy basis function $h_i(x)$. We can specify the matrices $\bar{A}_i(\kappa,\gamma)$ and $\bar{B}_{ij}, i = 1, ..., L$ so that $\sum_{i=1}^{L} h_i(x)\bar{A}_i(\kappa,\gamma)\bar{x}$ and $\sum_{i=1}^{L} h_i(x)\bar{B}_{ij}\bar{x}$ in (24) can approximate $\bar{f}(\bar{x},\kappa,\gamma)$ and $\bar{g}_j(\bar{x})$ in (14) by the fuzzy identification method [21], [22], respectively. After approximating the nonlinear stochastic system in (14) by using the T–S fuzzy system in (24), the nonlinear stochastic dynamic problem in (14) could be replaced by solving the fuzzy stochastic problem in (24) such that the stochastic-tracking design problem in (16) can be solved [38]. In the Proposition 1, based on the fuzzy approximation, if we choose the Lyapunov function $V(\bar{x}) = \bar{x}^T P\bar{x}$, where P is a positive-definite matrix, then we can get the following robust optimal reference-tracking design of synthetic gene networks by a fuzzy stochastic-tracking scheme.

Proposition 2: Based on the T–S fuzzy approximation, the suboptimal-tracking design in (16) for synthetic gene networks can be solved by the following constrained optimization:

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}] \\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} E\bar{x}^T(0)P\bar{x}(0) = \min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}] \\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} \mathrm{Tr}PR_0 \tag{26}$$

subject to

$$P>0$$
$$\begin{bmatrix} \bar{A}_i^T(\kappa,\gamma)P+P\bar{A}_i(\kappa,\gamma)+\bar{Q} & PS \\ +\sum_{j=1}^{m}\bar{B}_{ij}^T\bar{M}_j^TP\bar{M}_j\bar{B}_{ij} & \\ SP & -S \end{bmatrix}<0,\quad i=1,\ldots,L \tag{27}$$

where R_o denotes the covariance matrix $E\left[\bar{x}(0)\bar{x}^T(0)\right]$, $S=\begin{bmatrix} I & 0 \\ 0 & \left(c^2+1\right)I \end{bmatrix}$ with c defined in (8).

Proof: See Appendix B

In Proposition 2, the upper bound of tracking errors in (26) is a function of P, which is a positive-definite solution of LMIs in (27). Therefore, our robust optimal-tracking design is to specify κ and γ within allowable ranges such that the upper bound of tracking errors is as small as possible. ■

Remark 5:

1) The fuzzy basis function $h_i(x)$ in (24) can be replaced by the other interpolation functions, for example, the cubic spline function.
2) By fuzzy approximation, the HJI in (18) of the nonlinear stochastic-tracking design problem is replaced by a set of LMIs in (27), which can be more easily solved. Since these design parameters are constrained within the allowable ranges of kinetic parameters $\kappa\in[\underline{\kappa},\bar{\kappa}]$ and decay rates $\gamma\in[\underline{\gamma},\bar{\gamma}]$, there is no closed-form solution for the constrained optimization problem in (26) and (27). In this situation, the genetic algorithm should be combined with Robust Control Toolbox in MATLAB to solve this constrained optimization problem via convex optimization techniques [13].
3) In general, $S\geq I$ in (18) and (27). If the synthetic gene network is free of interactions with unknown molecules in cellular context, i.e., $l(x)=0$ or $c=0$, then $S=I$. Therefore, the synthetic gene network needs more effort to tolerate the uncertain interactions of unknown molecules to avoid their effects on the reference tracking.
4) If fuzzy approximation errors are considered in the design procedure [10], [11], some extra terms should be included in (27). However, since the main focus of this research is on the applications of the fuzzy theory to the robust-tracking design in the nascent field of synthetic gene networks, they are neglected in the design procedure. Further, the approximation errors can be merged into the external disturbance v and can be efficiently eliminated by the robust optimal-tracking design. As shown in the simulation example in the sequel, the effect of the approximation error can be efficiently attenuated by the proposed robust optimal-tracking design.

Remark 6:

In the genetic oscillator design case, the eigenvalues of A_r in reference model are always on the $j\omega$-axis to generate periodic signals, i.e., one half of eigenvalues of $\bar{A}_i$ are on the $j\omega$-axis (i.e., with zero real parts). In this situation, it is not easy to specify κ and γ to satisfy the LMIs in (27) and to solve the optimal-tracking design problems in (26) and (27). In order to overcome this difficulty, an eigenvalue-shifted technique is proposed as an expedient scheme to treat the oscillation-tracking design problem. Let us adjust the system variables in (24) by $\bar{x}_s(t)=e^{-\lambda t}\bar{x}(t)$ for a positive value λ, then $\bar{x}_s(t)$ can be obtained by the following eigenvalue-shifted system:

$$d\bar{x}_s=\sum_{i=1}^{L}h_i(x_s)\left[\left(\left[\bar{A}_i(\kappa,\gamma)-\lambda I\right]\bar{x}_s+\bar{l}(\bar{x}_s)\bar{v}_s\right)dt+\sum_{j=1}^{m}\bar{M}_j\bar{B}_{ij}\bar{x}_s dW_i\right] \tag{28}$$

where $\bar{v}_s=e^{-\lambda t}\bar{v}$.

For the eigenvalue-shifted system in (28), suppose we want to specify κ and γ to minimize the following tracking performance:

$$J(\kappa,\gamma)=E\int_0^{t_f}\bar{x}_s^T(t)\bar{Q}\bar{x}_s(t)dt \tag{29}$$

which is the same as (15), except that $\bar{x}(t)$ is replaced by $\bar{x}_s(t)$, i.e., we use the eigenvalue-shifted system (28) to replace (24) and use the tracking performance in (29) to replace the tracking performance in (15).

In this transformation case, the tracking design problem is relaxed how to specify κ and γ in (28) to minimize $J(\kappa,\gamma)$ in (29). By Proposition 2, the suboptimal-tracking design is modified as follows:

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}}E\left[\bar{x}_s^T(0)P\bar{x}_s(0)\right]=\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}}\mathrm{Tr}PR_0 \tag{30}$$

subject to

$$P>0$$
$$\begin{bmatrix} \left(\bar{A}_i(\kappa,\gamma)-\lambda I\right)^TP+P\left(\bar{A}_i(\kappa,\gamma)-\lambda I\right) & PS \\ +\bar{Q}+\sum_{j=1}^{m}\bar{B}_{ij}^T\bar{M}_j^TP\bar{M}_j\bar{B}_{ij} & \\ SP & -S \end{bmatrix}<0. \tag{31}$$

In the earlier expedient method, due to more negative eigenvalues of $\bar{A}_i(\kappa,\gamma)-\lambda I$ in (31), we have this more feasible way to solve the optimal design problem of tracking periodic reference signals. However, in order to avoid some distortions due to the signal transformation, λ should be selected as small as possible so that its influence on the tracking performance is as small as possible. In this situation, we could take $\lambda\in[0,\bar{\lambda}]$ as another performance to be minimized simultaneously and need to solve the following constrained optimization for the robust optimal-tracking design problem of the synthetic gene network:

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]\\ \lambda\in[0,\bar{\lambda}]}}\mathrm{Tr}PR_0+\lambda \tag{32}$$

subject to $P>0$, (31).

This eigenvalue-shifted technique will be confirmed and discussed in the design example in the sequel.

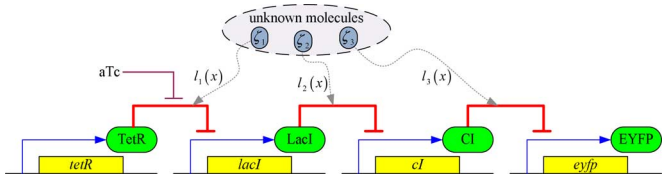

Fig. 2. Synthetic transcriptional cascade. TetR represses *lacI*, LacI represses *cI*, CI represses *eyfp*, and aTc activates the repression of *lacI* by TetR. The fluorescence of the protein EYFP is the output. In this cascade, there exist uncertain interactions with three unknown molecules $(\zeta_1, \zeta_2, \zeta_3)$ that intertwine with the synthetic gene network.

According to the aforementioned analysis, the robust optimal reference-tracking design procedure for synthetic gene networks under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external disturbances is summarized in the following.

Design procedure:

1) Construct the nonlinear stochastic dynamic model (7) for a synthetic gene network.
2) Provide the design specifications i)–iv).
3) Construct the T–S fuzzy model (24) to approximate nonlinear stochastic dynamic model (7).
4) Solve the constrained optimization problem in (26) and (27), or (30) and (31), or (32) for the design kinetic parameters κ and decay rates γ from the allowable parameter ranges.

Remark 7: The software package such as Robust Control Toolbox in MATLAB [14] can be employed to solve the LMI-constrained optimization problem in (26) and (27), or (30) and (31), or (32) easily.

V. Design Example *In Silico*

In this section, two examples are given to illustrate the design procedure and then to confirm the performance of the proposed robust optimal reference-tracking design method of synthetic gene networks. The first example is given to illustrate the robust optimal transient response tracking of a desired synthetic transcriptional cascade. The second example is a robust optimal-tracking design of a simple synthetic genetic oscillator with the desired amplitude and period.

Example 1: Consider synthesizing a cascade of transcriptional inhibitions built in *E. coli* [39]; a synthetic gene network is then represented in Fig. 2. It consists of four genes, i.e., *tetR*, *lacI*, *cI*, and *eyfp*, that code for the three repressor proteins TetR, LacI, CI, and the fluorescent protein EYFP, respectively [3]. In the synthetic transcriptional cascade, the protein TetR represses gene *lacI*, the protein LacI represses gene *cI*, and the protein CI represses gene *eyfp*. The fluorescence protein (i.e., protein EYFP) is the measured output. The system can be activated by the addition or removal of a small diffusible molecular aTc in the growth media. More precisely, aTc binds to TetR and relieves the repression of *lacI* [3]. The aTc concentration serves as an external input to the synthetic gene network. For the convenience of representation, let us denote $x = [x_{tetR},\ x_{lacI},\ x_{cI},\ x_{eyfp}]^T = [x_1,\ x_2,\ x_3,\ x_4]^T$ and the external input $u = u_{\mathrm{aTc}}$. The dynamic equations of the synthetic gene network in Fig. 2 are given as follows [1], [39]:

$$\begin{aligned}
\dot{x}_1 &= \kappa_{10} - \gamma_1 x_1 + v_1 \\
\dot{x}_2 &= \kappa_{20} + \kappa_2 (H_2(x_1) + H_a(u) \\
&\quad - H_2(x_1) H_a(u)) - \gamma_2 x_2 + v_2 \\
\dot{x}_3 &= \kappa_{30} + \kappa_3 H_3(x_2) - \gamma_3 x_3 + v_3 \\
\dot{x}_4 &= \kappa_{40} + \kappa_4 H_4(x_3) - \gamma_4 x_4 + v_4
\end{aligned} \tag{33}$$

where the vector $\kappa_0 = [\kappa_{10},\ \kappa_{20},\ \kappa_{30},\ \kappa_{40}]^T = [150, 587, 160, 3487]^T$ is a constant vector and denotes the basal levels of proteins, which are not easy to measure and can be considered as one kind of disturbances. $H_i(x)$, $i = 2, 3, 4$ are all Hill functions for repressors, which are decreasing S-shaped curves and can be described as the form $H_i(x) = \beta/(1 + (x/K_i)^n)$ with $\beta = 1$, $n = 2$, and $K_i = 2000$. $H_a(u)$ is a Hill function for activators (aTc is an activator), which is an increasing S-shaped curve and has the form $r(x) = x^n/(x^n + A^n)$ with $n = 2$ and $A = 2000$ [15], [16]. In summary, the term $H_2(x_1) + H_a(u) - H_2(x_1)H_a(u)$ is the regulation to model the logical ''OR'' function. The vector of $\kappa = [\kappa_2,\ \kappa_3,\ \kappa_4]^T$ and $\gamma = [\gamma_1,\ \gamma_2,\ \gamma_3,\ \gamma_4]^T$ contain the corresponding kinetic parameters and decay rates, respectively. The vector of $v = [v_1,\ v_2,\ v_3,\ v_4]^T$ denotes the environmental disturbances. Suppose we want the behaviors of the synthetic gene network to track the trajectories of a desired reference model by designing the kinetic parameter κ and the decay rate γ. $u = u_{\mathrm{aTc}}$, which is a constant, is the input to the synthetic gene network. We assume that the value of anhydrotetracycline input concentration is 10^4 (i.e., $u_{\mathrm{aTc}} = 10\,000$).

Suppose the synthetic gene network suffers from the stochastic parameter fluctuations with standard deviations and the bounded uncertain interactions $l(x)$ with unknown molecules $\zeta = [\zeta_1, \zeta_2, \zeta_3]^T$ in the host cell as follows:

$$\begin{aligned}
\Delta\kappa_0 &= (\Delta\kappa_{10}, \Delta\kappa_{20}, \Delta\kappa_{30}, \Delta\kappa_{40}) = (30, 50, 30, 50) \\
\Delta\kappa &= (\Delta\kappa_2, \Delta\kappa_3, \Delta\kappa_4) = (200, 50, 200) \\
\Delta\gamma &= (\Delta\gamma_1, \Delta\gamma_2, \Delta\gamma_3, \Delta\gamma_4) = (0.03, 0.03, 0.03, 0.03) \\
\|l(x)\| &\le 0.4.
\end{aligned} \tag{34}$$

Then, the synthetic gene network under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and extrinsic disturbances can be described in the host cell as follows:

$$dx = (f(x,\ \kappa,\ \gamma) + l(x)\zeta + v)dt + \sum_{j=1}^{4} M_j g_j(x,\ \kappa,\ \gamma) dW_j. \tag{35}$$

Suppose the biological allowable ranges of kinetic parameters and decay rates to be selected are given by [3]

$$\begin{aligned}
&\kappa_2 \in [70, 7000],\ \kappa_3 \in [75, 8000],\ \kappa_4 \in [30, 30\,000] \\
&\gamma_1 \in [0.1, 0.2],\ \gamma_2 \in [0.02,\ 0.07] \\
&\gamma_3 \in [0.8,\ 0.97],\ \gamma_4 \in [0.1,\ 0.95]
\end{aligned} \tag{36}$$

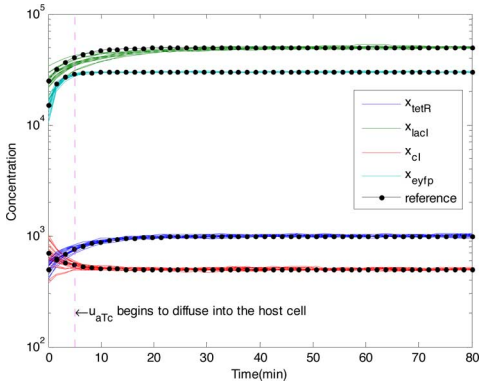

Fig. 3. In order to confirm the robust optimal reference tracking of the synthetic gene network in the *in silico* example, the synthetic gene network with the minimum error tracking is designed with optimal kinetic parameters $\kappa_o = (\kappa_2,\ \kappa_3,\ \kappa_4) = (3814.9, 75.4, 10910)$ and decay rates $\gamma_o = (\gamma_1,\ \gamma_2,\ \gamma_3,\ \gamma_4) = (0.15, 0.0734, 0.3202, 0.4585)$. The Monte Carlo simulation is given with 20 rounds and random initial conditions to demonstrate the robust ability of optimal reference tracking. It is obviously seen that the concentrations of the synthetic gene network can robustly track the trajectories of the reference model despite parameter fluctuations, uncertain interactions with unknown molecules, and environmental disturbances. While the external input u_{aTc} diffuses into the host cell, the system can track the reference system.

such that the synthetic gene network can optimally track the following reference model with desired behaviors:

$$dx_r = A_r x_r + r \tag{37}$$

where $A_r = \mathrm{diag}\left([-0.15, -0.2, -0.3, -0.5]\right)$ and $r = [150, 1000, 150, 15\,000]^T$. The reference model is set so that the synthetic gene network has desired transient behaviors and the desired steady states of x_r are with $[1000, 50\,000, 500, 30\,000]^T$ (see Fig. 3).

After developing the stochastic dynamic system in (35) for the synthetic gene network in Fig. 2, we need to design $\kappa = [\kappa_2,\ \kappa_3,\ \kappa_4]$ and $\gamma = [\gamma_1,\ \gamma_2,\ \gamma_3,\ \gamma_4]$ from the allowable ranges in (36) so that the states of the synthetic gene network can optimally track the desired states of the reference model in (37). Because it is difficult to solve the nonlinear HJI in (18), the T–S fuzzy model is developed to approximate the stochastic nonlinear dynamic system. For the convenience of design, the triangle-type membership functions are used for *Rules 1–5*, i.e., five triangle-type membership functions are employed for a nonlinear function to construct the T–S fuzzy model. The operating points for all states are all distributed from 0 to 10^5.

We have constructed the T–S fuzzy model to approximate the nonlinear stochastic dynamic model and to obtain the augmented system in (24). With the help of the fuzzy approximation method and the LMI technique, we can easily solve the constrained optimization problem in (26) and (27). Based on Proposition 2, by specifying design kinetic parameters κ and decay rates γ within the allowable ranges to solve the constrained optimization in (26), the minimum-tracking error can be achieved with the optimal design parameters $\kappa_o = (\kappa_2,\ \kappa_3,\ \kappa_4) = (3814.9, 75.4, 10\,910)$ and decay rates $\gamma_o = (\gamma_1,\ \gamma_2,\ \gamma_3,\ \gamma_4) = (0.15, 0.0734, 0.3202, 0.4585)$. The synthetic gene network is simulated with the designed parameter κ_o and decay rate γ_o accompanied by intrinsic parameter fluctuations and bounded uncertain interactions in (34). For the convenience of simulation, we consider the uncertain interactions

$$l(x) = \begin{bmatrix} 0 & 0 & 0 \\ 0.2\sin x_1 & 0 & 0 \\ 0 & 0.4\cos x_2 & 0 \\ 0 & 0 & 0.3\cos x_3 \end{bmatrix}$$

with unknown molecules $\zeta = [\zeta_1, \zeta_2, \zeta_3]^T$ and environmental disturbances $v(t) = [10n_1,\ 100n_2,\ 10n_3,\ 100n_4]^T$, where $\zeta_i, i = 1, 2, 3$ are unit-step functions, and n_i, $i = 1, \ldots, 4$ are independent Gaussian white noises with zero means and unit variances. In order to demonstrate the robust optimal reference tracking for the synthetic gene network, the example using Monte Carlo method with 20 rounds and with uncertain initial values is presented in Fig. 3. The initial values $x_1(0) - x_4(0)$ are assumed normal distributed random variables with means 500, 25 000, 700, and 15 000 and standard deviations 100, 5000, 140, and 3000, respectively. We obviously find that the concentrations of the synthetic gene network can track the trajectories of the reference system in spite of uncertain initial values, intrinsic parameter fluctuations, uncertain interactions, and external disturbances. From the simulation result, it is seen that the designed synthetic gene network by the proposed method has robust optimal reference-tracking ability and noise-filtering ability to tolerate intrinsic parameter fluctuations and to attenuate the external disturbances, respectively. Therefore, the robust optimal reference-tracking design for synthetic gene networks is an efficient method to properly engineer system parameters of synthetic gene networks in the host cell, in spite of intrinsic uncertainties and extrinsic disturbances.

Example 2: Suppose we want to synthesize a gene network with negative-feedback loop shown in Fig. 4 [40], [41]. The repressor protein LacI inhibits the transcription of the repressor gene *tetR*, whose protein product in turn inhibits the expression of the repressor gene *cI*. Finally, CI, the protein product of repressor gene *cI*, inhibits *lacI* 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 concentrations of proteins TetR, CI, and LacI, for convenience, are denoted by x_1, x_2, and x_3, respectively. The dynamic equations for the synthetic network are given as follows:

$$\begin{aligned} \dot{x}_1 &= \kappa_1 H(x_3) - \gamma_1 x_1 + v_1 \\ \dot{x}_2 &= \kappa_2 H(x_1) - \gamma_2 x_2 + v_2 \\ \dot{x}_3 &= \kappa_3 H(x_2) - \gamma_3 x_3 + v_3 \end{aligned} \tag{38}$$

where the vectors $\kappa = [\kappa_1,\ \kappa_2,\ \kappa_3]^T$, and $\gamma = [\gamma_1,\ \gamma_2,\ \gamma_3]^T$ contain the corresponding kinetic parameters and decay rates, respectively. The vector $v = [v_1,\ v_2,\ v_3]^T$ denotes the environmental disturbances on the host cell due to changing extracellular environments, interactions with the cellular context, etc. $H(\cdot)$ is a Hill function for repressors, which is characterized by decreasing S-shaped curves and can be described as the form $H(x) = \beta/(1 + (x/K))^n$ with $\beta = 1$, $n = 4$, and $K = 200$.

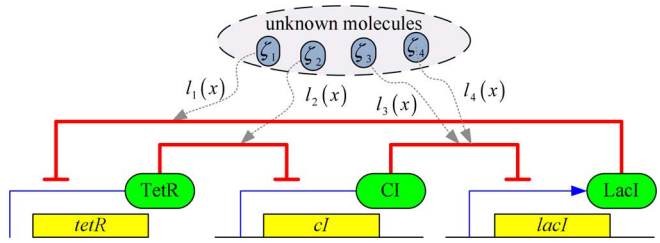

Fig. 4. Synthetic transcriptional cascade. TetR represses *lacI*, LacI represses *cI*, and CI represses *tetR*. They can build an oscillatory network. In this cascade, there exist uncertain interactions with unknown molecules $(\zeta_1, \zeta_2, \zeta_3, \zeta_4)$ that intertwine with the synthetic gene network.

Suppose the kinetic parameters κ and decay rates γ suffer from the parameter fluctuations with standard deviations and bounded uncertain interactions $l(x)$ in the host cell as follows:

$$\begin{aligned} \Delta\kappa &= (\Delta\kappa_1, \Delta\kappa_2, \Delta\kappa_3) = (10, 10, 10) \\ \Delta\gamma &= (\Delta\gamma_1, \Delta\gamma_2, \Delta\gamma_3) = (0.05, 0.05, 0.05) \\ \|l(x)\| &\leq 0.5. \end{aligned} \tag{39}$$

Then, the synthetic gene network under intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and environmental disturbances can be described in host cell as follows:

$$dx = [f(x, \kappa, \gamma) + l(x)\zeta + v]\,dt + \sum_{j=1}^{3} M_j g_j(x, \kappa, \gamma) dW_j. \tag{40}$$

The biological allowable ranges of kinetic parameters and decay rates of the synthetic gene network are given by

$$\begin{aligned} &\kappa_1 \in [100, 500], \kappa_2 \in [100, 500], \kappa_3 \in [100, 500] \\ &\gamma_1 \in [0.1, 0.9], \gamma_2 \in [0.1, 0.9], \gamma_3 \in [0.1, 0.9]. \end{aligned} \tag{41}$$

The purpose is to design a robust synthetic gene network, whose behaviors can track the following reference oscillation system with the desired amplitude and period, as shown in Fig. 5:

$$\begin{bmatrix} \dot{x}_{r1} \\ \dot{x}_{r2} \\ \dot{x}_{r3} \end{bmatrix} = \begin{bmatrix} 0 & 0.42339 & -0.48853 \\ -0.7873 & 0 & 0.66617 \\ 0.68232 & -0.50037 & 0 \end{bmatrix} \begin{bmatrix} x_{r1} \\ x_{r2} \\ x_{r3} \end{bmatrix} + \begin{bmatrix} 18.89 \\ 27.253 \\ -45.943 \end{bmatrix} \tag{42}$$

or $\dot{x}_r = A_r x_r + r$.

Since the eigenvalues of A_r are all on the $j\omega$-axis, the expedient design in (30) and (31) in Remark 5 must be employed to treat the oscillatory gene network design problem. First of all, we approximate the stochastic nonlinear system in (40) using the T–S fuzzy model approach. For the convenience of design, ten triangle-type membership functions are taken for Rules 1–10 and the operating points for all states are all distributed from 0 to 1000. The augmented system combined the T–S fuzzy model to approximate the synthetic gene network in (40) with the reference model in (42) is obtained as follows:

$$d\bar{x} = \sum_{i=1}^{10} h_i(\bar{x}) \left[\left(\bar{A}_i(\kappa, \gamma)\bar{x} + \bar{l}(\bar{x})\bar{v} \right) dt + \sum_{j=1}^{3} \bar{M}_j \bar{B}_{ij} \bar{x} dW_j \right] \tag{43}$$

where $\bar{A}_i(\kappa, \gamma)$ and $\bar{B}_{ij}$ are obtained by fuzzy approximating method.

Because one half of eigenvalues of the augmented system in (43) are on the $j\omega$-axis, the eigenvalue-shifted technique in Remark 6 can be employed to treat the robust oscillation-tracking design problem. The constrained optimization in (32) is employed to solve the optimal-tracking design problem with the allowable ranges of κ and γ in (41) and $\lambda \in [0, 0.1]$.

With the help of LMI technique, the constrained optimization problem in (32) can be solved easily. The simulation using Monte Carlo method with 20 rounds and with uncertain initial values is shown in Fig. 5, where the initial values $x_1(0) \sim x_3(0)$ are assumed normal distributed random variables with means 280, 180, and 185 and standard deviations 28, 18, and 18.5, respectively. Moreover, a common positive-definite symmetric matrix P for solving (30) and (31) is obtained as shown at the bottom of the next page, with the optimal kinetic parameters $\kappa_o = (\kappa_1, \kappa_2, \kappa_3) = (234.67, 439.38, 218.97)$, decay rates $\gamma_o = (\gamma_1, \gamma_2, \gamma_3) = (0.44452, 0.88719, 0.47455)$, and $\lambda_o = 0.088588$. Fig. 5 demonstrates the robust optimal-tracking result for synthetic gene network by the designed kinetic parameter κ_o and decay rate γ_o with intrinsic parameter

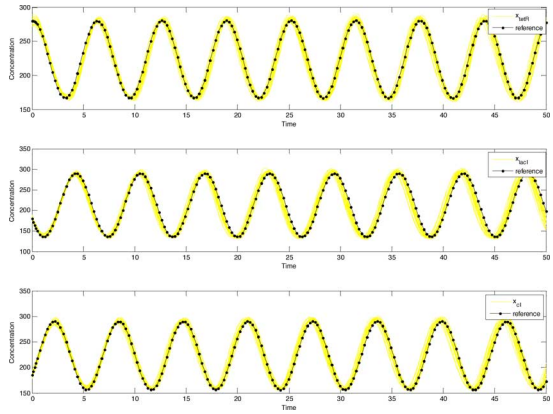

Fig. 5. Synthetic gene network is constructed by the minimum error tracking with optimal kinetic parameters $\kappa_o = (\kappa_1, \kappa_2, \kappa_3) = (234.67, 439.38, 218.97)$, decay rates $\gamma_o = (\gamma_1, \gamma_2, \gamma_3) = (0.44452, 0.88719, 0.47455)$, and $\lambda_o = 0.088588$ to meet the desired behaviors of reference model. The robust optimal-tracking performance of synthetic gene oscillator is confirmed with parameter fluctuations with standard deviations as $(\Delta\kappa_1, \Delta\kappa_2, \Delta\kappa_3) = (10, 10, 10)$, $(\Delta\gamma_1, \Delta\gamma_2, \Delta\gamma_3) = (0.05, 0.05, 0.05)$, uncertain interactions with unknown molecules $l(x)\zeta = (0.5\sin x_2\zeta_1, 0.1\cos x_1\zeta_2, 0.2\sin x_3\zeta_3 + 0.3\sin x_2\zeta_4)^T$, and environmental disturbances $v = (n_1, n_2, n_3)^T$, where $\zeta = [\zeta_1, \zeta_2, \zeta_3, \zeta_4]^T$ are unit-step functions, and n_i are standard zero-mean white noises. The Monte Carlo simulation is given with 20 rounds and random initial conditions to demonstrate the robust ability of optimal reference tracking. Unlike the Example 1, the asymptotical tracking cannot be achieved due to changing reference signals. As shown in the figures, the concentrations of the synthetic gene oscillators in the host cell can track the behaviors of the reference model in spite of intrinsic uncertainties, uncertain interactions, and extrinsic disturbances.

fluctuations in (39). For the convenience of simulation, we assume the uncertain interactions

$$l(x) = \begin{bmatrix} 0.5\sin x_2 & 0 & 0 & 0 \\ 0 & 0.1\cos x_1 & 0 & 0 \\ 0 & 0 & 0.2\sin x_3 & 0.3\sin x_2 \end{bmatrix}$$

with unknown molecules $\zeta = [\zeta_1, \zeta_2, \zeta_3, \zeta_4]^T$ and environmental disturbances $v(t) = [n_1,\ n_2,\ n_3]^T$, where ζ_i, $i = 1, 2, 3, 4$ are unit-step functions, and $n_i, i = 1, 2, 3$ are independent Gaussian white noises with zero means and unit variances.

From the simulation result shown in Fig. 5, we can observe that the designed synthetic gene network by the proposed method has robust optimal reference-tracking ability and noise-filtering ability to tolerate intrinsic parameter fluctuations and to attenuate the uncertain interactions and external disturbances, respectively. However, due to changing reference signal, the asymptotical tracking cannot be achieved as Example 1. It is observed that different initial values will cause different phases, but the period is the same, despite intrinsic noises and extrinsic disturbances. Therefore, the eigenvalue-shifted method is an efficient method to properly engineer system parameters of robust synthetic gene oscillators in the host cell, in spite of intrinsic uncertainties and extrinsic disturbances.

VI. Discussion

Unlike the conventional regulation design of synthetic gene networks to a desired steady state, the proposed robust optimal-tracking design could make synthetic gene network tracking a desired behavior like an oscillation or the other transient behavior. In these robust gene network design examples, the kinetic parameters κ and decay rates γ can be designed within the allowable ranges in (9) to satisfy the four design specifications to achieve the robust optimal tracking *in vivo*. As for the biological implementation, we could refer to standard biological parts in biological device datasheets to construct genetic circuits with fine-tuned parameters κ_i and decay rates γ_i. This way, synthetic biologists can increase efficiency of gene circuit design through registries of biological parts and standard datasheets, which are developed with proper packing and characterizing of ''modular'' biological activities so that these biological parts or devices with some desired characteristics may be efficiently assembled into the gene circuit [42].

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 the device might be used to meet the requirements of a system [42]. Such a determination is based on a set of standard characteristics of the device behavior, which are the product of engineering theory and experience. In the datasheets, the characteristics typically reported are common across a wide range of device types, such as sensors, logic elements, and actuators [43].

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 scheme [42], [43]. Because datasheets of biological parts or devices are an embodiment of engineering standard for synthetic biology, a good device standard should define sufficient information about biological parts or devices to allow the design of gene circuit systems with optimal parameters. Datasheets have contained a formal set of input–output transfer functions, dynamic behaviors, compatibility, requirements, and other details about a particular part or device [42], [43]. Since the kinetic parameters κ_i are combinations of transcription and translation rate, they could be measured from the input–output transfer functions and the dynamic behaviors of biological parts or devices in biological device datasheets. From properly characterized input–output transfer functions and dynamic behaviors of parts or devices in biological device datasheets, an engineer can estimate the corresponding parameters of biological parts or devices. When the biological parts and devices in datasheets become more complete in the future, we can rapidly select from a vast list the parts that will meet the design kinetic parameters κ_i. Therefore, we can ensure that devices selected from datasheets can fit the optimal parameters and systems synthesized from them can satisfy the requirements of design parameters for the robust optimal-tracking gene networks.

Recently, the directed evolution methods are also used to change the elasticity (kinetic property of κ_i) and will be useful techniques for biochemical circuit design [44]–[46]. The advances of implementation techniques of kinetic parameter κ_i and decay rate γ_i have made an engineering of synthetic gene networks possible in the near future [47].

Because the kinetic parameters κ_i are a combination of transcription rate, mRNA decay rate, and translation rate, there are some variations or uncertainties on the kinetic parameters κ_i. By the same way, the decay rates γ_i are full of some variations or uncertainties when the biologists rely on degradation tags. These variations or uncertainties of the kinetic parameters and decay rates are transformed to state-dependent noise terms $\sum_{i=1}^{m} M_i g(x)\, dW_i$ in (7). In our design method, two statistics of disturbances $v(t)$ can be unknown or uncertain. If the standard deviations of the kinetic parameters and decay rates to be tolerated are given in M_i, then the proposed robust synthetic genetic network not only can optimally achieve the desired

$$P = 1000 \times \begin{bmatrix} 15.819023 & -0.291797 & 0.20718 & -15.819023 & 0.291797 & -0.20718 \\ -0.291797 & 8.627381 & 0.104891 & 0.291797 & -8.627381 & -0.104891 \\ 0.20718 & 0.104891 & 11.565062 & -0.20718 & -0.104891 & -11.565062 \\ -15.819023 & 0.291797 & -0.20718 & 288.210581 & 8.88916 & -70.197667 \\ 0.291797 & -8.627381 & -0.104891 & 8.88916 & 155.948231 & -31.430748 \\ -0.20718 & -0.104891 & -11.565062 & -70.197667 & -31.430748 & 301.139633 \end{bmatrix}$$

trajectories of a reference model but can also tolerate the parameter fluctuations, uncertain interactions with unknown molecules, and environmental disturbances. How to select the proper kinetic parameters and decay rates in synthetic gene networks to satisfy the four design specifications is important for the robust optimal reference tracking of the desired behaviors. Specification iii) delivers the desired behaviors given in (10) to be tracked by synthetic gene networks. Nevertheless, how to select kinetic parameter κ_i and decay rate γ_i to achieve the reference model tracking in a realistic gene network is due to nature selection in the evolutionary response to the challenge of surviving in a changing environment [48]. However, the kinetic parameters κ_i and decay rates γ_i of the corresponding proteins for synthetic gene networks are selected by designers to tolerate parameter fluctuations, uncertain interactions with molecules, and environmental disturbances on the host cell. However, these parameters are hardly to keep invariant *in vivo* and environmental disturbances always exist on the host cell. Therefore, to remedy these uncertainties and disturbances on the host cell, specifications ii) and iv) deliver the standard deviations of parameter fluctuations to be tolerated and the optimal error tracking to the guaranty of robust tracking of synthetic gene networks. By using the earlier design specifications to achieve the robust optimal-tracking design purpose, biologists can select suitable kinetic parameters and decay rates to design a robust optimal reference tracking of synthetic gene networks to meet these design specifications. In the IC industry, due to high complexity and difficulty system design companies (like Intel) and system implementation companies [like Taiwan Semiconductor Manufacturing Company (TSMC)] perform very large scale integration products by division of labor. In the future, system designers should cooperate with implementation companies to produce complex synthetic gene networks. If this is the case, the development of systematic design tools is an important topic for synthetic gene networks. Therefore, the proposed robust optimal reference-tracking design method has potential applications to the synthetic gene network design in the near future.

Because of intrinsic perturbations and extrinsic disturbances on the host cell, the synthetic gene network engineered so far in bacteria to behave in a particular way seems decay rapidly in its behavior after a short time period [41], [49]. Therefore, how to develop a robust-tracking design scheme is an important topic for synthetic gene networks to work with desired reference behaviors in spite of intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and external environmental disturbances on the host cell. The contributions of this research are listed as follows: 1) We propose four design specifications for engineering synthetic gene networks to guarantee the robust optimal reference-tracking design purpose. 2) The T–S fuzzy model and the LMI technique are developed to simplify the design procedure of the robust optimal tracking of synthetic gene networks via the help of LMI toolbox in MATLAB. 3) In order to avoid the design difficulty of solving LMIs due to tracking desired oscillation systems, whose eigenvalues are all on $j\omega-$axis, an expedient eigenvalues-shifted method is also proposed to improve the design procedure for synthetic genetic oscillators. Two *in silico* examples are provided to illustrate the design procedure, in which the four design specifications are guaranteed for robust optimal-tracking design of synthetic gene networks. These design results can be confirmed through Monte Carlo simulation and shown in Figs. 3 and 5.

VII. Conclusion

We have presented a stochastic model to mimic the dynamical properties of synthetic gene networks in the host cell with parameter uncertainties, uncertain interactions with unknown molecules, and external disturbances. Then, four design specifications are introduced to guarantee that synthetic gene networks could optimally track the reference behaviors under intrinsic parameter fluctuations, uncertain interactions with unknown molecules in the cellular context, and external disturbances on the host cell from the robust optimal-tracking points of view. Finally, based on a nonlinear stochastic system, a systematic design method is proposed for synthetic gene networks to meet these design specifications to achieve robust optimal tracking of the desired reference behaviors in spite of intrinsic parameter fluctuations, uncertain interactions with unknown molecules, and extrinsic disturbances. To avoid solving nonlinear stochastic optimal reference-tracking design problem of robust synthetic gene networks directly, a fuzzy design technique is employed to approximate a nonlinear stochastic gene network to simplify the design procedure. Then, the robust optimal reference-tracking synthetic gene network design problem could be solved efficiently by a LMIs technique via LMI toolbox in MATLAB. The proposed design procedure can guarantee the synthetic gene network to satisfy the four design specifications so that the engineered gene network can achieve the robust optimal reference tracking in spite of intrinsic uncertainties and extrinsic disturbances on the host cell. An eigenvalue-shifted design method is also proposed as an expedient scheme to improve the optimal-tracking design procedure of synthetic gene oscillators. Therefore, the proposed systematic design method for the robust optimal reference tracking has much potential to robust-tracking design in the nascent field of synthetic gene networks in the future.

Appendix A
Proof of Proposition 1

Let us denote a Lyapunov energy function $V(\bar{x}) > 0$ for $\bar{x} \neq 0$ with $V(0) = 0$; then, (16) is equivalent to the following problem:

$$\begin{aligned} J(\kappa,\gamma) &= EV(\bar{x}(0)) - EV(\bar{x}(t_f)) \\ &\quad + E\int_0^{t_f}\left(\bar{x}^T\bar{Q}\bar{x} + \frac{dV(\bar{x})}{dt}\right)dt \\ &= EV(\bar{x}(0)) - EV(\bar{x}(t_f)) + E\int_0^{t_f}\left(\bar{x}^T\bar{Q}\bar{x}\right. \\ &\quad + \left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T \bar{f}(\bar{x},\kappa,\gamma) + \left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T \bar{l}(\bar{x})\bar{v} \\ &\quad \left. + \frac{1}{2}\sum_{j=1}^{m}\bar{g}_j^T(\bar{x})\bar{M}_j^T\frac{\partial^2 V(\bar{x})}{\partial \bar{x}^2}\bar{M}_j\bar{g}_j(\bar{x})\right)dt. \end{aligned}$$

The aforementioned equality is obtained by the Ito formula [8], [9].

By the fact $a^T b + b^T a \le \frac{1}{2}a^T a + \frac{1}{2}b^T b$ for any vector a and b, and $\bar{l}(\bar{x})\bar{l}(\bar{x})^T \le S$ [13], we obtain

$$\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T \bar{l}\,(\bar{x})\,\bar{v} \le \frac{1}{4}\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T \bar{l}\,(\bar{x})\,\bar{l}^T\,(\bar{x})\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right) + \bar{v}^T\bar{v} \le \frac{1}{4}\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T S\left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right) + \bar{v}^T\bar{v}$$

and then

$$J(\kappa,\gamma) \le EV(\bar{x}(0)) - EV(\bar{x}(t_f)) + E\int_0^{t_f}\left[\bar{x}^T\bar{Q}\bar{x} + \left(\frac{\partial V(\bar{x})}{\partial \bar{x}}\right)^T \bar{f}(\bar{x},\kappa,\gamma) + \frac{1}{4}\left(\frac{\partial V\,(\bar{x})}{\partial \bar{x}}\right)^T S\left(\frac{\partial V\,(\bar{x})}{\partial \bar{x}}\right) + \bar{v}^T\bar{v} + \frac{1}{2}\sum_{j=1}^{m} g_j^T(\bar{x})\bar{M}_j^T\frac{\partial^2 V(\bar{x})}{\partial\,\bar{x}^2}\bar{M}_j\,g_j(\bar{x})\right]dt.$$

By the inequalities in (18), we get

$$J\,(\kappa,\gamma) \le EV(\bar{x}(0)) - EV(\bar{x}(t_f)) + E\int_0^{t_f}\bar{v}^T\bar{v}dt \le EV\,(\bar{x}\,(0)) + E\int_0^{t_f}\bar{v}^T\bar{v}dt.$$

In other words, under the inequality constraint in (18), $EV(\bar{x}(0)) + E\int_0^{t_f}\bar{v}^T\bar{v}dt$ is the upper bound of $J(\kappa,\gamma)$. Then, the suboptimal-tracking design problem becomes how to minimize its upper bound subject to (18), i.e.,

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} J\,(\kappa,\gamma) \le \min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} EV\,(\bar{x}\,(0)) + E\int_0^{t_f}\bar{v}^T\bar{v}dt$$

subject to (18).

Since $\bar{v}$ is independent on the choice of the kinetic parameters κ and decay rates γ, the suboptimal-tracking problem becomes how to solve the following constrained optimization problem:

$$\min EV\,(\bar{x}\,(0))$$

subject to (18).

Appendix B

Proof of Proposition 2

As in the proof of Proposition 1, we choose a Lyapunov $V(\bar{x}) = \bar{x}^T P\bar{x}$ for a positive symmetric matrix $P > 0$

$$J(\kappa,\gamma) = E\int_0^{t_f}\bar{x}^T\bar{Q}\bar{x}dt = E\left\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(t_f)P\bar{x}(t_f) + \int_0^{t_f}\left(\bar{x}^T\bar{Q}\bar{x} + \frac{d\bar{x}^T P\bar{x}}{dt}\right)\right\}dt. \tag{B1}$$

By the Ito formula [50] and $E(dW_i/dt) = 0$, we have

$$\begin{aligned} J(\kappa,\gamma) &= E\left\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(t_f)P\bar{x}(t_f) + \int_0^{t_f}\sum_{i=1}^{L}h_i(x)\right.\\ &\quad\times\left[\bar{x}^T\bar{Q}\bar{x} + \left(\bar{A}_i(\kappa,\gamma)\bar{x} + \bar{l}(\bar{x})\bar{v}\right)^T P\bar{x}\right.\\ &\quad+\bar{x}^T P\left(\bar{A}_i(\kappa,\gamma)\bar{x} + \bar{l}(\bar{x})\bar{v}\right)\\ &\quad\left.\left.+\sum_{j=1}^{m}\bar{x}^T\bar{B}_{ij}^T\bar{M}_j^T P\bar{M}_j\,\bar{B}_{ij}\,\bar{x}\right]dt\right\}\\ &= E\left\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(t_f)P\bar{x}(t_f)\right.\\ &\quad+\int_0^{t_f}\sum_{i=1}^{L}h_i(x)\left[\bar{x}^T\bar{Q}\bar{x} + \bar{x}^T\left(\bar{A}_i^T(\kappa,\gamma)P + P\bar{A}_i(\kappa,\gamma)\right.\right.\\ &\quad+\bar{Q})\bar{x} + \bar{v}^T\bar{l}^T(\bar{x})P\bar{x} + \bar{x}^T P\bar{l}(\bar{x})\bar{v}\\ &\quad\left.\left.+\sum_{j=1}^{m}\bar{x}^T\bar{B}_{ij}^T\bar{M}_j^T P\bar{M}_j\,\bar{B}_{ij}\,\bar{x}\right]dt\right\}. \end{aligned} \tag{B2}$$

By the fact

$$\bar{x}^T P\bar{l}(\bar{x})\bar{v} + \bar{v}^T\bar{l}^T(\bar{x})P\bar{x} \le \bar{x}^T P\bar{l}(\bar{x})\bar{l}^T(\bar{x})P\bar{x} + \bar{v}^T\bar{v} \le \bar{x}^T PSP\bar{x} + \bar{v}^T\bar{v}$$

[13], then

$$J(\kappa,\gamma) \le E\left\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(t_f)P\bar{x}(t_f) + \int_0^{t_f}\sum_{i=1}^{L}h_i(x)\left[\bar{x}^T\left(\bar{A}_i^T(\kappa,\gamma)P + P\bar{A}_i(\kappa,\gamma) + \bar{Q} + PSP + \sum_{j=1}^{m}\bar{B}_{ij}^T\bar{M}_j^T P\bar{M}_j\,\bar{B}_{ij}\right)\bar{x} + \bar{v}^T\bar{v}\right]dt\right\}.$$

If the inequalities

$$\bar{A}_i^T(\kappa,\gamma)P + P\bar{A}_i(\kappa,\gamma) + \bar{Q} + PSP + \sum_{j=1}^{m}\bar{B}_{ij}^T\bar{M}_j^T P\bar{M}_j\,\bar{B}_{ij} < 0 \tag{B3}$$

hold, then we obtain

$$J(\kappa,\gamma) \le E\left\{\bar{x}^T(0)P\bar{x}(0) - \bar{x}^T(t_f)P\bar{x}(t_f) + \int_0^{t_f}\bar{v}^T\bar{v}dt\right\} \le E\left\{\bar{x}^T(0)P\bar{x}(0) + \int_0^{t_f}\bar{v}^T\bar{v}dt\right\}$$

i.e., if the inequalities in (B3) hold, then $E\{\bar{x}^T(0)P\bar{x}(0) + \int_0^{t_f}\bar{v}^T\bar{v}dt\}$ is the upper bound of $J(\kappa,\gamma)$.

Since $\bar{v}$ is independent on the choice of kinetic parameters κ and decay rates γ, the suboptimal-tracking problem is equivalent

to

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} E\left[\bar{x}^T(0)P\bar{x}(0)\right] = \min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} \mathrm{Tr}PR_0$$

subject to (B3), where $R_0 = E\left[\bar{x}(0)\bar{x}^T(0)\right]$.

By the Schur complement [13], the inequalities in (B3) are equivalent to the following LMIs:

$$\begin{bmatrix} \bar{A}_i^T(\kappa,\gamma)P + P\bar{A}_i(\kappa,\gamma) + \bar{Q} + \sum_{j=1}^{m}\bar{B}_{ij}^T\bar{M}_j^T P\bar{M}_j\bar{B}_{ij} & PS \\ SP & -S \end{bmatrix} < 0, \quad i = 1,\ldots,L \tag{B4}$$

Therefore, the suboptimal tracking is equivalent to solving the following constrained optimization-tracking problem:

$$\min_{\substack{\kappa\in[\underline{\kappa},\bar{\kappa}]\\ \gamma\in[\underline{\gamma},\bar{\gamma}]}} \mathrm{Tr}PR_0$$

subject to $P > 0$ and LMIs in (B4).

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