Parameter Estimation, Analysis, and Design of Synthetic Gene Switching Models: System Behavior- and Performance-based Approaches

Kwang-Ki K. Kim**, Kim Seng Cheong***, Kejia Chen*
Richard D. Braatz**

* University of Illinois at Urbana-Champaign, Urbana, IL, USA
** Massachusetts Institute of Technology, Cambridge, MA, USA
*** National University of Singapore

Abstract: This paper studies the parameter-dependent characteristics of a gene switching model that consists of dual positive feedback loops. Developing a predictive mathematical model for large regulatory networks in biological systems would be useful for their analysis and design. A parameter estimation scheme is proposed based on the observation of the steady-states for a gene switching model. Deterministic and stochastic stability are studied for this model, as well as other important system behaviors such as convergence rate to a stable equilibrium point, hysteresis induced by two time scales of the system model, and noise sensitivity with respect to the system parameters. Sensitivity of system performance indices with respect to the system parameters are analyzed in terms of $\mathcal{H}_\infty$ - and $\mathcal{H}_2$-norms of the linearized system model with their closed-form solutions. The presented qualitative and quantitative studies of the system characteristics enable the synthesis of a robust gene regulatory network that achieves desired static and dynamic responses.

Keywords: Process Applications; Modeling and Identification; Sensitivity Analysis.

1. INTRODUCTION

The development of predictive mathematical models of regulatory networks in living cells could revolutionize the study of complex diseases. Progress in the development of such mathematical models has been limited by both the incompleteness of the experimental data and the high complexity of the networks. This has motivated many researchers to take a ‘coarse-grained’ approach, in which the mathematical models are developed to describe the dynamical systems behavior without attempting to describe all of the molecular details [Boruhnoldt, 2005, Brandman et al., 2005]. This approach has been applied to gain insights into regulatory and signaling behavior for a wide variety of biological regulatory networks including those associated with mammalian circadian rhythm [Locke et al., 2008], the yeast cell-cycle network [Li et al., 2004], folding pathways in yeast [Hildebrandt et al., 2008], macrophages [Nykter et al., 2008], neutrophils [Brandman and Meyer, 2008], and somitogenesis oscillation in zebrafish [Horiakawa et al., 2006, Lewis, 2003].

Much of the research efforts have been in the identification of functional motifs that are connected to form larger regulatory networks [Brandman et al., 2005, Brandman and Meyer, 2008, Barabasi and Oltvai, 2004, Lee et al., 2002, Milo et al., 2002]. These motifs include switches, oscillators, biphasic amplitude filters, bandpass frequency filters, memory, noise filters, and noise amplifiers [Wolf and Arkin, 2003]. Motifs are especially relevant in synthetic biology, as well-characterized motifs can serve as a basis for the design of a synthetic genetic network to produce a desired dynamic behavior [McDaniel and Weiss, 2005]. Successful implementations of this motif-based approach is the design and construction of synthetic gene regulatory networks for toggle switches [Gardner et al., 2000, Atkinson et al., 2003], and oscillators with tunable parameters [Stricker et al., 2008, Elowitz and Leibler, 2000, Fung et al., 2005]. By coupling to cell-cell communication, such motifs have been used to implement built-in regulation of the cell population density in response to changes in the environment [You et al., 2004].

Several researchers have characterized the dynamics of certain classes of these motifs [Savageau, 2001], to assist synthetic biologists in their selection and the design of regulatory pathways. Quantitative relationships between the model parameters within a mathematical model of a motif and the dynamics can facilitate both the selection of parameters to obtain a desired regulatory network behavior, as well as the identification of parameters from experimental data where the mathematical model of a motif is used a coarse-grained model of a more complex regulatory network.

This paper characterizes the dynamics of a motif consisting of interlinked fast and slow positive feedback loops, which regulate polarization of budding yeast, calcium signaling, Xenopus oocyte maturation, and other processes [Brandman et al., 2005]. Interest in this motif as a component in synthetic genetic networks is that it provides a dual-time switch that can be rapidly and reliably induced while being relatively insensitive to noise in the stimulus [Brandman et al., 2005]. The derived analytical expressions enable the estimation of the motif model parameters from experimental data and the control-theoretic point of view on the gene switching model provides information that can be used to design robust biological circuit design of gene switches.
perform programmed desired behaviors in the presence of intrinsic and extrinsic perturbations.¹

2. MOTIF CHARACTERIZATION

This section characterizes the dynamics of the motif, including the relationship between the system model parameters and the output. The goal is to understand the general parameter-dependent characteristics of the dynamics of the motif.

2.1 Mathematical Models

Consider the mathematical model for the dual-positive feedback loops motif:

\[
\begin{align*}
\frac{dA}{dt} &= \tau_a \left( k_{\text{min}} - A + \frac{O^n}{O^n + \epsilon c_{50}} (1 - A) S \right), \\
\frac{dB}{dt} &= \tau_b \left( k_{\text{min}} - B + \frac{O^n}{O^n + \epsilon c_{50}} (1 - B) S \right), \\
\frac{dO}{dt} &= k_{\text{on}} (A + B)(1 - O) - k_{\text{off}} O + k_{\text{out}},
\end{align*}
\]

which has been normalized and nondimensionalized. The variables \( O, A, \) and \( B \) are concentrations between 0 and 1. \( O \) is activated by \( A \) and \( B \), with a nonlinear Hill function \( h(O) \triangleq \frac{O^n}{O^n + \epsilon c_{50}} \) that characterizes the relationship between the concentration of \( O \) and the rate of production of \( A \) and \( B \). The Hill coefficient is \( n \) and the concentration for half-maximum response for the feedback is \( \epsilon c_{50} \). The mutual activation \( O \) shared with \( A \) and \( B \) form the dual positive feedback loops. \( A \) and \( B \) are also activated by an external stimulus \( S \). The motif dynamics when an inner loop is suppressed is also of interest:

\[
\begin{align*}
\frac{dA}{dt} &= \tau_a \left( k_{\text{min}} - A + \frac{O^n}{O^n + \epsilon c_{50}} (1 - A) S \right), \\
\frac{dO}{dt} &= k_{\text{on}} A (1 - O) - k_{\text{off}} O + k_{\text{out}}.
\end{align*}
\]

where \( A \) represents either the faster or slower loop.

3. SYSTEM STABILITY ANALYSIS

3.1 Deterministic Stability

First consider the deterministic stability of the gene switching model (1) for which any intrinsic or extrinsic biochemical noise is ignored so that there is no source of stochasticity. Suppose that all the system parameters are positive. Stability analysis is straightforward for the switch-off case \((S = 0)\). The inner-loop dynamics for \( A \) and \( B \) are linear and globally exponentially stable, while the feedback from the output \( O \) to the inner-loop states \( A \) and \( B \). We also have \( dO/dt \leq -c O + d \) for some constants \( c, d > 0 \) satisfying \( d/c < 0 \), provided that the initial values of \( A \) and \( B \) are nonnegative. For any initial condition that satisfies \((A(0), B(0), O(0)) \in \mathbb{R}_+^3\) and \( S = 0 \), the system model parameters are positive, and the feedback is nonnegative, provided that the initial values of \( A \) and \( B \) are nonnegative. For any initial condition that satisfies \((A(0), B(0), O(0)) \in \mathbb{R}_+^3\), semi-global exponentially stability of the overall system follows from the comparison lemma [Khalil, 2002, Lem. 3.4]. For the switch-on case \((S = 1)\), the inner-loop dynamics for \( A \) and \( B \) have nonlinear terms, \( h(O)(1 - A) \) and \( h(O)(1 - B) \). The fact that the Hill function \( 0 \leq h(O) \leq 1 \) for all \( O \geq 0 \), implies that \( dA/dt \leq \tau_a (-A + k_{\text{min}} + c_A) \) and

\[
dB/dt \leq \tau_b (-B + k_{\text{min}} + c_B)
\]

where the constants \( c_A \) and \( c_B \) satisfy the relations \( k_{\text{min}} + c_A = A_{\text{ss}} \) and \( k_{\text{min}} + c_B = B_{\text{ss}} \), respectively. Similar to the switch-off case, there exist constants \( c, d > 0 \) such that \( dO/dt \leq -c O + d \) and \( d/c = O_{\text{ss}}, \) provided \((A(0), B(0)) \in \mathbb{R}_+^2\). Combining those upper bounds and applying the comparison lemma implies that the overall system is semi-global exponentially stable for any initial condition \((A(0), B(0), O(0)) \in \mathbb{R}_+^3\). It is easy to show that, for any positive system parameters, the nonnegative quadrant is a region of attraction to a unique equilibrium point for each case \( S = 0 \) or \( S = 1 \), and is a positively invariant set, i.e., once the solution trajectory enters the nonnegative quadrant, then it will stay inside the nonnegative quadrant for the entire future time and converge to a unique equilibrium point.

3.2 Stochastic Stability

Now consider intrinsic and extrinsic biochemical noise in the gene switching model (1). To study the stochastic stability properties of the system model, consider extrinsic noise in the stimulus \( S \) and in propagation of inner loop states \( A \) and \( B \) to the output. More precisely, the system dynamics change according to the stochastic differential equation (SDE):

\[
\begin{align*}
\frac{dA}{dt} &= \tau_a \left( k_{\text{min}} - A + \frac{O^n}{O^n + \epsilon c_{50}} (1 - A) S + w_a \right), \\
\frac{dB}{dt} &= \tau_b \left( k_{\text{min}} - B + \frac{O^n}{O^n + \epsilon c_{50}} (1 - B) S + w_b \right), \\
\frac{dO}{dt} &= k_{\text{on}} (A + B + v_a + v_b)(1 - O) - k_{\text{off}} O + k_{\text{out}},
\end{align*}
\]

where \([w_a, w_b, v_a, v_b]^T\) are jointly Gaussian random processes. This ODE can be written as \( \dot{X} = f(X) + G(X)W \), where \( X = [A, B, O]^T, W = [w_a, w_b, v_a, v_b]^T \), and the nonlinear functions \( F : \mathbb{R}^3 \rightarrow \mathbb{R}^3 \) and \( G : \mathbb{R}^3 \rightarrow \mathbb{R}_+^{3 \times 4} \) are appropriately defined with the support \( X \subset \mathbb{R}_+^3 \) of the state \( X \). Section 2 showed that the system \( X = F(X) \) is deterministically semi-global exponentially stable for all \( X \in \mathbb{R}_+^3 \). From a converse Lyapunov theorem (see [Khalil, 2002, Thm. 4.17], for example), there exists a smooth positive-definite function \( V(X) \) defined for all \( X \) that satisfies the inequalities

\[
\begin{align*}
\frac{d}{dt} V(\delta X) &\leq -\alpha V(\delta X), \\
\frac{\partial V}{\partial X}(\delta X) &\leq \beta \|\delta X\|, \\
\frac{\partial^2 V}{\partial X^2}(\delta X) &\leq \gamma,
\end{align*}
\]

where \( \alpha, \beta, \gamma > 0 \) are some constants and \( \delta X = X - X_{\text{ss}} \) refers to the deviation from the unique equilibrium point \( X_{\text{ss}} \). This result follows from the fact that the Jacobian \( \frac{dF}{dX} \) is bounded for all \( X \in \mathbb{R}_+^3 \). The results in [Samad and Khammash, 2004, Lem. 9], taking into account the fact that the nonlinear functions \( F \) and \( G \) satisfy the regularity conditions for the Cauchy problem (see [Lasota and Mackey, 1994], for details) imply that there exists a unique probability density function (pdf) \( p^* \) such that the pdf of the solution for the SDE (3) converges to \( p^* \). This implies stochastic asymptotic stability of the solution for

¹ In this paper, the term “robustness” is used to refer to any type of insensitivity to perturbations and unknowns, while performing desired or programmed functions.
Table 1. The exact and estimated time constants of the system under four distinct steady-states.

<table>
<thead>
<tr>
<th>Input = $S_{ss} = 0$</th>
<th>Output = $O_{ss}^0$</th>
<th>Output = $O_{ss}^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Estimated $\frac{1}{\tau}$</td>
<td>Exact $\frac{1}{\tau}$</td>
</tr>
<tr>
<td>Single slow loop</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Single fast loop</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dual loop</td>
<td>$(2, 125)$</td>
<td>$(2, 125)$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Input = $S_{ss} = 1$</th>
<th>Output = $O_{ss}^0$</th>
<th>Output = $O_{ss}^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Estimated $\frac{1}{\tau}$</td>
<td>Exact $\frac{1}{\tau}$</td>
</tr>
<tr>
<td>Single slow loop</td>
<td>183</td>
<td>138</td>
</tr>
<tr>
<td>Single fast loop</td>
<td>1.06</td>
<td>2</td>
</tr>
<tr>
<td>Dual loop</td>
<td>$(4.06, 109.2)$</td>
<td>$(2, 125)$</td>
</tr>
</tbody>
</table>

the SDE (3) in the presence of non-vanishing stochastic noise $W_t$. \(^2\)

4. PARAMETER ESTIMATION AND SYSTEM BEHAVIOR

4.1 Parameter Estimation

To relate the parameters to measurable quantities, the steady-state values of the output under different conditions are expressed in terms of the model parameters. The sets of algebraic equations for the steady-states of the dual-loop dynamics (1) and the single-loop dynamics (2) are

$$M_O \theta^k \Delta \begin{bmatrix} 0 & 2(1 - O_{ss}^{1d}) & 1 - O_{ss}^{1d} \\ 0 & 1 - O_{ss}^{0d} & 1 - O_{ss}^{0d} \\ 1 - O_{ss}^{1s} & 1 - O_{ss}^{1d} & 1 - O_{ss}^{1d} \\ 1 - O_{ss}^{0s} & 1 - O_{ss}^{1s} & 2 - 2O_{ss}^{1s} \end{bmatrix} \theta^k = 0, \quad (4)$$

where $\theta^k \Delta \begin{bmatrix} k_{\text{out}}^{\text{on}}, k_{\text{out}}^{\text{off}}, k_{\text{min}}, k_{\text{out}}^{\text{off}}, k_{\text{off}}^{\text{off}} \end{bmatrix}^T$, the subscript `ss` denotes steady-state values, the superscripts 0s and 0d denote single and dual loops with $S_{ss} = 0$, and the superscripts 1s and 1d denote single and dual loops with $S_{ss} = 1$. This equation uses the fact that Hill equation at the steady-state is $O_{ss}^k \approx 1$ due to $O_{ss}^{1s}, O_{ss}^{1d} \gg \epsilon c_{50}$.

Performing a singular value decomposition (SVD) on $M_O$ provides a more robust solution for $\theta^k$, which gives the relative values of $k_{\text{out}}^{\text{on}}, k_{\text{out}}^{\text{off}}, k_{\text{min}}$. The absolute value of $k_{\text{min}}$. The absolute value of $k_{\text{out}}^{\text{off}}$ equals the gradient of the output curve at $t = 0$, where $A$, $B$, and $O$ are all zero. It is only possible to estimate $k_{\text{out}}^{\text{on}}$ this way in the single slow-loop case because the gradient changes rapidly near $t = 0$ when there is a fast loop. The value for $\epsilon c_{50}$ can be estimated as twice the value at the transition point, which is when $O_{ss}^k / O_{ss}^{1s} + \epsilon c_{50}$ first exceeds 0.1.

To determine the time constant of the system, this nonlinear set of equations was linearized around its steady states. The time constants of the system under four distinct states were estimated and are summarized in Table 1. $\tau_a$ and $\tau_b$ can be estimated as the reciprocal of the time constants of the One Slow Loop and One Fast Loop response. Both the exact and the approximate values of the time constants are listed. The expressions for the approximate value of the time constants makes it easy to see how the model parameters affect the dynamics of the system. Table 2 summarizes

the data collected from the simulated responses and Table 3 compares the parameters estimated from those data with the exact value. The preceding parameter estimation was performed with the assumption that it is possible to suppress one of the dual loops at a time and that the steady-state value of the output is measurable. Parameter estimation will be more accurate when more information is available. For example, if either $A$ or $B$ is measurable, it is possible to calculate $\epsilon c_{50}$ from an analytical expression relating it to the gradient of the $A$ versus time plot at $t = 0$. The time constants can be also estimated without linearizing the system, since the differential equation is linear in $A$ when the stimulus is zero.

4.2 Convergence Rate

For a uniform time-scale dynamical system, the convergence rate (or response time) to a stable equilibrium point or an attracting limit cycle is governed by the eigenvalues of the eigenmodes corresponding to the slow dynamics. The case of dual-positive feedback loops motif (1), however, corresponds to a two-time-scale dynamical system in which the fast inner-loop dynamics has much smaller time constant, compared to time constant of the slow inner-loop dynamics. There are two distinct stages of system behavior and response to the input. For the switch-on ($S = 1$) case, behavior of the first stage is governed by the fast inner-loop dynamics and the solution trajectory reaches near the stable equilibrium point quickly during this stage and the

<table>
<thead>
<tr>
<th>Observables</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_{ss}^{0s}$</td>
<td>0.1206</td>
</tr>
<tr>
<td>$O_{ss}^{0d}$</td>
<td>0.06562</td>
</tr>
<tr>
<td>$O_{ss}^{1s}$</td>
<td>0.8675</td>
</tr>
<tr>
<td>$O_{ss}^{1d}$</td>
<td>0.7635</td>
</tr>
<tr>
<td>$\epsilon c_{50} / 2$</td>
<td>0.17</td>
</tr>
<tr>
<td>$\tau_{\text{fast}}$</td>
<td>0.0094</td>
</tr>
<tr>
<td>slope at $t = 0$</td>
<td>0.0094</td>
</tr>
</tbody>
</table>

| $\tau_{\text{fast}}$ | 4 |
| $\tau_{\text{slow}}$ | 154 |

Table 3. Parameter estimation errors.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimated</th>
<th>Exact</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\epsilon c_{50}$</td>
<td>0.34</td>
<td>0.35</td>
<td>3</td>
</tr>
<tr>
<td>$k_{\text{min}}$</td>
<td>0.011</td>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>$k_{\text{out}}^{\text{on}}$</td>
<td>0.0094</td>
<td>0.001</td>
<td>6</td>
</tr>
<tr>
<td>$k_{\text{out}}^{\text{off}}$</td>
<td>1.7</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>$k_{\text{off}}^{\text{off}}$</td>
<td>0.26</td>
<td>0.3</td>
<td>13</td>
</tr>
<tr>
<td>$\tau_{\text{slow}}$</td>
<td>0.25</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>$\tau_{\text{slow}}$</td>
<td>0.0065</td>
<td>0.0008</td>
<td>19</td>
</tr>
</tbody>
</table>
last of dynamic behavior is governed by the slow inner-loop dynamics for which the differential equation corresponding to the fast inner-loop dynamics can be replaced by an algebraic equation and the linearized system provides necessary information about the system during this stage of response. For the switch-off \((S = 0)\) case, similarly, behavior of the first stage is governed by the fast inner-loop dynamics, but the solution trajectory is far away from the new stable equilibrium point and the last dynamic behavior is governed by the slow inner-loop dynamics for which the differential equation corresponding to the fast inner-loop dynamics can be also replaced by an algebraic equation. This phenomena is observed in a simulation in Fig. 1 in which hysteresis curves with different values of \(\tau_a\) and \(\tau_b\) are provided and an appropriately scaled ramp input instead of the jump stimulus was applied to give clear comparisons between curves.

![Hysteresis curves of different time constants](image)

**Fig. 1.** Hysteresis curves of different time constants.

### 4.3 Hysteresis

The lag in the responses in Fig. 2 to stimulus (or input) is related to robustness against noise perturbation. As stated in the previous section, most of the time history of the solution trajectory is governed by a slow inner-loop dynamics, and the fast inner-loop dynamics only affects the system behavior during an initial short time period. Qualitatively, the overall system would be expected to be robust against additive noise perturbation in stimulus to the network motif for long time, when the system acts as a low-pass filter, while being sensitive to noise perturbation in short time.

An example hysteresis curve for the dual-positive feedback loops motif is shown in Fig. 2, for the time constants \((\tau_a = 0.5, \tau_b = 0.008)\). The shape of the hysteresis curves is related to the Hill coefficient. The duration for which the system is governed by the fast inner-loop dynamics is proportional to the Hill coefficient \(n\). The shape of a hysteresis curve is closer to a rectangular box as \(n\) increases, when the Hill function becomes steeper (figure not shown due to space constraints).

### 4.4 Noise Sensitivity

Consider noise perturbation \(w_t\) in the stimulus \(S\) and assume that the random process \(w_t\) is a Wiener process. Consider the case where \(S = 1\), since \(S = 0\) would imply no signal exchange for which no perturbation will propagate. To investigate the effect of this noise perturbation on the solution trajectory or the system output, first define a probability space. Let \(\Omega\) be a sample space equipped with a \(\sigma\)-algebra \(F\). Consider a probability measure \(\mu\) that is defined on \((\Omega, F)\) and the corresponding probability (measure) space defined by \((\Omega, F, \mu)\). The underlying probability space and measure are determined by the primitive random variables or processes.

Setting \(S = 1\), consider an autonomous stochastic differential equation (SDE)

\[
dx = f(x)dt + g(x)dw_t
\]

where \(x = [A, B]^T\),

\[
f(x) = \frac{\tau_a (k_{imin} - (1 + h(O)) A + h(O))}{\tau_b (k_{imin} - (1 + h(O)) B + h(O))},
\]

and

\[
g(x) = \frac{\tau_a h(O) (1 - A)}{\tau_b h(O) (1 - B)}.
\]

The goal is to study the probability density function of the solution \(x_t\), denoted by \(p(x(t); x_0, t_0)\) with the initial condition \(x_0 = x(t_0)\), and its sensitivity with respect to the system parameters. The Fokker-Planck equation (aka, the Kolmogorov forward equation) can be used for computing the probability density for this stochastic process. The pdf \(p(x(t); x_0, t_0)\), written in shorthand as \(p(x, t)\), solves

\[
\frac{\partial p(x, t)}{\partial t} = -\sum_{i=1}^{2} \frac{\partial}{\partial x_i} \left( f_i(x)p(x, t) \right) + \frac{1}{2} \sum_{i,j=1}^{2} \frac{\partial^2}{\partial x_i \partial x_j} \left( g_{ij}(x) p(x, t) \right)
\]

where \(\partial_i \triangleq \frac{\partial}{\partial x_i}\), \(\partial_{ij} \triangleq \frac{\partial^2}{\partial x_i \partial x_j}\), and the initial condition \(p(x_0, t_0)\) is assumed to be known and independent of the Wiener process \(w_t\). This is called the Cauchy problem in the partial differential equation (PDE) literature, that usually does not have a closed-form solution and can be hard to solve numerically for general systems.

To study the parameter sensitivity, instead of solving the Fokker-Planck equation to compute the evolution of the probability distribution of \(x_t\), use the linearized model of the motif dynamics (1):

\[
\frac{dX}{dt} = AX + Bw; \quad Y = CX
\]

where \(X \triangleq [\delta A, \delta B, \delta O]^T\) denotes the deviation of the steady-state and the system matrices are

\[
A = \begin{bmatrix}
-\tau_a (1 + h(y)u) & 0 & \tau_a (1 - x_1) h'(y) u \\
0 & -\tau_b (1 + h(y)u) & \tau_b (1 - x_1) h'(y) u \\
-k^{out}_{on} (1 - x_3) & k^{out}_{on} (1 - x_3) & -k^{out}_{off} (1 - x_1 + x_2)
\end{bmatrix},
\]

\[
B = [(1 - x_1) h(y) (1 - x_2) h(y)]^T, \quad C = [0 \ 0 \ 1];
\]

\[
h(y) \triangleq \frac{y^n}{y^n + \epsilon_{max}}, \quad h'(y) \triangleq \frac{dh(y)}{dy} = \frac{n y^{n-1} \epsilon_{max}}{(y^n + \epsilon_{max})^2}, \quad x_1 = x_2 = A_{ss} = B_{ss}, y = x_3 = O_{ss}, u = S_{ss}.
\]

For a Gaussian white noise \(w_t\) with the statistics \(\mathbb{E}[w_t] = 0\) and \(\mathbb{E}[w_t w_t^T] = \delta(t - s) \Sigma_{w}\), the solution trajectory of (7) is a
Gaussian random process and its mean \( \bar{X}_t \) and covariance \( \Sigma_{X,t} \) solve the linear system equations

\[
\begin{align*}
\frac{d\bar{X}}{dt} &= A\bar{X}; \quad \bar{X}_0 = \mathbf{E}[X_0] \\
\frac{d\Sigma_{X}}{dt} &= \Sigma_{X}A + A^T \Sigma_{X} + 2B\Sigma_u B^T; \quad \Sigma_{X,0} = \mathbf{E}[X_0 X_0^T]
\end{align*}
\]

and the system output is also a Gaussian random process, \( Y_t \sim \mathcal{N}(\bar{X}_t, \Sigma_{X,t}) \), where \( \Sigma_{X,t} = \Sigma_{X} + \bar{X}_t \Sigma_{X} \). The analysis goal is to quantify the sensitivity of \( \Sigma_{Y,t} \in \mathbb{R}^+ \) with respect to the system parameters. In particular, consider the system parameters \( \{\tau_\theta, \tau_b, k_{out}^{in}, k_{out}^{off}\} \), the steady-state solution \( \Sigma_{X,ss} \) for the second dynamic system in (8), i.e., \( \Sigma_{X,ss} \) satisfying the linear (Lyapunov) equation

\[
\Sigma_{X,ss}A + A^T \Sigma_{X,ss} + 2B\Sigma_u B^T = 0
\]

and the corresponding steady-state of the output covariance \( \Sigma_{Y,ss} = C \Sigma_{X,ss} C^T \). A solution for the Lyapunov equation (9) exists if and only if the matrix \( A \) is Hurwitz stable, i.e., \( \sigma(A) \subset \mathbb{C}^- \), and is unique if and only if the pair \( (A, \Sigma_{X,ss}^2) \) is controllable.

To analyze the sensitivity of the Lyapunov solution \( \Sigma_{X,ss} \) with respect to the variation of the system parameters \( \{\tau_\theta, \tau_b, k_{out}^{in}, k_{out}^{off}\} \), consider a variation in the matrix \( A \), denoted by \( \delta A \), such that the perturbed system matrix is \( A + \delta A \). This notation of variation can be used to represent the parametric perturbation in the linearized motif dynamics (7) due to the linear dependence of \( \delta A \) on the system parameters \( \{\tau_\theta, \tau_b, k_{out}^{in}, k_{out}^{off}\} \). With this perturbed matrix \( \delta A \), the steady-state covariance \( \delta \Sigma_{X,ss} \) satisfies a linear Lyapunov equation

\[
\delta \Sigma_{X,ss}A + A^T \delta \Sigma_{X,ss} + 2B\delta \Sigma_u B^T = 0.
\]

Suppose that \( A \) is Hurwitz stable and \((A, B)\) is controllable. If the perturbation \( \delta A \) is Hurwitz stable then \( \delta \Sigma_{X,ss} \geq \Sigma_{X,ss} \), and if the perturbation \( \delta A \) is anti-Hurwitz stable (i.e., \( -\delta A \) is Hurwitz stable) then \( \delta \Sigma_{X,ss} \leq \Sigma_{X,ss} \). More specifically, consider a parametric perturbation \( \theta = \theta + \delta \theta \) for \( \theta \in \{\tau_\theta, \tau_b, k_{out}^{in}, k_{out}^{off}\} \) in the matrix \( A \). The positive perturbation \( \delta \theta > 0 \) gives \( \delta \Sigma_{X,ss} \geq \Sigma_{X,ss} \) and the negative perturbation \( \delta \theta < 0 \) gives \( \delta \Sigma_{X,ss} \leq \Sigma_{X,ss} \) for any \( \theta \in \{\tau_\theta, \tau_b, k_{out}^{in}, k_{out}^{off}\} \), provided \( \theta > 0 \). In conclusion, the variation of the system output (or state) is a nondecreasing function of the system parameters \( \{\tau_\theta, \tau_b, k_{out}^{in}, k_{out}^{off}\} \). This result is consistent with intuition from the system dynamics, since, roughly speaking, those system parameters are linearly proportional to the inverse of the time constants.

5. SYSTEM PERFORMANCE ANALYSIS FOR LINEARIZED MODELS

This section considers the analysis of the sensitivity of certain parameters to functionalities of the biological system. Similar to Section 4, the linearized model (7) of the motif dynamics (1) is used to facilitate sensitivity analysis.

5.1 \( H_\infty \) Performance and Sensitivity Analysis

Observe that the system realization \((A, B, C)\) is a positive system in which \( A \) is a Metzler matrix, and \( B \) and \( C \) are nonnegative matrices, for all positive system parameters.

Computation of the \( H_\infty \) performance \(^4\) for this type of system can be performed without any frequency-dependent computation or solving a linear matrix inequality corresponding to the KYP lemma. In particular, [Rantzer, 2011] shows that, for any scalar transfer function \( G(s) = D + C(sI - A)^{-1}B \) with a positive realization \((A, B, C, D)\),

\[
\|G\|_\infty = D - CA^{-1}B,
\]

which is the steady-state gain of the system transfer function \( G(s) \). More generally, for any square transfer function \( G(s) = D + C(sI - A)^{-1}B \) with a positive realization \((A, B, C, D)\),

\[
\|G\|_\infty = \sigma(D - CA^{-1}B),
\]

which is the steady-state gain of the system transfer function \( G(s) \), computed in terms of the induced 2-norm. For the linearized system (7), we can derive that

\[
\|G\|_\infty = \frac{(1/\tau_a + 1/\tau_b)h(y)(1 - y)(1 - x)}{2h'(y)(1 - y)(1 - x)u - 2(2 + \frac{\sigma}{\tau_{A,B,C,D}})(1 + h(y)u)}.
\]

This closed-form for the \( H_\infty \)-norm of a system transfer function can be used to study the sensitivity of a system performance with respect to the system parameters. For example, consider the case when \( S_{ss} = 1 \) and a (deterministic) perturbation \( \delta S \). A biased step perturbation \( \delta S = \kappa \in \mathbb{R} \) in the stimulus will change the steady-state of the output by \( \delta O_{ss} = \kappa \|G\|_\infty \) so that the resultant output in the steady-state becomes \( O_{ss} + \delta O_{ss} \). Thus, the closed-form (10) can be used to quantify the sensitivity of the output in the steady-state with respect to the system parameters of interest, as well as to study qualitative behavior of the system performance in terms of the \( H_\infty \)-norm.

5.2 \( H_2 \) Performance and Sensitivity Analysis

Another commonly used system performance criterion is the \( H_2 \)-norm. Computation of \( H_2 \) performance of a linear time-invariant system is relatively easy in the sense that only a linear programming problem needs to be solved. For a strictly proper transfer function \( G(s) = C(sI - A)^{-1}B \),

\[
\|G\|_2 = \text{Tr}(B^T Y_o B)
\]

where \( Y_o \) is the observability Gramian given by

\[
Y_o = \int_0^\infty e^{At} C^T C e^{At} \, dt.
\]

This observability Gramian is a unique solution for the Lyapunov equation \( Y_o A + A^TY_o + C^T C = 0 \), provided that \( A \) is Hurwitz stable and the pair \((A, C)\) is observable. Similar to the \( H_\infty \)-norm computation, the closed-form solution for \( \|G\|_2 \) with a realization (7) can be derived (not shown here due to space constraints). However, the closed-form solution has a somewhat complex dependence on the system parameters and it is hard to gain much intuition from the expression. This observation motivates a different approach that is similar to the noise sensitivity analysis in Section 4.4, in which a Lyapunov equation is used to study how the Lyapunov solution changes as a system parameter changes. Suppose that \( A \) is Hurwitz stable and \((A, C)\) is observable. Consider a parametric perturbation \( \delta A \) with which the new system matrix is given by \( \delta A := A + \delta A \) and the corresponding system transfer function is defined by \( \hat{G} := C(sI - A)^{-1}B \). If the perturbation \( \delta A \) is Hurwitz stable then \( \hat{Y}_o \geq Y_o \), which implies \( \|\hat{G}\|_2 \geq \|G\|_2 \), and

\(^4\) Also known as the \( L_2 \)-gain.

This is a property of Lyapunov equation; see [Dullerud and Paganini, 2000, Prop. 4.4] for details.
if the perturbation $\delta A$ is anti-Hurwitz stable (i.e., $-\delta A$ is Hurwitz stable) then $\hat{Y}_o \leq Y_o$, which implies $\| \hat{G} \|_2 \leq \| G \|_2$.

More specifically, consider a parametric perturbation $\theta = \theta + \delta \theta$ for $\theta \in \{ \tau_a, \tau_b, k_{on}, k_{off} \}$ in the matrix $A$. The positive perturbation $\theta + \delta \theta \geq 0$ gives $\| \hat{G} \|_2 \geq \| G \|_2$ and the negative perturbation $\theta - \delta \theta \leq 0$ gives $\| \hat{G} \|_2 \leq \| G \|_2$ for any $\theta \in \{ \tau_a, \tau_b, k_{on}, k_{off} \}$, provided $\theta, \delta \theta > 0$. The $H_2$ norm can be used for a measure of performance when there is information about the spectral content of the input source and can be interpreted as (a) the output variance for a stationary stochastic noise applied at the input, (b) the system output energy variance for the impulse input, and (c) the output variance in the $L_{\infty}$ norm over time for an arbitrary input in the $L_2$ space (see [Dullerud and Paganini, 2000, Sec. 6] for details of $H_2$ control). The results in this section indicates that those measures of output variation incurred by an input of known spectrum are monotonically nondecreasing functions in the system parameters $\theta \in \{ \tau_a, \tau_b, k_{on}, k_{off} \}$ and can be used to tune such system parameters to achieve a desired $H_2$ performance of the system in a straightforward way.

6. DISCUSSION

Output responses to a noise-free and noisy stimulus are shown in Figs. 3c. All three components $O, A,$ and $B$ approach stable states when both the stimulus is on and when the stimulus is off. When the stimulus is turned on, the output speeds up after an initial slower phase, as the output $O$ is low and the value of the Hill term is close to zero. This is apparent from the “Two Slow Loop” case. It is harder to observe from the “Two Fast Loop” case because the total transition time from one state to another is very short. The dynamics of the output $O$ follows more closely the dynamics of the fastest loop when the stimulus $S$ is switched from off to on because the value of $1 - O$ decreases with time. On the other hand, when the stimulus is switched from on to off, the dynamics of the output $O$ follows more closely the dynamics of the slow loop as the value of $1 - O$ decreases with time. When the fast and the slow loop are coupled together, Fig. 3a shows that the system has fast response when turning on and slow response when turning off. A closer look in Fig. 3c shows the output response trajectories during the activation by the step stimulus without noise. The response time $t_r$ (that is, the time when the output reaches half of the steady-state) is compared for the models with different time constants. The response time of the Slow+Fast feedback loop is twice as slow as for Two Fast Loops, but almost nine times faster than for Two Slow Loops. The output $O$ for the Slow+Fast feedback loop is less sensitive than the Two Fast Loops to a pulse noise applied during the transition from the on-state to the off-state (see Fig. 3b).

7. CONCLUSION

Model equations for the dual-positive feedback loops regulating the gene switches in many biological systems are analyzed. This paper describes how the model parameters can be estimated from experimental data. Relating the kinetic parameters with the dynamic behavior, system performance, and sensitivity of the binary switches can help the prediction of the behavior of larger regulatory networks, which could be used from the design of gene switches to achieve the desired behavior.

REFERENCES