Preprints of the 19th World Congress
The International Federation of Automatic Control
Cape Town, South Africa. August 24-29, 2014

Robustness Analysis of Genetic Circuits Constructed by Bottom-up Strategy ⋆

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Abstract: We analyze the robustness of a system-level genetic circuit which is constructed by a bottom-up strategy. The latest trend in synthetic biology is to create large-scale and complex genetic circuits realizing desired programmable functions. An approach for the creation is bottom-up construction, that is, well-characterized small-scale circuits are coupled into large-scale system-level circuits involving desired programmable functions that can be predicted from the modules. However, in general, an intrinsic property in a circuit may be broken by internally connecting external circuits. We analyze a successful circuit created by a bottom-up construction strategy to identify the reason behind a successful synthesis. Utilizing the robustness analysis method based on the structured singular value, we can guarantee the validity of the bottom-up construction.

Keywords: Robustness; Bi-stability; Biocybernetics; Structured singular value; Uncertain dynamical systems.

1. INTRODUCTION

In synthetic biology, the researchers pursue, through design and construction of new biological circuits realizing desired functions, for fundamental understanding of vital functions in every living organisms and utilizing them to medical treatment. To achieve them, many module-level biological circuits, that is, circuits realizing desired properties, have been created, for example, genetic oscillators by Atkinson et al. [2003], Stricker et al. [2008], Del Vecchio et al. [2008], Kim & Winfree [2011], genetic toggle switches by Gardner et al. [2000], Atkinson et al. [2003], Loinger & Biham [2009], and so on. In the last decade, the trend in the research field has been changed from creating module-level circuits into assembling modules to create system-level circuits, that is, circuits realizing desired controllable functions Purnick & Weiss [2009]. However, few successful examples have been reported for the system-level circuit synthesis. In general, system-level circuits become larger and more complex than module-level circuits. Thus, it is difficult to obtain the exact mathematical models of the entire systems. In addition, there are many physical parameters we have to tune for large-scale circuits. There are only few system-level circuits constructed in a systematic way.

Guido et al. [2006] and Padirac et al. [2012] take bottom-up approaches in system-level circuit construction. The strategy is that well-characterized modules are coupled into complex large-scale systems involving desired programmable functions that can be predicted from the modules. A successful example by Padirac et al. [2012] is realized by construction of enzyme-catalyzed, DNA-based reactions in vitro. The purpose of Padirac et al. [2012] is to create biological switchable memories which emulate rewritable memory storages with 1-bit capacity, the motif of which is illustrated in Fig. 1. Although conventional module-level switches (Gardner et al. [2000], Atkinson et al. [2003], Loinger & Biham [2009]) only possess bi-stability properties, i.e., there exist two stable equilibria in the phase space, the system-level circuit created by Padirac et al. [2012] has an ability that a state near one equilibrium can be flipped to the other equilibrium by injecting an external molecular stimulus into the circuit. Successful switching behavior is observed in vitro experiments as well as numerical simulation.

⋆ This research is supported by the Aihara Innovative Mathematical Modelling Project, the Japan Society for the Promotion of Science (JSPS) through the Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program), initiated by the Council for Science and Technology Policy (CSTP).

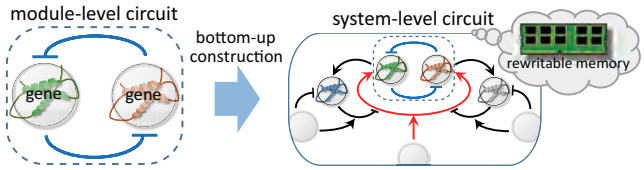

Fig. 1. System-level circuit constructed of modules

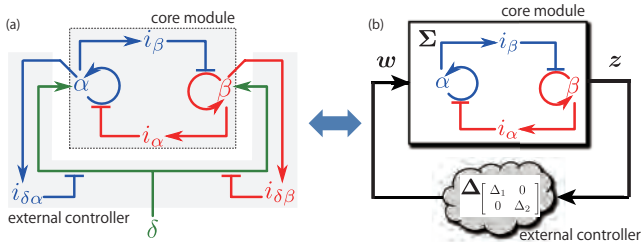

Fig. 2. Switchable memory circuit (a) is transformed to feedback form (b)

However, the reason behind the success of the construction is still unclear theoretically. In general the problem arises that bi-stability of a core circuit may be broken by an external circuit that is internally connected in the core. It may be expected that the bi-stability can be preserved in the entire system if the core circuit has high robustness against parametric variations, which is evaluated in the same way as many robustness analysis problems of biomolecular systems (see, e.g., Kim et al. [2006], Ghaemi et al. [2009], Chen et al. [2010], Waldherr & Allgöwer [2011], Cosentino & Bates [2012]). However, the robustness against parametric perturbations does not relate to that against dynamic perturbations and the feedback-type connection of external circuits cannot be represented by parametric variations of the core circuit but by dynamic perturbations. Therefore, we must evaluate robustness against the connection of circuits which is not determined at the step of construction.

In this paper, we analyze the switchable memory created in the previous work by Padirac et al. [2012] and reveal the reason behind the success. In order to evaluate the reasonability of the switchable memory system, we use the structured singular value to represent robustness against additional circuit connection. Then, we can guarantee the validity of the bottom-up construction. Finally, we propose a bottom-up strategy which assembles core circuits with high dynamic robustness to create system-level biomolecular circuits.

Notation: $\mathbb{R}_+ = [0, \infty)$. The poles (system poles) of a linear system $\dot{x} = Ax$ are defined by the roots of the characteristic polynomial $\phi(s) := \det(sI - A)$. In addition, an unstable pole is defined as poles lie in the open right half-plane.

2. SWITCHABLE MEMORY AND MOTIVATION

In this section, let us review the differential equation model of the switchable memory created by Padirac et al. [2012], which is illustrated in Fig. 2.

To construct the switchable memory systems, the paper by Padirac et al. [2012] starts with the design of the core circuit based on three enzyme-catalyzed, DNA-based reactions. The mathematical model of the core circuit is described by the Michaelis-Menten-type differential equations

$$\Sigma : \begin{cases} \dfrac{\mathrm{d}\alpha}{\mathrm{d}t} = \dfrac{t_\alpha \alpha}{1+\alpha+\lambda_\alpha i_\alpha} - \alpha + w_1, \\ \dfrac{\mathrm{d}\beta}{\mathrm{d}t} = \dfrac{t_\beta \beta}{1+\beta+\lambda_\beta i_\beta} - \beta + w_2, \\ \dfrac{\mathrm{d}i_\alpha}{\mathrm{d}t} = \dfrac{t_{i\alpha}\beta}{1+\beta} - i_\alpha, \\ \dfrac{\mathrm{d}i_\beta}{\mathrm{d}t} = \dfrac{t_{i\beta}\alpha}{1+\alpha} - i_\beta, \end{cases} \tag{1}$$

where α, β, i_α, $i_\beta \in \mathbb{R}_+$ are the state variables (concentrations of proteins and their inhibitors), w_1, $w_2 \in \mathbb{R}_+$ are the external signals, t_α, t_β, $t_{i\alpha}$, $t_{i\beta}$ are the scaled template concentrations with positive values, and λ_α and λ_β are the ratios of binding constants of activators over that of inhibitors with the values $(\lambda_\alpha, \lambda_\beta, t_{i\alpha}, t_{i\beta}) = (100, 100, 30, 30)$ and $t_\alpha, t_\beta \in [1, 30]$. Let $x = [\alpha, \beta, i_\alpha, i_\beta]^\mathsf{T}$.

For the differential equation model (1), there exist three equilibria in $\mathbb{R}^4_+$: the origin $x^e_0 = 0$,

$$x^e_1(t_\alpha) = \begin{bmatrix} -1+t_\alpha \\ 0 \\ 0 \\ \dfrac{3(-1+t_\alpha)}{10t_\alpha} \end{bmatrix}, \quad x^e_2(t_\beta) = \begin{bmatrix} 0 \\ -1+t_\beta \\ \dfrac{3(-1+t_\beta)}{10t_\beta} \\ 0 \end{bmatrix}.$$

We perform bifurcation analysis (see, e.g., Wiggins [2003], Kuznetsov [2004]), i.e., stability analysis of $x^e_1(t_\alpha)$ and $x^e_2(t_\beta)$ for all parameter values t_α, $t_\beta \in [1, 30]$. The result of the analysis is illustrated in the bifurcation diagram as Fig. 3 (a). The model is said to be bi-stable if both of the equilibria $x^e_1(t_\alpha)$ and $x^e_2(t_\beta)$ are stable, while we call mono-stable when one of them is stable and the other is unstable. The core circuit is supposed to have bi-stable equilibria depending on the value of the parameters. The volume of the bi-stability region is identified with the robustness of the bi-stability in conventional robustness analysis of biomolecular systems (Kim et al. [2006], Ghaemi et al. [2009], Chen et al. [2010], Waldherr & Allgöwer [2011], Hori et al. [2011], Cosentino & Bates [2012]). The bi-stability of the module is robust for parametric perturbations when the parameter region is sufficiently large. As illustrated in Fig. 3 (b), we have bi-stable behavior of the module on the red region in the parameter space $\mathcal{P} = \{(t_\alpha, t_\beta) \,|\, t_\alpha, t_\beta \in [1, 30]\}$, although all initial states converge to a unique fixed point on the blue region in $\mathcal{P}$. Fig. 3 (b) illustrates some state trajectories on the phase space of the core module model (1) for the parameter values $(t_\alpha, t_\beta) = (20, 20)$.

After the core circuit (1) with the bi-stability property is designed, the following external controller described by $\Delta = \mathrm{diag}\{\Delta_1, \Delta_2\}$ is connected to the module. The symbols Δ_1 and Δ_2 are represented by

$$\begin{aligned} \Delta_1 : \ & \frac{\mathrm{d}i_{\delta\alpha}}{\mathrm{d}t} = \frac{t_{i\delta\alpha}\alpha}{1+\alpha} - i_{\delta\alpha}, \quad w_1 = \frac{t_{\delta\alpha}\delta}{1+\delta+\lambda_{\delta\alpha}i_{\delta\alpha}}, \\ \Delta_2 : \ & \frac{\mathrm{d}i_{\delta\beta}}{\mathrm{d}t} = \frac{t_{i\delta\beta}\beta}{1+\beta} - i_{\delta\beta}, \quad w_2 = \frac{t_{\delta\beta}\delta}{1+\delta+\lambda_{\delta\beta}i_{\delta\beta}}, \end{aligned} \tag{2}$$

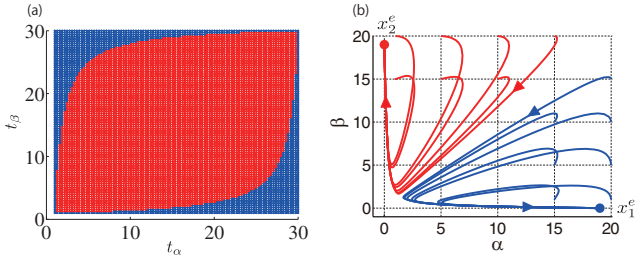

Fig. 3. Bifurcation diagram (a) and phase portrait (b) of core circuit.

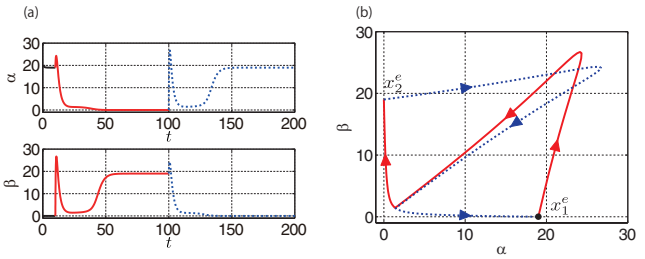

Fig. 4. Integral curve (a) and trajectory (b) of switchable memory.

where $\delta \in \mathbb{R}_+$ is the external stimulus which is used to control the core circuit (1), $i_{\delta\alpha}$, $i_{\delta\beta} \in \mathbb{R}_+$ are the states of Δ (concentrations of inhibitors for δ), and $t_{i\delta\alpha}$, $t_{i\delta\beta}$, $\lambda_{\delta\alpha}$, and $\lambda_{\delta\beta}$ are the positive constants. Then, a system-level circuit achieving desired programmable function is constructed as illustrated in Fig. 2 (a). The integral curve and trajectory of α and β of the switchable memory (Σ, Δ) from the initial state $x(0) = x_1^e$ are illustrated in Fig. 4. In the figure, we consider $(t_{i\delta\alpha}, t_{i\delta\beta}, t_{\delta\alpha}, t_{\delta\beta}, \lambda_{\delta\alpha}, \lambda_{\delta\beta}) = (5, 5, 120, 120, 30, 30)$ and the external stimulus δ is determined by the dynamics $\dot{\delta} = -\delta + \delta_{\rm imp}$. The states α and β of Σ can be controlled by δ. The sufficiently large impulsive stimulus $\delta_{\rm imp}$ is injected in the switchable memory (Σ, Δ) at the time $t = 10$ and 100. By the stimulus the state can be flipped from an equilibrium point to the other point. Due to such a function, the switchable memory can be utilized as a biological memory storage.

We discuss the implementability of the switchable memory in vitro and in vivo from a theoretical point of view. By Padirac et al. [2012], switching behavior is successfully observed in vitro experiment as well as the numerical simulation described above. However, the reason behind this experimental success and the perspective to those in vivo are still unclear. As shown in later sections, the external controller Δ of (1) can affect the desirable bi-stability of the core circuit Σ under the existence of persistent external stimulus δ. Actually, in vivo experiment, δ is fluctuated by the expression of other intracellular genes. In addition, we need to consider cell heterogeneity if the module Σ and controller Δ are implemented in intracellular genes. This heterogeneity can be represented by parametric variations of the module Σ, for example, the parameters t_α and t_β are affected by cell population dynamics, which are assumed to be fast (see e.g., Belta et al. [2001]). In view of this, we analyze the robustness of the bi-stability of Σ, i.e., the stability of $x_1^e(t_\alpha)$ and $x_2^e(t_\beta)$ and the instability of the origin, taking these dynamic uncertainties into an explicit account. This enables us to characterize the class of allowable external controllers Δ, and also to perform reliable switchable memory synthesis. To this end, we propose a dynamic robustness analysis method in the next section.

3. GENERAL ANALYSIS METHOD

3.1 Robust stability and instability

First, the switchable memory (Σ, Δ) is transformed into a feedback form as is illustrated in Fig. 2. In Fig. 2 (b), the signals w and z are given by $w = [w_1, w_2]^{\mathsf{T}}$ and $z = [\alpha, \beta]^{\mathsf{T}}$. For a general discussion of robustness analysis, we consider a general nonlinear feedback system $(\Sigma_{NL}, \Delta_{NL})$. The core circuit Σ of (1) is generalized to the nonlinear differential equations Σ_{NL}

$$\Sigma_{NL}: \ \dot{x} = f(x, w), \quad z = g(x, w),$$

where $x \in \mathbb{R}^n$ is the inner state vector and $w \in \mathbb{R}^m$ is the input. The symbol Δ_{NL} is the operator that represents the external circuit $w = \Delta_{NL} z$.

To discuss the local stability and instability of a specific steady state $(x, w) = (x^e, 0)$, the differential equation model of Σ_{NL} is linearized to

$$\Sigma_L: \dot{\tilde{x}} = A\tilde{x} + Bw, \quad z = C\tilde{x} + Dw,$$

where $A = \partial f/\partial x$, $B = \partial f/\partial w$, $C = \partial g/\partial x$, and $D = \partial g/\partial w$ at the steady state $(x, w) = (x^e, 0)$. We characterize the class of external circuits Δ_{NL} such that the stability and instability of the equilibria x_0^e, x_1^e, x_2^e of the core circuit Σ_{NL} are robustly preserved in the entire feedback system.

We review the robustness of the stability and instability of Σ_L against the dynamic perturbation $w = \Delta_{NL} z$. An approach to the robustness analysis is based on small-gain stability/instability theorems (Zames [1966], Zhou et al. [1996], Takeda & Bergen [1973], Desoer & Vidyasagar [1975], Inoue et al. [2013a,b,c]). For this, we give the conditional L_2 gain (Takeda & Bergen [1973], Desoer & Vidyasagar [1975]) as

$$\mu(\mathcal{S}) := \inf\{\, \mu > 0 : \ \|\mathcal{S}w\| \le \mu\|w\|, \ \forall w \in M(\mathcal{S}) \,\},$$

where $M(\mathcal{S})$ in L_2 is a linear manifold defined by

$$M(\mathcal{S}) := \{w \in L_2 : \ \mathcal{S}w \in L_2\}.$$

The manifold $M(\mathcal{S})$ is the class of all inputs that cancel all unstable modes in $\mathcal{S}$. We can define the linear manifold $M(\mathcal{S})$ in the L_2 space and have the conditional L_2 gain $\mu(\mathcal{S})$. When the system $\mathcal{S}$ is L_2 stable, $M(\mathcal{S})$ is equivalent to the L_2 space itself and $\mu(\mathcal{S})$ is equal to the L_2 gain.

Remark 1. When a system $\mathcal{S}$ is finite-dimensional and linear time-invariant, we can show that the gain $\mu(\mathcal{S})$ is equivalent to the $\mathcal{L}_\infty$ norm of $\mathcal{S}$ (see e.g., Zhou et al. [1996]). Suppose that $\mathcal{S}$ is represented by Σ_L with the Jacobian matrix A having no eigenvalue on the imaginary axis. Then, the $\mathcal{L}_\infty$ norm of Σ_L is defined by

$$\|\Sigma_L\|_{L_\infty} \triangleq \sup_{\omega \in \mathbb{R}} \sigma_{\max}\{C(j\omega I_n - A)^{-1}B + D\},$$

where $\sigma_{\max}\{\cdot\}$ is the maximum singular value of a matrix.

We consider the following assumptions on the external controller Δ_{NL} and the feedback system (Σ_L, Δ_{NL}).

A1) The external controller Δ_{NL} has the unique stable equilibrium ξ^e and its L_2 gain $\mu(\Delta_{NL})$ is finite.

A2) The feedback system (Σ_L, Δ_{NL}) is well-posed and its equilibrium is uniquely determined as $[\,0,\ \xi^e\,]^{\mathsf{T}}$.

The last part of Assumption A2 implies that the equilibrium x^e in Σ_{NL} on which we focus is independent of Δ_{NL}, and conversely δ^e of Δ_{NL} is independent of Σ_{NL}. Under Assumptions A1 and A2, we obtain the following theorem (Takeda & Bergen [1973], Inoue et al. [2013a,c]).

Theorem 2. Suppose that Σ_L is hyperbolic and its origin is stable (unstable). Then, the equilibrium $[\,0,\ \xi^e\,]^{\mathsf{T}}$ of the feedback system (Σ_L, Δ_{NL}) is stable (unstable) if

$$\mu(\Sigma_L)\mu(\Delta_{NL}) < 1 \tag{3}$$

holds.

Remark 3. The condition of the theorem is only a sufficient condition for the stability/instability of the feedback system (Σ_L, Δ_{NL}). However, in the following sense, the theorem provides a necessary and sufficient condition. Suppose that Σ_L is hyperbolic and the number of unstable poles is $P \geq 0$. Then, the number of unstable poles for the linearization of the feedback system (Σ_L, Δ_{NL}) at the equilibrium $[\,0,\ \xi^e\,]^{\mathsf{T}}$ is P if and only if (3) holds (see Inoue et al. [2013b] for details).

Remark 4. In assumption A1, the uniqueness of equilibria looks severe for large-scale bio-molecular systems. However, in this paper, we focus only on a bottom-up construction of a bio-molecular system by assembling smaller-scale circuits. We consider that Σ_{NL} is small-scale, for example it is module-level or part-level circuit. Therefore, we assume A1 in this paper. In addition, we can relax Assumption A2. The robustness analysis methods for the uncertain system $(\Sigma_{NL}, \Delta_{NL})$ with uncertain-independent equilibria are extended into that for an uncertain system with uncertain-dependent equilibria using the information on the steady state gain of Δ_{NL} (Inoue et al. [2014]). The details are omitted in this paper.

The inequality (3) is represented by $\mu(\Delta_{NL}) < 1/\mu(\Sigma_L)$. Therefore, the value $1/\mu(\Sigma_L)$ implies the maximum allowable gain of Δ_{NL} such that the stability or instability of Σ_L is preserved in the feedback system. In this sense, the reciprocal number of the conditional L_2 gain $\mu(\Sigma_L)$ is regarded as the robustness measure for the preservation of stability and instability of an equilibrium.

Remark 5. The conditional L_2 gain $\mu(\mathcal{S})$ of a nonlinear dynamical system can be evaluated by the combinations or multiplications of the gains of nonlinear static functions and linear time-invariant systems. This fact is illustrated by the external circuit Δ_1 of (2) as follows. The input-output relation $w_1 = \Delta_1\alpha$ is supposed to be decomposed to

$$\begin{aligned} S_1 &: u = \frac{t_{i\delta\alpha}\alpha}{1+\alpha}, \\ S_2 &: \frac{\mathrm{d}i_{\delta\alpha}}{\mathrm{d}t} = -i_{\delta\alpha} + u, \\ S_3 &: w_1 = \frac{t_{\delta\alpha}\delta}{1+\delta+\lambda_{\delta\alpha}i_{\delta\alpha}} \end{aligned}$$

Then, the gain $\mu(\Delta_1)$ of the nonlinear dynamical system Δ_1 can be evaluated by the multiplication $\mu(S_1)\mu(S_2)\mu(S_3)$. The gain of a nonlinear static function $y = h(z)$ such as S_1 and S_3 is given by the derivative as $\sup|\mathrm{d}h/\mathrm{d}z|$. In addition, the gain of a linear time-invariant system is given by the $\mathcal{L}_\infty$ norm. Therefore, we can evaluate the gain of a nonlinear dynamical system.

Remark 6. Further, for the analysis above, $\mu(\Sigma_L)$ can be replaced by the structured singular value, which is used in robustness analysis and robust controller synthesis of feedback systems (Doyle [1982], Packard & Doyle [1993], Balas et al. [1993]). In the value we can consider the information of the block-diagonal structure on Δ_{NL} to obtain more precise evaluation on the dynamic robustness of Σ_L. Although it is difficult to compute the value directly, we can obtain its upper bound as $\inf_M \mu(M\Sigma_L M^{-1})$ by using scaling technique with the matrix M that includes the structure on Δ_{NL} (see the papers by Doyle [1982], Packard & Doyle [1993], Balas et al. [1993] for details).

3.2 Robustness against activator/inhibitor connection

In the above, we assume that there is no a priori information on Δ_{NL} except for its gain. For additional information that Δ_{NL} is activator or inhibitor, that is, the feedback connection behaves as positive feedback or negative feedback, we can obtain more precise analysis results on the robustness. Suppose that Δ_{NL} behaves as an activator and it is represented as $\Delta_{NL} = \gamma(I - \Delta_{\mathrm{scaled}})$, where $\gamma > 0$ and Δ_{scaled} is the operator satisfying $\Delta_{\mathrm{scaled}} \in \mathcal{U}(1) := \{\mathcal{D}\,|\,\mu(\mathcal{D}) \leq 1 \text{ and A1, A2 hold}\}$. Then, the feedback system composed of Σ_L and such a Δ_{NL} can be equivalently rewritten as another feedback system composed of the linear dynamical system

$$\hat{\Sigma}_L : \begin{cases} \dot{\hat{x}} = \{A + \gamma B(I-\gamma D)^{-1}C\}\hat{x} + B(I-\gamma D)^{-1}\hat{w} \\ \hat{z} = (I-\gamma D)^{-1}(C\hat{x} + D\hat{w}) \end{cases}$$

and the perturbation

$$\hat{w} = \Delta_\gamma \hat{z}, \quad \Delta_\gamma := -\gamma\Delta_{\mathrm{scaled}} \in \mathcal{U}(\gamma).$$

Then, by utilizing the analysis method discussed above, we can evaluate the robustness against the connection of activators. Conversely, the uncertain inhibitor $\tilde{w} = \Delta_{NL}\tilde{z}$ is represented by $\tilde{w} = -\gamma(I - \Delta_{\mathrm{scaled}})\tilde{z}$. In a similar way, we can obtain the following system equivalently transformed from the feedback system composed of Σ_L and such a Δ_{NL}:

$$\hat{\Sigma}_L : \begin{cases} \dot{\hat{x}} = \{A - \gamma B(I+\gamma D)^{-1}C\}\hat{x} + B(I+\gamma D)^{-1}\hat{w} \\ \hat{z} = (I+\gamma D)^{-1}(C\hat{x} + D\hat{w}) \end{cases}$$

and $\hat{w} = \Delta_\gamma\hat{z}$, $\Delta_\gamma \in \mathcal{U}(\gamma)$ in order to evaluate the robustness against the connection of inhibitors.

4. ROBUSTNESS ANALYSIS OF SWITCHABLE MEMORY

4.1 Robust bifurcation analysis

We perform the robustness analysis of the switchable memory (Σ, Δ) of (1) and (2) to guarantee the validity of the bottom-up construction. Suppose that $\gamma < 1$. Then, we have the three equilibria $x_0^e = 0$ and

$$x_1^e(t_\alpha,\gamma)=\left[\begin{array}{cccc}\dfrac{-1+t_\alpha+\gamma}{1-\gamma} & 0 & 0 & \dfrac{3(-1+t_\alpha+\gamma)}{10t_\alpha}\end{array}\right]^{\mathsf{T}},$$

$$x_2^e(t_\beta,\gamma)=\left[\begin{array}{cccc}0 & \dfrac{-1+t_\beta+\gamma}{1-\gamma} & \dfrac{3(-1+t_\beta+\gamma)}{10t_\beta} & 0\end{array}\right]^{\mathsf{T}},$$

which are determined by the core module Σ and the external circuits $\Delta_i:\ w_i=\pm\gamma(1-\Delta_{i,\mathrm{scaled}})z$, $\Delta_{i,\mathrm{scaled}}\in\mathcal{U}(1)$, $i=1,2$. We analyze the robustness of the stability of $x_1^e(t_\alpha,\gamma)$ and $x_2^e(t_\beta,\gamma)$ against connecting Δ and the parametric variations in the core module which relates to the cell heterogeneity in order to characterize the class of the external controller Δ such that the bi-stability is preserved. To this end, we evaluate the robustness of the stability and instability against both dynamic and parametric perturbations using Theorem 2, Remark 6, and the discussion in Subsection 3.2.

The analysis results are illustrated in Fig. 5. The diagrams are called robust bifurcation diagrams (see Inoue et al. [2013c] for details). In the diagrams, we compute the maximum value of $\inf_M \mu(M\Sigma_L M^{-1})$ defined for the three equilibrium points and check the inequality condition $\max_{x^e}\inf_M \mu(M\Sigma_L M^{-1})<1/\gamma$ at each parameter point in $\mathcal{P}=\{(t_\alpha,t_\beta)\,|\,t_\alpha,t_\beta\in[1,30]\}$. The bi-stability is robust against dynamic uncertainty when the color is red, while the mono-stability has high robustness when the color is blue. Both bi-stability and mono-stability are fragile and easily broken on the white region.

Fig. 5 (a) and (b) indicate that the connected circuits $\Delta_{i,\mathrm{scaled}}$, $i=1,2$ activate α and β with the gain $\gamma=0.3$ and 0.5, respectively. The robust bi-stability regions are shrunk or completely broken compared with the nominal bi-stability region of Fig. 3 (a). Therefore, it is not guaranteed that the bi-stability is preserved in the entire system, if the external controller behaves as an activator. In addition, even though the bi-stability can be preserved, it is fragile for parametric perturbations of t_α and t_β. Fig. 5 (c) and (d) indicate that the connected circuits inhibit α and β with the gain $\gamma=0.3$ and 0.5, respectively. The robust bi-stability regions in the inhibition cases are almost equivalent to the bi-stability region in for the non-perturbation case illustrated in Fig. 3 (a).

We can see from the figures that there are some parameter points that the bi-stability is robust against both dynamic and parametric perturbations. In addition, the core circuit is robust for self-feedback-type inhibitions of α and β, although it is fragile for self-feedback-type activations of α and β. Since the external controller considered in Padirac et al. [2012] behaves as an inhibitor for the core circuit, the switchable memory is reasonable and we can support its validity theoretically. In Fig. 5, we choose the specific values of γ with no experimental evidence. However, from the analysis, we reveal the class of controllers that can be connected into the core circuit with bi-stability preserved. In addition, the analysis provides us a design guideline, e.g., the candidates of adequate parameter values for the core circuit.

4.2 Bottom-up strategy for further circuit construction

Based on the above discussion, we propose a construction strategy of system-level biomolecular circuits.

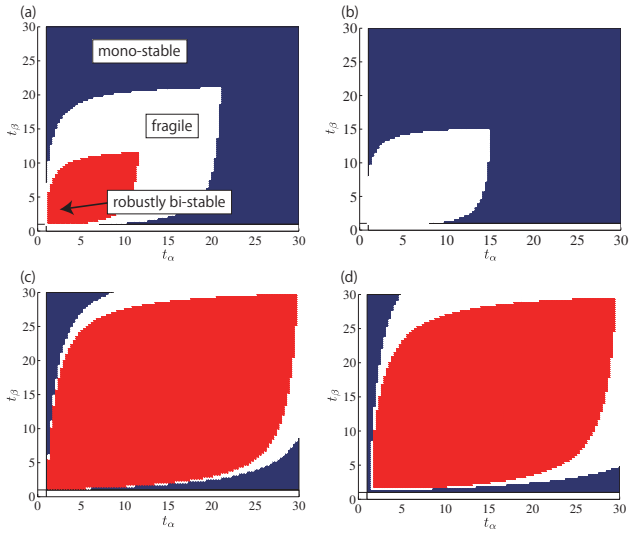

Fig. 5. Robust bi-stability region.

Step 1. Design a module-level circuit Σ that has a desired property that need to be controlled, such as bi-stability or oscillation.

Step 2. Evaluate the dynamic robustness of the module Σ. First, determine the connection port for the external controller. Then, perform bifurcation analysis including the evaluation of the dynamic robustness $1/\mu(\Sigma)$ against dynamic perturbations, which express dynamics caused by connecting the controller that is not known in this step. We can obtain the maximum allowable gain $\mu(\Delta)$ of the controller such that the property of the core module is preserved in the entire system (Σ,Δ).

Step 3. Design an external controller and tune its parameters such that the gain is less than the threshold evaluated in Step 2. Then, assemble the controller and the core module to construct a system-level circuit that realizes desired programmable functions.

5. CONCLUSION

We analyzed the switchable memory constructed by Padirac et al. [2012] and evaluates the reasonability of the bottom-up construction strategy. Furthermore, we proposed a novel bottom-up construction strategy for system-level circuits by assembling modules-level circuits with high dynamic robustness.

The construction procedure can be further employed for a variety of system-level biomolecular circuits by assembling modules-level circuits. For example, we expect the construction of a controllable oscillator by evaluating the robustness of the instability of an equilibrium.

ACKNOWLEDGEMENTS

MI gratefully acknowledges Professor Tetsuya J. Kobayashi, Dr. Masayasu Suzuki, and Tomonori Sadamoto for their suggestions and comments on this research.

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