Design of Genetic Switches with Only Positive Feedback Loops

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Abstract

We develop a new methodology to design synthetic genetic switch networks with multiple genes and time delays, by using monotone dynamical theory. We show that the networks with only positive feedback loops have no stable oscillation except equilibria whose stability is also independent of the time delays. Such systems have ideal properties for switch networks and can be designed without consideration of time delays, because the systems can be reduced from functional spaces to Euclidian spaces due to the independence to time delays. Specifically, we first prove the basic properties of the genetic networks composed of only positive feedback loops, and then propose a procedure to design the switches, which drastically simplifies analysis of the switches and makes theoretical analysis and designing tractable even for large scale systems. Finally, we demonstrate our theoretical results by designing a biologically plausible synthesized genetic switch with experimentally well investigated lacI, tetR, and cI genes.

Keywords: genetic network, switch, delay, stability, monotone dynamical system

1. Introduction

Recent development in genetic engineering has made the design and implementation of synthesized genetic networks realistic from both theoretical and experimental viewpoints, in particular for simple organisms, such as E.coli and yeast [1–4]. Actually, from the theoretical predictions, several simple genetic networks have been successfully constructed experimentally, i.e., genetic toggle switch [1], repressilator [2] and a single negative feedback loop network [3]. These experiments well agree with the predictions of mathematical models, which implies that mathematical model can be a powerful tool for designing synthesized genetic networks, especially when designing complicated switches with multiple genes [5, 6]. Such simple models represent a first step towards logical cellular control by manipulating and monitoring biological processes at the DNA level, and not only can be used as building blocks to synthesize artificial biological systems, but also have great potential for biotechnological and therapeutic applications [5].

However, the more complicated a synthetic network becomes, the more difficult it is to design and analyze the behaviors of the network, which are usually described by high-dimension nonlinear differential equations. Even for the design of a simple switch or oscillator in the previous works, many important physiological factors, such as translation processes and time delays are simply ignored or abbreviated without proper discussion, in order to reduce the dimensionality and complexity of the systems. It is well known that such factors may play important roles in the dynamics of genetic networks, and the theoretical models without consideration of these factors may even give wrong predictions [7–10]. One major obstacle to design multiple genetic networks with complicated dynamics is how to analyze the high-dimension nonlinear differential equations, in particular, differential equations with delays, which generally have infinite dimensions in a functional space [11].

In this paper, we develop a new methodology [10] to design genetic switch networks with multiple genes and time delays, by using monotone dynamical theory [12]. As indicated in this paper, the networks with only positive feedback loops have no oscillation except equilibria whose stability is also independent of the time delays. In other words, such systems have ideal properties for switch networks and can be designed without consideration of time delays, because the systems can be reduced from functional spaces to Euclidian spaces due to the independence to time delays. Specifically, we first show that synthesized genetic networks composed of only positive feedback loops have following desirable properties as genetic switches [10]:

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1. It is guaranteed that almost all orbits of a genetic network with only positive feedback loops converge to stable equilibria. In other words, there is no stable oscillation and other non-equilibrium attractors.

2. The systems with and without delays have the identical equilibria with the same stability, which implies that we can use ordinary differential equations rather than functional differential equations to design the synthetic networks.

3. The dimension of the model can be reduced by changing some differential equations (ODEs) into algebraic equations, keeping the equilibria and their stabilities invariant. This property makes analysis of a large-scale system tractable.

Then we propose a procedure [10] to design the switches, which drastically simplifies analysis of the switches and makes theoretical analysis and designing tractable even for large-scale systems.

Finally, to demonstrate our theoretical results, we give a biologically plausible example by designing a synthesized genetic switch with experimentally well investigated lacI, tetR, and cI genes for numerical simulation [10].

2. General settings

In this paper, we express a general genetic network by delayed differential equations or functional differential equations (FDEs). This model includes all essential properties of the genetic networks modeled in the previous works except stochasticity. Notice that most previous works on theoretical models of genetic networks ignore time delays resulted from transcription, translation and translocation processes, in spite of its importance.

Assume that there are \( n \) components (i.e. proteins, mRNAs, modified proteins, proteins at different locations in a carrier cell) in the network, and that the changes in their concentration are described by the following FDEs:

\[
\dot{x}(t) = f(x_t) - Dx(t) \equiv f(x_t),
\]

where \( \mathbb{R}^+ \) is the set of non negative real number and \( x(t) \in \mathbb{R}^+^n \) indicates the concentrations of all components at time \( t \in \mathbb{R} \). \( x_t \in \mathbb{C}^+ \equiv \mathbb{C}([-r, 0], \mathbb{R}^+^n) \) denotes the element of \( \mathbb{C}([-r, 0], \mathbb{R}^+^n) \), which is the space of continuous maps on \([-r, 0]\) into \( \mathbb{R}^+^n \). \( x_t(\theta) = x(t + \theta), -r \leq \theta \leq 0 \). When emphasizing the dependence of a solution on the initial data \( \phi \in \mathbb{C}^+ \), we write \( x(t, \phi) \) or \( x_t(\phi) \). \( D = diag(d_1, \ldots, d_n) \) is a \( n \times n \) diagonal matrix with \( n \) number of positive real diagonal components representing the degradation rates of each chemical component, and \( f : \mathbb{C}^+ \rightarrow \mathbb{R}^+^n \) indicates the synthesis rates of each component, belonging to \( \mathbb{C}^1(\mathbb{C}^+, \mathbb{R}^+^n) \). In addition, we define \( N = \{1, \ldots, n\} \).

We note that this model can describe not only synthesis and degradation reactions of the chemical components but also a variety of other chemical reactions, if required, such as enzymatic reactions, translocations and chemical modification of proteins.

With the settings above, we can easily show the following properties for this model:

1. Eqn. (1) generates a (local) semiflow \( \Phi \) on \( \mathbb{C}^+ \) by

\[
\Phi(t, \phi) = \Phi_t(\phi) = x_t(\phi), \quad \phi \in \mathbb{C}^+,
\]

for those \( t \) for which \( x_t(\phi) \) is defined [13]. A semiflow is an infinite-dimensional version of the usual flow in the theory of differential equations. The prefix “semi” indicates that generally we can derive the state of a genetic network from the present state only for the future but not for the past. In the rest of this paper, we assume that \( x_t(\phi) \) is defined for all \( t \geq 0 \), that is, the network never break down without external perturbation.

2. \( \mathbb{C}^+ \) is positively invariant for eqn. (1), that is, for arbitrary \( t \geq 0 \),

\[
\Phi(t, \mathbb{C}^+) \subset \mathbb{C}^+,
\]

holds [13]. This property means that the concentration of the \( n \) components never become negative, which is indispensable property for chemical concentration.

3. Define an orbit of eqn. (1) for initial data \( \phi \in \mathbb{C}^+ \) as

\[
\mathcal{O}^+(\phi) = \{x_t(\phi) : t \geq 0\}.
\]

If \( f \) is bounded on bounded subsets of \( \mathbb{C}^+ \) and \( \mathcal{O}^+(\phi) \) is bounded in \( \mathbb{C}^+ \), then \( \mathcal{O}^+(\phi) \) is precompact in \( \mathbb{C}^+ \) and the omega limit set, \( \omega(\phi) \), is defined by

\[
\omega(\phi) = \bigcap_{s \geq 0} \{x_t(\phi) : t \geq s\}.
\]

In addition, \( \omega(\phi) \) is nonempty, compact, connected and invariant [13].

The last assumption on the bounded \( f \) is naturally valid because \( f \) represents the synthesis rates of chemicals and generally has saturation property. Thus, if
that is, $f_i(x_t) = f_i(x_t/t, x_j(t - \tau_{ij}^k), x_j(t - \tau_{ij}^k))$, then $\frac{\partial f_i(x_t)}{\partial y} \geq 0$ on $\mathbb{C}^+$, or $\frac{\partial f_i(x_t)}{\partial y} \leq 0$ on $\mathbb{C}^+$ hold for all $i, j \in N$, where $x_t/t, x_j(t - \tau_{ij}^k)$ indicates all values of $x_t$ except $x_j(t - \tau_{ij}^k)$.

The basic mechanism of chemical reactions is stochastic collision of chemicals, and the probability that the collision occurs monotonously increases with the concentrations of chemicals involved in the reaction. Since the speed of a chemical reaction often inherits this monotonicity, assumption 2 is valid for most genetic networks. For instance, the following physiological reactions satisfy assumption 2: transcription activation, transcription inhibition, translation, phosphorylation, enzymatic reactions, normal chemical reactions and translocation. Next we define the types of an interaction:

**Definition 1 (Types of an interaction)**

Suppose that the concentration of the $j$-th chemical affects synthesis rate of the $i$-th chemical where $i \neq j$. Express $f_i$ as

$$f_i(x_t) = f_i(x_t/x_j, x_j(t - \tau_{ij}^k), \ldots, x_j(t - \tau_{ij}^k)),$$

where $x_t/x_j$ is $x_t$ without $x_j(t - \tau_{ij}^k), x_j(t - \tau_{ij}^k), \ldots, x_j(t - \tau_{ij}^k)$, and $l_{ij}$ means that the concentrations of the $j$-th chemical at $l_{ij}$ number of different time points affects the change in the concentration of the $i$-th chemical at $t$. Without loss of generality, let $\tau_{ij}^1 < \tau_{ij}^2 < \cdots < \tau_{ij}^{l_{ij}}$.

Focusing on the dependency of $f_i$ on $x_j(t - \tau_{ij}^k)$, we define $s_{ij}^k$, type of interaction between the $i$-th and the $j$-th components, as follows:

$$s_{ij}^k = \begin{cases} 
+1 & \text{if } \left(\frac{\partial f_i(x_t, x_j(t - \tau_{ij}^k), \ldots, x_j(t - \tau_{ij}^k))}{\partial x_j(t - \tau_{ij}^k)}\right)_{x_t = 0} > 0 \\
-1 & \text{if } \left(\frac{\partial f_i(x_t, x_j(t - \tau_{ij}^k), \ldots, x_j(t - \tau_{ij}^k))}{\partial x_j(t - \tau_{ij}^k)}\right)_{x_t = 0} < 0 \\
0 & \text{if } \left(\frac{\partial f_i(x_t, x_j(t - \tau_{ij}^k), \ldots, x_j(t - \tau_{ij}^k))}{\partial x_j(t - \tau_{ij}^k)}\right)_{x_t = 0} = 0 
\end{cases}$$

If $s_{ij}^k = 1 (-1)$, then the $j$-th chemical component is said to affect positively (negatively) the $i$-th component with time delay $\tau_{ij}^k$. In general, the $j$-th chemical component can affect the $i$-th component differently with different time delays, that is, $s_{ij}^k \neq s_{ij}^{k'}$ where $k \neq k'$.

However, since this situation is not so common for genetic networks, we assume that the $j$-th chemical component affects the $i$-th component either positively or negatively for the sake of simplicity, namely, $s_{ij}^k = s_{ij}^{k'}$ for $k, k' \in \{1, \ldots, l_{ij}\}$, and set $s_{ij} = s_{ij}^1$. For example, $s_{ij} = 1$ for $f_i = x_j(t - \tau_{ij}^k)/(1 + x_j(t - \tau_{ij}^k))$, and $s_{ij} = -1$ for $f_i = 1/(1 + x_j(t - \tau_{ij}^k))$.

Next, we define an interaction graph of the model of eqn. (1). This not only enables us to understand the relationship between the components intuitively but also gives us intuitive interpretation of theoretical results in this paper.

**Definition 2 (Interaction graph)**

An interaction graph, $IG(\mathcal{J})$, of a genetic network defined by eqn. (1) is a directed graph whose nodes represent individual chemical components of the genetic network and whose edges having an additional parameter represent the interaction between the nodes. When $s_{ij}^k \neq 0$ and $\tau_{ij}^k \geq 0$, that is, the $j$-th chemical affects the synthesis rate of the $i$-th chemical with time delay $\tau_{ij}^k$, the graph has an edge, $e_{ij}^k$, directed from the $j$-th node to the $i$-th node with an additional parameter, $(s_{ij}^k, \tau_{ij}^k)$.
It should be noticed that an edge from $j$-th node to $i$-th node is subscribed oppositely to the convention in graph theory. That is, an edge $e_{ij}$ in an interaction graph of eqn. (1) means from node-$j$ to node-$i$, which is related to the derivative of eqn. (1) by $x_j$, i.e., $\partial f_i(x_j(t - \tau_{ij}), \ast)/\partial x_j(t - \tau_{ij})$.

In addition, irreducibility of a graph is defined as follows.

**Definition 3 (Irreducibility)**

$IG(f)$ is said to be irreducible only when there is at least one path

$$p(i; j) = \{i = p_1 \to e_{p_2p_1} p_2 \to e_{p_3p_2} \ldots \to e_{p_{i-1}p_{i-2}} p_{i-1} \to e_{p_ip_{i-1}} p_i = j\},$$

from $i$-th node to $j$-th node for all $i, j \in N (i \neq j)$ where $p_1, \ldots, p_i \in N$ and $e_{p_ip_a}$ is the $k_{p_ip_a}$-th edge from node $p_a$ to node $p_i$.

This definition is different from the conventional definition of reducible or non-reducible graph in the graph theory. Under this definition, the interaction graph of a genetic network is irreducible when the network cannot be divided into several sub-networks, for which at least one sub-network affects the others but not vice versa. If a network is not irreducible, then it has at least one irreducible sub-networks. Thus, we can examine the irreducible sub-network first and then analyze the other sub-networks by taking the inputs from the irreducible sub-network as external inputs. Generally, analyzing each sub-network is much easier than the whole network. Therefore, we make the following assumption:

**Assumption 3**

$IG(f)$ of eqn. (1) is irreducible

Next, we define the types of feedback loops, which are quantitative characteristics of genetic networks.

**Definition 4 (Feedback loops and their types)**

If a path from the $i$-th node of an interaction graph to the same $i$-th node, $p(i; i) = \{i = p_1 \to e_{p_2p_1} p_2 \to e_{p_3p_2} \ldots \to e_{p_{i-1}p_{i-2}} p_{i-1} \to e_{p_ip_{i-1}} p_i = i\}$, then this path is said to be a feedback loop and furthermore be a direct feedback loop when $i$ is 1. In addition, this feedback loop is said to be positive (negative) if $\prod_{m=1}^{i-1} s_{p_{m+1}p_m} = 1 (= -1)$.

Figure 1 is an example of positive and negative feedback loops. The interaction graph in fig. 1 has one positive feedback loop, $1 \to 2 \to 3 \to 4 \to 5 \to 6 \to 1$, and one negative feedback loop, $1 \to 2 \to 5 \to 6$.

Finally, we have the following assumption:

**Assumption 4**

The interaction graph $IG(f)$ of the model (eqn. (1)) has only positive feedback loops.

This is one of the most important assumption in this paper, which, as shown in section 3, ensures that a network does not show non-equilibrium behaviors.

From assumption 4, we easily see that for arbitrary two nodes $i$ and $j$ of $IG(f)$, every paths from the $i$-th node to the $j$-th node have the same sign because of irreducibility of $IG(f)$ and the definition of types of an interaction and a feedback loop.

To apply theories of the monotone dynamical systems, we define a coordinate transformation as follows.

Choose a node of $IG(f)$ arbitrarily. Assume that the node is the $j$-th node. If the sign of a path from the $j$-th node to the $i$-th node ($i \in N$) is positive (negative), then set $\sigma_i = 1 (-1)$. $\sigma_i$ is well defined because all paths from the $j$-th node to the $i$-th node have the same sign. Using $\sigma_i$, we define a transformation $\mathbb{P}$ described by a matrix:

$$\mathbb{P} = \begin{pmatrix} \sigma_1 & 0 \\ \vdots & \ddots \\ 0 & \sigma_n \end{pmatrix}$$

Using $\mathbb{P}$, we define

$$\mathbb{C}' = \mathbb{P}(\mathbb{C}^+), \quad g = \mathbb{P} \circ f \circ \mathbb{P},$$

and

$$\dot{y}(t) = g(y_t) - Dy(t) \equiv \mathbb{g}(y_t) \quad y \in \mathbb{C}' . \quad (4)$$

![Figure 1: An example of feedback loops and their types. Signs + and – on an edge indicate respectively that s of the edge is 1 and –1.](image-url)
Since $P$ merely reverses the directions of some axes of coordinates, eqn. (4) has the same dynamical properties as eqn. (1). Thus, $C'$ is positively invariant for eqn. (4), and semiflow and omega limit set of eqn. (4) are defined in the same way as eqn. (1). In addition, $s_{ij}$ of $IG(g)$ is always non-negative because of the definition of $P$ [14].

Finally, we give the definitions of an equilibrium point, quasiconvergent point and convergent point that are used in our main theorems.

**Definition 5 (Equilibria)**
We define the set of equilibria for eqn. (1) as
\[ \mathcal{E}_f = \{ \phi \in \mathbb{C}^+ : \phi = \hat{x} \text{ for some } x \in \mathbb{R}^n \} \]
where $\hat{\cdot}$ is natural inclusion from $\mathbb{R}^n$ to $\mathbb{C}^+$. Then, the set of quasiconvergent point $Q_f$ and the set of convergent point $E_f$ are defined as
\[ Q_f = \{ \phi \in \mathbb{C}^+ : \omega(\phi) \subset \mathcal{E}_f \} \]
\[ E_f = \{ \phi \in \mathbb{C}^+ : \omega(\phi) = \{ \hat{x} \} \text{ for some } \hat{x} \in \mathcal{E}_f \} \]
The set of equilibria $E_g$, set of convergent point $E_g$ and set of quasiconvergent point $Q_g$ are similarly defined for eqn. (4), and $E_g = P(\mathcal{E}_f), E_f = P(\mathcal{E}_g), Q_g = P(Q_f), Q_f = P(Q_g), E_g = P(E_f)$ and $E_f = P(E_g)$ hold.

**Definition 6 (Quasi-positive matrix)**
A $n \times n$ matrix $B$ is said to be quasi-positive only when
\[ B + \lambda I_n \geq 0 \text{ for all sufficiently large } \lambda, \]
or when all components of $B$ except diagonal components are non-negative, where $I_n$ is $n \times n$ identity matrix. Note that a quasi-positive matrix is different from a positive semi-definite matrix.

**Definition 7 (Ordering $\leq_{B'}$)**
An ordered cone $\mathbb{K}_B$ of quasi-positive matrix $B$ is defined as
\[ \mathbb{K}_B = \{ \phi \in \mathbb{C}^n : \phi \geq 0 \text{ and } \phi(t) \geq e^B(t-s)\phi(s), -\tau \leq s \leq t \leq 0 \}. \]
$\mathbb{K}_B$ is closed in $\mathbb{C}^n$, closed under addition and under scalar multiplication by nonnegative scalars and $\mathbb{K}_B \cap (-\mathbb{K}_B) = \{0\}$. Moreover, $\mathbb{K}_B$ is a normal cone. The ordering $\leq_{B'}$ is also defined as
\[ \phi \leq_{B'} \psi \iff \psi - \phi \in \mathbb{K}_B \]
\[ \phi <_{B'} \psi \iff \phi \neq \psi \text{ and } \phi \leq_{B'} \psi \]
\[ \phi \ll_{B'} \psi \iff \phi_i \neq \psi_i \text{ for all } i \in N \]
\[ \text{and } \phi \leq_{B'} \psi. \]
For a smooth function $\phi$, $\phi \in \mathbb{K}_B$ is equivalent to
\[ \phi' \geq B\phi \text{ and } \phi \geq 0 \text{ on } [-\tau, 0]. \]

3. Main results

3.1. Convergence to equilibria

In this section, we prove that a genetic network with only positive feedback loops, which is also a monotone dynamical system [12] with cooperation, has no oscillation and strange attractors except equilibria.

**Definition 8 (Stable point)**
$\phi \in \mathbb{C}^+$ is a stable point of eqn. (1) if for every $\epsilon > 0$ there exists $\delta > 0$ such that $\|\Phi_t(\phi) - \Phi_t(\psi)\| < \epsilon$, for $t \geq 0$ whenever $\psi \in \mathbb{C}^+$ and $\|\phi - \psi\| < \delta$. We set $S_f$ to be the subset of stable points of eqn. (1). Observe that if $\phi \in S_f$ then points near $\phi$ have limit sets near $\omega(\phi)$. The set of stable points of eqn. (4), $S_g$, is similarly defined, and $S_g = P(S_f)$ and $S_f = P(S_g)$ hold.

Since all of results which are proven for eqn. (4) also hold for eqn. (1), we investigate only eqn. (4).

**Theorem 1**
Let eqn. (4) satisfies the following (T), (I'B) and (SM'B). Then $S_g \subset C_g$, and Int$C_g$ is dense in $C'$ for the semiflow generated by eqn. (4).

(T) $g$ maps bounded subsets of $C'$ to bounded subsets of $P(\mathbb{R}^+)$. For each $\phi \in C'$, $y(t, \phi)$ is defined for $t \geq 0$ and $\mathcal{O}^+(\phi)$ is bounded. For each compact subset $A_{C'}$ of $C'$, there exists a bounded set $B_{C'}$ of $C'$ such that $\omega(\phi) \subseteq B_{C'}$ for every $\phi \in A_{C'}$.

(SM'B) For every $\phi \in C'$ and every $\psi \in \mathbb{K}_B$, $\psi > 0$,
\[ dg(\phi)(\psi - B\psi(0)) > 0. \]

(I'B) If $\phi \in C'$, $\psi \in \mathbb{K}_B$, $I_J$ is a proper subset of $N$ such that $\psi_j > 0$ for $j \in I_J$ and $\psi_k(0) = 0$ for $k \notin I_J$ then for some $p \notin I_J$,
\[ dg(\phi)(\psi)_p > 0. \]

**Proof**: See theorem 4.1 in [13].

This theorem shows that if these assumptions are satisfied then there exists an open and dense set of convergent points, that is, almost all trajectories of eqn. (1) converge to equilibria. This property guarantees that a genetic switch has no attracting set except equilibria when constructed only with positive feedback loops.
However, the conditions \((T)\), \((SM'_B)\) and \((I'_B)\) are too abstract to understand. Thus, we rewrite these conditions.

\((T)\): From biological viewpoint, the saturation of \(g\) is naturally guaranteed because \(g\) describes synthesis rates of chemicals which cannot be unbounded. The boundedness of an orbit \(O^+(\phi)\) has already be assumed in the previous section. The existence of an attracting bounded set \(B_{C'}\) is also required, but it is biologically plausible to assume this condition.

\((SM'_B)\): To give a sufficient condition under which \((SM'_B)\) is satisfied, we modify corollary 3.9 in [13].

**Corollary 1**

Let \(dg(\phi) \in L(C, \mathbb{R}^n)\) be linearization of \(g\) at \(\phi \in C'\). If \(dg(\phi)\) is represented by the regular Borel measure \(\mu^\phi = (\mu^\phi_{ij})\) and if for every \(\phi \in C'\)

\[
\begin{align*}
(a) \quad & \int_{[-\tau,0]} d\mu^\phi(\theta)e^{B(\theta + \tau)} > Be^{B\tau} \\
(b) \quad & \int_{(s,0]} d\mu^\phi(\theta)e^{B\theta} \geq B, \quad -\tau \leq s < 0
\end{align*}
\]

then for \(\psi \in K_B\) and \(\phi \triangleright 0\)

\[dg(\phi)\psi - B\psi(0) \triangleright 0,
\]

holds.

**Proof:** By the equation (3.8) in [13], we have

\[
L(\phi) = G(0)e^{B\tau}\psi(-\tau) + \int_{[-\tau,0]} [G(0) - G(s)]d(e^{-Bs}\psi(s)).
\]

Since we assume \(\psi \triangleright 0\), if \(G(0)e^{B\tau} > 0\) and \(G(0) - G(s) \geq 0\) then \(L(\phi) > 0\) holds. Therefore, We have the proof of the corollary by applying to a linear operator \(L = dg(\phi) - BE\), where \(E\) is an operator satisfying \(E\psi = \psi(0)\).

Note that the matrix \(B\) should be quasi-positive in the corollary. Due to the definition of \(g\) and \(e^B > 0\), if we define \(b_{ij} = 0\) for \(i \neq j\) and \(b_{ii} < -d_i\) for \(i, j \in N\), clearly (a) and (b) are automatically satisfied.

\((I'_B)\): Due to \(\psi \in C'\) and \(s_{ij} \equiv s_{ij}^1 = \cdots = s_{ij}^{l_{ij}}\), it is easy to show that for every \(\psi \in K_B\) and for all \(i, j \in N, i \neq j, l = 1, 2, \ldots, l_{ij}\)

\[V_{ij}^l = \sum_{k=1}^l g_{ij}^k \psi_j(-\tau_{ij}^k) \geq 0.
\]

holds, which implies that \(V \equiv V_{ij}^l\) is quasi-positive. Therefore, \((I_B)\) holds if \(IG(g)\) is irreducible for all \(\psi \in K_B, \psi \triangleright 0\). In other words, there is no \(x_t \in C'\) at which the interaction graph of the network is decomposed into two irreducible sub-networks.

To summarize the discussion in this section, if a synthesized genetic switch, eqn. (1), is composed only of positive feedback loops and there is no \(x_t \in C'\) at which the interaction graph of the network is decomposed into two irreducible sub-networks, then eqn. (1) does not have any attracting set except equilibria. Actually, Such system is a monotone dynamical system with cooperation [12], which generates a strongly order preserving semiflow as shown in this paper.

### 3.2. Stability of equilibria

The stability of equilibria is one of the most important factors for designing a synthesized genetic switch because the states of the switch are stable equilibria. However, theorem 1 does not give any information on the stability of equilibria, although it shows that eqn. (4) does not have any attracting set except equilibria. In general, it is much more difficult to determine the stability of equilibria of FDEs than that of ODEs due to the transcendental characteristic equation of FDEs. Thus, it is better to avoid calculating the stability of equilibria of FDEs, if possible. Next theorem shows that the stability of equilibria is actually invariant even if we ignore the time delays.

**Theorem 2**

Let eqn. (7) be an associated ODE of eqn. (4) by ignoring all time delays

\[y(t) = G(y(t)) - Dy(t) \equiv \Phi(y(t))\]

where \(\tau_{ij}^k = 0\) for all \(i, j \in N\). Then eqn. (4) and (7) have identical equilibria. In addition, if eqn. (4) satisfies all conditions of Theorem 1, then each equilibrium of eqn. (4) and (7) has identical stability.

**Proof:** See Smith 1995 [12].

Theorem 2 implies that if \(v\) is an equilibrium and asymptotically stable (unstable) for eqn. (7), then it is asymptotically stable (unstable) for eqn. (4), and vice versa. Based on this theorem, instead of the complicated FDEs eqn. (4), we can only use an associated ODEs of eqn. (7) to design and analyze a genetic switch network only with positive feedback loops, which significantly reduce complexity of the problem and make designing of a large-scale multi-gene network tractable.

### 3.3. A reduction method

Although theorems 1-2 allow us to design or examine equilibria and their stability by much simple associated
ODEs of eqn. (4), it is still difficult to analyze nonlinear ODEs especially with high dimensions. To overcome this problem, we propose a reduction procedure to further simplify the ODEs to low dimensional ODEs with the same equilibria and stability as the original one.

Before showing the reduction procedure, we present the following theorem [12].

**Theorem 3**
Consider an ODE
\[ x'(t) = H(x(t)). \]
If this ODE has an equilibrium and the Jacobian matrix \( J \) of \( H(x) \) at the equilibrium is quasi-positive and irreducible then the spectral radius of \( J \), \( s(J) \), is a simple eigenvalue of \( J \) and satisfies
\[ s(J) < 0 \quad \text{if and only if} \quad \text{there exists } u > 0 \quad \text{such that} \quad Ju < 0. \] (8)

Next, we show that the Jacobian matrix of eqn. (7) is quasi-positive and irreducible. Since \( \sum_{i=1}^{l} g_{ij} \geq 0 \) holds for all \( l = 1, 2, \ldots, l_{ij}, \ i, j \in N, \ i \neq j \), the Jacobian matrix of eqn. (7) is quasipositive. Moreover, if \( \psi \) is irreducible for arbitrary \( \psi \in K_{B} \) and \( \phi > 0 \), that is, \( (I_{B}) \) is satisfied, then Jacobian matrix of \( G(y) \) is irreducible on \( R^{l} \) because of \( \psi = 1 \in K_{B} \). If a solution \( y(t, \phi) \) of eqn. (7) with initial condition \( \phi \in R^{l} \) has a compact closure for \( t \geq 0 \) then almost all solutions starting at a point on \( R^{l} \) converge to equilibria [12]. Actually the condition that the solution has a compact closure corresponds to the condition (T) of eqn. (10) and
\[ \mathfrak{A}' = J - D, \]

where \( G_{ij} = \partial G_{i}/\partial y_{j} \). We do not explicitly indicate the equilibrium point at which \( \mathfrak{A} \) and \( J \) are calculated for readability. In addition, we define \( J' \) to be a matrix obtained by removing the components of \( J \) enclosed by horizontal and vertical lines from \( J \) of eqn. (10) and
\[ \mathfrak{A}' = J' - \text{diag}(d_{1}, \ldots, d_{i-1}, d_{i+1}, \ldots, d_{n}). \]

Next, assume that \( G_{k}(y) = G_{k}(y_{i}, y') \) where \( y' = (y_{1}, \ldots, y_{1-i}, y_{i+1}, \ldots, y_{n}) \). By substituting \( y_{i} = G_{i}(y')/d_{i} \) into this equation, we obtain
\[ y_{k} = G'_{k}(y') - d_{k}y_{k} = G_{k}(1/d_{i}G_{i}(y'), y') - d_{k}y_{k}, \]
for \( k = 1, \ldots, i-1, i+1, \ldots, n \). The derivatives of this equation at an equilibrium point are
\[ G_{k} + \frac{1}{d_{i}}G_{ki}G_{ij} \geq 0 \quad \text{if} \quad j \neq k \]
\[ G_{kk} + \frac{1}{d_{i}}G_{ki}G_{ik} - d_{k} \quad \text{if} \quad j = k. \]

Thus, the Jacobian matrix of eqn. (9) is
\[ \mathfrak{A}' = J' - D' \]
\[ + \frac{1}{d_{i}} \begin{pmatrix} G_{1i} & \cdots & G_{1i} \\ \vdots & \ddots & \vdots \\ G_{ni} & \cdots & G_{ni} \end{pmatrix} \left( \begin{pmatrix} G_{1i} \\ \vdots \\ G_{ni} \end{pmatrix} \right)^{t}, \]
where \( t \) indicates transposition of a matrix. If \( s(\mathfrak{A}) < 0 \), there exists \( u > 0 \) such that \( \mathfrak{A}u < 0 \). Hence, \( \sum_{k=1}^{n} G_{jk}u_{k} - d_{j}u_{j} < 0 \) holds for all \( j \in N \). Let \( u' \)
be \(n-1\) vector obtained by removing \(u_i\) from \(u\). Note that \(u' > 0\). Then for all \(j \in \{1, \ldots, i-1, i+1, \ldots, n\}\),

\[
[\mathbf{A}'u']_j = \sum_{k=1}^{i-1} G_{jk}u_k + \sum_{k=i+1}^{n} G_{jk}u_k - d_j u_j + \frac{G_{ji}}{d_i} \left[ \sum_{k=1}^{i-1} G_{ik}u_k + \sum_{k=i+1}^{n} G_{ik}u_k \right]
\]

where we use the assumption that \(i\)-node does not have the edge \(e_{ii}\), that is, \(G_{ii} = 0\). According to this, \(\mathbf{A}u < 0\) and \(G_{ji} \geq 0\), we have \(\mathbf{A}'u' < 0\), which proved the theorem. 

By using this theorem, the associated ODEs can be reduced to a lower dimensional network step by step unless the all nodes of the interaction graph of the reduced network have direct feedback edges as schematically demonstrated in fig. 2. The finally obtained low dimensional ODEs are easy to analyze comparing to the original high dimensional ODEs. In addition, theorem 4 indicates that the dimensions of a genetic network can be reduced at least as low as the number of loops that the network has. Figure 2 demonstrates this method by the interaction graph \(IG(f)\). As defined above, each node corresponds to a variable of eqn. (1). By applying this method, a node in \(IG(f)\) without any direct feedback loop is eliminated, and the edges coming into this node and going out from this node are merged. Then, we finally obtain lower dimensional ODEs and the corresponding interaction graph with less number of nodes than original one (eqn. (1))

### 3.4. A procedure to design an artificial genetic switch

Theorem 4 tells us how we can reduce the dimensionality of a genetic network model to simplify the analysis and computation of the associated ODEs. In contrast, next theorem shows how we increase the dimensionality of a genetic network model while preserving equilibria and their stability.

**Theorem 5**

Let a transformation from eqn. (9) to eqn. (7) be

\[
y_i = G_i(y')/d_i \quad \iff \quad y_i = G_i(y) - d_i y_i.
\]

Assume that both Jacobian matrices of eqn. (7) and eqn. (9) are quasi-positive and irreducible, and that the positive orbits of eqn. (7) and eqn. (9) have a compact closure. Then eqn. (9) and eqn. (7) have the same equilibria and their stability is identical.

**Proof**: If \(s(\mathbf{A}') < 0\), there is a \(n-1\) vector \(u' = (u_1, \ldots, u_{i-1}, u_{i+1}, \ldots, u_n)\) such that \(\mathbf{A}'u' < 0\). If \(G_j\) of eqn. (7) does not depend on \(x_j\), that is \(G_{ji} = 0\), then \(\mathbf{A}u j < 0\) because of \(\mathbf{A}u_j = [\mathbf{A}'u'_j]\). Hence, we consider the case where \(G_{ji} > 0\). Then,

\[
[\mathbf{A}'u']_j = \sum_{k=1}^{i-1} G_{jk}u_k + \sum_{k=i+1}^{n} G_{jk}u_k - d_j u_j + \frac{G_{ji}}{d_i} \left[ \sum_{k=1}^{i-1} G_{ik}u_k + \sum_{k=i+1}^{n} G_{ik}u_k \right].
\]

Due to \(G_{ik} \geq 0\) and \(u_k > 0\),

\[
\sum_{k=1}^{i-1} G_{ik}u_k + \sum_{k=i+1}^{n} G_{ik}u_k > 0, \quad (10)
\]

holds for all \(k \in \{1, \ldots, i-1, i+1, \ldots, n\}\). Because of \(G_{ii} = 0\) and \(d_i > 0\), we have \(\mathbf{A}u_i = \sum_{k=1}^{i-1} G_{ik}u_k + \)
\[ \sum_{n=1}^{\infty} G_{ik} u_k - d_i u_i < 0 \]

In addition, for \([\mathcal{A}'u']_j = [\mathcal{A}u]_j + \sum_i G_{ji} [\mathcal{A}u]_i < 0\), we have \(u\) such that \([\mathcal{A}u]_j < 0\) and \([\mathcal{A}u]_i < 0\) by arranging \(u_i\) properly. Furthermore, it is possible to adjust \(u_i\) such that the previous inequality holds for all \(j\). Thus, we have \(u_i\) such that \([\mathcal{A}u]_j < 0\) for all \(j\), and \([\mathcal{A}u] < 0\), namely, \(s(\mathcal{A}) < 0\) is derived.

Theorem 5 guarantees that we can design a synthesized genetic switch by using the following procedure:

- Designing a switch with the simplest model to have the required configuration, equilibria and their stability, even such model may not realistic from biological viewpoint.
- Adding components one by one to the model according to theorem 5 to make the model more realistic and easy to implement experimentally. According to theorem 5, the enlarged model preserves the static properties of the system in terms of equilibria and their stability.

This procedure is schematically described in fig. 3. In fig. 3, starting with an abstract model of a genetic switch and the corresponding interaction graph, we obtain biologically realistic enlarged model by adding some components and edges to the interaction graph of the model. Although we have to incorporate time delays into the model to estimate the behaviors of the switch, theorems 1 - 5 guarantees that the more realistic model with time delays has the same equilibria with one designed with ODEs and their stability is identical to that obtained with ODEs.

**Figure 3:** A schematic diagram of the design procedure proposed in theorem 5.

4. Implementation and numerical simulation

We use an example in this section to verify our theoretical results.

First, we start with an abstract genetic switch composed of two components, as shown in fig. 4.

**Figure 4:** Two-component and three-feedback loops model. Each component has positive direct feedback loop (solid lines), and their mutual interactions form a positive feedback loop (broken lines).

Assume that all interactions are positive, which means that all feedback loops are also positive. Simple algebraic analysis shows that this network can have three stable equilibria, as indicated in fig. 5.

**Figure 5:** The null-clines of two-components and three feedback loops model equations. Each intersection of the two null-clines corresponds to an equilibrium point. In this case, the model has three stable equilibria.

In other words, the switch has three states. By applying the procedure proposed in previous section, we derive a realistic design of the switch depicted in fig. 6 with three genes and three promoters [15], where fig.
7 is a schematic diagram of the switch. In this switch, we use lacI, tetR, and ci genes, and Pt tetO − 1, P trc2, and P RM promoters, where P RM is mutated to disable O R RM site. In fact, lacI, tetR genes with Pt tetO − 1 and P trc2 promoters were artificially engineered [16] and were used to construct a synthesized genetic switch (two-state toggle switch) [1].

The binding site O R 1 of P RM promoter is artificially alternated (or mutated) so that ci protein cannot bind to O R 1. Although there is no detail experimental report for the mutated P RM, a plenty of quantitative experimental data on ci proteins and wild type P RM promoter allow us to predict its qualitative functions. Due to the mutated O R 2 site, ci proteins are expected only to activate the expression of the gene located downstream of the mutated P RM, by binding to O R 1 and O R 2 sites of the mutated P RM.

![Figure 6](image)

**Figure 6:** An implementation of two-component and three-feedback loops model with lacI, tetR, and ci. The signs indicate the types of interactions between the genes.

![Figure 7](image)

**Figure 7:** Schematic diagram of the implementation of two-component and three-feedback loops model. The broken line indicates the feedback loop with lacI and tetR genes which is identical to a toggle switch examined in [1]. The thick line indicates direct feedback loop of ci.

To formulate the mathematical equations, we distinguish tetR and ci genes with different ribosome binding site (RBS) by subscripts. Then, the FDEs of this network can be described as follows:

\[
\begin{align*}
\frac{dm_{lacI}}{dt} &= f_{P_r, tetO-1}(p_{tetR}(t - \tau_{P_r, tetO-1})) - d_{m_{lacI}} m_{lacI} \\
\frac{dp_{lacI}}{dt} &= s_{m_{lacI}} (m_{lacI}(t - \tau_{lacI})) - d_{p_{lacI}} p_{lacI} \\
\frac{dm_{tetR1}}{dt} &= f_{P_{trc2}}(p_{lacI}(t - \tau_{P_{trc2}})) - d_{m_{tetR1}} m_{tetR1} \\
\frac{dm_{tetR2}}{dt} &= f_{P_{trc3}}(p_{ci}(t - \tau_{P_{trc3}})) - d_{m_{tetR2}} m_{tetR2} \\
\frac{dp_{tetR}}{dt} &= s_{m_{tetR1}} (m_{tetR1}(t - \tau_{tetR1})) \\
&+ s_{m_{tetR2}} (m_{tetR2}(t - \tau_{tetR2})) - d_{p_{tetR}} p_{tetR} \\
\frac{dm_{ci1}}{dt} &= f_{P_{trc1}}(p_{ci}(t - \tau_{P_{trc1}})) - d_{m_{ci1}} m_{ci1} \\
\frac{dm_{ci2}}{dt} &= f_{P_{trc3}}(p_{ci}(t - \tau_{P_{trc3}})) - d_{m_{ci2}} m_{ci2} \\
\frac{dp_{ci}}{dt} &= s_{m_{ci1}} (m_{ci1}(t - \tau_{ci})) \\
&+ s_{m_{ci2}} (m_{ci2}(t - \tau_{ci})) - d_{p_{ci}} p_{ci}
\end{align*}
\]

where m and p indicate concentrations of mRNAs and proteins of genes assigned by subscripts. s and d indicate synthesis rates of proteins, and degradation rates of mRNAs and proteins, respectively. f indicates synthesis rate of mRNA enhanced by the corresponded promoters. \(\tau\) represents time delay due to transcription, translation and transportation.

By applying the proposed procedure in theorem 4 and normalizing the equations properly, we obtain the following two dimensional differential equations, which preserve equilibria and their stability of the network:

\[
\begin{align*}
\frac{dp_{tetR}}{dt} &= f_1(p_{tetR}) + \epsilon_{tetR} f_2(p_{ci}) - p_{tetR} \quad (11) \\
\frac{dp_{ci}}{dt} &= k [\epsilon_{ci} f_1(p_{tetR}) + f_2(p_{ci}) - \delta_{p_{ci}}] \quad (12)
\end{align*}
\]

where

\[
\begin{align*}
f_1(p_{tetR}) &= \frac{s_{m_{tetR1}}}{d_{m_{tetR1}} d_{p_{tetR}}} \\
&\times f_{P_{trc2}} \left( \frac{s_{m_{lacI}} f_{P_r, tetO-1}(p_{tetR})}{d_{m_{lacI}} d_{p_{lacI}}} \right) \\
f_2(p_{ci}) &= \frac{s_{m_{tetR2}}}{d_{m_{tetR2}} d_{p_{tetR}}} f_{P_{trc3}}(p_{ci}) \\
\end{align*}
\]

\[
\epsilon_{tetR} = \frac{s_{m_{tetR2}}}{s_{m_{tetR1}}}, \quad \epsilon_{ci} = \frac{s_{m_{ci1}}}{s_{m_{ci2}}}, \quad k = \frac{s_{m_{tetR1}} s_{m_{ci2}}}{d_{p_{tetR}} d_{m_{ci2}}} \text{ and } \delta = \frac{d_{p_{ci}}}{k d_{p_{tetR}}}.
\]

Since \(f_{P_{trc2}}(p_{lacI})\) and \(f_{P_r, tetO-1}(p_{tetR})\) are monotonously decreasing functions but \(f_{P_{trc3}}(p_{ci})\) is a monotonously increasing function, both \(f_1(p_{tetR})\) and
$f_2(p_{cI})$ are monotonously increasing functions, which satisfy the conditions of theorems 1-5. According to the previous work [1], $\frac{dp_{tetR}}{dt} = f_1(p_{tetR}) - p_{tetR}$ can have two stable equilibria by inserting proper RBS to $tetR_1$ and $lacI$ genes [1]. Therefore, if we set $\epsilon_{tetR}$ small enough, namely, we choose proper RBS for $tetR_2$, the null-cline of eqn. (11) can be as depicted by broken line in fig. 5. Similarly, $\frac{dp_{cl}}{dt} = \kappa [f_2(p_{cI}) - \delta_{p_{cl}}]$ has three stable equalibria or one stable equilibrium at low expression level. Note that changing order of two genes downstream of a promoter may result in a completely different switch due to inefficiency of poly-cistronic transcription. For instance, sometime the transcriptional efficiency of the second gene downstream of a promoter can be as low as $1/100$ as that of the first gene, although increasing translation efficiency (e.g. add or enhance RBS) of the gene can compensate this problem to some extent. However, the problem in our model is that activation of the protein $cI$ is so strong that $cI$ is always kept at high expression if gene $cI$ is located in the first place of promoters. Hence, although $\frac{dp_{cl}}{dt} = \kappa [f_2(p_{cI}) - \delta_{p_{cl}}]$ has only one stable equilibrium point at high expression level without any repressive factor, we could make $\frac{dp_{cl}}{dt} = \kappa [f_2(p_{cI}) - \delta_{p_{cl}}]$ to have three stable equilibria or one stable equilibrium at low expression level by locating $cI_2$ next to $tetR_2$ gene as shown fig. 6 due to inefficiency of poly-cistronic transcription (the transcription efficiency of $cI_2$ will decrease about 100 times). In addition, if we locate $cI_1$ genes as shown in fig. 6 and set $\epsilon_{cI}$ small enough by choosing proper RBS for $cI_1$, the null-cline of eqn. (12) can be as depicted by solid line in fig. 5.

Based on these qualitative observation, we conduct a numerical simulation for fig. 6 and calculate null-clines of eqn. (11) and eqn. (12) (fig. 8) where we set

\[
\begin{align*}
\frac{f_{P_{tetO}}(p_{tetR})}{d_{mi_{lacI}}} & = 0.1 + \frac{1}{0.7^2 + p_{tetR}^2} \\
\frac{s_{mi_{tetR}}f_{Ptrc2}(p_{lacI})}{d_{mi_{tetR}}d_{pi_{tetR}}} & = 0.1 + \frac{1}{0.8^4 + p_{lacI}^4} \\
\frac{s_{mi_{tetR}}f_{P_{AM}}(p_{cI})}{d_{mi_{tetR}}d_{pi_{tetR}}} & = 0.1 + \frac{2p_{cI}^2 + \delta_{p_{cI}}^2}{1 + p_{cI}^2},
\end{align*}
\]

and $\epsilon_{tetR} = 0.1$, $\epsilon_{cI} = 0.1$, $\kappa = 1$ and $\delta = 1$. The same geometry of null-clines of eqn. (11) and eqn. (12) as in fig. 5 is obtained. It is notable that it is not difficult to obtain this geometry by setting $\epsilon_{tetR}$ and $\epsilon_{cI}$ small. By inserting proper RBS to a gene, the translation of the gene is enhanced about 10-folds or more. Thus, in this sense, $\epsilon_{tetR} = 0.1$ and $\epsilon_{cI} = 0.1$ are reasonable, and this switch is expected to be implemented experimentally without much difficulty.

![Figure 8: Geometric structure of eqs. 11 and 12 calculated based on eqn. (13).](image)

## 5 Conclusion

In this paper, we have proven that genetic networks with only positive feedback loops have desirable properties as switches, and developed a new procedure to design synthetic genetic switch networks with multiple genes and time delays, by using monotone dynamical theory. We show that the networks with only positive feedback loops have no oscillation except equilibrium whose stability is also independent of the time delays. In other words, such systems have ideal properties for switch networks and can be designed without consideration of time delays, because the systems can be reduced from functional spaces to Euclidian spaces. Specifically, we first prove the basic properties of the synthesized genetic networks composed of only positive feedback loops, and then propose a procedure to design the switches, which drastically simplifies analysis of the switches and makes theoretical analysis and designing tractable even for large-scale systems. Finally, to demonstrate our theoretical result, we have designed a realistic synthetic switch with three states using experimentally well investigated $lacI$, $tetR$ and $cI$ genes and their corresponding promoters, with numerical simulation. This simulation shows that the design we proposed can have three states only by properly choosing only two parameters, RBS of $tetR_2$ and that of $cI_1$.

However, our model does not include stochastic ef-
fect of chemical reactions especially probabilistic attachment of regulatory proteins at their binding sites. A recent integrated study of theoretical model and a de novo synthesized genetic network shows that the cl positive feedback loop is easily subject to such stochastic effect than the toggle switch composed of lacI and tetR genes [17–19]. Therefore, it is important to clarify how stochastic effects influence the predicted behaviors of the switch by using a deterministic model in the future. In addition, it is also necessary to investigate how time delays affect stochastic nature of synthesized genetic networks via both theoretical and experimental approaches.

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