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著者	Nakakuki Takashi, Imura Jun-ichi
journal or	Automatica
publication title	
volume	114
page range	108826-1-108826-10
year	2020-01-17
URL	http://hdl.handle.net/10228/00008679

doi: https://doi.org/10.1016/j.automatica.2020.108826

${\bf Finite-time\ regulation\ property\ of\ DNA\ feedback\ regulator\ }^{\star}$

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Abstract

In dynamic DNA nanotechnology, the DNA strand displacement technique provides the cornerstone for the bottom-up design of a man-made DNA molecular system. Practically, a feedback controller for regulating the concentration of a target DNA strand to the desired level is indispensable for mediating the kinetic momentum of a molecular actuator. However, such a regulator system operates by consuming fuel strands, and requires sufficient supplies of these consumables for its normal execution, indicating that, in practice, optimal controller design requires the period of time during which the regulator proceeds with normal operation to be as long as possible. The fact that the system is naturally high dimensional and nonlinear complicates the analysis of properties emerging during a finite-time period in terms of their theoretical aspects. In this paper, we first define the new concept of a "finite-time regulation property" of DNA systems in the regulation problem. Then, to theoretically analyze this regulation property, we present two-time-scale modeling based on the difference in the initial distribution of the abundance of DNA strands. Focusing on the fast mode as a subsystem with a positive quadratic structure, we propose a new method for analyzing the regulation property observed in a finite period of time.

Key words: Complex systems, Nonlinear systems, Regulator theory, Singular perturbation method, Control system analysis

1 Introduction

Molecular computing is a research field that aims to program biochemical reactions equipped with a specific information-processing capability, thereby harnessing the chemical nature of biomolecules [3]. Especially, deoxyribonucleic acid (DNA) has attracted attention as a versatile biomolecule in the sense that it can be artificially synthesized, programmable, and applied to build structures, sensors, circuits, and actuators [27,25,8,15,19]. This is because the fundamental physical-chemical characteristics of DNA base pairs are fully understood, such that the molecular interaction is statically and kinetically controllable by designing the base sequences appropriately.

Recently, enthusiastic challenges have been expanded to establish technologies for molecular computing to control a full-fledged molecular system [1,29]. Especially,

the remarkable progress achieved by molecular logic circuits opened up the possibility for the rational design of functional circuits implemented on DNA strand displacement (DSD) reactions [23]. In principle, various kinds of information processing are realizable in vitro by suitably connecting an output strand of a DSD reaction to an input strand of another DSD reaction to successfully create an ensemble of connected DSD reactions, which provides the cornerstone for the bottom-up design of DSD reaction systems (hereafter referred to as a DSD system) [17]. It follows that a variety of DSD systems such as logic gates [23,30], arithmetic circuits [7], and more complicated combinatorial circuits [30,2] have been developed in cooperation with the growing availability of computer-aided software for base sequence design. Moreover, the practical problems pertaining to the reliability (e.g., leakage reactions) and performance (e.g., response time) of DSD systems are expected to drastically improve by using extensive leakless designs [26] and various photon-fueled DNA nanodevices [11].

One of the attractive applications of DSD system design is to develop a controller for a molecular machine such as a molecular robot. The concept of a molecular robot has emerged as a symbolic milestone in the crosscutting research field spanning nanotechnology, molecular computing, and robotics, and provides us with a

Preprint submitted to Automatica

31 December 2019

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^{*} This work was supported by JSPS KAKENHI Grant Number 15H01715, 15H00823, and 17K06500. The material in this paper was not presented at any conference. Corresponding author T. Nakakuki.

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fully new robotic system from the perspective of control theory [9]. A molecular robot is an autonomous mobile system comprising biological molecules, which is defined as having a compartmentalized interior space containing a reaction field for a DSD system, a sensing mechanism that converts our command from the external world into an internal signal, and a self-driven actuator. For example, an autonomous amoeba-like robot [20] has a capsule-like frame consisting of a phospholipid bilayer, and a modified kinesin actuator of which the driving force is generated by microtubule-based kinesin movement. Importantly, the microtubule-kinesin interaction that determines the kinetic momentum of the actuator is mediated by a DSD system, and this interaction can therefore be controlled by the concentration of a DNA strand as a control input released from a control circuit. Then, a typical control problem would be to design a feedback regulator that would be able to keep the number of microtubule-kinesin interactions constant. Such a controller design problem is generalized as the statement "Design a DSD system for regulating the concentration of a specific output strand to a desired level in a given reaction field". For the sake of argument, in this study, we refer to such a controller as a "DNA feedback regulator."

DSD-based feedback regulators can operate under a broad range of reaction conditions, which is strongly advantageous for application to an extensive range of molecular machines. One of the remarkable studies on the design of the DNA feedback regulator includes the PI-like controller, where the three fundamental linear I/O elements (integration, gain, and summation) made with DSD systems are introduced to realize the proportional and integral elements for the controller [12,28]. In addition, inspired by this study, the design of PID controllers containing differential elements has also been reported [16]. In other instances, a simple on/off-like controller, in which a DNA comparator and an autocatalytic amplifier are incorporated into the regulator, has been proposed [10]. Recently, a nonlinear quasisliding-mode feedback controller has also been proposed [21] on the basis of the unimolecular and bimolecular design that provides the cornerstone for the creation of arbitrary chemical reaction systems [24].

On the other hand, a DSD system operates while consuming fuel strands, which serve as a kind of driving energy, and requires sufficient supplies of these consumables for its normal execution (See Definition 1 for the details). Because it is not always possible to continually supply a molecular machine with the fuel strands from a peripheral system, the regulator operates normally within a limited period of time, indicating that, in practice, optimal controller design requires the period of normal operation of the regulator to be as long as possible. However, because the DNA feedback regulator system is a nonlinear and high-dimensional positive system characterized as a biochemical reaction mechanism, the regulation property of the closed-loop system observed within a finite-time period has not yet been theoretically analyzed, and remained an open problem after we completed our previous work [10]. This would have to be accomplished to refine the regulator for practical application to a molecular machine.

Therefore, in this study, we establish a framework for the theoretical analysis of the regulation property, with the aim of controlling a general DSD system more effectively. To this end, we first demonstrate that the regulation can be achieved only during a finite period of time under the condition that the fuel strands remain entirely in the reaction field. This is demonstrated by using a typical example of a design of a DNA feedback regulator. Then, the key property that we really have to discuss here in the regulation problem is clearly defined as a "finite-time regulation property." Subsequently, an analytical framework for the finite-time regulation property is established according to the biochemical reaction mechanism and the structural feature of the positive quadratic system [13,14]. To this end, (i) inspired by the nondimensional transformation in [22], we propose to model the DSD system on two time scales. This approach is based on the difference in the initial distribution of the abundance of DNA strands. In addition, (ii) the properties the slow and fast modes in this model with two time scales satisfy are investigated in the context of singular perturbation theory [6]. The finite-time regulation property is shown to correspond to the behavior of the fast modes. Importantly, the regulation phenomenologically observed with the successful simulation is theoretically confirmed by the proposed systematic methodology. Noteworthy is that the approach of using the singular perturbation theory along with twotime-scale modeling methods has a rich history of solving problems related to a variety of biological systems. Especially, a "non-standard" problem statement such that the algebraic equation in terms of a fast system fails to have a unique solution frequently appears for biochemical reaction systems [5,22]. Although our problem statement is partially similar to these studies, our results provide a powerful framework to practically overcome the feedback regulation problem of the DSD system, in which our theoretical derivations are simplified by utilizing the beneficial structure of the positive quadratic system [13,14] in the context of DSD systems.

2 Problem statements of DNA feedback regulator

2.1 Design and operating principle of DSD systems

The DSD reaction consists of the three main reaction processes denoted by bidirectional two-way or three-way arrows in Fig. 1: In step 1, a single-stranded input strand X_1 , binds to the double-stranded X_2 comprising output and substrate strands. The input strand is designed to



Fig. 1. DNA strand displacement reaction.

bind to the "toehold" sequence in the substrate strand by designing their toehold sequences such that they are complementary to each other. In step 2, a random process known as branch migration occurs thermodynamically in order to decrease the Gibbs free energy. This process results in the output strand on trimeric X_3 gradually becoming detached from the helical structure. This detachment eventually leads to X_4 , which is hijacked by the input strand. In step 3, trimeric X_4 dissociates into double-stranded X_5 and single-stranded output strand X_6 . In accordance with the fact that changes in the concentration of strands in a DSD reaction as a function of time can be modeled by ordinary differential equations based on chemical reaction kinetics [31], the DSD reaction is described by

$$\dot{\xi}_{1} = -k_{f_{1}}\xi_{1}\xi_{2} + k_{r_{1}}\xi_{3}
\dot{\xi}_{2} = -k_{f_{1}}\xi_{1}\xi_{2} + k_{r_{1}}\xi_{3}
\dot{\xi}_{3} = k_{f_{1}}\xi_{1}\xi_{2} - k_{r_{1}}\xi_{3} - k_{2}(\xi_{3} - \xi_{4})
\dot{\xi}_{4} = k_{2}(\xi_{3} - \xi_{4}) + k_{f_{3}}\xi_{5}\xi_{6} - k_{r_{3}}\xi_{4}
\dot{\xi}_{5} = -k_{f_{3}}\xi_{5}\xi_{6} + k_{r_{3}}\xi_{4}
\dot{\xi}_{6} = -k_{f_{3}}\xi_{5}\xi_{6} + k_{r_{3}}\xi_{4},$$
(1)

where ξ_i (i = 1, ..., 6) denotes the concentration of singleor double-stranded X_i . The coefficients k_{f_i} , k_{r_i} (i = 1, 3), and k_2 denote the association, dissociation, and branch migration rate constants, respectively. This small circuit generates the output strand X_6 upon the administration of the input strand X_1 where the strand X_2 contributes to providing fuel (energy) for the system. Thus, the initial concentration $\xi_2(0)$ should be a large value, and $\xi_2(0) \gg \xi_i(0), \forall i \in \{1, 3, 4, 5, 6\}$ under practical experimental conditions.

In general, the ordinary differential equations of the connected DSD reactions are simply constructed by integrating the equations of each DSD reaction, and mutually adding binding-unbinding reactions between the input and output strands to connected DSD reactions. Hence, a general form of a DSD system is given by

$$\xi = \psi(\xi), \ \xi(0) = \xi_0,$$
 (2)

where $\xi \in D_{\xi} \subset \mathbb{R}^{\zeta}$ is a state vector expressing the concentration of DNA strands, and $\psi : D_{\xi} \to \mathbb{R}^{\zeta}$ is a nonlinear function characterized by the mass action law as shown in (1).

Noting that the strands in a DSD system can be classified into two groups according to their initial concentrations, we now define both the signal and fuel strands in a DSD system. This definition provides an important concept that is used throughout the paper.

Definition 1 (Signal and fuel strands) Let I_{fuel} and I_{signal} be index sets such that $\xi_i(0) \gg \xi_j(0)$, $\forall i \in I_{\text{fuel}}$, $\forall j \in I_{\text{signal}}$ and $I_{\text{fuel}} \bigcup I_{\text{signal}} = \{1, ..., \zeta\}$. The symbol X_i is termed a signal strand for $i \in I_{\text{signal}}$, and a fuel strand for $i \in I_{\text{fuel}}$.

Remark 1 Information processing using signal strands is executed by continuously consuming fuel strands, which are prepared in sufficient amounts in the reaction field under the initial conditions [18]. It is a noticeable property that a DSD system operates normally for a certain period of time when the reaction fields are rich in fuel strands. Although it would be possible to supply the DSD system with a fuel strand from outside in an "in-vitro" open system, this would be technically difficult and unrealistic in a molecular robot as a closed capsule.

2.2 Motivating example

Fig. 2 shows a simple realization of a DNA feedback regulator system. The plant (denoted by Σ_P) is assumed to be a simple DSD system in this toy example. The control objective is to regulate the output y_p to the reference concentration r^* . For the sake of simplicity, we set the time when the reference concentration r^* is given in the system to t = 0, that is $r(0) = r^*$. The controller structure is based on the integral action with a pair of auto-catalytic DNA amplifiers for the reference r and the output y_p (denoted by $\Sigma_A^{(r)}$ and $\Sigma_A^{(y)}$) [17] along with a hybridization-based subtractor to calculate the control input u_p (See Section 4 for the detailed explanation).

Fig. 3 shows the short-term (A) and long-term (B) simulation results of the DNA feedback regulator. Despite the successful result in panel (A), perfect regulation is limited to a certain period of time as indicated in the long-term simulation in panel (B), where the output $y_p(t)$ gradually decreases to zero, diverging from the reference r(t) as the fuel in $\Sigma_A^{(r)}$ is depleted with time. This behavior results from the finiteness problem where $\Sigma_A^{(r,y)}$ needs a sufficient supply of fuel strands for their normal operations, and is an inevitable/natural property of a



Fig. 2. Schematic of a simple DNA feedback regulator.



Fig. 3. Simulations of the DNA feedback regulator.

DSD system (See Remark 1). Therefore, the meaning of "regulation" in a molecular machine would need to be re-defined in a wholly new manner.

2.3 Problem statements

Consider a DNA feedback regulator system given by

$$\begin{aligned} \dot{\xi} &= \psi_{\rm dfr}(\xi), \ \xi(0) = \xi_0, \\ y_p &= c_{\rm dfr}\xi, \end{aligned} \tag{3}$$

for a time period $t \in [0, t_1]$, where $\xi = [r \xi_{\bar{r}}]^T \in D_{\xi} \subset \mathbb{R}^{\zeta}$ is the state vector comprising the reference signal $r \in \mathbb{R}$ and other strands $\xi_{\bar{r}} \in \mathbb{R}^{\zeta-1}$, $y_p \in \mathbb{R}$ is the output signal, $\psi_{\text{dfr}} : D_{\xi} \to \mathbb{R}^{\zeta}$ is a nonlinear vector-valued function characterized by the mass action law, and $c_{\text{dfr}} \in \mathbb{R}^{1 \times \zeta}$ is a constant vector. We propose the following property the DNA feedback regulator needs to satisfy.

Definition 2 (Finite-time regulation property)

System (3) is said to have the finite-time regulation property if, for each of all e > 0 and $t_b \in (0, t_1)$, there exists a set of initial states $\Omega_{\xi} \subset D_{\xi}$ such that for all $\xi_0 \in \Omega_{\xi}$,

$$|y_p(t) - r(t)| \le e, \ \forall t \in [t_b, t_1].$$

$$\tag{4}$$

Then, the remaining problem is how to determine whether the DNA feedback regulator possesses the finitetime regulation property. Importantly, conventional stability theory such as the Lyapunov stability theory, which considers the trajectories to converge to the steady state as time goes to infinity, cannot be applied in this case. On the other hand, Fig. 3 implies that the output y_p temporarily stays at approximately r for a certain period of time, which reminds us of the concept of a quasi-steady state in singular perturbation theory [6]. Then, the key point is whether a DSD system can be transformed into a model with two time scales. Do fast and slow modes exist in a DSD system? If so, how can we separate the states into modes with two time scales? In the following section, we first provide a solution to these questions. Based on the transformation, we next propose a theoretical framework for analyzing the finite-time regulation property, taking advantage of singular perturbation theory.

3 Analysis framework for finite-time regulation property

3.1 Two-time-scale modeling for DSD system

Let us investigate the structure of the nonlinear function $\psi(\xi)$ in the DSD system (2). All the reactions consist of binding, unbinding, and branch migration. In the chemical reaction kinetics, the reaction rate of the binding process is modeled by a quadratic term (e.g., $k_{f_1}\xi_1\xi_2$ in (1)), and the reaction rates of the unbinding and branch migration processes are approximated by a linear term (e.g., $k_{r_1}\xi_3$ and $k_2(\xi_3 - \xi_4)$ in (1)). Since a binding process involving X_i reduces the amount of X_i while generating a new complex, the quadratic term always has a negative influence on the reaction velocity dX_i/dt , and a positive influence on the others. We can also make a similar statement about the linear term. Based on the above consideration, the general form (2) is formulated by

$$\dot{\xi}_{i} = \sum_{j=1}^{\zeta-1} \sum_{k=j+1}^{\zeta} \alpha_{j,k}^{(i)} k_{f_{j,k}}^{(i)} \xi_{j} \xi_{k} + \sum_{j=1}^{\zeta} \beta_{j}^{(i)} (k_{r_{j}}^{(i)} + k_{j}^{(i)}) \xi_{j}, (5)$$

with $\xi_i(0) = \xi_{i_0} \in D_{\xi_i} \subset D_{\xi}$ for $i \in \{1, ..., \zeta\}$, where $k_{f_{j,k}}^{(i)}$ is the association rate constant $(k_{f_{*,*}})$ between strands X_j and X_k , and $k_{r_j}^{(i)}$ and $k_j^{(i)}$ are the dissociation (k_{r_*}) and branch migration (k_*) rate constants of strand X_j as indicated by the subscript "j, k", respectively, acting on X_i as indicated by the superscript "(i)". The stoichiometric coefficients are defined as follows:

$$\alpha_{j,k}^{(i)} = \begin{cases} -1 \text{ if } i=j,k\\ 1 \text{ otherwise} \end{cases}, \ \beta_j^{(i)} = \begin{cases} -1 \text{ if } i=j\\ 1 \text{ otherwise} \end{cases}. (6)$$

Remark 2 (Positive quadratic) From a phenomenological perspective, it is obvious that the DSD system belongs to a class of positive systems. In fact, from (5), for all $\xi_1, \dots, \xi_{i-1}, \xi_{i+1}, \dots, \xi_{\zeta} > 0$ and $\xi_i = 0$ we have $d\xi_i/dt \ge 0$, which implies that the state ξ_i starting from a non-negative initial value never has a negative value. Hence, we can assume $D_{\xi_i} \subset \mathbb{R}_{\ge 0}$. Moreover, because the right-hand side of (5) comprises linear and quadratic terms at most, the DSD system belongs to a class of positive quadratic systems [14,13].

Next, we introduce a reasonable assumption by limiting the variation of the structures of reaction schemes that exclude some pathological strand designs while supporting the theoretical derivations.

Assumption 1 The DSD system (5) satisfies the following conditions:

(i) For each $i \in I_{\text{fuel}}$,

$$k_{f_{j,k}}^{(i)}=0,\ k_{r_{j}}^{(i)}=0,\ k_{j}^{(i)}=0,\ \forall j,k\in I_{\mathrm{fuel}}$$

- (ii) For each $i \in I_{\text{signal}}$, at least one of the following conditions holds:
 - (a) $\exists j \in I_{\text{signal}}, \exists k \in I_{\text{fuel}}, k_{f_{j,k}}^{(i)} \neq 0.$ (b) $\exists j \in I_{\text{fuel}}, \exists k \in I_{\text{signal}}, k_{f_{j,k}}^{(i)} \neq 0.$ (c) $\exists j \in I_{\text{signal}}, k_{r_j}^{(i)} \neq 0.$ (d) $\exists j \in I_{\text{signal}}, k_j^{(i)} \neq 0.$

Remark 3 Condition (i) states that fuel strands do not associate with other fuel strands, do not undergo selfdissociation into a pair of single strands, and do not cause themselves to undergo a branch migration reaction. Otherwise, in a test tube during sample preparation, a fuel strand as an energy resource would be drained rapidly while creating a large amount of waste by binding to other fuel strands, would be destroyed autonomously, or would undergo a structural transition, which implies that its structure would be too unstable to successfully implement the system in a wet experiment. Condition (ii) excludes a type of "pathological" reaction scheme with some special structures. It would be safe to say that these conditions do not reduce the size of the class of applicable DSD systems in a practical application.

Theorem 1 For the DSD system (5), consider the following procedure:

(i) Define the total concentrations by

$$T_f = \sum_{i \in I_{\text{fuel}}} \xi_i(0), \tag{7}$$

$$T_s = \sum_{i \in I_{\text{signal}}} \xi_i(0). \tag{8}$$

(ii) Normalize the state variables and time variables as

$$\bar{\xi}_i = \frac{\xi_i}{T_f}, \ \forall i \in I_{\text{fuel}},\tag{9}$$

$$\bar{\xi}_i = \frac{\xi_i}{T_s}, \ \forall i \in I_{\text{signal}}, \tag{10}$$

$$t_r = T_s k_f t, \tag{11}$$

where $k_f = \max_{i,j,k} (k_{f_{j,k}}^{(i)}).$

(iii) Classify the slow and fast modes: For some i, if the right-hand side of the transformed system under the coordinates of (ii) can be bracketed with T_s , that is,

$$k_f T_s \frac{d\bar{\xi}_i}{dt_r} = T_s \tilde{f}(\xi), \qquad (12)$$

where \tilde{f} is a function derived from (5), then the state $\bar{\xi}_i$ belongs to a slow mode, and otherwise to a fast mode, which determines the indexes $I_{\text{slow}} = \{i_{s_1}, ..., i_{s_n}\}$ for a slow mode and $I_{\text{fast}} = \{i_{f_1}, ..., i_{f_m}\}$ for a fast mode, respectively, where $n + m = \zeta$.

If Assumption 1 is satisfied, then the above procedure transforms system (5) into the following two-time-scale model:

$$\dot{x} = f(x, z, \varepsilon) = A_f(z)x + b_f(z, \varepsilon), \ x(0) = x_0 \in B_x, \ (13)$$

$$\varepsilon \dot{z} = g(x, z, \varepsilon) = A_g(x)z + \varepsilon b_g(z), \ z(0) = z_0 \in B_z, \ (14)$$

where $x = [x_1, ..., x_n]^T = [\bar{\xi}_{i_{s_1}}, ..., \bar{\xi}_{i_{s_n}}]^T \in D_x \subset \mathbb{R}^n_{\geq 0}$ and $z = [z_1, ..., z_m]^T = [\bar{\xi}_{i_{f_1}}, ..., \bar{\xi}_{i_{f_m}}] \in D_z \subset \mathbb{R}^m_{\geq 0}$ are the respective "dimensionless" slow and fast states, $B_x = \{x \in D_x | \sum_{i=1}^n x_i = 1\}$ and $B_z = \{z \in D_z | \sum_{i=1}^m z_i = 1\}$ are the sets of initial states, $A_f : D_z \to \mathbb{R}^{n \times n}$ and $A_g : D_x \to \mathbb{R}^{m \times m}$ are matrix-valued affine functions, $b_f : D_z \times \mathbb{R}_{>0} \to \mathbb{R}^n$ is composed of linear and/or quadratic terms with $b_f(0, \varepsilon) = 0$, $b_g : D_z \to \mathbb{R}^m$ consists of quadratic terms with $b_g(0) = 0$, and $\varepsilon = T_s/T_f > 0$ is a sufficiently small constant by (7) and (8) along with Definition 1.

Before proceeding to the proof, we claim the next Lemma.

Lemma 1 Under Assumption 1, $I_{\text{fuel}} = I_{\text{slow}}$ and $I_{\text{signal}} = I_{\text{fast}}$.

Proof: We devote this proof to showing that the two statements $I_{\text{fuel}} \subseteq I_{\text{slow}}$ and $I_{\text{signal}} \cap I_{\text{slow}} = \emptyset$. Then, the claim directly results from these two inclusion relations. Under Assumption 1 (i), the system (5) is rewritten as

$$\dot{\xi}_{i} = \sum_{j \in I_{s_{1}}} \sum_{k \in I_{f_{2}}} \alpha_{j,k}^{(i)} k_{f_{j,k}}^{(i)} \xi_{j} \xi_{k} + \sum_{j \in I_{f_{1}}} \sum_{k \in I_{s_{2}}} \alpha_{j,k}^{(i)} k_{f_{j,k}}^{(i)} \xi_{j} \xi_{k} + \sum_{j \in I_{s_{3}}} \sum_{k \in I_{s_{2}}} \alpha_{j,k}^{(i)} k_{f_{j,k}}^{(i)} \xi_{j} \xi_{k} + \sum_{j \in I_{s_{3}}} \beta_{j}^{(i)} (k_{r_{j}}^{(i)} + k_{j}^{(i)}) \xi_{j}, (15)$$

where $I_{s_1} = I_{\text{signal}} \cap \{1, ..., \zeta - 1\}, I_{s_2} = I_{\text{signal}} \cap \{j + 1, ..., \zeta\}, I_{s_3} = I_{\text{signal}} \cap \{1, ..., \zeta\}, I_{f_1} = I_{\text{fuel}} \cap \{1, ..., \zeta - 1\},$ and $I_{f_2} = I_{\text{fuel}} \cap \{j + 1, ..., \zeta\}.$

Let X_i be a fuel strand, that is, $i \in I_{\text{fuel}}$. By normalizing the state and time with (9) and (11), we obtain

$$k_{f}T_{s}\frac{d\bar{\xi}_{i}}{dt_{r}} = T_{s}\left(\sum_{j\in I_{s_{1}}}\sum_{k\in I_{f_{2}}}\alpha_{j,k}^{(i)}k_{f_{j,k}}^{(i)}\bar{\xi}_{j}\bar{\xi}_{k} + \sum_{j\in I_{f_{1}}}\sum_{k\in I_{s_{2}}}\alpha_{j,k}^{(i)}k_{f_{j,k}}^{(i)}\bar{\xi}_{j}\bar{\xi}_{k} + \sum_{j\in I_{s_{1}}}\sum_{k\in I_{s_{2}}}\alpha_{j,k}^{(i)}k_{f_{j,k}}^{(i)}\frac{T_{s}}{T_{f}}\bar{\xi}_{j}\bar{\xi}_{k} + \sum_{j\in I_{s_{3}}}\beta_{j}^{(i)}k_{r_{j}}^{(i)}\frac{1}{T_{f}}\bar{\xi}_{j}\right),$$
(16)

which implies that X_i is a slow mode, indicating that $I_{\text{fuel}} \subseteq I_{\text{slow}}$.

Next, let X_i be a signal strand, that is, $i \in I_{\text{signal}}$. Similarly, we have

$$k_{f}T_{s}\frac{d\bar{\xi}_{i}}{dt_{r}} = \sum_{j\in I_{s_{1}}}\sum_{k\in I_{f_{2}}}\alpha_{j,k}^{(i)}k_{f_{j,k}}^{(i)}T_{f}\bar{\xi}_{j}\bar{\xi}_{k}$$

+
$$\sum_{j\in I_{f_{1}}}\sum_{k\in I_{s_{2}}}\alpha_{j,k}^{(i)}k_{f_{j,k}}^{(i)}T_{f}\bar{\xi}_{j}\bar{\xi}_{k} + \sum_{j\in I_{s_{1}}}\sum_{k\in I_{s_{2}}}\alpha_{j,k}^{(i)}k_{f_{j,k}}^{(i)}T_{s}\bar{\xi}_{j}\bar{\xi}_{k}$$

+
$$\sum_{j\in I_{s_{3}}}\beta_{j}^{(i)}(k_{r_{j}}^{(i)} + k_{j}^{(i)})\bar{\xi}_{j}.$$
 (17)

This is because, under Assumption 1 (ii), the right-hand side of (17) cannot be bracketed with T_s , I_{signal} cannot be I_{slow} , that is, $I_{\text{signal}} \cap I_{\text{slow}} = \emptyset$.

Now, we are ready to prove Theorem 1.

Proof: We continue the discussion from the end of the proof of Lemma 1.

In the case of $i \in I_{\text{fuel}}$, by dividing both sides of (16) by $k_f T_s$ and using Lemma 1, we specify that the first two terms on the right-hand side comprise the cross terms $x_* z_*$, and the third and fourth terms comprise the quadratic term $\varepsilon z_* z_*$ and linear terms z_* , respectively. Likewise, in the case of $i \in I_{\text{signal}}$, by dividing both sides of (17) by $k_f T_f$, we also specify that the first two terms of the right-hand side comprise the cross terms $x_* z_*$, and the third and fourth terms comprise the quadratic term $\varepsilon z_* z_*$ and linear term z_* , respectively.

From (7) and (8), the fact $I_{\text{fuel}} = I_{\text{slow}}$ and $I_{\text{signal}} = I_{\text{fast}}$ directly specifies the sets of initial states by $B_x = \{x \in D_x | \sum_{i=1}^n x_i = 1\}$ and $B_z = \{z \in D_z | \sum_{i=1}^m z_i = 1\}$ under the normalization (9) and (10). Taken together, rewriting the time variable t_r as t enables us to obtain the two-time-scale model (13) and (14).

3.2 Singular Perturbation Theory for DSD system

The two-time-scale modeling (13) and (14) straightforwardly motivates us to introduce the singular perturbation theory [6]. For this scenario, by employing the new time variable $\tau = t/\varepsilon$ and then setting $\varepsilon = 0$ that freezes the x-system (13) at t = 0, we consider the following system in the τ time scale:

$$\frac{d\tilde{z}}{d\tau} = g(x_0, \tilde{z}, 0) = A_g(x_0)\tilde{z}, \ \tilde{z}(0) = \tilde{z}_0 \in B_z, \quad (18)$$

where $\tilde{z}(\tau) = z(\varepsilon\tau)$. Now, let us introduce the concept of the boundary-layer system [6] to (18), where we allow the frozen parameter x to vary in the region D_x . Then,

$$\frac{d\tilde{z}}{d\tau} = g(x, \tilde{z}, 0) = A_g(x)\tilde{z}, \ \tilde{z}(0) = \tilde{z}_0 \in B_z.$$
(19)

Remark 4 Under these circumstances with $\varepsilon = 0$ as a limiting case, the globally Lipschitz condition is satisfied for the system (19) because it is simply a linear system with the parameter $x \in D_x$. Because the initial state \tilde{z}_0 is well defined owing to the boundedness of the set of initial states B_z , the global existence of the solution is assured, indicating that the system (19) is well defined.

In what follows, we consider quite common conditions that are generally satisfied by DSD systems.

Assumption 2 The DSD system (5) satisfies the following conditions in addition to Assumption 1.

- (i) There is at least a mass conservation law among signal strands.
- (ii) The matrix-valued function $A_g(x)$ of the corresponding two-time scale model (14) has no eigenvalue with a positive real part for all $x \in D_x$.

In the context of the singular perturbation theory [6], $A_g(x)$ in (19) plays a key role in assuring the exponential stability of the boundary-layer system, and $A_g(x)$ is expected to be Hurwitz for all $x \in D_x$. However, a DSD system generally possesses the law of mass conservation among all or a part of the signal strands involved in a series of reactions, which implies that $A_g(x)$ has zero eigenvalues for all $x \in D_x$, that is, $A_g(x)$ is singular, as shown in Lemma 4.

The fact that the matrix $A_g(x)$ linked to the stability of the boundary layer system is not Hurwitz and has eigenvalues at the origin for any $x \in D_x$ prohibits us from simply applying the singular perturbation theory [6] to the two-time-scale model (13) and (14). To overcome the problem, we take advantage of an invariant manifold of the \tilde{z} -system (19).

Let the state x(t) be represented by $x_0 + \delta_x(t)$. Then, the z-system (14) is rewritten as

$$\varepsilon \dot{z} = A_g(x_0)z + J(z, \delta_x, \varepsilon), \ z(0) = z_0 \in B_z, \quad (20)$$

where

$$J(z, \delta_x, \varepsilon) = \tilde{A}_g(\delta_x) z + \varepsilon b_g(z).$$
(21)

The matrix-valued function $\tilde{A}_g : D_{\delta_x} \to \mathbb{R}^{m \times m}$ is linear, where $D_{\delta_x} \subset \mathbb{R}^n$ is a region containing the origin. Now, for a given $x_0 \in B_x$, consider a non-singular transformation $T = [t_1^T \cdots t_k^T t_{k+1}^T \cdots t_m^T]^T$ that transforms $A_q(x_0)$ into a Jordan form:

$$\Lambda := TA_g(x_0)T^{-1} = \begin{bmatrix} O & 0\\ 0 & A_2 \end{bmatrix},$$
(22)

where $O \in \mathbb{R}^{k \times k}$ is a zero matrix of which k eigenvalues are related to k left-eigenvectors $(t_i \in \mathbb{R}^m, i = 1, ..., k)$ that also link to the k mass conservation laws $(t_i^T \dot{\tilde{z}} \equiv 0, i = 1, ..., k)$ in the \tilde{z} -system (19) from Lemma 5, and $A_2 \in \mathbb{R}^{(m-k) \times (m-k)}$ is a Hurwitz matrix of which (m-k)eigenvalues are related to (m-k) left-eigenvectors $(t_i \in \mathbb{R}^m, i = k + 1, ..., m)$ from Lemma 6.

Let the change of coordinate be defined by

$$\begin{bmatrix} p\\q \end{bmatrix} \triangleq Tz,\tag{23}$$

where $p \in D_p \subset \mathbb{R}^k$ and $q \in D_q \subset \mathbb{R}^{(m-k)}$ are the new state vectors. By applying it to the system (20), we obtain

$$\varepsilon \dot{p} = \beta_p(p, q, \delta_x, \varepsilon), \ p(0) = p_0 \in B_p, \tag{24}$$

$$\varepsilon \dot{q} = A_2 q + \beta_q(p, q, \delta_x, \varepsilon), \ q(0) = q_0 \in B_q, \qquad (25)$$

where

$$\beta_{p}(p,q,\delta_{x},\varepsilon) = \begin{bmatrix} t_{1}^{T} \\ \vdots \\ t_{k}^{T} \end{bmatrix} J(T^{-1}[p \ q]^{T},\delta_{x},\varepsilon), \qquad (26)$$
$$\beta_{q}(p,q,\delta_{x},\varepsilon) = \begin{bmatrix} t_{k+1}^{T} \\ \vdots \\ t_{m}^{T} \end{bmatrix} J(T^{-1}[p \ q]^{T},\delta_{x},\varepsilon), \qquad (27)$$

and the sets of initial conditions, B_p and B_q , are defined as

$$B_p \times B_q = B_{pq} = \left\{ \begin{bmatrix} p \\ q \end{bmatrix} \in \mathbb{R}^m \left| \begin{bmatrix} 1 & \cdots & 1 \end{bmatrix} T^{-1} \begin{bmatrix} p \\ q \end{bmatrix} = 1 \right\}, \quad (28)$$

which contains $0 \in B_p$ and $0 \in B_q$.

On the other hand, for the \tilde{z} -system (19), from Lemma 5, we have

$$\frac{dp}{d\tau} = 0, \ p(0) = p_0 \in B_p, \tag{29}$$

$$\frac{dq}{d\tau} = A_2 q + \beta_q(p, q, \delta_x, 0), \ q(0) = q_0 \in B_q,$$
(30)

where the right-hand side of the *p*-system becomes the zero vector, that is, $\beta_p(p,q,\delta_x,0) = 0$, because the *i*-th element of $\beta_p(p,q,\delta_x,\varepsilon)$ is given by

$$\varepsilon \left(p^T \eta_{pp}^{(i)} p + p^T \eta_{pw}^{(i)} (w+h) + (w+h)^T \eta_{ww}^{(i)} (w+h) \right), (31)$$

where $\eta_{**}^{(i)}$ is defined appropriately, based on the fact that $\dot{p} = [t_1^T \cdots t_k^T]^T \dot{z} \equiv 0.$

Then, we can claim the following lemma that ensures the existence of a stable invariant manifold in the transformed system (29) and (30).

Lemma 2 Under Assumption 2, consider the system (29) and (30). Then, there exist neighborhoods $\overline{D}_{\delta_x} = \{\delta_x \in D_{\delta_x} | \|\delta_x\| < \gamma_{\delta_x}\}$ and $\overline{D}_q = \{q \in D_q | \|q\| < \gamma_q\}$ $(\gamma_{\delta_x} \text{ and } \gamma_q \text{ are small constants})$ and a smooth mapping $h: D_p \times \overline{D}_{\delta_x} \to \overline{D}_q$ such that the region

$$W(p,\delta_x) = \left\{ (p,q,\delta_x) \middle| q = h(p,\delta_x), p \in D_p, \delta_x \in \bar{D}_{\delta_x} \right\}, (32)$$

is an exponentially stable invariant manifold for the system.

Proof: Define the following function:

$$f_q(q, p, \delta_x) = A_2 q + \beta_q(p, q, \delta_x, 0).$$
(33)

Because the *i*-th element (i = k + 1, ..., m) of β_q is expressed by $\delta_x^T \eta_{xp}^{(i)} p + \delta_x^T \eta_{xq}^{(i)} q$, where $\eta_*^{(*)}$ is a constant matrix defined appropriately, it is established that the function f_q is smooth, and for each $p_0 \in D_p$,

$$f_q(0, p_0, 0) = 0, (34)$$

and the Jacobian matrix $[\partial f_q/\partial q](0, p_0, 0)$ is nonsingular because of the non-singularity of A_2 . Applying the implicit function theorem [6] leads to the existence of the neighborhoods \bar{D}_{δ_x} and \bar{D}_q and a smooth mapping h.

In addition, because $f_q(h(p, \delta_x), p, \delta_x) = 0$ on $D_p \times \overline{D}_{\delta_x}$, the region W is an invariant manifold for the system.

Next, let us introduce the following change of variables:

$$w = q - h(p, \delta_x). \tag{35}$$

Then, we have

$$\frac{dw}{d\tau} = A_2(w+h(p,\delta_x)) + \beta_q(p,w+h(p,\delta_x),\delta_x,0).$$
 (36)

Taking into account the identities on the invariant manifold

$$w(t) \equiv 0 \to \dot{w}(t) \equiv 0$$

results in

$$\frac{dw}{d\tau} = A_2 w + N_w(p, w, \delta_x, 0),$$

$$w(0) = w_0 = q_0 - h(p_0, 0) \in D_w, (37)$$

where $D_w \subset \mathbb{R}^{(m-k)}$ is a region containing the origin, and

$$N_{w}(p, w, \delta_{x}, \varepsilon) = \beta_{q}(p, w+h, \delta_{x}, \varepsilon) - \beta_{q}(p, h, \delta_{x}, \varepsilon) - \frac{\partial h}{\partial p} \{\beta_{p}(p, w+h, \delta_{x}, \varepsilon) - \beta_{p}(p, h, \delta_{x}, \varepsilon)\} - \varepsilon \frac{\partial h}{\partial \delta_{x}} \{(A_{f}(T^{-1}[p \ w+h]^{T}) - A_{f}(T^{-1}[p \ h]^{T}))(x_{0} + \delta_{x}) + b_{f}(T^{-1}[p \ w+h]^{T}, \varepsilon) - b_{f}(T^{-1}[p \ h]^{T}, \varepsilon)\}. (38)$$

Because N_w satisfies $N_w(p, 0, \delta_x, 0) = 0$, $N_w(p, w, 0, 0) = 0$, and $(\partial N_w / \partial w)(p, w, 0, 0) = 0$ for any small $k_w > 0$, there exists a neighborhood $\bar{D}'_{\delta_x} = \{\delta_x \in D_{\delta_x} | \|\delta_x\| < \gamma_{\delta'_x}\}$ ($\gamma_{\delta'_x}$ is a small constant) such that

$$\|N_w(p, w, \delta_x, 0)\| \le k_w \|w\|, \forall w \in D_w, \forall \delta_x \in \bar{D}'_{\delta_x}.$$
 (39)

Because A_2 is Hurwitz, for all $w_0 \in D_w$, there exist a neighborhood $\Omega_{\delta_x} \subseteq \overline{D}_{\delta_x} \cap \overline{D}'_{\delta_x}$, and positive constants α_1 and α_2 such that the solutions of (37) satisfy

$$\|w(\tau)\| \le \alpha_1 \|w_0\| \exp(-\alpha_2 \tau), \ \forall \tau \ge 0,$$
 (40)

for all $p \in D_p$ and $\delta_x \in \Omega_{\delta_x}$, indicating that the invariant manifold W is exponentially stable.

Remark 5 The beneficial structure of β_q , which ensures the existence of an exponentially stable invariant manifold, results from the fact that the DSD system is a quadratic system.

We consider applying singular perturbation theory (e.g., Theorem 11.1 in [6]) to the DSD system (13) and (14). Now, the system (37) is a possible candidate for a boundary-layer system with a solution satisfying (40).

Under the change of coordinates (23) and (35), the original systems (13) and (14) are translated into the following full system:

$$\delta_x = F(\delta_x, p, w, \varepsilon), \ \delta_x(0) = 0, \tag{41}$$

$$\varepsilon \dot{w} = G(\delta_x, p, w, \varepsilon), \ w(0) = w_0 \in D_w, \tag{42}$$

$$\varepsilon \dot{p} = H(\delta_x, p, w, \varepsilon), \ p(0) = p_0 \in B_p, \tag{43}$$

where $\delta_x = x - x_0$,

$$F(\delta_x, p, w, \varepsilon) = A_f(T^{-1}[p \ w+h]^T)(x_0 + \delta_x) + b_f(T^{-1}[p \ w+h]^T, \varepsilon), \qquad (44)$$
$$G(\delta_x, p, w, \varepsilon) = A_2 w + N_w(p, w, \delta_x, \varepsilon), \qquad (45)$$

$$H(\delta_x, p, w, \varepsilon) = \beta_p(p, w+h, \delta_x, \varepsilon).$$
(46)

The corresponding quasi-steady state model is given by

$$\delta_x = F(\delta_x, p_0, 0, 0).$$
 (47)

In what follows, we denote the solutions of (41), (42), (43), and (47) by $\delta_x(t,\varepsilon)$, $w(t,\varepsilon)$, $p(t,\varepsilon)$, and $\overline{\delta}_x(t)$, respectively. Then, $q(t,\varepsilon) = w(t,\varepsilon) + h(p,\delta_x)$.

Theorem 2 For the two-time-scale system (13) and (14) under Assumption 2, consider the full system (41)– (43). Assume that the quasi-steady-state model (47) has a unique solution $\overline{\delta}_x(t) \in S$ for a time period $t \in [0, t_1]$, where S is a compact subset of Ω_{δ_x} . Let Ω_w be a compact subset of D_w . Then, there exist positive constants k^* and ε^* such that for all $w_0 \in \Omega_w$ and $\varepsilon \in (0, \varepsilon^*)$,

$$\|\delta_x(t,\varepsilon) - \bar{\delta}_x(t)\| \le k^*\varepsilon, \ \forall t \in [0,t_1].$$
(48)

Moreover, for each of all $t_b \in (0, t_1)$, there exist positive constants k^{**} and ε^{**} ($\leq \varepsilon^*$) such that for all $w_0 \in \Omega_w$ and $\varepsilon \in (0, \varepsilon^{**})$,

$$\|q(t,\varepsilon) - h(p_0, \bar{\delta}_x(t))\| \le k^{**}\varepsilon, \ \forall t \in [t_b, t_1].$$

$$(49)$$

Proof: The description of the full system and the statement of the theorem are similar to the original version of the singular perturbation theory [6] except for the existence of the *p*-system (43). Fortunately, the effect of $H(\delta_x, p, w, \varepsilon)$ can be reduced by specifying a smaller value for ε according to (31), that is,

$$\|H(\delta_x, p, w, \varepsilon)\| \le L_H \varepsilon, \tag{50}$$

where L_H is a positive constant.

First, we evaluate the way in which the trajectory $w(t,\varepsilon)$ evolves depending on ε compared to (40). To

this end, consider the boundary-layer system (37). By virtue of the smoothness of $h(p, \delta_x)$, it can be verified that $N_w(p, w, \delta_x, 0)$ is continuously differentiable with respect to $(p, w, \delta_x) \in D_p \times D_w \times \Omega_{\delta_x}$, and satisfies

$$\left\|\frac{\partial N_w}{\partial w}(p, w, \delta_x, 0)\right\| \le L_1,\tag{51}$$

$$\left\|\frac{\partial N_w}{\partial \delta_x}(p, w, \delta_x, 0)\right\| \le L_{2\delta_x} \|w\|,\tag{52}$$

$$\left\|\frac{\partial N_w}{\partial p}(p, w, \delta_x, 0)\right\| \le L_{2p} \|w\|,\tag{53}$$

for some positive constants L_1 , $L_{2\delta_x}$, and L_{2p} . Using these estimates and the exponentially decaying trajectory (40), we conclude from the lemma (p.368 in [6]) that there is a Lyapunov function $V_1(\delta_x, p, w) : \Omega_{\delta_x} \times D_p \times$ $D_w \to \mathbb{R}$ such that

$$c_1 \|w\|^2 \le V_1(\delta_x, p, w) \le c_2 \|w\|^2, \tag{54}$$

$$\frac{\partial V_1}{\partial w} G(\delta_x, p, w, 0) \le -c_3 \|w\|^2, \tag{55}$$

$$\left\| \frac{\partial V_1}{\partial w} \right\| \le c_4 \|w\|, \left\| \frac{\partial V_1}{\partial p} \right\| \le c_{5p} \|w\|^2, \left\| \frac{\partial V_1}{\partial \delta_x} \right\| \le c_{5x} \|w\|^2, (56)$$

for all $w \in D_w$.

By differentiating V_1 along the trajectories of the full system (41)–(43), we have

$$\begin{split} \dot{V}_{1} &= \frac{1}{\varepsilon} \frac{\partial V_{1}}{\partial w} G(\delta_{x}, p, w, \varepsilon) \\ &+ \frac{1}{\varepsilon} \frac{\partial V_{1}}{\partial p} H(\delta_{x}, p, w, \varepsilon) + \frac{\partial V_{1}}{\partial \delta_{x}} F(\delta_{x}, p, w, \varepsilon) \\ &= \frac{1}{\varepsilon} \frac{\partial V_{1}}{\partial w} G(\delta_{x}, p, w, 0) + \frac{1}{\varepsilon} \frac{\partial V_{1}}{\partial w} \left\{ G(\delta_{x}, p, w, \varepsilon) - G(\delta, p, w, 0) \right\} \\ &+ \frac{1}{\varepsilon} \frac{\partial V_{1}}{\partial p} H(\delta_{x}, p, w, \varepsilon) + \frac{\partial V_{1}}{\partial \delta_{x}} F(\delta_{x}, p, w, \varepsilon) \\ &\leq -\frac{c_{3}}{\varepsilon} \|w\|^{2} + c_{4}L_{3} \|w\| + c_{5x}k_{F} \|w\|^{2} + c_{5p}L_{H} \|w\|^{2} \\ &\leq -\frac{c_{3}}{2\varepsilon} \|w\|^{2} + c_{4}L_{3} \|w\|, \ \forall \varepsilon \leq \frac{c_{3}}{2(c_{5x}k_{F} + c_{5p}L_{H})}, \end{split}$$
(57)

where the following estimates are employed:

 $\|G(\delta_x, p, w, \varepsilon) - G(\delta_x, p, w, 0)\| \le L_3 \varepsilon, \ \|F(\delta_x, p, w, \varepsilon)\| \le k_F.$

Using the same derivation employed in the proof in [6] (pp. 702–703), we conclude that

$$\|w(t,\varepsilon)\| \le k_1 \exp(-\alpha t/\varepsilon) + \varepsilon \delta, \ \forall t \in [0,t_1], \quad (58)$$

where k_1 , α , and δ are positive constants.

Next, we evaluate the gap between $F(\delta_x, p, w, \varepsilon)$ of the full system and $F(\delta_x, p_0, 0, 0)$ of the quasi-steady-state

model. Then, we have

$$||F(\delta_x, p, w, \varepsilon) - F(\delta_x, p_0, 0, 0)|| \le ||F(\delta_x, p, w, \varepsilon) - F(\delta_x, p, w, 0)|| + ||F(\delta_x, p, w, 0) - F(\delta_x, p, 0, 0)|| + ||F(\delta_x, p, 0, 0) - F(\delta_x, p_0, 0, 0)||.$$
(59)

A detailed calculation of the right-hand side shows that the first two terms are bounded by $L_4\varepsilon$ and $L_5||w||$ with positive constants L_4 and L_5 , respectively. As for the third term, it is also bounded by $L_6\varepsilon$ with a positive constant because of (50). Thus, we obtain

$$||F(\delta_x, p, w, \varepsilon) - F(\delta_x, p_0, 0, 0)|| \le (L_4 + L_6)\varepsilon + L_5 ||w||, (60)$$

the form of which is compatible with the derivation in the proof [6]. The remainder of the proof consistently follows the original version of the proof (pp.704-706 in [6]).

3.3 Finite-time Regulation Property

Fig. 3B indicates that the DNA feedback regulator functions normally only for a finite period of time. In other words, the regulation can be attained during the quasisteady state, rather than the "real" steady state after depletion of the fuels. Theorem 2 characterizes the behavior of the *fast* signal strands during the quasi-steady state by (49) along with the two-time-scale modeling in Theorem 1. Hence, applying Theorem 2 to the DNA feedback regulator (3) leads to the next main result.

Theorem 3 Consider the DNA feedback regulator system (3). Assume that all conditions in Theorems 1 and 2 are satisfied. If the invariant manifold $W(p, \delta_x)$ satisfies $y_p(t) = r(t)$, then the system has the finite-time regulation property.

Remark 6 Our framework might be able to provide additional benefits regarding the transient property and duration of normal operation. For the transient property, t_b in (49) corresponds to a kind of settling time, during which we can choose appropriate $\varepsilon \in (0, \varepsilon^{**})$ to improve the transient property. As for the duration of a normal operation, a larger t_1 is desired, where the time t_1 is linked to the behavior of the quasi-steady-state model (47).

Finally, Fig. 4 summarizes the story flow of Section 3.

4 Example study

The main purpose of this example study is to explain "theoretically" the regulation within the finite time observed in the numerical simulation of Fig. 3. To this end, the description here is devoted to providing a solid analytical explanation. Fig. 5 illustrates the detailed reaction scheme of the typical DNA feedback regulator designed in Fig. 2, where the superscript and subscript to



Fig. 4. Flow chart of the framework for finite-time regulation property.

the right of the strand name denote the circuit class (P, plant; R, amplifier for reference; Y, amplifier for output) and the state-variable number, respectively. A fuel strand with a large initial concentration is indicated in boldface. For example, $\mathbf{S}_{\underline{1}}^{\mathrm{P}}$ means the strand S, which is an element of the plant system Σ_{P} , is a kind of fuel strand, and is represented by the state variable ξ_1 in the ordinary differential equation. Then, the closed-loop system Σ_{DFR} is described as follows:

Plant
$$(\Sigma_P)$$
:
 $\dot{\xi}_1 = -k_{f_1}\xi_1\xi_{19} + k_{r_1}\xi_2$
 $\dot{\xi}_2 = k_{f_1}\xi_1\xi_{19} - k_{r_1}\xi_2 - k_2(\xi_2 - \xi_3)$
 $\dot{\xi}_3 = k_2(\xi_2 - \xi_3) + k_{f_3}\xi_4\xi_5 - k_{r_3}\xi_3$
 $\dot{\xi}_4 = -k_{f_3}\xi_4\xi_5 + k_{r_3}\xi_3$
 $\dot{\xi}_5 = -k_{f_3}\xi_4\xi_5 + k_{r_3}\xi_3 - k_{f_4}\xi_5\xi_6 + k_{r_4}\xi_7 - k_{f_9}\xi_5\xi_{14} + k_{r_9}\xi_{13}$

Amplifier for output $y_p(\Sigma_A^{(y)})$:



Amplifier for output $(\zeta_0, ..., \zeta_{14})$

Fig. 5. Reaction scheme of DNA feedback regulator $\Sigma_{\rm DFR}$.

$$\begin{split} \xi_6 &= -k_{f_4}\xi_5\xi_6 + k_{r_4}\xi_7 \\ \dot{\xi}_7 &= k_{f_4}\xi_5\xi_6 - k_{r_4}\xi_7 - k_5(\xi_7 - \xi_8) \\ \dot{\xi}_8 &= k_5(\xi_7 - \xi_8) + k_{f_6}\xi_9\xi_{10} - k_{r_6}\xi_8 \\ \dot{\xi}_9 &= -k_{f_6}\xi_9\xi_{10} + k_{r_6}\xi_8 - k_{f_16}\xi_9\xi_{19} \\ \dot{\xi}_{10} &= -k_{f_6}\xi_9\xi_{10} + k_{r_6}\xi_8 - k_{f_7}\xi_{10}\xi_{11} + k_{r_7}\xi_{12} \\ \dot{\xi}_{11} &= -k_{f_7}\xi_{10}\xi_{11} + k_{r_7}\xi_{12} \\ \dot{\xi}_{12} &= k_{f_7}\xi_{10}\xi_{11} - k_{r_7}\xi_{12} - k_8(\xi_{12} - \xi_{13}) \\ \dot{\xi}_{13} &= k_8(\xi_{12} - \xi_{13}) + k_{f_9}\xi_5\xi_{14} - k_{r_9}\xi_{13} \\ \dot{\xi}_{14} &= -k_{f_9}\xi_5\xi_{14} + k_{r_9}\xi_{13} \end{split}$$

$$\begin{split} & \text{Amplifier for reference } r \ (\Sigma_A^{(r)}): \\ & \dot{\xi}_{15} \!=\! -k_{f_{10}}\xi_{15}\xi_{16} + k_{r_{10}}\xi_{17} - k_{f_{15}}\xi_{15}\xi_{24} + k_{r_{15}}\xi_{23} \\ & \dot{\xi}_{16} \!=\! -k_{f_{10}}\xi_{15}\xi_{16} + k_{r_{10}}\xi_{17} \\ & \dot{\xi}_{17} \!=\! k_{f_{10}}\xi_{15}\xi_{16} - k_{r_{10}}\xi_{17} - k_{11}(\xi_{17} - \xi_{18}) \\ & \dot{\xi}_{18} \!=\! k_{11}(\xi_{17} - \xi_{18}) + k_{f_{12}}\xi_{19}\xi_{20} - k_{r_{12}}\xi_{18} \\ & \dot{\xi}_{19} \!=\! -k_{f_{12}}\xi_{19}\xi_{20} \!+\! k_{r_{12}}\xi_{18} \!-\! k_{f_{16}}\xi_{9}\xi_{19} \!-\! k_{f_{1}}\xi_{12}\xi_{19} \!+\! k_{r_{1}}\xi_{2} \\ & \dot{\xi}_{20} \!=\! -k_{f_{12}}\xi_{19}\xi_{20} \!+\! k_{r_{12}}\xi_{18} \!-\! k_{f_{13}}\xi_{20}\xi_{21} \!+\! k_{r_{13}}\xi_{22} \\ & \dot{\xi}_{21} \!=\! -k_{f_{13}}\xi_{20}\xi_{21} \!+\! k_{r_{13}}\xi_{22} \\ & \dot{\xi}_{22} \!=\! k_{f_{13}}\xi_{20}\xi_{21} \!+\! k_{r_{13}}\xi_{22} \!-\! k_{14}(\xi_{22} \!-\! \xi_{23}) \\ & \dot{\xi}_{23} \!=\! k_{14}(\xi_{22} \!-\! \xi_{23}) \!+\! k_{f_{15}}\xi_{15}\xi_{24} \!-\! k_{r_{15}}\xi_{23} \\ & \dot{\xi}_{24} \!=\! -k_{f_{15}}\xi_{15}\xi_{24} \!+\! k_{r_{15}}\xi_{23}, \end{split}$$

where $\xi_i \in D_{\xi_i} \subset \mathbb{R}$ is a state variable for i = 1, ..., 25, the reference is $r = \xi_{15}$ with the initial concentration $r(0) = r^*$, the control input is $u_p = \xi_{19}$ with $\xi_{19}(0) =$ 0, the output is $y_p = \xi_5$ with $y_p(0) = 0$, the initial concentrations of fuel strands $\xi_1(0), \xi_4(0), \xi_6(0), \xi_{11}(0),$ $\xi_{16}(0)$, and $\xi_{21}(0)$ have large values, and others are set to zero. Because the hybridization (reaction no. 16) is assumed to be an irreversible reaction, we exclude the state variable ξ_{25} from the system description.

Remark 7 For the demonstration simulation in Fig. 3, we employ realistic rate constants from a previous study [31] as follows: $k_{f_i} = 5 \times 10^{-4} n M^{-1} s^{-1}$, $k_{r_i} = 0.7 s^{-1}$ $(i = 1, 3), k_{f_j} = 5 \times 10^{-6} n M^{-1} s^{-1}$, $k_{r_j} = 2.1 s^{-1}$ (i = 4, 6, 7, 9, 10, 12, 13, 15), $k_{f_{16}} = 3 \times 10^{-3} n M^{-1} s^{-1}$ and $k_* = 1 s^{-1}$ for the kinetic constants, $\xi_1(0) = 2000 n M$ and $\xi_4(0) = 1000 n M$ for Σ_p , and $\xi_6(0) = \xi_{16}(0) = 1000$ n M and $\xi_{11}(0) = \xi_{21}(0) = 100000 n M$ for $\Sigma_A^{(r,y)}$; the other initial concentrations are set to 0 n M. The target r^* is set to 1.0, 2.0, and 0.5 n M at $t = 0, 5 \times 10^4$, and 10×10^4 s, respectively, as plotted by the dashed line.

According to Fig. 5, the fuel strands (denoted in bold font) are specified as $S^{\rm P}$, $I_3^{\rm P}$, $S^{\rm Y}$, $F^{\rm Y}$, $S^{\rm R}$, and $F^{\rm R}$, that is, $I_{\rm fuel} = \{1, 4, 6, 11, 16, 21\}$. Then, $I_{\rm signal} = \{1, ..., 24\} \setminus I_{\rm fuel}$. It is easily confirmed that Assumption 1 is satisfied from the ordinary differential equations of Σ_{DFR} . In accordance with the procedure in Theorem 1, the model with two time scales (13) and (14) is given by $f(x,z) = [-k'_{f_1}z_{14}x_1 + k'_{r_1}z_1, -k'_{f_3}z_{3}x_2 + k'_{r_3}z_2, -k'_{f_4}z_{3}x_3 + k'_{r_4}z_4, -k'_{f_7}z_{7}x_4 + k'_{r_7}z_8, -k'_{f_{10}}z_{11}x_5 + k'_{r_{10}}z_{12}, -k'_{f_{13}}z_{15}x_6 + k'_{r_{13}}z_{16}]^T$, and $g(x, z, \varepsilon) = [-k'_{2r1}z_1 + k'_{2}z_2 + k'_{f_1}x_{12}t_4, k'_{2}z_1 - k'_{2r_3}z_2 + k'_{f_3}x_{2}z_3, k'_{r_3}z_2 - (k'_{f_3}x_2 + k'_{f_4}x_3)z_3 + k'_{r_4}z_4 + k'_{r_9}z_9 - \varepsilon k'_{f_9}z_{3}z_{10}, k'_{f_4}x_{3}z_3 - k'_{5r_4}z_4 + k'_{55}z_5, k'_{52}z_4 - k'_{5r_6}z_5 + \varepsilon k'_{f_6}z_{6}z_7, k'_{r_6}z_5 - \varepsilon k'_{f_6}z_{6}z_7 - \varepsilon k'_{f_1}z_{6}z_{14}, k'_{r_6}z_5 - k'_{f_7}x_{427} + k'_{r_7}z_8 - \varepsilon k'_{f_6}z_{6}z_7, k'_{f_7}x_{427} - k'_{8r_7}z_8 + k'_{8}z_9, k'_{8}z_8 - k'_{8r_9}z_9 + \varepsilon k'_{f_9}z_{3}z_{10}, -\varepsilon k'_{f_9}z_{3}z_{10} + k'_{r_9}z_9, -k'_{f_{10}}x_{5}z_{11} + k'_{r_{10}}z_{12} + k'_{r_{15}}z_{17} - \varepsilon k'_{f_{15}}z_{11}z_{18}, k'_{f_{10}}z_{5}z_{11} - k'_{11r_{10}}z_{12} + k'_{11}z_{13}, k'_{11}z_{12} - k'_{11r_{12}}z_{13} + \varepsilon k'_{f_{12}}z_{14}z_{15}, k'_{r_{12}}z_{13} - k'_{f_{13}}x_{6}z_{15} + k'_{r_{13}}z_{16} - \varepsilon k'_{f_{12}}z_{14}z_{15}, k'_{r_{12}}z_{13} - k'_{f_{13}}x_{6}z_{15} + k'_{r_{13}}z_{16} - \varepsilon k'_{f_{12}}z_{14}z_{15}, k'_{f_{13}}z_{16} + k'_{4}z_{17}, k'_{4}z_{16} - k'_{4r_{15}}z_{17} + \varepsilon k'_{f_{15}}z_{11}z_{18}, -\varepsilon k'_{f_{15}}z_{11}z_{18} + k'_{r_{15}}z_{17}]^T$, where $k'_{f_i} = k_{f_i}/k_f$ and $k'_{r_i} = k_{r_i}/(k_fT_f)$ and $k'_{ir_j} = 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, k'_i = k_i/(k_fT_f)$ and $k'_{ir_j} = (k_i + k_{r_j})/(k_fT_f)$ for i = 2, 5, 8, 11, 14 and $j = 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, nd k'_{f_{16}} = k_{f_{16}}/k_f$. Then, it is easily obtained that $A_f(z) \in \mathbb{R}^{6\times 6}, A_g(x) \in \mathbb{R}^{18\times18}$, $b_f(z) \in \mathbb{R}^6$, and $b_g(z) \in \mathbb{R}^{18}$, where we can confirm Assumption 2 numerically. In what follows, for the sake of simplicity without dropping the essential argument, the respective kinetic parameters of the two amplifiers $(\Sigma_A^{(r)})$ and $\Sigma_A^{(r)})$ are assumed to be equal to each other, that is,

Now, let us investigate the trajectories of the z-system on the invariant manifold h. By analytically solving g(x, z, 0) = 0 instead of directly calculating h, we obtain

1

$$\begin{split} h &= \left\{ q \in \mathbb{R}^{13} \left| \begin{bmatrix} p \\ q \end{bmatrix} = Tz, z_1 \in \mathbb{R}, \\ z_2 &= z_1, z_3 = \frac{k_{r_3} z_1}{k_{f_3} x_2}, z_4 = \frac{k_{f_4} (k_5 + k_{r_6}) z_3 x_3}{k_5 k_{r_4} + k_5 k_{r_6} + k_{r_4} k_{r_6}}, \\ z_5 &= \frac{(k_5 + k_{r_4}) z_4 - k_{f_4} z_3 x_3}{k_5}, z_6 = \frac{k_{f_1} (k_{f_4} z_3 x_3 - k_{r_4} z_4) x_1}{k_{f_{16}} k_{r_1} z_1}, \\ z_7 &= \frac{(k_8 k_{r_7} + k_8 k_{r_9} + k_{r_7} k_{r_9}) (k_{f_4} z_3 x_3 - k_{r_4} z_4)}{k_8 k_{f_7} k_{r_9} x_4}, \\ z_8 &= \frac{k_{f_7} z_7 x_4 + k_{r_4} z_4 - k_{f_4} z_3 x_3}{k_{r_7}}, \\ z_9 &= \frac{k_{828} - k_{f_4} z_3 x_3 + k_{r_4} z_4}{k_8}, z_{10} = \frac{x_3 z_3}{x_5}, z_{11} = z_4, \\ z_{12} &= z_5, z_{13} = \frac{k_{r_1} z_1}{k_{f_1} x_1}, z_{14} = \frac{z_7 x_4}{x_6}, z_{15} = z_8, z_{16} = z_9 \right\}, (61) \end{split}$$

which implies that the relation $z_3 = (x_5/x_3)z_{10}$ is satisfied on the manifold h. It is noted that the transformation T can be obtained from $A_g(x_0)$ in a numerical analysis, where the initial condition x_0 is given, for example, as shown in Remark 7. Next, we calculate the corresponding quasi-steady-state model. It can be analytically shown that

$$\begin{split} \dot{\delta}_{x_1} &= \dot{\delta}_{x_2} = 0, \\ \dot{\delta}_{x_i} &= -\left(k_5 k_8 k_{f_4} k_{f_7} k_{r_6} k_{r_9} \ell(\delta_x)\right) \delta_{x_i} \text{ for } i = 3, 4, 5, 6, (62) \end{split}$$

where $\delta_x = [\delta_{x_1} \ \delta_{x_2} \ \delta_{x_3} \ \delta_{x_4} \ \delta_{x_5} \ \delta_{x_6}]^T$, and $\ell(\delta_x)$ is defined appropriately. Then, it becomes clear that $x_3(t) \equiv x_5(t)$ in the quasi-steady-state model with $x_3(0) = x_5(0)$. In addition, we can confine the solution $\overline{\delta}_x(t)$ of the quasisteady-state model (62) to a compact set $S \subset \Omega_{\delta_x}$ for all $t \in [0, t_1]$ by setting sufficiently small values for the parameters (e.g., k_5). Therefore, we conclude from Theorem 2 that for each of all $t_b \in (0, t_1)$, there exist positive constants k^{**} and ε^{**} such that for all $w_0 \in \Omega_w$ and $\varepsilon \in (0, \varepsilon^{**}), |z_3(t) - z_{10}(t)| \leq k^{**}\varepsilon, \ \forall t \in [t_b, t_1].$

Finally, for each of all e > 0, selecting the initial concentration of signal strands ($\xi_i(0)$ for $i \in I_{\text{signal}}$) and/or fuel strands ($\xi_i(0)$ for $i \in I_{\text{fuel}}$) to satisfy $\varepsilon = T_s/T_f \leq e/k^{**}$ leads to $|z_3(t) - z_{10}(t)| = |y_p(t) - r(t)| \leq e$ for all $t \in [t_b, t_1]$. Therefore, the DNA feedback regulator system Σ_{DFR} has the finite-time regulation property, which is consistent with Theorem 3. In this case, it is feasible to maintain r(t) as close as possible to $r(0) = r^*$ by increasing the initial concentration of fuel strands in the auto-catalytic amplifier Σ_A [17]. This confirms the validity of the simulation results in Fig. 3.

5 Conclusions

The new paradigm of DNA molecular design is creating an entirely new research field in control theory. The realization of the DNA feedback regulator is a symbolic milestone, and the finite-time regulation property defined in this study plays an important role in the molecular machine from both technical and theoretical perspectives. The technical highlight of our work is the conversion of a class of the DSD system to the two-time-scale model, which was accomplished by sophisticatedly taking advantage of the fundamental properties with which a DSD system, built on the biochemical reaction principle, is inherently equipped. We claimed that singular perturbation theory can be helpful for analyzing the finite-time regulation property.

Although our method was established with the aim to achieve the above-mentioned milestone, it seems to be possible to apply the method to a general DNA reaction system and even a general biochemical reaction system such as an intracellular signal transduction system. This would require the theory to be appropriately customized as necessary because all these reaction systems involve molecular interactions and the kinetic principles are similar.

A Properties of $A_g(x)$

Some important properties of $A_g(x)$, which plays an important role in Section 3.2, are summarized below.

Lemma 3 For any $x \in D_x$, the matrix $A_g(x)$ is a Metzler matrix.

Proof: This result is directly derived from (5) owing to the stoichiometric coefficients given by (6).

Lemma 4 For the two-time-scale model (13) and (14), $A_g(x)$ has zero eigenvalues for all $x \in D_x$ if the law of mass conservation exists among the signal strands.

Proof: Based on the fact $I_{\text{fast}} = I_{\text{signal}}$, let $\kappa = [\kappa_1, ..., \kappa_m]$ be a non-zero vector satisfying $\kappa \dot{z} \equiv 0$ for all $t \geq 0$, where $0 \leq \kappa_i < \infty$ is a non-negative integer for all $i \in \{1, ..., m\}$. Then, we have

$$\varepsilon \kappa \dot{z} = \kappa A_q(x) z + \kappa \varepsilon b_q(z) \equiv 0, \ \forall t \ge 0.$$
 (A.1)

Because the identical relation (A.1) holds for each of all $\varepsilon > 0$, it follows that $\kappa A_g(x)z = 0$ for all $t \ge 0$. Noting that this relation necessarily also holds at t = 0 for all $x_0 \in B_x$ and $z_0 \in B_z$, we can specify that $\kappa A_g(x) = 0$ for all $x \in D_x$. As κ is a non-zero vector, $A_g(x)$ has zero eigenvalues for all $x \in D_x$.

Lemma 5 For the \tilde{z} -system (19), $A_g(x)$ has zero eigenvalues for all $x \in D_x$ if and only if the law of mass conservation exists among the signal strands.

Proof: The sufficiency results directly from Lemma 4; hence, here we focus on the proof of the necessity. Assume that there exists a left eigenvector $\kappa \in \mathbb{R}^m$ such that $\kappa A_g(x) = 0$ for all $x \in D_x$. By multiplying both sides by any trajectory \tilde{z} of the system (19), we obtain

$$\kappa A_g(x)\tilde{z} = \kappa \frac{d\tilde{z}}{d\tau} = 0, \ \forall \tau \ge 0,$$
 (A.2)

which implies that the law of mass conservation exists among the signal strands in the \tilde{z} -system (19).

Lemma 6 Assume that the Frobenius eigenvalue of $A_g(x)$ is non-positive for all $x \in D_x$. Then, $A_g(x)$ has no eigenvalue on the imaginary axis except for the origin.

Proof: Because $A_g(x)$ is a Metzler matrix from Lemma 3, this result is directly derived from the fact that the Frobenius eigenvalue of a Metzler matrix is real and unique [4].

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