Multi-time-scale biomolecular 'quasi-integral' controllers for set-point regulation and trajectory tracking

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***Abstract*— Recent trends in synthetic biology to move from prototypes to applications have triggered higher expectations on the robustness, predictability and responsiveness of biomolecular circuits. Therefore, a systematic approach to designing biomolecular controllers for regulating gene expression is needed. Although a number of integral control motifs (ICMs) have been proposed for set-point regulation, their performance *in vivo* is challenged by integration leakiness due to dilution, which cannot be neglected in growing cells. In this paper, we study a class of *quasi-integral controllers* designed based on existing ICMs and multiple time-scale separations. We demonstrate that by engineering all controller reactions to be much faster than dilution, set-point regulation can be achieved even in the presence of a leaky integrator. Furthermore, by engineering controller parameters for a second layer of time-scale separation, arbitrarily small tracking error can be achieved under certain technical conditions. We demonstrate a realization of our design principle through a small RNA feedback circuit.**

I. INTRODUCTION

Rapid advances in synthetic biology in recent years have posed unprecedented opportunities and challenges to control engineers. On the one hand, the lack of robustness, predictability and modularity of biomolecular circuits remain major hurdles to the creation of larger systems with sophisticated functionalities [1]. Control theoretic tools have been proven instrumental in addressing these fundamental challenges [2]. On the other hand, emergent applications of synthetic biology set higher standards in the precision and responsiveness of biomolecular circuits. For example, in cancer immunotherapy, T cell activity needs to be tightly regulated to protect healthy cells [3] and in cell identity reprogramming, expression of several key transcription factors must track a given temporal profile (i.e., trajectory) [4]. While all these design requirements fit well into the established control-theoretic framework of regulation and tracking [5], [6], [7], finding their biomolecular realizations remains challenging. For instance, existing theory often relies on a combination of state observers and dynamic controllers to achieve disturbance decoupling and tracking [6], [7], and their biomolecular realizations are still at their infancy [8]. Nevertheless, recent developments in constructing biomolecular controllers have shown that biomolecular systems have a distinctive set of tool boxes, such as molecular sequestration and time-scale separation that can be exploited to the benefit of control design [2].

To address the regulation problem, in which the steady state output of a circuit must be regulated at a constant set-point regardless of constant disturbances and uncertainties, a natural control theoretic approach based on the internal model principle [9] is to construct biomolecular integral controllers. To this end, a few integral control motifs (ICMs) have been proposed [10], [11], [12]. However, the ability of these motifs to realize integral control all hinges on the idealized assumption that a biomolecular "memory species" does not dilute as the host cell grows. While this assumption is satisfied in cell free systems, it is not met in living cells, in which cell growth dictates dilution of all species in the circuit. As a consequence, performance of a circuit containing the ICM in practice cannot be guaranteed, and often needs to be evaluated computationally on a case-by-case basis [13]. Less work has investigated the trajectory tracking problem in biomolecular systems, in which the output of a circuit is required to follow a given temporal concentration profile. Nevertheless, in [14], Hsiao et al. first constructed a biomolecular concentration tracker in bacteria using negative feedback. Yet, lack of in-depth theoretical developments in this aspect has hampered our ability to derive generalizable design principles.

Here, we provide a general controller design approach to address the problem of set-point regulation and trajectory tracking in living cells. While ICMs cannot guarantee perfect set-point regulation due to the presence of dilution, we show that by selecting/engineering certain rate constants in a circuit containing an ICM to operate on three different time-scales, the closed loop output can be regulated arbitrarily close to a constant set-point even in the presence leaky integration. We call the resultant controllers with such time-scale separation property *quasi-integral controllers*. If the process to be controlled satisfies a passivity condition, we further demonstrate that a linearized quasi-integral control system can track a time-varying trajectory with error that decreases with the separation of time-scales. Our results establish a general approach for synthetic biologists to design biomolecular controllers based on "off-the-shelf" ICMs that can perform set-point regulation and trajectory tracking.

Notations: The ∞-norm of a signal $\mathbf{v}(t)$ defined for $t \geq 0$ is denoted by $\|\mathbf{v}\|_\infty := \sup_{\tau\in[0,\infty)}[|\mathbf{v}(\tau)|_\infty]$, where $|\cdot|_\infty$ represents the ∞-vector norm. When we consider a scalar time-varying input $u(t)$ to an n-th order dynamical system, we represent its derivatives by $\mathbf{u}(t) :=$

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$[u(t), \dot{u}(t), \cdots, u^{(n)}(t)]^T$.

II. MOTIVATING EXAMPLE

As a motivating example, we consider the antithetic ICM proposed in [10], in which two controller species c_1 and c_2 regulate the expression of a target gene to produce protein p. The controller takes as inputs a constant reference input u and the concentration of p, which is the output of the closed loop system and we denote it by p (*italic*). The production rates of two controller species c_1 and c_2 are engineered to be proportional to u and p, respectively. Furthermore, the two controller species can bind together and annihilate. These processes can be described by the following reactions:

$$\mathrm{u} \xrightarrow{\alpha} \mathrm{c}_1, \quad \mathrm{p} \xrightarrow{\alpha} \mathrm{p} + \mathrm{c}_2, \quad \mathrm{c}_1 + \mathrm{c}_2 \xrightarrow{\theta} \varnothing, \tag{1}$$

where, without loss of generality, we assume that the production of c_1 and c_2 have identical rate constant α. The biochemical process to be controlled contains the decay (i.e., dilution and degradation) of p with rate constant δ, and its production activated by the controller species c_1. These processes are described by the following reactions:

$$\mathrm{c}_1 \xrightarrow{\beta(d)} \mathrm{c}_1 + \mathrm{p}, \qquad \mathrm{p} \xrightarrow{\delta} \varnothing, \tag{2}$$

where $\beta(d) > 0$ models an external disturbance/parameter uncertainty d affecting the production rate of p. Such disturbance may arise, for example, from the competition of gene expression machinery by other genes in the cell [15]. These chemical reactions translate into the following mass-action kinetic model:

$$\dot{c}_1 = \alpha u - \theta c_1 c_2, \quad \dot{c}_2 = \alpha p - \theta c_1 c_2, \quad \dot{p} = \beta(d) c_1 - \delta p, \tag{3}$$

A coordinate transformation $z := c_1 - c_2$ reveals the integral action in this ICM since $\dot{z} := \alpha(u - p)$. The *memory variable* z guarantees that, under appropriate stability and reachability conditions, the closed loop system can reach set-point u and perfectly reject disturbance d [10], [16]. When a biomolecular circuit implements such a motif in living cells, the concentration of all biomolecules are subject to dilution due to cell growth (i.e., volume expansion). This unavoidable dilution effect has been neglected in the ICM model (1). To factor dilution into the model, we consider, in addition to (1), the reactions $\mathrm{c_i} \xrightarrow{\gamma} \varnothing$ $(i = 1, 2)$, where γ is the dilution rate constant proportional to the specific growth rate of the cell. In face of dilution, (3) must be modified to become

$$\begin{aligned} \dot{c}_1 &= \alpha u - \theta c_1 c_2 - \gamma c_1, \\ \dot{c}_2 &= \alpha p - \theta c_1 c_2 - \gamma c_2, \\ \dot{p} &= \beta(d) c_1 - \delta x. \end{aligned} \tag{4}$$

According to (4), the memory variable $z = c_1 - c_2$ is no longer integrating the error, rather, it carries out a *leaky integration:* $\dot{z} = \alpha(u - p) - \gamma z$, and consequently, perfect set-point regulation cannot be expected. We call ICMs that include dilution *leaky-ICMs*. While similar problems exist in engineering, they can often be solved by reducing the rate of leakiness γ of the integration component (e.g., a leaky capacitor). In biological context, however, decreasing γ must be accomplished by decreasing host cell growth, which is often undesirable [17]. Consequently, depending on the rest of the system parameters, the effect of leaky integration can be appreciable [13], [16], making the performance of ICMs under question in practice.

The class of quasi-integral biomolecular controllers we propose here are based on these idealized ICMs. However, the unwanted effects of leaky integration in these quasi-integral controllers can be mitigated through time-scale separation between all controller reactions and dilution. Additionally, we show that if a second layer of time-scale separation exists in the controller reactions, then the resultant linearized closed loop system can achieve trajectory tracking. In the next section, we first state the general control problem and provide a mathematical description of the closed loop system under consideration.

III. PROBLEM FORMULATION

In this paper, we consider a closed loop system $\Sigma^{(\epsilon,\mu)}$ composed of a plant Σ_p and a controller $\Sigma_c^{(\epsilon,\mu)}$ with an ordered pair of parameters (ϵ, μ). The closed loop system takes as inputs a scalar reference $\tilde{u}(t)$ and a constant disturbance d to produce a scalar output $y(t)$. The disturbance could also model parameter uncertainties. We describe two control problems in Section III-A and propose a multi-time-scale controller setup in Section III-B aimed to address them.

A. Control objectives

We are interested in two control problems closely related to synthetic biology applications. The first one is to design a controller to regulate the steady state behavior of the system when the reference input is constant: $\tilde{u}(t) \equiv \bar{u}$.

Definition 1: (Set-point regulation.) Assuming that $\Sigma^{(\epsilon,\mu)}$ has a unique locally asymptotically stable steady state, we say that $\Sigma^{(\epsilon,\mu)}$ achieves (ϵ, μ)-*set-point regulation* in an *admissible input set* $\mathbb{U} \times \mathbb{D}$ if for any pair of constant inputs $(\bar{u}, d) \in \mathbb{U} \times \mathbb{D}$, there exists a constant ϵ_1 and a class $\mathcal{K}$ function $\alpha_1(\cdot)$, both dependent on $\bar{u}$ and d, such that for all $0 < \epsilon < \epsilon_1$, the equilibrium output $\bar{y}(\bar{u}, d, \epsilon, \mu)$ satisfies

$$\lim_{\mu \to 0} |\bar{y}(\bar{u}, d, \epsilon, \mu) - \bar{u}| = \alpha_1(\epsilon). \tag{5}$$

Remark 1: Definition 1 resembles the concept of approximate integral control in [18], where an integrator appears in system dynamics when a small parameter approaches 0. However, this result is inapplicable to our setting as this would require us to set $\gamma = 0$, corresponding to cell death.

Adding upon set-point regulation, we consider next the problem of regulating the system output to track a smooth temporal trajectory with bounded derivatives.

Definition 2: (Trajectory tracking.) System $\Sigma^{(\epsilon,\mu)}$ has the (ϵ, μ)-*asymptotic tracking* property in an admissible input set $\mathcal{U} \times \mathbb{D} \subset \mathcal{L}_\infty^{n+1} \times \mathbb{R}$ if for every $(\tilde{\mathbf{u}}(t), d) \in \mathcal{U} \times \mathbb{D}$, there exists a positive constant ϵ_2 and a class $\mathcal{K}$ function $\alpha_2(\cdot)$, both dependent on $\tilde{\mathbf{u}}(t)$ and d, such that for all $0 < \epsilon < \epsilon_2$ and for some initial conditions,

$$\lim_{\mu \to 0} \limsup_{t \to \infty} |y(t, \tilde{u}, d, \epsilon, \mu) - \tilde{u}(t)| = \alpha_2(\epsilon). \tag{6}$$

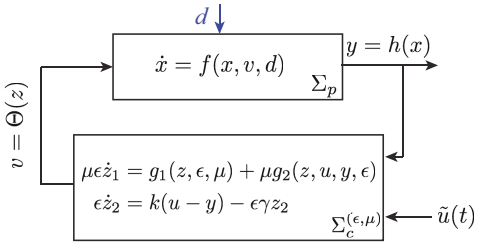

Fig. 1. Quasi-integral control system setup.

In the next section, we propose a general class of quasi-integral controllers with multiple time-scales. We will show in Section IV that these (nonlinear) controllers can achieve set-point regulation and their linearized models can achieve trajectory tracking, implying that the (nonlinear) controllers can track a time-varying input with small enough amplitude.

B. Multi-time-scale quasi-integral controller

We consider a feedback interconnection of a plant (i.e., biomolecular process to be controlled) and a biomolecular controller (see Fig. 1). The dynamics of the plant, Σ_p, can be written as

$$\Sigma_p: \quad \dot{x} = f(x, v, d), \quad y = h(x), \tag{7}$$

in which x represents the plant states (e.g., concentration of biomolecules), and $f(\cdot)$ describes the plant dynamics. The plant takes two inputs: v is the control input, and $d \in \mathbb{D}$ is a constant external disturbance input. The scalar output of the plant y is determined by $h(x)$. The plant is connected to a quasi-integral biomolecular controller $\Sigma_c^{(\epsilon,\mu)}$ parameterized by an ordered pair of small positive parameters (ϵ, μ). These parameters characterize the different time-scales present in the controller dynamics. The controller takes two scalar inputs: a) the output of the process y, and b) the reference input $\tilde{u}(t)$. It produces an output v to control the plant Σ_p. We assume that the controller is constructed based on an ICM with an intended "memory variable". However, we factor into its model the fact that integral action is leaky as the host cell grows with rate constant $\gamma > 0$. We therefore consider the following quasi-integral controller

$$\begin{aligned} \Sigma_c^{(\epsilon,\mu)}: \quad & \mu\epsilon\dot{z}_1 = g_1(z, \epsilon, \mu) + \mu g_2(z, u, y, \epsilon), \\ & \epsilon\dot{z}_2 = k(u - y) - \epsilon\gamma z_2, \quad v = \Theta(z), \end{aligned} \tag{8}$$

where $z := [z_1^T, z_2]^T$ are the controller states. Specifically, scalar z_2 is the intended memory variable that carries out the integral action with integral gain k when $\gamma = 0$, corresponding to an ideal situation where the host cell does not grow. We assume that the dynamics of z can be engineered to be much faster than dilution. This fact is captured by parameter $0 < \epsilon \ll 1$ in equations (8). The rest of the controller states are represented by $z_1 \in \mathbb{R}^m$. We assume that z_1 evolves on a faster time-scale compared to that of the leaky memory variable z_2. This is captured by the small parameter $0 < \mu \ll 1$ in (8). In the next section, we study the control performance of the closed loop system (7)-(8).

IV. SET-POINT REGULATION AND TRACKING

In this section, we study the closed-loop quasi-integral control system in Fig. 1. We first give explicit algebraic conditions under which the nonlinear closed loop system can achieve (ϵ, μ)-set-point regulation. We then consider a linearized model of the controller and a strictly positive real (SPR) plant to evaluate trajectory tracking performance.

A. Set-point regulation

Here, we study the equilibrium location of the quasi-integral control system (7)-(8). As we shall demonstrate, regardless of the presence of dilution, which breaks the intended integral control structure in $\Sigma_c^{(\epsilon,\mu)}$, the equilibrium output of the closed loop system $\bar{y}$ can approximately reach a constant set-point $\bar{u}$ regardless of disturbance d if the controller parameters ϵ and μ are small.

The steady state of $\Sigma^{(\epsilon,\mu)}$, $\bar{\xi} = \bar{\xi}(\bar{u}, d, \epsilon, \mu) := [\bar{x}^T(\bar{u}, d, \epsilon, \mu), \bar{z}^T(\bar{u}, d, \epsilon, \mu)]^T$, can be computed from the following algebraic equations:

$$\begin{aligned} & F(\bar{\xi}, d) = 0, \quad k[\bar{u} - h(\bar{x})] = \epsilon\gamma\bar{z}, \\ & g_1(\bar{z}, \epsilon, \mu) + \mu G_2(\bar{\xi}, \bar{u}, \epsilon) = 0, \end{aligned} \tag{9}$$

where $F(\bar{\xi}, d) := f(\bar{x}, \Theta(\bar{z}), d)$ and $G_2(\bar{\xi}, \bar{u}, \epsilon) := g_2(\bar{z}, \bar{u}, h(\bar{x}), \epsilon)$. To study how the solution of (9) changes with parameters (ϵ, μ), we make the following assumptions.

Assumption 1: The functions F, g_1, G_2 and h are $\mathcal{C}^1$ in all their arguments. Specifically, g_1 and G_2 are $\mathcal{C}^1$ in an open set around $(\epsilon, \mu) = (0, 0)$.

Assumption 2: When $(\epsilon, \mu) = (0, 0)$, there exists a unique solution $\xi^* := \bar{\xi}(\bar{u}, d, 0, 0)$ to (9), that is,

$$F(\xi^*, d) = 0, \quad g_1(z^*, 0, 0) = 0, \quad u - h(x^*) = 0. \tag{10}$$

Assumption 3: The following matrix is invertible:

$$D := \left.\begin{bmatrix} \partial F/\partial x & \partial F/\partial z \\ 0 & \partial g_1/\partial z \\ -k \cdot \partial h/\partial x & 0 \end{bmatrix}\right|_{(\bar{\xi}=\xi^*, \bar{u}, d, \epsilon=0, \mu=0)}.$$

Assumptions 1-3 guarantee that the equilibrium $\bar{y}$ is $\mathcal{C}^1$ in an open set containing $(\epsilon, \mu) = (0, 0)$.

Theorem 1: If $\Sigma^{(\epsilon,\mu)}$ has a unique locally asymptotically stable steady state, then it has (ϵ, μ)-set-point regulation property in $\mathbb{U} \times \mathbb{D}$ if Assumptions 1-3 are satisfied for all $(\bar{u}, d) \in \mathbb{U} \times \mathbb{D}$

Proof: For a given pair of $(\bar{u}, d) \in \mathbb{U} \times \mathbb{D}$, the steady state $\bar{\xi}(\bar{u}, d, \epsilon, \mu)$ of system $\Sigma^{(\epsilon,\mu)}$ can be solved from equations (9), which can be re-written as

$$\mathcal{F}(\bar{\xi}, \bar{u}, d, \epsilon, \mu) := \begin{bmatrix} F(\bar{\xi}, d) \\ g_1(\bar{z}, \epsilon, \mu) + \mu G_2(\bar{\xi}, \bar{u}, \epsilon) \\ k(\bar{u} - h(\bar{x})) - \epsilon\gamma\bar{z} \end{bmatrix} = 0. \tag{11}$$

For simplicity of notation, we drop the arguments $\bar{u}$ and d in (11) as they are constants in this problem, and instead write it as $\mathcal{F}(\bar{\xi}, \epsilon, \mu) = 0$ with slight abuse of notation. Based on Assumption 2, there exists ξ^* such that $\mathcal{F}(\xi^*, 0, 0) = 0$. Since $\mathcal{F}$ is $\mathcal{C}^1$ at $(\xi^*, 0, 0)$ (Assumption 1), and $\partial\mathcal{F}/\partial\xi|_{\xi^*,0,0}$ is invertible (Assumption 3), according to the implicit function theorem, we can write $\bar{\xi} = \bar{\xi}(\epsilon, \mu)$, where $\bar{\xi}$ is $\mathcal{C}^1$ in (ϵ, μ)

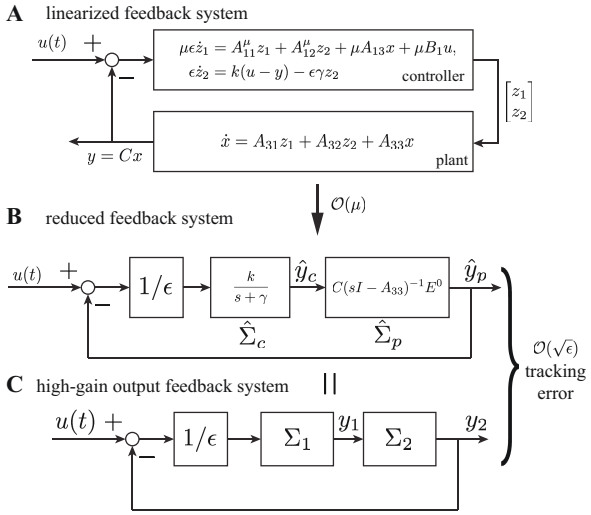

Fig. 2. Linearized quasi-integral control system. (A) Full model. (B) By setting $\mu = 0$, one obtains the reduced model, which contains high gain feedback connection of a reduced controller ($\hat{\Sigma}_c$) and a reduced plant ($\hat{\Sigma}_p$). (C) Theorem 2 establishes that high gain feedback of two SPR systems (Σ_1 and Σ_2) can achieve reference tracking with $\mathcal{O}(\sqrt{\epsilon})$ error.

in an open set containing $(\xi^*, 0, 0)$. Since $h(\cdot)$ is $\mathcal{C}^1$, from (10), we have $\lim_{(\epsilon,\mu)\to(0,0)} h(\bar{x}(\epsilon,\mu)) = h(x^*) = \bar{u}$. This implies (ϵ, μ)-set-point regulation. ■

B. Small amplitude trajectory tracking

In this section, we study the quasi-integral controller's ability to track a bounded time-varying reference input $\tilde{u}(t) \in \mathbb{U}$, which we decompose into $\tilde{u}(t) = \bar{u} + u(t)$. We assume that the time-varying part $u(t)$ is small enough so that we can infer the behavior of the nonlinear system (7)-(8) from its linearized counterpart at steady state $\bar{\xi}(\bar{u}, d, \epsilon, \mu)$, which can be written as follows:

$$\begin{aligned} \mu\epsilon\dot{z}_1 &= A_{11}^{\mu} z_1 + A_{12}^{\mu} z_2 + \mu A_{13} x + \mu B_1 u, \\ \epsilon\dot{z}_2 &= k(u-y) - \epsilon\gamma z_2, \\ \dot{x} &= A_{31} z_1 + A_{32} z_2 + A_{33} x, \quad y = Cx. \end{aligned} \tag{12}$$

where $A_{11}^{\mu} = \frac{\partial g_1}{\partial z_1} + \mu\frac{\partial G_2}{\partial z_1}, A_{12}^{\mu} = \frac{\partial g_1}{\partial z_2} + \mu\frac{\partial G_2}{\partial z_2}, A_{13} = \frac{\partial G_2}{\partial x}, A_{31} = \frac{\partial F}{\partial z_1}, A_{32} = \frac{\partial F}{\partial z_2}, A_{33} = \frac{\partial F}{\partial x}, B_1 = \frac{\partial G_2}{\partial u}, C = \frac{\mathrm{d}h}{\mathrm{d}x}$, with all derivatives evaluated at $\bar{\xi}$. With reference to Fig. 2A, regarding μ as the perturbation parameter, system (12) is in standard singular perturbation form [5]. We therefore consider the following *reduced system* of (12), obtained by setting $\mu = 0$:

$$\begin{aligned} \epsilon\dot{z}_{2r} &= k(u - y_r) - \epsilon\gamma z_{2r}, \\ \dot{x}_r &= A_{33} x_r + E^0 z_{2r}, \quad y_r = Cx_r, \end{aligned} \tag{13}$$

where $E^{\mu} := A_{32} - A_{31}(A_{11}^{\mu})^{-1} A_{12}^{\mu}$. To show closeness of y and y_r using singular perturbation on the infinite time interval [5], we make the following assumptions.

Assumption 4: There exists $\epsilon^* > 0$ such that matrices A_{11}^0 and $\begin{bmatrix} -\gamma & -kC/\epsilon \\ E^0 & A_{33} \end{bmatrix}$ are Hurwitz for all $0 < \epsilon < \epsilon^*$.

Lemma 1: (Thm. 11.2, [5]) If Assumption 4 is satisfied, then for any $0 < \epsilon < \epsilon^*$, there exists positive t_0, $\mu^*(\epsilon)$, and $K_0(\epsilon)$ such that $|y(t,u,\epsilon,\mu) - y_r(t,u,\epsilon)| = \mu K_0(\epsilon) + \mathcal{O}(\mu^2)$ holds for all $t \in [t_0, \infty)$ and $\mu < \mu^*$.

Given the closeness between system (12) and the reduced system (13), we study the tracking performance of the reduced system. With reference to Fig. 2B, the reduced system (13) can be decomposed into a *reduced controller* $\hat{\Sigma}_c$ and a *reduced plant* $\hat{\Sigma}_p$ in cascade with static output feedback gain $1/\epsilon$. Specifically, we have

$$\begin{aligned} \hat{\Sigma}_c: \quad \dot{z}_{2r} &= kv_1 - \gamma z_{2r}, \quad \hat{\Sigma}_p: \quad \dot{x}_r = A_{33} x_r + E^0 v_2, \\ \hat{y}_c &= z_{2r}, \qquad\qquad\quad \hat{y}_p = Cx_r = y_r. \end{aligned} \tag{14}$$

The two subsystems are interconnected according to $v_1 = (u - \hat{y}_p)/\epsilon$ and $v_2 = \hat{y}_c$ (Fig. 2B). Tracking of $\mathbf{u}(t) \in \mathcal{L}_\infty$ for a class of such systems have been studied in [19], which we state briefly in the following Theorem.

Theorem 2: ([19], Thm. 2.) Consider the static output feedback control system in Fig. 2C, where for $i = 1, 2$, the SISO subsystem Σ_i described by $\dot{x}_i = A_i x_i + B_i v_i, y_i = C_i x_i$, satisfies the following conditions: 1) The pair (A_i, B_i) is controllable, the pair (A_i, C_i) is observable; 2) Σ_i has strictly proper and SPR transfer function $H_i(s)$; and 3) $H_1(s)H_2(s)$ does not contain any pole-zero cancellation. If the closed loop system with $v_1 = (u - y_2)/\epsilon$ and $v_2 = y_1$ is subject to a reference input $u(t)$ with bounded derivatives (i.e., $\mathbf{u}(t) \in \mathcal{L}_\infty^{n+1}$), then for ϵ sufficiently small, there exists a $K = K(\|\mathbf{u}\|_\infty)$, independent of ϵ, such that the tracking error $e(t,\epsilon) = u(t) - y(t,u,\epsilon)$ satisfies

$$\limsup_{t\to\infty} |e(t,\epsilon)| = K\sqrt{\epsilon}. \tag{15}$$

To apply Theorem 2 to our context, we need the following assumptions on plant dynamics.

Assumption 5: The reduced plant $\hat{\Sigma}_p$ in (14) is controllable and observable. Its transfer function is strictly proper, SPR, and does not have a zero at $s = -\gamma$.

Lemma 2: If Assumption 5 is satisfied and $\mathbf{u}(t) \in \mathcal{L}_\infty^{n+1}$, then there exists positive constants ϵ^{**} and $K = K(\|\mathbf{u}\|_\infty)$, independent of ϵ, such that $\limsup_{t\to\infty} |y_r(t,u,\epsilon) - u(t)| = K\sqrt{\epsilon}$ for all $0 < \epsilon < \epsilon^{**}$.

Proof: The result follows from applying Theorem 2 directly to $\hat{\Sigma}_c$ and $\hat{\Sigma}_p$. ■

Since Lemma 1 establishes the closeness between the full order and the reduced order system, and 2 demonstrates the tracking performance of the reduced order system, we are ready to study the tracking performance of the linearized full system (12).

Theorem 3: If Assumptions 4-5 are satisfied and $\mathbf{u}(t) \in \mathcal{L}_\infty^{n+1}$, then there exists $\epsilon > 0$ sufficiently small and $K = K(\|\mathbf{u}\|_\infty) > 0$, independent of ϵ, such that the tracking error of (12) satisfies

$$\lim_{\mu\to 0} \limsup_{t\to\infty} |y(t,u,\epsilon,\mu) - u(t)| = K\sqrt{\epsilon}. \tag{16}$$

Therefore, the linearized system (12) can achieve (ϵ, μ)-asymptotic trajectory tracking.

Proof: According to Lemma 2, we can pick an $0 < \epsilon_0 < \min\{\epsilon^{**}, \epsilon^*\}$ such that $\limsup_{t\to\infty} |y_r(t,u,\epsilon_0) - u(t)| = K\sqrt{\epsilon_0}$. Given this ϵ_0, according to Lemma 1,

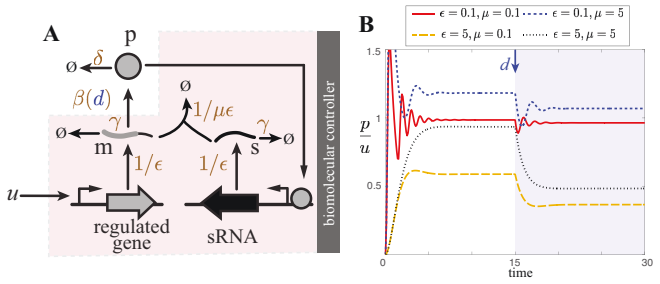

Fig. 3. A realization of quasi-integral controller. (A) Small RNA-based feedback circuit diagram. (B) Set-point regulation can be achieved when ϵ and μ are sufficiently small. Simulations are performed for $\gamma = \delta = 1$, $\bar{u} = 10$, and $\beta(d) = 5/(1+d)$, where disturbance is applied at $t = 15$.

there exists positive t_0, $\mu^*(\epsilon_0)$ and $K_0(\epsilon_0)$, such that for all $\mu < \mu^*$ and $t > t_0$, $\limsup_{t\to\infty} |y(t,u,\epsilon_0,\mu) - y_r(t,u,\epsilon_0)| = \mu K_0(\epsilon_0) + \mathcal{O}(\mu^2)$. Note that since $\lim_{\mu\to 0}\lim_{t\to\infty}|y_r(t,u,\epsilon_0) - u(t)| = 0$, by triangle inequality, we have $\lim_{\mu\to 0}\limsup_{t\to\infty}|y(t,u,\epsilon_0,\mu) - u(t)| = K\sqrt{\epsilon_0}$. ■

Remark 2: The two small parameters ϵ, μ serve different purposes in the multi-time-scale quasi-integral controller. Parameter ϵ serves as a high static feedback gain to overcome the leaky integration effect at steady state [16]. Decreasing the other small parameter μ allows z_1 dynamics to become much faster than leaky integration (z_2 dynamics). Therefore, a small μ serves to "hide" high order controller dynamics.

Remark 3: The name "quasi-integral controllers" stems from the fact that their designs are based on the ICMs proposed previously [10], [11], [12]. However, from a theoretical perspective, the underlying high gain mechanism of these quasi-integral controllers is very different from an integral controller.

V. EXAMPLE

In this section, we apply the results developed in Section IV to a realization of the leaky-ICM example in (4). In particular, we consider the small RNA-based feedback circuit in Fig. 3A, where two controller species mRNA (m) and a small RNA (s) regulate the translation of protein p [16], [20], [21]. In this example, the circuit is aimed to attenuate disturbance d affecting the translation rate of p. For example, when d is due to the translation of another resource-competition protein, $\beta(d)$ takes the form of $\beta(d) = \bar{\beta}/(1+d)$ [15], [20]. A simplified ODE model of this circuit can be written as

$$\begin{aligned} \dot{m} &= \tilde{u}(t)/\epsilon - ms/(\epsilon\mu) - \gamma m, \\ \dot{s} &= p/\epsilon - ms/(\epsilon\mu) - \gamma s, \\ \dot{p} &= \beta(d)m - \delta p. \end{aligned} \tag{17}$$

We refer the readers to [16], [20] for detailed derivation of this model from chemical reactions. Parameter ϵ can be decreased by simultaneously increasing the copy numbers of the regulated gene and that of the small RNA; parameter μ can be decreased by increasing the complementarity between the mRNA and the small RNA, which increases their binding affinity. This model can be manipulated into the form of (7)-(8). In particular, let $z_1 = m$, $z_2 = m - s$ and $x = p$, we have:

$$\begin{aligned} \mu\epsilon\dot{z}_1 &= \mu\tilde{u}(t) - z_1(z_1 - z_2) - \mu\epsilon\gamma z_1, \\ \epsilon\dot{z}_2 &= (\tilde{u}(t) - y) - \epsilon\gamma z_2, \\ \dot{x} &= \beta(d)z_1 - \delta x, \quad y = x. \end{aligned} \tag{18}$$

We analyze the set-point regulation and tracking behavior of (18) in the following sections.

A. Set-point regulation

We first study the set-point regulation problem, in which we assume the reference input is a positive constant $\tilde{u}(t) \equiv \bar{u}$. We have shown in [16] that this circuit has a unique locally exponentially stable steady state $\bar{\xi} = [\bar{z}_1, \bar{z}_2, \bar{x}]$ for any positive parameters (ϵ, μ) and $(\bar{u}, d) \in \mathbb{U} \times \mathbb{D} := \{\bar{u} > 0, \beta(d) > 0\}$. Moreover, $\bar{\xi}$ can be found from

$$\begin{aligned} &F(\bar{\xi}, d) = \beta(d)\bar{z}_1 - \delta\bar{x}, \quad g_1(\bar{z}) = -\bar{z}_1(\bar{z}_1 - \bar{z}_2), \\ &G_2(\bar{\xi}, \bar{u}, \epsilon) = \bar{u} - \epsilon\gamma\bar{z}_1, \quad h(\bar{x}) = \bar{x}. \end{aligned}$$

We first check that Assumptions 1-3 are satisfied. In particular, $F(z^*, x^*, d) = 0$, $G_1(z^*) = 0$ and $\bar{u} = h(x^*)$ has a solution at $\xi^* := [z_1^*, z_2^*, x^*] = [\delta\bar{u}/\beta(d), \delta\bar{u}/\beta(d), \bar{u}]$. The matrix

$$\begin{aligned} D &= \begin{bmatrix} \partial G_1/\partial z_1 & \partial G_1/\partial z_2 & 0 \\ 0 & 0 & -k\cdot \mathrm{d}h/\mathrm{d}x \\ \partial F/\partial z_1 & \partial F/\partial z_2 & \partial F/\partial x \end{bmatrix}\Bigg|_{\bar{\xi}=\xi^*,\bar{u},d,\epsilon=0,\mu=0} \\ &= \begin{bmatrix} -\delta\bar{u}/\beta(d) & \delta\bar{u}/\beta(d) & 0 \\ 0 & 0 & -1 \\ \beta & 0 & -\delta \end{bmatrix} \end{aligned}$$

is invertible. From Theorem 1 we claim that it can achieve (ϵ, μ)-set-point regulation in $\mathbb{U} \times \mathbb{D}$ (Fig. 3B).

B. Trajectory tracking

To study the ability of (18) to track a time-varying reference $\tilde{u}(t) = \bar{u} + u(t)$, where $u(t)$ has small amplitude and $\tilde{u}(t)$ has bounded derivatives (i.e., $\tilde{\mathbf{u}}(t) \in \mathcal{L}_\infty^{n+1}$). We linearize (18) around state $\bar{\xi}$ and inputs $(\bar{u}, d) \in \mathbb{U} \times \mathbb{D}$ to obtain

$$\begin{bmatrix} \mu\epsilon\dot{z}_1 \\ \epsilon\dot{z}_2 \\ \dot{x} \end{bmatrix} = \begin{bmatrix} A_{11}^\mu & A_{12}^\mu & 0 \\ 0 & -\epsilon\gamma & -k \\ A_{31} & A_{32} & A_{33} \end{bmatrix} \begin{bmatrix} z_1 \\ z_2 \\ x \end{bmatrix} + \begin{bmatrix} \mu B_1 \\ k \\ 0 \end{bmatrix} u, \tag{19}$$

where, following the notations in (12), we have $A_{11}^\mu = -2\bar{z}_1 + \bar{z}_2 - \mu\epsilon\gamma$, $A_{12}^\mu = \bar{z}_1$, $A_{31} = \beta(d) > 0$, $A_{32} = 0$, $A_{33} = -\delta$, $B_1 = 1$, $C = 1$ and $k = 1$. To check whether Assumption 4 is satisfied, note that matrices $A_{11}^0 = -\delta\bar{u}/\beta(d) + \mathcal{O}(\epsilon) + \mathcal{O}(\mu) < 0$ for small (ϵ, μ) and $\begin{bmatrix} -\gamma & -kC/\epsilon \\ E^0 & A_{33} \end{bmatrix} = \begin{bmatrix} -\gamma & -1/\epsilon \\ -\beta\bar{z}_1/A_{11}^0 & -\delta \end{bmatrix}$ is Hurwitz for all positive (ϵ, μ) and $(\bar{u}, d) \in \mathbb{U} \times \mathbb{D}$. By Lemma 1, we can therefore approximate (19) by its reduced order model:

$$\epsilon\dot{z}_{2r} = (u - x) - \epsilon\gamma z_{2r}, \qquad \dot{x} = \tilde{\beta}z_{2r} - \delta x, \tag{20}$$

in which $\tilde{\beta} = -\beta\bar{z}_1/(-2\bar{z}_1 + \bar{z}_2) = \beta + \mathcal{O}(\epsilon) > 0$ for small ϵ. Note that in (20), the reduced plant $\hat{\Sigma}_p : \dot{x} =$

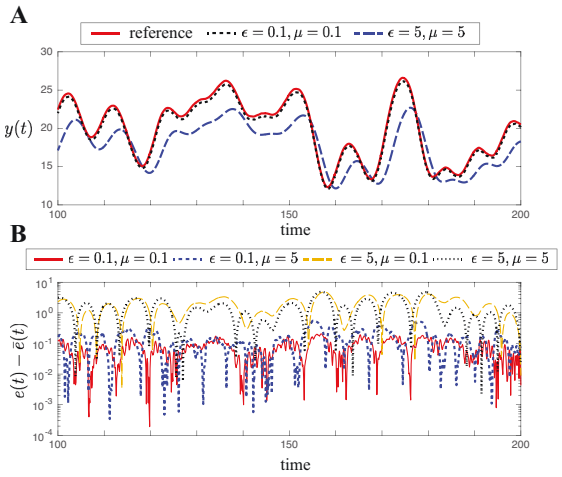

Fig. 4. Numerical simulation of the tracking performance of (17) with different parameters ϵ and μ. (A) Tracking of a randomly generated smooth trajectory. (B) Dynamic tracking error with DC component removed. Simulation parameters: $\gamma = \delta = 1$, $\beta = 5$ and $\bar{u} = 10$.

$\tilde{\beta} z_{2r} - \delta x$ satisfy Assumption 5. According to Theorem 3, we conclude that the linearized small RNA feedback system can achieve (ϵ, μ)-asymptotically tracking for every $d \in \mathbb{D}$ and $\tilde{\mathbf{u}}(t) \in \mathcal{U} := \{\tilde{\mathbf{u}}(t) \in \mathcal{L}_\infty^{n+1} : \tilde{u}(t) > 0, \forall t\}$. In Fig. 4, we simulate the tracking performance of the (nonlinear) small RNA feedback model (17) given a smooth reference trajectory generated by band-limited white noise and a moving average filter. Tracking performance of the circuit with different ϵ and μ values were evaluated. As it can be seen in Fig. 4, decreasing ϵ and μ both improves the tracking performance of the circuit.

VI. DISCUSSION AND FUTURE WORK

We propose a class of multi-time-scale quasi-integral biomolecular controllers that can achieve set-point regulation and trajectory tracking. A systematic design procedure is described as follows. First, one selects/engineers the reactants in an ICM such that the rate of all controller reactions are much greater than dilution. This creates a fast time-scale in the controller (ϵ) that effectively functions as a high-gain to compensate for any undesirable steady state effect due to the leaky integrator. Second, one selects/engineers all controller reactions other than the ones involved in leaky integration to the fastest time-scale ($\mu\epsilon$). This additional layer of time-scale separation "hides" all high-order dynamics in the controller to guarantee tracking in the closed loop system.

The results in this paper can be strengthened in a few directions. From a theoretical perspective, to study nonlinear tracking performance, it may be possible to generalize the result in [19] for SPR systems to more general nonlinear systems that satisfy certain passivity-type conditions. This may also help us develop an efficient tool to check uniform stability of the closed loop system with respect to ϵ and μ. Our numerical simulation in Fig. 4 suggests that tracking error is dominantly determined by ϵ. Therefore, analyzing the system's behavior when $\mu = \mathcal{O}(1)$ is of interest. However, in this case, with a single perturbation parameter ϵ, the boundary layer system does not have an equilibrium, and singular perturbation cannot be applied. Further theoretical developments maybe required towards solving this problem.

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