Robustness of networked systems to unintended interactions with application to engineered genetic circuits

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***Abstract*—A networked dynamical system is composed of subsystems interconnected through prescribed interactions. In many engineering applications, however, one subsystem can also affect others through "unintended" interactions that can significantly hamper the intended network's behavior. Although unintended interactions can be modeled as disturbance inputs to the subsystems, these disturbances depend on the network's states. As a consequence, a disturbance attenuation property of each isolated subsystem is, alone, insufficient to ensure that the network behavior is robust to unintended interactions. In this paper, we provide sufficient conditions on subsystem dynamics and interaction maps, such that the network's behavior is robust to unintended interactions. These conditions require that each subsystem attenuates constant external disturbances, is monotone or "near-monotone", the unintended interaction map is monotone, and the prescribed interaction map does not contain feedback loops. We employ this result to guide the design of resource-limited genetic circuits. More generally, our result provide conditions under which robustness of constituent subsystems is sufficient to guarantee robustness of the network to unintended interactions.**

I. Introduction

A networked system is the interconnection of input/output (I/O) subsystems through a prescribed interaction map. Many properties of networked systems can be determined using I/O properties of the constituent subsystems and the specified interaction map [1–7]. Here, we consider the case where a networked system, which we refer to as the "nominal network", is perturbed by unintended interactions among subsystems (Fig.1). These unintended interactions often arise from one subsystem physically perturbing the environment that comprises all other subsystems, thereby indirectly affecting their dynamics. For example, in close formation control of aerial vehicles, the vortex created by the propulsion force of the leading vehicle can severely affect the dynamics of its neighbors, creating instability [8–11]; in a wind farm with multiple turbines, the wake effect of one turbine alters the surrounding air flow, which, in turn, affects adjacent turbines, reducing efficiency [12, 13]; in building temperature control, the temperature difference between neighboring rooms induces thermal conduction, which results in deviation of each room's temperature from its set point [14]; in genetic circuits, increased expression of one gene decreases the amount of resources available to express other genes, unintentionally reducing their expression levels [15, 16].

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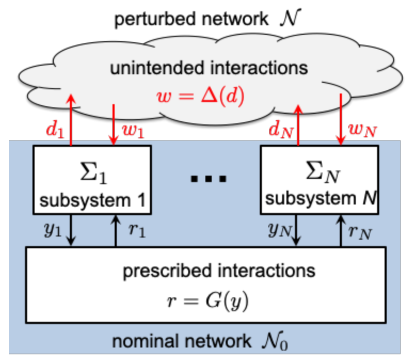

Fig. 1. **Schematic of a perturbed network $\mathcal{N}$.** It is composed of N subsystems interconnected via prescribed interaction map G and unintended interaction map Δ.

To retain the prescribed function of a network despite unintended interactions, one approach is to co-design all subsystems and their interactions monolithically [8, 12, 13, 16]. A different approach, taken in networked systems research, is to allow each subsystem to be designed independent of others, thus allowing scalable network analysis and design [1–7, 17–21]. Specifically, work in this direction has been concerned with deriving conditions on subsystems' I/O dynamics and interaction map for network stability, performance, and/or robustness to state-independent disturbances. In this paper, we take the networked systems research approach. In particular, we obtain conditions for robustness to an unintended interaction map (Δ in Fig. 1), rendering state-dependent disturbances. Our earlier work [22] has studied a simplified version of this problem where the subsystems are modeled as static I/O maps.

Here, with reference to Fig. 1, we provide mathematical conditions on the subsystems and interactions under which the behavior of the perturbed network (with unintended interactions) is arbitrarily close to that of the nominal network (without unintended interactions). Specifically, we are interested in the network's steady state behavior and thus we define a *network disturbance decoupling* (NDD) property, by which the steady state outputs from all subsystems become essentially independent of the unintended interactions. We prove that if (i) each constituent subsystem is monotone or near-monotone and it can asymptotically attenuate the effect of a constant external disturbance on its output, (ii) the prescribed interactions do not contain a feedback loop, and (iii) the unintended interaction map is cooperative, then the NDD property of a network can be entirely determined by the static I/O characteristics of the subsystems. We apply our theoretical results to guide the design of robust genetic circuits in living cells, where unintended interactions arise from resource competition and disrupt network behavior [16]. While solutions have appeared recently to make a single genetic subsystem robust to constant disturbances [23–28], it remains unclear the extent to which such solutions can be scaled up to enable robustness of a network of genetic subsystems to unintended interactions.

The organization of this paper is as follows: In Section II, we present a motivating example. In Section III, we formulate the NDD problem. Section IV studies networks composed of monotone subsystems and states conditions for NDD. Section V extends the result to non-monotone subsystems that can be reduced to a monotone system through timescale separation. Finally, in Section VI, we revisit the motivating example.

II. Motivating example

This paper is motivated by the problem of engineering robust genetic circuits (i.e., networks) in living cells [29–33]. These circuits allow to control the way in which a cell senses and responds to its environment, thereby offering tremendous opportunities in a number of applications, such as biomanufacturing [34], drug delivery and therapeutics [35], and regenerative medicine [36]. Although genetic circuits have been built and used in a number of settings already, lack of robustness remains a major hurdle hampering progress [32]. Among known causes of lack of robustness, competition for shared gene expression resources has appeared as a major player [15, 16]. In this example, we illustrate how this problem can be cast within the formulation of Fig. 1.

A genetic circuit is composed of N genetic subsystems. Each genetic subsystem contains a series of biochemical reactions that express gene i to produce a protein p_i as output. In particular, the gene is first transcribed to produce mRNA m_i at rate r_i, which is then translated to produce protein p_i at rate T_i. Using m_i and p_i (*italic*) to represent the concentrations of species m_i and p_i (roman), respectively, the state of a genetic subsystem is $x_i = [m_i, p_i]^\top$ and its output is $y_i = p_i$. Based on mass-action kinetics, the dynamics of subsystem i can be written as [37]:

$$\dot{m}_i = r_i - \delta_0 m_i, \qquad \dot{p}_i = T_i(m_i) - \delta p_i, \tag{1}$$

where δ_0 and δ are decay rate constants of the mRNA and the protein, respectively, and $T_i(m_i)$ is the translation rate increasing with mRNA concentration m_i. The transcription rate of a gene i, r_i, can be modulated by the concentration of other proteins in the network, a process called *transcriptional regulation* [37]. These prescribed interactions are often modeled by $r_i = G_i(y)$, where $y := [y_1, \cdots, y_N]^\top$ and $G_i(\cdot)$ is a nonlinear function called Hill function [37]. The above descriptive framework has become standard practice to design G and to tune parameters in each genetic subsystem to obtain prescribed circuit behavior, such as genetic oscillators, toggle switches, and logic gates [38–40].

A major challenge in engineering genetic circuits is the omnipresence of unintended interactions, which severely hamper a circuit's function [41]. One contributor to unintended interactions is resource competition [33]. In particular, translation of mRNA relies on the cellular resource ribosome, which is demanded by all mRNAs in the cell for translation. When mRNA m_j is transcribed in genetic subsystem j, it binds with free ribosome, reducing its availability to translate m_i, thus unintentionally decreasing the output of subsystem i. Accounting for N subsystems competing for a conserved pool of ribosome, the translation rate of each gene becomes (see [22] for derivation):

$$T_i = T_i(m_i, w_i) = \frac{\alpha_i \cdot (m_i/\kappa_i)}{1 + m_i/\kappa_i + w_i}, \; w_i = \sum_{j \neq i} \frac{m_j}{\kappa_j}, \tag{2}$$

where α_i is the translation rate constant, κ_i is the dissociation constant that decreases with the affinity of m_i with the ribosome, and w_i is the ribosome demand by all other subsystems in the circuit. Because translation rate T_i decreases with w_i, by substituting (2) into (1), we observe that the output $y_i = p_i$ now decreases with m_j. These create unintended interactions and give rise to unexpected circuit behavior [16]. Hence, a genetic circuit with ribosome competition can be regarded as a perturbed network with subsystem dynamics (1) with $T_i = T_i(m_i, w_i)$, with prescribed interaction (i.e., transcriptional regulation) map $G(\cdot)$, and with unintended interaction map $\Delta(\cdot)$: $w_i = \sum_{j \neq i} d_j$, where $d_i = m_i/\kappa_i$ is the disturbance output of subsystem i.

To reduce the dependence of each subsystem's output y_i on disturbance w_i, an additional molecule, called small RNA (sRNA), was introduced into each genetic subsystem to create a biomolecular feedback control mechanism [24]. The dynamics in such a *feedback-regulated subsystem* can be described by the following mass-action kinetic model:

$$\begin{aligned} \dot{m}_i &= \frac{1}{\varepsilon_i} r_i - \frac{1}{\varepsilon_i}\lambda_i m_i s_i - \delta_0 m_i, \\ \dot{s}_i &= \frac{1}{\varepsilon_i}\beta_i p_i - \frac{1}{\varepsilon_i}\lambda_i m_i s_i - \delta_0 s_i, \\ \dot{p}_i &= T_i(m_i, w_i) - \delta p_i, \end{aligned} \tag{3}$$

where s_i is the concentration of sRNA, λ_i, β_i are constant parameters, and ε_i is a small design parameter that can be decreased experimentally (see [24]). When w_i is a constant, state-independent disturbance, it has been shown that the steady state output of (3) satisfies $\lim_{\varepsilon_i \to 0^+} y_i = r_i/\beta_i$, which is independent of w_i. This asymptotic static disturbance attenuation property is attained if the constant reference input takes value in an admissible set $\bar{\mathcal{R}}_i := \{0 \leq r_i < \alpha_i/\beta_i\}$ [42]. The situation $r_i \geq \alpha_i/\beta_i$ physically corresponds to a scenario where the desired output cannot be reached even with all available ribosomes translating m_i.

Given that each subsystem can asymptotically reject disturbance w_i to reach set-point r_i/β_i, it is tempting to use multiple such feedback controllers, one in each genetic subsystem, to ensure that the output of multiple feedback-regulated subsystems become independent of w_i, that is, of ribosome usage. This approach, however, can fail depend-

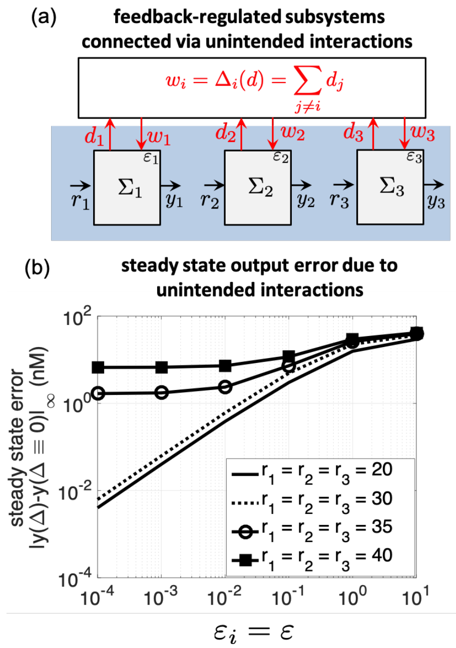

Fig. 2. **Network disturbance decoupling for feedback-regulated genetic subsystems with independent reference inputs.** (a) The nominal network $\mathcal{N}_0$ (shaded in blue) consists of three feedback-regulated genetic subsystems (3), each taking an independent but identical reference input $r_i = r_0$. The subsystems are coupled through unintended interactions arising from resource conservation ($w_i = \Delta_i(d)$), leading to the perturbed network $\mathcal{N}$. (b) Steady state error (vector ∞-norm) between the outputs of the perturbed and the nominal networks as $\varepsilon_i = \varepsilon$ varies. For every $\varepsilon_i = \varepsilon$ and $r_i = r_0$, the trajectory converges to an asymptotically stable equilibrium. Subsystems have identical parameters: $\alpha_i = 100$ nM/hr, $\lambda_i = 1$ $(\mathrm{nM}\cdot\mathrm{hr})^{-1}$, $\delta = 1$ hr^{-1}, $\beta_i = 1$ hr^{-1}, and $\kappa_i = 1$ nM for all i. Based on these parameters and for all r_i levels chosen, we find $r_i \in \bar{R}_i$ and hence each subsystem in isolation can asymptotically attenuate a constant disturbance as ε_i decreases.

ing on the value of reference input r_i to each subsystem. Specifically, we simulated the network in Fig.2a, which is composed of 3 feedback-regulated genetic subsystems with the dynamics in (3) but no prescribed interactions among them (i.e., $r_i(t) \equiv r_0$ for all i). We chose simulation parameters such that $r_i \in \bar{\mathcal{R}}_i$, hence each subsystem in isolation can asymptotically reject any constant disturbance as ε_i is decreased. However, as shown in Fig.2b, we found that decreasing ε_i for all subsystems fails to decrease the tracking error for large reference input values despite $r_i \in \bar{\mathcal{R}}_i$.

These simulation results demonstrate that even if all constituent subsystems of a network can attenuate constant, state-independent disturbances in isolation, this robustness property may be lost when disturbances are state-dependent through an unintended interaction map $w = \Delta(d)$. Specifically, in this case, the problem occurs because d_i reflects the "control effort" of the feedback regulation mechanism in subsystem i. Hence, when $\varepsilon_i \to 0^+$ to improve disturbance attenuation of subsystem i, depending on r_i level, disturbance output d_i may grow unbounded, leading to $w_j \to \infty$, which cannot be compensated by the control effort in subsystem j. The result in this paper allows us to place sufficient conditions on subsystem dynamics, Δ, and G such that this problem does not occur.

III. Problem Formulation

After introducing some notations, we present our system setup. Specifically, We describe mathematical conditions that restrict the class of subsystems we consider. We then formally define the NDD problem.

Notations: For a vector $v \in \mathbb{R}^n$, we denote $|v| := \max_i |v_i|$ for vector ∞-norm. For a signal $v(t) : \mathbb{R} \to \mathbb{R}^n$, its ∞-norm is denoted as $\|v\| := \sup_{t\geq 0} |v(t)|$. For a closed set $\mathcal{A}$ and a vector x, $\mathrm{dist}\{x, \mathcal{A}\} = \min_{s\in\mathcal{A}} |x - s|$. For a time-dependent function $x(t)$, we will use the following notations:

$$\lim_{t\to\infty} \mathrm{dist}\{x(t), \mathcal{A}\} = 0 \quad \Leftrightarrow \quad x(t) \to \mathcal{A},$$
$$\lim_{t\to\infty} \mathrm{dist}\{x(t), \mathcal{A}\} \leq \mu \quad \Leftrightarrow \quad x(t) \xrightarrow{\mu} \mathcal{A}.$$

The comparison operators $<$, $\leq$, as well as min and max operations are defined component-wise. The set $[a, b] := \{x \in \mathbb{R}^n : a \leq x \leq b\}$, where $a \leq b$, defines a box in $\mathbb{R}^n$. Concatenation of N a-dimensional vectors $x_1, \cdots, x_N$ is written as $x := [x_1^\top, \cdots, x_N^\top]^\top \in \mathbb{R}^{aN}$. Similarly, given N vector-valued functions $f_1(x_1), \cdots, f_N(x_N)$ with $f_i : \mathbb{R}^a \to \mathbb{R}^b$ for all i, we write the stacked function as $f(x) := [f_1^\top(x_1), \cdots, f_N^\top(x_N)]^\top : \mathbb{R}^{aN} \to \mathbb{R}^{bN}$. For sets $\mathcal{A}_1, \cdots, \mathcal{A}_N$, we write $\mathcal{A} := \prod_{i=1}^N \mathcal{A}_i$. A scalar continuous function $\alpha(x)$ with $\alpha(0) = 0$ is of class $\mathcal{K}_0$ ($\mathcal{K}$) if it is non-decreasing (strictly increasing) with x. For a $n \times m$ matrix A, $\mathrm{sign}(A)_{ij} = 1$ if $A_{ij} \geq 0$ and $\mathrm{sign}(A_{ij}) = -1$ otherwise. A function $f(x, y)$ is said to be Lipschitz continuous in $x \in \mathcal{X}$ uniformly in $y \in \mathcal{Y}$ if there exists a constant $L > 0$ such that for all $y \in \mathcal{Y}$, $|f(x^+, y) - f(x^-, y)| \leq L|x^+ - x^-|$ for any $x^-, x^+ \in \bar{\mathcal{X}}$. $\triangledown$

With reference to Fig.1, a *perturbed network* $\mathcal{N}$ is a tuple (Σ, G, Δ), where $\Sigma := (\Sigma_1, \cdots, \Sigma_N)$ is a set of N subsystems, and G and Δ describe the prescribed and unintended interaction maps, respectively. Each subsystem $\Sigma_i = \Sigma_i(\varepsilon_i)$ is parameterized by a positive parameter ε_i and follows the dynamics:

$$\dot{x}_i = f_i(x_i, r_i, w_i; \varepsilon_i), \quad y_i = l_i(x_i), \quad d_i = \rho_i(x_i), \qquad (4)$$

where x_i is the state variable evolving in $\mathcal{X}_i \subseteq \mathbb{R}^n$. Signals r_i and w_i are reference and disturbance inputs, respectively, taking values on sets $\mathcal{R}_i$ and $\mathcal{W}_i$ that contain the origin; y_i and d_i are prescribed and disturbance outputs, respectively, taking values on $\mathcal{Y}_i$ and $\mathcal{D}_i$. For each fixed ε_i, we assume the function f_i is differentiable and locally Lipschitz on $\mathcal{X}_i \times \mathcal{R}_i \times \mathcal{W}_i$. The output functions l_i, ρ_i are assumed to be differentiable and locally Lipschitz on $\mathcal{X}_i$. For simplicity, we consider I/O signals r_i, w_i, y_i and d_i to be scalars, and write $u_i := [r_i, w_i]^\top$ and $q_i := [y_i, d_i]^\top$. Because of this, with slight abuse of notation, for any function $f(\cdot)$ with vector argument $u_i = [r_i, w_i]^\top$, the notation $f(u_i)$ is used interchangeably with $f(r_i, w_i)$ for convenience.

Assumption 1. (Subsystem stability). There exists $\varepsilon_i^* > 0$ such that for each fixed $(r_i, w_i) \in \mathcal{R}_i \times \mathcal{W}_i$ and $0 < \varepsilon_i \leq$

ε_i^*, system (4) has a globally asymptotically stable (GAS) equilibrium $\varphi_i(r_i, w_i; \varepsilon_i)$, that is, for all initial conditions $x_i^0 \in \mathcal{X}_i$, $\lim_{t\to\infty} x_i(t, r_i, w_i; \varepsilon_i) = \varphi_i(r_i, w_i; \varepsilon_i)$. $\triangledown$

If Assumption 1 is satisfied, $\varphi_i(\cdot, \cdot; \varepsilon_i)$ is called the *static input/state (I/S) characteristic* of Σ_i. The corresponding *static I/O characteristic* for the prescribed output is:

$$y_i = h_i(r_i, w_i; \varepsilon_i) := l_i \circ \varphi_i(r_i, w_i; \varepsilon_i). \tag{5}$$

Assumption 2. (Subsystem disturbance attenuation). There exists class $\mathcal{K}$ functions $\alpha_i(\cdot)$ and $\alpha_i^0(\cdot)$, a non-empty compact set $\bar{\mathcal{R}}_i \subseteq \mathcal{R}_i$, a constant $\varepsilon_i^* > 0$, and a bounded function $H_i(r_i)$ such that

$$|h_i(r_i, w_i; \varepsilon_i) - H_i(r_i)| \leq \alpha_i(\varepsilon_i)|w_i| + \alpha_i^0(\varepsilon_i) \tag{6}$$

for every fixed $(r_i, w_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i$ and $0 < \varepsilon_i \leq \varepsilon_i^*$. $\triangledown$

We call $H_i(r_i)$ the *nominal static I/O characteristic* because it is independent of w_i. According to Assumption 2, for any bounded and fixed disturbance input w_i, the steady state prescribed output $y_i = h_i(r_i, w_i; \varepsilon_i)$ deviates at most $\mathcal{O}(\varepsilon_i)$ from $H_i(r_i)$. The set $\bar{\mathcal{R}}_i$ is the *admissible reference input set*, where (6) holds.

The subsystems are connected through a static intended interaction map

$$r = G(y). \tag{7}$$

In a perturbed network, the disturbance output of subsystem i, d_i, perturbs subsystem j through a disturbance input w_j. The dependence of w_j on d_i gives rise to unintended interactions among subsystems, which we model using a static unintended interaction map

$$w = \Delta(d). \tag{8}$$

We assume that both maps $G(\cdot)$ and $\Delta(\cdot)$ are globally Lipschitz. We use $y(t; \varepsilon, \Delta)$ to represent the stacked outputs of the perturbed network consisting of (4), (7), and (8), and write $y(t; \varepsilon, 0)$ for the stacked outputs of a *nominal network* $\mathcal{N}_0 = (\Sigma, G, \Delta \equiv 0)$ consisting of (4), (7), but without disturbance input (i.e., $w \equiv 0$).

Definition 1. (NDD). Given $\mu > 0$ and a fixed ε, the perturbed network $\mathcal{N}(\varepsilon) = (\Sigma(\varepsilon), G, \Delta)$ is said to have the μ-network disturbance decoupling (μ-NDD) property if

$$\limsup_{t\to\infty} |y(t; \varepsilon, \Delta) - y(t; \varepsilon, 0)| \leq \mu$$

for all initial conditions $x^0 \in \mathcal{X}$. $\triangledown$

For small μ, the output of $\mathcal{N}$ becomes close to that of the nominal network $\mathcal{N}_0$. The μ-NDD property therefore quantifies network robust performance with respect to the unintended interaction map Δ. In general, asymptotic static disturbance attenuation of the subsystems is insufficient to guarantee μ-NDD for arbitrarily small μ. For example, the unintended interactions may result in $\lim_{\varepsilon\to 0^+} |w(t; \varepsilon)| \to \infty$, as shown in the motivating example of Section II, or they may de-stabilize the network.

Problem Statement. Given a perturbed network $\mathcal{N}(\varepsilon) = (\Sigma(\varepsilon), G, \Delta)$ consisting of subsystems with the asymptotic static disturbance attenuation property (6), determine conditions on $\Sigma_i(\varepsilon_i)$, G, and Δ such that given any $\mu > 0$, μ-NDD can be achieved if ε_i is sufficiently small for every i. $\triangledown$

Solution to the NDD problem identifies a class of perturbed networks that are robust to unintended interactions, in the sense that any effect arising from unintended interactions can be mitigated by simply improving disturbance attenuation of the constituent subsystems (i.e., decreasing ε_i). As we demonstrate next, one class of such networks are those with certain monotonicity properties.

IV. Network disturbance decoupling with monotone subsystems

After introducing background on monotone systems, we provide mathematical conditions to solve the NDD problem for networks composed of monotone subsystems.

A. Technical background: Monotone systems

We present some basic concepts on monotone systems theory and mixed-monotone functions. A more complete and in-depth treatment of these topics can be found in [43–47].

Definition 2. ([46]). A function $f : \mathcal{X} \to \mathcal{Y}$ is *mixed-monotone* if there exists a function $\hat{f} : \mathcal{X}^2 \to \mathcal{Y}$, called a *decomposition function* of $f(\cdot)$, such that for all $x, x_1, x_2, z \in \mathcal{X}$ the following are satisfied: (i) $f(x) = \hat{f}(x, x)$, (ii) $x_1 \leq x_2 \Rightarrow \hat{f}(x_1, z) \leq \hat{f}(x_2, z)$, and (iii) $x_1 \leq x_2 \Rightarrow \hat{f}(z, x_2) \leq \hat{f}(z, x_1)$. $\triangledown$

According to the above definition, take any $x^- \leq x \leq x^+$, we have $\hat{f}(x^-, x^+) \leq f(x) \leq \hat{f}(x^+, x^-)$. A differentiable function $f : \mathbb{R}^m \to \mathbb{R}^n$ has *sign-stable* partial derivatives if there exists a matrix $\Lambda \in \mathbb{R}^{n\times m}$, whose elements Λ_{ij} take values in $\{1, -1\}$ and satisfy $\Lambda_{ij}(\partial f_i/\partial x_j) \geq 0$ for all i, j and x. If f has sign-stable partial derivatives, then one decomposition function of f can be found through Λ. In particular, let

$$\Lambda^- = -\min(0, \Lambda), \qquad \Lambda^+ = \mathbf{1}_{m\times n} - \Lambda^-, \tag{9}$$

define a vector function $\hat{f}(x^+, x^-) : \mathbb{R}^{2m} \to \mathbb{R}^n$ whose i-th element is:

$$\hat{f}_i(x^+, x^-) := f_i\left(\mathrm{diag}(\Lambda_i^+) \cdot x^+ + \mathrm{diag}(\Lambda_i^-) \cdot x^-\right), \tag{10}$$

where Λ_i^+ (or Λ_i^-) is the i-th row of Λ^+ (or Λ^-, respectively). Then, $\hat{f}$ is a decomposition function of f. In particular, we call $\hat{f}$ the *canonical decomposition function* of f.

Example 1. Given a constant matrix A, the function $f(x) = Ax$ is mixed-monotone. Its canonical decomposition function is $\hat{f}(x^+, x^-) = A^+x^+ + A^-x^-$, where

$$A_{ij}^- := \begin{cases} A_{ij}, & \text{if } A_{ij} < 0, \\ 0, & \text{otherwise,} \end{cases} \qquad A^+ := A - A^-.$$

For any $x^- \leq x \leq x^+$, it can be verified that $\hat{f}(x^-, x^+) = A^+x^- + A^-x^+ \leq f(x) = Ax \leq A^+x^+ + A^-x^- = \hat{f}(x^+, x^-)$. $\triangledown$

Lemma 1. Let f and g be two mixed-monotone functions with decomposition functions $\hat{f}$ and $\hat{g}$, respectively. Then

$h := f \circ g$ is also mixed-monotone and $\hat{h}(x_1, x_2) := \hat{f}(\hat{g}(x_1, x_2), \hat{g}(x_2, x_1))$ is a decomposition function of h. $\triangledown$

Now we consider a system with input $u(t)$ and output $q(t)$:

$$\dot{x} = f(x, u), \qquad q = L(x), \tag{11}$$

where $f : \mathbb{R}^n \times \mathbb{R}^m \to \mathbb{R}^n$ and $L : \mathbb{R}^n \to \mathbb{R}^b$ are differentiable and their partial derivatives with respect to x and u are sign-stable. We review the notion of (orthant) input/state (I/S) monotone systems [45].

Definition 3. ([44, 45]). System (11) is I/S monotone if there exists vectors $\sigma^u \in \mathbb{R}^m$ and $\sigma^x \in \mathbb{R}^n$, whose elements take values in $\{1, -1\}$, such that

$$\sigma_i^x \sigma_j^x \frac{\partial f_i}{\partial x_j}(x, u) \geq 0, \qquad \sigma_i^x \sigma_k^u \frac{\partial f_i}{\partial u_k}(x, u) \geq 0,$$

for all indices $i \neq j$, k, and for all x, u. Specifically, the system is said to be I/S monotone with respect to the partial order pair $(\sigma^u; \sigma^x)$. $\triangledown$

If for each fixed u, the I/S monotone system (11) has a GAS equilibrium $x = \varphi(u)$, then $\varphi(\cdot)$ is called the static I/S characteristic of (11). The static I/S characteristic of an I/S monotone system has sign-stable partial derivatives [44]. In particular, the sign pattern of $(\partial\varphi/\partial u)$ is $\Lambda = \sigma^x(\sigma^u)^\top$, and the canonical decomposition function of φ can then be found according to (10). An important property of I/S monotone systems is the following convergent-input-convergent-state/output property.

Definition 4. ([44, 45].) System (11) is convergent-input-convergent-state if there exists a function $\phi(\cdot, \cdot) : \mathbb{R}^{2m} \to \mathbb{R}^{2n}$, called an *I/S gain function* of (11), such that for any u^-, u^+, if $u(t) \to [u^-, u^+]$, then $x(t) \to [\phi(u^-, u^+), \phi(u^+, u^-)]$. Similarly, it is convergent-input-convergent-output if there exists a function $\psi(\cdot, \cdot) : \mathbb{R}^{2m} \to \mathbb{R}^{2b}$, called an *I/O gain function*, such that for any u^-, u^+, if $u(t) \to [u^-, u^+]$, then $q(t) \to [\psi(u^-, u^+), \psi(u^+, u^-)]$. $\triangledown$

A graphical representation of a convergent-input-convergent-output system with I/O gain function ψ is shown in Fig.3. If the input $u(t)$ eventually enters the box $[u^-, u^+]$, output $q(t)$ will eventually converge to the box $[\psi(u^-, u^+), \psi(u^+, u^-)]$.

Lemma 2. Suppose that (11) is monotone with a static I/S characteristic $x = \varphi(u)$, then it is convergent-input-convergent-state. Additionally, if the output function $L(x)$ is mixed-monotone with a decomposition function $\hat{L}(x^+, x^-)$, then (11) is convergent-input-convergent-output. Specifically, let $\hat{\varphi}$ be the canonical decomposition function of φ, then an I/O gain function of (11) is $\psi(u^+, u^-) := \hat{L}(\hat{\varphi}(u^+, u^-), \hat{\varphi}(u^-, u^+))$. $\triangledown$

Proof for convergence of $x(t)$ can be found in [45] (Lemma 2). Convergence of $q(t)$ is a consequence of Lemma 1. I/S monotonicity of system (11) can be determined by simple graphical conditions [48]. Specifically, the *incidence graph induced by f* is a signed digraph. Each element in the $(n+m)$-vector $\xi = [x^\top, u^\top]^\top$ is a node. There is a directed edge (ξ_i, x_j) from ξ_i to x_j if $\text{sign}(\partial f_j/\partial \xi_i) \neq 0$ for some ξ. Each edge (ξ_i, x_j) is associated with a sign defined as $\text{sign}(\partial f_j/\partial \xi_i)$. An *undirected cycle* is a sequence of nodes $\xi_{c_1}, \cdots, \xi_{c_k}$ such that $\xi_{c_1} = \xi_{c_k}$ and for each $1 \leq i \leq (k-1)$, either edge $(\xi_{c_i}, \xi_{c_{i+1}})$ exists or edge $(\xi_{c_{i+1}}, \xi_{c_i})$ exists. The sign of this cycle is the product of the signs of all edges constituting the cycle.

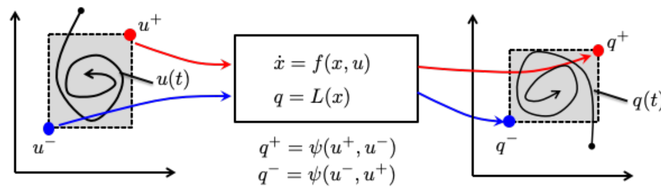

Fig. 3. **A graphical representation of the I/O gain function ψ for system (11)**. If the input $u(t)$ ultimately enters the box $[u^-, u^+]$, the output $q(t)$ ultimately converges to the box $[\psi(u^-, u^+), \psi(u^+, u^-)]$. This schematic assumes system (11) is cooperative, that is, $(\sigma^u; \sigma^x) = (1; \mathbf{1}_n)$, and $\partial L/\partial x \geq 0$ for all x.

Lemma 3. ([48, 49]). System (11) is I/S monotone if and only if the incidence graph induced by f does not contain an undirected negative cycle. $\triangledown$

B. Conditions on subsystems and interaction maps

Here we provide a set of sufficient conditions on the subsystem dynamics and prescribed/unintended interaction maps for NDD. These conditions are centered around the subsystems having the I/S monotonicity property, which we assume to hold on boxes $\mathcal{X}_i, \mathcal{R}_i, \mathcal{W}_i, \mathcal{Y}_i$, and $\mathcal{D}_i$. These boxes are Cartesian products of (possibly unbounded) closed real intervals. Additionally, we assume that for all $(r_i(t), w_i(t))$ taking values on $\mathcal{R}_i \times \mathcal{W}_i$ and for all $\varepsilon_i > 0$, the set $\mathcal{X}_i$ is positively invariant under the subsystems dynamics (4). For example, in biomolecular systems, $\mathcal{X}_i, \mathcal{R}_i, \mathcal{W}_i, \mathcal{Y}_i$, and $\mathcal{D}_i$ can be chosen as the non-negative orthant, because the state variables and I/O signals represent species concentrations and are thus non-negative.

Assumption 3. (Subsystem monotonicity). For every $\varepsilon_i \in (0, \varepsilon_i^*]$, each subsystem Σ_i in (4) is I/S monotone with respect to the partial orders $(\sigma^u; \sigma^x)$. The partial derivatives of output functions l_i and ρ_i are sign-stable. $\triangledown$

Due to Assumptions 1 and 3, the subsystem I/S characteristic φ_i is mixed-monotone. Let $\hat{\varphi}_i(u_i^+, u_i^-; \varepsilon_i)$ and $\hat{\rho}_i(x_i^+, x_i^-)$ be the canonical decomposition functions of $\varphi_i(u_i; \varepsilon_i)$ and $\rho_i(x_i)$, respectively. We follow Lemma 2 and define the disturbance I/O gain function of Σ_i as:

$$\psi_i(u_i^+, u_i^-; \varepsilon_i) := \hat{\rho}_i(\hat{\varphi}_i(u_i^+, u_i^-; \varepsilon_i), \hat{\varphi}_i(u_i^-, u_i^+; \varepsilon_i)). \tag{12}$$

We assume that increasing disturbance output from Σ_i does not decrease disturbance input to Σ_j. This is a mild assumption satisfied in many scenarios, including our motivating example in Section II, as we will show in Section VI.

Assumption 4. (Unintended interactions). The unintended interaction map $\Delta(\cdot)$ is cooperative, that is, $\Delta_i(d_j^-) \leq \Delta_i(d_j^+)$ for all i, j and $d_j^- \leq d_j^+$. $\triangledown$

The prescribed interaction map is assumed to have a simple structure.

Assumption 5. (Intended interaction). The intended interaction map $r = G(y)$ does not contain any feedback loop, that is, $\partial G_i/\partial y_j \equiv 0$ for all $j \geq i$. $\triangledown$

Given Assumptions 1 and 5, because G does not contain feedback loops, equation $r = G \circ H(r)$ has a unique solution $r^* = [r_1^*, \cdots, r_N^*]^\top$. We call r^* the *nominal reference input* to the network, since r^* is computed using G and the subsystem nominal static I/O characteristic $y_i = H_i(r_i)$, which is independent of w_i. We use

$$\psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i) := \psi_i(r_i^*, w_i^+, r_i^*, w_i^-; \varepsilon_i) \tag{13}$$

to represent a subsystem's disturbance I/O gain function for a fixed r_i^*. If $w_i \to [w_i^-, w_i^+]$ and $r_i \equiv r_i^*$, then the disturbance output d_i is ultimately bounded in the box $[\psi_i^*(w_i^-, w_i^+; r_i^*, \varepsilon_i), \psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i)]$. We will use ψ_i^* to elicit conditions for NDD and use $v^\pm$ to represent vector concatenation $v^\pm := [(v^-)^\top, (v^+)^\top]^\top$. Finally, we impose the following technical assumption on each subsystem's static characteristic and disturbance I/O gain function.

Assumption 6. (Subsystem Lipschitz conditions). The static I/O characteristic $h_i(r_i, w_i; \varepsilon_i)$ is Lipschitz continuous in $r_i \in \bar{\mathcal{R}}_i$ uniformly in $(w_i, \varepsilon_i) \in \mathcal{W}_i \times (0, \varepsilon_i^*]$. The disturbance I/O gain function $\psi_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i)$ is Lipschitz continuous in $r_i^-, r_i^+ \in \bar{\mathcal{R}}_i$ uniformly in $w_i^-, w_i^+ \in \mathcal{W}_i$ and $\varepsilon_i \in (0, \epsilon_i^*]$. In addition, ψ_i^* is sub-linear in that there exists a non-negative function $a_i(r_i)$ such that $|\psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i) - \psi_i^*(0, 0; r_i^*, \varepsilon_i)| \leq a_i(r_i^*)|w_i^\pm|$ uniformly in $\bar{\mathcal{R}}_i \times (0, \epsilon_i^*]$. $\triangledown$

C. NDD for networks composed of monotone subsystems

With reference to Fig.1, the perturbed network $\mathcal{N}$ can be regarded as a feedback interconnection of $\mathcal{N}_0$ and Δ. The nominal network $\mathcal{N}_0$, with input w and output d, has the convergent-input-convergent-output property. Specifically, its I/O gain function can be approximated by $\psi^* := [\psi_1^*, \cdots, \psi_N^*]^\top$, which is composed of subsystem I/O gain functions, as the next Lemma shows.

Lemma 4. Consider $\mathcal{N}_0$ under Assumptions 1-3,5,6, and suppose that the nominal reference input r^* satisfies $r_i^* \in \mathrm{int}(\bar{\mathcal{R}}_i)$ for all i. Then, there exists functions $P, Q : \mathbb{R}_{\geq 0} \times \mathbb{R}_{>0} \to \mathbb{R}_{\geq 0}$, such that if $w(t) \to [w^-, w^+]$, then

$$\begin{aligned} d(t) \to [&\psi^*(w^-, w^+; r^*, \varepsilon) - P(|w^\pm|; \varepsilon), \\ &\psi^*(w^+, w^-; r^*, \varepsilon) + P(|w^\pm|; \varepsilon)], \end{aligned} \tag{14a}$$

$$y(t) \to [H(r^*) - Q(|w^\pm|; \varepsilon), H(r^*) + Q(|w^\pm|; \varepsilon)]. \tag{14b}$$

Particularly, the functions P, Q can be decomposed as

$$\begin{aligned} P(|w^\pm|; \varepsilon) &:= p_1(\varepsilon)|w^\pm| + p_0(\varepsilon), \\ Q(|w^\pm|; \varepsilon) &:= q_1(\varepsilon)|w^\pm| + q_0(\varepsilon), \end{aligned} \tag{15}$$

where p_1, p_0, q_1, q_0 are non-negative scalar functions with the following property: for each i, given any $\mu > 0$, there exists $\varepsilon_i^{**} = \varepsilon_i^{**}(\mu, \varepsilon_{i+1}, \cdots, \varepsilon_N) > 0$, such that $p_1(\varepsilon), p_0(\varepsilon), q_1(\varepsilon), q_0(\varepsilon) \leq \mu$ if $0 < \varepsilon_i \leq \varepsilon_i^{**}(\mu, \varepsilon_{i+1}, \cdots, \varepsilon_N)$ for all i.

The proof of Lemma 4 is in Appendix Section VIII-A. Essentially, this property holds because each subsystem has the disturbance attenuation property (Assumption 2) and is monotone (Assumption 3). Equation (14a) allows us to approximate the disturbance I/O behavior of network $\mathcal{N}_0$ using the disturbance I/O gain functions (ψ_i^*) of the subsystems Σ_i with a constant reference input r_i^*. In addition, by (14b) and (15), for a constant $|w^\pm|$, the effect of disturbance input w on the prescribed output y can be arbitrarily diminished by decreasing each ε_i. Yet, for the perturbed network $\mathcal{N}$, we need to prove that $w = w(\varepsilon)$ does not grow as ε is decreased. To this end, we need some results on boundedness of discrete time systems. Specifically, consider

$$x(k+1) = F(x(k)), \tag{16}$$

where $x \in \mathbb{R}^n$, and without loss of generality, we assume that $F(0) = 0$. System (16) is said to be ultimately bounded [50] in a box $[x_*^-, x_*^+]$ if, for any initial condition $x(0)$, there exists a $k_* > 0$ such that $x(k) \in [x_*^-, x_*^+]$ for all $k \geq k_*$. We use $x(k) \to [x_*^-, x_*^+]$ to denote that $x(k)$ is ultimately bounded in $[x_*^-, x_*^+]$. We next introduce a Lyapunov characterization of the ultimate boundedness property that is robust to perturbations.

Definition 5. System (16) is said to be *exponentially ultimately bounded* if there exist positive constants c_1, c_2, c_3, r_0 and a function $V(\cdot) : \mathbb{R}^n \to \mathbb{R}$ such that

$$c_1|x|^2 \leq V(x) \leq c_2|x|^2, \tag{17a}$$

$$|V(x_1) - V(x_2)| \leq c_3|x_1 - x_2| \cdot (|x_1| + |x_2|), \tag{17b}$$

$$V(F(x)) - V(x) \leq -c_4|x|^2, \text{ for all } |x| \geq r_0. \tag{17c}$$

Specifically, if (17) is satisfied, system (16) is exponentially ultimately bounded in $[-r_*, r_*]$, where $r_* := c_1 r_0/c_2$. $\triangledown$

If (17) is satisfied with $r_0 = 0$, system (16) has an exponentially stable equilibrium point at $x = 0$. The boundedness property of an exponentially ultimately bounded system is robust to perturbations. In fact, consider a perturbation of the nominal system (16):

$$x(k+1) = F(x(k)) + p \cdot \delta(x(k)), \tag{18}$$

where p is a constant parameter and $|\delta(x)| \leq L_1|x| + L_2$ for all x. Assume that $F(x)$ is sub-linear, that is, there exists $L_F > 0$ such that $|F(x)| \leq L_F|x|$, then we can prove the following robust boundedness result for the perturbed discrete time system (18).

Lemma 5. Suppose the nominal system (16) is exponentially ultimately bounded in $[-r_*, r_*]$, then there exists $p_*, \kappa > 0$, such that for all $p \in [0, p_*]$, system (18) satisfies $x(k) \to [-r_* - \kappa p, r_* + \kappa p]$. $\triangledown$

The proof of Lemma 5 can be found in Appendix Section VIII-B. Now we are ready to state our first main result. It uses the monotonicity properties of Δ and the convergent-input-convergent-output of $\mathcal{N}_0$ in Lemma 4 to provide an ε-independent bound on $w(t; \varepsilon)$, which allows each subsystem to decrease ε_i for disturbance attenuation.

Theorem 1. Consider the perturbed network (4), (7), and

(8) under Assumptions 1-6. Suppose that there exists a set $\mathcal{R}_{\mathcal{N}} \subseteq \prod_{i=1}^{N} \bar{\mathcal{R}}_i$ and a positive constant vector $\bar{\varepsilon}_0 \leq \varepsilon^* := [\varepsilon_1^*, \cdots, \varepsilon_N^*]^\top$ such that for each fixed $0 < \varepsilon \leq \varepsilon_0$, $w(t;\varepsilon)$ is bounded for all t and that the discrete time dynamical system

$$\begin{aligned} w^-(k+1) &= \Delta \circ \psi^*(w^-(k), w^+(k); r^*, \varepsilon), \\ w^+(k+1) &= \Delta \circ \psi^*(w^+(k), w^-(k); r^*, \varepsilon) \end{aligned} \tag{19}$$

is exponentially ultimately bounded in an ε-independent set $[w_*^-(r^*), w_*^+(r^*)]$ for all $0 < \varepsilon \leq \varepsilon_0$ and for every $r^* \in \mathcal{R}_{\mathcal{N}}$. Then, there exists a positive function $\varepsilon_i^{**}(\mu, \varepsilon_{i+1}, \cdots, \varepsilon_N)$, such that for any $\mu > 0$, $\mathcal{N}$ has the μ-NDD property if $r^* \in \mathrm{int}(\mathcal{R}_{\mathcal{N}})$ and if $0 < \varepsilon_i \leq \varepsilon_i^{**}$ for all i. $\triangledown$

Proof. By Lemma 2, $\mathcal{N}_0$ has the convergent-input-convergent-output property. Since Δ is cooperative (Assumption 4) and $w(t)$ is bounded for all t, a small-gain theorem for convergent-input-convergent-output systems (Appendix Section VIII-D) shows that $w(t) \to [w_{**}^-, w_{**}^+]$ if the discrete time system

$$\begin{aligned} w^+(k+1) &= \Delta \circ [\psi^*(w^+, w^-; r^*, \varepsilon) + P(|w^\pm(k)|; \varepsilon)], \\ w^-(k+1) &= \Delta \circ [\psi^*(w^-, w^+; r^*, \varepsilon) - P(|w^\pm(k)|; \varepsilon)], \end{aligned} \tag{20}$$

is ultimately bounded in $[w_{**}^-, w_{**}^+]$. To show that w_{**}^- and w_{**}^+ can be chosen independent of ε, we treat (20) as a perturbation of the nominal discrete time system (19). By Lemma 5 and with reference to (15), there exists a $p^* > 0$, such that if (19) is exponentially ultimately bounded in an ε-independent set $[w_*^-, w_*^+]$ and $|p_1(\varepsilon)|, |p_0(\varepsilon)| \leq p^*$, then $[w_{**}^-, w_{**}^+]$ is ε-independent. Therefore, if $0 < \varepsilon_i \leq \min\{\bar{\varepsilon}_0, \varepsilon_i^{**}(p^*, \varepsilon_{i+1}, \cdots, \varepsilon_N)\}$ for every i, we can apply Lemma 4 to find

$$\begin{aligned} y(t; \varepsilon, \Delta) \to [&H(r^*) - q_1(\varepsilon)|w_{**}^\pm| - q_0(\varepsilon), \\ &H(r^*) + q_1(\varepsilon)|w_{**}^\pm| + q_0(\varepsilon)], \\ y(t; \varepsilon, 0) \to [&H(r^*) - q_0(\varepsilon), H(r^*) + q_0(\varepsilon)]. \end{aligned}$$

Hence, $\limsup_{t\to\infty} |y(t;\varepsilon,0) - y(t;\varepsilon,\Delta)| \leq q_1(\varepsilon)|w_{**}^\pm| + 2q_0(\varepsilon)$, where $w_{**}^\pm$ is ε-independent. This implies that, given any $\mu > 0$, μ-NDD can be achieved if each ε_i is taken sufficiently small such that $q_1(\varepsilon)|w_{**}^\pm| + 2q_0(\varepsilon) \leq \mu$. ■

Under the conditions of Theorem 1, NDD of the (nN)-dimensional continuous time system $\mathcal{N}$ can be certified if the $(2N)$-dimensional discrete time system (19) is ultimately bounded in an ε-independent set. This discrete time system can be constructed using the static disturbance I/O gain functions of the constituent subsystems and the unintended interaction Δ. It provides an upper bound for the "steady state amplification" of disturbance signals in the perturbed network. If the trajectory of (19) is ultimately bounded in an ε-independent set, then NDD can be achieved if each ε_i is sufficiently small. We call $\mathcal{R}_{\mathcal{N}}$ the *network admissible reference input set* because if the subsystems and the prescribed interactions are designed such that $r^* \in \mathcal{R}_{\mathcal{N}}$, then μ-NDD can be achieved for arbitrarily small μ by decreasing ε_i.

Remark 1. The discrete time dynamical system (19) does not explicitly involve the prescribed interaction map G. Instead, one can first compute r^* assuming no unintended interactions, and then substitute r^* into (19) to check if it leads to an ultimately bounded, ε-independent $w(k)$. $\triangledown$

Remark 2. Note that ε_i^* is a function of $\varepsilon_{i+1}, \cdots, \varepsilon_N$. This implies that the requirement on disturbance attenuation for an upstream subsystem i is generally stricter than its downstream subsystems $j \geq i+1$ to diminish propagation of the regulation error via prescribed interactions. In the special case where $G(y) \equiv r^*$ (i.e., no prescribed interactions), ε_i^* can be chosen independent of $\varepsilon_{i+1}, \cdots, \varepsilon_N$. $\triangledown$

Remark 3. The requirement for $w(t;\varepsilon)$ to be bounded for all t for each fixed ε is often satisfied in physical systems with nonlinear dynamics. For example, in biomolecular systems, the state variables represent molecular concentrations, which are often bounded above by conservation laws. If an ε-independent bound for $w(t;\varepsilon)$ can be easily found for $\mathcal{N}$, then there is no need to check the boundedness of (19). $\triangledown$

While many engineering subsystems have I/S monotone dynamics, the presence of controllers is often required for them to achieve asymptotic static disturbance attenuation. When a dynamic negative feedback controller is used to regulate a subsystem, the resultant dynamics of the regulated subsystem is often not monotone.

Example 2. Suppose that a plant has I/S monotone dynamics $\dot{x}_i = -x_i + u_i + w_i$, $y_i = x_i$, where $u_i = z_i$ is a control input arising from a dynamic feedback controller $\dot{z}_i = -z_i + (r_i - x_i)/\varepsilon_i$. It is easy to show that the regulated subsystem

$$\dot{x}_i = -x_i + z_i + w_i, \ \dot{z}_i = -z_i + (r_i - x_i)/\varepsilon_i \tag{21}$$

has the asymptotic static disturbance attenuation property with a nominal static I/O characteristic $H_i(r_i) = r_i$. However, the incidence graph induced by

$$f_i(x_i, z_i, r_i, w_i; \varepsilon_i) = \begin{bmatrix} -x_i + z_i + w_i \\ -z_i + (r_i - x_i)/\varepsilon_i \end{bmatrix}$$

contains a negative cycle $x_i \dashv z_i \to x_i$, indicating that it is non-monotone according to Lemma 3. $\triangledown$

Motivated by this example, in the next section, we seek conditions for networks composed of non-monotone subsystems to achieve NDD. In the context of Example 2, we show that if the dynamics of the feedback controller z_i is sufficiently fast, then (21) behaves like an I/S monotone system, thus, the results developed in this section hold with similar conditions.

V. Network disturbance decoupling with two-timescale non-monotone subsystems

Certain non-monotone systems can have dynamic properties similar to those of monotone systems [51, 52]. In particular, for autonomous systems, if the "non-monotone dynamics" in a two-timescale non-monotone system evolve at a sufficiently fast rate, certain convergence properties for monotone systems are preserved [51]. Based on similar reasonings, we provide conditions for NDD of networks composed of non-monotone subsystems.

A. Two-timescale subsystem setup

We consider subsystem Σ_i parameterized by an additional small positive parameter ν, which induces a timescale separation in the subsystems. For simplicity, we use the same ν for all subsystems, although the results are not restricted to this case. We now write $\Sigma_i = \Sigma_i(\varepsilon_i, \nu)$ as:

$$\begin{aligned} \dot{x}_i &= f_i(x_i, z_i, u_i; \varepsilon_i), \quad y_i = l_i(x_i) \\ \nu \dot{z}_i &= g_i(x_i, z_i, u_i; \varepsilon_i), \quad d_i = \rho_i(x_i, z_i), \end{aligned} \tag{22}$$

where $x_i \in \mathcal{X}_i \subseteq \mathbb{R}^n$, $z_i \in \mathcal{Z}_i \subseteq \mathbb{R}^m$ and the I/O signals $u_i = [r_i, w_i]^\top$ and $q_i = [y_i, d_i]^\top$ are defined as before in Section III. We assume that the prescribed output y_i is a function of the slow state x_i only, but the disturbance output d_i may depend on both x_i and z_i. Subsystem (22) is singularly perturbed by ν. In particular, in the fast time scale $\tau = t/\nu$, the boundary layer dynamics [50] of (22) are:

$$\mathrm{d}z_i/\mathrm{d}\tau = g_i(x_i, z_i, u_i; \varepsilon_i), \tag{23}$$

where x_i and u_i are treated as fixed parameters.

Assumption 7. (Subsystem boundary layer). For every fixed $(x_i, u_i) \in \mathcal{X}_i \times (\mathcal{R}_i \times \mathcal{W}_i)$ and $\varepsilon_i \in (0, \varepsilon_i^*]$, system (23) has a GAS equilibrium $\bar{z}_i = \Gamma_i(x_i, u_i; \varepsilon_i) \in \mathcal{Z}_i$. $\triangledown$

Substituting $z_i = \Gamma_i(x_i, u_i; \varepsilon_i)$ into (22), a candidate reduced model of (22) is:

$$\dot{\bar{x}}_i = \bar{f}_i(\bar{x}_i, u_i; \varepsilon_i), \;\; \bar{d}_i = \bar{\rho}_i(\bar{x}_i, u_i; \varepsilon_i), \;\; \bar{y}_i = l_i(\bar{x}_i), \tag{24}$$

where $\bar{f}_i(\bar{x}_i, u_i; \varepsilon_i) := f_i(\bar{x}_i, \Gamma_i(\bar{x}_i, u_i; \varepsilon_i), u_i; \varepsilon_i)$ and $\bar{\rho}_i(\bar{x}_i, u_i; \varepsilon_i) := \rho_i(\bar{x}_i, \Gamma_i(\bar{x}_i, u_i; \varepsilon_i))$. We denote system (24) by $\bar{\Sigma}_i$ and require it to have similar stability, disturbance attenuation, monotonicity, and Lipschitz continuity properties as specified for the subsystems in Section IV. These conditions are summarized below.

Assumption 8. Each $\bar{\Sigma}_i$ satisfies the following:

(i) It is I/S monotone with respect to the partial orders $(\sigma^u; \sigma^x)$ for all $\varepsilon_i \in (0, \varepsilon_i^*]$. The output functions $\bar{\rho}_i$ and l_i have sign-stable partial derivatives.

(ii) It is endowed with a well-defined I/S characteristic $\bar{\varphi}_i(u_i; \varepsilon_i)$. The I/O characteristics $h_i(u_i; \varepsilon_i)$ satisfies Assumption 2. $\triangledown$

By this assumption, the functions $\bar{\varphi}_i(u_i; \varepsilon_i)$ and $\bar{\rho}_i(x_i, z_i)$ have canonical decomposition functions $\hat{\varphi}_i(u_i^+, u_i^-; \varepsilon_i)$ and $\hat{\rho}_i(\bar{x}_i^+, u_i^+, \bar{x}_i^-, u_i^-; \varepsilon_i)$, respectively. The decomposition functions can be composed according to Lemma 1 to obtain the disturbance I/O gain function

$$\psi_i(u_i^+, u_i^-; \varepsilon_i) = \hat{\rho}_i(\hat{\varphi}_i(u_i^+, u_i^-; \varepsilon_i), u_i^+, \hat{\varphi}_i(u_i^-, u_i^+; \varepsilon_i), u_i^-; \varepsilon_i).$$

Similar to (13), we write

$$\psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i) := \psi_i(r_i^*, w_i^+, r_i^*, w_i^-; \varepsilon_i) \tag{25}$$

for the subsystem static disturbance I/O gain function for a fixed reference input r_i^*. Under mild technical conditions, the conditions to guarantee NDD in Theorem 1 for networks with monotone subsystems are also sufficient for networks composed of two-timescale subsystems (22). To show this, we extend the convergent-input-convergent-state/output results for monotone systems in Lemma 2 to singularly perturbed systems with monotone reduced dynamics. This requires an additional technical assumption as follows.

Assumption 9. There exists $M_1(\varepsilon) > 0$, independent of ν, such that $|u(t)| \leq M_1(\varepsilon)$ for all t. In addition, there exists $M_2(\varepsilon) > 0$, independent of ν, such that $\|\dot{u}\| \leq M_2(\varepsilon)$. $\triangledown$

Lemma 6. (Approximate convergent-input-convergent-output for singularly perturbed monotone systems). Consider system (22) and suppose that Assumptions 7-9 are satisfied. Then, given any $e > 0$, there exists $\nu^*(e; \varepsilon)$, such that for a fixed ε, if $0 < \nu \leq \nu^*$ and $u_i(t) \to [u_i^-, u_i^+]$, then

$$d_i(t) \xrightarrow{e} [\psi_i(u_i^-, u_i^+; \varepsilon_i), \psi_i(u_i^+, u_i^-; \varepsilon_i)]. \tag{26}$$

Proof. See Section VIII-C in the Appendix for details. ■

Remark 4. If g_i is not a function of $u_i(t)$, the requirement $\|\dot{u}_i\| \leq M_2$ in Assumption 9 can be removed. $\triangledown$

B. NDD for networks composed of two-timescale subsystems

Using Lemma 6, we can determine conditions for NDD of a perturbed network composed of two-timescale non-monotone subsystems.

Theorem 2. Consider the perturbed network (7), (8), and (22) under Assumptions 4-9. Suppose that there exists a set $\mathcal{R}_\mathcal{N} \subseteq \prod_{i=1}^N \bar{\mathcal{R}}_i$ and a positive constant $\bar{\varepsilon}_0 \leq \varepsilon^*$ such that for all $0 < \varepsilon \leq \varepsilon_0$ the discrete time system (19), where ψ^* is the I/O gain function of the reduced system defined in (25), is exponentially ultimately bounded in an ε-independent set $[w_*^-, w_*^+]$. Then, given any $\mu > 0$, μ-NDD can be achieved if $r^* \in \text{int}(\mathcal{R}_\mathcal{N})$ and if for all i

$$0 < \varepsilon_i \leq \varepsilon_i^{***}(\mu, \varepsilon_{i+1}, \cdots, \varepsilon_N), \; 0 < \nu \leq \nu^*(\mu, \varepsilon), \tag{27}$$

where ε_i^{***} and ν^{**} are both positive functions non-increasing with μ. $\triangledown$

Proof. (Sketch). The proof is similar to that of Theorem 1 but we need to keep track of the model reduction error arising from applying Lemma 6 to the subsystems. In particular, for a perturbed network composed of singularly perturbed monotone subsystems, there exists $\nu^{**}(\mu_1; \varepsilon)$ such that for all $0 < \nu \leq \nu^{**}$, the convergence result in (14) can be replaced by

$$\begin{aligned} d(t) &\xrightarrow{\mu_1} [\psi^*(w^-, w^+; r^*, \varepsilon) - P(|w^\pm|; \varepsilon), \\ &\qquad \psi^*(w^+, w^-; r^*, \varepsilon) + P(|w^\pm|; \varepsilon)], \\ y(t) &\xrightarrow{\mu_1} [H(r^*) - Q(|w^\pm|; \varepsilon), H(r^*) + Q(|w^\pm|; \varepsilon)], \end{aligned}$$

where P and Q have the same form as those in (15). If the discrete time system (19) converges to $[w_*^-, w_*^+]$, the small-gain theorem for approximate convergent-input-convergent-output systems (Lemma 11 in Appendix Section VIII-D) leads to $w(t) \xrightarrow{\alpha(\mu_1)} [w_{**}^-, w_{**}^+]$, where $\alpha(\cdot)$ is a class $\mathcal{K}_0$ function, and w_{**}^+ and w_{**}^- are ε-independent. The rest of the proof is similar to that of Theorem 1. One can take, for example, $\varepsilon_i^{***} := \varepsilon_i^{**}(\mu/2, \varepsilon_{i+1}, \cdots, \varepsilon_N)$ and $\nu^{**} = \nu^*(\mu/2, \varepsilon)$. ■

In summary, in addition to the conditions of Theorem 1, to achieve NDD for networks composed of non-monotone subsystems, Theorem 2 requires that the timescale separation in each subsystem is sufficiently large (ν is sufficiently small). This ensures that the behavior of Σ_i, which may be non-monotone, are sufficiently close to that of $\bar{\Sigma}_i$, which is monotone. Since ν^{**} depends on ε, when decreasing ε to achieve μ-NDD for a fixed μ, it is important to ensure that $\nu \leq \nu^{**}(\mu, \varepsilon)$ remains satisfied.

Remark 5. While a small ε ensures that the equilibrium location of $\mathcal{N}$ is close to that of $\mathcal{N}_0$, the value of parameter ν does not affect the equilibrium location of (22) and hence that of the perturbed network. The role of a small ν is to guarantee that $\mathcal{N}$ is dynamically "well-behaved". This is a consequence of the approximate convergent-input-convergent-state property for singularly perturbed monotone subsystems in Lemma 6. $\triangledown$

VI. Motivating example revisited

Here we apply Theorem 2 to a network composed of genetic feedback-regulated subsystems described in Section II. The feedback-regulated subsystem dynamics in (3) are not monotone, because the incidence graph induced by the dynamics in (3) contains a negative cycle: $s_i \dashv m_i \rightarrow p_i \rightarrow s_i$. However, if the decay rate constant δ_0 of the RNA species m_i and s_i can be made much larger than the decay rate constant δ of protein p_i, then the subsystem dynamics can be regarded as a two-timescale system [53]. In particular, the subsystem dynamics can be re-written as:

$$\nu \dot{m}_i = \frac{1}{\varepsilon_i} r_i - \frac{1}{\varepsilon_i}\lambda_i m_i s_i - \delta m_i, \tag{29a}$$

$$\nu \dot{s}_i = \frac{1}{\varepsilon_i}\beta_i p_i - \frac{1}{\varepsilon_i}\lambda_i m_i s_i - \delta s_i, \tag{29b}$$

$$\dot{p}_i = T_i(m_i, w_i) - \delta p_i, \tag{29c}$$

$$y_i = p_i,\ d_i = \rho_i(x_i, z_i) = m_i/\kappa_i. \tag{29d}$$

System (29) is in the form of (22), with fast state variables $z_i = [m_i, s_i]^\top$, slow state variable $x_i = p_i$, reference input r_i, disturbance input w_i, prescribed output y_i, and disturbance output d_i. In practice, the decay rate constant (δ_0) of mRNA and sRNA is often faster than that of protein (δ) [54]. To further increase δ_0 to reduce ν, one can (a) engineer the mRNA sequence to recruit additional RNase for its degradation or (b) produce an additional mRNA species m_i' that can bind and sequester sRNA s_i to effectively enhance its removal rate [53, 55]. The parameter ε_i can be decreased experimentally by increasing the amount of DNA that encodes m_i and s_i [24] and by rational design of the sRNA sequence [56]. In order to apply Theorem 2, the following section verifies Assumptions 4-9.

A. Verification of Assumptions 4-9

Recall from (2) that ribosome competition can be modeled as unintended interaction

$$w_i = \Delta_i(d) = \sum_{j \neq i} d_j, \tag{30}$$

which satisfies Assumption 4. The prescribed interactions G_i are Hill functions, which are globally Lipschitz. We only consider G that does not contain any feedback loops and, thus, satisfies Assumption 5. These interaction maps and subsystem dynamics (29) give rise to the perturbed gene network $\mathcal{N}$. The non-negative orthant is positvely invariant under the dynamics of $\mathcal{N}$. Hence, we have $\mathcal{X}_i, \mathcal{R}_i, \mathcal{W}_i = \mathbb{R}_{\geq 0}$ and $\mathcal{Z}_i = \mathbb{R}^2_{\geq 0}$. The required Lipschitz conditions in Assumption 6 are verified in Appendix Section VIII-F.

To verify Assumption 7, the boundary layer dynamics are:

$$\begin{aligned} \frac{\mathrm{d}}{\mathrm{d}\tau} m_i &= \frac{1}{\varepsilon_i}(r_i - \lambda_i m_i s_i) - \delta m_i, \\ \frac{\mathrm{d}}{\mathrm{d}\tau} s_i &= \frac{1}{\varepsilon_i}(\beta_i p_i - \lambda_i m_i s_i) - \delta s_i. \end{aligned} \tag{31}$$

For each fixed pair $(r_i, p_i) \in \mathcal{R}_i \times \mathcal{X}_i$ and positive ε_i, system (31) has a unique non-negative equilibrium $\bar{z}_i = [\bar{m}_i, \bar{s}_i]^\top \in \mathcal{Z}_i$, where

$$\bar{m}_i(p_i, r_i; \varepsilon_i) = \frac{A_i + \sqrt{A_i^2 + 4\varepsilon_i^2\delta^2\lambda_i r_i}}{2\varepsilon_i \delta \lambda_i}, \tag{32}$$

with $A_i(p_i, r_i) := r_i\lambda_i - \beta_i\lambda_i p_i - \delta^2\varepsilon_i^2$. GAS of $\bar{z}_i$ has been shown using a Lyapunov function [53, 57].

To verify Assumption 8, we substitute $\bar{m}_i$ into (29c) and (29d), the reduced subsystem dynamics $\bar{\Sigma}_i$ follow:

$$\dot{\bar{p}}_i = \bar{f}_i(\bar{p}_i, r_i, w_i; \varepsilon_i),\ \ \bar{y}_i = \bar{p}_i,\ \ \bar{d}_i = \bar{\rho}_i(\bar{p}_i, r_i; \varepsilon_i), \tag{33}$$

where

$$\bar{f}_i(\bar{p}_i, r_i, w_i; \varepsilon_i) = T_i(\bar{m}_i(\bar{p}_i, r_i; \varepsilon_i), w_i) - \delta\bar{p}_i, \tag{34a}$$

$$\bar{\rho}_i(\bar{p}_i, r_i; \varepsilon_i) = \bar{m}_i(\bar{p}_i, r_i; \varepsilon_i)/\kappa_i. \tag{34b}$$

According to (2) and (32), we have:

$$\frac{\partial T_i}{\partial \bar{m}_i} > 0, \quad \frac{\partial T_i}{\partial w_i} < 0, \quad \frac{\partial \bar{m}_i}{\partial r_i} > 0, \quad \frac{\partial \bar{m}_i}{\partial \bar{p}_i} < 0, \quad . \tag{35}$$

Hence, $\frac{\partial \bar{f}_i}{\partial r_i} = \frac{\partial \bar{f}_i}{\partial T_i} \cdot \frac{\partial T_i}{\partial \bar{m}_i} \cdot \frac{\partial \bar{m}_i}{\partial r_i} > 0$ and $\frac{\partial \bar{f}_i}{\partial w_i} = \frac{\partial \bar{f}_i}{\partial T_i} \cdot \frac{\partial T_i}{\partial w_i} < 0$. Consequently, $\bar{\Sigma}_i$ is I/S monotone with respect to the partial orders $(\sigma^r, \sigma^w; \sigma^x) = (1, -1; 1)$. Since the output functions have sign-stable partial derivatives, Assumption 8-(i) is satisfied. To verify Assumption 8-(ii), we first show that the scalar reduced dynamics (33) has a well-defined I/S characteristic. For each fixed $(r_i, w_i) \in \mathcal{R}_i \times \mathcal{W}_i$ and $\varepsilon_i > 0$, the function $\bar{f}_i(\bar{p}_i; r_i, w_i, \varepsilon_i)$ is monotonically decreasing in $\bar{p}_i$. In addition, since $\bar{f}_i(0, r_i, w_i; \varepsilon_i) \geq 0$ and $\lim_{\bar{p}_i \to +\infty} \bar{f}_i(\bar{p}_i, r_i, w_i; \varepsilon_i) = -\infty$, the scalar reduced system $\dot{\bar{p}}_i = \bar{f}_i(\bar{p}_i, r_i, w_i; \varepsilon_i)$ has a GAS equilibrium. Let

$$\bar{p}_i = \bar{\varphi}_i(r_i, w_i; \varepsilon_i) \tag{36}$$

be the static I/S characteristic of $\bar{\Sigma}_i$, since $\bar{y}_i = \bar{p}_i$, the subsystem static I/O characteristic is $h_i = \bar{\varphi}_i$. To verify the static disturbance attenuation property in Assumption 8-(ii), we show in Appendix Section VIII-E that there exists constants $K_i^1, K_i^2 > 0$ such that

$$|h_i(r_i, w_i; \varepsilon_i) - r_i/\beta_i| \leq \varepsilon_i(K_i^1|w_i| + K_i^2) \tag{37}$$

for all $(r_i, w_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i$ and for ε_i sufficiently small, where $\bar{\mathcal{R}}_i$ can be taken as any ε-independent compact subset of $(0, \alpha_i\beta_i/\delta)$. Hence, comparing (37) with equation (6), the nominal static I/O characteristic is $H_i(r_i) = r_i/\beta_i$, and $\bar{\mathcal{R}}_i$ is an admissible reference input set.

Finally, we verify Assumption 9, which requires $r(t)$ and $w(t)$ and their derivatives to be bounded. By (29c) and the comparison lemma, for any initial condition, $p_i(t)$ is globally attracted to the set $[0, \alpha_i/\delta]$. With reference to (29c), because $T_i(m_i, w_i)$ is bounded in $[0, \alpha_i]$, $\dot{p}_i$ is bounded in $[-\alpha_i, \alpha_i]$. Because $r_i = G_i(y) = G_i(p)$ and $G(\cdot)$ is a Hill function, $\dot{r}_i(t)$ and $r_i(t)$ are both bounded. Similarly, it is possible to verify from (29) that $[0, r_i/\varepsilon_i]$ is a globally attractive set for $m_i(t)$ and hence $w_i(t) = \sum_{j\neq i} d_j = \sum_{j\neq i} m_j/\kappa_j$ is bounded by a ν-independent constant. We do not need $\dot{w}(t)$ to be bounded by an ν-independent constant because the boundary layer dynamics (31) does not depend on $w(t)$.

B. Application of Theorem 2

Because $\bar{\Sigma}_i$ is I/S monotone with respect to $(\sigma^r, \sigma^w; \sigma^x) = (1, -1; 1)$, the canonical decomposition function of $\bar{\varphi}_i$, which we denote as $\hat{\varphi}_i$, is

$$\hat{\varphi}_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i) = \bar{\varphi}_i(r_i^+, w_i^-; \varepsilon_i). \tag{38}$$

For the disturbance output function $\bar{\rho}_i$, since $\partial\bar{\rho}_i/\partial\bar{p}_i < 0, \partial\bar{\rho}_i/\partial r_i > 0$, the canonical decomposition function $\hat{\rho}_i$ of $\bar{\rho}_i$ is:

$$\hat{\rho}_i(p_i^+, r_i^+, p_i^-, r_i^-; \varepsilon_i) = \bar{\rho}_i(p_i^-, r_i^+; \varepsilon_i). \tag{39}$$

Given (38)-(39), and according to Lemma 1, a static disturbance I/O gain function of $\bar{\Sigma}_i$ is $\psi_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i) := \bar{\rho}_i(\bar{\varphi}_i(r_i^-, w_i^+; \varepsilon_i), r_i^+; \varepsilon_i) = \bar{m}_i(\bar{\varphi}_i(r_i^-, w_i^+; \varepsilon_i), r_i^+; \varepsilon_i)/\kappa_i$. Because of this and by equation (25), for a fixed input r_i^*, we have:

$$\psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i) = \bar{m}_i(\bar{\varphi}_i(r_i^*, w_i^+; \varepsilon_i), r_i^*, \varepsilon_i)/\kappa_i. \tag{40}$$

On the other hand, when (33) reaches steady state, $\bar{m}_i$ necessarily satisfies

$$\alpha_i \frac{\bar{m}_i(\bar{\varphi}_i(r_i^*, w_i^+; \varepsilon_i), r_i^*, \varepsilon_i)/\kappa_i}{1 + \bar{m}_i(\bar{\varphi}_i(r_i^*, w_i^+; \varepsilon_i), r_i^*; \varepsilon_i)/\kappa_i + w_i^+} = \delta\bar{\varphi}_i(r_i^*, w_i^+; \varepsilon_i).$$

Substituting into (40), the disturbance I/O gain function of (33) can be written alternatively as:

$$\psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i) = \frac{\delta\bar{\varphi}_i(r_i^*, w_i^+; \varepsilon_i)(1 + w_i^+)}{\alpha_i - \delta\bar{\varphi}_i(r_i^*, w_i^+; \varepsilon_i)}. \tag{41}$$

With Assumptions 4-9 satisfied, we can apply Theorem 2 to determine if NDD can be achieved for genetic circuits composed of subsystems (29). Specifically, we find that the discrete time dynamical system (19), where ψ^* is given by (41), is exponentially ultimately bounded in an ε-independent set for some r^* values, and hence NDD can be guaranteed for some r^* according to Theorem 2.

Proposition 1. Let $\mathcal{R}_{\mathcal{N}}$ be an ε-independent compact subset of

$$\tilde{\mathcal{R}}_{\mathcal{N}} := \left\{ r_i^* \in \bar{\mathcal{R}}_i : \sum_{j\neq i} \frac{r_j^*\delta}{\alpha_j\beta_j - \delta r_j^*} < 1, \forall i \right\}. \tag{42}$$

Given any $\mu > 0$, the perturbed network (7), (29), and (30) has the μ-NDD property if $r^* \in \mathcal{R}_{\mathcal{N}}$ and ν and each ε_i are sufficiently small. $\triangledown$

Proof. With all assumptions in Theorem 2 satisfied, we only need to verify that the discrete time system (19) is exponentially ultimately bounded in an ε-independent set. Given the form of ψ_i^* in (41), we find that the dynamics of w^+ and w^- in (19) will be completely decoupled. Hence, it is sufficient to show that the trajectory of the following N-dimensional discrete time system is exponentially ulitmately bounded in an ε-independent set:

$$\begin{aligned} w_i(k+1) =& \Delta_i \circ \psi^*(w(k); r^*, \varepsilon) \\ =& \sum_{j\neq i} \frac{\delta\bar{\varphi}_j(r_j^*, w_j(k); \varepsilon_j)(1 + w_j(k))}{\alpha_j - \delta\bar{\varphi}_j(r_j^*, w_j(k); \varepsilon_j)}. \end{aligned} \tag{43}$$

To this end, we define

$$\eta_i(r_i, w_i; \varepsilon_i) := \frac{\delta\bar{\varphi}_i(r_i, w_i; \varepsilon_i)}{\alpha_i - \delta\bar{\varphi}_i(r_i, w_i; \varepsilon_i)},\ \eta_i^*(r_i) := \frac{\delta r_i}{\alpha_i\beta_i - \delta r_i},$$

and note that for all $(r_i, w_i; \varepsilon_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i \times (0, \varepsilon_i^*]$, the followings are satisfied: (i) $\eta_i(r_i, w_i; \varepsilon_i) > 0$, (ii) $\eta_i(r_i, w_i; \varepsilon_i) < \eta_i(r_i, 0; \varepsilon_i)$, and (iii) $\alpha_i - \delta h_i(r_i, w_i; \varepsilon_i)$ is bounded away from 0, and thus by (37), there exists constant $k_i > 0$ such that $|\eta_i(r_i, 0; \varepsilon_i) - \eta_i^*(r_i)| \le k_i\varepsilon_i$. Using these properties, we consider $V(k) := |w(k)|^2$ as a candidate Lyapunov function, which satisfies

$$\begin{aligned} V(k+1) =& \left| \sum_{j\neq i} \eta_j(r_j^*, w_j(k); \varepsilon_j)(1 + w_j(k)) \right|^2 \\ \le& (1 + |w(k)|)^2 \left[\sum_{j\neq i} \eta_j^*(r_j^*) + k_j\varepsilon_j \right]^2. \end{aligned} \tag{44}$$

Because $r^* \in \mathcal{R}_{\mathcal{N}}$ and $\varepsilon \in (0, \varepsilon_i^*]$, there exists an ε-independent constant $0 < \vartheta < 1$, such that $\sum_{j\neq i}(\eta_j^*(r_j^*) + k_j\varepsilon_j) \le 1 - \vartheta$ for all i. Thus, we have $V(k+1) - V(k) \le (1-\vartheta)(1+|w|)^2 - |w|^2 \le -\vartheta|w|^2 + 2(1-\vartheta)|w| + (1-\vartheta) \le -\vartheta|w|^2/2$ if $|w| \ge w_* := \max\{1, 6(1-\vartheta)/\vartheta\}$, where w_* is ε-independent. This proves that (43) is exponentially ultimately bounded in $[0, w_*]$. ∎

Therefore, if G and Σ_i are designed such that $r^* \in \mathcal{R}_{\mathcal{N}}$, then the network behavior can be made independent of Δ (i.e., NDD is achieved) by making ε_i sufficiently small in each subsystem. Our result thus provides an analytical robustness performance limit for genetic circuits composed of feedback-regulated genetic subsystems.

Remark 6. According to (42), because $\frac{r_i^*\delta}{\alpha_i\beta_i - \delta r_i^*}$ is positive for $r_i \in \bar{\mathcal{R}}_i$, as the number of subsystems increases, the reference input each subsystem can take for the network to maintain NDD decreases.

C. Example: Network without prescribed interactions

We first consider a network consisting of three identical feedback-regulated subsystems with reference input $r_i^* = r_0$ for all i (see Fig. 2a). Recall that in Fig.2b, our simulations show that NDD can only be achieved for certain reference input levels. To explain this, we apply Proposition 1 and find that any compact subset of $\tilde{\mathcal{R}}_{\mathcal{N}} := \{0 < r_0 < 100/3\}$ is a network admissible input set, which we denote by $\mathcal{R}_{\mathcal{N}}$. In accordance with the simulation in Fig.2b, NDD can be achieved by decreasing ε_i if $r^* \in \mathcal{R}_{\mathcal{N}}$. On the other hand, decreasing ε_i does not improve the network's robustness to unintended interactions if $r^* \notin \tilde{\mathcal{R}}_{\mathcal{N}}$, indicating that our result is not conservative. The value of ν does not affect NDD

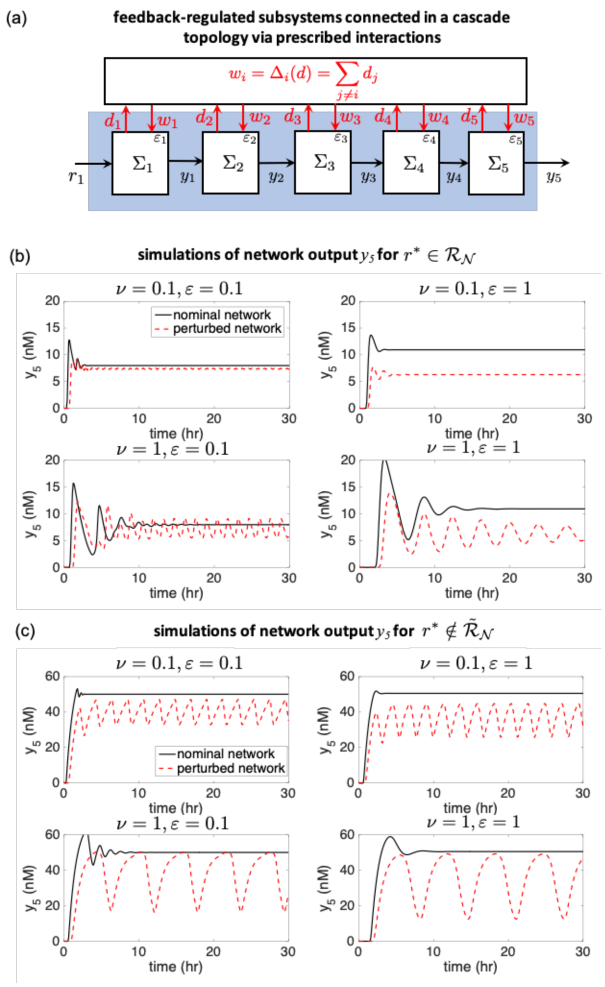

Fig. 4. **Network disturbance decoupling for a cascade of feedback-regulated genetic subsystems.** (a) Schematic of a genetic circuit composed of five feedback-regulated subsystems connected in a cascade topology. (b) Simulation results for the network when $r_i^* \in \mathcal{R}_{\mathcal{N}}$. (c) Simulation results for the network when $r_i^* \notin \tilde{\mathcal{R}}_{\mathcal{N}}$. Simulation parameters are identical for all subsystems: $\alpha_i = 70$ nM/hr, $\lambda_i = 5$ $(\mathrm{nM}\cdot\mathrm{hr})^{-1}$, $\delta = 0.5$ hr^{-1}, $\beta_i = 1$ hr^{-1}, $\kappa_i = 10$ nM, and $\varepsilon_i = \varepsilon$ for all i. The prescribed interactions follow equation (45) with parameters: $n_i = 4$, $k_i = 6$ nM, and and $r_1^* = 10$ nM/hr. For panel (b) $B_i = 10$ nM/hr for all $i \geq 2$ and for panel (c) $B_i = 10$ nM/hr for $i = 2$ and $B_i = 50$ nM/hr for $i = 3, 4, 5$.

property of $\mathcal{N}$. In fact, local stability of this network can be shown for any $\nu > 0$ through linearization [58]. Thus, with reference to Remark 5, there is no need to decrease ν to ensure network stability and the requirement for ν to be sufficiently small in Proposition 1 is conservative in this special case.

D. Example: Network with cascade-like prescribed interactions

We study another network $\mathcal{N}$ composed of five genetic feedback-regulated subsystems connected in a cascade topology through prescribed interactions, that is, through transcriptional regulation (see Fig. 4a). In particular, we model prescribed interactions as Hill functions [37]:

$$r_i = G_i(y_{i-1}) = \begin{cases} B_i \frac{(y_{i-1}/k_i)^{n_i}}{1+(y_{i-1}/k_i)^{n_i}}, & \text{if } i \neq 1, \\ r_1^*, & \text{if } i = 1, \end{cases} \tag{45}$$

where B_i quantifies the maximum transcription rate from gene i, k_i is a dissociation constant whose value decreases with the binding affinity between protein p_{i-1} and the promoter of gene i, and n_i is describes the binding cooperativity. Using (45) and the subsystem nominal static I/O characteristic $y_i = r_i/\beta_i$, we can compute the nominal reference input r^*. Simulation results for network $\mathcal{N}$ with different (ν, ε) pairs are shown in Fig. 4b-c. For the simulations in Fig. 4b, we choose parameters for the subsystems and the prescribed interaction map such that $r^* \in \mathcal{R}_{\mathcal{N}}$. We therefore apply Proposition 1 to claim that for arbitrarily small μ, μ-NDD of $\mathcal{N}$ can be achieved by decreasing both ε and ν, which is consistent with simulations in Fig. 4b. In contrast, when the parameters are chosen such that $r^* \notin \tilde{\mathcal{R}}_{\mathcal{N}}$, as shown in Fig. 4c, decreasing ε and ν does not lead to NDD.

VII. Discussion and Future Work

In this paper, we have studied networked dynamical systems, in which unintended interactions among subsystems perturb the prescribed network's behavior. We have provided conditions on subsystem dynamics, the intended and the unintended interaction maps to achieve network disturbance decoupling (NDD), where the steady state outputs from all subsystems become essentially independent of the unintended interactions. While NDD may be addressed by designing the entire network monolithically, we find that, under certain conditions, NDD can be obtained by simply improving each subsystem's robustness to a constant, state-independent disturbance. Specifically, these conditions require that (i) all subsystems are I/S monotone, (ii) the prescribed interactions among subsystems do not contain feedback loops, and (iii) the unintended interactions are cooperative. When the subsystem dynamics are non-monotone, the same result holds with similar conditions if the subsystem dynamics have a timescale separation property, such that each reduced subsystem dynamics are monotone. We apply our theoretical result to guide the design of genetic circuits that are robust to context. In particular, we show that a recently implemented biomolecular feedback controller [24], which enables a single genetic subsystem to asymptotically attenuate a constant disturbance, can theoretically be used to regulate multiple genes in a network to reach NDD.

Experimental validation of the results in Section VI is underway. In the future, we plan to consider NDD problems for a larger class of unintended interactions Δ, including, for example, Δ that contain dynamics. We also plan to extend this study to multi-stable networks and to consider intended interaction maps that contain feedback loops. These studies may provide guidance to engineer networked systems to function robustly in different contexts.

VIII. Appendix

A. Proof of Lemma 4

To prove Lemma 4, we note that the I/O gain function of each subsystem has the following property.

Lemma 7. Suppose that Assumptions 1,2,6 are satisfied and let $\hat{h}_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i)$ be the canonical decomposition function of $h_i(r_i, w_i; \varepsilon_i)$, then, for any $e_i > 0$ such that $r_i - e_i, r_i + e_i \in \bar{\mathcal{R}}_i$, the function $\hat{h}_i$ satisfies:

$$\begin{aligned}|\hat{h}_i(r_i+e_i, w_i^+, r_i-e_i, w_i^-; \varepsilon_i) - H_i(r_i)| \\ \leq L_h|e_i| + \alpha_i(\varepsilon_i)|w_i^{\pm}| + \alpha_i^0(\varepsilon_i),\end{aligned} \quad (46)$$

where $L_h > 0$ is the Lipschitz constant of h_i . $\triangledown$

Proof. Due to Assumption 3, the static I/O characteristic $y_i = h_i(r_i, w_i; \varepsilon_i)$ is also sign-stable. For $s = r, w$, define $\Lambda_s := \text{sign}(\partial h_i / \partial s_i)$, and let Λ_s^+ and Λ_s^- be defined according to (9). By equation (10), let $\Lambda_{s,j}$ be the j-th row of matrix Λ_s, the canonical decomposition function

$$\hat{h}_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i) := h_i(\mathfrak{p}_r(r_i^{\pm}), \mathfrak{p}_w(w_i^{\pm}); \varepsilon_i), \quad (47)$$

where

$$\begin{aligned}\mathfrak{p}_{r,j}(r^{\pm}) &:= \text{diag}(\Lambda_{r,j}^+)r^+ + \text{diag}(\Lambda_{r,j}^-)r^-, \\ \mathfrak{p}_{w,j}(w^{\pm}) &:= \text{diag}(\Lambda_{w,j}^+)w^+ + \text{diag}(\Lambda_{w,j}^-)w^-,\end{aligned} \quad (48)$$

are the j-th elements of the vector-valued functions $\mathfrak{p}_r$ and $\mathfrak{p}_w$, respectively. Note that (48) satisfies $|\mathfrak{p}_{s,j}(s^{\pm})| \leq |s^{\pm}|$. Therefore,

$$\begin{aligned}|\hat{h}_i(r_i, w_i^+, r_i, w_i^-; \varepsilon_i) - H_i(r_i)| &= |h_i(r_i, \mathfrak{p}_w(w_i^{\pm}); \varepsilon_i) - H_i(r_i)| \\ &\leq \alpha_i(\varepsilon_i)|\mathfrak{p}_w(w_i^{\pm})| + \alpha_i^0(\varepsilon_i) \\ &\leq \alpha_i(\varepsilon_i)|w_i^{\pm}| + \alpha_i^0(\varepsilon_i).\end{aligned} \quad (49)$$

On the other hand, by the definition of $\hat{h}_i$ in (47) and the Lipschitz property of h_i in Assumption 6, the decomposition function $\hat{h}_i$ is Lipschitz continuous in $r_i^{\pm} \in (\bar{\mathcal{R}}_i)^2$ uniformly in $w_i^{\pm}$ and ε_i with a Lipschitz constant L_h. Hence, we have

$$\begin{aligned}|\hat{h}_i(r_i+e_i, w_i^+, r_i-e_i, w_i^-; \varepsilon_i) - \hat{h}_i(r_i, w_i^+, r_i, w_i^-; \varepsilon_i)| \\ \leq L_h|e_i|.\end{aligned} \quad (50)$$

Combining (49) and (50), we have (46) proven by triangle inequality. ■

Proof. (Lemma 4). We prove Lemma 4 through induction. In particular, given $w(t) \to [w^-, w^+]$, we find the ultimate bound for each element of $d(t)$ using the disturbance I/O gain function of each subsystem in (12), the subsystem static disturbance attenuation property (6), and Assumptions 5 and 6. For $i = 1$, according to Assumption 5, we necessary have $r_1(t) \equiv r_1^*$, which is independent of the state of all other subsystems. Since Σ_1 is I/S monotone and the prescribed output function l_i has sign-stable Jacobian, the static I/O characteristic h_i is necessarily equipped with a canonical decomposition function $\hat{h}_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i)$ that serves as the I/O gain function for the prescribed output y. Thus, if $w_1(t) \to [w_1^-, w_1^+]$, then we have

$$\begin{aligned}y_1(t) &\to [\hat{h}_1(r_1^*, w_1^-, r_1^*, w_1^+; \varepsilon_1), \hat{h}_1(r_1^*, w_1^+, r_1^*, w_1^-; \varepsilon_1)], \\ d_1(t) &\to [\psi_1(r_1^*, w_1^-, r_1^*, w_1^+; \varepsilon_1), \psi_1(r_1^*, w_1^+, r_1^*, w_1^-; \varepsilon_1)].\end{aligned} \quad (51)$$

Let $y_1^* := H_1(r_1^*)$, by Lemma 7, we can write

$$y_1(t) \to [y_1^* - Q_1(w_1^{\pm}; \varepsilon_1), y_1^* + Q_1(w_1^{\pm}; \varepsilon_1)], \quad (52)$$

where $Q_1(w_1^{\pm}; \varepsilon_1) := \alpha_1(\varepsilon_1)|w_1^{\pm}| + \alpha_1^0(\varepsilon_1)$. On the other hand, by the definition of ψ_i^* in (13), the convergence result for $d_1(t)$ in (51) can be re-written as $d_1(t) \to [\psi_1^*(w_1^-, w_1^+; r_1^*, \varepsilon_1), \psi_1^*(w_1^+, w_1^-; r_1^*, \varepsilon_1)]$. Due to Assumption 5, the reference input $r_2 = G_2(y)$ to Σ_2 is only a function of y_1. Let $r_2^* := G_2(y_1^*)$, let L_G be the Lipschitz constant of $G(\cdot)$, we have

$$r_2(t) \to [r_2^* - L_G Q_1(w_1^{\pm}; \varepsilon_1), r_2^* + L_G Q_1(w_1^{\pm}; \varepsilon_1)]. \quad (53)$$

We use $r_2^- := r_2^* - L_G Q_1(w_1^{\pm}; \varepsilon_1)$ and $r_2^+ := r_2^* + L_G Q_1(w_1^{\pm}; \varepsilon_1)$ to denote the ultimate bounds for $r_2(t)$. Since $r_2^* \in \text{int}(\bar{\mathcal{R}}_2)$, for sufficiently small ε_1, $r_2^{\pm} \in \bar{\mathcal{R}}_2$. Similar to our treatment in (51) for Σ_1, we have

$$\begin{aligned}y_2(t) &\to [\hat{h}_2(r_2^-, w_2^-, r_2^+, w_2^+; \varepsilon_2), \hat{h}_2(r_2^+, w_2^+, r_2^-, w_2^-; \varepsilon_2)], \\ d_2(t) &\to [\psi_2(r_2^-, w_2^-, r_2^+, w_2^+; \varepsilon_2), \psi_2(r_2^+, w_2^+, r_2^-, w_2^-; \varepsilon_2)].\end{aligned} \quad (54)$$

By the subsystem disturbance attenuation property (6), let $y_2^* := H_2(r_2^*)$, we have

$$y_2(t) \to [y_2^* - Q_2(w_{\leq 2}^{\pm}; \varepsilon_{\leq 2}), y_2^* + Q_2(w_{\leq 2}^{\pm}; \varepsilon_{\leq 2})] \quad (55)$$

where

$$Q_2(w_{\leq 2}^{\pm}; \varepsilon_{\leq 2}) := L_h L_G Q_1(w_1^{\pm}; \varepsilon_1) + \alpha_2(\varepsilon_2)|w_2^{\pm}| + \alpha_2^0(\varepsilon_2),$$

according to Lemma 7. Also due to Assumption 6, the convergence of $d_2(t)$ in (54) can be re-written as:

$$d_2(t) \to [\psi_2^*(w_2^-, w_2^+; r_2^*; \varepsilon_2) - P_2, \psi_2^*(w_2^+, w_2^-; r_2^*; \varepsilon_2) + P_2],$$

where

$$\begin{aligned}P_2 = P_2(w_1^{\pm}; \varepsilon_2) :&= L_\psi(\varepsilon_2) L_G Q_1(w_1^{\pm}; \varepsilon_1) \\ &= L_\psi(\varepsilon_2)[\alpha_1(\varepsilon_1)|w_1^{\pm}| + \alpha_1^0(\varepsilon_1)],\end{aligned}$$

and $L_\psi(\varepsilon)$ is the Lipschitz constant of ψ_i for variables r_i^- and r_i^+ as stated in Assumption 6. Since we do not assume the Lipschitz property of ψ_i to hold uniformly in ε_i, L_ψ is in general dependent on ε_i. Note that, for a fixed ε_2, since α_1 and α_1^0 are class $\mathcal{K}$ functions, P_2 can be made arbitrarily small if ε_1 is sufficiently small. Using (52) and (55) to determine $r_3^{\pm}$, we can continue the iteration to find the boxes that bounds $r_3(t)$ and $d_3(t)$. After k iterations, let $w_{\leq k} := [w_1, \cdots, w_k]^\top$ and $\varepsilon_{\leq k} := [\varepsilon_1, \cdots, \varepsilon_k]^\top$, we have

$$y_k(t) \to [y_k^* - Q_k, y_k^* + Q_k],$$

$$d_k(t) \to [\psi_k^*(w_k^-, w_k^+; r_k^*, \varepsilon_k) - P_k, \psi_k^*(w_k^+, w_k^-; r_k^*, \varepsilon_k) + P_k],$$

where $y_k^* = H_k(r_k^*)$

$$Q_k(w_{\leq k}^{\pm}; \varepsilon_{\leq k}) := \sum_{i=1}^{k} (L_h L_G)^{k-i} \cdot (\alpha_i(\varepsilon_i)|w_i^{\pm}| + \alpha_i^0(\varepsilon_i)),$$

$$P_k(w_{\leq k}^{\pm}; \varepsilon_{\leq k}) := L_\psi(\varepsilon_k) \sum_{i=1}^{k-1} L_h^{k-1-i} L_G^{k-i} \cdot (\alpha_i(\varepsilon_i)|w_i^{\pm}| + \alpha_i^0(\varepsilon_i)).$$

Note $Q(w^{\pm}; \varepsilon)$ and $P(w^{\pm}; \varepsilon)$ can be arranged as in (15).

Specifically, let

$$p_{1,k}(\varepsilon_{\leq k}) := L_\psi(\varepsilon_k)\sum_{i=1}^{k-1} L_h^{k-1-i} L_G^{k-i}\alpha_i(\varepsilon_i),$$
$$p_{0,k}(\varepsilon_{\leq k}) := L_\psi(\varepsilon_k)\sum_{i=1}^{k-1} L_h^{k-1-i} L_G^{k-i}\alpha_i^0(\varepsilon_i),$$

we have $p_j(\varepsilon) = [p_{j,1}, \cdots, p_{j,N}]^\top$ for $j = 0, 1$. Since α_i and α_i^0 are class $\mathcal{K}$ functions, for each k, given any $\mu > 0$, $p_{1,k} \leq \mu$, and hence $p_1 \leq \mu$, can be satisfied if

$$\varepsilon_i \leq \alpha_i^{-1}\left(\frac{\mu L_\psi^{-1}(\varepsilon_k)}{(k-1)L_h^{k-1-i}L_G^{k-1}}\right) =: \varepsilon_{i,k}^{**}(\mu, \varepsilon_k)$$

$\forall i \leq k-1$, $\forall k$. We can then take

$$\varepsilon_i^{**}(\mu, \varepsilon_{\geq i+1}) := \min_{k=i+1,\cdots,N} \varepsilon_{i,k}^{**}(\mu, \varepsilon_k).$$

A similar upper bound ε^{**} can be established for $p_0, q_1, q_0 \leq \mu$ to be satisfied. This completes the proof. ∎

B. Proof of Lemma 5

Proof. Consider $V(x)$ in (17) as a candidate Lyapunov function for the perturbed system, then we have

$$\begin{aligned} &\Delta V := V(F(x) + p\delta(x)) - V(x) \\ =&V(F(x) + p\delta(x)) - V(F(x)) + V(F(x)) - V(x) \\ \leq& c_3 p\delta(x)(|F(x)| + |F(x) + p\delta(x)|) - c_4|x|^2,\ \forall |x| \geq r_0 \\ \leq& (-c_4 + pa(p))|x|^2 + pb(p)|x| + pc(p),\ \forall |x| \geq r_0 \end{aligned} \quad (56)$$

where $a(p) = c_3(2L_1L_F + pL_1^2)$, $b(p) = 2c_3L_2(pL_1 + L_F)$, and $c(p) = c_3pL_2^2$. By (56), there exists $p_* > 0$, such that $\Delta V \leq -c_4|x|^2/2 + p(b(p)|x| + c(p))$ for all $p \in [0, p_*]$. For such a fixed p, take $r_p := p \cdot \max\left(2L_2\sqrt{\frac{2c_3}{c_4}}, 8\frac{b(p_*)}{c_4}\right)$, one can verify that $pb(p)|x|, pc(p) \leq c_4|x|^2/8$ for all $|x| \geq r_p$. Hence, $\Delta V \leq -c_4|x|^2/4$ for all $|x| \geq r_0 + r_p$. By Definition 5, for all $p \in [0, p_*]$, the perturbed system (18) is exponentially ultimately bounded in $[-c_1(r_0 + r_p)/c_2, c_1(r_0 + r_p)/c_2]$. ∎

C. Proof of Lemma 6

We first show that the reduced system is ISS after a coordinate translation, which allows us to use a singular perturbation result for ISS systems [59] to compute the model reduction error for the fast variable z_i. This is then used to compute the model reduction error for the slow variable x_i. Since ν is the singular perturbation parameter and ε_i is treated as a constant, we do not explicitly spell out ε_i in the sequel. We also suppress the subscript i for simplicity in this section. For example, we will write x instead of x_i.

Recall $\bar{\varphi}(u)$ is the static I/S characteristic of the reduced system. We let $\tilde{x} := \bar{x} - \bar{\varphi}(0)$ and write the translated reduced system as:

$$\dot{\tilde{x}} = \tilde{f}(\tilde{x}, u(t)) := \bar{f}(\tilde{x} + \bar{\varphi}(0), u(t)). \quad (57)$$

Lemma 8. Under the assumptions of Lemma 6, the translated reduced system (57) is ISS. ▽

Proof. To show that (57) is ISS, we first show that it has the asymptotic gain property (see [60]), that is, there exists a class $\mathcal{K}_0$ function $\gamma(\cdot)$ such that $\limsup_{t\to\infty} |\tilde{x}| \leq \gamma(\|u\|)$. According to Theorem 1 in [60], this asymptotic gain property, combined with the fact that (57) is GAS when $u \equiv 0$, is equivalent to (57) being being ISS. Given Assumption 8, let $\hat{\varphi}(\cdot, \cdot)$ be the canonical decomposition function of $\bar{\varphi}(\cdot)$ and suppose that $\mathcal{U} := [\underline{u}, \overline{u}]$. Let $u^-(\|u\|) := \max(-\mathbf{1}_n\|u\|, \underline{u})$, $u^+(\|u\|) := \min(\mathbf{1}_n\|u\|, \overline{u})$, where $\mathbf{1}_n$ is an n-vector with all elements being 1. Therefore, the input $u(t)$ to (57) satisfies $u(t) \to [u^-(\|u\|), u^+(\|u\|)]$, and by Lemma 2, we have $\tilde{x} \to [\tilde{\varphi}^-(\|u\|), \tilde{\varphi}^+(\|u\|)]$, where $\tilde{\varphi}^-(\|u\|), \tilde{\varphi}^+(\|u\|) : \mathbb{R} \to \mathbb{R}^n$ are defined as:

$$\tilde{\varphi}^+(\|u\|) := \hat{\varphi}(u^+(\|u\|), u^-(\|u\|)) - \bar{\varphi}(0),$$
$$\tilde{\varphi}^-(\|u\|) := \hat{\varphi}(u^-(\|u\|), u^+(\|u\|)) - \bar{\varphi}(0).$$

Let $\gamma(\|u\|) = \max_{v\leq\|u\|}\max\{|\tilde{\varphi}^+(v)|, |\tilde{\varphi}^-(v)|\}$. Since $\gamma(0) = 0$ and it is non-decreasing, it is an asymptotic gain of (57). The GAS property of (57) when $u = 0$ is a consequence of the existence of the I/S characteristic for all $u \in \mathcal{U}$. ∎

Since the convergent-input-convergent-state property we aim to prove is translation-invariant, we will assume in the sequel that $\bar{\varphi}(0) = 0$ and hence the reduced system $\bar{\Sigma}$ is ISS.

Lemma 9. Under the assumptions of Lemma 6, given any $\mu > 0$, there exists $\nu^* = \nu^*(\mu)$, such that

$$\limsup_{t\to\infty} |z(t) - \Gamma(x, u(t))| \leq \mu \quad (58)$$

for all $0 < \nu \leq \nu^*$. In addition, the trajectory of (22) is bounded (by an μ-independent constant) for all $t \geq 0$. ▽

Lemma 9 is adopted from [59], according to which the boundedness condition for $\|\dot{u}\|$ can be removed if g is independent of u. To show the convergent-input-convergent-output property in Lemma 6, let $y_b(t) := z(t) - \Gamma(x(t), u(t))$. The dynamics of x in (22) can be written as:

$$\dot{x} = F(x, y_b(t), u(t)) := f(x, \Gamma(x, u) + y_b, u). \quad (59)$$

We treat (59) as a perturbation of the reduced system, whose dynamics follow

$$\dot{x} = F(x, 0, u(t)) = f(x, \Gamma(x, u), u). \quad (60)$$

Let $x(t, y_b(t), u(t))$ be the trajectory of (59), we aim to show that it is close to $x(t, 0, u(t))$, the trajectory of (60), as $t \to \infty$ for small ν. Given that $u(t) \to [u^-, u^+]$, because both systems are I/S monotone with respect to the input $u(t)$, there exists u_*^- and u_*^+, which are two corners of the box set $[u^-, u^+]$, such that for all t

$$x(t, y_b(t), u_*^-) \leq x(t, y_b(t), u(t)) \leq x(t, y_b(t), u_*^+), \quad (61a)$$
$$x(t, 0, u_*^-) \leq x(t, 0, u(t)) \leq x(t, 0, u_*^+). \quad (61b)$$

Specifically, $u_*^- = u_*^-(u^-, u^+)$ and $u_*^+ = u_*^+(u^-, u^+)$ can be found according to (9)-(10). The trajectories of the nominal system satisfies $\lim_{t\to\infty} x(t, 0, u_*^-) = \hat{\varphi}(u^-, u^+)$, and $\lim_{t\to\infty} x(t, 0, u_*^+) = \hat{\varphi}(u^+, u^-)$. We now show that $\lim_{\nu\to 0}\limsup_{t\to 0} |x(t, y_b(t), u_*^-) - x(t, 0, u_*^-)| = 0$. To this end, we introduce the following lemma.

Lemma 10. Consider the nominal system $\dot{x} = F(x, 0)$ with a GAS equilibrium x^* and the perturbed system $\dot{x}_p = F(x_p, v(t))$. Suppose that F is continuous and locally

Lipschitz, and the trajectory of the perturbed system is bounded. For any $e > 0$, there exists $\delta > 0$, such that if $\limsup_{t\to\infty} |v(t)| < \delta$, then $\limsup_{t\to\infty} |x_p(t) - x^*| \le e$. $\triangledown$

This lemma can be derived from Proposition II.4 in [61]. Since the perturbed system is bounded as a consequence of Lemma 9, we can apply Lemma 10. Because of (58), we have that for any $\mu > 0$, there exists sufficiently small ν such that $\limsup_{t\to\infty} |x(t, y_b(t), u_*^-) - x(t, 0, u_*^-)| = \limsup_{t\to\infty} |x(t, y_b(t), u_*^-) - \hat{\varphi}(u^-, u^+)| \le \mu$. The same claim can be made for $x(t, y_b(t), u_*^+)$. This shows that for any given $\mu > 0$, $x(t) \xrightarrow{\mu} [\hat{\varphi}(u^-, u^+), \hat{\varphi}(u^+, u^-)]$ for sufficiently small ν. Consequently, the disturbance output satisfies $d(t) \xrightarrow{\mu} [\psi(u^-, u^+), \psi(u^+, u^-)]$ for sufficiently small ν because the output function ρ is assumed to be Lipschitz and sign-stable. ∎

D. Small-gain theorem for (approximate) convergent-input-convergent-output system

We state and prove the small-gain theorem for (approximate) convergent-input-convergent-output (CICO) systems. For generality, we consider system (11) with input $u(t)$ and output $q(t)$. This system is interconnected with a cooperative function $u = \Delta(q)$, where $\Delta(\cdot)$ is globally Lipschitz with Lipschitz constant L_Δ.

Lemma 11. Suppose that system (11) has the following approximate CICO property: for any u^-, u^+, if $u(t) \to [u^-, u^+]$, then $q(t) \xrightarrow{\mu} [\psi(u^-, u^+), \psi(u^+, u^-)]$, where $\mu > 0$ is a parameter. Assume that there exists u_0^+ and u_0^- such that $u(t) \in [u_0^-, u_0^+]$ for all t in the interconnected system. If the discrete time dynamical system

$$\begin{aligned} u^-(k+1) &= \Delta \circ \psi(u^-(k), u^+(k)), \\ u^+(k+1) &= \Delta \circ \psi(u^+(k), u^-(k)). \end{aligned} \tag{62}$$

is exponentially ultimately bounded in $[u_*^-, u_*^+]$, then there exists $\mu^*, \kappa > 0$, such that $u(t) \xrightarrow{\kappa\mu} [u_*^-, u_*^+]$ for all $\mu \in (0, \mu^*]$. $\triangledown$

Proof. The proof is similar to that of Theorem 1 in [45]. Since the closed loop $u(t)$ is bounded in $[u^-(0), u^+(0)] := [u_0^-, u_0^+]$, we have

$$q(t) \xrightarrow{\mu} [\psi(u^-(0), u^+(0)), \psi(u^+(0), u^-(0))],$$

By the cooperativity and Lipschitz property of Δ, we have that $u(t) \to [u^-(1), u^+(1)]$, where

$$\begin{aligned} u^-(1) &:= \Delta \circ \psi(u^-(0), u^+(0)) - L_\Delta \mu, \\ u^+(1) &:= \Delta \circ \psi(u^+(0), u^-(0)) + L_\Delta \mu. \end{aligned}$$

After $(k+1)$-iterations, $u(t) \to [u^-(k+1), u^+(k+1)]$, where

$$\begin{aligned} u^-(k+1) &= \Delta \circ \psi(u^-(k), u^+(k)) - L_\Delta \mu, \\ u^+(k+1) &= \Delta \circ \psi(u^+(k), u^-(k)) + L_\Delta \mu. \end{aligned} \tag{63}$$

To study convergence of the this discrete time iteration, We treat it as a perturbation of the nominal system (62). Since (62) is exponentially ultimately bounded in $[u_*^-, u_*^+]$, we apply Lemma 5 to prove ultimate boundedness of (63). This provides a bound for the trajectory of the continuous time interconnected system because $u(t) \to [u^-(k), u^+(k)]$ for every integer $k \ge 0$. ∎

Since the singularly perturbed system (22) has the approximate CICO property as shown in Lemma 6, this small-gain theorem is directly applicable to study its feedback interconnection with a cooperative function $\Delta(\cdot)$. On the other hand, if the conditions for Lemma 11 are satisfied with $\mu = 0$, then we have $u(t) \to [u_*^-, u_*^+]$.

E. Disturbance attenuation of feedback-regulated subsystems

We show that $|h_i(r_i, 0; \varepsilon_i) - r_i/\beta_i|$ and $|h_i(r_i, w_i; \varepsilon_i) - h_i(r_i, 0; \varepsilon_i)|$ are both small in the following claims. Inequality (37) can then be obtained via triangle inequality.

Claim 1. There exists $K_i^* > 0$, independent of r_i, such that

$$|h_i(r_i, 0; \varepsilon_i) - r_i/\beta_i| \le K_i^* \varepsilon_i \tag{64}$$

for all $r_i \in \bar{\mathcal{R}}_i$ and for ε_i sufficiently small. $\triangledown$

The proof for a constant r_i can be found in [42], and K_i^* can be chosen independent of r_i because $\bar{\mathcal{R}}_i$ is compact.

Claim 2. Consider system (33), there exists a positive constant k_i^*, independent of r_i, such that for any fixed pair $(r_i, w_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i$,

$$|h_i(r_i, w_i; \varepsilon_i) - h_i(r_i, 0; \varepsilon_i)| \le k_i^* \varepsilon_i |w_i| + K_i^* \varepsilon_i \tag{65}$$

for ε_i sufficiently small, where K_i^* is as defined in Claim 1.

Proof. To show Claim 2, we prove that $\limsup_{t\to\infty} |\bar{y}_i(t) - h_i(r_i, 0; \varepsilon_i)| \le \varepsilon_i k_i^* |w_i| + K_i^* \varepsilon_i$. This is sufficient because we know $\bar{\Sigma}_i$ has a GAS equilibrium. We first fix a $r_i \in \bar{\mathcal{R}}_i$, and let $y_i^* = h_i(r_i, 0; \varepsilon_i)$ and $\tilde{y}_i := \bar{y}_i - y_i^*$. The dynamics of $\tilde{y}_i$ follow:

$$\dot{\tilde{y}}_i = T_i(\tilde{y}_i, r_i, w_i) - \delta(y_i^* + \tilde{y}_i), \tag{66}$$

where

$$T_i(\tilde{y}_i, r_i, w_i) := \alpha_i \frac{\bar{m}_i(\tilde{y}_i + y_i^*, r_i; \varepsilon_i)/\kappa_i}{1 + \bar{m}_i(\tilde{y}_i + y_i^*, r_i; \varepsilon_i)/\kappa_i + w_i}.$$

and because $T_i(0, r_i, 0) - \delta y_i^* = 0$, we have $\bar{m}_i(y_i^*, r_i; \varepsilon_i) = \kappa_i \delta y_i^* / (\alpha_i - \delta y_i^*)$. Let $k_i(y_i^*) := \frac{\delta \kappa_i y_i^*}{\alpha_i - \delta y_i^*} \cdot \frac{2\delta}{\beta_i}$, we show that the trajectory of (66) is ultimately bounded in the set $\mathcal{P}_i(y_i^*) := \{-k_i \varepsilon_i w_i - K_i^* \varepsilon_i \le \tilde{y}_i \le 0\}$ using the Lyapunov function $V_i(\tilde{y}_i) = \tilde{y}_i^2/2$. For $\tilde{y}_i \ge 0$, since $\partial T_i/\partial w_i, \partial T_i/\partial \tilde{y}_i < 0$, we have $\dot{V}_i = \tilde{y}_i[T_i(\tilde{y}_i, w_i, r_i) - \delta x_i^* - \delta \tilde{y}_i] \le \tilde{y}_i[T_i(0, 0, r_i) - \delta y_i^* - \delta \tilde{y}_i] = -2\delta V_i$. By Claim 1, $y_i^* \le r_i/\beta_i + K_i^* \varepsilon_i$, and therefore, for $\tilde{y}_i \le -k_i \varepsilon_i w_i - K_i^* \varepsilon_i < 0$, we have $\bar{y}_i = \tilde{y}_i + y_i^* \le r_i/\beta_i - k_i \varepsilon_i w_i$. We can use this to find that $\partial \bar{m}_i / \partial \tilde{y}_i \le -\frac{\beta_i}{2\varepsilon_i \delta}$ for all $\tilde{y}_i \le -k_i \varepsilon_i w_i - K_i^* \varepsilon_i$, and therefore $\bar{m}_i(y_i^* + \tilde{y}_i, r_i; \varepsilon_i) \ge \bar{m}_i(y_i^*, r_i; \varepsilon_i)(1 + w_i)$ by mean value theorem. Substituting into (66), we obtain

$$T_i(\tilde{y}_i, r_i, w_i) \ge \alpha_i \frac{\gamma_i^1(y_i^*, r_i; \varepsilon_i)/\kappa_i}{1 + \gamma_i^1(y_i^*, r_i; \varepsilon_i)/\kappa_i} = T_i(0, r_i, 0)$$

if $\tilde{y}_i \le -k_i \varepsilon_i w_i - K_i^* \varepsilon_i$. Thus, $\dot{V}_i = \tilde{y}_i[T_i(\tilde{y}_i, r_i, w_i) - \delta y_i^* - \delta \tilde{y}_i] \le \tilde{y}_i[T_i(0, r_i, 0) - \delta y_i^* - \delta \tilde{y}_i] = -2\delta V_i$. Hence, we have shown that $\tilde{y}_i(t)$ eventually enters $\mathcal{P}_i$ for any fixed $(r_i, w_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i$. Since $\bar{\mathcal{R}}_i$ is compact, due to Claim 1, y_i^* is also bounded in a compact set. Thus, there exists $k_i^* \ge k_i(y_i^*)$ for all y_i^*. ∎

F. Lipschitz properties of subsystem characteristics

Since $\psi_i(r_i^+, w_i^+, r_i^-, w_i^-; \varepsilon_i) = \bar{\rho}_i(\bar{\varphi}_i(r_i^-, w_i^+; \varepsilon_i), r_i^+; \varepsilon_i)$, to show Assumption 6 is satisfied, we prove that $\bar{\rho}_i$ and $\bar{\varphi}_i = h_i$ each satisfies the Lipschitz conditions below.

Claim 3. There are positive functions $c_x(\cdot)$, $c_r(\cdot)$ such that: $|\bar{\rho}_i(p_i^+, r_i, w_i; \varepsilon_i) - \bar{\rho}_i(p_i^-, r_i, w_i; \varepsilon_i)| \le c_p(\varepsilon_i)|p_i^+ - p_i^-|$, $|\bar{\rho}_i(p_i, r_i^+, w_i; \varepsilon_i) - \bar{\rho}_i(p_i, r_i^-, w_i; \varepsilon_i)| \le c_r(\varepsilon_i)|r_i^+ - r_i^-|$, $\forall (p_i, r_i, w_i; \varepsilon_i) \in \mathcal{X}_i \times \bar{\mathcal{R}}_i \times \mathcal{W}_i \times (0, \varepsilon_i^*]$. In addition, $h_i(r_i, w_i; \varepsilon_i)$ is Lipschitz in $r_i \in \bar{\mathcal{R}}_i$ uniformly in $(w_i, \varepsilon_i) \in \mathcal{W}_i \times (0, \varepsilon_i^*]$. $\triangledown$

Proof. We first show the Lipschitz property of $h_i(r_i, w_i; \varepsilon_i)$. Since $\bar{\mathcal{R}}_i$ is an ε_i-independent compact subset of $(0, \alpha_i\beta_i/\delta)$, we let $\bar{\mathcal{R}}_i := [\vartheta_i^1, \alpha_i\beta_i/\delta - \vartheta_2^i]$, where $0 < \vartheta_1^i, \vartheta_2^i < \alpha_i\beta_i/\delta$ are ε_i-independent constants. Setting the dynamics of (29) to steady state, the equilibrium m_i is the solution to

$$\begin{aligned}\mathfrak{F}_i(m_i, r_i, w_i) :=& \frac{\alpha_i\beta_i}{\delta}\frac{m_i/\kappa_i}{1 + m_i/\kappa_i + w_i} - r_i \\ &+ \varepsilon_i\delta m_i - \varepsilon_i\frac{\delta r_i}{\lambda_i m_i} + \frac{\varepsilon_i^2\delta}{\lambda_i} = 0,\end{aligned}$$

and the equilibrium output $y_i = p_i$ can be subsequently determined via

$$y_i = \mathfrak{G}_i(m_i, w_i) = \frac{\alpha_i}{\delta}\frac{m_i/\kappa_i}{1 + m_i/\kappa_i + w_i}.$$

Using chain rule and the implicit function theorem, we have $\frac{\partial h_i}{\partial r_i} = \frac{\partial \mathfrak{G}_i}{\partial m_i} \cdot \frac{\partial m_i}{\partial r_i} = -\frac{\partial \mathfrak{G}_i}{\partial m_i}\frac{\partial \mathfrak{F}_i}{\partial r_i}\left(\frac{\partial \mathfrak{F}_i}{\partial m_i}\right)^{-1}$, from which we find $0 < \frac{\partial h_i}{\partial r_i} \le \frac{1}{\beta_i} + \frac{\alpha_i}{2\delta\vartheta_i^1}$ for all $(r_i, w_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i$. To show the Lipschitz properties of $\bar{\rho}_i$ are satisfied, we use (32) to find the following uniform bounds: $0 < \frac{\partial \bar{m}_i}{\partial r_i} \le \frac{1}{2\delta\varepsilon_i}$ and $-\frac{\beta_i}{2\delta\varepsilon_i} \le \frac{\partial \bar{m}_i}{\partial p_i} < 0$. Since $\bar{\rho}_i = \bar{m}_i/\kappa_i$, we can take $c_r(\varepsilon_i) = \frac{1}{2\delta \min_i(\kappa_i)\varepsilon_i}$ and $c_p(\varepsilon_i) = \frac{\max_i(\beta_i)}{2\min_i(\kappa_i)\delta\varepsilon_i}$. ∎

Claim 3 implies that ψ_i is Lipschitz in $(r_i^+, r_i^-) \in (\bar{\mathcal{R}}_i)^2$ uniformly in $w_i^-, w_i^+ \in \mathcal{W}_i$ with Lipschitz constant $L_\psi(\varepsilon_i) = c_r(\varepsilon_i) + c_x(\varepsilon_i)L_h$. The I/O gain function ψ_i^* is sublinear because, according to (41), $\psi_i^*(w_i^+, w_i^-; r_i^*, \varepsilon_i) = \eta_i(r_i^*, w_i^+; \varepsilon_i)(1 + w_i^+)$ and, as we have shown in the proof of Proposition 1, η_i is positive and bounded for $(r_i^*, w_i^+; \varepsilon_i) \in \bar{\mathcal{R}}_i \times \mathcal{W}_i \times (0, \varepsilon_i^*]$.

References

[1] P. Moylan and D. Hill. Stability criteria for large-scale systems. *IEEE Trans. Autom. Control*, 23(2):143–149, 1978.

[2] J.A. Fax and R.M. Murray. Information flow and cooperative control of vehicle formations. *IEEE Trans. Autom. Control*, 49(9):1465–1476, 2004.

[3] Mu. Arcak and E. D. Sontag. Diagonal stability of a class of cyclic systems and its connection with the secant criterion. *Automatica*, 42(9):1531–1537, 2006.

[4] U. T. Jonsson and C.-Y. Kao. A scalable robust stability criterion for systems with heterogeneous LTI components. *IEEE Trans. Autom. Control*, 55(10):2219–2234, 2010.

[5] S. Dashkovskiy, B. S. Rüffer, and F. R. Wirth. An ISS small gain theorem for general networks. *Math. Control. Signals, Syst.*, 2007.

[6] Z.-P. Jiang and Y. Wang. A generalization of the nonlinear small-gain theorem for large-scale complex systems. In *2008 7th World Congress on Intelligent Control and Automation*, 2008.

[7] A. Rantzer. Scalable control of positive systems. *European Journal of Control*, 24:72–80, 2015.

[8] A. Proud, M. Pachter, and J. D'Azzo. Close formation flight control. In *Guidance, Navigation, and Control Conference and Exhibit*, 1999.

[9] S. N. Singh, P. Chandler, C. Schumacher, S. Banda, and M. Pachter. Nonlinear adaptive close formation control of unmanned aerial vehicles. *Dynamics and Control*, 10(2):179–194, 2000.

[10] C.J. Schumacher and Rajeeva Kumar. Adaptive control of UAVs in close-coupled formation flight. In *Proceedings of the American Control Conference*, 2000.

[11] R. Fierro, C. Belta, J.P. Desai, and V. Kumar. On controlling aircraft formations. In *Proceedings of the IEEE Conference on Decision and Control*, 2001.

[12] E. Bitar and P. Seiler. Coordinated control of a wind turbine array for power maximization. In *Proceedings of the American Control Conference*, 2013.

[13] L. Buccafusca, J. P. Jansch-Porto, G. E. Dullerud, and C. L. Beck. An application of nested control synthesis for wind farms. *IFAC-PapersOnLine*, 52(20):199–204, 2019.

[14] Y. Ma, A. Kelman, A. Daly, and F. Borrelli. Predictive control for energy efficient buildings with thermal storage: Modeling, stimulation, and experiments. *IEEE Control Systems*, 32(1):44–64, 2012.

[15] A. Gyorgy, J. I. Jiménez, J. Yazbek, H.-H. Huang, H. Chung, R. Weiss, and D. Del Vecchio. Isocost lines describe the cellular economy of genetic circuits. *Biophys. J.*, 109(3):639–646, 2015.

[16] Y. Qian, H.-H. Huang, J. I. Jiménez, and D. Del Vecchio. Resource competition shapes the response of genetic circuits. *ACS Synth. Biol.*, 6(7):1263–1272, 2017.

[17] D. D. Siljak. Stability of large-scale systems under structural perturbations. *IEEE Trans. Syst. Mat. Cybern.*, SMC-2(5):657–663, 1972.

[18] P. Moylan. A connective stability result for interconnected passive systems. *IEEE Trans. Autom. Control*, 25(4):812–813, 1980.

[19] P. Ioannou. Decentralized adaptive control of interconnected systems. *IEEE Trans. Autom. Control*, 31(4):291–298, 1986.

[20] Y. Guo, Z.-P. Jiang, and D. J. Hill. Decentralized robust disturbance attenuation for a class of large-scale nonlinear systems. *Systems & Control Letters*, 37(2):71–85, 1999.

[21] M. Andreasson, D. V. Dimarogonas, H. Sandberg, and Johansson K. H. Distributed control of networked dynamical systems: Static feedback, integral action and consensus. *IEEE Trans. Autom. Control*, 59(7):1750–1764, 2014.

[22] Y. Qian and D. Del Vecchio. Mitigation of ribosome competition through distributed sRNA feedback. In *Proceedings of the IEEE Conference on Decision and Control*, 2016.

[23] C. L. Kelly, A. W. K. Harris, H. Steel, E. J. Hancock, J. T. Heap, and A. Papachristodoulou. Synthetic negative feedback circuits using engineered small RNAs. *Nucleic Acids Res.*, 46(18):9875–9889, 2018.

[24] H.-H. Huang, Y. Qian, and D. Del Vecchio. A quasi-integral controller for adaptation of genetic modules to variable ribosome demand. *Nat. Commun.*, 9(1), 2018.

[25] S. K. Aoki, G. Lillacci, A. Gupta, A. Baumschlager, D. Schweingruber, and M. Khammash. A universal biomolecular integral feedback controller for robust perfect adaptation. *Nature*, 570(7762):533–537, 2019.

[26] D. K. Agrawal, X. Tang, A. Westbrook, R. Marshall, C. S. Maxwell, J. Lucks, V. Noireaux, C. L. Beisel, M. J. Dunlop, and E. Franco. Mathematical modeling of RNA-based architectures for closed loop control of gene expression. *ACS Synth. Biol.*, 7(5):1219–1228, 2018.

[27] D. K. Agrawal, R. Marshall, V. Noireaux, and E. D Sontag. In vitro implementation of robust gene regulation in a synthetic biomolecular integral controller. *Nat. Commun.*, 10(1), 2019.

[28] N. Olsman, A.-A. Baetica, F. Xiao, Y. P. Leong, R. M. Murray, and J. C. Doyle. Hard limits and performance tradeoffs in a class of antithetic integral feedback networks. *Cell Systems*, 9(1):49–63.e16, 2019.

[29] D. Del Vecchio, A. J. Dy, and Y. Qian. Control theory meets synthetic biology. *J. R. Soc. Interface*, 13(120):20160380, 2016.

[30] Y. Qian, C. McBride, and D. Del Vecchio. Programming cells to work for us. *Annual Review of Control, Robotics, and Autonomous Systems*, 1(1):411–440, 2018.

[31] V. Hsiao, A. Swaminathan, and R. M. Murray. Control theory for synthetic biology: Recent advances in system characterization, control design, and controller implementation for synthetic biology. *IEEE Control Systems*, 38(3):32–62, 2018.

[32] D. Del Vecchio, Y. Qian, R. M. Murray, and E. D. Sontag. Future systems and control research in synthetic biology. *Annual Reviews in*

Control, 45:5–17, 2018.
[33] C. McBride, R. Shah, and D. Del Vecchio. The effect of loads in molecular communications. *Proceedings of the IEEE*, 107(7):1369–1386, 2019.
[34] P. P. Peralta-Yahya, F. Zhang, S. B. del Cardayre, and J. D. Keasling. Microbial engineering for the production of advanced biofuels. *Nature*, 488:320–328, 2012.
[35] W. C. Ruder, T. Lu, and J. J. Collins. Synthetic biology moving into the clinic. *Science*, 333(6047):1248–1252, 2011.
[36] M. B. Johnson, A. R. March, and L. Morsut. Engineering multicellular systems: Using synthetic biology to control tissue self-organization. *Current Opinion in Biomedical Engineering*, 4:163–173, 2017.
[37] D. Del Vecchio and R. M. Murray. *Biomolecular Feedback Systems*. Princeton University Press, Princeton, 2014.
[38] M. B. Elowitz and S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403(6767):335–338, 2000.
[39] T. S. Gardner, C. R. Cantor, and J. J. Collins. Construction of a genetic toggle switch in escherichia coli. *Nature*, 403(6767):339–342, 2000.
[40] A. A. K. Nielsen, B. S. Der, J. Shin, P. Vaidyanathan, V. Paralanov, E. A. Strychalski, D. Ross, D. Densmore, and C. A. Voigt. Genetic circuit design automation. *Science*, 352(6281):aac7341–aac7341, 2016.
[41] T. W. Grunberg and D. Del Vecchio. Modular analysis and design of biological circuits. *Curr. Opin. Biotech.*, 63:41–47, 2020.
[42] Y. Qian and D. Del Vecchio. Realizing 'integral control' in living cells: how to overcome leaky integration due to dilution? *J. R. Soc. Interface*, 15(139):20170902, 2018.
[43] Hal L. Smith. *Monotone Dynamical Systems: An Introduction to the Theory of Competitive and Cooperative Systems*. American Mathematical Society, 1995.
[44] D. Angeli and E.D. Sontag. Monotone control systems. *IEEE Trans. Autom. Control*, 48(10):1684–1698, 2003.
[45] D. Angeli, G. A. Enciso, and E. D. Sontag. A small-gain result for orthant-monotone systems under mixed feedback. *Systems & Control Letters*, 68:9–19, 2014.
[46] Samuel Coogan and Murat Arcak. Efficient finite abstraction of mixed monotone systems. In *Proceedings of the 18th International Conference on Hybrid Systems Computation and Control*, 2015.
[47] H.L. Smith. Global stability for mixed monotone systems. *Journal of Difference Equations and Applications*, 14(10-11):1159–1164, 2008.
[48] H. Kunze and D. Siegel. A graph theoretical approach to monotonicity with respect to initial conditions II. *Nonlinear Analysis: Theory, Methods & Applications*, 35(1):1–20, 1999.
[49] D. Angeli and E. Sontag. Interconnections of monotone systems with steady-state characteristics. In *Optimal Control, Stabilization and Nonsmooth Analysis*, pages 135–154. Springer, 2004.
[50] H. K. Khalil. *Nonlinear systems*. Prentice Hall, Upper Saddle River, New Jersey, 3rd edition, 2002.
[51] L. Wang and E. D. Sontag. Singularly perturbed monotone systems and an application to double phosphorylation cycles. *J. Nonlinear Sci.*, 2008.
[52] A. Sootla and A. Mauroy. Operator-theoretic characterization of eventually monotone systems. *IEEE Control Systems Letters*, 2018.
[53] T. W. Grunberg and D. Del Vecchio. Time-scale separation based design of biomolecular feedback controllers. In *Proceedings of the IEEE Conference on Decision and Control*, 2019.
[54] E. Levine, Z. Zhang, T. Kuhlman, and T. Hwa. Quantitative characteristics of gene regulation by small RNA. *PLoS Biol.*, 5(9):e229, 2007.
[55] D. Del Vecchio, H. Abdallah, Y. Qian, and J. J. Collins. A blueprint for a synthetic genetic feedback controller to reprogram cell fate. *Cell Systems*, 4(1):109–120.e11, 2017.
[56] D. Na, S. M. Yoo, H. Chung, H. Park, J. H. Park, and S. Y. Lee. Metabolic engineering of escherichia coli using synthetic small regulatory RNAs. *Nat. Biotechnol.*, 31(2):170–174, 2013.
[57] F. Blanchini and E. Franco. Structurally robust biological networks. *BMC Systems Biology*, 5(1):74, 2011.
[58] Y. Qian and D. Del Vecchio. The "power network" of genetic circuits. In *Lecture Notes in Control and Information Sciences - Proceedings*, pages 109–121. Springer International Publishing, 2018.
[59] P.D. Christofides and A.R. Teel. Singular perturbations and input-to-state stability. In *Proceedings of 3rd European Control Conference*, pages 1845–1850, 1995.
[60] E.D. Sontag and Yuan Wang. New characterizations of input-to-state stability. *IEEE Trans. Autom. Control*, 41(9):1283–1294, 1996.
[61] E.D. Sontag. A remark on the converging-input converging-state property. *IEEE Trans. Autom. Control*, 48(2):313–314, 2003.