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Mitigation of ribosome competition through distributed sRNA feedback

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***Abstract*—A current challenge in the robust engineering of synthetic gene networks is context dependence, the unintended interactions among genes and host factors. Ribosome competition is a specific form of context dependence, where all genes in the network compete for a limited pool of translational resources available for gene expression. Recently, theoretical and experimental studies have shown that ribosome competition creates a hidden layer of interactions among genes, which largely hinders our ability to predict design outcomes. In this work, we establish a control theoretic framework, where these hidden interactions become disturbance signals. We then propose a distributed feedback mechanism to achieve disturbance decoupling in the network. The feedback loop at each node consists of the protein product transcriptionally activating a small RNA (sRNA), which forms a translationally inactive complex with mRNA rapidly. We illustrate that with this feedback mechanism, protein production at each node is only dependent on its own transcription factor inputs, and almost independent of hidden interactions arising from ribosome competition.**

I. INTRODUCTION

Context dependence is a recurrent challenge in the bottom-up design of large scale synthetic gene networks [1]. In particular, although input/output (i/o) responses of simple genetic parts can be well-characterized in isolation, their behaviors may change significantly when connected in a network [2],[3]. Such behaviors, which are often referred to as lack of modularity [4], largely hinder our capability to carry out predictive design at the system level. In order to preserve modularity of circuit modules, recently there has been an increasing interest in finding methods to mitigate various forms of context dependence [2],[5],[6].

In this paper, we focus on competition of translational resources (ribosomes) as a special form of context dependence in gene (transcription) networks. In a gene network, each node consists of a gene that is expressed to produce proteins, which serve as transcription factors (TFs) that regulate gene expression at other nodes. Gene expression relies on the availability of ribosomes, which are molecular machines that are found in limited amount in cells at constant growth rate [7]. Limited access to free ribosomes has been identified as a major bottleneck in genetic circuits [3]. As all genes in the network compete for a common pool of ribosomes, a hidden layer of interactions among nodes arises, which can significantly change network behavior [8].

In order to engineer the cells to mitigate the effects of ribosome competition, An and Chin [9] propose the use of orthogonal ribosomes (O-ribosomes) to decouple ribosome usage of endogenous mRNAs and synthetic mRNAs. However, the problem of mitigating the coupling among synthetic mRNAs remains. Negative feedback has been widely used to enhance reliability and robustness of gene networks (see [10] for a comprehensive review). In [11], the authors compare performance of three negative feedback mechanisms that increase robustness of steady state expression of a constitutive gene with respect to resource competition.

In this paper, we propose a distributed sRNA feedback mechanism to mitigate the effects of ribosome competition on protein production in a gene network. By modeling competition-induced hidden interactions as disturbances among nodes, we formulate a static network disturbance decoupling problem, whose aim is to attenuate the static effects of disturbances on the output of each node i (y_i), so that y_i only depends on its own reference input. Attenuating external disturbances through distributed control has been widely studied in control literature (see [12], for example). However, in our case, disturbance input to each node is produced by the rest of the network. Thus, to achieve network disturbance decoupling, we require each node to possess a disturbance attenuation property, and that the network doesn't amplify the disturbances as we increase disturbance attenuation at individual nodes. The requirement on the network can be verified if an interconnection matrix, constructed by the static node i/o gains and the interconnection rule, is diagonally dominant. Such a requirement is related to the network small-gain criteria in [13]. We show that in a gene network with distributed sRNA feedback, when reference inputs to all nodes fall into an admissible input set, the key node and network properties are satisfied. Explicit expression of the admissible input set is given in terms of physical parameters to educate our ongoing experimental implementation.

The rest of the paper is organized as follows. In Section II, we model hidden interactions arising from ribosome competition as disturbances. In Section III, we formulate the static network disturbance decoupling problem, and provide sufficient conditions that guarantee network disturbance decoupling. In Section IV, we propose an sRNA mediated distributed feedback design, through which network disturbance decoupling can be achieved. We test our design with an activation cascade example in Section V. Discussion and conclusions are in Section VI.

Notations: Let $\mathbf{y} = [y_1, \cdots, y_n]^T$ be a vector in $\mathbb{R}^n$, we define $\mathbf{y}_{-i}$ as the vector $[y_1, \cdots, y_{i-1}, y_{i+1}, \cdots, y_n]^T$. When there is no risk of ambiguity, $\bar{\mathbf{x}}$ stands for the steady state of signal $\mathbf{x}$ under some dynamics of interests. $\mathbf{y}_{(i)}$ represents the i-th element of vector $\mathbf{y}$, and $\mathbf{A}_{(j,k)}$ is the (j,k)-th element of matrix $\mathbf{A}$. The positive orthant is denoted by $\mathbb{R}^n_+$.

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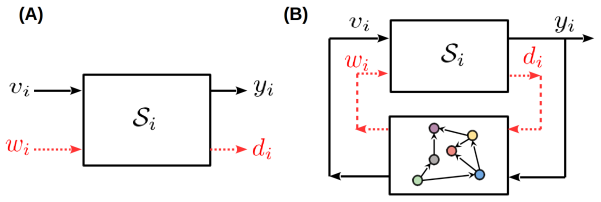

Fig. 1. (A) Node i in isolation. The black solid lines represent the reference i/o signals, and the red dashed lines represent disturbance i/o signals. (B) In a network, disturbance input to node i is produced by the rest of the network, whose dynamics are also affected by disturbance output of node i.

II. DISTURBANCES ARISING FROM RIBOSOME COMPETITION

A. Gene Expression with Limited Ribosomes

A transcriptional component (node) is a fundamental building block in gene networks. Here, we consider a node i taking a TF $\mathrm{u_i}$ as input that binds with the promoter region of gene i ($\mathrm{p_i}$) with cooperativity n_i to produce a protein $\mathrm{x_i}$ as output. Depending on the type of the TF (activator or repressor), $\mathrm{u_i}$ can either promote or inhibit gene transcription to produce mRNAs ($\mathrm{m_i}$). mRNAs are then translated by ribosomes (z) to produce protein ($\mathrm{x_i}$). At a constant growth rate, the total amount of ribosomes are conserved [7]. Assuming that binding reactions are much faster than transcription and translation [14], and thus can be set to quasi-steady state, each node can be described by its mRNA and protein concentrations: $[m_i, x_i]^T \in \mathbb{R}^2_+$.

If node i is the only node in the network, all ribosomes are available for its translation, and the ribosome conservation law is $z_t = z + z_i$, where z_t (z) is the total (free) amount of ribosomes, and z_i is the amount of ribosomes bound with $\mathrm{m_i}$. Using standard reaction rate equations for transcriptional regulation [15], simplified dynamics of node i can be written as:

$$\dot{m}_i = T_i v_i - \delta m_i, \quad \dot{x}_i = R_i \frac{m_i/\kappa_i}{1 + m_i/\kappa_i} - \gamma x_i, \tag{1}$$

where T_i is the basal transcription rate of node i when $u_i \equiv 0$, δ (γ) is the dilution/degradation rate of mRNA (protein), R_i is a lumped translation rate constant that is proportional to z_t, and κ_i is the dissociation constant between ribosomes and mRNA ribosome binding site (RBS). Smaller κ_i indicates stronger binding. We call $v_i = v_i(u_i)$ as the *reference input* to node i. The reference input describes regulation effect of TF u on the transcription rate of node i, and is defined as

$$v_i = v_i(u_i) := \frac{1 + \frac{T_i'}{T_i}(\frac{u_i}{k_i})^{n_i}}{1 + (\frac{u_i}{k_i})^{n_i}}, \tag{2}$$

where k_i is the dissociation constant between $\mathrm{u_i}$ and $\mathrm{p_i}$, and T_i' is the transcription rate of node i when $u_i \to \infty$. Therefore, $T_i < T_i'$ if $\mathrm{u_i}$ is a repressor, otherwise $\mathrm{u_i}$ is an activator. Detailed derivation of (1) can be found in [8]. Note that according to (1), the output of each node, x_i, is only dependent on v_i, and consequently only on u_i.

B. Ribosome Usage as Disturbances in a Network

We consider a network consisting of n nodes. Each node takes a constant reference input v_i. When the network has multiple nodes, due to the ribosome conservation law, $z_t = z + \sum_{i=1}^n z_i$, the node dynamics become ([8]):

$$\dot{m}_i = T_i v_i - \delta m_i,$$
$$\dot{x}_i = R_i \frac{m_i/\kappa_i}{1 + m_i/\kappa_i + \sum_{j\neq i} m_j/\kappa_j} - \gamma x_i. \tag{3}$$

Note that in (3), dynamics of node i are not only dependent on its own reference input v_i, but also on the concentration of mRNA transcripts of other nodes in the network (m_j), which is undesirable for predictable design. In this sense, we regard

$$w_i := \sum_{j\neq i} m_j/\kappa_j \tag{4}$$

as a disturbance input to node i. Without w_i, dynamics of (3) are identical to those in (1), which are the dynamics of node i in the absence of other ribosome-competing nodes. According to (4), ribosome demand of node i, which we define as $d_i := m_i/\kappa_i$, is a disturbance output of node i and acts as disturbance input to all other nodes.

As a consequence, we can regard each node as a system with two inputs and two outputs as shown in Fig. 1. The black solid arrows are reference input (v_i) and reference output (y_i), while the red dashed arrows represent disturbance input (w_i) and output (d_i). In (3), the reference output is defined as $y_i = x_i$. Previous theoretical study [8], and experimental results [3] have demonstrated significant effects of competition-induced hidden interactions on steady state gene expression. It is thus desirable to design a feedback mechanism to mitigate the effect of disturbances, so that expression of each node only responds to its own TF input. In the following section, we formulate a general control theoretic framework to address this problem.

III. NETWORK DISTURBANCE DECOUPLING

Our objective is to have the steady state reference output of each node (y_i) be only dependent on its own reference input (v_i), while independent of the reference inputs to other nodes (v_j,$j \neq i$), which enter dynamics of node i through disturbances. Therefore, we expect the i/o response of each node to be as if they were the only nodes in the network. We refer to this problem as *static network disturbance decoupling problem*.

Here, we propose sufficient conditions that guarantee static network disturbance decoupling. These conditions fall into two categories: properties of the node and of the network. In particular, when a node is viewed in isolation (Fig.1(A)), by decreasing a suitable small parameter ϵ, y_i should become arbitrarily insensitive to w_i (node disturbance attenuation). When node i is part of a network (Fig.1(B)), w_i is determined by the network, and may depend on ϵ. We therefore require that w_i does not increase dramatically as we decrease ϵ (network ϵ-well-posedness). Algebraic conditions are given for both conditions in what follows.

A. Disturbance Attenuation of a Node

Consider a node $\mathcal{S}_i^\epsilon$ that takes two inputs: a constant external reference input v_i taking values on a set $\mathcal{V}_i$, and a constant external disturbance input w_i taking values on $\mathcal{W}_i$. We call $\mathcal{V}_i$ the *admissible reference input set*, and $\mathcal{W}_i$ the *admissible disturbance input set*. The system produces two

outputs: a reference output y_i and a disturbance output d_i (refer to Fig.1(A)). System $\mathcal{S}_i^\epsilon$ is parameterized by a small parameter ϵ.

A1 We assume that each node $\mathcal{S}_i^\epsilon$ has a well-defined static i/o map:

$$y_i = h_i(v_i, w_i, \epsilon), \qquad d_i = g_i(v_i, w_i, \epsilon), \tag{5}$$

where functions $h_i(\cdot)$ and $g_i(\cdot)$ are $\mathcal{C}^2$ in ϵ for $(v_i, w_i, \epsilon) \in \mathcal{V}_i \times \mathcal{W}_i \times (-\epsilon^*, \epsilon^*)$ with $\mathcal{V}_i \times \mathcal{W}_i \subseteq \mathbb{R}_+^2$, and $0 < \epsilon^* \ll 1$

A2 We assume each subsystem is i/o positive: for all $(v_i, w_i, \epsilon) \in \mathcal{V}_i \times \mathcal{W}_i \times (-\epsilon^*, \epsilon^*)$, we have $d_i > 0$ and $y_i > 0$.

Due to **A1**, for ϵ^* sufficiently small, the i/o characteristics (5) can be written as Taylor series in ϵ:

$$y_i = h_i(v_i, w_i, \epsilon) = h_i(v_i, w_i, 0) + \epsilon \tilde{h}_i(v_i, w_i, 0) + \mathcal{O}(\epsilon^2),$$
$$d_i = g_i(v_i, w_i, \epsilon) = g_i(v_i, w_i, 0) + \epsilon \tilde{g}_i(v_i, w_i, 0) + \mathcal{O}(\epsilon^2),$$

where

$$\tilde{h}_i(v_i, w_i, 0) := \left.\frac{\partial h_i}{\partial \epsilon}\right|_{(v_i, w_i, 0)}, \quad \tilde{g}_i(v_i, w_i, 0) := \left.\frac{\partial g_i}{\partial \epsilon}\right|_{(v_i, w_i, 0)}.$$

***Definition** 1:* (Node disturbance attenuation). Node i is said to have the *ϵ-static disturbance attenuation property* in $\mathcal{V}_i$ if $h_i(v_i, w_i, 0) \equiv h_i(v_i, 0, 0)$ for all $v_i \in \mathcal{V}_i$ and $w_i \in \mathcal{W}_i$.

For a node with ϵ-static disturbance attenuation property, any contribution from the disturbance input to the reference output is attenuated by a factor of ϵ. However, in a network setting, disturbance input w_i is generated by other nodes in the network, and in principle, it may even grow unbounded as $\epsilon \to 0$. Therefore, the next requirement is that the disturbance signals are smooth in ϵ as it approaches 0, which we refer to as the network ϵ-well-posedness property.

B. Network Disturbance Decoupling

Consider a network $\mathcal{N}^\epsilon$ composed of n nodes with static i/o maps in (5). We denote by $\mathcal{I}$ the index set $\{1, \cdots, n\}$. Let $\mathbf{v} = [v_1, \cdots, v_n]^T$, $\mathbf{y} = [y_1, \cdots, y_n]^T$, $\mathbf{w} = [w_1, \cdots, w_n]^T$, and $\mathbf{d} = [d_1, \cdots, d_n]^T$ be concatenations of reference input, reference output, disturbance input and disturbance output signals at all nodes. The following set notations are used: $\mathbf{V} = \mathcal{V}_1 \times \cdots \times \mathcal{V}_n$, and $\mathbf{W} = \mathcal{W}_1 \times \cdots \times \mathcal{W}_n$. We assume disturbance coupling takes the following form.

A3 For all $i \in \mathcal{I}$, $w_i = \sum_{j \neq i} d_j$.

***Definition** 2:* (Network ϵ-well-posedness): Let $\mathcal{V}_\mathcal{N} \subseteq \mathbf{V}$, under **A3**, network $\mathcal{N}^\epsilon$ is *locally ϵ-well-posed* in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$ if there exists an open set $\mathcal{W} \subseteq \mathbf{W}$, and $\epsilon^* > 0$ such that there is an interconnection signal $\mathbf{w}(\mathbf{v}, \epsilon) \in \mathcal{W}$ that satisfies

$$w_i = \sum_{j \neq i} g_j(v_j, w_j, \epsilon), \ \forall i \in \mathcal{I}. \tag{6}$$

Furthermore, $\mathbf{w}(\mathbf{v}, \epsilon)$ is continuously differentiable in ϵ for all $(\mathbf{v}, \mathbf{w}, \epsilon) \in \mathcal{V}_\mathcal{N} \times \mathcal{W} \times (-\epsilon^*, \epsilon^*)$.

A locally ϵ-well-posed network has static interconnection signal $\mathbf{w}(\mathbf{v}, \epsilon) \in \mathcal{W}$. Static i/o characteristics of each node in the network can be found as

$$y_i = H_i(v_i, \mathbf{v}_{-i}, \epsilon) := h_i(v_i, \mathbf{w}(\mathbf{v}, \epsilon), \epsilon). \tag{7}$$

Similar to the single node case, we define an ϵ-disturbance decoupling property for the network.

***Definition** 3:* (Network disturbance decoupling). Network $\mathcal{N}^\epsilon$ is said to have *local ϵ-network disturbance decoupling property* in $\mathcal{V}_\mathcal{N} \times \tilde{\mathcal{W}}$ if there exists a ϵ^* and an open set $\tilde{\mathcal{W}} \subseteq \mathbf{W}$ such that for all $i \in \mathcal{I}$, $H_i(v_i, \mathbf{v}_{-i}, 0) \equiv H_i(v_i, \mathbf{0}, 0)$ for all $(\mathbf{v}, \mathbf{w}, \epsilon) \in \mathcal{V}_\mathcal{N} \times \tilde{\mathcal{W}} \times (-\epsilon^*, \epsilon^*)$.

For a network with such property, static reference output of each node is practically independent of the reference input to other nodes ($\mathbf{v}_{-i}$).

***Claim** 1:* Network $\mathcal{N}^\epsilon$ has local ϵ-network disturbance decoupling property in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$ if (i) each node i has ϵ-static disturbance attenuation property in $\mathcal{V}_i \times \mathcal{W}_i$, and (ii) the network is locally ϵ-well-posed in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$, with $\mathcal{W} \subseteq \mathbf{W}$.

Proof of Claim 1 can be found in [16]. Now we provide sufficient conditions to certify that the network is locally ϵ-well-posed in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$. We assume disturbance output can be written as an affine function of disturbance input when $\epsilon = 0$.

A4 For all $(\mathbf{v}, \mathbf{w}) \in \mathcal{V}_\mathcal{N} \times \mathcal{W}$, we have $g_i(v_i, w_i, 0) = g_i(v_i) + \hat{g}_i(v_i) w_i$.

According to **A2**, $d_i = g_i(v_i, w_i, 0) > 0$ for all positive v_i, w_i, the above assumption thus also implies $g_i(v_i) > 0$ and $\hat{g}_i(v_i) > 0$ for all $\mathbf{v} \in \mathcal{V}_\mathcal{N}$.

A5 Admissible disturbance input set $\mathcal{W}_i = \mathbb{R}_+$ for all $i \in \mathcal{I}$.

We introduce the interconnection matrix $\mathbf{A}(\mathbf{v})$ and a positive vector $\mathbf{\Phi}(\mathbf{v})$. The (j,k)-th element of $\mathbf{A}(\mathbf{v})$ is defined as

$$\mathbf{A}_{(j,k)}(\mathbf{v}) := \begin{cases} 1, & \text{if } j = k, \\ -\hat{g}_k(v_k), & \text{if } j \neq k. \end{cases} \tag{8}$$

and the i-th element of vector $\mathbf{\Phi}(\mathbf{v})$ is:

$$\mathbf{\Phi}_{(i)}(\mathbf{v}) = \sum_{j \neq i} g_j(v_j).$$

The following claim provides sufficient conditions for a network to be locally ϵ-well-posed.

***Claim** 2:* Based on Assumptions **A1**-**A5**, if we pick $\mathcal{V}_\mathcal{N}$ such that matrix $\mathbf{A}(\mathbf{v})$ is diagonally dominant for all $\mathbf{v} \in \mathcal{V}_\mathcal{N}$, then there exists an open set $\mathcal{W}$ such that network $\mathcal{N}^\epsilon$ is locally ϵ-well-posed in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$.

The proof consists of showing that when $\epsilon = 0$, the interconnection signal $\mathbf{w}$ can be found by solving $\mathbf{A}(\mathbf{v})\mathbf{w} = \mathbf{\Phi}(\mathbf{v})$. It can be shown that $\mathbf{A}$ belongs to a class of inverse-positive matrices [17], we have $\mathbf{w} \in \mathbf{W}$. We can further use implicit function theorem to show $\mathbf{w} = \mathbf{w}(\mathbf{v}, \epsilon)$ is $\mathcal{C}^1$ in ϵ. Details can be found in [16]. Our definition of local disturbance decoupling does not rule out the existence of an interconnection signal $\mathbf{w}(\mathbf{v}, \epsilon) \notin \mathcal{W}$ that does not satisfy $H_i(v_i, \mathbf{v}_{-i}, 0) = H_i(v_i, \mathbf{0}, 0)$. For global disturbance decoupling, we need to show all $\mathbf{w}(\mathbf{v}, \epsilon) \in \mathbf{W}$ that satisfy (6) are $\mathcal{C}^1$ in ϵ (global ϵ-well-posedness), which is discussed in detail in the extended version of this paper [16].

Our results apply to networks where reference inputs and outputs are connected (*i.e.* $v_i = v_i(\mathbf{y})$). As long as $\mathbf{v} \in \mathcal{V}_\mathcal{N}$ and bounded, the network has ϵ-disturbance decoupling property, and its steady-state can be found by solving simultaneously the i/o maps of all nodes, which are derived for each of them in isolation (refer to [16]).

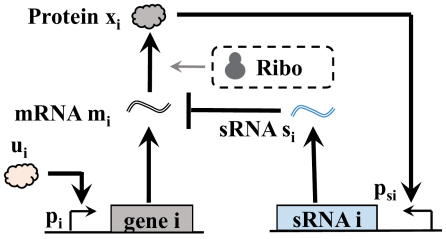

Fig. 2. A schematic of the sRNA feedback acting on node i.

IV. DISTURBANCE DECOUPLING REALIZED THROUGH DISTRIBUTED sRNA FEEDBACK

Small RNAs have been recognized as critical regulators in gene expression [18]. In this section, we propose a distributed sRNA feedback design that achieves ϵ-network disturbance decoupling described in Section III.

A. sRNA Feedback Setup

A diagram of the sRNA feedback mechanism for node i is shown in Fig.2. To attenuate disturbances arising from ribosome competition, sRNA-enabled mRNA inhibition creates an effective negative feedback loop around the translation process: the output protein ($\mathrm{x_i}$) transcriptionally activates the production of sRNA ($\mathrm{s_i}$), which forms a translationally inactive complex with mRNA. The complex then degrades rapidly. Recent experimental results suggest that sRNA is a potent repressor for target gene expression, inhibiting target gene expression by up to 150 folds [19].

When ribosome availability decreases, for instance, $\mathrm{x_i}$ production decreases, down-regulating sRNA production, which in turn up-regulates $\mathrm{m_i}$, and consequently $\mathrm{x_i}$, compensating for the loss in $\mathrm{x_i}$ production due to ribosome limitation. To compensate for the decrease in gene (sRNA) expression due to feedback, we need to maintain sufficiently high transcription rates for both species. Due to the comparative short length of sRNA nucleotide chains, average sRNAs' transcription rate is about 10 times larger than mRNAs' [20]. Meanwhile, we can increase gene transcription by increasing its plasmid copy number p_i. Assuming binding reactions have reached quasi-steady state, node i can be described by $\mathbf{x}_i = [m_i, s_i, x_i]^T$. Based on reaction rate equations and ribosome conservation law, we derive an ODE model for a node with sRNA feedback:

$$\begin{aligned} \dot{m}_i &= GT_i v_i - Gm_i s_i - \delta m_i, \\ \dot{s}_i &= GT_{si}\frac{x_i/k_{si}}{1 + x_i/k_{si}} - Gm_i s_i - \delta s_i, \\ \dot{x}_i &= R_i \frac{m_i/\kappa_i}{1 + m_i/\kappa_i + w_i} - \gamma x_i. \end{aligned} \tag{9}$$

We refer the readers to [16] for detailed derivation of this model. In equations (9), $G := \beta/k_*$ is defined as the effective repression of translation, where β is the degradation rate of the mRNA-sRNA complex, and k_* is the dissociation constant between sRNA and mRNA. Magnitude of G can be tuned by rational design of the sRNA target-binding sequence [18]. Parameter k_{si} is the dissociation constant between activator $\mathrm{x_i}$ and sRNA promoter $\mathrm{p_{si}}$. Parameters κ_i, δ and γ are defined identically as in (1). Other lumped parameters are defined as follows:

$$T_i := \frac{p_i^t \pi_{i0}}{G}, \qquad T_{si} := \frac{p_{si}^t \pi_{si}}{G}, \qquad R_i := \theta_i z_t,$$

where p_i^t (p_{si}^t) is the gene (sRNA gene) copy number, π_{i0} (π_{si}) is the mRNA (sRNA) basal transcription rate constant, θ_i is the mRNA translation rate constant, and z_t is the total number of ribosome available. T_i (T_{si}) can be made constant as we tune G by changing p_i^t (p_{si}^t).

In what follows, we verify that a gene network $\mathcal{N}_s^\epsilon$ consisting of nodes with distributed sRNA feedback has local ϵ-network disturbance decoupling property defined in Definition 3. Following Claim 1, in the next two subsections, we first verify the node disturbance attenuation property, and then ϵ-well-posedness of the network.

B. Node Disturbance Attenuation

Here, we view node i in isolation, and treat w_i as an external input. By studying static i/o characteristics of (9), we show that it has the desired node disturbance attenuation property within a suitable admissible input set. We let $\epsilon := \delta/G \ll 1$ be a small positive parameter that can be decreased by increasing G. Setting the time derivatives in (9) to zero, according to [21], when $v_i \in \mathcal{V}_i$, we can find its unique positive steady state $\bar{\mathbf{x}}_i = [\bar{m}_i, \bar{s}_i, \bar{x}_i]^T$:

$$\begin{aligned} \bar{m}_i &= \frac{T_i \kappa_i k_{si} \gamma v_i (1 + w_i)}{T_{si} R_i - (\gamma k_{si} + R_i) T_i v_i} + \mathcal{O}(\epsilon), \\ \bar{s}_i &= \frac{T_{si} R_i - (\gamma k_{si} + R_i) T_i v_i}{\kappa_i k_{si} \gamma v_i (1 + w_i)} + \mathcal{O}(\epsilon), \\ \bar{x}_i &= \frac{T_i k_{si} v_i}{T_{si} - T_i v_i} + \mathcal{O}(\epsilon), \end{aligned} \tag{10}$$

where $\mathcal{V}_i$ is the set in which the approximation in (10) is valid. In particular, we have

$$\mathcal{V}_i := \{0 < v_i \le v_i^{\max}\}, \text{ with } v_i^{\max} < \frac{T_{si} R_i}{T_i(\gamma k_{si} + R_i)}. \tag{11}$$

Note that in (10), the zeroth order approximation of reference output $\bar{x}_i$ is independent of w_i. We therefore verify each node i has the desired ϵ-static disturbance attenuation property in $\mathcal{V}_i$, which is defined in Definition 1. In Fig. 3, we simulate the static i/o characteristics of (9). As G increases (and therefore ϵ decreases), static i/o characteristic from v_i to $\bar{x}_i$ becomes closer to the zeroth order approximation in (10) (Fig.3(A)). In addition, static output $\bar{x}_i$ becomes insensitive to disturbance w_i as G increases (Fig.3(B)).

C. Network Disturbance Coupling with sRNA Feedback

Now we consider a gene network $\mathcal{N}_s^\epsilon$ consisting of n nodes. Each node has a local sRNA feedback in the form of (9). In order to study the local ϵ-well-posedness property of $\mathcal{N}_s^\epsilon$, we first verify **A3**-**A5**, and then find a network admissible input set $\mathcal{V}_\mathcal{N}$, where Claim 2 can be applied. We defined before in (4) that $w_i = \sum_{j \neq i} d_j$, therefore, **A3** is satisfied. According to (10), when $\mathbf{v} \in \mathbf{V} = \mathcal{V}_1 \times \cdots \times \mathcal{V}_n$, we have

$$d_i = \frac{\bar{m}_i}{\kappa_i} = \frac{T_i k_{si} \gamma v_i (1 + w_i)}{T_{si} R_i - (\gamma k_{si} + R_i) T_i v_i} + \mathcal{O}(\epsilon), \tag{12}$$

for all $i \in \mathcal{I}$, which satisfies **A4** with

$$g_i(v_i) = \hat{g}_i(v_i) = \frac{T_i k_{si} \gamma v_i}{T_{si} R_i - (\gamma k_{si} + R_i) T_i v_i}.$$

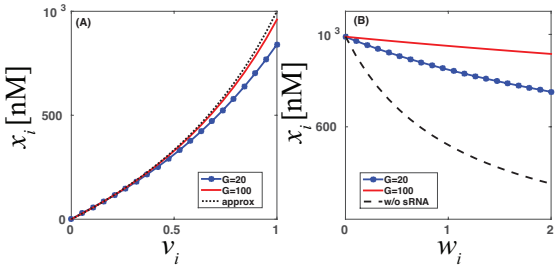

Fig. 3. Simulation of static i/o characteristics of node i in isolation with sRNA feedback using (9). (A) Static i/o characteristic from reference input (v_i) to reference output ($\bar{x}_i$). (B) Static i/o characteristic from disturbance input (w_i) to reference output ($\bar{x}_i$). Simulation parameters: $T_{si} = 1000[\text{nM}]^2$, $\gamma = 1[\text{hr}]^{-1}$, $\delta = 10[\text{hr}]^{-1}$, $\kappa_i = 1000[\text{nM}]$, $R_i = 10^4[\text{nM/hr}]$. In (A), $T_i = 500[\text{nM}]^2$, $w_i = 0$. In (B), for comparison purpose, value of T_i is taken such that $\bar{x}_i$ is the same at $w_i = 0$ for all three cases.

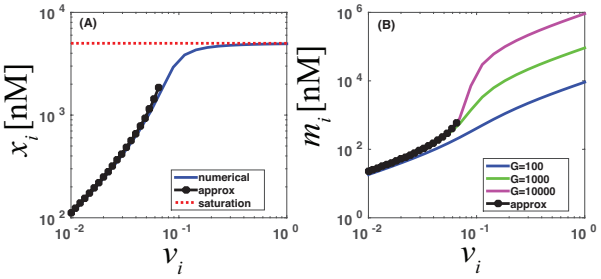

Fig. 4. Static i/o characteristics of a node with $\mathcal{V}_i = [0, 0.08]$. Approximate analytical solution within $\mathcal{V}_i$ and numerical solution for $v_i \in [0, 1]$ are given in (A) and (B) for protein and mRNA concentrations, respectively.

Assumption **A5** is naturally satisfied due to the positivity of biological signals. In order to find the network admissible reference input set $\mathcal{V}_\mathcal{N} \subseteq \mathbf{V}$, according to Claim 2, we need to satisfy the strictly diagonally dominant requirement of the interconnection matrix defined in (8). To ensure $\mathbf{A}(\mathbf{v})$ is strictly diagonally dominant in $\mathcal{V}_\mathcal{N}$, we define $\mathcal{V}_\mathcal{N}$ as:

$$\mathcal{V}_\mathcal{N} := \left\{ \mathbf{v} \in \mathbf{V} : \sum_{j \neq i} \hat{g}_j(v_j) < 1, \forall i \in \mathcal{I} \right\}. \tag{13}$$

According to Claim 2, network $\mathcal{N}_s^\epsilon$ is locally ϵ-well-posed in $\mathcal{V}_\mathcal{N}$. Since disturbance attenuation property of each node has been shown, as an immediate application of Claim 1, $\mathcal{N}_s^\epsilon$ has local ϵ-network disturbance decoupling property in $\mathcal{V}_\mathcal{N}$. In [16], we prove that the positive steady state of the network is unique, and therefore global ϵ-network disturbance decoupling can be obtained. In addition, stability of the steady state is also shown.

D. Admissible Reference Input Set

We have picked $\mathcal{V}_i$ defined in (11) as the admissible input set for each node throughout our analysis. Here, we first emphasize the necessity of $v_i \in \mathcal{V}_i$, by studying the undesirable consequences of $v_i \notin \mathcal{V}_i$. To facilitate experimental implementation, we then discuss what physical parameters enlarge the size of $\mathcal{V}_i$.

When $v_i \notin \mathcal{V}_i$, the steady state in series expansion of ϵ becomes

$$\bar{m}_i = \frac{T_i v_i(\gamma k_{si} + R_i) - T_{si} R_i}{T_i v_i \epsilon} + \mathcal{O}(1), \quad \bar{x}_i = \frac{R_i}{\gamma} + \mathcal{O}(\epsilon). \tag{14}$$

In (14), static reference output $\bar{x}_i$ becomes independent of the reference input v_i, and mRNA concentration is on the scale of $\mathcal{O}(1/\epsilon)$ (see Fig. 4 (A), (B)). In this scenario, target protein production specified by v_i is beyond the maximum gene expression capability of the node: although a large amount of $\mathrm{m_i}$ (control input) has been produced, target protein production still couldn't be reached due to limitation of ribosomes (actuator saturation). This is a biological analogy to integrator windup in the control literature [22].

Similarly, in a network setting, according to (13), a fundamental trade-off in our design is that increasing the number of nodes n shrinks the size of $\mathcal{V}_\mathcal{N}$. This is due to the fact that free ribosomes become more scarce as we increase the number of nodes.

According to (11), the size of $\mathcal{V}_i$ increases with the maximum transcription rate of sRNA (T_{si}), while decreases with the basal transcription rate of gene i (T_i). Both parameters (T_i and T_{si}) can be tuned by gene (sRNA) copy number and promoter strength. The size of $\mathcal{V}_i$ also increases with the total amount of ribosomes ($\propto R_i$), and the binding strength of $\mathrm{x_i}$ with $\mathrm{p_{si}}$ ($1/k_{si}$).

V. APPLICATION TO AN ACTIVATION CASCADE

A two-stage activation cascade is composed of a TF input (u) activating node $\mathrm{x_1}$, which serves as a transcription activator for the output node $\mathrm{x_2}$. With only transcriptional regulations, an activation cascade is expected to have positive i/o response from u to $\mathrm{x_2}$ [14]. However, in [8], we showed that hidden interactions arising from resource limitations can make the response of a two-stage activation cascade to become biphasic.

To demonstrate the effects of sRNA distributed feedback, we compare the static i/o characteristics of four activation cascades: Σ_{OL}, Σ_{OL}^c, Σ_{CL} and Σ_{CL}^c, shown in Fig. 5 (A)-(D), respectively. Σ_{OL} is a fictitious activation cascade where nodes are not competing for ribosomes. Σ_{OL}^c is the cascade where ribosome are shared among nodes. Dynamics of node i ($i = 1, 2$) in Σ_{OL} and Σ_{OL}^c are in the form of (1) and (3), respectively. Similarly, Σ_{CL} (Σ_{CL}^c) represents a cascade with distributed sRNA feedback without (with) ribosome competition. Assuming that activation is not leaky (no protein production without the activator), for all four systems, the reference inputs are specified by

$$v_1 = v_1(u) = \frac{(\frac{u}{k_1})^{n_1}}{1 + (\frac{u}{k_1})^{n_1}}, \quad v_2 = v_2(x_1) = \frac{(\frac{x_1}{k_2})^{n_2}}{1 + (\frac{x_1}{k_2})^{n_2}},$$

where k_i is the dissociation constant of activator with DNA promoter region, and n_i is the cooperativity of activation at stage i. In Fig. 5 (E)-(F), we simulate the static i/o responses of the four systems. Due to the presence of ribosome competition, response of Σ_{OL}^c becomes significantly different from that of Σ_{OL}, whose model is usually used to guide design. On the contrary, responses of systems Σ_{CL} and Σ_{CL}^c are highly similar, implying that with the feedback, ribosome competition plays an almost negligible role in the static i/o response of the cascade. The benefit of distributed sRNA feedback thus lies in the fact that it preserve modularity with respect to ribosome competition. Namely, they can

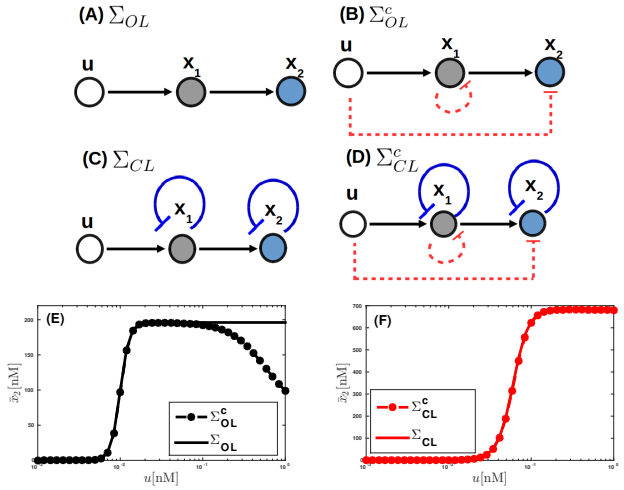

Fig. 5. (A)-(D) Interaction graph of the four networks we simulated. Black arrows represent transcriptional regulations, red dashed arrows are the hidden interactions arising from ribosome limitations, and blue arrows represent the feedback loops through sRNA. (E) Static i/o characteristic of systems Σ_{OL} and Σ^c_{OL}. (F) Static i/o characteristics of systems Σ_{CL}, Σ^c_{CL}. Simulation parameters: $T_1 = 1000[\text{nM}]^2$, $T_2 = 100[\text{nM}]^2$, $T_{s1} = 1200[\text{nM}]^2$, $T_{s2} = 120[\text{nM}]^2$, $R_1 = R_2 = 10^4[\text{nM/hr}]$, $k_{s1} = k_{s2} = 200[\text{nM}]$, $\kappa_1 = 100[\text{nM}]$, $\kappa_2 = 10^3[\text{nM}]$, $\delta = 5[\text{hr}]^{-1}$, $\gamma = 1[\text{hr}]^{-1}$, $k_1 = 1[\text{nM}]$, $k_2 = 2[\text{nM}]$, $n_1 = 2$, $n_2 = 4$.

be connected together in a "plug-and-play" fashion through transcriptional regulation, and hidden interactions generated by ribosome competition can be neglected.

VI. DISCUSSION AND CONCLUSIONS

In this paper, we model each node in a gene network as a system with two inputs and two outputs. In addition to reference input and protein production output, ribosome demand by the rest of the network is modeled as a disturbance input to node i, and ribosome usage of node i as its disturbance output. We view the mitigation of ribosome competition effects as a static network disturbance decoupling problem, where static output of node i needs to be practically independent of the reference input to other nodes in the network. By studying the static i/o maps of each node, and the interconnection rule, we show that sRNA feedback can achieve static network disturbance decoupling, given that the reference inputs stay within an admissible input set $\mathcal{V}_{\mathcal{N}}$. Implementation of our feedback design relies on a few additional considerations that are not included here. In particular, although competition for transcriptional resources such as RNA polymerases is found to play a minor role in gene expression [3], it may become noticeable when p_i increases. Increased p_i demands more transcriptional resources, leading to their depletion. Furthermore, this paper only considers static i/o signals, if promoter p_i is regulated by a time varying input produced by node j, then large amount of p_i may slow down node j dynamics [6]. In future works, we will analyze to what extent these considerations need to be factored into the model.

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