# Mitigation of ribosome competition through distributed sRNA feedback (extended version)

Yili Qian and Domitilla Del Vecchio

Department of Mechanical Engineering
Massachusetts Institute of Technology

**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.

# 1 INTRODUCTION

This paper is an extended version of a paper of the same title accepted to Proceedings of the 55th IEEE Conference on Decision and Control (2016) [1].

Context dependence is a recurrent challenge in the bottom-up design of large scale synthetic gene networks [2]. 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 [3],[4]. Such behaviors, which are often referred to as lack of modularity [5], 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 of various forms of context dependence [3],[6],[7].

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 [8]. Limited access to free ribosomes has been identified as a major bottleneck in genetic circuits [4]. 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 [9].

In order to engineer the cells to mitigate the effects of ribosome competition, An and Chin [10] 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 [11] for a comprehensive review). In [12], 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 [13], 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 [14]. 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 2, we model hidden interactions arising from ribosome competition as disturbances. In Section 3, we formulate the static network disturbance decoupling problem, and provide sufficient conditions that guarantee network disturbance decoupling. In Section 4, 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 5. Discussion and conclusions are in Section 6.

*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 $\mathbb{R}^n_+$.

# 2 DISTURBANCES ARISING FROM RIBOSOME COMPETITION

## 2.1 Gene Expression with Limited Ribosomes

A transcriptional component (node) is a fundamental building block in gene networks. It takes a number of TFs as inputs to regulate the production of protein $\mathrm{x_i}$ as output. Here, we consider a node $i$ taking a TF $\mathrm{u_i}$ as input that bind with the promoter region of gene $i$ ($\mathrm{p_i}$) with cooperativity $n_i$. Depending on the type of TFs (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 [8]. Assuming that binding reactions are much faster than transcription and translation [15], and thus can be set to quasi-steady state, each node can be described by the concentrations of its mRNA and protein: $[m_i, x_i]^T \in \mathbb{R}^2_+$.

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 [16], simplified dynamics of node $i$ can be written as:

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

where $T_i$ is the basal transcription rate of node $i$ when $u_i \equiv 0$, $\delta$ ($\gamma$) is the dilution/degradation rate of mRNA (protein), $R_i$ is a lumped translation rate constant that is proportional to $z_t$, and $\kappa_i$ is the dissociation constant between ribosomes and mRNA ribosome binding site (RBS). Smaller $\kappa_i$ 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,

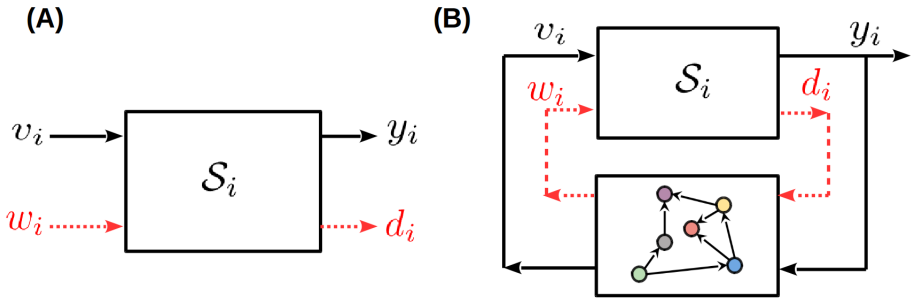


Figure 1: (A) Node $i$ in isolation. The black solid lines represent the reference i/o signals, and the red dashed lines represent disturbances i/o signals. (B) In a network, inputs to node $i$ are produced by the rest of the network, whose dynamics are also affected by disturbance output of node $i$.

otherwise $u_i$ is an activator. Detailed derivation of (1) can be found in [9]. Note that according to (1), the output of each node, $x_i$, is only dependent on $v_i$, and consequently only on $u_i$.

## 2.2 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,

$$z_t = z + \sum_{i=1}^{n} z_i, \tag{3}$$

the node dynamics can be written as ([9]):

$$\begin{aligned} \dot{m}_i &= T_i v_i - \delta m_i, \\ \dot{x}_i &= R_i \frac{m_i/\kappa_i}{1 + m_i/\kappa_i + \sum_{j \neq i} m_j/\kappa_j} - \gamma x_i. \end{aligned} \tag{4}$$

Note that in (4), 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

$$w_i := \sum_{i \neq j} m_j/\kappa_j \tag{5}$$

as a disturbance input to node $i$. Without $w_i$, dynamics of (4) are identical to those in (1), which are the dynamics of node $i$ in the absence of other ribosome-competing nodes. According to (5), ribosome demand of node $i$, which we define as

$$d_i := m_i/\kappa_i, \tag{6}$$

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 (4), the reference output is defined as $y_i = x_i$. Previous theoretical study [9], and experimental results [4] 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.

# 3 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 reponse 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 $\epsilon$, $y_i$ 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 $\epsilon$. We therefore require that $w_i$ dose not increase dramatically as we decrease $\epsilon$ (network $\epsilon$-well-posedness). Algebraic conditions are given for both conditions in what follows.

## 3.1 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 $\mathcal{V}_i \subseteq \mathbb{R}$, and a constant external disturbance input $w_i$ taking values on $\mathcal{W}_i \subseteq \mathbb{R}$. 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 $y_i \in \mathbb{R}$ and a disturbance output $d_i \in \mathbb{R}$ (refer to Fig.1(A)). System $\mathcal{S}_i^\epsilon$ is parameterized by a small parameter $\epsilon$.

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

$$y_i = h_i(v_i, w_i, \epsilon), \qquad d_i = g_i(v_i, w_i, \epsilon), \tag{7}$$

where functions $h_i(\cdot)$ and $g_i(\cdot)$ are $\mathcal{C}^2$ in $\epsilon$ 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 $\epsilon^*$ sufficiently small, the i/o characteristics (7) can be written as Taylor series in $\epsilon$:

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

where

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

**Definition 1.** *(Node disturbance attenuation). Node $i$ is said to have the $\epsilon$-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 $\epsilon$-static disturbance attenuation property, any contribution from the disturbance input to the reference output is attenuated by a factor of $\epsilon$. 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 $\epsilon$ as it approaches 0, which we refer to as the network $\epsilon$-well-posedness property.

## 3.2 Network Local Disturbance Decoupling

Consider a network $\mathcal{N}^\epsilon$ composed of $n$ nodes with static i/o maps in (7). 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 local $\epsilon$-well-posedness): Let $\mathcal{V}_\mathcal{N} \subseteq \mathbf{V}$, under **A3**, network $\mathcal{N}^\epsilon$ is locally $\epsilon$-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*

$$w_i = \sum_{j \neq i} g_j(v_j, w_j, \epsilon), \ \forall i \in \mathcal{I}. \tag{8}$$

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

A locally $\epsilon$-well-posed network has static interconnection signal $\mathbf{w}(\mathbf{v}, \epsilon) \in \mathcal{W}$ which is $\mathcal{C}^1$ in $\epsilon$. Therefore, static i/o characteristics of each node in the network can be found as

$$y_i = H_i(v_i, \mathbf{v}_{-i}, \epsilon) := h_i(v_i, \mathbf{w}(\mathbf{v}, \epsilon), \epsilon). \tag{9}$$

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

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

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 $\epsilon$-network disturbance decoupling property in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$ if (i) each node $i$ has $\epsilon$-disturbance attenuation property in $\mathcal{V}_i \times \mathcal{W}_i$, and (ii) the network is locally $\epsilon$-well-posed in $\mathcal{V}_\mathcal{N} \times \mathcal{W}$.

*Proof.* Consider the static i/o response of $\mathcal{S}_i^\epsilon$, which has $\epsilon$-disturbance attenuation property in $\mathcal{V}_i \times \mathcal{W}_i$:

$$\begin{aligned} y_i &= h_i(v_i, \mathbf{w}_{(i)}(\mathbf{v}, \epsilon), 0) + \epsilon \tilde{h}_i(v_i, \mathbf{w}_{(i)}(\mathbf{v}, \epsilon), 0) + \mathcal{O}(\epsilon^2) \\ &= h_i(v_i, 0, 0) + \epsilon \tilde{h}_i(v_i, \mathbf{w}_{(i)}(\mathbf{v}, \epsilon), 0) + \mathcal{O}(\epsilon^2). \end{aligned} \tag{10}$$

Since the network is locally $\epsilon$-well-posed, we can write

$$w_i(\mathbf{v}, \epsilon) = w_i(\mathbf{v}, 0) + \mathcal{O}(\epsilon). \tag{11}$$

Substituting (11) into (10), we have

$$y_i = h_i(v_i, 0, 0) + \epsilon \tilde{h}_i(v_i, w_i(\mathbf{v}, 0), 0) + \mathcal{O}(\epsilon^2).$$

The zeroth order approximation of $y_i$ only depends on $v_i$. □

Now we provide sufficient conditions to certify that the network is locally $\epsilon$-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 $i$.

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

$$\mathbf{A}_{(j,k)}(\mathbf{v}) := \begin{cases} 1, & \text{if } j = k, \\ -\hat{g}_k(v_k), & \text{if } j \neq k. \end{cases} \tag{12}$$

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

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

We shall introduce the following lemma [17], which gives sufficient conditions for a class of matrices to be inverse-positive.

**Lemma 1.** *If $\mathbf{B} \in \mathbb{R}^{p \times p}$ is a strictly diagonally dominant matrix, where $|\mathbf{B}_{(i,i)}| > \sum_{j \neq i} |\mathbf{B}_{(i,j)}|$ for all $i, j \in \{1, \cdots, p\}$, then $\mathbf{B}$ is non-singular. Furthermore, let $\boldsymbol{\zeta} \in \mathbb{R}^p$, and $\boldsymbol{\zeta} > \mathbf{0}$. If $\mathbf{B}_{(i,i)} > 0$ and $\mathbf{B}_{(i,j)} < 0$ for all $i \neq j$, then $\mathbf{B}^{-1}\boldsymbol{\zeta} > \mathbf{0}$.*

**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 $\epsilon$-well-posed in $\mathcal{V}_{\mathcal{N}} \times \mathcal{W}$.*

*Proof.* Based on the definition of local $\epsilon$-well-posedness, we need to verify the existence and smoothness properties of the solution to (8). Note that when $\epsilon = 0$, according to Assumption **A4**, we have

$$w_i = \sum_{j \neq i} \bar{g}_j(v_j, w_j, 0) = \sum_{j \neq i} g_j(v_j) + \hat{g}_j(v_j) w_j, \ \forall i.$$

This equation is linear in $\mathbf{w}$, and can be re-written as

$$\mathbf{A}(\mathbf{v})\mathbf{w} = \mathbf{\Phi}(\mathbf{v}). \tag{13}$$

Since matrix $\mathbf{A}(\mathbf{v})$ is diagonally dominant, and thus invertible, we have $\mathbf{w} = \mathbf{A}^{-1}(\mathbf{v})\mathbf{\Phi}(\mathbf{v})$. Moreover, since $\mathbf{\Phi} > 0$, $\mathbf{A}_{(i,i)} > 0$ and $\mathbf{A}_{(i,j)} < 0$ $(j \neq i)$, according Lemma 1, we have $\mathbf{w} > \mathbf{0}$. Due to **A5**, $\mathbf{w} \in \mathbf{W}$. Let

$$F_i(\mathbf{v}, \mathbf{w}, \epsilon) := \mathbf{w}_i - \sum_{j \neq i} \bar{g}_j(v_j, w_j, \epsilon),$$

and $\mathbf{F}(\mathbf{v}, \mathbf{w}, \epsilon) := [F_1(\mathbf{v}, \mathbf{w}, \epsilon), \cdots, F_n(\mathbf{v}, \mathbf{w}, \epsilon)]^T$. We have shown that $(\mathbf{v}, \mathbf{A}^{-1}(\mathbf{v})\mathbf{\Phi}(\mathbf{v}), 0)$ is a solution to $\mathbf{F}(\mathbf{v}, \mathbf{w}, \epsilon) = 0$. Due to **A1**, $\mathbf{F}(\mathbf{v}, \mathbf{w}, \epsilon)$ is $\mathcal{C}^2$ in $\epsilon$. Since

$$\frac{\partial \mathbf{F}}{\partial \mathbf{w}}(\mathbf{v}, \mathbf{w}, \epsilon) \Big|_{\mathbf{v}, \mathbf{A}^{-1}(\mathbf{v})\mathbf{\Phi}(\mathbf{v}), 0} = \mathbf{A}^{-1}(\mathbf{v}) \text{ is non-singular,}$$

we can apply implicit function theorem [18] to claim that there exists an open set $\mathcal{V}_{\mathcal{N}} \times \mathcal{W} \times (-\epsilon^*, \epsilon)$ containing $(\mathbf{v}, \mathbf{A}^{-1}(\mathbf{v})\mathbf{\Phi}(\mathbf{v}), 0)$ in which we have a unique $\mathcal{C}^1$ mapping $\mathcal{V}_{\mathcal{N}} \times (-\epsilon^*, \epsilon^*) \mapsto \mathcal{W}$ such that $\mathbf{w} = \mathbf{w}(\mathbf{v}, \epsilon)$. □

**Remark:** Our results can be easily to more complex networks, where reference inputs and outputs are connected:

$$v_i = \mathcal{F}_i(\mathbf{y}), \quad \forall i, \tag{14}$$

where $\mathcal{F}_i(\cdot)$ is a bounded function describing the reference interconnection rules. (*i.e.* how outputs of nodes in the network affects reference input to node $i$.) As long as $\mathbf{v} \in \mathcal{V}_{\mathcal{N}}$ for all $i \in \mathcal{I}$, our result guarantees that

$$y_i = \mathcal{H}_i(\mathbf{y}) + \mathcal{O}(\epsilon), \quad \forall i \in \mathcal{I} \tag{15}$$

where

$$\mathcal{H}_i(\mathbf{y}) := H_i(\mathcal{F}_i(\mathbf{y}), \mathbf{0}, 0).$$

Estimating the solution to (15) may become hard, depending on the form of reference interconnection rule $\mathcal{F}_i(\cdot)$. We leave it to the designer of the network to specify the reference interconnection rules that achieve their functions of interests. Our aim here, however, is to ensure that equation (15) does not change with the change in disturbance signals in the network, arising from, for example, adding of new nodes to the network. As long as the reference inputs stay in the admissible reference input set $\mathcal{V}_{\mathcal{N}}$, the behavior of the network can be predicted reliably by solving (15).

## 3.3 Network Global Disturbance Decoupling

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 $H_i(v_i, \mathbf{v}_{-i}, 0) = H_i(v_i, \mathbf{0}, 0)$. To show global disturbance decoupling, we need to show all $\mathbf{w}(\mathbf{v}, \epsilon) \in \mathbf{W}$ that satisfy (8) are $\mathcal{C}^1$ in $\epsilon$ (global $\epsilon$-well-posedness). The definitions of global $\epsilon$-well-posedness and global $\epsilon$-network disturbance decoupling are stated as follows.

**Definition 4.** (Network global $\epsilon$-well-posedness): Let $\mathcal{V}_{\mathcal{N}} \subseteq \mathbf{V}$, network $\mathcal{N}^{\epsilon}$ is *globally $\epsilon$-well-posed* in $\mathcal{V}_{\mathcal{N}}$ if there exists an $\epsilon^* > 0$ such that all interconnection signals $\mathbf{w}(\mathbf{v}, \epsilon) \in \mathbf{W}$ that satisfy

$$w_i = \sum_{j \neq i} g_j(v_j, w_j, \epsilon), \ \forall i. \tag{16}$$

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

**Definition 5.** (Network global disturbance decoupling): Network $\mathcal{N}^{\epsilon}$ is said to have *global $\epsilon$-network disturbance decoupling property* in $\mathcal{V}_{\mathcal{N}}$ if there exists $\epsilon^* > 0$ such that for all $i$, $H_i(v_i, \mathbf{v}_{-i}, 0) \equiv H_i(v_i, \mathbf{0}, 0)$ for all $(\mathbf{v}, \epsilon) \in \mathcal{V}_{\mathcal{N}} \times (-\epsilon^*, \epsilon^*)$.

Accordingly, we have the sufficient condition to obtain global disturbance decoupling.

**Claim 3.** Network $\mathcal{N}^{\epsilon}$ has global $\epsilon$-network disturbance decoupling property in $\mathcal{V}_{\mathcal{N}}$ if (i) each node $i$ has $\epsilon$-disturbance attenuation property in $\mathcal{V}_i \times \mathcal{W}_i$, and (ii) the network is globally $\epsilon$-well-posed in $\mathcal{V}_{\mathcal{N}}$.

*Proof.* Since the network is globally $\epsilon$-well-posed, suppose (16) has $p$ solutions $\mathbf{w}_1\mathbf{v}, \epsilon), \cdots, \mathbf{w}_p\mathbf{v}, \epsilon)$, all of which are $\mathcal{C}^1$ in $\epsilon$, the proof of Claim 3 can be obtained by applying the proof for Claim 1 $p$ times to each solution of (16). □

A potential scenario that gives rise to a locally $\epsilon$-well-posed but not globally $\epsilon$-well-posed network is when the algebraic equation (8) is singular in $\epsilon$ (i.e. the highest order of $w$ is multiplied by $\epsilon$). In this case, the number of solutions obtained in (8) by setting $\epsilon = 0$ is different from the number of solutions obtained when $\epsilon \neq 0$ but small, and implicit function theorem doesn't tell any information about the smoothness of these emergent solutions. To rule out such scenarios, it is sufficient to ask that (8) has a unique solution in $\mathcal{V}_{\mathcal{N}} \times \mathbf{W} \times (-\epsilon^*, \epsilon^*)$. This requirement, albeit hard to show in general, can be proven for our system of interests, which we discuss in detail in Section 4.3.

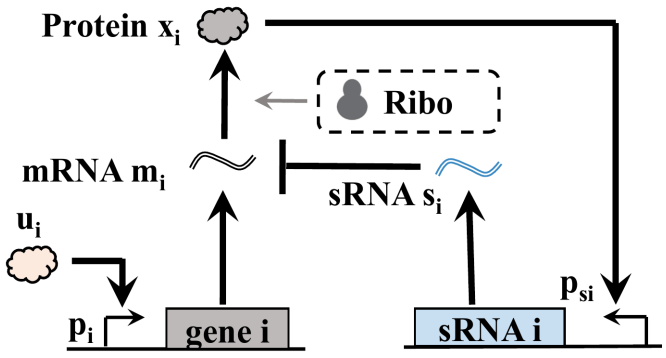


Figure 2: A schematic of the sRNA feedback acting on node $i$.

# 4 DISTURBANCE DECOUPLING REALIZED THROUGH DISTRIBUTED sRNA FEEDBACK

Small RNAs have been recognized as critical regulators in gene expression [19]. In this section, we propose a distributed sRNA feedback design that achieves the static network disturbance decoupling described in Section 3.

## 4.1 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 ($x_i$) transcriptionally activates the production of sRNA ($s_i$), 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 [20].

When ribosome availability decreases, for instance, $x_i$ production decreases, down-regulating sRNA production, which in turn up-regulates $m_i$, and consequently $x_i$, compensating for the loss in $x_i$ production due to ribosome limitation. To compensate for the decrease in gene (sRNA) expression due the feedback, we need to maintain sufficiently high transcription rates for both species. Due to the comparative short length of sRNA nucleotide

chains, average mRNAs' transcription rate is about 10 times larger than mRNAs' [21]. Meanwhile, we can increase gene transcription by increasing its plasmid copy number $p_i$.

When there is no ambiguity, we follow the notations used in Section 2 to derive our model. We consider a node $i$ taking a TF input ($u_i$) that form complexes $\mathrm{c_i}$ with $\mathrm{p}_i$. The complexes are then transcribed at a rate $\pi_{ij}$ to produce mRNA ($\mathrm{m_i}$). mRNA can also be transcribed at a basal rate $\pi_{i0}$, and it is diluted and degraded by RNase at a rate $\delta$:

$$\mathrm{p_i} + \mathrm{n_i} \cdot \mathrm{u_i} \underset{\mathrm{k_i^-}}{\overset{\mathrm{k_i^+}}{\rightleftharpoons}} \mathrm{c_i} \xrightarrow{\pi_\mathrm{i}} \mathrm{c_i} + \mathrm{m_i}, \qquad \mathrm{p_i} \xrightarrow{\pi_{\mathrm{i0}}} \mathrm{p_i} + \mathrm{m_i}, \qquad \mathrm{m_i} \xrightarrow{\delta_\mathrm{i}} \emptyset.$$

The mRNA then binds with free ribosome (z) to produce a translationally active complex $\mathrm{M_i}$, which is translated at rate $\theta_i$ to produce the protein $\mathrm{x_i}$. The protein is diluted and degraded by protease at a rate $\gamma$:

$$\mathrm{m_i} + \mathrm{z} \underset{\kappa_\mathrm{i}^-}{\overset{\kappa_\mathrm{i}^+}{\rightleftharpoons}} \mathrm{M_i}, \qquad \mathrm{M_i} \xrightarrow{\theta_\mathrm{i}} \mathrm{m_i} + \mathrm{z} + \mathrm{x_i}, \qquad \mathrm{x_i} \xrightarrow{\gamma_\mathrm{i}} \emptyset.$$

sRNA in node $i$ ($\mathrm{s_i}$) binds with mRNA to form a translationally inactive complex $\mathrm{C}_{i*}$ that degrades rapidly:

$$\mathrm{s_i} + \mathrm{m_i} \underset{\mathrm{k_{i*}^-}}{\overset{\mathrm{k_{i*}^+}}{\rightleftharpoons}} \mathrm{C_{i*}}, \qquad \mathrm{C_{i*}} \xrightarrow{\beta_\mathrm{i}} \emptyset.$$

sRNA is activated by protein $\mathrm{x_i}$ to produce a complex $\mathrm{c_{si}}$, which is transcribed to produce an sRNA ($\mathrm{s_i}$) at rate $\pi_{si}$. We assume that the activation has cooperativity 1. $\mathrm{s_i}$ is diluted and degraded by RNase at a rate $\delta_i$:

$$\mathrm{p_{si}} + \mathrm{x_i} \underset{\mathrm{k_{si}^-}}{\overset{\mathrm{k_{si}^+}}{\rightleftharpoons}} \mathrm{c_{si}}, \qquad \mathrm{c_{si}} \xrightarrow{\pi_{\mathrm{si}}} \mathrm{c_{si}} + \mathrm{s_i}, \qquad \mathrm{s_i} \xrightarrow{\delta_\mathrm{i}} \emptyset.$$

Consequently, the concentration of each species can be described by the fol-

lowing ODEs:

$$\dot{c}_i = k_i^+ p_i u_i^{n_i} - k_i^- c_i, \tag{17a}$$
$$\dot{M}_i = \kappa_i^+ m_i z - \kappa_i^- M_i - \theta_i M_i, \tag{17b}$$
$$\dot{c}_{si} = k_{si}^+ p_{si} x_i - k_{si}^- c_{si}, \tag{17c}$$
$$\dot{C}_{i*} = k_{i*}^+ s_i m_i - k_{i*}^- C_{i*} - \beta_i C_{i*}, \tag{17d}$$
$$\begin{aligned}\dot{m}_i = {} & \pi_i c_i + \pi_{i0} p_i - \delta m_i - \kappa_i^+ m_i z + \kappa_i^- M_i \\ & + \theta_i M_i - k_{i*}^+ s_i m_i + k_{i*}^- C_{i*},\end{aligned} \tag{24e}$$
$$\dot{s}_i = \pi_{si} c_{si} - \delta s_i - k_{i*}^+ s_i m_i + k_{i*}^- C_{i*}, \tag{24f}$$
$$\dot{x}_i = \theta_i M_i - \gamma x_i - k_{si}^+ p_{si} x_i + k_{si}^- c_{si}. \tag{24g}$$

Assuming $p_i^t$ and $p_{si}^t$ are constants [15], we have

$$p_i^t = p_i + c_i, \qquad \text{and} \qquad p_{si}^t = p_{si} + c_{si}. \tag{25}$$

Setting equations (17a) to (17d) to quasi-steady state, complex concentrations can be obtained as follows:

$$c_i = \frac{p_i u_i^{n_i}}{k_i}, \qquad M_i = \frac{m_i z}{\kappa_i}, \qquad c_{si} = \frac{p_{s_i} x_i}{k_{si}}, \qquad C_{i*} = \frac{s_i m_i}{k_{i*}}, \tag{26}$$

and we have defined the following effective dissociation constants:

$$k_i := \frac{k_i^-}{k_i^+}, \qquad \kappa_i := \frac{\kappa_i^- + \theta_i}{\kappa_i^+}, \qquad k_{si} := \frac{k_{si}^-}{k_{si}^+}, \qquad k_{i*} := \frac{k_{i*}^- + \beta_i}{k_{i*}^+}.$$

Using equations (26) and (25), the dynamics of our target node can be rewritten as:

$$\begin{aligned}\dot{m}_i &= p_i^t \frac{\pi_{i0} + \pi_i (u_i/k_i)^{n_i}}{1 + (u_i/k_i)^{n_i}} - \delta m_i - \frac{\beta_i}{k_{i*}} m_i s_i, \\ \dot{s}_i &= p_{si}^t \pi_{si} \frac{x_i/k_{si}}{1 + x_i/k_{si}} - \delta s_i - \frac{\beta_i}{k_{i*}} m_i s_i, \\ \dot{x}_i &= \frac{\theta_i}{\kappa_i} m_i z - \gamma x_i.\end{aligned} \tag{27}$$

Due to ribosome competition, the free amount of ribosome $z$ can be written as

$$z = \frac{z_t}{1 + m_i/\kappa_i + \sum_{j \neq i} m_j/\kappa_j}. \tag{28}$$

Substitute (28) into (27), let $G_i := \beta_i/k_{i*}$ be the effective sRNA repression rate, we obtain the model in (29). For simplicity of analysis, we assume that $G_i = G$ for all $i$, and the dilution rates of mRNA and protein are the same for all nodes.

$$\begin{aligned} \dot{m}_i &= GT_i v_i(u_i) - Gm_i s_i - \delta m_i, \\ \dot{s}_i &= GT_{si}\frac{x_i/k_{si}}{1 + x_i/k_{si}} - Gm_i s_i - \delta s_i, \\ \dot{x}_i &= R_i \frac{m_i/\kappa_i}{1 + m_i/\kappa_i + w_i} - \gamma x_i. \end{aligned} \tag{29}$$

Since $\beta$ 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 [19]. We have also defined the following lumped parameters:

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

and the reference input $v_i(u_i)$ is defined as:

$$v_i(u_i) = \frac{1 + \pi_i/\pi_{i0}(u_i/k_i)^{n_i}}{1 + (u_i/k_i)^{n_i}}. \tag{30}$$

$T_i$ ($T_{si}$) 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 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 local $\epsilon$-well-posedness of the network. By further demonstrating that the network always has a unique solution in the positive orthant, we show that the disturbance decoupling property is global.

## 4.2 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 (29), 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 (29) to zero, we can find its

steady state $\bar{\mathbf{x}}_i = [\bar{m}_i, \bar{s}_i, \bar{x}_i]^T$ using:

$$T_{si}\frac{R_i\bar{m}_i}{\gamma k_{si}\kappa_i(1+w_i)+(\gamma k_{si}+R_i)\bar{m}_i} - \epsilon\frac{T_i v_i}{\bar{m}_i} + \epsilon\bar{m}_i = T_i v_i - \epsilon^2, \tag{31}$$

$$\bar{x}_i = \frac{R_i}{\gamma}\cdot\frac{\bar{m}_i/[\kappa_i(1+w_i)]}{1+\bar{m}_i/[\kappa_i(1+w_i)]}, \qquad \bar{s}_i = \frac{T_i\bar{v}}{\bar{m}_i} - \epsilon. \tag{32}$$

**Remark:** System (29) has a well-defined static i/o characteristic since its steady state $\bar{\mathbf{x}}_i$ is unique. For all positive $v_i$, $w_i$ and $\epsilon$, according to (32), $\bar{s}_i, \bar{x}_i$ are bijective functions of $\bar{m}_i$, hence, we only need to show uniqueness of $\bar{m}_i$. The left hand side of (31) increases monotonically with $\bar{m}_i$, and ranges $\mathbb{R}$, while the right hand side of (31) is a constant. Therefore, there is a unique steady state $\bar{\mathbf{x}}_i$ in the positive orthant. According to [22], for $\epsilon$ sufficiently small and $v_i \in \mathcal{V}_i$, the steady state of (29) can be written as

$$\begin{aligned}
\bar{m}_i &= \frac{T_i\kappa_i k_{si}\gamma v_i(1+w_i)}{T_{si}R_i - (\gamma k_{si}+R_i)T_i v_i} + \mathcal{O}(\epsilon),\\
\bar{s}_i &= \frac{T_{si}R_i - (\gamma k_{si}+R_i)T_i v_i}{\kappa_i k_{si}\gamma v_i(1+w_i)} + \mathcal{O}(\epsilon),\\
\bar{x}_i &= \frac{T_i k_{si} v_i}{T_{si} - T_i v_i} + \mathcal{O}(\epsilon),
\end{aligned} \tag{33}$$

where $\mathcal{V}_i$ is the set in which the approximation in (33) is valid. In particular, we have

$$\mathcal{V}_i := \left\{0 < v_i \le v_i^{\max}\right\}, \text{ with } v_i^{\max} < \frac{T_{si}R_i}{T_i(\gamma k_{si}+R_i)}. \tag{34}$$

Note that in (33), the zeroth order approximation of reference output $\bar{x}_i$ is independent of $w_i$. We therefore verify each node $i$ has the desired $\epsilon$-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 (29). As $G$ increases (and therefore $\epsilon$ decreases), static i/o characteristic from $v_i$ to $\bar{x}_i$ becomes closer to the zeroth order approximation in (33) (Fig.3(A)). In addition, static output $\bar{x}_i$ becomes insensitive to disturbance $w_i$ as $G$ increases (Fig.3(B)).

## 4.3 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 (29). In order to study the local $\epsilon$-well-

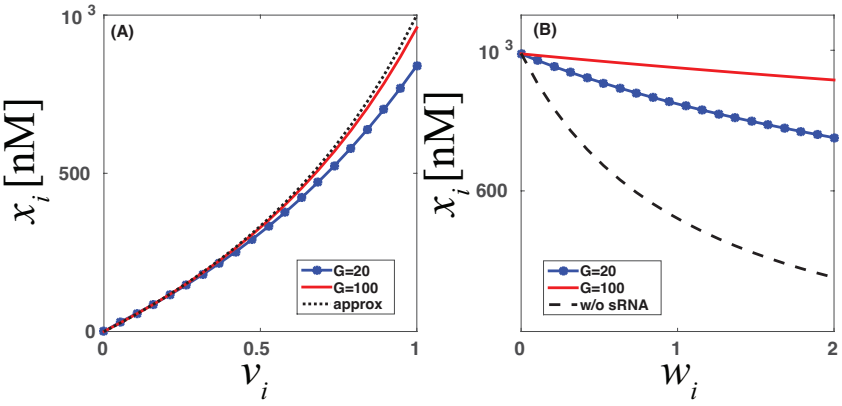


Figure 3: Simulation of static i/o characteristics of node $i$ in isolation with sRNA feedback using (29). (A) Static i/o characteristic from reference input ($v_i$) to reference output ($\bar{x}_i$). (B) Static i/o characteristic from disturbance input ($w_i$) to reference output ($\bar{x}_i$). Simulation 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.

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 (5) that $w_i = \sum_{j\neq i} d_j$, therefore, **A3** is satisfied. According to (33), when $\mathbf{v} \in \mathbf{V} = \mathcal{V}_1 \times \cdots \times \mathcal{V}_n$, for all $i \in \mathcal{I}$, we have

$$d_i = \frac{\bar{m}_i}{\kappa_i} = \frac{T_i k_{si}\gamma v_i(1+w_i)}{T_{si}R_i - (\gamma k_{si} + R_i)T_i v_i} + \mathcal{O}(\epsilon), \tag{35}$$

which satisfies **A4** with

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

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 (12). To ensure $\mathbf{A}(\mathbf{v})$ is strictly diagonally dominant in $\mathcal{V}_\mathcal{N}$, we define $\mathcal{V}_\mathcal{N}$ as:

$$\mathcal{V}_\mathcal{N} := \left\{ \mathbf{v} \in \mathbf{V} : \sum_{j\neq i} \hat{g}_j(v_j) < 1, \forall i \in \mathcal{I} \right\}. \tag{36}$$

According to Claim 2, network $\mathcal{N}_s^\epsilon$ is locally $\epsilon$-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 $\epsilon$-static network disturbance disturbance decoupling property in $\mathcal{V}_\mathcal{N}$. In order to show that $\mathcal{N}_s^\epsilon$ indeed has global $\epsilon$-network disturbance decoupling property, we show that $\mathcal{N}_s^\epsilon$ has a unique positive steady state.

**Claim 4.** Given a constant $\mathbf{v}$, network $\mathcal{N}_s^\epsilon$ has a unique positive steady state $\bar{\mathbf{x}} = [\bar{\mathbf{x}}_1^T, \cdots, \bar{\mathbf{x}}_n^T]^T$ for all positive integer $n$.

*Proof.* Let

$$F_i(m_1, \cdots, m_n) := -\epsilon \frac{T_i \bar{v}_i}{m_i} + \epsilon m_i - T_i \bar{v}_i + \epsilon^2 + \frac{T_{si} R_i m_i}{\gamma k_{si} \kappa_i (1 + \sum_{j \neq i} m_j/\kappa_j) + (\gamma k_{si} + R_i) m_i}, \tag{37}$$

steady state mRNA concentration of each node $i$, $\bar{m}_i$, can be found from the following $n$ equations:

$$F_i(\bar{m}_1, \bar{m}_2, \cdots, \bar{m}_n) = 0, \quad \forall i \in \mathcal{I}. \tag{38}$$

This can be seen by combining equations (31), (32), and (5). To show (38) has a unique solution for any positive integer $n$, we use induction. The idea is to use the first $k$ ($1 \leq k \leq n$) equations, to uniquely find $\bar{m}_k$ as a function of $\bar{m}_{k+1}, \cdots, \bar{m}_n$. When we continue the induction to $k = n$, we have a unique solution of $\bar{m}_n$, and consequently $\bar{m}_1, \cdots, \bar{m}_{n-1}$, using results from previous induction steps.

When $k = 1$, regarding $\bar{m}_2, \cdots, \bar{m}_n$ as positive parameters, we have seen in the isolated node case that there exists function $f_1^1(\cdot) : \mathbb{R}^{n-1} \to \mathbb{R}_+$ such that $\bar{m}_1 = f_1^1(\bar{m}_2, \cdots, \bar{m}_n)$. Now suppose that using the first $(k-1)$ equations in (38), we can find functions $f_j^{k-1} : \mathbb{R}^{n-k+1} \mapsto \mathbb{R}_+$ such that

$$\bar{m}_j = f_j^{k-1}(\bar{m}_k, \bar{m}_{k+1}, \cdots, \bar{m}_n), \quad \forall j = 1, \cdots, (k-1).$$

Regarding $\bar{m}_{k+1}, \cdots, \bar{m}_n$ as positive parameters, with abuse of notation, we write $\bar{m}_j = f_j^{k-1}(\bar{m}_k)$, and $F_j(\bar{m}_1, \cdots, \bar{m}_n) = F_j(\bar{m}_1, \cdots, \bar{m}_k)$. To continue the induction, we need to show that there exists $f_k^k$ such that

$$\bar{m}_k = f_k^k(\bar{m}_{k+1}, \cdots, \bar{m}_n).$$

For this purpose, if we can show

$$\frac{\mathrm{d}}{\mathrm{d}\bar{m}_k} F_k(\bar{m}_1, \cdots, \bar{m}_k) > 0 \tag{39}$$

for all positive $\bar{m}_k$, since range$(F_k) = \mathbb{R}$, there exists a unique positive $\bar{m}_k$ such that $F_k(\bar{m}_1, \cdots, \bar{m}_k) = F_k(f_1^{k-1}(\bar{m}_k), \cdots, \bar{m}_k) = 0$. Note that according to the definition of $F_i$, if we have $P_j^k(\bar{m}_k) := \bar{m}_j/\bar{m}_k = f_j^k(\bar{m}_k)/\bar{m}_k$ decreasing monotonically with $\bar{m}_k$ for all $j = 1, \cdots, (k-1)$, then $F_j$ increases monotonically with $\bar{m}_k$. Differentiating $P_j^k$ with respect to $\bar{m}_k$, it is sufficient to show

$$X_j^k := \frac{\mathrm{d}\bar{m}_j}{\mathrm{d}\bar{m}_k} \cdot \frac{\bar{m}_k}{\bar{m}_j} < 1, \quad \forall j = 1, \cdots, (k-1),$$

to guarantee that (39) holds. Applying implicit function theorem for the first $(k-1)$ equations in (38), $F_1 = 0, \cdots, F_{k-1} = 0$, we obtain

$$\begin{bmatrix} \frac{\partial F_1}{\partial \bar{m}_1}\bar{m}_1 & \cdots & \frac{\partial F_1}{\partial \bar{m}_{k-1}}\bar{m}_{k-1} \\ \vdots & \ddots & \vdots \\ \frac{\partial F_{k-1}}{\partial \bar{m}_1}\bar{m}_1 & \cdots & \frac{\partial F_{k-1}}{\partial \bar{m}_{k-1}}\bar{m}_{k-1} \end{bmatrix} \begin{bmatrix} X_1^k \\ \vdots \\ X_{k-1}^k \end{bmatrix} = - \begin{bmatrix} \frac{\partial F_1}{\partial \bar{m}_k}\bar{m}_k \\ \vdots \\ \frac{\partial F_{k-1}}{\partial \bar{m}_k}\bar{m}_k \end{bmatrix}. \tag{40}$$

We define the following positive constants,

$$\mathcal{D}_i := [(\gamma k_{si} + R_i)\bar{m}_i + \gamma k_{si}\kappa_i + \sum_{j \neq i} \bar{m}_j/\kappa_j]^2,$$

$$\Gamma_{ij} := \frac{T_{si}R_i\gamma k_{sj}\bar{m}_i\bar{m}_j}{\mathcal{D}_i}, \Delta_i := \frac{T_{si}R_i\gamma k_{si}\kappa_i\bar{m}_i}{\mathcal{D}_i} + \epsilon + \epsilon\frac{T_i\bar{v}_i}{\bar{m}_i^2}.$$

Equation (40) can be written as

$$(\boldsymbol{\Gamma} + \boldsymbol{\Delta})\mathbf{X} = \boldsymbol{\eta}, \tag{41}$$

where $\mathbf{X}_{(i)} := X_i^k$, $\boldsymbol{\eta}_{(i)} := \Gamma_{ik}$, and

$$\boldsymbol{\Gamma}_{(i,j)} = \begin{cases} \sum_{q \neq i}^k \Gamma_{iq}, & i = j, \\ -\Gamma_{ij}, & i \neq j, \end{cases} \qquad \boldsymbol{\Delta} := \mathrm{diag}(\Delta_i).$$

Note that since $(\boldsymbol{\Gamma} + \boldsymbol{\Delta})$ is strictly diagonally dominant, we have $\mathbf{X} > 0$. Furthermore, $\boldsymbol{\Gamma}\mathbf{e} = \boldsymbol{\eta}$, where $\mathbf{e} = [1, \cdots, 1]^T$. Therefore the solution satisfies,

$$\mathbf{X} = \boldsymbol{\Gamma}^{-1}\boldsymbol{\eta} - \boldsymbol{\Delta}\mathbf{X} < \mathbf{e}.$$

Therefore, we have shown (39) holds, and we have a unique positive $\bar{m}_k = f_k^k(\bar{m}_{k+1}, \cdots, \bar{m}_n)$. □

Since $\mathcal{N}_s^\epsilon$ has a unique positive steady state (there is one unique disturbance signal), and we have shown that the network is locally $\epsilon$-well-posed (there exists one disturbance signal that is $\mathcal{C}^1$ in $\epsilon$), this implies that the network is globally $\epsilon$-well-posed (all disturbance signals are $\mathcal{C}^1$ in $\epsilon$). We have shown that each node has $\epsilon$-static disturbance attenuation property, as a direct application of Claim 3, the network has global $\epsilon$-network disturbance decoupling property in $\mathcal{V}_\mathcal{N}$.

## 4.4 Local Stability of the Steady State

By proving that the network has global $\epsilon$-network disturbance decoupling property. We have shown that the static i/o response of each node becomes essentially decoupled from ribosome competition when sRNA feedback is applied. Here, we demonstrate that the steady state we found are locally asymptotically stable. In particular, we consider stability for a special case where the network is homogeneous (*i.e.* all nodes have same parameters and same reference input). Due to Claim 4, the network has a unique steady state. Furthermore, due to homogeneity, it must lie on the diagonal of the $3 \times n$ state space (i.e. $\bar{\mathbf{x}}_1 = \bar{\mathbf{x}}_2 = \cdots = \bar{\mathbf{x}}_n$). Linearizing the system around $\bar{\mathbf{x}} := [\bar{\mathbf{x}}_1, \cdots, \bar{\mathbf{x}}_n]^T$ gives the following linearized subsystems:

$$\begin{aligned} \dot{m}_i &= -(G\bar{s}_i + \delta)m_i - G\bar{m}_i s_i, \\ \dot{s}_i &= GT_{si}f_{si}x_i - G\bar{s}_i m_i - (G\bar{m}_i + \delta)s_i, \\ \dot{x}_i &= R_i q_{ii} m_i + R_i \sum_{j\neq i} q_{ij} m_j - \gamma x_i, \end{aligned} \tag{42}$$

where we have defined

$$f_{si} := \frac{\mathrm{d}}{\mathrm{d}x_i}\frac{x_i/k_{si}}{1 + x_i/k_{si}}\bigg|_{\bar{\mathbf{x}}_i} = \frac{1/k_{si}}{(1 + \bar{x}_i/k_{si})^2}, \tag{43}$$

$$q_{ii} := \frac{\partial}{\partial m_i}\frac{m_i/\kappa_i}{1 + m_i/\kappa_i + \sum_{j\neq i} m_j/\kappa_j}\bigg|_{\bar{\mathbf{x}}_i} = \frac{(1 + \sum_{j\neq i} \bar{m}_j/\kappa_j)/\kappa_i}{(1 + \bar{m}_i/\kappa_i + \sum_{j\neq i} \bar{m}_j/\kappa_j)^2}, \tag{44}$$

$$q_{ij} := \frac{\partial}{\partial m_j}\frac{m_i/\kappa_i}{1 + m_i/\kappa_i + \sum_{j\neq i} m_j/\kappa_j}\bigg|_{\bar{\mathbf{x}}_i} = -\frac{\bar{m}_i/\kappa_i\kappa_j}{(1 + \bar{m}_i/\kappa_i + \sum_{j\neq i} \bar{m}_j/\kappa_j)^2}. \tag{45}$$

To show stability of the network, we consider the following result previously applied to vehicle formation control [23]. Consider $N$ identical linear

subsystems, whose dynamics are defined as

$$\dot{X}_i = P_A X_i + P_B u_i, \tag{46}$$
$$y_i = P_{C1} X_i, \tag{47}$$
$$z_{ij} = P_{C2}(X_i - X_j),\ j \in \mathcal{J}_i, \tag{48}$$

where $i \in [1, N]$ is the index of subsystems, $\mathcal{J}_i \subseteq [1, N] - \{i\}$ represents the set of subsystems that communicate with subsystem $i$. $y_i$ and $z_{ij}$ represent the absolute measurement and relative measurements taken by subsystem $i$, respectively. Denote by $z_i$

$$z_i = \frac{1}{|\mathcal{J}_i|} \sum_{j \in \mathcal{J}_i} z_{ij}. \tag{49}$$

Define a decentralized control law which maps $y_i$, $z_i$ to $u_i$:

$$\begin{aligned} \dot{V}_i &= K_A V_i + K_{B1} y_i + K_{B2} z_i, \\ u_i &= K_C V_i + K_{D1} y_i + K_{D2} z_i. \end{aligned} \tag{50}$$

We now consider the dynamics of the network with $N$ subsystems, using the hat notation $\hat{A} := I_N \otimes A$ to represent matrix $A$ repeated $N$ times along the diagonal, where $\otimes$ is the Kronecker product. Using this notation, the dynamics of the network can be represented as follows:

$$\begin{aligned} \dot{X} &= \hat{P}_A X + \hat{P}_B \hat{K}_{D1} \hat{P}_{C1} X + \hat{P}_B \hat{K}_{D2} \hat{P}_{C2} L_n X + \hat{P}_B \hat{K}_C V, \\ \dot{V} &= \hat{K}_A V + \hat{K}_{B1} \hat{P}_{C1} X + \hat{K}_{B2} \hat{P}_{C2} L_n X, \end{aligned} \tag{51}$$

where $n$ is the dimension of $x_i$, and $L_n := L \otimes I_n$. The graph Laplacian $L$ is defined as follows:

$$L_{(ii)} = 1, \tag{52}$$
$$L_{(ij)} = \begin{cases} -\frac{1}{|\mathcal{J}_i|},\ j \in \mathcal{J}_i \\ 0, \qquad j \notin \mathcal{J}_i. \end{cases} \tag{53}$$

**Lemma 2.** *A local controller (50) stabilizes the dynamics in equation (51) if and only if it simultaneously stabilizes the set of $N$ subsystems*

$$\begin{aligned} \dot{X} &= P_A X + P_B u \\ y &= P_{C1} X \\ z &= \lambda_i P_{C2} X \end{aligned} \tag{54}$$

*where $\lambda_i$ are the $N$ eigenvalues of $L$.*

Local stability of our sRNA feedback network can be implied as a direct application of Lemma 2. Particularly, we take

$$\begin{aligned}
&X_i = [m_i, s_i]^T, \quad && V_i = x_i, && P_A = \begin{bmatrix} -G\bar{s}_i - \delta & -G\bar{m}_i \\ -G\bar{s}_i & -G\bar{m}_i - \delta \end{bmatrix}, \\
&P_B = \begin{bmatrix} 0 \\ GT_{si}f_{si} \end{bmatrix}, && P_{C1} = \begin{bmatrix} 1 & 0 \end{bmatrix}^T, && P_{C2} = \begin{bmatrix} -R_i q_{ij} & 0 \end{bmatrix}^T, \\
&K_A = -\gamma, && K_C = 1, && K_{D1} = 0, \\
&K_{D2} = 0, && K_{B1} = R_i[q_{ii} + (n-1)q_{ij}], && K_{B2} = n-1
\end{aligned} \quad . \tag{55}$$

The graph of our network is fully connected, due to the nature of resource competition. Therefore, the graph Laplacian is

$$L = \begin{bmatrix} 1 & -\frac{1}{n-1} & -\frac{1}{n-1} & \cdots & -\frac{1}{n-1} \\ -\frac{1}{n-1} & 1 & -\frac{1}{n-1} & \cdots & -\frac{1}{n-1} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ -\frac{1}{n-1} & -\frac{1}{n-1} & -\frac{1}{n-1} & \cdots & 1 \end{bmatrix}, \tag{56}$$

which has an eigenvalue of 0 and repeated eigenvalues of $1 + \frac{1}{n-1}$. Theorem 2 implies that stability of the network can be inferred by the following lower dimensional systems

$$\dot{X} = (P_A + P_B K_{D1} P_{C1} + \lambda_L K_{D2} P_{C2})X + P_B K_C P_{C2} V, \tag{57}$$

$$\dot{V} = (K_{B1} P_{C1} + \lambda_L K_{B2} P_{C2})X + K_A V, \tag{58}$$

where $\lambda_L$ are the eigenvalues of $L$. Substitute (55) into (57), stability of (57) can be shown by testing the Hurwitz stability of the following two matrices $A^1_{\text{equiv}}$ and $A^2_{\text{equiv}}$, corresponding to $\lambda_L = 0$ and $\lambda_L = 1 + 1/(n-1)$, respectively:

$$A^1_{\text{equiv}} = \begin{bmatrix} -G\bar{s}_i - \delta & -G\bar{m}_i & 0 \\ -G\bar{s}_i & -G\bar{m}_i - \delta & GT_{si}f_{si} \\ R_i[q_{ii} + (n-1)q_{ij}] & 0 & -\gamma \end{bmatrix}. \tag{59}$$

$$A^2_{\text{equiv}} = \begin{bmatrix} -G\bar{s}_i - \delta & -G\bar{m}_i & 0 \\ -G\bar{s}_i & -G\bar{m}_i - \delta & GT_{si}f_{si} \\ R_i(q_{ii} - q_{ij}) & 0 & -\gamma \end{bmatrix}. \tag{60}$$

Both matrices are Hurwitz stable using Routh-Hurwitz condition, and the steady state found in the previous section.

### 4.5 Admissible Reference Input Set

We have picked $\mathcal{V}_i$ defined in (34) 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 $v_i \notin \mathcal{V}_i$, solution of (31) in series expansion of $\epsilon$ becomes

$$\bar{m}_i = \frac{T_i v_i(\gamma k_{si} + R_i) - T_{si} R_i}{T_i v_i \epsilon} + \mathcal{O}(1), \qquad \bar{x}_i = \frac{R_i}{\gamma} + \mathcal{O}(\epsilon). \tag{61}$$

In (61), 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 [24].

Similarly, in a network setting, according to (36), a fundamental trade-off in our design is that increasing the number of nodes $n$ shrinks the size $\mathcal{V}_{\mathcal{N}}$. This is due to the fact that free ribosomes become more scarce as we increase the number of nodes.

According to (34), 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 $\mathrm{p_{si}}$ ($1/k_{si}$).

# 5 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 $\mathrm{x_2}$ [15]. However, in [9], we showed that hidden interactions arising from resource limitations can make the response

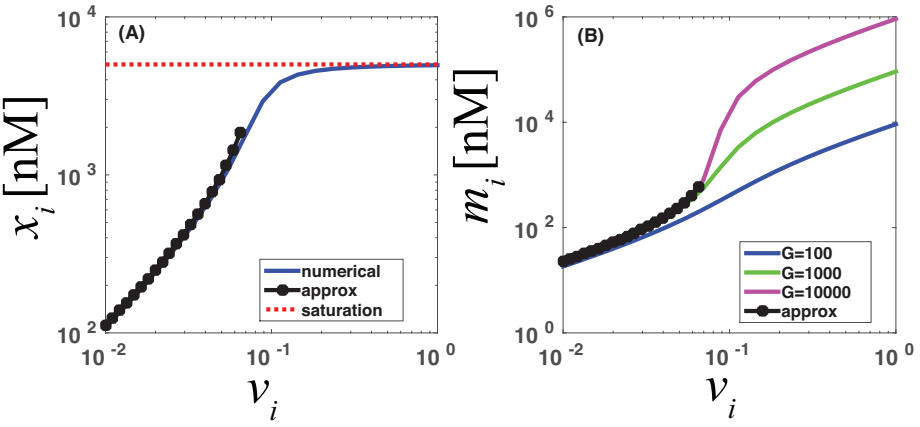


Figure 4: Static i/o characteristics of a node with $\mathcal{V}_i = [0, 0.08]$. Approximate analytical solution within $\mathcal{V}_i$ and numerical solution for $v_i \in [0, 1]$ are given in (A) and (B) for protein and mRNA concentrations, respectively.

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: $\Sigma_{OL}$, $\Sigma^c_{OL}$, $\Sigma_{CL}$ and $\Sigma^c_{CL}$, shown in Fig. 5 (A)-(D), respectively. $\Sigma_{OL}$ is a fictitious activation cascade where nodes are not competing for ribosomes. $\Sigma^c_{OL}$ is the cascade where ribosome are shared among nodes. Dynamics of node $i$ ($i = 1, 2$) in $\Sigma_{OL}$ and $\Sigma^c_{OL}$ are in the form of (1) and (4), respectively. Similarly, $\Sigma_{CL}$ ($\Sigma^c_{CL}$) 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

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

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 $\Sigma^c_{OL}$ becomes significantly different from that of $\Sigma_{OL}$, whose model is usually used to guide design. On the contrary, responses of systems $\Sigma_{CL}$ and $\Sigma^c_{CL}$ are highly similar, implying that with the feedback, ribosome competition plays an almost negligible role in the

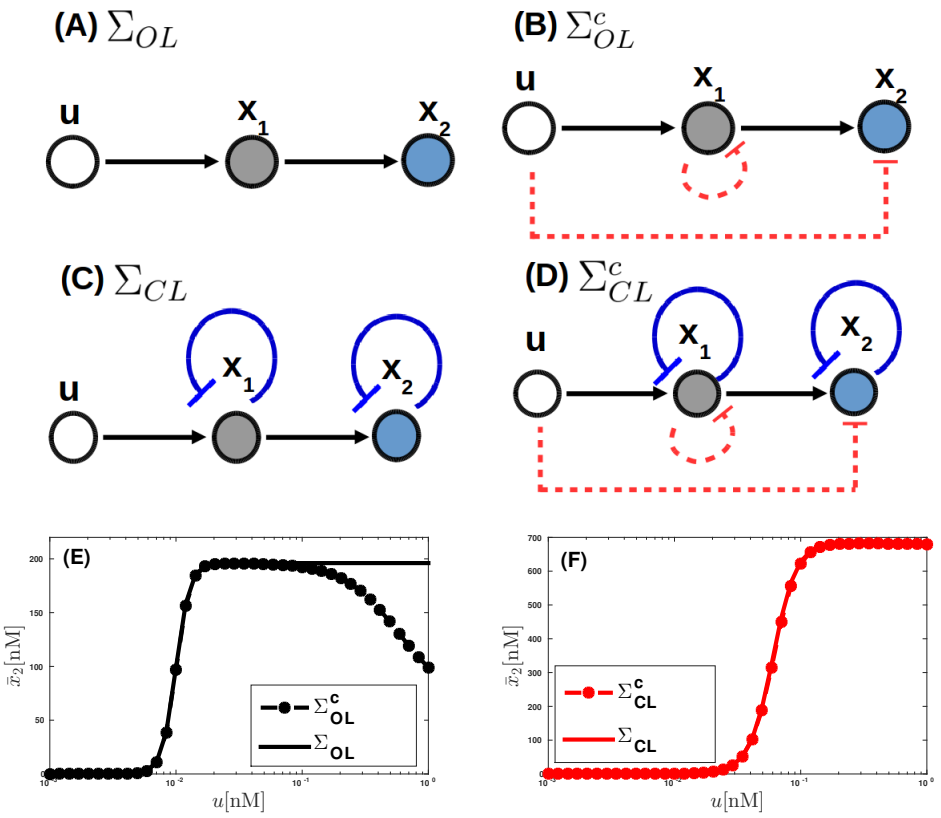


Figure 5: (A)-(D) Interaction graph of the four networks we simulated. Black arrows represent transcriptional regulations, red dashed arrows are the hidden interactions arising from ribosome limitations, and blue arrows represent the feedback loops through sRNA. (E) Static i/o characteristic of systems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

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

# 6 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 $i$ is 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 and $\sigma$-factors is found to play a minor role in gene expression [4], it may become noticeable when $p_i$ increases since increased $p_i$ demands more transcriptional resources, leading to their depletion. Furthermore, this paper only considers static i/o signals, if promoter $\mathrm{p_i}$ is regulated by a time varying input produced by node $j$, then large amount of $\mathrm{p_i}$ may significantly slows down node $j$ dynamics [7]. In future works, we will analyze to what extent these considerations need to be factored into the model.

*Acknowledgement*: We thank Eduardo D. Sontag, Mohammad Naghnaeian, Aaron Dy, and Abdullah Hamadeh for helpful discussions and suggestions.

# References

[1] Y. Qian and D. Del Vecchio, "Mitigation of ribosome competition through distributed sRNA feedback," in *Proceedings of the 55th IEEE*

*Conference on Decision and Control*, Las Vegas, NV, 2016.

[2] S. Cardinale and A. P. Arkin, "Contextualizing context for synthetic biology- identifying causes of failure of synthetic biological systems," *Biotechnol. J.*, vol. 7, pp. 856–866, 2012.

[3] D. Mishra, P. M. Rivera, A. Lin, D. Del Vecchio, and R. Weiss, "A load driver device for engineering modularity in biological networks," *Nat. Biotechnol.*, vol. 32, pp. 1268–1275, 2014.

[4] 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 gengene circuits," *Biophys. J.*, vol. 109, no. 3, pp. 639–646, 2015.

[5] D. Del Vecchio, "Modularity, context-dependence, and insulation in engineered biological circuits," *Trends Biotechnol.*, vol. 33, no. 2, pp. 111–119, 2015.

[6] T. H. Segall-Shapiro, A. J. Meyer, A. D. Ellington, E. D. Sontag, and C. A. Voigt, "A "resource allocator" for transcription based on a highly fragmented T7 RNA polymerase," *Mol. Syst. Biol.*, vol. 10, p. 742, 2014.

[7] K. S. Nilgiriwala, J. I. Jiménez, P. M. Rivera, and D. Del Vecchio, "Synthetic tunable amplifying buffer circuit in *e. coli*," *ACS Synth. Biol.*, vol. 4, no. 5, p. 577584, 2015.

[8] H. Bremer and P. P. Dennis, "Modulation of chemical composition and other parameters of the cell by growth rate," in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, F. C. Neidhardt, Ed. ASM Press, 1996.

[9] Y. Qian and D. Del Vecchio, "Effective interaction graphs arising from resource limitations in gene networks," in *Proceedings of the American Control Conference*, Chicago, IL, 2015, pp. 4417–4423.

[10] W. An and J. W. Chin, "Synthesis of orthogonal transcription-translation networks," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 106, no. 21, pp. 8477–8482, 2009.

[11] D. Del Vecchio, A. J. Dy, and Y. Qian, "Control theory meets synthetic biology," *Journal of The Royal Society Interface*, vol. 13, no. 120, 2016.

[12] A. Hamadeh and D. Del Vecchio, "Mitigation of resource competition in synthetic genetic circuits through feedback regulation," in *Proceedings of the 53rd Conference on Decision and Control*, Los Angeles, CA, 2014, pp. 3829–3834.

[13] Y. Guo, Z.-P. Jiang, and D. J. Hill, "Decentralized robust disturbance attenuation for a class of large-scale nonlinear systems," *Syst. Control. Lett.*, vol. 37, no. 2, pp. 71–85, 1999.

[14] M. Vidyasagar, *Input-output analysis of large-scale interconnected systems*, ser. Lecture Notes in Control and Information Sciences. New York: Springer-Verlag, 1981, vol. 29.

[15] U. Alon, *An Introduction to Systems Biology: Design Principles of Biological Circuits*. Chapman & Hall/CRC Press, 2006.

[16] D. Del Vecchio and R. M. Murray, *Biomolecular Feedback Systems*. Princeton: Princeton University Press, 2014.

[17] M. Fiedler and V. Pták, "On matrices with non-positive off-diagonal elements and positive principal minors," *Czechoslovak Mathematical Journal*, vol. 12, no. 3, pp. 382–400, 1962.

[18] S. G. Krantz and H. R. Parks, *The implicit function theorem: history, theory, and applications*. Birkhäuser, 2002.

[19] 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.*, vol. 31, pp. 170–174, 2013.

[20] V. Sharma, Y. Sakai, K. A. Smythe, and Y. Yokobayashi, "Knockdown of recA gene expression by artificial small RNAs in Escherichia coli," *Biochem. Biophys. Res. Commun.*, vol. 430, no. 1, pp. 256–259, 2013.

[21] R. Hussein and H. N. Lim, "Direct comparison of small RNA and transcription factor signaling," *Nucleic Acids Res.*, vol. 40, no. 15, pp. 7269–7279, 2012.

[22] J. G. Simmonds and J. E. Mann, *A first look at perturbation theory*. New York: Dovers publications, 1997.

[23] J. A. Fax, "Optimal and cooperative control of vehicle formations," Ph.D. dissertation, California Institute of Technology, 2002.

[24] A. Saberi, Z. Lin, and A. R. Teel, "Control of linear systems with saturating actuators," *IEEE Trans. Automat. Contr.*, vol. 41, no. 3, pp. 368–378, 1994.