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Limitations and Trade-offs in Gene Expression due to Competition for Shared Cellular Resources

Andras Gyorgy and Domitilla Del Vecchio

Abstract—Gene circuits share transcriptional and translational resources in the cell. The fact that these common resources are available only in limited amounts leads to unexpected couplings in protein expressions. As a result, our predictive ability of describing the behavior of gene circuits is limited. In this paper, we consider the simultaneous expression of proteins and describe the coupling among protein concentrations due to competition for RNA polymerase and ribosomes. In particular, we identify the limitations and trade-offs in gene expression by characterizing the attainable combinations of protein concentrations. We further present two application examples of our results: we show that even in the absence of regulatory linkages, genes can seemingly behave as repressors, and surprisingly, as activators to each other, purely due to the limited availability of shared cellular resources.

I. INTRODUCTION

One of the major bottlenecks in systems and synthetic biology is context-dependence [1], as it hinders our ability to accurately predict the behavior of complex systems from that of the composing modules [2]. This lack of modularity is particularly important when engineering biological systems using smaller components [3], as it often leads to a lengthy and ad hoc re-design process every time the context changes [4]. Context-dependence arises due to a number of different factors: unknown regulatory linkages; loading effects due to known regulatory interactions between components, a phenomenon known as retroactivity [5], [6]; metabolic burden [7]; cell growth [8]; and competition for shared cellular resources [9].

In this paper, we focus on the effects of competition for transcriptional and translational resources on gene expression. Since these resources are available only in limited amounts, they have to be reallocated every time new genes are introduced into the cell, or when the activity of already present genes changes. Due to the reallocation of these common resources, the over-expression of one gene can affect the growth rate of the cell [8], and it can decrease the expression of other genes [10]. As a result, the expression of different genes become coupled, even in the absence of regulatory linkages among them. To accurately predict and control the behavior of gene circuits, we must determine the distribution of shared resources, that is, the cellular economy of gene expression.

Here, we characterize how the expression of different genes become coupled due to competition for RNA polymerase (RNAP) and ribosomes. We focus on RNAP and ribosomes as their availability is considered to be the major limiting factor in transcription [11] and translation [10], respectively. We prove that due to the limited availability of these cellular resources, the attainable protein concentrations lie within the intersection of simplexes, and we show how these simplexes depend on various biochemical parameters, such as ribosome binding site (RBS) strength and DNA copy number. Building upon our results, we show that even in the absence of regulatory linkages, genes can seemingly repress and activate each other, as a result of the reallocation of limited resources. In particular, we first consider two genes and show that activating one decreases the expression of the other. We further demonstrate that this effect can be interpreted employing isocost lines, a tool originating from microeconomics to describe the affordable combinations of different products having only a limited budget. Second, in the case of three genes, we show that increasing the production of one protein can surprisingly increase the concentration of a second one by reallocating resources from the expression of the third protein.

Our work is closely related to recent efforts investigating the effects of shared cellular resources on gene circuits. In particular, in [12] and [13] the authors detail the effects of the limited availability of ribosomes causing translational crosstalk, a phenomenon verified experimentally in [14] in cell-free systems. A general framework for studying the effects of resource competition is presented in [15] using Metabolic Control Analysis [16], yielding response coefficients that describe local flux sensitivities in a gene network. Our work complements these results as we consider the role of both RNAP and ribosomes to characterize the global limitations and trade-offs in protein expression for $n$ genes. Some of these results have been validated in vivo for two genes [17].

This paper is organized as follows. In Section II, the system of interest is introduced, together with the motivation and research question: Having $n$ genes, what are the limitations and trade-offs in gene expression due to competition for shared cellular resources? In Section III, we determine the attainable protein concentrations and characterize how various biochemical parameters affect the interdependence in gene expression. In Section IV, we present two implications of the limited availability of RNAP and ribosomes on gene expression. Finally, we conclude our results and present future research directions in Section V.
II. SYSTEM MODEL AND PROBLEM FORMULATION

We consider a system in which \( n \) genes are expressed. In particular, each gene is first transcribed by RNAP to mRNA, then mRNA is translated by ribosomes to protein (Fig. 1A).

Furthermore, we focus on the case when the transcription of each gene is regulated by a transcription factor (TF) as follows. In the case of gene \( i \) expressing protein \( p_i \), TF \( u_i \) first binds to the empty promoter \( b_i^e \) forming the promoter complex \( b_i \). Then, the binding of RNAP x to \( b_i \) can form the transcriptionally active promoter complex \( c_i \), resulting in the production of mRNA \( m_i \) at rate \( \gamma_i \) (encompassing the elongation reactions). Finally, mRNA decays at rate \( \delta_i \). Consequently, the reactions describing the transcriptional processes for gene \( i \) are as follows:

\[
\begin{align*}
&u_i + b_i^e \xrightarrow{\zeta_i} b_i, \quad b_i + x \xrightarrow{\kappa^+_i} c_i, \quad c_i \xrightarrow{\gamma_i} b_i + x + m_i, \quad m_i \xrightarrow{\delta_i} 0.
\end{align*}
\]

Translation of \( m_i \) is initialized by the ribosome \( y \) binding to the RBS of the mRNA \( m_i \), forming the translationally active complex \( d_i \). The degradation of mRNA when bound to the ribosome occurs with rate constant \( a_i \delta_i \) where \( 0 < a_i \leq 1 \) (\( a_i \to 0 \) represents the case when the ribosome-bound mRNA is protected from degradation, whereas \( a_i = 1 \) models the scenario when ribosomes provide no protection against degradation, which is considered in what follows). Protein \( p_i \) is degraded at rate \( \lambda_i \), whereas elongation and production are lumped together in one step with effective production rate constant \( \pi_i \). Therefore, the reactions describing the translation processes for gene \( i \) are given by

\[
\begin{align*}
&m_i + y \xrightarrow{\kappa^+_i} d_i, \quad d_i \xrightarrow{\pi_i} m_i + y + p_i, \quad p_i \xrightarrow{\lambda_i} 0.
\end{align*}
\]

Consequently, the corresponding differential equation model for \( i = 1, 2, \ldots, n \) is given by

\[
\begin{align*}
\dot{b}_i &= (\zeta_i u_i b_i^e - \gamma_i c_i) - (\kappa^+_i x b_i - \kappa^-_i c_i) + \gamma_i c_i, \\
\dot{c}_i &= (\kappa^+_i x b_i - \kappa^-_i c_i) - \gamma_i c_i, \\
\dot{m}_i &= \gamma_i c_i - \delta_i m_i - (\kappa^+_i m_i y - \kappa^-_i d_i) + \pi_i d_i, \\
\dot{d}_i &= (\kappa^+_i m_i y - \kappa^-_i d_i) - \pi_i d_i - a_i \delta_i d_i, \\
\dot{p}_i &= \pi_i d_i - \lambda_i p_i.
\end{align*}
\]

(A) **RNAP and Ribosome Demand at the Steady State**

Introduce the dissociation constants \( k_i = (\kappa^-_i + \gamma_i)/\kappa^+_i \) and \( k_i = (\kappa^-_i + \pi_i + \delta_i)/\kappa^+_i \) for \( i = 1, 2, \ldots, n \). Given that protein production and decay are much slower than binding and unbinding reactions [18], we have \( \gamma_i \ll \kappa^-_i \) and \( \pi_i, \delta_i \ll \kappa^-_i \), so that \( k_i \approx \kappa^-_i / \kappa^+_i \). The stronger the binding of RNAP to the promoter, the smaller \( k_i \), and similarly, the stronger the binding of ribosome to the RBS, the smaller \( k_i \). Next, define

\[
\begin{align*}
h_i &= \frac{\gamma_i \eta_i}{\delta_i}, \quad q_i = \frac{\pi_i}{\lambda_i} h_i, \quad \text{and} \quad \mu_i = \frac{\chi_i}{\zeta_i}.
\end{align*}
\]

where \( \mu_i \) is the dissociation constant of the TF \( u_i \) to the promoter of gene \( i \). Furthermore let

\[
\epsilon_i = \frac{n_i}{\eta_i} \left( 1 + \frac{x}{\kappa^-_i} \right), \quad \text{for } i = 1, 2, \ldots, n.
\]

Assuming that DNA concentration is constant [19], we have that \( \eta_i = b_i^e + b_i + c_i \), where \( \eta_i \) is the total concentration of the promoter of gene \( i \). We further have \( \epsilon_i = (b_i + c_i)/\eta_i \), so that \( \epsilon_i \in (0, 1) \) is the fraction of the promoter of gene \( i \) activated by \( u_i \). At the steady state of (1), we have

\[
\begin{align*}
c_i &= \epsilon_i \eta_i \frac{x}{x + \kappa^-_i}, \\
d_i &= \epsilon_i \eta_i \frac{x}{x + \kappa^-_i + y}, \\
\end{align*}
\]

whereas the concentration of protein \( p_i \) is

\[
\begin{align*}
p_i &= \epsilon_i q_i \frac{x}{x + \kappa^-_i + y + \kappa^-_i}, \quad \text{for } i = 1, 2, \ldots, n.
\end{align*}
\]

We call \( c_i \) and \( d_i \) in (4) the **RNAP and ribosome demand** of gene \( i \) at the steady state, respectively, as they represent the concentration of RNAP and ribosomes bound to the promoter and mRNA, respectively. The protein concentrations \( p_i \) for \( i = 1, 2, \ldots, n \) in (5) are implicitly coupled as the free concentration \( x \) and \( y \) of RNAP and ribosomes, respectively, depend on the demand by the genes (Fig. 1B), as we detail in the next section.
B. Modeling the Limited Availability of RNAP & Ribosomes

According to [20], RNAP can be divided into four main categories: immature RNAP, free RNAP, and RNAP bound specifically (and transcribing) and non-specifically to the chromosome. Based on [21], the cell has approximately 1500 RNAP molecules ($x_T = 1500\text{nM}$), among which about 200 are actively transcribing endogenous genes ($x_S = 200\text{nM}$) at low growth rate. Furthermore, [20] suggests that the ratio of immature RNAP is negligible, and the remaining 1300 molecules are partitioned as follows: 100 of them are free ($x = 100\text{nM}$), whereas 1200 are non-specifically bound ($x_N = 1200\text{nM}$). Consequently, the conservation law for RNAP without the additional genes of Fig. 1B is given by

$$x_T = x + x_S + x_N. \quad (6)$$

As for ribosomes, [21] reports that the number of ribosomes per cell is 6800 ($y_T = 6800\text{nM}$), 80% of which is active, that is, approximately 5500 ($y_S = 5500\text{nM}$) at low growth rate. According to [22], the concentration of free ribosomes is approximately 15%, so that the ratio of non-specifically bound ribosomes and immature ribosomes is about 5%. This is negligible compared to the fraction of active and free ribosomes, unlike in the case of RNAP. For simplicity, we treat this last 5% as if they belonged to the pool of free ribosomes (so that we slightly under-estimate the effect of competition for ribosomes). As a result, the conservation law for ribosomes without the additional genes of Fig. 1B is given by

$$y_T = y + y_S. \quad (7)$$

In [17], several exogenous genes are constitutively expressed with the simultaneous activation of an inducible gene. Since there is no appreciable change in growth rate even when using high copy number plasmids, we assume that $x_S$ and $y_S$, representing the resources allocated to the gene expression of the host, are constant (however, if an overexpressed protein is toxic to the cell, the growth rate may decrease [8] and $x_S$ and $y_S$ might also be affected). Therefore, introduce $X = x_T - x_S$ and $Y = y_T - y_S$ denoting the concentration of available RNAP and ribosomes, respectively. To model the non-specific binding of RNAP, introduce the “RNAP sink” described by the reactions

$$\tilde{b} + x \xrightarrow{k^-} \tilde{c},$$

where $\tilde{b} + \tilde{c}$ is the DNA concentration of this “RNAP sink”. At the steady state we obtain that the concentration of RNAP sequestered by this sink is $\tilde{c} = \tilde{b}x / (x + \tilde{k})$ with $\tilde{k} = k^- / k^+$, and since the non-specific binding of RNAP is weak ($\tilde{k} \ll x$ by [23]), we obtain that $x_N = \tilde{c} \approx x \tilde{b} / \tilde{k}$.

The RNAP and ribosome demand of gene $i$ is given by $c_i$ and $d_i$ in (4), respectively. Introduce $\hat{N} = 1 + \frac{\tilde{b}}{\tilde{k}}$, so that upon addition of genes $i (i = 1, 2, \ldots, n)$, (6)–(7) become

$$X = \hat{N}x + \sum_{i=1}^n c_i \eta_i \frac{x}{x + \kappa_i}, \quad (8)$$

$$Y = y + \sum_{i=1}^n c_i \eta_i \frac{y}{x + \kappa_i + \frac{x}{y + \bar{k}_i}}. \quad (9)$$

Let $\epsilon = (\epsilon_1, \epsilon_2, \ldots, \epsilon_n)^T$ and $u = (u_1, u_2, \ldots, u_n)^T$, and write (3) as

$$\epsilon = E(u, x), \quad (10)$$

so that (8) and (9) can be written with (10) as

$$X = F_\epsilon(\epsilon, x) \quad \text{and} \quad Y = G_\epsilon(\epsilon, x, y), \quad (11)$$

respectively, and (5) with $p = (p_1, p_2, \ldots, p_n)^T$ as

$$p = H_\epsilon(\epsilon, x, y). \quad (12)$$

C. Problem Formulation

Define

$$F(u, x) = F_\epsilon(E(u, x), x),$$

$$G(u, x, y) = G_\epsilon(E(u, x), x, y),$$

$$H(u, x, y) = H_\epsilon(E(u, x), x, y), \quad (13)$$

using (10)–(12), and introduce the sets $\mathcal{U} = [0, \infty)^n$ and

$$\mathcal{P} = \{ p \mid p = H(u, x, y), X = F(u, x), Y = G(u, x, y), x \in [0, X], y \in [0, Y], u \in \mathcal{U} \}, \quad (14)$$

so that $\mathcal{P}$ is the set of protein concentrations attainable at the steady state. Therefore, we call $\mathcal{P}$ the realizable region. Here, we seek an explicit characterization of $\mathcal{P}$ solely in terms of $p$, instead of the definition in (14) involving $u$, $x$ and $y$ in the form of implicit constraints. As a result, we can answer the following questions. How does the concentration of protein $p_j$ change upon activation of gene $i$ for $j \neq i$? To what extent is it possible to increase the concentration of $p_i$ without affecting the concentration of $p_j$? In other words, we seek to characterize the limitations and trade-offs in protein production due to the limited availability of RNAP and ribosomes.

III. REALIZABLE REGION

We characterize the realizable region $\mathcal{P}$ through a series of intermediate results. In particular, we first focus on the activation level $c_i$ of gene $i$ for $i = 1, 2, \ldots, n$. Then, we consider a biologically reasonable approximation of (5) and (8)–(9) and characterize the corresponding set $\mathcal{S}$ of attainable protein concentrations. Finally, we prove that $\mathcal{P} \subseteq \mathcal{S}$.

A. Activation Level of Genes

Claim 1. Take $F(u, x)$ and $G(u, x, y)$ defined in (13). For $u \in \mathcal{U}$, there is a unique $(x, y) \in [0, X] \times [0, Y]$ such that $F(u, x) = X$ and $G(u, x, y) = Y$. As a result, there exist functions $f, g : \mathbb{R}^n \to \mathbb{R}$ such that $x = f(u)$ and $y = g(u)$.

Proof: According to (13), we have

$$F(u, x) = \hat{N}x + \sum_{i=1}^n \frac{u_i}{\mu_i} \left(1 + \frac{x}{\kappa_i}\right) \eta_i \frac{x}{x + \kappa_i} - X. \quad (15)$$
Fix $u \in \mathcal{U}$. Since $F(u, x)$ is continuous and $F(u, 0) = 0$ and $F(u, X) > X$ by (15), there is at least one $x \in [0, X]$ such that $F(u, x) = X$, according to the Intermediate Value Theorem [24]. Furthermore, since $F(u, x)$ in (15) is strictly increasing with $x$, there is exactly one $x \in [0, X]$ such that $F(u, x) = X$. Then, let $f : \mathbb{R}^n \rightarrow \mathbb{R}$ be the function that maps $u$ to this unique $x$, that is, $F(u, f(u)) = X$. The proof for $G$ can be constructed similarly.

With $H(u, x, y)$ defined in (13), introduce $A : \mathbb{R}^n \rightarrow \mathbb{R}^n$ as $A(u) = H(u, f(u), g(u))$, so that (14) can be written as

$$
P = \{p \mid p = A(u), u \in \mathcal{U}\}. \quad (16)$$

Claim 2. Let $\mathcal{E} = [0, 1]^n$. Take $F_\epsilon(x, y)$ and $G_\epsilon(x, y)$ from (11). For $\epsilon \in \mathcal{E}$, there is a unique $(x, y) \in [0, X] \times [0, Y]$ such that $F_\epsilon(x, y) = X$ and $G_\epsilon(x, y) = Y$. As a result, there exist functions $f_\epsilon, g_\epsilon : \mathbb{R}^n \rightarrow \mathbb{R}$ such that $x = f_\epsilon(\epsilon)$ and $y = g_\epsilon(\epsilon)$.

Proof: Similar to the proof of Claim 1.

Claim 3. Take $u \in \mathcal{U}$, the functions $f$ and $g$ defined in Claim 1, together with $f_\epsilon$ and $g_\epsilon$ defined in Claim 2. Furthermore, consider $\epsilon = E(u, f(u))$ from (10) with $x = f(u)$. Then $f(u) = f_\epsilon(\epsilon)$ and $g(u) = g_\epsilon(\epsilon)$.

Proof: By Claim 1, we have $X = F(u, f(u))$, yielding $X = F(u, f(u)) = F_\epsilon(E(u, f(u)), f(u))$ from (13), and since $\epsilon = E(u, f(u))$ by assumption, we obtain $X = F_\epsilon(f(u))$. We further have $X = F_\epsilon(f_\epsilon(\epsilon))$ by Claim 2. As a result, we obtain that $x = f(u)$ and $f_\epsilon(\epsilon)$ are both solutions of $X = F_\epsilon(x, y)$, and since it has a unique solution by Claim 2, we conclude that $f(u) = f_\epsilon(x)$. The proof of $g(u) = g_\epsilon(\epsilon)$ can be constructed similarly.

With $H_\epsilon(x, y)$ defined in (12), introduce the function $A_\epsilon : \mathbb{R}^n \rightarrow \mathbb{R}^n$ as $A_\epsilon(\epsilon) = H_\epsilon(f_\epsilon(\epsilon), g_\epsilon(\epsilon))$ and the set

$$
P_\epsilon = \{p \mid p = A_\epsilon(\epsilon), \epsilon \in \mathcal{E}\}. \quad (17)$$

Lemma 1. With $\mathcal{P}$ and $\mathcal{P}_\epsilon$ given in (16) and (17), respectively, we obtain that $\mathcal{P} \supseteq \mathcal{P}_\epsilon$.

Proof: Let $x = f(u)$ and $y = g(u)$ denote the unique solutions of $F(u, x) = X$ and $G(u, y) = Y$ with $(x, y) \in [0, X] \times [0, Y]$ for $u \in \mathcal{U}$, respectively (Claim 1). Referring to (11), let $x = f_\epsilon(\epsilon)$ and $y = g_\epsilon(\epsilon)$ denote the unique solutions of $F_\epsilon(x, y) = X$ and $G_\epsilon(x, y) = Y$ with $(x, y) \in [0, X] \times [0, Y]$ for $\epsilon \in \mathcal{E}$, respectively (Claim 2).

To prove that $\mathcal{P} \supseteq \mathcal{P}_\epsilon$, we show that for every $u \in \mathcal{U}$ there is an $\epsilon \in \mathcal{E}$ such that $A(u) = A_\epsilon(\epsilon)$. First, consider $\epsilon = E(u, f(u))$, and given that $f(u) \in [0, X]$, we conclude that $\epsilon \in [0, 1]$, by (3), so that $\epsilon \in \mathcal{E}$ by the definition of $\mathcal{E}$. Second, considering (13) implies $A(u) = H(u, f(u), g(u)) = H_\epsilon(E(u, f(u)), f(u), g(u))$, so that $\epsilon = E(u, f(u))$ together with $f(u) = f_\epsilon(\epsilon)$ and $g(u) = g_\epsilon(\epsilon)$ from Claim 3 yield $A(u) = H_\epsilon(\epsilon, f_\epsilon(\epsilon), g_\epsilon(\epsilon)) = A_\epsilon(\epsilon)$, where we used the definition of $A_\epsilon(\epsilon)$.

Similarly, to show that $\mathcal{P}_\epsilon \subseteq \mathcal{P}$ it is sufficient to prove that for every $\epsilon \in \mathcal{E}$ there is a $u \in \mathcal{U}$ such that $A(u) = A_\epsilon(\epsilon)$. Since (3) yields $u_\epsilon = \epsilon, \mu, \kappa_i / ((1 - \epsilon_i)(\kappa_i + f_\epsilon(\epsilon)))$, and given that $\epsilon_i \in (0, 1]$ as $\epsilon \in \mathcal{E}$, we obtain $u_\epsilon \in [0, \infty)$, so that $u \in \mathcal{U}$. The part $A(u) = A_\epsilon(\epsilon)$ can be shown as above.

By Lemma 1, in order to find the realizable region $\mathcal{P}$, it is sufficient to consider (5) with (8)–(9) for $\epsilon \in \mathcal{E}$, instead of considering (5) with (8)–(9) and with (3) for $u \in \mathcal{U}$.

B. Approximate Model & Approximate Realizable Region $\mathcal{S}$

As an intermediate step to characterize the realizable region $\mathcal{P}$, consider the (biologically reasonable, see Appendix) approximations $x < k_i$ and $y < k_i$ for $i = 1, 2, \ldots, n$, so that (8)–(9) and (5) take the form

$$
X = N x + \sum_{i=1}^{n} \epsilon_i \eta_i \kappa_i x, \quad Y = y + \sum_{i=1}^{n} h_i \kappa_i x, \quad (18)
$$

$$
p_i = \epsilon_i \eta_i \kappa_i x. \quad (19)
$$

Expressing $x$ and $y$ from (18) as a function of $\epsilon$ and substituting these expressions into (19) yields

$$
p_i = \frac{Q_i \epsilon_i}{N + \sum_{i=1}^{n} H_i \epsilon_i}, \quad \text{for } i = 1, 2, \ldots, n \quad (20)
$$

with

$$
Q_i = \frac{q_i \kappa_i}{\eta_i \kappa_i} X Y \quad \text{and} \quad H_i = \frac{h_i \kappa_i}{\eta_i \kappa_i} X. \quad (21)
$$

Furthermore, let $\hat{A} : \mathbb{R}^n \rightarrow \mathbb{R}^n$ be the function mapping $\epsilon$ to $p$ according to (20), so that $p = \hat{A}(\epsilon)$. Next, define

$$
p_i^{\max} = \frac{Q_i}{N + H_i}, \quad \text{and} \quad p_i^{\infty} = \frac{Q_i}{H_i}. \quad (22)
$$

and introduce the simplexes $S_i$ for $i = 1, 2, \ldots, n$ as

$$
S_i = \left\{ p \mid p \geq 0 \text{ and } \frac{p_i}{p_i^{\max}} + \sum_{j=1}^{n} \frac{p_{ij}}{p_{ij}^{\infty}} < 1 \right\}. \quad (23)
$$

Lemma 2. Let

$$
\mathcal{S} = \{ p \mid p = \hat{A}(\epsilon), \epsilon \in \mathcal{E} \}. \quad (24)
$$

Then, we obtain $\mathcal{S} \subseteq \mathcal{S}_i$ where $\mathcal{S}_i$ is defined in (23).

Proof: We first show $\mathcal{S} \subseteq \bigcap_{i=1}^{n} \mathcal{S}_i$ as follows. Introduce $\mathcal{E}_i = \{ \epsilon \mid \epsilon_i \in [0, 1] \}$ and $\epsilon_j \in [0, \infty)$ for $j \neq i$ and let $P_i = Q_i / (N + H_i \epsilon_i)$, so that we have $P_i < p_i^{\max}$ by (22). Furthermore, $p = \hat{A}(\epsilon)$ satisfies

$$
\frac{p_i}{p_i^{\max}} + \sum_{j=1}^{n} \frac{p_{ij}}{p_{ij}^{\infty}} = 1 \quad (25)
$$

by substitution of (20) into (25). The fact that $\epsilon \in \mathcal{E}$ yields $p \geq 0$ by (19), and $P_i < p_i^{\max}$ with (25) result in

$$
\frac{p_i}{p_i^{\max}} + \sum_{j=1}^{n} \frac{p_{ij}}{p_{ij}^{\infty}} < \frac{P_i}{p_i^{\max}} + \sum_{j=1}^{n} \frac{P_{ij}}{p_{ij}^{\infty}} = 1,
$$

so that $p \in \mathcal{S}_i$ by (23) for $\epsilon \in \mathcal{E}_i$. Combining this together with the fact that $\epsilon \in \mathcal{E} = \bigcap_{i=1}^{n} \mathcal{E}_i$ yields that $\mathcal{S} \subseteq \bigcap_{i=1}^{n} \mathcal{S}_i$. 
Second, we prove $\cap_{i=1}^{n}S_{i} \subseteq S$ by showing that for any $p \in \cap_{i=1}^{n}S_{i}$, there exists an $\epsilon \in \mathcal{E}$ such that $p = \hat{A}(\epsilon)$. To this end, pick $p \in \cap_{i=1}^{n}S_{i}$ and define

$$P_{i} = \frac{p_{i}}{1 - \sum_{j \neq i}^{n} \frac{p_{j}}{p_{j}^{\infty}}}, \quad \epsilon_{i} = \frac{-NP_{i}}{Q_{i} - H_{i}P_{i}}$$

for $i = 1, 2, \ldots, n$. Substituting $\epsilon$ into (20) we obtain that $p = \hat{A}(\epsilon)$. Therefore, it is only left to show that $\epsilon \in \mathcal{E}$. Given that $p \in \cap_{i=1}^{n}S_{i}$, we obtain by (23) that

$$0 \leq p_{i} < p_{i}^{\max} \quad \text{and} \quad 0 \leq \sum_{j \neq i}^{n} \frac{p_{j}}{p_{j}^{\infty}} < 1.$$  

Combining this together with (26) yields that $P_{i} \in [0, p_{i}^{\max})$. Having $P_{i} = 0$ and $P_{i} = p_{i}^{\max}$ result in $\epsilon_{i} = 0$ and $\epsilon_{i} = 1$ in (26) by (22). Furthermore, as $\epsilon_{i}$ in (26) is a strictly increasing function of $P_{i}$ for $P_{i} \in [0, p_{i}^{\max})$, we conclude that $\epsilon_{i} \in [0, 1]$ for $i = 1, 2, \ldots, n$, so that $\epsilon \in \mathcal{E}$.

The realizable region $S$ of protein concentrations when $x \ll \kappa_{i}$ and $y \ll k_{i}$ is given as $S = \cap_{i=1}^{n}S_{i}$ by Lemma 2, where $S_{i}$ is the $n$-dimensional simplex defined by the following $n + 1$ vertices: the origin, $p_{i}^{\max}$ on the $p_{i}$-axis and $p_{j}^{\infty}$ on the $p_{j}$-axis for $j \neq i$ (see Fig. 2). Furthermore, the dependence of $S_{i}$ on the biochemical parameters is given by the expressions of $p_{i}^{\max}$ and $p_{i}^{\infty}$ in (22). For instance, both $p_{i}^{\max}$ and $p_{i}^{\infty}$ increase as $k_{i}$ decreases (stronger RBS for gene $i$), and $p_{i}^{\max}$ increases while $p_{i}^{\infty}$ remains unaffected as $\kappa_{i}$ decreases (stronger promoter for gene $i$).

C. The Realizable Region $\mathcal{P}$ Lies Inside $S$

We next show that even when the approximations $x \ll \kappa_{i}$ and $y \ll k_{i}$ do not hold, the set of attainable protein concentrations given by $\mathcal{P}$ in (16) lie within $S$ in (24).

**Theorem 1.** Considering $\mathcal{P}$ and $S$ defined in (16) and (24), respectively, we obtain that $\mathcal{P} \subseteq S$.

**Proof:** With $\mathcal{P}_{c}$ defined in (17), we have $\mathcal{P}_{c} = \mathcal{P}$ by Lemma 1, so that it is sufficient to show that $\mathcal{P}_{c} \subseteq S$ to prove $\mathcal{P} \subseteq S$. To this end, fix $\epsilon \in \mathcal{E}$ and let $p = A_{\epsilon}(\epsilon)$. If we can show that $p \in S$, for $i = 1, 2, \ldots, n$, it implies that $p \in S$ since $S = \cap_{i=1}^{n}S_{i}$ by Lemma 2, yielding $\mathcal{P}_{c} \subseteq S$.

To show that $p \in S$, for $i = 1, 2, \ldots, n$, define

$$\alpha_{i} = \frac{\kappa_{i}}{x + \kappa_{i}}, \quad \beta_{i} = \frac{1}{y + k_{i}}, \quad \epsilon_{i}' = \alpha_{i}\beta_{i}\epsilon_{i},$$

so that (4)–(5) and (8)–(9) become

$$c_{i} = \frac{\beta_{i}^{2}}{\epsilon_{i}'}, \quad d_{i} = \frac{h_{i}}{\kappa_{i}k_{i}}xy, \quad p_{i} = \frac{q_{i}}{\kappa_{i}k_{i}}xy,$$

$$x = \frac{X}{N + \sum_{i=1}^{n} \frac{h_{i}}{\kappa_{i}k_{i}}}, \quad y = \frac{Y}{1 + \sum_{i=1}^{n} \frac{h_{i}}{k_{i}}}, \quad \epsilon_{i}'.$$

As a result, with $Q_{i}$ from (21) and with $H_{i}' = h_{i}X/(\kappa_{i}k_{i}) + \eta_{i}/(\beta_{i}\kappa_{i})$, we can write $p_{i}$ in (28) with (29) as

$$p_{i} = \frac{Q_{i}\epsilon_{i}'}{N + \sum_{i=1}^{n} H_{i}'\epsilon_{i}'}.$$

Furthermore, we introduce $\hat{p}_{i} = (Q_{i}\epsilon_{i}'/(N + \sum_{i=1}^{n} H_{i}'\epsilon_{i}'))$ and let $\hat{p}_{i} = (\hat{p}_{1}, \hat{p}_{2}, \ldots, \hat{p}_{n})^{T}$. The fact that $\alpha_{i}, \beta_{i} \in (0, 1)$ yields $\epsilon_{i}' \in [0, \epsilon_{i}]$ by (27) and $H_{i} \in (0, H_{i}')$ by (21). Since $\epsilon_{i}' \in [0, \epsilon_{i}]$ implies $\hat{p}_{i} < \hat{p}_{i}$, and similarly, $H_{i} \in (0, H_{i}')$ yields $p_{i} < \hat{p}_{i}$, we obtain

$$0 \leq p_{i} < \hat{p}_{i} < \hat{p}_{i}.$$

Furthemore, from Lemma 2 we have

$$\frac{\hat{p}_{i}}{p_{i}^{\max}} + \sum_{j=1}^{n} \frac{\hat{p}_{j}}{p_{j}^{\infty}} - 1 < 0,$$

and combining (31)–(32) yields

$$\frac{\hat{p}_{i}}{p_{i}^{\max}} + \sum_{j=1}^{n} \frac{\hat{p}_{j}}{p_{j}^{\infty}} < 1.$$
We have $p_i \geq 0$ by (31). Together with (33) this implies that $p \in S_i$ for $i = 1, 2, \ldots, n$ by (23), concluding the proof. ■

Introduce $x_0$ and $y_0$ such that

$$X = F_\epsilon(0, x_0) \quad \text{and} \quad Y = G_\epsilon(0, x_0, y_0),$$

that is, $x_0$ and $y_0$ denote the concentration of free RNAP and ribosomes, respectively, when none of the genes in Fig. 1B are activated ($\epsilon_i = 0$ for $i = 1, 2, \ldots, n$). Next, define

$$B = \{ p \mid p = H(\epsilon, x_0, y_0), \, \epsilon \in \{0, 1\}\},$$

representing the set of attainable protein concentrations without considering competition for RNAP and ribosomes (so that $x = x_0$ and $y = y_0$), see Fig. 3 for a particular example when $n = 2$. Since $S \subset B$ in Fig. 3, if $(p_1, p_2) \in B \setminus S$ then $(p_1, p_2) \notin P$. As a result, without considering competition for RNAP and ribosomes we would erroneously conclude that the protein concentrations $(p_1, p_2)$ are attainable.

IV. PRACTICAL IMPLICATIONS OF THE LIMITED AVAILABILITY OF RNAP AND RIBOSOMES

Here, we present two examples of how protein concentrations become coupled due to competition for shared resources, and we detail the resulting limitations and trade-offs building upon our results from the previous section. In particular, we show that proteins can seemingly behave both as repressors and as activators, purely as an effect of the limited availability of RNAP and ribosomes.

A. Lateral Inhibition with Two Genes

Consider two genes, and for simplicity, focus on the biologically reasonable approximations (see Appendix) $x \ll \kappa_i$ and $y \ll k_i$ for $i = 1, 2$, so that the realizable set of protein concentrations $P$ is equal to $S$ by Lemma 2. We investigate the limitations and trade-offs in protein expression, i.e., how $p_2$ changes as the expression of $p_1$ increases.

Fix the activation $\epsilon_2$ of gene 2 ($\epsilon_2 = \epsilon_2^*$), while increasing the activation $\epsilon_1$ of gene 1. Without considering competition for the shared resources, the set of attainable protein concentrations $B$ given in (35), see Fig. 4A. In this case, the concentration $x$ and $y$ of free RNAP and ribosomes, respectively, are independent of the value of $\epsilon_1$ and $\epsilon_2$ ($x = x_0$ and $y = y_0$, see (34)). As a result, $p_1$ increases while $p_2$ remains unaffected when increasing the activation $\epsilon_1$ of gene 1 by (16). That is, the attainable pairs $(p_1, p_2)$ lie along a horizontal line (Fig. 4A). However, due to the limited availability of resources, $p_2$ decreases by (20) as the activation $\epsilon_1$ of gene 1 increases, since some of the resources have to be reallocated from gene 2 to gene 1. Referring to (20), the pair $(p_1, p_2)$ satisfies the linear constraint

$$\left( A_1 + \frac{B_1}{X} \right) p_1 + \left( A_2 + \frac{B_2 + C/\epsilon_2^*}{X} \right) p_2 = Y \quad (36)$$

with $A_1 = \lambda_1/\pi_1$, $B_1 = \delta_1k_1A_1/\gamma_1$ and $C = B_2\kappa_2\kappa_3N/\eta_2$. Since $\frac{\partial p_1}{\partial \epsilon_1} > 0$ and $\frac{\partial p_2}{\partial \epsilon_1} < 0$ from (20), the pair $(p_1, p_2)$ moves along the line (36) from left to right by increasing $\epsilon_1$ (red line in Fig. 4B). The top boundary of $S$ in Fig. 4B is given by (36) when $\epsilon_2 = 1$ (gene 2 is fully activated), and similarly, the right boundary of $S$ corresponds to the case when $\epsilon_1 = 1$ (gene 1 is fully activated).

The linear constraint in (36) can be interpreted as an isocost line [25], a concept introduced in microeconomics to describe what combinations of two products can be purchased with a limited budget. Here, the products are $p_1$ and $p_2$ with prices $\alpha$ and $\beta$, respectively, whereas the budget $Y$ is the concentration of available ribosomes. Increasing the availability of resources (RNAP and ribosomes) allows for purchasing more products: the value of $p_1$ and $p_2$ can be increased simultaneously. In particular, increasing the concentration $X$ of available RNAP molecules decreases the prices $\alpha$ and $\beta$, whereas increasing the concentration of available ribosomes $Y$ increases the budget. Furthermore, the isocost line describes how changing the biochemical parameters of a gene affects the extent of competition due to the limited availability of resources. In particular, the slope of the isocost line is $-\alpha/\beta$ by (36), so that producing an extra $p_1$ decreases the concentration of $p_2$ by $\alpha/\beta$. The “more expensive” $p_1$ compared to $p_2$ (the greater $\alpha/\beta$), the more $p_2$ have to be sacrificed in order to purchase an additional unit of $p_1$. For instance, $\alpha$ decreases with the dissociation constant $k_1$, so that stronger RBS for gene 1 makes the isocost line flatter by (36), verified in vivo in [17].

Without competition for shared resources, increasing the activation $\epsilon_1$ of gene 1 does not affect $p_2$ (Fig. 4A). However, due to the limited availability of RNAP and ribosomes, the
expression of gene 2 decreases when activating \( p_1 \) according to (36), as \( \frac{\partial p_2}{\partial \epsilon_1} < 0 < \frac{\partial p_1}{\partial \epsilon_1} \) by (20). As a result, to increase \( p_2 \) and keep \( p_1 \) unaffected (phase #1 in Fig. 4C), we must increase the activation of both genes: by (20), increasing \( \epsilon_1 \) yields greater expression of \( p_1 \), and the resulting decrease in \( p_2 \) can be compensated by increasing \( \epsilon_2 \). However, when gene 2 becomes fully activated (\( \epsilon_2 \rightarrow 1 \)), compensation is no longer possible, so that further increasing the activation \( \epsilon_1 \) of gene 1 decreases \( p_1 \) (phase #2 in Fig. 4C). Finally, when gene 1 becomes fully activated (\( \epsilon_1 \rightarrow 1 \)), the concentration of \( p_1 \) cannot be further increased while keeping gene 2 fully activated. Instead, we must decrease \( \epsilon_2 \) so that resources can be reallocated to the expression of \( p_1 \) (phase #3 in Fig. 4C).

**B. Lateral Activation with Three Genes**

Since genes compete for the shared resources, one would expect that activating one gene decreases the expression of a different one. Here, we show that this is not always the case, and that counter-intuitively, unconnected genes can behave as activators to each other due to the limited availability of resources. To this end, consider \( \kappa_2 \ll x \ll \kappa_1, \kappa_3 \) and \( y \ll \kappa_1, \kappa_2, \kappa_3 \), so that the promoter of gene 2 is saturated with RNAP. Furthermore, we focus on the case when genes 2 and 3 are fully activated (\( \epsilon_2, \epsilon_3 \rightarrow 1 \)). Considering (8)–(9) when \( \kappa_2 \ll x \ll \kappa_1, \kappa_3 \) and \( y \ll \kappa_1, \kappa_2, \kappa_3 \), and taking the derivative of \( p_2 \) in (5) with respect to \( \epsilon_1 \) yields

\[
\text{sgn} \left( \frac{dp_2}{d\epsilon_1} \right) = \text{sgn} \left( \frac{\eta_1 h_3}{\kappa_1 \kappa_3} - \left( \frac{\eta_3}{\kappa_3} \right) \frac{h_1}{\kappa_1 \kappa_1} \right).
\]

As a result, \( \frac{dp_2}{dp_1} > 0 \) if, for instance, \( k_1 \) is sufficiently large (the RBS of the mRNA encoding \( p_1 \) is sufficiently weak).

In this case, activating gene 1 increases the concentration of \( p_2 \), despite gene 2 being already fully activated (\( \epsilon_2 \rightarrow 1 \)). This seemingly counter-intuitive result can be explained as follows. Activating gene 1 results in an increased demand for RNAP, consequently, less RNAP is available for the other two genes by (8). However, since the promoter of gene 2 is particularly strong (\( \kappa_2 \ll x \)), it stays saturated with RNAP, that is, the concentration of mRNA encoding \( p_2 \) remains about the same. By contrast, the promoter of gene 3 is weak (\( \kappa_3 \gg x \)), so that less mRNA encoding \( p_3 \) is produced, i.e., the demand for ribosomes by gene 3 decreases by (4). In the meantime, if the RBS of the mRNA encoding \( p_3 \) is weak (\( k_1 \) is sufficiently large), the demand for ribosomes by gene 1 is negligible by (4). Consequently, the ribosomes not used by gene 3 can be used by gene 2. In summary, the key features for obtaining the phenomenon are weak RBS in gene 1, strong promoter in gene 2 and weak promoter in gene 3. This lateral activation phenomenon is demonstrated in Fig. 5 (without using the approximations \( \kappa_2 \ll x \ll \kappa_1, \kappa_3 \) and \( y \ll \kappa_1, \kappa_2, \kappa_3 \)).

**V. DISCUSSION**

In this paper, we have characterized how the concentration of proteins become coupled due to competition for shared cellular resources, even in the absence of regulatory linkages. In particular, we showed that the realizable region \( P \) of protein concentrations lies within \( S \), which is a biologically reasonable outer approximation (see Appendix) easily calculated from (23)–(24). Building on this result, we determined the limitations and trade-offs in gene expression due to the limited availability of RNAP and ribosomes, and how they depend on various biochemical parameters. Furthermore, we demonstrated that the coupling in protein concentrations due to competition for RNAP and ribosomes can be interpreted using isostoc lines, a concept introduced in microeconomics to describe the attainable combinations of products having a limited budget. Finally, we presented the counter-intuitive phenomenon of lateral activation, in which inducing the expression of one protein can increase the production of a second one, by reallocating resources from a third, serving as a buffer for shared resources.

A natural extension of the results presented here is considering regulatory linkages among genes, thus enabling the description of how the limited availability of resources couples the expression of different proteins in arbitrary gene networks. We are further working on the extension of the presented framework to describe the dynamic behavior of gene circuits. A particularly interesting research direction is combining the results of [6], describing the effects of sharing transcription factors on the dynamics of modules, and the result presented here, characterizing the stationary effects of the limited availability of transcriptional and translational machinery. As a result, one could account for two of the major causes of context-dependence in systems and synthetic biology in a unified mathematical framework, allowing a more detailed understanding of natural systems, and the design of multi-module systems with predictable behavior.
**TABLE I**

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<td>hr$^{-1}$</td>
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VI. APPENDIX

The dissociation constant of the T7 RNA polymerase to its promoter is approximately 200 nM [26], and since this binding is considerably stronger than that of bacterial RNA polymerase, we conclude that $\kappa_i \gg 200$ nM, suggesting $x \ll \kappa_i$ as $x \approx 100$ nM.

According to [27], as many as 20 RNA polymerase molecules can simultaneously transcribe a gene. Instead of having one gene recruiting a maximum of $\omega$ RNA polymerases, we consider $\omega$ genes allowed to recruit at most one RNA polymerase at a time, as if the DNA copy number was $\omega \eta$ instead of $\eta$ (we use a low-range value of $\omega = 5$ denoting the number of RNA polymerase molecules simultaneously transcribing a gene). Similarly, according to [21], several ribosomes can simultaneously translate each mRNA, up to a few dozen depending on the growth rate. Instead of having $m$ mRNA molecules, each of which can be bound to $\phi$ ribosomes at any given time, we consider $\phi m$ mRNA molecules allowed to be bound to a single ribosome. This can be achieved by considering the effective production rate $\phi \gamma$ instead of $\gamma$ (we use a low-range value of $\phi = 5$ denoting the number of translations per mRNA).

Considering the typical value of biochemical parameters given in Tab. I with $k = 1000$ nM, we obtain $p \approx 10 \mu$M, which is comparable to the concentration of one of the most abundant proteins in *E. coli* [28]. Therefore, we approximate the binding of ribosomes to the RBS of the mRNA to be significantly weaker than 1000 nM, so that $\kappa_i \gg 1000$ nM. Combining this with the fact that the concentration of free ribosomes is $y = 1300$ nM suggests that $y \ll \kappa_i$.

REFERENCES