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Mitigation of resource competition in synthetic genetic circuits through feedback regulation

Abdullah Hamadeh*, Domitilla Del Vecchio*

Abstract—A recurring challenge in the design of genetic circuits is context dependence, the fact that the behavior of a functional module is influenced by the state of the wider cellular milieu with which it interacts. One key player in context dependence is the scarcity of shared cellular resources, especially those required for transcription and translation during gene expression. Because of competition for these limited resources, the behavior of modules becomes coupled in subtle ways, preventing circuits from working as expected. This paper proposes a classical feedback control approach to mitigate the steady state effects of the competition for resources necessary for gene expression. In particular, we analyze and compare the ability of several inhibitory feedback regulation architectures to reduce the interdependence between different gene expression processes due to resource limitations.

I. INTRODUCTION

The emergence of the field of synthetic biology [1] has given rise to novel challenges in the design of feedback systems. A particular feature of this design environment is context dependence [2]; the fact that functional biomolecular modules behave very differently in different settings, their behavior being largely dependent on what other modules they ‘connect’ with via shared pools of reagents. This property can make the operation of synthetic circuits unpredictable, and therefore poses a significant design challenge.

The nature of this design challenge has direct analogues in modern control design, one example being the dependence of the behavior of electrical loads on power demand in a circuit. There is therefore ample scope for the adaptation of fundamental control theoretic ideas to design questions that arise in synthetic biology. Questions of how feedback regulation may be employed by biochemical systems to reduce the effect of parametric uncertainties has received much attention since the 1970s, with seminal works such as [3]. Recent work has also focused on negative feedback motifs that attenuate the influence of intrinsic and extrinsic sources of noise on gene expression [4], [5]. The question of context dependence has been addressed via notions of retroactivity [6] and methods to construct ‘insulation devices’ that shield genetic and signal transduction modules from their environment so that their input-output properties are preserved regardless of upstream or downstream processes with which they share reagents and products.

In this paper, we are concerned with the problem of context dependence arising from the competition for cellular resources during gene expression [7], [8], [9], [10]. In this process, RNA polymerase (RNAP) transcribes genes into mRNA, and ribosomes translate mRNA into protein. Ribosomes and RNAP exist in limited numbers throughout the lifetime of the cell [11], [12]. As demonstrated in [13], among the various gene expression processes taking place simultaneously throughout the cell, they therefore behave as scarce resources, placing limits on the achievable space of protein product outputs.

Our aim in this work is to design synthetic genetic circuits in which impact of the competition for resources at steady state is mitigated. To achieve this, we model the dynamics of the simultaneous expression of two genes. The two processes are coupled through their use of the shared RNAP and ribosomes, and the total concentration of each resource is assumed to be fixed. Regarding the uptake of resources by either one of the two processes as being an external signal acting upon the other, we will investigate the effectiveness of negative feedback to de-sensitize the circuits to this disturbance. We propose to implement three different negative (inhibitory) feedback loops, illustrated in Figure 1: inhibition of a gene’s transcription by its own protein product (A in Figure 1), inhibition of a gene’s transcription by an RNA species that is co-expressed with its mRNA transcript (B in Figure 1), and inhibition of mRNA translation by its protein product (C in Figure 1).

This paper is organized as follows, in Section II we present the gene expression model. In Section III we model the proposed feedback mechanisms that will be used to mitigate the effects of resource competition. In Section IV we present the framework through which we will make a fair comparison between the different feedback architectures and we derive the limits on the lowest achievable competition for each of the four feedback structures. Finally, in Sections V and VI we illustrate the effectiveness of our proposed designs, concluding with a discussion of the relative qualities of the different feedback structures and avenues for future work.

Fig. 1. The feedback mechanisms considered.

II. PROBLEM FORMULATION

A. Gene expression model

Our system of interest consists of two gene circuits, indexed $i = 1, 2$. The genes on each circuit are respectively expressed upon activation by transcription factors $u_1$, $u_2$. The transcription factors bind to the promoter region of the DNA $p_i^*$ to form a complex $p_i$. This reaction, denoted R-1 in Table I obeys the differential equation

$$\dot{p}_i = \chi_i p_i - \zeta_i u_i p_i^*$$

(1)
TABLE I
REACTIONS OF THE GENE EXPRESSSION MODEL.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-1</td>
<td>$u_i + p_i^* \rightarrow \frac{\gamma_i}{x_i} p_i$</td>
</tr>
<tr>
<td>R-2</td>
<td>$p_i + x \rightarrow \frac{\gamma_i}{x_i} c_i$</td>
</tr>
<tr>
<td>R-3</td>
<td>$c_i \rightarrow m_i + n_i + x$</td>
</tr>
<tr>
<td>R-4</td>
<td>$m_i \rightarrow \varnothing$</td>
</tr>
<tr>
<td>R-5</td>
<td>$n_i \rightarrow \varnothing$</td>
</tr>
<tr>
<td>R-6</td>
<td>$m_i + y \rightarrow \frac{\gamma_i}{x_i} d_i$</td>
</tr>
<tr>
<td>R-7</td>
<td>$d_i \rightarrow y$</td>
</tr>
<tr>
<td>R-8</td>
<td>$d_i \rightarrow \pi P_i + m_i + y$</td>
</tr>
<tr>
<td>R-9</td>
<td>$P_i \rightarrow \varnothing$</td>
</tr>
<tr>
<td>R-10</td>
<td>$\pi P_i + p_i + e_i = \eta_i$</td>
</tr>
<tr>
<td>R-11</td>
<td>$x + c_i + b_i = x_T$</td>
</tr>
<tr>
<td>R-12</td>
<td>$y + d_i + d_2 = y_T$</td>
</tr>
</tbody>
</table>

The complex $p_i$ will draw free RNA polymerase $x$ to form a complex $c_i$, initiating the transcription of gene $i$, producing the mRNA $m_i$ and the microRNA $n_i$ at a rate $\gamma_i$. This reaction, R-2 in Table I, is modeled as

$$\dot{c}_i = \alpha_i p_i x - \beta_i c_i + \gamma_i c_i$$

and relation R-10 represents the conservation of DNA. The microRNA $n_i$ will be used as a transcriptional regulator in Section III. It is assumed to decay at a rate $\theta_i$ (reaction R-5) and its dynamics obey

$$\dot{n}_i = \gamma_i c_i - \theta_i n_i$$

The mRNA $m_i$ decays at a rate $\delta_i$. Free ribosomes $y$ bind to the ribosomal binding site (RBS) on the mRNA molecule to form a complex $d_i$. The production and decay of mRNA $m_i$ and its binding reaction with ribosomes are given by reactions R-3, R-4 and R-6 in Table I. The mRNA-ribosome complex decays at a rate $a_i\delta_i$ (reaction R-7), where $a_i \in [0,1]$, modeling the ability of ribosomes to protect mRNA from decay. The complex also translates the mRNA to produce the protein $P_i$ at a rate $\pi_i$ (reaction R-8). The protein $P_i$ decays at a rate $\lambda_i$ (reaction R-9). The dynamics of the concentrations of the mRNA $m_i$, the mRNA-ribosome complex $d_i$, and the protein $P_i$ are described by the ordinary differential equations

$$\dot{m}_i = \gamma_i c_i - \delta_i m_i - \rho_i m_i y + \sigma_i d_i + \pi_i d_i$$

$$\dot{d}_i = \rho_i m_i y - \sigma_i d_i - \pi_i d_i - a_i \delta_i d_i$$

$$\dot{p}_i = \pi_i d_i - \lambda_i P_i$$

The binding of RNAP to the promoter yields

$$\frac{p_i x}{c_i} = \frac{\beta_i + \gamma_i}{\alpha_i} = K_i$$

where $K_i$ is the dissociation constant between RNAP and the promoter $i$. Furthermore, from (5), at steady state we have

$$\frac{m_i y}{d_i} = \frac{\sigma_i + \pi_i + a_i \delta_i}{\rho_i} = Q_i$$

where $Q_i$ is the ribosome-mRNA dissociation constant.

B. Signaling cross-talk due to resource competition

The two gene circuits described in Section II-A share the same limited pool of transcriptional and translational resources, respectively, RNAP and ribosomes. The total amount of each resource is given in R-11, R-12 in Table I. The sharing of these resources indirectly couples the dynamics of the two circuits. As an illustration, Figure 2 shows that when the transcription factor $u_i$ binds the promoter $p_i^*$, there is a change in the level of both $P_i$ and $P_2$, even though there is no change in the activation level of the promoter responsible for $P_2$. This effect is due to expression of gene 1 sequestering RNAP and ribosomes from promoter $p_2$ and mRNA $n_2$, which are responsible for producing protein $P_2$. Our aim is therefore to design a regulatory scheme for these circuits that mitigates this effect.

III. MITIGATION OF RESOURCE COMPETITION THROUGH FEEDBACK

We propose to employ negative feedback to de-sensitize the steady state of the circuits to the disturbances arising from changes in the availability of resources by lowering the degree of competition, $|dP_2/dP_1|$. We will analyze the ability of four different inhibitory feedback control schemes to achieve this aim. The four regulation schemes are:

A: Repression of transcription of gene $i$ via protein $P_i$, modeled by reaction F-1 in Table II, denoted by $\tau_{pi}$, in Figure 3. Protein $P_i$ will bind to the free promoter $p_i$ to produce the complex $I_{pi}$, the concentration of which obeys

$$\dot{I}_{pi} = \phi_{pi} p_i P_i - \phi_{pi} I_{pi}$$

B: Repression of transcription of gene $i$ via microRNA $n_i$, as proposed in [14], modeled by reaction F-2 in Table II, denoted by $\tau_{Bn}$, in Figure 3. This feedback loop consists of microRNA $n_i$ inhibiting its own transcription by binding to promoter $p_i$ to form a complex $I_{ni}$, which obeys the ODE

$$\dot{I}_{ni} = \phi_{ni} p_i n_i - \phi_{ni} I_{ni}$$

C: Repression of translation of mRNA $m_i$ via protein $P_i$, as proposed in [15], modeled by reaction F-3 in Table II, denoted by $\tau_{Cm}$, in Figure 3. Protein $P_i$ binds the mRNA $m_i$, forming the complex $I_{mi}$ and preventing translation. When the mRNA in the complex $I_{mi}$ decays at the rate $\phi_{mi}$, the protein $P_i$ is released. The complex $I_{mi}$ satisfies

$$\dot{I}_{mi} = \phi_{mi} m_i P_i - \phi_{mi} I_{mi} - \phi_{mi} I_{mi}$$
BC: This feedback configuration simultaneously combines the microRNA regulation of transcription and the protein regulation of translation in cases B and C above.

These regulatory reactions modify (1)-(6) so that the dynamics of the two circuits satisfy

\[ p_i^* = \chi_i p_i - \zeta_i u_i p_i \]

\[ c_i = \alpha_i p_i x - \beta_i c_i - \gamma_i c_i \]

\[ n_i = \gamma_i c_i - \theta_i p_i + \phi_i n_i + \phi_d I_i \]

\[ m_i = \gamma_i c_i - \delta_i m_i - \rho_i m_i y + \sigma_i d_i + \pi_i d_i - \phi_i m_i I_m - \phi_d m_i P_i \]

\[ d_i = \rho_i m_i y - \sigma_i d_i - \pi_i d_i - a_i \delta_i d_i \]

\[ P_i = \pi_i d_i - \lambda_i P_i - \phi_1 p_i P_i + \phi_2 I_i, P_i - \phi_3 m_i P_i + \phi_6 I_i_1 + \phi_7 I_i_2 \]

with the feedback regulation (9), subject to the new DNA conservation relation

\[ p_i^* + p_i + I_{n_i} + I_{p_i} + c_i = \eta_i \]

as well as the same RNAP and ribosome conservation relations as in the un-regulated circuit

\[ x + c_1 + c_2 = x_T \quad y + d_1 + d_2 = y_T \]

where \( F_i := \frac{\lambda_i}{\xi_i} \), \( D_i := \frac{\lambda_i}{\xi_i} \), \( B_i \) and \( u_i \) are determined by

\[ a_i(u_i) := \left[ \frac{\lambda_i}{\xi_i} x_T + B_i(u_i) \right] \delta_i Q_i \]

\[ b_i(u_i) := \left[ \tau_{C_i} B_i(u_i) + \tau_{A_i} D_i \right] \delta_i Q_i \]

\[ \tilde{c}_i := \tau_{A_i} \tau_{C_i} D_i Q_i \]

\[ \tilde{G}_{i,j} := \left[ \frac{\tau_{B_i} \tau_{A_i} \tau_{C_i} Q_i^2}{|y_T - (A_i/x_i) P_i - (B_i/y_i)|^2} \right] \]

where we define the feedback strengths, respectively, resulting from the reactions (9a), (9b), (9c) as \( \tau_{A_i} := \frac{\phi_1}{\phi_2} \), \( \tau_{B_i} := \frac{\phi_3}{\phi_6 + \phi_7} \). The proof of this proposition comes simply from combining the steady state relations of (9) and (10) with the constraints (11), applying the approximation of Assumption 1 and eliminating \( u_1 \) and all states except \( P_1 \) and \( P_2 \).

IV. ANALYSIS OF FEEDBACK DESIGNS

Equation (12) in Proposition 1 presents the relationship between the proteins \( P_1 \) and \( P_2 \) that holds for a given concentration of the transcription factor input \( u_2 \). As \( u_1 \) is varied, the steady state will move along (12). This relation therefore describes the degree of competition for resources between the processes expressing \( P_1 \) and \( P_2 \). Note that in the case of little competition we can expect \( |dP_2/dP_1| \) to be small. Equation (12) is also a function of the strengths of the three feedbacks, \( \tau_{A_1}, \tau_{B_1}, \tau_{C_1} \), defined in Proposition 1. Equation (12) can therefore be used to analyze and compare the ability of the three feedbacks to reduce the effects of resource competition by evaluating the impact of strengthening the three feedbacks on \( |dP_2/dP_1| \).

When no feedback regulates the circuits \( \tau_{A_1} = \tau_{B_1} = \tau_{C_1} = 0 \) and the concentration of transcription factor \( u_2 \) is held fixed, it can be seen from (12) that \( P_1 \) and \( P_2 \) satisfy

\[ x_T y_T - \tilde{a}_2(u_2) P_2 = \tilde{c}_1 P_1 \]

as the concentration of transcription factor \( u_1 \) is varied. From (14), we can quantify the competition for resources as \( |dP_2/dP_1| = \tilde{c}_1/\tilde{a}_2 \).

Setting \( \tau_{A_1} > 0 \) and \( \tau_{B_1} = \tau_{C_1} = 0 \) (feedback scheme A), the dependence of \( P_1 \) on \( P_2 \) is transformed into the parabolic relation \( x_T y_T - \tilde{a}_2(u_2) P_2 - \tilde{b}_2 P_2^2 = \tilde{c}_1 P_1 \), with only the \( \tilde{a}_2 \) coefficient dependent on \( u_2 \). Figure 4 shows the change of the \( P_1 - P_2 \) relationship from linear (under no feedback) to parabolic (under the feedback scheme A). In this Figure, the concentration of transcription factor input \( u_2 \) is fixed at 1 \( \mu \)M while that of transcription factor input \( u_1 \) is varied from \( u_1 = 0 \) \( \mu \)M to \( u_1 = 1 \) \( \mu \)M. Note that the feedback A reduces \( |dP_2/dP_1| \), thus diminishing the impact on \( P_2 \) of an increase in the production of \( P_1 \).

To simplify our analysis of the feedback regulated circuits, we make the following assumption:

Assumption 2: The two circuits indexed \( i = 1, 2 \), modeled by the ODEs (10) and the regulatory feedback ODEs (9) have identical parameters.

Although Assumption 2 is not realistic, it allows us to demonstrate the feasibility of the proposed feedback regulation approach to reduce the effects of resource competition. Using this assumption, we will henceforth drop the \( i \) notation except where a subscript is required to denote species belonging to the different circuits.

\[ F_1 := \frac{\lambda_1}{\xi_1}, D_1 := \frac{\lambda_1}{\xi_1} \]

\[ a_i(u_i) := \left[ \frac{\lambda_i}{\xi_i} x_T + B_i(u_i) \right] \delta_i Q_i \]

\[ b_i(u_i) := \left[ \tau_{C_i} B_i(u_i) + \tau_{A_i} D_i \right] \delta_i Q_i \]

\[ \tilde{c}_i := \tau_{A_i} \tau_{C_i} D_i Q_i \]

\[ \tilde{G}_{i,j} := \left[ \frac{\tau_{B_i} \tau_{A_i} \tau_{C_i} Q_i^2}{|y_T - (A_i/x_i) P_i - (B_i/y_i)|^2} \right] \]
A. A mathematically controlled comparison

Note that the measure of competition, $|dP_2/dP_1|$ is a local measure, depending on the particular point along the curve (12) at which it is taken. If we set $u_2 = \bar{u}$, where $\bar{u}$ is a fixed level of input transcription factor, then the competition measure $|dP_2/dP_1|$ is maximized over $u_1 \in [0, \bar{u}]$ at $u_1 = \bar{u}$. This is due to the fact that, from (12), $\frac{d^2P_2}{du^2} < 0$ and $\frac{d^2P_1}{du^2} > 0$ and that increasing $u_1$ necessarily increases $P_1$. Also note that since the two circuits are identical and assumption, the point along (12) where $u_1 = u_2 = \bar{u}$, where competition is maximized, is such that $P_1 = P_2$.

The addition of feedback regulation results in a reduction of the circuits’ steady states. To provide a fair comparison between the different regulation schemes we consider, each circuit will be analyzed at the same steady state $P$. which we define to be the steady state that is exhibited by the circuit with no feedback ($\tau_A = \tau_B = \tau_C = 0$), at a fixed RBS dissociation constant $Q = \bar{Q}$, under large transcription factor inputs, $u_1 = u_2 = \bar{u} \rightarrow \infty$. From (14), this steady state is given by

$$P := \frac{x_Ty_T}{2 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 2)}$$

Restoration of the steady state protein outputs of the regulated circuits to (15) will be achieved by sufficiently increasing the ribosomal affinity for mRNA; for each regulated circuit, the dissociation constant $Q$ will be reduced from $Q = \bar{Q}$ to a level which is such that when the circuits are subject to inputs $u_1 = u_2 = \bar{u}$ we have $\lim_{\bar{u} \rightarrow \infty} P_2 = \bar{P}$. The measure of competition will then be $\lim_{\bar{u} \rightarrow \infty} \frac{dP_2}{dP_1}$. In the case where there is no feedback and $Q = \bar{Q}$, we have, from (14),

$$\lim_{\bar{u} \rightarrow \infty} \frac{dP_2}{dP_1} = \frac{1 + \frac{\bar{Q}}{\bar{u}}}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$$

To assess the effectiveness of the different feedback schemes in reducing competition, we will demonstrate that $|dP_2/dP_1|$ is a decreasing function of the feedback gains $\tau_A$, $\tau_B$, $\tau_C$ and we will evaluate this measure as the feedback gains are made large.

The following claim gives the RBS dissociation constants $Q$ that achieve the steady state $P$ for each of the four feedback regulation schemes.

**Claim 1:** Under Assumption 1, constraints (11) and input $u_1 = u_2 = \bar{u}$ the steady state of system (10) satisfies $\lim_{\bar{u} \rightarrow \infty} P_1 = \lim_{\bar{u} \rightarrow \infty} P_2 = P$ if for

- **Feedback A:** $Q = Q_A(\tau_A) := \frac{Q}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$
- **Feedback B:** $Q = Q_B(\tau_B) := \frac{Q}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$
- **Feedback C:** $Q = Q_C(\tau_C) := \frac{Q}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$
- **Feedback BC:** $Q_{BC}(\tau_B, \tau_C) := \frac{Q}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$

where $\beta(\tau_B) := \frac{Q}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$

**Proof:** Note that because the two circuits $i = 1, 2$ are identical and (12) is such that $\frac{dP_2}{dP_1} < 0$ over the domain $P_1 \geq 0$, setting $u_1 = u_2 = \bar{u}$ results in a unique steady state protein output satisfying $P_1 = P_2$. The modified RBS strengths $Q_A, Q_B, Q_C, Q_{BC}$ are required to be such that when $u_1 = u_2 = \bar{u}$ we have $\lim_{\bar{u} \rightarrow \infty} P_1 = \lim_{\bar{u} \rightarrow \infty} P_2 = P$. For each regulation scheme, the modified RBS strength is obtained by substituting the appropriate values of $\tau_A$, $\tau_B$, $\tau_C$ into (12), setting $u_2 = \bar{u}$ and $P_1 = P_2 = P$, solving for $Q$ and evaluating its limit as $\bar{u} \rightarrow \infty$.

When the feedback gains $\tau_A$, $\tau_B$, $\tau_C$ are zero, each of the modified RBS strengths in Claim 1 is restored to $Q = \bar{Q}$.

1) **Transcription repression through protein feedback:** In this case, the feedback gains in equations (12), (13) satisfy $\tau_A > 0$, $\tau_B > 0$, $\tau_C = 0$. When $u_2 = \bar{u}$ is fixed and $u_1 \in [0, \bar{u}]$, the steady state of the two circuits will satisfy

$$x_Ty_T - \bar{a}(\bar{u})P_2 - \tilde{b}P_2^2 = \tilde{c}P_1$$

**Proposition 2:** With RBS dissociation constant $Q_A(\tau_A)$ and transcription factor input $u_1 = u_2 = \bar{u}$ the competition measure $\left| \frac{dP_2}{dP_1} \right|$ is such that $\frac{dP_2}{dP_1} < 0$ and

$$\lim_{\bar{u} \rightarrow \infty} \left| \frac{dP_2}{dP_1} \right| = \frac{1}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$$

**Proof:** We first implicitly differentiate (17) with respect to $P_1$ and substitute $\bar{a}(\bar{u})P_2 - \tilde{b}P_2^2 = \tilde{c}P_1$ and

$$\lim_{\bar{u} \rightarrow \infty} \left| \frac{dP_2}{dP_1} \right| = \frac{x_T + \frac{\bar{Q}}{\bar{u}} Q_A(\tau_A) \left( \frac{k}{y} + 1 + 2\tau_A \bar{P} \right)}{x_T + \frac{\bar{Q}}{\bar{u}} Q_A(\tau_A) \left( \frac{k}{y} + 1 + 2\tau_A \bar{P} \right)}$$

The first result of the claim follows by differentiating (19) with respect to $\tau_A$. The second result follows by taking the limit of (19) as $\tau_A \rightarrow \infty$.

2) **Transcription repression through miRNA feedback:** Here, the feedback gains are such that $\tau_A = 0$, $\tau_B > 0$, $\tau_C = 0$. Hence, with $u_2 = \bar{u}$ fixed and $u_1 \in [0, \bar{u}]$, (12) becomes

$$x_Ty_T - \bar{a}(\bar{u})P_2 - \tilde{G}_{1,2}(P_1, P_2) = \tilde{e}P_1$$

**Proposition 3:** With RBS dissociation constant $Q_B(\tau_B)$ and transcription factor input $u_1 = u_2 = \bar{u}$ the competition measure $\left| \frac{dP_2}{dP_1} \right|$ is such that $\frac{d^2P_2}{dP_1^2} < 0$ and

$$\lim_{\bar{u} \rightarrow \infty} \left| \frac{dP_2}{dP_1} \right| = \frac{1}{1 + \frac{\bar{Q}}{\bar{u}} (\frac{k}{y} + 1)}$$

**Proof:** We first implicitly differentiate (20) with respect to $P_1$ and substitute $Q = Q_B(\tau_B)$. Setting $u_1 = u_2 = \bar{u}$, we have $\lim_{\bar{u} \rightarrow \infty} P_1 = \lim_{\bar{u} \rightarrow \infty} P_2 = P$ and
the steady state protein concentrations $P_0$ have this case, the feedback gains satisfy $\tau_\omega > 0$. With $u_2 = \bar{u}$ fixed and $u_1 \in [0, \bar{u}]$, from (12), the steady state protein concentrations $P_1, P_2$ lie along

$$x_T y_T - \bar{a}(\bar{u}) P_2 - \bar{b}(\bar{u}) P_2^2 = \varepsilon P_1 + f P_1^2$$

(23)

**Proposition 4:** With RBS dissociation constant $Q_C(\tau_C)$ and transcription factor input $u_1 = u_2 = \bar{u}$, the competition measure $\frac{dP_2}{dP_1}$ is such that

$$\lim_{\bar{u} \to \infty} \left[ \lim_{\tau_C \to \infty} \frac{dP_2}{dP_1} \right] = \frac{1 + 2 \frac{\delta Q}{\tau_T}}{1 + 2 \frac{\delta Q}{\tau_T} \left( \frac{K}{\eta} + 1 \right)}$$

(24)

**Proof:** We first implicitly differentiate (23) with respect to $P_1$ and substitute $Q = Q_C(\tau_C)$. Setting $u_1 = u_2 = \bar{u}$, we have $\lim_{\bar{u} \to \infty} P_1 = \lim_{\tau_C \to \infty} P_2 = \bar{P}$ and

$$\lim_{\bar{u} \to \infty} \left[ \lim_{\tau_C \to \infty} \frac{dP_2}{dP_1} \right] = \frac{\frac{x_T + \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1)}{x_T + \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1) + 2 \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1) + 2 \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1)}}{x_T + \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1) + 2 \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1) + 2 \frac{\delta Q C}{\tau_T} (\frac{K}{\eta} + 1)}$$

(25)

The first result of the claim follows by differentiating (25) with respect to $\tau_C$. The second result follows by taking the limit of (25) as $\tau_C \to \infty$.

**4) Combined miRNA and protein translation feedback:** In this case, the feedback gains satisfy $\tau_A = 0$, $\tau_B > 0$, $\tau_C > 0$. From (12), with $u_2 = \bar{u}$ fixed and $u_1 \in [0, \bar{u}]$, the steady state protein levels $P_1, P_2$ lie along the curve

$$x_T y_T - \bar{a}(\bar{u}) P_2 - \bar{b}(\bar{u}) P_2^2 - \bar{C}_{1,2}(P_1, P_2) = \bar{\varepsilon} P_1 + \bar{f} P_1^2$$

(26)

**Proposition 5:** With RBS dissociation constant $Q = Q_{BC}(\tau_B, \tau_C)$ and transcription factor input $u_1 = u_2 = \bar{u}$, the competition measure $\frac{dP_2}{dP_1}$ is such that

$$\lim_{\bar{u} \to \infty} \left[ \lim_{\tau_B \to \infty} \left[ \lim_{\tau_C \to \infty} \frac{dP_2}{dP_1} \right] \right] = \frac{1}{1 + 2 \frac{\delta Q}{\tau_T} \left( \frac{K}{\eta} + 1 \right)}$$

(27)

**Proof:** We first implicitly differentiate (26) with respect to $P_1$ and substitute $Q = Q_{BC}(\tau_B, \tau_C)$. Setting $u_1 = u_2 = \bar{u}$, we have $\lim_{\bar{u} \to \infty} P_1 = \lim_{\tau_B \to \infty} P_2 = \bar{P}$ and

$$\lim_{\bar{u} \to \infty} \left[ \lim_{\tau_B \to \infty} \left[ \lim_{\tau_C \to \infty} \frac{dP_2}{dP_1} \right] \right] = \frac{h_1(\tau_C) P_B Q_{BC} + 2 \tau_C \frac{\delta Q C}{\tau_T} Q_{BC} P + x_T + \frac{\delta Q C}{\tau_T} Q_{BC}}{h_2(\tau_C) P_B Q_{BC} + 2 \tau_C \frac{\delta Q C}{\tau_T} Q_{BC} P + x_T + \frac{\delta Q C}{\tau_T} Q_{BC}}$$

(28)

where

$$h_1(\tau_C) = \frac{1}{\tau_T} \frac{K}{\eta} \left( \frac{\delta + \tau_C P}{\omega} \right)^2 \omega, \quad h_2(\tau_C) = \frac{1}{\tau_T} \frac{K}{\eta} \left( \frac{\delta + \tau_C P}{\omega} \right)^2 \omega$$

The result follows by taking the limit of (28) as $\tau_B \to \infty$ and $\tau_C \to \infty$.

**V. EXAMPLES**

We present two sets of simulations demonstrating our proposed designs. In both, the concentration of transcription factor input $u_2$ is held fixed at 1 nM and the model is simulated for concentrations of the transcription factor input $u_1$ ranging from 0 to 1 mM. The resulting steady state concentrations of proteins $P_1$ and $P_2$ are then plotted against each other. This is done for the unregulated circuits and for the four proposed feedback schemes.

In the first set of simulations we compare the circuits in a high RNAP competition regime, characterized by a low promotor dissociation constant $\gamma$ and high promoter concentration. The second set of simulations is carried out in a low RNAP competition regime. The parameters common to both sets of simulations are as follows: from [17] we have $\frac{1}{\tau_T} = 200$ nM and therefore we set $\frac{1}{\tau_B} = 1$ nM.hour$^{-1}$, $\frac{1}{\tau_C} = 200$ hour$^{-1}$. From [12], the elongation rate of a peptide chain is around 20 amino acids per second. GFP, a typical protein that may be used in an experimental setup to test this work, is composed of approximately 240 amino acids. Therefore we set $\tau_T = 300$ hour$^{-1}$. From [18] we obtain $\delta = \theta = 10$ per hour. We assume that ribosomes binding to mRNA offer no protection from the RNAase, and therefore complexes $d_i$ decay at the same rate as mRNA, giving $a = 1$. The approximately 1 hour half-life of protein [19] sets $\lambda = 1$ per hour. From [21], a typical RBS dissociation constant is $10^4$ nM, and since $Q = \frac{\sigma + \tau + \delta a}{\gamma} \approx \frac{\delta a}{\gamma}$, we take $\sigma = 100$ hour$^{-1}$ and $\rho = 100$ (nM.hour)$^{-1}$. We take the total concentration of RNAP that is available to the two competing circuits to be $\gamma_T = 27$ nM and that of ribosomes to be $\gamma_T = 1.35$ nM. The feedback parameters in Table II are chosen so that $\tau_A = 10$ (nM)$^{-1}$, $\tau_B = 10$ (nM)$^{-1}$ and $\tau_C = 10$ (nM.hour)$^{-1}$ when non-zero.

**A. Example 1: High demand for RNAP**

In this first example, we take $\eta = 0.6 \mu M, \gamma = 250$ hour$^{-1}$ (from [12]), $\alpha = 20$ (nM.hour)$^{-1}$, $\beta = 6000$ hour$^{-1}$, yielding a promotor dissociation constant of $K = 312.5$ nM. Simulation results are shown in Figure 5.
A machinery required for gene expression.

synthetic genetic circuits that mitigates the effects of we have presented a methodology for the design of \( \alpha = 10 \) (nM.hour\(^{-1} \)), \( \beta = 6000 \) hour\(^{-1} \), yielding a promoter dissociation constant of \( K = 2475 \) nM. Simulation results are shown in Figure 6.

### B. Example 2: Low demand for RNAP

In this example we take \( \eta = 0.5 \) \( \mu \)M, \( \gamma = 18750 \) hour\(^{-1} \), \( \alpha = 10 \) (nM.hour\(^{-1} \)), \( \beta = 6000 \) hour\(^{-1} \), yielding a promoter dissociation constant of \( K = 2475 \) nM. Simulation results are shown in Figure 6.

Fig. 5. Simulations for Example 1, showing competition for resources under different feedback structures.

Fig. 6. Simulations for Example 2, showing competition for resources under a low RNAP competition regime.

**VI. DISCUSSION & FUTURE WORK**

Using ideas from classical feedback control theory, we have presented a methodology for the design of synthetic genetic circuits that mitigates the effects of resource competition arising from the scarcity of the cellular machinery required for gene expression.

As seen in Figure 5, we find that, as expected from Propositions 2, 5, schemes A and BC achieve the lowest competition, outperforming B. All three of these schemes give lower competition than C, which may be expected since the circuit in this example is operating in a regime where the promoter concentration is high, leading to high competition for RNAP, which C does not regulate against.

In Figure 6, schemes A and BC again achieve the lowest competition. However, here we have relatively low competition for RNAP and, since scheme C regulates against competition for ribosomes, it performs better than scheme B, which only regulates against competition for RNAP. This therefore shows that if it is known that a particular disturbance is more likely to affect one part of the genetic circuit than another, we can make a judicious choice about where a feedback control loop can be most effective.

In future work we will extend this analysis to non-identical circuits. Further work is also needed to develop techniques for the implementation of schemes B and C. These are most likely to involve the use of siRNA to implement such feedbacks in an indirect manner. The long term aim of this work is to advance the tools available for the design and implementation of reliable synthetic genetic circuits, enabling them to perform accurate logical operations, which would be a significant step towards the development of robust cellular programming techniques that find application in medicine and biotechnology.

**REFERENCES**


