Mitigation of resource competition in synthetic genetic circuits through feedback regulation

Citation: Hamadeh, Abdullah, and Domitilla Del Vecchio. "Mitigation of Resource Competition in Synthetic Genetic Circuits through Feedback Regulation." 53rd IEEE Conference on Decision and Control (December 2014), Los Angeles, CA, USA, Institute of Electrical and Electronics Engineers (IEEE), 2014.

As Published: http://dx.doi.org/10.1109/CDC.2014.7039985

Publisher: Institute of Electrical and Electronics Engineers (IEEE)

Persistent URL: http://hdl.handle.net/1721.1/119171

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

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 reactants. 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 reactants 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.

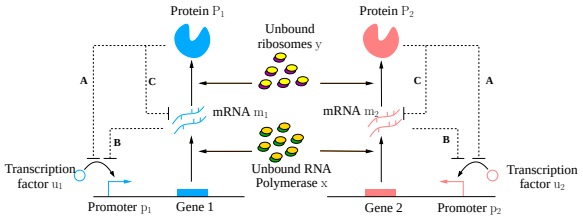

Fig. 1. The feedback mechanisms considered.

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

$$\dot{p}_i^* = \chi_i p_i - \zeta_i u_i p_i^* \tag{1}$$

* Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, 02139, USA. Emails: ahamadeh@mit.edu (Abdullah Hamadeh), ddv@mit.edu (Domitilla Del Vecchio). This work was funded by ONR Award # N000141310074.

R-1	$\mathrm{u}_i + \mathrm{p}_i^* \underset{\chi_i}{\overset{\zeta_i}{\rightleftharpoons}} \mathrm{p}_i$	Transcription factor binds DNA
R-2	$\mathrm{p}_i + \mathrm{x} \underset{\beta_i}{\overset{\alpha_i}{\rightleftharpoons}} \mathrm{c}_i$	RNAP binds DNA
R-3	$\mathrm{c}_i \xrightarrow{\gamma_i} \mathrm{m}_i + \mathrm{n}_i + \mathrm{x}$	Transcription with mRNA and microRNA outputs
R-4	$\mathrm{m}_i \xrightarrow{\delta_i} \varnothing$	mRNA decay
R-5	$\mathrm{n}_i \xrightarrow{\theta_i} \varnothing$	microRNA decay
R-6	$\mathrm{m}_i + \mathrm{y} \underset{\sigma_i}{\overset{\rho_i}{\rightleftharpoons}} \mathrm{d}_i$	Ribosome binds mRNA
R-7	$\mathrm{d}_i \xrightarrow{a_i\delta_i} \mathrm{y}$	mRNA-ribosome complex decay
R-8	$\mathrm{d}_i \xrightarrow{\pi_i} \mathrm{P}_i + \mathrm{m}_i + \mathrm{y}$	Translation of mRNA
R-9	$\mathrm{P}_i \xrightarrow{\lambda_i} \varnothing$	Protein decay
R-10	$\mathrm{p}_i^* + \mathrm{p}_i + \mathrm{c}_i = \eta_i$	DNA conservation
R-11	$x + c_1 + c_2 = x_T$	RNAP conservation
R-12	$y + d_1 + d_2 = y_T$	Ribosome conservation

TABLE I
REACTIONS OF THE GENE EXPRESSION MODEL.

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 γ_i. This reaction, R-2 in Table I, is modeled as

$$\dot{c}_i = \alpha_i p_i x - \beta_i c_i - \gamma_i c_i \tag{2}$$

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 θ_i (reaction R-5) and its dynamics obey

$$\dot{n}_i = \gamma_i c_i - \theta_i n_i \tag{3}$$

The mRNA m_i decays at a rate δ_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 π_i (reaction R-8). The protein P_i decays at a rate λ_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

$$\dot{m}_i = \gamma_i c_i - \delta_i m_i - \rho_i m_i y + \sigma_i d_i + \pi_i d_i \tag{4}$$

$$\dot{d}_i = \rho_i m_i y - \sigma_i d_i - \pi_i d_i - a_i\delta_i d_i \tag{5}$$

$$\dot{P}_i = \pi_i d_i - \lambda_i P_i \tag{6}$$

The binding of RNAP to the promoter yields

$$\frac{p_i x}{c_i} = \frac{\beta_i + \gamma_i}{\alpha_i} =: K_i \tag{7}$$

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

$$\frac{m_i y}{d_i} = \frac{\sigma_i + \pi_i + a_i\delta_i}{\rho_i} =: Q_i \text{ at steady state} \tag{8}$$

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_1 binds the promoter p_1^*, there is a change in the level of *both* P_1 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 m_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.

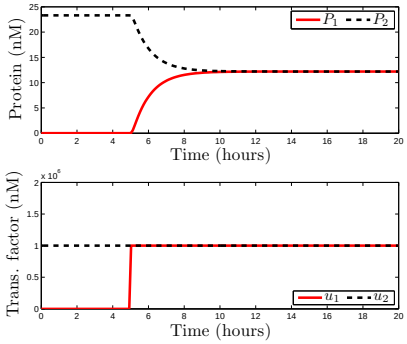

Fig. 2. Effect of competition for limited RNAP and ribosomes: expression of the two genes is coupled through a common pool of transcriptional and translational resources. Activation of gene 1 using transcription factor u_1 causes a rise in P_1 and a fall in P_2. Simulation shown for a rise in u_2 concentration from $u_1 = 10\mu$M to $u_1 = 20\mu$M at time $t = 5$ hours, with $u_2 = 10\mu$M fixed for the duration of the simulation. Simulation parameters for $i = 1, 2$ (see Section V for references: $\zeta_i = 1$ nM.hour^{-1}, $\chi_i = 200\zeta_i$ hour^{-1}, $\alpha_i = 20$ (nM.hour)$^{-1}$, $\beta_i = 6000$ hour^{-1}, $\gamma_i = 250$ hour^{-1}, $\theta_i = 10$ hour^{-1}, $\delta_i = 10$ hour^{-1}, $\rho_i = 100$ (nM.hour)$^{-1}$, $\sigma_i = 10^6$ hour^{-1}, $a_i = 1$ $\pi_i = 300$ hour^{-1} , $\lambda_i = 1$ hour^{-1}, $\eta_i = 500$ nM, $x_T = 27$ nM, $y_T = 13.5$ nM

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 τ_{A_i} in Figure 3. Protein P_i will bind to the free promoter p_i to produce the complex I_{P_i}, the concentration of which obeys

$$\dot{I}_{P_i} = \phi_{1_i} p_i P_i - \phi_{2_i} I_{P_i} \tag{9a}$$

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 τ_{B_i} 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_{n_i}, which obeys the ODE

$$\dot{I}_{n_i} = \phi_{3_i} p_i n_i - \phi_{4_i} I_{n_i} \tag{9b}$$

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 τ_{C_i} in Figure 3. Protein P_i binds the mRNA m_i, forming the complex I_{m_i} and preventing translation. When the mRNA in the complex I_{m_i} decays at the rate ϕ_{7_i}, the protein P_i is released. The complex I_{m_i} satisfies

$$\dot{I}_{m_i} = \phi_{5_i} m_i P_i - \phi_{6_i} I_{m_i} - \phi_{7_i} I_{m_i} \tag{9c}$$

F-1	$\mathrm{P}_i + \mathrm{p}_i \underset{\phi_{2_i}}{\overset{\phi_{1_i}}{\rightleftharpoons}} \mathrm{I}_{\mathrm{P}_i}$	Protein binds to DNA to inhibit transcription
F-2	$\mathrm{n}_i + \mathrm{p}_i \underset{\phi_{4_i}}{\overset{\phi_{3_i}}{\rightleftharpoons}} \mathrm{I}_{\mathrm{n}_i}$	miRNA binds to DNA to inhibit transcription
F-3	$\mathrm{m}_i + \mathrm{P}_i \underset{\phi_{6_i}}{\overset{\phi_{5_i}}{\rightleftharpoons}} \mathrm{I}_{\mathrm{m}_i} \xrightarrow{\phi_{7_i}} \mathrm{P}_i$	Protein binds to mRNA to inhibit translation

TABLE II

REACTIONS IMPLEMENTING THREE OF THE FEEDBACK CONTROLLED SCHEMES.

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

$$\dot{p}_i^* = \chi_i p_i - \zeta_i u_i p_i^* \tag{10a}$$
$$\dot{c}_i = \alpha_i p_i x - \beta_i c_i - \gamma_i c_i \tag{10b}$$
$$\dot{n}_i = \gamma_i c_i - \theta_i n_i - \phi_{3_i} p_i n_i + \phi_{4_i} I_{n_i} \tag{10c}$$
$$\dot{m}_i = \gamma_i c_i - \delta_i m_i - \rho_i m_i y + \sigma_i d_i + \pi_i d_i + \phi_{6_i} I_m - \phi_{5_i} m_i P_i \tag{10d}$$
$$\dot{d}_i = \rho_i m_i y - \sigma_i d_i - \pi_i d_i - a_i \delta_i d_i \tag{10e}$$
$$\dot{P}_i = \pi_i d_i - \lambda_i P_i - \phi_{1_i} p_i P_i + \phi_{2_i} I_{P_i} - \phi_{5_i} m_i P_i + \phi_{6_i} I_{m_i} + \phi_{7_i} I_{m_i} \tag{10f}$$

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

$$p_i^* + p_i + I_{n_i} + I_{P_i} + c_i = \eta_i \tag{11a}$$

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

$$x + c_1 + c_2 = x_T \qquad y + d_1 + d_2 = y_T \tag{11b}$$

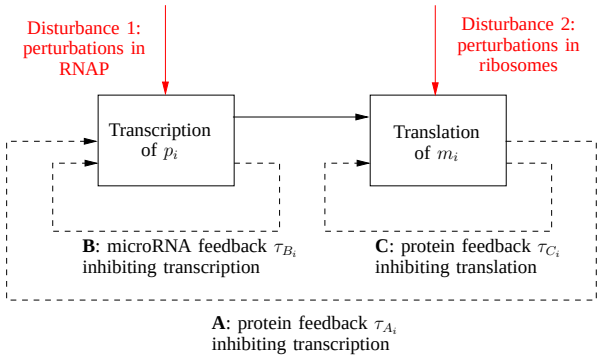

Fig. 3. Block diagram of the three individual inhibitory feedback loops.

Based on free RNAP and ribosome concentrations from [11], [16] and K_i , Q_i values form [20], [21], we make the following assumption.

Assumption 1: Steady state concentrations of free RNAP x and free ribosomes y satisfy $x \ll K_1,\ K_2,\ y \ll Q_1,\ Q_2$.

Proposition 1: Under Assumption 1, the steady state of system (10), at a given transcription factor level u_2 and under the feedback regulation scheme (9) and the constraints (11) satisfies the relation

$$x_T y_T - \tilde{a}_2(u_2) P_2 - \tilde{b}_2(u_2) P_2^2 - \tilde{c}_2 P_2^3 - \tilde{G}_{1,2}(P_1, P_2) = \tilde{e}_1 P_1 + \tilde{f}_1 P_1^2 \tag{12}$$

where $F_i := \frac{\lambda_i}{\pi_i}\frac{1}{\gamma_i}$, $D_i := \frac{\lambda_i}{\pi_i}\frac{K_i}{\eta_i \gamma_i}$, $B_i(u_i) := F_i + D_i\left[1 + \frac{\chi_i}{\zeta_i}\frac{1}{u_i}\right]$

$$\tilde{a}_i(u_i) := \left[\frac{\lambda_i}{\pi_i} x_T + B_i(u_i)\delta_i Q_i\right] \qquad \tilde{e}_i := \left[\frac{\lambda_i}{\pi_i} x_T + F_i \delta_i Q_i\right]$$
$$\tilde{b}_i(u_i) := \left[\tau_{C_i} B_i(u_i) + \tau_{A_i} D_i \delta_i\right] Q_i \qquad \tilde{f}_i := \tau_{C_i} F_i Q_i$$
$$\tilde{c}_i := \tau_{A_i}\tau_{C_i} D_i Q_i \qquad \tilde{G}_{i,j} := \frac{\tau_{B_j} D_j F_j Q_j^2 \left[\delta_j + \tau_{C_j} P_j\right]^2 P_j^2}{\left[y_T - (\lambda_i/\pi_i) P_i - (\lambda_j/\pi_j) P_j\right]} \tag{13a}$$

where we define the feedback strengths, respectively, resulting from the reactions (9a), (9b), (9c) as $\tau_{A_i} := \frac{\phi_{1_i}}{\phi_{2_i}}$, $\tau_{B_i} := \frac{\phi_{3_i}}{\phi_{4_i}}\frac{\gamma_i}{\theta_i}$, $\tau_{C_i} := \frac{\phi_{5_i}\phi_{7_i}}{\phi_{6_i}+\phi_{7_i}}$

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, τ_{A_i}, τ_{B_i}, τ_{C_i}, 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 ($\tau_A = \tau_B = \tau_C = 0$) and the concentration of transcription factor u_2 is held fixed, it can be seen from (12) that P_1 and P_2 satisfy

$$x_T y_T - \tilde{a}_2(u_2) P_2 = \tilde{e}_1 P_1 \tag{14}$$

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

Setting $\tau_{A_i} > 0$ and $\tau_{B_i} = \tau_{C_i} = 0$ (feedback scheme **A**), the dependence of P_1 on P_2 is transformed into the parabolic relation $x_T y_T - \tilde{a}_2(u_2) P_2 - \tilde{b}_2 P_2^2 = \tilde{e}_1 P_1$, 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 μM while that of transcription factor input u_1 is varied from $u_1 = 0$ μM to $u_1 = 1$ μM. 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.

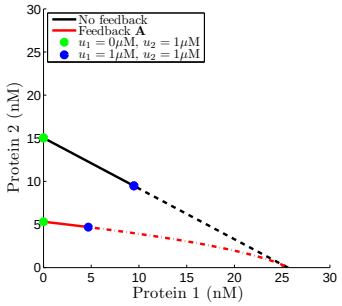

Fig. 4. Effect of adding feedback **A** on competition for resources. With transcription factor $u_2 = 1$ μM and concentration of u_1 varied from $u_1 = 0$ μM to $u_1 = 1$ μM, the steady state concentrations of proteins P_1, P_2 lie on a linear curve when no feedback regulation is used (black dashed curve) and on a parabolic curve when feedback **A** is applied (red dash-dotted curve). The parameters of feedback **A** used here are $\phi_{1_i} = 1$(nM.hour)$^{-1}$, $\phi_{2_i} = 1$ hour^{-1}. All other parameters are the same as those in Figure 2.

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), $\frac{dP_2}{dP_1} < 0$ and $\frac{d^2P_2}{dP_1^2} < 0$ and that increasing u_1 necessarily increases P_1. Also note that since the two circuits are identical by 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 $\bar{P}$, 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, $u_1 = u_2 = \bar{u} \to \infty$. From (14), this steady state is given by

$$\bar{P} := \frac{x_T y_T}{2\frac{\lambda}{\pi}x_T + \frac{\lambda}{\pi}\frac{\delta\bar{Q}}{\gamma}\left(\frac{K}{\eta}+2\right)} \tag{15}$$

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 $\lim_{\bar{u}\to\infty} P_1 = \lim_{\bar{u}\to\infty} P_2 = \bar{P}$. The measure of competition will then be $\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|$. In the case where there is no feedback and $Q = \bar{Q}$, we have, from (14),

$$\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right| = \frac{1+\frac{\delta\bar{Q}}{\gamma x_T}}{1+\frac{\delta\bar{Q}}{\gamma x_T}\left(\frac{K}{\eta}+1\right)} \tag{16}$$

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 τ_A, τ_B, τ_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 $\bar{P}$ 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 $\lim_{\bar{u}\to\infty} P_1 = \lim_{\bar{u}\to\infty} P_2 = \bar{P}$ if for

Feedback **A**: $Q = Q_A(\tau_A) := \bar{Q}\frac{\left(\frac{K}{\eta}+2\right)}{\left(\frac{K}{\eta}+2\right)+\tau_A\frac{K}{\eta}\bar{P}}$

Feedback **B**: $Q = Q_B(\tau_B) := \bar{Q}\beta(\tau_B)$

Feedback **C**: $Q = Q_C(\tau_C) := \bar{Q}\frac{\delta}{\delta+\tau_C\bar{P}}$

Feedback **BC**: $Q_{BC}(\tau_B,\tau_C) := \bar{Q}\frac{\delta}{\delta+\tau_C\bar{P}}\beta(\tau_B)$

where $\beta(\tau_B) := \frac{\left(\frac{K}{\eta}+2\right)\left(\sqrt{4\tau_B x_T\frac{K}{\eta}+\left(\frac{K}{\eta}+2\right)^2}-\left(\frac{K}{\eta}+2\right)\right)}{\left(2\frac{K}{\eta}\tau_B x_T\right)}$

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 $\lim_{\bar{u}\to\infty} P_1 = \lim_{\bar{u}\to\infty} P_2 = \bar{P}$. For each regulation scheme, the modified RBS strength is obtained by substituting the appropriate values of τ_A, τ_B, τ_C into (12), setting $u_2 = \bar{u}$ and $P_1 = P_2 = \bar{P}$, solving for Q and evaluating its limit as $\bar{u} \to \infty$. ■

When the feedback gains τ_A, τ_B, τ_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

$$x_T y_T - \tilde{a}(\bar{u})P_2 - \tilde{b}P_2^2 = \tilde{e}P_1 \tag{17}$$

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 $\frac{d}{d\tau_A}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] < 0$ and

$$\lim_{\tau_A\to\infty}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] = \frac{1}{1+2\frac{\delta\bar{Q}}{\gamma x_T}\left(\frac{K}{\eta}+2\right)} \tag{18}$$

Proof: We first implicitly differentiate (17) with respect to P_1 and substitute $Q = Q_A(\tau_A)$. Setting $u_1 = u_2 = \bar{u}$, we have $\lim_{\bar{u}\to\infty} P_1 = \lim_{\bar{u}\to\infty} P_2 = \bar{P}$ and

$$\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right| = \frac{x_T+\frac{1}{\gamma}\delta Q_A(\tau_A)}{x_T+\frac{1}{\gamma}\delta Q_A(\tau_A)\left(\frac{K}{\eta}+1+2\tau_A\bar{P}\right)} \tag{19}$$

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

■

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

$$x_T y_T - \tilde{a}(\bar{u})P_2 - \tilde{G}_{1,2}(P_1, P_2) = \tilde{e}P_1 \tag{20}$$

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 $\frac{d}{d\tau_B}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] < 0$ and

$$\lim_{\tau_B\to\infty}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] = \frac{1}{1+\frac{\delta\bar{Q}}{\gamma x_T}\left(\frac{K}{\eta}+2\right)} \tag{21}$$

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 $\lim_{\bar{u}\to\infty} P_1 = \lim_{\bar{u}\to\infty} P_2 = \bar{P}$ and

$$\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|=\frac{x_T+\frac{1}{\gamma}\delta Q_B+\frac{x_T\frac{K}{\eta}}{\bar{Q}^2\left(\frac{K}{\eta}+2\right)^2}\tau_B Q_B^2}{x_T+\frac{1}{\gamma}\left(\frac{K}{\eta}+1\right)\delta Q_B+2\frac{\delta x_T\frac{K}{\eta}}{\gamma\bar{Q}\left(\frac{K}{\eta}+2\right)}\tau_B Q_B^2+\frac{x_T\frac{K}{\eta}}{\bar{Q}^2\left(\frac{K}{\eta}+2\right)^2}\tau_B Q_B^2} \quad (22)$$

The first result of the claim follows by differentiating (22) with respect to τ_B. The second result follows by taking the limit of (22) as $\tau_B \to \infty$.

■

3) Translation repression through protein feedback: In this case, the feedback gains are such that $\tau_A = 0,\ \tau_B = 0,\ \tau_C > 0$. 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

$$x_T y_T - \tilde{a}(\bar{u})P_2 - \tilde{b}(\bar{u})P_2^2 = \tilde{e}P_1 + \tilde{f}P_1^2 \quad (23)$$

Proposition 4: With RBS dissociation constant $Q_C(\tau_C)$ and transcription factor input $u_1 = u_2 = \bar{u}$ the competition measure $\left|\frac{dP_2}{dP_1}\right|$ is such that $\frac{d}{d\tau_C}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] < 0$ and

$$\lim_{\tau_C\to\infty}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] = \frac{1+2\frac{\delta\bar{Q}}{\gamma x_T}}{1+2\frac{\delta\bar{Q}}{\gamma x_T}\left(\frac{K}{\eta}+1\right)} \quad (24)$$

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 $\lim_{\bar{u}\to\infty} P_1 = \lim_{\bar{u}\to\infty} P_2 = \bar{P}$ and

$$\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|=\frac{x_T+\delta Q_C(\tau_C)\frac{1}{\gamma}+2\tau_C Q_C(\tau_C)\bar{P}\frac{1}{\gamma}}{x_T+\delta Q_C(\tau_C)\frac{1}{\gamma}\left(\frac{K}{\eta}+1\right)+2\tau_C Q_C(\tau_C)\bar{P}\frac{1}{\gamma}\left(\frac{K}{\eta}+1\right)} \quad (25)$$

The first result of the claim follows by differentiating (25) with respect to τ_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

$$x_T y_T - \tilde{a}(\bar{u})P_2 - \tilde{b}(\bar{u})P_2^2 - \tilde{G}_{1,2}(P_1, P_2) = \tilde{e}P_1 + \tilde{f}P_1^2 \quad (26)$$

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 $\left|\frac{dP_2}{dP_1}\right|$ is such that

$$\lim_{\tau_C\to\infty}\lim_{\tau_B\to\infty}\left[\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|\right] = \frac{1}{1+2\frac{\delta\bar{Q}}{\gamma x_T}\left(\frac{K}{\eta}+2\right)} \quad (27)$$

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

$$\lim_{\bar{u}\to\infty}\left|\frac{dP_2}{dP_1}\right|=\frac{h_1(\tau_C)\tau_B Q_{BC}^2+2\tau_C\frac{1}{\gamma}Q_{BC}\bar{P}+x_T+\frac{1}{\gamma}\delta Q_{BC}}{h_2(\tau_C)\tau_B Q_{BC}^2+2\tau_C\frac{1}{\gamma}\left(\frac{K}{\eta}+1\right)Q_{BC}\bar{P}+x_T+\frac{1}{\gamma}\left(\frac{K}{\eta}+1\right)\delta Q_{BC}} \quad (28)$$

where $h_1(\tau_C) = \frac{1}{\gamma^2}\frac{K}{\eta}\left(\delta+\tau_C\bar{P}\right)^2\omega$, $h_2(\tau_C) = \frac{1}{\gamma^2}\frac{K}{\eta}\left(\delta+\tau_C\bar{P}\right)\omega\left((2+\omega)\left(\delta+\tau_C\bar{P}\right)+2\tau_C\bar{P}\right)$ and $\omega = \frac{\gamma x_T}{\delta\bar{Q}\left(\frac{K}{\eta}+2\right)}$. The result follows by taking the limit of (28) as $\tau_B \to \infty$ and $\tau_C \to \infty$.

■

B. Comparison of feedback schemes

Equations (18), (21), (24), (27) give the degree of competition that is achieved using each regulation scheme when the feedback gains are made large. All achieve competition measures that are lower than that of the unregulated circuit in (16). It is interesting to note that schemes **A** and **BC**, each of which regulates against both RNAP and ribosome competition, achieve the same limit. Practically, scheme **A** may offer a better design since the extra feedback in **BC** provides an additional source of noise and may therefore yield poorer performance when factors other than resource competition are considered.

We also find that **A** and **BC** both achieve lower competition measures than **B** and **C**, which is not surprising since each of the latter two regulates against disturbances arising from competition for only one of the two resources. Comparing schemes **B** and **C**, we find that **B** can achieve lower competition than **C** when $\bar{Q} > \frac{\gamma x_T}{\delta}\frac{\frac{K}{\eta}-2}{2\left(\frac{K}{\eta}\right)+4}$. That is, when the unregulated circuit experiences both low competition for ribosomes (due to weak RBS and thus high Q) and high competition for RNAP (due to strong promoters in high concentrations, thus low K and high η), adding the feedback **B** to regulate against RNAP competition achieves a lower competition measure than adding feedback **C**. The converse is true when there is high competition for ribosomes and low competition for RNAP.

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 mM and the model is simulated for concentrations of the transcription factor input u_1 ranging from 0μM 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 promoter dissociation constant K 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 that $\frac{\chi}{\zeta} = 200$nM and therefore we set $\zeta = 1$ (nM.hour)$^{-1}$, $\chi = 200$ 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 $\pi = 300$ 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 10^4nM, and since $\bar{Q} = \frac{\sigma+\pi+a\delta}{\rho} \approx \frac{\sigma}{\rho}$, we take $\sigma = 10^6$ 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 x_T =27nM and that of ribosomes to be y_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 η =0.6 μM, γ =250 hour^{-1} (from [12]), $\alpha = 20$ (nM.hour)$^{-1}$, $\beta = 6000$ hour^{-1}, yielding a promoter dissociation constant of $K = 312.5$ nM. Simulation results are shown in Figure 5.

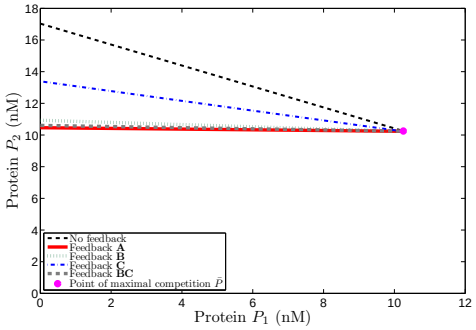

Fig. 5. Simulations for Example 1, showing competition for resources under different feedback structures.

B. Example 2: Low demand for RNAP

In this example we take η =0.5 μM, γ =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.

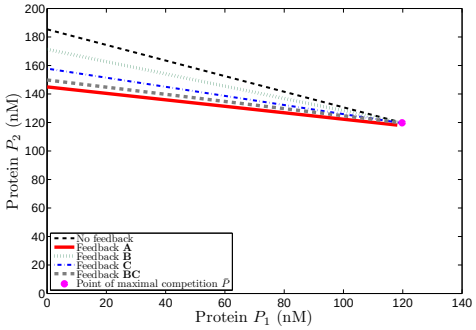

Fig. 6. Simulations for Example 2, showing competition for resources under a low RNAP competition regime.

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

[1] P. E. M. Purnick, R. Weiss, "The second wave of synthetic biology: from modules to systems," Nature Reviews Molecular Cell Biology 10:410-422, 2009.

[2] S. Cardinale, A.P. Arkin, "Contextualizing context for synthetic biology - identifying causes of failure of synthetic biological systems," Biotechnol J 7(7):856-866, 2012.

[3] M. A. Savageau, "Biochemical Systems Analysis: A Study of Function and Design in Molecular Biology", Addison-Wesley, Reading, Mass, USA, 1976.

[4] V. Shimoga, J. White, Y. Li, E. Sontag, L. G. Bleris, "Synthetic mammalian transgene negative autoregulation", Nature/EMBO Molecular Systems Biology, 9:670, 2013.

[5] Abhyudai Singh,"Negative Feedback Through mRNA Provides the Best Control of Gene-Expression Noise," IEEE Transactions on Nanobioscience 10(3):194-200, 2011.

[6] D. Del Vecchio, A.J. Ninfa, E.D. Sontag, "Modular Cell Biology: Retroactivity and Insulation," Nature Molecular Systems Biology, 4:161, 2008.

[7] N.A. Cookson, W.H. Mather, T. Danino, O. Mondragn-Palomino, R.J. Williams, L.S. Tsimring, J. Hasty, "Queueing up for enzymatic processing: correlated signaling through coupled degradation," Mol Syst Biol 7:561, 2011.

[8] D. Siegal-Gaskins, V. Noireaux, R.M. Murray, "Biomolecular resource utilization in elementary cell-free gene circuits," American Control Conference 1531-1536, 2013.

[9] J. Vind, M.A. Sorensen, M.D. Rasmussen, S. Pedersen, "Synthesis of proteins in *Escherichia coli* is limited by the concentration of free ribosomes: expression from reporter genes does not always reflect functional mRNA levels," J Mol Biol 231(3):678-688, 1993.

[10] D. Del Vecchio and R. M. Murray, "Biomolecular Feedback Systems," Princeton University Press, 2014.

[11] S. Klumpp, T. Hwa, "Growth-rate-dependent partitioning of RNA polymerases in bacteria," Proc Natl Acad Sci USA 105(51):20245-20250, 2008.

[12] H. Bremer and P. P. Dennis. "Modulation of chemical composition and other parameters of the cell by growth rate. *Escherichia coli* and *Salmonella*: cellular and molecular biology", 2, 1996.

[13] A. Gyorgy and D. Del Vecchio, "Limitations and Trade-offs in Gene Expression due to Competition for Shared Cellular Resources," IEEE Conference on Decision and Control 2014.

[14] M.-X. Zhang, H. Ou, Ying H. Shen, J. Wang, J. Wang, J. Coselli, and X. L. Wang, "Regulation of endothelial nitric oxide synthase by small RNA," Proc Natl Acad Sci USA, 102(47): 1696716972, 2005.

[15] J. A. Stapleton, K. Endo, Y. Fujita, K. Hayashi, M. Takinoue, H. Saito, and T. Inoue, "Feedback Control of Protein Expression in Mammalian Cells by Tunable Synthetic Translational Inhibition," ACS Synthetic Biology, vol. 1(3), pp. 83-88, 2012.

[16] A.D. Tadmor, T. Tlusty, "A coarse-grained biophysical model of *E. coli* and its application to perturbation of the rRNA operon copy number", PLOS Computational Biology, vol. 4, 2008.

[17] M. Weber and J. Buceta. "Dynamics of the quorum sensing switch: stochastic and non-stationary effects". BMC Systems Biology, 7(6), 2013.

[18] J. A. Bernstein, A. B. Khodursky, P.-H. Lin, S. Lin-Chao, S. N. Cohen. "Global analysis of mRNA decay and abundance in *Escherichia coli* at single-gene resolution using two-color fluorescent DNA microarrays". PNAS, 99(15), 2002.

[19] G. Kramer, R. R. Sprenger, M. A. Nessen, W. Roseboom, D. Speijer, L.n de Jong, M. J. T. de Mattos, J. W. Back, and C. G. de Koster. "Proteome-wide alterations in *Escherichia coli* translation rates upon anaerobiosis". Molecular & Cellular Proteomics, 9(11), 2010.

[20] A. G. Perez, V. E. Angarica, J. Collado-Vides, and A. T. R. Vasconcelos. "From sequence to dynamics: the effects of transcription factor and polymerase concentration changes on activated and repressed promoters". BMC Molecular Biology, 10(92), 2009.

[21] D. Na, S. Lee, and D. Lee. "Mathematical modeling of translation initiation for the estimation of its efficiency to computationally design mRNA sequences with desired expression levels in prokaryotes". BMC Systems Biology, 4(71), 2010.