On the design of integral translational resource allocation controllers for synthetic cellular circuitry

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***Abstract*— Competition for gene expression resources within cellular systems limits the modularity of synthetic circuits, and can lead to the emergence of hidden regulatory interactions between different circuit genes. Experimental evidence suggests that the finite number of free ribosomes in the cell limits protein synthesis capacity and can create unforeseen coupling between co-expressed circuit genes that can result in performance degradation or even circuit failure. Recent work has shown that the cell's ribosome population can be subdivided into host-specific and circuit-specific functions by the production of quasi-orthogonal ribosomes. In this paper, we investigate the design of an integral feedback controller which acts to dynamically allocate ribosomes between host and circuit genes in order to reduce circuit-circuit coupling. We show that whilst the controller is able to successfully allocate resources and improve circuit performance, a non-zero steady state error remains. We show that interactions between the host cell's physiology and the synthetic circuitry act to prevents perfect integral action for the proposed controller architecture.**

I. Introduction

To control cellular processes, synthetic biologists and biotechnologists design regulatory interactions between genes; by regulating the activity of genes (i.e. controlling transcription), it is assumed protein levels will follow. However, the use of a common pool of gene expression resources results in the emergence of hidden interactions, which we term couplings, between genes which are not immediately apparent from circuit topologies. This results in a breakdown in the relationship between transcriptional regulation (input) and protein levels (output) [1]. Experimental evidence suggests that it is the number of free ribosomes which limits protein synthesis capacity and therefore creates these non-regulatory linkages (e.g. [2], [3], [4]). The effect of resource limitation is demonstrated in Fig. 2 (open loop); we consider a simple two gene circuit where one gene is induced (increasing ω_A) and a second is constitutively expressed (constant ω_B). Utilisation of the same ribosome pool results in the emergence of coupling between the genes: as the first gene is induced its mRNA number increases and sequesters more ribosomes, reducing their availability for the second gene hence resulting in the decrease in its protein levels (p_B) while its mRNA remains constant. This coupling effect can be quantified by considering the gradient of the resulting isocost line where steady state protein levels are plotted (Fig. 2).

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We have recently shown that the the cell's ribosome population can be subdivided into host-specific and circuit-specific functions by the production of quasi-orthogonal ribosomes ('o-ribosomes') [5]. These circuit-specific ribosomes can be created by expressing a synthetic version of the 16S rRNA which mediates mRNA ribosome binding site recognition (e.g. [6]). By producing the rRNA in response to the circuit's demand for translational capacity, resource allocation controllers can be designed to match demand with supply and hence reduce resource-mediated gene coupling. In this paper we consider the design of an integral controller which acts to dynamically allocate ribosomes between host and circuit genes in order to reduce circuit-circuit coupling.

Integral control is receiving increasing interest in synthetic biology as it allows a system to undergo perfect adaptation [7], [8]. Biological implementation of such designs usually takes the form of a process which produces an activator which itself activates an inhibitor, which inhibits the original process. If the process output is increased by a disturbance then the process is subjected to greater inhibition. If the process output falls, the level of inhibitor production falls and so the process input increases, allowing adaptation. We review recent work on implementing integral controllers using chemical reaction networks in Section II, and then in Section III we propose an architecture for an integral resource allocation controller for synthetic circuits. We develop a mathematical model of the controller which takes into account key host processes, and in Section IV we investigate the ability of the integral controller to decouple genes in a simple two gene circuit. In Section V we demonstrate that host-circuit interactions prevent the controller from achieving perfect adaptation.

II. Realising integral control in a chemical reaction network

In [8], Briat *et al.* propose the following chemical reaction network which implements perfect integral control: Some output X is produced in response to the input reference species Z_1 by some function f (Eq. 2). This activates the production of a sensor species Z_2 by some function g and therefore the output is measured via the concentration of a sensor species Z_2 (Eq. 3). Both f and g are monotonically increasing functions. The action of the controller is through a 'comparison' reaction in which Z_1 and Z_2 are eliminated (Eq. 4). This elimination reaction is the proposed source of

the integral action:

$$\emptyset \xrightarrow{\mu} Z_1 \tag{1}$$

$$\emptyset \xrightarrow{f(Z_1)} X \tag{2}$$

$$\emptyset \xrightarrow{g(X)} Z_2 \tag{3}$$

$$Z_1 + Z_2 \xrightarrow{\eta} \emptyset \tag{4}$$

This chemical reaction network acts to reject disturbances to the species X.

III. Integral control for translational resource allocation

The architecture for integral control described above requires the use of multiple proteins. Given that additional protein production would impose even greater competition for o-ribosomes (and reduce cellular growth rate through increased 'burden'), in our proposed controller architecture we instead propose the use of small RNAs. The expression of small RNAs requires only transcription by RNA polymerase and not translation by ribosomes. Experimental evidence shows that in most cases RNA polymerase is not limiting and that the production of RNAs is also less energy intensive than the production of proteins, so further reducing the whole cell burden of their expression [9]. Taking inspiration from [8], [10], we propose that the required inhibitory action takes the form of sequestration of the o-rRNA by a synthetic small RNA.

A. A chemical reaction network implementing integral resource allocation

The proposed topology for our putative controller is depicted in Fig. 1. We first consider the production of o-ribosomes. The synthetic rRNA (ρ) is consitutively expressed. This co-opts host ribosomal components (referred to here as the 'naive ribosome', p_R) to produce o-ribosomes (P) (Eq. 5):

$$\emptyset \xrightarrow{\omega_\rho} \rho \qquad p_R + \rho \underset{u_\rho}{\overset{b_\rho}{\rightleftharpoons}} P \tag{5}$$

The input species, equivalent to Z_1, is the o-rRNA. The $f(\cdot)$ function is carried out by the following chemical reactions: (i) the mRNA m_G is constitutively expressed (Eq. 6), (ii) this reversibly binds o-ribosomes to form a translation complex (c_G) and (iii) translation produces the functional activatory transcription factor protein p_G (Eq, 7).

$$\emptyset \xrightarrow{\omega_G} m_G \tag{6}$$

$$m_G + P \underset{u_G}{\overset{b_G}{\rightleftharpoons}} c_G \xrightarrow{T_L(c_G, e)} m_G + P + p_G \tag{7}$$

The protein p_G takes the place of species X above; use of the o-ribosome pool for the translation of circuit genes will result in a resource-mediated perturbation to the level of p_G. In this way p_G acts a sensor of o-ribosome demand as its levels are determined only by competition for translational resources (see Fig. 3b).

The sensor species (equivalent to Z_2 in Eq. 3) takes the form of a small RNA (sRNA, denoted s). The transcription of this RNA is activated by (p_G) (where $\mathcal{H}$ is a function describing the activation, see Eq. 29):

$$\emptyset \xrightarrow{\omega_s \mathcal{H}(p_G)} s \tag{8}$$

Hence p_G carries out the $g(\cdot)$ function action in Briat *et al.* The comparison reaction (equivalent to Eq. 4) takes the form of a bimolecular reaction between ρ and s to form the RNA duplex (d) which is degraded (Eq. 9). Note that we consider the comparison reaction to be reversible.

$$\rho + s \underset{u_s}{\overset{b_s}{\rightleftharpoons}} d \xrightarrow{\delta_d} \emptyset \tag{9}$$

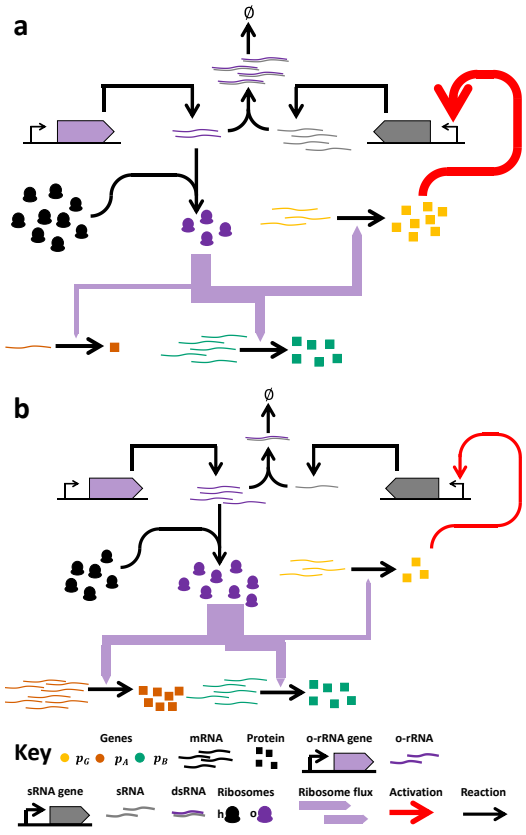

Fig. 1. **Structure and function of the controller.** (a) Low demand circuit. When competition is low, p_G expression is high and so sRNA transcription is activated resulting in high sequestration of, and hence degradation of, the o-rRNA. Therefore co-option of ribosomes to the o-ribosome pool is low. (b) High demand circuit. As p_A is induced, the o-ribosome pool redistributes across circuit and controller genes (width of purple ribosome flux lines) due to competition between mRNAs. This reduces translation of p_G and hence reduced activation of s transcription. This results in less sequestration of the o-rRNA and so increased co-option of ribosomes to the orthogonal pool. The ribosome flux for m_B translation despite the increase in m_A is maintained.

B. Host-circuit interaction model

We have recently developed and validated a model of microbial physiology which includes the production of o-ribosomes [5]. This model captures (i) that energy production is limited by enzymatic activity, (ii) that ribosomes are autocatalytic, (iii) that genes compete for ribosomes (i.e. there is a finite translational capacity), (iv) that the proteome mass

is finite (competition for space) and, (v) the 'quasi' nature of orthogonal ribosomes due to competition for the naive protein component. It consists of 16 differential equations which track the time evolution of a simple metabolism (including production of an 'energy' species, e, from a substrate s_i) and proteome, which consists of metabolic enzymes (p_T and p_E), host factors (p_H), the large protein-component of the 'naive' ribosome (p_R), functional ribosomes (host, R, and orthogonal, P) and the rRNAs (host, r, and orthogonal, ρ). The naive ribosomes are specified by interaction with the rRNAs which we model as a reversible reaction (Eq. 5). See [5] for a full description of model derivation:

The dynamics of the metabolism describe the import of an external substrate (s_e) to become an internal substrate (s_i) by a transporter enzyme (p_T). This is then converted into an 'energy' species (e) by an enzyme (p_E). We model the enzymatic reactions using Michaelis-Menton kinetics:

$$\frac{ds_i}{dt} = \frac{v_T p_T s_e}{k_T + s_e} - \frac{v_E p_E s_i}{k_E + s_i} - \lambda s_i \tag{10}$$

$$\frac{de}{dt} = \varphi_e \frac{v_E p_E s_i}{k_E + s_i} - \sum_X \Big(n_X T_L(c_X, e) \Big) - \lambda e \tag{11}$$

mRNAs are born spontaneously at a rate proportional to the energy status of the cell ($T_X(e)$) and any regulatory interactions (represented by Hill functions, $\mathcal{H}(\cdot)$). Free (host or orthogonal) ribosomes (denoted $\mathcal{R}$) bind mRNAs reversibly to form translation complexes (c_X). Proteins are produced from translational complexes at the rate $T_L(c_X,\ e)$. Protein production liberates mRNA and free ribosomes. All species are degraded and/or diluted by cell growth (λ). Applying the law of mass action to these interactions yields the following dynamics:

$$\frac{dm_X}{dt} = \omega_X \mathcal{H} T_X(e) - b_X \mathcal{R} m_X + u_X c_X ... - (\delta_{m_X} + \lambda) m_X \tag{12}$$

$$\frac{dc_X}{dt} = b_X \mathcal{R} m_X - u_X c_X - (\delta_R + \lambda) c_X \tag{13}$$

$$\frac{dp_X}{dt} = T_L(c_X,\ e) - (\delta_{p_X} + \lambda) p_X \tag{14}$$

If translated by the host pool then $\mathcal{R} = R$ and if by the orthogonal pool $\mathcal{R} = P$. For the host genes ($X \in \{T,\ E,\ R,\ H\}$) the regulatory interactions are:

$$\mathcal{H}(\cdot) = 1 \qquad \text{where } X \in \{T, E, R\} \tag{15}$$

$$\mathcal{H}(\cdot) = \frac{1}{1 + (p_H / k_H)^{h_H}} \qquad \text{where } X = H \tag{16}$$

The expressions describing the energy dependence of transcription and translation are given by:

$$T_X(e) = \frac{e}{o_X + e} \tag{17}$$

$$T_L(c_X,\ e) = \frac{1}{n_X} \Big(\frac{\gamma_{max} e}{\kappa_\gamma + e} \Big) c_X \tag{18}$$

In addition to the reactions described above the naive ribosome (p_R) reacts reversibly with either the host (r) or orthogonal (ρ) 16S-rRNA to produce functional free host (R) or orthogonal (P) ribosomes:

$$\frac{dp_R}{dt} = T_L(c_R, e) - (\delta_R + \lambda) p_R \ ... - b_r p_R r + u_r R \ ... - b_\rho p_R \rho + u_\rho P \tag{19}$$

The dynamics of the host rRNA and free host ribosomes are:

$$\frac{dr}{dt} = \omega_r T_X(e) - b_r p_R r + u_r R - (\delta_r + \lambda) r \tag{20}$$

$$\frac{dR}{dt} = b_r p_R r - u_r R - (\delta_R + \lambda) R \ ... + \sum_X \Big(T_L(c_X, e) - b_X R m_X + u_X c_X \Big) \tag{21}$$

where the $\sum$ term represents the dynamics of translating the host genes ($X \in \{T,\ E,\ H,\ R\}$). The dynamics of the orthogonal rRNA and free host ribosomes are:

$$\frac{d\rho}{dt} = \omega_\rho T_X(e) - b_\rho p_R \rho + u_\rho P - (\delta_\rho + \lambda) \rho \tag{22}$$

$$\frac{dP}{dt} = b_\rho p_R \rho - u_\rho P - (\delta_R + \lambda) P \ ... + \sum_Y \Big(T_L(c_Y, e) - b_Y P m_Y + u_Y c_Y \Big) \tag{23}$$

where Y is the set of genes translated by the o-ribosome pool, such as circuit genes (in this paper denoted A and B).

All components are diluted at the cell's growth rate (λ). λ is dynamically calculated within the model based on the number of translation complexes translated by the host ribosomes (the set X) and the o-ribosome pool (the set Y):

$$\lambda = \frac{1}{M} \Big(\frac{\gamma_{max} e}{\kappa_\gamma + e} \Big) \Big(\sum_X \big(c_X \big) + \sum_Y \big(c_Y \big) \Big) \tag{24}$$

The parametrisation of this host model is given in Table I.

C. Controller model

To introduce the proposed controller described in Section III-A we implemented the following ODEs into the host model above. We first introduced the additional equations describing the production of the new transcription factor p_G. Applying the law of mass action to Eq. 6 and 7 (and including dilution and decay of the species) results in:

$$\frac{dm_G}{dt} = \omega_G T_X(e) - b_G m_G P + u_G c_G ... - (\lambda + \delta_{m_G}) m_G \tag{25}$$

$$\frac{dc_G}{dt} = b_G m_G P - u_G c_G - (\lambda + \delta_{p_R}) c_G \tag{26}$$

$$\frac{dp_G}{dt} = T_L(c_G,\ e) - (\lambda + \delta_{p_G}) p_G \tag{27}$$

We modify $d\rho/dt$ (Eq. 22) by applying the law of mass action to Eq. 9 to include the dynamics due to the comparison reaction:

$$\frac{d\rho}{dt} = \omega_\rho T_X(e) - b_\rho p_R \rho ... + u_\rho P - b_s \rho s + u_s d - (\delta_\rho + \lambda) \rho \tag{28}$$

TABLE I
HOST MODEL PARAMETERS [5]

Parameter	Value	Units
s_e	10^4	molecules
φ_e	0.5	–
v_T	728	molecules·min^{-1}
v_E	5800	molecules·min^{-1}
$k_{\{T,E\}}$	1000	molecules
$\omega_{\{T,E\}}$	4.14	molecules·min^{-1}
ω_H	948.93	molecules·min^{-1}
ω_R	930	molecules·min^{-1}
ω_r	3170	molecules·min^{-1}
ω_ρ	$*$	molecules·min^{-1}
$o_{\{T,E,H,\rho\}}$	4.38	molecules
$o_{\{R,r\}}$	426.87	molecules
$b_{\{T,E,H,R\}}$	1	molecules^{-1}·min^{-1}
$u_{\{T,E,H,R\}}$	1	molecules^{-1}
$b_{\{r,\rho\}}$	1	molecules^{-1}·min^{-1}
$u_{\{r,\rho\}}$	1	min^{-1}
$\delta_{m_{\{T,E,H,R,r,\rho\}}}$	0.1	min^{-1}
$\delta_{p_{\{T,E,H\}}}$	0	min^{-1}
δ_R	0	min^{-1}
$n_{\{T,E,H\}}$	300	amino acids
n_R	7459	amino acids
k_H	152219	molecules
h_H	4	–
γ_{max}	1260	amino acids·min^{-1}·(e molecules)$^{-1}$
κ_γ	7	(e molecules)
M	10^8	aa

$*$, ω_ρ is optimised as part of the controller design process.

The dynamics of the free orthogonal ribosome pool remain unchanged bar the addition of the controller gene G to the set Y. The dynamics of the small RNA (s) are:

$$\frac{ds}{dt} = \omega_s T_X(e)\left(\frac{(p_G/k_G)^{h_G}}{1+(p_G/k_G)^{h_G}}\right)... \\ - b_s\rho s + u_s d - (\delta_s + \lambda)s \tag{29}$$

(note the additional scaling of ω_s by the activatory Hill function – the p_G term – as referred to in Section III-A). The production dynamics of RNA duplex (d) and its degradation/dilution are given by:

$$\frac{dd}{dt} = b_s\rho s - u_s d - (\delta_d + \lambda)d \tag{30}$$

We assume the interaction between the o-rRNA and the small RNA is diffusion limited and reversible (such that $b_s = u_s = 1$, Eq. 9). We account for the rapid degradation of double stranded RNA by setting the decay constant (δ_{r_d}) to 0.5 molecules per minute (~ 5 times greater than single stranded RNAs). Assuming the small RNA has the same kinetics as the host's mRNAs we set $o_s = 4.38$ and $\delta_s = 0.1$.

We assume that all protein-encoding genes of the circuit and controller (i.e. $Y \in \{A, B, G\}$), have characteristics similar to that of the host factor gene p_H and therefore set $o_Y = 4.38$, $b_Y = 1$, $u_Y = 1$, $n_Y = 300$, $\delta_{m_Y} = 0.1$ and $\delta_{p_Y} = 0$. Other parameters were optimised as described below.

The host equations describing the energy status and growth rate were also modified to take account of the new controller genes as described in [5].

D. Controller design process

To achieve specific designs (i.e. controller paramerisations for this fixed topology), we utilised the *ga* function from MATLAB's Global Optimisation Toolbox (version 3.4), together with the Parallel Computing Toolbox (version 6.8), to optimise the experimentally variable parameters (i.e., ω_ρ, ω_s, ω_G, b_G, k_G and h_G) within biologically feasible ranges to minimise gene coupling. To design our controller, we consider the behaviour of two genes, p_1 and p_2. The first is induced (i.e., ω_1 is increased) while the induction of p_2 is constant (ω_2 is constant). We quantify coupling by taking logs of the varying input ω_1 and the two outputs p_1 and p_2. Using the in-built *polyfit* function we fit lines through these transformed points. The impact of p_1 induction on p_2 is given by the gradient, Δ, of the line $\log_{10}(\omega_1)$ v $\log_{10}(p_2)$. In the absence of p_2, p_1 should increase linearly with increasing ω_1. The impact of p_2 on the induction profile of p_1 is quantified by observing the deviation of the simulated values $\log_{10}(p_1)$ (ϕ_{sim}) from those expected by fitting a line through the points $\log_{10}(\omega_1)$ v $\log_{10}(p_1)$ (ϕ_{fit}). Individual input–output responses, where ω_2 is constant, were scored as:

$$\text{score}(\boldsymbol{\omega_1}, \omega_2) = \sum\left((\phi_{fit} - \phi_{sim})^2\right) + \sum\left(\Delta^2\right) \tag{31}$$

To ensure the controller can function across a range of different circuit inductions we simulate a number of different ω_2 values, we define $\boldsymbol{\omega_2}$ as a vector of N induction values. We use the cost function in the optimisation:

$$\text{cost}(\boldsymbol{\omega_1}, \boldsymbol{\omega_2}) = \sum_1^N \text{score}(\boldsymbol{\omega_1}, \boldsymbol{\omega_2}) \tag{32}$$

All models were simulated using *ode15s* in MATLAB 2016b using a time span of $[0...10^4]$ minutes. Simulations were deemed to have reached steady state if the maximum value of the derivative was less than 1 (which is negligible on the scale of protein production in the model, total protein at steady state is 10^8).

IV. ANALYSIS OF CONTROLLER PERFORMANCE

The optimal controller successfully decouples co-expressed genes in a simple two gene circuit (Fig. 2). We simulate the response of a constitutively expressed gene (p_B) as a second gene is induced (p_A). In the absence of the controller the resulting isocost line has a gradient of -0.98 (meaning approximately one p_B molecule is lost for each p_A molecule gained). In the presence of the controller, p_B is almost unaffected, with an isocost gradient of only -0.0032.

To demonstrate the changing distribution of controller components and begin to investigate the dynamic properties of the controller, we consider the induction point when approximately equal amounts of protein are being made ($\omega_A = \omega_B = 100$ mRNAs per min) (Fig. 3). As before, we consider the response of a constitutively expressed gene

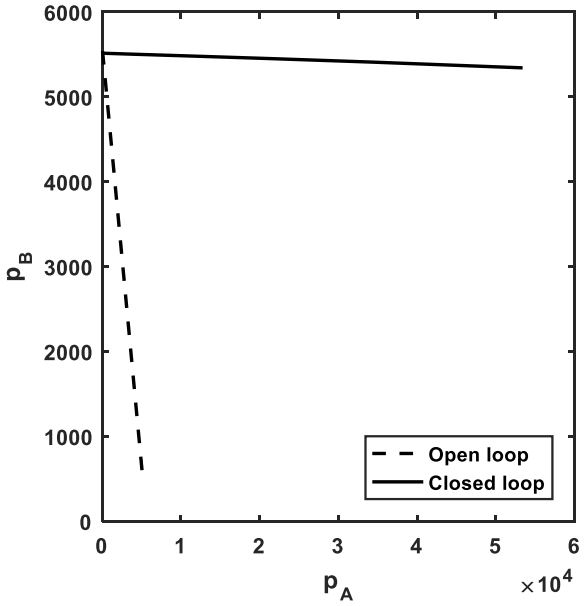

Fig. 2. **The proposed controller decouples co-expressed genes.** Simulation of the action of the controller. ω_A is varied between 1 and 10^4 mRNAs per minute. ω_B is held constant at 100 mRNAs per minute throughout. The simulation time span is increased until it reaches steady state. Controller parameters: $\omega_\rho = 200$ rRNAs per min; $\omega_s = 10^3$ sRNAs per min; $\omega_G = 10^3$ mRNAs per min; Hill function parameters $k_G = 8 \times 10^4$ and $h_G = 4$. Open loop $\omega_\rho = 1.8$ rRNAs per min (ω_ρ is set such that the initial p_B levels are equivalent).

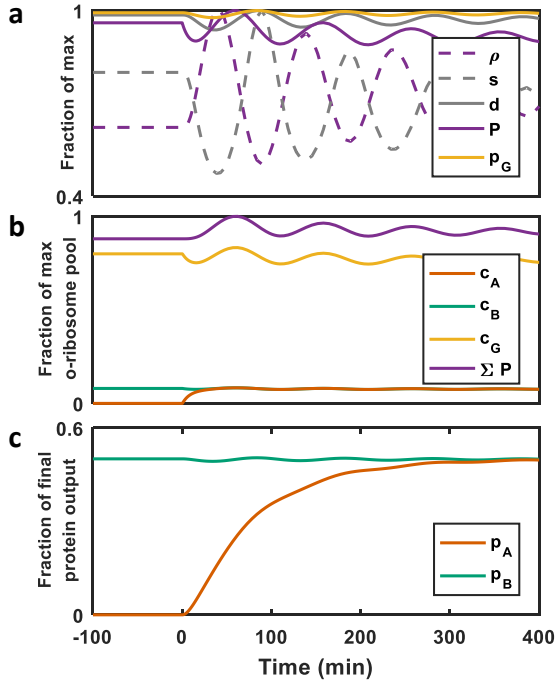

Fig. 3. **Controller dynamics.** p_A is induced at $t = 0$. $\omega_A = \omega_B = 100$ mRNAs per min. The system reaches steady state at $t = 10^3$ min. **(a)** Changing distribution of the controller components. ρ, o-16S rRNA; s, sRNA; d, RNA duplex (dsRNA); P, free o-ribosome; p_G, controller protein. Normalised by their maximum value. **(b)** Changing distribution of the translation complexes over time, c_Y, in response to p_A induction. $\Sigma\, P$, sum of all o-ribosomes. Normalised by maximum $\Sigma\, P$. **(c)** Protein output over time normalised by sum of the final circuit protein concentration.

(p_B) to the induction of second (p_A). When circuit demand is low, the expression of p_G is high. This results in high expression of the sRNA (r_i) and so high sequestration of the o-rRNA (Figure 3c). Upon induction of the second gene (and so an increase in demand), the expression of p_G falls due to a decrease in translation because of resource competition. This results in a large decrease in sRNA production and so liberates o-rRNA, whose levels rise by nearly 40%. This results in greater co-option of host ribosomes to the o-ribosome pool and therefore decouples the co-expressed genes, although note that a error persists at steady state – i.e. p_A does not reach its pre-p_B induction value (Fig. 3c). The o-ribosome pool (i.e. the controlled species) is not maintained in the presence of the disturbance but the perturbation is reduced from 50% (in the absence of control) to only 6% (in the presence of the controller).

V. Host-circuit interactions cause loss of perfect integral action

From Fig. 3, we note that after the induction of the second gene, the first does not fully recover – a small steady state error remains, indicating that this design is not functioning as a perfect integral controller. We propose that this loss of integral control may be due to the effects of additional host factors that are included in our model. To test this hypothesis, we gradually remove host factors to derive a simple model which *does* show perfect integral action.

We initially removed the host enzymes and other proteins to reduce competition and metabolism-based feedback by setting Eq. 10–14 (where $X \in \{T, E, H, R\}$) to zero. We set the initial value of internal energy levels (e) and this is held constant throughout the simulation. This removes the production of p_R, which we reintroduce considering the spontaneous resource-free production of p_R:

$$\emptyset \xrightarrow{\gamma_R} p_R \tag{33}$$

We parameterised the new reduced model using the same values as before and we scaled the transcription rates (ω terms) by the $T_X(\tilde{e})$ where $\tilde{e}$ is the value of the internal energy molecule in the host model. We calculated γ_R as $T_L(\tilde{c}_R, \tilde{e})$, where $\tilde{c}_R$ is the steady state concentration of the translation complex of the naive ribosomes in the host model. We set the protein decay rates δ_{p_X} to 0.022 per min. Therefore the dynamics of the naive ribosome in the reduced model (with species denoted with a $\sim$ above) are:

$$\frac{d\tilde{p}_R}{dt} = \gamma_R - \delta_{p_R}\tilde{p}_R - b_r\tilde{p}_R\tilde{r} + u_r\tilde{R} - b_\rho\tilde{p}_R\tilde{\rho} + u_\rho\tilde{P} \tag{34}$$

All other controller and circuit equations remain unchanged. The simple removal of host components in this manner does not restore perfect integral action. To remove further host-circuit interactions we also removed competition for the p_R species by setting the host rRNA transcription rate ω_r to zero throughout (therefore $\tilde{r} = 0$ throughout). This model still does not exhibit perfect integral action.

We note that in the model described in Section II the reference and sensor species (Z_1 and Z_2) are only removed

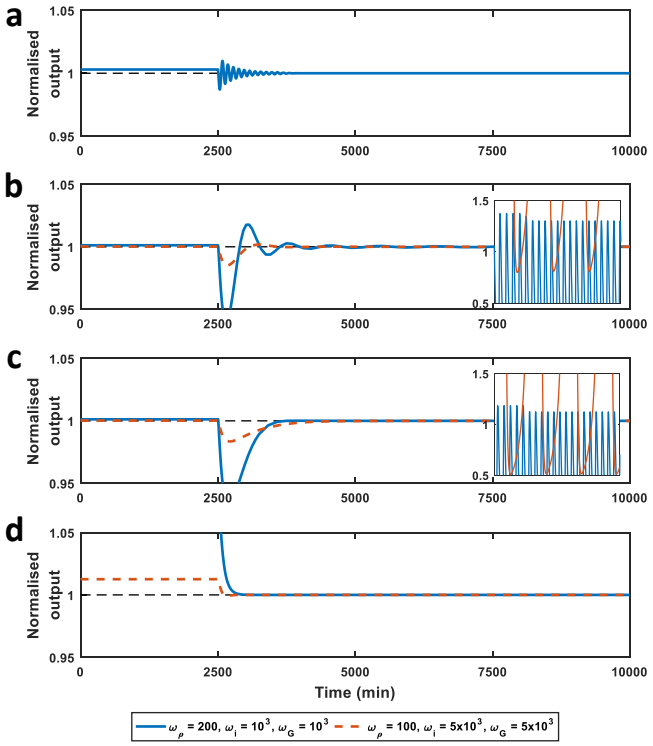

Fig. 4. **Integral action is lost due to host-circuit interactions.** Simulations showing the change in p_A in response to the induction of a second gene p_B. Both genes induced at $\omega_A = \omega_B = 100$ mRNAs per min. p_B is induced at 2500 minutes. **(a)** Simulation of the full model with all host reactions. **(b)** Simulations of the reduced model. Host processes are removed as described in the main text. λ is approximated for the protein species. The reversible reactions are removed (i.e. $u_i = u_\rho = 0$). Inset, $\omega_r = 3,1750$ host rRNAs per min. **(c)** Same simulation as in (b) but with reversible reactions reinstated ($u_\rho = u_i = 1$). Inset, $\omega_r = 3,1750$ host rRNAs per min. **(d)** Same simulations as in (b) but with the o-rRNA and sRNA degradation reactions reintroduced ($\delta_\rho = \delta_s = 0.1$ per min.)

due to the 'comparison' reaction, and there interaction is uni-directional not reversible (Eq. 4). To replicate this we removed the dynamic nature of cellular growth by setting the growth rate, λ, to zero. We set the decay rates of the o-rRNA and sRNA to zero ($\delta_\rho = \delta_s = 0$) and remove the reversibility of their reactions. We also set the reverse reaction rates to zero, i.e., $u_\rho = u_s = 0$. To maintain the removal of the protein species we set the decay rates $\delta_R = \delta_{p_G} = \delta_{p_A} = \delta_{p_B} = 0.022$ per min. Perfect integral action is now obtained (Fig. 4b).

If the assumption that the sRNA–o-rRNA and o-rRNA–'empty' ribosome binding reactions are reversible is restored (i.e. $u_\rho = u_i = 1$) then perfect integral action is maintained provided that the transcription rate of the sequestering sRNA is increased (Fig. 4c). However, reinstating the RNA decay rates ($\delta_\rho = \delta_s = 0.1$ per min) results in loss of perfect integral action (Fig. 4d).

VI. Conclusions

In this paper we proposed a novel architecture for an integral feedback controller which acts to dynamically allocate ribosomes between host and circuit genes in order to reduce circuit-circuit coupling. We showed that the controller is able to successfully allocate resources and significantly improve circuit performance. Our analysis showing that interactions between the host cell's physiology and the synthetic circuitry act to prevents perfect integral action corroborates the recent results reported in [11], which show that the dilution of the sequestration components (here the o-rRNA and sRNA) due to growth results in a 'leak' effect which destroys the integral action of this proposed topology. It was shown in [11] that this controller topology functions as an integral controller in the absence of this dilution effect, and that the dilution effect can be overcome to create a *quasi*-integral action by increasing the transcription rates of the controller components (as we have show in the context of resource allocation here) and increasing the affinity of the RNA target species (which we have not varied as we assume it is diffusion limited). If competition for naive ribosomes (the p_R species) is reconsidered by relaxing the $\omega_r = 0$ condition this results in sustained oscillations regardless of removal of the other host protein species (*insets* in Fig. 4b and c). This suggests that the non-linearities caused by these host-circuit interactions may make the emergence of oscillatory behaviour or even instability more likely, as suggested in our previous work [12]. Much work remains for control theorists to do in analysing the suitability of this and other possible controller architectures for providing robust and reliable resource allocation in complex synthetic circuits.

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