

Robust set-point regulation of gene expression using resource competition couplings in mammalian cells

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Abstract—Gene expression depends on the cellular context. One major contributor to gene expression variability is competition for limited transcriptional and translational resources, which may induce indirect couplings among otherwise independently-regulated genes. Here, we apply control theoretical concepts and tools to design an incoherent feedforward loop (iFFL) biomolecular controller operating in mammalian cells using translational-resource competition couplings. Harnessing a resource-aware mathematical model, we demonstrate analytically and computationally that our resource-aware design can achieve near-constant set-point regulation of gene expression whilst ensuring robustness to plasmid uptake variation. We also provide an analytical condition on the model parameters to guide the design of the resource-aware iFFL controller ensuring robustness and performance in set-point regulation. Our theoretical design based on translational-resource competition couplings represents a promising approach to build more sophisticated resource-aware control circuits operating at the host-cell level.

I. INTRODUCTION

The implementation of novel functionalities in mammalian cells is a key aspect of synthetic biology. Despite recent advances in the engineering of complex biosystems, the design of synthetic biomolecular circuits in mammalian cells is still a painstakingly slow and *ad hoc* process requiring several iterations of the design-build-test-learn cycle [1]. Considering that such a process often leads to numerous interactions of the cycle, it is natural to test and characterise the synthetic parts comprising synthetic constructs and the behaviour of the resulting system using transient transfection of DNA plasmids in the desired mammalian cell lines [1]. Transient transfection typically generates large variability in the number of plasmids transfected in individual mammalian cells [1]–[4]. This large variability in plasmid copy number alters the expression levels of the genetic parts thereby impacting the proper behaviour of engineered biomolecular constructs. In the last decade, feedforward biomolecular controllers have been successfully used to make gene expression robust to DNA copy number variation in bacteria [5] and mammalian cells [2], [3], [6]. Such controllers are typically based on an incoherent feedforward loop (iFFL) motif (Fig. 1) [4], [7], in which an output species (e.g., a target gene) is

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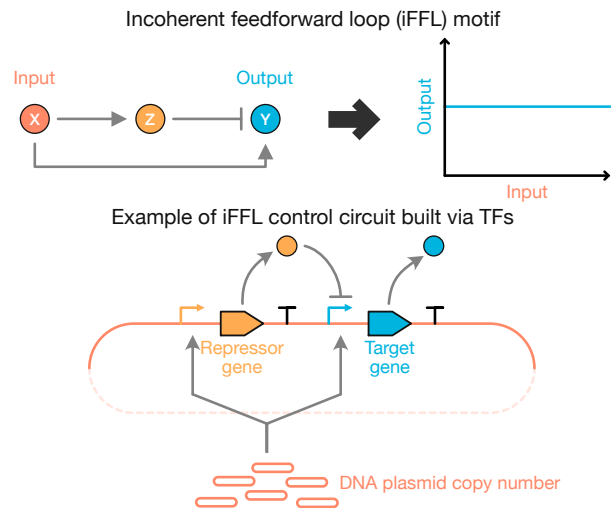


Fig. 1. Top: Incoherent feedforward loop (iFFL) motif. The output molecular species Y is upregulated and downregulated (incoherent) by the input molecular species X and controller species Z, respectively. The controller species Z is used to realise the incoherent branch between the input species X and the output species Y. If the two branches are compensating each other, such a motif ensures set-point regulation of the output species Y despite variations in the input species X (e.g., gene copy number variation). Bottom: Example of an iFFL control circuit built using TFs to reject perturbations in plasmid copy number, thereby reducing variation in the expression level of a target gene [2], [5].

simultaneously upregulated and downregulated (incoherent) by the input to the iFFL (e.g., the DNA plasmid copy number). An intermediate controller species is used to realise the incoherent branch between the input and the output. Perfect adaptation to input variation is achieved if the two branches are exactly compensating each other [7]. To date, iFFL controllers able to achieve near-perfect adaptation to DNA copy number variation have been built using transcription factors (TFs) [2], [5], microRNAs (miRNAs) [2], [3], or endoRNases [6] as controller species [4]. An example of iFFL circuit implemented via TF is illustrated in Fig. 1.

More generally, transient gene expression typically depends on the cellular context [1], [4], [6]. In particular, competition for limited cellular resources (e.g., RNA polymerases or ribosomes) may induce couplings among otherwise independently-regulated gene expression processes [1], [6], [8], [9]. In turn, such couplings can impact the host cell growth or lead to a drop in the transient gene expression levels [1], [6], [8], [9]. Recently, resource-aware designs [10] have been proposed to mitigate the side effects caused by gene expression burden in bacteria [11]–[13] and mammalian cells [1], [6]. The development of several resource-aware

mathematical models has enabled the study of gene expression in resource-limited contexts [1], [6], [8], [9], [11], [13]–[19]. Motivated by these previous works, it is thus natural to ask whether resource competition couplings may be used to design synthetic biomolecular circuits operating at the host-cell level.

Here, we present the theoretical design of a feedforward biomolecular control circuit designed to operate in mammalian cells using translational-resource competition couplings. In particular, the biomolecular controller is based on an iFFL motif in which the incoherent branch is realised via translational-resource-competition couplings established between two independently-regulated genes. Using a resource-aware deterministic model, we demonstrate analytically that our design can achieve near-constant set-point regulation of gene expression whilst ensuring robustness to plasmid uptake variation. We then provide a condition on the controller gene’s parameters to design a resource-aware controller that ensures robustness and performance in set-point regulation. Finally, we present a numerical validation of our design simulating the resource-aware iFFL controller in different operating regimes.

II. PROBLEM FORMULATION

We consider a synthetic biomolecular circuit made of two independently-regulated genes as illustrated in Fig. 2. The two genes are constitutively transcribed from their promoters to produce mRNA species m_y and m_z , which in turn are translated to produce the protein species p_y and p_z . mRNA and protein species are subjected to decay through either degradation or dilution reactions. As mRNA degradation dynamics typically happen (much) faster than dilution dynamics in slowly dividing cells (e.g., mammalian cells), we assume that mRNA species do not undergo dilution reactions arising from cell division. We also assume first-order degradation kinetics for all species.

We consider the case in which the two-gene circuit is transfected in individual mammalian cells using a single DNA plasmid. The two genes thus have the same DNA copy number, which we denote by C . Considering the simplest gene construct involving two genes that are not directly regulating each other, we assume that no other mechanisms are present in the two-gene circuit to regulate transient gene expression at the transcriptional and translational levels.

Our control objective is to regulate the target species p_y so as to robustly maintain a constant expression level despite variation in the plasmid copy number C . In this case, the synthetic circuit can be analysed as a single-input single-output (SISO) system, where the molecular species p_y is the system output, whilst the plasmid copy number C is the system input. We leverage the controller species p_z to achieve the control objective using possible couplings induced by competition for limited cellular resources. In what follows, we denote concentrations of molecular species in *italics* and use the subscripts y and z to denote parameters and molecular species associated to the target and controller gene, respectively.

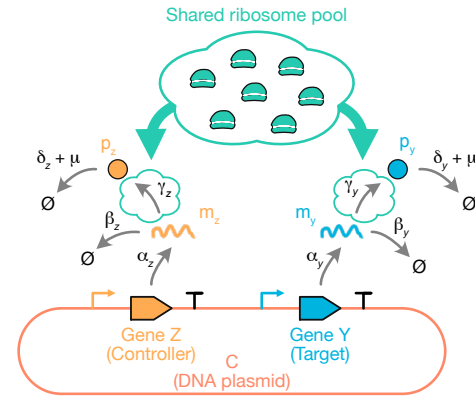


Fig. 2. Representation of the synthetic two-gene circuit. Two independently-regulated genes are transcribed from their constitutive promoters to produce mRNA species m_y and m_z , which in turn are translated to produce the protein species p_y and p_z . Expression of the two genes has an impact on cellular resources. In this case, the two mRNA species m_y and m_z are translated via a finite pool of ribosomes. Competition for the limited pool of ribosomes induces indirect interactions between the two genes (interactions not shown here). mRNA and protein species are subjected to decay through either degradation or dilution reactions. As mRNA degradation dynamics happen (much) faster than dilution dynamics, we assume that mRNA species do not undergo dilution reactions arising from cell division. The two-gene circuit is transfected in individual mammalian cells using a single DNA plasmid. Therefore the two genes have the same DNA copy number C .

III. RESOURCE-AWARE MODELLING FRAMEWORK

Although the two genes do not interact at the circuit level, they may interact at the host-cell level. Potential interactions between the two genes can be induced due to competition for shared cellular resources (e.g., RNA polymerases and ribosomes). There exists a number of potential interactions between a genetic circuit and a host mammalian cell [4], but the key cause of resource competition in mammalian cells still largely remains an open question. We here focus on interactions arising from competition for shared translational resources as ribosomes are currently considered to be one of the prime contributors to gene expression burden [1]. We note that some recent results emphasise RNA polymerases as potentially important contributors to resource competition [6], and that our modelling framework can also be extended to include transcriptional resources – this is however beyond the scope of this paper and something we plan to do in future work. In the present work we thus assume that the translational machinery dominates resource competition and consider that mRNA species m_y and m_z are translated using a finite pool of ribosomes. Therefore, the mRNA species compete for a limited number of ribosomes, thus establishing interactions at the host-cell level as illustrated in Fig. 2.

Often, host-level interactions are treated as external disturbances altering the behaviour of synthetic circuits [1], [6], [8], [9]. In contrast, we here harness these interactions to design a resource-aware biomolecular controller operating at the host-cell level. In what follows, we analyse the behaviour of the two-gene circuit at the host-cell level.

A. Resource-aware mathematical model

To analyse the behaviour of the synthetic circuit at the host-cell level, we need a mathematical model that considers indirect interactions between the two independently-regulated genes. Over the past years, several mathematical models have been developed to investigate gene expression in a resource-limited context [1], [6], [8], [9], [11], [14]–[19]. Hereafter, we decided to use a resource-aware deterministic model that has been recently developed to investigate burden due to translational resource sharing in mammalian cells [1]. In particular, the model includes indirect couplings arising from competition for a limited pool of ribosomes.

Using the resource-aware modelling framework presented in [1], we derive the following deterministic nonlinear ODE model describing the synthetic circuit illustrated in Fig. 2:

$$\dot{m}_y = c \alpha_y - \beta_y m_y, \quad (1a)$$

$$\dot{p}_y = \gamma_y \frac{m_y/\kappa_y}{1 + m_y/\kappa_y + m_z/\kappa_z} r_0 - (\delta_y + \mu) p_y, \quad (1b)$$

$$\dot{m}_z = c \alpha_z - \beta_z m_z, \quad (1c)$$

$$\dot{p}_z = \gamma_z \frac{m_z/\kappa_z}{1 + m_y/\kappa_y + m_z/\kappa_z} r_0 - (\delta_z + \mu) p_z. \quad (1d)$$

where c is the plasmid concentration in the host cell, m_y and m_z are the concentrations of mRNA species, p_y and p_z are the concentrations of the protein species, α_y and α_z are the transcription rates, β_y and β_z are the mRNA degradation rates, γ_y and γ_z are the translation rates, δ_y and δ_z are the protein degradation rates, and μ is the dilution rate. The binding affinity between the mRNA species and the ribosome species is characterised by the lumped parameters κ_y and κ_z , whilst r_0 captures the limited amount of available translational resources (i.e., ribosomes) in the host cell. We assume that the concentration r_0 is conserved and remains constant at the time scale of the considered reactions. We also assume that all model parameters are strictly positive. Finally, we assume that protein degradation dynamics happen faster than dilution dynamics. Such an assumption is reasonable in slow-growing mammalian cell lines if we consider synthetic gene constructs implemented using short half-life proteins. Indeed, a mammalian cell typically divides approximately every 24 hours, whilst destabilised proteins typically have half-lives in the order of a few hours or sometimes even shorter than one hour (e.g., half-life of HES7 is around 22.3 min [20]). Under the previous assumption, the protein decay rates in (1b) and (1d) can be approximated by $\delta_y + \mu \approx \delta_y$ and $\delta_z + \mu \approx \delta_z$, respectively. In what follows, we consider only the decay rates terms δ_y and δ_z in (1b) and (1d).

A full description of the translational-resource-aware model, also including a list of biochemical reactions and model assumptions, is available in [1].

B. Quasi-steady-state approximation of mRNA dynamics

Assuming that the mRNA concentrations m_y and m_z reach their steady state faster than the protein concentrations p_y and p_z , we can make a quasi-steady-state approximation for the

mRNA dynamics in the resource-aware model. Such an assumption is biologically reasonable since mRNA species are typically produced and degraded faster than protein species. Therefore, assuming a quasi-steady-state approximation of the mRNA concentrations (i.e., $\dot{m}_y \approx 0$ and $\dot{m}_z \approx 0$), we obtain the following steady-state mRNA concentrations:

$$\bar{m}_y = c \frac{\alpha_y}{\beta_y}, \quad \bar{m}_z = c \frac{\alpha_z}{\beta_z}. \quad (2)$$

Substituting these mRNA steady-state concentrations in the resource-aware model yields the reduced model:

$$\dot{p}_y = c w_y \gamma_y \frac{r_0}{1 + c w_y + c w_z} - \delta_y p_y, \quad (3a)$$

$$\dot{p}_z = c w_z \gamma_z \frac{r_0}{1 + c w_y + c w_z} - \delta_z p_z, \quad (3b)$$

where the lumped parameters $w_y = \frac{\alpha_y}{\beta_y \kappa_y}$ and $w_z = \frac{\alpha_z}{\beta_z \kappa_z}$ denote the ribosome demand of each gene with respect to the total amount of translational resources (i.e., ribosomes) available in the cell host. An increase in the ribosome demand corresponds to a decrease in the effective translation rate of that gene.

We note that the resource-aware reduced system is now linear in the state $\mathbf{x} = [p_y, p_z]^T$, but nonlinear in the input c . The Jacobian matrix calculated from (3) is not dependent on the input c and its eigenvalues are all strictly negative and correspond to the protein degradation rates. Therefore, we can conclude that the reduced resource-aware model is asymptotically stable if the input c is maintained constant over time.

C. Steady-state analysis of the reduced model

Under the previous considerations, we analyse the reduced resource-aware model at steady state (i.e., $\dot{p}_y = 0$ and $\dot{p}_z = 0$), obtaining the steady-state concentrations:

$$\bar{p}_y = c \frac{w_y \gamma_y}{\delta_y} \frac{r_0}{1 + c w_y + c w_z}, \quad (4a)$$

$$\bar{p}_z = c \frac{w_z \gamma_z}{\delta_z} \frac{r_0}{1 + c w_y + c w_z}. \quad (4b)$$

As we can observe, the steady-state concentration of the target protein \bar{p}_y depends nonlinearly on the concentration c .

IV. RESOURCE-AWARE CONTROL DESIGN

To achieve the control objective, we need to remove the dependency of \bar{p}_y from c in (4a). We provide hereafter a condition for this.

We first make the following assumption:

Assumption 1: There exists a minimum plasmid concentration c_{min} such that

$$c \geq c_{min} \gg \frac{1}{w_y + w_z}. \quad (5)$$

Therefore, c_{min} is the minimum admissible value for the plasmid concentration c in the host cell.

We are now ready to propose a condition that ensures set-point regulation of the target protein p_y despite variations in the gene concentration c .

Proposition 1: Consider the reduced model in (3). Under *Assumption 1*, we have that the steady-state protein concentration \bar{p}_y can be approximated by

$$\bar{p}_y = \frac{w_y \gamma_y r_0}{\delta_y (w_y + w_z)}, \quad (6)$$

which is independent of the plasmid concentration c , meaning that the resource-aware model in (3) achieves robust set-point regulation since the target species remains constant despite variations in gene copy number.

Proof: The proof is trivial. If the system's parameters satisfy *Assumption 1*, then it holds that $1 \ll c_{min} w_y + c_{min} w_z \leq c w_y + c w_z$. Hence, the steady-state concentration in (4a) can be approximated by (6), which is independent on the plasmid concentration c . ■

A. Parameter space design

To realise our proposed resource-aware control strategy, we need to tune the parameters of the two-gene circuit such that the design condition in (5) is satisfied given an operating range for the plasmid concentration c . Tuning the parameters of the target gene may be unfeasible or undesirable. Indeed, the target gene represents the plant of our control scheme and should therefore not be modified to achieve the control objectives. Thereby, the key design principle relies on tuning the parameters of the controller gene (i.e., gene Z in Fig. 2) to fulfil the design condition in (5). At the same time, the controller gene can be used to change the set-point level of the target protein p_y . To avoid the trivial case in which the target gene satisfies the design condition in (5) without the contribution of the controller gene, we make the following assumption:

Assumption 2: In absence of the controller gene, that is when $w_z = 0$, the parameter w_y alone does not satisfy the design condition in (5) given an operating range for the plasmid concentration c .

To achieve (5) in more stringent operating ranges, we can increase the ribosome demand coefficient $w_z = \frac{\alpha_z}{\beta_z \kappa_z}$. According to the definition of w_z , this can be achieved by increasing the transcription rate α_z , or by decreasing the effective dissociation constant κ_z or the mRNA degradation rate β_z .

If we look at the dynamics of the target protein concentration p_y , we observe that the controller gene indirectly downregulates the target gene via the lumped parameter $w_z = \frac{\alpha_z}{\beta_z \kappa_z} = \frac{\bar{m}_z}{\kappa_z}$. Hence, an increase in the steady-state concentration of the controller mRNA corresponds to a decrease in the effective translational rate of the target mRNA. Thereby, the controller mRNA acts like a repressor on the target protein due to resource competition as illustrated in Fig. 3 (dashed green line). These interactions resemble those featured in the iFFL motif illustrated in Fig. 1. We thus conclude that our resource-aware design behaves similarly to an iFFL controller. The novelty of our design lies in the fact that the incoherent branch is realised using indirect couplings caused by competition for shared translational resources as shown in Fig. 3.

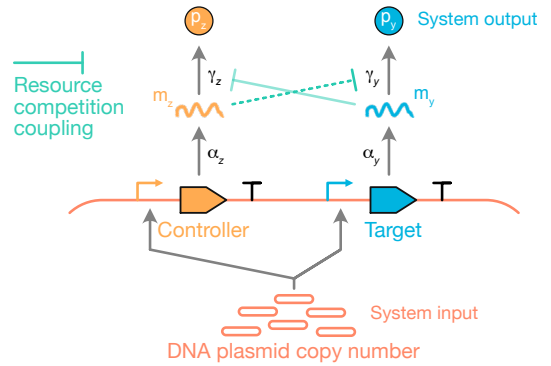


Fig. 3. Illustration of the resource-aware iFFL controller. Competition for limited translational resources induces indirect couplings (green lines) between the two genes. In particular, the controller mRNA negatively regulates the effective translation rate of the target mRNA (dashed green line). Hence, the controller mRNA m_z acts like a repressor on the target protein p_y . Thanks to this repression realised via a resource competition coupling, our resource-aware design resembles the iFFL controller shown in Fig. 1.

B. Robustness analysis

To quantify how robust our resource-aware iFFL controller is at reducing the impact of gene copy number, we calculate the stabilisation error E [5], which is defined as the relative change in the target protein level as the plasmid concentration c increases from the minimum value c_{min} to $+\infty$:

$$E = \frac{\lim_{c \rightarrow \infty} \bar{p}_y}{\bar{p}_y|_{c=c_{min}}} - 1 = \frac{\frac{w_y \gamma_y r_0}{\delta_y (w_y + w_z)}}{c_{min} \frac{w_y \gamma_y r_0}{\delta_y (1 + c_{min} w_y + c_{min} w_z)}} - 1 = \frac{1}{c_{min} (w_y + w_z)}.$$

Hence, stabilisation error decreases as the ribosome demand coefficients w_y and w_z increase. This also implies that higher ribosome demand coefficients are required for better set-point performance at lower plasmid copy numbers. Focusing on the controller gene Z, we can design the expression construct for Z to increase the ribosome demand coefficient w_z so as to decrease the stabilisation error E .

Note that an increase in w_z results in a decrease in the target protein level. The decrease can be quantified by calculating the stabilised promoter strength S [5]:

$$S = \frac{\bar{p}_y|_{c=c_{min}}}{\bar{p}_y|_{c=c_{min}, w_z=0}} = \frac{c_{min} \frac{w_y \gamma_y r_0}{\delta_y (1 + c_{min} w_y + c_{min} w_z)}}{c_{min} \frac{w_y \gamma_y r_0}{\delta_y (1 + c_{min} w_y)}} = \frac{1 + c_{min} w_y}{1 + c_{min} w_y + c_{min} w_z} = \frac{E}{E + 1} (1 + c_{min} w_y).$$

Hence, the relative strength S decreases as w_z increases. This implies a trade-off between the stabilised error E and the stabilised promoter strength S .

V. NUMERICAL VALIDATION OF THE RESOURCE-AWARE FEEDFORWARD CONTROLLER

In what follows, we simulate the resource-aware iFFL controller to assess its performance in achieving the control

TABLE I

NUMERICAL PARAMETERS USED TO SIMULATE THE RESOURCE-AWARE MODEL.

Parameter	Value(s)	Units	Source
μ	0.029	h^{-1}	[6]
r_0	1000	nM	[6]
α_y	1	h^{-1}	[6]
β_y	1	h^{-1}	[6]
γ_y	1	h^{-1}	[6]
δ_y	0.347	h^{-1}	EGFP half-life [21]
κ_y	8	nM	[6]
α_z	1	h^{-1}	[6]
β_z	1	h^{-1}	[6]
γ_z	1	h^{-1}	[6]
δ_z	0.347	h^{-1}	EGFP half-life [21]
κ_z	[1, 2, 4, 8, 16]	nM	[6]

objective in different operating regimes. In particular, we present a series of simulation results reporting the steady-state concentration \bar{p}_y when the plasmid concentration c and the ribosome demand coefficient w_z are varied. The reported simulation results are obtained using the resource-aware model presented in (1) and the numerical parameters reported in Table I. We observe that such parameters are suitable to describe the two-gene circuit in a resource-limited context in mammalian cells.

Our simulation results illustrate robustness in set-point regulation of the steady-state concentration of the protein of interest \bar{p}_y when the plasmid copy number is varied in the range $0.1 \leq c \leq 100$ nM. We also present simulation results that illustrate the trade-off between the stabilised error E and the stabilised promoter strength S when the ribosome demand coefficient w_z is modified by varying the dissociation constant κ_z . This latter parameter can be tuned in mammalian cells by decreasing the translation initiation rate [6]. This can be done by placing, for example, variable numbers of upstream open-reading frames (uORFs) in the 5' UTR of the controller gene transcription unit [6] as an increase in the number of uORFs effectively increases the dissociation constant κ_z . We therefore simulate the resource-aware model using six different values for the dissociation constant κ_z : $\kappa_z = [1, 2, 4, 8, 16, +\infty]$ nM. The latter value ($\kappa_z = +\infty$) corresponds to the worst-case scenario in which the controller gene is absent in the gene circuit.

It is easy to verify analytically that each set of numerical parameters fulfils the design condition (5) in different operating ranges of the plasmid concentration c . Numerical simulations confirm that the two-gene circuit behaves like an iFFL controller as can be seen in Fig. 4. Indeed, the output of the two-gene circuit adapts to input variations if the plasmid concentration c fulfils the design condition in (5). Moreover, the numerical simulations also confirm that the output level of the target protein depends on the lumped parameter w_z . Figure 5 shows six illustrative simulations, one for each value of the parameter κ_z reported in Fig. 4, subject to constant perturbations in the plasmid concentration $c(t)$. Specifically, the plasmid concentration is initialised at the value $c(0) = 100$ nM and is then subject to a constant

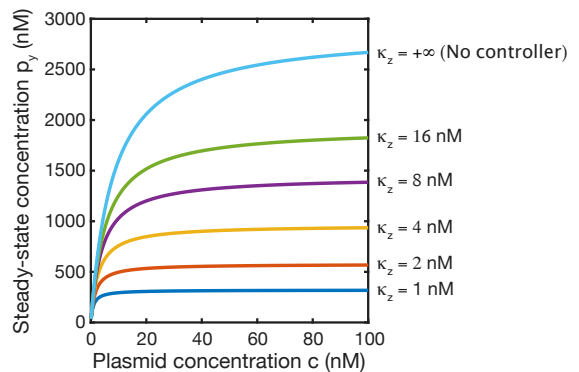


Fig. 4. Steady-state concentrations of the target species \bar{p}_y in response to variations in plasmid copy number with and without the proposed resource-aware iFFL controller. Set-point regulation is assessed by looking at variations of the steady-state concentration \bar{p}_y when varying the plasmid copy number c and the effective dissociation constant κ_z . The cyan line represents the worst-case scenario in which the controller gene is absent ($w_z = 0$). All simulations are run using the numerical parameters reported in Table I.

perturbation every 50 h, i.e., it is set to $c = 10$ nM at $t = 50$ h, to $c = 50$ nM at $t = 100$ h, and finally to $c = 25$ nM at $t = 150$ h. The trajectories shown in Fig. 5 confirm that the resource-aware controller can effectively reject constant disturbances affecting the input c for lower values of κ_z .

In Fig. 6, we present simulation results that illustrate the trade-off between the stabilised error E and the stabilised promoter strength S when the lumped parameter w_z is varied in the range $0.01 \leq w_z \leq 100$ nM^{-1} . We set $c_{min} = 1$ nM to calculate the two performance indices E and S . As can be seen in Fig. 6, an increase in the ribosome demand coefficient w_z corresponds to a decrease in the stabilised error, but also to a decrease in the stabilised promoter strength.

VI. CONCLUSIONS

Here, we have presented the theoretical design of a translational-resource-aware iFFL control circuit designed to operate in mammalian cells. Differently from other iFFL biomolecular controllers, our control design relies on indirect resource-based couplings to regulate the target gene. Indirect couplings may be induced via competition for limited cellular resources at transcriptional and translational levels, e.g., RNA polymerases and ribosomes. Using a resource-aware ODE model [1], we proved analytically and computationally that our iFFL controller can achieve robust regulation of steady-state protein expression levels despite variations in gene copy number. Finally, we showed the existence of a trade-off between robustness and maximum steady-state protein expression levels achievable in set-point regulation.

In contrast to other iFFL circuits, our approach does not require to engineer direct interactions between the genetic components of the iFFL circuit. This may lead to a reduction in time and resources. Nevertheless, the design of indirect couplings requires a reliable model of gene expression in limited-resource contexts. Therefore, the performance of our control design depends on the chosen resource-aware modelling framework. Although we here made the assumption

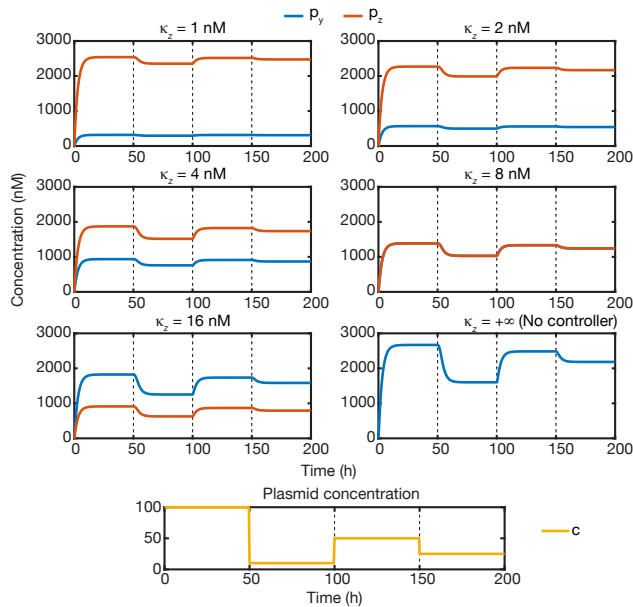


Fig. 5. Illustrative simulations of the resource-aware iFFL controller with $\kappa_z = [1, 2, 4, 8, 16, +\infty]$ nM. Trajectories of p_y (blue lines) and p_z (red lines) show the response of the translational-resource-aware two-gene circuit to constant disturbances (step perturbations) in the input parameter c (yellow line). The input parameter c is initialised at the value $c_0 = 100$ nM and is subject to a constant disturbance every 50 h: at $t = 50$ h, $c(t) = 10$ nM; at $t = 100$ h, $c(t) = 50$ nM; at $t = 150$ h, $c(t) = 25$ nM. Simulations are run using the numerical parameters reported in Table I.

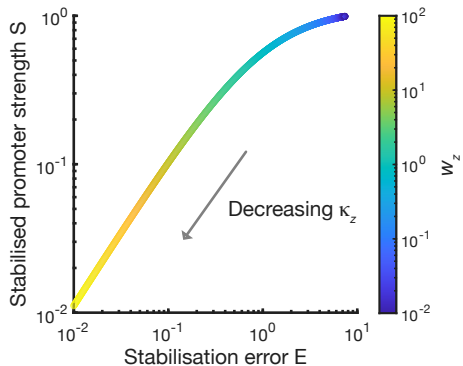


Fig. 6. Trade-off between the stabilised error E and the stabilised promoter strength S when varying the lumped parameter w_z in the range $[0.01, 100]$ nM⁻¹. We set $c_{min} = 1$ nM to calculate the stabilised error E and the relative strength S . All simulations are run using the resource-aware model in (1) and the numerical parameters reported in Table I.

that the ribosomes are the dominant limiting resources, the core of our resource-aware control design would not change if we made different assumptions on the key mechanism behind resource competition – for example, if transcription and not translational machinery dominated the resource sequestration in mammalian cells. In a possible extension of the present work we could consider both transcriptional and translational resources to design more effective resource-aware controllers.

Our approach paves the way to the design of more sophisticated resource-aware control circuits operating at the host-cell level.

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