Feedforward growth rate control mitigates gene activation burden

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Heterologous gene activation causes non-physiological burden on cellular resources that cells are unable to adjust to. Here, we introduce a feedforward controller that actuates growth rate upon activation of a gene of interest (GOI) to compensate for such a burden. The controller achieves this by activating a modified SpoT enzyme (SpoTH) with sole hydrolysis activity, which lowers ppGpp level and thus increases growth rate. An inducible RelA+ expression cassette further allows to precisely set the basal level of ppGpp, and thus nominal growth rate, in any bacterial strain. Without the controller, activation of the GOI decreased growth rate by more than 50%. With the controller, we could activate the GOI to the same level without growth rate defect. A cell strain armed with the controller in co-culture enabled persistent population-level activation of a GOI, which could not be achieved by a strain devoid of the controller. The feedforward controller is a tunable, modular, and portable tool that allows dynamic gene activation without growth rate defects for bacterial synthetic biology applications.

In bacterial synthetic genetic circuits, genes work in orchestration to accomplish a variety of functions, from monitoring stress level and releasing drugs in the gut1–4, to sensing environmental pollutants in soil or water5–9. In these circuits, genes become dynamically activated and repressed, depending on the environment and on the circuit’s state. When a gene of interest (GOI) is activated, cellular resources that the cell would otherwise devote to growth are used by the GOI’s expression. This burden on cellular resources decreases growth rate and leads to physiological changes with poorly predictable outcomes that generally hinder the intended performance of the engineered cell10–17. Decreased growth rate upon a GOI activation has especially severe consequences when engineered bacteria are in co-culture with other strains. In fact, co-existence of multiple strains in co-culture is contingent on tightly matching growth rates, wherein small growth rate differences between the strains typically lead to extinction of the slower growing strain18–20. Therefore, growth rate reduction of an engineered bacterial strain upon a GOI activation, by leading to this strain’s extinction in co-culture, also leads to loss of the population-level expression of the GOI.

To mitigate the consequences of gene expression burden, researchers have devised methods that make synthetic gene expression robust to changes in the availability of cellular resources21–25. Complementary approaches have also used orthogonal ribosomes for heterologous expression through synthetic ribosomal RNA (rRNA)26. However, none of these tools can control growth rate. The problem of controlling growth rate has been addressed by a feedback controller that senses gene expression burden and reduces the GOI’s expression to low levels such that growth rate is not affected27. While this approach is ideal to maximize protein yield in batch-production, it is not suitable when the GOI needs to be dynamically activated to a specific and possibly high level as in biosensors and genetic logic gates28,29. Here, we introduce a feedforward controller that allows the activation of a GOI to any desired level while keeping growth rate constant. The controller co-expresses SpoTH, a modified version of SpoT with only hydrolysis activity, with the GOI.

When the GOI is activated, SpoTH is also expressed and catalyzes the hydrolysis of ppGpp, thereby de-repressing ribosomal rRNA and increasing ribosome level and growth rate30–32. We induce the

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expression of RelA+, a variant of RelA protein that exhibits constitutive ppGpp synthesis activity, to elevate ppGpp level in any strain of interest. Therefore, we use RelA+ to modulate basal ppGpp level and thus achieve a desired growth rate. We then control the expression of SpoTH to maintain that desired growth rate as a GOI is activated. The controller achieves a constant growth rate as a GOI is activated in multiple strains, at different nominal growth rates, and also in co-culture.

Results

Growth rate actuation via SpoTH in strains with elevated ppGpp levels

During balanced exponential growth, ppGpp is the primary regulator of both rRNA and growth rate and, in particular, there is an inverse relationship between basal ppGpp level and both rRNA transcription rate, and growth rate. Furthermore, during exponential growth, rRNA production rate is the rate-limiting step in the process of ribosome production. The RelA/SpoT Homolog (RSH) proteins are responsible for catalyzing the synthesis and hydrolysis of ppGpp as shown in Fig. 1a and mathematical model in Supplementary notes 2 and 3. The growth rate versus SpoTH expression as ppGpp concentration varies, as predicted by our mathematical model (Supplementary notes 2 and 3), is shown in Fig. 1b. Expression of SpoTH also places a load on the cell via resource (e.g., ribosome) sequestration. Thus, once a sufficient amount of ppGpp has been removed through SpoTH expression, the burden effects from further SpoTH expression overwhelm the upregulation in growth rate due to the removal of ppGpp. In turn, this leads to a non-monotonic response between SpoTH expression and growth rate as observed in Fig. 1b.

We experimentally characterized the ability of SpoTH expression to actuate growth rate in three different strains carrying mutations in the spoT gene, resulting in different basal levels of ppGpp. Specifically, we tested the CF944 (spoT202 allele), CF945 (spoT203 allele), and CF946 (spoT204 allele) strains, where the basal ppGpp levels are lowest for CF944 and highest for CF946. Alongside these strains, we also characterized the growth rate response to SpoTH expression in the wild-type MG1655 strain (WT). The genetic circuit used to express SpoTH in these strains is shown in Fig. 2a, b. In particular, Fig. 2b shows how SpoTH expression affects growth rate. For CF945 and CF946, activation of the spoT gene increased growth rate by up to 80% and 60%, respectively (Fig. 2c). For MG1655 and CF944, which have lower basal level of ppGpp, we were unable to positively actuate growth rate as the spoT gene was activated. Strain CF945 provides the most relative growth rate actuation and thus it is the strain we proceeded with.

For a fixed strain, an additional method to tune basal ppGpp level is via the growth medium composition, specifically through the carbon source. Consequently, we also tested four common carbon sources: glucose, glycerol, fructose, and lactose. The nominal growth rate without SpoTH expression was ~0.35 hr⁻¹, ~0.32 hr⁻¹, ~0.2 hr⁻¹, and ~0.12 hr⁻¹ and can be increased by up to ~45%, ~50%, ~85%, and ~75% by expressing SpoTH with glucose, fructose, glycerol, and lactose, respectively (Fig. 2d, e).

These data indicate that there is a tradeoff between nominal growth rate and the relative growth rate increase that can be achieved by SpoTH expression (Fig. 2e). This tradeoff occurs because the extent to which growth rate can be increased is directly tied to the amount of ppGpp available to be hydrolyzed. That is, high basal ppGpp, yielding lower basal growth rate, allows for larger growth rate increase upon SpoTH expression (Supplementary Fig. 13 and Supplementary note 3). However, the maximum achievable growth rate when SpoTH is expressed should be lower than that when there is no ppGpp and no SpoTH expression (e.g., MG1655 with no SpoTH expression Fig. 2c) since SpoTH expression places a load on cellular resources. Thus, once a sufficient amount of ppGpp has been removed through SpoTH expression, the burden effects from further SpoTH expression overwhelm the upregulation in growth rate due to the removal of ppGpp. In turn, this leads to a non-monotonic response between SpoTH expression and growth rate as observed in Fig. 1b.

Feedforward control of growth rate in the CF945 strain

The feedforward growth rate controller co-expresses SpoTH with the red fluorescent protein (RFP) GOI (Fig. 3a). We refer to this system as the closed loop (CL) system. The open loop (OL) system is a configuration...
where SpoTH is missing (Fig. 3b). Addition of AHL activates the RFP gene, which sequesters cellular resources, including ribosomes, and negatively affects growth rate (upper branch in Fig. 3c). In the CL system, however, addition of AHL also increases SpoTH expression (lower branch in Fig. 3c), which increases ribosome level and growth rate, thereby compensating for the growth rate reduction caused by RFP gene activation. The mathematical model predicts that if the ribosome binding site (RBS) of SpoTH is appropriately tuned, then the availability of ribosomes increases exactly to match the demand for ribosomes by RFP gene activation (Fig. 3d and Supplementary notes 2 and 3). Therefore, we designed four SpoTH RBSs for the CL system with varying strengths (Supplementary note 4).

In fructose, the OL system growth rate drops by over 25% when activating the RFP gene, while for the CL system with RBS 2, the growth rate remains nearly constant when the RFP gene is activated to the same level (Fig. 3e). In glycerol, the OL system growth rate drops by over 45% when activating the RFP gene, while for the CL system with RBS 2, the growth rate remains nearly constant for the same RFP gene activation (Fig. 3f). Finally, in lactose, the OL system growth rate drops at most by 10% when we activate the RFP gene, while for the CL system with RBS 1, the growth rate drops at most by 10% when we activate the RFP gene. The circuit yields even lower growth rates than those of the OL system (Supplementary Fig. 4). This is expected since CJB expression sequesters cellular resources, adding to the burden of activating the RFP gene.

Taken together, these data indicate that the feedforward controller can be easily tuned across different nominal growth rates, which we achieved here by different carbon sources, to ensure no growth rate decrease upon the GOI’s activation.

**Feedforward control of ribosomes in common bacterial strain**

To extend the feedforward controller to common bacterial strains, we introduced an inducible RelA+ gene expression cassette to elevate the ppGpp level in any strain of interest (Fig. 4a, b). The *E. coli* RelA+ variant, containing the N-terminal 455 residues of wild-type RelA protein, has constitutive ppGpp-synthesizing activity and its expression has been shown to directly increase ppGpp levels [13, 14]. The genetic construct used to express RelA+ and SpoTH is shown in Fig. 4a, b. As expected from the
ability of RelA+ to increase the level of ppGpp, increased levels of RelA+
in MG1655 (WT), TOP10, and NEB strains lead to lower growth rate
(Fig. 4c). For a level of RelA+ expression that halves the nominal strain
growth rate, SpoTH gene activation upregulates growth rate close to
the level with no RelA+ for all three strains (compare growth rate for
maximal aTc in Fig. 4d to that for no SAL in Fig. 4c). We conclude that,
with constitutive RelA+ expression, SpoTH gene activation allows to
increase growth rate in common laboratory strains, thereby enabling
transportability of the feedforward controller.

We next evaluated the ability of the feedforward controller to
keep growth rate constant as the RFP gene is activated in a
TOP10 strain (Fig. 5a, b). To this end, we established three OL systems
at different nominal growth rates by transforming the OL system cir-
cuit of Fig. 3a in CF944, CF945, and CF946. We then evaluated three
genetically identical CL systems all using RBS 2 (Fig. 5a), each with
nominal growth rate matching that of the corresponding OL systems,
which we obtained by adjusting the RelA+ expression level (insets of
Fig. 5c–e). This way, both OL and CL systems have matching growth
rates before the RFP gene is activated.

When the RFP gene is activated, the growth rate of the OL system
drops by 20%, 55%, and 40% in the CF944, CF945, and CF946 strains,
respectively (Fig. 5c–e). In contrast, the growth rates of the associated
CL systems, only drop by 5%, 7%, and 15%, respectively, when the RFP
gene is activated to the same level (Fig. 5c–e). The RBS of the CL system
in Fig. 5e, can be further tuned to prevent a growth rate drop as the RFP
gene is activated (Supplementary note 6). The growth rate versus RFP
production rate for all CL RBS values tested is shown in Supplemen-
tary Fig. 6.

Taken together, these data show that RelA+ expression sets the
nominal desired growth rate for the CL system in a strain of interest,
and that the SpoTH co-activation with the GOI maintains this pre-set
nominal growth rate as the GOI is activated.

**Feedforward controller for persistent GOI expression in co-
culture**

Engineered bacteria that dynamically express a GOI are often deployed
in environments where other microbes are already present. Examples
include engineered bacteria delivering biotherapeutics in the gut
microbiome or acting as biosensors for water contaminants7. If the
activation of the GOI leads to growth rate defects, then environmental
faster-growing organisms will overtake the population, leading to loss of
the GOI population-level expression18,20. This, in turn, hinders the sensing
or drug delivery functionality of the engineered cell strain. Similarly, in engineered consortia, where multiple strains are programmed to each accomplish a different but complementary function, the different strains’ growth rates should remain sufficiently close to one another despite dynamic activation of genes\(^\text{50-51}\). Here, we tackle this problem by employing the feedforward controller to activate the GOI such that the strain’s growth rate does not change upon GOI activation.

Specifically, we compare the performance of the OL strain expressing inducible RFP (GOI) to that of the CL strain armed with the feedforward controller, when co-cultured with a “competitor strain” that constitutively expresses blue fluorescent protein (BFP) (Fig. 6a–c). The performance metric that we use for this comparison is the temporal population-level expression of RFP after its activation, that is, the intensity of RFP normalized by the OD of the co-culture. When grown in isolation and post induction of RFP, the growth rates of the OL and CL strains are initially close to each other and to that of the competitor strain. However, as time progresses, the growth rate of the OL strain drops to about 50% of its original value while that of the CL strain maintains the initial growth rate over time (Fig. 6d).

As a consequence, when OL and competitor strains are in co-culture and the GOI is activated, the population-level intensity of BFP increases, while that of RFP ultimately decreases (Fig. 6e). This dynamic change in the population-level intensity of RFP and BFP can be attributed to the competitor strain overtaking the population due to its higher growth rate (compare blue line to dark gray bars in Fig. 6d). To further verify that this population-level dynamic change was not due to a dynamic change in the expression level of BFP and RFP, we tracked the same biological replicates as in Fig. 6 in monoculture, which showed constant BFP and RFP intensity throughout the time course (Supplementary Fig. 8). When the CL and competitor strains are in co-culture and the GOI is activated, the population-level intensity of BFP and RFP settle to a constant level (Fig. 6f), consistent with the adaptation of the growth rate of the CL strain to its initial value post induction of the GOI (Fig. 6d, light gray bars). Therefore, we conclude that the CL strain, by preventing a steady decrease in growth rate upon activation of the GOI, also allows persistent GOI population-level expression.

Discussion

The alarmone ppGpp has been referred to as the “CEO of the cell”, whose job is to optimally regulate resources for growth based on environmental conditions and current translational activity\(^\text{27,30}\). In this paper, we exploited the inverse relationship between ppGpp level and growth rate\(^\text{17-19}\) to engineer an actuator that upregulates growth rate (Fig. 1). Specifically, the actuator exogenously expresses a modified version of SpoT with only hydrolysis activity (SpoTH) and, in strains with elevated basal ppGpp level, activation of the SpoTH gene upregulates growth rate (Fig. 2). We demonstrated growth rate actuation first in strains with elevated basal ppGpp level and by tuning the carbon source in the growth media (Fig. 2). Other methods such as tuning the amino acid concentration in the media could also be considered\(^\text{10}\). We then made the actuator portable to common laboratory strains by artificially raising ppGpp’s level through the expression of the RelA+ enzyme (Figs. 4 and 5).

We employed the actuator to create a feedforward controller of growth rate that compensates for the burden on cellular resources observed in the form of growth rate defects due to activating a GOI (Figs. 3 and 5). The controller co-expresses SpoTH with the GOI (RFP); therefore, when the GOI is activated, SpoTH is also activated, which increases growth rate. This increase in growth rate, when the SpoTH RBS is well tuned, exactly compensates for the burden on cellular resources. The design is also tunable and modular.
While the primary focus of this study was to exploit the inverse relationship between ppGpp level and growth rate to create a growth rate controller, there is also an inverse relationship between ppGpp level and ribosome production rate. Given that ribosomes are a key resource in protein translation, we expect that our controller can also actuate protein production rate. The ability to control protein production rates can be leveraged to address the critical issue that the cellular resources responsible for protein production (e.g., ribosomes) are sequestered from free ribosomes by a protein’s expression. RelA+ activation via SAL sets the basal level of ppGpp and thus the nominal growth rate. 

For conditions associated with low nominal growth rates (e.g., cells growing in lactose), we observed the greatest relative protein production of a constitutively expressed GFP gene for the experiment associated with Fig. 2c. Specifically, GFP production rate increases for MG1655, CF944, CF945, and CF946, by 22%, 45%, 90%, and 65%, respectively, when SpoTH is expressed. However, this change in ppGpp also directly modulates the concentration and growth rate. Dashed edges represent sequestration of free ribosomes by a protein’s expression. RelA+ activation via SAL sets the basal level of ppGpp and thus the nominal growth rate. 

The SpoTH actuator can also mitigate the growth defects caused by activation of a toxic protein, such as dCas9. With the SpoTH actuator, we could reach without growth defects a dCas9 production constant as the GOI is activated. Specifically, for the data associated with Fig. 3 and Fig. 5, for low nominal growth rates, the CL system allows also GFP production rate to stay approximately constant when the RFP gene is activated, where it otherwise drops by over 70% for the OL system (Supplementary Fig. 3 and Supplementary Fig. 5). However, the SpoTH RBS that keeps protein production rate constant as the GOI is expressed is not the same as the one that keeps growth rate constant. A constant protein production rate implies that as the GOI is expressed, the changes in ppGpp due to SpoTH expression render a net zero change in free cellular resources responsible for protein production (e.g., ribosomes). However, this change in ppGpp also directly modulates the concentration of growth-related proteins. As a consequence, growth rate can increase while protein production rates decrease as SpoTH is expressed (compare Fig. 2 and Supplementary Fig. 1 and see also mathematical model in Supplementary note 3). In future applications, the feedforward controller may be used synergistically with previously engineered controllers that maintain protein production rate constant once a resource competitor (GOI) is activated, but cannot maintain growth rate constant. In fact, the concurrent implementation of the SpoTH feedforward controller with these controllers will maintain both a constant growth rate and constant protein production rate of a protein of interest, when a GOI is activated.

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rate that would otherwise cause a 40% decrease in growth rate without SpoTH expression (Supplementary Notes 7). We estimate that this production rate is at least four times higher than that reachable without growth defects without SpoTH expression (Supplementary Notes 7). These results have direct applications to CRISPRi-based genetic circuits where dCas9 should be at high concentrations to minimize the effects of its sequestration by multiple sgRNAs\textsuperscript{58–60}. However, dCas9 toxicity limits its concentration to ranges where sequestration effects are prominent\textsuperscript{66}.

Persistent population-level expression of a GOI in a strain that shares the environment with competing organisms is hampered by growth rate imbalances that follow the GOI activation\textsuperscript{4,20}. We applied the feedforward controller to achieve persistent population-level expression of RFP (GOI) in a strain co-cultured with a competitor strain (Fig. 6). In applications, we can use the RelA\textsuperscript{+} inducible expression cassette to set the strain (Fig. 6). In applications, we can use the RelA\textsuperscript{+} inducible expression cassette to set the strain constitutively expresses BFP. The CL strain consists of P\textsubscript{BFP} in TOP10. This strain expresses RFP using the AHL inducible Plux promoter. The competitor strain has P\textsubscript{BFP} in TOP10. This strain constitutively expresses BFP. Temporal responses of growth rate for the OL and CL strains grown in isolation post activation of the RFP gene (GOI) through AHL induction. The growth rate of the competitor strain grown in isolation is shown with a blue line (see Supplementary Fig. 8 for raw data). e, f Temporal responses of RFP (red) and BFP (blue) fluorescence normalized by the total OD of the co-culture (OD\textsubscript{total}) for the OL and competitor strains co-culture in e, and for the CL and competitor strains co-culture in f. AHL\textsuperscript{+} denotes media containing the AHL inducer at 27.5 nM concentration. The growth rate and fluorescence of each strain for all biological replicates was simultaneously tracked in isolation (Supplementary Fig. 8). Data are shown as the mean ± one standard deviation (N = 3, three biological replicates). Individual experimental values are presented as black dots. All experiments were performed in media with glycerol as the sole carbon source. The complete experimental protocol is provided in the Materials and Methods section. Plasmid description, plasmid map, and essential DNA sequences are provided in the Supplementary Information section Plasmid maps and DNA sequences.

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co-exist with environmental species or with other engineered strains, while running circuits in which genes become dynamically activated.

**Methods**

**Bacterial strain and growth**
The bacterial strain used for genetic circuit construction was E. coli NEB10B (NEB, C30199) and LB broth Lennox was the growth medium used during construction. Characterization was performed using the CF494, CF495, CF946, MG1655 (CGSC, 6300), and TOP10 strains. Characterization experiments were done using M9 minimal medium supplemented with 0.2% casamino acids, 1 mM thiamine hydrochloride, ampicillin (100 μg/mL), and either 0.4% glucose, 0.4% fructose, 0.4% glycerol, or 2 g/L lactose (the specific carbon source used for each experiment is specified in the figure caption).

**Microplate photometer protocol**
This protocol was used to generate the data in all figures in the main text except that of the co-culture experiment (Fig. 6). Cultures were prepared by streaking cells from a 15% glycerol stock stored at −80 °C onto a LB (Lennox) agar plate containing 100 μg/mL ampicillin and incubated at 37 °C. Isolated colonies were picked and grown in 2 ml of growth medium in culture tubes (VWR, 60818-667) for 12–24 hours at 30 °C and 220 rpm in an orbital shaker. Cultures were then diluted to an OD at 600 nm (OD600nm) of 0.0075 and grown for an additional 6 hours in culture tubes to ensure exponential growth before induction. Cultures were then induced and plated onto 96-well plate (Falcon, 351172). The 96-well plate was incubated at 37 °C in a Synergy MX (Biotek, Winooski, VT) microplate reader (BioTek Gen 5 wavelength) to monitor GFP fluorescence at 485 nm (bandwidth = 9 nm), respectively and the Sensitivity = 80. Excitation and emission wavelengths to monitor RFP fluorescence are 532 nm (bandwidth = 10 nm) and 619 nm (bandwidth = 13.5 nm), respectively, and the Sensitivity = 100.

**Calculating growth rate and protein production rates**
The media background OD (0.08 OD600 nm), GFP (100 AU), and BFP (28000 AU) were subtracted from the data prior to any calculations. To ensure the data analyzed was coming from cells in exponential growth, only OD values (adjusted for background) of OD600 nm = 0.06 and OD600 nm = 0.14 were considered except for experiments done in lactose where the range was OD600 nm = 0.06 and OD600 nm = 0.1, since cells growing in lactose entered stationary phase at lower OD values.

To dampen noise before differentiating, the data was then filtered using a moving average filter. Given a signal with n measurements \( y = \{y_1, y_2, ..., y_n\} \) sampled at a constant period \( \Delta t \), we apply the moving average filter as follows:

\[
d_{j} = \frac{1}{2n-1} \sum_{r=-n}^{n} y_{j+r}, \quad j \in [3,4, ..., n-1],
\]

where \( d = [d_1, d_2, ..., d_{n-1}] \) is our filtered signal with boundary points identical to those of \( y = [y_1, y_2, ..., y_n] \).

The growth rate is calculated from the filtered OD signal by performing linear regression (in a least-squares sense) on the log of the signal and taking the slope of the fit. The temporal growth rate data from Fig. 6d was calculated by partitioning the OD versus time data (Supplementary Fig. 9) into the time intervals shown in Fig. 6d and then calculating the growth rate of each individual partition per the above method.

The RFP and GFP production rates were calculated in a similar manner as above. Denoting GFP(t) and RFP(t) as the filtered GFP and RFP signal measured by the plate reader at time t, the GFP production rate \( a_{GFP}(t) \) and RFP production rate \( a_{RFP}(t) \) are given by

\[
a_{GFP}(t) = \frac{\text{GFP}(t_{i+1}) - \text{GFP}(t_{i-1})}{2(t_{i+1} - t_{i-1})OD(t_i)}, \quad a_{RFP}(t) = \frac{\text{RFP}(t_{i+1}) - \text{RFP}(t_{i-1})}{2(t_{i+1} - t_{i-1})OD(t_i)},
\]

where \( OD(t) \) is the filtered OD level.

**Genetic circuit construction**
The genetic circuit construction was based on Gibson assembly. DNA fragments to be assembled were amplified by PCR using Phusion High-Fidelity PCR Master Mix with GC Buffer (NEB, M0532S), purified with gel electrophoresis and Zymo clean Gel DNA Recovery Kit (Zymo Research, D4002), quantified with the nanophotometer (Implen, F330), and assembled with Gibson assembly protocol using NEBuilder HiFi DNA Assembly Master Mix (NEB, E2621S). Assembled DNA was transformed into competent cells prepared by the CCMB80 buffer (TekNova, C3132). Plasmid DNA was prepared by the plasmid miniprep-classic kit (Zymo Research, D4015). DNA sequencing used Quintarabio DNA basic sequencing service. Primers and gBlocks were obtained from Integrated DNA Technologies. The list of constructs and essential DNA sequences can be found in Supplementary Information section Plasmid maps and DNA sequences. The lists of plasmids and primers are provided in Supplementary Data.

**Reporting summary**
Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

**Data availability**
Simulation, fluorescence, and growth rate data generated or analyzed during this study are included in the paper and its Supplementary Information files. A reporting summary for this article is available as a
Supplementary Information file. Source data are provided with this paper.

Code availability
Custom MATLAB (The MathWorks, Inc., Natick, MA, USA) codes are used to process the experimental data. A Supplementary Software file is provided.

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Author contributions
D.D.V. and C.B. designed the study; H.H., J.G., L.S., and C.B. designed and built the genetic circuits; C.B. performed the experiments; C.B. analyzed the data; C.B. developed the mathematical models; C.B. and D.D.V. wrote the paper.

Competing interests
The authors declare no competing interests.

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