Synthetic mixed-signal computation in living cells

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Living cells implement complex computations on the continuous environmental signals that they encounter. These computations involve both analogue- and digital-like processing of signals to give rise to complex developmental programs, context-dependent behaviours and homeostatic activities. In contrast to natural biological systems, synthetic biological systems have largely focused on either digital or analogue computation separately. Here we integrate analogue and digital computation to implement complex hybrid synthetic genetic programs in living cells. We present a framework for building comparator gene circuits to digitize analogue inputs based on different thresholds. We then demonstrate that comparators can be predictably composed together to build band-pass filters, ternary logic systems and multi-level analogue-to-digital converters. In addition, we interface these analogue-to-digital circuits with other digital gene circuits to enable concentration-dependent logic. We expect that this hybrid computational paradigm will enable new industrial, diagnostic and therapeutic applications with engineered cells.
Analogical and digital computation each have distinct advantages for cellular computing. Digital computation in synthetic and natural biological systems is useful for signal integration given its relative robustness to noise and is exemplified by decision-making circuits, such as those in developmental programs that lead cells into differentiated states. Analogue computation is useful for signal processing in synthetic or natural biological systems when the output needs to be dependent on graded information or continuous functions of the inputs, such as the sum or ratio of energy sources or signalling molecules. However, analogue signal integration is susceptible to noise, making it challenging to design robust synthetic genetic programs. Here we combine the benefits of analogue signal processing with digital signal integration to create artificial mixed-signal gene networks that carry out new hybrid functions in living cells.

Our approach is to process signals from front-end analogue sensors with composable input-discretization devices that are analogous to electronic comparators. The outputs of these devices can then be processed in a digital manner with downstream circuits. This strategy of explicitly digitizing analogue signals followed by digital computing stages is conceptually different than other mixed-signal computing approaches, such as fuzzy logic, neural networks and hybrid automata, in which analogue and digital processing are intricately coupled. However, the components developed here may be useful for future gene circuits implementing these other forms of hybrid computing. Electronic comparators compare analogue voltages between two terminals and output a digital OFF or ON signal (or ‘LO’ or ‘HI’) if $V_+ < V_-$ or $V_+ > V_-$, respectively. Rather than voltage, our genetic comparators take the concentration of an activated transcription factor as their input. The transcription factor acts a front-end sensor for continuous information (for example, the concentration of a small molecule), and should ideally operate over a wide input dynamic range to enable multiple genetic comparators with different thresholds to discretize the same input into multiple distinct outputs. In contrast to previously developed thresholding circuits that module continuous levels of gene expression in response to molecular concentration and could be used as comparators, our comparators convert molecular concentration into digital gene expression. This enabled us to create higher-order mixed-signal circuits that also take on digital gene expression states, such as 2-bit analogue-to-digital converters (ADCs) and ternary logic circuits, in contrast to previous mixed-signal circuits, such as filters that are essentially 1-bit ADCs.

The first component is the threshold module. It includes a promoter, which is regulated by the transcription factor, and a ribosome-binding site (RBS) that together set the expression level of the downstream recombinase gene and determine the threshold for comparator activation. This is in contrast to electronic comparators, where a second input can dynamically set the threshold (for example, V). The second module is the digitization module, which is composed of a recombinase whose expression is controlled by the threshold module. The recombinase digitizes the input value by inverting the orientation of a targeted DNA segment maintained at a very-low-copy number. The third module is the DNA that is inverted by the recombinase, which can contain a gene or gene-regulatory elements, such as transcriptional promoters or terminators, to alter expression of the desired output(s).

The digitization aspect of the comparator relies on recombinases, and thus we explored how the number of sites targeted by recombinases affects signal digitization into two distinct gene expression states within individual cells. The serine integrases (recombinases) we used flip, excise or integrate DNA depending on the orientation of attB and attP recombinase-recognition sites, and their activity is unidirectional unless co-factors are present. Recombinases have been used to build digital counters, integrate logic and memory, and amplify input–output transfer functions. To discretize H$_2$O$_2$ input levels, we placed the Bxb1 recombinase under the control of the oxySp promoter on a low-copy plasmid. To keep the basal level of bxb1 minimal such that there is little recombinase activity in the cell in the uninduced state, we added a ClpXP-mediated degradation tag to the 3’ end of the bxb1-coding sequence (Supplementary Fig. 3a). We tested two options as reporters for recombinase activity: a medium-copy plasmid (MCP, maintained at 20–30 copies per cell) and a bacterial artificial chromosome (BAC, maintained at 1–2 copies per cell), each of which contained a constitutive promoter upstream of an inverted gfp gene flanked by oppositely oriented attB and attP sites.

We induced bxb1 expression at different concentrations of H$_2$O$_2$ and measured GFP expression via flow cytometry (Supplementary Fig. 3b–d). We set a threshold for calling cells GFP ‘ON’ or ‘OFF’, and used this threshold to calculate the per cent of cells that was ON (%ON) at each concentration of H$_2$O$_2$ (Supplementary Note 1). The %ON versus H$_2$O$_2$ concentration data were fit to a sigmoidal function to generate input–output transfer functions (Supplementary Note 1; Supplementary Table 3). The MCP and BAC reporters had similar transfer functions, although cells using the MCP reporter had a higher per cent of cells ON at the basal H$_2$O$_2$ concentration (Supplementary Fig. 3b). However, GFP expression in cells with the MCP reporter exhibited a multi-modal distribution especially at intermediate concentrations of H$_2$O$_2$, which suggests partial plasmid flipping and thus mixed GFP expression levels in different cells (Supplementary Fig. 3d). This effect was further demonstrated by increases in the geometric mean of GFP levels with increasing H$_2$O$_2$ in the ON population (Supplementary Fig. 3f). In contrast, cells with the BAC reporter only exhibited a bi-modal distribution (Supplementary Fig. 3c), and the geometric mean of the ON population only marginally increased with H$_2$O$_2$ concentration (Supplementary Fig. 3e). Thus, we concluded that the BAC reporter converts the input concentration of H$_2$O$_2$ into digital OFF and ON gene expression states within individual cells better than the MCP reporter.

We further sought to demonstrate that our analogue-to-digital comparator circuits could be used to drive downstream circuits in a trans-acting manner. To construct a cascade, we replaced gfp in the BAC expression operon with tetR and placed gfp under the control of the TetR-regulated promoter pLetO on a
MCP (Supplementary Fig. 4). In the absence of H$_2$O$_2$, the majority of cells expressed gfp and were in the ON state. In the presence of H$_2$O$_2$, gfp expression from pLtetO was efficiently repressed and the majority of cells were switched into an OFF state. These results demonstrate that recombinase circuits can be used together with trans-acting regulation to assemble functional cascades. We also developed a method to simplify the quantification of OFF versus ON, since fluorescent gene expression levels from the BAC are low and can result in overlapping OFF and ON gene expression distributions in flow cytometry. This method amplifies the copy number of the reporter from low to high, but preserves the bi-modal nature of the OFF and ON populations, thus confirming the digital flipping of the BAC (Supplementary Fig. 5).

The threshold module of the comparator can be used to shift the discretization threshold. We created comparators with different thresholds and transition bands (for example, the input dynamic range) by assembling combinations of promoters with different transcription factor affinities, RBSs and recombinases (Fig. 1). We defined the transition band as the range of H$_2$O$_2$ concentrations across which the per cent of cells expressing the output fluorophore is between 10 and 90% as interpolated from the transfer function (though on a single cell level, gene expression is binary), and we calculated the ‘relative input range’ of the transition band to define its width (Supplementary Note 1). A narrow relative input range is indicative of low variability across the cell population around the input threshold for state switching, which is important for robustness to noise 41.

The low-threshold comparator used the Bxb1 recombinase and oxySp promoter, which is activated at low H$_2$O$_2$ concentrations. We screened different RBSs in this construct and found that none of these circuits turned ON below 1 nM H$_2$O$_2$ without also exhibiting a high basal level of recombinase activity (Fig. 1a). To address this issue and reduce basal Bxb1 expression, we used a strong RBS (RBS30) and randomly mutated the −10 region of the oxySp promoter to create a low-threshold comparator that had a transition band between 0.91 and 6.44 nM H$_2$O$_2$, giving it a relative input range of 7.10 (Fig. 1b; Supplementary Fig. 2a). To create a medium-threshold comparator, we tested different RBSs controlling phiC31 recombinase translation from the katGp promoter (Fig. 1c). A circuit with RBS31 had a transition band of 6.50–25.13 nM, which is a relative input range of 3.87 (Fig. 1d; Supplementary Fig. 2b). To create a high-threshold comparator, we used tp901 recombinase and screened different RBS and promoter combinations (Fig. 1e). We first tried the ahpCp promoter, but found that this promoter–recombinase combination had an intermediate activation threshold. We instead turned to the katGp promoter and tested different RBSs. Use of RBS33 yielded a circuit with improved behaviour, with a transition band of 15.19–85.49 nM H$_2$O$_2$ and relative input range of 5.63 (Fig. 1f; Supplementary Fig. 2c).

**Signal-processing circuits composed of genetic comparators.** Comparators with different thresholds can be composed together to build more complex signal-processing circuits in living cells (Figs 2 and 3). For example, circuits that turn gene expression ON with increasing input concentrations (as in Fig. 1) can be considered high-pass circuits (since they allow high-concentration inputs to ‘pass’ or be outputted). Next, to create low-pass circuits (which only allow low-concentration inputs to ‘pass’), we built a gene expression cassette that was ON in the basal state and used an inducible recombinase circuit to turn the output gene OFF by inverting the upstream promoter. Then, to create band-pass filters (Fig. 2), we combined a low-threshold high-pass circuit with either a medium- or high-threshold low-pass circuit (Fig. 2a,c), thus implementing the logic in Fig. 2e. The band-pass circuits switched GFP expression ON at low concentrations of H$_2$O$_2$ and switched GFP OFF at either medium or high concentrations of H$_2$O$_2$, depending on the threshold of the low-pass circuit (Fig. 2b,d; Supplementary Figs 7 and 8). The transfer function of each band-pass circuit could be predicted from straightforward addition of the transfer function of the high-pass circuit with the transfer function of the low-pass circuit that composed it (Supplementary Note 1). To determine the transfer functions of the high-pass and low-pass circuits, we measured GFP activation by the comparators using the same reporters for each recombinase as in Fig. 1 (Supplementary Figs 7 and 8). We defined the bandwidth of a band-pass filter as the relative input range over which the circuit switched from 50% ON to 50% OFF. The band-pass circuit composed of the low-threshold high pass and medium-threshold low pass had a relative input range of 3.16; the band-pass circuit composed of the low-threshold high pass and high-threshold low pass had a wider relative input range of 7.34. This circuit architecture can be adapted to create band-stop filters by making the low-threshold circuit a low pass and making the high-threshold circuit a high pass.

Higher-order signal-processing circuits can be designed to convert a single analogue input into multiple distinct outputs. For instance, we built ADCs that convert input H$_2$O$_2$ into the expression of multiple genes (Fig. 3). For example, we built a circuit that can be used to output a pair of signals that encode the information of a ternary output. The circuit measures input H$_2$O$_2$ concentration and converts it into three gene expression states that represent a confirmed low concentration (‘−1’), an intermediate concentration (‘0’) or a confirmed high concentration (‘+1’). To construct this circuit (Fig. 3a,b), we altered the band-pass circuit in Fig. 2a such that gfp was initially expressed by the proD promoter, but would be shut off by Bxb1 production. We then added a copy of rfp that could be activated by inversion of the promoter by PhiC1 production. We defined the ‘−1’ state as when >90% of cells were GFP positive and the ‘1’ state as when >90% of cells were RFP positive. This resulted in three distinct gene expression states within the cells that were toggled at different H$_2$O$_2$ concentrations (Fig. 3c; Supplementary Fig. 9). In future work, the rfp and gfp outputs could be replaced by other genetic regulators that feed into downstream computing circuits. These types of circuits could be extended to implement ternary logic, to report inequalities (such as <, = and >), or to encode distinct outputs at low or high input levels to actuate downstream circuits.

We also built a circuit where multiple comparators with different thresholds were each used to drive expression of a different fluorophore, thus implementing an ADC (Fig. 3d,e). This circuit classified H$_2$O$_2$ concentrations into one of four gene expression states in each cell ([gfp, rfp, bfp] = 000, 100, 110, 111) due to successive Bxb1, PhiC31 and TP901 expression with increasing H$_2$O$_2$, thereby encoding 2 bits of information (Fig. 3f; Supplementary Fig. 10). The relative input ranges of the threshold circuits (horizontal lines in Fig. 3f) were 7.79, 5.08 and 6.42 for gfp, rfp and bfp expression, respectively, demonstrating that the ADC operates similarly in each concentration range. The resolution of an electronic ADC is a measure of the number of output discrete values encoded across a continuous input voltage range 42. We created an analogous figure of merit for genetic ADCs, where we measure the number of bits encoded across the ADC relative input range (Supplementary Note 1). We calculated this relative resolution for our ADC to be 3.84. Adding XOR (exclusive OR) and buffer gates downstream of the current GFP, RFP and BFP outputs should implement a canonical 2-bit ADC that generates a binary 2-bit output.
**Figure 1 | Genetic comparators with different activation thresholds.** (a) The low-threshold H$_2$O$_2$ comparator circuit. OxyR is constitutively expressed from a low-copy plasmid (LCP) and activates transcription of bxb1 recombinase from either the oxySp or oxySp* promoter on the same LCP in response to H$_2$O$_2$. Bxb1 translation is altered by the strength of the ribosome-binding site (RBS). Bxb1 inverts the gfp expression cassette located between inversely oriented attB and attP sites (triangles) on a bacterial artificial chromosome (BAC), thus turning on GFP expression. The gfp cassette has a ribozyme sequence for cleaving the 5’-untranslated region of an mRNA transcript (Riboj)61, a computationally designed RBS62, the gfp-coding sequence and a transcriptional terminator. (b) The percentage of GFP-positive cells at different H$_2$O$_2$ concentrations as measured by flow cytometry. Different combinations of the oxySp and oxySp* promoters, and RBSs exhibit different H$_2$O$_2$ thresholds and basal levels for GFP activation. The oxySp* and RBS30 combination (red diamonds) had the lowest threshold and a narrow transition band (shaded region). (c) The medium-threshold H$_2$O$_2$ comparator circuit. The same as a, except with the katGp promoter instead of the oxySp or oxySp* promoters, and phiC31 recombinase and its att inversion sites instead of bxb1 recombinase and its att inversion sites. (d) Different combinations of the katGp promoter and RBSs had different H$_2$O$_2$ thresholds and basal levels for GFP activation. The katGp and RBS31 combination (red triangles) had a medium H$_2$O$_2$ threshold and narrow transition band (shaded region). (e) The high-threshold H$_2$O$_2$ comparator circuit. The same as a, except with either the katGp promoter or ahpCp promoter instead of the oxySp or oxySp* promoters, and tp901 recombinase and its att inversion sites instead of bxb1 recombinase and its att inversion sites. (f) Different combinations of katGp and ahpCp promoters and RBSs exhibited different H$_2$O$_2$ thresholds for GFP activation. The katGp and RBS33 combination (red diamonds) had the highest threshold and a narrow transition band (shaded region). Lines are sigmoidal fits to the data (Supplementary Note 1). The errors (s.d.) are derived from flow cytometry experiments of three biological replicates, each of which involved n > 30,000 gated events.

**A mixed-signal-processing gene circuit.** Analogue-to-digital circuits can be further interfaced with digital circuits to form mixed-signal-processing circuits (Fig. 4). We built a variant of the band-pass circuit where the low-threshold comparator and medium-threshold comparator circuits both flip the directionality of gfp. This resulted in an analogue-to-digital circuit where only...
intermediate H$_2$O$_2$ levels enable GFP production, which is analogous to an XOR gate on H$_2$O$_2$ concentrations digitized using two different thresholds (Fig. 4a,b). In addition, we placed tp901 under control of the TetR-repressed pLtetO promoter and constitutively expressed tetR, thereby making tp901 digitaly inducible by anhydrotetracycline (aTc) 49. We then used tp901 to control the direction of the promoter driving transcription of gfp.

We assayed GFP levels at different H$_2$O$_2$ concentrations in the presence and absence of aTc, and found a majority of GFP-positive cells only at intermediate concentrations of H$_2$O$_2$ and when aTc was absent (Fig. 4b), thus implementing the concentration-dependent logic shown in Fig. 4c. Concentration-dependent logic could allow cells to carry out distinct activities at intermediate input levels, as opposed to extreme ones, and to encode a greater density of information into biological signals.

**Discussion**

We have shown that cells can be engineered to implement synthetic computations that convert continuous information into discrete information. These computations rely on gene circuits that threshold and discretize signals from sensors, analogous to comparators in electronics. Our basic comparator design should be adaptable to other cellular contexts and for sensing inputs besides chemical concentration, such as light 13 or contact 10. There are other known ways to implement thresholding circuits 20–23 and to dynamically alter thresholds 24, suggesting that it would be possible to implement a negative input terminal analogous to that in electronic comparators, rather than the fixed threshold that we implemented here.

Our comparators can be composed together to build multi-threshold ADCs. In contrast to previously described genetic
band-pass filters\textsuperscript{25–28}, our band-pass filters convert continuous information into distinct gene expression states instead of altering continuous gene expression. Furthermore, the outputs from our ADCs can be integrated with other digital circuits (Fig. 4). Alternatively, multiple analogue signals could be integrated at the front end to calculate complex analogue functions\textsuperscript{11} before feeding the output(s) into downstream ADCs. We have engineered the outputs of our circuits to be Boolean (Figs. 2 and 4),
ternary (Fig. 3a–c) or multi-state digital (Fig. 3d–f). It may be possible to further increase ADC resolution by increasing the number of comparators across the same range of H$_2$O$_2$ or by adding comparators that can respond to lower or higher concentrations of H$_2$O$_2$.

There are a number of potential challenges involved in scaling mixed-signal gene circuits. First, it is important that comparators do not substantially affect cell growth. We found that the number of plasmids on which comparator circuits are encoded impacted cell growth more than the number of comparator circuits (Supplementary Figs 13 and 14). Thus, to scale mixed-signal computation, it will be important to decrease the number of episomal DNA constructs, for example, by moving comparators to the chromosome. Furthermore, to increase ADC resolution,
comparators will need to have sharper thresholds at the population level (that is, more consistency in the behaviour of each cell around the threshold point). We surmise that this may be possible by implementing negative feedback in the analogue sensor circuit, which can reduce population-level heterogeneity\(^44\). Screening large promoter/RBS/recombinase libraries could enable the identification of circuits that implement various thresholds on a given analogue input. Using novel orthogonal recombinases could aid in the scaling of mixed-signal gene circuits\(^14\). For certain analogue inputs, it may also be necessary to implement a graded positive-feedback\(^11\) or negative-feedback loop\(^45\) to enable wide input dynamic range activation of the sensor transcription factor.

ADCs are the complement of digital-to-analogue converters (DACs): ADCs convert an analogue input signal into discrete output signals, whereas DACs convert discrete input signals into analogue output signals (Supplementary Fig. 12a–c). For example, DACs that we previously implemented in living cells accepted two digital inputs and produced four different gene expression levels as outputs depending on the specific combination of inputs (Supplementary Fig. 12d)\(^{36}\). Here we built ADCs that translate a single analogue input in the form of inducer concentration to multiple discrete outputs, represented by triggering the expression of different genes (Supplementary Fig. 12e).

These mixed-signal circuits constitute a first step towards advanced analogue–digital hybrid computational approaches. For instance, to implement an artificial neural network circuit, multiple analogue inputs could be fed into the promoter controlling recombinase expression, and the weights of the analogue inputs could be tuned via their binding affinity to the promoter. Linking these artificial circuits together could allow the creation of artificial neuronal networks in living cells. The comparators could also be used in a hybrid automaton if they were integrated with state machines, wherein states switch based on analogue thresholds. In addition, the ternary logic circuit (Fig. 3a) could be used to implement fuzzy logic by converting the ‘0’ state into the expression of a third gene.

We envision that mixed-signal processing will enable a wide range of industrial\(^46\), diagnostic and therapeutic applications using engineered cells\(^47,48\). For example, cells could be designed to produce quorum-sensing signals that trigger multiple distinct production pathways as the quorum-sensing molecules accumulate in a bioreactor. The first phase could be focused on biomass accumulation, the second phase dedicated to secreting the desired product, such as a biologic protein drug fused to a secretion tag, and the third committed to secreting product-modifying enzymes, such as a protease to separate the secretion tag from the active drug. Such behaviour could be programmed with an ADC that senses the concentration of an accumulating quorum-sensing molecule as an input and triggers successive circuits with higher concentrations, similar to the system shown in Fig. 3d,e. As a first step towards such industrial applications, we scaled up the operational volume of the ADC circuit by 100 × and found the circuit functioned, albeit with shifted thresholds (Supplementary Fig. 15).

In addition, cells could be designed to detect continuous quantities of multiple biomarkers, integrate these signals to diagnose disease conditions and produce reporter output(s) for non-invasive biosensing applications. For instance, probiotic or commensal\(^49\) bacteria could be engineered to sense the concentration of multiple biomarkers for inflammatory bowel disease (for example, reactive oxygen species, nitric oxide and blood), discretize the magnitude of each of these analogue signals using ADCs with a range of thresholds, integrate the resulting information with Boolean logic (for example, a multi-input AND gate) to decide whether a disease flare-up is occurring and how severe it is, and produce discrete reporters that can be detected outside of the body. Reporting on disease states and severity with digitized outputs (for example, different fluorescent or colorimetric reporters) could be more robust than analogue outputs (for example, a single fluorescent reporter expressed at different levels), since the latter is more susceptible to noise. Our ADCs could also be used as peak detectors due to the inherent memory feature of recombinase-based switches. For instance, probiotic bacteria could be engineered to remember the maximum concentration of a biomarker that they detected while passing through the intestine. Similar circuits could be used to create environmental sensors that sense and record maximum pollutant levels\(^50\).

Mixed-signal circuits could also be useful for engineering cell therapies whose therapeutic outputs are regulated by quantitative levels of disease biomarkers. For example, mammalian gene circuits could be designed such that blood glucose levels below the normal region (‘−1’ in a ternary logic system) would switch on glucagon secretion, blood glucose levels in the desired region (‘0’ in a ternary logic system) would result in no hormone secretion and blood glucose levels above the normal region (‘1’ in a ternary logic system) would trigger insulin secretion. The ability to trigger distinct outputs in response to different conditions could enable new ‘homeostatic’ therapies. As a first step towards such applications, we tested the temporal response of the ternary logic circuit (Fig. 3a) and found that the cells likely induce recombination well before a change in fluorescent protein gene expression is observed (Supplementary Fig. 16). Such applications would benefit from resettable mixed-signal circuits, which could be implemented using transcriptional regulators, rather than the permanent-memory mixed-signal circuits described here.

In summary, mixed-signal gene circuits merge analogue and digital signal processing to enable both continuous information sensing and robust multi-signal integration, and computing in living cells. Ultimately, we expect that this hybrid analogue–digital computational paradigm will allow synthetic biological systems to begin to approach the nuanced complexities found in natural biological systems\(^14,15,18,49,50–60\).

**Methods**

**Strains and plasmids.** All plasmids were constructed using PCR and Gibson assembly starting from DNA sources as referenced in Supplementary Table 2 or from gBlocks manufactured by IDT. E. coli EP300 (F− mcrA Δ[mmr-hsdRMS-mcrBC] Δbsd2 Δ[λ2amu263 lacX74 recA1 endA1 Δ864 proA1 araD139 [F− proA1 endA1 Δ864 (araD139 proA1 lacIqp lacZΔM15 Δ(λpro) lacY1 Δ[lacX74 proA1 araD139 XylA araE1 trpE1 lacY1])lacY1]) was used for all experiments. Plasmid and plasmid DNA can be obtained at Addgene under Addgene ID numbers 78211–78229.

**Circuit characterization.** Plasmids were transformed into chemically competent E. coli EP300, plated on LB medium with appropriate antibiotics and grown overnight at 37 °C. Antibiotic concentrations were carbenicillin (50 μg ml\(^−1\)), kanamycin (30 μg ml\(^−1\)) and chloramphenicol (35 μg ml\(^−1\)). The next day, single colonies were inoculated into Tekno Hi-Def Azure Media with appropriate antibiotics and 0.2% glucose, and incubated shaking aerobically for 16–18 h at 37 °C. Cultures were then diluted 2,500 × into fresh Hi-Def Azure Media with appropriate antibiotics and 0.2% glucose, and shaken for 20 min aerobically at 37 °C. After 20 min, 200 μl of culture was transferred to a 96-well plate, and H₂O₂ (Sigma–Aldrich H201009-100ML) was added at appropriate concentration via serial dilution. For the experiment in Fig. 4, aTc (Cayman Chemical 10009542) was added to a final concentration of 75 ng ml\(^−1\). Plates were incubated aerobically with shaking for 20h at 30 °C for all experiments except those in Supplementary Fig. 1, in which plates were incubated for 3 h. After incubation, the optical densities of cultures were measured at 600 nm in a plate reader. For experiments in Supplementary Figs 3–5, cells were then assayed on the flow cytometer. For all other experiments (Figs 1–4; Supplementary Figs 6–11), cells were washed with PBS, diluted 8 × into fresh Hi-Def Azure Media with appropriate antibiotics, 0.4% glucose and 1 × CopyControl Induction Solution (Epicentre), and incubated shaking aerobically for a further 10 h at 30 °C. After this incubation, the optical densities of cultures were measured at 600 nm in a plate....
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Data availability. Plasmids described in this study have been deposited into the Addgene repository under Addgene ID numbers 78211–78229. The authors declare that all other data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the corresponding author upon request.

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Author contributions

J.R.R. and T.K.L. conceived the study. J.R.R. and G.S. performed the experiments and collected the data. All authors analysed the data, discussed the results and wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: J.R.R., G.S. and T.K.L. have filed a provisional patent application based on this work (‘Analogue to Digital Computations in Biological Systems’, PCT/US2015/067381).

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