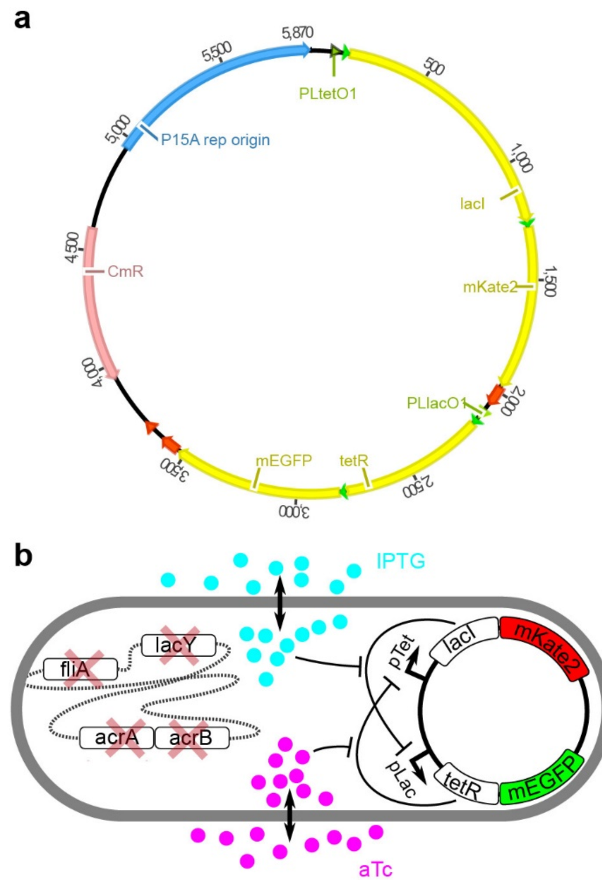
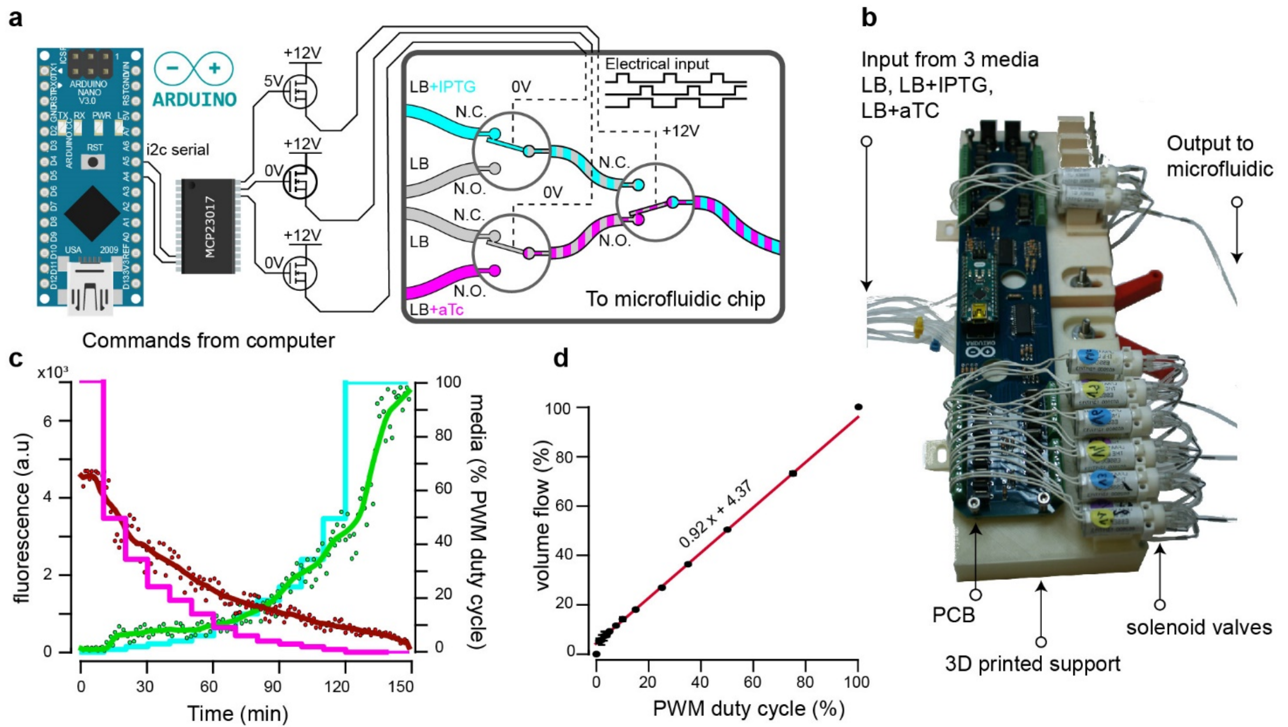
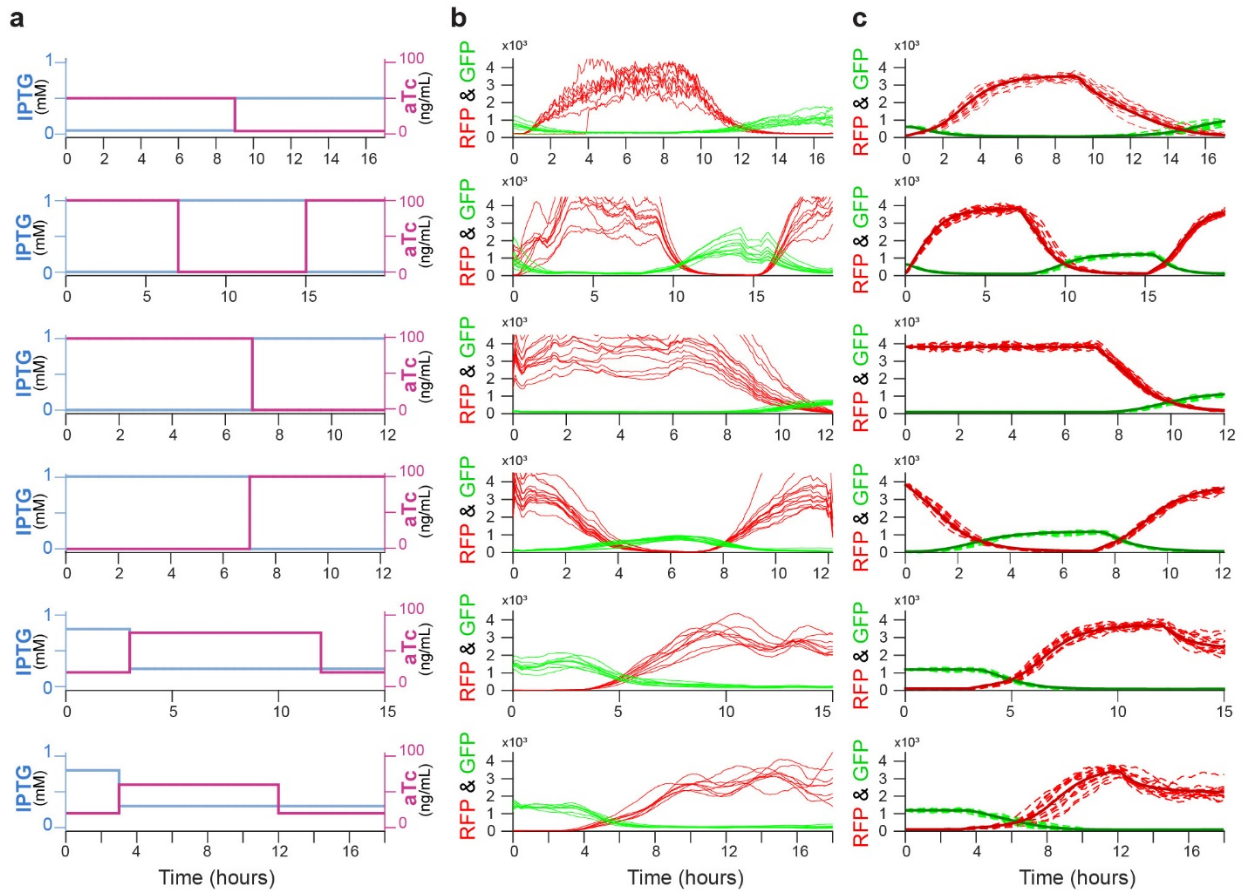


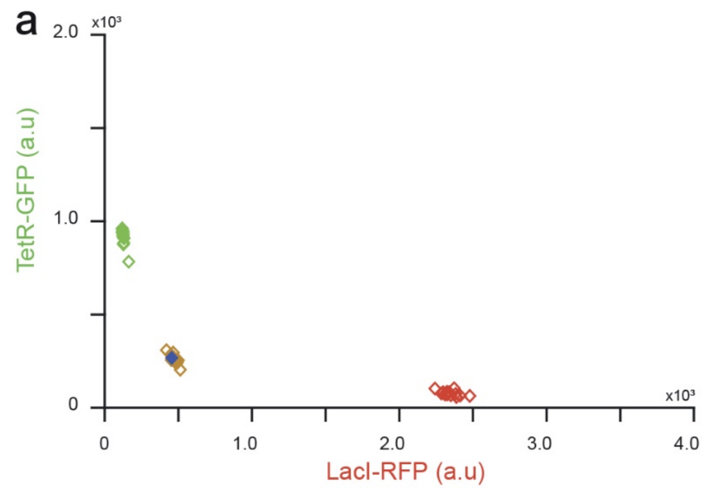
Supplementary Figure 1. Toggle switch cells. a. Map of the toggle switch plasmid. Yellow arrows are coding sequences (*lacI-mKate2* and *tetR-mEGFP*). Light green arrows are promoters (pLac and pTet). Dark green arrows represent ribosome binding sites (RBS). Red arrows represent terminators. The chloramphenicol resistance cassette and plasmid origin of replication are shown in pink and blue, respectively. **b**. The cellular chassis is based on the K-12 BW25113 background with the *fliA*, *lacY*, *acrA* and *acrB* genes knocked out. IPTG and aTc enter the cell by passive diffusion through the cellular membrane and interact with the LacI and TetR proteins. The cellular state is observed by monitoring the fluorescence levels of mEGPF (TetR) and mKate2 (LacI).



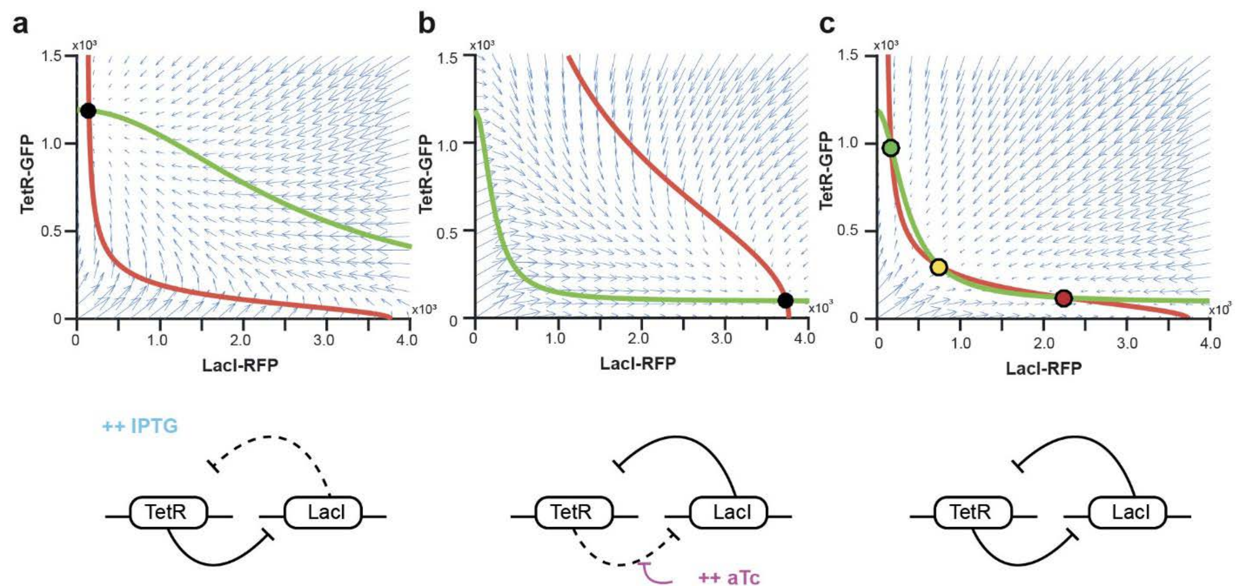
Supplementary Figure 2. Characterization of mixing device. **a**. Sketch of the electronic circuit controlling the fluidic valve and the principle of mixing in Pulse Width Modulation (PWM) mode. **b**. Actual mixing device showing the two triplets of valves required to run two experiments in parallel. **c**. Various concentrations of fluorescein and rhodamine were flowed through the main microfluidic device and the levels of fluorescence in the chambers were monitored. The fluorescence levels closely follow the mixing levels imposed by the valve system. **d**. Calibration experiment: PWM mixing was run over extended periods of time at different duty cycles, and volume consumption was measured.



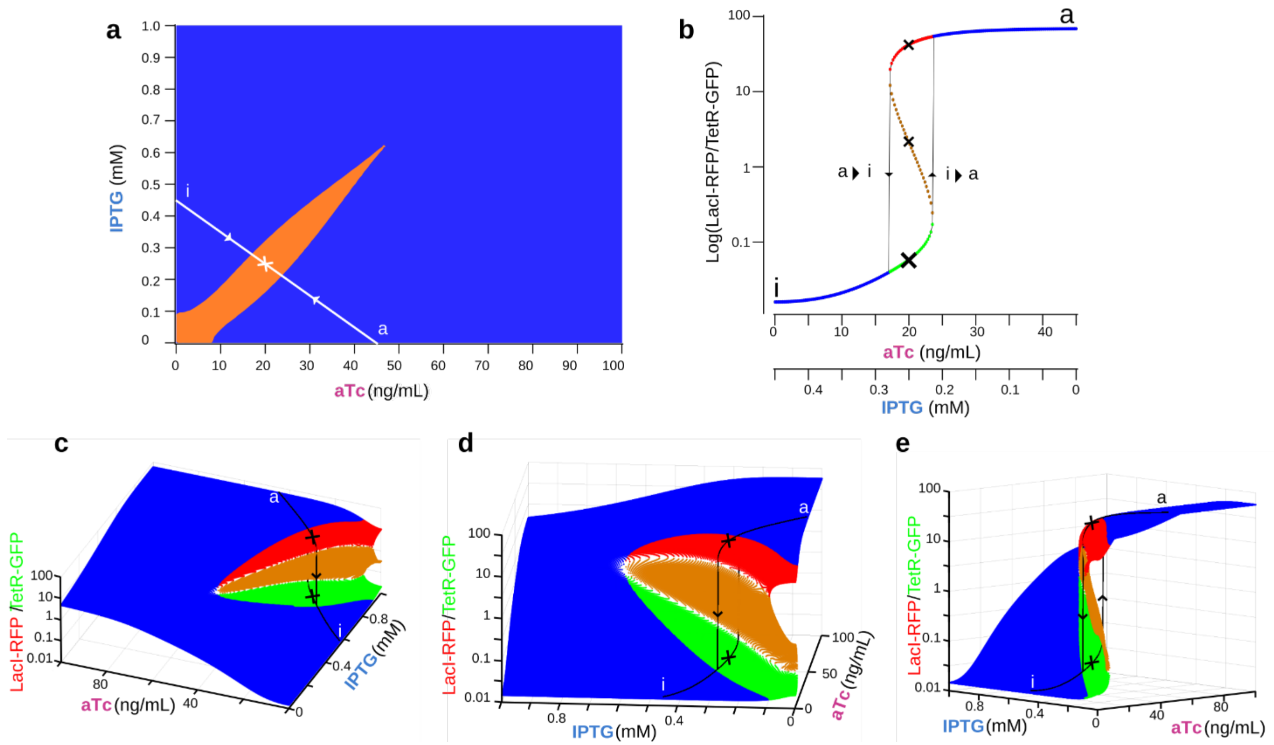
Supplementary Figure 3. Model calibration. a. Several temporal profiles using different levels of aTc and IPTG inducers were used to construct an experimental data set to characterize the behavior of the system. **b**. Corresponding temporal evolution of single cells (n ~ 10 cells per experiments). Red and green lines correspond to single-cell LacI-RFP and TetR-GFP fluorescence levels in arbitrary units. **c.** Predictions obtained using the model fitted to the average behavior of all experiments. Thick red and green curves correspond to the behavior of the deterministic model. Thinner dotted lines correspond to predictions obtained using the stochastic implementation of the model. Naturally, as the stochastic model only captures intrinsic variability and as all simulations started from the same initial state, we expected that the variability of the simulated cells was smaller than the one of the observed cells.



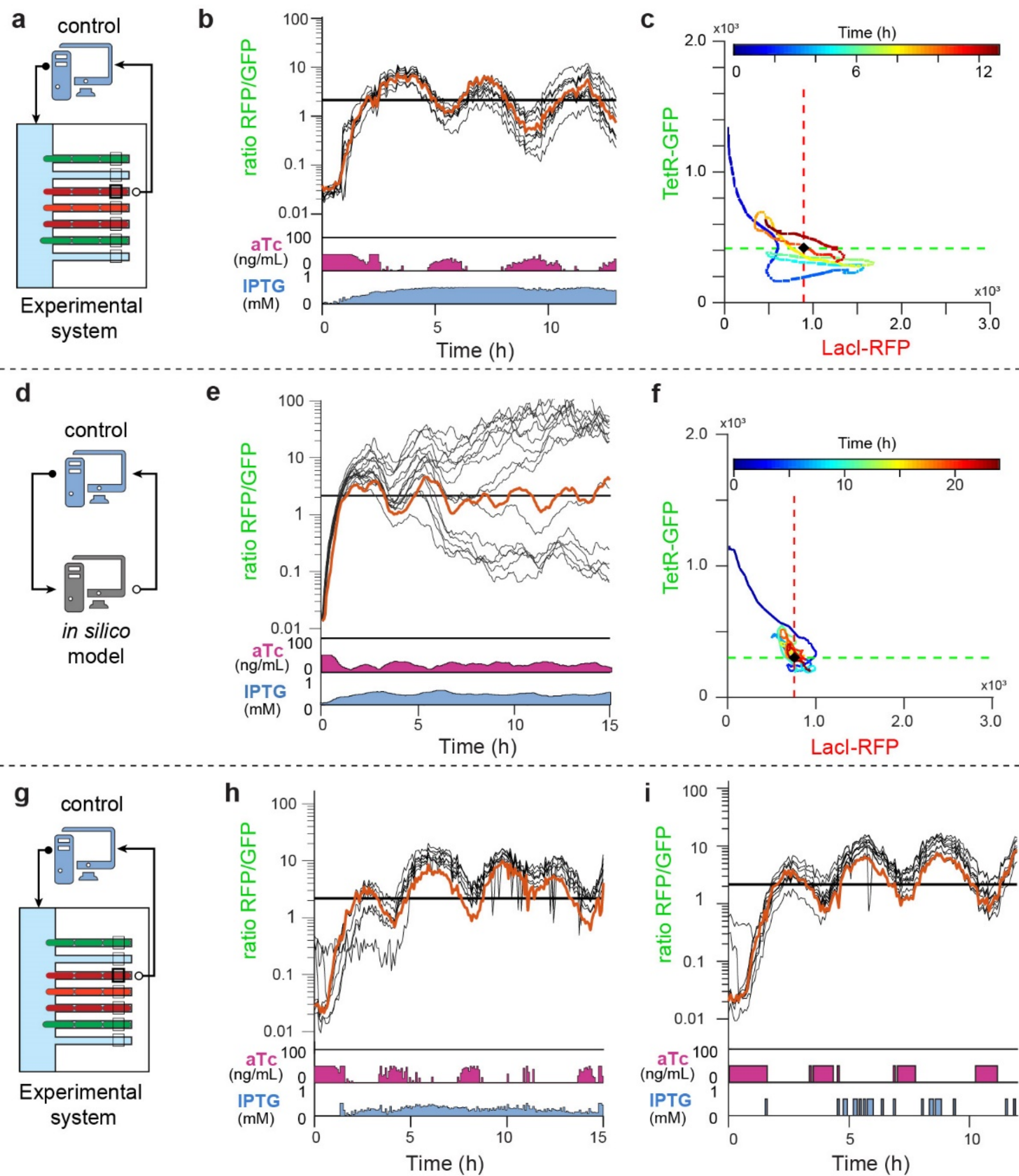
Supplementary Figure 4. Capacity of the characterization data to constrain the locations of the equilibrium points of the deterministic model. Distribution of the equilibrium points in the protein space under reference conditions as predicted using the 15 best-fitting parameter sets when the optimization was iterated 30 times. LacI-dominant and TetR-dominant stable equilibria are represented by red and green diamonds, whereas unstable equilibria are represented by yellow diamonds. The set-point used in control experiments is represented by a blue square.



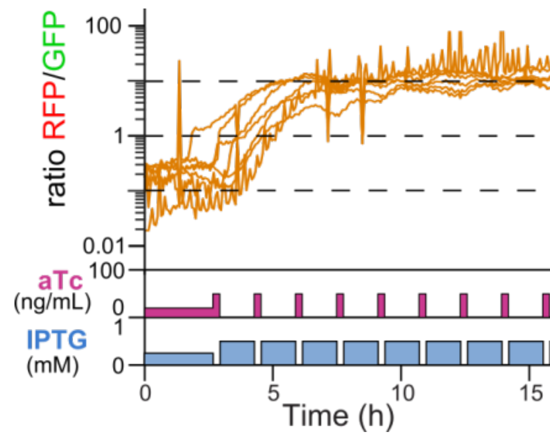
Supplementary Figure 5. Influence of IPTG and aTc on the bistable behavior of the toggle switch. a. State space in the presence of an excess of IPTG (1 mM) as predicted by the numerical model. Addition of IPTG turns off the repression of the *tetR* gene by LacI. As a result, TetR is fully expressed and the system only presents one stable, attractive state: all cells will turn green eventually. **b.** Similarly, an excess of aTc (100 ng.mL^{-1}) turns off the repression of the *lacI* gene by TetR. As a result, LacI is fully expressed and cells will eventually reach the unique stable state (high level of LacI-RFP). **c.** An interesting case occurs when both branches are in competition (aTc = 20 ng.mL^{-1}, IPTG = 0.25 mM), so that there are two stable equilibria and one unstable equilibrium (see Figure 1 and Main text) and reproduced here numerically using a deterministic model. The red and green curves shown in (**a-c**) are the LacI and TetR nullclines computed using the fitted model under different operating conditions. By definition, equilibria are located at nullcline intersections.



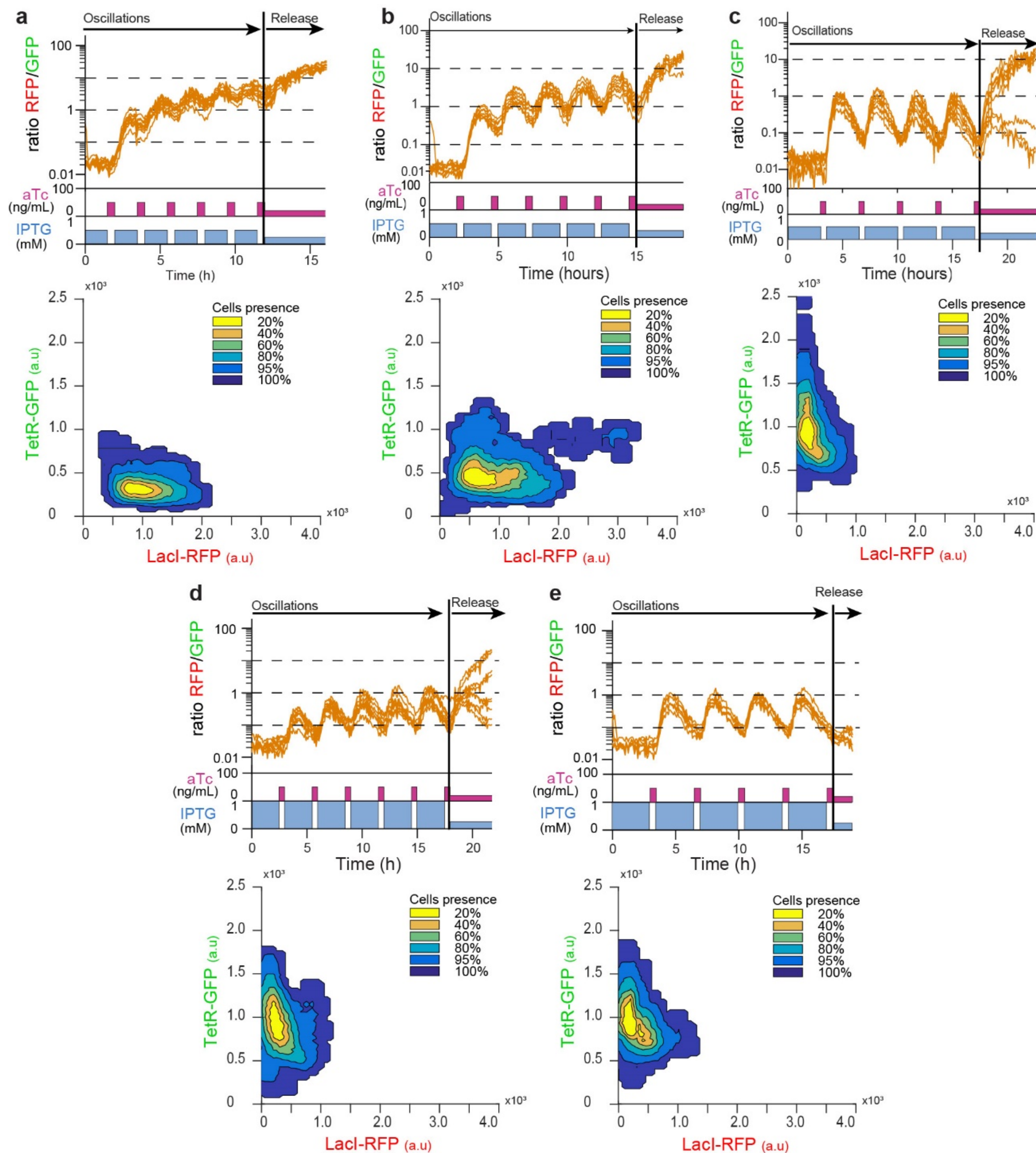
Supplementary Figure 6. Stability analysis of the toggle switch circuit in different environments. a. Representation of the stability of the circuit as a function of inducer concentrations. Blue and orange regions represent the conditions under which the system is mono- or bi-stable, respectively. The white segment going from point a (full aTc; aTc = 45 ng.mL^{-1}, IPTG = 0) to point i (full IPTG; aTc = 0, IPTG = 0.45 mM) passes through the reference concentrations for this study (white cross, aTc = 20 ng.mL^{-1} and IPTG = 0.25 mM). **b**. Logarithm of the LacI-RFP/TetR-GFP ratio of the stable and unstable equilibrium points are represented for different concentrations of aTc and IPTG along the white segment of panel **(a)**. Monostable points are represented in blue. LacI-dominant and TetR-dominant stable points of the bistable region are represented in red and green, respectively. Unstable equilibrium points of the bistable region are represented in orange. Along this line, the system shows hysteresis. **c-e**. Same representation of the stability of the equilibria of system as in **(b)** represented in the aTc/IPTG plane. Different views of the cusp-catastrophe curve. The hysteresis line from panel **(a)** and **(b)** is represented in black.



Supplementary Figure 7. Other example of PI control experiments. a. Experimental control runs. **b.** Ratio of red (LacI-RFP) and green (TetR-GFP) fluorescence levels as a function of time using a double PI controller to drive protein expressions. The parameters of the PI dual controller are $K_P^L = 5.0\ 10^{-2}$, $K_I^L = 2.0\ 10^{-4}\ s^{-1}$, $K_P^T = 2.5\ 10^{-2}$, $K_I^T = 6.94\ 10^{-4}\ s^{-1}$ (see Methods). Thick orange and thin black lines represent controlled and non-controlled cells (n = 10), respectively. **c**. Trajectory of the controlled cells in the state space. The cell starts from a high TetR-GFP state and then moves towards the target point (black diamond) while describing large oscillations around the target. Time is color coded from *t* = 0 h (blue) to t = 12 h (red). **d.** *In silico* control runs. **e**. Ratio of red (LacI-RFP) and green (TetR-GFP) fluorescence levels as a function of time using a double PI controller to drive simulated cells (n = 20). The PI parameters are $K_P^L = 5.0\ 10^{-2}$, $K_I^L = 2.0\ 10^{-4}\ s^{-1}$, $K_P^T = 2.5\ 10^{-2}$, $K_I^T = 6.94\ 10^{-4}\ s^{-1}$. **f**. Trajectory of the controlled cell for *in silico* control experiments. **g.** Experimental control runs. **h.** Ratio of red (LacI-RFP) and green (TetR-GFP) fluorescence levels of single cells (n = 8) as a function of time using a double PI controller to drive protein expressions. The PI parameters are $K_P^L = 5.0\ 10^{-2}$, $K_I^L = 2.0\ 10^{-4}\ s^{-1}$, $K_P^T = 2.5\ 10^{-2}$, $K_I^T = 6.94\ 10^{-4}\ s^{-1}$. **i.** Ratio of red (LacI-RFP) and green (TetR-GFP) fluorescence levels of single cells (n = 8) as a function of time using a double Bang-bang controller to drive protein expressions.



Supplementary Figure 8. Too Fast periodic forcing experiments. Ratio of the red (LacI-RFP) and green (TetR-GFP) fluorescence levels of cells subjected to periodic stimulations with alternating high aTc and high IPTG concentrations. The cells were grown in 0.25 mM IPTG and 20 ng.mL^{-1} aTc prior to the experiment and during the first 2.5 hours. They were then subjected to alternations of 0.5 mM IPTG for 75 min, 50 ng.mL^{-1} aTc for 15 min. The cells do not remain in a state of balanced expression, and instead drift to the LacI-RFP high state.



Supplementary Figure 9. Additional periodic forcing experiments. Ratio of the red (LacI-RFP) and green (TetR-GFP) fluorescence levels of cells subjected to periodic stimulations with alternating high aTc and high IPTG concentrations. All cells present a similar behavior and remain in a state of balanced expression. Cell probability of presence for the two last input periods is shown below. Experimental conditions were: **(a)** 0.5 mM IPTG for 90 min, 50 ng.mL^{-1} aTc for 30 min, n=12; **(b)** 0.5 mM IPTG for 120 min, 50 ng.mL^{-1} aTc for 30 min, n = 10; **(c)** 0.5 mM IPTG for 180 min, 50 ng.mL^{-1} aTc for 30 min, n = 10; **(d)** 1.0 mM IPTG for 150 min, 50 ng.mL^{-1} aTc for 30 min, n = 9; **(e)** 1.0 mM IPTG for 180 min, 50 ng.mL^{-1} aTc for 30 min, n = 7. As expected, the average ratio shifted to RFP when the periodic forcing favored aTc over IPTG. At some point, periodic forcing was stopped to observe release of the cells. Cells were **(a-b)** subsequently attracted to the RFP dominant state; **(c-d)** split into two sub-populations attracted to either RFP or GFP dominant states; or **(e)** attracted to the GFP dominant state.

Supplementary Table 1

Transcription rates (mRNA min^{-1})	κ_L^{m0}	3.20e-2 (3.045e-1)	*plac* regulation by TetR	θ_{LacI}	31.94 (124.9)
	κ_T^{m0}	1.19e-1 (3.313e-1)		η_{LacI}	2.00 (2.00)
	κ_L^{m}	8.30 (13.01)		θ_{IPTG}	9.06e-2 (2.926e-1)
	κ_T^{m}	2.06 (5.055)		η_{IPTG}	2.00 (2.00)
Translation rates (a.u. mRNA^{-1} min^{-1})	κ_L^{p}	9.726e-1 (6.606e-1)	*ptet* regulation by LacI	θ_{tetR}	30.00 (76.40)
	κ_T^{p}	1.170 (5.098e-1)		η_{TetR}	2.00 (2.152)
Degradation rates (min^{-1})	g_L^{m}	1.386e-1		θ_{aTc}	11.65 (35.98)
	g_T^{m}	1.386e-1		η_{aTc}	2.00 (2.00)
	g_L^{p}	1.65e-2	IPTG exchange rate (min^{-1})	$\kappa_{IPTG}^{\text{in}}$	2.75e-2 (4.00e-2)
	g_T^{p}	1.65e-2		$\kappa_{IPTG}^{\text{out}}$	1.11e-1 (4.00e-2)
			aTc exchange rate (min^{-1})	κ_{aTc}^{in}	1.62e-1 (N/A)
				$\kappa_{aTc}^{\text{out}}$	2.00e-2 (N/A)

Supplementary Table 1. Parameters for the toggle switch model. Threshold parameters are expressed in arbitrary fluorescence units (a.u.) for proteins (θ_{LacI} and θ_{TetR}), ng.mL^{-1} for aTc (θ_{aTc}), and mM for IPTG (θ_{IPTG}). The values of 4 parameters were fixed. mRNA degradation rates were fixed based on typical half-life of 5 minutes for mRNAs in *E. coli* (1). Protein apparent degradation rates were set based on estimates of cell cycle times in our experiments (~42 min). We therefore assumed that dilution due to growth dominates over actual protein degradation. Note that the significant difference between the mRNA and protein apparent degradation rates allowed us to employ a quasi-steady state assumption in which mRNAs are assumed to be at the steady state levels set by the concentration of the repressors. The dynamics of the 4D system could then be represented in the 2D protein space. For the sake of simplicity, in the main text the latter is called the state space. Realistic initial values for the search were obtained as explained below. Previous work has quantified that the number of mRNAs produced by a fully induced *lac* promoter is ~60 per cell (2). Then the comparison of regulatory ranges and leakage levels given in (3) led to us estimate that the transcription rate of a fully induced *tet* promoter is ~25% higher than the one of *plac*. This provided us with estimates of transcription rates. Lastly, an estimate of translation rates in arbitrary fluorescence units can be deduced from fluorescence levels at maximal induction (3700 a.u. for LacI and 1200 a.u. for TetR) and previous parameter estimates. Global optimization was then used to fit model predictions to calibration data, leading to a first model (parameter values in parenthesis). The addition of non-identical exchange rates in and out of the cell called for a recalibration of the parameters. This has been done by global optimization followed by limited manual adjustments, and has led to the reference model. Lastly, initial conditions were the steady state concentrations that correspond to the pre-incubation media (1 mM IPTG and no aTc, unless specified otherwise).

Supplementary Note 1

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