Supplemental Information

S.1 Comparison of TS and TES circuits

S.1.1 Equations for bistable TES

This set of equations describes the bistable TES in figure 1 of the main paper. The parameters values used to produce the rate-balance plot in figure 1e is shown in S.1.3

$$\frac{d}{dt}m_{cI} = V_{P_{RM}} NC \left[\frac{1}{1+\left(\frac{K_{CI}}{p_{CI}}\right)^{n_r}} + \ell_{P_{RM}} \right] - \gamma_m m_{cI} \quad (1)$$

$$\frac{d}{dt}p_{CI} = \epsilon_{CI} m_{cI} - \gamma_{CI} p_{CI} - \frac{p_L k_{cat}}{1+\frac{K_M}{p_{CI}}} \quad (2)$$

$$\frac{d}{dt}m_t = V_{P_{RM}} NC \left[\frac{1}{1+\left(\frac{K_{CI}}{p_{CI}}\right)^{n_r}} + \ell_{P_{RM}} \right] - \gamma_m m_t \quad (3)$$

$$\frac{d}{dt}p_T = \epsilon_T m_t - \gamma_T p_T \quad (4)$$

$$\frac{d}{dt}m_l = V_{P_{LtetO-1}} NC \left[\frac{1}{1+\left(\frac{p_T}{K_T}\right)^{n_t}} + \ell_{P_T} \right] - \gamma_m m_l \quad (5)$$

$$\frac{d}{dt}p_L = \epsilon_L m_l - \gamma_L p_L \quad (6)$$

S.1.2 Equations for bistable TS

This set of equations describes the bistable TS show in S.1.4 and rate-balance plot S.1.5. The parameters values used to produce the rate balance plot in S.1.5 are shown in S.1.2.

$$\frac{d}{dt}m_{cI} = V_{P_{RM}}NC\left[\frac{1}{\left\{1+\left(\frac{K_{CI}}{p_{CI}}\right)^{n_r}\right\}\left\{1+\left(\frac{p_{CI434}}{K_{434}}\right)^{n_{434}}\right\}} + \ell_{P_{RM}}\right] - \gamma_m m_{cI} \quad (1)$$

$$\frac{d}{dt}p_{CI} = \epsilon_{CI}m_{cI} - \gamma_{CI}p_{CI} \quad (2)$$

$$\frac{d}{dt}m_t = V_{P_{RM}}NC\left[\frac{1}{\left\{1+\left(\frac{K_{CI}}{p_{CI}}\right)^{n_r}\right\}\left\{1+\left(\frac{p_{CI434}}{K_{434}}\right)^{n_{434}}\right\}} + \ell_{P_{RM}}\right] - \gamma_m m_t \quad (3)$$

$$\frac{d}{dt}p_T = \epsilon_T m_t - \gamma_T p_T \quad (4)$$

$$\frac{d}{dt}m_{CI434} = V_{P_{LtetO-1}}NC\left[\frac{1}{1+\left(\frac{p_T}{K_T}\right)^{n_t}} + \ell_{P_T}\right] - \gamma_m m_{CI434} \quad (5)$$

$$\frac{d}{dt}p_{CI434} = \epsilon_{CI434}m_{cI434} - \gamma_{CI434}p_{CI434} \quad (6)$$

S.1.3 List of parameters

Parameters and Variables	Description	Units	Value for TES	Value for TS
m_{cI}	mRNA concentration for *cI*	M		
m_t	mRNA concentration for *tetR*	M		
m_l	mRNA concentration for *mf-lon*	M		
p_{CI}	Protein concentration for CI	M		
p_T	Protein concentration for TetR	M		
p_L	Protein concentration for *mf*-Lon	M		
$V_{P_{RM}}$	Velocity for mRNA production for P_{RM}	$M \cdot s^{-1}$	.3 [46]	.3 [46]
$V_{P_{LtetO-1}}$	Velocity for mRNA production for $P_{LtetO-1}$	$M \cdot s^{-1}$	.3 [30]	.3 [30]
$\ell_{P_{RM}}$	mRNA leakage of P_{RM} promoter	$M \cdot s^{-1}$	1/10 [47]	1/10 [47]
ℓ_{P_T}	mRNA leakage of $P_{LtetO-1}$ promoter	$M \cdot s^{-1}$	1/5050 [30]	1/5050 [30]
K_{CI}	Disassociation constant of CI to P_{RM}	M	2.5×10^{-8} [33]	2.5×10^{-8} [33]
K_{CI434}	Disassociation constant of CI434 to P_{RM}	M		2.5×10^{-8} [32]
K_T	Disassociation constant of TetR to $P_{LtetO-1}$	M	1.79×10^{-10} [48]	1.79×10^{-10} [48]
K_m	Michaelis constant for *mf*-Lon	M	3.7×10^{-6} [25]	3.7×10^{-6} [25]
k_{cat}	Catalytic rate of *mf*-Lon	s^{-1}	.071 [25]	.071 [25]
γ_m	Degradation rate of mRNA	s^{-1}	2.38×10^{-3} [49]	2.38×10^{-3} [49]
γ_{CI}	Degradation rate of CI	s^{-1}	2×10^{-4}	2×10^{-4}
γ_{CI434}	Degradation rate of CI434	s^{-1}		2×10^{-4}
γ_T	Degradation rate of TetR	s^{-1}	7×10^{-4} [39]	2×10^{-4}
γ_L	Degradation rate of *mf*-Lon	s^{-1}	7×10^{-4} [39]	2×10^{-4}
ϵ_{CI}	Translation rate for CI	s^{-1}	4.5×10^{-5}	3×10^{-5}
ϵ_{CI434}	Translation rate for CI	s^{-1}		5×10^{-5}
ϵ_T	Translation rate for TetR	s^{-1}	3×10^{-8}	6×10^{-7}
ϵ_L	Translation rate for *mf*-Lon	s^{-1}	3.5×10^{-5}	
n_r	Hill coefficient for P_{RM}		2[50]	2[50]
n_{434}	Hill coefficient for P_{RM}		1[50]	1[50]
n_t	Hill coefficient for $P_{LtetO-1}$		2[50]	2[50]
N	Plasmid copy number for psc101		3	3
C	Concentration of a single protein/mRNA in a typical bacterium	M	1.5×10^{-9}[51]	1.5×10^{-9}[51]

It should be noted the degradation rate of all proteins in TS is the doubling time of the cell, indicating the absence of *ssrA* degradation tags.

S.1.4 Schematic for TS

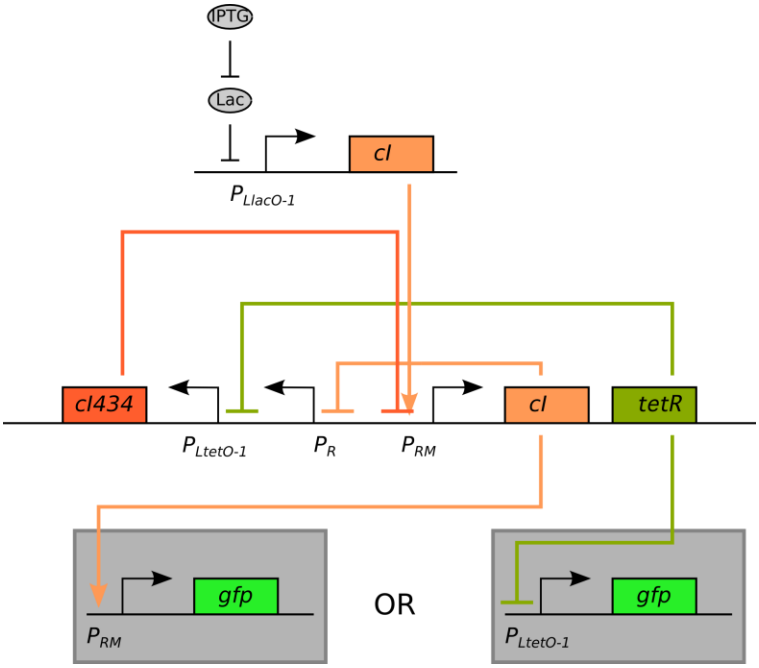

Figure S.1 – Bistable TS

The full transcriptional bistable switch consists of two promoters and three genes, and is based off of our bistable TES from figure 1 of the main paper. The repressor CI434 is used in place of *mf*-Lon. In order for CI434 to repress P_{RM}, O_R3 needs to be changed to a CI-434 binding site [32]. Just as in TES, the main positive feedback loop is the P_{RM}-CI loop. The second loop consists of $P_{LtetO-1}$ making CI434, repressing P_{RM}.

S.1.5 Rate balance plot for TS plot

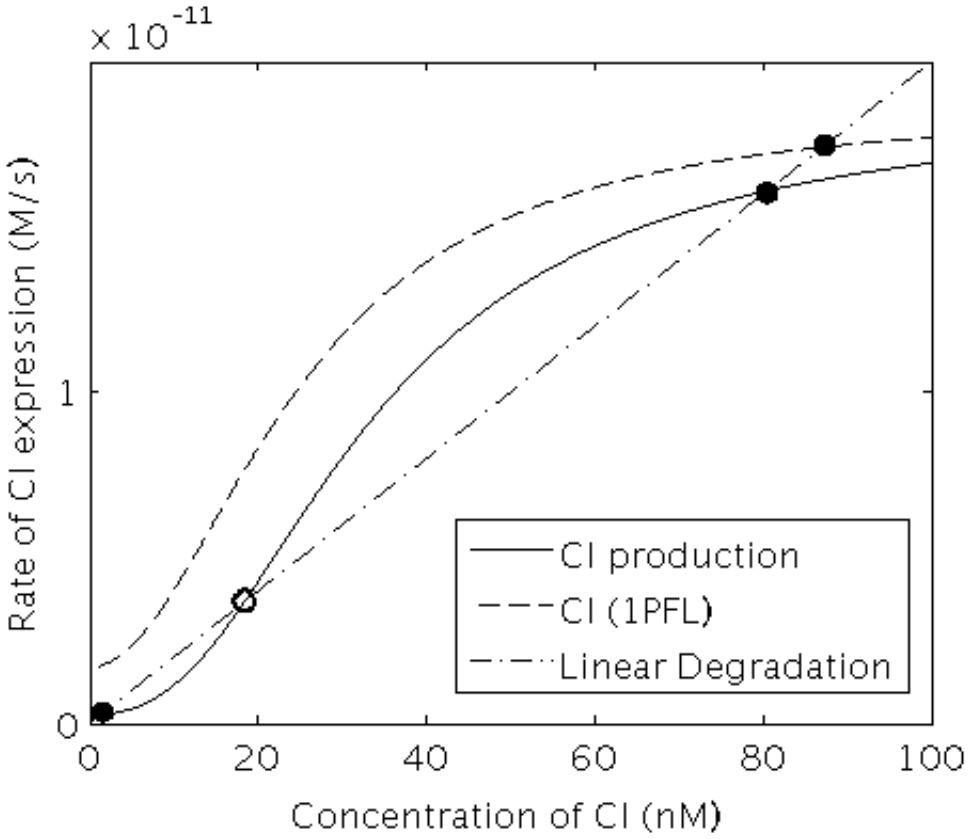

Figure S.2 – Rate-balance plot of a bistable TS

Just like for the bistable TES, having only the P_{RM}-CI positive feedback loop, bistabile behavior is not possible. Bistable behavior is conferred once the second positive feedback loop. In the degradative TES, the linear degradation of CI is changed into a nonlinear degradation to create a bistable condition (figure 1e). With the TS, bistability was created by adding more nonlinearity into the CI production rate (figure S.2, dashed line).

S.2 Kinetic Data for SW5 and SW6 reporter

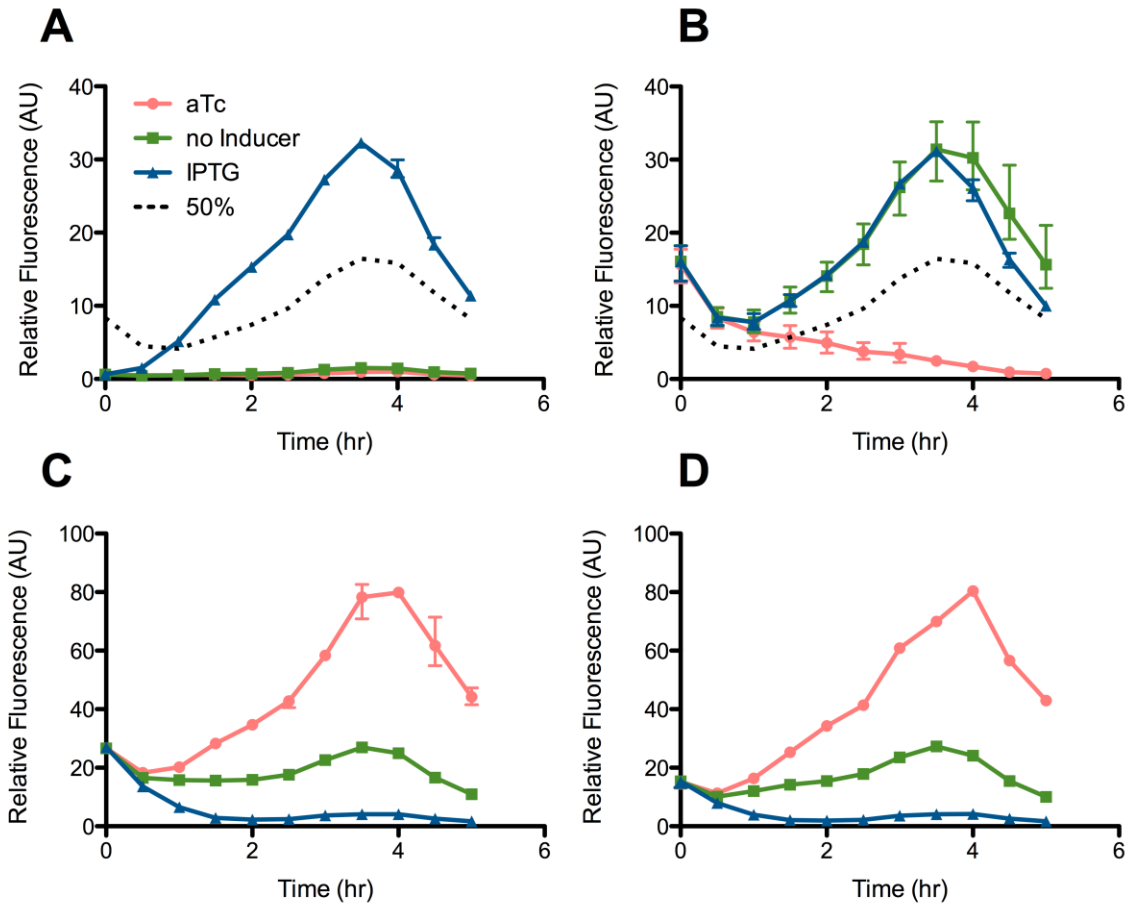

Figure S.3 - Transient switching behavior for SW5

Kinetic behavior of SW5 is shown using both types of reporters (figure S.3). (A) and (B) report using PRM-GFP. (C) and (D) report using PLtetO-1-GFP. For (A) and (C) strains are in the "on" state at time t=0. For (B) and (D) strains are initially in the "off" state. At time t=0 inducers are added into the culture to either reinforce the state, flip the state, or hold the state (no inducer added).

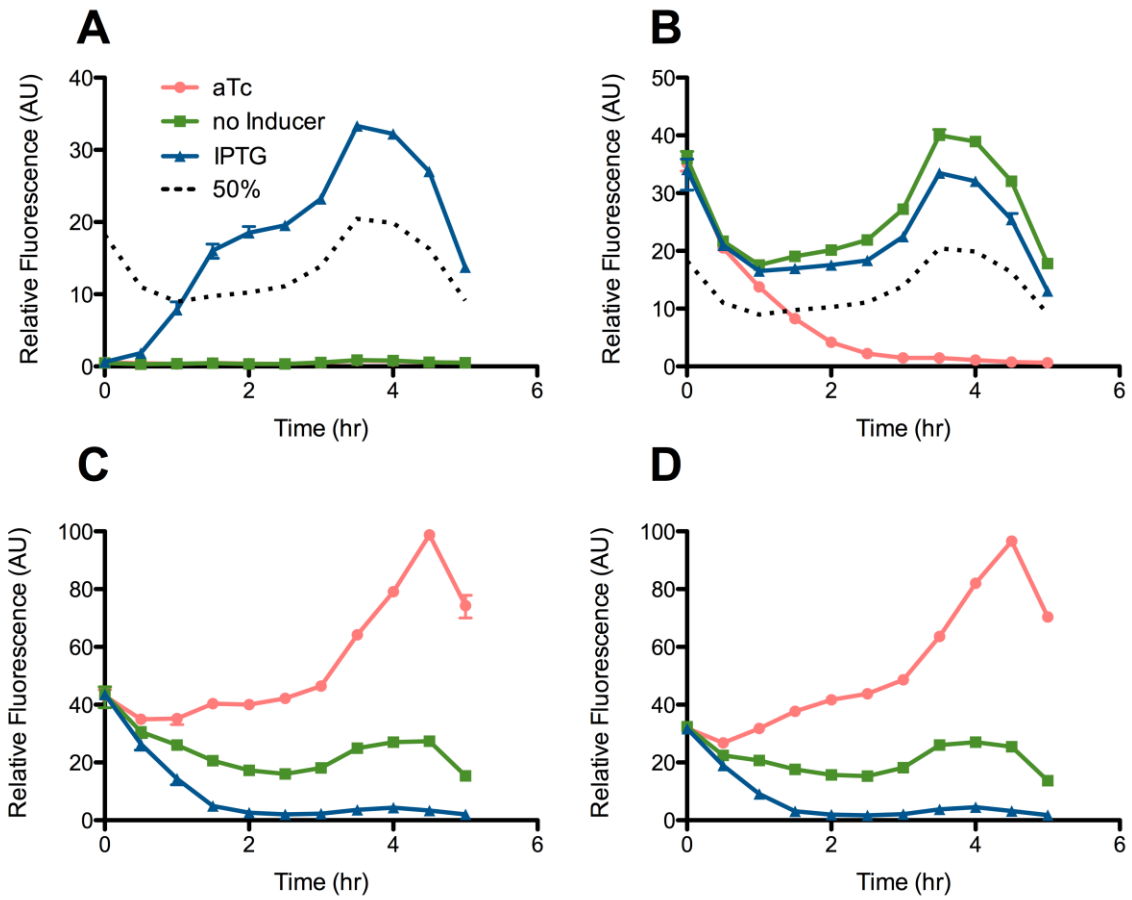

Figure S.4 - Transient switching behavior for SW6

Transient behavior of SW6 (figure S.4). The plots for SW6 are similar to figure 8 in the paper and figure S.3.

S.3 Flow Cytometry

S.3.1 Deactivation

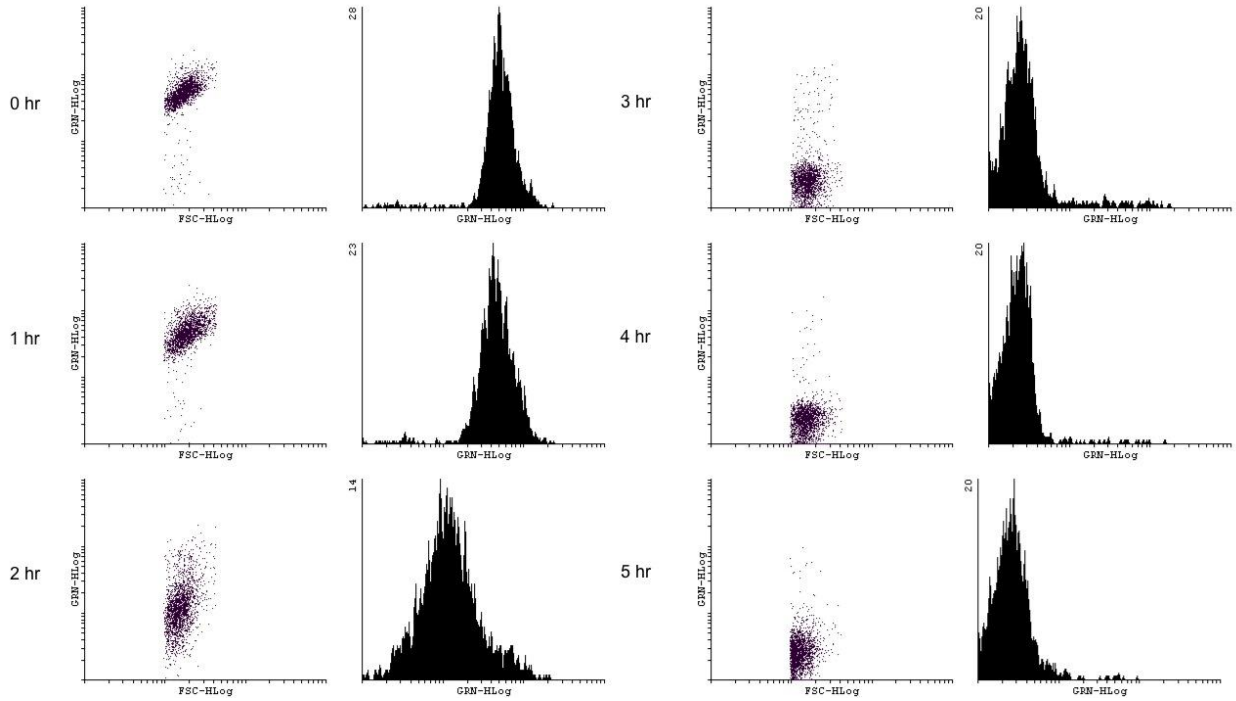

Figure S.5 – Flow cytometry of switch deactivation

This series of flow cytometry plots shows a time course for SW6 switching from the "on" to "off" state. GFP expression uses the PRM-GFP reporter. For all scatter plots, the vertical axis is GFP fluorescence measurement and the horizontal axis is the forward scattering measurement. For all histograms, the vertical axis represents the number of counts and the horizontal axis represents GFP fluorescence. During the "high" to "low" transition, usually we see a bimodal distribution representing both the "on" and "off" population. As time progresses, the "on" population shrinks and the "off" population grows. Instead of observing a bimodal distribution during the transition we see the whole "on" population move to an intermediate state, then move into the "off" state.

S.3.2 Inducers and $P_{LtetO-1}$

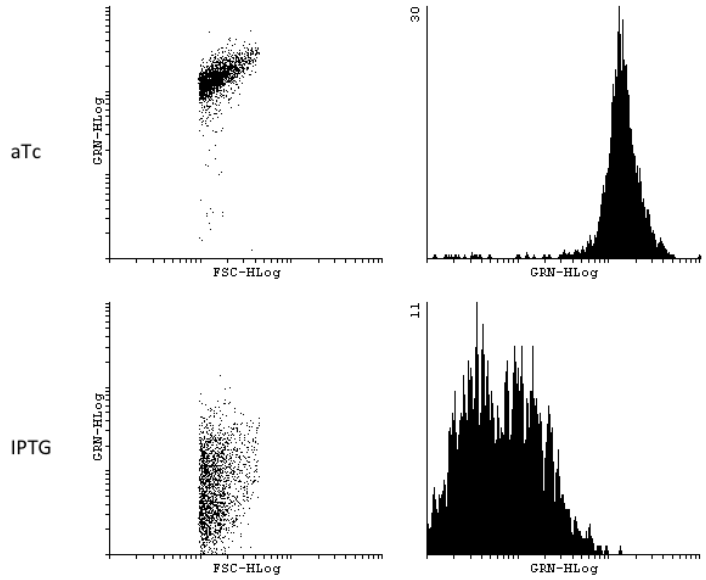

Figure S.6 – Flow cytometry with full induction by inducers

In these plots we observed the effect of adding inducers on $P_{LtetO-1}$. In the plots above GFP is reported using the PLtetO-1-GFP reporter. When aTc is added we observed high expressions of GFP shing that $P_{LtetO-1}$ is fully active (top two plots). When IPTG is added a bimodal population distribution was observed (bottom two plots). One population has no GFP expression, the other population shows that PLtetO-1 is partially active. This indicates that TetR is unable to completely shut off pLtetO-1.

S.3.3 No inducers added

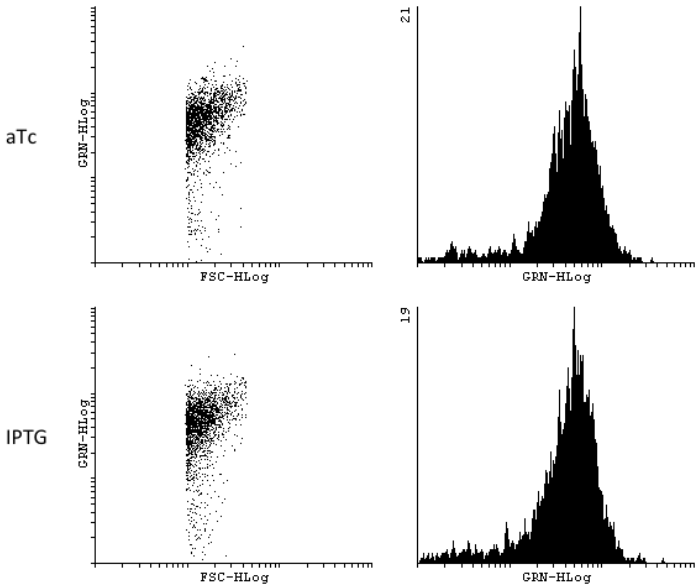

Figure S.7 – Flow cytometry without added inducers

In these plots we observed the behavior of $P_{LtetO\text{-}1}$ at steady-state. Once again the PLtetO-1-GFP reporter is used. When inducers are removed and cultures are allowed to come to steady-state a strains initially cultured overnight in aTc appear identical to strains initially cultured in IPTG.

S.4 Single Feedback Loop Bistable System

It may be possible to create a bistable system with only the P_{RM}-CI positive feedback loop, and a constitutive expression of *mf*-Lon. This however would require the Michaelis constant for *mf*-Lon to be significantly smaller that the measured valued.

S.4.1 Equations

$$\frac{d}{dt}m_{cI} = V_{P_{RM}}NC\left[\frac{1}{1+\left(\frac{K_{CI}}{p_{CI}}\right)^{n_r}} + \ell_{P_{RM}}\right] - \gamma_m m_{cI} \quad (1)$$

$$\frac{d}{dt}p_{CI} = \epsilon_{CI}m_{cI} - \gamma_{CI}p_{CI} - \frac{p_L k_{cat}}{1+\frac{K_M}{p_{CI}}} \quad (2)$$

$$p_L = constant \quad (6)$$

S.4.2 List of parameters

Parameters and Variables	Description	Units	Value
m_{cI}	mRNA concentration for *cI*	M	
p_{CI}	Protein concentration for CI	M	
p_L	Protein concentration for *mf*-Lon	M	1×10^{-10}
$V_{P_{RM}}$	Velocity for mRNA production for P_{RM}	$M \cdot s^{-1}$	.3 [46]
$\ell_{P_{RM}}$	mRNA leakage of P_{RM} promoter	$M \cdot s^{-1}$	1/10 [47]
ℓ_{P_T}	mRNA leakage of $P_{LtetO-1}$ promoter	$M \cdot s^{-1}$	1/5050 [30]
K_{CI}	Disassociation constant of CI to P_{RM}	M	2.5×10^{-8} [33]
K_m	Michaelis constant for *mf*-Lon	M	3.7×10^{-9}
k_{cat}	Catalytic rate of *mf*-Lon	s^{-1}	.071 [25]
γ_m	Degradation rate of mRNA	s^{-1}	2.38×10^{-3} [49]
γ_{CI}	Degradation rate of CI	s^{-1}	2×10^{-4}
ϵ_{CI}	Translation rate for CI	s^{-1}	5×10^{-5}
n_r	Hill coefficient for P_{RM}		2[50]
n_t	Hill coefficient for $P_{LtetO-1}$		2[50]
N	Plasmid copy number for psc101		3
C	Concentration of a single protein/mRNA in a typical bacterium	M	1.5×10^{-9}[51]

S.4.3 Schematic

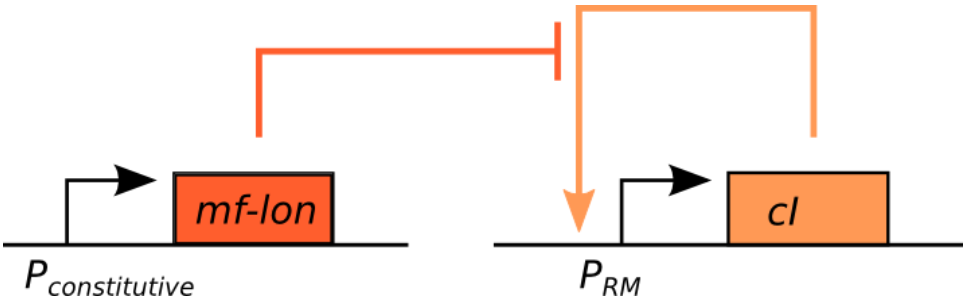

Figure S.8 – A P_{RM}-CI single positive feedback loop bistable circuit

S.4.4 Rate-Balance plot

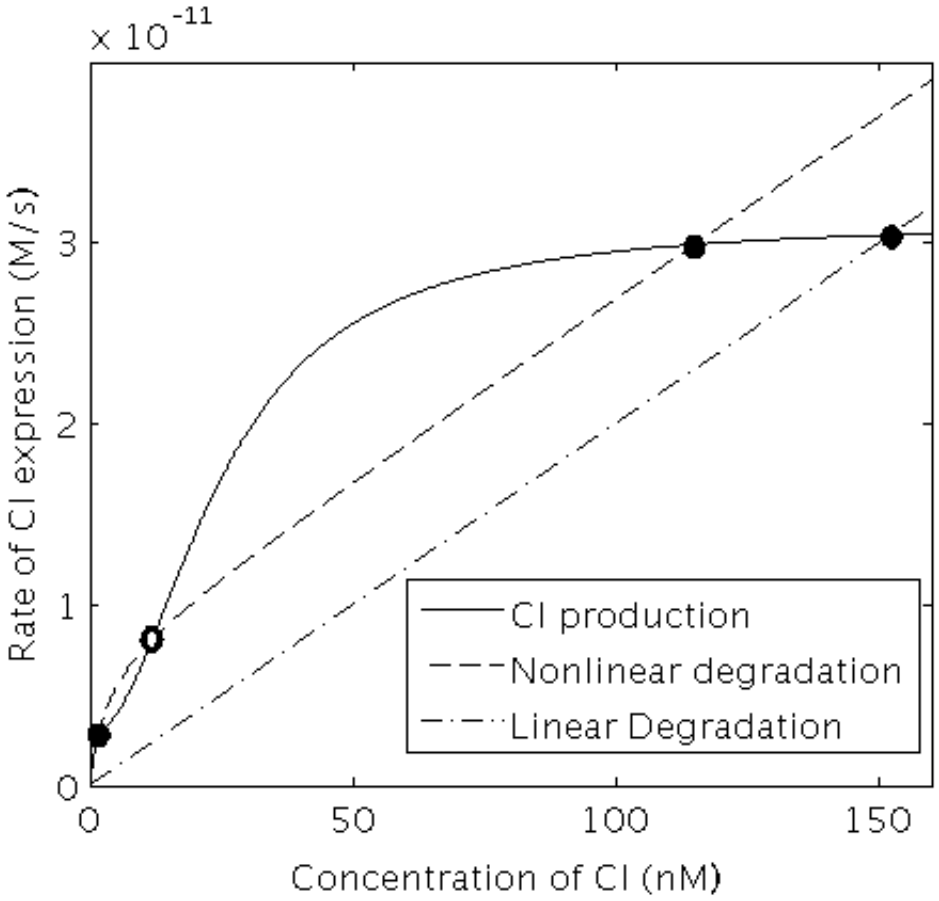

Figure S.9 – Rate-balance plot for a P_{RM}-CI bistable circuit

S.5 Accession Numbers

Accession numbers are registered with GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Name	Accession Number
PRM-GFP	JX155239
PLtetO-1-GFP	JX155236
PLlacO-1-CIwt	JX155233
PLlacO-1-CILVA	JX155231
PLlacO-1-CImf	JX155232
RFP1	JX155240
RFP2	JX155241
RFP3	JX155242
RFP4	JX155243
RFP5	JX155244
RFP6	JX155245
RFP7	JX155246
SW1	JX155247
SW2	JX155248
SW3	JX155249
SW4	JX155250
SW5	JX155251
SW6	JX155252
SW7	JX155253
PRM-CIwt-TetR	JX155238
PRM-CILVA-TetR	JX155236
PRM-CImf-TetR	JX155237
PLlacO-1-mfLon	JX155234