Supporting Text

This *Supporting Text* gives additional simulation results for the regulatory network of the genetic toggle switch interfaced with the SOS pathway. Unlike the assumption in the main text that the cell volume is a constant, here it is assumed that the cell volume depends on the processes of cell growth and cell division.

We use the stochastic model

$$u(t+\tau) = u(t) + P[\varepsilon(\alpha_1 + \frac{\beta_1 K_1^3}{K_1^3 + v(t)^3})\tau] - P[(d_1 + \frac{\gamma s}{1+s})u(t)\tau]$$
$$v(t+\tau) = v(t) + P[\varepsilon(\alpha_2 + \frac{\beta_2 K_2^3}{K_2^3 + u(t)^3})\tau] - P[d_2 v(t)\tau]$$

with the same reaction rates as before, namely $\alpha_1 = \alpha_2 = 0.2$ μM•min^{-1}, $\beta_1 = \beta_2 = 4$ μM•min^{-1}, $\varepsilon = 1$, $d_1 = d_2 = 1$ min^{-1}, $K_1 = K_2 =$ 1 μM, and $\gamma = 1$ min^{-1}. Here we consider the processes of cell growth and cell division. If each cell cycle that lasts time T, the cell volume in each cell cycle is

$$V(t) = (1 + \frac{t}{T})V(0), \qquad t \in [0, T].$$

It has been estimated that *Escherichia coli* takes 50 h for the growth of 50~60 generations (1). For simplicity we assume that it takes 1 h for a cell to double its volume ($T = 60$), and the cell growth rate is 0.0115 min^{-1}. Every 60 min, a cell will divide into two daughter cells, and the molecular numbers in the two daughter cells are assumed to be half of those in the mother cell. It has been estimated that *E. coli* has a volume in the range from 6.0×10^{-16} to 9.8×10^{-16} liters (2), and we assumed that the volume of a daughter cell immediately after division is $V(0) = 5.0 \times 10^{-16}$ liters, and a cell would divide into two daughter cells if its volume reaches $V(T) = 10.0 \times 10^{-16}$ liters. Similar to

the calculation in ref. 2, we estimated that ≈320 and 640 molecules per cell in *E. coli* led to a concentration of 1 μM when the cell volume is 5.0×10^{-16} and 10.0×10^{-16} liters, respectively. Similar molecular numbers can be obtained for other values of the cell volume. In addition, the kinetic rates α_1, α_2, β_1, β_2, K_1, and K_2, are associated with the concentration and thus depend on the values of the cell volume in an obvious manner.

Each stochastic simulation gives the dynamics of molecular numbers in one single cell, and we trace the dynamics of one of the two daughter cells after each cell division. Fig. 7 gives two simulations with $s = 1.6$ for successful switching and unsuccessful switching, respectively. In these simulations, mitomycin C (MMC) was assumed to be applied for 15 h. The degradation rate of λ CI is

$$d = \begin{cases} d_1 (= 1) & t \in [0,60] \ \textit{and} \ \ t \geq 960 \\ d_1 + \dfrac{\gamma s}{s+1} & t \in [60,960] \ \textit{and} \ \ s \in [1.4,1.8]. \end{cases}$$

As indicated in Fig. 8*C*, all simulations showed unsuccessful genetic switching when $s = 1.4$, whereas all simulations gave successful genetic switching when $s = 1.8$.

We predicted the system outputs under different experimental conditions by assuming that MMC was applied for 5, 10, 15, and 20 h, respectively. Note that in experiments MMC was applied for 15 h. For each case, we simulated the stochastic model with different values of *s* ranging from 1.4 to 1.95 and measured the percentages of successful genetic switching based on 1,000 simulations. Fig. 8 gives the simulated percentages and these values can be approximated by a Hill function (best fit)

$$f(s) = a \frac{(s - s_0)^n}{K^n + (s - s_0)^n} \times 100\% \quad \textbf{[1]}$$

with $s_0 = 1.4$. Here *a* is a parameter for matching the value of the Hill function to the simulated percentage when it is 100%. Different values of the Hill coefficient *n* were

tested, and we found that $n = 4$ could best fit the simulated percentages. In addition, values of K = 0.365, 0.31, 0.28, 0.25 were estimated for the MMC application times 5, 10, 15, and 20 h, respectively. Simulation results predicted that the behavior of genetic switching should be in ultrasensitive response to the degradation parameter *s*.

To test the impact of cell volume on system outputs, we simulated the stochastic model based on the assumption of constant cell volume. Similar to the simulations in Fig. 8, it was assumed again that MMC was applied for 5, 10, 15, and 20 h, respectively. We simulated the stochastic model with values of *s* ranging from 1.5 to 2.0. Simulated percentages of successful genetic switching based on 1,000 simulations are presented in Fig. 9. Simulated percentages also can be approximated by the Hill function (1), and the same Hill coefficient of $n = 4$ is estimated based on the best fit of the simulated percentages. In this case, $s_0 = 1.5$ and $K = 0.31, 0.26, 0.25, 0.22$ when MMC was applied for 5, 10, 15, and 20 h, respectively.

Simulation results in Figs. 8 and 9 indicate that the assumptions about the nature of the cell volume do have impact on the dynamics of genetic regulatory networks that may lead to different estimated values of the same parameters in the model. However, because both sets of data are very similar qualitatively, we are confident that our modeling and simulation approach is accurate and effective. Fig. 10 gives a direct comparison between constant and variable cell volume of the simulated percentages of switched cells that have been presented in Figs. 8 and 9. For different MMC application times, there is a drift of the values of *s* for realizing the same percentages of switched cells. For the regulatory network of the genetic toggle switch interfaced with the SOS pathway, two implementations based on different assumptions of the cell volume can make similar predictions of the ultrasensitive response of the genetic switching by using the different estimated values of *s* in a number of experimental conditions.

1. Kobayashi, H., Kærn, M., Araki, M., Chung, K., Gardner, T. S., Cantor, C. R. & Collins, J. J. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 8414–8419.
2. Santillán, M. & Mackey, M. C. (2004) *Biophys. J.* **86,** 1282–1292.