

# Repressilator and Toggle Switch

## Deterministic simulations

The Repressilator and the Toggle Switch systems are two simple models of gene networks, that have been experimentally constructed and validated. These combine theoretical and experimental works pioneered the field of synthetic biology. The Toggle Switch is a synthetic gene regulatory network which confers bistability, while the Repressilator produces oscillations.

In these practicals, we will analyse, through numerical simulations and bifurcation analyses, properties of these dynamical systems.

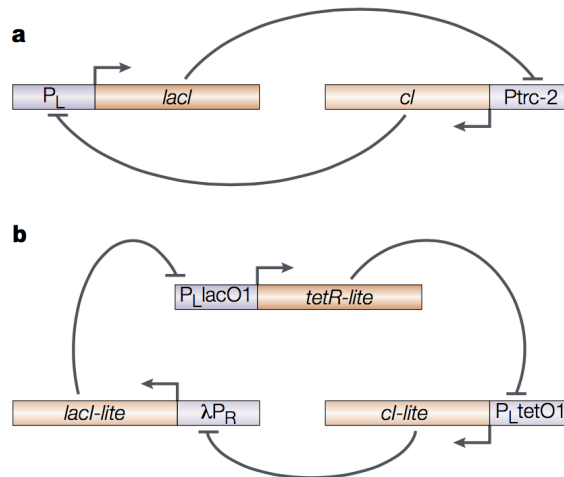


Figure 1: (a) Scheme of the Toggle switch circuit. (b) Scheme of the Repressilator (Figure from Hasty et al, Nat Rev Genet 2001).

## References

- Gardner TS, Cantor CR, Collins JJ (2000) Construction of a genetic toggle switch in *Escherichia coli*. *Nature* 403: 339-42.
- Elowitz MB, Leibler S (2000) A synthetic oscillatory network of transcriptional regulators. *Nature* 403: 335-8.

## Toggle Switch

Equations of the original model for the Toggle switch (Gardner et al, 2000):

$$\frac{dx}{dt} = \alpha_1 \frac{K_1^n}{K_1^n + y^n} - \delta_1 x \quad (1)$$

$$\frac{dy}{dt} = \alpha_2 \frac{K_2^n}{K_2^n + x^n} - \delta_2 y \quad (2)$$

Parameter values:  $\alpha_1 = \alpha_2 = 2$ ,  $K_1 = K_2 = 1$ ,  $n = 4$ ,  $\delta_1 = \delta_2 = 1$ .

Modified version of the Toggle switch model, which incorporates the effect of IPTG ( $S$ ):

$$\frac{dx}{dt} = \alpha_1 \frac{K_1^n}{K_1^n + \left( \frac{y}{(1 + S/K_s)^m} \right)^n} - \delta_1 x \quad (3)$$

$$\frac{dy}{dt} = \alpha_2 \frac{K_2^n}{K_2^n + x^n} - \delta_2 y \quad (4)$$

Parameter values:  $K_s = 5$ ,  $m = 2$ , ( $S = 0.01$ ).

## Questions

1. Simulate Eqs. (1)-(2) for different initial conditions (with  $x(0) \neq y(0)$ ) and show that the system is bistable.
2. Draw the nullclines in the phase plane and discuss the effect of  $\alpha_1$  and  $\alpha_2$  on the bistability (cf. Figs. 2a and 2b of the paper).
3. Draw the stability diagram as a function of parameters  $\alpha_1$  and  $\alpha_2$  for  $n = 2$  and  $n = 4$  (cf. Figs. 2c and 2d of the paper).
4. Discuss the effect of  $\delta_1$  and  $\delta_2$  on the bistability (note that these two parameters are implicitly assumed to be equal in the paper).
5. Draw the bifurcation diagram as a function of IPTG (taken constant) and then study the effect of a pulse of IPTG (cf. Fig. 5 of the paper).
6. Study the effect of a pulse of  $\delta_1$  (which would correspond to a pulse of temperature). Is the switch much faster than the opposite switch induced by a pulse of IPTG, as claimed in the paper (cf. Fig. 6 of the paper)?
7. Run a simulation with a pulse of IPTG ( $S$ ) and, subsequently, a pulse of temperature ( $\delta_1$ ) (cf. Fig. 4 of the paper).

## Repressilator

Equations of the original model for the Repressilator (Elowitz and Leibler, 2000):

$$\frac{dm_1}{dt} = \alpha_1 \frac{K_1^n}{K_1^n + p_3^n} + \alpha_0 - \gamma m_1 \quad (5)$$

$$\frac{dm_2}{dt} = \alpha_2 \frac{K_2^n}{K_2^n + p_1^n} + \alpha_0 - \gamma m_2 \quad (6)$$

$$\frac{dm_3}{dt} = \alpha_3 \frac{K_3^n}{K_3^n + p_2^n} + \alpha_0 - \gamma m_3 \quad (7)$$

$$\frac{dp_1}{dt} = \beta m_1 - \delta p_1 \quad (8)$$

$$\frac{dp_2}{dt} = \beta m_2 - \delta p_2 \quad (9)$$

$$\frac{dp_3}{dt} = \beta m_3 - \delta p_3 \quad (10)$$

Parameter values:  $\alpha_1 = \alpha_2 = \alpha_3 = 100$ ,  $\alpha_0 = 0$ ,  $K_1 = K_2 = K_3 = 1$ ,  $n = 2$ ,  $\gamma = 1$ ,  $\beta = 5$ ,  $\delta = 5$ .

## Questions

1. Simulate the Repressilator (Eqs. 5-10) starting from different initial conditions (cf. Fig. 1c of the paper).
2. What happens if  $\alpha_1$  is changed? Draw the bifurcation diagram as a function of parameter  $\alpha_1$ .
3. Draw the stability diagram as a function of  $\alpha = \alpha_1 = \alpha_2 = \alpha_3$  and  $\beta$  for various values of  $\alpha_0$  (cf. Fig. 1b of the paper, but note that in the paper  $\beta = \delta$ ).
4. In the paper, the authors write that the period of the oscillations is determined mainly by protein stability. This can be checked by plotting the period as a function of  $\alpha$  vs  $\delta$ .
5. How would you modify the equations to incorporate the effect of IPTG ( $S$ )? What is the effect of a continuous injection of IPTG? What is the effect of a pulse of IPTG?
6. In the experimental construction, the level of expression is measured by the GFP, which is under the control of one of the three promoters of the repressilator. Propose an evolution equation for GFP mRNA and protein concentration and show that for low GFP degradation rate the average level of GFP tends to increase (cf. Fig. 2 of the paper).