

## V Synthetic genetic switches

### Reference:

1. T. S. Gardner, C. R. Cantor, and J. J. Collins. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* **403**, 339-342 (2000).

In this paper Gardner *et al.* engineered a plasmid containing two repressor genes mutually controlling each other's expression. In Box 1 the authors postulate the two equations describing the toggle switch:

$$\begin{aligned}\frac{du}{dt} &= \frac{\alpha_1}{1+v^\beta} - u \\ \frac{dv}{dt} &= \frac{\alpha_2}{1+u^\gamma} - v\end{aligned}\tag{V.1}$$

Let's derive this equation and explore which assumptions were made during the derivation. The fast equilibrium reactions for this problem are:



The first two reactions model the binding of the repressors to the two promoters inhibiting transcription. We will assume that there is only one binding site for the repressor (in contrast to the lambda phage promoters). However the repressor monomers can form multimers. Repressor 1 multimerizes with  $\gamma$  subunits and repressor 2 with  $\beta$  subunits. Note that  $K_3$  and  $K_4$  are the effective association constants for multimerization. The units of  $K_3$  and  $K_4$  are  $(M)^{1-\gamma}$  and  $(M)^{1-\beta}$  respectively. The last two equations assume that intermediate states are not allowed. This is the Hill approximation as we discussed before (see for example [II.22]). Remember that the total number of promoter site is conserved, and the total concentration of both promoters is identical since they are on the same plasmid:

$$[P^T] = [P_1^T] = [P_1] + [P_1 R_2^\beta] = [P_2^T] = [P_2] + [P_2 R_1^\gamma] \quad [V.3]$$

Now the rate of synthesis of repressor 1 and 2 can be written as:

$$\begin{aligned} R_{\text{gen1}} &= k_1 [P^T] \frac{[P_1]}{[P_1] + [P_1 R_2^\beta]} = k_1 [P^T] \frac{1}{1 + K_1 [R_2]^\beta} = \frac{k_1 [P^T]}{1 + K_1 K_4 [R_2]^\beta} \\ R_{\text{gen2}} &= k_2 [P^T] \frac{[P_2]}{[P_2] + [P_2 R_1^\gamma]} = k_2 [P^T] \frac{1}{1 + K_2 [R_1]^\gamma} = \frac{k_2 [P^T]}{1 + K_2 K_3 [R_1]^\gamma} \end{aligned} \quad [V.4]$$

The rates  $k_1$  and  $k_2$  are the effective synthesis rates (including RNA polymerase binding, transcription, translation, and folding) of repressor protein 1 and 2, respectively).

Assuming a first order decay process with rate  $\delta$ , the kinetic equations are:

$$\begin{aligned} \frac{d[R_1]}{dt} &= \frac{k_1 [P^T]}{1 + K_1 K_4 [R_2]^\beta} - \delta [R_1] \\ \frac{d[R_2]}{dt} &= \frac{k_2 [P^T]}{1 + K_2 K_3 [R_1]^\gamma} - \delta [R_2] \end{aligned} \quad [V.5]$$

When we introduce a dimensionless time  $\tilde{t} = t\delta$ , equation (50) becomes:

$$\begin{aligned} \frac{d[R_1]}{d\tilde{t}} &= \frac{1}{\delta} \frac{k_1 [P^T]}{1 + K_1 K_4 [R_2]^\beta} - [R_1] \\ \frac{d[R_2]}{d\tilde{t}} &= \frac{1}{\delta} \frac{k_2 [P^T]}{1 + K_2 K_3 [R_1]^\gamma} - [R_2] \end{aligned} \quad [V.6]$$

if we use the following dimensionless concentrations:

$$\begin{aligned} u &= [R_1] (K_2 K_3)^{1/\gamma} \\ v &= [R_2] (K_1 K_4)^{1/\beta} \end{aligned} \quad [V.7]$$

Equation [V.6] becomes:

$$\begin{aligned} \frac{du}{d\tilde{t}} &= \frac{1}{\delta} \frac{k_1 [P^T] (K_2 K_3)^{1/\gamma}}{1 + v^\beta} - u \\ \frac{dv}{d\tilde{t}} &= \frac{1}{\delta} \frac{k_2 [P^T] (K_1 K_4)^{1/\beta}}{1 + u^\gamma} - v \end{aligned} \quad [V.8]$$

Finally by defining  $\alpha_1$  and  $\alpha_2$  as:

$$\begin{aligned} \alpha_1 &\equiv \frac{k_1 [P^T] (K_2 K_3)^{1/\gamma}}{\delta} \\ \alpha_2 &\equiv \frac{k_2 [P^T] (K_1 K_4)^{1/\beta}}{\delta} \end{aligned} \quad [V.9]$$

we recover the 'Box' equation:

$$\begin{aligned}\frac{du}{d\tilde{t}} &= \frac{\alpha_1}{1+v^\beta} - u \\ \frac{dv}{d\tilde{t}} &= \frac{\alpha_2}{1+u^\gamma} - v\end{aligned}\tag{V.10}$$

In steady state both these equations equal zero:

$$\begin{aligned}u &= \frac{\alpha_1}{1+v^\beta} \\ v &= \frac{\alpha_2}{1+u^\gamma}\end{aligned}\tag{V.11}$$

## VI Stability analysis

Consider the following two coupled differential equations:

$$\begin{aligned}\dot{x} &= f(x, y) \\ \dot{y} &= g(x, y)\end{aligned}\tag{VI.1}$$

The nullclines are defined as:

$$\begin{aligned}\dot{x} = 0 &\rightarrow f(x_o, y_o) = 0 \\ \dot{y} = 0 &\rightarrow g(x_o, y_o) = 0\end{aligned}\tag{VI.2}$$

in order to solve [VI.2] we linearize around the fixed point  $(x_o, y_o)$ :

$$\begin{aligned}\tilde{x} &\equiv x - x_o \\ \tilde{y} &\equiv y - y_o\end{aligned}\tag{VI.3}$$

If  $f(x, y)$  and  $g(x, y)$  are approximated by a first order Taylor expansion, [VI.2] can be written as:

$$\begin{aligned}\dot{x} &\approx \tilde{x} \left. \frac{\partial f}{\partial x} \right|_{(x_o, y_o)} + \tilde{y} \left. \frac{\partial f}{\partial y} \right|_{(x_o, y_o)} \equiv a\tilde{x} + b\tilde{y} \\ \dot{y} &\approx \tilde{x} \left. \frac{\partial g}{\partial x} \right|_{(x_o, y_o)} + \tilde{y} \left. \frac{\partial g}{\partial y} \right|_{(x_o, y_o)} \equiv c\tilde{x} + d\tilde{y}\end{aligned}\tag{VI.4}$$

or in matrix notation:

$$\begin{aligned}\vec{\dot{X}} &= A\vec{X} \\ A &= \begin{bmatrix} a & b \\ c & d \end{bmatrix} \\ \vec{\dot{X}} &= \begin{bmatrix} \dot{x} \\ \dot{y} \end{bmatrix} \\ \vec{X} &= \begin{bmatrix} \tilde{x} \\ \tilde{y} \end{bmatrix}\end{aligned}\tag{VI.5}$$

The matrix A is characterized by its trace and the determinant:

$$\begin{aligned}\tau &= \text{trace}(A) = a + d \\ \Delta &= \det(A) = ad - bc\end{aligned}\tag{VI.6}$$

Let's try to find a solution of the convenient form:

$$\vec{\dot{v}} = \lambda \vec{v} = A \vec{v} \quad [\text{VI.7}]$$

This vector is called the eigenvector,  $\lambda$  is the corresponding eigenvalue. [VI.7] can be solved by:

$$\det \begin{bmatrix} a - \lambda & b \\ c & d - \lambda \end{bmatrix} = 0 \quad [\text{VI.8}]$$

leading to:

$$\lambda_1 = \frac{\tau + \sqrt{\tau^2 - 4\Delta}}{2} \quad [\text{VI.9}]$$

$$\lambda_2 = \frac{\tau - \sqrt{\tau^2 - 4\Delta}}{2}$$

or

$$\Delta = \lambda_1 \lambda_2 \quad [\text{VI.10}]$$

$$\tau = \lambda_1 + \lambda_2$$

For a stable fixed point both  $\lambda_1$  and  $\lambda_2$  should be negative. Therefore a stable fixed point is characterized by:

$$\Delta > 0 \quad [\text{VI.11}]$$

$$\tau < 0$$

Now let us use the evaluate the stability of the toggle switch (Chapter V) as an example:

$$\dot{u} = f(u, v) = \frac{\alpha_1}{1 + v^\beta} - u \quad [\text{VI.12}]$$

$$\dot{v} = g(u, v) = \frac{\alpha_2}{1 + u^\gamma} - v$$

The fixed points are:

$$u = \frac{\alpha_1}{1 + v^\beta} \quad [\text{VI.13}]$$

$$v = \frac{\alpha_2}{1 + u^\gamma}$$

The matrix A is given by:

$$A = \begin{bmatrix} -1 & \frac{-\alpha_1 \beta v^{\beta-1}}{(1+v^\beta)^2} \\ \frac{-\alpha_2 \gamma u^{\gamma-1}}{(1+u^\gamma)^2} & -1 \end{bmatrix} \quad [\text{VI.14}]$$

The trace of this matrix is always negative so the only requirement for stability is that the determinant of this matrix is larger than zero. Let us focus on the conditions for which the determinant equals zero. This would define the boundary in parameter space that separates the bistable from monostable region. Setting the determinant to zero gives:

$$\frac{\alpha_1 \beta v^{\beta-1}}{(1+v^\beta)^2} \frac{\alpha_2 \gamma u^{\gamma-1}}{(1+u^\gamma)^2} = 1 \quad [\text{VI.15}]$$

Using the conditions for the fixed points [VI.13] gives:

$$\beta \gamma v^{\beta+1} u^{\gamma+1} = \alpha_1 \alpha_2 \quad [\text{VI.16}]$$

To be able to solve [VI.16] we have to make some assumptions. We assume that we are working with strong promoters and therefore  $\alpha_1$  and  $\alpha_2$  are large and that expression ratio between the ON and OFF state is large. In the case where  $u \gg v$ , the fixed points are approximated by:

$$\begin{aligned} u &\approx \alpha_1 \\ v &\approx \frac{\alpha_2}{\alpha_1^\gamma} \end{aligned} \quad [\text{VI.17}]$$

Condition [VI.16] now becomes:

$$\beta \gamma \alpha_2^\beta \alpha_1^{-\beta\gamma} = 1 \Leftrightarrow \log \alpha_1 \approx \frac{1}{\gamma} \log \alpha_2 \quad [\text{VI.18}]$$

Consistent with Fig. 2c-d in the toggle switch paper. The other boundary is obtained by realizing that the system is symmetry. By replacing  $\alpha_1$  by  $\alpha_2$  and  $\gamma$  by  $\beta$ , the other boundary is found:

$$\log \alpha_1 \approx \beta \log \alpha_2 \quad [\text{VI.19}]$$