## IV 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 others expression. In Box 1 postulate the two equations describing the toggle switch:

$$\begin{aligned}\frac{du}{dt} &= \frac{\alpha_1}{1+v^{\beta}} - u \\ \frac{dv}{dt} &= \frac{\alpha_2}{1+u^{\gamma}} - v\end{aligned} \qquad \textbf{[IV.1]}$$

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

$$\begin{aligned} P_1 + R_2^{\beta} &\xleftrightarrow{K_1} P_1R_2^{\beta} \\ P_2 + R_1^{\gamma} &\xleftrightarrow{K_2} P_2R_1^{\gamma} \\ \gamma R_1 &\xleftrightarrow{K_3} R_1^{\gamma} \\ \beta R_2 &\xleftrightarrow{K_4} R_2^{\beta} \end{aligned} \qquad \textbf{[IV.2]}$$

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 γ subunits and repressor 2 with β 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:

$$[P^T] = [P_1^T] = [P_1] + [P_1R_2^{\beta}] = [P_2^T] = [P_2] + [P_2R_1^{\gamma}] \qquad \textbf{[IV.3]}$$

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

$$R_{gen1} = k_1[P^T]\frac{[P_1]}{[P_1]+[P_1R_2^\beta]} = k_1[P^T]\frac{1}{1+K_1[R_2^\beta]} = \frac{k_1[P^T]}{1+K_1K_4[R_2]^\beta}$$
$$R_{gen2} = k_2[P^T]\frac{[P_2]}{[P_2]+[P_2R_1^\gamma]} = k_2[P^T]\frac{1}{1+K_2[R_1^\gamma]} = \frac{k_2[P^T]}{1+K_2K_3[R_1]^\gamma} \quad \textbf{[IV.4]}$$

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:

$$\frac{d[R_1]}{dt} = \frac{k_1[P^T]}{1+K_1K_4[R_2]^\beta} - \delta[R_1]$$
$$\frac{d[R_2]}{dt} = \frac{k_2[P^T]}{1+K_2K_3[R_1]^\gamma} - \delta[R_2] \quad \textbf{[IV.5]}$$

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

$$\frac{d[R_1]}{d\tilde{t}} = \frac{1}{\delta}\frac{k_1[P^T]}{1+K_1K_4[R_2]^\beta} - [R_1]$$
$$\frac{d[R_2]}{d\tilde{t}} = \frac{1}{\delta}\frac{k_2[P^T]}{1+K_2K_3[R_1]^\gamma} - [R_2] \quad \textbf{[IV.6]}$$

if we use the following dimensionless concentrations:

$$u = [R_1](K_2K_3)^{1/\gamma}$$
$$v = [R_2](K_1K_4)^{1/\beta} \quad \textbf{[IV.7]}$$

Equation [IV.6] becomes:

$$\frac{du}{d\tilde{t}} = \frac{1}{\delta}\frac{k_1[P^T](K_2K_3)^{1/\gamma}}{1+v^\beta} - u$$
$$\frac{dv}{d\tilde{t}} = \frac{1}{\delta}\frac{k_2[P^T](K_1K_4)^{1/\beta}}{1+u^\gamma} - v \quad \textbf{[IV.8]}$$

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

$$\alpha_1 \equiv \frac{k_1[P^T](K_2K_3)^{1/\gamma}}{\delta}$$
$$\alpha_2 \equiv \frac{k_2[P^T](K_1K_4)^{1/\beta}}{\delta} \quad \textbf{[IV.9]}$$

we recover the 'Box' equation:

$$\frac{du}{d\tilde{t}} = \frac{\alpha_1}{1+v^{\beta}} - u$$
$$\frac{dv}{d\tilde{t}} = \frac{\alpha_2}{1+u^{\gamma}} - v \qquad \textbf{[IV.10]}$$

In steady state both these equations equal zero:

$$u = \frac{\alpha_1}{1+v^{\beta}}$$
$$v = \frac{\alpha_2}{1+u^{\gamma}} \qquad \textbf{[IV.11]}$$

## V Stability analysis

Consider the following two coupled differential equations:

$$\begin{aligned} \dot{x} &= f(x,y) \\ \dot{y} &= g(x,y) \end{aligned} \qquad \textbf{[V.1]}$$

The nullclines are defined as:

$$\begin{aligned} \dot{x} = 0 &\rightarrow f(x_o,y_o) = 0 \\ \dot{y} = 0 &\rightarrow g(x_o,y_o) = 0 \end{aligned} \qquad \textbf{[V.2]}$$

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

$$\begin{aligned} \tilde{x} &\equiv x - x_o \\ \tilde{y} &\equiv y - y_o \end{aligned} \qquad \textbf{[V.3]}$$

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

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

or in matrix notation:

$$\begin{aligned} \dot{\vec{X}} &= A\vec{X} \\ A &= \begin{bmatrix} a & b \\ c & d \end{bmatrix} \\ \dot{\vec{X}} &= \begin{bmatrix} \dot{x} \\ \dot{y} \end{bmatrix} \\ \vec{X} &= \begin{bmatrix} \tilde{x} \\ \tilde{y} \end{bmatrix} \end{aligned} \qquad \textbf{[V.5]}$$

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

$$\begin{aligned} \tau &= trace(A) = a + d \\ \Delta &= \det(A) = ad - bc \end{aligned} \qquad \textbf{[V.6]}$$

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

$$\dot{\vec{v}} = \lambda\vec{v} = A\vec{v} \qquad \textbf{[V.7]}$$

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

$$\det\begin{bmatrix} a-\lambda & b \\ c & d-\lambda \end{bmatrix} = 0 \qquad \textbf{[V.8]}$$

leading to:

$$\lambda_1 = \frac{\tau + \sqrt{\tau^2 - 4\Delta}}{2}$$
$$\lambda_2 = \frac{\tau - \sqrt{\tau^2 - 4\Delta}}{2} \qquad \textbf{[V.9]}$$

or

$$\Delta = \lambda_1 \lambda_2$$
$$\tau = \lambda_1 + \lambda_2 \qquad \textbf{[V.10]}$$

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

$$\Delta > 0$$
$$\tau < 0 \qquad \textbf{[V.11]}$$