# BE 150: Design Principles of Genetic Circuits

Justin Bois

Caltech

Spring, 2018

Caltech

Donna and Benjamin M.
Rosen Bioengineering Center

This document was prepared at Caltech with financial support from the Donna and Benjamin M. Rosen Bioengineering Center.

# 8 The repressilator

Today we will talk about an oscillating genetic circuit developed by Elowitz and Leibler and published in 2000, called the repressilator. As we work through this example, we will learn a valuable technique for analyzing dynamical systems, including many of those we encounter in systems biology, **linear stability analysis**.

## 8.1 Design of the repressilator

The repressilator consists of three repressors on a plasmid, as shown in Fig. 7. They are TetR, λ cI, and LacI. For our analysis here, the names are unimportant, and we will call them repressor 1, 2, and 3. Repressor 1 represses production of repressor 2, which in turn represses production of repressor 3. Finally, repressor 3 represses production or repressor 1, completing the loop.

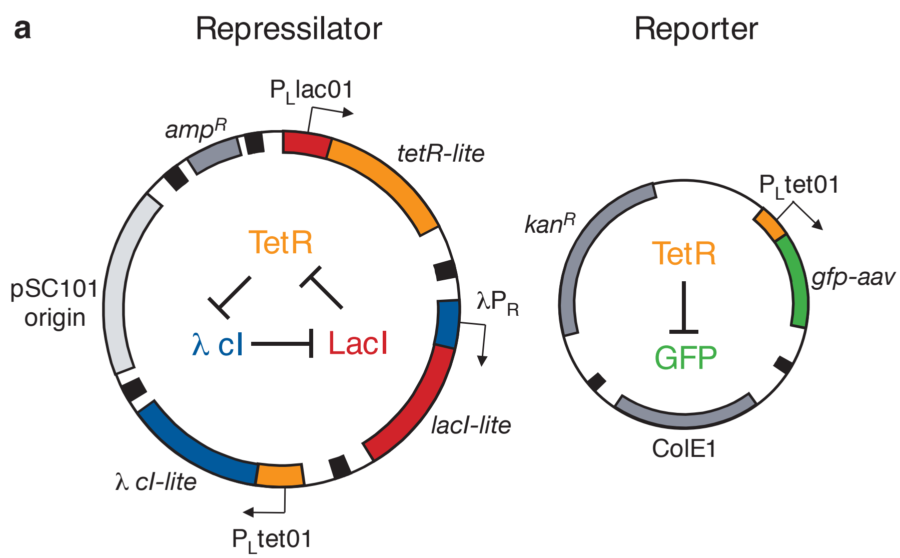


Figure 7: Schmematic of the plasmids used to construct the repessilator in *E. coli*. The three repressors TetR, λ cI, and LacI, are on the same plasmid and form a cycle of repression. Additionally, TetR represses GFP, which is found on another plasmid. The *lite* suffix on the repressors signifies that they have a destruction tage to decrease their stability. The *aav* suffix on the GFP indicates that it is a variant of intermediate stability. Taken from Elowitz and Leibler, *Science*, **403**, 335–338, 2000.

We might work out the dynamics of this system by reasoning. We might get a stable steady state, where all three levels of repressor are tuned to reasonably repress the others. Conversely, we might image a dynamic scenario. Say that initially repressor 1 has high copy number and repressors 2 and 3 are low. The high copy of number of repressor 1 will keep the numbers of repressor 2 down. This means that repressor 3 is free to be expressed. As its copy number grows, it will start to repress repressor 1. As repressor 1 goes down, repressor 2 is expressed in higher numbers. The increased repressor 2 copy number leads to less repressor 3. Then, repressor 1 comes back up again. So, we see a cycle, where repressor 1 is high, then repressor 3, and finally repressor 2. Since repressor 1 represses GFP, we will see an oscillation in GFP as repressor 1 goes up and down.

## 8.2 Dynamical equations for the repressilator

To analyze the repressilator, we will write down our usual dynamical expressions. For simplicity, we will assume symmetry among the species and will consider only protein dynamics, ignoring mRNA.

$$\frac{\mathrm{d}x_1}{\mathrm{d}t} = \frac{\beta}{1+(x_3/k)^n} - \gamma x_1, \tag{8.1}$$

$$\frac{\mathrm{d}x_2}{\mathrm{d}t} = \frac{\beta}{1+(x_1/k)^n} - \gamma x_2, \tag{8.2}$$

$$\frac{\mathrm{d}x_3}{\mathrm{d}t} = \frac{\beta}{1+(x_2/k)^n} - \gamma x_3. \tag{8.3}$$

In dimensionless units, this is

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \frac{\beta}{1+x_j^n} - x_i, \qquad \text{with } i,j \text{ pairs } (1,3),(2,1),(3,2). \tag{8.4}$$

### 8.2.1 Fixed point

To find the fixed point of the repressilator, we solve for $x_i$ with $\dot{x}_i = 0\ \forall i$. We get that

$$x_1 = \frac{\beta}{1+x_3^n}, \tag{8.5}$$

$$x_2 = \frac{\beta}{1+x_1^n}, \tag{8.6}$$

$$x_3 = \frac{\beta}{1+x_2^n}. \tag{8.7}$$

We can substitute the expression for $x_3$ into that for $x_1$ to get

$$x_1 = \frac{\beta}{1+\left(\dfrac{\beta}{1+x_2^n}\right)^n}. \tag{8.8}$$

We can then substitute the expression for for $x_2$ to get

$$x_1 = \frac{\beta}{1+\left(\dfrac{\beta}{1+\left(\dfrac{\beta}{1+x_1^n}\right)^n}\right)^n}. \tag{8.9}$$

This looks like a gnarly expression, but we can write it conveniently as a **composite function**. Specifically,

$$x_1 = f(f(f(x_1))) \equiv fff(x_1), \tag{8.10}$$

where

$$f(x) = \frac{\beta}{1+x^n}. \tag{8.11}$$

By symmetry, this relation holds for repressors 2 and 3 as well, so we have

$$x_i = fff(x_i). \tag{8.12}$$

Writing the relationship for the fixed point with a composite function is useful because we can easily compute the derivatives of the composite function using the chain rule.

$$ff'(x) = f'(f(x)) \cdot f'(x), \tag{8.13}$$

$$fff'(x) = f'(ff(x)) \cdot ff'(x) = f'(f(f(x))) \cdot f'(f(x)) \cdot f'(x). \tag{8.14}$$

Now, since $f(x)$ is monotonically decreasing, $f'(x) < 0$, and also $f'(f(x)) < 0$. This means that $ff'(x) > 0$, so $ff(x)$ is monotonically increasing. Now, $f'(ff(x)) < 0$, since $f'(\text{anything monotonically increasing}) < 0$. this means that $fff(x)$ is monotonically decreasing. Since $x_i$ is increasing, there is a single fixed point with $x = fff(x)$ (see Fig. 8). So, we have a unique fixed point with

$$x_1 = x_2 = x_3 \equiv x_0 = \frac{\beta}{1 + x_0^n}, \tag{8.15}$$

or

$$\beta = x_0(1 + x_0^n). \tag{8.16}$$

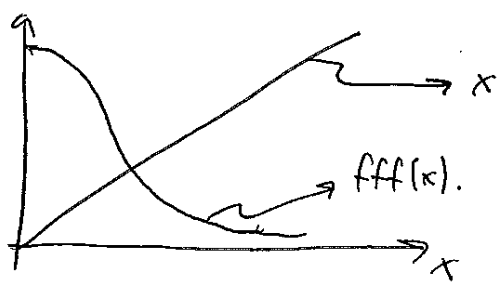


Figure 8: Sketch of the fixed point of a repressilator.

Because we have a single fixed point, we cannot have bistability in the repressilator. What happens at this fixed point? To answer this question, we turn to **linear stability analysis**.

## 8.3 Linear stability analysis

We first give an introduction to the technique of linear stability analysis generically. The basic idea is that we approximate a nonlinear dynamical system by its Taylor series to first order near the fixed point and then look at the behavior of the simpler linear system. The Hartman-Grobman theorem (which we will not derive here) ensures that the linearized system faithfully represents the phase portrait of the full nonlinear system near the fixed point.

Say we have a dynamical system with variables $\mathbf{u}$ with

$$\frac{\mathrm{d}\mathbf{u}}{\mathrm{d}t} = f(\mathbf{u}), \tag{8.17}$$

where $f(\mathbf{u})$ is a vector-valued function, i.e.,

$$f(\mathbf{u}) = (f_1(u_1, u_2, \ldots), f_2(u_1, u_2, \ldots), \ldots). \tag{8.18}$$

Say that we have a fixed point $\mathbf{u}_0$. Then, linear stability analysis proceeds with the following steps.

1) Linearize about $\mathbf{u}_0$, defining $\delta\mathbf{u} = \mathbf{u} - \mathbf{u}_0$. To do this, expand $f(\mathbf{u})$ in a Taylor series about $\mathbf{u}_0$ to first order.

$$f(\mathbf{u}) = f(\mathbf{u}_0) + \nabla f(\mathbf{u}_0) \cdot \delta\mathbf{u} + \cdots, \tag{8.19}$$

where $\nabla f(\mathbf{u}_0) \equiv \mathsf{A}$ is the Jacobi matrix,

$$\nabla f(\mathbf{u}_0) \equiv \mathsf{A} = \begin{pmatrix} \frac{\partial f_1}{\partial u_1} & \frac{\partial f_1}{\partial u_2} & \cdots \\ \frac{\partial f_2}{\partial u_1} & \frac{\partial f_2}{\partial u_2} & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix}. \tag{8.20}$$

Thus, we have

$$\frac{\mathrm{d}\mathbf{u}}{\mathrm{d}t} = \frac{\mathrm{d}\mathbf{u}_0}{\mathrm{d}t} + \frac{\mathrm{d}\,\delta\mathbf{u}}{\mathrm{d}t} = f(\mathbf{u}_0) + \mathsf{A} \cdot \delta\mathbf{u} + \text{higher order terms}. \tag{8.21}$$

Since

$$\frac{\mathrm{d}\mathbf{u}_0}{\mathrm{d}t} = f(\mathbf{u}_0) = 0, \tag{8.22}$$

we have, to linear order,

$$\frac{\mathrm{d}\,\delta\mathbf{u}}{\mathrm{d}t} = \mathsf{A} \cdot \delta\mathbf{u}. \tag{8.23}$$

2) Compute the eigenvalues, $\lambda$ of $\mathsf{A}$.
3) 
   - If $\mathrm{Re}(\lambda) < 0$ for all $\lambda$, then the fixed point $\mathbf{u}_0$ is linearly stable.
   - If $\mathrm{Re}(\lambda) > 0$ for any $\lambda$, then the fixed point $\mathbf{u}_0$ is linearly unstable.
   - If $\mathrm{Re}(\lambda) = 0$ for one or more $\lambda$, with the rest having $\mathrm{Re}(\lambda) < 0$, then the fixed point $\mathbf{u}_0$ lies at a bifurcation.

So, if we can assess the dynamics of the linearized system near the fixed point, we can get an idea what is happening with the full system.

To do the linearization, we need to do Taylor expansions of Hill functions. We do this so often, this is worth stating here and memorizing for future use.

$$\frac{x^n}{1 + x^n} = x_0 + \frac{n x_0^{n-1}}{(1 + x_0^n)^2}\, \delta x + \text{higher order terms}, \tag{8.24}$$

$$\frac{1}{1 + x^n} = x_0 - \frac{n x_0^{n-1}}{(1 + x_0^n)^2}\, \delta x + \text{higher order terms}. \tag{8.25}$$

## 8.4 Linear stability analysis for the repressilator

To perform linear stability analysis for the repressilator, we begin by writing the linearized system.

$$\frac{\mathrm{d}\,\delta x_1}{\mathrm{d}t} \approx -\frac{\beta n x_0^{n-1}}{(1+x_0^n)^2}\,\delta x_3 - \delta x_1, \tag{8.26}$$

$$\frac{\mathrm{d}\,\delta x_2}{\mathrm{d}t} \approx -\frac{\beta n x_0^{n-1}}{(1+x_0^n)^2}\,\delta x_1 - \delta x_2, \tag{8.27}$$

$$\frac{\mathrm{d}\,\delta x_3}{\mathrm{d}t} \approx -\frac{\beta n x_0^{n-1}}{(1+x_0^n)^2}\,\delta x_2 - \delta x_3. \tag{8.28}$$

Defining

$$a = \frac{\beta n x_0^{n-1}}{(1+x_0^n)^2}, \tag{8.29}$$

we can write this in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\delta x_1\\ \delta x_2\\ \delta x_3\end{pmatrix} = -\mathsf{A}\cdot\begin{pmatrix}\delta x_1\\ \delta x_2\\ \delta x_3\end{pmatrix}, \tag{8.30}$$

with

$$\mathsf{A} = -\begin{pmatrix}1 & 0 & a\\ a & 1 & 0\\ 0 & a & 1\end{pmatrix}. \tag{8.31}$$

To compute the eigenvalues of $\mathsf{A}$, we compute the characteristic polynomial using cofactors,

$$(1+\lambda)(1+\lambda)^2 + a(a^2) = (1+\lambda)^3 + a^3 = 0. \tag{8.32}$$

This is solved to give

$$\lambda = -1 + a\sqrt[3]{-1}. \tag{8.33}$$

Recalling that there are three cube roots of $-1$, we get our three eigenvalues.

$$\lambda = -1-a,\ -1+\frac{a}{2}(1+i\sqrt{3}),\ -1+\frac{a}{2}(1-i\sqrt{3}). \tag{8.34}$$

The first eigenvalue is always real and negative. The second two have a positive real part if $a > 2$, or

$$\frac{\beta n x_0^{n-1}}{(1+x_0^n)^2} > 2. \tag{8.35}$$

Now,

$$\beta = x_0(1+x_0^n), \tag{8.36}$$

which we found when computing the fixed point, so

$$a = \frac{n x_0^n}{1+x_0^n}. \tag{8.37}$$

So, $a > 2$ only if $n > 2$, meaning that we must have ultrasensitivity for the fixed point to be unstable.

At the bifurcation,

$$a = \frac{nx_0^n}{1 + x_0^n} = 2, \tag{8.38}$$

so

$$x_0^n = \frac{2}{n-2}. \tag{8.39}$$

Using $\beta = x_0(1 + x_0^n)$, we can write

$$\beta = \frac{n}{2}\left(\frac{n}{2} - 1\right)^{-\frac{n+1}{n}} \tag{8.40}$$

at the bifurcation. So, for $n > 2$ and

$$\beta > \frac{n}{2}\left(\frac{n}{2} - 1\right)^{-\frac{n+1}{n}}, \tag{8.41}$$

we have imaginary eigenvalues with positive real parts. This is an **oscillatory instability**.

## 8.5 Numerical solution of the repressilator dynamics

We can solve for the dynamics of the repressilator numerically. This is done in the Jupyter notebook accompanying this lecture. Importantly, we see that we get a succession of peaks, $1 \to 2 \to 3 \to 1 \to 2 \to 3 \cdots$. This is like a clock. Can we have a clock with 12 peaks ("hours")?