BE 150: Design Principles of Genetic Circuits

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9 Delay oscillators

We continue our discussion of oscillators by considering a very simple oscillator, perhaps even simpler than the repressilator. We will consider a simple, single-component circuit with negative feedback **with delay**. The basic idea has been put forward many times, notably by Julian Lewis in 2003, describing transcriptional delay.

9.1 A simple delay oscillator

For the simplest picture, consider an autoinhibitory gene. The inhibition of transcription if realized by the protein product, which takes a while to be made after transcription. So, there is a time delay between the onset of production of the mRNA transcript and the effect of self-repression. We will call this time delay τ. So, we can write an ODE describing the dynamics of expression of this gene (which we will call X) as

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\beta}{1 + \left(\frac{x(t-\tau)}{k}\right)^n} - \gamma x. \tag{9.1}$$

In other words, we are stating that the expression of X at time t is dependent on its expression level at time $t - \tau$.

Doing our usual nondimensionalization, setting $\beta/\gamma \to \beta$, $\gamma t \to t$, $x/k \to x$, and $\gamma\tau \to \tau$, we have

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\beta}{1 + (x(t-\tau))^n} - x. \tag{9.2}$$

We can solve this numerically (see accompanying Jupyter notebook), to get oscillations.

The principle behind the presence of oscillations is simple, and illustrated in Fig. 9. Because the rate of expression is determined by past protein levels, high expression occurs when protein levels were low in the past and low expression occurs when protein levels were high in the past.

9.2 An example of delay in a biological circuit

To investigate how delay can give rise to oscillations, we consider a multistep process of the production and action of a repressor. The delay in transcriptional regulation in real cells comes from processes such as translation, trans-nuclear transport, etc. We will consider a simple version of this, shown in Fig. 10, in which r is some intermediate state en route to functioning protein. We can write the dynamics as

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{\beta_m}{1 + (p/k)^n} - \gamma_m m, \tag{9.3}$$

$$\frac{\mathrm{d}r}{\mathrm{d}t} = \beta_r m - \gamma_r r, \tag{9.4}$$

$$\frac{\mathrm{d}p}{\mathrm{d}t} = \beta_p r - \gamma_p p, \tag{9.5}$$

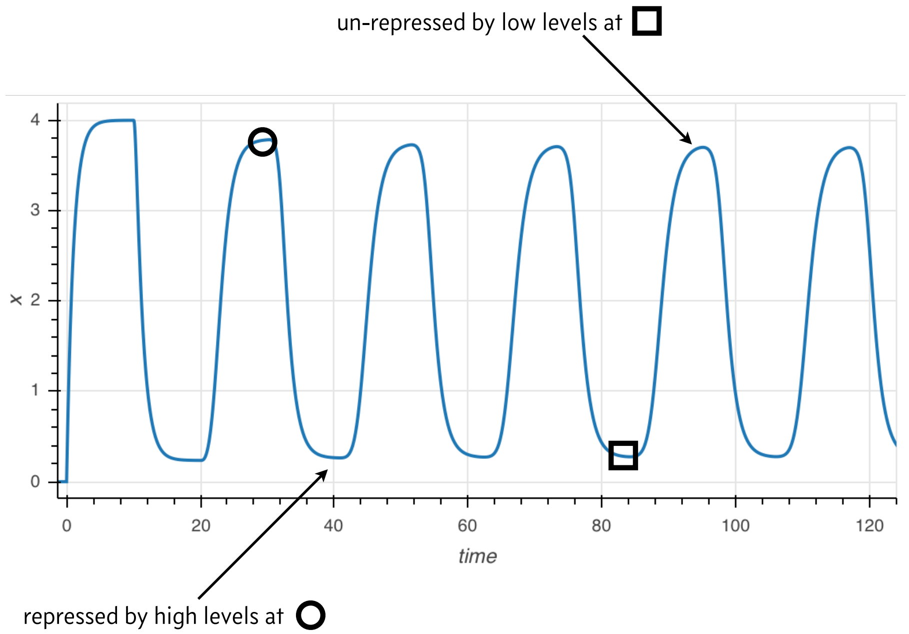

Figure 9: Schematic of how delayed repression can give oscillations.

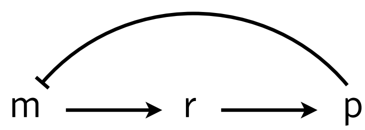

Figure 10: Delayed repression as a result of slow mRNA processing.

where for simplicity we have assumed mass action type kinetics for all processes other than repression. These equations can be nondimensionalized by renaming parameters and variables as $\gamma_m t \to t, p/k \to p$, $\beta_r\beta_p m/\gamma_m^2 k \to m$, $\beta_p r/\gamma_m k \to r$, $\beta_m\beta_r\beta_p/\gamma_m^2 k \to \beta$ and $\gamma_p/\gamma_m \to \gamma$ to give

$$\frac{\mathrm{d}m}{\mathrm{d}t} = \frac{\beta}{1+p^n} - m, \tag{9.6}$$

$$\frac{\mathrm{d}r}{\mathrm{d}t} = m - r, \tag{9.7}$$

$$\frac{\mathrm{d}p}{\mathrm{d}t} = r - \gamma p. \tag{9.8}$$

There is a single fixed point (m_0, r_0, p_0) for this system,

$$m_0 = r_0 = \gamma p_0, \tag{9.9}$$

$$\frac{\beta}{\gamma} = p_0(1+p_0^n). \tag{9.10}$$

We can perform linear stability analysis about this fixed point, just as we did in the last lecture.

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\delta m\\ \delta r\\ \delta p\end{pmatrix} = \mathsf{A}\cdot\begin{pmatrix}\delta m\\ \delta r\\ \delta p\end{pmatrix}, \tag{9.11}$$

with

$$\mathsf{A} = \begin{pmatrix}-1 & 0 & -a\\ 1 & -1 & 0\\ 0 & 1 & -\gamma\end{pmatrix}, \tag{9.12}$$

where

$$a = \frac{\beta n p_0^{n-1}}{(1+p_0^n)^2} = \frac{\gamma n p_0^n}{1+p_0^n}. \tag{9.13}$$

To find the eigenvalues, we write the characteristic polynomial as

$$-(1+\lambda)^2(\gamma+\lambda) - a = -\lambda^3 - (2+\gamma)\lambda^2 - (1+2\gamma)\lambda - \gamma - a = 0. \tag{9.14}$$

This polynomial has no positive real roots and either one or three negative real roots according to the Descartes Sign Rule. We are interested in the case where we have one negative real root and two complex conjugate roots. If the real part of these complex conjugate roots is positive, we have an oscillatory instability.

We can compute the eigenvalues of the linear stability matrix for various values of β and γ for fixed Hill coefficient n. This is done in the accompanying Jupyter notebook. Importantly, we see that we must have very high ultrasensitivity (n about 9 or 10) to get oscillatory instabilities for reasonable values of β and γ. Furthermore, the sliver of parameter space that gives an oscillatory instability is small (Fig. 11). Such a simple description shows that the ability of oscillations is not robust to parameter values and that high sensitivity is necessary.

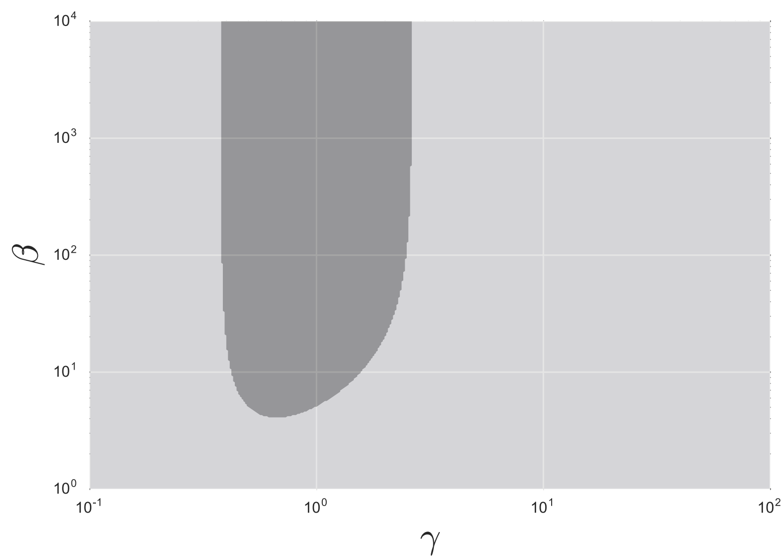

Figure 11: Linear stability diagram for a delay due to mRNA processing for $n = 10$. The dark gray region has parameter values that give oscillatory instability.

To gain better insights on how the delay affect stability, we will consider the stability of the simple picture described by equation (9.1). First, we lay the groundwork for linear stability of delayed differential equations.

9.3 Linear stability analysis of a delayed differential equation

Consider a system of delay differential equations,

$$\frac{\mathrm{d}\mathbf{u}}{\mathrm{d}t} = f(\mathbf{u}(t), \mathbf{u}(t-\tau)). \tag{9.15}$$

Here, we have written that the time derivative explicitly depends on the value of $\mathbf{u}$ at the present time and also at some time τ time units ago in the past. A steady state of the delay differential equations, $\mathbf{u}_0$ satisfies $\mathbf{u}_0(t) = \mathbf{u}_0(t-\tau) \equiv \mathbf{u}_0$ by the definition of a steady state. So, the steady state $\mathbf{u}_0$ satisfies $f(\mathbf{u}_0, \mathbf{u}_0) = 0$. We will perform a linear stability analysis about this steady state.

To linearize, we now have the f is a function of two sets of variables, present and past $\mathbf{u}$. We then write a Taylor series expansion with respect to both of these variables to linearize.

$$f(\mathbf{u}(t), \mathbf{u}(t-\tau)) \approx f(\mathbf{u}, \mathbf{u}_0) + \mathsf{A}\cdot(\mathbf{u}(t) - \mathbf{u}_0) + \mathsf{A}_\tau \cdot (\mathbf{u}(t-\tau) - \mathbf{u}_0), \tag{9.16}$$

where

$$\mathsf{A} = \begin{pmatrix} \frac{\partial f_1}{\partial u_1(t)} & \frac{\partial f_1}{\partial u_2(t)} & \cdots \\ \frac{\partial f_2}{\partial u_1(t)} & \frac{\partial f_2}{\partial u_2(t)} & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix} \tag{9.17}$$

and

$$\mathsf{A}_\tau = \begin{pmatrix} \frac{\partial f_1}{\partial u_1(t-\tau)} & \frac{\partial f_1}{\partial u_2(t-\tau)} & \cdots \\ \frac{\partial f_2}{\partial u_1(t-\tau)} & \frac{\partial f_2}{\partial u_2(t-\tau)} & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix}, \tag{9.18}$$

where all derivatives in both matrices are evaluated at $\mathbf{u}(t) = \mathbf{u}(t-\tau) = \mathbf{u}_0$. Defining $\delta\mathbf{u} = \mathbf{u} - \mathbf{u}_0$, our linearized differential equations are

$$\frac{\mathrm{d}\,\delta\mathbf{u}}{\mathrm{d}t} = (\mathsf{A} + \mathsf{A}_\tau)\cdot\delta\mathbf{u}. \tag{9.19}$$

To solve the linear system, we insert the ansatz, $\delta\mathbf{u} = \mathbf{w}\,\mathrm{e}^{\lambda t}$, giving

$$\lambda\,\mathrm{e}^{\lambda t}\,\mathbf{w} = \mathsf{A}\cdot\mathbf{w}\,\mathrm{e}^{\lambda t} + \mathsf{A}_\tau\cdot\mathbf{w}\,\mathrm{e}^{\lambda(t-\tau)}, \tag{9.20}$$

or, upon simplifying,

$$\lambda\,\mathbf{w} = (\mathsf{A} + \mathrm{e}^{-\lambda\tau}\mathsf{A}_\tau)\cdot\mathbf{w}. \tag{9.21}$$

So, λ is an eigenvalue and $\mathbf{w}$ the corresponding eigenvector for the matrix $(\mathsf{A} + \mathrm{e}^{-\lambda\tau}\mathsf{A}_\tau)$.

In general, there are infinitely many values of λ that satisfy equation (9.21). Nonetheless, if we can show that the real part of all possible values of λ is less than zero, then the fixed point is stable. Otherwise, if the real part of any values of λ are positive, the fixed point is locally unstable. If λ has an imaginary part for the eigenvalues where the real part is positive, the instability is oscillatory.

9.4 Linear stability analysis of delayed autorepression

We consider now the simple case of an autorepressor with delay. The governing ODE is

$$\frac{\mathrm{d}x(t)}{\mathrm{d}t} = \frac{\beta}{1 + (x(t-\tau)/k)^n} - \gamma x(t). \tag{9.22}$$

We nondimensionalize by setting $\gamma t \to t$, $\gamma\tau \to \tau$, $\beta/k\gamma \to \beta$, and $x/k \to x$. Our dimensionless equation is

$$\frac{\mathrm{d}x(t)}{\mathrm{d}t} = \frac{\beta}{1 + (x(t-\tau))^n} - x(t). \tag{9.23}$$

The steady state is given by setting the time derivative to zero,

$$\beta = x_0(1 + x_0^n). \tag{9.24}$$

The x_0 that satisfies this equality is unique because the right hand is monotonically increasing from zero, so it only crosses a value of β once. So, we will consider the stability of this unique steady state.

We linearize the right hand side of the ODE around the fixed point. The matrices A and A_τ are scalars in this case because we have a single species. Remember that the eigenvalue of a scalar is just the scalar itself. So, we have

$$\mathsf{A} = -1 \tag{9.25}$$

$$\mathsf{A}_\tau = -\frac{\beta n x_0^{n-1}}{(1+x_0^n)^2} = -\frac{n x_0^n}{1+x_0^n} \equiv -a_\tau, \tag{9.26}$$

where we have define a_τ for convenience. Thus, our characteristic equation is

$$\lambda = -1 - a_\tau \mathrm{e}^{-\lambda\tau}. \tag{9.27}$$

There are infinitely many λ that satisfy this equation in general.

We can still make progress. To further investigate the dynamics, we write $\lambda = a + ib$, where a is the real part at b is the imaginary part. Then, the characteristic equation is

$$a + ib = -a_\tau \mathrm{e}^{-a\tau}\mathrm{e}^{-ib\tau} - 1 = -a_\tau \mathrm{e}^{-a\tau}(\cos b\tau - i\sin b\tau) - 1. \tag{9.28}$$

This can be written as two equations be equating the real and imaginary parts of both sides of the equality.

$$a = -(1 + a_\tau \mathrm{e}^{-a\tau}\cos b\tau) \tag{9.29}$$

$$b = a_\tau \mathrm{e}^{-a\tau}\sin b\tau. \tag{9.30}$$

Right away, we can see that if a is positive, we must have $a_\tau > 1$, since $|\mathrm{e}^{-a\tau}\cos bt| < 1$. Recall our expression for a_τ,

$$a_\tau = \frac{n x_0^n}{1+x_0^n}. \tag{9.31}$$

Because $x_0^n/(1+x_0^n) < 1$, we must have $n > 1$ in order to have the eigenvalue have a positive real part and therefore have an instability. So, **ultrasensitivity is a requirement for a delay oscillator.**

To investigate when we get an oscillatory instability, we will compute the values of a_τ and τ that lie on the bifurcation line between a stable steady state and an oscillatory instability, a so-called Hopf bifurcation. To do this, we solve for the line with $a = 0$ and $b \neq 0$. In this case, the characteristic equation gives

$$-a_\tau \cos bt = 1 \tag{9.32}$$

$$a_\tau \sin bt = b. \tag{9.33}$$

Squaring both equations and adding gives

$$a_\tau^2 = 1 + b^2, \tag{9.34}$$

Thus,

$$b = \sqrt{a_\tau^2 - 1}, \tag{9.35}$$

which only holds for $a_\tau > 1$, which we already found was a requirement for an oscillatory instability. To find τ, we have

$$-\,a_\tau \cos b\tau = -a_\tau \cos \sqrt{a_\tau^2 - 1}\,t = 1 \tag{9.36}$$

$$\Rightarrow \quad \tau = \frac{\pi - \cos^{-1}\left(a_\tau^{-1}\right)}{\sqrt{a_\tau^2 - 1}}. \tag{9.37}$$

The region of stability is below the bifurcation line in the τ-β plane, since a smaller τ serves to decrease the real part of λ. We can compute the linear stability diagram in the τ-β plane. This is done in the accompaying Jupyter notebook, and the result is shown in Fig. 12 for various degrees of ultrasensitivity. The longer the time delay, the more robust to variations in other parameters is the oscillatory behavior.

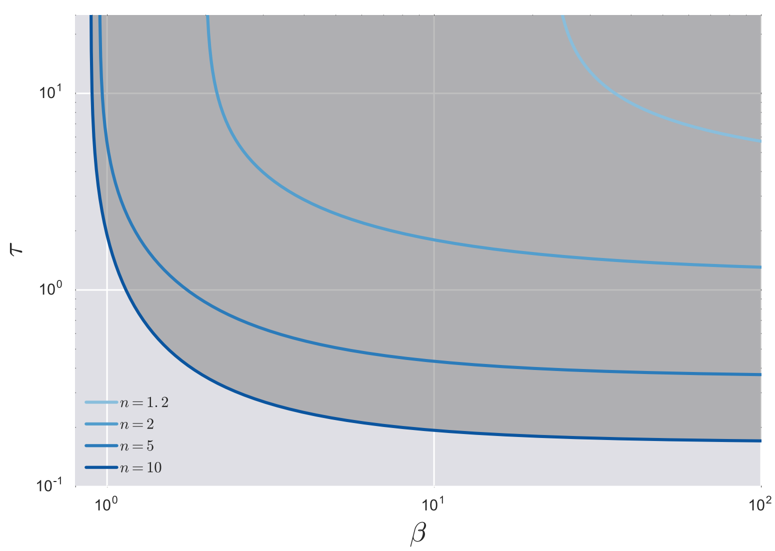

Figure 12: Linear stability diagram for a single component delay oscillator. The dark shaded region has an oscillatory instability, though only above the respective bifurcation lines.

9.5 Stabilization of a delay oscillator with a toggle

Finally, we note that we can make the oscillations more robust by connecting a delay oscillator to a toggle. This was done by Stricker, et al., *Nature*, **456**, 516–519, 2008. The circuit diagram is shown in Fig. 13.

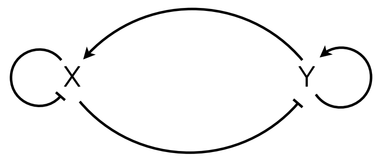

Figure 13: Two delay oscillators (X delayed autorepression and Y delayed repression via X) coupled to a bistable circuit (autoactivated Y).

X and Y are coupled delay oscillators. X oscillates because of delayed repression, which we have been studying. Y oscillates via its X-mediated self-repression. Finally, the autoactivation of Y has bistability, a high and a low state as we saw in the first week of class. The toggle due to the bistability tends to push the oscillator to extremes, stabilizing the limit cycle. This is more difficult to explore mathematically, and Stricker, et al., do it numerically. They designed and built robust circuits using this architecture.