Biomolecular Feedback Systems

Domitilla Del Vecchio
MIT

Richard M. Murray
Caltech

Version 1.0b, September 14, 2014

This is the electronic edition of *Biomolecular Feedback Systems*, available from
http://www.cds.caltech.edu/~murray/BFSwiki.

Printed versions are available from Princeton University Press,
http://press.princeton.edu/titles/10285.html.

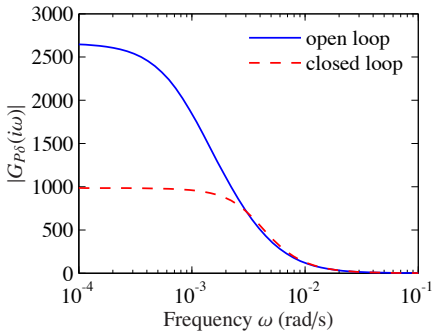


Figure 3.7: Attenuation of perturbations in a genetic circuit with linearization given by equation (3.8). The parameters of the closed loop system are given by $\alpha = 800$ μM/s, $\alpha_0 = 5 \times 10^{-4}$ μM/s, $\gamma = 0.001$ s^{-1}, $\delta_0 = 0.005$ s^{-1}, $\kappa = 0.02$ s^{-1}, $n = 2$, and $K = 0.025$ μM. For the open loop system, we have set $\alpha = P_e\delta_0/(\kappa/\gamma)$ to make the steady state values of open loop and closed loop systems the same.

3.2 Robustness

The term "robustness" refers to the general ability of a system to continue to function in the presence of uncertainty. In the context of this text, we will want to be more precise. We say that a given function (of the circuit) is robust with respect to a set of specified perturbations if the sensitivity of that function to perturbations is small. Thus, to study robustness, we must specify both the function we are interested in and the set of perturbations that we wish to consider.

In this section we study the robustness of the system

$$\frac{dx}{dt} = f(x, \theta, u), \qquad y = h(x, \theta)$$

to various perturbations in the parameters θ and disturbance inputs u. The function we are interested in is modeled by the outputs y and hence we seek to understand how y changes if the parameters θ are changed by a small amount or if external disturbances u are present. We say that a system is robust with respect to these perturbations if y undergoes little change as these perturbations are introduced.

Parametric uncertainty

In addition to studying the input/output transfer curve and the stability of a given equilibrium point, we can also study how these features change with respect to changes in the system parameters θ. Let $y_e(\theta_0, u_0)$ represent the output corresponding to an equilibrium point x_e with fixed parameters θ_0 and external input u_0, so that $f(x_e, \theta_0, u_0) = 0$. We assume that the equilibrium point is stable and focus here on understanding how the value of the output, the location of the equilibrium point,

and the dynamics near the equilibrium point vary as a function of changes in the parameters θ and external inputs u.

We start by assuming that $u = 0$ and investigate how x_e and y_e depend on θ; we will write $f(x,\theta)$ instead of $f(x,\theta,0)$ to simplify notation. The simplest approach is to analytically solve the equation $f(x_e,\theta_0) = 0$ for x_e and then set $y_e = h(x_e,\theta_0)$. However, this is often difficult to do in closed form and so as an alternative we instead look at the linearized response given by

$$S_{x,\theta} := \left.\frac{dx_e}{d\theta}\right|_{\theta_0}, \qquad S_{y,\theta} := \left.\frac{dy_e}{d\theta}\right|_{\theta_0},$$

which are the (infinitesimal) changes in the equilibrium state and the output due to a change in the parameter. To determine $S_{x,\theta}$ we begin by differentiating the relationship $f(x_e(\theta),\theta) = 0$ with respect to θ:

$$\frac{df}{d\theta} = \frac{\partial f}{\partial x}\frac{dx_e}{d\theta} + \frac{\partial f}{\partial \theta} = 0 \quad \Longrightarrow \quad S_{x,\theta} = \frac{dx_e}{d\theta} = -\left(\frac{\partial f}{\partial x}\right)^{-1}\left.\frac{\partial f}{\partial \theta}\right|_{(xe,\theta_0)}. \tag{3.9}$$

Similarly, we can compute the output sensitivity as

$$S_{y,\theta} = \frac{dy_e}{d\theta} = \frac{\partial h}{\partial x}\frac{dx_e}{d\theta} + \frac{\partial h}{\partial \theta} = -\left.\left(\frac{\partial h}{\partial x}\left(\frac{\partial f}{\partial x}\right)^{-1}\frac{\partial f}{\partial \theta} - \frac{\partial h}{\partial \theta}\right)\right|_{(xe,\theta_0)}.$$

These quantities can be computed numerically and hence we can evaluate the effect of small (but constant) changes in the parameters θ on the equilibrium state x_e and corresponding output value y_e.

A similar analysis can be performed to determine the effects of small (but constant) changes in the external input u. Suppose that x_e depends on both θ and u, with $f(x_e,\theta_0,u_0) = 0$ and θ_0 and u_0 representing the nominal values. Then

$$\left.\frac{dx_e}{d\theta}\right|_{(\theta_0,u_0)} = -\left(\frac{\partial f}{\partial x}\right)^{-1}\left.\frac{\partial f}{\partial \theta}\right|_{(xe,\theta_0,u_0)}, \qquad \left.\frac{dx_e}{du}\right|_{(\theta_0,u_0)} = -\left(\frac{\partial f}{\partial x}\right)^{-1}\left.\frac{\partial f}{\partial u}\right|_{(xe,\theta_0,u_0)}.$$

The sensitivity matrix can be normalized by dividing the parameters by their nominal values and rescaling the outputs (or states) by their equilibrium values. If we define the scaling matrices

$$D^{x_e} = \mathrm{diag}\{x_e\}, \qquad D^{y_e} = \mathrm{diag}\{y_e\}, \qquad D^{\theta} = \mathrm{diag}\{\theta\},$$

then the scaled sensitivity matrices can be written as

$$\bar{S}_{x,\theta} = (D^{x_e})^{-1} S_{x,\theta} D^{\theta}, \qquad \bar{S}_{y,\theta} = (D^{y_e})^{-1} S_{y,\theta} D^{\theta}. \tag{3.10}$$

The entries in these matrices describe how a fractional change in a parameter gives a fractional change in the state or output, relative to the nominal values of the parameters and state or output.

Example 3.7 (Transcriptional regulation)**.** Consider again the case of transcriptional regulation described in Example 3.6. We wish to study the response of the protein concentration to fluctuations in its parameters in two cases: a *constitutive promoter* (open loop) and self-repression (closed loop).

For the case of open loop we have $F(P) = \alpha$, and the system has the equilibrium point at $m_e = \alpha/\delta$, $P_e = \kappa\alpha/(\gamma\delta)$. The parameter vector can be taken as $\theta = (\alpha, \delta, \kappa, \gamma)$ and the state as $x = (m, P)$. Since we have a simple expression for the equilibrium concentrations, we can compute the sensitivity to the parameters directly:

$$\frac{\partial x_e}{\partial \theta} = \begin{pmatrix} \frac{1}{\delta} & -\frac{\alpha}{\delta^2} & 0 & 0 \\ \frac{\kappa}{\gamma\delta} & -\frac{\kappa\alpha}{\gamma\delta^2} & \frac{\alpha}{\gamma\delta} & -\frac{\kappa\alpha}{\delta\gamma^2} \end{pmatrix},$$

where the parameters are evaluated at their nominal values, but we leave off the subscript 0 on the individual parameters for simplicity. If we choose the parameters as $\theta_0 = (0.00138, 0.00578, 0.115, 0.00116)$, then the resulting sensitivity matrix evaluates to

$$S^{\text{open}}_{x_e,\theta} \approx \begin{pmatrix} 173 & -42 & 0 & 0 \\ 17300 & -4200 & 211 & -21100 \end{pmatrix}. \tag{3.11}$$

If we look instead at the scaled sensitivity matrix, then the open loop nature of the system yields a particularly simple form:

$$\bar{S}^{\text{open}}_{x_e,\theta} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 1 & -1 & 1 & -1 \end{pmatrix}. \tag{3.12}$$

In other words, a 10% change in any of the parameters will lead to a comparable positive or negative change in the equilibrium values.

For the case of negative regulation, we have

$$F(P) = \frac{\alpha}{1 + (P/K)^n} + \alpha_0,$$

and the equilibrium points satisfy

$$m_e = \frac{\gamma}{\kappa} P_e, \qquad \frac{\alpha}{1 + P_e^n/K^n} + \alpha_0 = \delta m_e = \frac{\delta\gamma}{\kappa} P_e. \tag{3.13}$$

In order to make a proper comparison with the previous case, we need to choose the parameters so that the equilibrium concentrations m_e, P_e match those of the open loop system. We can do this by modifying the promoter strength α and/or the RBS strength, which is proportional to κ, so that the second formula in equation (3.13) is satisfied or, equivalently, choose the parameters for the open loop case so that they match the closed loop steady state protein concentration (see Example 2.2).

Rather than attempt to solve for the equilibrium point in closed form, we instead investigate the sensitivity using the computations in equation (3.13). The state, dynamics and parameters are given by

$$x = \begin{pmatrix} m & P \end{pmatrix}, \qquad f(x,\theta) = \begin{pmatrix} F(P) - \delta m \\ \kappa m - \gamma P \end{pmatrix}, \qquad \theta = \begin{pmatrix} \alpha_0 & \delta & \kappa & \gamma & \alpha & n & K \end{pmatrix}.$$

Note that the parameters are ordered such that the first four parameters match the open loop system. The linearizations are given by

$$\frac{\partial f}{\partial x} = \begin{pmatrix} -\delta & F'(P_e) \\ \beta & -\gamma \end{pmatrix}, \qquad \frac{\partial f}{\partial \theta} = \begin{pmatrix} 1 & -m_e & 0 & 0 & \partial F/\partial\alpha & \partial F/\partial n & \partial F/\partial K \\ 0 & 0 & m_e & -P_e & 0 & 0 & 0 \end{pmatrix},$$

where again the parameters are taken to be at their nominal values and the derivatives are evaluated at the equilibrium point. From this we can compute the sensitivity matrix as

$$S_{x,\theta} = \begin{pmatrix} -\frac{\gamma}{\gamma\delta-\kappa F'} & \frac{\gamma m}{\gamma\delta-\kappa F'} & -\frac{mF'}{\gamma\delta-\kappa F'} & \frac{PF'}{\gamma\delta-\kappa F'} & -\frac{\gamma\partial F/\partial\alpha}{\gamma\delta-\kappa F'} & -\frac{\gamma\partial F/\partial n}{\gamma\delta-\kappa F'} & -\frac{\gamma\partial F/\partial K}{\gamma\delta-\kappa F'} \\ -\frac{\kappa}{\gamma\delta-\kappa F'} & \frac{\kappa m}{\gamma\delta-\kappa F'} & -\frac{\delta m}{\gamma\delta-\kappa F'} & \frac{\delta P}{\gamma\delta-\kappa F'} & -\frac{\kappa\partial F/\partial\alpha_1}{\gamma\delta-\kappa F'} & -\frac{\kappa\partial F/\partial n}{\gamma\delta-\kappa F'} & -\frac{\kappa\partial F/\partial K}{\gamma\delta-\kappa F'} \end{pmatrix},$$

where $F' = \partial F/\partial P$ and all other derivatives of F are evaluated at the nominal parameter values and the corresponding equilibrium point. In particular, we take nominal parameters as $\theta = (5\cdot 10^{-4}, 0.005, 0.115, 0.001, 800, 2, 0.025)$.

We can now evaluate the sensitivity at the same protein concentration as we use in the open loop case. The equilibrium point is given by

$$x_e = \begin{pmatrix} m_e \\ P_e \end{pmatrix} = \begin{pmatrix} 0.239 \\ 23.9 \end{pmatrix}$$

and the sensitivity matrix is

$$S^{\text{closed}}_{x_e,\theta} \approx \begin{pmatrix} 76 & -18 & -1.15 & 115 & 0.00008 & -0.45 & 5.34 \\ 7611 & -1816 & 90 & -9080. & 0.008 & -45 & 534 \end{pmatrix}.$$

The scaled sensitivity matrix becomes

$$\bar{S}^{\text{closed}}_{x_e,\theta} \approx \begin{pmatrix} 0.159 & -0.44 & -0.56 & 0.56 & 0.28 & -3.84 & 0.56 \\ 0.159 & -0.44 & 0.44 & -0.44 & 0.28 & -3.84 & 0.56 \end{pmatrix}. \tag{3.14}$$

Comparing this equation with equation (3.12), we see that there is reduction in the sensitivity with respect to most parameters. In particular, we become less sensitive to those parameters that are not part of the feedback (columns 2–4), but there is higher sensitivity with respect to some of the parameters that are part of the feedback mechanism (particularly n). ∇

More generally, we may wish to evaluate the sensitivity of a (non-constant) solution to parameter changes. This can be done by computing the function $dx(t)/d\theta$, which describes how the state changes at each instant in time as a function of (small) changes in the parameters θ. This can be used, for example, to understand how we can change the parameters to obtain a desired behavior or to determine the most critical parameters that determine a specific dynamical feature of the system under study.

Let $x(t, \theta_0)$ be a solution of the nominal system

$$\dot{x} = f(x, \theta_0, u), \qquad x(0) = x_0.$$

To compute $dx/d\theta$, we write a differential equation for how it evolves in time:

$$\frac{d}{dt}\left(\frac{dx}{d\theta}\right) = \frac{d}{d\theta}\left(\frac{dx}{dt}\right) = \frac{d}{d\theta}(f(x, \theta, u)) = \frac{\partial f}{\partial x}\frac{dx}{d\theta} + \frac{\partial f}{\partial \theta}.$$

This is a differential equation with $n \times m$ states given by the entries of the matrix $S_{x,\theta}(t) = dx(t)/d\theta$ and with initial condition $S_{x,\theta}(0) = 0$ (since changes to the parameters do not affect the initial conditions).

To solve these equations, we must simultaneously solve for the state x and the sensitivity $S_{x,\theta}$ (whose dynamics depend on x). Thus, letting

$$M(t, \theta_0) := \frac{\partial f}{\partial x}(x, \theta, u)\bigg|_{x=x(t,\theta_0),\theta=\theta_0}, \qquad N(t, \theta_0) := \frac{\partial f}{\partial \theta}(x, \theta, u)\bigg|_{x=x(t,\theta_0),\theta=\theta_0},$$

we solve the set of $n + nm$ coupled differential equations

$$\frac{dx}{dt} = f(x, \theta_0, u), \qquad \frac{dS_{x,\theta}}{dt} = M(t, \theta_0)S_{x,\theta} + N(t, \theta_0), \tag{3.15}$$

with initial condition $x(0) = x_0$ and $S_{x,\theta}(0) = 0$.

This differential equation generalizes our previous results by allowing us to evaluate the sensitivity around a (non-constant) trajectory. Note that in the special case in which we are at an equilibrium point and the dynamics for $S_{x,\theta}$ are stable, the steady state solution of equation (3.15) is identical to that obtained in equation (3.9). However, equation (3.15) is much more general, allowing us to determine the change in the state of the system at a fixed time T, for example. This equation also does not require that our solution stay near an equilibrium point; it only requires that our perturbations in the parameters are sufficiently small. An example of how to apply this equation to study the effect of parameter changes on an oscillator is given in Section 5.4.

Several simulation tools include the ability to do sensitivity analysis of this sort, including COPASI and the MATLAB SimBiology toolbox.

Adaptation and disturbance rejection

In this section, we study how systems can keep a desired output response even in the presence of external disturbances. This property is particularly important for biomolecular systems, which are usually subject to a wide range of perturbations. These perturbations or disturbances can represent a number of different physical entities, including changes in the circuit's cellular environment, unmodeled/undesired interactions with other biological circuits present in the cell, or parameters whose values are uncertain.

point of half-maximal value of the Hill function $\beta/(1+(A/K_A)^n)$ to the right. As a consequence, the nullclines will intersect at one point only, in which the value of B is high and the value of A is low (Figure 5.4a). The opposite will occur when u_2 is high and $u_1 = 0$, leading to only one intersection point in which B is low and A is high (Figure 5.4b).

5.4 The repressilator

Elowitz and Leibler constructed an oscillatory genetic circuit consisting of three repressors arranged in a ring fashion and called it the "repressilator" [26] (Figure 5.1). The repressilator exhibits sinusoidal, limit cycle oscillations in periods of hours, slower than the cell-division time. Therefore, the state of the oscillator is transmitted between generations from mother to daughter cells.

A dynamical model of the repressilator can be obtained by composing three transcriptional modules in a loop fashion. The dynamics can be written as

$$\begin{aligned} \frac{dm_{\mathrm{A}}}{dt} &= F_1(C) - \delta m_{\mathrm{A}}, & \frac{dm_{\mathrm{B}}}{dt} &= F_2(A) - \delta m_{\mathrm{B}}, & \frac{dm_{\mathrm{C}}}{dt} &= F_3(B) - \delta m_{\mathrm{C}}, \\ \frac{dA}{dt} &= \kappa m_{\mathrm{A}} - \gamma A, & \frac{dB}{dt} &= \kappa m_{\mathrm{B}} - \gamma B, & \frac{dC}{dt} &= \kappa m_{\mathrm{C}} - \gamma C, \end{aligned} \tag{5.7}$$

where we take

$$F_1(P) = F_2(P) = F_3(P) = F(P) = \frac{\alpha}{1+(P/K)^n},$$

and assume initially that the parameters are the same for all the three repressor modules. The structure of system (5.7) belongs to the class of cyclic feedback systems that we have studied in Section 3.3. In particular, the Mallet-Paret and Smith Theorem 3.5 and Hastings et al. Theorem 3.4 can be applied to infer that if the system has a unique equilibrium point and this equilibrium is unstable, then the system admits a periodic solution. Therefore, to apply these results, we determine the number of equilibria and their stability.

The equilibria of the system can be found by setting the time derivatives to zero. Letting $\beta = (\kappa/\delta)$, we obtain

$$A_{eq} = \frac{\beta F_1(C_{eq})}{\gamma}, \qquad B_{eq} = \frac{\beta F_2(A_{eq})}{\gamma}, \qquad C_{eq} = \frac{\beta F_3(B_{eq})}{\gamma},$$

which combined together yield

$$A_{eq} = \frac{\beta}{\gamma} F_1\left(\frac{\beta}{\gamma} F_3\left(\frac{\beta}{\gamma} F_2(A_{eq})\right)\right) =: g(A_{eq}).$$

The solution to this equation determines the set of equilibria of the system. The number of equilibria is given by the number of crossings of the two functions

$h_1(A) = g(A)$ and $h_2(A) = A$. Since h_2 is strictly monotonically increasing, we obtain a unique equilibrium if h_1 is monotonically decreasing. This is the case when $g'(A) = dg(A)/dA < 0$, otherwise there could be multiple equilibrium points. Since we have that

$$\text{sign}(g'(A)) = \prod_{i=1}^{3} \text{sign}(F_i'(A)),$$

it follows that if $\Pi_{i=1}^{3}\text{sign}(F_i'(A)) < 0$ the system has a unique equilibrium. We call the product $\Pi_{i=1}^{3}\text{sign}(F_i'(A))$ the *loop sign*.

It follows that any cyclic feedback system with negative loop sign will have a unique equilibrium. In the present case, system (5.7) is such that $F_i' < 0$, so that the loop sign is negative and there is a unique equilibrium. We next study the stability of this equilibrium by studying the linearization of the system.

Letting P denote the equilibrium value of the protein concentrations for A, B, and C, the Jacobian matrix of the system is given by

$$J = \begin{pmatrix} -\delta & 0 & 0 & 0 & 0 & F_1'(P) \\ \kappa & -\gamma & 0 & 0 & 0 & 0 \\ 0 & F_2'(P) & -\delta & 0 & 0 & 0 \\ 0 & 0 & \kappa & -\gamma & 0 & 0 \\ 0 & 0 & 0 & F_3'(P) & -\delta & 0 \\ 0 & 0 & 0 & 0 & \kappa & -\gamma \end{pmatrix},$$

whose characteristic polynomial is given by

$$\det(sI - J) = (s+\gamma)^3(s+\delta)^3 - \kappa^3 \prod_{i=1}^{3} F_i'(P). \tag{5.8}$$

The roots of this characteristic polynomial are given by

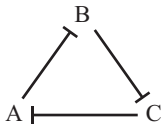
$$(s+\gamma)(s+\delta) = r,$$

in which $r \in \{\kappa F'(P), -(\kappa F'(P)/2)(1 - i\sqrt{3}), -(\kappa F'(P)/2)(1 + i\sqrt{3})\}$ and $i = \sqrt{-1}$ represents the imaginary unit. In order to invoke Hastings et al. Theorem 3.4 to infer the existence of a periodic orbit, it is sufficient that one of the roots of the characteristic polynomial has positive real part. This is the case if

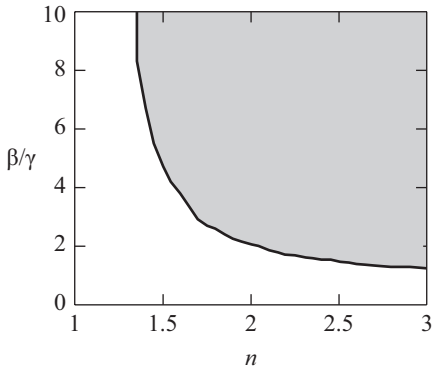
$$\kappa|F'(P)| > 2\gamma\delta, \qquad |F'(P)| = \alpha \frac{n(P^{n-1}/K^n)}{(1 + (P/K)^n)^2},$$

in which P is the equilibrium value satisfying the equilibrium condition

$$P = \frac{\beta}{\gamma} \frac{\alpha}{1 + (P/K)^n}.$$



(a) Repressilator



(b) Parameter space

Figure 5.5: Parameter space for the repressilator. (a) Repressilator diagram. (b) Space of parameters that give rise to oscillations. Here, we have set $K = 1$ for simplicity.

One can plot the pair of values $(n,\beta/\gamma)$ for which the above two conditions are satisfied. This leads to the plot of Figure 5.5b. When n increases, the existence of an unstable equilibrium point is guaranteed for larger ranges of β/γ. Of course, this "behavioral" robustness does not guarantee that other important features of the oscillator, such as the period, are not changed when parameters vary.

A similar result for the existence of a periodic solution can be obtained when two of the Hill functions are monotonically increasing and only one is monotonically decreasing:

$$F_1(P) = \frac{\alpha}{1+(P/K)^n}, \qquad F_2(P) \quad = \frac{\alpha(P/K)^n}{1+(P/K)^n}, \qquad F_3(P) = \frac{\alpha(P/K)^n}{1+(P/K)^n}.$$

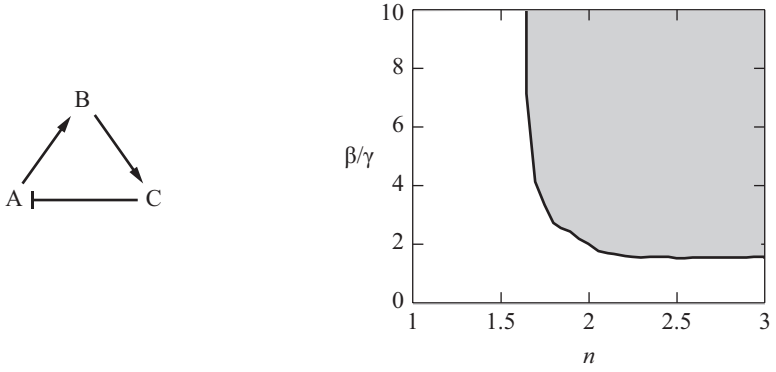
That is, two interactions are activations and one is a repression. We refer to this as the "non-symmetric" design. Since the loop sign is still negative, there is only one equilibrium point. We can thus obtain the condition for oscillations again by establishing conditions on the parameters that guarantee that at least one root of the characteristic polynomial (5.8) has positive real part, that is,

$$\kappa(|F_1'(P_3)F_2'(P_1)F_3'(P_2)|)^{(1/3)} > 2\gamma\delta, \tag{5.9}$$

in which P_1, P_2, P_3 are the equilibrium values of A, B, and C, respectively. These equilibrium values satisfy:

$$P_2 = \frac{\beta}{\gamma}\frac{(P_1/K)^n}{1+(P_1/K)^n}, \qquad P_3 = \frac{\beta}{\gamma}\frac{(P_2/K)^n}{1+(P_2/K)^n}, \qquad P_1(1+(P_3/K)^n) = \frac{\beta}{\gamma}.$$

Using these expressions numerically and checking for each combination of the parameters $(n,\beta/\gamma)$ whether (5.9) is satisfied, we can plot the combinations of n and β/γ values that lead to an unstable equilibrium. This is shown in Figure 5.6b.



(a) Loop oscillator (b) Parameter space

Figure 5.6: Parameter space for a loop oscillator. (a) Oscillator diagram. (b) Space of parameters that give rise to oscillations. As the value of n is increased, the range of the other parameter for which a periodic cycle exists becomes larger. Here, we have set $K = 1$.

From this figure, we can deduce that the qualitative shape of the parameter space that leads to a limit cycle is the same in the repressilator and in the non-symmetric design. One can conclude that it is then possible to design the circuit such that the parameters land in the filled region of the plots.

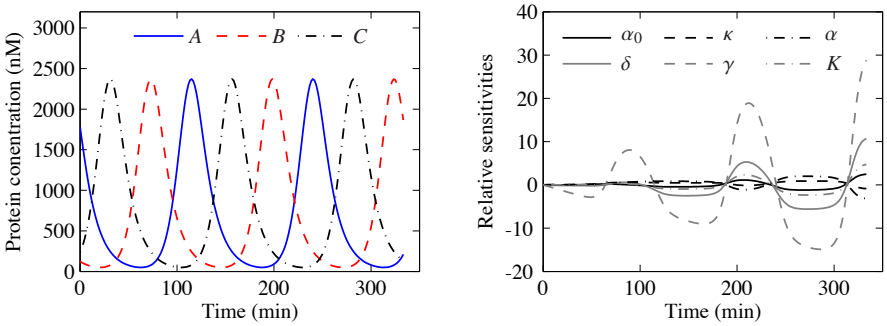
In practice, values of the Hill coefficient n between one and two can be obtained by employing repressors that have cooperativity higher than or equal to two. There are plenty of such repressors, including those originally used in the repressilator design [26]. However, values of n greater than two may be hard to reach in practice. To overcome this problem, one can include more elements in the loop. In fact, it is possible to show that the value of n sufficient for obtaining an unstable equilibrium decreases when the number of elements in the loop is increased (see Exercise 5.6). Figure 5.7a shows a simulation of the repressilator.

In addition to determining the space of parameters that lead to periodic trajectories, it is also relevant to determine the parameters to which the system behavior is the most sensitive. To address this question, we can use the parameter sensitivity analysis tools of Section 3.2. In this case, we model the repressilator Hill functions adding the basal expression rate as it was originally done in [26]:

$$F_1(P) = F_2(P) = F_3(P) = \frac{\alpha}{1 + (P/K)^n} + \alpha_0.$$

Letting $x = (m_A, A, m_B, B, m_C, C)$ and $\theta = (\alpha_0, \delta, \kappa, \gamma, \alpha, K)$, we can compute the sensitivity $S_{x,\theta}$ along the limit cycle corresponding to nominal parameter vector θ_0 as illustrated in Section 3.2:

$$\frac{dS_{x,\theta}}{dt} = M(t, \theta_0) S_{x,\theta} + N(t, \theta_0),$$



(a) Protein concentration (b) Sensitivity

Figure 5.7: Repressilator parameter sensitivity analysis. (a) Protein concentrations as functions of time. (b) Sensitivity plots. The most important parameters are the protein and mRNA decay rates γ and δ. Parameter values used in the simulations are $\alpha = 800$ nM/s, $\alpha_0 = 5 \times 10^{-4}$ nM/s, $\delta = 5.78 \times 10^{-3}$ s^{-1}, $\gamma = 1.16 \times 10^{-3}$ s^{-1}, $\kappa = 0.116$ s^{-1}, $n = 2$, and $K = 1600$ nM.

where $M(t, \theta_0)$ and $N(t, \theta_0)$ are both periodic in time. If the dynamics of $S_{x,\theta}$ are stable then the resulting solutions will be periodic, showing how the dynamics around the limit cycle depend on the parameter values. The results are shown in Figure 5.7, where we plot the steady state sensitivity of A as a function of time. We see, for example, that the limit cycle depends strongly on the protein degradation and dilution rate δ, indicating that changes in this value can lead to (relatively) large variations in the magnitude of the limit cycle.

5.5 Activator-repressor clock

Consider the activator-repressor clock diagram shown in Figure 5.1. The activator A takes two inputs: the activator A itself and the repressor B. The repressor B has the activator A as the only input. Let $\mathrm{m_A}$ and $\mathrm{m_B}$ represent the mRNA of the activator and of the repressor, respectively. Then, we consider the following four-dimensional model describing the rate of change of the species concentrations:

$$\begin{aligned} \frac{dm_\mathrm{A}}{dt} &= F_1(A,B) - \delta_A m_\mathrm{A}, & \frac{dm_\mathrm{B}}{dt} &= F_2(A) - \delta_B m_\mathrm{B}, \\ \frac{dA}{dt} &= \kappa_A m_\mathrm{A} - \gamma_A A, & \frac{dB}{dt} &= \kappa_B m_\mathrm{B} - \gamma_B B, \end{aligned} \tag{5.10}$$

in which the functions F_1 and F_2 are Hill functions and given by

$$F_1(A,B) = \frac{\alpha_A (A/K_A)^n + \alpha_{A0}}{1 + (A/K_A)^n + (B/K_B)^m}, \qquad F_2(A) = \frac{\alpha_B (A/K_A)^n + \alpha_{B0}}{1 + (A/K_A)^n}.$$

The Hill function F_1 can be obtained through a combinatorial promoter, where there are sites both for an activator and for a repressor. The Hill function F_2 has the