Biomolecular Feedback Systems

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Chapter 3
Analysis of Dynamic Behavior

In this chapter, we describe some of the tools from dynamical systems and feedback control theory that will be used in the rest of the text to analyze and design biological circuits. We focus here on deterministic models and the associated analyses; stochastic methods are given in Chapter 4.

3.1 Analysis near equilibria

As in the case of many other classes of dynamical systems, a great deal of insight into the behavior of a biological system can be obtained by analyzing the dynamics of the system subject to small perturbations around a known solution. We begin by considering the dynamics of the system near an equilibrium point, which is one of the simplest cases and provides a rich set of methods and tools.

In this section we will model the dynamics of our system using the input/output modeling formalism described in Chapter 1:

$$\frac{dx}{dt} = f(x, \theta, u), \qquad y = h(x, \theta), \tag{3.1}$$

where $x \in \mathbb{R}^n$ is the system state, $\theta \in \mathbb{R}^p$ are the system parameters and $u \in \mathbb{R}^q$ is a set of external inputs (including disturbances and noise). The system state x is a vector whose components will represent concentration of species, such as transcription factors, enzymes, substrates and DNA promoter sites. The system parameters θ are also represented as a vector, whose components will represent biochemical parameters such as association and dissociation rate constants, production rate constants, decay rate constants and dissociation constants. The input u is a vector whose components will represent concentration of a number of possible physical entities, including kinases, allosteric effectors and some transcription factors. The output $y \in \mathbb{R}^m$ of the system represents quantities that can be measured or that are of interest for the specific problem under study.

Example 3.1 (Transcriptional component)**.** Consider a promoter controlling a gene g that can be regulated by a transcription factor Z. Let m and G represent the mRNA and protein expressed by gene g. We can view this as a system in which $u = Z$ is the concentration of transcription factor regulating the promoter, the state $x = (x_1, x_2)$ is such that $x_1 = m$ is the concentration of mRNA and $x_2 = G$ is the

concentration of protein, which we can take as the output of interest, that is, $y = G = x_2$. Assuming that the transcription factor regulating the promoter is a repressor, the system dynamics can be described by the following system:

$$\frac{dx_1}{dt} = \frac{\alpha}{1+(u/K)^n} - \delta x_1, \qquad \frac{dx_2}{dt} = \kappa x_1 - \gamma x_2, \qquad y = x_2, \tag{3.2}$$

in which $\theta = (\alpha, K, \delta, \kappa, \gamma, n)$ is the vector of system parameters. In this case, we have that

$$f(x,\theta,u) = \begin{pmatrix} \dfrac{\alpha}{1+(u/K)^n} - \delta x_1 \\ \kappa x_1 - \gamma x_2 \end{pmatrix}, \qquad h(x,\theta) = x_2.$$

∇

Note that we have chosen to explicitly model the system parameters θ, which can be thought of as an additional set of (mainly constant) inputs to the system.

Equilibrium points and stability [1]

We begin by considering the case where the input u and parameters θ in equation (3.1) are fixed and hence we can write the dynamics of the system as

$$\frac{dx}{dt} = f(x). \tag{3.3}$$

An *equilibrium point* of the dynamical system represents a stationary condition for the dynamics. We say that a state x_e is an equilibrium point for a dynamical system if $f(x_e) = 0$. If a dynamical system has an initial condition $x(0) = x_e$, then it will stay at the equilibrium point: $x(t) = x_e$ for all $t \geq 0$.

Equilibrium points are one of the most important features of a dynamical system since they define the states corresponding to constant operating conditions. A dynamical system can have zero, one or more equilibrium points.

The *stability* of an equilibrium point determines whether or not solutions nearby the equilibrium point remain close, get closer or move further away. An equilibrium point x_e is *stable* if solutions that start near x_e stay close to x_e. Formally, we say that the equilibrium point x_e is stable if for all $\epsilon > 0$, there exists a $\delta > 0$ such that

$$\|x(0) - x_e\| < \delta \quad \Longrightarrow \quad \|x(t) - x_e\| < \epsilon \quad \text{for all } t > 0,$$

where $x(t)$ represents the solution to the differential equation (3.3) with initial condition $x(0)$. Note that this definition does not imply that $x(t)$ approaches x_e as time increases but just that it stays nearby. Furthermore, the value of δ may depend on ϵ, so that if we wish to stay very close to the solution, we may have to start very, very

[1] The material of this section is adopted from Åström and Murray [1].

close ($\delta \ll \epsilon$). This type of stability is also called *stability in the sense of Lyapunov*. If an equilibrium point is stable in this sense and the trajectories do not converge, we say that the equilibrium point is *neutrally stable*.

An example of a neutrally stable equilibrium point is shown in Figure 3.1. The

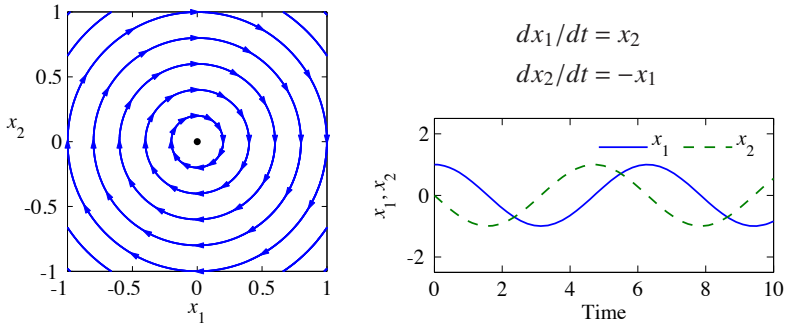


Figure 3.1: Phase portrait (trajectories in the state space) on the left and time domain simulation on the right for a system with a single stable equilibrium point. The equilibrium point x_e at the origin is stable since all trajectories that start near x_e stay near x_e.

figure shows the set of state trajectories starting at different initial conditions in the two-dimensional state space, also called the *phase plane*. From this set, called the *phase portrait*, we see that if we start near the equilibrium point, then we stay near the equilibrium point. Indeed, for this example, given any ϵ that defines the range of possible initial conditions, we can simply choose $\delta = \epsilon$ to satisfy the definition of stability since the trajectories are perfect circles.

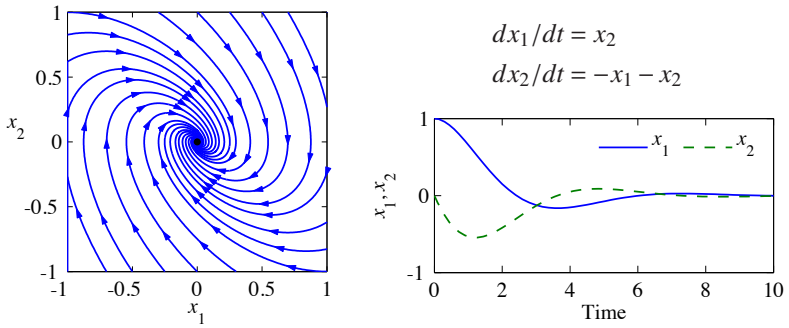


Figure 3.2: Phase portrait and time domain simulation for a system with a single asymptotically stable equilibrium point. The equilibrium point x_e at the origin is asymptotically stable since the trajectories converge to this point as $t \to \infty$.

An equilibrium point x_e is *asymptotically stable* if it is stable in the sense of Lyapunov and also $x(t) \to x_e$ as $t \to \infty$ for $x(0)$ sufficiently close to x_e. This corresponds to the case where all nearby trajectories converge to the stable solution for large time. Figure 3.2 shows an example of an asymptotically stable equilibrium

point. Note from the phase portraits that not only do all trajectories stay near the equilibrium point at the origin, but that they also all approach the origin as t gets large (the directions of the arrows on the phase portrait show the direction in which the trajectories move).

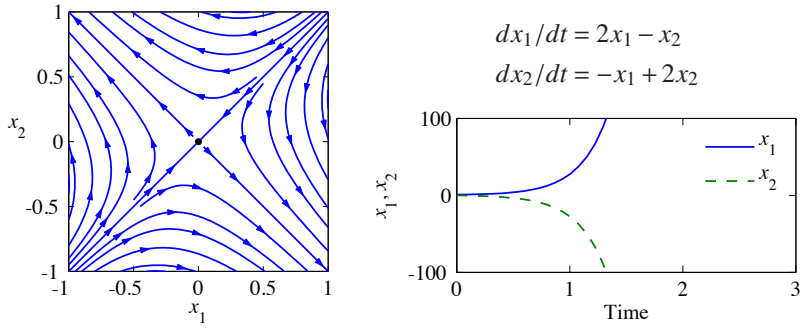


Figure 3.3: Phase portrait and time domain simulation for a system with a single unstable equilibrium point. The equilibrium point x_e at the origin is unstable since not all trajectories that start near x_e stay near x_e. The sample trajectory on the right shows that the trajectories very quickly depart from zero.

An equilibrium point x_e is *unstable* if it is not stable. More specifically, we say that an equilibrium point x_e is unstable if given some $\epsilon > 0$, there does *not* exist a $\delta > 0$ such that if $\|x(0) - x_e\| < \delta$, then $\|x(t) - x_e\| < \epsilon$ for all t. An example of an unstable equilibrium point is shown in Figure 3.3.

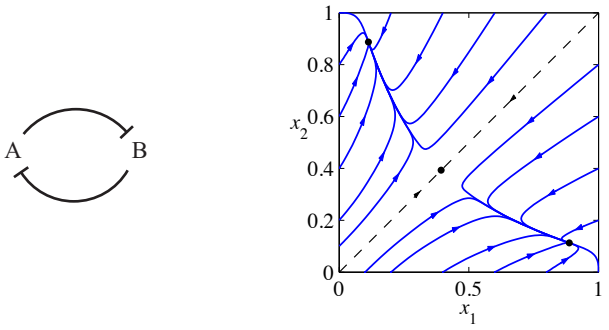
The definitions above are given without careful description of their domain of applicability. More formally, we define an equilibrium point to be *locally stable* (or *locally asymptotically stable*) if it is stable for all initial conditions $x \in B_r(a)$, where

$$B_r(a) = \{x : \|x - a\| < r\}$$

is a ball of radius r around a and $r > 0$. A system is *globally stable* if it is stable for all $r > 0$. Systems whose equilibrium points are only locally stable can have interesting behavior away from equilibrium points (see [1], Section 4.4).

To better understand the dynamics of the system, we can examine the set of all initial conditions that converge to a given asymptotically stable equilibrium point. This set is called the *region of attraction* for the equilibrium point. In general, computing regions of attraction is difficult. However, even if we cannot determine the region of attraction, we can often obtain patches around the stable equilibria that are attracting. This gives partial information about the behavior of the system.

For planar dynamical systems, equilibrium points have been assigned names based on their stability type. An asymptotically stable equilibrium point is called a *sink* or sometimes an *attractor*. An unstable equilibrium point can be either a *source*, if all trajectories lead away from the equilibrium point, or a *saddle*, if some trajectories lead to the equilibrium point and others move away (this is the situ-



(a) Bistable circuit (b) Phase portrait

Figure 3.4: (a) Diagram of a bistable gene circuit composed of two genes. (b) Phase portrait showing the trajectories converging to either one of the two possible stable equilibria depending on the initial condition. The parameters are $\beta_1 = \beta_2 = 1$ μM/min, $K_1 = K_2 = 0.1$ μM, and $\gamma = 1$ min^{-1}.

ation pictured in Figure 3.3). Finally, an equilibrium point that is stable but not asymptotically stable (i.e., neutrally stable, such as the one in Figure 3.1) is called a *center*.

Example 3.2 (Bistable gene circuit)**.** Consider a system composed of two genes that express transcription factors repressing each other as shown in Figure 3.4a. Denoting the concentration of protein A by x_1 and that of protein B by x_2, and neglecting the mRNA dynamics, the system can be modeled by the following differential equations:

$$\frac{dx_1}{dt} = \frac{\beta_1}{1 + (x_2/K_2)^n} - \gamma x_1, \qquad \frac{dx_2}{dt} = \frac{\beta_2}{1 + (x_1/K_1)^n} - \gamma x_2.$$

Figure 3.4b shows the phase portrait of the system. This system is bistable because there are two (asymptotically) stable equilibria. Specifically, the trajectories converge to either of two possible equilibria: one where x_1 is high and x_2 is low and the other where x_1 is low and x_2 is high. A trajectory will approach the first equilibrium point if the initial condition is below the dashed line, called the separatrix, while it will approach the second one if the initial condition is above the separatrix. Hence, the region of attraction of the first equilibrium is the region of the plane below the separatrix and the region of attraction of the second one is the portion of the plane above the separatrix. ∇

Nullcline analysis

Nullcline analysis is a simple and intuitive way to determine the stability of an equilibrium point for systems in $\mathbb{R}^2$. Consider the system with $x = (x_1, x_2) \in \mathbb{R}^2$

described by the differential equations

$$\frac{dx_1}{dt} = f_1(x_1, x_2), \qquad \frac{dx_2}{dt} = f_2(x_1, x_2).$$

The nullclines of this system are given by the two curves in the x_1, x_2 plane in which $f_1(x_1, x_2) = 0$ and $f_2(x_1, x_2) = 0$. The nullclines intersect at the equilibria of the system x_e. Figure 3.5 shows an example in which there is a unique equilibrium.

The stability of the equilibrium is deduced by inspecting the direction of the trajectory of the system starting at initial conditions x close to the equilibrium x_e. The direction of the trajectory can be obtained by determining the signs of f_1 and f_2 in each of the regions in which the nullclines partition the plane around the equilibrium x_e. If $f_1 < 0$ ($f_1 > 0$), we have that x_1 is going to decrease (increase) and similarly if $f_2 < 0$ ($f_2 > 0$), we have that x_2 is going to decrease (increase). In Figure 3.5, we show a case in which $f_1 < 0$ on the right-hand side of the nullcline $f_1 = 0$ and $f_1 > 0$ on the left-hand side of the same nullcline. Similarly, we have chosen a case in which $f_2 < 0$ above the nullcline $f_2 = 0$ and $f_2 > 0$ below the same nullcline. Given these signs, it is clear from the figure that starting from any point x close to x_e the vector field will always point toward the equilibrium x_e and hence the trajectory will tend toward such equilibrium. In this case, it then follows that the equilibrium x_e is asymptotically stable.

Example 3.3 (Negative autoregulation)**.** As an example, consider expression of a gene with negative feedback. Let x_1 represent the mRNA concentration and x_2 represent the protein concentration. Then, a simple model (in which for simplicity we have assumed all parameters to be 1) is given by

$$\frac{dx_1}{dt} = \frac{1}{1 + x_2} - x_1, \qquad \frac{dx_2}{dt} = x_1 - x_2,$$

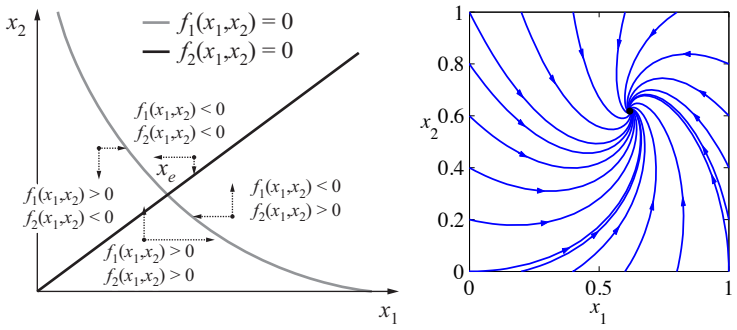
so that $f_1(x_1, x_2) = 1/(1 + x_2) - x_1$ and $f_2(x_1, x_2) = x_1 - x_2$. Figure 3.5a exactly represents the situation for this example. In fact, we have that

$$f_1(x_1, x_2) < 0 \quad \Longleftrightarrow \quad x_1 > \frac{1}{1 + x_2}, \qquad f_2(x_1, x_2) < 0 \quad \Longleftrightarrow \quad x_2 > x_1,$$

which provides the direction of the vector field as shown in Figure 3.5a. As a consequence, the equilibrium point is stable. The phase portrait of Figure 3.5b confirms the fact since the trajectories all converge to the unique equilibrium point. ∇

Stability analysis via linearization

For systems with more than two states, the graphical technique of nullcline analysis cannot be used. Hence, we must resort to other techniques to determine stability.



(a) Nullclines (b) Phase portrait

Figure 3.5: (a) Example of nullclines for a system with a single equilibrium point x_e. To understand the stability of the equilibrium point x_e, one traces the direction of the vector field (f_1, f_2) in each of the four regions in which the nullclines partition the plane. If in each region the vector field points toward the equilibrium point, then such a point is asymptotically stable. (b) Phase portrait for the negative autoregulation example.

Consider a linear dynamical system of the form

$$\frac{dx}{dt} = Ax, \quad x(0) = x_0, \tag{3.4}$$

where $A \in \mathbb{R}^{n\times n}$. For a linear system, the stability of the equilibrium at the origin can be determined from the eigenvalues of the matrix A:

$$\lambda(A) = \{s \in \mathbb{C} : \det(sI - A) = 0\}.$$

The polynomial $\det(sI - A)$ is the *characteristic polynomial* and the eigenvalues are its roots. We use the notation λ_j for the jth eigenvalue of A and $\lambda(A)$ for the set of all eigenvalues of A, so that $\lambda_j \in \lambda(A)$. For each eigenvalue λ_j there is a corresponding eigenvector $v_j \in \mathbb{C}^n$, which satisfies the equation $Av_j = \lambda_j v_j$.

In general λ can be complex-valued, although if A is real-valued, then for any eigenvalue λ, its complex conjugate λ^* will also be an eigenvalue. The origin is always an equilibrium point for a linear system. Since the stability of a linear system depends only on the matrix A, we find that stability is a property of the system. For a linear system we can therefore talk about the stability of the system rather than the stability of a particular solution or equilibrium point.

The easiest class of linear systems to analyze are those whose system matrices are in diagonal form. In this case, the dynamics have the form

$$\frac{dx}{dt} = \begin{pmatrix} \lambda_1 & & & 0 \\ & \lambda_2 & & \\ & & \ddots & \\ 0 & & & \lambda_n \end{pmatrix} x. \tag{3.5}$$

It is easy to see that the state trajectories for this system are independent of each other, so that we can write the solution in terms of n individual systems $\dot{x}_j = \lambda_j x_j$. Each of these scalar solutions is of the form

$$x_j(t) = e^{\lambda_j t} x_j(0).$$

We see that the equilibrium point $x_e = 0$ is stable if $\lambda_j \leq 0$ and asymptotically stable if $\lambda_j < 0$.

Another simple case is when the dynamics are in the block diagonal form

$$\frac{dx}{dt} = \begin{pmatrix} \sigma_1 & \omega_1 & & 0 & 0 \\ -\omega_1 & \sigma_1 & & 0 & 0 \\ 0 & 0 & \ddots & \vdots & \vdots \\ 0 & 0 & & \sigma_m & \omega_m \\ 0 & 0 & & -\omega_m & \sigma_m \end{pmatrix} x.$$

In this case, the eigenvalues can be shown to be $\lambda_j = \sigma_j \pm i\omega_j$. We once again can separate the state trajectories into independent solutions for each pair of states, and the solutions are of the form

$$\begin{aligned} x_{2j-1}(t) &= e^{\sigma_j t}(x_{2j-1}(0)\cos\omega_j t + x_{2j}(0)\sin\omega_j t), \\ x_{2j}(t) &= e^{\sigma_j t}(-x_{2j-1}(0)\sin\omega_j t + x_{2j}(0)\cos\omega_j t), \end{aligned}$$

where $j = 1, 2, \ldots, m$. We see that this system is asymptotically stable if and only if $\sigma_j = \operatorname{Re}\lambda_j < 0$. It is also possible to combine real and complex eigenvalues in (block) diagonal form, resulting in a mixture of solutions of the two types.

Very few systems are in one of the diagonal forms above, but some systems can be transformed into these forms via coordinate transformations. One such class of systems is those for which the A matrix has distinct (non-repeating) eigenvalues. In this case there is a matrix $T \in \mathbb{R}^{n\times n}$ such that the matrix TAT^{-1} is in (block) diagonal form, with the block diagonal elements corresponding to the eigenvalues of the original matrix A. If we choose new coordinates $z = Tx$, then

$$\frac{dz}{dt} = T\dot{x} = TAx = TAT^{-1}z$$

and the linear system has a (block) diagonal A matrix. Furthermore, the eigenvalues of the transformed system are the same as the original system since if v is an eigenvector of A, then $w = Tv$ can be shown to be an eigenvector of TAT^{-1}. We can reason about the stability of the original system by noting that $x(t) = T^{-1}z(t)$, and so if the transformed system is stable (or asymptotically stable), then the original system has the same type of stability.

This analysis shows that for linear systems with distinct eigenvalues, the stability of the system can be completely determined by examining the real part of the eigenvalues of the dynamics matrix. For more general systems, we make use of the following theorem, proved in [1]:

Theorem 3.1 (Stability of a linear system)**.** *The system*

$$\frac{dx}{dt} = Ax$$

is asymptotically stable if and only if all eigenvalues of A all have a strictly negative real part and is unstable if any eigenvalue of A has a strictly positive real part.

In the case in which the system state is two-dimensional, that is, $x \in \mathbb{R}^2$, we have a simple way of determining the eigenvalues of a matrix A. Specifically, denote by $\mathrm{tr}(A)$ the trace of A, that is, the sum of the diagonal terms, and let $\det(A)$ be the determinant of A. Then, we have that the two eigenvalues are given by

$$\lambda_{1,2} = \frac{1}{2}\left(\mathrm{tr}(A) \pm \sqrt{\mathrm{tr}(A)^2 - 4\det(A)}\right).$$

Both eigenvalues have negative real parts when (i) $\mathrm{tr}(A) < 0$ and (ii) $\det(A) > 0$.

An important feature of differential equations is that it is often possible to determine the local stability of an equilibrium point by approximating the system by a linear system. Suppose that we have a nonlinear system

$$\frac{dx}{dt} = f(x)$$

that has an equilibrium point at x_e. Computing the Taylor series expansion of the vector field, we can write

$$\frac{dx}{dt} = f(x_e) + \left.\frac{\partial f}{\partial x}\right|_{x_e} (x - x_e) + \text{higher-order terms in } (x - x_e).$$

Since $f(x_e) = 0$, we can approximate the system by choosing a new state variable $z = x - x_e$ and writing

$$\frac{dz}{dt} = Az, \qquad \text{where} \quad A = \left.\frac{\partial f}{\partial x}\right|_{x_e}. \tag{3.6}$$

We call the system (3.6) the *linear approximation* of the original nonlinear system or the *linearization* at x_e. We also refer to matrix A as the *Jacobian matrix* of the original nonlinear system.

The fact that a linear model can be used to study the behavior of a nonlinear system near an equilibrium point is a powerful one. Indeed, we can take this even further and use a local linear approximation of a nonlinear system to design a feedback law that keeps the system near its equilibrium point (design of dynamics). Thus, feedback can be used to make sure that solutions remain close to the equilibrium point, which in turn ensures that the linear approximation used to stabilize it is valid.

Example 3.4 (Negative autoregulation)**.** Consider again the negatively autoregulated gene modeled by the equations

$$\frac{dx_1}{dt} = \frac{1}{1+x_2} - x_1, \qquad \frac{dx_2}{dt} = x_1 - x_2.$$

In this case,

$$f(x) = \begin{pmatrix} \frac{1}{1+x_2} - x_1 \\ x_1 - x_2 \end{pmatrix},$$

so that, letting $x_e = (x_{1,e}, x_{2,e})$, the Jacobian matrix is given by

$$A = \left.\frac{\partial f}{\partial x}\right|_{x_e} = \begin{pmatrix} -1 & -\frac{1}{(1+x_{2,e})^2} \\ 1 & -1 \end{pmatrix}.$$

It follows that $\mathrm{tr}(A) = -2 < 0$ and that $\det(A) = 1 + 1/(1+x_{2,e})^2 > 0$. Hence, independently of the value of the equilibrium point, the eigenvalues both have negative real parts, which implies that the equilibrium point x_e is asymptotically stable. ∇

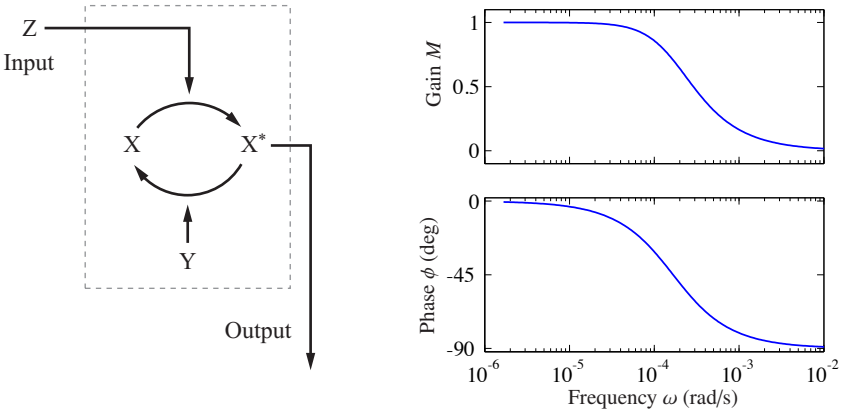
Frequency domain analysis

Frequency domain analysis is a way to understand how well a system can respond to rapidly changing input stimuli. As a general rule, most physical systems display an increased difficulty in responding to input stimuli as the frequency of variation increases: when the input stimulus changes faster than the natural time scales of the system, the system becomes incapable of responding. If instead the input is changing much more slowly than the natural time scales of the system, the system will have enough time to respond to the input. That is, the system behaves like a "low-pass filter." The cut-off frequency at which the system does not display a significant response is called the *bandwidth* and quantifies the dominant time scale. To identify this dominant time scale, we can perform input/output experiments in which the system is excited with periodic inputs at various frequencies. Then, we can plot the amplitude of response of the output as a function of the frequency of the input stimulation to obtain the "frequency response" of the system.

Example 3.5 (Phosphorylation cycle)**.** To illustrate the basic ideas, we consider the frequency response of a phosphorylation cycle, in which enzymatic reactions are each modeled by a one-step reaction. Referring to Figure 3.6a, we have that the one-step reactions involved are given by

$$\mathrm{Z} + \mathrm{X} \xrightarrow{k_1} \mathrm{Z} + \mathrm{X}^*, \qquad \mathrm{Y} + \mathrm{X}^* \xrightarrow{k_2} \mathrm{Y} + \mathrm{X},$$

with conservation law $X + X^* = X_{\mathrm{tot}}$. Let Y_{tot} be the total amount of phosphatase. We assume that the kinase Z has a time-varying concentration, which we view as the *input* to the system, while X^* is the *output* of the system.



(a) Phosphorylation cycle (b) Frequency response

Figure 3.6: (a) Diagram of a phosphorylation cycle, in which Z is the kinase, X is the substrate, and Y is the phosphatase. (b) Bode plot showing the magnitude M and phase lag ϕ for the frequency response of a one-step reaction model of the phosphorylation system on the left. The parameters are $\beta = \gamma = 0.01$ min^{-1}.

The differential equation model for the dynamics is given by

$$\frac{dX^*}{dt} = k_1 Z(t)(X_{\text{tot}} - X^*) - k_2 Y_{\text{tot}} X^*.$$

If we assume that the cycle is weakly activated ($X^* \ll X_{\text{tot}}$), the above equation is well approximated by

$$\frac{dX^*}{dt} = \beta Z(t) - \gamma X^*, \tag{3.7}$$

where $\beta = k_1 X_{\text{tot}}$ and $\gamma = k_2 Y_{\text{tot}}$. To determine the frequency response, we set the input $Z(t)$ to a periodic function. It is customary to take sinusoidal functions as the input signal as they lead to an easy way to calculate the frequency response. Let then $Z(t) = A_0 \sin(\omega t)$.

Since equation (3.7) is linear in the state X^* and input Z, it can be directly integrated to yield

$$X^*(t) = \frac{A_0\beta}{\sqrt{\omega^2+\gamma^2}} \sin(\omega t - \tan^{-1}(\omega/\gamma)) - \frac{A_0\beta\omega}{(\omega^2+\gamma^2)} e^{-\gamma t}.$$

The second term dies out for t large enough. Hence, the steady state response is given by the first term. In particular, the amplitude of response is given by $A_0\beta/\sqrt{\omega^2+\gamma^2}$, in which the gain $\beta/\sqrt{\omega^2+\gamma^2}$ depends both on the system parameters and on the frequency of the input stimulation. As the frequency of the input stimulation ω increases, the amplitude of the response decreases and approaches zero for very high frequencies. Also, the argument of the sine function shows a

negative phase shift of $\tan^{-1}(\omega/\gamma)$, which indicates that there is an increased lag in responding to the input when the frequency increases. Hence, the key quantities in the frequency response are the magnitude $M(\omega)$, also called *gain* of the system, and phase lag $\phi(\omega)$ given by

$$M(\omega) = \frac{\beta}{\sqrt{\omega^2 + \gamma^2}}, \qquad \phi(\omega) = -\tan^{-1}\left(\frac{\omega}{\gamma}\right).$$

These are plotted in Figure 3.6b, a type of figure known as a *Bode plot*.

The bandwidth of the system, denoted ω_B, is the frequency at which the gain drops below $M(0)/\sqrt{2}$. In this case, the bandwidth is given by $\omega_B = \gamma = k_2 Y_{\text{tot}}$, which implies that the bandwidth of the system can be made larger by increasing the amount of phosphatase. However, note that since $M(0) = \beta/\gamma = k_1 X_{\text{tot}}/(k_2 Y_{\text{tot}})$, increased phosphatase will also result in decreased amplitude of response. Hence, if we want to increase the bandwidth of the system while keeping the value of $M(0)$ (also called the *zero frequency gain*) unchanged, one should increase the total amounts of substrate and phosphatase in comparable proportions. Fixing the value of the zero frequency gain, the bandwidth of the system increases with increased amounts of phosphatase and substrate. ∇

More generally, the *frequency response* of a linear system with one input and one output

$$\dot{x} = Ax + Bu, \qquad y = Cx + Du$$

is the response of the system to a sinusoidal input $u = a\sin\omega t$ with input amplitude a and frequency ω. The *transfer function* for a linear system is given by

$$G_{yu}(s) = C(sI - A)^{-1}B + D$$

and represents the steady state response of a system to an exponential signal of the form $u(t) = e^{st}$ where $s \in \mathbb{C}$. In particular, the response to a sinusoid $u = a\sin\omega t$ is given by $y = Ma\sin(\omega t + \phi)$ where the gain M and phase lag ϕ can be determined from the transfer function evaluated at $s = i\omega$:

$$G_{yu}(i\omega) = Me^{i\phi}, \qquad \begin{aligned} M(\omega) &= |G_{yu}(i\omega)| = \sqrt{\text{Im}(G_{yu}(i\omega))^2 + \text{Re}(G_{yu}(i\omega))^2}, \\ \phi(\omega) &= \tan^{-1}\left(\frac{\text{Im}(G_{yu}(i\omega))}{\text{Re}(G_{yu}(i\omega))}\right), \end{aligned}$$

where $\text{Re}(\cdot)$ and $\text{Im}(\cdot)$ represent the real and imaginary parts of a complex number. For finite dimensional linear (or linearized) systems, the transfer function can be written as a ratio of polynomials in s:

$$G(s) = \frac{b(s)}{a(s)}.$$

The values of s at which the numerator vanishes are called the *zeros* of the transfer function and the values of s at which the denominator vanishes are called the *poles*.

The transfer function representation of an input/output linear system is essentially equivalent to the state space description, but we reason about the dynamics by looking at the transfer function instead of the state space matrices. For example, it can be shown that the poles of a transfer function correspond to the eigenvalues of the matrix A, and hence the poles determine the stability of the system. In addition, interconnections between subsystems often have simple representations in terms of transfer functions. For example, two systems G_1 and G_2 in series (with the output of the first connected to the input of the second) have a combined transfer function $G_{\text{series}}(s) = G_1(s)G_2(s)$, and two systems in parallel (a single input goes to both systems and the outputs are summed) has the transfer function $G_{\text{parallel}}(s) = G_1(s) + G_2(s)$.

Transfer functions are useful representations of linear systems because the properties of the transfer function can be related to the properties of the dynamics. In particular, the shape of the frequency response describes how the system responds to inputs and disturbances, as well as allows us to reason about the stability of interconnected systems. The Bode plot of a transfer function gives the magnitude and phase of the frequency response as a function of frequency and the *Nyquist plot* can be used to reason about stability of a closed loop system from the open loop frequency response ([1], Section 9.2).

Returning to our analysis of biomolecular systems, suppose we have a system whose dynamics can be written as

$$\dot{x} = f(x, \theta, u)$$

and we wish to understand how the solutions of the system depend on the parameters θ and input disturbances u. We focus on the case of an equilibrium solution $x(t; x_0, \theta_0) = x_e$. Let $z = x - x_e$, $\tilde{u} = u - u_0$ and $\tilde{\theta} = \theta - \theta_0$ represent the deviation of the state, input and parameters from their nominal values. Linearization can be performed in a way similar to the way it was performed for a system with no inputs. Specifically, we can write the dynamics of the perturbed system using its linearization as

$$\frac{dz}{dt} = \left(\frac{\partial f}{\partial x}\right)_{(x_e,\theta_0,u_0)} \cdot z \quad + \quad \left(\frac{\partial f}{\partial \theta}\right)_{(x_e,\theta_0,u_0)} \cdot \tilde{\theta} \quad + \quad \left(\frac{\partial f}{\partial u}\right)_{(x_e,\theta_0,u_0)} \cdot \tilde{u}.$$

This linear system describes small deviations from $x_e(\theta_0, u_0)$ but allows $\tilde{\theta}$ and $\tilde{u}$ to be time-varying instead of the constant case considered earlier.

To analyze the resulting deviations, it is convenient to look at the system in the frequency domain. Let $y = Cx$ be a set of values of interest. The transfer functions between $\tilde{\theta}$, $\tilde{u}$ and y are given by

$$G_{y\tilde{\theta}}(s) = C(sI - A)^{-1}B_\theta, \qquad G_{y\tilde{u}}(s) = C(sI - A)^{-1}B_u,$$

where

$$A = \left.\frac{\partial f}{\partial x}\right|_{(x_e,\theta_0,u_0)}, \qquad B_\theta = \left.\frac{\partial f}{\partial \theta}\right|_{(x_e,\theta_0,u_0)}, \qquad B_u = \left.\frac{\partial f}{\partial u}\right|_{(x_e,\theta_0,u_0)}.$$

Note that if we let $s = 0$, we get the response to small, constant changes in parameters. For example, the change in the outputs y as a function of constant changes in the parameters is given by

$$G_{y\tilde{\theta}}(0) = -CA^{-1}B_\theta.$$

Example 3.6 (Transcriptional regulation)**.** Consider a genetic circuit consisting of a single gene. The dynamics of the system are given by

$$\frac{dm}{dt} = F(P) - \delta m, \qquad \frac{dP}{dt} = \kappa m - \gamma P,$$

where m is the mRNA concentration and P is the protein concentration. Suppose that the mRNA degradation rate δ can change as a function of time and that we wish to understand the sensitivity with respect to this (time-varying) parameter. Linearizing the dynamics around the equilibrium point (m_e, P_e) corresponding to a nominal value δ_0 of the mRNA degradation rate, we obtain

$$A = \begin{pmatrix} -\delta_0 & F'(P_e) \\ \kappa & -\gamma \end{pmatrix}, \qquad B_\delta = \begin{pmatrix} -m_e \\ 0 \end{pmatrix}. \tag{3.8}$$

For the case of no feedback we have $F(P) = \alpha$ and $F'(P) = 0$, and the system has the equilibrium point at $m_e = \alpha/\delta_0$, $P_e = \kappa\alpha/(\gamma\delta_0)$. The transfer function from δ to P, after linearization about the steady state, is given by

$$G^{\text{ol}}_{P\delta}(s) = \frac{-\kappa m_e}{(s+\delta_0)(s+\gamma)},$$

where "ol" stands for open loop. For the case of negative regulation, we have

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

and the resulting transfer function is given by

$$G^{\text{cl}}_{P\delta}(s) = \frac{\kappa m_e}{(s+\delta_0)(s+\gamma) + \kappa\sigma}, \qquad \sigma = -F'(P_e) = \frac{n\alpha P_e^{n-1}/K^n}{(1 + P_e^n/K^n)^2},$$

where "cl" stands for closed loop.

Figure 3.7 shows the frequency response for the two circuits. To make a meaningful comparison between open loop and closed loop systems, we select the parameters of the open loop system such that the equilibrium point for both open loop and closed loop systems are the same. This can be guaranteed if in the open loop system we choose, for example, $\alpha = P_e\delta_0/(\kappa/\gamma)$, in which P_e is the equilibrium value of P in the closed loop system. We see that the feedback circuit attenuates the response of the system to perturbations with low-frequency content but slightly amplifies perturbations at high frequency (compared to the open loop system). ∇

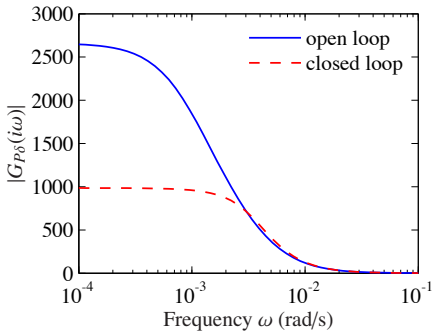


Figure 3.7: Attenuation of perturbations in a genetic circuit with linearization given by equation (3.8). The parameters of the closed loop system are given by $\alpha = 800$ μM/s, $\alpha_0 = 5 \times 10^{-4}$ μM/s, $\gamma = 0.001$ s^{-1}, $\delta_0 = 0.005$ s^{-1}, $\kappa = 0.02$ s^{-1}, $n = 2$, 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.

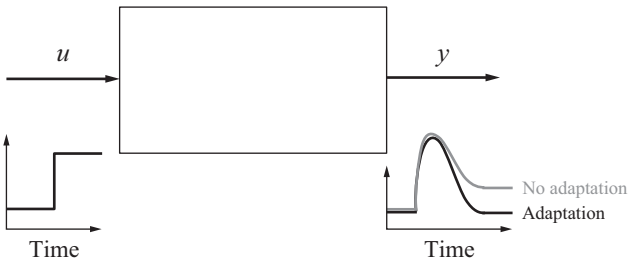


Figure 3.8: Adaptation property. The system is said to have the adaptation property if the steady state value of the output does not depend on the steady state value of the input. Hence, after a constant input perturbation, the output returns to its original value.

Here, we represent the disturbance input to the system of interest by u and we will say that the system adapts to the input u when the steady state value of its output y is independent of the (constant) nonzero value of the input (Figure 3.8). That is, the system's output is robust to the disturbance input. Basically, after the input changes to a constant nonzero value, the output returns to its original value after a transient perturbation. Adaptation corresponds to the concept of *disturbance rejection* in control theory. The full notion of disturbance rejection is more general, depends on the specific disturbance input and is often studied using the internal model principle [17].

We illustrate two main mechanisms to attain adaptation: integral feedback and incoherent feedforward loops (IFFLs). Here, we follow a similar treatment as that of [89]. In particular, we study these two mechanisms from a mathematical standpoint to illustrate how they achieve adaptation. Possible biomolecular implementations are presented in later chapters.

Integral feedback

In integral feedback systems, a "memory" variable z accounts for the accumulated error between the output of interest $y(t)$, which is affected by an external perturbation u, and its nominal (or desired) steady state value y_0. This accumulated error is then used to change the output y itself through a gain k (Figure 3.9). If the input perturbation u is constant, this feedback loop brings the system output back to the desired value y_0.

To understand why in this system the output $y(t)$, after any constant input perturbation u, tends to y_0 for $t \to \infty$ independently of the (constant) value of u, we write the equations relating the accumulated error z and the output y as obtained from the block diagram of Figure 3.9. The equations representing the system are given by

$$\frac{dz}{dt} = y_0 - y, \qquad y = kz + u,$$

so that the equilibrium is obtained by setting $\dot{z} = 0$, from which we obtain $y = y_0$.

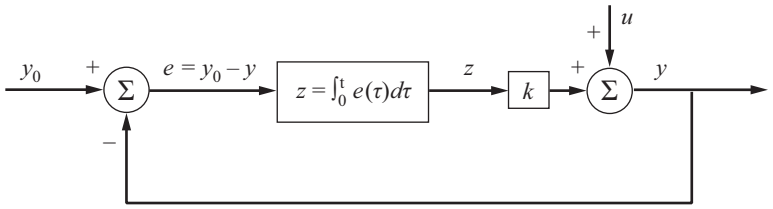


Figure 3.9: A basic block diagram representing a system with integral action. In the diagram, the circles with $\sum$ represent summing junctions, such that the output arrow is a signal given by the sum of the signals associated with the input arrows. The input signals are annotated with a "+" if added or a "−" if subtracted. The desired output y_0 is compared to the actual output y and the resulting error is integrated to yield z. This error is then used to change y. Here, the input u can be viewed as a disturbance input, which perturbs the value of the output y.

That is, the steady state of y does not depend on u. The additional question to answer is whether, after a perturbation u occurs, $y(t)$ tends to y_0 for $t \to \infty$. This is the case if and only if $\dot{z} \to 0$ as $t \to \infty$, which is satisfied if the equilibrium of the system $\dot{z} = -kz - u + y_0$ is asymptotically stable. This, in turn, is satisfied whenever $k > 0$ and u is a constant. Hence, after a constant perturbation u is applied, the system output y approaches its original steady state value y_0, that is, y is robust to constant perturbations.

More generally, a system with integral action can take the form

$$\frac{dx}{dt} = f(x,u), \quad u = (u_1, u_2), \quad y = h(x), \quad \frac{dz}{dt} = y_0 - y, \quad u_2 = k(x,z),$$

in which u_1 is a disturbance input and u_2 is a control input that takes the feedback form $u_2 = k(x,z)$. The steady state value of y, being the solution to $y_0 - y = 0$, does not depend on the disturbance u_1. In turn, y tends to this steady state value for $t \to \infty$ if and only if $\dot{z} \to 0$ as $t \to \infty$. This is the case if z tends to a constant value for $t \to \infty$, which is satisfied if u_1 is a constant and the steady state of the above system is asymptotically stable.

Integral feedback is recognized as a key mechanism of perfect adaptation in biological systems, both at the physiological level and at the cellular level, such as in blood calcium homeostasis [24], in the regulation of tryptophan in *E. coli* [94], in neuronal control of the prefrontal cortex [71], and in *E. coli* chemotaxis [102].

Incoherent feedforward loops

Feedforward motifs (Figure 3.10) are common in transcriptional networks and it has been shown that they are overrepresented in *E. coli* gene transcription networks, compared to other motifs composed of three nodes [4]. Incoherent feedforward circuits represent systems in which the input u directly helps promote the production of the output $y = x_2$ and also acts as a delayed inhibitor of the output

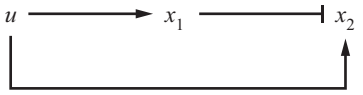


Figure 3.10: Incoherent feedforward loop. The input u affects the output $y = x_2$ through two channels: it indirectly represses it through an intermediate variable x_1 while directly activating it through a different path.

through an intermediate variable x_1. This incoherent counterbalance between positive and negative effects gives rise, under appropriate conditions, to adaptation. A large number of incoherent feedforward loops participate in important biological processes such as the EGF to ERK activation [75], the glucose to insulin release [76], ATP to intracellular calcium release [67], micro-RNA regulation [93], and many others.

Several variants of incoherent feedforward loops exist for perfect adaptation. Here, we consider two main ones, depending on whether the intermediate variable promotes degradation of the output or inhibits its production. An example where the intermediate variable promotes degradation is provided by the "sniffer," which appears in models of neutrophil motion and *Dictyostelium* chemotaxis [101]. In the sniffer, the intermediate variable promotes degradation according to the following differential equation model:

$$\frac{dx_1}{dt} = \alpha u - \gamma x_1, \qquad \frac{dx_2}{dt} = \beta u - \delta x_1 x_2, \qquad y = x_2. \tag{3.16}$$

In this system, the steady state value of the output x_2 is obtained by setting the time derivatives to zero. Specifically, we have that $\dot{x}_1 = 0$ gives $x_1 = \alpha u/\gamma$ and $\dot{x}_2 = 0$ gives $x_2 = \beta u/(\delta x_1)$. In the case in which $u \neq 0$, these can be combined to yield $x_2 = (\beta\gamma)/(\delta\alpha)$, which is a constant independent of the input u. The linearization of the system at the equilibrium is given by

$$A = \begin{pmatrix} -\gamma & 0 \\ -\delta(\beta\gamma)/(\delta\alpha) & -\delta(\alpha u/\gamma) \end{pmatrix},$$

which has eigenvalues $-\gamma$ and $-\delta(\alpha u/\gamma)$. Since these are both negative, the equilibrium point is asymptotically stable. Note that in the case in which, for example, u goes back to zero after a perturbation, as it is in the case of a pulse, the output x_2 does not necessarily return to its original steady state. That is, this system "adapts" only to constant nonzero input stimuli but is not capable of adapting to pulses. This can be seen from equation (3.16), which admits multiple steady states when $u = 0$. For more details on this "memory" effect, the reader is referred to [91].

A different form for an incoherent feedforward loop is one in which the intermediate variable x_1 inhibits production of the output x_2, such as in the system:

$$\frac{dx_1}{dt} = \alpha u - \gamma x_1, \qquad \frac{dx_2}{dt} = \beta \frac{u}{x_1} - \delta x_2, \qquad y = x_2. \tag{3.17}$$

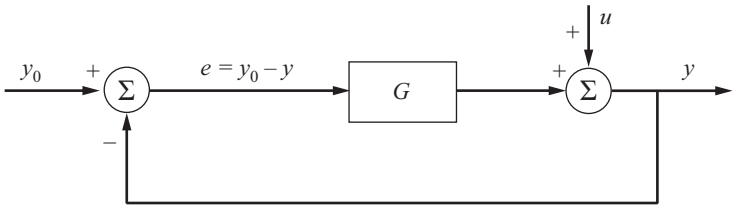


Figure 3.11: High gain feedback. A possible mechanism to attain disturbance attenuation is to feedback the error $y_0 - y$ between the desired output y_0 and the actual output y through a large gain G.

The equilibrium point of this system for a constant nonzero input u is given by setting the time derivatives to zero. From $\dot{x}_1 = 0$, we obtain $x_1 = \alpha u/\gamma$ and from $\dot{x}_2 = 0$ we obtain that $x_2 = \beta u/(\delta x_1)$, which combined together result in $x_2 = (\beta\gamma)/(\delta\alpha)$, which is again a constant independent of the input u.

By calculating the linearization at the equilibrium, one obtains

$$A = \begin{pmatrix} -\gamma & 0 \\ -\gamma^2/(\alpha^2 u) & -\delta \end{pmatrix},$$

whose eigenvalues are given by $-\gamma$ and $-\delta$. Hence, the equilibrium point is asymptotically stable. Further, one can show that the equilibrium point is globally asymptotically stable because the x_1 subsystem is linear, stable, and x_1 approaches a constant value (for constant u) and the x_2 subsystem, in which $\beta u/x_1$ is viewed as an external input is also linear and asymptotically stable.

High gain feedback

Integral feedback and incoherent feedforward loops provide means to obtain exact rejection of constant disturbances. Sometimes, exact rejection is not possible, for example, because the physical constraints of the system do not allow us to implement integral feedback or because the disturbance is not constant with time. In these cases, it may be possible to still attenuate the effect of the disturbance on the output of interest by the use of negative feedback with high gain. To explain this concept, consider the diagram of Figure 3.11.

In a high gain feedback configuration, the error between the output y, perturbed by some exogenous disturbance u, and a desired nominal output y_0 is fed back with a negative sign to produce the output y itself. If $y_0 > y$, this will result in an increase of y, otherwise it will result in a decrease of y. Mathematically, one obtains from the block diagram that

$$y = \frac{u}{1+G} + y_0 \frac{G}{1+G},$$

so that as G increases the (relative) contribution of u on the output of the system can be arbitrarily reduced.

High gain feedback can take a much more general form. Consider a system with $x \in \mathbb{R}^n$ in the form $\dot{x} = f(x)$. We say that this system is *contracting* if any two trajectories starting from different initial conditions exponentially converge to each other as time increases to infinity. A sufficient condition for the system to be contracting is that in some set of coordinates, with matrix transformation denoted Θ, the symmetric part of the linearization matrix (Jacobian)

$$\frac{1}{2}\left(\frac{\partial f}{\partial x} + \frac{\partial f}{\partial x}^T\right)$$

is negative definite. We denote the largest eigenvalue of this matrix by $-\lambda$ for $\lambda > 0$ and call it the contraction rate of the system.

Now, consider the nominal system $\dot{x} = Gf(x)$ for $G > 0$ and its perturbed version $\dot{x}_p = Gf(x_p) + u(t)$. Assume that the input $u(t)$ is bounded everywhere in norm by a constant $C > 0$. If the system is contracting, we have the following robustness result:

$$\|x(t) - x_p(t)\| \leq \chi \|x(0) - x_p(0)\| e^{-G\lambda t} + \frac{\chi C}{\lambda G},$$

in which χ is an upper bound on the condition number of the transformation matrix Θ (ratio between the largest and the smallest eigenvalue of $\Theta^T\Theta$) [62]. Hence, if the perturbed and the nominal systems start from the same initial conditions, the difference between their states can be made arbitrarily small by increasing the gain G. Therefore, the contribution of the disturbance u on the system state can be made arbitrarily small.

A comprehensive treatment of concepts of stability and robustness can be found in standard references [55, 90].

Scale invariance and fold-change detection

Scale invariance is the property by which the output $y(t)$ of the system does not depend on the absolute amplitude of the input $u(t)$ (Figure 3.12). Specifically, consider an adapting system and assume that it preadapted to a constant background input value a, then apply input $a + b$ and let $y(t)$ be the resulting output. Now consider a new background input value pa and let the system preadapt to it. Then apply the input $p(a + b)$ and let $\bar{y}(t)$ be the resulting output. The system has the scale invariance property if $y(t) = \bar{y}(t)$ for all t. This also means that the output responds in the same way to inputs changed by the same multiplicative factor (fold), hence this property is also called *fold-change detection*. Looking at Figure 3.12, the outputs corresponding to the two indicated inputs are identical since the fold change in the input value is equal to b/a in both cases.

Some incoherent feedforward loops can implement the fold-change detection property [35]. As an example, consider the feedforward motif represented by equations (3.17), in which the output is given by $y = x_2$, and consider two inputs:

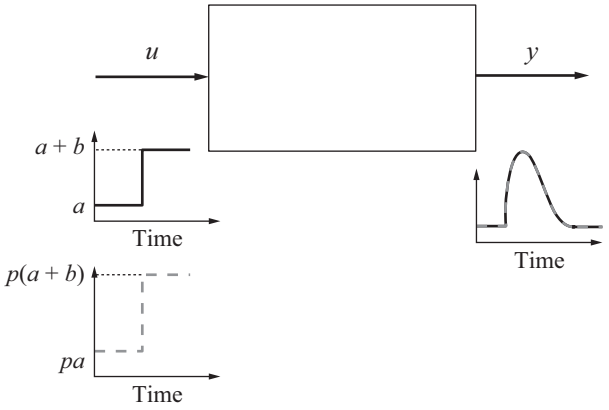


Figure 3.12: Fold-change detection. The output response does not depend on the absolute magnitude of the input but only on the fold change of the input.

$u_1(t) = a$ for $t < t_0$ and $u_1(t) = a + b_1$ for $t \geq t_0$, and $u_2(t) = pa$ for $t < t_0$ and $u_2(t) = pa + pb_1$ for $t \geq t_0$. Assume also that at time t_0 the system is at the steady state, that is, it is preadapted. Hence, we have that the two steady states from which the system starts at $t = t_0$ are given by $x_{1,1} = a\alpha/\gamma$ and $x_{1,2} = pa\alpha/\gamma$ for the x_1 variable and by $x_{2,1} = x_{2,2} = (\beta\gamma)/(\delta\alpha)$ for the x_2 variable. Integrating system (3.17) starting from these initial conditions, we obtain for $t \geq t_0$

$$x_{1,1}(t) = a\frac{\alpha}{\gamma}e^{-\gamma(t-t_0)} + (a+b)(1-e^{-\gamma(t-t_0)}),$$
$$x_{1,2}(t) = pa\frac{\alpha}{\gamma}e^{-\gamma(t-t_0)} + p(a+b)(1-e^{-\gamma(t-t_0)}).$$

Using these in the expression of $\dot{x}_2$ in equation (3.17) gives the differential equations that $x_{2,1}(t)$ and $x_{2,2}(t)$ obey for $t \geq t_0$ as

$$\frac{dx_{2,1}}{dt} = \frac{\beta(a+b)}{a\frac{\alpha}{\gamma}e^{-\gamma(t-t_0)} + (a+b)(1-e^{-\gamma(t-t_0)})} - \delta x_{2,1}, \qquad x_{2,1}(t_0) = (\beta\gamma)/(\delta\alpha)$$

and

$$\frac{dx_{2,2}}{dt} = \frac{p\beta(a+b)}{pa\frac{\alpha}{\gamma}e^{-\gamma(t-t_0)} + p(a+b)(1-e^{-\gamma(t-t_0)})} - \delta x_{2,2}, \qquad x_{2,2}(t_0) = (\beta\gamma)/(\delta\alpha),$$

which gives $x_{2,1}(t) = x_{2,2}(t)$ for all $t \geq t_0$. Hence, the system responds exactly the same way after changes in the input of the same fold. The output response is not dependent on the scale of the input but only on its shape.

3.3 Oscillatory behavior

In addition to equilibrium behavior, a variety of cellular processes involve oscillatory behavior in which the system state is constantly changing, but in a repeating

pattern. Two examples of biological oscillations are the cell cycle and circadian rhythm. Both of these dynamic behaviors involve repeating changes in the concentrations of various proteins, complexes and other molecular species in the cell, though they are very different in their operation. In this section we discuss some of the underlying ideas for how to model this type of oscillatory behavior, focusing on those types of oscillations that are most common in biomolecular systems.

Biomolecular oscillators

Biological systems have a number of natural oscillatory processes that govern the behavior of subsystems and whole organisms. These range from internal oscillations within cells to the oscillatory nature of the beating heart to various tremors and other undesirable oscillations in the neuro-muscular system. At the biomolecular level, two of the most studied classes of oscillations are the cell cycle and circadian rhythm.

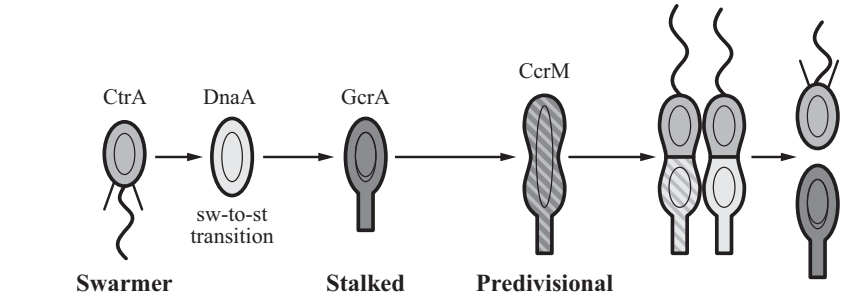
The cell cycle consists of a set of "phases" that govern the duplication and division of cells into two new cells:

- G1 phase - gap phase, terminated by "G1 checkpoint";
- S phase - synthesis phase (DNA replication);
- G2 phase - gap phase, terminated by "G2 checkpoint";
- M - mitosis (cell division).

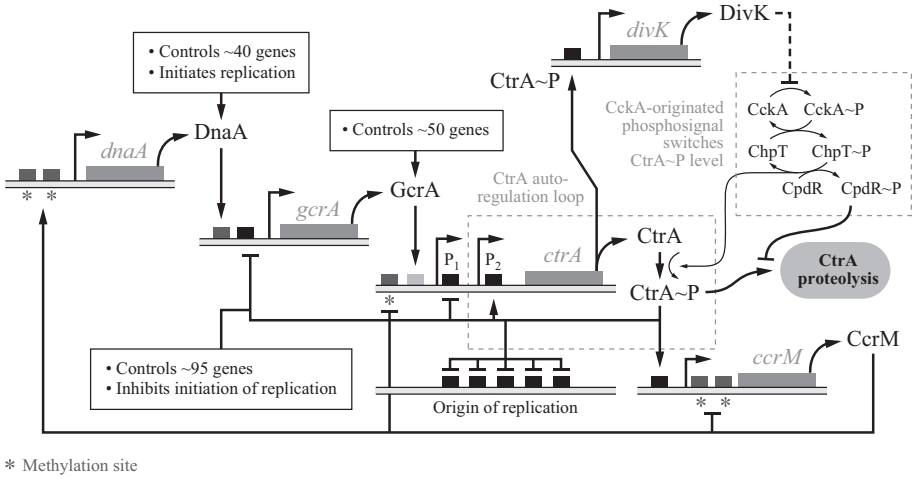
The cell goes through these stages in a cyclical fashion, with the different enzymes and pathways active in different phases. The cell cycle is regulated by many different proteins, often divided into two major classes. *Cyclins* are a class of proteins that sense environmental conditions internal and external to the cell and are also used to implement various logical operations that control transition out of the G1 and G2 phases. *Cyclin dependent kinases* (CDKs)are proteins that serve as "actuators" by turning on various pathways during different cell cycles.

An example of the control circuitry of the cell cycle for the bacterium *Caulobacter crescentus* (henceforth *Caulobacter*) is shown in Figure 3.13 [59]. This organism uses a variety of different biomolecular mechanisms, including transcriptional activation and repression, positive autoregulation (CtrA), phosphotransfer and methylation of DNA. The cell cycle is an example of an oscillator that does not have a fixed period. Instead, the length of the individual phases and the transitioning of the different phases are determined by the environmental conditions. As one example, the cell division time for *E. coli* can vary between 20 and 90 minutes due to changes in nutrient concentrations, temperature or other external factors.

A different type of oscillation is the highly regular pattern encoding in circadian rhythm, which repeats with a period of roughly 24 hours. The observation of circadian rhythms dates as far back as 400 BCE, when Androsthenes described observations of daily leaf movements of the tamarind tree [69]. There are three



(a) Overview of cell cycle



(b) Molecular mechanisms controlling cell cycle

Figure 3.13: The *Caulobacter crescentus* cell cycle. (a) *Caulobacter* cells divide asymmetrically into a stalked cell, which is attached to a surface, and a swarmer cell that is motile. The swarmer cells can become stalked cells in a new location and begin the cell cycle anew. The transcriptional regulators CtrA, DnaA and GcrA are the primary factors that control the various phases of the cell cycle. (b) The genetic circuitry controlling the cell cycle consists of a large variety of regulatory mechanisms, including transcriptional regulation and post-translational regulation. Figure adapted from [59].

defining characteristics associated with circadian rhythm: (1) the time to complete one cycle is approximately 24 hours, (2) the rhythm is endogenously generated and self-sustaining, and (3) the period remains relatively constant under changes in ambient temperature. Oscillations that have these properties appear in many different organisms, including microorganisms, plants, insects and mammals. Some common features of the circuitry implementing circadian rhythms in these organisms is the combination of positive and negative feedback loops, often with the positive elements activating the expression of clock genes and the negative elements repressing the positive elements [11]. Figure 3.14 shows some of the different organisms in which circadian oscillations can be found and the primary genes

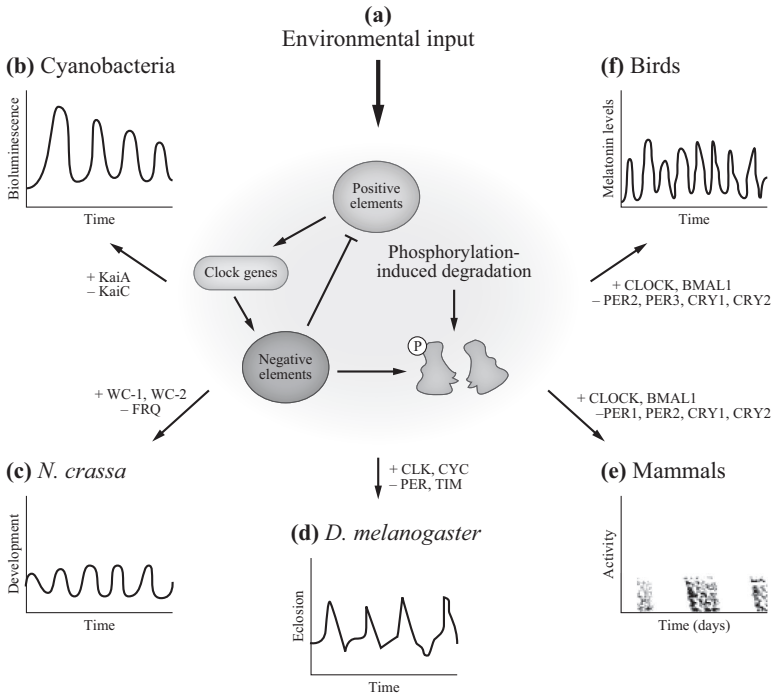


Figure 3.14: Overview of mechanisms for circadian rhythm in different organisms. Circadian rhythms are found in many different classes of organisms. A common pattern is a combination of positive and negative feedback, as shown in the center of the figure. Driven by environmental inputs (a), a variety of different genes are used to implement these positive and negative elements (b–f). Figure adapted from [11].

responsible for different positive and negative factors.

Clocks, oscillators and limit cycles

To begin our study of oscillators, we consider a nonlinear model of the system described by the differential equation

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

where $x \in \mathbb{R}^n$ represents the state of the system, $u \in \mathbb{R}^q$ represents the external inputs, $y \in \mathbb{R}^m$ represents the (measured) outputs and $\theta \in \mathbb{R}^p$ represents the model parameters. We say that a solution $(x(t), u(t))$ is *oscillatory with period* T if $y(t + T) = y(t)$. For simplicity, we will often assume that $p = q = 1$, so that we have a single input and single output, but most of the results can be generalized to the multi-input, multi-output case.

There are multiple ways in which a solution can be oscillatory. One of the simplest is that the input $u(t)$ is oscillatory, in which case we say that we have a *forced*

oscillation. In the case of a stable linear system with one input and one output, an input of the form $u(t) = A \sin \omega t$ will lead, after the transient due to initial conditions has died out, to an output of the form $y(t) = M \cdot A \sin(\omega t + \phi)$ where M and ϕ represent the gain and phase of the system (at frequency ω). In the case of a nonlinear system, if the output is periodic with the same period then we can write it in terms of a set of harmonics,

$$y(t) = B_0 + B_1 \sin(\omega t + \phi_1) + B_2 \sin(2\omega t + \phi_2) + \cdots .$$

The term B_0 represents the average value of the output (also called the bias), the terms B_i are the magnitudes of the ith harmonic and ϕ_i are the phases of the harmonics (relative to the input). The *oscillation frequency* ω is given by $\omega = 2\pi/T$ where T is the oscillation period.

A different situation occurs when we have no input (or a constant input) and still obtain an oscillatory output. In this case we say that the system has a *self-sustained oscillation*. This type of behavior is what is required for oscillations such as the cell cycle and circadian rhythm, where there is either no obvious forcing function or the forcing function is removed but the oscillation persists. If we assume that the input is constant, $u(t) = A_0$, then we are particularly interested in how the period T (or equivalently the frequency ω), amplitudes B_i and phases ϕ_i depend on the input A_0 and system parameters θ.

To simplify our notation slightly, we consider a system of the form

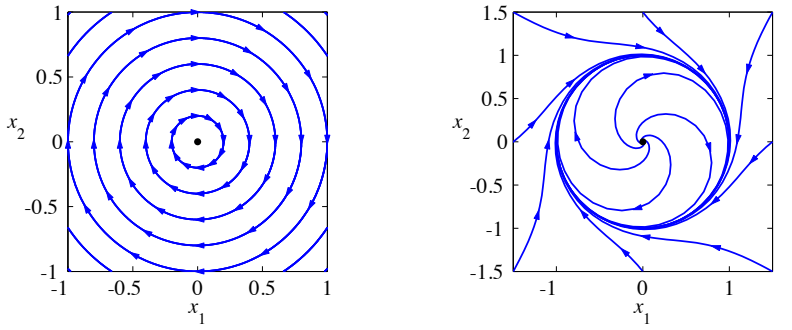
$$\frac{dx}{dt} = f(x, \theta), \qquad y = h(x, \theta), \tag{3.18}$$

where the input is ignored (or taken to be one of the constant parameters) in the analysis that follows. We have focused on the oscillatory nature of the output $y(t)$ thus far, but we note that if the states $x(t)$ are periodic then the output is as well, and this is the most common case. Hence we will often talk about the *system* being oscillatory, by which we mean that there is a solution for the dynamics in which the state satisfies $x(t + T) = x(t)$.

More formally, we say that a closed curve $\Gamma \in \mathbb{R}^n$ is an *orbit* if trajectories that start on Γ remain on Γ for all time and if Γ is not an equilibrium point of the system. As in the case of equilibrium points, we say that the orbit is *stable* if trajectories that start near Γ stay near Γ, *asymptotically stable* if in addition nearby trajectories approach Γ as $t \to \infty$ and *unstable* if it is not stable. The orbit Γ is periodic with period T if for any $x(t) \in \Gamma$, $x(t + T) = x(t)$.

There are many different types of periodic orbits that can occur in a system whose dynamics are modeled as in equation (3.18). A *harmonic oscillator* references to a system that oscillates around an equilibrium point, but does not (usually) get near the equilibrium point. The classical harmonic oscillator is a linear system of the form

$$\frac{d}{dt}\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} = \begin{pmatrix} 0 & \omega \\ -\omega & 0 \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix},$$



(a) Linear harmonic oscillator (b) Nonlinear harmonic oscillator

Figure 3.15: Examples of harmonic oscillators.

whose solutions are given by

$$\begin{pmatrix} x_1(t) \\ x_2(t) \end{pmatrix} = \begin{pmatrix} \cos\omega t & \sin\omega t \\ -\sin\omega t & \cos\omega t \end{pmatrix} \begin{pmatrix} x_1(0) \\ x_2(0) \end{pmatrix}.$$

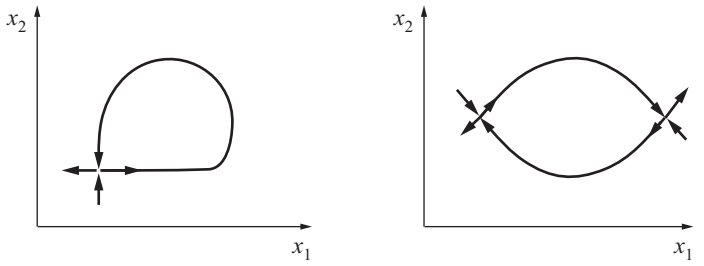
The frequency of this oscillation is fixed, but the amplitude depends on the values of the initial conditions, as shown in Figure 3.15a. Note that this system has a single equilibrium point at $x = (0,0)$ and the eigenvalues of the equilibrium point have zero real part, so trajectories neither expand nor contract, but simply oscillate.

An example of a nonlinear harmonic oscillator is given by the equation

$$\frac{dx_1}{dt} = x_2 + x_1(1 - x_1^2 - x_2^2), \qquad \frac{dx_2}{dt} = -x_1 + x_2(1 - x_1^2 - x_2^2). \tag{3.19}$$

This system has an equilibrium point at $x = (0,0)$, but the linearization of this equilibrium point is unstable. The phase portrait in Figure 3.15b shows that the solutions in the phase plane converge to a circular trajectory. In the time domain this corresponds to an oscillatory solution. Mathematically the circle is called a *limit cycle*. Note that in this case, the solution for any initial condition approaches the limit cycle and the amplitude and frequency of oscillation "in steady state" (once we have reached the limit cycle) are independent of the initial condition.

A different type of oscillation can occur in nonlinear systems in which the equilibrium points are saddle points, having both stable and unstable eigenvalues. Of particular interest is the case where the stable and unstable orbits of one or more equilibrium points join together. Two such situations are shown in Figure 3.16. The figure on the left is an example of a *homoclinic orbit*. In this system, trajectories that start near the equilibrium point quickly diverge away (in the directions corresponding to the unstable eigenvalues) and then slowly return to the equilibrium point along the stable directions. If the initial conditions are chosen to be precisely on the homoclinic orbit Γ then the system slowly converges to the equilibrium



(a) Homoclinic orbit (b) Heteroclinic orbit

Figure 3.16: Homoclinic and heteroclinic orbits.

point, but in practice there are often disturbances present that will perturb the system off of the orbit and trigger a "burst" in which the system rapidly escapes from the equilibrium point and then slowly converges again. A somewhat similar type of orbit is a *heteroclinic orbit*, in which the orbit connects two different equilibrium points, as shown in Figure 3.16b.

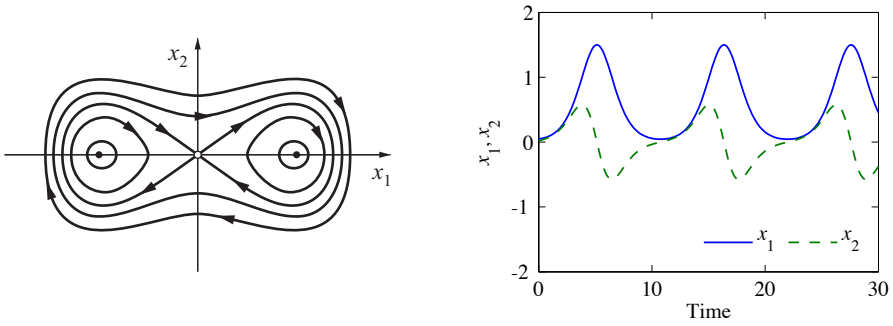
An example of a system with a homoclinic orbit is given by the system

$$\frac{dx_1}{dt} = x_2, \qquad \frac{dx_2}{dt} = x_1 - x_1^3. \tag{3.20}$$

The phase portrait and time domain solutions are shown in Figure 3.17. In this system, there are periodic orbits both inside and outside the two homoclinic cycles (left and right). Note that the trajectory we have chosen to plot in the time domain has the property that it rapidly moves away from the equilibrium point and then slowly reconverges to the equilibrium point, before being carried away again. This type of oscillation, in which one slowly returns to an equilibrium point before rapidly diverging is often called a *relaxation oscillation*. Note that for this system, there are also oscillations that look more like the harmonic oscillator case described above, in which we oscillate around the unstable equilibrium points at $x = (\pm 1, 0)$.

Example 3.8 (Glycolytic oscillations)**.** Glycolysis is one of the principal metabolic networks involved in energy production. It is a sequence of enzyme-catalyzed reactions that converts sugar into pyruvate, which is then further degraded to alcohol (in yeast fermentation) and lactic acid (in muscles) under anaerobic conditions, and ATP (the cell's major energy supply) is produced as a result. Both damped and sustained oscillations have been observed. Damped oscillations were first reported by [23] while sustained oscillations in yeast cell free extracts were observed in [42, 81].

Here we introduce the basic motif that is known to be at the core of this oscillatory phenomenon. Specifically, a substrate S is converted to a product P, which, in turn, acts as an enzyme catalyzing the conversion of S into P. This is an example of autocatalysis, in which a product is required for its own production. A simple



(a) Phase portrait (b) Time domain solution

Figure 3.17: Example of a homoclinic orbit.

differential equation model of this system can be written as

$$\frac{dS}{dt} = v_0 - v_1, \qquad \frac{dP}{dt} = v_1 - v_2, \tag{3.21}$$

in which v_0 is a constant production rate and

$$v_1 = S\,F(P), \quad F(P) = \frac{\alpha(P/K)^2}{1+(P/K)^2}, \qquad v_2 = k_2 P,$$

where $F(P)$ is the standard Hill function. Under the assumption that $K \gg P$, we have $F(P) \approx k_1 P^2$, in which we have defined $k_1 := \alpha/K^2$. This second-order system admits a stable limit cycle under suitable parameter conditions (Figure 3.18). ∇

One central question when analyzing the dynamical model of a given system is to establish whether the model constructed admits sustained oscillations. This

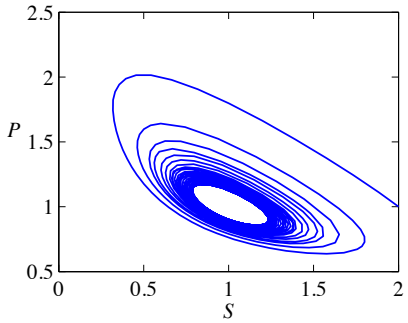


Figure 3.18: Oscillations in the glycolysis system. Parameters are $v_0 = 1$, $k_1 = 1$, and $k_2 = 1.00001$.

way we can validate or disprove models of biomolecular systems that are known to exhibit sustained oscillations. At the same time, we can provide design guidelines for engineering biological circuits that function as clocks, as we will see in Chapter 5. With this respect, it is particularly important to determine parameter conditions that are required and/or sufficient to obtain periodic behavior. To analyze these sorts of questions, we need to introduce tools that allow us to infer the existence and robustness of a limit cycle from a differential equation model.

In order to proceed, we first introduce the concept of ω-limit set of a point p, denoted $\omega(p)$. Basically, the ω-limit set $\omega(p)$ represents the set of all points to which the trajectory of the system starting from p tends as time approaches infinity. This is formally defined in the following definition.

Definition 3.1. A point $\bar{x} \in \mathbb{R}^n$ is called an *ω-limit point* of $p \in \mathbb{R}^n$ if there is a sequence of times $\{t_i\}$ with $t_i \to \infty$ for $i \to \infty$ such that $x(t_i, p) \to \bar{x}$ as $i \to \infty$. The *ω-limit set* of p, denoted $\omega(p)$, is the set of all ω-limit points of p.

The ω-limit set of a system has several relevant properties, among which are the facts that it cannot be empty and that it must be a connected set.

Limit cycles in the plane

Before studying periodic behavior of systems in $\mathbb{R}^n$, we study the behavior of systems in $\mathbb{R}^2$. Several high-dimensional systems can often be well approximated by systems in two dimensions by, for example, employing quasi-steady state approximations. For systems in $\mathbb{R}^2$, we will see that there are easy-to-check conditions that guarantee the existence of a limit cycle.

The first result provides a simple check to rule out periodic solutions for systems in $\mathbb{R}^2$. Specifically, let $x \in \mathbb{R}^2$ and consider

$$\frac{dx_1}{dt} = f_1(x_1, x_2), \qquad \frac{dx_2}{dt} = f_2(x_1, x_2), \tag{3.22}$$

in which the functions $f_i : \mathbb{R}^2 \to \mathbb{R}^2$ for $i = 1, 2$ are smooth. Then, we have the following:

Theorem 3.2 (Bendixson's criterion)**.** *Let D be a simply connected region in $\mathbb{R}^2$ (i.e., there are no holes in D). If the expression*

$$\frac{\partial f_1}{\partial x_1} + \frac{\partial f_2}{\partial x_2}$$

is not identically zero and does not change sign in D, then system (3.22) *has no closed orbits that lie entirely in D.*

Example 3.9. Consider the system

$$\frac{dx_1}{dt} = -x_2^3 + \delta x_1^3, \qquad \frac{dx_2}{dt} = x_1^3,$$

with $\delta \geq 0$. We can compute

$$\frac{\partial f_1}{\partial x_1} + \frac{\partial f_2}{\partial x_2} = 3\delta x_1^2,$$

which is not identically zero and does not change sign over all of $\mathbb{R}^2$ when $\delta \neq 0$. If $\delta \neq 0$, we can thus conclude from Bendixson's criterion that there are no periodic solutions. We leave it as an exercise to investigate what happens when $\delta = 0$ (Exercise 3.5). ∇

The following theorem completely characterizes the ω-limit set of any point for a system in $\mathbb{R}^2$.

Theorem 3.3 (Poincaré-Bendixson)**.** *Let M be a bounded and closed positively invariant region for the system $\dot{x} = f(x)$ with $x(0) \in M$ (i.e., any trajectory that starts in M stays in M for all $t \geq 0$). Assume that there are finitely many equilibrium points in M. Let $p \in M$, then one of the following possibilities holds for $\omega(p)$:*

(i) $\omega(p)$ is an equilibrium point;

(ii) $\omega(p)$ is a closed orbit;

(iii) $\omega(p)$ consists of a finite number of equilibrium points and orbits, each starting (for $t = 0$) and ending (for $t \to \infty$) at one of the fixed points.

This theorem has two important consequences:

1. If the system does not have equilibrium points in M, since $\omega(p)$ is not empty, it must be a periodic solution;

2. If there is only one equilibrium point in M and it is unstable and not a saddle (i.e., the eigenvalues of the linearization at the equilibrium point are both positive), then $\omega(p)$ is a periodic solution.

We will employ this result in Chapter 5 to determine parameter conditions under which activator-repressor circuits admit sustained oscillations.

Limit cycles in $\mathbb{R}^n$

The results above hold only for systems in two dimensions. However, there have been extensions of the Poincaré-Bendixson theorem to systems with special structure in $\mathbb{R}^n$. In particular, we have the following result, which can be stated as follows under some mild technical assumptions, which we omit here.

Theorem 3.4 (Hastings et al. [40])**.** *Consider a system $\dot{x} = f(x)$, which is of the form*

$$\dot{x}_1 = f_1(x_n, x_1),$$
$$\dot{x}_j = f_j(x_{j-1}, x_j),\ 2 \leq j \leq n$$

on the set M defined by $x_i \geq 0$ for all i with the following inequalities holding in M:

(i) $\frac{\partial f_i}{\partial x_i} < 0$ *and* $\frac{\partial f_i}{\partial x_{i-1}} > 0$, *for* $2 \leq i \leq n$, *and* $\frac{\partial f_1}{\partial x_n} < 0$;

(ii) $f_i(0,0) \geq 0$ *and* $f_1(x_n, 0) > 0$ *for all* $x_n \geq 0$;

(iii) The system has a unique equilibrium point $x^* = (x_1^*, ..., x_n^*)$ *in* M *such that* $f_1(x_n, x_1) < 0$ *if* $x_n > x_n^*$ *and* $x_1 > x_1^*$, *while* $f_1(x_n, x_1) > 0$ *if* $x_n < x_n^*$ *and* $x_1 < x_1^*$;

(iv) $\frac{\partial f_1}{\partial x_1}$ *is bounded above in* M.

Then, if the Jacobian of f at x^* has no repeated eigenvalues and has any eigenvalue with positive real part, then the system has a non-constant periodic solution in M.

This theorem states that for a system with cyclic structure in which the cycle "has negative loop gain," the instability of the equilibrium point (under some technical assumption) is equivalent to the existence of a periodic solution. This theorem, however, does not provide information about whether the orbit is attractive or not, that is, whether it is an ω-limit set of any point in M. This stability result is implied by a general theorem, which can be stated as follows under some mild technical assumptions, which we omit here.

Theorem 3.5 (Mallet-Paret and Smith [64])**.** *Consider the system $\dot{x} = f(x)$ with the following cyclic feedback structure*

$$\begin{aligned} \dot{x}_1 &= f_1(x_n, x_1), \\ \dot{x}_j &= f_j(x_{j-1}, x_j), \quad 2 \leq j \leq n \end{aligned}$$

on a set M defined by $x_i \geq 0$ for all i with all trajectories starting in M bounded for $t \geq 0$. Then, the ω-limit set $\omega(p)$ of any point $p \in M$ can be one of the following:

(i) An equilibrium point;

(ii) A non-constant periodic orbit;

(iii) A set of equilibrium points connected by homoclinic or heteroclinic orbits.

As a consequence of the theorem, we have that for a system with cyclic feedback structure that admits one equilibrium point only and at which the linearization has all eigenvalues with positive real part, the ω-limit set must be a periodic orbit.

In Chapter 5, we will apply these results to determine parameter conditions that make loop circuits with state in $\mathbb{R}^n$ admit a limit cycle.

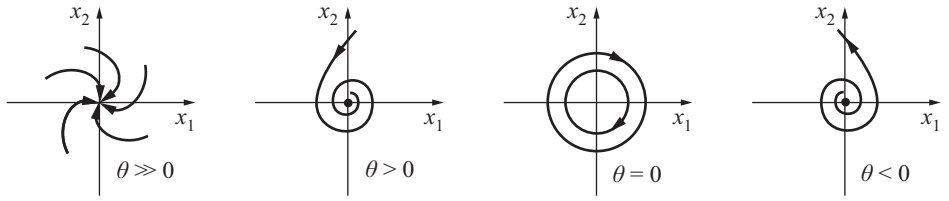


Figure 3.19: Phase portraits for a linear system as parameter θ changes. When θ is positive and large in absolute value, the eigenvalues are real and negative, and the response is not oscillatory (overdamped). When θ is positive but not too large in absolute value, the eigenvalues are complex with negative real part and damped oscillations arise (underdamped). When $\theta = 0$, the system displays oscillatory solutions, while when $\theta < 0$, the equilibrium point becomes unstable and trajectories diverge.

3.4 Bifurcations

Another important property of nonlinear systems is how their behavior changes as the parameters governing the dynamics change. We can study this in the context of models by exploring how the location of equilibrium points, their stability, their regions of attraction and other dynamic phenomena, such as limit cycles, vary based on the values of the parameters in the model.

Parametric stability

Consider a differential equation of the form

$$\frac{dx}{dt} = f(x,\theta), \quad x \in \mathbb{R}^n, \theta \in \mathbb{R}^p, \tag{3.23}$$

where x is the state and θ is a set of parameters that describe the family of equations. The equilibrium solutions satisfy

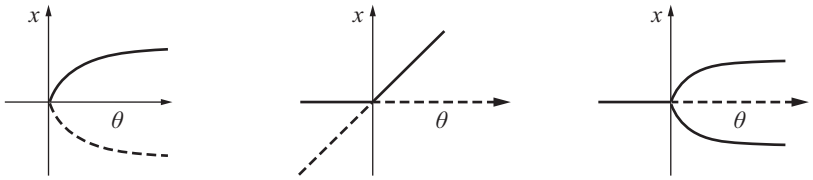
$$f(x,\theta) = 0,$$

and as θ is varied, the corresponding solutions $x_e(\theta)$ can also vary. We say that the system (3.23) has a *bifurcation* at $\theta = \theta^*$ if the behavior of the system changes qualitatively at θ^*. This can occur either because of a change in stability type or because of a change in the number of solutions at a given value of θ.

As an example of a bifurcation, consider the linear system

$$\frac{dx_1}{dt} = x_2, \qquad \frac{dx_2}{dt} = -kx_1 - \theta x_2,$$

where $k > 0$ is fixed and θ is our bifurcation parameter. Figure 3.19 shows the phase portraits for different values of θ. We see that at $\theta = 0$ the system transitions



Saddle node bifurcation
$\dot{x} = \theta - x^2$

Transcritical bifurcation
$\dot{x} = \theta x - x^2$

Pitchfork bifurcation
$\dot{x} = \theta x - x^3$

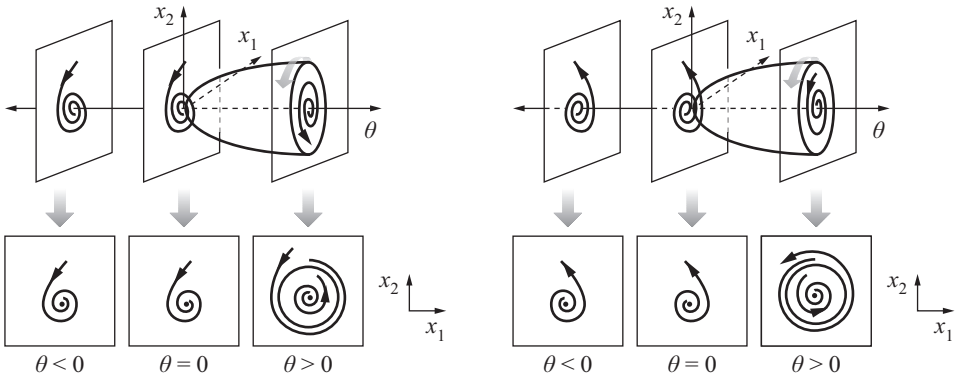
Figure 3.20: Bifurcation diagrams for some common bifurcations. In a *saddle node bifurcation*, as θ decreases a stable and an unstable equilibrium point approach each other and then "collide" for $\theta = 0$ and annihilate each other. In a *transcritical bifurcation*, a stable and an unstable equilibrum point approach each other, and then intersect at $\theta = 0$, swapping their stability. In a *pitchfork bifurcation*, a unique stable equilibrium point for $\theta < 0$ gives rise to three equilibria at the point $\theta = 0$, of which two are stable and one is unstable.

from a single stable equilibrium point at the origin to having an unstable equilibrium. Hence, as θ goes from negative to positive values, the behavior of the system changes in a significant way, indicating a bifurcation.

A common way to visualize a bifurcation is through the use of a *bifurcation diagram*. To create a bifurcation diagram, we choose a function $y = h(x)$ such that the value of y at an equilibrium point has some useful meaning for the question we are studying. We then plot the value of $y_e = h(x_e(\theta))$ as a function of θ for all equilibria that exist for a given parameter value θ. By convention, we use dashed lines if the corresponding equilibrium point is unstable and solid lines otherwise. Figure 3.20 shows examples of some common bifurcation diagrams. Note that for some types of bifurcations, such as the pitchfork bifurcation, there exist values of θ where there is more than one equilibrium point. A system that exhibits this type of behavior is said to be *multistable*. A common case is when there are two stable equilibria, in which case the system is said to be *bistable*. We will see an example of this in Chapter 5.

Hopf bifurcation

The bifurcations discussed above involved bifurcation of equilibrium points. Another type of bifurcation that can occur is when a system with an equilibrium point admits a limit cycle as a parameter is changed through a critical value. The Hopf bifurcation theorem provides a technique that is often used to understand whether a system admits a periodic orbit when some parameter is varied. Usually, such an orbit is a small amplitude periodic orbit that is present in the close vicinity of an unstable equilibrium point.



(a) Supercritical (b) Subcritical

Figure 3.21: Hopf bifurcation. (a) As θ increases a stable limit cycle appears. (b) As θ increases a periodic orbit appears but it is unstable. Figure adapted from [100].

Consider the system dependent on a parameter α:

$$\frac{dx}{dt} = g(x, \alpha), \qquad x \in \mathbb{R}^n, \qquad \alpha \in \mathbb{R},$$

and assume that at the equilibrium point x_e corresponding to $\alpha = \alpha_0$ (i.e., $g(x_e, \alpha_0) = 0$), the linearization $\partial g/\partial x$ evaluated at (x_e, α_0) has a pair of (nonzero) imaginary eigenvalues with the remaining eigenvalues having negative real parts. Define the new parameter $\theta := \alpha - \alpha_0$ and redefine the system as

$$\frac{dx}{dt} = f(x, \theta) =: g(x, \theta + \alpha_0),$$

so that the linearization $\partial f/\partial x$ evaluated at $(x_e, 0)$ has a pair of (nonzero) imaginary eigenvalues with the remaining eigenvalues having negative real parts. Denote by $\lambda(\theta) = \beta(\theta) + i\omega(\theta)$ the eigenvalue such that $\beta(0) = 0$. Then, if $\partial\beta/\partial\theta$ evaluated at $\theta = 0$ is not zero, the system admits a small amplitude almost sinusoidal periodic orbit for θ small enough and the system is said to go through a Hopf bifurcation at $\theta = 0$. If the small amplitude periodic orbit is stable, the Hopf bifurcation is said to be *supercritical*, while if it is unstable it is said to be *subcritical*. Figure 3.21 shows diagrams corresponding to these bifurcations.

In order to determine whether a Hopf bifurcation is supercritical or subcritical, it is necessary to calculate a "curvature" coefficient, for which there are formulas (Marsden and McCracken [66]) and available bifurcation software, such as AUTO. In practice, it is often enough to calculate the value $\bar{\alpha}$ of the parameter at which the Hopf bifurcation occurs and simulate the system for values of the parameter α close to $\bar{\alpha}$. If a small amplitude limit cycle appears, then the bifurcation is most likely supercritical.

Example 3.10 (Glycolytic oscillations)**.** Recalling the model of glycolytic oscillations given in (3.21), we ask whether such an oscillator goes through a Hopf bifurcation. In order to answer this question, we consider again the expression of the eigenvalues

$$\lambda_{1,2} = \frac{\mathrm{tr}(J) \pm \sqrt{\mathrm{tr}(J)^2 - 4\det(J)}}{2},$$

in which

$$\mathrm{tr}(J) = k_2 - k_1\left(\frac{v_0}{k_2}\right)^2 \quad \text{and} \quad \det(J) = k_1\left(\frac{v_0}{k_2}\right)^2.$$

The eigenvalues are imaginary if $\mathrm{tr}(J) = 0$, that is, if $k_1 = k_2^3/v_0^2$. Furthermore, the frequency of oscillations is given by $\omega = \sqrt{4\det(J)} = \sqrt{4k_1(v_0/k_2)^2}$. Therefore, this system goes through a Hopf bifurcation as the parameter k_1 approaches k_2^3/v_0^2. When $k_1 \approx k_2^3/v_0^2$, an approximately sinusoidal oscillation appears. When k_1 is large, the Hopf bifurcation theorem does not imply the existence of a periodic solution. This is because the Hopf theorem provides only local results. ∇

The Hopf bifurcation theorem is based on center manifold theory for nonlinear dynamical systems. For a rigorous treatment of Hopf bifurcation it is thus necessary to study center manifold theory first, which is outside the scope of this text. For details, the reader is referred to standard text in dynamical systems [100, 39].

In Chapter 5, we will illustrate how to employ Hopf bifurcation to understand one of the key design principles of clocks based on two interacting species, an activator and a repressor.

3.5 Model reduction techniques

The techniques that we have developed in this chapter can be applied to a wide variety of dynamical systems. However, many of the methods require significant computation and hence we would like to reduce the complexity of the models as much as possible before applying them. In this section, we review methods for doing such a reduction in the complexity of models. Most of the techniques are based on the common idea that if we are interested in the slower time scale dynamics of a system, the fast time scale dynamics can be approximated by their equilibrium solutions. This idea was introduced in Chapter 2 in the context of reduced order mechanisms; we present a more detailed mathematical analysis of such systems here.

The mathematical analysis of systems with multiple time scales and the consequent model order reduction is called *singular perturbation theory*. In particular, we are concerned with systems that have processes evolving on both fast and slow time scales and that can be written in a standard form, which we now introduce.

Let $(x,y) \in D := D_x \times D_y \subset \mathbb{R}^n \times \mathbb{R}^m$ and consider the vector field

$$\begin{aligned} \frac{dx}{dt} &= f(x,y,\epsilon), \qquad x(0) = x_0, \\ \epsilon\frac{dy}{dt} &= g(x,y,\epsilon), \qquad y(0) = y_0, \end{aligned} \tag{3.24}$$

in which $0 < \epsilon \ll 1$ is a small parameter and both $f(x,y,0)$ and $g(x,y,0)$ are well-defined. Since $\epsilon \ll 1$, the rate of change of y can be much larger than the rate of change of x, resulting in y dynamics that are much faster than the x dynamics. That is, this system has a slow time scale evolution (in x) and a fast time scale evolution (in y), so that x is called the slow variable and y is called the fast variable.

If we are interested only in the slower time scale, then the above system can be approximated (under suitable conditions) by the *reduced system*

$$\begin{aligned} \frac{d\bar{x}}{dt} &= f(\bar{x},\bar{y},0), \qquad \bar{x}(0) = x_0, \\ 0 &= g(\bar{x},\bar{y},0), \end{aligned}$$

in which we have set $\epsilon = 0$. Let $y = h(x)$ denote the locally unique solution of $g(x,y,0) = 0$. The manifold of (x,y) pairs where $y = h(x)$ is called the *slow manifold*. The *implicit function theorem* [65] shows that this solution exists whenever $\partial g/\partial y$ is, at least locally, nonsingular. In fact, in such a case we have

$$\frac{dh}{dx} = -\frac{\partial g}{\partial y}^{-1}\frac{\partial g}{\partial x}.$$

We can rewrite the dynamics of x in the reduced system as

$$\frac{d\bar{x}}{dt} = f(\bar{x},h(\bar{x}),0), \qquad \bar{x}(0) = x_0.$$

We seek to determine under what conditions the solution $x(t)$ is "close" to the solution $\bar{x}(t)$ of the reduced system. This problem can be addressed by analyzing the fast dynamics, that is, the dynamics of the system in the fast time scale $\tau = t/\epsilon$. In this case, we have that

$$\frac{dx}{d\tau} = \epsilon f(x,y,\epsilon), \qquad \frac{dy}{d\tau} = g(x,y,\epsilon), \qquad (x(0),y(0)) = (x_0,y_0),$$

so that when $\epsilon \ll 1$, $x(\tau)$ does not appreciably change. Therefore, the above system in the τ time scale can be well approximated by the system

$$\frac{dy}{d\tau} = g(x_0,y,0), \qquad y(0) = y_0,$$

in which x is "frozen" at the initial condition x_0. This system is usually referred to as the *boundary layer* system. For this system, the point $y = h(x_0)$ is an equilibrium point. Such an equilibrium point is asymptotically stable if $y(\tau)$ converges

to $h(x_0)$ as $\tau \to \infty$. In this case, the solution $(x(t), y(t))$ of the original system approaches $(\bar{x}(t), h(\bar{x}(t)))$. This qualitative explanation is more precisely captured by the following singular perturbation theorem under some mild technical assumptions, which we omit here [55].

Theorem 3.6. *Assume that*

$$Real\left(\lambda\left(\frac{\partial}{\partial y}g(x,y)\Big|_{y=h(x)}\right)\right) < 0$$

uniformly for $x \in D_x$. Let the solution of the reduced system be uniquely defined for $t \in [0, t_f]$. Then, for all $t_b \in (0, t_f]$ there are constants $\epsilon^* > 0$ and $M > 0$, and a set $\Omega \subseteq D$ such that

$$\|x(t) - \bar{x}(t)\| \leq M\epsilon \text{ for } t \in [0, t_f],$$
$$\|y(t) - h(\bar{x}(t))\| \leq M\epsilon \text{ for } t \in [t_b, t_f],$$

provided $\epsilon < \epsilon^*$ and $(x_0, y_0) \in \Omega$.

Example 3.11 (Hill function)**.** In Section 2.1, we obtained the expression of the Hill function by making a quasi-steady state approximation on the dynamics of reversible binding reactions. Here, we illustrate how Hill function expressions can be derived by a formal application of singular perturbation theory. Specifically, consider the simple binding scenario of a transcription factor X with DNA promoter sites p. Assume that such a transcription factor is acting as an activator of the promoter and let Y be the protein expressed under promoter p. Assume further that X dimerizes before binding to promoter p. The reaction equations describing this system are given by

$$\mathrm{X} + \mathrm{X} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{X}_2, \qquad \mathrm{X}_2 + \mathrm{p} \underset{d}{\overset{a}{\rightleftharpoons}} \mathrm{C}, \qquad \mathrm{C} \xrightarrow{k_\mathrm{f}} \mathrm{m}_\mathrm{Y} + \mathrm{C},$$

$$\mathrm{m}_\mathrm{Y} \xrightarrow{\kappa} \mathrm{m}_\mathrm{Y} + \mathrm{Y}, \qquad \mathrm{m}_\mathrm{Y} \xrightarrow{\delta} \emptyset, \qquad \mathrm{Y} \xrightarrow{\gamma} \emptyset, \qquad p + C = p_{\mathrm{tot}}.$$

The corresponding differential equation model is given by

$$\frac{dX_2}{dt} = k_1 X^2 - k_2 X_2 - a X_2 (p_{\mathrm{tot}} - C) + dC, \quad \frac{dm_\mathrm{Y}}{dt} = k_\mathrm{f} C - \delta m_\mathrm{Y},$$
$$\frac{dC}{dt} = a X_2 (p_{\mathrm{tot}} - C) - dC, \qquad \frac{dY}{dt} = \kappa m_\mathrm{Y} - \gamma Y,$$

in which we view $X(t)$ as an input to the system. We will see later in Chapter 6 that the dynamics of the input $X(t)$ will be "perturbed" by the physical process of reversible binding that makes it possible for the system to take X as an input.

Since all the binding reactions are much faster than mRNA and protein production and decay, we have that $k_2, d \gg k_f, \kappa, \delta, \gamma$. Let $K_m := k_2/k_1$, $K_d := d/a$, $c := k_2/d$, and $\epsilon := \gamma/d$. Then, we can rewrite the above system by using the substitutions

$$d = \frac{\gamma}{\epsilon}, \qquad a = \frac{\gamma}{K_d \epsilon}, \qquad k_1 = c\frac{\gamma}{K_m \epsilon}, \qquad k_2 = c\frac{\gamma}{\epsilon},$$

so that we obtain

$$\begin{aligned} \epsilon \frac{dX_2}{dt} &= c\frac{\gamma}{K_m}X^2 - c\gamma X_2 - \frac{\gamma}{K_d}X_2(p_{tot} - C) + \gamma C, & \frac{dm_Y}{dt} &= k_f C - \delta m_Y, \\ \epsilon \frac{dC}{dt} &= \frac{\gamma}{K_d}X_2(p_{tot} - C) - \gamma C, & \frac{dY}{dt} &= \kappa m_Y - \gamma Y. \end{aligned}$$

This system is in the standard singular perturbation form (3.24). As an exercise, the reader can verify that the slow manifold is locally asymptotically stable (see Exercise 3.10). The slow manifold is obtained by setting $\epsilon = 0$ and determines X_2 and C as functions of X. These functions are given by

$$X_2 = \frac{X^2}{K_m}, \qquad C = \frac{p_{tot}X^2/(K_m K_d)}{1 + X^2/(K_m K_d)}.$$

As a consequence, the reduced system becomes

$$\begin{aligned} \frac{dm_Y}{dt} &= k_f \frac{p_{tot}X^2/(K_m K_d)}{1 + X^2/(K_m K_d)} - \delta m_Y, \\ \frac{dY}{dt} &= \kappa m_Y - \gamma Y, \end{aligned}$$

which is the familiar expression for the dynamics of gene expression with an activator as derived in Section 2.1. Specifically, letting $\alpha = k_f p_{tot}$ and $K = \sqrt{K_m K_d}$, we have that

$$F(X) = \alpha \frac{(X/K)^2}{1 + (X/K)^2}$$

is the standard Hill function expression. ∇

Example 3.12 (Enzymatic reaction)**.** Recall the enzymatic reaction

$$\mathrm{E} + \mathrm{S} \underset{d}{\overset{a}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k} \mathrm{E} + \mathrm{P},$$

in which E is an enzyme, S is the substrate to which the enzyme binds to form the complex C, and P is the product resulting from the modification of the substrate S due to the binding with the enzyme E. The corresponding system of differential equations is given by

$$\begin{aligned} \frac{dE}{dt} &= -aES + dC + kC, & \frac{dC}{dt} &= aES - (d+k)C, \\ \frac{dS}{dt} &= -aES + dC, & \frac{dP}{dt} &= kC. \end{aligned} \tag{3.25}$$

By considering that binding and unbinding reactions are much faster than the catalytic reactions, mathematically expressed by $d \gg k$, we showed before that by approximating $dC/dt = 0$, we obtain $C = E_{\text{tot}}S/(S + K_m)$, with $K_{\text{m}} = (d + k)/a$ and $dP/dt = V_{max}S/(S + K_{\text{m}})$ with $V_{max} = kE_{\text{tot}}$. From this, it also follows that

$$\frac{dE}{dt} \approx 0 \qquad \text{and} \qquad \frac{dS}{dt} \approx -\frac{dP}{dt}. \tag{3.26}$$

How good is this approximation? By applying the singular perturbation method, we will obtain a clear answer to this question. Specifically, define $K_{\text{d}} := d/a$ and convert the system to standard singular perturbation form by defining the small parameter $\epsilon := k/d$, so that $d = k/\epsilon$, $a = k/(K_{\text{d}}\epsilon)$, and the system becomes

$$\begin{aligned} \epsilon\frac{dE}{dt} &= -\frac{k}{K_{\text{d}}}ES + kC + \epsilon kC, & \epsilon\frac{dC}{dt} &= \frac{k}{K_{\text{d}}}ES - kC - \epsilon kC, \\ \epsilon\frac{dS}{dt} &= -\frac{k}{K_{\text{d}}}ES + kC, & \frac{dP}{dt} &= kC. \end{aligned}$$

We cannot directly apply singular perturbation theory on this system because from the linearization of the first three equations, we see that the boundary layer dynamics are not locally asymptotically stable since there are two zero eigenvalues. This is because the three variables E, S, C are not independent. Specifically, $E = E_{\text{tot}} - C$ and $S + C + P = S(0) = S_{\text{tot}}$, assuming that initially we have S in amount $S(0)$ and no P and C in the system. Given these conservation laws, the system can be rewritten as

$$\epsilon\frac{dC}{dt} = \frac{k}{K_{\text{d}}}(E_{\text{tot}} - C)(S_{\text{tot}} - C - P) - kC - \epsilon kC, \qquad \frac{dP}{dt} = kC.$$

Under the assumption made in the analysis of the enzymatic reaction that $S_{\text{tot}} \gg E_{\text{tot}}$, we have that $C \ll S_{\text{tot}}$ so that the equations finally become

$$\epsilon\frac{dC}{dt} = \frac{k}{K_{\text{d}}}(E_{\text{tot}} - C)(S_{\text{tot}} - P) - kC - \epsilon kC, \qquad \frac{dP}{dt} = kC.$$

We can verify (see Exercise 3.11) that in this system the boundary layer dynamics are locally asymptotically stable, so that setting $\epsilon = 0$ one obtains

$$\bar{C} = \frac{E_{\text{tot}}(S_{\text{tot}} - \bar{P})}{(S_{\text{tot}} - \bar{P}) + K_{\text{m}}} =: h(\bar{P}),$$

and thus that the reduced system is given by

$$\frac{d\bar{P}}{dt} = V_{max}\frac{(S_{\text{tot}} - \bar{P})}{(S_{\text{tot}} - \bar{P}) + K_{\text{m}}}.$$

This system is the same as that obtained in Chapter 2. However, $dC(t)/dt$ and $dE(t)/dt$ are not close to zero as obtained earlier. In fact, from the conservation

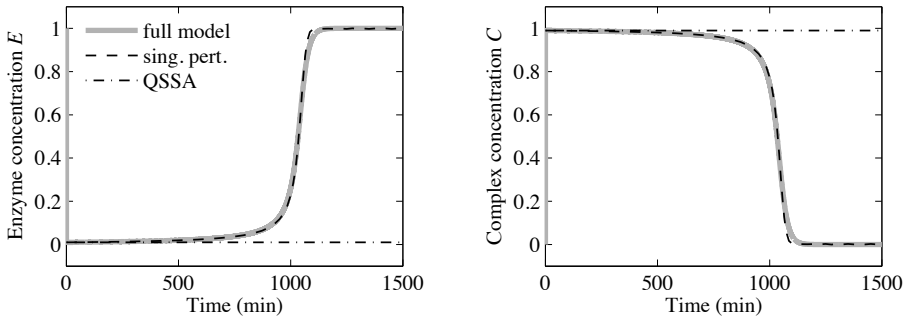


Figure 3.22: Simulation results for the enzymatic reaction comparing the approximations from singular perturbation and from the quasi-steady state approximation (QSSA). Here, we have $S_{\text{tot}} = 100$ nM, $E_{\text{tot}} = 1$ nM, $a = 10$ nM^{-1} min^{-1}, $d = 10$ min^{-1}, and $k = 0.1$ min^{-1}. The full model is the one in equations (3.25).

law $\bar{S} + \bar{C} + \bar{P} = S(0) = S_{\text{tot}}$, we obtain that $d\bar{S}/dt = -d\bar{P}/dt - d\bar{C}/dt$, in which now $d\bar{C}/dt = \partial h/\partial P(\bar{P}) \cdot d\bar{P}/dt$. Therefore, we have that

$$\frac{d\bar{E}}{dt} = -\frac{d\bar{C}}{dt} = -\frac{\partial h}{\partial P}(\bar{P})\frac{d\bar{P}}{dt}, \qquad E(0) = E_{\text{tot}} - h(\bar{P}(0)), \tag{3.27}$$

and

$$\frac{d\bar{S}}{dt} = -\frac{d\bar{P}}{dt}(1 + \frac{\partial h}{\partial P}(\bar{P})), \qquad \bar{S}(0) = S_{\text{tot}} - h(\bar{P}(0)) - \bar{P}(0), \tag{3.28}$$

which are different from expressions (3.26).

These expressions are close to those in equation (3.26) only when $\partial h/\partial P$ is small enough. In the plots of Figure 3.22, we show the time trajectories of the original system, of the Michaelis-Menten quasi-steady state approximation (QSSA), and of the singular perturbation approximation. In the original model (solid line in Figure 3.22), $E(t)$ starts from a unit concentration and immediately collapses to zero as the enzyme is all consumed to form the complex C by the substrate, which is in excess. Similarly, $C(t)$ starts from zero and immediately reaches the maximum possible value of one.

In the quasi-steady state approximation, both $E(t)$ and $C(t)$ are assumed to stabilize immediately to their (quasi) steady state and then stay constant. This is depicted by the dash-dotted plots in Figure 3.22, in which $E(t)$ stays at zero for the whole time and $C(t)$ stays at one for the whole time. This approximation is fairly good as long as there is an excess of substrate. When the substrate concentration goes to zero as it is all converted to product, the complex concentration C goes back to zero (see solid line of Figure 3.22). At this time, the concentrations of complex and enzyme substantially change with time and the quasi-steady state approximation is unsatisfactory. By contrast, the reduced dynamics obtained from the singular perturbation approach well represent the dynamics of the full system even during

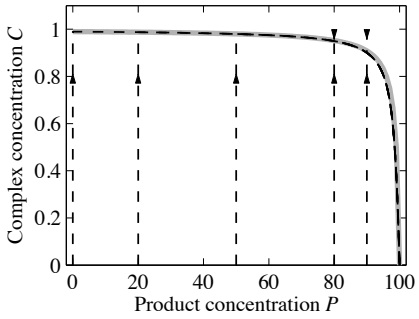


Figure 3.23: The slow manifold of the system $C = h(P)$ is shown by the solid line. The dashed lines show the trajectories of the full system (3.25). These trajectories collapse into an ϵ-neighbor of the slow manifold.

this transient time. Hence, while the quasi-steady state approximation is good only as long as there is an excess of substrate in the system, the reduced dynamics obtained by the singular perturbation approach are a good approximation even when the substrate concentration goes to zero.

In Figure 3.23, we show the curve $C = h(P)$ and the trajectories of the full system. All of the trajectories of the system immediately collapse into an ϵ-neighbor of the curve $C = h(P)$. From this plot, it is clear that $\partial h/\partial P$ is small as long as the product concentration P is small enough, which corresponds to a substrate concentration S large enough. This confirms that the quasi-steady state approximation is good only as long as there is excess of substrate S. ∇

Exercises

3.1 (Frequency response of a phosphorylation cycle) Consider the model of a covalent modification cycle as illustrated in Chapter 2 in which the kinase Z is not conserved, but it is produced and decays according to the reaction $\mathrm{Z} \underset{u(t)}{\overset{\gamma}{\rightleftharpoons}} \emptyset$. Let $u(t)$ be the input stimulus of the cycle and let X^* be the output. Determine the frequency response of X^* to u, determine its bandwidth, and make plots of it. What parameters can be used to tune the bandwidth?

3.2 (Design for robustness) Consider a one-step reaction model for a phosphorylation cycle as seen in Section 2.4, in which the input stimulus is the time-varying concentration of kinase $Z(t)$. When found in the cellular environment, this cycle is subject to possible interactions with other cellular components, such as the nonspecific or specific binding of X^* to target sites, to noise due to stochasticity of the cellular environment, and to other crosstalk phenomena. For now, we can think of

these disturbances as acting like an aggregate rate of change on the output protein X^*, which we call $d(t)$. Hence, we can model the "perturbed" cycle by

$$\frac{X^*}{dt} = Z(t)k_1 X_{\text{tot}}\left(1 - \frac{X^*}{X_{\text{tot}}}\right) - k_2 Y_{\text{tot}} X^* + d(t).$$

Assume that you can tune all the parameters in this system. Can you tune these parameters so that the response of $X^*(t)$ to $d(t)$ is arbitrarily attenuated while the response of $X^*(t)$ to $Z(t)$ remains arbitrarily large? If yes, explain how these parameters should be tuned to reach this design objective.

3.3 (Design limitations) This problem illustrates possible limitations that are involved in any realistic design question. Here, we examine this through the open loop and negative feedback transcriptional component. Specifically, we want to compare the robustness of these two topologies to perturbations. We model these perturbations as a time-varying disturbance affecting the production rate of mRNA m and protein P. To slightly simplify the problem, we focus only on disturbances affecting the production of protein. The open loop model becomes

$$\frac{dm_{\text{P}}}{dt} = \alpha_0 - \delta m_{\text{P}}, \qquad \frac{dP}{dt} = \kappa m_{\text{P}} - \gamma P + d(t),$$

and the negative feedback system becomes

$$\frac{dm_{\text{P}}}{dt} = \alpha_0 + \frac{\alpha}{1 + (P/K)^n} - \delta m_{\text{P}}, \qquad \frac{dP}{dt} = \kappa m_{\text{P}} - \gamma P + d(t).$$

Answer the following questions:

(i) After performing linearization about the equilibrium point, determine analytically the frequency response of P to d for both systems.

(ii) Sketch the magnitude plot of this response for both systems, compare them, and determine what happens as κ and α increase (note: if your calculations are correct, you should find that what really matters for the negative feedback system is the product $\alpha\kappa$, which we can view as the *feedback gain*). Is increasing the feedback gain the best strategy to decrease the sensitivity of the system to the disturbance?

(iii) Pick parameter values and use MATLAB to draw plots of the frequency response magnitude and phase as the feedback gain increases and validate your predictions in (b). (Suggested parameters: $\delta = 1$ hrs^{-1}, $\gamma = 1$ hrs^{-1}, $K = 1$ nM, $n = 1$, $\alpha\kappa = \{1, 10, 100, 1000, ...\}$.)

(iv) Investigate the answer to (c) when you have $\delta = 20$ hrs^{-1}, that is, the timescale of the mRNA dynamics becomes faster than that of the protein dynamics. What changes with respect to what you found in (c)?

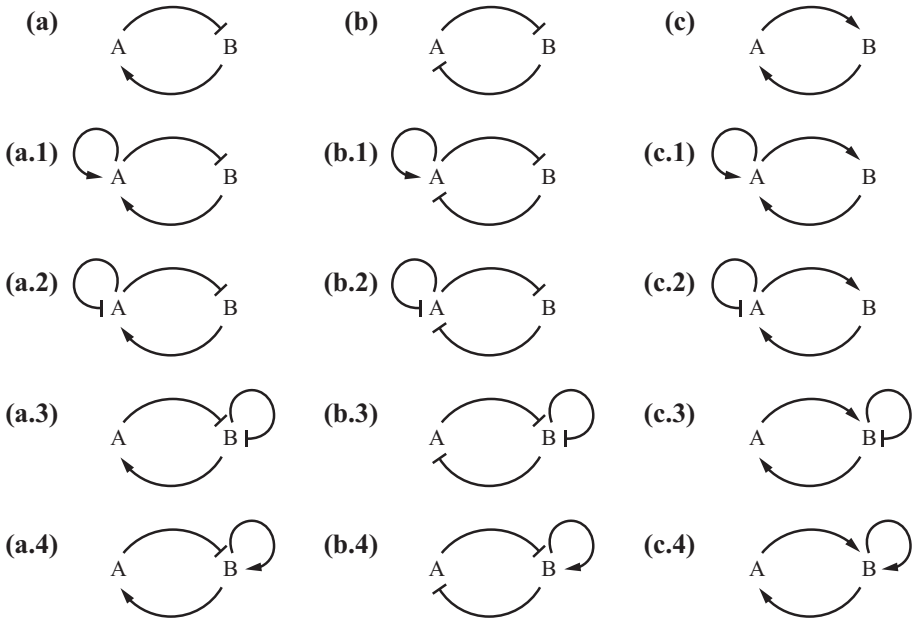


Figure 3.24: Circuit topologies with two proteins: A and B.

(v) When δ is at least 10 times larger than γ, you can approximate the m dynamics to the quasi-steady state. So, the two above systems can be reduced to one differential equation. For these two reduced systems, determine analytically the frequency response to d and use it to determine whether arbitrarily increasing the feedback gain is a good strategy to decrease the sensitivity of response to the disturbance.

3.4 (Adaptation) Show that the dynamics of the "sniffer" in equation (3.16) can be taken into the standard integral feedback form through a suitable change of coordinates.

3.5 (Bendixson criterion) Consider the system

$$\frac{dx_1}{dt} = -x_2^3 + \delta x_1^3, \qquad \frac{dx_2}{dt} = x_1^3.$$

When $\delta > 0$, Bendixson's criterion rules out the existence of a periodic solution in $\mathbb{R}^2$. Assume now $\delta = 0$, and determine whether the system admits a limit cycle in $\mathbb{R}^2$. (Hint: consider the function $V = x_1^2 + x_2^2$ and determine the behavior of $V(t)$ when $x_1(t)$ and $x_2(t)$ are solutions to the above system.)

3.6 (Bendixson criterion) Consider the possible circuit topologies of Figure 3.24, in which A and B are proteins and activation ($\rightarrow$) and repression ($\dashv$) interactions

represent transcriptional activation or repression. Approximate the mRNA dynamics at the quasi-steady state. Use Bendixson's criterion to rule out topologies that cannot give rise to closed orbits.

3.7 (Two gene oscillator) Consider the feedback system composed of two genes expressing proteins A (activator) and R (repressor), in which we denote by A, R, m_{A}, and m_{R}, the concentrations of the activator protein, the repressor protein, the mRNA for the activator protein, and the mRNA for the repressor protein, respectively. The differential equation model corresponding to this system is given by

$$\frac{dm_{\mathrm{A}}}{dt} = \frac{\alpha}{1+(R/K_1)^n} - \delta m_{\mathrm{A}}, \qquad \frac{dm_{\mathrm{R}}}{dt} = \frac{\alpha(A/K_2)^m}{1+(A/K_2)^m} - \delta m_{\mathrm{R}},$$
$$\frac{dA}{dt} = \kappa m_{\mathrm{A}} - \gamma A, \qquad \frac{dR}{dt} = \kappa m_{\mathrm{R}} - \gamma R.$$

Determine parameter conditions under which this system admits a stable limit cycle. Validate your findings through simulation.

3.8 (Goodwin oscillator) Consider the simple set of reactions

$$\mathrm{X}_1 \xrightarrow{k} \mathrm{X}_2 \xrightarrow{k} \mathrm{X}_3 \ldots \xrightarrow{k} \mathrm{X}_{\mathrm{n}}.$$

Assume further that X_{n} is a transcription factor that represses the production of protein X_1 through transcriptional regulation (assume simple binding of X_{n} to DNA). Neglecting the mRNA dynamics of X_1, write the differential equation model of this system and determine conditions on the length n of the cascade for which the system admits a stable limit cycle. Validate your findings through simulation.

3.9 (Phosphorylation via singular perturbation) Consider again the model of a covalent modification cycle as illustrated in Section 2.4 in which the kinase Z is not constant, but it is produced and decays according to the reaction

$$\mathrm{Z} \underset{u(t)}{\overset{\gamma}{\rightleftharpoons}} \emptyset.$$

(i) Consider that $d \gg k, \gamma, u(t)$ and employ singular perturbation with small parameter $\epsilon = \gamma/d$ to obtain the approximated dynamics of $Z(t)$ and $X^*(t)$. How is this different from the result obtained in Exercise 2.12?

(ii) Simulate these approximated dynamics when $u(t)$ is a periodic signal with frequency ω and compare the responses of Z of these approximated dynamics to those obtained in Exercise 2.12 as you change ω. What do you observe? Explain.

3.10 (Hill function via singular perturbation) Show that the slow manifold of the following system is asymptotically stable:

$$\epsilon\frac{dX_2}{dt} = c\frac{\gamma}{K_\mathrm{m}}X^2 - c\gamma X_2 - \frac{\gamma}{K_\mathrm{d}}X_2(p_\mathrm{tot} - C) + \gamma C, \qquad \frac{dm_\mathrm{Y}}{dt} = \alpha C - \delta m_\mathrm{Y},$$
$$\epsilon\frac{dC}{dt} = \frac{\gamma}{K_\mathrm{d}}X_2(p_\mathrm{tot} - C) - \gamma C, \qquad \frac{dY}{dt} = \beta m_\mathrm{Y} - \gamma Y.$$

3.11 (Enzyme dynamics via singular perturbation) Show that the slow manifold of the following system is asymptotically stable:

$$\epsilon\frac{dC}{dt} = \frac{k}{K_\mathrm{d}}(E_\mathrm{tot} - C)\cdot(S_\mathrm{tot} - P) - kC - \epsilon kC, \qquad \frac{dP}{dt} = kC.$$