Biomolecular Feedback Systems

Domitilla Del Vecchio
MIT

Richard M. Murray
Caltech

Classroom Copy v0.6c, July 11, 2012

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, building on tools already described in AM08. We focus here on deterministic models and the associated analyses; stochastic methods are given in Chapter 4.

Prerequisites. Readers should have a understanding of the tools for analyzing stability of solutions to ordinary differential equations, at the level of Chapter 4 of AM08. We will also make use of linearized input/output models in state space, based on the techniques described in Chapter 5 of AM08 and the frequency domain techniques described in Chapters 8–10.

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:

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

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 proteins, kinases, DNA promoter sites, inducers, allosteric effectors, etc. The system parameters θ is also a vector, whose components will represent biochemical parameters such as association and dissociation rates, production rates, decay rates, dissociation constants, etc. The input u is a vector whose components will represent a number of possible physical entities, including the concentration of transcription factors, DNA concentration, kinases concentration, etc. The output $y \in \mathbb{R}^m$ of the system represents quantities that can be measured or that are used to interconnect subsystem models to form larger models.

Example 3.1 (Transcriptional component)**.** Consider a promoter controlling a gene g that can be regulated by a transcription factor Z. Let m_G and G represent the mRNA and protein expressed by gene g. This system can be viewed 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 $x_1 = m_G$ is the concentration of mRNA and $x_2 = G$ is the concentration of protein, and $y = G = x_2$ is the concentration of protein G. Assuming that the transcription factor regulating the promoter is a repressor, the system dynamics can be described by the following system

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

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

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

∇

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 [AM08]

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 a 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 the the differential equation (3.3) with initial condition $x(0)$. Note that this definition does not imply that $x(t)$ approaches x_e as

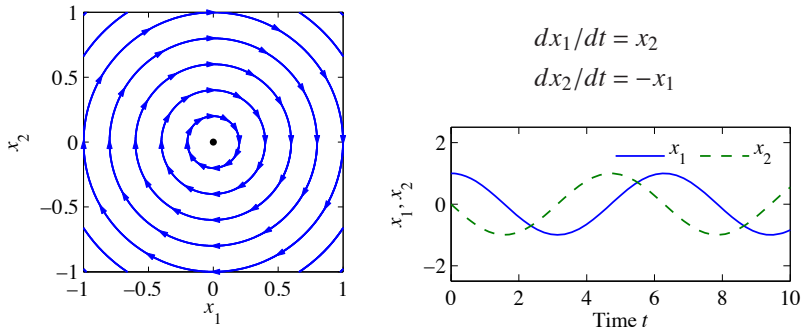


Figure 3.1: Phase portrait (trajectories in the state space) on the left and time domain simulation on the right for a system with a single stable equilibrium point. The equilibrium point x_e at the origin is stable since all trajectories that start near x_e stay near x_e.

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 close ($\delta \ll \epsilon$). This type of stability, which is illustrated in Figure 3.1, 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. From 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.

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).

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 $x \in B_r(a)$,

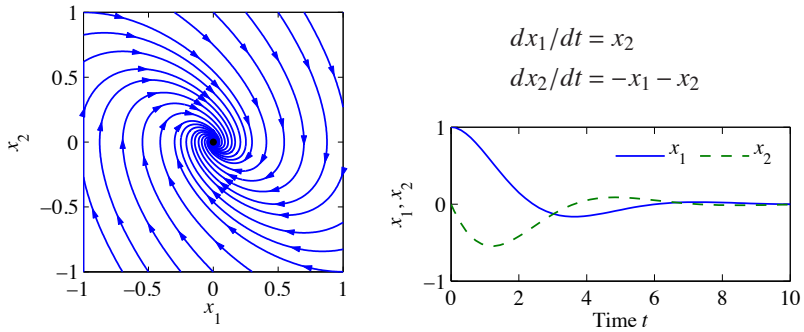


Figure 3.2: Phase portrait and time domain simulation for a system with a single asymptotically stable equilibrium point. The equilibrium point x_e at the origin is asymptotically stable since the trajectories converge to this point as $t \to \infty$.

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, as we explore in the next section.

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 situation 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 that repress each other as shown in Figure 3.4. 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:

$$\frac{dx_1}{dt} = \frac{\alpha_1}{(x_2^n/K_2) + 1} - \delta x_1, \qquad \frac{dx_2}{dt} = \frac{\alpha_2}{(x_1^n/K_1) + 1} - \delta x_2.$$

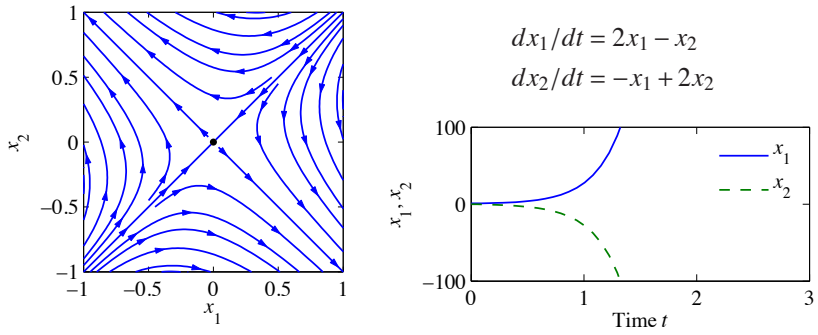


Figure 3.3: Phase portrait and time domain simulation for a system with a single unstable equilibrium point. The equilibrium point x_e at the origin is unstable since not all trajectories that start near x_e stay near x_e. The sample trajectory on the right shows that the trajectories very quickly depart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

Figure 3.4(b) shows the phase portrait of the system. This system is bi-stable 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 one 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. ∇

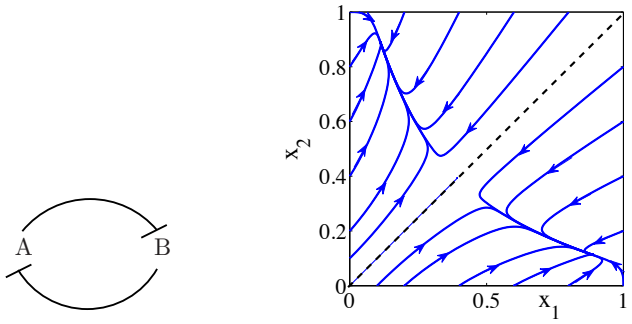
Nullcline Analysis

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



(a) (b)

Figure 3.4: (a) Diagram of a bistable gene circuit composed of two genes. (b) Phase plot showing the trajectories converging to either one of the two possible stable equilibria depending on the initial condition. The parameters are $\alpha_1 = \alpha_2 = 1$, $K_1 = K_2 = 0.1$, and $\delta = 1$.

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 (see 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 3.5(a) 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 3.5. As a consequence, the equilibrium point is stable. The phase plot of Figure 3.5(b) confirms this 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.

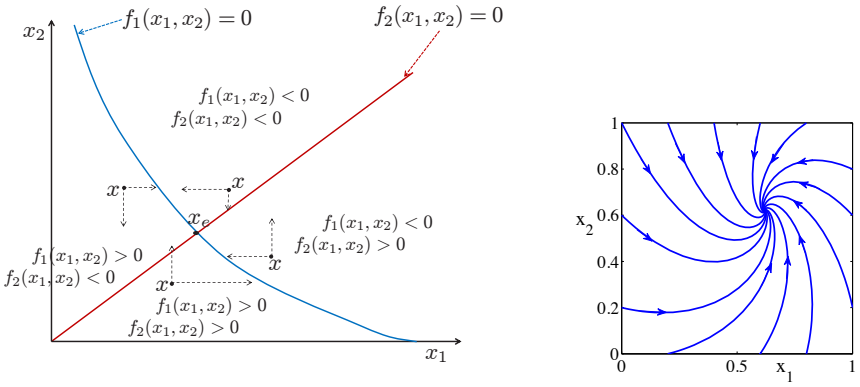


Figure 3.5: (a) Example of nullclines for a system with a single equilibrium point x_e. To understand the stability of the equilibrium point x_e, one traces the direction of the vector field (f_1, f_2) in each of the four regions in which the nullcline partition the plane. If in each region the vector field points toward the equilibrium point, then such a point is asymptotically stable. (b) Phase plot diagram 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 $v_j \in \mathbb{R}^n$, 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 dynamics 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 dynamics 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 the next chapter:

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 (1) $\mathrm{tr}(A) < 0$ and (2) $\det(A) > 0$. By contrast, if condition (2) is satisfied but $\mathrm{tr}(A) > 0$, the eigenvalues have positive real parts.

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

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

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

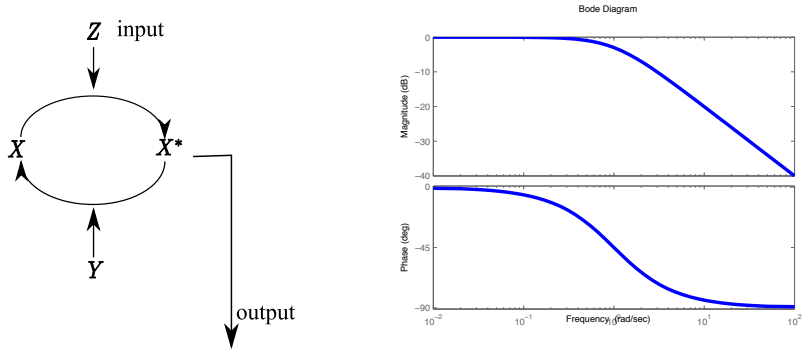
Frequency domain analysis

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 stimulus is changing much slower than the natural time scales of the system, the system will respond very accurately. 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 input at various frequencies.

Example 3.5 (Phosphorylation cycle)**.** To illustrate the basic ideas, we consider the frequency response of a phosphorylation cycle, in which enzymatic reactions are modeled by a first order 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) Reaction daigram (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 and phase lag for the frequency response of a one step reaction model of the phosphorylation system on the left. The magnitude is plotted in decibels (dB), in which $M|_{dB} = 20\log_{10}(M)$. The parameters are $\beta = \delta = 1$.

The differential equation model 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

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

where $\beta = k_1 X_{\text{tot}}$ and $\delta = k_2 Y_{\text{tot}}$. 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 lead to

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

The second term dies out for t large enough. Hence, the steady state response is given by the first term. The amplitude of response is thus given by $A_0\beta/\sqrt{\omega^2 + \delta^2}$, in which the gain $\beta/\sqrt{\omega^2 + \delta^2}$ depends on the system parameters and on the frequency of the input stimulation.

As this frequency increases, the amplitude decreases and approaches zero for infinite frequencies. Also, the argument of the sine function shows a negative phase shift of $\tan^{-1}(\omega/\delta)$, which indicates that there is an increased delay in responding

to the input as the frequency increases. Hence, the key quantities in the frequency response are the magnitude gain $M(\omega)$ and phase lag $\phi(\omega)$ given by

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

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 magnitude gain drops below $M(0)/\sqrt{2}$. In this case, the bandwidth is given by $\omega_B = \delta = k_2 Y_{\text{tot}}$, which implies that the bandwidth of the system can be made larger by increasing the amount of phosphatase. However, note that since $M(0) = \beta/\delta = k_1 X_{\text{tot}}/(k_2 Y_{\text{tot}})$, increased phosphatase will also result in decreased amplitude of response. Hence, if one wants 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 kinase. ∇

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 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 shift ϕ can be determined from the transfer function evaluated at $s = i\omega$:

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

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 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 response 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 (AM08, Section 9.2).

Returning to our analysis of biomolecular systems, suppose we have a systems 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

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

This linear system describes small deviations from $x_e(\theta_0, w_0)$ but allows $\tilde{\theta}$ and $\tilde{w}$ 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 $\tilde{\theta}$, $\tilde{w}$ and y are given by

$$H_{y\tilde{\theta}}(s) = C(sI - A)^{-1}B_\theta, \qquad H_{y\tilde{w}}(s) = C(sI - A)^{-1}B_w,$$

where

$$A = \left.\frac{\partial f}{\partial x}\right|_{(x_e,\theta_0,w_0)}, \qquad B_\theta = \left.\frac{\partial f}{\partial \theta}\right|_{(x_e,\theta_0,w_0)}, \qquad B_w = \left.\frac{\partial f}{\partial w}\right|_{(x_e,\theta_0,w_0)}.$$

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

$$H_{y\tilde{\theta}}(0) = CA^{-1}B_\theta = CS_{x,\theta}.$$

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

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

where m is the mRNA concentration and P is the protein concentration. Suppose that the mRNA degradation rate γ 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 an equilibrium point

$$A = \begin{pmatrix} -\gamma & F'(p_e) \\ \beta & -\delta \end{pmatrix}, \qquad B_\gamma = \begin{pmatrix} -m_e \\ 0 \end{pmatrix}.$$

For the case of no feedback we have $F(P) = \alpha_0$, and the system has an equilibrium point at $m_e = \alpha_0/\gamma$, $P_e = \beta\alpha_0/(\delta\gamma)$. The transfer function from γ to p, after linearization about the steady state, is given by

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

where γ_0 represents the nominal value of γ around which we are linearizing. 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

$$G^{\text{cl}}_{P\gamma}(s) = \frac{\beta m_e}{(s+\gamma_0)(s+\delta) + \beta\sigma}, \qquad \sigma = -F'(P_e) = \frac{n\alpha P_e^{n-1}/K^n}{(1 + P_e^n/K^n)^2}.$$

Figure 3.7 shows the frequency response for the two circuits. We see that the feedback circuit attenuates the response of the system to disturbances with low-frequency content but slightly amplifies disturbances at high frequency (compared to the open loop system). ∇

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

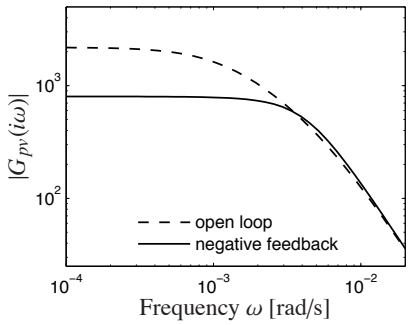


Figure 3.7: Noise attenuation in a genetic circuit.

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

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

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 changes 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 w.

We start by assuming that $u = 0$ and investigating how x_e and y_e depend on θ. 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

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

which is the (infinitesimal) change 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:

$$\frac{df}{d\theta} = \frac{\partial f}{\partial x}\frac{dx_e}{d\theta} + \frac{\partial f}{\partial \theta} = 0 \quad \Longrightarrow \quad S_{x,\theta} = \frac{dx_e}{d\theta} = -\left(\frac{\partial f}{\partial x}\right)^{-1} \frac{\partial f}{\partial \theta}\bigg|_{(xe,\theta_0)}. \tag{3.8}$$

Similarly, we can compute the change in the output sensitivity as

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

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

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

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

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

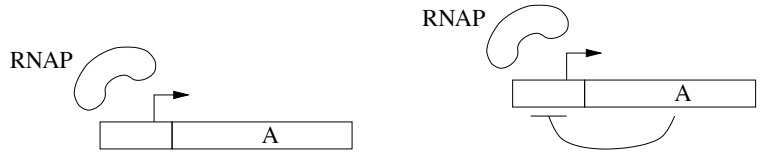
Then the scaled sensitivity matrices can be written as

$$S_{x,\theta} = (D^{x_e})^{-1} S_{x\theta} D^{\theta}, \qquad S_{y,\theta} = (D^{y_e})^{-1} S_{y\theta} D^{\theta}. \tag{3.9}$$

The entries in this matrix describe how a fractional change in a parameter gives a fractional change in the output, relative to the nominal values of the parameters and outputs.

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* (no regulation) and self-repression (negative feedback), illustrated in Figure 3.8. For the case of no feedback we have $F(p) = \alpha_0$, and the system has an equilibrium point at $m_e = \alpha_0/\gamma$, $P_e = \beta\alpha_0/(\delta\gamma)$. The parameter vector can be taken as $\theta = (\alpha_0,\gamma,\beta,\delta)$. Since we have a simple expression for the equilibrium concentrations, we can compute the sensitivity to the parameters directly:

$$\frac{\partial x_e}{\partial \theta} = \begin{pmatrix} \frac{1}{\gamma} & -\frac{\alpha_0}{\gamma^2} & 0 & 0 \\ \frac{\beta}{\delta\gamma} & -\frac{\beta\alpha_0}{\delta\gamma^2} & \frac{\alpha_0}{\delta\gamma} & -\frac{\beta\alpha_0}{\gamma\delta^2} \end{pmatrix},$$



(a) Open loop (b) Negative feedback

Figure 3.8: Parameter sensitivity in a genetic circuit. The open loop system (a) consists of a constitutive promoter, while the closed loop circuit (b) is self-regulated with negative feedback (repressor).

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

$$S^{\text{open}}_{x_e,\theta} \approx \begin{pmatrix} 170 & -41 & 0 & 0 \\ 17000 & -4100 & 210 & -21000 \end{pmatrix}. \tag{3.10}$$

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

$$S^{\text{open}}_{x_e,\theta} = \begin{pmatrix} 1 & -1 & 0 & 0 \\ 1 & -1 & 1 & -1 \end{pmatrix}. \tag{3.11}$$

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

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

and the equilibrium points satisfy

$$m_e = \frac{\delta}{\beta} P_e, \qquad \frac{\alpha}{1 + P_e^n/K} + \alpha_0 = \gamma m_e = \frac{\gamma\delta}{\beta} P_e. \tag{3.12}$$

In order to make a proper comparison with the previous case, we need to be careful to choose the parameters so that the equilibrium concentration P_e matches that of the open loop system. We can do this by modifying the promoter strength α or the RBS strength β so that the second formula in equation (3.12) 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 2.3).

Rather than attempt to solve for the equilibrium point in closed form, we instead investigate the sensitivity using the computations in equation (3.12). The state, dynamics and parameters are given by

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

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

$$\frac{\partial f}{\partial x} = \begin{pmatrix} -\gamma & F'(P_e) \\ \beta & -\delta \end{pmatrix}, \qquad \frac{\partial f}{\partial \theta} = \begin{pmatrix} 1 & -m & 0 & 0 & \frac{1}{1+P^n/K} & \frac{K\alpha P^n \log(P)}{(K+P^n)^2} & \frac{\alpha}{(1+P^n/K)^2} \\ 0 & 0 & m & -P & 0 & 0 & 0 \end{pmatrix},$$

where again the parameters are taken to be their nominal values. From this we can compute the sensitivity matrix as

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

where $F' = \partial F / \partial P$ and all other derivatives of F are evaluated at the nominal parameter values.

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

$$x_e = \begin{pmatrix} m_e \\ P_e \end{pmatrix} = \begin{pmatrix} \frac{\alpha_0}{\gamma} \\ \frac{\alpha_0 \beta}{\delta\gamma} \end{pmatrix} = \begin{pmatrix} 0.239 \\ 23.9 \end{pmatrix}$$

and the sensitivity matrix is

$$S^{\text{closed}}_{x_e,\theta} \approx \begin{pmatrix} 76.1 & -18.2 & -1.16 & 116. & 0.134 & -0.212 & -0.000117 \\ 7610. & -1820. & 90.8 & -9080. & 13.4 & -21.2 & -0.0117 \end{pmatrix}.$$

The scaled sensitivity matrix becomes

$$S^{\text{closed}}_{x_e,\theta} \approx \begin{pmatrix} 0.16 & -0.44 & -0.56 & 0.56 & 0.28 & -1.78 & -3.08 \times 10^{-7} \\ 0.16 & -0.44 & 0.44 & -0.44 & 0.28 & -1.78 & -3.08 \times 10^{-7} \end{pmatrix}. \quad (3.13)$$

Comparing this equation with equation (3.11), 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 mechanisms (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. We assume $u = 0$ for simplicity of exposition.

Let $x(t; x_0, \theta_0)$ be a solution of the dynamics with initial condition x_0 and parameters θ_0. To compute $dx/d\theta$, we write down a differential equation for how it evolves in time:

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

This is a differential equation with $n \times m$ states $S_{ij} = dx_i/d\theta_j$ and with initial condition $S_{ij}(0) = 0$ (since changes to the parameters to not affect the initial conditions).

To solve these equations, we must simultaneously solve for the state x and the sensitivity S (whose dynamics depend on x). Thus, we must solve the set of $n + nm$ coupled differential equations

$$\frac{dx}{dt} = f(x,\theta,u), \qquad \frac{dS_{x\theta}}{dt} = \frac{\partial f}{\partial x}(x,\theta,u)S_{x\theta} + \frac{\partial f}{\partial \theta}(x,\theta,u). \tag{3.14}$$

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 that we are at an equilibrium point and the dynamics for $S_{x,\theta}$ are stable, the steady state solution of equation (3.14) is identical to that obtained in equation (3.8). However, equation (3.14) 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.

Example 3.8 (Repressilator)**.** Consider the example of the repressilator, which was described in Example 2.2. The dynamics of this system can be written as

$$\begin{aligned}
\frac{dm_1}{dt} &= F_{\text{rep}}(P_3) - \gamma m_1 & \frac{dP_1}{dt} &= \beta m_1 - \delta P_1 \\
\frac{dm_2}{dt} &= F_{\text{rep}}(P_1) - \gamma m_2 & \frac{dP_2}{dt} &= \beta m_2 - \delta P_2 \\
\frac{dm_3}{dt} &= F_{\text{rep}}(P_2) - \gamma m_2 & \frac{dP_3}{dt} &= \beta m_3 - \delta P_2,
\end{aligned}$$

where the repressor is modeled using a Hill function

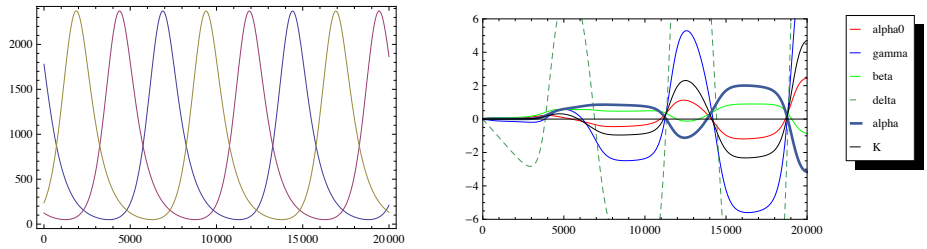
$$F_{\text{rep}}(P) = \frac{\alpha}{1 + (P/K)^n} + \alpha_0.$$

The dynamics of this system lead to a limit cycle in the protein concentrations, as shown in Figure 3.9a.

We can analyze the sensitivity of the protein concentrations to changes in the parameters using the sensitivity differential equation. Since our solution is periodic, the sensitivity dynamics will satisfy an equation of the form

$$\frac{dS_{x,\theta}}{dt} = A(t)S_{x,\theta} + B(t),$$

where $A(t)$ and $B(t)$ are both periodic in time. Letting $x = (m_1, P_1, m_2, P_2, m_3, P_3)$ and $\theta = (\alpha_0, \gamma, \beta, \delta, \alpha, K)$, we can compute $S_{x,\theta}$ along the limit cycle. If the dynamics for $S_{x,\theta}$ are stable then the resulting solutions will be periodic, showing how the dynamics around the limit cycle depend on the parameter values. The results are



(a) (b)

Figure 3.9: Repressilator sensitivity plots

shown in Figure 3.9b, where we plot the steady state sensitivity of P_1 as a function of time. We see, for example, that the limit cycle depends strongly on the protein degradation and dilution rate γ, indicating that changes in this value can lead to (relatively) large variations in the magnitude of the limit cycle.

∇

Several simulation tools include the ability to do sensitivity analysis of this sort, including `COPASI`.

Adaptation and disturbance rejection

A system is said to adapt to the input u when the steady state value of its output y is independent of the actual (constant) value of the input (Figure 3.10). Basically, after the input changes to a constant 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 and depends on the specific disturbance input and it is studied using the internal model principle [89].

For example, for adaptation to constant signals, the internal model principle requires integral feedback. The internal model principle is a powerful way to uncover biochemical structures in natural networks that are known to have the adaptation property. An example of this is the bacterial chemotaxis described in more detail in Chapter 5.

We illustrate two main mechanisms to attain adaptation: integral feedback and incoherent feedforward loops (IFFLs). We next 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 keeps track of the accumulated difference between $y(t)$ and its nominal steady state value y_0. A comparison is

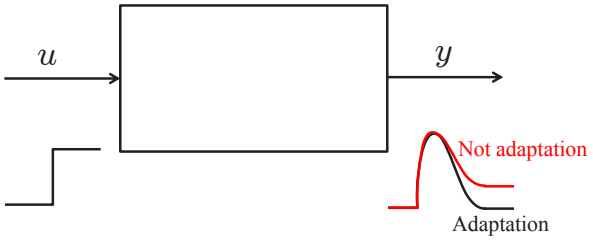


Figure 3.10: Adaptation property. The system is said to have the adaptation property if the steady state value of the output does not depend on the steady state value of the input. Hence, after a constant input perturbation, the output returns to its original value.

performed between this memory variable and the current input u, providing an error term that is used to drive the feedback mechanism that brings the system output back to the desired value y_0 (Figure 3.11).

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. The equations representing the system are given by:

$$\frac{dz}{dt} = y_1, \qquad y_1 = y - y_0, \qquad y = k(u - z),$$

so that the equilibrium is obtained by setting $\dot{z} = 0$, from which we obtain $y = y_0$. That is, the steady state of y does not depend on u. The additional question to answer is whether, after a perturbation u occurs, $y_1(t)$ tends to zero 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

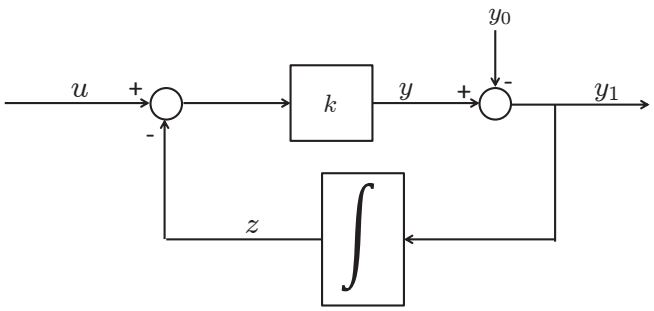


Figure 3.11: Basic block diagram representing a system with integral action.

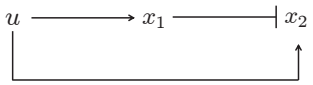


Figure 3.12: Incoherent feedforward loop. The input u affects the output through two channels. It indirectly represses it through an intermediate variable x_1 and it activates it directly.

of the system $\dot{z} = -kz + ku - y_0$ 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 back 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

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

in which the steady state value of y, being the solution to $y - y_0 = 0$, does not depend on u. 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, in turn, is the case if z tends to a constant value for $t \to \infty$, which is satisfied if u is a constant and the steady state of the above system is asymptotically stable.

Integral feedback is recognized as a key mechanism of perfectly adapting biological systems, both at the physiological level and at the cellular level, such as in blood calcium homeostasis [25], in the regulation of tryptophan in *E. coli* [94], in neuronal control of the prefrontal cortex [67], and in *E. coli* chemotaxis [102].

Incoherent feedforward loops

Feedforward motifs (Figure 3.12) are common in transcriptional networks and it has been shown they are over-represented in *E. coli* gene transcription networks, compared to other motifs composed of three nodes [3]. These are systems in which the input u directly helps promote the production of the output x_2 and also acts as a delayed inhibitor of the output 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 [72], the glucose to insulin release [75], ATP to intracellular calcium release [64], microRNA regulation [93], and many others.

Several variants of incoherent feedforward loops exist for perfect adaptation. The "sniffer", for example, is one in which the intermediate variable promotes

degradation:

$$\frac{dx_1}{dt} = \alpha u - \delta x_1, \qquad \frac{dx_2}{dt} = \beta u - \gamma x_1 x_2. \tag{3.15}$$

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 $\dot{x}_1 = 0$ given $x_1 = \alpha u/\delta$ and $\dot{x}_2 = 0$ gives $x_2 = \beta u/(\gamma x_1)$, which combined together result in $x_2 = (\beta\delta)/(\gamma\alpha)$, which is a constant independent of the input u. The linearization of the system at the equilibrium is given by

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

which has eigenvalues $-\delta$ and $-\gamma(\alpha u/\delta)$. Since these are both negative, the equilibrium point is asymptotically stable. The sniffer appears in models of neutrophil motion and *Dictyostelium* chemotaxis [101].

Another form for a feedforward loop is one in which the intermediate variable x_1 inhibits production of the output x_2, such as in the system:

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

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

By calculating the linearization at the equilibrium, one obtains

$$A = \begin{pmatrix} -\delta & 0 \\ -\frac{u}{x_1^2} & -\gamma \end{pmatrix},$$

whose eigenvalues are given by $-\delta$ and $-\gamma$. 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 exponentially stable.

Scale Invariance and fold-change detection

Scale invariance is the property by which the output $x_2(t)$ of the system does not depend on the amplitude of the input $u(t)$ (Figure 3.13). Specifically, consider an adapting system and assume that it pre-adapted to a constant background value a, then apply input $a + b$ and let $x_2(t)$ be the resulting output. Now consider a new background value $p\,a$ for the input and let the system pre-adapt to it. Then apply the input $p(a + b)$ and let $x_2(t)$ be the resulting output. The system has the scale

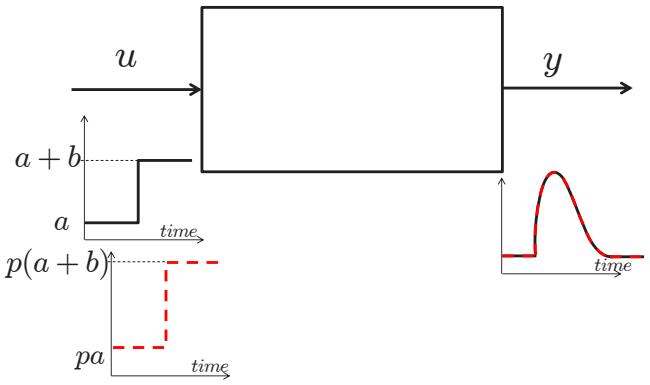


Figure 3.13: Fold-change detection. The output response does not depend on the absolute magnitude of the input but only on the fold change of the input.

invariance property if $x_2(t) = x_2(t)$. This also means that the output responds in the same way to inputs changing by the same multiplicative factor (fold), hence this property is also called fold-change detection. Looking at Figure 3.13, the output would present different pulses for different fold changes b/a.

Incoherent feedforward loops can implement the fold-change detection property [36]. As an example, consider the feedforward motif represented by the sniffer and consider two inputs: $u_1(t) = a + b_1(t - t_0)$ and $u_2(t) = pa + pb_1(t - t_0)$. Assume also, as said above, that at time t_0 the system is at the steady state, that is, it pre-adapted. Hence, we have that the two steady states from which the system starts at $t = t_0$ are given by $x_{1,1} = a\alpha/\delta$ and $x_{1,2} = pa\alpha/\delta$ for the x_1 variable and by $x_{2,1} = x_{2,2} = (\beta\delta)/(\gamma\alpha)$ for the x_2 variable. Integrating system (3.16) starting from these initial conditions, we obtain for $t \geq t_0$

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

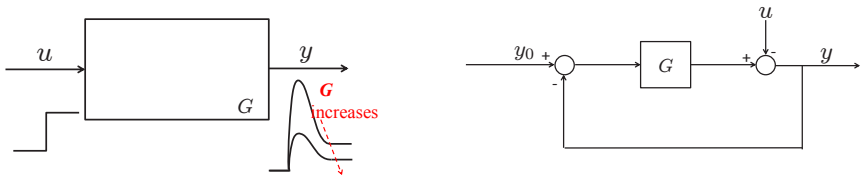
Using these in the expression of $\dot{x}_2$ in equation (3.16) gives the differential equations to which $x_{2,1}(t)$ and $x_{2,2}(t)$ obey for $t \geq t_0$ as

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

and

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

which give $x_{2,1}(t) = x_{2,2}(t)$ 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.



(a) (b)

Figure 3.14: (a) Disturbance attenuation. A system is said to have the disturbance attenuation property if there is an internal system parameter G such that the system output response becomes arbitrarily close to a nominal output (independent of the input u) by increasing the value of G. (b) High gain feedback. A possible mechanism to attain disturbance attenuation is to feedback the error between the nominal output y_0 and the actual output y through a large gain G.

Disturbance attenuation

A system has the property of disturbance attenuation if there is a system parameter G such that the output response $y(t)$ to the input $u(t)$ can be made arbitrarily small as G is increased (Figure 3.14a). A possible mechanism for disturbance attenuation is high gain feedback (Figure 3.14b). 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 $\dot{x} = F(x,t)$. We say that this system is *contracting* if any two trajectories starting from different initial conditions tend to each other as time increase 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) is negative definite. That is, that the largest eigenvalue of

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

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

Now, consider the nominal system $\dot{x} = Gf(x,t)$ for $G > 0$ and its perturbed version $\dot{x}_p = Gf(x_p,t) + u(t)$. 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 (ratio between the largest and the smallest eigenvalue of $\Theta^T\Theta$) of the transformation matrix Θ [60]. 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. Hence, the system has the disturbance attenuation property.

3.3 Analysis of Reaction Rate Equations

The previous section considered analysis techniques for general dynamical systems with small perturbations. In this section, we specialize to the case where the dynamics have the form of a reaction rate equation:

$$\frac{ds}{dt} = Nv(x,\theta), \tag{3.17}$$

where x is the vector of species concentrations, θ is the vector of reaction parameters, N is the stoichiometry matrix and $v(x,\theta)$ is the reaction rate (or flux) vector.

Reduced reaction dynamics

When analyzing reaction rate equations, it is often the case that there are conserved quantities in the dynamics. For example, conservation of mass will imply that if all compounds containing a given species are captured by the model, the total mass of that species will be constant. This type of constraint will then give a conserved quantity of the form $c_i = H_i x$ where H_i represents that combinations of species in which the given element appears. Since c_i is constant, it follows that $dc_i/dt = 0$ and, aggregating the set of all conserved species, we have

$$0 = \frac{dc}{dt} = H\frac{ds}{dt} = HNv(x,\theta) \quad \text{for all } x.$$

If we assume that the vector of fluxes spans $\mathbb{R}^m$ (the range of $v : \mathbb{R}^n \times \mathbb{R}^p \to \mathbb{R}^m$), then this implies that the conserved quantities correspond to the left null space of the stoichiometry matrix N.

It is often useful to remove the conserved quantities from the description of the dynamics and write the dynamics for a set of independent species. To do this, we transform the state of the system into two sets of variables:

$$\begin{pmatrix} x_i \\ x_d \end{pmatrix} = \begin{pmatrix} P \\ H \end{pmatrix} x. \tag{3.18}$$

The vector $x_i = Px$ is the set of independent species and is typically chosen as a subset of the original species of the model (so that the rows P consists of all zeros and a single 1 in the column corresponding to the selected species). The matrix H should span the left null space of N, so that x_d represents the set of dependent concentrations. These dependent species do not necessarily correspond to individual species, but instead are often combinations of species (for example, the total concentration of a given element that appears in a number of molecules that participate in the reaction).

Given the decomposition (3.18), we can rewrite the dynamics of the system in terms of the independent variables x_i. We start by noting that given x_i and x_d, we can reconstruct the full set of species x:

$$x = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} x_i \\ x_d \end{pmatrix} = Lx_i + c_0, \qquad L = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} I \\ 0 \end{pmatrix}, \quad c_0 = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} 0 \\ c \end{pmatrix}$$

where c_0 represents the conserved quantities. We now write the dynamics for x_i as

$$\frac{dx_i}{dt} = P\frac{dx}{dt} = PNv(Lx_i + c_0, \theta) = N_r v_r(x_i, c_0, \theta), \tag{3.19}$$

where N_r is the *reduced stoichiometry matrix* and v_r is the rate vector with the conserved quantities separated out as constant parameters.

The reduced order dynamics in equation (3.19) represent the evolution of the independent species in the reaction. Given x_i, we can reconstruct the full set of species from the dynamics of the independent species by writing $x = Lx_i + c_0$. The vector c_0 represents the values of the conserved quantities, which must be specified in order to compute the values of the full set of species. In addition, since $x = Lx_i + c_0$, we have that

$$\frac{dx}{dt} = L\frac{dx_i}{dt} = LN_r v_r(x_i, c_0, p) = LN_r v(x, \theta),$$

which implies that

$$N = LN_r.$$

Thus, L also reconstruct the reduced stoichiometry matrix from the reduced space to the full space.

Example 3.9 (Enzyme kinetics)**.** Consider an enzymatic reaction

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

whose full dynamics can be written as

$$\frac{d}{dt}\begin{pmatrix} S \\ E \\ C \\ P \end{pmatrix} = \begin{pmatrix} -1 & 1 & 0 \\ -1 & 1 & 1 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} aE \cdot S \\ dC \\ kC \end{pmatrix}.$$

The conserved quantities are given by

$$H = \begin{pmatrix} 0 & 1 & 1 & 0 \\ 1 & -1 & 0 & 1 \end{pmatrix}.$$

The first of these is the total enzyme concentration $E_{\text{tot}} = E + C$, while the second asserts that the concentration of product P is equal to the free enzyme concentration E minus the substrate concentration S. If we assume that we start with substrate concentration S_0, enzyme concentration E_{tot} and no product or bound enzyme, then the conserved quantities are given by

$$c = \begin{pmatrix} E + C \\ S - E + P \end{pmatrix} = \begin{pmatrix} E_{\text{tot}} \\ S_0 - E_{\text{tot}} \end{pmatrix}.$$

There are many possible choices for the set of independent species $x_i = Px$, but since we are interested in the substrate and the product, we choose P as

$$P = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}.$$

Once P is chosen then we can compute

$$L = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} I \\ 0 \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 1 & 1 \\ -1 & -1 \\ 0 & 1 \end{pmatrix}, \qquad c_0 = \begin{pmatrix} P \\ H \end{pmatrix}^{-1} \begin{pmatrix} 0 \\ c \end{pmatrix} = \begin{pmatrix} 0 \\ E_{\text{tot}} - S_0 \\ S_0 \\ 0 \end{pmatrix},$$

The resulting reduced order dynamics can be computed to be

$$\begin{aligned} \frac{d}{dt} \begin{pmatrix} S \\ P \end{pmatrix} &= \begin{pmatrix} -1 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} a(P + S + E_{\text{tot}} - S_0)S \\ d(-P - S + S_0) \\ k(-P - S + S_0) \end{pmatrix} \\ &= \begin{pmatrix} -a(P + S + E_{\text{tot}} - S_0)S - d(P + S - S_0) \\ k(S_0 - S - P) \end{pmatrix}. \end{aligned}$$

A simulation of the dynamics is shown in Figure 3.15. We see that the dynamics are very well approximated as being a constant rate of production until we exhaust the substrate (consistent with the Michaelis-Menten approximation).

∇

Metabolic control analysis

Metabolic control analysis (MCA) focuses on the study of the sensitivity of steady state concentrations and fluxes to changes in various system parameters. The basic concepts are equivalent to the sensitivity analysis tools described in Section 3.1,

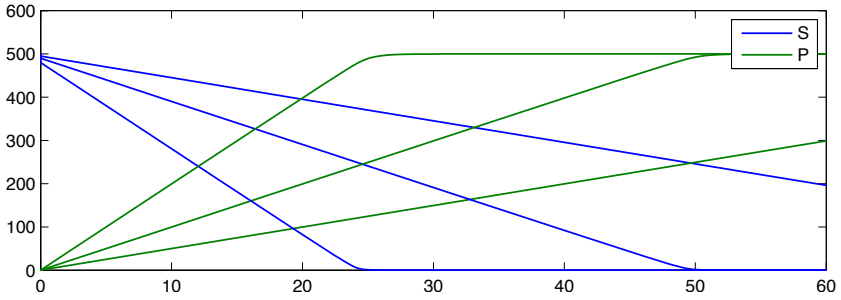


Figure 3.15: Enzyme dynamics. The simulations were carried out $a = d = 10$, $k = 1$, $S_0 = 500$ and $E_{\text{tot}} = 5, 1020$. The top plot shows the concentration of substrate S and product P, with the fastest case corresponding to $E_{\text{tot}} = 20$. The figures on the lower left zoom in on the substrate and product concentrations at the initial time and the figures on the lower right at one of the transition times.

specialized to the case of reaction rate equations. In this section we provide a brief introduction to the key ideas, emphasizing the mapping between the general concepts and MCA terminology (as originally done by [47]).

Consider the reduced set of chemical reactions

$$\frac{dx_i}{dt} = N_r v_r(x_i, \theta) = N_r v(Lx_i + c_0, \theta).$$

We wish to compute the sensitivity of the equilibrium concentrations x_e and equilibrium fluxes v_e to the parameters θ. We start by linearizing the dynamics around an equilibrium point x_e. Defining $z = x - x_e$, $u = \theta - \theta_0$ and $f(z, u) = N_r v(x_e + z, \theta_0 + u)$, we can write the linearized dynamics as

$$\frac{dx}{dt} = Ax + Bu, \qquad A = \left(N_r \frac{\partial v}{\partial s} L\right), \quad B = \left(N_r \frac{\partial v}{\partial p}\right), \tag{3.20}$$

which has the form of a linear differential equation with state z and input u.

In metabolic control analysis, the following terms are defined:

$$\epsilon_\theta = \left.\frac{dv}{d\theta}\right|_{x_e, \theta_o} \qquad \epsilon_\theta = \text{flux control coefficients}$$

$$R_\theta^x = \frac{\partial x_e}{\partial \theta} = C^x \epsilon_\theta \qquad \begin{aligned} R_\theta^x &= \\ C^x &= \text{concentration control coefficients} \end{aligned}$$

$$R_\theta^v = \frac{\partial v_e}{\partial \theta} = C^v \epsilon_\theta \qquad \begin{aligned} R_\theta^v &= \\ C^v &= \text{rate control coefficients} \end{aligned}$$

These relationships describe how the equilibrium concentration and equilibrium rates change as a function of the perturbations in the parameters. The two control

matrices provide a mapping between the variation in the flux vector evaluated at equilibrium,

$$\left(\frac{\partial v}{\partial \theta}\right)_{x_e,\theta_0},$$

and the corresponding differential changes in the equilibrium point, $\partial x_e/\partial\theta$ and $\partial v_e/\partial\theta$. Note that

$$\frac{\partial v_e}{\partial \theta} \neq \left(\frac{\partial v}{\partial \theta}\right)_{x_e,\theta_0}.$$

The left side is the relative change in the equilibrium rates, while the right side is the change in the rate function $v(x,\theta)$ evaluated at an equilibrium point.

To derive the coefficient matrices C^x and C^v, we simply take the linear equation (3.20) and choose outputs corresponding to s and v:

$$y_x = Ix, \qquad y_v = \frac{\partial v}{\partial x}Lx + \frac{\partial v}{\partial \theta}u.$$

Using these relationships, we can compute the transfer functions

$$H_x(s) = (sI - A)^{-1}B = \Big[(sI - N_r\frac{\partial v}{\partial x}L)^{-1}N_r\Big]\frac{\partial v}{\partial \theta},$$
$$H_v(s) = \frac{\partial v}{\partial s}L(sI - A)^{-1}B + \frac{\partial v}{\partial p} = \Big[\frac{\partial v}{\partial x}L(sI - N_r\frac{\partial v}{\partial x}L)^{-1}N_r + I\Big]\frac{\partial v}{\partial \theta}.$$

Classical metabolic control analysis considers only the equilibrium concentrations, and so these transfer functions would be evaluated at $x = 0$ to obtain the equilibrium equations.

These equations are often normalized by the equilibrium concentrations and parameter values, so that all quantities are expressed as fractional quantities. If we define

$$D^x = \text{diag}\{x_e\}, \qquad D^v = \text{diag}\{v(x_e,\theta_0)\}, \qquad D^\theta = \text{diag}\{\theta_0\},$$

then the normalized coefficient matrices (without the overbar) are given by

$$C^x = (D^x)^{-1}C^xD^v, \qquad C^v = (D^v)^{-1}C^vD^v,$$
$$R^x_\theta = (D^x)^{-1}R^x_\theta D^\theta, \qquad R^v_\theta = (D^v)^{-1}R^v_\theta D^\theta.$$

Flux balance analysis

Flux balance analysis is a technique for studying the relative rate of different reactions in a complex reaction system. We are most interested in the case where there may be multiple pathways in a system, so that the number of reactions m is greater than the number of species n. The dynamics

$$\frac{dx}{dt} = Nv(x,\theta)$$

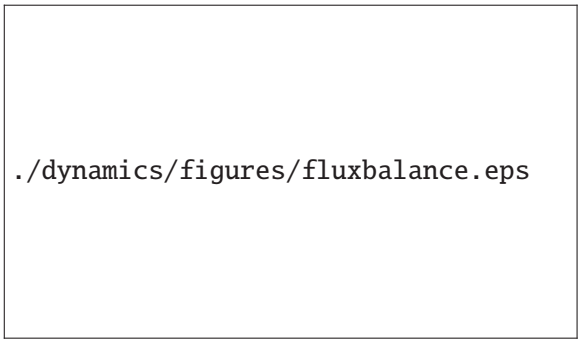


Figure 3.16: Flux balance analysis.

thus have the property that the matrix N has more columns that rows and hence there are multiple reactions that can produce a given set of species. Flux balance is often applied to pathway analysis in metabolic systems to understand the limiting pathways for a given species and the the effects of changes in the network (e.g., through gene deletions) to the production capacity.

To perform a flux balance analysis, we begin by separating the reactions of the pathway into internal fluxes v_i versus exchanges flux v_e, as illustrated in Figure 3.16. The dynamics of the resulting system now be written as

$$\frac{dx}{dt} = Nv(x,\theta) = N\begin{pmatrix} v_i \\ v_e \end{pmatrix} = Nv_i(x,\theta) - b_e,$$

where $b_e = -Nv_e$ represents the effects of external fluxes on the species dynamics. Since the matrix N has more columns that rows, it has a *right* null space and hence there are many different internal fluxes that can produce a given change in species.

In particular, we are interested studying the steady state properties of the system. In this case, we have that $dx/dt = 0$ and we are left with an algebraic system

$$Nv_i = b_e.$$

Material to be completed. Review

3.4 Oscillatory Behavior

In addition to equilibrium behavior, a variety of cellular procesess 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 "phases" that govern the duplication and division of cells into two new cells:

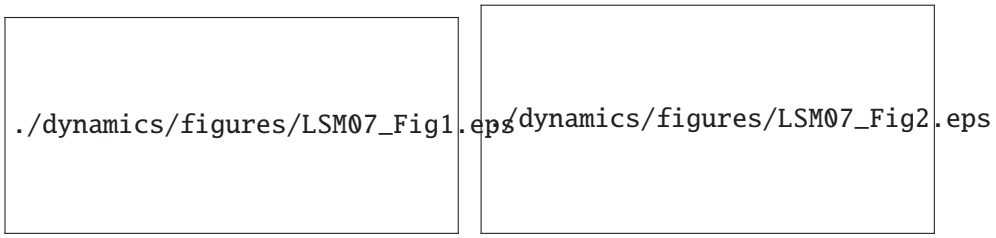
- G1 phase - gap phase, terminated by "G1 checkpoint"
- S phase - synthesis phase (DNA replication)
- G2 phase - gap phase, terminated by "G2 checkpoint"
- M - mitosis (cell division)

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. *Cyclinscyclins* 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 3.17 [57]. This organism uses a variety of different biomolecular mechanisms, including transcriptional activation and represssion, positive autoregulation (CtrA), phosphotransfer and methlylation 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 minutes 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 tamirind tree [65]. There are three defining characteristics associated with circadian rhythm: (1) the time to complete one cycle is approximately 24 hours, (2) the rhythm is endogenously generated and



(a) Overview of cell cycle (b) Molecular mechanisms

Figure 3.17: The *Caulobacter crescentus* cell cycle. (a) *Caulobacter* cells divide asymmetrically into a stalked cell, which is attached to a surface, and a swarmmer 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, described in more detail in the text. Figure obtained from [57] (permission TBD).

self-sustaning and (3) the period remains relatively constant under changes in ambient temperature. Oscillations that have these properties appaer in many different organisms, including micro-organisms, plants, insects and mammals. Some common features of the circuitry implementing circadian rhythms in these organisms is the combination of postive 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 3.18 shows some of the different organisms in which circadian oscillations can be found and the primary genes responsible for different postive 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

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

where $x \in \mathbb{R}^n$ represents the state of the system (typically concentrations of various proteins and other species and complexes), $u \in \mathbb{R}^q$ represents the external inputs, $y \in \mathbb{R}^p$ represents the (measured) outputs and $\theta \in \mathbb{R}^K$ 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 linear system, an input of the form $u(t) = A\sin\omega t$ then

Figure 3.18: *Caption omitted pending permission*. (Figure and caption from [11])

we now already the output will be 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 then we can write it in terms of a set of harmonics,

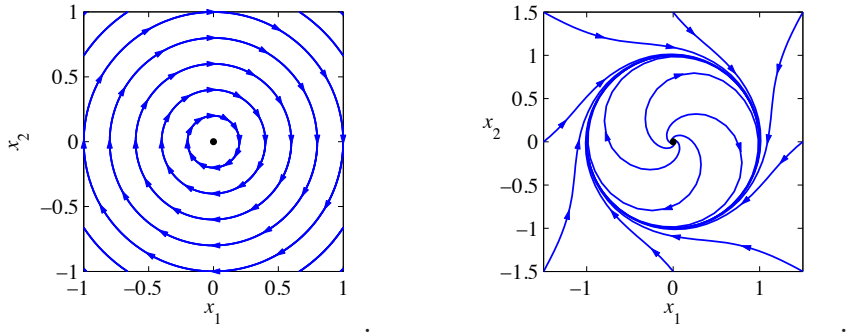
$$y(t) = B_0 + B_1 \sin(\omega t + \phi_1) + B_2 \sin(2\omega t + \phi_2) + \cdots$$

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 by 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 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

$$\frac{dx}{dt} = F(x, \theta), \qquad y = h(x, \theta) \tag{3.21}$$



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

Figure 3.19: Examples of harmonic oscillators.

where $F(x,\theta) = f(x,u,\theta)$ reflects the fact that 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, as 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 $x(t) \in \Gamma$, $x(t+T) = x(t)$.

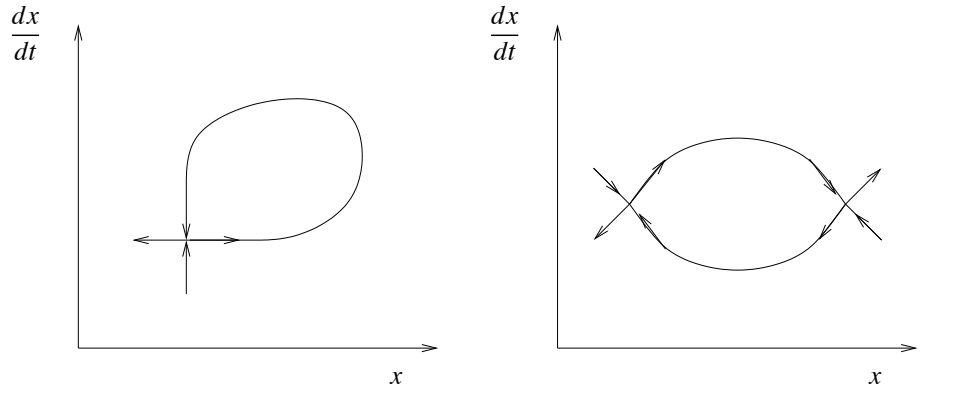
There are many different types of periodic orbits that can occur in a system whose dynamics are modeled as in equation (3.21). A *harmonic oscillator* references to a system that oscillates around an equilibrium point, but does not (usually) get near the equilibrium point. The classical harmonic oscillator is a linear system of the form

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

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 3.19. 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.



(a) Homoclinic orbit (b) Heteroclinic orbit

Figure 3.20: Homoclinic and heteroclinic orbits.

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

$$\frac{dx_1}{dt} = x_2 + x_1(1 - x_1^2 - x_2^2), \qquad \frac{dx_2}{dt} = -x_1 + x_2(1 - x_1^2 - x_2^2). \tag{3.22}$$

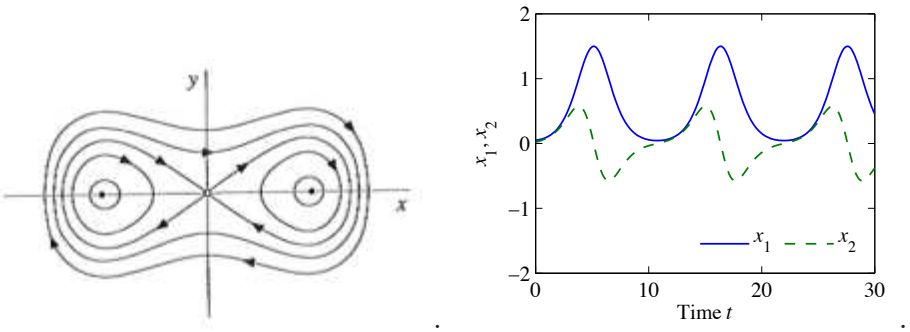
This system has an equilibrium point at $x = (0,0)$, but the linearization of this equilibrium point is unstable. The phase portrait in Figure 3.19b shows that the solutions in the phase plane converge to a circular trajectory. In the time domain this corresponds to an oscillatory solution. Mathematically 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 equlibrium 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 sitautions are shown in Figure 3.20. 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 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 3.20b.

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

$$\frac{dx_1}{dt} = x_2, \qquad \frac{dx_2}{dt} = x_1 - x_1^3. \tag{3.23}$$



(a) (b)

Figure 3.21: Example of a homoclinic orbit.

The phase portrait and time domain solutions are shown in Figure 3.21. 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 re-converges to the equilibrium point, before begin 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 equilibirum points at $x = (\pm 1, 0)$.

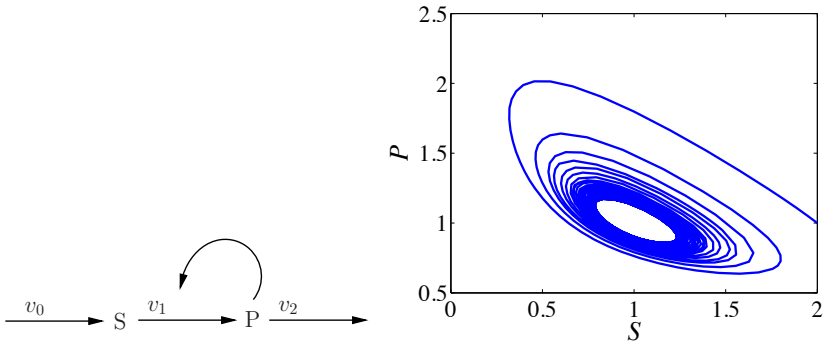
Example 3.10 (Glycolytic oscillations)**.** Glycolysis is one of the principal metabolic networks involved in energy production. It is a sequence of enzyme-catalyzed reactions that coverts sugar into pyruvate, which is then further degraded to alcohol (in yeast fermentation) and lactic acid (in muscles) in 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 [24] while sustained oscillations in yeast cell free extracts were observed when glucose-6-phosphate (G6P), fructose-6-phosphate (F6P) [43] or trehalose [79] were used as substrates.

Here, we introduce the fundamental motif which is known to be at the core of these oscillatory phenomenon. This is depicted in Figure 3.22 (a). A simple model for the system is given by the two differential equations

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

in which

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



(a) (b)

Figure 3.22: (a) The Glycolisis pathway. "S" is a substrate, which is converted into product "P". This, in turn, is activating its own production by enhancing the rate v_2. (b) Oscillations in the glycolisis pathway. Parameters are $v_0 = 1$, $k_1 = 1$, and $k_2 = 1.00001$.

where $f(P)$ is the Hill function. Under the assumption that $K \gg P^2$, we have $f(P) \approx k_1 P^2$, in which we have defined $k_1 := \alpha/K$. This second order system admits a stable limit cycle under suitable parameter conditions (Figure 3.22(b)). ∇

The example above illustrates some of the types of questions we would like to answer for oscillatory systems. For example, Under what parameter conditions do oscillations occur in the glycolitic system? How much can the parameter change before the limit cycle disappears? To analyze these sorts of questions, we need to introduce tools that allow to infer the existence and robustness of limit cycle behavior from a differential equation model. The objective of this section is to address these questions.

Consider the system $\dot{x} = F(x)$ and let $x(t, x_0)$ denote its solution starting at x_0 at time $t = 0$, that is, $\dot{x}(t, x_0) = F(x(t, x_0))$ and $x(0, x_0) = x_0$. We say that $x(t, x_0)$ is a *periodic solution* if there is $T > 0$ such that $x(t, x_0) = x(t + T, x_0)$ for all $t \in \mathbb{R}$. Here, we seek to answer two questions: (a) when does a system $\dot{x} = F(x)$ admit periodic solutions? (b) When are these periodic solutions stable or asymptotically stable?

In order to provide the main result to state the existence of a stable periodic solution, we need the concept of omega-limit set of a point p, denoted $\omega(p)$. Basically, the omega-limit set $\omega(p)$ denotes 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 $x \in \mathbb{R}^n$ is called an *omega-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 $x(t_i, p) \to x$ as $i \to \infty$. The *omega limit set* of p, denoted $\omega(p)$, is the set of all omega-limit points of p.

The omega-limit set of a system has several relevant properties, among which the fact 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 $\mathbb{R}^2$ as several high dimensional systems can be often 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 that we next give provides a simple check to rule out periodic solutions for system in $\mathbb{R}^2$. Specifically, let $x \in \mathbb{R}^2$ and consider

$$\dot{x}_1 = F_1(x_1, x_2) \qquad \dot{x}_2 \quad = F_2(x_1, x_2), \tag{3.24}$$

in which the functions $F : \mathbb{R}^2 \to \mathbb{R}^2$ is smooth. Then, we have the following result:

Theorem 3.2 (Bendixson's criterion)**.** *If on a simply connected region $D \subset \mathbb{R}^2$ (i.e., there are no holes in it) 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, then system (3.24) *has no closed orbits that lie entirely in D.*

Example 3.11. Consider the system

$$\dot{x}_1 = -x_2^3 + \delta x_1^3, \qquad \dot{x}_2 \quad = x_1^3,$$

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 positive in all $\mathbb{R}^2$ if $\delta \neq 0$. If $\delta \neq 0$, we can thus conclude from Bendixson's criterion that there are no periodic solutions. Investigate as an exercise what happens when $\delta = 0$. ∇

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

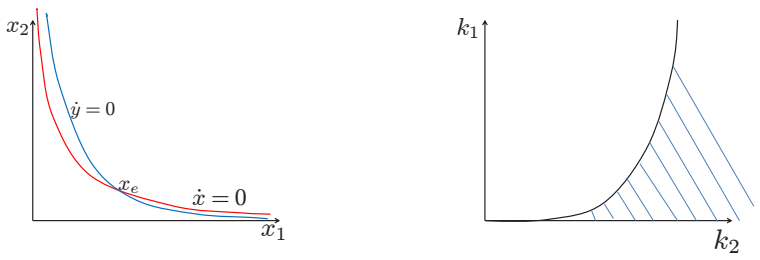
Theorem 3.3 (Poincarè-Bendixson)**.** *Let M be a bounded and closed positively invariant region for the system $\dot{x} = F(x)$ with $x \in$ (i.e., any trajectory that starts in M stays in M for all $t \geq 0$). Let $p \in M$, then one of the following possibilities holds for $\omega(p)$:*

(i) *$\omega(p)$ is a steady state;*

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

(iii) *$\omega(p)$ consists of a finite number of steady states 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:



(a) Nullclines (b) Stability diagram

Figure 3.23: (a) The nullclines and the equilibrium of the system. (b) Parameter space leading to oscillatory behavior.

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

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

Example 3.12 (Glycolytic oscillations)**.** Consider again the glycolysis example. Let $x_1 = S$ and $x_2 = P$ and rewrite the system (3.10) as

$$\frac{dx_1}{dt} = v_0 - k_1 x_1 x_2^2 =: F_1(x_1, x_2), \qquad \frac{dx_2}{dt} = k_1 x_1 x_2^2 - k_2 x_2 =: F_2(x_1, x_2).$$

As a first step, we need to determine the number of steady states. From $\dot{x} = 0$, we obtain

$$x = \frac{v_0}{k_1 y^2},$$

while from $\dot{y} = 0$, we obtain

$$x = \frac{k_2}{k_1 y}.$$

The intersection between these two curves (the nullclines) in the (x_1, x_2) plane gives rise to one steady state only (Figure 3.23a). The reader can determine a positively invariant region that is compact. Then, it is enough to verify that the steady state $(x_{1,e}, x_{2,e})$ is unstable and not a saddle to guarantee the existence of a stable limit cycle. Thus,

$$J = \begin{pmatrix} \frac{\partial F_1}{\partial x_1} & \frac{\partial F_1}{\partial x_2} \\ \frac{\partial F_2}{\partial x_1} & \frac{\partial F_2}{\partial x_2} \end{pmatrix}\Bigg|_{(x_{1,e}, x_{2,e})} = \begin{pmatrix} -k_1 x_{2,e}^2 & -2k_1 x_{1,e} x_{2,e} \\ k_1 x_{2,e}^2 & -k_2 + 2k_1 x_{1,e} x_{2,e} \end{pmatrix},$$

in which $x_{1,e} = k_2^2/(k_1 v_0)$ and $x_{2,e} = v_0/k_2$. The eigenvalues are such that

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

in which

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

Since $\det(J) > 0$, in order to have an unstable equilibrium that is not a saddle, it is necessary and sufficient to have $\mathrm{tr}(J) > 0$, which leads to

$$k_1 < k_2^3/v_0^2.$$

This region is depicted in Figure 3.23b. Hence, if k_2 is large enough (i.e., the outflux is large enough compared to the strength of the self activation) a stable limit cycle arises. ∇

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

The results above holds only for systems in two dimensions. However, there have been recent extensions of this theorem to systems with special structure in $\mathbb{R}^n$. In particular, we have the following result due to Hastings et al. (1977).

Theorem 3.4 (Hastings et al. 1977)**.** *Consider a system $\dot{x} = F(x)$, which is of the form*

$$\dot{x}_1 = F_1(x_n, x_1)$$
$$\dot{x}_j = F_j(x_{j-1}, x_j),\ 2 \le j \le n$$

on the set M defined by $x_i \ge 0$ 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* $2 \le i \le n$, *and* $\frac{\partial F_1}{\partial x_n} < 0$;

(ii) $F_i(0,0) \ge 0$ *and* $F_1(x_n, 0) > 0$ *for all* $x_n \ge 0$;

(iii) The system has a unique steady state $x^* = (x_1^*, ..., x_n^*)$ 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 gain", the instability of the steady state (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, of whether it is an omega-limit set of any point in M. This stability result is implied by a more recent theorem due to Mallet-Paret and Smith (1990), for which we provide a simplified statement as follows.

Theorem 3.5 (Mallet-Paret and Smith, 1990)**.** *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:

(a) A steady state;

(b) A non-constant periodic orbit;

(c) A set of steady states 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 steady state only and at which the linearization has all eigenvalues with positive real part, the omega limit set must be a periodic orbit.

Let for some $\delta_i \in \{1, -1\}$ be $\delta_i \frac{\partial F_i(x, x_{i-1})}{\partial x_{i-1}} > 0$ for all $0 \leq i \leq n$ and define $\Delta := \delta_1 \cdot ... \cdot \delta_n$. One can show that the sign of Δ is related to whether the system has one or multiple steady states.

In Chapter 6, we will apply these results to determine the parameter space that makes the repressilator (see Example 2.2) oscillate.

3.5 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

$$\frac{dx}{dt} = F(x, \theta), \quad x \in \mathbb{R}^n, \theta \in \mathbb{R}^k, \tag{3.25}$$

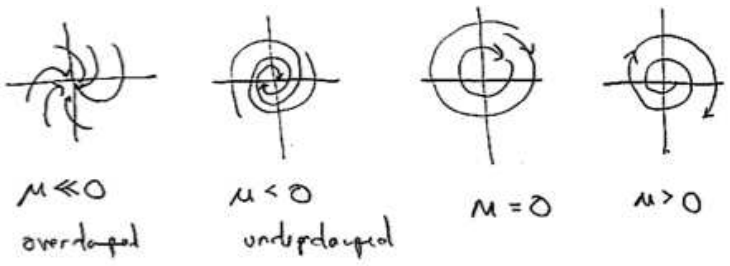


Figure 3.24: Phase portraits for a simple bifurcation.

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 (3.25) 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 a change in the number of solutions at a given value of θ.

As an example of a bifurcation, consider the linear system

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

where $k > 0$ is fixed and θ is our bifurcation parameter. Figure 3.24 shows the phase portraits for different values of θ. We see that at $\theta = 0$ the system transitions from a single stable equilibrium point at the original 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 3.25 shows examples of some common bifurcation diagrams. Note that for some types of bifucations, 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 that there are two stable equilibria, in which case the system is said to be *bistable*.

Another type of diagram that is useful in understanding parametric dependence is a *parametric stability diagram*, an example of which was shown in Figure 3.23. In this type of diagram, we pick one or two (or sometimes three) parameters in the

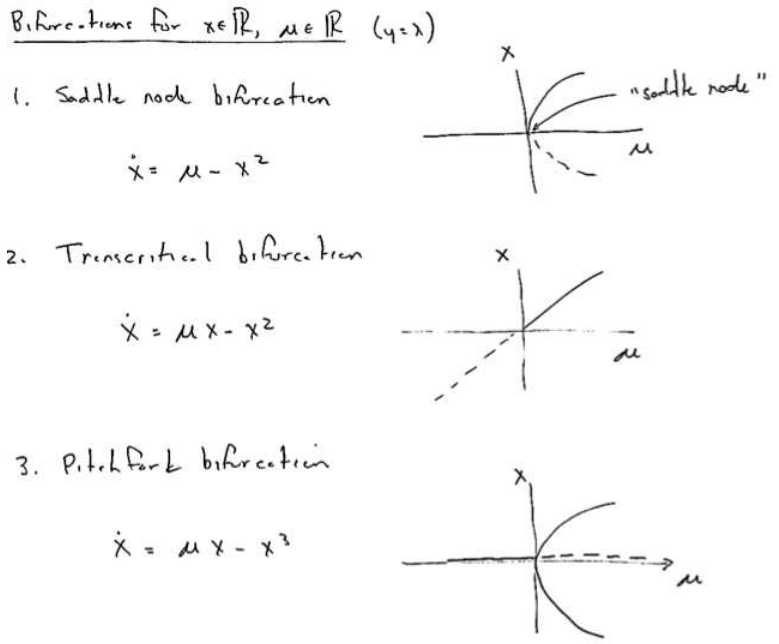
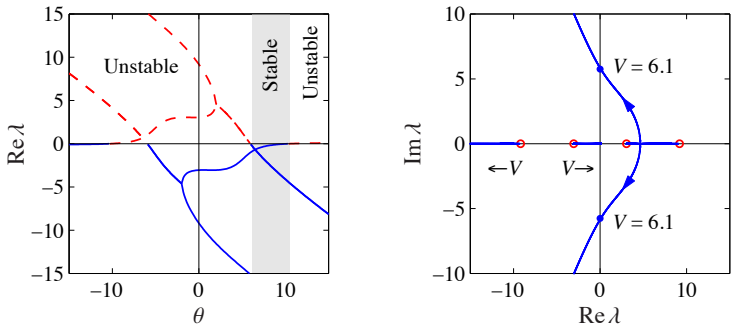


Figure 3.25: Bifurcation diagrams for some common bifurcations

system and then analyze the stability type for the system over all possible combinations of those parameters. The resulting diagram shows those regions in parameter space where the system exhibits qualitatively different behaviors; an example is shown in Figure 3.26a.

A particular form of bifurcation that is very common when controlling linear systems is that the equilibrium remains fixed but the stability of the equilibrium changes as the parameters are varied. In such a case it is revealing to plot the eigenvalues of the system as a function of the parameters. Such plots are called *root locus diagrams* because they give the locus of the eigenvalues when parameters change. An example is shown in Figure 3.26b. Bifurcations occur when parameter values are such that there are eigenvalues with zero real part. Computing environments such LabVIEW, MATLAB and Mathematica have tools for plotting root loci.

Parametric stability diagrams and bifurcation diagrams can provide valuable insights into the dynamics of a nonlinear system. It is usually necessary to carefully choose the parameters that one plots, including combining the natural parameters of the system to eliminate extra parameters when possible. Computer programs such as `AUTO`, `LOCBIF` and `XPPAUT` provide numerical algorithms for producing stability and bifurcation diagrams.



(a) Stability diagram (b) Root locus diagram

Figure 3.26: Stability plots a nonlinear system. The plot in (a) shows the real part of the system eigenvalues as a function of the parameter θ. The system is stable when all eigenvalues have negative real part (shaded region). The plot in (b) shows the locus of eigenvalues on the complex plane as the parameter θ is varied and gives a different view of the stability of the system. This type of plot is called a *root locus diagram*.

Hopf bifurcation

The bifurcations discussed above involved bifurcation of equilibrium points. Another type of bifurcation that can occur is that 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 steady state.

Consider the system dependent on a parameter α:

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

and assume that at the steady state x corresponding to $\alpha = \alpha$ (i.e., $g(x,\alpha) = 0$), the linearization $\partial g/\partial x(x,\alpha)$ has a pair of (non zero) imaginary eigenvalues with the remaining eigenvalues having negative real parts. Define the new parameter $\theta := \alpha - \alpha$ and re-define the system as

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

so that the linearization $\partial f/\partial x(x,0)$ has a pair of (non zero) 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 $\frac{\partial \beta}{\partial \theta}(0) \neq 0$ 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

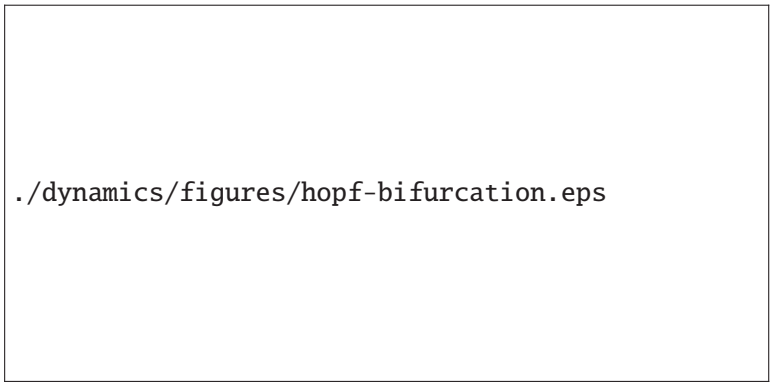


Figure 3.27: Hopf Bifurcation. On the left hand, as θ increases a stable limit cycle appears. On the right hand side, as θ increases a limit cycle appears but it is unstable.

orbit is stable, the Hopf bifurcation is said *supercritical*, while if it is unstable it is said *subcritical*. Figure 3.27 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 McCrocken, 1976) and available bifurcation software, such as AUTO. In practice, it is often enough to calculate the value α of the parameter at which Hopf bifurcation occurs and simulate the system for values of the parameter α close to α. If a small amplitude limit cycle appears, then the bifurcation must be supercritical.

Example 3.13 (Glycolytic oscillations)**.** Recalling the model (3.10) for the glycolytic oscillator, 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 $\omega = \sqrt{4\det(J)} = 4k_1(v_0/k_2)^2$. 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. For obtaining global results, one has to apply other tools, such as the Poincarè-Bendixson theorem. ∇

The Hopf bifurcation theorem is based on center manifold theory for nonlinear dynamical systems. For a rigorous treatment of Hopf bifurcation 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, 41].

3.6 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 the 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 mathematical analysis of such systems here.

Singular perturbation analysis

Singular perturbation techniques apply to systems that have processes that evolve 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

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

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 absolute value of the time derivative of y can be much larger than the time derivative 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).

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

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

Let $y = \gamma(x)$ denote *slow manifold* given by the locally unique solution of $g(x, y, 0) = 0$. The *implicit function theorem* [63] shows that this solution exists whenever $\partial g / \partial y$ is non singular. Furthermore, the theorem also shows that

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

We can now approximate the dynamics in x (i.e., on the slow manifold) as

$$\frac{dx}{dt} = f(x, \gamma(x), 0), \qquad x(0) = x_0.$$

We seek to determine under what conditions the solution $x(t)$ is "close" to the solution $x(t)$ of the reduced system. This problem can be addressed by analyzing the fast dynamics. Letting $\tau = t/\epsilon$ be the fast time scale, 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 approximated by

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

in which x is "frozen" at the initial condition. This system is usually referred to as the *boundary layer* system. If for all x_0, we have that $y(\tau)$ converges to $\gamma(x_0)$, then for $t > 0$ we will have that the solution $x(t)$ is well approximated by the solution $x(t)$ to the reduced system. This qualitative explanation is more precisely captured by the following theorem [54].

Theorem 3.6. *Assume that*

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

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 is a constant $\epsilon^* > 0$ and set $\Omega \subseteq D$ such that

$$x(t) - x(t) = O(\epsilon) \text{ uniformly for } t \in [0, t_f],$$
$$y(t) - \gamma(x(t)) = O(\epsilon) \text{ uniformly for } t \in [t_b, t_f],$$

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

Example 3.14 (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 binding. Here, we illustrate how Hill function expressions can be derived by a formal application of singular perturbation. 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

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

$$\mathrm{m_Y} \xrightarrow{\beta} \mathrm{m_Y} + \mathrm{Y}, \qquad \mathrm{m_Y} \xrightarrow{\gamma} \emptyset, \qquad \mathrm{Y} \xrightarrow{\delta} \emptyset, \qquad p + C = p_{\mathrm{tot}}.$$

The corresponding differential equation model is given by

$$\begin{aligned}
\frac{dX_2}{dt} &= k_1 X^2 - k_2 X_2 - a X_2 (p_{\mathrm{tot}} - C) + dC \\
\frac{dC}{dt} &= a X_2 (p_{\mathrm{tot}} - C) - dC \\
\frac{dm_Y}{dt} &= \alpha C - \gamma m_Y \\
\frac{dY}{dt} &= \beta m_Y - \delta Y.
\end{aligned}$$

Since all the binding reactions are much faster than mRNA and protein production and decay, we have that $k_1, k_2, a, d \gg \alpha, \beta, \gamma, \delta$. Let $k_{\mathrm{m}} := k_2/k_1$, $K_{\mathrm{d}} := d/a$, $c := k_2/d$, and $\epsilon := \delta/d$. Then, we can re-write the above system by using the substitutions

$$d = \frac{\delta}{\epsilon}, \; a = \frac{\delta}{K_{\mathrm{d}} \epsilon}, \; k_2 = c\frac{\delta}{\epsilon}, \; k_1 = c\frac{\delta}{k_{\mathrm{m}} \epsilon},$$

so that we obtain

$$\begin{aligned}
\epsilon \frac{dX_2}{dt} &= c\frac{\delta}{k_{\mathrm{m}}} X^2 - c\delta X_2 - \frac{\delta}{K_{\mathrm{d}}} X_2 (p_{\mathrm{tot}} - C) + \delta C \\
\epsilon \frac{dC}{dt} &= \frac{\delta}{K_{\mathrm{d}}} X_2 (p_{\mathrm{tot}} - C) - \delta C \\
\frac{dm_Y}{dt} &= \alpha C - \gamma m_Y \\
\frac{dY}{dt} &= \beta m_Y - \delta Y.
\end{aligned}$$

This system is in the standard singular perturbation form (3.6). As an exercise, the reader can verify that the slow manifold is locally asympotically stable (see Exercises). The slow manifold is obtained by setting $\epsilon = 0$ and determines X_2 and C as functions of X. These functions are given by

$$X_2 = \frac{X^2}{k_{\mathrm{m}}}, \qquad C = \frac{p_{\mathrm{tot}} X^2/(k_{\mathrm{m}} K_{\mathrm{d}})}{1 + X^2/(k_{\mathrm{m}} K_{\mathrm{d}})}.$$

As a consequence, the reduced system becomes

$$\begin{aligned}
\frac{dm_Y}{dt} &= \alpha \frac{p_{\mathrm{tot}} X^2/(k_{\mathrm{m}} K_{\mathrm{d}})}{1 + X^2/(k_{\mathrm{m}} K_{\mathrm{d}})} - \gamma m_Y \\
\frac{dY}{dt} &= \beta m_Y - \delta Y,
\end{aligned}$$

which is the familiar expression for the dynamics of gene expression with an activator as derived in Section 2.1. ∇

Example 3.15 (Enzymatic reaction)**.** Let's go back to 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

$$\frac{dE}{dt} = -aE\cdot S + dC + kC, \qquad \frac{dC}{dt} = aE\cdot S - (d+k)C, \tag{3.26}$$

$$\frac{dS}{dt} = -aE\cdot S + dC, \qquad \frac{dP}{dt} = kC. \tag{3.27}$$

By assuming that $a, d \gg k$, we obtained before that approximately $dC/dt = 0$ and thus that $C = E_{\mathrm{tot}}S/(S+K_m)$, with $k_{\mathrm{m}} = (d+k)/a$ and $dP/dt = V_{max}S/(S+k_{\mathrm{m}})$ with $V_{max} = kE_{\mathrm{tot}}$. From this, it also follows that

$$\frac{dE}{dt} \approx 0 \text{ and } \frac{dS}{dt} \approx -\frac{dP}{dt}. \tag{3.28}$$

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

$$\epsilon\frac{dE}{dt} = -\frac{k}{K_{\mathrm{d}}}E\cdot S + kC + \epsilon kC, \qquad \epsilon\frac{dC}{dt} = \frac{k}{K_{\mathrm{d}}}E\cdot S - kC - \epsilon kC,$$

$$\epsilon\frac{dS}{dt} = -\frac{k}{K_{\mathrm{d}}}E\cdot S + kC, \qquad \frac{dP}{dt} = kC.$$

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

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

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

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

One can verify (see Exercises) that in this system, the boundary layer dynamics is locally exponentially stable, so that setting $\epsilon = 0$ one obtains

$$C = \frac{E_{\text{tot}}(S_{\text{tot}} - P)}{(S_{\text{tot}} - P) + k_{\text{m}}} =: \gamma(P)$$

and thus that the reduced system is given by

$$\frac{dP}{dt} = V_{max} \frac{(S_{\text{tot}} - P)}{(S_{\text{tot}} - P) + k_{\text{m}}}.$$

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 law $S + C + P = S(0) = S_{\text{tot}}$, we obtain that $\frac{dS}{dt} = -\frac{dP}{dt} - \frac{dC}{dt}$, in which now $\frac{dC}{dt} = \frac{\partial \gamma}{\partial P}(P) \cdot \frac{dP}{dt}$. Therefore

$$\frac{dS}{dt} = -\frac{dP}{dt}(1 + \frac{\partial \gamma}{\partial P}(P)),\ S(0) = S_{\text{tot}} - \gamma(P(0)) - P(0) \tag{3.29}$$

and

$$\frac{dE}{dt} = -\frac{dC}{dt} = -\frac{\partial \gamma}{\partial P}(P)\frac{dP}{dt},\ E(0) = E_{\text{tot}} - \gamma(P(0)), \tag{3.30}$$

which are different from expressions (3.28).

These expressions are close to those in equation (3.28) only when $\partial\gamma/\partial P(P)$ is small enough. In the plots of Figure 3.28, 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 full model (solid line in Figure 3.28), $E(t)$ 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 QSSA, 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 dotted plots in Figure 3.28, 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, also the complex concentration C goes to zero (see solid line of Figure 3.28). At this time, the concentrations of complex and enzyme substantially change with time and the QSSA is unsatisfactory. By contrast, the reduced dynamics obtained from the singular perturbation approach well represent the dynamics of the full system even during this transient behavior. Hence, while the QSSA is a good approximation only as long as there is excess of substrate in the system, the reduced dynamics obtained by singular perturbation is a good approximation even when the substrate concentration goes to zero.

In Figure 3.29, we show the curve $C = \gamma(P)$ (in red) and the trajectories of the full system in black. All of the trajectories of the system immediately collapse into

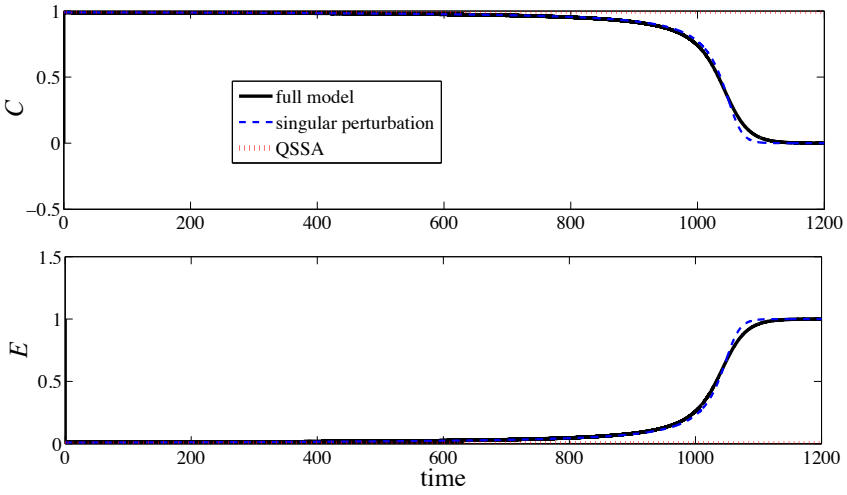


Figure 3.28: Simulation results for the enzymatic reaction comparing the approximations from singular perturbation and from the quasi-steady state approximation (QSSA). Here, we have $S_{\text{tot}} = 100$, $E_{\text{tot}} = 1$, $a = d = 10$, and $k = 0.1$. The full model is the one in equations (3.27).

an ϵ-neighbor of the curve $C = \gamma(P)$. From this plot, it is clear that $\partial\gamma/\partial P$ 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 QSSA 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 constant, but it is produced and decays according to the reaction $\text{Z} \underset{u(t)}{\overset{\delta}{\rightleftharpoons}}$. 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 Homework 1, 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 non-specific or specific binding of X* to target sites, to noise due to stochasticity of the cellular environment, and to other cross-talk phenomena. We will come back to these "disturbances" later during the course. For now, we can think of these distur-

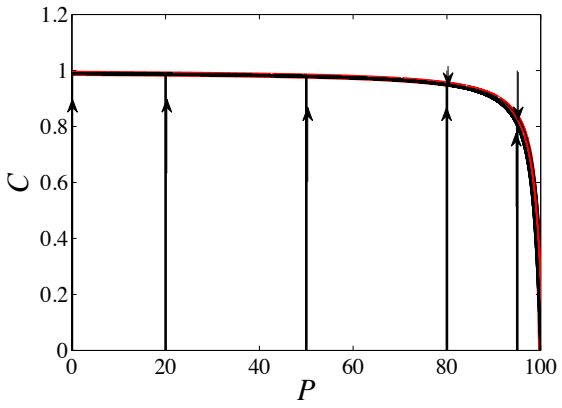


Figure 3.29: The slow manifold of the system $C = \gamma(P)$ is shown in red. In black, we show the trajectories of the the full system. These trajectories collapse into an ϵ-neighbor of the slow manifold. Here, we have $S_{\text{tot}} = 100$, $E_{\text{tot}} = 1$, $a = d = 10$, and $k = 0.1$.

bances 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

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

which is the same as you found in Homework 1, except for the presence of the disturbance $d(t)$. Assume that you can tune all the parameters in this system (we will see later that this is actually possible to large extent by suitably fabricating genetic circuits). 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 and justify your answer through a careful mathematical reasoning using the tools introduced in class.

3.3 (Adaptation) Show that the equation of the sniffer 3.15 can be taken into the standard integral feedback form through a suitable change of coordinates.

3.4 (Design limitations) This problem is meant to have you think about possible trade-offs and limitations that are involved in any realistic design question (we will come back to this when we start design). Here, we examine this through the open loop and negative feedback transcriptional component seen in class (see Figure 3-8 in the Lecture Notes). Specifically, we want to compare the robustness of these two topologies to cellular noise, crosstalk, and other cellular interactions. As performed in Problem 1, we model these phenomena 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

$$\dot{m} = \alpha_0 - \gamma m \qquad \dot{P} = \beta m - \delta P + d(t)$$

and the negative feedback system becomes

$$\dot{m} = \alpha_0 + \frac{\alpha}{K + P^n} - \gamma m \qquad \dot{P} = \beta m - \delta P + d(t).$$

Answer the following questions:

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

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

(c) Pick parameter values and use Matlab to draw Bode plots as the feedback gain increases and validate your predictions of (b). (Suggested parameters: $\gamma = 1, \delta = 1, K = 1, n = 1, \alpha\beta = \{1, 10, 100, 1000, ...\}$). Note: in Matlab, once you have determined the matrices A, B, C, and D for the linearization, you can just do: `SYS=ss(A,B,C,D); bode(SYS)` and the Bode plot will pop up.

(d) Investigate the answer to (c) when you have $\gamma = 20$, that is, the timescale of the mRNA dynamics becomes faster than that of the protein dynamics. What does change with respect to what you found in (c)? Note: when γ increases you are reducing the (phase) lag within the negative feedback loop...

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

3.5 (Bendixson criterion) Consider the possible circuit topologies of Figure 3.30, in which A and B are transcriptional components. Model each transcriptional component by a first order system, in which you have approximated the mRNA dynamics at the quasi-steady state. Hence, each topology will be represented by a dynamical system in the plane $\mathbb{R}^2$. Use Bendixson criterion to rule out topologies that cannot give rise to closed orbits.

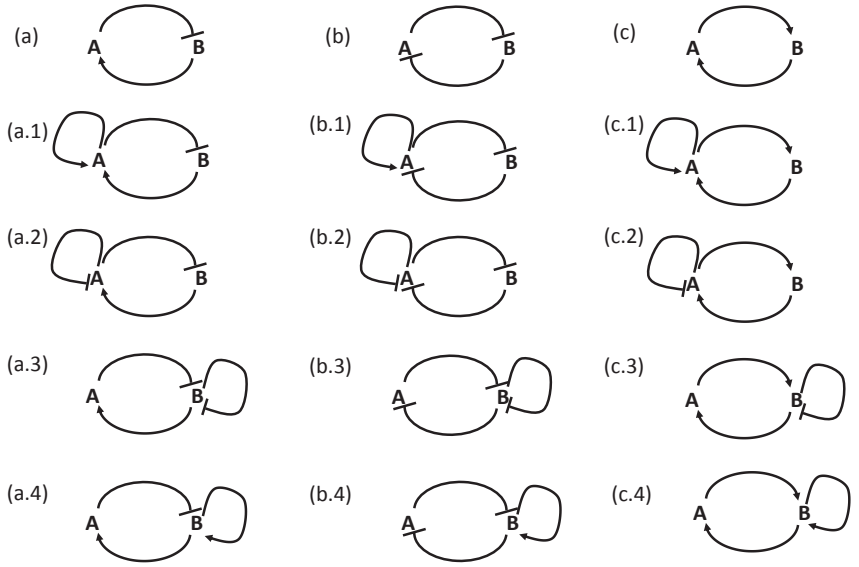


Figure 3.30: Circuit topologies with two components (proteins): A and B.

3.6 (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 ODE model corresponding to this system is given by

$$\frac{dm_A}{dt} = \frac{\alpha_0}{K_1 + R^n} - \gamma m_A \qquad \frac{dm_R}{dt} = \frac{\alpha A^m}{K_2 + A^m} - \gamma m_R$$
$$\frac{dA}{dt} = \beta m_A - \delta A \qquad \frac{dR}{dt} = \beta m_R - \delta R.$$

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

3.7 (Goodwin oscillator) Consider the simple set of reactions

$$\mathrm{X}_1 \xrightarrow{k} X_2 \xrightarrow{k} X_3 .... \xrightarrow{k} \mathrm{X}_\mathrm{n}.$$

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_1 to DNA). Neglecting the mRNA dynamics of X_1, write down the ODE 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 finding through simulation.

3.8 (Activator-repressor clock) A well known oscillating motif is given by the activator-repressor clock by Atkinson et al. [5] in which an activator protein A activates its own production and the one of a repressor protein R, which in turn acts as a repressor for A. The ODE model corresponding to this clock is given by

$$\frac{dm_A}{dt} = \frac{\alpha A^m + \alpha_0}{K_1 + R^n + A^m} - \gamma m_A \qquad \frac{dm_R}{dt} = \frac{\alpha A^m}{K_2 + A^m} - \gamma m_R$$
$$\frac{dA}{dt} = \mu(\beta m_A - \delta A) \qquad \frac{dR}{dt} = \beta m_R - \delta R,$$

in which $\mu > 0$ models the difference of speeds between the dynamics of the activator and that of the repressor. Indeed a key requirement for this system to oscillate is that the dynamics of the activator are sufficiently faster than that of the repressor. Demonstrate that this system goes through a Hopf Bifurcation with bifurcation parameter μ. Validate your findings with simulation by showing the small amplitude periodic orbit.

3.9 (Phosphorylation via singular perturbation) Consider again the model of a covalent modification cycle as illustrated in Chapter 2 in which the kinase Z is not constant, but it is produced and decays according to the reaction $\mathrm{Z} \underset{u(t)}{\overset{\delta}{\rightleftharpoons}} \emptyset$.

(a) Consider that $k_f, k_r \gg k_{\text{cat}}, \delta, u(t)$ and employ singular perturbation with small parameter, for example, $\epsilon = \delta/k_r$ to obtain the approximated dynamics of $Z(t)$ and $X^*(t)$. How is this different from the result obtained in Exercise 2.9? Explain.

(b) Simulate these approximated dynamics when $u(t)$ is a periodic signal with frequency ω and compare the responses of Z of this approximated dynamics to those obtained in Exercise 2.9 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:

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

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

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

3.12 (BE 150, Winter 2011; Based on Alon 4.6—Shaping the pulse) Consider a situation where X in an I1-FFL begins to be produced at time t=0, so that the level of protein X gradually increases. The input signal S_x and S_y are present throughout.

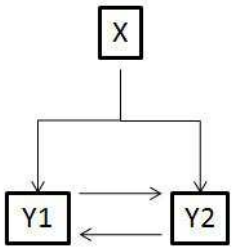
(a) How does the pulse shape generated by the I1-FFL depend on the thresholds K_{xz}, K_{xy}, and K_{yz}, and on β, the production rate of protein X? (i.e. How does increasing or decreasing these parameters change the height or position of the pulse peak, the slope of the rise of the pulse, etc?)

(b) Analyze a set of genes $Z_1, Z_2, ..., Z_n$, all regulated by the same X and Y in I1-FFLs. Design thresholds such that the genes are turned ON in the rising phase of the pulse in a certain temporal order and turned OFF in the declining phase of the pulse with the same order.

(c) Design thresholds such that the turn-OFF order is opposite the turn-ON order. Plot the resulting dynamics.

3.13 (BE 150, Winter 2011; Based on Alon 5.6—Bi-fan dynamics) Consider a bi-fan in which activators X_1 and X_2 regulate genes Z_1 and Z_2. The input signal of X_1, S_X2, appears at time t=0 and vanishes at time t=D. The input signal of X_2, S_X2, appears at time t=D/2 and vanishes at t=2D. Plot the dynamics of the promoter activity of Z_1 and Z_2 given that the input functions of Z_1 and Z_2 are AND and OR logic, respectively.

3.14 (BE 150, Winter 2011; Based on Alon 6.1—Memory in the regulated-feedback network motif) Transcription factor X activates transcription factor Y_1 and Y_2. Y_1 and Y_2 mutually activate each other. The input function at the Y_1 and Y_2 promoters is an OR gate (Y_2 is activated when either X or Y_1 binds the promoter). At time t=0, X begins to be produced from an initial concentration of X=0. Initially $Y_1 = Y_2 = 0$. All production rates are $\beta = 1$ and degradation rates are $\alpha = 1$. All of the activation thresholds are K=0.5. At time t=3, production of X stops.



(a) Plot the dynamics of X, Y_1, Y_2. What happens to Y_1 and Y_2 after X decays away?

(b) Consider the same problem, but now Y_1 and Y_2 repress each other and X activates Y_1 and represses Y_2. At time t=0, X begins to be produced and the initial

levels are $X = 0, Y_1 = 0, Y_2 = 1$. At time t=3, X production stops. Plot the dynamics of the system. What happens after X decays away?

3.15 (BE 150, Winter 2011; Repressilator) Simulate the following simplified version of the repressilator:

$$\frac{dm_1}{dt} = \frac{k_p}{1+(\frac{p_3}{K_M})^n} - k_{mdeg}m_1 \qquad \frac{dp_1}{dt} = k_{trans}m_1 - k_{pdeg}p_1$$
$$\frac{dm_2}{dt} = \frac{k_p}{1+(\frac{p_1}{K_M})^n} - k_{mdeg}m_2 \qquad \frac{dp_2}{dt} = k_{trans}m_2 - k_{pdeg}p_2$$
$$\frac{dm_3}{dt} = \frac{k_p}{1+(\frac{p_2}{K_M})^n} - k_{mdeg}m_3 \qquad \frac{dp_3}{dt} = k_{trans}m_3 - k_{pdeg}p_3$$

(a) Simulate the system using the following parameters: $k_p = 0.5, n = 2, K_M = 40, k_{mdeg} = 0.0058, k_{pdeg} = 0.0012, k_{trans} = 0.116$.

(b) Suppose the protein half-life suddenly decreases by half. Which parameter(s) will change and how? Simulate what happens. What if the protein half-life is doubled? How do these two changes affect the oscillatory behavior?

(c) Now assume that there is leakiness in the transcription process. How does the system's ODE change? Simulate the system with a small leakiness (say, 5e-3) and comment on how it affects the oscillatory behavior.

3.16 (BE 150, Winter 2011; Glycolytic oscillations) In almost all living cells, glucose is broken down into the cell's energy currency, ATP, via the glycolysis pathway. Glycolysis is autocatalytic in the sense that ATP must first be consumed in the early steps before being produced later and oscillations in glycolytic metabolites have been observed experimentally. We will look at a minimal model of glycolysis:

$$\frac{dX}{dt} = \frac{2Vy^a}{1+y^h} - kx \qquad \frac{dY}{dt} = (q+1)kx - q\frac{2Vy^a}{1+y^h} - 1$$

Note that this system has been normalized such that $Y_{ss} = 1$.

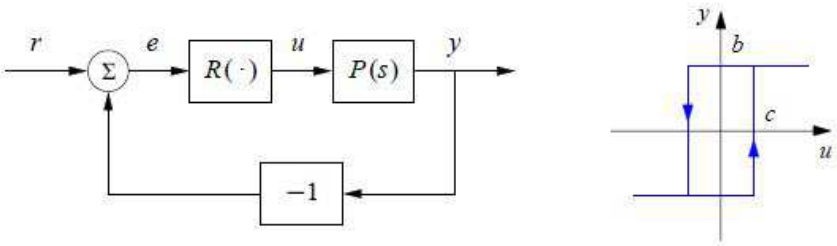
(a) While a system may have the potential to oscillate, the behavior still depends on the parameter values. The glycolysis system undergoes multiple *bifurcations* as the parameters are varied. Using linear stability analysis, find the parameter conditions where the system is stable vs. unstable. Next, find the conditions where the system has eigenvalues with nonzero imaginary parts.

(b) Let *q=k=V=1*. Find the relationship between *h* and *a* where the system is stable or not. Draw the stability diagram and mark the regions where the system is stable vs. unstable. In the same plot, mark the regions where the system has eigenvalues with nonzero imaginary parts.

(c) Let $q=k=V=1$. Choose h and a such that the eigenvalues are unstable and have nonzero imaginary parts. Use these parameter values and simulate the nonlinear system in MATLAB. Sketch the time response of the system starting with initial condition X(0) =1.2, Y(0) = 0.5 (you may use MATLAB or sketch by hand). Comment on what you see compared to what linear stability analysis told you about the system.

3.17 (BE 150, Winter 2011) Finding limit cycles for nonlinear systems and understanding how changes in parameters affect the amplitude and period of the oscillation is difficult to do in analytical form. A graphical technique that gives some insight into this problem is the use of *describing functions*, which is described in *Feedback Systems*, Section 9.5. In this problem we will use describing functions for a simple feedback system to approximate the amplitude and frequency of a limit cycle in analytical form.

Consider the system with the block diagram shown below. The block R is a relay



with hysteresis whose input/output response is shown on the right and the process transfer function is $P(s) = e^{-s\tau}/s$. Use describing function analysis to determine frequency and amplitude of possible limit cycles. Simulate the system and compare with the results of the describing function analysis.

3.18 (BE 150, Winter 2011) In this problem we will compare the model with single methylation site vs. double methylation sites. The model with a single methylation site is given by:

$$\frac{d(X+X*)}{dt} = V_R R - \frac{V_B B X*}{K+X*}$$

where the *activity* is given by $A = X*$. The model with two methylation sites is given by

$$\frac{d(X_2+X_2*)}{dt} = \frac{RV_R X_1}{X_1+X_0} - BV_B X_2*$$

$$\frac{d(X_1+X_1*)}{dt} = BV_B X_2 * + \frac{RV_R X_0}{X_1+X_0} - \frac{RV_R X_1}{X_1+X_0} - BV_B X_1*$$

$$\frac{dX_0}{dt} = -\frac{RV_R X_0}{X_0+X_1} + BV_B X_1*$$

and the activity is given by $A = X_1* + X_2*$. Let $K = 10, V_R R = 1, V_B B = 2$. Derive the parameter sensitivities of the activities ($\frac{dA}{dp_i}$) for both the single and double methylation models. Comment on which parameter each model is most robust and most sensitive to.

3.19 (BE 150, Winter 2011) Consider a toy model of protein production:

$$\frac{dm}{dt} = f(p) - \gamma m \qquad\qquad \frac{dp}{dt} = g(p) - \delta p$$

(a) Assume that there is transcriptional self-regulation ($f(p) = \frac{\alpha}{K+p^n}$). We now know that the mRNA transcription process and thus we want to understand the sensitivity with respect to the mRNA transcription rate α_0. Compute the transfer function from α to p. Plot this transfer function for $\alpha = 0.002, \beta_0 = 0.1, \gamma = 0.005, \delta = 0.001, K = 0.002$. Compare it with the transfer function from α_0 to p without regulation ($f(p) = \alpha_0 = 0.001$). (Note: As a reminder on how to compute these transfer functions, see BFS chapter 3 page 3-11).

(b) Now assume that there is no transcriptional regulation ($f(p) = \alpha_0$) but there is translational self-regulation such that $g(p) = \frac{\beta m}{K+p^n}$. Computer the transfer function from α_0 to p when $\beta = 0.2$. Compare again with the case with no regulation.

3.20 (BE 150, Winter 2011) Consider a simple model of chemotaxis:

$$\frac{dX_m}{dt} = k_R R + k^f(L) X_m^* - k^r X_m$$

$$\frac{dX_m^*}{dt} = -k_B B^p \frac{X_m^*}{K_{X_m^*} + X*_m} - k^f(L) X_m^* + k^r X_m$$

where X_m is the concentration of methylated receptor complex, and X_m^* is the concentration of activated, methylated receptor complex. Ligand concentration enters into the equation through the rate $k^f(L)$. In this model, $CheR$ (R) and $CheB^P$ (B^P) concentrations are constant. (BFS, Section 5)

(a) Pick parameter values such that $k_B B^p > k_R R$ and plot the dynamics, doubling the ligand concentration at time t=20. Compare to figure 5.12 in BFS.

(b) Now assume that CheR no longer acts in saturation. Rederive the dynamics and plot. Comment on how this assumption affects adaptation.